Structural and synthetic studies of sesquiterpenoids and flavonoids isolated from *Helichrysum* species

by

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Natura in minima maxima

Nature is the greatest in the smallest things

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– Gloria Patri –

Abstract

The genus *Helichrysum* (Asteraceae) consists of approximately 500 species worldw with 245 indigenous to South Africa. As a result of the large number of species, the chemistry and biological activity of several species have not yet been investigated. The aim of this project was to investigate the phytochemistry of three species and propose a synthetic route to one of the antibacterial compounds isolated.

An extensive literature review regarding the widespread traditional uses, biological activity and phytochemistry of the South African *Helichrysum* species is provided.

From *Helichrysum splendidum*, a plant used traditionally to treat rheumatism, two monomeric guaianolides and a dimeric guaianolide, helisplendidilactone, were isolated. The stereochemistry of these known compounds was confirmed and the NMR assignments for certain peaks of helisplendidilactone were corrected. An X-ray structure for helisplendidilactone was obtained for the first time.

The phytochemistry of *Helichrysum montanum* was investigated for the first time and new diastereoisomers of known guaianolides were isolated. The phytochemistry of *H. splendidum* and *H. montanum* is remarkably similar and supports their morphological classification in the same taxonomic group. The chloroform:methanol extract of *H. montanum* yielded a new dimeric guaianolide, 13'-epihelisplendidilactone, which is related to helisplendidilactone, as well as three monomeric guaianolides (of which one is a new diastereomer of a known compound). The extract also yielded spathulenol (a sesquiterpene), umbelliferone (a coumarin) and 4',5,7-trihydroxy-3,3',8-trimethoxyflavone (a flavonoid).

Thirty-five *Helichrysum* species were screened for antimicrobial activity against six micro-organisms and a preliminary cytotoxic assay, which included the use of "normal" and cancer cell lines, was performed. *H. excisum* was selected for further study based on the fact that it exhibited promising antimicrobial activity and relative low toxicity. Furthermore, with the exception of the essential oil, the phytochemistry of this species has not been investigated.

From the aerial parts of *H. excisum*, five flavonoids, identified as pinocembrin, gnaphaliin, lepidissipyrone, 5-hydroxy-7,8-dimethoxyflavone and isoscutellarein 7-*O*-β-glucoside were isolated. Four of these flavonoids have an unsubstituted B-ring, a phenomenon often observed in flavonoids isolated from *Helichrysum* species. The active antimicrobial component of *H. excisum* has been identified as lepidissipyrone.

Owing to the interesting biological activities reported for phloroglucinol α-pyrones and the synthetic challenges associated with these molecules, lepidissipyrone was selected for a synthetic study. Both the flavanone and pyrone moieties present in lepidissipyrone have been successfully synthesised. A successful strategy towards the CH_2 linker between the two units has been illustrated. The strategy could be used to synthesise similar phloroglucinol-derived pyrones.

Table of Contents

Chapter 3: The antimicrobial activity and cytotoxicity of selected *Helichrysum* extracts

Chapter 4: The phytochemistry of *Helichrysum splendidum*

List of Figures

Chapter 1: Why do Natural Product Research?

Chapter 2: South African *Helichrysum* species: A Review of the Traditional uses, Biological activity and Phytochemistry

Chapter 3: The antimicrobial activity and cytotoxicity of selected *Helichrysum* extracts

Chapter 4: The phytochemistry of *Helichrysum splendidum*

Chapter 5: The phytochemistry of *Helichrysum montanum*

Chapter 6: The antimicrobial activity and phytochemistry of *Helichrysum excisum*

Chapter 7: Synthetic approaches towards the synthesis of lepidissipyrone

List of Tables

List of Schemes

Chapter 4: The phytochemistry of *Helichrysum splendidum*

Chapter 5: The phytochemistry of *Helichrysum montanum*

Chapter 7: Synthetic approaches towards the synthesis of lepidissipyrone

List of abbreviations

AIDS	:	acquired immune deficiency syndrome
AM1	:	Austin Model 1
approx.	:	approximately
ATCC	:	American Type Culture Collection
BuLi	:	butyl lithium
Bn	:	benzyl
br	:	broad
calc.	:	calculated
CFU	:	colony forming units
cf.	:	probably
COSY	:	Correlation spectroscopy
d	:	doublet
DBU	:	1,8-diazabicyclo[5.4.0]undecene-7
dd	:	doublet of doublets
ddd	:	doublet of doublet of doublets
DMF	:	dimethylformamide
DMSO	:	dimethyl sulfoxide
ex.	:	collected by
Et	:	ethyl
FCS	:	fetal calf serum
ft.	:	feet above sea level
GC	:	gas chromatography
HIV	:	human immunodeficiency virus
HSQC	:	heteronuclear single quantum correlation
HMQC	:	heteronuclear multiple quantum correlation
INT	:	*p*-iodo-nitrotetrazolium violet
IR	:	infra red
LDA	:	lithium diisopropylamide
m	:	multiplet
Me	:	methyl

MEM	:	methoxyethoxymethyl ether
MIC	:	minimum inhibitory concentration
MOM	:	methoxy methyl
MS	:	mass spectrometry
NCI	:	National Cancer Institute (USA)
NCTC	:	National Collection of Type Cultures
NF-κB	:	nuclear factor-κB
NHLS	:	National Health Laboratory Service
NMR	:	nuclear magnetic resonance
NOESY	:	nuclear Overhauser effect spectroscopy
NS	:	not susceptible
NU	:	University of KwaZulu-Natal herbarium
PBS	:	phosphate buffer saline
Ph	:	phenyl
q	:	quartet
RPMI	:	Roswell Park Memorial Institute
RT	:	retention time
s	:	singlet
S.N.	:	unknown locality
SRB	:	sulforhodamine B
subsp.	:	subspecies
t	:	triplet
TBDMSCl	:	*t*-butyldimethylsilyl chloride
TCA	:	trichloroacetic acid
THF	:	tetrahydrofuran
TLC	:	thin layer chromatography
Tris	:	tris(hydroxymethyl)aminomethane
TSB	:	tryptone soya broth

CHAPTER 1

Why do Natural Product Research?

"A small collection of smart compounds may be more valuable than a much larger hodgepodge collection mindlessly assembled'' – S.J. Danishefsky

1.1 Natural products as drugs

Natural products formed the basis of most early medicines and still serve as an important source of new pharmaceutical agents today. Novel drugs approved for use in oncology, as anti-infectives, as anti-inflammatories and drugs used in the treatment of cardiovascular and metabolic diseases are often natural products, natural product derivatives, or inspired by natural products (Butler, 2005; Newman et al., 2003; Newman and Cragg, 2007; Wilson and Danishefsky, 2007).

The advent of computers, automation, robotics, the sequencing of the human and pathogenic genomes, and the introduction of high-throughput screens based on large numbers of molecular targets, led to a demand for huge libraries of compounds to satisfy the capacity of the screens. This meant that chemistry became the rate-limiting step in the drug discovery process (Newman et al., 2003; Newman and Cragg, 2007; Rouhi, 2003a). The tedious and expensive nature of traditional natural products isolation, legalities associated with source (e.g. plant) collection and difficult intellectual property issues surrounding compounds obtained from natural products are further deterrents associated with natural product research (Balunas and Kinghorn, 2005; Cordell, 1995). The advantages offered by rational drug design and combinatorial chemistry have therefore prompted most pharmaceutical companies to reduce or terminate their natural product drug discovery programmes (Butler, 2005; Gullo and Hughes, 2005, Newman et al., 2003; Newman and Cragg, 2007, Rouhi, 2003a).

Combinatorial chemistry has, however, failed as a source of *de novo* synthetic small molecules that were developed from hit to lead to approved drug. For the period of 1981-

2006, Newman and Cragg (2007) stated that "Although combinatorial chemistry in one or more of its manifestations has now been used as a discovery source for approximately 70% of the time covered by this review, to date, we can find only one *de novo* new chemical entity (NCE) reported in the public domain as resulting from this method of chemical discovery and approved for drug use anywhere". The decline in the number of new active substances (and new natural product templates) in the past decade correlates with a decreased interest in natural product drug discovery by pharmaceutical companies (Butler, 2005).

Despite the current low level of natural product drug discovery programmes of pharmaceutical companies, natural products still play an important role as a source of drugs. The recent survey by Newman and Cragg (2007) showed that approximately 70% of the 974 small-molecule NCE's introduced as drugs during 1981-2006 can be traced to or were inspired by natural products. Even the best-selling drug, atorvastatin (Lipitor), is a direct descendent of a natural product (Newman and Cragg, 2007). Natural products feature in high percentages especially in approvals as antimicrobials (77% of small chemical entities) and anticancer drugs (78% of small chemical entities).

The reason for the success of natural products as drugs can be attributed to their unique structural characteristics. These compounds have diverse skeletons, often contain numerous stereogenic centres (associated with stereospecific binding), frequently have many rings (fused and bridged), and are therefore rigid. This rigidity is associated with stronger protein binding due to a thermodynamic advantage over more flexible compounds that can form the same pattern of hydrogen bonds and hydrophobic interactions. They generally contain more nitrogen and oxygen atoms than combinatorial compounds, which is important for hydrogen bonding during ligand-receptor binding. Another interesting difference between natural products and combinatorial compounds is their lipophilicity, a property that affects the pharmacokinetics of a drug. Combinatorial compounds are generally more hydrophilic than both drugs and natural products (Feher and Schmidt, 2003; Lee and Schneider, 2001).

Although still labour intensive, practical difficulties associated with natural product drug discovery are increasingly overcome by advances in screening, separation and structural elucidation technologies. Smaller biotechnology companies in particular, are exploring

natural resources as drug candidates, filling the gap left by large pharmaceutical companies (Gullo and Hughes, 2005; Rouhi, 2003a).

1.2 Plants as sources of drugs

In a 2000 report by Newman et al., it was stated that approximately 119 chemical substances obtained from 90 plant species were important drugs in one or more countries. Of these, 74% was the result of chemical studies directed at isolation of active components from plants used in traditional medicine (Newman et al., 2000). Plants provided humankind with some of the best-known medicines, including quinine, aspirin, and morphine while important new drugs such as taxol and vincristine were both recently isolated from plants (Van Wyk and Wink, 2004; Balunas and Kinghorn, 2005). In 2006 for example, the botanical preparation Hemoxin® was approved as a treatment for sickle cell anaemia in Nigeria. This preparation includes a mixture of plants directly derived from indigenous knowledge and may be classified as a true "ethnobotanical preparation". According to Meinwald, of the 250 000 described flowering plants, only about 10% have been examined chemically and many have been investigated decades ago, when techniques were relatively crude (Rouhi, 2003b).

South Africa has a remarkable biodiversity with more than 30 000 species of flowering plants (accounting for 10% of the world's higher plants), 3000 of which are used medicinally (Van Wyk et al., 2000; Van Wyk and Gericke, 2000). World renowned South African plant remedies include Cape aloes (*Aloe ferox*), "buchu" (*Agathosma betulina*) and devil's claw (*Harpagophytum procumbens*). If the remarkable number of South African plant species is taken into account, the almost complete absence of drug leads obtained from South African plants is quite surprising. Two examples of lead compounds obtained from South African plants are that of the appetite suppressant P57 isolated from *Hoodia gordonii* (Van Heerden et al., 2007; Van Wyk and Wink, 2004) and anticancer compound combretastatin, isolated from *Combretum caffrum* (Griggs et al., 2001; Tron et al., 2006) (Fig. 1.1).

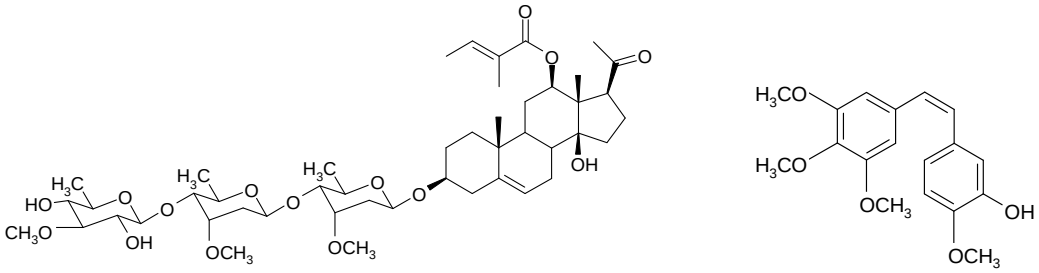


Figure 1.1 Structures of P57 from *Hoodia gordonii* and combretastatin from *Combretum caffrum*

1.3 Plants as sources of antimicrobials and resistance-modifying agents

There is a vast amount of literature available on plant-derived antimicrobial compounds. Compound classes with antimicrobial activity include terpenes, phenylpropanoids and stilbenoids, simple phenols and tropolones, flavonoids, alkaloids, polyketides, poly-ynes, sulfur-containing compounds, and acylphloroglucinols. Despite the large amount of literature available on the antibacterial activity of extracts and their compounds, no single plant-derived chemical entity is used clinically as an anti-staphylococcal agent - most likely because of perceived resupply issues (Gibbons, 2004).

It is postulated that secondary metabolites are produced by plants as defence against microbes and other enemies in their environment (Schreiber, quoted by Rouhi, 2003a)**,** thus explaining the presence of antimicrobial compounds. Micro-organisms, however, have the natural ability to acquire resistance against antimicrobials and this may have caused plants to evolve biosynthetic routes to synthesise compounds which evade these multi-drug resistant mechanisms. Consequently, compounds isolated from plants may play an important role as resistance-modifying agents, acting for instance on membrane proteins responsible for efflux of antibiotics out of cells that create ineffective intracellular concentrations of drugs (Gibbons, 2004). For example, epicatechin gallate, a polyphenolic constituent of green tea inhibits the synthesis of penicillin binding protein 2', thus reversing methicillin resistance in *Staphylococcus aureus* (Yam et al., 1998; Anderson et al., 2005).

Significant characteristics associated with compounds that modify multi-drug resistance include a large size and high degree of lipophilicity, important for solubility in bacterial membranes and binding to efflux transporters (Gibbons, 2004). Isoflavones isolated from *Lupinus argenteus*, for example, have been shown to potentiate the activity of berberine and norfloxacin by inhibiting multi-drug resistance (MDR) pumps (Morel et al., 2003).

1.4 Why investigate the genus *Helichrysum*?

The Asteraceae is the largest plant family in southern Africa with 253 genera encompassing 2253 species. In this family, there are relatively few large genera and only three genera (representing 1% of the Asteraceae) have more than 100 taxa, namely *Senecio* L., *Helichrysum* Mill and *Euryops* Cass. (Koekemoer, 1996). In South Africa, the genus *Helichrysum* encompass a large number of species that is used traditionally, often in conditions associated with infections. There are numerous reports on the antimicrobial activity and other activities of extracts of these species and the vast and interesting number of compounds isolated from the South African *Helichrysum* species. However, due to the large number of species and the taxonomic complexity of the genus, the phytochemistry and biological activity of many species have not been investigated. Furthermore, most of the phytochemical research was done in the 1970's and 1980's by the group of Bohlmann and often no connection was established between compounds isolated and any biological activity (See Chapter 2 for an extensive literature review).

1.5 Natural product synthesis

Compound availability has in the past been a serious concern associated with natural product-based drug discovery (Wilson and Danishefsky, 2007). The low supply, especially in the case of anti-infectives, may be overcome by using recombinant technology (biosynthesis) or by organic synthesis. Total synthesis, semi-synthesis and in some cases, manipulation of biosynthesis, also provide ways to obtain natural product derivatives with improved therapeutic indexes and enables the assembly of compounds inspired by the original natural product (Gullo and Hughes, 2005; Nicolaou and Snyder, 2004; Wilson and Danishefsky, 2007). By combining synthesis and a process known as "dynamic combinatorial screening" for example, a set of vancomycin dimers was synthesised that

showed improved activity when compared to vancomycin, an antibiotic used against methicillin-resistant strains of *S. aureus* (Nicolaou and Snyder, 2004). In the synthesis of cycloproparadicicol (inspired by the natural resorcinylic macrolide, radicicol), the replacement of an epoxide functionality with a cyclopropyl group resulted in improved *in vivo* anticancer activity (Wilson and Danishefsky, 2007).

A complex total synthesis of a natural product may lead to the additional reward of developing novel methodologies and still provides the absolute proof of the assigned structure. For example, efforts directed towards the total synthesis of quinine led to the knowledge of the construction of heteroaromatic systems, while attempts to synthesise progesterone provided insights into the formation and cleaving of carbon-carbon bonds (Nicolaou and Snyder, 2004). In recent years, there has been a huge increase in the capacity of chemical synthesis. These advances include recognising the importance transition metals and the generation of enantiopure substances through reagent control. Due to advances made in synthetic methodology, synthesis of compounds with a high level of complexity is now possible and timelines for accomplishing total syntheses have been significantly improved. Creative approaches during retrosynthetic analysis, such as "pattern recognition" using the concept of identifying substructural motifs, have further enabled medicinal chemists to effectively synthesise complex natural products, such as the anticancer agent migrastatin (Wilson and Danishefsky, 2007).

In 2003 and again in 2007, Newman and Cragg reported that there was a tendency to move away from large combinatorial libraries and emphasis was placed on small, focused collections that were structurally more natural product-like in terms of their combination of heteroatoms and significant numbers of stereogenic centers within a single molecule. It is postulated that scaffolds of natural origin, which presumably have undergone evolutionary selection over time, might confer favourable bioactivities and bioavailibilities to library members (Rouhi, 2003c, Nicolaou and Snyder, 2004). Small combinatorial libraries have yielded derivatives of taxol for example (Xiao et al., 1997), while a combinatorial library of 10 000 structures based on the privileged structure of the 2,2-dimethylbenzopyran scaffold that occurs widely in nature and are associated with a wide range of biological activities was recently successfully completed (Nicolaou et al., 2000). A possible solution to the low yield of small novel chemical entities approved as drugs might lie in this

approach that combines the advantages offered by nature with those offered by synthetic and combinatorial chemistry and rational drug design.

1.6 Aims of this study

This investigation aims to combine plant natural product research with organic synthesis, with the main focus on the South African species of the medicinally important genus *Helichrysum*.

The main aims of the project are:

- To isolate and identify the secondary metabolites of three South African *Helichrysum* species, namely *H. splendidum*, *H. montanum* and *H. excisum*.
- To identify biologically-active components present in *Helichrysum* species
- To develop synthetic methodologies for the synthesis of a bio-active molecule isolated from *Helichrysum*.

1.7 References

Anderson, J.C., Headley, C., Stapleton, P.D., Taylor, P.W., 2005. Asymmetric total synthesis of B-ring modified (-)-epicatechin gallate analogues and their modulation of β-lactam resistance in *Staphylococcus aureus*. Tetrahedron 61, 7703–7711.

Balunas, M.J., Kinghorn, A.D., 2005. Drug discovery from medicinal plants. Life Sciences 78, 431-441.

Butler, M.S., 2005. Natural products to drugs: natural product derived compounds in clinical trials. Natural Product Reports 22, 162-195.

Cordell, G.A., 1995. Changing strategies in natural products chemistry. Phytochemistry 40, 1585-1612.

Feher, M., Schmidt, J.M., 2003. Property distributions: differences between drugs, natural products, and molecules from combinatorial chemistry. Journal of Chemical Information and Computer Sciences 43, 218-227.

Gibbons, S., 2004. Anti-staphylococcal plant natural products. Natural Product Reports 21, 263-277.

Griggs, J., Metcalfe, J.C., Hesketh, R., 2001. Targeting tumour vasculature: the development of combretastatin A4. The Lancet Oncology 2, 82–87.

Gullo, V.P., Hughes, D.E., 2005. Exploiting new approaches for natural product drug discovery in the biotechnology industry. Drug Discovery Today: Technologies 2, 281-286.

Koekemoer, M., 1996. An overview of the Asteraceae of Southern Africa. In: Compositae: Systematics Eds. Hind, D.J.N., Beentjie, H.J., The Royal Botanic Gardens, Kew. pp. 95-110.

Lee, M-L., Schneider, G., 2001. Scaffold architecture and pharmacophoric properties of natural products and trade drugs: Application in the design of natural product-based combinatorial libraries. Journal of Combinatorial Chemistry 3, 284-289.

Morel, C.C., Stermitz, F.R., Tegos, G., Lewis, K., 2003. Isoflavones as potentiators of antibacterial activity. Journal of Agriculture and Food Chemistry 51, 6577-6579.

Newman, D.J., Cragg, G.M., Snader, K.M., 2000. The influence of natural products upon drug discovery. Natural Product Reports 17, 215-234.

Newman, D.J., Cragg, G.M., 2007. Natural products as sources of drugs over the last 25 years. Journal of Natural Products 70, 461-477.

Newman, D.J., Cragg, G.M., Snader, K.M., 2003. Natural products as sources of new drugs over the period 1981-2002. Journal of Natural Products 66, 1022-1037.

Nicolaou, K.C., Pfefferkorn, J.A., Roecker, A.J., Cao, G.-Q., Barluenga, S., Mitchell, H.J., 2000. Natural product-like combinatorial libraries based on privileged structures. 1. General principles and solid-phase synthesis of benzopyrans. Journal of the American Chemical Society 122, 9939-9953.

Nicolaou, K.C., Snyder, S.A., 2004. The essence of total synthesis. Proceedings of the National Academy of Sciences of the United States of America 101, 11929-11936.

Rouhi, A.M., 2003a. Rediscovering natural products. Chemical and Engineering News 81, 77-78, 82-83, 86, 88-91.

Rouhi, A.M., 2003b. The case for natural product research. Chemical and Engineering News 81, 77-78, 82-83, 86, 88-91.

Rouhi, A.M. 2003c. Moving beyond natural products. Chemical and Engineering News 81, 104-107.

Tron, G.C., Pirali, T., Sorba, G., Paliai, F., Busacca, S., Genazzani, A.A., 2006. Medicinal chemistry of combretastatin A4: Present and future directions. Journal of Medicinal Chemistry 49, 3033-3044.

Van Heerden, F.R., Horak R.M., Maharaj, V.J., Vleggaar, R., Senabe, J.V., Gunning, P.J., 2007. An appetite suppressant from *Hoodia* species. Phytochemistry 68, 2545–2553.

Van Wyk, B-E., Gericke, N., 2000. People's Plants. A Guide to Useful Plants of Southern Africa, 1st ed. Briza, Pretoria, p. 166.

Van Wyk, B-E., Van Oudtshoorn, B., Gericke, N., 2000. Medicinal Plants of South Africa, 2nd ed. Briza, Pretoria, p.148.

Van Wyk, B-E., Wink, M., 2004. Medicinal Plants of the World. Briza, Pretoria, p. 171.

Wilson, R.M., Danishefsky, S.J., 2007. Pattern recognition in retrosynthetic analysis: snapshots in total synthesis. Journal of Organic Chemistry 72, 4293-4305.

Xiao, X-Y., Parandoosh, Z., Nova, M.P., 1997. Design and synthesis of a taxoid library using radiofrequency encoded combinatorial chemistry. Journal of Organic Chemistry 62, 6029-6033.

Yam, T., Hamilton-Miller J., Shah S., 1998. The effect of a component of tea (*Camellia sinensis*) on methicillin resistance, PBP2'synthesis, and β-lactamase production in *Staphylococcus aureus*. Journal of Antimicrobial Chemotherapy 42, 211-216.

CHAPTER 2

South African *Helichrysum* species: A review of the traditional uses, biological activity, and phytochemistry

2.1 Introduction

The genus *Helichrysum* Mill. derives its name from the Greek words *helios* (sun) and *chrysos* (gold) which is appropriate considering the attractive yellow flowers displayed by several species (Pooley, 2003). It belongs to the family Asteraceae, tribe Inuleae and subtribe Gnaphaliinae (Hilliard, 1983). This large genus consists of approximately 500 species and although *Helichrysum* species are also found in southern Europe, south-west Asia, southern India, Sri Lanka (previously Ceylon), and Australia, most species occur in Africa, including Madagascar (Hilliard, 1983). In South Africa (including Namibia), the ca. 244-250 species are widely distributed and the tremendous morphological diversity displayed by these species resulted in their division into 30 morphological groups, using the shape and size of the flower heads as differentiating characteristics (Hilliard, 1983). The flower heads occur as either solitary or in compact or spreading inflorescences. The aerial parts are usually hairy or woolly and plants occur as herbs or shrublets that are sometimes dwarfed and cushion forming. They are also often aromatic (Pooley, 1998; Pooley, 2003; Van Wyk et al., 2000).

In South Africa, the genus is widely used in traditional medicine. The uses are well documented although renaming of species and the resulting confusing taxonomic nomenclature may cause uncertainty as to which specific species was referred to in some reports. The chemistry of the genus has been studied extensively and there are several reports on the biological activity diplayed by extracts and compounds isolated from South African species.

The aims of this chapter are:

- To present a collated and coherent overview of the documented traditional uses of South African *Helichrysum* species and the compounds isolated from them.
- To summarise biological activities of extracts (excluding essential oils) and isolated compounds from South African *Helichrysum* species.

2.2 Traditional uses

Several *Helichrysums* are widely used in Southern African traditional medicine as summarised in Table 2.1. The first written record of the medicinal use of *Helichrysum* dates back to 1727 when Boerhaave (Professor of Botany and Medicine at the University of Leiden from 1701 to 1738) noted that a *Helichrysum* species was used to treat nervousness and hysteria. The report of a *Helichrysum* species in the early literature could have been based on knowledge acquired from the local Khoi and San people, but is most probably due to the fact that European botanists used their knowledge of medicinal properties of European genera (Scott and Hewett, 2008). In European medicine, flower heads from *Helichrysum arenarium* (*Helichrysi flos*; synon. *Flores Stoechados citrinae* or *Flores Gnaphalii arenarii*) are used to treat peptic discomfort and are also used as an additive to tea mixtures, mainly to improve their appearance. This diuretic tea has value in the supportive treatment of cholecystitis and spastic disorders of the gall bladder and urinary tract (Van Wyk and Wink, 2004). Other species such as *H. angustifolium*, *H. italicum* and *H. stoechas* are well known in folk medicine and are used for their anti-inflammatory and anti-allergenic properties (Carini et al., 2001).

2.2.1 Ambiguities in nomenclature

As is the case for all ethnobotanical data, the fact that plant names are changed (Germishuizen and Meyer, 2003) and frequently incorrectly cited (Arnold et al., 2002) is quite problematic. To complicate matters further, variation in spelling of names also occurs. Special care needs to be taken to consult the original texts to unambiguously confirm that a plant selected for a particular study is in fact the same species cited by, for example, Watt and Breyer-Brandwijk (1962). In Table 2.1, current names are given and

previously accepted names are shown in parenthesis. For the sake of clarity, the name as it appears in the reference is sometimes indicated in brackets after the reference.

In some cases, one species name was changed to another, for example *H. adscendens* Less. var. *cephaloideum* Moeser. in Watt and Breyer-Brandwijk (1962) is now known as *H. cephaloideum* DC. In other instances, a *Helichrysum* species now belongs to a different genus for example, *H. capillaceum* (Thunb.) Less (Watt and Breyer-Brandwijk, 1962) is now classified as *Troglophyton capillaceum* subsp. *capillaceum* (Hilliard, 1983).

Sometimes the same species name with only a different author name refers to a different species, for example *H. calocephalum* Schltr., which is now classified as *H. ecklonis* and not *H. calocephalum* Klatt (Germishuizen and Meyer, 2003; Gibbs Russell et al., 1987). Batten and Bokelmann, (1966), Jacot Guillarmod, (1971), Phillips, (1917) and Watt and Breyer-Brandwijk (1962) all used *H. calocephalum* Schltr., which is now recognised as *H. ecklonis*, but in Arnold et al. (2002) there is no reference to *H. ecklonis* yet the above-mentioned sources are used as references under *H. calocephalum* Klatt.

The specific *Helichrysum* species referred to when *H. crispum* is used in ethnobotanical literature is also ambiguous. Germishuizen and Meyer (2003) stated that *H. crispum* of authors other than (L.) D Don is *H. patulum* (L.) D. Don. and not *H. crispum* (L.) D. Don. In Watt and Breyer-Brandwijk (1962) and Smith (1966), the name appears as *H. crispum* Less. therefore indicating *H. patulum*, although Arnold et al. (2002) cited the name *H. crispum* (L.) D. Don (with reference to Smith, 1966) as well as *H. patulum* with reference to Watt and Breyer-Brandwijk (1962). Roberts (1990) used *H. crispum* without an author name, causing uncertainty as to which particular species is referred to; the cited medicinal uses are however similar to those indicated by Watt and Breyer-Brandwijk (1962) for *H. crispum* Less. Salie and co-workers (1996) determined that *H. crispum* (L.) D. Don had moderate (10 mg/ml) antimicrobial activity against *Pseudomonas aeruginosa*. Both Salie et al. (1996) and Swanepoel (1997) use the name *H. crispum* (L.) D. Don., but when indicating its traditional uses refer to Watt and Breyer-Brandwijk (1962). Scott et al. (2004) showed that *H. patulum* had antimicrobial activity against *Staphylococcus aureus* in the disc diffusion assay that was comparable to that of the ciprofloxacin control, while the traditional uses indicated correspond very well to those of Watt and Breyer-Brandwijk for

H. crispum Less. Both species occur in the same region making exclusion of one species on the basis of distribution impossible.

H. pedunculare DC. is another name with an unfortunate and confusing history. In this case, it seems that *H. pedunculare* DC. in ethnobotanical literature could refer to either *H. pedunculatum* Hilliard and Burtt or *H. nudifolium* var *pilosellum* (previously known as *H. pedunculare* (L.) DC. var. *pilosellum,* (Germishuizen and Meyer, 2003; Hilliard, 1983; Arnold et al., 2002). The vernacular name and uses indicated by e.g. Watt and Breyer-Brandwijk (1962) for *H. pedunculare* DC. and Bhat and Jacobs (1995) for *H. pedunculatum* Hilliard and Burtt. are similar. According to Hilliard (1983), *H. pedunculare* (L.) DC. is also a synonym for *H. odoratissimum* (L.) Sweet.

In some instances it is impossible to decide to which species an author refers to, for example *H. agrostophilum* Klatt (Watt and Breyer-Brandwijk, 1962) that was in part changed to *H. pallidum* DC. and in part to *Helichrysum griseum* Sond (Germishuizen and Meyer, 2003).

2.2.2 Administration routes

Plant parts used include the leaves, stems, flowers, roots, and sometimes the whole plant. The plant remedies are administered in different ways, including the preparation of teas, inhalation of smoke and vapours, and placement of leaves in the form of a poultice on wounds to prevent infection (Table 2.1). Several of these species are known by the same vernacular names, for example *H. cymosum*, *H. nudifolium*, *H. odoratissimum* and *H. petiolare* are all known as *imphepho* which indicates that they can be used interchangeably, as Van Wyk et al., (2000), noted that “use often depends on local availability rather than preference for a particular species”.

2.2.3 Traditional uses of South African Helichrysum species

The traditional uses of *Helichrysum* in South Africa are summarised in Table 2.1. There are several recurring South African traditional uses for plants from this genus. Smoke is often inhaled to induce trances or to invoke the goodwill of the ancestors. They are often used to treat respiratory conditions and leaves are often applied as wound dressings. They are used in the treatment of gastro-intestinal disorders such as abdominal pain and colic and also eye conditions. They also seem to have an effect on the relief of pain and

inflammation as they are used to treat menstrual pain, rheumatism, and headaches. The plants are used to fumigate huts and also used as bedding to repel insects.

2.2.4 Correlation between medicinal uses and morphological groups

Plants from almost all morphological groups are used medicinally and the broad spectrum of uses seems to cross the different morphological groups. In some cases there does seem to be a relationship between the morphological group (according to Hilliard, 1983) or adjacent morphological classes and the traditional uses of these plants. *H. epapposum* (Group 3), *H. natalitium* (Group 3) *H. gymnocomum* (Group 4) and *H. odoratissimum* (Group 4) all share the Zulu/Xhosa name *imphepho* and all are burnt as incense to invoke the goodwill of the ancestors. However, this particular use applies to many species for example, *H. cymosum* (Group 8), *H. petiolare* (Group 18), *H. dregeanum* (Group 30) and in some sources *H. nudifolium* (Group 23) share the same vernacular name and use. *H. cymosum* (Group 8), *H. kraussii* (Group 8), *H. melanacme* (Group 8), *H. athrixiifolium* (Group 9) and *H. dregeanum* (Group 9) are all used to treat respiratory complaints such as coughs and colds. The administration route of these remedies does however vary; for *H. arthrixiifolium* the leaf is smoked, for *H. kraussii* the dried flowers and seeds are smoked in a pipe, for *H. cymosum* a decoction of leaves is drunk, for *H. dregeanum* the leaf is smoked and the administration route is not indicated in the source for *H. melanacme*.

South African species are not often used to treat heart and kidney ailments. Both *H. pandurifolium* and *H. patulum* belongs to Group 18, and are indicated in the treatment of kidney disease and heart disorders. Both are also used to treat backpain and respiratory conditions by the same administration route. Plants from Groups 23 and 24 are often used to treat wounds. The leaves of *H. miconiifolium* (Group 23), *H. nudifolium* (Group 23), *H. pedunculatum* (Group 23), *H. appendiculatum* (Group 24) and *H. longifolium* (Group 24) are all used as wound dressings. However, *H. foetidum* (Group 30) is mentioned as a replacement for *H. pedunculatum* in the treatment of circumcision wounds (Gerstner, 1938). The species constituting Group 23 are also used for respiratory conditions including, *H. mundtii*, *H. nudifolium* and *H. pedunculatum*. Root decoctions of both *H. adenocarpum* and *H. ecklonis* belonging to Group 28 are used to treat diarrhoea in children, while a root infusion from *H. argyrophyllum* (Group 29) is used to treat intestinal troubles. It is interesting to note that the only two species indicated for the treatment of snakebite both belong to Group 30, namely *H. cooperi* and *H. setosum*.

2.3 Phytochemistry

The chemistry of this genus is complex with a wide variety of chemical classes occurring as is illustrated in the three major publications by Bohlmann and Jakupovic (Bohlmann et al., 1980b; Jakupovic et al., 1986; Jakupovic et al., 1989b) in which the phytochemistry of a total of 63 South African *Helichrysum* species was investigated. The classes of compounds isolated from the South African *Helichrysum* species are summarised in Table 2.2 and Figures 2.1 to 2.11. Acylphloroglucinols are commonly found, often with prenyl or geranyl side chains. The replacement of the cinnamic acid starter unit by other acyl CoA derivatives in the biosynthesis of the main constituents seems to be characteristic (Jakupovic et al., 1989b). The appearance of humulone derivatives, such as helihumulone (**49**) is also widespread (Jakupovic et al., 1989b).

Flavonoids derived from phloroglucinol are very common and often have unsubstituted B rings (Bohlmann and Abraham, 1979a; Bohlmann and Abraham, 1979d; Jakupovic et al., 1986) which is a characteristic feature of plants from the Inuleae tribe (Harborne, 1977). The presence of 6- and 8-hydroxyflavonols and their methyl ethers are also frequent as in other members of the tribe (Harborne, 1977). A wide variety of chalcones are also found, including those substituted with prenyl or geranyl groups, dihydrochalcones and pyranochalcones. As in other Inuleae species, these chalcones are often accompanied by their structurally and biogenetically related flavanones (Harborne, 1977) as can be seen for *H. acutatum* (Bohlmann and Abraham, 1979c), *H. cymosum* (Jakupovic et al., 1989b) and *H. oreophilum* (Jakupovic et al., 1986).

The presence of α-pyrones is rather common (they occur in plants from morphologic Groups 1, 2, 4, 12, 15, 18, 19 and 24) and are often isolated from the roots of these plants (Hänsel et al., 1980; Jakupovic et al., 1986; Jakupovic et al., 1989b). Different types of diterpenes occur; these include the kaurenoic acid type (Jakupovic et al., 1989b) as well as helifulvenic acid derivatives (Bohlmann et al., 1980a). Sesquiterpenes from a variety of skeletal types occur, as is characteristic for the rest of the family (Hegnauer, 1977). Some skeletal types, such as the humulenes, are widely distributed across the genus, whereas others such as the guaianolides are restricted to a few species (morphological Groups 10 and 22). *Helichrysum* species are known for their aromaticity and a variety of

monoterpenes are reported in the essential oils of some species (Lourens et al., 2004; Van Vuuren et al., 2006; Frum and Viljoen, 2006; Asekun et al., 2007). Squalene (**309**) is the most common triterpene found and is often present in high concentration.

Other unusual compounds that occur is thiophene derivatives which have been isolated from the roots of these plants (*H. acutatum* and *H. tenuifolium*). These thiophenes are the result of addition reactions of a common chloro-acetylene precursor with H_2S (Bohlmann and Abraham, 1979b; Bohlmann and Abraham, 1979c). Simple polyacetylenes are widespread and acetylenics with pyran and furan moieties, some with epoxy and/or chlorine substitution, occur in these plants and are characteristic of the Gnaphalineae (Harborne, 1977).

As for the traditional uses, one particular class of compound is not restricted to a particular morphological group. However, there are some compounds that occur mainly in a particular morphological class. For example, phloroglucinols (excluding those belonging to the flavonoid class) feature as major compounds in morphological classes 2, 3, 4, 12, 14, 15, 20, 24 and 28. Flavonoids are present in basically all morphologic groups, but a large number are found in plants from Groups 8, 9 and 27. Diterpenes were isolated in large quantities from species in Groups 23, 25 and 30 (all 10 plants investigated in this group had this type of compound as the major chemical species). *H. umbraculigerum* from Group 5 seems to be the only species investigated that contains compounds of the cannabigerol type as the major constituent and *H. dasyanthum* (Group 10) and *H. splendidum* (Group 22) contain mainly sesquiterpenes of the guaianolide type (which is absent in the other species). Plants from Groups 6, 18 and 19 are also rich in sesquiterpenes (Table 2.2).

Although there seem to be similarities in the chemistry of the European and South African species, the Australian species are chemically different from their South African counterparts (Jakupovic et al., 1989a; Jakupovic et al., 1989b).

2.4 Biological activity

2.4.1 Anti-infective activity

Considering the traditional uses of this genus (specifically the treatment of wounds and respiratory infections and the application as a fumigant for example), there seems to be a strong indication that these plants and their compounds should exhibit antimicrobial activity. A large amount of antimicrobial work on *Helichrysum* species was done by the group of Meyer from the University of Pretoria, South Africa. Extracts of several species (Table 2.1) were submitted to antibacterial testing using a group of randomly selected bacteria which normally included the Gram-positive bacteria: *Bacillus cereus*, *Bacillus pumilis*, *Bacillus subtilis*, *Micrococcus kristinae, Staphylococcus aureus,* and the Gram-negatives: *Enterobacter cloaceae, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Serratia marcescens.* Acetone extracts were mostly tested, but in a few cases methanol, water and dichloromethane extracts were used. Assays involving agar dilution and direct autobiography were employed (Afolayan and Meyer, 1997; Bremner and Meyer, 2000; Dilika et al., 1997; Mathekga and Meyer, 1998; Mathekga et al., 2000; Mathekga, 2001; Meyer and Dilika, 1996; Meyer and Afolayan, 1995).

For example, when dichloromethane, acetone, methanol and water extracts of *H. aureonitens* were tested against the above-mentioned micro-organisms, the water extract displayed no activity whereas the dichloromethane extract showed the most promising activity against Gram-positive organisms such as *B. cereus* and *M. kristinae* at a concentration of 0.5 mg/ml (Meyer and Afolayan, 1995, Mathekga, 2001). Since 0.5 mg/ml was the lowest concentration tested, the MIC for this extract could in fact be lower than that. An acetone extract of *H. caespititium* was active against all the above-mentioned organisms at 1 mg/ml (the highest concentration tested) except *K. pneumoniae* and *S. marcescens* (Mathekga et al., 2000). Acetone extracts of *H. appendiculatum, H. argyrosphaerum, H. aureonitens, H. bellum, H. caespititium, H. callicomum, H. candolleanum, H. chionosphaerum, H. decorum, H. glomeratum, H. herbaceum, H. hypoleucum, H. kraussii, H. longifolium, H. melanacme, H. miconiifolium, H. montanum, H. monticola, H. nudifolium, H. odoratissimum, H. oreophilum, H. pilosellum, H. psilolepis, H. rugulosum, H. simillimum, H. sutherlandi, H. trilineatum,* and *H. umbraculigerum* were tested against the same organisms and again activity was mainly

observed against the Gram-positive bacteria. *K. pneumoniae* and *S. marscecens* were resistant to all the extracts. *H. glomeratum, H. montanum, H. monticola, H. oreophilum* and *H. pilosellum* were all inactive at the maximum concentration tested against all the bacterial species used in the assay (Mathekga and Meyer, 1998; Mathekga, 2001). Antifungal activity was also determined for the 28 acetone extracts mentioned above. Extracts were tested against *Aspergillus flavus, Aspergillus niger, Cladosporium cladosporioides, Cladosporium cucumerinum, Cladosporium sphaerospermum* and *Phytophthora capsici* (Mathekga, 2001). The most active extracts were those of *H. caespititium, H. callicomum, H. glomeratum* (which showed no antibacterial activity), *H. hypoleucum, H. melanacme, H. oreophilum,* and *H. rugulosum,* all of which had MIC's of 0.01 mg/ml against one or more of the fungal species tested (Mathekga, 2001).

A dichloromethane extract of *H. pedunculatum* displayed activity against three *Bacillus* species (10 mg/ml), *M. kristinae* (10 mg/ml), *S. aureus* (20 mg/ml) and the Gram-negative *E. cloaceae* (50 mg/ml) after incubation on agar plates (Meyer and Dilika, 1996). The lowest concentration tested was 10 mg/ml which may indicate that lower concentrations could still inhibit the growth of these micro-organisms. Antibacterial activity was also observed against *S. aureus* for both *H. pedunculatum* and *H. longifolium* with a direct autobiography method on thin-layer chromatograms (2 mg of extract applied). It is however difficult to deduct qualitative data from this paper (Dilika et al., 1997). Gerstner (1938) stated that *H. foetidum* is sometimes used as an alternative for *H. pedunculatum*. Methanol and water extracts of *H. foetidum* had MIC's of more than 4 mg/ml against *S. aureus, Streptococcus pyogenes, E. coli* and *P. aeruginosa* in the 96-well plate assay (Steenkamp et al., 2004) indicating that this plant has weak (Gibbons, 2004) *in vitro* antibacterial activity.

In a disc diffusion assay, extracts of solvents with different polarity of *H. crispum* displayed antibacterial activity against the Gram-negative *P. aeruginosa, Mycobacterium smegmatis,* and the yeast *Candida albicans,* but was surprisingly enough inactive against *S. aureus*. MIC's of 10 mg/ml were reported against *P. aeruginosa* and *C. albicans* (Salie et al., 1996).

Activity ranging from 0.078 mg/ml – 0.3 mg/ml against Gram-positive and Gram-negative bacteria and yeasts was also reported for a *H. cymosum* extract (Van Vuuren et al., 2006).

Acetone and methanol extracts of *H. odoratissimum* (incorrectly identified as *H. dasyanthum* in Lourens et al., 2004), *H. excisum*, *H. felinum*, and *H. petiolare* displayed activity against *S. aureus* and *B. cereus*. The species with the best activity was the acetone extract of *H. odoratissimum* with an MIC of 0.016 mg/ml against *S. aureus* (which correlates well with the values obtained by Matheka and Meyer, 1998).

Helichrysum species are often used to treat respiratory conditions and tuberculosis (Table 2.1). Extracts of *H. odoratissimum* and *H. melanacme* showed activity against *Mycobacterium tuberculosis* at concentrations of 0.5 mg/ml (Lall and Meyer, 1999; Lall et al., 2006). The acetone extract of *H. caespititium* inhibited a drug-sensitive strain of *M. tuberculosis* at a concentration of 0.5 mg/ml in the agar plate method and a MIC of 0.1 mg/ml was observed using the rapid radiometric method (Meyer et al., 2002). The water extract caused partial inhibition at the highest concentration of 5 mg/ml.

In some cases the antimicrobial activity of isolated compounds was determined. Flavonoids are generally one of the largest classes of antibacterial compounds (Gibbons, 2004). Galangin (**54**) isolated from *H. aureonitens* (Meyer and Afolayan, 1995), inhibited the growth of four Gram-positive bacteria (three *Bacillus* species and *M. kristinae*) as well as the Gram-negative *E. cloaceae* (Afolayan and Meyer, 1997). The highest activity observed was against *B. cereus, M. kristinae* and *E. cloaceae* at 0.1 mg/ml. In other studies by Cushnie et al. (2003) and Cushnie and Lamb (2006), the activity of galangin (**54**) was shown against six strains of β-lactam sensitive and resistant strains of *S. aureus* and 16 strains of 4-quinolone resistant strains of the bacterium at MIC's of approximately 50 μg/ml. Galangin (**54**) also displays some antifungal activity against fungi such as *Aspergillus tamari* (35% growth inhibition at 0.5 mg/ml) (Afolayan and Meyer, 1997). These results support the use of *H. aureonitens* in the treatment of skin infections, often caused by *S. aureus*.

Another flavonoid, 3-*O*-methylquercetin (**64**) was isolated from *H. odoratissimum* and antimicrobial activity determined for a broad range of micro-organisms including Gram-negative bacteria such as *Salmonella typhimurium* (MIC = 50 μg/ml), Gram-positive bacteria, such as *S. aureus* (MIC = 6.25 μg/ml) and fungi, for example *C. albicans* (MIC = 12.5 μg/ml), in the microdilution method (Van Puyvelde et al., 1989). Bremner and Meyer

(1998) also reported on the anti-staphylococcal activity for pinocembrin chalcone (**22**) (isolated from *H. trilineatum*), as well as pinocembrin (**1**) that was obtained as an artifact during the isolation procedure.

Flavonoids (**21**, **35**) isolated from the flowers of *H. gymnocomum* exhibited promising antimicrobial activity against a wide variety of Gram-positive and Gram-negative organisms as well as yeasts. An MIC of 8 μg/ml was for example observed against *Cryptococcus neoformans* for 5,7-dibenzyloxyflavanone (**35**) (Drewes and Van Vuuren, 2008). Two chalcones (**23**, **39**) isolated from *H. melanacme* exhibited MIC's of 0.05 mg/ml against the drug sensitive H37Rv strain of *M. tuberculosis*. The activity of the chalcones was higher than the extract but a combination of the two chalcones did not result in an improved MIC (Lall et al., 2006).

A study on the structure-activity relationships responsible for the anti-staphylococcal activity of flavonoids indicated that chalcones are generally more active than flavones, which has similar or better activity than flavanones. The most important structural feature for activity is the presence of the α,β-unsaturated carbonyl group (in chalcones and flavones) that favours π-electron delocalization of phenyl ring B. Furthermore, a hydroxy group at postion 2' of chalcones and 5 of flavones improves antibacterial activity (Alcaráz et al., 2000). A hydroxy group exerts an effect opposite to that of a methoxy group, while the presence of OH groups induce and enhance activity against methicillin resistant *S. aureus*, replacing them with methoxy groups drastically reduces or even eliminates anti-staphylococcal activity (Alcaráz et al., 2000).

There are also reports on the antimicrobial activity of compounds other than flavonoids. Activity against Gram-positive bacteria was observed for both linoleic and oleic acids, isolated from antibacterial extracts of *H. pedunculatum* (a plant used to treat circumcision wounds, Dilika et al., 2000). The MIC of both fatty acids was 1.0 mg/ml for *S. aureus* and *M. kristinae* in the agar diffusion assay. The MIC's was 0.05 mg/ml of each fatty acid when they were administered at the same time (Dilika et al., 2000).

Kaurenoic acid (**195**) (a diterpene), isolated from *H. kraussii*, exhibited a MIC as low as 1 μg/ml against *E. coli* and MIC's of 10 μg/ml against *B. cereus, B. subtilis, S. aureus* and *S.*

marcescens (Bremner and Meyer, 2000). Significant antimicrobial activity was also observed for monomeric and dimeric diterpenes (**228-232**) from *H. tenax* var *tenax.* MIC values as low as 3.1 and 3.6 µg/ml were determined against *B. cereus* whereas MIC's as low as 41.5 µg/ml were determined for a Gram-negative organism such as *P. aeruginosa* (Drewes et al., 2006).

MIC's of 100 µg/ml were observed for a prenylated butyrylphloroglucinol (**90**) isolated from *H. kraussii* against *B. cereus, B. pumilis, B. subtilis, M. kristinae, S. aureus, S. marcescens* and *E. coli* in an agar diffusion assay (Bremner and Meyer, 2000). The same phloroglucinol analogue (**90**) was isolated from *H. gymnocomum* and MIC's of below 100 µg/ml (6 - 45 µg/ml) were reported for *Enterococcus faecalis, Staphylococcus epidermidis, S. aureus, B. cereus, P. aeruginosa, C. neoformans,* and *C. albicans* (Drewes and Van Vuuren, 2008). A difference in assays employed, inoculum size and possibly different strains of the micro-organism used may account for the observed difference in activity. A structurally related phloroglucinol (**102**) also exhibited promising antibacterial activity against *E. faecalis, S. aureus P. aeruginosa,* and *C. neoformans* (Drewes and Van Vuuren, 2008). Caespitin (**127**) and caespitate (**98**) (both phloroglucinols) exhibited antimicrobial activity against several bacteria as well as fungi (Dekker et al., 1983; Mathekga et al., 2000). Caespitin (**127**) was active against *S. aureus, Streptococcus pyogenes, C. neoformans, Trichophyton rubrum, T. mentagrophytes,* and *Microsporum canis* although neither the method used, nor the level of activity, are indicated in the relevant article (Dekker et al., 1983). Caespitate (**98**) exhibited antibacterial activity against the Gram-positive *B. cereus, B. pumilis, B. subtilis, M. kristinae* and *S. aureus* at concentrations of 0.5 µg/ml in the agar dilution method (Mathekga et al., 2000). This compound also exhibited antifungal activity which ranged from 0.5 - 1.0 µg/ml against *A. flavus, A. niger, C. cladosporioides, C. cucumerinum, C. sphaerospermum,* and *Phytophtora capsici* (Mathekga et al., 2000). Caespitate (**98**) was also active against several *M. tuberculosis* strains at a concentration of 0.1 mg/ml which was similar to the MIC observed for the crude extract of *H. caespititium* (Meyer et al., 2002). Several caespitin derivatives were synthesised with MIC values as low as 2 µg/ml against *Staphylococcus aureus* and *Streptococcus pyogenes*. These compounds also exhibit antifungal activity. The possible development of antimicrobial resistance was examined as well as the development of cross resistance with known antimicrobials (Van der Schyff et al., 1986).

For helihumulone (**49**), a phloroglucinol derivative of the humulone type, activity was exhibited for a broad range of micro-organisms with some promising results, for example 16 µg/ml against *P. aeruginosa*. The antimalarial activity of this compound was determined to be 15 µg/ml (Van Vuuren et al., 2006). As previously mentioned, the South African *Helichrysums* contain a large amount of phloroglucinol derivatives and considering the promising antimicrobial activity observed for this type of compound, it seems a class well worth investigating.

Aqueous extracts of *H. aureonitens* exhibited antiviral activity against the *Herpes simplex* virus type I *in vitro* at a concentration of 1.35 mg/ml (Meyer et al., 1996). The flavone, galangin (**54**), isolated from this plant also exhibited antiviral activity against *H. simplex* virus type I and the *Coxsackie* virus at concentrations of 6 µg/ml (Meyer et al., 1997). The antiviral activity was also determined for a crude ethanolic extract of *H. melanacme* and its isolated constituents. The activities of the isolated prenylated chalcone (**23**) and a pyranochalcone (**39**) were lower (IC_{50} = 0.1 mg/ml) against the *Influenza A* virus than that of the crude extract (0.01 mg/ml) although a combination of the two chalcones resulted in an improved IC_{50} (0.01 mg/ml, Lall et al., 2006).

In summary, the crude extracts generally show some degree of antimicrobial activity, which is usually higher against Gram-positive organisms than against Gram-negative organisms. Although the antibacterial and antifungal activities of these plants are well documented, antimalarial, antimycobacterial, and antiviral data are scarce. Isolated compounds sometimes exhibit more superior activity when compared to the crude extract, but often the crude extract has similar activity. Correct identification of plant material is crucial as misidentification of plant material can lead to incorrect reporting (Lourens et al., 2004). The selected range of concentrations is often on the high side (Gibbons, 2004, considered values of below 1 mg/ml for extracts and 64 µg/ml for single chemical entities as significant); for example a range of 10-100 mg/ml was used for *H. pedunculatum* extracts (Meyer and Dilika, 1996). Positive controls (antibiotics) are absent in some of the assays (Mathekga et al., 2000), making it difficult to comparatively assess the activity of a particular extract or compound. The fact that different assays are employed impairs comparison of data between different laboratories (assays relying on diffusion are especially suspect since a low rate of diffusion would present a low activity, which is not

always a true representation). Microbial strains are often not referenced and the number of colony forming units not mentioned (Meyer and Afolayan, 1995). Extracts also often do not dissolve completely in the solvents used and as Cushnie and co-workers (2003) illustrated with galangin (**54**), this can have a profound effect on the MIC's observed (Cushnie et al., 2003). Chemical classes such as the flavonoids, acylphloroglucinols, and diterpenes from South African *Helichrysum* species exhibit promising antimicrobial activity and plants that contain these compounds seems potential candidates for further study.

2.4.2 Other biological data

Unpublished work done by Noristan laboratories indicates that fractions of the extract of *H. caespititium* exhibits anti-inflammatory activity of up to 82% at 360 mg/kg in the carrageenan test done on rats and prevents platelet aggregation (Swanepoel, 1997). Ethanolic extracts of *H. subglomeratum* and *H. nudifolium* inhibited cyclo-oxygenase enzymes *in vitro* by 69% and 96% (50 µg of plant extract used) respectively, indicating inhibition of prostaglandin synthesis (Jäger et al., 1996). The group at Noristan determined that fractions of a *H. nudifolium* extract also reduced edema in the carrageenan assay by approximately 30% at 300 mg/kg in rats (Swanepoel, 1997). These results indicate that *H. nudifolium* has both *in vitro* and *in vivo* anti-inflammatory activity, probably partly due to the inhibition of the cyclo-oxygenase enzymes.

The group at Noristan observed that the second of three fractions obtained after gradient column chromatography (using petroleum ether, ethyl acetate and methanol) of a dichloromethane/methanol extract from *H. panduratum* showed a 79% reduction in pain experienced in the writhing pain test at 500 mg/kg. Edema was also reduced by 50% in the carrageenan test indicating that this plant has both anti-inflammatory and analgesic properties. It was also antihypertensive (a reduction of 6% in mean blood pressure was observed after administering a dose of 300 mg/kg) and weakly antimicrobial (Swanepoel, 1997).

A fraction from a dichloromethane/methanol extract of *H. petiolare* investigated by the Noristan group determined that administration of 300 mg/kg of extract to mice reduced mean blood pressure by 21% and resulted in a 6% reduction in heart rate (Swanepoel, 1997). Acetone extracts of *H. excisum* (IC_{50} = 35 µg/ml) and *H. felinum* (IC_{50} = 39 µg/ml)

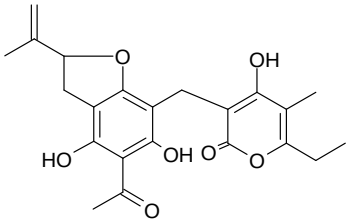
inhibited the 5-lipoxygenase enzyme which also plays a role in inflammation. Antioxidant activity (as indicated with the DPPH assay) of acetone and methanol extracts of *H. odoratissimum, H. excisum, H. felinum* and *H. petiolare* was comparable to that of vitamin C, as expected for species rich in phenolic compounds (Lourens et al., 2004).

European research further highlights the antioxidant and anti-inflammatory effects displayed by plants from this genus. It is quite often the flowers that are investigated, a plant part that are seldom investigated in South African research (Drewes and Van Vuuren, 2008; Table 2.2). Anti-oxidant activity was reported for flower extracts from *H. stoechas* (Carini et al., 2001), *H. arenarium* (Czinner et al., 2000; Czinner et al., 2001) and *H. italicum* (Facino et al., 1990). *In vivo* (topical) anti-inflammatory activity comparable to that of the indomethacin standard was observed for an acetophenone derivative, gnaphaliin (**67**) and ursolic acid isolated from *H. stoechas* (Recio et al., 1991). *In vivo* and *in vitro* anti-inflammatory activity was also observed for acetophenone glucosides, flavonoids, and other compounds isolated from *H. italicum* (Sala et al., 2001; Sala et al., 2002; Sala et al., 2003a, Sala et al., 2003b) as well as for extracts from *H. compactum* (Süzgeç, et al., 2005). These promising results indicate that more research should be undertaken on the anti-inflammatory activity of South African species, as many similar compounds appear in the South African and European species.

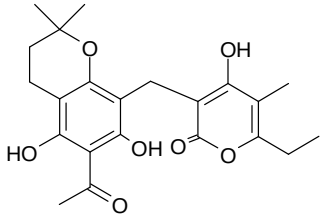
As previously mentioned, *Helichrysum* species are often burnt as incense to invoke the goodwill of the ancestors, in protective and other charms, and to induce trances. It is also used in the treatment of insanity, possession, used as a sedative to treat insomnia, and as a protective cleanser (Table 2.1). Their traditional uses indicate that these plants may exhibit psychotropic effects. Stafford et al. (2005) determined the GABA-receptor binding effect of extracts from *H. argyrolepis, H. herbaceum, H. nudifolium, H. ruderale, H. rugulosum, H. simillimum,* and *H. umbraculigerum* by using the ^{3}H-Ro 15-1788 binding assay. *H. ruderale* and *H. umbraculigerum* exhibited the most pronounced effects, while *H. herbaceum, H. rugulosum,* and *H. simillimum* showed moderate to good dose dependant activity.

There appears to be a large divide between the rich chemical data available and biological testing on compounds isolated from the South African species. One chemical class, the α-pyrones will be discussed as an example. By our rough estimate, 28 different pyrones were

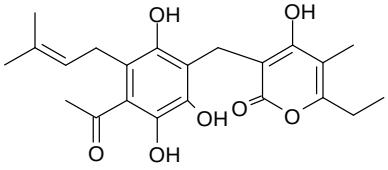
isolated from South African *Helichrysum* species. The same class of compound was isolated from European species and rather interesting biological activity was observed. Italipyrone (**370**), plicatipyrone (**371**), and a mixture of homoarenol (**372**) and arenol (**373**) were all active against *B. subtilis*, *S. aureus*, *S. epidermidis*, and *Mycobacterium phlei* using the agar diffusion method with the highest MIC being 25 μg/ml and the lowest 3 μg/ml (Ríos et al., 1991).



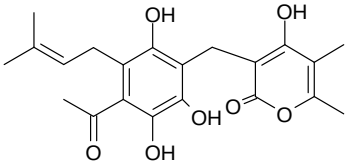
370



371

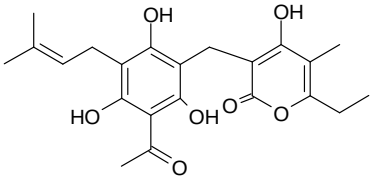


372

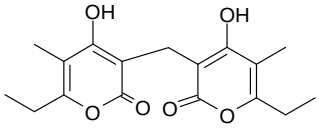


373

Antifungal activity was also reported for α-pyrones isolated from *H. decumbens* (Tomás-Lorente et al., 1989). Pyrones [like arzanol (**374**) and helipyrone (**375**)] showed significant anti-oxidant activity and arzanol was not toxic at all concentrations tested (Rosa et al., 2007). Most interesting though, are the findings by Appendino et al. (2007) that arzanol (**374**) inhibits HIV-I replication in T-cells and inhibited NF-κB (IC50 = 5 μg/ml) indicating that this group of compounds may exhibit both antiviral and anti-inflammatory properties. To our knowledge, none of the unique pyrones isolated from South African species were evaluated for biological activity.



374



375

Most concerning is the almost complete absence of toxicity data for the South African species of this genus. In very few cases, for example where antiviral and antimalarial activity was determined (Lall et al., 2006; Meyer et al., 1996; Meyer et al., 1997; Van Vuuren et al., 2006) toxicity is mentioned. Toxicity of the diterpenes is well known (for example IC_{50} values of below 4 µg/ml was reported for three diterpene lactones from *Parinari capensis*; Uys et al., 2002), and several *Helichrysum* species contain high amounts of these compounds. Furthermore, Reid et al. (2006) screened 42 medicinal South African plants for mutagenicity, which included *H. herbaceum*, *H. nudifolium*, *H. ruderale*, *H. rugulosum*, *H. simillimum*, and *H. umbraculigerum*. The only three plants that showed mutagenic activity were all *Helichrysums*, namely *H. herbaceum* (at 5 mg/ml), *H. rugulosum* (at 5 mg/ml), and *H. simillimum* (at 0.05 mg/ml). These results highlight both the need and importance of toxicity and safety data for plants of this genus. In general, there also seems to be a large need for *in vivo* validation of *in vitro* results since the effectiveness of these extracts and their compounds have not been validated in living organisms.

2.5 Conclusion

Helichrysum species are used extensively in ethnomedicine in South Africa and many of the uses are associated with the treatment of infections, e.g. it is used widely for treatment of respiratory diseases and wound dressing (Table 2.1). The large morphological diversity of the genus is mirrored by the diversity of chemical compounds isolated from the genus. However, determining the biological activity of the plant extracts and isolated compounds has, up to now, not received the same attention as the phytochemical studies. Furthermore, there are sometimes confusion as to exactly which species have been used and in any studies based on reported traditional uses, the identity of the species should be verified first. There is an interesting relationship between the morphological classification and the classes of chemical compounds isolated from a specific morphological group and there are certain classes of compounds, e.g. diterpenes, guaianolides, acylated phloroglucinols and α-pyrone derivatives, for which one can predict in which species they are most likely to occur. This may be important in the search of new plant-derived drugs, e.g. acylated phloroglucinols show potential as anti-staphylococcal drug leads (Gibbons, 2004) and α-pyrone derivatives have anti-HIV properties (McClacken and Fairlamb, 2005; Appendino

et al., 2007). It is clear that *Helichrysum* is an interesting genus from an ethnobotanical, phytochemical and pharmacological perspective but that biological data to correlate the ethnobotany to the chemistry is often still lacking. To complete the picture, a multidisciplinary approach involving botanists, chemists and biologists is required.

2.6 References

Afolayan, A.J., Meyer, J.J.M., 1997. The antimicrobial activity of 3,5,7-trihydroxyflavone isolated from the shoots of *Helichrysum aureonitens.* Journal of Ethnopharmacology 57, 177-181.

Alcaráz, L.E., Blanco, S.E., Puig, O.N., Tomás, F., Ferretti, F.H., 2000. Antibacterial flavonoids against methicillin resistant *Staphylococcus aureus* strains. Journal of Theoretical Biology 205, 231-240.

Appendino, G., Ottino, M., Marquez, N., Bianchi, F., Giana, A., Ballero, M., Sterner, O., Fiebich, B.L., Munoz, E., 2007. Arzanol, an anti-inflammatory and anti-HIV-1 phloroglucinol α-pyrone from *Helichrysum italicum* ssp. *microphyllum*. Journal of Natural Products 70, 608-612.

Arnold, T.H., Prentice, C.A., Hawker, L.C., Snyman, E.E., Tomalin, M., Crouch, N.R., Pottas-Bircher, C., 2002. Medicinal and Magical Plants of Southern Africa: an Annotated Checklist. National Botanical Institute, Pretoria, pp. 32-34.

Asekun, O.T., Grierson, D.S., Afolayan, A.J., 2007. Characterization of essential oils from *Helichrysum odoratissimum* using different drying methods. Journal of Applied Sciences 7, 1005-1008.

Batten, A., Bokelmann, H., 1966. Wild flowers of the Eastern Cape Province. Books of Africa, Cape Town, pp.149-160.

Bhat, R.B., Jacobs, T.V., 1995. Traditional herbal medicine in Transkei. Journal of Ethnopharmacology 48, 7-12.

Bohlmann, F., Abraham, W-F., 1979a. Neue Diterpene und weitere Inhaltsstoffe aus *Helichrysum calliconum* und *Helichrysum heterolasium.* Phytochemistry 18, 889-891.

Bohlmann, F., Abraham, W-F., 1979b. Neue, chlorsubstituierte Thiophenacetylenverbindungen mit ungewöhnlicher Struktur aus *Helichrysum-arten*. Phytochemistry 18, 839-842.

Bohlmann, F., Abraham, W-R., 1979c. Neue Diterpene aus *Helichrysum acutatum*. Phytochemistry 18, 1754-1756.

Bohlmann, F., Abraham, W-F., 1979d. Neue Prenylflavanone aus *Helichrysum hypocephalum*. Phytochemistry 18, 1851-1853.

Bohlmann, F., Abraham, W-F., Sheldrick, W.S., 1980a. Weitere Diterpene mit Helifulvan-gerüst und andere Inhaltsstoffe aus *Helichrysum chionosphaerum*. Phytochemistry 19, 869-871.

Bohlmann, F., Ates, N., 1984. Three prenylated flavanoids from *Helichrysum athrixiifolium*. Phytochemistry 23, 1338-1339.

Bohlmann, F., Hartono, L., Jakupovic, J., 1985. A diterpene related to erythroxydiol from *Helichrysum refluxum*. Phytochemistry 24, 611-612.

Bohlmann, F., Hoffmann, E., 1979. Cannabigerol-ähnliche Verbindungen aus *Helichrysum umbraculigerum*. Phytochemistry 18, 1371-1374.

Bohlmann, F., Knauf, W., Misra, L.N., 1984a. Structures and synthesis of chlorophenol derivatives from *Helichrysum* species. Tetrahedron 40, 4987-4989.

Bohlmann, F., Mahanta, P.K., 1979. Weitere Phloroglucin-derivate aus *Helichrysum gymnoconum*. Phytochemistry 18, 348-350.

Bohlmann, F., Mahanta, P.K., Zdero, C., 1978a. Neue Chalkon-derivate aus Südafrikanischen *Helichrysum*-arten. Phytochemistry 17, 1935-1937.

Bohlmann, F., Misra, L.N., 1984. New prenylflavanones and chalcones from *Helichrysum rugulosum*. Planta Medica 50, 271-272.

Bohlmann, F., Misra, L.N., Jakupovic, J., 1984b. Weitere Phloroglucin- und α-pyrone-derivate aus *Helichrysum* arten. Planta Medica 50, 174-176.

Bohlmann, F., Suwita, A., 1978. Neue Phloroglucin-derivate aus *Leontonyx*-arten sowie weitere Verbindungen aus verretern der Tribus Inuleae. Phytochemistry 17, 1929-1934.

Bohlmann, F., Suwita, A., 1979a. Weitere Phloroglucin-derivate aus *Helichrysum*-arten. Phytochemistry 18, 2046-2049.

Bohlmann, F., Suwita, A., 1979b. Ein neues Guajanolid und ein Secoguajanolid aus *Helichrysum splendidum*. Phytochemistry 18, 885-886.

Bohlmann, F., Zdero, C., 1973. Über ein neues Azulen aus *Helichrysum bracteatum* (Vent.) Willd. Chemische Berichte 106, 1337-1340.

Bohlmann, F., Zdero, C., 1979. Neue Phloroglucin-derivate aus *Helichrysum natalitium* und *Helichrysum bellum*. Phytochemistry 18, 641-644.

Bohlmann, F., Zdero, C., 1980a. Neue Phloroglucin-derivate aus *Helichrysum*-arten. Phytochemistry 19, 153-155.

Bohlmann, F., Zdero, C., 1980b. Neue Geranylphloroglucin-derivate aus *Helichrysum monticola*. Phytochemistry 19, 683-684.

Bohlmann, F., Zdero, C., 1983. Flavanones from *Helichrysum thapsus*. Phytochemistry 22, 2877-2878.

Bohlmann, F., Zdero, C., Abraham, W-R., Suwita, A., Grenz, M., 1980b. Neue Diterpene und neue Dihydrochalcon-derivate sowie weitere Inhaltsstoffe aus *Helichrysum* arten. Phytochemistry 19, 873-879.

Bohlmann, F., Zdero, C., Hoffmann, E., Mahanta, P.K., Dorner, W., 1978b. Neue Diterpene und Sesquiterpene aus Südafrikanischen *Helichrysum*-arten. Phytochemistry 17, 1917-1922.

Bohlmann, F., Zdero, C., Zeisberg, R., Sheldrick, W.S., 1979a. Helifulvanolsäure - ein neues Diterpen mit anomalem Kohlenstoffgerüst aus *Helichrysum fulvum*. Phytochemistry 18, 1359-1362.

Bohlamnn, F., Zdero, C., Ziesche, J., 1979b. Neue Flavone und Phloroglucin-derivate aus *Helichrysum herbaceum* und *Helichrysum chrysargyrum*. Phytochemistry 18, 1375-1378.

Bohlmann, F., Ziesche, J., 1979. Ein ungewöhnliches Tetrahydrofuran-derivat aus *Helichrysum aureo-nitens*. Phytochemistry 18, 664-665.

Bohlmann, F., Ziesche, J., Mahanta, P.K., 1979b. Neue Chalkon-derivate und humulon-ähnliche Verbindungen aus *Helichrysum* arten. Phytochemistry 18, 1033-1036.

Bremner, P.D., Meyer, J.J.M., 1998. Pinocembrin chalcone: an antibacterial compound from *Helichrysum trilineatum*. Planta Medica 64, 777.

Bremner, P.D., Meyer, J.J.M., 2000. Prenyl-butyrylphloroglucinol and kaurenoic acid: two antibacterial compounds from *Helichrysum kraussii*. South African Journal of Botany 66, 115-117.

Candy, H.A., Laing, M., Weeks, C.M., 1975. The crystal structure of helichrysoside, a new acylated flavonoid glycoside from *Helichrysum kraussii*. Tetrahedron Letters 14, 1211-1214.

Candy, H.A., Wright, W., 1975. Helichrysoside: A new acylated flavonoid glycoside from *Helichrysum kraussii* Sch. Bip. Journal of the South African Chemical Institute 28, 215-219.

Carini, M., Aldini, G., Furlanetto, S., Stefani, R., Facino, R.M., 2001. LC coupled to ion-trap MS for the rapid screening and detection of polyphenol antioxidants from *Helichrysum stoechas*. Journal of Pharmaceutical and Biomedical Analysis 24, 517-526.

Cunningham, A., 1988. An Investigation of the Herbal Medicine Trade in Natal/KwaZulu. Institute of Natural Resources Investigational Report 29. University of Natal, Pietermaritzburg.

Cushnie, T.P.T., Hamilton, V.E.S., Lamb, A.J., 2003. Assessment of the antibacterial activity of selected flavonoids and consideration of discrepancies between previous reports. Microbiological Research 158, 281-289.

Cushnie, T.P.T., Lamb, A.J., 2006. Assessment of the antibacterial activity of galangin against 4-quinolone resistant strains of *Staphylococcus aureus*. Phytomedicine 13, 187-191.

Czinner, E., Hagymási, K., Blázovics, A., Kéry, Á., Szőke, É., Lemberkovics, É., 2001. The *in vitro* effect of *Helichrysi flos* on microsomal lipid peroxidation. Journal of Ethnopharmacology 77, 31-35.

Czinner, E., Hagymási, K., Blázovics, A., Kéry, Á., Szőke, É., Lemberkovics, É., 2000. *In vitro* antioxidant properties of *Helichrysum arenarium* (L.) Moench. Journal of Ethnopharmacology 73, 437-443.

Dekker, T.G., Fourie, T.G., Snyckers, F.O., Van der Schyf, C.J., 1983. Studies of South African medicinal plants. Part 2. Caespitin, a new phloroglucinol with antimicrobial properties from *Helichrysum caespititium*. South African Journal of Chemistry 36, 114-116.

Dilika, F., Afolayan, A.J., Meyer, J.J.M., 1997. Comparative antibacterial activity of two *Helichrysum* species used in male circumcision in South Africa. South African Journal of Botany 63, 158-159.

Dilika, F., Bremner, P.D., Meyer, J.J.M., 2000. Antibacterial activity of linoleic and oleic acids isolated from *Helichrysum pedunculatum*: a plant used during circumcision rites. Fitoterapia 71, 450-452.

Dlamini, B., 1981. Swaziland Flora: Their Local Names and Uses. Ministry of Agriculture and Co-operatives: Forestry Section, Mbabane, Swaziland.

Drewes, S.E., Mudau, K.E., Van Vuuren, S.F., Viljoen, A.M., 2006. Antimicrobial monomeric and dimeric diterpenes from the leaves of *Helichrysum tenax* var *tenax*. Phytochemistry 67, 716-722.

Drewes, S.E., Van Vuuren, S.F., 2008. Antimicrobial acylphloroglucinols and dibenzyloxyflavonoids from flowers of *Helichrysum gymnocomum*. Phytochemistry 69, 1745-1794.

Facino, R.M., Carini, M., Franzoi, L., Pirola, O., Bosiso, E., 1990. Phytochemical characterization and radical scavenger activity of flavonoids from *Helichrysum italicum* G. Don (Compositae). Pharmacological Research 22, 709-720.

Frum, Y., Viljoen, A.M., 2006. *In vitro* 5-lipoxygenase activity of 3 indigenous South African aromatic plants used in traditional healing and the stereospecific activity of limonene in the 5-lipoxygenase assay. Journal of Essential Oil Research 18, 85-88.

Gelfand, M., Mavi, S., Drummond, R.B., Ndemera, B., 1985. The Traditional Medical Practitioner in Zimbabwe. Mambo Press, Gweru, Zimbabwe, pp.83-84, 87, 94-95, 235, 238, 289, 332.

Germishuizen, G., Meyer, N.L., 2003. Strelitzia 14. Plants of Southern Africa: An Annotated Checklist. National Botanical Institute, Pretoria, pp. 235-246.

Gerstner, J., 1938. A preliminary checklist of Zulu names of plants with short notes. Bantu Studies 12, 321-342.

Gibbons, S., 2004. Anti-staphylococcal plant natural products. Natural Product Reports 21, 263-277.

Gibbs Russell, G.E., Welman, W.G., Retief, E., Immelman, K.L., Germishuizen, G., Pienaar, B.J., Van Wyk, M., Nicholas, A., 1987. Memoirs of the Botanical Survey of Southern Africa. List of Species of Southern African Plants. No 56, ed.2, part 2. Botanical Research Institute. Department of Agriculture and Water Supply.

Githens, T.S., 1949. Drug Plants of Africa. African Handbooks 8. University of Pennsylvania Press. The University Museum, Philadelphia, pp. 90-91.

Hänsel, R., Cybulski, E-M., Çubuçku, B., Meriçli, A.H., Bohlmann, F., Zdero, C., 1980. Neue pyron-derivate aus *Helichrysum*-arten. Phytochemistry 19, 639-644.

Harborne, J., 1977. Inuleae - Chemical Review. In: Heywood, V.H., Harborne, J.B., Turner, B.L. (Eds.), The Biology and Chemistry of the Compositae. Academic Press, London, pp. 603-619.

Hegnauer, R., 1977. The chemistry of the Compositae. In: Heywood, V.H., Harborne, J.B., Turner, B.L. (Eds.), The biology and chemistry of the Compositae. Academic Press, London, pp. 283-335.

Hilliard, O.M., 1983. Asteraceae. In: O.A. Leistner Flora of Southern Africa., Vol. 33, part 7 (Inuleae), Botanical Research Institute of South Africa, Pretoria, pp. 7,2:61-7,2:317.

Hulme, M., 1954. Wild Flowers of Natal. Shuter and Shooter, Pietermaritzburg, plate 41, 46, 47.

Hutchings, A., Johnson, C.T., 1986. Glimpses of a Xhosa herbal. Veld and Flora 72, 59-62.

Hutchings, A., Scott, A.H., Lewis, G., Cunningham, A., 1996. Zulu Medicinal Plants. Natal University Press, Pietermaritzburg.

Hutchings, A., Van Staden, J., 1994. Plants used for stress related ailments in traditional Zulu, Xhosa and Sotho medicine. Part 1. Plants used for headaches. Journal of Ethnopharmacology 43, 89-124.

Jacot Guillarmod, A., 1971. Flora of Lesotho (Basutoland). Cramer, Lehre, pp. 34, 38, 40-46, 267-281, 432-434.

Jäger, A.K., Hutchings, A., Van Staden, J., 1996. Screening of Zulu medicinal plants for prostaglandin-synthesis inhibitors. Journal of Ethnopharmacology 52, 95-100.

Jakupovic, J., Kuhnke, J., Schuster, A., Metwally, M.A., Bohlmann, F., 1986. Phloroglucinol derivatives and other constituents from South African *Helichrysum* species. Phytochemistry 25, 1133-1142.

Jakupovic, J., Schuster, A., Sun, H., Bohlmann, F., Bhakuni, D.S., 1987. Prenylated phtalides from *Anaphalis araneosa* and *Helichrysum platypterum*. Phytochemistry 26, 580-581.

Jakupovic, J., Schuster, A., Bohlmann, F., Ganzer, U., King, R.M., Robinson, H., 1989a. Diterpenes and other constituents from Australian *Helichrysum* and related species. Phytochemistry 28, 543-551.

Jakupovic, J., Zdero, C., Grenz, M., Tsichritzis, F., Lehmann, L., Hashemi-Nejad, S.M., Bohlmann, F., 1989b. Twenty-one acylphloroglucinol and further constituents from South African *Helichrysum* species. Phytochemistry 28, 1119-1131.

Lall, N., Hussein, A.A., Meyer, J.J.M., 2006. Antiviral and antituberculous activity of *Helichrysum melanacme* constituents. Fitoterapia 77, 230-232.

Lall, N., Meyer, J.J.M., 1999. *In vitro* inhibition of drug-resistant and drug-sensitive strains of *Mycobacterium tuberculosis* by ethnobotanically selected South African plants. Journal of Ethnopharmacology 66, 347-354.

Lourens, A.C.U., Reddy, D., Başer, K.H.C., Viljoen, A.M., Van Vuuren, S.F., 2004. *In vitro* biological activity and essential oil composition of four indigenous South African *Helichrysum* species. Journal of Ethnopharmacology 95, 253-258.

Lucas, A., Pike, B., 1971. Wild Flowers of the Witwatersrand. Purnell, Cape Town, pp. 94-95.

Mabogo, D.E.N., 1990. The Ethnobotany of the Vhavenda. MSc Thesis, University of Pretoria, South Africa.

Mathekga, A.D.M., 2001. Antimicrobial Activity of *Helichrysum* Species and the Isolation of a New Phloroglucinol from *Helichrysum caespititium.* PhD dissertation. University of Pretoria, South Africa.

Mathekga, A.D.M., Meyer, J.J.M., 1998. Antibacterial activity of South African *Helichrysum* species. South African Journal of Botany 64, 293-295.

Mathekga, A.D.M., Meyer, J.J.M., Horn, M.M., Drewes, S.E., 2000. An acylated phloroglucinol with antimicrobial properties from *Helichrysum caespitium*. Phytochemistry 53, 93-96.

McGlacken, G.P., Fairlamb, I.J.S., 2005. 2-Pyrone natural products and mimetics: isolation, characterisation and biological activity. Natural Product Reports 22, 369-385.

Meyer, J.J.M., Afolayan, A.J., 1995. Antibacterial activity of *Helichrysum aureonitens*. Journal of Ethnopharmacology 47, 109-111.

Meyer, J.J.M., Afolayan, A.J., Taylor, M.B., Engelbrecht, L., 1996. Inhibition of *Herpes simplex* virus type 1 by aqueous extracts from shoots of *Helichrysum aureonitens* (Asteraceae). Journal of Ethnopharmacology 52, 41-43.

Meyer, J.J.M., Afolayan, A.J., Taylor, M.B., Erasmus, D., 1997. Antiviral activity of galangin isolated from the aerial parts of *Helichrysum aureonitens*. Journal of Ethnopharmacology 56, 165-169.

Meyer, J.J.M., Dilika, F., 1996. Antibacterial activity of *Helichrysum pedunculatum* used in circumcision rites. Journal of Ethnopharmacology 53, 51-54.

Meyer, J.J.M., Lall, N., Mathekga, A.D.M., 2002. *In vitro* inhibition of drug-resistant and drug-sensitive strains of *Mycobacterium tuberculosis* by *Helichrysum caespititium*. South African Journal of Botany 68, 90-93.

Neuwinger, H.D., 1996. African Ethnobotany. Poisons and Drugs. Chapman and Hall, Weinheim, pp. 253-255.

Phillips, E.P., 1917. A contribution to the flora of the Leribe Plateau and environs: with a discussion on the relationships of the floras of Basutoland, the Kalahari, and the south-eastern regions. Annals of the South African Museum 16, 123-135.

Pooley, E., 1998. A field guide to the wild flowers. KwaZulu-Natal and the Eastern region, 1st ed. Natal Flora Publications Trust, Durban, pp. 82, 212-215, 310-317, 442-443.

Pooley, E., 2003. Mountain flowers: A Field Guide to the Flora of the Drakensberg and Lesotho, 1st ed. The Flora Publications Trust, Durban, pp. 44, 102-110, 146-157, 222-225.

Recio, M.C., Giner, R., Terencio, M.C., Sanz, M.J., Ríos, J.L., 1991. Anti-inflammatory activity of *Helichrysum stoechas*. Planta Medica 57, A56.

Reid, K.A., Maes, J., Maes, A., Van Staden, J., De Kimpe, N., Mulholland, D.A., Verschaeve, L., 2006. Evaluation of the mutagenic effects of South African plants. Journal of Ethnopharmacology 106, 44-50.

Ríos, J.L., Recio, M.C., Villar, A., 1991. Isolation and identification of the antibacterial compounds from *Helichrysum stoechas*. Journal of Ethnopharmacology 33, 51-55.

Roberts, M., 1990. Indigenous Healing Plants. Southern Book Publishers, Johannesburg, pp. 76-78.

Rood, B., 1994. Uit die Veldapteek. Tafelberg Publishers, Cape Town, pp. 32-33.

Rosa, A., Deiana, M., Atzeri, A., Corona, G., Incani, A., Melis, M.P., Appendino, G., Dessí, M.A., 2007. Evaluation of the antioxidant and cytotoxic activity of arzanol, a prenylated α-pyrone-phloroglucinol etherodimer from *Helichrysum italicum* subsp. *Microphyllum*. Chemico-Biological Interactions 165, 117-126.

Sala, A., Recio, M.D.C., Giner, R.M., Máñez, S., Ríos, J-L., 2001. New acetophenone glucosides isolated from extracts of *Helichrysum italicum* with anti-inflammatory activity. Journal of Natural Products 64, 1360-1362.

Sala, A., Recio, M.D.C., Giner, R.M., Máñez, S., Tournier, H., Schinella, G., Ríos, J-L., 2002. Anti-inflammatory and anti-oxidant properties of *Helichrysum italicum*. Journal of Pharmacy and Pharmacology 54, 365-371.

Sala, A., Recio, M.C., Schinella, G.R., Máñez, S., Giner, R.M., Cerdá-Nicolás, M., Ríos, J-L., 2003a. Assessment of the anti-inflammatory activity and free radical scavenger activity of tiliroside. European Journal of Pharmacology 461, 53-61.

Sala, A., Recio, M.C., Schinella, G.R., Máñez, S., Giner, R.M., Ríos, J-L., 2003b. A new dual inhibitor of arachidonate metabolism isolated from *Helichrysum italicum*. European Journal of Pharmacology 460, 219-226.

Salie, F., Eagles, P.F.K., Leng, H.M.J., 1996. Preliminary antimicrobial screening of four South African Asteraceae species. Journal of Ethnopharmacology 52, 27-33.

Scott, G., Hewett, M.L., 2008. Pioneers in ethnopharmacology: The Dutch East India Company (VOC) at the Cape from 1650 to 1800. Journal of Ethnopharmacology 115, 339-360.

Scott, G., Springfield, E.P., Coldrey, N., 2004. A pharmacognostical study of 26 South African plant species used as traditional medicines. Pharmaceutical Biology 42, 186-213.

Smith, A., 1895. A contribution to South African Material Medica, Juta, Cape Town, pp. 72, 81, 97, 100-101.

Smith, C.A., 1966. Common Names of South African Plants. Memoirs of the Botanical Survey of South Africa No. 35. Department of Agricultural and Technical Services, Pretoria, pp. 62, 85, 90, 118-119, 206, 208, 211, 225, 246, 251, 266, 286, 298, 306, 376, 400, 415-416, 430, 443, 458, 465, 476, 478, 489, 517-518, 614, 615.

Stafford, G.I., Jäger, A.K., Van Staden, J., 2005. Activity of traditional South African sedative and potentially CNS-acting plants in the GABA-benzodiazepine receptor assay. Journal of Ethnopharmacology 100, 210-215.

Steenkamp, V., Mathivha, E., Gouws, M.C., Van Rensburg, C.E.J., 2004. Studies on the antibacterial, antioxidant and fibroblast growth stimulation of wound healing remedies from South Africa. Journal of Ethnopharmacology 95, 353-357.

Süzgeç, S., Meriçli, A.H., Houghton, P.J., Çubukçu, B., 2005. Flavonoids of *Helichrysum compactum* and their antioxidant and antibacterial activity. Fitoterapia 76, 269-272.

Swanepoel, D.P., 1997. The Medicinal Value of the Southern African Asteraceae. MSc dissertation. University of Pretoria, South Africa.

Tomás-Lorente, F., Iniesta-Sanmartín, E., Tomás-Barberán, F.A., Trowitzsch-Kienast, W., Wray, V., 1989. Antifungal phloroglucinol derivatives and lipophilic flavonoids from *Helichrysum decumbens*. Phytochemistry 28, 1613-1615.

Uys, A.C.U., Malan, S.F., Van Dyk, S., Van Zyl, R.L., 2002. Antimalarial compounds from *Parinari capensis*. Bioorganic and Medicinal Chemistry Letters 12, 2167-2169.

Van der Schyf, C.J., Dekker, T.G., Fourie, T.G., Snyckers, F., 1986. Synthesis and antimicrobial activity of a series of caespitin derivitaves. Antimicrobial Agents and Chemotherapy 30, 375-381.

Van Puyvelde, L., De Kimpe, N., Costa, J., Munyjabo, V., Nyirankuliza, S., Hakizamungu, E., Schamp, N., 1989. Isolation of flavonoids and a chalcone from *Helichrysum odoratissimum* and synthesis of helichrysetin. Journal of Natural Products 52, 629-633.

Van Vuuren, S.F., Viljoen, A.M., Van Zyl., R.L., Van Heerden, F.R., Başer, K.H.C., 2006. The antimicrobial and toxicity profiles of helihumulone, leaf essential oil and extracts of *Helichrysum cymosum* (L.) D. Don subsp. *cymosum*. South African Journal of Botany 72, 287-290.

Van Wyk, B-E., Van Oudtshoorn, B., Gericke, N., 2000. Medicinal Plants of South Africa, 2nd ed. Briza, Pretoria, p.148.

Van Wyk, B-E., Gericke, N., 2000. People's Plants. A Guide to Useful Plants of Southern Africa, 1st ed. Briza, Pretoria, p. 166.

Van Wyk, B-E., Van Heerden, F.R., Van Oudtshoorn, B., 2002. Poisonous Plants of South Africa, 1st ed. Briza, Pretoria, pp. 122-123.

Van Wyk, B-E., Wink, M., 2004. Medicinal Plants of the World. 1st edition. Briza, Pretoria, pp. 168, 360, 361, 412.

Von Koenen, E., 2001. Medicinal, Poisonous and Edible Plants in Namibia. Klaus Hess publishers, Verlag, p. 127.

Walker, J., 1996. Wild Flowers of KwaZulu-Natal. W.R. Walker Family Trust, Pinetown, pp. 169-173.

Watt, J.M., Breyer-Brandwijk, M.G., 1962. The Medicinal and Poisonous Plants of Southern and Eastern Africa, 2nd ed. Livingstone, London, pp. 237-240.

Wright, W.G., 1976. South African plant extractives. Part 3. Helichrysin, a new chalcone glucoside from a *Helichrysum* species. Journal of the Chemical Society, Perkin Transactions 1, 1819-1820.

Table 2.1 Traditional uses and biological activities reported for *Helichrysum* species

Species[a]	**Plant part used**	**Dosage form**	**Traditional Use**	**Classification of use**[b]	**Biological activity**[b]	**References**
H. acutatum DC. **21**[c]			Widely used as traditional medicine, sold commercially in large quantities	NS		Arnold et al., 2002; Cunningham, 1988; Hutchings et al., 1996.
H. adenocarpum DC. **28**	Root	Decoction	Used to treat diarrhoea and vomiting in children.	GIT		Arnold et al., 2002; Jacot Guillarmod, 1971; Neuwinger, 1996; Phillips, 1917; Pooley, 2003; Walker, 1996; Watt and Breyer-Brandwijk, 1962.
H. appendiculatum (L.f.) Less. **24**	Leaf	Eaten raw	Chest problems or infection of the respiratory tract	Resp Infec Anth W P	[d]B, [d]F	Arnold et al., 2002; Githens, 1949; [e]Mathekga, 2001; Smith, 1895; Smith, 1966; Swanepoel, 1997; Walker, 1996; Watt and Breyer-Brandwijk, 1962.
	Plant		Smallpox			
	Plant		Anthelmintic			
	Root		Coughs and colds and applied externally on wounds			
	Leaf	Wound dressing	Applied externally to wounds. Ground leaves are rubbed into areas which cramps or on wounds			
	Roots		Ground and burnt and smeared on body to relax body and to reduce swelling			
	Leaf		Used medicinally as tea			
H. argyrophyllum DC. **29**	Root	Infusion	Intestinal troubles	GIT		Arnold et al., 2002; Batten and Bokelmann, 1966; Smith, 1966; Walker, 1996; Watt and Breyer-Brandwijk, 1962.
			Not grazed by stock, preventing soil erosion in overgrazed areas.			
H. argyrosphaerum DC. **15**			Browsed by animals but poisonous if large quantities is ingested.	Poi	[d]B, [d]F	Hutchings et al., 1996; [e]Mathekga, 2001; Pooley, 1998; Van Wyk et al., 2002.
H. asperum (Thunb.)			The plants are casually browsed by sheep and	Poi		Smith, 1966 [f], (*H. ericaefolium* DC.).

Hilliard and Burtt. (= *H. ericifolium* Less) **12**			said to be a cause of "Geilsiekte".			
H. athrixiifolium (Kuntze) Moeser **9**	Leaf	Smoked	Chest complaints.	Resp		Arnold et al., 2002; Jacot Guillarmod, 1971 [f](*H. athrixifolium* O. Hoffm.); Phillips, 1917 [f](*H. athrixiifolium* O. Hoffm.); Watt and Breyer-Brandwijk, 1962 [f](*H. athrixifolium* O. Hoffm.).
H. aureonitens Sch. Bip. **8**	Leaves and stems	Burnt as incense	Used to invoke the goodwill of the ancestors and to induce trances	Psy Psyc Infect Insect	[d]B, [d]F, V	[e]Afolayan and Meyer, 1997; Cunningham, 1988; Hutchings et al., 1996; Jacot Guilllarmod, 1971; [e]Mathekga, 2001; [e]Meyer and Afolayan, 1995; [e]Meyer et al., 1996; [e]Meyer et al., 1997; Phillips, 1917; Pooley, 1998; Pooley, 2003; Swanepoel, 1997; Walker, 1996, Watt and Breyer-Brandwijk, 1962.
	Leaves and stems		Commercially sold			
		Decoction	A remedy for inuresis in children			
		Extracts	Used topically for skin infections especially against *Herpes zoster* and infections associated with *Herpes simplex*			
			Used to keep red mites away			
			Used as tinder to start fire, used to make hats.			
[g]*H. aureum* Houtt. Merr. var. *aureum*/ *monocephalum* (= *H. fulgidum* (L.f.) Willd.) **30**		Decoction	Used for washing sore eyes.	Eye		Arnold et al., 2002 [f](*H. fulgidum* L.f.) Willd.); Batten and Bokelmann, 1966 [f](*H. fulgidum* Willd.); Jacot Guillarmod, 1971 [f](*H. fulgidum* (L.) Willd.); Phillips, 1917 [f](*H. fulgidum* Willd.).
H. bellum Hilliard **28**					[d]B, [d]F	[e]Mathekga, 2001.
H. caespititium (DC.) Harv **12**	Plant	Crushed and burnt and smoke inhaled	Used to treat head and chest colds (headaches)	Resp Infect GIT Vi W	[d]B, [d]F, I, My	Arnold et al., 2002; [e]Dekker et al., 1983; Gelfand et al., 1985 [f](*H. caespitium* Sond.); Hutchings and Van Staden, 1994; Jacot Guillarmod, 1971 [f](*H. caespitium* Sond.); [e]Mathekga et al., 2000; [e]Mathekga, 2001; [e]Meyer et al., 2002; Neuwinger, 1996; Phillips, 1917 [f](*H. caespitium* Sond); Pooley, 1998; Pooley, 2003; [e]Swanepoel, 1997; Watt and Breyer-Brandwijk, 1962.
	Plant	Decoction	Drunk by the Kwena and the Kgatla to treat gonorrhoea			
	Root	Decoction	Nausea			
	Roots		Virility			
	Plant	Ointment	Ointment is applied to the roof of the mouth for a depressed fontanelle			
			Used as dressing for open wounds during			

			circumcision rites			
H. callicomum Harv **2**			Protective charm. Mixed with *Aster bakerianus* (*hispidis*) and *H. rugulosum*	M GIT	[d]B, [d]F	Arnold et al., 2002; Jacot Guillarmod, 1971; [e]Mathekga and Meyer, 1998; [e]Mathekga, 2001; Phillips, 1917; Pooley, 2003; Watt and Breyer-Brandwijk, 1962.
			Used for fuel in winter			
		Enema	Used as an ingredient in an enema for colic.			
H. calocephalum Klatt **23**						Arnold et al., (2002) refers to others using *H. calocephalum* Schltr, which is classified as *H. ecklonis* Sond (Germishuizen and Meyer, 2003).
Helichrysum calophalum Klatt **23**	Root		Used for hyperfunction of the lower gastro-intestinal tract.	GIT		Swanepoel, 1997, information obtained from TRAMED database. It is not clear to these authors whether this use pertains to *H. calocephalum* Klatt or *H. ecklonis* Sond.
H. candolleanum Buek **15**					[d]B, [d]F	[e]Mathekga, 2001
H. chionosphaerum DC. **25**					[d]B, [d]F	[e]Mathekga, 2001
H. cephaloideum DC. (= *H. adscendens* Less. var. *cephaloideum* Moes.) **24**			Irritant poisoning in sheep demonstrated. Known to be poisonous to sheep (symptoms similar to that of poisoning caused by *Geigera*).	Poi		Van Wyk et al., 2002; Watt and Breyer-Brandwijk, 1962.
H. cochleariforme DC. (= *H. imbricatum* Less) **15**		Tea, infusion	Demulcent in coughs and other pulmonary affections. In the Western Cape area the plant is used to treat whooping cough, other coughs, bronchial catarrh and bronchitis	Resp		Arnold et al., 2002; Neuwinger 1996; Smith, 1966; Swanepoel 1997; Watt and Breyer-Brandwijk, 1962.
	Whole plant	Decoction	Drunk for infections of the respiratory tract			
H. cooperi Harv. **30**	Leaf	Ointment, applied after bathing	Used as love charm. The ointment is applied after bathing and as a result the desired lady finds the man irresistible	M Fum Snakebite		Arnold et al., 2002; Hutchings et al., 1996; Pooley, 1998; Pooley, 2003; Walker, 1996; Watt and Breyer-Brandwijk, 1962.

H. cooperi Harv. **30**	Leaves		Used to make Zulu headdress distinctive to married women			
			Used as a fumigant and as part of a traditional remedy for snakebite.			
H. crispum (L.) D. Don **17**			Used medicinally as a calming tea. Coughs, bronchitis, urinary tract infections and tuberculosis.	Resp Renal	B	Arnold et al., 2002 (with reference to Smith, 1966); Kling as quoted by [e]Salie et al., 1996; Roberts, 1990 [f](*H. crispum*). These authors are not certain whether Kling is referring to *H. crispum* (L.) D. Don or *H. crispum* Less.
H. cymosum (L.) D. Don **8**			Used to invoke the goodwill of the ancestors and to induce trances	M Psy Resp GIT P	[d]B, [d]F, Pl	Arnold et al., 2002; Bhat and Jacobs, 1995; Kokwaro as quoted by Neuwinger, 1996; Neuwinger, 1996; Pooley, 2003; [e]Van Vuuren at el., 2006; Van Wyk et al., 2000.
	Leaf	Decoction /tea	Used to treat colds and coughs			
	Root	Extract	Used as emetic and purgative			
	Leaf		Filtrate drunk to treat colds and fever			
	Leaf	Boiled, and vapours inhaled	Vapour bath used to treat headaches.			
H. dasymallum Hilliard (= *H. lanatum* Harv.) **21**			Used as medicinal tea Woolly coat used for tinder boxes.	NS		Arnold et al., 2002; Lucas and Pike, 1971; Smith, 1966.
H. decorum DC. **30**	Plant	Burned and smoke inhaled	Used to induce trances.	Psy	[d]B, [d]F	Arnold et al., 2002; Hutchings et al., 1996; [e]Mathekga, 2001; Neuwinger, 1996.
H. dregeanum Sond. and Harv. **9**	Leaf	Smoked	Used to treat head colds	Resp GIT		Arnold et al., 2002; Hutchings and Van Staden, 1994; Jacot Guillarmod, 1971; Neuwinger, 1996; Phillips, 1917; Smith, 1966; Watt and Breyer-Brandwijk, 1962.
		Infusion	Used to treat hiccups.			
			Browsed by stock.			
H. ecklonis Sond (= *H. calocephalum*			Used by the Xhosas to ward of evil magic spells, which follow on seeing *iChanti*, the	M GIT		Batten and Bokelmann, 1966; Jacot Guillarmod, 1971; Phillips, 1917;

Schltr.) **28**			water snake			Pooley, 2003; Watt and Breyer-Brandwijk, 1962.
	Root	Decoction	Used to treat diarrhoea in children.			
H. epapposum Bolus **3**	Leaves and stems	Burned as incense	Used to invoke the goodwill of the ancestors , commercially sold	M		Arnold et al., 2002; Cunningham, 1988; Hutchings et al., 1996.
H. excisum (Thunb.) Less **12**					[d]B, I	[e]Lourens et al., 2004
H. felinum Less **17**					[d]B, I	[e]Lourens et al., 2004
H. flanaganii Bolus **13**	Leaves	Burned	Incense	M		Walker, 1996.
H. foetidum (L.) Moench **30**	Plant	Extract is drunk/ smoke inhaled	Used to induce trances	Psy Infect Resp W Eye P	B	Arnold et al., 2002; Batten and Bokelmann, 1966 [f](*H. foetidum* Cass.); Gerstner, 1938 [f](*H. foetidum* Cass); Hulme, 1954; Hutchings et al., 1996; Kokwaro quoted by Neuwinger, 1996; Neuwinger, 1996 Roberts, 1990; Rwangabo, quoted by Neuwinger, 1996; [e]Steenkamp et al., 2004; Swanepoel 1997; Van Wyk and Gericke, 2000; Watt and Breyer-Brandwijk, 1962 [f](*H. foetidum* Cass.).
	Leaf	Extract	Used to treat flu (influenza)			
	Leaf	Wound dressing	Used to treat circumcision and infected wounds (festering sores)			
	Leaf	Preparation	Applied to treat *Herpes*			
	Root	Extract	Eye problems, used to bath eyes			
	Leaf		Used in making headdress distinctive of married women			
	Plant		Aromatic and astringent (used to draw out infection).			
			Used to treat menstrual pain			
H. glomeratum Klatt **6**					B, [d]F	[e]Mathekga and Meyer, 1998; [e]Mathekga, 2001.
[g]*H. griseum* Sond (= *H. agrostophilum* Klatt) **23**			Preventative charm against illness Burnt as fuel in winter.	M		Arnold et al., 2002, Phillips, 1917.

H. gymnocomum DC. **4**	Stems and leaves	Burned as incense	Used to invoke the goodwill of the ancestors	Skin M Fum	[d]B, [d]F	Cunningham, 1988; [e]Drewes and Van Vuuren, 2008; Hutchings et al., 1996; Phillips, 1917.
		Ointment	Mixed with fat, only the wives of chiefs were previously allowed to use it			
			Used to fumigate sick rooms.			
			Commercially sold			
H. herbaceum (Andrews) Sweet **29**	Stems and leaves	Burned as incense	Used to invoke the goodwill of the ancestors	M	[d]B, [d]F	Arnold et al., 2002; Cunningham, 1988; Hutchings et al., 1996; [e]Mathekga, 2001; Neuwinger, 1996; Pooley, 1998; Pooley, 2003.
	Stems and leaves		Commercially sold			
H. hypoleucum Harv **16**					[d]B, [d]F	[e]Mathekga and Meyer, 1998; [e]Mathekga, 2001.
H. indicum (L.) Grierson (= *H. expansum* (Thunb.) Less) **15**	Plant	Burned and crushed	Mixed with *Conyza pinnata*. Crushed and burnt to drive sickness from a room.	M		Arnold et al., 2002; Jacot Guillarmod, 1971.
H. kraussii Sch. Bip **8**	Leaf	Decoction	Use to wash keloid scars	Skin M Resp Infect	[d]B, [d]F	Arnold et al., 2002; Arnold and Gulumian as quoted by Neuwinger, 1996; [e]Bremner and Meyer, 2000; [e]Mathekga, 2001; Gelfand et al., 1985; Mabogo, 1990; Neuwinger, 1996; Swanepoel, 1997; Walker, 1996; Watt and Breyer-Brandwijk, 1962.
	Root and leaves	Infusion	Used to drive bad spirits away, used to wash body			
	Dried flower and seed	Smoked in a pipe	The Karanga smoke this as a remedy for coughs and pulmonary tuberculosis			
	Plant	Burnt, salt is added to ash and ingested	Cough			
H. kraussii Sch. Bip **8**	Root		Venereal disease			Arnold et al., 2002; Arnold and Gulumian as quoted by Neuwinger, 1996; [e]Bremner and Meyer, 2000; [e]Mathekga, 2001; Gelfand et al., 1985; Mabogo, 1990; Neuwinger, 1996; Swanepoel, 1997; Walker, 1996; Watt and Breyer-Brandwijk, 1962.
	Root	Mixed with salt and other ingredients	Applied to child's side with small amount given orally			

H. lepidissimum S. Moore **19**		Powder or ointment	Used as a body perfume	Skin		Dlamini, 1981; Watt and Breyer-Brandwijk, 1962.
H. litorale Bolus (= *Leontonyx angustifolius* DC. = *Leontonyx spathulatus* Less) **14**	Plant		Dried and pounded or mixed with lard or fat, was used for applying to ulcers In the Western Cape Province an ointment for boils, carbuncles and abscesses is made from this plant, *Cyanella lutea* and "tiendaegeneesbossie"	W Skin		Smith, 1966; Swanepoel, 1997; Watt and Breyer-Brandwijk, 1962.
Helichrysum longifolium DC. **24**	Leaf		Used by the Pondos to treat circumcision wounds. The leaves are heated over very hot ash before being used as a bandage for the treatment of wounds after circumcision.	W	[d]B, [d]F	[e]Dilika et al., 1997; [e]Mathekga, 2001.
H. lucilioides Less. **12**			Excellent stock feed			Smith, 1966.
H. melanacme DC. **8**			Used as bedding Used medicinally as tea Used for cough, fever, headache, colds and chest pain.	Resp P	[d]B, [d]F, My, V	Arnold et al., 2002; [e]Lall and Meyer, 1999; [e]Lall et al., 2006; [e]Mathekga, 2001; Smith, 1966.
H. miconiifolium DC. **23**		Tea	Used medicinally as tea	P Anthel	[d]B, [d]F	Smith, 1966 [f](*H. miconiaefolium* DC); Arnold et al., 2002; [e]Mathekga, 2001; Swanepoel, 1997.
	Leaf		The Xhosa grind and boil the leaves and use it as a wash for pain after circumcision.			
	Root		The powdered root is used for intestinal parasites and for ticks on poultry.			
H. montanum DC. **22**					B, [d]F	[e]Mathekga, 2001.
H. monticola Hilliard **28**					B, [d]F	[e]Mathekga, 2001.
Helichrysum mundtii Harv. **23**	Plant	Decoction	Chest complaints	Resp		Arnold et al., 2002; Jacot Guillarmod, 1971; Pooley, 1998; Pooley, 2003; Phillips, 1917 [f](*H. mundii*, Harv.); Watt and Breyer-Brandwijk, 1962.

Helichrysum natalitium DC. **3**	Leaves and stems	Burnt as incense	Used to invoke the goodwill of the ancestors	M		Arnold et al., 2002; Cunningham, 1988; Hutchings et al., 1996; Pooley, 2003.
	Leaves and stems		Commercially sold			
H. nudifolium (L.) Less var. *nudifolium (= H coriaceum* Harv. = also *H. gerberifolium* A. Rich, = also *H. leiopodium* DC. = also *H. nudifolium* var. *quinquenerve* = also *H. nudifolium* var. *leiopodium*) **23**	Leaf	Burnt as incense	To invoke the goodwill of the ancestors	M Resp W Infect P Skin GIT	[d]B, [d]F, I	Arnold et al., 2002; Gerstner, 1938 [f](*H. undifolium*, also *H. leiopodium* DC.); Githens, 1949 [f](*H. nudifolium*, also *H. leiopodium*); Glover et al quoted by Neuwinger, 1996; Hulme, 1954; Hutchings et al., 1996; Hutchings and Johnson, 1986; Hutchings and Van Staden, 1994; Jacot Guillarmod, 1971 [f](*H. nudifolium* var. *leiopodium*); [e]Jäger et al., 1996; Mabogo, 1990; Phillips 1917 [f](*H. leiopodium* DC.); Rood, 1994; Smith, 1895 [f](*H. nudiflorum*), Smith, 1966 [f](*H. coriaceum* Sond. and *H. nudifolium* var. *quinquenerve)*; [e]Swanepoel 1997 [f](*H. gerberifolium*), Van Wyk et al., 2000; Neuwinger, 1996 (also [f]*H. gerberifolium* Sch. Bip); Watt and Breyer-Brandwijk, 1962 [f](*H. gerberaefolium* Sch. Bip. Ex A.Rich).
		Infusion	Colds (Zulu and Khoi – administration route not indicated)			
	Leaf	Eaten raw	Used to treat colds by the Xhosa			
	Plant	Infusion	Regarded as demulcent, used to treat catarrh, phthisis and other pulmonary affections			
	Leaf/ plant		Respiratory infections			
	Root		Coughs and colds			
	Leaf	Wound dressing	Wounds			
	Root/ Leaf		Applied to sores on the genitalia by the Xhosa			
	Plant/ leaf	Smoke inhaled	Headache			
	Leaf	Infusion	Rectal prolapse			
H. nudifolium (L.) Less var. *nudifolium (H coriaceum* Harv.= also *H. gerberifolium* A. Rich, = also *H. leiopodium* DC. = also *H. nudifolium* var. *quinquenerve* = also *H. nudifolium* var. *leiopodium*) **23**		Powder mixed, eaten with butter	Protection of children from illness	M Resp W Infect P Skin GIT	[d]B, [d]F, I	Arnold et al., 2002; Gerstner, 1938 [f](*H. undifolium*, also *H. leiopodium* DC.); Githens, 1949 [f](*H. nudifolium*, also *H. leiopodium*); Glover et al., quoted by Neuwinger, 1996; Hulme, 1954; Hutchings et al., 1996; Hutchings and Johnson, 1986; Hutchings and Van Staden, 1994; Jacot Guillarmod, 1971 [f](*H. nudifolium* var. *leiopodium*); [e]Jäger et al., 1996; Mabogo, 1990; Phillips 1917 [f](*H. leiopodium* DC.); Rood, 1994; Smith, 1895 [f](*H. nudiflorum*), Smith, 1966 [f](*H. coriaceum* Sond. and *H.*
	Root	Decoction	Chest problems, used as emetic by the Zulu			
	Leaf	Decoction	To encourage weaning in babies			
	Leaf	Infusion	Diseases in goats			
	Plant	Infusion on hot stones	Used as steam bath to treat fever and nightmares			
	Plant	Poultice	Swellings			

		Decoction	Colic in children (administered as enema)			*nudifolium* var. *quinquenerve)*; [e]Swanepoel 1997 [f](*H. gerberifolium*), Van Wyk et al., 2000; Neuwinger, 1996 (also [f]*H. gerberifolium* Sch. Bip); Watt and Breyer-Brandwijk, 1962 [f](*H. gerberaefolium* Sch. Bip. Ex A.Rich).
			Rubbed into scarifications over bruises Used as tea			
	Root	Decoction	Internal sores (intestinal ulceration)			
H. *nudifolium* var. *oxyphyllum* (= *H. oxyphyllum* DC. = also *H. undatum* Less.) **23**			Protective charm against thunder			
H. nudifolium var. *pilosellum* (= *H. latifolium* (Thunb.) Less. = *H. pilosellum* (L.f.) Less) **23**			Used for "doctoring' people who wish some deed concealed and who are afraid of being found out	M GIT	B, [d]F	Arnold et al., 2002 [f](*H. pilosellum*); Hulme, 1954 [f](*H. latifolium*); Hutchings et al., 1996 [f](*H. pilosellum* (L.f.) Less); Jacot Guillarmod, 1971 [f](*H. latifolium* (Thunb.) Less.); [e]Mathekga and Meyer, 1998; [e]Mathekga, 2001; Neuwinger, 1996 [f](*H. pilosellum* (L.f.) Less); Phillips, 1917 [f](*H. latifolium* Less.); Phillips, 1917 [f](*H. latifolium* Less.); Pooley, 2003 [f](*H. pilosellum*); Swanepoel 1997; Walker, 1996 [f](*H. pilosellum* (L.f.) Less); Watt and Breyer-Brandwijk, 1962 [f](*H. latifolium* Less.).
			Ingredient in colic remedy			
	Leaf	Infusion	Stomach ache in children			
	Roots	Ground and burnt	Ground and burnt near cattle suffering from black leg			
[g]*H. nudifolium* var. *pilosellum* (= *H. pilosellum* (L.f.) Less = *H. pedunculare* (L.) DC. var. *pilosellum*) **23**			As an antiseptic and to induce fast healing: Used after circumcision to prevent inflammation externally Also externally applied to wounds and used for infections of the respiratory tract As an antiseptic Stomach ailments	W Resp GIT		Arnold et al., 2002; the sources below are indicated in Arnold et al., under *H. pedunculare* DC.: Batten and Bokelmann, 1966 [h](*isicwe*, *isiGqutsi*); Githens, 1949 [f](*H. pedunculare)*; Smith, 1895 [h](*isi-Cwe*.); Smith, 1966 .
H. odoratissimum (L.) Sweet **4**	Leaf/ ground plants	Used as wound dressing/ leaf pulp	Wounds and burns	W Fum Psy Psyc M	[d]B, [d]F, My	Adjanohoun quoted by Neuwinger, 1996; Arnold et al. 2002; Baerts and Lehmann quoted by Neuwinger, 1996; Cunningham, 1988; Dlamini, 1981; Hutchings and Johnson, 1986;
	Plant		The Southern Sotho use this plant to fumigate			

H. odoratissimum (L.) Sweet **4**			huts	Resp Eye GIT P		Hutchings et al., 1996; Hutchings and Van Staden, 1994; Jacot Guillarmod, 1971; Kokwaro quoted by Neuwinger, 1996; [e]Lall and Meyer, 1999; [e]Lourens et al., 2004; [e]Mathekga and Meyer, 1998; [e]Mathekga, 2001; Neuwinger, 1996; Pooley, 1998; Pooley, 2003; Rwangabo quoted by Neuwinger, 1996; Smith, 1966; Swanepoel, 1997; Van Puyvelde et al., 1989; Van Wyk et al., 2000; Van Wyk and Gericke, 2000; Watt and Breyer-Brandwijk, 1962.
		Ointment	It is mixed with fat to form pleasantly smelling ointment, formerly only used by wives of chiefs			
	Leaf	Ash is rubbed into scarifications	Insanity, possession.			
		Burnt as incense	Used to invoke the goodwill of the ancestors, protective charm			
		Tea	Aids sleep, relieves muscle tension and cramps			
	Plant, leaf, stems	Smoke inhaled	Used as a sedative and to treat insomnia and as protective cleanser.			
	Root		Colds, coughs			
	Leafy twigs	Ash is eaten	Coughs			
	Leaf and twigs	Extract or sap used as eye drop	Conjunctivitis			
		Decoction	Abdominal pain			
	Aerial parts	Extract	Used to treat dehydration			
	Leaf	Sap	Heartburn, flatulence			
	Root	Extract	Purgative (extract is drunk)			
	Leaf	Ash is eaten	Vomiting			
		Tea	Colic and stitch			
	Leaf	Decoction	Febrile convulsions (part of preparation)			
	Leaf	Smoke inhaled	Headache			
	Leaf	Infusion	Fever (Also used as wash)			
	Leaf and twigs	Decoction	Used to treat female sterility, menstrual pain and eczema in Rwanda			

	Leafy twigs	Decoction	Tonic for pregnant women			
	Leaf	Decoction	Galactagoque.			
			Used a bedding material since it is an effective insect repellent. Sold commercially. The Xhosa also use the plant for spiritual purposes, as a fumigant when a baby is born			
H. oreophilum Klatt **21**					B, [d]F	[e]Mathekga and Meyer, 1998; [e]Mathekga, 2001.
[g]*H. pallidum* DC. (= *H. agrostophilum* Klatt (in part) = *H. undatum* (Thunb.) Less var. *agrostophilum* (Klatt) Moeser = *H. undatum* var. *pallidum* **23**			Preventative charm for illness.	M		Arnold et al., 2002; Jacot Guillarmod, 1971 [f](*H. undatum* var. *agrostophilum*); Phillips, 1917 [f](*H. undatum* Less., var. *pallidum* and *H. agrostophilum* Klatt).
			Burnt as fuel in winter			
	Roots	Bathing in decoction	The act of forgetting, The bath is suppose to make a person invisible/or forgotten by his enemies, witchcraft.			
H. panduratum O. Hoffm. **18**	Leaf	Decoction	Febrile convulsions in children (part of a preparation)	P Infect	A, I	Adjanohoun quoted by Neuwinger, 1996; Haerdi quoted by Neuwinger, 1996; Neuwinger, 1996; Neuwinger, 1996; Pooley, 1998; [e]Swanepoel, 1997.
	Plant	Sap	Used to treat malaria in children			
			Used to make herbal tea			
H. pandurifolium Schrank. (= *Helichrysum auriculatum* Less.) **18**	Infusion, demulcent		Respiratory conditions	Resp P Ca Renal		Arnold et al. 2002; Roberts, 1990 [f](*H. auriculatum* Less.); Rood, 1994; Smith, 1966 [f](*H. auriculatum* Less.); Swanepoel, 1997; Watt and Breyer-Brandwijk, 1962 [f](*H. auriculatum* Less.).
			Backpain, heart trouble, kidney disease, kidney stones			
			Historically been used as a tea.			
H. patulum (L.). Don (= *H. crispum* Less) **18**			Heart trouble, backache, kidney disease, also 'heart weakness' (also heart treatment in animals). Stress and fatigue.	P Ca Renal Resp		Neuwinger, 1996 [f](*H. crispum* Less.); Roberts, 1990 [f](*H. crispum*); Scott et al., 2004; Smith, 1966 [f](*H. crispum* Less.); Watt and Breyer-Brandwijk, 1962 [f](*H. crispum* Less.).
		Infusion	Hyperpiesa, (hyperpepsia is probably a spelling error in Neuwinger), coronary thrombosis, bladder conditions/infections.			
			Asthma, Influenza			

			Gynaecological disorders			
			Used as bedding.			
H. pedunculatum Hilliard and Burtt (= *H. pedunculare* DC.) **23**	Leaf and root		As an antiseptic and to induce fast healing: Used after circumcision to prevent inflammation externally. Also externally applied to wounds and used for infections of the respiratory tract As an antiseptic Stomach ailments	W Resp Infect GIT	B	Arnold et al., 2002; Batten and Bokelmann, 1966 ([f]*H. pedunculare* DC., [h]*isiCwe*, [h]*isiGqutsi*, Xhosa); Bhat and Jacobs, 1995 ([f]*H. pedunculatum* Hilliard and Burtt, [h]*isiCwe*, [h]*siGgutsi*, Xhosa); [e]Dilika et al., 1997; Gerstner, 1938 ([f]*H. pedunculare* DC., [h]*isiCwe*, Zulu); Githens, 1949 ([f]*H. pedunculare*, [h]*isicwe*, Zulu); Hutchings et al., 1996 ([f]*H. pedunculatum* Hilliard et Burtt); [e]Meyer and Dilika, 1996; Rood, 1994 ([f]*H. pedunculatum*, [h]*ery'kue*, Fingo); Smith, 1895 ([f]*H. pedunculare* DC., [e]*isi-Cwe*); Smith, 1966 ([f]*H. pedunculare* DC.); Neuwinger, 1996 ([f]*H. pedunculatum* Hilliard and Burtt); Swanepoel, 1997; Watt and Breyer-Brandwijk, 1962 ([f]*H. pedunculare* DC.).
H. petiolare Hilliard and Burtt **18**			Coughs, colds, catarrh, headache, fever, menstrual disorders, urinary tract infections..		[d]B	Arnold et al. 2002; [e]Lourens et al., 2004; Kirstenbosch Botanical Garden; Neuwinger, 1996; Roberts, 1990 [f](*H. peteolatum*); Scott et al., 2004; Smith, 1966 [f](*H. petiolatum* DC.); Van Wyk et al., 2000.
	Leaf		Antiseptic wound dressing			
		Tea	Tea taken for heart conditions, stress, hypertension, anxiety and over-excitement.			
			Used as bedding			
H. platypterum DC. **20**	Root	Decoction	Renew virility in men	Vi		Arnold et al. 2002; Jacot Guillarmod, 1971; Phillips, 1917; Watt and Breyer-Brandwijk, 1962.
	Root	Crushed and sucked				

H. psilolepis Harv. **22**	Root	Decoction	Dysmenorrhoea	P	[d]B, [d]F	Arnold et al., 2002; Jacot Guillarmod, 1971; [e]Mathekga, 2001; Phillips, 1917; Neuwinger, 1996; Watt and Breyer-Brandwijk, 1962.
			Used to weave hats			
H. rotundatum (= *H coriaceum* (DC.) Harv.)			Used as tea	NS		Smith, 1966 [f](*H. coriaceum* Sond.).
Helichrysum rugulosum Less. **9**			Protective charm (with *H. callicomum* and *Aster bakerianus*;	M GIT Fum	[d]B, [d]F	Arnold et al., 2002; Dlamini, 1981; Jacot Guillarmod, 1971; [e]Mathekga and Meyer, 1998; [e]Mathekga, 2001; Phillips, 1917; Pooley, 1998; Pooley, 2003; Watt and Breyer-Brandwijk, 1962.
		Enema	Colic (an ingredient)			
			Used to fumigate huts when children are ill (cold).			
H. setosum Harv. **30**			Love potion	M Epilepsy Fum Snakebite		Chabra quoted by Neuwinger, 1996; Jacot Guillarmod, 1971; Lucas and Pike, 1971; Neuwinger, 1996; Phillips, 1917; Watt and Breyer-Brandwijk, 1962.
	Leaf	Decoction	Epilepsy			
			Fumigate rooms			
	Root	Powdered and rubbed into the wound	Snakebite, roots are also mixed with the flesh of the snake and put in the patient's porridge			
H. simillimum DC. **8**					[d]B, [d]F	[e]Mathekga, 2001.
H. splendidum (Thunb.) Less. **22**	Roots		Used to treat rheumatism	P Skin		Arnold et al., 2002; Dlamini, 1981; Jacot Guillarmod, 1971; Pooley, 2003; Swanepoel 1997.
			Fuel plant in the mountains			
	Leaf		The leaves are boiled and the steam inhaled to induce sweating.			
			It is used together with *Senecio* species to treat pimples.			

H. subglomeratum Less. **6**	Aerial parts	Smoke inhaled	Headaches	P	I	[e]Jäger et al., 1996.
Helichrysum sutherlandii Harv. **17**	Plant	Burnt, powdered plant material	Powder applied to cuts in the skin of a sick person	M	[d]B, [d]F	Arnold et al., 2002; Jacot Guillarmod, 1971; [e]Mathekga, 2001; Phillips, 1917; Pooley, 1998; Pooley, 2003; Watt and Breyer-Brandwijk, 1962.
[g]*H. tenax* M.D. Hend var. *tenax* (= *H. fulgidum* (L.f) Willd. **30**		Decoction	Used for washing sore eyes.	Eye	[d]B, [d]F	Arnold et al., 2002 [f](*H. fulgidum* (L.f) Willd.); Batten and Bokelmann, 1966 [f](*H. fulgidum* Willd.); [e]Drewes et al., 2006; Jacot Guillarmod, 1971 [f](*H. fulgidum* (L.) Willd.); Phillips, 1917 [f](*H. fulgidum* Willd.).
H. tomentosulum (Klatt) Merxm **1**			Used as a perfume (subsp.) *aromaticum*	P Renal		Neuwinger, 1996; Van Wyk and Gericke, 2000; Von Koenen, 2001.
	Twigs	Extract	Twigs are pounded in water and used as mouth wash for tooth ache			
	Plant	Smoke inhaled	The entire plant is placed on red hot coals and smoke inhaled for body pain. The same treatment is used by pregnant women suffering from antepartum haemorrhage			
	Root	Decoction	Bladder problems (dribbling)			
			Used as thatching.			
H. trilineatum DC. **22**					[d]B, [d]F	[e]Bremner and Meyer, 1998; [e]Mathekga, 2001.
H. umbraculigerum Less. **5**			Heavily grazed		[d]B, [d]F	[e]Mathekga, 2001; Pooley, 1998.
Helichrysum uninervium Burtt Davy **12**[i]			The Swazi use the plant as a purgative or an emetic. They add one teaspoon of the plant to soft porridge which is then eaten by the patient.	GIT		Swanepoel, 1997.

[a] Where the species name has been changed, the previously accepted name is given in brackets. The following species are no longer classified as *Helichrysum*: *H. capillaceum* (Thunb.) Less (Phillips, 1917; Jacot Guillarmod, 1971; Watt Breyer-Brandwijk, 1962) accepted name now *Troglophyton capillaceum* subsp. *capillaceum* (Thunb.) Hilliard and B.L.Burtt (Gibbs Russell et al., 1987); *H. orbiculare* (Thunb.) Druce (Smith, 1966) accepted name now *Plecostachys serpyllifolia* (P.J,Bergius) Hilliard and B.L.Burtt (Gibbs Russell et al., 1987); *H. sesamoides* Willd. (Smith, 1966) accepted name now *Edmondia sesamoides* (L.) Hilliard (Gibbs Russell et al., 1987); *H. vestitum* (L.) Willd.

(Smith 1966) accepted name now *Syncarpha vestita* (L.) B.Nord. (Gibbs Russell et al., 1987); *H. hochstetteri* (A. Rich) Hook. F.) (Githens, 1949), *H. stenopterum* DC (Dlamini, 1981) accepted name now *Achyrocline stenoptera* (DC.) Hilliard (Gibbs Russell et al., 1987).

[b]Abbreviations used:
A = Analgesic activity determined
Anth = Anthelmintic
B = Antibacterial activity determined

Ca = Cardiac conditions
Eye = Used in eye conditions
F = Antifungal activity determined
Fum = Used as fumigant, often plants are burnt in room of a sick person
GIT = Gastrointestinal conditions, which include mainly colic, nausea and vomiting, diarrhoea and stomach pain
I = Anti-inflammatory activity determined
Infect = Conditions associated with infections, such as gonorrhoea and smallpox
Inflam = Conditions associated with inflammation such as swelling, menstrual pain
Insect = Plants are used to deter insects such as red mites
M = Used in a magical sense, to invoke the goodwill of the ancestors and as charms (protective, love)
My = Antimycobacterial activity determined
NS = Not specified
P = Conditions associated with pain, inflammation and fever, which include headache, convulsions and dysmenorrhoea
Pl = Antiplasmodial (antimalarial) activity determined
Psy = Psychotropic use – plants that are used to induce trances
Psyc = Psychological conditions such as inuresis in children and insomnia
Poi = Possible poison, mainly when stock ingest excessive amounts
Renal = Conditions associated with kidney and bladder problems

Resp = Respiratory conditions, which include colds, coughs, flu, tuberculosis
Skin = Used for skin conditions such as keloid scars, abscesses, as ointments
W = Used to dress wounds
V = Antiviral activity determined
Vi = Used for virility in men

[c] The number refers to the morphological group according to Hilliard (1983).
[d] Antimicrobial activity of 1 mg/ml or less observed for one or more micro-organisms.
[e] Reference associated with biological activity
[f] In some cases the author name as indicated in the source, is not present in either Hilliard (1983) or Germishuizen and Meyer (2003). The current author was then chosen.
[g] In cases where the name in the source and the current name differ, the name used in the source is indicated in brackets for clarification.
[h] In cases where the old name is used to describe two different species in the current system, the uses are indicated under both the current names
[i]Vernacular name

Table 2.2 Compounds isolated from South African *Helichrysum* species

Species	Plant group	Phenolic Derivatives						Phloro-glucinols	Pyrones	Diterpenes	Terpenes	Other	Reference
		a	b	c	d	e	f						
[g]*H. acutatum* DC. Roots and aerial parts	21	1	22							240, 241 242	309	328 349	Bohlmann and Abraham, 1979c
[g]*H. adenocarpum* DC. Roots and aerial parts	28											338 340	Bohlmann et al., 1980b
H. albirosulatum Killick Roots and aerial parts	6									192, 193, 194	284		Bohlmann et al., 1980b Bohlmann et al., 1978b
H. allioides Less Roots	23											334	Bohlmann and Zdero, 1973
H. anomalum Less Aerial parts	9							91, 94			250		Jakupovic et al., 1989b
[g]*H. appendiculatum* (L.f.) Less Roots and aerial parts	24										309		Bohlmann et al., 1980b
H. argentissimum J.M. Wood. Roots	28									195	305	338	Bohlmann et al., 1980b
H. argyrolepis MacOwan Aerial parts	29		23	42 48 49									Bohlmann et al., 1984b
[g]*H. argyrophyllum* DC. Aerial parts and roots	29				56 57					247	268	319, 334 341, 342 343, 344 345,346 353	Jakupovic et al., 1989b Bohlmann and Zdero, 1973

Species	Plant group	Phenolic Derivatives						Phloro-glucinols	Pyrones	Diterpenes	Terpenes	Other	Reference
		a	b	c	d	e	f						
H. asperum (Thunb.) Hilliard et Burtt. var. *albidulum* (DC) Hilliard aerial parts	12							90, 93, 96, 97, 98, 99 100, 105, 110, 112, 113, 119, 121, 122, 123, 124, 125, 126, 128, 131, 133, 134, 135					Jakupovic et al., 1989b
[g]*H. athrixiifolium* (Kuntze) Moeser Roots and aerial parts	9	6 17 18	23 25 34										Bohlmann and Ates, 1984
[g]*H. aureonitens* Sch. Bip. Roots and aerial parts	8				54						250, 253, 264, 265 309	323	Bohlmann and Ziesche, 1979; Afolayan and Meyer, 1997; Meyer et al., 1997
[g]*H. aureum* (Houtt.) Merr. Aerial parts and roots	30									195, 204 206, 207 209	280		Jakupovic et al., 1989b (stach-15-en-19-oic acid also isolated)
H. aureum (Houtt.) Merr. var *monocephalum* (DC.) Hilliard Aerial parts and roots	30									195, 196 197, 198	256		Bohlmann et al., 1978b
H. auriceps Hilliard Roots	24							171 173	171 173			339	Bohlmann and Zdero, 1980a
H. bellum Hilliard Aerial parts and roots	28							166 167		195	270	339	Bohlmann and Zdero, 1979

Species	Plant group	Phenolic Derivatives						Phloro-glucinols	Pyrones	Diterpenes	Terpenes	Other	Reference
		a	b	c	d	e	f						
[g]*H. caespititium* DC. Harv. Whole plant Aerial parts	12							98 127					Dekker et al., 1983 Mathekga et al., 2000
[g]*H. callicomum* Harv. (= *H. calliconum*) Aerial parts and roots	2	1						89, 103, 137, 168, 169	*184 *185 *186	243 244 245	252 270 309 310	338	Bohlmann and Abraham, 1979a Bohlmann et al., 1984b, *structures drawn without double bonds in reference, but names indicate pyrones
H. candolleanum H. Buek. Aerial parts	15							87 128		226			Jakupovic et al., 1989b
H. cephaloideum DC. Roots and aerial parts	24				59			106, 115, 138, 139 171, 173 174, 175 177, 178 179, 180	171, 173 174, 175 177, 178 179, 180		270		Hänsel et al., 1980; Bohlmann and Zdero, 1980a Jakupovic et al., 1986
H. cerastioides DC. (1984) Aerial parts	15							88, 141 181	181			345 347	Bohlmann et al., 1984b Jakupovic et al., 1989b [h](*H. cerastroides* DC. supsp. a*urosicum* Merxm. et A. Schreiber)
H. chionosphaerum DC. Aerial parts and roots	25				68					195, 199 201, 207 209, 212 224, 225 226, 227	250 255 257 261	338 354 355	Bohlmann et al., 1980a Jakupovic et al., 1989b

Species	Plant group	Phenolic Derivatives						Phloro-glucinols	Pyrones	Diterpenes	Terpenes	Other	Reference
		a	b	c	d	e	f						
H. chrysargyrum Moeser Aerial parts and roots	22				61			145, 146, 147, 148 149, 150			270 309 311		Bohlmann et al., 1979b
H. confertum N.E.Br Roots	17									195, 213 214, 215 221			Bohlmann et al., 1978b
[g]*H. cooperi* Harv. Roots and aerial parts	30					79				195, 197 198			Bohlmann et al., 1978b
H. cooperi ps. aff. H. *cooperi* Harv.	?		32										Wright, 1976 (Helichrysin- 30a) (also luteolin-7-*O*-glucoside, and 2-*O*-methylchiroinositol)
[g]*H. cymosum* (L.) D. Don. Aerial parts	8	1, 2 7 13 14	22 23	49	56 65							350	Jakupovic et al., 1989b
[g]*H. cymosum* (L.) Don ssp. *calvum*, 1979b Roots and aerial parts	8		22 24 28 38	49							250 270 309		Bohlmann et al., 1979c
[g]*H. cymosum* (L.) D. Don ssp. *cymosum*, 2006)	8			49									Van Vuuren et al., 2006
H. dasyanthum (Willd.) Sweet Aerial parts	10				58					201 202	265, 273 274, 286 292, 293 294, 295 296	351	Jakupovic et al., 1989b
H. dasymallum Hilliard (=*H. lanatum* Harv.) roots	21											334	Bohlmann and Zdero, 1973 [h](*H. lanatum* DC.)

Species	Plant group	Phenolic Derivatives						Phloro-glucinols	Pyrones	Diterpenes	Terpenes	Other	Reference
		a	b	c	d	e	f						
[g]*H. decorum* DC. aerial parts	30		30										Bohlmann et al., 1980b
H. drakensbergense Killick Roots and aerial parts	19								188	233	256, 258 271, 309	353	Bohlmann and Suwita, 1979a
[g]*H. dregeanum* Sond. & Harv. Aerial parts	9		23		58			140			250, 264		Jakupovic et al., 1989b
H. excisum (Thunb.) Less. Aerial parts	12	1 19				67 72 80			19				Lourens et al., in preparation
H. felinum Less Aerial parts	17		23		58			140					Jakupovic et al., 1989b
[g]*H. flanaganii* Bolus Aerial parts and roots	13							90 105					Bohlmann et al., 1980b
[g]*H. foetidum* (L.) Moench. Roots	30											338	Bohlmann and Zdero, 1973
H. fulvum N. E. Br. Aerial parts and roots	30									195, 203 210, 211		338	Bohlmann et al., 1979a
H. glaciale Hilliard Aerial parts	27	14 15											Bohlmann et al., 1980b
H. glomeratum Klatt Aerial parts	6		36							233	250, 256 258, 309 318		Bohlmann and Suwita, 1979a
H. grandiflorum (L.) D. Don. Roots	17											338	Bohlmann and Zdero, 1973
[g]*H. gymnocomum* DC Roots and aerial parts	4							90, 95 101, 105, 107, 111			312		Bohlmann and Mahanta, 1979 [h](*H. gymnoconum* DC.)
[g]*H. gymnocomum* DC Flowers	4	21	35					90, 102					Drewes and Van Vuuren, 2008

Species	Plant group	Phenolic Derivatives						Phloro-glucinols	Pyrones	Diterpenes	Terpenes	Other	Reference
		a	b	c	d	e	f						
[g]*H. herbaceum* (Andrews) Sweet Aerial parts	29	16				74 75 76 77 78					254, 256, 309		Bohlmann et al., 1979b
H. heterolasium Hilliard Aerial parts	30		31		60					197, 198 200, 204 205	248, 249 254, 267 286, 287	338	Bohlmann and Abraham, 1979a
H. hyphocephalum Hilliard Aerial parts and roots	27	2, 3 4, 5 9					82				309	324	Bohlmann and Abraham, 1979d [h] (*H. hypocephalum)*
[g]*H. indicum* (L.) Grierson Aerial parts	15							90 105					Jakupovic et al., 1989b
H. infaustum J.M. Wood. & M.S. Evans Aerial parts	4							90, 91 105			250, 266 267		Bohlmann and Suwita, 1979a
[g]*H. kraussii* Sch. Bip. Aerial parts, flowers and roots	8		22 28 39		58 64 69	70 71		90		195	250, 251 261, 263 264, 265		Jakupovic et al., 1989b Bremner and Meyer, 2000 Candy et al., 1975; Candy and Wright, 1975.
H. krebsianum Less Aerial parts	23									234, 235			Bohlmann et al., 1980b
H. krookii Moeser Roots and aerial parts	5							91, 92 108, 109			270 309	338	Bohlmann et al., 1980b
H. lepidissimum S. Moore Aerial parts	19	19 20					83		19 20			325 326	Jakupovic et al., 1989b
[g]*H. litorale* Bolus *(= Leontonyx angustifolius* DC., *= Leontonyx spathulatus* Less.)	14							120, 129 132, 136 157			270		Bohlmann and Suwita, 1978 [h] (*Leontonyx spathulatus* Less.)

Species	Plant group	Phenolic Derivatives						Phloro-glucinols	Pyrones	Diterpenes	Terpenes	Other	Reference
		a	b	c	d	e	f						
[g]*H. melanacme* (DC.) Harv. Shoots	8		23 39		63 64								Lall et al., 2006
[g]*H. miconiifolium* DC. Roots	23									195, 205			Bohlmann et al., 1980b
H. mimetes S. Moore Aerial parts	19					74			189	246	262, 266 267, 268 272 309		Jakupovic et al., 1986
H. mixtum (Kuntze.) Moeser Roots	24							138, 139 172, 176 179, 180 182, 183	172, 176 179, 180 182, 183				Jakupovic et al., 1986
H. moeseranium Thell. Aerial parts	22							90, 105 136		195			Jakupovic et al., 1989b
H. montanum DC.	22										286 300, 301 304, 307		Lourens et al., in preparation
H. monticola Hilliard Aerial parts and roots	28			45 46 47				91, 108 129, 130 156			309		Jakupovic et al., 1989b Bohlmann and Zdero, 1980b
[g]*H. mundtii Harv.* Roots and aerial parts	23			51 52 53		73 81				236	313 314		Bohlmann et al., 1978a, Bohlmann et al., 1980b [h]*(H. mundii* Harv.)
H. nanum Klatt Aerial parts	6							104		236	250 318		Bohlmann and Suwita, 1979a
[g]*H. natalitium* DC. Aerial parts and roots	3							91, 92 108, 109			309	338	Bohlmann and Zdero, 1979

Species	Plant group	Phenolic Derivatives						Phloro-glucinols	Pyrones	Diterpenes	Terpenes	Other	Reference
		a	b	c	d	e	f						
[g]*H. nudifolium* L. Less var. *nudifolium* Aerial parts and roots	23							142, 143 144, 155, 158		222, 237 239	261, 267 276, 277 278, 279 281, 282 288, 289 290, 291 309	334 336	Jakupovic et al., 1986 Bohlmann et al., 1978b [h](*H. nudifolium* L. Less) Bohlmann and Zdero, 1973 [h](*H.nudifolium* L. Less)
H.nudifolium L.Less var. *nudifolium* (= *H. coriaceum* Harv.) Roots	23									216, 217	276 283	335	Bohlmann et al., 1984a
[g]*H. nudifolium* var. *oxyphyllum* (= *H. oxyphyllum* DC. Aerial parts	23	1	28 29							235	265		Bohlmann et al., 1980b [h](*H. oxyphyllum* Klatt)
[g]*H. nudifolium var. pilosellum* *(=H. latifolium* Less.)	23											334	Bohlmann and Zdero, 1973 [h](*H. latifolium* Less.)
H. nudifolium var. pilosellum *(= H. pilosellum* (L.f.) Less) Roots	23									195, 196 197, 204 205			Jakupovic et al., 1986
[g]*H. odoratissimum* (L.) Sweet Aerial parts and roots	4		31		58 64			90, 105 170, 172	170 172	234 237		334 337	Van Puyvelde et al., 1989; Hänsel et al., 1980; Bohlmann and Zdero, 1973
H. oreophilum Klatt. Aerial parts	21	1	22 28 29					87		235 238	251		Jakupovic et al., 1986 Bohlmann et al., 1980b
H. pagophilum M.D. Hend. Aerial parts	27				58								Bohlmann et al., 1980b
[g]*H. pallidum* DC. Aerial parts and roots	23									195, 197 205, 208	259 309		Bohlmann et al., 1980b
[g]*H. panduratum* O.Hoffm. Aerial parts	18										256 314		Bohlmann and Abraham, 1979b

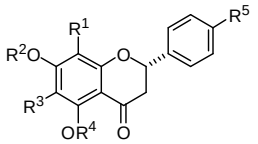
Species	Plant group	Phenolic Derivatives						Phloro-glucinols	Pyrones	Diterpenes	Terpenes	Other	Reference
		a	b	c	d	e	f						
[g]*H. patulum* (L.) D. Don (= *H. crispum* Less) Aerial parts	18							110, 117 129, 136			285		Bohlmann and Suwita, 1979a [h] (*H. crispum* Less)
[g]*H. pedunculatum* Hilliard & Burtt Leaves	23												Dilika et al., 2000 Linoleic and oleic acids
[g]*H. petiolare* Hilliard & B.L.. Burtt. Aerial parts	18		28			72		140	190		250, 251 265, 275 286, 287	334	Jakupovic et al., 1989b Bohlmann and Zdero, 1973 (*H. petiolatum* DC.)
[g]*H. platypterum* DC. Aerial parts and roots	20				55	70	83	90, 105 107, 114, 116, 151, 152, 153, 154, 159, 160, 161, 163, 164 165		195 196		321 338 339	Jakupovic et al., 1986 Bohlmann et al., 1980b Bohlmann and Zdero, 1979a Jakupovic et al., 1987
H. polycladum Klatt Aerial parts and roots	8	12 9	22 23 28	43 44 51				162				330	Bohlmann et al., 1980b
H. populifolium DC. Roots	16											331	Bohlmann et al., 1980b
H. reflexum N. E. Br. *(=H. refluxum* N. E. Br.*)* Aerial parts	29									195 223	261 309 **		Bohlmann et al., 1985 **other terpenes also isolated, see reference
H. revolutum (Thunb.) Less Aerial parts	9				58			140					Jakupovic et al., 1989b
H. retortoides N.E. Br. Aerial parts	26											338	Bohlmann et al., 1980b
H. rosum (Berg.) Less. Aerial parts	9							140				322	Jakupovic et al., 1989b

Species	Plant group	Phenolic Derivatives						Phloro-glucinols	Pyrones	Diterpenes	Terpenes	Other	Reference
		a	b	c	d	e	f						
H. ruderale Hilliard & B.L. Burtt. Aerial parts	30									196 197			Bohlmann et al., 1980b
[g]*H. rugulosum* Less. Aerial parts and roots	9	6, 8 9 10 11	23 24 25 26										Bohlmann and Misra, 1984
H. scabrum (Thunb.) Less Aerial parts	9		22					140					Jakupovic et al., 1989b
[g]*H. setosum* Harv. Aerial parts	30									218, 219 220			Jakupovic et al., 1986
H. spiralepis Hilliard and Burtt. *(= Leontonyx squarrosus)*	14							93, 110, 118, 131, 136, 157				332	Bohlmann and Suwita, 1978
[g]*H. splendidum* (Thunb.) Less. Aerial parts and roots	22			40	66						254, 256 272, 286 297, 298 299, 302 303, 304 306	330	Bohlmann and Suwita, 1979b Jakupovic et al., 1989b
H. subfalcatum Hilliard Aerial parts	6									236	308		Bohlmann et al., 1980b
[g]*H. subglomeratum* Less Aerial parts	6		22	49							264 272		Jakupovic et al., 1989b
[g]*H. sutherlandii* Harv. Aerial parts and roots	17		33 37	50						213		338	Bohlmann et al., 1978a Bohlmann et al., 1980b (*H. suterlandii* Harv.)
H. swynnertonii S. Moore Aerial parts and roots	25											348	Bohlmann et al., 1980b

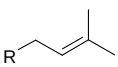
Species	Plant group	Phenolic Derivatives						Phloro-glucinols	Pyrones	Diterpenes	Terpenes	Other	Reference
		a	b	c	d	e	f						
H. tenax var *tenax* M.D. Hend. leaves	30									228, 229 230, 231,232			Drewes et al., 2006
H. tenuiculum DC. Aerial parts and roots	8	2	23 24 38	48							250, 315 309		Bohlmann et al., 1979c
H. tenuifolium Killick. Aerial parts and roots	22	1		41	54 55	70	83				254, 256, 317	327, 329 338, 352	Bohlmann and Abraham, 1979b; Bohlmann et al.,1984a
H. thapsus (O. Kuntze) Moeser Aerial parts	23	9					84 85 86						Bohlmann and Zdero, 1983.
H. tomentosulum Klatt. Merxm subsp. *aromaticum* (Dinter) Merxm. Aerial parts	1		25		62								Jakupovic et al., 1989b
H. tricostatum (Thunb.) Less Aerial parts	11				58 66								Jakupovic et al., 1989b
H. trilineatum DC. Shoots and roots	22										316	327 333	Bremner and Meyer, 1998; Bohlmann et al., 1980b
H. umbraculigerum Less. Aerial parts	5	2	27								309	320, 356 357, 358 359, 360 361, 362, 363, 364, 365, 366, 367, 368, 369	Bohlmann and Hoffmann, 1979
H. vernum Hilliard Roots	28									193, 195	260		Bohlmann et al., 1980b
H. zeyheri Less. Aerial parts	1							89 103	185, 187 191		250, 264		Jakupovic et al., 1986

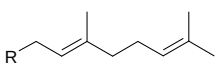
a: Flavanone, b: Chalcone, c: Dihydrochalcone, d: Flavonol, e: Flavone, f: Other flavonoids, [g]Used traditionally, [h]Name used in reference

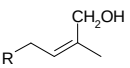
Figure 2.1 Flavanones (a)

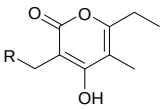


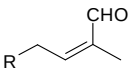
	R^1	R^2	R^3	R^4	R^5	Plant
1	H	H	H	H	H	*H. acutatum* *H. callicomum* *H. cymosum* *H. excisum* *H. oreophilum* *H. oxyphyllum* *H. tenuifolium*
2	Isopentenyl	H	H	H	H	*H. cymosum* *H. hyphocephalum* *H. tenuiculum* *H. umbraculigerum*
3	Hydroxy-isopentenyl	H	H	H	H	*H. hyphocephalum*
4	Oxo-isopentenyl	H	H	H	H	*H. hyphocephalum*
5	Geranyl	H	H	H	H	*H. hyphocephalum*
6	H	Isopentenyl	H	H	H	*H. arthrixiifolium* *H. rugulosum*
7	OCH_3	Isopentenyl	H	H	H	*H. cymosum*
8	H	Isopentenyl	H	CH_3	H	*H. rugulosum*
9	H	H	Isopentenyl	H	H	*H. hyphocephalum* *H. polycladum* *H. rugulosum* *H. thapsus*
10	Isopentenyl	Isopentenyl	H	H	H	*H. rugulosum*
11	Isopentenyl	H	Isopentenyl	H	H	*H. rugulosum*
12	H	CH_3	H	H	H	*H. polycladum*
13	OH	CH_3	H	H	H	*H. cymosum*
14	OCH_3	H	H	H	H	*H. cymosum* *H. glaciale*
15	H	H	OCH_3	H	H	*H. glaciale*
16	H	H	H	CH_3	H	*H. herbaceum*
17	Isopentenyl	H	H	H	OH	*H. arthrixiifolium*
18	H	Isopentenyl	H	H	OH	*H. arthrixiifolium*
19	H	H	CH_2-lactone	H	H	*H. excisum* *H. lepidissimum*
20	Isopentenyl	H	CH_2-lactone	H	H	*H. lepidissimum*
21	H	Benzyl	H	Benzyl	H	*H. gymnocomum*

Isopentenyl = 

Geranyl = 

Hydroxyisopentenyl = 

CH_2-lactone = 

Oxoisopentenyl = 

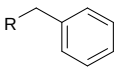
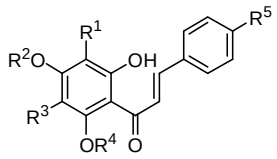
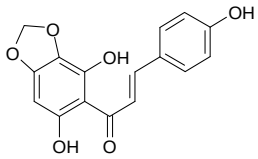
Benzyl = 

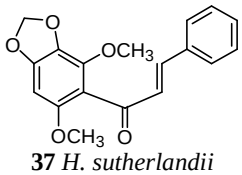
Figure 2.2 Chalcones (b)



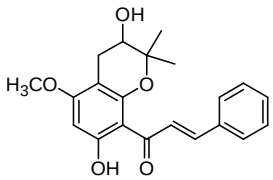
	R^1	R^2	R^3	R^4	R^5	Plant
22	H	H	H	H	H	*H. acutatum* *H. cymosum* *H. kraussii* *H. oreophilum* *H. polycladum* *H. scabrum* *H. subglomeratum*
23	Isopentenyl	H	H	H	H	*H. argyroplepis* *H. athrixiifolium* *H. dregeanum* *H. cymosum* *H. felinum* *H. melanacme* *H. polycladum* *H. revolutum* *H. rugulosum* *H. tenuiculum*
24	Isopentenyl	CH_3	H	H	H	*H. cymosum* *H. rugulosum* *H. tenuiculum*
25	H	Isopentenyl	H	H	H	*H. athrixiifolium* *H. rugulosum* *H. tomentosulum*
26	H	Isopentenyl	H	CH_3	H	*H. rugulosum*
27	H	H	Geranyl	H	H	*H. umbraculigerum*
28	H	CH_3	H	H	H	*H. cymosum* *H. kraussii* *H. oreophilum* *H. oxyphyllum* *H. petiolare* *H. polycladum*
29	H	CH_3	H	CH_3	H	*H. oreophilum* *H. oxyphyllum*
30	H	CH_3	H	CH_3	OH	*H. decorum*
31	H	H	H	CH_3	OH	*H. heterolasium* *H. odoratissimum*
32	H	Glucose	H	CH_3	OH	*H. ps. Aff. H.cooperi Harv.*
33	H	CH_3	OCH_3	CH_3	H	*H. sutherlandii*
34	H	Isopentenyl	H	H	OH	*H. athrixiifolium*
35	H	Benzyl	H	Benzyl	H	*H.gymnocomum*



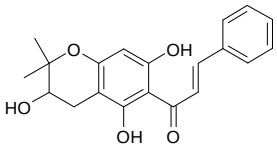
36 *H. glomeratum*



37 *H. sutherlandii*

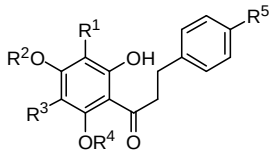


38 *H. cymosum, H. tenuiculum*

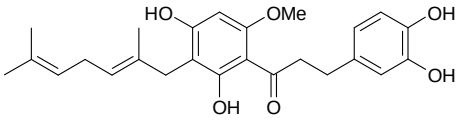


39 *H. kraussii, H. melanacme*

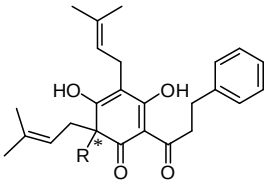
Figure 2.3 Dihydrochalcones (c)



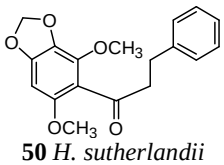
	R^1	R^2	R^3	R^4	R^5	R^6	Plant
40	H	H	H	H	OH	H	*H. splendidum*
41	H	H	H	H	H	H	*H. tenuifolium*
42	H	H	Isopentenyl	H	H	H	*H. argyrolepis*
43	H	Isopentenyl	H	H	H	H	*H. polycladum*
44	Isopentenyl	H	Isopentenyl	H	H	H	*H. polycladum*
45	E-Geranyl	H	OH	H	OH	H	*H. monticola*
46	Z-Geranyl	H	OH	H	OH	H	*H. monticola*



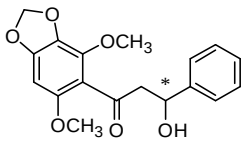
47 *H. monticola*



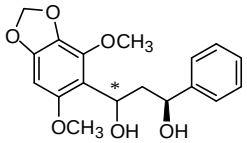
48 R = $CH_2CH=C(CH_3)_2$
H. tenuiculum,H. argyrolepis
49 R = OCH_3
H. argyrolepis, H. cymosum, H. subglomeratum



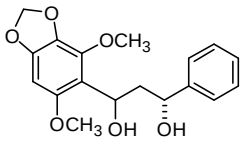
50 *H. sutherlandii*



51 *H. mundtii, H. polycladum*

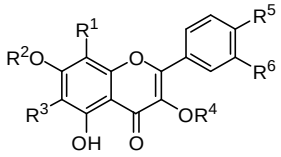


52 *H. mundtii*

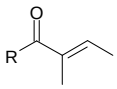


53 *H. mundtii*

Figure 2.4 Flavonols (d)



	R^1	R^2	R^3	R^4	R^5	R^6	Plant
54	H	H	H	H	H	H	*H. aureonitens* *H. tenuifolium*
55	H	H	H	CH_3	H	H	*H. platypterum* *H. tenuifolium*
56	OCH_3	H	H	CH_3	H	H	*H. argyrophyllum* *H. cymosum*
57	OCH_3	H	H	Angeloyl	H	H	*H. argyrophyllum*
58	OCH_3	CH_3	OCH_3	H	H	H	*H. dasyanthum* *H. dregeanum* *H. felinum* *H. kraussii* *H. odoratissimum* *H.pagophilum* *H. revolutum* *H. tricostatum*
59	OCH_3	CH_3	OCH_3	CH_3	H	H	*H. cephaloideum*
60	H	H	OCH_3	CH_3	H	H	*H. heterolasium*
61	H	CH_3	OH	CH_3	H	H	*H. chrysargyrum*
62	H	CH_3	OCH_3	CH_3	OH	H	*H. tomentosulum*
63	H	H	H	H	OH	OH	*H. melanacme*
64	H	H	H	CH_3	OH	OH	*H. kraussii* *H. melanacme* *H. odoratissimum*
65	OCH_3	CH_3	H	H	OH	H	*H. cymosum*
66	OCH_3	CH_3	H	CH_3	OH	OH	*H. splendidum* *H. tricostatum*
67	OCH_3	H	H	OCH_3	H	H	*H. excisum*
68	H	CH_3	OCH_3	CH_3	OCH_3	OCH_3	*H. chionosphaerum*
69	H	H	H	*p*-coumaroyl glucoside	OH	OH	*H. kraussii*

Angeloyl = 

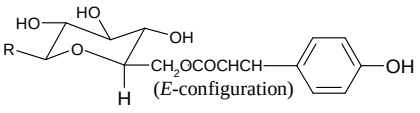
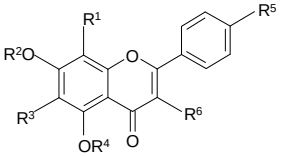
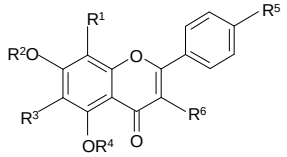
p-Coumaroyl glucoside = 

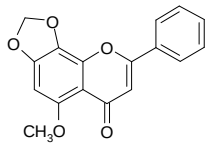
Figure 2.5 Flavones (e)



	R^1	R^2	R^3	R^4	R^5	R^6	Plant
70	H	H	H	H	H	H	*H. kraussii* *H. platypterum* *H. tenuifolium*

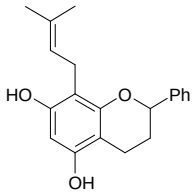


71	H	CH_3	H	H	H	H	*H. kraussii*
72	OCH_3	CH_3	H	H	H	H	*H. excisum* *H. petiolare*
73	OCH_3	CH_3	H	CH_3	H	H	*H. mundtii*
74	OH	CH_3	OCH_3	H	H	H	*H. herbaceum* *H. mimetes*
75	OCH_3	CH_3	OCH_3	CH_3	H	H	*H. herbaceum*
76	OCH_3	H	OCH_3	CH_3	OCH_3	H	*H. herbaceum*
77	H	CH_3	H	CH_3	H	H	*H. herbaceum*
78	OH	CH_3	OCH_3	CH_3	H	H	*H. herbaceum*
79	H	H	H	H	OH	H	*H. cooperi*
80	OH	Glucose	H	H	OH	H	*H. excisum*

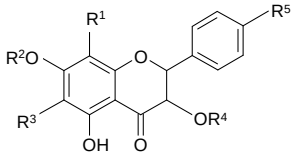


81 *H. mundtii*

Figure 2.6 Other flavonoids (f)



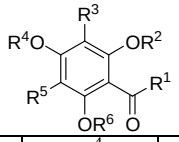
82 *H hyphocephalum*



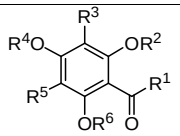
83-86

	R^1	R^2	R^3	R^4	R^5	Plant
83	H	H	H	H	H	*H. lepidissimum* *H. platypterum* *H. tenuifolium*
84	H	H	Isopentenyl	H	H	*H. thapsus*
85	H	H	Geranyl	H	H	*H. thapsus*
86	H	H	Isopentenyl	Ac	H	*H. thapsus*

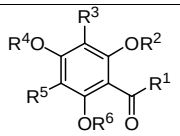
Figure 2.7 Phloroglucinol derivatives (excluding flavonoids)



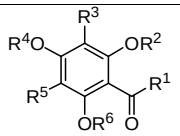
	R^1	R^2	R^3	R^4	R^5	R^6	Plant
87	CH_3	H	Isopentenyl	H	H	H	*H. candolleanum* *H. oreophilum*
88	CH_3	H	Geranyl	H	H	CH_3	*H. cerastioides*
89	$CH(CH_3)_2$	H	H	H	H	H	*H. callicomum* *H. zeyheri*



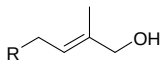
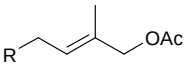
	R^1	R^2	R^3	R^4	R^5	R^6	Plant
90	$CH(CH_3)_2$	H	Isopentenyl	H	H	H	*H. asperum* *H. flanagani* *H. gymnoconum* *H. indicum* *H. infaustum* *H. kraussii* *H. moeseranium* *H. odoratissimum* *H. platypterum*
91	$CH(CH_3)_2$	H	E-Geranyl	H	H	H	*H. anomalum* *H. infaustum* *H. krookii* *H. monticola* *H. natalitium*
92	$CH(CH_3)_2$	H	(*Z*)-Geranyl	H	H	H	*H. krookii* *H. natalitium*
93	$CH(CH_3)_2$	H	H	Isopentenyl	H	H	*H. asperum* *H. spiralepis*
94	$CH(CH_3)_2$	H	H	Geranyl	H	H	*H. anomalum*
95	$CH(CH_3)_2$	CH_3	H	Geranyl	H	H	*H. gymnocomum*
96	$CH(CH_3)_2$	H	H	Hydroxy-isopentenyl	H	H	*H. asperum*
97	$CH(CH_3)_2$	H	H	Acetoxy isopentenyl	H	H	*H. asperum*
98	$CH(CH_3)_2$	H	Acetoxy-isopentenyl	H	H	H	*H. asperum* *H. caespititium*
99	$CH(CH_3)_2$	H	H	CH_2CH_2CH-$(CH_3)COOH$	H	H	*H. asperum*
100	$CH(CH_3)_2$	H	H	CH_2CH_2CH-$(CH_3)COOMe$	H	H	*H. asperum*
101	$CH(CH_3)_2$	H	Isopentenyl	H	H	CH_3	*H. gymnocomum*
102	$CH(CH_3)_2$	H	$CH_2CH(OH)$ $C(CH_3)=CH_2$	H	H	H	*H. gymnocomum*
103	$CH(CH_3)CH_2$ CH_3	H	H	H	H	H	*H. callicomum* *H. zeyheri*
104	$CH(CH_3)CH_2$ CH_3	CH_3	CH_3	H	H	H	*H. nanum*
105	$CH(CH_3)CH_2$ CH_3	H	Isopentenyl	H	H	H	*H. asperum* *H. flanagani* *H. gymnocomum* *H. indicum* *H. infaustum* *H. moeseranium* *H. odoratissimum* *H. platypterum*
106	$CH(CH_3)CH_2$ CH_3	H	Isopentenyl	CH_3	H	H	*H. cephaloideum*
107	$CH(CH_3)CH_2$ CH_3	H	Isopentenyl	H	H	CH_3	*H. gymnocomum* *H. platypterum*

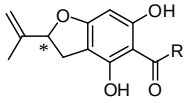


	R^1	R^2	R^3	R^4	R^5	R^6	Plant
108	$CH(CH_3)CH_2CH_3$	H	(*E*)-Geranyl	H	H	H	*H. krookii* *H. monticola* *H. natalitium*
109	$CH(CH_3)CH_2CH_3$	H	(*Z*)-Geranyl	H	H	H	*H. krookii* *H. natalitium*
110	$CH(CH_3)CH_2CH_3$	H	H	Isopentenyl	H	H	*H. asperum* *H.patulum (H. crispum* in reference) *H. spiralepis*
111	$CH(CH_3)CH_2CH_3$	CH_3	H	Geranyl	H	H	*H. gymnocomum*
112	$CH(CH_3)CH_2CH_3$	H	H	Hydroxy-isopentenyl	H	H	*H. asperum*
113	$CH(CH_3)CH_2CH_3$	H	H	Acetoxy-isopentenyl	H	H	*H. asperum*
114	$CH_2CH(CH_3)_2$	H	Isopentenyl	H	H	H	*H. platypterum*
115	$CH_2CH(CH_3)_2$	H	Isopentenyl	CH_3	H	H	*H. cephaloideum*
116	$CH_2CH(CH_3)_2$	H	Isopentenyl	H	H	CH_3	*H. platypterum*
117	$CH{=}C(CH_3)_2$	H	H	Isopentenyl	H	H	*H. patulum* (*H. crispum* in reference)
118	CH_2CH_3	H	H	Isopentenyl	H	H	*H. spiralepis*
119	$CH_2CH_2CH_3$	H	Isopentenyl	H	H	H	*H. asperum*
120	$CH_2CH_2CH_3$	OCH_3	Isopentenyl	H	OCH_3	H	*H. litorale*
121	$CH_2CH_2CH_3$	H	Acetoxy-isopentenyl	H	H	H	*H. asperum*
122	$CH_2CH_2CH_3$	H	H	Isopenteny	H	H	*H. asperum*
123	$CH_2CH_2CH_3$	H	H	Hydroxy-isopentenyl	H	H	*H. asperum*
124	$CH_2CH_2CH_3$	H	H	Acetoxy-isopentenyl	H	H	*H. asperum*
125	$CH_2CH_2CH_3$	H	H	Dihydroxy-isopentenyl	H	H	*H. asperum*
126	$CH_2CH_2CH_3$	H	H	CH_2CH_2CH-$(CH_3)COOH$	H	H	*H. asperum*
127	$CH_2CH_2CH(CH_3)_2$	H	Isopentenyl	H	H	H	*H. caespititium*
128	Ph	H	Isopentenyl	H	H	H	*H. asperum* *H. candolleanum*
129	Ph	H	E-Geranyl	H	H	H	*H. litorale* *H. monticola* *H. patulum* (*H. crispum* in ref)
130	Ph	H	Z-Geranyl	H	H	H	*H. monticola*
131	Ph	H	H	Isopentenyl	H	H	*H. asperum* *H. spiralepis*
132	Ph	H	H	Geranyl	H	H	*H. litorale*

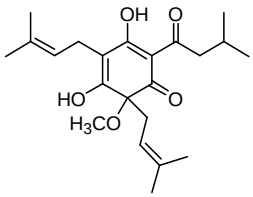


133	Ph	H	H	Hydroxy-isopentenyl	H	H	*H. asperum*
134	Ph	H	H	CH_2CH_2CH-$(CH_3)COOH$	H	H	*H. asperum*
135	Ph	H	H	CH_2CH_2CH-$(CH_3)CO_2CH3$	H	H	*H. asperum*
136	Ph	H	Isopentenyl	H	Isopen-tenyl	H	*H. litorale* *H. moeseranium* *H.patulum* *(H. crispum* in reference) *H. spiralepis*

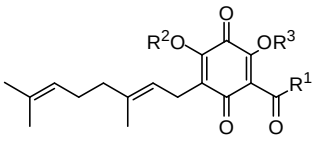
Hydroxyisopentenyl =  Acetoxyisopentenyl = 

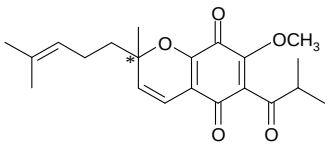


	R	Plant
137	$CH(CH_3)_2$	*H. callicomum*
138	$CH(CH_3)CH_2CH_3$	*H. cephaloideum* *H. mixtum*
139	$CH_2CH(CH_3)_2$	*H. cephaloideum* *H. mixtum*



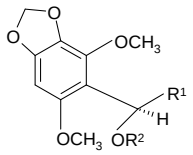
140 *H. dreagenaum, H. felinum, H. petiolare, H. revolutum, H. rosum, H. scabrum*



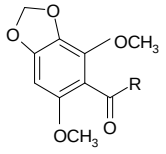


144 *H. nudifolium*

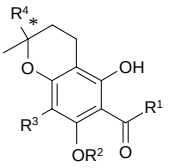
	R^1	R^2	R^3	Plant
141	CH_3	CH_3	H	*H. cerastioides*
142	$CH(CH_3)_2$	H	H	*H. nudifolium*
143	$CH(CH_3)_2$	H	CH_3	*H. nudifolium*

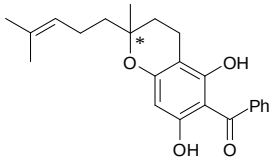


	R^1	R^2	Plant
145	$CH(CH_3)_2$	H	*H. chrysargyrum*
146	$CH(CH_3)_2$	CH_3	*H. chrysargyrum*
147	$CH(CH_3)CH_2CH_3$	H	*H. chrysargyrum*
148	$CH(CH_3)CH_2CH_3$	CH_3	*H. chrysargyrum*

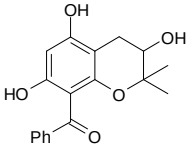


	R	
149	$CH(CH_3)_2$	*H. chrysargyrum*
150	$CH(CH_3)CH_2CH_3$	*H. chrysargyrum*



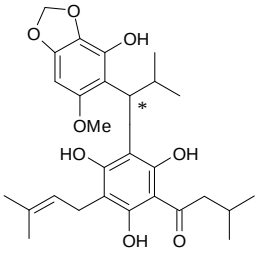


156 *H monticola*

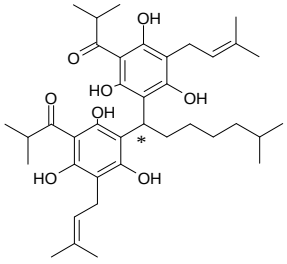


157 *H. litorale, H. spiralepis*

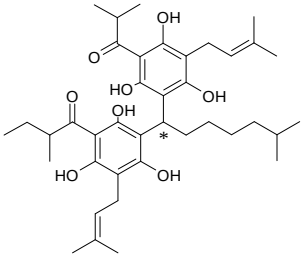
	R^1	R^2	R^3	R^4	Plant
151	$CH(CH_3)CH_2CH_3$	H	H	CH_3	*H. platypterum*
152	$CH(CH_3)CH_2CH_3$	CH_3	H	CH_3	*H. platypterum*
153	$CH_2CH(CH_3)_2$	H	H	CH_3	*H. platypterum*
154	$CH_2CH(CH_3)_2$	CH_3	H	CH_3	*H. platypterum*
155	$CH_2CH(CH_3)_2$	CH_3	OH	CH_2CH_2 $CH{=}C(CH_3)_2$	*H. nudifolium*



158 *H. nudifolium*

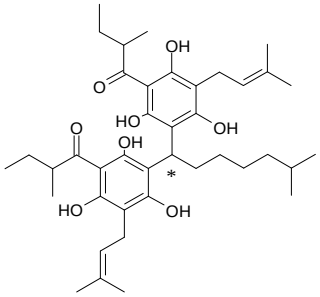


159 *H. platypterum*

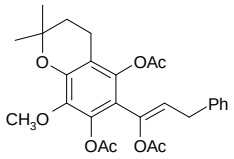


160 *H. platypterum*

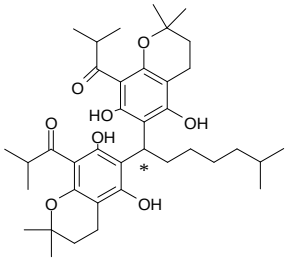
* Configuration not indicated in original source



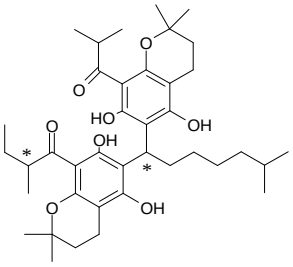
161 *H. platypterum*



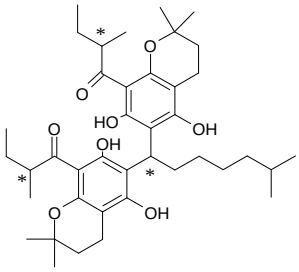
162 *H. polycladum*



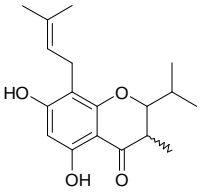
163 *H. platypterum*



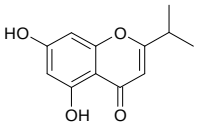
164 *H. platypterum*



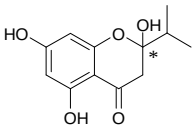
165 *H. platypterum*



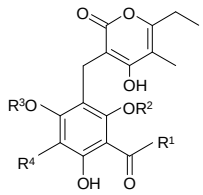
2 isomers. **166**; **167** *H. bellum*



168 *H. callicomum*

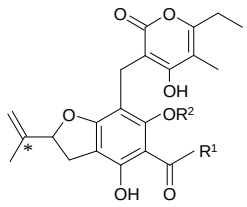


169 *H. callicomum*

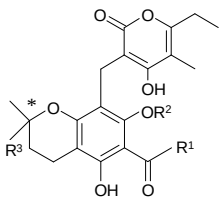


	R^1	R^2	R^3	R^4	
170	$CH(CH_3)_2$	H	H	Isopentenyl	*H. odoratissimum*
171	$CH(CH_3)_2$	CH_3	H	Isopentenyl	*H. auriceps* *H. cephaloideum*
172	$CH(CH_3)CH_2CH_3$	H	H	Isopentenyl	*H. odoratissimum* *H. mixtum*
173	$CH(CH_3)CH_2CH_3$	CH_3	H	Isopentenyl	*H. auriceps* *H. cephaloideum*
174	$CH(CH_3)CH_2CH_3$	H	CH_3	Isopentenyl	*H. cephaloideum*
175	$CH(CH_3)CH_2CH_3$	H	CH_3	H	*H. cephaloideum*
176	$CH_2CH(CH_3)_2$	H	H	Isopentenyl	*H. mixtum*
177	$CH_2CH(CH_3)_2$	H	CH_3	H	*H. cephaloideum*
178	$CH_2CH(CH_3)_2$	H	CH_3	Isopentenyl	*H. cephaloideum*

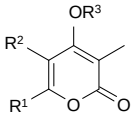
*Configuration not indicated in original source



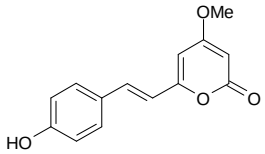
	R^1	R^2	
179	$CH(CH_3)CH_2CH_3$	H	*H. cephaloideum* *H. mixtum*
180	$CH_2CH(CH_3)_2$	H	*H. cephaloideum* *H. mixtum*



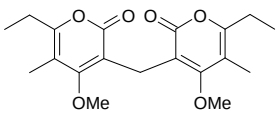
	R^1	R^2	R^3	
181	CH_3	H	$CH_2CH_2CHC(CH_3)_2$	*H. cerastioides*
182	$CH(CH_3)CH_2CH_3$	H	CH_3	*H. mixtum*
183	$CH_2CH(CH_3)_2$	H	CH_3	*H. mixtum*



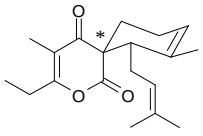
	R^1	R^2	R^3	
184	CH_2CH_3	CH_3	CH_3	*H. callicomum*
185	$CH(CH_3)_2$	CH_3	CH_3	*H. callicomum* *H. zeyheri*
186	$CH(CH_3)_2$	CH_2OH	CH_3	*H. callicomum*
187	$CH(CH_3)_2$	CH_3	H	*H. zeyheri*



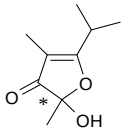
188 *H. drakenbergense*



189 *H. mimetes*



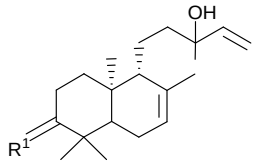
190 *H. petiolare*



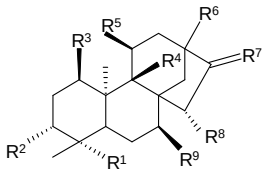
191 *H. zeyheri*

*Configuration not indicated in original source

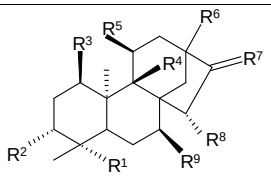
Figure 2.8 Diterpenes



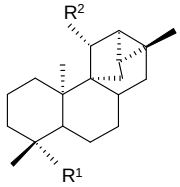
	R^1	
192	O	*H. albirosulatum*
193	H	*H. albirosulatum* *H. vernum*
194	OH, H	*H. albirosulatum*



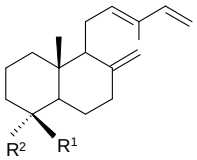
	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8	R^9	Species
195	COOH	H	H				CH_2			*H. argentissimum* *H. aureum* *H. bellum* *H. chionosphaerum* *H. confertum* *H. cooperi* *H. fulvum* *H. kraussii* *H. miconiifolium* *H. moeseranium* *H. pallidum* *H. pilosellum* *H. platypterum* *H. reflexum* *H. vernum*
196	COOH	H	H				CH_2	9,11-double bond		*H. aureum* *H. pilosellum* *H. platypterum* *H. ruderale*
197	COOH		H		OCO CH_3		CH_2			*H. aureum* *H. cooperi* *H. heterolasium* *H. pallidum* *H. pilosellum* *H. ruderale*
198	COOH	OCO CH_3					CH_2			*H. aureum* *H. cooperi* *H. heterolasium*
199	COOH		OCO CH_3				CH_2			*H. chionosphaerum*
200	COOH						CH_2	$OCOCH_3$		*H. heterolasium*

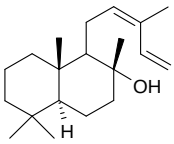


	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8	R^9	Species
201	COOH						CH_2	OH		*H. chionosphaerum* *H. dasyanthum*
202	COOH			OH			CH_2	OH	OH	*H. dasyanthum*
203	COOH					CH_3	H	H		*H. fulvum* *15,16 double bond
204	CH_2O H	H	H				CH_2			*H. aureum* *H. heterolasium* *H. pilosellum*
205	CHO						CH_2			*H. heterolasium* *H. miconiifolium* *H. pallidum* *H. pilosellum*
206	CH_2OC OCH_2C OOH	H	H				CH_2			*H. aureum*
207	CH_3					OH	CH_2 OH			*H.aureum* *H. chionosphaerum*
208	CH_3						H, OH			*H. pallidum*

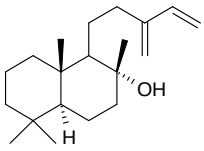


	R^1	R^2	
209	COOH	H	*H. aureum* *H. chionosphaerum*
210	COOH	OH	*H. fulvum*
211	COOH	$OCOCH_3$	*H. fulvum*
212	CH_2OH	H	*H. chionosphaerum*



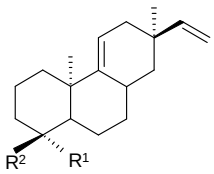


216 abienol, *H. nudifolium* (*H. coriaceum* in reference)

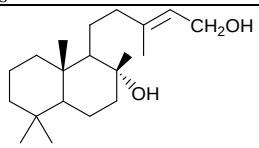


217 (isoabienol, *H. nudifolium* (*H. coriaceum* in reference)

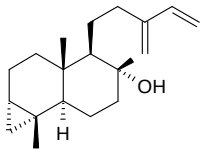
	R^1	R^2	
213	CH_3	CH_3	*H. confertum* *H. sutherlandii*
214	H	$COOCH_3$	*H. confertum*
215	$COOCH_3$	H	*H. confertum*



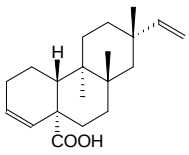
	R^1	R^2	
218	CH_3	CH_2OH	*H. setosum*
219	CH_2OCOCH_2COOH	CH_3	*H. setosum*
220	CH_3	CH_2OCOCH_2COOH	*H. setosum*



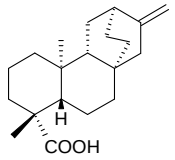
221 *H. confertum*



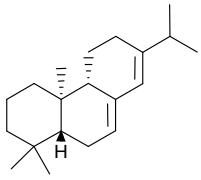
222 *H. nudifolium*



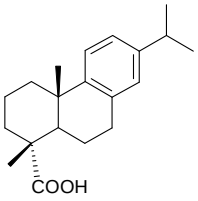
223 *H. refluxum*



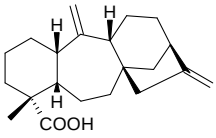
224
H. chionospaerum



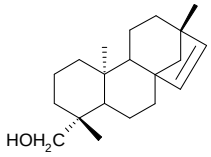
225
H. chionospaerum



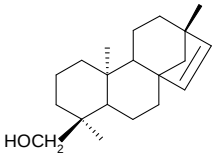
Dehydroabietic acid
226 *H. chionospaerum, H. candolleanum*



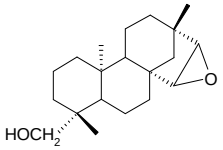
227
H. chionospaerum



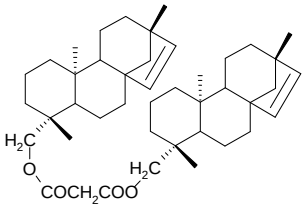
228 *H. tenax*



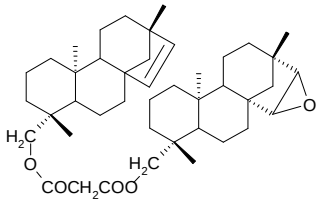
229 *H. tenax*



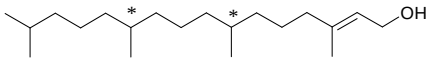
230 *H. tenax*



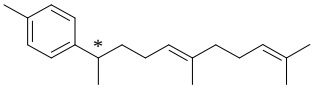
231 *H. tenax*



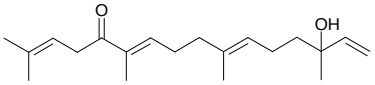
232 *H. tenax*



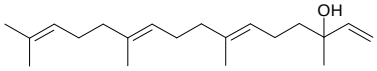
233 *H. drakenbergense, H. glomeratum*



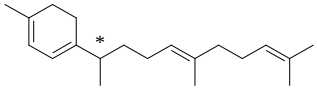
234 *H. krebsianum, H. odoratissimum*



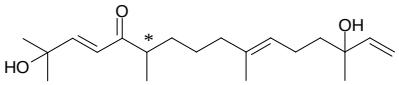
12-Oxo-geranyl linalool
235 *H. krebsianum, H. oreophilum, H. oxyphyllum*



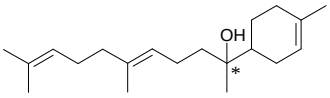
geranyl linalool
236 *H. mundtii, H. nanum, H. subfalcatum*



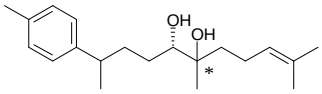
237 *H. nudifolium, H. odoratissimum*



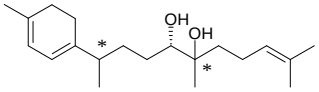
238 *H. oreophilum*



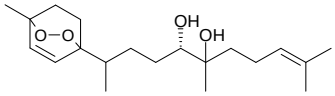
239 *H. nudifolium*



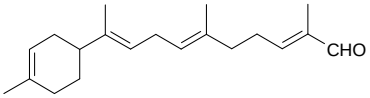
240 *H. acutatum*



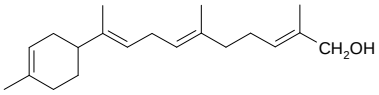
241 *H. acutatum*



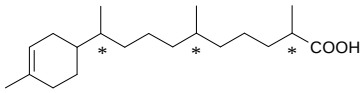
242 *H. acutatum*



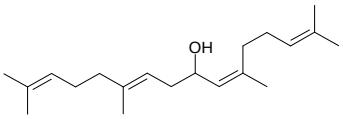
243 *H. callicomum*



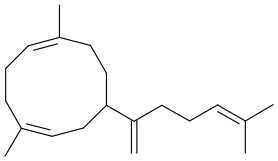
244 *H. callicomum*



245 *H. callicomum*

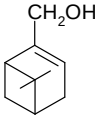


246 geranyl nerol
H. mimetes

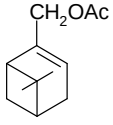


247 *H. argyrophyllum*

Figure 2.9 Monoterpenes



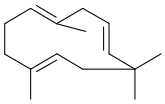
248 *H. heterolasium*



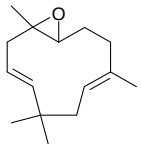
249 *H. heterolasium*

*Configuration not indicated in source

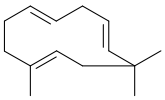
Figure 2.10 Sesquiterpenes



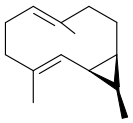
250 α-humulene = humulene
H. anomalum
H. aureonitens
H. chionospaerum
H. cymosum
H. dregeanum
H. glomeratum
H. infaustum
H. kraussii
H. nanum
H. petiolare
H. tenuiculum
H. zeyheri



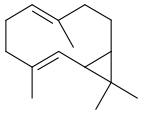
251 α-humulene-epoxide
H. kraussi
H. petiolare
H. oreophilum



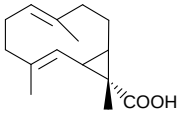
252
H. callicomum



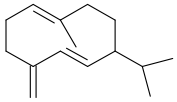
253
H. aureonitens



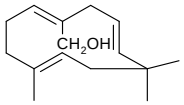
254
H. herbaceum
H. heterolasium
H. splendidum
H. tenuifolium



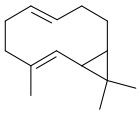
255
H. chionospaerum



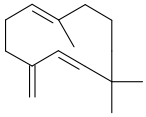
256
H. aureum
H. drakenbergense
H. glomeratum
H. herbaceum
H. panduratum
H. splendidum
H. tenuifolium



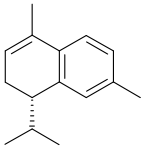
257
H. chionospaerum



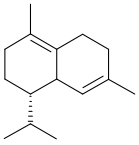
258 *H drakenbergense*
H. glomeratum



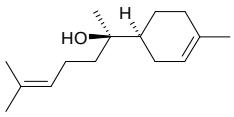
259 *H. pallidum*



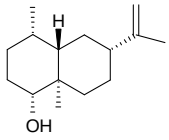
260
H. vernum



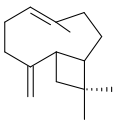
261
H. chionospaerum
H. kraussi
H. nudifolium
H. reflexum



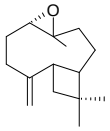
262
H. mimetes



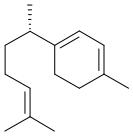
263
H. krausii



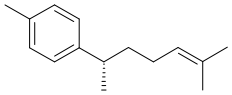
264
H. aureonitens,
H. dregeanu
H. kraussi,i H. zeyheri
H. subglomeratum



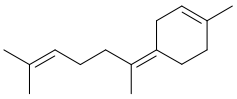
265
H. aureonitens
H. dasyanthum,
H. kraussi,i H.petiolare
H.oxyphyllum



266
H. infaustum
H. mimetes

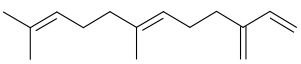


267
H. heterolasium,
H. infaustum, H.mimetes
H. nudifolium



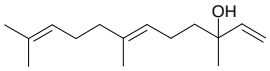
γ-curcumene endoperoxide
268
H. mimetes

269
H. argyrophyllum
H. mimetes

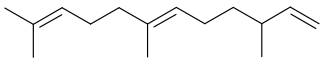


270

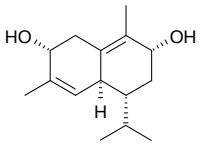
H. bellum, H. callicomum, H. cephaloideum, H. chrysargyrum, H. cymosum, H. krookii, H. litorale



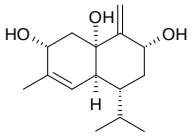
nerolidol
271
H. mimetes, H. splendidum, H. subglomeratum



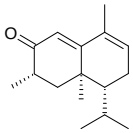
272
H. drakenbegense



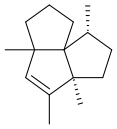
273 *H. dasyanthum*



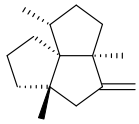
274 *H. dasyanthum*



275 *H. petiolare*

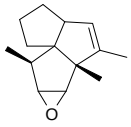


276 *H. nudifolium*



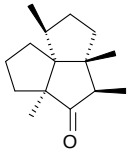
β-isocomene

277 *H. nudifolium*

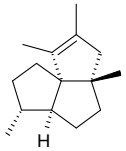


isocomene-5,6-epoxide

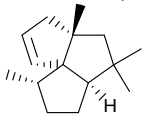
278 *H. nudifolium*



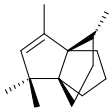
279 *H. nudifoliu*m



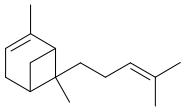
silphiperfolene
280 *H. aureum*



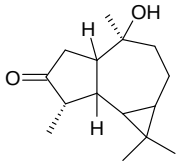
silphinene
281 *H. nudifolium*



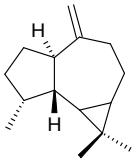
modhephene
282 *H. nudifolium*



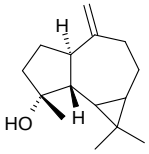
α-bergamotene
283 *H. coriaceum*



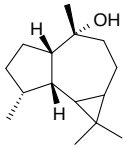
284
H. albirosulatum



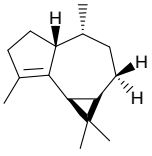
285
H. crispum



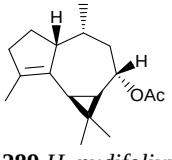
spathulenol
286
H. dasyanthum
H. heterolasium,
H. montanum,
H. petiolare
H. splendidum



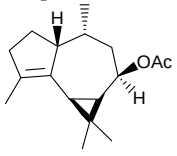
ledol
287
H. heterolasium
H. petiolare



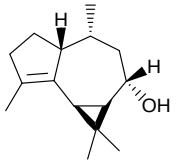
288 *H. nudifolium*



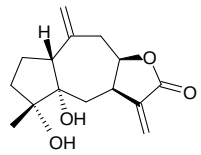
289 *H. nudifolium*



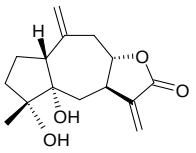
290 *H. nudifolium*



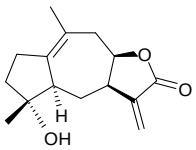
291 *H. nudifolium*



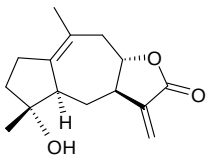
292 *H. dasyanthum*



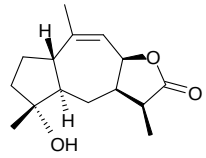
293 *H. dasyanthum*



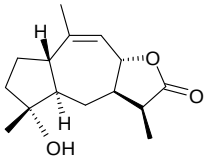
294 *H. dasyanthum*



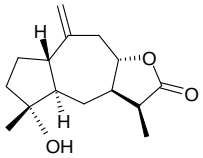
295 *H. dasyanthum*



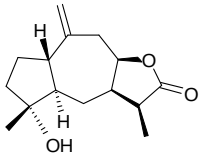
296 *H. dasyanthum*



297 *H. splendidum*



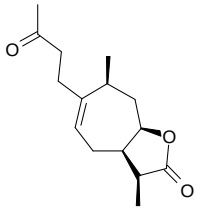
298 *H. splendidum*



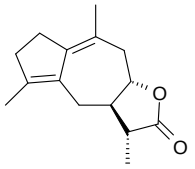
299
H. splendidum

300* stereoisomer of 298, from *H. montanum*

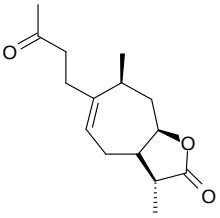
301* stereoisomer of 299 from *H.montanum*



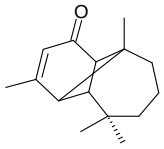
302
H. splendidum



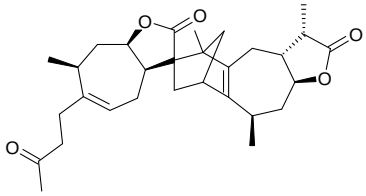
303
H. splendidum



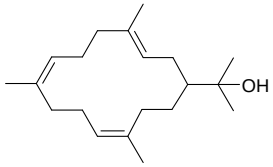
304
H. splendidum,
H. montanum



305
H. argentissimum



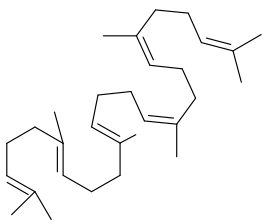
306 *H. splendidum*
307* Stereoisomer of 306, from *H. montanum*



308 *H. subfalcatum* (diterpene)

* These compounds are described in Chapter 5.

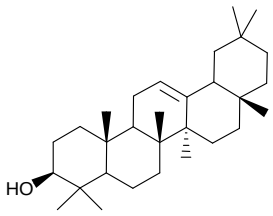
Figure 2.11 Triterpenes



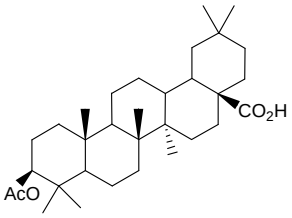
squalene

309

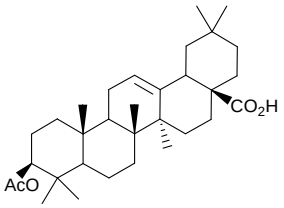
H. acutatum, H. appendiculatum, H. aureonitens, H. callicomum, H. chrysargyrum, H. cymosum, H. drakenbergense, H. glomeratum, H. hypocephalum, H. krookii, H. mimetes, H. monticola, H. natalitium, H. nudifolium, H. pallidum, H. tenuiculum, H. umbraculigerum



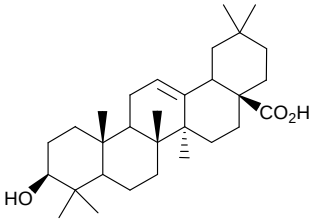
310 *H. callicomum*



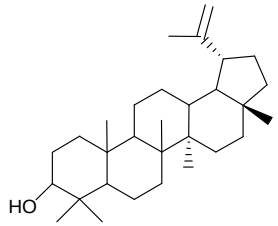
311 *H. chrysargyrum*



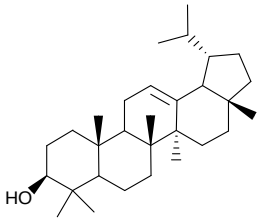
312 *H. gymnocomum*



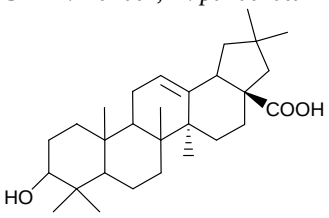
313 *H. mundtii*



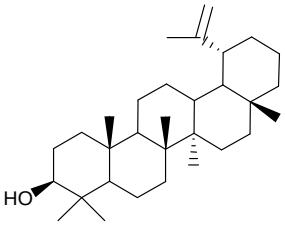
314 *H. mundtii, H. panduratum*



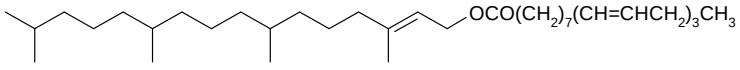
315 *H. tenuiculum*



316 *H. trilineatum*

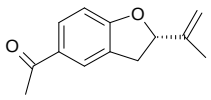


317 *H. tenuifolium*

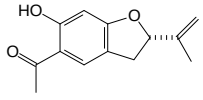


318 *H. glomeratum, H. nanum*

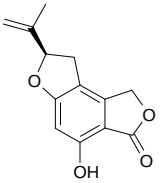
Figure 2.12 Other



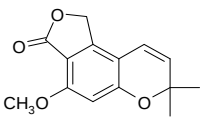
319
H. argyrophyllum



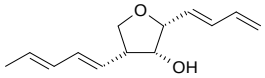
320
H. umbraculigerum



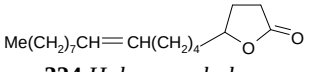
321 *H. platypterum*



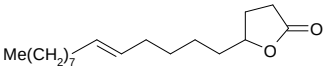
322 *H. rosum*



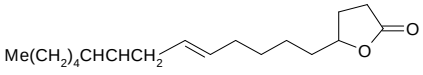
323 *H. aureonitens*



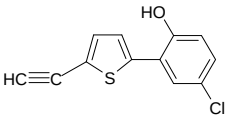
324 *H. hypocephalum*



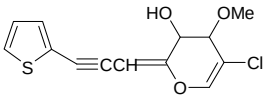
325 *H. lepidissimum*



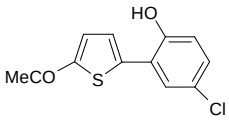
326 *H. lepidissimum*



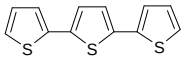
327 *H. tenuifolium*
H. trilineatum



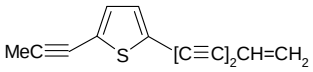
328 *H. acutatum* *



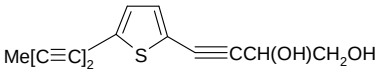
329 *H. tenuifolium*



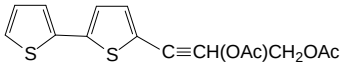
330 *H. polycladum,*
H. splendidum



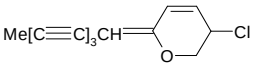
331 *H. populifolium**



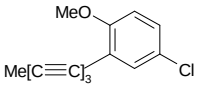
332 *H. spiralepis*



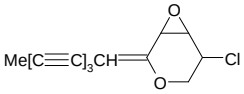
333 *H. trilineatum*



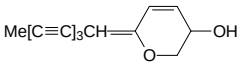
334
*H. allioides, H. argenteum, H. argyrophyllum, H. lanatum, H. latifolium, H. nudifolium, H. odoratissimum, H. paniculatum, H. petiolatum, H. serotinum**



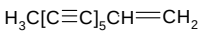
335
H. coriaceum



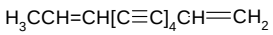
336 *H. nudifolium**



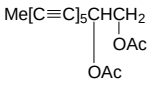
337 *H. odoratissimum**



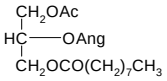
338 *H. adenocarpum, H. argentissimum, H. callicomum, H. chionospaerum, H. foetidum, H. fulvum, H. grandiflorum, H. heterolasium, H. krookii, H. natalitium, H. platypterum, H. retortoides, H. sutherlandii, H. tenuifolium**



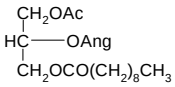
339 *H. auriceps, H. bellum, H. Platypterum**



340 *H. adenocarpum* *



341
*H. argyrophyllum**



342
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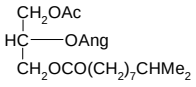
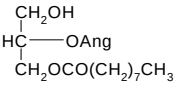
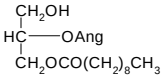


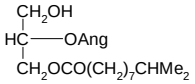
343
*H. argyrophyllum**



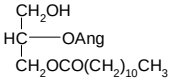
344
*H. argyrophyllum**



345 *H. argyrophyllum*
*H. cerastroides**

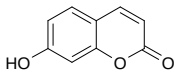


346
H. argyrophyllum

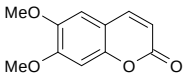


347 *H. cerastroides***

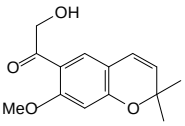
* Configuration at double bonds and stereocentres not indicated in original sources



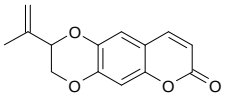
348 *H. swynnertonii*



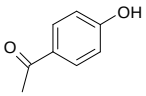
349 *H. acutatum*



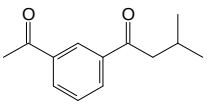
350 *H. cymosum*



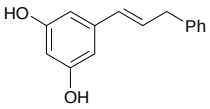
351 obliquin
H. dasyanthum



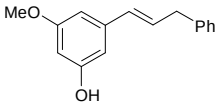
352
H. drakenbergense
H. tenuifolium



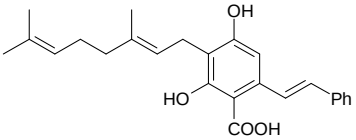
353
H. argyrophyllum



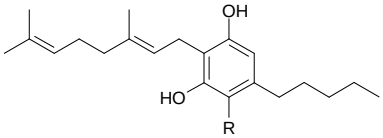
354
H. chionospaerum



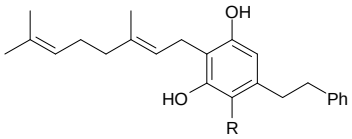
355
H. chionospaerum



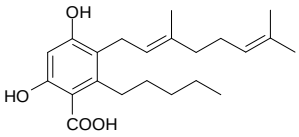
356 *H. umbraculigerum*



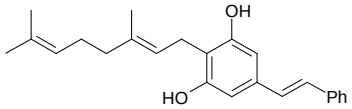
357 R =H,
358 R = COOH *H. umbraculigerum*



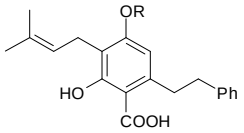
359 R = H,
360 R = COOH *H. umbraculigerum*



361 *H. umbraculigerum*

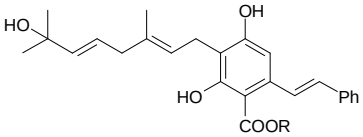


362 *H. umbraculigerum*



363	**364**	**365**	**366**	**367**
R H	*i*Bu	*i*Val	MeBu	Ang

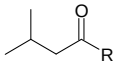
(*H. umbraculigerum*)



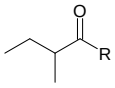
368 R = H
369 R = Me *H. umbraculigerum*

*i*Bu = isobutyryl =

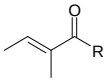
*i*Val = isovaleryl =



MeBu = 2-methylbutyryl =



Ang = angeloyl =



CHAPTER 3

The antimicrobial activity and cytotoxicity of selected *Helichrysum* extracts

3.1 Introduction

There are two main reasons why researchers should concern themselves with antimicrobial research. Firstly, it is well established that resistance of bacteria, especially those associated with serious infections, are increasing against antibiotics worldwide (Alanis, 2005; Chander et al., 2007; Jansen et al., 2006; Overbye and Barret, 2005). Secondly, another alarming trend is the global decrease in antibacterial and antibiotic research by large pharmaceutical companies. There are various reasons for this decrease: 1) development of resistance against any antimicrobial drug is almost guaranteed as the most resistant individuals in a bacterial population is naturally selected, 2) antibiotics are taken as short courses and are therefore at an economic disadvantage when compared to drugs directed at chronic conditions such as cardiovascular disease which is taken daily over a long period of time, and 3) due to the AIDS epidemic shared anti-infective resources has been mostly used for antiviral research, decreasing the funding available for antimicrobial research. Furthermore, an increase in regulatory requirements have led to an increase in developmental costs, patents expire soon after market introduction and the reservation of new drugs for special situations result in decreased revenues (Overbye and Barret, 2005; Norrby et al., 2005). Some of the most useful antibiotics have been obtained from natural products (Butler, 2005; Silverman, 1992) and it seems reasonable to use nature as a source of inspiration to search for chemical entities in the fight against the ever evolving microbes.

Even if compounds isolated from plants cannot claim the same success in the clinical setting as those isolated from soil bacteria for instance, several phytochemicals exhibit antimicrobial activity (Gibbons, 2004). As set out in Chapter 2, the antimicrobial activities of extracts from the genus *Helichrysum* have been determined by different laboratories and

this genus is rich in anti-infective compounds such as phloroglucinols, diterpenes, and flavonoids (Dekker et al., 1983; Drewes et al., 2006; Drewes and Van Vuuren, 2008; Süzgeç et al., 2005). *Helichrysum* species are also widely used in traditional medicine to treat ailments associated with antimicrobial infections. It is often used to dress wounds and to treat patients with respiratory diseases. These facts indicate that the genus is a potential promising source of antimicrobial compounds. Moreover, although there are several reports on the antimicrobial activities of *Helichrysum* extracts, comparative toxicity values are often not available (Drewes et al., 2006; Lourens et al., 2004; Mathekga and Meyer, 1998), indicating the importance of a study to determine relative toxicity.

The aims of this chapter are:

- To report on the antimicrobial activity of extracts of selected *Helichrysum* species
- To report on preliminary observations regarding cytotoxicity against "normal" and cancer cell lines for extracts of the selected *Helichrysum* species
- Based on biological activity, select *Helichrysum* species for phytochemical investigations.

3.2 Results and discussion

Thirty-five indigenous *Helichrysum* species were collected on the basis of availability. Plant material was collected in various provinces of South Africa during 2002 to 2004. The plant species, voucher numbers and localities are indicated in Table 3.1. Some of the species are illustrated in Fig. 3.1.

H. herbaceum

H. cf. *swynnertonii*

H. oreophilum

H. cephaloideum

H. krebsianum

H. odoratissimum

Figure 3.1 *Helichrysum* species (Photos: L.Lourens)

The antimicrobial and cytotoxicity of extracts of the species tested are collated in Table 3.2. Of the 35 species screened, seven (*H. aureum*, *H. excisum*, *H.* cf. *foetidum*, *H. kraussii*, *H. odoratissimum*, *H. platypterum*, and the *H. rugulosum* flower extract) exhibited MIC's ≤ 0.1 mg/ml against one or more micro-organisms. Activity was mainly observed against the two Gram-positive micro-organisms, *S. aureus* and *B. cereus*. A few extracts had activity against *S. epidermidis* (*H. excisum* and the *H. rugulosum* flower extract), *K. pneumoniae* (*H. rugulosum* flower extract) and the yeast *C. neoformans* (*H. herbaceum* and *H. rugulosum* flower extract). None of the extracts were active against *P. aeruginosa*. These results support the trend observed by Mathekga and Meyer (1998) that *Helichrysum* extracts are generally more active against Gram-positive than Gram-negative micro-organisms.

The extracts of *H. herbaceum* and *H. rugulosum* flowers were the only extracts active against the yeast *C. neoformans* and exhibited MIC's of 0.5 and 1.0 mg/ml, respectively. The flower extract of *H. rugulosum* showed much better activity against *S. aureus*, *S. epidermidis*, and *K. pneumoniae* than the extract of the leaves and stems of the same plant. Since this flower extract had much better activity than the stems and leaves, investigating the flower extracts of other species may yield interesting results in a future study. There are reports on the traditional use of six of the seven most active species relating to antimicrobial use. For example, *H. foetidum* is used to treat festering sores while *H. odoratissimum* is used to treat wounds and burns, indicating that the traditional use should be considered in species selection.

Although the MIC values observed for some species seem promising, in several cases toxicity (inhibition of Graham cell growth) was also observed at 0.1 mg/ml. The MIC's for *H. aureum* extract, for example, are 0.02 and 0.01 mg/ml against *S. aureus* and *B. cereus*, respectively, but only 5% Graham cell growth is observed at a concentration of 0.1 mg/ml. Species with potential toxicity include *H. acutatum*, *H. aureum* var *aureum*, *H. platypterum* and *H. rugulosum*, since less than 10% Graham cell growth was observed at the test concentration (100 μg/ml).

With extracts of *H. adenocarpum*, *H. appendiculatum*, *H. cephaloideum* and *H. indicum* more than 80% growth was observed for the Graham cells, a 'normal' cell line, while the growth of the MCF-7 breast cancer cells were less than half of that at the same

concentration of extract, indicating a degree of selectivity. In general, the MCF-7 cells were more sensitive towards the extracts than either the Graham or SF-268 cells.

3.3 Conclusion

The antimicrobial activities of 35 *Helichrysum* species have been shown in relation to their cytoxicity. The results of this study enabled us to select *Helichrysum* species for further study. Criteria used for selection included biological activity, availability of plant material, whether phytochemical studies have been performed on the species previously, and the presence of promising phytochemicals in related species. Based on these criteria, the species selected for further study included *Helichrysum splendidum, H. montanum,* and *H. excisum.*

Although the extract of *H. splendidum* did not exhibit potent antimicrobial or anticancer activity (the extract was relatively non-toxic to normal cells) an interesting class of compounds, namely guaianolides, was previously isolated from this species. These types of compounds are used as anticancer agents and exhibit anti-inflammatory activity, which is interesting considering its traditional use to treat rheumatism. The ambiguities that exist in literature regarding the stereochemistry of these isolated compounds prompted us to reinvestigate this species (Chapter 4).

It was proposed that the morphologically related species, *H. montanum*, would exhibit phytochemistry similar to that of *H. splendidum.* No previous phytochemical investigations have been carried out on this species and it was anticipated that it would be a source of exciting sesquiterpenoids. Furthermore, this species inhibited the growth of all three cell lines in the cytotoxicity assay, indicating the possible presence of cytotoxic compounds (Chapter 5).

Helichrysm excisum was selected for further study based on the fact that it exhibited promising antimicrobial activity, relative low toxicity and except for the essential oil, has not been studied phytochemically (Chapter 6).

3.4 Experimental

3.4.1 Plant collection

Localities and voucher numbers of collected plant material is displayed in Table 3.1 Specimens of each plant were sent to the South African National Biodiversity Institute in Pretoria for identification by Ms M. Welman and Ms. J.A. Ready. Voucher specimens were deposited in the University of KwaZulu-Natal herbarium (NU) in Pietermaritzburg.

3.4.2 Instrumentation and chemicals

Culture media and fetal calf serum (FCS) were obtained from Highveld Biological, while other chemicals were obtained from Sigma and Saarchem. An automated spectrophotometric plate reader (Labsystems iEMS reader MF), connected to Ascent version 2.4 software, was used to read absorbences in the sulforhodamine B (SRB) assay.

3.4.3 General extraction

Plant material was air dried (protected from sunlight), ground and extracted twice with chloroform:methanol (1:1) for 24 hours at room temperature where after the solvent was removed under vacuum.

3.4.4 Antimicrobial bioassays

Determination of minimum inhibitory concentration (MIC) values

Six microorganisms, including three Gram-positive bacteria (*Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 12600, and *Staphylococcus epidermidis* ATCC 2223), two Gram-negative bacteria (*Klebsiella pneumoniae* NCTC 9633 and *Pseudomonas aeruginosa* ATCC 9027, and a yeast (C*ryptococcus neoformans* ATCC 90112) were used in the screening of the crude extracts. Selection of test organisms were undertaken according to the traditional use of species of the genus i.e. treatment of respiratory disorders and wounds. The reference stock cultures were obtained from the National Health Laboratory Service (NHLS) (Johannesburg) and were maintained in the microbiology laboratory of the Department of Pharmacy and Pharmacology, University of the Witwatersrand, Johannesburg, South Africa. Cultures were maintained in tryptone soya broth (TSB) prepared by dissolving 30 g in 750 ml of sterile water. The agar was sterilised by autoclaving for 15 minutes at 121 ºC.

MIC values were determined by the 96-well microplate method (Fig. 3.2) as described by Eloff (1988) and adapted by Magee et al. (2006). Stock solutions were prepared by dissolving samples in DMSO (64 mg/ml) since dissolution problems occurred with solvents such as acetone. Sterile water (100 µl) was added to all wells of the microtitre plate. The stock solutions (100 µl) of 10 different extracts were added to the first row (wells A1-A10) of the 96-well plate, mixed with the water and 100 µl of the resulting solution transferred to the next row of the plate. This process of serial dilution was repeated for all rows until the last row, where the remaining 100 µl was discarded. A fixed bacterial culture (100 µl) of approximate inoculum size of 1×10^6 colony forming units (CFU)/ml in TSB was added to all wells to obtain a concentration range of 16 to 0.125 mg/ml of extract. Further dilutions were prepared when activity below these ranges were observed.

The plates were incubated at 37 ºC for 24 h for bacteria and 48 h for the yeast. MIC values were determined at least in duplicate. Positive controls included ciprofloxacin for bacteria and amphotericin B for the yeast (column 11). A solvent control (column 12) was included with the assay since DMSO has antimicrobial activity and samples with MIC values equal to that found for DMSO were considered not susceptible (NS, Table 5.2). After the incubation period, 50 µl of a 0.4 mg/ml *p*-iodonitrotetrazolium violet (INT) solution was added to all wells and left for 6 h (except *Cryptococcus* which was left for 12 h) at room temperature before reading the MIC's.

The method is based on the principle that the colourless tetrazolium compound (INT) acts as an electron acceptor and is reduced to a coloured product by biologically active organisms (Eloff, 1998). The MIC value was determined as the lowest concentration of sample required to inhibit the growth of test organisms. Wells where bacterial growth occurred stained red in the presence of INT and the concentration in the first unstained well was taken as the MIC.

Figure 3.2 Example of a 96 well plate used to determine MIC's. Wells where red staining occurs are indicative of bacterial growth. In clear (yellow) wells no bacterial growth occurs. The concentration in the first clear well is taken as the MIC. Column 11 is used for the negative control (solvent) and column 12 for the positive control (e.g. ciprofloxacin).

3.4.5 Cytotoxicity screening against normal and cancer cell lines

Cytotoxicity was determined with the sulforhodamine B (SRB) assay (Monks et al., 1991; Wu et al., 1993; Kamatou et al., 2008). SRB is a water-soluble dye that binds to basic amino acids of cellular proteins that are only synthesised by viable cells (Voigt, 2005). The absorbance can therefore be used as an indication of cell growth.

Cell lines

Breast adenocarcinoma (MCF-7) and neuronal (glioblastoma) (SF-268) cell lines were obtained from the Division of Cancer Treatment and Diagnosis, National Cancer Institute (NCI), Fairview Centre, Frederick Maryland in the United States of America. Transformed human kidney epithelial cells (Graham cells) were obtained from Dr. Robyn van Zyl from the Department of Pharmacy and Pharmacology, University of the Witwatersrand.

Preparation of media and solutions

The Graham cells were cultured in Ham F10 media containing 5% (v/v) heat inactivated fetal calf serum (FCS) and 0.1% gentamicin. The SF-268 and MCF-7 cells were maintained in RPMI-1640 media containing 5% FCS and 0.01% L-glutamine, while media used in the assay included 0.1% gentamicin. A 0.4% (w/v) SRB solution was prepared using 1% acetic acid and a 50% trichloroacetic acid (TCA) solution prepared with distilled Millipore water. The phosphate buffer saline (PBS) (pH 7.4) was prepared with NaCl (8 g),

KCl (0.3 g), $Na_2HPO_4.2H_2O$ (0.73 g) and KH_2PO_4 (0.2 g) in one litre of distilled Millipore water which was then autoclaved. Trypan blue was prepared at a concentration of 2 mg/ml in PBS and the solution of tris(hydroxymethyl)aminomethane (Tris base) was prepared at a concentration of 10 mM (pH 10.5) with distilled Millipore water. All solutions were stored at 4 ºC until required.

Preparation of cells

The cells were maintained at 37 ºC in a 5% CO_2 humidified incubator. The media was replaced three times a week and the cells were trypsinised weekly, and then allowed to reach confluency before being used in the assay. After trypsinisation, a single cell suspension was obtained, stained with trypan blue and cell density determined on a haemacytometer. The stock cell suspension was then diluted to the required cell concentration with culture media. The experiment was carried out only when at least 95% of cells were viable. The Graham cells were seeded at 25 000 cells/well, while the MCF-7 and SF-268 cells were seeded at 15 000 cells/well.

Preparation of samples for the SRB assay

Crude extracts were weighed and dissolved in dimethyl sulfoxide (DMSO) to prepare stock solutions of ca. 10 mg/ml. These solutions were diluted further with RPMI-1640 media containing 0.1% gentamicin sulfate (a 50 fold dilution was prepared, e.g. 20 µl of stock solution and 980 µl of media).

SRB assay

A volume of 100 µl of the prepared cell suspension was plated out in all wells of the 96 well plate except rows A and H. Wells A_1, A_2, H_1 and H_2 served as blanks, while wells A_3-A_{12} and H_3-H_{12} were used as colour controls for samples. Media without cells were therefore transferred to these wells. A duplicate plate was prepared to serve as a time zero plate (reference plate to indicate cell growth at time of sample addition). The plates were then incubated for 24 hours at 37 °C in 5% CO_2 at 100% relative humidity to allow for attachment of cells. After incubation, 100 µl of the prepared samples was added to the appropriate wells (Fig 3.3) to obtain concentrations of 100 µg of sample per well. Samples were plated out in triplicate or quadruplicate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Blank	C1	C2	C3	C	C	C	C	C	C	C
B	Cell control		S1	S1								
C	Cell control		S1	S1								
D	Cell control		S2	S2								
E	Cell control		S2	S2								
F	Cell control		S3	S3								
G	Cell control		S3	S3								
H	Blank	Blank	C1	C2	C3	C	C	C	C	C	C	C

Figure 3.3 Representation of a microtitre plate used in the SRB assay. Blank = only culture media without cells; Cell control = cells with only media and no sample; C = sample control, sample and culture media, no cells; S = cells and sample.

Plates were then incubated for a further 48 hours. The time zero plate was however, not incubated, but the cells were fixed after the first 24 hour incubation period to establish that adequate cell growth occurred before addition of samples. At the end of the 48 hour incubation period, cells were fixed with TCA. Ice-cold TCA (50 µl) was added to all wells and the plates incubated for a further hour at 4 °C, where after the supernatant was discarded and the plates washed copiously (five times) to remove all TCA, growth medium and extract residues. Plates were then air dried at room temperature. To stain the fixed cells, the SRB solution (100 µl) was added to all wells and left for approximately 10 minutes, where after the SRB solution was discarded and the plates washed thoroughly with 1% acetic acid to remove all unbound dye. The plates were again air dried. The bound dye was solubilised through the addition of 200 µl Tris base. The absorbance was read at 492 nm against a Tris base blank after the plates were shaken at 960 rpm for 3 minutes.

Determination of percentage cell growth

The percentage cell growth in reference to control growth was calculated as follows:

$$\% \text{ cell growth} = \frac{(\text{mean abs test sample} - \text{mean abs background}) * 100}{(\text{mean abs control} - \text{mean abs blank})}$$

where "mean abs test sample" refers to the mean absorbance at 492 nm obtained for the three similar sample wells (100 µl cell suspension + 100 µl sample)

"mean abs background" refers to the mean absorbance at 492 nm of the wells containing only media (100 µl) and sample (100 µl)

"mean abs control" refers to absorbance of wells containing only cell suspension and no sample.

"mean abs blank" refers to wells containing only media and neither cells nor sample.

3.5 References

Alanis, A.J., 2005. Resistance to antibiotics: Are we in the post-antibiotic era? Archives of Medical Research 36, 697-705.

Butler, M.S., 2005. Natural products to drugs: natural product derived compounds in clinical trials. Natural Product Reports 22, 162-195.

Chander, Y., Gupta, S.C., Goyal, S.M., Kumar, K., 2007. Perspective. Antibiotics: has the magic gone? Journal of the Science of Food and Agriculture 87, 739-742.

Dekker, T.G., Fourie, T.G., Snyckers, F.O., Van der Schyf, C.J., 1983. Studies of South African medicinal plants. Part 2. Caespitin, a new phloroglucinol derivative with antimicrobial properties from *Helichrysum caespititium*. South African Journal of Chemistry 36, 114-116.

Drewes, S.E., Mudau, K.E., Van Vuuren, S.F., Viljoen, A.M., 2006. Antimicrobial monomeric and dimeric diterpenes from the leaves of *Helichrysum tenax* var *tenax*. Phytochemistry 67, 716-722.

Drewes, S.E., Van Vuuren, S.F., 2008. Antimicrobial acylphloroglucinols and dibenzyloxyflavonoids from flowers of *Helichrysum gymnocomum*. Phytochemistry 69, 1745-1794.

Eloff, J.N., 1998. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Medica 64, 711-713.

Gibbons, S., 2004. Anti-staphylococcal plant natural products. Natural Product Reports 21, 263-277.

Jansen, W.T.M., Van der Bruggen, J.T., Verhoef, J., Fluit, A.C., 2006. Bacterial resistance: A sensitive issue. Complexity of the challenge and containment strategy in Europe. Drug Resistance Updates 9, 123-133.

Kamatou, G.P.P., Van Zyl, R.L., Davids, H., Van Heerden, F.R, Lourens, A.C.U., Viljoen, A.M., 2008. Antimalarial and anticancer activities of selected South African *Salvia* species and isolated compounds from *S. radula.* South African Journal of Botany 74, 238-243.

Lourens, A.C.U., Reddy, D., Başer, K.H.C., Viljoen, A.M., Van Vuuren, S.F., 2004. *In vitro* biological activity and essential oil composition of four indigenous South African *Helichrysum* species. Journal of Ethnopharmacology 95, 253-258.

Magee, A.R., Van Wyk, B-E., Van Vuuren, S.F., 2006. Ethnobotany and antimicrobial activity of sieketroos (*Arctopus* species). South African Journal of Botany 73, 159-162.

Mathekga, A.D.M., Meyer, J.J.M., 1998. Antibacterial activity of South African *Helichrysum* species. South African Journal of Botany 64, 293-295.

Monks, A., Scudiero, D., Skehan, P., Shoemaker, R., Paull, K.D., Vistica, D., Hose, C., Langley, J., Cronise, P., Vaigro-Wolff, A., Gary-Goodrich, M., Campbell, H., Mayo, J., Boyd, R., 1991. Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumour cell lines. Journal of the National Cancer Institute 183, 757−766.

Norrby, S.R., Nord, C.E., Finch, R., 2005. Lack of development of new antimicrobial drugs: a potential serious threat to public health. The Lancet: Infectious Diseases 5, 115-119.

Overbye, K.M., Barret, J.F., 2005. Antibiotics: where did we go wrong? Drug Discovery Today 10, 45-52.

Silverman, R.B., 1992. The Organic Chemistry of Drug Design and Drug Action. Academic Press: London, pp. 5, 8.

Süzgeç, S., Meriçli, A.H., Houghton, P.J., Çubukçu, B., 2005. Flavonoids of *Helichrysum compactum* and their antioxidant and antibacterial activity. Fitoterapia 76, 269-272.

Voigt, W., 2005. Sulforhodamine B assay and chemosensitivity. Methods in Molecular Medicine 110**,** 39−48.

Wu, F.Y.H., Liao, W.C., Chang, H.M., 1993. Comparison of antitumour activity of vitamin K1, K2, and K3 on human tumour cells by two (MTT and SRB) cell viability assays. Life Sciences 52, 1797−1804.

Table 3.1 *Helichrysum* species collected.

	Plant species	Voucher number	Locality/ date collected
1.	*H. acutatum* DC.	A. Lourens and A. Viljoen 1	Mpumulanga, 4 km from Graskop, on the road to Hazyview, Kowyns pass. S 24 57 54.0; E 30 51 08.1; 4756 ft.; 27/09/2003
2.	*H. adenocarpum* DC. subsp. *adenocarpum*	S.N.	Unknown locality
3.	*H. appendiculatum* (L.f.) Less.	A. Lourens and A. Viljoen 28	Mpumalanga, approximately 2 km on God's Window turn-off (R534). Turn-off from road from Blyderivierspoort to Graskop; 25/01/2004
4.	*H. aureonitens* Sch. Bip.	J. E. Victor 2439	Amatolas
5.	*H. aureum* (Houtt.) Merril var. *aureum*	J. E. Victor 2428	Amatolas
6.	*H. cephaloideum* DC.	A. Lourens and A. Viljoen 20	KwaZulu-Natal, approximately 5 km from Underberg on road to Bulwer. S 29 18 57.2; E 29 32 41.5; 4759 ft.; 10/01/2004
7.	*H. callicomum* Harv.	DBH 6565	Transkei
8.	*H. dasyanthum* (Willd.) Sweet	A. Lourens 33 (ex. J. Manning)	Western Cape, Cape Agulhas, Red Hill; 01/2004
9.	*H. excisum* (Thunb.) Less.	J. Vlok 2830 and J. Vlok 2830A	Western Cape, northern base of Robinson pass, near Paardebont; 20/11/2002; 23/08/2004
10.	*H. felinum* Less.	J. Vlok 2828	Western Cape, northern base of Robinson pass; 11/2002
11.	*H.* cf. *foetidum* (L.) Moench.	J. Vlok 2833	Eastern Cape, George
12.	*H. herbaceum* (Andr.) Sweet	A. Lourens and A. Viljoen 17	KwaZulu-Natal, Alpine Heath, on walk to waterfall; 04/01/2004
13.	*H. indicum* (L.) Grierson	A. Lourens 34 (ex. J. Manning)	Cape Agulhas
14.	*H. kraussii* Sch. Bip.	A. Lourens and A. Viljoen 4	Mpumalanga, Robber's pass. Shortly after turn-off to Pelgrim's Rest. (Sign 6/6). Everywhere along pass. S 24 53 57.9; E 30 37 20.4; 4402 ft.; 03/10/2003
15.	*H. melanacme* DC.	A. Lourens and A. Viljoen 12	Free State, 2 km from Clarens on road to Koster. S 28 31 12; E 28 26 45; 6062 ft.; 03/01/2004
16.	*H miconiifolium* DC.	A. Lourens and A. Viljoen 24	Kwa-Zulu Natal, On gravel road from Loteni to Himeville. S 29 36 49.5; E 29 33 48.6; 4439 ft.; 10/01/2004
17.	*H. montanum* DC.	F. van Heerden 1	Western Cape, Koueveld mountains, on farm Weltevrede Murraysburg; 25/12/2003
18.	*H. nudifolium* (L.) Less.	A. Lourens and A. Viljoen 13	Free State, 2 km from Clarens on road to Koster. S 28 31 12; E 28 26 45; 6062 ft.; 03/01/2004
19.	*H. odoratissimum* (L.) Sweet	A. Lourens and A. Viljoen 6	Mpumalanga, Robbers pass, approx. 2 km after Crystal Springs turn-off on road from Lydenburg to Pelgrims Rest (approx. 9 km from Pelgrim's Rest); 04/10/2004
20.	*H. oreophilum* Klatt.	A. Lourens and A. Viljoen 10	Mpumalanga, farm Aantree, Draaikraal; 14/12/2003
21.	*H. pallidum* DC.	A. Lourens and A. Viljoen 7	Mpumalanga, On road from Graskop to Sabie, approx. 1 km after turn-off to Sabie, just after "The Bonnet". S 24 56 18.2; E 30 48 41.6; 4833 ft.; 04/10/2004

	Plant species	Voucher number	Locality/ date collected
22.	*H. pandurifolium* Schrank	A. Lourens 35 (ex. J. Manning)	Cape Agulhas; 01/2004
23.	*H. patulum* (L.) D. Don.	A. Lourens 36 (ex. J. Manning)	Cape Agulhas; 01/2004
24.	*H. petiolare* Hilliard and Burtt	J. Vlok 2827	Western Cape, Oudtshoorn, base of Robinson pass, near farm Moerasrivier; 20/12/2002
25.	*H. platypterum* DC.	A. Lourens and A. Viljoen 30	Mpumalanga, Just outside Dullstroom on road to Belfast. (Sign 56/0). Farm dam on left hand side of road. Approx. 500 m from Uitvlugt turn-off; 25/01/2004
26.	*H. psilolepis* Harv.	A. Lourens and A. Viljoen 11	Free State, 2 km from Clarens on road to Koster. S 28 31 12; E 28 26 45; 6062 ft.; 03/01/2004
27.	*H. retortum* (L.) Willd.	A. Lourens 37 (ex. J. Manning)	Cape Agulhas; 01/2004
28.	*H. rosum* (Berg.) Less. cf. var. *rosum*	J. E. Victor 2436	Amatolas
29.	*Helichrysum ruderale* Hilliard and Burtt	A. Lourens and A. Viljoen 32	KwaZulu-Natal, Hilton, Weir street; 14/11/2004
30.	*H. rugulosum* Less.	A. Lourens and A. Viljoen 8	North West, on Derby road, from Klerkskraal dam, 19 km from turn-off. Along road side. S 26 05 33; E 27 07 04; 4933 ft.; 12/12/2003.
31.	*H scitilum* Hiliard and Burtt	F. van Heerden 2	Western Cape, Koueveld mountains, on farm Weltevrede Murraysburg; 25/12/2003.
32.	*H. simillimum* DC.	A. Lourens and A. Viljoen 23	KwaZulu-Natal, on road from Loteni to Himeville. Near Loteni stock theft unit. S 29 33 44.3; E 29 35 45.6; 4652 ft.; 10/01/2004
33.	*H. splendidum* (Thunb.) Less.	A. Lourens and A. Viljoen 2	Mpumalanga, 2.6 km from God's Window at stop point near "Staircase", 27/09/2003
34.	*H. wilmsii* Moeser	A. Lourens and A. Viljoen 5	Mpumalanga, Robbers pass, approx. 2 km after crystal Springs turn-off on road from Lydenburg to Pelgrims Rest. (Approx. 9 km from Pelgrims Rest). S 24 52 34.2; E 30 42 13.8; 04/10/2004
35.	*H. zeyheri* Less.	F. van Heerden 3	Western Cape, Koueveld mountains, on farm Weltevrede Murraysburg

Table 3.2 Antimicrobial activity (MIC, mg/ml) and cytotoxicty (at 0.1 mg/ml ± standard deviation) of *Helichrysum* extracts

Plant Species	**Antimicrobial activity[a] Test organisms**						**Cytotoxicity[a,b]**		
	C. neoformans	*P. aeruginosa*	*S. aureus*	*B. cereus*	*S. epidermidis*	*K. pneumoniae*	% Growth of Graham cells	% Growth of SF-268 cells[b]	% Growth of MCF-7 cells[b]
H. acutatum DC.	NS[c]	NS	0.5	1	NS	NS	0.1±0.1	15.8±1.0	6.6±0.1
H. adenocarpum DC.	NS	NS	NS	NS	NS	NS	98.3±0.6	88.1±3.6	37.2±3.2
H. appendiculatum (L.f.) Less	NS	NS	NS	4	NS	NS	91.7±2.1	81.0±2.8	37.5±2.4
H. aureonitens Sch. Bip.	NS	NS	4	4	4	NS	75.6±0.9	76.0±4.7	37.5±3.7
H. aureum (Houtt.) Merril	NS	NS	0.02	0.01	NS	NS	5.0±0.2	35.9±1.9	7.0[d]
H. callicomum Harv.	NS	NS	1	0.25	4	NS	27.3±2.9	56.1±2.8	11.2[d]
H. cephaloideum DC.	NS	NS	NS	1	4	NS	93.7±4.6	83.8±2.6	46.1[d]
H. dasyanthum (Willd.) Sweet	NS	NS	NS	4	NS	NS	91.3±0.7	81.7±3.6	52.8[d]
H. excisum (Thunb.) Less	NS	NS	0.2	0.03	0.1	NS	63.6±1.1	71.9±3.1	31.4±0.8
H. felinum Less	NS	NS	2	0.16	2	NS	48.0±1.4	54.1±2.3	23.2±0.2
H. cf. *foetidum* (L.) Moench	NS	NS	0.5	0.01	NS	NS	32.7±2.1	57.8±2.1	24.9±0.4
H. herbaceum (Andr.) Sweet	0.5	NS	0.5	1	4	NS	50.8±3.0	46.4±1.6	22.9±0.3
H. indicum (L.) Grierson	NS	NS	1	0.5	4	NS	81.1±2.3	70.6±4.2	35.8±0.8
H. kraussii Sch. Bip.	NS	NS	0.5	0.004	4	2	28.6±1.4	45.2±4.4	9.0[d]
H. melanacme DC.	NS	NS	0.5	0.25	4	2	18.1±0.4	53.3±2.7	12.2[d]
H. miconiifolium DC.	NS	NS	2	1	4	NS	37.9±6.6	52.4±1.8	20.9[d]
H. montanum DC.	NS	NS	1	4	NS	NS	29.4±0.7	46.1±4.0	19.2[d]
H. nudifolium (L.) Less	NS	NS	NS	4	NS	NS	73.1±2.5	83.9±1.8	35.3±0.4

Plant Species	Antimicrobial activity[a] Test organisms						Cytotoxicity[a,b]		
	C. neoformans	*P. aeruginosa*	*S. aureus*	*B. cereus*	*S. epidermidis*	*K. pneumoniae*	% Growth of Graham cells	% Growth of SF-268 cells	% Growth of MCF-7 cells
H. odoratissimum (L.) Sweet	NS	NS	0.02	0.03	4	2	17.5±0.4	48.2±1.4	7.4±0.7
H. oreophilum Klatt	NS	NS	4	4	NS	NS	63.4±2.2	82.9±3.0	34.8±0.1
H. pallidum DC.	NS	NS	NS	NS	NS	NS	88.6±0.9	83.9±9.7	49.0±1.7
H. pandurifolium Schrank	NS	NS	2	4	NS	NS	57.1±1.2	71.8±0.8	34.2±0.1
H. patulum (L.) D. Don	NS	NS	4	4	NS	NS	63.8±1.3	75.2±2.0	37.8[d]
H. petiolare Hilliard and Burtt	NS	NS	4	2	NS	NS	59.3±3.4	76.6±3.0	33.4[d]
H. platypterum DC.	NS	NS	0.05	0.5	NS	NS	0.8±0.3	35.1±1.5	4.6
H. psilolepis Harv.	NS	NS	1	1	NS	NS	25.9±1.9	58.4±7.4	23.1[d]
H. retortum (L.) Willd.	ND[e]	NS	4	NS	NS	NS	79.6±3.4	87.3±4.8	ND[e]
H. rosum (Berg.) Less.	NS	NS	1	1	4	NS	47.5±1.8	54.8±5.0	22.5±0.7
H. ruderale Hilliard & Burtt.	ND	NS	2	1	NS	NS	45.1±1.2	75.8±3.9	NDc
H. rugulosum Less.	NS	NS	0.5	0.25	4	NS	3.0±1.2	44.7±3.6	12.7±2.7
H. rugulosum Less. Flowers	1.0	NS	0.01	0.33	0.01	0.22	ND	ND	ND
H. scitilum Hilliard & Burtt.	NS	NS	NS	NS	4	NS	58.6±1.2	85.3±6.8	46.8[d]
H. simillimum DC.	NS	NS	1	4	4	NS	54.6±2.6	66.8±3.9	26.8[d]
H. splendidum (Thunb.) Less	NS	NS	4	NS	NS	NS	82.3±3.2	72.8±3.3	49.2±2.5
H. wilmsii Moeser	NS	NS	1	1	4	NS	22.5±2.1	64.1±6.1	15.3±0.5
H. zeyheri Less.	NS	NS	2	4	NS	NS	54.4±9.5	58.2±3.1	37.4[d]

Plant Species	Antimicrobial activity[a] Test organisms						Cytotoxicity[a,b]		
	C. neoformans	*P. aeruginosa*	*S. aureus*	*B. cereus*	*S. epidermidis*	*K. pneumoniae*	% Growth of Graham cells	% Growth of SF-268 cells	% Growth of MCF-7 cells
Antimicrobial control[f]		0.3×10^{-3}	0.3×10^{-3}	1.0×10^{-3}	2.5×10^{-3}	0.7×10^{-3}			
Antimicrobial control[f]	2.5×10^{-3}								
DMSO control[g]	2	4	8	8	16	4			

[a]Experiments done at least in duplicate. Exceptions include determination of cytotoxicty against MCF-7 cells for extracts of *H. aureum, H. calliconum, H. cephaloideum, H. dasyanthum, H. kraussii, H. melanacme, H. miconiifolium, H. montanum, H. patulum, H. petiolare, H. platypterum, H. psilolepis, H. scitilum, H. simillimum.*
[b]At a concentration of 0.1 mg/ml of 5-flourouracil, more than 80% growth was observed for the SF-268 cells, while an IC_{50} of 1.1 µg/ml was determined for the same drug against the MCF-7 cell line (Kamatou et al., 2008).
[c]Not susceptible, MIC observed equal to that of solvent control
[d]Experiments done only once, using the SRB assay
[e]Not determined
[f]Ciprofloxacin used as a positive control against bacteria and Amphotericin B used as a positive control against the yeast
[g]Solvent control

CHAPTER 4

The phytochemistry of *Helichrysum splendidum*

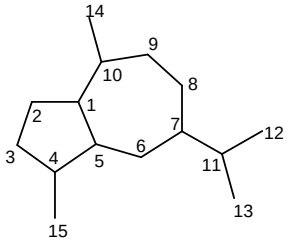
4.1 Introduction

Helichrysum splendidum (Thunb.) Less. (Fig. 4.1) is a slender, erect aromatic shrub which is distributed widely on the high mountains of East Africa and Malawi to Zimbabwe. In South Africa the plant occurs in the eastern parts of the country, with distribution ranging from the highlands of Mpumalanga to the Swartberg near Oudtshoorn and George (Hilliard, 1983). This plant is used traditionally to treat rheumatism (Pooley, 2003; Jacot-Guillarmod, 1971) and is also used as fuel and as women's perfume (Dlamini, 1981).

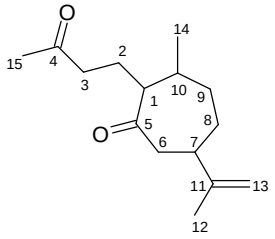
Figure 4.1 Habitat and flowers of *H. splendidum*

The phytochemistry of *H. splendidum* has been studied previously by Bohlmann and Suwita (1979) and Jakupovic and co-workers (1989). In addition to the normal terpenes and flavones, guaianolides and secoguaianolides were isolated from the plant (Bohlmann and Suwita, 1979, Jakupovic et al., 1989). Guaianolides rarely occur in *Helichrysum* species and apart from *H. splendidum*, the only other *Helichrysum* species from which guaianolides were isolated was *H. dasyanthum* (Jakupovic et al., 1989). Guaianolides are a

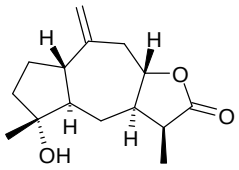
large class of sesquiterpenoids which can be divided into simple guaianes, 12,8-guaianolides, 12,6-guaianolides (introduction of a lactone moiety changes the name from guaiane to guaianolide), guaiane-dimers and seco-, cyclo- and abeo-guaianes (Dictionary of Natural Products, Fig. 4.2).



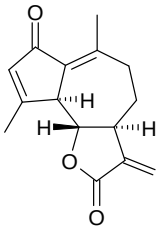
Guaiane (Dictionary of Natural Products)



Secoguaiane from *Nephthea chabrolii* (Su et al., 2007)



12,8-Guaianolide from *H. splendidum* (Jakupovic et al., 1989)



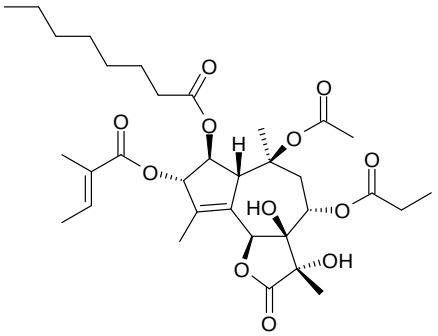
12,6-Guaianolide from *Artemisia myriantha* (Wong and Brown., 2002)

Figure 4.2 Examples of different guaianes

Various biological activities, such as anti-inflammatory (Hilmi et al., 2003; Siedle et al., 2003), cytotoxic (Hilmi et al., 2002), and antiviral effects (Kim et al., 2002; Hwang et al., 2006) have been attributed to guaianolides. In the case of guaianolides from *Warionia saharae* and *Artemisia argyi*, all of the cytotoxic guaianolides have an *exo*-methylene moiety adjacent to the lactone carbonyl (Hilmi et al., 2002, Kim et al., 2002), which probably results in the alkylation of biological nucleophiles (Kim et al., 2002). The same interaction is believed to be responsible for the inhibition of NF-κB (Jin et al., 2004) associated with anti-inflammatory effects of these compounds.

The medical relevance of the guaianolides is illustrated by the almost 10 000 publications on the subject of thapsiargin (**376**), a highly oxygenated guaianolide which acts as a selective, irreversible inhibitor of sarco-endoplasmic reticulum Ca^{2+} ATP dependant

pumps, thus initiating apoptosis and rendering it an efficient anticancer agent (Ball et al., 2007). Arglabin (**377**), a guaianolide isolated from *Artemisia myriantha*, is also used clinically as an anticancer agent (Wong and Brown, 2002; Newman et al., 2003).



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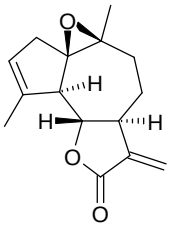


377

This chapter describes the confirmation of the structures of guaianolides isolated from *H. splendidum*, a species that was previously studied (Bohlmann and Suwita, 1979; Jakupovic et al., 1989). An important part of this project was the investigation of the sesquiterpenoids of *H. montanum* (Chapter 4). However, problems experienced with the determination of the relative configuration of the sesquiterpenes in this species and the ambiguity that exists in literature on the stereochemistry and NMR assignments of the isolated guaianolides from *H. splendidum* prompted us to reinvestigate this species. An initial TLC (thin-layer chromatographic) investigation of the extracts of *H. splendidum* and *H. montanum* revealed the presence of compounds that stained red with anisaldehyde spray reagent (Fig. 4.3). These sesquiterpenoids were targeted during this investigation.

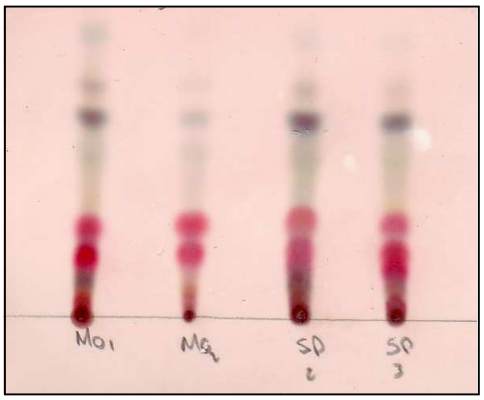


Figure 4.3 TLC of extracts of *H. montanum* (MO_1 locality unknown, MO_2 collected in the Koueveld Mountains) and *H. splendidum* (SP_2 and SP_3 both collected in Mpumalanga).

To study the conformations of these compounds, molecular modelling was employed. The most stable conformation of different stereoisomers, determined with the AM1 method, was used in conjunction with observed NOESY correlations to determine the relative configuration of the isolated sesquiterpenoids.

4.2 Results and discussion

The chloroform:methanol (1:1) extract of *H. splendidum* yielded the monomeric guaianolides, **302** and **304,** as well as the dimeric guaianolide helisplendidilactone (**306)**.

High-resolution mass spectrometry of compound **302** confirmed the molecular formula of $C_{15}H_{22}O_3$. The presence of 15 carbons in the ^{13}C NMR spectrum (Plate 2) indicated a possible sesquiterpene structure. To the best of our knowledge, this is the first report on the ^{13}C data of compound **302**. Inspection of the DEPT NMR spectrum (Plate 4) revealed the presence of two CH_3, four CH_2, six CH and three quaternary carbons. There were no aromatic protons present in the ^1H NMR spectrum (Plate 1), but a doublet of doublets was observed in the region characteristic for alkenes (δ_H 5.44). A deshielded proton was also observed at (δ_H 4.61) indicating the proximity of an oxygen atom to this proton. The remaining protons were present in the aliphatic region, confirming the proposed sesquiterpene structure.

After careful consideration of the COSY (Plate 3), HSQC (Plate 5) and HMQC (Plate 6, Fig. 4.4) NMR spectra, the structure of compound **302** was assigned as 11α,13-dihydroxanthalongin, previously isolated from *H. splendidum* (Jakupovic et al., 1989) and *Arnica mollis* (Marcinek-Hüpen-Bestendonk et al., 1990).

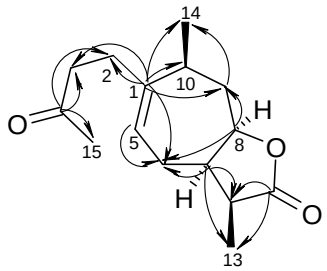


Figure 4.4 Selected HMQC correlations observed for compound **302**

The 7,8-*cis*-configuration of the lactone ring was unequivocally established by the observed NOESY correlations (Plate 7) in conjunction with the AM1 calculated structure (Fig. 4.5). A NOESY experiment captures the through-space nuclear Overhauser effect (NOE) between a proton and its neighbouring proton that are within a distance of approximately 5 Å (Claridge, 1999). Important NOESY correlations for the assignment of the relative configuration of 11α,13-dihydroxanthalongin (**302**) are those observed between H-8 (δ_H 4.61) and H-7 (δ_H 2.65) as well as between H-11 (δ_H 2.79) and H-7 (δ_H 2.65). Furthermore, the NOESY correlation observed between H-10 (δ_H 2.34) and H-8 (δ_H 4.61) confirmed the α-orientation of H-10.

The assignment of the stereochemistry of 11α,13-dihydroxanthalongin (**302**) is the same as that proposed by Jakupovic et al. (1989) and Marcinek-Hüpen-Bestendonk et al. (1990). Based on NMR data, the relative configuration of the compounds is unambiguous. However, we do not have proof for the absolute configuration. The absolute configurations of guaianolides rest on the assumption that H-7 always has α-orientation if the structure is drawn with the lactone on the right-hand side and the 14-methyl at the top. For a few guaianolides (Ulubelen et al., 1995; Hamada et al., 1980) the absolute configuration was confirmed, but in most of the published literature, this is just assumed without any proof. One method of determining the absolute configuration would have been to reduce the 4-ketone to a hydroxy group and prepare the corresponding Mosher ester. However, we did not have enough material to prepare the Moshers ester and, furthermore, C-4 is so far away from the other stereogenic centres that it would be difficult to correlate the stereochemistry on this carbon with the other stereogenic centres. For this investigation on the structures of guaianolides present in *Helichrysum* species, we have assumed the relative configuration at C-7 is *R*.

In the AM1 calculated structure, the distance between H-7 and H-8 is 2.31 Å, the distance between H-11 and H-7 is 2.35 Å and the distance between H-8 and H-10 is 2.28 Å, confirming that at least the calculated distance between these atoms complies with the requirements for observing a NOESY correlation (Fig. 4.5). The structure of compound **302** was unambiguously established as 11α,13-dihydroxanthalongin.

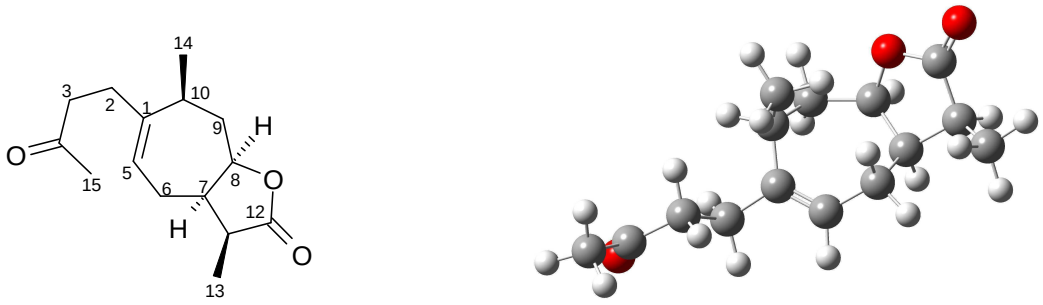


Figure 4.5 Structure of 11α,13-dihydroxanthalongin (**302)** and the AM1 calculated three-dimensional structure of this compound.

Initial inspection of the ^{1}H and ^{13}C NMR spectra of compound **304** revealed that this compound was a sesquiterpene lactone closely related to **302**. High-resolution mass spectrometry confirmed that the molecular formula $C_{15}H_{22}O_3$ (*m/z* 251.1659 $[M+H]^+$) of compound **304** was similar to that of compound **302** (*m/z* 251.1654 $[M+H]^+$). However, clear differences were observed in the chemical shifts of certain protons and carbons. C-6, 7, 10 and 13 (Plate 9) all shifted with 3 ppm or more downfield, while C-5 shifted upfield (approximately 3 ppm). In the ^{1}H NMR spectrum (Plate 8) both H-7 and H-11 has shifted upfield, while H-13 has shifted downfield. Data obtained from analysis of two-dimensional experiments (COSY, HSQC and HMQC, Plates 10, 12, 13) confirmed that the basic structure of compound **304** was similar to that of compound **302.**

Confirming the relative configuration of compound **304** was less straightforward than for compound **302.** This was due to the overlapping signals observed for H-10 and H-7 (δ_H 2.37), as well as for H-11 and H-6 (δ_H 2.24). A NOESY correlation (Plate 14, Fig. 4.6a) is observed between the protons at δ_H 2.36 (assigned to H-7, H-10) and H-8 (δ_H 4.46). This could therefore be a correlation between either H-8 and H-7 or H-8 and H-10, but is most probably a correlation between H-7 and H-8. Due to the overlapping signals of H-7 and H-10, a correlation between H-8 and H-10 can however not be excluded. This NOESY correlation of H-8 could therefore not be used to determine whether the lactone ring had a 7,8-*cis* or 7,8-*trans* configuration.

If one considers the AM1 calculated model (Fig. 4.6b) of compound **304** in conjunction with the NOESY spectrum, the NOESY correlation observed between the signal at δ_H 2.36

(assigned as H-7, H-10) and δ_H 1.22 (H-13) is most likely a correlation between H-13 and H-7 as H-10 is spatially too far away from H-13 (6.56 Å in the AM1 calculated structure), confirming that H-7 and the H-13 are on the same side of the ring. As no NOESY correlation is observed between H-8 (δ_H 4.46) and the multiplet that is assigned to H-11 (δ_H 2.24), it can be concluded that H-8 must also be on the same side of the ring as H-7 and H-13, resulting in the 7,8-*cis* assignment of the lactone ring (Fig 4.6).

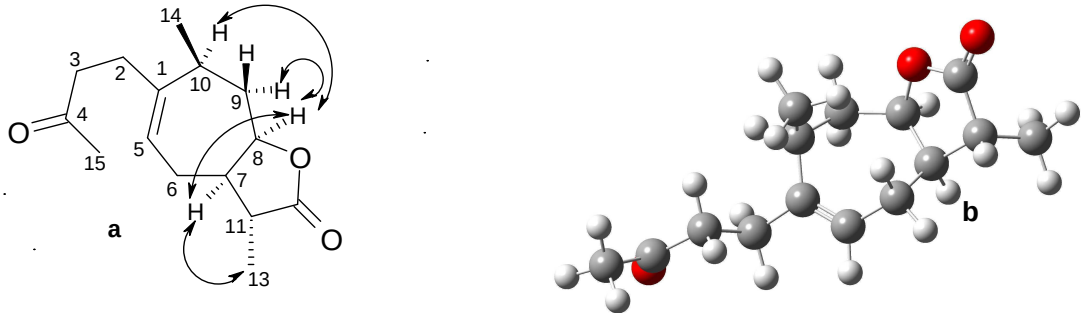


Figure 4.6 a) Important NOESY correlations observed for compound **304** and **b**) AM1 calculated structure for compound **304**.

Cycloheptene, as a pseudo six-membered ring, is believed to exist in stable chair or boat conformers (Leong et al., 1998) (Fig. 4.7). From the AM1 calculated structures of both compounds **302** and **304**, it appears as if the cycloheptene rings of these compounds adapt chair conformations.

Figure 4.7 a) Chair conformation of cycloheptene ring b) Boat conformation of cycloheptene ring

Compound **304** was previously isolated from *Arnica mollis* (Marcinek-Hüpen-Bestendonk et al., 1990) and *H. splendidum* (Bohlmann and Suwita, 1979). The relative configuration was incorrectly assigned as 7,8-*trans* in the latter paper (the configuration was assigned based on comparison of chemical shifts and coupling constants of similar lactones) but was

corrected by Jakupovic and co-workers (1989), although no NMR data was supplied for this compound in this particular publication. A compound with NMR data identical to compound **304**, with the configuration assigned as 7,8-*trans*, was also isolated from *Tithonia diversifolia* (Kuo and Lin, 1999). However, the authors refer to Bohlmann and Suwita (1979), indicating a possible incorrect assignment of configuration at C-8, emphasising the importance of independent stereochemical assignments, even though these are known compounds.

The structure of the dimeric helisplendidilactone (**306**), previously isolated by Jakupovic et al. (1989), was based on extensive one- and two-dimensional NMR experiments (Plates 15-21), high-resolution mass spectrometry, and X-ray structural analysis. The ^{1}H NMR assignments (except for H-14 and H-14') made by Jakupovic et al. (1989) were confirmed. Two interesting ^{1}H,^{1}H couplings were observed in the ^{1}H NMR spectrum. The first was a homoallylic coupling between Hα-6' and H-10'. Homoallylic coupling occurs over five bonds (^{5}J) and is normally weaker than allylic coupling. In compound **306**, both C-6' and C-10' C-H bonds are co-planar with the π-bond, which allow for maximum overlap of the σ-bonds with the π-bond and therefore a detectable homoallylic coupling (Pavia et al., 1996). A long-range W (^{4}J) coupling is also observed between Ha-3' and Hα-13. The rigid, strained ring system has a favourable geometry for the overlap involved (Pavia et al., 1996).

Our ^{13}C NMR assignments for helisplendidilactone (**306**) differ at certain positions from those previously reported (Jakupovic et al., 1989). From the HSQC (Plate 19), it was clear that the assignments of C-9 and C-9' had to be interchanged because the carbon signal at δ_C 40.3 (assigned by Jakupuvic et al., 1989 as C-9) showed HSQC correlations with the protons at δ_H 1.54 and δ_H 2.28 assigned as the H-9' protons. The assignment of the protons is correct as a COSY correlation is observed between these protons and the signal at δ_H 4.14 (H-8'). The HSQC correlation between the proton singlet at δ_H 1.22 and the carbon at δ_C 13.4 indicates that the carbon signal at δ_C 13.4 can be assigned to C-15', while the carbon signal at δ_C 10.6 correlating with the proton doublet at δ_H 1.23 can be assigned to C-13'. Thus, the assignments of C-13' and C-15' has to be exchanged as well.

According to Jakupovic et al. (1989), the C-1 carbon has a chemical shift of δ_C 150.1 while

C-1' appears at δ_C 144.5. However, the HMQC correlation (Plate 20, Fig. 4.7) observed between the carbon at δ_C 144.5 and the broad proton triplet at δ_H 2.10 (H-2, which shows a COSY correlation with H-3, δ_H 2.53) indicates that the signal at δ_C 144.5 should be assigned to C-1 and the signal at δ_C 150.1 to C-1'. This change in assignment is further supported by the assignment of the signal at δ_C 144.7 as C-1 of the dihydroxanthalongin monomers (**302**, **304**) by Marcinek-Hüpen-Bestendonk and co-workers (1990). HMQC correlations (Fig. 4.8) between the proton doublet at δ_H 1.11 (H-14' according to Jakupovic et al., 1989) and the carbons at δ_C 144.7 (C-1) and 35.8 (C-10) resulted in our assignment of this proton as H-14. Similar HMQC correlations between the proton doublet at δ_C 1.25 (H-14' by our assignment) and the carbons at δ_C 150.1 (C-1'), δ_C 40.4 (C-9') and δ_C 29.3 (C-10') support this change.

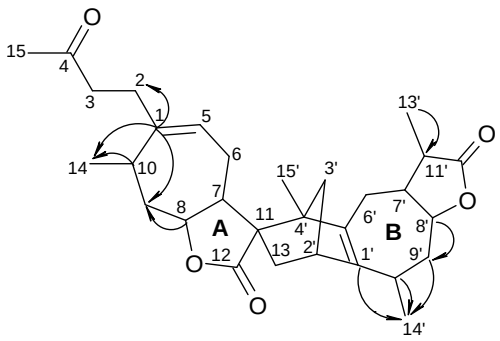


Figure 4.8 Selected HMQC couplings observed for helisplendidilactone (**306**) to support changes in assignments from those of Jakupovic et al. (1989).

Confirmation of the relative configuration of helisplendidilactone (**306**) was challenging due to the complexity of the proton NMR spectrum (Plate 15) in which several overlapping signals were observed. The NOESY correlation observed between H-7 (δ_H 2.44) and H-8 (δ_H 4.48), confirmed the *cis*-configuration of the dihydroxanthalongin lactone ring. A NOESY correlation is observed between H_α-9 (δ_H 1.77) and H-14 (δ_H 1.11), while a NOESY is neither present between H_α-9 (δ_H 1.77) and H-8 (δ_H 4.48) nor between H-14 (δ_H 1.11) and H-8 (δ_H 4.48), which indicates that these protons (H-8 and H-14) were on opposite sides of the ring (Fig. 4.9).

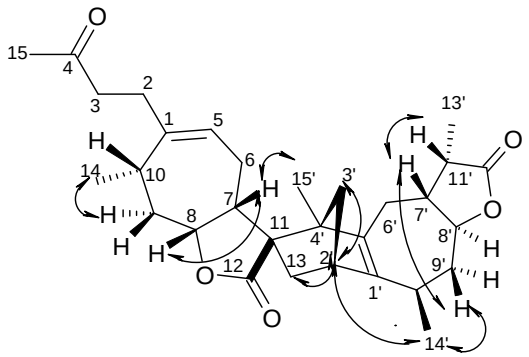


Figure 4.9 Important NOESY correlations observed for helisplendidilactone (**306**)

The absence of a NOESY correlation between H-7' (δ_H 2.10) and H-8' (δ_H 4.14) indicated 7'8'-*trans* configuration for the second lactone ring, while a clear NOESY correlation is observed between H-7' (δ_H 2.10) and H-11' (δ_H 2.72), showing that these protons are cofacial. This is confirmed by the absence of a NOESY correlation between H-8' (δ_H 4.14) and H-11' (δ_H 2.72) (indicating that these protons are on opposite sides of the ring), while a NOESY is observed between H-8' (δ_H 4.14) and H-13' (δ_H 1.23).

The relative configuration at C-14' could be determined indirectly by using the NOESY correlations observed between H-7' (δ_H 2.10) and H-14' (δ_H 1.25) with H_β-9' (δ_H 1.49). H-14' (δ_H 1.25) is on the same side of the ring as H-7' (δ_H 2.10), as both H-7' and H-14' shows NOESY correlations with H_β-9' (δ_H 1.49), while no correlation is observed between H-8' and H_β-9'. A NOESY correlation observed between the signal at δ_H 2.28 (assigned as Hb-6, H-10, Hb-3', H_α-9', H-10') and H-8' (δ_H 4.14) could either be between H-8' and H_α-9' or H-8' and H-10' as these signals (H_α-9', H-10') overlap.

The relative configuration of the stereogenic centres of the two monomer moieties could therefore be determined with NOESY correlations (Fig. 4.9). However, the orientation of the bridge was not absolutely clear from the NMR data obtained and confirmation of the complex configurational assignments necessitated the X-ray analysis of helisplendidilactone (**306)** (Fig. 4.10 and Fig. 4.11).

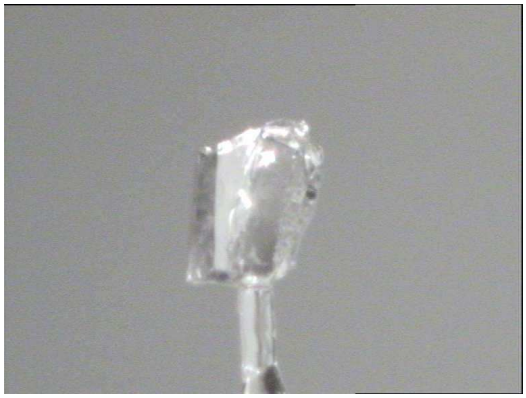


Figure 4.10 Crystal used for data collection

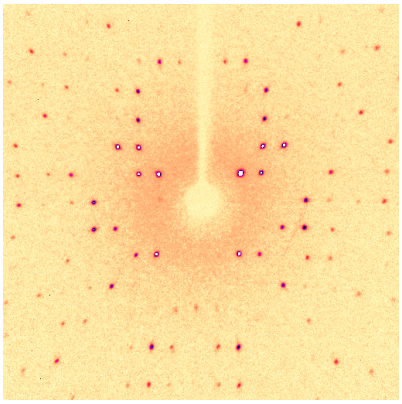


Figure 4.11 *b*-Axis axial photo indicating mirror symmetry consistent with monoclinic lattice.

The X-ray structure of helisplendidilactone (**306**) has not been reported in the literature. Crystal data of helisplendidilactone (**306**) obtained in this study is given in Table 4.1 and Table 4.2 and indicates that all bond lengths and bond angles are in agreement with the expected values (Table 4.2) for a good X-ray-diffraction structure refinement. The X-ray structure is summarised as follows: Monoclinic, *P*21, *a* = 9.0738(5), *b* = 11.4764(7), *c* = 12.2960(9) Å, α = 90, β = 96.933(5), γ = 90°, *V* = 1271.08(14) Å^3, and Z = 2 (Fig. 4.12, 4.13).[a]

The X-ray structure confirmed that both the backbone structure and relative configuration (we were unable to determine the absolute configuration) were correctly assigned by NMR spectroscopic methods by Jakupovic et al. (1989). In the illustration of helisplendidilactone (**306**) in the paper by Jakupovic and co-workers (1989), the stereochemical assignment at C-11 is not clear. Herewith, we provide an unambiguous assignment of the relative configuration at C-11.

[a] Crystal data collected and analysed by Prof. Orde Munro of the University of KwaZulu-Natal, Pietermaritzburg

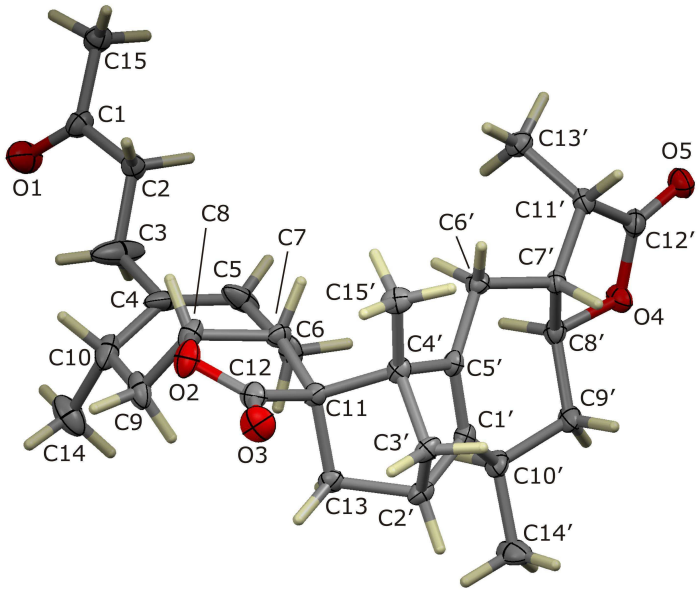


Figure 4.12 Colour thermal ellipsoid plot for helisplendidilactone (**306**) (50% probability level), with H atoms not labelled for clarity.

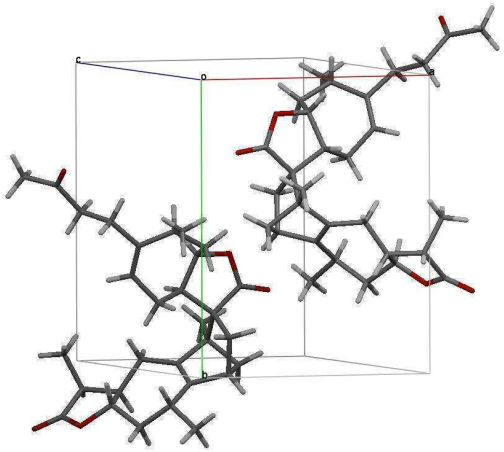


Figure 4.13 Unit cell contents.

The AM1 model of helisplendidilatone was obtained using the atomic coordinates obtained from the X-ray structure as input. The calculated structure was overlayed with the X-ray structure to determine whether there was a good correlation between the calculated and experimental structures. A reasonably good fit was obtained between the calculated and X-ray structure, illustrating the usefulness of these models (Fig. 4.14). The differences observed are possibly due to the fact that the atoms of the side chain are more tightly packed in the crystal structure, while more free rotation is experienced in the gas phase

molecular model. In both cases the cycloheptene ring of part A of the dimer adapts a boat-like conformation, while that of the B unit derived from guaia-1,4-dien-12,8β-olide is more chair like.

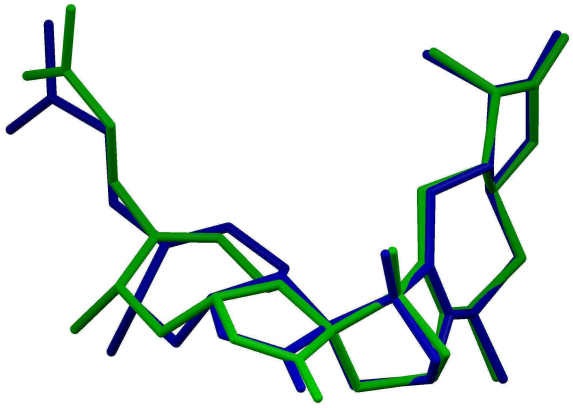
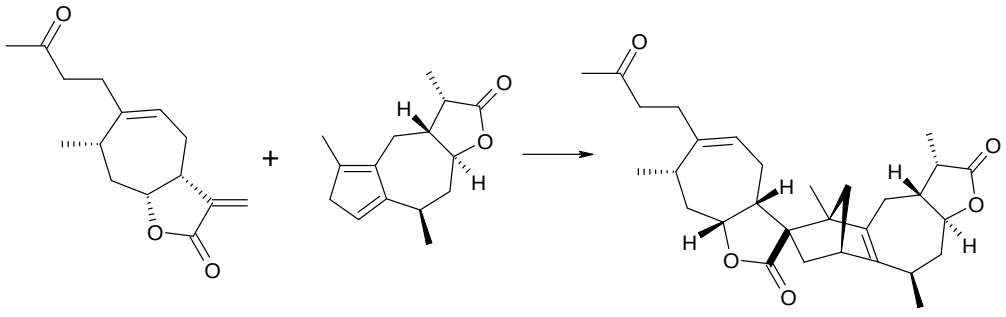


Figure 4.14 Helisplendidilactone (**306**) X-ray structure (blue) vs. AM1-calculated helisplendidilactone structure

Biosynthetically, the dimeric guaianolide **306** is most likely formed by a Diels-Alder reaction between xanthalongin (tomentosin) (**378**), and **379** (Scheme 4.1), neither of which have been isolated from *H. splendidum* (Jakupovic et al., 1989). The diene is probably very reactive, as is the diene acting as a precursor for absinthin, another dimeric guaianolide, which is only observed in Diels-Alder adducts as reported by Jakupovic et al. (1989). Dimeric guaianolides, like absinthin, occur in *Artemisia* species (Bohlmann et al., 1985, Kim et al., 2002, Jin et al., 2004, Beauhaire et al., 1981, Wong and Brown, 2002) and in *Geigera* species (Zdero and Bohlmann, 1989).



378 **379**

Scheme 4.1 Diels-Alder reaction yielding helisplendidilactone (**306**).

4.3 Conclusion

Three guaianolides, were isolated from *H. splendidum*. The determination of the relative configuration proved to be challenging due to overlapping signals. The assignment of the configuration as done by Jakupovic et al (1989) for these compounds was confirmed, while the NMR assignments for certain peaks of helisplendidilactone (**306**) was corrected. An X-ray structure for helisplendidilactone (**306**) was obtained for the first time. The examples of incorrect assignments of configuration associated with these types of compounds (Bohlmann and Suwita, 1979; Kuo and Lin, 1999) emphasise that extreme care needs to be taken in the assignment of the configuration of these compounds, even if they are known. This study has also shown the value of AM1 calculated models during the determination of the complex configuration exhibited by these compounds.

4.4 Experimental

4.4.1 General experimental procedures

^{1}H and ^{13}C NMR spectra of all compounds were recorded on a Varian Unity Inova 500 spectrometer with a 5 mm SW/Z-PFG probe (all spectra recorded at 25 °C, operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C spectra). Spectra were measured in $CDCl_3$ for all compounds. Structures were determined by analysis of 2D (HSQC, HMQC, COSY and NOESY) NMR spectra and by comparison of ^{1}H and ^{13}C NMR spectra with reported. Mass spectrometry data was collected on a time-of-flight Waters LCT Premier mass spectrometer using electrospray ionization in the positive or negative mode. Optical rotation was determined with a Perkin Elmer 241 polarimeter for compound **306**. X-ray structural analysis were carried out on an Oxford Xcalibur 2 CCD diffractometer, (MoKα) radiation.

Separations were done using open column chromatography and a chromatotron (Model 7924, Harrison Research). Silica gel ($60F_{254,}$ 40-63 μm, Merck) was used for column chromatography, while silica gel Merck 7749 with gypsum binding agent was used for preparation of chromatotron plates. Thin-layer chromatography (TLC) was done on precoated Kieselgel 60 F_{254} plates (Merck or Machery-Nagel). Detection was done by UV (254 nm) followed by staining with an anisaldehyde solution, prepared as follows:

Absolute ethanol (465 ml) was cooled in an ice bath. Acetic acid (5 ml), sulphuric acid (17 ml) and *p*-anisaldehyde (13 ml) was added and the solution mixed and stored in the fridge.

4.4.2 Plant material

H. splendidum was collected on the Steenkampsberg pass in Mpumalanga in January 2004. The plant was identified by Ms M. Welmann from the South African National Botanical Institute, Pretoria. Voucher specimens were deposited at the University of Kwazulu-Natal herbarium NU (*H. splendidum*, A. Lourens and A. Viljoen 2).

4.4.3 Extraction and isolation

Air-dried and ground leaves and stems of *H. splendidum* (107 g) were extracted with a mixture of chloroform:methanol (1:1) at room temperature for 48 hours to obtain approximately 8 g of extract. After running a column with hexanes:ethyl acetate (2:1), thirteen fractions (A1-A13) were obtained. A chromatotron of A9 and A10 using dichloromethane:diethyl ether (19:1) as eluent resulted in the collection of five fractions (B1-B5), B1 being the clean compound **302** (8.4 mg). A chromatotron of A6 and A7, using the same eluent, led to the isolation of compound **304** (6.4 mg). A short column was run on another 8 g of extract with dichloromethane:methanol (99:1) to obtain 10 fractions (D1-D17). Fraction D9-D10 was repeatedly cleaned up with the chromatotron using petroleum ether:ethyl acetate (2:1) and finally dichloromethane:diethyl ether (19:2) to obtain 5.7 mg of helisplendidilactone (**306**). The green solid obtained after purification was slowly crystallised at room temperature in an NMR tube with hexanes:dichloromethane to yield colourlesss crystals.

4.4.4 Physical data of isolated compounds

Compound **302**, a red brown gum, was identified as 11α,13-dihydroxanthalongin (Jakupovic et al., 1989; Marcinek-Hüpen-Bestendonk et al., 1990), ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.13 (3H, *d*, $J_{14,10}$ = 7.1 Hz, H-14), 1.15 (3H, *d*, $J_{13,11}$ = 7.4 Hz, H-13), 1.86 (1H, *ddd*, $J_{6\alpha,6\beta}$ = 15.7 Hz, $J_{6\alpha,5}$ = 9.4 Hz, $J_{6\alpha,7}$ = 2.7 Hz, H_α-6), 2.04 (2H, *m*, H_α-9, H_β-9), 2.14 (3H, *s*, H-15), 2.22 (2H, *m*, H-2, H_β-6), 2.34 (2H, *m*, H_a-2, H-10), 2.43 (1H, *ddd*, $J_{3a,3b}$ = 16.4 Hz, $J_{3a,2}$ = 9.1 Hz, $J_{3a,2}$ = 6.7 Hz, H_a-3), 2.55 (1H, *ddd*, $J_{3a,3b}$ = 16.4 Hz, $J_{3b,2}$ = 9.3 Hz, $J_{3b,2}$ = 5.7 Hz, H_b-3), 2.65 (1H, *dddd*, $J_{7,11}$ = 8.9 Hz, $J_{7,6\beta}$ = 9.1 Hz, $J_{7,8}$ = 6.2 Hz, $J_{7,6\alpha}$ = 2.7 Hz, H-7), 2.79 (1H, *dq*, $J_{11,7}$ = 8.7 Hz, $J_{11,13}$ = 7.5 Hz, H-11), 4.61 (1H, *ddd*, $J_{8,9\beta}$ = 11.2

Hz, $J_{8,7}$ = 6.2 Hz, H-8), 5.44 (1H, *br dd*, $J_{5,6\alpha}$ = 9.4 Hz, $J_{5,6\beta}$ = 2.2 Hz, H-5); ^{13}C NMR (125 MHz, $CDCl_3$): δ 10.8 (CH_3, C-13), 21.3 (CH_3, C-14), 21.8 (CH_2, C-6), 29.8 (CH_3, C-15), 31.0 (CH_2, C-2), 32.8 (CH, C-10), 36.9 (CH_2, C-9), 38.9 (CH, C-11), 42.2 (CH, C-7), 42.7 (CH_2, C-3), 80.5 (CH, C-8), 122.6 (CH, C-5), 144.1 (C, C-1), 179.1 (C, C-12), 208.2 (C, C-4); HRESIMS (positive ionization mode), *m/z* 251.1654 $[M+H]^+$ (calc. for $C_{15}H_{23}O_3$ 251.1647).

Compound **304**, a red-brown gum, was identified as 11β,13-dihydroxanthalongin (Bohlmann and Suwita, 1979; Marcinek-Hüpen-Bestendonk et al., 1990, ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.14 (3H, *d*, $J_{14,10}$ = 7.1 Hz, H-14), 1.22 (3H, *d*, $J_{13,11}$ = 7.1 Hz, H-13), 1.85 (1H, *ddd*, $J_{9\beta,9\alpha}$ = 13.7, $J_{9\beta,8}$ = 12.2 Hz, $J_{9\beta,10}$ = 11.1 Hz, H_β-9), 1.97 (1H, *ddd*, $J_{9\alpha,9\beta}$ = 13.7 Hz, $J_{9\alpha,10}$ = 6.9 Hz, $J_{9\alpha,8}$ = 2.6 Hz, H_α-9), 2.15 (3H, *s*, H-15), 2.17 (1H, *m*, H_a-6), 2.24 (3H, *m*, H_b-6, H-2, H-11), 2.37 (2H, *m*, H-7, H-10), 2.47 (1H, *ddd*, $J_{3a,3b}$ = 15.7 Hz, $J_{3a,2}$ = 8.9 Hz, $J_{3a,2}$ = 6.8 Hz, H_a-3), 2.56 (1H, *ddd* $J_{3b,3a}$ = 15.2, $J_{3b,2}$ = 8.7 Hz, $J_{3b,2}$ = 6.3 Hz, H_b-3), 4.46 (1H, *ddd*, *J* = $J_{8,9\beta}$ = 10.9 Hz, $J_{8,7}$ = 8.5 Hz, $J_{8,9\alpha}$ = 2.5 Hz, H-8), 5.36 (1H, *br dd*, $J_{5,6\beta}$ = 5.8 Hz, $J_{5,6\alpha}$ = 8.7 Hz, H-5); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.1 (*q*, C-13), 20.8 (*q*, C-14), 26.4 (*t*, C-6), 30.1 (*q*, C-15), 30.5 (*t*, C-2), 35.4 (*t*, C-9), 35.7 (*d*, C-10), 39.4 (*d*, C-11), 42.9 (*t*, C-3), 45.2 (*d*, C-7), 79.3 (*d*, C-8), 119.8 (*d*, C-5), 144.8 (*s*, C-1), 179.6 (*s*, C-12), 208.3 (*s*, C-4); HRESIMS (positive ionization mode), *m/z* 251.1659 $[M+H]^+$ (calc. for $C_{15}H_{23}O_3$ 251.1647).

Compound **306**, colourless plates, crystallised from dichloromethane:hexanes was identified as helisplendidilactone (Jakupovic et al., 1989). $[\alpha]_D$ = -45 (CH_2Cl_2, c = 0.805) [Lit. Jakupovic et al., 1989, -64^o ($CHCl_3$, c = 0.21)]; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.08 (1H, *dt*, $J_{3'a,3'b}$ = 8.5 Hz, $J_{3'a,2'}$ = $J_{3'a,13\alpha}$ = 2.4 Hz, Ha-3'), 1.11 (3H, *d*, $J_{14,10}$ = 7.0 Hz, H-14), 1.17 (1H, *m*, Ha-6), 1.22 (3H, *s*, H-15'), 1.23 (3H, *d*, $J_{13',11'}$ = 7.9 Hz, H-13'), 1.25 (3H, *d*, $J_{10',14'}$ = 7.0 Hz, H-14'), 1.49 (1H, ***ddd*,** $J_{9'\beta,8'= 9'\beta,9'\alpha = 9'\beta,10'}$ = 12.4 Hz, H_β-9'), 1.57 (1H, *dd*, $J_{13\alpha,13\beta}$ = 12.1 Hz, $J_{13\alpha,2'}$ = $J_{13\alpha,3'a}$ = 2.5 Hz, H_α-13), 1.68 (1H, *ddd*, $J_{6'\alpha,6'\beta}$ = 15.2 Hz, $J_{6'\alpha,7'}$ = 12.0 Hz, $J_{6'\alpha,10'}$ = 2.6 Hz, H_α-6'), 1.77 (1H, *ddd*, $J_{9\alpha,8\ =\ 9\alpha,9\beta\ =\ 9\alpha,10}$ = 12.2 Hz, H_α-9), 1.98 (1H, *br d*, $J_{13\beta,13\alpha\ =\ 13\beta,2'}$ = 12.0 Hz, H_β-13), 1.98 (1H, *m*, H_β-9), 2.10 (1H, *br ddd*, $J_{7',8'}$ ~$J_{7',6'\alpha}$ = 11.0 Hz, $J_{7',11'}$ = 7.5 Hz, $J_{7',6'\beta}$ = 2.8 Hz, H-7'), 2.13 (3H, *s*, H-15), 2.19 (2H, *br t*, $J_{2,3}$ = 7.7 Hz, H-2), 2.28 (5H, *m*, Hb-6, H-10, Hb-3', H_α-9', H-10'), 2.44 (3H, *m*,

Ha-3, H_{β}-6', H-7), 2.53 (1H, *ddd*, $J_{2,3}$ = 7.0 and 6.6 Hz**,** $J_{3a,3b}$ = 16.5 Hz, Hb-3) 2.72 (1H, *dq*, $J_{11',13'}$ = 7.8 Hz, $J_{11',7'}$ = 7.5 Hz, H-11'), 2.94 (1H, *br s*, H-2'), 4.14 (1H, *ddd*, $J_{8',7'=}$ $J_{8',9'\beta}$ = 11.0 Hz, $J_{8',9'\alpha}$ = 2.2 Hz, H-8'), 4.48 (1H, *ddd*, $J_{8,9\alpha}$ =12.3 Hz, $J_{8,7}$ = 9.0 Hz, $J_{8,9\beta}$ = 2.2 Hz, H-8), 5.19 (1H, *dd*, $J_{5,6}$ = 9.0 Hz, $J_{5,6}$ = 5.5 Hz, H-5); ^{13}C NMR (125 MHz, $CDCl_3$): δ 10.6 (*q*, C-13'), 13.4 (*q*, C-15'), 20.7 (*q*, C-14), 21.2 (*q*, C-14'), 25.0 (*t*, C-6'), 25.3 (*t*, C-6), 29.3 (*d*, C-10'), 29.8 (*q*, C-15), 30.3 (*t*, C-2), 35.8 (*d*, C-10), 36.2 (*t*, C-13), 37.1 (*t*, C-9), 39.7 (*d*, C-11'), 40.4 (*t*, C-9'), 41.8 (*d*, C-7), 42.5 (*t*, C-3), 43.2 (*d*, C-2'), 45.5 (*d*, C-7'), 50.7 (*t*, C-3'), 54.0 (*s*, C-4'), 63.0 (*s*, C-11), 78.5 (*d*, C-8), 84.6 (*d*, C-8'), 119.7 (*d*, C-5), 140.7 (*s*, C-5'), 144.5 (*s*, C-1), 150.2 (*s*, C-1'), 179.1 (*s*, C-12'), 181.7 (*s*, C-12), 207.8 (*s*, C-4); HRESIMS (positive ionization mode), *m/z* 479.280 $[M-H]^-$ (calc. for $C_{30}H_{39}O_5$ 479.2797).

4.4.5 Molecular modelling

AM1 geometry optimisations were performed with Gaussian® 03W, version 6.1 (Gaussian Inc. Carnegie Office Park Building 6, Pittsburgh, PA 15106, USA, Copyright 1995-2004), while overlays were done in Hyperchem®, release 6.03 for Windows (Hypercube Inc., copyright 2000).

4.5 References

Ball, M., Andrews, S.P., Wierschen, F., Cleator, E., Smith, M.D., Ley, S.V., 2007. Total synthesis of thapsigargin, a potent SERCA pump inhibitor. Organic Letters 9, 663-666.

Beauhaire, J., Fourrey, J.L., Lallemand, J.Y., Vuilhorgne, M., 1981. Dimeric sesquiterpene lactone. Structure of isoabsinthin. Acid isomerization of absinthin derivatives. Tetrahedron Letters 22, 2269-2272.

Bohlmann, F., Ang, W., Trinks, C., Jakupovic, J., Huneck, S., 1985. Dimeric guaianolides from *Artemisia sieversiana*. Phytochemistry 24, 1009-1015.

Bohlmann, F., Suwita, A., 1979. Ein neues Guajanolid und ein Secoguajanolid aus *Helichrysum splendidum*. Phytochemistry 18, 885-886.

Claridge, T.D.W., 1999. High Resolution NMR Techniques in Organic Chemistry. Pergamon, Oxford, p.285.

Dictionary of Natural Products, 2003. CHCD Dictionary of Natural Products on CD-ROM. Chapman and Hall, London, UK.

Dlamini, B., 1981. Swaziland Flora: Their Local Names and Uses. Ministry of Agriculture and Co-operatives: Forestry Section, Mbabane, Swaziland.

Hamada, F., Ohta, T., Shigeo, N., 1980. CD study of guaianolide sesquiterpenes having 8-fused lactone: The absolute configuration of helenium lactone and pleniradin. Heterocycles 14, 429-432.

Hilliard, O.M., 1983. Asteraceae. In: O.A. Leistner Flora of Southern Africa., Vol. 33, part 7 (Inuleae), Botanical Research Institute of South Africa, Pretoria, pp. 7,2:61-7,2:317.

Hilmi, F., Sticher, O., Heilmann, J., 2002. New cytotoxic 6,7-*cis* and 6,7-*trans* configured guaianolides from *Warionia saharae*. Journal of Natural Products 65, 523-526.

Hilmi, F. Gertsch, J., Bremner, P., Valovic, S., Heinrich, M., Sticher, O., Heilmann, J., 2003. Cytotoxic versus anti-inflammatory effects in HeLa, Jurkat T and human peripheral blood cells caused by guaianolide-type sesquiterpene lactones. Bioorganic and Medicinal Chemistry 11, 3659-3663.

Hwang, D-R., Wu, Y-S., Chang, C-W., Lien, T-W., Chen, W-C., Tan, U-K., Hsu, J.T.A., Hsieh, H-P., 2006. Synthesis and antiviral activity of a series of sesquiterpene lactones and analogues in the subgenomic HCV replicon system. Bioorganic and Medicinal Chemistry 14, 83-91.

Jacot Guillarmod, A., 1971. Flora of Lesotho (Basutoland). Cramer, Lehre, pp. 34, 38, 40-46, 267-281, 432-434.

Jakupovic, J., Zdero, C., Grenz, M., Tsichritzis, F., Lehmann, L., Hashemi-Nejad, S.M., Bohlmann, F., 1989. Twenty-one acylphloroglucinol and further constituents from South African *Helichrysum* species. Phytochemistry 28, 1119-1131.

Jin, H.Z., Lee, J.H., Homg, Y.S., Kim, Y.H., Lee, J.L., 2004. Inhibitors of the LPS-induced NF-κB activation from *Artemisia sylvatica*. Phytochemistry 65, 2247-2253.

Kim, J.H., Kim, H-K., Jeon, S.B., Son, K-H., Kim, E.H., Kang, S.K., Sung, N-D., Kwon, B-M. 2002. New sesquiterpene-monoterpene lactone, artemisolide, isolated from *Artemisia argyi*. Tetrahedron Letters, 43, 6205-6208.

Kuo, Y-H., Lin, B-Y., 1999. A new dinorxanthane and chromone from the root of *Tithonia diversifolia*. Chemical and Pharmaceutical Bulletin. 47, 428-429.

Leong, M.K., Mastryukov, V.S., Boggs, J.E., 1998. Structure and conformation of cyclopentene, cycloheptene and *trans*-cyclooctene. Journal of Molecular Structure 445, 149-160.

Marcinek-Hüpen-Bestendonk, C., Willuhn, G., Steigel, A., Wendisch, D., Middelhauve, B., Wiebcke, M., Mootz, D., 1990. Germacranolide, Guaianolide und Xanthanolide aus Blüten von *Arnica mollis* und Röntgenstrukturanalyse von Baileyinacetat. Planta Medica 56, 104-110.

Newman, D.J., Cragg, G.M., Snader, K.M., 2003. Natural products as sources of new drugs over the period 1981-2002. Journal of Natural Products 66, 1022-1037

Pavia, D.L., Lampman, G.M., Kriz, G.S., 1996. Introduction to Spectroscopy. Harcourt Brace College Publishers: Orlando, Florida. Pp.240-243.

Pooley, E., 2003. Mountain Flowers: A Field Guide to the Flora of the Drakensberg and Lesotho, 1st ed. The Flora Publications Trust, Durban, pp. 44, 102-110, 146-157, 222-225.

Siedle, B., Gustavsson, L., Johansson, S., Murillo, R., Castro, V., Bohlin, L., Merfort, I., 2003. The effect of sesquiterpene lactones on the release of human neutrofil elastase. Biochemical Pharmacology 65, 897-903.

Su, J-H., Dai, C-F., Huang, H-H., Wu, Y-C., Sung, P-J., Hsu, C-H., Sheu, J-H., 2007. Terpenoid-related metabolites from a Formosan soft coral *Nephthea chabrolii*. Chemical and Pharmaceutical Bulletin. 55, 594-597.

Ulubelen, A., Gören, N., Jiang, T-Y., Scott, L., Tianasoa-Ramomojy, M., Snyder, J.K., 1995. NMR assignments and absolute stereochemistry of two guaianolide sesquiterpenes from *Tanacetum densum* subsp. Amani. Magnetic Resonance in Chemistry 33, 900-904.

Wedge, D.E., Galindo, J.C.G., Macoas, F.A., 2000. Fungicidal activity of natural and synthetic sesquiterpene lactone analogs. *Phytochem*. 53: 747-757.

Wong, H-F., Brown, G.D., 2002. Dimeric guaianolides and a fulvenoguaianolide from *Artemesia myriantha*. Journal of Natural Products 65, 481-486.

Zdero, C., Bohlmann, F., 1989. Sesquiterpene lactones and other terpenes from Geigera species. Phytochemistry 28, 3105-3120.

Table 4.1 Crystal data and structure refinement for helisplendidilactone (**306**).

Molecular formula	C_{30} H_{40} O_5
Formula weight	480.62
Temperature	100 (2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	*P*21
Unit cell dimensions	*a* = 9.0738(5) Å
	b = 11.4764(7) Å
	c = 12.2960(9) Å
	α = 90°
	β = 96.933(5)˚
	γ = 90°
Volume	1271.08(14) $Å^3$
Z	2
Density (calculated)	1.256 mg/m^3
Absorption coefficient	0.084 mm^{-1}
F(100)	520
Crystal size	0.40 x 0.30 x 0.25 mm^3
θ Range for data collection	4.43 to 28.06°
Index ranges	-12<=h<=12, -15<=k<=15, -16<=l<=16
Reflections collected	16002
Independent reflections	6126 [R(int) = 0.0276]
Completeness to θ = 25.00°	99.2 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9935 and 0.9224
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	6126 / 13 / 321
Goodness-of-fit on F^2	1.089
Final R indices [I>2sigma(I)]	R_1 = 0.0516, wR_2 = 0.1073
R indices (all data)	R_1 = 0.0524, wR_2 = 0.1077
Absolute structure parameter	0.1(10)
Largest diff. peak and hole	0.608 and -0.455 e $Å^{-3}$

Table 4.2 Bond lengths [Å] and angles [°] for helisplendidilactone (**306**).

Bond lengths:

C(1)-O(1)	1.216(3)	C(7')-C(8')	1.524(3)
C(1)-C(15)	1.496(3)	C(7')-C(18)	1.533(3)
C(1)-C(2)	1.519(3)	C(7)-C(8)	1.536(3)
C(1')-C(5')	1.346(3)	C(7)-C(11)	1.557(3)
C(1')-C(10')	1.522(3)	C(8')-O(4)	1.463(2)
C(1')-C(2')	1.525(3)	C(8')-C(9')	1.515(3)
C(2)-C(3)	1.487(3)	C(8)-O(2)	1.455(3)
C(2')-C(3')	1.527(3)	C(8)-C(9)	1.525(3)
C(2')-C(13)	1.558(3)	C(9')-C(10')	1.534(3)
C(3)-C(4)	1.524(3)	C(9)-C(10)	1.534(4)
C(3')-C(4')	1.541(3)	C(10')-C(14’)	1.527(3)
C(4')-C(15')	1.518(3)	C(10)-C(14)	1.534(3)
C(4')-C(5')	1.537(3)	C(11)-C(12)	1.522(3)
C(4')-C(11)	1.593(3)	C(11)-C(13)	1.564(3)
C(4)-C(5)	1.320(4)	C(12)-O(3)	1.205(3)
C(4)-C(10)	1.505(4)	C(12)-O(2)	1.344(3)
C(5')-C(6')	1.504(3)	C(13’)-C(11’)	1.532(3)
C(5)-C(6)	1.527(3)	C(11’)-C(12’)	1.517(3)
C(6)-C(7)	1.539(3)	C(12’)-O(5)	1.197(3)
C(6')-C(7')	1.517(3)	C(12’)-O(4)	1.358(3)

Table 4.2 (continued) Bond lengths [Å] and angles [°] for helisplendidilactone (**306**).

Bond angles:

O(1)-C(1)-C(15)	121.42(19)	O(4)-C(8')-C(9')	108.53(16)
O(1)-C(1)-C(2)	121.5(2)	O(4)-C(8')-C(7')	104.14(15)
C(15)-C(1)-C(2)	117.03(18)	C(9')-C(8')-C(7')	115.96(17)
C(5')-C(1')-C(10')	130.62(19)	O(2)-C(8)-C(9)	109.12(19)
C(5')-C(1')-C(2')	106.52(17)	O(2)-C(8)-C(7)	105.00(16)
C(10')-C(1')-C(2')	121.49(17)	C(9)-C(8)-C(7)	114.87(18)
C(3)-C(2)-C(1)	113.08(19)	C(8')-C(9')-C(10')	113.24(16)
C(1')-C(2')-C(3')	101.54(16)	C(8)-C(9)-C(10)	113.4(2)
C(1')-C(2')-C(13)	106.25(16)	C(1')-C(10')-C(14')	112.99(18)
C(3')-C(2')-C(13)	100.53(16)	C(1')-C(10')-C(9')	115.54(17)
C(2)-C(3)-C(4)	118.4(2)	C(14')-C(10')-C(9')	108.93(17)
C(2')-C(3')-C(4')	94.39(15)	C(4)-C(10)-C(9)	116.90(19)
C(15')-C(4')-C(5')	117.26(17)	C(4)-C(10)-C(14)	111.1(2)
C(15')-C(4')-C(3')	117.13(16)	C(9)-C(10)-C(14)	110.0(2)
C(5')-C(4')-C(3')	99.43(15)	C(12)-C(11)-C(7)	102.27(16)
C(15')-C(4')-C(11)	115.17(16)	C(12)-C(11)-C(13)	109.33(16)
C(5')-C(4')-C(11)	104.58(15)	C(7)-C(11)-C(13)	118.03(16)
C(3')-C(4')-C(11)	100.75(16)	C(12)-C(11)-C(4')	109.65(16)
C(5)-C(4)-C(10)	126.0(2)	C(7)-C(11)-C(4')	115.49(16)
C(5)-C(4)-C(3)	123.8(3)	C(13)-C(11)-C(4')	102.02(15)
C(10)-C(4)-C(3)	110.2(2)	O(3)-C(12)-O(2)	119.6(2)
C(1')-C(5')-C(6')	131.92(18)	O(3)-C(12)-C(11)	128.9(2)
C(1')-C(5')-C(4')	107.82(17)	O(2)-C(12)-C(11)	111.44(18)
C(6')-C(5')-C(4')	120.02(16)	C(2')-C(13)-C(11)	103.07(16)
C(4)-C(5)-C(6)	126.8(2)	C(12')-C(11')-C(13')	108.50(17)
C(5)-C(6)-C(7)	113.28(19)	C(12')-C(11')-C(7')	102.31(16)
C(5')-C(6')-C(7')	114.76(16)	C(13')-C(11')-C(7')	115.94(16)
C(6')-C(7')-C(8')	113.18(16)	O(5)-C(12')-O(4)	121.42(19)
C(6')-C(7')-C(18)	116.32(16)	O(5)-C(12')-C(11')	128.3(2)
C(8')-C(7')-C(18)	102.83(15)	O(4)-C(12')-C(11')	110.27(17)
C(8)-C(7)-C(6)	110.87(17)	C(12)-O(2)-C(8)	111.39(16)
C(8)-C(7)-C(11)	103.87(16)	C(12')-O(4)-C(8')	110.08(15)
C(6)-C(7)-C(11)	118.21(16)		

CHAPTER 5

The phytochemistry of *Helichrysum montanum*

5.1 Introduction

Helichrysum montanum DC. is a mat-forming dwarf shrub that occurs mainly at high altitudes (1800 - 2500 m) such as the high mountains of KwaZulu-Natal (Drakensberg), Lesotho, and the Eastern Cape. Its branches are very short, congested and densely leafy, and the leaves are distinctly striped and appear greyish-white and woolly. This species is morphologically closely related to *H. splendidum* (Fig. 5.1), both species belonging to Hilliard's morphological Group 22. *H. montanum* is distinguished from *H. splendidum* by its dense growth pattern and by its leaves, which is always broadest near the tip. It generally grows at higher altitudes than *H. splendidum* and flowers later. *H. montanum* flowers between January and April, while the flowering time of *H. splendidum* is between October and January (Hilliard, 1983; Pooley, 1998).

Figure 5.1 a) Flower and leaf of *H. montanum* b) Flower and leaf of *H. splendidum*

A preliminary investigation by thin-layer chromatography (TLC) revealed that extracts of both *H. montanum* and *H. splendidum* (Fig. 5.2) exhibited red staining of certain compounds with anisaldehyde spray reagent. To the best of our knowledge, the phytochemistry of *H. montanum* has not been investigated previously and the TLC results of these plant extracts indicated that *H. montanum* may exhibit an interesting chemical profile, similar to that of *H. splendidum*.

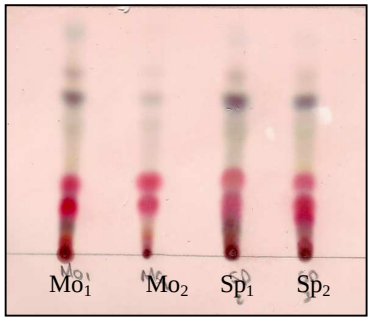


Figure 5.2 TLC of extracts of *H. montanum* (MO_1 locality unknown, MO_2 collected in the Koueveld Mountains) and *H. splendidum* (SP_1 and SP_2 both collected in Mpumalanga).

The aims of this chapter are:

- To report on the isolation and characterisation of secondary metabolites from *H. montanum.*
- To determine whether the phytochemistry of *H. montanum* supports the close morphological relationship observed with *H. splendidum*.

5.2 Results and discussion

The chloroform:methanol (1:1) extract of *H. montanum* yielded two new compounds, 13'-epihelisplendidilactone (**307**) and the monomeric guaianolide **301**, both of which are stereoisomers of compounds isolated from *H. splendidum*. The extract also yielded five known compounds, namely spathulenol (**286**, a sesquiterpene), umbelliferone (**348**, a coumarin), the guaianolide 11β,13-dihydroxanthalongin (**304**) (also isolated from *H. splendidum*), 1β,5α,7α,8β,11β)-4α-hydroxyguai-10(14)-en-8,12α-olide (**300**), a stereoisomer of a guaianolide isolated from *H. splendidum*, and a flavone, 4',5,7-trihydroxy-3,3',8-trimethoxyflavone (**381**).

The ^{1}H NMR (Plate 22) and ^{13}C NMR (Plate 23) spectra of compound **307** indicated that the spectra were similar to those of helisplendidilactone (**306**), previously isolated from *H. splendidum* (Thunb.) Less. (Jakupovic et al., 1989) and reisolated in our laboratory (Chapter 4). The multiplicity of each of the 30 carbons of compound **307** was determined with a DEPT experiment (Plate 25), which indicated the presence of eight quaternary, nine CH, eight CH_2 and five CH_3 carbons, supporting the molecular formula of $C_{30}H_{40}O_5$ as

determined by high-resolution mass spectrometry. Consideration of two-dimensional spectra [(HSQC (Plate 26), COSY (Plate 24) and HMQC (Plate 27, Fig. 5.3)] and comparison with the NMR data (Plates 15 - 21) obtained for helisplendidilactone (**306**) (Chapter 4, Jakupovic et al., 1989) revealed that compound **307**, which we named 13'-epihelisplendidilactone, had a backbone similar to that of helisplendidilactone (Table 5.1).

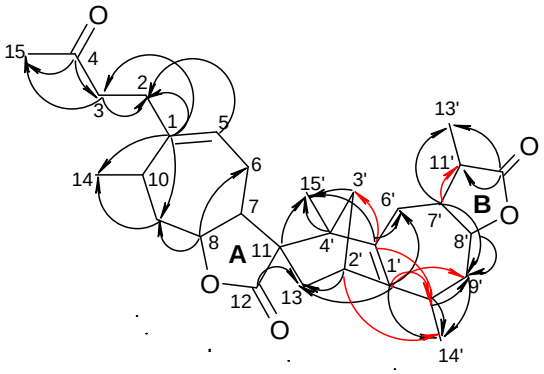


Figure 5.3 HMQC correlations observed for 13'-epihelisplendidilactone (**307**) (arrows from ^{13}C to ^{1}H). Arrows in red are probable HMQC correlations as overlap of signals occur where these correlations are observed.

Table 5.1 ^{1}H and ^{13}C NMR data ($CDCl_3$) of helisplendidilactone (**306**) and 13'-epihelisplendidilactone (**307**)[a].

	Helisplendidilactone (306) δ_H,(multiplicity, *J* in Hz)	**13'-epi-helisplendidilactone (307)** δ_H (multiplicity, *J* in Hz)	**Helisplendidilactone (306)** δ_C	**13'-epi-helisplendidilactone (307)** δ_C
1			144.5 C	144.4 C
2	2.19 (*br t*, 7.7)	2.20 (*br t*, 7.9)	30.3 CH_2	30.4 CH_2
3a	2.44 (*m*)	2.44 (*m*)	42.5 CH_2	42.4 CH_2
3b	2.53 (*ddd*, 7.0, 6.6 16.5)	2.55 (*ddd*, 6.7, 6.4 16.5)		
4			207.8 C	207.9 C
5	5.19 (*dd*, 9.0, 5.5)	5.23 (*dd*, 8.9, 5.2)	119.7 CH	119.9 CH
6a	1.17 (*m*)	1.19 (*m*)	25.3 CH_2	25.3 CH_2
6b	2.28 (*m*)	2.30 (*m*)		
7	2.44 (*m*)	2.44 (*m*)	41.8 CH	42.02 CH
8	4.48 (*ddd*, 12.3, 9.0 2.2)	4.48 (*ddd*, 12.1 Hz, 8.9, 2.6)	78.5 CH	78.6 CH
9α	1.77 (*ddd*, 12.2)	1.78 (*ddd*, 12.4)	37.1 CH_2	37.1 CH_2

9β	1.98 (*m*)	1.98 (*ddd*, 12.1, 6.7, 2.3)		
10	2.28 (*m*)	2.30 (*m*)	35.8 CH	35.6 CH
11			63.0 C	62.9 C
12			181.7 C	181.6 C
13 α	1.57 (*dd*, 12.1, 2.5)	1.56 (*dd*, 12.2, 2.6)	36.2 CH_2	36.1 CH_2
13b	1.98 (*br d*, 12.0)	1.98 (*dd*, 12.1, 3.8)		
14	1.11 (*d*, 7.0)	1.11 (*d*, 6.8)	20.7 CH_3	20.7 CH_3
15	2.13 (*s*)	2.13 (*s*)	29.8 CH_3	29.8 CH_3
1’			150.2 C	150.5 C
2’	2.94 (*br s*)	2.94 (*br s*)	43.2 CH	43.3 CH
3’a	1.08 (*br* dd, 8.5, 2.1)	1.08 (*br dd*, 8.5**,** 2.1)	50.7 CH_2	50.6 CH_2
3'b	2.28 (*m*)	2.30 (*m*)		
4’			54.0 C	54.3 C
5’			140.7 C	140.3 C
6’α	1.68 (*ddd*, 15.2, 2.6)	1.65 (*m*)	25.0 CH_2	28.5 CH_2
6’β	2.44 (*m*)	2.64 (*br d*, 13.5)		
7’	2.10 (*br ddd*, 11.0 7.5, 2.8)	1.65 (*m*)	45.5 CH	50.7 CH
8’	4.14 (*ddd*, 11.0, 2.2)	3.93 (*ddd*, 11.0, 2.2)	84.6 CH	84.9 CH
9’β	1.49 (*ddd***,** 12.4)	1.49 (*ddd*, 11.5)	40.4 CH_2	40.2 CH_2
9’α	2.28 (*m*)	2.30 (*m*)		
10’	2.28 (*m*)	2.30 (*m*)	29.3 CH	29.5 CH
11’	2.72 (*dq*, 7.8, 7.5)	2.30 (*m*)	39.7 CH	42.00 CH
12’			179.1 C	178.3 C
13’	1.23 (*d*, 7.9)	1.260 (*d*, 7.0)	10.6 CH_3	12.8 CH_3
14’	1.25 (*d*, 7.0)	1.257 (*d*)	21.2 CH_3	21.3 CH_3
15’	1.22 (*s*)	1.23 (*s*)	13.4 CH_3	13.5 CH_3

[a] Positions where significant differences in chemical shifts are observed are indicated in red.

The changes observed in the chemical shifts of certain signals in the ^{1}H and ^{13}C NMR spectra of helisplendidilactone (**306**) and 13’-epihelisplendidilactone (**307**) suggested that a stereoisomer of helisplendidilactone was isolated. Significant differences observed in the NMR data of the two compounds include those for H_{β}-6’ ($\Delta\delta_H$ = -0.2), H-7’ ($\Delta\delta_H$ = +0.48), H-8’ ($\Delta\delta_H$ = +0.21), H-11’ ($\Delta\delta_H$ = +0.42) and C-6’ ($\Delta\delta$c = -3.5), C-7’ ($\Delta\delta_C$ = -5.2), C-11’ ($\Delta\delta_C$ = -2.3), and C-13’ ($\Delta\delta_C$ = -2.2) (Table 5.1). These differences indicate the two molecules differ in the stereochemistry of the B guaianolide unit (Fig 5.3).

Consideration of the NOESY spectrum (Plate 28) and comparison with the NMR data obtained for helisplendidilactone (**306**) revealed that 13'-epihelisplendidilactone (**307**) had a 7,8-*cis* configuration like helisplendidilactone for the A guaianolide unit and that the bridge moiety was identical for the two compounds. The chemical shifts for the "left-hand side" of the molecule, which encompasses the A guaianolide monomer as well as the bridge, is almost identical for the two compounds. Important NOESY correlations are those observed between H-7 (δ_H 2.44) and H-8 (δ_H 4.48), confirming the 7,8-*cis*-configuration of the A unit, while the absence of a NOESY correlation between H-8 (δ_H 4.48) and H-14 (δ_H 1.11) indicates that this methyl group must be *trans* to H-8 (δ_H 4.48). This is confirmed by the NOESY correlation observed between H-14 (δ_H 1.11) and H_α-9 (δ_H 1.78) and the absence of a correlation between H-8 (δ_H 4.48) and H_α-9 (δ_H 1.78), similar to what was observed for helisplendidilactone (**307**).

A NOESY correlation is also observed between H-8 (δ_H 4.48) and most probably H-10 (δ_H 2.30, although the H-10 signal overlaps with one of the H-6 proton signals, which means the observed NOESY may also be between H-8 and H-6). The NOESY correlation observed between H-7 (δ_H 2.44) and H-15' (δ_H 1.23) for helisplendidilactone (**306**), is also observed for 13'-epihelisplendidilactone (**307**). The relative configuration of the left-hand side of the molecule could therefore be determined with relative ease.

This was not the case for the right-hand side of the molecule. Analysis of the NMR spectra, especially the ^{1}H NMR spectrum (Plate 22) of 13'-epihelisplendidilactone (**307**) proved to be more challenging than that of helisplendidilactone (**306**). For 13'-epihelisplendidilactone (**307**), the signals for both H-7' (δ_H 1.65) and H-11' (δ_H 2.30) overlap with the signals of other protons which complicate the assignment of the stereochemistry at C-7' and C-11'.

In an attempt to improve the resolution (in other words, to separate overlapping signals), a chiral shift reagent, Eu(fod)$_3$ was added to the compound. This reagent was selected as it was used to improve the resolution of the signals of a related guaianolide (Bohlmann et al., 1977). Although this addition did improve the resolution between H_β-9 and H_β-13, the signals for H-6$_\alpha$'/7' and H-10'/11' were still overlapping (Plate 29). However, the addition

did enable us to observe a NOESY correlation between H_β-9' and H_α-6'/H-7' not seen in the spectrum where no addition occurred (Plate 28 vs. Plate 30).

NMR experiments were also performed on a 600 MHz NMR spectrometer[a] in further efforts directed at enhancement of resolution. However, there was no significant improvement in the spectra when compared to those obtained from the 500 MHz instrument. Further specialised NMR experiments, such as a double quantum COSY, a gradient multiple quantum filtered COSY, and a homonuclear-2dj-resolved experiment were also performed. These experiments were done to determine whether coupling constants of individual protons belonging to multiplets could not be obtained, but due to the complexity of these signals unambiguous conclusions could not be drawn.

Finally, NOESY correlations used in conjunction with AM1 calculated structures of the most stable conformations of different possible stereoisomers provided a solution. An important correlation that was absent in the NOESY spectrum of **307** was a correlation observed for **306** (helisplendidilactone), between H-8' (δ_H 3.93) and H-13' (δ_H 1.26). This indicated that in 13'-epihelisplendidilactone (**307**), the 13'-methyl group was *trans* to H-8'. Therefore, two alternative structures were proposed for compound **307:** a) where the B-lactone ring has 7',8'-*cis* configuration with the 13'-methyl group in the α-position (Fig. 5.4a) or b) where the B-lactone ring has a 7',8'-*trans* configuration as in helisplendidilactone (**306**), but with the 13'-methyl group in the β-position (Fig.5.4b).

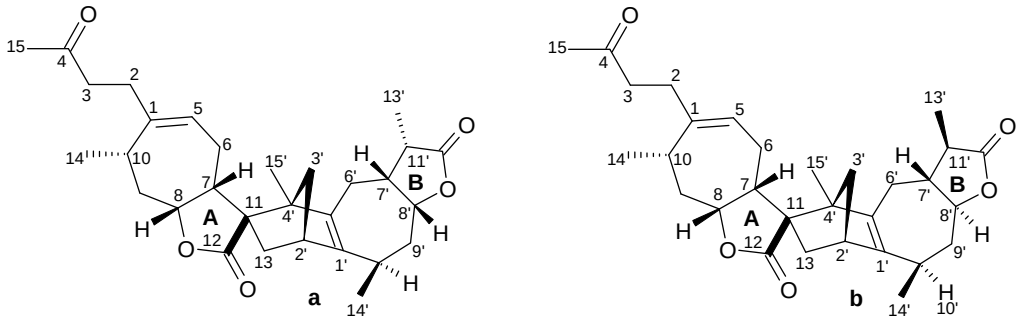


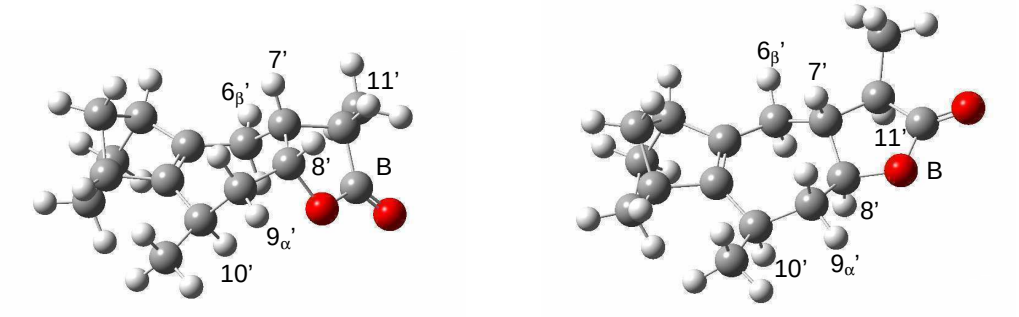
Figure 5.4 Alternative structures possible for 13'-epihelisplendidilactone (**307**). a) Structure with 7',8'-*cis* configuration. b) Structure with 7',8'-*trans* configuration

[a] NMR experiments on the 600 MHz Varian instrument were performed by Ms. Jean McKenzie of the University of Stellenbosch

As mentioned before, the major difficulty in assigning the relative configuration at C-7' and C-11' lies in the fact that in the ^{1}H NMR spectrum, the signals for H-11' and H-10' as well as those of H_α-6' and H-7' are overlapping. This extensive overlap of signals was not observed for helisplendidilactone (**306**).

If the AM1 calculated structures of 13'-epihelisplendidilactone (**307**) (Fig. 5.5, only the B-unit is displayed for the sake of clarity) is considered, the following arguments can be made to differentiate between the stereoisomers: If there was a 7',8'-*cis* configuration, the NOESY correlations observed for H-8' (δ_H 3.93) is most probably with H-11' (δ_H 2.30) and H-7' (δ_H 1.65) (Fig. 5.5a). On the other hand, if one considers the 7',8'-*trans* possibility, these same NOESY correlations indicate a close proximity of H-8' and H-11'/H-10' and H-8' and only H_α-6' (also observed for helisplendidilactone) (Fig. 5.5b). These correlations did therefore not allow unambiguous assignment of the relative configuration at C-7' or C-11' (Fig.5.6).

As direct NOESY correlations could not be used to determine the relative configuration at C-7' and C-11', correlations observed with other protons had to be considered. A NOESY correlation is observed between H_β-9' and H_α-6'/7', which is most likely a correlation between H-7' and H_β-9', as a correlation between H_α-6' and H_β-9' does not seem feasible for both possible structures. Furthermore, if the model of the 7',8'-*cis* structure is considered, a NOESY correlation is expected between H_β-9' and H-8', however no correlation is seen between these protons which are resolved in the ^{1}H NMR spectrum. It is therefore concluded that 13'-epihelisplendidilactone (**307**) has the 7',8'-*trans* structure, thus differing from helisplendidilactone only in the orientation of the 13'-methyl group (Fig. 5.4b and 5.5b). A NOESY correlation is also observed between H-13'/14' and H_α-6'/7'. This is probably a correlation between H-7' with H-13', further supporting this assignment (Fig. 5.6).



a **b**

Figure 5.5 a) B guaianolide unit with 7',8'-*cis* configuration. b) B guaianolide with 7'8'-*trans* configuration (Models calculated with Gaussian).

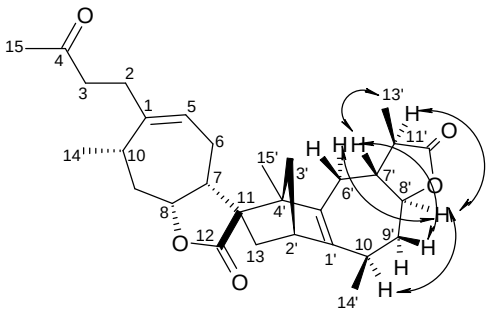
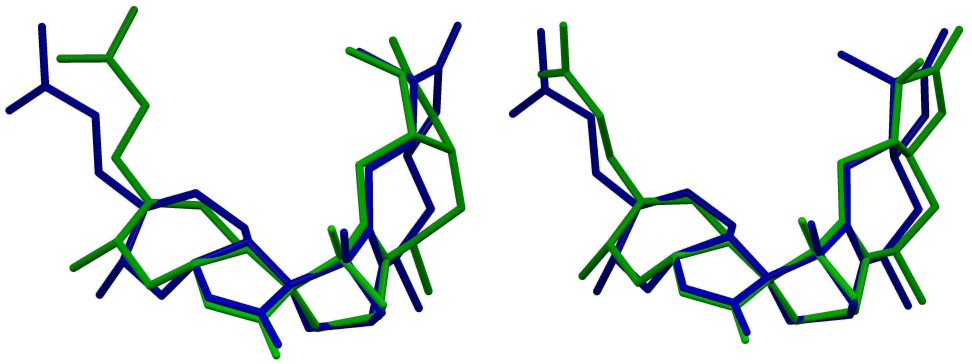


Figure 5.6 NOESY correlations observed for the second lactone ring of 13'-epihelisplendidilactone (**307**).

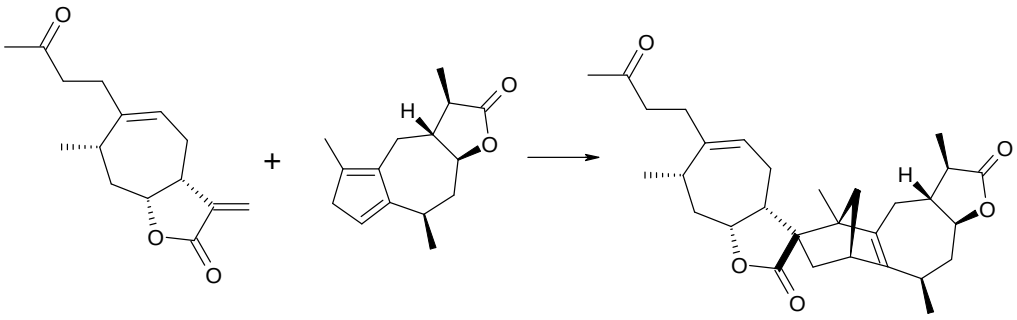
The AM1 calculated structures were obtained by using the atomic coordinates of the X-ray structure of helisplendidilactone (**306**), then changing the stereochemistry to obtain the isomers possible for 13'-epihelisplendidilactone (**307**), followed by AM1 geometry optimisations in Gaussian^{R}. These models were then overlayed with the X-ray structure of helisplendidilactone (**306**) in Hyperchem^{R} to observe whether any conformational changes occurred. A much better fit was obtained with the X-ray structure of helisplendidilactone and the AM1 calculated structure of the 7',8'*trans* 13'-epihelisplendidilactone structure than with the 7'8'-*cis* structure (Fig. 5.7).



a **b**

Figure 5.7 a) Overlay of the AM1 calculated structure of the proposed 7'8'-*cis* stereoisomer of 13'-epihelisplendidilactone with the X-ray structure of helisplendidilactone (**306**). b) Overlay of the AM1 calculated structure of the 7'8'-*trans* stereoisomer of 13'-epihelisplendidilactone (**307**) with the X-ray structure of helisplendidilactone (**306**).

As previously mentioned for helisplendidilactone (**306**), 13'-epihelisplendidilactone (**307**) is most likely a product of the Diels-Alder reaction between **378** and guaia-1,4-dien-12,8β-olide (**380**) (Jakupovic et al., 1989), neither of which was isolated from *H. montanum* (Scheme 5.1).



378 **380**

Scheme 5.1 Diels-Alder reaction yielding 13'-epihelisplendidilactone (**307**).

The second new guaianolide isolated from *H. montanum* was assigned structure **301**. A known guaianolide **300** (Bohlmann et al., 1982; Rustaiyan, 1987) was also isolated. The ^{1}H and ^{13}C NMR spectra of compounds **300** (Plates 37, 38) and **301** (Plates 56, 57) were similar. These compounds also exhibited the same blue staining with anisaldehyde spray

reagent. Both compounds had 15 carbons (acetone is present as an impurity in **300**), indicating the presence of two closely related sesquiterpenes. This was confirmed by high-resolution mass spectrometry which indicated that both compounds had a molecular formula of $C_{15}H_{22}O_3$. Two characteristic proton signals at approximately δ_H 5.0 and δ_H 4.9 indicating the presence of an exomethylene group were observed, while protons typical of those next to the oxygen in a lactone ring occurred at δ_H 4.26 (**300**) and δ_H 4.45 (**301**).

The normal two-dimensional experiments [(COSY (Plates 39, 58), HSQC (Plates 41, 60), HMQC (Plates 42, 61, Fig. 5.8) and NOESY (Plates 43, 62)] were performed and it was determined that compounds **300** and **301** are two stereoisomers of 4-hydroxyguai-10(14)-en-12,8-olide. To our knowledge six stereoisomers of this structure have been described (Table 5.2) and these compounds were isolated from *H. splendidum* (Jakupovic et al., 1989), *Gnephosis brevifolia* (Jakupovic et al., 1988), *Geigera aspera* Harv. var. *aspera* (Bohlmann et al., 1982), and *Dittrichia graveolens* (Rustaiyan et al., 1987). A synthetic route was also developed for two of these isomers (Blay et al., 2000).

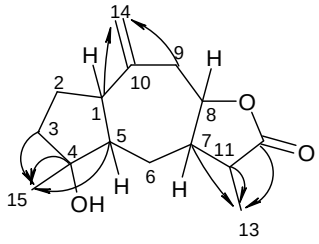


Figure 5.8 HMQC correlations observed for both compound **300** and **301**.

The proton NMR shifts observed for compound **300** were an exact match to those observed by Bohlmann et al. (1982) for 11β,13-dihydroinoviscolide. The structure of this compound was later revised by Rustaiyan and co-workers (1987) who corrected the relative configuration from the 1α,8α-stereoisomer to the 1β,8βH-stereoisomer as indicated in Figure 5.10. In our hands, NOESY correlations were observed between H-1 and H-8, while no correlations were observed between H-8 and H-7 or H-1 and H-5, indicating *trans*-1,5 and *trans*-7,8 configuration. Other correlations confirming this configuration are those between H_β-6 and H-15, H_β-6 and H-11 and between H-7 and H_α-6 (Fig. 5.9). The NOESY correlations observed for compound **300** used in conjunction with the AM1-calculated

models of the structures confirmed the configuration as assigned by Rustaiyan and co-workers (1987). Therefore, **300** has the same structure as the guaianolide previously isolated from *Geigera aspera* var. *aspera* (Bohlmann et al., 1982) and *Dittrichia graveolens* (Rustaiyan et al., 1987), both members of the Asteraceae.

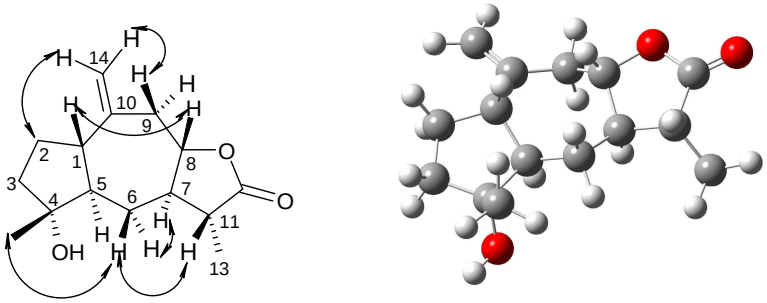
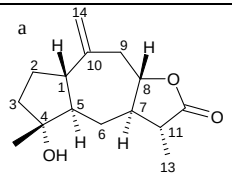
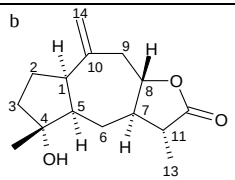
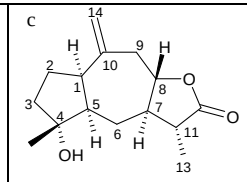
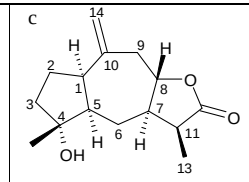
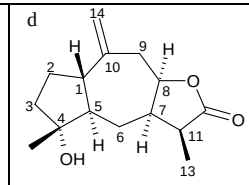
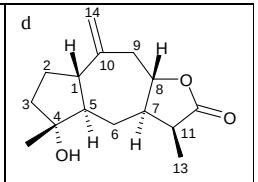


Figure 5.9 a) NOESY correlations observed for compound **300** b) AM1 Gaussian model of compound **300**.

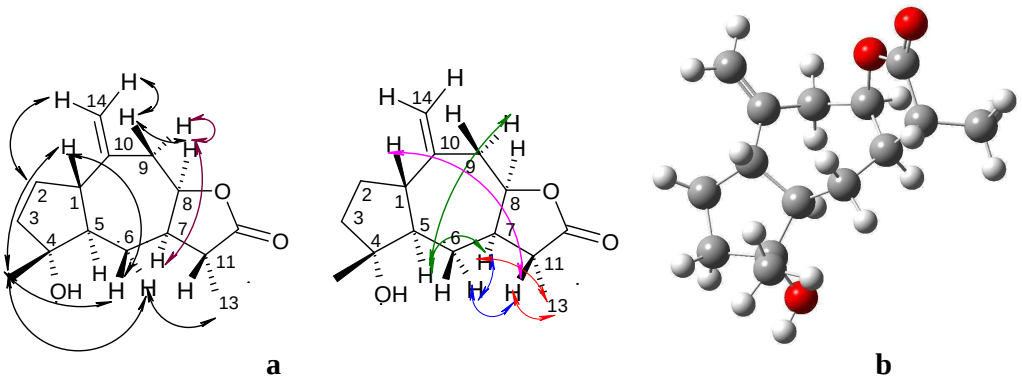
As previously mentioned, compound **301** was identified as a new guaianolide. The shifts of several protons (H-1, H-5, H_β-6, H-7, H-8, H-9) in the ^{1}H NMR spectrum of compound **301** differed from those in the proton spectrum of compound **300,** although all HSQC, COSY and HMQC correlations supported the assignment of a similar carbon backbone. The relative configuration was again assigned using NOESY correlations (Fig. 5.9). However, as more overlap occurred for signals associated with stereogenic centres in **301**, determining the configuration was more challenging.

The difference between compound **301** and compound **300** is the relative configuration at C-8. If one considers the AM1 calculated models of these compounds, a large difference in the most stable conformations is observed, with the five-membered lactone ring in compound **301** bending up towards the central cycloheptane ring when compared to compound **300,** providing a possible explanation for the chemical shift changes observed between the two compounds. NOESY correlations relevant to the determination of the configuration include those observed between H-1 and H-15, H-5 and H-8, H_α-6 and H-13, H-8 and H-7/H_α-9 and the absence of a correlation between H-8 and H-1 (Fig. 5.10). Compound **301** is a new C-11 epimer of a similar guaianolide previously isolated from *H. splendidum* (Jakupovic et al., 1989).

Table 5.2 Structures and [1]H NMR shifts of 4-hydroxyguai-10(14)-en-12,8-olide isomers previously isolated and synthesised, compared to compounds **300** and **301**.

Proton	**301**	**300**	a 	b 	c 	c 	d 	d 
1	2.04	2.15	2.18	3.03	2.96-3.05[e]		2.05	2.13
2	1.77	1.77	1.80	1.75	1.84-1.60[e]	1.85-1.60[e]	1.70	1.78
		1.95		1.89		1.92-1.80[e]		1.92
3_1	1.77	1.77	1.73	1.73			1.75	1.82, 1.72
3_2	1.72							
5	1.35	1.59	1.60	2.13			1.67	1.60
6α	2.14	2.15	2.14	1.80	2.15-2.06[e]	2.12[e]	1.85	1.96
6β	1.35	1.11	1.12	1.08	1.07	1.02	1.70	1.17
7	2.35	1.77	1.75	1.71	1.92-1.80[e]	2.20-2.08[e]	2.50	2.23
8	4.45	4.26	4.26	3.79	3.78	3.97	4.57	4.40
9	2.63	3.17	3.17	3.04	3.03	3.04	2.96	3.17
9’	2.35	2.51	2.51	2.17	2.14	3.00	2.19	2.54
11	2.35	2.30	2.30	2.27	2.26	2.65	2.86	2.71
13	1.29	1.25	1.25	1.24	1.23	1.16	1.22	1.21
14	4.98	4.94	4.95	5.05	5.03	5.02	4.93	4.95
	4.91	5.04	5.04	5.09	5.07	5.08		5.02
15	1.18 s	1.20 s	1.20	1.16	1.14	1.13	1.20	1.20

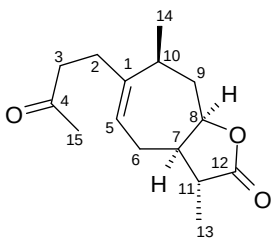
[a] Rustaiyan et al., 1987 (revision of Bohlmann et al., 1982); [b] Rustaiyan et al., 1987, [c] Blay et al., 2000, [d] Jakupovic et al., 1989 [e] Signals interchangeable within column, as peaks were not assigned in publication



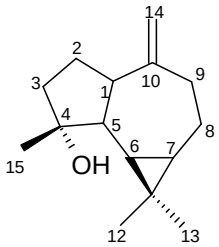
a **b**

Figure 5.10 (a) NOESY correlations observed for compound **301**, arrows in identical colours (other than black) indicate where overlap of signals occur and more than one possibility is likely for the observed NOESY correlation (b) AM1 calculated structure of **301**.

The fourth compound identified in the extract of *H. montanum* is the seco-guaianolide compound **304**, which was also isolated from *H. splendidum***.** It is interesting to note that where as both compounds **304** and **302** (with the 13-methyl in the β-position) were isolated from *H. splendidum*, only **304** (with the 13-methyl in the α-position) were isolated from *H. montanum*. The same phenomenon is observed for compounds **300** and **301**, which are the C-11 α-methyl isomers of similar guaianolides isolated from *H. splendidum*. Helisplendidilactone (**306**) also differs from 13'-epihelisplendidilactone (**307**) only in the orientation of the 13'-methyl group. Biosynthetically, it therefore seems as though *H. montanum* synthesises metabolites with the 13-methyl group *cis* relative to H-7, while *H. splendidum* is able to synthesise compounds with the 13-methyl in both the α and β positions.



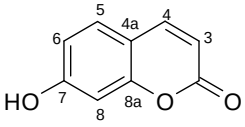
304



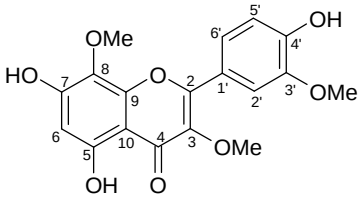
286

Except for flavonoid **381**, the other compounds isolated from *H. montanum* have all previously been isolated from *Helichrysum* species. Spathulenol (**286)** is recognisable by the broad singlets at δ_H 4.67 and δ_H 4.69 in the ^{1}H NMR spectrum (Plate 31), which are characteristic of the exomethylene group. The three singlets upfield (δ_H 1.04, 1.05 and 1.25) each integrating for three protons are assigned to the three methyl groups. The ^{13}C NMR spectrum (Plate 32) exhibited 15 major peaks, typical of a sesquiterpene, in a pattern related to that exhibited by compounds **300, 301** and **304**. The compound was further identified using the DEPT (Plate 34) and two-dimensional experiments (Plates 33, 35, 36), as well as by comparison with literature values (Tringali et al., 1995, Ulebelen et al., 1994). Spathulenol was previously isolated from *H. splendidum* (Bohlmann and Suwita, 1979), *H. dasyanthum*, *H. petiolare* (Jakupovic et al., 1989) and *H. heterolasium* (Bohlmann and Abraham, 1979a).

Umbelliferone (**348**) was identified by the coupling patterns of protons in the aromatic region, consisting of one set of ABX protons (δ_H 7.45, 6.79 and 6.71) (Plate 44) and two coupled protons typical of a coumarin (δ_H 6.18, 7.84). The data obtained from the ^{13}C (Plate 45), DEPT (Plate 47) and two-dimensional NMR spectra (Plates 46, 48, 49) supported this assignment. Coumarins are not very often isolated from *Helichrysum* species. Umbelliferone (**348**) was previously isolated from *H. swynnertonii* (Bohlmann et al., 1980), while other coumarins were isolated from *H. cymosum* (Jakupovic et al., 1989) and *H. acutatum* (Bohlmann and Abraham, 1979b).



348



381

For the polyoxygenated flavonoid **381** a ^{1}H NMR spectrum (Plate 50) characteristic for a flavonoid was obtained (NMR spectra, Plates 52-55). Due to the limited amount of material, the quality of the ^{13}C, HMQC and HSQC spectra were not very good. Therefore, these spectra played a limited role in the determination of this structure. However, data obtained from the NOESY spectrum enabled us to propose a structure. Based on ^{1}H NMR

chemical shift data, the compound seemed to be the same as a flavone isolated from *H. splendidum*, 3',4',5-trihydroxy-3,7,8-trimethoxyflavone, by Bohlmann and Suwita (1979).

However, for **381**, no NOESY correlation was observed between any of the methoxy groups, between a methoxy group and the aromatic singlet (δ_H 6.26), or a methoxy group and an *ortho*-coupled B-ring proton (δ_H 7.00, 7.75). In the NOESY spectrum, two of the methoxy groups (δ_H 3.83, 3.96), assigned to 3-OMe and 8-OMe correlates with both H-2' and H-6', whereas the third methoxy group (δ_H 3.92, assigned as 3'-OMe) correlates with only H-2'. These correlations unambiguously confirmed the assignment of the flavone as 4',5,7-trihydroxy-3,3',8-trimethoxyflavone (Seaman et al., 1972). It is possible that the flavonoid isolated by Bohlmann and Suwita (1979) from *H. splendidum* is the same compound, but the substitution pattern was assigned wrongly by these authors.

5.3 Conclusion

The phytochemistry of *Helichrysum montanum* was investigated for the first time and two new guaianolides were isolated. The occurrence of guaianolides is very rare in this genus; this type of compound has previously only been isolated from *H. dasyanthum* (Jakupovic et al., 1989) and *H. splendidum* (Bohlmann and Suwita, 1979; Jakupovic et al., 1989). The phytochemistry of *H. splendidum* and *H. montanum* is remarkably similar as initial TLC results indicated and supports their morphological classification in the same taxonomic group. However, interesting differences were observed in the stereochemistry of similar compounds isolated from these plants. It is also the configurational assignments of these compounds that poses a challenge to the chemist. Overlapping signals and errors in literature complicate stereochemical assignments and great care needs to be taken when elucidating these structures. The isolation of these remarkable compounds again illustrates the fascinating chemical diversity exhibited by South African *Helichrysum* species.

5.4 Experimental

5.4.1 General experimental procedures and instruments

^{1}H and ^{13}C NMR spectra of all compounds were recorded on a Varian Unity Inova 500 spectrometer with a 5 mm SW/Z-PFG probe (all spectra recorded at 25 °C, operating at

500 MHz for ^{1}H and 125 MHz for ^{13}C spectra). Spectra were measured in $CDCl_3$ for all compounds except for umbelliferone and the flavonoid, where spectra were obtained in CD_3OD. Structures were determined by analysis of 2D (HSQC, HMBC, COSY and NOESY) NMR spectra. Mass spectrometry data was collected on a time-of-flight Waters LCT Premier mass spectrometer using electrospray ionization in the positive or negative mode. Optical rotation was determined with a Perkin Elmer 241 polarimeter. Separations were done using open column chromatography and a chromatotron (Model 7924, Harrison Research). Silica gel ($60F_{254,}$ 40-63 μm, Merck) was used for column chromatography, while silica gel MERCK 7749 with gypsum binding agent was used for manufacturing of chromatotron plates. Thin-layer chromatography (TLC) was done on precoated Kieselgel 60 F_{254} plates (Merck or Machery-Nagel). Detection was done by UV (254 nm) followed by staining with an anisaldehyde solution, prepared as follows: Absolute ethanol (465 ml) was cooled in an ice bath. Acetic acid (5 ml), sulphuric acid (17 ml) and *p*-anisaldehyde (13 ml) was added and the solution mixed and stored in the fridge.

5.4.2 Plant material

Leaves and stems of *H. montanum* were collected in the Koueveld Mountains in 2004. These plants were identified by Ms. M. Welmann from the South African National Botanical Institute, Pretoria. Voucher specimens were deposited at the University of KwaZulu-Natal herbarium NU (*H. montanum*, F. van Heerden 1)

5.4.3 Extraction and isolation

Air dried and ground leaves and stems of *H. montanum* (216 g) were extracted with a mixture of chloroform:methanol (1:1) at room temperature for 48 hours to yield 8 g of extract. Initial clean up of 4 g of the extract was done by consecutively running a column with hexanes:dichloromethane (9:1), dichloromethane:methanol (6:1), dichloromethane: methanol (2:1) and methanol to obtain four initial fractions (F1-F4). Fraction F2 was further separated on a column using hexanes:ethyl acetate:methanol (7:5:1) resulting in seven fractions (F2.1–F2.7). Centrifugal chromatography (dichloromethane: hexanes:diethyl ether (4:2:1) of fraction 2.3 yielded compound **307** (6.5 mg).

A short column of a further 1 g of extract with sequentially using dichloromethane:methanol (99:1) and dichloromethane:methanol (95:5) resulted in 10 fractions (G1-G10). A chromatotron of fraction G3 and G4 with hexanes:ethyl acetate

(10:2) yielded compound **286** (spathulenol) as a mixture (12.5 mg). A chromatotron ran with dichloromethane: hexanes:diethyl ether (4:2:1) on fraction G7 followed by a hexanes:ethyl acetate (2:1) chromatotron of G7.2 (second of nine fractions) resulted in the isolation of compound **304** (1 mg), also isolated from helisplendidilactone. Compound **301** was isolated from fraction G8 after a chromatotron using hexanes: ethyl acetate (2:1) as eluent (fraction four of six fractions). Compound **348** (umbelliferone, 0.6 mg) was obtained by running consecutive chromatotrons on G9 with hexanes:ethyl acetate:methanol (9:4:1) and G9.4 (fourth of five fractions) with hexanes:ethyl acetate (4:1).

Compound **376** (the flavonoid, 3 mg) was obtained by consecutive chromatotrons of fraction G7.9 and G9.5 with hexanes:ethyl acetate (2:1) and (1:1). Column fractionation of another 2 g of extract with hexanes:ethyl acetate (2:1), yielded seven fractions (H1-H7). Chromatotrons on H5 with hexanes:ethyl acetate and (H5.3 of three fractions) with hexanes:ethyl acetate (5:3) resulted in the isolation of compound **300** (1 mg) (Figure 5.11).

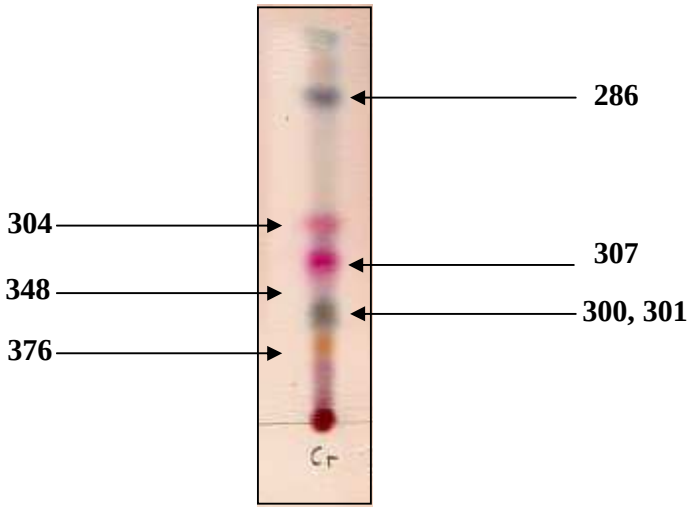


Figure 5.11 Thin-layer chromatography plate, indicating compounds isolated from *H. montanum*

5.4.4 Physical data of isolated compounds

Compound **307**, a red-brown gum, was identified as 13'-epihelisplendidilactone $[\alpha]_D$ = -11 (CH_3OH, c = 0.08); ^1H NMR (500 MHz, $CDCl_3$): δ_H 1.08 (1H, *br dd*, $J_{3'a,3'b}$ 8.5 Hz, $J_{3'a,2'}$ = $J_{3'a,13'}$ = 2.1 Hz, Ha-3'), 1.11 (3H, *d*, $J_{14,10}$ = 6.8 Hz, H-14), 1.19 (1H, *m*, Ha-6), 1.23 (3H, *s*, H-15'), 1.257 (3H, *d*, $J_{10',14'}$ = 7.0 Hz, H-14'), 1.260 (3H, *d*, $J_{13',11'}$ = 7.0 Hz, H-13'),

1.49 (1H, *ddd*, $J_{9'\beta,8'=9'\beta,9'\alpha=9'\beta,10'}$ = 11.5 Hz, H_β-9'), 1.56 (1H, *dd*, $J_{13\alpha,13\beta}$ = 12.2 Hz, $J_{13\alpha,2'}$ = 2.6 Hz, H_α-13), 1.65 (2H, *m*, H_α-6', H-7'), 1.78 (1H, *ddd*, $J_{9\alpha,8=9\alpha,9\beta=9\alpha,10}$ = 12.4 Hz, H_α-9), 1.98 (1H, *ddd*, $J_{9\beta,9\alpha}$ = 12.1 Hz, $J_{9\beta,10}$ = 6.7 Hz, $J_{9\beta,8}$ = 2.3 Hz, H_β-9), 1.98 (1H, *dd*, $J_{13\beta13\alpha}$ = 12.1 Hz, $J_{13\beta2'}$ = 3.8 Hz, H_β-13), 2.13 (3H, *s*, H-15), 2.20 (2H, *br t*, $J_{2,3}$ = 7.9 Hz, H-2), 2.30 (6H, *m*, Hb-6, H-10, Hb-3', H_α-9', H-10', H-11'), 2.44 (2H, *m*, Ha-3, H-7), 2.55 (1H, *ddd*, $J_{2,3}$ = 6.7 and 6.4 Hz**,** $J_{3a,3b}$ = 16.5 Hz, Hb-3), 2.64 (1H, *br d*, $J_{6\alpha6\beta=J_{6\alpha7'}}$ = 13.5 Hz, H_β-6'), 2.94 (1H, *br s*, H-2'), 3.93 (1H, *ddd*, $J_{8'7'=8'9'\beta}$ = 11.0 Hz, $J_{8',9'\alpha}$ = 2.2 Hz, H-8'), 4.48 (1H, *ddd*, $J_{8,9\alpha}$ =12.1 Hz, $J_{8,7}$ = 8.9 Hz, $J_{8,9\beta}$ = 2.6 Hz, H-8), 5.23 (1H, *dd*, $J_{5,6}$ = 8.9 Hz, $J_{5,6}$ = 5.2 Hz, H-5); ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 12.8 (*q*, C-13'), 13.5 (*q*, C-15'), 20.7 (*q*, C-14), 21.3 (*q*, C-14'), 25.3 (*t*, C-6), 28.5 (*t*, C-6'), 29.5 (*d*, C-10'), 29.8 (*q*, C-15), 30.4 (*t*, C-2), 35.6 (*d*, C-10), 36.1 (*t*, C-13), 37.1 (*t*, C-9), 40.2 (*t*, C-9'), 42.00 (*d*, C-11'), 42.02 (*d*, C-7), 42.4 (*t*, C-3), 43.3 (*d*, C-2'), 50.6 (*t*, C-3'), 50.7 (*d*, C-7'), 54.3 (*s*, C-4'), 62.9 (*s*, C-11), 78.6 (*d*, C-8), 84.9 (*d*, C-8'), 119.9 (*d*, C-5), 140.3 (*s*, C-5'), 144.4 (*s*, C-1), 150.5 (*s*, C-1'), 178.3 (*s*, C-12'), 181.6 (*s*, C-12), 207.9 (*s*, C-4), HRESIMS (negative ionization mode), *m/z* 479.2777 $[M-H]^-$ (calc. for $C_{30}H_{39}O_5$ 479.2797).

Compound **286**, a white amorphous solid was identified as a mixture containing spathulenol as the main component (Tringali et al., 1995, Ulubelen et al., 1994), ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 0.47 (1H, *dd*, *J* = 11.3 Hz, 9.5 Hz, H-6), 0.70 (1H, *m*, H-7), 1.04 (3H, *s*, H-13), 1.05 (3H, *s*, H-12), 1.25 (3H, *s*, H-15), 1.30 (1H, *m*, H-5), 1.58 (2H, *m*, H-2a, H-3a), 1.77 (1H, *m*, H-3b), 1.89 (1H, *m*, H-2b), 2.01 (2H, *m*, H-9a, H-8), 2.20 (1H, *m*, H-1), 2.42 (1H, *dd*, *J* = 5.9 Hz, 12.9 Hz, H-9b), 4.67 (1H, *br s*, H-14), 4.69 (1H, *br s*, H-14); ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 16.3 (*q*, C-13), 20.3 (*s*, C-11), 24.8 (*t*, C-8), 26.1 (*q*, C-15), 26.7 (*t*, C-2), 27.5 (*d*, C-7), 28.6 (*q*, C-12), 29.9 (*d*, C-6), 38.8 (*t*, C-9), 41.7 (*t*, C-3), 53.4 (*d*, C-1*), 54.3 (*d*, C-5*), 80.9 (*s*, C-4), 106.2 (*t*, C-14), 153.4 (*s*, C-10); HRESIMS (neagtive ionization mode), *m/z* 219.1750 $[M-H]^-$ (calc. for $C_{15}H_{23}O$219.1749). *Signals interchangeable.

Compound **301**, a white solid, was identified as 1β,5α,7α,8α,11β*H*-4α-hydroxyguai-10(14)-en-8,12α-olide, $[\alpha]_D$ = +35 (CH_3OH, c = 0.13); ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 1.18 (3H, *s*, H-15), 1.29 (3H, *d*, $J_{13,11}$= 6.8 Hz, H-13), 1.35 (2H, *m*, H-5, H_β-6), 1.77 (4H, *m*, H-2, H-2, H-3, H-3), 2.04 (1H, *br s*, H-1), 2.14 (1H, *br dd*, $J_{6\alpha,6\beta}$ = 12.6 Hz, $J_{6\alpha,5}$ = $J_{6\alpha,7}$ = 5.3 Hz, H_α-6), 2.35 (3H, *m*, H-7, H_β-9, H-11), 2.63 (1H, *dd*, $J_{9\alpha,9\beta}$ = 12.7 Hz, 3.1 Hz,

H_α- 9), 4.45 (1H, *ddd*, $J_{8,9\alpha}$ = 3.0 Hz, $J_{8,7}$ = 8.2 Hz, $J_{8,9\beta}$ =12.1 Hz, H-8), 4.91 (1H, *s*, H-14), 4.98 (1H, *s*, H-14); ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 14.6 (*q*, C-13), 24.2 (*q*, C-15), 24.4 (*t*, C-2), 29.3 (*t*, C-6), 39.4 (*t*, C-9), 39.5 (*t*, C-3), 41.1 (*d*, C-11), 45.2 (*d*, C-7), 50.9 (*d*, C-1), 54.8 (*d*, C-5), 80.1 (*s*, C-4), 81.7 (*d*, C-8), 112.0 (*t*, C-14), 145.9 (*s*, C-10), 178.9 (*s*, C-12); HRESIMS (negative ionization mode), *m/z* 249.1480 $[M-H]^-$ (calc. for $C_{15}H_{21}O_3$ 249.1491).

Compound **304**, a red brown gum, was identified as 11β,13-dihydroxanthalongin (Bohlmann and Suwita, 1979; Marcinek-Hüpen-Bestendonk et al., 1990), assignment, see Chapter 3.

Compound **348**, a white amorphous solid was identified as umbelliferone (Nath et al., 2005), ^{1}H NMR (500 MHz, CD_3OD, $CDCl_3$): δ_H 6.18 (1H, *d*, $J_{3,4}$ = 9.5 Hz, H-3), 6.71 (1H, *d*, $J_{8,6}$ = 2.2 Hz, H-8), 6.79 (1H, *dd*, $J_{6,5}$ = 8.5 Hz, $J_{6,8}$ = 2.3 Hz, H-6), 7.45 (1H, *d*, $J_{5,6}$ = 8.5 Hz, H-5), 7.84 (1H, *d*, $J_{4,3}$ = 9.3 Hz, H-4); ^{13}C NMR (125 MHz, CD_3OD, $CDCl_3$): δ_C 103.4 (*d*, C-8), 112.3 (*d*, C-3), 113.1 (*s*, 4a), 114.5 (*d*, C-6), 130.6 (*d*, C-5), 146.0 (*d*, C-4), 156.5* (*s*, 8a), 162.5* (*s*, C-7), 163.5* (*s*, C-2); HRESIMS (positive ionization mode), *m/z* 163.0398 $[M+H]^+$ (calc. for $C_9H_7O_3$ 163.0395).*Estimated from HMQC.

Compound **381**, a yellow amorphous solid was identified as 4',5,7-trihydroxy-3,3',8-trimethoxyflavone (Wang et al., 1989), ^{1}H NMR (500 MHz, CD_3OD: δ_H 3.83 (3H, *s*, 3-OMe), 3.92 (3H, *s*, 3'-OMe), 3.96 (3H, *s*, 8-OMe), 4.58 (1H, *s*, OH), 6.26 (1H, *s*, H-6), 7.00 (1H, *d*, $J_{5',6'}$ = 8.5 Hz, H-5'), 7.75 (1H, *dd*, $J_{6',2'}$ = 2.1 Hz, $J_{6',5'}$ = 8.5 Hz, H-6'), 7.80 (1H, *d*, $J_{2',6'}$ = 1.9 Hz, H-2'); ^{13}C NMR (125 MHz, CD_3OD): δ_C 55.3 (OMe, C-3'), 59.4 (OMe, C-3), 60.8 (OMe, C-8), 98.9 (*d*, C-6)*, 102.7 (*s*, C-10), 111.5 (*d*, C-2'), 115.8/115.4 (*d*, C-5'), 121.8 (*s*, C-1'), 122.6 (*d*, C-6'), 128.3 (s, C-8), 138.5 (*s*, C-3), 147.9 (*s*, C-4')*, 149.1 (*s*, C-3'), 150.0 (*s*, C-9)*, 156.3 (C-2)*, 156.9 (*s*, C-5)*, 157.5 (*s*, C-7)*, 179.0 (C, C-4); HRESIMS (negative ionization mode), *m/z* 359.0754 $[M-H]^-$ (calc. for $C_{18}H_{15}O_8$ 359.0767). Ambiguous assignment due to poor quality of spectra.

Compound **300**, a white solid, was identified as 1β,5α,7α,8β,11β*H*-4α-hydroxyguai-10(14)-en-8,12α-olide (Bohlmann et al., 1982; Rustaiyan et al., 1987); $[\alpha]_D$ = +47 [lit. Bohlmann et al., 1982, +28 ^{o} ($CHCl_3$, c = 0.13)]; ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 1.11

(1H, *dd*, $J_{6\beta,6\alpha} = J_{6\beta,5} = J_{6\beta,7}$ = 12.4 Hz, H_β-6), 1.20 (3H, s, H-15), 1.24 (3H, *d*, $J_{13,11}$= 7.2 Hz, H-13), 1.59 (1H, *m*, H-5), 1.77 (4H, *m*, Ha-3, H-7, Ha-2, Hb-3), 1.95 (1H, *m*, H-2), 2.15 (2H, *m*, H_α-6, H-1), 2.30 (1H, *ddd*, $J_{11,13}$ = 7.0 Hz, $J_{11,7}$ = 12.0 Hz, H-11), 2.51 (1H, *dd*, $J_{9\alpha,8}$ = 10.4 Hz, $J_{9\alpha,9\beta}$ = 15.8 Hz, H_α-9), 3.17 (1H, *dd*, $J_{9\beta,9\alpha}$ = 15.7 Hz, $J_{9\beta,8}$ = 5.6 Hz, H_β-9), 4.26 (1H, *td*, $J_{8,7} = J_{8,9\alpha}$ = 10.3 Hz, $J_{8,9\beta,}$ = 5.6 Hz, H-8), 4.94 (1H, *br s*, H-14), 5.04 (1H, *br s*, H-14); ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 13.0 (*q*, C- 13), 24.0 (*q*, C-15), 29.7 (*t*, C-2), 31.1 (*t*, C-6), 40.8 (*t*, C-9), 41.2 (*t*, C-3), 42.3 (*d*, C-11), 47.6 (*d*, C-1), 50.3 (*d*, C-7), 58.3 (*d*, C-5), 80.5 (*s*, C-4), 82.0 (*d*, C-8), 111.3 (*t*, C-14), 146.7 (*s*, C-10), 178.4 (*s*, C-12); HRESIMS (negative ionization mode), *m/z* 249.1500 $[M-H]^-$ (calc. for $C_{15}H_{21}O_3$ 249.1491).

5.4.5 Molecular modelling

AM1 geometry optimisations were performed with Gaussian^R 03W, version 6.1 (Gaussian Inc. Carnegie Office Park Building 6, Pittsburgh, PA 15106, USA, Copyright 1995-2004), while overlays were done in Hyperchem^R, release 6.03 for Windows (Hypercube Inc., copyright 2000).

5.5 References

Blay, G., Bargues, V., Cardona, L., Collado, A.M., García, B., Muñoz, M.C., Pedro, J.R., 2000. Stereoselective synthesis of 4α-hydroxy-8,12-guaianolides from santonin. Journal of Organic Chemistry 65, 2138-2144.

Bohlmann, F., Abraham, W-F., 1979a. Neue Diterpene und weitere Inhaltsstoffe aus *Helichrysum calliconum* und *Helichrysum heterolasium*. Phytochemistry 18, 889-891.

Bohlmann, F., Abraham, W-R., 1979b. Neue Diterpene aus *Helichrysum acutatum*. Phytochemistry 18, 1754-1756.

Bohlmann, F., Czerson, H., Schőneweiß, S., 1977. Neue inhaltsstoffe aus *Inula viscosa* Ait. Chemische Berichte 110, 1330-1334.

Bohlmann, F., Suwita, A., 1979. Ein neues Guajanolid und ein Secoguajanolid aus *Helichrysum splendidum*. Phytochemistry 18, 885-886.

Bohlmann, F., Zdero, C., Abraham, W-R., Suwita, A., Grenz, M., 1980. Neue Diterpene und neue Dihydrochalcon-derivate sowie weitere Inhaltsstoffe aus *Helichrysum* arten. Phytochemistry 19, 873-879.

Bohlmann, F., Zdero, C., Ahmed, M., 1982. New sesquiterpene lactones, geranyllinalol derivatives and other constituents from *Geigera* species. Phytochemistry 21, 1679-1691.

Hilliard, O.M., 1983. Asteraceae. In: O.A. Leistner Flora of Southern Africa., Vol. 33, part 7 (Inuleae), Botanical Research Institute of South Africa, Pretoria, pp. 7,2:61-7,2:317.

Jakupovic, J., Schuster, A., Bohlmann, F., King, R.M., Lander, N.S., 1988. Sesquiterpene lactones from *Gnephosis* species. Phytochemistry 27, 3181-3185.

Jakupovic, J., Zdero, C., Grenz, M., Tsichritzis, F., Lehmann, L., Hashemi-Nejad, S.M., Bohlmann, F., 1989. Twenty-one acylphloroglucinol and further constituents from South African *Helichrysum* species. Phytochemistry 28, 1119-1131.

Nath, M., Jairath, R., Eng, G., Song, X., Kumar, A., 2005. New diorganotin(IV) derivatives of 7-hydroxycoumarin (umbelliferone) and their adducts with 1,10-phenanthroline. Spectrochimica Acta Part A 61, 3155–3161.

Pooley, E., 1998. A Field Guide to the Wild Flowers. KwaZulu-Natal and the Eastern Region, 1st ed. Natal Flora Publications Trust, Durban, pp. 82, 212-215, 310-317, 442-443.

Rustaiyan, A., Jakupovic, J., Chau-Thi, T.V., Bohlmann, F., Sadjadi, A., 1987. Further sesquiterpene lactones from the genus *Dittrichia*. Phytochemistry 26, 2603-2606.

Seaman, F., Rodriguez, E., Carman, N.J., Mabry, T.J., 1972. A new flavonoid from Ambrosia dumosa. Phytochemistry 11, 2626-2627.

Tringali, C., Piatelli, M., Spatafora, C., 1995. Sesquiterpenes and geranylgeranylglycerol from the brown algae *Taonia lacheana* and *Taonia atomaria* f. *ciliata*: their chemotaxonomic significance. Phytochemistry 40, 827-831.

Ulubelen, A., Topcu, G., Eriş, C., Sönmez, U., Kartal, M., Kurucu, S., Bozok-Johansson, C., 1994. Terpenoids from *Salvia sclarea*. Phytochemistry 36, 971-974.

Wang, Y., Hamburger, M., Gueho, H., Hostettmann, K., 1989. Antimicrobial flavonoids from *Psiadia trinervia* and their methylated and acetylated derivatives. Phytochemistry 28, 2323-2327.

CHAPTER 6

The antimicrobial activity and phytochemistry of *Helichrysum excisum*

6.1 Introduction

Helichrysum excisum (Thunb.) Less. (Fig 6.1) is a highly aromatic, densely twiggy dwarf shrub with yellow flowers endemic to the Western Cape Province of South Africa (Fig. 6.1, Fig. 6.2). Based on morphological characters, it is placed in Group 12 (Hilliard, 1983) (Paragraph 2.1). This morphologic group contains sixteen species. The phytochemistry of two of these species (*H. caespititium* and *H. asperum* var. *albidulum*) has been investigated. *H. caespititium* is used in traditional medicine to treat colds, headaches, gonorrhoea and nausea occurs widely in the central parts of South Africa (Hutchings and Van Staden, 1994; Watt and Breyer-Brandwijk, 1962; Hilliard, 1983).

a

b

Figure 6.1 a) *Helichrysum excisum,* plant habitat b) *H. excisum,* flower (Photos: J. Vlok)

Acylated phloroglucinols were isolated from both *H. caespititium* (Dekker et al., 1983, Mathekga et al., 2000) and *H. asperum* var. *albidulum*, a plant found in the Western Cape (Jakupovic et al., 1989, Hilliard, 1983). Various phloroglucinols exhibit biological activity

such as antibacterial (Gibbons, 2004) and antiviral (Appendino et al., 2007; Nakane et al., 1991) activity. In a previous study, Lourens et al. (2004) reported on the antimicrobial activity of crude extracts of *H. excisum* against Gram-positive organisms such as *Staphylococcus aureus* and *Bacillus cereus*. To the best of our knowledge, there is no reference in ethnobotanical literature relating to the use of *H. excisum*. However, the interesting chemistry and medicinal use of morphologically related species served as motivation for the investigation of this species. Furthermore, apart from the composition of the steam-distilled oil (Lourens et al., 2004), no phytochemical studies have been reported on *H. excisum.*

The aims of this chapter are:

- To investigate the phytochemistry of *H. excisum* and to determine which compounds are responsible for the antibacterial activity of the extract.
- To establish whether the chemistry of *H. excisum* is similar to the other species investigated in the same morphologic group (whether any chemotaxonomic conclusions can be drawn).

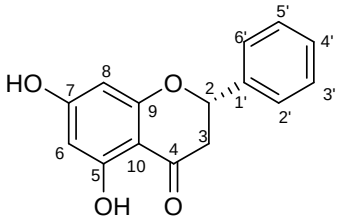
6.2 Results and discussion

From the aerial parts of *H. excisum*, five flavonoids, identified as pinocembrin (**1**), gnaphaliin (**67**), lepidissipyrone (**19**), 5-hydroxy-7,8-dimethoxyflavone (**72**) and isoscutellarein 7-*O*-β-glucoside (**80**), were isolated. Four of these compounds have an unsubstituted B-ring, a trend often observed in the flavonoids isolated from *Helichrysum* species (Chapter 2).

The molecular formula of **1**, $C_{15}H_{12}O_4$, was confirmed by high-resolution mass spectrometry. In the ^{13}C NMR spectrum (Plate 64), the presence of 15 carbon atoms, of which 12 are aromatic, two aliphatic, and one carbonyl are observed. This suggests that the compound might be a flavonoid.

In the ^{1}H NMR spectrum (Plate 63), the chemical shifts and coupling constants of two aromatic protons δ_H 5.90 (1H, *d*, *J* = 2 Hz) and δ_H 5.94 (1H, *d*, *J* = 2 Hz) are characteristic of two *meta*-coupled protons of the A ring of a 5,7-dioxygenated flavonoid. The remaining

five aromatic protons were assigned to a monosubstituted aromatic ring. In the aliphatic region, two non-equivalent methylene protons at δ_H 2.78 (1H, *dd*, *J* = 17 Hz, *J* = 3 Hz) and δ_H 3.09 (1H, *dd*, *J* = 17 Hz, *J* = 13 Hz) which are coupled to a doublet of doublets at δ_H 5.46 (1H, *dd*, *J* = 13 Hz, *J* = 3 Hz) suggests a flavanone structure. From the evidence mentioned, the structure was assigned as pinocembrin (**1**). The assignment was confirmed by analysis of two-dimensional NMR data (Plates 65-68) and comparison of the NMR data with literature values (Fukui et al., 1988, Jung and McLaughlin, 1990).



Pinocembrin (**1**)

The compound showed laevorotatory rotation like the majority of natural flavanones and is assigned a 2*S* configuration (Bohlmann and Zdero, 1983; Slade et al., 2005), with the C-2 phenyl substituent in the α-equatorial position. Taking the configuration of the stereocentre at C-2 and the coupling constants of the two H-3 geminal protons into account, the proton at δ_H 2.78 could be assigned as H_β-3 and the proton at δ_H 3.09 as H_α-3. It is interesting to note that in the HMQC (Plate 68), H_α-3 shows correlations with C-2, C-4 and C-1', while H_β-3 only shows a correlation with C-4 (Fig. 6.2).

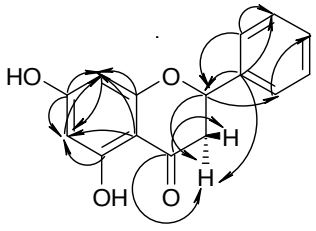
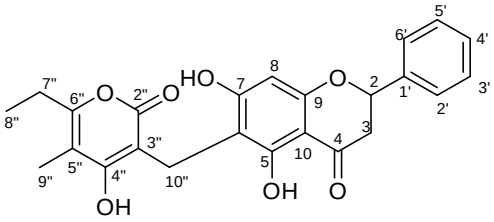


Figure 6.2 Correlations seen in HMQC of pinocembrin (indicated by arrows from ^{13}C to ^{1}H).

Pinocembrin (**1**) and its derivatives (often prenyl derivatives) occur frequently in the South African *Helichrysum* species, especially in plants from Hilliard's morphological Groups 8, 9, 21, 23 and 27 (Bohlmann et al., 1980; Bohlmann et al., 1979; Bohlmann and Ates, 1984;

Bohlmann and Misra, 1984; Jakupovic et al., 1986; Bohlmann and Abraham, 1979c,d; Bohlmann and Zdero, 1983). This compound has been isolated from *H. cymosum* (Jakupovic et al., 1989), *H. oreophilum, H. zeyheri* (Jakupovic et al., 1986), *H. oxyphyllum* (Bohlmann et al., 1980), *H. callicomum* (Bohlmann and Abraham, 1979a), *H. tenuifolium* (Bohlmann and Abraham, 1979b) and from the roots of *H. acutatum* (Bohlmann and Abraham, 1979c). High concentrations are also found in the Australian species *H. stirlingii* (Jakupovic et al., 1987) and European species such as *H. italicum* (Sala et al., 2003).

Lepidissipyrone (**19**) precipated as a white solid from chloroform. Its structure was based on NMR data (Plates 69-74, Table 6.1), combined with high-resolution mass spectrometry which indicated a molecular formula of $C_{24}H_{24}O_7$. As for pinocembrin (**1**), the set of doublet of doublet signals at δ_H 2.84 (1H, *dd*, *J* = 17 Hz, *J* = 3 Hz, H-3) and δ_H 3.27 (1H, *dd*, *J* = 17 Hz, *J* = 13 Hz, H-3), coupling to the doublet of doublets at δ_H 5.53 (1H, *dd*, *J* = 13 Hz, *J* = 3 Hz, H-2), in the ^{1}H NMR spectrum (Plate 69) indicated the presence of a flavanone moiety. Integration of the signal at δ_H 7.51 indicated that there were five aromatic protons, suggesting an unsubstituted aromatic B ring. The shift of the proton at δ_H 6.21 is in the region characteristic for protons on the A ring of flavonoids.



Lepidissipyrone (**19**)

The presence of a 6-ethyl-4-hydroxy-5-methylpyrone moiety was deduced from the appearance of the triplet at δ_H 1.18 (3H, *t*, *J* = 8 Hz) coupled to the quartet at δ_H 2.54 (2H, *q*, *J* = 8 Hz), the methyl singlet at δ_H 1.86 in the ^{1}H NMR spectrum (Plate 69) and the presence of a second carbonyl signal at δ_C 169.0 in the ^{13}C NMR spectrum. This substitution pattern is commonly found in pyrones isolated from *Helichrysum* species (Bohlmann et al., 1984; Bohlmann and Zdero, 1980; Hänsel et al., 1980; Jakupovic et al., 1986). The ^{13}C (Plate 70) and DEPT NMR (Plate 71) spectra showed the presence of 24 carbons (the signals at δ_C 127.0 and δ_C 129.3 each representing two carbons), which included two CH_3, three CH_2, five CH, and 12 quaternary carbons. Two-dimensional NMR

experiments (COSY – Plate 71, HSQC – Plate 73, HMQC - Plate 74) were employed in further analysis of the structure (Table 6.1).

Table 6.1 ^{1}H and ^{13}C NMR ($CDCl_3$) of lepidissipyrone (**19**).

Position	δ_H [a]	δ_C [b]	Correlation (δ_C) observed in HMQC	^{13}C NMR assignment of HMQC correlated carbon
2	5.53 (dd, 13.3, 2.8)	81.5 (CH)	194.8, 136.5, 127.0	4, 1', 2' and 6'
3a	2.84 (dd, 17.2, 2.8)	42.7 (CH_2)	194.8, 102.3	4, 10
3b	3.27 (dd, 17.2, 13.3)	42.7 (CH_2)	194.8, 136.5, 81.5	4, 1', 2
4		194.8 (C)		
5		157.7 (C)		
6		104.9 (C)		
7		163.2 (C)		
8	6.21 (s)	99.7 (CH)	165.8, 163.2, 104.9, 102.3	9, 7, 6, 10
9		165.8 (C)		
10		102.3 (C)		
1'		136.5 (C)		
2',6'	7.51 (5H m)	127.0 (CH)	136.5, 129.3, 130.1, 81.5	1', 3' and 5', 4', 2
3'5'	7.51 (5H m)	129.3 (CH)		
4'	7.51 (5H m)	130.1 (CH)		
2"		169.0 (C)		
3"		101.2 (C)		
4"		166.9 (C)		
5"		107.6 (C)		
6"		161.9 (C)		
7"	2.54 (2H q, 7.6)	24.3 (CH_2)	161.9, 107.6, 11.5	6", 5", 8"
8"	1.18 (3H t, 7.6)	11.5 (CH_3)	161.9, 24.3	6", 7"
9"	1.86 (3H s)	9.2 (CH_3)	107.6, 161.9, 166.9	5", 6", 4"
10"	3.57 (2H s)	17.4 (CH_2)	169.0, 166.9, 157.7, 104.9, 101.2	2", 4", 5, 6, 3"
4"-OH	8.09 (s)		166.9, 107.6, 101.2	4", 5", 3"
5-OH	11.89 (s)		102.3	10
7-OH	10.61 (s)		163.2	**7**

[a] Integral (if not 1H), multiplicity and coupling constant(s) (in Hz) indicated in parentheses.

[b] Multiplicity determined by DEPT indicated in parentheses.

HMQC (Plate 74) correlations indicated that the methylene group connecting the pyrone and flavanone moiety was attached to C-6 of the flavanone A ring and C-3" of the pyrone. ^{2}J correlations were observed between H-10" δ_H 3.57 and C-6 δ_C 104.9 and C-3" δ_C 101.2, while ^{3}J correlations were observed between H-10" δ_H 3.57 and carbons resonating at δ_C 169.0, 166.9 and 157.7, assigned to carbons C-2", C-4" and C-5, respectively.

The aromatic proton at δ_H 6.21 showed HMQC correlations to δ_C 165.8 (C-9), 163.2 (C-7), 104.9 (C-6), 102.3 (C-10) confirming its assignment as H-8 of the aromatic A ring. Considering the HMQC correlations, the singlet at δ_H 8.09 could be assigned to the 4"-OH, while the singlet at δ_H 11.89 most likely belongs to the 5-OH and the singlet at δ_H 10.61 to the 7-OH. It may be of interest to note that as for pinocembrin, a HMQC correlation was only observed between the more downfield H-3 (δ_H 3.27) and C-1' as well as C-2, while these correlations were absent for the upfield H-3 (δ_H 2.84). However, this compound decomposed in the chloroform solution before the optical rotation could be obtained. This compound was previously only isolated from *H. lepidissimum* and the proton data is in agreement with that reported by Jakupovic et. al (1989).

Similar pyrones were isolated from other plants in this genus, for example *H. mixtum*, *H. cephaloideum* (Jakupovic et al., 1986), *H. auriceps* (Bohlmann and Zdero, 1980), *H. cerastioides* (Bohlmann et al., 1984) and *H. odoratissimum* (Hänsel et al., 1980), although the pyrone side chain is usually combined with a phloroglucinol and not with a flavanone moiety. The antimicrobial activity of lepidissipyrone (**19**) is summarised in Table 6.2.

Table 6.2 Mean MIC (μg/ml) values for the chloroform:methanol (1:1) extract of *Helichrysum excisum*, lepidissipyrone (**19**) and isoscutellarein 7-*O*-glucoside (**80**).

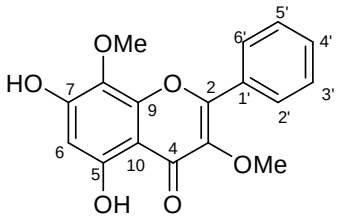
	S. aureus[a] ATCC 12600	*S. epidermidis*[a] ATCC 2223	*B. cereus*[a] ATCC 11778	*C. neoformans*[a] ATCC 90112	*K. pneumoniae*[a] NCTC 9633	*P. aeruginosa*[a] ATCC 9027
Crude extract	200	100	33	2000	4000	NS
Lepidissipyrone	156 (370 μM)	78 (185 μM)	78 (185 μM)	156 (370 μM)	312 (739 μM)	156 (370 μM)
Isoscutellarein 7-*O*-glucoside	NS[b]	ND[c]	NS	ND	ND	ND
Positive Control[d] (μg/ml)	0.39 (1.18 μM)	0.78 (2.35 μM)	2.5 (7.54 μM)	0.78 (0.84 μM)	2.5 (7.54 μM)	2.5 (7.54 μM)
DMSO	8000	8000	8000	4000	8000	4000

[a] MIC values at least determined in duplicate

[b] Not susceptible, activity observed equivalent to DMSO control

[c] Not determined

[d] Ciprofloxacin was used as positive control for bacteria and amphotericin B as positive control for the yeast.



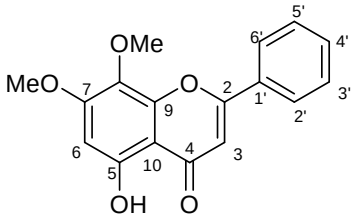
Gnaphaliin (**67**)

The structure of gnaphaliin (5,7-dihydroxy-3,8-dimethoxyflavone) (**67**) could be deduced from the ^{1}H and ^{13}C NMR spectra (Plates 75, 76) and is characteristic of a flavone. The two singlets at δ_H 3.88 and δ_H 4.00 indicated the presence of two methoxy groups, while the splitting pattern and the integration of the signals at δ_H 7.54 and δ_H 8.10 showed the presence of an unsubstitued B-ring. The chemical shift of the aromatic proton at δ_H 6.44 was comparable to those of similar flavones (Tomás-Lorente et al., 1991) and its HMQC correlations (Plate 79) with C-5/C-7, C-8 and C-10 used to determine its position. Since only one aromatic proton was present on the A and C rings, it was proposed that two hydroxy and two methoxy substituents were present on these rings.

The singlet at δ_H 12.4 is typical for a hydrogen-bonded hydroxy, implying the presence of a C-5 hydroxy group. The NOESY correlations (Plate 80) observed between both the methoxy groups and H-2'and H-6', confirmed their relative positions as only substitution on C-3 and C-8 allowed both methoxy groups to be in close proximity to both the H-2' and 6' protons. The ^{13}C (Plate 76) and DEPT NMR spectra showed 17 carbons, two methyl groups, six CH, and nine quaternary carbons. The assignment of our ^{13}C NMR spectrum of gnaphaliin differs slightly from that of Tomás-Lorente (1991), with the signal at δ_C 62.0 being assigned as the 8-methoxy group and the signal at δ_C 60.4 assigned to the 3-methoxy group, based on HSQC (Plate 78) and HMQC correlations (Plate 79). Gnaphaliin (**67**), was previously isolated from *H. cymosum* and *H. argyrophyllum* (Jakupovic et al., 1989) and European species such as *H. picardii* (Tomás-Lorente et al., 1991) and *H. italicum* (Sala et al., 2003).

The ^{1}H (Plate 81) and ^{13}C NMR (Plate 82) spectra of 5-hydroxy-7,8-dimethoxyflavone (**72**) were similar to those obtained for gnaphaliin (**67**). For this compound, the ^{1}H NMR signals assigned to the two methoxy groups were closer to each other (δ_H 3.95 and 3.96),

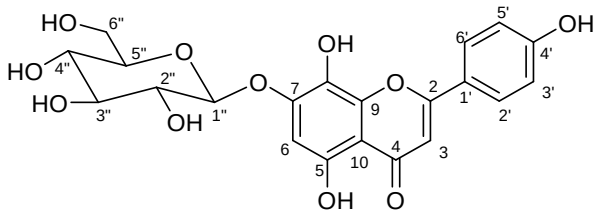
while an extra proton was present in the aromatic region (δ_H 6.68) and an extra CH signal present in the DEPT spectrum (Plate 84). This suggested that **72** contains one hydroxy substituent less than gnaphaliin (**67**). This was confirmed by the high-resolution mass data (*m/z* 297.0757 $[M-H]^-$), which indicated a molecular formula of $C_{17}H_{14}O_5$. The singlet at δ_H 12.6 was again indicative of a hydroxy substitution on C-5, while the presence of an unsubstituted B ring could be derived from the integration and splitting pattern of the aromatic protons at δ_H 6.68 and 7.95. Important HMQC correlations (Plate 86) observed were those between the proton at δ_H 6.45 (H-6) and the carbons at δ_C 105.0 (C-10), 128.9 (C-8) and 158.7 (C-7) and the proton at δ_H 6.68 (H-3) and the carbons at δ_C 105.0 (C-10), 131.4 (C-1'), 163.9 (C-2), confirming the positions of the unsubstituted aromatic protons as H-6 and H-3. The spectroscopic data obtained are identical to that reported by Kuroyanagi et al. (1987) for 5-hydroxy-7,8-dimethoxyflavone except for the assignments of C-5 and C-7. The HMQC correlation observed between the 7 or 8 methoxy signal at δ_H 3.96 and the carbon at δ_C 158.7 (thus C-7) resulted in the revised assignment. This change is in agreement with the assignments of C-5 and C-7 as reported by Horie et al. (1998) for related flavones. An extract of the South African *H. petiolare* as well as the Kenyan *H. schimperi* previously yielded this compound (Jakupovic et al., 1989, Jakupovic et al., 1990).



7,8-Dimethoxy-5-hydroxyflavone (**72**)

The ^1H (Plate 88) and ^{13}C NMR (Plate 89) spectral data of compound **80** again suggested the presence of a flavone. The substitution pattern in ^1H NMR spectrum for compound **80** differed from the other isolated flavonoids in certain aspects. The presence of two *ortho*-coupled doublets (J = 8.8 Hz) at δ_H 6.98 and δ_H 8.01 assigned to H-3',5' and H-2',6' respectively, indicated a *para*-substituted B ring. Several signals in the aliphatic region of the ^1H NMR indicated the presence of a sugar moiety. The coupling constant of the proton at C-1" was calculated as 7.3 Hz which agrees with the value obtained for glucose by Lenherr et al. (1984). Further evidence for the presence of a glucose group and its localization at C-7 was obtained by comparing and correlating ^{13}C NMR data of our

compound with other 7-glycosylated 8-hydroxyapigenins (Albach et al., 2003; Lenherr et al., 1984; Nakanishi et al., 2004). The existence of a β-linkage was confirmed by the anomeric proton doublet at δ_H 4.97 with a large coupling constant (J = 7.3 Hz). HMQC correlations (Plate 93) confirmed the assignments of the singlet at δ_H 6.67 as H-6 (correlations with δ_C 105.2 (C-10), 127.0 (C-8), 151.2 (C-5), 152.4 (C-7)) and the singlet at δ_H 6.85 as H-3 (correlations with carbons resonating at δ_C 105.2 (C-10), 101.3 (C-1'), 164.0 (C-2) and 182.4 (C-4). All other NMR spectroscopic data as well as the high-resolution mass results (*m/z* 447.0923 $[M-H]^-$, indicating a molecular formula of $C_{21}H_{20}O_{11}$ were in agreement with the proposed structure.



Isoscutellarein 7-*O*-β-glucoside (**80**)

Isoscutellarein 7-*O*-glucoside was previously found in different species of the primitive order of liverworts, Calobryales, from Antarctic *Bryum* species (Markham 1977, Markham and Given, 1988) and from *Rosmarinus officinalis* (Del Bano et al., 2003). To the best of our knowledge, this is the first time this glycoside has been isolated from the genus *Helichrysum.*

The main class of compounds isolated from *H. excisum* was therefore not of the acylated phloroglucinol type as for *H. caespititium* (Dekker et al., 1983, Mathekga et al., 2000) and *H. asperum* var. *albidulum* (Jakupovic et al., 1989) (the other two Group 12 species investigated), but mainly flavonoids, indicating that *H. excisum* is chemically not closely related to *H. caespititium* and *H. asperum* var. *albidulum*. It would be interesting to investigate the other species classified morphologically as Group 12, but collection of plant material is hampered by the fact that several of these species are narrowly endemic (Hilliard, 1983).

As mentioned previously, *H. excisum* extracts exhibited antimicrobial activity against Gram-positive organisms such as *S. aureus*, *S. epidermidis*, and *B. cereus*. The bio-

autographic assay of initial column fractions, obtained after fractionation of the extract, as well as of the isolated compounds, indicated that the antibacterial activity of the extract could be attributed mainly to the presence of lepidissipyrone (**19**). MIC values were therefore determined for this compound (Table 6.2). Unfortunately, only moderate activity was observed (MIC values of between 78 and 312 µg/ml observed for the screened micro-organisms). As expected, lepidissipyrone (**19**) has higher activity towards the Gram-negative organisms and *C. neoformans* than the crude extract, but only a slight improvement was observed against *S. aureus* and *S. epidermidis.* The decrease in activity observed against *B. cereus*, when considering the activity of the crude extract, indicates that lepidissipyrone may be acting synergistically with other chemical entities. Although none of the other flavonoids exhibited observable activity in the bio-autographic assay, varying anti-staphylococcal activity has been recorded for pinocembrin (**1**) (666 µg/ml, Alcaráz et al. 2000; 0.1 µg/plate, Bremner and Meyer, 1998; 125 µg/disc, Fukui et al., 1988; 50 µg/ml, Hufford and Laswell, 1978). It was also reported that gnaphaliin (**67**) inhibits the growth of several microorganisms (Tomás-Lorente et al., 1991; Mendoza et al., 1997). These discrepancies in observed activity may be due to several factors including differences in assays used (Cushnie et al., 2003, Cushnie and Lamb, 2005).

The modest activity observed for lepidissipyrone was unexpected since a much higher antimicrobial activity, with MIC's of 12.5 µg/ml against *Bacillus* species and *S. epidermidis*, was reported for a phloroglucinol with a similar lactone side chain (Tomás-Barberán, 1990). Other similar lactones also strongly inhibited several Gram-positive microorganisms (Ríos et al., 1991). Lepidissipyrone (**19**) decomposed on standing in chloroform, which indicated that the pyrone ring present might be acid sensitive. This instability might explain the MIC values observed for the isolated compound.

6.3 Conclusion

One of the active antimicrobial components of *H. excisum* was identified as lepidissipyrone (**19**), a flavanone containing an α-pyrone substituent on the A-ring. The phytochemical investigation of this species has shown that, based on chemical evidence, *H. excisum* does not belong in the same taxonomic group as *H. caespititium* and *H. asperum* var. *albidulum.* Further investigation of species within morphological Group 12 is required

to determine whether this morphological group is characterised by flavonoids or phloroglucinols as major chemical constituents.

6.4 Experimental

6.4.1 Plant collection

Helichrysum excisum (Thunb.) Less was collected on Robinson's pass (near Oudtshoorn) during November 2002 (Jan Vlok, 2830, NU) and August 2004 (Jan Vlok 2830A, NU) in the Western Cape, South Africa. The identity of the plant material was confirmed at the South African National Biodiversity Institute in Pretoria and voucher specimens deposited in the University of KwaZulu-Natal herbarium (NU) in Pietermaritzburg, South Africa.

6.4.2 Instrumentation and chemicals

Column (silica gel 60) and centrifugal chromatography (chromatotron model 7924, Harrison Research) were used for the purification of extracts and fractions. The circular plates for use on the chromatotron were coated with preparative silica gel (1 or 2 mm thickness). Thin-layer chromatography (TLC) plates (Kieselgel 60 F_{254}, 0.25 mm) were used for examination of fractions and visualization of spots was achieved by staining with anisaldehyde/H_2SO_4 and heating. The anisaldehyde solution was prepared as follows: 465 ml absolute ethanol was chilled in an ice bath. Acetic acid (5 ml), sulfuric acid (17 ml) and *p*-anisaldehyde (13 ml) was added and the solution mixed and stored in the refrigerator.

^{1}H and ^{13}C-NMR spectra were recorded on a Varian Unity Inova 500 spectrometer with a 5 mm SW/Z-PFG probe (all spectra recorded at 25 °C) (500 MHz for ^{1}H) at room temperature in solutions of either deuterated chloroform or methanol. Spectra for isoscutellarein 7-*O*-glucoside (**80**) were recorded on a Varian Inova 2000 (300 MHz for ^{1}H) in DMSO-d_6 using TMS as internal standard. Structures were determined by analysis of 2D (HSQC, HMBC, COSY and NOESY) NMR spectra and by comparison of ^{1}H and ^{13}C NMR spectra with literature values. Mass spectrometry data was collected on a time-of-flight Waters LCT Premier mass spectrometer using electrospray ionization in the positive mode. Optical rotation was determined with a Perkin Elmer 241 polarimeter.

6.4.3 General extraction

Plant material was air dried (protected from sunlight) and ground. Plant material was extracted twice with solvent for 24 hours at room temperature where after the solvent was removed under vacuum. Chloroform:methanol (1:1), dichloromethane and methanol extracts were prepared. Extraction of the air-dried, ground aerial parts (250 g) of *H. excisum* (Jan Vlok 2830A) with chloroform: methanol (1:1) yielded 16 g of extract. A dichloromethane extract (5 g) was obtained by extracting 100 g dried, ground plant material (Jan Vlok 2830A) at room temperature. A methanol extract (17.3 g) was prepared from ground, air-dried aerial parts (160 g, Jan Vlok, 2830).

6.4.4 Antimicrobial bioassays

Determination of minimum inhibitory concentration (MIC) values

See Paragraph 5.2.4. The antimicrobial activity for lepidissipyrone (**19**) was determined against *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 12600), *Staphylococcus epidermidis* (ATCC 2223), *Klebsiella pneumoniae* (NCTC 9633), *Pseudomonas aeruginosa* (ATCC 9027), and *Cryptococcus neoformans* (ATCC 90112). Antimicrobial activity for isoscutellarein 7-*O*-glucoside (**80**) was determined for *B. cereus* (ATCC 11778) and *S. aureus* (ATCC 12600).

Bio-autography on thin-layer chromatography (TLC) plates

Bioassay-guided isolation of antibacterial compounds from *Helichrysum excisum* was done by direct bio-autography on TLC (Van Vuuren et al., 2006). The crude chloroform: methanol extract, the fractions obtained after initial column chromatography of the crude extract and the isolated compounds were spotted on TLC plates and developed with hexanes:ethyl acetate 2:1 as solvent system. A duplicate TLC served as reference plate. A tryptone soya agar base layer was poured into a sterile Petri dish and the developed TLC plates placed onto its surface after setting. A second agar layer, inoculated with *S. aureus* (ATCC 12600) was poured onto the TLC plate. After incubation for 24 hours at 37 °C inhibition zones were visualized by spraying with a 0.4 mg/ml INT solution. Clear zones on the pink background indicated compounds with anti-staphylococcal activity. The corresponding compounds could be identified with the reference TLC (Figure 6.3).

Figure 6.3 Bio-autographic assay of compounds isolated from *H. excisum*. The clear zone is observed for lepidissipyrone

6.4.5 Fractionation and isolation of compounds

The crude chloroform: methanol extract (6 g) was fractionated on silica gel by sequential elution with hexanes:ethyl acetate (2:1), hexanes:ethyl acetate (1:1), and ethyl acetate (100%) to yield 12 fractions (A1-A12). Fraction A4 (680 mg) was further fractionated using chloroform (100%), chloroform:acetone (50:1), chloroform:acetone (25:1), chloroform: acetone (10:1), and acetone (100%) to give 7 fractions (B1-B7). Fraction B4 (51 mg) was purified on a chromatotron with hexanes:ethyl acetate (2:1) as mobile phase to yield compound **1** (2 mg), which precipitated when the dried fraction obtained from the chromatotron was dissolved in chloroform. Compound **67** (1 mg) was obtained from fraction B3 (70 mg) after separation on a chromatotron with hexanes:ethyl acetate (2:1) as eluent. Purification of fraction B2 (180 mg) on the chromatotron with hexanes:ethyl acetate (2:1) yielded compound **19** (5 mg) which precipitated as a white solid from a fraction that contained a mixture of two compounds.

The crude dichloromethane extract (5 g) was fractionated on silica gel using hexanes:ethyl acetate (9:1), hexanes:ethyl acetate (8.5:1), hexanes:ethyl acetate (8:2), and hexanes:ethyl acetate (6:4) followed by washing the column with methanol as eluent to obtain 8 fractions (C1-C8). Fraction C4 was further fractionated with a chromatotron with hexanes:ethyl acetate (2:1) as eluent to yield compound **72** as a yellow solid (2 mg).

The methanol extract (17.3 g) was dissolved in methanol and subjected to column chromatography using silica gel and subsequent mobile phases consisting of hexanes:

dichloromethane (9:1), dichloromethane:methanol (6:1), dichloromethane:methanol (3:1), and methanol (100%). Isoscutallarein 7-*O*-glucoside (**80**) precipitated as a yellow solid (85 mg).

6.4.6 Physical data of compounds

Compound **1**, a white amorphous solid, was identified as (*S*)-pinocembrin (Fukui et al., 1988; Jung and McLaughlin, 1990). $[\alpha]_D$ = -53 (MeOH, c = 0.09) [lit. Fukui et al., 1988, -52 (MeOH, c = 0.188); Su et al., 2003, -58.5 (MeOH, c = 0.91); Hsieh et al., 1998, -47.3 (MeOH, c = 5.48)].^{1}H NMR (500 MHz, CD_3OD): δ_H 2.78 (1H, *dd*, $J_{3\alpha,3\beta}$ = 17 Hz, $J_{2,3\alpha}$ = 3 Hz, H_β-3), 3.09 (1H, *dd*, $J_{3\alpha,3\beta}$ = 17 Hz, $J_{2,3\beta}$ = 13 Hz, H_α-3), 5.46 (1H, *dd*, $J_{2,3\beta}$ = 13 Hz, $J_{2,3\alpha}$ = 3 Hz, H-2), 5.90 (1H, *d*, $J_{6,8}$ = 2 Hz, H-6), 5.94 (1H, *d*, $J_{6,8}$ = 2 Hz, H-8), 7.36-7.43 (3H, *m*, H-3',4',5'), 7.50 (2H, *d*, *J* = 8 Hz, H-2' and H-6'); ^{13}C NMR (125 MHz, CD_3OD): δ_C 44.2 (*t*, C-3), 80.5 (*d*, C-2), 96.3 (*d*, C-8), 97.2 (*d*, C-6), 103.4 (*s*, C-10), 127.4 (*d*, C-2' and C-6'), 129.6 (*d*, C-4'), 129.7 (*d*, C-3' and C-5'), 140.5 (*s*, C-1'), 164.7 (*s*, C-9), 165.5 (*s*, C-5), 168.6 (*s*, C-7), 197.3 (*s*, C-4); HRESIMS (negative ionization mode), *m/z* 255.0652 $[M-H]^-$ (calc. for $C_{15}H_{11}O_4$ 255.0657).

Compound **67**, a yellow amorphous solid, was identified as gnaphaliin (Tomás-Lorente et al., 1991), ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 3.88 (3H, *s*, 3-OMe), 4.00 (3H, *s*, 8-OMe), 6.28 (1H, *s*, 7-OH), 6.44 (1H, *s*, H-6), 7.54 (3H, *m*, H-3',H-4' and H-5'), 8.10 (2H, *m*, H-2' and H-6'), 12.4 (1H, *s*, 5-OH); ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 60.4 (*q*, 3-OMe), 62.0 (*q*, 8-OMe), 98.5 (*d*, C-6), 105.8 (*s*, C-10), 126.7 (*s*, C-8), 128.3 (*d*, C-2',C-6'), 128.8 (*d*, C-3' and C-5'), 130.5 (*s*, C-1'), 131.1 (*d*, C-4'), 139.7 (*s*, C-3), 155.1 (*s*, C-2), 155.5 (*s*, C-5), 157.6 (*s*, C-7), 179.1 (*s*, C-4); HRESIMS (negative ionization mode), *m/z* 313.0712 $[M-H]^-$ (calc. for $C_{17}H_{13}O_6$ 313.0712).

Compound **19**, a white amorphous solid, was identified as lepidissipyrone (Jakupovic et al., 1989; Hua and Wang, 2004), ^{1}H NMR (500 MHz, $CDCl_3$): See Table 6.1; ^{13}C NMR (125 MHz, $CDCl_3$): See Table 6.1; HRESIMS (negative ionization mode), *m/z* 421.1273 $[M-H]^-$ (calc. for $C_{24}H_{21}O_7$ 421.1287).

Compound **72**, a yellow amorphous solid, was identified as 5-hydroxy-7,8-dimethoxyflavone (Gupta et al., 1983; Kuroyanagi et al., 1987), ^{1}H NMR (500 MHz,

$CDCl_3$): δ_H 3.95 (3H, *s*, 7 or 8-OMe), 3.96 (3H, *s*, 7 or 8-OMe), 6.45 (1H, *s*, H-6), 6.68 (1H, *s*, H-3), 7.56 (3H, *m*, H-3', H-4' and H-5'), 7.95 (2H, *m*, H-2' and H-6'), 12.6 (1H, *s*, 5-OH), ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 56.4 (*q*, 7 or 8-OMe), 61.7 (*q*, 7 or 8-OMe), 95.8 (*d*, C-6), 105.0 (*s*, C-10), 105.4 (*d*, C-3), 126.3 (*d*, C-2'and C-6'), 128.9 (*s*, C-8), 129.2 (*d*, C-3' and 5'), 131.4 (*s*, C-1'), 131.9 (*d*, C-4'), 149.4 (*s*, C-9), 157.6 (*s*, C-5), 158.7 (*s*, C-7), 163.9 (*s*, C-2), 182.7 (*s*, C-4), HRESIMS (negative ionization mode) *m/z* 297.0757 $[M-H]^-$ (calc. for $C_{17}H_{13}O_5$ 297.0763).

Compound **80**, a yellow amorphous solid, was identified as isoscutellarein 7-*O*-β-glucoside (8-hydroxyapigenin 7-*O*-β-glucoside) (Albach et al., 2003). ^{1}H NMR (300 MHz, DMSO-d_6,): δ_H 3.22 (1H, *t*, $J_{3",4"} = J_{4",5"}$ = 8.5 Hz, H-4"), 3.54-3.32 (4H, *m*, H-2", H-3", H-5" and H-6"), 3.78 (1H, *m*, H-6"), 4.68 (1H, *br s*, OH), 4.97 (1H, *d*, *J* = 7.3 Hz, H-1", β-glucoside), 5.11 (2H, *br s*, OH), 6.67 (1H, *s*, H-6), 6.85 (1H, *s*, H-3), 6.98 (2H, *d*, *J* = 8.8 Hz, H-3' and H-5'), 8.01 (2H, *d*, *J* = 8.8 Hz, H-2' and H-6'), ^{13}C NMR (75 MHz, DMSO-d_6): δ_C 60.7 (*t*, C-6"), 69.7 (*d*, C-4"), 73.2 (*d*, C-2"), 75.7 (*d*, C-3"), 77.3 (*d*, C-5"), 98.7 (*d*, C-6), 101.3 (*s*, C-1''), 102.7 (*d*, C-3), 105.2 (*s*, C-10), 116.0 (*d*, C-3' and C-5'), 121.3 (*s*, C-1'), 127.0 (*s*, C-8), 128.7 (*d*, C-2' and C-6'), 144.3 (*s*, C-9), 151.2 (*s*, C-5), 152.4 (*s*, C-7), 161.3 (*s*, C-4'), 164.0 (*s*, C-2), 182.4 (*s*, C-4), HRESIMS (negative ionization mode) *m/z* 447.0923 $[M-H]^-$ (calc. for $C_{21}H_{19}O_{11}$ 447.0927).

6.5 References

Alcaráz, L.E., Blanco, S.E., Puig, O.N., Tomás, F., Ferretti, F.H., 2000. Antibacterial activity of flavonoids against methicillin-resistant *Staphylococcus aureus* strains. Journal of Theoretical Biology 205, 231-240.

Albach, D.C., Grayer, R.J., Jensen, S.R., Özgőkce, F., Veitch, N.C., 2003. Acylated flavone glycosides from *Veronica*. Phytochemistry 64, 1295-1301.

Appendino, G., Ottino, M., Marquez, N., Bianchi, F., Giana, A., Ballero, M., Sterner, O., Fiebich, B.L., Munoz, E., 2007. Arzanol, an anti-inflammatory and anti-HIV-1 phloroglucinol α-pyrone from *Helichrysum italicum* ssp. *microphyllum*. Journal of Natural Products 70, 608-612.

Bohlmann, F., Abraham, W-R., 1979a., Neue diterpene und weitere inhaltsstoffe aus *Helichrysum calliconum* und *Helichrysum heterolasium*. Phytochemistry 18, 889-891.

Bohlmann, F., Abraham, W-R., 1979b. Neue, chlorsubstituierte thiophenacetylenverbindungen mit ungewöhnlicher struktur aus Helichrysum-arten. Phytochemistry 18, 839-842.

Bohlmann, F., Abraham, W-R., 1979c. Neue diterpene aus *Helichrysum acutatum*. Phytochemistry 18, 1754-1756.

Bohlmann, F., Abraham, W-R., 1979d. Neue prenylflavanone aus *Helichrysum hypocephalum*. Phytochemistry 18, 1851-1853.

Bohlmann, F., Ates, N., 1984. Three prenylated flavanoids from *Helichrysum athrixiifolium.* Phytochemistry 23, 1338-1339.

Bohlmann, F., Misra, L.N., 1984. New prenylflavanones and chalcones from *Helichrysum rugulosum*. Planta Medica 50, 271-272.

Bohmann, F., Misra, L.N., Jakupovic, J., 1984. Weitere phloroglucin- und α-pyrone-derivate aus *Helichrysum*-arten. Planta Medica 50, 174-176.

Bohlmann, F., Zdero, C., 1980. Neue phloroglucin-derivate aus *Helichrysum*-arten. Phytochemistry 19, 153-155.

Bohlmann, F., Zdero. C., 1983. Flavanones from *Helichrysum thapsus*. Phytochemistry 22, 2877-2878.

Bohlmann, F., Zdero, C., Abraham, W-R., Suwita, A., Grenz, M., 1980. Neue diterpene und neue dihydrochalkon-drivate sowie weitere inhaltsstoffe aus *Helichrysum* arten. Phytochemistry 19, 873-879.

Bohlmann, F., Ziesche, J., Mahanta, P.K., 1979. Neue chalkon-derivate und humulon-ähnliche verbindungen aus *Helichrysum* arten. Phytochemistry 18, 1033-1036.

Bremner, P.D., Meyer, J.J.M., 1998. Pinocembrin chalcone: an antibacterial compound from *Helichrysum trilineatum*. Planta Medica 64, 777.

Cushnie, T.P.P., Hamilton, V.E.S., Lamb, A.J., 2003. Assesment of the antibacterial activity of selected flavonoids and consideration of discrepancies between previous reports. Microbiological Research 158, 281-289.

Cushnie, T.P.P., Lamb, A.J., 2005. Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents 26, 324-356.

Dekker, T.G., Fourie, T.G., Snyckers, F.O., Van der Schyf, C.J., 1983. Studies of South African medicinal plants. Part 2. Caespitin, a new phloroglucinol derivative with antimicrobial properties from *Helichrysum caespititium*. South African Journal of Chemistry 36, 114-116.

Del Bano, M.J., Lorente, J., Castillo, J., Benavente-Garciá, O., Del Río, J.A., Ortuńo, A., Quirin, K-W., Gerard, D., 2003. Phenolic diterpenes, flavones, and rosmarinic acid distribution during the development of leaves, flowers, stems, and roots of *Rosmarinus officinalis*. Antioxidant activity. Journal of Agricultural and Food Chemistry 51, 4247-4253.

Fukui, H., Goto, K., Tabata, M., 1988. Two antimicrobial flavanones from the leaves of *Glycyrrhiza glabra*. Chemical and Pharmaceutical Bulletin 36, 4174-4176.

Gibbons, S., 2004. Anti-staphylococcul plant natural products. Natural Product Reports 21, 263-277.

Gupta, K.K., Taneja, S.C., Dhar, K.L., Atal, C.K., 1983. Flavonoids of *Andrographis paniculata.* Phytochemistry 22, 314-315.

Hänsel, R., Cybulski, E-M., Cubukcu, B., Mercli, A.H., Bohlmann, F., Zdero, C., 1980. Neue pyron-derivate aus *Helichrysum* arten. Phytochemistry 19, 639-644.

Hilliard, O.M., 1983. In: Flora of Southern Africa. Ed. O.A. Leistner, Vol. 33, part 7(2) (Asteraceae). Botanical Research Institute of South Africa. pp. 61, 131-132.

Horie, T., Ohtsuru,Y., Shibata, K., Yamashita, K., Tsukayama, M., Kawamura, Y., 1998. ^{13}C NMR spectral assignment of the A-ring of polyoxygenated flavones. Phytochemistry 47, 865-874.

Hufford, C.D., Laswell, W.L., 1978. Antimicrobial actvities of constituents of *Uvaria chamae*. Lloydia 41, 156-160.

Hsieh, Y-L., Fang, J-M., Cheng, Y-S., 1998. Terpenoids and flavonoids from *Psuedotsuga wilsoniana*. Phytochemistry 47, 845 850.

Hua, Y., Wang, H-Q., 2004. Chemical components of *Anaphalis sinica* Hance. Journal of the Chinese Chemical Society 51, 409-415.

Hutchings, A., Van Staden, J., 1994. Plants used for stress-related ailments in traditional Zulu, Xhosa and Sotho medicine. Part 1. Plants used for headaches. Journal of Ethnopharmacology 43, 89-124.

Jakupovic, J., Grenz, M., Bohlmann, F., Mungai, G.M., 1990. 12β-Hydroxyabieta-7,13-diene and other constituents from East African *Helichrysum* species. Phytochemistry 29, 1589-1590.

Jakupovic, J., Kuhnke, J., Schuster, A., Metwally, M.A., Bohlmann, F., 1986. Phloroglucinol derivatives and other constituents from South African *Helichrysum* species. Phytochemistry 25, 1133-1142.

Jakupovic, J., Pathak, V.P., Bohlmann, F., King, R.M., Robinson, H., 1987. Obliquin derivatives and other constituents from Australian *Helichrysum* species. Phytochemistry 26, 803-807.

Jakupovic, J., Zdero, C., Grenz, M., Tsichritzis, F., Lehmann, L., Hashemi-Nejad, S.M., Bohlmann, F., 1989. Twenty-one acylphloroglucinol and further constituents from South African *Helichrysum* species. Phytochemistry 28, 1119-1131.

Jung, J.H., Mclaughlin, J.L., 1990. ^{13}C-^{1}H NMR long range coupling and deuterium isoptope effects of flavanones, Phytochemistry 29, 1271-1275.

Kuroyanagi, M., Sato, M., Ueno, A., Nishi, K., 1987. Flavonoids from *Andrographis paniculata*. Chemical Pharmaceutical Bulletin 35, 4429-4435.

Lenherr, A., Lahloub, M.F., Sticher, O., 1984. Three flavonoid glycosides containing acetylated allose from *Stachys recta*. Phytochemistry 23, 2343-2345.

Lourens, A.C.U., Reddy, D., Başer, Viljoen, A.M., Van Vuuren, S.F., 2004. In vitro biological activity and essential oil composition of four indigenous South African *Helichrysum* species. Journal of Ethnopharmacology 95, 253-258.

Markham, K.R., 1977. Flavonoids and phylogeny of the 'primitive' New Zealand hepatic, *Haplomitrium gibbsiae*. Phytochemistry 16, 617-619.

Markham, K.R., Given, D.R., 1988. The major flavonoids of an Antarctic *Bryum*. Phytochemistry 27, 2843-2845.

Mathekga, A.D.M., Meyer, J.J.M., Horn, M.M., Drewes, S.E., 2000. An acylated phloroglucinol with antimicrobial properties from *Helichrysum caespititium*. Phytochemistry 53, 93-96.

Mendoza, L., Wilkens, M., Urzúa, A., 1997. Antimicrobial study of the resinous exudates and of diterpenoids and flavonoids isolated from some Chilean *Pseudognaphalium* (Asteraceae). Journal of Ethnopharmacology 58, 85-88.

Nakane, H., Arisawa, M., Fujita, A., Koshimura, S., Ono, K., 1991. Inhibition of HIV-transcriptase activity by some phloroglucinol derivatives. Federation of European Biochemical Societies (FEBS) 286, 83-85.

Nakanishi, T., Iida, N., Inatomi, Y., Murata, H., Inada, A., Murata, J., Lang, F.A., Iinuma, M., Tanaka, T., 2004. Neolignan and flavonoid glycosides in *Juniperus communis* var. *depressa*. Phytochemistry 65, 207-213.

Ríos, J.L., Recio, M.C., Villar, A., 1991. Isolation and identification of the antibacterial compounds from *Helichrysum Stoechas*. Journal of Ethnopharmacology 33, 51-55.

Sala, A., Recio, M.C., Schinella, G.R., Máñez, S., Giner, R.M., Cerdá-Nicolás, M., Ríos, J-L., 2003. Assessment of the anti-inflammatory activity and free radical scavenger activity of tiliroside. European Journal of Pharmacology 461, 53-61.

Slade, D., Ferreira, D., Marais, J.P.J., 2005. Circular dichroism, a powerful tool for the assessment of absolute configuration of flavonoids. Phytochemistry 66, 2177-2215.

Su, B-N., Park, E.J., Vigo, J.S., Graham, J.G., Cabieses, F., Fong, H.H.S., Pezzuto, J.M., Kinghorn, A.D., 2003. Activity guided isolation of the chemical constituents of Muntingia calabura using a quinine reductase induction assay. Phytochemistry 63, 335-341.

Tomás-Barberán, F., Iniesta-Sanmartín, E., Tomás-Lorente, F., Rumbero, A., 1990. Antimicrobial phenolic compounds from three Spanish *Helichrysum* species. Phytochemistry 29, 1093-1095.

Tomás-Lorente, F., Iniesta-Sanmartín, E., Tomás-Barberán, F.A., 1991. Antimicrobial phenolics from *Helichrysum picardii*. Fitoterapia 62, 521-3.

Van Vuuren, S.F., Viljoen, A.M., Van Zyl, R.L., Van Heerden, F.R., Başer, K.H.C., 2006. The antimicrobial, antimalarial and toxicity profiles of helihumulone, leaf essential oil and extracts of *Helichrysum cymosum* (L.) D. Don subsp. *cymosum*. South African Journal of Botany 72, 287-290

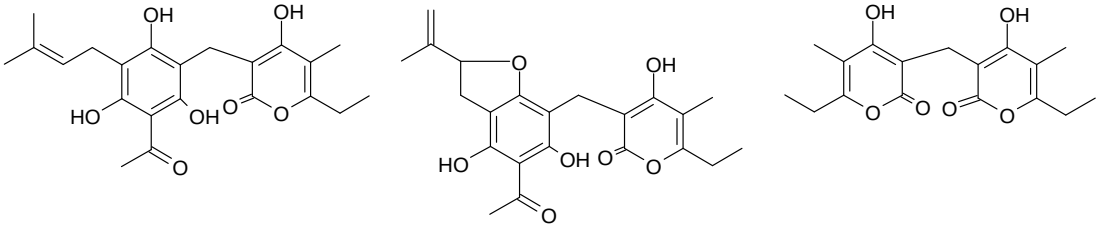
Watt, J.M., Breyer-Brandwijk, M.G., 1962**.** The Medicinal and Poisonous Plants of Southern and Eastern Africa. 2nd edition. Livingstone, London, pp. 237-240.

CHAPTER 7

Synthetic approaches towards the synthesis of lepidissipyrone

7.1 Introduction

Several phloroglucinol α-pyrones have been isolated from South African and European *Helichrysum* species (Fig. 7.1). The phloroglucinol moieties of these compounds are often prenylated while the 4-hydroxy-2-pyrone unit is usually alkylated in both the 5- and 6-positions (Bohlmann et al., 1984; Bohlmann and Zdero, 1980; Hänsel et al., 1980; Jakupovic et al., 1986, Vrkoč et al., 1971). These compounds exhibit noticable biological activity. Phloroglucinol pyrones isolated from *H. decumbens* exhibited antifungal (Tomás-Lorente et al., 1989) and antibacterial activity (Tomás-Barberan et al., 1990), while those isolated from *H. stoechas* exhibited antimicrobial activity (Ríos et al., 1991).



Arzanol (**374**) (*H. italicum*) | Italipyrone (**372**) (*H. stoechas*) | Helipyrone (**375**) (*H. decumbens*)

Figure 7.1 Phloroglucinol-derived α-pyrones isolated from *Helichrysum* species

Arzanol (**374**), isolated from *H. italicum* showed significant *in vitro* antioxidant activity and had a protective effect against oxidative degradation of linoleic acid and cholesterol, oxidisable substances present in membranes. The compound was also found to be non-toxic in the MTT assay against VERO cells (a line of fibroblasts derived from monkey kidney) at concentrations of 0.5-40 μM (Rosa et al., 2007). The most consequential

findings are those by Appendino and co-workers (2007) who reported on the NF-$_K$B inhibitory activity of arzanol (**374**) as well as its inhibition of HIV-1 replication in T-cells.

Pyrones are an important class of naturally-occurring lactones and the 4-hydroxy-2-pyrone (α-pyrone) motif can be found in a wide range of medicinally significant natural products (Whang et al., 1990; Carney et al., 2002; Zhang and Danishefsky, 2002; Kurdyumov et al., 2006). The biological activities associated with pyrones include the inhibition of HIV protease (Thaisrivongs et al., 1996; Tummino et al., 1996; De Clercq, 2002), acetylcholinesterase, and acyl-CoA-cholesterol acyltransferase (McGlacken and Fairlamb 2005 and references therein). The 2-pyrones also display antimicrobial (Fairlamb et al., 2004), antiparasitic (Shimamura et al., 2007), and anticancer activity (Ridley and Khosla, 2007) and have been shown to inhibit cyclo-oxygenase (Rao et al., 2003; McGlacken and Fairlamb, 2005) indicating their usefulness as analgesic and anti-inflammatory agents. Flavanones on the other hand, also possess a broad array of biological activity, which include antitumour (Ren et al., 2003), antibacterial (Tsuchiya et al., 1996, Urgaonkar et al., 2005), and anti-inflammatory activities (Benavente-García and Castillo, 2008). Flavanones also exhibit activity as estrogen receptor modulators (Chen et al., 2004).

A limited number of synthetic routes have been published for the synthesis of substituted 4-hydroxy-2-pyrones. These routes normally involve β-keto ester (Banerjee and Achari, 1993; Köster and Hoffmann, 1987; Zhang and Danishefsky, 2002; Katritzky et al., 2005; Kurdyumov et al., 2006) and/or acid chloride precursors (Ali et al., 1982; Lokot et al., 1999; Sartori et al., 1991; Kurdyumov et al., 2006). The synthesis of flavanones is well established and usually involves one of two routes. The first route involves the coupling of a C-6 phenolic unit (e.g. phloroglucinol) to a C3-C6 unit (cinnamic residue) to form the flavonoid skeleton (Nay et al., 2000, Solladíe et al., 1999). The more commonly used route consists of a condensation between an acetophenone derivative and a benzaldehyde to yield the chalcone (Nay et al., 2001; Drexler and Amiridis, 2003, Solladíe et al., 1999). Although a number of routes have recently been developed for the asymmetric synthesis of flavanones (Hodgetts, 2005, Biddle et al., 2007), the enantioselective synthesis of flavanones that controls the C-2 configuration remains a challenge due to the potential for reversible phenoxide elimination to form the achiral 2'-hydroxy chalcones (Kabbe and Widdig, 1982).

One of the compounds isolated from *Helichrysum excisum* (Thunb.) Less. is a flavanone substituted with a 4-hydroxy-2-pyrone moiety (lepidissipyrone, **19**) with antimicrobial activity (Chapter 6). Although a number of syntheses for α-pyrones have been published, only a single reference to the preparation of a phloroglucinol or flavanone substituted α-pyrone could be found (Vrkoč and Sorm, 1975b). Based on the interesting biological activities associated with and the shortage of synthetic routes towards these compounds, the decision was made to attempt the synthesis of lepidissipyrone.

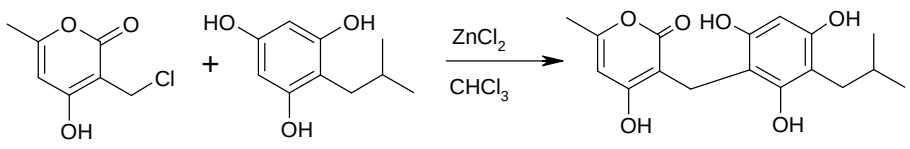
The aims of this chapter are:

- To investigate a strategy towards the synthesis of lepidissipyrone (**19**)
- To develop synthetic methods that could also lead to the synthesis of the other α-pyrone derivatives listed in Fig. 7.1.

7.2 Results and discussion

7.2.1 Retrosynthetic analysis of lepidissipyrone (**19**)

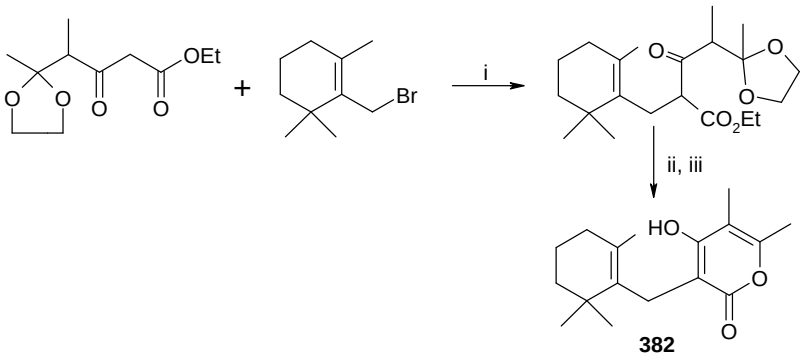
The synthetic challenges associated with the total synthesis of lepidissipyrone (**19**) are obvious. Firstly, there is little literature precedent for the coupling of the 4-hydroxy-2-pyrone unit *via* a CH_2 bridge to a phloroglucinol moiety. A patent by Vrkoč and Sorm (1975a) mentions the preparation of 3-chloromethyl-4-hydroxy-pyrone derivatives that are coupled to phloroglucinol derivatives to yield phloroglucinol pyrones with antibacterial activity (Scheme 7.1) (Vrkoč and Sorm, 1975b). However, according to Hagiwara et al. (2002), introduction of the methyl halide side chain (via formylation) to the 4-hydroxypyrone is troublesome as the pyrone has a tendency to dimerise in the presence of Lewis acids or bases to yield helipyrone (**375**).



Scheme 7.1 Synthetic route of phloroglucinol-related α-pyrones synthesised by Vrkoč and Sorm (1975b).

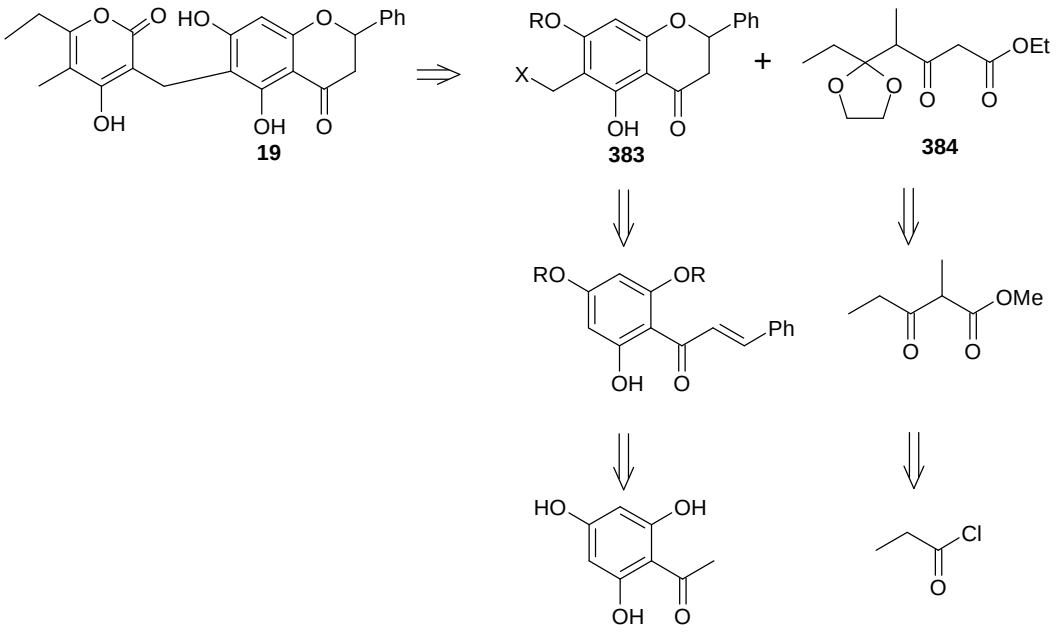
Phloroglucinols are also notoriously difficult to work with due to the high electron density of the phloroglucinol ring and its tendency to degrade (Gissot et al., 2004, Van Vuuren et al., 2006). Flavanones are known to undergo ring opening reactions to form chalcones under acidic and basic conditions (Cisak and Mielczarek, 1992; Zhang et al., 2008) and in this case, the pyrone moiety has to be attached selectively at position 6, and not position 8 of the A-ring of the flavanone. Therefore, the challenge is to prepare a heavily substituted α-pyrone moiety joined to C-6 of the electron-rich phloroglucinol moiety of a flavanone.

Banerjee and Achari (1993) prepared α-pyrone intermediate **382** in the total synthesis of lygodinolide, using an alkyl bromide and a β-keto ester (Scheme 7.2).



Scheme 7.2 Intermediates in the total synthesis of lygodinolide. Reagents (i) NaH, THF, 0-25 °C, 16 h, then reflux 3 h (ii) Pd(II) chloride-acetonitrile, acetone, rt, 24 h (iii) DBU, benzene, reflux, 14 h (Banerjee and Achari, 1993).

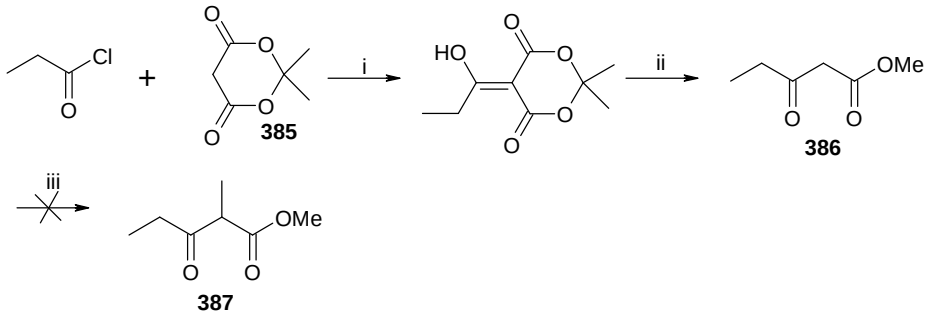
Based on this route, the following retrosynthetic analysis (Scheme 7.3) for lepidissipyrone (**19**) was proposed:



Scheme 7.3 Retrosynthesis of lepidissipyrone (**19**)

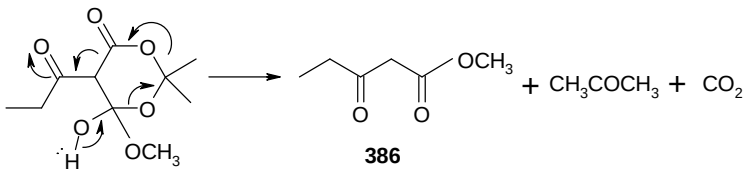
7.2.2 Preparation of ethyl 4-(2-ethyl-1,3-dioxolan-2-yl)-3-oxopentanoate (**384**)

The original route selected for the synthesis of the β-keto ester moiety is shown in Scheme 7.4 and was based on the method used for the preparation of β-keto esters by Lokot and co-workers (1999) and Scribner et al. (1978). The first step was the acylation of Meldrum's acid (**385**), followed by alcoholysis in methanol to obtain β-keto ester **386**. It was anticipated that alkylation of ester **386** would afford the desired methylated β-keto ester **387** (Lee and Kim, 2002).



Scheme 7.4 Initial route towards synthesis of β-keto ester **387**. Reagents. (i) Pyridine, CH_2Cl_2, -15 - 5 °C, 2 h (ii) MeOH, reflux, 3 h, 67% (iii) K_2CO_3, CH_3I.

The acidity of Meldrum's acid (**385**) (p*K*a 4.97) allows it to react with electrophiles in the absence of strong bases and acylation occurs in the presence of pyridine. The methanolysis proceeds readily due to the enolization of the acyl group, which enables nucleophilic attack by the alcohol (Scribner et al., 1978, Scheme 7.5).

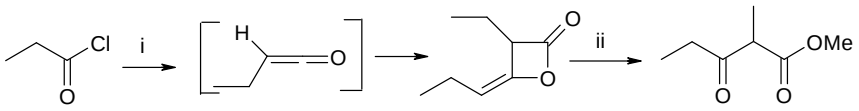


386

Scheme 7.5 Methanolysis of acylated Meldrum's acid (Scribner et al., 1978).

Although β-keto ester **386** was synthesised successfully, alkylation resulted in the formation of multiple products and this prompted the decision to use an alternative route. Claisen condensation is a well-known method for the general synthesis of β-keto esters (Clayden et al., 2001). A self condensation of methyl propionate using potassium hydride as base was therefore attempted (Kamal et al., 2005). However, this reaction was unsuccessful.

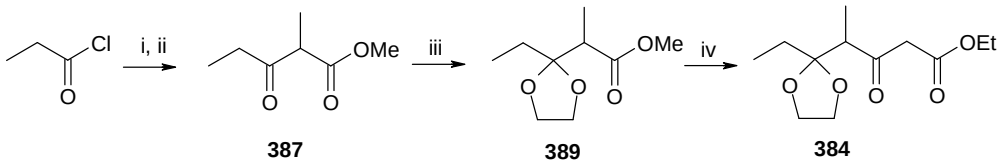
In another attempt to synthesise β-keto ester **384**, propionyl chloride was treated with triethylamine as base according to the method of Sung and Wu (2001). The base removes a proton on the α-carbon of the acid chloride to generate a ketene *in situ* that proceeds with the precipitation of triethylammonium chloride. Dimerisation of the unstable ketene results in the formation of β-lactone **388**. Refluxing of the lactone in the presence of an excess of methanol and sodium acetate as catalyst afforded β-keto ester **387** in 52% yield (Sung and Wu, 2001, Scheme 7.6).



388 **387**

Scheme 7.6 Synthesis of β-keto ester **387.** Reagents: (i) Et_3N, ether, 0 °C - rt, 2 days (ii) NaOAc, MeOH, reflux, 1 day, 52%.

The ketone functionality in compound **387** was protected by ketalisation to afford the protected ester **389** (Bates et al., 1988). This protecting group was selected based on the simplicity of the protection procedure and the availability of starting materials. A Claisen condensation of ketal ester **389** with ethyl acetate in the presence of LiHMDS (Banerjee and Achari, 1993) resulted in an extremely low yield of the desired product and the reaction was repeated with LDA to afford ester **384** (Plates 95, 96) (Shone et al., 1986) (Scheme 7.7)



387 **389** **384**

Scheme 7.7 Synthesis of β-keto ester **384**. Reagents: (i) Et_3N, ether, 0 °C - rt, 2 days (ii) NaOAc, MeOH, reflux, 1 day, 52%; (iii) TsOH, 1,2-ethanediol, toluene, reflux overnight, 66% (iv) LDA, EtOAc, THF, 0 °C - 15 °C, 2 h, 37%.

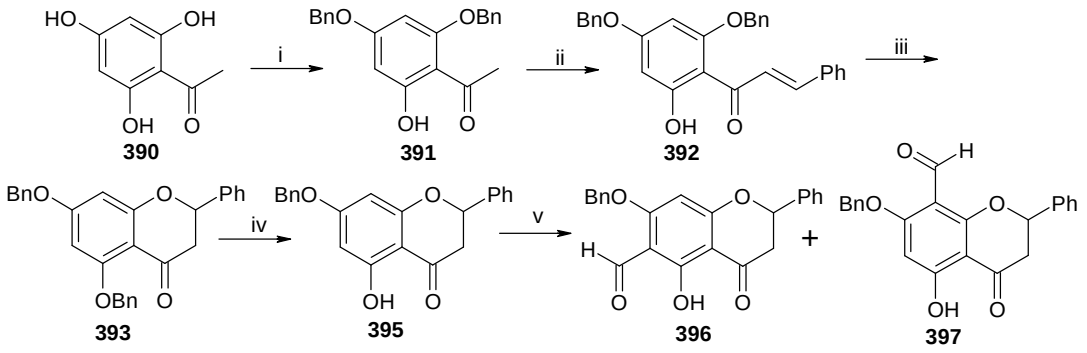
7.2.3 Preparation of the flavanone moiety

After obtaining β-keto ester **384**, the next step was to synthesise a flavanone with a "suitable arm" (**383**), such as an alkyl halide for coupling with the ester (Scheme 7.8). Three options were considered for the introduction of the C-6 substituent of the flavanone: a) direct methylbromination using reagents such as HBr and formaldehyde, b) hydroxymethylation followed by halogenation and c) introduction of a 6-formyl group that could be transformed into the required substituent.

In a model reaction on 2,4-dibenzyloxy-6-hydroxyphloroacetophenone (**391**), direct methylbromination using HBr and formaldehyde (Biali et al., 1997; Van der Made et al., 1993) was unsuccessful. In a second model reaction on the same substrate, employing a two step approach to the bromomethylphloroacetophenone, a hydroxymethylation followed by bromination was attempted. Although the desired alcohol was obtained by hydroxymethylation under basic conditions (Ducho et al., 2003), the yields for this reaction were low. The reaction never went to completion and when left for extended times or heated, a product formed that could not be isolated. This was not totally unexpected as the

Bakelite-type polymerisation of phenols is well known and the trihydroxylated system is electron rich. Subsequently, the introduction of a formyl group at C-6 of the flavanone was planned, followed by reduction and bromination. Scheme 7.8 indicates the first proposed route for the synthesis of the flavanone moiety, which could be coupled to the β-keto ester.

Starting material for this route was phloroacetophenone (**390**) (Scheme 7.8). Due to the multiple hydroxy groups present on the phloroacetophenone ring, selective protection was required. Benzyl protecting groups were chosen based on the ease of formation of benzyl ethers, their stability under various conditions and their removal by a variety of deprotection methods, including the use of Lewis acids, catalytic hydrogenolysis (Greene, 1991) and sodium in the presence of butanol (Odejinmi and Wiemer, 2005). MOM-protection was considered, as it is often utilised in this type of synthesis (Bu et al., 1997, Tan et al., 2002, Urgaonkar et al., 2005) but difficulties in obtaining commercial MOM-chloride and the toxicity associated with these preparations (Kishore et al., 2006) ruled out the use of this reagent.

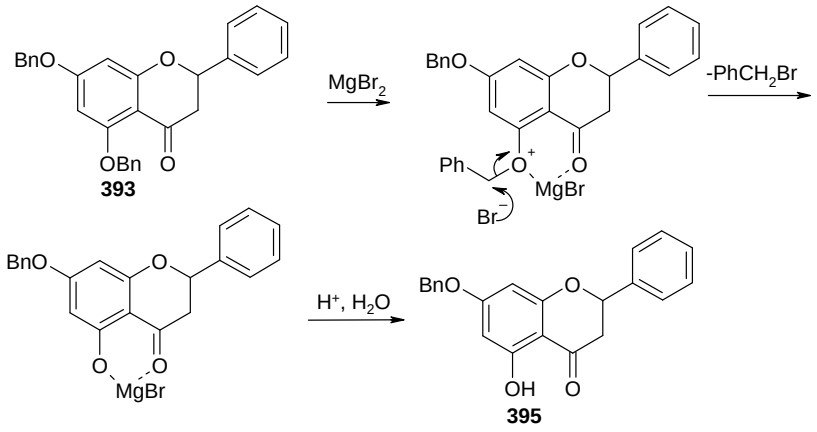


Scheme 7.8 Flavanone synthesis, deprotection and formylation. Reagents: (i) BnCl, K_2CO_3, DMF, 80 °C, 2 h, 82% (ii) PhCHO, NaH, DMF, 0 °C, 45 min, 100% (iii) AcOH, reflux, 6 h, 32% or DBU, CH_2Cl_2, rt, 2 days, 49% (iv) H_2, Pd/C, CH_2Cl_2/EtOAc, rt, 45 min, 90% (v) a) $POCl_3$, DMF, CH_3CN, 18 h, rt, b) MeOH/H_2O, 9% (**396**), 37%, (**397**).

It was anticipated that the free hydroxy group would have a stronger *ortho*-directing effect than the benzyloxy group, enabling monosubstitution of the flavanone A-ring. Selective benzylation of 2,4,6-trihydroxyacetophenone afforded **391**. Condensation of ketone **391** and benzaldehyde was attempted in the presence of sodium hydroxide (Ali and Ilyas, 1986; Suzuki et al., 2004), potassium hydroxide (Ichino et al., 1988) and sodium hydride

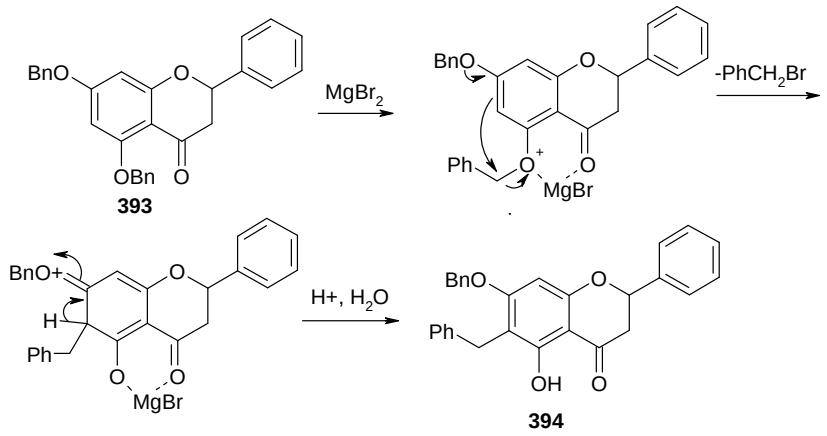
(Hatakeyama et al., 2005) in order to obtain chalcone **392**. The reaction with sodium hydride at 0 °C gave quantitative yields of chalcone **392** (Plates 97, 98). Synthesis of flavanone **393** was quite problematic since an equilibrium between the flavanone and chalcone exists (Urgaonkar et al., 2005). Both the acid (acetic acid) (Solladie et al., 1999) and base (DBU) catalysed reactions gave rather low yields, although the starting material could be reisolated and the reaction repeated (Scheme 7.8).

Haraldsson and Baldwin (1997) have illustrated that benzyl ethers adjacent to carbonyl groups can be selectively cleaved in the presence of other benzyl ethers using magnesium bromide. Mechanistically this involves the formation of a six-membered chelated ring (Scheme 7.9).



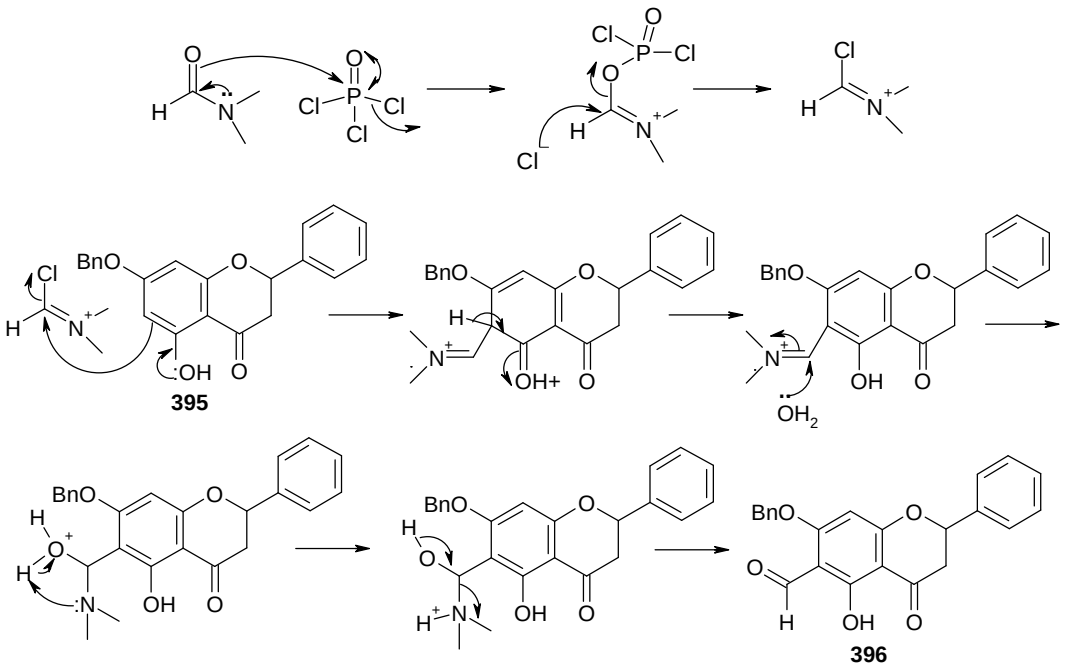
Scheme 7.9 Mechanism suggested for selective debenzylation of 5-hydroxy group (Haraldsson and Baldwin, 1997).

This method seemed ideally suited for this synthesis, as selective deprotection of the 5-hydroxy group of flavanone **393** was required. However, debenzylation using magnesium bromide led to the formation of an unforeseen side product **394**. It is proposed that the reactive phloroglucinol ring reacts with the benzyl bromide formed during the deprotection reaction, resulting in the *C*-benzylated (**394**, Scheme 7.10) as well as the *O*-debenzylated product (**395**). Selective debenzylation of flavanone **393** was successfully achieved in high yields using catalytic hydrogenation over 10% palladium on charcoal to afford flavanone **395** (Plates 99, 100).



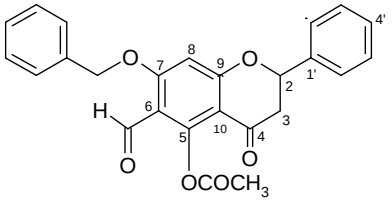
Scheme 7.10 Mechanism suggested for *C*-benzylation of flavanone **392**.

Before formylation of the flavanone was performed, hydroxymethylation was attempted. This reaction proved to be even more problematic than the similar benzylated phloroacetophenone (**391**) hydroxymethylation reaction. The yield was very low and methylation of the aliphatic hydroxy occurred as a side reaction. Since direct hydroxymethylation did not seem to be effective, a Vilsmeier-Haack formylation of the flavanone was proposed (Graybill et al., 1999, Scheme 7.11). Alternative formylation methods were considered, but as formylation using $MgCl_2$ (Hansen and Skattebol, 2005) was unsuccessful for phloroglucinol, the Vilsmeier formylation seemed the better choice.



Scheme 7.11 Mechanism of Vilsmeier formylation of flavanone **395**.

Attempts to use procedures where all the reagents were mixed together simultaneously resulted in a solution from which no product could be isolated. The procedure followed thus involved the separate preparation of the Vilsmeier reagent, to which the flavanone was added. The yields were also low and instead of the major product being substituted in the 6-position (**396**), 8-substitution was preferred (**397**), resulting in the formation of an undesired major product (Scheme 7.8). The position of the aldehyde was determined by acylation of the free hydroxy groups of the two products and determining the NOESY correlations of the 6- (**398**) and 8-acylated (**399**) aldehydes (Plates 101, 102).



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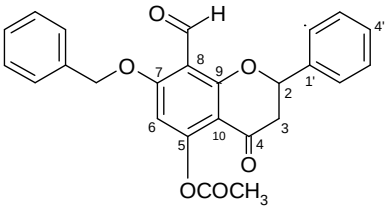


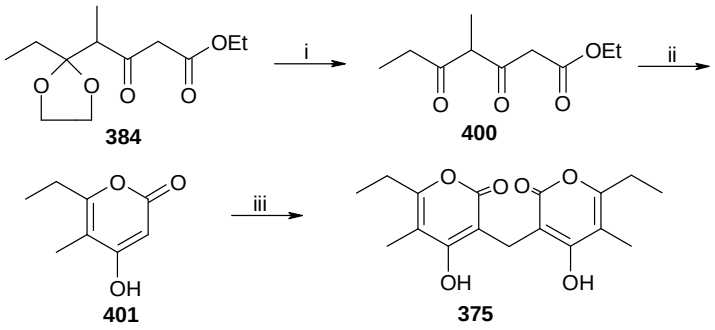
399

Condensation of ester **384** and aldehyde **397** was attempted in the presence of sodium hydride, which (as expected) resulted in the decomposition of the flavanone to the chalcone. The flavanone in general seemed unstable and converted back to the chalcone even in the presence of 50% toluenesulfonic acid (when refluxed). Coupling of the completed flavanone moiety to the ester therefore does not seem feasible.

7.2.4 Preparation of the pyrone moiety

As an alternative, the possibility of attaching the side chain necessary for coupling to the completed pyrone moiety (obtained from β-keto ester **384**) (Scheme 7.12) was investigated. Deprotection of ester **384** was first attempted in the presence of 2 M HCl (Zong et al. 2006) which resulted in a mixture of products. Stirring with 80% acetic acid (Babler et al., 1978) provided much better yields and afforded β-keto ester **400**. Lactone **401** (Plates 103, 104) was obtained in the presence of DBU (Cervello et al., 1990). However, chloromethylation with formaldehyde and HCl did not yield the desired chloromethylated pyrone, but the dimeric lactone **375** (Plates 105, 106) was obtained. Helipyrone (**375**) was also previously synthesised by Ali and co-workers (1982). Hagiwara and co-workers (2001, 2002) experienced similar problems with attaching a halomethyl side chain to the 2-pyrone and eventually obtained the 3-hydroxymethylpyrone *via* a

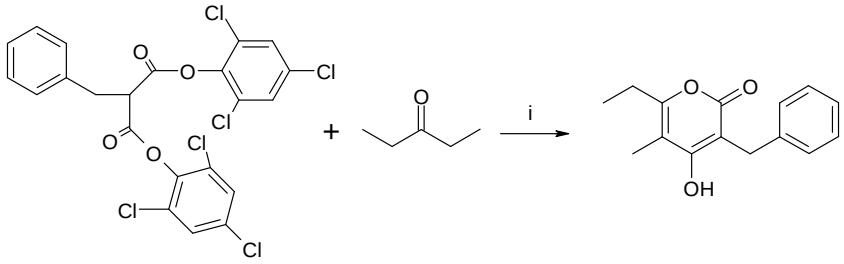
phenylthiomethyl pyrone intermediate (Hagiwara et al., 2001; Hagiwara et al., 2002). As this route would add several more steps to the total synthesis, it was not pursued further.



Scheme 7.12 Synthesis of helipyrone (**375**). Reagents: (i) 80% AcOH, 50 °C, 48 h, 67% (ii) DBU, benzene, reflux, overnight, 53% (iii) $(CH_2O)_n$, HCl, dioxane, rt, 2h, 22%.

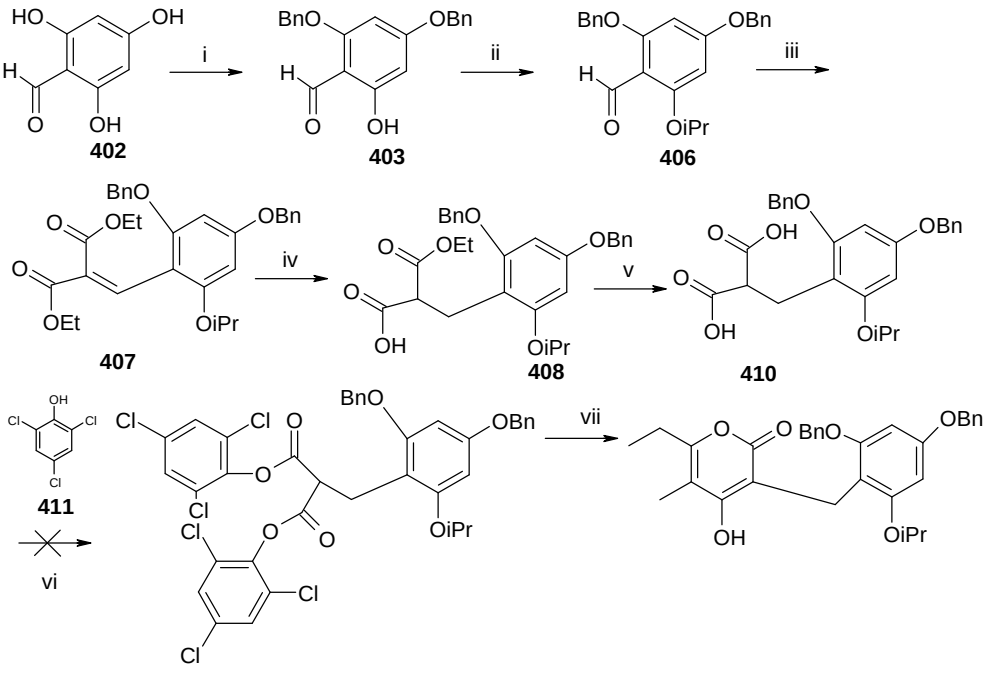
7.2.5 Alternative approach towards pyrone synthesis

A different approach used the route by Kappe and Schmidt (1970) for the synthesis of 3-benzyl-6-ethyl-4-hydroxy-5-methyl-2-pyrone (Scheme 7.13). Diethyl ketone and bis(2,4,6-trichlorophenyl) benzylmalonate is reacted by fusion at approximately 250 °C. Similar reactions were described by Stadlbauer et al. (2001). The reported yields for this type of reaction are very low (5 - 41%, Stadlbauer et al., 2001; Kappe and Schmidt, 1970).



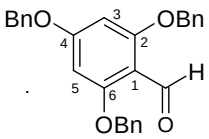
Scheme 7.13 Synthesis of 3-benzyl-6-ethyl-4-hydroxy-5-methyl-2-pyrone by Kappe and Schmidt (1970). Reagents (i) 240 - 245 °C, 30 min.

Based on this approach, the route below was suggested, using 2,4,6-trihydroxybenzaldehyde (**402**) as starting material (Scheme 7.14):



Scheme 7.14 Synthesis of 2-(2,4-dibenzyloxy-6-isopropoxybenzyl)malonic acid (**410**)**.** Reagents: (i) BnBr, K_2CO_3, DMF, rt, overnight, 89% (ii) K_2CO_3, $(CH_3)_2CHBr$, DMF, 71% (iii) AcOH, pyrrolidine, toluene, reflux, 4 h, 63% (iv) $NaBH_4$, EtOH, 50% (v) NaOH, EtOH, H_2O, reflux, 6 h, 83%; (vi) $POCl_3$ (vii) diethylketone, 250 °C, 20 min.

Selective benzylation of 2,4,6-trihydroxybenzaldehyde (**402**) with benzyl bromide in DMF afforded protected aldehyde **403** (Anderson et al., 2005) (a small amount of tribenzylated benzaldehyde **404,** was also isolated). A NOESY experiment was performed on the acetylated dibenzylated product **405** to confirm the substitution pattern of the benzyloxy groups.



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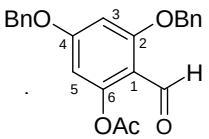
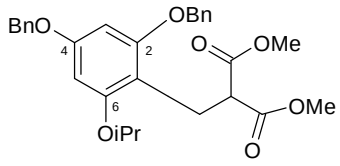


405

Since the remaining free hydroxy-group of **403** would attack the ester and result in the formation of a coumarin during coupling with the ester, isopropyl protection of this group was done to afford 2,4-dibenzyloxy-6-isopropoxybenzaldehyde (**406**). At first, TBDMS

protection in the presence of both diisopropylamine or sodium hydride was attempted for the remaining hydroxy group of **403**, but possibly due to the bulkiness of the group and the hydrogen bonding between this hydroxy and the carbonyl, this protection was not successful. A Knoevenagel condensation of **406** was done with diethyl malonate in the presence of acetic acid and pyrrolidine (Antonioletti et al., 2002) affording alkene **407** (Plates 107-108).

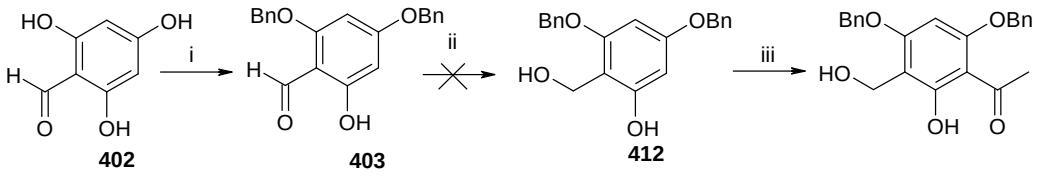
The double bond could not be reduced via hydrogenation, since it would cause cleavage of the benzyl ethers, again resulting in the formation of coumarin side products. The double bond was therefore reduced with sodium borohydride in ethanol. The reaction did not proceed at room temperature, as starting material was reisolated after stirring for 24 h. Refluxing for 3 hours results in the isolation of a mono ester **408** as major product where one of the ester groups is already hydrolysed by the sodium borohydride, while **409** is isolated as minor product. Hydrolysis occurs readily and with high yields when refluxing any of the esters in ethanol with sodium hydroxide to afford diacid **410**. The reaction of the diacid and trichlorophenol **(411)** was attempted, using phosphoryl chloride. This resulted in complete decomposition of the diacid. This route was therefore not pursued any further.



409

7.2.6 Synthesis of alkene **415**

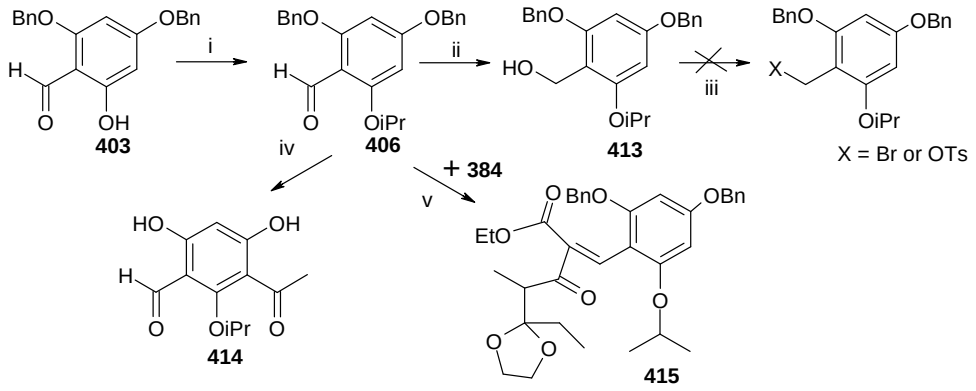
Another approach that was investigated involved starting with 2,4,6-trihydroxybenzaldehyde (**402**), followed by selective benzylation to yield **403**, subsequent reduction of the aldehyde to afford alcohol **412**, and then a Friedel-Crafts acylation reaction to obtain the ketone (Scheme 7.15).



Scheme 7.15 Route utilising 2,4,6-trihydroxybenzaldehyde as starting material. Reagents: (i) BnBr, K_2CO_3, DMF, rt, 12 h, 89% (ii) $NaBH_4$, EtOH (iii) AcCl, $ZnCl_2$.

Selective benzylation was done in the presence of benzyl bromide in DMF (Anderson et al., 2005), to afford the protected aldehyde **403.** However, the reduction of the aldehyde was problematic. Although two 'products' were observed on TLC during the reaction with both sodium borohydride and lithium aluminium hydride, quenching with even mild acid resulted in a very polar product that could not be isolated. The reaction was only quenched with water and the products isolated. The NMR spectra of these products indicated that neither was the desired alcohol, but rather a polymerised product. Reduction of 2,4-dibenzyloxy-6-hydroxybenzaldehyde (**403**) was thus not feasible. Isopropyl protection of the remaining hydroxy was performed to afford 2,4-dibenzyloxy-6-isopropoxybenzaldehyde (**406**) and reduction of this aldehyde yielded the desired alcohol **413** in high yields. However, neither bromination (with several different methods) nor tosylation of this alcohol was successful.

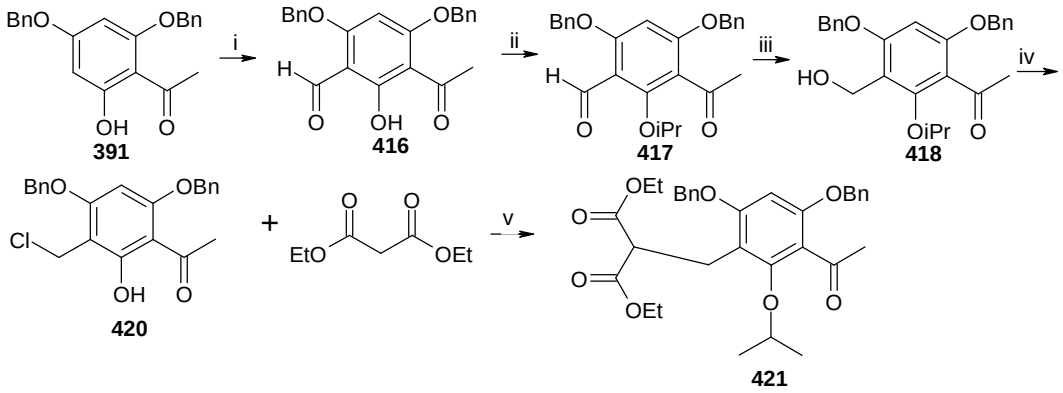
Reactions aimed at acylation of the aromatic ring of **406** also did not yield the desired product - in one case the debenzylated keto-aldehyde **414** was obtained. Arnaudinaud and co-workers (2001) illustrated debenzylation in the presence of $TiCl_4$, so the deprotection observed with $SnCl_4$ was not surprising. Condensation with ester **384** did give the desired alkene **415** (Scheme 7.16, Plates 109, 110). Hydrogenation to reduce the double bond selectively in the presence of the benzyl groups was attempted**,** in the hope that it would be possible to reduce the double bond without debenzylation. After hydrogenation for 2 h at room temperature at 120 kPa, the starting material was recovered quantitatively. In this case reduction with sodium borohydride is not feasible, as it will most probably result in reduction of the ketone.



Scheme 7.16 Synthesis of alkene **415**. Reagents (i) $(CH_3)_2CHBr$, K_2CO_3, DMF, 71% (ii) $NaBH_4$, CH_2Cl_2, -78 - 0 °C, 1 h, 90% (iii) CBr_4, PPh_3, CH_2Cl_2 or TsCl, di-isopropylamine/NaH (iv) $SnCl_4$, CH_2Cl_2, v) AcOH, pyrrolidine, toluene, reflux, 4 h, 28%

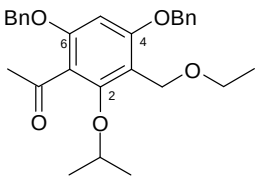
7.2.7 Coupling of phloroacetophenone and β-keto ester moieties

Finally, a decision was made to do a Vilsmeier formylation of ketone **391**, then to attempt selective reduction of the aldehyde of **416** to obtain alcohol **418** followed by bromination or tosylation (Scheme 7.17). It was anticipated that the electron withdrawing acyl group would decrease the reactivity of the ring, enabling halogenation or tosylation of the alcohol, a reaction that was not successful for **413**.



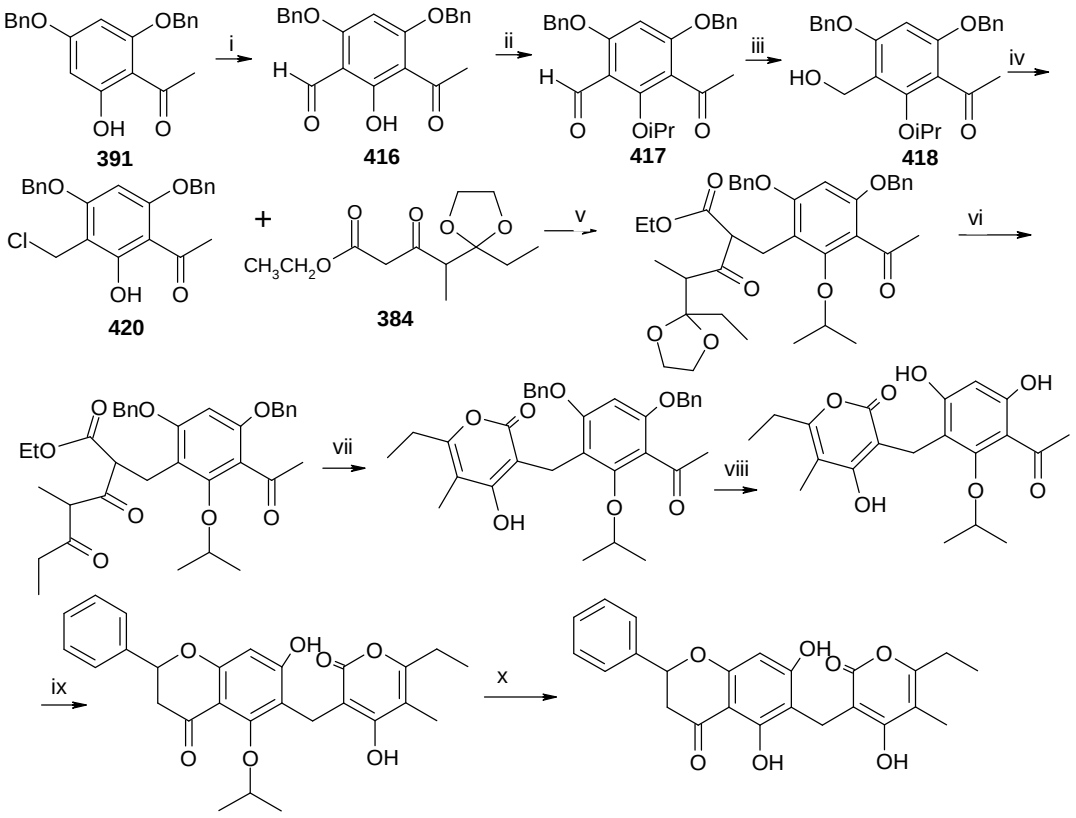
Scheme 7.17 Synthetic route illustrating coupling of phloroglucinol moiety to β-keto ester moiety. Reagents (i) a) DMF, $POCl_3$, CH_3CN, rt 18 h, b) MeOH, H_2O, rt, 4 h , 42% (ii) K_2CO_3, $(CH_3)_2CHBr$, DMF, 58% (iii) $NaBH_4$, CH_2Cl_2, -78-0 °C, 1 h, 62% (iv) $SOCl_2$, CH_2Cl_2, 45 minutes, (quantitative) (v) LDA, THF, 0 °C-rt, 2 days, 54%.

The Vilsmeier formylation gave keto-aldehyde **416** in a 42% yield, while isopropyl protection resulted in the isolation of **417**. Selective reduction of the aldehyde in the presence of the ketone was successful with sodium borohydride at reduced temperatures in the presence of a mixed solvent system. Alcohol **418** (Plates 111-112) was obtained in 62% yield (Ward and Rhee, 1988). Bromination (using several different reagents) was attempted, but failed, as did tosylation. In this case the product from the bromination reaction could be isolated and was identified as the ethyl ether **419**. A possible explanation for this ether formation seems to be that the bromide or tosylate does in fact form, but that it is so reactive that the solvent (ethanol) is nucleophilic enough to displace the bromide and to form the ether. Therefore, it was decided to attempt the formation of a chloride. Indeed,, exposure of alcohol **418** to thionyl chloride afforded chloride **420**. This chloride was not very stable and converts back to the alcohol in the presence of water, or atmospheric moisture.



419

A model reaction, using diethyl malonate, was performed to illustrate the coupling between the chloride **420** and diethyl malonate. This reaction involved the *in situ* preparation of chloride **420** and its immediate addition to a solution of diethyl malonate and LDA in THF. The coupled product **421** (Plates 113-114) was isolated in a 54% yield. It should be feasible to synthesise lepidissipyrone (**19**) by using the route given in Scheme 7.18. We have shown that precursors **420** and **384** can be prepared and that a 1,3-dicarbonyl derivative can be linked to precursor **420** (Scheme 7.7, Scheme 7.19). However, due to time limitations, the final steps in this synthesis could not be completed.



Scheme 7.18 Synthetic route proposed for the synthesis of lepidissipyrone (**19**). Reagents (i) a) DMF, $POCl_3$, CH_3CN, rt 18 h, b) MeOH, H_2O, rt, 4 h , 42% (ii) $(CH_3)_2CHBr$, K_2CO_3, DMF, 58% (iii) $NaBH_4$, CH_2Cl_2, -78-0 °C, 1 h, 62% (iv) $SOCl_2$, CH_2Cl_2, 45 minutes, (quantitative) (v) LDA, THF, 0 °C-rt, (vi) 80% AcOH (vii) DBU, benzene (viii) a) PhCHO, NaH, 0 °C b) DBU, CH_2Cl_2, rt (ix) isopropyl deprotection.

7.3 Conclusion

The aim of this investigation was to investigate a methodology that would provide access to the synthesis of lepidissipyrone (**19**) and other phloroglucinol-substituted α-pyrones (Fig. 7.1). The highly reactive nature of the electon-rich phloroglucinol nucleus posed several problems during this synthesis and any proposed synthetic route should take cognisance of this problem. One of the pyrones, helipyrone (**375**), has been prepared successfully. A successful strategy towards the coupling of a 1,3-dicarbonyl derivative to the benzylic position of a phloroglucinol derivative has been illustrated. Both the flavanone and pyrone moieties present in lepidissipyrone (**19**) have been synthesised successfully. It

is believed that this approach can be used to synthesise phloroglucinol-pyrones that are associated with several biological activities.

7.4 Experimental

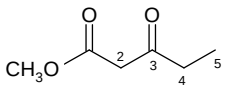
7.4.1 General materials and methods

Solvents were dried by distillation under N_2. DMF was distilled from molecular sieves and CH_2Cl_2 and CH_3CN from CaH_2. These solvents were stored under nitrogen on molecular sieves. THF and Et_2O were freshly distilled from sodium with benzophenone ketyl as indicator for THF. Dry reactions were performed in oven-dried glassware under an atmosphere of N_2. Reagents were purchased from Sigma Aldrich. Column chromatography was performed on Merck silica gel 60. A chromatotron (model 7924, Harrison Research) was used for centrifugal chromatography. Thin-layer chromatography (TLC) was done on precoated Kieselgel 60 F_{254} plates (Merck or Machery-Nagel). Detection was done by UV (254 nm) followed by staining with an anisaldehyde solution, prepared as follows: Absolute ethanol (465 ml) was cooled in an ice bath. Acetic acid (5 ml), sulphuric acid (17 ml) and *p*-anisaldehyde (13 ml) was added and the solution mixed and stored in a fridge.

Melting points were determined with a Kofler hot-stage apparatus and are uncorrected. Mass spectrometric data were collected on a time-of-flight Waters LCT Premier mass spectrometer using electrospray ionization in the positive or negative mode. Mass spectrometric data for some of the ester precursors was obtained using GC-MS (Thermo Finnigan PolarisQ Ion Trap with TRACE GC/MS). Infrared spectra were recorded on a Perkin-Elmer Spectrum 1 Series FT-IR spectrophotometer using KBr discs for solids and sodium chloride discs for oils or directly on a Bruker Alpha FT-IR. NMR spectra were recorded on a Varian Unity Inova 500 spectrometer with a 5 mm SW/Z-PFG probe (all spectra recorded at 25 °C) or Bruker Avance III 400 or 500 spectrometers with 5 mm BBO-Z probes, all spectra recorded at 30 °C). ^{1}H NMR spectra were recorded at 400 or 500 MHz and ^{13}C spectra at 100 or 125 MHz. Chemical shifts (δ) are quoted in parts per million (ppm) and referenced to a residual solvent peak ($CDCl_3$, 7.26 and 77.0 ppm or CD_3OD, 3.31 and 49 ppm or DMSO-d_6, 2.5 and 39.5 ppm).

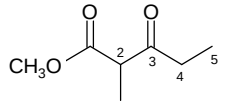
7.4.2 Experimental procedures and physical data of compounds

Methyl 3-oxopentanoate (**386)**



To a solution of Meldrum's acid (2 g, 13.9 mmol) in 25 ml dichloromethane at -20 °C was added pyridine (2.26 ml, 28.0 mmol) and dropwise, propionyl chloride (24 ml, 28.0 mmol) under nitrogen. The reaction was stirred for 1 h between -10 °C and -20 °C and then for another hour at 5 °C. The reaction mixture was then acidified with 1 M HCl and extracted with dichloromethane. The dichloromethane layers were combined, washed with 1 M HCl and dried over anhydrous magnesium sulphate. The solvent was evaporated and the unpurified product was used in the next stage. The acylated Meldrum's acid was refluxed for 3 h in methanol and after evaporation of the solvent the crude product was purified on silica using dichloromethane:petroleum ether 8:2 as eluent to yield 1.2 g (67%) of β-oxoester **386** as a yellow oil (method from Scribner et al., 1978; Lokot et al., 1999): ^{1}H NMR (lit. Jung and Hagenah, 1987) (500 MHz, $CDCl_3$) δ_H 1.07 (3H, *t*, *J* = 7.3 Hz H-5,), 2.56 (2H, *q*, *J* = 7.30 Hz, H-4), 3.45, (2H, *s*,H-2), 3.72 (3H, *s*, $OC\underline{H_3}$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 7.5 (C-5), 36.3 (C-4), 48.7 (C-2), 52.3 (OCH_3), 167.7 (C-1), 203.2 (C-3).

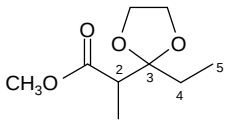
Methyl 2-methyl-3-oxopentanoate (387)



The method of Sung and Wu (2001) was modified to synthesise compound **387**. To a solution of propionyl chloride (3 ml, 34.5 mmol) dissolved in 10 ml anhydrous ether, was added triethylamine (4.8 ml, 34.5 mmol) in anhydrous ether (69 ml) dropwise over a period of 1 hour with vigorous stirring at 0 °C under nitrogen. The solution was stirred for two days at room temperature. Triethylammonium chloride precipitated as a thick white solid. The ether was evaporated and the residue dissolved in methanol and sodium acetate (4 mg) was added. The resulting solution was then refluxed under nitrogen for one more day. After evaporation of the methanol, the residue was dissolved in water and ether. The organic and aqueous layers were separated and the aqueous layer extracted twice more with ether. After combining of the ether fractions and drying with magnesium sulphate, the

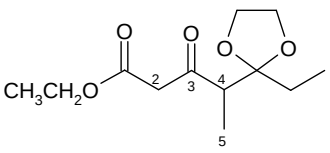
product was purified by chromatotron with a mobile phase of hexanes:ethyl acetate 15:1 to give β-keto ester **387** as a colourless oil 2.58 g (52%): ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 1.04 (3H, *t*, *J* = 7.4 Hz, H-5), 1.31 (3H, *d*, *J* = 7.2 Hz, 2-C$\underline{H}_3$), 2.54 (2H, *dq*, *J* = 14.5 Hz, 7.2 Hz, H-4), 3.52 (1H, *q*, *J* = 7.2 Hz, H-2), 3.70 (3H, *s*, OC$\underline{H}_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 7.6 (C-5), 12.8 (2-CH_3), 34.6 (C-4), 52.27 (C-2), 52.31 (O$\underline{C}H_3$), 171.0 (C-1), 206.3 (C-3); GC-MS *m/z* 144.03 (calculated for $C_7H_{12}O_3$ 144.07864); RT = 11.37 min.

Methyl 2-(2-ethyl-1,3-dioxolan-2-yl)propanoate (389)



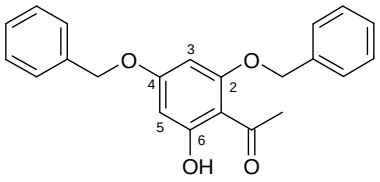
A solution of β-keto ester **387** (950 mg, 6.6 mmol), ethane-1,2-diol (1.1 ml, 19.8 mmol) and toluene-4-sulfonic acid monohydrate (10 mg) was heated to reflux in toluene using a Dean Stark apparatus overnight (a modified method of Bates et al., 1988). The cooled solution was neutralised with saturated aqueous sodium hydrogen carbonate and the organic and aqueous layers separated. The aqueous layer was further extracted with dichloromethane. The combined organic phases were dried over anhydrous magnesium sulphate and the solvent evaporated to give the crude acetal. Chromatography of this through silica gel, using hexanes:ethyl acetate (15:1) as eluent yielded 819 mg (66%) of the desired product **389** as a colourless oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 0.89 (3H, *t*, *J* = 7.4 Hz, H-5), 1.19 (3H, *d*, *J* = 7.2 Hz, 2-C$\underline{H}_3$), 1.77 (2H, *m*, H-4), 2.85 (1H, *q*, *J* = 7.2 Hz, H-2), 3.68 (3H, *s*, OC$\underline{H}_3$), 3.95-4.03 (4H, *m*, OC$\underline{H}_2$C$\underline{H}_2$O); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 7.2 (C-5), 12.5 (2-$\underline{C}H_3$), 27.9 (C-4), 46.6 (C-2), 51.7 (O$\underline{C}H_3$), 65.6 (O$\underline{C}H_2\underline{C}H_2$O), 65.7, 111.5 ($\underline{C}CH_2CH_3$), 173.9; HRESIMS (positive ionization mode), *m/z* 189.1132 $[M+H]^+$ (calc. for $C_9H_{17}O_4$ 189.11268); IR (NaCl) ν 2981, 1738, 1212, 1078 cm^{-1}.

Ethyl 4-(2-ethyl-1,3-dioxolan-2-yl)-3-oxopentanoate (384)



Ethyl acetate (2.0 ml, 21.2 mmol), in THF (5 ml) was added dropwise to a solution of lithium diisopropylamide (prepared *in situ* from 4.5 ml of diisopropylamine and 21 ml of a 1.5 M solution BuLi) in dry THF (50 ml) at -78 °C under nitrogen. The resulting solution was stirred for 1 hour at 0 °C and cooled to -78 °C before β-keto-ester **389** (2.0 g, 10.6 mmol) dissolved in THF (5 ml) was added dropwise. The temperature was allowed to rise to 0 °C and the reaction mixture stirred for 4 hours. The reaction was quenched with water (50 ml) and diluted with ether (50 ml). The layers were separated and the aqueous layer was extracted twice with 50 ml ether. The aqueous layer was then acidified with 1 M HCl and extracted with ether twice more. The combined ether extracts were washed with water, brine, dried (over anhydrous magnesium sulphate) and concentrated *in vacuo* to afford a yellow oil. The desired product was obtained after purification on two subsequent chromatorons using hexanes:ethyl acetate (199:1) as eluent to afford 962 mg (37%) of the colourless oil **384**: ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 0.86 (3H, *t*, *J* = 7.4 Hz, $CCH_2C\underline{H}_3$), 1.13 (3H, *d*, *J* = 6.9 Hz, H-5), 1.26 (3H, *t*, *J* = 7.1 Hz, $OCH_2C\underline{H}_3$), 1.65 (2H, *dq*, *J* = 7.3 Hz, $C\underline{CH}_2CH_3$), 3.10 (1H, *q*, *J* = 6.9 Hz**,** H-4), 3.61 (2H, *dd*, *J* = 15.9 Hz, H-2), 3.95-3.99 (4H, *m*, $OC\underline{H}_2C\underline{H}_2O$), 4.17 (2H, *dq*, *J* = 7.4 Hz, $O\underline{CH}_2CH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 7.09 ($CCH_2\underline{C}H_3$), 11.68 (C-5), 14.1 ($OCH_2\underline{C}H_3$), 27.8 ($C\underline{C}H_2CH_3$), 50.1 (C-4), 52.5 (C-2), 61.1 ($O\underline{C}H_2CH_3$), 65.2, 65.4 ($O\underline{CH}_2\underline{CH}_2O$), 111.9 ($\underline{C}CH_2CH_3$), 167.6 (C-1), 204.4 (C-3); GC-MS *m/z* 215.13 $[M-29]^-$, (calculated for $C_{10}H_{15}O_5$ 215.09); RT = 16.07 min; IR (NaCl) ν 2981, 1741, 1713, 1308, 1033 cm^{-1}.

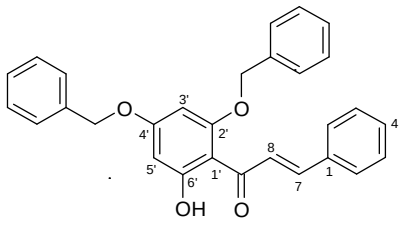
2,4-Dibenzyloxy-6-hydroxyphloroacetophenone (391)



2,4-Dibenzyloxy-6-hydroxyphloroacetophenone **391** was prepared according to the method of Tsukayama et al. (2004). K_2CO_3 (4 g, 29.8 mmol) was added to a solution of 2,4,6-trihydroxyphloroacetophenone (1 g, 5.9 mmol, dried by leaving the monohydrate at 120 °C in an oven for 24 h) in DMF (9 ml), followed by the dropwise addition of a solution of

benzyl chloride (1.45 ml, 12.6 mmol) in DMF (2 ml) while stirring under nitrogen. The reaction was heated to 80 - 90 °C, stirred for 2 h, cooled down and quenched by the addition of 1 M HCl (until the reaction mixture was acidic, as determined with litmus paper) and diluted with dichloromethane. The layers were separated and the aqueous phase extracted with dichloromethane (3 x). The dichloromethane fractions were pooled and washed with copious amounts of water (5 x) to remove the DMF and dried by the addition of anhydrous $MgSO_4$. The solvent was evaporated and the resulting product purified with column chromatography using hexanes:ethyl acetate (9:1) as eluent to afford 1.7 g (82%) of **391** as a white solid, recrystallised from acetone:methanol (1:1) to afford colourless needles: mp 93-94 °C, (lit. Tsukayama et al., 1989, 100-102°C); (lit. Kumazawa et al., 2001, 108-109°C), (lit. Dulcire et al., 1990, 112-115°C); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 2.56 (3H, *s*, COC<u>H</u>$_3$), 5.060 (2H, *s*, OC<u>H</u>$_2$Ph), 5.065 (2H, *s*, OC<u>H</u>$_2$Ph), 6.10 (1H, *d*, *J* = 2.3 Hz, H-3 or H-5), 6.17 (1H, *d*, *J* = 2.3 Hz, H-3 or H-5), 7.34 – 7.42 (10H, *m*, ArH), 14.03 (1H, *s*, OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 33.2 (CO<u>C</u>H$_3$), 70.2 (O<u>C</u>H$_2$Ph), 71.1 (O<u>C</u>H$_2$Ph), 92.3 (C-3 or C-5), 94.7 (C-3 or C-5), 106.3 (C-1), 127.6, 127.9, 128.3, 128.4, 128.66, 128.69 (Ar<u>C</u>), 135.6, 135.8 (CH$_2$<u>C</u>Ar), 161.9, 165.0, 167.5 (C-2, C-4, C-6), 203.1 ($COCH_3$); HRESIMS (positive ionization mode), *m/z* 349.1430 $[M+H]^+$ (calc. for $C_{22}H_{21}O_4$ 349.1440); IR (KBr) ν 1619; 1593; 1271; 1171; 1104 cm^{-1}.

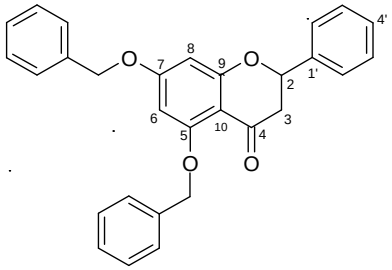
2'4'-Dibenzyloxy-6'-hydroxychalcone (392)



The method of Hatakeyama et al., 2005 was used in the preparation of compound **392**. 2,4-Dibenzyloxy-6-hydroxyacetophenone (**391**) (6.2 g, 17.8 mmol) was dissolved in 50 ml DMF under nitrogen. The solution was cooled down to 0 °C (in an ice bath) and sodium hydride (1.28 g, 53.5 mmol) was slowly added while stirring. Benzaldehyde (2.2 ml, 21.4 mmol) dissolved in 5 ml DMF was added dropwise. The reaction was stirred for a further 45 minutes at 0 °C. It was quenched by the addition of ice and acidified with 1 M HCl (until blue litmus turned red). The bright yellow filtrate that was formed were filtered to

quantitatively (7.8 g) yield the product **392** that was recrystallised from hexanes:ethyl acetate to afford yellow plates: mp 120-122 °C, (lit. Drewes et al., 2008, 119°C); ^{1}H NMR ($CDCl_3$) δ_H 5.07 (2H, *s*, OC<u>H</u>$_2$Ph), 5.12 (2H, *s*, OC<u>H</u>$_2$Ph), 6.18 (1H, *d*, *J* = 2.3 Hz, H-3'), 6.23 (1H, *d*, *J* = 2.3 Hz, H-5'), 7.06-7.51 (15H, *m*, Ar<u>H</u>), 7.72 (1H, *d*, *J* = 15.7 Hz, H-7), 7.88 (1H, *d*, *J* = 15.7 Hz, H-8), 14.6 (1H, *s*, ArO<u>H</u>); ^{13}C NMR ($CDCl_3$) δ_C 70.3 (O<u>C</u>H$_2$Ph), 71.4 (O<u>C</u>H$_2$Ph), 92.5 (C-3'), 95.0 (C-5'), 106.3 (C-1'), 127.5 (C-8), 127.6, 128.4, 128.6, 128.71, 128.75, 128.9 (Ar<u>C</u>), 135.3, 135.4, 135.8 (C-1, OCH$_2$<u>C</u>Ar), 142.7 (C-7), 161.7 (C-2'), 165.2 (C-4'), 168.8 (C-6'), 192.6 (C=O) (lit. Drewes et al., 2008); HRESIMS (positive ionization mode), *m/z* 437.1747 $[M+H]^+$ (calc. for $C_{29}H_{25}O_4$ 437.1753); IR (KBr) ν 1624; 1344; 1226; 1207; 1155, 739 cm^{-1}.

5,7-Dibenzyloxyflavanone (393)



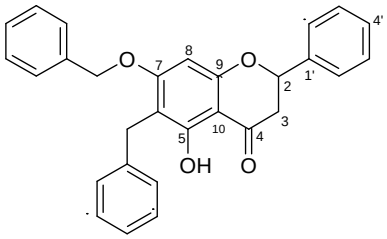
Chalcone **392** (630 mg, 1.44 mmol) was dissolved in acetic acid (40 ml) and refluxed for 6 hours (Solladíe et al., 1999). The reaction was quenched by the addition of water (40 ml). The aqueous phase was extracted five times with dichloromethane. The organic phase was washed five times with ice water, the fractions combined and dried with magnesium sulphate and the solvent evaporated. Since the starting material was still present, the product was purified with a chromatotron using hexanes:ethyl acetate (14:1) to afford **393** as a white solid 201 mg (32%).

<u>Alternative method</u>:

Chalcone **392** (7.69 g, 17.6 mmol) was dissolved in dichloromethane (50 ml) and stirred for 10 minutes before DBU (2.44 ml, 17.6 mmol) was added dropwise. The reaction was stirred for 2 days and was quenched by acidifying with 1 M HCl. The organic and aqueous layers were separated and the aqueous layer further extracted with dichloromethane. The organic layers were combined, washed with water and brine and dried over magnesium

sulphate. The solvent was evaporated and the product cleaned with a chromatotron using hexanes:ethyl acetate (14:1) as eluent to yield 3.8 g of compound **393** (49%) (2.5 g of chalcone **392** was recovered). Flavanone **393** was recrystallised from dichloromethane: hexanes to afford fine white needles: mp 122-124 °C (lit. Drewes et al., 2008, 115°C); ^{1}H NMR ($CDCl_3$) δ_H 2.81 (1H, *dd*, *J* = 16.6 Hz, 3.0 Hz, H-3a), 3.05 (1H, *dd*, *J* = 16.6 Hz, 13.3 Hz, H-3b), 5.05 (2H, *s*, OC<u>H</u>$_2$Ph), 5.17 (2H, *s*, OC<u>H</u>$_2$Ph), 5.43 (1H, *dd*, *J* = 13.3 Hz, 3.0 Hz , H-2), 6.24 (1H, *d*, *J* = 2.3 Hz, H-6), 6.26 (1H, *d*, *J* = 2.3 Hz, H-8), 7.29-7.59 (15H, *m*, Ar<u>H</u>); ^{13}C NMR ($CDCl_3$) δ_C 45.7 (C-3) 70.3 (O<u>C</u>H$_2$Ph), 70.4 (O<u>C</u>H$_2$Ph), 79.3 (C-2), 94.8 (C-8), 95.2 (C-6), 106.5 (C-10), 126.2, 126.5, 127.6, 127.7, 128.4, 128.6, 128.69, 128.72, 128.8 (Ar<u>C</u>), 135.8, 136.4 (CH$_2$<u>C</u>Ar), 138.8 (C-1'), 161.1 (C-5), 164.9 (C-7, C-9), 188.9 (C-4); HRESIMS (positive ionization mode), *m/z* 437.1772 $[M+H]^+$ (calc. for $C_{29}H_{25}O_4$ 437.1753); IR (KBr) ν 1667; 1606; 1572; 1259; 1213; 1164; 1120; 696 cm^{-1}.

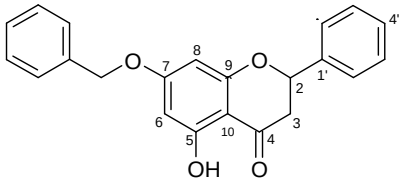
6-Benzyl-7-benzyloxy-5-hydroxy-2-phenylchroman-4-one (**394**)



The methods employed by Baldwin et al. (1986) and Haraldsson and Baldwin (1997) were employed for the deprotection of flavanone **393**. Anhydrous ether (2 ml) and benzene (2 ml) was added to magnesium turnings (80 mg, 3.3 mmol). The flask was cooled to 0 °C, and bromine (84 μl, 1.6 mmol) was added carefully. When the reaction has started (effervescence after the addition of a few drops of bromine), stirring was commenced and the addition of the bromine continued until complete. The ice bath was removed and the solution gently refluxed for several minutes until colourless, and then allowed to cool. The magnesium bromide solution was then slowly added to the protected flavanone **393** (717 mg, 1.6 mmol) in dry benzene (10 ml) at room temperature. During addition the reaction mixture was stirred very efficiently and after completion of the addition the reaction was refluxed overnight. The reaction was then cooled down and 1 M HCl (20 ml) was added and the resulting solution boiled for 30 minutes. The mixture was allowed to cool and

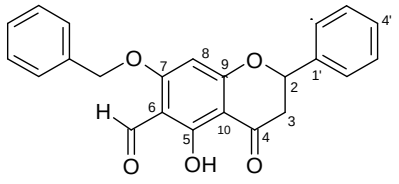
extracted with dichloromethane to yield 343 mg (50% C-benzylated **394** and 50% O-debenzylated product **395** , yields approximated using NMR); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 2.87 (1H, dd, *J* = 17 Hz, 3 Hz, H-3a), 3.07 (1H, *dd*, *J* = 17 Hz, 13 Hz, H-3b), 3.97 (2H, *dd*, *J* = 14.4 Hz, CC$\underline{H_2}$Ph), 5.10 (2H, *s*, OC$\underline{H_2}$Ph), 5.43 (1H, *dd*, *J* = 13 Hz, 3.3 Hz, H-2), 6.20 (1H, *s*, H-6 or H-8), 7.17-7.48 (10H, *m*, Ar$\underline{H}$), 12.18 (1H, *s*, OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 28.4 (C$\underline{C}H_2$Ph), 70.4 (O$\underline{C}H_2$Ph), 78.8 (O$\underline{C}H_2$Ph), 93.7 (C-6 or C-8), 125.6, 125.9, 127.3, 128.0, 128.1, 128.56, 128.59, 128.60, 128.75 (Ar$\underline{C}$), 135.8, 138.7, 141.3 (C-1', $CH_2\underline{C}$Ar), 159.2, 162.9, 164.8 (C-5, C-7, C-9), 196.3 ($\underline{C}OCH_3$); HRESIMS (negative ionization mode), *m/z* 435.1589 $[M-H]^-$ (calc. for $C_{29}H_{23}O_4$ 435.1596).

7-Benzyloxy-5-hydroxyflavanone (395)



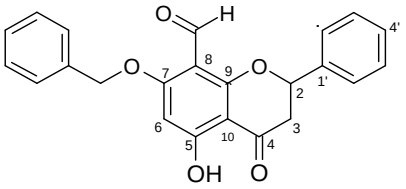
The dibenzylated flavanone **393** (1.4 g, 3.2 mmol) was dissolved in dichloromethane:ethyl acetate (1:1) (10 ml). A 5% palladium on carbon catalyst was added and the reaction stirred at room temperature under an atmosphere of hydrogen (130 kPa) for 25 minutes. The catalyst was filtered off and the product cleaned with a chromatotron, using hexanes: ethyl actetate (14:1) as solvent system to yield 996 mg (90%) of **395** that was recrystallised from hexanes:ethyl acetate to afford colourless needles: mp 84-85 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 2.82 (1H, *dd*, *J* = 17.1 Hz, 3.1 Hz, H-3a), 3.09 (1H, *dd*, *J* = 17.1 Hz, 13.0 Hz, H-3b), 5.07 (2H, *s*, O$\underline{CH_2}$Ph), 5.42 (1H, *dd*, *J* = 13.0 Hz, 3.0 Hz, H-2), 6.14 (1H, *d*, *J* = 2.3 Hz, H-6 or H-8), 6.16 (1H, *d*, *J* = 2.3 Hz, H-6 or H-8), 7.33-7.47 (10 H, *m*, Ar$\underline{H}$), 12.0 (1H, *s*, Ar$\underline{OH}$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 43.4 (C-3), 70.3 (O$\underline{C}H_2$Ph), 79.2 (C-2), 94.9, 96.0 (C-6 and C-8), 103.3 (C-10), 126.1, 127.4, 128.3, 128.7, 128.8 (Ar$\underline{C}$), 135.7, 138.3 ($CH_2\underline{C}$Ar, C-1'), 162.8, 164.1, 167.0 (C-5, C-7, C-9), 195.7 (C-4); HRESIMS (positive ionization mode), *m/z* 347.1286 $[M+H]^+$ (calc. for $C_{22}H_{19}O_4$ 347.1283) IR (KBr) ν 1635; 1574; 1374; 1301; 1160 cm^{-1}.

7-Benzyloxy-5-hydroxy-4-oxo-2-phenylchroman-6-carbaldehyde (396)



N,*N*-Dimethylformamide (250 µl, 3.2 mmol) was cooled to 0 °C and distilled $POCl_3$ (297 µl, 3.2 mmol) was added. The light orange solution was stirred vigorously for approximately 20 minutes until the reagent solidified. Distilled acetonitrile (2 ml) was added until the white solid dissolved. The reaction mixture was stirred for a further 45 minutes at room temperature under nitrogen. Flavanone **395** (552 mg, 1.6 mmol) in acetonitrile (2 ml) was added *via* syringe to the flask and the mixture was stirred for 18 h at room temperature, where after 5 ml of a 2:1 methanol:water solution was added for imine hydrolysis that occurred over 4 h at room temperature. The product was purified on a chromatotron with hexanes:ethyl acetate 14:1 as eluent. Compound **396** was obtained as the minor product (53.8 mg, 9% of cream solid). ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 2.89 (1H, dd, *J* = 17.0 Hz, 3.1 Hz, H-3a), 3.12 (1H, *dd*, *J* = 17.0 Hz, 13.2 Hz, H-3b), 5.19 (2H, *s*, OC<u>H</u>$_2$Ph), 5.50 (1H, *dd*, *J* = 13.2 Hz, 3.1 Hz, H-2), 6.14 (1H, *s*, H-8), 7.36-7.46 (10H, *m*, Ar<u>H</u>), 10.34 (1H, *s*, C<u>H</u>O); ^{13}C NMR (125 MHz, $CDCl_3$) 71.0 (O<u>C</u>H$_2$Ph), 79.9 (C-2), 92.4 (C-8), 126.2, 127.2, 128.6, 128.9, 129.0, 129.2 (Ar<u>C</u>), 134.8, 137.5 (C-1', CH_2<u>C</u>Ar), HRESIMS (positive ionization mode), *m/z* 375.1227 $[M+H]^+$ (calc. for $C_{23}H_{19}O_5$ 375.12325).

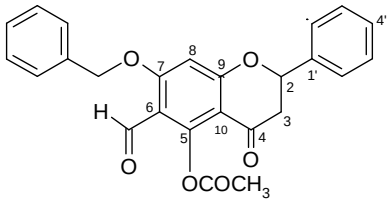
7-Benzyloxy-5-hydroxy-4-oxo-2-phenylchroman-8-carbaldehyde (397)



N,*N*-Dimethylformamide (250 µl, 3.2 mmol) was cooled to 0 °C and distilled $POCl_3$ (297 µl, 3.2 mmol) was added. The light orange solution was stirred vigorously for approximately 20 minutes until the reagent solidified. Distilled acetonitrile (2 ml) was

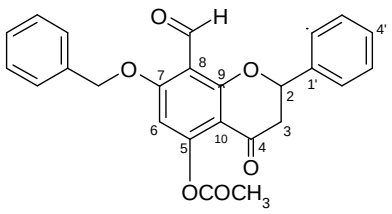
added until the white solid dissolved. The reaction mixture was stirred for a further 45 minutes at room temperature under nitrogen. Flavanone **395** (552 mg, 1.6 mmol) in acetonitrile (2 ml) was added *via* syringe to the flask and the mixture was stirred for 18 h at room temperature, where after 5 ml of a 2:1 methanol: water solution was added for imine hydrolysis that occurred over 4 h at room temperature. The product was purified on a chromatotron with hexanes: ethyl acetate 14:1 as eluent. Aldehyde **397** was obtained as an amorphous cream solid 195 mg (37 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 3.01 (1H, *dd*, *J* = 17.2 Hz, 3.6 Hz, H-3a), 3.13 (1H, *dd*, *J* = 17.2 Hz, 12.1 Hz, H-3b), 5.21 (2H, *s*, OC$\underline{H}_2$Ph), 5.62 (1H, *dd*, *J* = 12.1 Hz, 3.6 Hz, H-2), 6.17 (1H, *s*, H-6), 7.33 – 7.51 (10H, *m*, Ar$\underline{H}$), 10.39 (1H, *s*, C$\underline{H}$O), 12.71 (1H, *s*, OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 42.3 (C-3), 71.0 (O$\underline{C}H_2$Ph), 79.4 (C-2), 94.1 (C-6), 102.6 (C-10), 125.8, 127.0, 127.2, 127.3, 128.3, 128.6, 128.8, 128.9, 129.0 (Ar$\underline{C}$), 135.1, 137.5 (C-1', $CH_2\underline{C}$Ar), 164.7, 167.9, 168.4 (C-5, C-7, C-9), 185.9 (C-4), 196.0 ($\underline{C}$HO); HRESIMS (positive ionization mode), *m/z* 375.1218 $[M+H]^+$ (calc. for $C_{23}H_{19}O_5$ 375.1232); IR (directly) ν 1683, 1628, 1572, 732, 693 cm^{-1}.

7-Benzyloxy-6-formyl-4-oxo-2-phenylchroman-5-yl acetate (398)



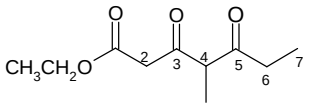
Aldehyde **396** (50 mg, 0.2 mmol) was stirred overnight with pyridine (2 ml) and acetic anhydride (2 ml). Ice was added to the reaction and the precipitate washed with cold water to quantitatively yield **398** as a light brown solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 2.45 (3H, *s*, COC$\underline{H}_3$), 2.80 (1H, *dd*, *J* = 16.6 Hz, 2.6 Hz, H-3a), 3.07 (1H, *dd*, *J* = 16.6 Hz, 13.3 Hz, H-3b), 5.19 (1H, *s*, OC$\underline{H}_2$Ph), 5.52 (1H, *dd*, 13.3 Hz, 2.6 Hz, H-2), 6.57 (1H, *s*, H-8), 7.37-7.47 (10H, *m*, Ar$\underline{H}$), 10.36 (1H, *s*, C$\underline{H}$O); HRESIMS (negative ionization mode), *m/z* 415.1185 $[M-H]^-$ (calc. for $C_{25}H_{19}O_6$ 415.1182).

7-Benzyloxy-8-formyl-4-oxo-2-phenylchroman-5-yl acetate (399)



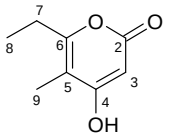
Aldehyde **397** (100 mg, 0.3 mmol) was stirred overnight with pyridine (2 ml) and acetic anhydride (2 ml). Ice was added to the reaction and the precipitate washed with cold water to yield 98 mg (79%) of **399** as a light brown solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 2.41 (3H, *s*, COC<u>H</u>$_3$), 2.89 (1H, *dd*, *J* = 16.7 Hz, 3.1 Hz, H-3a), 3.04 (1H, *dd*, *J* = 16.7 Hz, 13.0 Hz, H-3b), 5.23 (2H, *s*, OC<u>H</u>$_2$Ph), 5.61 (1H, *dd*, *J* = 13.0 Hz, 3.1 Hz, H-2), 6.43 (1H, *s*, H-6), 7.30-7.49 (10H, *m*, Ar<u>H</u>), 10.48 (1H, *s*, C<u>H</u>O); HRESIMS (positive ionization mode), *m/z* 417.1348 $[M+H]^+$ (calc. for $C_{25}H_{21}O_6$ 417.1338).

Ethyl 4-methyl-3,5-dioxoheptanoate (400)



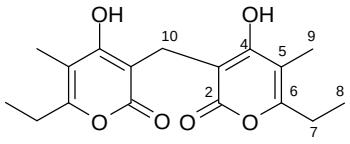
β-keto-ester **384** (393 mg, 1.6 mmol) was dissolved in 80% acetic acid (10 ml) and stirred at 50 °C for 2 days (Reaction progress monitored by GC-MS). Cold water (10 ml) and ether (20 ml) was then added and the layers separated. The aqueous layer was extracted twice more with ether. The ether layers were combined and the excess water removed with magnesium sulphate. The solvent was evaporated the product purified on the chromatotron with petroleum ether:ethyl acetate (19:1) to yield a yellow oil, 397 mg (67%) of **400**: ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 0.96 (3H, *t*, *J* = 7.2 Hz, H-7), 1.17 (3H, *t*, *J* = 7.2 Hz, OCH_2C<u>H</u>$_3$), 1.24 (3H, *d*, *J* = 7.1 Hz, 4-C<u>H</u>$_3$), 2.41 (2H, *dq*, H-6), 3.41 (2H, *dd*, *J* = 15.8 Hz, H-2), 3.77 (1H, *q*, *J* = 7.0 Hz, H-4), 4.08 (2H, *q*, O<u>C</u>$H_2$$CH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 7.5 (C-7), 12.6 (4-<u>C</u>H_3), 14.0 (OCH_2<u>C</u>H_3), 34.7 (C-6), 47.7 (C-2), 60.0, 61.4, (O<u>C</u>H_2CH_3, C-4), 167.0 (C-1), 199.8 (C-3), 207.4 (C-5); GC-MS *m/z* 185.01 $[M-15]^-$ (calculated for $C_9H_{13}O_4$ 185.08), RT = 16.07 min; IR (NaCl) ν 2984, 1727, 1259 cm^{-1}.

6-Ethyl-4-hydroxy-5-methyl-2-pyrone (401)



To β-ketoester **400** (278 mg, 1.4 mmol) in benzene was added 1.2 equivalents of DBU (250 µl, 1.7 mmol). The mixture was refluxed overnight and quenched by acidifying with 1 M HCl. The aqueous phase was extracted with ethyl acetate, the organic fractions combined and dried with magnesium sulphate. The product 313 mg (53%) was obtained after cleaning with a short silica column using hexanes and then ethyl acetate as eluents. It was recrystallised from dioxane to yield **401** as colourless needles: mp 112-113°C (lit. Kappe and Schmidt, 1970, 156-157°C; lit. Ali et al., 1982, 155°C); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 1.19 (3H, *t*, *J* = 7.5 Hz, H-8), 1.93 (3H, *s*, H-9), 2.56 (2H, *q*, *J* = 7.5 Hz, H-7), 5.70 (1H, *s*, H-3), 11.19 (1H, *s*, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 9.0 (C-9), 11.4 (C-8), 24.5 (C-7), 89.7 (C-3), 108.4 (C-5), 163.7 (C-6), 167.9 (C-4), 173.0 (C-2). (Ali et al., 1982); HRESIMS (negative ionization mode), *m/z* 153.0550 $[M-H]^-$ (calc. for $C_8H_9O_3$ 153.0552); IR (KBr) ν 1673, 1561, 1334, 762 cm^{-1}.

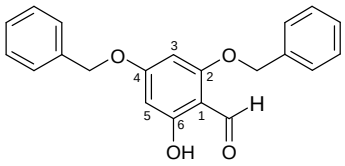
Helipyrone (375)



Lactone **401** (105 mg, 0.7 mmol) was dissolved in dioxane and a 37% solution of formaldehyde (277 µl, 3.4 mmol) and HCl (32%, 230 µl, 2 mmol) was added. The solution was stirred while HCl gas (H_2SO_4 added dropwise to NaCl) was bubbled through for 2 h at room temperature. After completion, ice was added to the reaction mixture and the aqueous phase extracted with dichloromethane. The organic phase was washed with brine before drying with magnesium sulphate. The solvent was removed under vacuum to yield 48 mg (22%) of **375** that was recrystallised from ethyl acetate to afford colourless plates: mp 191-193°C (lit. Kappe and Schmidt, 1970, 217-218 °C; lit. Ali et al., 1982, 218-220

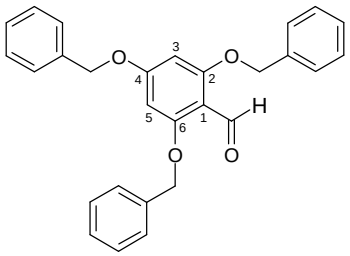
°C); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 1.19 (6H, *t*, *J* = 7.5 Hz, H-7 (x2)), 1.96 (6H, *s*, H-9 (x2)), 2.56 (4H, *q*, *J* = 7.5 Hz, H-7 (x2)), 3.54 (2H, *s*, H-10), 11.19 (2H, *s*, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 9.4 (C-9), 11.6 (C-8), 19.1 (C-10), 24.3 (C-7), 101.7 (C-3), 108.8 (C-5), 161.3 (C-6), 168.7 (C-4), 169.6 (C-2) (Ali et al., 1982, Appendino et al., 2007); HRESIMS (positive ionization mode), *m/z* 321.1331 $[M+H]^+$ (calc. for $C_{17}H_{21}O_6$ 321.1338); IR (KBr) ν 1672, 1561, 1334, 762 cm^{-1}.

2,4-Dibenzyloxy-6-hydroxybenzaldehyde (403)



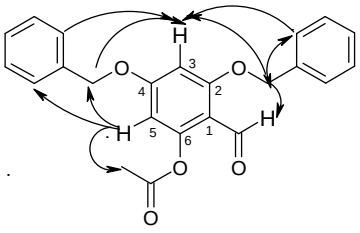
Aldehyde **403** was prepared according to the method of Anderson et al. (2005). Anhydrous K_2CO_3 (3.6 g, 26.0 mmol), was added to a stirred solution of 2,4,6-trihydroxybenzaldehyde (2.0 g, 13.0 mmol) in DMF (20 ml). Benzyl bromide (3 ml, 26.0 mmol) was added dropwise to this solution and the reaction mixture was stirred overnight at 40 °C. The mixture was then diluted with ethyl acetate (40 ml) and water (40 ml). The aqueous and organic layers were separated and the aqueous layer further extracted with ethyl acetate. To ensure complete removal of the product, 40 ml of a 1 M HCl was added to the aqueous layer and it was again extracted with ethyl acetate. The organic layers were combined and dried with magnesium sulphate before removal of the solvent under vacuum. The product was purified using column chromatography with hexanes:ethyl acetate (9:1) as eluent to yield 3.9 g of **403** as a white solid (89%) that was recrystallised from hexanes:ethyl acetate (9:1) to afford white needles: mp 82–83 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 5.07 (2H, *s*, OC$\underline{H_2}$Ph), 5.08 (2H, *s*, OC$\underline{H_2}$Ph), 6.08 (1H, *d*, *J* = 2.0 Hz, H-3 or H-5), 6.11 (1H, d, *J* = 2.1 Hz, H-3 or H-5), 7.36 –7.41 (10H, *m*, Ar$\underline{H}$), 10.2 (1H, *s*, C$\underline{H}$O), 12.50 (1H, *s*, OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 70.4 (O$\underline{C}H_2$Ph), 70.5 (O$\underline{C}H_2$Ph), 92.3, 94.1 (C-3, C-5), 106.3 (C-1), 127.4, 127.6, 128.36, 128.42, 128.71, 128.72, 135.57, 135.64 (Ar$\underline{C}$), 162.6, 166.3, 167.1 (C-2, C-4, C-6), 191.9 ($\underline{C}$HO); HRESIMS (positive ionization mode), *m/z* 335.1272 $[M+H]^+$ (calc. for $C_{21}H_{19}O_4$ 335.12833); IR (KBr) ν 1644; 1621; 1579; 1279; 1225; 1204; 1160; 1113; 734 cm^{-1}.

2,4,6-Tribenzyloxybenzaldehyde (404)



For the method of preparation see compound **403.** After column chromatography 276 mg (5%) of compound **404** was obtained The compound was recrystallised from petroleum ether:dichloromethane to afford colourless needles: mp 113-114 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 5.03 (2H, *s*, OC<u>H</u>$_2$Ph), 5.14 (4H, *s*, OC<u>H</u>$_2$Ph), 6.22 (2H, *s*, H-3, H-5), 7.32 – 7.46 (15H, *m*, Ar<u>H</u>), 10.5 (1H, *s*, C<u>H</u>O); ^{13}C NMR (125 MHz, $CDCl_3$); δ_C 70.3 (O<u>C</u>H$_2$Ph), 70.6 (O<u>C</u>H$_2$Ph), 93.0 (C-3, C-5), 126.9, 127.5, 128.0, 128.4, 128.6, 128.7 (Ar<u>C</u>), 135.7, 136.1 (CH$_2$<u>C</u>Ar), 162.6, 164.9, 167.3 (C-2, C-4, C-6), 187.5 (<u>C</u>HO); HRESIMS (positive ionization mode), *m/z* 425.1747 $[M+H]^+$ (calc. for $C_{28}H_{25}O_4$ 425.1753); IR (KBr) ν 1599, 1236, 1101 cm^{-1}.

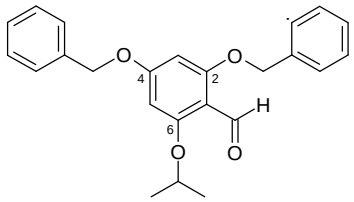
2,4-Dibenzyloxy-6-acetoxybenzaldehyde (405)



2,4-Dibenzyloxy-6-hydroxyabenzaldehyde **403** (205 mg, 0.6 mmol) was refluxed in acetic anhydride for 1.5 h. The excess acetic anhydride was removed *in vacuo* and the crude product purified with hexanes:ethyl acetate (9:1) on the chromatotron to yield 96 mg (43%) of **405** as amorphous solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.37 (3H, *s*, OCOC<u>H</u>$_3$), 5.07 (2H, *s*, OC<u>H</u>$_2$Ph), 5.11 (2H, *s*, OC<u>H</u>$_2$Ph, benzyl group on C-2), 6.32 (1H, d, *J* = 2.2 Hz, H-5), 6.52 (1H, *d*, 2.15 Hz, H-3), 7.38 –7.41 (10H, *m*, Ar<u>H</u>), 10.33 (1H, s, <u>C</u>HO); HRESIMS (positive ionization mode), *m/z* 377.1398 $[M+H]^+$ (calc. For $C_{23}H_{21}O$

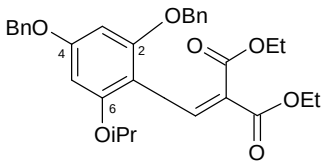
377.1389). Arrows indicating NOESY correlations used to determine positions of benzyloxy groups.

2,4-Dibenzyloxy-6-isopropoxybenzaldehyde (406)



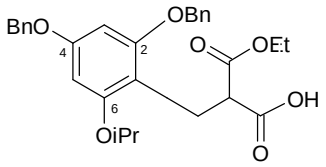
The dibenzylated aldehyde **403** (2 g, 6.0 mmol) was dissolved in 45 ml DMF and stirred under nitrogen. Anhydrous potassium carbonate (2.5 g, 18.0 mmol) and 2-bromopropane (2 ml, 21.0 mmol) was added. The reaction was stirred for 2 days at 80 - 90 °C and then cooled down. It was quenched by the addition of 1 M HCl (until reaction mixture was acidic, blue litmus turned red) and 40 ml water. The aqueous layer was extracted five times with ethyl acetate. The organic layer was washed copiously with water before drying with magnesium sulphate. The solvent was removed under vacuum and the product purified with column chromatography using successively hexanes:ethyl acetate 9:1, 8:2 and 2:1 to afford 1.6 g (71%) of **406** that was recrystallised from hexanes:ethyl acetate to yield white needles: mp 80°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 1.36 (6H, *d*, *J* = 6.1 Hz, CH(C$\underline{H}_3$)$_2$), 4.54 (1H, *heptet*, *J* = 6.1 Hz, C$\underline{H}$(CH$_3$)$_2$), 5.06 (2H, *s*, OC$\underline{H}_2$Ph), 5.13 (2H, *s*, OC$\underline{H}_2$Ph), 6.16 (1H, *d*, *J* = 2.2 Hz, H-3 or H-5), 6.19 (1H, d, *J* = 2.2 Hz, H-3 or H-5), 7.29 – 7.48 (10H, *m* CH$_2$Ar$\underline{H}$), 10.42 (C$\underline{H}$O); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 22.0 (CH($\underline{C}$H$_3$)$_2$), 70.3, 70.6, 71.8 (O$\underline{C}$H$_2$Ph, ($\underline{C}$H(CH$_3$)$_2$), 92.8, 93.9 (C-3, C-5), 110.6 (C-1), 126.9, 127.5, 127.9, 128.4, 128.6, 128.8 (Ar$\underline{C}$), 136.0, 136.4 (CH$_2\underline{C}$Ar), 162.5, 163.0, 164.8 (C-2, C-4, C-6), 187.8 ($\underline{C}$=O); HRESIMS (positive ionisation mode), *m/z* 377.1747 $[M+H]^+$ (calc. for $C_{24}H_{25}O_4$377.1753). IR (KBr) ν 2981, 1709, 1604, 1250, 1119 cm^{-1}.

Diethyl 2-(2,4-dibenzyloxy-6-isopropoxybenzylidene)malonate (407)



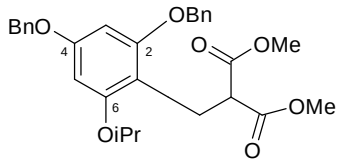
Aldehyde **406** (1.6 g, 4.3 mmol) was dissolved in 20 ml toluene. Diethylmalonate (695 mg, 4.3 mmol), acetic acid (69 mg, 1.2 mmol) and pyrrolidine (40 μL, 0.5 mmol) were added to this solution. The reaction was refluxed for 4 h and then cooled. The reaction was quenched by the addition of 20 ml water. The organic and aqueous phases were separated and the aqueous layer was extracted twice with ethyl acetate. The organic layers were combined and washed twice with water before drying with magnesium sulphate. The solvent was evaporated and the product purified with column chromatograpy using eluents with increasing polarity, hexanes:ethyl acetate 9:1, 8:2, 2:1 to afford 1.38 g (63%) of **407** as solid that was recrystallised from hexanes:ethyl acetate to afford white needles: mp 77-78 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 1.12 (3H, *t*, *J* = 7.1 Hz, $OCH_2C\underline{H}_3$), 1.30 (6H, *d*, *J* = 6.1 Hz, $CH(C\underline{H}_3)_2$), 1.32 (3H, *t*, *J* = 7.1 Hz, $OCH_2C\underline{H}_3$), 4.11 (2H, *q*, *J* = 7.1 Hz, $OC\underline{H}_2CH_3$), 4.29 (2H, *q*, *J* = 7.1 Hz, $OC\underline{H}_2CH_3$), 4.44 (1H, heptet, *J* = 6.1 Hz, $C\underline{H}(CH_3)_2$), 4.99 (2H, *s*, $OC\underline{H}_2Ph$), 5.07 (2H, *s*, $OC\underline{H}_2Ph$), 6.16 (1H, d, *J* = 2.2 Hz, H-3 or H-5), 6.18 (1H, d, *J* =2.2 Hz, H-3 or H-5), 7.27 – 7.40 (10H, *m*, $CH_2Ar\underline{H}$), 7.96 (1H, *s*, $C\underline{H}$=C); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 13.9 ($OCH_2\underline{C}H_3$), 14.2 ($OCH_2\underline{C}H_3$), 22.0 ($CH(\underline{C}H_3)_2$), 60.4 $O\underline{C}H_2CH_3$), 61.0 ($O\underline{C}H_2CH_3$), 70.2, 70.6, 72.3 ($O\underline{C}H_2Ph$, ($\underline{C}H(CH_3)_2$), 93.3, 94.7 (C-3, C-5), 108.1 (C-1), 125.7, 126.0, 126.9, 127.5, 127.8, 128.2, 128.5, 128.7, 136.4, 136.6, 137.7 (Ar$\underline{C}$, $\underline{C}$H=$\underline{C}$), 158.4, 158.7, 161.9 (C-2, C-4,C-6), 165.8, 166.1 ($\underline{C}$OOEt); HRESIMS (positive ionisation mode), *m/z* 519.2368 $[M+H]^+$ (calc. for $C_{31}H_{35}O_7$ 519.2383); IR (KBr) ν 1711, 1604, 1227, 1102 cm^{-1}.

2-(2,4-Dibenzyloxy-6-isopropoxybenzyl)-3-ethoxy-3-oxopropanoic acid (408)



Alkene **407** (1.3 g, 2.6 mmol) was dissolved in 10 ml ethanol:methanol (1:1). The solution was stirred and sodium borohydride (390 mg, 10.3 mmol) was added portion wise. The reaction was refluxed for 6 h, cooled down and quenched by the addition of 2 ml of a 1 M HCl solution. The solvent was removed under vacuum, and the residue diluted with ethyl acetate. This fraction was washed three times with water and dried over magnesium sulphate before removal of the solvent. The product was purified using a chromatotron and a mobile phase consisting of hexanes:ethyl acetate 9:1 to afford 635 mg (50%) of **408** as yellow gum: ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 1.12 (3H, *t*, *J* = 7.0 Hz, $CH_2C\underline{H}_3$), 1.28 (3H, *d*, *J* = 6.2 Hz, $CH(C\underline{H}_3)_2$), 1.30 (3H, *d*, *J* = 6.2 Hz, $CH(C\underline{H}_3)_2$), 3.29 (2H, *dd*, *J* = 7.5 Hz, 3.6 Hz, $CHC\underline{H}_2$), 3.77 (1H, *t*, *J* = 7.5 Hz, $C\underline{H}CH_2$), 4.07 (2H, *q*, *J* = 7.0 Hz, $C\underline{H}_2CH_3$), 4.47 (1H, heptet, *J* = 6.2 Hz, $C\underline{H}(CH_3)_2$), 4.99 (2H, *s*, $OC\underline{H}_2Ph$), 5.01 (2H, *s*, $OC\underline{H}_2Ph$), 6.17 (1H, *d*, *J* =2.2 Hz, H-3 or H-5), 6.21 (1H, *d*, *J* =2.2 Hz, H-3 or H-5), 7.28-7.42 (10H, *m*, Ar$\underline{H}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 13.9 ($CH_2\underline{C}H_3$), 21.9 ($CH\underline{C}H_2$), 22.1 ($CH(\underline{C}H_3)_2$), 50.7 ($C\underline{H}CH_2$), 61.4 ($\underline{C}H_2CH_3$), 70.15, 70.22, 70.24 ($O\underline{C}H_2Ph$, ($\underline{C}H(CH_3)_2$), 92.4, 93.6 (C-3, C-5), 107.8 (C-1), 126.0, 127.0, 127.5, 127.7, 128.0, 128.5, 128.6, 128.7, 128.9 (Ar$\underline{C}$), 137.0, 137.1 ($CH_2\underline{C}Ar$), 157.4, 158.3, 159.2 (C-2, C-4, C-6), 170.4 ($\underline{C}OOEt$), 173.8 (COOH); HRESIMS (positive ionisation mode), *m/z* 493.2215 $[M+H]^+$ (calc. for $C_{29}H_{33}O_7$ 493.2226); IR (KBr) ν 1711, 1605, 1234, 1151, 1112 cm^{-1}.

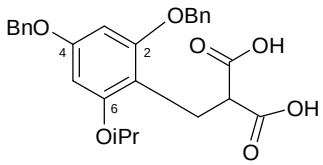
Dimethyl 2-(2,4-dibenzyloxy-6-isopropoxybenzyl)malonate (409)



Alkene **407** (1.3 g, 2.6 mmol) was dissolved in 10 ml ethanol:methanol (1:1). The solution was stirred and sodium borohydride (390 mg, 10.3 mmol) was slowly added where after the reaction was refluxed for 6 h. The reaction was quenched by the addition of 2 ml of a 1 M HCl solution. The organic solvent was removed under vacuum, and the residue diluted with ethyl acetate. The organic fraction was washed three times with water and dried over magnesium sulphate before removal of the solvent. The product was purified using a chromatotron and a mobile phase consisting of hexanes:ethyl acetate 9:1 to yield **409** (28%) as a yellow gum: ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 1.35 (6H, *d*, *J* = 6.1 Hz,

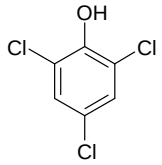
$CH(C\underline{H}_3)_2$), 3.36 (2H, *d*, *J* = 7.5 Hz, $CHC\underline{H}_2$), 3.65 (6H, *s*, OCH_3), 3.84 (1H, *t*, *J* = 7.5 Hz, $C\underline{H}CH_2$), 4.51 (1H, heptet, *J* = 6.1 Hz, $C\underline{H}(CH_3)_2$), 5.03 (2H, *s*, $OC\underline{H}_2Ph$), 5.06 (2H, *s*, $OC\underline{H}_2Ph$), 6.23 (1H, *d*, *J* = 2.2 Hz, H-3 or H-5), 6.26 (1H, *d*, *J* = 2.2 Hz, H-3 or H-5), 7.34 – 7.47 (10, *m*, Ar$\underline{H}$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 21.9 ($CH(\underline{C}H_3)_2$), 22.7 ($CH\underline{C}H_2$), 50.9 ($C\underline{H}CH_2$), 52.0 (OCH_3), 70.17 ($\underline{C}H(CH_3)_2$), 70.22 ($O\underline{C}H_2Ph$), 92.4, 93.6 (C-3, C-5), 108.1 (C-1), 126.8, 127.4, 127.6, 127.9, 128.3, 128.4 (Ar$\underline{C}$), 136.8, 137.0 ($CH_2\underline{C}Ar$), 157.2, 158.1, 158.9 (C-2, C-4, C-6), 169.9 (C=O); HRESIMS (positive ionisation mode), *m/z* 493.2242 $[M+H]^+$ (calc. for $C_{29}H_{33}O_7$ 493.22263); IR (KBr) ν 1733, 1602, 1151, 1100 cm^{-1}.

2-(2,4-Dibenzyloxy-6-isopropoxybenzyl)malonic acid (410)



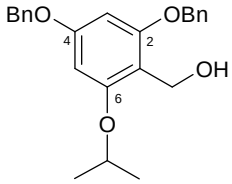
Di-ester **409** (320 mg, 0.7 mmol) was dissolved in 19 ml ethanol and 1 ml water. Sodium hydroxide (54 mg, 1.4 mmol) was added and the reaction refluxed for 6 h. The ethanol was removed under vacuum and the residue diluted with ethyl acetate (10 ml). After the addition of 10 ml of a 1 M HCl solution and 10 ml water, the organic and aqueous layers were separated. The aqueous layer was extracted four times with ethyl acetate. The ethyl acetate layers were combined and washed twice with water and brine. It was dried using magnesium sulphate and the solvent evaporated under vacuum to afford **410** as white solid 250 mg (83%) (Wu et al., 2004): ^{1}H NMR (500 MHz, CD_3OD) δ_H 1.28 (6H, *d*, *J* = 6.0 Hz, $CH(C\underline{H}_3)_2$), 3.21 (2H, *d*, *J* = 6.9 Hz, $CHC\underline{H}_2$), 3.67 (1H, *t*, *J* = 6.9 Hz, $C\underline{H}CH_2$), 4.50 (1H, heptet, *J* = 6.0 Hz, $C\underline{H}(CH_3)_2$), 5.00 (2H, *s*, $OC\underline{H}_2Ph$), 5.03 (2H, *s*, $OC\underline{H}_2$), 6.21 (1H, *d*, *J* = 2.2 Hz, H-3 or H-5), 6.26 (1H, *d*, *J* = 2.2 Hz, H-3 or H-5), 7.26-7.44 (10H, *m*, Ar$\underline{H}$); ^{13}C NMR (125 MHz, CD_3OD) δ_C 19.4 $CH(\underline{C}H_3)_2$, 20.9 $CH\underline{C}H_2$, 49.7 ($C\underline{H}CH_2$), 68.1 ($\underline{C}H(CH_3)_2$), 68.3 ($O\underline{C}H_2Ph$), 68.4 ($O\underline{C}H_2Ph$), 91.0, 91.9 (C-3, C-5), 106.7 (C-1), 125.3, 125.6, 125.7, 125.9, 126.5, 126.6 (Ar$\underline{C}$), 135.9, 136.0 ($CH_2\underline{C}Ar$), 155.6, 156.7, 157.5 (C-2, C-4, C-6), 170.6 (C=O); HRESIMS (positive ionisation mode), *m/z* 465.1926 $[M+H]^+$ (calc. for $C_{27}H_{29}O_7$ 465.1913); IR 9KBr) ν 1702, 1588, 1096 cm^{-1}.

2,4,6-Trichlorophenol (411)



To phenol (500 mg, 5.3 mmol) and HCl (3.52 g, 11 ml, 98.0 mmol) in acetonitrile (20 ml) in a 100 ml flat bottomed flask, H_2O_2 (30% v/v, 4.5 g, 15 ml, 0.1 mol) was added dropwise (15 minutes) at room temperature. The solution was stirred for 4 h and then poured into ice cold water. The aqueous phase was extracted with ethyl acetate, the organic phase washed with brine and dried with magnesium sulphate to afford 746 mg of **411** as cream solid (73%) (Bhatkhande et al., 2002). The product was recrystallised from chloroform to afford white needles: mp 63°C (lit. Bhatkhande et al., 2002, 64-65 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 5.80 (2H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 121.6, 125.4, 128.1, 146.9; HRESIMS (negative ionization mode), *m/z* 194.9168 $[M-H]^-$ (calc. for $C_6H_2Cl_3O$ 194.9171).

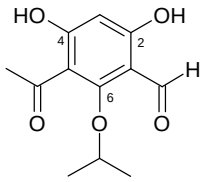
(2,4-Dibenzyloxy-6-isopropoxyphenyl)methanol (413)



Aldehyde **406** (1 g, 2.7 mmol) was dissolved in 5 ml dichloromethane and 4 ml ethanol. The solution was cooled down to 0 °C in an ice bath before sodium borohydride (151 mg, 4.0 mmol) was slowly added. The reaction was stirred for 1 h at 0 °C before quenching it by the addition of brine (10 ml). The aqueous and organic layers were separated, and the aqueous layer extracted with dichloromethane (4 x). The dichloromethane layers were combined and washed with water (2 x) and brine (2 x) before drying with $MgSO_4$. The solvent was evaporated and the white solid **413**, 905 mg (90%) was used as is in the next step. NB: Do not quench the reaction with acid, as it results in a mixture of products and decreases the yield for the reaction: ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 1.22 (6H, *d*, *J* = 6.0 Hz, C<u>H</u>$(CH_3)_2$, 4.39 (1H, heptet, *J* = 6.0 Hz, C<u>H</u>$(CH_3)_2$), 4.66 (2H, *s*, C<u>H$_2$</u>OH), 4.91 (2H, *s*,

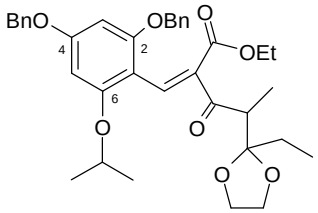
$OC\underline{H}_2Ph$), 4.93 (2H, *s*, $OC\underline{H}_2Ph$), 6.12 (1H, d, *J* = 2.1 Hz, H-3 or H-5), 6.16 (1H, d, *J* = 2.1 Hz, H-3 or H-5), 7.19-7.32 (10H, *m*, Ar<u>H</u>); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 22.1 ($(\underline{C}H_3)_2CH$), 54.9 (CH_2OH), 70.2, 70.4, 70.9 (OCH_2Ph, $OCH(CH_3)_2$), 93.0 , 94.4 (C-3, C-5), 112.0 (C-1). 127.2, 127.4, 127.9, 128.0, 128.5, 128.6, 136.7 (Ar<u>C</u>), 157.5, 158.2, 159.8 (C-2, C-4, C-6); HRESIMS (negative ionisation mode), *m/z* 377.1748 $[M-H]^-$ (calc. for $C_{24}H_{25}O_4$ 377.1753); IR (KBr) ν 2976, 1606, 1149, 1116 cm^{-1}.

3-Acetyl-4,6-dihydroxy-2-isopropoxybenzaldehyde (414)



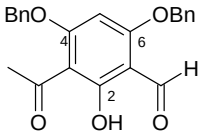
Tin(IV)chloride ($SnCl_4$, 195 µl, 0.6 mmol) and acetyl chloride (53 µl, 0.7 mmol) was added dropwise at -10 °C to a stirring solution of aldehyde **406** in dry CH_2Cl_2. The reaction mixture was stirred until completion (monitored by TLC) and poured on ice. The separated aqueous phase was extracted with dichloromethane and the combined organic phases washed with saturated sodium bicarbonate, dried over anhydrous magnesium sulphate and the solvent evaporated to afford compound **414** as part of a mixture: ^1H NMR (400 MHz, $CDCl_3$) δ_H 1.41 (6H, *d*, *J* = 6.16 Hz, $CH(C\underline{H}_3)_2$), 2.71 (3H, *s*, $C\underline{H}_3O$), 4.68 (1H, heptet, *J* = 6.10 Hz, $C\underline{H}(CH_3)_2$, 5.90 (1H, *s*, Ar<u>H</u>), 10.03 (1H, *s*, C<u>H</u>O), 14.57 (1H, *s*, OH), 14.85 (1H, *s*, OH); HRESIMS (positive ionisation mode), *m/z* 237.0766 $[M+H]^+$ (calc. for $C_{12}H_{13}O_5$ 237.0763).

Ethyl 2-(2,4-Dibenzyloxy-6-isopropoxybenzylidene)-4-(2-ethyl-[1,3]dioxolan-2-yl)-3-oxo-pentanoate (415)



Aldehyde **406** (870 mg, 2.3 mmol) was dissolved in 20 ml toluene. Ester **384** (577 mg, 2.4 mmol), acetic acid (40 µl, 0.6 mmol) and pyrrolidine (22 µL, 0.3 mmol) were added to this solution. The reaction was refluxed for 4 h and then cooled. The reaction was quenched by the addition of 20 ml water. The organic and aqueous phases were separated and the aqueous layer was extracted twice with ethyl acetate. The organic layers were combined and washed twice with water before drying with magnesium sulphate. The solvent was evaporated and the product purified with column chromatograpy using hexanes:ethyl acetate 9:1 as eluent to afford 388 mg (28%) of brown gum that contained **415** as major component: ^{1}H NMR (400 MHz, $CDCl_3$) δH 0.84 (3H, *t*, *J* = 7.3 Hz, CCH_2CH_3), 1.07 (3H, *t*, *J* = 7.2 Hz, OCH_2CH_3), 1.14 (3H, *d*, *J* = 6.8 Hz, $CHCH_3$), 1.21 (3H, *d*, *J* = 6.1 Hz, $CH(CH_3)_2$), 1.27 (3H, *d*, *J* = 6.14 Hz, $CH(CH_3)_2$), 1.67 (2H, *m*, CCH_2CH_3), 3.78 (4H, *m*, OCH_2CH_2O), 3.83 (1H, *m*, $CHCH_3$), 4.05 (2H, *m*, OCH_2CH_3), 4.41 (1H, heptet, *J* = 6.1 Hz, $CH(CH_3)_2$), 5.01 (4H, *br s*, OCH_2Ph), 6.16 (1H, *d*, *J* = 2.1 Hz, H-3 or H-5), 6.19 (1H, d, *J* = 2.1 Hz, H-3 or H-5), 7.28-7.39 (10H, *m*, ArH), 7.67 (1H, *s*, CH=C); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 7.2 (CCH_2CH_3), 12.2 ($CHCH_3$), 14.0 (OCH_2CH_3), 21.7, 21.9 ($CH(CH_3)_2$), 28.4 (CCH_2CH_3), 47.8 ($CHCH_3$), 60.1 (OCH_2CH_3), 65.2, 65.4 (OCH_2CH_2O), 70.1, 70.5 (OCH_2Ph), 72.0 ($CH(CH_3)_2$), 93.1, 94.6 (C-3, C-5), 107.5 (C-1), 112.7 (OCO), 126.9, 127.1, 127.3, 127.4, 127.7, 128.0, 128.3, 128.3, 128.4, 128.52, 128.54 (ArC, CH=C), 136.6 (C=CH)*, 136.47, 136.43 (CH_2CAr)*, 157.4, 158.3, 161.5 (C-2, 4, 6), 167.4 (ester), 200.3 (ketone); HRESIMS (positive ionisation mode), *m/z* 603.2946 $[M+H]^+$ (calc. for $C_{36}H_{43}O_8$ 603.2958); IR (KBr) ν 2980, 1700, 1600, 1156, 1101 cm^{-1}.*Signals interchangeable.

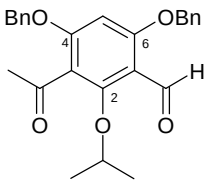
3-Acetyl-4,6-dibenzyloxy-2-hydroxybenzaldehyde (416)



The product was prepared according to the method of Graybill et al. (1999). *N*,*N*-Dimethylformamide (553 µl, 7.1 mmol) was cooled to 0 °C and distilled $POCl_3$ (665 µl, 7.1 mmol) was added. The light orange solution was stirred for approximately 20 minutes until the reagent solidified. Distilled acetonitrile (5 ml) was added until the white solid dissolved. The reaction mixture was stirred for a further 45 minutes at room temperature

under nitrogen. Ketone **377** (1.66 g, 4.8 mmol) in acetonitrile (5 ml) was added *via* syringe to the flask and the mixture was stirred for 18 h at room temperature. Imine hydrolysis in 2:1 methanol: water (20 ml) took place over 4 h at room temperature. The solution was concentrated and chromatographed on a silica column eluting with increasing polarity using hexanes: ethyl acetate 9:1, 8:2, 2:1, 1:1 as mobile phase to yield a cream solid 758 mg (42%) that was recrystallised from hexanes: ethyl acetate to afford colourless needles: mp 135 -136°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 2.51 (3H, *s*, C$\underline{H_3}$CO), 5.12 (2H, *s*, C$\underline{H_2}$OPh), 5.13 (2H, *s*, C$\underline{H_2}$OPh), 6.05 (1H, *s*, H-5), 7.34-7.40 (10H, *m*, Ar$\underline{H}$), 10.26 (1H, *s*, C$\underline{H}$O), 13.34 (1H, *s*, ArO$\underline{H}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 32.7 (C$\underline{H_3}$CO), 70.8 (C$\underline{H_2}$OPh), 71.0 (C$\underline{H_2}$OPh), 88.9 (C-5), 106.6 (C-1, C-3), 127.2, 127.3, 128.6, 128.9, 135.1, 135.2, 164.5, 164.6 (C-2, C-4, C-6), 190.7 (C$\underline{H}$O), 200.5 ($CH_3\underline{C}$O); HRESIMS (positive ionisation mode), *m/z* 377.1386 $[M+H]^+$ (calc. for $C_{23}H_{21}O_5$ 377.1389); IR (KBr) ν 1671, 1606, 1177, 1132 cm^{-1}.

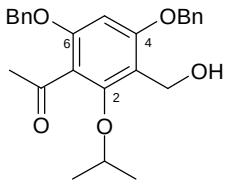
3-Acetyl-4,6-dibenzyloxy-2-isopropoxybenzaldehyde (417)



The dibenzylated keto-aldehyde **416** (1.5 g, 4.0 mmol) was dissolved in 30 ml DMF and stirred under nitrogen. Anhydrous potassium carbonate (1.64 g, 11.9 mmol) and 2-bromopropane (1 ml, 11.9 mmol) was added. The reaction was stirred for 18 hours at 80 - 90 °C and then cooled down. It was quenched by the addition of ice (approximately 30 ml). The aqueous layer was extracted five times with CH_2Cl_2. The dichloromethane layers were combined and washed with water (copiously) and brine before drying with anhydrous magnesium sulphate. The solvent was removed under vacuum and the product purified with column chromatography using hexanes:ethyl acetate 9:1 to afford 970 mg (58%) of **417** as yellow gum: ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 1.23 (6H, *d*, *J* = 6.10 Hz, CH(C$\underline{H_3}$)$_2$), 2.45 (3H, *s*, C$\underline{H_3}$CO), 4.31 (1H, heptet, *J* = 6.11 Hz, C$\underline{H}$(CH$_3$)$_2$), 5.06 (2H, *s*, C$\underline{H_2}$OPh), 5.11 ((2H, *s*, C$\underline{H_2}$OPh), 6.35 (1H, *s*, H-5), 7.29-7.40 (10H, *m*, Ar$\underline{H}$), 10.34 (1H, *s*, C$\underline{H}$O); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 21.9 (CH(C$\underline{H_3}$)$_2$), 32.4 (C$\underline{H_3}$CO), 70.5, 70.8 (C$\underline{H_2}$OPh),

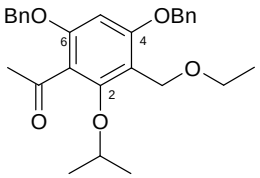
79.4 (CH(CH_3)$_2$), 93.8 (C-5), 113.6, 120.4 (C-1, C-3), 126.2, 126.7, 126.87, 126.89, 128.09, 128.15, 128.6, 128.8, 129.3, 130.2 (ArC), 158.6, 160.6, 163.1 (C-2, C-4, C-6), 187.3 (CHO), 200.8 (CH_3CO); HRESIMS (positive ionisation mode), *m/z* 419.1870 $[M+H]^+$ (calc. for $C_{26}H_{27}O_5$ 419.1858); IR (KBr) ν 1675, 1583, 1385, 1167, 1094 cm^{-1}.

1-(4,6-Dibenzyloxy-3-hydroxymethyl-2-isopropoxyphenyl)ethanone (418)



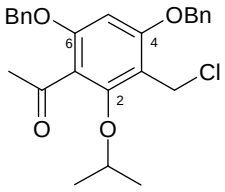
Sodium borohydride (400 mg, 10.6 mmol) was dissolved in 7 ml dichloromethane and 3 ml ethanol and the solution cooled down to -78 °C. The aldehyde (870 mg, 2.1 mmol) was dissolved in 7 ml dichloromethane and 3 ml ethanol and added dropwise to the sodium borohydride solution. The reaction was stirred for 1 h at 0 °C before quenching it by the addition of brine (10 ml). The aqueous and organic layers were separated, and the aqueous layer further extracted with dichloromethane (4 x). The dichloromethane layers were combined and washed with water (2 x) and brine (2 x) before drying with $MgSO_4$. The product 47 mg (62%) was used as is in the next step. It was crystallised from hexanes:ethyl acetate to afford **418** as fine white needles: mp 112-114°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 1.26 (6H, *d*, *J* = 6.04 Hz, CH(CH_3)$_2$), 2.49 (3H, *s*, CH_3CO), 4.25 (1H, heptet, *J* = 6.00 Hz, CH(CH$_3$)$_2$), 4.71 (2H, *s*, CH_2OH), 5.03 (2H, *s*, CH_2OPh), 5.06 (2H, *s*, CH_2OPh), 6.38 (1H, *s*, H-5), 7.30-7.39 (10H, *m*, ArH); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 22.1 (CH(CH_3)$_2$), 32.4 (CH_3CO), 55.4 (CH_2OH), 70.5, 70.7 (CH_2OPh), 78.4 (CH(CH$_3$)$_2$), 94.2 (C-5), 116.7, 119.8 (C-1, C-3), 127.0, 127.1, 127.9, 128.2, 128.5, 128.6, 136.0, 136.2 (ArC), 154.4, 156.0, 159.1 9c-2, C-4, C-6), 201.7 (CH_3CO); HRESIMS (positive ionisation mode), *m/z* 421.2025 $[M+H]^+$ (calc. for $C_{26}H_{29}O_5$ 421.2015); IR (KBr) ν 2977, 1684, 1594, 1166, 1097 cm^{-1}.

1-(4,6-Dibenzyloxy-3-ethoxymethyl-2-isopropoxyphenyl)ethanone (419)



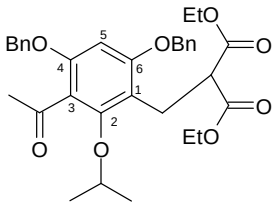
Alcohol **418** (500 mg, 1.2 mmol) was dissolved in 2 ml ethanol and hydrobromic acid (48%, 1 ml) was added at 0 - 5 °C, where after the reaction mixture was stirred overnight at room temperature. The reaction was quenched by the addition of ice and the mixture extracted with dichloromethane (3 x 20 ml). The organic layer was washed with $NaHCO_3$, water, brine and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the residue chromatographed with a chromatotron using hexanes:ethyl acetate 9:1 as eluent (Kumar et al., 2005) to yield a white solid 353 mg (66%) **419** that was recrystallised from hexanes:ethyl acetate to afford fine white needles: mp 51-53°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 1.21 (3H, *t*, *J* = 7.04 Hz, $OCH_2C\underline{H}_3$), 1.25 (6H, *d*, *J* = 6.01 Hz, $CH(C\underline{H}_3)_2$), 2.49 (3H, *s*, $C\underline{H}_3CO$), 3.57 (2H, *q*, *J* = 7.01 Hz, $OC\underline{H}_2CH_3$), 4.33 (1H, heptet, $C\underline{H}(CH_3)_2$), 6.03 Hz, 4.49 (2H, *s*, $C\underline{H}_2OEt$), 5.04 (2H, *s*, $OC\underline{H}_2Ph$), 5.06 (2H, *s*, $OC\underline{H}_2Ph$), 6.34 (1H, *s*, H-5), 7.30-7.43 (10H, *m*, Ar$\underline{H}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 15.1 ($CH_2\underline{C}H_3$), 22.2 ($CH(\underline{C}H_3)_2$), 32.5 ($\underline{C}H_3CO$), 61.5 ($\underline{C}H_2OCH_2CH_3$), 65.7 ($CH_2O\underline{C}H_2CH_3$), 7.03, 70.6 ($\underline{C}H_2OPh$), 78.3 ($\underline{C}H(CH_3)_2$), 94.3 (C-5), 114.2, 119.9 (C-1, C-3), 127.0, 127.86, 127.84, 128.4, 128.46, 128.52, 136.3, 136.6 (ArC), 155.7, 156.3, 159.9 (C-2, C-4, C-6), 201.9 (C=O); HRESIMS (negative ionisation mode), *m/z* 447.2167 $[M-H]^-$ (calc. for $C_{28}H_{31}O_5$ 447.2171); IR (KBr) ν 1711, 1604, 1576, 1227, 1102 cm^{-1}.

1-(4,6-Dibenzyloxy-3-chloromethyl-2-isopropoxyphenyl)ethanone (420)



To a chilled solution of alcohol **418** (300 mg, 0.7 mmol) in dry CH_2Cl_2 (2 ml) was added dropwise under stirring a solution of $SOCl_2$ (0.09 ml, 1.8 mmol) dissolved in dry CH_2Cl_2 (2 ml). The reaction was stirred at 0 °C for approximately 45 minutes (followed by TLC) until all the starting material was consumed. The solvent and unreacted $SOCl_2$ was removed under vacuum and the resulting precipitate dissolved in dry THF. The crude product **420** was used without purification in the next step (Amato et al., 2007). Quantitative (100%), crude used in next step: ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 1.28 (6H, *d*, *J* = 6.02 Hz, CH(C<u>H</u>$_3$)$_2$), 2.48 (3H, *s*, C<u>H</u>$_3$CO), 4.34 (1H, heptet, *J* = 6.01 Hz, C<u>H</u>(CH$_3$)$_2$), 4.72 (2H, *s*, CH_2Cl), 5.02 (2H, *s*, C<u>H</u>$_2$OPh), 5.10 (2H, *s*, C<u>H</u>$_2$OPh), 6.33 (1H, *s*, H-5), 7.31-7.45 (10H, *m*, Ar<u>H</u>); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 22.3 (CH(<u>C</u>H$_3$)$_2$), 32.5 (<u>C</u>H$_3$CO), 36.6 (CH_2Cl), 70.4, 70.8 (<u>C</u>H$_2$OPh), 78.0 (<u>C</u>H(CH$_3$)$_2$), 94.0 (C-5), 113.6, 119.4 (C-1, C-3), 127.0, 127.1, 128.0, 128.1, 128.6, 136.1, 136.2 (Ar<u>C</u>), 155.0, 157.0, 159.3 (C-2, C-4, C-6), 201.5 (C=O); HRESIMS (positive ionisation mode), *m/z* 439.1675 $[M+H]^+$ (calc. for $C_{26}H_{28}O_4Cl$ 439.1676); IR (KBr) ν 1700, 1597, 1168, 1097, 734 cm^{-1}.

Diethyl 2-(3-Acetyl-4,6-dibenzyloxy-2-isopropoxybenzyl)malonate (421)



The alcohol **418** (300 mg, 0.7 mmol) was dissolved in 2 ml dry dichloromethane. The solution was cooled down to 0 °C in an ice bath and $SOCl_2$ (0.09 ml, 1.8 mmol) dissolved in 2 ml dry dichloromethane added dropwise. The reaction was stirred at 0 °C for approximately 45 minutes (followed by TLC) until all the starting material was consumed. The solvent and unreacted $SOCl_2$ was removed under vacuum and the resulting precipitate dissolved in dry THF.

Diisopropyl amine (0.2 ml, 1.4 mmol) was dissolved in 2 ml dry THF and the solution cooled down to -78 °C. Butyl lithium (0.95 ml, 1.4 mmol) was slowly added while stirring and the solution allowed to warm up to 0 °C. The solution was allowed to stir for 30 minutes at this temperature to allow the LDA to form and then cooled down to -78 °C

again. Diethyl malonate (0.13 ml, 0.9 mmol) was dissolved in 2 ml dry THF and added dropwise to the LDA solution. The reaction mixture was stirred for 30 minutes while the temperature was allowed to rise to 0 °C. The solution was cooled to -10 °C and the chloride, dissolved in THF added. The reaction was stirred at room temperature for 2 days. The THF was evaporated and the crude product purified on a chromatotron using hexanes:ethyl acetate 4:1 as eluent to afford **421** as cream solid 214 mg (54%) (over 2 steps). It was recrystalised from acetone to afford cream needles: mp 97-99°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 1.17 (6H, *t*, *J* = 7.11 Hz, $OCH_2C\underline{H}_3$), 1.22 (6H, *d*, *J* = 6.04 Hz, $CH(C\underline{H}_3)_2$), 2.45 (3H, *s*, $C\underline{H}_3CO$), 3.27 (2H, *d*, *J* = 7.47 Hz, $C\underline{H}CH_2$,), 3.77 (1H, *t*, *J* = 7.47 Hz, $CHC\underline{H}_2$,), 4.10 (4H, *dd*, *J* = 7.2 Hz, 1.52 Hz, $OC\underline{H}_2CH_3$), 4.14 (1H, heptet, *J* = 6.08 Hz, $C\underline{H}(CH_3)_2$), 4.99 (2H, *s*, $C\underline{H}_2OPh$), 5.03 (2H, *s*, $C\underline{H}_2OPh$), 6.30 (1H, *s*, H-5), 7.30-7.39 (10H, *m*, Ar$\underline{H}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 13.9 ($OCH_2\underline{C}H_3$), 22.2 ($CH(C\underline{H}_3)_2$), 23.5 ($CH\underline{C}H_2$), 32.4 ($\underline{C}H_3CO$), 51.3 ($\underline{C}HCH_2$), 61.07 ($O\underline{C}H_2CH_3$), 70.4, 70.8 ($\underline{C}H_2OPh$), 77.7 ($\underline{C}H(CH_3)_2$), 94.2 (C-5), 113.9, 119.6 (C-1, C-3), 127.0, 127.1, 127.9, 128.0, 128.5, 128.6, 136.5 (ArC), 154.9, 155.2, 158.7 (C-2, C-4, C-6), 169.4 ($\underline{C}OOEt$), 202.0 ($\underline{C}H_3CO$); HRESIMS (positive ionisation mode), *m/z* 563.2643 $[M+H]^+$ (calc. for $C_{33}H_{39}O_8$ 563.2645); IR ν 1735, 1686, 1596, 1254, 1100 cm^{-1}.

7.5 References

Ali, E., Bagchi, D., Pakrashi, S.C., 1982. Helipyrone from *Anaphalis araneosa* and its synthesis. Phytochemistry 21, 243-244.

Ali, S.M., Ilyas, M., 1986. Biomimetic approach to biflavonoids: Oxidative coupling of 2'-hydroxychalcones with I_2 in alkaline methanol. Journal of Organic Chemistry 51, 5415-5417.

Amato, M.E., Ballistreri, F.P., Pappalardo, A., Sciotto, D., Tomaselli, G.A., Toscano, R.M., 2007. Synthesis and conformational aspects of 20- and 40-membered macrocyclic mono and dinuclear uranyl complexes incorporating salen and (R)-BINOL units. Tetrahedron 63, 9751-9757.

Anderson, J.C., Headley, C., Stapleton, P. D., Taylor, P.W., 2005. Asymmetric total synthesis of B-ring modified (-)-epicatechin gallate analogues and their modulation of β-lactam resistance in *Staphylococcus aureus*. Tetrahedron 61, 7703-7711.

Antonioletti, R., Bovicelli, P., Malancona, S., 2002. A new route to 2-alkenyl-1,3-dicarbonyl compounds, intermediates in the synthesis of dihydrofurans. Tetrahedron 58, 589-596.

Appendino, G., Ottino, M., Marquez, N., Bianchi, F., Giana, A., Ballero, M., Sterner, O., Fiebich, B. L., Munoz, E., 2007. Arzanol, an anti-inflammatory and anti-HIV-1 phloroglucinol α-pyrone from *Helichrysum italicum* ssp. *microphyllum*. Journal of Natural Products 70, 608-612.

Arnaudinaud, V., Nay, B., Nuhrich, A., Deffieux, G., Mérillon, J-M., Monti, J-P., Vercauteren, J., 2001. Total synthesis of isotopically labeled flavonoids. Part 3. ^{13}C-labelled (-)-procyanidin B3 from 1-[^{13}C]-acetic acid. Tetrahedron Letters 42, 1279-1281.

Babler, J.H., Malek, N.C., Coghlan, M.J., 1978. Selective hydrolysis of α,β-and β,γ-unsaturated ketals: A method for deconjugation of β,β-disubstituted α,β-unsaturated ketones. Journal of Organic Chemistry 43, 1821-1823.

Baldwin, J.E., Haraldsson, G.G., 1986. The selective cleavage of aromatic benzyl ethers with magnesium bromide via neighbouring group effect. Acta Chemica Scandinavica B 40, 400-401.

Banerjee, A.K., Achari, B., 1993. Regiocontrolled synthesis of spiro furapyrone-cyclohexane ring systems: approach to the pyranoditerpene lygodinolide. Tetrahedron Letters 34, 1209-1210.

Bates, M.A., Sammes, P.G., Thomson, G.A., 1988. Synthesis of the C-glycoside fragment of nogalamycin and some nogalamycin precursors. Journal of the Chemical Society, Perkin Transactions 1, 3037-3045.

Benavente-García, O., Castillo, J., 2008. Update on uses and properties of citrus flavonoids: New findings in anticancer, cardiovascular, and anti-inflammatory activity. Journal of Agricultural and Food Chemistry 56, 6185–6205

Bhatkhande, B.S., Adhikari, M.V., Samant, S.D., 2002. Sonochemical chloro-oxidation of phenols using HCl-H_2O_2. Ultrasonics Sonochemistry 9, 31-35.

Biali, S.E., Bohmer, V., Brenn, J., Frings, M., Thondorf, I., Vogt, W., Wohnert, J., 1997. Conformation, inversion barrier, and solvent-induced shift in *exo*-and *endo/exo*-calix[4]arenes. Journal of Organic Chemistry 62, 8350-8360.

Biddle, M.M., Lin, M., Scheidt, K.A., 2007. Catalytic enantioselective synthess of flavanones and chromanones. Journal of the American Chemical Society 129, 3830-3831.

Bohlmann, F., Misra, L.N., Jakupovic, J., 1984. Weitere Phloroglucin- und α-Pyrone-derivate aus *Helichrysum*-arten. Planta Medica 50, 174-176.

Bohlmann, F., Zdero, C., 1980. Neue Phloroglucin-derivate aus *Helichrysum*-arten. Phytochemistry 19, 153-155.

Bu, X., Zhao, L., Li, Y., 1997. A facile synthesis of 6-*C*-prenylflavanones. Synthesis 1246-1248.

Carney, J.R., Krenisky, J.M., Williamson, R. Th., Luo, J., 2002. Achyrofuran, a new antihyperglycemic dibenzofuran from the South American medicinal plant *Achyrocline satureioides*. Journal of Natural Products 65, 203-205.

Cervello, J., Marquet, J., Moreno-Manas., 1990. Copper and cobalt mediated regioselective alkylation of polyketide models: Methyl 3,5-dioxohexanoate and triacetic acid lactone. Tetrahedron 46, 2035-2046.

Chen, H.Y., Dykstra, K.D., Birzin, E.T., Frisch, K., Chan, W., Yang, Y.T., Mosley, R.T., DiNinno, F., Rohrer, S.P., Schaeffer, J.M., Hammond, M.L., 2004. Estrogen receptor ligands. Part 1: The discovery of flavonoids with subtype selectivity. Bioorganic and Medicinal Chemistry Letters 14, 1417-1421.

Cisak, A., Mielczarek, C., 1992. Practical and theoretical aspects of flavanone-chalcone isomerisations. Journal of the Chemical Society. Perkin transactions 2 1603-1607.

Clayden, J., Greeves, N., Warren, S., Wothers, P., 2001. Organic Chemistry. Oxford, Oxford University Press, p. 723-733.

De Clercq, E., 2002. New anti-HIV agents and targets. Medical Research Reviews 22, 531-565.

Drewes, S.E., Van Vuuren, S.F., 2008. Antimicrobial acylphloroglucinols and dibenzyloxy flavonoids from flowers of *Helichrysum gymnocomum*. Phytochemistry 69, 1745-1749.

Drexler, M.T., Amiridis, M.D., 2003. The effect of solvents on the heterogeneous synthesis of flavanone over MgO. Journal of Catalysis 214, 136-145.

Ducho, C., Wendicke, S., Gőrbig, U., Balzarini, J., Meier, C., 2003. 3,3-Di-(*tert*-butyl)-6-fluoro-cycloSal-d4TMP – a pronucleotide with a considerably improved masking group. European Journal of Organic Chemistry 4786-4791.

Dulcire, B., Winternitz, P.F., 1990. Synthesis of {Ring B-$^{13}C_6$}diosmin. Journal of Natural Products 53, 696-698.

Fairlamb, I.J.S., Marrison, L.R., Dickinson, J.M., Lu, F-J., Schmidt, J.P., 2004. 2-Pyrones possessing antimicrobial and cytotoxic activities. Bioorganic and Medicinal Chemistry 12, 4285-4299.

Gissot, A., Wagner, A., Mioskowski, C., 2004. Buffer-induced, selective *C*-alkylation of phloroglucinol: application to the synthesis of an advanced intermediate of catechin. Tetrahedron 60, 6807-6812.

Graybill, T. L., Casillas, E. G., Pal, K., Townsend, C. A., 1999. Bisfuran intermediates of aflatoxin biosynthesis. Journal of the American Chemical Society, 121, 7729-7746

Greene, T.W., Wuts, P.G.M., 1991. Protective Groups in Organic Synthesis; 2nd ed, John Wiley and Sons: New York.

Hagiwara, H., Kobayashi, K., Hoshi, T., Suzuki, T., Ando, M., 2001. A new protocol for synthesis of 3-hydroxymethyl-4-methoxy-2H-pyrone derivatives. Tetrahedron 57, 5039-5043.

Hagiwara, H., Kobayashi, K., miya, S., Hoshi, T., Suzuki, T., Ando, M., Okamoto, T., Kobayashi, M., Yamamoto, I., Ohtsubo, S., Kato, M., Uda, H., 2002. First total synthesis of the phytotoxins solanopyrones D and E via the Domino Michael protocol. Journal of Organic Chemistry 67, 5969-5976.

Hänsel, R., Cybulski, E-M., Cubukcu, B., Mercli, A.H., Bohlmann, F., Zdero, C., 1980. Neue pyron-derivate aus *Helichrysum* arten. Phytochemistry 19, 639-644.

Hansen, T.V., Skattebol, l., 2005. A high yielding one-pot method for the preparation of salen ligands. Tetrahedron Letters 46, 3829-3830.

Haraldsson, G.G., Baldwin, J.E., 1997. A selective cleavage of aromatic benzyl ethers located ortho to a carbonyl group by magnesium bromide. Tetrahedron 53, 215-224.

Hatakeyama, K., Ohmori, K., Suzuki, K., 2005. Synthesis of (±)-lotthanongine, a novel natural product with a flavan-indole hybrid structure. Synlett 8, 1311-1315.

Hodgetts, K.J., 2005. Inter-and intramolecular Mitsunobu rection based approaches to 2-substituted chromans and chroman-4-ones. Tetrahedron 61, 6860-6870.

Ichino, K., Tanaka, H., Ito, K., 1988. Synthesis of helilandin B, pashanone, and their isomers. Journal of Natural Products 51, 906-914.

Jakupovic, J., Kuhnke, J., Schuster, A., Metwally, M.A., Bohlmann, F., 1986. Phloroglucinol derivatives and other constituents from South African *Helichrysum* species. Phytochemistry 25, 1133-1142

Jung, M.E., Hagenah, J.A., 1987. Synthetic approach to aklavinone using 2-0~0-2H-pyran-5-carboxylate (coumalate) intermediates'. Journal of Organic Chemistry 52, 1889-1902.

Kabbe, H.J., Widdig, A. 1982. Synthesis and reactions of 4-chromanones. Angewandte Chemie International Edition 21, 247-256.

Kamal, A., Shaik, A.A., Sinha, R., Yadav, J.S., Arora, S.K., 2005. Antitubercular agents. Part 2. New thiolactomycin analogues active against *Mycobacterium tuberculosis*. Bioorganic and Medicinal Chemistry Letters 15, 1927-1929.

Kappe, T., Schmidt, H., 1970. Eine synthese des helipyrons. Tetrahedron Letters 58, 5105-5106.

Katritzky, A.R., Wang, Z., Wang, M., Hall, C.D., Suzuki, K., 2005**.** Facile syntheses of 2,2-dimethyl-6-(2-oxoalkyl)-1,3-dioxin-4-ones and the corresponding 6-substituted 4-hydroxy-2-pyrones. Journal of Organic Chemistry 70, 4854-4856.

Kishore, P.S., Viswanathan, B., Varadarajan, T.K., 2006. Heteropolyacid-catalyzed synthesis of chloromethyl methyl ether. Tetrahedron Letters 47, 429-431.

Köster, G., Hoffmann, R.W., 1987. Functionalization of 3,5,6-trialkyl-4-hydroxy-2-pyrones in the 6-α position. Liebigs Annalen Chemie, 987-990.

Kumar, S.K., Amador, M., Hidalgo, M., Bhat, S.V., Khan, S.R., 2005. Design, synthesis and biological evaluation of novel riccardiphenol analogs. Bioorganic and Medicinal Chemistry 13, 2873-2880.

Kumazawa, T., Kimura, T., Matsuba, S., Sato, S., Onodera, J-I., 2001. Synthesis of 8-C-glucosylflavones. Carbohydrate Research 334, 183-193.

Kurdyumov, A., Muñoz, R.L.P., Hsung, R.P., 2006. Total syntheses of daldiniapyrone, annularin B, and (±)-annularin F. Synthesis 11, 1787-1790.

Lee, M., Kim, D.H., 2002. Syntheses and kinetic evaluation of racemic and optically active 2-benzyl-2-methyl-3,4-epoxybutanoic acids as irreversible inactivators for carboxypeptidase A. Bioorganic and Medicinal Chemistry 10, 913-922.

Lokot, I.P., Pashkovsky, F.S., Lakhvich, F.A., 1999. A new approach to the synthesis of 3,6- and 5,6-dialkyl derivatives of 4-hydroxy-2-pyrone. Synthesis of *rac*-germicidin. Tetrahedron 55, 4783-4792.

McGlacken, G.P., Fairlamb, I.J.S., 2005. 2-Pyrone natural products and mimetics: isolation, characterisation and biological activity. Natural Product Reports 22, 369-385.

Nay, B., Arnaudinand, V., Peyrat, J-F., Nuhrich, A., Deffieux, G., Mérillon, J-M., Vercauteren, J., 2000. Total synthesis of isotopically labeled flavonoids, ^{13}C labelled (±)-catechin from potassium [^{13}C]cyanide. European Journal of Organic Chemistry 1279-1283.

Nay, B., Arnaudinand, V., Vercauteren, J., 2001. Gram-scale production and applications of optically pure ^{13}C-labelled (+)-catechin and (-)-epicatechin. European Journal of Organic Chemistry 2379-2384.

Odejinmi, S.I., Wiemer, D.F., 2005. Application of benzyl protecting groups in the synthesis of prenylated aromatic compounds. Tetrahedron Letters 46, 3871-3874.

Rao, P.N.P., Amini, M., Li, H., Habeeb, A.G., Knaus, E.E., 2003. 6-Alkyl, alkoxy, or alkylthio-substituted 3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones: A novel class of diarylheterocyclic selective cyclooxygenase-2 inhibitors. Bioorganic and Medicinal Chemistry Letters 13, 2205-S2209.

Ren, W., Qiao, Z., Wang, H., Zhu, L., Zhang, L., 2003. Flavonoids: Promising anticancer agents. Medicinal Research Reviews 23, 519-534.

Ridley, C.P., Khosla, C., 2007. Synthesis and biological activity of novel pyranopyrones derived from engineered aromatic polyketides. American Chemical Society Chemical Biology 2, 104-108.

Ríos, J.L., Recio, M.C., Villar, A., 1991. Isolation and identification of the antibacterial compounds from *Helichrysum Stoechas*. Journal of Ethnopharmacology 33, 51-55.

Rosa, A., Deiana, M., Atzeri, A., Corona, G., Incani, A., Melis, M.P., Appendino, G., Dessì, M.A., 2007. Evaluation of the antioxidant and cytotoxic activity of arzanol, a prenylated α-pyrone-phloroglucinol etherodimer from *Helichrysum italicum* subsp. *microphyllum*. Chemico-Biological Interactions 165, 117-126.

Sartori, G., Casnati, G., Bigi, F., Baraldi, D., 1991. Friedel-Crafts coordinated processes: selective cycloologomerization of acyl chlorides. Tetrahedron Letters 32, 2153-2156.

Scribner, J.D., Smith, D.L., McCloskey, J.A., 1978. Meldrum's acid in organic synthesis. 2. General and versatile synthesis of β-keto esters. Journal of Organic Chemistry 43, 2087-2088.

Shimamura, H., Sunazuka, T., Izuhara, T., Hirose, T., Shiomi, K., Omura, S., 2007. Total synthesis and biological evaluation of verticipyrone and analogues. Organic Letters 9, 65-67.

Shone, R.L., Deason, J.R., Miyano, M., 1986. A short synthesis of elasnin. Journal of Organic Chemistry 51, 268-270.

Solladie, G., Gehrold, N., Maignan, J., 1999. Biomimetic synthesis of the flavanone leridol, revision of the structure of the natural product. European Journal of Organic Chemistry, 2309-2314.

Stadlbauer, W., Badawey, E-S. Hojas, G., Roschger, P., Kappe, T., 2001. Malonates in cyclocondensation reactions**.** Molecules 6, 338-352

Sung, K., Wu, S-Y., 2001. Convenient and efficient syntheses of β-keto esters and β-keto amides directly from β-alkylacetyl chlorides. Synthetic Communications 31, 3069-3074.

Suzuki, K., Tsuruga, K., Hiranuma, K., Yamaura, M., 2004. Synthesis of 7,8-dihydroxy-5-hydroxymethyl-2-phenyl-chroman-4-one; the aglycon of actinoflavoside. Synlett 1, 116-118.

Tan, W-F., Li, W-D.Z., Li, Y-L., 2002. First total synthesis of (±)-glyflavanone-A. Synthetic Communications 32, 1077-1083.

Thaisrivongs, S., Romero, L., Tommasi, R.A., Janakiraman, M.N., Strohbach, J.W., Turner, S.R., Biles, C., Morge, R.R., Johnason, P.D., Aristoff, P.A., Tomich, P.K., Lynn, J.C., Horng, M-M., Chong, K-T., Hinshaw, R.R., Howe, W.J., Finzel, B.C., Watenpaugh, K.D., 1996. Structure-based design of HIV protease inhibitors: 5,6-dihydro-4-hydroxy-2-pyrones as effective, nonpeptidic inhibitors. Journal of Medicinal Chemistry 39, 4630-4642.

Tomás-Barberán, F., Iniesta-Sanmartín, E., Tomás-Lorente, F., Rumbero, A., 1990. Antimicrobial phenolic compounds from three Spanish *Helichrysum* species. Phytochemistry 29, 1093-1095

Tomás-Lorente, F., Iniesta-Sanmartín, E., Tomás-Barberán, F.A., Trowitzsch-Kienast, W., Wray, V., 1989. Antifungal phloroglucinol derivatives and lipophilic flavonoids from *Helichrysum decumbens*. Phytochemistry 28, 1613-1615.

Tsuchiya, H., Sato, M., Miyazaki, T., Fujiwara, S., Tanigaki, S., Ohyama, M., Tanaka, T., Iinuma, M., 1996. Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant *Staphylococcus aureus*. Journal of Ethnopharmacology 50, 27-34.

Tsukayama, M., Kawamura Y., Tamaki, H., Kubo, T., Horie, T., 1989. Synthesis of pyranoisoflavones from pyronochalcones: Synthesis of elongatin and its angular isomer. Bulletin of the Chemical Society of Japan 62, 826-832.

Tsukayama, M., Wada, H., Kawamura, Y., Yamashita, K., Nishiuchi, M., 2004. Regioselective synthesis of 6-alkyl- and 6-prenylpolyhydroxyisoflavones and 6-alkylcoumaronochromone derivatives. Chemical Pharmaceutical Bulletin 52, 1285-1289.

Tummino, P.J., Prasad, J.V.N.V., Ferguson, D., Nouhan, C., Graham, N., Domagala, J.M., Ellsworth, E., Gajda, C., Hagen, S.E., Lunney, E.A., Para, K.S., Tait, B.D., Pavlovsky, A., Erickson, J.W., Gracheck, S., McQuade, T.J., Hupe, D.J., 1996. Discovery and optimization of nonpeptide HIV-1 protease inhibitors. Bioorganic and Medicinal Chemistry 4, 1401-1410.

Urgaonkar, S., La Pierre, H.S., Meir, I., Lund, H., RayChaudhuri, D., Shaw, J.T., 2005. Synthesis of antimicrobial natural products targeting FtsZ: (±)-dichamanetin and (±)-2"'-hydroxy-5"-benzylisouvarinol-B. Organic Letters 7, 5609-5612.

Van der Made, A.W., Van der Made, R.H., 1993. A convenient procedure for bromomethylation of aromatic compounds. Selective mono-, bis-, or trisbromomethylation. Journal of Organic Chemistry 58, 1262-1263.

Van Vuuren, S.F., Viljoen, A.M., Van Zyl, R.L., Van Heerden, F.R., Başer, K.H.C., 2006. The antimicrobial, antimalarial and toxicity profiles of helihumulone, leaf essential oil and extracts of *Helichrysum cymosum* (L.) D. Don subsp. *cymosum*. South African Journal of Botany 72, 287-290

Vrkoč, J., Dolejš, Sedmera, P., Vašíčková, S., Šorm, F., 1971. The structure of arenol and homoarenol, α-pyrone derivatives from *Helichrysum arenarium* (L.) Moench. Tetrahedron Letters 3, 247-250.

Vrkoč, J., Sorm, F., 1975a. 3-Chloromethyl-4-hydroxy-2H-pyran-2-one derivatives. (Czech.). Czech 2 pp. CODEN: CZXXA9 CS 161395 19751115 Patent written in Czech. Application: CS 72-8962 19721227.

Vrkoč, J., Sorm, F., 1975b. 3-(2,4,6-Trihydroxybenzyl)-4-hydroxy-2H-pyran-2-one derivatives Czech. 3 pp. CODEN: CZXXA9 CS 161396 19751115 Patent written in Czech. Application: CS 73-72 19730103.

Ward, D.E., Rhee, C.K., 1988. Chemoselective reduction with sodium borohydride. Aldehydes vs. ketones. Synthetic Communications 18, 1927-1933.

Whang, K., Cooke, R.J., Okay, G., Cha, J.K., 1990. Total synthesis of (+)-verrucosidin. Journal of the American Chemical Society 112, 8985-8987.

Wu, Z., Minhas, G. S., Wen, D., Jiang, H., Chen, K., Zimniak, P., Zheng, J., 2004. Design, synthesis, and structure-activity relationships of haloenol lactones: Site-directed and isozyme-selective glutathione *S*-transferase inhibitors. Journal of Medicinal Chemistry 47, 3282-3294.

Zhang, F., Danishefsky, S.J., 2002. An efficient stereoselective total synthesis of DL-sesquicillin, a glucocorticoid antagonist. Angewandte Chemie Interantional Edition 41, 1434-1437.

Zhang, Y., Zhang, P., Cheng, Y., 2008. Structural characterization of isoprenylated flavonoids from Kushen by electrospray ionization multistage tandem mass spectrometry. Journal of Mass Spectrometry 43, 1421-1431.

Zong, R., Wang, D., Hammit, R., Thummel, R.P., 2006. Synthetic approaches to polypyridyl bridging ligands with proximal multidentate binding sites. Journal of Organic Chemistry 71, 167-175.

CHAPTER 8

Conclusion

Species from the morphologically diverse genus *Helichrysum* are used extensively in ethnomedicine in South Africa. The morphological diversity of the genus is mirrored by the variety of chemical compounds isolated from its species, which include diterpenes, flavonoids, guaianolides, acylated phloroglucinols, and α-pyrone derivatives (Chapter 2). Extracts and isolated compounds from this genus are also associated with a variety of biological activities. These facts indicate that plants from this genus may be important in the search for new plant-derived drugs.

Interesting observations were made regarding correlations observed between morphology and traditional use as well as phytochemistry of the South African species: There are indications that morphological relationships can serve as a guide towards expected phytochemical composition of related species.

Three *Helichrysum* species were investigated during this study. *H. splendidum*, a species previously studied by Bolmann and Suwita (1979) and Jakupovic and co-workers (1989) was reinvestigated to clarify the complex stereochemistry associated with its main chemical constituents. The phytochemistry of the morphologically related *H. montanum* was explored for the first time. New guaianolides, diastereomers of those found in *H. splendidum* were isolated, while several known compounds were also identified. The phytochemical similarities observed between *H. montanum* and *H. splendidum* supports their close morphological relationship. Furthermore, the isolation of guaianolides from *H. montanum* are of taxonomical importance as this type of compound very rarely occur in plants of this genus (Jakupovic et al., 1989).

In antimicrobial and cytotoxicity assays, extracts of the thirty-five *Helichrysum* species that were assayed exhibited activity mostly against Gram-positive micro-organisms. There are reports on the traditional use of six of the seven most active species relating to antimicrobial use, indicating that traditional use should be taken into account during species selection before a phytochemical study. The preliminary cytotoxicity results

obtained for these extracts are quite disturbing, considering the wide medicinal use of these plants. However, the cytotoxicity assays were only performed at one concentration (in duplicate) and needs to be explored further before final conclusions can be made regarding their toxicity and potential as anticancer agents.

Based on the findings obtained from the antimicrobial assays, *H. excisum* was selected as a suitable candidate for further study. This is the first phytochemical study on the non-volatile components of this species. Bio-activity guided fractionation led to the isolation of lepidissipyrone, a flavanone α-pyrone previously isolated from *H. lepidissimum* (Jakupovic et al., 1989). Several flavonoids, mostly with an unsubstituted B-ring, were also identified. The antimicrobial activity of lepidissipyrone was determined and bio-activity established for the first time, although the observed antimicrobial activity was a bit disappointing. Literature indications are however, that phloroglucinol α-pyrones generally exhibit promising antimicrobial activity (Tomás-Barberán, 1990; Ríos et al., 1991) and similar compounds (Hänsel et al., 1980; Bohlmann and Zdero, 1980; Jakupovic et al., 1986; Bohlmann et al., 1984; Jakupovic et al., 1989) that occur in South African *Helichrysum* species, is well worth investigating.

One of the reasons why *H. excisum* was an exciting candidate was the fact that phloroglucinols were previously isolated from other plants belonging to the same morphologic group. It was hypothesised that the extract of *H. excisum* would yield mostly phloroglucinols, as this type of compound featured as main chemical constituent in two morphologically related species. However, this was not the case, as flavonoids were isolated as main chemical component of this species. Although flavonoids and acylphloroglucinols are biosynthetically related, in general, the acylphloroglucinols are more active as anti-infective compounds. Using the morphological relationship to predict the phytochemistry of a particular species was therefore not so successful in this case. Further investigation of the phytochemistry of the other ten Group 12 species is required to clarify the main chemical constituents of this morphological group.

The biological activity associated with phloroglucinol α-pyrones and the synthetic challenges posed by these compounds prompted the development of a strategy towards the synthesis of lepidissipyrone. Several different approaches were investigated. A route which

involves the coupling of a chloromethylated phloroacetophenone derivative and a β-keto ester precursor was developed. We believe this strategy could be used to synthesise other phloroglucinol α-pyrone analogues for further biological evaluation.

References

Bohlmann, F., Misra, L.N., Jakupovic, J., 1984. Weitere Phloroglucin- und α-pyrone-derivate aus *Helichrysum* arten. Planta Medica 50, 174-176.

Bohlmann, F., Suwita, A., 1979. Ein neues Guajanolid und ein Secoguajanolid aus *Helichrysum splendidum*. Phytochemistry 18, 885-886.

Bohlmann, F., Zdero, C., 1980. Neue Phloroglucin-derivate aus *Helichrysum*-arten. Phytochemistry 19, 153-155.

Hänsel, R., Cybulski, E-M., Çubuçku, B., Meriçli, A.H., Bohlmann, F., Zdero, C., 1980. Neue pyron-derivate aus *Helichrysum*-arten. Phytochemistry 19, 639-644.

Jakupovic, J., Kuhnke, J., Schuster, A., Metwally, M.A., Bohlmann, F., 1986. Phloroglucinol derivatives and other constituents from South African *Helichrysum* species. Phytochemistry 25, 1133-1142.

Jakupovic, J., Zdero, C., Grenz, M., Tsichritzis, F., Lehmann, L., Hashemi-Nejad, S.M., Bohlmann, F., 1989. Twenty-one acylphloroglucinol and further constituents from South African *Helichrysum* species. Phytochemistry 28, 1119-1131

Ríos, J.L., Recio, M.C., Villar, A., 1991. Isolation and identification of the antibacterial compounds from *Helichrysum Stoechas*. Journal of Ethnopharmacology 33, 51-55.

Tomás-Barberán, F., Iniesta-Sanmartín, E., Tomás-Lorente, F., Rumbero, A., 1990. Antimicrobial phenolic compounds from three Spanish *Helichrysum* species. Phytochemistry 29, 1093-1095.

Appendix 1

NMR spectra of selected compounds

Plate 1: ^{1}H NMR spectrum of compound 302 in $CDCl_3$

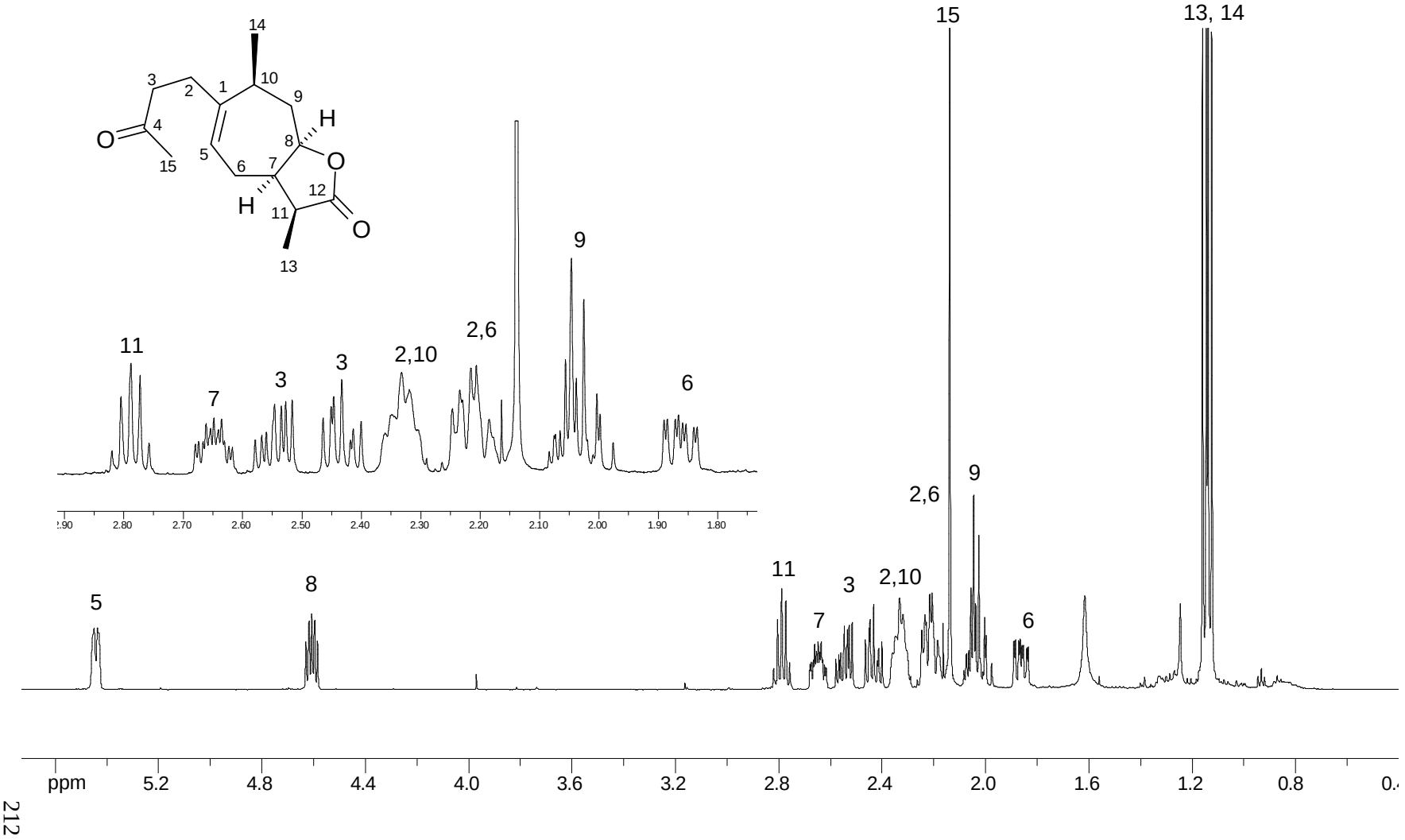
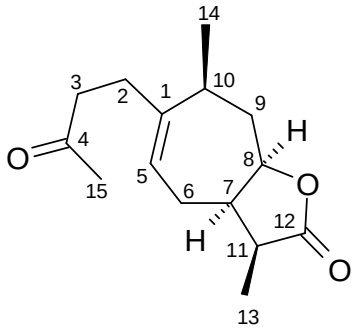


Plate 2: ^{13}C NMR spectrum of compound 302 in $CDCl_3$



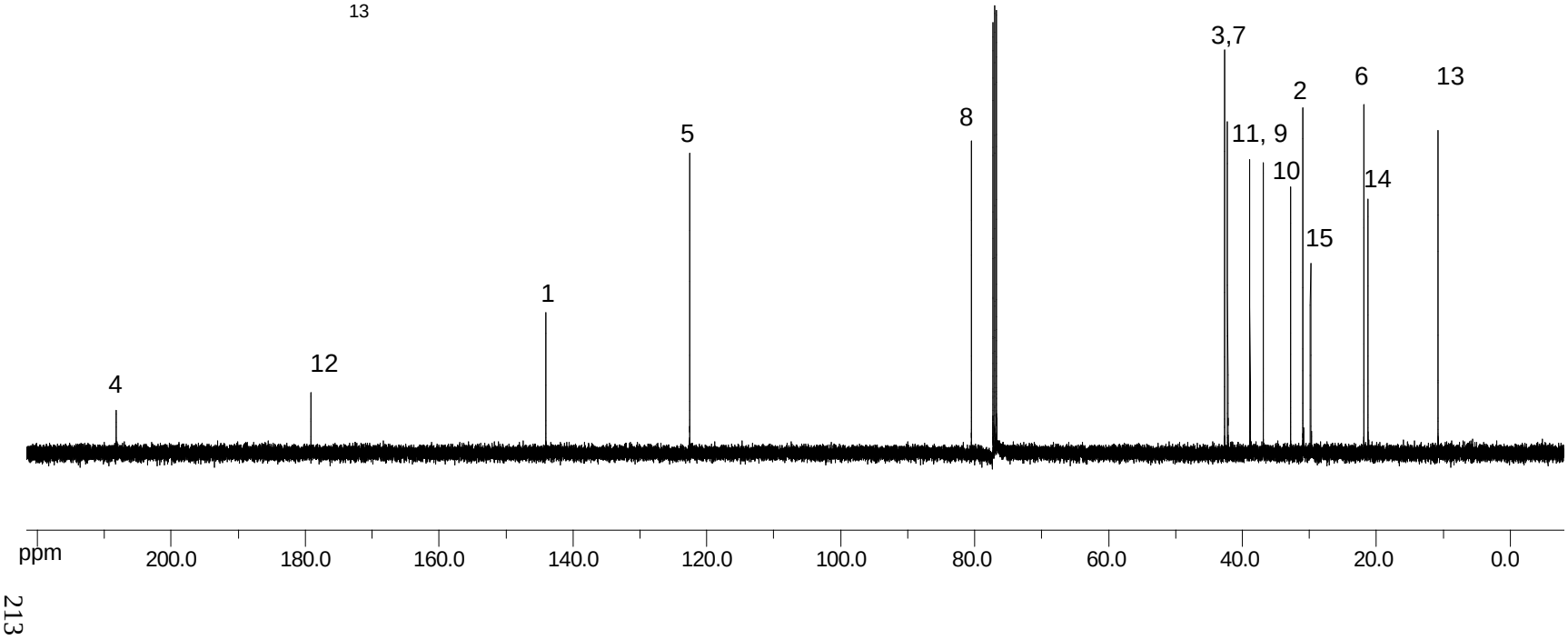


Plate 3: COSY NMR spectrum of compound 302 in $CDCl_3$

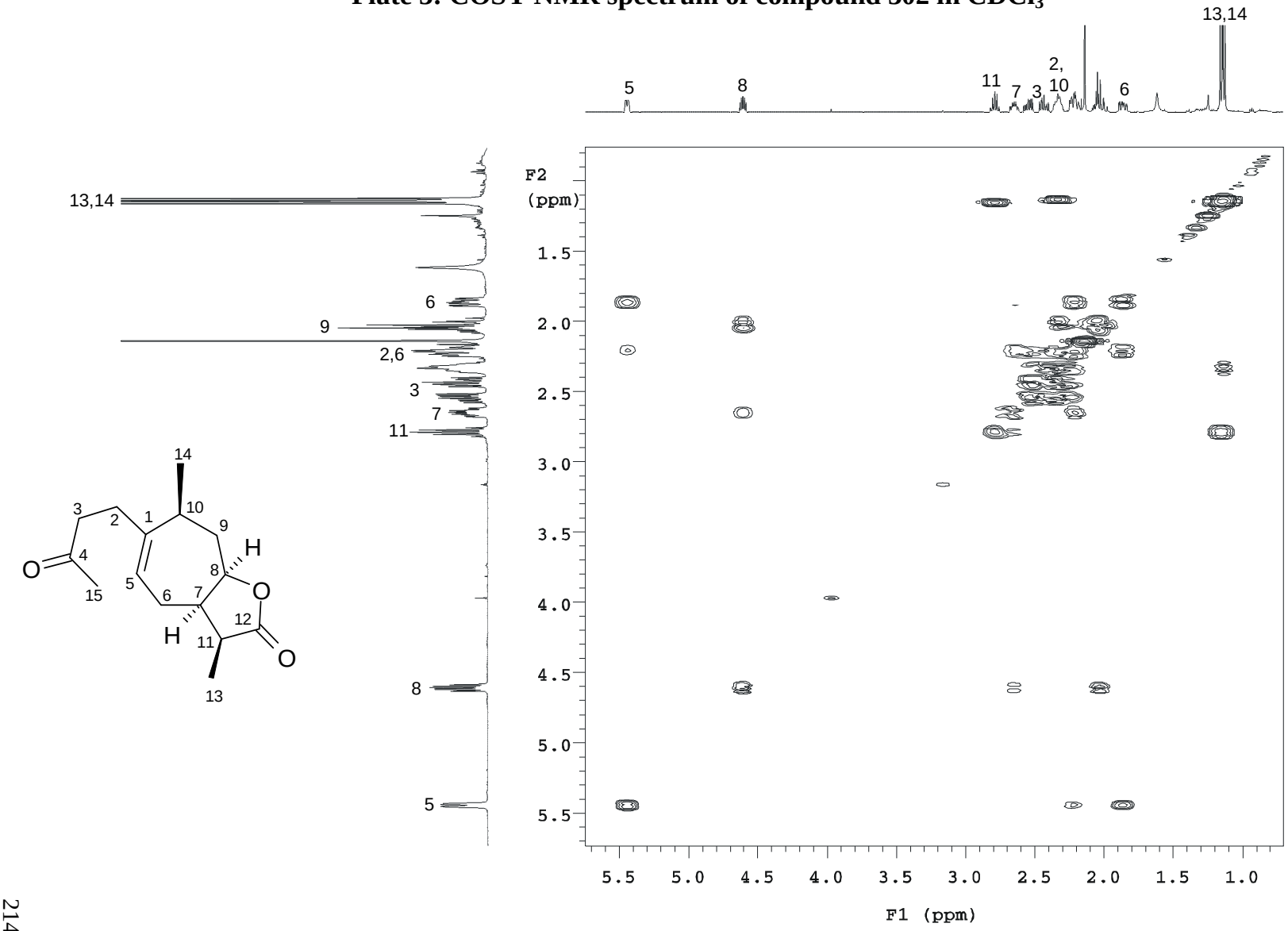


Plate 4: DEPT NMR spectrum of compound 302 in $CDCl_3$

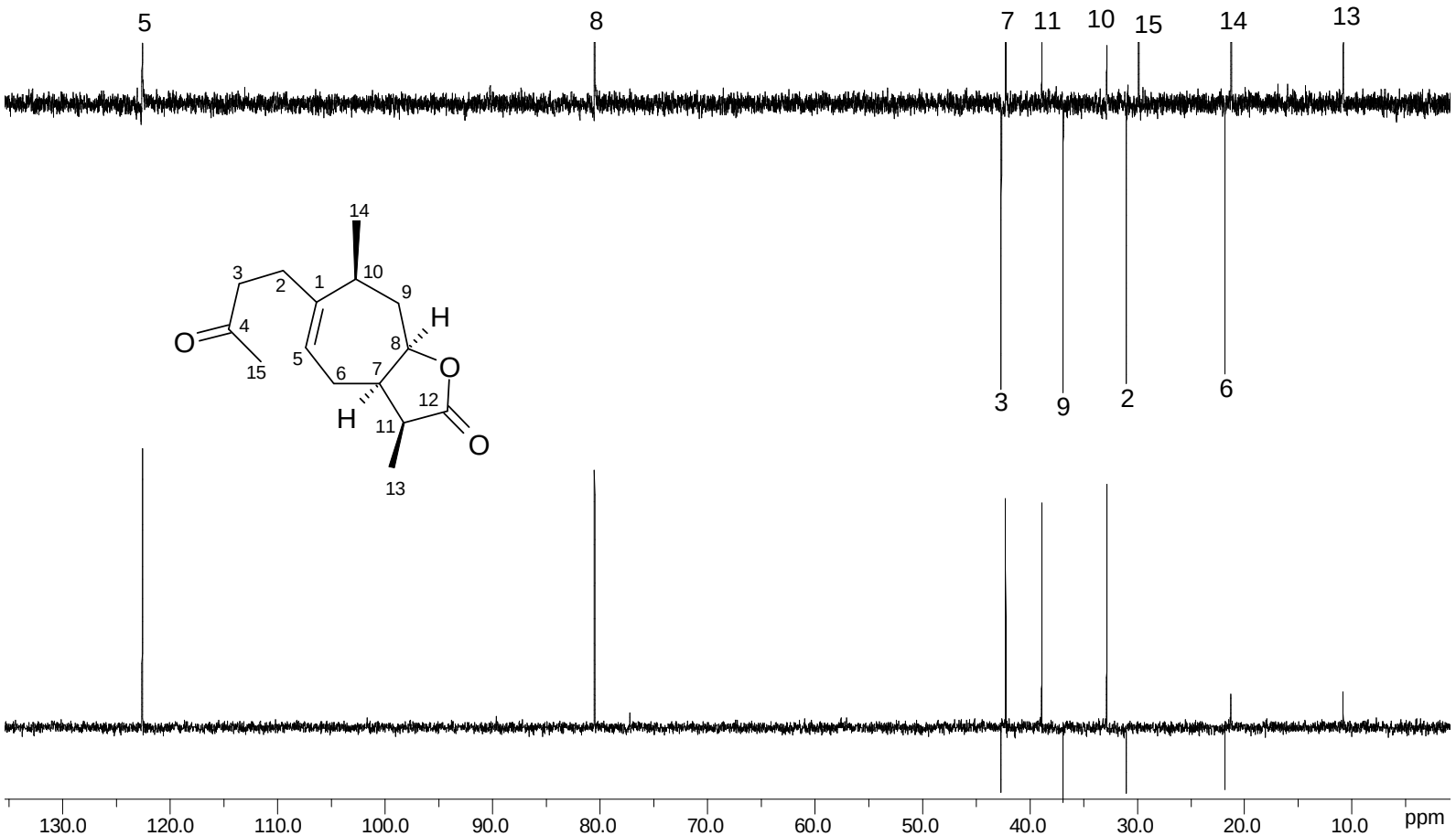


Plate 5: HSQC NMR spectrum of compound 302 in $CDCl_3$

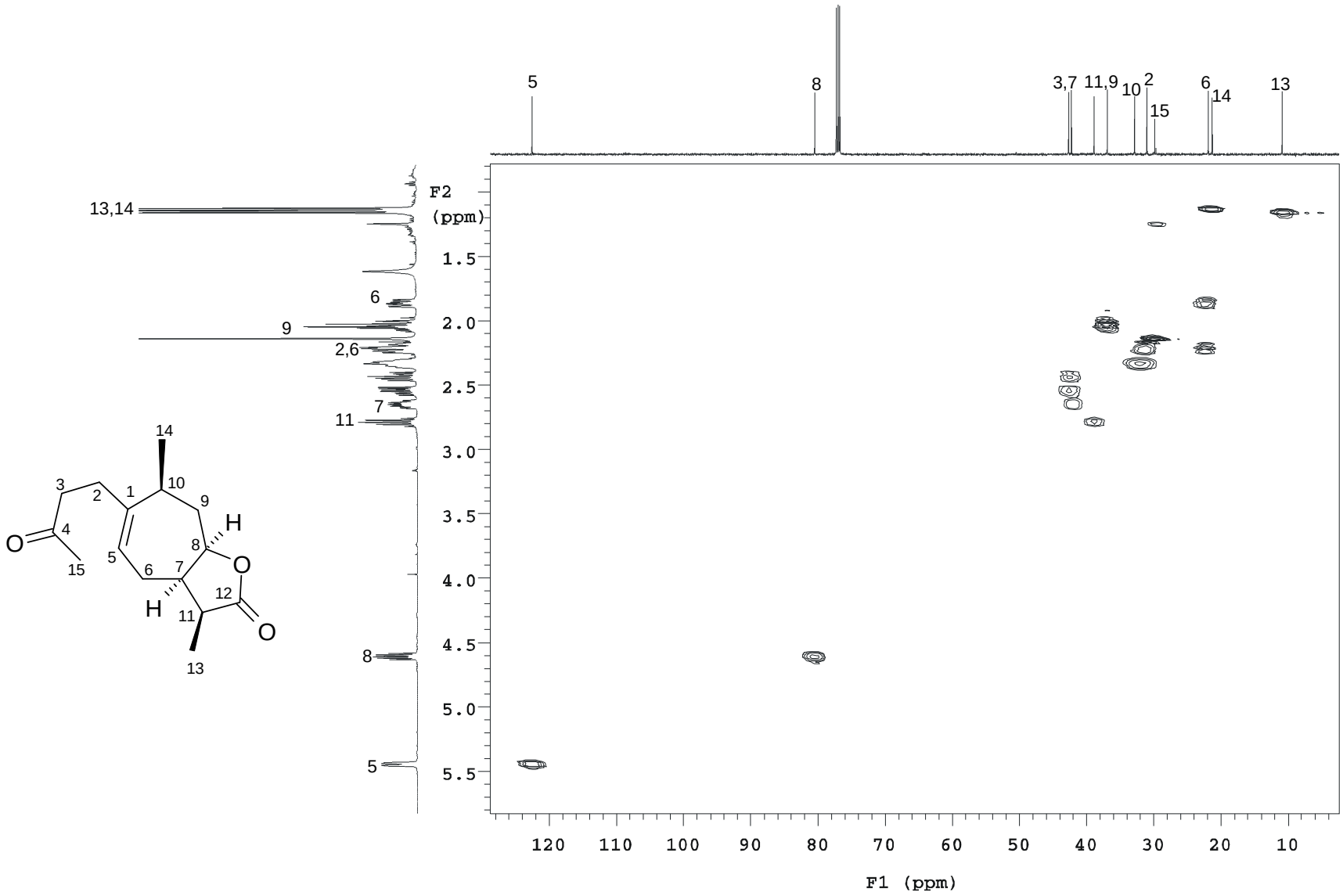


Plate 6: HMQC NMR spectrum of compound 302 in $CDCl_3$

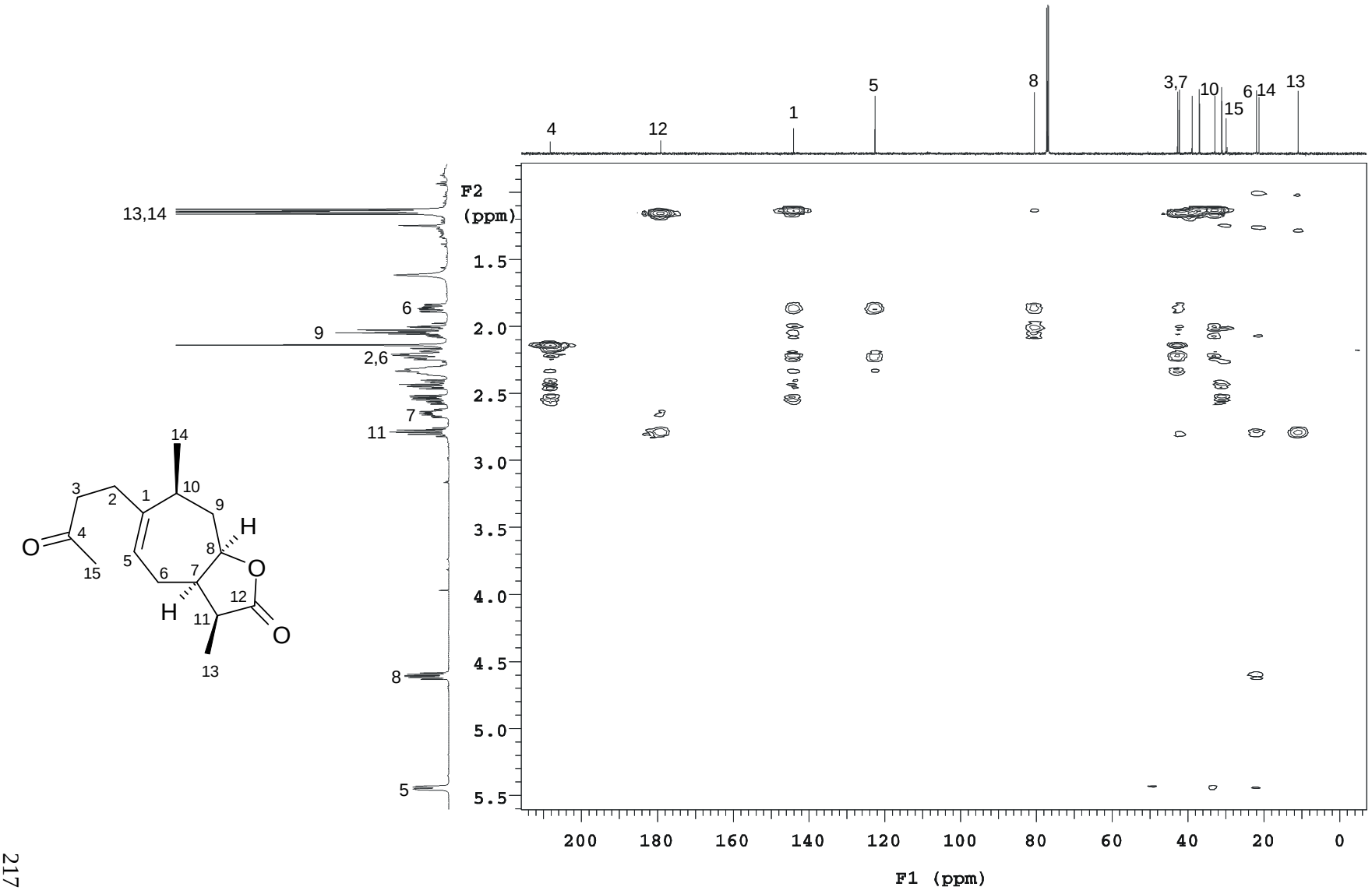


Plate 7: NOESY NMR spectrum of compound 302 in $CDCl_3$

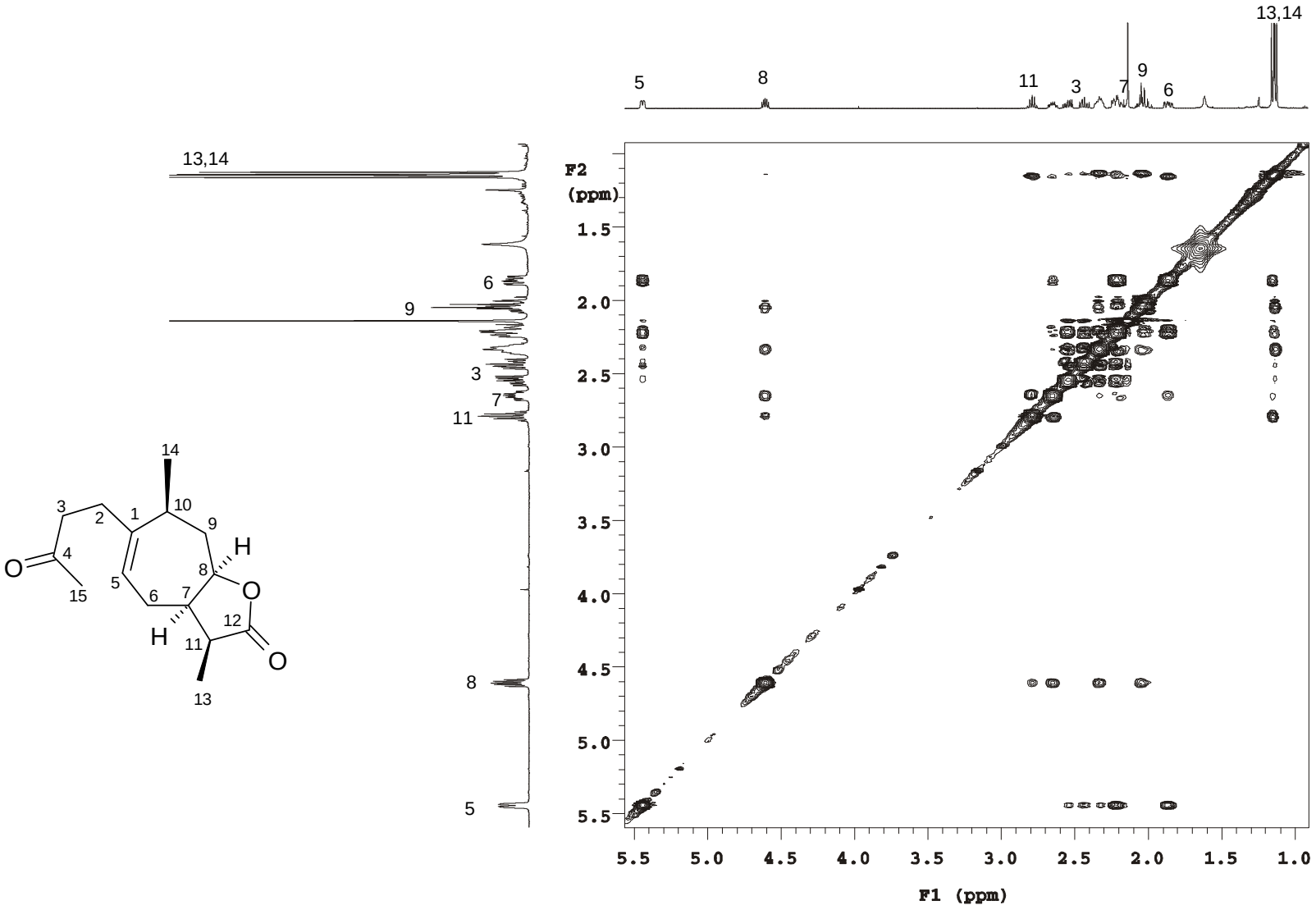


Plate 8: ^{1}H NMR spectrum of compound 304 in $CDCl_3$

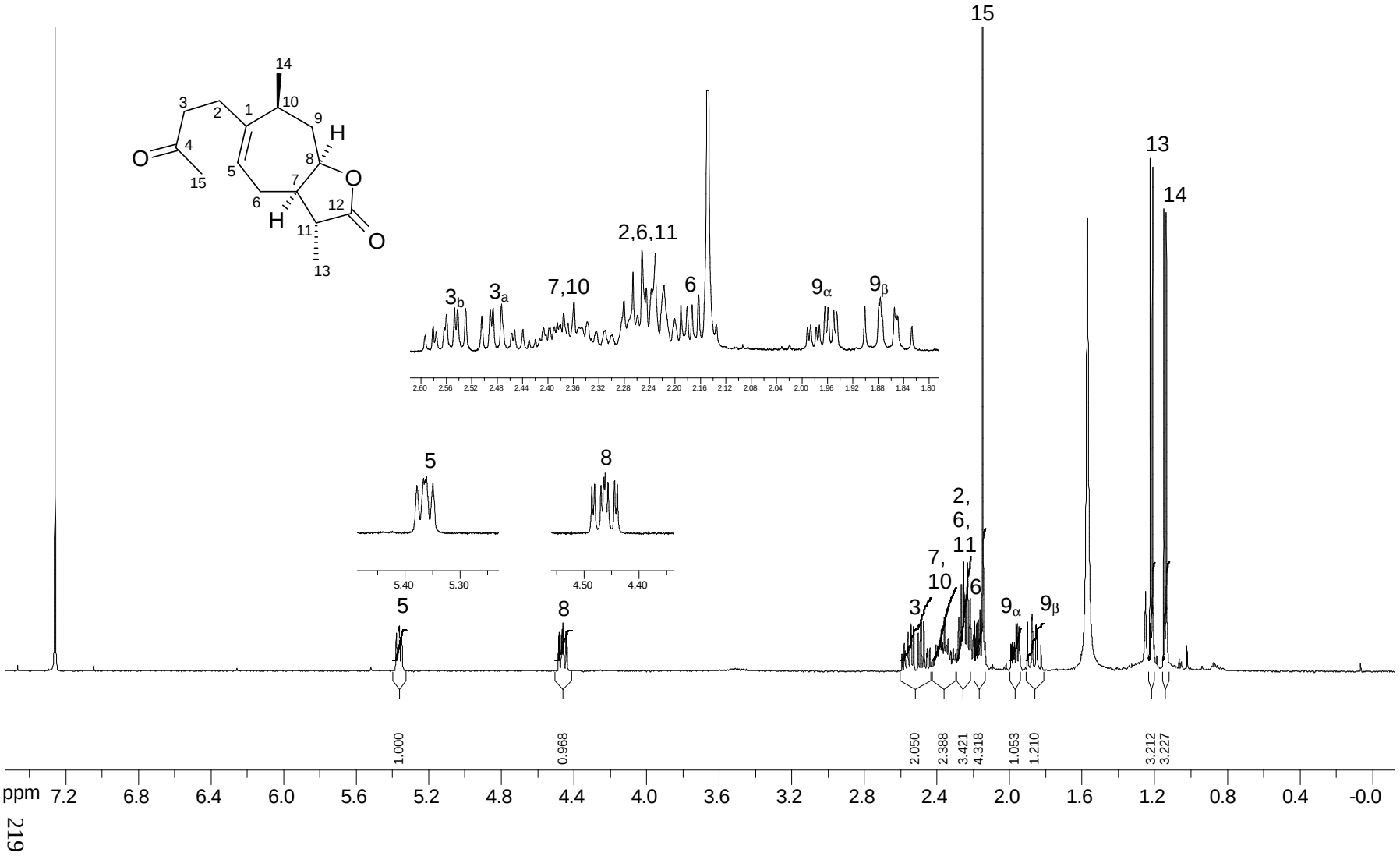


Plate 9: ^{13}C NMR spectrum of compound 304 in $CDCl_3$

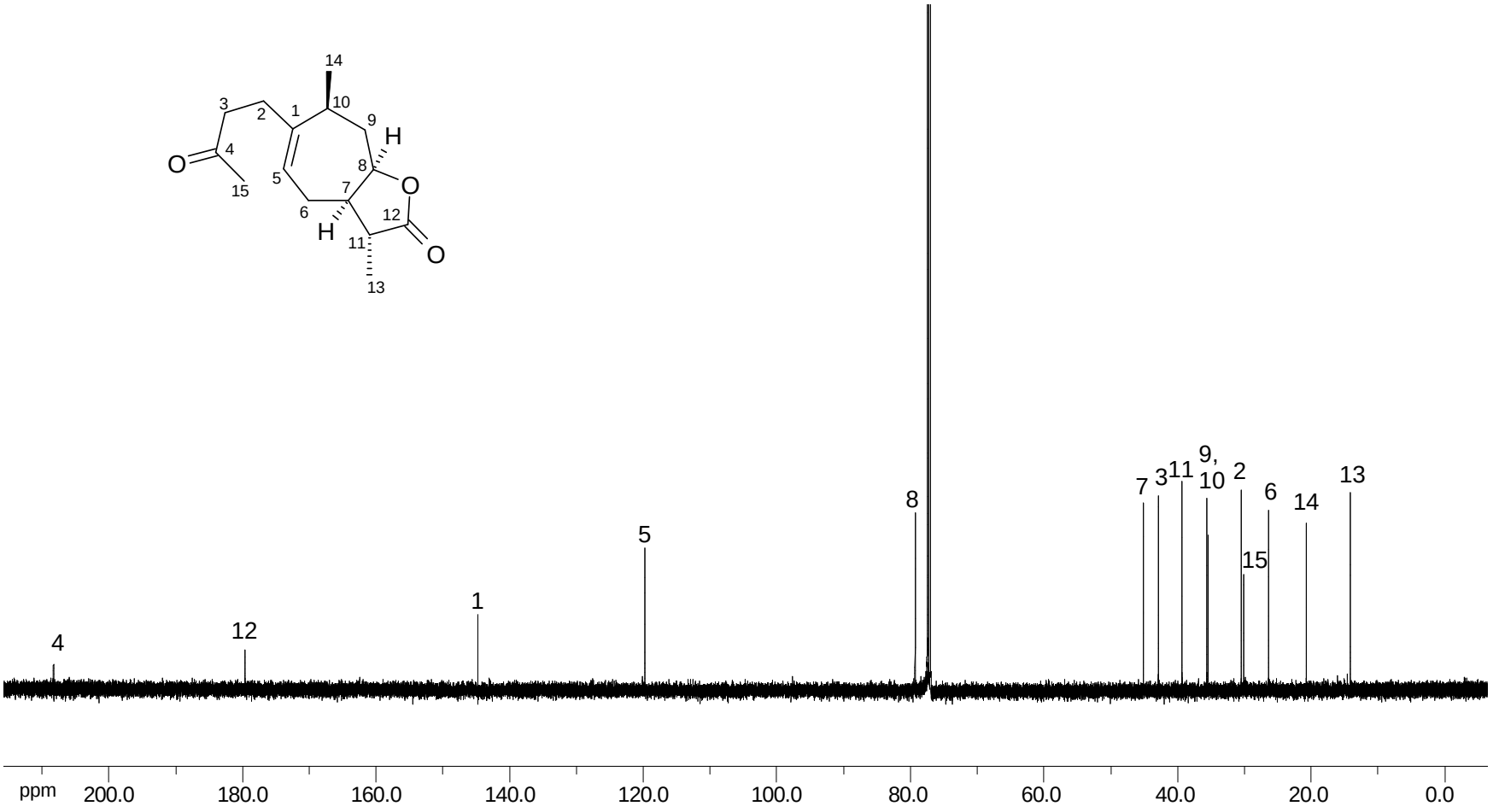


Plate 10: COSY NMR spectrum of compound 304 in $CDCl_3$

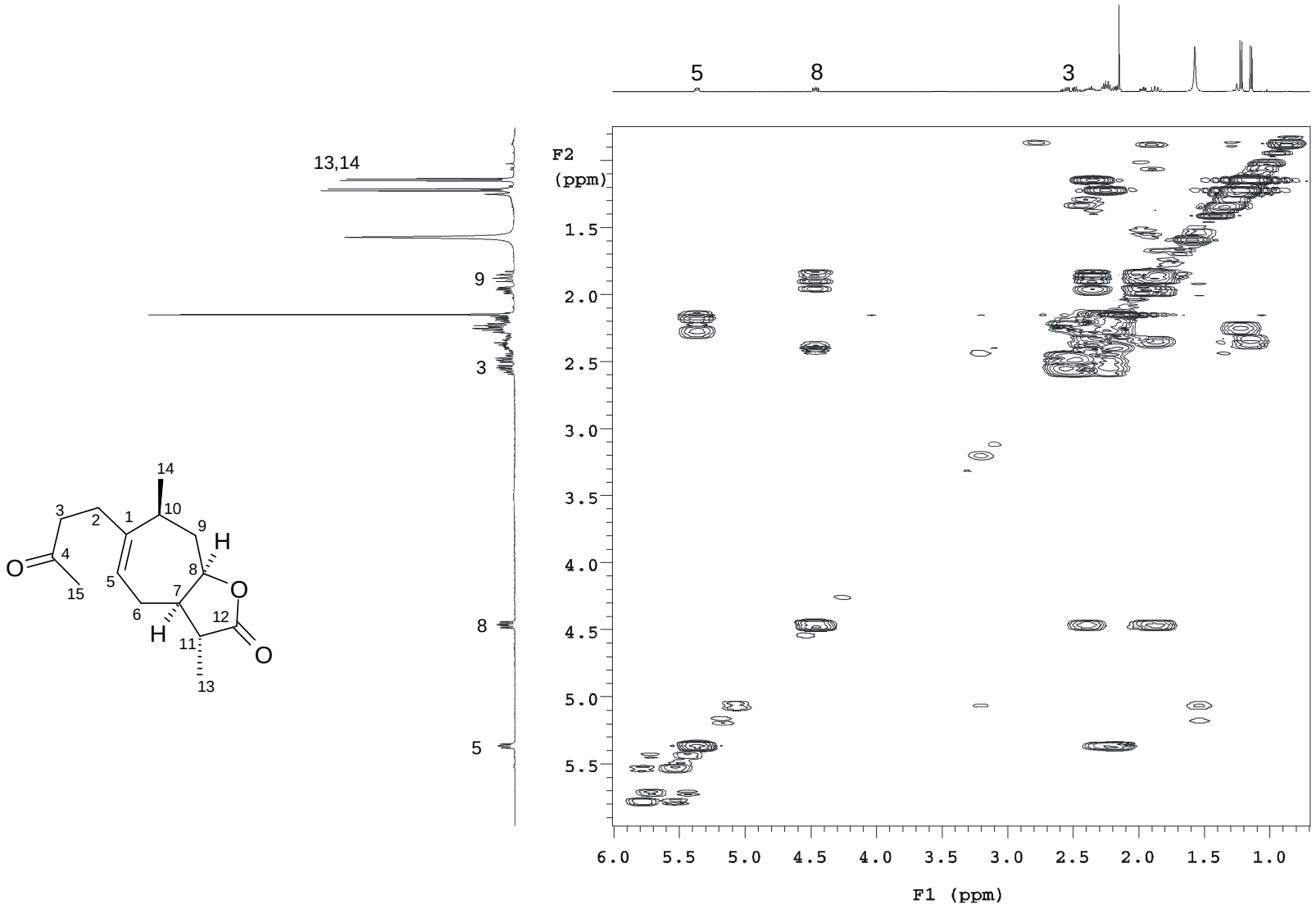


Plate 11: DEPT NMR spectrum of compound 304 in $CDCl_3$

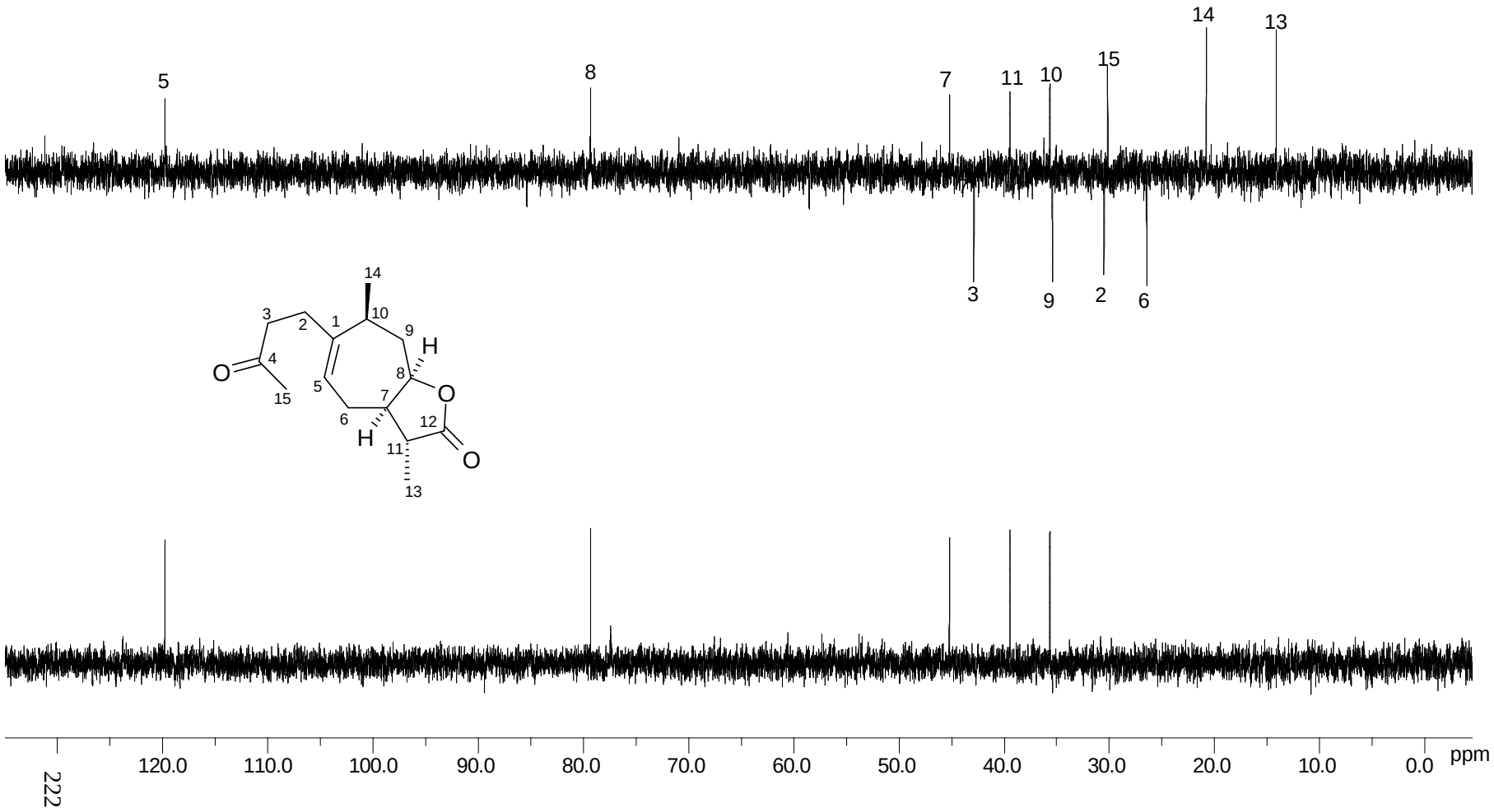


Plate 12: HSQC NMR spectrum of compound 304 in $CDCl_3$

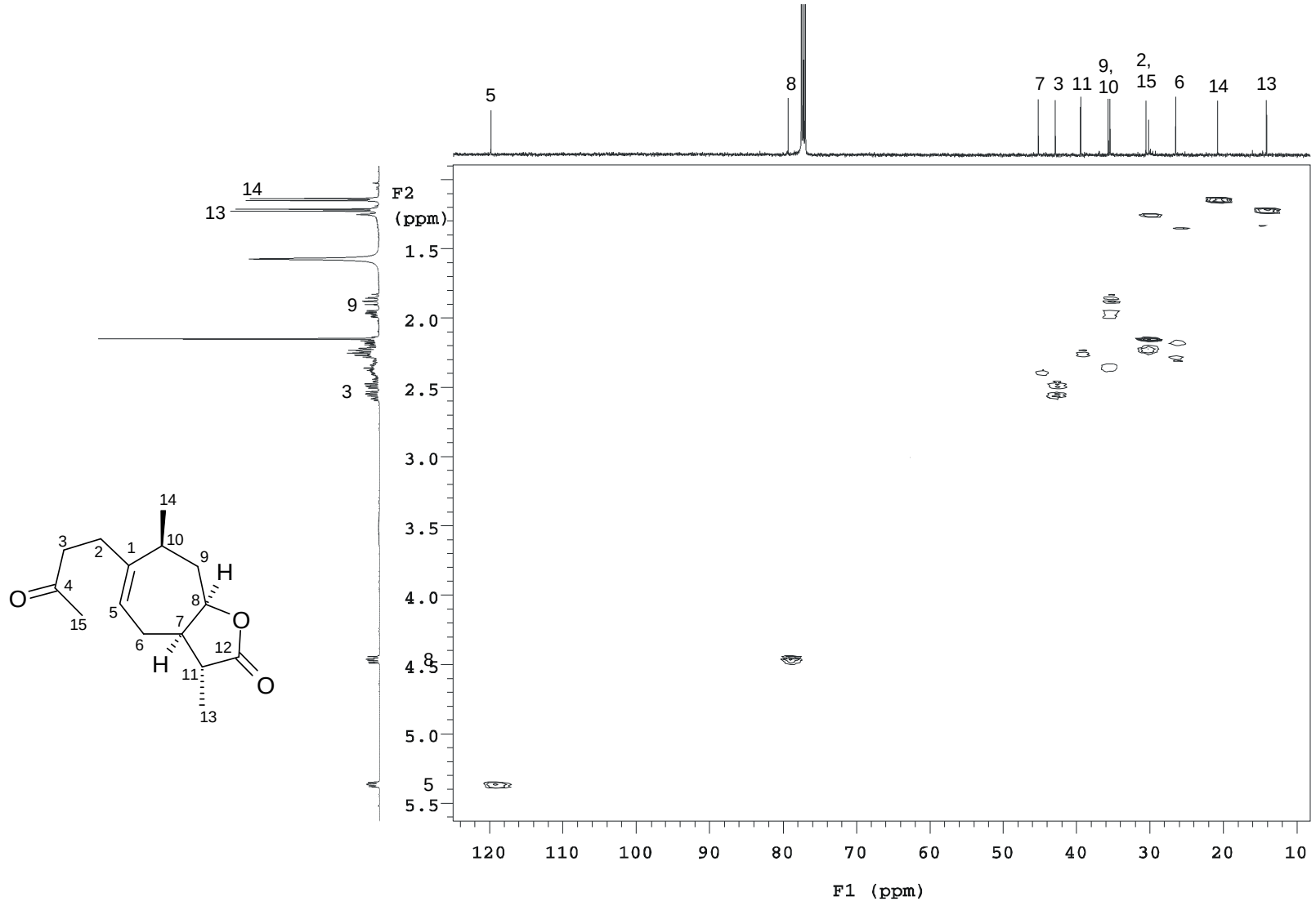


Plate 13: HMQC NMR spectrum of compound 304 in $CDCl_3$

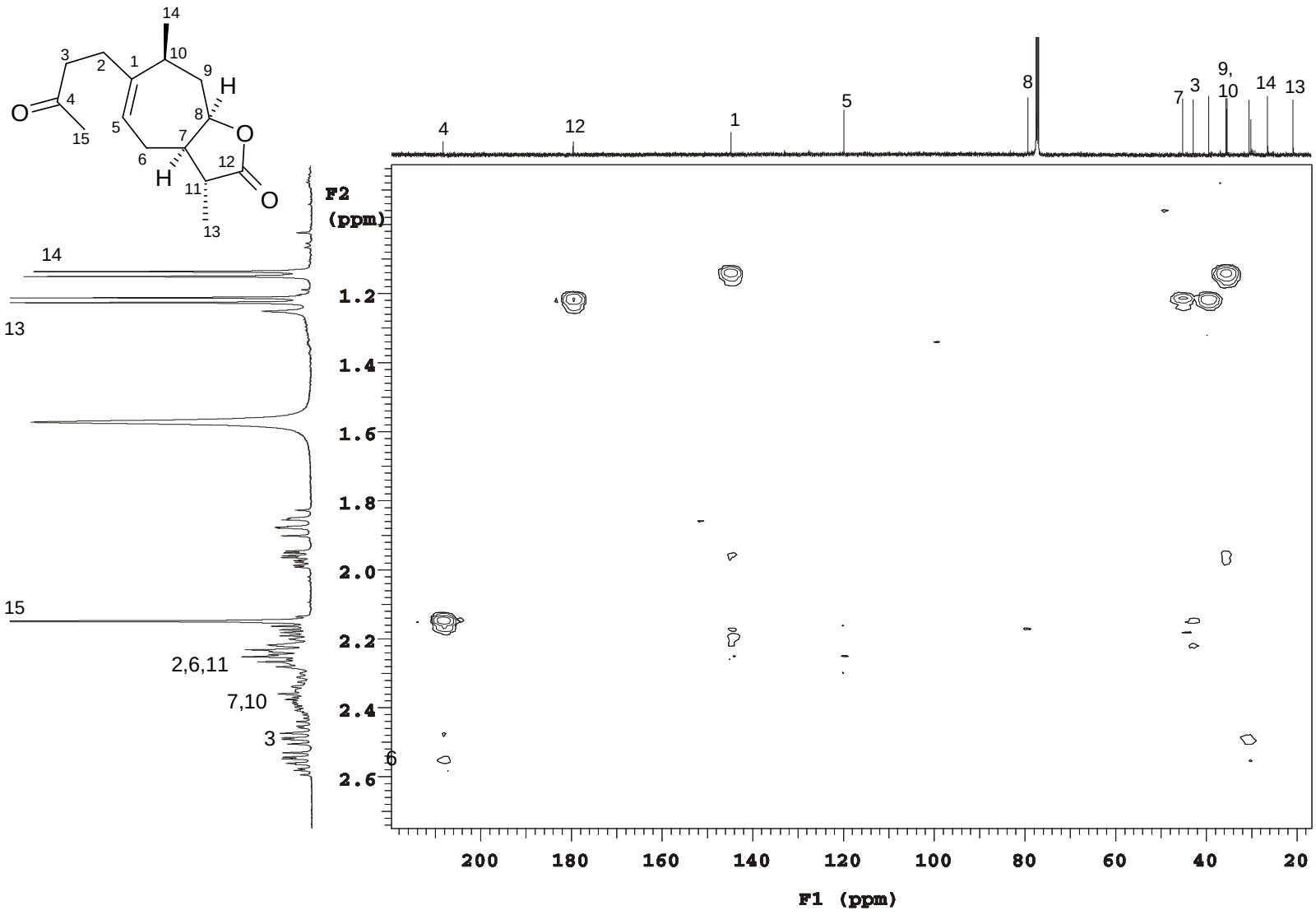


Plate 14: NOESY NMR spectrum of compound 304 in $CDCl_3$

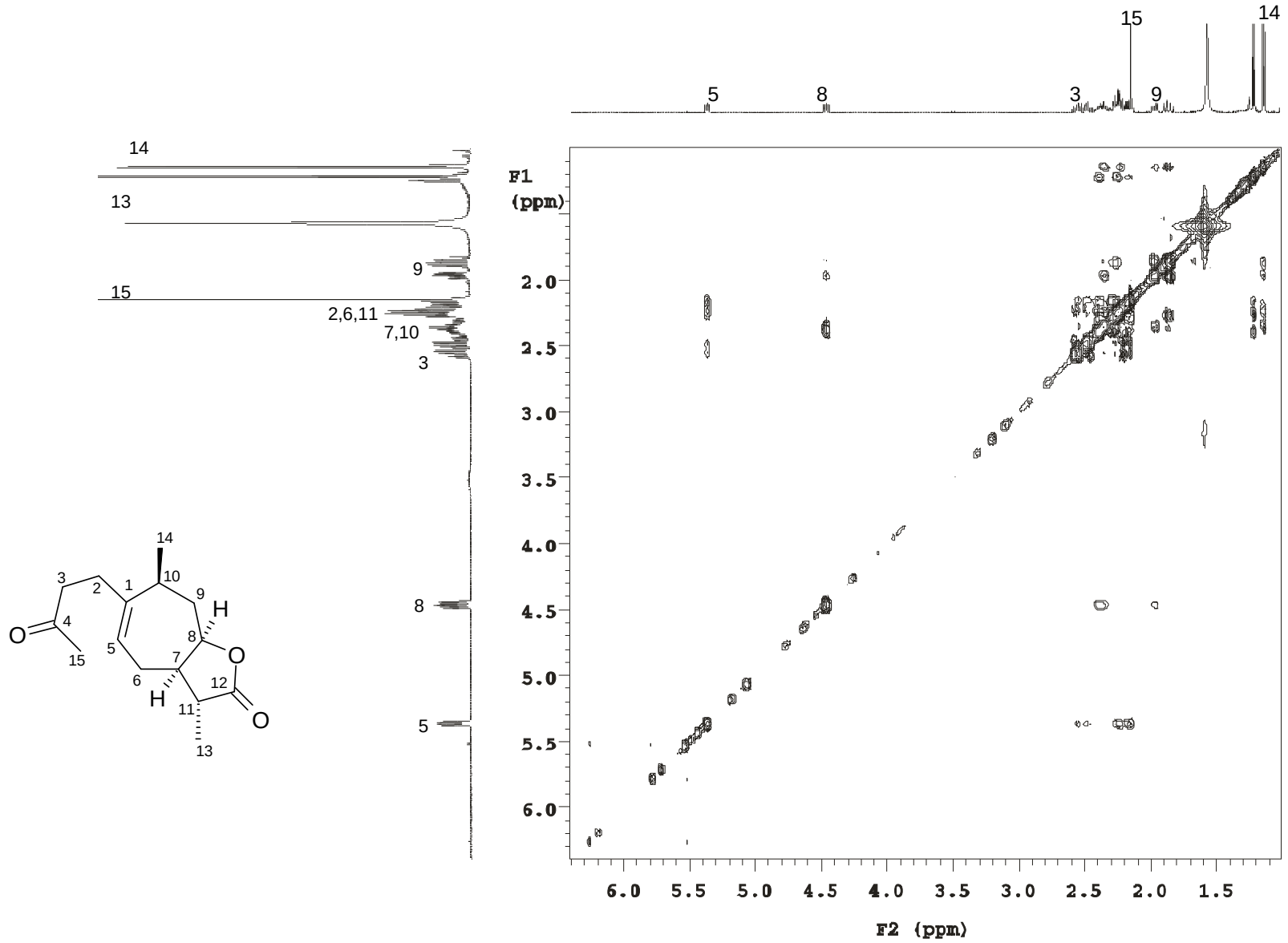


Plate 15: ^{1}H NMR spectrum of helisplendidilactone (306) in $CDCl_3$

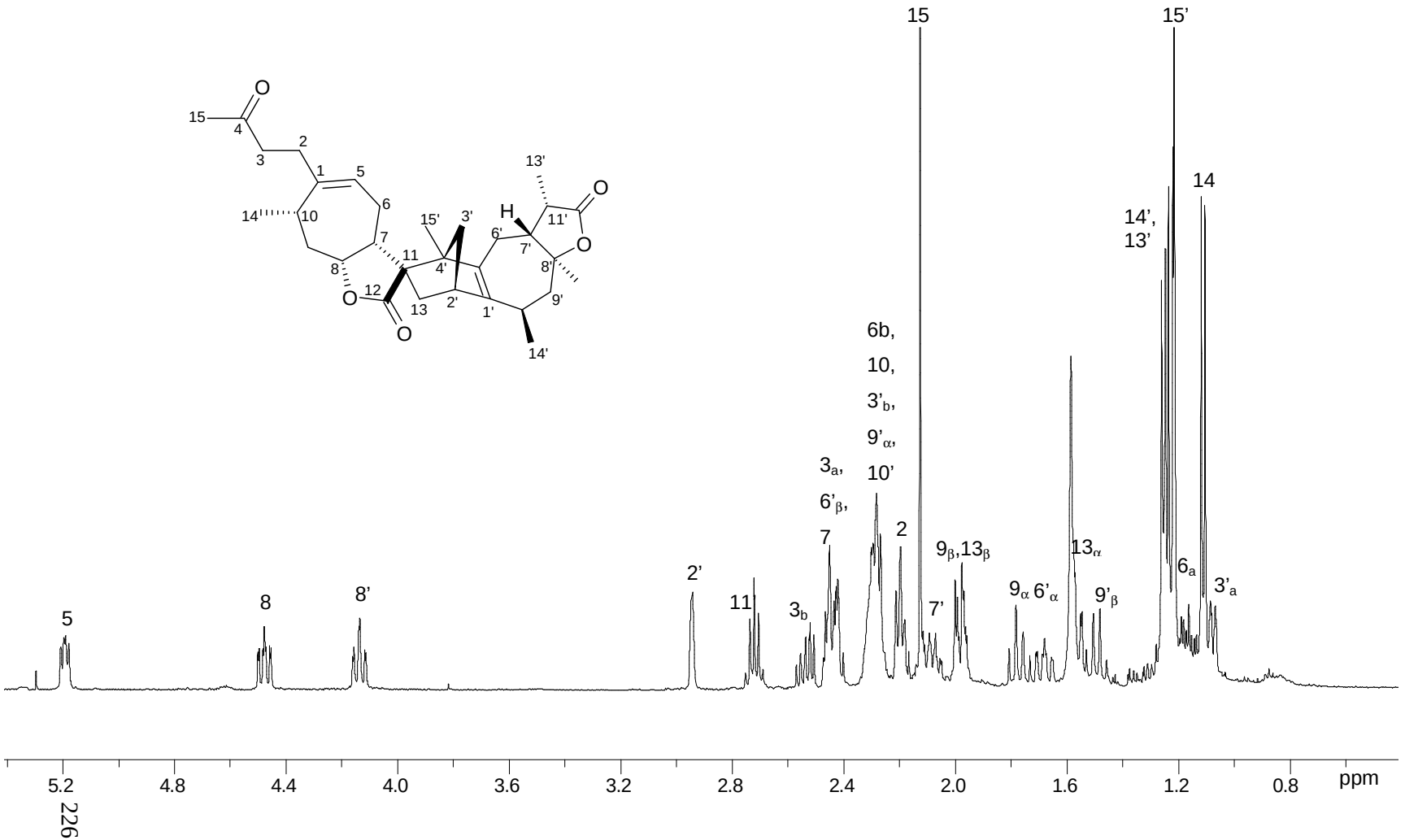


Plate 16: ^{13}C NMR spectrum of helisplendidilactone (306) in $CDCl_3$

Carbon	Position		Carbon	Position
4	207.8		7	41.8
12	181.7		9'	40.4
12'	179.1		11'	39.7
1'	150.2		9	37.1
1	144.5		13	36.2
5'	140.7		10	35.8
5	119.7		2	30.3
8'	84.6		15	29.8
8	78.5		10'	29.3
11	63.0		6	25.3
4'	54.0		6'	25.0
3'	50.7		14'	21.2
7'	45.5		14	20.7
2'	43.2		15'	13.4
3	42.5		13'	10.6

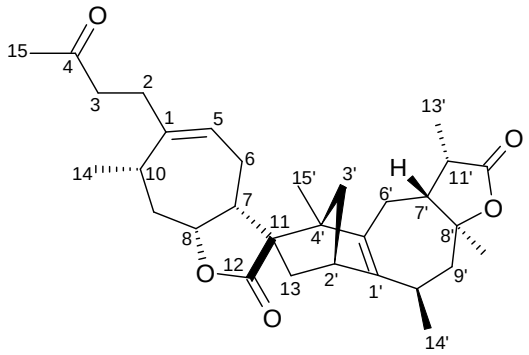


Plate 17: COSY NMR spectrum of helisplendidilactone (306) in $CDCl_3$

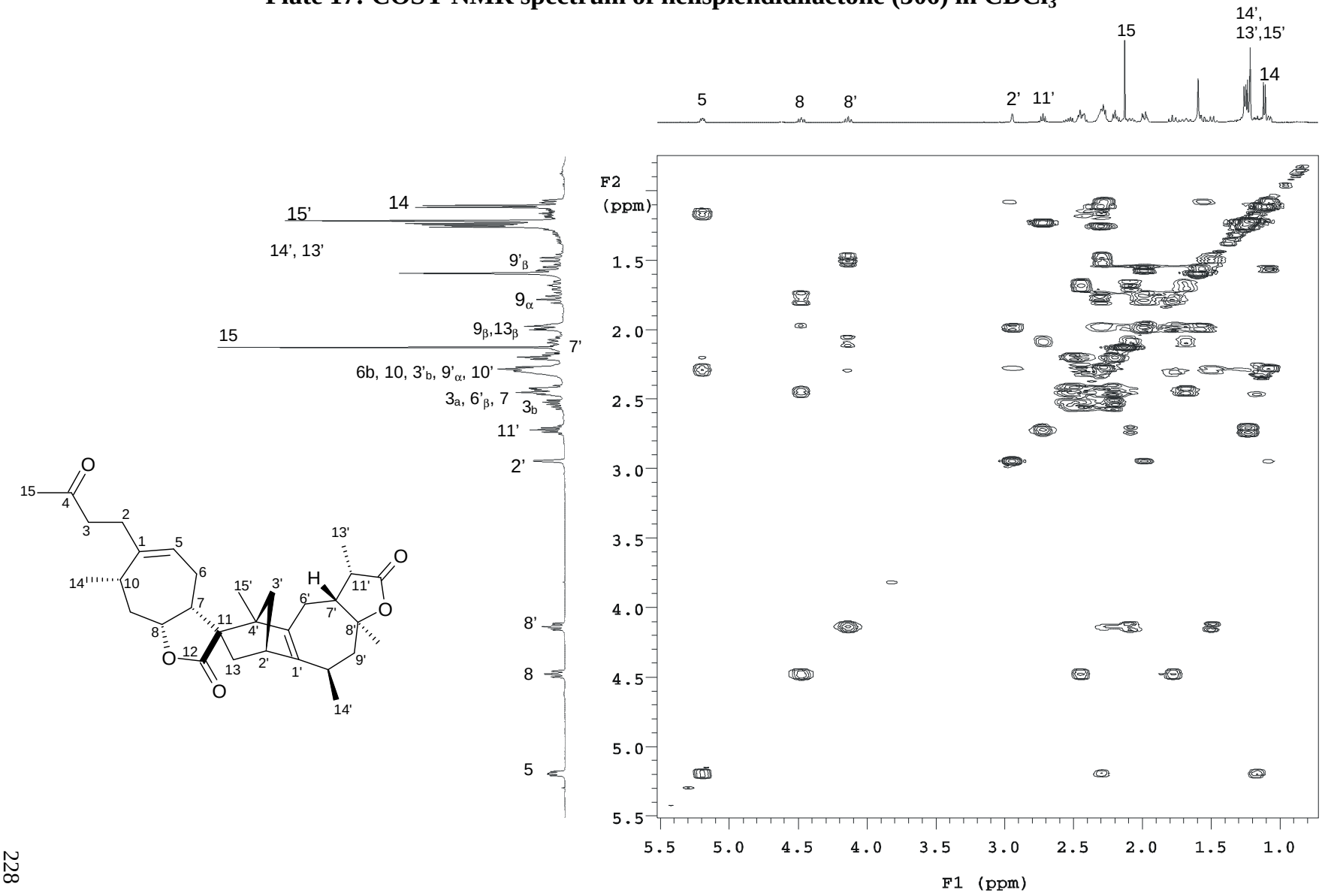


Plate 18: DEPT NMR spectrum of helisplendidilactone (306) in $CDCl_3$

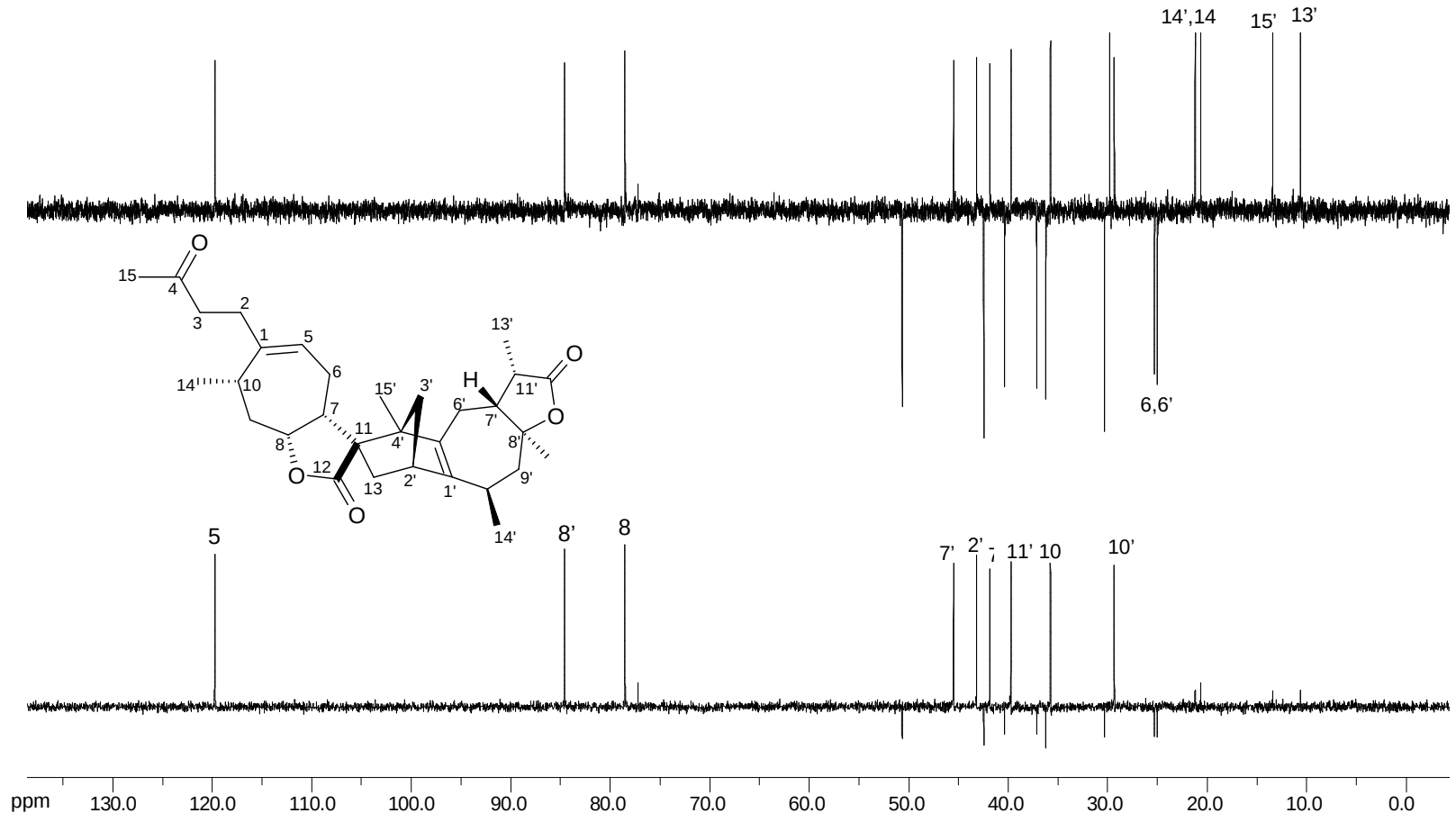


Plate 19: HSQC NMR spectrum of helisplendidilactone (306) in $CDCl_3$

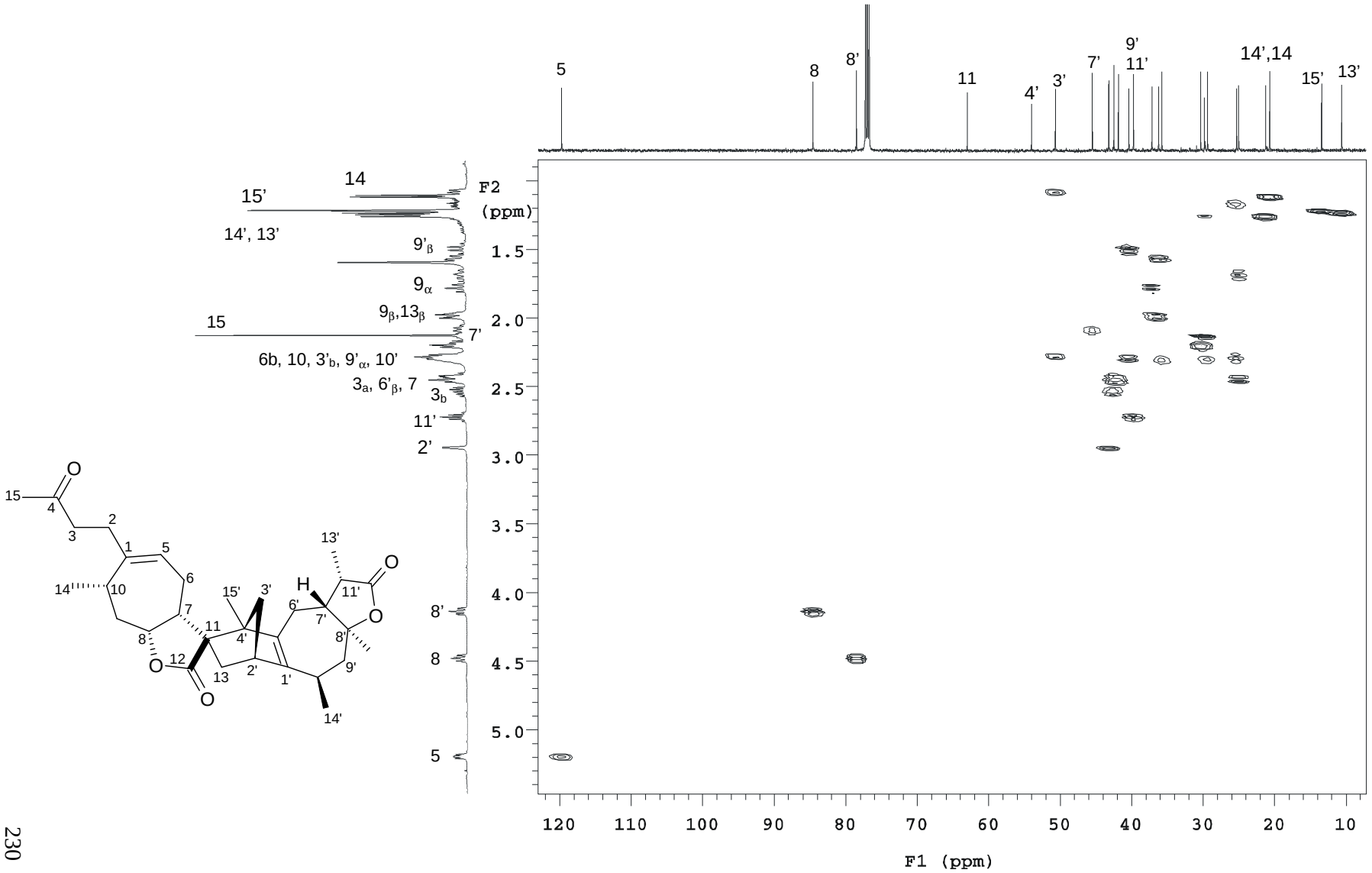


Plate 20: HMQCNMR spectrum of helisplendidilactone (306) in $CDCl_3$

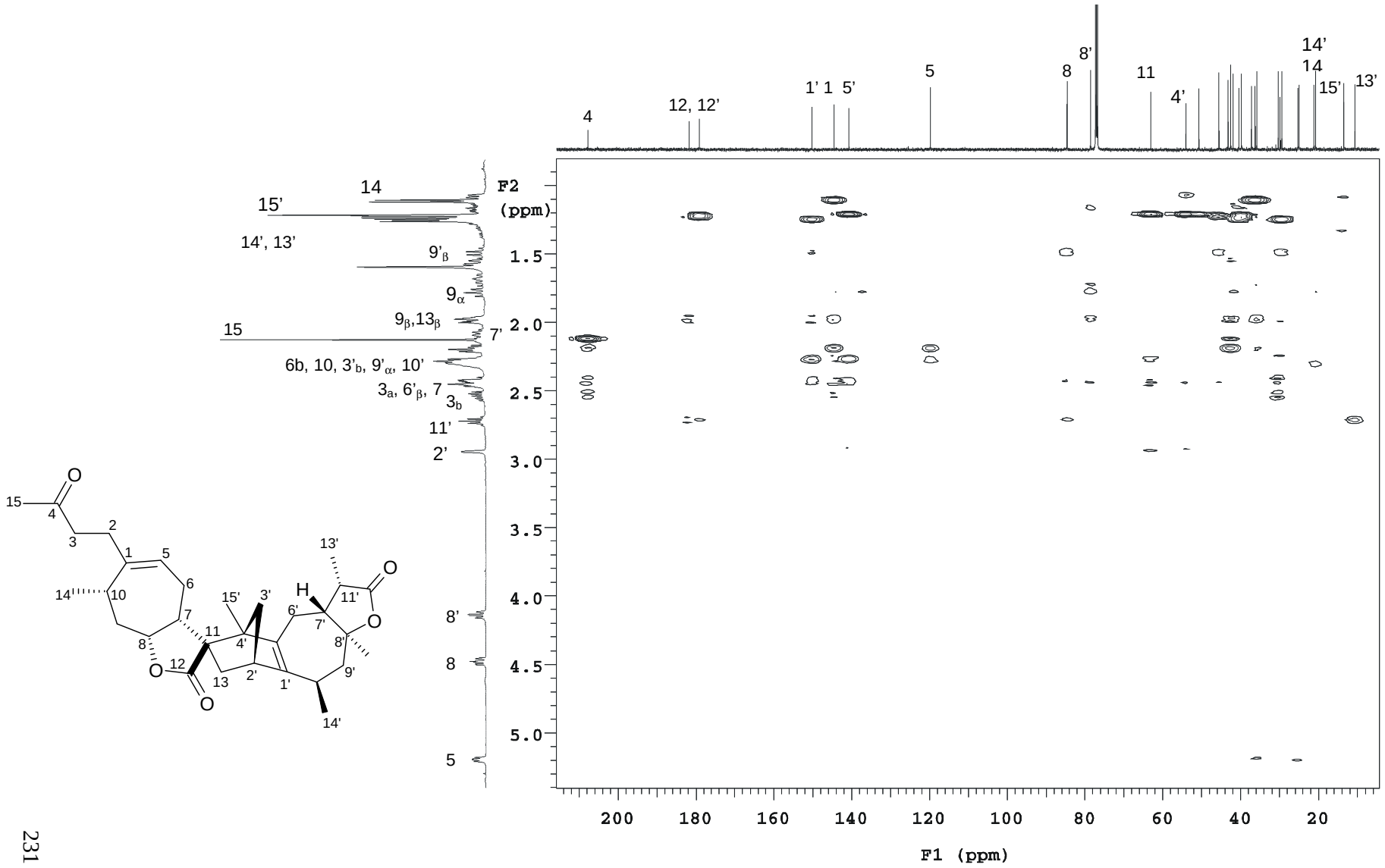


Plate 21: NOESY NMR spectrum of helisplendidilactone (306) in $CDCl_3$

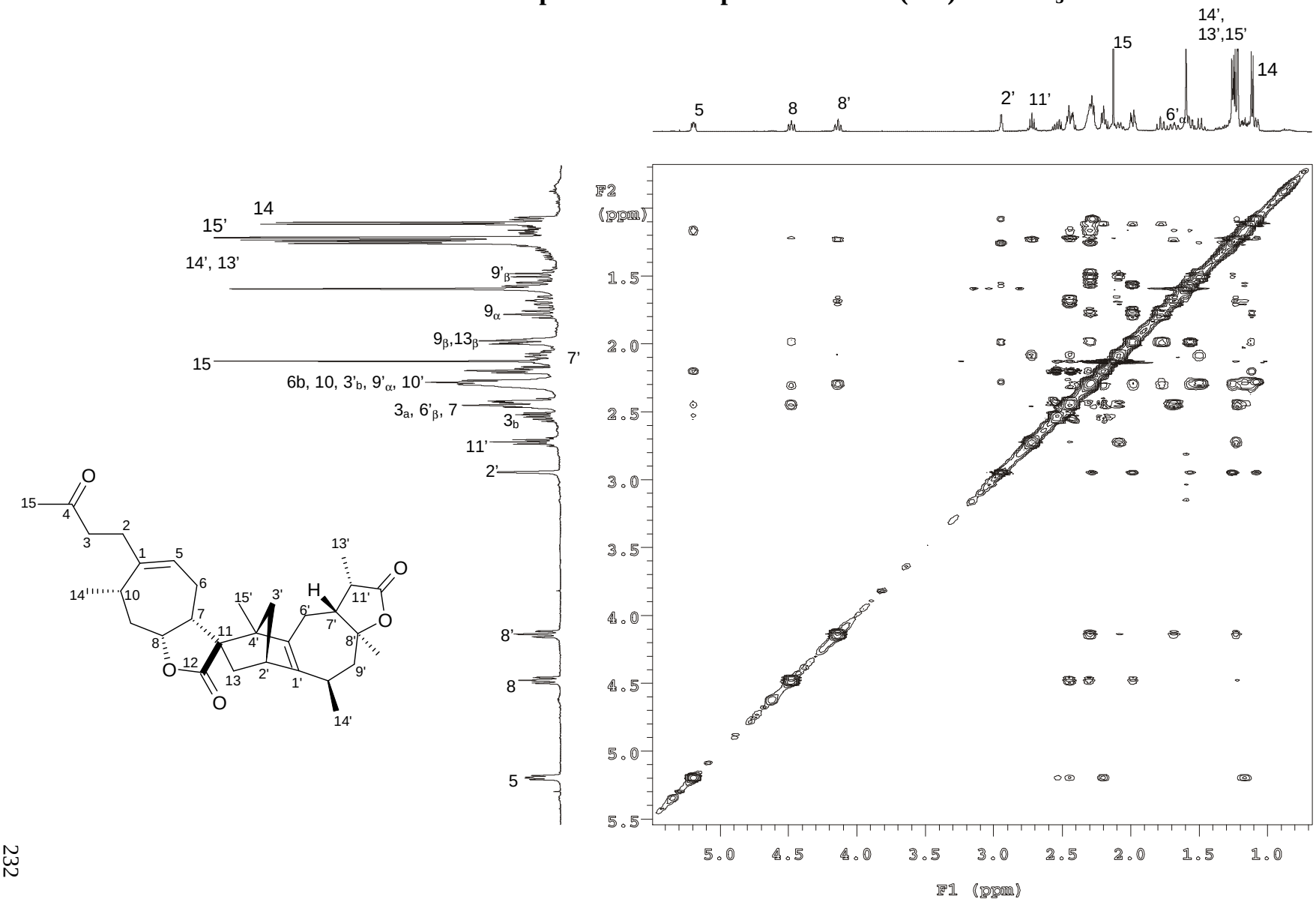


Plate 22: ^{1}H NMR spectrum of helimontanilactone (307) in $CDCl_3$

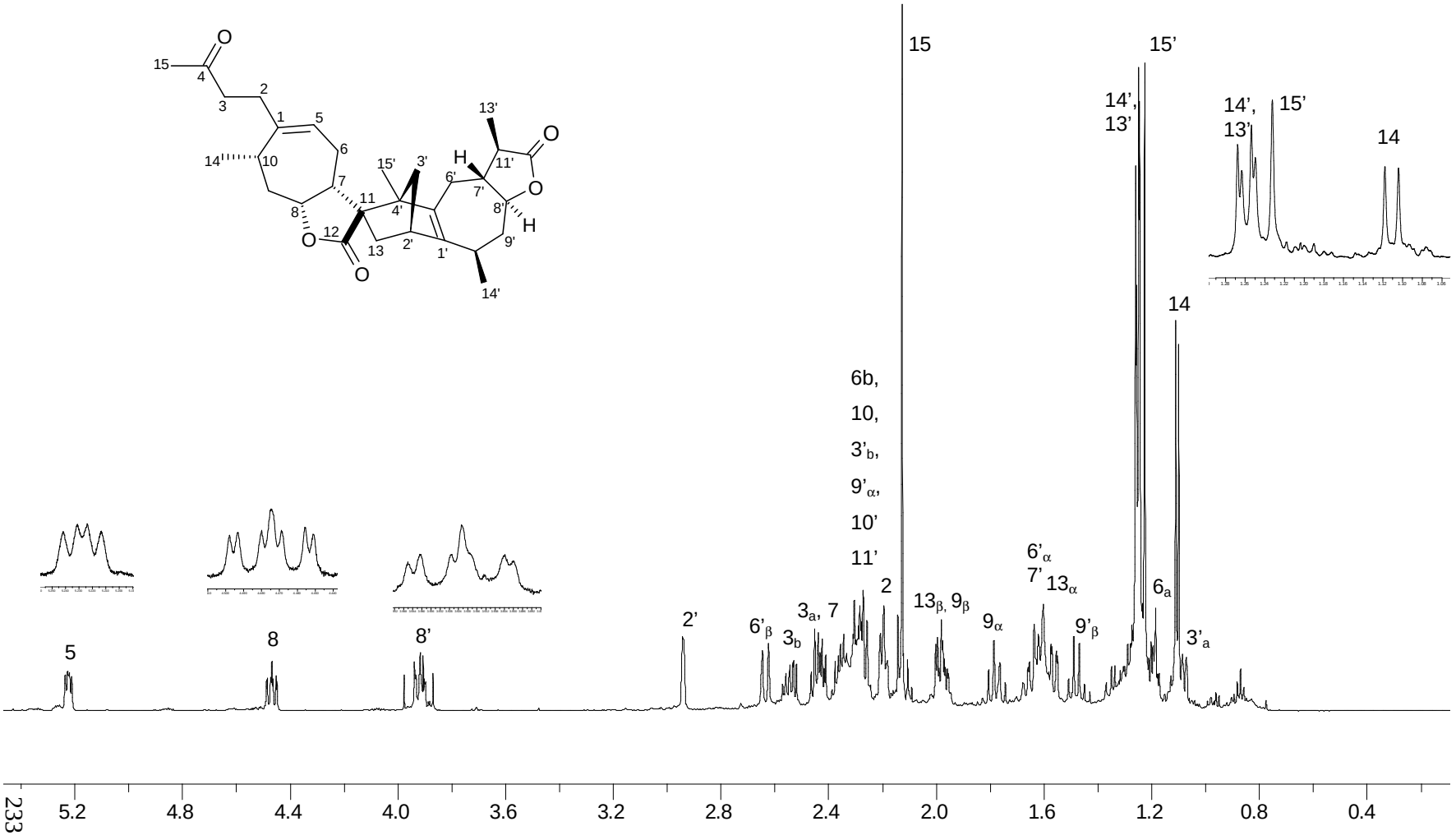


Plate 23: ^{13}C NMR spectrum of helimontanilactone (307) in $CDCl_3$

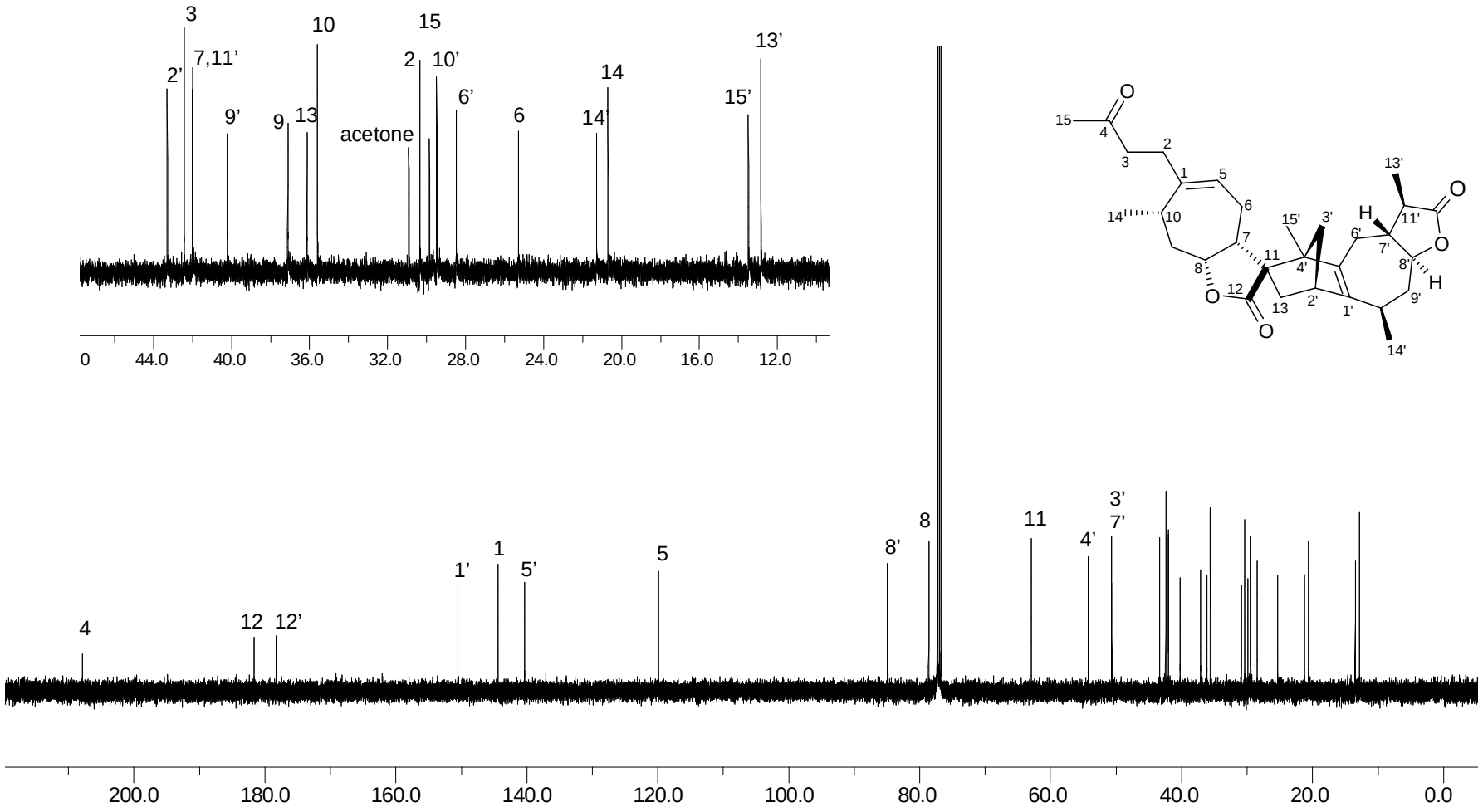


Plate 24: COSY NMR spectrum of helimontanilactone (307) in $CDCl_3$

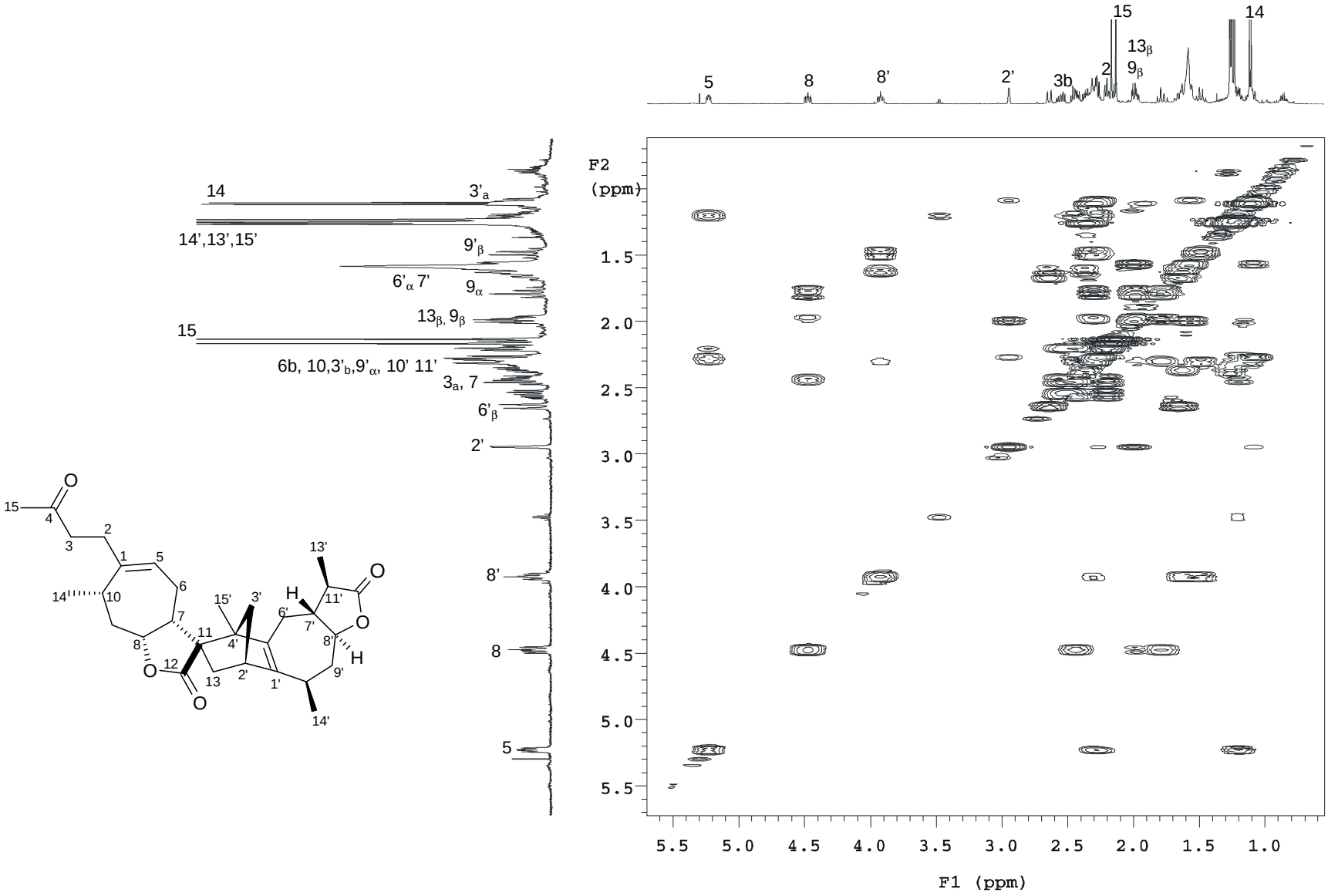


Plate 25: DEPT NMR spectrum of helimontanilactone (307) in $CDCl_3$

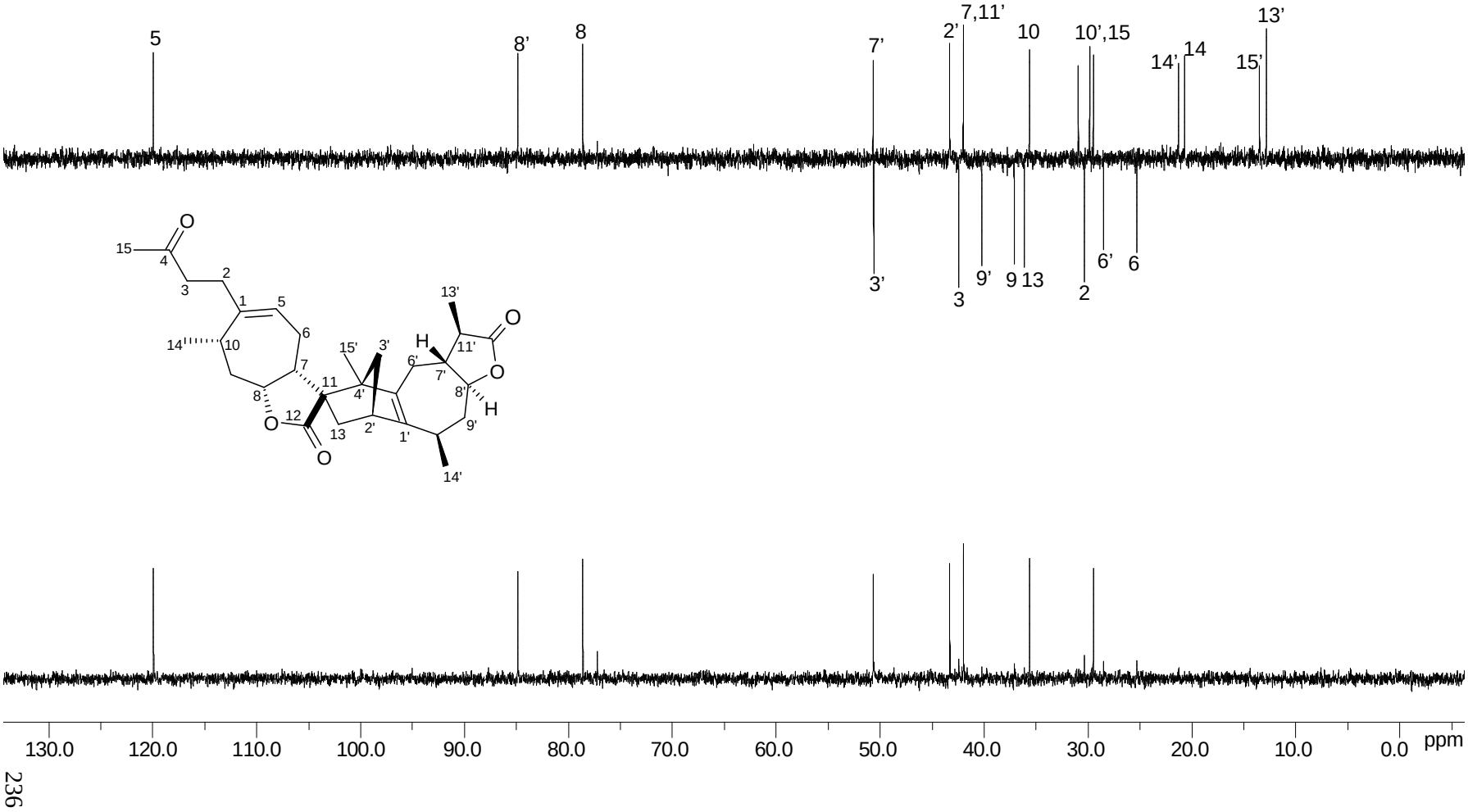


Plate 26: HSQC NMR spectrum of helimontanilactone in (307) in $CDCl_3$

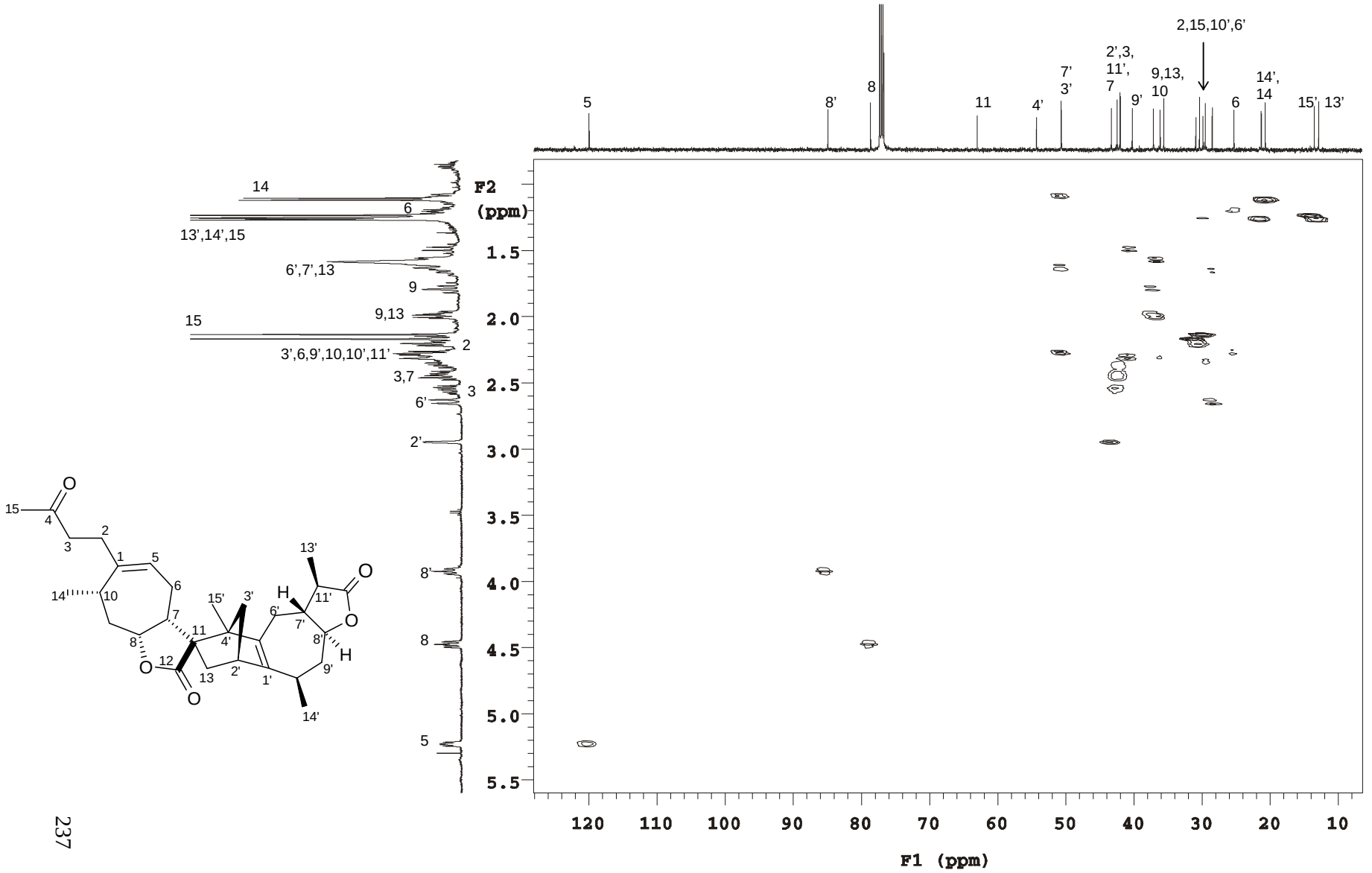


Plate 27: HMQC NMR spectrum of helimontanilactone (307) in $CDCl_3$

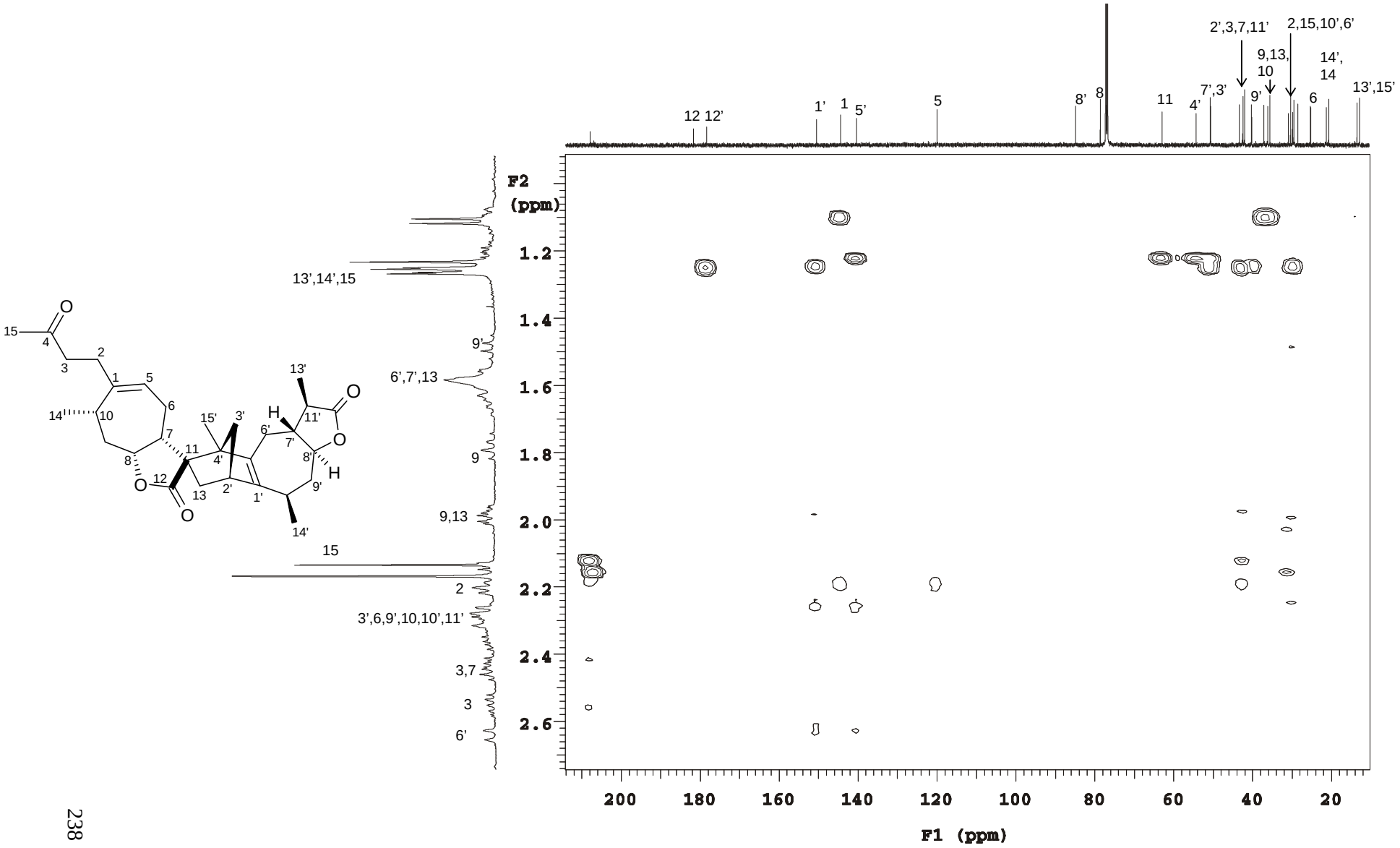


Plate 28: NOESY NMR spectrum of helimontanilactone (307) in $CDCl_3$

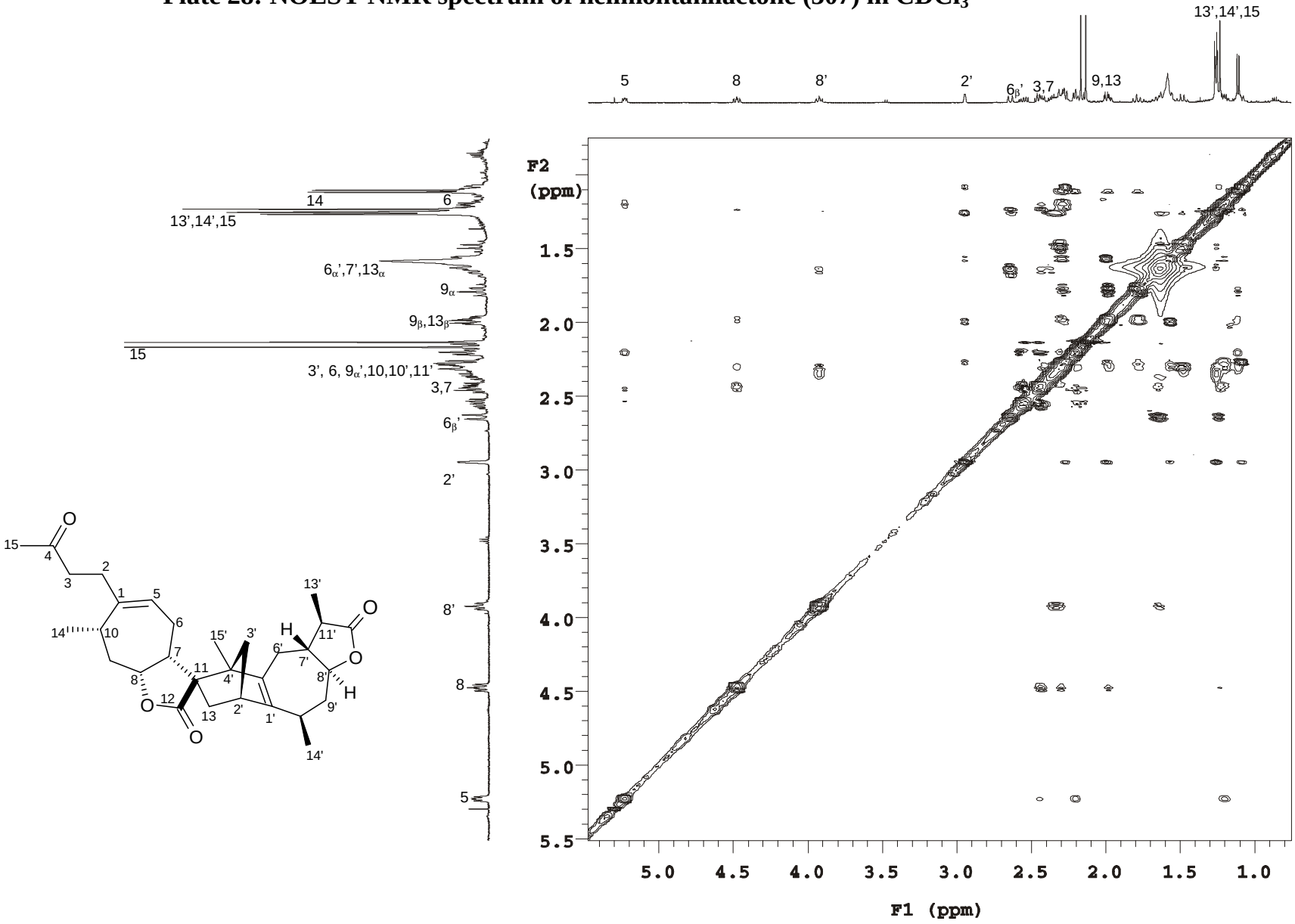


Plate 29: ^{1}H NMR spectrum of helimontanilactone (307) in $CDCl_3$ with $Eu(fod)_3$

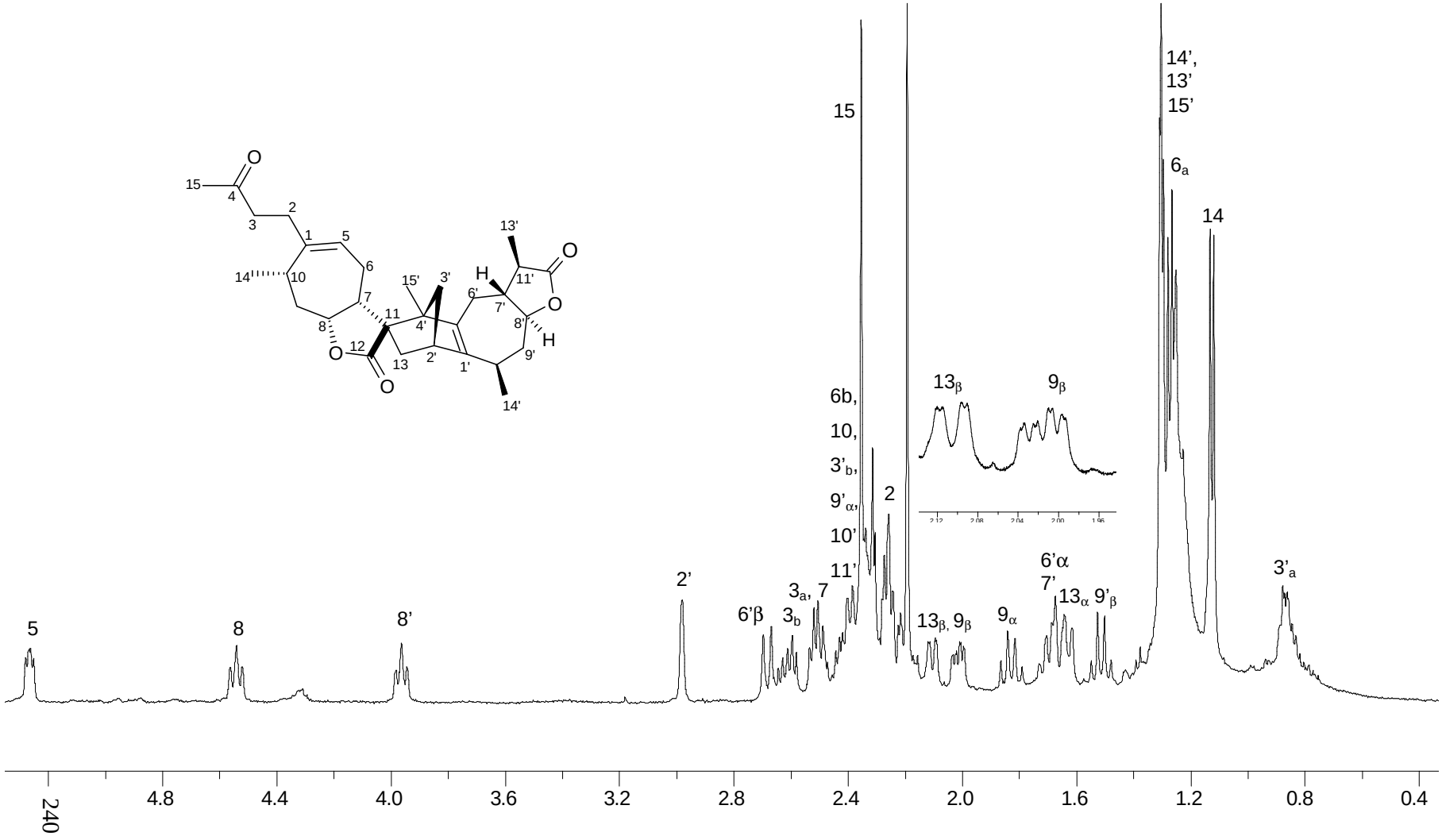


Plate 30: NOESY NMR spectrum of helimontanilactone (307) in $CDCl_3$ with $Eu(fod)_3$

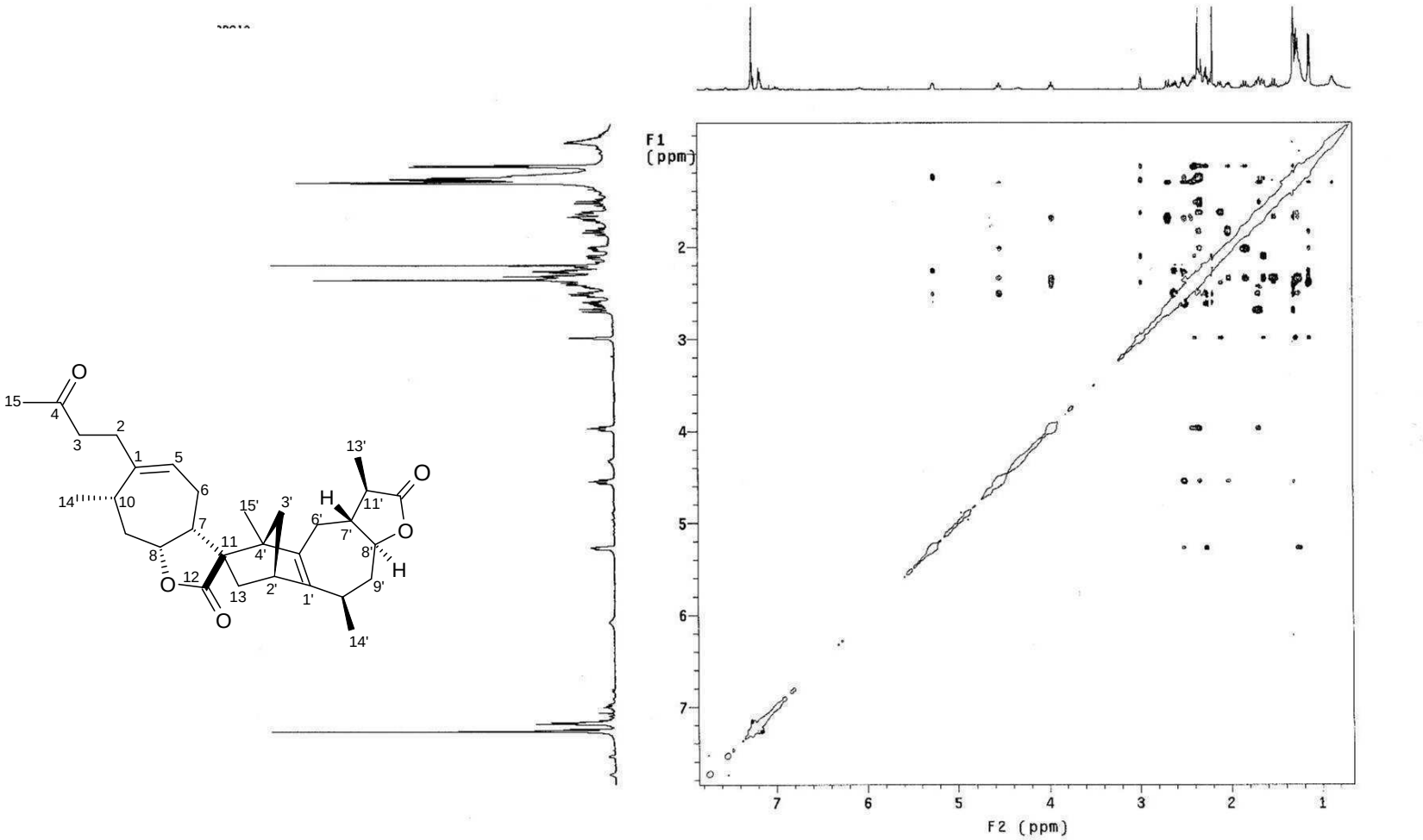


Plate 31: ^{1}H NMR spectrum of spathulenol (286) in $CDCl_3$

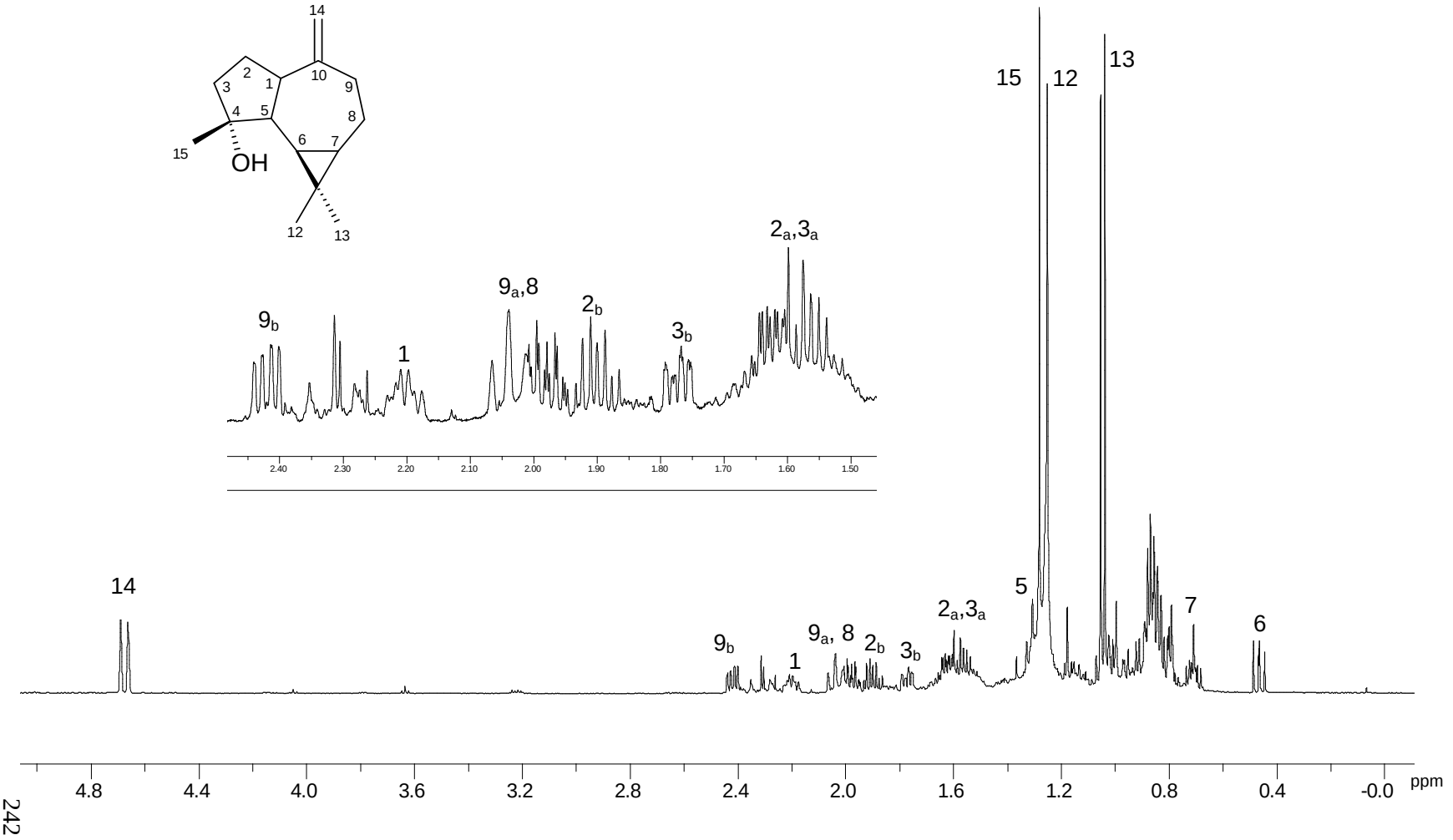


Plate 32: ^{13}C NMR spectrum of spathulenol (286) in $CDCl_3$

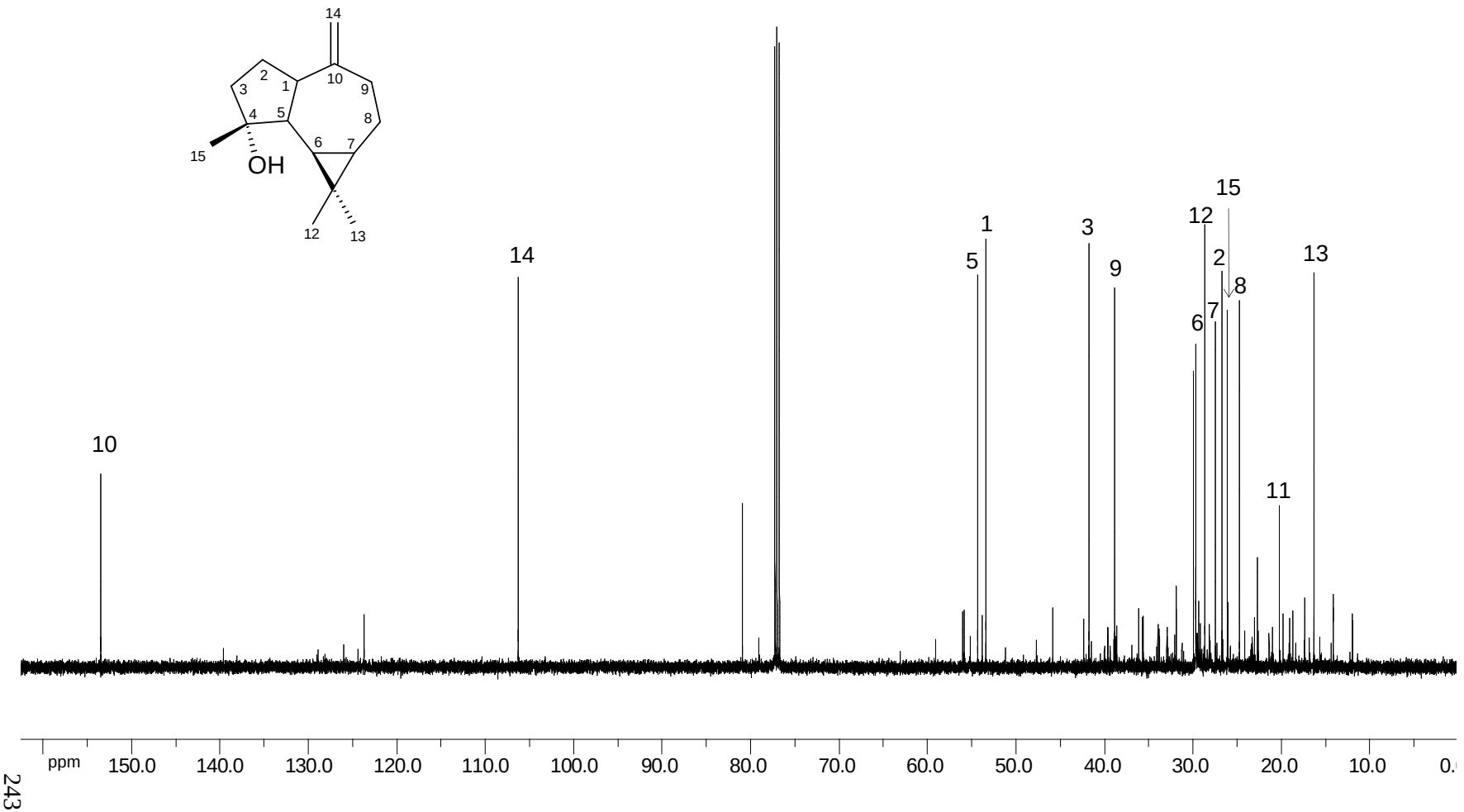


Plate 33: COSY NMR spectrum of spathulenol (286) in $CDCl_3$

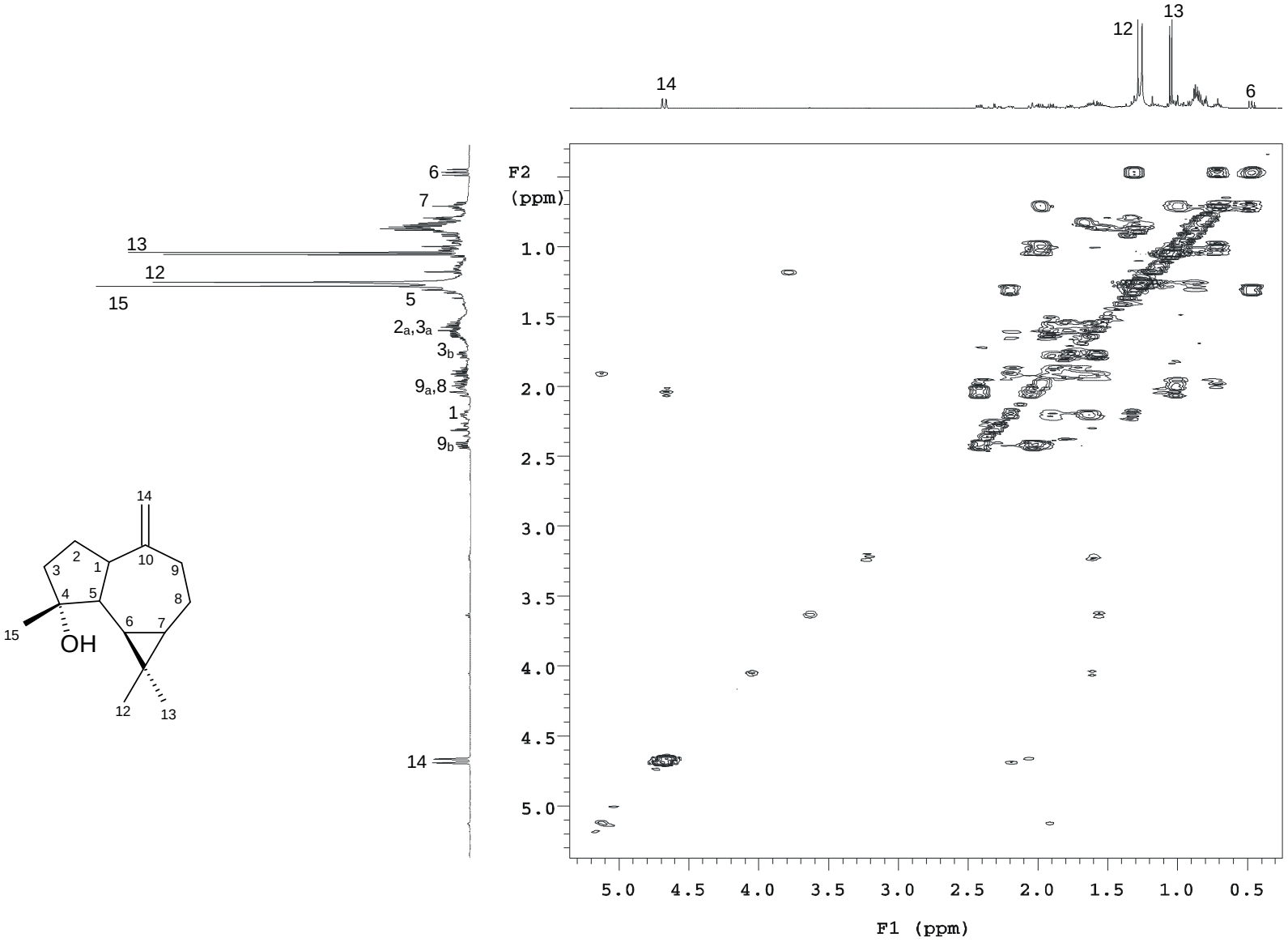


Plate 34: DEPT NMR spectrum of spathulenol (286) in $CDCl_3$

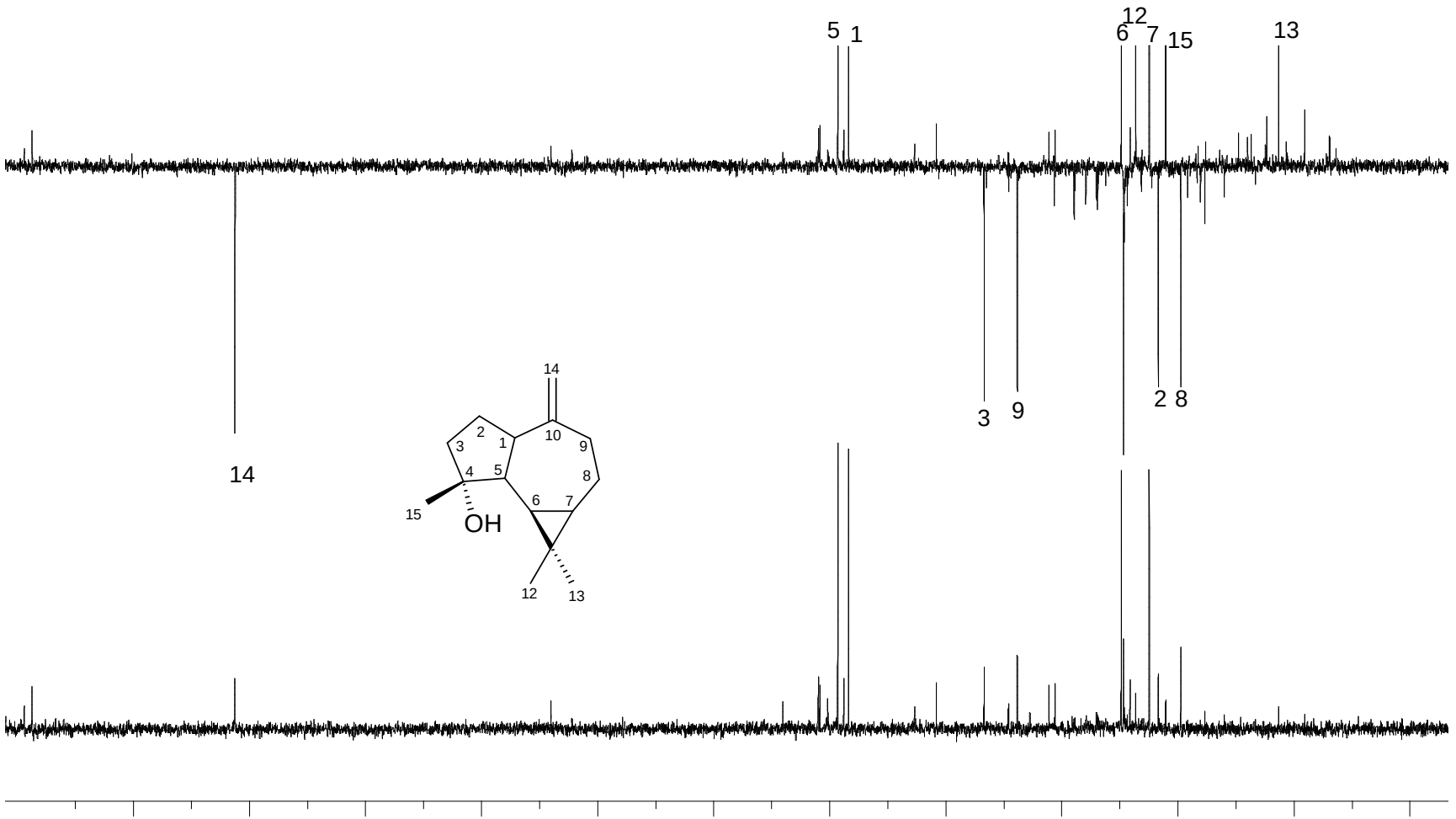


Plate 35: HSQC NMR spectrum of spathulenol (286) in $CDCl_3$

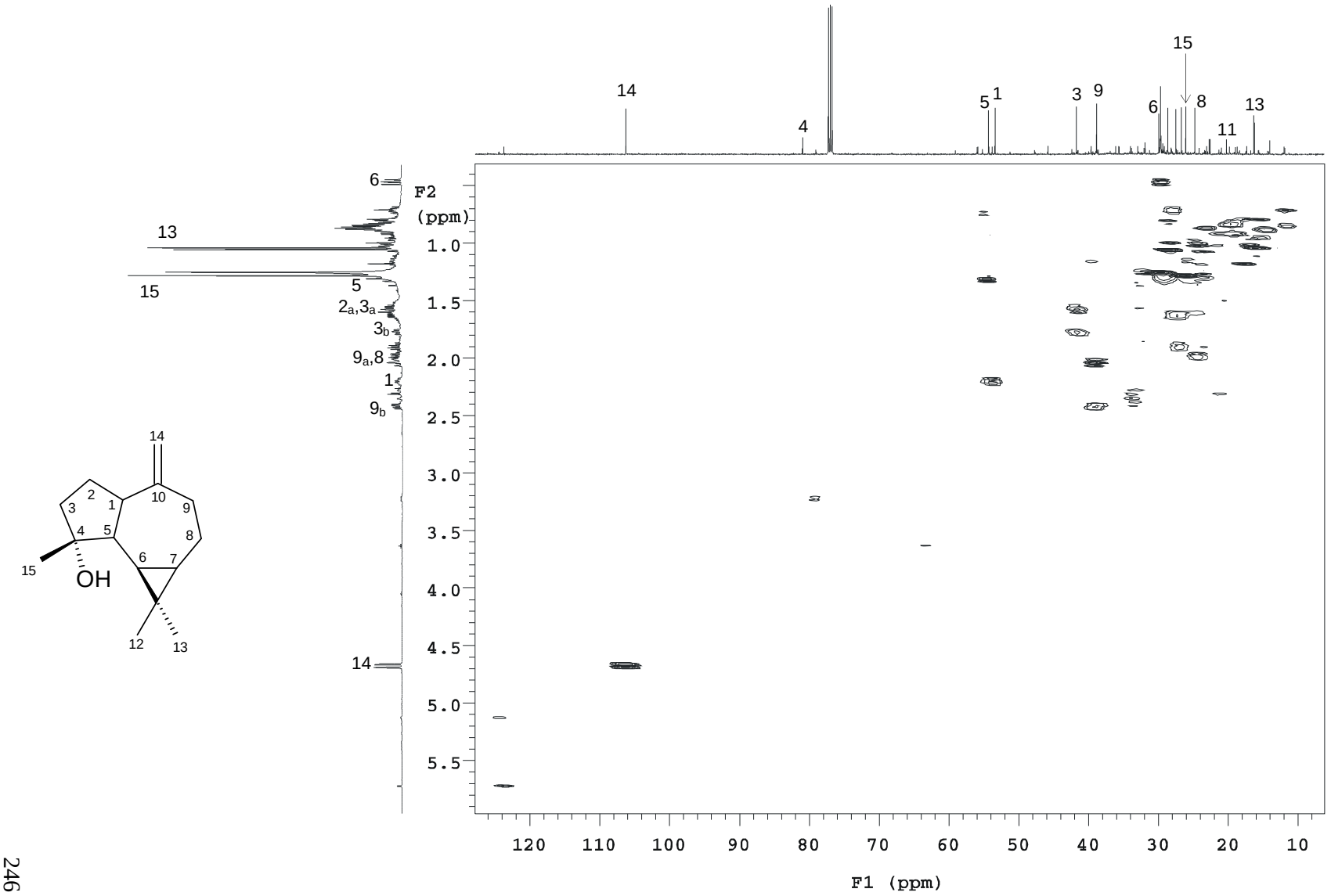


Plate 36: HMQC NMR spectrum of spathulenol (286) in $CDCl_3$

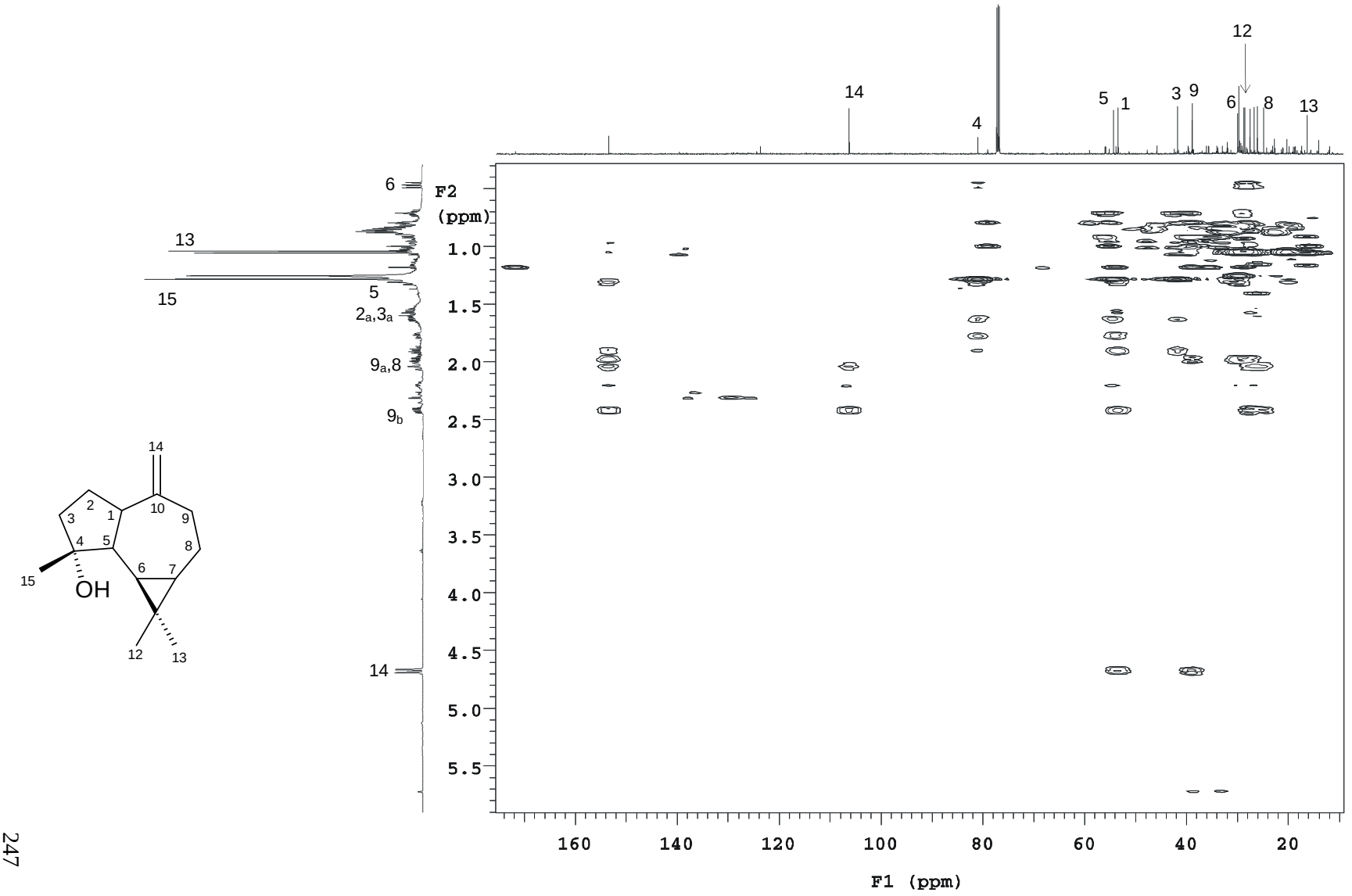


Plate 37: ^{1}H NMR spectrum of compound 301 in $CDCl_3$

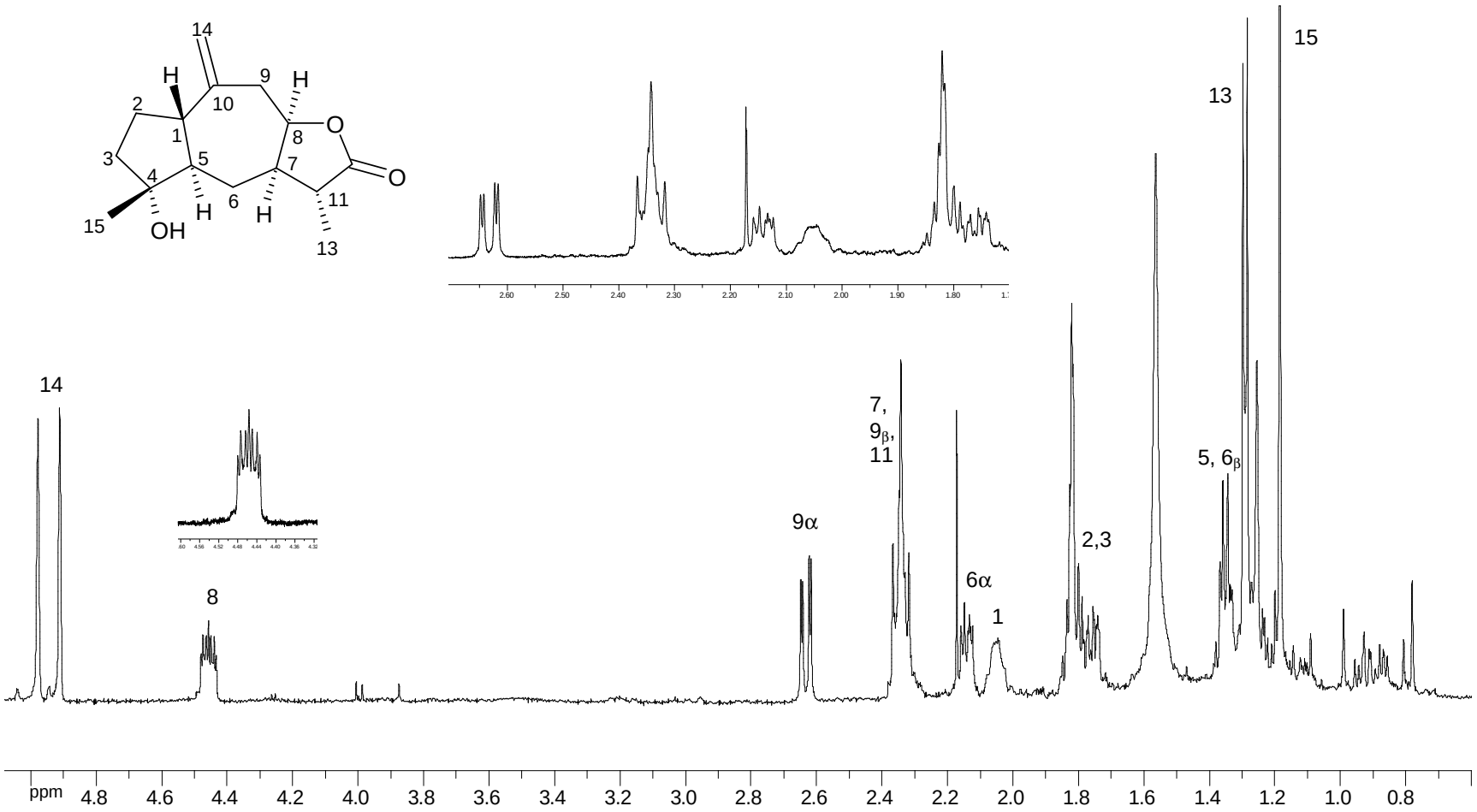


Plate 38: ^{13}C NMR spectrum of compound 301 in $CDCl_3$

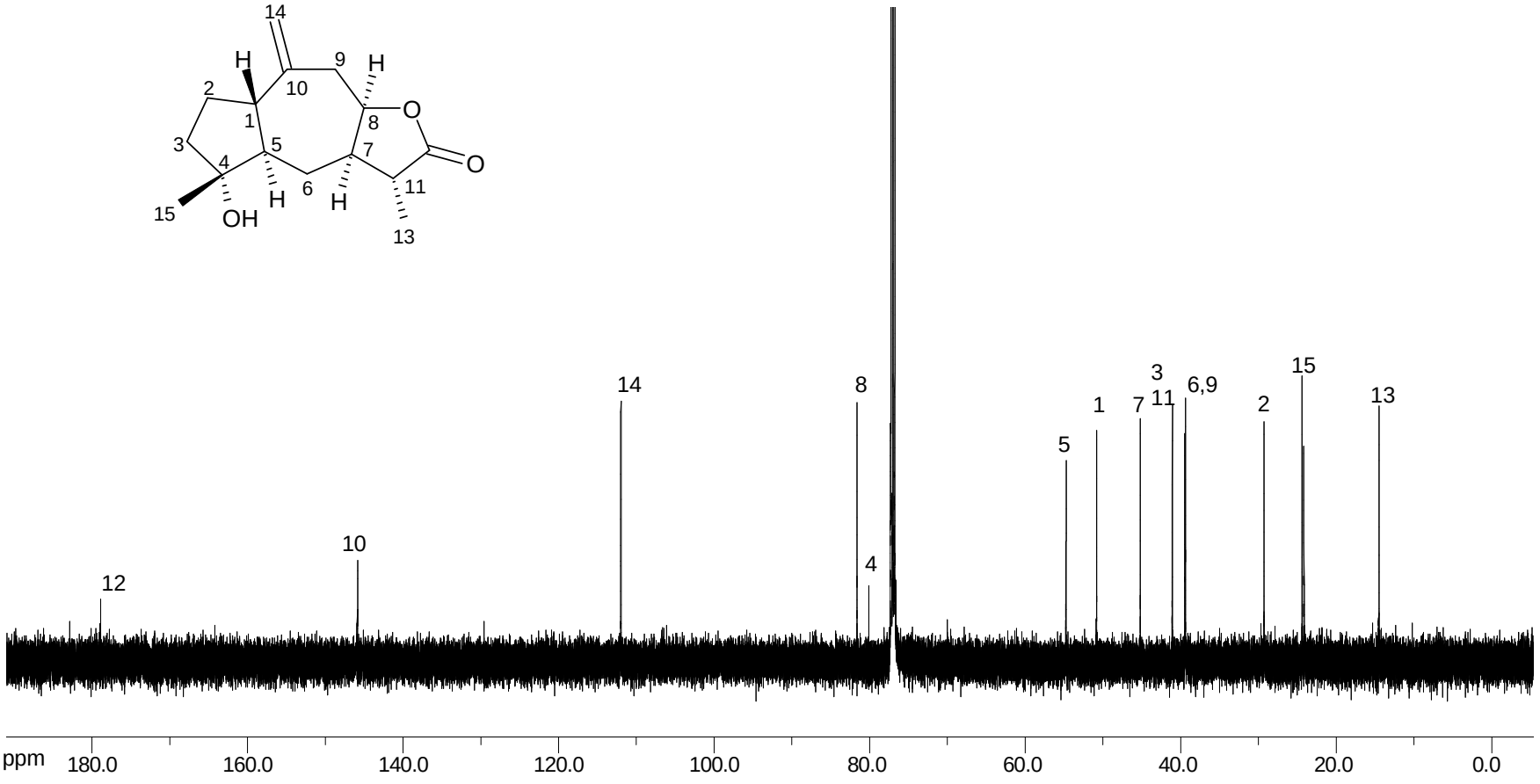


Plate 39: COSY NMR spectrum of compound 301 in $CDCl_3$

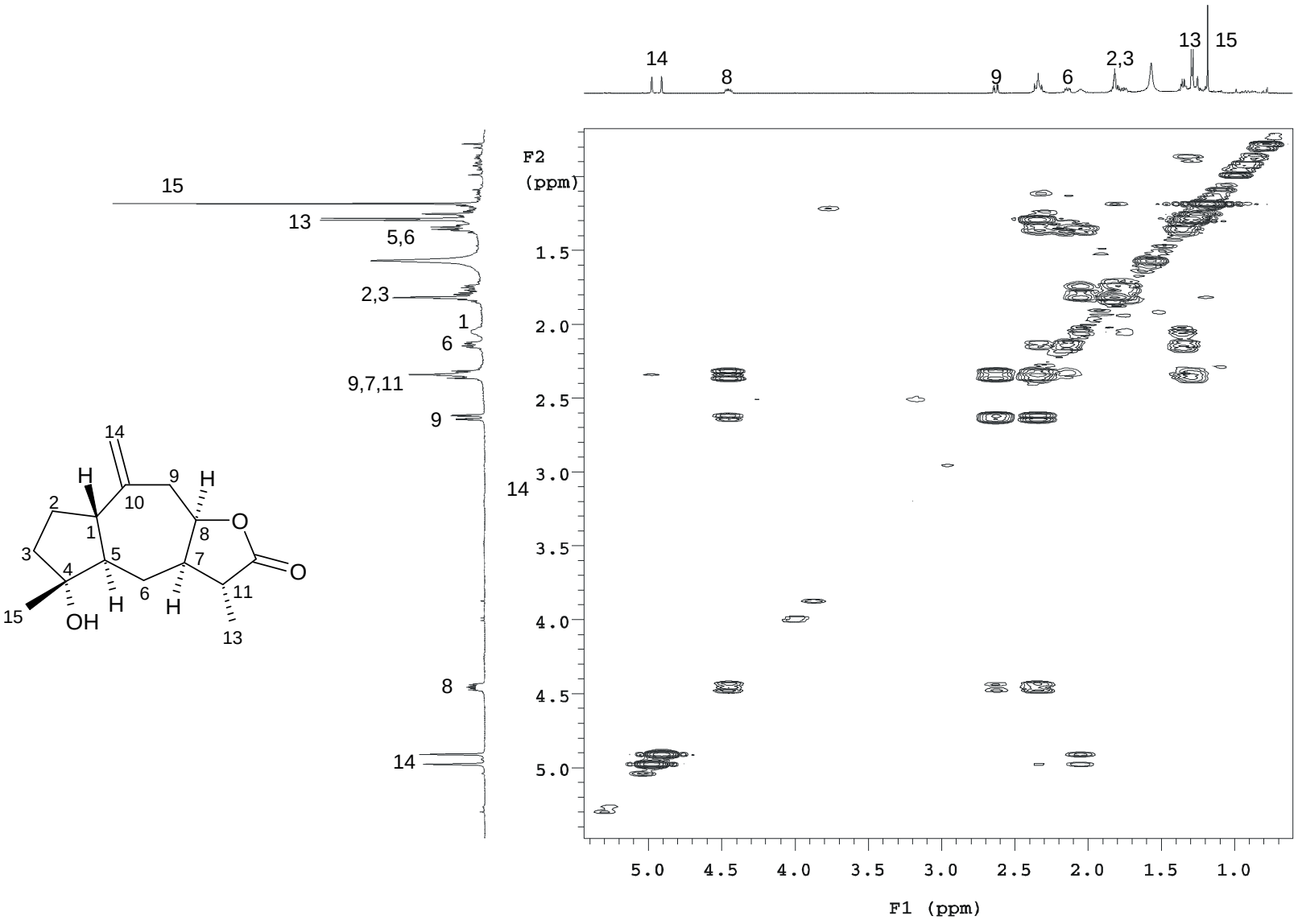


Plate 40: DEPT NMR spectrum of compound 301 in $CDCl_3$

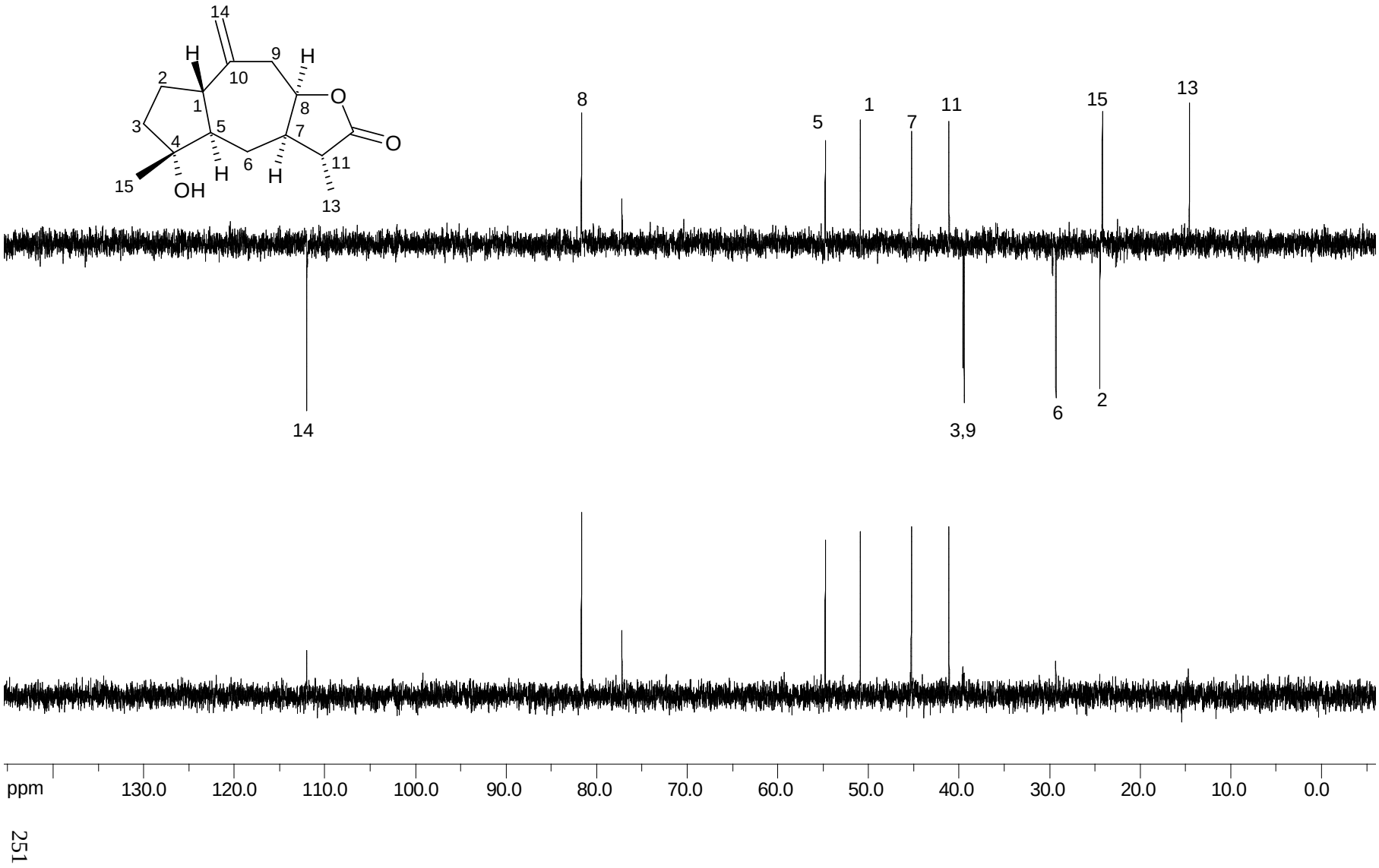


Plate 41: HSQC NMR spectrum 301 of in $CDCl_3$

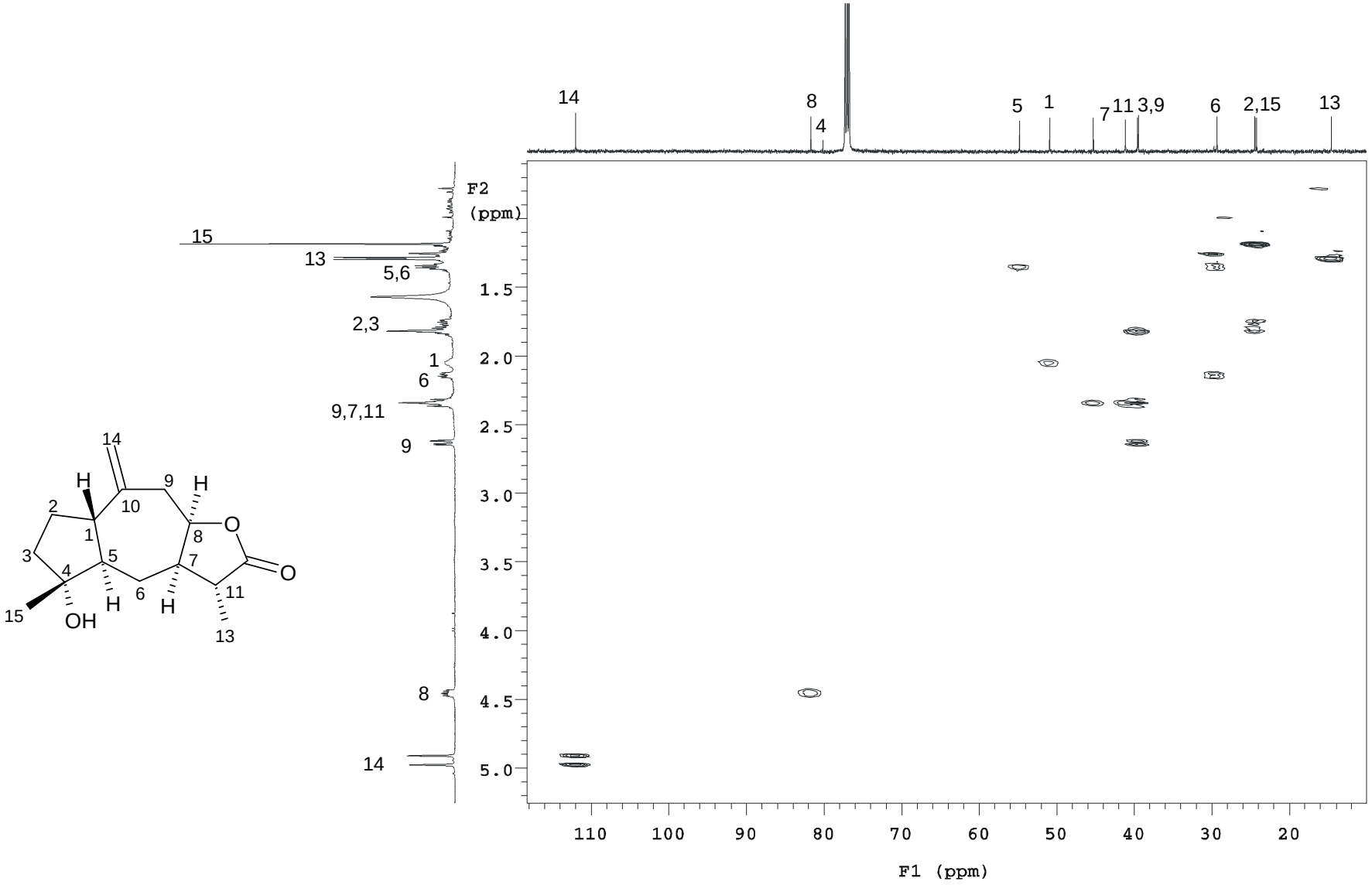


Plate 42: HMQC NMR spectrum of 301 in $CDCl_3$

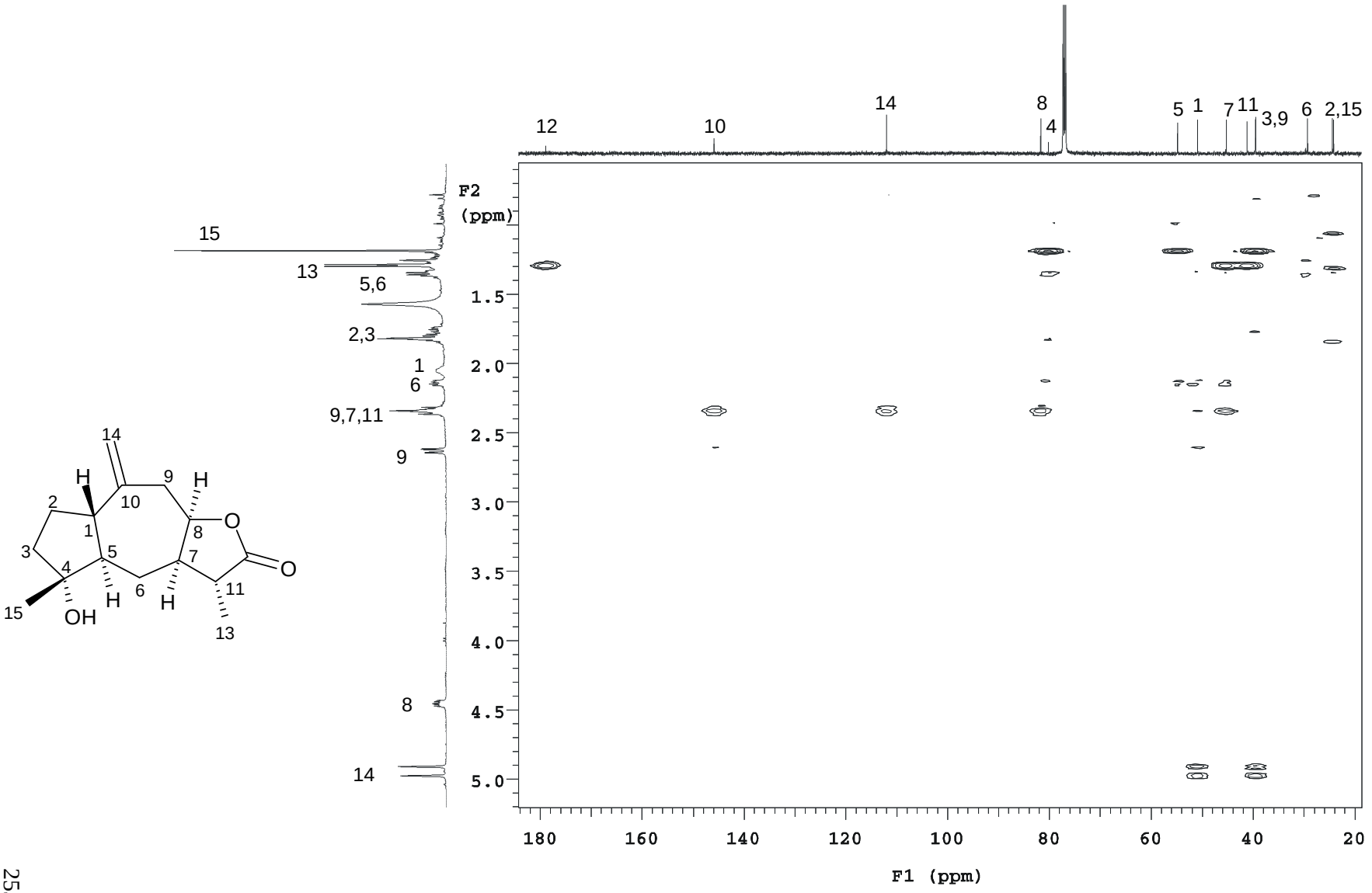


Plate 43: NOESY NMR spectrum of 301 in $CDCl_3$

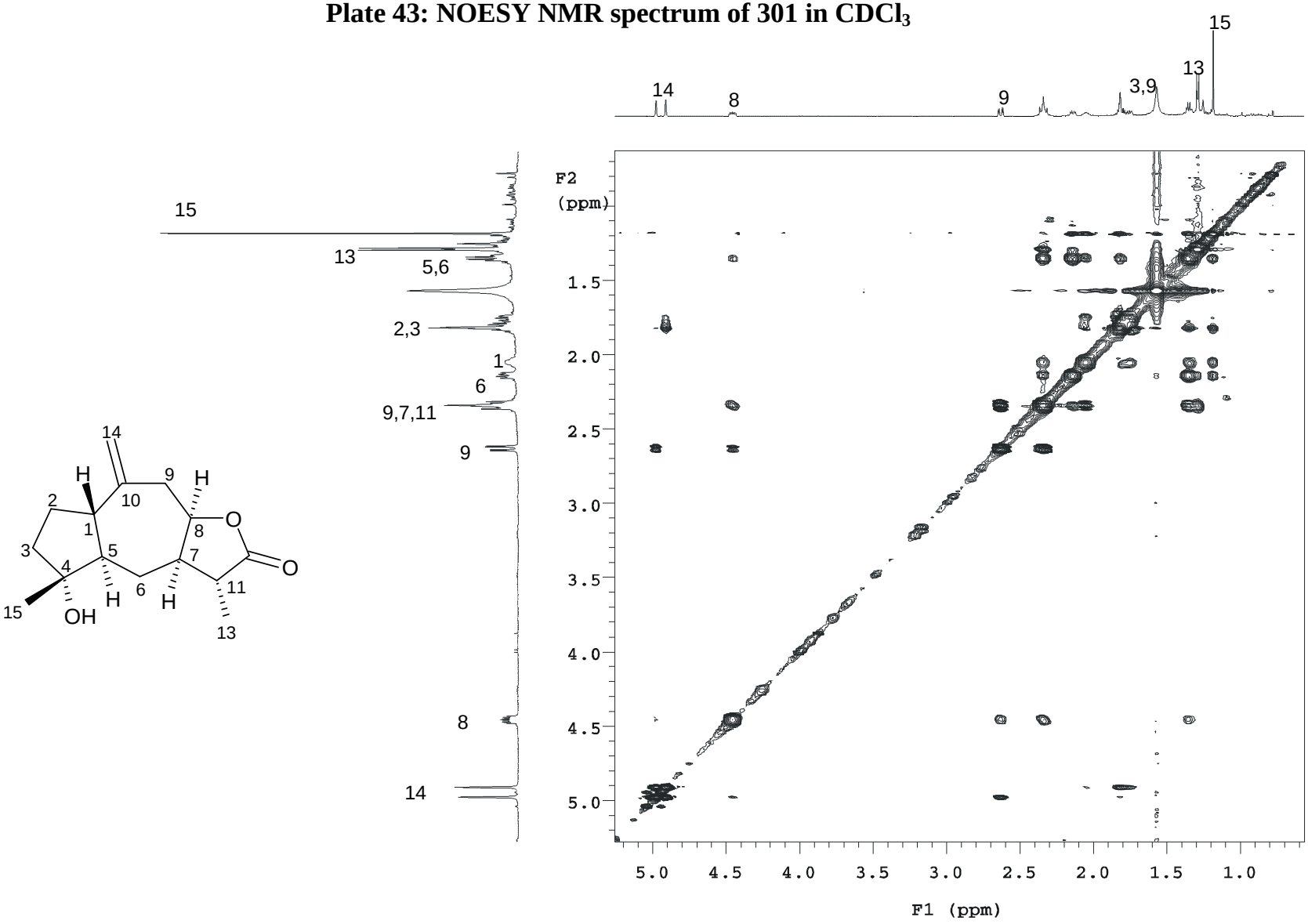


Plate 44: ^{1}H NMR spectrum of umbelliferone (348) in CD_3OD, $CDCl_3$

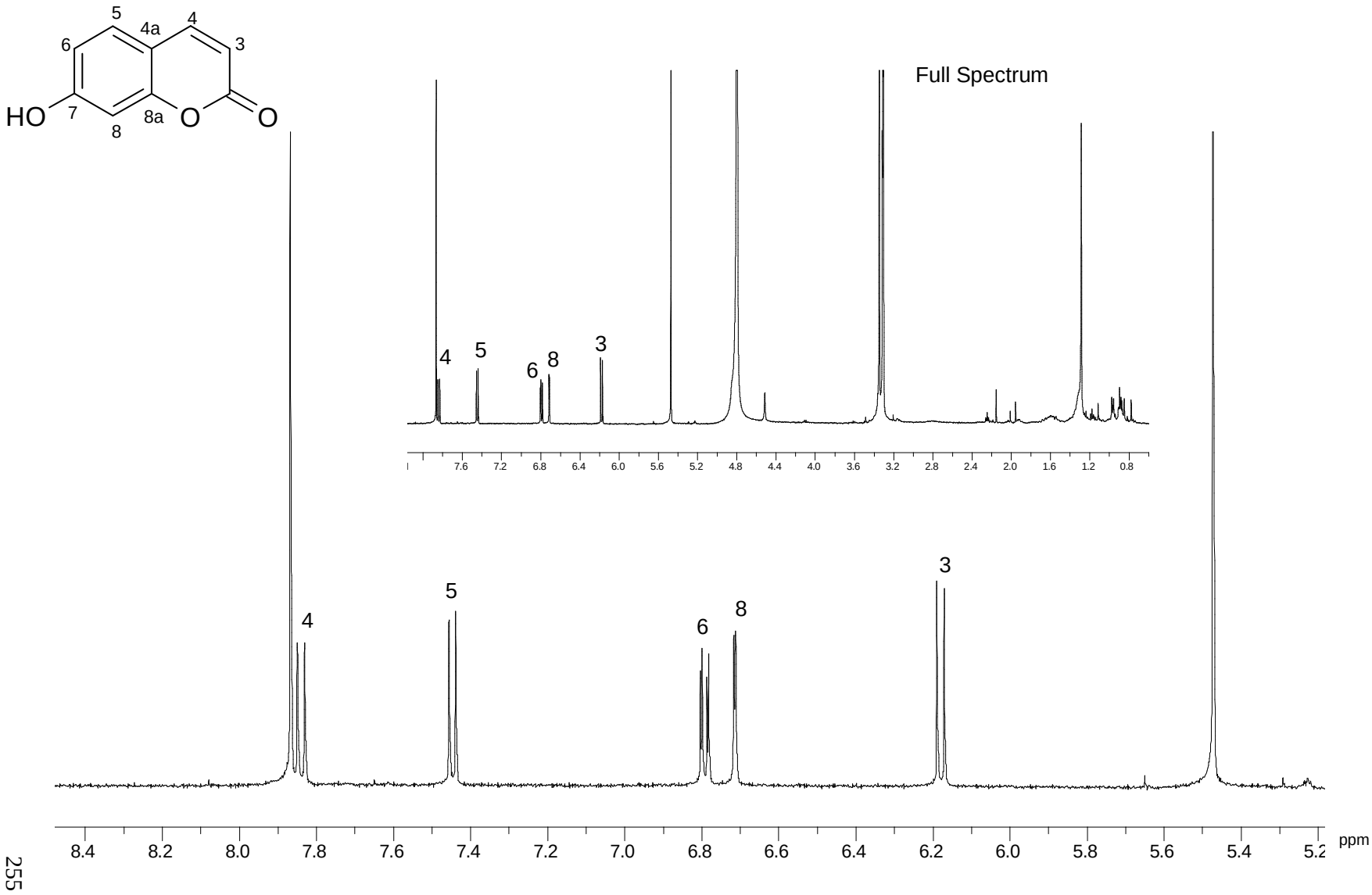


Plate 45: ^{13}C NMR spectrum of umbelliferone (348) in CD_3OD, $CDCl_3$

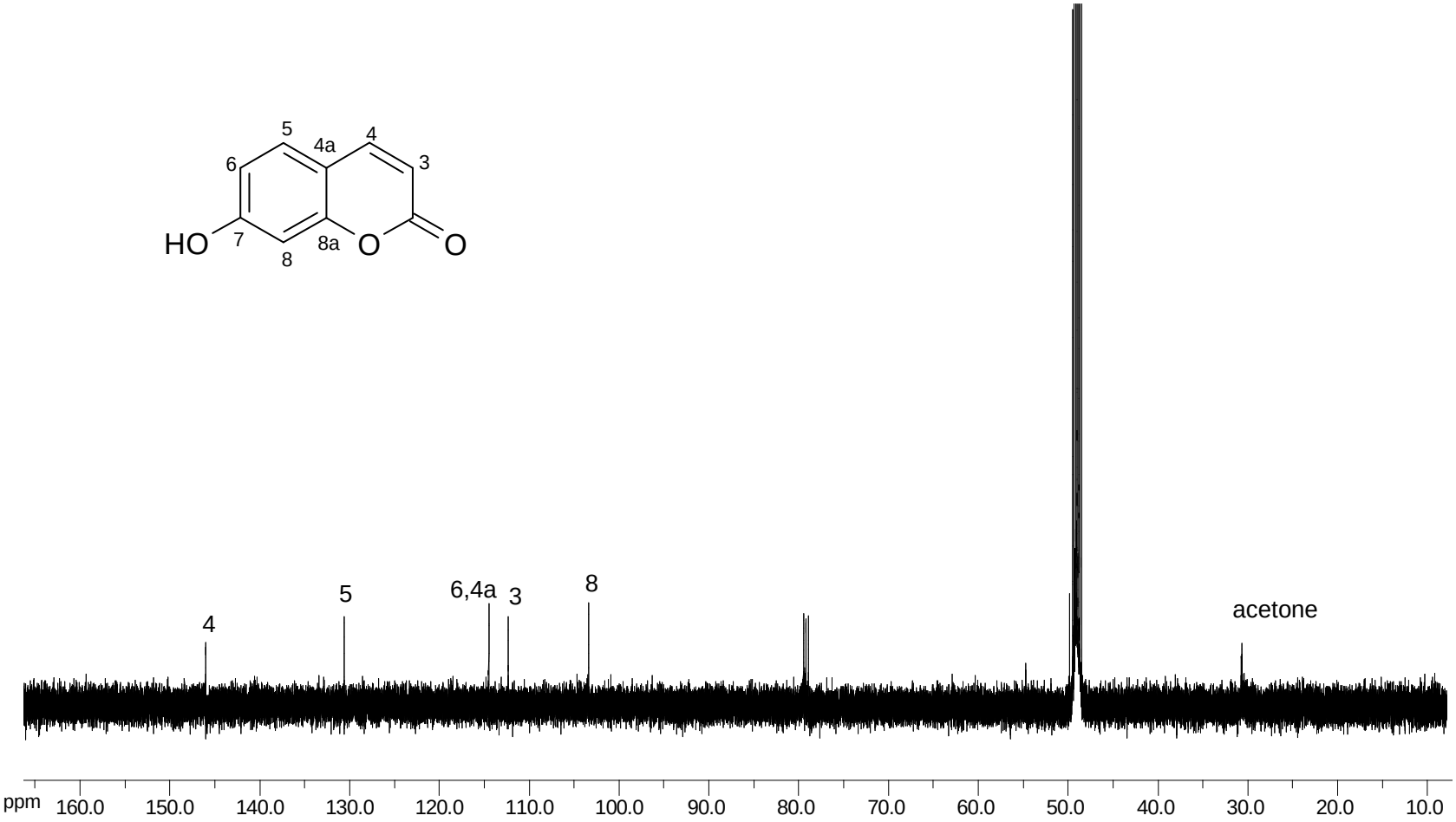


Plate 46: COSY NMR spectrum of umbelliferone (348) in CD_3OD, $CDCl_3$

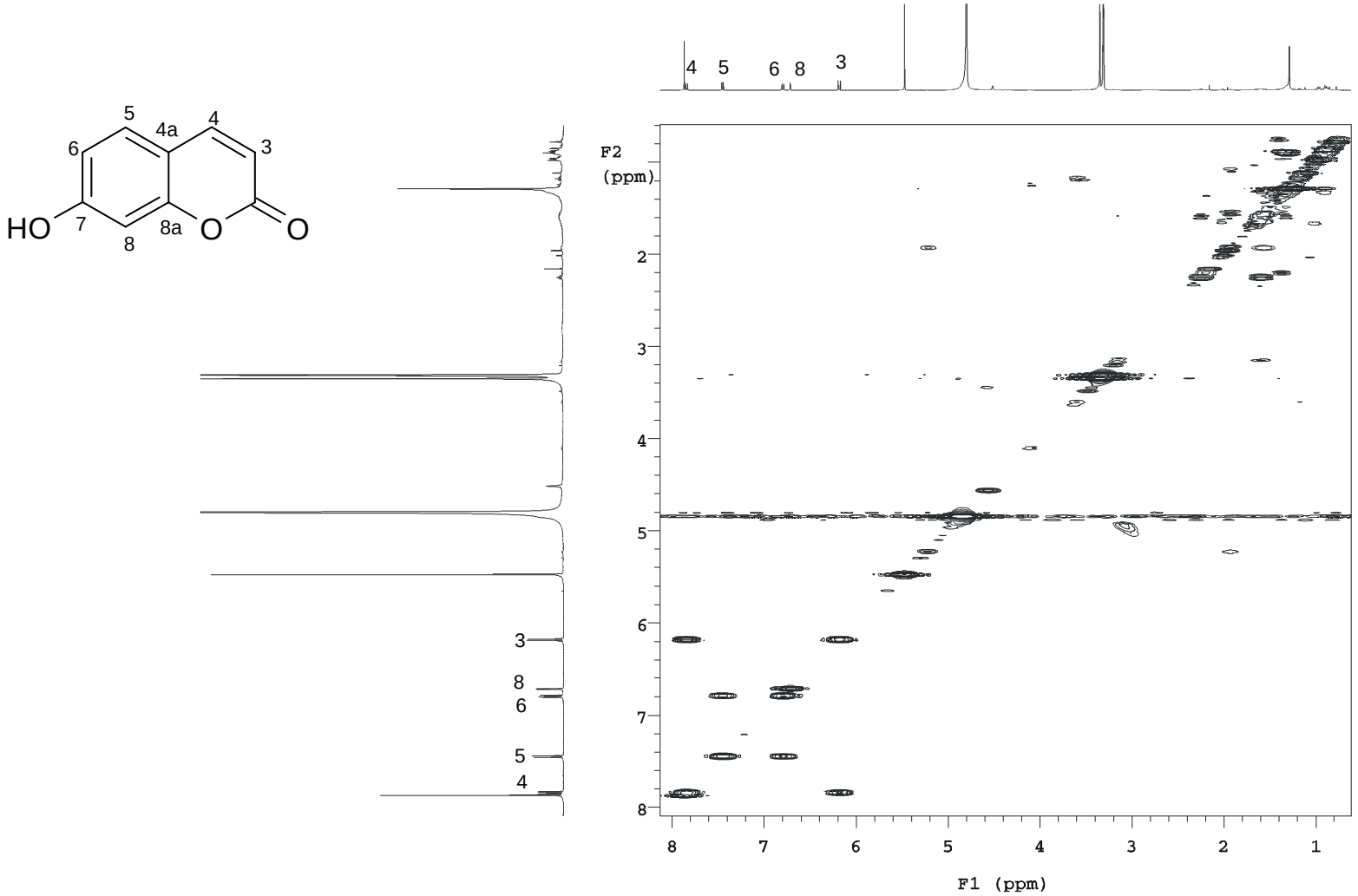


Plate 47: DEPT NMR spectrum of umbelliferone (348) in CD_3OD, $CDCl_3$

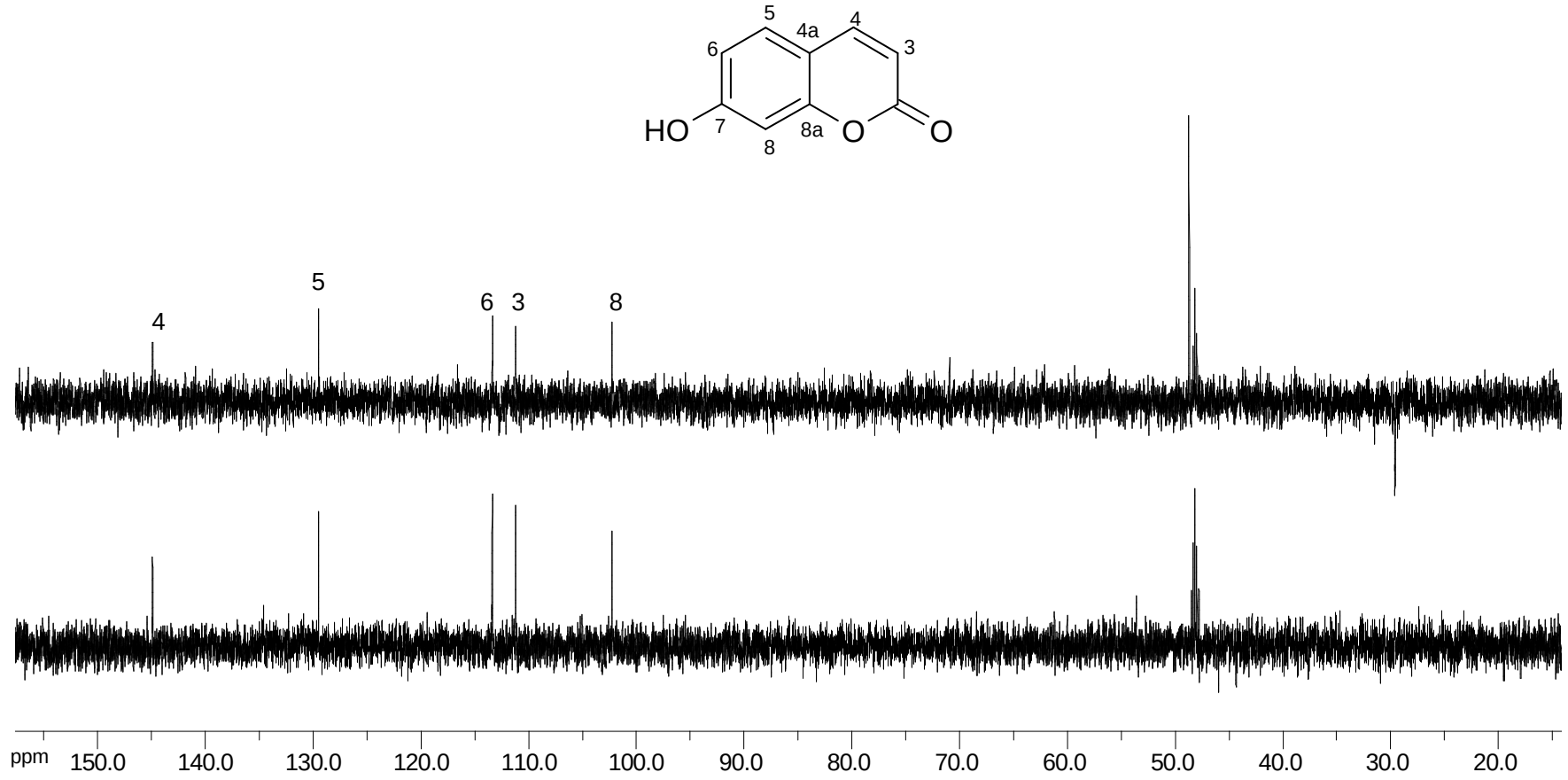


Plate 48: HSQC NMR spectrum of umbelliferone (348) in CD_3OD, $CDCl_3$

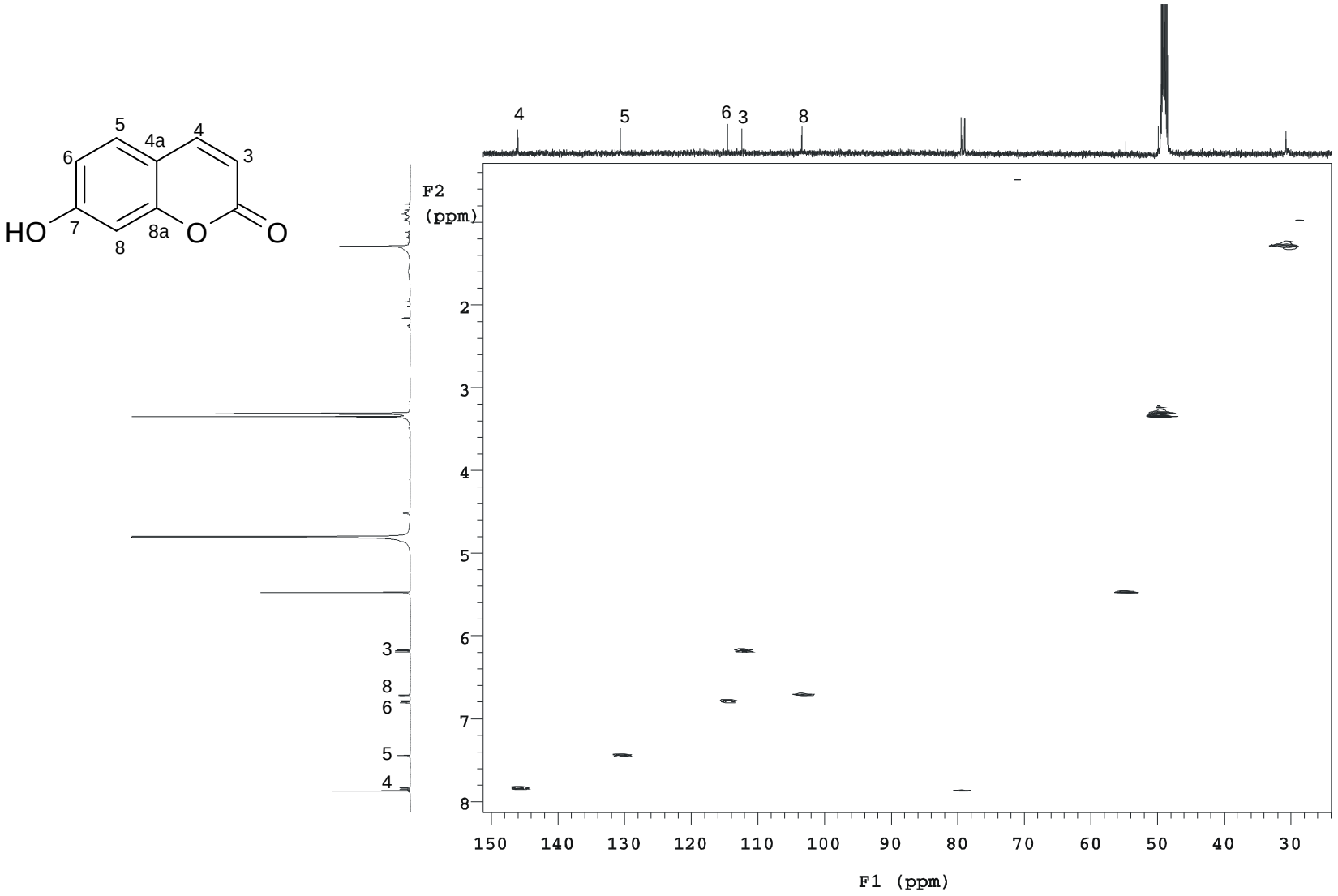


Plate 49: HMQC NMR spectrum of umbelliferone (348) in CD_3OD, $CDCl_3$

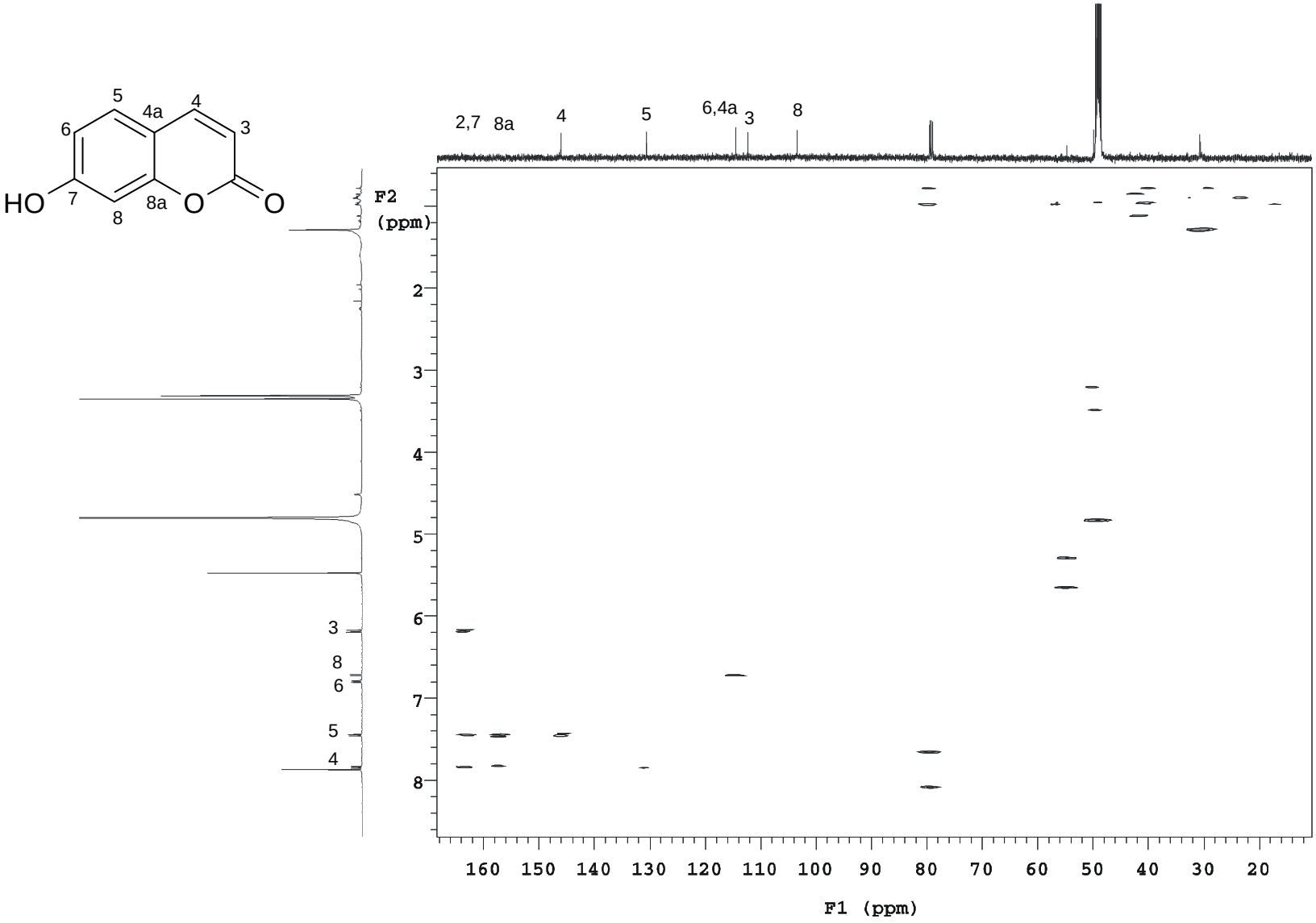


Plate 50: ^{1}H NMR spectrum of 4’,7,5-trihydroxy-3,3’,8-trimethoxyflavone (381) in CD_3OD

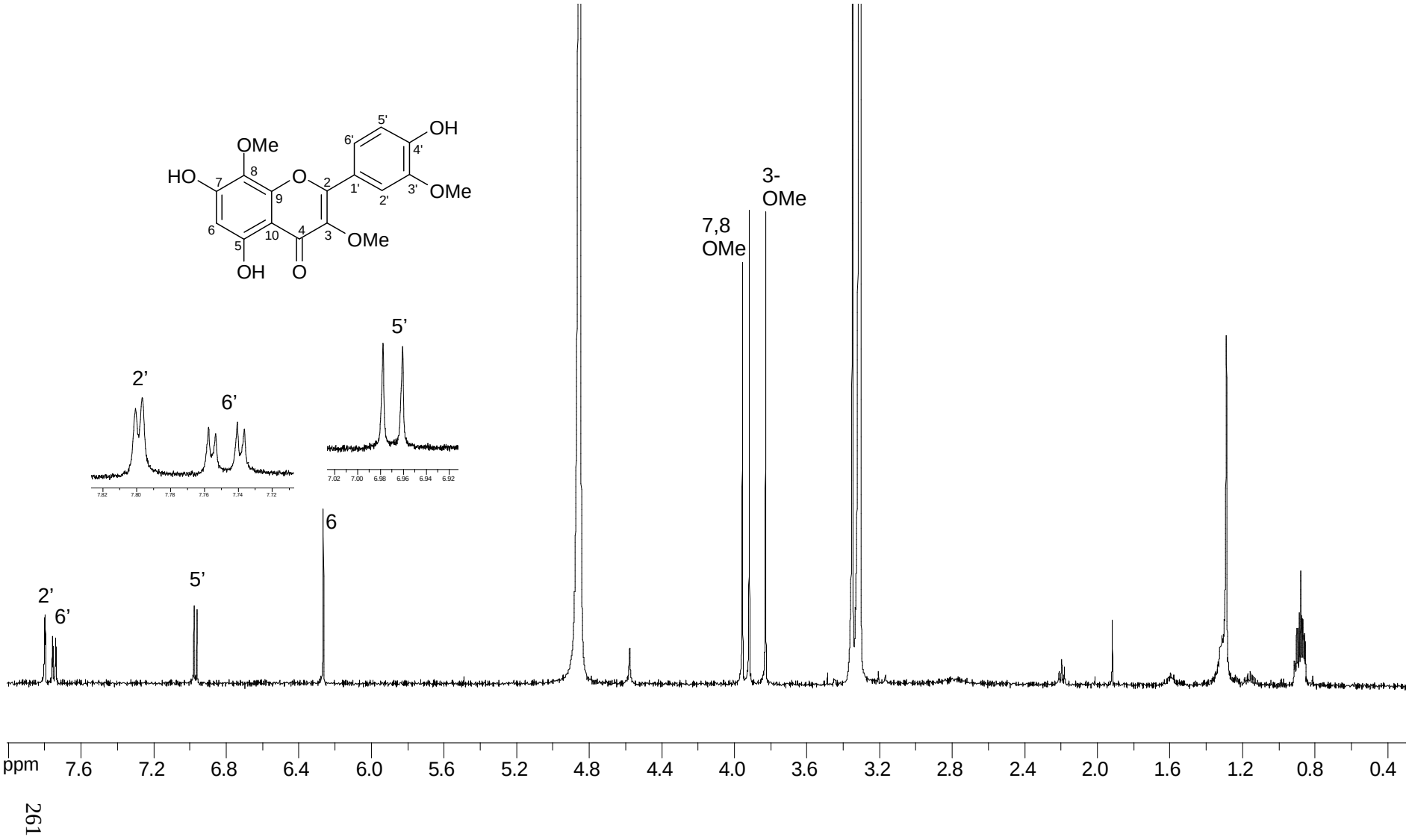


Plate 51: ^{13}C NMR spectrum of 4',7,5-trihydroxy-3,3',8-trimethoxyflavone 3 (381) in CD_3OD

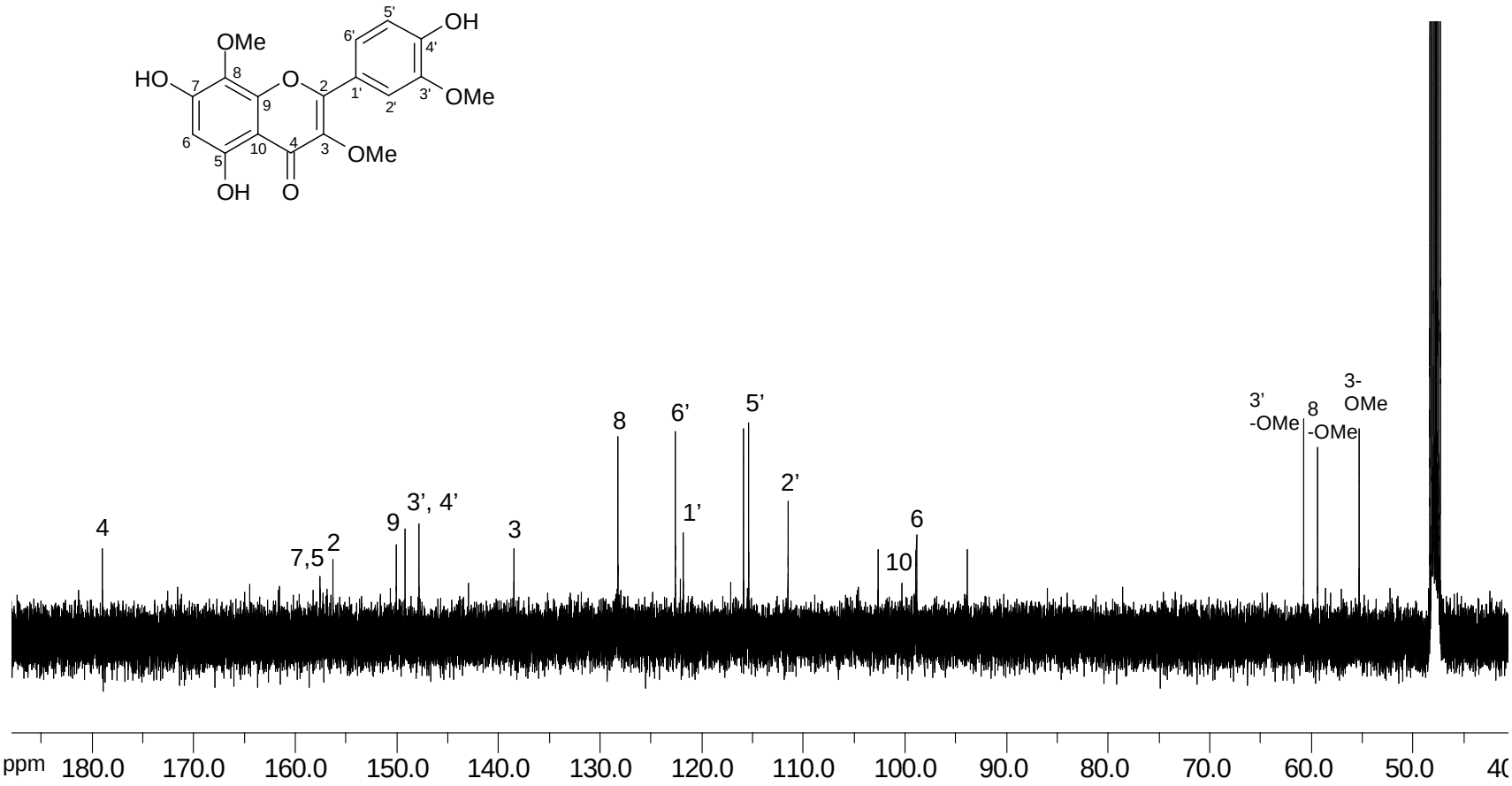


Plate 52: COSY NMR spectrum of 4',7,5-trihydroxy-3,3',8-trimethoxyflavone (381) in CD_3OD

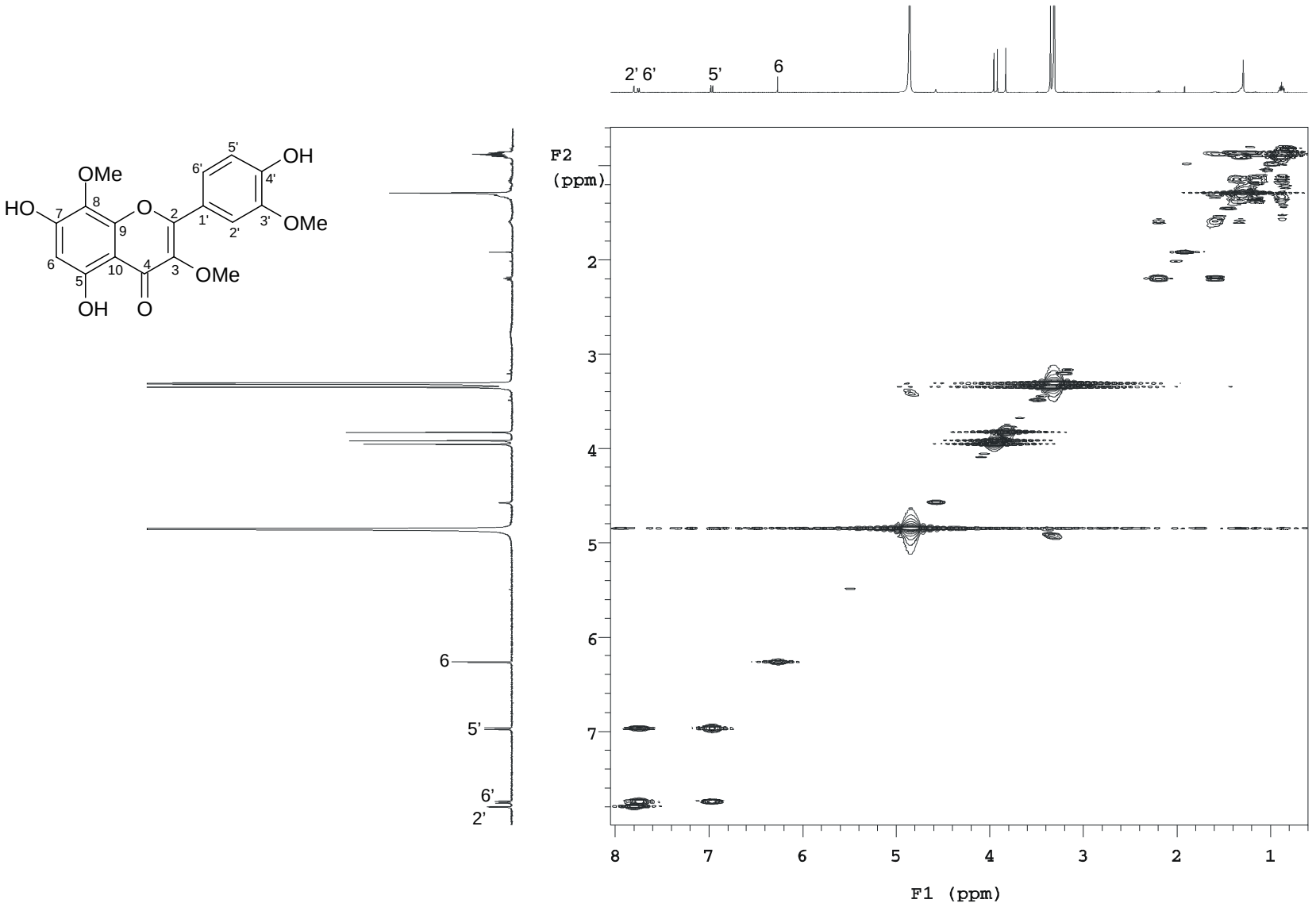


Plate 53: HSQC NMR spectrum of 4',7,5-trihydroxy-3,3',8-trimethoxyflavone (381) in CD_3OD

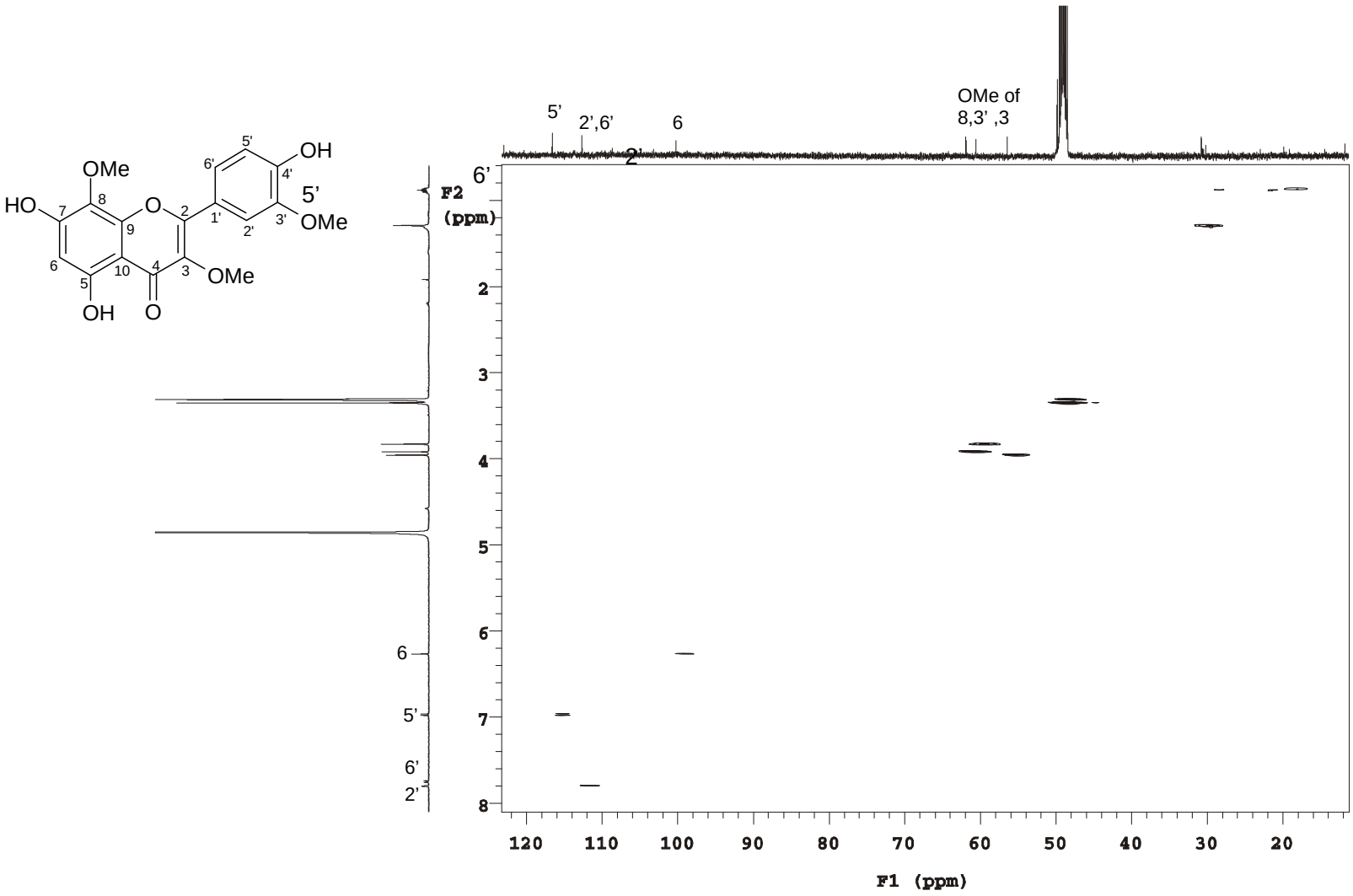


Plate 54: HMQC NMR spectrum of 4',7,5-trihydroxy-3,3',8-trimethoxyflavone (381) in CD_3OD

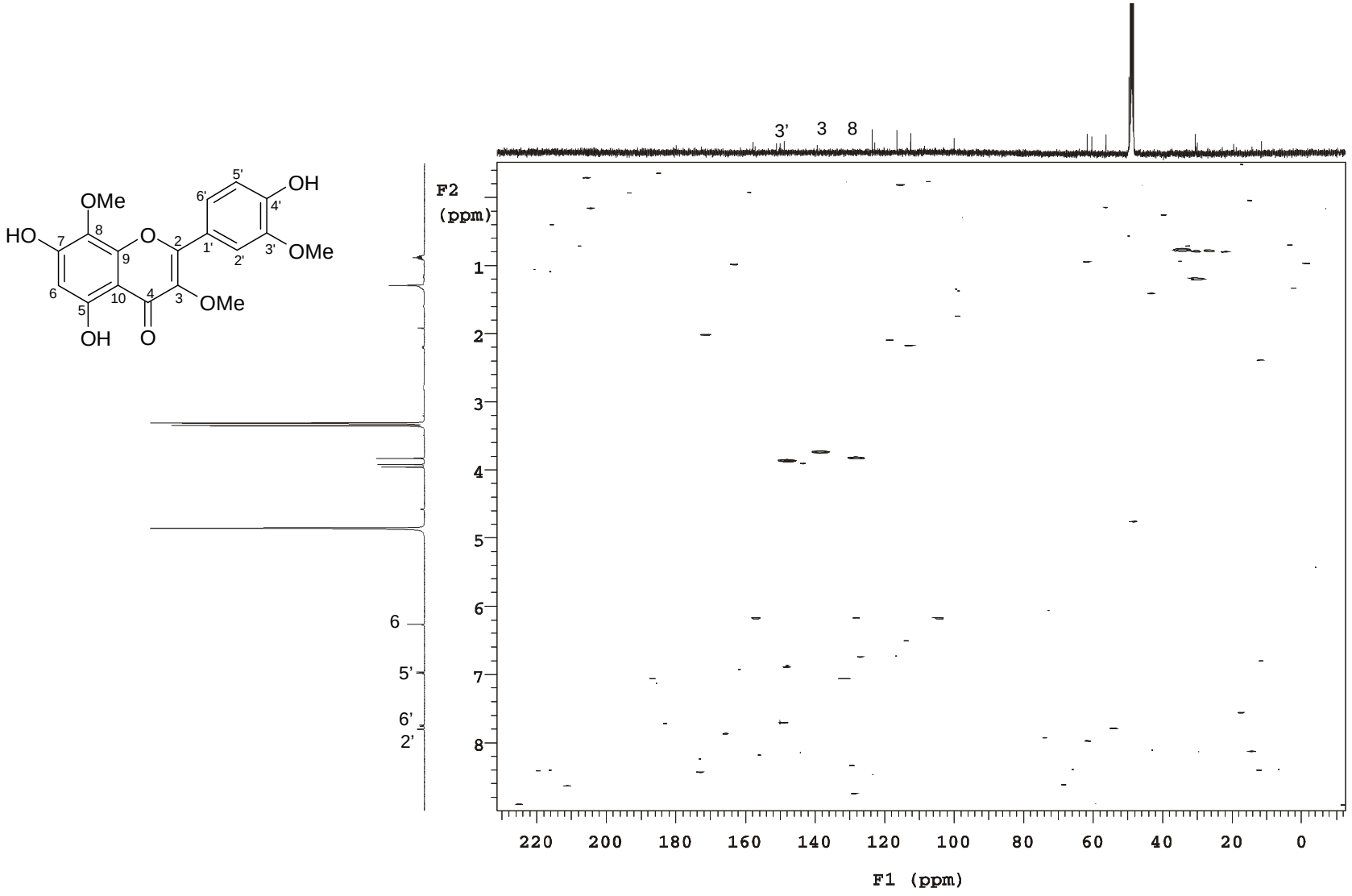


Plate 55: NOESY NMR spectrum of 4',7,5-trihydroxy-3,3',8-trimethoxyflavone (381) in CD_3OD

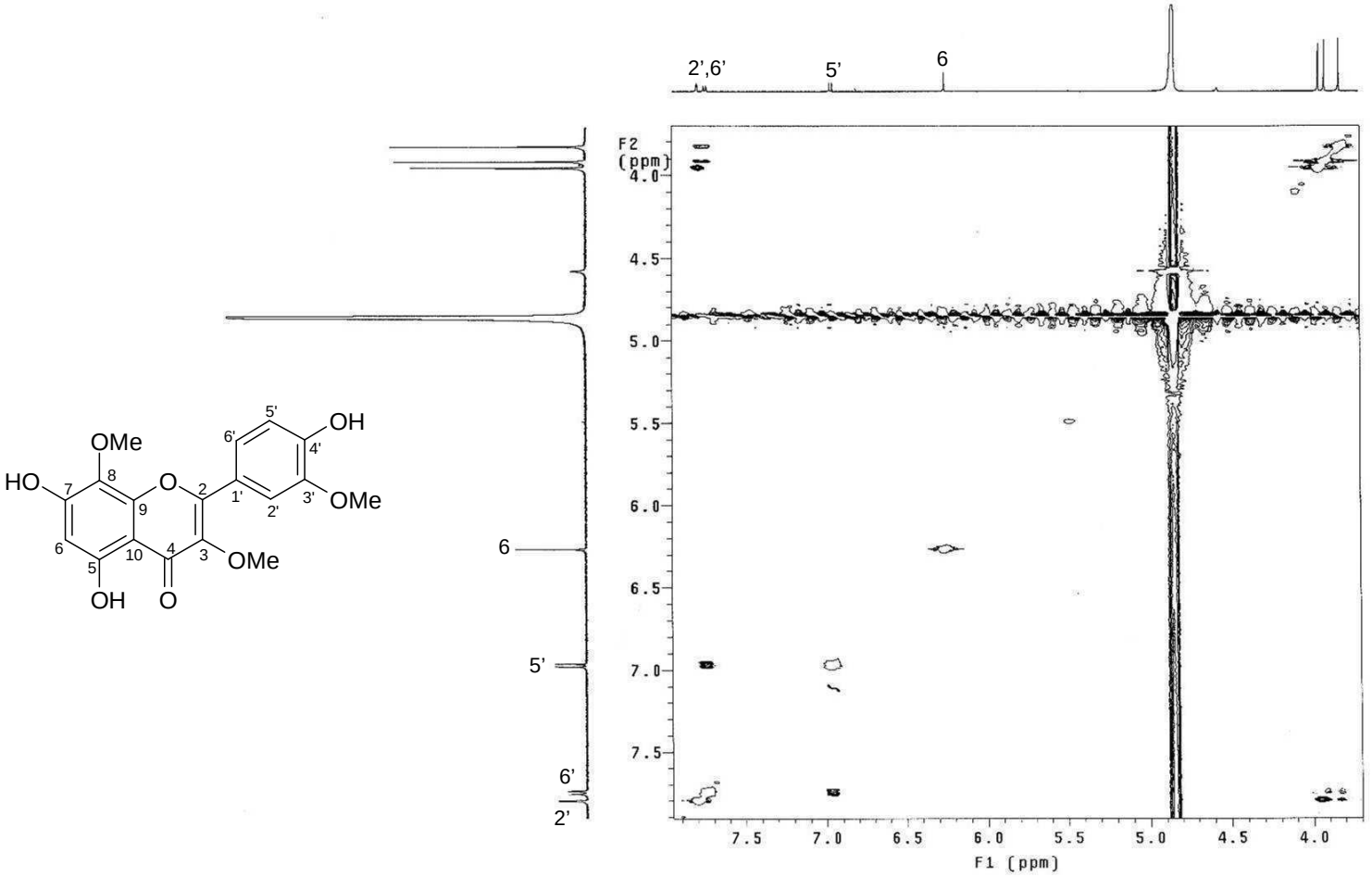


Plate 56: ^{1}H NMR spectrum of compound 300 in $CDCl_3$

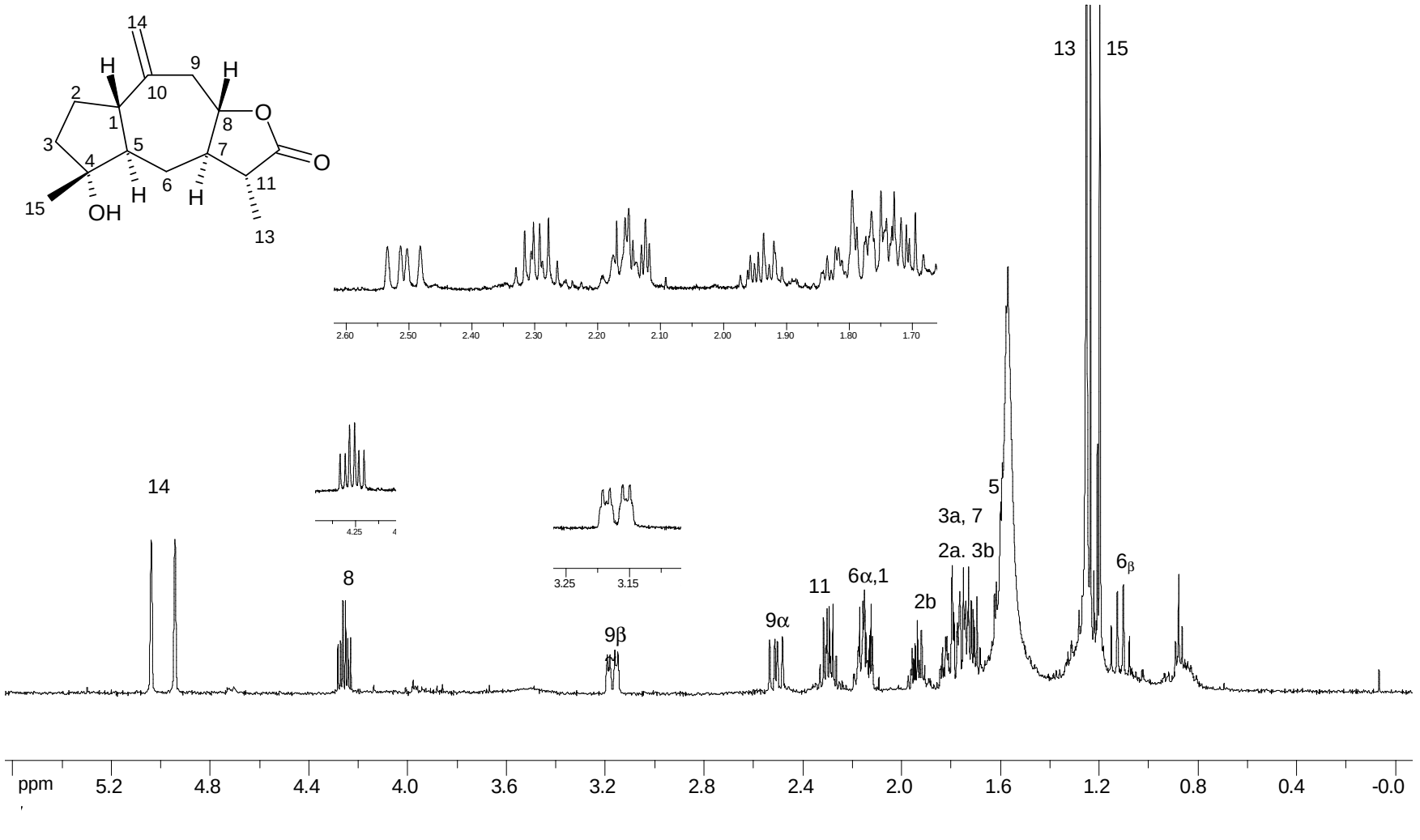


Plate 57: ^{13}C NMR spectrum of compound 300 in $CDCl_3$

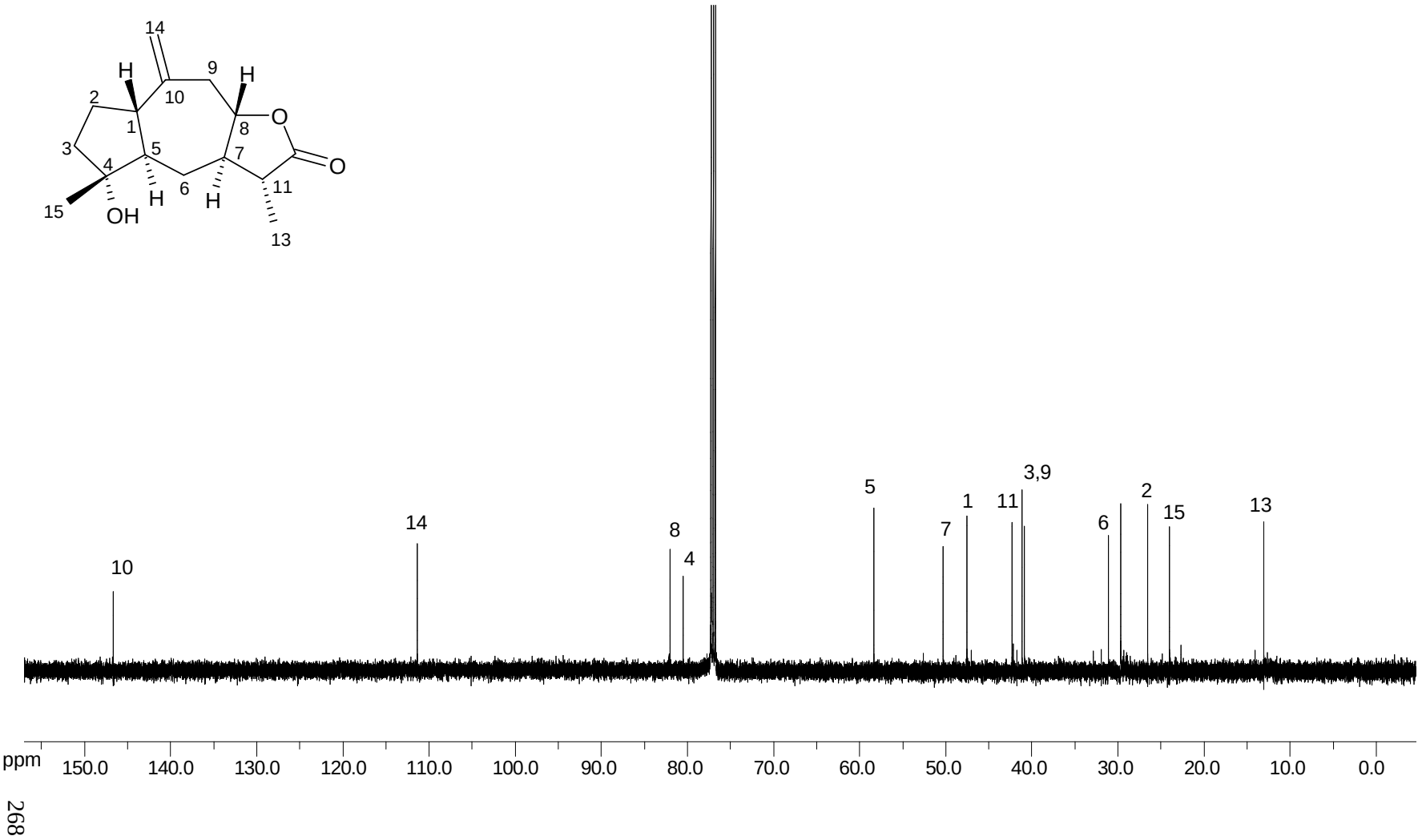


Plate 58: COSY NMR spectrum of compound 300 in $CDCl_3$

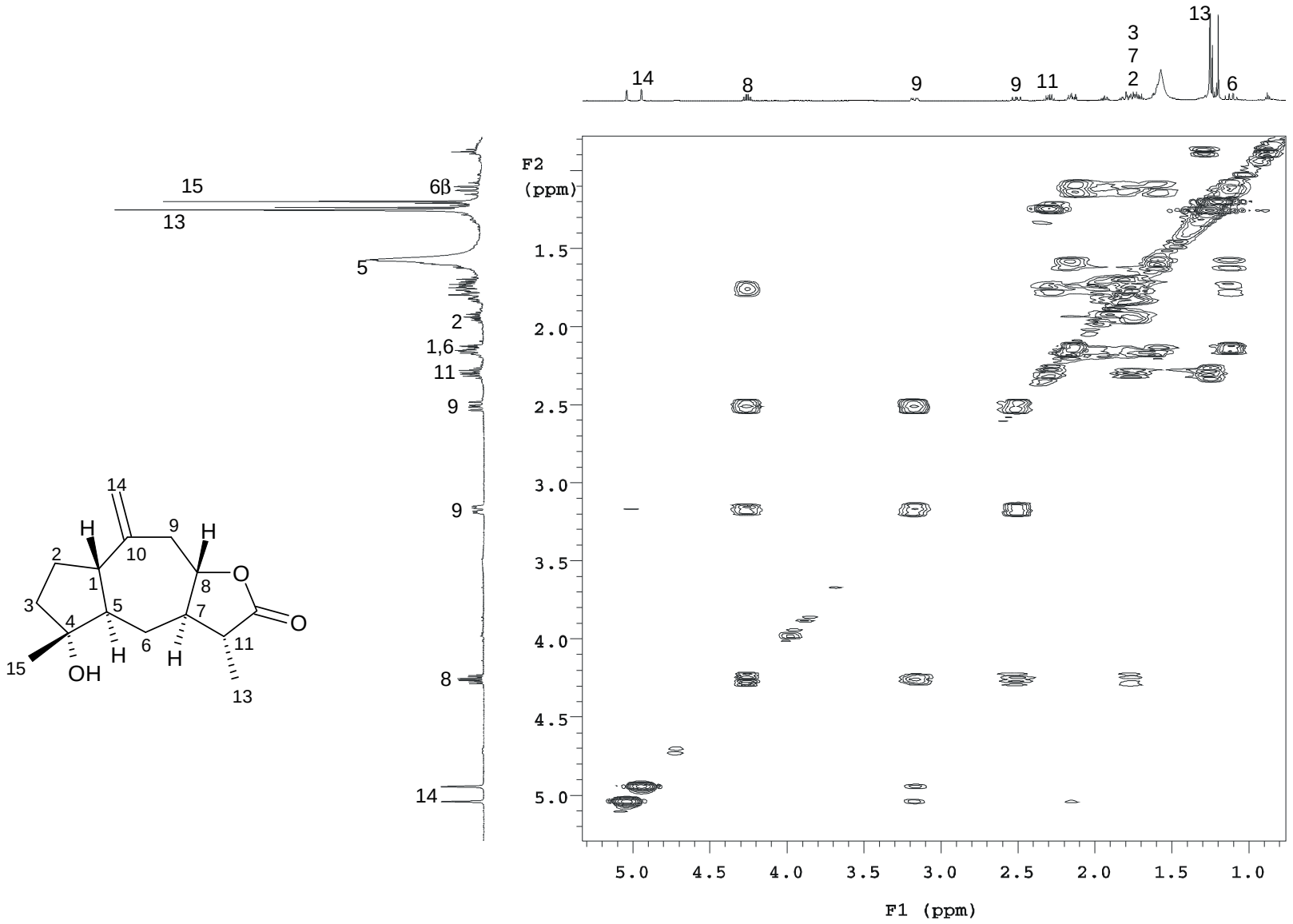


Plate 59: DEPT NMR spectrum of compound 300 in $CDCl_3$

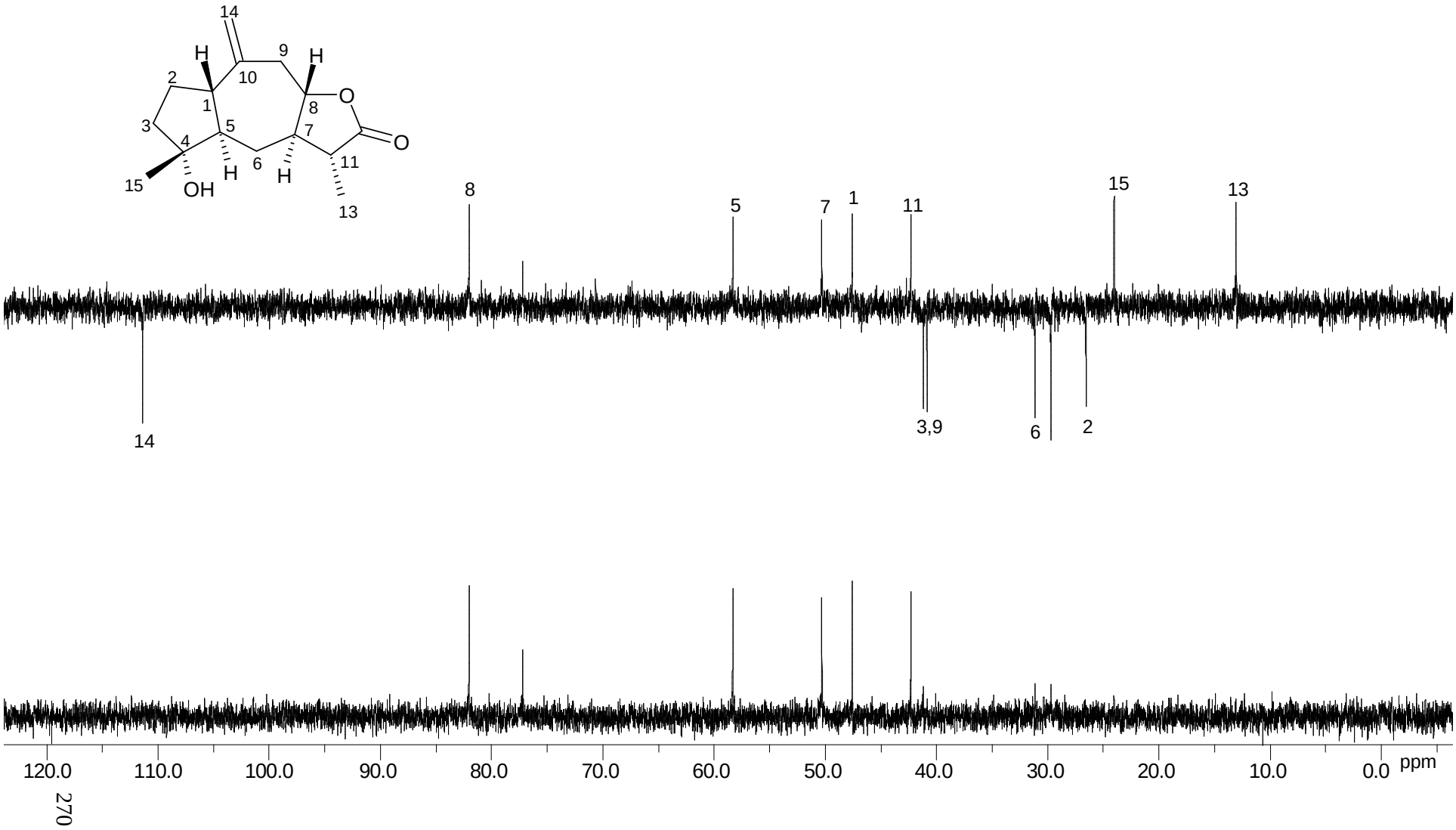


Plate 60: HSQC NMR spectrum of compound 300 in $CDCl_3$

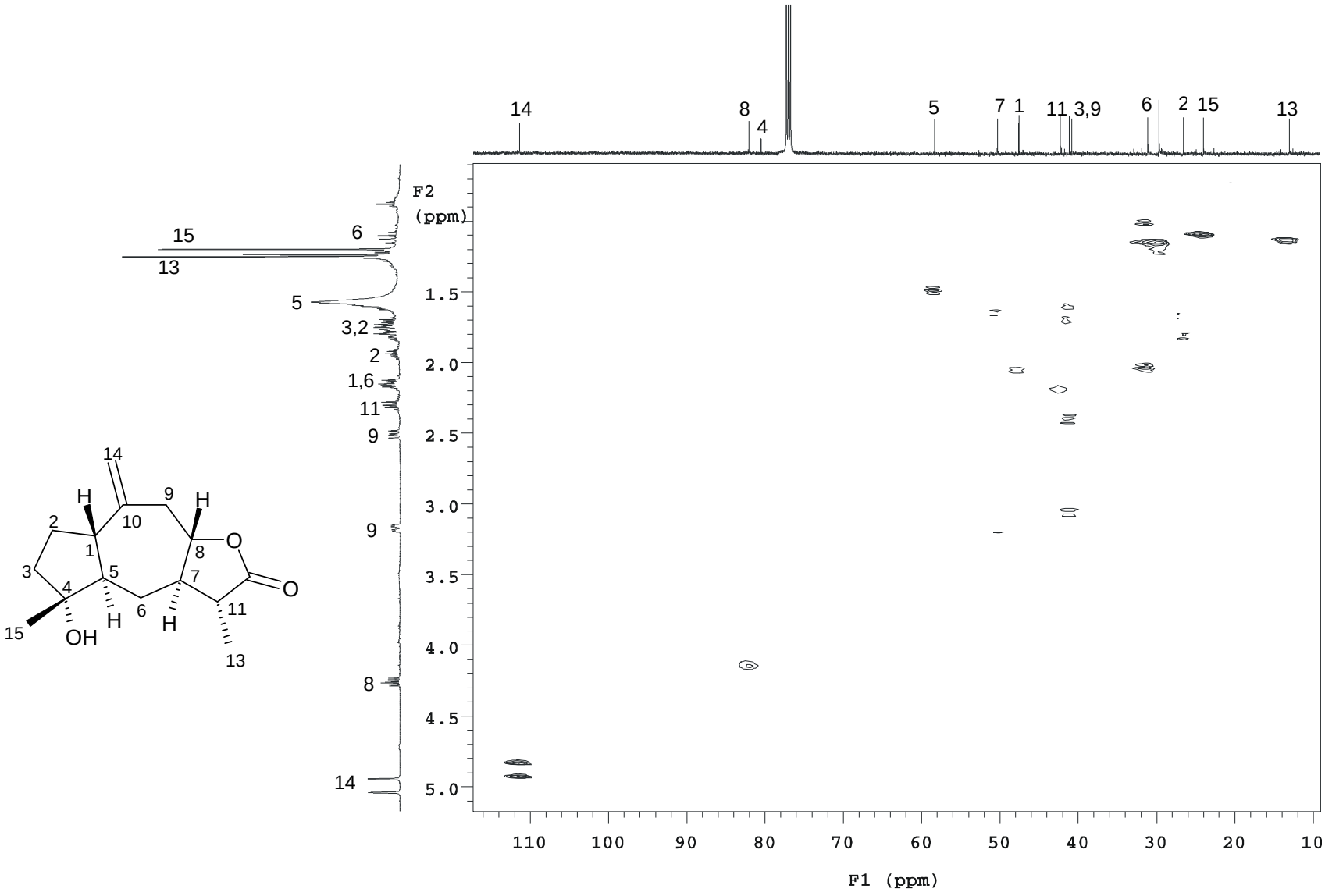


Plate 61: HMQC NMR spectrum of compound 300 in $CDCl_3$

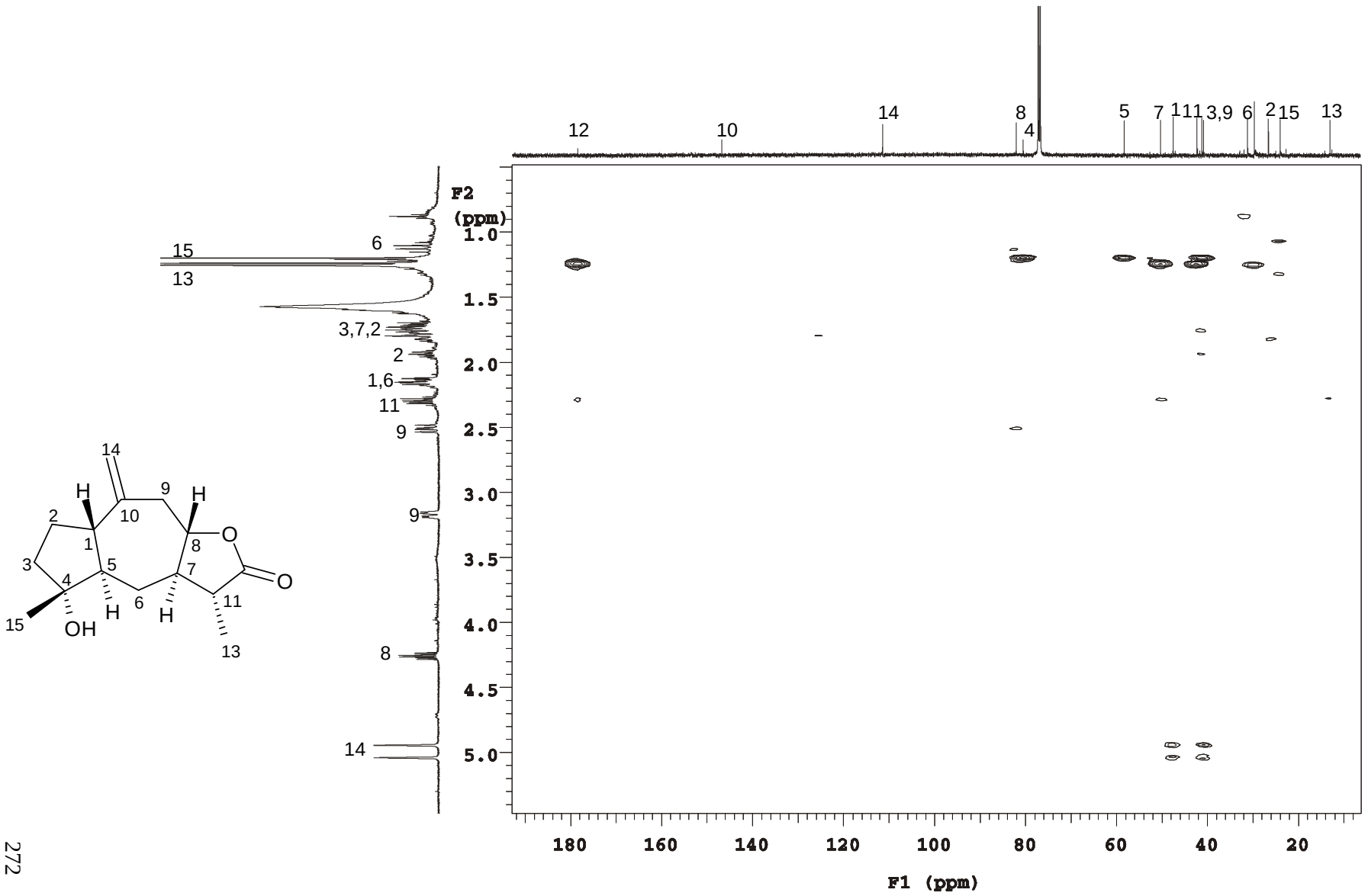


Plate 62: NOESY NMR spectrum of compound 300 in $CDCl_3$

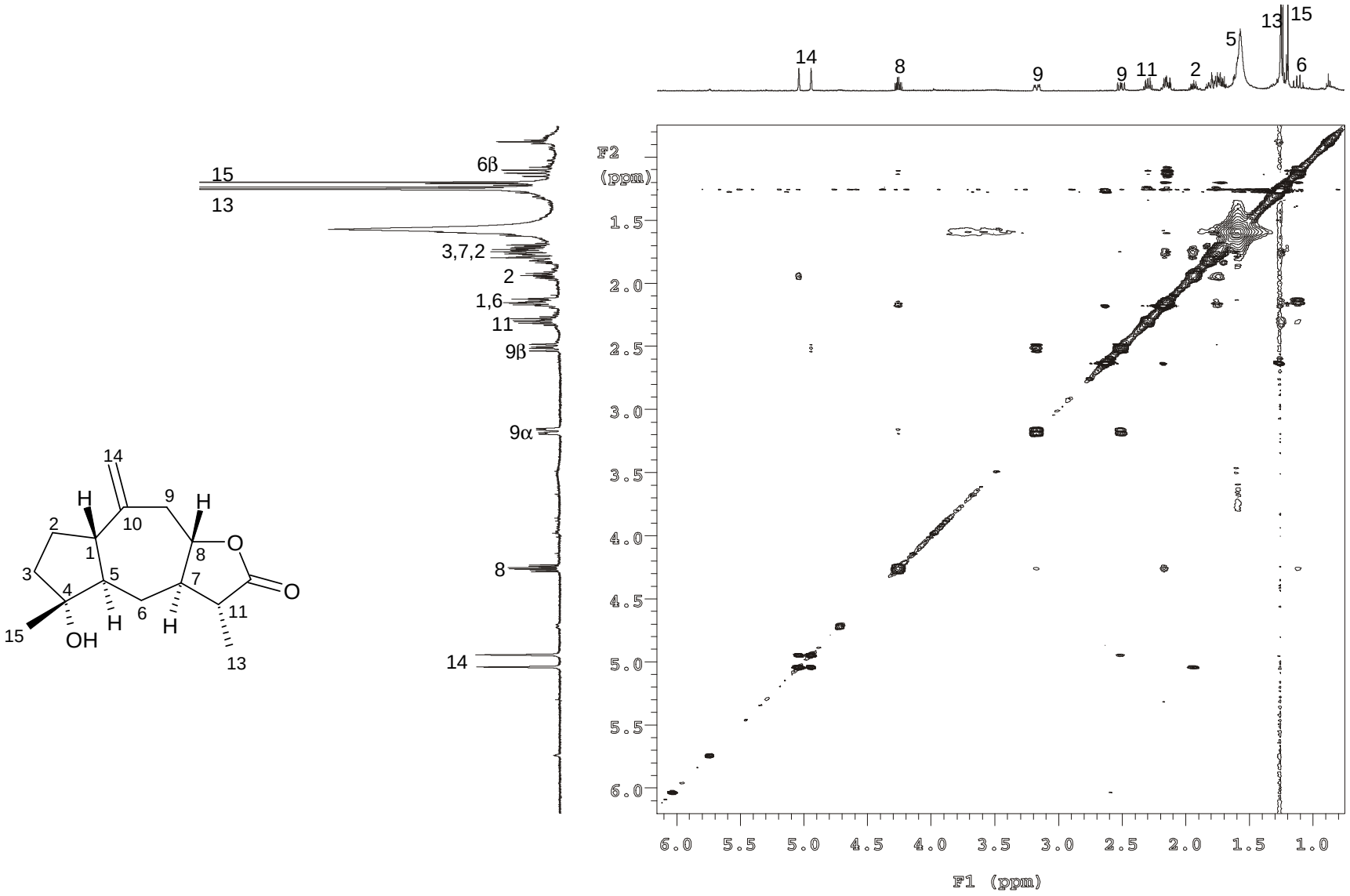


Plate 63: ^{1}H NMR spectrum of pinocembrin (1) in CD_3OD

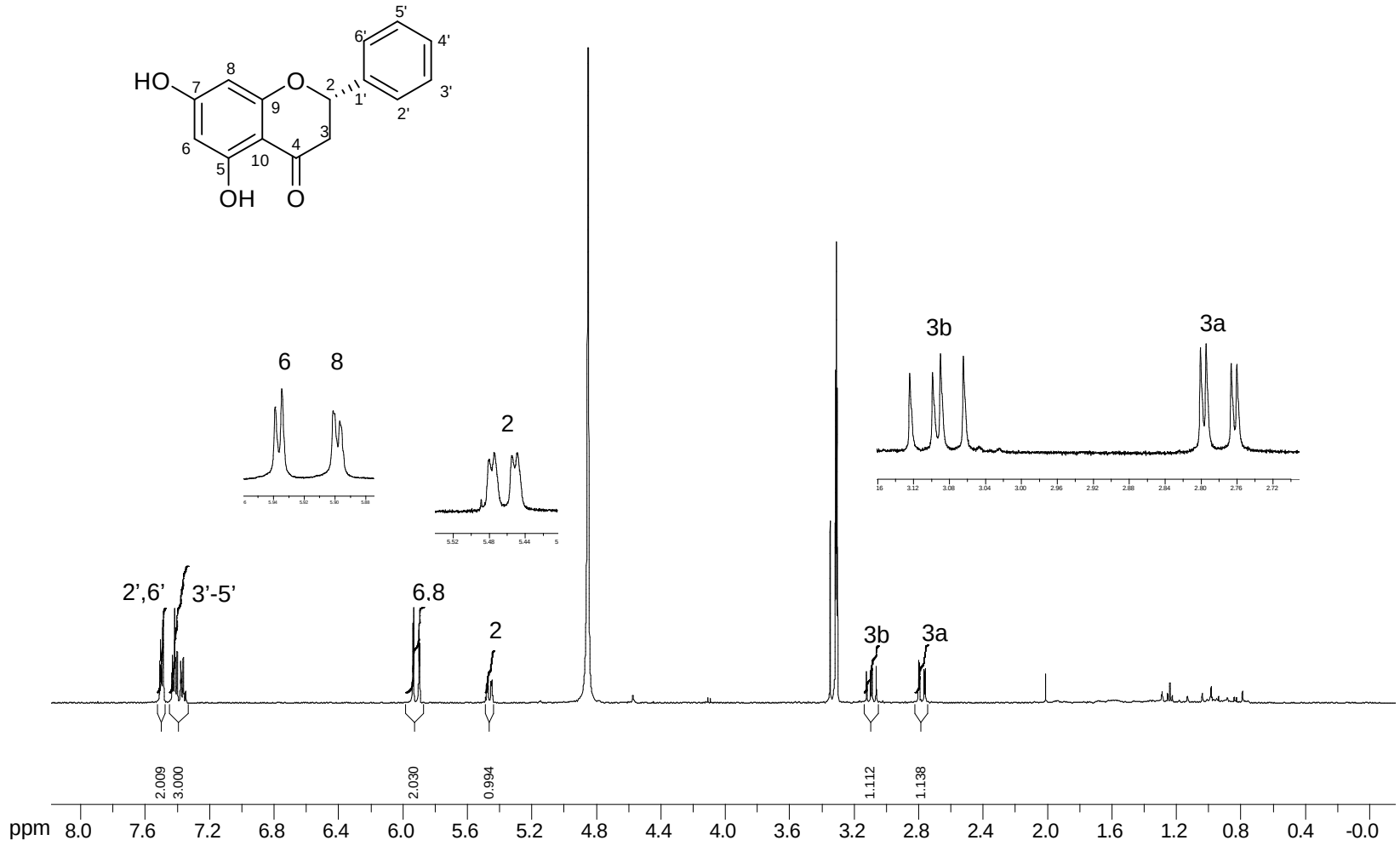


Plate 64: ^{13}C NMR spectrum of pinocembrin (1) in CD_3OD

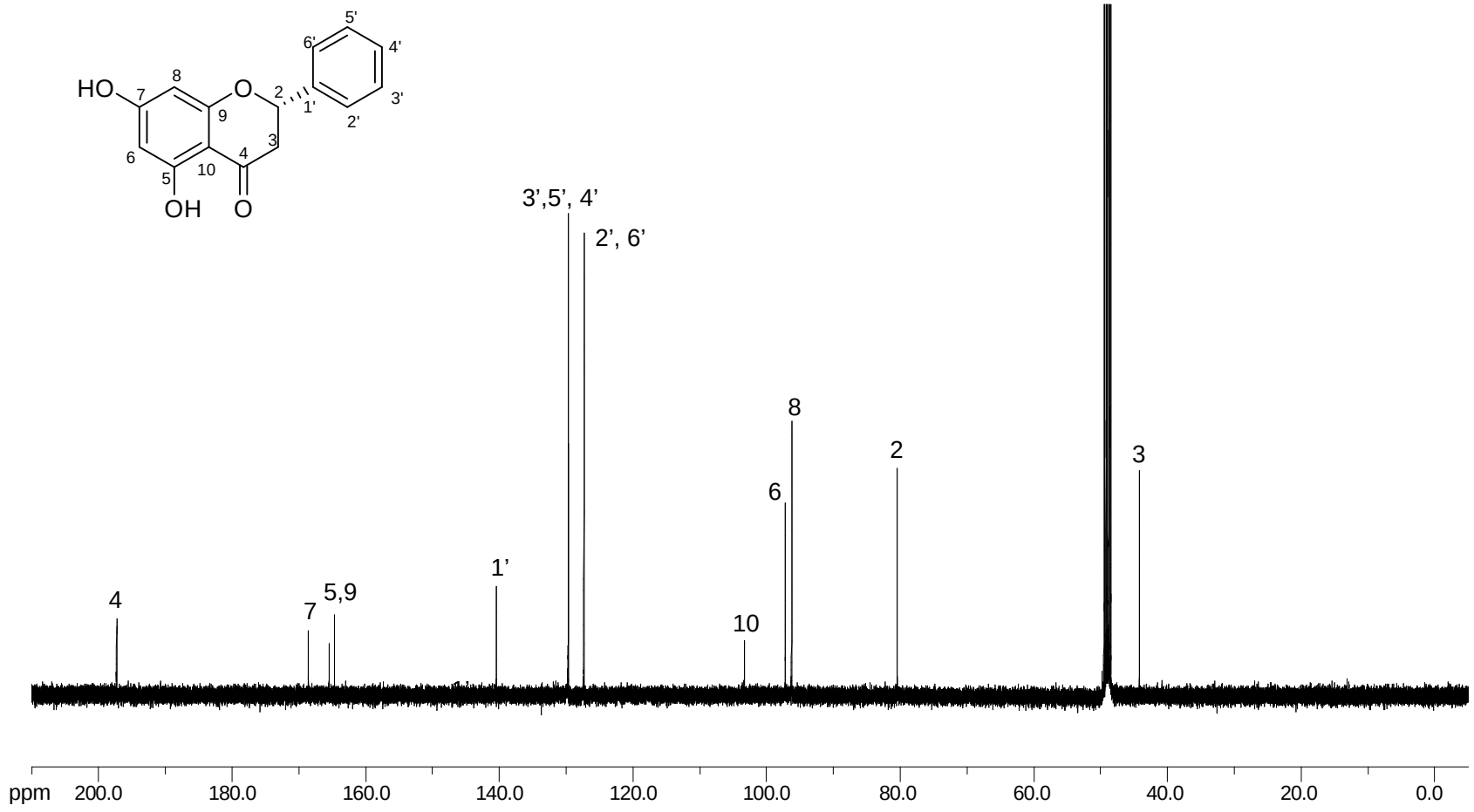


Plate 65: COSY NMR spectrum of pinocembrin (1) in CD_3OD

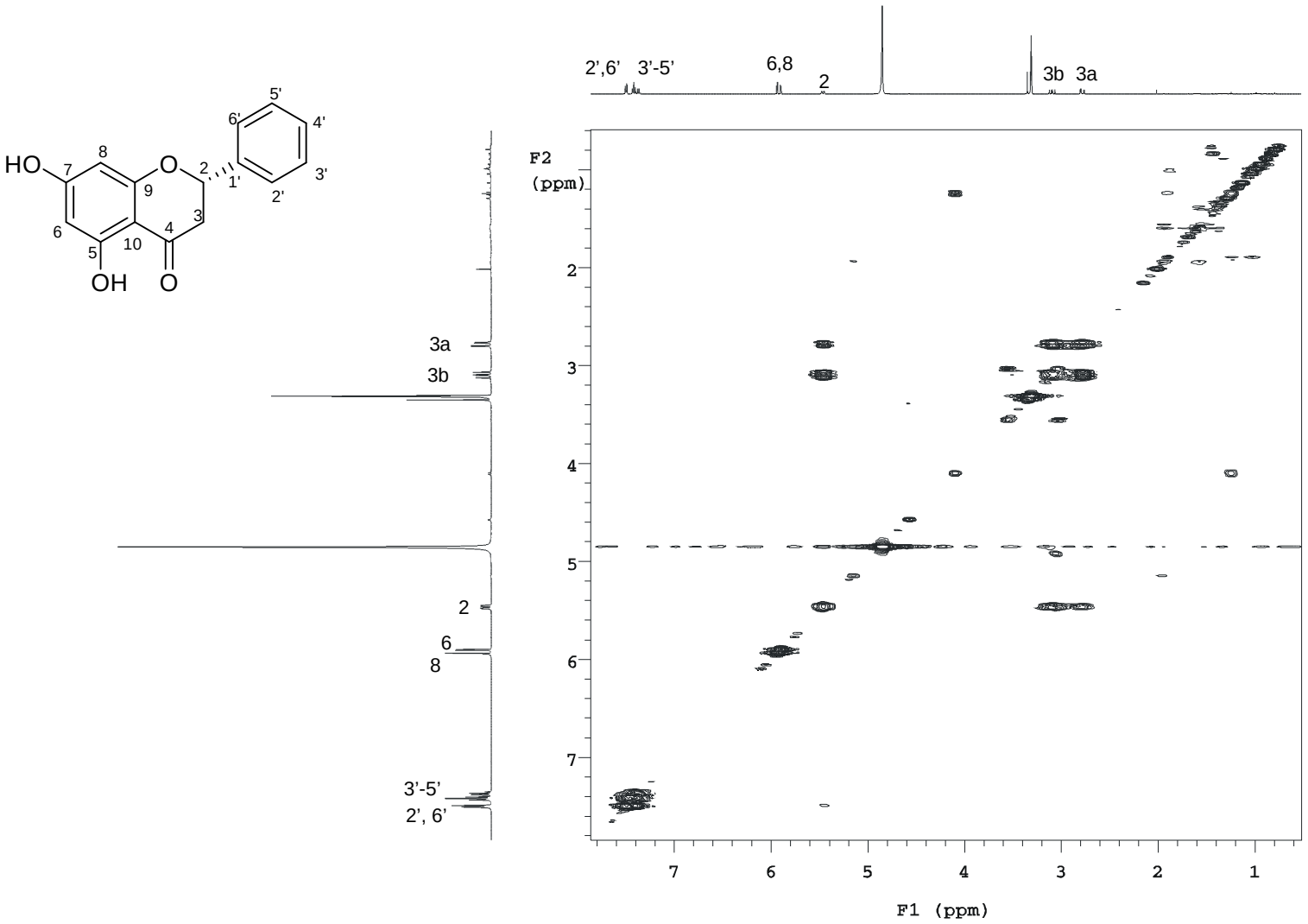


Plate 66: DEPT NMR spectrum of pinocembrin (1) in CD_3OD

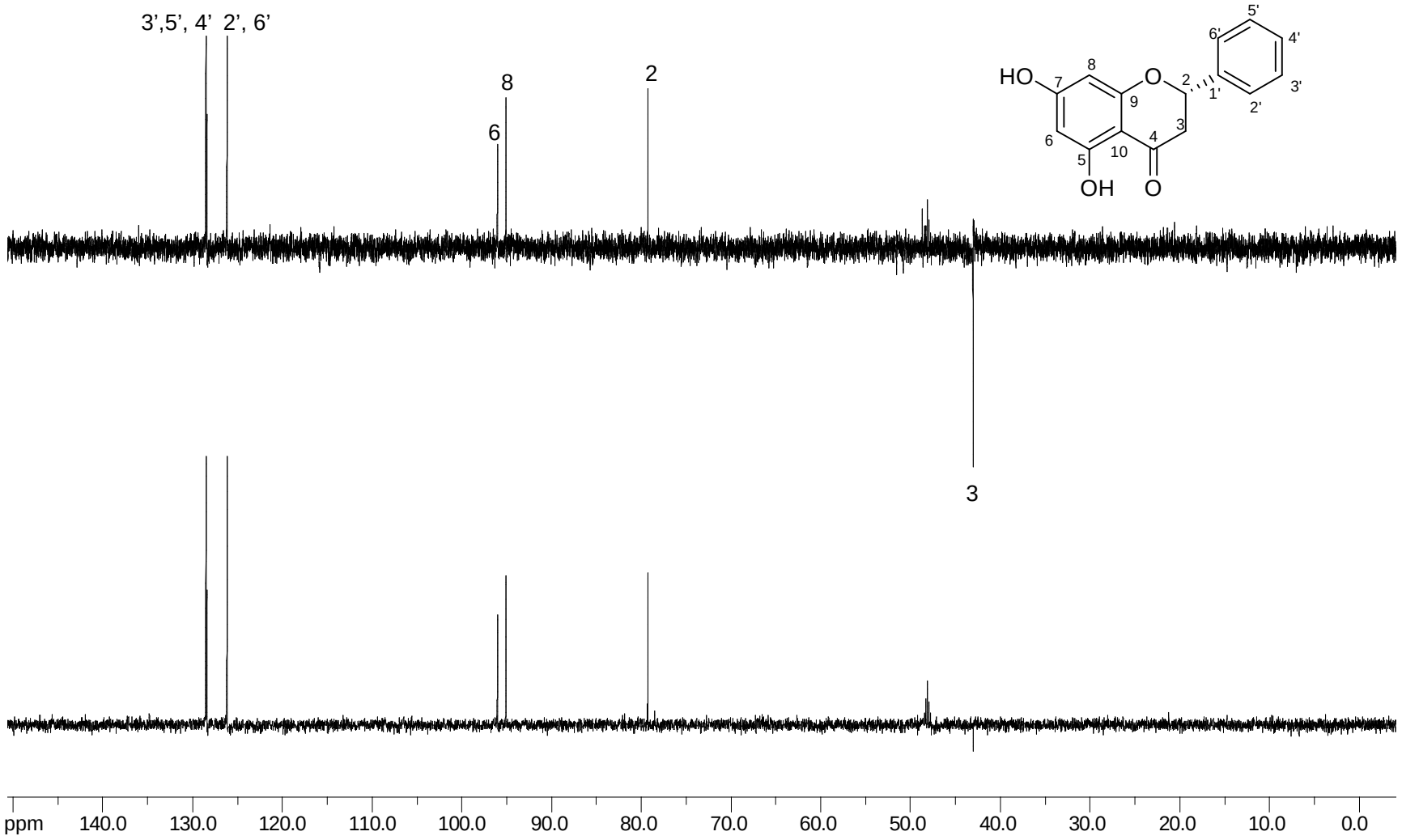


Plate 67: HSQC NMR spectrum of pinocembrin (1) in CD_3OD

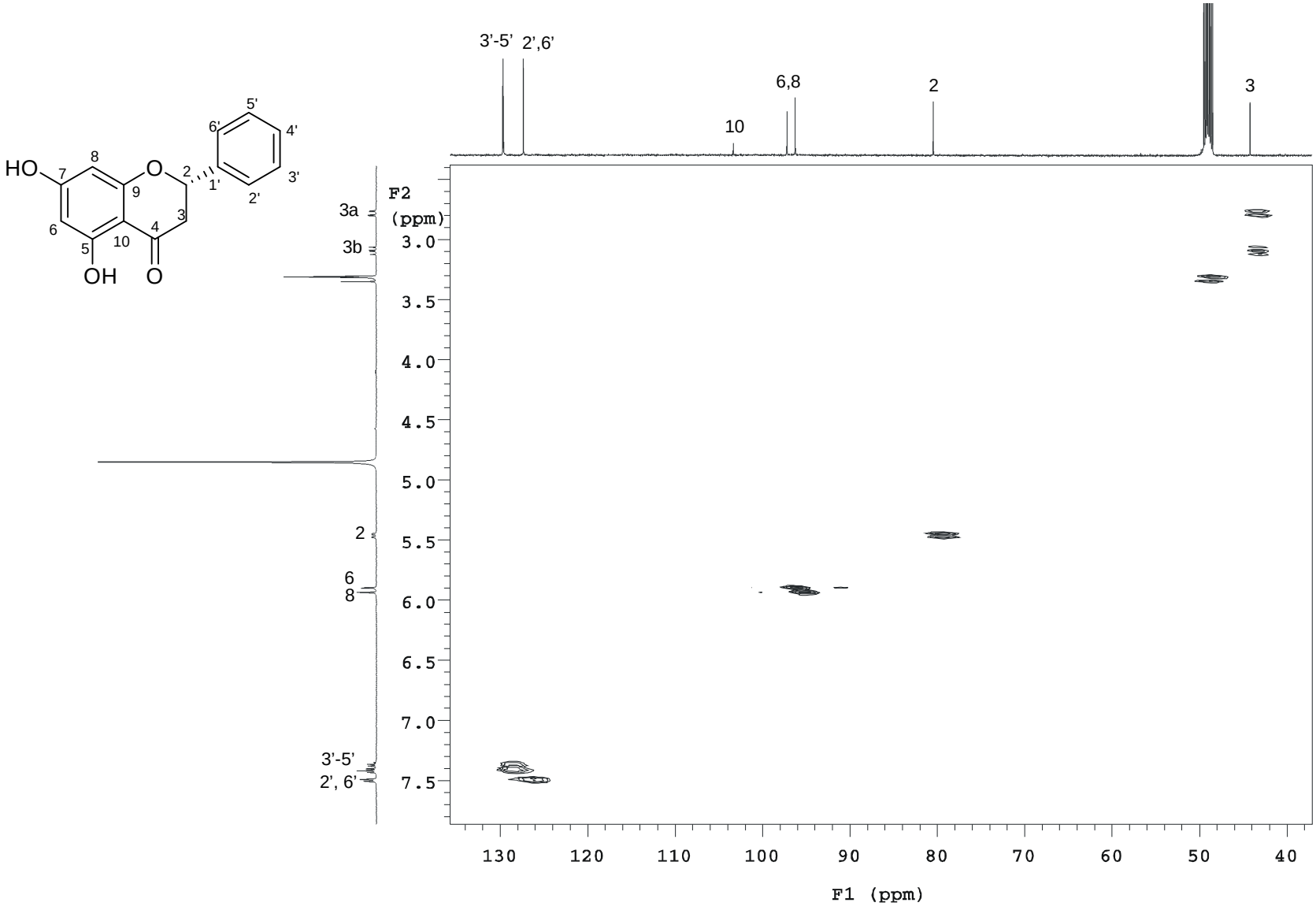


Plate 68: HMQC NMR spectrum of pinocembrin (1) in CD_3OD

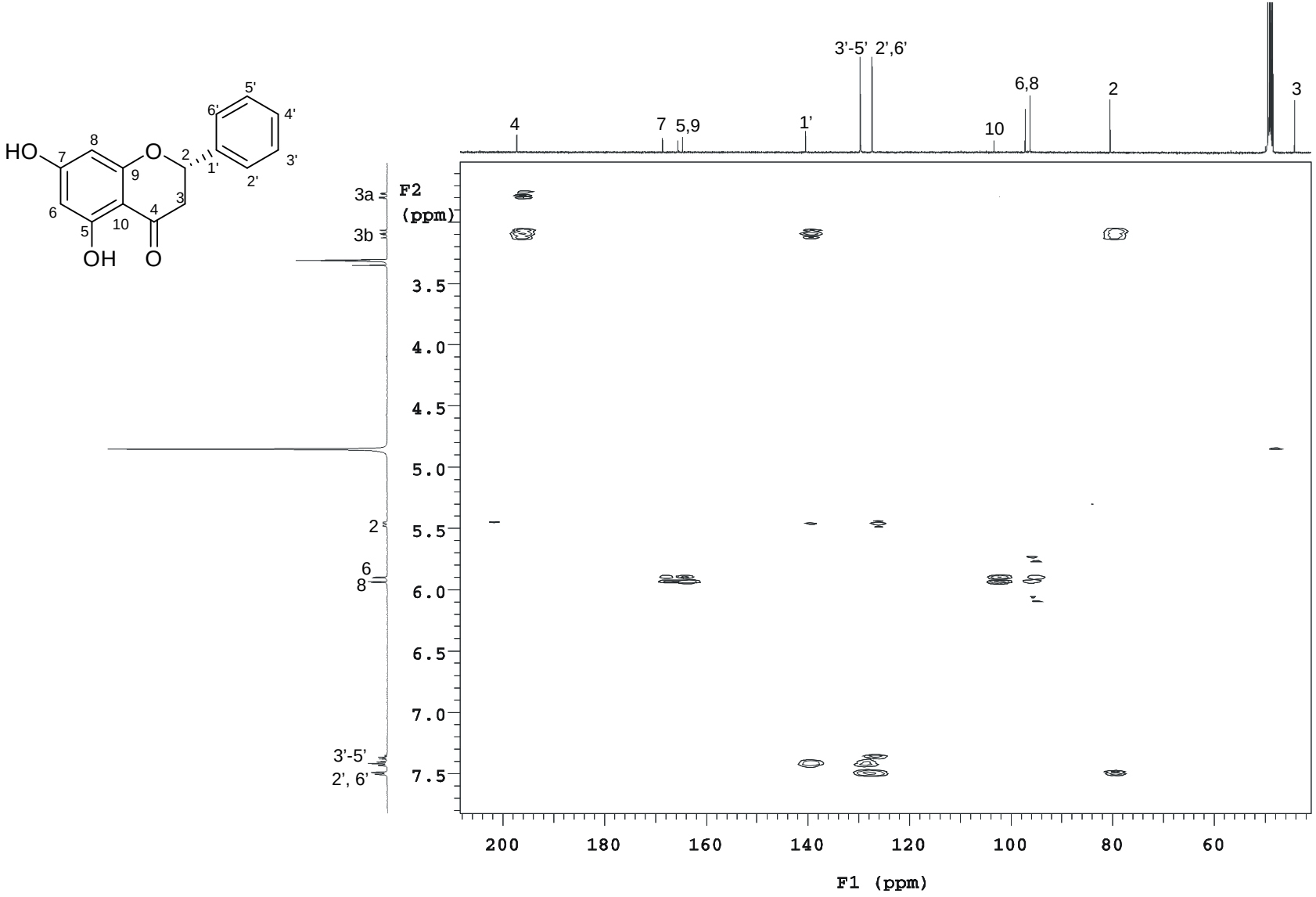


Plate 69: ^{1}H NMR spectrum of lepidissipyrone (19) in $CDCl_3$

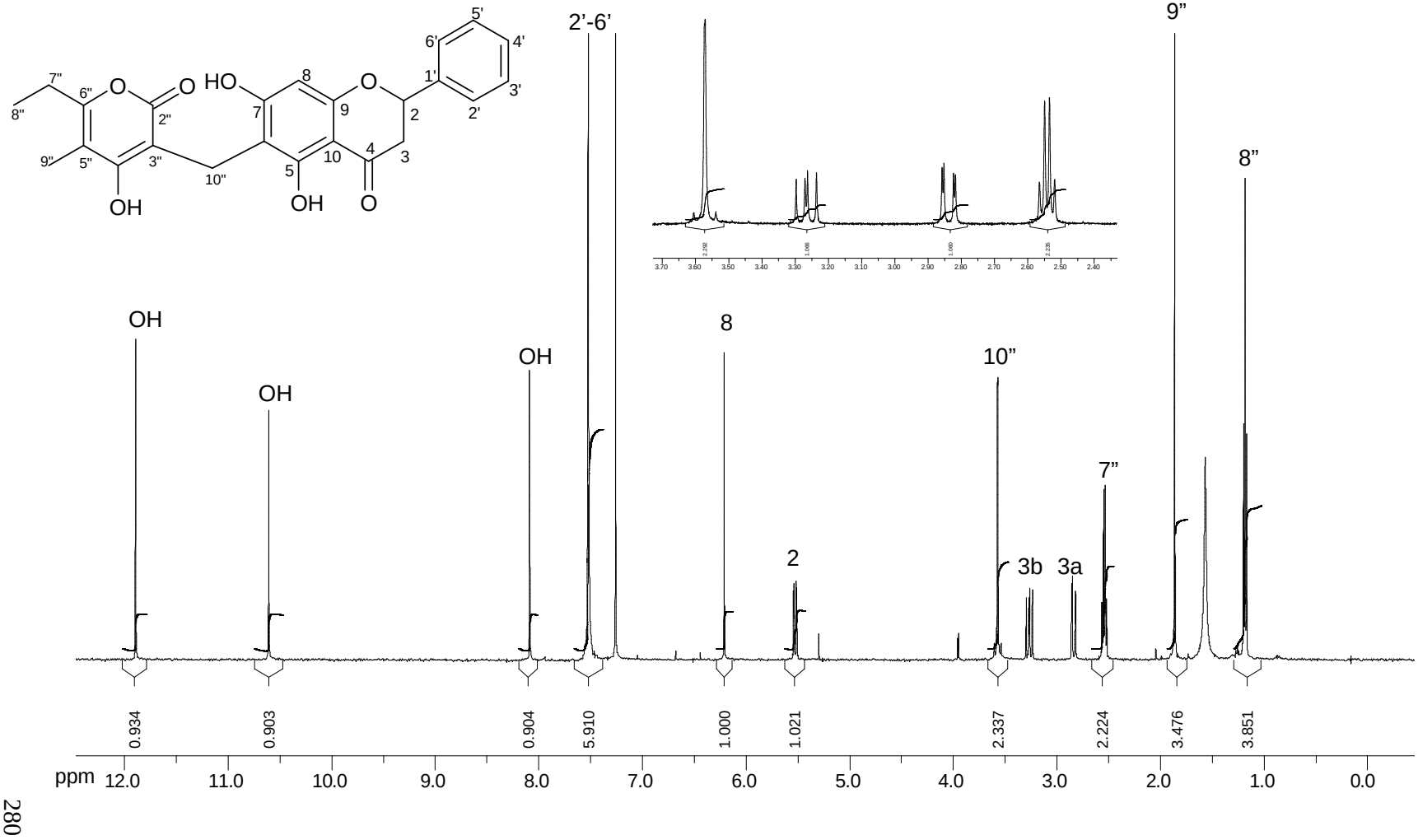


Plate 70: ^{13}C NMR spectrum of lepidissipyrone (19) in $CDCl_3$

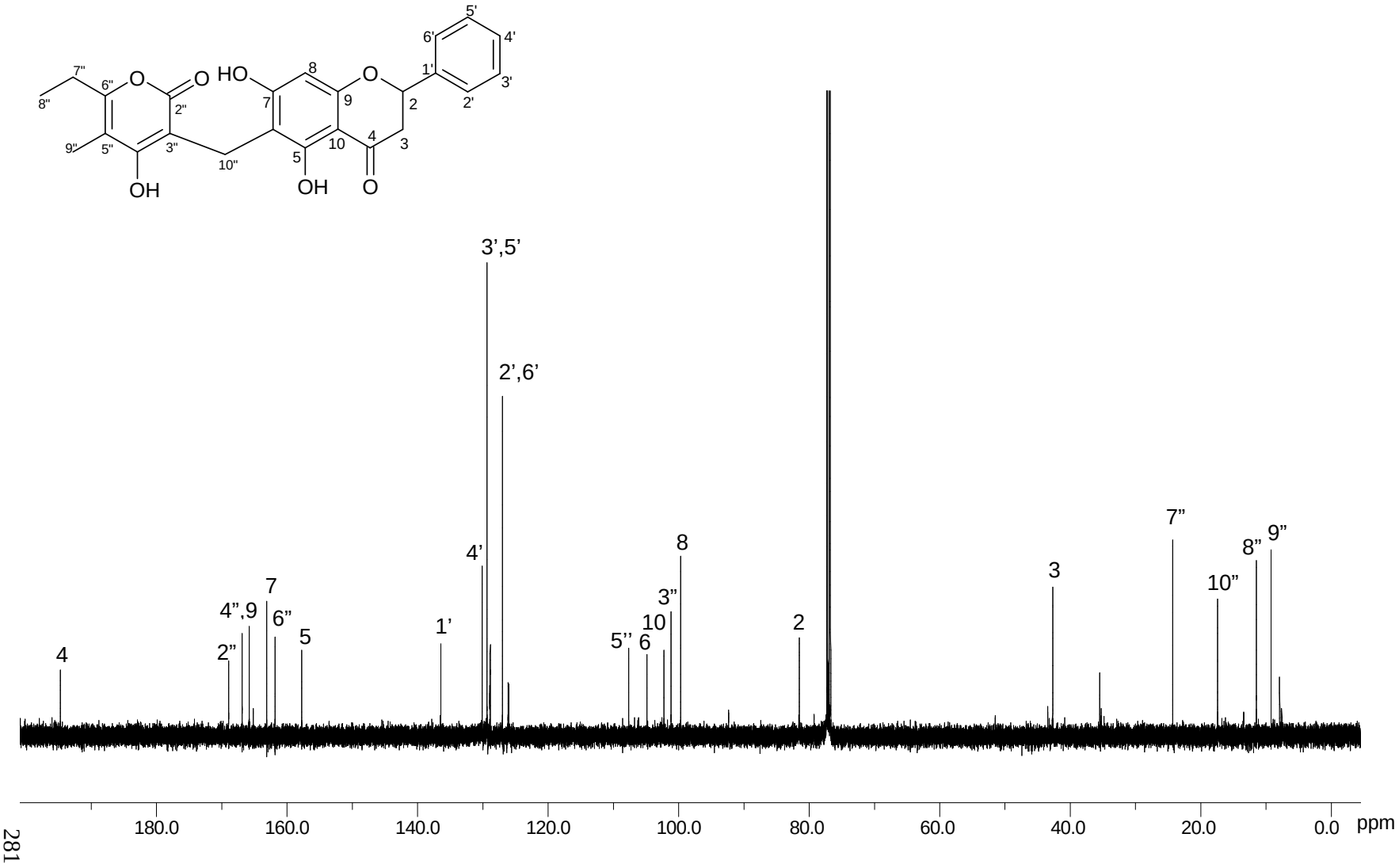


Plate 71: COSY NMR spectrum of lepidissipyrone (19) in $CDCl_3$

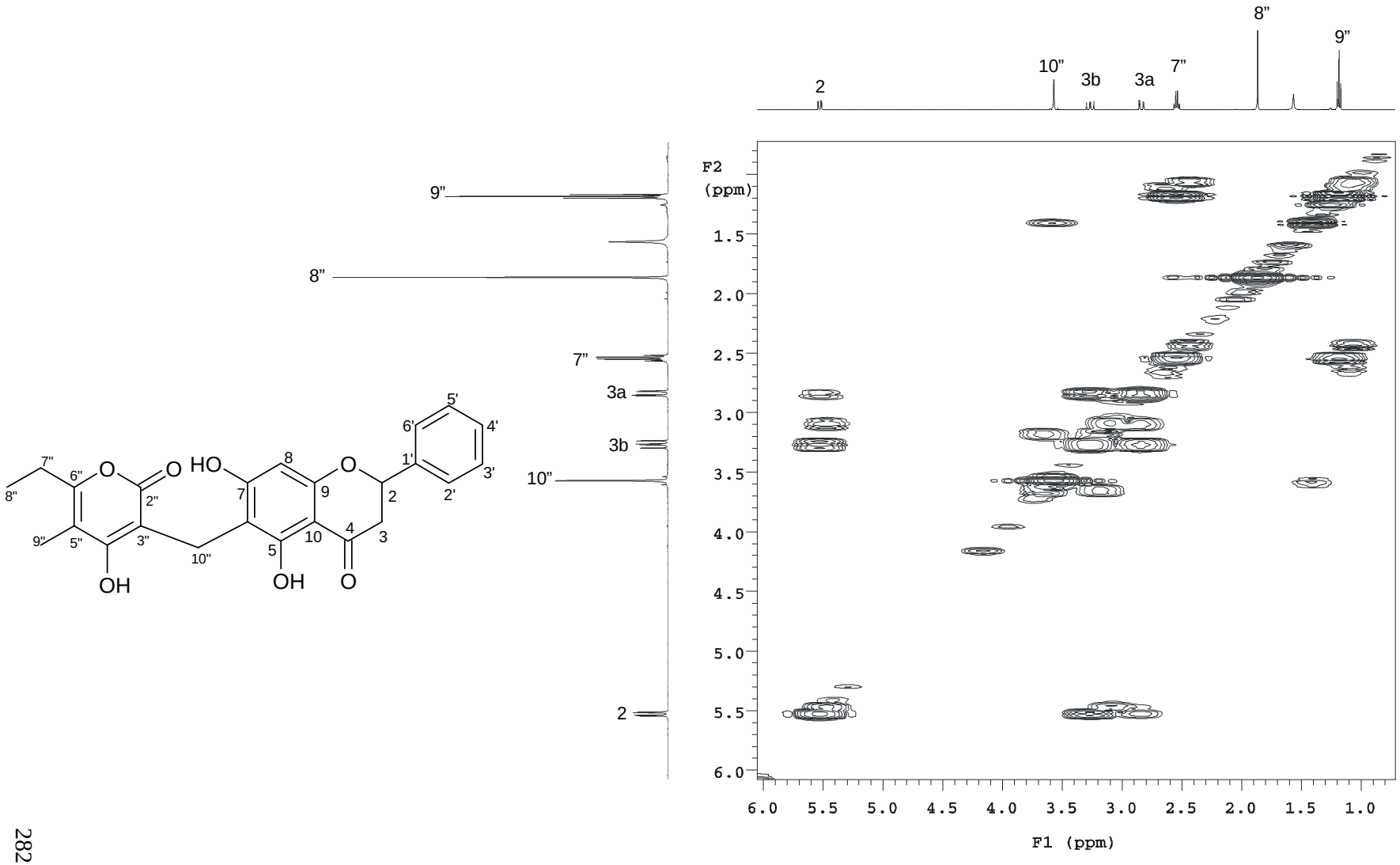


Plate 72: DEPT NMR spectrum of lepidissipyrone (19) in $CDCl_3$

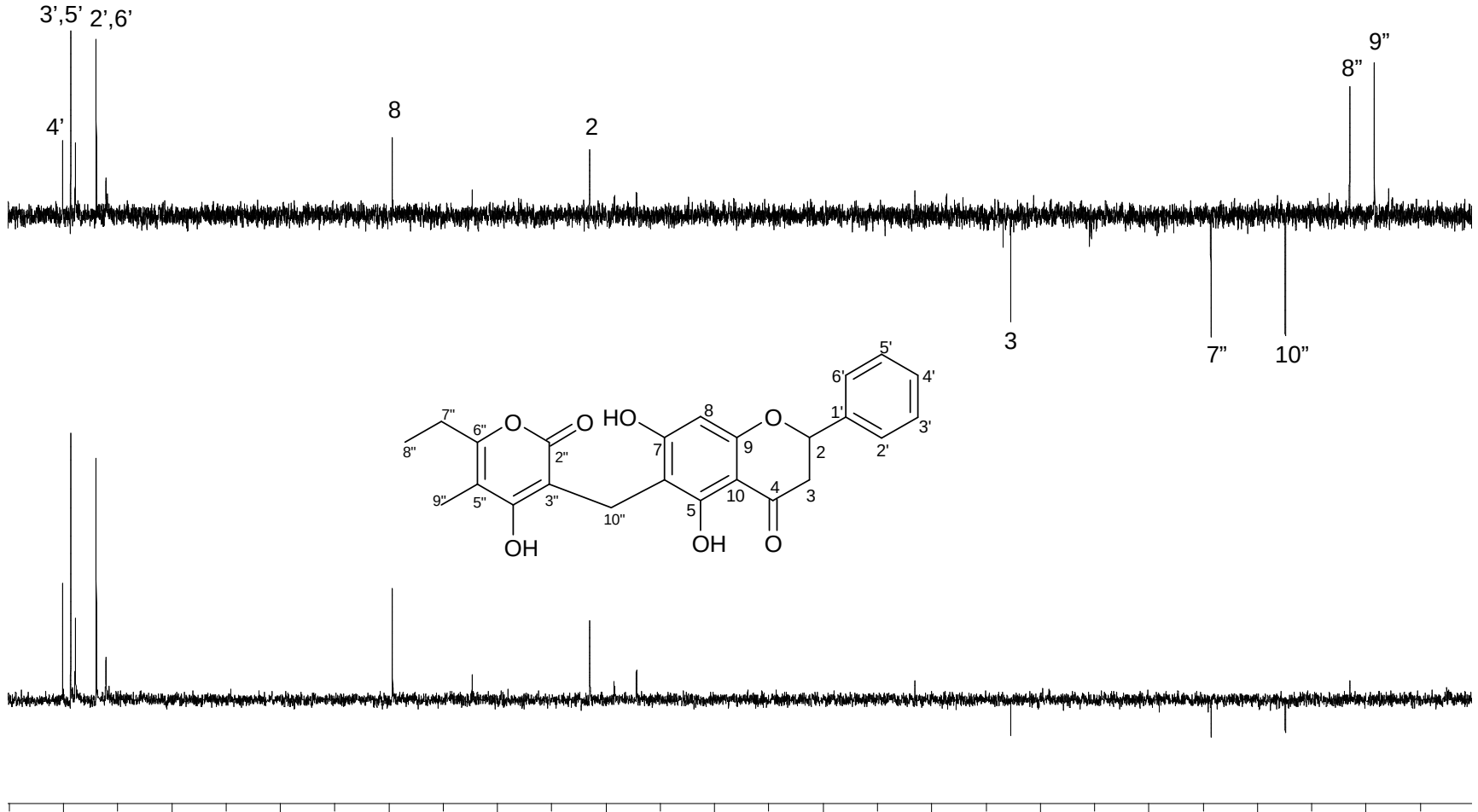


Plate 73: HSQC NMR spectrum of lepidissipyrone (19) in $CDCl_3$

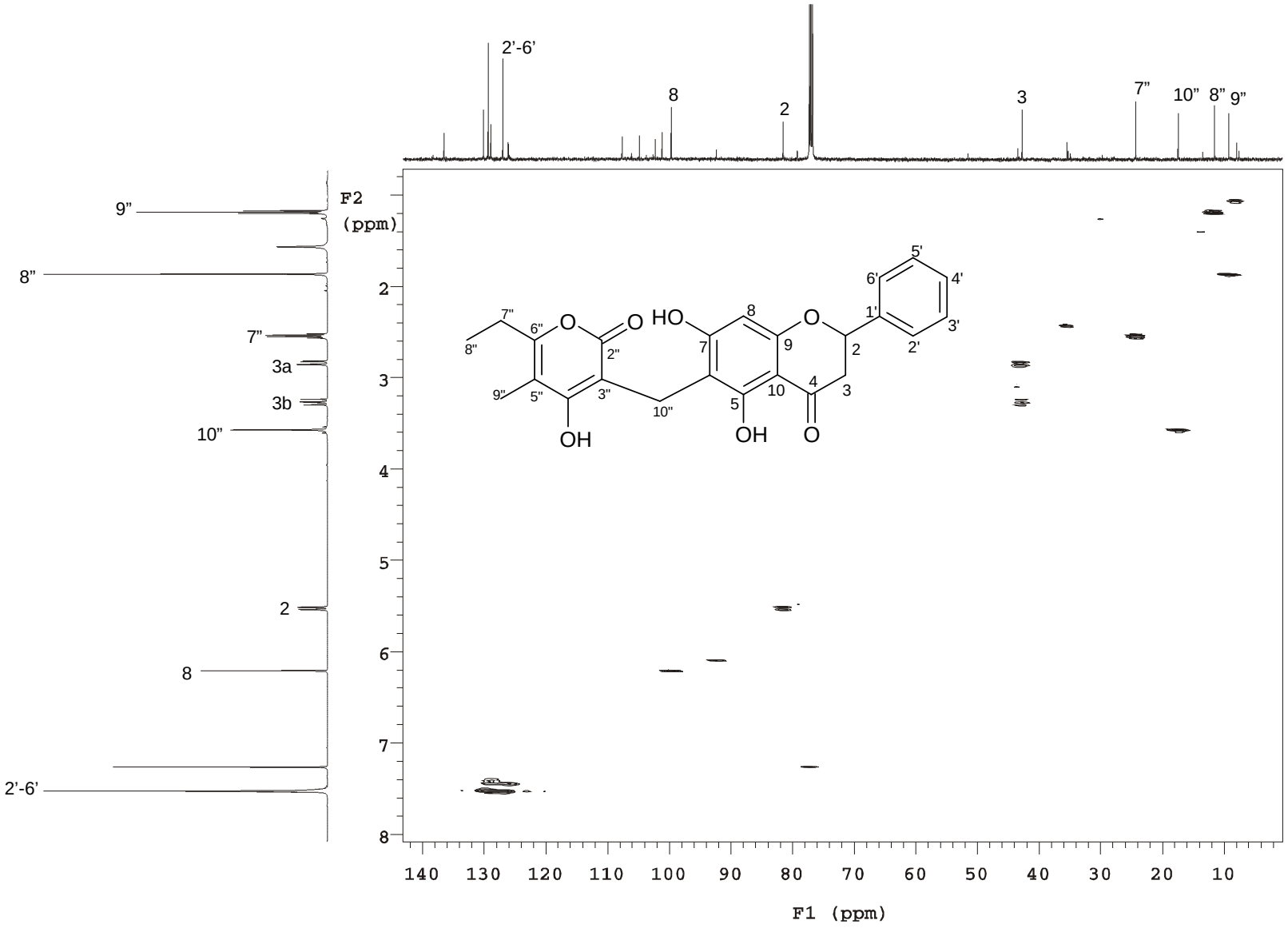


Plate 74: HMQC NMR spectrum of lepidissipyrone (19) in $CDCl_3$

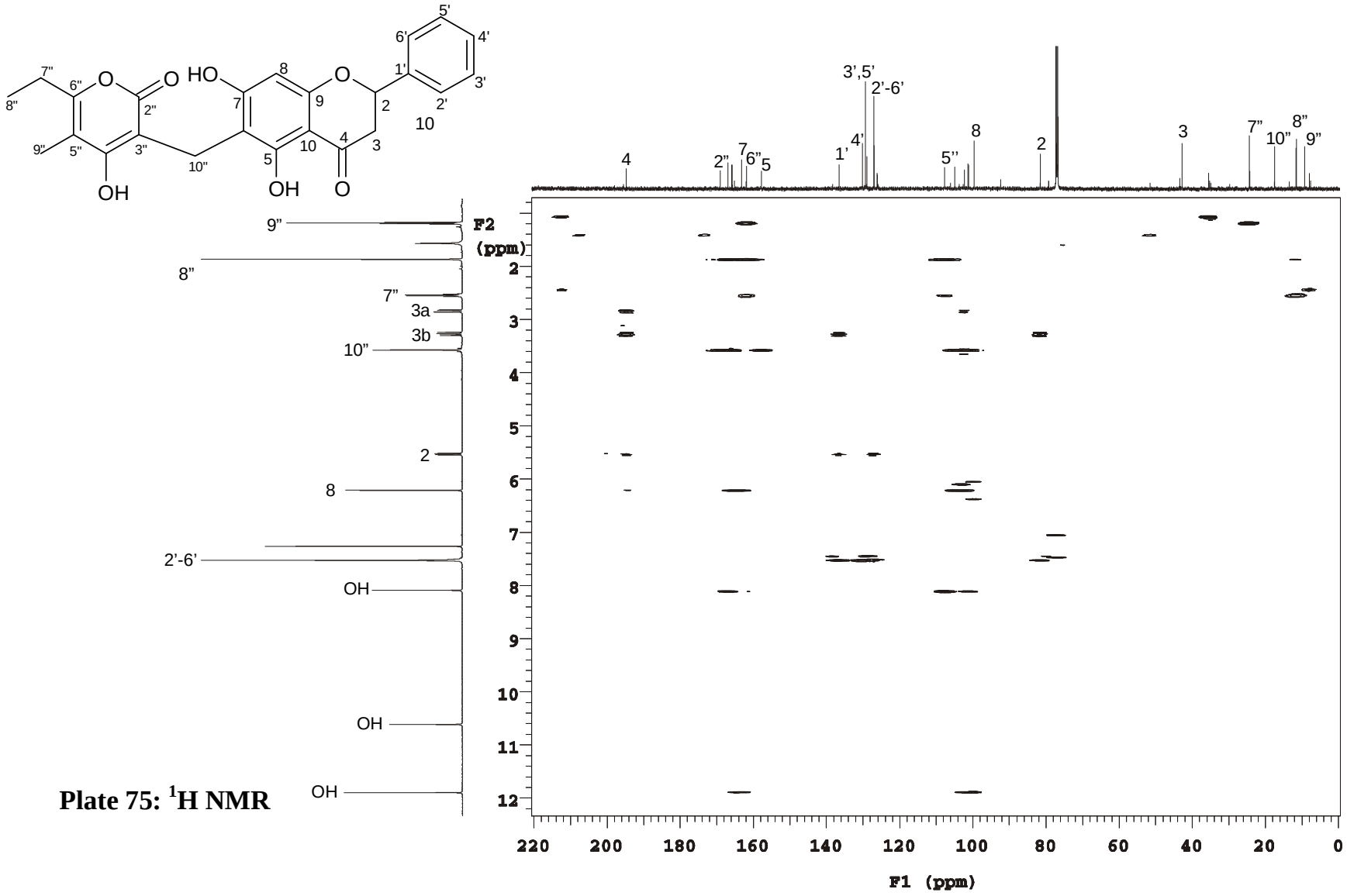


Plate 75: ^{1}H NMR

spectrum of gnaphaliin (67) in $CDCl_3$

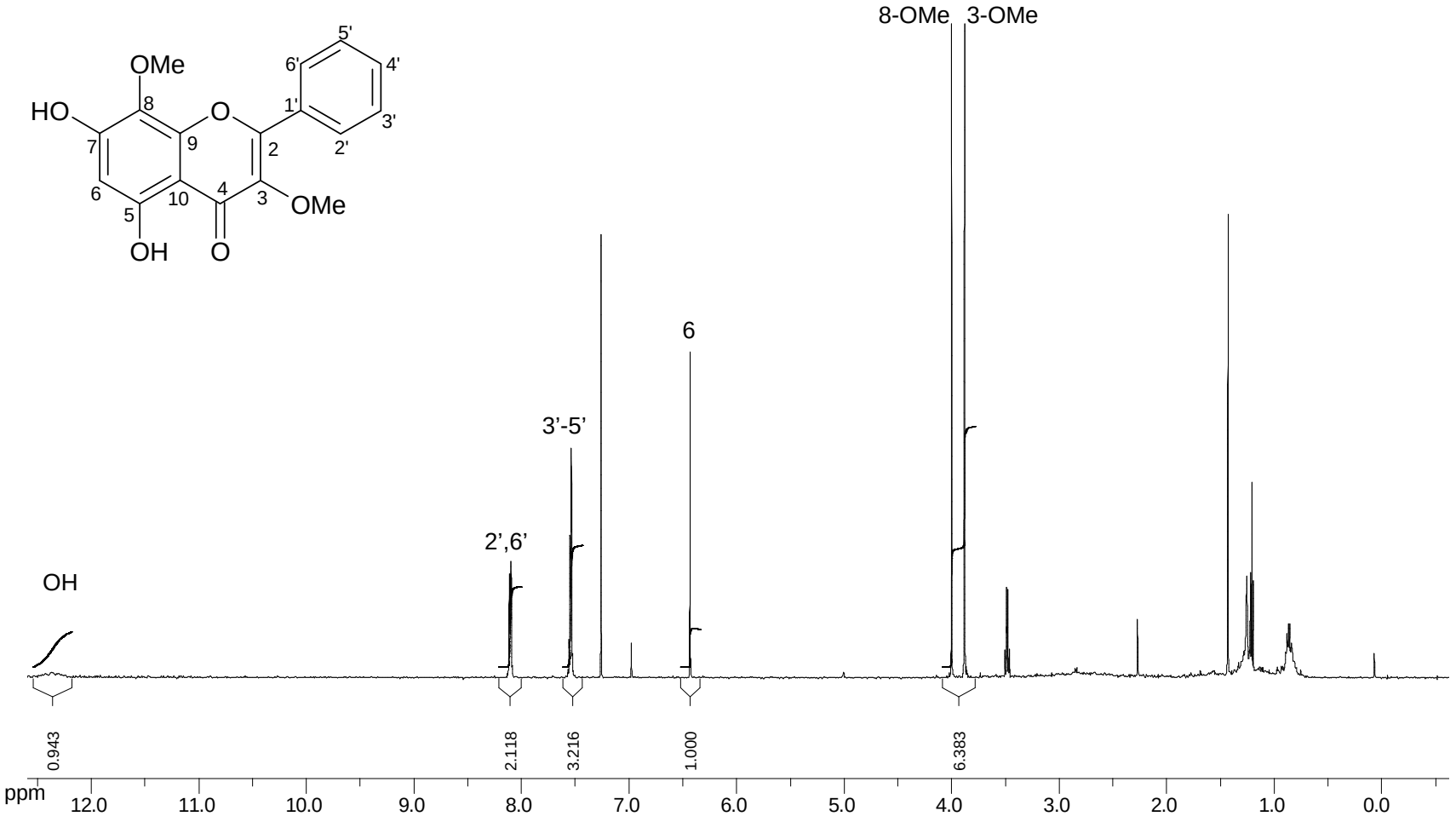


Plate 76: ^{13}C NMR spectrum of gnaphaliin (67) in $CDCl_3$

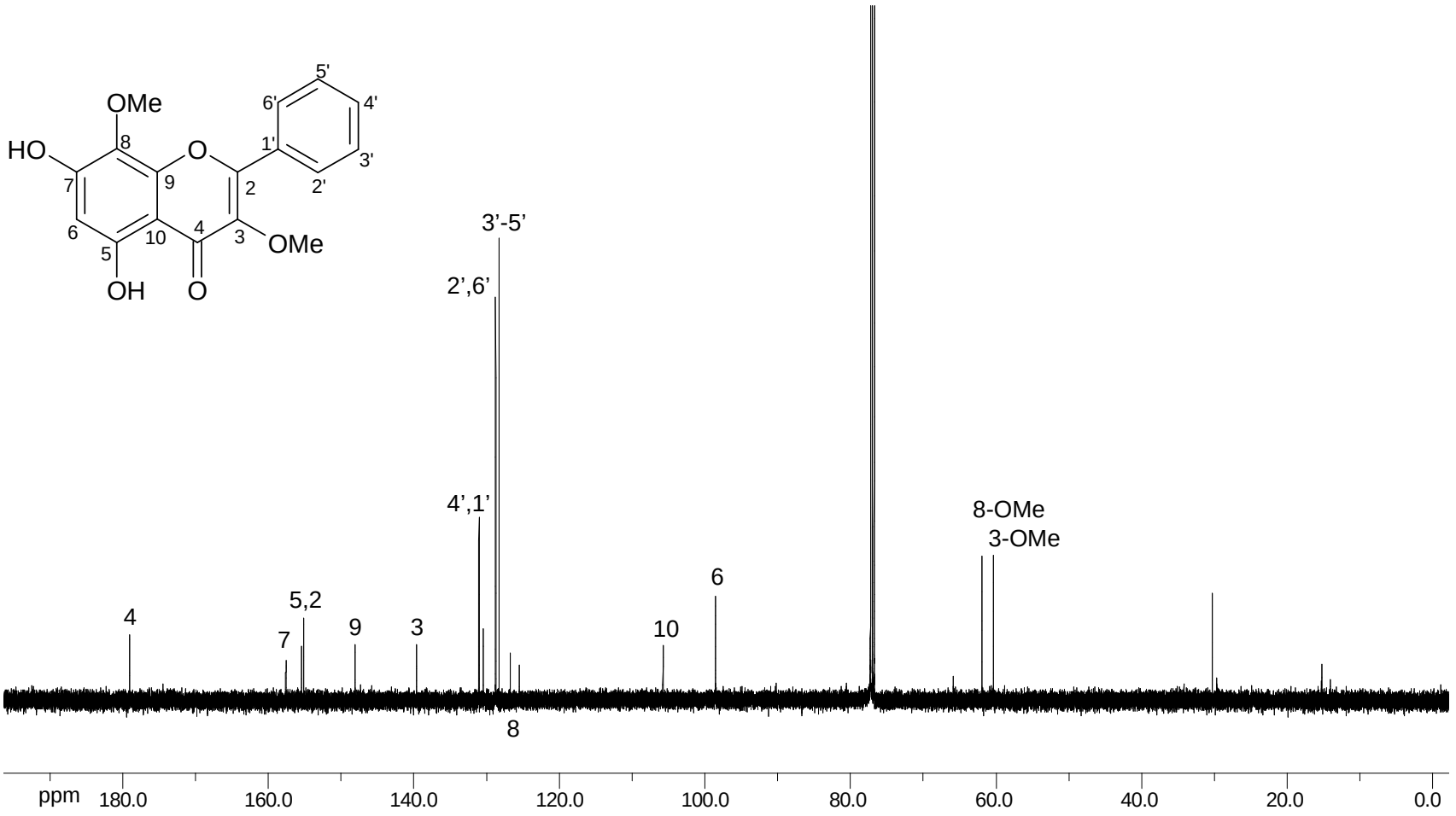


Plate 77: COSY NMR spectrum of gnaphaliin (67) in $CDCl_3$

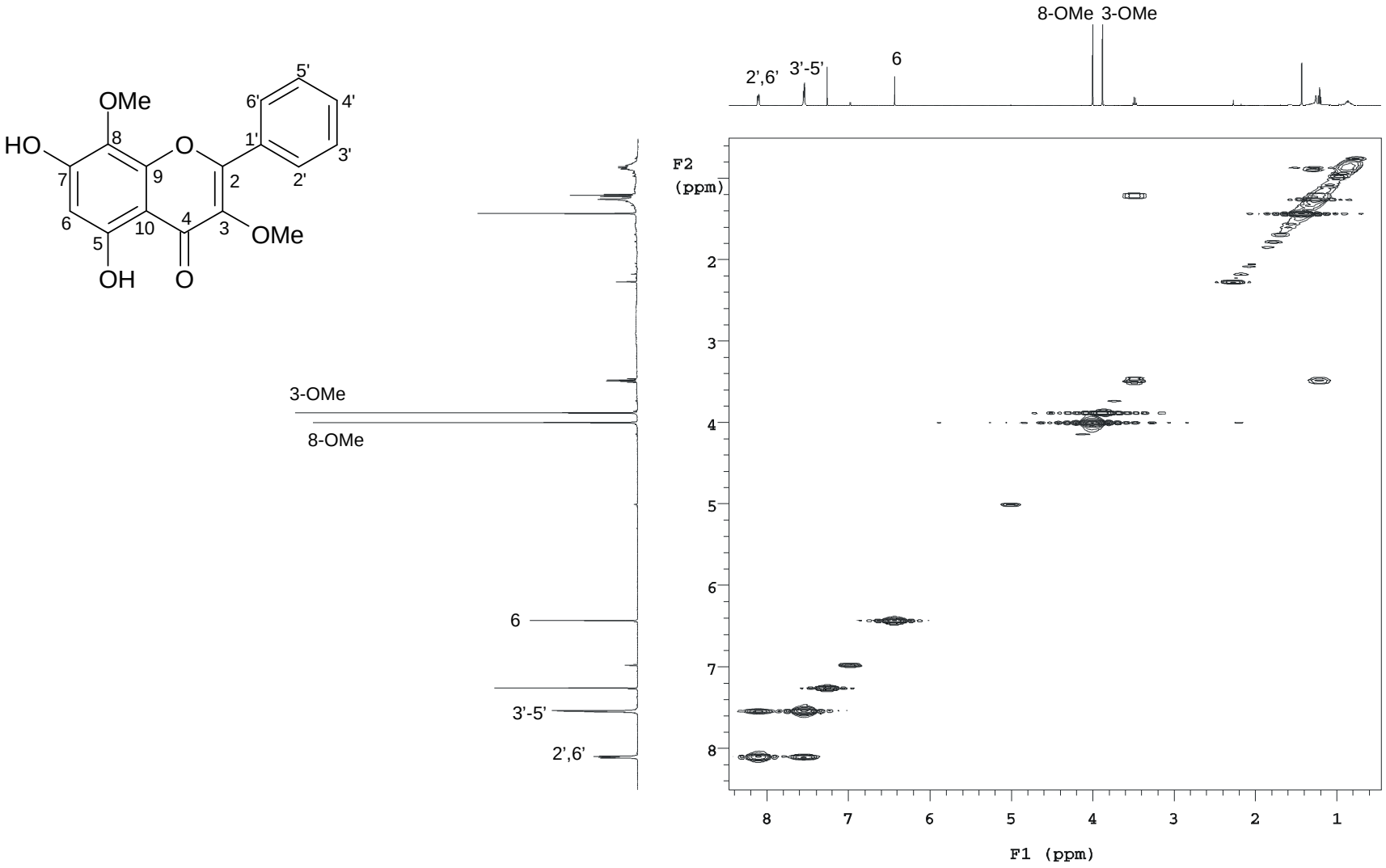


Plate 78: HSQC NMR spectrum of gnaphaliin (67) in $CDCl_3$

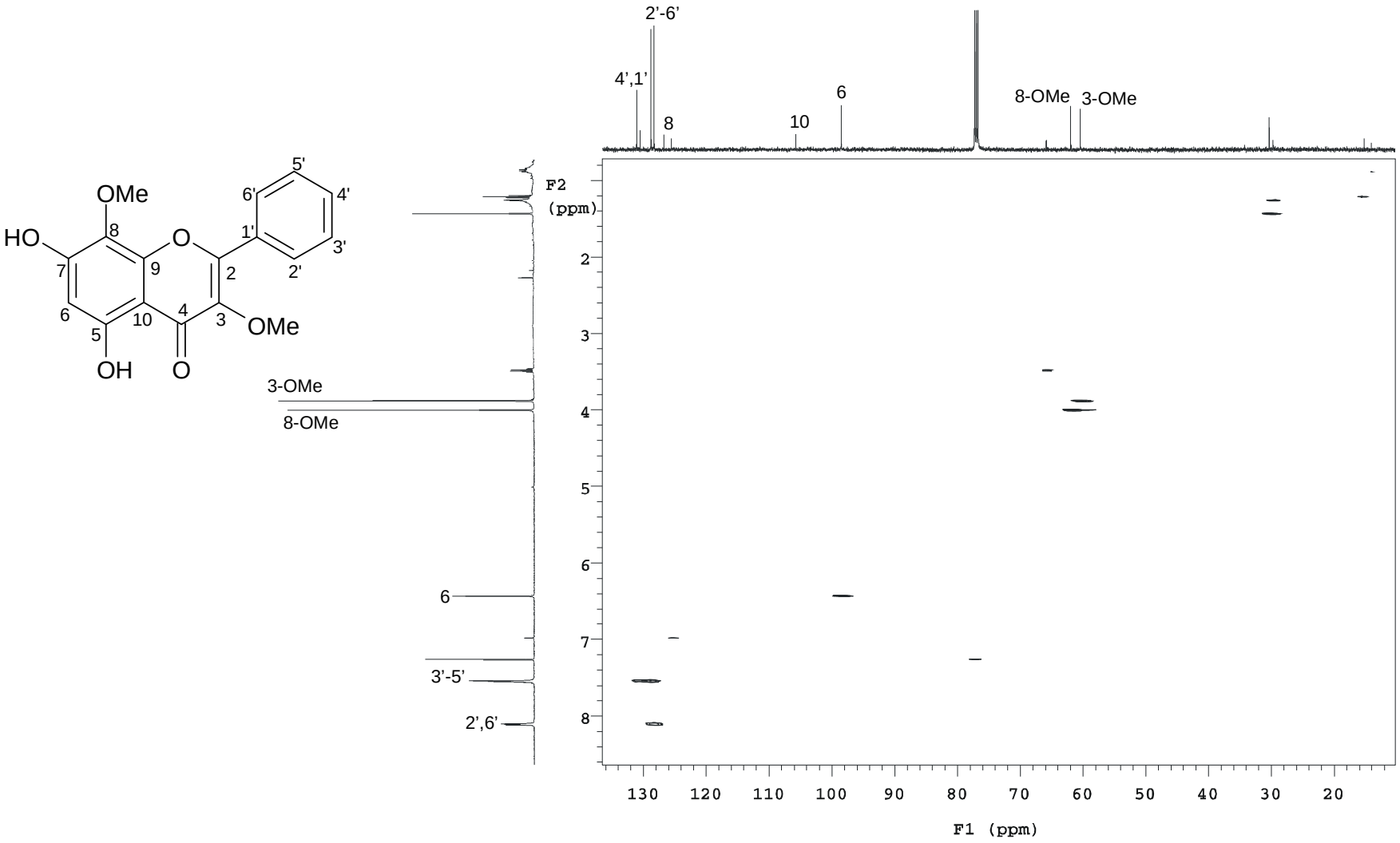


Plate 79: HMQC NMR spectrum of gnaphaliin (67) in $CDCl_3$

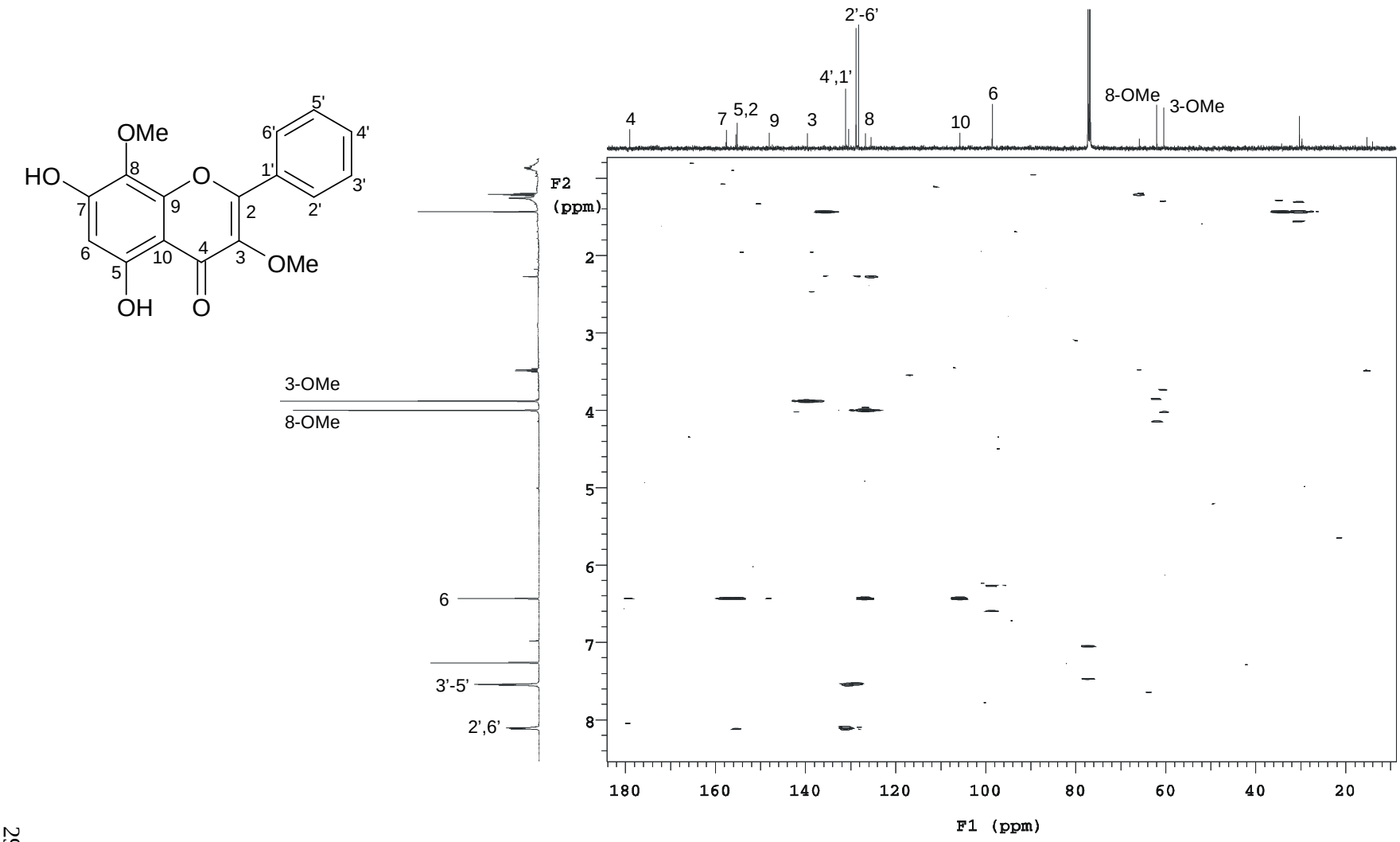


Plate 80: NOESY NMR spectrum of gnaphaliin (67) in $CDCl_3$

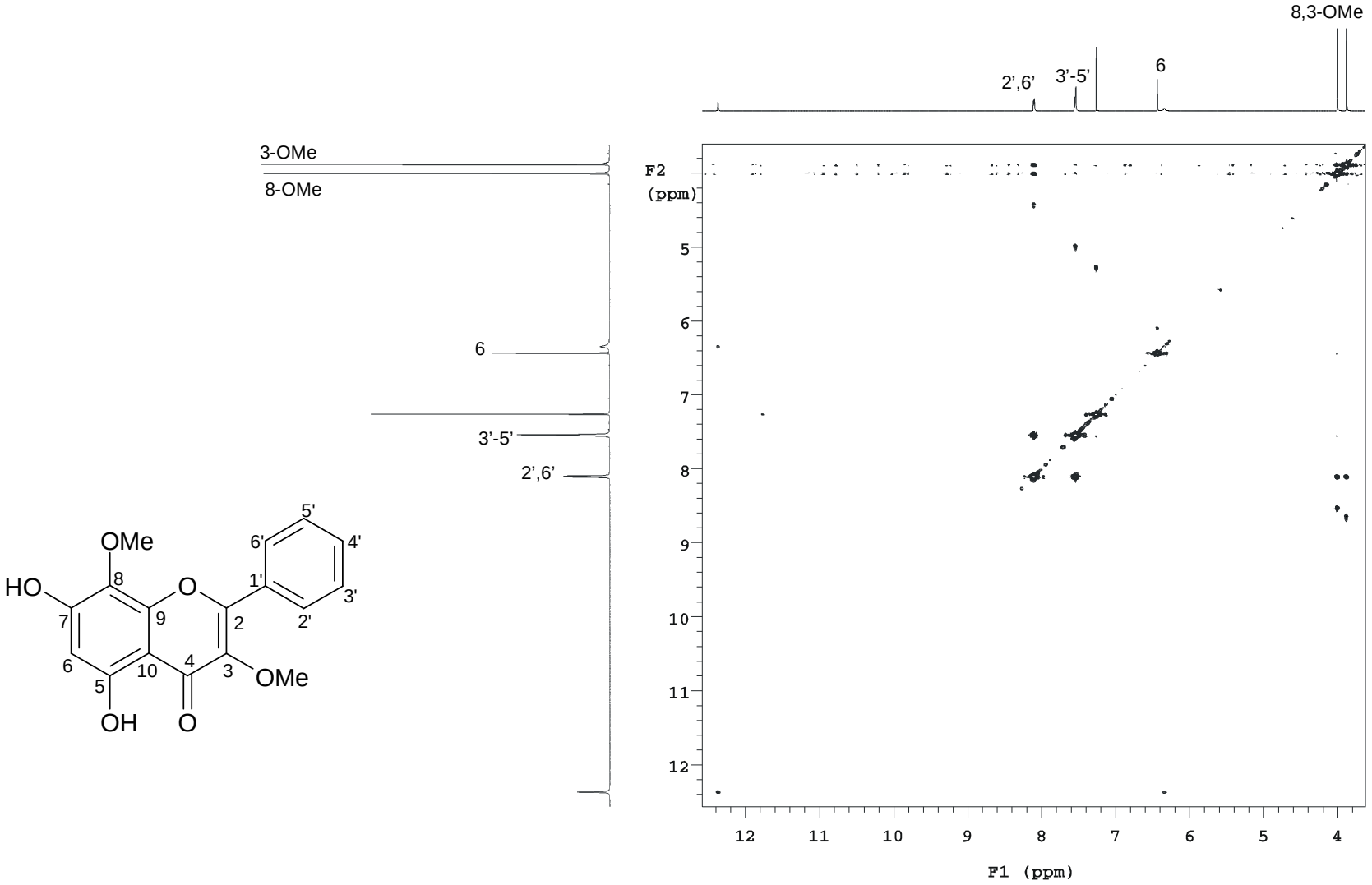


Plate 81: ^{1}H NMR spectrum of 5-hydroxy-7,8-dimethoxyflavone (72) in $CDCl_3$

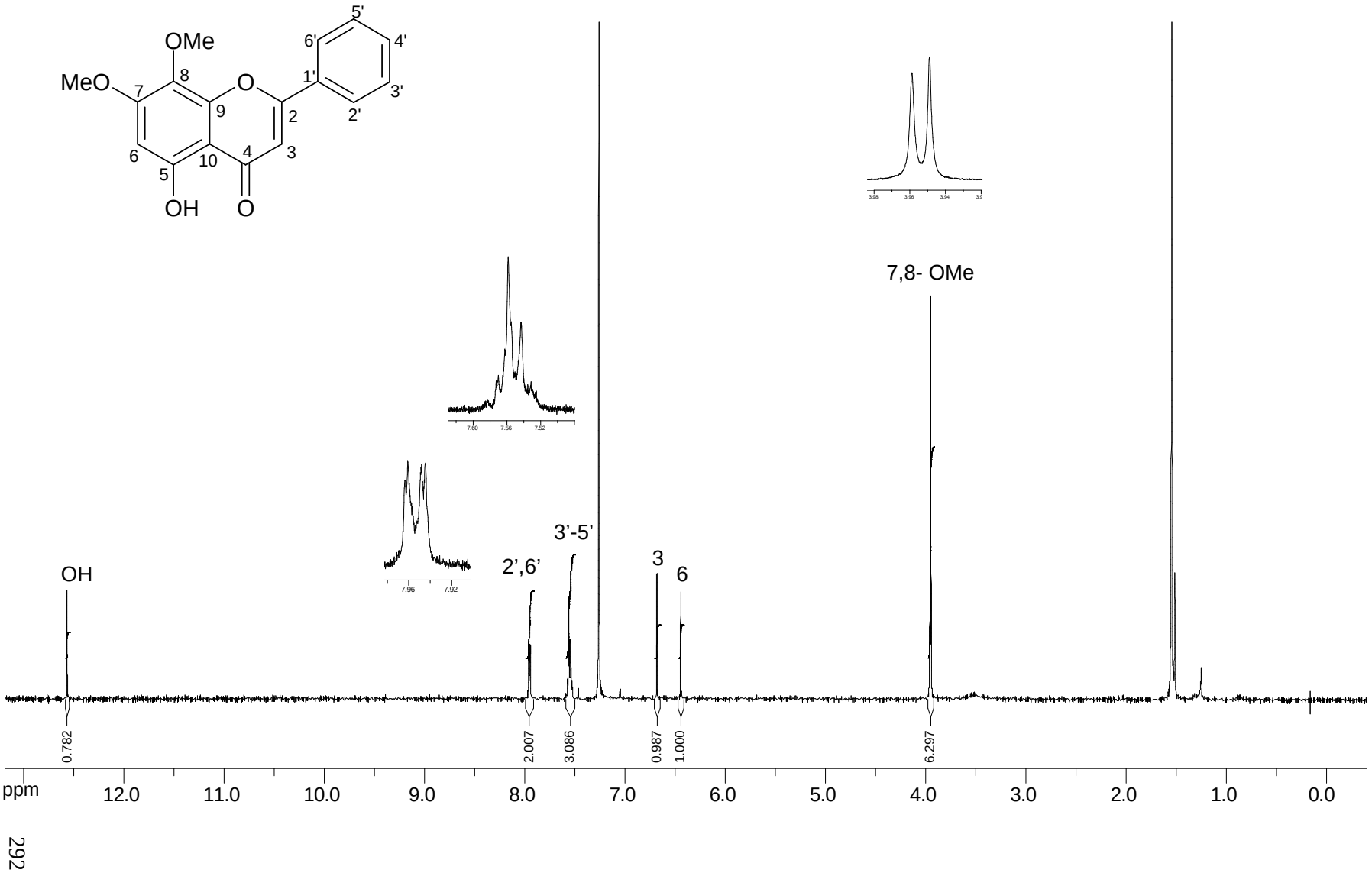


Plate 82: ^{13}C NMR spectrum of 5-hydroxy-7,8-dimethoxyflavone (72) in $CDCl_3$

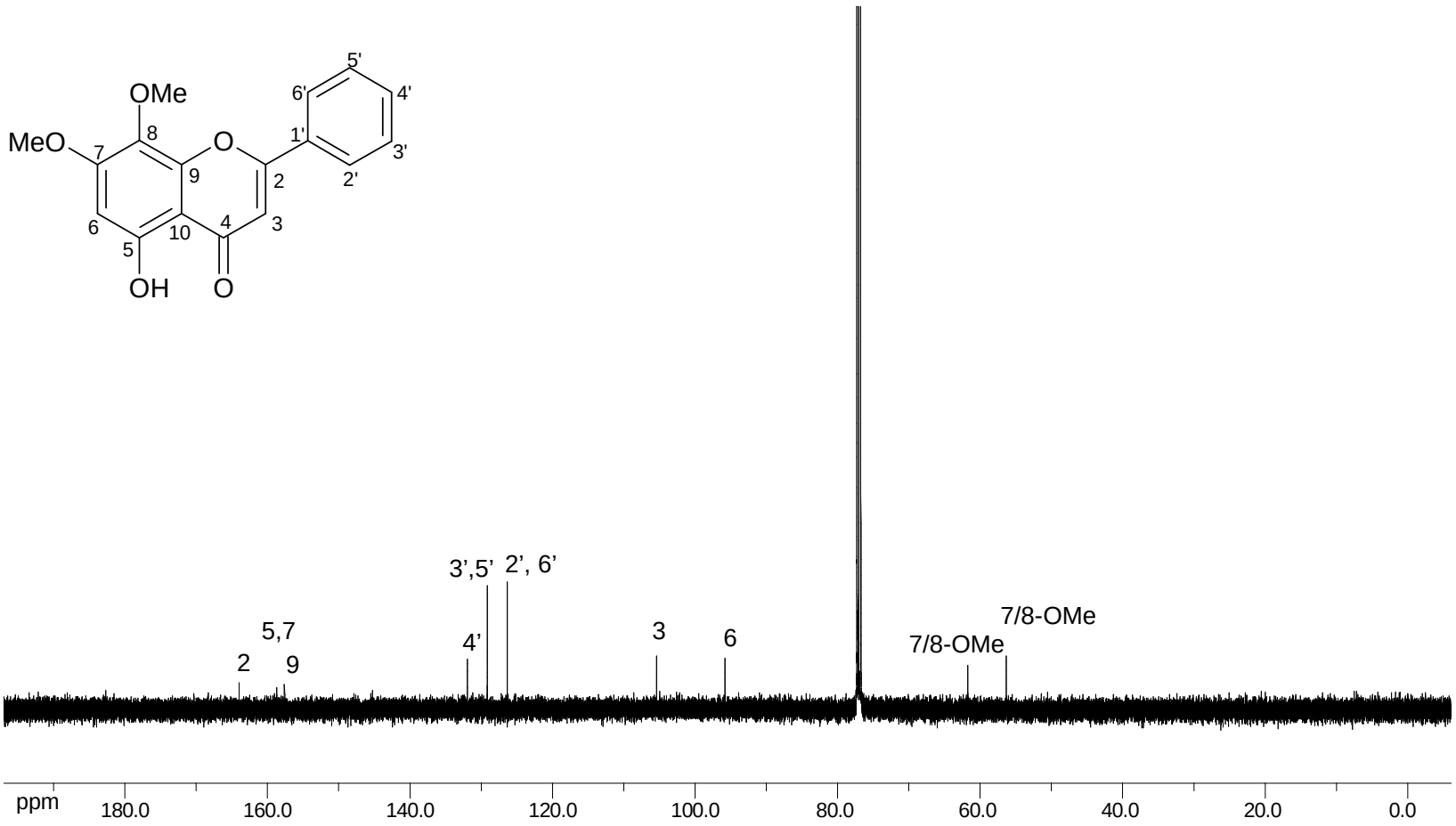


Plate 83: COSY NMR spectrum of 5-hydroxy-7,8-dimethoxyflavone (72) in $CDCl_3$

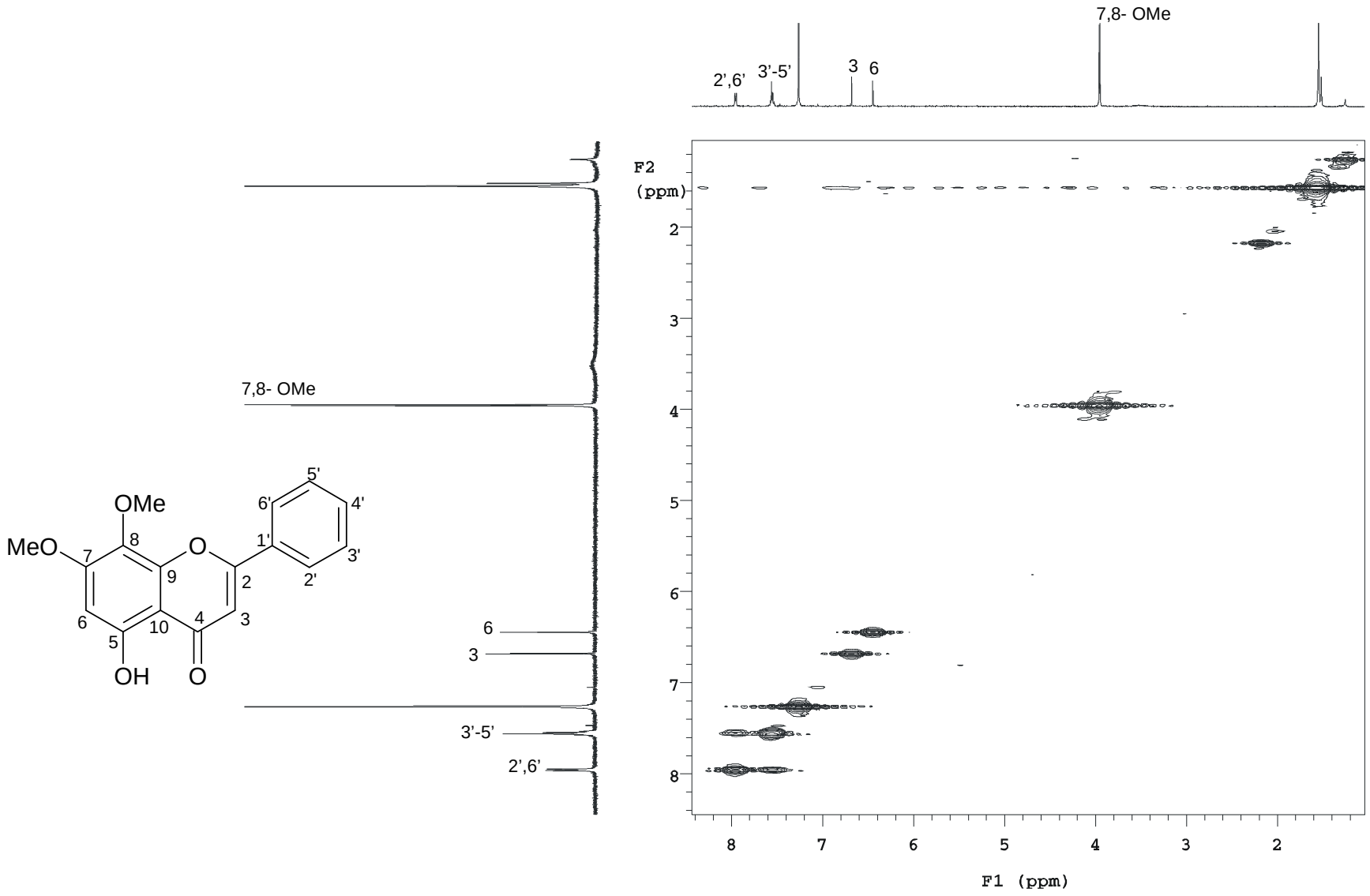


Plate 84: DEPT NMR spectrum of 5-hydroxy-7,8-dimethoxyflavone (72) in $CDCl_3$

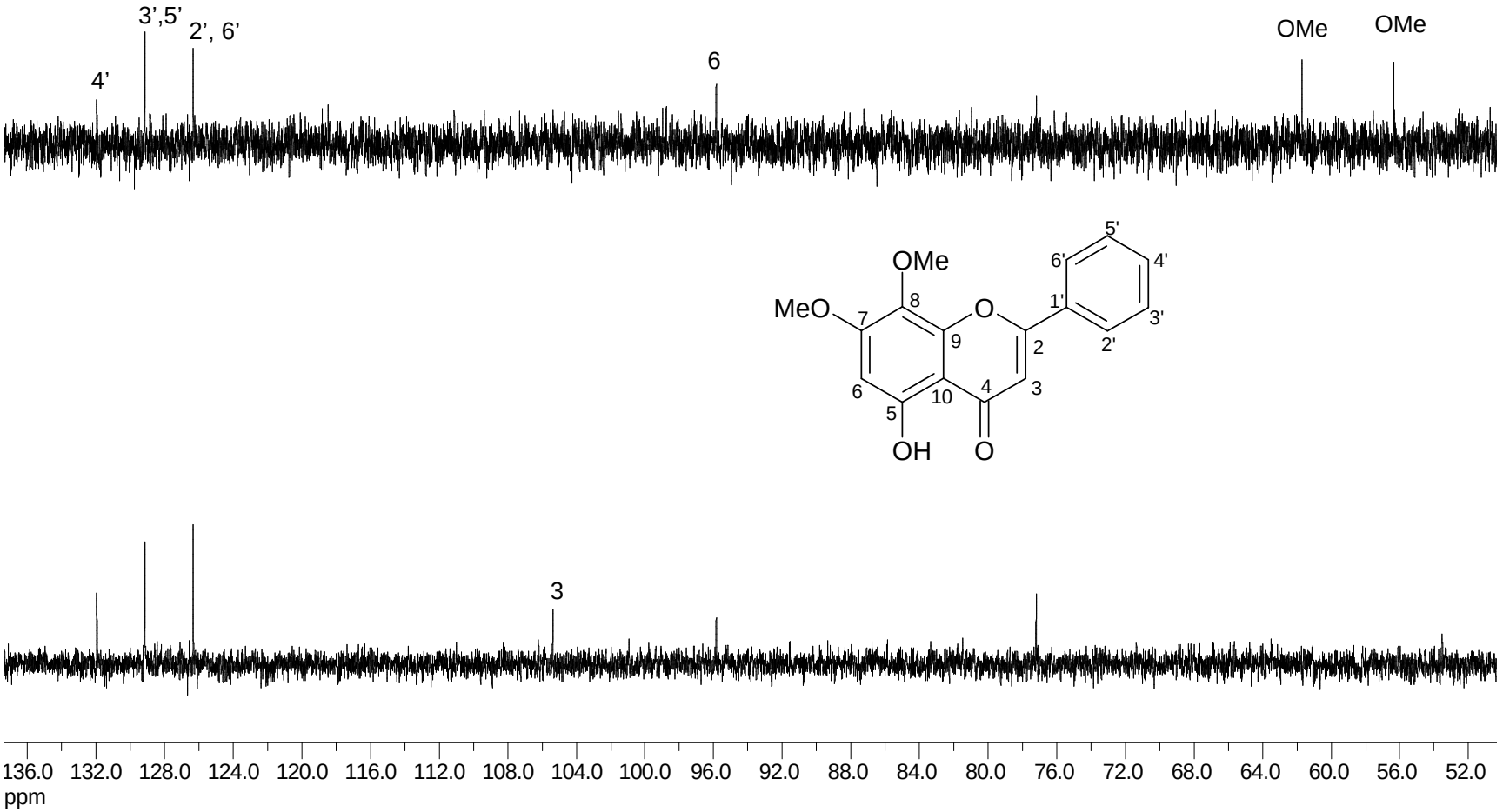


Plate 85: HSQC NMR spectrum of 5-hydroxy-7,8-dimethoxyflavone (72) in $CDCl_3$

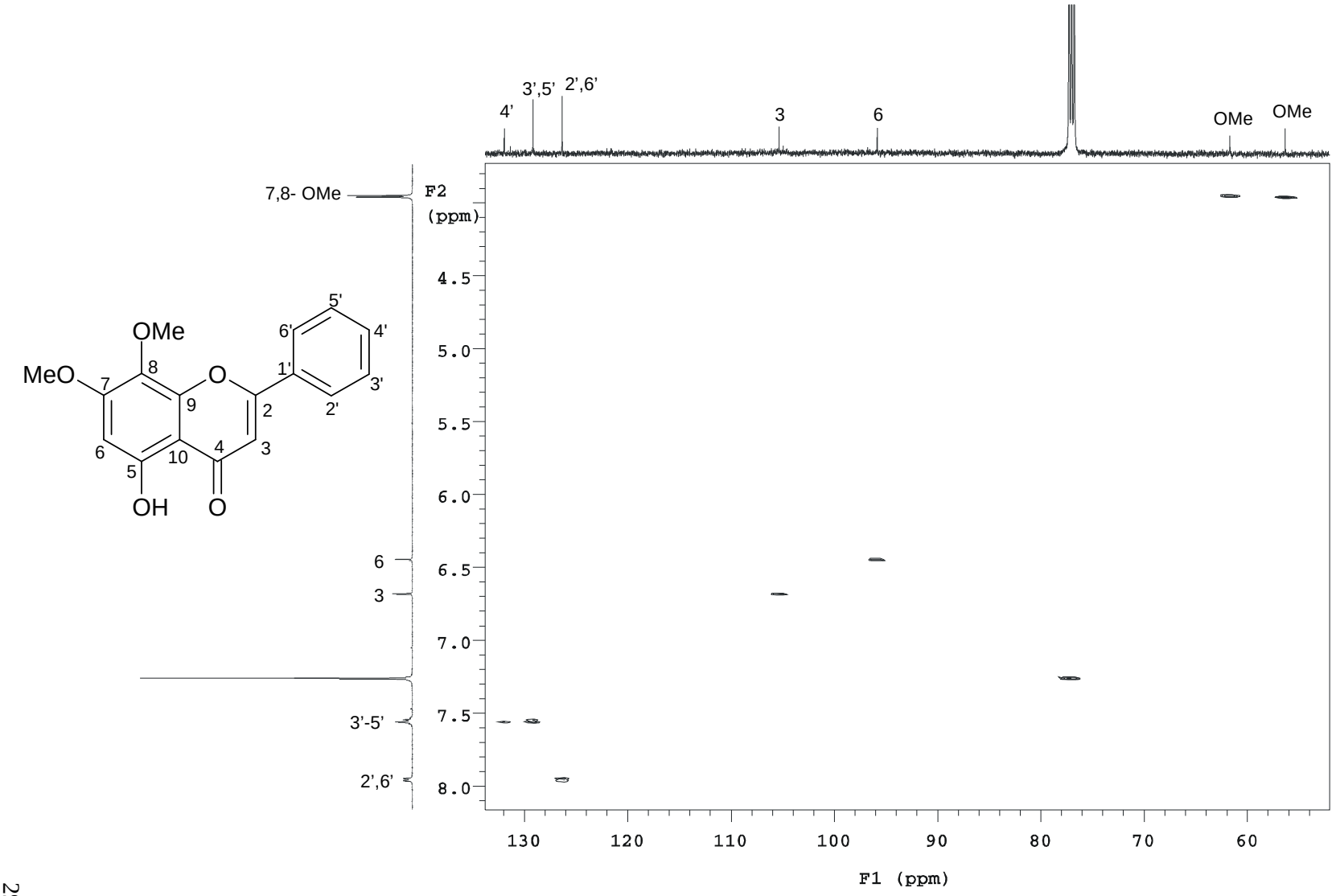


Plate 86: HMQC NMR spectrum of 5-hydroxy-7,8-dimethoxyflavone (72) in $CDCl_3$

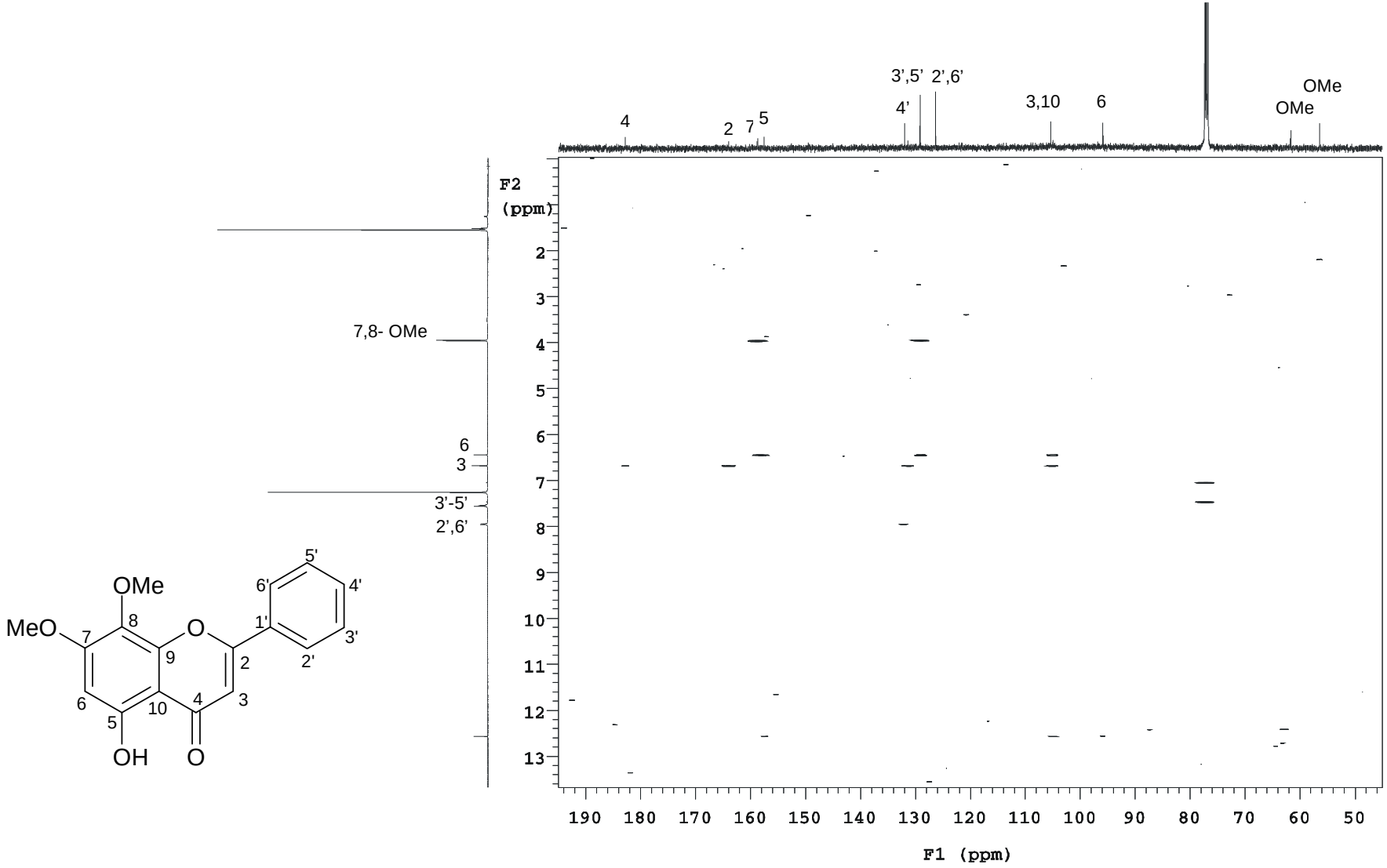


Plate 87: NOESY NMR spectrum of 5-hydroxy-7,8-dimethoxyflavone (72) in $CDCl_3$

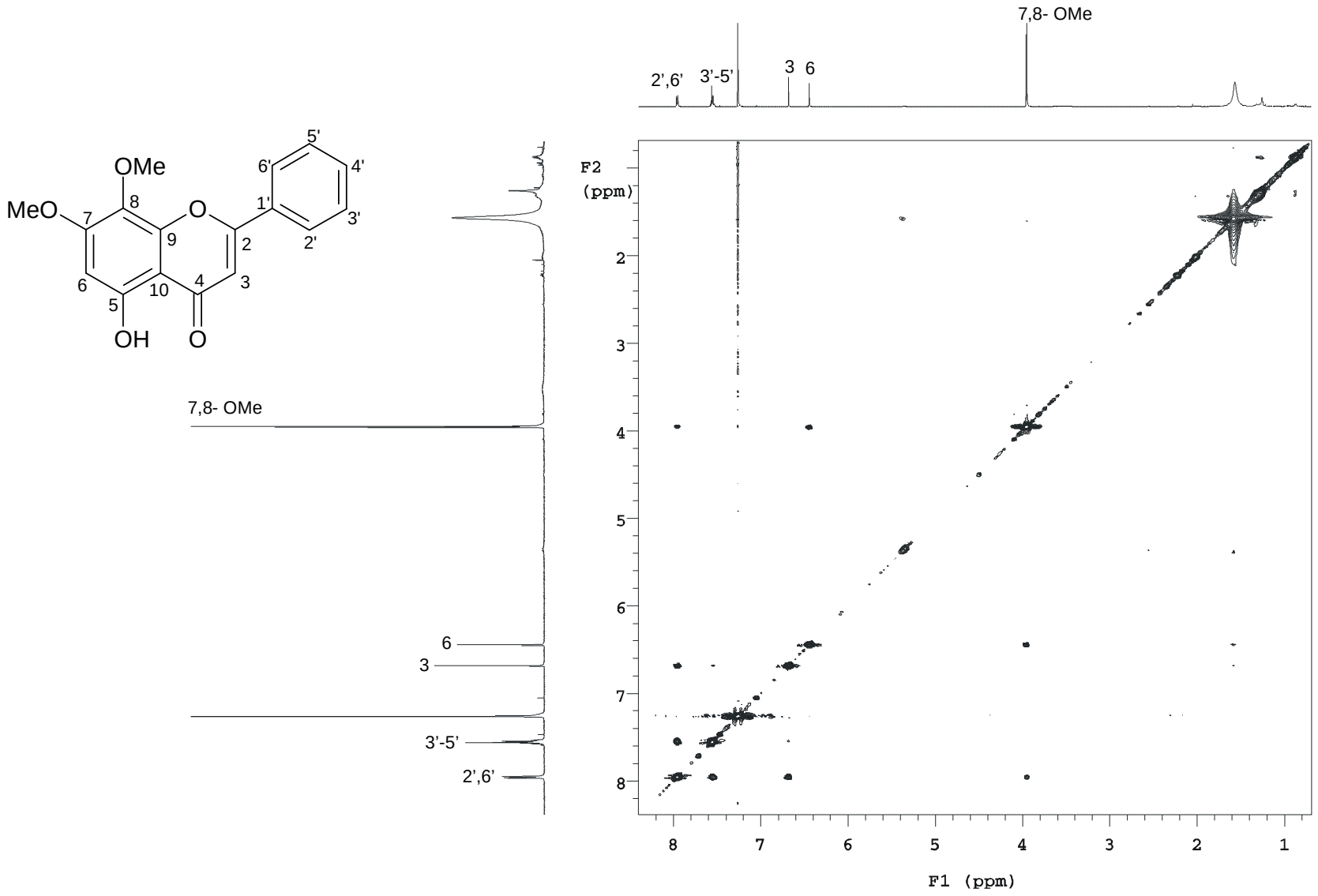


Plate 88: ^{1}H NMR spectrum of isoscutellarein 7-O-β-glucoside (80) in CD_3OD

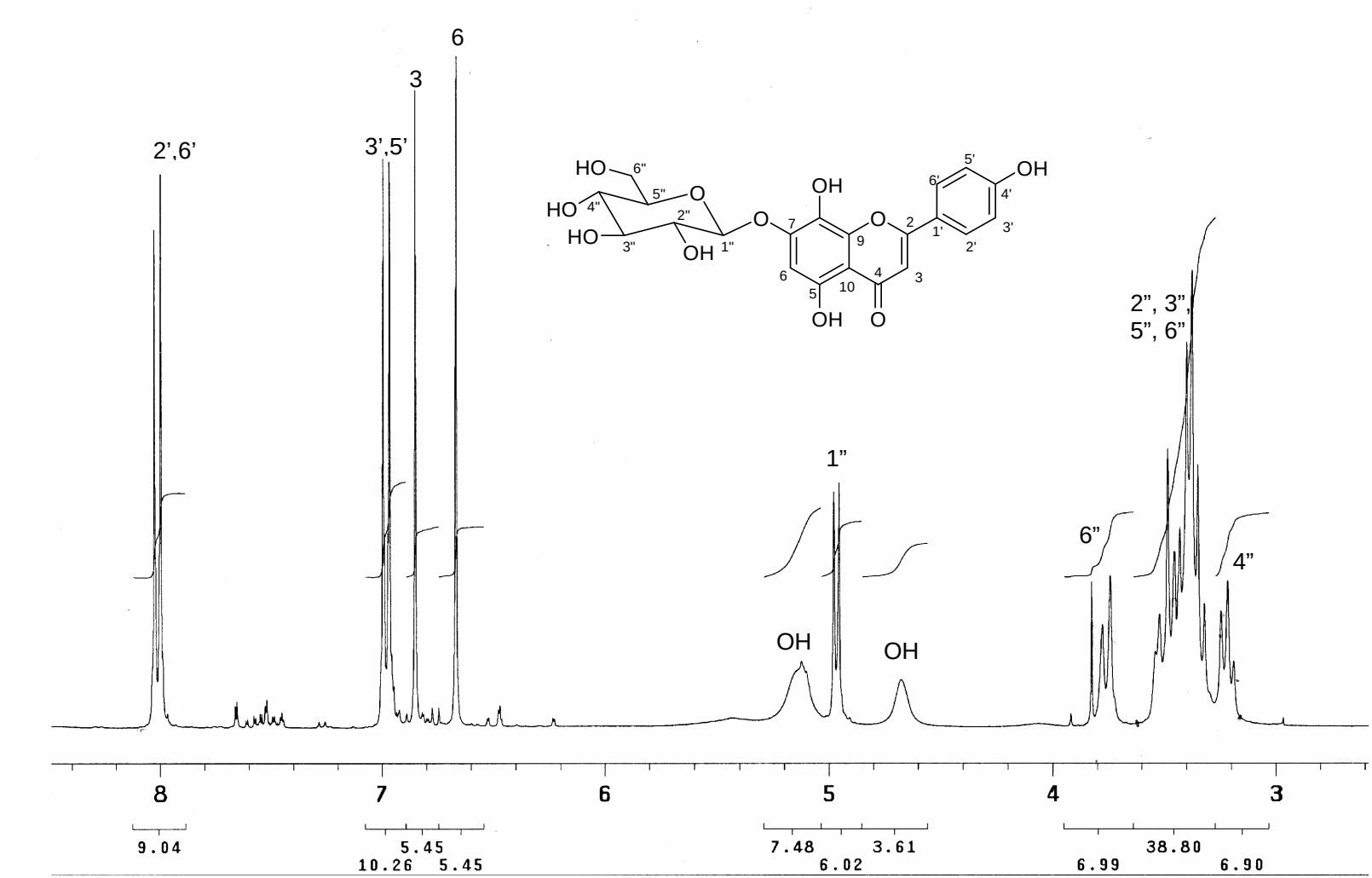


Plate 89: ^{13}C NMR spectrum of isoscutellarein 7-O-β-glucoside (80) in CD_3OD

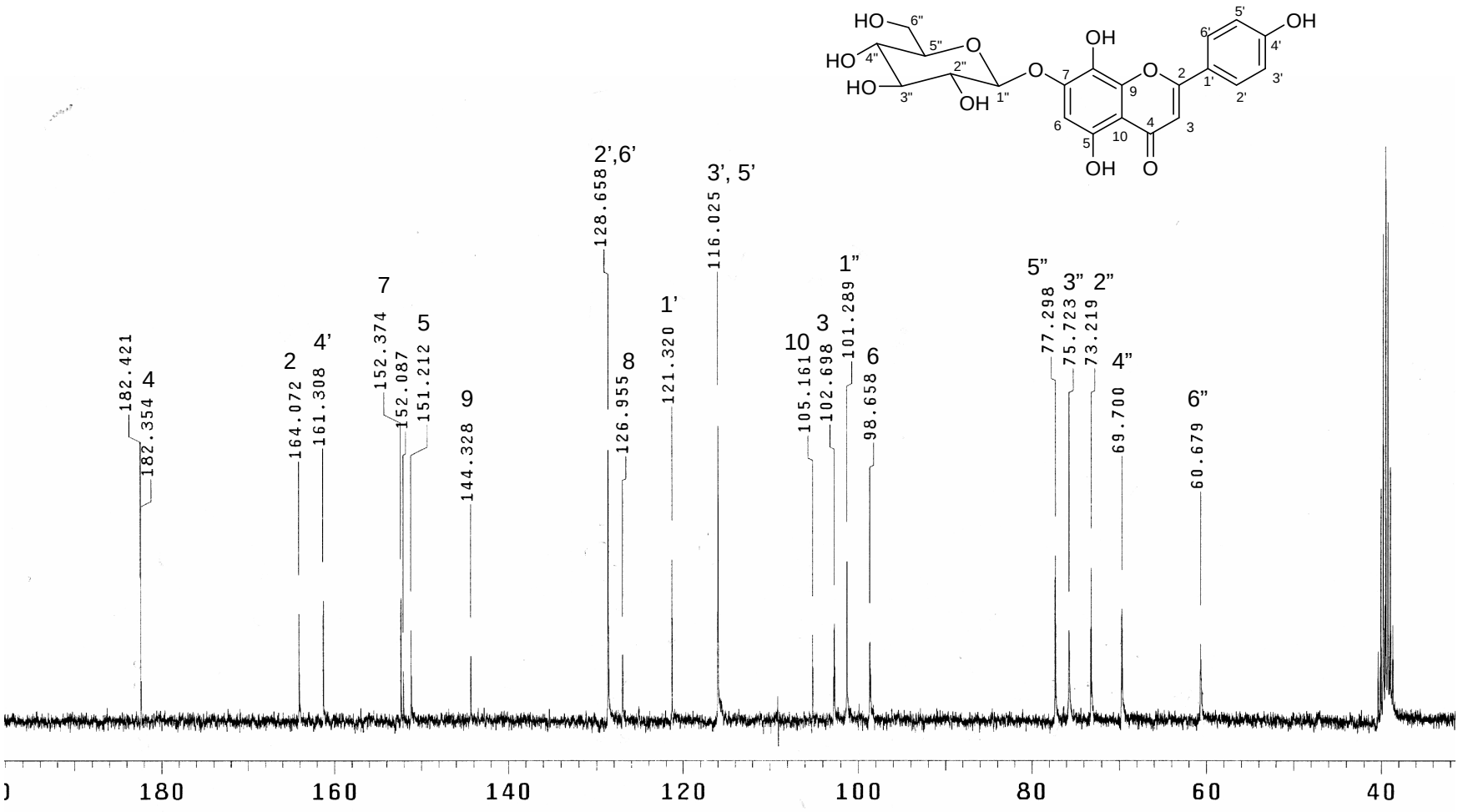


Plate 90: COSY NMR spectrum of isoscutellarein 7-O-glucoside (80) in CD_3OD

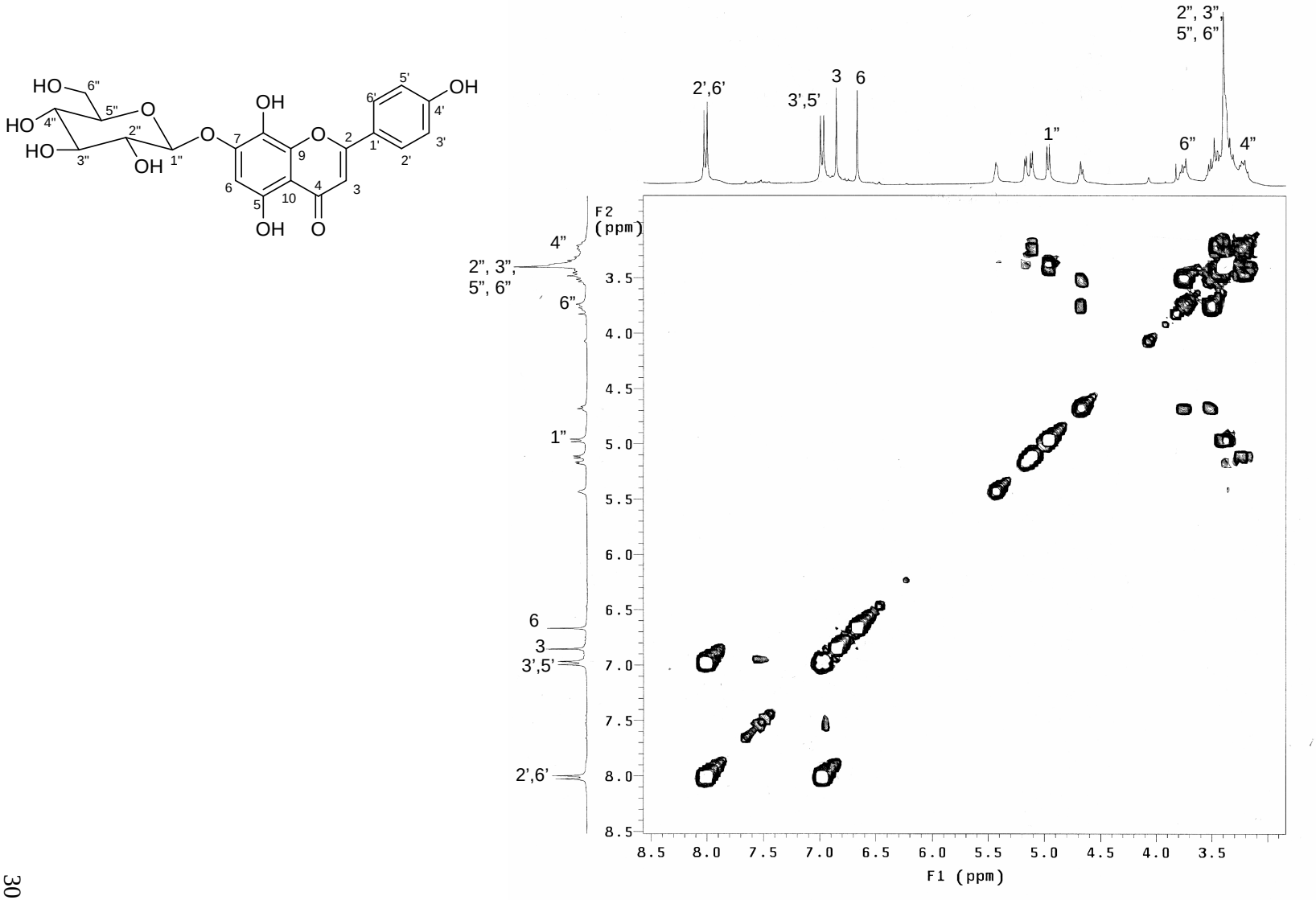


Plate 91: DEPT NMR spectrum of isoscutellarein 7-O-glucoside (80) in CD_3OD

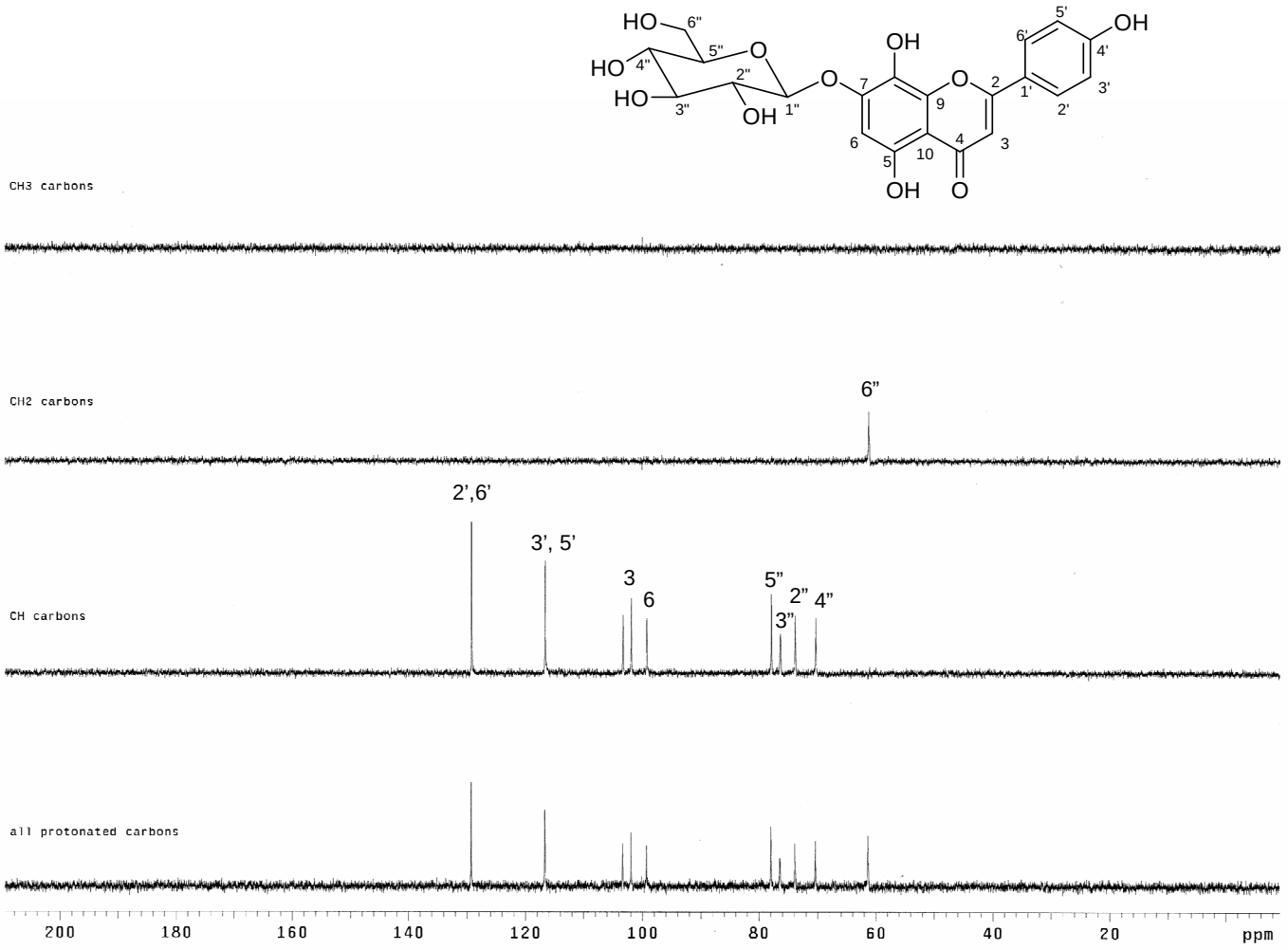


Plate 92: HSQC NMR spectrum of isoscutellarein 7-O-glucoside (80) in CD_3OD

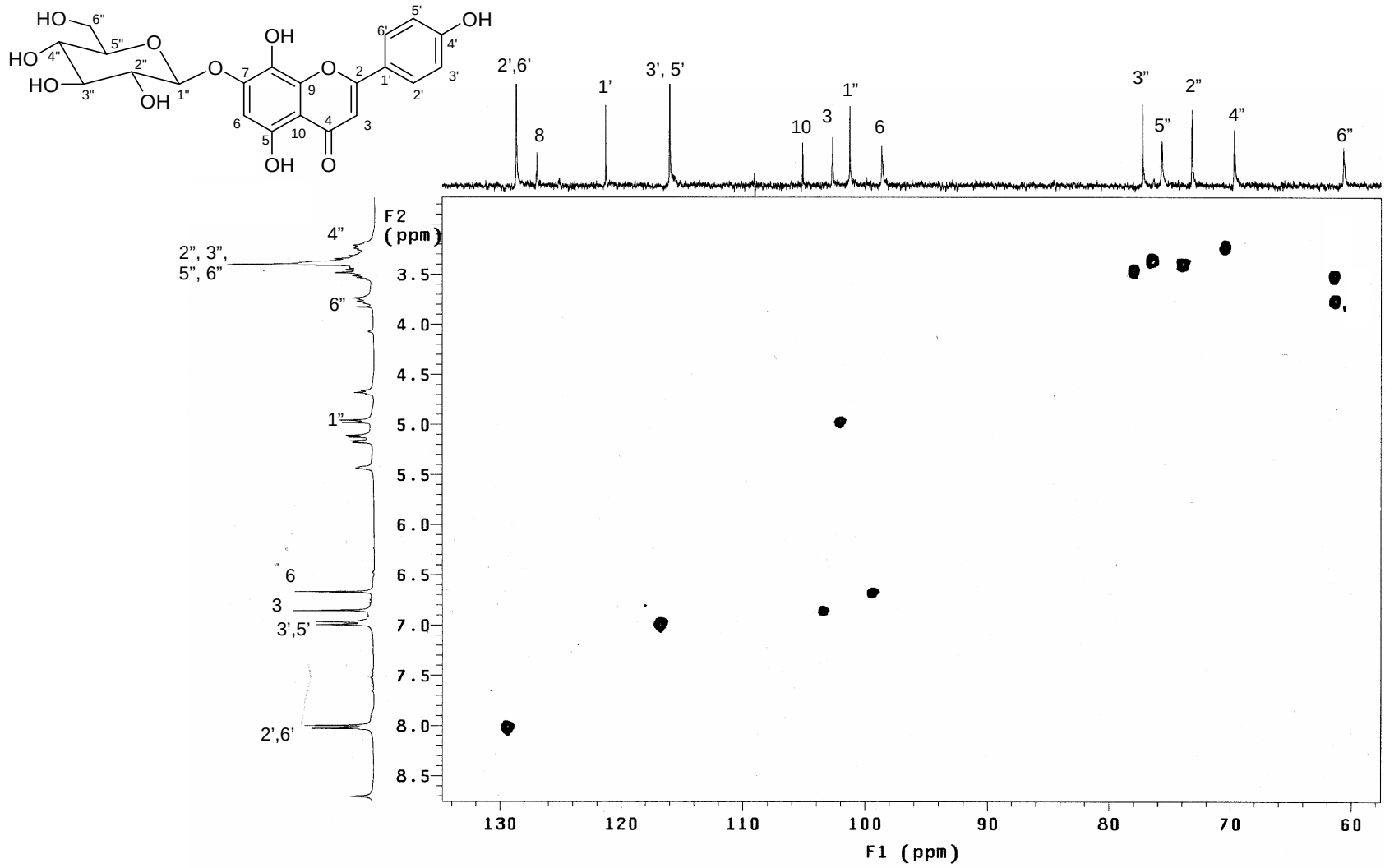


Plate 93: HMQC NMR spectrum of isoscutellarein 7-O-glucoside (80) in CD_3OD

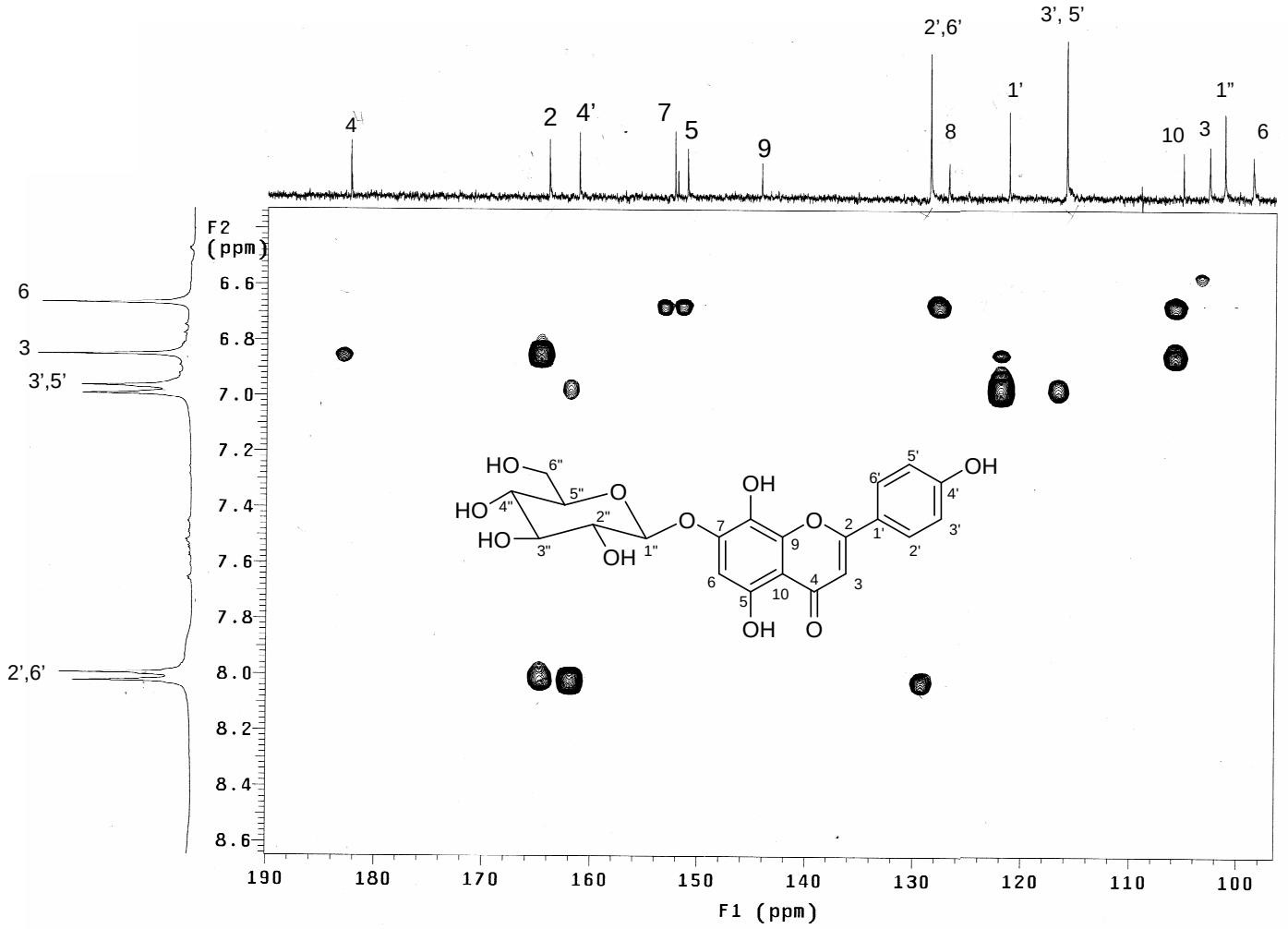


Plate 94: NOESY NMR spectrum of isoscutellarein 7-O-glucoside (80) in CD_3OD

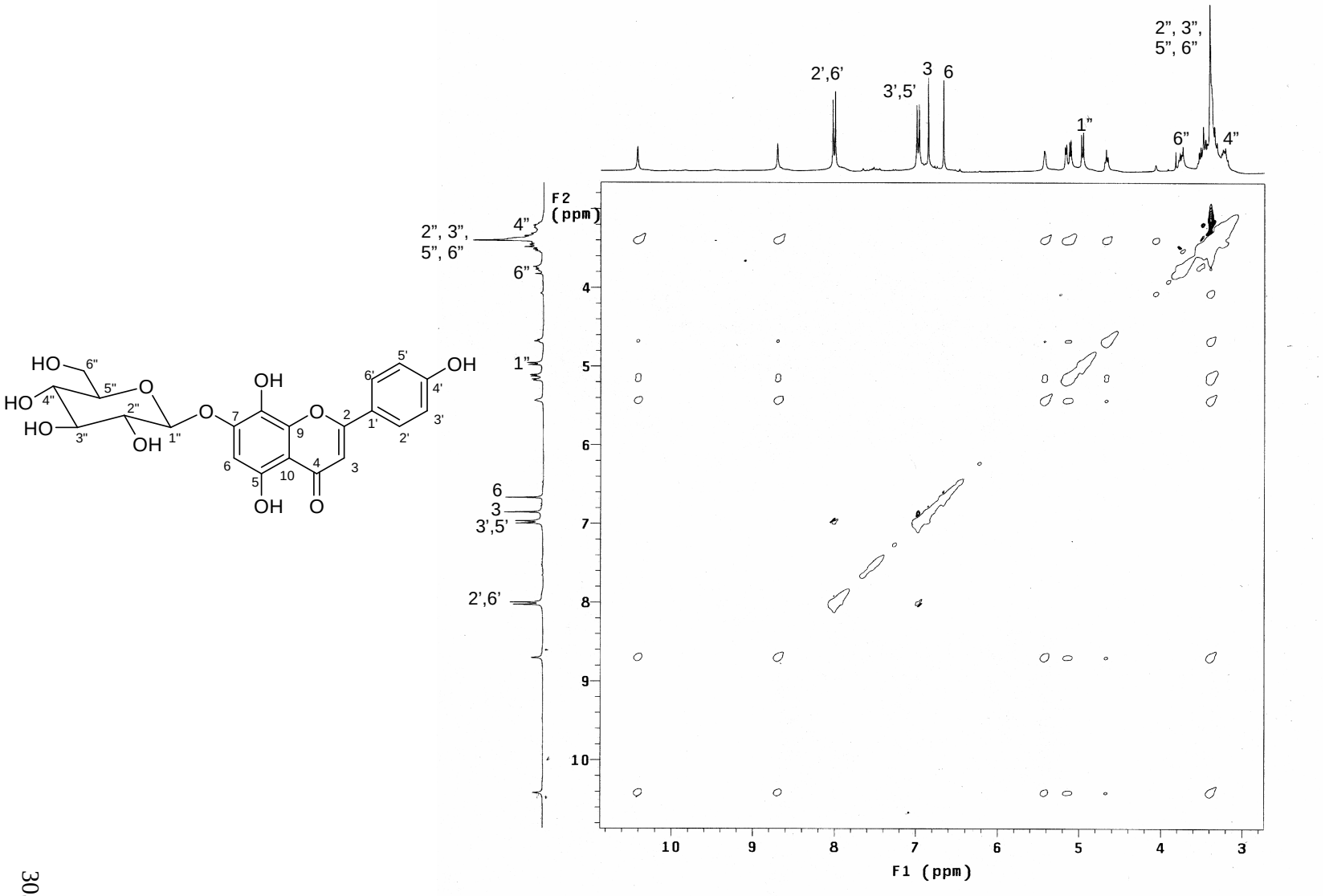


Plate 95: ^{1}H NMR spectrum of β-keto ester 384 in $CDCl_3$

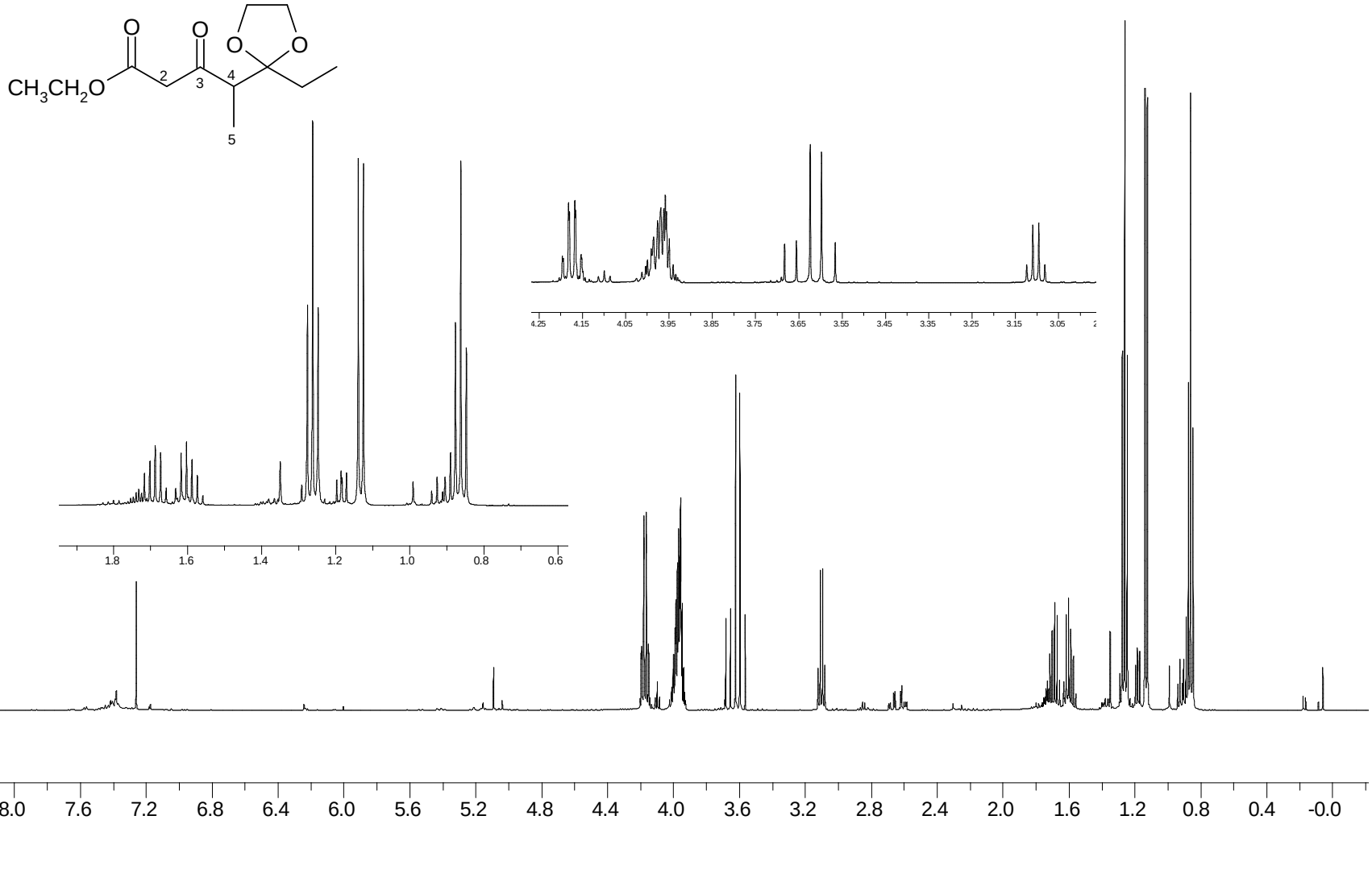


Plate 96: ^{13}C NMR spectrum of β-keto ester 384 in $CDCl_3$

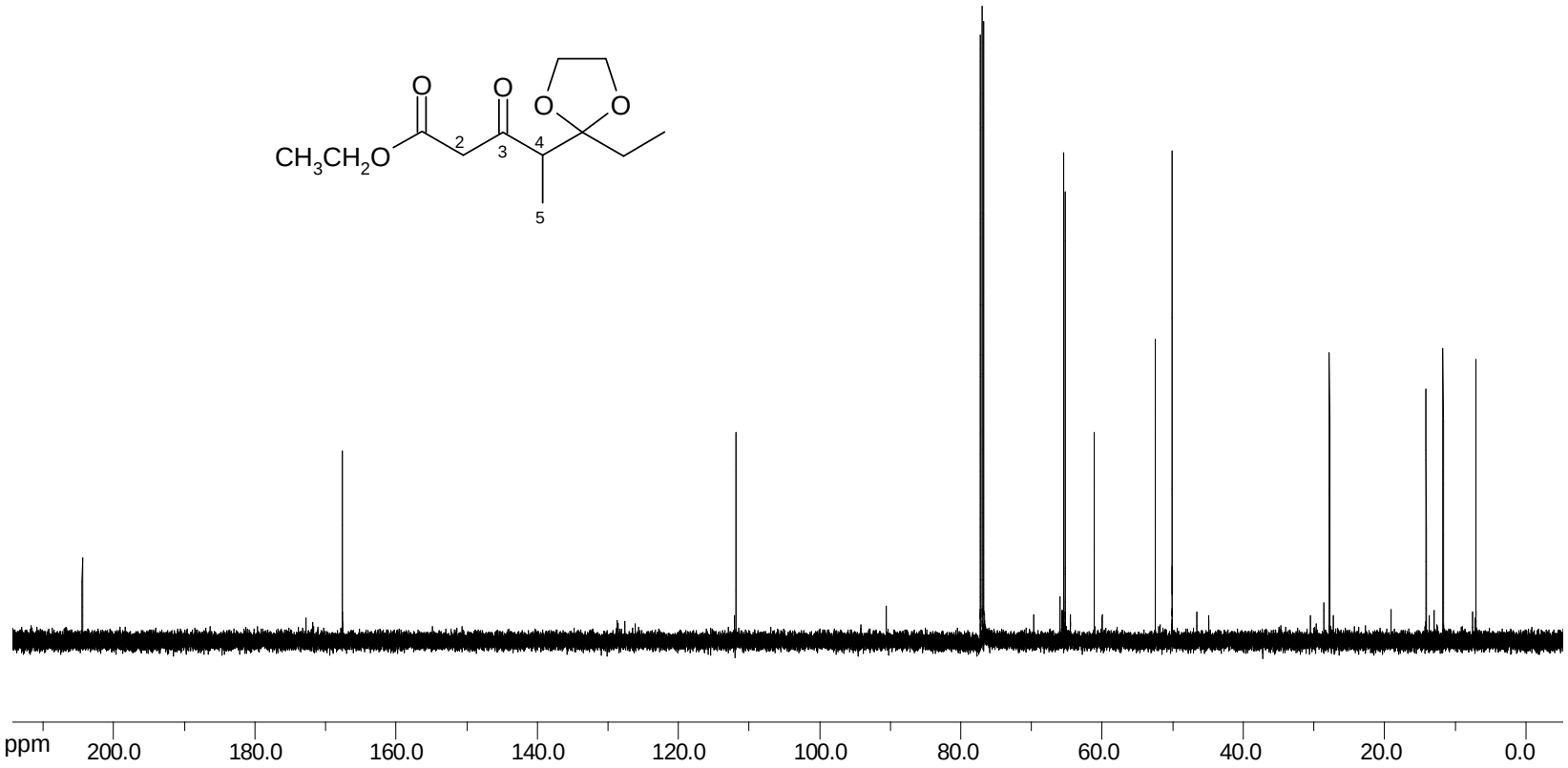


Plate 97: ^{1}H NMR spectrum of chalcone 392 in $CDCl_3$

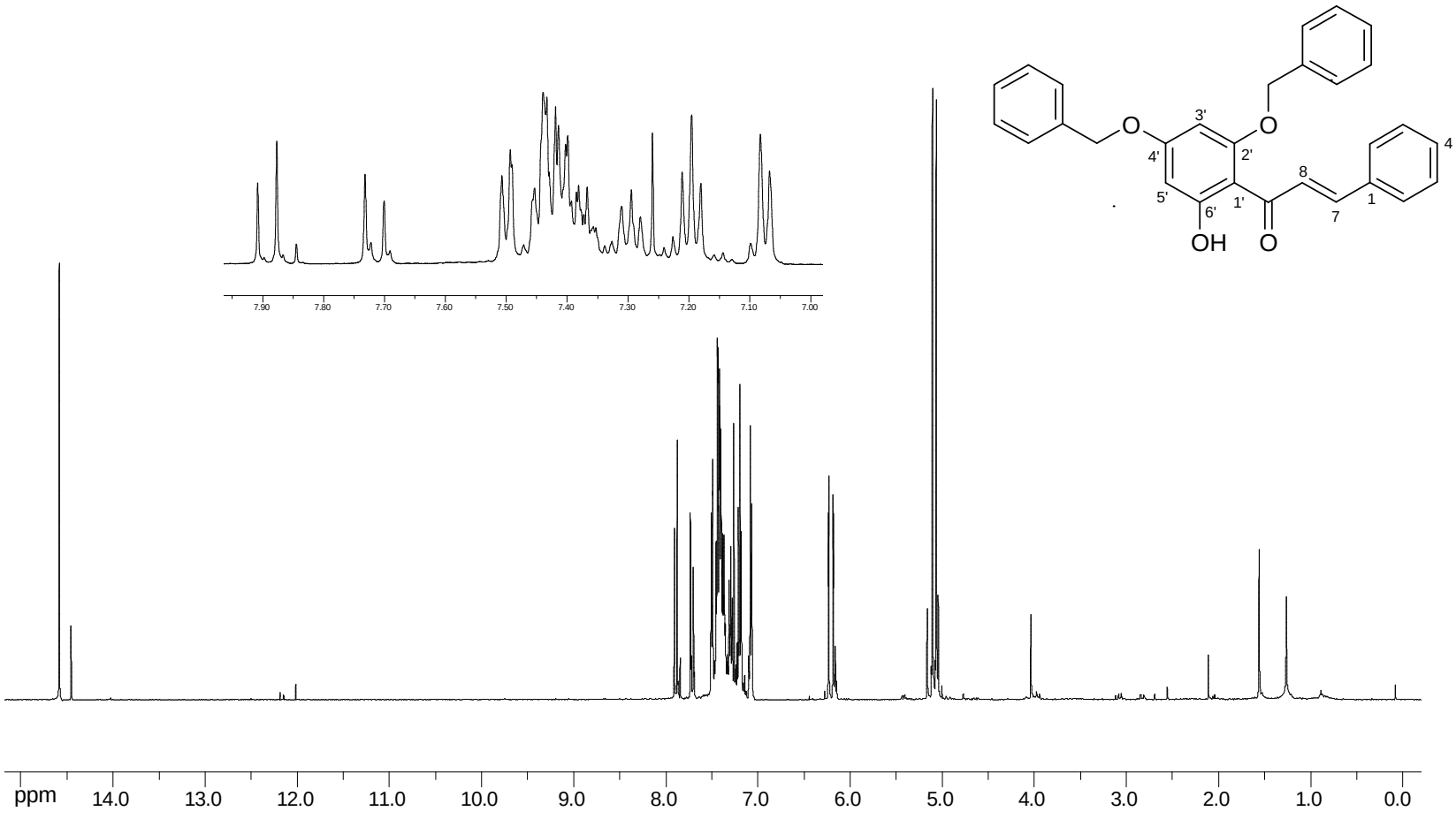


Plate 98: ^{13}C NMR spectrum of chalcone 392 in $CDCl_3$

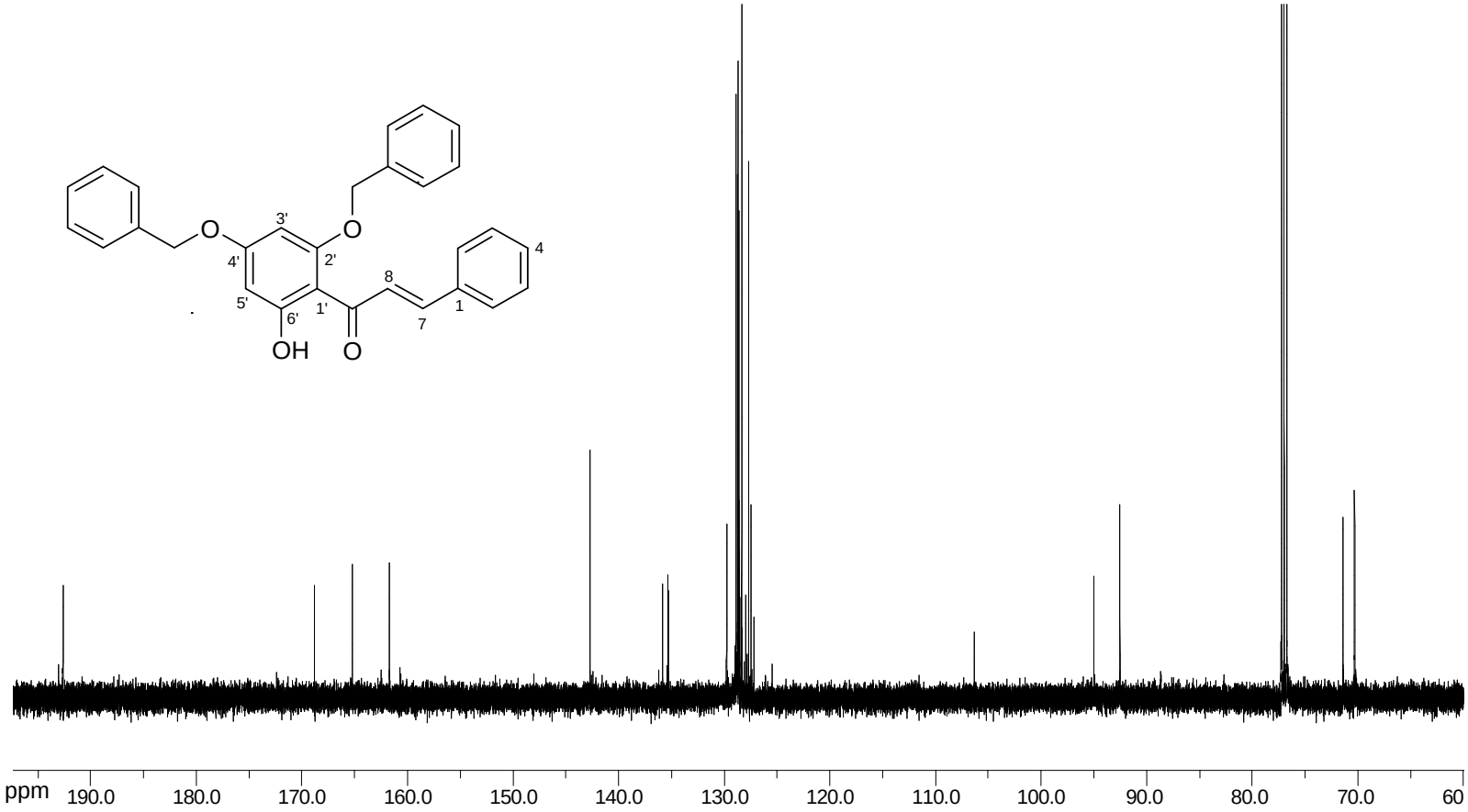


Plate 99: ^{1}H NMR spectrum of flavanone 395 in $CDCl_3$

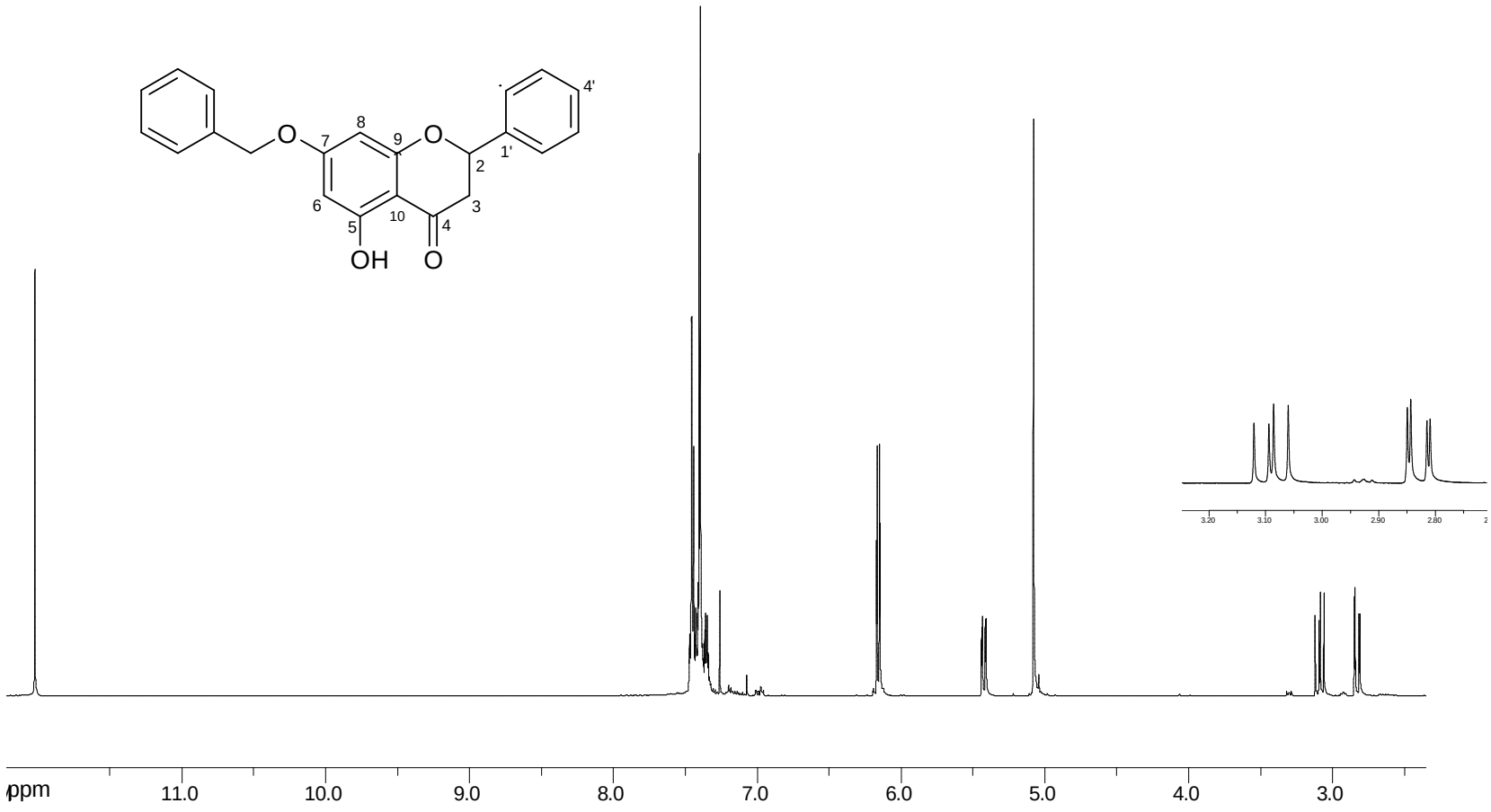


Plate 100: ^{13}C NMR spectrum of flavanone 395 in $CDCl_3$

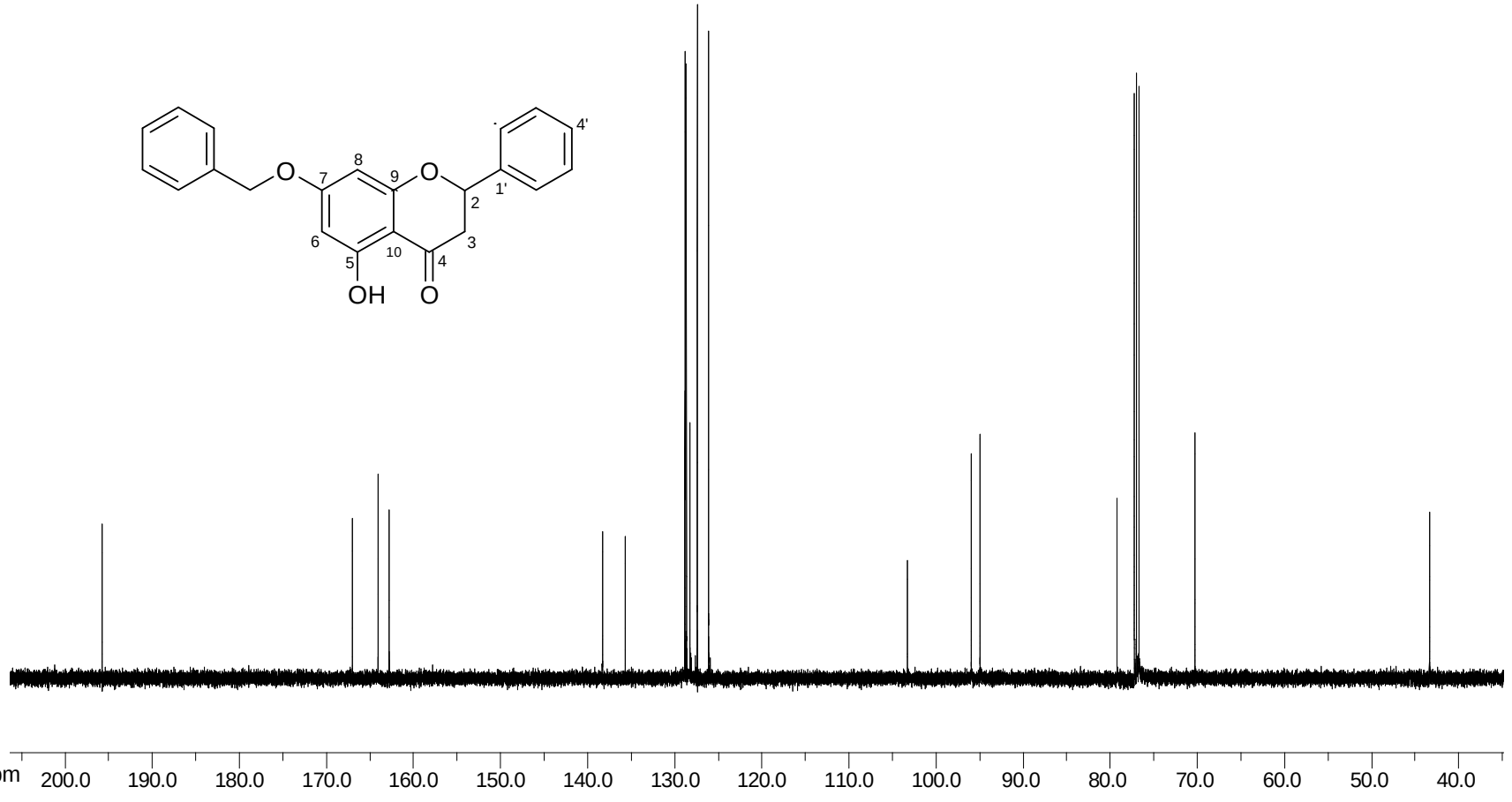


Plate 101: NOESY NMR spectrum of flavanone 398 in $CDCl_3$

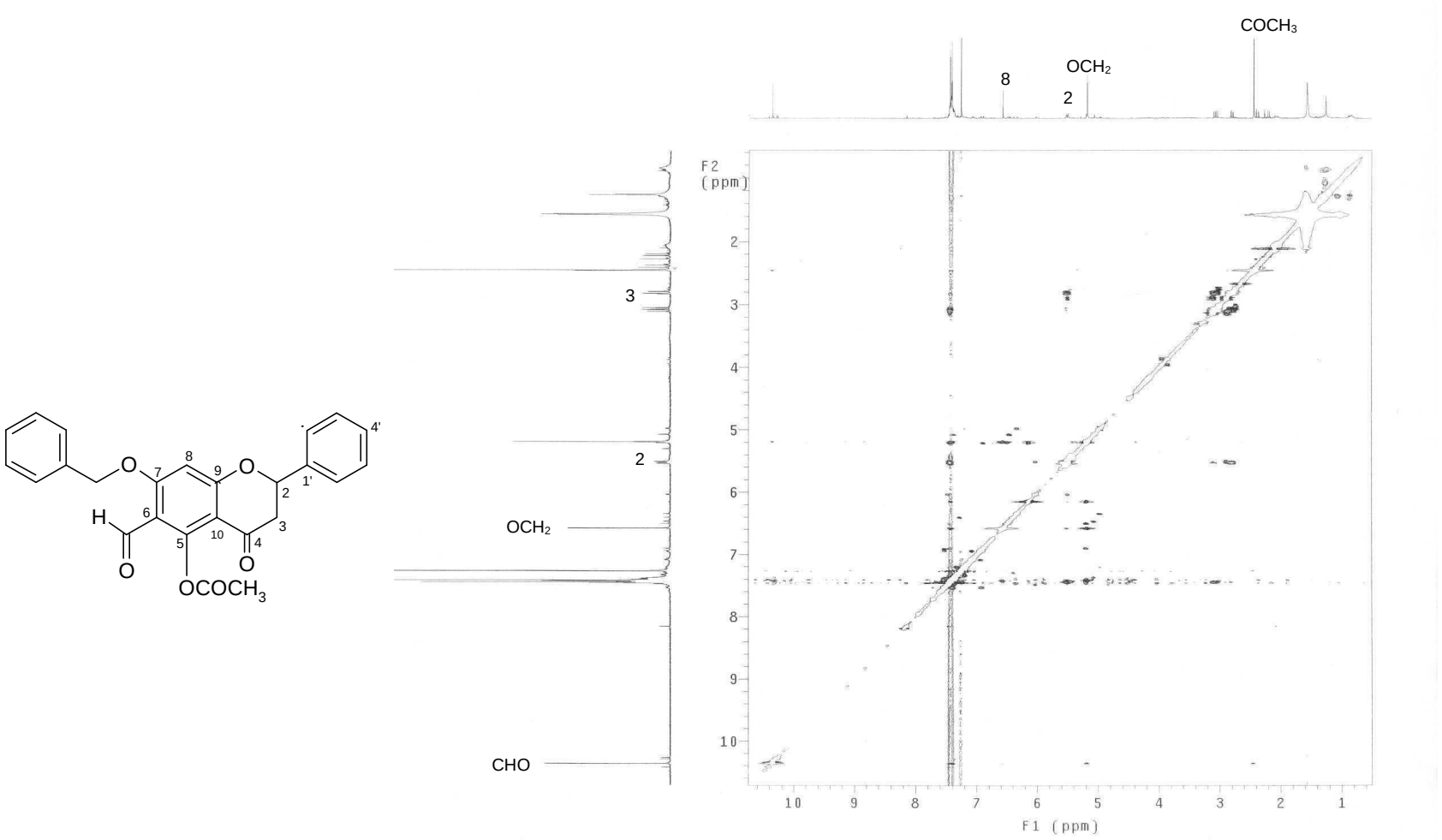


Plate 102: NOESY NMR spectrum of flavanone 399 in $CDCl_3$

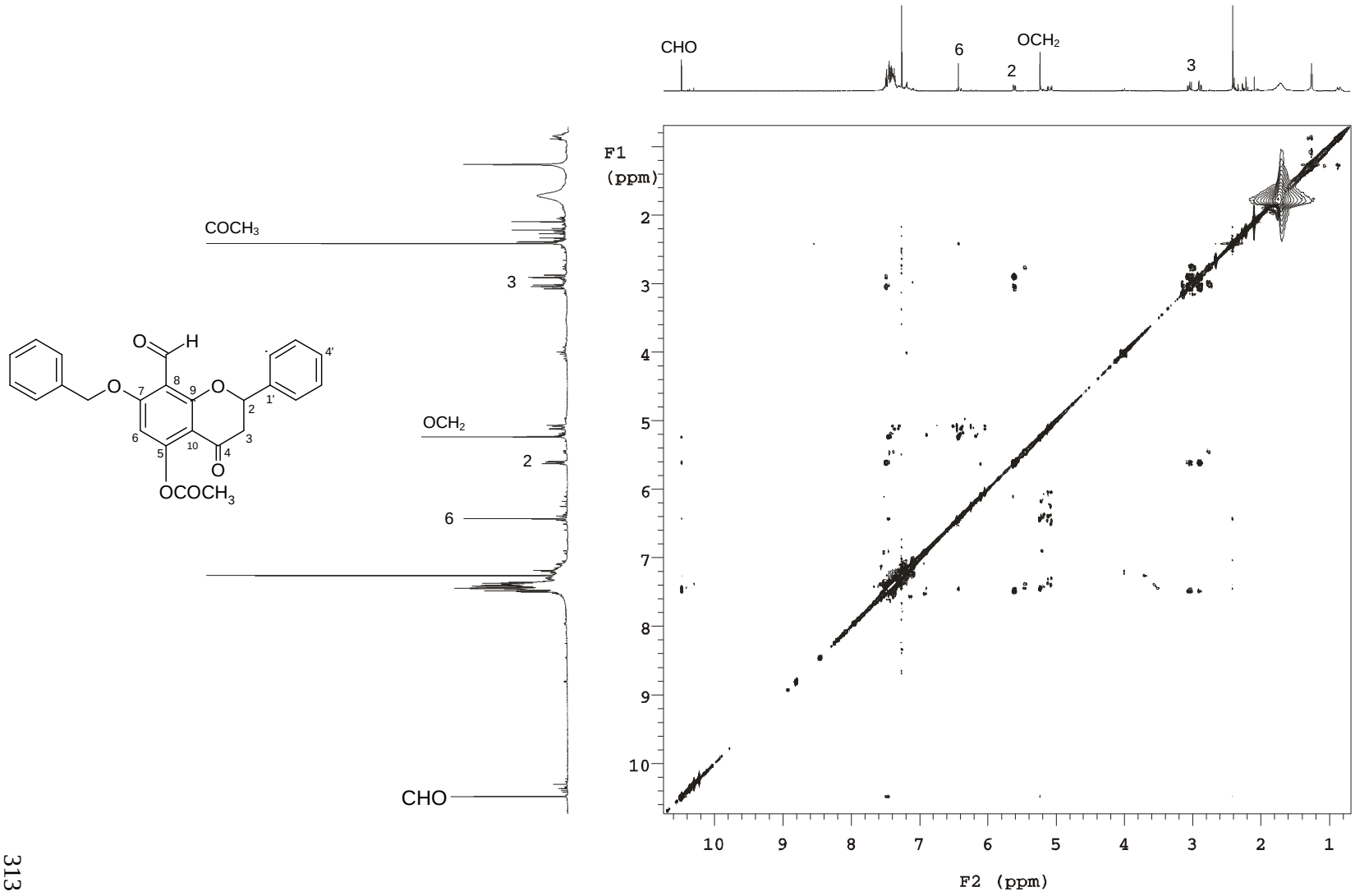


Plate 103: ^{1}H NMR spectrum of pyrone 401

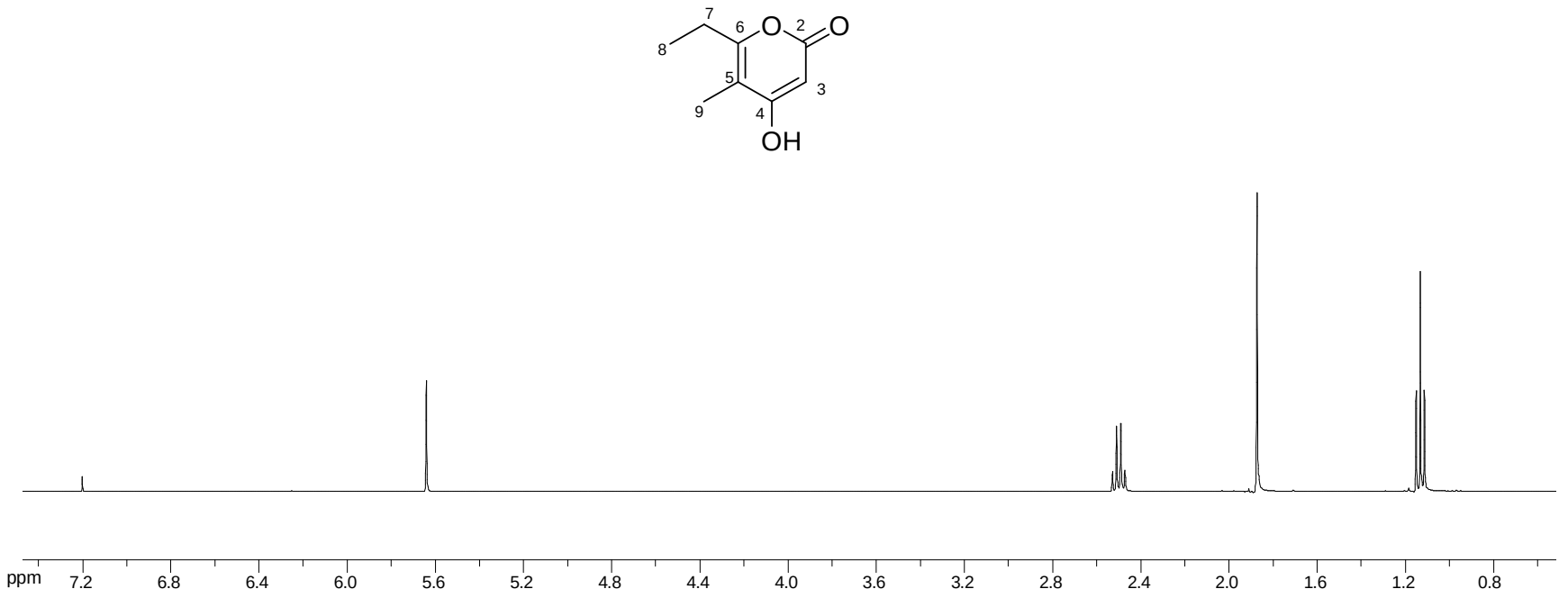


Plate 104: ^{13}C NMR spectrum of pyrone 401

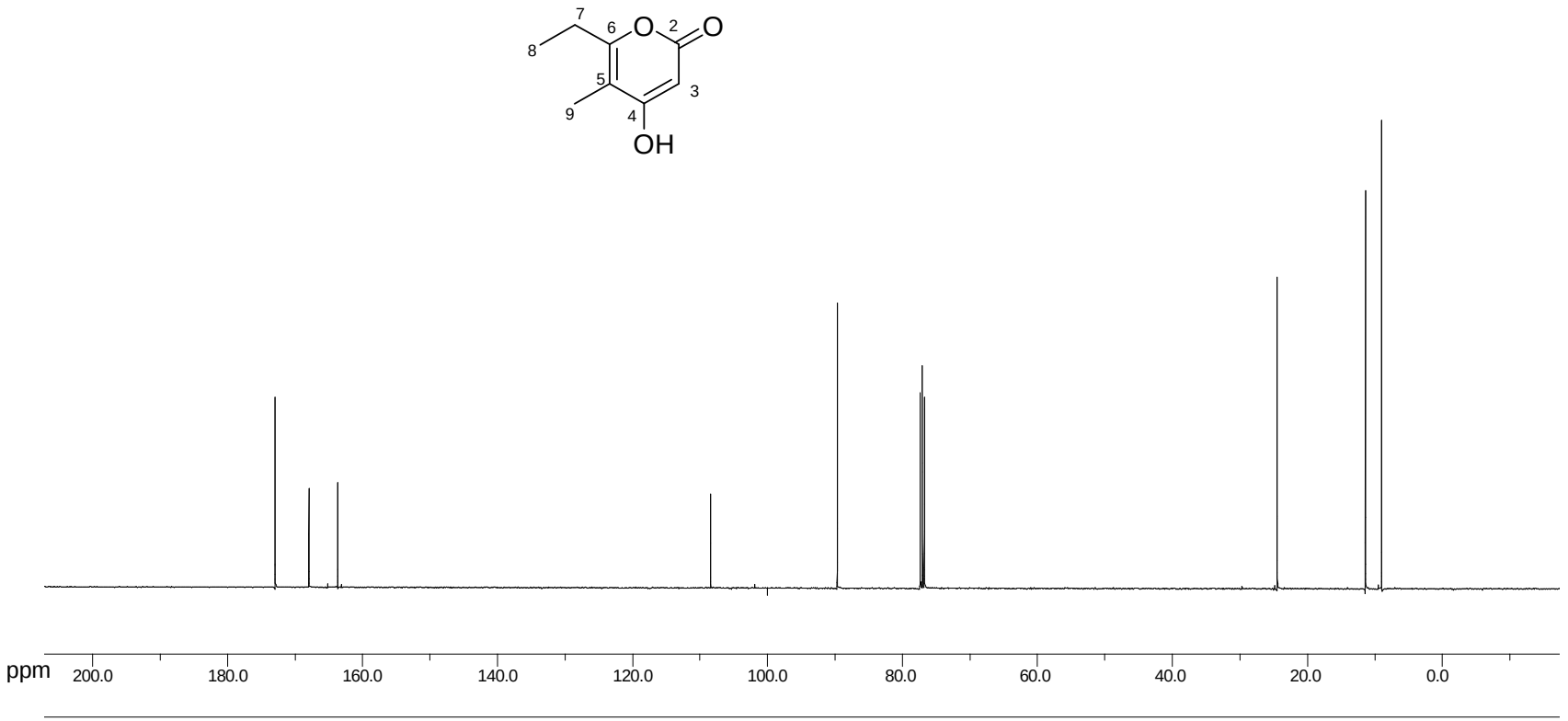


Plate 105: ^{1}H NMR spectrum of pyrone 375

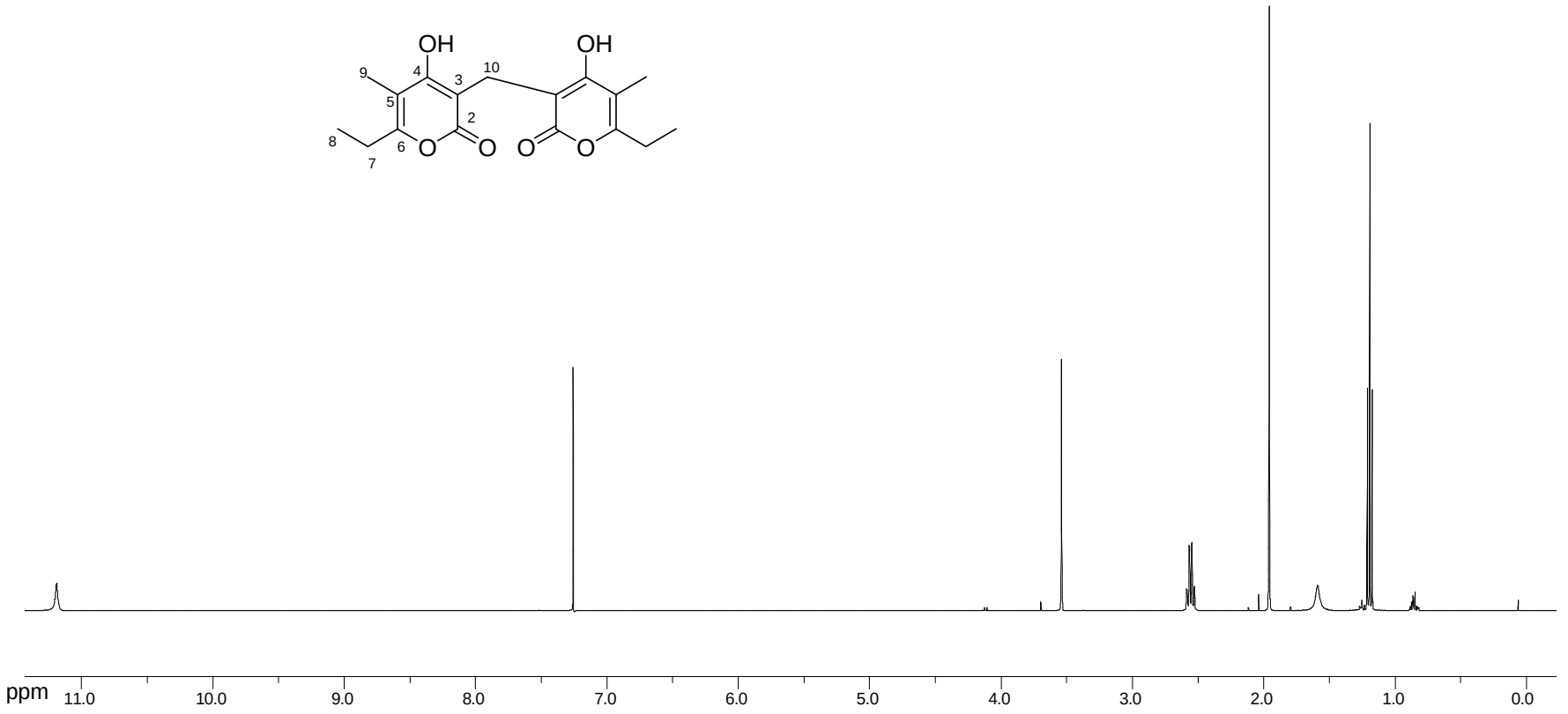


Plate 106: ^{13}C NMR spectrum of pyrone 375

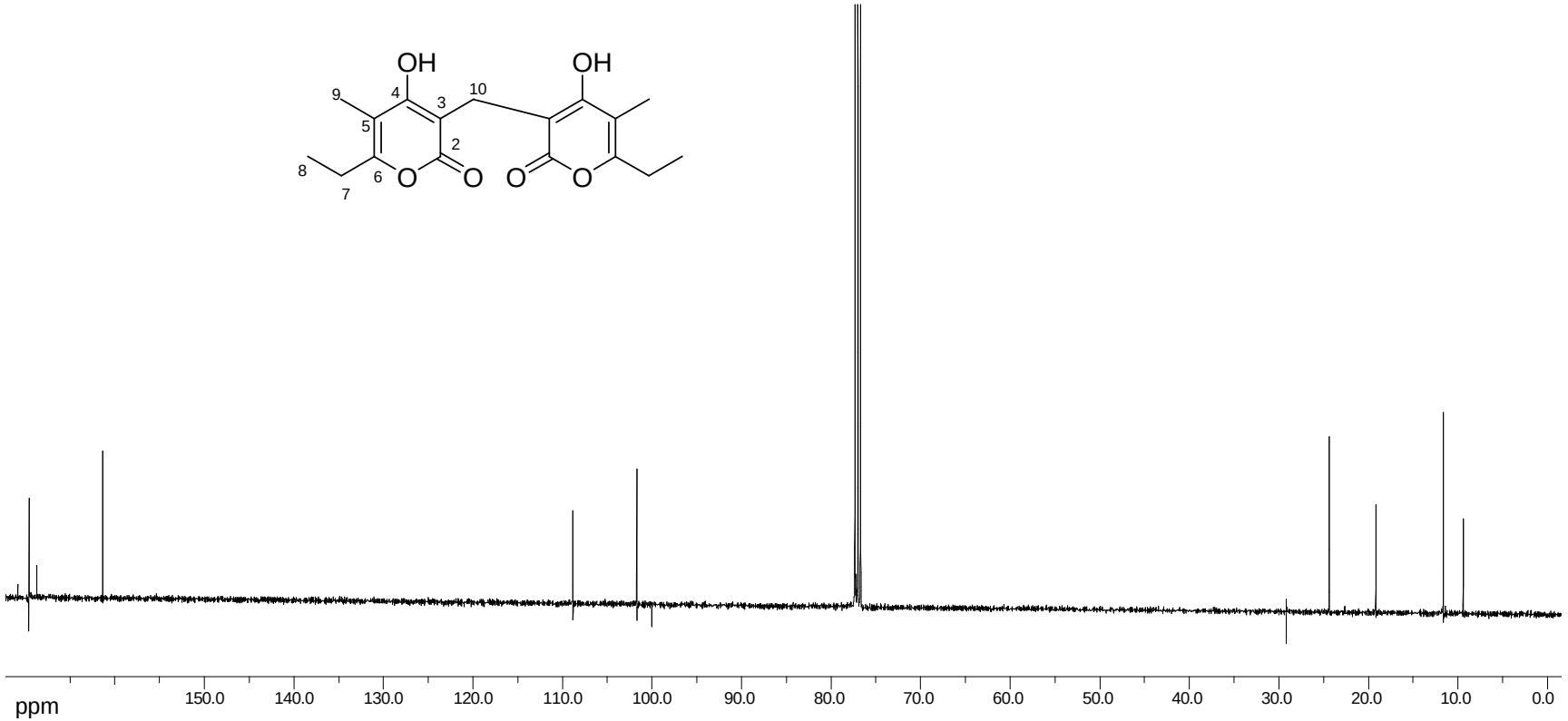


Plate 107: ^{1}H NMR spectrum of alkene 407

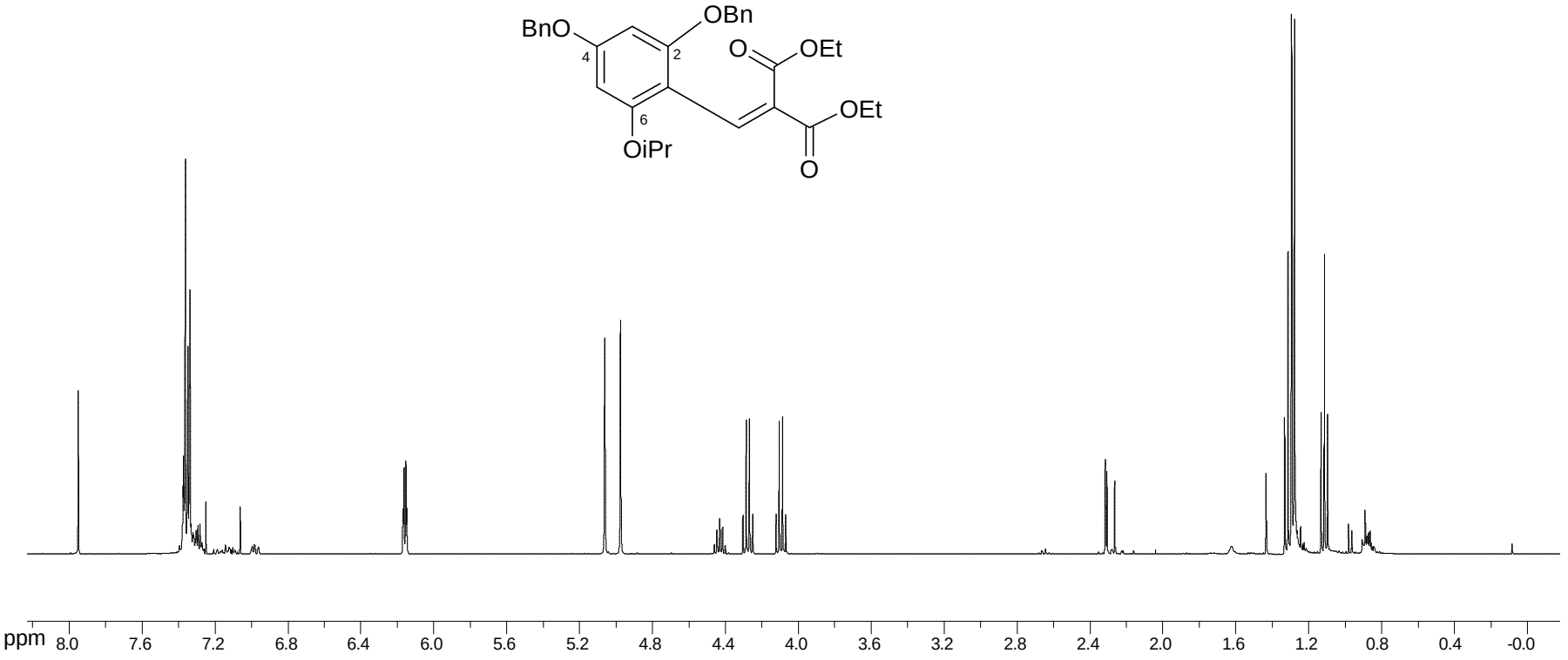


Plate 108: ^{13}C NMR spectrum of alkene 407

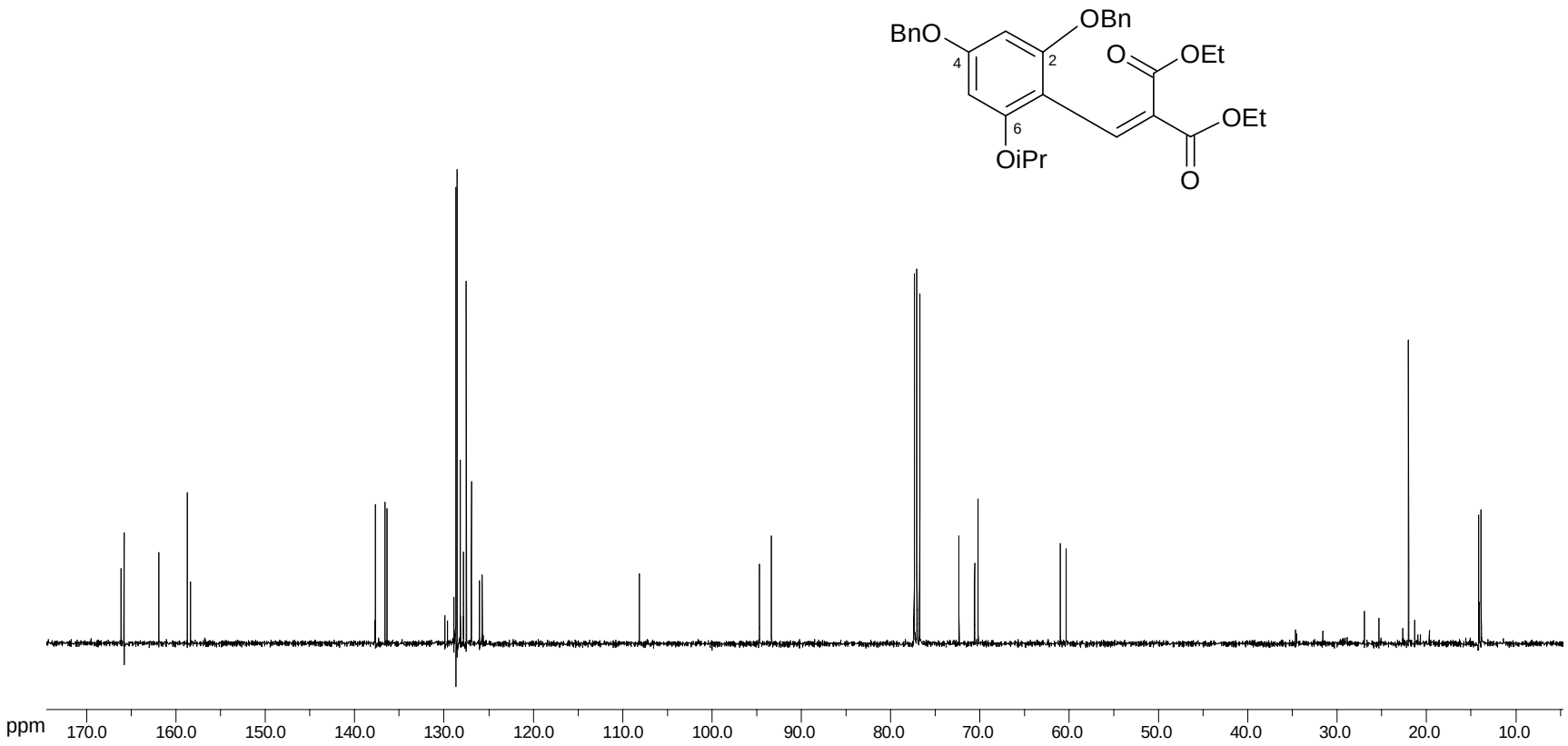


Plate 109: ^{1}H NMR spectrum of alkene 415

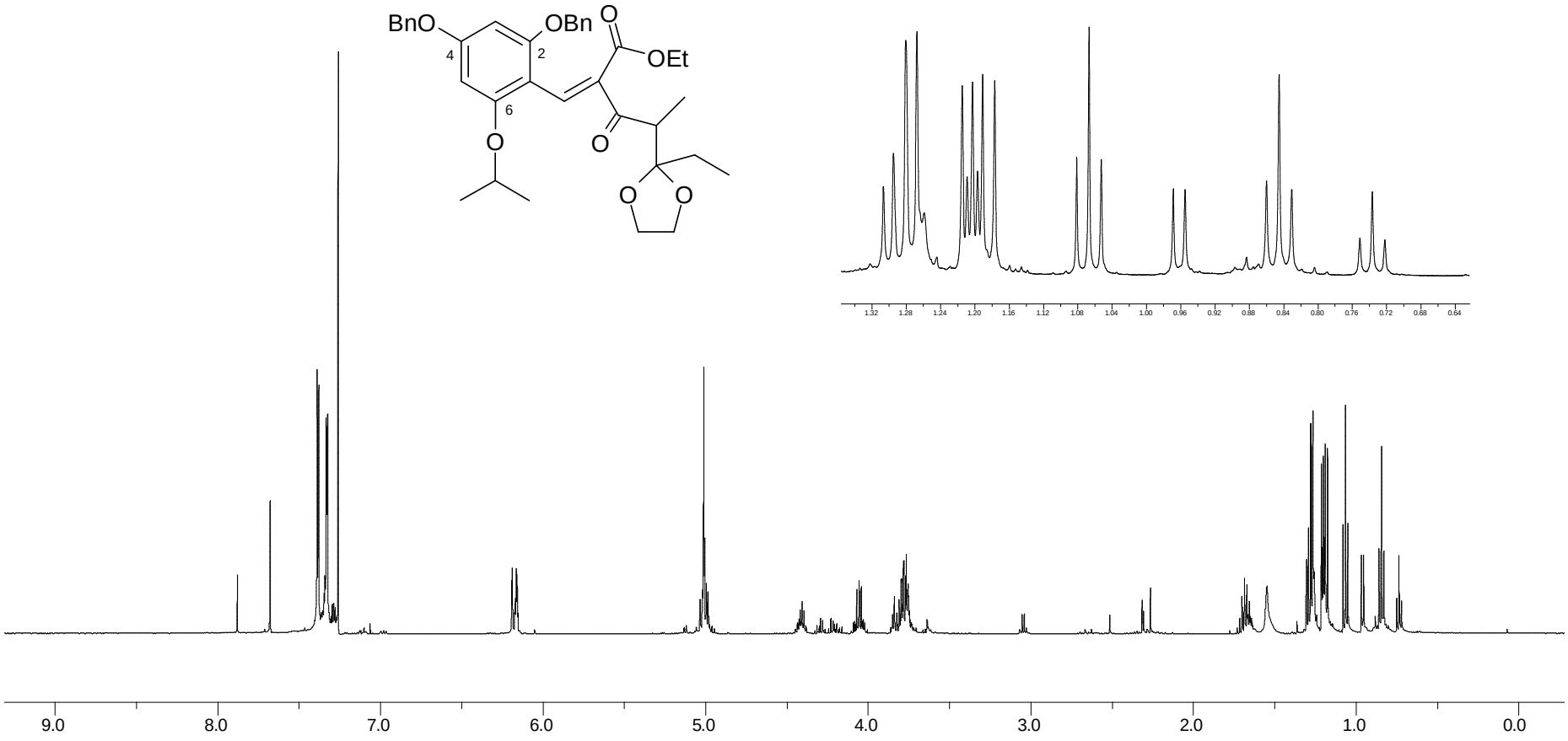


Plate 110: ^{13}C NMR spectrum of alkene 415

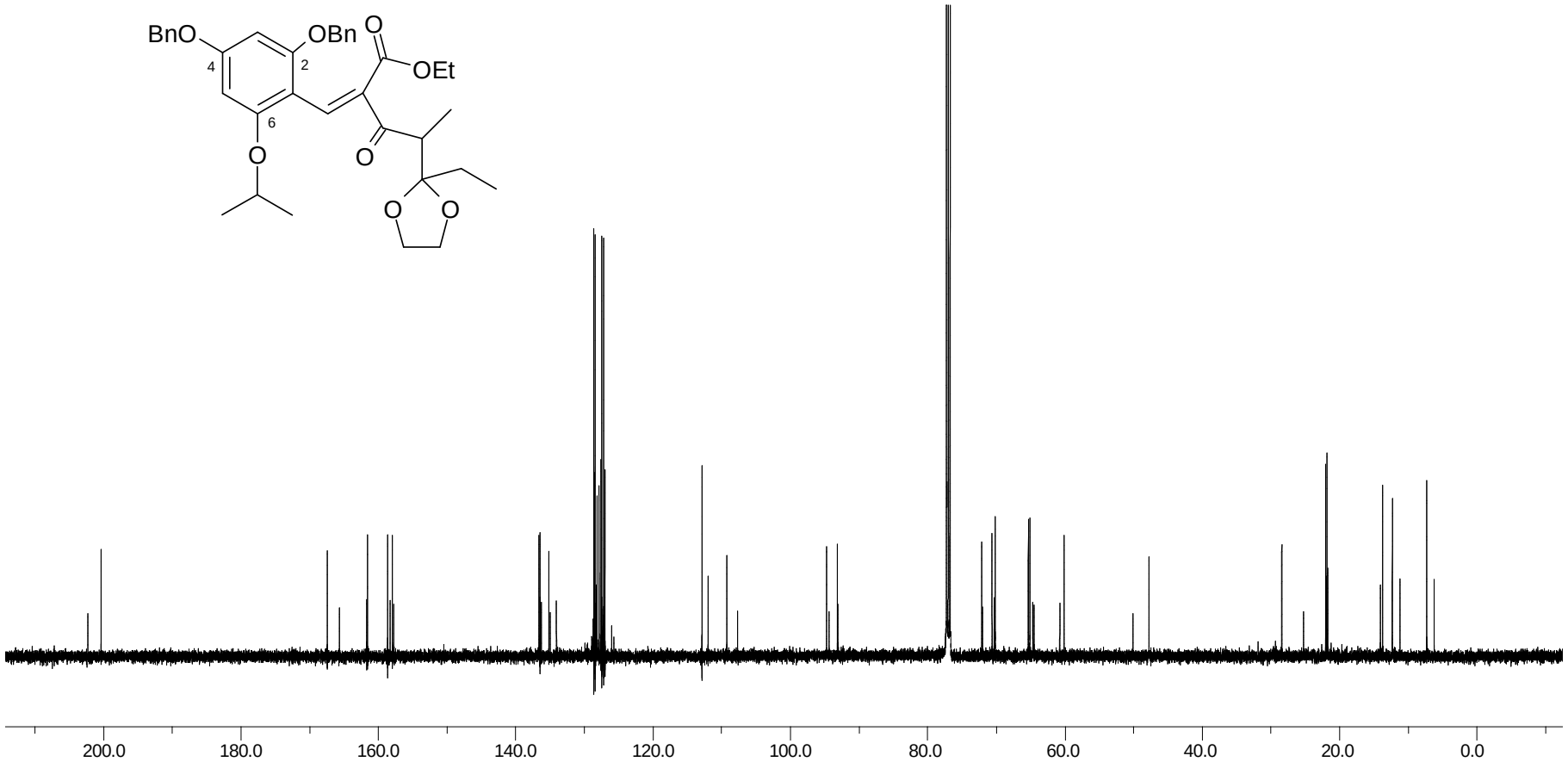


Plate 111: ^{1}H NMR spectrum of alcohol 418

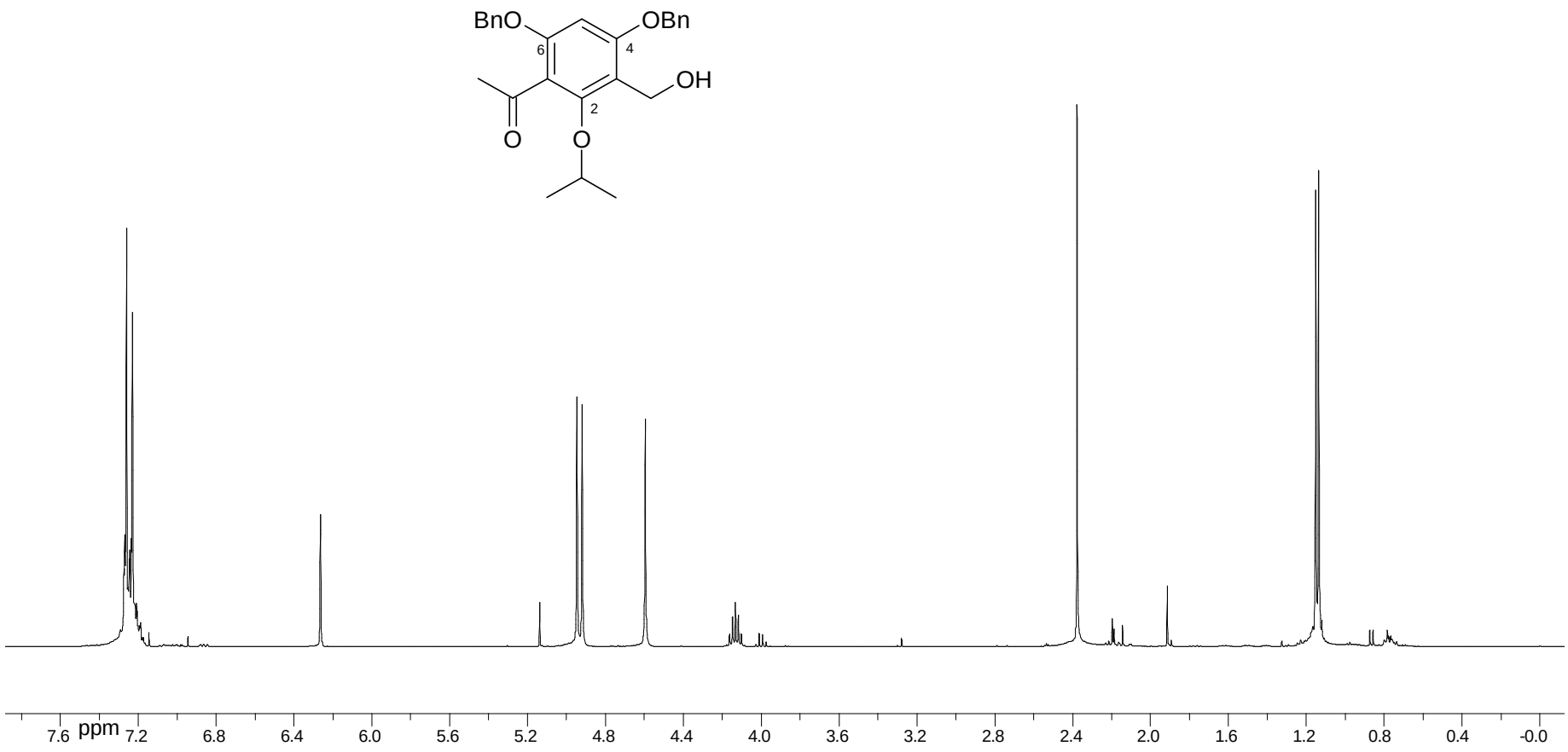


Plate 112: ^{13}C NMR spectrum of alcohol 418

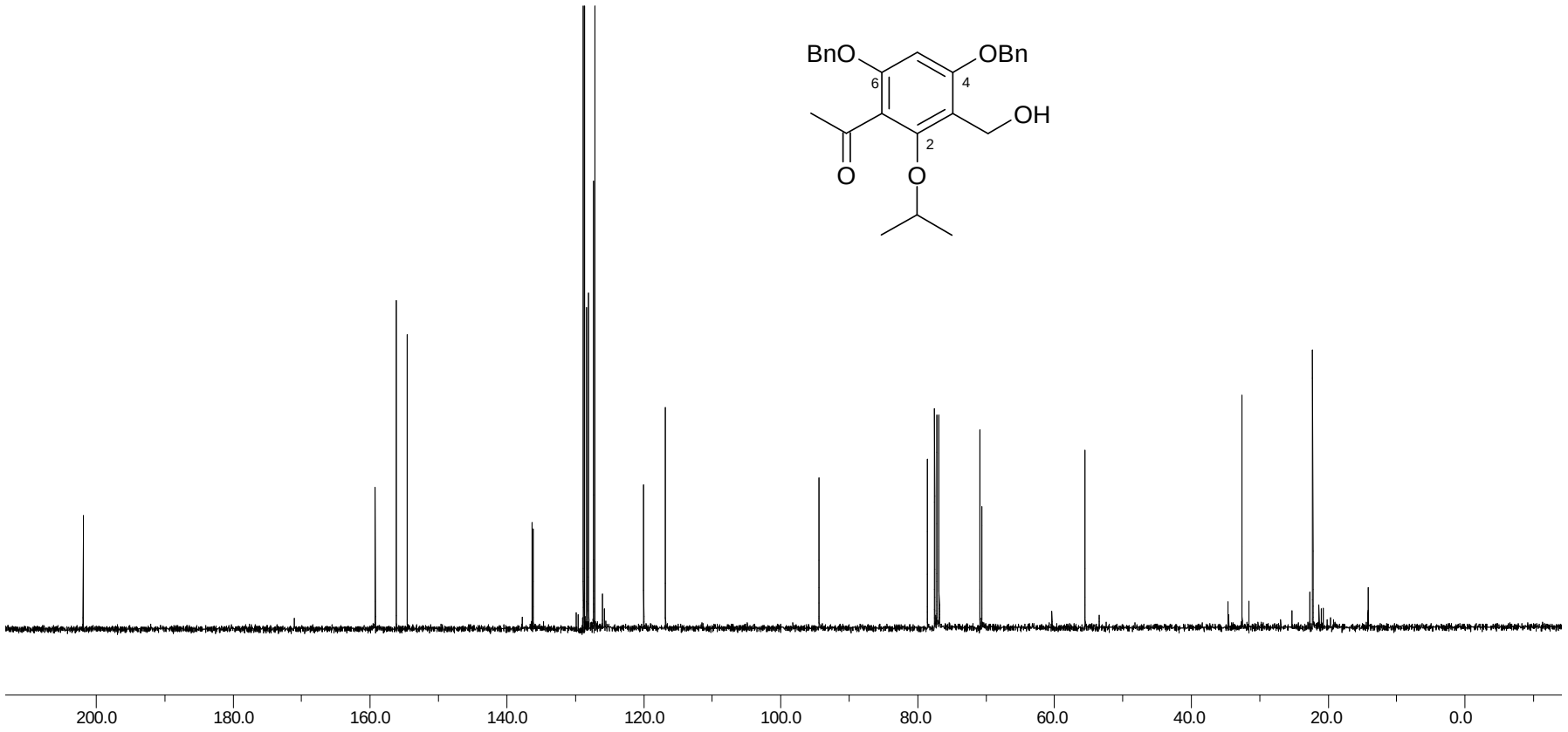


Plate 113: ^{1}H NMR spectrum of compound 421

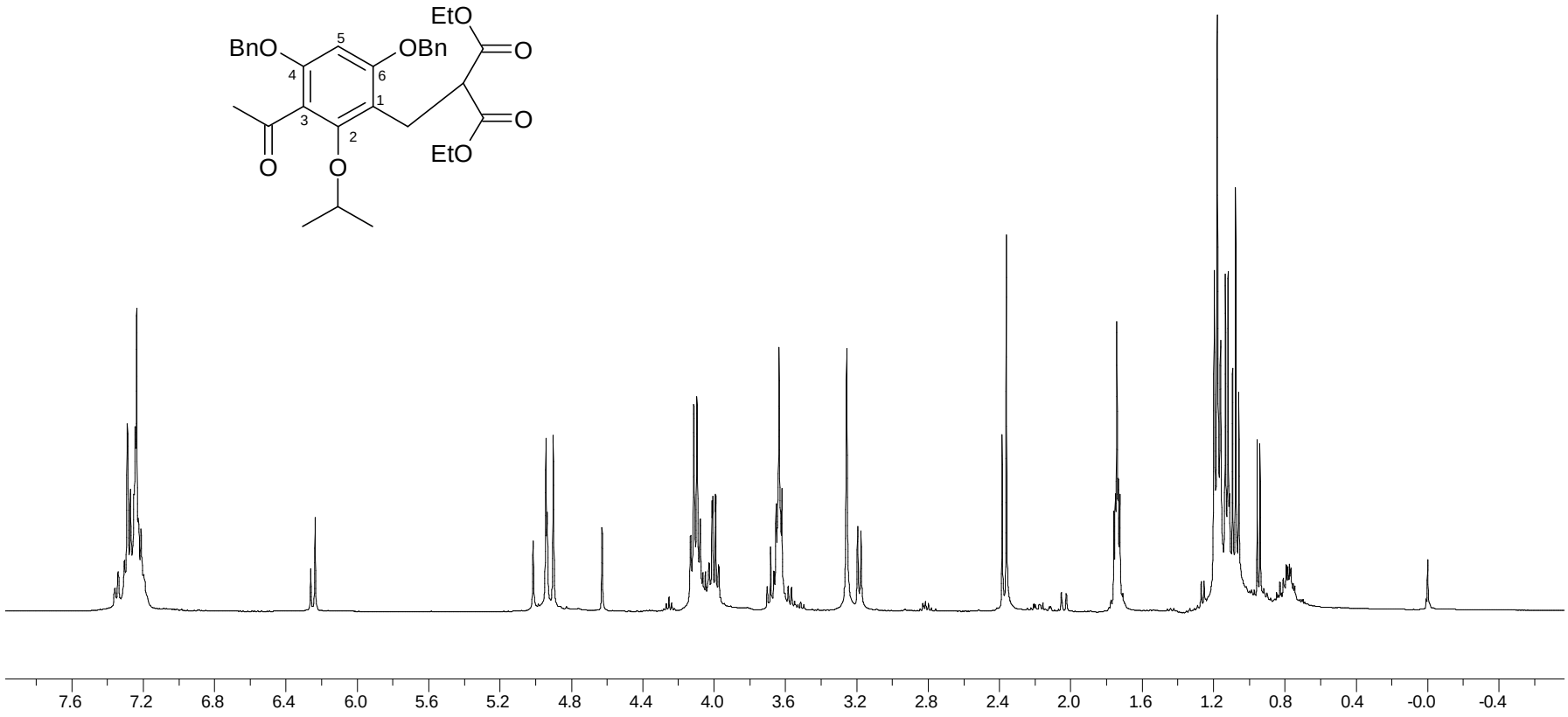
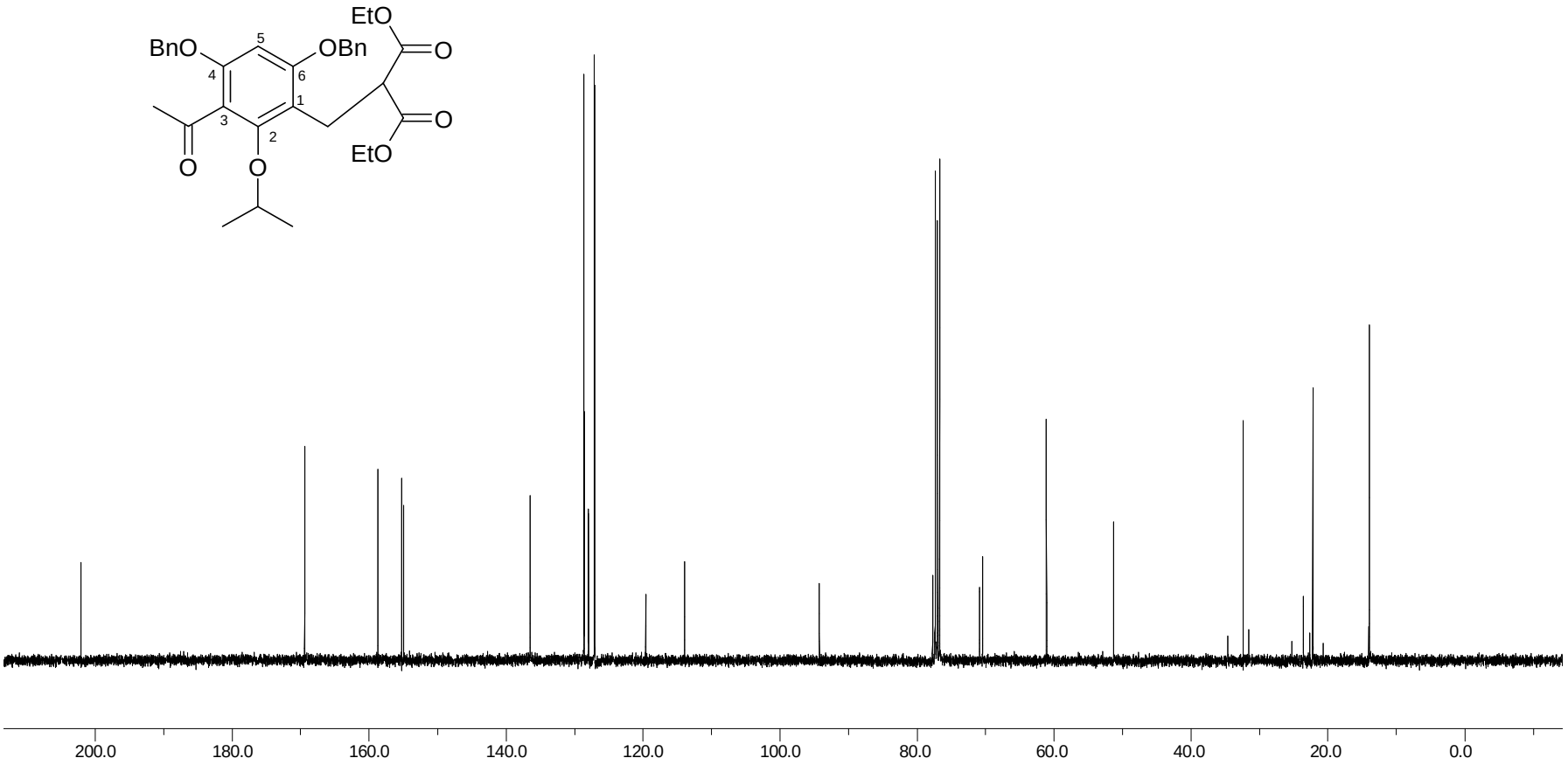


Plate 114: ^{13}C NMR spectrum of compound 421



Appendix 2

Additional Crystal Structure Information for Helisplendidilactone

1. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for helisplendidilactone. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
C(1)	-2598(2)	3885(2)	6827(2)	23(1)
C(1')	3548(2)	10665(2)	7422(2)	17(1)
C(2)	-1399(2)	4805(2)	7070(2)	21(1)
C(2')	5001(2)	10350(2)	6986(2)	20(1)
C(3)	-635(4)	5099(3)	6103(2)	58(1)
C(3')	6015(2)	10110(2)	8046(2)	20(1)
C(4')	4909(2)	9282(2)	8512(2)	18(1)
C(4)	676(3)	5937(2)	6257(2)	33(1)
C(5')	3511(2)	10048(2)	8349(2)	17(1)
C(5)	640(3)	6960(2)	6744(2)	33(1)
C(6)	1840(2)	7900(2)	6822(2)	23(1)
C(6')	2347(2)	9935(2)	9116(2)	19(1)
C(7')	1425(2)	11023(2)	9222(2)	16(1)
C(7)	3259(2)	7556(2)	7567(2)	19(1)
C(8')	514(2)	11379(2)	8151(2)	18(1)
C(8)	3795(2)	6340(2)	7267(2)	24(1)
C(9')	1326(2)	12082(2)	7367(2)	21(1)
C(9)	3483(3)	6028(2)	6056(2)	30(1)
C(10')	2331(2)	11339(2)	6728(2)	21(1)
C(10)	1970(3)	5441(2)	5752(2)	32(1)
C(11)	4660(2)	8338(2)	7558(2)	18(1)
C(12)	5914(2)	7449(2)	7695(2)	24(1)
C(13)	4795(2)	9093(2)	6515(2)	19(1)
C(14)	1622(3)	5359(3)	4502(2)	37(1)
C(15')	5360(2)	8792(2)	9650(2)	23(1)
C(15)	-3565(2)	3644(2)	7704(2)	24(1)
C(14')	2922(3)	12096(2)	5855(2)	33(1)
C(13')	-507(2)	9768(2)	10095(2)	22(1)
C(11')	254(2)	10952(2)	10020(2)	18(1)
C(12')	-897(2)	11829(2)	9540(2)	22(1)
O(1)	-2764(2)	3367(2)	5958(2)	43(1)

O(2)	5392(2)	6354(1)	7587(1)	31(1)
O(3)	7232(2)	7612(2)	7884(2)	35(1)
O(4)	-680(2)	12096(1)	8495(1)	23(1)
O(5)	-1886(2)	12257(1)	9964(1)	28(1)

2. Anisotropic displacement parameters ($Å^2 x\ 10^3$)for helisplendidilactone. The anisotropic displacement factor exponent takes the form: $-2p^2[\ h^2a^{*2}U^{11} + ... + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
C(1)	23(1)	20(1)	24(1)	0(1)	1(1)	-4(1)
C(1')	18(1)	14(1)	20(1)	0(1)	1(1)	1(1)
C(2)	18(1)	21(1)	23(1)	-5(1)	5(1)	-3(1)
C(2')	21(1)	16(1)	22(1)	4(1)	3(1)	-3(1)
C(3)	75(2)	80(2)	19(1)	2(1)	2(1)	-56(2)
C(3')	17(1)	18(1)	26(1)	3(1)	0(1)	-4(1)
C(4')	13(1)	18(1)	21(1)	2(1)	0(1)	2(1)
C(4)	42(1)	41(1)	16(1)	2(1)	5(1)	-27(1)
C(5')	17(1)	14(1)	20(1)	0(1)	1(1)	2(1)
C(5)	21(1)	54(2)	26(1)	-13(1)	11(1)	-14(1)
C(6)	13(1)	22(1)	35(1)	-8(1)	5(1)	1(1)
C(6')	19(1)	17(1)	19(1)	4(1)	3(1)	4(1)
C(7')	18(1)	13(1)	16(1)	-2(1)	1(1)	1(1)
C(7)	18(1)	17(1)	23(1)	1(1)	5(1)	-3(1)
C(8')	19(1)	16(1)	19(1)	0(1)	0(1)	4(1)
C(8)	29(1)	15(1)	28(1)	3(1)	-2(1)	-1(1)
C(9')	27(1)	17(1)	19(1)	3(1)	2(1)	7(1)
C(9)	36(1)	24(1)	29(1)	-5(1)	-2(1)	16(1)
C(10')	25(1)	17(1)	19(1)	2(1)	0(1)	6(1)
C(10)	49(2)	16(1)	28(1)	0(1)	-7(1)	2(1)
C(11)	14(1)	16(1)	23(1)	2(1)	4(1)	2(1)
C(12)	24(1)	23(1)	26(1)	4(1)	5(1)	7(1)
C(13)	13(1)	19(1)	24(1)	2(1)	5(1)	1(1)
C(14)	33(1)	49(2)	28(1)	-12(1)	-3(1)	13(1)
C(15')	18(1)	27(1)	23(1)	6(1)	-1(1)	1(1)
C(15)	20(1)	22(1)	31(1)	1(1)	5(1)	0(1)
C(14')	41(1)	35(1)	26(1)	14(1)	12(1)	15(1)

C(13')	22(1)	19(1)	24(1)	3(1)	2(1)	2(1)
C(11')	19(1)	18(1)	17(1)	-2(1)	1(1)	3(1)
C(12')	27(1)	16(1)	22(1)	-2(1)	2(1)	3(1)
O(1)	58(1)	44(1)	26(1)	-12(1)	7(1)	-25(1)
O(2)	31(1)	19(1)	41(1)	1(1)	-3(1)	9(1)
O(3)	17(1)	38(1)	50(1)	4(1)	8(1)	10(1)
O(4)	25(1)	24(1)	22(1)	3(1)	5(1)	12(1)
O(5)	32(1)	25(1)	28(1)	-1(1)	10(1)	10(1)

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Appendix 3

Conference presentations

2007 55th International Congress and the Annual Meeting of the Society for Medicinal Plant Research. (2-6 September, Graz, Austria)

Oral presentation: Presented at workshop for Young Researchers. Isolation and characterisation of guaianolides from *Helichrysum montanum* and *H. splendidum*

Poster presentation: Isolation and characterisation of guaianolides from *Helichrysum montanum* and *H. splendidum*

Abstract published in: *Planta Medica*, 2007, **9**, 815.

2006 The 38th National Convention of the South African Chemical Institute (3-8 December, University of KwaZulu-Natal, Durban)

Oral presentation: Isolation and synthesis of polyphenolic compounds from *Helichrysum excisum*

4th International Conference on Pharmaceutical and Pharmacological Sciences (ICPPS). (20-23 September, Riverside Hotel, Vanderbijlpark)

Oral Presentation: Isolation of selected bio-active lactones from South African *Helichrysum* species

9th Frank Warren Conference on Organic Chemistry
(22 -25 January, University of Cape Town)

Poster Presentation: Bio-active lactones from *Helichrysum* species (Book prize received for one of three best posters)

2005 Suid-Afrikaanse Akademie vir Wetenskap en Kuns: studentesimposium (28 October, University of South Africa, Pretoria).

Oral presentation: Fitochemie en antimikrobiese aktiwiteit van *Helichrysum excisum*. (Section winner. Abstract published in: Suid-Afrikaanse Tydskrif vir Natuurwetenskap en Tegnologie, 25 (1), 51).

Indigenous Plant Use Forum (IPUF) (27-30 June, Rhodes University, Grahamstown)

Oral presentation: Phytochemistry of *Helichrysum montanum*.

Appendix 4

Publication

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South African *Helichrysum* species: A review of the traditional uses, biological activity and phytochemistry

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ABSTRACT

Aims of the study: In South Africa, the genus *Helichrysum* is widely used in traditional medicine. The uses are well documented although renaming of species and the resulting confusing taxonomic nomenclature may cause uncertainty as to which specific species was referred to in some reports. The aim of this paper is to present a collated and coherent overview of the documented traditional uses of *Helichrysum* species and to update the botanical identity of previously studied species.
Materials and methods: Databases (Scifinder, ISI Web of Knowledge) and several books were used to collect in information on South African *Helichrysum* species.
Results: The traditional uses, chemistry and biological activity of *Helichrysum* species have been summarized. It was attempted to give clarity as to exactly which species is refer to in the ethnobotanical literature.
Conclusions: Although a large number of ethnopharmacological uses have been documented and the chemistry of the genus has been studied extensively, only a few South African species have been investigated for their biological activity.

1. Introduction

The genus *Helichrysum* Mill. derives its name from the Greek words *helios* (sun) and *chrysos* (gold) which is appropriate considering the attractive yellow flowers displayed by several species (Pooley, 2003). The genus belongs to the Asteraceae family, tribe Inuleae and subtribe Gnaphaliinae (Hilliard, 1983). This large genus consists of approximately 500–600 species and although *Helichrysum* species are also found in southern Europe, south-west Asia, southern India, Sri Lanka (previously Ceylon) and Australia, most species occur in Africa, including Madagascar (Hilliard, 1983). In South Africa (including Namibia), the ca. 244–250 species are widely distributed and the tremendous morphological diversity displayed by these species resulted in their subdivision into 30 morphological groups, using the shape and size of the flower heads as differentiating characteristics (Hilliard, 1983). The flower heads are either solitary or occur in compact or spreading inflorescences. The aerial parts are usually hairy or woolly and plants occur as herbs or shrublets that are sometimes dwarfed and cushion forming. They are often aromatic (Pooley, 1998, 2003; Van Wyk et al., 2000).

2. Traditional uses

Several *Helichrysums* are widely used in Southern African traditional medicine as summarised in Table 1. The first written record of the medicinal use of *Helichrysum* dates back to 1727 when Boerhaave noted that a *Helichrysum* species was used to treat nervousness and hysteria. The report of a *Helichrysum* species in the early literature could have been based on knowledge acquired from the local Khoi and San people, but is most probably due to the fact that European botanists used their knowledge of medicinal properties of European genera (Scott and Hewett, 2008).

2.1. Ambiguities in nomenclature

As is the case for all ethnobotanical data, the fact that plant names are changed (Germishuizen and Meyer, 2003) and frequently incorrectly cited (Arnold et al., 2002) is quite problematic. To complicate matters further, variation in spelling of names also occurs. Special care needs to be taken when consulting the original texts to unambiguously confirm that a plant selected for a particular study is in fact the same species cited by, for example, Watt and Breyer-Brandwijk (1962). In Table 1, current names are given and previously accepted names are shown in parenthesis. For the sake of clarity, the name as it appears in the reference is sometimes indicated in brackets after the reference.

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Table 1
Traditional uses and biological activities reported for *Helichrysum* species

Species[a]	Plant part used	Dosage form	Traditional use	Classification of use[b]	Biological activity[b]	References
Helichrysum acutatum DC. **21**[c]			Widely used as traditional medicine, sold commercially in large quantities	NS		Arnold et al. (2002), Cunningham (1988), Hutchings et al. (1996)
Helichrysum adenocarpum DC. **28**	Root	Decoction	Used to treat diarrhoea and vomiting in children.	GIT		Arnold et al. (2002), Jacot Guillarmod (1971), Neuwinger (1996), Phillips (1917), Pooley (2003), Walker (1996), Watt and Breyer-Brandwijk (1962)
Helichrysum appendiculatum (L.f.) Less. **24**	Leaf	Eaten raw	Chest problems or infection of the respiratory tract	Resp, Infec, Anth, W, P	B[d], F[d]	Arnold et al. (2002), Githens (1949), Mathekga (2001)[e], Smith (1895), Smith (1966), Swanepoel (1997), Walker (1996), Watt and Breyer-Brandwijk (1962)
	Plant		Smallpox			
	Plant		Anthelmintic			
	Root		Coughs and colds and applied externally on wounds			
	Leaf	Wound dressing	Applied externally to wounds. Ground leaves are rubbed into areas which cramps or on wounds			
	Roots		Ground and burnt and smeared on body to relax body and to reduce swelling			
	Leaf		Used medicinally as tea			
Helichrysum argyrophyllum DC. **29**	Root	Infusion	Intestinal troubles	GIT		Arnold et al. (2002), Batten and Bokelmann (1966), Smith (1966), Walker (1996), Watt and Breyer-Brandwijk (1962)
			Not grazed by stock, preventing soil erosion in overgrazed areas			
Helichrysum argyrosphaerum DC. **15**			Browsed by animals but poisonous if large quantities is ingested	Poi	B[d], F[d]	Hutchings et al. (1996), Mathekga (2001)[e], Pooley (1998), Van Wyk et al. (2002)
Helichrysum asperum (Thunb.) Hilliard and Burtt. (=*Helichrysum ericifolium* Less.) (Hilliard, 1983) **12**[f]			The plants are casually browsed by sheep and said to be a cause of "Geilsiekte"	Poi		Smith (1966) (*Helichrysum ericaefolium* DC.)[g]
Helichrysum athrixiifolium (Kuntze) Moeser **9**[f]	Leaf	Smoked	Chest complaints.	Resp		Arnold et al. (2002), Jacot Guillarmod (1971) (*Helichrysum athrixifolium* O. Hoffm.)[g], Phillips (1917) (*Helichrysum athrixiifolium* O. Hoffm.)[g], Watt and Breyer-Brandwijk (1962) (*Helichrysum athrixifolium* O. Hoffm.)[g]
Helichrysum aureonitens Sch. Bip. **8**	Leaves and stems	Burnt as incense	Used to invoke the goodwill of the ancestors and to induce trances	Psy, Psyc, Infect, Insect	B[d], F[d], V	Afolayan and Meyer (1997)[e], Cunningham (1988), Hutchings et al. (1996), Jacot Guillarmod (1971), Mathekga (2001)[e], Meyer and Afolayan (1995)[e], Meyer et al. (1996)[e], Meyer et al. (1997)[e], Phillips (1917), Pooley (1998), Pooley (2003), Swanepoel (1997), Walker (1996), Watt and Breyer-Brandwijk (1962)
	Leaves and stems		Commercially sold			
		Decoction	A remedy for inuresis in children			
		Extracts	Used topically for skin infections especially against *Herpes zoster* and infections associated with *Herpes simplex*			
			Used to keep red mites away			
			Used as tinder to start fire, used to make hats.			
Helichrysum aureum Houtt. Merr. var. *aureum/monocephalum* (=*Helichrysum fulgidum* (L.f.) Willd.) **30**[h]		Decoction	Used for washing sore eyes	Eye		Arnold et al. (2002) (*Helichrysum fulgidum* L.f.) Willd.)[g], Batten and Bokelmann (1966) (*Helichrysum fulgidum* Willd.)[g], Jacot Guillarmod (1971) (*Helichrysum fulgidum* (L.) Willd.)[g], Phillips (1917) (*Helichrysum fulgidum* Willd.)[g]

Table 1 (*Continued*)

Species[a]	Plant part used	Dosage form	Traditional use	Classification of use[b]	Biological activity[b]	References
Helichrysum bellum Hilliard **28**					B[d], F[d]	Mathekga (2001)[e]
Helichrysum caespititium (DC.) Harv **12**[f]	Plant	Crushed and burnt and smoke inhaled	Used to treat head and chest colds (headaches)	Resp, Infect, GIT, Vi, W	B[d], F[d], I, My	Arnold et al. (2002), Dekker et al. (1983)[e], Gelfand et al. (1985) (*Helichrysum caespitium* Sond.)[g], Hutchings and Van Staden (1994), Jacot Guillarmod (1971) (*Helichrysum caespitium* Sond.)[g], Mathekga et al. (2000)[e], Mathekga (2001)[e], Meyer et al. (2002)[e], Neuwinger (1996), Phillips (1917) (*Helichrysum caespitium* Sond)[g], Pooley (1998), Pooley (2003), Swanepoel (1997)[e], Watt and Breyer-Brandwijk (1962)
	Plant	Decoction	Drunk by the Kwena and the Kgatla to treat gonorrhoea			
	Root	Decoction	Nausea			
	Roots		Virility			
	Plant	Ointment	Ointment is applied to the roof of the mouth for a depressed fontanelle Used as dressing for open wounds during circumcision rites			
Helichrysum callicomum Harv **2**			Protective charm. Mixed with *Aster bakerianus* (*hispidis*) and *Helichrysum* Used for fuel in winter	M, GIT	B[d], F[d]	Arnold et al. (2002), Jacot Guillarmod (1971), Mathekga and Meyer (1998)[e], Mathekga (2001)[e], Phillips (1917), Pooley (2003), Watt and Breyer-Brandwijk (1962)
		Enema	Used as an ingredient in an enema for colic			
Helichrysum calocephalum Klatt **23**						Arnold et al. (2002) refers to others using *Helichrysum calocephalum* Schltr, which is classified as *Helichrysum ecklonis* Sond (Germishuizen and Meyer, 2003)
Helichrysum calophalum Klatt **23**	Root		Used for hyperfunction of the lower gastro-intestinal tract	GIT		Swanepoel (1997), information obtained from TRAMED database. It is not clear to these authors whether this use pertains to *Helichrysum calocephalum* Klatt or *Helichrysum ecklonis* Sond
Helichrysum candolleanum Buek **15**					B[d], F[d]	Mathekga (2001)[e]
Helichrysum chionosphaerum DC. **25**					B[d], F[d]	Mathekga (2001)[e]
Helichrysum cephaloideum DC. (=*Helichrysum adscendens* Less. var. *cephaloideum* Moes.) **24**			Irritant poisoning in sheep demonstrated. Known to be poisonous to sheep (symptoms similar to that of poisoning caused by *Geigera*)	Poi		Van Wyk et al. (2002), Watt and Breyer-Brandwijk (1962)
Helichrysum cochleariforme DC. (=*Helichrysum imbricatum* Less.) **15**		Tea, infusion	Demulcent in coughs and other pulmonary affections. In the Western Cape area the plant is used to treat whooping cough, other coughs, bronchial catarrh and bronchitis	Resp		Arnold et al. (2002), Neuwinger (1996), Smith (1966), Swanepoel (1997), Watt and Breyer-Brandwijk (1962)
	Whole plant	Decoction	Drunk for infections of the respiratory tract			
Helichrysum cooperi Harv. **30**	Leaf	Ointment, applied after bathing	Used as love charm. The ointment is applied after bathing and as a result the desired lady finds the man irresistible	M, Fum, Snakebite		Arnold et al. (2002), Hutchings et al. (1996), Pooley (1998), Pooley (2003), Walker (1996), Watt and Breyer-Brandwijk (1962)
	Leaves		Used to make Zulu headdress distinctive to married women Used as a fumigant and as part of a traditional remedy for snakebite.			

Helichrysum crispum (L.) D. Don. **17**			Used medicinally as a calming tea Coughs, bronchitis, urinary tract infections and tuberculosis.	Resp, Renal	B	Arnold et al. (2002) (with reference to Smith, 1966), Kling as quoted by Salie et al., 1996[e], Roberts (1990) (*Helichrysum crispum*)[g] These authors are not certain whether Kling is referring to *Helichrysum crispum* (L.) D. Don. or *Helichrysum crispum* Less.
Helichrysum cymosum (L.) D. Don. **8**			Used to invoke the goodwill of the ancestors and to induce trances	M, Psy, Resp, GIT, P	B[d], F[d], Pl	Arnold et al. (2002), Bhat and Jacobs (1995), Kokwaro as quoted by Neuwinger (1996), Neuwinger (1996), Pooley (2003), Van Vuuren et al. (2006)[e], Van Wyk et al. (2000)
	Leaf	Decoction/tea	Used to treat colds and coughs			
	Root	Extract	Used as emetic and purgative			
	Leaf		Filtrate drunk to treat colds and fever			
	Leaf	Boiled, and vapours from boiling leaves inhaled	Vapour bath used to treat headaches			
Helichrysum dasymallum Hilliard (=*Helichrysum lanatum* Harv.) **21**			Used as medicinal tea. Woolly coat used for tinder boxes	NS		Arnold et al. (2002), Lucas and Pike (1971), Smith (1966)
Helichrysum decorum DC. **30**	Plant	Burned and smoked inhaled	Used to induce trances	Psy	B[d], F[d]	Arnold et al. (2002), Hutchings et al. (1996), Mathekga (2001)[e], Neuwinger (1996)
Helichrysum dregeanum Sond. and Harv. **9**	Leaf	Smoked	Used to treat head colds	Resp, GIT		Arnold et al. (2002), Hutchings and Van Staden (1994), Jacot Guillarmod (1971), Neuwinger (1996), Phillips (1917), Smith (1966), Watt and Breyer-Brandwijk (1962)
		Infusion	Used to treat hiccups			
			Browsed by stock			
Helichrysum ecklonis Sond (=*Helichrysum calocephalum* Schltr.) **28**			Used by the Xhosas to ward of evil magic spells, which follow on seeing *iChanti*, the water snake	M, GIT		Batten and Bokelmann (1966), Jacot Guillarmod (1971), Phillips (1917), Pooley (2003), Watt and Breyer-Brandwijk (1962)
	Root	Decoction	Used to treat diarrhoea in children.			
Helichrysum epapposum Bolus **3**	Leaves and stems	Burned as incense	Used to invoke the goodwill of the ancestors	M		Arnold et al. (2002), Cunningham (1988), Hutchings et al. (1996)
	Leaves and stems		Commercially sold			
Helichrysum excisum (Thunb.) Less. **12**					B[d], I	Lourens et al. (2004)[e]
Helichrysum felinum Less. **17**					B[d], I	Lourens et al. (2004)[e]
Helichrysum flanaganii Bolus **13**	Leaves	Burned	Incense	M		Walker (1996)
Helichrysum foetidum (L.) Moench **30**[f]	Plant	Extract is drunk/smoke inhaled	Used to induce trances	Psy, Infect, Resp, W, Eye, P	B	Arnold et al. (2002), Batten and Bokelmann (1966) (*Helichrysum foetidum* Cass.)[g], Gerstner (1938) (*Helichrysum foetidum* Cass)[g], Hulme (1954), Hutchings et al. (1996), Kokwaro quoted by Neuwinger (1996), Neuwinger (1996) Roberts (1990), Rwangabo, quoted by Neuwinger (1996), Steenkamp et al. (2004)[e], Swanepoel (1997), Van Wyk and Gericke (2000), Watt and Breyer-Brandwijk (1962) (*Helichrysum foetidum* Cass.)[g]
	Leaf	Extract	Used to treat flu (influenza)			
	Leaf	Wound dressing	Used to treat circumcision and infected wounds (festering sores)			
	Leaf	Preparation	Applied to treat *Herpes*			
	Root	Extract	Eye problems, used to bath eyes			
	Leaf		Used in making headdress distinctive of married women			
	Plant		Aromatic and astringent (used to draw out infection). Used to treat menstrual pain			
Helichrysum glomeratum Klatt **6**					B, F[d]	Mathekga and Meyer (1998)[e], Mathekga (2001)[e]
Helichrysum griseum Sond (=*Helichrysum agrostophilum* Klatt) **23**[h]			Preventative charm against illness. Burnt as fuel in winter	M		Arnold et al. (2002), Phillips (1917)
Helichrysum gymnocomum DC. **4**	Stems and leaves	Burned as incense	Used to invoke the goodwill of the ancestors	Skin, M, Fum	B[d], F[d]	Cunningham (1988), Drewes and Van Vuuren (2008)[e], Hutchings et al. (1996), Phillips (1917)
		Ointment	Mixed with fat, only the wives of chiefs were previously allowed to use it			
			Used to fumigate sick rooms			
			Commercially sold			
Helichrysum herbaceum (Andrews) Sweet **29**	Stems and leaves	Burned as incense	Used to invoke the goodwill of the ancestors	M	B[d], F[d]	Arnold et al. (2002), Cunningham (1988), Hutchings et al. (1996), Mathekga (2001)[e], Neuwinger (1996), Pooley (1998), Pooley (2003)
	Stems and leaves		Commercially sold			

Table 1 (*Continued*)

Species[a]	Plant part used	Dosage form	Traditional use	Classification of use[b]	Biological activity[b]	References
Helichrysum hypoleucum Harv **16**					B[d], F[d]	Mathekga and Meyer (1998)[e], Mathekga (2001)[e]
Helichrysum indicum (L.) Grierson (=*Helichrysum expansum* (Thunb.) Less.) **15**	Plant	Burned and crushed	Mixed with *Conyza pinnata*. Crushed and burnt to drive sickness from a room	M		Arnold et al. (2002), Jacot Guillarmod (1971)
Helichrysum kraussii Sch. Bip **8**	Leaf	Decoction	Use to wash keloid scars	Skin, M, Resp, Infect	B[d], F[d]	Arnold et al. (2002), Arnold and Gulumian as quoted by Neuwinger (1996), Bremner and Meyer (2000)[e], Mathekga (2001)[e], Gelfand et al. (1985), Mabogo (1990), Neuwinger (1996), Swanepoel (1997), Walker (1996), Watt and Breyer-Brandwijk (1962)
	Root and leaves	Infusion	Used to drive bad spirits away, used to wash body			
	Dried flower and seed	Smoked in a pipe	The Karanga smoke this as a remedy for coughs and pulmonary tuberculosis			
	Plant	Burnt, salt is added to ash and ingested by mouth	Cough			
	Root		Venereal disease			
	Root	Mixed with salt and other ingredients	Applied to child's side with small amount given orally			
Helichrysum lepidissimum S. Moore **19**		Powder or ointment	Used as a body perfume	Skin		Dlamini (1981), Watt and Breyer-Brandwijk (1962)
Helichrysum litorale Bolus (=*Leontonyx angustifolius* DC. =*Leontonyx spathulatus* Less.) **14**	Plant		Dried and pounded or mixed with lard or fat, was used for applying to ulcers. In the Western Cape province an ointment for boils, carbuncles and abscesses is made from this plant, *Cyanella lutea* and "tiendaegeneesbossie"	W, Skin		Smith (1966), Swanepoel (1997), Watt and Breyer-Brandwijk (1962)
Helichrysum longifolium DC. **24**	Leaf		Used by the Pondos to treat circumcision wounds. The leaves are heated over very hot ash before being used as a bandage for the treatment of wounds after circumcision	W	B[d], F[d]	Dilika et al. (1997)[e], Mathekga (2001)[e]
Helichrysum lucilioides Less. **12**			Excellent stock feed			Smith (1966)
Helichrysum melanacme DC. **8**			Used as bedding. Used medicinally as tea. Used for cough, fever, headache, colds and chest pain	Resp, P	B[d], F[d], My, V	Arnold et al. (2002), Lall and Meyer (1999)[e], Lall et al. (2006)[e], Mathekga (2001)[e], Smith (1966)
Helichrysum miconiifolium DC. **23**		Tea	Used medicinally as tea	P, Anthel	B[d], F[d]	Smith (1966) (*Helichrysum miconiaefolium* DC.)[g], Arnold et al. (2002), Mathekga (2001)[e], Swanepoel (1997)
	Leaf		The Xhosa grind and boil the leaves and use it as a wash for pain after circumcision			
	Root		The powdered root is used for intestinal parasites and for ticks on poultry			
Helichrysum montanum DC. **22**					B, F[d]	Mathekga (2001)[e]
Helichrysum monticola Hilliard **28**					B, F[d]	Mathekga (2001)[e]
Helichrysum mundtii Harv. **23**	Plant	Decoction	Chest complaints	Resp		Arnold et al. (2002), Jacot Guillarmod (1971), Pooley (1998), Pooley (2003), Phillips (1917) (*Helichrysum mundii*, Harv.)[g], Watt and Breyer-Brandwijk (1962)
Helichrysum natalitium DC. **3**	Leaves and stems	Burnt as incense	Used to invoke the goodwill of the ancestors	M		Arnold et al. (2002), Cunningham (1988), Hutchings et al. (1996), Pooley (2003)
	Leaves and stems		Commercially sold			

Helichrysum nudifolium (L.) Less. var. *nudifolium* =*H coriaceum* Harv.[f] =also *Helichrysum gerberifolium* A. Rich, =also *Helichrysum leiopodium* DC. =also *Helichrysum nudifolium* var. *quinquenerve* =also *Helichrysum nudifolium* var. *leiopodium*) **23**	Leaf	Burnt as incense	To invoke the goodwill of the ancestors	M, Resp, W, Infect, P, Skin, GIT	B[d], F[d], I	Arnold et al. (2002), Gerstner (1938) (*Helichrysum undifolium*, also *Helichrysum leiopodium* DC.)[g], Githens (1949) (*Helichrysum nudifolium*, also *Helichrysum leiopodium*)[g], Glover et al. quoted by Neuwinger (1996), Hulme (1954), Hutchings et al. (1996), Hutchings and Johnson (1986), Hutchings and Van Staden (1994), Jacot Guillarmod (1971) (*Helichrysum nudifolium* var. *leiopodium*)[g], Jäger et al. (1996)[e], Mabogo (1990), Phillips (1917) (*Helichrysum leiopodium* DC.)[g], Rood (1994), Smith (1895) (*Helichrysum nudiflorum*)[g], Smith (1966) (*Helichrysum coriaceum* Sond. and *Helichrysum nudifolium* var. *quinquenerve*)[g], Swanepoel (1997)[e] (*Helichrysum gerberifolium*)[g], Van Wyk et al. (2000), Neuwinger (1996) (also *Helichrysum gerberifolium* Sch. Bip)[g], Watt and Breyer-Brandwijk (1962) (*Helichrysum gerberaefolium* Sch. Bip. Ex A.Rich)[g]
		Infusion	Colds (Zulu and Khoi—administration route not indicated)			
	Leaf	Eaten raw	Used to treat colds by the Xhosa			
	Plant	Infusion	Regarded as demulcent, used to treat catarrh, phthisis and other pulmonary affections			
	Leaf/plant		Respiratory infections			
	Root		Coughs and colds			
	Leaf	Wound dressing	Wounds			
	Root/Leaf		Applied to sores on the genitalia by the Xhosa			
	Plant/leaf	Smoke inhaled	Headache			
	Leaf	Infusion	Rectal prolapse			
		Powder mixed with butter and eaten	Protection of children from illness			
	Root	Decoction	Chest problems, used as emetic by the Zulu			
	Leaf	Decoction	To encourage weaning in babies			
	Leaf	Infusion	Diseases in goats			
	Plant	Infusion on hot stones	Used as steam bath to treat fever and nightmares			
	Plant	Poultice	Swellings			
		Decoction	Colic in children (administered as enema)			
			Rubbed into scarifications over bruises. Used as tea			
	Root	Decoction	Internal sores (intestinal ulceration)			
Helichrysum nudifolium var. *oxyphyllum* (=*Helichrysum oxyphyllum* DC. =also *Helichrysum undatum* Less.) **23**			Protective charm against thunder	M		Arnold et al. (2002), Gertsner (1938), Hutchings et al. (1996)
Helichrysum nudifolium var. *pilosellum* (=*Helichrysum latifolium* (Thunb.) Less. =*Helichrysum pilosellum* (L.f.) Less.) **23**			Used for "doctoring' people who wish some deed concealed and who are afraid of being found out	M, GIT	B, F[d]	Arnold et al. (2002) (*H. pilosellum*)[g], Hulme (1954) (*Helichrysum latifolium*)[g], Hutchings et al. (1996) (*Helichrysum pilosellum* (L.f.) Less.)[g], Jacot Guillarmod (1971) (*Helichrysum latifolium* (Thunb.) Less.)[g], Mathekga and Meyer (1998)[e], Mathekga (2001)[e], Neuwinger (1996) (*Helichrysum pilosellum* (L.f.) Less.)[g], Phillips (1917) (*Helichrysum latifolium* Less.)[g], Phillips (1917) (*Helichrysum latifolium* Less.)[g], Pooley (2003) (*Helichrysum pilosellum*)[g], Swanepoel (1997), Walker (1996) (*Helichrysum pilosellum* (L.f.) Less.)[g], Watt and Breyer-Brandwijk (1962) (*Helichrysum latifolium* Less.)[g]
			Ingredient in colic remedy			
	Leaf	Infusion	Stomach ache in children			
	Roots	Ground and burnt	Ground and burnt near cattle suffering from black leg			
Helichrysum nudifolium var. *pilosellum* (=*Helichrysum pilosellum* (L.f.) Less. =*Helichrysum pedunculare* (L.) DC. var. *pilosellum*) **23**[h]			As an antiseptic and to induce fast healing: used after circumcision to prevent inflammation externally	W, Resp, GIT		Arnold et al. (2002), the sources below are indicated in Arnold et al., under *Helichrysum pedunculare* DC.: Batten and Bokelmann (1966) (*isicwe*, *isiGqutsi*)[i], Githens (1949) (*Helichrysum pedunculare*)[g], Smith (1895) (*isi-Cwe.*)[i], Smith (1966)
			Also externally applied to wounds and used for infections of the respiratory tract			
			As an antiseptic			
			Stomach ailments			

Table 1 (*Continued*)

Species[a]	Plant part used	Dosage form	Traditional use	Classification of use[b]	Biological activity[b]	References
Helichrysum odoratissimum (L.) Sweet **4**	Leaf/ground plants	Used as wound dressing/leaf pulp	Wounds and burns	W, Fum, Psy, Psyc, M, Resp, Eye, GIT, P	B[d], F[d], My	Adjanohoun quoted by Neuwinger (1996), Arnold et al. (2002), Baerts and Lehmann quoted by Neuwinger (1996), Cunningham (1988), Dlamini (1981), Hutchings and Johnson (1986), Hutchings et al. (1996), Hutchings and Van Staden (1994), Jacot Guillarmod (1971), Kokwaro quoted by Neuwinger (1996), Lall and Meyer (1999)[e], Lourens et al. (2004)[e], Mathekga and Meyer (1998)[e], Mathekga (2001)[e], Neuwinger (1996), Pooley (1998), Pooley (2003), Rwangabo quoted by Neuwinger (1996), Smith (1966), Swanepoel (1997), Van Puyvelde et al., 1989, Van Wyk et al. (2000), Van Wyk and Gericke (2000), Watt and Breyer-Brandwijk (1962)
	Plant		The Southern Sotho use this plant to fumigate huts			
		Ointment	It is mixed with fat to form pleasantly smelling ointment, formerly only used by wives of chiefs			
	Leaf	Ash is rubbed into scarifications	Insanity, possession			
		Burnt as incense	Used to invoke the goodwill of the ancestors, protective charm			
		Tea	Aids sleep, relieves muscle tension and cramps			
	Plant, leaf, stems	Smoke inhaled	Used as a sedative and to treat insomnia and as protective cleanser.			
	Root		Colds, coughs			
	Leafy twigs	Ash is eaten	Coughs			
	Leaf and twigs	Extract or sap used as eye drop	Conjunctivitis			
		Decoction	Abdominal pain			
	Aerial parts	Extract	Used to treat dehydration			
	Leaf	Sap	Heartburn, flatulence			
	Root	Extract	Purgative (extract is drunk)			
	Leaf	Ash is eaten	Vomiting			
		Tea	Colic and stitch			
	Leaf	Decoction	Febrile convulsions (part of preparation)			
	Leaf	Smoke inhaled	Headache			
	Leaf	Infusion	Fever (also used as wash)			
	Leaf and twigs	Decoction	Used to treat female sterility, menstrual pain and eczema in Rwanda			
	Leafy twigs	Decoction	Tonic for pregnant women			
	Leaf	Decoction	Galactagoque			
			Used a bedding material since it is an effective insect repellent. Sold commercially. The Xhosa also use the plant for spiritual purposes, as a fumigant when a baby is born			
Helichrysum oreophilum Klatt **21**					B, F[d]	Mathekga and Meyer (1998)[e], Mathekga (2001)[e]
Helichrysum pallidum DC. (=*Helichrysum agrostophilum* Klatt (in part) =*Helichrysum undatum* (Thunb.) Less. var. *agrostophilum* (Klatt) Moeser =*Helichrysum undatum* var. *pallidum* **23**[h]			Preventative charm for illness	M		Arnold et al. (2002), Jacot Guillarmod (1971) (*Helichrysum undatum* var. *agrostophilum*)[g], Phillips (1917) (*Helichrysum undatum* Less., var. *pallidum* and *Helichrysum agrostophilum* Klatt)[g]
	Roots	Bathing in decoction	Burnt as fuel in winter The act of forgetting, The bath is suppose to make a person invisible/or forgotten by his enemies, witchcraft			
Helichrysum panduratum O. Hoffm. **18**	Leaf	Decoction	Febrile convulsions in children (part of a preparation)	P, Infect	A, I	Adjanohoun quoted by Neuwinger (1996), Haerdi quoted by Neuwinger (1996), Neuwinger (1996), Neuwinger (1996), Pooley (1998), Swanepoel (1997)[e]
	Plant	Sap	Used to treat malaria in children Used to make herbal tea			
Helichrysum pandurifolium Schrank. (=*Helichrysum auriculatum* Less.) **18**	Infusion, demulcent		Respiratory conditions	Resp, P, Ca, Renal		Arnold et al. (2002), Roberts (1990) (*Helichrysum auriculatum* Less.)[g], Rood (1994), Smith (1966) (*Helichrysum auriculatum* Less.)[g], Swanepoel (1997), Watt and Breyer-Brandwijk (1962) (*Helichrysum auriculatum* Less.)[g]
			Backpain, heart trouble, kidney disease, kidney stones Historically been used as a tea			

Helichrysum patulum (L.). Don. (=*Helichrysum crispum* Less.) **18**			Heart trouble, backache, kidney disease, also 'heart weakness' (also heart treatment in animals). Stress and fatigue	P, Ca, Renal, Resp		Neuwinger (1996) (*Helichrysum crispum* Less.)[g], Roberts (1990) (*Helichrysum crispum*)[g], Scott et al. (2004), Smith (1966) (*Helichrysum crispum* Less.)[g], Watt and Breyer-Brandwijk (1962) (*Helichrysum crispum* Less.)[g]
		Infusion	Hyperpiesa, (hyperpepsia is probably a spelling error in Neuwinger), coronary thrombosis, bladder conditions/infections Asthma, Influenza Gynaecological disorders Used as bedding			
Helichrysum pedunculatum Hilliard and Burtt (=*Helichrysum pedunculare* DC.) **23**	Leaf and root		As an antiseptic and to induce fast healing: used after circumcision to prevent inflammation externally	W, Resp, Infect, GIT	B	Arnold et al. (2002), Batten and Bokelmann (1966) (*Helichrysum pedunculare* DC.[g], *isiCwe*[i], *isiGqutsi*[i], Xhosa), Bhat and Jacobs (1995) (*Helichrysum pedunculatum* Hilliard and Burtt[g], *isiCwe*[i], *siGgutsi*[i], Xhosa), Dilika et al. (1997)[e], Gerstner (1938) (*Helichrysum pedunculare* DC.[g], *isiCwe*[i], Zulu), Githens (1949) (*Helichrysum pedunculare*[g], *isicwe*[i], Zulu), Hutchings et al. (1996) (*Helichrysum pedunculatum* Hilliard et Burtt)[g], Meyer and Dilika (1996)[e], Rood (1994) (*Helichrysum pedunculatum*[g], *ery'kue*[i], Fingo), Smith (1895) (*Helichrysum pedunculare* DC.[g], *isiCwe*[e]), Smith (1966) (*Helichrysum pedunculare* DC.)[g], Neuwinger (1996) (*Helichrysum pedunculatum* Hilliard and Burtt)[g], Swanepoel (1997), Watt and Breyer-Brandwijk (1962) (*Helichrysum pedunculare* DC.)[g]
			Also externally applied to wounds and used for infections of the respiratory tract As an antiseptic Stomach ailments			
Helichrysum petiolare Hilliard and Burtt **18**			Coughs, colds, catarrh, headache, fever, menstrual disorders, urinary tract infections		B[d]	Arnold et al. (2002), Lourens et al. (2004)[e], Kirstenbosch Botanical Garden, Neuwinger (1996), Roberts (1990) (*Helichrysum peteolatum*)[g], Scott et al. (2004), Smith (1966) (*Helichrysum petiolatum* DC.)[g], Van Wyk et al. (2000)
	Leaf		Antiseptic wound dressing			
		Tea	Tea taken for heart conditions, stress, hypertension, anxiety and over-excitement Used as bedding			
Helichrysum platypterum DC. **20**	Root	Decoction	Renew virility in men	Vi		Arnold et al. (2002), Jacot Guillarmod (1971), Phillips (1917), Watt and Breyer-Brandwijk (1962), Jakupovic et al., 1987
	Root	Crushed and sucked				
Helichrysum psilolepis Harv. **22**	Root	Decoction	Dysmenorrhoea	P	B[d], F[d]	Arnold et al. (2002), Jacot Guillarmod (1971), Mathekga (2001)[e], Phillips (1917), Neuwinger (1996), Watt and Breyer-Brandwijk (1962)
			Used to weave hats			
Helichrysum rotundatum (=*H coriaceum* (DC.) Harv.)[f]			Used as tea	NS		Smith (1966) (*Helichrysum coriaceum* Sond.)[g]
Helichrysum rugulosum Less. **9**			Protective charm (with *Helichrysum callicomum* and *Aster bakerianus*	M, GIT, Fum	B[d], F[d]	Arnold et al. (2002), Dlamini (1981), Jacot Guillarmod (1971), Mathekga and Meyer (1998)[e], Mathekga (2001)[e], Phillips (1917), Pooley (1998), Pooley (2003), Watt and Breyer-Brandwijk (1962)
		Enema	Colic (an ingredient) Used to fumigate huts when children are ill (cold)			
Helichrysum setosum Harv. **30**			Love potion	M, Epilepsy, Fum, Snakebite		Chabra quoted by Neuwinger (1996), Jacot Guillarmod (1971), Lucas and Pike (1971), Neuwinger (1996), Phillips (1917), Watt and Breyer-Brandwijk (1962)
	Leaf	Decoction	Epilepsy Fumigate rooms			
	Root	Powdered and rubbed into the wound	Snakebite, roots are also mixed with the flesh of the snake and put in the patient's porridge			

Table 1 (*Continued*)

Species[a]	Plant part used	Dosage form	Traditional use	Classification of use[b]	Biological activity[b]	References
Helichrysum simillimum DC. **8**					B[d], F[d]	Mathekga (2001)[e]
Helichrysum splendidum (Thunb.) Less. **22**	Roots		Used to treat rheumatism Fuel plant in the mountains	P, Skin		Arnold et al. (2002), Dlamini (1981), Jacot Guillarmod (1971), Pooley (2003), Swanepoel (1997)
	Leaf		The leaves are boiled and the steam inhaled to induce sweating It is used together with *Senecio* species to treat pimples			
Helichrysum subglomeratum Less. **6**	Aerial parts	Smoke inhaled	Headaches	P	I	Jäger et al. (1996)[e]
Helichrysum sutherlandii Harv. **17**	Plant	Burnt, powdered plant material	Powder applied to cuts in the skin of a sick person	M	B[d], F[d]	Arnold et al. (2002), Jacot Guillarmod (1971), Mathekga (2001)[e], Phillips (1917), Pooley (1998), Pooley (2003), Watt and Breyer-Brandwijk (1962)
Helichrysum tenax M.D. Hend var. *tenax* (=*Helichrysum fulgidum* (L.f) Willd. **30**[h]		Decoction	Used for washing sore eyes	Eye	B[d], F[d]	Arnold et al. (2002) (*Helichrysum fulgidum* (L.f) Willd.)[g], Batten and Bokelmann (1966) (*Helichrysum fulgidum* Willd.)[g], Drewes et al. (2006)[e], Jacot Guillarmod (1971) (*Helichrysum fulgidum* (L.) Willd.)[g], Phillips (1917) (*Helichrysum fulgidum* Willd.)[g]
Helichrysum tomentosulum (Klatt) Merxm **1**			Used as a perfume (subsp.) *aromaticum*	P, Renal		Neuwinger (1996), Van Wyk and Gericke (2000), Von Koenen (2001)
	Twigs	Extract	Twigs are pounded in water and used as mouth wash for tooth ache			
	Plant	Smoke inhaled	The entire plant is placed on red hot coals and smoke inhaled for body pain. The same treatment is used by pregnant women suffering from antepartum haemorrhage			
	Root	Decoction	Bladder problems (dribbling) Used as thatching.			
Helichrysum trilineatum DC. **22**					B[d], F[d]	Bremner and Meyer (1998)[e], Mathekga (2001)[e]
Helichrysum umbraculigerum Less. **5**			Heavily grazed		B[d], F[d]	Mathekga (2001)[e], Pooley (1998)
Helichrysum uninervium Burtt Davy **12**			The Swazi use the plant as a purgative or an emetic. They add one teaspoon of the plant to soft porridge which is then eaten by the patient	GIT		Swanepoel (1997)

[a] Where the species name has been changed, the previously accepted name is given in brackets. The following species are no longer classified as *Helichrysum*: *Helichrysum capillaceum* (Thunb.) Less. (Phillips, 1917; Jacot Guillarmod, 1971; Watt and Breyer-Brandwijk, 1962) accepted name now *Troglophyton capillaceum* subsp. *capillaceum* (Thunb.) Hilliard and B.L. Burtt (Gibbs Russell et al., 1987); *Helichrysum orbiculare* (Thunb.) Druce (Smith, 1966) accepted name now *Plecostachys serpyllifolia* (P.J, Bergius) Hilliard and B.L.Burtt (Gibbs Russell et al., 1987); *Helichrysum sesamoides* Willd. (Smith, 1966) accepted name now *Edmondia sesamoides* (L.) Hilliard (Gibbs Russell et al., 1987); *Helichrysum vestitum* (L.) Willd. (Smith, 1966) accepted name now *Syncarpha vestita* (L.) B. Nord. (Gibbs Russell et al., 1987); *Helichrysum hochstetteri* (A. Rich) Hook. F. (Githens, 1949) and *Helichrysum stenopterum* DC. (Dlamini, 1981) accepted name now *Achyrocline stenoptera* (DC.) Hilliard (Gibbs Russell et al., 1987).

[b] Abbreviations used: A = analgesic activity determined; Anth = anthelmintic; B = antibacterial activity determined; Ca = cardiac conditions; Eye = used in eye conditions; F = antifungal activity determined; Fum = used as fumigant, often plants are burnt in room of a sick person; GIT = gastrointestinal conditions, which include mainly colic, nausea and vomiting, diarrhoea and stomach pain; I = anti-inflammatory activity determined; Infect = conditions associated with infections, such as gonorrhoea and smallpox; Inflam = conditions associated with inflammation such as swelling, menstrual pain; Insect = plants are used to deter insects such as red mites; M = used in a magical sense, to invoke the goodwill of the ancestors and as charms (protective, love); My = antimycobacterial activity determined; NS = not specified; P = conditions associated with pain, inflammation and fever, which include headache, convulsions and dysmenorrhoea; Pl = antiplasmodial (antimalarial) activity determined; Psy = psychotropic use—plants that are used to induce trances; Psyc = psychological conditions such as inuresis in children and insomnia; Poi = possible poison, mainly when stock ingest excessive amounts; Renal = conditions associated with kidney and bladder problems; Resp = respiratory conditions, which include colds, coughs, flu, tuberculosis; Skin = used for skin conditions such as keloid scars, abscesses, as ointments; W = used to dress wounds; V = antiviral activity determined; Vi = used for virility in men.

[c] The number refers to the morphological group according to Hilliard (1983).

[d] Antimicrobial activity of 1 mg/ml or less observed for one or more micro-organisms.

[e] Reference associated with biological activity.

[f] In some cases the author name, as indicated in the source, is not present in either Hilliard (1983) or Germishuizen and Meyer (2003). The current author is then chosen.

[g] In cases where the name in the source and the current name differ, the name used in the source is indicated in brackets for clarification.

[h] In cases where the old name is used to describe two different species in the current system, the uses are indicated under both the current names.

[i] Vernacular name.

In some cases, one species name was changed to another, for example *Helichrysum adscendens* Less. var. *cephaloideum* Moeser. in Watt and Breyer-Brandwijk (1962) is now known as *Helichrysum cephaloideum* DC. In other instances, a *Helichrysum* species now belongs to a different genus for example, *Helichrysum capillaceum* (Thunb.) Less. (Watt and Breyer-Brandwijk, 1962) is now classified as *Troglophyton capillaceum* subsp. *capillaceum* (Hilliard, 1983).

Sometimes the same species name with only a different author name refers to a different species, for example *Helichrysum calocephalum* Schltr, which is now classified as *Helichrysum ecklonis* and not *Helichrysum calocephalum* Klatt (Gibbs Russell et al., 1987; Germishuizen and Meyer, 2003). Batten and Bokelmann (1966), Jacot Guillarmod (1971), Phillips (1917) and Watt and Breyer-Brandwijk (1962) all used *Helichrysum calocephalum* Schltr., which is now recognised as *Helichrysum ecklonis*, but in Arnold et al. (2002) there is no reference to *Helichrysum ecklonis* yet the above-mentioned sources are used as references under *Helichrysum calocephalum* Klatt.

The specific *Helichrysum* species referred to when *Helichrysum crispum* is used in ethnobotanical literature is also ambiguous. Germishuizen and Meyer (2003) stated that *Helichrysum crispum* of authors other than (L.) D. Don. is *Helichrysum patulum* (L.) D. Don. and not *Helichrysum crispum* (L.) D. Don. In Watt and Breyer-Brandwijk (1962) and Smith (1966), the name appears as *Helichrysum crispum* Less. therefore indicating *Helichrysum patulum*, although Arnold et al. (2002) cited the name *Helichrysum crispum* (L.) D. Don. (with reference to Smith, 1966) as well as *Helichrysum patulum* with reference to Watt and Breyer-Brandwijk (1962). Roberts (1990) used *Helichrysum crispum* without an author name, causing uncertainty as to which particular species is referred to; the cited medicinal uses are however similar to those indicated by Watt and Breyer-Brandwijk (1962) for *Helichrysum crispum* Less. Salie and co-workers (1996) determined that *Helichrysum crispum* (L.) D. Don. had weak (10 mg/ml) antimicrobial activity against *Pseudomonas aeruginosa*. Both Salie et al. (1996) and Swanepoel (1997) use the name *Helichrysum crispum* (L.) D. Don., but when indicating its traditional uses refer to Watt and Breyer-Brandwijk (1962). Scott et al. (2004) showed that *Helichrysum patulum* had antimicrobial activity against *Staphylococcus aureus* in the disc diffusion assay that was comparable to that of the ciprofloxacin control, while the traditional uses indicated correspond very well to those reported in Watt and Breyer-Brandwijk (1962) for *Helichrysum crispum* Less. Both species occur in the same region making exclusion of one species on the basis of distribution impossible.

Helichrysum pedunculare DC. is another name with an unfortunate and confusing history. In this case, it seems that *Helichrysum pedunculare* DC. in ethnobotanical literature could refer to either *Helichrysum pedunculatum* Hilliard and Burtt or *Helichrysum nudifolium* var. *pilosellum* (previously known as *Helichrysum pedunculare* (L.) DC. var. *pilosellum*) (Hilliard, 1983; Arnold et al., 2002; Germishuizen and Meyer, 2003). The vernacular name and uses indicated by for example Watt and Breyer-Brandwijk (1962) for *Helichrysum pedunculare* DC. and Bhat and Jacobs (1995) for *Helichrysum pedunculatum* Hilliard and Burtt. is similar. According to Hilliard (1983), *Helichrysum pedunculare* (L.) DC. is also a synonym for *Helichrysum odoratissimum* (L.) Sweet.

In some instances it is impossible to decide to which species an author refers, for example *Helichrysum agrostophilum* Klatt (Watt and Breyer-Brandwijk, 1962) that was in part changed to *Helichrysum pallidum* DC. and in part to *Helichrysum griseum* Sond (Germishuizen and Meyer, 2003).

2.2. Administration routes

Plant parts used include the leaves, stems, flowers, roots and sometimes the whole plant. The plant remedies are administered in different ways, including the preparation of teas, inhalation of smoke and vapours and placement of leaves in the form of a poultice on wounds to prevent infection (Table 1). Several of these species are known by the same vernacular names, for example *Helichrysum cymosum*, *Helichrysum nudifolium*, *Helichrysum odoratissimum* and *Helichrysum petiolare* are all known as *imphepho* which indicates that they can be used interchangeably, as Van Wyk et al. (2000), noted that "use often depends on local availability rather than preference for a particular species".

2.3. Traditional uses of South African Helichrysum species

The traditional uses of *Helichrysum* in South Africa are summarised in Table 1. There are several recurring South African traditional uses for plants from this genus. Smoke is often inhaled to induce trances or to invoke the goodwill of the ancestors. They are often used to treat respiratory conditions and leaves are often applied as wound dressings. They are used in the treatment of gastro-intestinal disorders such as abdominal pain and colic and also eye conditions. They also seem to have an effect on the relief of pain and inflammation as they are used to treat menstrual pain, rheumatism and headaches. The plants are used to fumigate huts and also used as bedding to repel insects.

2.4. Correlation between medicinal uses and morphological groups

Plants from almost all morphological groups are used medicinally and the broad spectrum of uses are not restricted to a specific morphological group. In some cases there does seem to be a relationship between the morphological group (according to Hilliard, 1983) or adjacent morphological classes and the traditional uses of these plants. *Helichrysum epapposum* (group 3), *Helichrysum natalitium* (group 3), *Helichrysum gymnocomum* (group 4) and *Helichrysum odoratissimum* (group 4) all share the Zulu/Xhosa name *imphepho* and all are burnt as incense to invoke the goodwill of the ancestors. However, this particular use applies to many species such as, *Helichrysum cymosum* (group 8), *Helichrysum petiolare* (group 18), *Helichrysum dregeanum* (30) and in some sources *Helichrysum nudifolium* (23) share the same vernacular name and use. *Helichrysum cymosum* (group 8), *Helichrysum kraussii* (group 8), *Helichrysum melanacme* (group 8), *Helichrysum athrixiifolium* (group 9) and *Helichrysum dregeanum* (group 9) are all used to treat respiratory complaints such as coughs and colds. The administration route of these remedies do however vary; for *Helichrysum arthrixiifolium* the leaf is smoked, for *Helichrysum kraussii* the dried flowers and seeds are smoked in a pipe, for *Helichrysum cymosum* a decoction of leaves is drunk, for *Helichrysum dregeanum* the leaf is smoked and the administration route is not indicated in the source for *Helichrysum melanacme*.

South African species are not often used to treat heart and kidney ailments. Both *Helichrysum pandurifolium* and *Helichrysum patulum* belongs to group 18, and are indicated in the treatment of kidney disease and heart disorders. Both are also used to treat backpain and respiratory conditions by the same administration route. Plants from groups 23 and 24 are often used to treat wounds. The leaves of *Helichrysum miconiifolium* (group 23), *Helichrysum nudifolium* (group 23), *Helichrysum pedunculatum* (group 23), *Helichrysum appendiculatum* (group 24) and *Helichrysum longifolium* (group 24) are all used as wound dressings. However, *Helichrysum foetidum* (group 30) is mentioned as a replacement for *Helichrysum pedun-*

culatum in the treatment of circumcision wounds (Gerstner, 1938). The species constituting group 23 are also used for respiratory conditions including, *Helichrysum mundtii, Helichrysum nudifolium* and *Helichrysum pedunculatum*. Root decoctions of both *Helichrysum adenocarpum* and *Helichrysum ecklonis* belonging to group 28 are used to treat diarrhoea in children, while a root infusion from *Helichrysum argyrophyllum* (group 29) is used to treat intestinal troubles. It is interesting to note that the only two species indicated for the treatment of snakebite both belong to group 30, namely *Helichrysum cooperi* and *Helichrysum setosum*.

3. Phytochemistry

The chemistry of this genus is complex with a wide variety of chemical classes occurring as is evident from the three major publications by Bohlmann and Jakupovic (Bohlmann and Zdero, 1980a; Jakupovic et al., 1986; Jakupovic et al., 1989) in which a total of 63 South African *Helichrysum* species were investigated chemically. The classes of compounds isolated from the South African *Helichrysum* species are summarised in Table 2 and Fig. 1. Acylphloroglucinols (**1**–**3**) are common, often with prenyl or geranyl side chains. The replacement of the cinnamic moiety by other acyl CoA derivatives in the biosynthesis of the main constituents seem to be characteristic (Jakupovic et al., 1989). The presence of humulone derivatives, such as helihumulone (**4**) is also widespread (Jakupovic et al., 1989).

Flavonoids (**5**–**11**) derived from phloroglucinol are very common and often have unsubstituted B rings (Bohlmann and Abraham, 1979a,d; Jakupovic et al., 1986) which is a characteristic feature of plants from the Inuleae tribe (Harborne, 1977). The presence of 6- and 8-hydroxyflavonols and their methyl ethers (**7**) are also frequent as in other members of the tribe (Harborne, 1977). A wide variety of chalcones (**8**–**10**) are also found, including dihydrochalcones (**11**), pyranochalcones (**10**) and those substituted with prenyl (**9**) or geranyl groups. As in other Inuleae species, these chalcones are often accompanied by their structurally and biogenetically related flavanones (Harborne, 1977) as can be seen for *Helichrysum acutatum* (**5** and **8**) (Bohlmann and Abraham, 1979c), *Helichrysum cymosum* (Jakupovic et al., 1989) and *Helichrysum oreophilum* (Jakupovic et al., 1986).

The presence of α-pyrones (**12**) is rather common (they occur in plants from morphologic groups 1, 2, 4, 12, 15, 18, 19 and 24) and they are often isolated from the roots of these plants (Hänsel et al., 1980; Jakupovic et al., 1986, 1989). Different types of diterpenes occur; these include the kaurenoic acid type (**15**, Jakupovic et al., 1989) as well as those derived from helifulvanic acid (**13**, Bohlmann et al., 1980b). Sesquiterpenes representing a variety of skeletal types occur, as is characteristic for the rest of the family (Hegnauer, 1977). Some skeletal types, such as the humulenes, are widely distributed across the genus, whereas others such as the guaianolides (**16**) are restricted to a few species (morphological groups 10 and 22). *Helichrysum* species are known for their aromaticity and a variety of monoterpenes are reported in the essential oils of some species (Lourens et al., 2004; Frum and Viljoen, 2006; Van Vuuren et al., 2006; Asekun et al., 2007). Squalene is the most common triterpene found and is often in high concentration.

Another unusual type of compound that occurs is thiophene derivatives (**17**, **18**) which have been isolated from the roots of species such as *Helichrysum acutatum* and *Helichrysum tenuifolium*. These thiophenes are the result of addition reactions of a common chloro-acetylene precursor with H_2S (Bohlmann and Abraham, 1979b,c). Simple polyacetylenes (**20**) are widespread. Acetylenics with pyran (**19**) and furan moieties, some with epoxy and/or chlorine substitution, occur in these plants and is characteristic of the Gnaphaliineae (Harborne, 1977).

As for the traditional uses, one particular class of compound is not restricted to a particular morphologic group. However, there are some compounds that occur mainly in a specific morphological class. For example, phloroglucinols (excluding those belonging to the flavonoid class) feature as major compounds in morphological classes 2, 3, 4, 12, 14, 15, 20, 24 and 28. Flavonoids are present in almost all of the morphological groups, but a large number are found in plants from groups 8, 9 and 27. Diterpenes were isolated in large quantities from species in groups 23, 25 and 30 (all 10 plants investigated in this group had this type of compound as the major chemical species). *Helichrysum umbraculigerum* from group 5 seems to be the only species investigated that contains compounds of the cannabigerol type (**21**) as the major constituent and *Helichrysum dasyanthum* (group 10) and *Helichrysum splendidum* (group 22) contain mainly sesquiterpenes of the guaianolide type (which is absent in the other species). Plants from groups 6, 18 and 19 are also rich in sesquiterpenes (Table 2).

Although there seem to be similarities in the chemistry of the European and South African species, the Australian species are chemically different from their South African counterparts (Jakupovic et al., 1989, 1989a).

4. Biological activity

4.1. Anti-infective activity

Considering the traditional uses of this genus (specifically the treatment of wounds and respiratory tract infections and the application as a fumigant for example), there seems to be a strong indication that these plants and their compounds should exhibit antimicrobial activity. Several studies on the antimicrobial activity of *Helichrysum* species was done by the group of Meyer from the University of Pretoria, South Africa. Extracts of several species (Table 1) were submitted to antibacterial testing using a group of randomly selected bacteria which normally included the Gram-positive bacteria: *Bacillus cereus*, *Bacillus pumilis*, *Bacillus subtilis*, *Micrococcus kristinae*, *Staphylococcus aureus* and the Gram-negatives: *Enterobacter cloaceae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Serratia marcescens*. Acetone extracts were mostly tested, but in a few cases methanol, water and dichloromethane extracts were used. Assays involving agar dilution and direct autobiography were employed (Meyer and Afolayan, 1995; Meyer and Dilika, 1996; Afolayan and Meyer, 1997; Dilika et al., 1997; Mathekga and Meyer, 1998; Bremner and Meyer, 2000; Mathekga et al., 2000; Mathekga, 2001). Antifungal activity was also determined for fungi such as *Aspergillus flavus*, *Aspergillus niger*, *Cladosporium cladosporioides*, *Cladosporium cucumerinum*, *Cladosporium sphaerospermum* and *Phytophthora capsici* (Mathekga, 2001).

Reports on antimicrobial activities from other laboratories include the relatively weak antimicrobial activity (Gibbons, 2004) documented for extracts of *Helichrysum foetidum* (MIC's of more than 4 mg/ml against all selected bacteria in the 96-well plate assay, Steenkamp et al. (2004) and *Helichrysum crispum* (L.) D. Don. where MIC's of 10 mg/ml were reported against *Pseudomonas aeruginosa* and *Candida albicans* (Salie et al., 1996). Activities ranging from 0.078 to 0.3 mg/ml against Gram-positive bacteria, Gram-negative bacteria and yeasts were also reported for a *Helichrysum cymosum* extract (Van Vuuren et al., 2006). Acetone and methanol extracts of *Helichrysum odoratissimum* (incorrectly identified as *Helichrysum dasyanthum* in Lourens et al., 2004), *Helichrysum excisum*, *Helichrysum felinum* and *Helichrysum petiolare* displayed activity against *Staphylococcus aureus* and *Bacillus cereus*. The species with the best

Table 2
Classes of compounds isolated from South African *Helichrysum* species

Species	Morphologic group	Flavonoid derivatives[a,b]						Phloroglucinols[b]	Pyrones[b]	Diterpenes[b]	Terpenes[b]	Other[b]	Reference
		A	B	C	D	E	F						
Helichrysum acutatum DC.[c] Roots and aerial parts	21	X	X							X	X	X	Bohlmann and Abraham (1979c)
Helichrysum adenocarpum DC.[c] Roots and aerial parts	28											X	Bohlmann et al. (1980a)
Helichrysum albirosulatum Killick Roots and aerial parts	6									X	X		Bohlmann et al. (1980a), Bohlmann et al. (1978a)
Helichrysum allioides Less. Roots	23											X	Bohlmann and Zdero (1973)
Helichrysum anomalum Less. Aerial parts	9							X			X		Jakupovic et al. (1989)
Helichrysum appendiculatum (L.f.)[c] Less. Aerial parts	24										X		Bohlmann et al. (1980a)
Helichrysum argentissimum J.M. Wood. Roots	28									X	X	X	Bohlmann et al. (1980a)
Helichrysum argyrolepis MacOwan Aerial parts	29		X	X									Bohlmann et al. (1984)
Helichrysum argyrophyllum DC.[c] Aerial parts and roots	29				X					X	X	XX	Jakupovic et al. (1989), Bohlmann and Zdero (1973)
Helichrysum asperum (Thunb.) Hilliard et Burtt. var. *albidulum* (DC.) Hilliard Aerial parts	12							XXXX					Jakupovic et al. (1989)
Helichrysum athrixiifolium (Kuntze)[c] Moeser Aerial parts and roots	9	X	X										Bohlmann and Ates (1984)
Helichrysum aureonitens Sch. Bip.[c] Roots and aerial parts	8				X						XX	X	Bohlmann and Ziesche (1979), Afolayan and Meyer (1997), Meyer et al. (1997)
Helichrysum aureum (Houtt.) Merr.[c] Aerial parts and roots	30									XX	X		Jakupovic et al. (1989)
Helichrysum aureum (Houtt.) Merr. var. *monocephalum* (DC.) Hilliard Aerial parts and roots	30									XX	X		Bohlmann et al. (1978a)
Helichrysum auriceps Hilliard Roots	24							X	X			X	Bohlmann and Zdero (1980)
Helichrysum bellum Hilliard Aerial parts and roots	28							X		X	X	X	Bohlmann and Zdero (1979a)
Helichrysum caespititium DC. Harv.[c] Whole plant Aerial parts	12							X					Dekker et al. (1983), Mathekga et al. (2000)

Table 2 (*Continued*)

Species	Morphologic group	Flavonoid derivatives[a,b]						Phloroglucinols[b]	Pyrones[b]	Diterpenes[b]	Terpenes[b]	Other[b]	Reference
		A	B	C	D	E	F						
Helichrysum callicomum Harv.[c] Aerial parts and roots	2	X						XX	X	X	XX	X	Bohlmann and Abraham (1979a) (*Helichrysum calliconum*[d]) Bohlmann et al. (1984)
Helichrysum candolleanum H. Buek Aerial parts	15							X		X			Jakupovic et al. (1989)
Helichrysum cephaloideum DC. Roots and aerial parts	24				X			XXX	XX		X		Hänsel et al. (1980), Bohlmann and Zdero (1980), Jakupovic et al. (1986)
Helichrysum cerastioides DC. Aerial parts	15							X	X			X	Bohlmann et al. (1984), Jakupovic et al. (1989) (*Helichrysum cerastroides* DC. supsp. a*urosicum* Merxm. et A. Schreiber[d])
Helichrysum chionosphaerum DC. Aerial parts and roots	25				X					XXX	XX	X	Bohlmann et al. (1980b), Jakupovic et al. (1989)
Helichrysum chrysargyrum Moeser Aerial parts and roots	22				X			XX			X		Bohlmann et al. (1979a)
Helichrysum confertum N.E.Br Roots	17									XX			Bohlmann et al. (1978a)
Helichrysum cooperi Harv.[c] Roots and aerial parts	30					X				X			Bohlmann et al. (1978a)
Helichrysum cooperi ps. aff. *Helichrysum cooperi* Harv.	?		X										Wright (1976)
Helichrysum cymosum (L.) D. Don.[c] Aerial parts	8	XX	X	X	X							X	Jakupovic et al. (1989)
Helichrysum cymosum (L.) Don. ssp.[c] *calvum* Roots and aerial parts	8		XX	X							X		Bohlmann et al. (1979b)
Helichrysum cymosum (L.) D. Don.[c] ssp. *cymosum*	8			X									Van Vuuren et al. (2006)
Helichrysum dasyanthum (Willd.) Sweet Aerial parts	10				X					X	XX	X	Jakupovic et al. (1989)
Helichrysum dasymallum Hilliard (=*Helichrysum lanatum* Harv.) Roots	21											X	Bohlmann and Zdero (1973) (*Helichrysum lanatum* DC.[d])
Helichrysum decorum DC.[c] Aerial parts	30		X										Bohlmann et al. (1980a)
Helichrysum drakensbergense Killick Roots and aerial parts	19								X	X	XX	X	Bohlmann and Suwita (1979)
Helichrysum dregeanum Sond. and Harv.[c] Aerial parts	9		X		X			X			X		Jakupovic et al. (1989)
Helichrysum felinum Less. Aerial parts	17		X		X			X					Jakupovic et al. (1989)

Helichrysum flanaganii Bolus[c] Aerial parts and roots	13						X						Bohlmann et al. (1980a)
Helichrysum foetidum (L.) Moench.[c] Roots	30											X	Bohlmann and Zdero (1973)
Helichrysum fulvum N. E. Br. Aerial parts and roots	30								XX			X	Bohlmann et al. (1979c)
Helichrysum glaciale Hilliard Aerial parts	27	X											Bohlmann et al. (1980a)
Helichrysum glomeratum Klatt Aerial parts	6		X						X	XX			Bohlmann and Suwita (1979)
Helichrysum grandiflorum (L.) D. Don. Roots	17											X	Bohlmann and Zdero (1973)
Helichrysum gymnocomum DC.[c] Roots and aerial parts	4						XX			X			Bohlmann and Mahanta (1979) (*Helichrysum gymnoconum* DC.[d])
Helichrysum gymnocomum DC.[c] Flowers	4	X	X				X						Drewes and Van Vuuren (2008)
Helichrysum herbaceum (Andrews) Sweet[c] Aerial parts	29	X			XX					X			Bohlmann et al. (1979a)
Helichrysum heterolasium Hilliard Aerial parts	30		X	X					XX	XX		X	Bohlmann and Abraham (1979a)
Helichrysum hyphocephalum Hilliard Aerial parts and roots	27	XX				X				X		X	Bohlmann and Abraham (1979d) (*Helichrysum hypocephalum* Hilliard[d])
Helichrysum indicum (L.) Grierson[c] Aerial parts	15						X						Jakupovic et al. (1989)
Helichrysum infaustum J.M. Wood. and M.S. Evans Aerial parts	4						X			X			Bohlmann and Suwita (1979)
Helichrysum kraussii Sch. Bip.[c] Aerial parts, flowers and roots	8		X	X	X		X		X	XX			Jakupovic et al. (1989), Bremner and Meyer (2000), Candy et al. (1975), Candy and Wright (1975)
Helichrysum krebsianum Less. Aerial parts	23								X				Bohlmann et al. (1980a)
Helichrysum krookii Moeser Roots and aerial parts	5						XX			X		X	Bohlmann et al. (1980a)
Helichrysum lepidissimum S. Moore Aerial parts	19	X				X		X				X	Jakupovic et al. (1989)
Helichrysum litorale Bolus[c] (=*Leontonyx angustifolius* DC., =*Leontonyx spathulatus* Less.)	14						XX			X			Bohlmann and Suwita (1978) (*Leontonyx spathulatus* Less.[d])

Table 2 (*Continued*)

Species	Morphologic group	Flavonoid derivatives[a,b]						Phloroglucinols[b]	Pyrones[b]	Diterpenes[b]	Terpenes[b]	Other[b]	Reference
		A	B	C	D	E	F						
Helichrysum melanacme (DC.) Harv.[c] Shoots	8		X		X								Lall et al. (2006)
Helichrysum miconiifolium DC.[c] Roots	23									X			Bohlmann et al. (1980a)
Helichrysum mimetes S. Moore Aerial parts	19					X			X	X	XX		Jakupovic et al. (1986)
Helichrysum mixtum (Kuntze.) Moeser Roots	24							XX	XX				Jakupovic et al. (1986)
Helichrysum moeseranium Thell. Aerial parts	22							X		X			Jakupovic et al. (1989)
Helichrysum monticola Hilliard Aerial parts and roots	28			X				XX			X		Jakupovic et al. (1989), Bohlmann and Zdero (1980a)
Helichrysum mundtii Harv.[c] (=*Helichrysum mundii* Harv.)[d] Roots and aerial parts	23			X		X				X	X		Bohlmann et al. (1978b), Bohlmann et al. (1980a)
Helichrysum nanum Klatt Aerial parts	6							X		X	X		Bohlmann and Suwita (1979)
Helichrysum natalitium DC.[c] Aerial parts and roots	3							XX			X	X	Bohlmann and Zdero (1979a)
Helichrysum nudifolium L. Less. var. *nudifolium*[c] Aerial parts and roots	23							XX		X	XXX	X	Jakupovic et al. (1986), Bohlmann et al. (1978a) (*Helichrysum nudifolium* L. Less.[d]) Bohlmann and Zdero (1973) (*Helichrysum nudifolium* L. Less.[d])
Helichrysum nudifolium var. *oxyphyllum* (=*Helichrysum oxyphyllum* DC.)[c] Aerial parts	23	X	X							X	X		Bohlmann et al. (1980a) (*Helichrysum oxyphyllum* Klatt[d])
Helichrysum nudifolium var. *pilosellum* (=*Helichrysum latifolium* Less.)[c]	23											X	Bohlmann and Zdero (1973) (*Helichrysum latifolium* Less.[d])
Helichrysum nudifolium var. *pilosellum* (=*Helichrysum pilosellum* (L.f.) Less.) Roots	23									XX			Jakupovic et al. (1986) (*Helichrysum pilosellum* (L.f.) Less.[d])
Helichrysum odoratissimum (L.) Sweet[c] Aerial parts and roots	4		X		X			XX	X	X		X	Van Puyvelde et al. (1989), Hänsel et al. (1980), Bohlmann and Zdero (1973)
Helichrysum oreophilum Klatt. Aerial parts	21	X	X					X		X	X		Jakupovic et al. (1986), Bohlmann et al. (1980a)
Helichrysum pagophilum M.D. Hend. Aerial parts	27				X								Bohlmann et al. (1980a)

Helichrysum pallidum DC.[c] Aerial parts and roots	23										XX	X		Bohlmann et al. (1980a)
Helichrysum panduratum O.Hoffm.[c] Aerial parts	18											X		Bohlmann and Abraham (1979b)
Helichrysum patulum (L.) D. Don.[c] (=*Helichrysum crispum* Less.) Aerial parts	18							XX				X		Bohlmann and Suwita (1979) (*Helichrysum crispum* Less.[d])
Helichrysum pedunculatum Hilliard and Burtt[c] Leaves	23												X	Dilika et al. (2000)
Helichrysum petiolare Hilliard and B.L. Burtt.[c] Aerial parts	18		X			X		X	X			XX	X	Jakupovic et al. (1989), Bohlmann and Zdero (1973) (*Helichrysum petiolatum* DC.[d])
Helichrysum platypterum DC.[c] Aerial parts and roots	20				X	X	X	XXX			X		X	Jakupovic et al. (1986), Bohlmann et al. (1980a), Bohlmann and Zdero (1979a), Jakupovic et al., 1987
Helichrysum polycladum Klatt Aerial parts and roots	8	X	X	X				X					X	Bohlmann et al. (1980a)
Helichrysum populifolium DC. Roots	16												X	Bohlmann et al. (1980a)
Helichrysum reflexum N. E. Br. Aerial parts	29										XX	XX		Bohlmann et al. (1985) (*Helichrysum refluxum* N. E. Br.[d])
Helichrysum revolutum (Thunb.) Less. Aerial parts	9		X		X			X						Jakupovic et al. (1989)
Helichrysum retortoides N.E. Br. Aerial parts	26												X	Bohlmann et al. (1980a)
Helichrysum rosum (Berg.) Less. Aerial parts	9							X					X	Jakupovic et al. (1989)
Helichrysum nudifolium L. Less. var. nudifolium (=Helichrysum coriaceum Harv.) Roots	23										XX	X	X	Bohlmann et al. (1984a) (*Helichrysum coriaceum* Harv.[d])
Helichrysum ruderale Hilliard and B.L. Burtt. Aerial parts	30										X			Bohlmann et al. (1980a)
Helichrysum rugulosum Less.[c] Aerial parts and roots	9	XX	XX											Bohlmann and Misra (1984)
Helichrysum scabrum (Thunb.) Less. Aerial parts	9		X					X						Jakupovic et al. (1989)
Helichrysum setosum Harv.[c] Aerial parts	30										XX			Jakupovic et al. (1986)
Helichrysum spiralepis Hilliard and Burtt. (=*Leontonyx squarrosus*)	14							XX					X	Bohlmann and Suwita (1978) (*Leontonyx squarrosus*[d])

Table 2 (*Continued*)

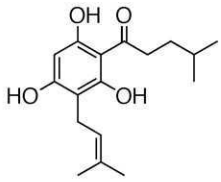
Species	Morphologic group	Flavonoid derivatives[a,b]						Phloroglucinols[b]	Pyrones[b]	Diterpenes[b]	Terpenes[b]	Other[b]	Reference
		A	B	C	D	E	F						
Helichrysum splendidum (Thunb.) Less.[c] Aerial parts and roots	22			X	X						XXX	X	Bohlmann and Suwita (1979a), Jakupovic et al. (1989)
Helichrysum subfalcatum Hilliard Aerial parts	6									X	X		Bohlmann et al. (1980a)
Helichrysum subglomeratum Less.[c] Aerial parts	6		X	X							X		Jakupovic et al. (1989)
Helichrysum sutherlandii Harv. (=*Helichrysum suterlandii* Harv.)[c] Aerial parts and roots	17		X	X						X		X	Bohlmann et al. (1978b), Bohlmann et al. (1980a)
Helichrysum swynnertonii S. Moore Aerial parts and roots	25											X	Bohlmann et al. (1980a)
Helichrysum tenax var. *tenax* M.D. Hend. Leaves	30									XX			Drewes et al. (2006)
Helichrysum tenuiculum DC. Aerial parts and roots	8	X	X	X							X		Bohlmann et al. (1979b)
Helichrysum tenuifolium Killick. Aerial parts and roots	22	X		X	X	X	X				X	XX	Bohlmann and Abraham (1979b)
Helichrysum thapsus (O. Kuntze) Moeser Aerial parts	23	X					X						Bohlmann and Zdero (1983)
Helichrysum tomentosulum Klatt. Merxm subsp. *aromaticum* (Dinter) Merxm.[c] Aerial parts	1		X		X								Jakupovic et al. (1989)
Helichrysum tricostatum (Thunb.) Less. Aerial parts	11				X								Jakupovic et al. (1989)
Helichrysum trilineatum DC. Shoots and roots	22										X	X	Bremner and Meyer (1998), Bohlmann et al. (1980a)
Helichrysum umbraculigerum Less. Aerial parts	5		X								X	XXX	Bohlmann and Hoffmann (1979)
Helichrysum vernum Hilliard Roots	28									X	X		Bohlmann et al. (1980a)
Helichrysum zeyheri Less. Aerial parts	1							X	X		X		Jakupovic et al. (1986)

[a] A = flavanone, B = chalcone, C = dihydrochalcone, D = flavonol, E = flavone, F = other flavonoids.

[b] X = 3 or less compounds isolated; XX = 4–9 compounds isolated; XXX = 10 or more compounds isolated, XXXX = more than 20 compounds isolated.

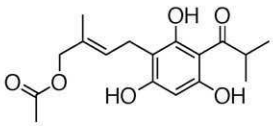
[c] Used in traditional medicine.

[d] Name as used in reference.



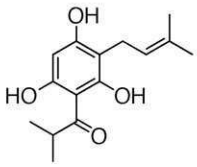
1

Acylphloroglucinol, from *H. caespititium*



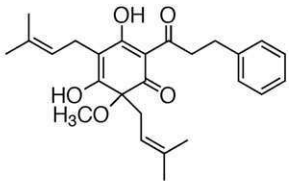
2

Acylphloroglucinol, from *H. caespititium*



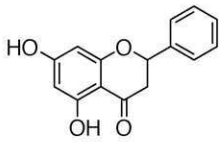
3

Acylphloroglucinol from *H. asperum, H. flanaganii, H. gymnocomum, H. infaustum*



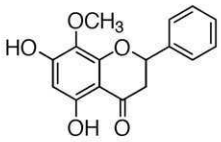
4

Helihumulone from *H. argyrolepis, H. cymosum, H. subglomeratum*



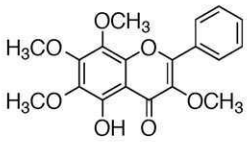
5

Flavanone from *H. acutatum, H. callicomum, H. cymosum, H. oreophilum*



6

Flavanone from *H. cymosum*

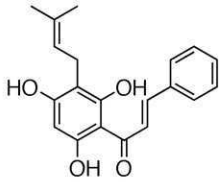


7

Flavone from *H. cephaloideum*

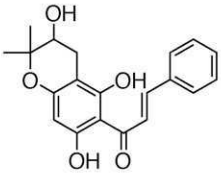
8

Chalcone from *H. acutatum, H. cymosum, H. kraussii, H. oreophilum*



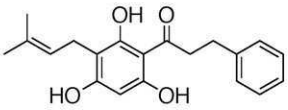
9

Prenylated chalcone from *H. melanacme*



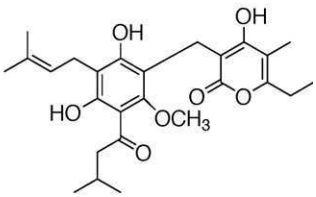
10

Pyranochalcone from *H. melanacme*



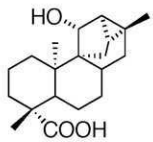
11

Dihydrochalcone from *H. argyrolepis*



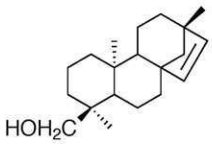
12

Phloroglucinol with pyrone moiety from *H. cephaloideum*



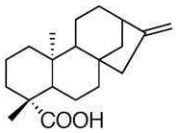
13

Diterpene (helifulvanic acid type) from *H. fulvum*



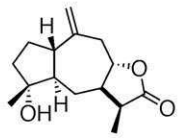
14

Diterpene (beyerenoic acid type) from *H. tenax*



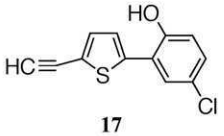
15

Diterpene (kaurenoic acid) from *H. argentissimum, H. kraussii*



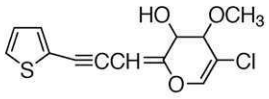
16

Sesquiterpene of the guaianolide type from *H. splendidum*



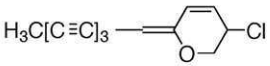
17

Acetylenic thiophene from *H. tenuifolium, H. trilineatum*



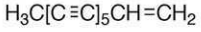
18

Acetylenic thiophene from *H. acutatum*



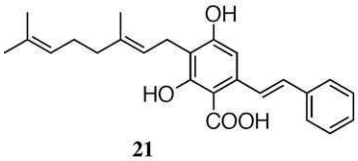
19

Acetylene from *H. allioides, H. argenteum*



20

Acetylene from *H. adenocarpum, H. callicomum*



21

Cannibigerol type compound from *H. umbraculigerum*

Fig. 1. Compounds isolated from South African *Helichrysum* species.

activity was the acetone extract of *Helichrysum odoratissimum* with an MIC of 0.016 mg/ml against *Staphylococcus aureus* (which correlates well with the values obtained by Mathekga and Meyer, 1998).

Helichrysum species are often used to treat respiratory conditions and tuberculosis (Table 1). Extracts of *Helichrysum odoratissimum* and *Helichrysum melanacme* showed activity against *Mycobacterium tuberculosis* at concentrations of 0.5 mg/ml (Lall and Meyer, 1999; Lall et al., 2006). The acetone extract of *Helichrysum caespititium* inhibited a drug sensitive-strain of *Mycobacterium tuberculosis* at a concentration of 0.5 mg/ml in the agar plate method and a MIC of 0.1 mg/ml was observed using the rapid radiometric method (Meyer et al., 2002). The water extract caused partial inhibition at the highest concentration of 5 mg/ml.

In some cases the antimicrobial activity of isolated compounds was determined. Flavonoids are generally one of the largest classes of antibacterial compounds (Gibbons, 2004). Galangin (3,5,7-trihydroxyflavone) isolated from *Helichrysum aureonitens* (Meyer and Afolayan, 1995), inhibited the growth of four Gram-positive bacteria (three *Bacillus* species and *Micrococcus kristinae*) as well as the Gram-negative *Enterobacter cloaceae* (Afolayan and Meyer, 1997). The highest activity observed was against *Bacillus cereus*, *Micrococcus kristinae* and *Enterobacter cloaceae* at 0.1 mg/ml. In other studies by Cushnie et al. (2003) and Cushnie and Lamb (2006), the activity of galangin was shown against six strains of β-lactam sensitive and resistant strains of *Staphylococcus aureus* and 16 strains of 4-quinolone resistant strains of the bacterium at MIC's of approximately 50 μg/ml. Galangin also displays some antifungal activity against fungi such as *Aspergillus tamari* (35% growth inhibition at 0.5 mg/ml) (Afolayan and Meyer, 1997). These results support the use of *Helichrysum aureonitens* in the treatment of skin infections, often caused by *Staphylococcus aureus*.

Another flavonoid, 3-*O*-methylquercetin was isolated from *Helichrysum odoratissimum* and antimicrobial activity determined for a broad range of micro-organisms including Gram-negative bacteria such as *Salmonella typhimurium* (MIC = 50 μg/ml), Gram-positive bacteria, such as *Staphylococcus aureus* (MIC = 6.25 μg/ml) and fungi, for example *Candida albicans* (MIC = 12.5 μg/ml), in the microdilution method (Van Puyvelde et al., 1989). Bremner and Meyer (1998) also reported on the anti-staphylococcal activity for pinocembrin chalcone (**8**, isolated from *Helichrysum trilineatum*), as well as pinocembrin (**5**) that was obtained as an artifact during the isolation procedure. Flavonoids isolated from the flowers of *Helichrysum gymnocomum* exhibited promising antimicrobial activity against a wide variety of Gram-positive and Gram-negative organisms as well as yeasts. An MIC of 8 μg/ml was for example observed against *Cryptococcus neoformans* for 5,7-dibenzyloxyflavanone (Drewes and Van Vuuren, 2008). Two chalcones isolated from *Helichrysum melanacme* (**9**, **10**) exhibited MIC's of 0.05 mg/ml against the drug sensitive H37Rv strain of *Mycobacterium tuberculosis*. The activity of the chalcones was higher than that of the crude extract but a combination of the two chalcones did not result in an improved MIC (Lall et al., 2006).

There are also reports on the antimicrobial activity of compounds other than flavonoids. Activity against Gram-positive bacteria was observed for both linoleic and oleic acids, isolated from antibacterial extracts of *Helichrysum pedunculatum* (a plant used to treat circumcision wounds, Dilika et al., 2000). The MIC of both fatty acids was 1.0 mg/ml for *Staphylococcus aureus* and *Micrococcus kristinae* in the agar diffusion assay. The MIC's was 0.05 mg/ml of each fatty acid when they were administered at the same time (Dilika et al., 2000).

Kaurenoic acid (**15**, a diterpene), isolated from *Helichrysum kraussii*, exhibited a MIC as low as 1 μg/ml against *Escherichia coli* and MIC's of 10 μg/ml against *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus* and *Serratia marcescens* (Bremner and Meyer, 2000). Significant antimicrobial activity was also observed for monomeric (**14**) and dimeric diterpenes from *Helichrysum tenax* var. *tenax*. MIC values as low as 3.1 and 3.6 μg/ml were determined against *Bacillus cereus* whereas MIC's as low as 41.5 μg/ml were determined for a Gram-negative organism such as *Pseudomonas aeruginosa* (Drewes et al., 2006).

MIC's of 100 μg/ml were observed for prenylated butyrylphloroglucinol (**3**) isolated from *Helichrysum kraussii* against *Bacillus cereus*, *Bacillus pumilis*, *Bacillus subtilis*, *Micrococcus kristinae*, *Staphylococcus aureus*, *Serratia marcescens* and *Escherichia coli* in an agar diffusion assay (Bremner and Meyer, 2000). The same phloroglucinol was isolated from *Helichrysum gymnocomum* and MIC's of below 100 μg/ml (6–45 μg/ml) were reported for *Enterococcus faecalis*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Cryptococcus neoformans* and *Candida albicans* (Drewes and Van Vuuren, 2008). A difference in assays employed, inoculum size and possibly different strains of the same micro-organism used may account for the observed difference in activity. A structurally related phloroglucinol also exhibited promising antibacterial activity against *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Cryptococcus neoformans* (Drewes and Van Vuuren, 2008). Caespitin (**1**) and caespitate (**2**) (both phloroglucinols) exhibited antimicrobial activity against several bacteria as well as fungi (Dekker et al., 1983; Mathekga et al., 2000). Caespitin (**1**) was active against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Cryptococcus neoformans*, *Trichophyton rubrum*, *Trichophyton mentagrophytes* and *Microsporum canis* although neither the method used nor the level of activity, are indicated in the relevant article (Dekker et al., 1983). Caespitate (**2**), exhibited antibacterial activity against the Gram-positive *Bacillus cereus*, *Bacillus pumilis*, *Bacillus subtilis*, *Micrococcus kristinae* and *Staphylococcus aureus* at concentrations of 0.5 μg/ml in the agar dilution method (Mathekga et al., 2000). This compound also exhibited antifungal activity which ranged from 0.5 to 1.0 μg/ml against *Aspergillus flavus*, *Aspergillus niger*, *Cladosporium cladosporioides*, *Cladosporium cucumerinum*, *Cladosporium sphaerospermum* and *Phytophtora capsici* (Mathekga et al., 2000). Caespitate (**2**) was also active against several *Mycobacterium tuberculosis* strains at a concentration of 0.1 mg/ml which was similar to the MIC observed for the crude extract of *Helichrysum caespititium* (Meyer et al., 2002).

Several caespitin derivatives were synthesised with MIC values as low as 2 μg/ml against *Staphylococcus aureus* and *Streptococcus pyogenes*. These compounds also exhibit antifungal activity. The possible development of antimicrobial resistance was examined as well as the development of cross resistance with known antimicrobials (Van der Schyf et al., 1986). For helihumulone (**4**), a phloroglucinol of the humulone type, activity was exhibited for a broad range of micro-organisms with some promising results, for example 16 μg/ml against *Pseudomonas aeruginosa*. The antimalarial activity of this compound was determined to be 15 μg/ml (Van Vuuren et al., 2006). As previously mentioned, the South African *Helichrysums* contain a large amount of phloroglucinol derivatives and considering the promising antimicrobial activity observed for this type of compound, it seems a class well worth investigating.

Aqueous extracts of *Helichrysum aureonitens* exhibited antiviral activity against the *Herpes simplex* virus type I *in vitro* at a concentration of 1.35 mg/ml (Meyer et al., 1996). The flavone, galangin, isolated from this plant also exhibited antiviral activity against *Herpes simplex* virus type I and the *Coxsackie* virus at concentrations of 6 μg/ml (Meyer et al., 1997). The antiviral activity was also determined for a crude ethanolic extract of *Helichrysum melanacme* and its isolated constituents. The activity of the isolated prenylated chalcone (**9**) and a pyranochalcone (**10**) was lower

(IC_{50} = 0.1 mg/ml) against the *Influenza A* virus than that of the crude extract (0.01 mg/ml) although a combination of the two chalcones resulted in an improved IC_{50} (0.01 mg/ml, Lall et al., 2006).

In summary, the crude extracts generally show some degree of antimicrobial activity, which is usually higher against Gram-positive organisms than against Gram-negative organisms. Although the antibacterial and antifungal activities of these plants are well documented, antimalarial, antimycobacterial and antiviral data are scarce. Isolated compounds sometimes exhibit more superior activity when compared to the crude extract, but often the crude extract has similar activity. Correct identification of plant material is crucial as misidentification of plant material can lead to incorrect reporting (Lourens et al., 2004). The selected range of concentrations is often on the high side (Gibbons, 2004, considered values of below 1 mg/ml for extracts and 64 μg/ml for single chemical entities as significant); for example a range of 10–100 mg/ml was used for *Helichrysum pedunculatum* extracts (Meyer and Dilika, 1996). Positive controls (antibiotics) are absent in some of the assays (Mathekga et al., 2000), making it difficult to comparatively assess the activity of a particular extract or compound. The fact that different assays are employed impairs comparison of data between different laboratories (assays relying on diffusion are especially suspect since a low rate of diffusion would present a low activity, which is not always a true representation). Microbial strains are often not referenced and the number of colony forming units not mentioned (Meyer and Afolayan, 1995). Extracts also often do not dissolve completely in the solvents used and as Cushnie illustrated with galangin (2003) this can have a profound effect on the MIC's observed (Cushnie et al., 2003). Chemical classes such as the flavonoids, acylphloroglucinols and diterpenes from South African *Helichrysum* species exhibit promising antimicrobial activity and plants that contain these compounds seems potential candidates for further study.

4.2. Other biological data

Unpublished work done by Noristan laboratories indicates that fractions of the extract of *Helichrysum caespititium* exhibits anti-inflammatory activity of up to 82% at 360 mg/kg in the carrageenan test done on rats and prevents platelet aggregation (Swanepoel, 1997). Ethanolic extracts of *Helichrysum subglomeratum* and *Helichrysum nudifolium* inhibited prostaglandin synthesis *in vitro* by 69 and 96% (50 μg of plant extract used), respectively (Jäger et al., 1996). The group at Noristan determined that fractions of a *Helichrysum nudifolium* extract also reduced edema in the carrageenan assay by approximately 30% at 300 mg/kg in rats (Swanepoel, 1997). These results indicate that *Helichrysum nudifolium* has both *in vitro* and *in vivo* anti-inflammatory activity, possibly due to the inhibition of the cyclooxygenase enzymes.

The group at Noristan observed that the second of three fractions obtained after gradient column chromatography (using petroleum ether, ethyl acetate and methanol) of a dichloromethane/methanol extract from *Helichrysum panduratum* showed a 79% reduction in pain experienced in the writhing pain test at 500 mg/kg. Edema was also reduced by 50% in the carrageenan test indicating that this plant has both anti-inflammatory and analgesic properties. It was also antihypertensive (a reduction of 6% in mean blood pressure was observed after administering a dose of 300 mg/kg) and weakly antimicrobial (Swanepoel, 1997). A fraction from a dichloromethane/methanol extract of *Helichrysum petiolare* investigated by the group from Noristan determined that administration of 300 mg/kg of extract to mice reduced mean blood pressure by 21% and resulted in a 6% reduction in heart rate (Swanepoel, 1997). Acetone extracts of *Helichrysum excisum* (IC_{50} = 35 μg/ml) and *Helichrysum felinum* (IC_{50} = 39 μg/ml) inhibited the 5-lipoxygenase enzyme which also plays a role in inflammation. Antioxidant activity (as indicated with the DPPH assay) of acetone and methanol extracts of *Helichrysum odoratissimum*, *Helichrysum excisum*, *Helichrysum felinum* and *Helichrysum petiolare* was comparable to that of vitamin C, as expected for species rich in phenolic compounds (Lourens et al., 2004).

European research further highlights the antioxidant and anti-inflammatory effects displayed by plants from this genus. It is quite often the flowers that are investigated, a plant part that are seldom investigated in South African research (Drewes and Van Vuuren, 2008; Table 2). Antioxidant activity was reported for flower extracts from *Helichrysum stoechas* (Carini et al., 2001), *Helichrysum arenarium* (Czinner et al., 2000; Czinner et al., 2001) and *Helichrysum italicum* (Facino et al., 1990). *In vivo* (topical) anti-inflammatory activity comparable to that of the indomethacin standard was observed for an acetophenone derivative, gnaphaliin (a flavonoid) and ursolic acid isolated from *Helichrysum stoechas* (Recio et al., 1991). *In vivo* and *in vitro* anti-inflammatory activity was also observed for acetophenone glucosides, flavonoids and other compounds isolated from *Helichrysum italicum* (Sala et al., 2001, 2002, 2003a,b) as well as for extracts from *Helichrysum compactum* (Süzgeç et al., 2005). These promising results indicate that more research should be undertaken on the anti-inflammatory activity of South African species, as many similar compounds appear in the South African and European species.

As previously mentioned, *Helichrysum* species are often burnt as incense to invoke the goodwill of the ancestors, in protective and other charms and to induce trances. It is also used in the treatment of insanity, possession, used as a sedative to treat insomnia and as a protective cleanser (Table 1). Their traditional uses indicate that these plants may exhibit psychotropic effects. Stafford et al. (2005) determined the GABA-receptor binding effect of extracts from *Helichrysum argyrolepis*, *Helichrysum herbaceum*, *Helichrysum nudifolium*, *Helichrysum ruderale*, *Helichrysum rugulosum*, *Helichrysum simillimum* and *Helichrysum umbraculigerum* by using the ^{3}H-Ro 15-1788 binding assay. *Helichrysum ruderale* and *Helichrysum umbraculigerum* exhibited the most pronounced effects, while *Helichrysum herbaceum*, *Helichrysum rugulosum* and *Helichrysum simillimum* showed moderate to good dose dependant activity.

There appears to be a large divide between the rich chemical data available and biological testing on compounds isolated from the South African species. One chemical class, the α-pyrones will be discussed as an example. By our rough estimate, 28 different pyrones were isolated from South African *Helichrysum* species. The same type of compounds was isolated from European species and rather interesting biological activity was observed. Italipyrone, plicatipyrone, a mixture of helipyrones and a mixture of homoarenol and arenol were all active against *Bacillus subtilis*, *Staphylococcus aureus*, *S. epidermidis* and *Mycobacterium phlei* using the agar diffusion method with the highest MIC being 25 μg/ml and the lowest 3 μg/ml (Ríos et al., 1991). Antifungal activity was also reported for α-pyrones isolated from *Helichrysum decumbens* (Tomás-Lorente et al., 1989). Pyrones (like arzanol and helipyrone) showed significant antioxidant activity and arzanol was not toxic at all concentrations tested (Rosa et al., 2007). Most interesting though is the findings by Appendino et al. (2007) that arzanol inhibits HIV-I replication in T-cells and inhibited NF-κB (IC_{50} = 5 μg/ml) indicating that this group of compounds may exhibit both antiviral and anti-inflammatory properties. To our knowledge, none of the unique pyrones isolated from South African species were evaluated for biological activity.

Most concerning is the almost complete absence of toxicity data for the South African species of this genus. In very few cases, for example where antiviral and antimalarial activities were determined (Meyer et al., 1996, 1997; Lall et al., 2006; Van Vuuren

et al., 2006) toxicity is mentioned. Toxicity of the diterpenes is well known (for example IC_{50} values of below 4 μg/ml was reported for three diterpene lactones from *Parinari capensis*; Uys et al., 2002), and several *Helichrysum* species contain high amounts of these compounds, to name but one example. Furthermore, Reid et al. (2006) screened 42 medicinal South African plants for mutagenicity, which included *Helichrysum herbaceum*, *Helichrysum nudifolium*, *Helichrysum ruderale*, *Helichrysum rugulosum*, *Helichrysum simillimum* and *Helichrysum umbraculigerum*. The only three plants that showed mutagenic activity were all *Helichrysums*, namely *Helichrysum herbaceum* (at 5 mg/ml), *Helichrysum rugulosum* (at 5 mg/ml) and *Helichrysum simillimum* (at 0.05 mg/ml). These results highlight both the need and importance of toxicity and safety data for plants of this genus. In general, there also seems to be a large need for *in vivo* validation of *in vitro* results since the effectiveness of these extracts and their compounds have not been validated in living organisms.

5. Conclusion

Helichrysum species are used extensively in ethnomedicine in South Africa and many of the uses are associated with the treatment of infections, e.g. it is used widely for treatment of respiratory diseases and wound dressing (Table 1). The large morphological diversity of the genus is complemented by chemical diversity as illustrated by the range of novel compounds isolated from the genus. Despite the extensive past and present traditional uses, the unrivalled botanical diversity, and the chemical complexity, it remains ironic that explorations of the biological activities of indigenous species are comparatively poorly studied. The genus is notoriously challenging from a taxonomic perspective and several examples have been highlighted to emphasise the importance of correct botanical identification when embarking on ethnopharmacological and phytochemical studies. There is an interesting relationship between the morphological classification and the classes of chemical compounds isolated from a specific morphological group and there are certain classes of compounds, e.g. diterpenes, guaianolides, acylated phloroglucinols and α-pyrone derivatives, for which one can predict in which species they are most likely to occur. This may be important in the search of new plant-derived drugs, e.g. acylated phloroglucinols show potential as anti-staphylococcal drug leads (Gibbons, 2004) and α-pyrone derivatives have anti-HIV properties (McGlacken and Fairlamb, 2005; Appendino et al., 2007). It is clear that *Helichrysum* is an interesting genus from an ethnobotanical, phytochemical and pharmacological perspective but that biological data to correlate the ethnobotany to the chemistry are often still lacking. To advance our knowledge on this fascinating genus a multidisciplinary approach involving botanists, chemists and ethnopharmacologists is required.

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References

Afolayan, A.J., Meyer, J.J.M., 1997. The antimicrobial activity of 3,5,7-trihydroxyflavone isolated from the shoots of *Helichrysum aureonitens*. Journal of Ethnopharmacology 57, 177–181.

Appendino, G., Ottino, M., Marquez, N., Bianchi, F., Giana, A., Ballero, M., Sterner, O., Fiebich, B.L., Munoz, E., 2007. Arzanol, an anti-inflammatory and anti-HIV-1 phlroglucinol α-pyrone from *Helichrysum italicum* ssp. *microphyllum*. Journal of Natural Products 70, 608–612.

Arnold, T.H., Prentice, C.A., Hawker, L.C., Snyman, E.E., Tomalin, M., Crouch, N.R., Pottas-Bircher, C., 2002. Medicinal and Magical Plants of Southern Africa: an Annotated Checklist. National Botanical Institute, Pretoria, pp. 32–34.

Asekun, O.T., Grierson, D.S., Afolayan, A.J., 2007. Characterization of essential oils from *Helichrysum odoratissimum* using different drying methods. Journal of Applied Sciences 7, 1005–1008.

Batten, A., Bokelmann, H., 1966. Wild Flowers of the Eastern Cape Province. Books of Africa, Cape Town, pp. 149–160.

Bhat, R.B., Jacobs, T.V., 1995. Traditional herbal medicine in Transkei. Journal of Ethnopharmacology 48, 7–12.

Bohlmann, F., Abraham, W.-F., 1979a. Neue Diterpene und weitere Inhaltsstoffe aus *Helichrysum calliconum* und *Helichrysum heterolasium*. Phytochemistry 18, 889–891.

Bohlmann, F., Abraham, W.-F., 1979b. Neue, chlorsubstituierte Thiophenacetylen-verbindungen mit ungewöhnlicher Struktur aus *Helichrysum-arten*. Phytochemistry 18, 839–842.

Bohlmann, F., Abraham, W.-R., 1979c. Neue Diterpene aus *Helichrysum acutatum*. Phytochemistry 18, 1754–1756.

Bohlmann, F., Abraham, W.-F., 1979d. Neue Prenylflavanone aus *Helichrysum hypocephalum*. Phytochemistry 18, 1851–1853.

Bohlmann, F., Abraham, W.-F., Sheldrick, W.S., 1980b. Weitere Diterpene mit Helifulvan-gerüst und andere Inhaltsstoffe aus *Helichrysum chionosphaerum*. Phytochemistry 19, 869–871.

Bohlmann, F., Ates, N., 1984. Three prenylated flavanoids from *Helichrysum athrixiifolium*. Phytochemistry 23, 1338–1339.

Bohlmann, F., Hartono, L., Jakupovic, J., 1985. A diterpene related to erythroxydiol from *Helichrysum refluxum*. Phytochemistry 24, 611–612.

Bohlmann, F., Hoffmann, E., 1979. Cannabigerol-ähnliche Verbindungen aus *Helichrysum umbraculigerum*. Phytochemistry 18, 1371–1374.

Bohlmann, F., Knauf, W., Misra, L.N., 1984a. Structures and synthesis of chlorophenol derivatives from *Helichrysum* species. Tetrahedron 40, 4987–4989.

Bohlmann, F., Mahanta, P.K., 1979. Weitere Phloroglucin-derivate aus *Helichrysum gymnoconum*. Phytochemistry 18, 348–350.

Bohlmann, F., Mahanta, P.K., Zdero, C., 1978b. Neue Chalkon-derivate aus Südafrikanischen *Helichrysum*-arten. Phytochemistry 17, 1935–1937.

Bohlmann, F., Misra, L.N., 1984. New prenylflavanones and chalcones from *Helichrysum rugulosum*. Planta Medica 50, 271–272.

Bohlmann, F., Misra, L.N., Jakupovic, J., 1984. Weitere Phloroglucin- und α-Pyrone-derivate aus *Helichrysum* arten. Planta Medica 50, 174–176.

Bohlmann, F., Suwita, A., 1978. Neue Phloroglucin-derivate aus *Leontonyx*-arten sowie weitere Verbindungen aus verretern der Tribus Inuleae. Phytochemistry 17, 1929–1934.

Bohlmann, F., Suwita, A., 1979. Weitere Phloroglucin-derivate aus *Helichrysum*-arten. Phytochemistry 18, 2046–2049.

Bohlmann, F., Suwita, A., 1979a. Ein neues Guajanolid und ein Secoguajanolid aus *Helichrysum splendidum*. Phytochemistry 18, 885–886.

Bohlmann, F., Zdero, C., 1973. Über ein neues Azulen aus *Helichrysum bracteatum* (Vent.) Willd. Chemische Berichte 106, 1337–1340.

Bohlmann, F., Zdero, C., 1979a. Neue Phloroglucin-derivate aus *Helichrysum natalitium* und *Helichrysum bellum*. Phytochemistry 18, 641–644.

Bohlmann, F., Zdero, C., 1980. Neue Phloroglucin-derivate aus *Helichrysum*-arten. Phytochemistry 19, 153–155.

Bohlmann, F., Zdero, C., 1980a. Neue Geranylphloroglucin-derivate aus *Helichrysum monticola*. Phytochemistry 19, 683–684.

Bohlmann, F., Zdero, C., 1983. Flavanones from *Helichrysum thapsus*. Phytochemistry 22, 2877–2878.

Bohlmann, F., Zdero, C., Abraham, W.-R., Suwita, A., Grenz, M., 1980a. Neue Diterpene und neue Dihydrochalcon-derivate sowie weitere Inhaltsstoffe aus *Helichrysum* arten. Phytochemistry 19, 873–879.

Bohlmann, F., Zdero, C., Hoffmann, E., Mahanta, P.K., Dorner, W., 1978a. Neue Diterpene und Sesquiterpene aus Südafrikanischen *Helichrysum*-arten. Phytochemistry 17, 1917–1922.

Bohlmann, F., Zdero, C., Zeisberg, R., Sheldrick, W.S., 1979c. Helifulvanolsäure—ein neues Diterpen mit anomalem Kohlenstoffgerüst aus *Helichrysum fulvum*. Phytochemistry 18, 1359–1362.

Bohlmann, F., Zdero, C., Ziesche, J., 1979a. Neue Flavone und Phloroglucin-derivate aus *Helichrysum herbaceum* und *Helichrysum chrysargyrum*. Phytochemistry 18, 1375–1378.

Bohlmann, F., Ziesche, J., 1979. Ein ungewöhnliches Tetrahydrofuran-derivat aus *Helichrysum aureo-nitens*. Phytochemistry 18, 664–665.

Bohlmann, F., Ziesche, J., Mahanta, P.K., 1979b. Neue Chalkon-derivate und humulon-ähnliche Verbindungen aus *Helichrysum* arten. Phytochemistry 18, 1033–1036.

Bremner, P.D., Meyer, J.J.M., 1998. Pinocembrin chalcone: an antibacterial compound from *Helichrysum trilineatum*. Planta Medica 64, 777.

Bremner, P.D., Meyer, J.J.M., 2000. Prenyl-butyrylphloroglucinol and kaurenoic acid: two antibacterial compounds from *Helichrysum kraussii*. South African Journal of Botany 66, 115–117.

Candy, H.A., Laing, M., Weeks, C.M., 1975. The crystal structure of helichrysoside, a new acylated flavonoid glycoside from *Helichrysum kraussii*. Tetrahedron Letters 14, 1211–1214.

Candy, H.A., Wright, W., 1975. Helichrysoside: a new acylated flavonoid glycoside from *Helichrysum kraussii* Sch. Bip. Journal of the South African Chemical Institute 28, 215–219.

Carini, M., Aldini, G., Furlanetto, S., Stefani, R., Facino, R.M., 2001. LC coupled to ion-trap MS for the rapid screening and detection of polyphenol antioxidants

from *Helichrysum stoechas*. Journal of Pharmaceutical and Biomedical Analysis 24, 517–526.
Cunningham, A., 1988. An Investigation of the Herbal Medicine Trade in Natal/KwaZulu. Institute of Natural Resources Investigational Report 29. University of Natal, Pietermaritzburg.
Cushnie, T.P.T., Hamilton, V.E.S., Lamb, A.J., 2003. Assessment of the antibacterial activity of selected flavonoids and consideration of discrepancies between previous reports. Microbiological Research 158, 281–289.
Cushnie, T.P.T., Lamb, A.J., 2006. Assessment of the antibacterial activity of galangin against 4-quinolone resistant strains of *Staphylococcus aureus*. Phytomedicine 13, 187–191.
Czinner, E., Hagymási, K., Blázovics, A., Kéry, Á., Szőke, É., Lemberkovics, É., 2001. The *in vitro* effect of *Helichrysi flos* on microsomal lipid peroxidation. Journal of Ethnopharmacology 77, 31–35.
Czinner, E., Hagymási, K., Blázovics, A., Kéry, Á., Szőke, É., Lemberkovics, É., 2000. *In vitro* antioxidant properties of *Helichrysum arenarium* (L.) Moench. Journal of Ethnopharmacology 73, 437–443.
Dekker, T.G., Fourie, T.G., Snyckers, F.O., Van der Schyf, C.J., 1983. Studies of South African medicinal plants. Part 2. Caespitin, a new phloroglucinol with antimicrobial properties from *Helichrysum caespititium*. South African Journal of Chemistry 36, 114–116.
Dilika, F., Afolayan, A.J., Meyer, J.J.M., 1997. Comparative antibacterial activity of two *Helichrysum* species used in male circumcision in South Africa. South African Journal of Botany 63, 158–159.
Dilika, F., Bremner, P.D., Meyer, J.J.M., 2000. Antibacterial activity of linoleic and oleic acids isolated from *Helichrysum pedunculatum*: a plant used during circumcision rites. Fitoterapia 71, 450–452.
Dlamini, B., 1981. Swaziland Flora: their Local Names and Uses. Ministry of Agriculture and Co-operatives: Forestry Section, Mbabane, Swaziland.
Drewes, S.E., Mudau, K.E., Van Vuuren, S.F., Viljoen, A.M., 2006. Antimicrobial monomeric and dimeric diterpenes from the leaves of *Helichrysum tenax* var. *tenax*. Phytochemistry 67, 716–722.
Drewes, S.E., Van Vuuren, S.F., 2008. Antimicrobial acylphloroglucinols and dibenzyloxyflavonoids from flowers of *Helichrysum gymnocomum*. Phytochemistry 69, 1745–1794.
Facino, R.M., Carini, M., Franzoi, L., Pirola, O., Bosiso, E., 1990. Phytochemical characterization and radical scavenger activity of flavonoids from *Helichrysum italicum* G. Don (Compositae). Pharmacological Research 22, 709–720.
Frum, Y., Viljoen, A.M., 2006. *In vitro* 5-lipoxygenase activity of 3 indigenous South African aromatic plants used in traditional healing and the stereospecific activity of limonene in the 5-lipoxygenase assay. Journal of Essential Oil Research 18, 85–88.
Gelfand, M., Mavi, S., Drummond, R.B., Ndemera, B., 1985. The Traditional Medical Practitioner in Zimbabwe. Mambo Press, Gweru, Zimbabwe, pp. 83–84, 87, 94–95, 235, 238, 289, 332.
Germishuizen, G., Meyer, N.L., 2003. Strelitzia 14. Plants of Southern Africa: an Annotated Checklist. National Botanical Institute, Pretoria, pp. 235–246.
Gerstner, J., 1938. A Preliminary Checklist of Zulu Names of Plants with Short Notes. Bantu Studies 12, 321–342.
Gibbons, S., 2004. Anti-staphylococcal plant natural products. Natural Product Reports 21, 263–277.
Gibbs Russell, G.E., Welman, W.G., Retief, E., Immelman, K.L., Germishuizen, G., Pienaar, B.J., Van Wyk, M., Nicholas, A., 1987. Memoirs of the Botanical Survey of Southern Africa. List of Species of Southern African Plants. No. 56, 2nd ed., Part 2. Botanical Research Institute. Department of Agriculture and Water Supply.
Githens, T.S., 1949. Drug Plants of Africa. African Handbooks 8. University of Pennsylvania Press, The University Museum, Philadelphia, pp. 90–91.
Hänsel, R., Cybulski, E.-M., Çubuçku, B., Meriçli, A.H., Bohlmann, F., Zdero, C., 1980. Neue pyron-derivate aus *Helichrysum*-arten. Phytochemistry 19, 639–644.
Harborne, J., 1977. Inuleae—chemical review. In: Heywood, V.H., Harborne, J.B., Turner, B.L. (Eds.), The Biology and Chemistry of the Compositae. Academic Press, London, pp. 603–619.
Hegnauer, R., 1977. The chemistry of the Compositae. In: Heywood, V.H., Harborne, J.B., Turner, B.L. (Eds.), The Biology and Chemistry of the Compositae. Academic Press, London, pp. 283–335.
Hilliard, O.M., 1983. Asteraceae. In: Leistner, O.A. (Ed.), Flora of Southern Africa, vol. 33, part 7 (Inuleae). Botanical Research Institute of South Africa, Pretoria, pp. 7,2:61–7,2:317.
Hulme, M., 1954. Wild Flowers of Natal. Shuter and Shooter, Pietermaritzburg, Plate 41, 46, 47.
Hutchings, A., Johnson, C.T., 1986. Glimpses of a Xhosa herbal. Veld and Flora 72, 59–62.
Hutchings, A., Scott, A.H., Lewis, G., Cunningham, A., 1996. Zulu Medicinal Plants. Natal University Press, Pietermaritzburg.
Hutchings, A., Van Staden, J., 1994. Plants used for stress related ailments in traditional Zulu, Xhosa and Sotho medicine. Part 1. Plants used for headaches. Journal of Ethnopharmacology 43, 89–124.
Jacot Guillarmod, A., 1971. Flora of Lesotho (Basutoland). Cramer, Lehre, pp. 34, 38, 40–46, 267–281, 432–434.
Jäger, A.K., Hutchings, A., Van Staden, J., 1996. Screening of Zulu medicinal plants for prostaglandin-synthesis inhibitors. Journal of Ethnopharmacology 52, 95–100.
Jakupovic, J., Kuhnke, J., Schuster, A., Metwally, M.A., Bohlmann, F., 1986. Phloroglucinol derivatives and other constituents from South African *Helichrysum* species. Phytochemistry 25, 1133–1142.
Jakupovic, J., Schuster, A., Sun, H., Bohlmann, F., Bhakuni, D.S., 1987. Prenylated phtalides from *Anaphalis araneosa* and *Helichrysum platypterum*. Phytochemistry 26, 580–581.
Jakupovic, J., Schuster, A., Bohlmann, F., Ganzer, U., King, R.M., Robinson, H., 1989a. Diterpenes and other constituents from Australian *Helichrysum* and related species. Phytochemistry 28, 543–551.
Jakupovic, J., Zdero, C., Grenz, M., Tsichritzis, F., Lehmann, L., Hashemi-Nejad, S.M., Bohlmann, F., 1989. Twenty-one acylphloroglucinol and further constituents from South African *Helichrysum* species. Phytochemistry 28, 1119–1131.
Lall, N., Hussein, A.A., Meyer, J.J.M., 2006. Antiviral and antituberculous activity of *Helichrysum melanacme* constituents. Fitoterapia 77, 230–232.
Lall, N., Meyer, J.J.M., 1999. *In vitro* inhibition of drug-resistant and drug-sensitive strains of *Mycobacterium tuberculosis* by ethnobotanically selected South African plants. Journal of Ethnopharmacology 66, 347–354.
Lourens, A.C.U., Reddy, D., Başer, K.H.C., Viljoen, A.M., Van Vuuren, S.F., 2004. *In vitro* biological activity and essential oil composition of four indigenous South African *Helichrysum* species. Journal of Ethnopharmacology 95, 253–258.
Lucas, A., Pike, B., 1971. Wild Flowers of the Witwatersrand. Purnell, Cape Town, pp. 94–95.
Mabogo, D.E.N., 1990. The Ethnobotany of the Vhavenda. MSc Thesis, University of Pretoria, South Africa.
Mathekga, A.D.M., 2001. Antimicrobial Activity of *Helichrysum* Species and the Isolation of a New Phloroglucinol from *Helichrysum caespititium*. PhD dissertation. University of Pretoria, South Africa.
Mathekga, A.D.M., Meyer, J.J.M., 1998. Antibacterial activity of South African *Helichrysum* species. South African Journal of Botany 64, 293–295.
Mathekga, A.D.M., Meyer, J.J.M., Horn, M.M., Drewes, S.E., 2000. An acylated phloroglucinol with antimicrobial properties from *Helichrysum caespitium*. Phytochemistry 53, 93–96.
McGlacken, G.P., Fairlamb, I.J.S., 2005. 2-Pyrone natural products and mimetics: isolation, characterisation and biological activity. Natural Product Reports 22, 369–385.
Meyer, J.J.M., Afolayan, A.J., 1995. Antibacterial activity of *Helichrysum aureonitens*. Journal of Ethnopharmacology 47, 109–111.
Meyer, J.J.M., Afolayan, A.J., Taylor, M.B., Engelbrecht, L., 1996. Inhibition of *Herpes simplex* virus type 1 by aqueous extracts from shoots of *Helichrysum aureonitens* (Asteraceae). Journal of Ethnopharmacology 52, 41–43.
Meyer, J.J.M., Afolayan, A.J., Taylor, M.B., Erasmus, D., 1997. Antiviral activity of galangin isolated from the aerial parts of *Helichrysum aureonitens*. Journal of Ethnopharmacology 56, 165–169.
Meyer, J.J.M., Dilika, F., 1996. Antibacterial activity of *Helichrysum pedunculatum* used in circumcision rites. Journal of Ethnopharmacology 53, 51–54.
Meyer, J.J.M., Lall, N., Mathekga, A.D.M., 2002. *In vitro* inhibition of drug-resistant and drug-sensitive strains of *Mycobacterium tuberculosis* by *Helichrysum caespititium*. South African Journal of Botany 68, 90–93.
Neuwinger, H.D., 1996. African Ethnobotany. Poisons and Drugs. Chapman and Hall, Weinheim, pp. 253–255.
Phillips, E.P., 1917. A contribution to the flora of the Leribe Plateau and environs: with a discussion on the relationships of the floras of Basutoland, the Kalahari, and the south-eastern regions. Annals of the South African Museum 16, 123–135.
Pooley, E., 1998. A Field Guide to the Wild Flowers. KwaZulu-Natal and the Eastern Region, 1st ed. Natal Flora Publications Trust, Durban, pp. 82, 212–215, 310–317, 442–443.
Pooley, E., 2003. Mountain Flowers: A Field Guide to the Flora of the Drakensberg and Lesotho, 1st ed. The Flora Publications Trust, Durban, pp. 44, 102–110, 146–157, 222–225.
Recio, M.C., Giner, R., Terencio, M.C., Sanz, M.J., Ríos, J.L., 1991. Anti-inflammatory activity of *Helichrysum stoechas*. Planta Medica 57, A56.
Reid, K.A., Maes, J., Maes, A., Van Staden, J., De Kimpe, N., Mulholland, D.A., Verschaeve, L., 2006. Evaluation of the mutagenic effects of South African plants. Journal of Ethnopharmacology 106, 44–50.
Ríos, J.L., Recio, M.C., Villar, A., 1991. Isolation and identification of the antibacterial compounds from *Helichrysum stoechas*. Journal of Ethnopharmacology 33, 51–55.
Roberts, M., 1990. Indigenous Healing Plants. Southern Book Publishers, Johannesburg, pp. 76–78.
Rood, B., 1994. Uit die Veldapteek. Tafelberg Publishers, Cape Town, pp. 32–33.
Rosa, A., Deiana, M., Atzeri, A., Corona, G., Incani, A., Melis, M.P., Appendino, G., Dessí, M.A., 2007. Evaluation of the antioxidant and cytotoxic activity of arzanol, a prenylated α-pyrone-phloroglucinol etherodimer from *Helichrysum italicum* subsp. *Microphyllum*. Chemico-Biological Interactions 165, 117–126.
Sala, A., Recio, M.D.C., Giner, R.M., Máñez, S., Ríos, J.-L., 2001. New acetophenone glucosides isolated from extracts of *Helichrysum italicum* with anti-inflammatory activity. Journal of Natural Products 64, 1360–1362.
Sala, A., Recio, M.D.C., Giner, R.M., Máñez, S., Tournier, H., Schinella, G., Ríos, J.-L., 2002. Anti-inflammatory and anti-oxidant properties of *Helichrysum italicum*. Journal of Pharmacy and Pharmacology 54, 365–371.
Sala, A., Recio, M.C., Schinella, G.R., Máñez, S., Giner, R.M., Cerdá-Nicolás, M., Ríos, J.-L., 2003a. Assessment of the anti-inflammatory activity and free radical scavenger activity of tiliroside. European Journal of Pharmacology 461, 53–61.
Sala, A., Recio, M.C., Schinella, G.R., Máñez, S., Giner, R.M.M., Ríos, J.-L., 2003b. A new dual inhibitor of arachidonate metabolism isolated from *Helichrysum italicum*. European Journal of Pharmacology 460, 219–226.
Salie, F., Eagles, P.F.K., Leng, H.M.J., 1996. Preliminary antimicrobial screening of four South African Asteraceae species. Journal of Ethnopharmacology 52, 27–33.

Scott, G., Hewett, M.L., 2008. Pioneers in ethnopharmacology: The Dutch East India Company (VOC) at the Cape from 1650 to 1800. Journal of Ethnopharmacology 115, 339–360.
Scott, G., Springfield, E.P., Coldrey, N., 2004. A pharmacognostical study of 26 South African plant species used as traditional medicines. Pharmaceutical Biology 42, 186–213.
Smith, A., 1895. A Contribution to South African Material Medica. Juta, Cape Town, pp. 72, 81, 97, 100–101.
Smith, C.A., 1966. Common Names of South African Plants. Memoirs of the Botanical Survey of South Africa No. 35. Department of Agricultural and Technical Services, Pretoria, pp. 62, 85, 90, 118–119, 206, 208, 211, 225, 246, 251, 266, 286, 298, 306, 376, 400, 415–416, 430, 443, 458, 465, 476, 478, 489, 517–518, 614, 615.
Stafford, G.I., Jäger, A.K., Van Staden, J., 2005. Activity of traditional South African sedative and potentially CNS-acting plants in the GABA-benzodiazepine receptor assay. Journal of Ethnopharmacology 100, 210–215.
Steenkamp, V., Mathivha, E., Gouws, M.C., Van Rensburg, C.E.J., 2004. Studies on the antibacterial, antioxidant and fibroblast growth stimulation of wound healing remedies from South Africa. Journal of Ethnopharmacology 95, 353–357.
Süzgeç, S., Meriçli, A.H., Houghton, P.J., Çubukçu, B., 2005. Flavonoids of *Helichrysum compactum* and their antioxidant and antibacterial activity. Fitoterapia 76, 269–272.
Swanepoel, D.P., 1997. The Medicinal Value of the Southern African Asteraceae. MSc Dissertation. University of Pretoria, South Africa.
Tomás-Lorente, F., Iniesta-Sanmartín, E., Tomás-Barberán, F.A., Trowitzsch-Kienast, W., Wray, V., 1989. Antifungal phloroglucinol derivatives and lipophilic flavonoids from *Helichrysum decumbens*. Phytochemistry 28, 1613–1615.
Uys, A.C.U., Malan, S.F., Van Dyk, S., Van Zyl, R.L., 2002. Antimalarial compounds from *Parinari capensis*. Bioorganic and Medicinal Chemistry Letters 12, 2167–2169.
Van der Schyf, C.J., Dekker, T.G., Fourie, T.G., Snyckers, F., 1986. Synthesis and antimicrobial activity of a series of caespitin derivitaves. Antimicrobial Agents and Chemotherapy 30, 375–381.
Van Puyvelde, L., De Kimpe, N., Costa, J., Munyjabo, V., Nyirankuliza, S., Hakizamungu, E., Schamp, N., 1989. Isolation of flavonoids and a chalcone from *Helichrysum odoratissimum* and synthesis of helichrysetin. Journal of Natural Products 52, 629–633.
Van Vuuren, S.F., Viljoen, A.M., Van Zyl, R.L., Van Heerden, F.R., Başer, K.H.C., 2006. The antimicrobial and toxicity profiles of helihumulone, leaf essential oil and extracts of *Helichrysum cymosum* (L.) D. Don subsp. *cymosum*. South African Journal of Botany 72, 287–290.
Van Wyk, B.-E., Van Oudtshoorn, B., Gericke, N., 2000. Medicinal Plants of South Africa, 2nd ed. Briza, Pretoria, p. 148.
Van Wyk, B.-E., Gericke, N., 2000. People's Plants. A Guide to Useful Plants of Southern Africa, 1st ed. Briza, Pretoria, p. 166.
Van Wyk, B.-E., Van Heerden, F.R., Van Oudtshoorn, B., 2002. Poisonous Plants of South Africa, 1st ed. Briza, Pretoria, pp. 122–123.
Von Koenen, E., 2001. Medicinal, Poisonous and Edible Plants in Namibia. Klaus Hess Publishers, Verlag, p. 127.
Walker, J., 1996. Wild Flowers of KwaZulu-Natal. W.R. Walker Family Trust, Pinetown, pp. 169–173.
Watt, J.M., Breyer-Brandwijk, M.G., 1962. The Medicinal and Poisonous Plants of Southern and Eastern Africa, 2nd ed. Livingstone, London, pp. 237–240.
Wright, W.G., 1976. South African plant extractives. Part 3. Helichrysin, a new chalcone glucoside from a *Helichrysum* species. Journal of the Chemical Society, Perkin Transactions 1, 1819–1820.