Structure, Synthesis and Biological Activities of Biflavonoids Isolated from *Ochna serrulata* (Hochst.) Walp.

By

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Declaration

This study represents original work by the author and has not been submitted in any form for any degree or diploma to any University. Where the use of published information from other authors has been made, it is duly acknowledged in the text.

Signed ……………………...

Monica Mbaraka Ndoile

I hereby certify that this statement is correct.

Signed ………………………………….

Professor Fanie R. van Heerden (**Supervisor**)

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Completing a PhD is one of life's greatest experience; first and foremost, I would like to express my sincere gratitude to my rock, tower of refuge and strength, my salvation- Jesus! If ever I loved thee, my Jesus 'tis now!

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Abstract

The phytochemistry of *Ochna serrulata* (Hochst.) Walp. was investigated for the first time; two new dimeric chalcones (5-deoxyurundeuvine C and serrulone A) and two new biflavonoid derivatives (4,4',7-tri-*O*-methylisocampylospermone A and 4'''-de-*O*-methylafzelone A) were isolated. These compounds were isolated along with the known compounds lophirone A, afzelone B, campylospermone A, isocampylospermone A, ochnaflavone, 2",3"-dihydroochnaflavone, lophirone C, psilosin, 3'-*O*-methylpsilosin, a cyanoglucoside, epicatechin, (2',4'-dihydroxyphenyl)acetic acid, methyl (2',4'-dihydroxyphenyl)acetate, irisolone 4'-methyl ether, iriskumaonin 3'-methyl ether, 3',4'-dimethoxy-6,7-methylenedioxyisoflavone, lophirone L, syringaresinol and 16α,17-dihydroxy-*ent*-kauran-19-oic acid.

The growth inhibitory effect of these compounds was evaluated against three cancer cell line panel of TK 10 (renal), UACC62 (melanoma) and MCF7 (breast) using a sulforhodamine B (SRB) assay. Ochnaflavone and 3'-methoxypsilosin demonstrated selectivity and only inhibited the growth of melanoma cancer cells. However, ochnaflavone showed higher activity by totally inhibiting the growth of melanoma cancer cells at 12.91 µM, whereas, 3'-*O*-methylpsilosin has this effect at a concentration of 14.11 µM. Lophirone C, a dimeric chalcone, demonstrated the highest cytotoxic activity amongst all isolated compounds against renal, melanoma and breast cancer cells with TGI at 35.63 µM, 11.67 µM and 30.35 µM, respectively. Lophirone A, a rearranged biflavonoid, showed TGI against these cancer cells at 58.96 µM, 26.23 µM and 40.01 µM, respectively. The rest of the compounds showed no significant cytotoxicity against the three cancer cells.

The new biflavonoid, 4,4',7-tri-*O*-methylisocampylospermone A demonstrated the highest antimalarial activity against chloroquine-resistant strains of *Plasmodium falciparum* (FCR-3) with IC$_{50}$ of 11.46 µM, followed by ochnaflavone (17.25 µM).

Serrulone A (26.52 µM), lophirone A (29.78 µM), 5-deoxyurundeuvine C (31.07 µM), lophirone C (35.31 µM), 4'''-de-*O*-methylafzelone A (38.43 µM), afzelone B (39.54 µM), irisolone 4'-methyl ether (40.72 µM) and syringaresinol (42.66 µM) were moderately active. The following compounds exhibited the lowest antimalarial activity, 2",3"-dihydroochnaflavone (61.86 µM), iriskumaonin 3'-*O*-methyl ether (93.69 µM),3'-*O*-methylpsilosin (106.35 µM) and16α,17-dihydroxy-ent-kauran-19-oic acid (106.48 µM).

Owing to the observed and reported biological/pharmacological activity, ochnaflavone (an ether-linked biflavone consisting of apigenin and luteolin moieties) was selected for synthetic studies. An older method, nucleophilic aromatic substitution (S_NAr) was successfully applied in the construction of the diary ether. Oxidative ring cyclization of the ether-linked dimeric chalcone was achieved by using heated pyridine and iodine. The two methods can be extended further in the synthesis of other novel biflavones with ether linkage.

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List of Abbreviations

AIDS	:	acquired immune deficiency syndrome
br	:	broad
calc.	:	calculated
COSY	:	correlation spectroscopy
d	:	doublet
DBU	:	1,8-diazabicyclo[5.4.0]undecene-7
DCM	:	dichloromethane
dd	:	doublet of doublets
DDQ	:	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DEPT	:	distortionless enhancement by polarization transfer
DMF	:	*N,N*-dimethylformamide
DMSO	:	dimethyl sulfoxide
DNA	:	deoxyribonucleic acid
EtOAc	:	ethyl acetate
FDA	:	Food and drug administration (United States of America)
Fig.	:	figure
GI	:	percentage growth inhibition
HIV	:	human immunodeficiency virus
HMBC	:	heteronuclear multiple-bond correlation
HRESIMS	:	high-resolution electrospray ionization mass spectrometry
HSQC	:	heteronuclear single-quantum correlation
Hz	:	Hertz
IC	:	inhibitory concentration

IR	:	infrared
LRMS	:	low-resolution mass spectrometry
m	:	multiplet
MeOH	:	methanol
MS	:	mass spectrometry
m/z	:	mass-to-charge ratio
NCI	:	National Cancer Institute
NF-κB	:	nuclear factor-κB
NMR	:	nuclear magnetic resonance
NOESY	:	nuclear Overhauser effect spectroscopy
q	:	quartet
s	:	singlet
t	:	triplet
TGI	:	total growth inhibition
THF	:	tetrahydofuran
TLC	:	thin-layer chromatography
UKZN	:	University of KwaZulu-Natal
WHO	:	World Health Organization
℃	:	degrees Celsius
1D	:	one-dimensional
2D	:	two-dimensional

CHAPTER 1

Introduction and Aims of the Study

1.1   Novel Drugs Derived from Plant Natural Products

Since time immemorial, humans have relied on plants for the provision of medication, food, clothing, shelter and transportation (Cragg and Newman, 2009). For thousands of years plants have been the foundation of traditional medicine systems where the knowledge on the plants has been passed on from generation to generation (Koehn and Carter, 2005). In African, Indian and Chinese communities, plants have formed the main ingredient of traditional medicines (Gurib-Fakim, 2006; Magassouba et al., 2007).

Over 80% of the population residing in developing countries were estimated by WHO in 2008 to be depending directly on plants for their primary medical requirements (WHO, 2008); this is attributed to the fact that plant-derived medicines can be easily accessed and are also cheap (Amin and Mousa, 2007; Ramawat and Goyal, 2008; WHO, 2008). Even the people of the developed world are also dependent directly or indirectly on plants for their health care. In the United States, 25% of the prescriptions given from community pharmacies consisted of plant extracts or active ingredients of plant origin (Cragg and Newman, 2005). In Dar es Salaam (the biggest city in Tanzania), 21% of patients who visited public hospitals have consulted a traditional healer before they come to hospital (de Boer *et al.*, 2005). Plant-derived medicines are offered/taken in the form of tinctures, teas, poultices and powders, depending on the knowledge of the use and application method of a particular plant for a given ailment (Balunas and Kinghorn, 2005; Fennell et al., 2004).

Plants produce compounds of varying diversity as a means of defence against bacteria, fungi, pests and predators, hence the plants are efficient natural chemical factories, producing compounds of various structures that result in different physiological effects in the body once ingested (Edeoga *et al.*, 2005). In 1985, Farnsworth *et al.* estimated that at least 119 substances isolated from plants were used as important drugs in one or more countries and that 74% of these compounds were discovered as a result of phytochemical studies on plants used for medicinal purposes (Farnsworth *et al.*, 1985). The development of some synthetic drugs also uses plant-derived structures as leads (Ganesan, 2008; Vuorela et al., 2004). An analysis of the origin of new drugs that came onto the market during the period 1981-2006 indicated that only 37% of 974 new chemical entities were devoid of natural origin (i.e., purely synthetic) and that the remaining drugs are either a natural product or have a natural origin by having pharmacophores derived from a natural product (Newman and Cragg, 2007).

The isolation and characterisation of natural products began over 200 years ago, when morphine (**1**) was first isolated from *Papaver somniferum* L. by a 21-year-old pharmacist's trainee called Friedrich Sertürner. This was the first plant-derived pure compound with biological activity to be isolated and it formulated a foundation for alkaloid chemistry which consequently resulted in the development of more effective analgesic agents (Ramoutsaki *et al.*, 2002). After this discovery, an era began where purification and administration of plant-derived drugs were made possible.

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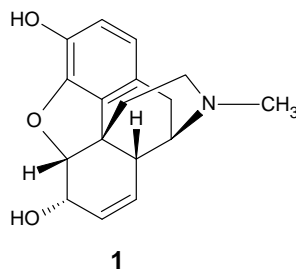
In 1820, the French researchers, Caventou and Pelletier isolated quinine (**2**), an antimalarial drug, from the bark of *Cinchona* L. trees (Guerra, 1977). The bark of these trees has been used by indigenous people of the Amazon region for the

treatment of fevers and in the early 1600s the bark was first introduced into Europe for the treatment of malaria. The bark was not only used for malaria treatment but also for treatment of lupus and arthritis. Nocturnal leg cramps was known to be treated by quinine, however, recently the FDA has regarded this kind of treatment as uncertain (FDA, 2010). The discovery of **2** laid the foundation for the synthesis of chloroquine (**3**) and mefloquine (**4**), which were commonly used antimalarial drugs for several years. The more effective antimalarial drug artemisinin (**5**) was first isolated from *Artemisia annua* L. (Quinhaosu), a plant that has long been used as a remedy for fever in Chinese traditional medicine. Artemisinin (**5**) then served as a lead structure for the development of the semisynthetic drugs artemether (**6**) and arteether (**7**) (Cragg and Newman, 2005; SEAQUAMAT, 2005).
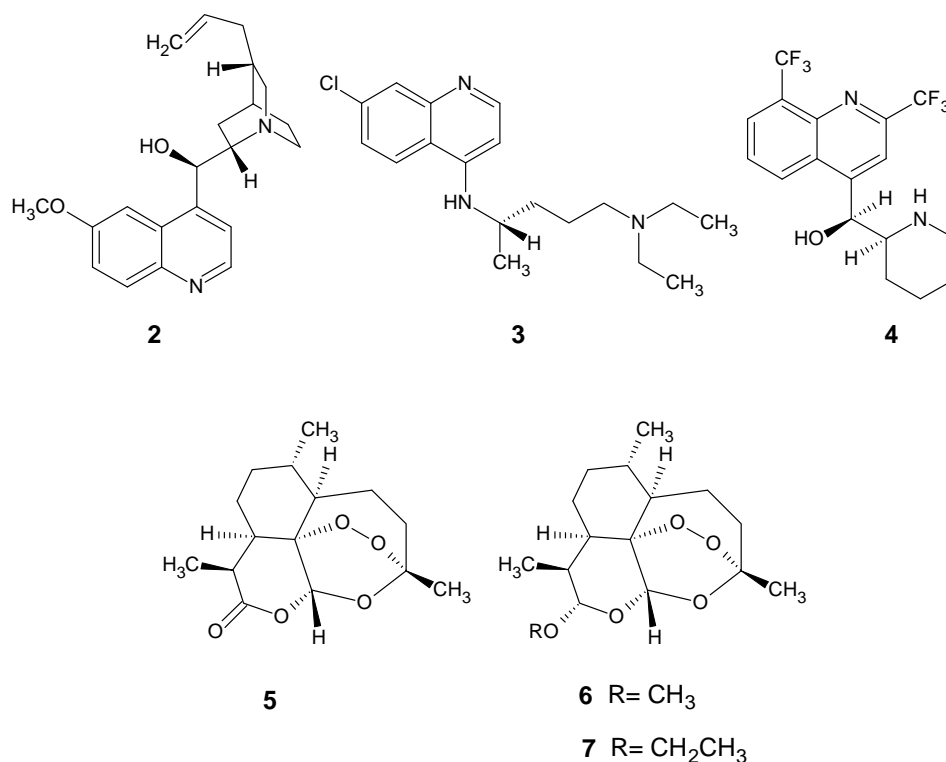
Strychnine (**8**), a colourless crystalline alkaloid was isolated from the seed extract of *Strychnos* L. species (Loganiaceae) native to India and Southeast Asia by the French botanist Leschenault de la Tour (1773-1826). The powdered seeds and decoction have been used by Hindu physicians in the treatment of certain types of cancer, digestive disorders, and diseases of the nervous system, heart diseases,

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respiratory and circulatory problems and in the treatment of cutaneous diseases (ulcers infested with maggots). The plant has long been used as an arrow poison in Java, Indonesia. The structure of **8** was first elucidated by Sir Robert Robinson (Robinson, 1945) and its total synthesis was first accomplished in 1954 by Robert W. Woodward (Woodward and Brehm, 1948; Woodward et al., 1947; Woodward et al., 1954). Small doses of **8** help in improving appetite and provide a generally strong and hopeful feeling. When dosage is increased, **8** is known to quicken and deepen the respiration and to increase pulse and blood pressure. At poisonous dosages (30 to 120 mg.Kg^{-1}), **8** affects the nervous system, particularly the spinal cord and medulla (Daly, 2005; Gupta, 2009).
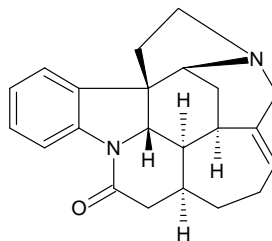
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The genus *Strophanthus* DC. (Apocynaceae) is found in tropical Africa and Asia, where for hundreds of years the natives have used the seeds of these plants to make poisonous arrow heads for hunting. The isolation of G-strophanthin (**9**) (also known as milk for the ageing heart) and its formulation into a cardiotonic agent established a remedy for cardiac diseases. G-strophanthin is a steroidal glycoside isolated from the dried ripe seeds of *Strophanthus* species (Apocynaceae). The compound is known to strengthen contraction of cardiac muscles and has predominant systolic action, therefore an excellent remedy for patients with pronounced cardiac dilation (Aperia, 2007; Gao et al., 2002; Kracke, 2004).
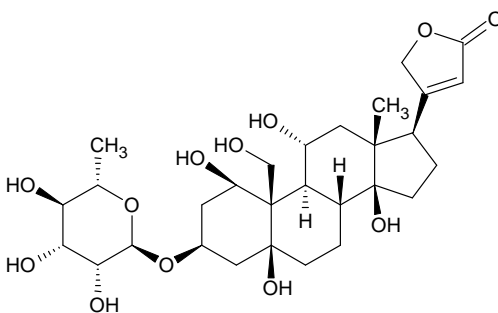
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Salicylic acid (**10**) was first isolated from the trees of the genus *Salix* commonly called willow trees, but later the compound was also isolated from the flowers of *Filipendula ulmaria* (L.) (meadowsweet). The meadowsweet plant has been used as a remedy for flu, rheumatism, arthritis and fevers in some communities of western Asia and Europe. The plant is also known to have anti-ulcerogenic, anti-tumour, anti-microbial, anti-carcinogenic, antioxidant and anti-coagulant activities. Salicylic acid, the active principle of the meadowsweet flowers has a disagreeable irritating taste when taken orally and its side effects, which limits its usage. The acetate derivative of **10**, acetylsalicylic acid (**11**), was first synthesized in 1850 and this has become the prototypical aspirin of today (Vane and Botting, 2003; Yildirim and Turker, 2009).
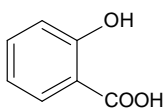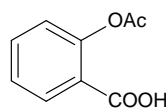
10   **11**

The antihypertensive agent, reserpine (**12**), an indole alkaloid, was developed from the traditionally-used medicinal plant *Rauwolfia serpentine* Benth*.* The plant is locally used in Ayurvedic medicine for the treatment of snakebites. Reserpine (**12**) has been used for a long time as an anti-psychotic and anti-hypertensive drug. However, due to its side effects and the evolution of better drugs for the mentioned purposes, **12** is rarely used currently (Carlsson, 2001; Halberstein, 2005).

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In 1887, an alkaloid ephedrine (**13**) was first isolated from *Ephedra sinica* Stapf. (Ma Huang), a plant with a history of being used for the treatment of the common cold, hay fever and asthma in Chinese traditional medicine. Ephedrine (**13**), an appetite suppressant in combination with herbs containing caffeine, is known to enhance weight loss and/or to improve athletic performance. Structurally, **13** is similar to its semi-synthetic derivatives amphetamine and methamphetamine. The structure of **13** as a bronchodilator, has laid the foundation where anti-asthma compounds (beta agonists) were synthesized, *viz.* salbutamol (**14**) and salmeterol (**15**) (Abourashed et al., 2003; Hackman et al., 2006; Shekelle et al., 2003).

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14

15

A benzylisoquinoline, tubocurarine (**16**), was isolated from *Chondrodendron* Ruiz & Pav. species. The compound is a neuromuscular-blocking drug or skeletal muscle relaxant and it is used in anaesthesia to provide skeletal muscle relaxation during surgery. *Chondrodendron* species have long been used by the indigenous people of the Amazon as arrow poisons (Lee, 2005; Tuba et al., 2002).

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A large number of chemotherapeutic agents used in cancer treatment are derived from natural products. Similarly, several laboratories throughout the world have directed considerable efforts towards discovering new chemotherapeutic agents of natural origin. Vinblastine (**17**) and vincristine (**18**), also called vinca alkaloids, were first isolated from *Catharanthus roseus* L. (Madagascar periwinkle). These compounds were discovered during a search for potential sources of hypoglycaemic drugs; this was due to the fact that the plant was traditionally used for the treatment of diabetes. Vinca alkaloids are known to bind to tubulin dimers thereby inhibiting the assembly of microtubule structures and hence affect cell division of all rapidly dividing cells including cancer cells (Gigant et al., 2005; Okouneva et al., 2003; Takimoto and Calvo, 2008).
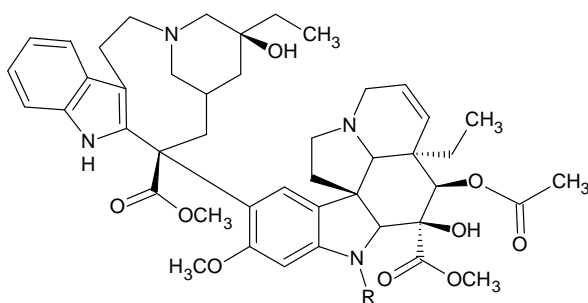
17 R=CH₃

18 R=CHO

Podophyllum (L.) (meaning 'shield-formed') species, also known as May apples, are originating from the Himalayan region where several other medicinal plants are found. The plants have been used locally for the treatment of skin cancers, warts, and as a purgative and emetic for several hundreds of years (Alam *et al.*, 2008).

The roots of the plants were used both as a medicine and poison by Asians, in some cases they were used as a suicide agent (Alam *et al.*, 2008). Two compounds, epipodophyllotoxin (**19**) and podophyllotoxin (**20**) are non-alkaloidal toxic lignans isolated from the roots and rhizomes of *Podophyllum* species. These compounds have anticancer activity by arresting mitotic division of the cell, however, the clinical use of **20** was largely affected by its undesirable gastrointestinal toxicity (Damayanthi and Lown, 1998). Due to this side effect, the synthetic glycosides of *Podophyllum* lignans and the semisynthetic derivatives of **20**, *viz.* etoposide (**21**) and teniposide (**22**) were discovered (Canel et al., 2000; Cragg and Newman, 2005).

Compounds **21** and **22** have a unique mechanism of action, they are DNA topo-isomerase II inhibitors and hence arrest the growth of cancer cells by causing double strand breaks in DNA (van Maanen *et al.*, 1988).
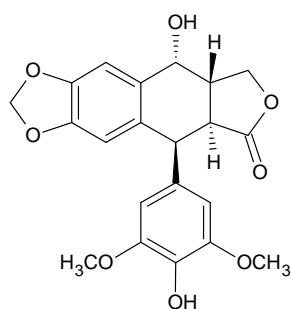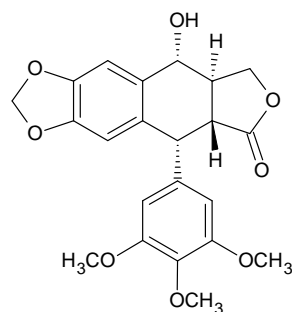
19   **20**

However **20**, which is the parent compound, has no inhibitory effect against DNA topoisomerase II, but is a potent inhibitor of microtubule formation and thereby inhibit cell division (Canel et al., 2000; Cragg and Newman, 2005; Gordaliza et al., 2004; Petersen and Alfermann, 2001).
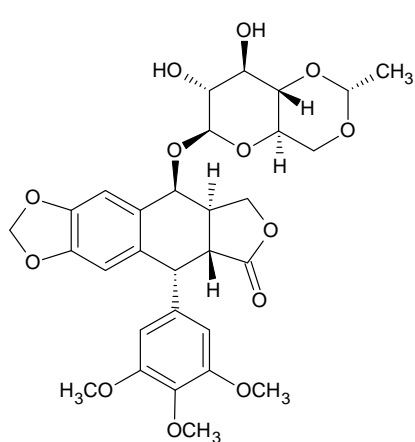
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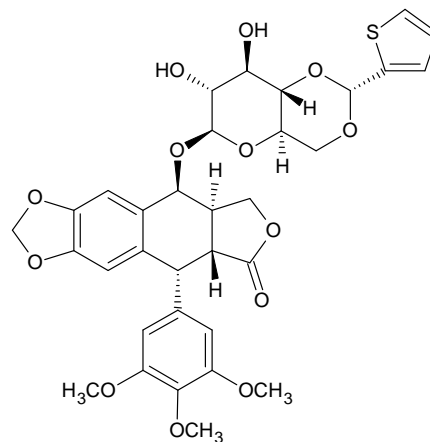
22

Paclitaxel (**23**) (Taxol®) was isolated from the bark of *Taxus brevifolia* Nutt., *T. canadensis* Marsh. and *T. baccata* L. These species are known to be used by Native Americans for treatment of noncancerous conditions. Paclitaxel (**23**) is a diterpenoid alkaloid with anticancer activities; the compound is known to disrupt the microtubule functioning and hence inhibit the process of cell division. The compound is effective in the treatment of cancers of the ovary, breast, lung, oesophagus, bladder, endometrium and cervix (Chan and Yang, 2000; Takimoto and Calvo, 2008; Zhong, 2002).
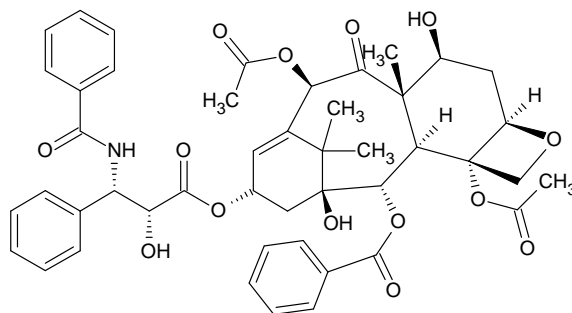
23

Like any other growing cell, cancer cells rely on a developing vasculature to meet their needs in terms of oxygen, nutrients and depuration. This implies that if the vascular bed that has developed within the tumoural mass can be made to collapse, then tumoural growth can be significantly hampered. The

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combretastatins (**24** and **25**) act as anticancer agents by shutting down the vascular systems in tumour cells thereby causing tumour necrosis. These compounds were isolated from *Combretum caffrum* (Eckl. & Zeyh.), a Zulu medicinal plant locally called South African bush willow. Sodium phosphate salt of **24** and **25** were developed to overcome the problem of their insolubility in water. The sodium phosphate form **26** act as a prodrug which when subjected to endogenous non-specific phosphatase enzymes, cleaves to its active form **25**.
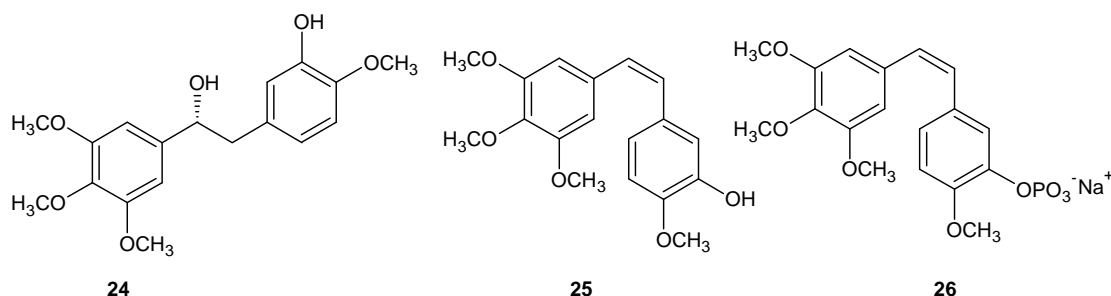
In search of more efficient anticancer drugs, a considerable number of compounds were synthesized based on the combrestatin skeleton (Cragg and Newman, 2005; Holwell et al., 2002; Li and Sham, 2002; Pettit and Lippert, 2000; Tozer et al., 2002; Tron et al., 2006; West and Price, 2004).

Ancistrocladus korupensis (D. W. Thomas & Gereau) (Ancistrocladaceae), a plant native to Korup National Park in Cameroon is the main source of michellamine B (**27**). The compound is an anti-HIV naphthalene-tetrahydroisoquinoline alkaloid and the leaves of this plant are the only known source of the compound. Michellamine B (**27**) is known to inhibit HIV-1 in its early T-lymphocyte viral infection phase and also inhibits HIV-2 in MT-2 cells. The studies done on dogs showed that the effective anti-HIV *in vivo* concentration of the compound is reached close to its neurotoxic concentration level. Thus, despite its *in vitro* activity against a wide range of HIV-1 and HIV-2 strains, the small difference between the toxic concentration and the concentration required for efficient antiviral activity has resulted in the discontinuation of further studies designed for its clinical development (Alves and Rosa, 2007; Cheng et al., 2008; McMahon et al., 1995; Singh et al., 2005; Tandon and Chhor, 2005; Yang et al., 2001).
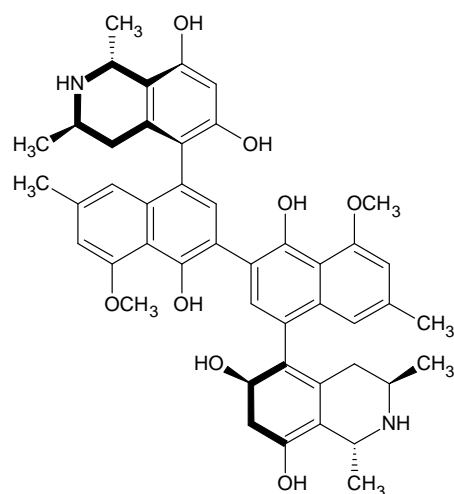
27

(+)-Calanolide A (**28**), an anti-HIV coumarin derivative, was isolated from the leaves and twigs of *Calophyllum lanigerum* Miq., a rare plant found in Sarawak, Malaysia. The compound act by inhibiting HIV-1 reverse transcriptase and has been chosen for preclinical trials by the US National Cancer Institute. However, the sources of **28** from plant material are problematic since the original population of plants was destroyed (Flavin et al., 1996; Spino et al., 1998). Other populations of the same species yielded only a small amount of **28**. However, the latex of another species, *C. teysmanii* with major anti-HIV activity, contained (–)-calanolide B (**30**) as the active ingredient. Although **30** was slightly less active than **28**, it was regarded as a better alternative due to the availability of the plant and because the latex can easily be obtained by just making small slashes on the mature tree bark without harming the plant. The studies focused on the isolation of active compounds from *Calophyllum* species yielded two enantiomers of calanolide A (**28** and **29**), two enantiomers of calanolide B (**30** and **31**) and (-)-dihydrocalanolide B (**32**) (Singh et al., 2005; Tandon and Chhor, 2005; Yang et al., 2001).

28   **29**   **30**

31   **32**
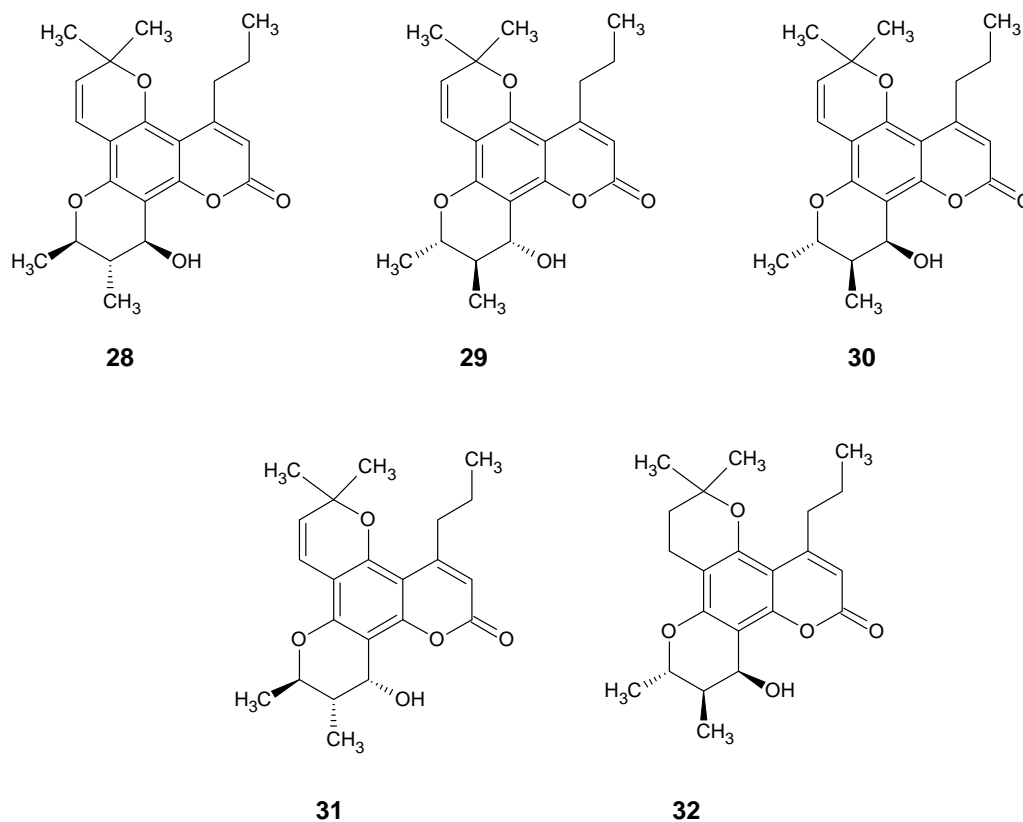
Homalanthus nutan (G. Forst.) Guill. is a plant found in Samoa where it is locally known as the mamala tree. Indigenous people use a concoction made from the bark for the treatment of hepatitis. Research has lead to the isolation of a protein kinase C activator prostratin (**33**) from the plant. The compound is a phorbol ester, but unlike other phorbol esters (*viz.* phorbol myristate acetate (**34**)), **33** is not a tumour promoter (Gustafson *et al.*, 1992). The antiviral activity of **33** was discovered during research on the traditional knowledge of Samoan healers in Falealupo village by ethnobotanist Paul Cox. Prostratin (**33**) showed antiviral activity against severe infection by numerous strains of HIV and also it activates gene expression of the virus from the latently infected cells (Anderson et al., 2006; Biancotto et al., 2004; Wender et al., 2008; Williams et al., 2004).

33　　　　　　　　　　　**34**
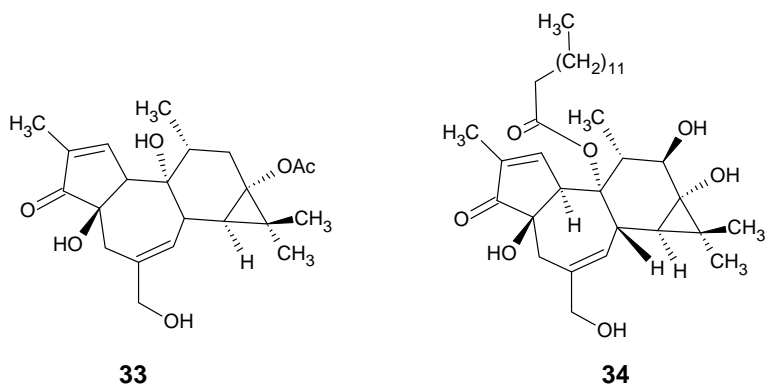
To conclude, many compounds with medicinal value have been isolated from plants that are traditionally used for medicinal purposes. Some of these compounds are currently in use clinically or have established a base for the synthesis of other compounds with better activities.

1.2　Aims of the Study

This investigation is aimed at studying the structures, biological activities and synthesis of the compounds present in the Zulu medicinal plant, *Ochna serrulata* Hochst.) Walp.

The main aims of this project were:

- To isolate and characterise the compounds from the stem, leaves and roots of *O. serrulata*.
- To establish antimalarial and anticancer activities of the isolated compounds.
- To develop a synthetic methodology for the synthesis of the most active compound.

The isolation and characterisation of the compounds from the stem and leaves are discussed in Chapter 3 of this thesis, whereas Chapter 4 discusses compounds from the roots of *O. serrulata*.

The biological activities of the isolated compounds are reported in Chapter 5, and the synthesis of the most potent compound is reported in Chapter 6 of this thesis. Final conclusions and recommendations are given in Chapter 7 of this thesis.

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CHAPTER 2

The Family Ochnaceae - An Overview

2.1   Introduction

The observed resistance of pathogens and unwanted side effects exhibited by currently used drugs, necessitate a continuous search for new drugs against a wide variety of ailments (Verschaeve and Van Staden, 2008). It has been shown in Chapter 1 that many compounds with medicinal value are derived from traditionally used medicinal plants. In this Chapter, an overview of the documented traditional uses of plants from the family Ochnaceae, will be presented together with the compounds isolated from this family and their biological/pharmacological activities.

2.2   Distribution, Economic Importance and Medicinal Uses of Ochnaceae plants

The family Ochnaceae is mainly comprised of trees and shrubs with about 30 genera and 450 species which are highly distributed around the globe. Tropical Africa, Asia, Australia, Madagascar, the Mascarene islands and America as shown in the map below (Fig 2.1), are the regions where these species are mostly found (Coates, 2002; Mabberley, 2008). Species of the family Ochnaceae flourish in areas ranging from open to semi-open and in evergreen forests, they are characterized by evergreen petiole leaves which sometimes becomes leathery, specifically for the genus *Ochna* L. (Gardner *et al.*, 2000).
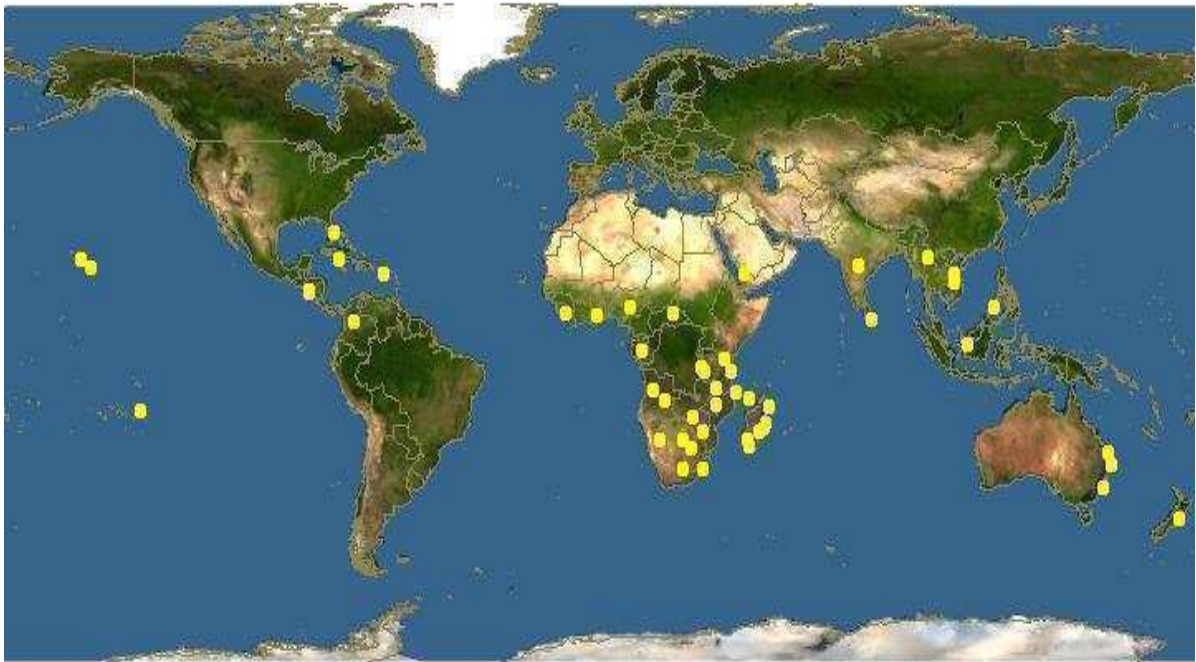
Fig 2.1: Distribution of Ochnaceae plant species

(Map: http://www.discoverlife.org/mp/20q?search=Ochna&guide=Guianas_flora)

The two genera, *Brackenridgea* and *Ochna* are commonly found in South Africa, Zimbabwe and Mozambique, with a total of 32 native species. For the genus *Brackenridgea,* about 12 species are known to be native to Southern Africa including Zimbabwe and Mozambique and 20 species of the genus *Ochna* occur in this area (Coates, 2002; Mabberley, 2008).

Economic importance of members of Ochnaceae

Some species of the family Ochnaceae are known to have economic value. Since species of the genus *Ochna* are known to have very attractive yellow flowers and beautiful fruits, the genus have been cultivated for ornamental purposes. Members of the genus *Sauvagesia* are used for making tea in Lesser Antilles, whereas members of *Ouratea* and *Lophira* are used as a source of a valuable oil, commonly called meni oil, from their seeds. Meni oil has gained popularity in Nigeria and some other West African countries where it is used for cooking, as a remedy for lice and as a hair

lotion. The seeds of these species also serve as food, regardless of their bitter and caustic taste. *Lophira* species found in West Africa are an excellent source of commercially valuable timber known by different names such as African oak, azobe, ekki, bongossi or red ironwood. The yellow dye from the bark of *Brackenridgea zanguebarica* Oliv. is used for dying mats to give a beautiful bright yellow colour (Lohlum et al., 2010; Mabberley, 2008; Moller et al., 2006; Rosalina, 2006).

Medicinal uses

In various traditional medicine systems, members of the family Ochnaceae are popularly used as medicines for different ailments. The species of the genus *Lophira* also known as Meni oil trees, locally called *beung* in Chamba language and *namijin kadanya* in Hausa language, are known to be helpful to pregnant women during labour. The leaves of *Lophira* plants are boiled in water and the resulting extract is used for drinking and washing for an easy labour. The inner bark of these plants is used for pain relieving purposes, especially for the treatment of headaches. The bark decoction is reported to be used to stop vaginal discharge and also for the treatment of diarrhoea, ovarian cyst and typhoid fever where one small cup is consumed three times a day (Jiofack et al., 2010; Lohlum et al., 2010).

The genus *Ouratea* is used in Brazil and many West African countries in traditional medicine systems, where the leaves extracts are used for the treatment of upper respiratory tract infections, dysentery, and diarrhoea and as pain-relieving agents, especially for treatment of tooth ache. The extracts from the epicarp fruit commonly called *folha-de-serra* (Brazil) are used for the treatment of liver and skin infections (Brandão et al., 2011; Pegnyemb et al., 2005).

The genus *Brackenridgea* is important as part of East African traditional medicine systems where the plants are used for the treatment of anaemia. The powdered roots of these species have been used to treat swollen parts of the body and for mental illnesses and worms. The powdered stem bark of these plants have been applied on

wounds and a decoction of the bark of these species is used as an eye wash. It is also effective against conjunctivitis and a decoction can be used in porridge preparation for a person with jaundice to drink in overcoming jaundice. The roots of the *Brackenridgea* plants are known as mumino in Muda (Mozambique) and are macerated in water and taken orally for the treatment of diarrhoea, to induce or to speed up the delivery process, miscarriage and treatment of venereal diseases (Bruschi et al., 2011; Moller et al., 2006).

2.3   Genus *Ochna:* Local Uses

More than 85 plant species are known to belong to the genus *Ochna*. The genus comprises of trees and shrubs which are distributed worldwide from tropical Africa, Asia to America. In Southern Africa *Ochna* species are found in South Africa (Limpopo, Mpumalanga, KwaZulu-Natal and Eastern Cape provinces), Swaziland, Zimbabwe and Mozambique. The genus was named by Linnaeus in 1753, the name *Ochna* originated from a *Greek* word, *Ochne,* which means "wild pear"; this is because the leaves of these species resemble those of the wild pear. Species of this genus are usually known as Ochnas or mickey mouse plants, this name is a result of the appearance of their black drupelets fruits sitting on a red receptacle mimicking the face of mickey mouse as shown in Fig 2.2 below (Coates, 2002; Mabberley, 2008).

Ochna species, also called '*sunari*' or '*yerra juvvi*' in Indian traditional medicine systems, are regarded as one of the important drugs used in the treatment of different ailments such as constipation, ulcers, sores, cancers, epilepsy, menstrual complaints, lumbago, asthma, and as an antidote to snake bites in India. While the root bark of the plant is used as a digestive tonic, the boiled leaves are considered to be a key element in the treatment of lumbago and ulcers. The decoction of the roots of these plants in Indian traditional medicine are used in the treatment of menstrual complaints and asthma (Imam *et al.*, 2003).

Fig 2.2: *Ochna serrulata* leaves and fruits

(Photo: http://www.biodiversityexplorer.org/plants/ochnaceae/ochna.htm)

Ochna integerrima Merr. is a common plant which is widely distributed in Thailand, where in their traditional medicine systems, the bark of this plant is used as a digestive tonic. In Indonesia an infusion of the roots and leaves are used as an anti-dysenteric and an antipyretic agent. The flowers are the most celebrated flowers in Vietnam. During "Tet" festivals, Vietnamese New Year festivals, the bright yellow flowers of the plant are used for decoration. In Vietnamese culture it is regarded as a must to grow plants for "tet" celebrations, bringing good luck and prosperity to the family (Kaewamatawong et al., 2002; Likhitwitayawuid et al., 2001).

In Swazi traditional medicine systems, the bark of *Ochna arborea* Burch. Ex DC, also called *Cape Plane Tree* or *sifubasenkhala* in siSwati, is used for healing bone fractures and pain relieving especially for the cure of headaches. For the same healing purpose, *O. gamostigmata (Bird's-eye Bush), O. holstii* Engl. *(red ironwood, umthelelo), O. natalitia* Meisn. Walp. *(Natal plane, lincedza) and O. serrulata (Carnival bush, umvuma)* are also used. *Ochna arborea* is used to make handles and carved sticks to support old people and the plant is thought to be a protective charm to drive off evil spirits. *Ochna natalitia* is suspected to hybridize with *O. serrulata* in the southern-eastern parts of Mpumalanga (Schmidt *et al.*, 2002).

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The indigenous people of Central Africa use the stem bark of *O. calodendron* for pain relieving purposes and for the treatment of liver infections and dysentery (Messanga *et al.*, 2001). In Zimbabwe, the mature leaves of *O. pulchra* Hook., also called '*umnyelenyele*' or '*muparamhosva*' in their vernacular language, is assumed to be good for cattle feeding, but its juvenile leaves are believed to be poisonous to cattle. In Zimbabwean traditional medicine systems, the plant is extensively used to treat blood parasites and various skin diseases (Sibanda *et al.*, 1990). The Pare society of Kilimanjaro in Tanzania use the wood of *O. holstii*, locally known as '*kitakwa*', for making handles, spears and firewood (de Boer *et al.*, 2005).

O. afzelii is a common tree of West Africa where it has various names in different West African countries. In Ghana the plant is locally known as *adangme* or *okoliawatso*, in Senegal as *mandingbambara* or *mananitiana* and in Togo as *Tem* or *fanam*. Various parts (leaves, roots, wood, bark) of the plant are used by the natives to treat different health problems including female sterility, menstrual complaints, jaundice, lumbago and dysentery (Bouquet, 1969).

The stem bark of *O. lanceolata*, a semi-evergreen tree found widely in central and peninsular India is commonly used as an abortifacient and also for treating gastric complaints and menstrual disorders (Mathew, 1981; Muthukumarasamy et al., 2003). The Zulu people in South Africa use a decoction of the root of *Ochna serrulata* which is commonly known as fynblaarrooihout (Afr.), Umbomvane (Zulu), iliTye (Xhosa) for the treatment of bone diseases and gangrenous proctitis (Hutchings et al., 1996; Hutchings and Van Staden, 1994).

In New South Wales and southern Queensland, Australia, *O. serrulata* has been declared a noxious weed, class 4. The plant is described to be a tough and adaptable weed which grows in areas ranging from sunny and open positions to the shade of deep forest. It is believed to cause the disappearance of some indigenous species due to its adaptability nature and easy spread. The plant has been widely cultivated outside South Africa as an excellent ornamental garden plant (Wolff, 1999).

2.4 The Genus *Ochna*: Phytochemistry and Biological Activities

From the previous phytochemical studies on the genus *Ochna,* it has been revealed that the genus is rich in phenolic biflavonoids (Khalivulla et al., 2008; Likhitwitayawuid et al., 2001; Pegnyemb et al., 2001, 2003b). Flavonoids, an abundant class of plant constituents, are acknowledged to exhibit numerous biological/pharmacological activities such as anticancer, antibacterial, antiviral, anti-inflammatory, immunomodulatory activities, antimalarial and anti-HIV activities (Ichino et al., 2006; Murakami et al., 1991a; Murakami et al., 1991b; Murakami et al., 1992; Reutrakul et al., 2007).

The rearranged biflavonoids, calodenone (**35**), lophirone A (**36**) and afzelone D (**37**), were isolated for the first time from the methanol extracts of the stem bark of *O. calodendron*, *Lophira lanceolata* and *O. afzelii,* respectively (Ghogomu et al., 1987; Messanga et al., 1992; Pegnyemb et al., 2003a). The compounds represent a very unusual skeleton of a biflavonoid (Ghogomu *et al.,* 1987). Lophirone A (**36**) has been reported to be an inhibitor of Epstein-Barr virus and has shown inhibitory properties against tumor promotion. To date there are many reports on the isolation of **36** from *Ochna* species (Anuradha et al., 2006; Kaewamatawong et al., 2002; Kittisak et al., 2005; Pegnyemb et al., 2003b). There are no filed reports on the biological activities of **35** and **37**.

Dimeric chalcones have also been isolated from the genus *Ochna*, the report filed by Messanga and co-workers indicated the isolation and characterization of lophirone K (**38**) and lophirone C (**39**) from *Lophira lanceolata*. Calodenin A (**40**) and calodenin B (**41**) were isolated from *O. calodendron* (Messanga et al., 1994; Tih et al., 1989).
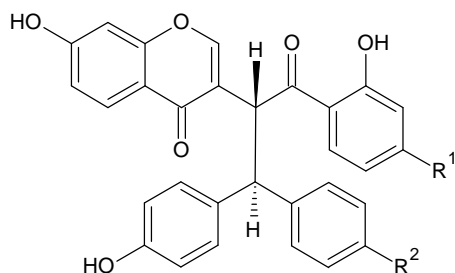
35 R^1 = OH, R^2 = OMe

36 R^1 = OH, R^2 = OH

37 R^1 = OMe R^2 = OH

The other dimeric chalcones reported from *O. afzelii* are isolophirone C (**42**) and dihydrolophirone C (**43**). The roots of *Lophira lanceolata* yielded three dimeric chalcones namely lophirone F (**44**), lophirone G (**45**) and lophirone H (**46**) (Tih *et al.*, 1990).
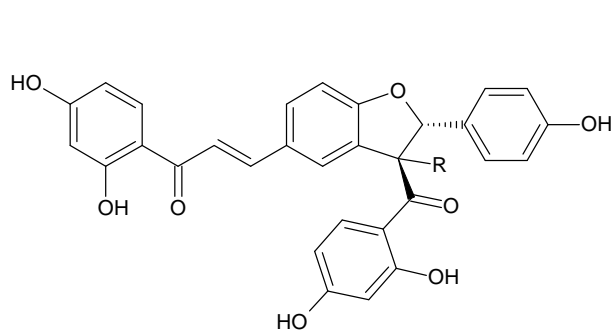
38   R=OH
39   R= H
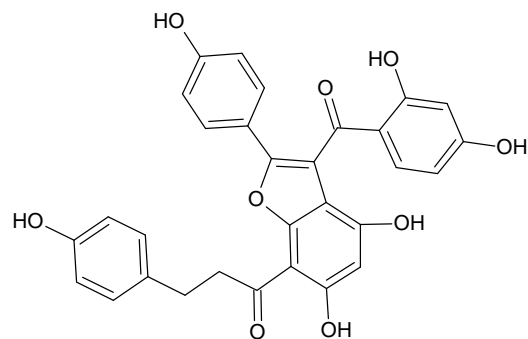
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It is of interest to note that the relative configuration of lophirone C (**39**), G (**45**) and F (**44**) are known but the absolute configurations have not been determined. There are no reports on the biological activities of the mentioned compounds.
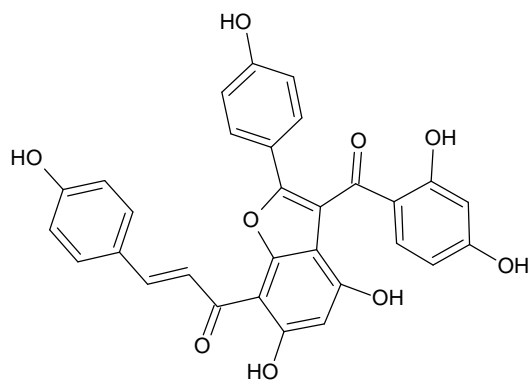
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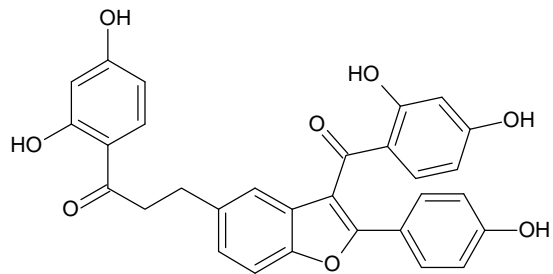
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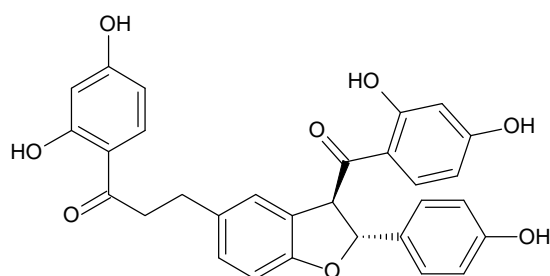
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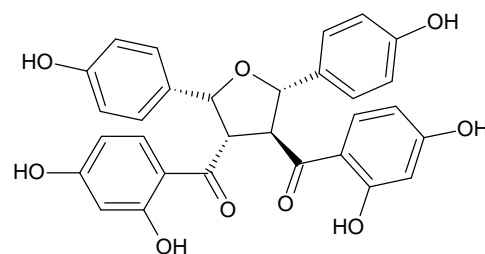
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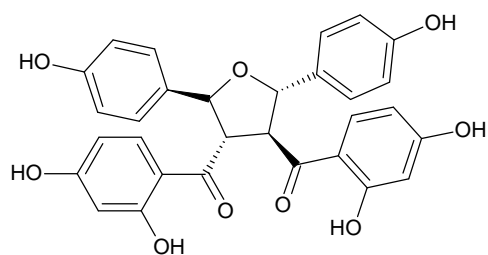
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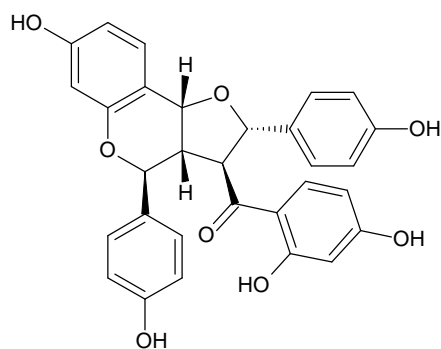
46

From the stem bark of *O. integerrima,* a derivative of lophirone B (**47a**), 6'''-hydroxylophirone B (**47b**) was isolated together with an *O*-glucosylated chalcone 6'''-hydroxylophirone-B 4'''-*O*-β-glucoside (**48**) (Kaewamatawong et al., 2002; Tih et al., 1989).

47a R^1 = H, R^2 =OH

47b R^1 =OH, R^2 = OH

48 R^1 =OH, R^2 = O-Glucoside
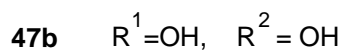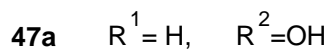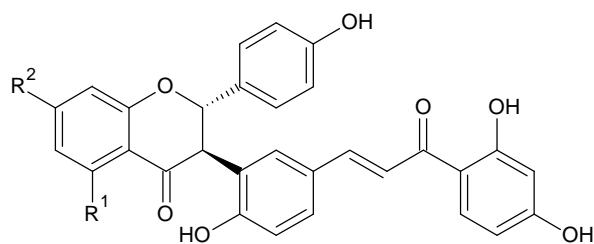
The stem bark of *O. afzelii*, a plant commonly found in tropical Africa, yielded two biflavonoids, namely a flavanone/chalcone hybrid, afzelone A (**49**) and a biflavanone, afzelone B (**50**) (Pegnyemb *et al.*, 2001, 2003b). The biological activities of these compounds have not been determined.
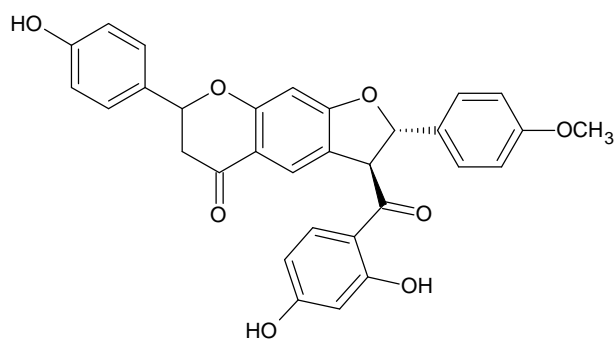
49
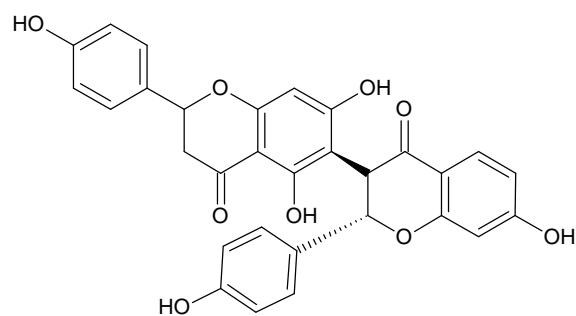
50

The aerial parts of *Ochna squrrosa* yielded an ether-linked biflavone, ochnaflavone (**51**) (Okigawa *et al.*, 1976). The compound has also been isolated from aerial parts of *Lonicera japonica* (Caprifoliaceae) (Kun *et al.*, 1992). Derivatives of ochnaflavone have been isolated from other plant species, these are 7-methoxy-2,3-dihydroochnaflavone (**52**) and 2,3-dihydroochnaflavone (**53**) from the leaves of *O. obtusata* (Rao *et al.*, 1997).

| **51** | **52** R=OMe | **54** |

53 R=OH
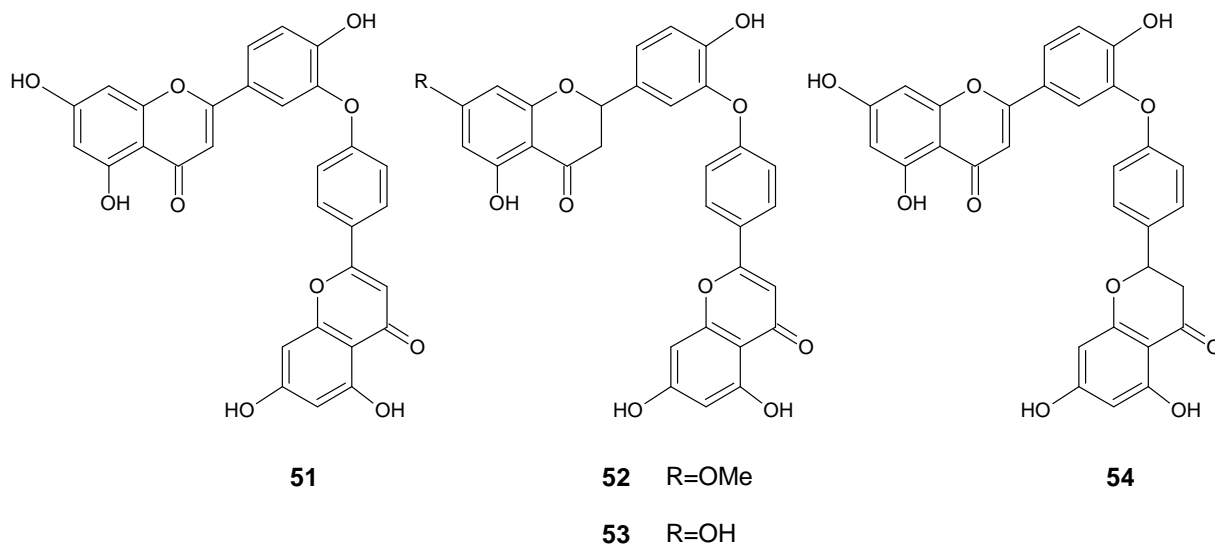
2'',3''-Dihydroochnaflavone (**54**), another ochnaflavone derivative, was isolated from the leaves and twigs of *O. integerrima* (Likhitwitayawuid *et al.*, 2001). Ochnaflavone (**51**) has a broad range of biological/pharmacological activities including anti-inflammatory, anticancer, anti-HIV and anti-atherogenic activities (Suh et al., 2006a; Suh et al., 2006b; Suh et al., 2006c).

Reutrakul and group reported the isolation and anti-HIV activity of the derivatives of **51** *viz.* 7''-methoxyochnaflavone (**55**) and 7''-methoxy-2'',3''-dihydroochnaflavone (**56**). The compounds were isolated from the leaves of *O. integerrima* and were found to exhibit a potent anti-HIV activity with EC_{50} values of 2.0 and 0.9 µg/mL, respectively (Reutrakul *et al.*, 2007). The two compounds **55** and **56** were also found to inhibit the HIV-1 reverse transcriptase (RT) enzyme with IC_{50} values of 2.0 and 2.4 µg/mL, respectively (Reutrakul *et al.*, 2007).
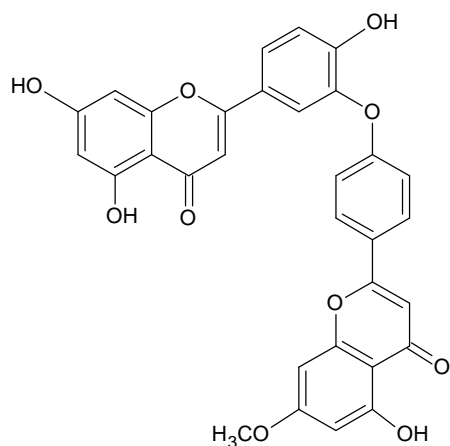
55
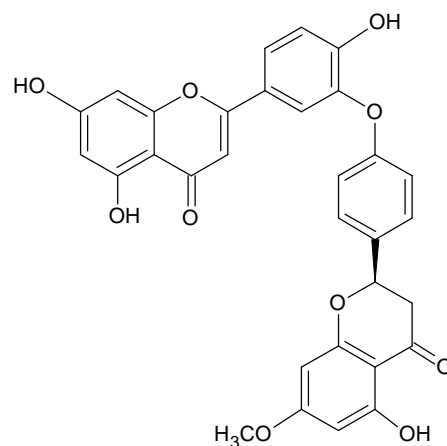
56

O. integerrima yielded prenylated and glucosylated flavonoids with significant anti-HIV activities. These compounds include 6-γ,γ-dimethylallyldihydrokaempferol 7-*O*-β-D-glucoside (**57**), 6-γ,γ-dimethylallylquercetin 7-*O*-β-D-glucoside (**58**), 6-(3-hydroxy-3-methylbutyl)taxfolin 7-*O*-β-D-glucoside (**59**), 6-(3-hydroxy-3-methylbutyl)quercetin 7-*O*-β-D-glucoside (**60**). The anti-HIV activities of these compounds range from EC_{50} 14 - 102.4 µg/ml (Reutrakul *et al.*, 2007).

57 $R^1 =$ $R^2 = H$

58 $R^1 =$ $R^2 = OH$

59 $R^1 =$ $R^2 = OH$

60 $R^1 =$ $R^2 = OH$
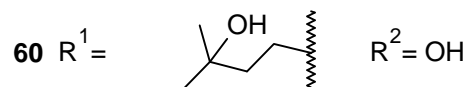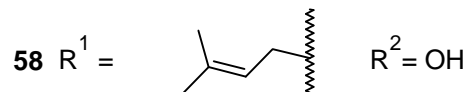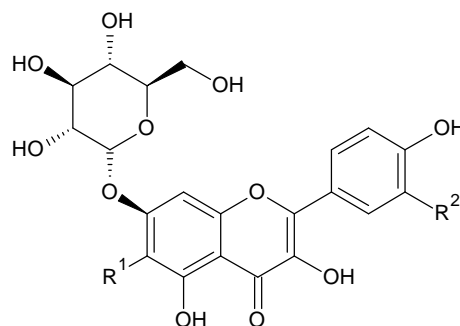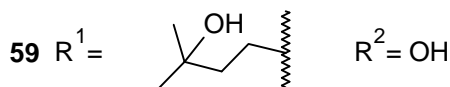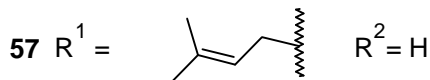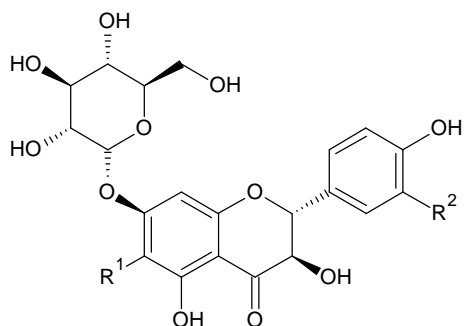
Potent antimalarial activities have been shown by isocampylospermone A (**61**) and campylospermone A (**62**); these compounds are diastereomers isolated for the first

time from the stem bark of *O. integerrima*. Biflavanoids **61** and **62** have shown to be significantly active against *Plasmodium falciparum* multidrug resistant strains (K1) at 0.08 and 5.2 µg/mL, respectively, and multidrug sensitive strains (FCR3) at 0.26 and 4.5 µg/mL for **61** and **62**, respectively (Ichino *et al.*, 2006).

61   **62**
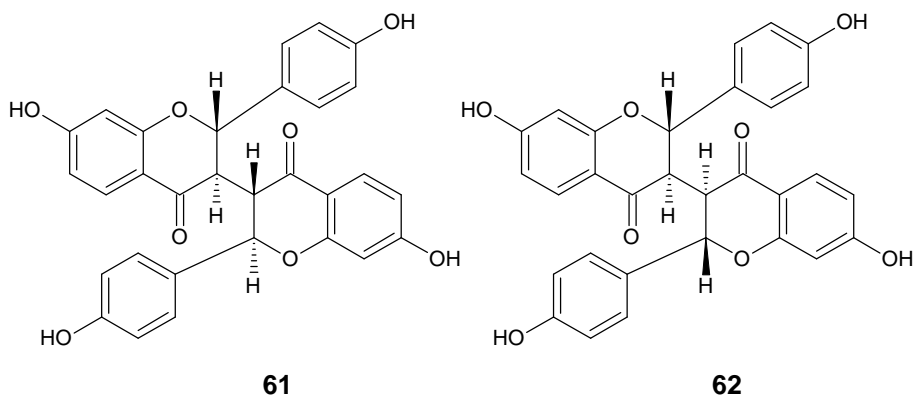
Kaemferol 3-*O*-α-rhamnoside (**63**) and ruixianglangdusu B (**64**) were isolated from the leaves of *O. lanceolata* (Khalivulla et al., 2008; Kun et al., 1992), there are no filed reports on the biological activities of these compounds.

63   **64**
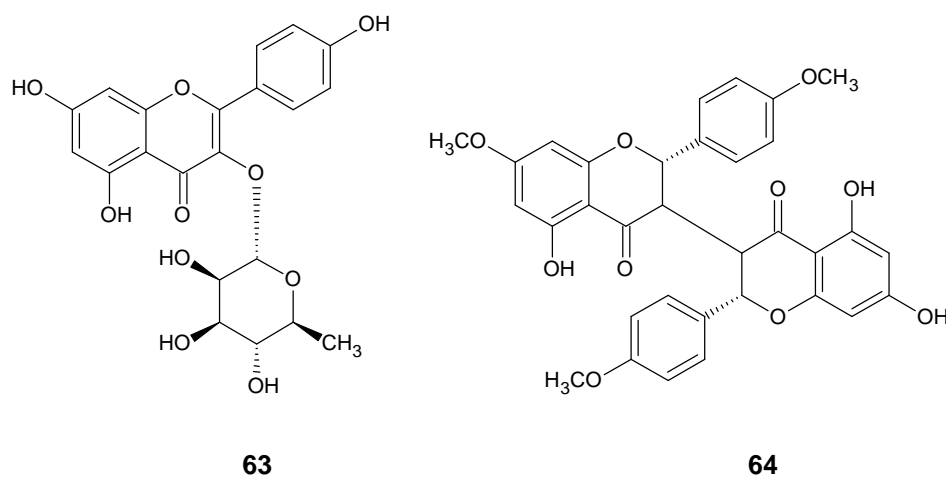
In 1987, Kamil *et al.* reported the isolation of tetrahydroamentoflavone (**65**) and amentoflavone 7"-*O*-methyl ether (**66**) from the leaves and twigs of *O. pumila* (Kamil *et al.*, 1987). There are no filed reports on the biological activities of these compounds.
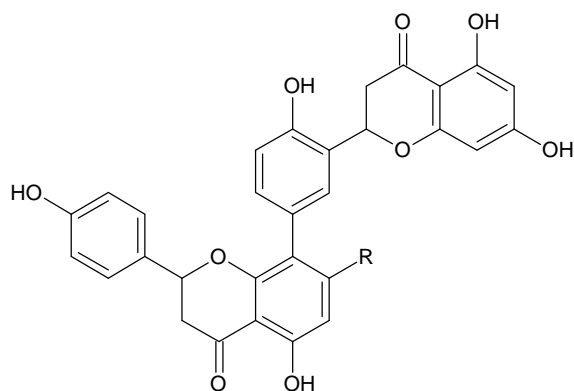
65 R=OH

66 R=OMe

From the roots of *O. pulchra* (Ochnaceae), a woody plant found in southern and central Africa, Sibanda and co-workers reported the isolation of vismiones from the genus *Ochna* for the first time. The compounds isolated from the plant were ochnabianthrone (**67**) together with vismiones L (**68**), M (**69**), D (**70**), acetylvismione D (**71**) and 3-*O*-geranylemodinanthrone (**72**) (Sibanda *et al.*, 1990, 1993). The biological activities of these compounds are not known yet.

67

68

69

70 R = H

71 R = Ac

72
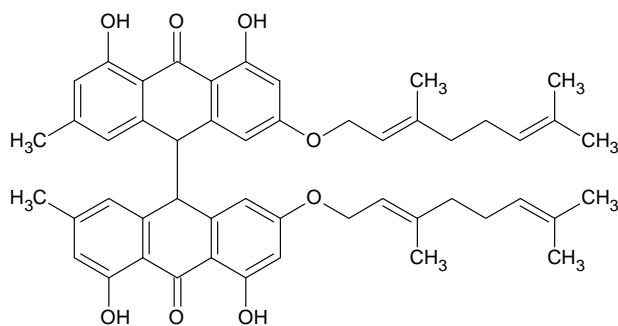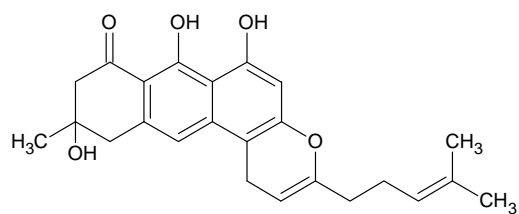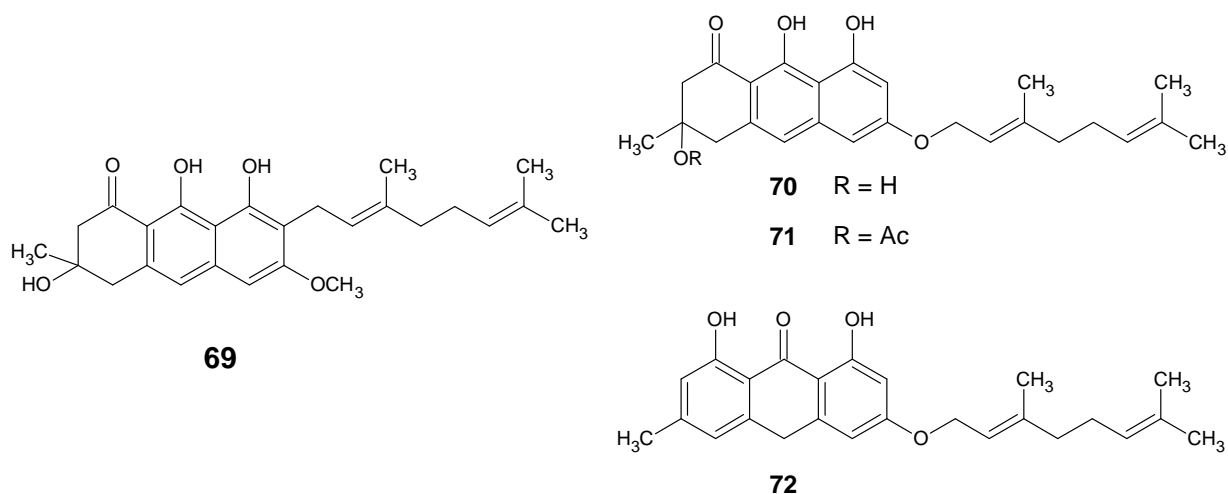
2.5   Conclusion

Members of the family Ochnaceae are widely used in traditional medicine systems of different cultures in the treatment of dysentery, liver infections and blood parasites. The family is also known for its use in curing toothaches, lumbago, ulcers, menstrual complaints, cancer and sores. Conditions like constipation, asthma and epilepsy are known to be cured by the use of Ochnaceae plants. There is a large diversity in the compounds that have been isolated from Ochnaceae species ranging from simple flavonoids to complex flavonoids. However, only the relative configuration of these compounds is known and the absolute configuration has not been determined. The biological/pharmacological activities of these compounds isolated from Ochnaceae species have up to now not received enough attention. This thesis therefore intends to report the isolation, structural elucidation as well as biological activities of the compounds from *O. serrulata*. In the next chapter, the isolation and characterization of compounds from the EtOAc extract of the stem bark and leaves of *O. serrulata* will be discussed.

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CHAPTER 3

Compounds Isolated from the Aerial Parts of *Ochna serrulata*

3.1 Introduction

The phytochemistry of some species of *Ochna* has been studied as discussed in Chapter 2 of this thesis. However, the phytochemistry of *O. serrulata* has not been investigated, therefore, in Chapter 3 and 4 of this thesis, the structures of the compounds present in the stem, leaves and roots of *O. serrulata* are reported for the first time.

3.1.1 *Ochna serrulata*

O. serrulata is a plant with bright yellow flowers accompanied by a very unusual persistent calyx that enlarges and becomes bright red when in fruit. The fruits are also colourful with black drupelets sitting on a bright red, open receptacle (Golding, 2002). The species name of the plant is derived from the Latin word *serrula* meaning "little saw", referring to its fine-toothed leaf margins (Simpson, 1979).

O. serrulata is a small shrub mostly growing to a height of 1 to 2 m; occasionally the plant may become a small tree of up to 6 m high. Its leaves are narrow and shiny green with fine toothed serrations along its edges. In spring, the fragrant yellow flowers of the plant blossom but the petals have a tendency of dropping soon after they bloom and five or six fruits grow together attached to the sepals. During the fruit development, the sepals tend to enlarge and its colour changes to bright red (Mark, 1999). In Southern Africa, *O. serrulata* are found in South Africa

(Limpopo, Mpumalanga, KwaZulu-Natal and Eastern Cape), Swaziland, Zimbabwe and Mozambique (Golding, 2002).

The aims of this Chapter are:

- To report the isolation of the compounds from the stem bark and leaves of *O. serrulata*
- To report the structures of the isolated compounds from the stem and leaves of the plant.

3.2   Results and Discussion

The EtOAc extracts of the pulverized stem and leaves of *O. serrulata* afforded a rearranged biflavonoid lophirone A (**36**), lophirone C (**39**), three biflavonoids afzelone B (**50**), ochnaflavone (**51**), 2",3"-dihydroochnaflavone (**54**), a mixture of isocampylospermone A (**61**) and campylospermone A (**62**), methyl (2',4'-dihydroxyphenyl)acetate (**73**), (2',4'-dihydroxyphenyl)acetic acid (**74**), epicatechin (**75**), 5-deoxyurundeuvine C (**76**), two *C*-glucosylated flavones psilosin (**77**) and 3'-*O*-methylpsilosin (**78**) and a cyanoglucoside (**79**). Compounds **61** and **62** were also isolated from the roots of the plant and their structural elucidation will be discussed in Chapter 4.

3.2.1   Methyl (2',4'-dihydroxyphenyl)acetate (73)

73
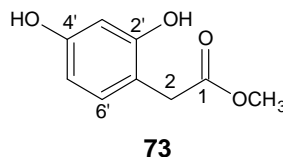
In the ^{1}H NMR and ^{1}H, ^{1}H COSY spectra of **73** (Plate 1a and 1b) an ABX aromatic spin system at δ_H 6.28 (d), 6.23 (dd) and 6.87 (d) was identified. Methylene protons resonated at δ_H 3.48 (*s*, 2H) and a methoxy group was observed at δ_H 3.64 (*s*, 3H).

The ^{13}C NMR spectrum (Plate 1c) showed the presence of nine peaks corresponding to nine carbons, consistent with the proposed structure. Two peaks at δ_C 157.3 and 156.1 are characteristic of aromatic carbons bonded to oxygen atoms and the peak at δ_C 173.8 suggesting the presence of an ester carbonyl carbon. The peak at δ_C 50.7 correlated with the methoxy protons in the HSQC spectrum. Furthermore, the HMBC spectrum (Plate 1d) pointed out the correlation between the methoxy group and the carbonyl at δ_C 173.8, confirming the presence of a methyl ester group. The long-range correlations between the methylene protons and C-1' of the aromatic ring implied that the methylene group is linked to C-1' of the aromatic ring.

The HMBC spectrum indicated ^{1}H/^{13}C correlations shown in Fig. 3.1 confirmed the structure of compound **73** as methyl (2',4'-dihydroxyphenyl)acetate.

73
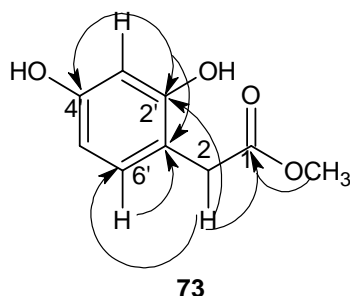
Fig. 3.1: HMBC correlations in structure 73

The compound was isolated for the first time from *Madhuca pasquiery* and later from the seeds of *Ilex aquifolium*. It is reported to have antioxidant activity at 2.55 x 10^{-3} mg.mL^{-1} (Nahar et al., 2005; Taylor et al., 2011). This is the first report on the isolation of methyl (2',4'-dihydroxyphenyl)acetate from *Ochna* species.

3.2.2 (2',4'-Dihydroxyphenyl)acetic acid (74)

74
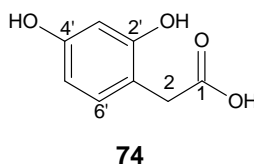
Careful observation of the ^{1}H NMR and ^{1}H, ^{1}H COSY spectra of **74** (Plate 2a and 2b) showed the presence of an ABX aromatic spin system at δ_H 6.34 (d), 6.28 (dd) and 6.87 (d) as well as methylene protons which resonated at δ_H 3.42 (s, 2H). Essentially, **74** showed same features in the NMR spectra as **73**, with the only difference being the absence of a methoxy group in **74**.

The ^{13}C NMR spectrum showed the presence of eight carbons consistent with the proposed structure, where the peak at δ_C 177.2 signified the presence of a carbonyl group and the two oxygen-linked aromatic carbons resonated at δ_C 156.3 and 157.6. A DEPT-135 experiment (Plate 2f) established the presence of a methylene carbon at δ_C 36.6. The correlations between the methylene protons and carbons resonating at δ_C 177.2 (C-1), δ_C 156.3 (C-2'), δ_C 130.9 (C-6') and δ_C 113.3 (C-1') showed that the methylene carbon is linked to the aromatic ring and at the same time to the carbonyl carbon resonating at δ_C 177.2.

From the rationalization of the NMR data, structures **74** and **74a** are possible.

| **74** | **74a** |
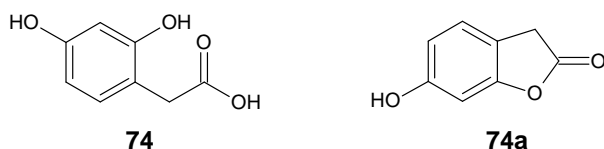
Structures **74** and **74a** will give the same splitting pattern in their NMR spectra but **74a** is not the correct structure since the downfield resonance of the carbonyl carbon at δ_C 177.2 is not consistent with the presence of a lactone functionality (Iqbal *et al.*, 1995). The ^{1}H/^{13}C correlations from the HMBC spectrum (Plate 2e) are given in Fig. 3.2 and hence confirmed **74** to be (2',4'-dihydroxyphenyl)acetic acid.

74
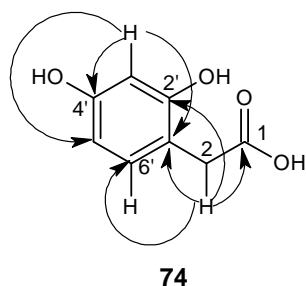
Fig. 3.2: HMBC correlations in structure 74

This compound has previously been identified as a constituent of a spider (*Nephila clavata*) toxin; it acts by inhibiting binding of L-glutamic acid to the brain synaptic membranes and hence causes paralysis of the prey (Kim et al., 1998; Pan-Hou et al., 1987; Taylor et al., 2011). The compound was also isolated from the seeds of *Ilex aquifolium* by Nahar and co-workers who reported an antioxidant activity at 1.5 x 10^{-3} mg.mL^{-1} for the compound (Nahar *et al.*, 2005). In the Ochnaceae, the compound was isolated for the first time from the MeOH extract of the branches of *Ouratea hexasperma* (de Carvalho *et al.*, 2008). The observed difference between the NMR data of the reported compound and the one reported in this thesis is attributed by different solvents used for NMR experiments. The isolation of (2',4'-dihydroxyphenyl)acetic acid from an *Ochna* species is reported here for the first time.

3.2.3 Epicatechin (75)

75
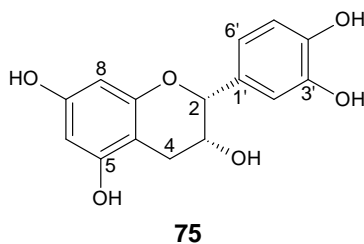
The ^{1}H NMR (Plate 3a) and ^{1}H, ^{1}H COSY (Plate 3b) spectra showed the presence of an ABX aromatic spin system at δ_H 6.96 (d, *J*=1.9 Hz), 6.78 (dd, *J*=1.9, 8.1 Hz) and 6.70 (d, *J*=8.1 Hz). The presence of a tetrasubstituted aromatic system was revealed by the two *m*-coupled protons at δ_H 5.93 and 5.91 for H-6 and H-8,

respectively, of a flavonoid derivative. Two chemically different methine protons were present at δ_H 4.79 (H-2) and 4.16 (m, H-3) (H-2 was overlapping with the solvent signal in ^{1}H NMR spectrum but its chemical shift was obtained from the HSQC spectrum). Methylene protons resonated at δ_H 2.84 (J=17.0, 4.6 Hz, H-4) and 2.72 (J=17.0, 4.6 Hz, H-4) in ^{1}H NMR and ^{1}H, ^{1}H COSY spectra.

In the ^{13}C NMR spectrum (Plate 3c) there were fourteen signals and the peaks at δ_C 157.9, 157.6, 157.3, 145.9 and 145.7 are in agreement with the presence of five oxygen-linked aromatic carbons in the proposed structure. The absence of peaks at δ_C 170-190 showed the absence of carbonyl carbons in the compound. In the HMBC spectrum (Plate 3e), the long-range correlations between H-2', H-6' and C-2 implied that the ABX aromatic ring system was connected to the pyran ring at C-2. The two m-coupled aromatic protons at δ_H 5.91 (1H, d, J=2.4 Hz) and 5.93 (1H, d, J=2.4 Hz) showed long-range ^{1}H/^{13}C correlations [HMBC spectrum (Plate 3e)] with C-7, C-4a and C-8a at δ_C 157.3, 100.1 and 145.7, respectively, confirmed the presence of the benzopyran system of the flavonoid unit. The connectivity of the carbon skeleton was confirmed by the HMBC correlations as shown in Fig. 3.3.

75
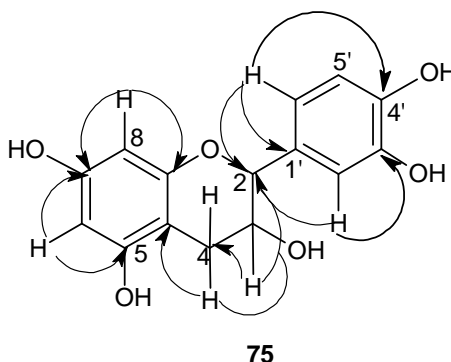
Fig. 3.3: HMBC correlations in structure 75

The *pseudo*-molecular ion peak [M+Na]$^+$ at *m/z* 313.0688 in the positive-ion mass spectrum is consistent with the molecular formula of $C_{15}H_{14}O_6$. Based on the spectroscopic evidence, compound **75** was assigned as epicatechin.

Epicatechin (**75**) is known for its antioxidant and anticarcinogenic activities, it is also an anti-artherogenic and antitumor agent (Xu *et al.*, 2004). The compound

was initially called *kakaool* due to the fact that it is found in large quantities in cacao beans (Freudenberg *et al.*, 1932). There are several reports on the isolation of this compound from *Ochna* species (de Carvalho et al., 2008; Rao et al., 1997; Reddy et al., 2008).

3.2.4 Lophirone A (36)

36
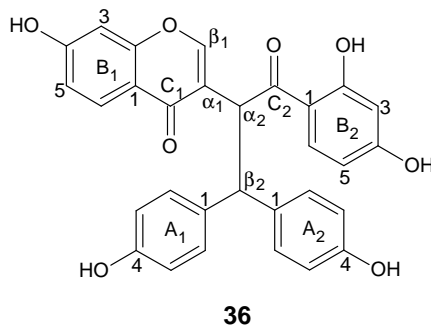
A yellow amorphous solid, compound **36**, produced a characteristic red coloured spot when stained with anisaldehyde/sulfuric acid and heat. Structural determination of **36** was achieved by careful analysis of 1D (^{1}H, ^{13}C) and 2D (HMBC, HSQC, COSY) NMR spectra.

The ^{1}H, ^{1}H COSY (Plate 4b) and the ^{1}H NMR spectra (Plate 4a), showed the presence of two *p*-substituted aromatic rings and two trisubstituted phenyl ring systems. A singlet at δ_H 8.28 is characteristic of H-2 of a 3-substituted benzopyran-4-one moiety (such as an isoflavone) and the peaks at δ_H 6.15 and 4.79 revealed the presence of an AX system. The large coupling (*J*=12.1 Hz) observed between the latter two protons suggested that they are in an antiperiplanar conformation.

In the ^{13}C NMR spectrum (Plate 4c) there are 22 peaks corresponding to 26 carbons with chemical shifts between δ_C 100 to 170 implying the presence of four aromatic ring systems in addition to the pyran-4-one ring. The methine carbons at δ_C 53.6 and 44.1 were correlated to protons at δ_H 4.79 and 6.15, respectively, in the HSQC spectrum (Plate 4d). The two carbonyls resonated at δ_C 204.7 and 175.4 and the up-field resonance of the later carbonyl suggested that it is part of a 4-pyrone system.

The position of the two *p*-substituted aromatic ring systems (AA'XX') was unambiguously assigned by to the long-range heteronuclear correlation [HMBC spectrum (Plate 4e)] between H-β_2 at δ_H 4.79 and C-1A$_2$ and C-1A$_1$ at δ_C 135.8 and 134.7 of ring A$_1$ and A$_2$, respectively, indicative of the connection of both rings to C-β_2, therefore, validating partial structure **36a** (Fig. 3.4).
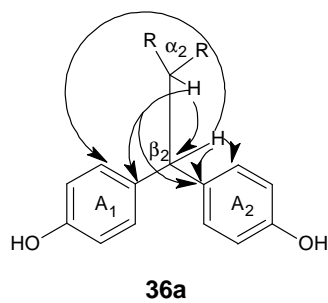
Fig. 3.4: HMBC correlations in partial structure 36a

The ^{1}H NMR spectrum showed the presence of a singlet at δ_H 11.67 signifying that one of the trisubstituted aromatic rings has a hydroxy group *peri* to a carbonyl group. The downfield resonance of H-6B$_1$ (at δ_H 7.94) shows its close proximity to the carbonyl and therefore, the second trisubstituted aromatic ring (ring B$_1$) is not substituted at C-5 (Abraham *et al.*, 2003).

The presence of a singlet at around δ_H 8.28 (H-β_1) evidenced that C-2 of the benzopyran moiety is not substituted. The long-range correlation between H-α_2 (at δ_H 6.15) and C-α_1 (at δ_C 122.3) confirms that C-α_2 is linked to the benzopyran moiety through C-α_1. The position of the first trisubstituted aromatic system was confirmed by the long-range correlations between H-6B$_1$ (at δ_H 7.94) and the carbonyl at δ_C 175.4 suggesting that this particular ring (B$_1$) is part of a benzopyran moiety and therefore forms ring A of a flavonoid skeleton.

Long-range correlations between H-6B$_2$ (δ_H 8.33) and a carbonyl at δ_C 204.7 (C-2) suggested the connectivity of the second trisubstituted aromatic ring to this carbonyl, which in turn was also correlated to H-α_2 as shown in partial structure **36b** (Fig. 3.5).

The assignment of **36** as lophirone A was confirmed further by the positive ion mass spectrum which exhibited a *pseudo*-molecular ion peak [M+Na]$^+$ at *m/z* 533, in agreement with molecular formula of $C_{30}H_{22}O_8$. The compound was previously isolated from the stem bark of *Lophira lanceolata* Van Tiegh Keay (Ochnaceae) by Ghogomu and coworkers. The NMR data of **36** was in agreement with those reported for lophirone A (Ghogomu *et al.*, 1987). The proposed structure of **36** is rather unusual as a biflavonoid since common biflavonoids have two C_6-C_3-C_6 units, but in **36** an aryl of one of the units have migrated to the other moiety.

36b
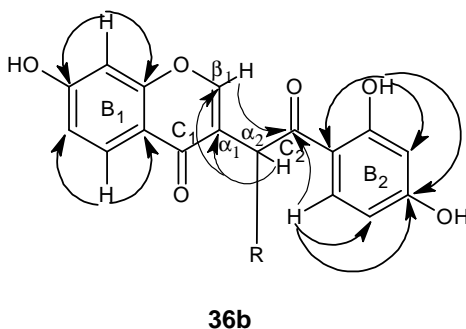
Fig. 3.5: HMBC correlations in the partial structure 36b

Lophirone A (**36**) has been reported to be an inhibitor of Epstein-Barr virus and has also shown inhibitory properties against tumor promotion. To date there are many reports on the isolation of **36** from *Ochna* species (Anuradha et al., 2006; Kaewamatawong et al., 2002; Kittisak et al., 2005; Pegnyemb et al., 2003). In an attempt to determine the absolute configuration of the compound at C-α_2, Murakami *et al.* have reported the crystal structure of a *p*-bromobenzoate derivative of **36**, but they could not determine the absolute configuration because racemic crystals were obtained (Murakami *et al.*, 1991).

The biosynthesis of **36** (Fig. 3.6) is believed to involve a rearrangement of a dimeric flavanone. The first step is the protonation of the oxygen atom of the benzopyran system followed by the formation of a phenol (in red) and a new bond (in red) between the *p*-hydroxy aromatic ring and one of the methine carbons. The second step is marked by the removal of a hydrogen atom from the C-3 of the remaining benzopyran system, thereby resulting into the breaking of the bond between the *p*-hydroxy aromatic system and the benzopyran system hence a total

shift of the ring and formation of the 3-substituted benzopyrone unit (in red) (Masatake *et al.*, 1984).

It is interesting to note that from the stems and roots of *O. serrulata*, dimeric flavanones (campylospermone type) and lophirone A have both been isolated showing that the proposed biogenesis is feasible.
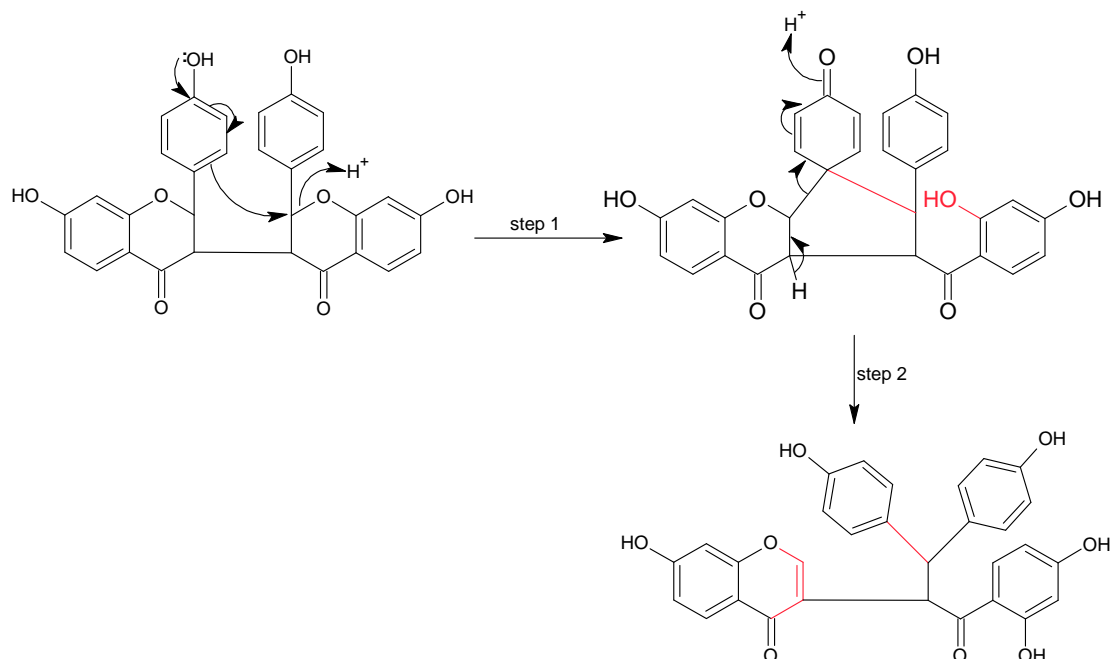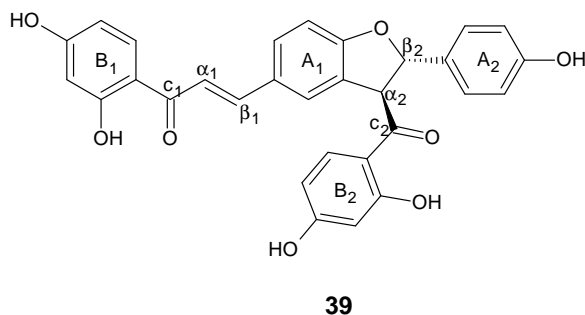

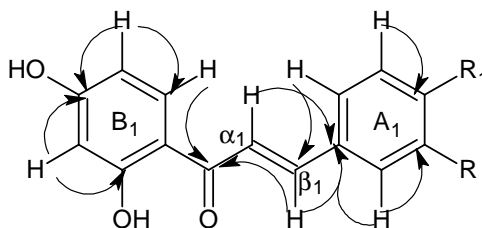**Fig. 3.6: Proposed biogenesis of 36 (Masatake *et al.*, 1984)**

3.2.5 Lophirone C (39)

39

The structural determination of **39** was achieved through a combination of different spectroscopic techniques including MS, ^{1}H, ^{13}C, COSY, HMBC and HSQC.

Careful examination of the ^{1}H, ^{1}H COSY (Plate 5a) and the ^{1}H NMR spectra (Plate 5b) showed the presence of a *p*-hydroxy aromatic ring and three trisubstituted aromatic ring systems together with an α,β-unsaturated carbonyl moiety at δ_H 7.72 (1H, d, $J_{\alpha1, \beta1}$ =15.4 Hz, H-β_1) and 7.51 (1H, d, $J_{\alpha1, \beta1}$ =15.4 Hz, H-α_1). In addition, the spectra showed the presence of two *vicinal* methine protons at δ_H 6.09 and 5.31, each integrating for one proton.

The ^{13}C NMR (Plate 5c) spectrum showed the presence of 30 carbons with peaks at δ_C 167.7, 166.6, 163.7 (2C) and 159.3 (2C) indicating the presence of six oxygen-linked quaternary aromatic carbons. The signal for the C-α_2 could not be observed in the ^{13}C NMR spectrum. The signal for H-α_2 was disappearing with time, while that of H-β_2 was changing from doublet to singlet over time. However, the compound was isolated as a clean sample from the plant. The ^{1}H/^{13}C correlation present in HMBC spectrum (Plate 5e) between H-6B$_1$ and the carbonyl at δ_C 193.4 confirms that a 2,4-dihydroxy aromatic ring system (ring B$_1$) is linked to this carbonyl (C$_1$). The mentioned carbonyl was in turn correlated to the H-α_1 and H-β_1 at δ_H 7.51 and 7.72, indicating the linkage of these *sp*^{2} carbons to the mentioned carbonyl as shown in the proposed structure. Furthermore, these protons attached to *sp*^{2}-hybridized carbons correlated to C-1A$_1$ (at δ_C 129.1), C-2A$_1$ and C-6A$_1$ (at δ_C 126.9 and 132.5 respectively), verifying the linkage of the second trisubstituted aromatic ring system (ring A$_1$) to the two *sp*^{2} carbons (C-α_1 and C-β_1). This information enabled the construction of partial structure **39a** in Fig. 3.7.

39a

Fig. 3.7: HMBC correlations in partial structure 39a

The carbonyl at δ_C 201.7 (C_2) correlated with the methine proton attached to the sp^3 carbon at δ_H 6.09 (H-β_2). The mentioned carbonyl was further correlated to H-$6B_2$ at δ_H 7.74 indicating that the third 2,4-dihydroxyphenyl ring (ring B_2) was linked to it as shown in the proposed structure **39b**.

The presence of a benzofuran instead of a benzopyran moiety was evidenced by the downfield resonance of the carbonyl at δ_C 201.7; if the carbonyl was present in a ring as in the 4-pyranone systems, the resonance would have been around δ_C 180-190 (Pegnyemb *et al.*, 2001).

The ^{1}H/^{13}C correlation between H-β_2 and C-$1A_2$ (of AA'XX' system) as well as the correlation between H-2, $6A_2$ at δ_H 7.21 and C-β_2 at δ_C 89.2 pointed out the connectivity of *p*-hydroxyphenyl ring to C-β_2. Thus the NMR data allowed assignment of the partial structure **39b** in Fig. 3.8.

39b

Fig. 3.8: HMBC correlations in partial structure 39b

The negative ion mass spectrum exhibited a *pseudo*-molecular ion peak [M-H]$^-$ at *m/z* 510, in agreement with a molecular formula of $C_{30}H_{22}O_8$. The NMR data of the compound correlated with that reported for lophirone C, previously isolated from the stem bark of *Lophira lanceolata* Van Tiegh Keay (Ochnaceae) (Pegnyemb *et al.*, 2001). Biosynthetically, **39** is proposed to originate from 2',4',4-trihydroxychalcone (**39c**). After conjugate addition of 2',4',4-trihydroxychalcone on itself to form a dimer (**39d**), cyclization of the intermediate **39e** gives the dihydrofuran ring in **39** (Fig. 3.9) (Ghogomu et al., 1989; Shimamura et al., 1996).
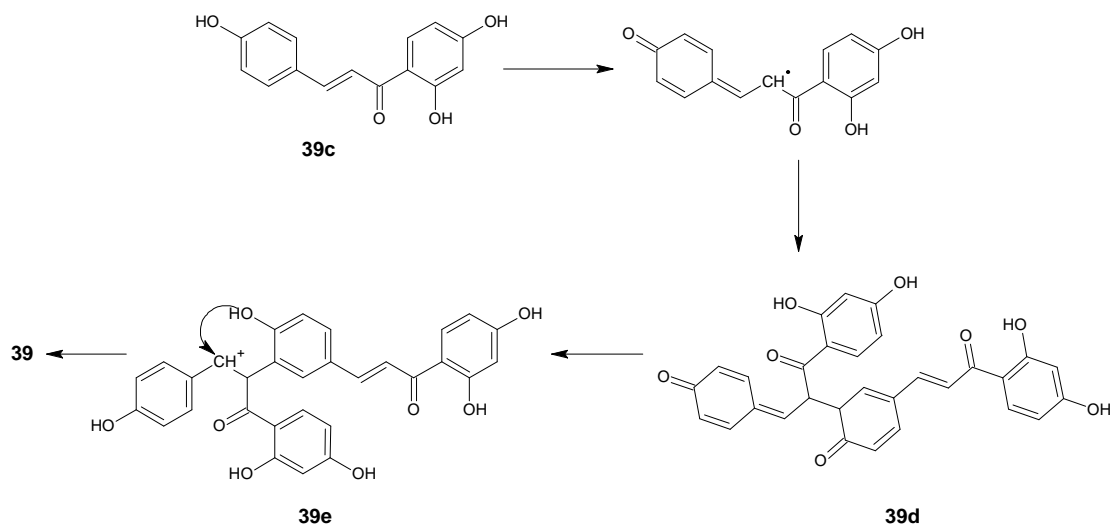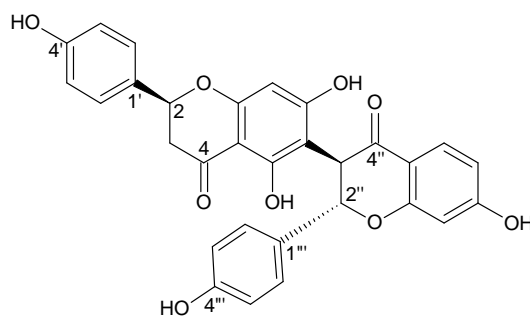
Fig. 3.9: Proposed biogenesis of 39 (Ghogomu et al., 1989; Shimamura et al., 1996)

There are no filed reports on the biological activities of lophirone C (**39**). It is of interest to note that the relative configuration of **39** is known but the absolute configuration has not been reported.

3.2.6 Afzelone B (50)

50

The presence of two *p*-hydroxy aromatic systems, one trisubstituted aromatic system and a pentasubstituted aromatic ring system were established from the detailed analysis of ^{1}H NMR (Plate 6a) and ^{1}H, ^{1}H COSY (Plate 6b) spectra. These spectra also depicted two *vicinal* diaxial protons as part of a pyrano ring at δ_H 5.84 and 4.62 and an AMX spin system at δ_H 3.03 and 2.61 for H-3, and 5.27

52

for H-2, indicating the presence of two flavanone moieties, one of which substituted at C-3".

Twenty-four signals were observed in the ^{13}C NMR spectrum (Plate 6c) corresponding to 29 carbons as per proposed structure, the signal for C-3" could not be detected in the spectrum, but could be observed in HSQC spectrum. The presence of seven oxygen-linked quaternary aromatic carbons was evidenced by the peaks at δ_C 166.7, 165.7, 163.9, 159.17, 159.16 and 158.8 (2C), consistent with the proposed structure.

The connectivity of the above-mentioned systems was established by ^{1}H/^{13}C correlations present in HMBC spectrum (Plate 6e). The correlation between H-2 (δ_H 5.27) and C-2',6' (δ_C 129.2) indicated that one of the *p*-hydroxy aromatic system was linked to the benzopyran system through C-2 as expected in flavanones.

The substitution in the pentasubstituted aromatic system can be according to structure **50a**, **50b** or **50c** below, where all three structures will give the similar splitting pattern in their ^{1}H NMR spectra.

50a   **50b**   **50c**
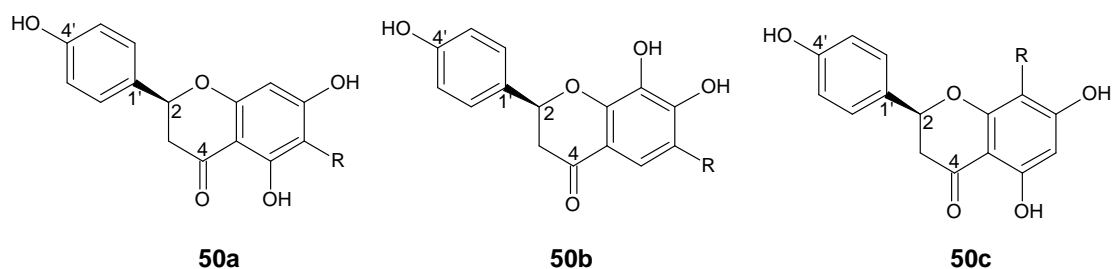
A singlet at δ_H 5.80 belongs to the pentasubstituted aromatic ring and the high-field resonance indicated that the proton is not *peri* to the carbonyl because if it were, then it would have had a lower field resonance at around δ_H 7.5-8.0. This was further supported by the presence of the hydrogen-bonded proton at δ_H 11.8 which showed that C-5 was hydroxy substituted and therefore structure **50b** is not the correct structure for **50** (Abraham *et al.*, 2003). NOE correlation observed between H-2, H-3" (at δ_H 5.27, 4.62) and the aromatic singlet H-8 (at δ_H 5.80) supports the

presence of a proton on C-8. The above-mentioned facts confirm that partial structure **50a** is correct.
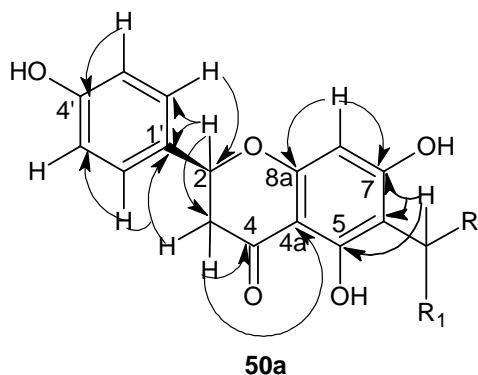

Fig. 3.10: HMBC correlations in partial structure 50a

The position of the second *p*-substituted aromatic system was assigned to be linked to C-2" due to the $^1H/^{13}C$ long-range correlations between H-2" (δ_H 5.84) and C-2''',6''' (δ_C 130.1) as expected in flavanones (Fig. 3.10). The *ABX* aromatic system was part of the benzopyran system thereby forming the second flavanone unit as was established by the long-range correlations between H-5" at δ_H 7.75 and C-4a" at δ_C 115.1, C-4" at δ_C 194.6.

The interflavanoid linkage involved C-3" of ring C and C-6 of the pentasubstituted aromatic ring was supported by the long-range correlations between H-3" at δ_H 4.62 and C-5, 6 and 7 at δ_C 159.16, 106.2 and 166.7 respectively, according to partial structure **50d** (Fig. 3.11).
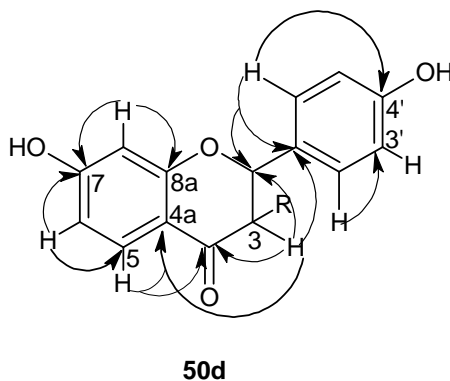

50d

Fig. 3.11: HMBC correlations in partial structure 50d

Relative configuration of this molecule was established by observations in the ^{1}H NMR spectrum where a large coupling constant was observed between H-2" and H-3" (J=13.0 Hz) indicating that they are in *trans*-diaxial positions as indicated in the structure **50**. The configuration was supported by the weak NOE interaction in NOESY spectrum (Plate 6f) between H-2" and H-3". NOE interaction between H-2, H-3" and H-8 confirmed the position of the pentasubstituted aromatic ring as indicated in the structure. These NOE interactions are as summarized in Fig. 3.12.

The negative ion mass spectrum of **50** exhibited a *pseudo*-molecular ion peak [M-H]$^-$ at *m/z* 526, which is in agreement with $C_{30}H_{22}O_9$ as the molecular formula of the compound. The compound was isolated previously from the stem bark of *Ochna afzelii* (Ochnaceae) (Pegnyemb *et al.*, 2003), none the less, the biological activity of **50** have not been reported.
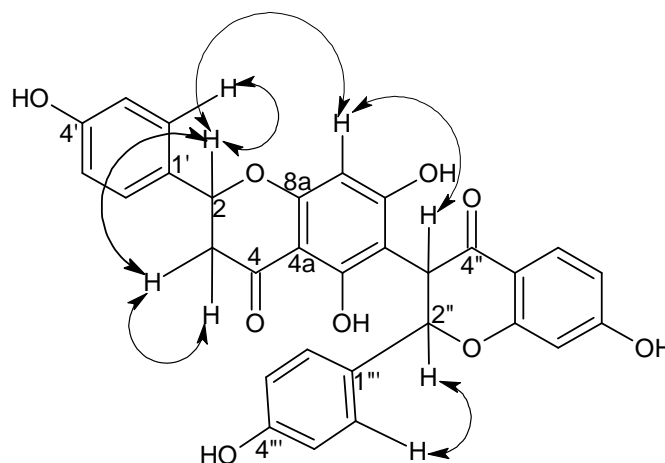
50

Fig. 3.12 NOE Interactions in structure 50

3.2.7 5-Deoxyurundeuvine C (76)
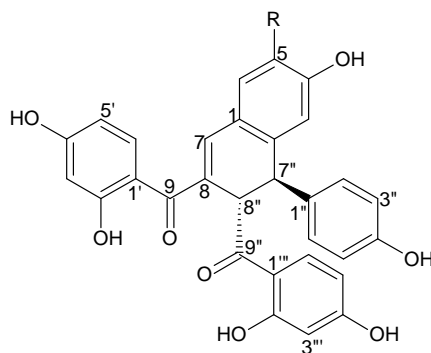

76 R= H

80 R=OH

The structure of compound **76** was characterized based on MS and 1D (^{1}H, ^{13}C) and 2D (COSY, HMBC, HSQC) NMR analysis. The ^{1}H NMR (Plate 7a) and ^{1}H, ^{1}H COSY (Plate 7b) spectra showed the following aromatic systems: one *p*-substituted benzene ring and three protons in a benzene ring showing an ABX spin system. Furthermore, a trisubstituted alkene and two *vicinal* methine protons were identified by the singlet peak at δ_H 7.24 for olefinic proton and two *trans*-coupled doublets at δ_H 4.99 and 4.34 (*J*=7.9 Hz) (Bandeira *et al.*, 2003).

In the ^{13}C NMR spectrum (Plate 7c) the presence of six oxygen-linked quaternary aromatic carbons (δ_C 166.9, 166.6, 166.3, 166.2, 161.4 and 157.8) and two carbonyls at δ_C 206.2 and 200.0 were observed. The chemical shifts of the carbonyls indicated that neither of them was part of a pyran-4-one ring because if they were, their resonances would be around δ_C 180-190 ppm. The heteronuclear multiple bond correlation (HMBC) spectrum (Plate 7e) displayed ^{1}H/^{13}C correlations between H-7 at δ_H 7.24 (s) and a carbonyl at δ_C 200.0 (C-9), C-2 at δ_C 133.1, C-8 and C-1 at δ_C 125.3, signifying that one of the three trisubstituted aromatic systems is linked to both C-7 and C-7''.

The linkage of the second ABX aromatic system to a carbonyl was established by the long-range ^{1}H/^{13}C correlations between H-6' at δ_H 7.68 and the carbonyl (C-9)

at δ_C 200.0. These facts lead to the construction of the partial structure **76a** in Fig. 3.13.
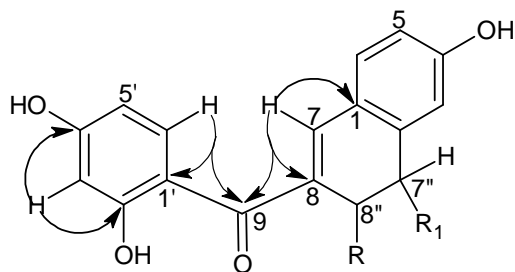
76a

Fig. 3.13: HMBC correlations in partial structure 76a

The *p*-substituted aromatic system was shown to be linked to C-8" due to the $^1H/^{13}C$ correlations between H-7" at δ_H 4.34 and C-1" at δ_C 133.1 and C-2", 6" at δ_C 130.8. Correlations between H-8" at δ_H 4.99 and both carbonyls [δ_C 206.2 (C-9") and 200.0 (C-9)], C-7 (δ_C 141.7), C-1" and C-2 (δ_C 133.1) and C-7" (δ_C 49.6) confirmed the position of C-8" and H-7" in the proposed structure.

The link of trisubstituted ring B' was derived from the long-range $^1H/^{13}C$ correlations between H-6"' and the carbonyl (C-9") at δ_C 206.2 which in turn was correlated to H-8" at δ_H 4.99. These correlations allowed the construction of partial structure **76b** (Fig. 3.14).
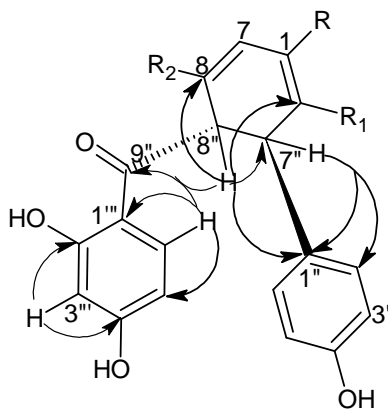
76b

Fig. 3.14: HMBC correlations in partial structure 76b

The relative configuration of **76** was established based on the value of the coupling constant (*J*=7.9 Hz) corresponding to *vicinal* spin-spin interactions between H-8" (δ_H 4.99 (1H, d, *J*=7.9 Hz)) and H-7" (δ_H 4.34 (1H, d, *J*=7.9 Hz)) which is a typical axial-axial coupling (Bandeira *et al.*, 2003). The absolute configuration of **76** was not determined.

A *pseudo*-molecular ion peak [M-H]⁻ at *m/z* 509 was present in the mass spectrum of **76** in agreement with a molecular formula of $C_{30}H_{22}O_8$ for **76**.

Table 3.1: ^{13}C and ^1H – NMR spectral data for 76 as compared to 80 in CD₃OD

80 δ ^{13}C	C (state)	76 δ ^{13}C	C (state)	δ H, *m* (*J*)
204.7	9" (q)	206.2	9" (q)	-
199.2	9 (q)	200.0	9 (q)	-
166.9	4'(q)	166.9	4'(q)	-
166.4	4'''(q)	166.6	4'''(q)	-
165.9	2'(q)	166.3	2'(q)	-
165.7	2'''(q)	166.2	2'''(q)	-
157.3	4''(q)	161.4	4''(q)	-
148.7	4(q)	157.8	4(q)	-
145.2	5(q)	115.4	5(CH)	6.67 (3H, dd, *J*=2.3, 8.2 Hz)
141.6	7 (CH)	141.7	7 (CH)	7.24 (1H, s)
135.7	6' (CH)	135.9	6' (CH)	7.68 (1H, d, *J*=8.8 Hz)
134.7	1" (q)	133.1	1" (q)	-
134.0	6''' (CH)	134.5	6''' (CH)	7.72 (1H, d, *J*=9.1 Hz)
131.4	2 (q)	133.1	2 (q)	-
130.2	2", 6" (CH)	130.8	2", 6" (CH)	7.05 (2H, d, *J*=8.5 Hz)
124.8	8,1 (q)	125.3	8,1 (q)	-
117.31	6 (CH)	132.5	6 (CH)	7.18 (1H, d, *J*=8.2 Hz)
116.8	3 (CH)	103.8	3 (CH)	6.27 (2H, d, *J*=2.3 Hz)
116.3	3", 5" (CH)	116.7	3", 5" (CH)	6.67 (3H, d, *J*=8.5 Hz)
113.4	1' (q)	114.2	1' (q)	-

113.1	1''' (q)	114.0	1''' (q)	-
109.0	5''' (CH)	109.3	5''' (CH)	6.27 (2H, dd, *J*=2.3, 9.1 Hz)
108.6	5'(CH)	108.9	5'(CH)	6.35 (2H, dd, *J*=2.4, 8.8 Hz)
103.9	3' (CH)	104.2	3' (CH)	6.16 (1H, d, *J*=2.4 Hz)
103.8	3'''(CH)	116.8	3'''(CH)	6.35 (2H, d, *J*=2.4 Hz)
51.1	8'' (CH)	51.8	8'' (CH)	4.99 (1H, d, *J*=7.9 Hz)
48.4	7'' (CH)	49.6	7'' (CH)	4.34 (1H, d, *J*=7.9 Hz)

The spectroscopic data of **76** were compared to those reported in the literature for urundeuvine C (**80**) isolated from the stem bark of *Astronium urundeuva* Engl. (Anacardiaceae) (Bandeira *et al.*, 2003). The difference between compound **76** and **80** is that whereas **76** has three trisubstituted aromatic rings and one *p*-substituted aromatic system, **80** has two trisubstituted aromatic rings, one tetrasubstituted aromatic ring and one *p*-substituted aromatic ring.
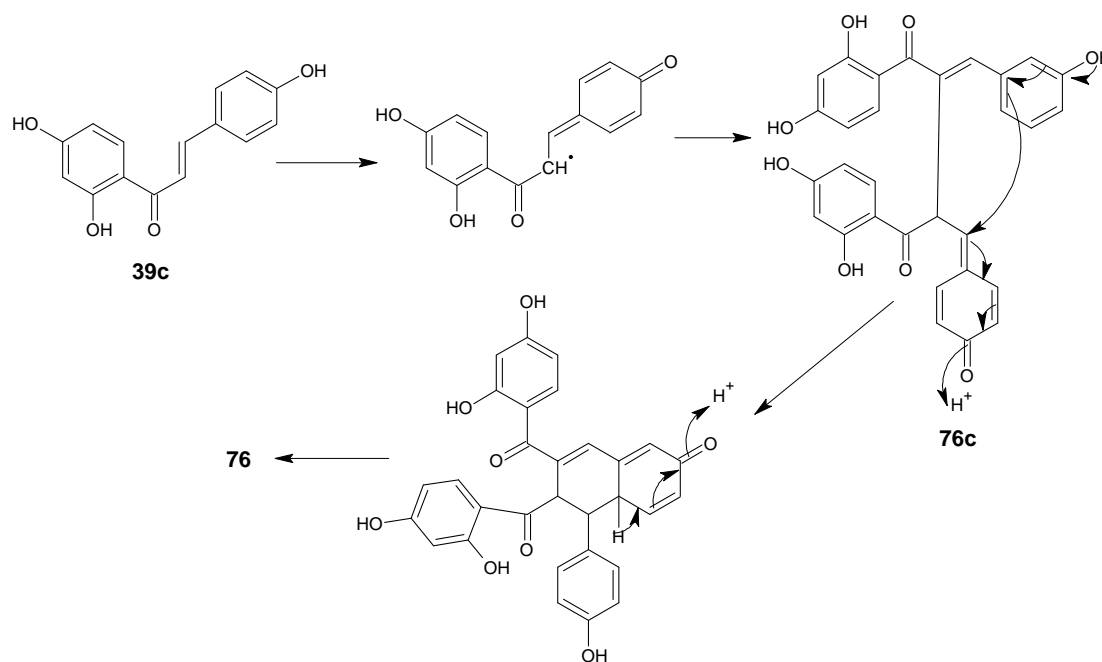

Fig. 3.15: Proposed biogenesis of 76 (Bai *et al.*, 2003)

Biogenetically, **76** is proposed to originate from 2',4',4-trihydroxychalcone (**39c**). After conjugate addition of **39c** on itself to form a dimer, followed by dienone-phenol rearrangement to yield an intermediate **76c**. Cyclization of **76c** gives compound **76** (Fig. 3.15) (Bai *et al.*, 2003). This compound has not been

described previously and this is the first report on the isolation and characterization of **76**, which was named 5-deoxyurundeuvine C.

3.2.8 Ochnaflavone (51)
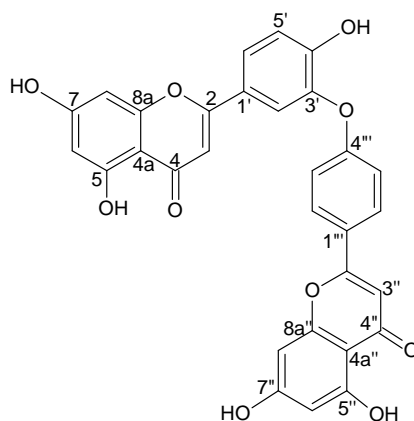
51

The presence of a *p*-substituted aromatic ring, a trisubstituted aromatic ring and two tetrasubstituted aromatic rings were established by careful examination of ^{1}H NMR (Plate 8a) and the ^{1}H, ^{1}H COSY (Plate 8b) spectra of **51**. This compound was clearly a biflavonoid which consisted of two flavone units. This is due to the presence of a singlet at δ_H 6.81 (2H) in the ^{1}H NMR spectrum which is characteristic of H-3 of flavones. In the HSQC spectrum this signal correlated with carbons resonating at δ_C 103.9 and 104.2 (Burns *et al.*, 2007).

In the ^{13}C NMR spectrum (Plate 8c), the presence of two carbonyls was indicated by the peaks at δ_C 182.2 and 182.1. Furthermore, 11 peaks appearing in the δ_C 165 to 140 resonance region accounting for oxygen-linked quaternary aromatic carbons (9 peaks) and the two β-carbons of α,β-unsaturated pyran ring system. The DEPT 135 (Plate 8f) spectrum showed the presence of 13 methine carbons with no methylene carbons in accordance with the proposed structure.

The ^{1}H NMR spectrum showed the presence of two singlets at δ_H 11.76 and 11.73 indicating that **51** is a 5,5"-dihydroxybiflavonoid (Markham, 1989). This is further supported by peaks at δ_C 94.6 and 99.4 in ^{13}C NMR spectrum for C-6, 6" and C-8,

8", respectively; these peaks are typical for 5,7-dihydroxyflavone derivatives which usually give a difference >4 between the resonance of C-6 and C-8 (Chari *et al.*, 1977).

The two aromatic rings with *m*-coupled protons are part of the benzopyran moieties as indicated by the long-range correlations between H-6, H-8 (H-6", H-8") and C-4a, C-4a" (partial structure **51a** and **51b**). The position of the *p*-hydroxyphenyl ring was derived from the long-range HMBC (Plate 8e) correlations between H-3" at δ_H 6.81 and C-1''' at δ_C 124.8, confirming that *p*-hydroxyphenyl ring is linked to the benzopyran moiety at C-2". Furthermore, the HMBC correlations between H-2''',6''' (δ_H 8.01) and C-2" (δ_C 163.2) supported partial structure **51a** (Fig. 3.16).

51a
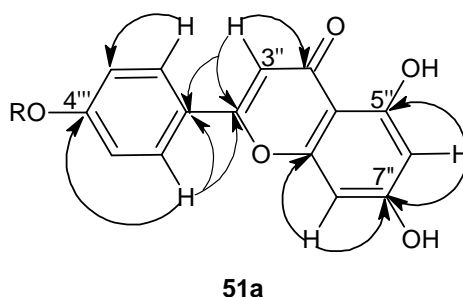
Fig. 3.16: HMBC correlations in partial structure 51a

The trisubstituted aromatic ring was attached to C-2 of the second benzopyran moiety based on the correlations between H-6' (δ_H 7.87) and C-2 (δ_C 163.6), also H-2' (δ_H 7.84) correlated to C-2. Fig. 3.17 summarizes the correlations as provided for partial structure **51b**.
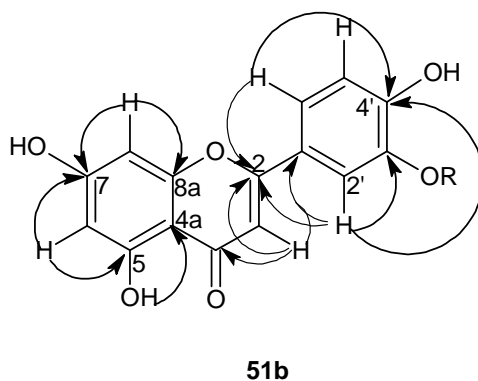

51b

Fig. 3.17: HMBC correlations in partial structure 51b

An ether linkage between the two flavone units was proposed due to the fact that H-2' showed long-range correlations with C-6', C-3', C-4' and C-2; H-3'''/H-5''' showed correlations with C-1''' and C-4'''. The absence of long-range correlations between the protons in the *p*-substituted and trisubstituted aromatic rings indicated that the two rings are not C-C linked. This was further confirmed by MS where an extra oxygen atom was observed and hence confirmed the linkage. Also, the down-field resonance of the C-3' signal as compared to the unsubstituted flavones, suggests that C-3' and C-4''' are involved in the interflavonoid linkage.

The positive ion mass spectrum of **51** shows the presence of the *pseudo-*molecular ion peak [M+Na]$^+$ at *m/z* 561 consistent with the molecular formula $C_{30}H_{18}O_{10}$. Therefore, **51** was identified as ochnaflavone, which was previously isolated from the aerial parts of *Lonicera japonica* (Kun *et al.*, 1992). To date there are many reports on the isolation of **51** from Ochnaceae plants (Flamini et al., 1997; Jayaprakasam et al., 2000; Rao et al., 1997). Ochnaflavone (**51**) has a broad range of biological/pharmacological activities including anti-inflammatory, anticancer, anti-HIV and anti-atherogenic activities (Suh et al., 2006a; Suh et al., 2006b; Suh et al., 2006c).

3.2.9 2'',3''-Dihydroochnaflavone (54)

54
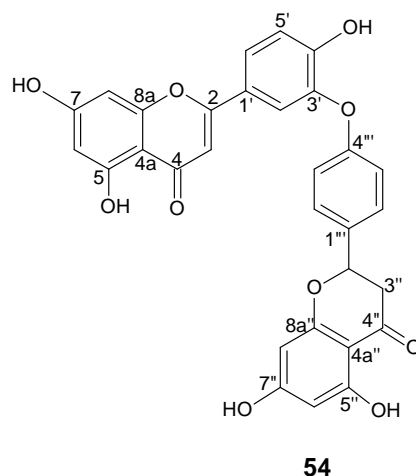
The ^{1}H NMR spectrum (Plate 9a) showed two intramolecular hydrogen-bonded hydroxy signals at δ_H 12.83 and 12.16 indicating that **54** is a 5,5''-dihydroxybiflavonoid (Markham, 1989). Upon careful observation of the ^{1}H NMR

and ^{1}H, ^{1}H COSY (Plate 9b) spectra, it was clear that substitution pattern of the aromatic rings is similar to that of ochnaflavone (**51**). The only difference was the presence of one ABX spin system of a flavanone moiety at δ_H 5.44 (dd, J=3.1, 12.6 Hz) and 3.12 (dd, J=12.6, 17.8 Hz), 2.78 (dd, J=3.1, 17.8 Hz) for H-2" and 3", respectively, indicating that **54** is a dihydro derivative of **51**.

The flavanone moiety can be linked to the other half either at C-3' (**54a**) or C-4'" (**54b**) and the two will give a similar splitting pattern in their ^{1}H NMR spectra.

54a **54b**
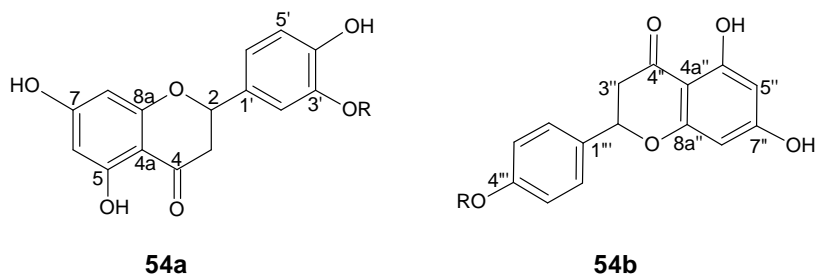
HMBC (Plate 9e) correlations between H-2" and C-1'", C-2'"/C-6'" implied that the *p*-substituted aromatic system was linked to C-2" and thereby partial structure **54b** was supported by the NMR spectroscopic data. More proof for the linkage of *p*-substituted system was provided by the correlations between H-3" and C-1'" as shown in partial structure **54b** in Fig. 3.18.


54b
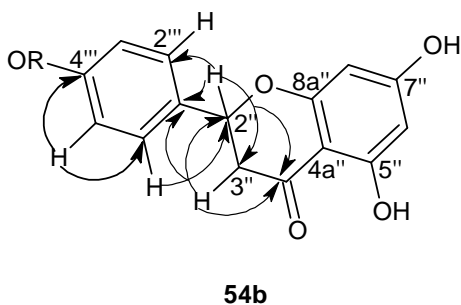
Fig. 3.18: HMBC correlations in partial structure 54b

The trisubstituted aromatic system was found to be linked to C-2 of benzopyran moiety, this is due to the correlations between H-2'/H-6' and C-2 at δ_C 163.8. Figures 3.18 and 3.19 summarize the ^{1}H/^{13}C correlations for partial structures **54b**

and **54c**. The flavones moiety was linked to the flavanone moiety by ether linkage as observed in **51**.
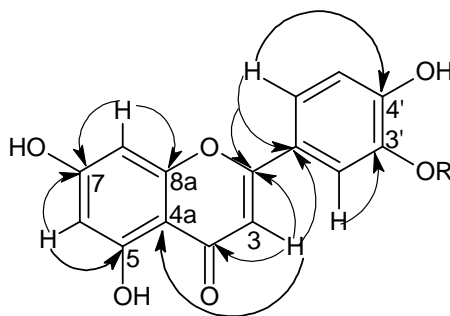
54c

Fig. 3.19: HMBC correlations in partial structure 54c

In the negative ion mass spectrum, the *pseudo*-molecular ion peak [M-H]- at *m/z* 539 consistent with the molecular formula $C_{30}H_{20}O_{10}$ was present, indicating that **54** has two additional hydrogens as compared to **51**. The compound was reported for the first time from the leaves of *Ochna integerrima* (Likhitwitayawuid *et al.*, 2001). The absolute configuration at C-2" and biological activities of **54** have not been reported.

3.2.10   Psilosin (77)
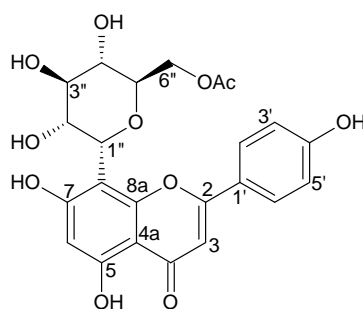
77

The ^{1}H NMR (Plate 10a) and ^{1}H, ^{1}H COSY (Plate 10b) spectra of compound **77** were analyzed and a *p*-substituted aromatic ring, a pentasubstituted aromatic system and flavone skeleton were identified. Hydrogen bonded hydroxy group at

δ_H 13.29 were also visible in the ^{1}H NMR spectrum, indicating that **77** is a 5-hydroxyflavone (Markham, 1989).

^{13}C NMR spectrum (Plate 10c) gave six signals due to the *C*-glucoside substituent at δ_C 77.0, 75.3, 74.0, 69.7, 68.2 and 65.2, suggesting that **77** is a flavone with a *C*-glucoside moiety. Peaks at δ_C 164.4, 162.8, 161.2 and 160.6 established the presence of four oxygen-linked quaternary aromatic carbons. Furthermore, the spectrum showed the presence of a carbonyl at δ_C 170.6 correlating to a methyl carbon at δ_C 20.8 (HMBC, Plate 10e) implying the presence of an acetyl group. Whereas the DEPT-135 spectrum (Plate 10f) indicated the presence of a methylene carbon at δ_C 65.2 belonging to C-6" of the glucoside moiety, the DEPT-90 spectrum pointed to the presence of 11 CH's in agreement with the proposed structure.

The *p*-substituted aromatic ring is linked to C-2 of the benzopyran moiety of a flavones as evidenced by the correlations between H-3 and C-1' of the *p*-substituted aromatic ring at δ_C 121.1, furthermore, H-2'/6' at δ_H 8.30 correlated to C-2 at δ_C 164.4 (Fig. 3.20).

The H-6" of the glucoside moiety correlated with the acetyl group, and therefore showing the linkage of the acetyl group to C-6". The linkage of the sugar moiety to the aglycone in **77** was assigned to be at C-8, this being supported by the fact that the C-8 signal appeared at high field (at δ_C 104.4) and also due to the correlations between the anomeric proton of the sugar at δ_H 4.68 and C-7 (δ_C 162.8) and C-8a (δ_C 156.3) while the singlet proton signal (H-6) correlates with C-5 and C-7. The summarized ^{1}H/^{13}C correlations for **77** are illustrated in Fig. 3.20.
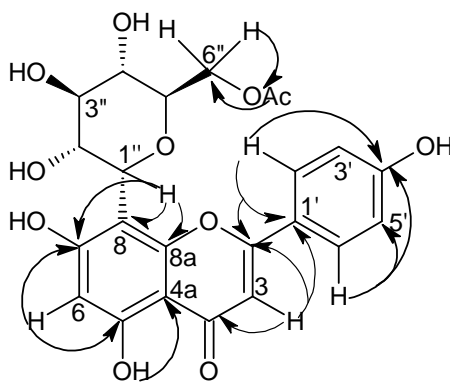
77

Fig. 3.20: HMBC correlations in structure 77

The *pseudo*-molecular ion peak [M-H]⁻ at *m/z* 473 from the negative ion mass spectrum of **77** corresponds to the molecular formula of $C_{23}H_{22}O_{11}$. The NMR data for **77** agreed with the data reported for psilosin, a compound previously isolated from the leaves of *Crataegus pinnatifida* Bge. Var. major N.E.Br (Zhang and Xu, 2003). The isolation of **77** from the Ochnaceae has not been reported and there are also no filed reports on the biological/pharmacological activities of this compound.

3.2.11   3'-*O*-methylpsilosin (78)
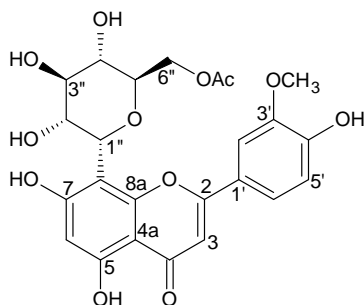
78

The ¹H NMR spectrum (Plate 11a) showed an intramolecular hydrogen-bonded hydroxy proton signal at δ_H 13.35 suggesting that **78** is a 5-hydroxyflavonoid (Markham, 1989). Upon examination of the ¹H NMR and ¹H, ¹H COSY (Plate 11b), ¹³C (Plate 11c), HSQC (Plate 11d) and HMBC spectra (Plate 11e), showed that

compound **78** is a flavone *C*-glucoside with an acetyl group in the sugar moiety, similar to **77**. The only difference between these two flavones being the presence of a methoxy group and a trisubstituted aromatic system in **78** instead of a *p*-substituted aromatic ring in **77**.

The trisubstituted aromatic system was linked to the benzopyran moiety at C-2 (at δ_C 164.6) as shown by the correlations between H-3 at δ_H 6.95 and C-1' at δ_C 121.8. Further confirmation for this attachment was provided by the correlation between H-2' at δ_H 7.54 and C-2 at δ_C 164.6. The position of the methoxy group on the trisubstituted aromatic ring was shown by the correlations between H-2' at δ_H 7.54 and the carbon bearing the methoxy group (C-3') at δ_C 148.3.

A series of six peaks from δ_C 77.3 to δ_C 65.4 in the ^{13}C NMR spectrum was indicative of the presence of a *C*-glucoside system. The *C*-glycone moiety was linked to C-8 due to the correlations between the anomeric sugar proton and C-8a and C-7 of the pentasubstituted aromatic ring system.

Fig. 3.21 gives the summary of the ^{1}H/^{13}C long-range correlations for **78**.
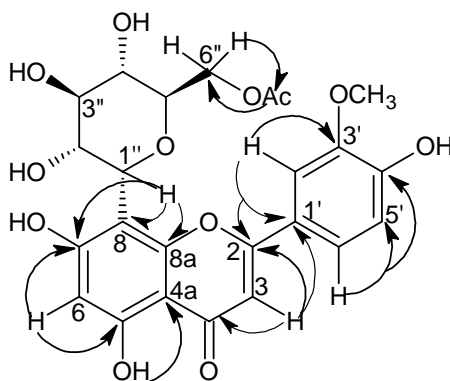
78

Fig. 3.21: HMBC correlations in structure 78

The *pseudo*-molecular ion peak [M-H]⁻ at *m/z* 503 consistent with the $C_{24}H_{24}O_{12}$ molecular formula for **78** specified the presence of additional methoxy group as compared to **77**. The compound was isolated from the leaves of *Sarothamnus scoparius* (Brum-Bousquet *et al.*, 1977). The 3'-hydroxy derivative of **78** has been

isolated from the flowers of *Trollius ledebouri* and from the fern *Odontosoria gymnogrammoides* (Hori et al., 1987; Zou et al., 2005). Neither the isolation of **78** from the Ochnaceae species nor the biological/pharmacological activities of the compound have been reported previously.

3.2.12 (2*Z*)-[(4*R*,5*R*,6*S*)-6-(β-D-Glucopyranosyloxy)-4,5-dihydroxycyclohex-2-en-1-ylidene]ethanenitrile (79)
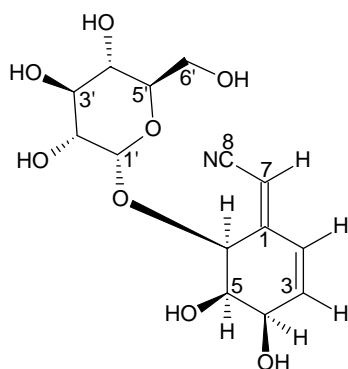
79

Structure of **79** was established from analyses of the 1D [^{1}H (Plate 12a), ^{13}C (Plate 12c) and DEPT-135 (Plate 12f)] and 2D [^{1}H, ^{1}H COSY (Plate 12b), NOESY (Plate 12g), HMBC (Plate 12e) and HSQC (Plate 12d)] spectra.

The ^{1}H NMR together with ^{1}H, ^{1}H COSY spectra showed the presence of two doublets due to two olefinic protons at δ_H 6.18 and δ_H 5.92 (*J*=10.0 Hz), a singlet due to trisubstituted alkene at δ_H 5.71s (H-7). The presence of an *O*-glucosyl moiety was established by the overlapping multiplets between δ_H 3.65 to δ_H 2.65; further evidence to this was provided by the anomeric proton of the sugar which appeared as a doublet at δ_H 4.38 (d, *J*=7.8 Hz) and correlated to a carbon signal at δ_C 103.3 in the HSQC. The sharp absorption at 2225 cm^{-1} in the IR spectrum is characteristic of nitriles. The long-range correlations between H-7 at δ_H 5.71 and C-8 and C-1 (at δ_C 117.8 and 154.3) implied the connection of the three carbons as shown in the proposed structure.
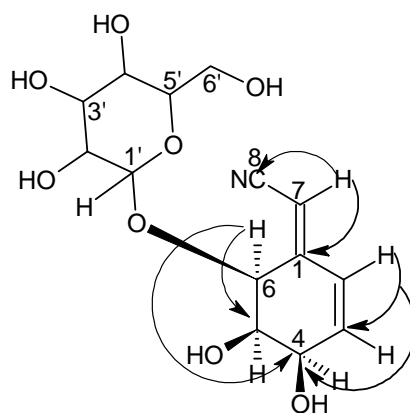
79

Fig. 3.22: HMBC correlations in structure 79

The relative configuration of the molecule was established based on observations in the NOESY spectrum. Strong NOE correlations between H-4, H-5 and H-6 suggested that these protons "are on the same plane" and therefore have *cis* configuration. The structure above was further confirmed by the HMBC (Fig. 3.22) and NOESY correlations (Fig. 3.23).
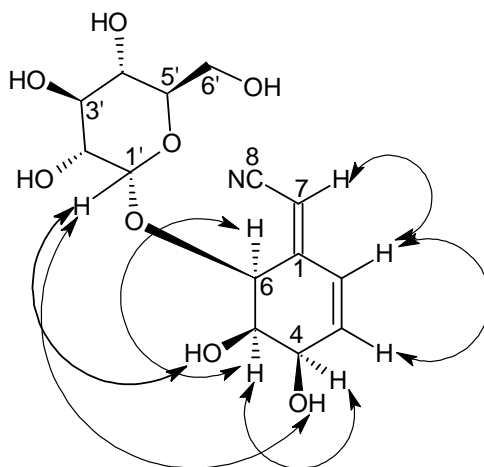
79

Fig. 3.23: NOE Interactions in structure 79

The proposed structure **79** was further confirmed by MS analysis which showed the presence of a *pseudo*-molecular ion peak [M+Na]$^+$ at *m/z* 352 consistent with the molecular formula of $C_{14}H_{19}O_8N$. The compound was first isolated from the

roots of *Ilex* species and later from *Semiaqilegia* species (Ranunculaceae). Its total synthesis was reported in 2007 (Lefebvre and Drian, 2007; Niu et al., 2006). Nitrile containing natural products from plants are known to be derived from amino acids. These acids undergo *N*-hydroxylation and decarboxylation to afford aldoximes, followed by enzymatic conversion of aldoximes to the corresponding nitriles (Fraiser, 1999). In plants, cyanogenic glucosides containing a cyanohydrin group, are known to occur widely. However, cynoglucosides bearing cyanomethylene group such as **79** which are non-cyanogenic, are rare (Murakami *et al.*, 1993). The presence of the cyano group in **76** may indicate the defensive nature of the plants (Fraiser, 1999), but on the contrary, the compound did not show any significant cytotoxicity activity when tested. This compound has not been isolated previously from *Ochna* species.

3.3   Conclusion

The phytochemical studies on the stem bark and leaves of *O. serrulata* were performed for the first time and one novel compound (5-deoxyurundeuvine C) was isolated. The isolation of a cyanoglucoside, psilosin and 3'-*O*-methylpsilosin is hereby reported for the first time from an *Ochna* species. The rest of the compounds were previously isolated from other *Ochna* species. *O. serrulata,* in common with other *Ochna* species, is rich in biflavonoids.

3.4   Experimental

3.4.1   General

The solvents used in the reported investigations included hexanes (mixture of hexane isomers), EtOAc, DCM, MeOH and acetone were of analytical grade.

3.4.2   General experimental procedure

For purification of fractions, centrifugal chromatography (chromatotron model 7924, Harrison Research) was used. The circular chromatotron plates were coated

with preparative silica gel (2 or 4 mm thickness, Merck 7749 with gypsum binding agent). Silica gel 60F$_{254}$, (40-63 µm, Merck) was also used for the purification of the compounds by column chromatography. Thin-layer chromatography (TLC) plates (Kieselgel 60 F$_{254}$, 0.25mm) stained with anisaldehyde/ sulfuric acid and heat were used for monitoring separation of the isolates. The stain solution was made as follows: In a 1 L volumetric flask, 465 ml of methanol were cooled in an ice bath, while shaking to ensure homogeneity, 5 ml of acetic acid was added followed by addition of 17 ml of concentrated sulfuric acid and 13 ml of *p*-anisaldehyde and stored in a refrigerator.

Bruker Avance III 500 or Bruker Avance III 400 spectrometers were used for the ^{1}H, ^{1}H, ^{1}H COSY, HSQC, HMBC and ^{13}C NMR spectra recording at 500 MHz/400 MHz for ^{1}H and 125 MHz/100 MHz for ^{13}C. All spectra were recorded in deuterated solvents like chloroform, DMSO, acetone and MeOH, at 30 $^{\circ}$C using either a 5 mm BBOZ probe or a 5 mm TBIZ probe. Chemical shifts (δ) are given in parts per million (ppm) and referenced to a residual solvent peak (*e.g.* DMSO-d$_6$: ^{1}H, 2.50 ppm, ^{13}C, 39.5 ppm; CDCl$_3$: ^{1}H 7.26 ppm, ^{13}C, 77.0 ppm, methanol-d$_4$: ^{1}H 3.31 ppm, ^{13}C, 49.1 ppm, acetone-d$_6$ ^{1}H 2.05 ppm, ^{13}C, 29.9 ppm). Peak multiplicities are designated as *s* for singlet, *d* for doublet, *m* for multiplet. Coupling constants (*J*) are given in Hz. Mass spectral data were collected on the time-of-flight Waters LCT Premier mass spectrometer using electrospray ionization in the positive or negative mode. Infrared spectra were recorded on a Perkin Elmer FT-IR spectrometer with universal ATR sampling accessory and optical rotations were determined using ADP 440+ model polarimeter manufactured by Bellingham and Stanley.

3.4.3 Plant material

The stem, leaves and roots of *Ochna serrulata* were collected from the UKZN Botanical Garden in September 2009. The plant was identified by Allison Young from UKZN Botanical Garden, voucher specimen (NU, M. Ndoile 01) deposited at the University of KwaZulu-Natal herbarium.

3.4.4 Extraction and isolation (Stem bark)

Oven–dried (30 ℃) and ground stem bark of *O. serrulata* (0.92 kg) was extracted with MeOH at room temperature for 48 hours to obtain 9.7 g extract. The extract consisted of mixtures of polar components and was dissolved in EtOAc:H_2O (8:2). This was followed by extraction with 3 x 100 mL EtOAc aiming at extracting moderately polar organic compounds and 7.0 g of the extract was obtained.

The EtOAc extract (7.0 g) was fractionated in a short silica gel column eluted with increasing polarity of EtOAc:hexanes, where 5 fractions were obtained (1-5), the 5th being with MeOH wash. Fraction 3 (500 mg) was fractionated on a silica gel column eluted with EtOAc:hexanes (1:1), where 2 fractions were obtained (3.1 and 3.2). Fraction 3.1 was further purified on a chromatotron with EtOAc:hexanes (3:7) resulting in the isolation of 12 mg of **73**.

The remaining fractions of 3.1 and fraction 3.2 were combined with fraction 4 (1.6 g) and the mixture was subjected to chromatography on a short silica gel column eluted with increasing polarity of EtOAc:hexanes, 6 fractions were obtained (4.1-4.6). Fraction 4.3 (400 mg) was combined with fraction 4.4 (200 mg) and was purified repeatedly on a chromatotron with DCM:EtOAc (2:1) and resulted in the isolation of 60 mg of **75**, 50 mg of **36**, 52 mg of **39**, 30 mg of **50**, 28 mg of a mixture of **61** and **62** and 50 mg of **76**.

3.4.5 Extraction and Isolation (Leaves)

Oven–dried (30 ℃) and ground leaves of *O. serrulata* (1.98 kg) were extracted with MeOH at room temperature for 48 hours to obtain 40 g of the MeOH leaves extract. The extract was then fractionated by a short silica gel column eluted with DCM followed by EtOAc and finally washed with MeOH, yielding 8.2 g of DCM extract, 8.8 g of EtOAc extract and 20 g of MeOH extract.

The EtOAc extract (8.8 g) was then fractionated by using a short silica gel column eluted with increasing polarity of hexanes:EtOAc solvent ratios to obtain five fractions (L1-5), the fifth being with MeOH wash. Silica gel column

chromatography with EtOAc:DCM (7:3) on L4 (1.0 g) followed by a chromatotron with MeOH:DCM (1:9) afforded 12 mg of **74** and 48 mg of **51**. The remaining of fraction L4 were combined with L5 and purified by a silica gel column chromatography with EtOAc:DCM (7:3) followed by a chromatotron with MeOH:DCM (1:9) to afford 6 mg of **74**, 16 mg of **51** and 28 mg of **54** together with two fractions (L5.3 and L5.4). A chromatotron ran on a combined fraction (L5.3 and L5.4) with MeOH:DCM (1:9) afforded 18 mg of **77** and 13 mg of **78**.

The flow diagram for the isolation of the compounds from the stem and leaves are as provided in Fig. 3.24 and 3.25 respectively.

Fig. 3.24: Isolation of compounds from EtOAc extract of the stem back of *O. serrulata*
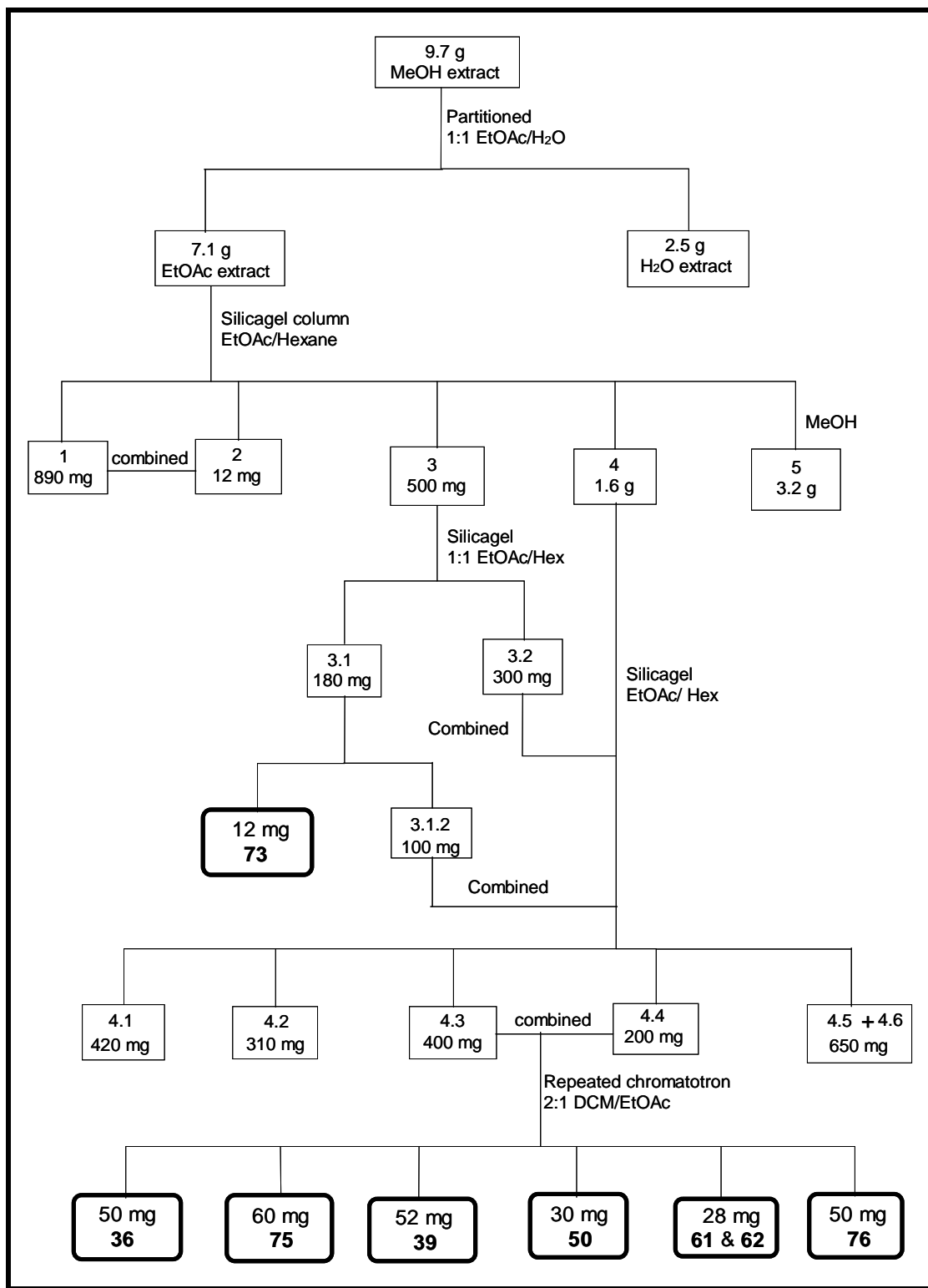
Fig. 3.25: Isolation of compounds from the EtOAc extract of the leaves of *O. serrulata*
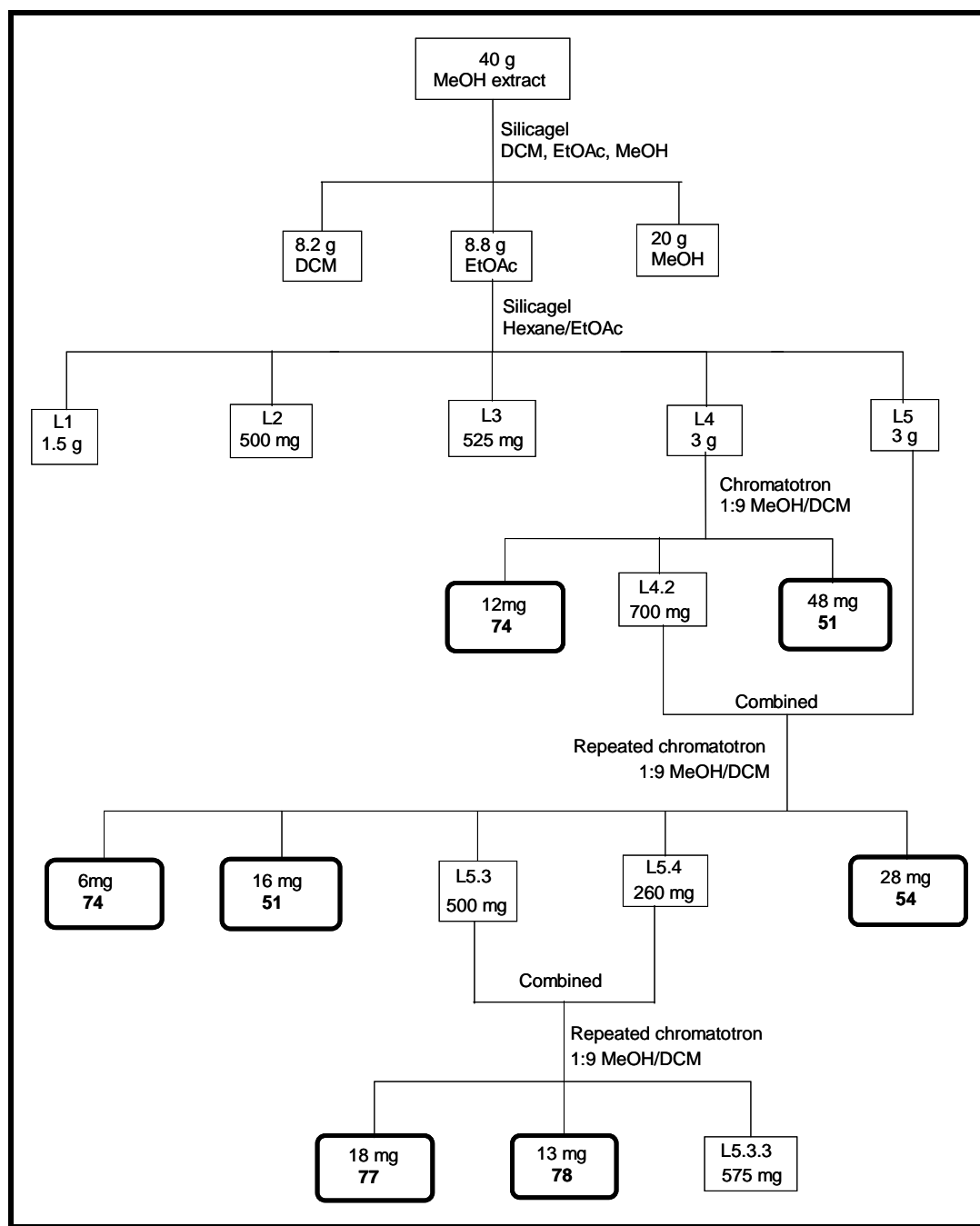
Physical data of the isolated compounds

Methyl (2',4'-dihydroxyphenyl)acetate (73) was isolated as a yellow solid substance. ^{1}H NMR (400 MHz, CD$_3$OD): δ_H 6.87 (1H, d, $J_{5',6}$=8.0 Hz, H-6'), 6.28 (1H, *d*, $J_{3',5}$=2.4 Hz, H-3'), 6.23 (1H, *dd*, $J_{5',6}$=8.0 Hz, $J_{3',5}$=2.4 Hz, H-5'), 3.64 (3H, s, OMe), 3.48 (2H, *s*, H-2) (Plate 1a). ^{13}C NMR (100 MHz, CD$_3$OD): δ_C 173.8 (CO), 156.1 (C-2'), 157.3 (C-4'), 130.9 (C-6'), 112.0 (C-1'), 106.0 (C-5'), 101.9 (C-3'), 50.7 (OMe), 34.5 (C-2) (Plate 1c).

(2',4'-Dihydroxyphenyl)acetic acid (74) was isolated as a yellow non-crystalline solid. ^{1}H NMR (400 MHz, CD$_3$OD): δ_H 6.87 (1H, d*, $J_{5',6}$=8.2 Hz, H-6'), 6.34 (1H, d, $J_{3',5}$=2.4 Hz, H-3'), 6.28 (1H, dd, $J_{5',6}$=8.2 Hz, $J_{3',5}$=2.4 Hz, H-5'), 3.42 (2H,s, H-2) (Plate 2a). ^{13}C NMR (100 MHz, CD$_3$OD): δ_C 177.2 (CO), 157.6 (C-4'), 156.3 (C-2'), 130.9 (C-6'), 113.3 (C-1'), 106.5 (C-5'), 102.6 (C-3'), 36.6 (C-2) (Plate 2c).

Epicatechin (75) isolated as a brown non-crystalline solid. ^{1}H NMR (400 MHz, CD$_3$OD): δ_H 6.96 (1H, *d*, $J_{2',6}$=1.9 Hz, H-2'), 6.78 (1H, *dd*, $J_{5',6}$= 8.1 Hz, $J_{6',2}$=1.9 Hz, H-6'), 6.70 (1H, d, $J_{5',6}$=8.1 Hz, H-5'), 5.93 (1H, *d*, $J_{6,8}$=2.4 Hz, H-6), 5.91 (1H, d, $J_{6,8}$=2.4 Hz, H-8), 4.79 (1H, *d*, $J_{2,3}$=4.4 Hz, H-2), 4.16 (1H, m, H-3), 2.84, (1H, *dd*, $J_{3,4}$=17.0, 4.6 Hz, H-4), 2.72 (1H, *dd*, $J_{3,4}$=17.0, 4.6 Hz, H-4) (Plate 3a). ^{13}C NMR (100 MHz, CD$_3$OD): δ_C 157.9 (C-3'), 157.6 (C-4'), 157.3 (C-7), 145.9 (C-5), 145.7 (C-8a), 132.3 (C-1'), 119.4 (C-6'), 115.9 (C-5'), 115.3 (C-2'),100.1 (C-4a), 96.4 (C-8), 95.9 (C-6), 78.5 (C-2), 66.3 (C-3), 29.5 (C-4) (Plate 3c). HRESIMS (positive ionization mode), *m/z* 313.0688 [M+Na]$^+$ (calculated for C$_{15}$H$_{14}$NaO$_6$ 313.0688).

Lophirone A (36) was isolated as a yellow non-crystalline solid. $[\alpha]^{20}_D$= +70.9 (*c* 0.4, MeOH) (lit. +74.7 (MeOH (Murakami *et al.*, 1991)). ^{1}H NMR (400 MHz, (CD$_3$)$_2$CO): δ_H 8.33 (1H, *d*, $J_{5,6}$=8.9 Hz, H-6B2), 8.28 (1H, *s*, H- β1), 7.94 (1H, *d*, $J_{5,6}$=8.9 Hz, H- 6B1), 7.27 (2H, *d*, $J_{2,3}$=8.4 Hz, H-2,6A1), 7.24 (2H, *d*, $J_{2,3}$=8.4 Hz, H-2,6 A2), 6.91 (1H, *dd*, $J_{5,6}$=8.9 Hz, $J_{3,5}$=2.2 Hz, H-5B1), 6.77 (1H, *d*, $J_{3,5}$=2.2 Hz, H-3B1), 6.66 (2H, *d*, $J_{2,3}$=8.4 Hz, H-3,5A1), 6.61 (2H, *d*, $J_{2,3}$=8.4 Hz, H-3,5 A2), 6.45 (1H, *dd*, $J_{5,6}$=8.9 Hz, $J_{3,6}$ =2.2 Hz, H-5B2), 6.21 (1H, *d*, $J_{3,5}$=2.2 Hz, H-3B2), 6.15 (1H, *d*, $J_{\alpha2,\beta2}$=12.1 Hz, H-α2), 4.79 (1H, *d*, $J_{\beta2,\alpha2}$=12.1 Hz, H-β2) (Plate 4a).

^{13}C NMR (100 MHz, (CD$_3$)$_2$CO): δ_C 204.7 (C-2), 175.4 (C-1), 166.9 (C-2B2), 166.2 (C-4B2), 163.5 (C-4B1), 158.7 (C-2B1), 156.6 (C-4A2,A1), 156.4 (C-β1) 135.8 (C-1A2), 134.7 (C-1A1), 134.5 (C-6B2), 130.1 (C-2,6A1), 129.6 (C-2,6A2), 128.3 (C-6B1), 122.3 (C-α1), 117.4 (C-1B2), 116.0 (C-3,5A1), 115.9 (C-5B1), 115.9 (C-3,5A2), 114.3 (C-1B1), 109.1 (C-5B2), 103.5 (C-3B2), 103.3 (C-3B1), 53.6 (C-β2), 44.1 (C-α2), (Plate 4c). HRESIMS (positive ionization mode), *m/z* 533.1235 [M+Na]$^+$ (calculated for C$_{30}$H$_{22}$NaO$_8$ 533.1212).

Lophirone C (39) was isolated as a yellow solid substance. $[\alpha]^{22}_D$= -29.7 (*c* 0.3, MeOH) (lit. -16.4 (acetone) (Pegnyemb *et al.*, 2001)). ^{1}H NMR (400 MHz, CD$_3$OD): δ_H 7.85 (1H, d, $J_{6,5}$=9.0 Hz, H-6B$_1$), 7.74 (1H, d, $J_{5,6}$=8.9 Hz, H-6B$_2$), 7.72 (1H, d, $J_{\alpha1,\beta1}$=15.4 Hz Hβ$_1$), 7.66 (1H, dd, $J_{6,5}$=8.5 Hz, $J_{2,6}$=1.8 Hz, H6A$_1$), 7.51 (1H, d, $J_{\alpha1,\beta1}$=15.4 Hz, Hα$_1$), 7.41 (1H, d, $J_{2,6}$=1.8 Hz, H-2A$_1$), 7.21 (2H, d, $J_{2,3}$=8.6 Hz, H-2,6A$_2$), 6.94 (1H, d, $J_{6,5}$=8.5 Hz, H-5A$_1$), 6.80 (2H, d, $J_{2,3}$=8.6 Hz, H-3,5A$_2$), 6.44 (1H, dd, $J_{5,3}$=2.3 Hz, $J_{6,5}$=8.9 Hz,H-5B$_2$), 6.39 (1H, dd $J_{5,3}$=2.3 Hz, $J_{6,5}$=9.0 Hz, H-5B$_1$), 6.35 (1H, d, $J_{5,3}$=2.3 Hz, H-3B$_2$), 6.28 (1H, d, $J_{5,3}$=2.3 Hz, H-3B$_1$), 6.09 (1H, d, $J_{\alpha2,\beta2}$=7.2 Hz, Hβ$_2$), 5.31 (1H, d, $J_{\alpha2,\beta2}$=7.2 Hz, Hα$_2$) (Plate 5a). ^{13}C NMR (100 MHz, CD$_3$OD): δ_C 201.7 (C$_2$), 193.4 (C$_1$), 167.7 (C-2B$_1$), 166.6 (C-2B$_2$), 163.7 (C-2B$_1$, 4B$_2$), 159.3 (C-4A1,A$_2$), 145.2 (C-β$_1$), 134.8 (C-6B$_2$), 133.5 (C-6B$_1$), 132.5 (C-6A$_1$), 132.4 (C-3A1), 129.8 (C-1A$_2$), 129.1 (C-1A$_1$), 128.8 (C-2,6A$_2$), 126.9 (C-2A1), 119.3 (C-α$_1$), 116.8 (C-3,5A$_2$), 114.8 (C-1B$_1$),113.9 (C-1B$_2$), 111.3 (C-5A$_1$), 110.0 (C-5B$_2$), 109.5 (C-5B$_1$), 104.1 (C-3B$_2$), 103.9 (C-3B$_1$), 89.2 (C-β$_2$) (Plate 5c). LRMS (negative ionization mode), *m/z* 510.1202 [M-H]$^-$.

Afzelone B (50) was isolated as a yellow solid. $[\alpha]^{24}_D$= -22 (*c* 0.3, MeOH) (lit. -19.0 (acetone) (Pegnyemb *et al.*, 2003)). ^{1}H NMR (400 MHz, CD$_3$OD): δ_H 7.75 (1H, d, $J_{5'',6''}$=8.7 Hz, H-5"), 7.25 (2H, d, $J_{2',3}$=8.5 Hz, H-2',6'), 7.19 (2H, $J_{2''',3'''}$=8.5 Hz, H-2''',6'''), 6.76 (2H, d, $J_{2',3}$=8.5 Hz, H-3',5'), 6.63 (2H, d, $J_{2''',3'''}$=8.5 Hz, H-3''',5'''), 6.5 (1H, dd, $J_{8'',6''}$=2.2 Hz, $J_{5'',6''}$=8.7 Hz, H-6"), 6.33 (1H, d, $J_{6'',8''}$=2.2 Hz, H-8"), 5.84 (1H, d, $J_{2'',3''}$=13.0 Hz, H-2"), 5.8 (s, 1H, H-8), 5.27 (1H, dd, $J_{2,3}$=3.2, 13.0 Hz, H-2), 4.62 (1H, d, $J_{2'',3''}$=13.0 Hz, H-3"), 3.03 (1H, m), 2.61 (1H, m, H-3) (Plate 6a). ^{13}C NMR (100 MHz, CD$_3$OD): δ_C 197.9 (C4), 194.6 (C4"), 166.7 (C-7), 165.7 (C-7"), 163.9 (C-8a"), 159.17 (C-8a), 159.16 (C-5), 158.8 (C-4',4'''), 131.3 (C-1'), 130.9

(C-1'''). 130.5 (C-5''), 130.1 (C-2''',6'''), 129.2 (C-2',6'), 116.5 (C-3',5'), 115.8 (C-3''',5''') 115.1 (C-4a''), 111.9 (C-6''), 106.2 (C-6), 103.9 (C-8''), 103.8 (C-4a), 96.1 (C-8), 82.9 (C-2''), 78.1 (C-2), 42.1 (C-3) (Plate 6c). LRMS (negative mode), m/z = 526.1120 [M-H]⁻.

5-Deoxyurundeuvine C (**76**) was isolated as a yellow non-crystalline solid substance. $[\alpha]^{23}_D$= -5.2 (c 0.2, MeOH). ^{1}H NMR (400 MHz, CD₃OD): δ_H 7.72 (1H, d, $J_{5''',6''}$=9.1 Hz, H-6'''), 7.68 (1H, d, $J_{5',6}$=8.8 Hz, H-6'), 7.24 (1H, s, H-7), 7.18 (1H, d, $J_{5,6}$=8.2 Hz, H-6), 7.05 (2H, d, $J_{2'',3'}$=8.5 Hz, H-2'',6''), 6.67 (3H, dd, $J_{3,5}$=2.3 Hz, $J_{5,6}$=8.2 Hz, H-5), 6.67 (3H, d, $J_{2'',3'}$=8.5 Hz, H- 3'',5''), 6.35 (2H, dd, $J_{3',5}$=2.4 Hz, $J_{5',6}$=8.8 Hz, H-5'), 6.35 (2H, d, $J_{3''',5''}$=2.4 Hz, H-3'''), 6.27 (2H, d, $J_{3,5}$=2.3 Hz, H-3), 6.27 (2H, dd, $J_{3''',5''}$=2.3 Hz, $J_{5''',6''}$=9.1 Hz, H-5'''), 6.16 (1H, d, $J_{3',5}$=2.4 Hz, H-3'), 4.99 (1H, d, $J_{7'',8'}$=7.9 Hz, H- 8''), 4.34 (1H, d, $J_{7'',8'}$=7.9 Hz, H-7'') (Plate 7a). ^{13}C NMR (100 MHz, CD₃OD): δ_C 206.2 (C-9''), 200 (C-9), 166.9 (C-4'), 166.6 (C-4'''), 166.3 (C-2'), 166.2 (C-2'''), 161.4 (C-4'') 157.8 (C-4), 141.7 (C-7), 135.9 (C-6'), 134.5 (C-6'''), 133.1 (C-1'',2), 132.5 (C-6), 130.8 (C-2'',6''), 125.3 (C-1,8), 116.8 (C-3'''), 116.7 (C-3'',5''), 115.4 (C-5), 114.2 (C-1'), 114.0 (C-1''), 109.3 (C-5'''), 108.9 (C-5'), 104.2 (C-3'),103.8 (C-3), 51.8 (C-8''), 49.6 (C-7'') (Plate 7c). HRESIMS (negative ionization mode), m/z 509.1242 [M-H]⁻ (calculated for $C_{30}H_{21}O_8$ 509.1236).

Ochnaflavone (**51**) was isolated as a yellow solid substance. ^{1}H NMR (400 MHz, (CD₃)₂SO): δ_H 8.01 (2H, d, $J_{2''',3''}$=8.9 Hz, H-2''',6'''), 7.87 (1H, dd, $J_{5',6}$=8.9 Hz, $J_{2',6}$=2.3 Hz, H-6'), 7.84 (1H, d, $J_{2',5}$=2.3Hz, H-2'), 7.15 (1H, d, $J_{5',6}$=8.6 Hz, H-5'), 7.02 (2H, d, $J_{5''',6''}$=8.9 Hz, H-3''',5'''), 6.81 (1H, s, H-3,3''), 6.48 (2H, d, $J_{6,8}$=1.9 Hz, H-6,6''), 6.19 (2H, d, $J_{6,8}$=1.9 Hz, H-8,8'') (Plate 8a). ^{13}C NMR (100 MHz, (CD₃)₂SO): δ_C 182.2 (C-4), 182.1 (4''), 164.9 (C-7''), 164.7 (C-7), 163.6 (C-2), 163.2 (C-2''), 161.8 (C-5), 161.8 (C-5''), 161.3 (C-4'''), 157.8 (C-8a), 157.8 (C-8a''), 154.1 (C-4'), 142.2 (C-3'), 128.9 (C-2''',6'''), 125.8 (C-6'), 124.8 (C-1'''), 122.5 (C-1'), 121.6 (C-2'), 118.5 (C-5'), 116.6 (C-3''',5'''), 104.4 (C-4a''), 104.2 (C-3''), 104.1 (C-4a), 103.9 (C-3) 99.4 (C-8,8''), 94.6 (C-6,6'') (Plate 8c). HRESIMS (positive ionization mode), m/z 561.0796 [M+Na]⁺ (calculated for $C_{30}H_{18}NaO_{10}$ 561.0798).

2",3"-Dihydroochnaflavone (**54**) was isolated as a yellow non-crystalline solid substance. $[\alpha]^{22}_D$= +5.9 (*c* 0.4, MeOH) (lit. +6.8 (MeOH) (Likhitwitayawuid *et al.*, 2001)). ^{1}H NMR (400 MHz, (CD$_3$)$_2$CO): δ_H 7.88 (1H, dd, $J_{5',6}$=8.5 Hz, $J_{6',2}$=2.2 Hz, H-6'), 7.80 (1H, d, $J_{6',2}$=2.2 Hz, H-2'), 7.58 (2H, d, $J_{2''',3'''}$=8.3 Hz, H-2''',6'''), 7.25 (1H, d, $J_{5',6}$=8.5, H-5'), 7.07 (2H, d, $J_{5''',6'''}$=8.3 Hz, H-3''',5'''), 6.69 (1H, s, H-3), 6.54 (1H, d, $J_{6,8}$=2.2 Hz, H-8), 6.27 (1H, d, $J_{6,8}$=2.2 Hz, H-6), 6.01 (1H, d, $J_{6'',8''}$=2.1 Hz, H-8"), 5.99 (1H, d, $J_{6'',8''}$=2.1 Hz, H-6"), 5.44 (1H, dd, $J_{2'',3'}$=3.1, 12.6 Hz, H-2"), 3.12 (1H, dd, $J_{2'',3'}$=12.6, 17.8 Hz, H-3"), 2.78 (1H, dd, $J_{2'',3'}$=3.1, 17.8 Hz, H-3") (Plate 9a). ^{13}C NMR (100 MHz, (CD$_3$)$_2$CO): δ_C 195.9 (C-4"), 182.4 (C-4), 167.0 (C-7"), 164.7 (C-7), 164.0 (C-5"), 163.8 (C-2), 163.2 (C-8a"), 161.8 (C-5), 158.0 (C-4'''), 157.9 (C-8a), 153.2 (C-4'), 143.6 (C-3'), 133.4 (C-1'''), 127.7 (C-2''',6'''), 123.9 (C-6'), 122.8 (C-1'), 119.4 (C-2'), 117.5 (C-5'), 116.6 (C-3''',5'''), 103.9 (C-4a), 103.9 (C-3), 103.1 (C-4a"), 98.8 (C-6), 95.8 (C-6"), 94.8 (C-8"), 93.7 (C-8), 78.6 (C-2"), 42.7 (C-3") (Plate 9c). LRMS (negative mode), *m/z* = 539.0824 [M-H]$^-$.

Psilosin (**77**) was isolated as a yellow gum. ^{1}H NMR (500 MHz, (CD$_3$)$_2$SO): δ_H 8.3 (2H, d, $J_{2',3}$=8.7 Hz, H-2',6'), 6.88 (2H, d, $J_{2',3}$=8.7 Hz, H-3',5'), 6.81 (1H, *s*, H-3), 6.28 (1H, *s*, H-6), 4.68 (1H, *d*, $J_{1'',2'}$=9.6 Hz, H-1"), 4.21 (2H, *m*, H-5",6"), 4.08 (1H, *m*, H-6"), 3.90 (1H, m, H-2"), 3.78 (1H, *m*, H-4"), 3.40 (2H, m, H-3"), 1.97 (3H, *s*, Me) (Plate 10a). ^{13}C NMR (125 MHz, (CD$_3$)$_2$SO): δ_C 182.3 (C-4), 170.6 (CO), 164.4 (C-2), 162.8 (C-7), 161.2 (C-5), 160.6 (C-4'), 156.3 (C-8a), 129.8 (C-2',6'), 121.1 (C-1'), 116.2 (C-3',5'), 104.4 (C-8), 104.2 (C-4a), 102.0 (C-3), 98.4 (C-6), 77.0 (C-4"), 75.3 (C-3"), 74.0 (C-1"), 69.7 (C-2"), 68.2 (C-5"), 65.2 (C-6"), 20.8 (Me) (Plate 10c). LRMS (negative mode), *m/z* = 473.0880 [M-H]$^-$.

3'-*O*-methylpsilosin (**78**) was isolated as a yellow solid substance. ^{1}H NMR (500 MHz, (CD$_3$)$_2$SO): δ_H 8.23 (1H, *dd*, $J_{6',5}$=8.2 Hz, $J_{2',6}$=1.8 Hz, H-6'), 7.54 (1H, *d*, $J_{2',6}$=1.8 Hz, H-2'), 6.95 (1H, *s*, H-3), 6.88 (1H, *d*, $J_{5',6}$=8.2 Hz, H-5'), 6.28 (1H, *s*, H-6), 4.6 (1H, d, $J_{1'',2'}$=9.7 Hz, H-1"), 4.2 (2H, *m*, H-5",6"), 4.06 (1H, *m*, H-6"), 3.88 (3H, *s*, OCH$_3$), 3.77 (1H, *m*, H-4"), 3.4 (2H, *m*, H-3",2"), 1.97 (3H, *s*, Me) (Plate 11a). ^{13}C NMR (125 MHz, (CD$_3$)$_2$SO): showed the signals at δ_C 182.7 (C-4), 170.9 (CO), 164.6 (C-2), 163.1 (C-7), 161.0 (C-5), 156.6 (C-8a), 151.3 (C-4'), 148.3 (C-3'), 123.3 (C-6'), 121.8 (C-1'), 116.4 (C-5'), 110.5 (C-2'), 104.7 (C-8), 104.5 (C-4a), 102.9 (C-3), 98.6 (C-6), 77.3 (C-4"), 75.5 (C-3"), 74.3 (C-1"), 69.9 (C-2"), 68.3 (C-

5"), 65.4 (C-6''), 56.6 (OMe), 21.2 (Me) (Plate 11c). LRMS (negative mode), *m/z* = 503.0924 [M-H]⁻.

(2*Z*)-[(4*R*,5*R*,6*S*)-6-(β-D-Glucopyranosyloxy)-4,5-dihydroxycyclohex-2-en-1-ylidene]ethanenitrile (79) was isolated as off-white fluffy solids. $[\alpha]^{20}_D$= -136 (*c* 0.4, DMSO) (lit. -148 (H_2O) (Niu *et al.*, 2006)). ^1H NMR (400 MHz, $(CD_3)_2SO$): δ_H 6.18 (1H, *d*, $J_{2,3}$=10.0 Hz, H-2), 5.92 (1H, *d*, $J_{2,3}$=10.0 Hz, H-3), 5.71 (1H, *s*, H-7), 4.56 (1H, m, H-6), 4.45 (1H, br s, H-4), 4.38 (1H, *d*, $J_{1',2}$=7.8 Hz, H-1'), 4.04 (1H, br s, H-5), 3.65 (1H, m, H-6'), 3.49 (1H, m, H-6'), 3.15 (2H, m, H-4', 3'), 3.09 (1H, m, H-5'), 2.65 (1H, m, H-2') (Plate 12a). ^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ_C 154.3 (C-1), 139.7 (C-3), 125.3 (C-2), 117.8 (C-8), 103.3 (C-1'), 98.9 (C-7), 76.8 (C-4'), 76.7 (C-3'), 76.5 (C-6), 73.5 (C-2'), 70.2 (C-5'), 68.8 (C-5), 65.7 (C-4), 61.4 (C-6'), (Plate 12c). HRESIMS (positive ionization mode), *m/z* 352.1009 [M+Na]⁺ (calculated for $C_{14}H_{19}NO_8Na$ 352.1008).

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CHAPTER 4

Isolation of Secondary Metabolites from the Roots of
Ochna serrulata

4.1   Introduction

The roots of plants are in contact with microorganisms in the soil and, therefore, the plants need to develop a defense mechanism against these organisms. Thus it is no surprise that antimicrobial compounds are often isolated from the roots of plants. Not much has been reported on the phytochemistry of the roots of *Ochna* species. In this Chapter the structures of the compounds present in the roots of *O. serrulata* are discussed.

4.2   Results and Discussion

The methanol extract of the roots of *O. serrulata* yielded three isoflavonoid derivatives, irisolone 4'-methyl ether (**81**), 3',4'-dimethoxy-6,7-methylenedioxyisoflavone (**83**), iriskumaonin 3'-methyl ether (**84**), four biflavonoid derivatives [4,4',7-tri-*O*-methylisocampylospermone A (**85**), a mixture of isocampylospermone A (**61**) and campylospermone A (**62**), 4'''-de-*O*-methylafzelone A (**86**)], two dimeric chalcones [serrulone (**87**) and lophirone L (**88**)], a furofuran lignan [syringaresinol (**89**)] and a diterpene of *ent*-kauranoic acid type [16α,17-dihydroxy-*ent*-kauran-19-oic acid (**90**)]. In the following section, the detailed structural elucidation of these compounds will be discussed.

4.2.1　Irisolone 4'-methyl ether (81)
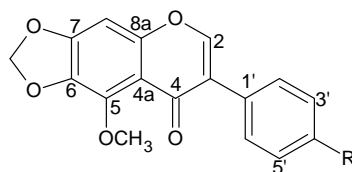
81 R= OCH$_3$

82 R= OH

The structure of **81** was determined by a combination of spectroscopic methods including MS and 1D [[1]H (Plate 13a), ^{13}C (Plate 13c)] and 2D [COSY (Plate 13b), HSQC (Plate 13d) and HMBC (Plate 13e)] NMR spectra.

The methylenedioxy protons exhibited a resonance at the expected position *viz.* δ_H 6.07 (s) correlating to a ^{13}C-signal at δ_C 102.1. A *p*-substituted aromatic system was observed in the ^{1}H NMR and ^{1}H,^{1}H COSY spectra as two doublets at δ_H 7.48 and 6.96, each integrating for two protons. A one-proton singlet at δ_H 6.64 correlating in the HSQC spectrum to a carbon at δ_C 93.2, indicate the presence of a pentasubstituted aromatic ring. An indication that the compound might be an isoflavone was provided by a one-proton singlet at δ_H 7.79 in the ^{1}H NMR spectrum which correlated (HSQC) to carbon at δ_C 150.2 in the ^{13}C NMR spectrum.

Inspection of the ^{1}H NMR and HSQC spectra revealed the presence of two singlets due to two methoxy groups at δ_H 4.09 and 3.85 which correlated (HSQC) to carbons at δ_C 61.2 and 55.3. These chemical shifts suggest that the former methoxy group is sterically hindered and must be placed on a carbon atom where both *ortho* carbons are substituted, whereas the latter must be placed on a carbon atom where at least one of the *ortho* carbons is unsubstituted (Fleischer *et al.*, 1998). Therefore, the methoxy group at δ_C 55.3 was placed at C-4 of the *p*-substituted aromatic ring while the other was placed on the pentasubstituted aromatic ring (ring B).

There are different positions where the methoxy group can be in the A-ring, the most likely being position 5 (**81a**) and position 8 (**81b**), both partial structures will give similar splitting pattern in the ^{1}H NMR spectra.

81a **81b**
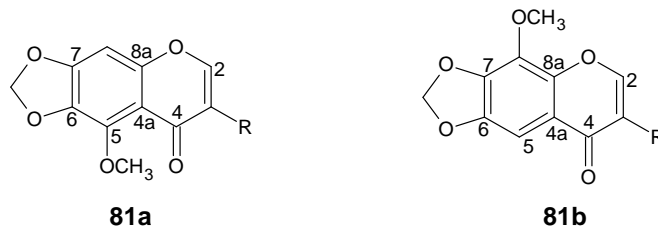
The up-field resonance of H-8 at δ_H 6.64 indicated that it is not *peri* to the carbonyl and therefore confirming partial structure **81a** (Abraham *et al.*, 2003). Further evidence for this substitution pattern is the down-field shift of the methoxy group resonating at δ_C 61.2 showing its close proximity to a carbonyl group (Kalla *et al.*, 1978).

The singlet due to a proton on the pentasubstituted aromatic ring (ring A) was found to show long-range correlations with signals at δ_C 135.6 and 152.8, these carbons were in turn correlated to the methylenedioxy protons at δ_H 6.07. Thus, the methylenedioxy group was linked to the pentasubstituted aromatic ring at the mentioned positions as pointed out in partial structure **81c** (Fig. 4.1). The correlation of H-8 with C-8a, C-4a confirmed that the pentasubstituted ring forms part of the benzopyran system and is at the same time linked to the methylenedioxy group. ^{1}H/^{13}C correlations as observed in the HMBC spectrum are summarized in Fig. 4.1 and 4.2 for partial structures **81c** and **81d**, respectively.

81a
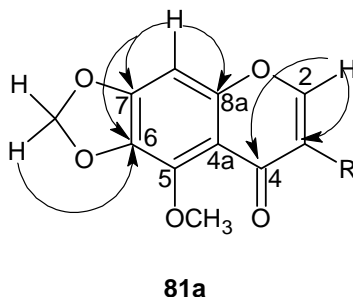
Fig. 4.1: HMBC correlations in partial structure 81a

The remaining partial structure was confirmed from the long-range ^{1}H/^{13}C correlations between the *p*-substituted (B ring) aromatic protons at δ_H 7.48 (H-2'/6') and C-3 at δ_C 125.4, which uncovered the connection of the B-ring to the benzopyran ring *via* C-3. Further proof for this linkage was provided by the ^{1}H/^{13}C correlations between H-2 of benzopyran ring and C-1' of the *p*-substituted ring. These ^{1}H/^{13}C correlations are summarized in partial structure **81d** in Fig. 4.2 below.
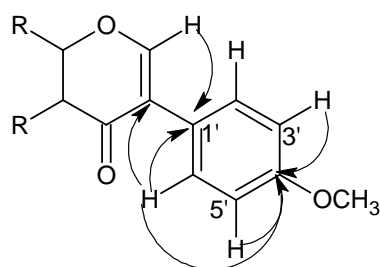
81c

Fig. 4.2: HMBC correlations in partial structure 81c

The mass spectrum of **81** gave a *pseudo*-molecular ion peak [M+Na]$^+$ at m/z 349.0671 in agreement with a molecular formula of C$_{18}$H$_{14}$O$_6$. Compound **81** was assigned as 4'-methoxyirisolone, an isoflavone previously isolated from various plants of the genus *Iris* (El Emary et al., 1980; Hiroyuki et al., 1996; Syeda et al., 2009). The compound was also previously reported from the leaves of *O. integerrima* (Reutrakul *et al.*, 2007).

4.2.2   3',4'-Dimethoxy-6,7-methylenedioxyisoflavone (83)

83
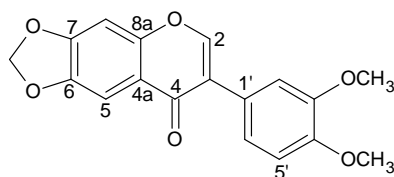
The structure of **83** was determined by a combination of spectroscopic methods including 1D [^{1}H (Plate 14a), ^{13}C (Plate 14c), DEPT-135 (Plate 14f)] and 2D [COSY (Plate 14b), HSQC (Plate 14d), HMBC (Plate 14e)] NMR and MS.

The ^{1}H and ^{13}C NMR spectra exhibited signals due to two methoxy groups, a methylenedioxy group, a trisubstituted aromatic ring, two aromatic singlet protons and an isoflavone nucleus. The position of the two methoxy groups resonating at δ_H 3.94 and 3.93 was based on the correlations observed between the protons of the trisubstituted aromatic ring and the carbons bearing the methoxy groups. Further proof for the positions of the methoxy groups was provided by the absence of chemical shifts of sterically hindered methoxy groups which are resonating downfield (above δ_C 60), suggesting that both methoxy groups are linked to the trisubstituted aromatic ring.

The trisubstituted aromatic ring was linked to the benzopyran system *via* C-3, this was confirmed by the ^{1}H/^{13}C correlations between H-2', 6' and C-3. A correlation was also observed between H-2 and C-1', which signified the stated connectivity. These correlations are summarized in Fig. 4.3 below for partial structure **83a**.
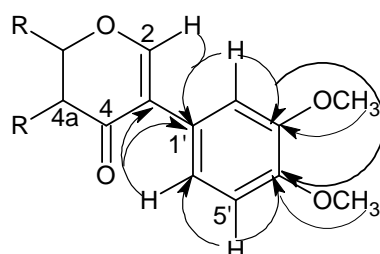
83a

Fig. 4.3: HMBC correlations in partial structure 83a

It was confirmed readily that the second aromatic ring (ring A) is linked to both the pyran ring and the methylenedioxy group by the observed correlation of the aromatic proton singlets (at δ_H 7.63 and 6.89) with C-6, C-7, C-8a and C-4a. The methylenedioxy protons at δ_H 6.12 were correlated to C-7 and C-6. The downfield peak for H-5 at δ_H 7.63 suggested its close proximity to the carbonyl. The above-mentioned facts allow the assignment of the partial structure **83b** in Fig. 4.4.
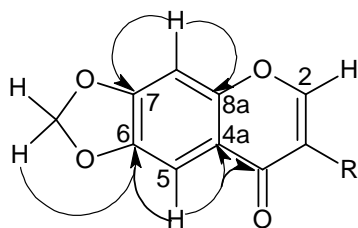
83b

Fig. 4.4: HMBC correlations in partial structure 83b

The mass spectrum of **83** gave a *pseudo*-molecular ion peak [M+Na]$^+$ at m/z 349.0671 which is in agreement with a molecular formula of $C_{18}H_{14}O_6$ for **83**. The structure of **83** was characterized as 3',4'-dimethoxy-6,7-methylenedioxyisoflavone.

The 5-hydroxy and 5-methoxy derivatives of **83** have been isolated from the leaves of *O. squarrosa*, these are squarrosin and 3'-*O*-methyliriskumaonin, respectively (Anuradha *et al.*, 2006). Compound **83** was first isolated from species of Leguminosae family and later from *Ateleia* species (Veitch *et al.*, 2003). The first total synthesis of the compound was reported in 1977 (Bharadwaj and Jain, 1977). The compound has been established to have moderate antioxidant activity (Voss *et al.*, 1992).

4.2.3   Iriskumaonin 3'-methyl ether (84)
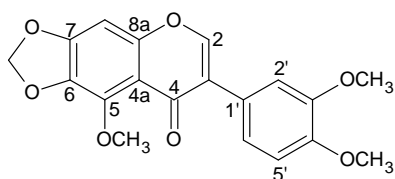
84

The ^{1}H NMR (Plate 15a) and the ^{1}H,^{1}H COSY (Plate 15b) spectra of **84** revealed the presence of three methoxy groups (at δ_H 3.91, 3.93 and 4.10), a methylenedioxy group at δ_H 6.08, a 1,3,4-trisubstituted aromatic system at δ_H 6.91 (d), 7.21 (d) and 7.02 (dd), a pentasubstituted aromatic ring with the only proton resonating at δ_H 6.65 and H-2 of an isoflavone at δ_H 7.81.

In the ^{13}C NMR (Plate 15c) the presence of three non-equivalent methoxy groups at δ_C 56.0, 55.9 and 61.3 was observed. The latter methoxy group has a downfield resonance, which is due to steric hindrance due to substituents present on both carbons at the *o*-positions. Therefore, this methoxy group could be placed at C-5 or C-8 of the pentasubstituted ring. The down-field resonance of the signal for this methoxy group at δ_H 4.10 indicates that it is in the *peri* position to a carbonyl, as indicated in partial structure **84a** (Fig. 4.5).

The partial structure **84a** was readily assembled due to its similarity to partial structure **81a** (Fig. 4.1) as observed in the ^{1}H and ^{13}C NMR spectra. The summary of the $^{1}H/^{13}C$ interactions for partial structure **84a** as observed in the HMBC spectrum (Plate 15e) of **84** is given in Fig. 4.5.

84a
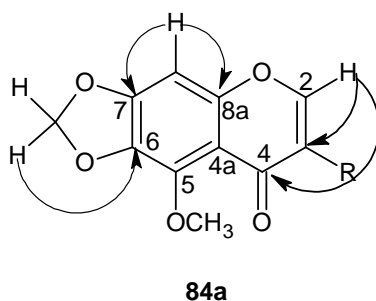
Fig. 4.5: HMBC correlations in partial structure 84a

The partial structure **84b** bear similarity to partial structure **83a** (Fig. 4.3). The $^{1}H/^{13}C$ correlations for **84b** in the HMBC spectrum of **84** are summarized in Fig 4.6. The mass spectrum of the compound gave a *pseudo*-molecular ion peak [M+Na]$^+$ at m/z 379.0786 in agreement with the molecular formula $C_{19}H_{16}O_7$. Thus, compound **84** was identified as 3'-*O*-methyliriskumaonin. The compound was first isolated from the rhizomes of *Iris* species (Hiroyuki et al., 1996; Kachroo et al., 1990; Kalla et al., 1978; Veitch et al., 2003).
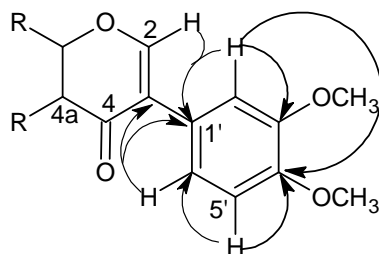
84b

Fig. 4.6: HMBC correlations in partial structure 84b

Compound **84** was later isolated from the leaves of *Ateleia herbertsmithii*, where the compound was reported to be the most abundant component in the leaves of the plant (Rahman et al., 2004; Veitch et al., 2003). Iriskumaonin 3'-methyl ether (**84**) was isolated from the leaves of *O. obtusata* and *O. integerrima* (Rao et al., 1997; Reutrakul et al., 2007; Reynaud et al., 2005). The compound has been reported to exhibit anti-cholinesterase activity at 200 µg/mL and moderate antioxidant activity (Hacıbekiroglu and Kolak, 2011).

4.2.4 Isocampylospermone A (61) and Campylospermone A (62)
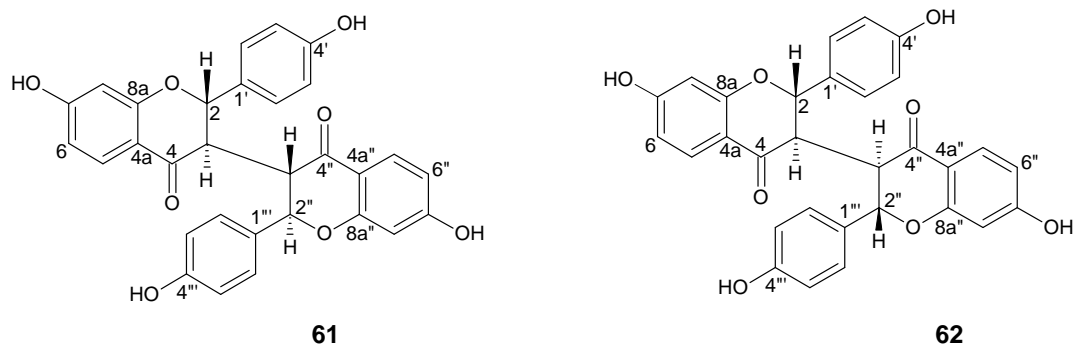
Isocampylospermone A (**61**) was isolated from the stem bark of *O. serrulata* as a 2:1 mixture with campylospermone A (**62**). However, the compound was also isolated from the MeOH extract of the root of *O. serrulata*. In this isolate, the ratio of **62** to **61** was 1:4, making it easier to assign the structure of **62** from the NMR spectra of the mixture. Purification of these compounds was not possible on silica gel since TLC (Kieselgel 60 F$_{254}$, 0.25mm) showed one spot, despite the evidence of two compounds in the ^{1}H NMR spectrum.

The two compounds were reported for the first time from the outer bark of *O. integerrima* by Ichino et al. (2006), naming them as biflavanone 1 and biflavanone 2. Biflavanone 2 was later published as campylospermone A isolated from the stem bark of *Campylospermum mannii* (Elo Manga et al., 2009; Ichino et al., 2006). The relative configuration of C3-C3" as assigned by Ichino *et al.* (2006) was based on optical rotation. Campylospermone A (**62**) had a significant specific rotation (+83.2 in MeOH) and is chiral, where as **61** has a very small specific rotation (+6) and was assigned as a *meso* isomer (the small specific rotation observed may have been the result of some epimerization of the compound). In analogy to chamaejasmin where the *meso* isomer was named isochamaejasmin, we herewith propose the name isocampylospermone A be assigned to compound **61**.

The structures of both compounds (**61** and **62**) were established from MS, 1D (^{1}H, ^{13}C) and 2D (COSY, HSQC and HMBC) NMR data. In the ^{1}H NMR (Plate 16a) and ^{1}H,^{1}H COSY spectra (Plate 16b) of the mixture of **61** and **62**, the presence of two *p*-substituted aromatic systems and two *o,p*-disubstituted aromatic systems were observed. The flavanone skeleton of **61** and **62** was characterized by the presence of two doublets at δ_H 5.85 ($J_{2,3}$=12.0 Hz, H-2, 2") and 2.66 ($J_{2,3}$=12.0 Hz, H-3, 3") each integrating for two protons. Both compounds were observed to be highly symmetrical dimers, showing only one set of signals in their NMR spectra. The ^{1}H NMR spectrum of **61** did not show the peaks for two C-ring protons at 30 $^\circ$C, this might be due to the restricted rotation (fixed conformation) at the mentioned temperature, as was also reported by Ichino *et al.* The presence of a downfield doublet signal in the ^{1}H NMR spectra of both compounds illustrated both compounds to be 5, 5"-deoxybiflavonoids.

The ^{13}C NMR spectrum (Plate 16c) indicated the presence of three peaks due to oxygen-linked aromatic carbons at δ_C 166.8, 165.2 and δ_C 159.6 for C-7,7", C-8a, 8a" and C-4',4''' respectively; confirming both compounds to be phenolic. The two carbonyls were observed at δ_C 193.8. The HMBC spectra (Plate 16e) of both **61** and **62** showed ^{1}H/^{13}C correlations between H-2',6' and the C-2'; between H-2,2" and C-1',1''' indicating the attachment of the two *p*-substituted aromatic systems to

the benzopyran system through C-2 forming ring B of the flavonoid moiety as shown in Fig. 4.7.

The two *o,p*-disubstituted aromatic rings were linked to the pyran ring and therefore form a benzopyran system of the flavonoid moiety; this was due to the $^1H/^{13}C$ correlations between H-5, 5" and the carbonyls. The interflavonoid bond for **62** was found to involve the two C-rings; this was provided by the long-range correlations observed between H-3 at δ_H 2.66 and C-3" at δ_C 52.6.
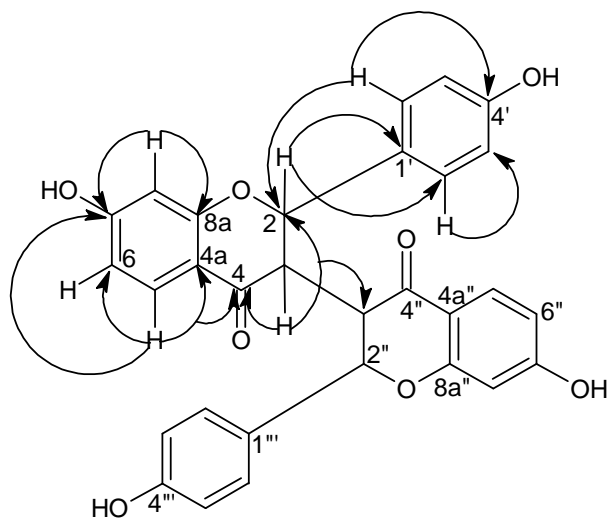
61 & **62**

Fig. 4.7: HMBC correlations in structure 61 and 62

From the large value of coupling constant (12.0 Hz) between H-2, 2" and H-3, 3"; the relative configuration of **62** was deduced to be *trans-trans*.

The negative ion mass spectra of both compounds exhibited a *pseudo*-molecular ion peak [M-H]⁻ at *m/z* 509, confirming $C_{30}H_{22}O_8$ to be the molecular formula of both **61** and **62**. The NMR data of both **61** and **62** are in agreement with those reported by Ichino *et al.* for the two compounds (Ichino *et al.*, 2006). Isocampylospermone A (**61**) is a *meso* compound, campylospermone A (**62**) is chiral but the absolute configuration is not known. As discussed in Chapter 2 of this thesis, isocampylospermone A (**61**) with IC$_{50}$ of 0.08 μg/mL was significantly

more active than campylospermone A (**62**) with IC_{50} of 5.2 µg/mL against malaria parasites (Ichino *et al.*, 2006).

4.2.5   4,4',7-tri-*O*-methylisocampylospermone A (85)
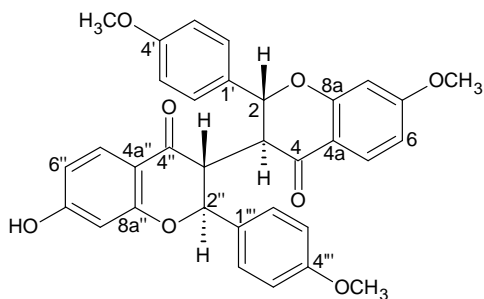
85

The ^{1}H NMR (Plate 17a) and ^{1}H,^{1}H COSY (Plate 17b) spectra of **85** revealed the molecule to be a 3,3"-biflavanone. Two *p*-substituted aromatic rings signified by peaks centred at δ_H 7.21 and 6.98 (4H each), two trisubstituted aromatic rings as illustrated by signals at δ_H 6.59, 6.28, 7.77 and 7.80, 6.65, 6.38, respectively, and three methoxy groups at δ_H 3.84 and 3.82, the latter signal represents two methoxy groups, were also observed in the ^{1}H NMR and COSY spectra. The signals of H-2 and H-3 of the two flavanone moieties were observed as unresolved broad signals which almost disappear into the base line. The appearance of these signals was similar to the heterocyclic proton signals of isocampylospermone A (**61**). This is probably due to restricted rotation at 30 °C (Ichino *et al.*, 2006). The presence of downfield proton doublets at δ_H 7.80 and 7.77 indicated that both 5 and 5" positions are unsubstituted.

The ^{13}C spectrum (Plate 17c) exposed the presence of two carbonyls at δ_C 190.7 and 190.5, six oxygen-linked quaternary aromatic carbons at δ_C 167.2, 165.4, 164.3, 164.3 and 161.6 (for two carbon atoms) consistent with the proposed structure of **85**.

The two *p*-substituted aromatic rings were linked to C-2 and C-2" of the two benzopyran systems as expected for flavanone dimers; this was due to the

correlations observed in the HMBC spectrum (Plate 17e) between H-2'/2''' and C-2, 2''.

Of the three methoxy groups observed in the ^{1}H NMR spectrum, two have the same chemical shift value indicating similar chemical environments, indicating that both might be linked to the two trisubstituted aromatic rings or, alternatively, to C-4'/C-4''' of the B-rings. ^{1}H/^{13}C correlations (HMBC) between these methoxy protons and C-4'/C-4''' at δ_C 161.6 signified their connectivity to these carbon atoms and therefore confined the remaining methoxy group to one of the A-rings. The position of this methoxy group was derived from the long-range correlations observed in the HMBC spectrum between H-6 (at δ_H 6.65), H-8 (at δ_H 6.38) and the carbon bearing this methoxy group (C-7 at δ_C 167.2).
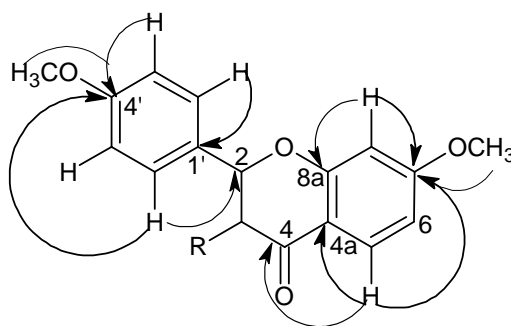
85a

Fig. 4.8: HMBC correlations in partial structure 85a

Long-range correlations observed between H-5/H-5'' of the A-rings and the two carbonyls, C-4a implied that the trisubstituted rings are linked to the pyran rings and therefore form the A-rings of the flavonoid units. The above discussed correlations confirm that partial structures **85a** and **85b** in Fig. 4.8 and 4.9 are correct.
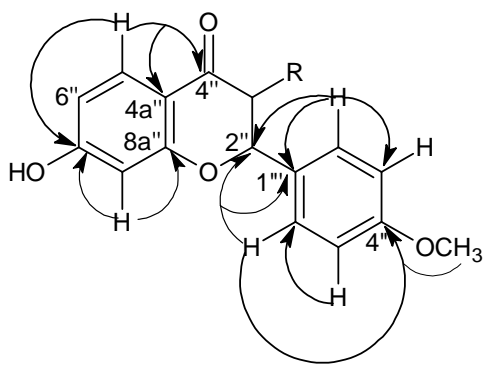
85b

Fig. 4.9: HMBC correlations in partial structure 85b

The molecular composition of **85** was found to be $C_{33}H_{28}O_8$ by mass spectrometry which gave a molecular ion peak [M-H]⁻ at m/z = 551. The structure of **85** was assigned as 4,4',7-tri-*O*-methylisocampylospermone A (or its antipode), which is a new compound.

4.2.6   4'''-De-*O*-methylafzelone A (86)
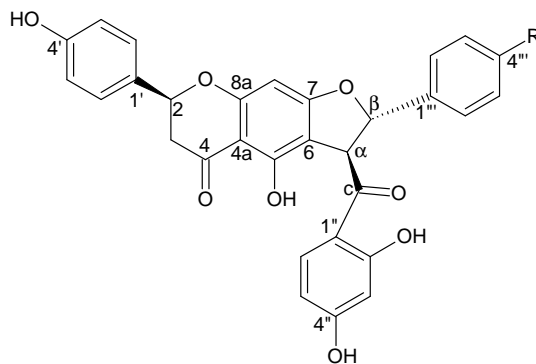
| **49** | R= OCH₃ |
| **86** | R= OH |

The structure of **86** was determined by 1D and 2D NMR spectroscopy together with MS and IR. The IR spectrum of the compound gave a very broad absorption band extending from 2800- 3700 cm⁻¹suggesting that **86** is a phenolic compound and absorption at 1650 cm⁻¹ established the presence of a ketone carbonyl.

The [1]H (Plate 18a) and ^{1}H,^{1}H COSY (Plate 18b) spectra pointed out evidence of an aliphatic AB system at δ_H 5.75 (1H, d, J=5.7 Hz, H-β) and 5.22 (1H, d, J=5.7 Hz, H-α) and the ABX spin system at δ_H 3.15 (1H, m) and 2.72 (1H, m) for H-3 and δ_H 5.39 (1H, m) for H-2 confirming the presence of a flavanone moiety. The two NMR spectra also exposed the presence of two *p*-substituted phenyl rings, one *o,p*-disubstituted aromatic ring and a pentasubstituted benzoyl system.

Thirty peaks were observed in the ^{13}C NMR spectrum (Plate 18c). Two carbonyls at δ_C 202.7 and 198.5 (C$_c$, C-4) and seven oxygen-linked aromatic carbons at δ_C 170.3, 167.5, 167.4, 166.5, 160.2, 159.5 and δ_C 159.2 are consistent with the proposed structure. A DEPT-135 experiment uncovered the presence of one CH_2 at δ_C 44.0, and sixteen CHs, the remaining thirteen carbons were quaternary carbons.

The presence of the furan ring instead of pyran ring was favored by the fact that the carbonyl that experienced strong correlations with the aliphatic AB protons had a pronounced downfield resonance at δ_C 202.7. The pyrone carbonyl resonates at around δ_C 190 to 199 ppm, consistent with the resonance of C-4 of the flavanones (Pegnyemb *et al.*, 2003). The absence of a downfield proton signal below *ca.* δ_H 7.5 indicated that C-5 of the pentasubstituted ring is substituted and therefore confine the signal at δ_C 6.06 to H-8.

The connectivities in this compound were confirmed by the HMBC spectrum (Plate 18e). The pentasubstituted aromatic ring is linked to the furan ring as well as pyran ring, based on correlations observed between H-8 and C-8a, C-4a, C-7. Further evidence for the position of the furan ring was provided by the long-range correlations of H-α and C-β, C-1''', C-5, C-6, C-7 and C-c, eliminating the possibility that the structure is the angular isomer as in partial structure **86a**. Structure **86a** was also unlikely due to the fact that there were no correlations observed between a singlet proton signal and C-5.

86a
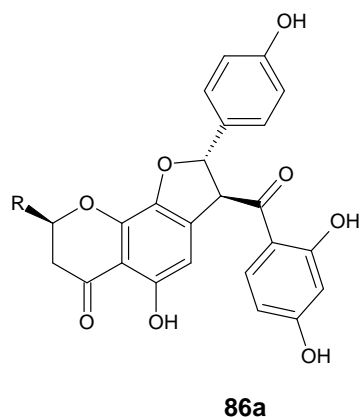
The *p*-substituted aromatic system was found to be linked to C-2 of the benzopyran system due to correlations observed between H-2 and C-1' at δ_C 131.0, also H-2', 6' and C-2 at δ_C 81.0 as shown in partial structure **86b** (Fig. 4.10).

86b
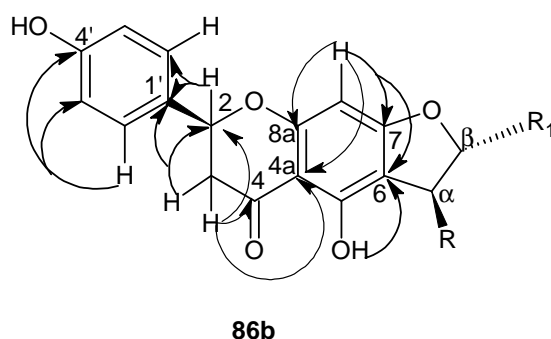
Fig. 4.10: HMBC correlations in partial structure 86b

The second *p*-substituted aromatic ring was shown to be linked to C-β of the furan ring by the long-range correlation observed between H-β and C-1''' at δ_C 131.9, also H-2''',6''' at δ_H 7.17 and C- β at δ_C 92.3. The carbonyl at δ_C 202.7 correlated to H-α which was in turn correlated to the *o,p*-disubstituted aromatic system and therefore confirm the position of *o,p*-disubstituted aromatic system to be linked to the mentioned carbonyl. The summary of these correlations in partial structure **86c** are as provided in Fig. 4.11.
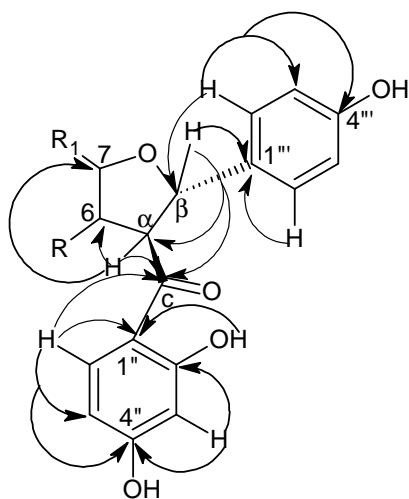
86c

Fig. 4.11: HMBC correlations in partial structure 86c

Compound **86** had a molecular formula of $C_{30}H_{22}O_9$ in agreement with a *pseudo*-molecular ion peak [M-H]$^-$ at *m/z* 525. Based on this data, the structure of **86** was assigned as 4'''-de-*O*-methylafzelone A, a derivative of afzelone A (Pegnyemb *et al.*, 2003). Compound **86** is hereby reported for the first time.

A 5-deoxy analogue of compound **86** has been isolated from the stem of *Caesalpinia ferrea* (Pau-Ferro) found in Middle and South America. It is reported to be the active ingredient in the stem of the plant having inhibitory effect on type II DNA topoisomerase and as a result, the compound inhibits cell growth and induces apoptosis (Hiroshi *et al.*, 2009).

4.2.7 Serrulone A (87)
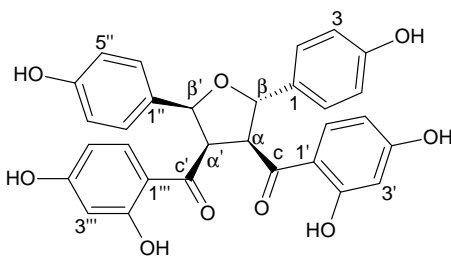
87

Analysis of the [1]H (Plate 19a) and ^{1}H,^{1}H COSY spectra (Plate 19b) of **87** indicated the presence of two *p*-substituted aromatic rings due to doublet signals at δ_H 7.39, 6.83, 7.20 and 6.59 (2H each), two trisubstituted rings (protons with *o*-, *o*/*m*-, and *m*-coupling) and an array of four methine protons constituting a tetrahydrofuran ring having a substituent on each carbon. Signals for two strongly hydrogen-bonded hydroxy protons were observed at δ_H 12.5 and 12.47, indicating their *peri*-position to the carbonyl and therefore belong to the hydroxy groups at C-2' and C-2'''.

The ^{13}C NMR spectrum (Plate 19c) of **87** uncovered the presence of two carbonyls at δ_C 202.5 (c) and 201.1 (c'), six aromatic carbons that are oxygen-linked at δ_C 167.1 (C-2'), 166.4 (C-4'), 166.2 (C-2'''), 165.7 (C-4''') 157.8 (4''), and δ_C 157.7 (C-4) as per proposed structure. The ^{1}H/^{13}C correlations (HMBC spectrum, Plate 19e) between H-2/H-6 at δ_H 7.39 and C-β at δ_C 85.7 lead to the unambiguous linkage of one of the two *p*-oxygenated aromatic rings to this carbon *viz.* C-β. In the same spectrum, the connection of one of the *o,p*-dioxygenated aromatic rings to the carbonyl which in turn correlated to H-α at δ_H 5.47 was observed leading to the conclusion that the *o,p*- dioxygenated system must be linked to carbonyl which in turn linked to C-α. The above discussed correlations led to the confirmation of partial structure **87a** in Fig. 4.12.
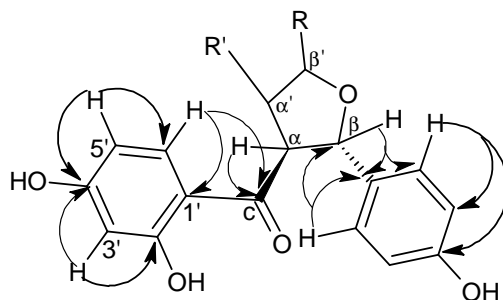
87a

Fig. 4.12: HMBC correlation in partial structure 87a

The second *p*-oxygenated phenyl ring was unambiguously established to be linked to C-β' due to the HMBC correlations between H-2'', 6'' at δ_H 7.20 and C-β' at δ_C 85.7 (Fig 4.13). The second disubstituted benzoyl ring was bonded to the carbonyl

at δ_C 202.5 (c') which in-turn had a long-range correlation with H-α' at δ_H 4.51, thus validating the connectivity as shown in partial structure **87b** (Fig. 4.13).
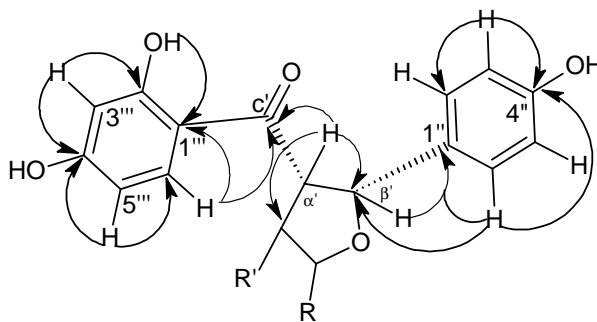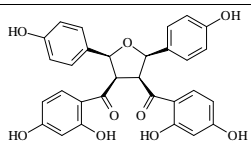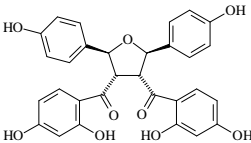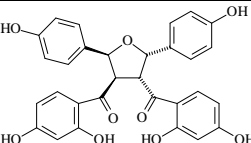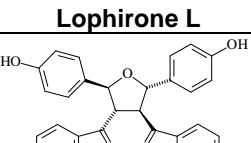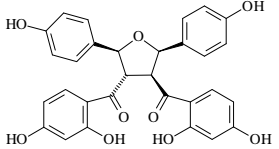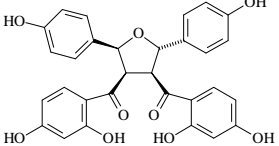
87b

Fig. 4.13: HMBC correlations in partial structure 87b

Compound **87** exhibited a *pseudo*-molecular ion [M-H]⁻ peak at *m/z* 527 in the mass spectrum consistent with a molecular formula of $C_{30}H_{24}O_9$. Based on the spectroscopic data discussed, six possible structures (Table 4.1) were possible for this compound.

Table 4.1:   Possible structures for 87 from the observed NMR data

Structure	Configuration	[α]$_D$	¹³C NMR signals[*]
	cis-cis-cis-isomer plane of symmetry *meso*-compound	0	13
	trans-cis-trans-isomer plane of symmetry *meso*-compound	0	13
Lophirone L	*cis-trans-cis*-isomer c_2-symmetry chiral	≠0	13
Lophirone G	*trans-trans-trans*-isomer c_2-symmetry chiral	≠0	13

Lophirone F	*cis-trans-trans*-isomer no symmetry chiral	≠0	26
	cis-cis-trans-isomer no symmetry chiral	≠0	26

In Table 4.13, the first four structures are symmetrical (expected to show 13 carbon signals) and therefore could not be the structure of **87** which is not symmetrical (it showed 26 carbon signals). This reduces the possibilities to the last two structures are the only options for **87**. It was observed that the spectroscopic data of **87** are not in agreement with those of lophirone F (**44**), isolated from the stem bark of *Lophira lanceolata* (Tih *et al.*, 1990). Therefore, the only possibility remaining for **87** is the last structure with a *cis-cis-trans* configuration around the furan ring. The structure of **87** was therefore assigned as *cis-cis-trans*-3,4-bis(2,4-dihydroxybenzoyl)-2,5-bis(4-hydroxyphenyl)furan and is given the common name serrulone A. The absolute configuration of **87** has not yet been established and the isolation and characterization of **87** have not been previously reported.

4.2.8   Lophirone L (88)
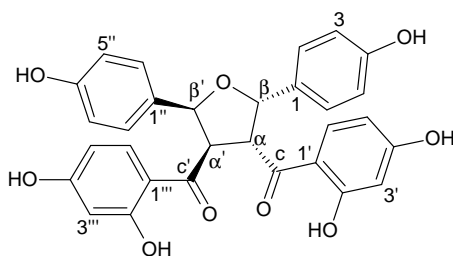
88

Comparison of the ^{1}H, ^{13}C NMR and MS of **87** and **88**, indicated that the two compounds are isomers. The only difference was that **88** is symmetrical while **87** is not. The [1]H (Plate 20a) and ^{1}H,^{1}H- COSY (Plate 20b) spectra of **88** showed the presence of two symmetrical *p*-oxygenated phenyl rings (AA'XX' system), two

symmetrical trisubstituted aromatic rings and a set of protons belonging to a symmetrical furan ring substituted on each carbon.

The ^{13}C NMR spectrum of **88** has 15 carbon signals and revealed the presence of a carbonyl at δ_C 202.7 and three oxygen-linked aromatic carbons at δ_C 166.7, 166.5 and 158.8 suggesting that both *p*-substituted and trisubstituted aromatic rings are phenolic (oxygen-linked). The linkage of *p*-substituted aromatic system to C-β was confirmed by the ^{1}H/^{13}C correlations observed (HMBC spectrum, Plate 20e) between the mentioned carbon *viz.* C-β and H-2, 6 at δ_H 7.36 (Fig. 4.14). The protons of the trisubstituted aromatic ring (δ_H 6.07, 6.03, 7.36) had a strong correlation to the carbonyl at δ_C 202.7, which was in turn correlated to H-α at δ_H 4.56, thus implied that the mentioned aromatic system was linked to the carbonyl which in turn was linked to H-α as summarized in Fig. 4.14 below.
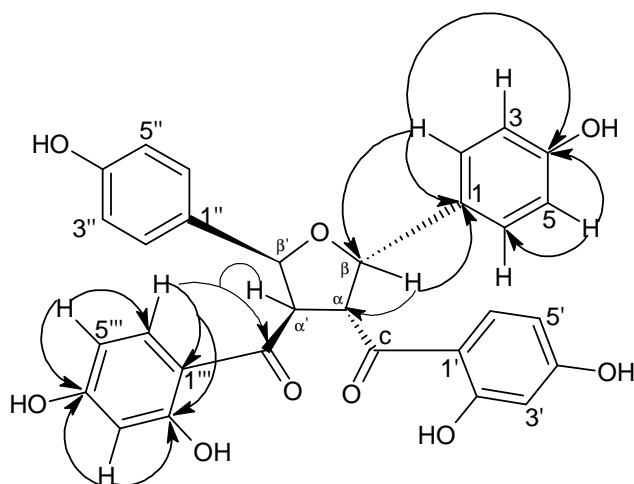
88

Fig. 4.14: HMBC correlations in structure 88

Compound **88** exhibited a *pseudo*-molecular ion [M-H]⁻ peak at *m/z* 527 consistent with $C_{30}H_{24}O_9$ molecular formula. Compound **88** has a specific rotation of -11.9 and is chiral. Therefore, it must be either lophirone G ([α]$_D$ +113 in acetone) (Tih *et al.*, 1990) or lophirone L ([α]$_D$ -6.4 in EtOH) (Anuradha *et al.*, 2006) (Table 4.1). NMR data of **88** were identical to those reported for lophirone L (Anuradha *et al.*, 2006). Based on comparison of the NMR data, the structure of **88** was assigned

as lophirone L, which was previously isolated from the roots of *O. squarrosa* (Anuradha *et al.*, 2006).

4.2.5   Syringaresinol (89)
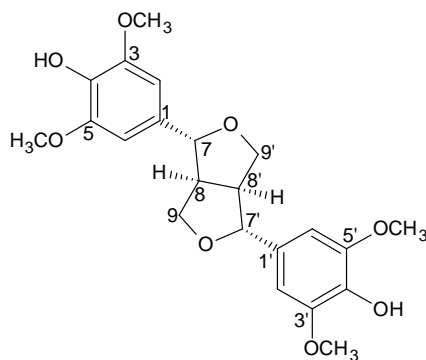
89

1D (^{1}H, ^{13}C, DEPT-135) and 2D (COSY, HSQC, HMBC) NMR spectra were used to determine the structure of syringaresinol (**89**). Observations in the mass spectrum in combination with ^{1}H (Plate 21a), ^{1}H,^{1}H COSY (Plate 21b) and ^{13}C (Plate 21c) NMR spectra of **89**, indicated that **89** was a symmetrical dimeric molecule. The ^{1}H NMR spectrum (Plate 21a) showed only one singlet at δ_H 6.69 (4H) suggesting that the four aromatic protons are equivalent as pointed out in the proposed structure.

The presence of a bis-tetrahydrofuran ring was signified by the ^{1}H-^{1}H interactions between the benzylic proton (H-7/H-7') at δ_H 4.68 and H-8/H-8' at δ_H 3.10 which were in turn interacting with CH$_2$-9/9' at δ_H 4.24 as observed in COSY spectra. The HMBC (Plate 21e) spectrum showed the presence of this ring by the correlations between H-8 and C-7, H-7 and C-9, H-9 and C-8, C-7. The linkage of the bis-tetrahydrofuran ring to the aromatic ring was confirmed by the long-range ^{1}H/^{13}C correlations between H-7/H-7' and C-1/C-1', C-2/C-2' and C-6/C-6'. The methoxy group protons were found to correlate to C-3 and 5 (δ_C 147.8) which in turn were also correlated to H-2 and H-6. The discussed correlations are summarized in Fig. 4.15.
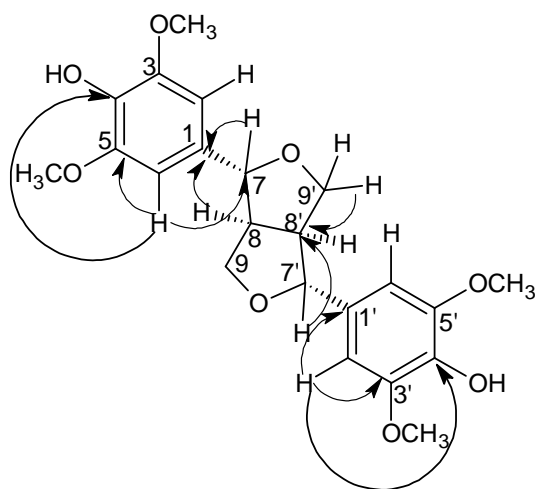
89

Fig. 4.15: **HMBC correlations in structure 89**

The molecular formula of **89** was found to be $C_{22}H_{26}O_8$ by mass spectrometry which gave a *pseudo*-molecular ion peak [M+Na]$^+$ at *m/z* = 441 in agreement with the proposed structure. The discussed correlations/interactions agree with the proposed structure thereby confirming **89** to be syringaresinol (El-Hassan *et al.*, 2003).

Syringaresinol (**89**) is reported to be a major constituent of *Lilac* species (the common name for the genus *Ryringa*) (Deyama, 1983). Later the compound was reported to be isolated from Thymelaceae and *Gmelina* species. The glucosylated syringaresinol was reported to be isolated and fully characterized for the first time from *Cressa cretica* and *Eucommia ulmoides* (Deyama, 1983; Shahat et al., 2004). The compound is reported to posses strong cytotoxicity, anti-inflammatory and antioxidant activities (El-Desouky and Gamal-Eldeen, 2009). In another study, the compound inhibited proliferation of leukemic cells by induction of apoptosis and the cell arrest mechanism (Park *et al.*, 2008). The compound was also found to inhibit the motility of *Helicobacter pylori* (Miyazawa *et al.*, 2006). The first total synthesis of syringaresinol (**89**) was reported in 2004 (Yan *et al.*, 2004).
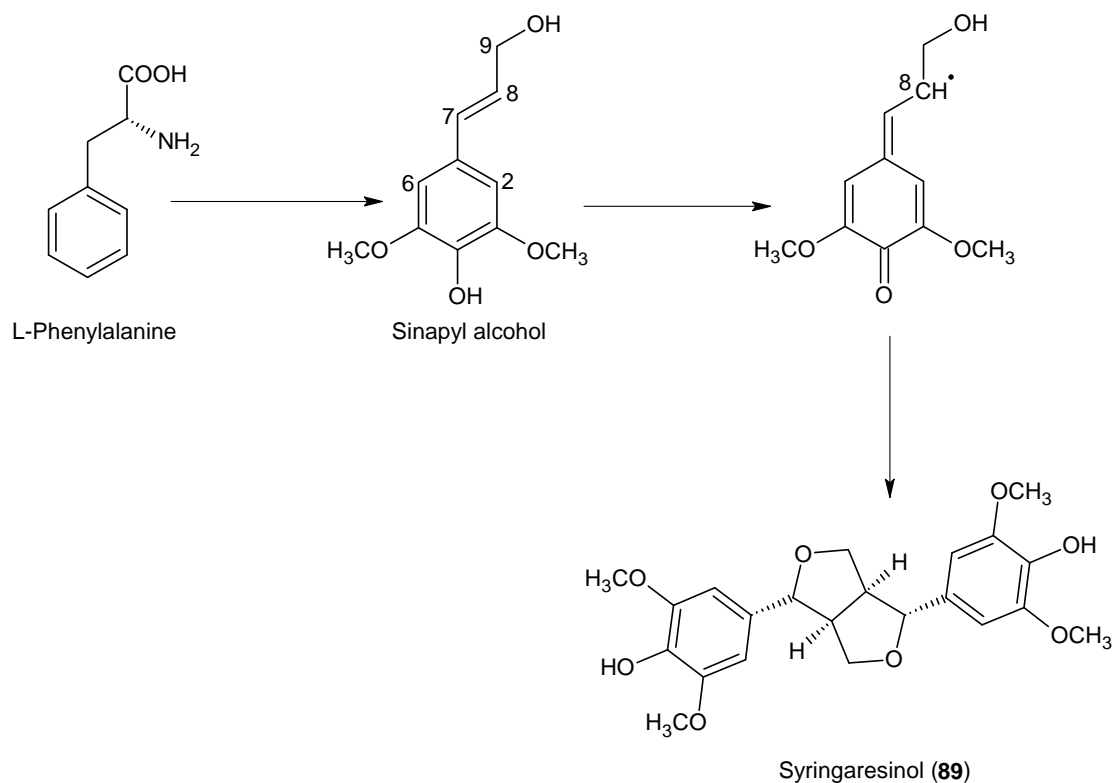
Fig. 4.16: Biosynthesis of syringaresinol (89)

The compound is proposed to be biosynthesized (Fig. 4.16) from sinapyl alcohol, which undergoes dimerization to give syringaresinol (**89**) (Katayama and Ogaki, 2001). This is the first report on the isolation of this type of lignan from *Ochna* species.

4.2.10   16α,17-Dihydroxy-*ent*-kauran-19-oic acid (90)

90
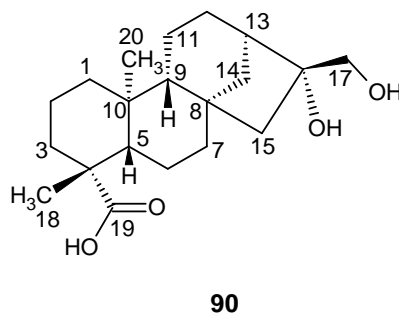
The negative ion mass spectrum exhibited a *pseudo*-molecular ion peak [M-H]⁻ at *m/z* 335, suggesting that **90** is a diterpene with the molecular formula of $C_{20}H_{32}O_4$.

The infrared spectrum of this compound showed an absorption at 1710 cm^{-1} due to the carboxylic acid carbonyl group and the carboxylic acid O-H stretch was observed at 3200 cm^{-1} while the alcohol O-H stretch was observed at 3400 cm^{-1} (Solomons and Fryhle, 2004).

The ^{13}C NMR spectrum (Plate 22c) of **90** showed the presence of 20 carbons and the DEPT-135 spectrum (Plate 22f) pointed out these are 5 quaternary carbons, 3 methine, 10 methylene and 2 methyl carbons.

The ^{1}H NMR spectrum (Plate 22a) of **90** showed the presence of two tertiary methyl groups at δ_H 1.67 and 0.88. These chemical shifts are characteristic of the equatorial C-18 and axial C-20 methyl groups of a kaurane diterpene with a C-19 axial carboxylic acid. The structure was further supported by ^{13}C NMR resonances at δ_C 178.9 (C-19), 15.4 (C-20) and 26.7 (C-18), which are typical of the axial C-20 and equatorial C-18 in diterpene with a C-19 axial carboxylic acid (Wang *et al.*, 2009). Other major features of the ^{1}H and ^{13}C NMR spectra were the presence of a hydroxy group at C-16 (δ_C 78.8) and two doublets centered at δ_H 3.23 and 3.13 (*J*=11.0 Hz) typical for 17-methylene of a 16α,17-dihydroxy moiety (Harinantenaina *et al.*, 2002). 16α,17-Dihydroxy-*ent*-kaurane has been reported to show resonances for these protons at δ_H 3.37 and 3.51 (*J*=12 Hz) and in that of 16α,17-dihydroxy-*ent*-kauran-19-oic acid at δ_H 3.32 and 3.43 (*J*=10.7 Hz) positions, comparable to the observed in **90** spectrum. Therefore, the isolated compound **90** was identified as 16α,17-dihydroxy-*ent*-kauran-19-oic acid (Hertz and Kulanthaivel, 1984; Wu et al., 1996). The ^{1}H/^{13}C correlations as observed in the HMBC spectrum (Plate 22e) pointed out the correlations shown below in Fig. 4.17.

90
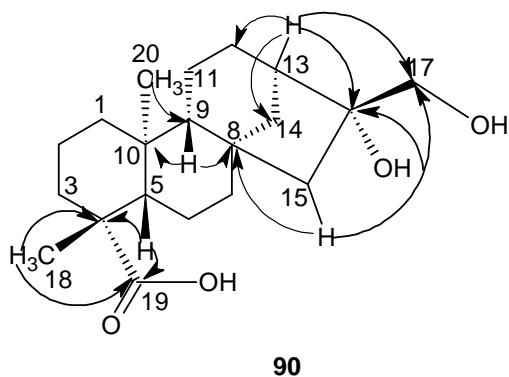
Fig. 4.17: HMBC correlations in structure 90

The compound has been isolated from various *Helianthus* species (Hertz and Kulanthaivel, 1984) and *Annona* species (Wu *et al.*, 1996). It was reported that the compound exhibited significant activity against HIV replication with EC_{50} value of 0.8 mg.mL^{-1} (Wu *et al.*, 1996). The isolation of this compound from *Ochna* species is reported here for the first time.

4.3   Conclusion

Three new compounds [4,4',7-tri-*O*-methylisocampylospermone A (**85**), 4'''-de-*O*-methylafzelone A (**86**) and serrulone A (**87**)] were isolated from the roots of *O. serrulata* along with three simple isoflavonoids [irisolone 4'-methyl ether (**81**), 3',4'-dimethoxy-6,7-methylenedioxyisoflavone (**83**), iriskumaonin 3'-methyl ether (**84**)], a diterpene of *ent*-kauranoic acid type [16α,17-dihydroxy-*ent*-kauran-19-oic acid (**90**)], a furofuran lignan [syringaresinol (**89**)] and biflavonoids [lophirone L (**88**), and a mixture of isocampylospermone A (**61**) and campylospermone A (**62**)]. This is the first report on the isolation of a furofuran lignan and a diterpene of *ent*-kauranoic acid type from *Ochna* species.

4.4   Experimental

4.4.1 Extraction and isolation

Oven–dried (30 °C) and ground roots of *O. serrulata* (1.2 kg) were extracted with MeOH at room temperature for 48 h to yield 15 g of extract. Initial fractionation of the extract was done by running a short silica gel column eluting with EtOAc:hexanes mixtures with increasing polarities and finally washed with MeOH. Five fractions were obtained (R.1-R.5), the fifth being the MeOH wash.

Compound **90** precipitated out when 850 mg of fraction R.2 was dissolved in MeOH. It was then washed further with MeOH to afford 10 mg of a white powder of **90**. The filtrate was subjected to repeated silica gel chromatography with DCM:hexanes (1:1) to afford the isoflavonoids **81** (4.1 mg), **83** (5.0 mg) and **84** (6.2 mg) and a novel biflavonoid **85** (4.2 mg).

Silica gel column chromatography of fraction R.3 (1.2 g) followed by repeated purification on a chromatotron with DCM:EtOAc (1:1) yielded 8.1 mg of **36**, 4.7 mg of **39**, 4.1 mg of **50** and 18.0 mg of **76** and 4.0 mg of **90** as a white powder.

Fraction R.4 (2.5 g) was subjected to silica gel column chromatography (DCM:EtOAc, 1:1) to obtain four fractions (R4.1-R4.4). R4.1 was purified further on a chromatotron with EtOAc:DCM (1:1) to afford 5.0 mg of an off-white solid (**89**). R4.2 was combined with the remaining R4.1 and subjected to purification procedure as above to yield **89** (1.5 mg), **86** (4.7 mg) as a yellow amorphous solid and **87** (5.1 mg). R4.3 was combined with R4.4 and repeatedly purified on a chromatotron with EtOAc:DCM (6:4) to afford **87** (1.1 mg), **88** (2.2 mg), **61** and **62** (5.4 mg) as yellow non-crystalline solids.

The flow diagram for the isolation of the compounds from the roots of *O. serrulata* is as given in Fig. 4.18 below.

Fig. 4.18: Isolation of compounds from methanol extract of the roots of *O. serrulata*
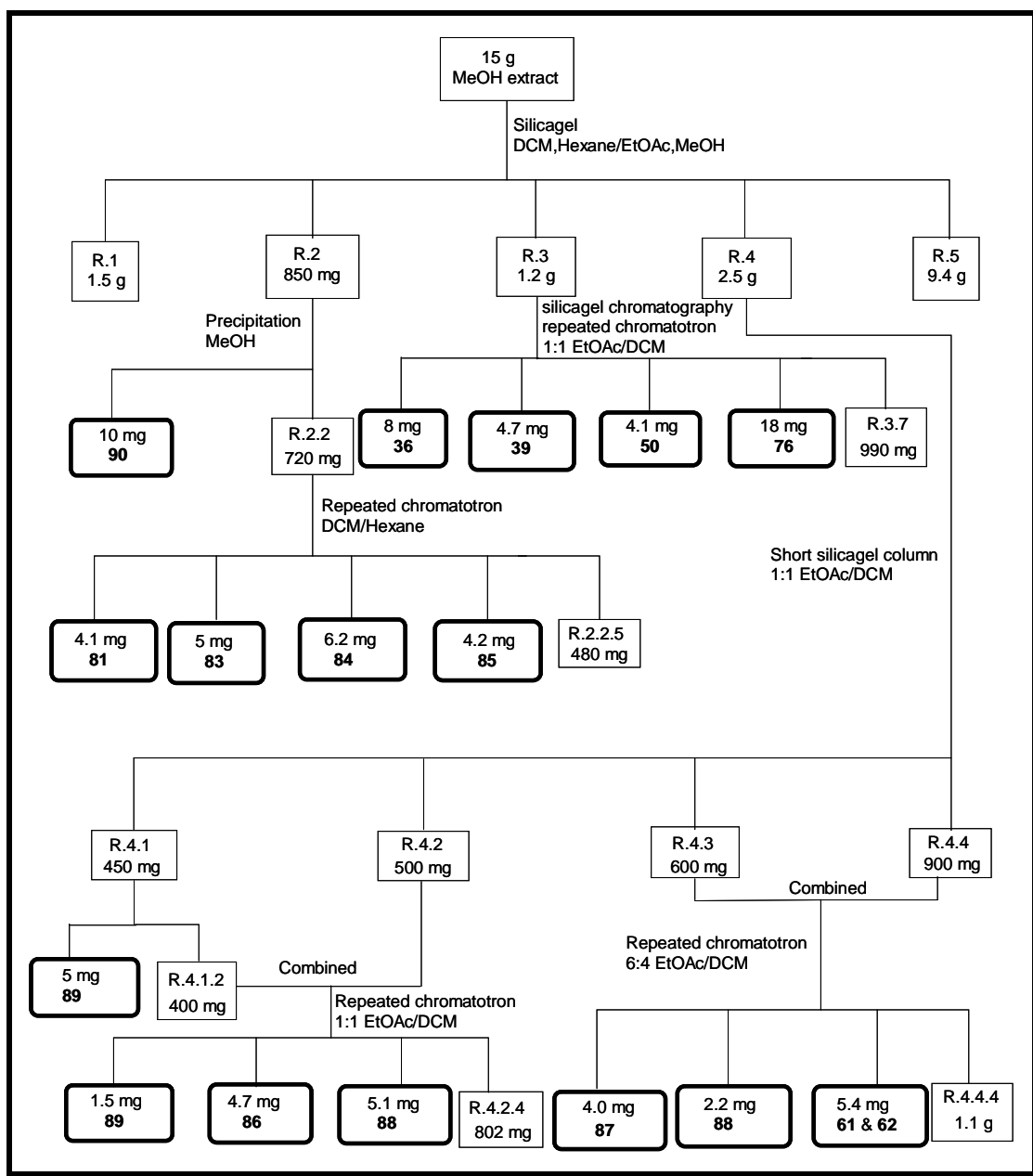
4.4.2 Physical data of the isolated compounds

Irisolone 4'-methyl ether (**81**) was isolated as a white non-crystalline solid substance. ^{1}H NMR (400 MHz, CDCl$_3$): δ_H 7.79 (1H, s, H-2), 7.48 (2H, d, $J_{2',3}$=8.8 Hz, H-2', 6'), 6.96 (2H, d, $J_{5',6}$=8.8 Hz, H-3',5'), 6.64 (1H, s, H-8), 6.07 (2H, s, OCH$_2$O), 4.09 (3H, s, OMe), 3.85 (3H, s, OMe) (Plate 13a). ^{13}C NMR (100 MHz, CDCl$_3$): δ_C 175.5 (C-4), 159.56 (C-4'), 152.8 (C-7), 151.9 (C-8a), 150.2 (C-2),141.7 (C-5), 135.6 (C-6), 130.9 (C-2', 6'), 125.4 (C-3), 124.1 (C-1'), 113.8 (C-4a), 113.9 (C-3', 5'), 102.1 (OCH$_2$O), 93.2 (C-8), 61.2 (OMe), 55.3 (OMe) (Plate 13c). HRESIMS (positive ionization mode), m/z 349.0671 [M+Na]$^+$ (calculated for C$_{15}$H$_{14}$NaO$_6$ 349.0688).

3',4'-Dimethoxy-6,7-methylenedioxyisoflavone (**83**) was isolated as a white amorphous solid substance. ^{1}H NMR (400 MHz, CDCl$_3$): δ_H 7.96 (1H, s, H-2), 7.63 (1H, s, H-5), 7.23 (1H, d, $J_{2',6}$=2.1 Hz, H-2'), 7.06 (1H dd $J_{5',6}$=8.4 Hz, $J_{2',6}$=2.1 Hz, H-6'), 6.94 (1H, d $J_{5',6}$=8.4 Hz, H-5'), 6.89 (1H, s, H-8), 6.12 (2H, s, OCH$_2$O), 3.94 (3H, s, OMe), 3.93 (3H, s, OMe), (Plate 14a). ^{13}C NMR (100 MHz, CDCl$_3$): δ_C 175.4 (C-4), 153.5 (C-8a), 152.7 (C-7), 151.9 (C-2), 149.1 (C-4'), 148.9 (C-3'), 146.3 (C-6), 124.6 (C-1'), 124.4 (C-3), 121.0 (C-6'), 119.6 (C-4a), 112.6 (C-2'), 111.2 (C-5'), 102.8 (C-5), 102.4 (OCH$_2$O), 97.8 (C-8), 56.0 (2xOMe) (Plate 14c). HRESIMS (positive ionization mode), m/z 349.0671 [M+Na]$^+$ (calculated for C$_{15}$H$_{14}$NaO$_6$ 349.0688).

Iriskumaonin 3'-methyl ether (**84**) isolated as a white amorphous solid substance. ^{1}H NMR (400 MHz, CDCl$_3$): δ_H 7.81 (1H, s, H-2), 7.21 (1H, d, $J_{2',6}$=2.0 Hz, H-2'), 7.02 (1H dd $J_{5',6}$=8.3 Hz, $J_{2',6}$=2.0 Hz, H-6'), 6.91 (1H, d $J_{5',6}$=8.3 Hz, H-5'), 6.65 (1H, s, H-8), 6.08 (2H, s, OCH$_2$O), 4.10 (3H, s, OMe), 3.93 (3H, s, OMe), 3.91 (3H, s, OMe) (Plate 15a). ^{13}C NMR (100 MHz, CDCl$_3$): δ_C 174.4 (C-4), 154.7 (C-8a), 152.8 (C-7), 150.4 (C-2), 149.1 (C-4'), 148.7 (C-3'), 141.8 (C-5), 135.5 (C-6), 125.4 (C-3), 124.6 (C-1'), 121.4 (C-6'), 113.8 (C-4a), 112.9 (C-2'), 111.1 (C-5'), 102.2 (OCH$_2$O), 93.2 (C-8), 61.3 (OMe), 56.0 (OMe), 55.9 (OMe) (Plate 15c). HRESIMS (positive ionization mode), m/z 379.0786 [M+Na]$^+$ (calculated for C$_{15}$H$_{14}$NaO$_6$ 379.0794).

Isocampylospermone A (61) was isolated as a yellow amorphous solid substance in a mixture with **campylospermone A (62)**.

Isocampylospermone A (61)

^{1}H NMR (400 MHz, CD$_3$OD): δ_H 7.74 (2H, d, $J_{6'',5''}$=8.7 Hz, H-5,5''), 7.02 (4H, d, $J_{2''',3''}$=8.5 Hz, H-2',6',2''',6'''), 6.77 (4H, d, $J_{2''',3''}$=8.5 Hz, H-3',5',3''',5'''), 6.49 (2H, dd, $J_{8,6}$=2.2 Hz, $J_{6,5}$=8.7 Hz, H-6,6''), 6.20 (2H, d, $J_{8,6}$=2.2 Hz, H-8,8'') (Plate 17a). ^{13}C NMR (100 MHz, CD$_3$OD): δ_C 192.7 (C-4,4''), 166.9 (C-7,7''), 165.2 (C-8a,8a''), 159.8 (C-4',4'''), 131.8 (C-5,5''), 130.9 (C-2',6', 2''',6'''), 130.4 (C-1',1'''), 116.6 (C-3',5',3''',5'''), 114.7 (C-4a,4a''), 112.1 (C-6,6''), 103.9 (C-8,8''), 83.8 (C-2,2''), 55.1 (C-3,3'') (Plate 17c).

Campylospermone A (62)

^{1}H NMR (400 MHz, CD$_3$OD): δ_H 7.67 (2H, d, $J_{6'',5''}$=8.6 Hz, H -5,5''), 6.90 (4H, d, $J_{2',3}$=8.5 Hz, H-2''',6''',2',6'), 6.76 (4H, d, $J_{3',2}$=8.5 Hz, H-3',5',3''',5'''), 6.48 (2H, dd, $J_{8'',6'}$=2.2 Hz, $J_{6'',5'}$=8.7 Hz, H- 6,6''), 6.25 (2H, d, $J_{8,6}$=2.2 Hz, H-8), 5.85 (2H, d, $J_{2,3}$=12.0 Hz, H-2,2''), 2.66 (2H, d, $J_{2,3}$=12.0 Hz, 3,3'') (Plate 17a). ^{13}C NMR (100 MHz, CD$_3$OD): δ_C 193.9 (C- 4,4''), 166.8 (C-7,7''), 165.2 (C-8a,8a''), 159.6 (C-4',4'''), 130.2 (C-5,5''), 129.7 (C-1',1'''), 130.4 (C-2',6', 2''',6'''), 116.6 (C-3',5',3''',5'''), 115.8 (C-4a,4a''), 112.1 (C-6,6''), 103.9 (C-8,8''), 85.7 (C-2,2''), 52.6 (C-3,3'') (Plate 17c). LRMS (negative ionization mode), *m/z* 509 [M-H]⁻.

4,4',7-Tri-*O*-methylisocampylospermone A (85)

4,4',7-Tri-*O*-methylisocampylospermone A (85) was isolated as yellow amorphous solid substance (Ichino *et al.*, 2006). $[\alpha]^{21}_D$= -101 (*c* 0.1, MeOH). ^{1}H NMR (500 MHz, (CD$_3$)$_2$CO): δ_H 7.80 (1H, d, $J_{5,6}$=8.8 Hz, H-5), 7.77 (1H, d, $J_{5'',6'}$=8.6 Hz, H-5''), 7.21 (4H, d, $J_{2',3}$=8.5 Hz, H-2',6',2''',6'''), 6.98 (4H, d, $J_{2',3}$=8.5 Hz, H-3',5'), 6.97 (2H, d, $J_{2''',3''}$=8.7 Hz, H-3''',5'''), 6.65 (1H, dd, $J_{6,8}$=2.3 Hz, $J_{5,6}$=8.8 Hz, H-6), 6.59 (1H, dd, $J_{6'',8'}$=2.1 Hz, $J_{5'',6'}$=8.6 Hz, H-6''), 6.38 (1H, d, $J_{6,8}$=2.3 Hz, H-8), 6.28 (1H, d, $J_{6'',8'}$=2.2 Hz, H-8''), 4.99 (2H, d, H-2,2''), 3.84 (3H, s, OMe), 3.82 (6H, s, OMe), 3.72 (2H, d, H-3,3'') (Plate 16a). ^{13}C NMR (125 MHz, (CD$_3$)$_2$CO): δ_C 190.7 (C-4), 190.5 (C-4''), 167.2 (C-7), 165.4 (C-7''), 164.3 (C-8a), 164.3 (C-8a''), 161.6 (C-4',4'''), 130.7 (C-2', 6', 2''',6'''), 130.4 (C-1'), 130.3 (C-1'''), 130.2 (C-5''), 129.7 (C-5), 115.2 (C-3', 5'), 115.0 (C-3''', 5'''), 114.7 (C-4a), 114.7 (C-4a''), 111.6 (C-6''), 111.1 (C-6), 103.5 (C-8''), 101.6 (C-8), 82.8 (C-2), 82.6 (C-

2"), 56.3 (OMe), 55.8 (OMe), 50.0 (C-3), 49.9 (C-3"), (Plate 16c). LRMS (negative ionization mode), *m/z* 551 [M-H]⁻.

4'''-De-*O*-methylafzelone A (86) was isolated as a yellow powder (Pegnyemb *et al.*, 2003). $[\alpha]^{22}_D$= +89.3 (*c* 0.4, MeOH). ¹H NMR (400 MHz, CD₃OD): δ_H 7.54 (1H, d, $J_{5'', 6''}$=8.8 Hz, H-6"), 7.34 (2H, d, $J_{3',2'}$=8.4 Hz, H-2',6'), 7.17 (2H, d, $J_{3''',2'''}$=8.3 Hz, H-2''',6'''), 6.83 (2H, d, $J_{3''',2'''}$=8.3 Hz, H-3''',5'''), 6.79 (2H, d, $J_{3',2'}$=8.4 Hz, H-3',5'), 6.29 (1H, d, $J_{3'',5''}$=2.2 Hz, H-3"), 6.26 (1H, dd, $J_{5'',6''}$=8.8 Hz, $J_{3'',5''}$=2.2 Hz, H-5"), 6.06 (1H, s H-8), 5.39 (1H, m, H-2), 5.75 (1H, d, $J_{\beta,\alpha}$=5.7 Hz, H-β), 5.22 (1H, d, $J_{\beta,\alpha}$=5.7 Hz, H-α), 3.15 (1H, m, H-3), 2.72 (1H, m, H-3) (Plate 18a). ¹³C NMR (100 MHz, CD₃OD): δ_C 202.7 (C-4"), 198.5 (C-4), 170.3 (C-7), 167.5 (C-4"), 167.4 (C-2"), 166.5 (C-8a), 160.2 (C-5), 159.5 (C-4'''), 159.2 (C-4'), 134.6 (C-6"), 131.9 (C-1'''), 131.0 (C-1'), 129.2 (C-2',6'), 128.9 (C-2''',6'''), 116.8 (C-3',5'), 116.5 (C-3''',5'''), 113.9 (C-1"), 109.6 (C-5"), 106.9 (C-6), 104.5 (C-4a), 103.9 (C-3"), 92.3 (C-β), 91.5 (C-8), 81.0 (C-2), 55.2 (C-α), 44.0 (C-3) (Plate 18c). LRMS (positive ionization mode), *m/z* 549 [M+Na]⁺.

Serrulone A (87) was isolated as a yellow solid. $[\alpha]^{21}_D$= +55.9 (*c* 0.3, MeOH). ¹H NMR (400 MHz, (CD₃)₂CO): δ_H 7.99 (1H, d, $J_{5',6}$=8.9 Hz, H-6'), 7.46 (1H, d, $J_{5'',6'''}$=8.8 Hz, H-6'''), 7.39 (2H, d, $J_{3,2}$=8.5 Hz, H-2,6), 7.20 (2H, d, $J_{3'', 2'}$=8.5 Hz, H-2",6"), 6.83 (2H, d, $J_{3,2}$=8.5 Hz, H-3,5), 6.59 (2H, d, $J_{3'',2'}$=8.5 Hz, H-3",5"), 6.3 (2H, dd, $J_{5',6}$=8.9 Hz, $J_{3',5}$=2.3 Hz, H-5'), 6.3 (2H, d, $J_{\beta',\alpha}$=8.8 Hz, H-β'), 6.18 (1H, d, $J_{3',5}$=2.3, H-3'), 6.08 (1H, dd, $J_{5'',6'''}$=8.8 Hz, $J_{3'',5''}$=2.2 Hz, H-5'''), 6.0 (1H, d, $J_{3'',5''}$=2.2, H-3'''), 5.94 (1H, d, $J_{\beta,\alpha}$=5.7 Hz, H-β), 5.47 (1H, t, $J_{\beta,\alpha}$ = 6.3 Hz, H-α), 4.51 (1H, dd, $J_{\beta',\alpha'}$ = 8.4, 7.5 Hz, H- α') (Plate 19a). ¹³C NMR (100 MHz, (CD₃)₂CO): δ_C 202.5 (C'), 201.1 (C), 167.1 (C-2'), 166.4 (C-4'), 166.2 (C-2'''), 165.7 (C-4'''), 157.8 (C-4"), 157.7 (C-4), 133.9 (C-6'''), 133.4 (C-6'), 129.8 (C-1), 128.8 (C-1"), 128.4 (C-2,6), 128.3 (C-2",6"), 116.3 (C-5),116.1 (C-3), 116.1 (C-3"), 114.9 (C-5"), 113.8 (C-1'''), 112.8 (C-1'), 108.9 (C-5'), 108.4 (C-5'''), 103.7 (C-3'), 103.2 (C-3'''), 85.7 (C-β), 81.6 (C- β'), 61.4 (C- α'), 56.4 (C-α) (Plate 19c). LRMS (negative ionization mode), *m/z* 527 [M-H]⁻.

Lophirone L (**88**) was isolated as a yellow powdery substance. $[\alpha]^{21}_D$= -11.9 (*c* 0.1, MeOH) (lit. -6.4 (EtOH) (Anuradha *et al.*, 2006)). ^{1}H NMR (400 MHz, CD$_3$OD): δ_H 7.36 (6H, d, $J_{2',3}$=8.5 Hz, H-2,6,2",6",6',6'''), 6.79 (4H, d, $J_{2'',3'}$=8.6 Hz, H-3,5,3",5"), 6.07 (2H, d, $J_{5',3}$=2.2 Hz, H-3',3'''), 6.03 (2H, dd, $J_{5',3}$=2.2 Hz, $J_{5',6}$=8.5 Hz, H-5',5'''), 5.39 (2H, dd, $J_{\alpha,\beta}$=2.3, 5.3 Hz, H-β,β'), 4.56 (2H, dd, $J_{\alpha,\beta}$=2.3, 5.3 Hz, H-α,α') (Plate 20a). ^{13}C NMR (100 MHz, CD$_3$OD): δ_C 202.7 (C,C'), 166.7 (C-2',2'''), 166.5 (C-4',4'''), 158.8 (C-4,4"), 133.9 (C-6',6'''), 132.2 (C-1,1"), 129.4 (C-2,6,2",6"), 116.4 (C-3,5,3",5"), 114.9 (C-1',1'''), 109.0 (C-5',5'''), 103.6 (C-3',3'''), 85.4 (C-β,β'), 59.9 (C-α,α') (Plate 20c). LRMS (negative ionization mode), *m/z* 526 [M-H]$^-$.

Syringaresinol (**89**) was isolated as a white solid. $[\alpha]^{21}_D$= -54 (*c* 0.2, MeOH) (lit. -36, CHCl$_3$) (Bryan and Fallon, 1976). ^{1}H NMR (500 MHz, (CD$_3$)$_2$CO): δ_H 6.69 (4H, s, H-2',6',2,6), 4.68 (2H, d, $J_{7,8}$=4.3 Hz, H-7,7'), 4.24 (dd, 2H, $J_{8,9}$=7.0, 15.8 Hz, H-9,9'), 3.85 (2H, d, $J_{8,9}$=3.8 Hz, H-9, 9'), 3.83 (12H, s, 4 x OMe), 3.10 (2H, m, H-8',8) (Plate 21a). ^{13}C NMR (125 MHz, (CD$_3$)$_2$CO): δ_C 147.8 (C-3,5,3',5'), 135.4 (C-4',4), 132.3 (C-1', 1), 103.7 (C-2',6', 2,6), 85.9 (C-7,7'), 71.5 (C-9,9'), 55.8 (4 x OMe), 54.5 (C-8,8') (Plate 21c). LRMS (positive ionization mode), *m/z* 441 [M+Na]$^+$.

16α,17-Dihydroxy-*ent*-kauran-19-oic acid (**90**) was isolated as a white powder. $[\alpha]^{20}_D$= -50.9 (*c* 0.2, DMSO) (lit. -53, MeOH, (Hsieh *et al.*, 2004)). ^{1}H NMR (500 MHz, (CD$_3$)$_2$SO): δ_H 3.23 (1H, d, $J_{17a,b}$=11.0 Hz, H-17), 3.13 (1H, d, $J_{17a,b}$=11.0 Hz, H-17), 2.5 (4H, m, H-1,15), 1.99 (1H, m, H-7), 1.91 (1H, m, H-13), 1.67 (5H, m, H-12, 18), 1.44 (2H, m, H-11), 1.33 (4H, m, H-3,6), 1.26 (1H, m, H-9), 1.08 (2H, m, H-14), 0.98 (3H, m, H-2, 5), 0.88 (3H, s, H-20) (Plate 22a). ^{13}C NMR (125 MHz, (CD$_3$)$_2$SO): δ_C 178.9 (C-19), 78.8 (C-16), 69.2 (C-17), 56.2 (C-2), 55.9 (C-5), 52.3 (C-9), 43.2 (C-4), 42.9 (C-8), 41.7 (C-3), 40.5 (C-13), 40.4 (C-15), 39.5 (C-1), 37.8 (C-7), 30.8 (C-10), 28.7 (C-14), 26.7 (C-18), 21.6 (C-12), 18.9 (C-6), 18.6 (C-11),15.4 (C-20) (Plate 22c). LRMS (negative ionization mode), *m/z* 335 [M-H]$^-$.

4.5   References

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CHAPTER 5

Cytotoxicity and Antimalarial Activities of the Isolated Compounds

5.1   Introduction

The combined effects of the health-related challenges due to the rapidly growing population, the failure of modern medicine to provide effective treatment, chronic diseases and the emergence of the multi-drug resistant pathogens have led to the increased use of herbal medicines in recent years. It is of importance to establish a scientific basis for the use and validation of medicinal plants through biological screening.

In this investigation, the major compounds extracted from *O. serrulata* were identified as biflavonoids, as was reported in Chapter 3 and 4. In this Chapter, a brief literature overview of the structure and biological activities of biflavonoids is presented, followed by a report on the antimalarial and cytotoxic activities of the compounds isolated in this investigation.

5.2   Biflavonoids: An Overview of Structure and Biological Activities

From the previous phytochemical studies on the genus *Ochna* as discussed in Chapter 2 of this thesis, it is clear that the genus is rich in biflavonoids (Khalivulla et al., 2008; Likhitwitayawuid et al., 2001; Pegnyemb et al., 2001, 2003).

Normally flavonoids are composed of a C6-C3-C6 central skeleton with a total of 15 carbons (Fig. 5.1). Different substituents can occur on the basic skeleton and thereby contribute to the diversity of flavonoids (Lia et al., 2007; Lin et al., 2001; López-Lázaro, 2002).

Biflavonoids are typical polyphenolic flavonoid dimers (Fig. 5.1). The individual flavonoid units in these compounds are joined in a symmetrical or unsymmetrical manner and can either be identical or non-identical. In addition, there are two ways how the two flavonoid units are commonly linked; either through a C-C bond or C-O-C bond. The bond connecting the two units may be at various positions, *e.g.* 3-4''', 4'-4''', etc. In this class of compounds, remarkable diversity is expected due to the substitution of different functional groups such as hydroxy, methoxy and carbonyl groups and stereogenic centers that are positioned unpredictably on the main biflavonoid unit. This class of compounds are not broadly distributed and indeed, there are few plant species that have biflavonoids as major secondary metabolites (Kim et al., 2008; Lin et al., 2001).

Flavanoid　　　　　　　　　**Biflavanoid**
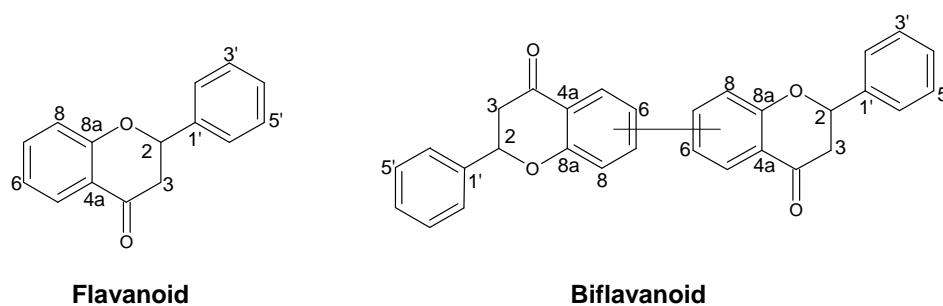
Fig. 5.1: General structures of flavonoids and biflavonoids

The beginning of the isolation and characterization of biflavonoids was marked by Furukawa who extracted a yellow pigment from the leaves of the maidenhair tree, *Ginko biloba* L in 1929. The pigment was later recognized as a biflavonoid, ginkgetin (**91**) (Baker and Simmonds, 1940; Lin et al., 1997). The variety of biological activities, structural diversity and low toxicity shown by biflavonoids has resulted in an interest in this class of compounds (Ariyasena et al., 2004; Machado and Lopes, 2008; Parveena et al., 2004; Zhao et al., 2006).

The biological activities observed for biflavonoids include antimicrobial (Jung et al., 2006; Mbwambo et al., 2006; Weniger et al., 2006), anti-inflammatory (Banerjee et al., 2002; Chen et al., 2006; Kim et al., 1998; Lim et al., 2006; Park et al., 2006; Son et al., 2006; Suh et al., 2006b; Woo et al., 2006), antiviral (Lee et al., 2006; Ma et al., 2001), anticancer (Son et al., 2006; Yoon et al., 2006), anti-clotting (Lale *et al.*, 1996), vasorelaxant (Dell'Agli et al., 2006; Kang et al., 2004) and other activities (Lee et al., 2006; Suh et al., 2006a; Suh et al., 2006b; Xu et al., 2001; Yamaguchi et al., 2005).

91 $R^1=R^2=R^3=R^4=OH$, $R^5=R^6=OCH_3$

92 $R^1=R^2=R^3=R^4=R^5=R^6=OH$

93 $R^1=R^2=R^4=R^5=OH$, $R^3=R^6=OCH_3$
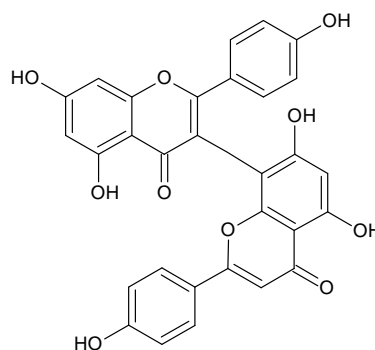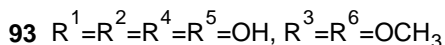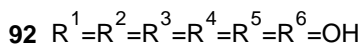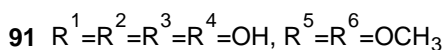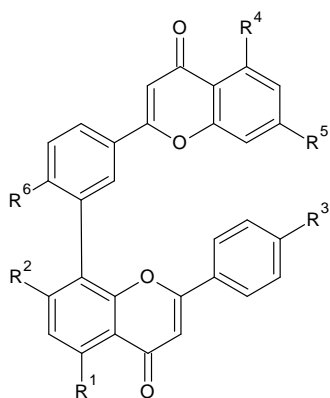
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Although a large number of biflavonoids have been isolated from plants, the assessment of their biological/pharmacological activities has not yet been given enough attention. A small number of biflavonoids have been investigated for different activities; these include ginkgetin (**91**), amentoflavone (**92**), isoginkgetin (**93**), morelloflavone (**94**), robustaflavone (**95**) and ochnaflavone (**51**).

Ochnaflavone (**51**), a biflavonoid mainly isolated from members of the Ochnaceae, has shown anti-atherogenic activity. Atherogenesis is the formation of plaque made up of fatty materials which gradually constrict the arteries hence cause high blood pressure and cardiovascular diseases. The antiatherogenic activity of **51** is related to its ability to inhibit human vascular smooth muscle cell proliferation (Suh *et al.*, 2006b).
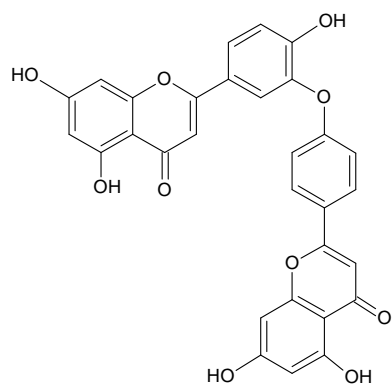
51
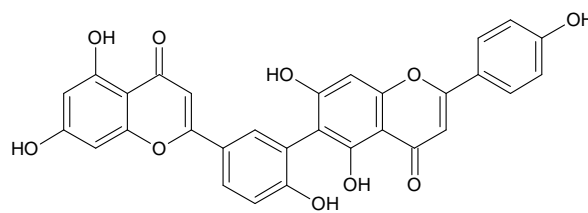
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Compounds **51**, **91**, **92** and **94** inhibit phospholipase A$_2$ and cyclooxygenase-2 enzymes activities thereby resulting into a decreased biosynthesis of the key mediators of inflammation (the prostaglandins) (Ariyasena et al., 2004; Chen et al., 2006; Lim et al., 2006; Son et al., 2006; Yoon et al., 2006).

In addition, biflavonoids like **51** are reported to suppress the activation of nuclear factor–κB (NF-κB), which is widely used by eukaryotic cells as a regulator of genes that control cell proliferation and cell survival. As such, in many tumours NF-κB is misregulated and so it remains active thereby turning on the gene expression, cell proliferation and protection against all conditions that would otherwise kill the cell. The suppression of NF-κB will result into downregulation of the gene expression and cell proliferation hence killing the particular cell (Banerjee et al., 2002; Jayaprakasam et al., 2000; Park et al., 2006; Suh et al., 2006b).

Amentoflavone (**92**) has shown to have different mechanisms of activity against cancer cells. The compound inhibits the activity of topoisomerase enzymes and thereby impairs the winding and unwinding activity of DNA in the cell. This will consequently lead to cell death (Grynberg *et al.*, 2002). Compound **92** also inhibited the activity of vascular endothelial growth factors (VEGF) thereby result in the death of the tumour cell by shutting down the vascular system (Tarallo *et al.*, 2011). Human cytochrome P450 enzymes are the main enzymes that are responsible for drug metabolism. However, highly reactive intermediate formed by these enzymes can bind to DNA and the resulting DNA adduct can cause

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mutation, in the presence of relevant gene this DNA adduct can initiate cancer (Goth-Goldstein *et al.*, 2010). Amentoflavone (**92**) inhibits these enzymes with a nanomolar potency (von Moltke *et al.*, 2004). The compound has shown to efficiently and specifically inhibit polymerization of amyloid peptides and thereby reduce the effects of Alzheimer's disease as compared to the monoflavonoid apigenin which is less effective and less specific (Thapa *et al.*, 2011).

5.3   Results and Discussion

5.3.1   Cytotoxicity activities

The cytotoxicity assays for all compounds isolated from *O. serrulata* were performed at CSIR, Pretoria. The growth inhibitory effects of the compounds were tested in the 3-cell line panel consisting of TK10 (renal), UACC62 (melanoma) and MCF7 (breast) by sulforhodamine B (SRB) assay. The assay was first developed by Skehan and colleagues in 1990 in the NCI (USA) anticancer drug discovery program. The binding of the dye to proteins is pH dependent and therefore, the clonogenicity, drug-induced cytotoxicity and cell proliferation are measured by the assay which basically utilizes the capability of the sulforhodamine B dye (Acid Red 52) to bind to the protein basic amino acid residues of the cells pretreated with trichloroacetic acid. Unbounding the dye from the protein amino acids of the cell is achieved by changing the pH to basic and therefore solubilize the dye that can be measured (Skehan *et al.*, 1990).

The obtained results are presented as dose-response curves for each cancer cell line. The dose-response curve is a plot of percentage growth versus concentration of the test compound. For each tested compound, three response parameters, GI_{50} (50% growth inhibition which signifies the growth inhibitory power of the test agent), TGI (which is the drug concentration resulting in total growth inhibition and signifies the cytostatic effect of the test agent) and LC_{50} (lethal concentration, killing 50% of the cells and signifies the cytotoxic effect of the test agent), were calculated for each cell line. These values interpolated from the mentioned graphs

represent the concentration at which percentage growth is +50, 0 and -50, respectively.

Four compounds, lophirone A (**36**), lophirone C (**39**), ochnaflavone (**51**) and 3'-*O*-methylpsilosin (**78**), exhibited potent cytotoxic activity against the three mentioned cancer cells, the remaining compounds were only slightly active.

Lophirone C (**39**) demonstrated higher cytotoxic activity against melanoma cancer cell lines (UACC62) than the other two cancer cell lines. Its activity against renal (TK10) and breast (MCF7) cancer cell lines was virtually the same. As shown in Fig. 5.2 and Table 5.1, the cytotoxic activity of **39** was concentration-dependent.
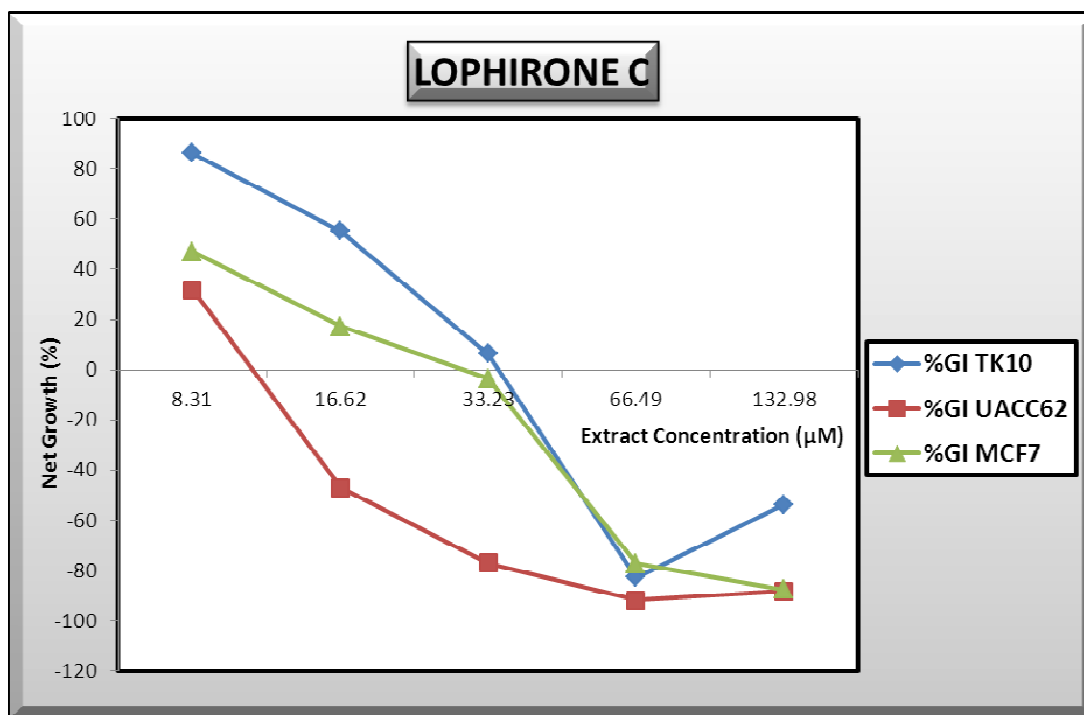
Fig. 5.2: Dose-response curve of lophirone C (39)

Table 5.1: Cytotoxic activity of 39

Activities	TK10 (µM)	UACC62 (µM)	MCF7 (µM)
GI_{50}	18.39	N/A	N/A
TGI	35.63	11.67	30.35
LC_{50}	54.27	18.25	54.19

There are no published reports on the activity of lophirone C (**39**) prior to this work. A concentration-dependent cytotoxic activity of lophirone A (**36**) was observed (Table 5.2), the compound reached its optimum activity against the 3 cancer cell lines at a concentration of 72.45 µM as illustrated in Fig. 5.3 below. The compound was highly active against melanoma (UACC62) cancer cells, with lesser activity against breast (MCF7) and renal (TK10) cancer cells.
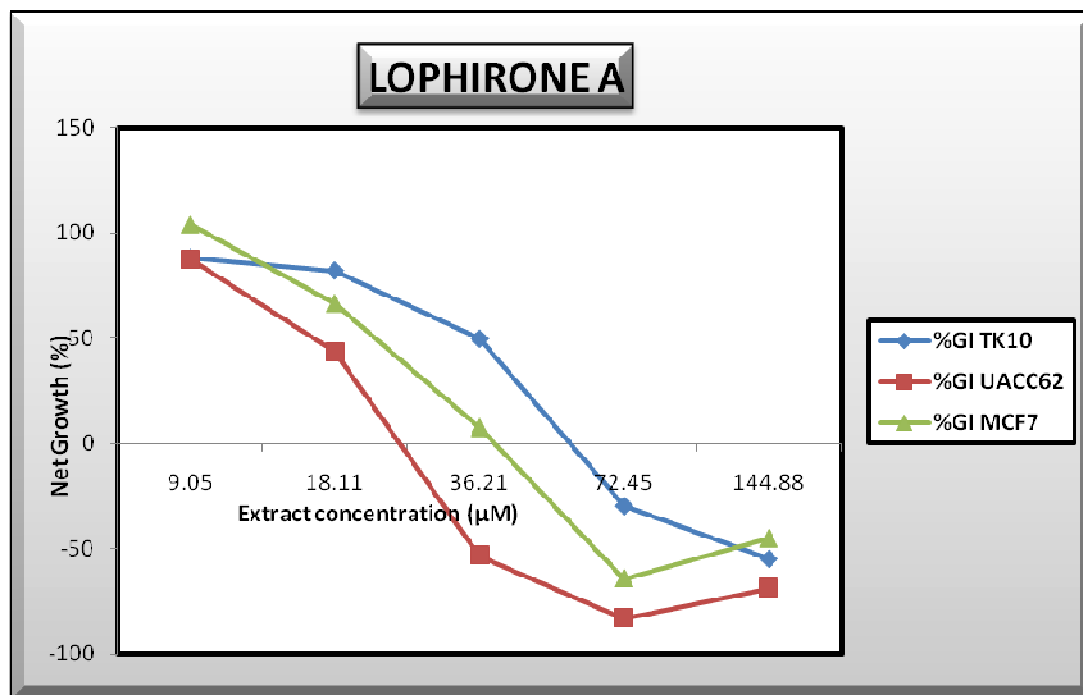
Fig. 5.3: Dose-response curve of lophirone A (36)

Table 5.2: Cytotoxic activity of 36

Activities	TK10 (µM)	UACC62 (µM)	MCF7 (µM)
GI_{50}	36.14	16.78	23.19
TGI	58.96	26.23	40.01
LC_{50}	131.29	35.59	62.25

It was observed that the cytotoxic activity of lophirone A (**36**) correlated well with the reported activity of the compound. As described in Chapter 2 of this thesis, **36** has been reported to be an inhibitor of Epstein-Barr virus and has shown inhibitory properties against tumour promotion.

A higher selectivity was demonstrated by ochnaflavone (**51**), where the compound inhibited the growth of melanoma cancer cells only (UACC62). The activity of the compound against the mentioned cancer cells was concentration-dependent (Table 5.3 and Fig. 5.4). The growth inhibitory power of ochnaflavone (**51**) against renal and breast cancer cells was shown to be the same, the compound totally inhibited the growth of renal (TK10), melanoma (UACC62) and breast (MCF7) cancer cells at a concentration of 137.34, 12.91 and 137.34 µM, respectively.

As described earlier in this Chapter, ochnaflavone (**51**) has shown a broad range of activities including anti-inflammatory, anticancer and anti-atherogenic activities. The observed cytotoxic activity of **51** correlates with the reported activity, however, the mechanism of the selectivity demonstrated by the compound needs to be investigated further.

A *C*-glucosylated flavone, 3'-*O*-methylpsilosin (**78**) showed higher selectivity as shown in Table 5.4. The compound inhibited the growth melanoma cancer cells (UACC62) only at a concentration of 14.11 µM, comparable to that of ochnaflavone (**51**) (12.91 µM). There are no filed reports on the biological activity of 3'-*O*-methylpsilosin (**78**).
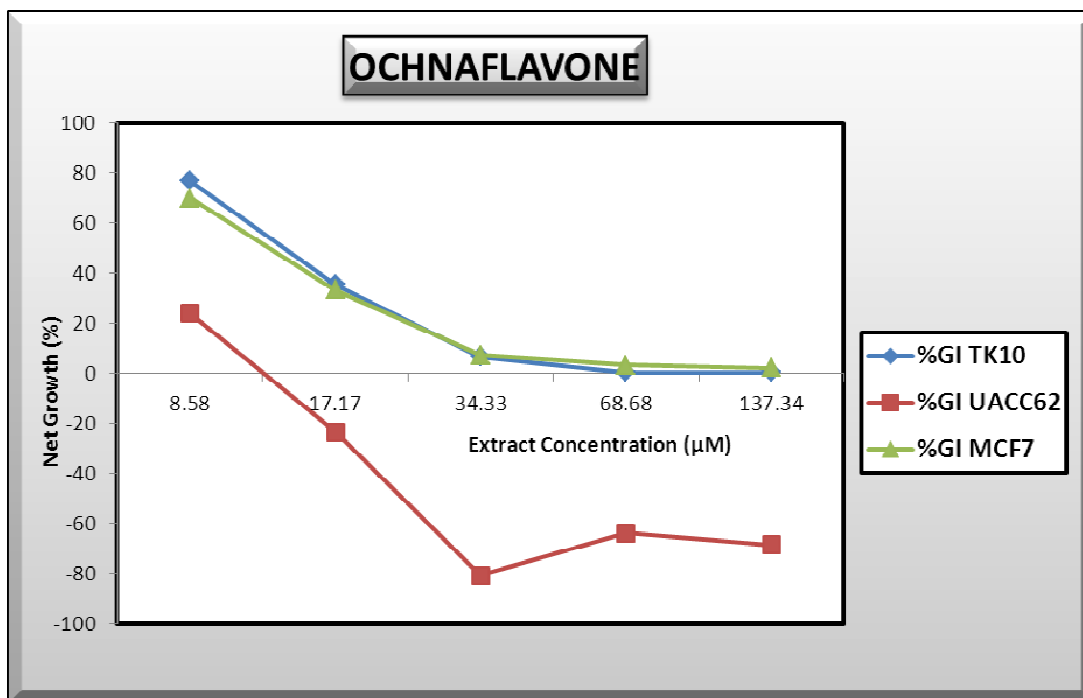
Fig. 5.4: Dose-response curve of ochnaflavone (51)

Table 5.3: Cytotoxic activity of 51

Activities	TK10 (µM)	UACC62 (µM)	MCF7 (µM)
GI_{50}	14.16	N/A	13.27
TGI	N/A	12.91	N/A
LC_{50}	N/A	25.16	N/A

Table 5.4: Cytotoxic activity of 78

Compound	%GI TK10	%GI UACC62	%GI MCF7
3'-*O*-methylpsilosin	8.02	-41.88	7.6

Note: Fixed concentration (19.84 µM) of 3'-*O*-methylpsilosin (**78**) was used.

Etoposide (**21**), a topoisomerase inhibitor that is used clinically for cancer treatment, was taken as a standard. It demonstrated almost the same level of cytotoxic activity against the three cancer cell lines (Fig. 5.5 and Table 5.5). The compound also exhibited a concentration-dependent activity, the sharp increment in its activity was observed at a concentration of 169.82 µM.

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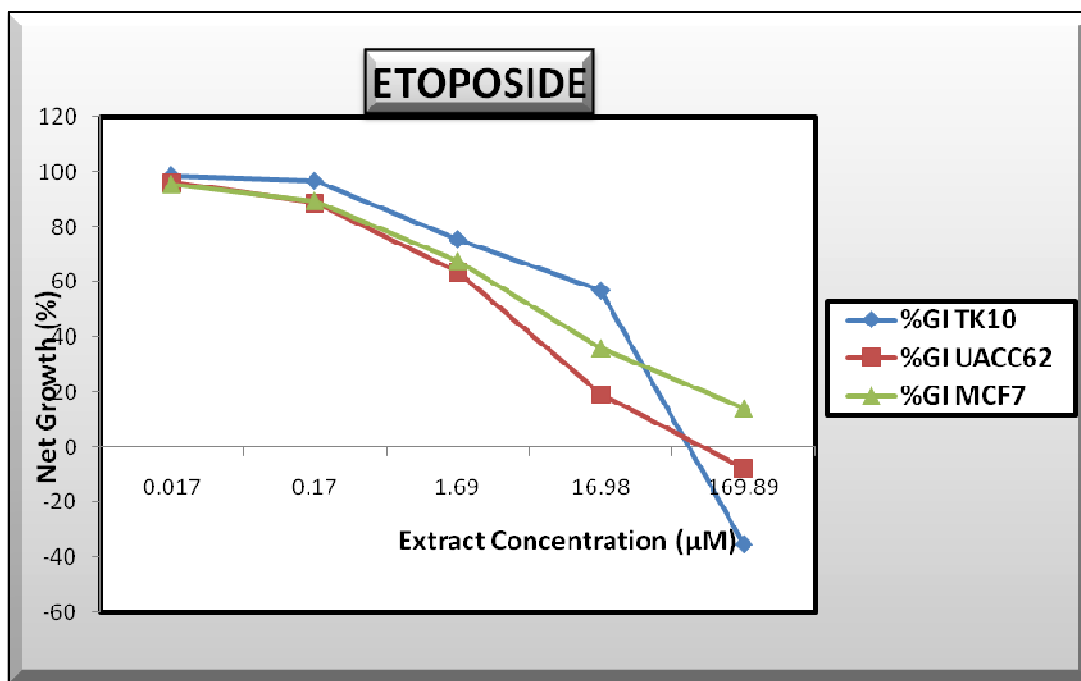
Fig. 5.5: Dose-response curve of etoposide (standard)

Table 5.5: Cytotoxic activity of etoposide (standard)

Activities	TK10 (µM)	UACC62(µM)	MCF7(µM)
GI_{50}	16.80	3.77	5.98
TGI	65.48	74.06	N/A
LC_{50}	N/A	N/A	N/A

All compounds [lophirone A (**36**) and C (**39**), ochnaflavone (**51**) and etoposide (**21**)] exhibited a concentration-dependant activity where, as their concentration increased their cytotoxic activities also increased. Lophirone C (**38**) showed a total growth inhibition (TGI) against renal (TK10), melanoma (UACC62) and breast (MCF7) cancer cells at 35.63 µM, 11.67 µM and 30.35 µM, respectively. Lophirone A (**36**) is less active and exhibited TGI against renal (TK10), melanoma (UACC62) and breast (MCF7) cancer cells at 58.96 µM, 26.23 µM and 40.01 µM, respectively.

Higher selectivity was observed for ochnaflavone (**51**) and 3'-*O*-methylpsilosin (**78**) where only the growth of melanoma cancer cells (UACC62) was inhibited. Ochnaflavone (**51**) and 3'-*O*-methylpsilosin (**78**) showed TGI against melanoma (UACC62) cancer cells at 12.91 µM and 14.11 µM, respectively.

To summarize, for the renal cancer cell line (TK10), the activity observed was **39** > **36** > **51** > **78**. For the melanoma cell line (UACC62) the activity was **39** > **51** > **78** > **36** and for the breast cancer cell line (MCF7) the activity observed was **39** > **36** > **51** >**78**.

It is of interest to note the difference in cytotoxic activity of the four compounds **51**, **36**, **39** and **78**. Thus, whereas the structurally-related compounds **51** and **78** have shown comparable and virtually the same pattern of cytotoxic activities, compound **39** has shown to be about as twice as active as **36**.

If the total growth inhibition (TGI) capacity of the compounds is compared with the standard [etoposide (**21**)], then, lophirone C (**39**) showed almost twice the activity of etoposide (**21**) and lophirone A (**36**) against renal cancer cell lines (TK10). Against melanoma cancer cells (UACC62), **39** and **51** showed virtually the same level of activity which was twice as much as that of **36** and almost seven times higher as compared to that of **21**. The TGI of **21** and **51** against breast cancer cells (MCF7) were not determined but the activity of **36** and **39** against these cancer cells was virtually the same.

To summarize, therefore, the trend in TGI (total growth inhibition) of the tested compounds against the renal cancer cell line (TK10) was **39** > **36** > **21**, for a melanoma cell line (UACC62) it was observed that **39** > **51** > **36** > **21** and for a breast cancer cell line (MCF7) **39** > **36**.

5.3.2 Antimalarial activities

With the exception of isocampylospermone A (**61**) and campylospermone A (**62**), the rest of the compounds isolated from the leaves, stem and roots of *O. serrulata* as reported in Chapter 3 and 4 of this thesis, have not been investigated for their antimalarial activity prior to this report. These two compounds (**61** and **62**) are active against *Plasmodium falciparum* multidrug resistant strains (K1) at 0.16 and 10.19 µM, respectively, multidrug sensitive strains (FCR3) at 0.51 and 8.82 µM for **61** and **62**, respectively (Ichino *et al.*, 2006).

The antimalarial tests for all compounds isolated from *O. serrulata* were performed by Prof. Robyn van Zyl at the Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of Witwatersrand, Johannesburg. The growth inhibitory effects of the compounds were evaluated against the chloroquine-resistant strains of *Plasmodium falciparum* (FCR-3). The Table below summarizes antimalarial activities of some of the compounds that showed moderate to weak activity. The rest of the compounds isolated from *O. serrulata* as reported in Chapter 3 and 4 of this thesis did not show any significant activity.

The observed antimalarial activity of a mixture of isocampylospermone A (**61**) and campylospermone A (**62**) (64.31 µM) did not correlate with the reported activity of these two compounds (0.16 and 10.19 µM, respectively). The published activity is much higher than what has been observed here. However, individual pure compounds were used for the reported activity, while the mixture of the two compounds was used in this assay.

Table 5.6   Antimalarial activity of the isolated compounds

S/No	Compound	IC_{50} (µM)
1	4,4',7-tri-*O*-methylisocampylospermone A (**85**)	11.46
2	Ochnaflavone (**51**)	17.25
3	Serrulone A (**87**)	26.52
4	Lophirone A (**36**)	29.78
5	5-Deoxyurundeuvine C (**76**)	31.07
6	Lophirone C (**39**)	35.31
7	4'''-De-*O*-methylafzelone A (**86**)	38.43
8	Afzelone B (**50**)	39.54
9	Irisolone 4'-methyl ether (**81**)	40.72
10	Syringaresinol (**89**)	42.66
11	2",3"-dihydroochnaflavone (**54**)	61.86
12	Mixture of isocampylospermone A (**61**) and campylospermone A (**62**)	64.31
13	Iriskumaonin 5-*O*-methyl ether (**84**)	93.69

| 14 | 3'-O-methylpsilosin (**78**) | 106.35 |
| 15 | 16α,17-dihydroxy-ent-kauran-19-oic acid (**90**) | 106.48 |

It is important to note the difference in antimalarial activity of the structurally-related 4,4',7-tri-O-methylisocampylospermone A (**85**) with IC$_{50}$ value of 11.46 µM and a mixture of isocampylospermone A (**61**) and campylospermone A (**62**) with IC$_{50}$ value of 64.31 µM. 4,4',7-Tri-O-methylisocampylospermone A (**85**) has a six-fold higher antimalarial activity than a mixture of **61** and **62**. None of the mentioned compounds showed any significant cytotoxic activity against the three tested cancer cells.

With regards to a structure-activity relationship, it is difficult to make any conclusive remarks at this stage because no trend could be observed. For example, ochnaflavone (**51**), 2",3" dihydroochnaflavone (**54**) and 3'-O-methylpsilosin (**78**) are all flavones, but the antimalarial activity of **51** was found to be four times higher than that of **54** and six times higher than that of **78**. In terms of the cytotoxic activity, **51** was comparable to **78**, but **54** was less active against all the three cancer cells.

The isomeric compounds, serrulone A (**87**) and lophirone L (**88**) exhibited completely different level of antimalarial activity. Compound **87** showed antimalarial activity at 26.52 µM, while **88** showed no significant activity. These compounds are stereoisomers and this indicates that relative and/or absolute configuration is important. On the other hand, lophirone C (**39**), serrulone A **87** and a 5-deoxyurundeuvine C (**76**) exhibited virtually the same activity against *P. falciparum* with IC$_{50}$ values of 35.31 µM, 26.52 µM and 31.07 µM, respectively.

The level of antimalarial activity of 4,4',7-tri-O-methylisocampylospermone A (**85**) and ochnaflavone (**51**) is considered to be moderate, however the suitability of these compounds as antimalarial agents that can be used in the treatment of malaria will depend on toxicological, specificity and biodegradability properties of the compounds. These factors therefore need to be investigated further.

5.4 Conclusion

The observed cytotoxic activity of lophirone A (**36**) is comparable to the reported tumour growth inhibition activity reported for this compound. A literature search indicated that a 5-deoxy analogy of 4'''-de-*O*-methylafzelone A (**86**) have been patented for its anticancer activities by Japanese scientists (Hiroshi *et al.*, 2009).

4,4',7-tri-*O*-methylisocampylospermone A (**85**) isolated from the roots of *O. serrulata* showed the highest antimalarial activity, whereas lophirone C (**39**) isolated from the stem and roots of the same plant showed potent cytotoxic activity against the mentioned three cancer cells.

Unlike lophirone C (**39**), ochnaflavone (**51**) showed comparable activity in both tests, where higher selectivity was observed in cytotoxic tests. Higher selectivity was also shown by 3'-*O*-methylpsilosin (**78**), but **51** stands superior in that it showed consistency in its activity. The observed and reported biological activities of **51** were the motivation to synthesize compound **51** from commercially available starting materials; therefore, Chapter 6 of this thesis describes the synthesis of ochnaflavone (**51**).

5.5 Materials and Methods

Cytotoxicity tests were performed at the CSIR, South Africa in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute. Pure compounds were assayed in 3-cell line panel consisting of TK10 (renal), UACC62 (melanoma) and MCF7 (breast).

For screening experiment, the cells were inoculated in a 96-well microtiter plates at plating densities of 7-10 000 cells/well and were incubated for 24 h. After 24 hr one plate was fixed with trichloroacetic acid to represent a measurement of the cell population for each cell line at the time of drug addition (T0).The other plates with cells were treated with the compounds which were previously dissolved in DMSO and diluted in medium to produce 5 concentrations (6.25-100 ppm). Cells without

drug addition serves as control. The blank contained complete medium without cells and etoposide was used as a standard. The plates were incubated for 48 hr after addition of the compounds.

The optical density of the test well after 48 hr period of exposure to compound is T, the optical density at time zero is T0 and the control optical density is C. The TGI is the concentration of test drug where $100 \times (T-T0)/(C-T0) = 0$

The results of five dose screening are reported as TGI (total growth inhibition). The biological activities of the compounds were categorised into 4: inactive (TGI > 50 µg/ml or TGI >100µM), weak activity (15 µg/ml < TGI < 50 µg/ml or 30 µM < TGI <100 µM, moderate activity (6.25 µg/ml < TGI <15 µg/ml or 10 µM < TGI <30 µM and potent activity (TGI <6.25 µg/ml or TGI <10 µM).

In vitro antimalarial tests as described in Chemaly *et al.* were performed by Prof. Robyn van Zyl at the Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of Witwatersrand (Chemaly *et al.*, 2007).

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CHAPTER 6

Synthesis of Ochnaflavone

6.1 General Introduction

This chapter describes the synthesis of ochnaflavone (**51**), a non-symmetrical biflavone consisting of apigenin and luteolin moieties. The compound exhibits diverse biological activities including anticancer, anti-inflammatory and anti-HIV activities as described in Chapter 5 of this thesis. Based on the broad range of biological activities and the observed selectivity in the cytotoxicity tests (as reported in Chapter 5), the decision was made to synthesize the compound.

The total synthesis of ochnaflavone has not been reported, although Okigawa et al. (1976) have published the preparation of the pentamethyl ether of this compound. Therefore, the aim of this Chapter is to report the first total synthesis of ochnaflavone (**51**) using an approach different from that of Okigawa et al.

6.1.1 Ochnaflavone

Ochnaflavone (**51**) is present in the leaves and stems of plants belonging to the family Ochnaceae (Kang et al., 2005). Most biflavones consist of an interflavone linkage between ring B of one moiety and the ring A of the other flavone moiety (AB type), between two A rings (AA type), or between two C rings (3,3"-CC type); but the most rare biflavones are the ones with the inter-flavone linkage through the two B rings. Ochnaflavone (**51**) has an ether link between an apigenin and luteolin

moieties (Sagrera *et al.,* 2011). Ochnaflavone (**51**) contains a diaryl ether functionality which is widely found in biologically active molecules and natural products (Evans et al., 2001; Nicolau et al., 1999). This structural feature is part of various significant pharmaceuticals with antibiotic activities, such as vancomycin, teicoplanin, the antiviral peptide K-13 and the antitumoral bouvardin (Bigot et al., 2000; Nicolaou et al., 2002; Theil, 1999; Zhu, 1997).

Ochnaflavone (**51**) has diverse biological activities such as anticancer, anti-inflammatory and anti-HIV activities, as discussed in Chapter 5 of this thesis. The broad range of biological activities, the observed cytotoxicity activity and the absence of a filed report on the synthesis of **51** have sparked interest in synthesizing this compound (Chang et al., 1994; Lee et al., 1995; Moon et al., 2006; Reutrakul et al., 2007; Son et al., 2006; Suh et al., 2006a; Suh et al., 2006b; You-Jin et al., 2009).
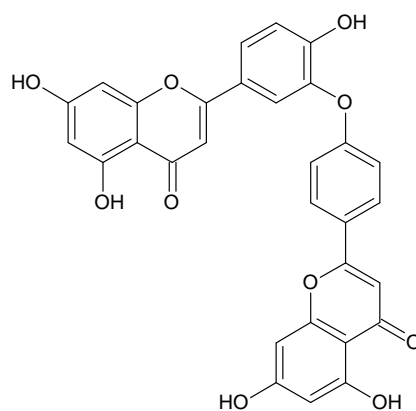
51

The two most important features of the synthesis of ochnaflavone (**51**) are the formation of the diaryl ether linkage and the assembly of the flavone nucleus. In the next paragraphs, different synthetic methods employed in the synthesis of diaryl ethers and flavones will be discussed

6.2   Synthesis of Diaryl Ethers

The most logical approach to the synthesis of **51** is to start with the preparation of the diaryl ether. This functionality is present in many other natural products, therefore it is not surprising that a great amount of research has been focused on the development and improvement of methods used to form diaryl ethers. In the next paragraphs, the most important methods to form diaryl ethers will be discussed.

6.2.1   Ullmann condensation

The Ullmann ether synthesis is a variation of the Ullmann reaction where phenolic compound is coupled with an electron-deficient aryl halide in the presence of a copper compound, the reaction is named after Fritz Ullmann. Recently, there have been extensive research efforts to develop copper-based catalysts in cross-coupling reactions of aryl halides with various nucleophiles so as to replace the traditional Ullmann-type reaction conditions. These traditional copper-based reactions typically involve the coupling of an aromatic halide with an amine (Goldberg reactions) or phenol (Ullmann reactions) for the synthesis of an aryl amine and an aryl ether, respectively, as illustrated in **Scheme 6.1** (Kunz *et al.*, 2003).

The terminology "Ullmann condensation" usually describes the copper-catalyzed reactions of aromatic halides with phenol salts or anilines for the synthesis of aryl ethers and amines. On the other hand, "Ullmann coupling" refers to reactions aiming at synthesizing biaryls from aromatic halides.

These reactions have offered a foundation for the preparation of diaryl ethers but typical Ullmann's reaction conditions experience the disadvantages of high reaction temperatures, the use of toxic solvents (e.g. hexamethylphosphoramide) and also intolerance to a wide variety of functional groups. The major weakness of the classical Ullmann reaction comes from inconsistent products obtained from the

use of different copper sources (Amberg et al., 1993; Capdevielle and Maumy, 1993; Peeters et al., 1994; Zhu et al., 2000).
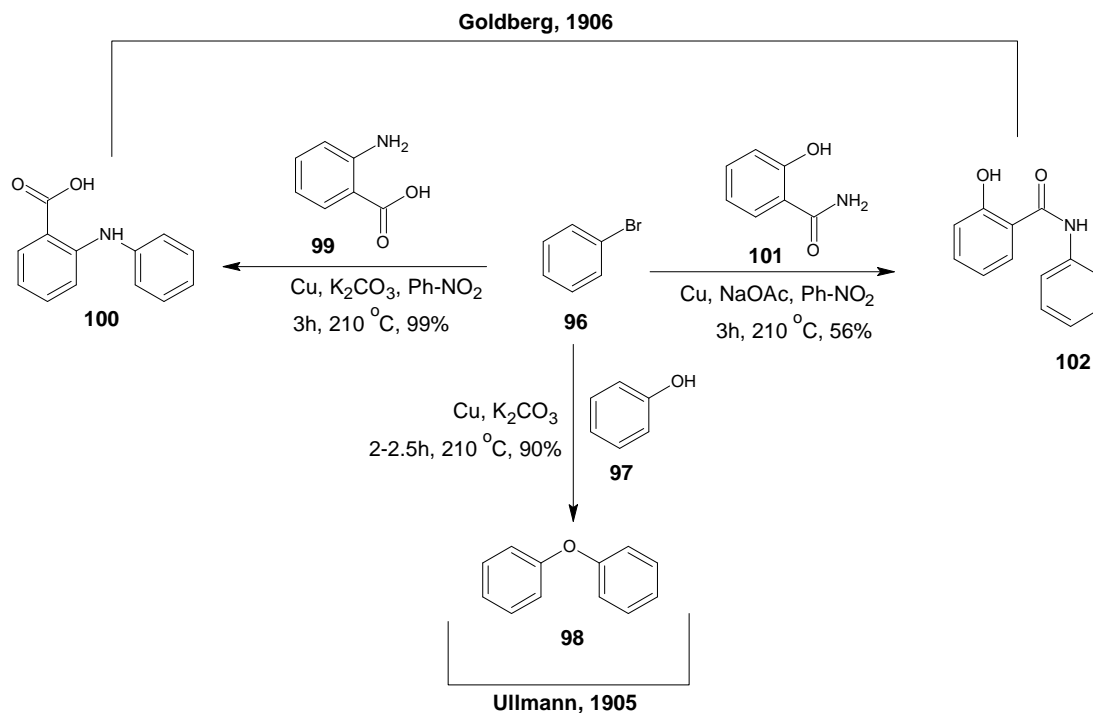
Scheme 6.1: Examples of Ullmann and Goldberg coupling reactions

Therefore, palladium-based catalysts were developed, however, due to the high costs associated with palladium-based catalyst systems, the past decade has seen the development of a multitude of copper catalysts for Ullmann-type *O*-arylation reactions (Boger and Yohannes, 1989; Chang et al., 2008; Chen et al., 2008; Naidu et al., 2008).

Applications of Ullmann condensation in synthesis:
In 1991, D. L. Boger and group utilized the intramolecular Ullmann condensation reaction to form a 15-membered macrocyclic ring of a cytotoxic natural product, combretastatin D-2. The iodo-part of the molecule was cyclized with the phenolic-part of the molecule **103** to form diaryl ether **104** which was later demethylated to yield combretastatin D-2 (Boger *et al.*, 1991). The compound has unusual *meta* and *para*-cyclophane subunits, the cyclization was mediated by methyl copper as illustrated in **Scheme 6.2**.

141
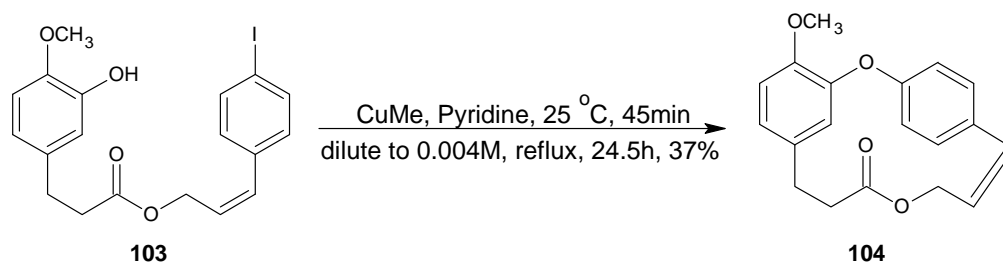
Scheme 6.2: Intramolecular Ullmann synthesis of 104 as reported by Boger
***et al.,* 1991**

(+)-Diepoxin δ (**105**), a highly oxygenated anticancer natural product, was synthesized in the Wipf laboratory. The key step in the synthesis of **105** was the building of the diaryl ether linkage which was accomplished via Ullmann condensation of phenolic compound (**106**) and 1-iodo-8-methoxynaphthalene (**107**). The condensation of the two precursors was carried out in refluxing pyridine in the presence of copper(I) oxide as illustrated in **Scheme 6.3** (Wipf and Jung, 2000).
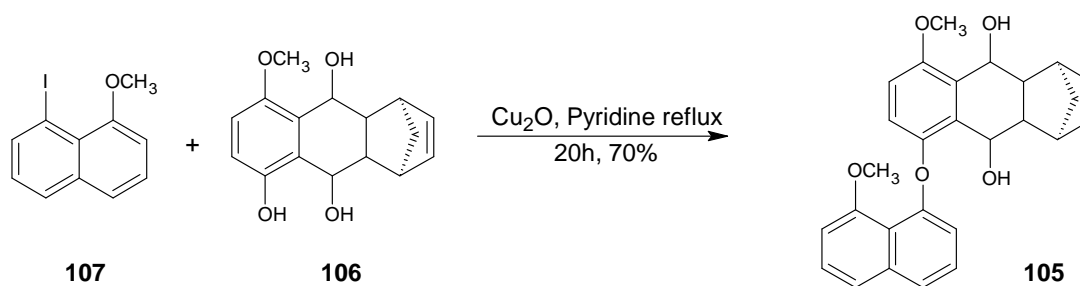
Scheme 6.3: Intermolecular Ullmann synthesis of 105 as reported by Wipf
and Jung, 2000

6.2.2 Nucleophilic aromatic substitution (S$_N$Ar) reactions

An older method for synthesis of diaryl ethers is the use of nucleophilic aromatic substitution (S$_N$Ar) reactions. This involves the direct nucleophilic coupling of a phenol to an electron-deficient aromatic system. This method is simple to use and has been applied in the synthesis of vancomycin, piperazinomycin, the combretastatins and other natural products. Nucleophilic coupling of phenols to different electron-deficient aryl frameworks has been known for some time, but the

application of this reaction method in the synthesis of diaryl ether-containing natural products has recently stimulated the development of modifications (Beugelmans et al., 1994a; Beugelmans et al., 1994b).

Applications of nucleophilic aromatic substitution in synthesis:

The construction of vancomycin and other diaryl ether natural products utilizes the intramolecular S_NAr macrocyclization. The Boger group illustrated the simplicity of the S_NAr macrocyclization in the synthesis of the important fragment of the antitumor agents deoxybouvardin and RA-VII (Carrington *et al.*, 1999). Compound **108** was successfully cyclized to the 14-membered macrocycle **109** in 50 - 60% yields, where sodium hydride was used to initiate ring cyclization as illustrated in **Scheme 6.4**.
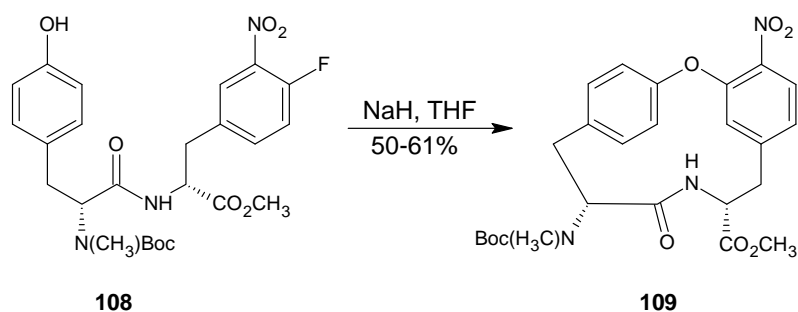
Scheme 6.4: Intramolecular S_NAr synthesis of 109 as reported by Carrington *et al.*, 1999

Caesium fluoride has also been used in S_NAr conditions for initiating intramolecular ring cyclization as shown in **Scheme 6.5**. Compound **110** was successfully ring cyclized to **111**, which is a derivative of the natural product alkaloid cadabicine (Carrington et al., 1999; Rao et al., 1997; Rao et al., 1995).
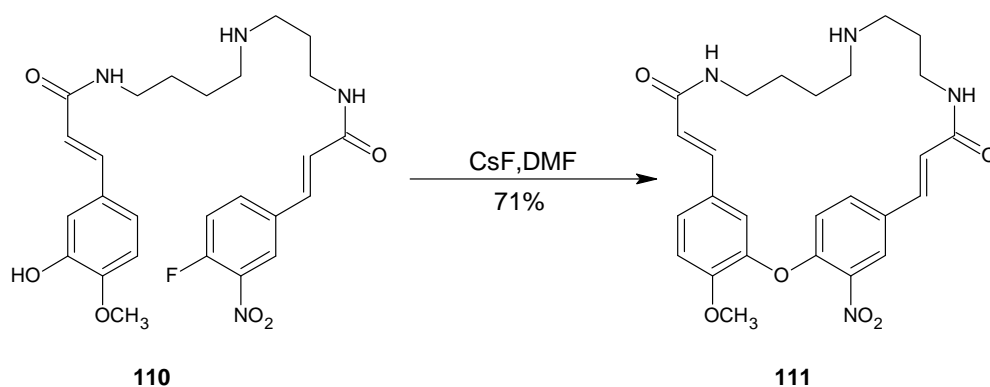
Scheme 6.5: Intramolecular S$_N$Ar synthesis of 111 as reported by Rao *et al.*, 1997

6.2.3 Other methods

Other methods have also been developed and one method involves the application of the Diels-Alder reaction. Good yields of diaryl ether were obtained by the cycloaddition reaction between a substituted aryloxy-1,3-butadiene and acetylenic electrophile, followed by aromatization. **Scheme 6.6** indicates an example of the stated reaction where diene **112** was reacted with methyl propiolate and the resulting product was oxidized with DDQ to yield diaryl ether **113** in 64% yield (Olsen et al., 1995; Sawyer, 2000).
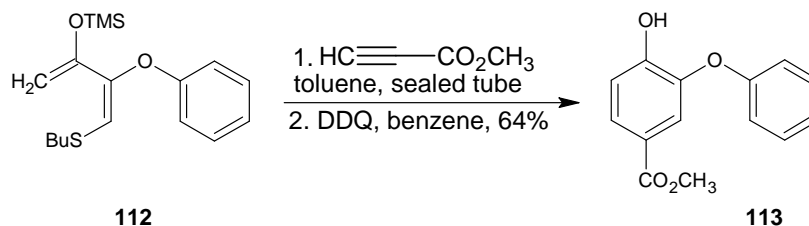
Scheme 6.6: Synthesis of 113 by Diels-Alder reaction as reported by Olsen *et al.*, 1995, Sawyer, 2000

Chan and Evans showed that diaryl ethers can also be prepared at room temperature using copper(II) acetate as catalyst and triethylamine as a base. The diaryl ether **116** is formed from a phenol **115** and an arylboronic acid **114** as illustrated in **Scheme 6.7** (Chan et al., 1998; Evans et al., 1998).
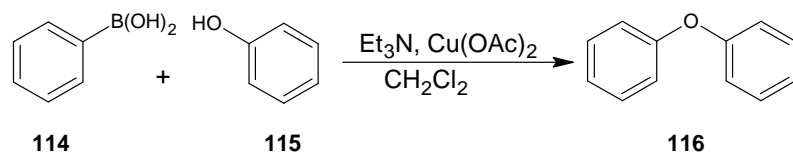
Scheme 6.7: Synthesis of 116 as reported by Chan *et al.*, 1998, Evans *et al.*, 1998

6.3   Synthesis of Flavones

The interesting biological activities of flavones have resulted into intense synthetic efforts towards the synthesis of various flavones. There are a number of methods reported for the synthesis of flavones.

6.3.1   The Baker-Venkataraman method

The Baker-Venkataraman approach involves the conversion of 2'-hydroxyacetophenone (**117**) to an ester **118** by acylation, followed by a rearrangement via an intramolecular Claisen condensation in the presence of potassium hydroxide (a base) and pyridine to give a diketone **119**. From the diketone, a flavone **120** is expected to be obtained under rather harsh conditions either by treating with concentrated sulfuric acid or heating with glacial acetic acid as in **Scheme 6.8** (Baker, 1933; Ganguly et al., 2005).
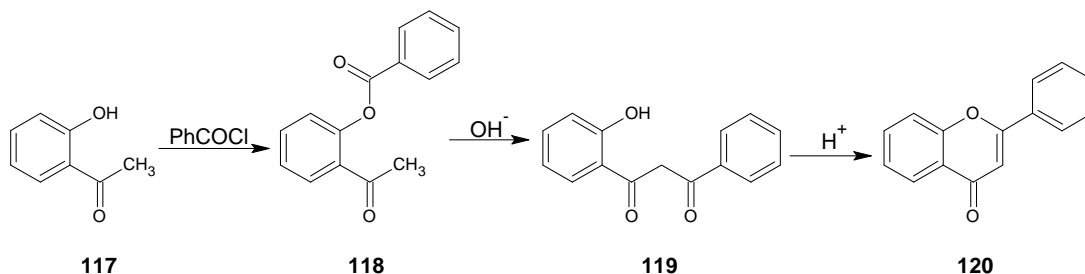
Scheme 6.8: Synthesis of flavones by Baker-Venkataraman Method

Proposed mechanism for diketone formation in Baker-Venkataraman Method

The first step is the removal of a hydrogen atom from acetophenone to form an enolate. The formed enolate attacks the ester carbon forming a cyclic alkoxide which finally opens up into a phenolate and protonation by acid gives the phenol as illustrated in **Scheme 6.9**.
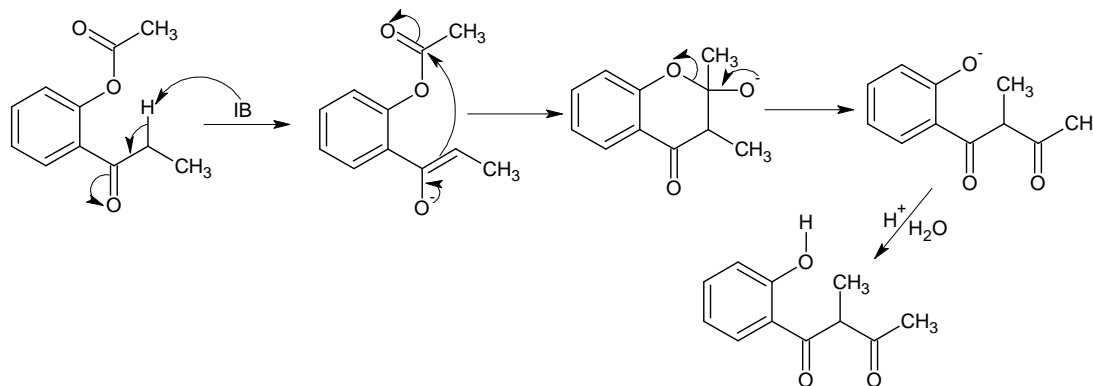
Scheme 6.9: Proposed mechanism for diketone formation

Modification of the Baker-Venkataraman method for flavone synthesis

The modification of the Baker-Venkataraman method of synthesizing flavones was published by Ganguly and co-workers (2005). In the presence of a base catalyst (DBU) and pyridine, the mixture of 2',4'-dihydroxyacetophenone (**121**) and benzoyl chloride (**122**) were heated to 80-90 °C as shown in **Scheme 6.10** to yield the respective flavone (Ganguly *et al.*, 2005). However, in an excess benzoyl chloride, the phenolic group of the formed β-diketone (Baker-Venkataraman product) undergoes esterification followed by rearrangement to a triketone; cyclodehydration of the triketone yields **123** (Chee *et al.*, 2011).
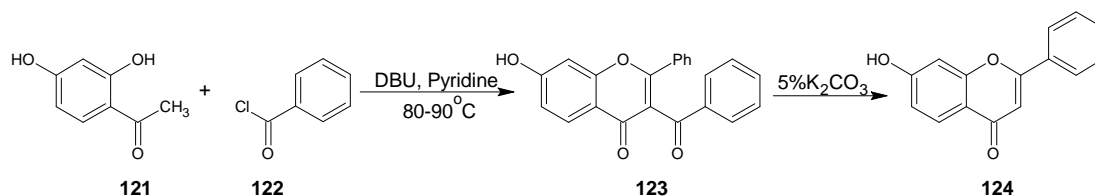
Scheme 6.10: Synthesis of flavones by Ganguly's method

6.3.2 The Konstanecki – Robinson method

In this method, *o*-hydroxyaryl ketones (**125**) are converted to chromones and/ or coumarins with aliphatic acid anhydrides in the presence of the sodium salt of the corresponding acid. On the other hand, if an aromatic acid anhydride (**126**) is used in the presence of the salt of the corresponding acid **127**, a flavone **128** is formed as shown in **Scheme 6.11** (Ellis, 1977; Nakanishi, 1974). The method for the formation of chromones and coumarins is called the Robinson method and for the formation of flavones, is called the Von Konstanecki method (Hepworth et al., 1996; Wagner and Farkas, 1975).
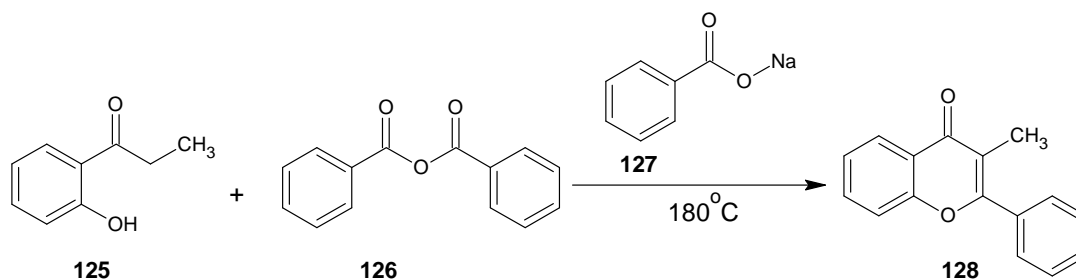
Scheme 6.11: Synthesis of flavones by the Von Konstanecki method

Modifications of the Von Konstanecki method for flavone synthesis
The synthesis of flavones has been modified over the years; one of the primary modifications has been substituting the carboxylate salt with other bases. While Kohn and Low demonstrated that catalytic amounts of triethylamine allowed for the reaction to be run at 160 °C (Kohn and Low, 1944), Looker and co-workers went further by using amines as solvents and thus reduce the reaction temperatures. They found that the reactions could be run at the refluxing temperature of the amines (Looker *et al.*, 1978).

Processes involved: 1: Enolization;   2: Acylation

Scheme 6.12: Mechanism of flavones synthesis by the Von Konstanecki method

Another group that contributed to the modification of this method is Grover and coworkers, they showed that the reaction proceed well even when potassium carbonate is used as a base. They also showed that the reaction can be performed with refluxing acetone as a solvent and so reduce greatly the temperature at which the reaction proceeds (Grover *et al.*, 1963).

6.3.3 Oxidative ring cyclization of chalcones

Another methodology most commonly used to prepare flavones involves the cyclization of substituted 2-hydroxychalcones, obtained by an aldol condensation reaction between a 2'-hydroxyacetophenone and an aldehyde. These cyclizations have been carried out under numerous conditions such as using iodine in DMSO/triethyleneglycol/pyridine and heat. It has been demonstrated that the role of DMSO/triethyleneglycol/pyridine in these reactions is to act as an oxidative

agent as well as a medium of energy transfer (Gupta *et al.*, 2000). The use of mild conditions such as ferric chloride in absolute ethanol has also been reported, iron(III) in these reactions acts as oxidizing agent facilitating the 2-hydroxychalcone ring closure. Catalysts such as $CuCl_2$, SeO_2 and silica have shown to be effective in catalyzing 2-hydroxychalcone ring cyclization. DDQ, a strong oxidant, which has been used for dehydrogenating reactions, have been reported to be effective in dehydrating flavanones to flavones and also ring cyclizing 2-hydroxychalcones. Since DDQ decomposes in water, the reactions are reported to be carried out in refluxing dry dioxane (Gupta et al., 2000; Huang et al., 2003; Imafuku et al., 1987; Kabalka and Mereddy, 2005; Mavel et al., 2006; Miyake et al., 2003; Zambare et al., 2009).

6.4   Previous Syntheses of Ether-linked Biflavones

The only report on the synthesis of an ether-linked biflavone was provided by Okigawa in 1976. The group synthesized 5,5",7,7",4'-pentamethoxyochnaflavone (**129**) by Baker-Vankataraman rearrangement. In their method, 5,5",7,7",4'-pentamethoxyochnaflavone (**129**) was obtained from dibenzoic acid **130** as a starting material that was converted to the diester. The obtained ester underwent rearrangement in the presence of a base to yield β–diketone (**131**). This was followed by ring closure when treated with concentrated sulfuric acid to yield 5,5",7,7",4'-pentamethoxyochnaflavone (**129**) as illustrated in **Scheme 6.13** (Okigawa *et al.*, 1976).

130   **131**   **129**
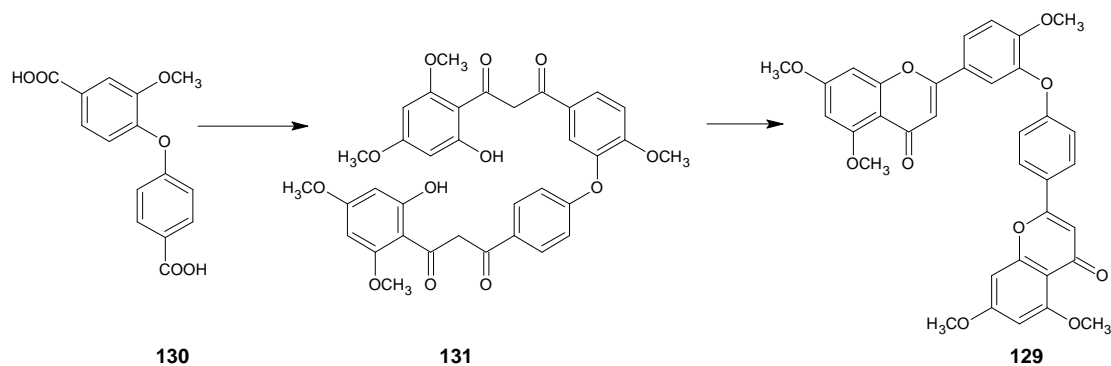
Scheme 6.13: Synthesis of 129 by Baker-Vankataraman method

6.5   Retrosynthetic Analysis of Ochnaflavone (51)

Although Okigawa and group reported the synthesis of the pentamethoxyochnaflavone (**129**), there are no filed reports on the total synthesis of ochnaflavone (**51**) and therefore in this Chapter the total synthesis of the compound will be reported. It was our aim to synthesize **51** by first generating the ether linkage between the two aromatic aldehydes to obtain diaryl ether **132**, followed by base-catalyzed aldol condensation with 2'-hydroxy-4',6'-dimethoxyacetophenone (**133**) to form a pentamethoxy dimeric chalcone **134**. The oxidative ring cyclization of **134** was expected to yield 5,5",7,7",4'-pentamethoxyochnaflavone (**129**), followed by demethylation of **129** to yield **51** as illustrated in **Scheme 6.14**.
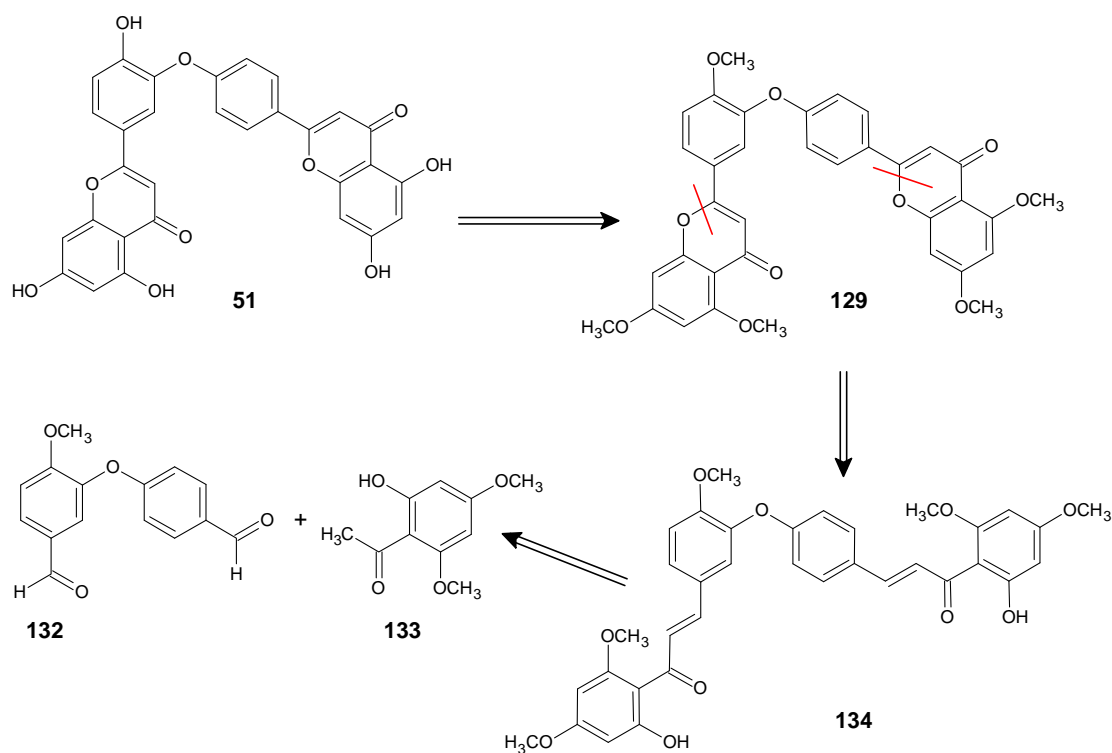
Scheme 6.14: Retrosynthesis of 51

6.6 Results and Discussion

6.6.1 Synthesis of diaryl ether (132)

The synthetic approach to diaryl ether **132** is illustrated in **Scheme 6.15**. This compound could be obtained from the Ullmann condensation and other applicable reactions but as stated earlier, these reactions require harsh conditions and result into mixtures of products. On the other hand, a base-initiated intermolecular nucleophilic aromatic substitution reaction was used. The reaction between isovanillin (**135**) and *p*-fluorobenzaldehyde (**136**) in a heated DMF gave diaryl ether **132** as a light yellow solid in 89% yield.
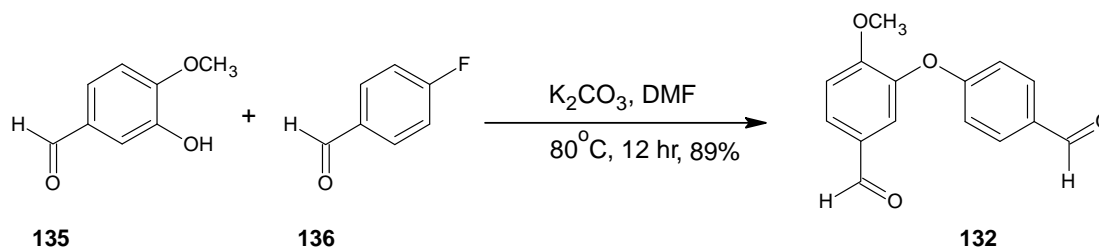
Scheme 6.15: Synthesis of 132 by intermolecular nucleophilic aromatic substitution

The ^{1}H NMR spectrum (Plate 23a) of the diaryl ether **132** showed the presence of two singlets at δ_H 9.94 and 9.93, typical for aldehydes and a *para*-substituted aromatic system could be identified by the presence of two doublets with coupling of 8.8 Hz at δ_H 7.89 and 7.06, each one integrating to two protons. Methoxy protons were observed at δ_H 3.92 and an ABX aromatic system was present (δ_H 7.89*dd*, 7.68*d* and 7.38*d*).

The ^{13}C NMR spectrum (Plate 23b) showed the presence of 13 signals, with two carbonyls at δ_C 190.5 and 190.0 and a methoxy carbon at δ_C 55.9. Three signals due to oxygen linked aromatic carbons were observed at δ_C 162.8, 156.9 and 143.4, consistent with the structure. Signals due to *p*-substituted and *o,p*-disubstituted aromatic rings were observed in their normal resonance regions.

6.6.2 Synthesis of 2'-hydroxy-4',6'-dimethoxyacetophenone (133)

Two different methods were employed in the synthesis of precursor **133** (2'-hydroxy-4',6'-dimethoxyacetophenone).

Method 1:

Commercially available 1,3,5-trihydroxybenzene (**137**) was methylated by using methyl iodide under basic condition in acetone for 6 h as indicated in **Scheme 6.16** to yield 1,3,5-trimethoxybenzene (**138**) in 89% yield as a white solid. The ^{1}H NMR spectrum (Plate 24a) of the product showed the presence of only two peaks "accounted to" the three equivalent aromatic protons and nine equivalent methoxy protons for the three methoxy groups. The signal for the aromatic protons was observed at δ_H 6.11 (3H, s) and the three methoxy groups were observed at δ_H 3.79 (9H, s). The presence of the three methoxy groups indicated that compound **138** was successfully methylated in the three respective positions.

This was followed by acylation, where **138** was acetylated by using acetyl chloride, anhydrous zinc chloride and dry dichloromethane for 6 h as indicated in **Scheme 6.16**. The white solid 2',4',6'-trimethoxyacetophenone (**139**) was obtained in 70% yield.

The ^{1}H NMR spectrum (Plate 25a) of **139** showed the presence of four peaks, being a singlet for the two equivalent aromatic protons observed at δ_H 6.05 (2H, s), the methoxy groups at δ_H 3.75 (3H, s) and 3.72 (6H, s) and a methyl group at δ_H 2.38 (3H, s). The observed methyl group indicated that the acylation reaction was successful.

Selective deprotection of the methoxy group *ortho* to the carbonyl in **139** was performed by using boron tribromide in dry DCM under nitrogen as per **Scheme 6.16**. The deprotected product, **133** obtained here showed the same NMR features as the one obtained in the second procedure described below.

Method 2:

Selective methylation of 2',4',6'-trihydroxyacetophenone (**140**) was accomplished by using methyl iodide and potassium carbonate in refluxing acetone as shown in **Scheme 6.16**. 2'-Hydroxy-4',6'-dimethoxyacetophenone (**133**) was isolated as a white solid in 50% yield. The methylation reaction occurred via the nucleophilic substitution reaction (S_N2) of the phenoxide ion and methyl iodide.

The ^{1}H NMR spectrum (Plate 26a) of **133** showed two singlets at δ_H 3.83 (3H) and δ_H 3.79 (3H) due to the two methoxy groups at C-4 and C-6, respectively. The phenolic proton signal was observed as a sharp singlet at δ_H 13.99, the appearance of this deshielded phenolic proton signal supported the presence of a hydroxy group at the C-2' position. The peaks corresponding to the aromatic ring appeared at δ_H 6.02 and δ_H 5.89 due to the two *meta*-coupled protons.

The ^{13}C NMR spectrum (Plate 26b) displayed nine different signals resulting from ten carbons, the signals for two methoxy carbons were overlapping at δ_C 55.5. The signal due to carbonyl carbon was observed at δ_C 203.1 and oxygen-linked aromatic signals were observed at δ_C 167.5, 166.1 and 162.9. Other aromatic signals were observed in their normal resonance regions.
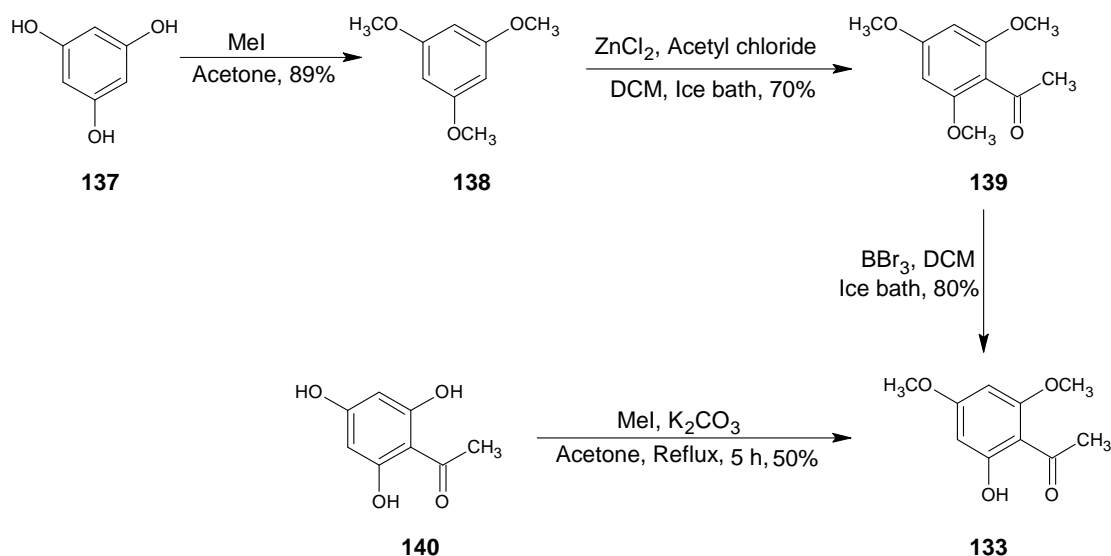
Scheme 6.16: The two synthetic routes to 133

6.6.3 Synthesis of a dimeric chalcone 134 by Claisen-Schmidt condensation

The Claisen-Schmidt condensation of 2-hydroxy-4,6-dimethoxyacetophenone (**133**) with diaryl ether **132** under basic condition (KOH) in EtOH proceeded smoothly to furnish dimeric chalcone **134** in 80% yield as indicated in **Scheme 6.17**.
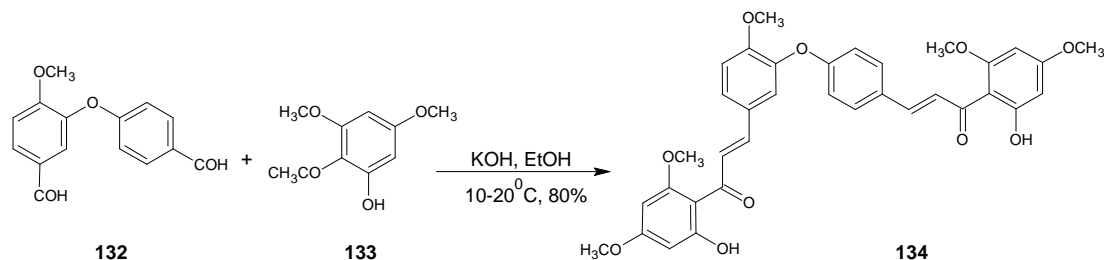
Scheme 6.17: Synthesis of 134 by Claisen-Schmidt condensation

The dimeric chalcone was obtained as a yellow powder, its structure was confirmed by the ^{1}H, ^{13}C NMR and MS. The presence of hydroxy groups at the two C-2 positions was supported by appropriately deshielded phenolic proton signals at δ_H 14.34 and 14.31 (1H, s) in its ^{1}H NMR spectrum.

The ^{1}H NMR spectrum (Plate 27a) of **134** displayed five singlets at δ_H 3.92, 3.89, 3.85, 3.83 and 3.83 each integrating for 3H due to the methoxy groups. The *m*-coupled protons of the two A-rings appeared at δ_H 5.97 (1H, d, *J* = 2.3 Hz), 6.12 (1H, d, *J* = 2.3 Hz), 6.10 (1H, d, *J* = 2.3 Hz) and δ_H 5.93 (1H, d, *J* = 2.3 Hz). The three aromatic protons of the *ABX*-ring were observed at δ_H 7.05 (1H, d, *J* = 8.6 Hz), 7.33 (1H, d, *J* = 2.0 Hz) and δ_H 7.42 (1H, dd, *J* = 8.6 and 2.0 Hz). The *para*-substituted aromatic ring was confirmed to be present due to the presence of doublet signals at δ_H 7.59 (2H, d, *J* = 8.6 Hz) and 7.00 (2H, d, *J* = 8.6 Hz). The α,β-protons of the one chalcone moiety appeared as two doublets at δ_H 7.85 and 7.78 with the characteristic trans coupling of 15.6 Hz. However the two protons of the second chalcone moiety overlap and appear as a broad singlet at δ_H 7.73.

The ^{13}C NMR spectrum (Plate 27b) of **134** showed the presence of 30 signals. The signals for the methoxy carbons were observed from δ_C 56.1 to 55.6. The

presence of a chalcone moiety was confirmed by signals at δ_C 141.7 (C-β_2), 141.4(C-β_1), 126.2 (C-α_1,α_2); low field due to the anisotropic effect of the carbonyl. The [M+H]$^+$ peak observed at *m/z* 611.1920 is consistent with the molecular formula $C_{35}H_{32}O_{10}$ (calc. 611.1917).

6.6.4 Oxidative cyclization of a dimeric chalcone 134 to biflavone 129

The last but one step in the synthesis of ochnaflavone (**51**) involves ring cyclization of the above synthesized dimeric chalcone **134** into 5,7,4',5",7"-pentamethoxyochnaflavone (**129**) as indicated previously in **Scheme 6.14**. The cyclization of **134** into the corresponding biflavone **129** was initially carried out by using a catalytic amount of iodine in DMSO at 130 - 140 $^\circ$C. However, at this specified temperature, the reaction resulted into decomposition of the starting material. Alternatively, the same reaction was repeated under microwave conditions to decrease the time in which **134** is exposed to a high temperature, however, **134** still decomposed (Menezes *et al.*, 2009). The same conditions (DMSO/I$_2$) were repeated at various temperatures. It was observed that at lower temperatures, longer time was taken for the reaction to complete and it also resulted into an un-isolatable mixture of products. The cyclization reaction was repeated, but this time using DDQ in refluxing dry dioxane (101 $^\circ$C), the starting material decomposed, but when the temperature of the reaction was lowered to 80 $^\circ$C, it was observed that the reaction resulted in a mixture of products as shown in **Table 6.1** (Imafuku *et al.*, 1987). At this point, it was established that the dimeric chalcone decomposes at temperatures exceeding 100 °C. It was also observed that while ring cyclization of most chalcones reported in literature to yield flavones, DMSO is regarded as a suitable solvent, it was not the case for ether-linked dimeric chalcone **134**.

Oxalic acid as a catalyst in absolute ethanol at 80 $^\circ$C as described by Zambare et al, did not yield the desired compound and instead a biflavanone **141** was obtained in 25% yield (Zambare *et al.*, 2009). When the reaction was left to continue for 72 h the starting materials could still be observed on TLC, this was due to the inter-convertibility of the two compounds through the equilibrium existing between the chalcone and flavanone and therefore prevents further

conversion of the dimeric chalcone to biflavanone (Andrzej and Cecylia, 1992; Bathélémy et al., 2008; González et al., 2002).

The ^{1}H NMR spectrum (Plate 28a) of **141** indicated that the two deshielded phenolic protons characterized by singlets at δ_H 12 – 14 were no longer present and this indicated that the ring closure reaction was successful. The presence of one *para*-substituted system was characterized by the protons doublet signals at δ_H 7.40 and 6.98 each integrating to two protons. The ABX aromatic spin system was easily identified due to the presence of the coupling signals at δ_H 7.26dd, 7.16d and 7.06d. The *meta*-coupled protons were observed at δ_H 6.16, 6.14, 6.11 and 6.10 each integrating for one proton. Singlets due to the five methoxy groups were observed at δ_H 3.90 (6H), 3.87 (3H) and 3.83 (6H). The presence of the two flavanone systems was confirmed by the ABX spin system at δ_H 3.02 (m) and 2.8 (m) for H-3, H-3" and 5.37 (m) for H-2, H-2".

The dimeric chalcone **134** was then subjected to a ring cyclization reaction in the presence of a catalytic amount of iodine in heated (80 °C) pyridine to yield the desired product (**129**) in 70% yield as shown in **Scheme 6.18** and **Table 6.1**. The characteristic signals of the hydrogen-bonded phenolic protons (δ_H 12 – 14) and the *trans*-alkene protons were not observed in the ^{1}H NMR spectrum (Plate 29a) indicating that ring closure reaction was successful. The formation of **129** was further supported by the presence of two singlet signals at δ_H 6.59 and 6.55, each integrating to one proton, of the typical flavone unit. The four *meta*-coupled aromatic protons were shifted down field to δ_H 6.54, 6.51, 6.36 and 6.35 in **129** from δ_H 6.12, 6.10, 5.97 and 5.93 in the dimeric chalcone **134**. The spectrum showed the presence of one ABX aromatic spin system, one *para*-substituted aromatic ring and five methoxy groups.

The ^{13}C NMR spectrum (Plate 29b) showed the presence of two carbonyls at δ_C 177.5 and 177.4. Peaks for nine oxygen bonded aromatic carbons were also observed at δ_C 164.0, 164.0, 160.9, 160.35, 160.3, 159.8, 159.7, 159.6 and 154.1. The five methoxy groups were observed at δ_C 56.4 (2xOCH$_3$), 56.1 (OCH$_3$), 55.7 (OCH$_3$) and δ_C 55.7 (OCH$_3$) consistent to the structure of **129**.

Table 6.1: Different conditions for ring cyclization reaction of 134

Entry	Starting material	Solvent/ Reagent	Reaction temp./cond.	Products	% yield
1	134	DMSO/I$_2$	130-140 $^\circ$C	a	0
2	134	DMSO/I$_2$	80-90 $^\circ$C	b	0
3	134	DMSO/I$_2$	130-140 $^\circ$C Microwave,5 min	a	0
4	134	Dry dioxane/DDQ	Reflux (101 $^\circ$C)	a	0
5	134	Dry dioxane/DDQ	80-90 $^\circ$C	b	0
6	134	EtOH/oxalic acid	80-90 $^\circ$C	**141**	25
7	134	Pyridine/I$_2$	80 $^\circ$C	**129**	70%

a- Decomposition of **134**, b- Mixture of un-isolatable products
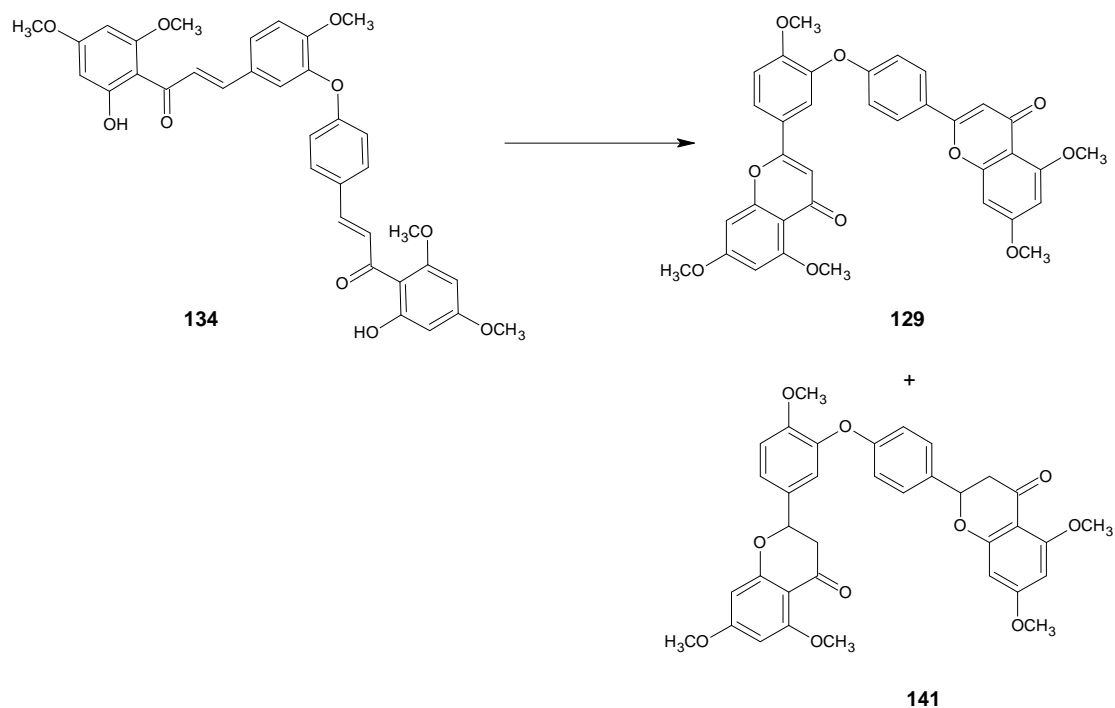
Scheme 6.18: Oxidative ring cyclization of dimeric chalcone 134

6.6.5 Deprotection of 129 by using BBr$_3$ in dry DCM

Ochnaflavone (**51**) was obtained as a yellow solid after purification of the reaction mixture obtained from the reaction of boron tribromide and **129** in dry DCM as illustrated in **Scheme 6.19**. The product obtained had the same NMR spectra as the isolated product from the leaves of *O. serrulata* (Plates 30a and b).

129   **51**
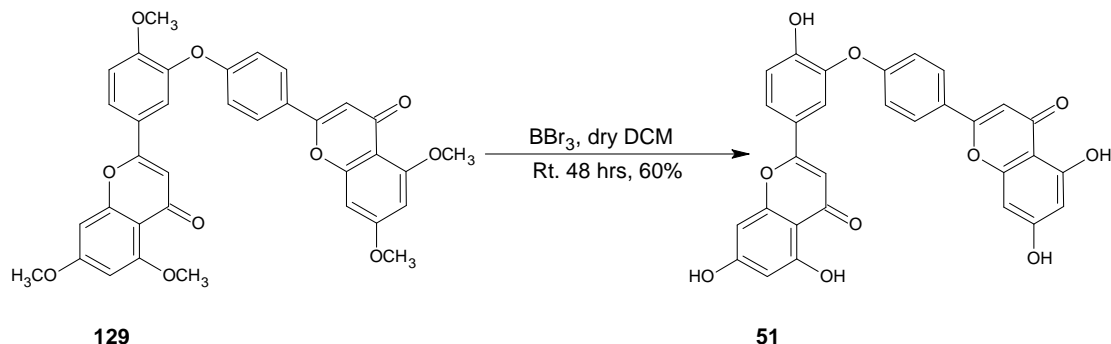
Scheme 6.19: Deprotection of 129 in BBr$_3$ in dry DCM

6.7   Conclusion

In this Chapter, the synthesis of a biflavone (ochnaflavone) with an ether linkage between apigenin and luteolin moieties is described. The synthesis of ochnaflavone (**51**) was carried out successfully from 2'-hydroxy-4',6'-dimethoxyacetophenone (**100**) and diaryl ether **132**. Base-catalyzed aldol condensation of **132** and **133** resulted in the formation of the dimeric chalcone **134**, which underwent oxidative cyclization in iodine/pyridine to give the pentamethoxy dimeric flavone **129**. Biflavone **129** was then deprotected by using boron tribromide in dry DCM to yield ochnaflavone (**51**). In the oxidative ring cyclization of the dimeric chalcone **134** several methods were employed, including iodine in DMSO, DDQ in dry dioxane and iron(III) chloride in absolute ethanol, but none of these reactions were successful. However, it was found that oxalic acid in absolute ethanol produced biflavanone **141** as the sole product, whereas reaction with iodine in pyridine gave the desired product **129** as a major product. This Chapter therefore successfully describes the synthesis of a rare type of ether-

linked natural biflavone (ochnaflavone) with anti-cancer, antitumor, anti-inflammatory and anti HIV activities.

6.8   Experimental

6.8.1   General materials and methods

Anhydrous solvents used for the reactions reported in this Chapter was collected from the PURE SOLV^{TM} – MD (Multiple Dispensing System) system manufactured by Innovative Technology Inc. Reagents were bought from Sigma Aldrich and other materials and methods are as described in the experimental section, Chapter 3 of this thesis.

6.8.2   Synthesis of diaryl ether (132)
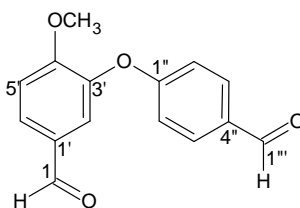
132

To a solution of isovanillin (**135**) (500 mg, 3.29 mmol) and anhydrous potassium carbonate (681 mg, 4.93 mmol) in 15 ml of dry DMF under nitrogen, 448 mg (3.61 mmol) of *p*-fluorobenzaldehyde (**136**) were added. The mixture was heated at 80 °C with stirring until the starting material was co nsumed (monitored by TLC). After all the starting material was consumed, the reaction was left to cool to room temperature, 20 ml of cold water was added, followed by extraction with 3 X 20 mL CHCl$_3$. The obtained organic layer was dried over anhydrous magnesium sulfate and the solvent was removed in *vacuo*. The residue obtained was purified by silica gel column chromatography with EtOAc:hexanes (3:7) as eluent to yield 748 mg (89%) of **132** as a light yellow solid.

^{1}H NMR (400 MHz, CDCl$_3$): δ_H 9.94 and 9.93 (2H, s, 2HCO), 7.89 (2H, d, $J_{2'',3''}$ = 8.8 Hz, H-3'',5''), 7.89 (1H, dd, $J_{5',6'}$ = 8.5 Hz, $J_{2',6'}$ 2.0 Hz, H-6'), 7.69 (1H, d, $J_{2',6'}$ = 2.0 Hz, H-2'), 7.38 (1H, d, $J_{5',6'}$ = 8.5 Hz, H-5'), 7.06 (2H, d, $J_{3'',2''}$ = 8.8 Hz, H-2'',6''), 3.91 (3H, s, OCH$_3$) (Plate 23a).

^{13}C NMR (100 MHz, CDCl$_3$): δ_C 190.6 (C-1), 190.0 (C-1'''), 162.6 (C-1''), 156.9 (C-4'), 143.4 (C-3'), 131.9 (C-3'',5'',4''), 130.9 (C-1'), 129.5 (C-6'), 122.3 (C-2'), 116.3 (C-2'',6''), 113.4 (C-5'), 55.9 (OCH$_3$) (Plate 23b).

6.8.3 Synthesis of trimethoxybenzene (138)
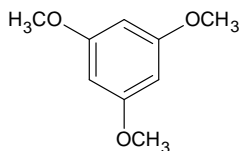
138

To a stirred mixture of 1.9 g (13.88 mmol) of potassium carbonate and 500 mg (3.97 mmol) of phloroglucinol (**137**) in 25 mL acetone, 1.97 g (13.88 mmol) of methyl iodide was added. The reaction was left to stir for 6 h while monitoring by TLC. The reaction was left to cool to room temperature after completion of the reaction and then 30 mL of cold distilled water was added followed by extraction with 3 X 30 mL CHCl$_3$. The chloroform extract was washed once with dilute hydrochloric acid, dried over anhydrous magnesium sulfate and the solvent removed in *vacuo*. The resulting crude mixture was purified by silica gel column chromatography with EtOAc:hexanes (1:9) to afford 558 mg (89%) of **138** as a white solid.

^{1}H NMR (400 MHz, CDCl$_3$), Plate : δ_H 6.11 (3H, s, H-2, 4, 6), 3.79 (9H, s, 3xOCH$_3$) (Plate 24a).

6.8.4 Synthesis of 2',4',6'-trimethoxyacetophenone (139)
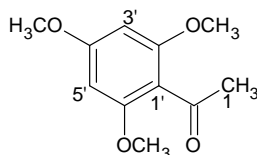
139

In a round-bottomed flask containing 442 mg (3.24 mmol) of anhydrous zinc chloride, 15 mL of DCM were added. The system was then flushed with nitrogen, followed by cooling the solution in an ice bath. A solution of acetyl chloride (305 mg (3.89 mmol)) in 5 mL DCM was added drop wise over 10 minutes. This was followed by addition of **138** (512 mg, 3.24 mmol)) dissolved in 5 mL DCM to the reaction mixture, the addition rate was adjusted so the solution did not boil excessively. After addition was complete, the ice bath was removed and the reaction was allowed to reach room temperature. After stirring for an additional 15 minutes at room temperature, the reaction mixture was poured carefully and slowly, with stirring, into a beaker containing ice and 6M HCl. At this stage **139** precipitated out as a white solid which was collected by filtration to give 476 mg (70%) of **139**.

^{1}H NMR (400 MHz, CDCl$_3$): δ_H 6.05 (2H, s, H-3', 5'), 3.75 (3H, s, OCH$_3$), 3.72 (6H, s, 2xOCH$_3$), 2.38 (3H, s, CH$_3$) (Plate 25a).

6.8.5 Synthesis of 2'-Hydroxy-4',6'-dimethoxyacetophenone (133)
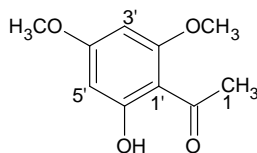
133

Two different procedures were used to synthesize **133**, the first being selective demethylation of 2',4',6'-trimethoxyacetophenone (**139**) and the second is selective methylation of 2',4',6'-trihydroxyacetophenone (**140**).

Method 1:

A solution of 805 mg (3.214 mmol) boron tribromide in 5 mL dry DCM was slowly added with a syringe to a stirred solution of **139** (450 mg, 2.14 mmol) in 10 mL dry DCM under nitrogen. After complete addition of BBr$_3$, the reaction mixture was left at room temperature for 12 hrs and then poured into 50 mL ice water. The mixture was then shaken with ice water to hydrolyse the excess BBr$_3$ and boron complexes. The product was extracted with 3 X 20 mL DCM. The obtained organic layers were dried over anhydrous magnesium sulfate and the solvent was removed in *vacuo* to obtain a dry crude reaction mixture. Purification by a silica gel column chromatography with EtOAc:hexanes (1:9), yielded 336 mg (80%) of 2'-hydroxy-4',6'-dimethoxyacetophenone (**133**) as a white solid.

Method 2:

To a well-stirred mixture of 2,4,6-trihydroxyacetophenone (**140**) (500 mg, 2.97 mmol) and methyl iodide (1.06 g, 7.43 mmol) in 20 mL acetone, 1.02 g (7.43 mmol) of anhydrous potassium carbonate was added. The reaction mixture was refluxed for 5 hours while monitoring by TLC until all the starting material was consumed. The reaction mixture was left to cool and 40 mL of cold acidified distilled water was added, followed by extraction with 3 X 20 mL CHCl$_3$. The obtained organic layer was dried over anhydrous magnesium sulfate and solvent removed in *vacuo*. The residue was purified by silica gel column chromatography with EtOAc:hexanes (1:9) to afford 291 mg (50%) of 2'-hydroxy-4',6'-dimethoxyacetophenone (**133**) as a white solid.

^{1}H NMR (400 MHz, CDCl$_3$): δ_H 13.99 (1H, s, OH), 6.02 (1H, d, $J_{3',5'}$ = 2.1 Hz, H-3'), 5.89 (1H, d, $J_{3',5'}$ = 2.1 Hz, H-5'), 3.83 (3H, s, OCH$_3$), 3.79 (3H, s, OCH$_3$), 2.58 (3H, s, CH$_3$) (Plate 26a).

^{13}C NMR (100 MHz, CDCl$_3$): δ_C 203.1 (C-2), 167.5 (C-2'), 166.1 (C-6'), 162.9 (C-4'), 105.9 (C-1'), 93.5 (C-3'), 90.6 (C-5'), 55.5 (2xOCH$_3$), 32.9 (C-1) (Plate 26b).

6.8.6 Synthesis of dimeric chalcone 134

134
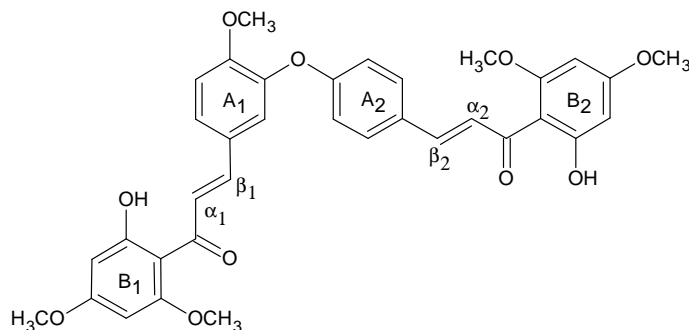
To a solution of 404 mg (1.55 mmol) of diaryl ether **132** in EtOH (50 mL), 620 mg (3.16 mmol) of 2'-hydroxy-4',6'-dimethoxyacetophenone (**133**) was added. The solution was cooled in an ice bath followed by addition of powdered 353 mg (6.31 mmol) of KOH. After overnight stirring, the reaction mixture was diluted with ice cold water (50 mL) and acidified with 6 M HCl. The formed **134** precipitated out as yellow solids and was filtered off under reduced pressure, washed with water. Dry yellow solids of **134** (772 mg, 80%) were obtained.

^{1}H NMR (400 MHz, CDCl$_3$): δ_H 14.34 (OH), 14.31 (OH), 7.85 (1H, d, $J_{\alpha1,\beta1}$ = 15.7 Hz, H-β_2), 7.79 (1H, d, $J_{\alpha2,\beta2}$ = 15.7 Hz, H-α_2), 7.73 (2H, s, H-α_1, β_1), 7.59 (2H, d, $J_{2,3}$ = 8.5 Hz, H-2, 6A$_2$), 7.42 (1H, dd, $J_{5,6}$ = 8.2 Hz, $J_{2,6}$ = 2.0 Hz, H-6A$_1$), 7.33 (1H, d, $J_{2,6}$ = 2.0 Hz, H-2A$_1$), 7.05 (1H, d, $J_{5,6}$ = 8.2, 2.0 Hz, H-5A$_1$), 7.0 (2H, d, $J_{2,3}$ = 8.5 Hz, H-3, 5A$_2$), 6.12 (1H, d, $J_{3,5}$ = 2.2 Hz, H-5B$_1$), 6.10 (1H, $J_{3,5}$ = 2.2 Hz, H-5B$_2$), 5.97 (1H, d, $J_{3,5}$ = 2.2 Hz, H-3B$_1$), 5.94 (1H, d, $J_{3,5}$ = 2.2 Hz, H-3B$_2$), 3.9 (3H, s, OCH$_3$), 3.89 (3H, s, OCH$_3$), 3.85 (3H, s, OCH$_3$), 3.83 (6H, s, 2xOCH$_3$) (Plate 27a).

^{13}C NMR (100 MHz, CDCl$_3$): δ_C 192.5 (C=O), 192.3 (C=O), 168.4 (C-6B$_1$,B$_2$), 166.2 (C-2B$_1$,B$_2$), 162.5 (C-4B$_1$,B$_2$), 159.4 (C-4A$_2$), 153.1 (C-4A$_1$), 144.6 (C-3A$_1$), 141.8 (C-β_2), 141.4 (C-β_1), 130.3 (C-2,6A$_2$), 130.0 (C-1A$_2$), 129.2 (C-1A$_1$), 126.9 (C-6A$_1$), 126.2 (C-α_1,α_2), 120.2 (C-2A$_1$), 117.4 (C-3,5A$_2$), 112.9 (C-5A$_1$), 106.4 (C-1B$_1$), 106.3 (C-1B$_2$), 93.9 (C-3B$_1$,B$_2$), 91.3 (C-5B$_1$), 91.2 (C-5B$_2$), 56.1 (OCH$_3$), 55.8 (2xOCH$_3$), 55.6 (2xOCH$_3$) (Plate 27b).

6.8.7 Synthesis of Biflavanone 141

141
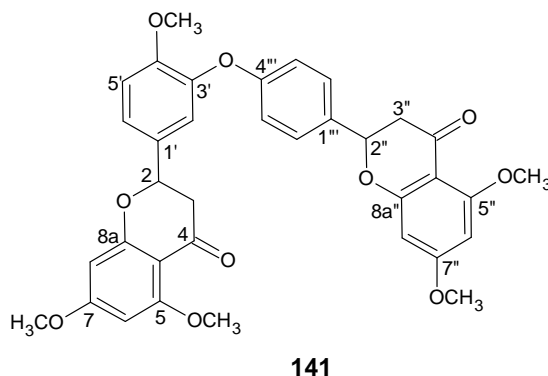
To a mixture of oxalic acid (10 mol%) and **134** (400 mg, 0.65 mmol), 20 mL of absolute ethanol was added. The stirred reaction mixture was heated at 80 °C for 72 h while monitoring the reaction by TLC. The reaction mixture was then cooled to room temperature and the solvent evaporated under reduced pressure. This was followed by extraction using 50 mL of water and 3 X 30 mL DCM and the organic layer collected was dried over anhydrous magnesium sulfate. DCM was removed in *vacuo* and the resulting residue was purified by silica gel column chromatography with EtOAc:hexanes (1:1) to afford 100 mg (25%) of the biflavanone **141** as a light yellow solid.

^{1}H NMR (400 MHz, CDCl$_3$): δ_H 7.40 (2H, d, $J_{2''',3'''}$ = 8.5 Hz, H-2''', 6'''), 7.26 (1H, dd, $J_{5',6'}$ = 8.5 Hz, $J_{2',6'}$ = 2.0 Hz, H-6'), 7.16 (1H, d, $J_{2',6'}$ = 2.0 Hz, H-2'), 7.06 (1H, d, $J_{5',6'}$ = 8.5 Hz, H-5'), 6.99 (2H, d, $J_{2''',3'''}$ = 8.5 Hz, H-3''', 5'''), 6.16 (1H, d, $J_{6,8}$ = 2.2 Hz, H-6), 6.14 (1H, d, $J_{6'',8''}$ = 2.0 Hz, H-6''), 6.10 (2H, d, $J_{6,8}$ = 2.0 Hz, H-8, 8''), 5.37 (2H, m, H-2, 2''), 3.9 (6H, s, 2OMe), 3.87 (3H, s, OMe), 3.83 (6H, s, 2OMe), 3.02 (2H, m, H-2, 2''), 2.8 (2H, m, H-3, 3'') (Plate 28a).

6.8.8 Synthesis of pentamethoxybiflavone 129
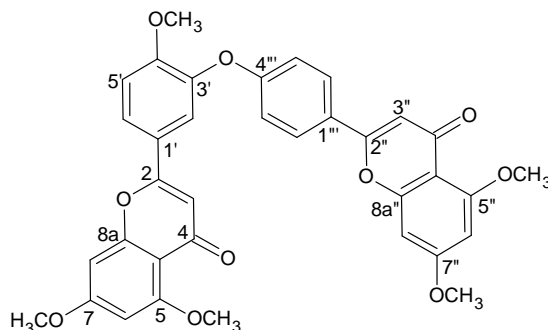
129

To a stirred solution of dimeric chalcone **134** (600 mg, 0.98 mmol) in 15 mL pyridine, 498 mg (1.96 mmol) of iodine was added and the mixture was heated to 80 °C while stirring for 24 h. The reaction was left to cool to room temperature and a cold solution of sodium thiosulfate was added to the reaction mixture until all excess iodine was consumed. This was followed by extraction with 3 X 30 mL DCM, the obtained organic layer was washed with 30 mL of water and dried over anhydrous magnesium sulfate. The solvent was removed in *vacuo* and the crude reaction mixture was purified by silica gel column chromatography with EtOAc:hexanes (8:2) to obtain **129** (417 mg, 70%) as yellow solid subtance.

^{1}H NMR (400 MHz, CDCl$_3$): δ_H 7.82 (2H, d, $J_{2''',3'''}$ = 8.8 Hz, H-2''', 6'''), 7.73 (1H, dd, $J_{5',6'}$ = 8.7 Hz, $J_{2',6'}$ = 2.3 Hz, H-6'), 7.61 (1H, d, $J_{2',6'}$ = 2.3 Hz, H-2'), 7.12 (1H, d, $J_{5',6'}$ = 8.7 Hz, H-5'), 7.02 (2H, d, $J_{2''',3'''}$ = 8.8 Hz, H-3''', 5'''), 6.54 (1H, d, $J_{6,8}$ = 2.2 Hz, H-6), 6.51 (1H, d, $J_{6'',8''}$ = 2.4 Hz, H-6''), 6.36 (1H, d, $J_{6,8}$ = 2.2 Hz, H-8), 6.35 (1H, d, $J_{6'',8''}$ = 2.4 Hz, H-6''), 6.59 (1H, s, H-3), 6.55 (1H, s, H-3''), 3.94 (3H, s, OMe), 3.93 (3H, s, OMe), 3.89 (3H, s, OMe), 3.88 (3H, s, OMe), 3.87 (3H, s, OMe) (Plate 29a).

^{13}C NMR (100 MHz, CDCl$_3$): δ_C 177.5 (C=O), 177.4 (C=O), 164.0 (C-7''), 164.0 (C-7), 160.9 (C-5,5''), 160.3 (C-2), 160.3 (C-2''), 159.8 (C-4'''), 159.7 (C-8a), 159.6 (C-8a''), 154.0 (C-4'), 143.8 (C-3'), 127.7 (C-2''',6'''), 125.8 (C-1'''), 124.7 (C-6'), 124.0 (C-1'), 119.8 (C-2'), 116.6 (C-3''', 5'''), 113.0 (C-5'), 109.2 (C-4a''), 109.1 (C-4a),

108.2 (C-3, 3"), 96.2 (C-6), 96.1 (C-6"), 92.8 (C-8", 8), 56.4 (2xOCH$_3$), 56.1 (OCH$_3$), 55. 7 (2xOCH$_3$) (Plate 29b).

6.8.9 Synthesis of ochnaflavone (51)
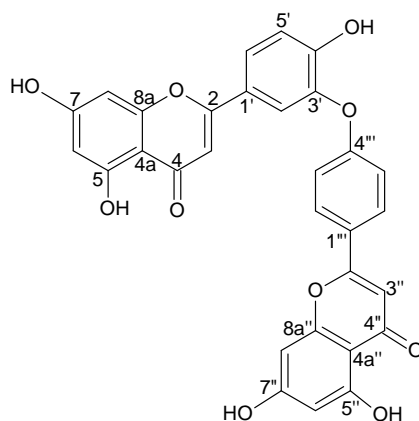
51

A solution of boron tribromide (206 mg, 0.82 mmol) in 5 mL dry DCM was slowly added with a syringe to a stirred solution of 40 mg (0.07 mmol) **129** in 10 mL DCM under nitrogen. After complete addition of BBr$_3$, the reaction mixture was left to stir at room temperature for 72 h and then poured into 30 mL ice water. The mixture was then shaken to hydrolyse the excess BBr$_3$ and boron complexes; a phenolic product was obtained by extraction with 3 X 20 mL EtOAc. The obtained organic layer was dried over anhydrous magnesium sulfate and the solvent was removed in *vacuo* to obtain a dry crude reaction mixture. Purification of the reaction mixture by a silica gel column chromatography with MeOH:CHCl$_3$ (1:9) afforded 21.2 mg (60%) of **51** as yellow solids.

^{1}H NMR (400 MHz, (CD$_3$)$_2$CO): δ_H 12.95 (5-OH), 12.94 (5"-OH), 8.08 (2H, d, $J_{2''',3'''}$ = 8.9 Hz, H-2''', 6'''), 7.903 (1H, dd, $J_{(5',6')}$ = 8.5 Hz, $J_{2',6'}$ = 2.1 Hz, H-6'), 7.89 (1H, d, $J_{2',6'}$ = 2.1 Hz, H-2'), 7.29 (1H, d, $J_{5',6'}$ = 8.5 Hz, H-5'), 7.16 (2H, d, $J_{2''',3'''}$ = 8.9 Hz, H-3''', 5'''), 6.73 (2H, s, H-3, 3"), 6.56 (1H, d, $J_{6,8}$ = 2.0 Hz, H-6), 6.55 (1H, d, $J_{6'',8''}$ = 2.4 Hz, H-6"), 6.28 (1H, d, $J_{6,8}$ = 2.0 Hz, H-8), 6.27 (1H, d, $J_{6'',8''}$ = 2.0 Hz, H-8") (Plate 30a).

^{13}C NMR (100 MHz, (CD$_3$)$_2$CO): δ_C 182.2 (C-4), 182.2 (C-4"),164.2 (C-7"), 164.18 (C-7), 163.5 (C-2), 163.1 (C-2"), 162.5 (C-5, 5"), 161.1 (C-4"'), 157.9 (C-8a, 8a"), 154.1 (C-4'), 142.4 (C-3'), 128.3 (C-2"',6"'), 125.4 (C-1"'), 123.6 (C-1'), 120.7 (C-2'), 118.1 (C-5'), 116.6 (C-3"', 5"'), 104.3 (C-4a), 104.52 (C-4a"), 104.5 (C-3"), 104.0 (C-3), 98.9 (C-6, 6"), 93.9 (C-8", 8) (Plate 30b).

6.9   References

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CHAPTER 7

Conclusion and recommendations

This thesis reports the phytochemical investigations of stem, leaves and roots of *O. serrulata* belonging to the family Ochnaceae. The Zulu people use the root decoction of this plant commonly known as fynblaarrooihout (Afr.), umbomvane (Zulu) or iliTye (Xhosa) for the treatment of bone diseases and gangrenous proctitis (Hutchings et al., 1996; Hutchings and Van Staden, 1994). The study is comprised of the isolation and identification of the compounds from the EtOAc and MeOH extracts of the plant, followed by subsequent screening of the isolated compounds for their cytotoxic and antimalarial activities and the synthesis of the most cytotoxic compound.

Four new compounds [two dimeric chalcones (5-deoxyurundeuvine C and serrulone A and two biflavonoids (4,4',7-tri-*O*-methylisocampylospermone A and 4'''-de-*O*-methylafzelone A)] were isolated along with 18 known compounds namely, one rearranged biflavonoid (lophirone A), five biflavonoid derivatives (afzelone B, ochnaflavone, 2",3"-dihydroochnaflavone, isocampylospermone A and campylospermone A), two dimeric chalcones (lophirone C and lophirone L), two *C*-glucosylated flavones (psilosin and 3'-*O*-methylpsilosin), a cyanoglucoside, (2',4'-dihydroxyphenyl)acetic acid, methyl (2',4'-dihydroxyphenyl)acetate, epicatechin, three isoflavonoid derivatives (irisolone 4'-methyl ether, iriskumaonin 3'-methyl ether, 3',4'-dimethoxy-6,7-dioxymethyleneisoflavone), a furofuran lignan (syringaresinol) and a diterpene (16α,17-dihydroxy-*ent*-kauran-19-oic acid) from the pulverized extracts of the stem, leaves and roots of *O. serrulata*. The structural elucidation of these compounds was achieved by a combination of spectroscopic methods (^{1}H, ^{1}H,^{1}H COSY, HSQC, HMBC, DEPT-135, 90, NOESY, ^{13}C NMR, mass spectrometry and infrared spectroscopy).

The isolation of psilosin, 3'-O-methylpsilosin, syringaresinol and 16α,17-dihydroxy-ent-kauran-19-oic acid from *Ochna* species is reported for the first time in this thesis.

Four compounds exhibited promising cytotoxic activities; the most potent compound was lophirone C with a total growth inhibition (TGI) against renal (TK10), melanoma (UACC62) and breast (MCF7) cancer cells at 35.63 µM, 11.67 µM and 30.35 µM, respectively. Lophirone A exhibited TGI against the same cancer cells at 58.96 µM, 26.23 µM and 40.01 µM, respectively. Ochnaflavone and 3'-O-methylpsilosin inhibited the growth of melanoma cancer cells (UACC62) only, where both compounds showed TGI against melanoma (UACC62) cancer cells at 12.91 µM and 14.11 µM, respectively.

A new biflavonoid (4,4',7-tri-O-methylisocampylospermone A) showed moderate activity against chloroquine-resistant strains of *Plasmodium falciparum* (FCR-3) with IC_{50} of 11.46 µM, followed by ochnaflavone (17.25 µM). Weakly active compounds were serrulone A (26.52 µM), lophirone A (29.78 µM), 5-deoxyurundeuvine C (31.07 µM), lophirone C (35.31 µM), 4'''-de-O-methylafzelone A (38.43 µM), afzelone B (39.54 µM), irisolone 4'-methyl ether (40.72 µM) and syringaresinol (42.66 µM)

This is the first report on the isolation and antimalarial activity of 4,4',7-tri-O-methylisocampylospermone A, structural modification of this compound is recommended for further studies to enhance its activity. Ochnaflavone and lophirone C can further be developed as new anticancer drug leads and structural modifications may improve their activity.

Ochnaflavone, an ether-linked biflavone consisting of apigenin and luteolin moieties, was synthesized in this study. Oxidative ring cyclization of a 2,2"-dihydroxy dimeric chalcone in iodine and heated pyridine was utilized to obtain the biflavone. The use of the described method for synthesis of the ether-linked dimeric flavone reported here can be extended to the synthesis of other compounds with a diaryl ether functionality. This functionality has been found to be

an important structural feature that plays a significant role in enhancing biological activity of a given molecule.

The study supports the notion that biologically-active compounds and potential pharmaceuticals can be isolated from African medicinal plants. As was found for other Ochnaceae species, the majority of the compounds isolated from the plant were biflavonoids. Many of these compounds have been isolated only from this family and they may be used as chemotaxonomical markers for the family.

To conclude, the main objectives of this study (to isolate, characterize, evaluate the biological activities of the compounds present in *O. serrulata* and synthesize the most potent compound) have been achieved.

APPENDIX

NMR spectra of the isolated and synthesized compounds

Plate 1a: ^{1}H NMR Spectrum of methyl (2',4'-dihydroxyphenyl)acetate (73) in CD$_3$OD
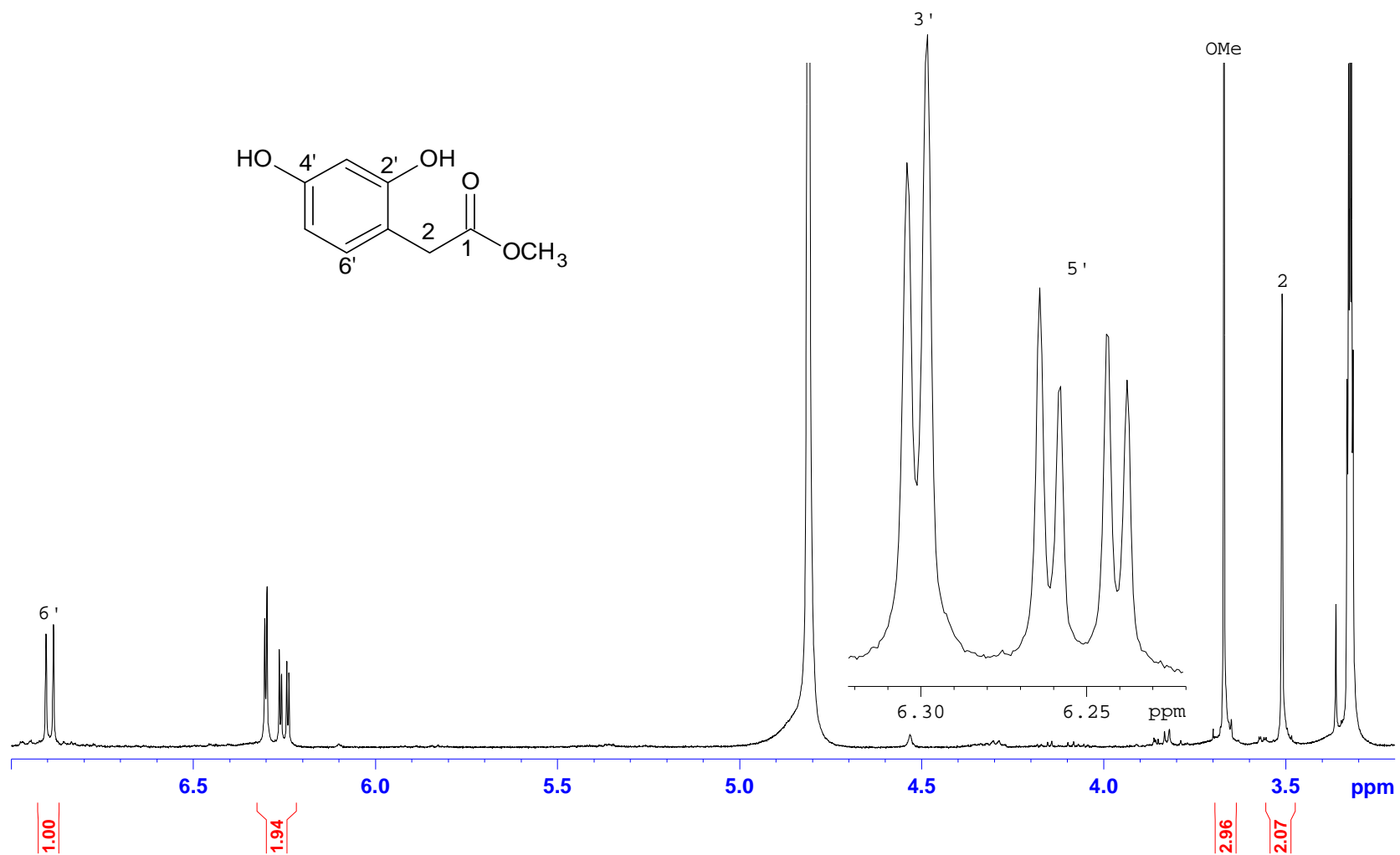
Plate 1b: COSY NMR Spectrum of methyl (2',4'-dihydroxyphenyl)acetate (73) in CD₃OD
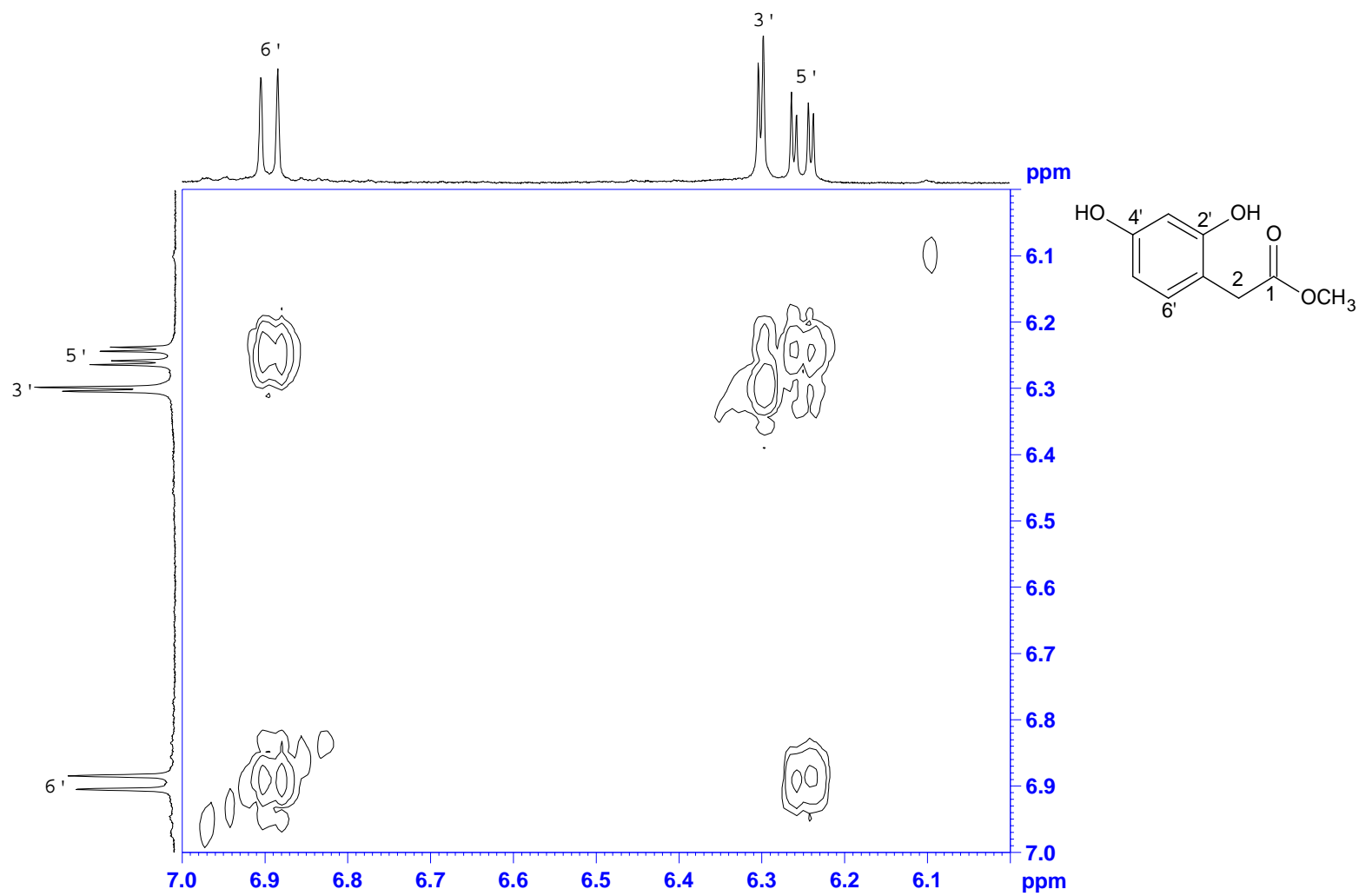
Plate 1c: ^{13}C NMR Spectrum of methyl (2',4'-dihydroxyphenyl)acetate (73) in CD$_3$OD

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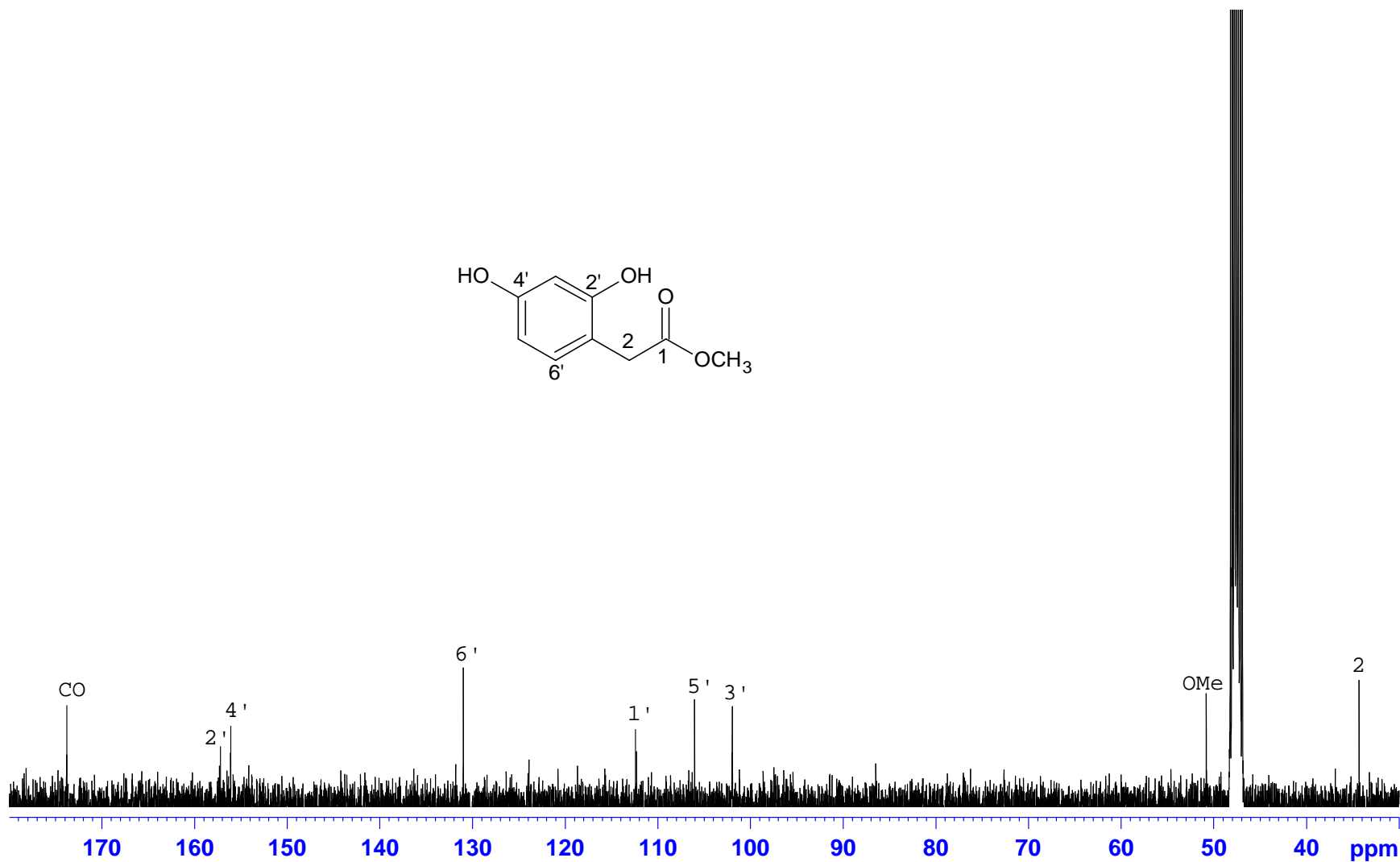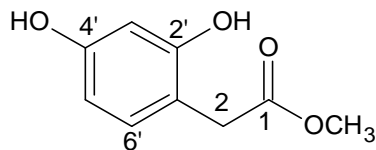
Plate 1d: HMBC NMR Spectrum of methyl (2',4'-dihydroxyphenyl)acetate (73) in CD₃OD
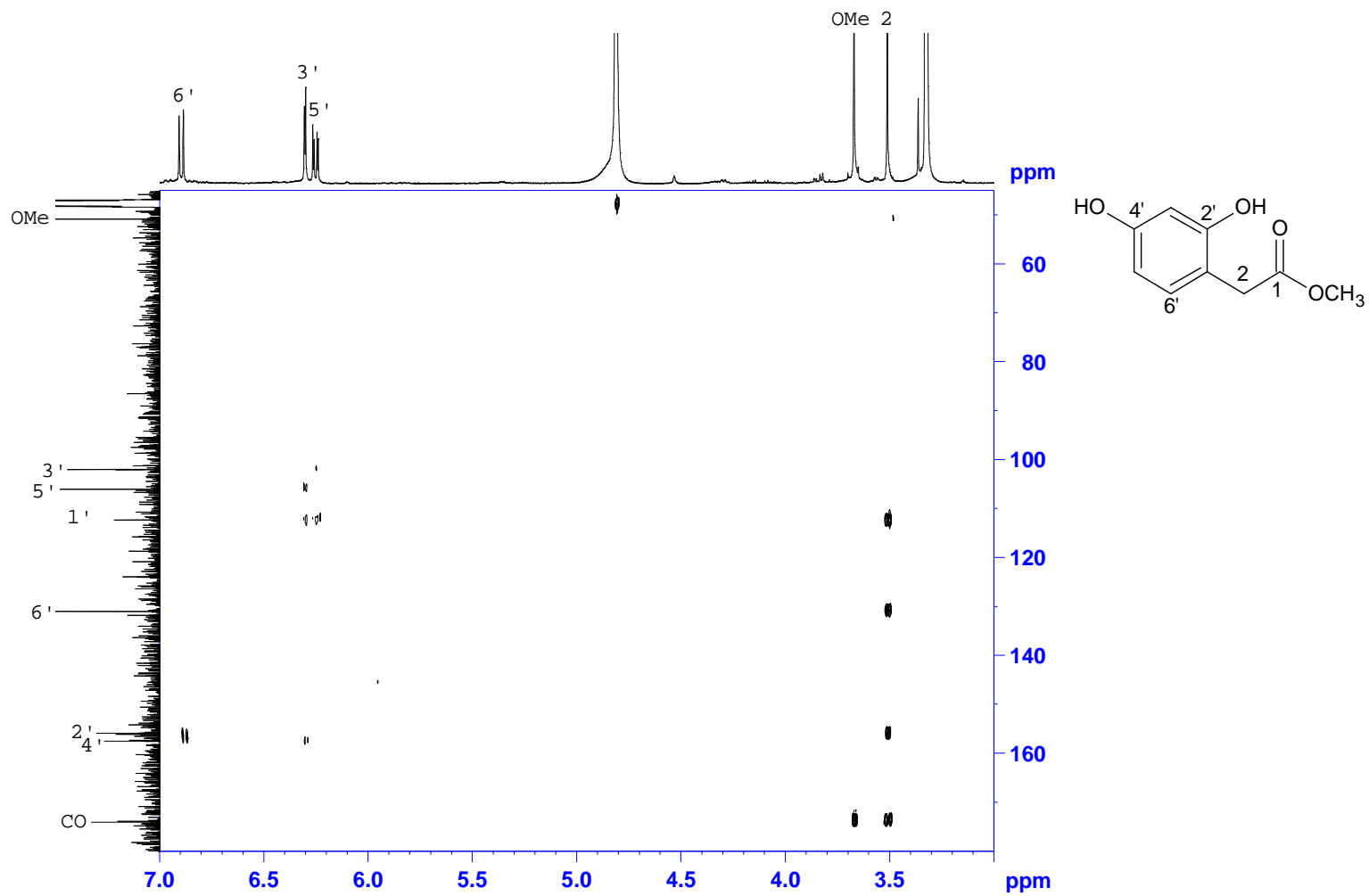
Plate 2a: ^{1}H NMR Spectrum of (2',4'-dihydroxyphenyl)acetic acid (74) in CD$_3$OD
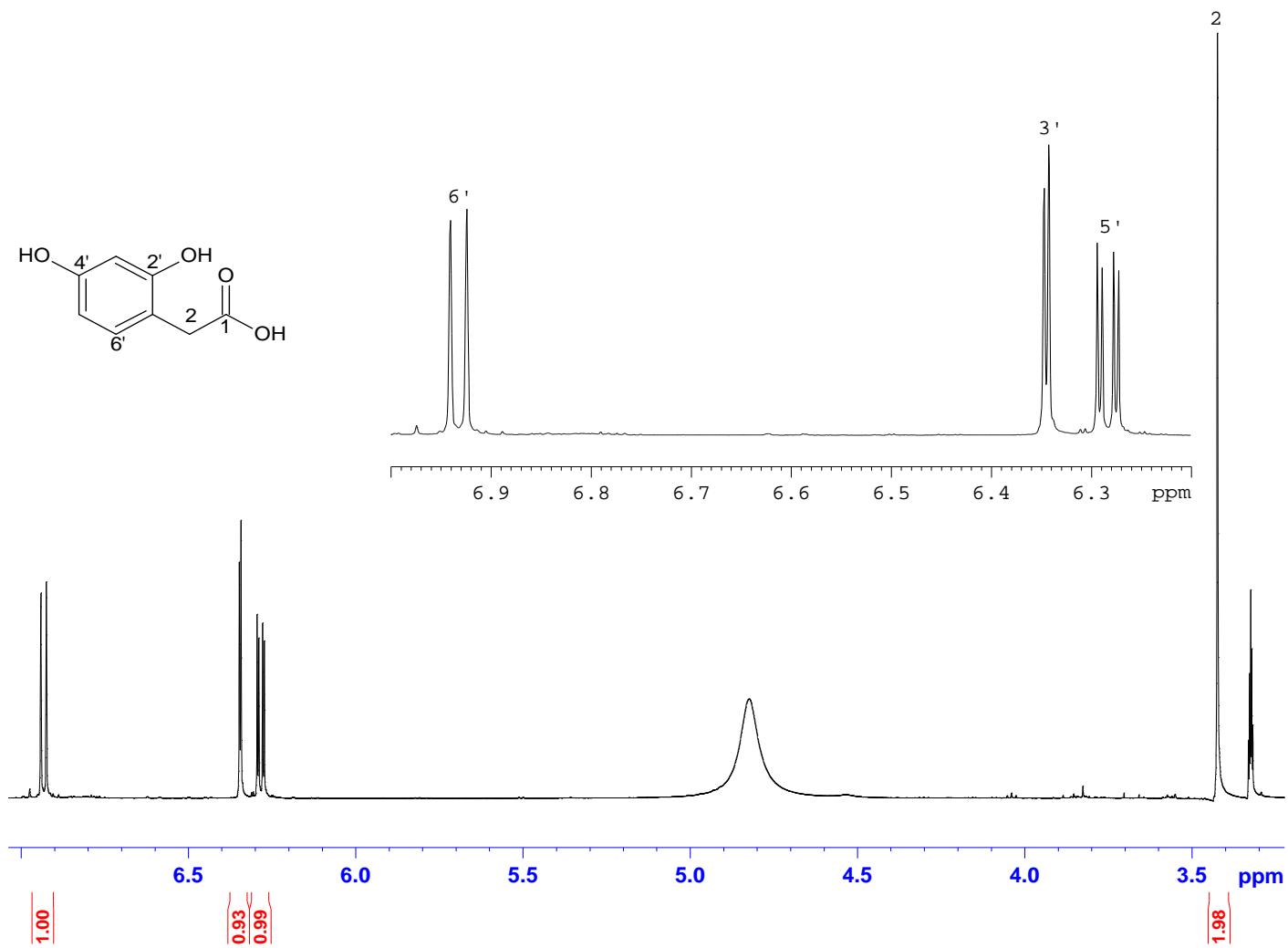
Plate 2b: COSY NMR Spectrum of (2',4'-dihydroxyphenyl)acetic acid (74) in CD$_3$OD
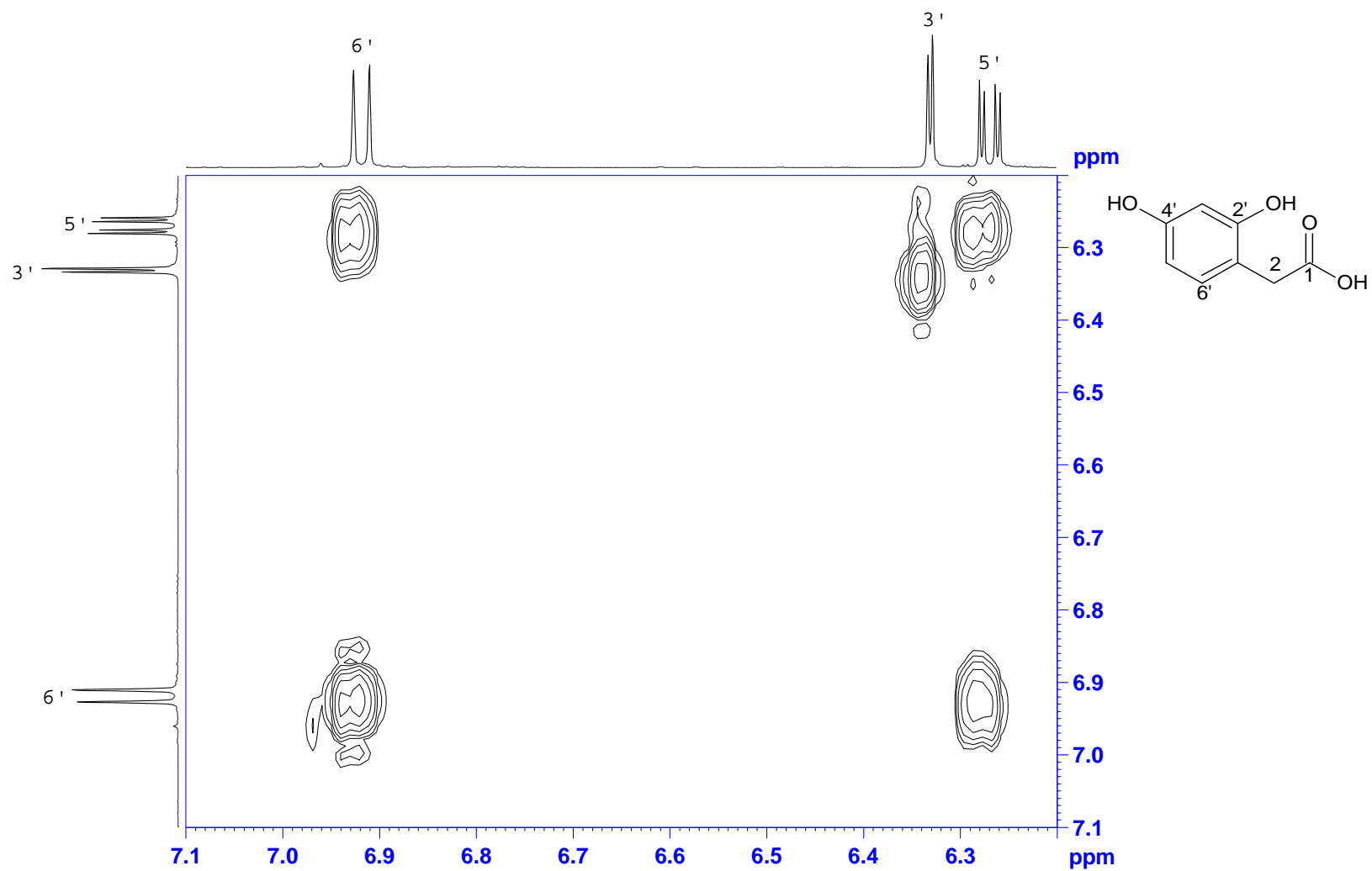
Plate 2c: ^{13}C NMR Spectrum of (2',4'-dihydroxyphenyl)acetic acid (74) in CD$_3$OD
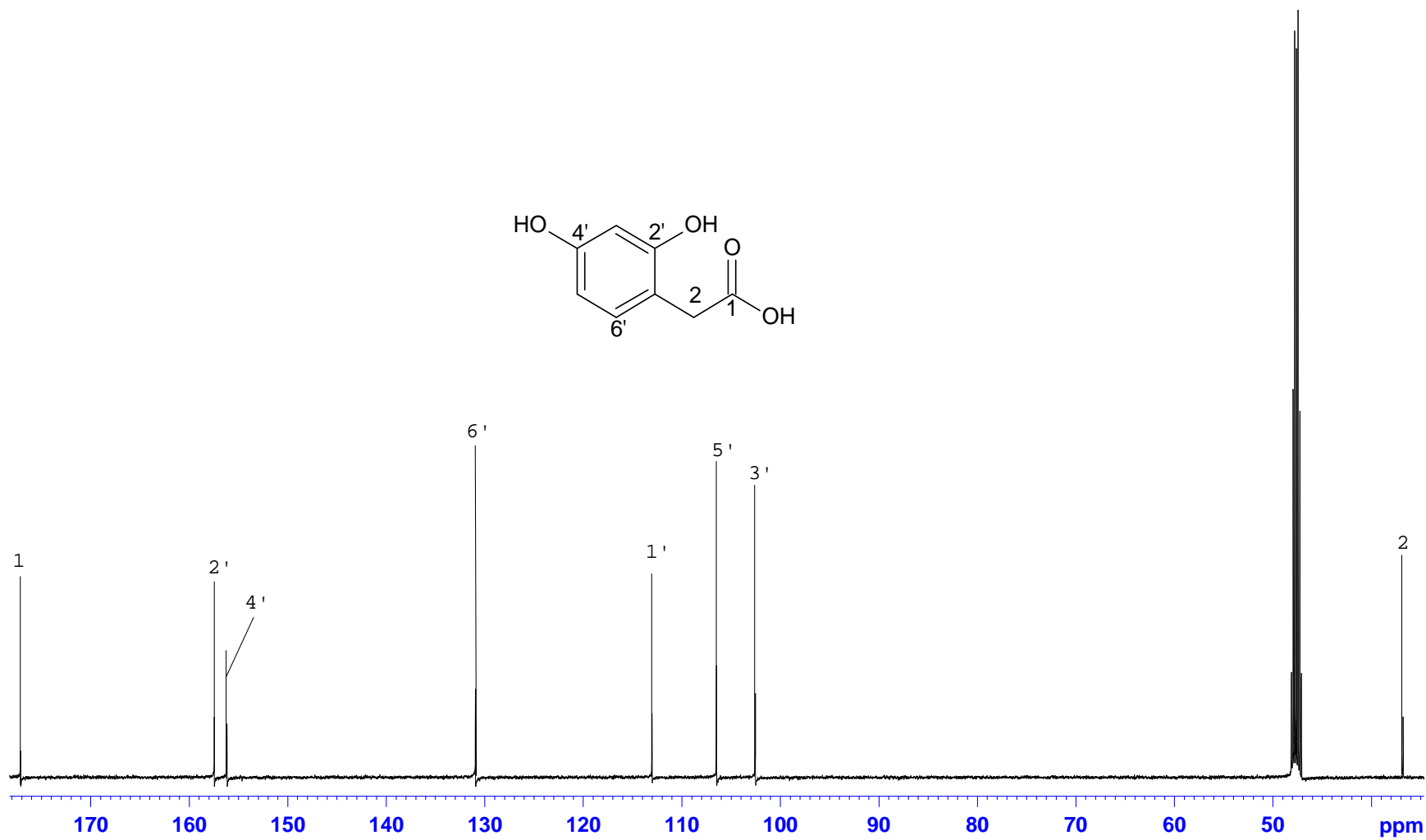
Plate 2d: HSQC NMR Spectrum of (2',4'-dihydroxyphenyl)acetic acid (74) in CD₃OD
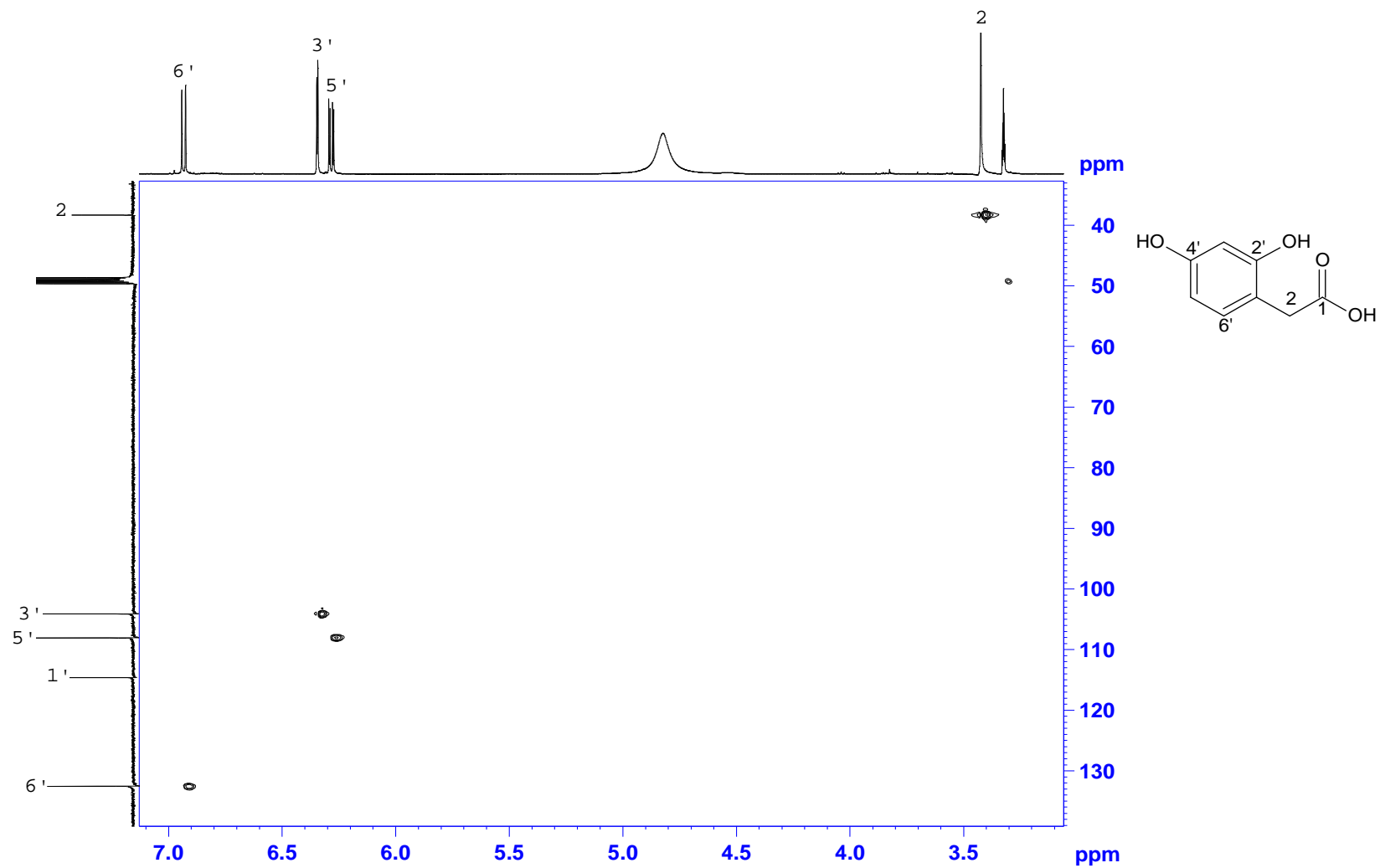
Plate 2e: HMBC NMR Spectrum of (2',4'-dihydroxyphenyl)acetic acid (74) in CD₃OD
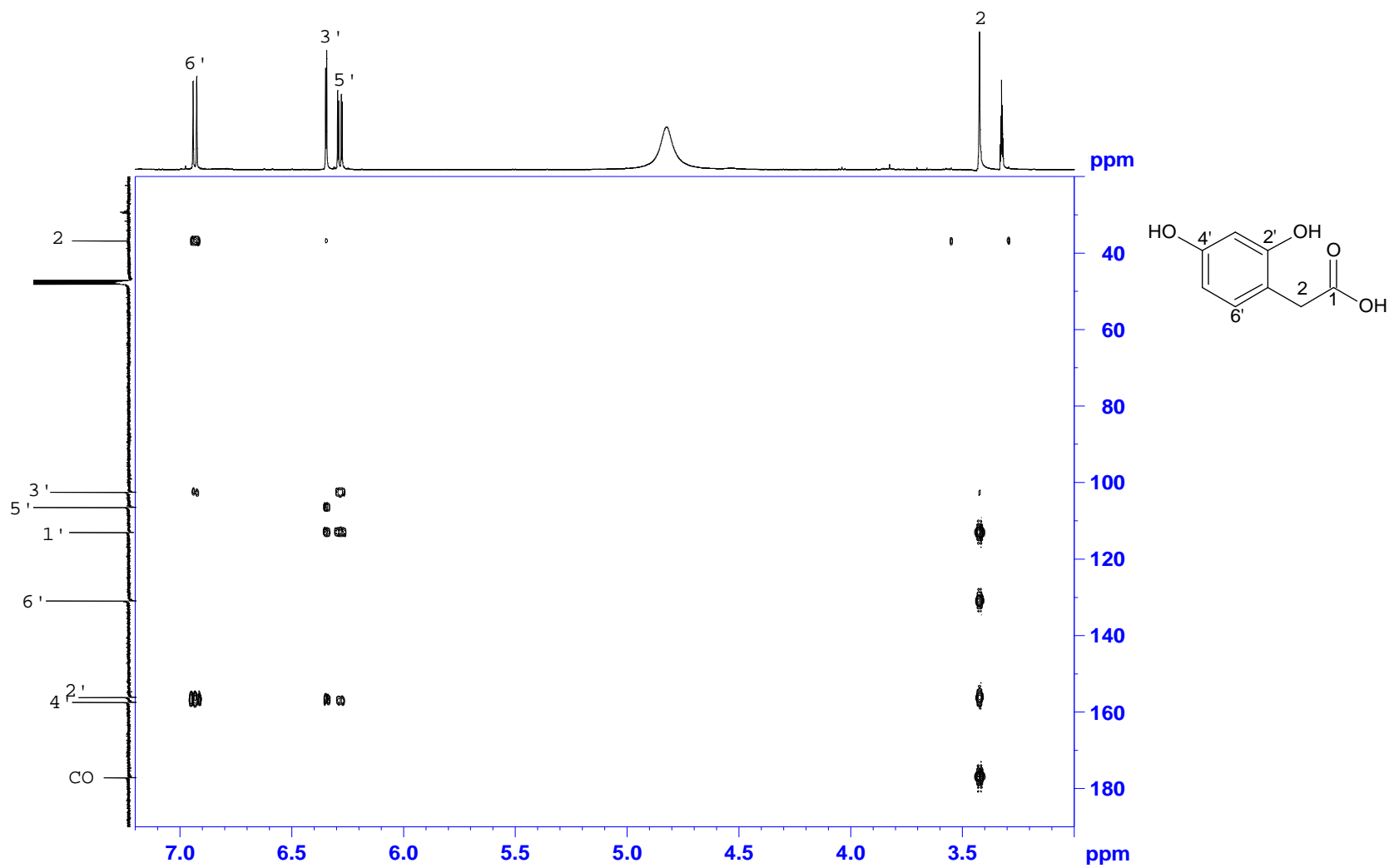
Plate 2f: DEPT-135 and 90 NMR Spectra of (2',4'-dihydroxyphenyl)acetic acid (74) in CD₃OD
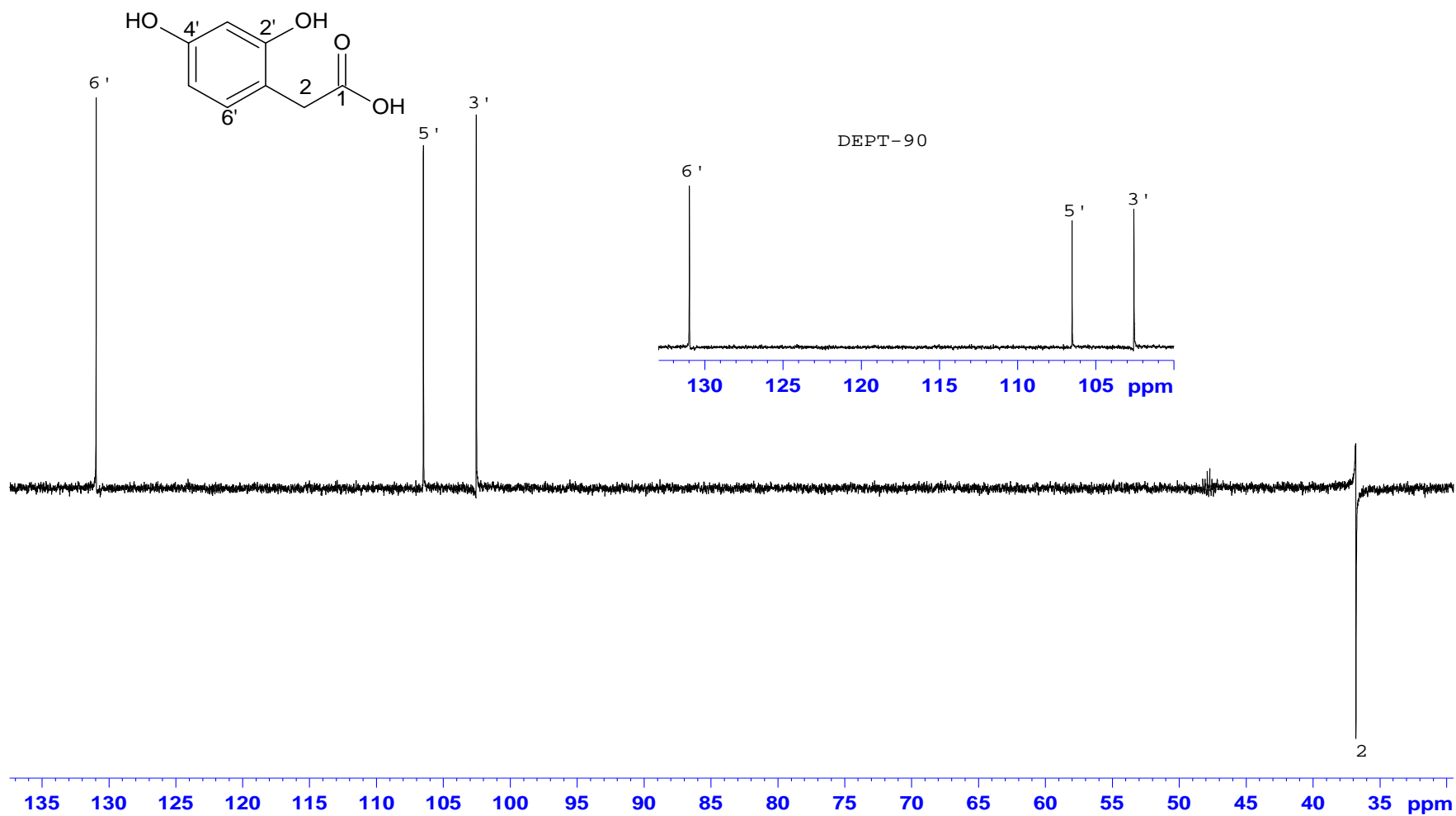
Plate 3a: ^{1}H NMR Spectrum of epicatechin (75) in CD$_3$OD
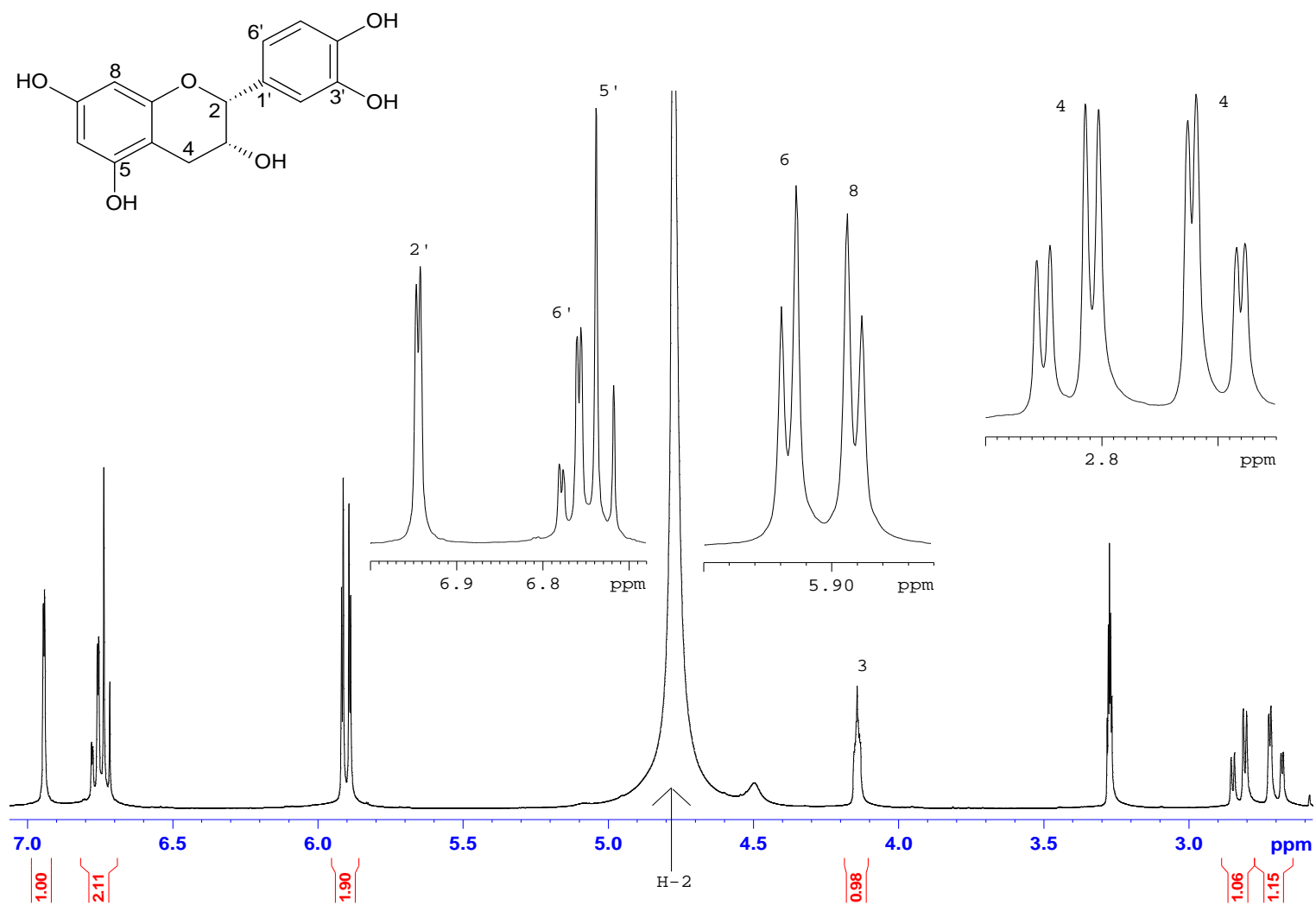
Plate 3b: COSY NMR Spectrum of epicatechin (75) in CD$_3$OD
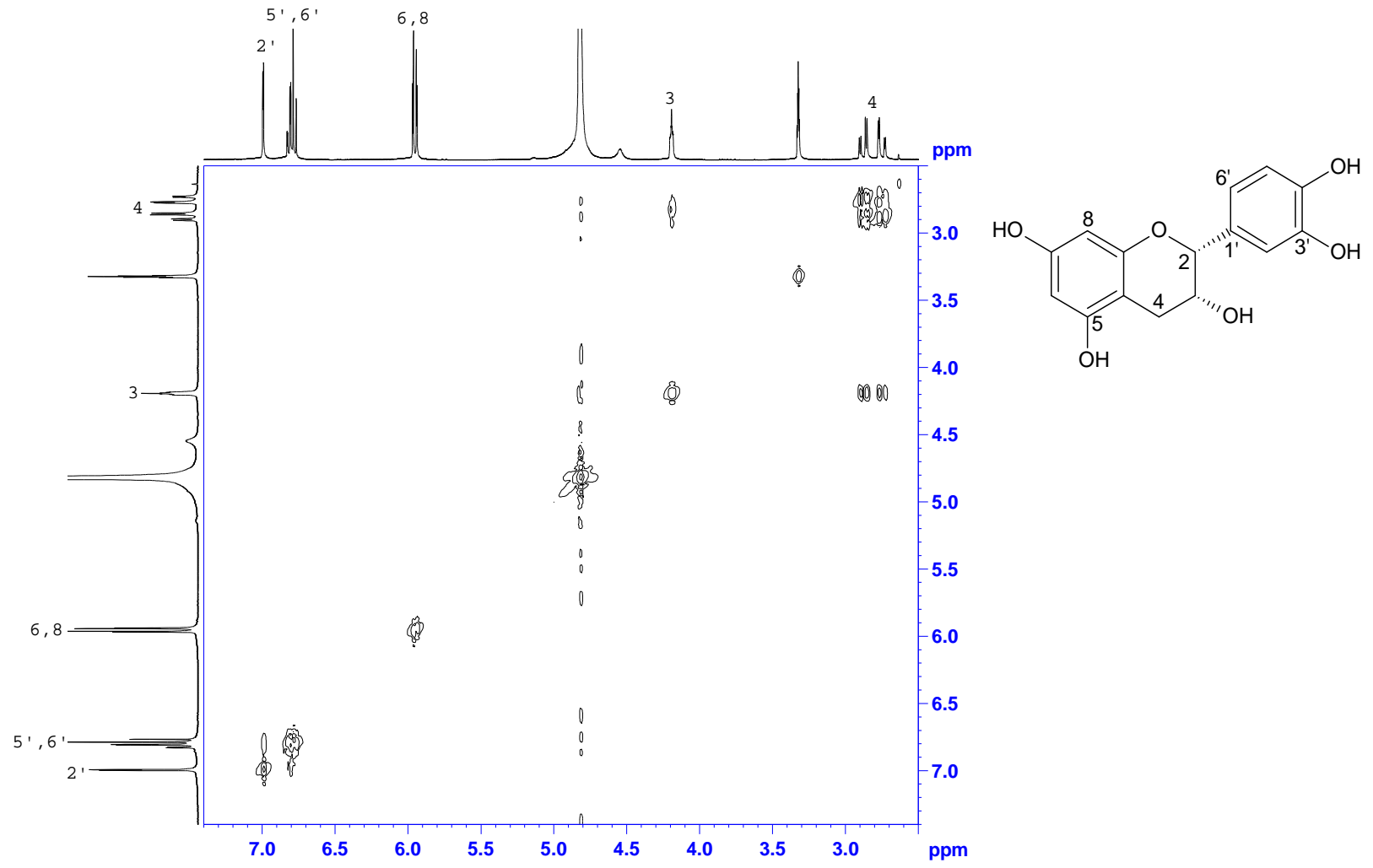
Plate 3c: ^{13}C NMR Spectrum of epicatechin (75) in CD$_3$OD
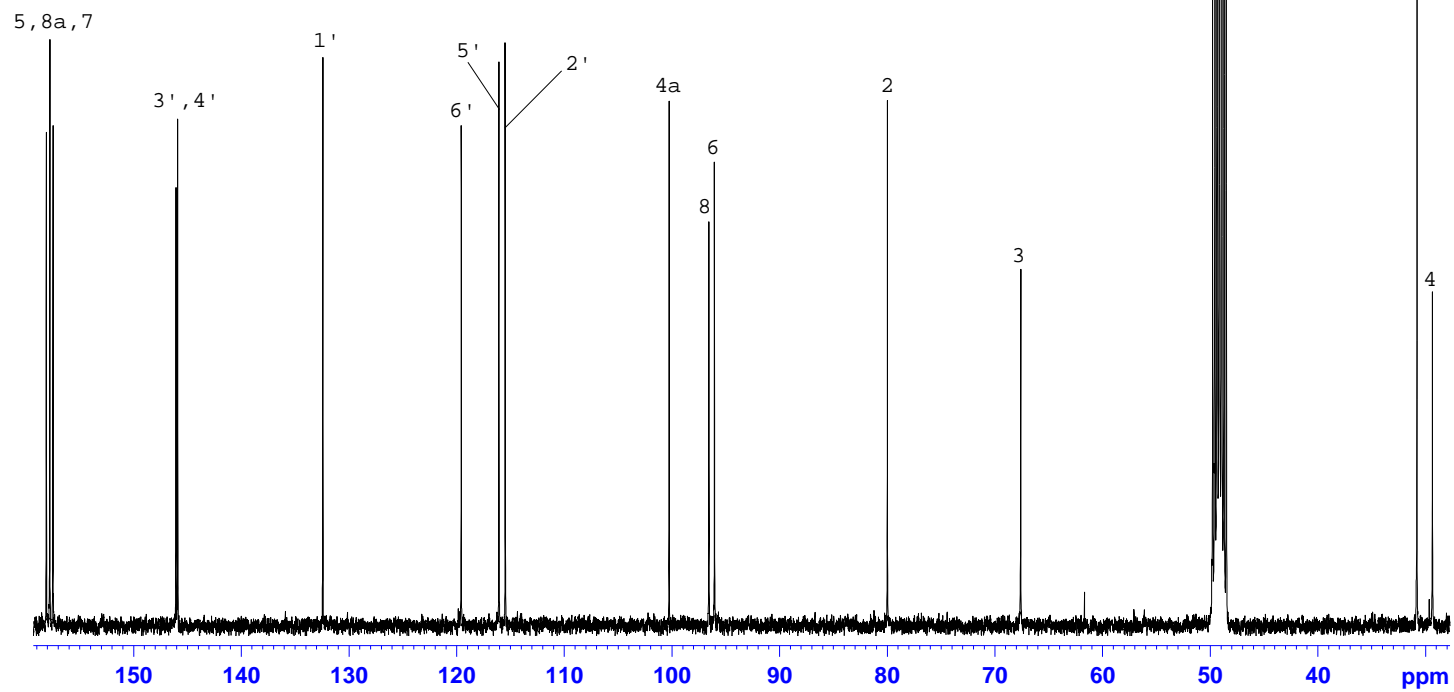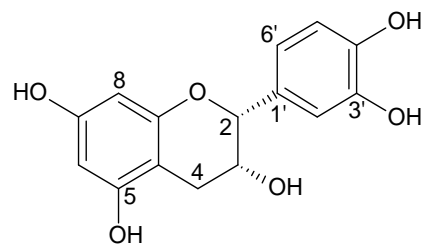
Plate 3d: HSQC NMR Spectrum of epicatechin (75) in CD$_3$OD
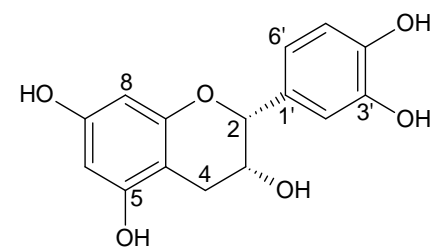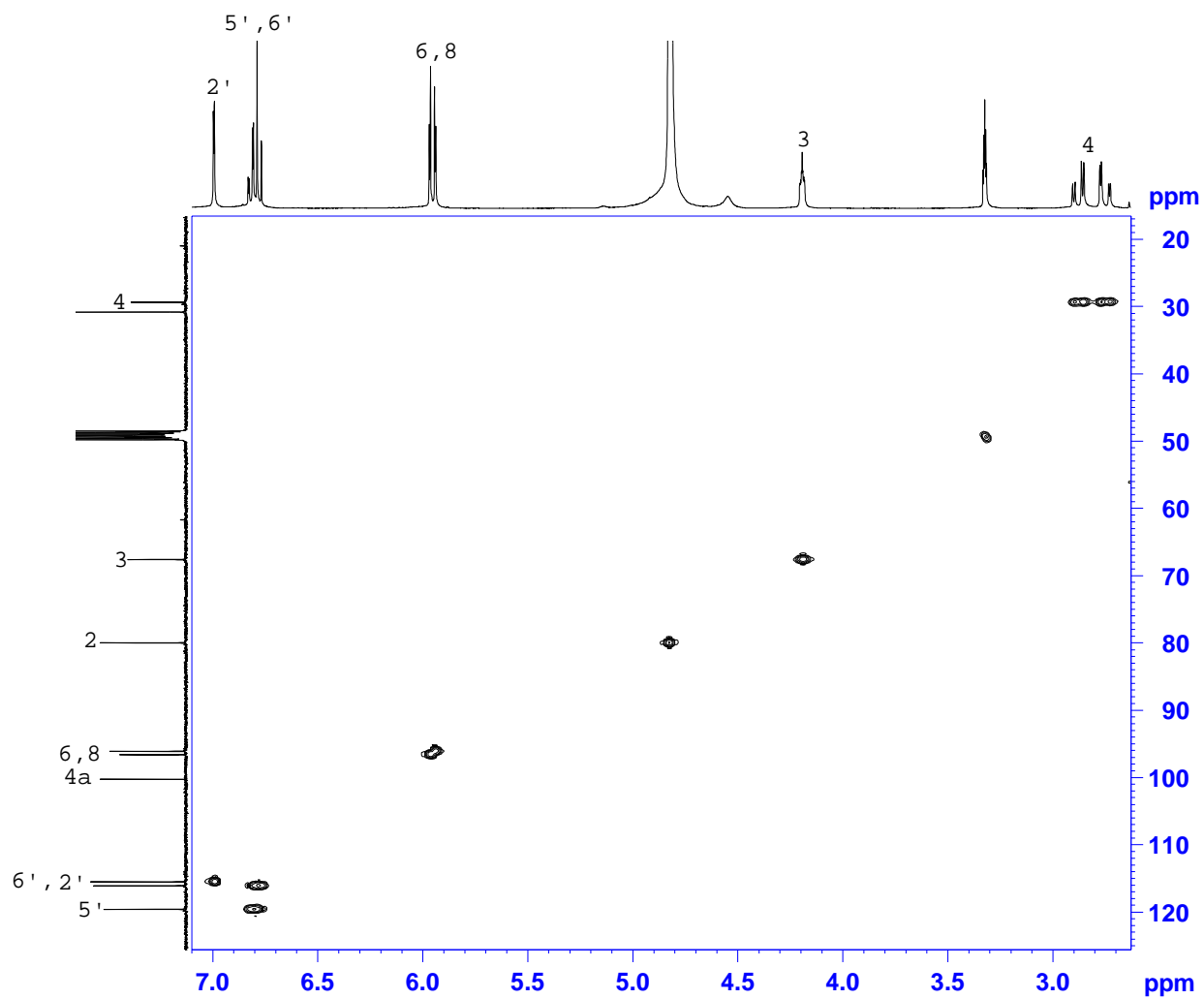
Plate 3e: HMBC NMR Spectrum of epicatechin (75) in CD₃OD
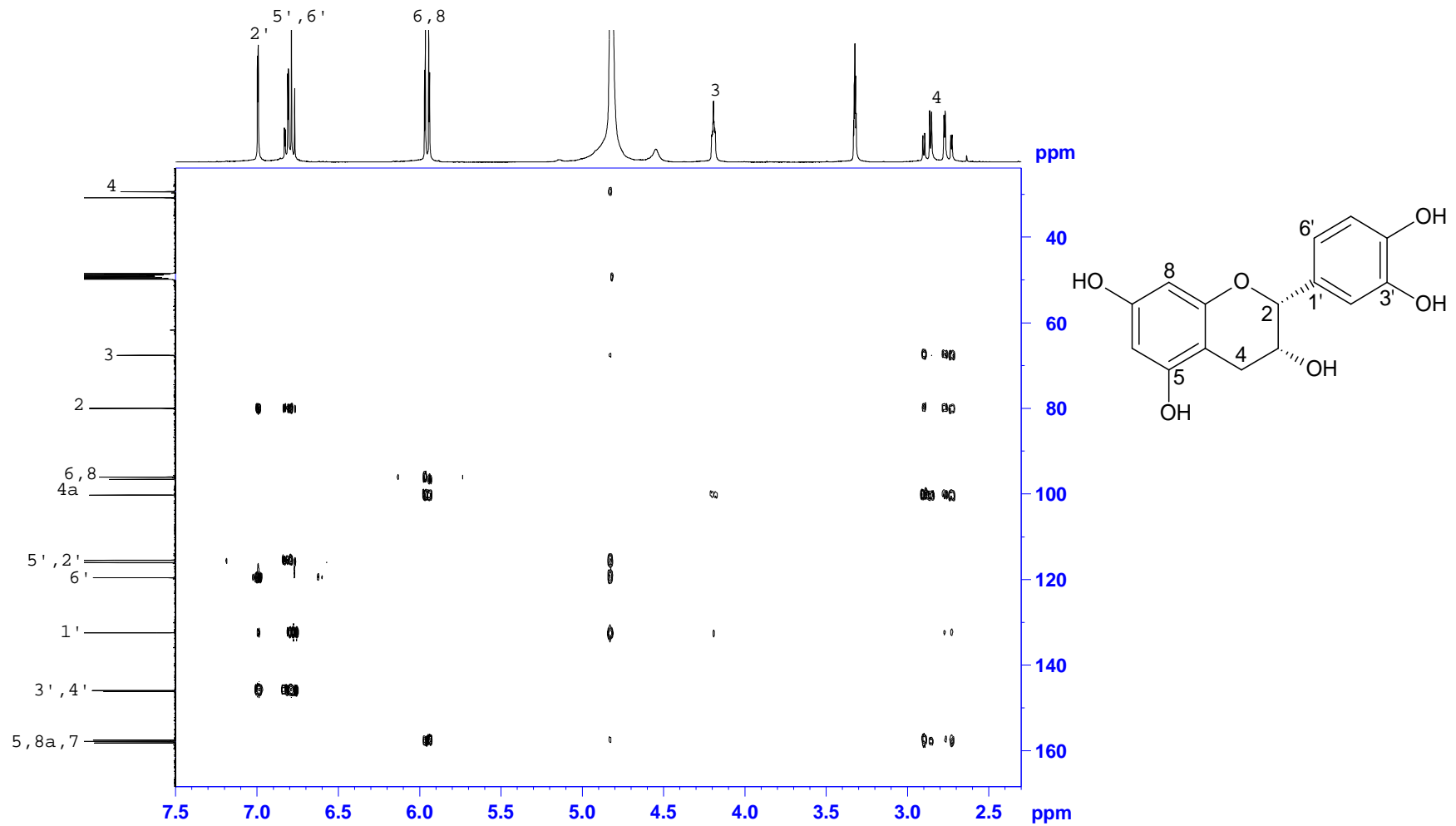
Plate 4a: ^{1}H NMR Spectrum of Iophirone A (36) in $(CD_3)_2CO$
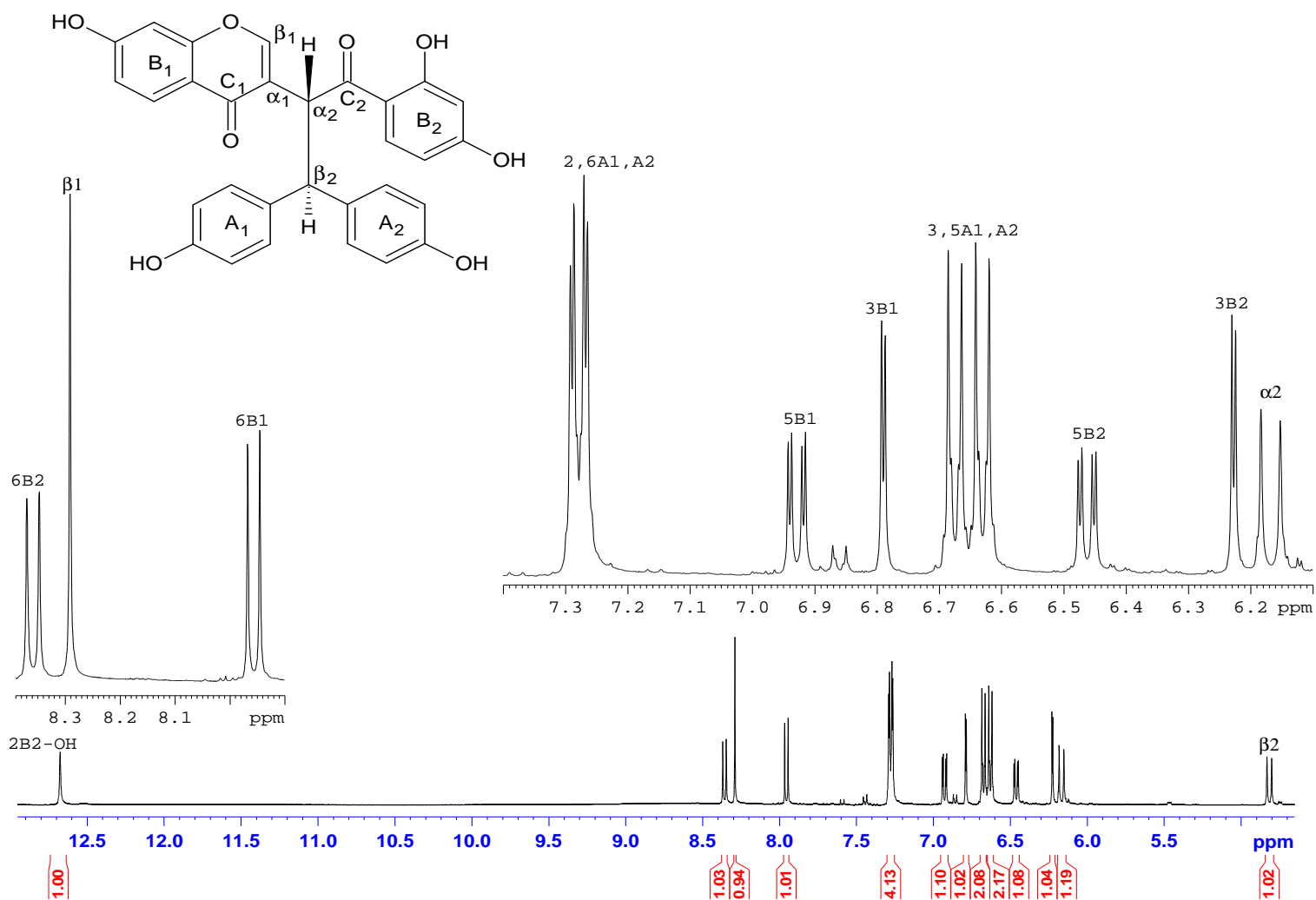
Plate 4b: COSY NMR Spectrum of Iophirone A (36) in (CD$_3$)$_2$CO
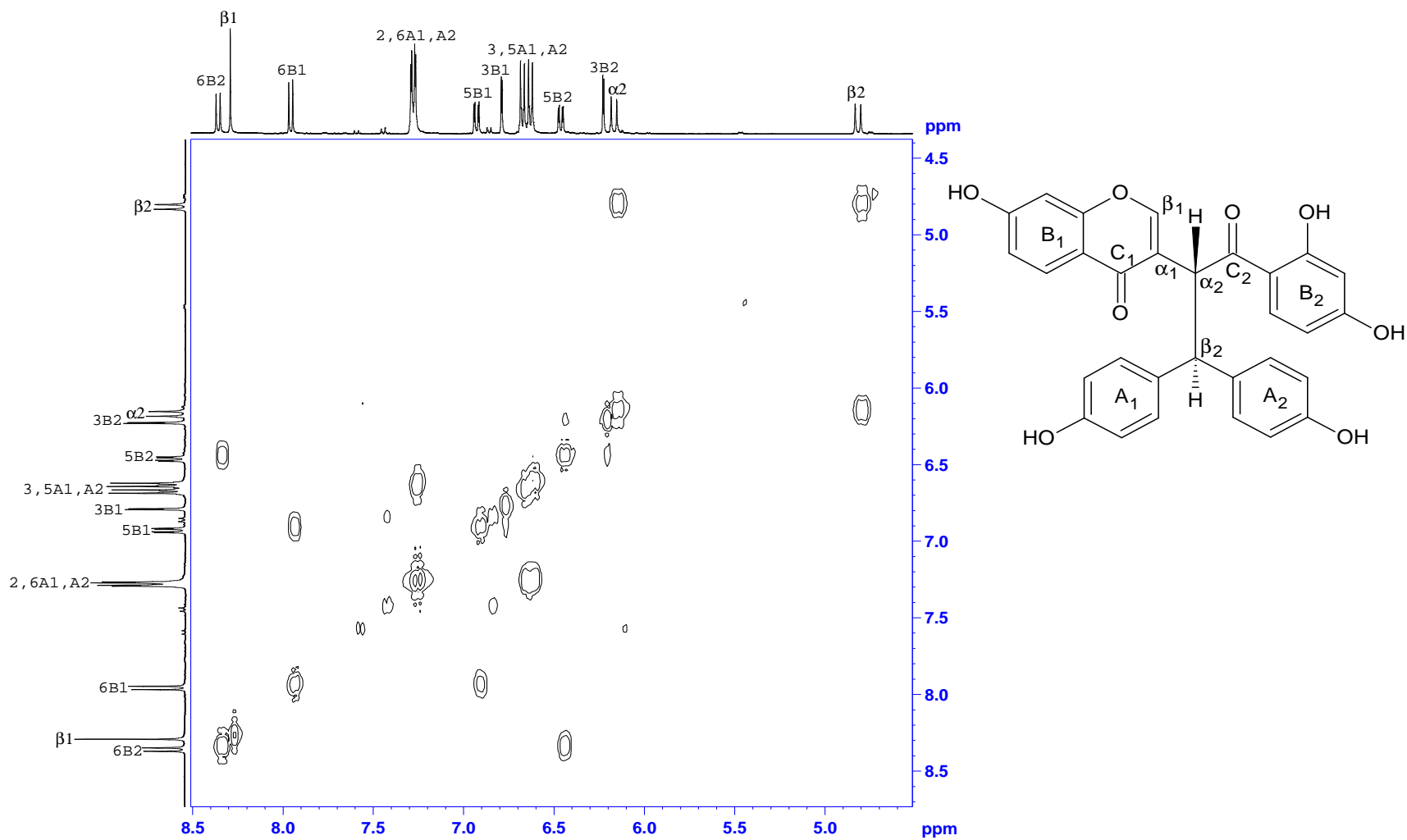
Plate 4c: ^{13}C NMR Spectrum of Iophirone A (36) in $(CD_3)_2CO$
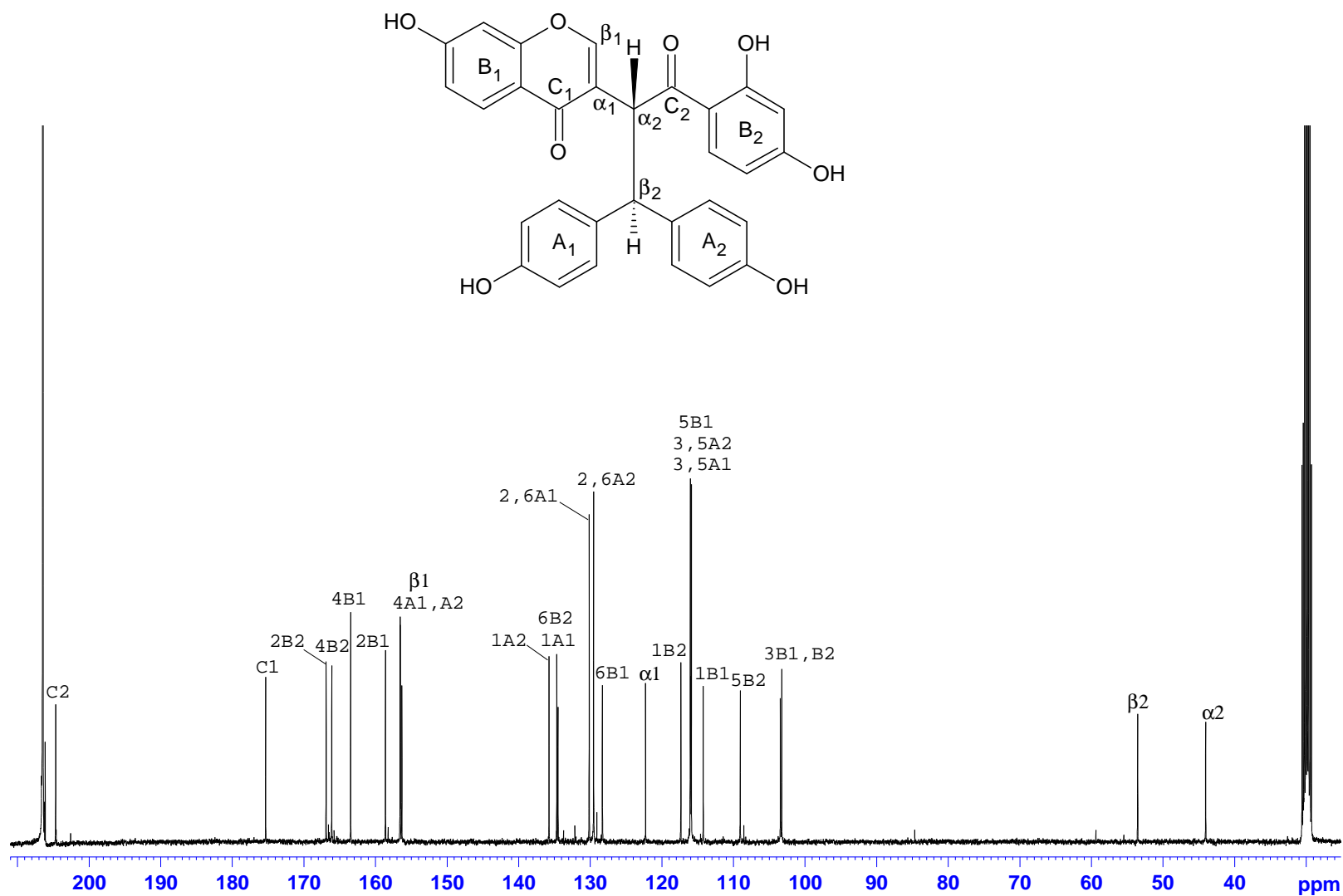
Plate 4d: HSQC NMR Spectrum of Iophirone A (36) in $(CD_3)_2CO$
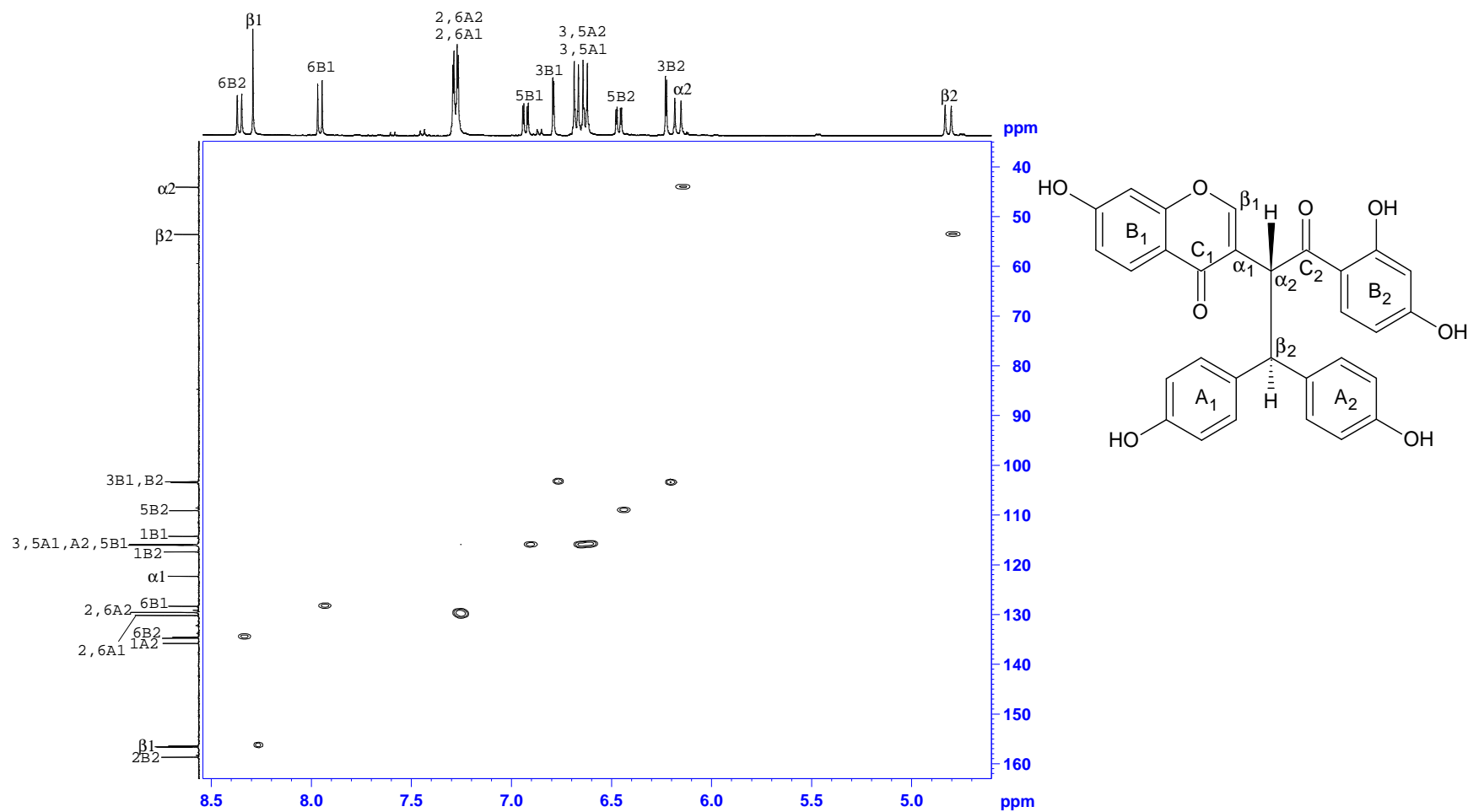
Plate 4e: HMBC NMR Spectrum of Iophirone A (36) in (CD$_3$)$_2$CO
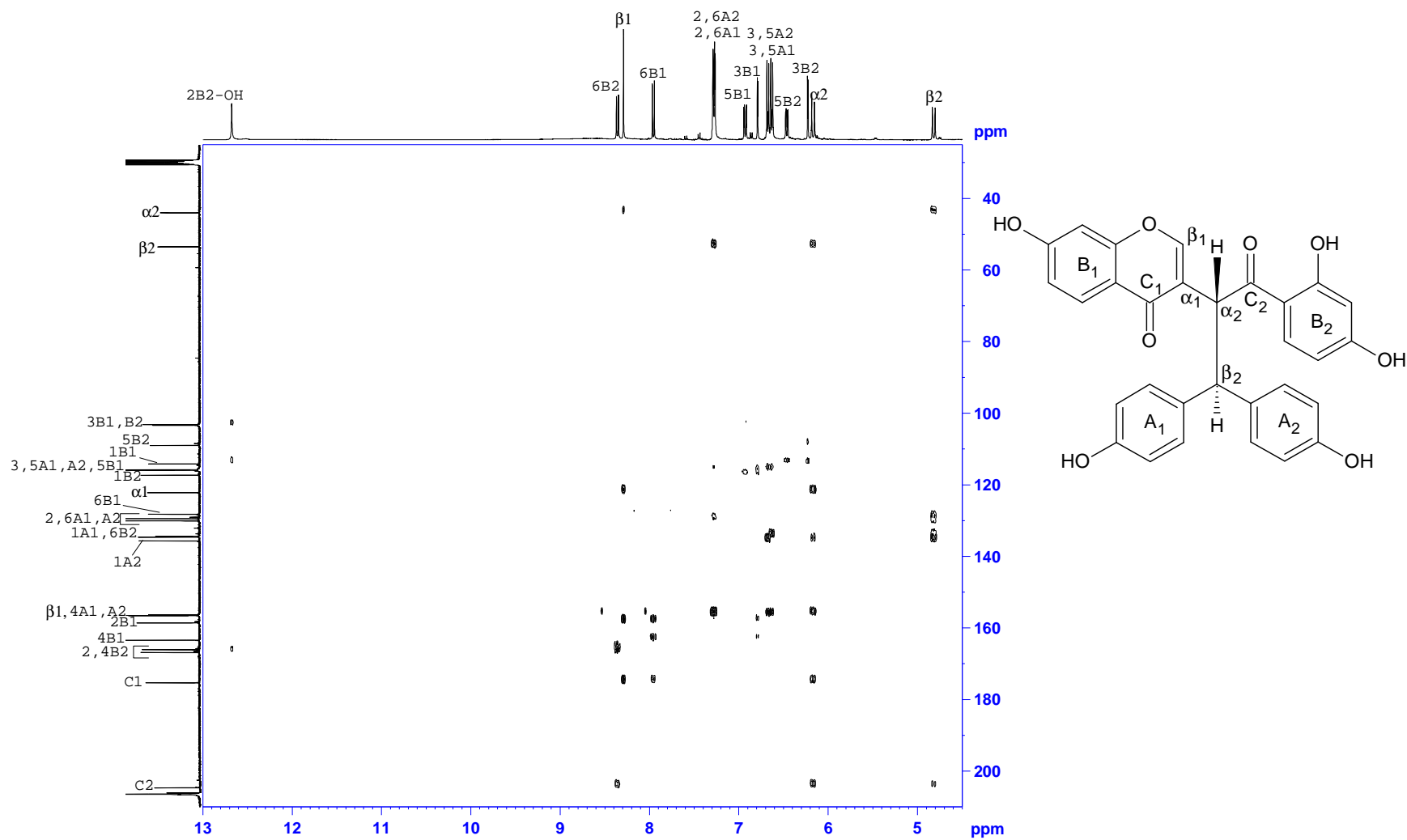
Plate 5a: ^{1}H NMR Spectrum of Iophirone C (39) in CD$_3$OD
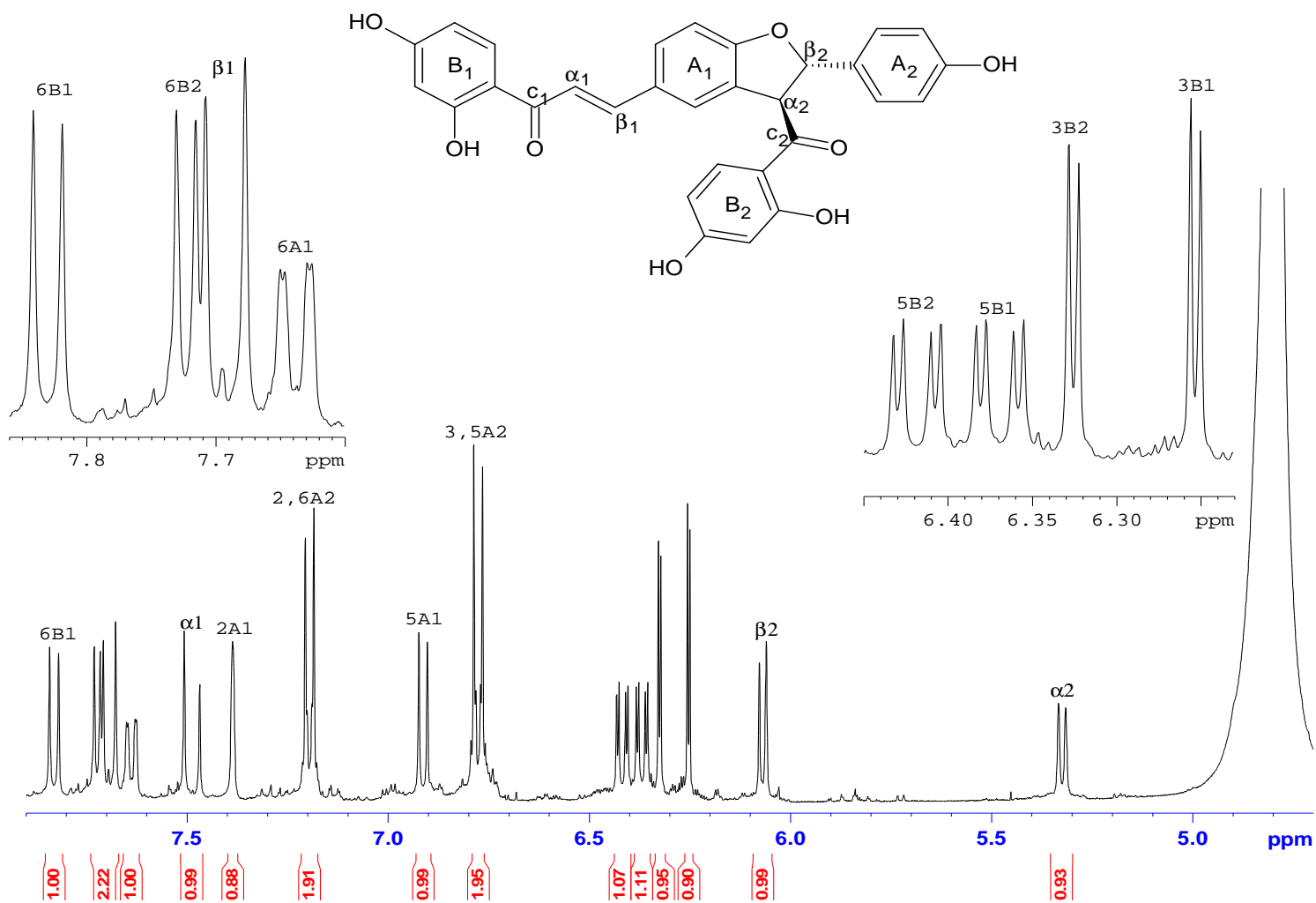
Plate 5b: COSY NMR Spectrum of Iophirone C (39) in CD$_3$OD
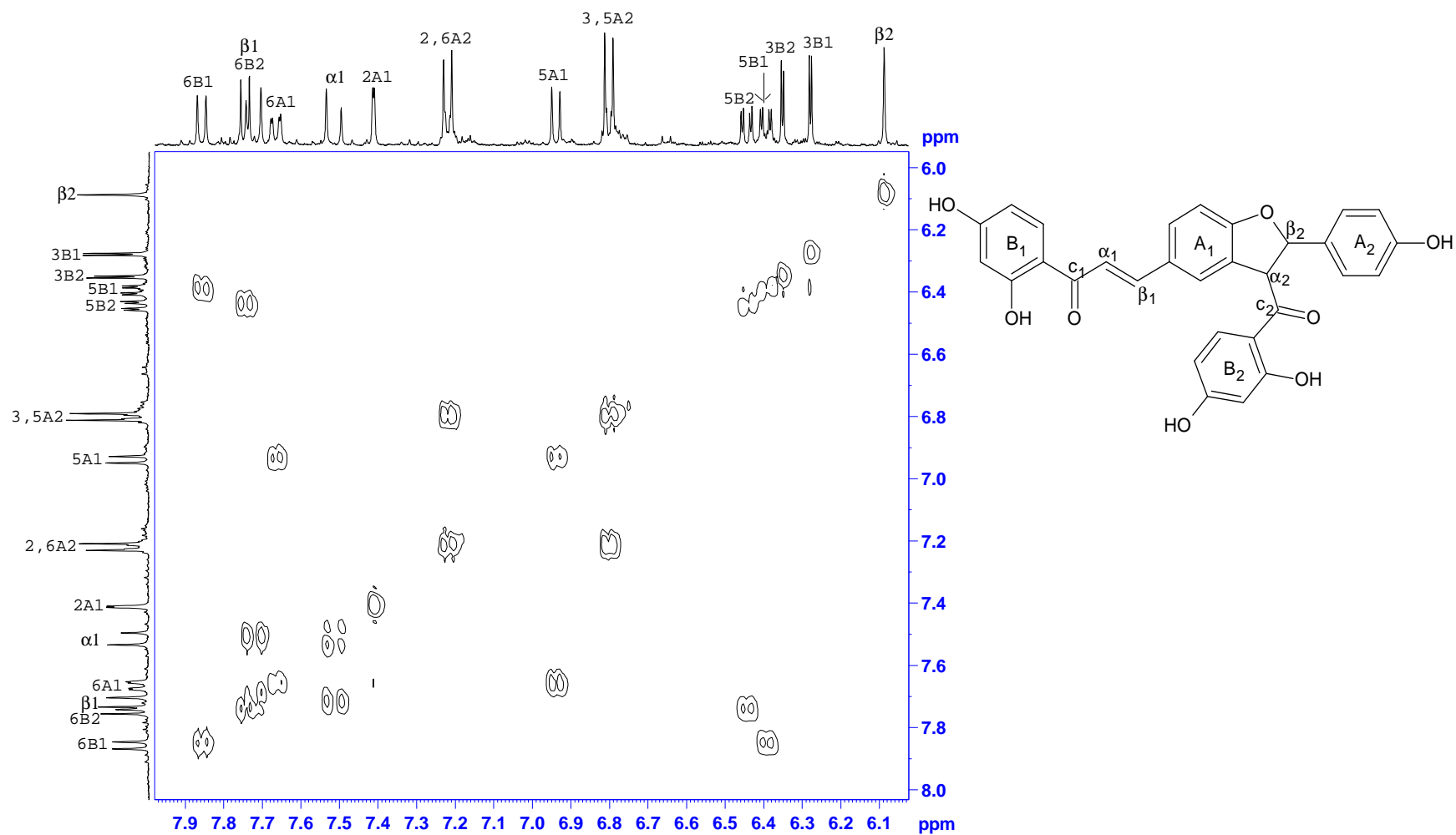
Plate 5c: ^{13}C NMR Spectrum of Iophirone C (39) in CD$_3$OD
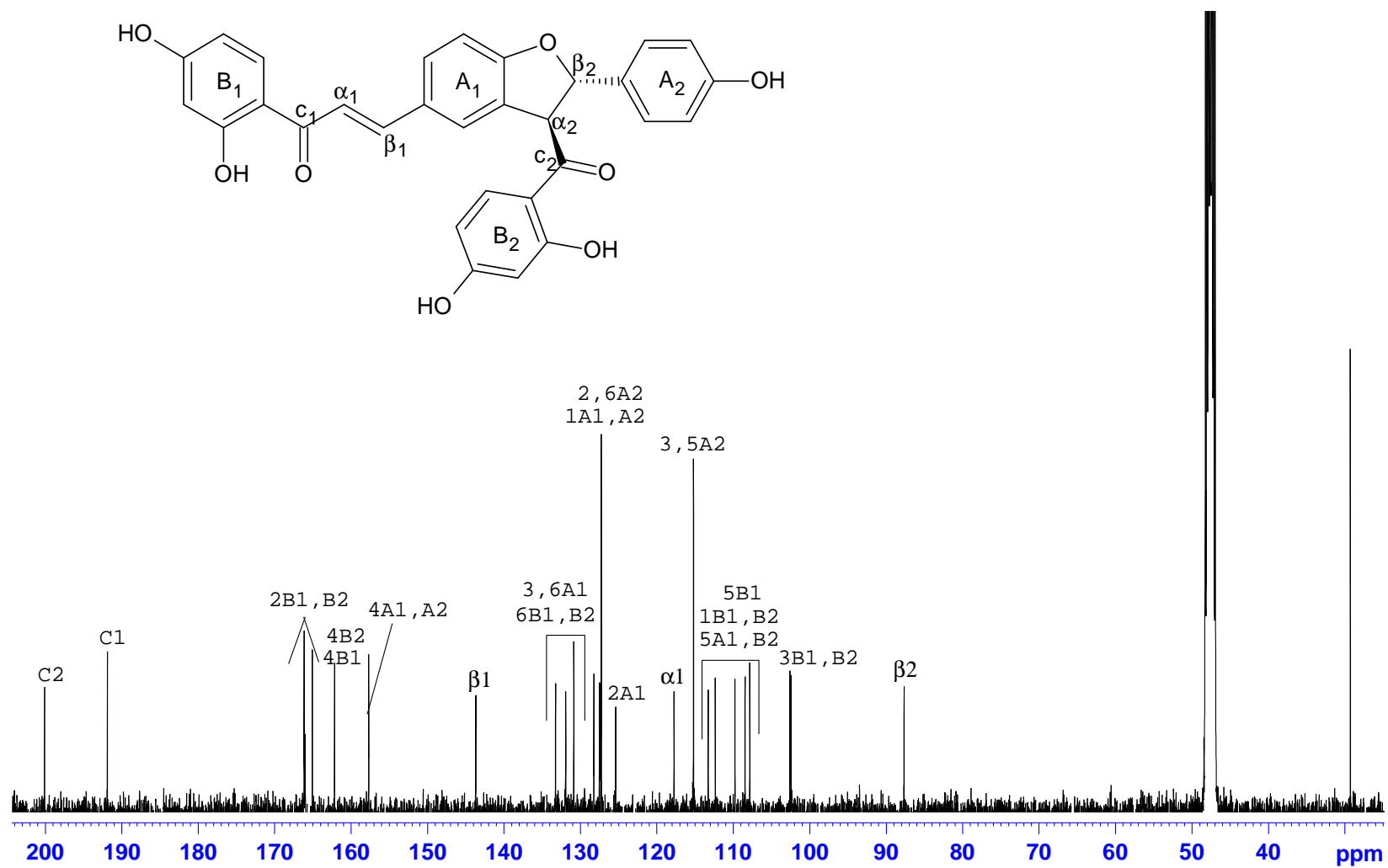
Plate 5d: HSQC NMR Spectrum of Iophirone C (39) in CD$_3$OD
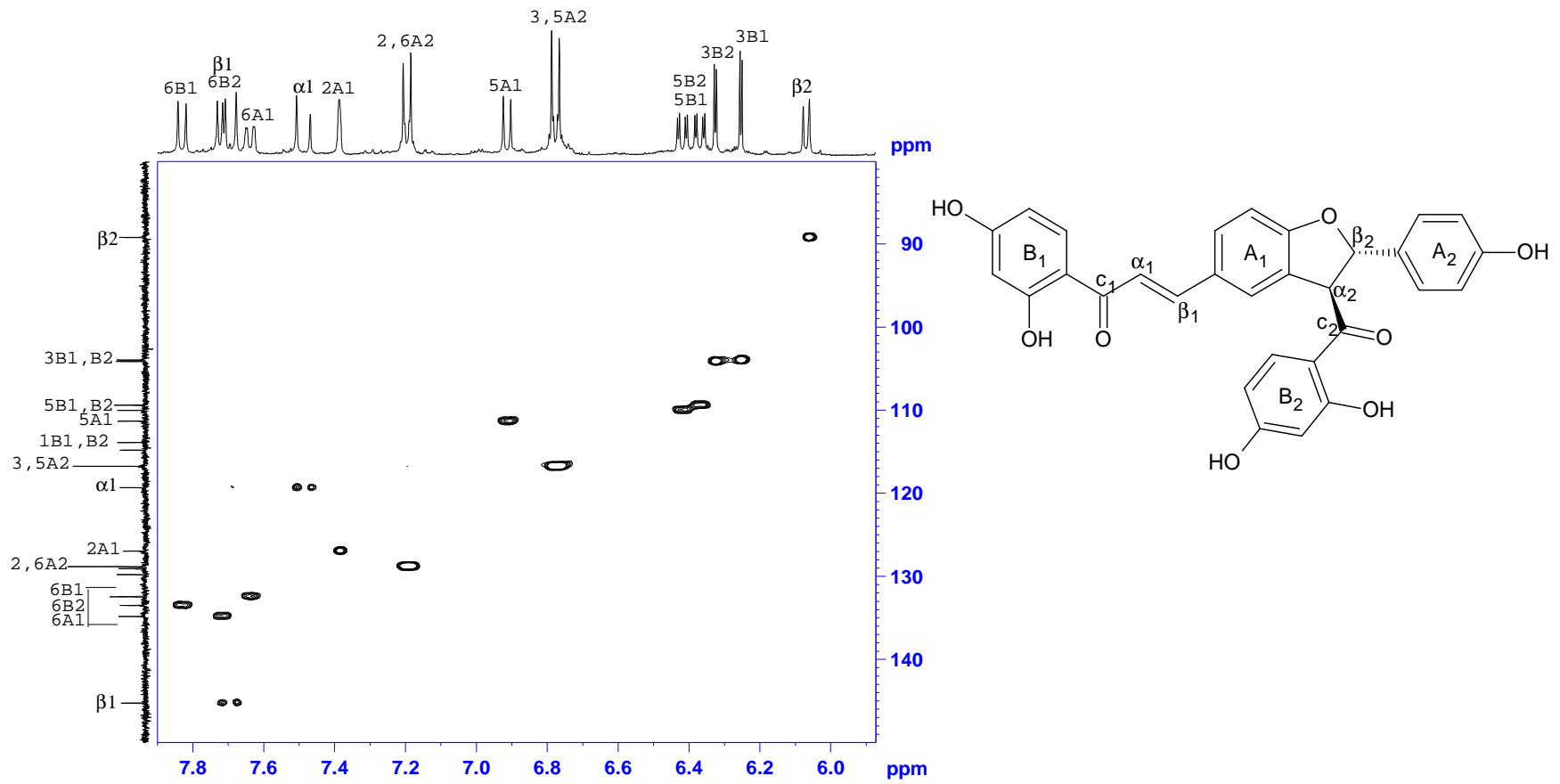
Plate 6a: ^{1}H NMR Spectrum of afzelone B (50) in CD$_3$OD
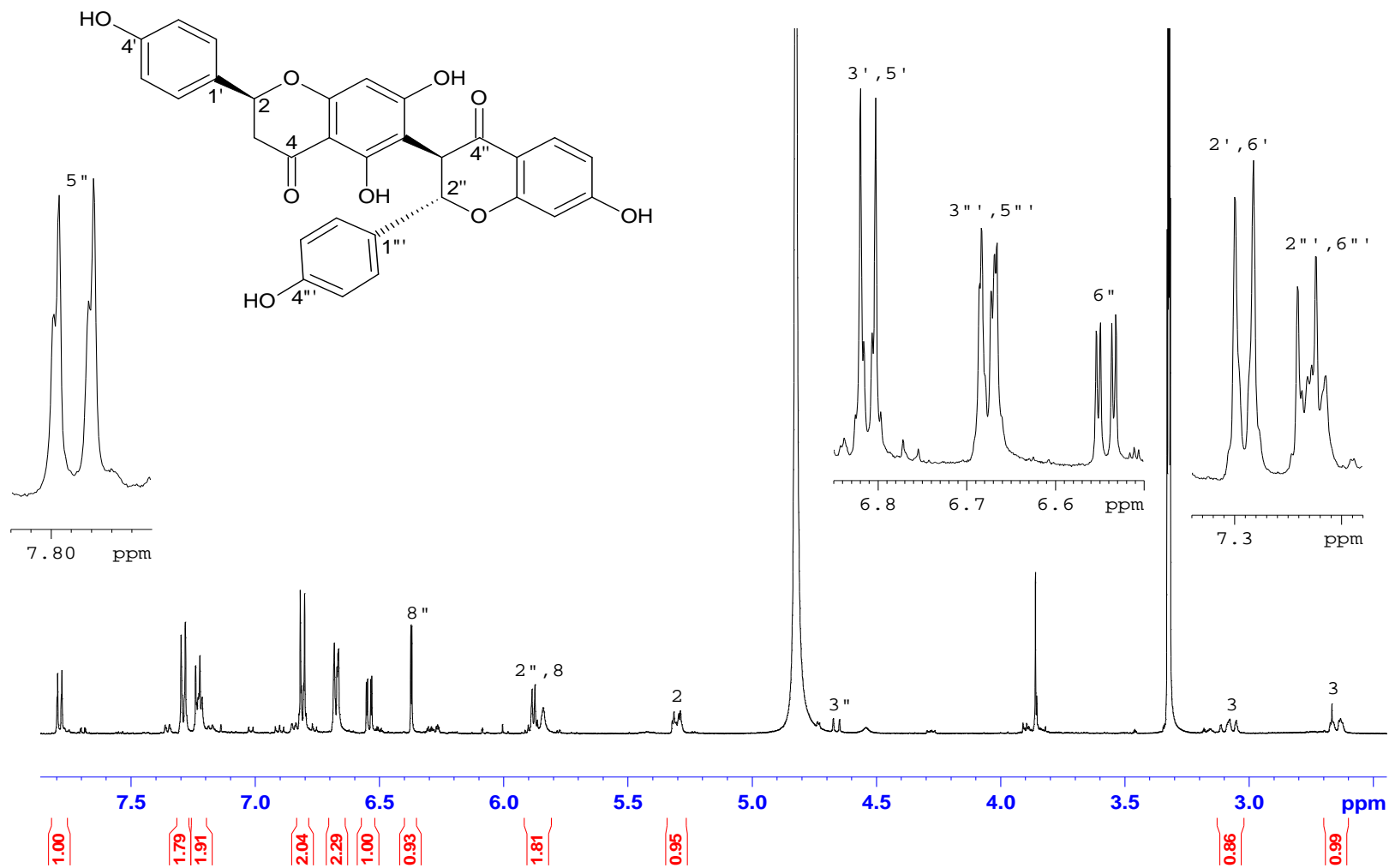
Plate 6b: COSY NMR Spectrum of afzelone B (50) in CD$_3$OD
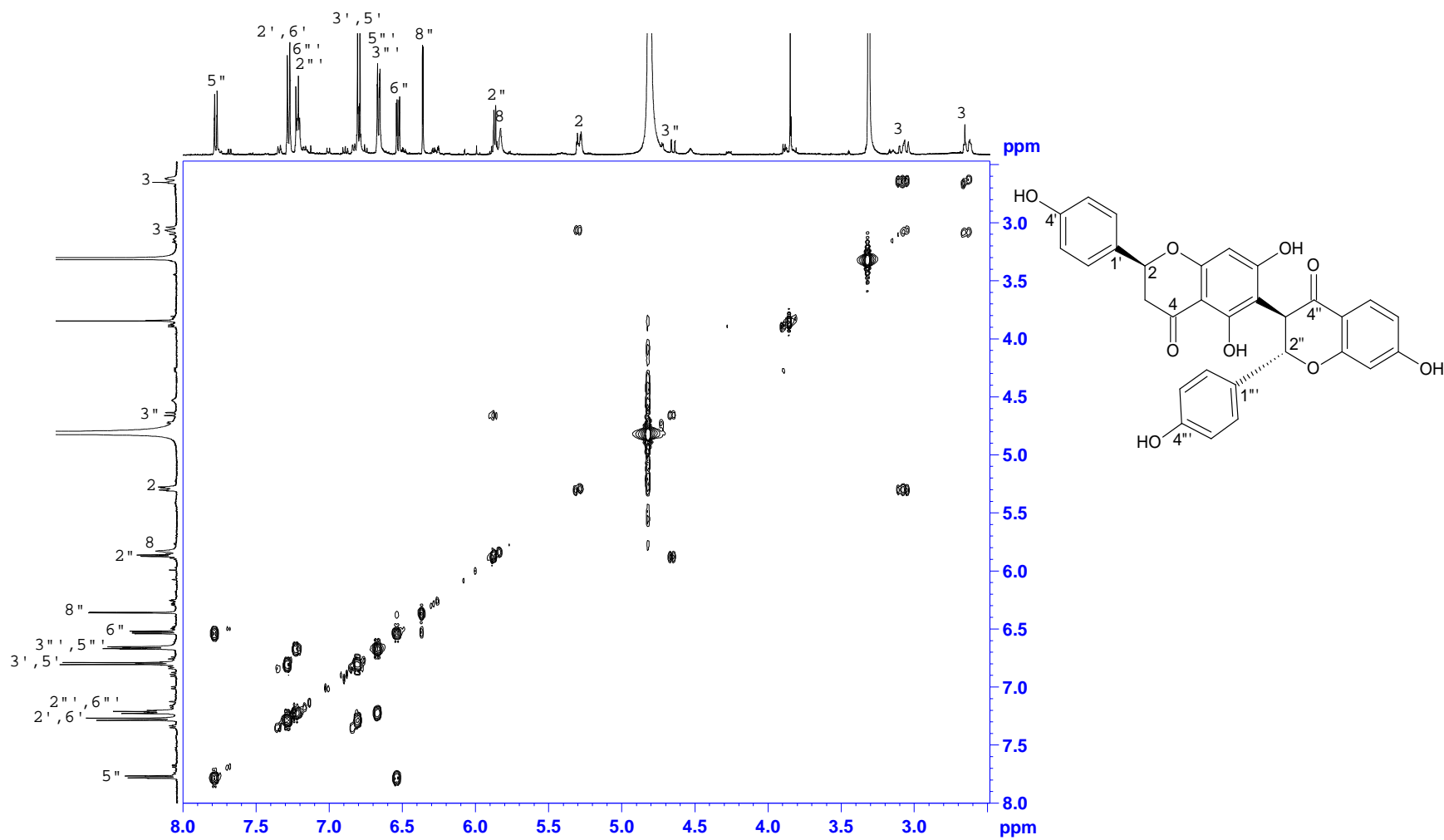
Plate 6c: ^{13}C NMR Spectrum of afzelone B (50) in CD$_3$OD
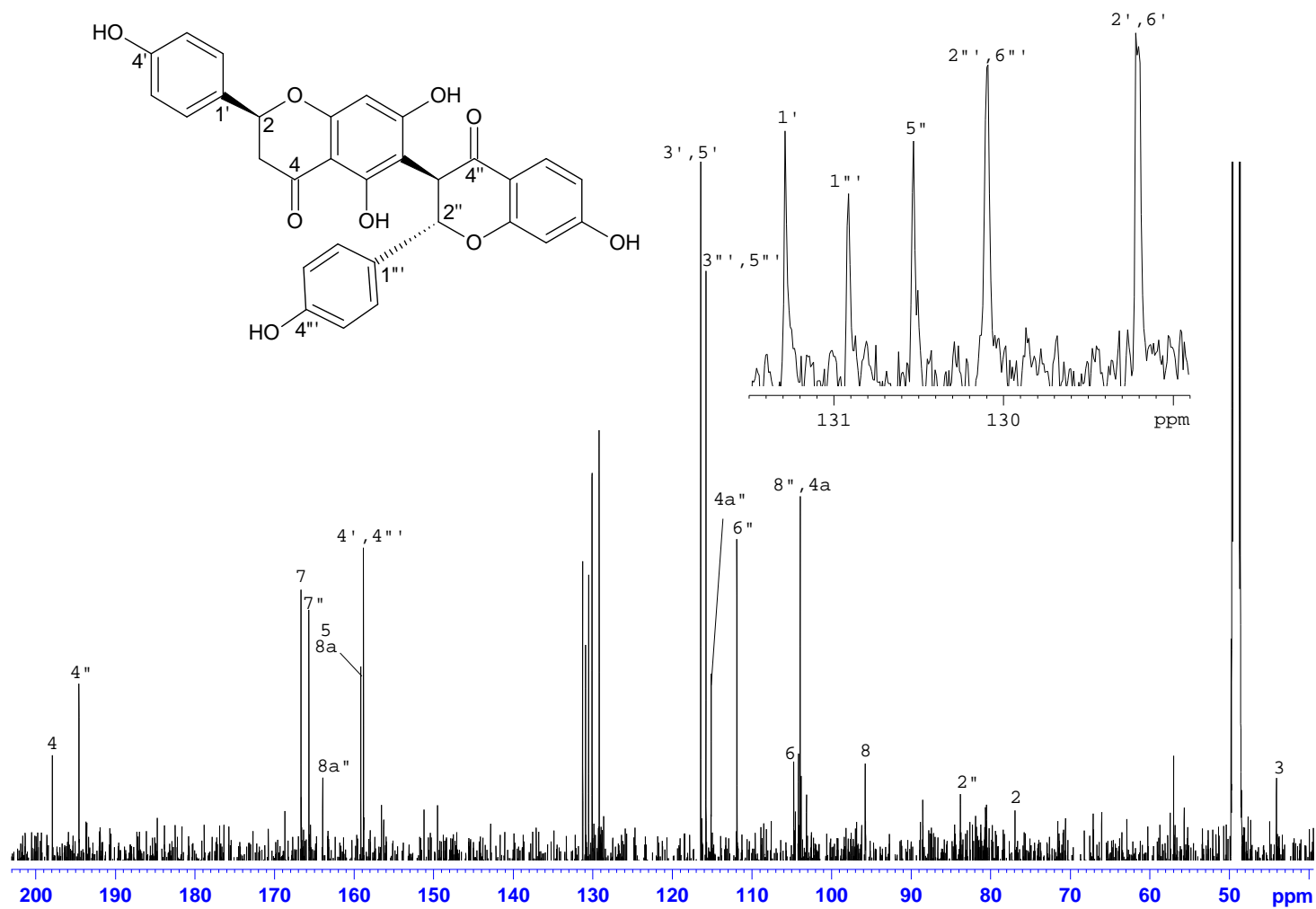
Plate 6d: HSQC NMR Spectrum of afzelone B (50) in CD$_3$OD
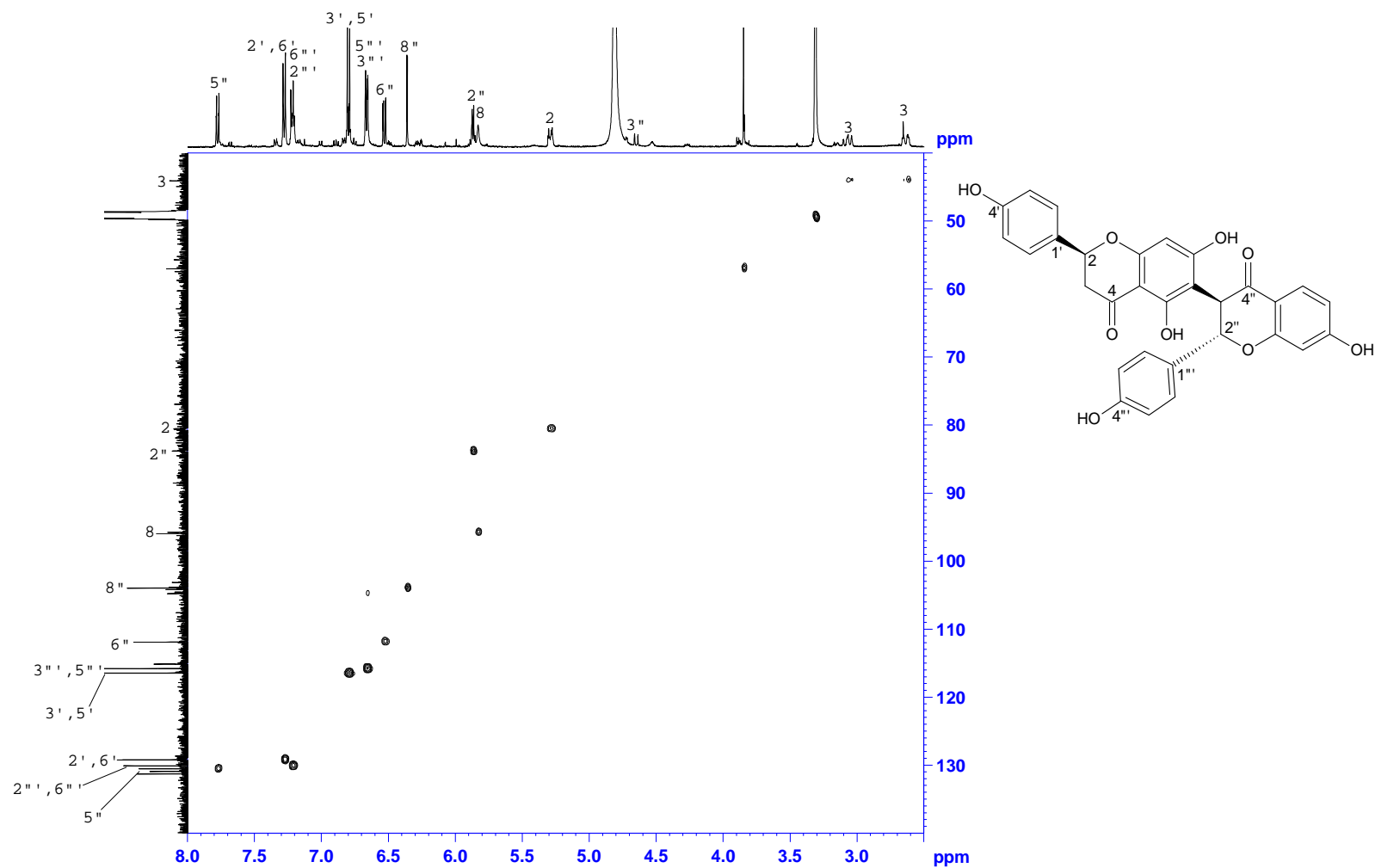
Plate 6e: HMBC NMR Spectrum of afzelone B (50) in CD₃OD
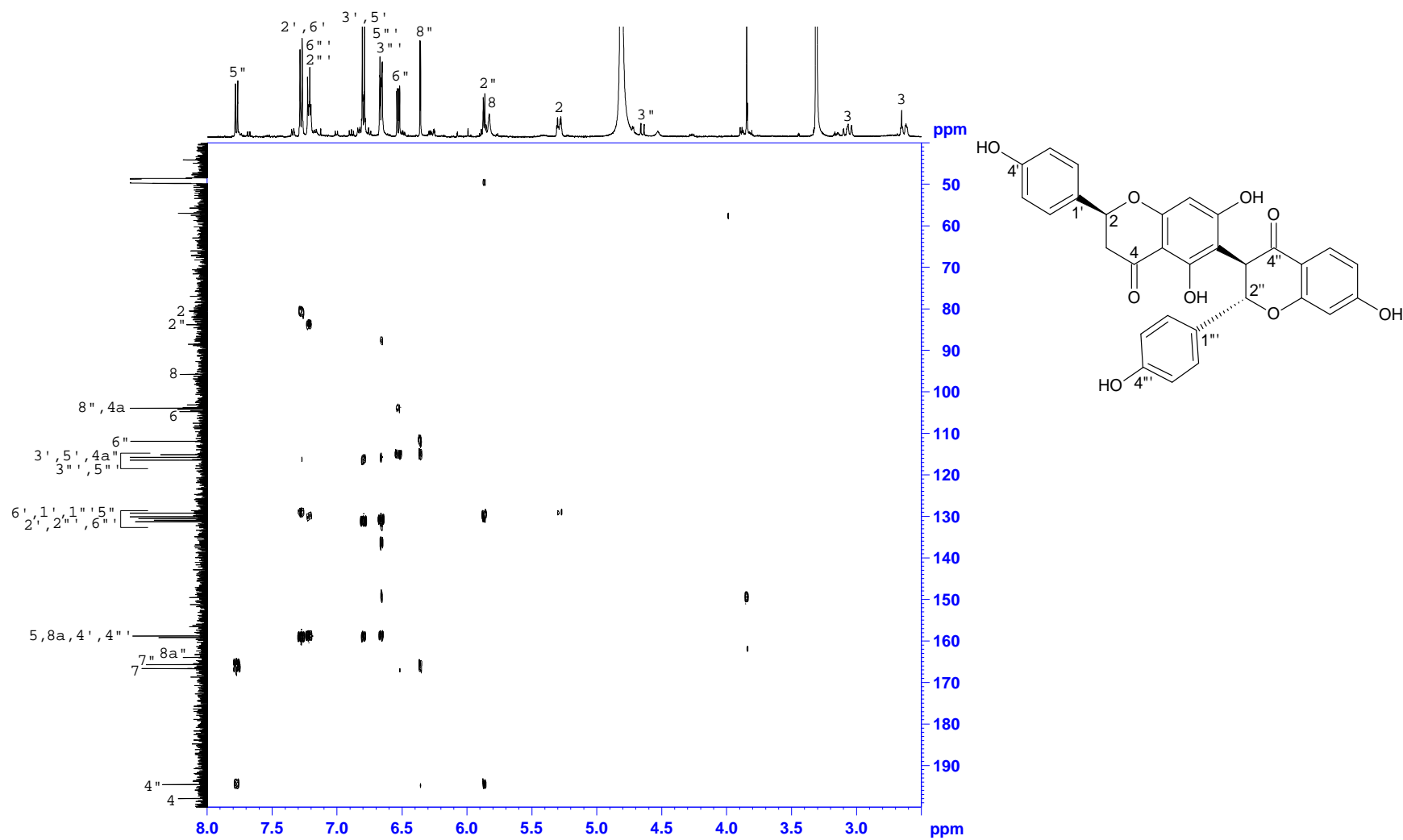
Plate 6f: NOESY NMR Spectrum of afzelone B (50) in CD₃OD
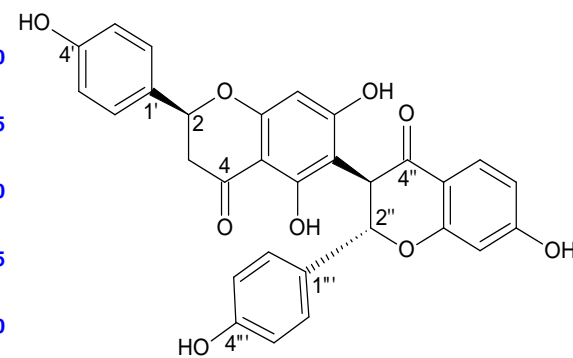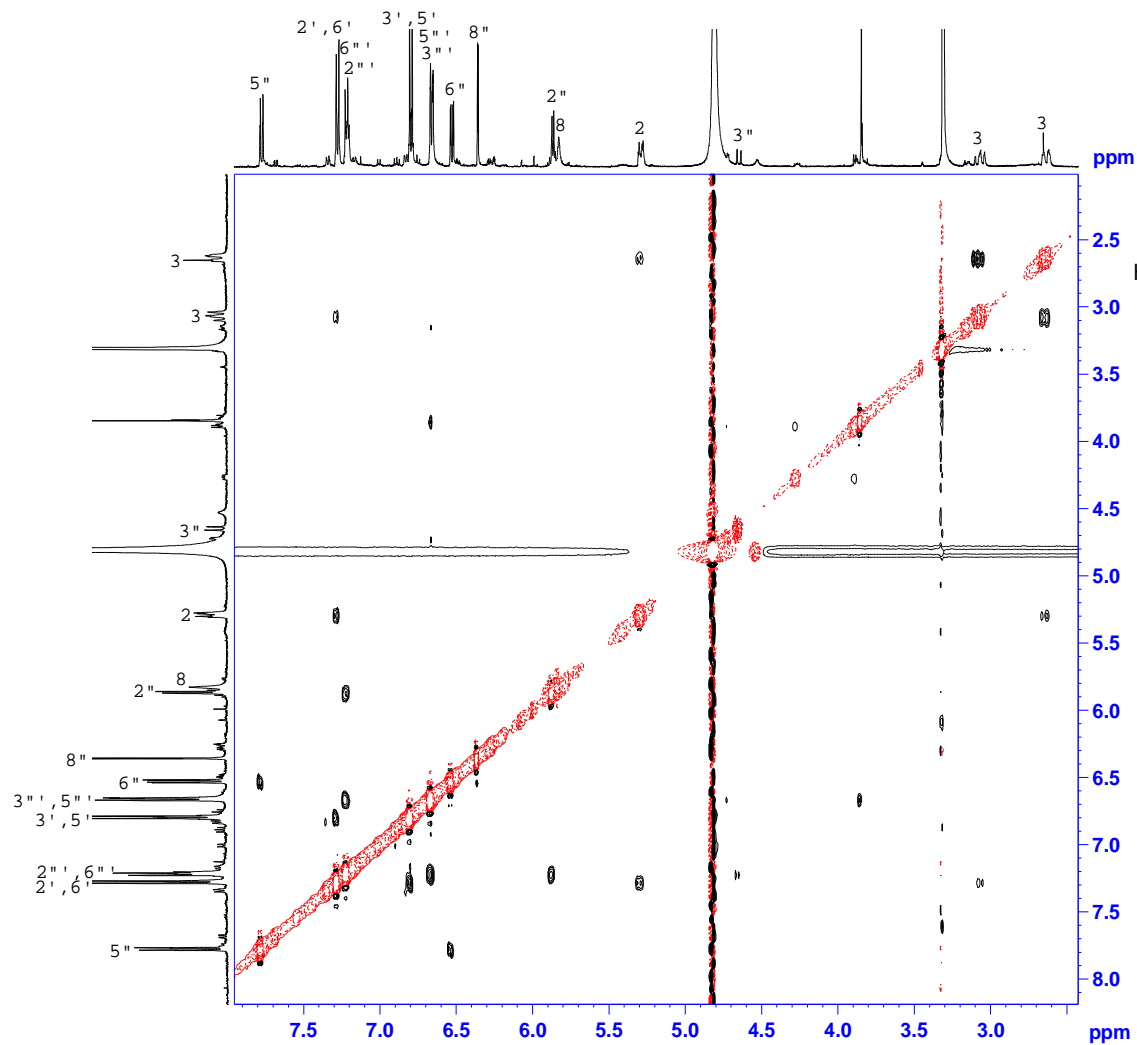
Plate 7a: ^{1}H NMR Spectrum of 5-deoxyurundeuvine C (76) in CD$_3$OD
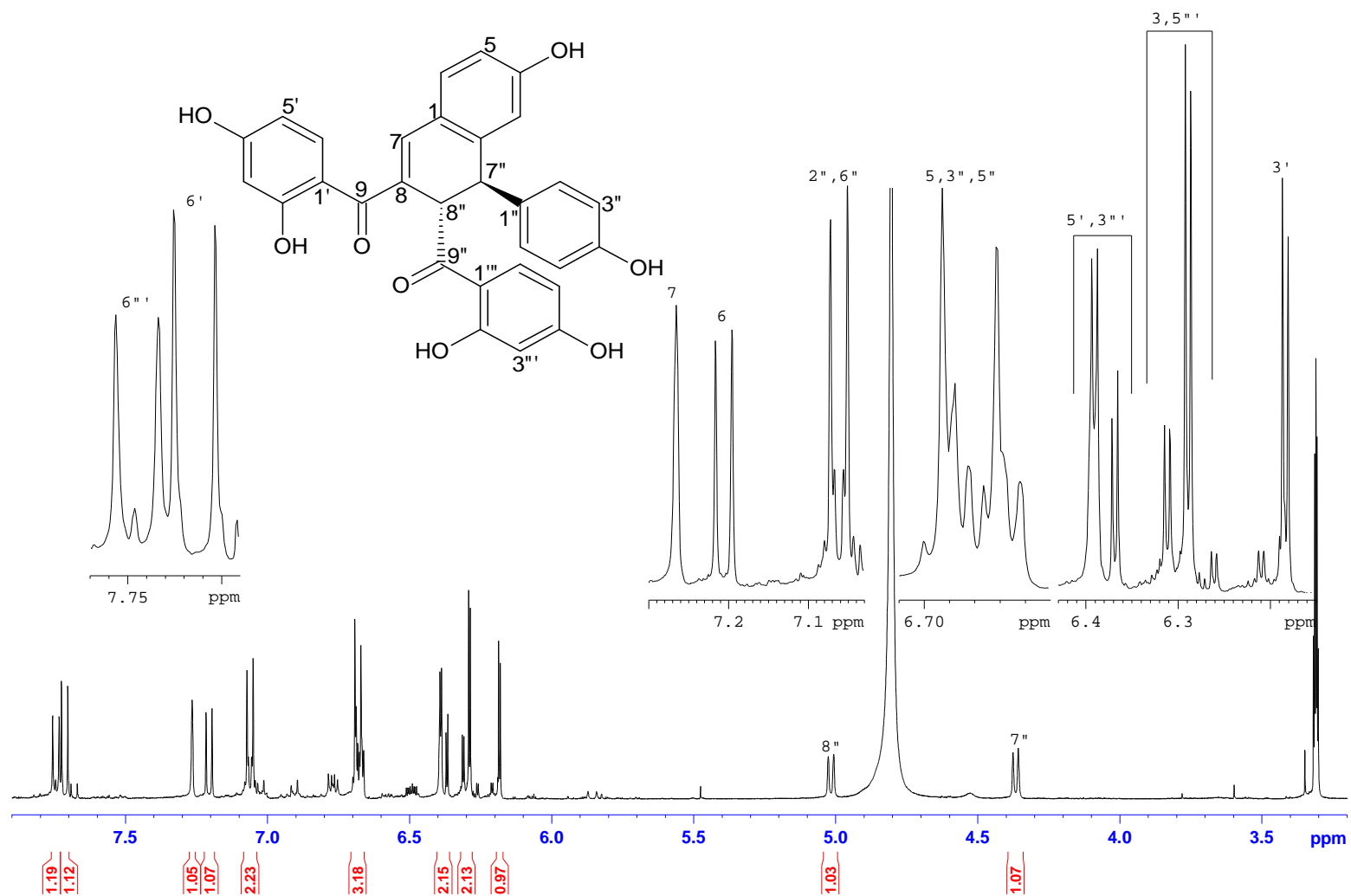
Plate 7b: COSY NMR Spectrum of 5-deoxyurundeuvine C (76) in CD₃OD
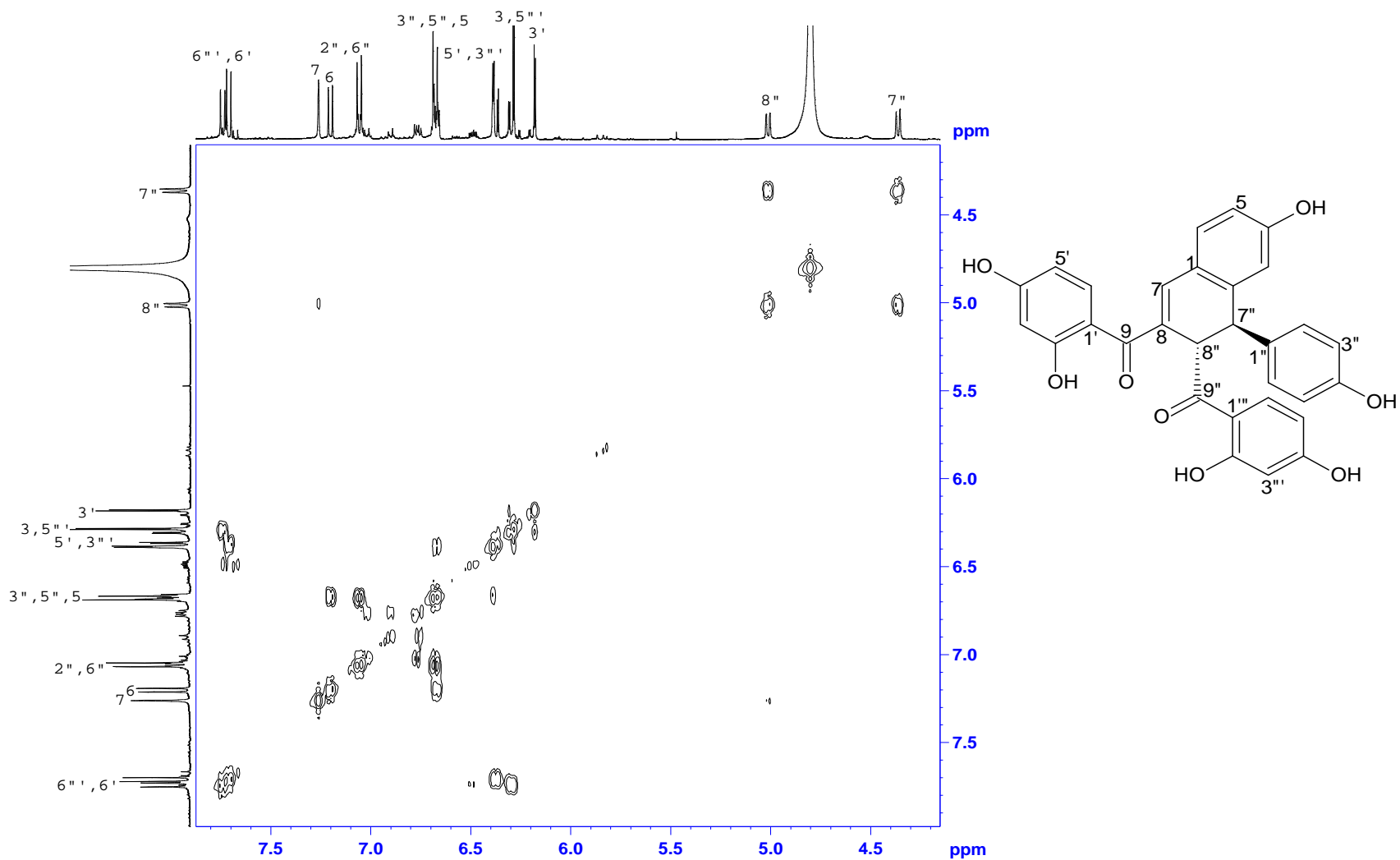
Plate 7c: ^{13}C NMR Spectrum of 5-deoxyurundeuvine C (76) in CD$_3$OD

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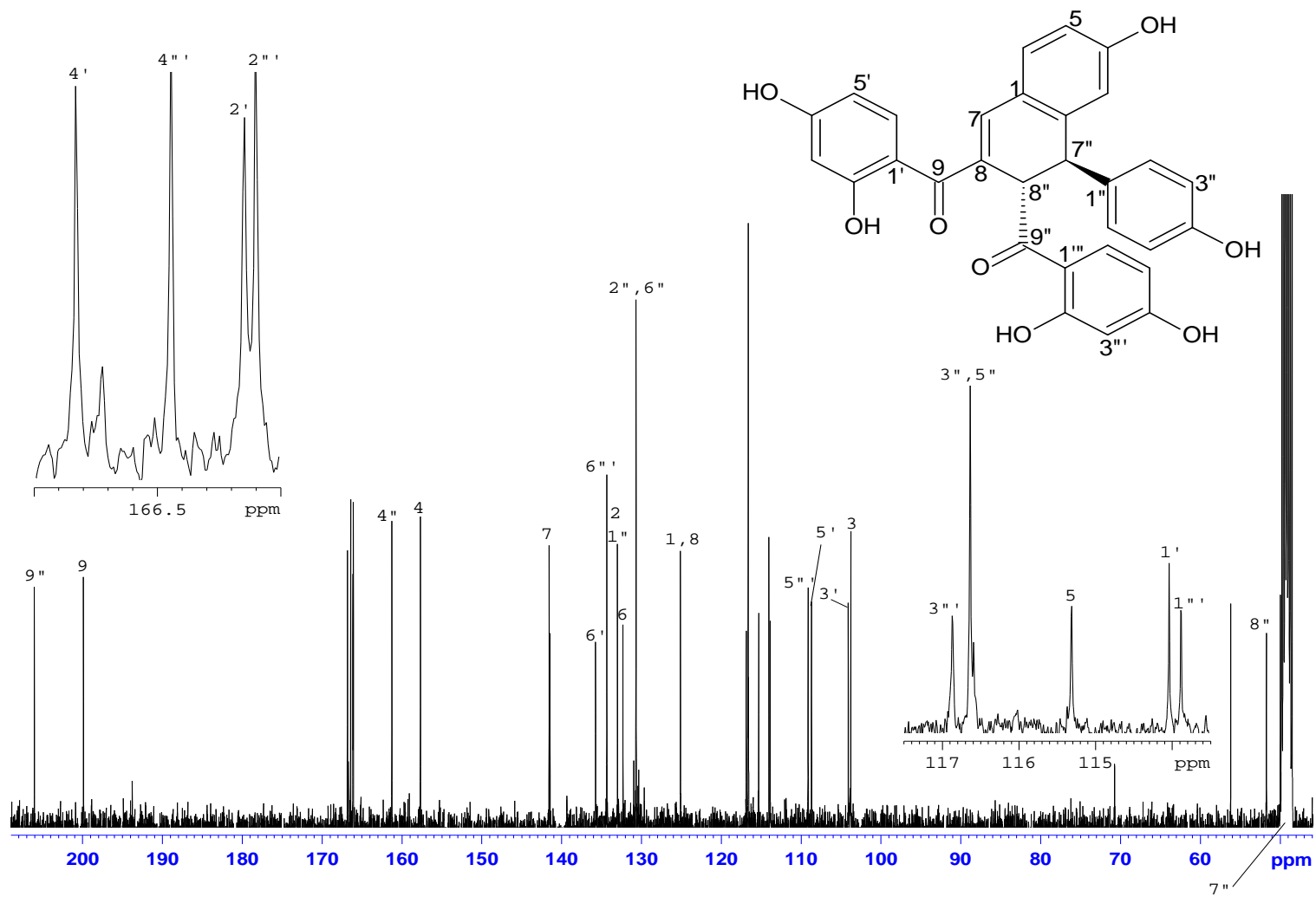
Plate 7d: HSQC NMR Spectrum of 5-deoxyurundeuvine C (76) in CD$_3$OD
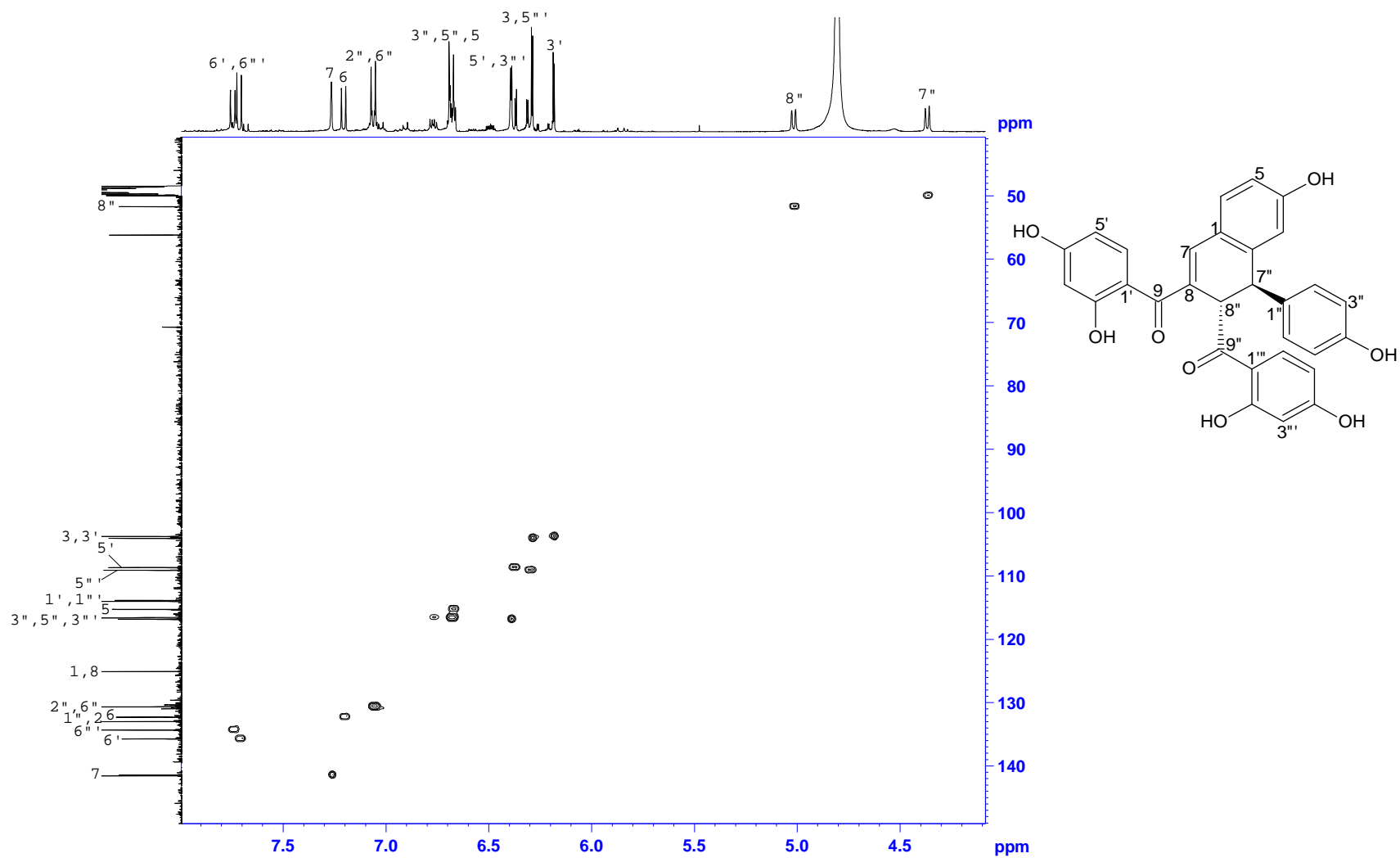
Plate 7e: HMBC NMR Spectrum of 5-deoxyurundeuvine C (76) in CD$_3$OD
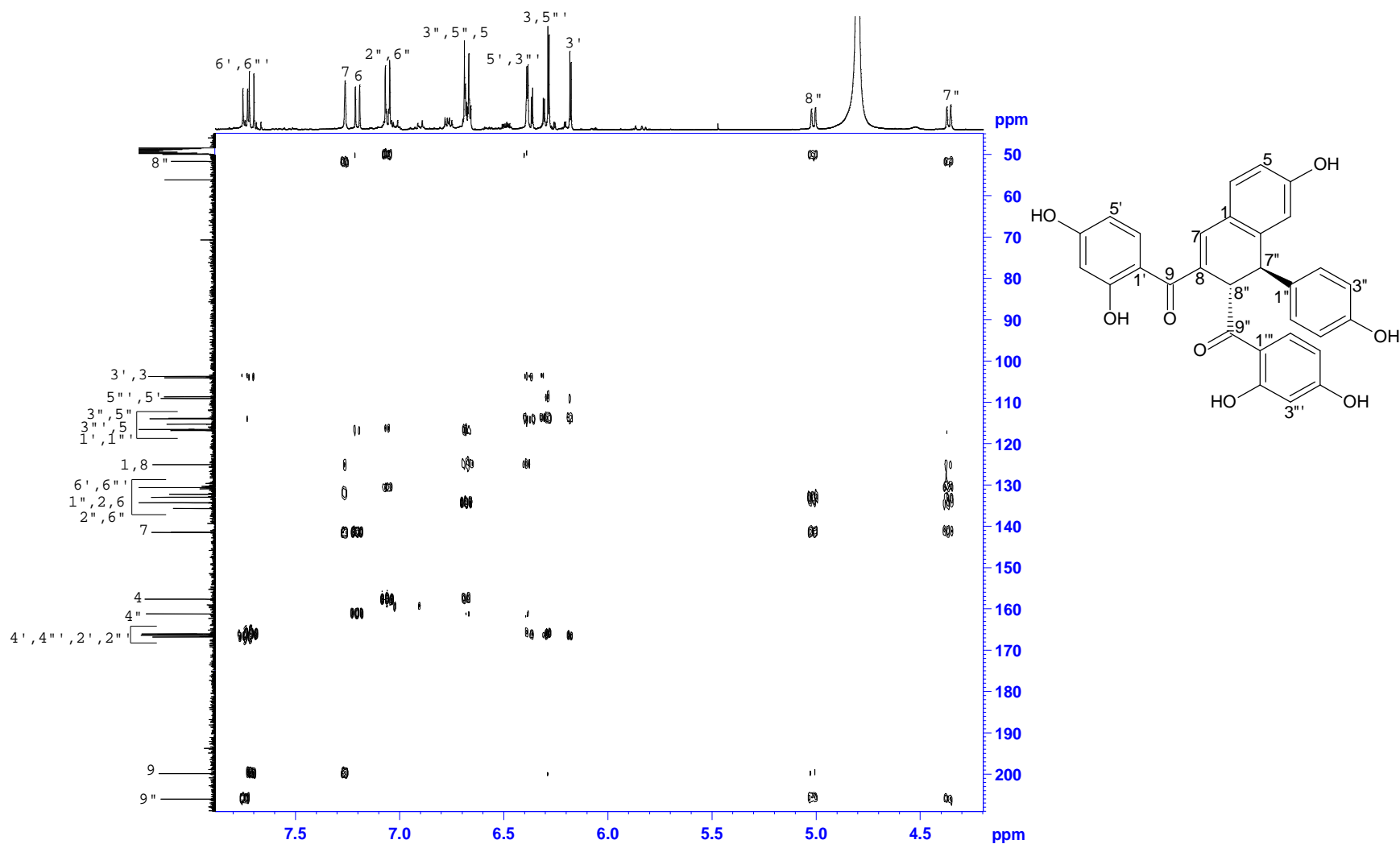
Plate 8a: ^{1}H NMR Spectrum of ochnaflavone (51) in $(CD_3)_2SO$
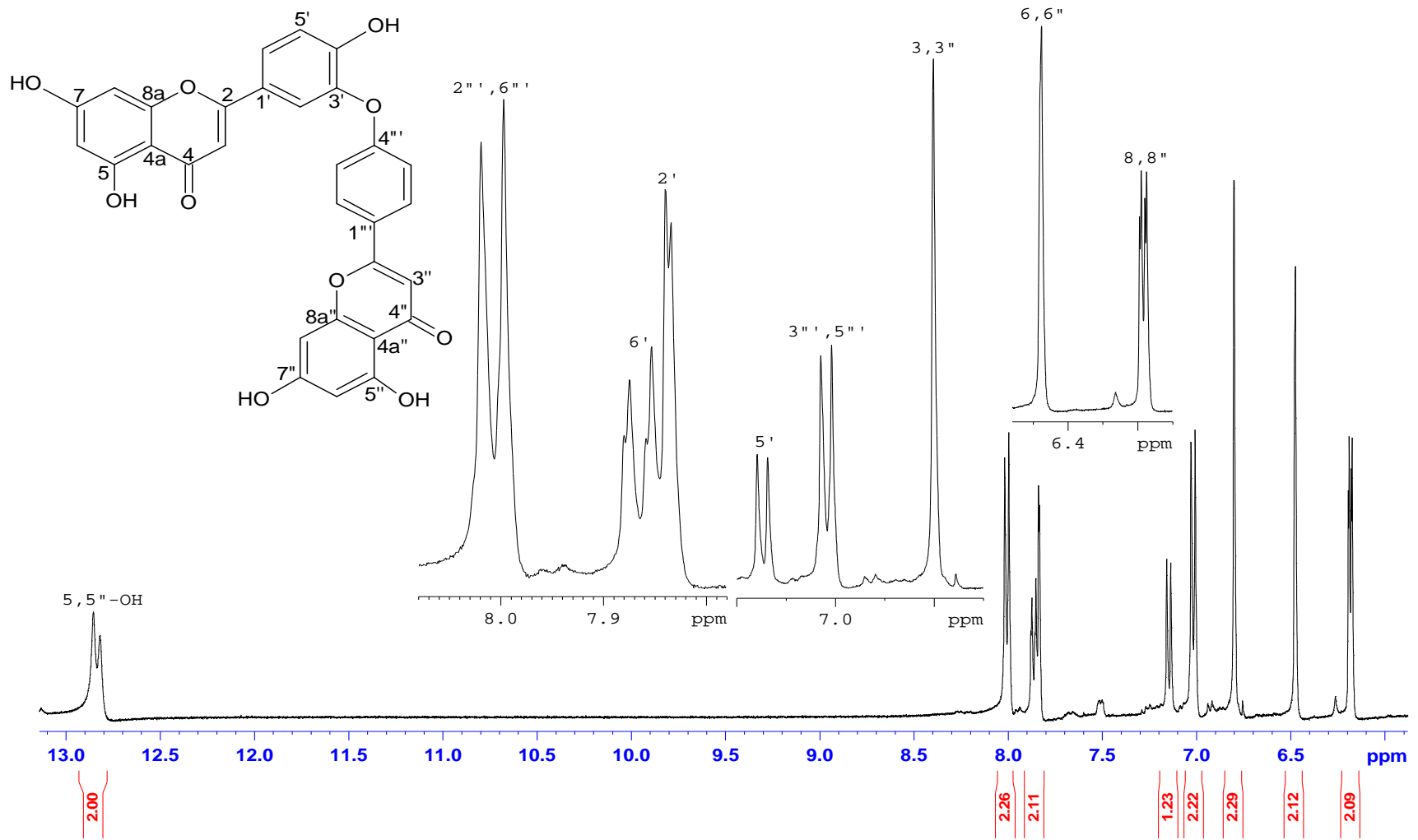
Plate 8b: COSY NMR Spectrum of ochnaflavone (51) in (CD$_3$)$_2$SO
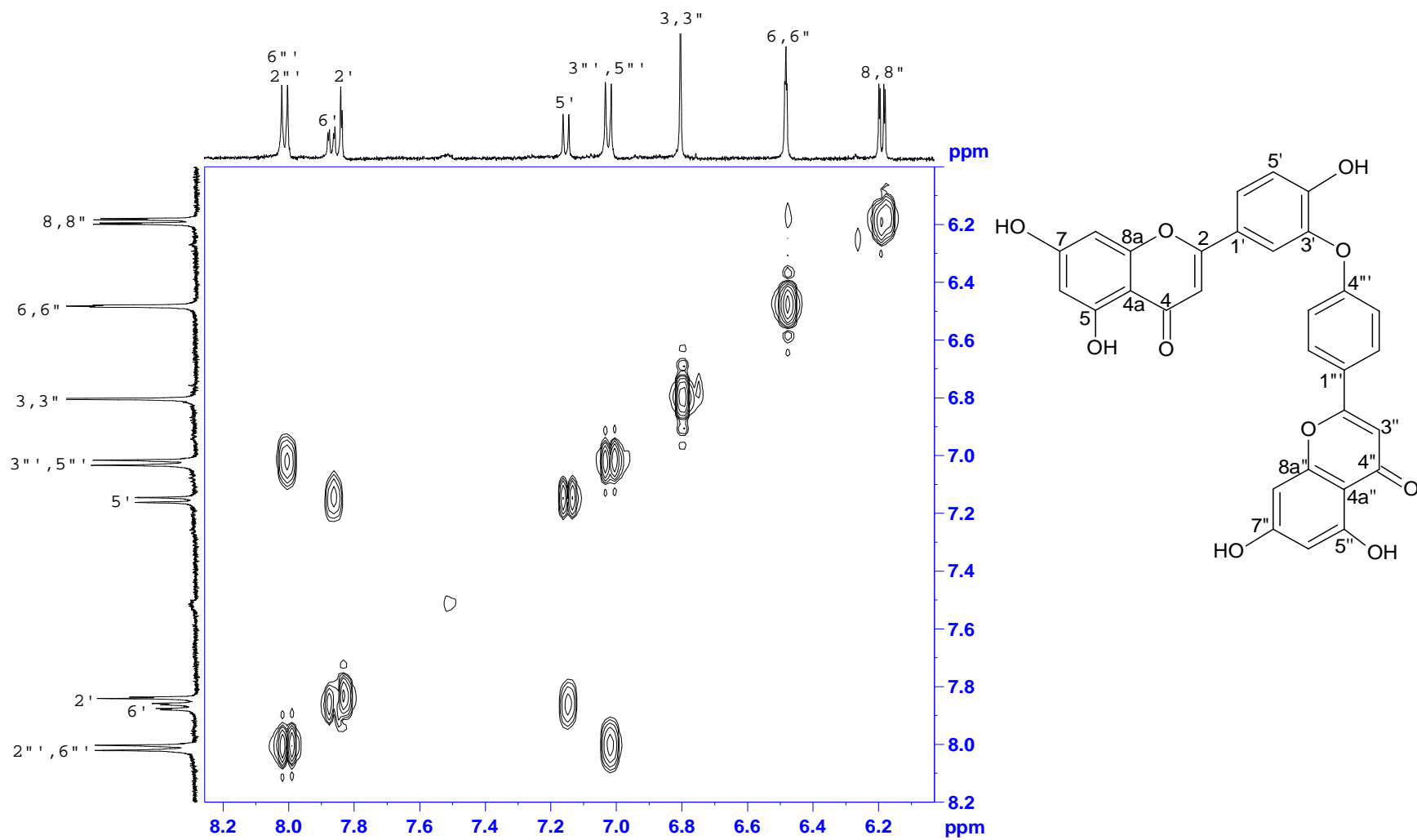
Plate 8c: ^{13}C NMR Spectrum of ochnaflavone (51) in $(CD_3)_2SO$
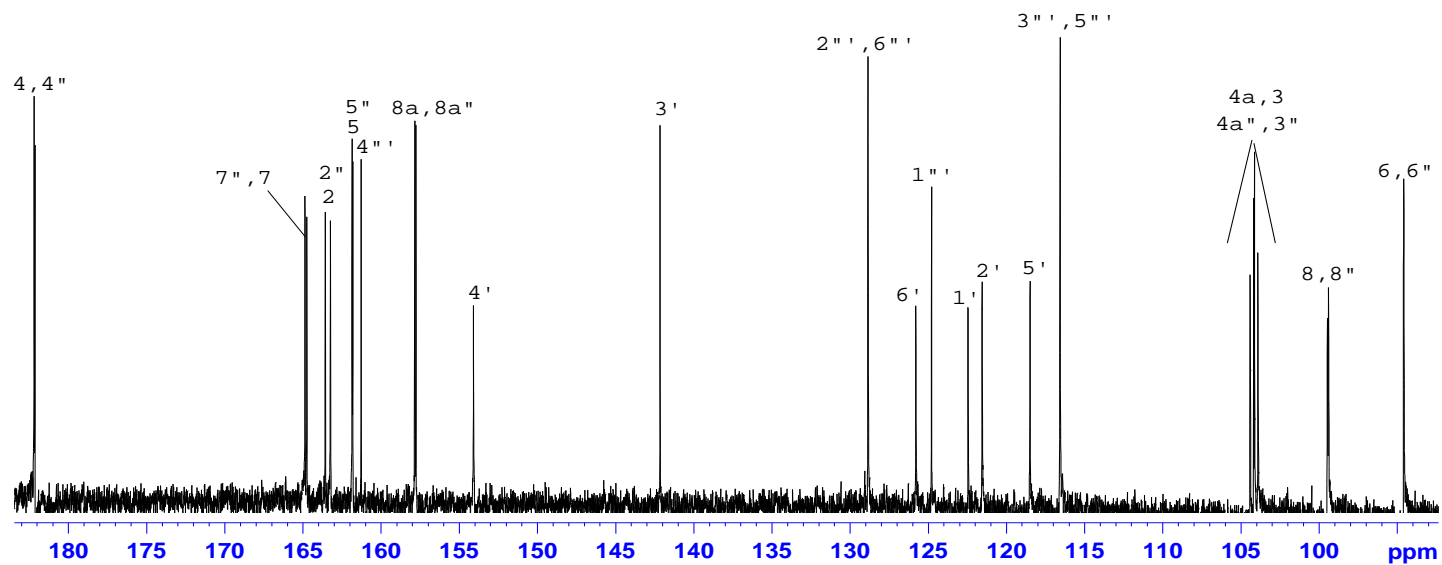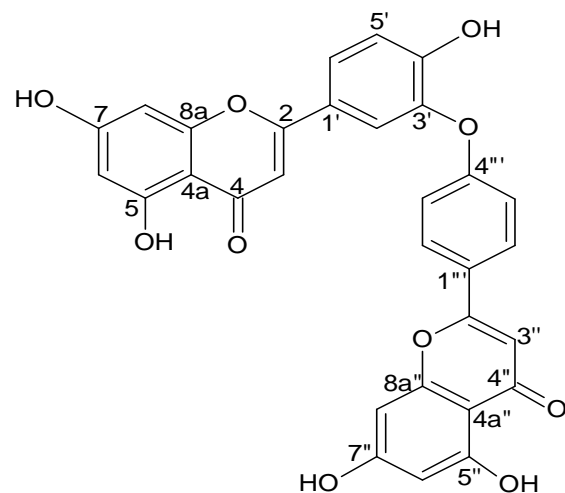
Plate 8d: HSQC NMR Spectrum of ochnaflavone (51) in $(CD_3)_2SO$
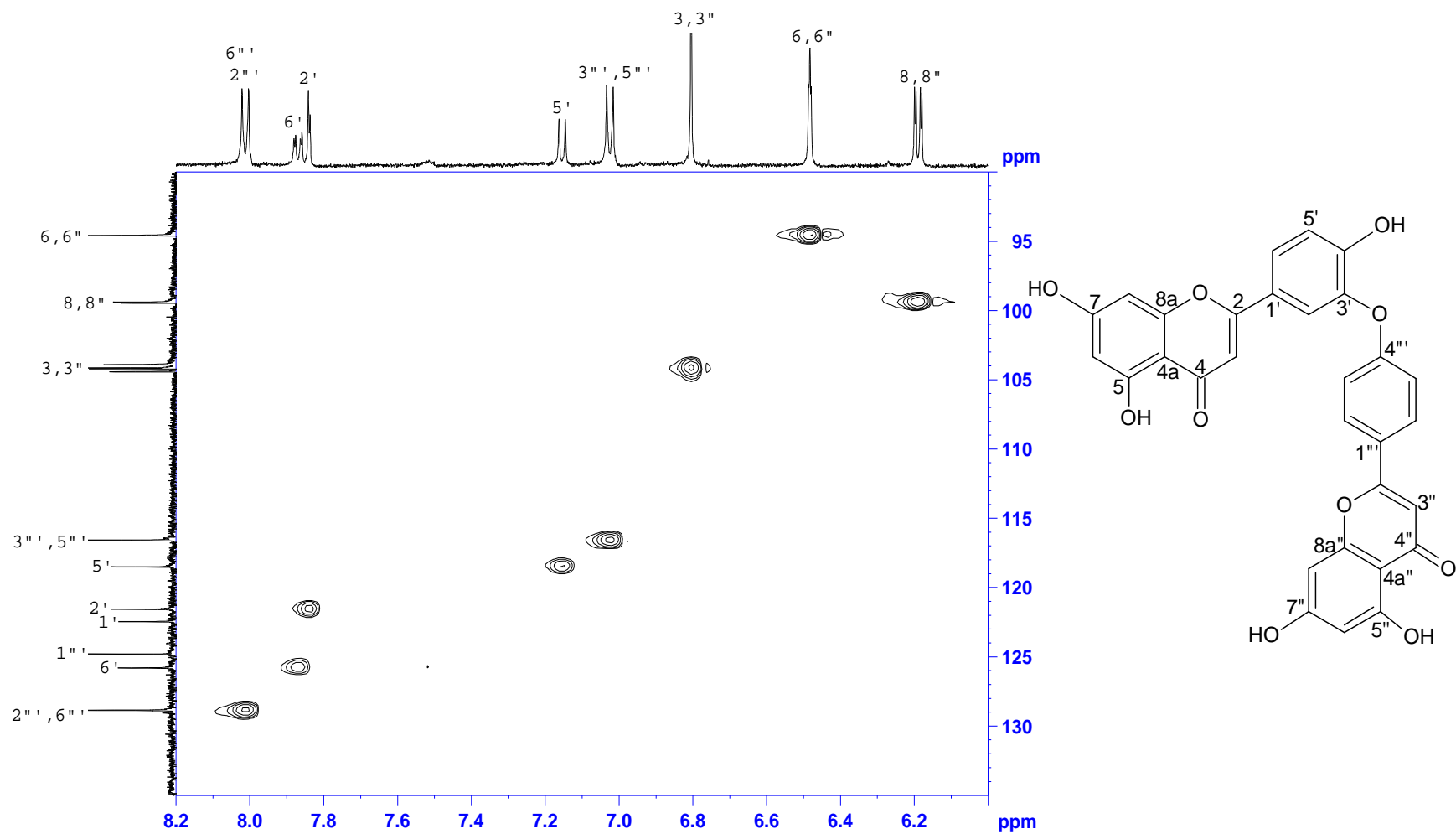
Plate 8e: HMBC NMR Spectrum of ochnaflavone (51) in (CD$_3$)$_2$SO
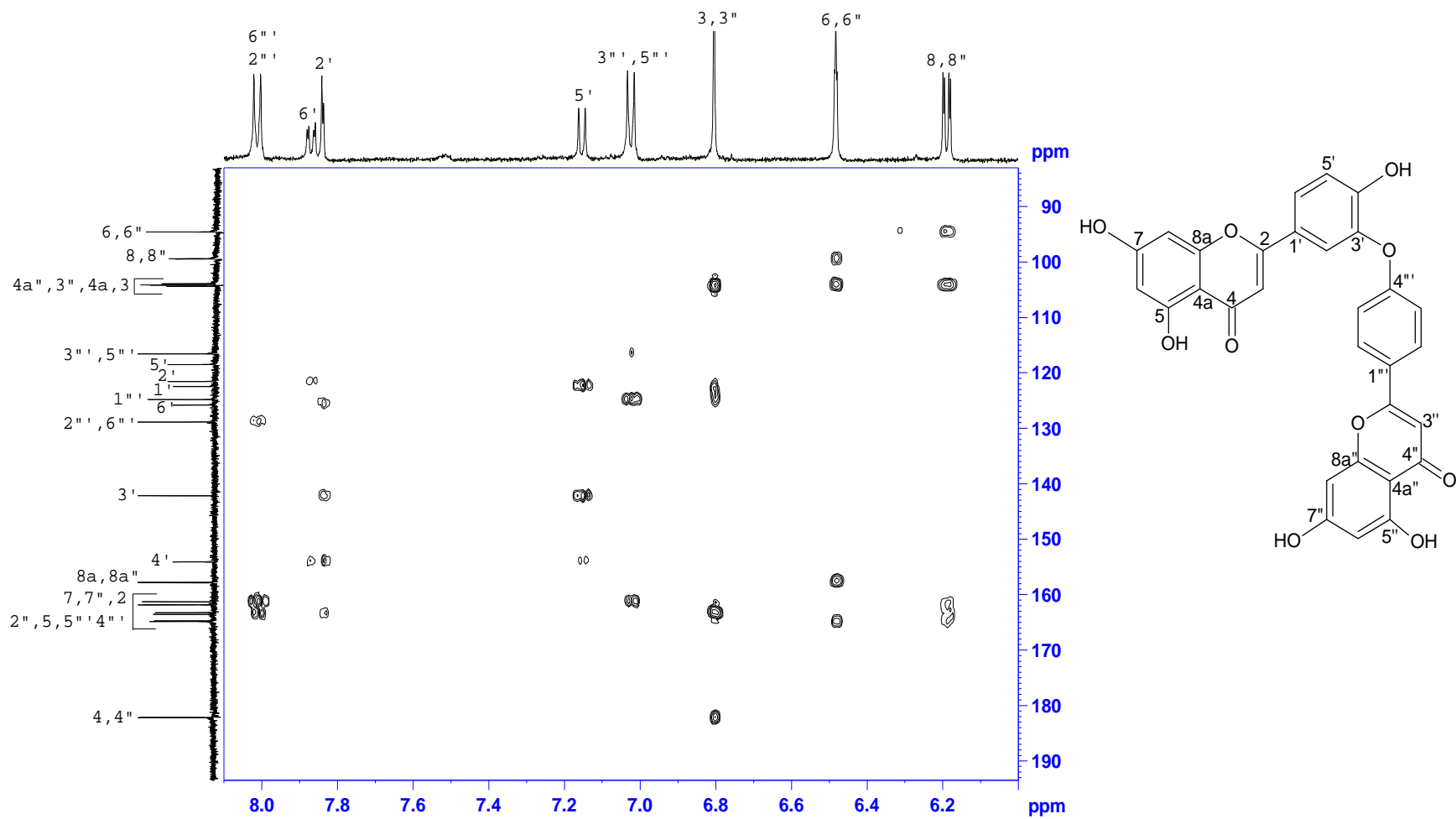
Plate 8f: DEPT-135 NMR Spectrum of ochnaflavone (51) in $(CD_3)_2SO$
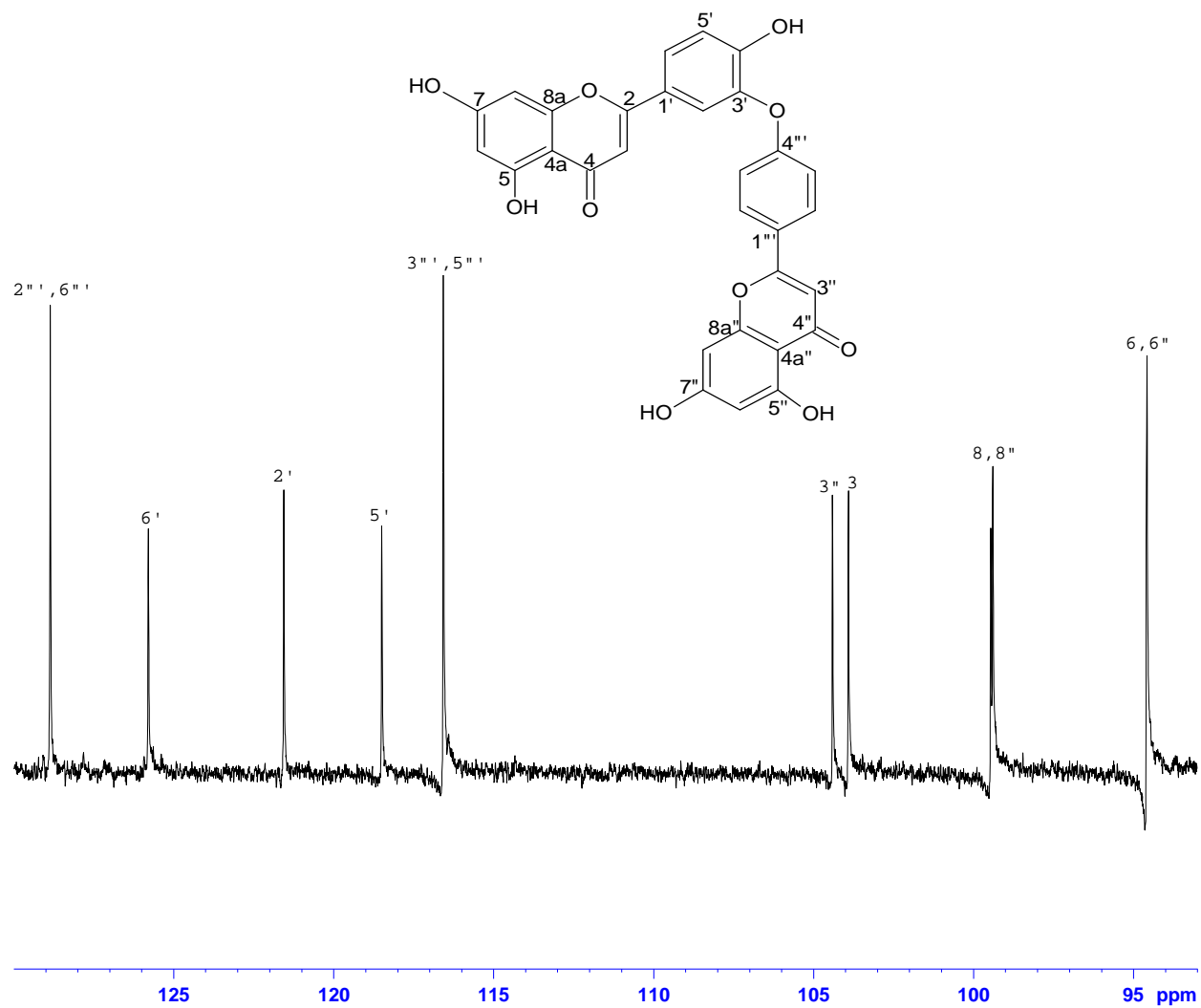
Plate 9a: ^{1}H NMR Spectrum of 2'',3''-dihydroochnaflavone (54) in (CD$_3$)$_2$CO
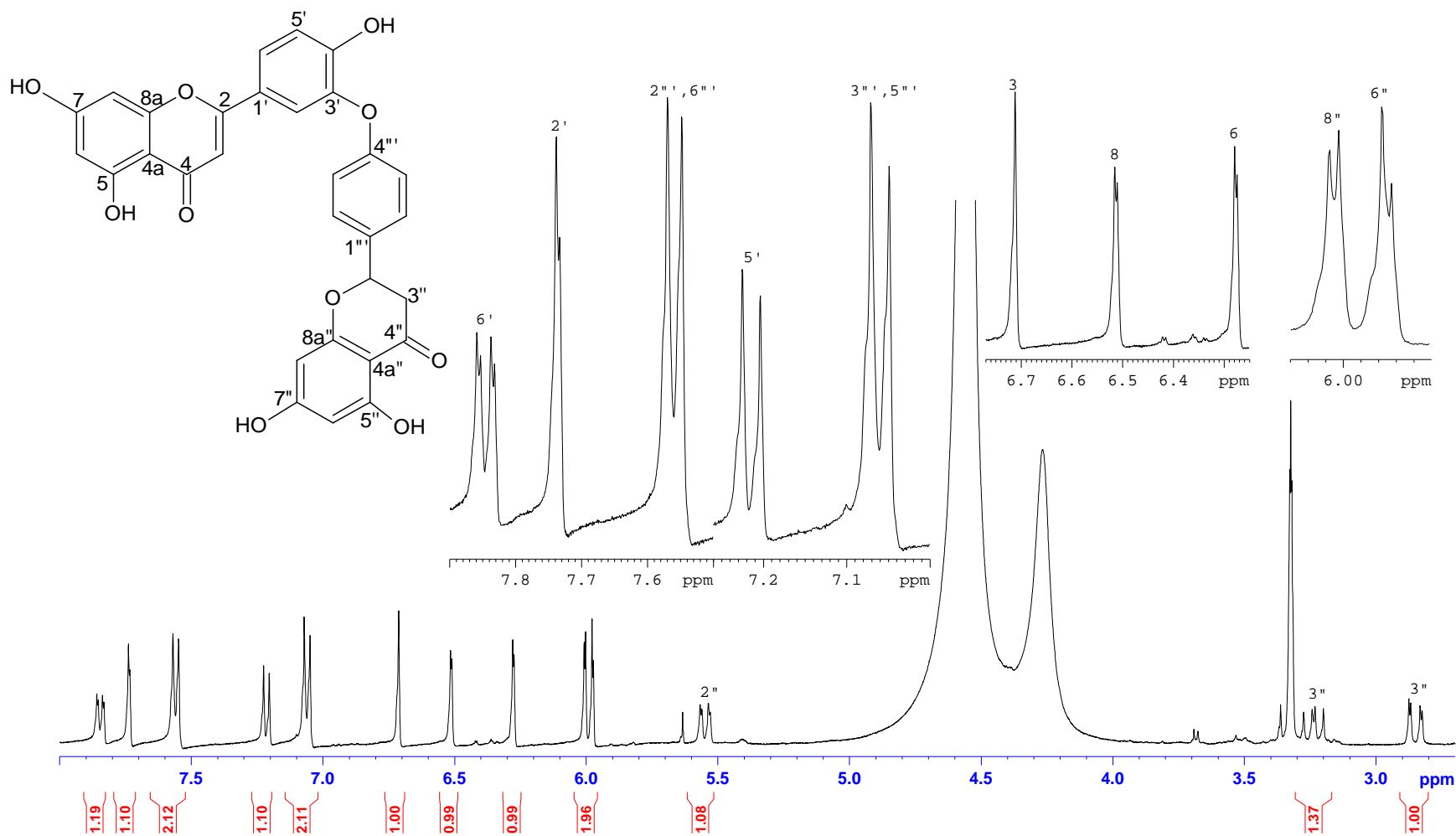
Plate 9b: COSY NMR Spectrum of 2'',3''-dihydroochnaflavone (54) in (CD$_3$)$_2$CO
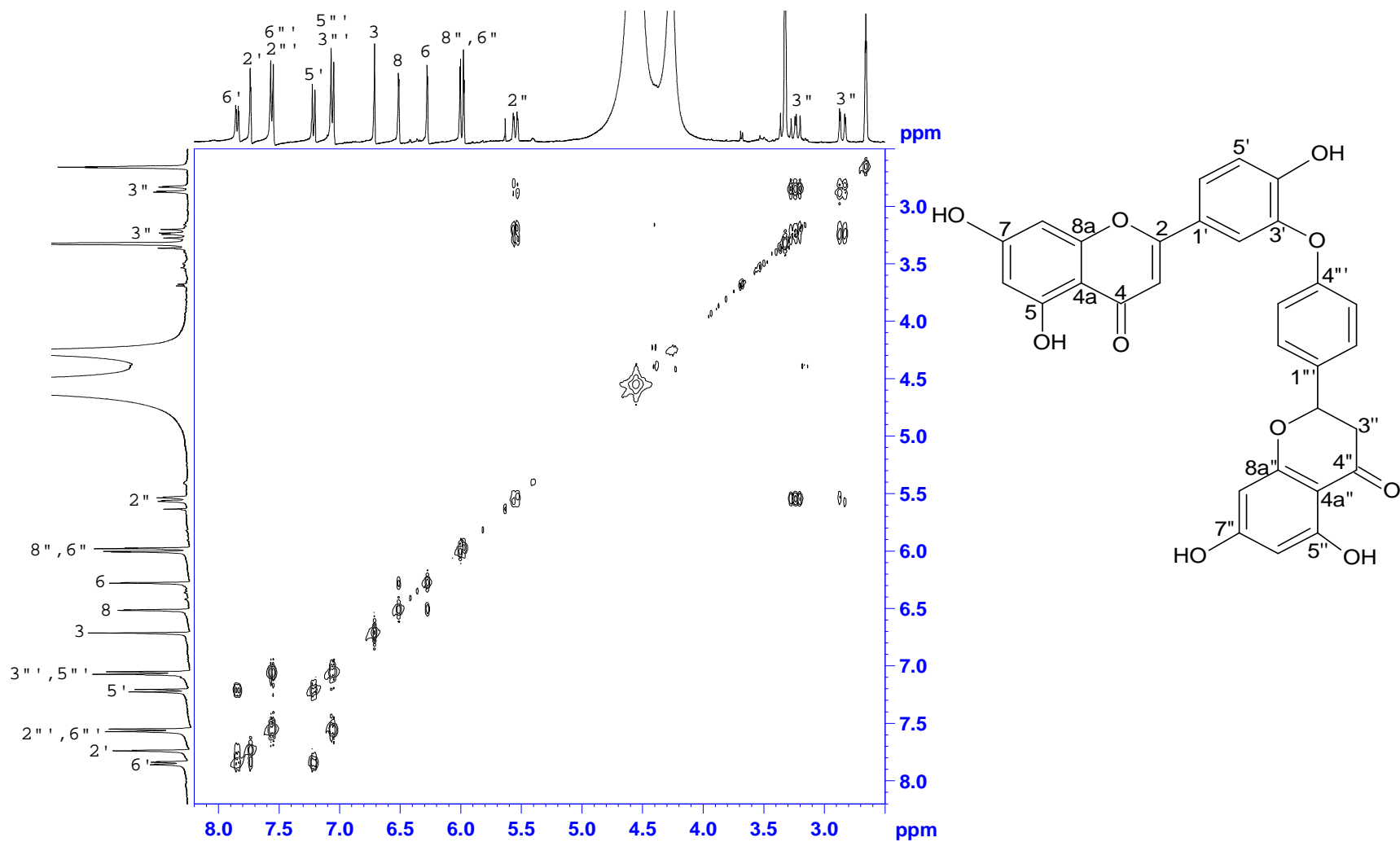
Plate 9c: ^{13}C NMR Spectrum of 2'',3''-dihydroochnaflavone (54) in $(CD_3)_2CO$
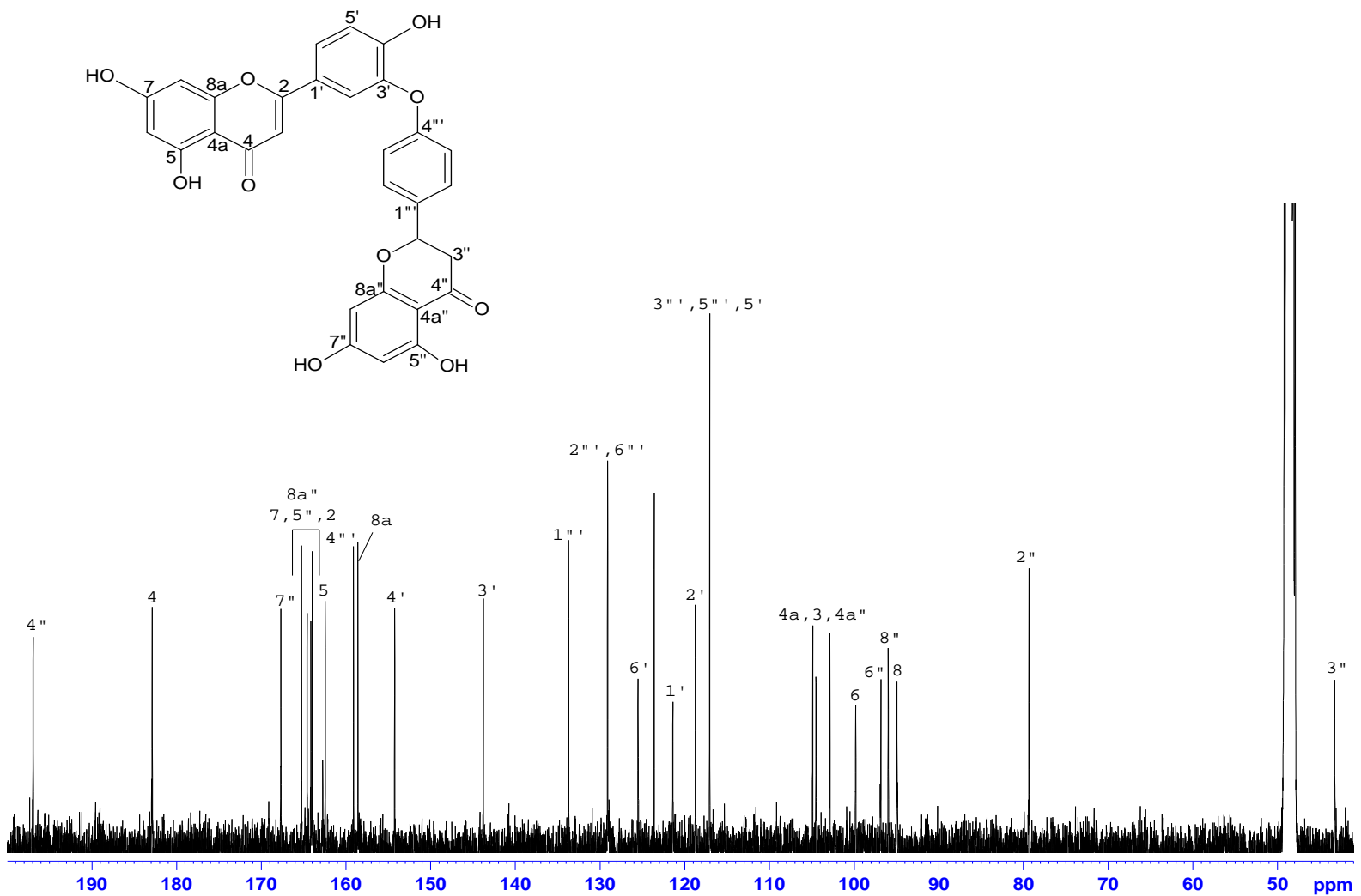
Plate 9d: HSQC NMR Spectrum of 2'',3''-dihydroochnaflavone (54) in $(CD_3)_2CO$
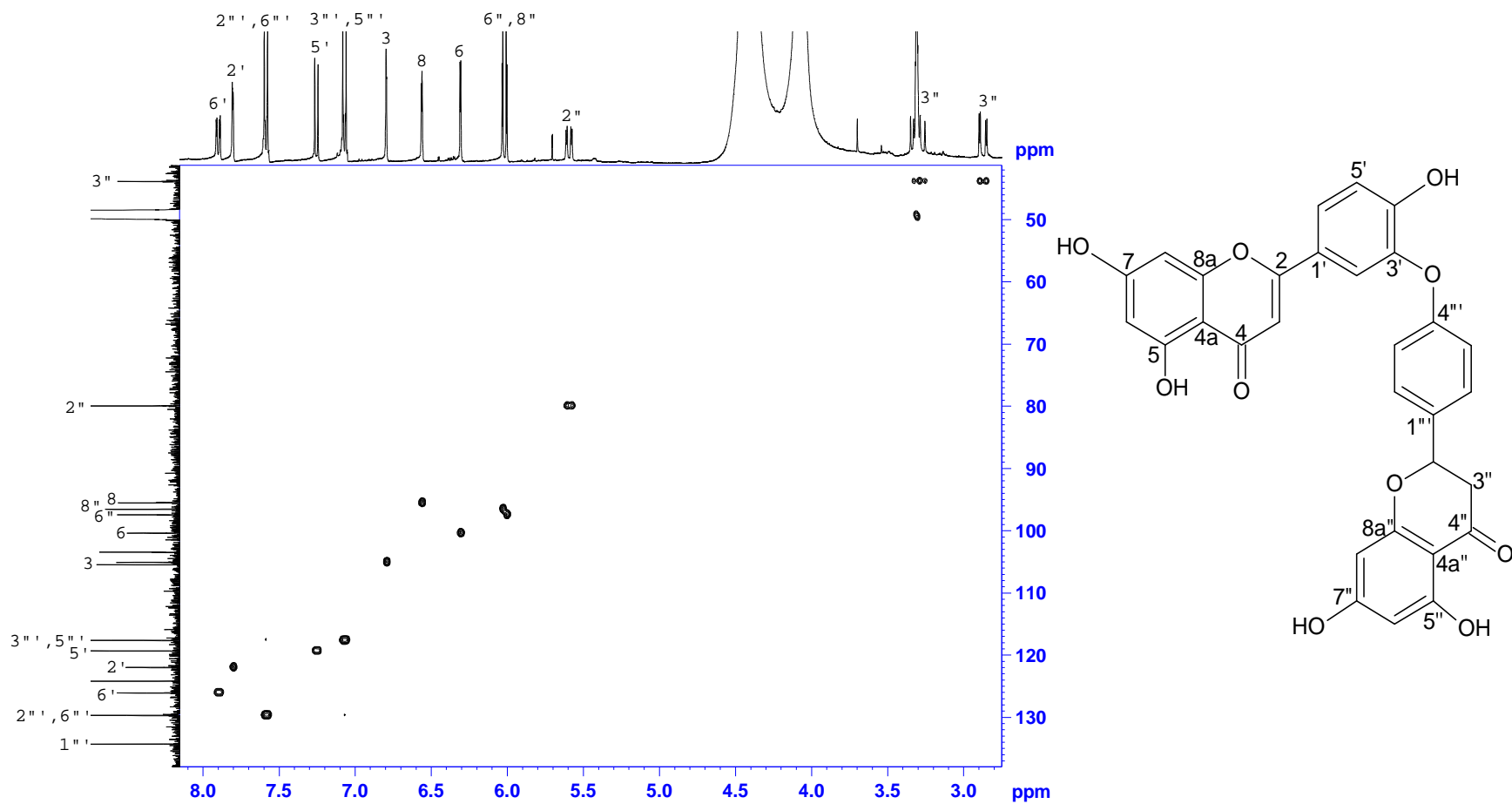
Plate 9e: HMBC NMR Spectrum of 2'',3''-dihydroochnaflavone (54) in $(CD_3)_2CO$
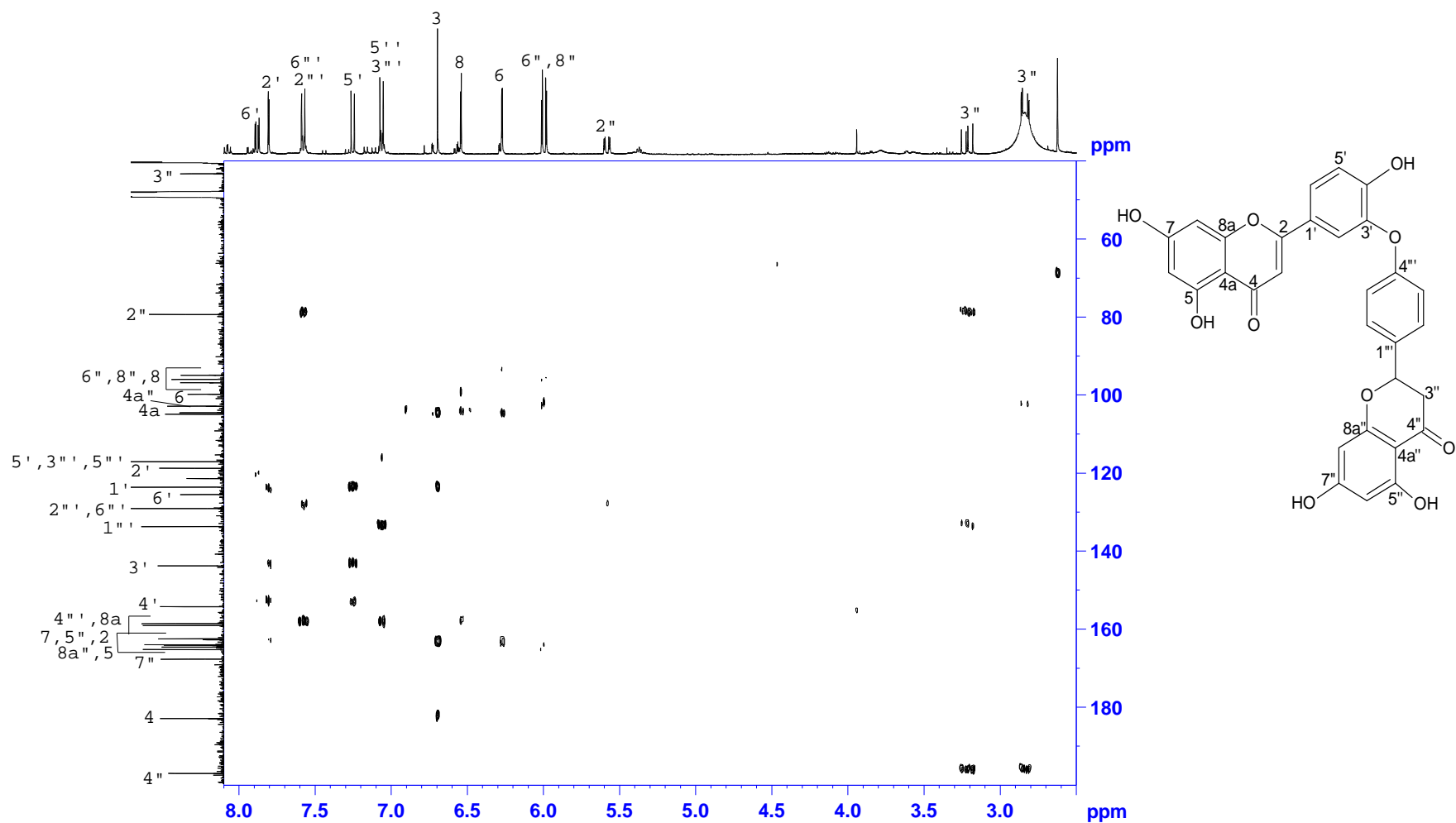
Plate 10a: ^{1}H NMR Spectrum of psilosin (77) in $(CD_3)_2SO$
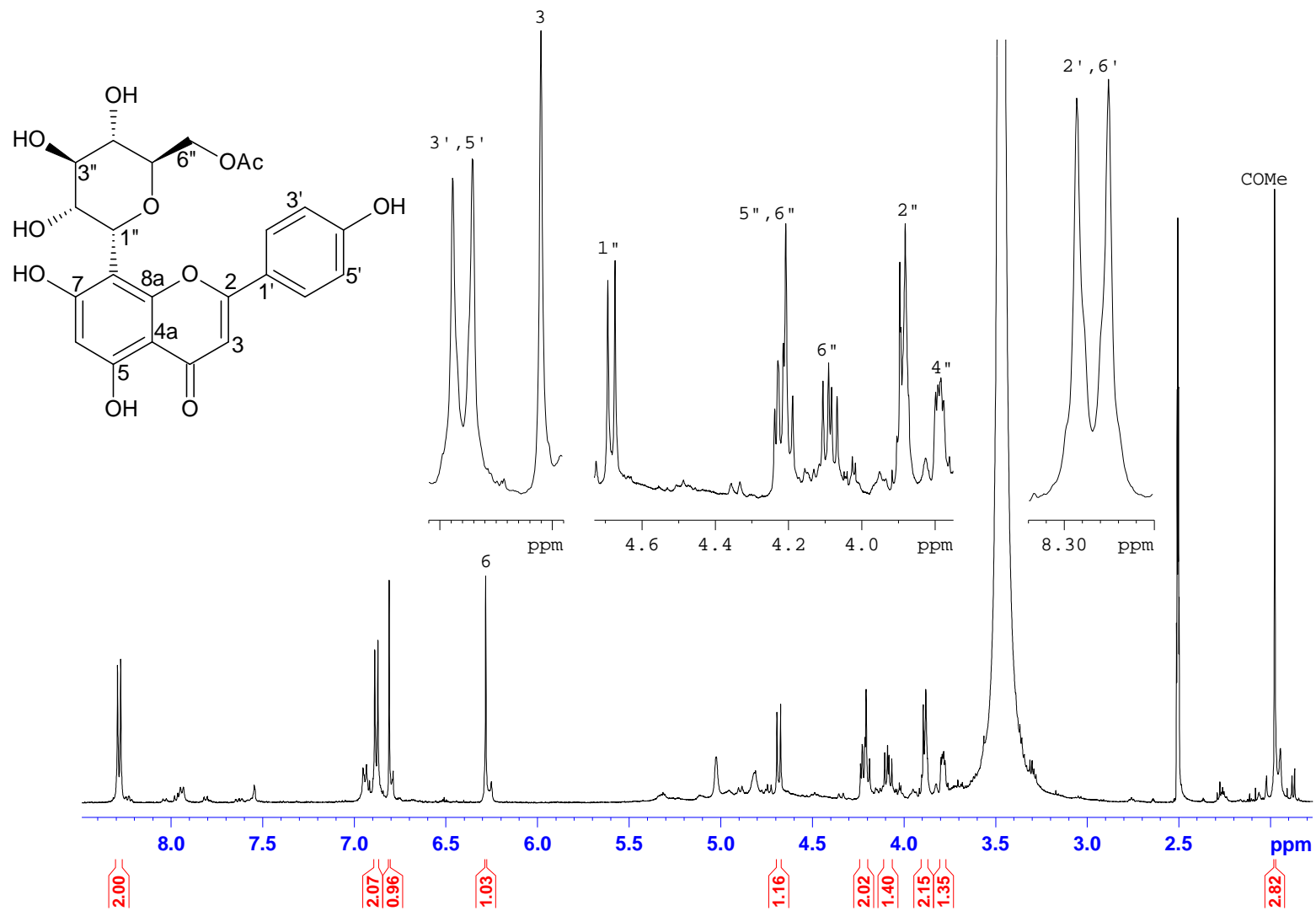
Plate 10b: COSY NMR Spectrum of psilosin (77) in $(CD_3)_2SO$
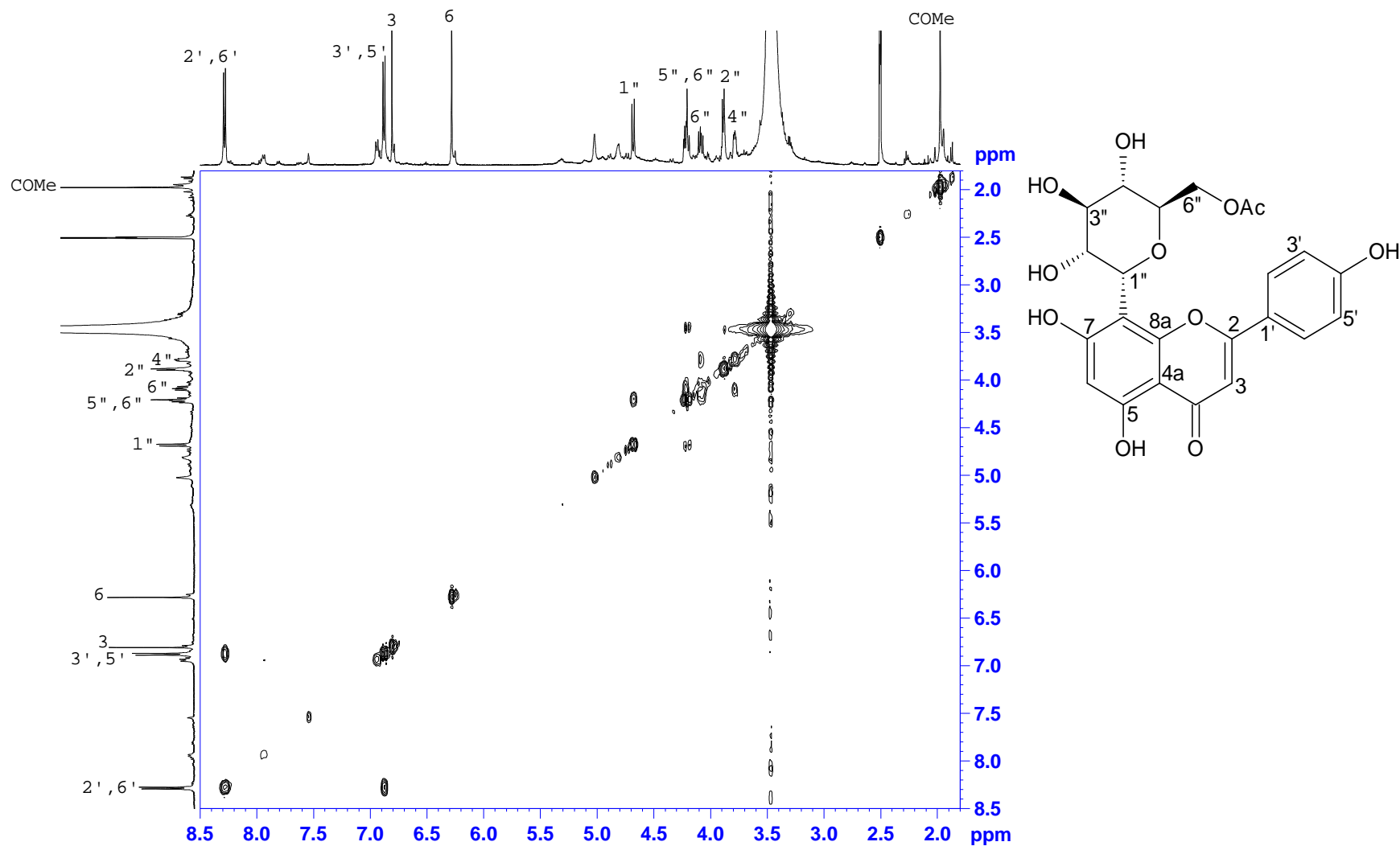
Plate 10c: ^{13}C NMR Spectrum of psilosin (77) in (CD$_3$)$_2$SO
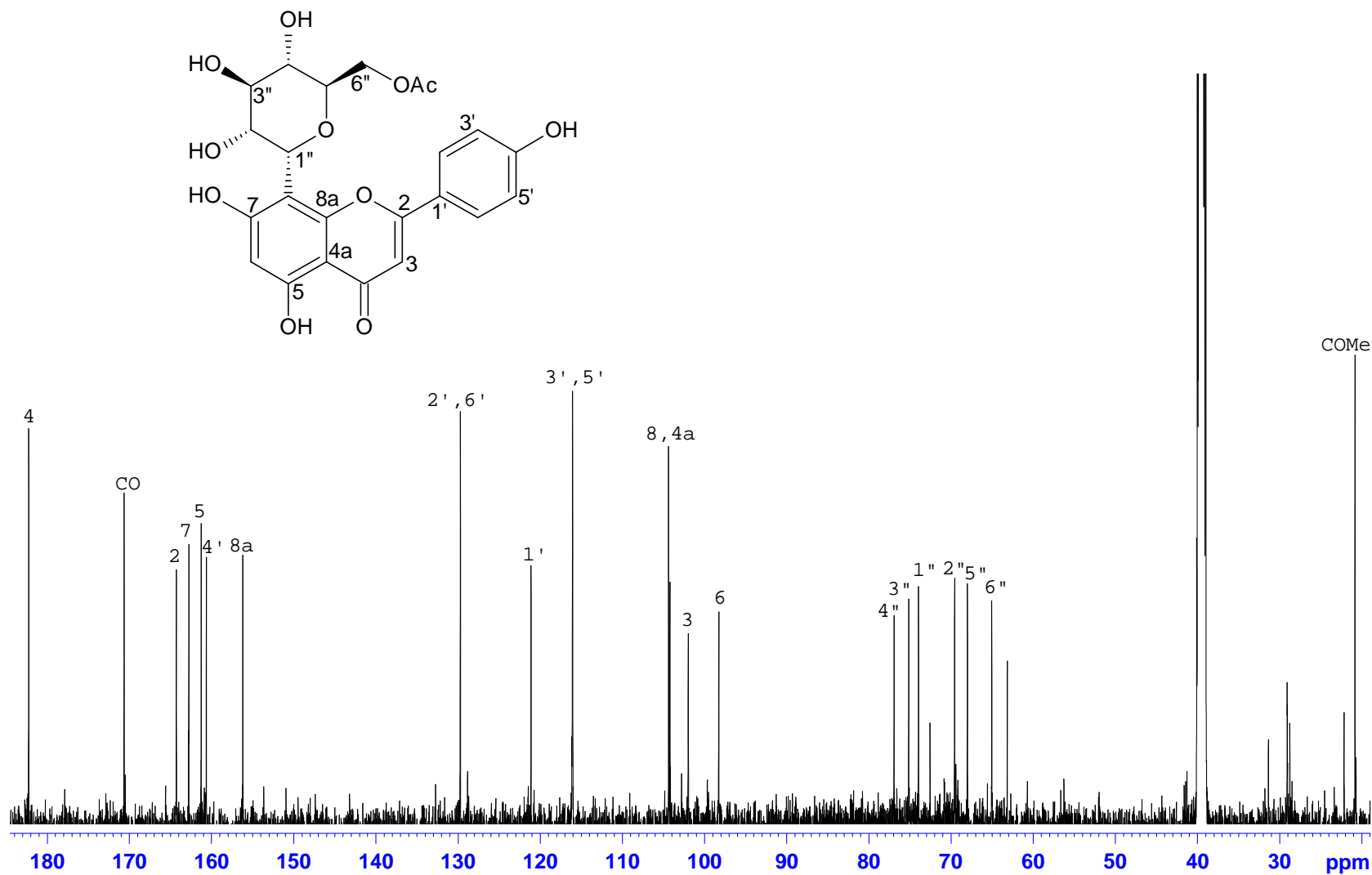
Plate 10d: HSQC NMR Spectrum of psilosin (77) in $(CD_3)_2SO$
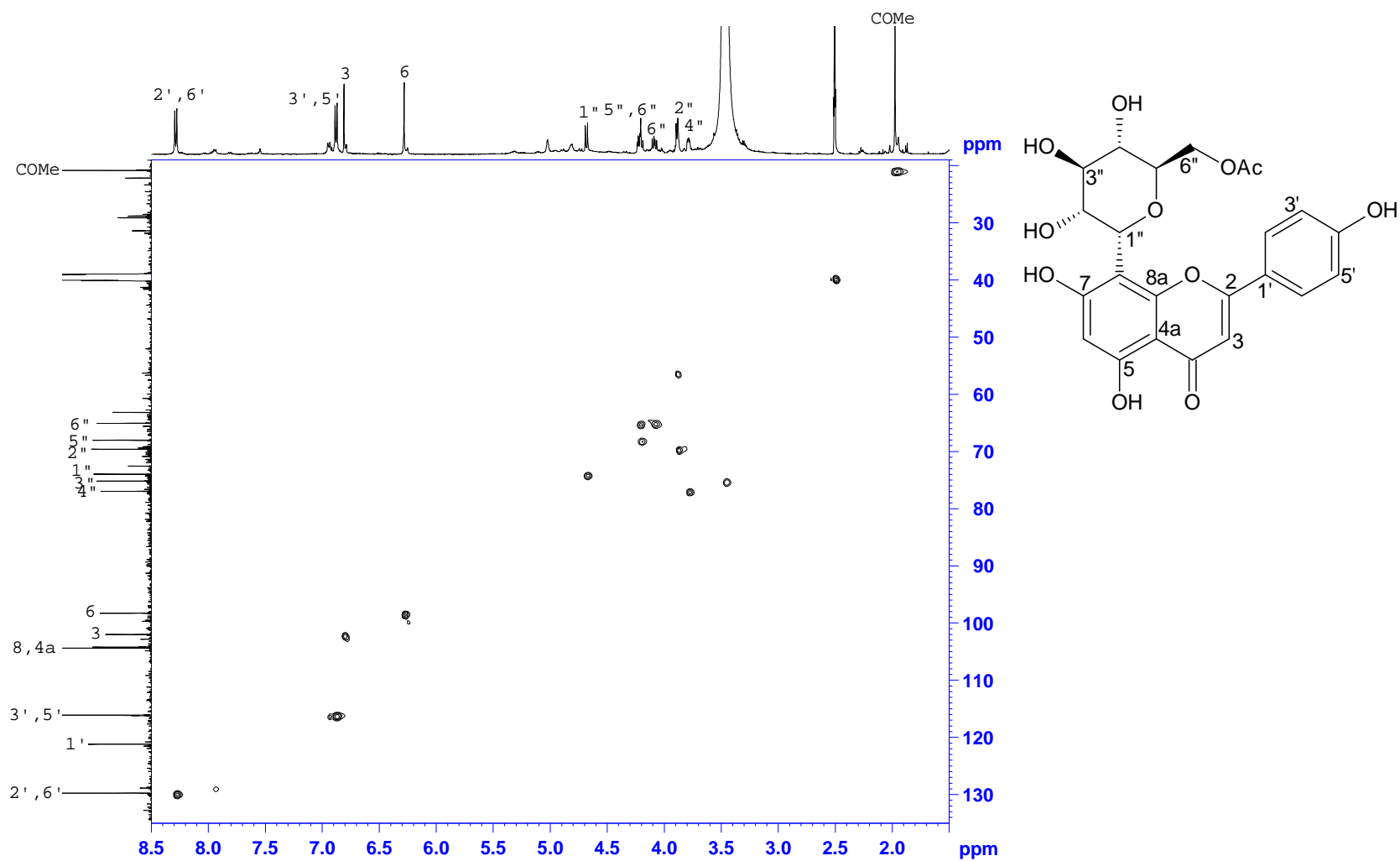
Plate 10e: HMBC NMR Spectrum of psilosin (77) in $(CD_3)_2SO$
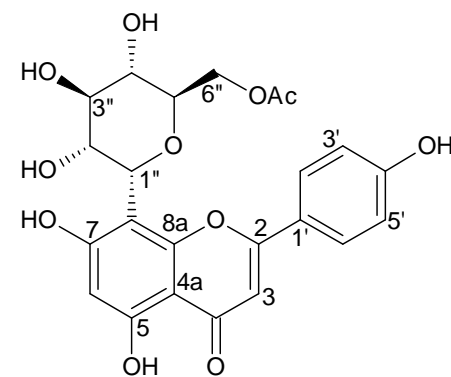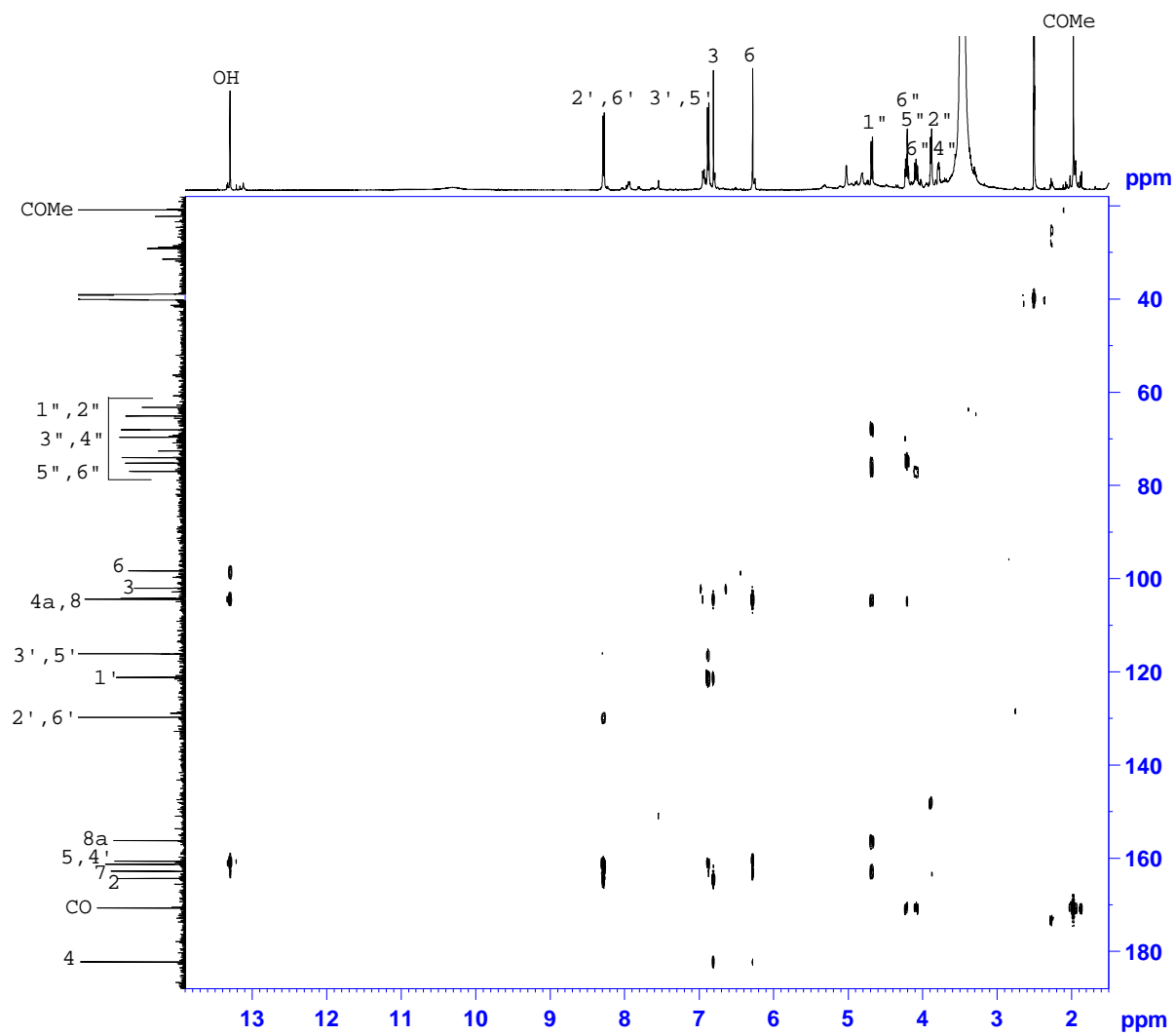
Plate 10f: DEPT-135, 90 NMR Spectrum of psilosin (77) in (CD₃)₂SO

DEPT-90

DEPT-135

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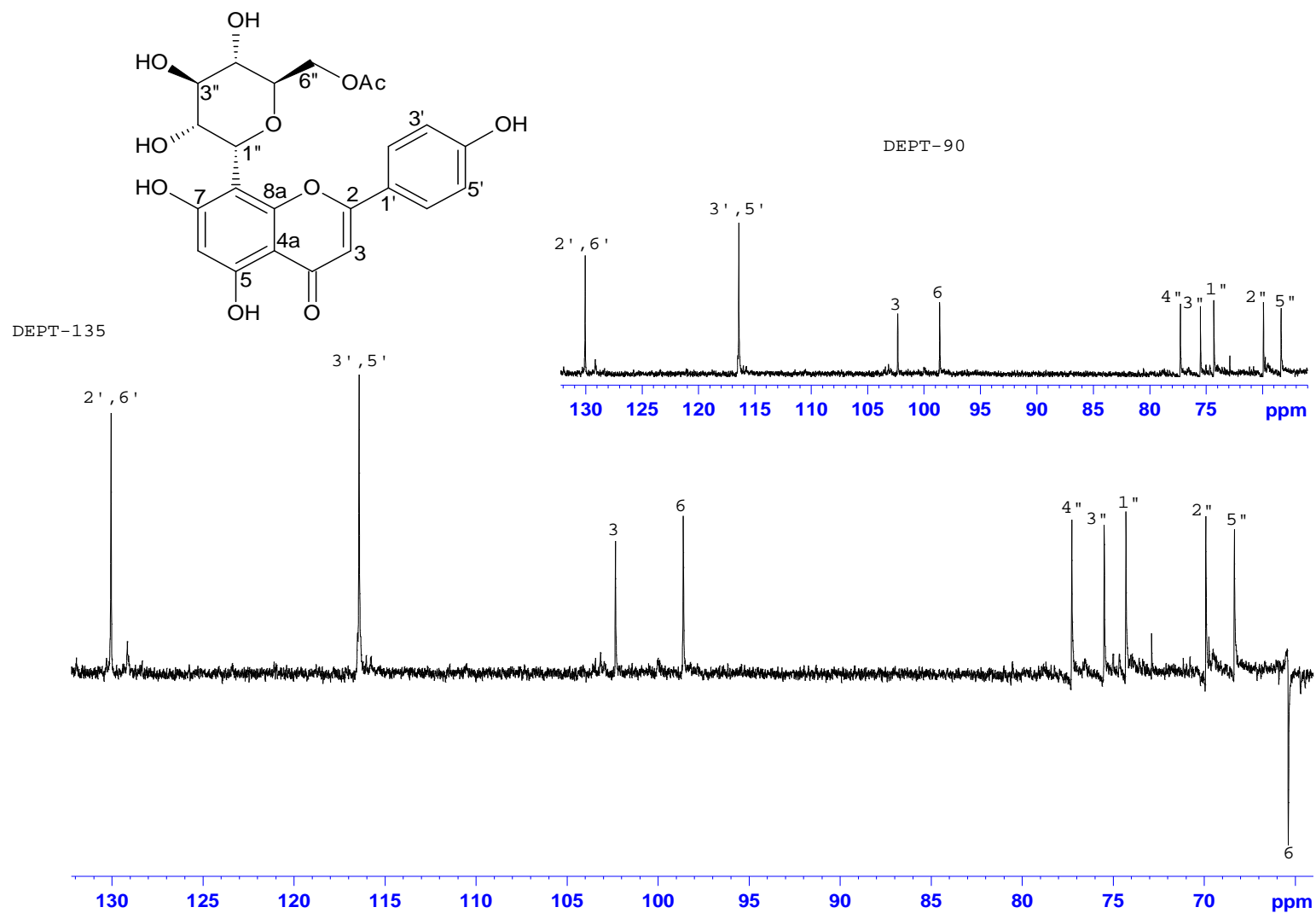
Plate 11a: ^{1}H NMR Spectrum of 3'-methoxypsilosin (78) in (CD$_3$)$_2$SO
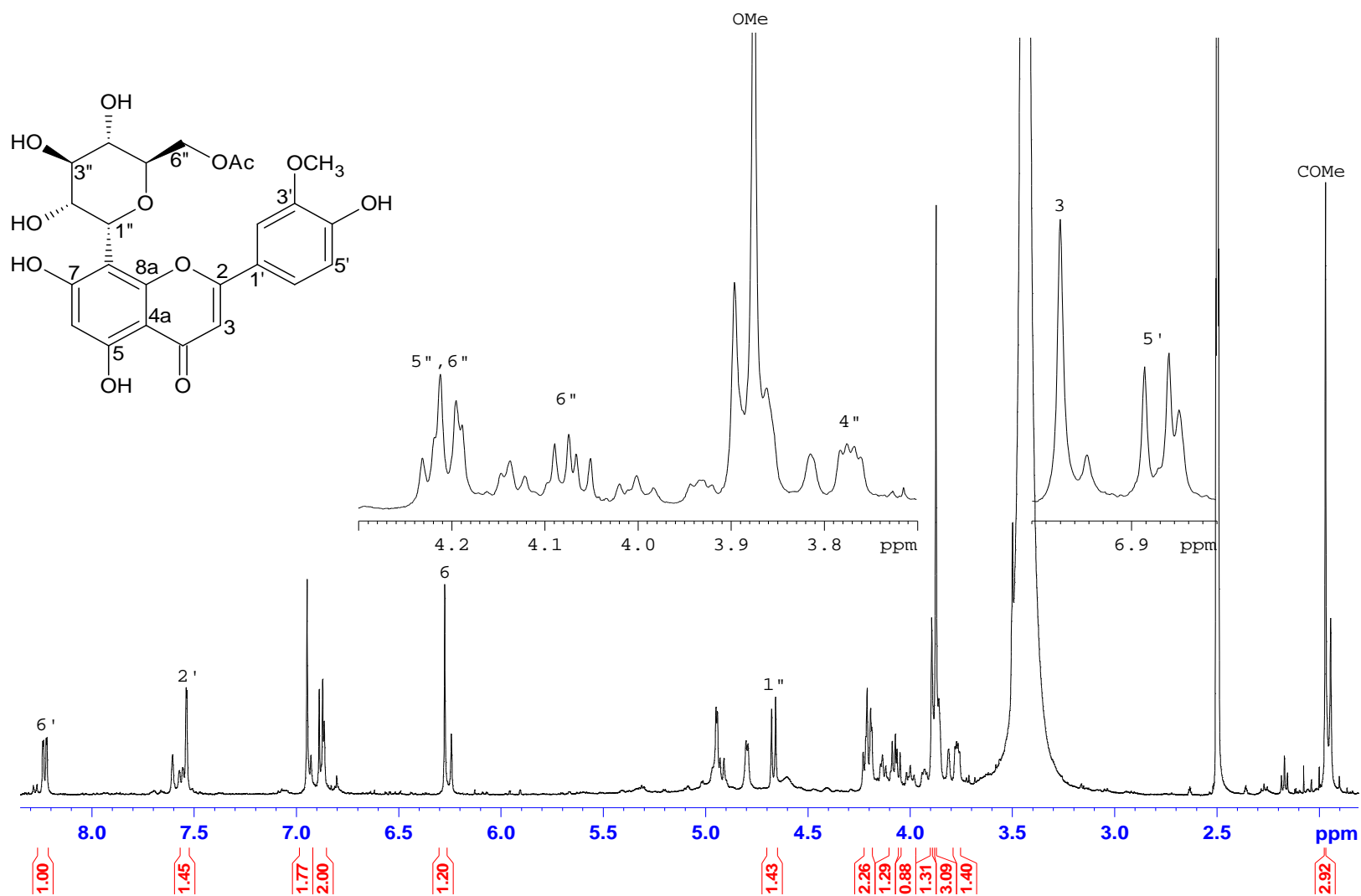
Plate 11b: COSY NMR Spectrum of 3'-methoxypsilosin (78) in $(CD_3)_2SO$
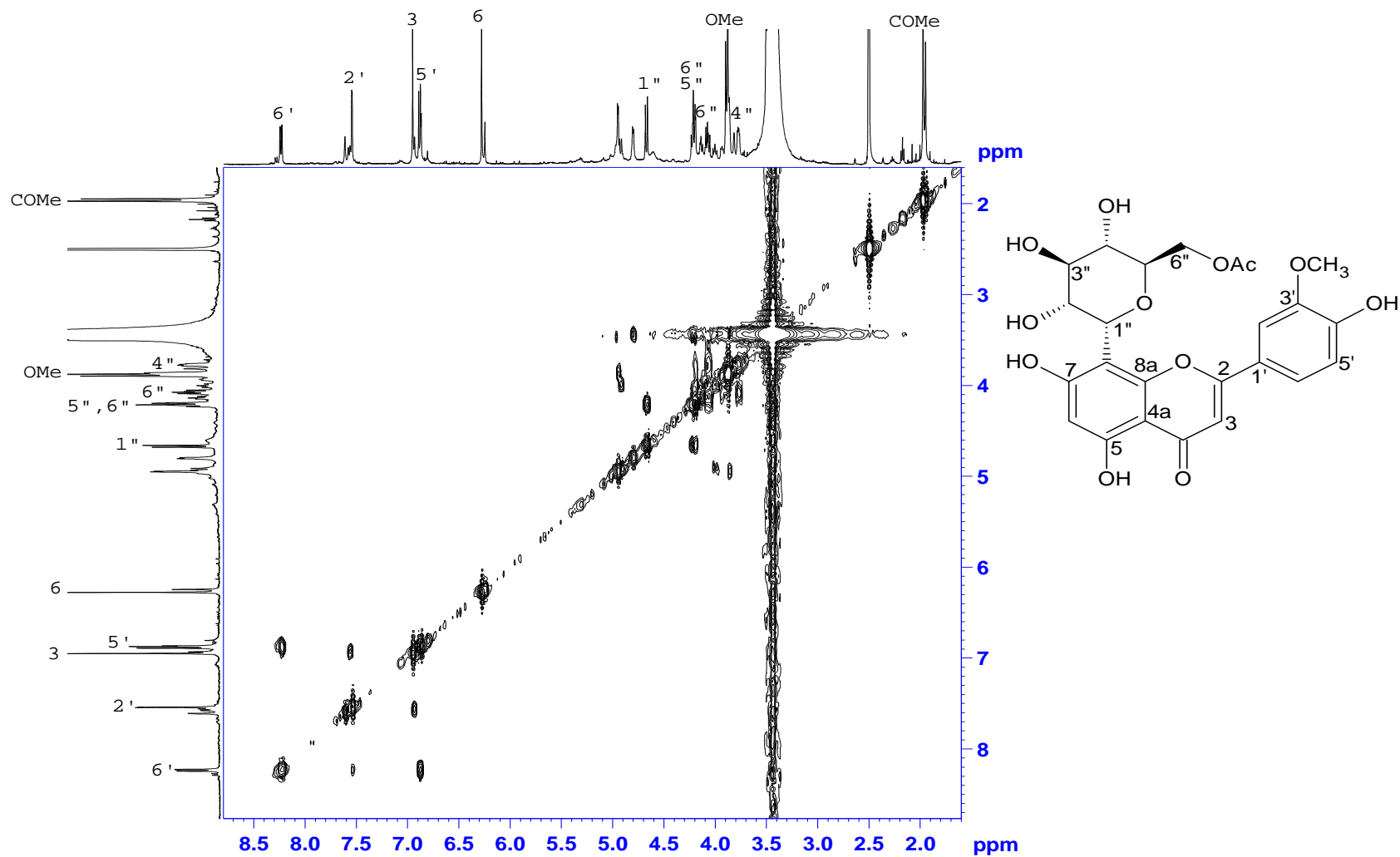
Plate 11c: ^{13}C NMR Spectrum of 3'-methoxypsilosin (78) in $(CD_3)_2SO$

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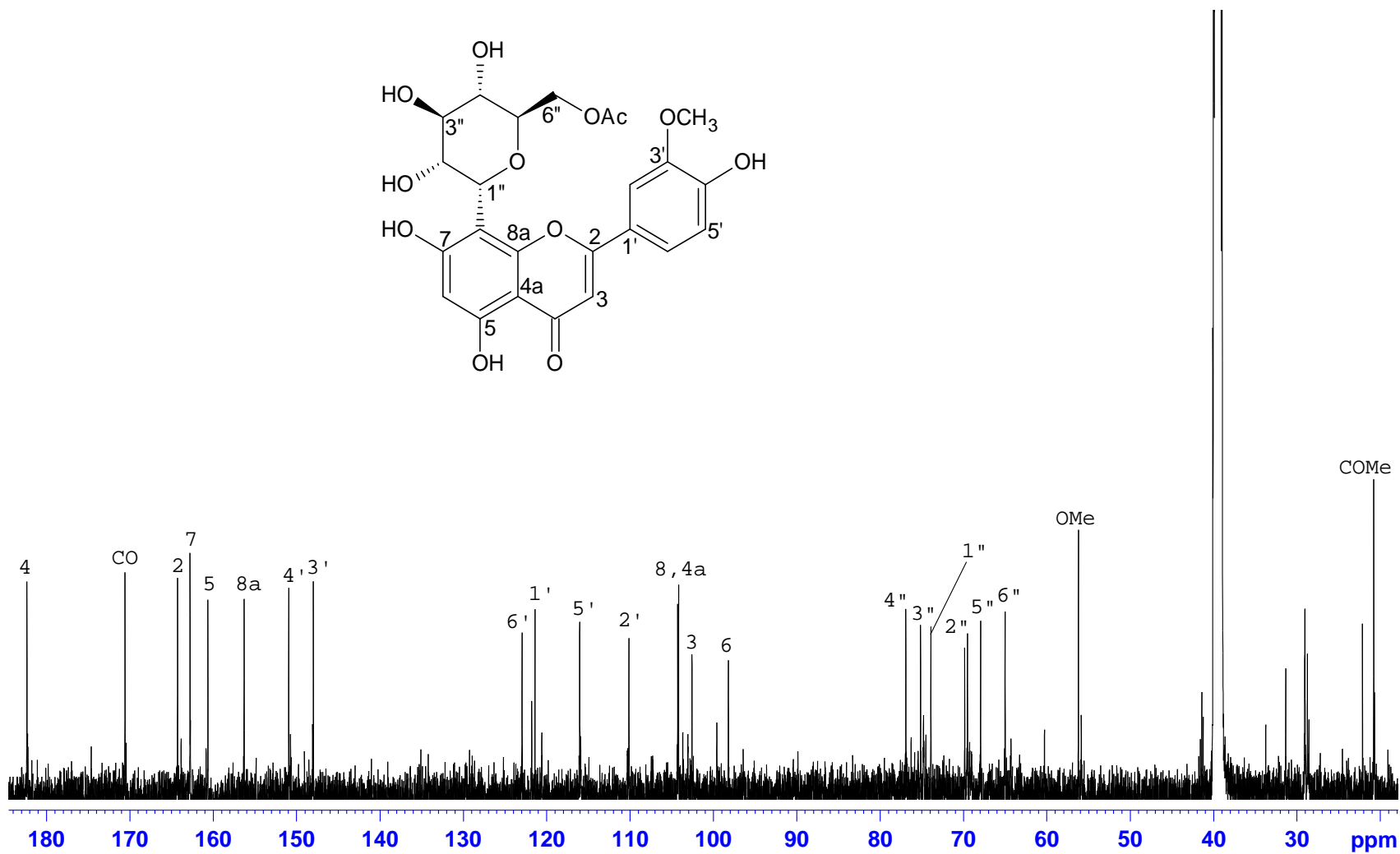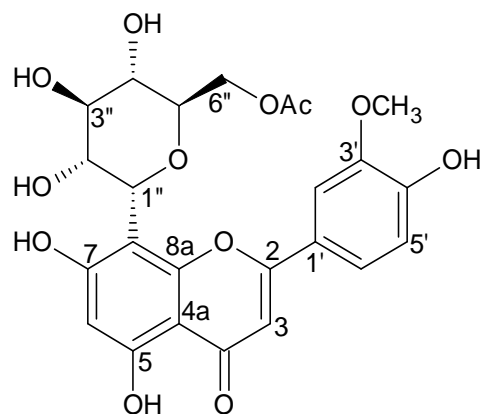
Plate 11d: HSQC NMR Spectrum of 3'-methoxypsilosin (78) in (CD$_3$)$_2$SO
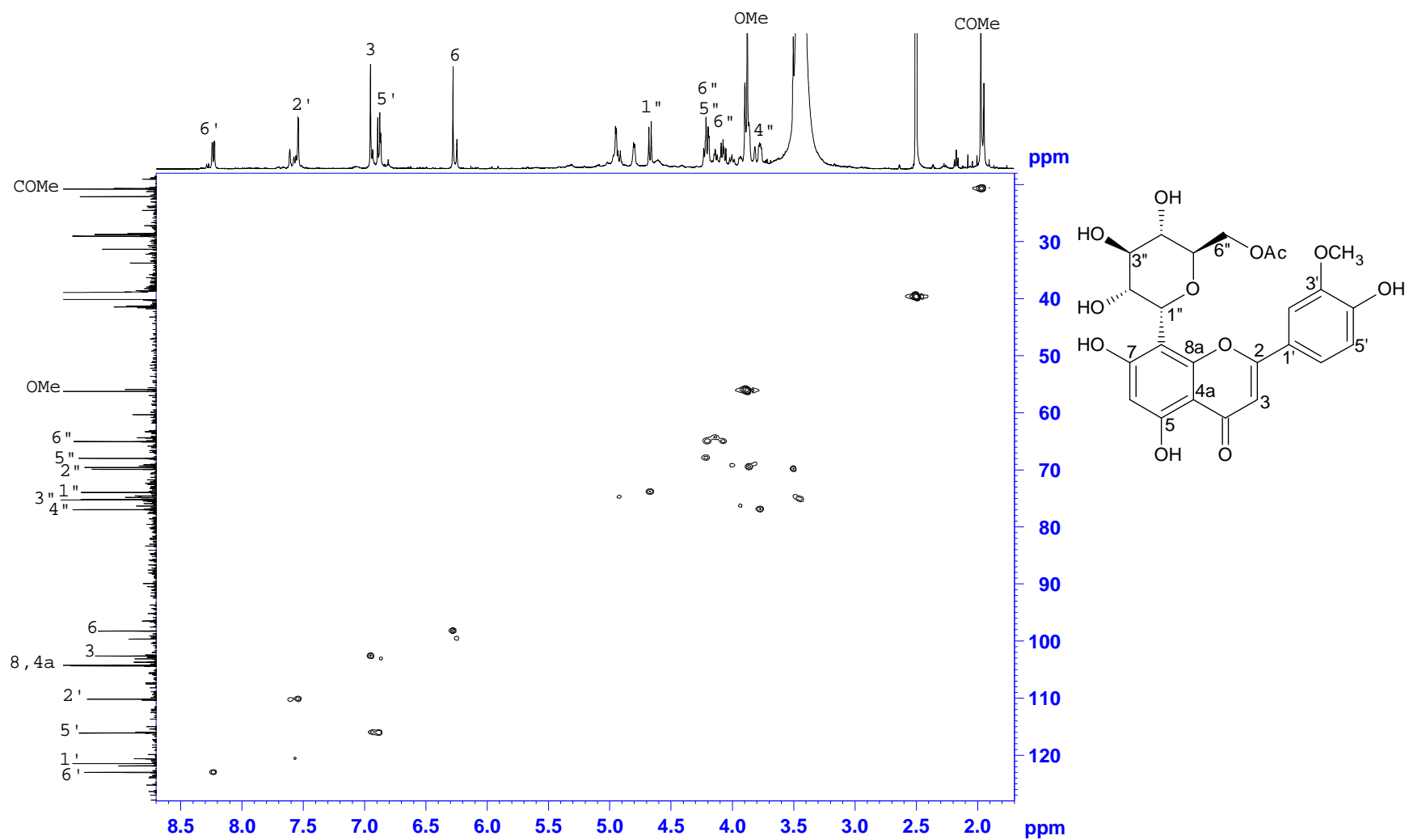
Plate 11e: HMBC NMR Spectrum of 3'-methoxypsilosin (78) in (CD$_3$)$_2$SO
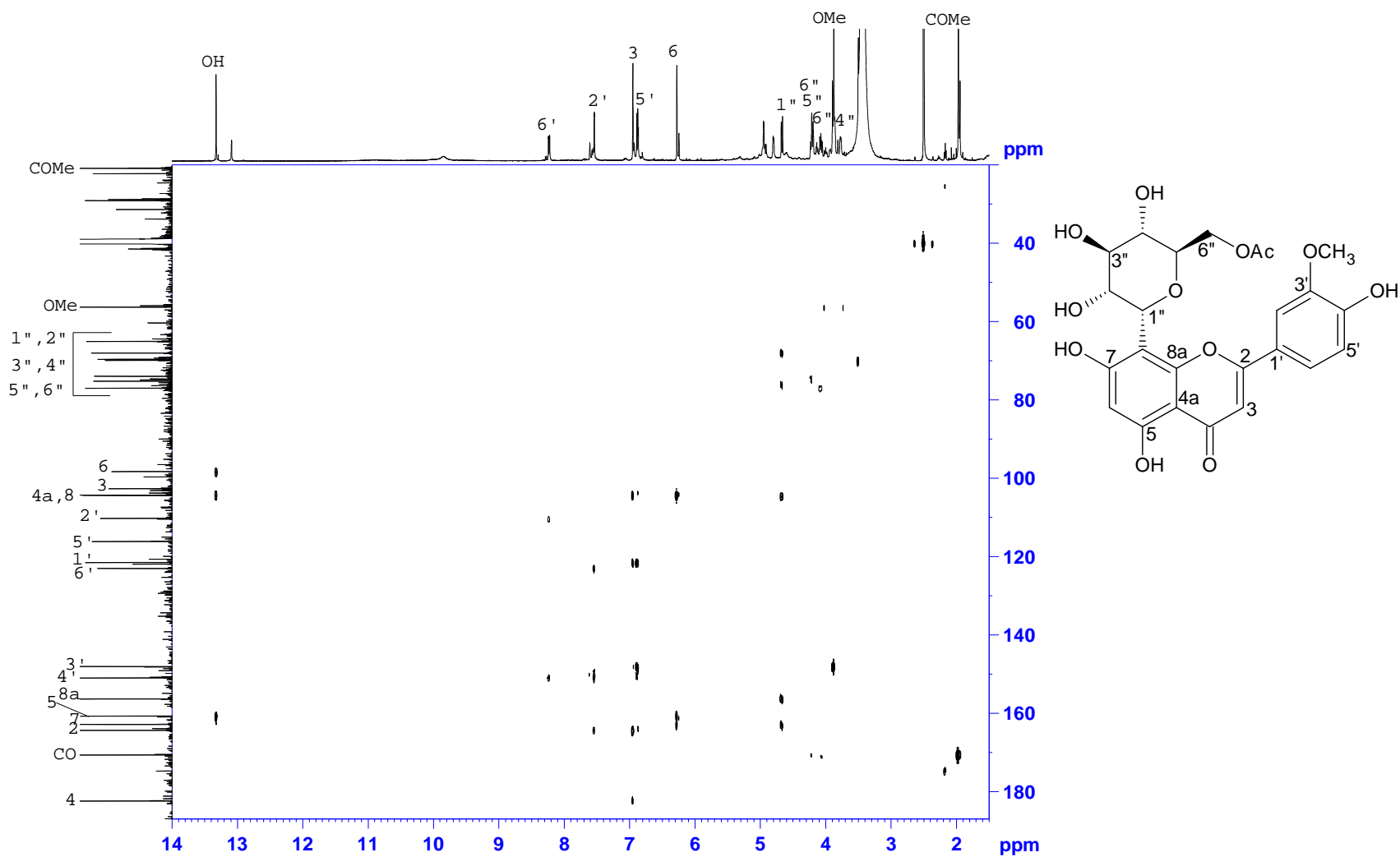
Plate 11f: DEPT 135 NMR Spectrum of 3'-methoxypsilosin (78) in (CD$_3$)$_2$SO
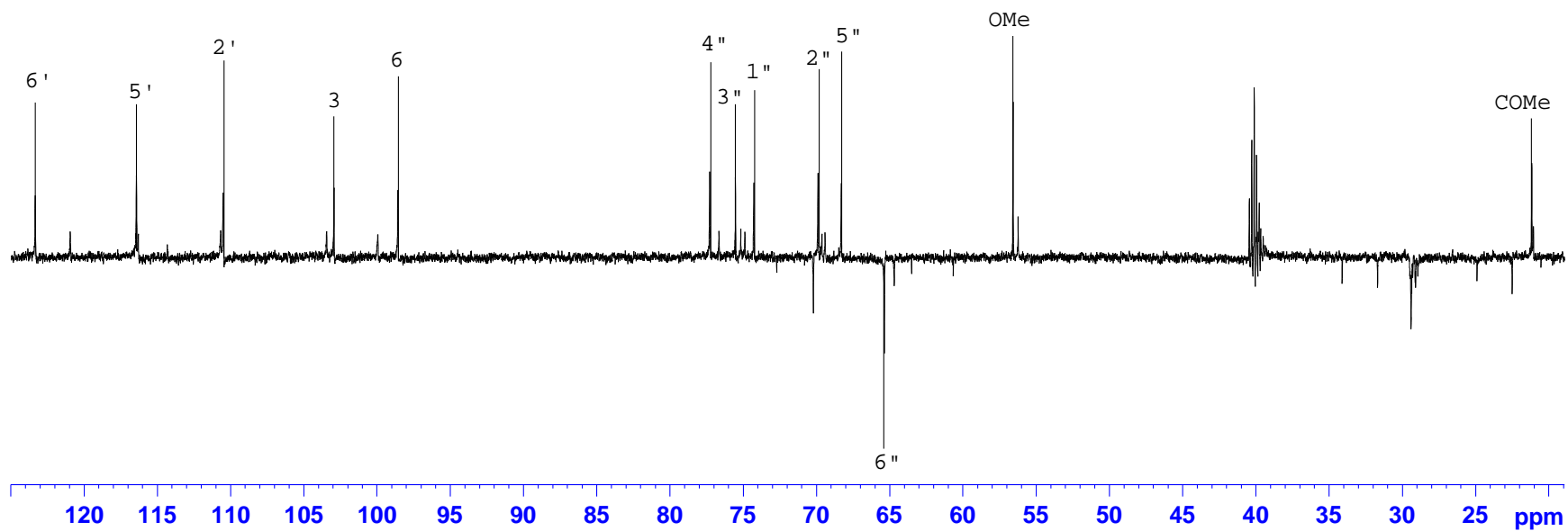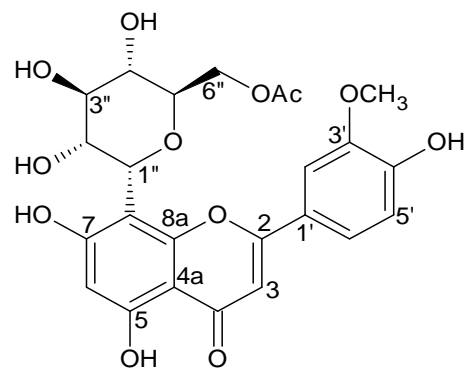
Plate 11g: DEPT 90 NMR Spectrum of 3'-methoxypsilosin (78) in (CD₃)₂SO
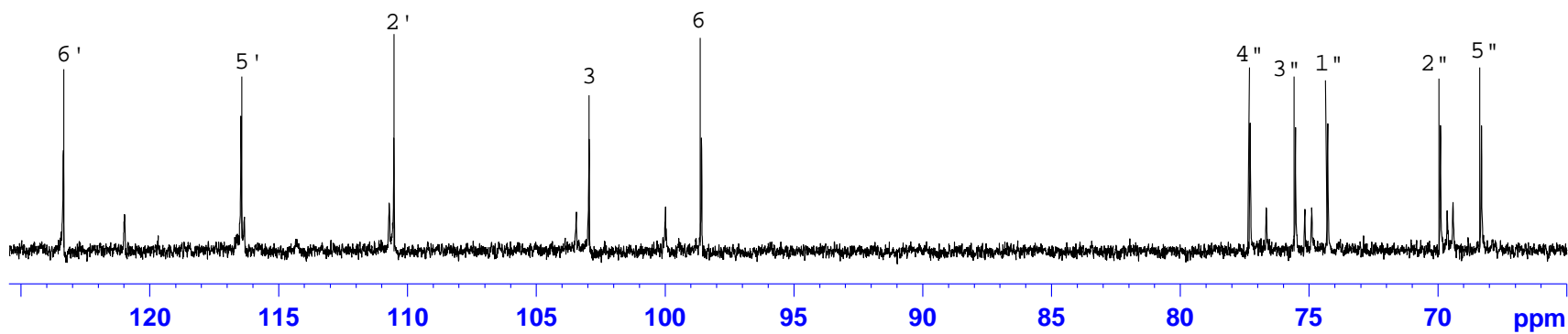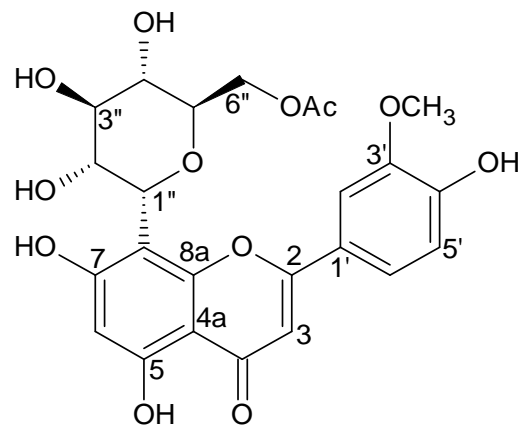
Plate 12a: ^{1}H NMR Spectrum of (2*Z*)-[(4*R*,5*R*,6*S*)-6-(β-*D*-glucopyranosyloxy)-4,5-dihydroxycyclohex-2-en-1-ylidene]ethanenitrile (79) in (CD$_3$)$_2$SO
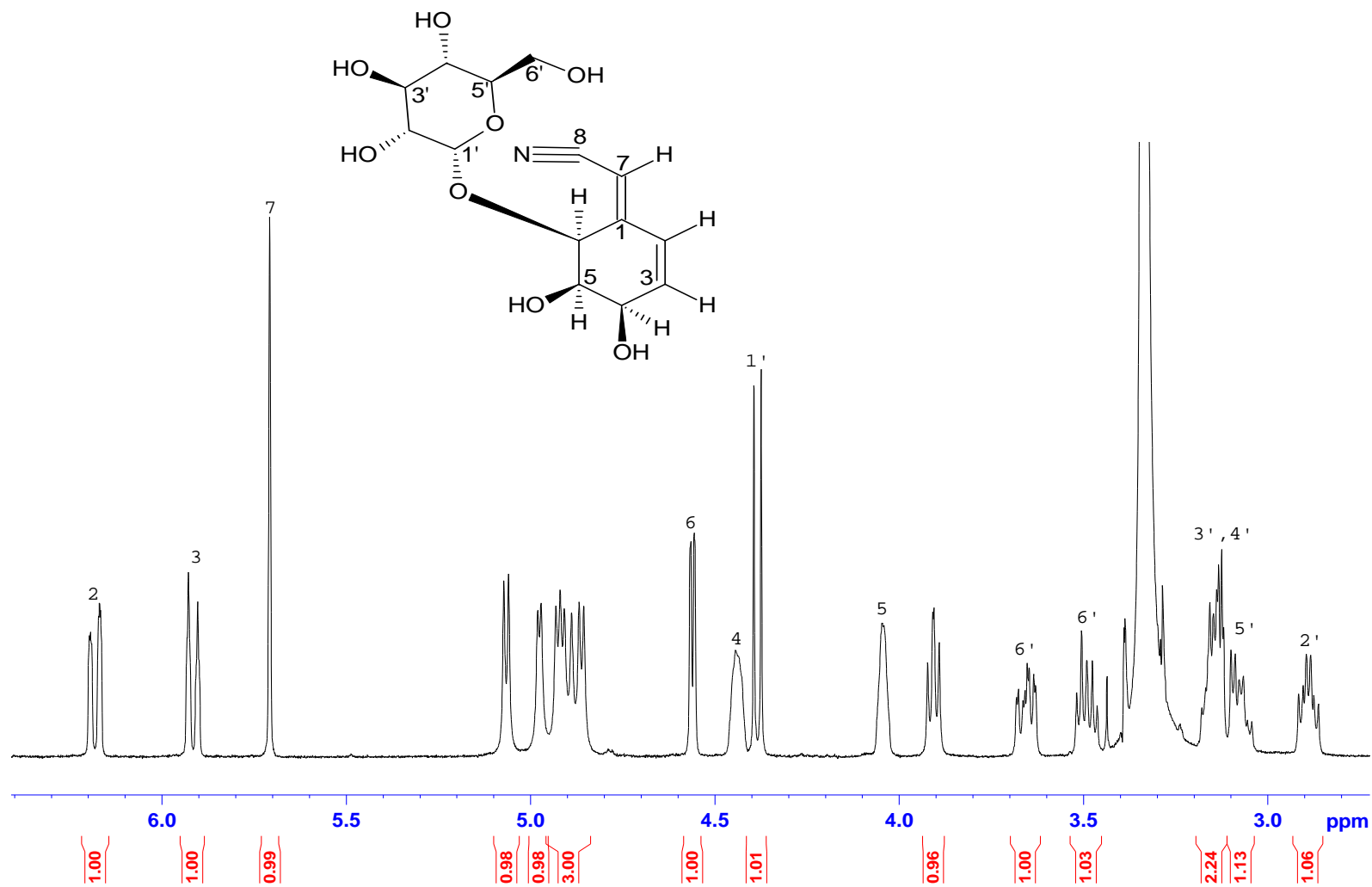
Plate 12b: COSY NMR Spectrum of (2Z)-[(4R,5R,6S)-6-(β-D-glucopyranosyloxy)-4,5-dihydroxycyclohex-2-en-1-ylidene]ethanenitrile (79) in (CD₃)₂SO
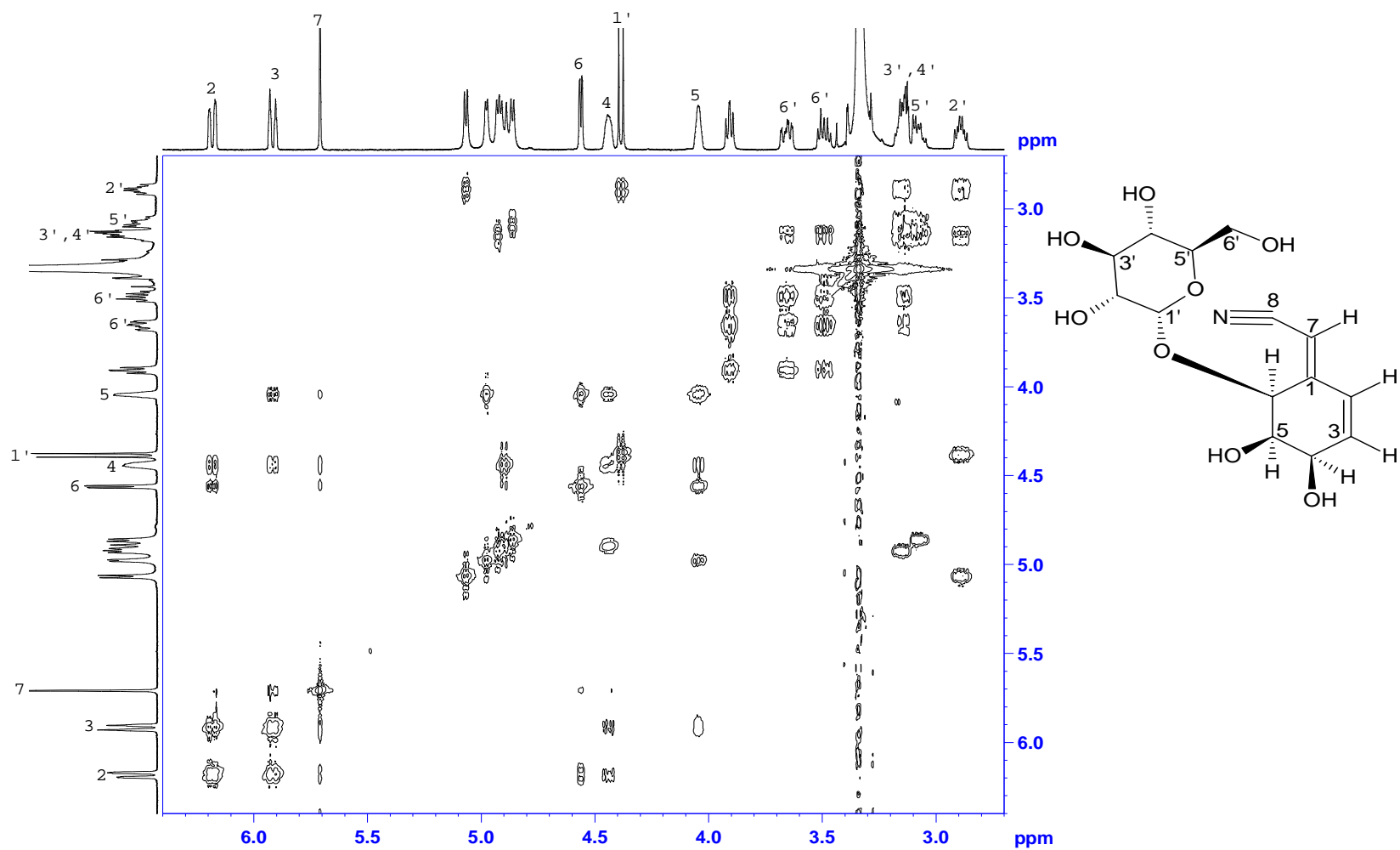
Plate 12c: ^{13}C NMR Spectrum of (2*Z*)-[(4*R*,5*R*,6*S*)-6-(β-*D*-glucopyranosyloxy)-4,5-dihydroxycyclohex-2-en-1-ylidene]ethanenitrile (79) in (CD$_3$)$_2$SO
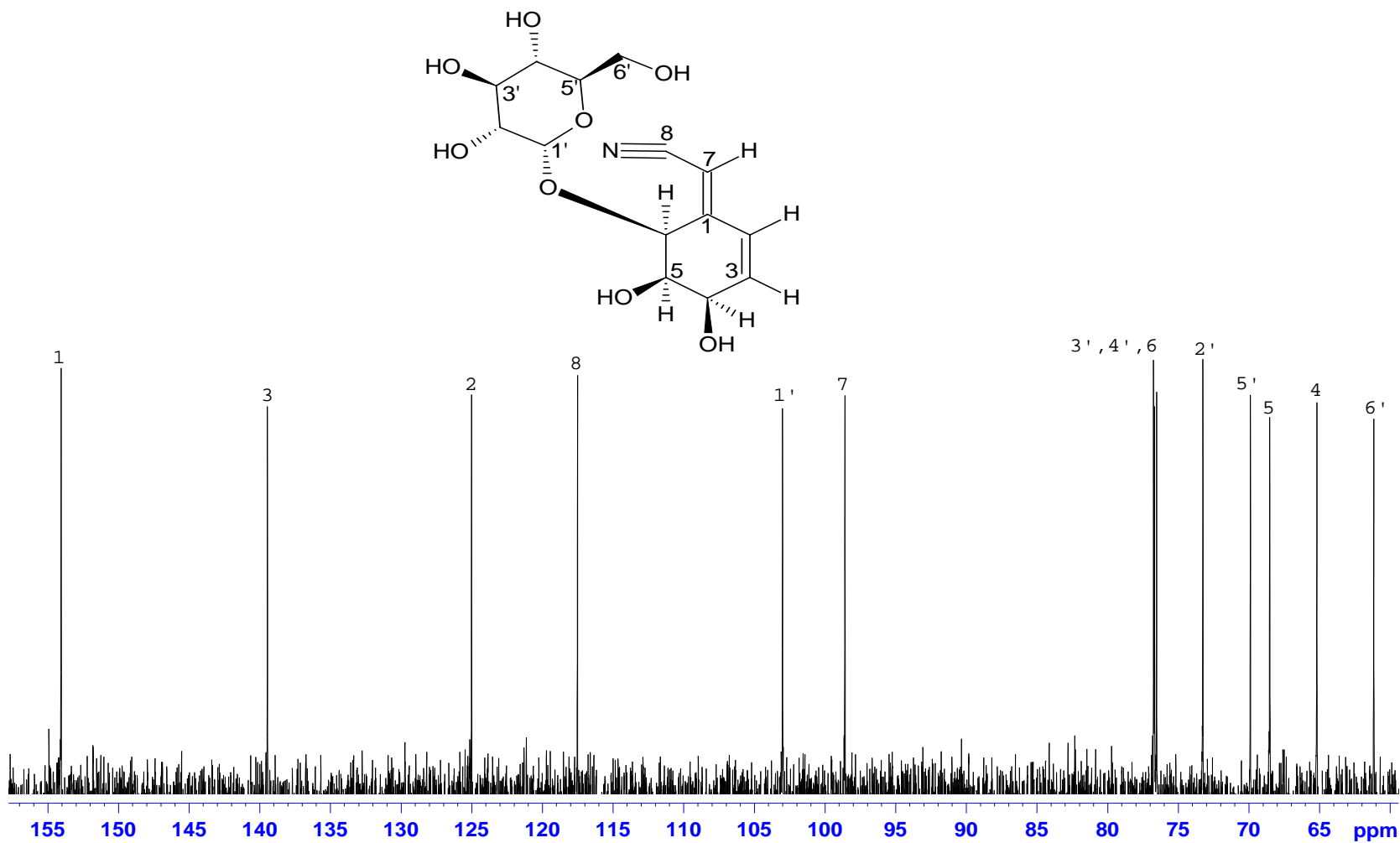
Plate 12d: HSQC NMR Spectrum of (2*Z*)-[(4*R*,5*R*,6*S*)-6-(*β-D*-glucopyranosyloxy)-4,5-dihydroxycyclohex-2-en-1-ylidene]ethanenitrile (79) in (CD₃)₂SO
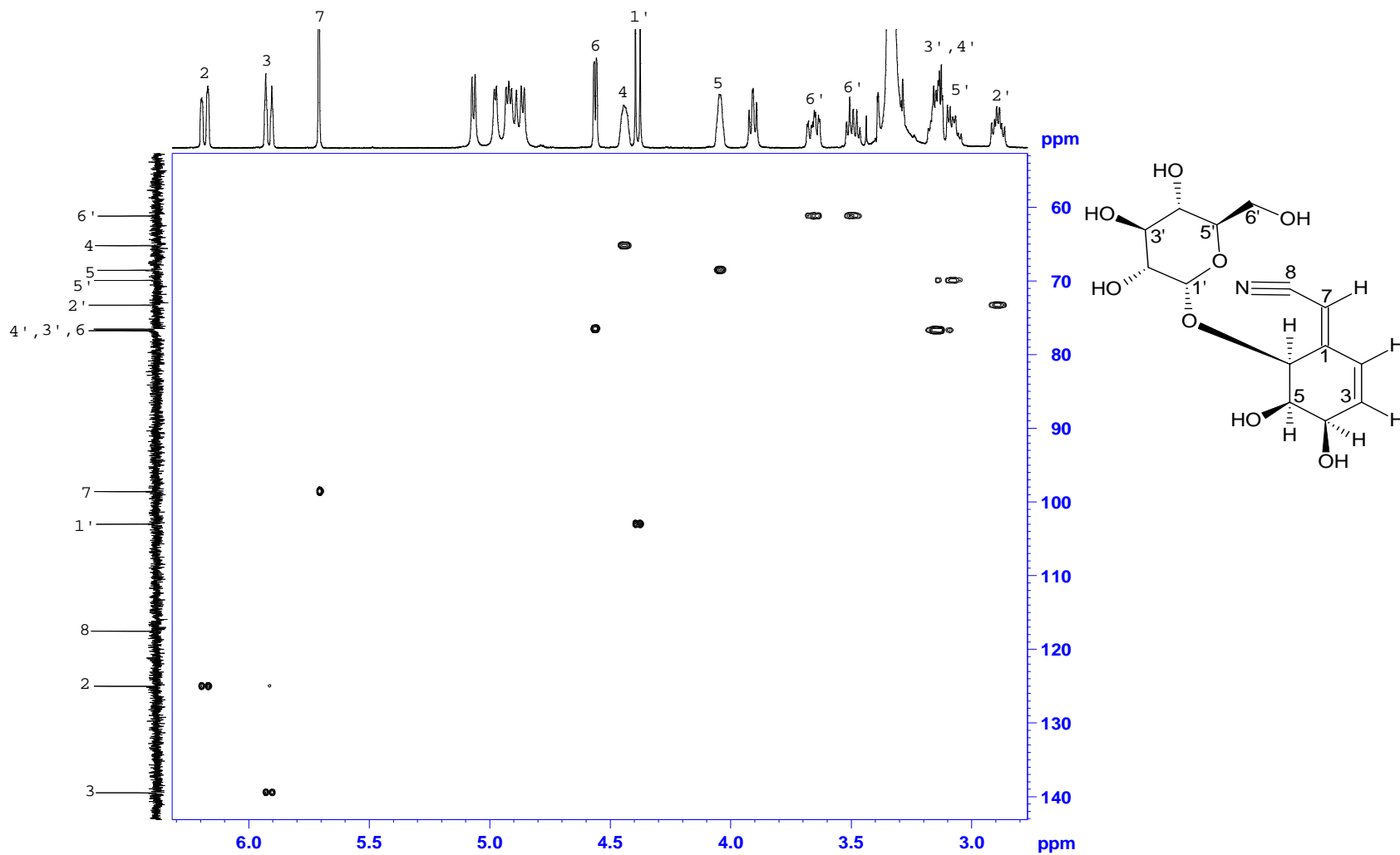
Plate 12e: DEPT -90 and 135 NMR Spectrum of (2*Z*)-[(4*R*,5*R*,6*S*)-6-(*β*-*D*-glucopyranosyloxy)-4,5-dihydroxycyclohex-2-en-1-ylidene]ethanenitrile (79) in (CD₃)₂SO
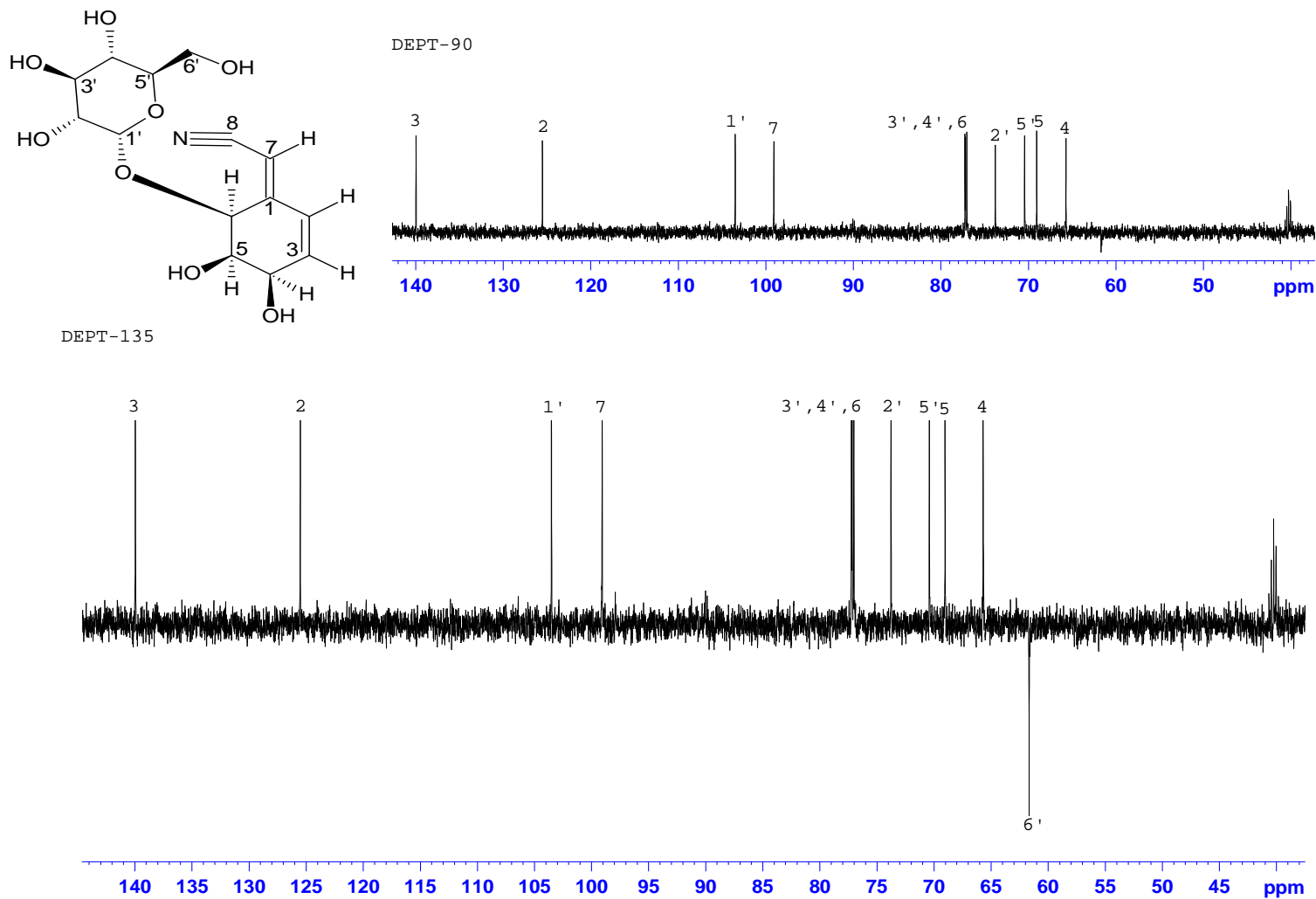
Plate 12g: NOESY NMR Spectrum of (2*Z*)-[(4*R*,5*R*,6*S*)-6-(*β*-D-glucopyranosyloxy)-4,5-dihydroxycyclohex-2-en-1-ylidene]ethanenitrile (79) in (CD₃)₂SO
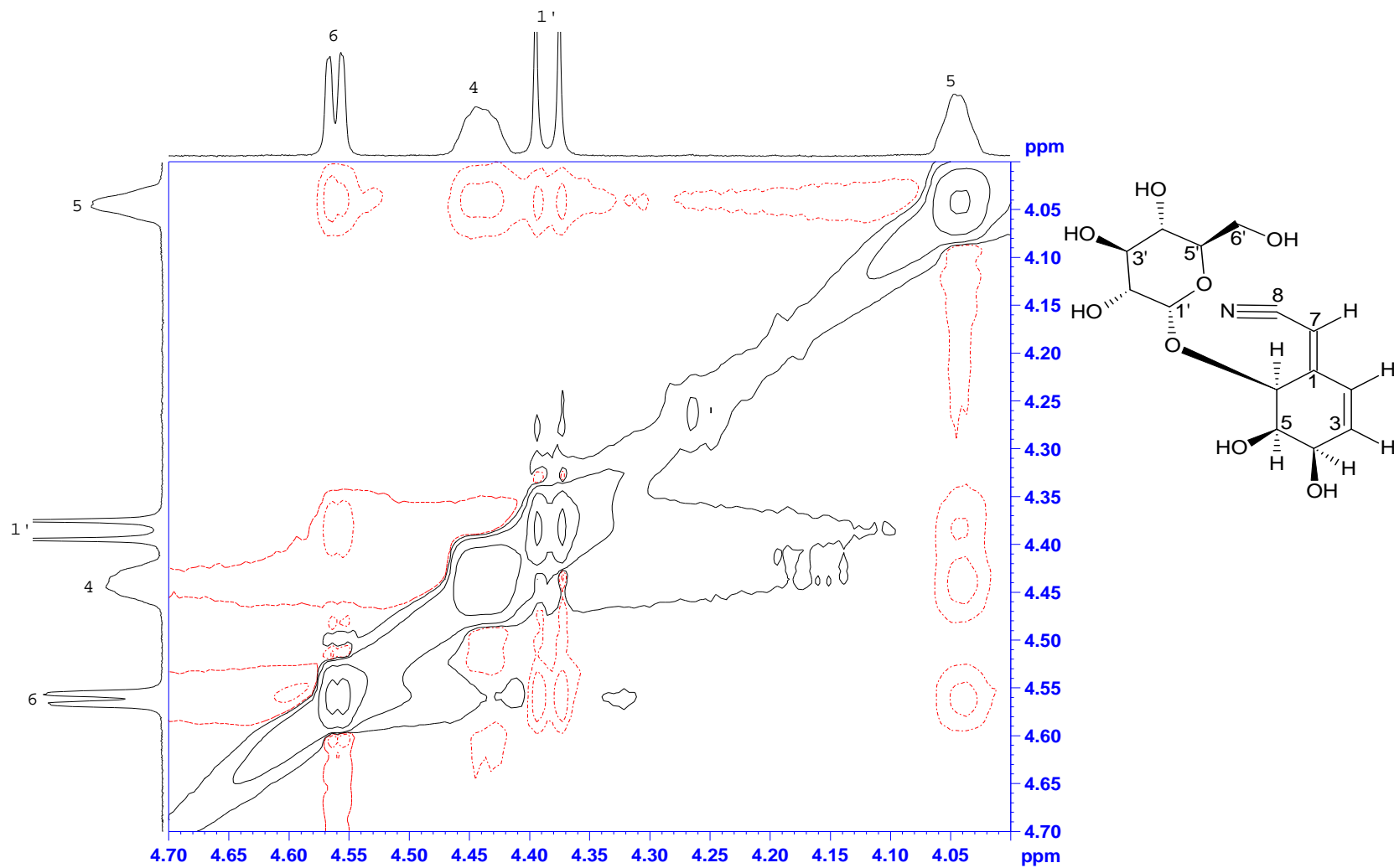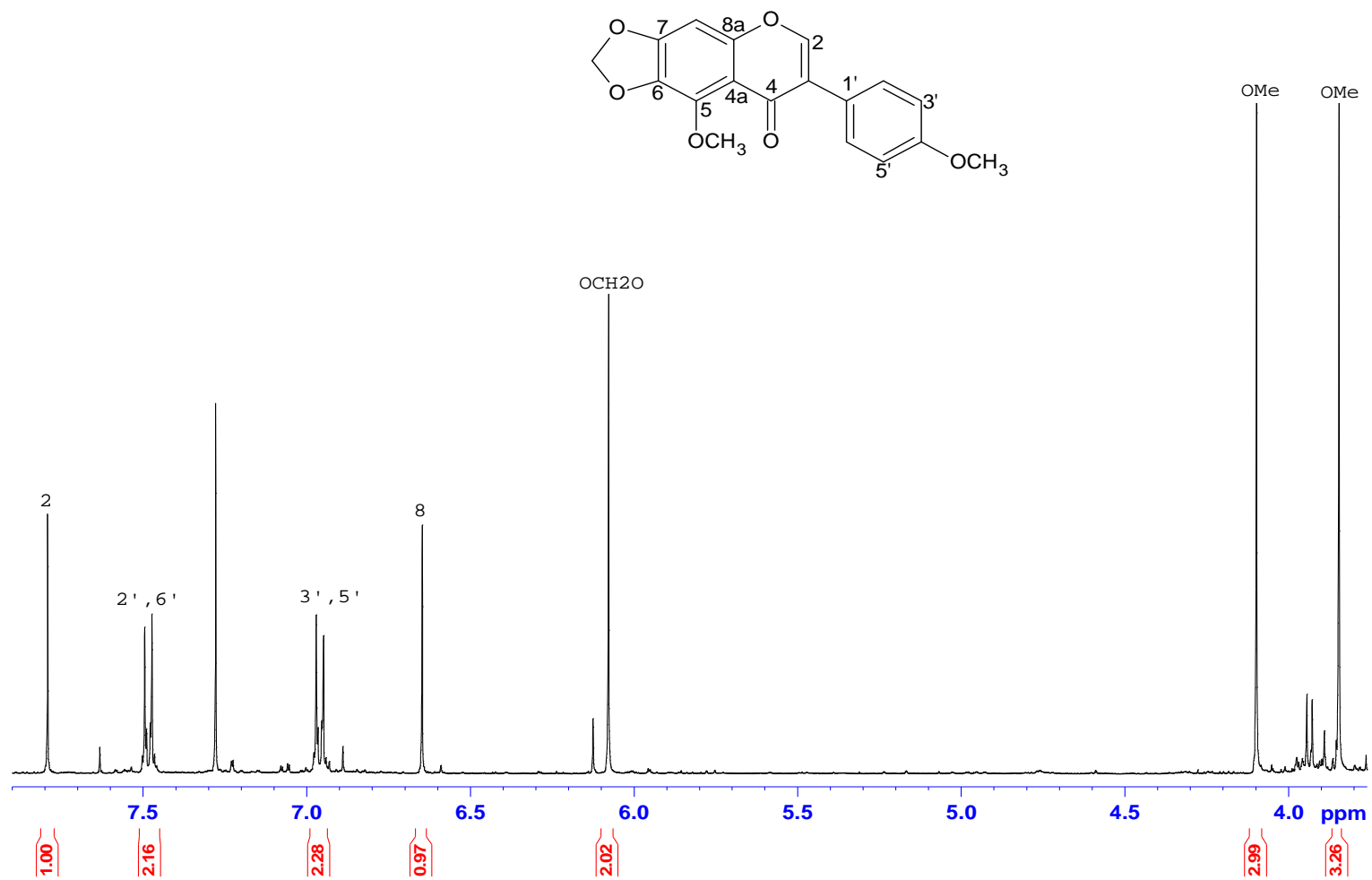
Plate 13b: COSY NMR Spectrum of irisolone-4'-methyl ether (81) in CDCl₃
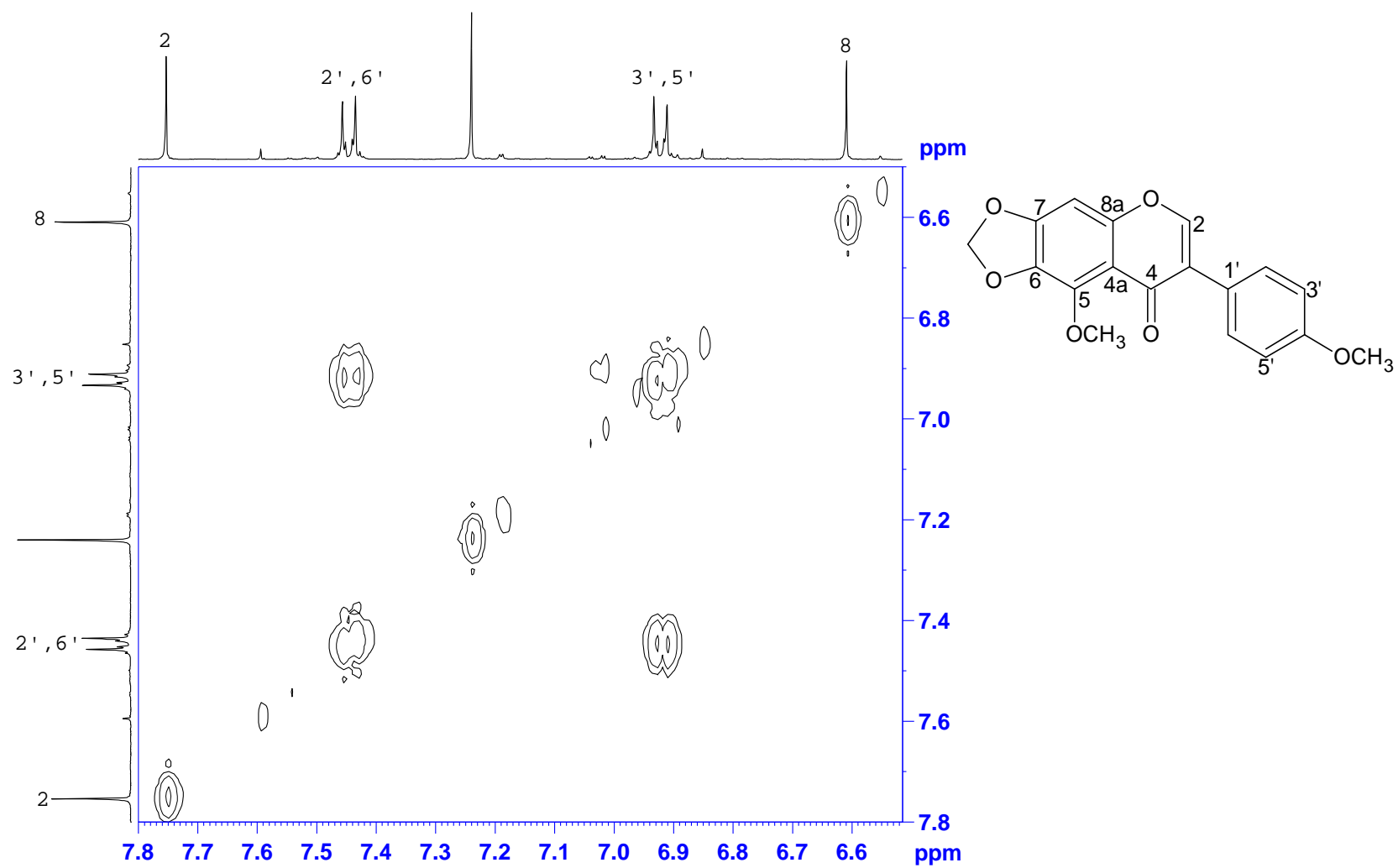
Plate 13c: ^{13}C NMR Spectrum of irisolone-4'-methyl ether (81) in CDCl$_3$
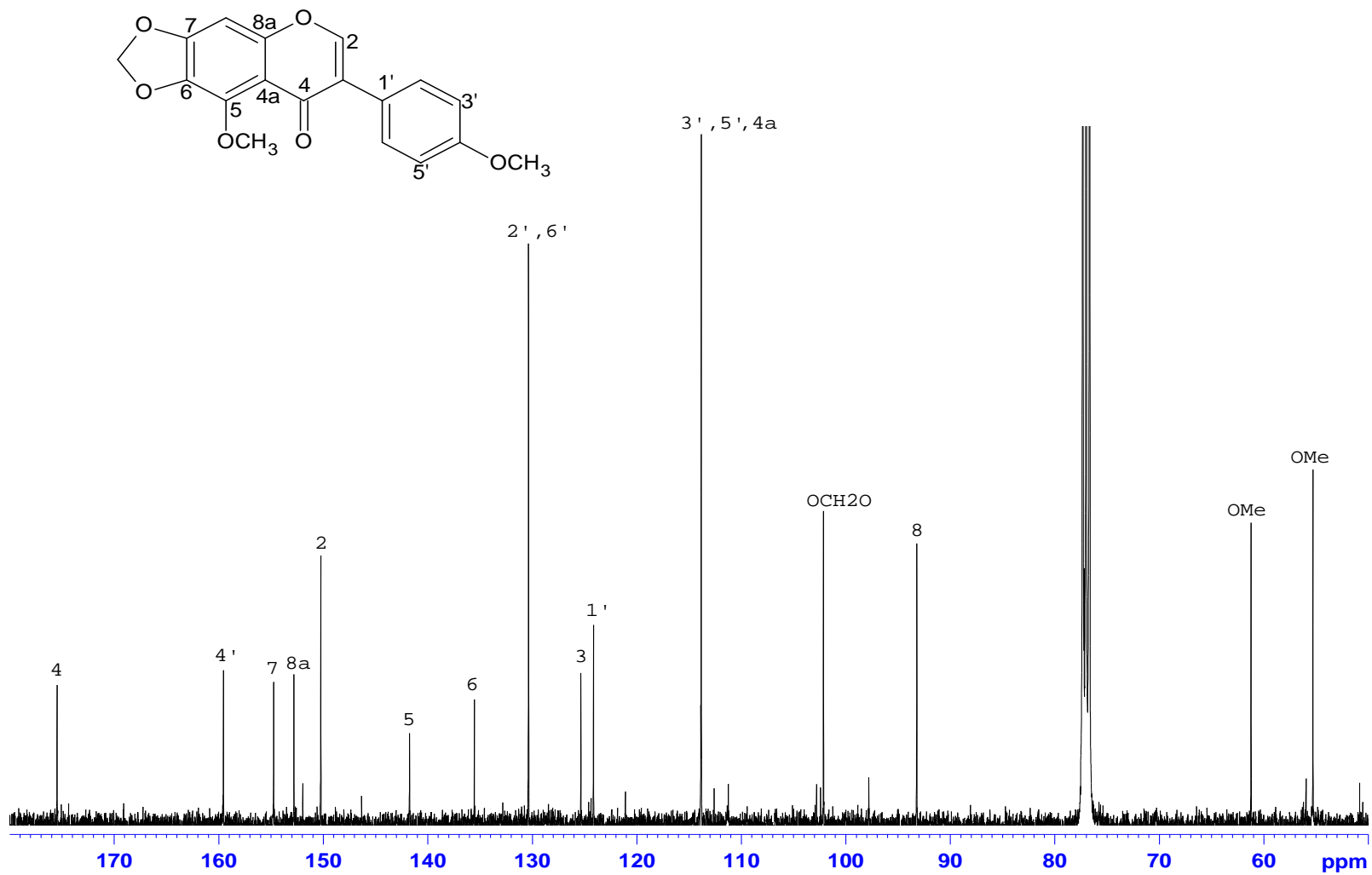
Plate 13d: HSQC NMR Spectrum of irisolone-4'-methyl ether (81) in CDCl₃
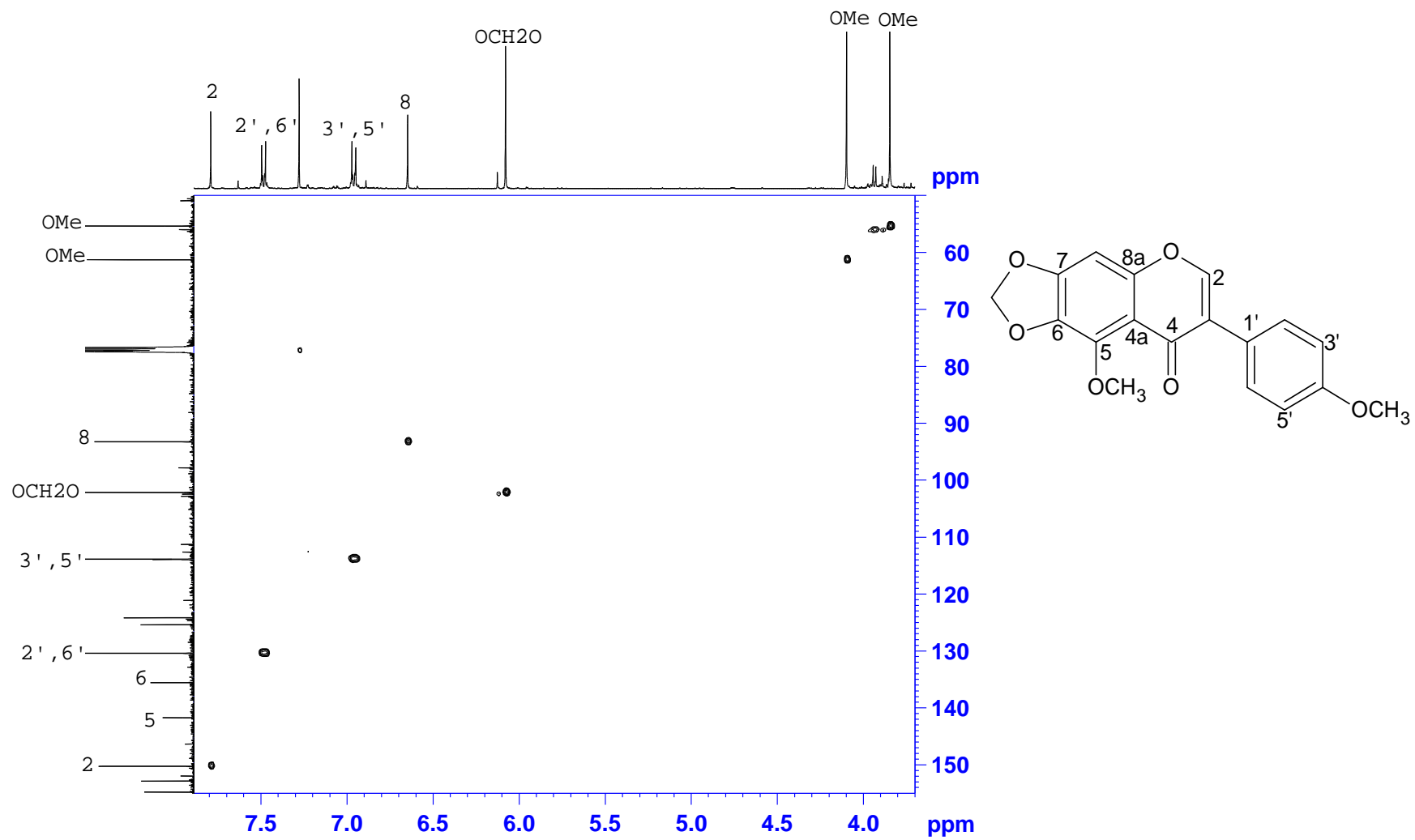
Plate 13e: HMBC NMR Spectrum of irisolone-4'-methyl ether (81) in CDCl₃
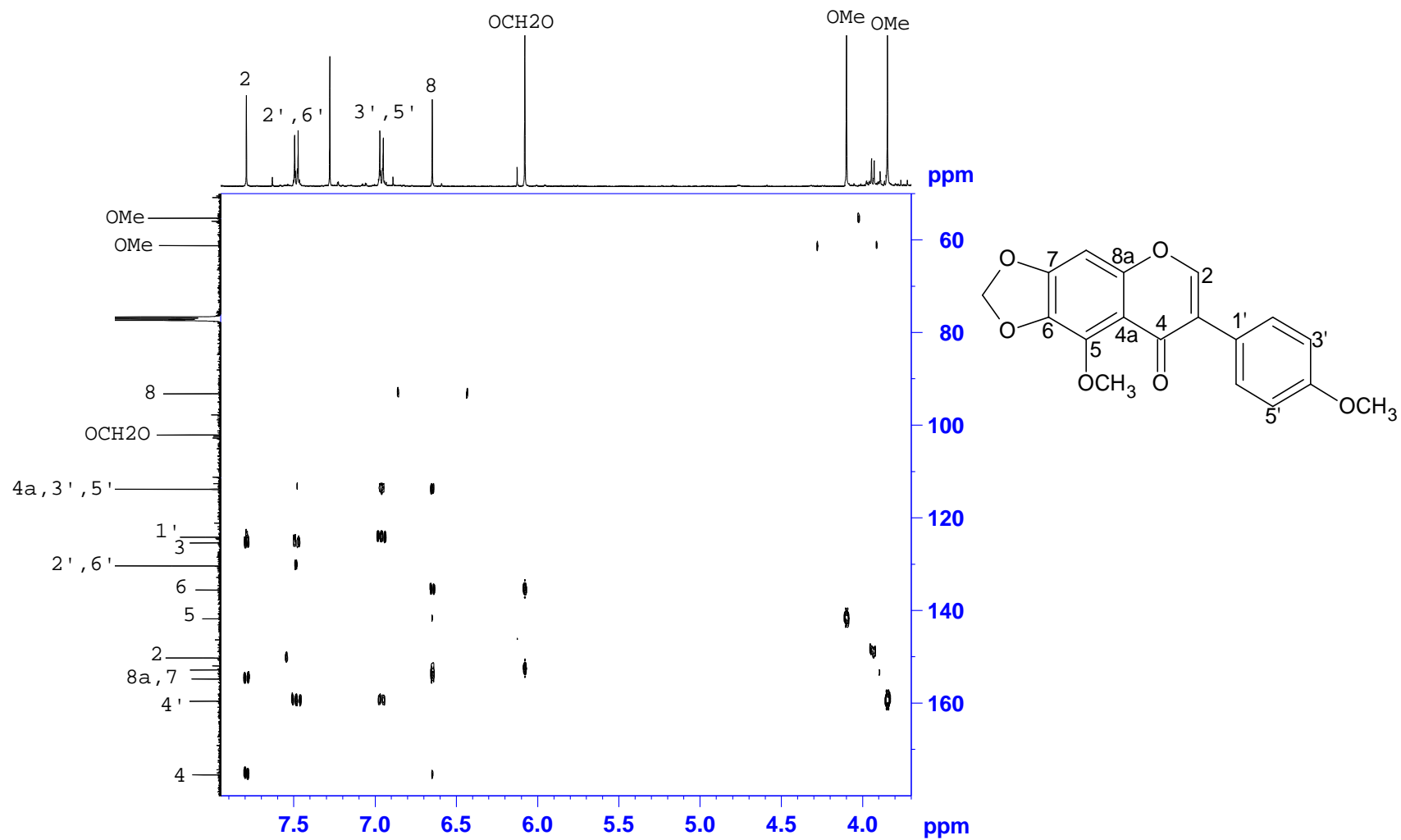
Plate 14a: ^{1}H NMR Spectrum of 3',4'-dimethoxy-6,7-methylenedioxyisoflavone (83) in CDCl$_3$
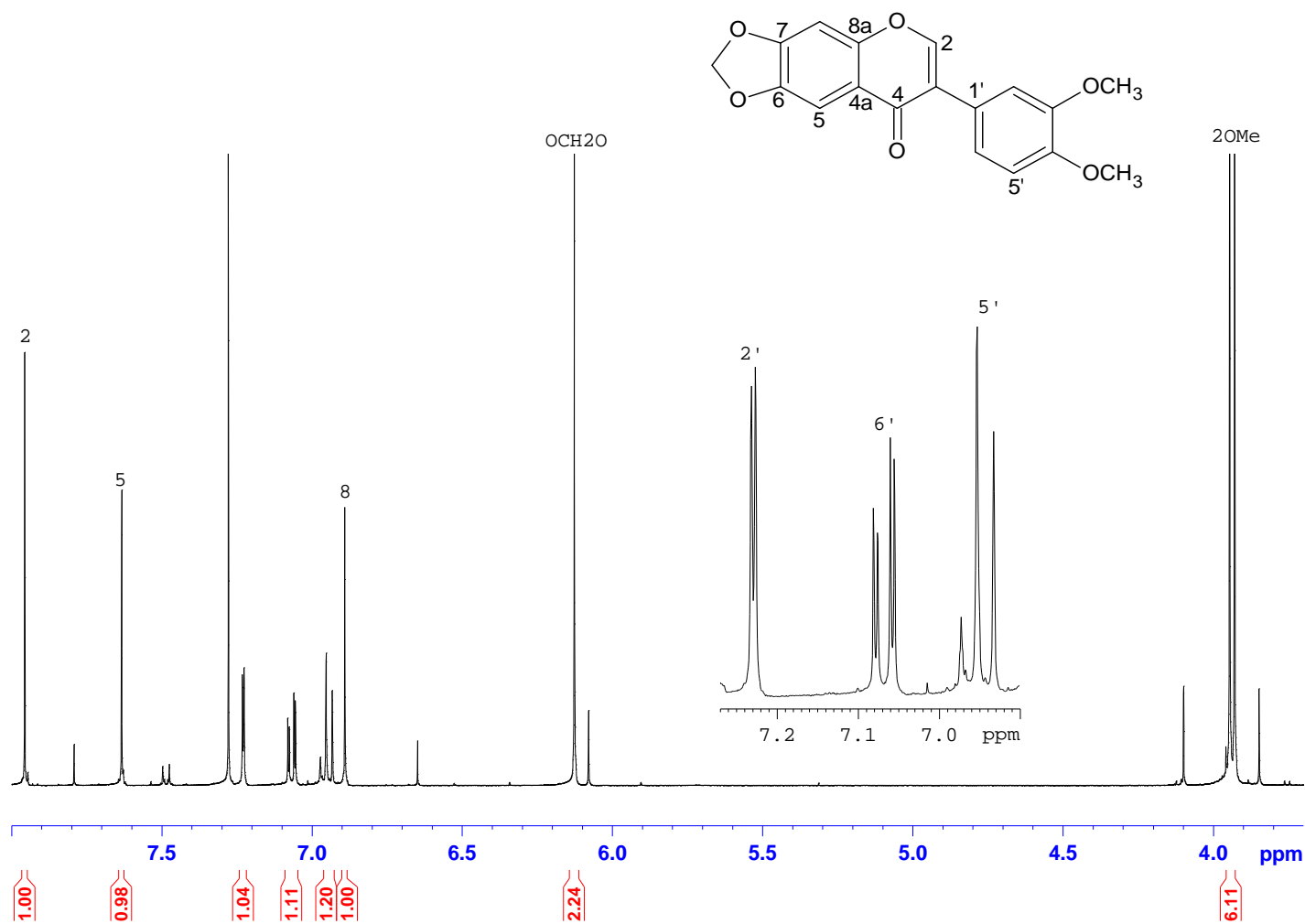
Plate 14b: COSY NMR Spectrum of 3',4'-dimethoxy-6,7-methylenedioxyisoflavone (83) in CDCl₃
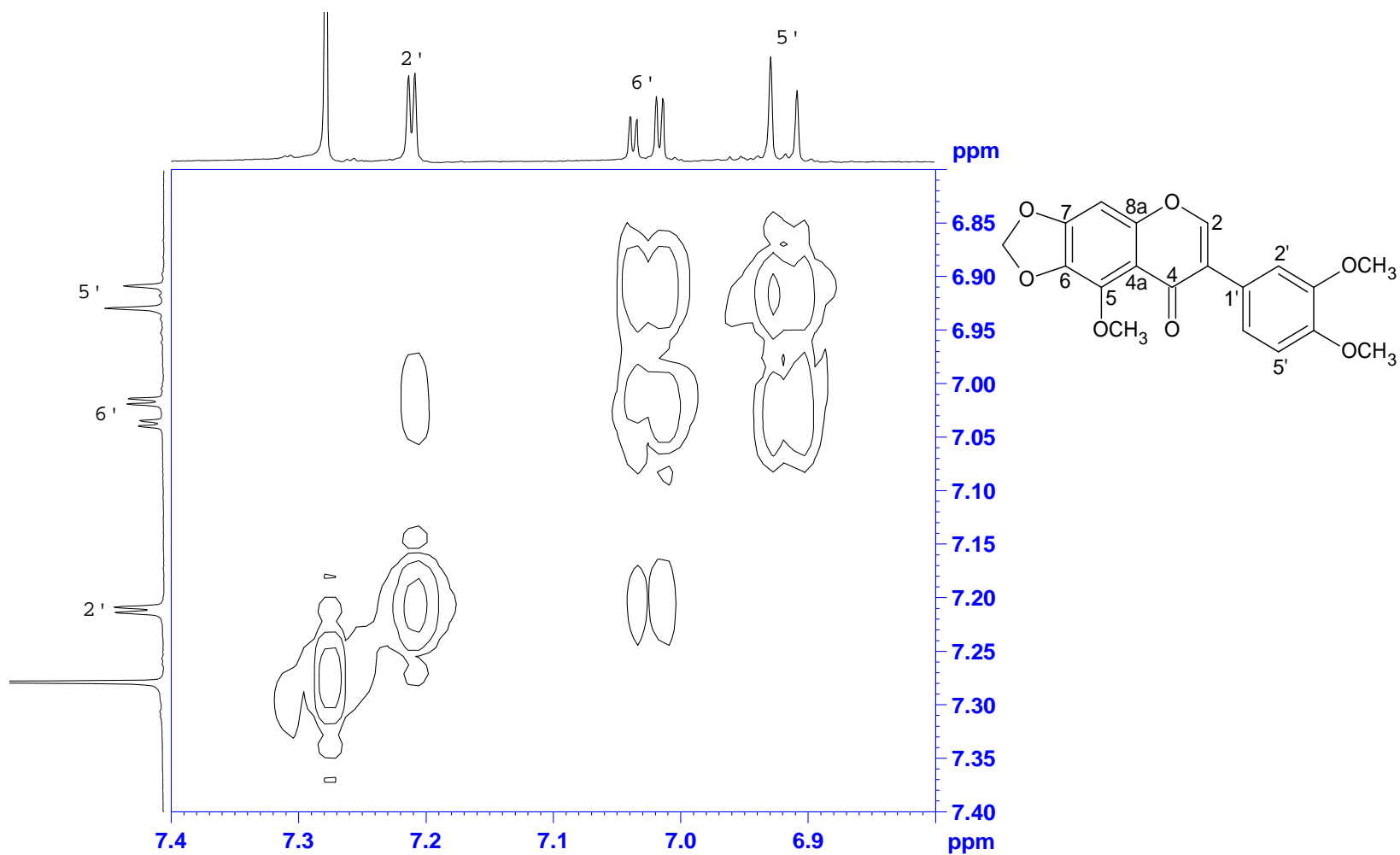
Plate 14c: ^{13}C NMR Spectrum of 3',4'-dimethoxy-6,7-methylenedioxyisoflavone (83) in CDCl$_3$

249
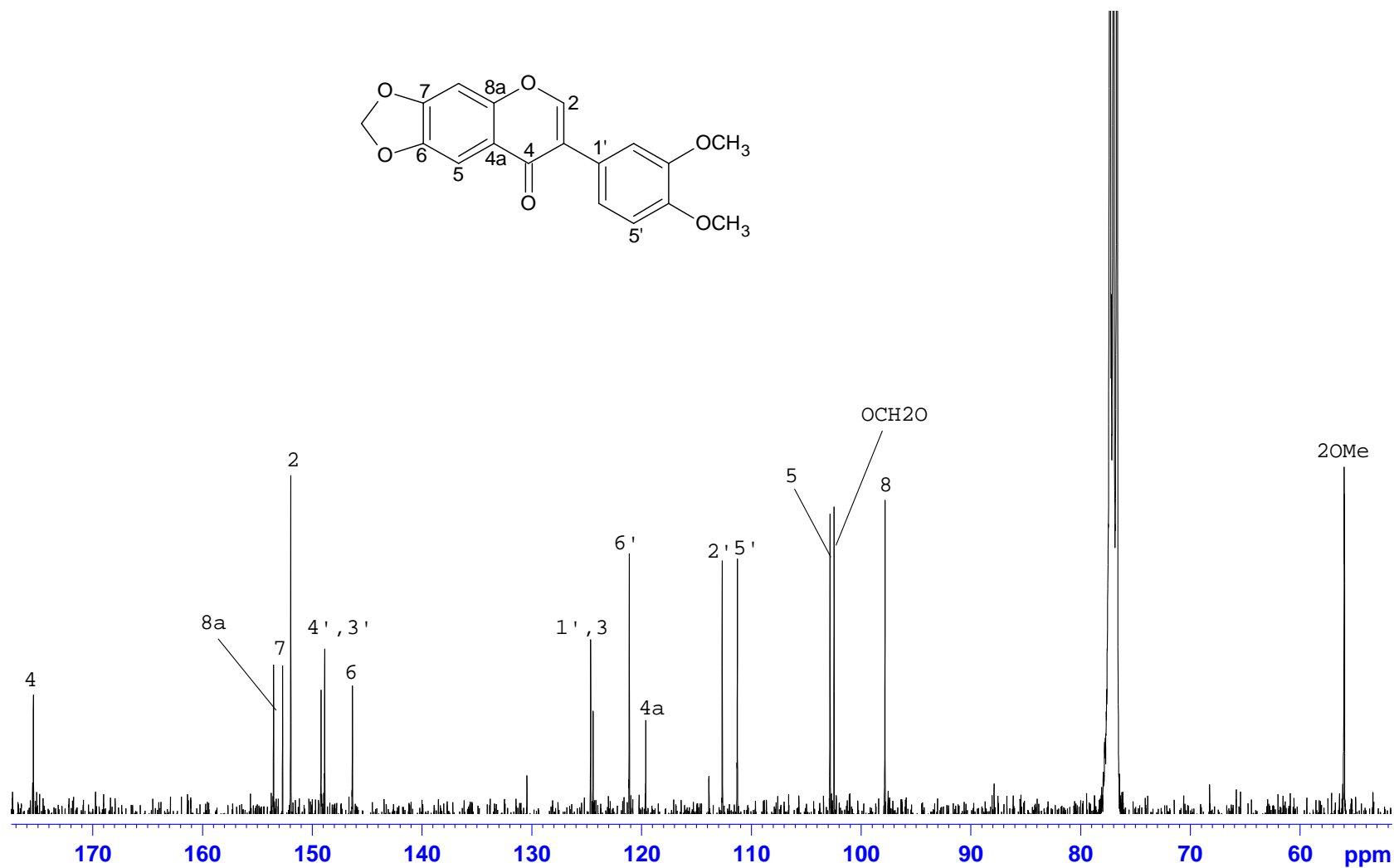
Plate 14d: HSQC NMR Spectrum of 3',4'-dimethoxy-6,7-methylenedioxyisoflavone (83) in CDCl₃
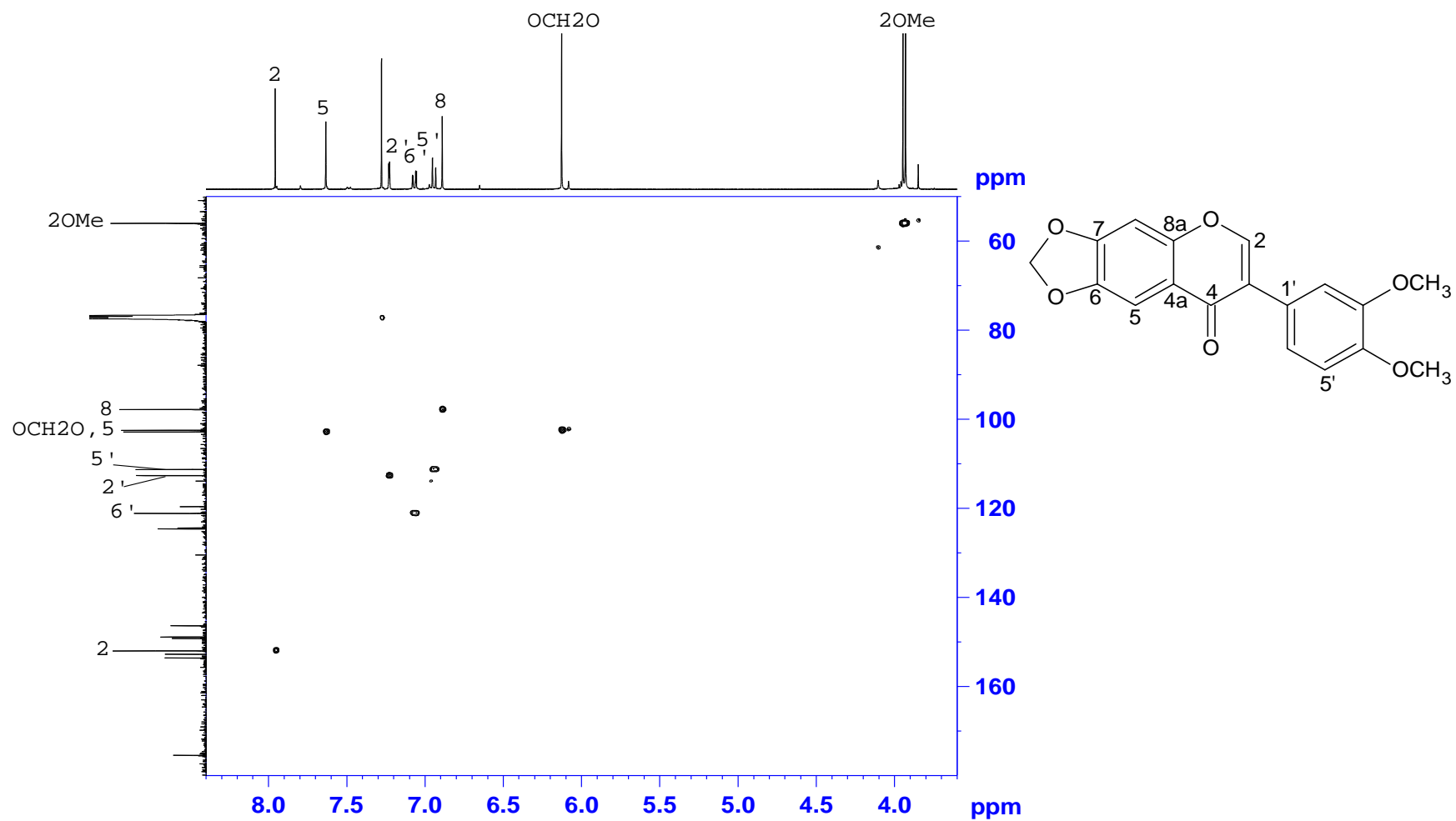
Plate 14e: HMBC NMR Spectrum of 3',4'-dimethoxy-6,7-methylenedioxyisoflavone (83) in CDCl$_3$
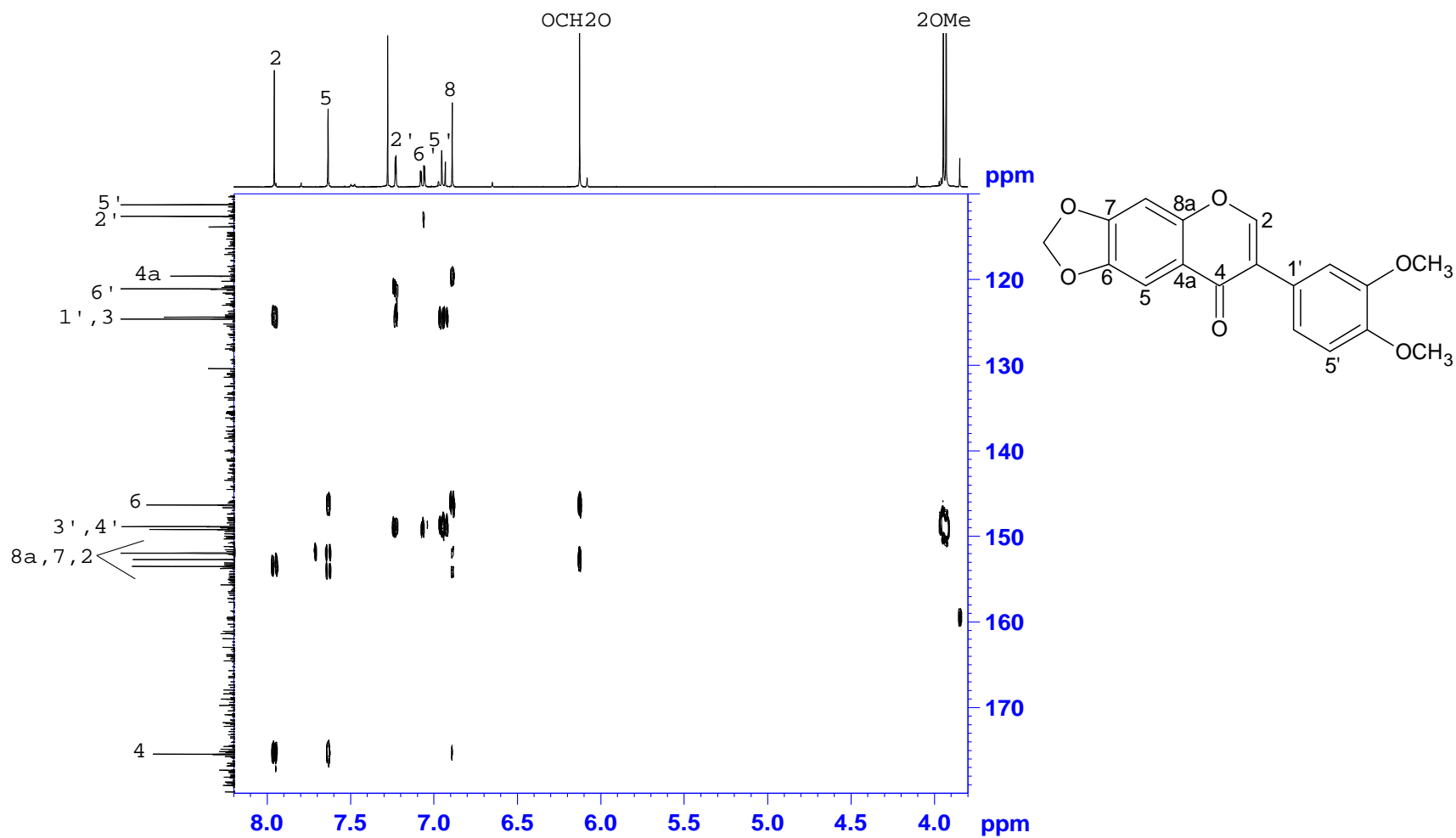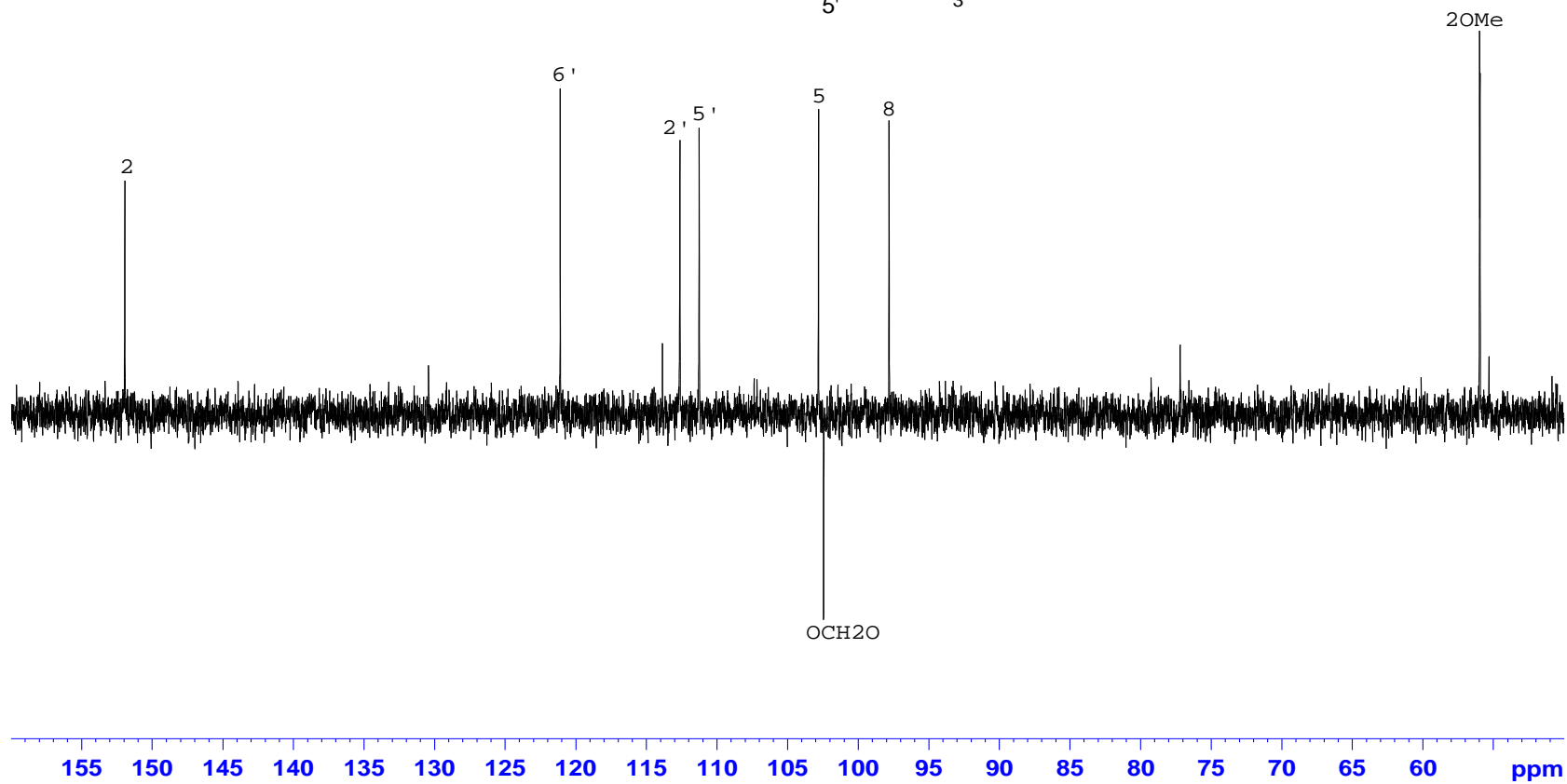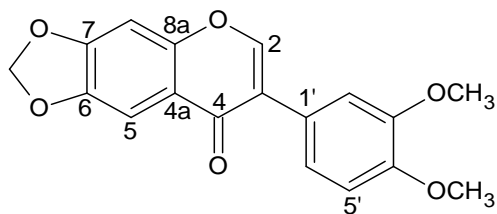
Plate 15a: ^{1}H NMR Spectrum of iriskumaonin-3'-methyl ether (84) in CDCl$_3$
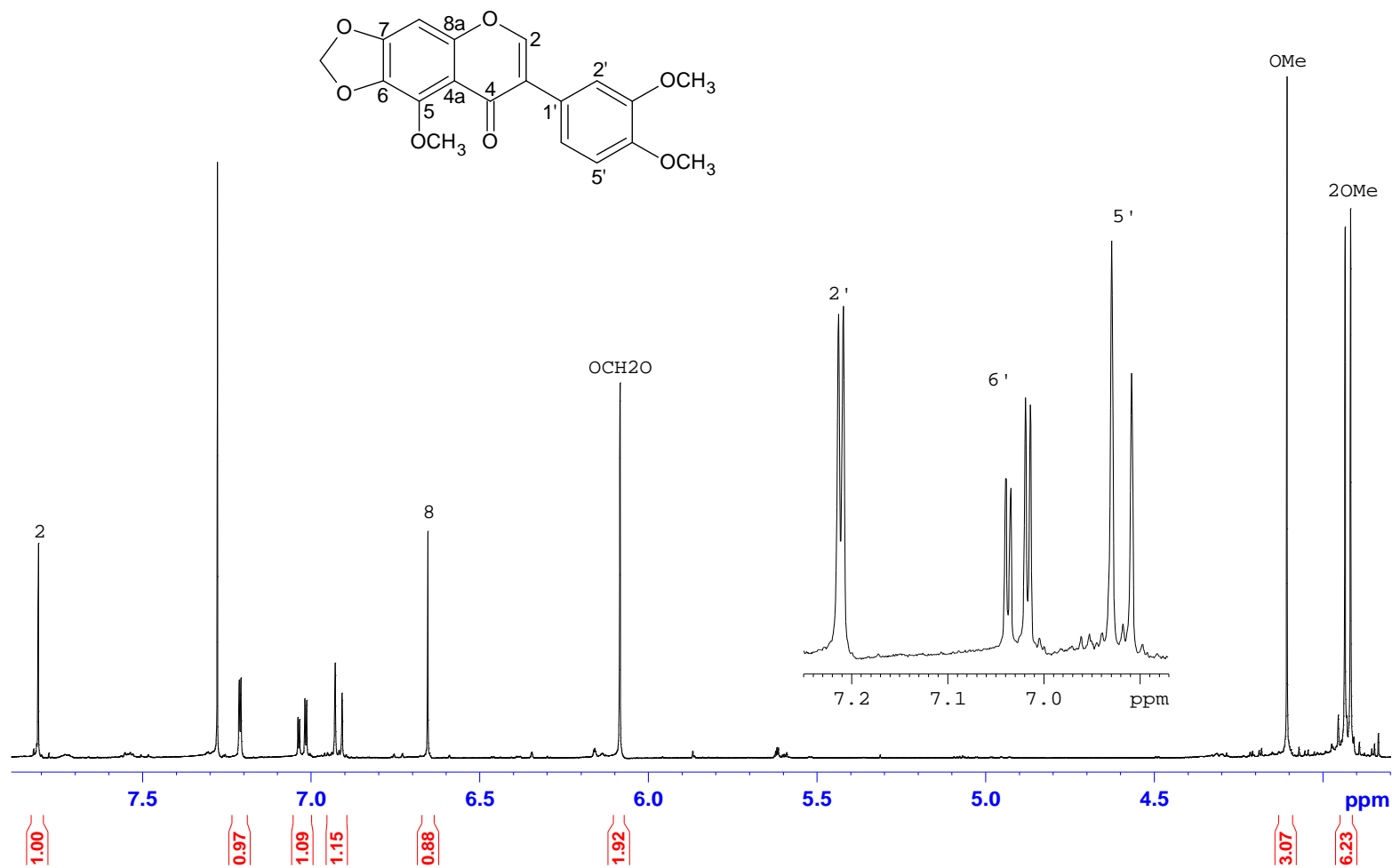
Plate 15b: COSY NMR Spectrum of iriskumaonin-3'-methyl ether (84) in CDCl₃

254
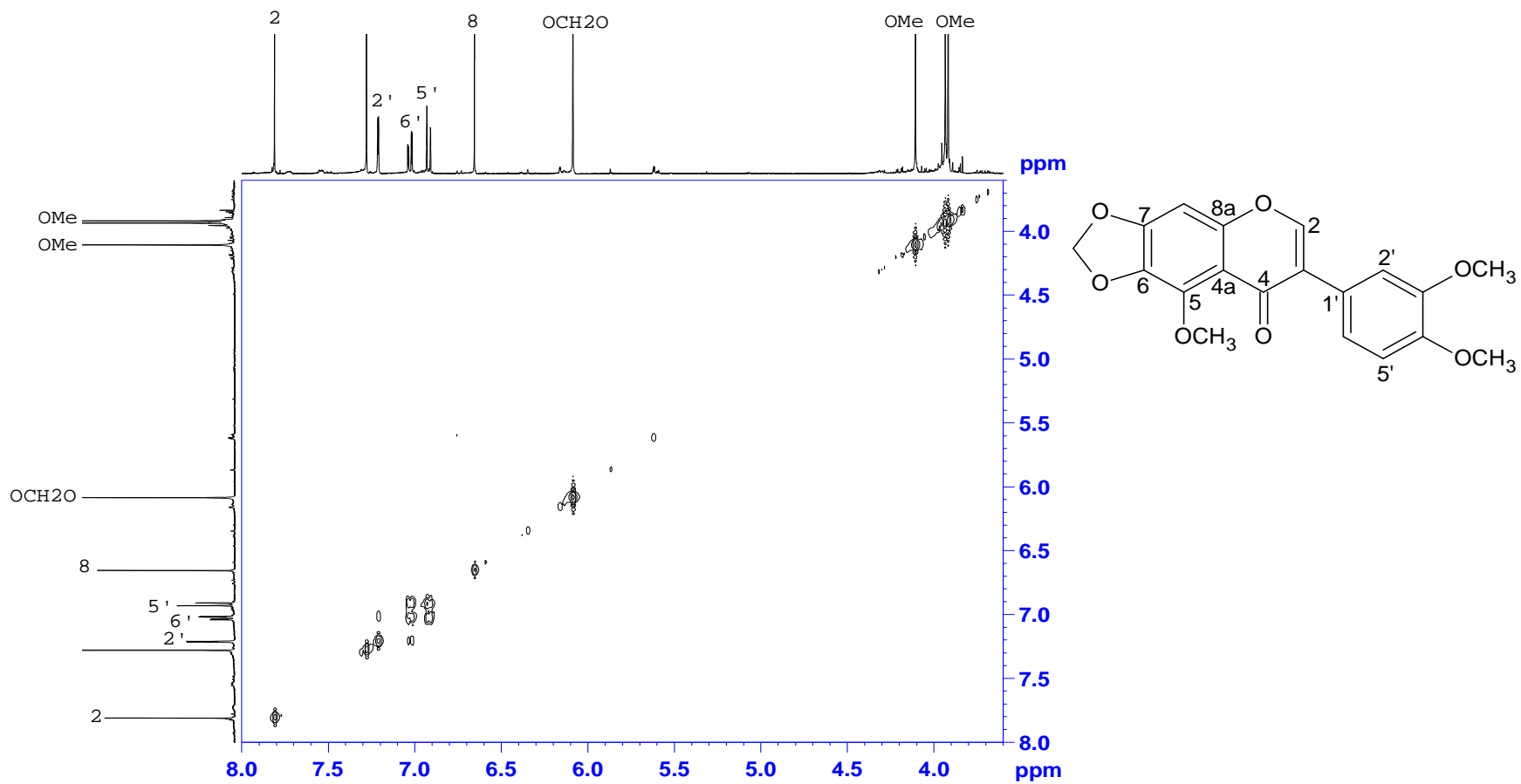
Plate 15c: ^{13}C NMR Spectrum of iriskumaonin-3'-methyl ether (84) in CDCl$_3$
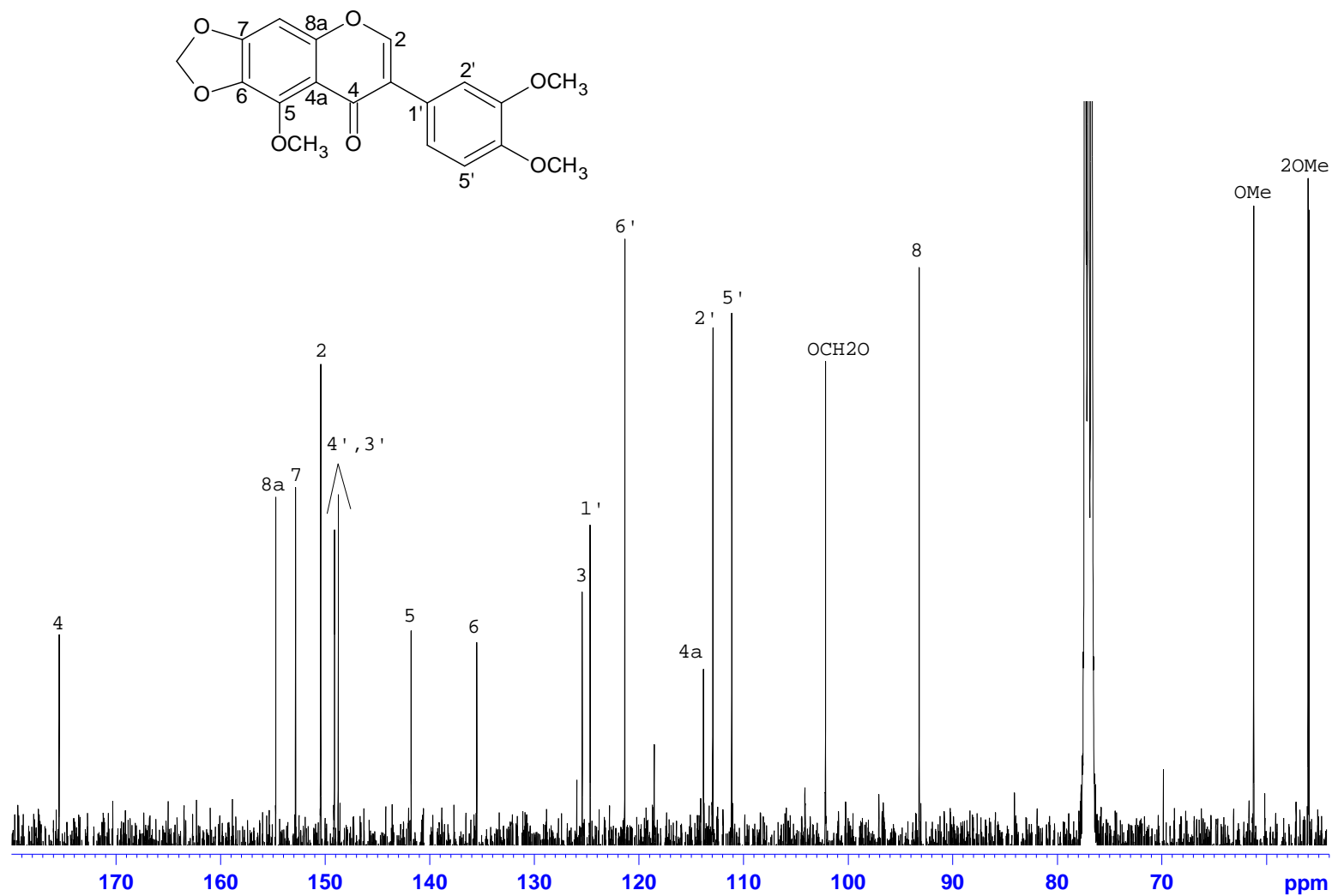
Plate 15d: HSQC NMR Spectrum of iriskumaonin-3'-methyl ether (84) in CDCl$_3$
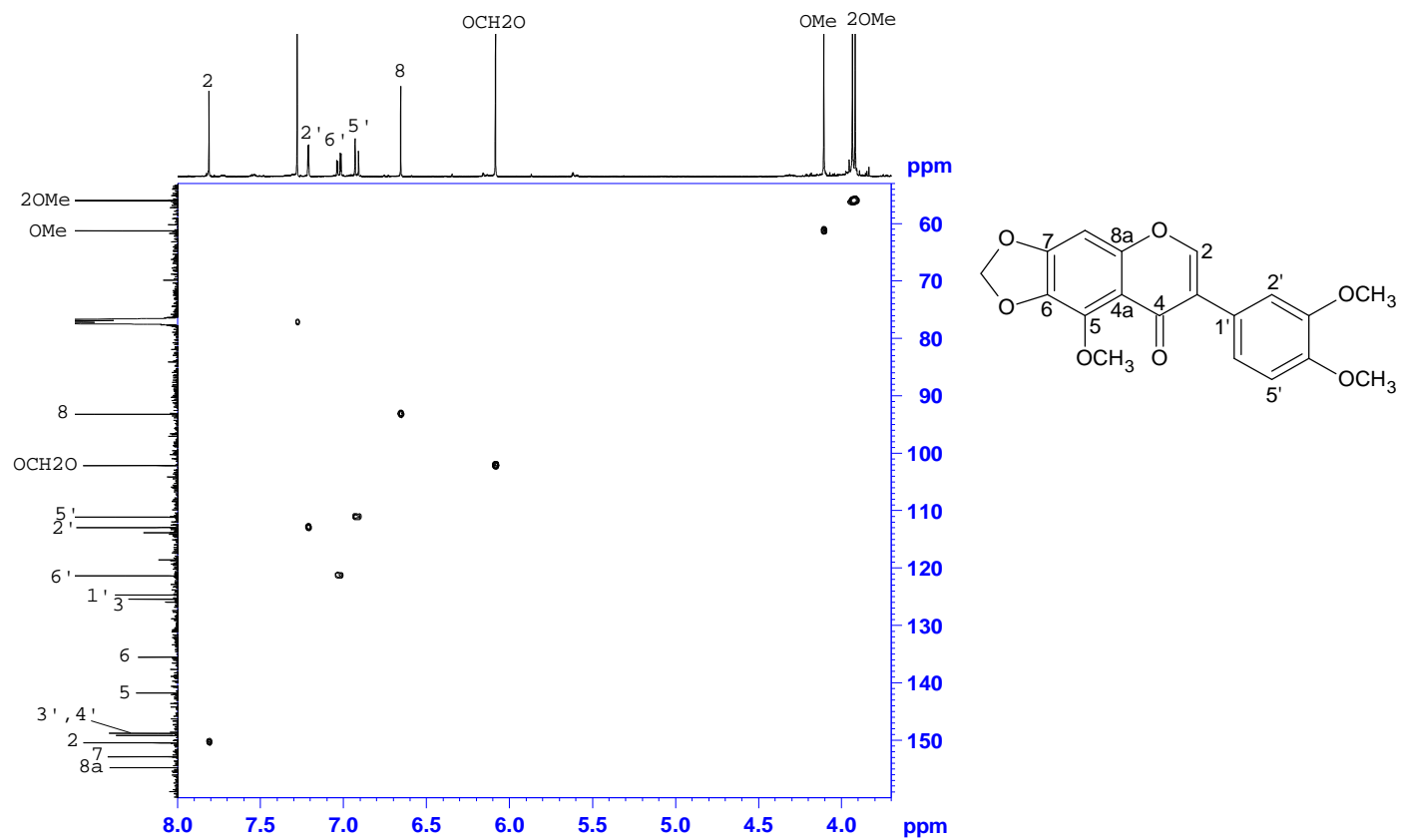
Plate 15e: HMBC NMR Spectrum of iriskumaonin-3'-methyl ether (84) in CDCl$_3$

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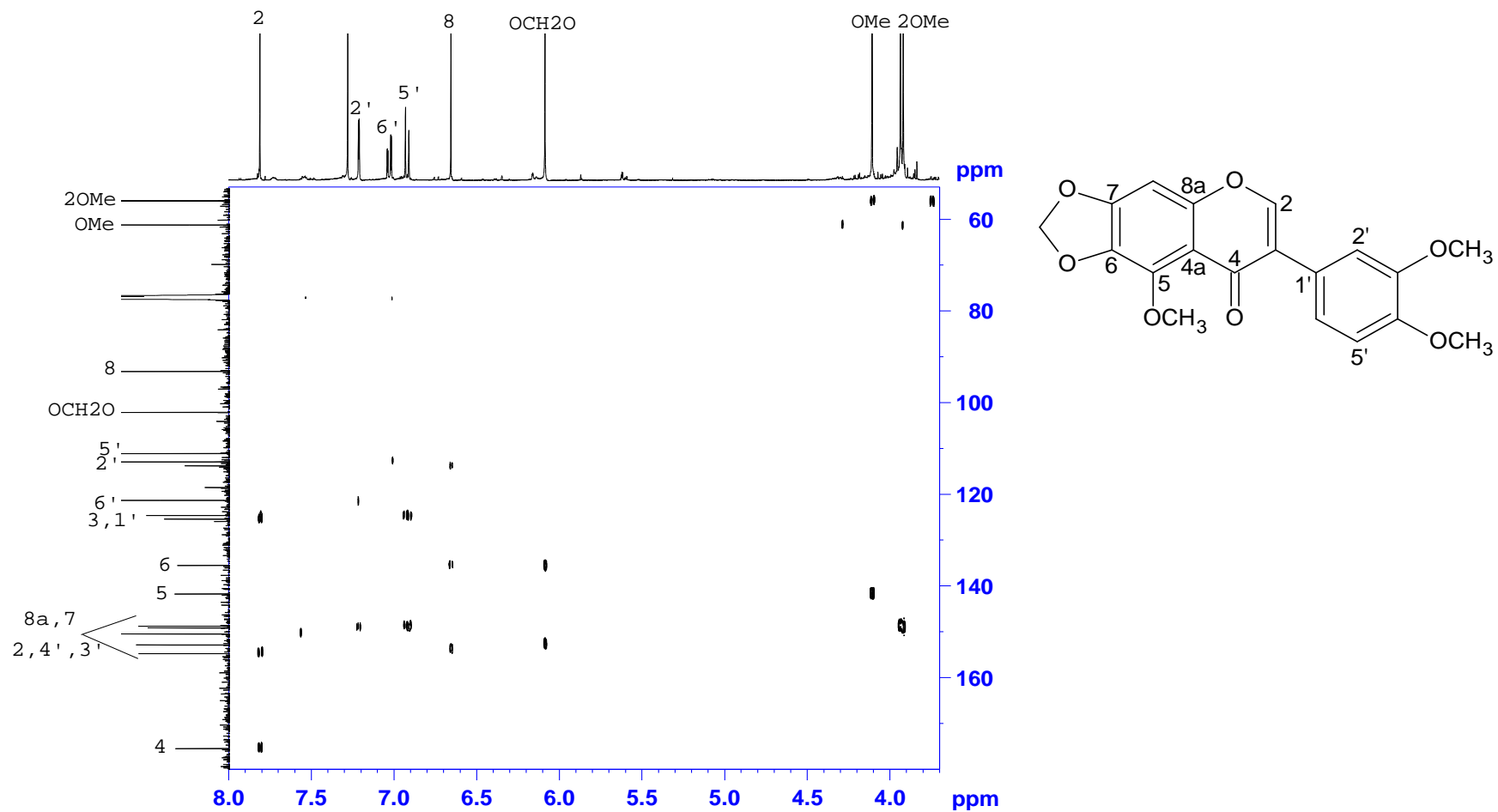
Plate 15f: DEPT-135 Spectrum of iriskumaonin-3'-methyl ether (84) in CDCl$_3$
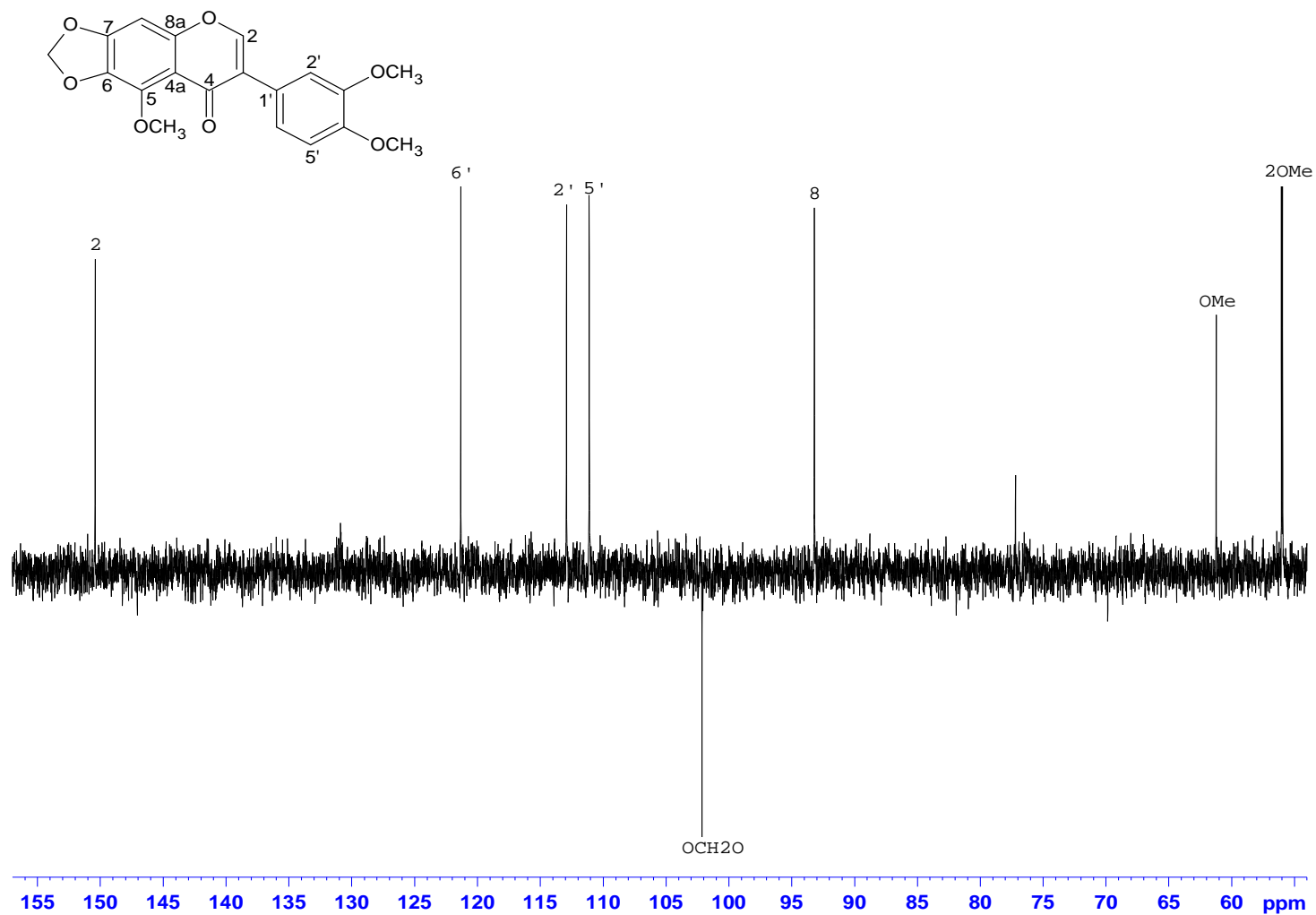
Plate 16a: ^{1}H NMR Spectrum of isocampylospermone A (61) and campylospermone A (62) in CD$_3$OD
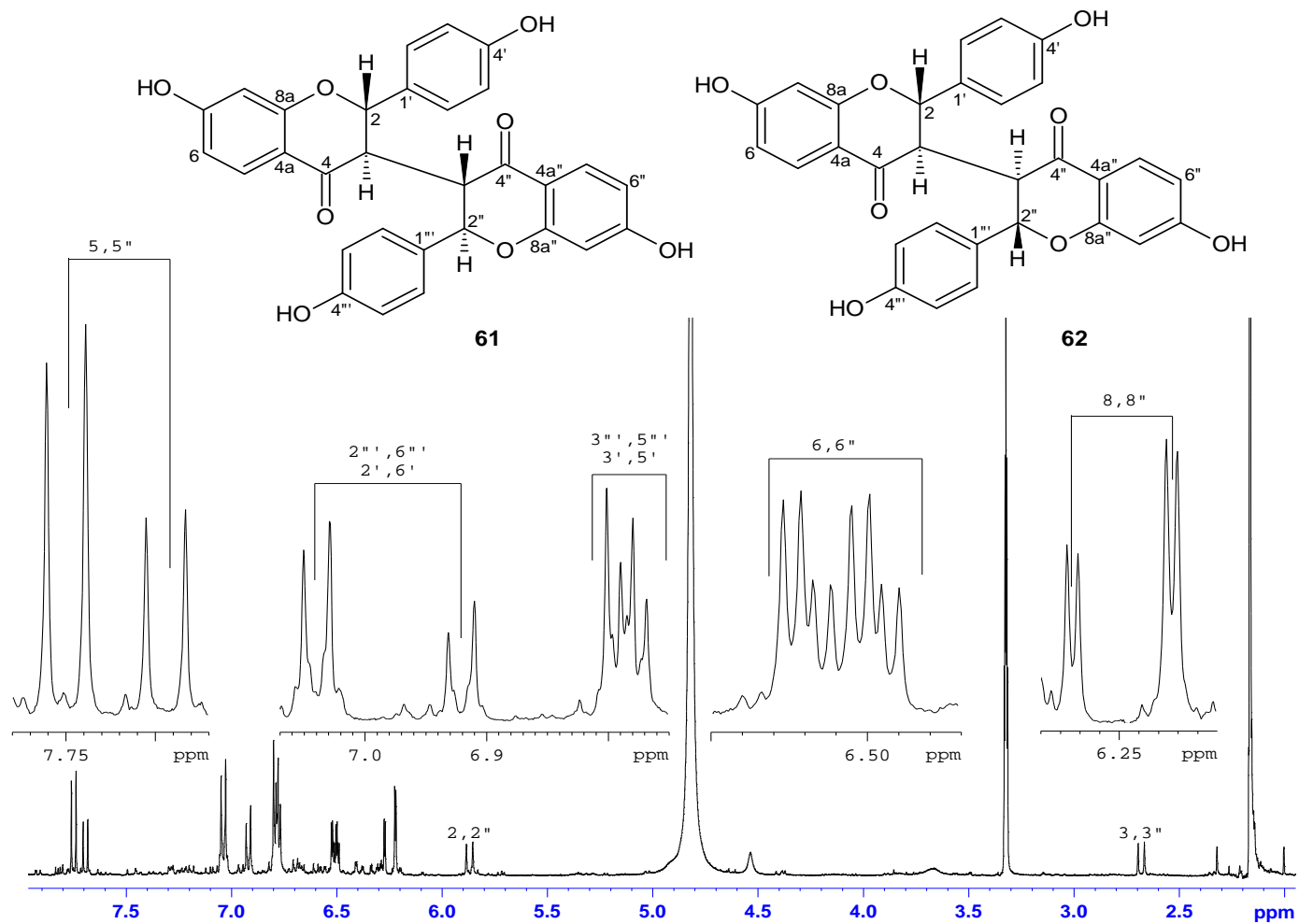
Plate 16b: COSY NMR Spectrum of isocampylospermone A (61) and campylospermone A (62) in CD₃OD
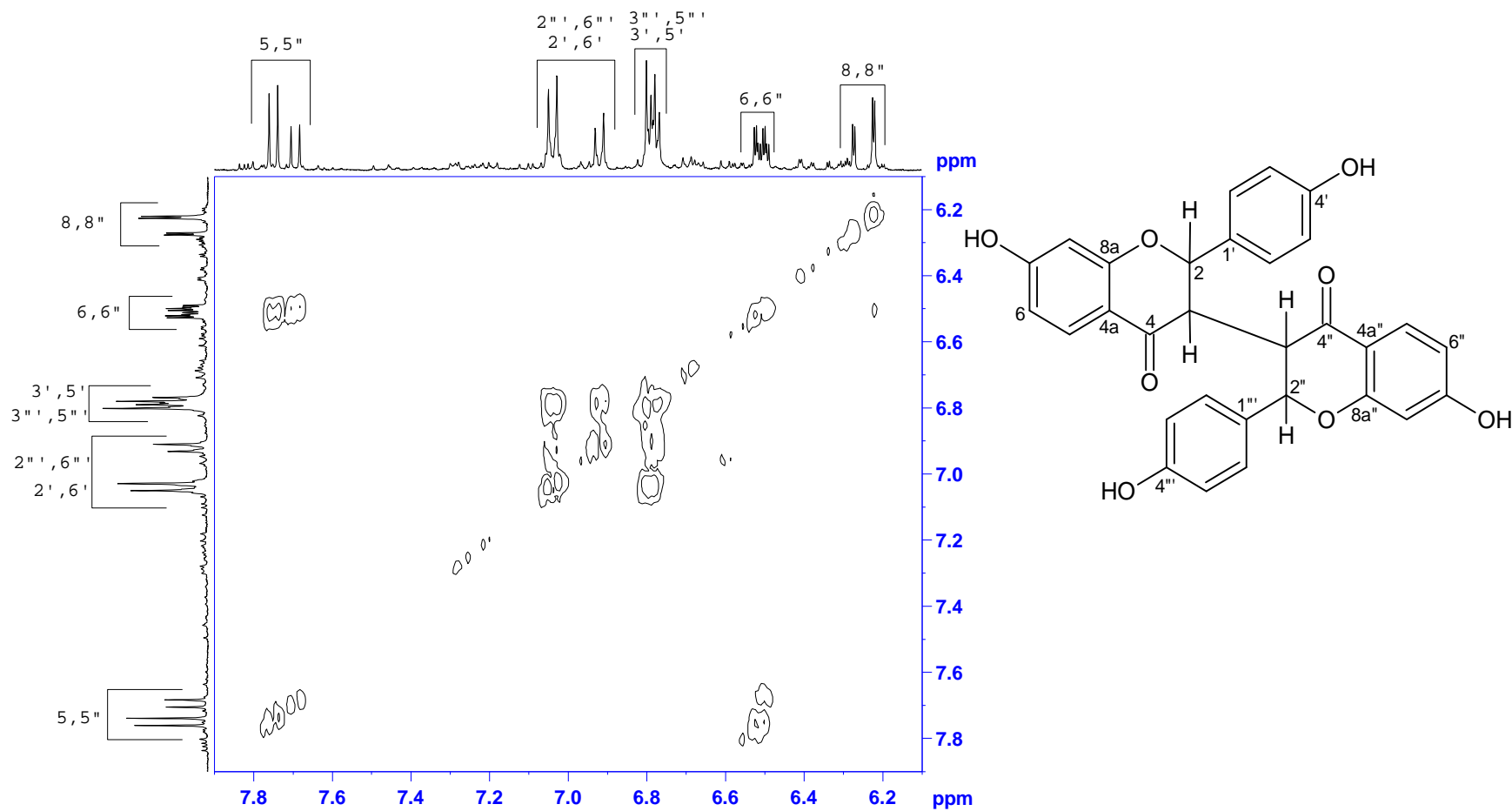
Plate 16c: ^{13}C NMR Spectrum of isocampylospermone A (61) and campylospermone A (62) in CD$_3$OD
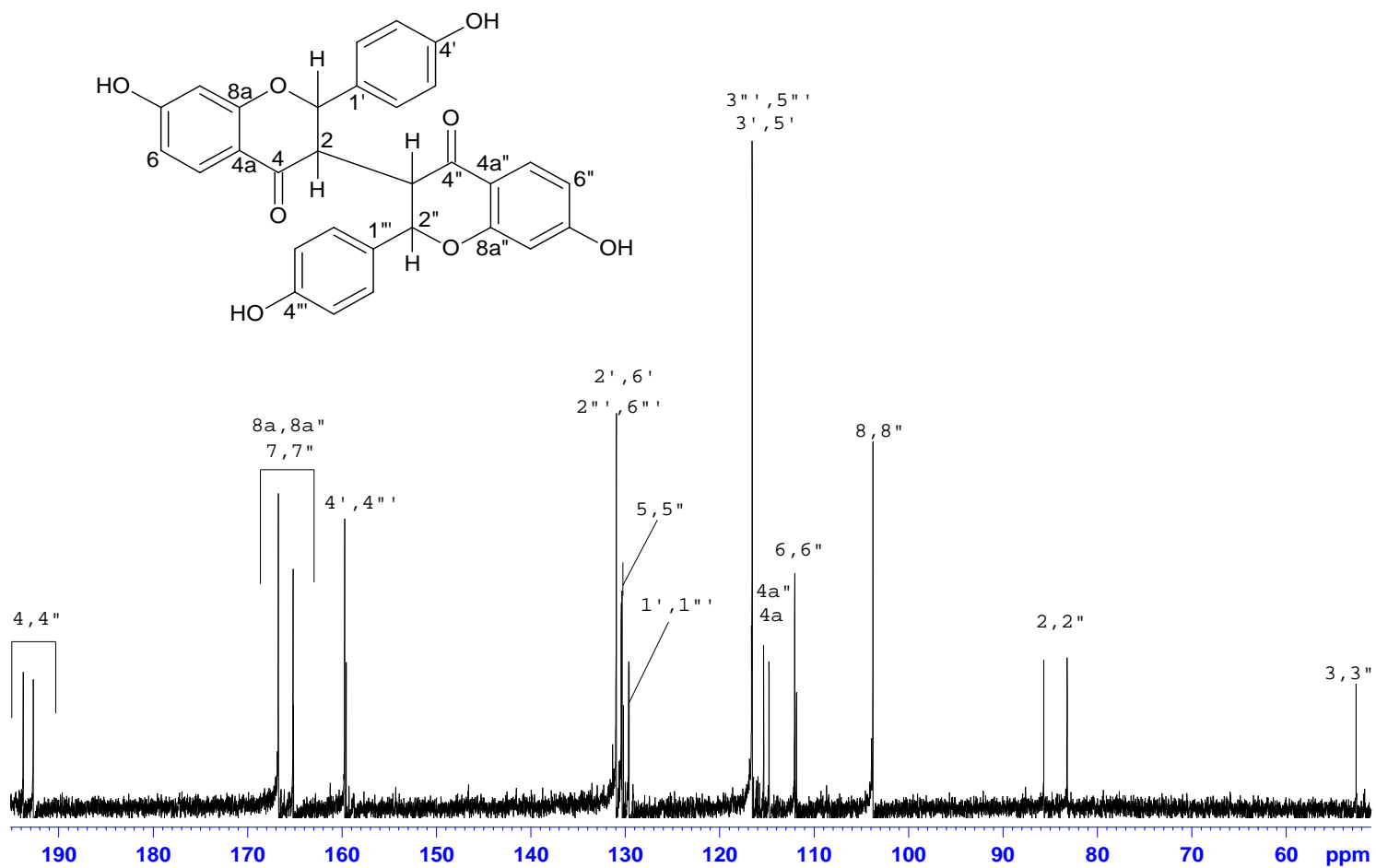
Plate 16d: HSQC NMR Spectrum of isocampylospermone A (61) and campylospermone A (62) in CD₃OD
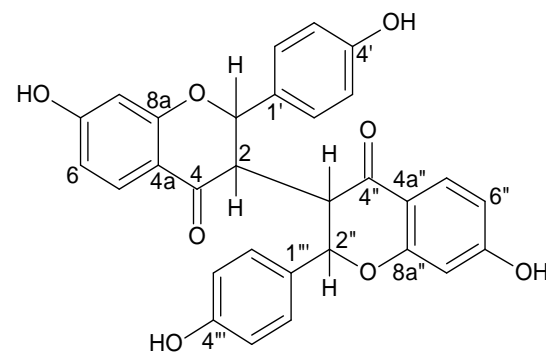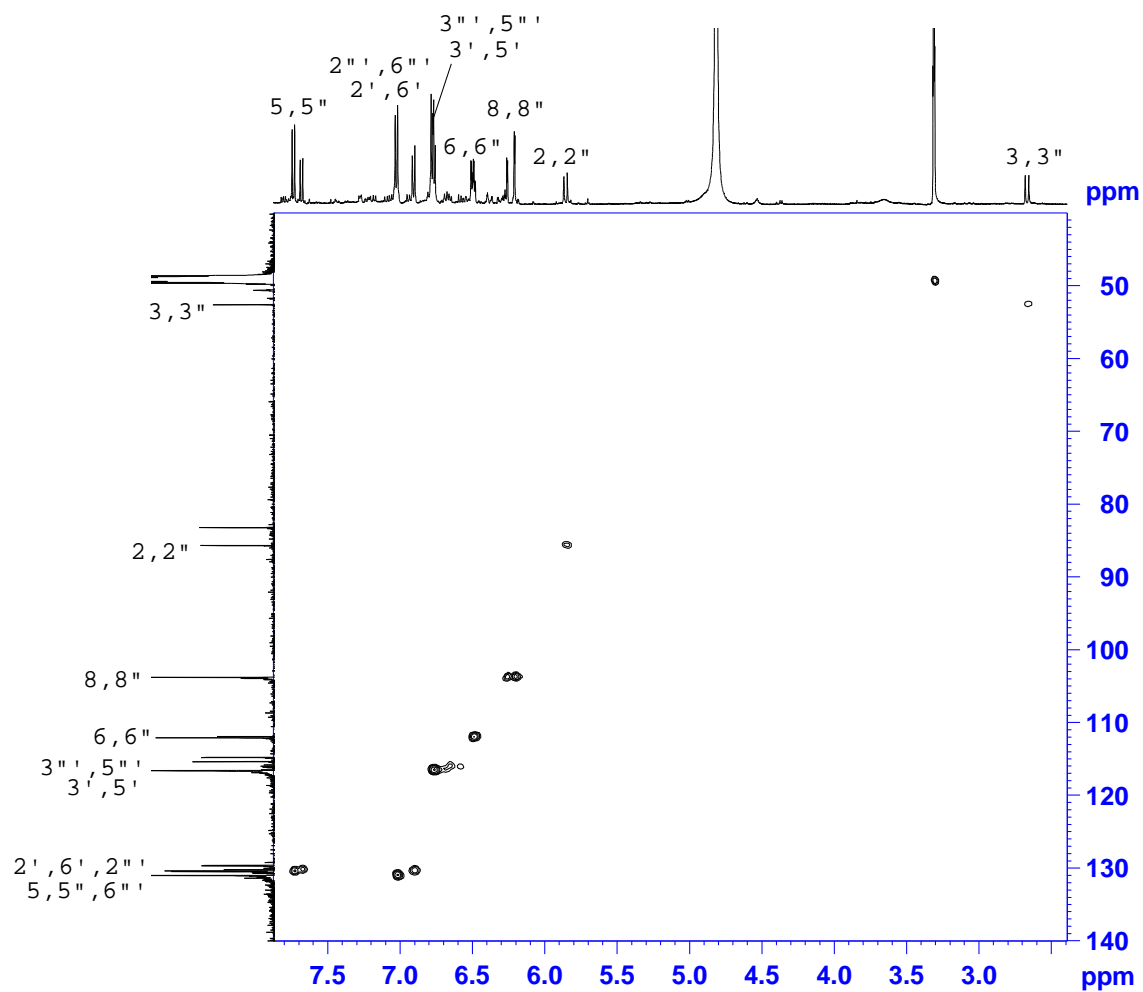
Plate 16e: HMBC NMR Spectrum of isocampylospermone A (61) and campylospermone A (62) in CD₃OD
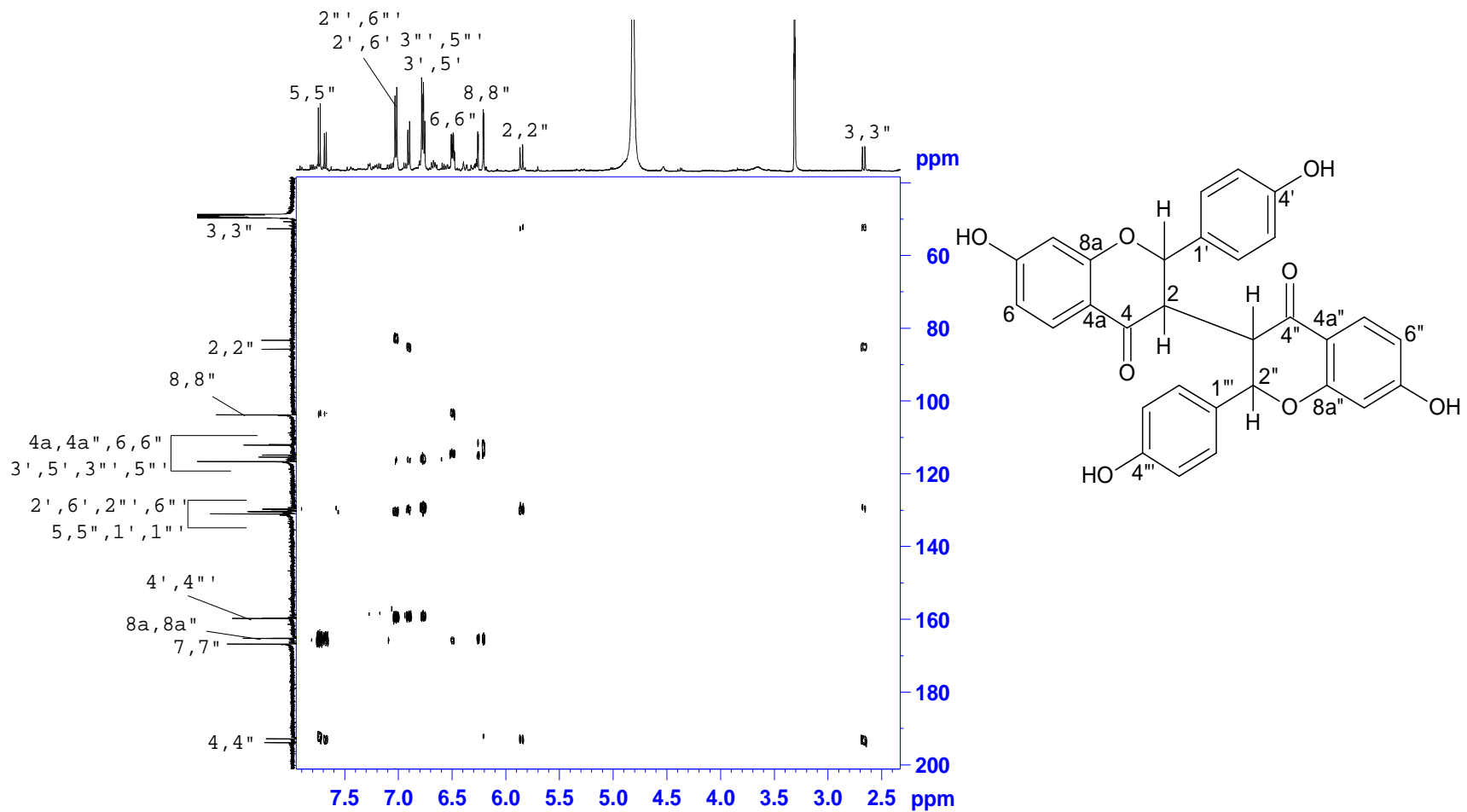
Plate 17a: ^{1}H NMR Spectrum of 4,4',7-tri-*O*-methylisocampylospermone A (85) in (CD$_3$)$_2$CO
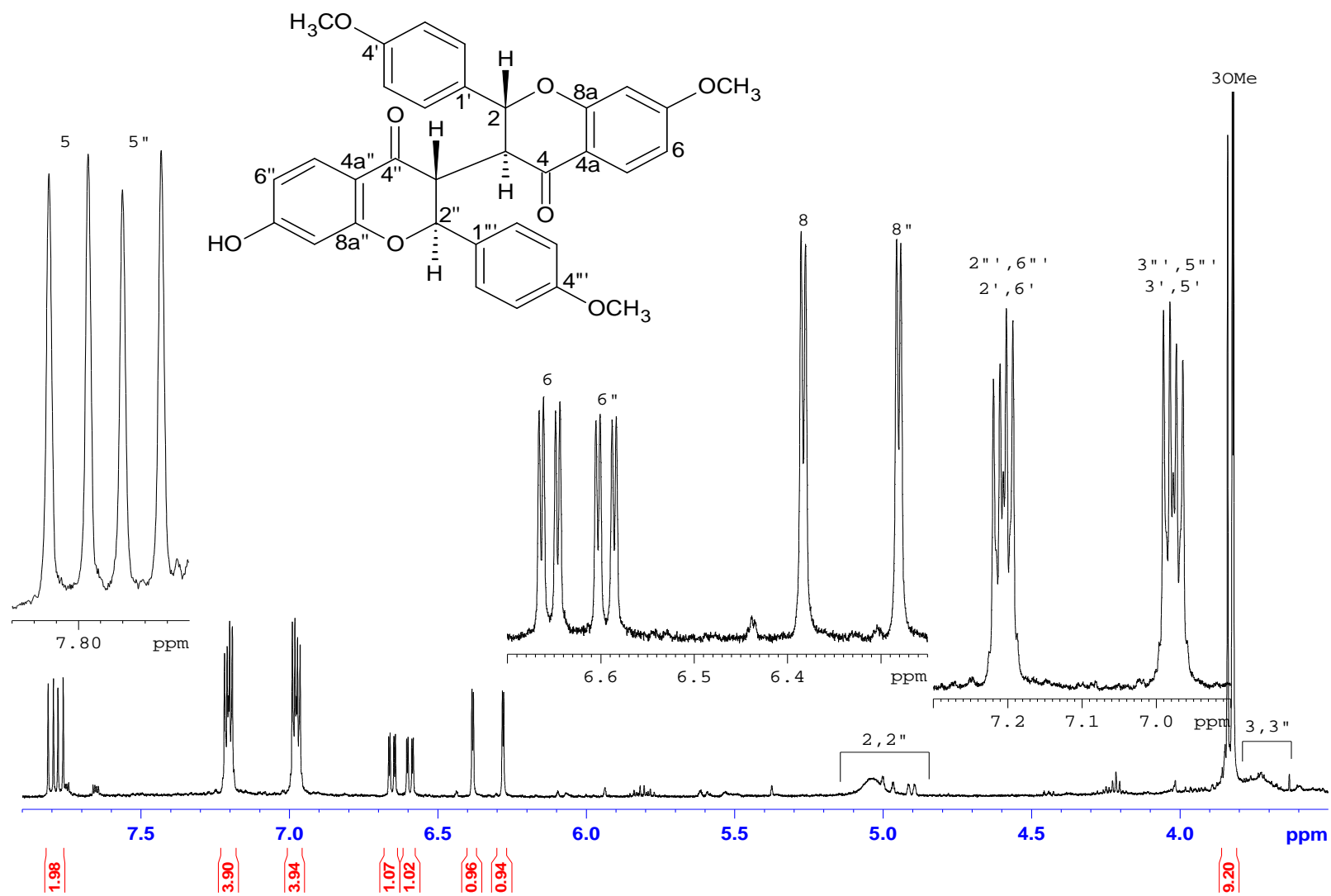
Plate 17b: COSY NMR Spectrum of 4,4',7-tri-*O*-methylisocampylospermone A (85) in (CD$_3$)$_2$CO
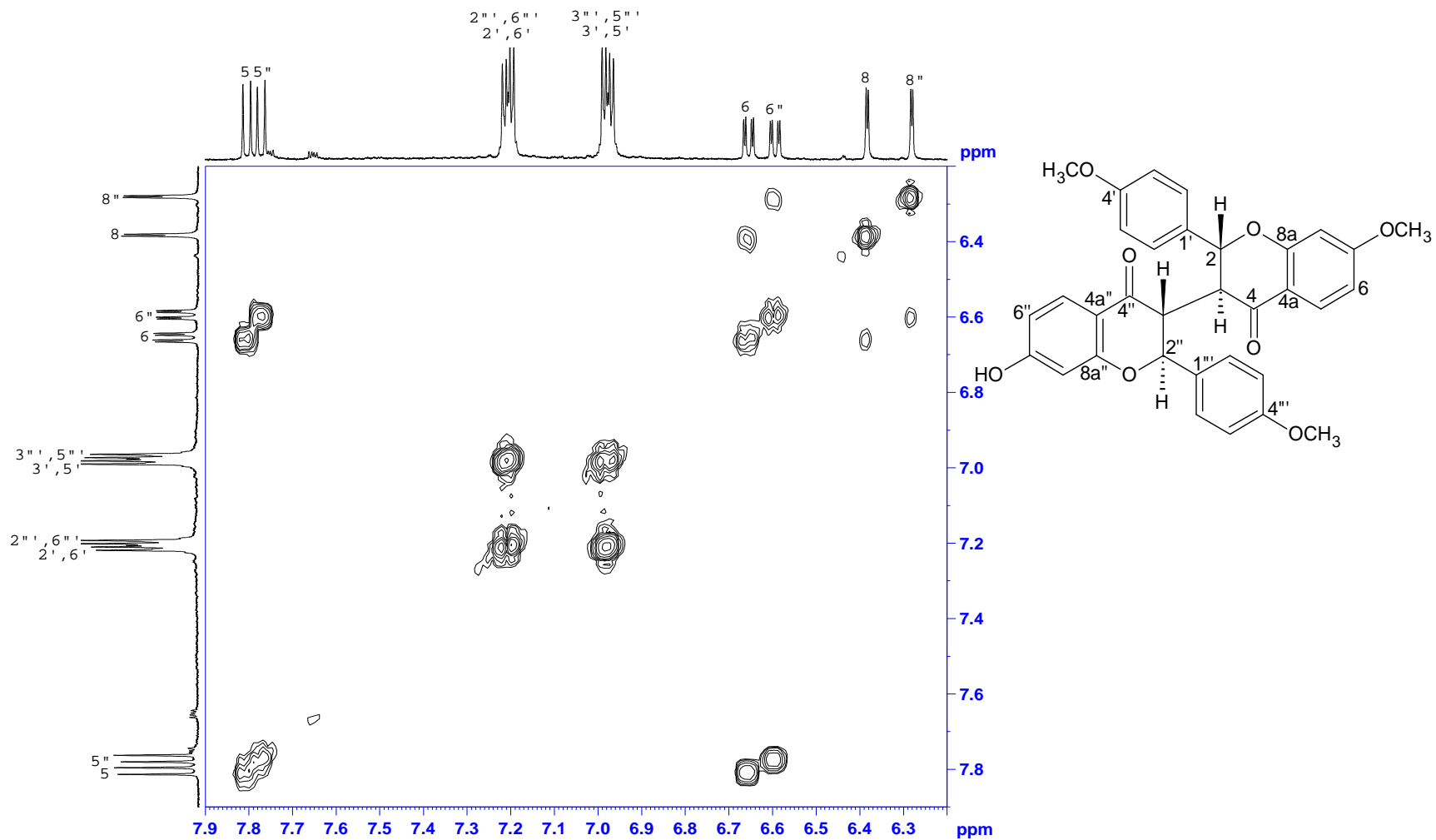
Plate 17c: ^{13}C NMR Spectrum of 4,4',7-tri-*O*-methylisocampylospermone A (85) in (CD$_3$)$_2$CO
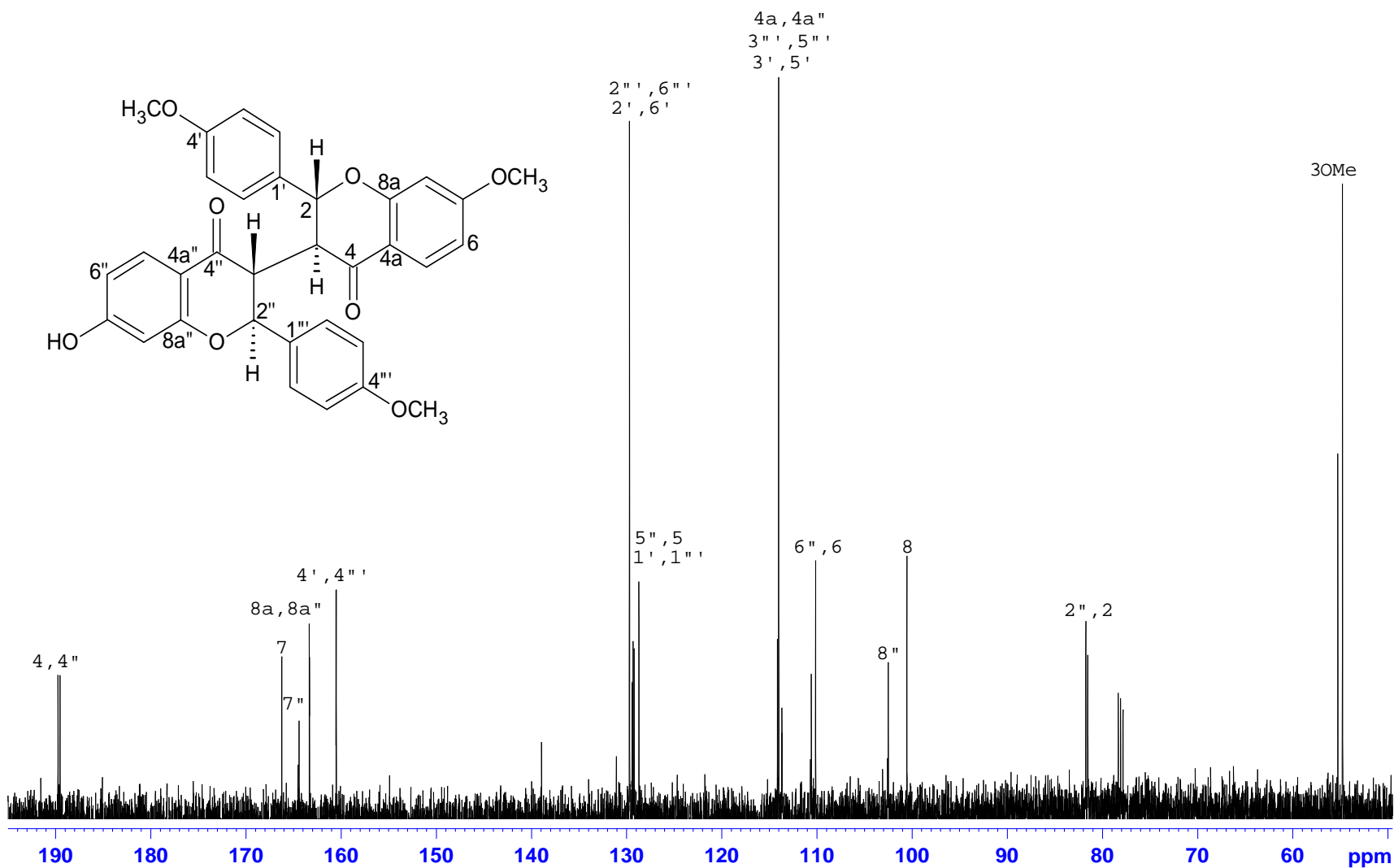
Plate 17d: HSQC NMR Spectrum of 4,4',7-tri-*O*-methylisocampylospermone A (85) in (CD$_3$)$_2$CO
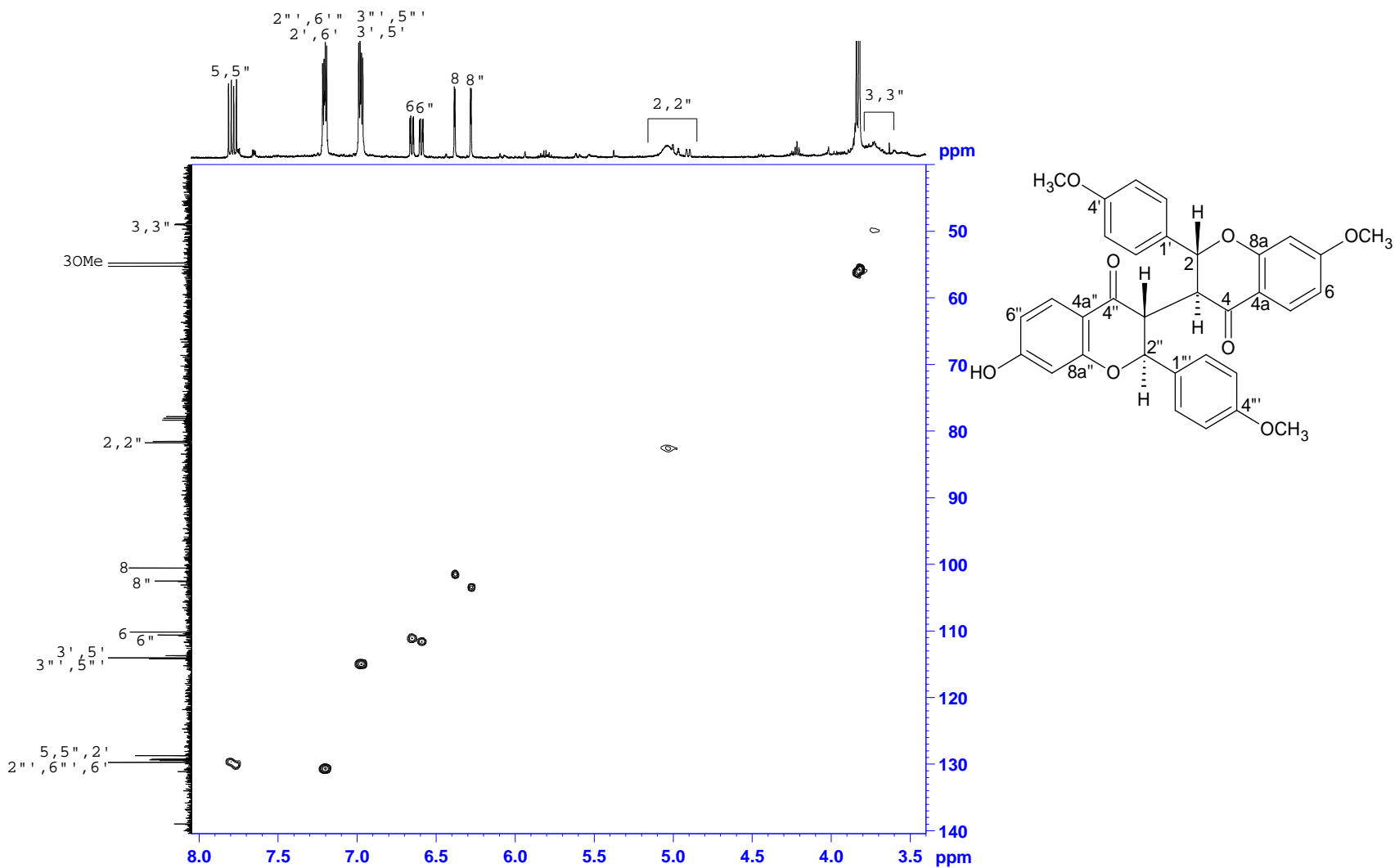
Plate 17e: HMBC NMR Spectrum of 4,4',7-tri-*O*-methylisocampylospermone A (85) in (CD₃)₂CO
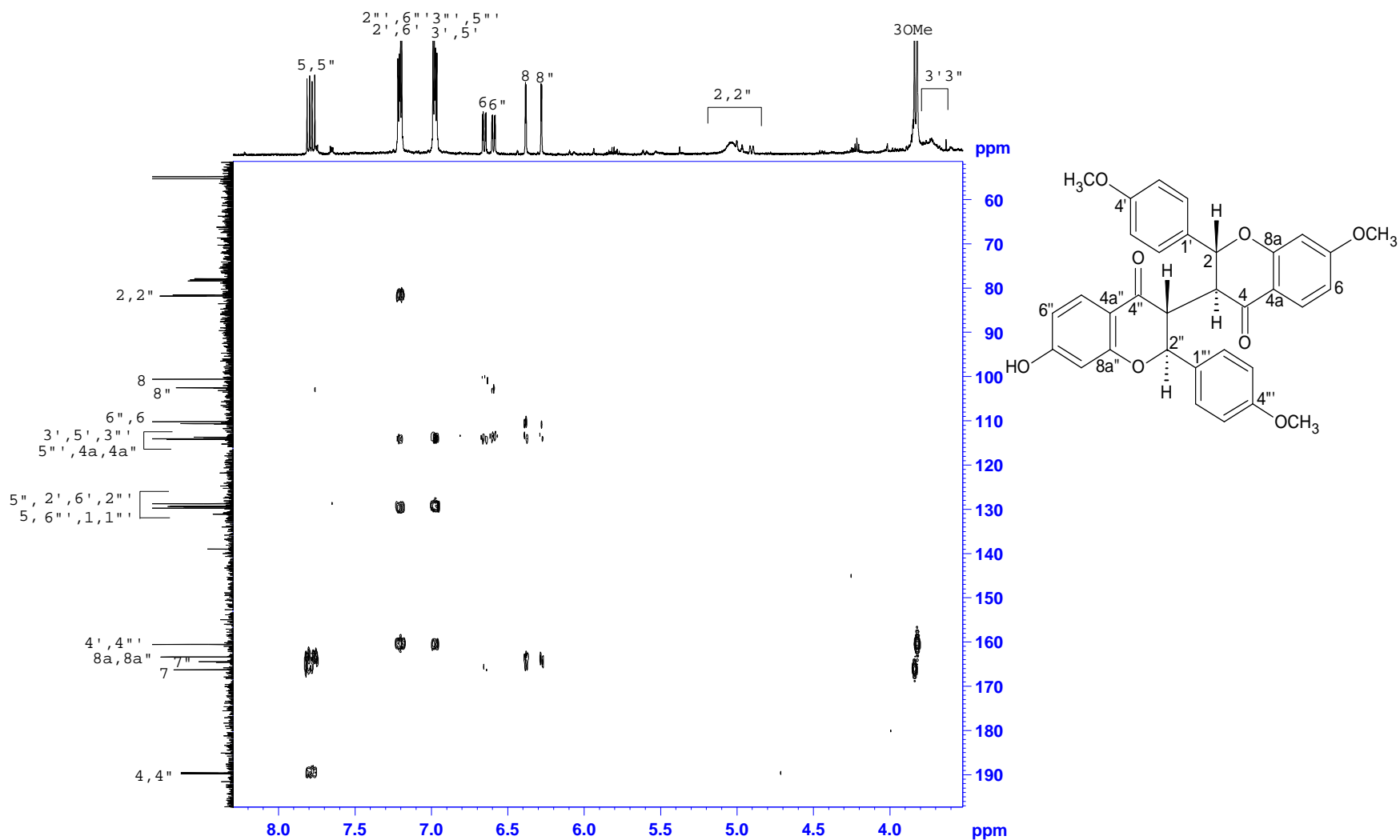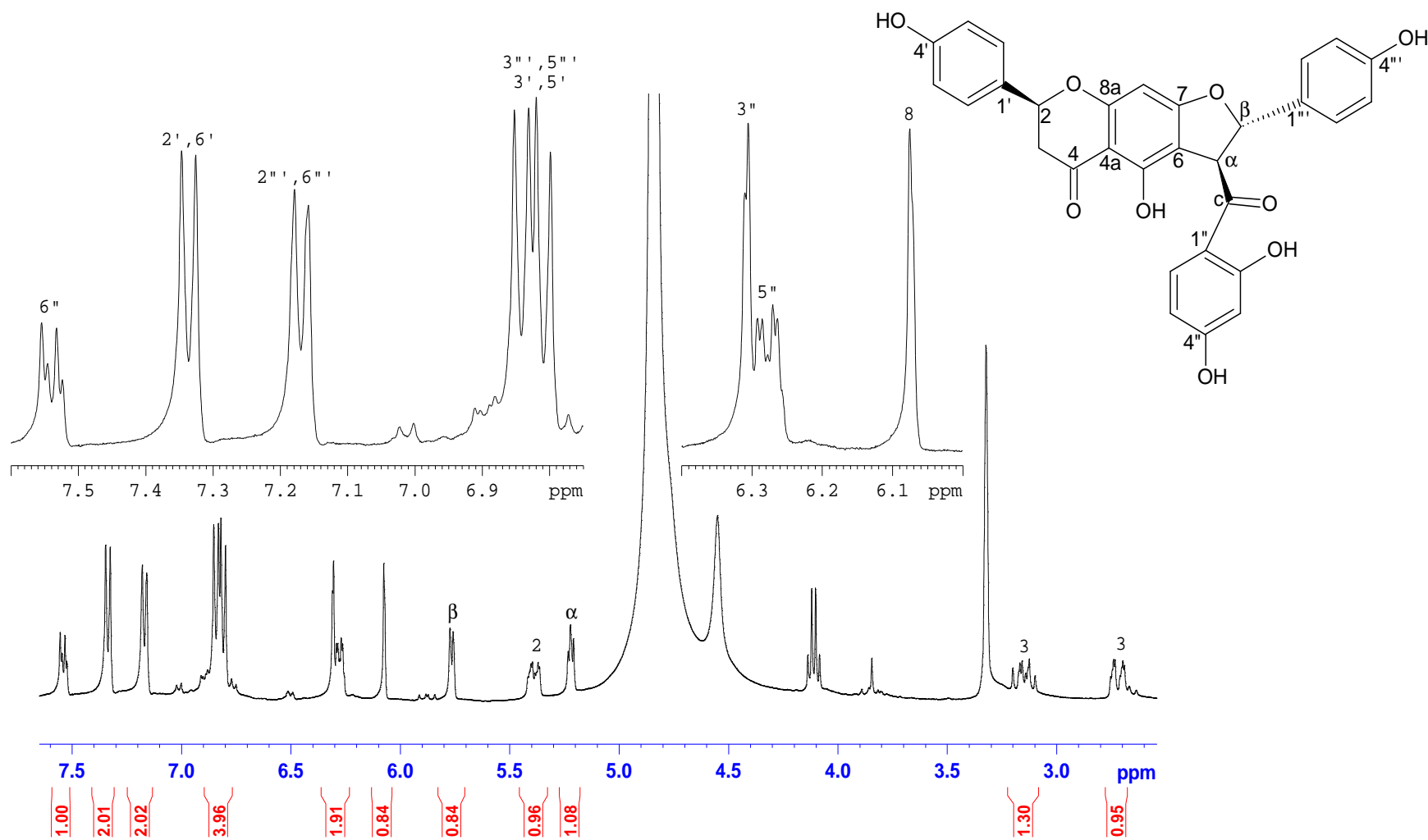
Plate 18b: COSY NMR Spectrum of 4"'-De-*O*-methylafzelone A (86) in CD₃OD

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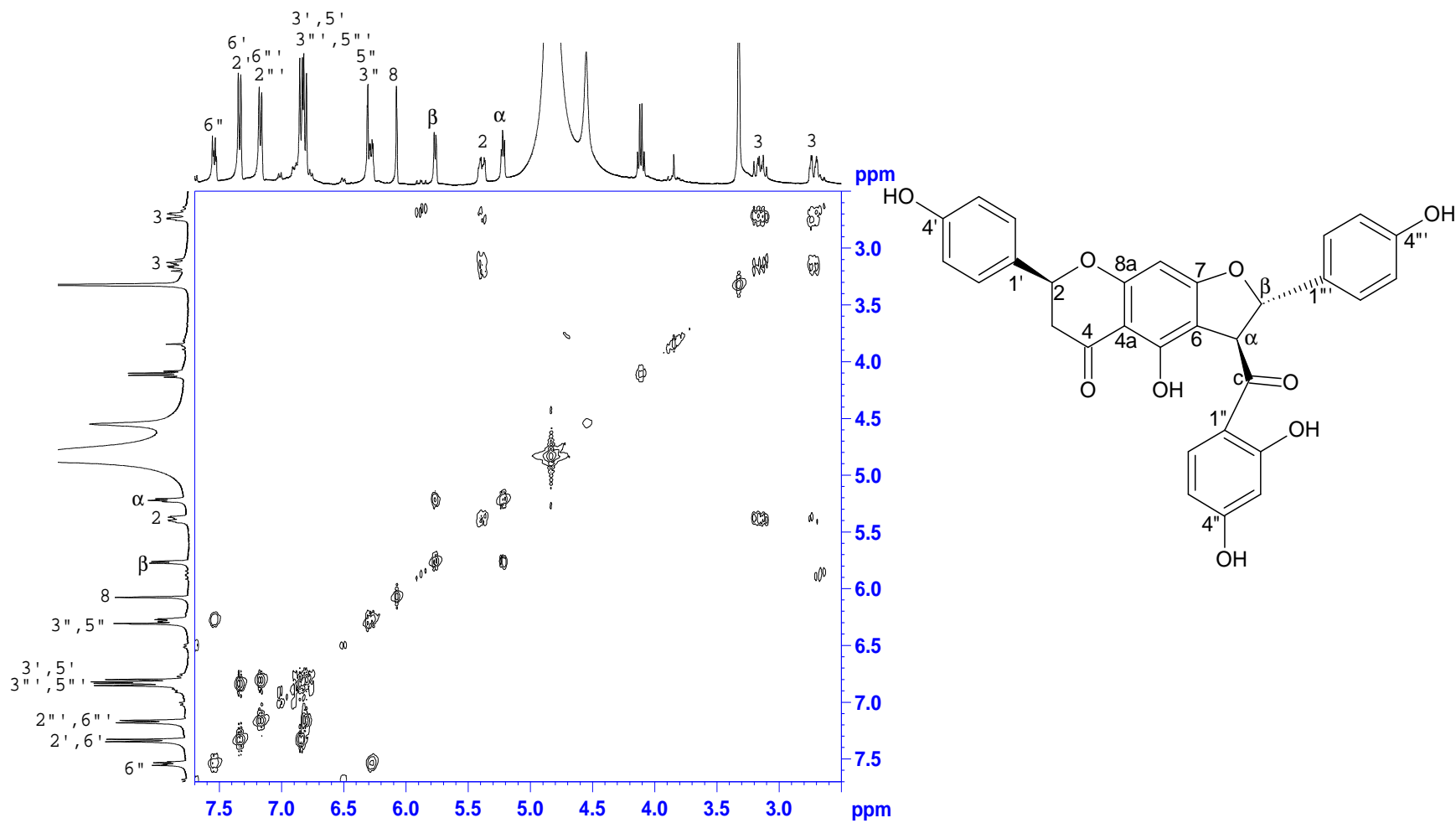
Plate 18c: ^{13}C NMR Spectrum of 4'''-De-*O*-methylafzelone A (86) in CD$_3$OD

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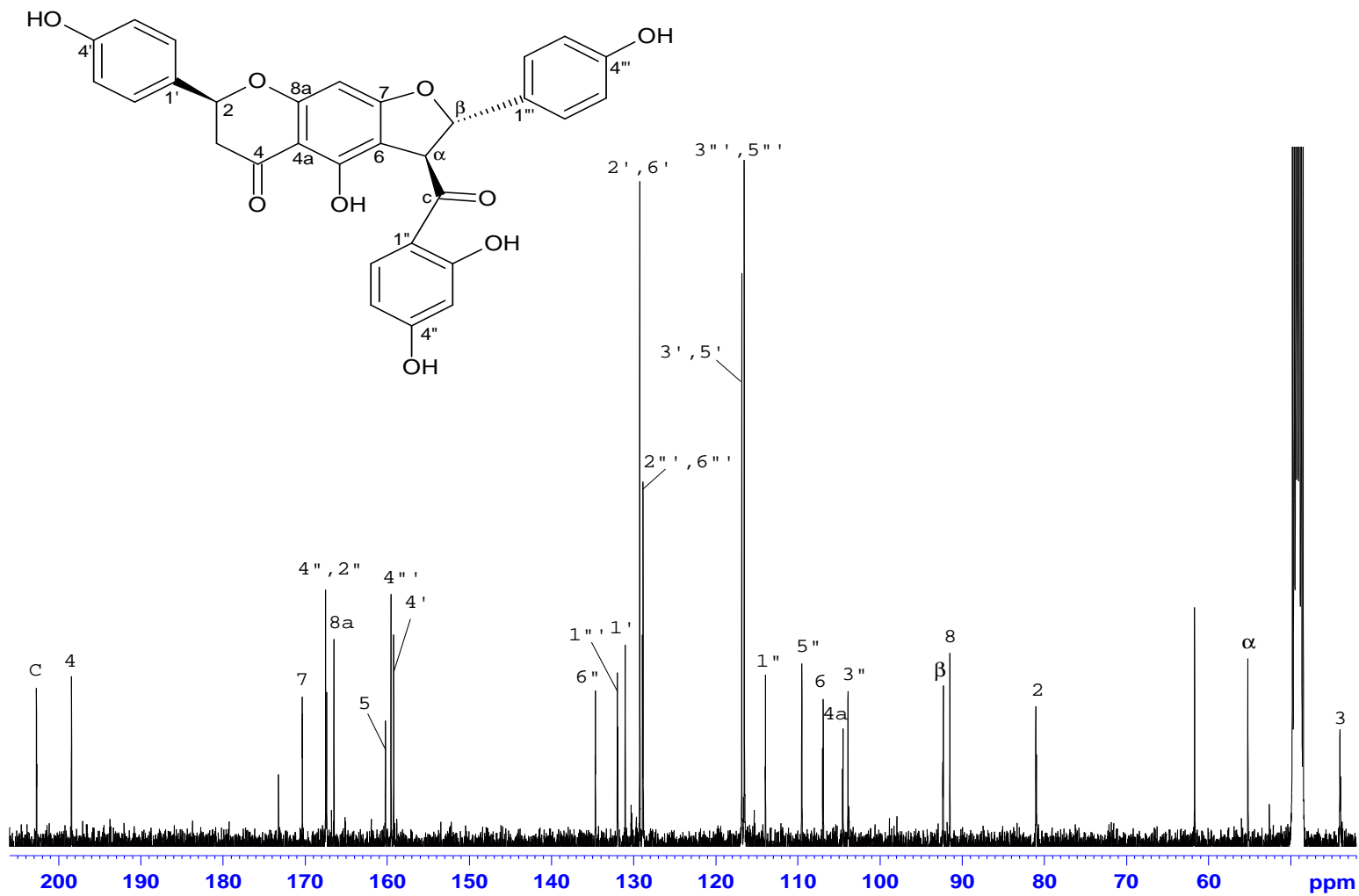
Plate 18e: HMBC NMR Spectrum of 4'''-De-*O*-methylafzelone A (86) in CD₃OD
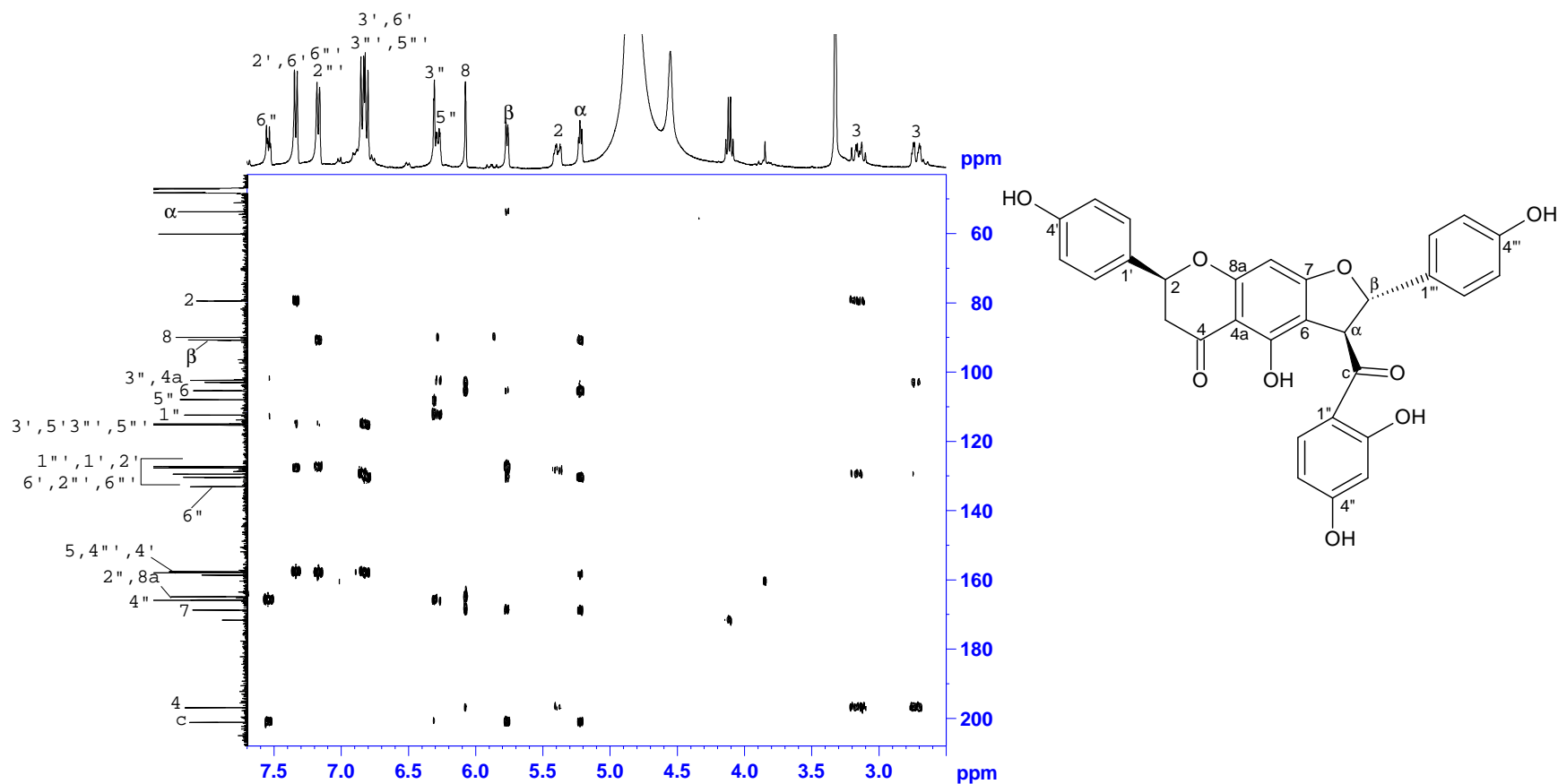
Plate 18f: DEPT-135 Spectrum of 4'''-De-*O*-methylafzelone A (86) in CD₃OD
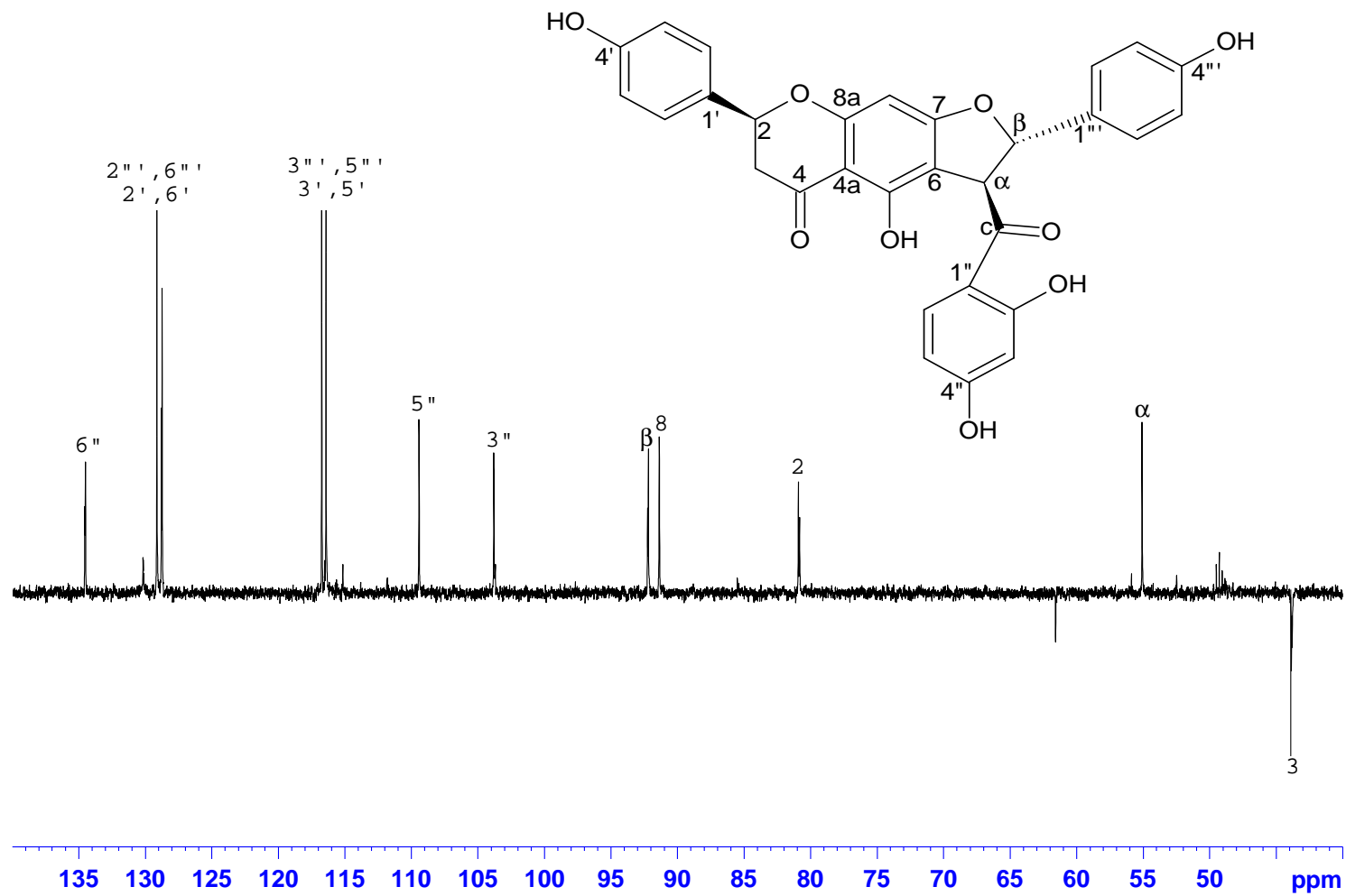
Plate 19a: ^{1}H NMR Spectrum of serrulone (87) in $(CD_3)_2CO$

275
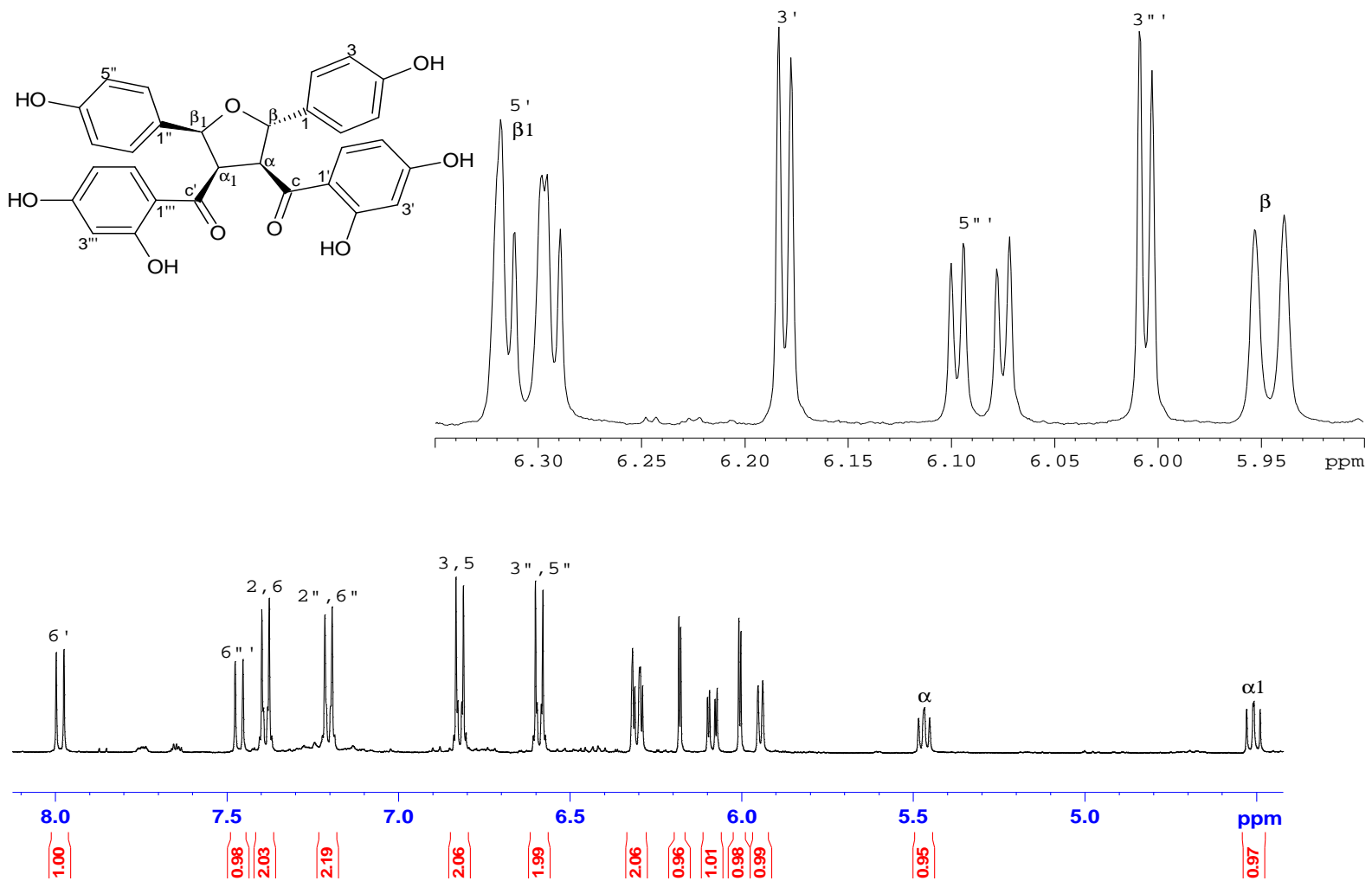
Plate 19b: COSY NMR Spectrum of serrulone (87) in $(CD_3)_2CO$
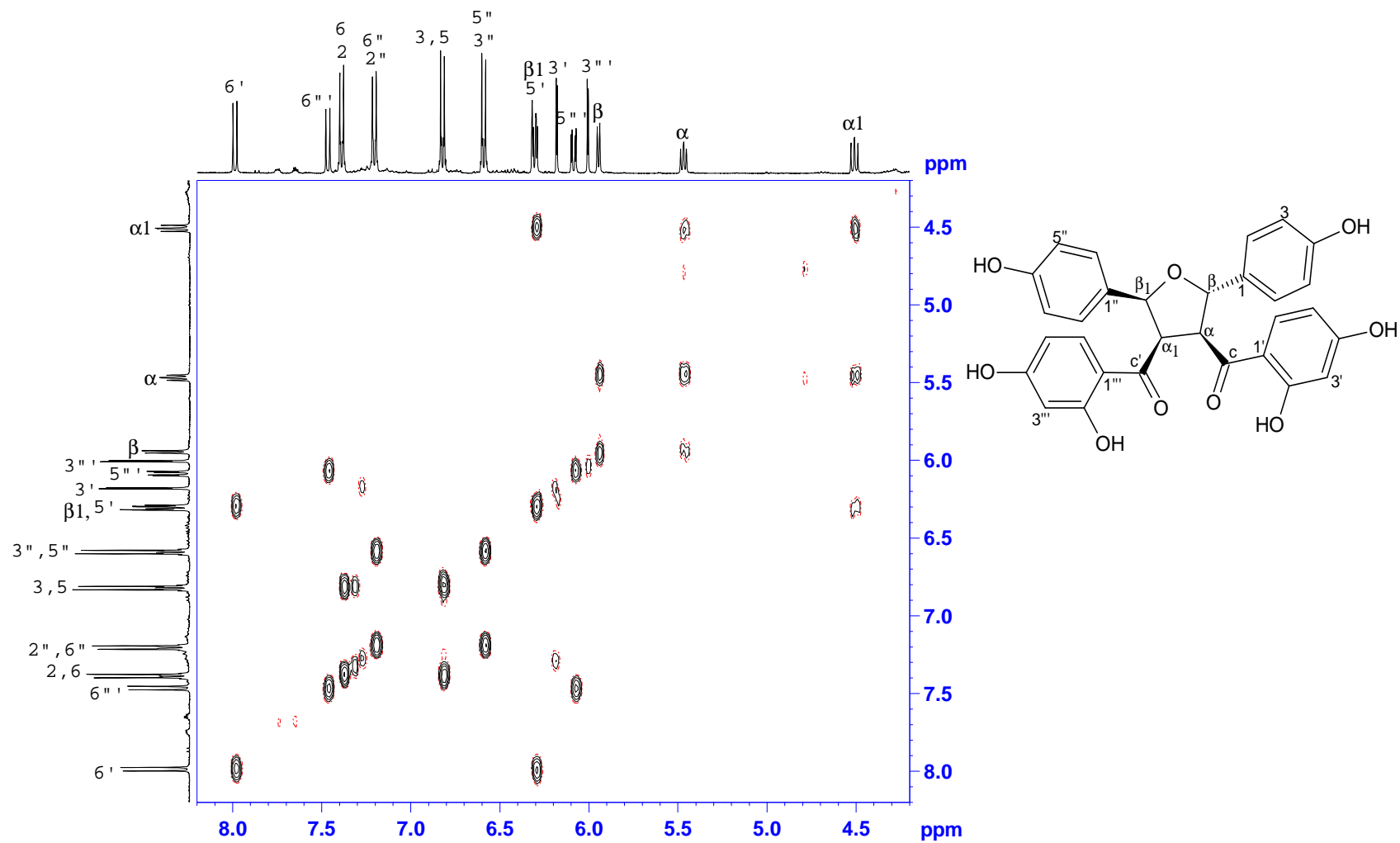
Plate 19c: ^{13}C NMR Spectrum of serrulone (87) in $(CD_3)_2CO$
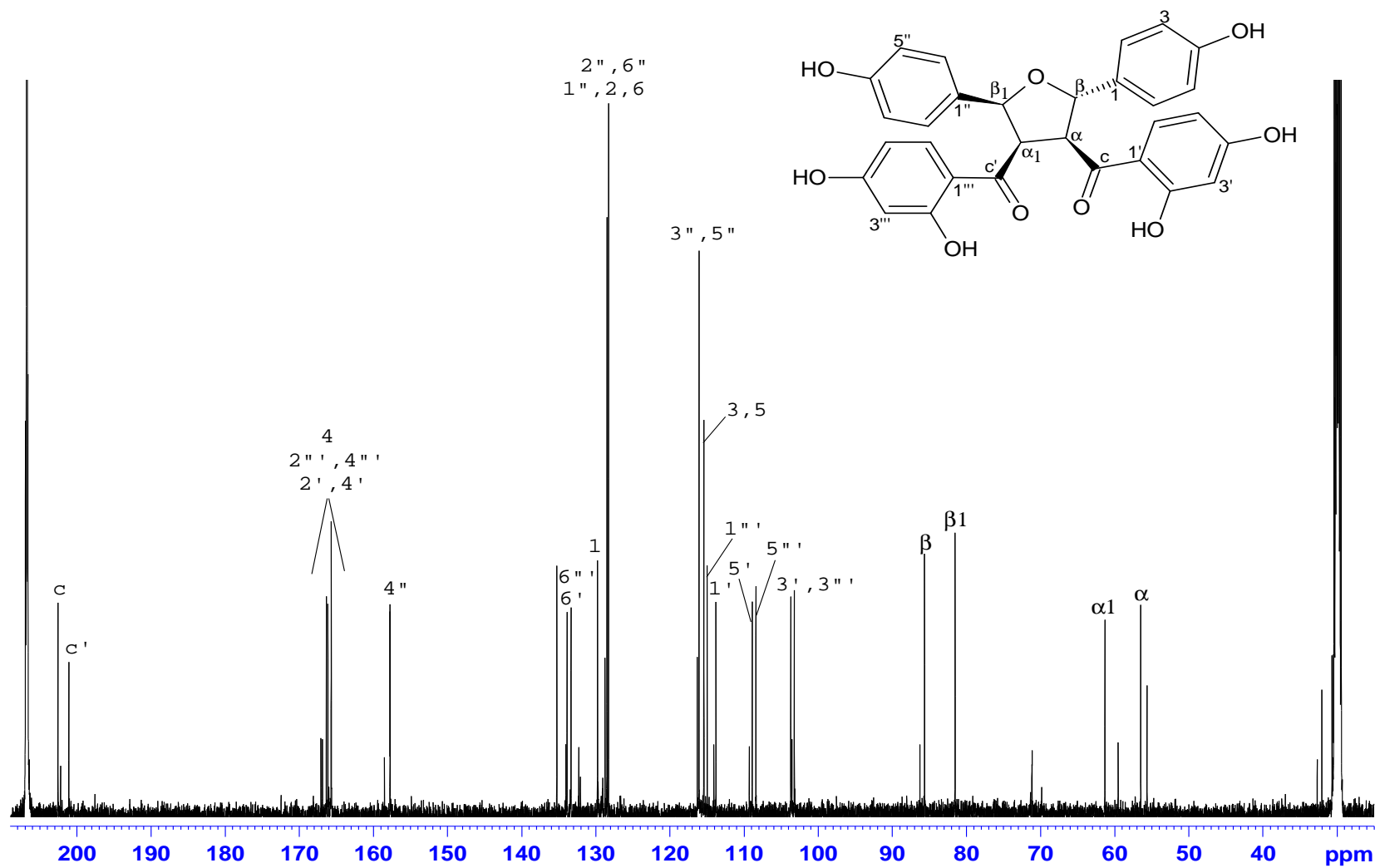
Plate 19d: HSQC NMR Spectrum of serrulone (87) in (CD$_3$)$_2$CO

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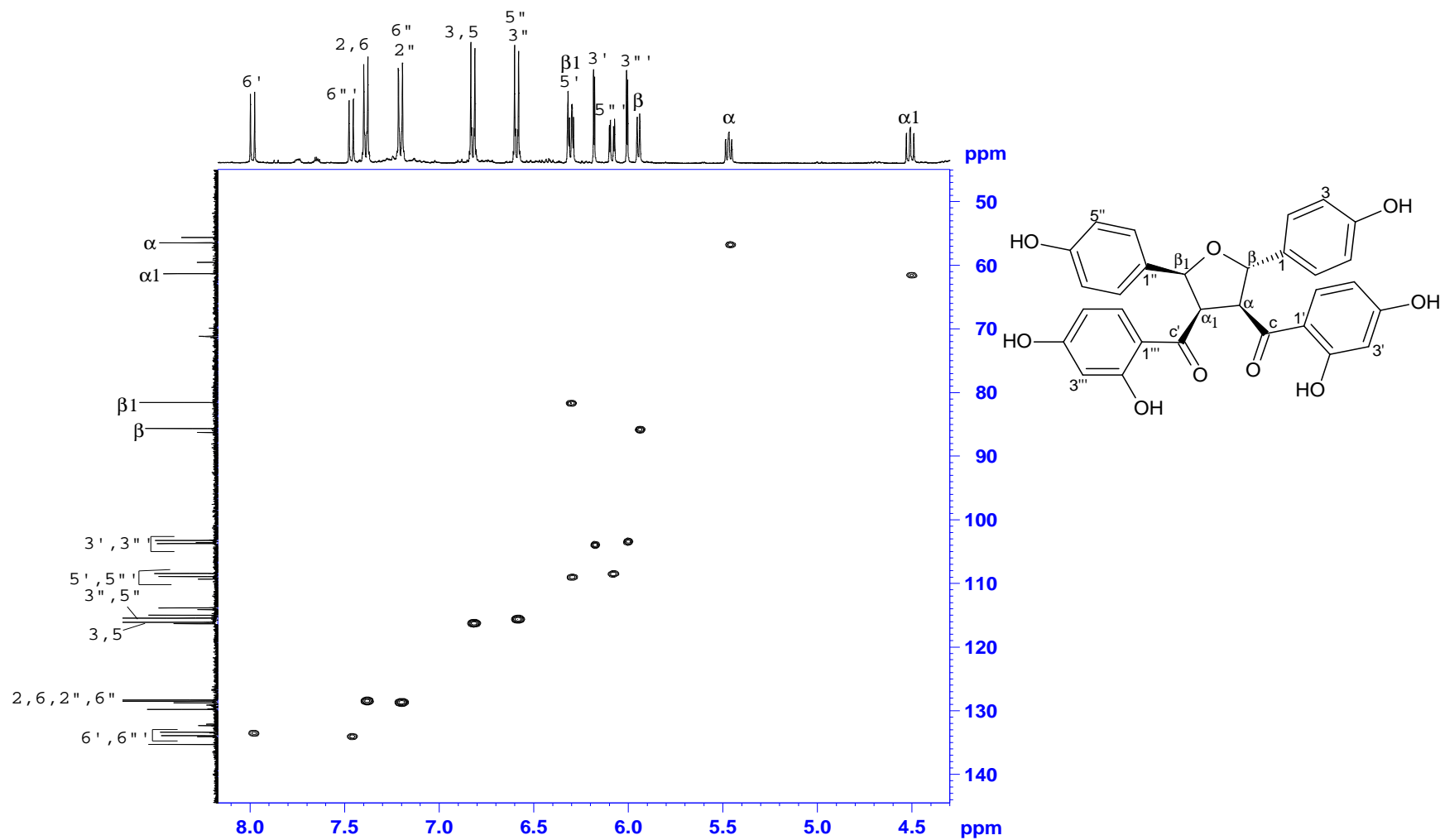
Plate 19e: HMBC NMR Spectrum of serrulone (87) in $(CD_3)_2CO$
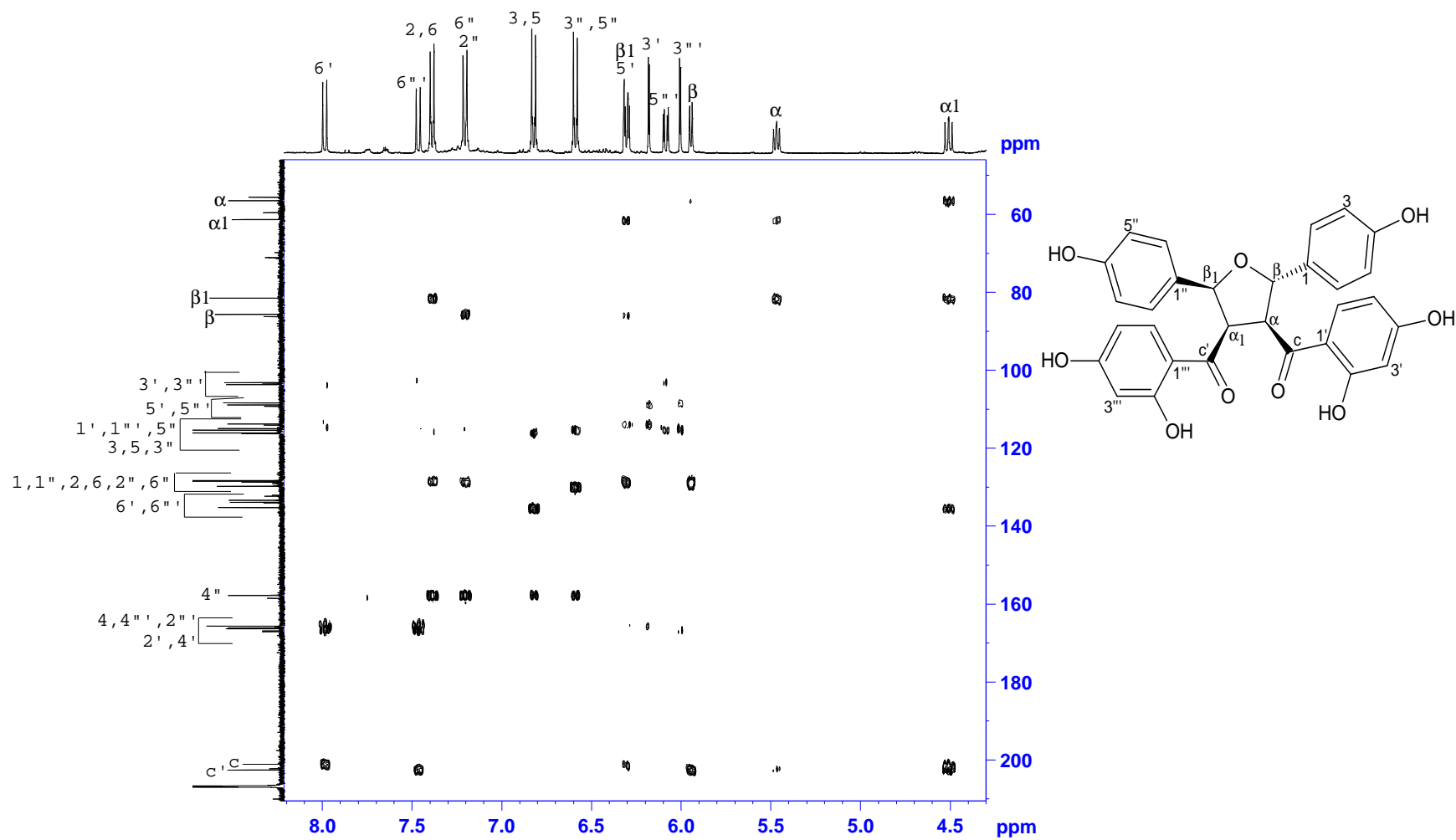
Plate 20a: ^{1}H NMR Spectrum of Lophirone L (88) in CD$_3$OD
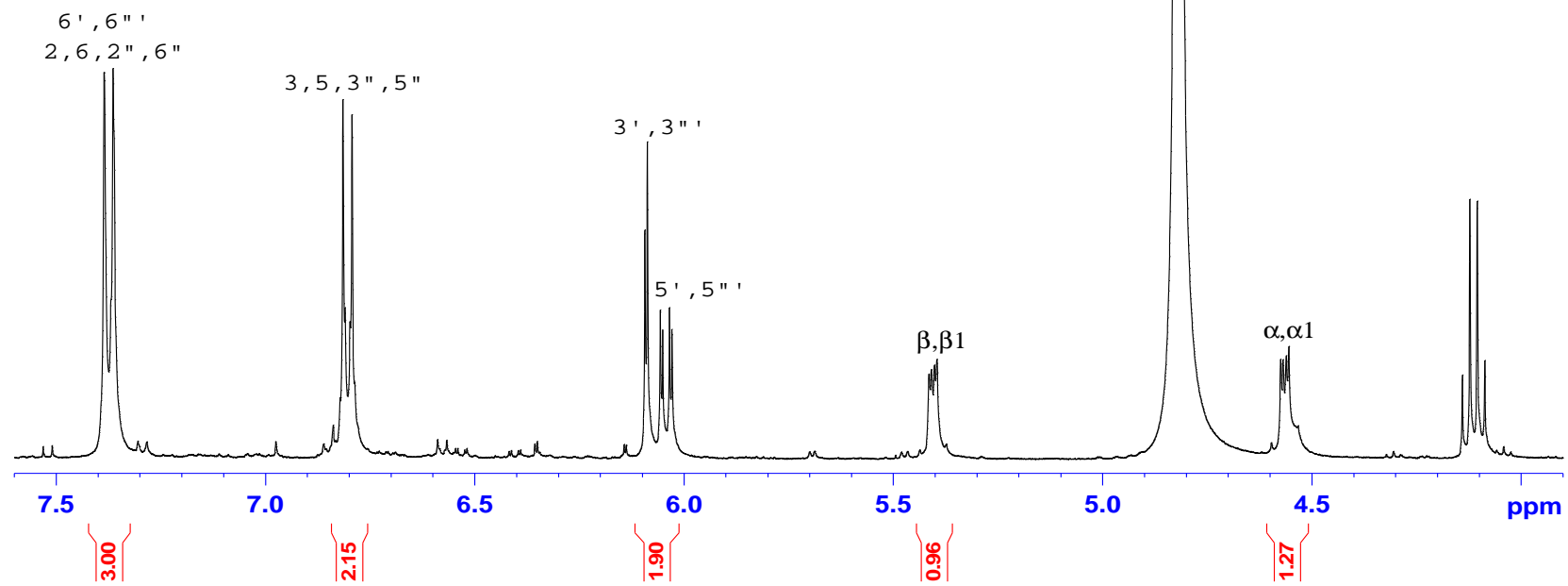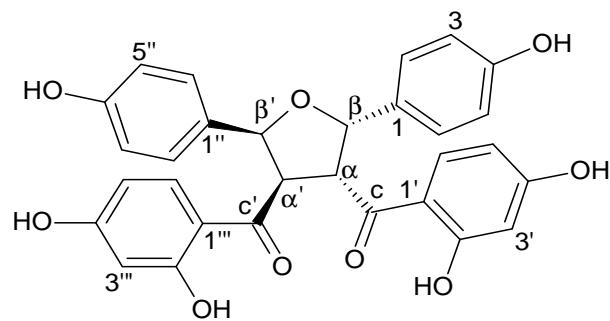
Plate 20b: COSY NMR Spectrum of Lophirone L (88) in CD₃OD
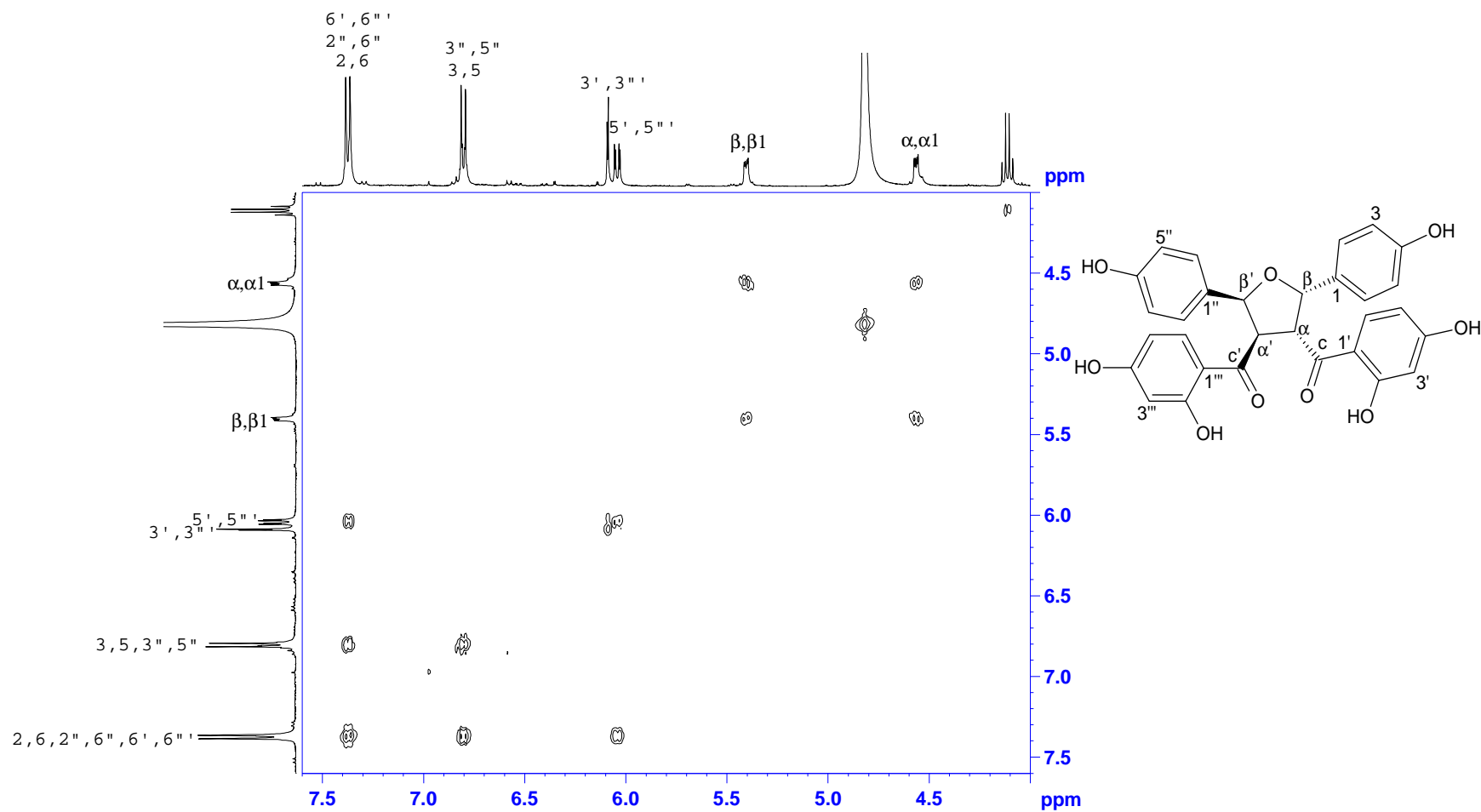
Plate 20c: ^{13}C NMR Spectrum of Lophirone L (88) in CD$_3$OD
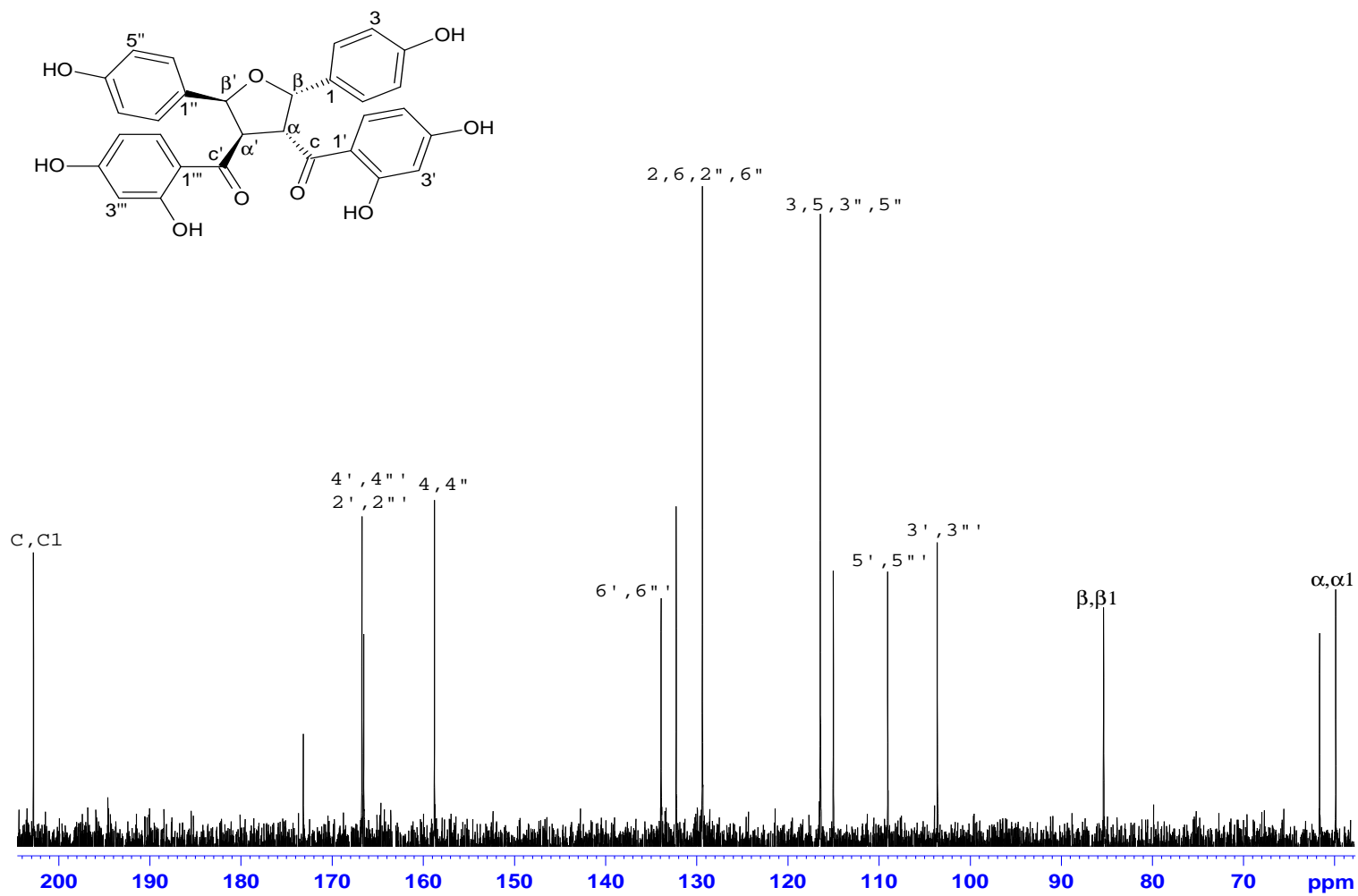
Plate 20d: HSQC NMR Spectrum of Lophirone L (88) in CD₃OD
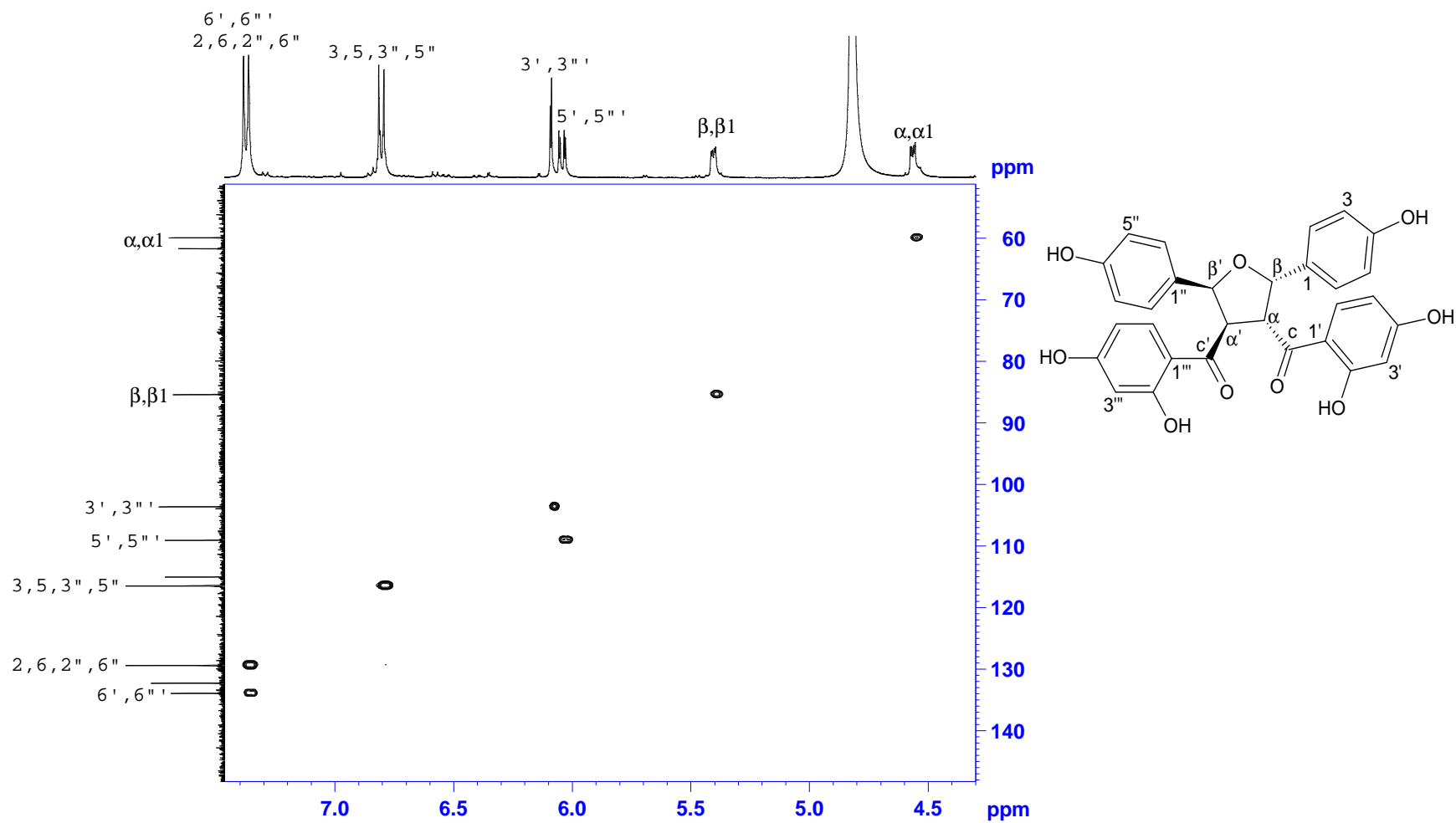
Plate 20e: HMBC NMR Spectrum of Lophirone L (88) in CD₃OD
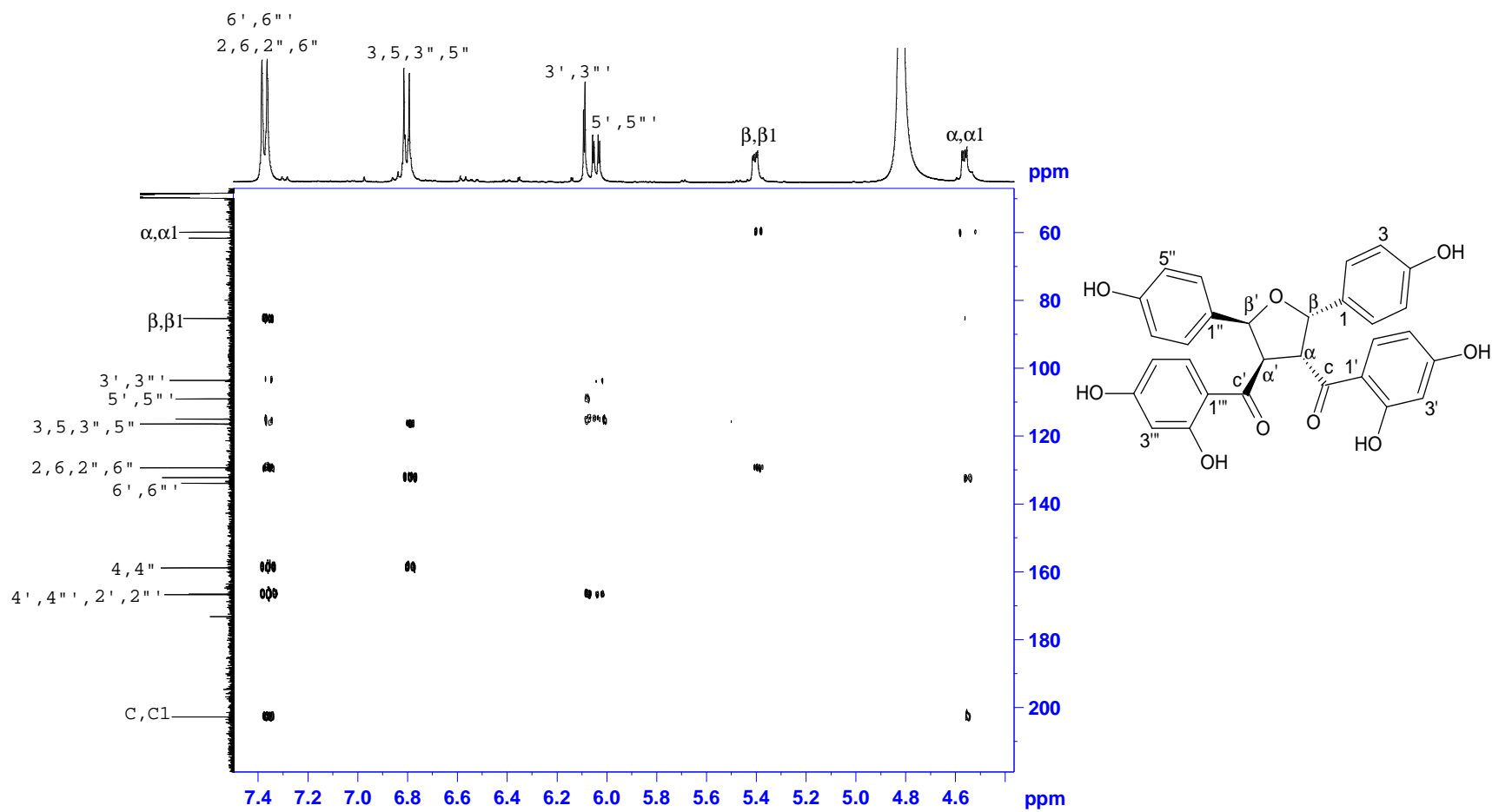
Plate 21a: ^{1}H NMR Spectrum of Syringaresinol (89) in (CD$_3$)$_2$CO
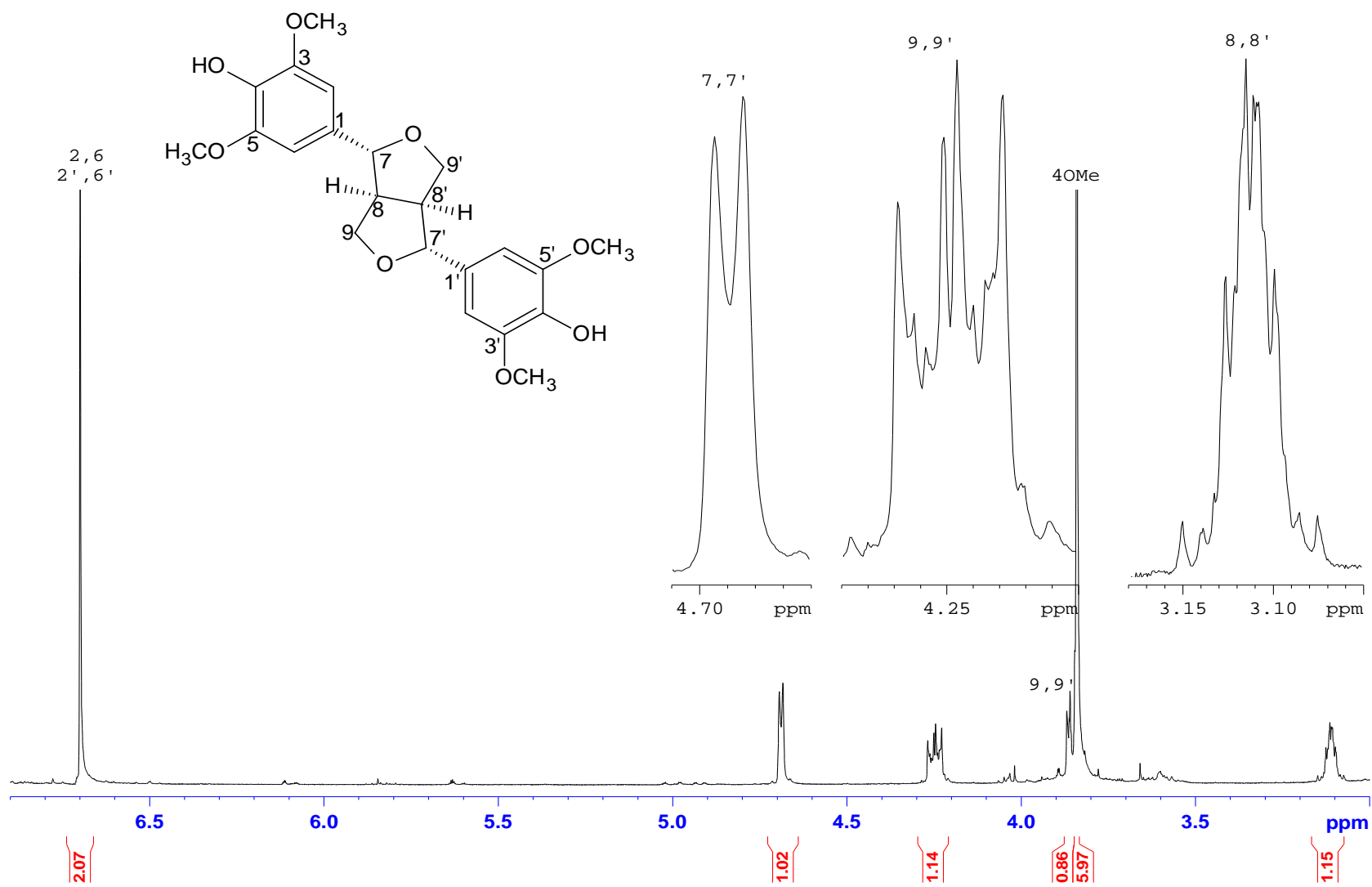
Plate 21b: COSY NMR Spectrum of Syringaresinol (89) in $(CD_3)_2CO$
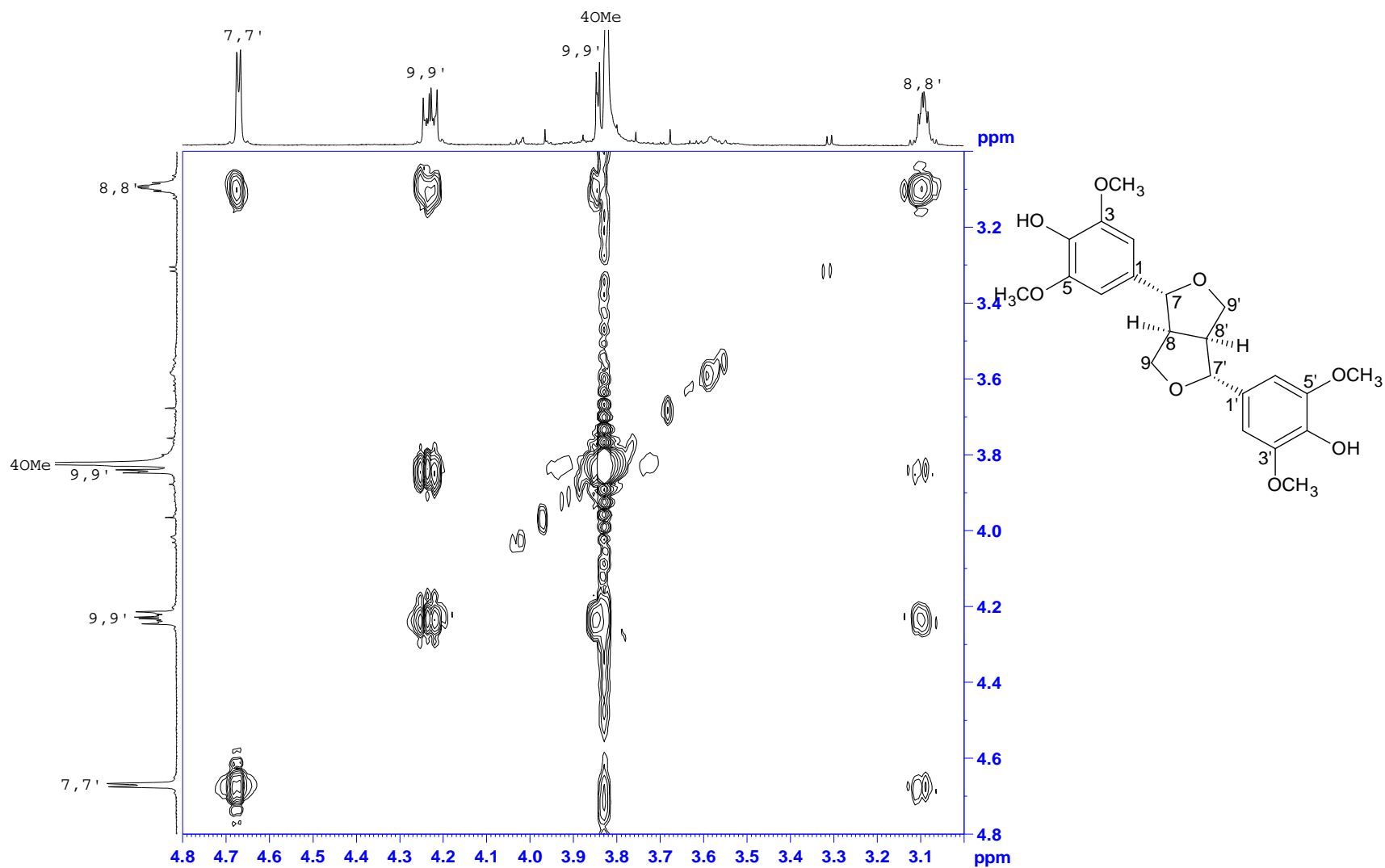
Plate 21c: ^{13}C NMR Spectrum of Syringaresinol (89) in (CD$_3$)$_2$CO
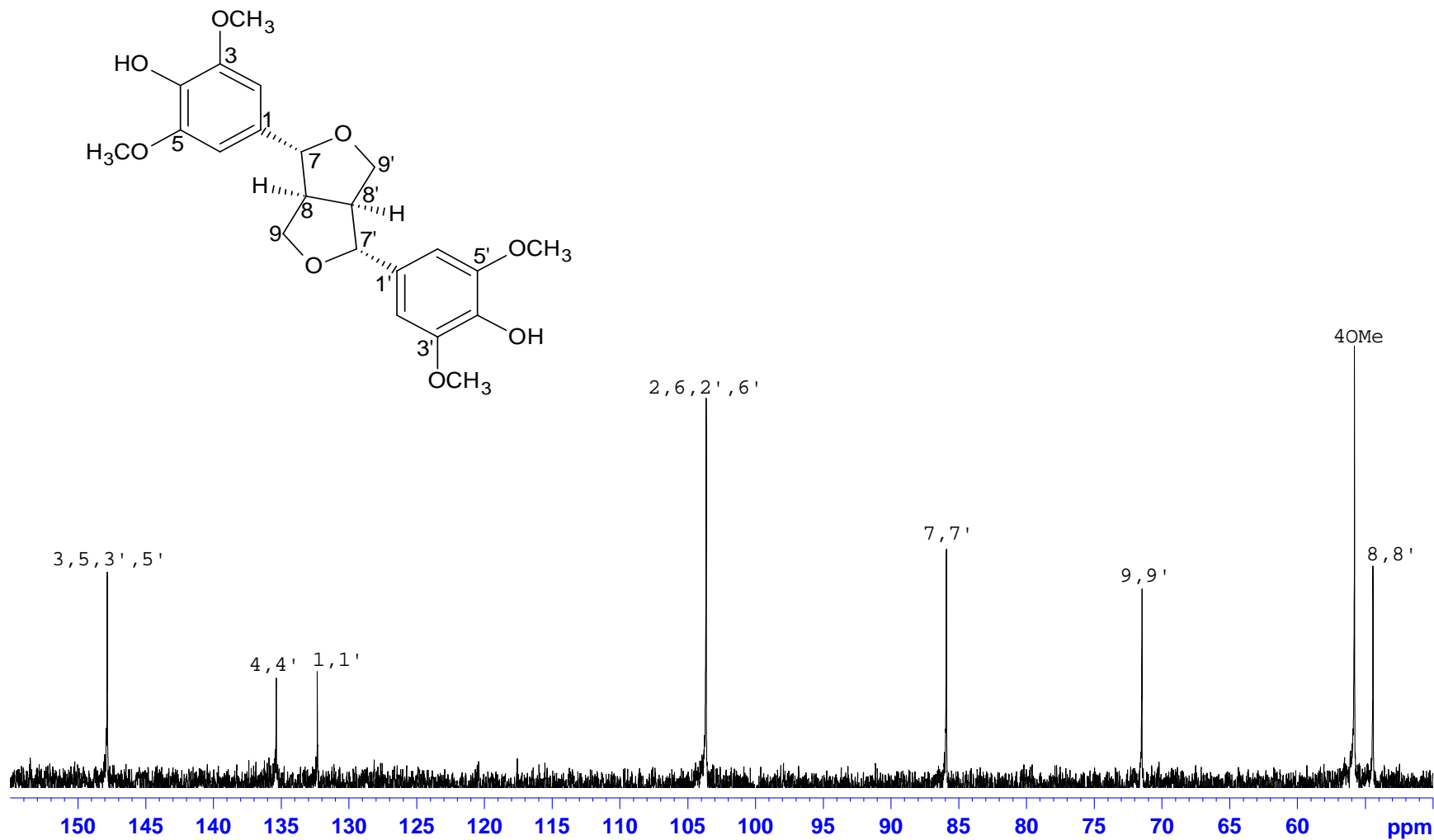
Plate 21d: HSQC NMR Spectrum of Syringaresinol (89) in $(CD_3)_2CO$
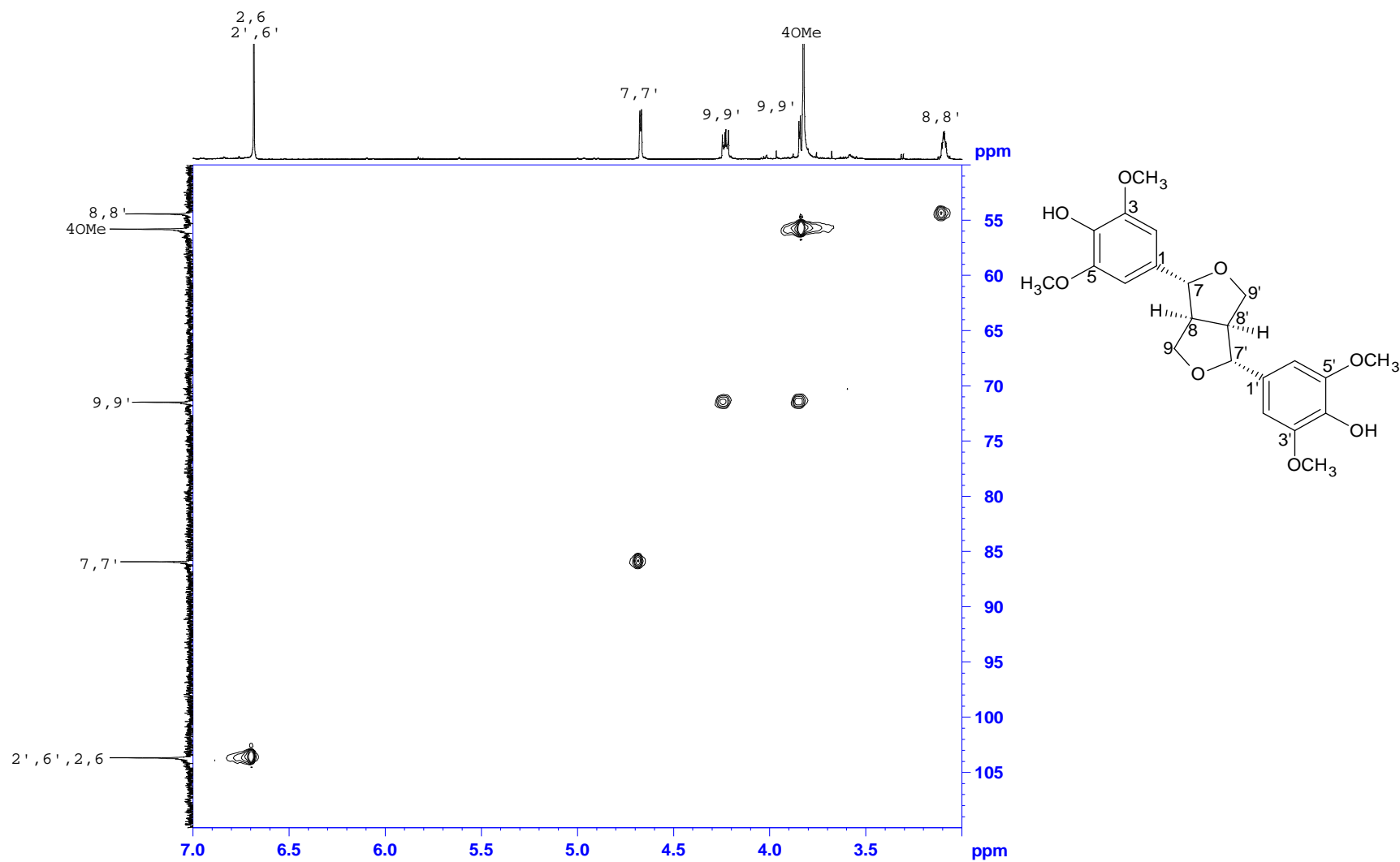
Plate 21e: HMBC NMR Spectrum of Syringaresinol (89) in $(CD_3)_2CO$

289
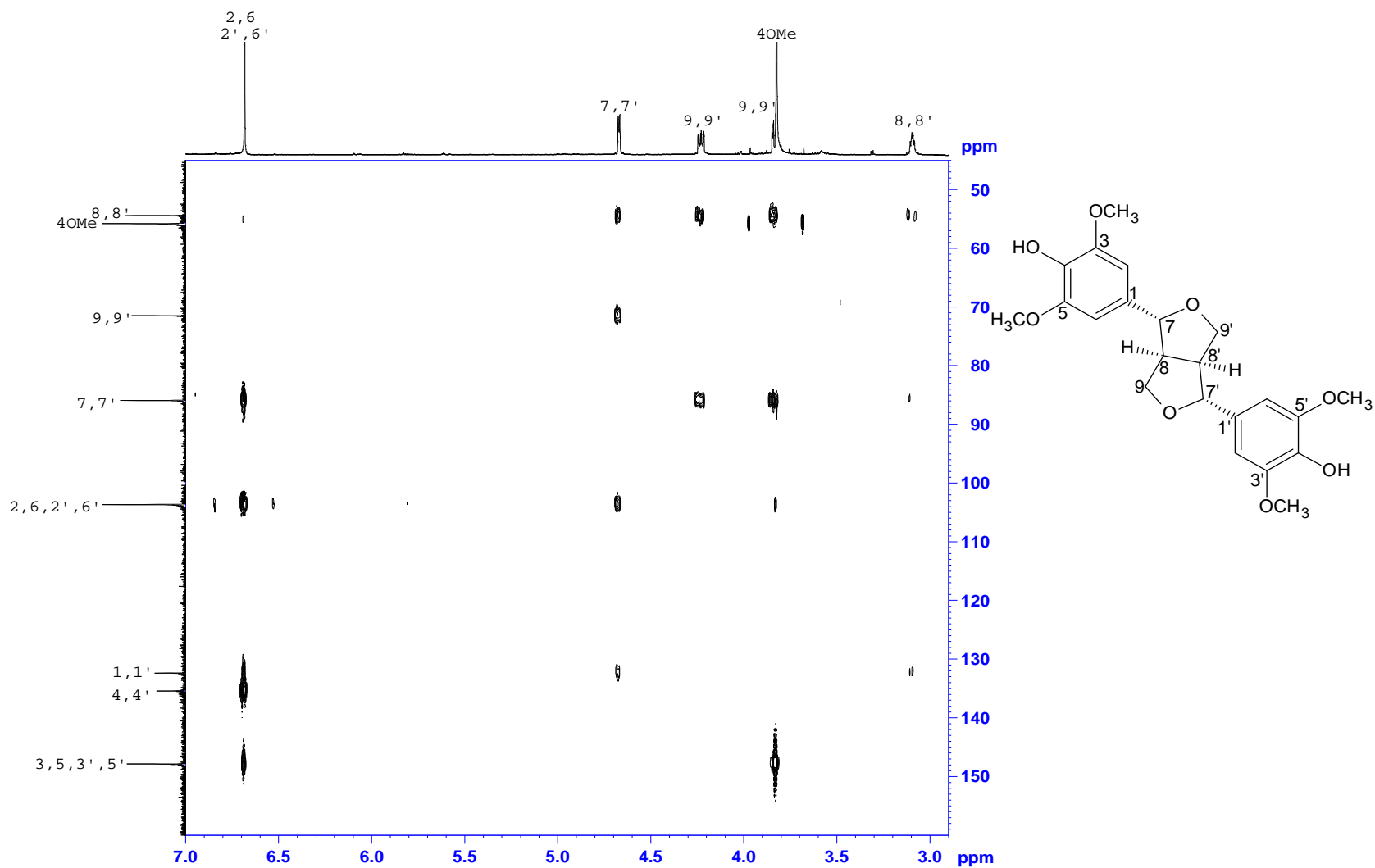
Plate 22a: ^{1}H NMR Spectrum of 16α,17-dihydroxy-*ent*-kauran-19-oic acid (90) in $(CD_3)_2SO$
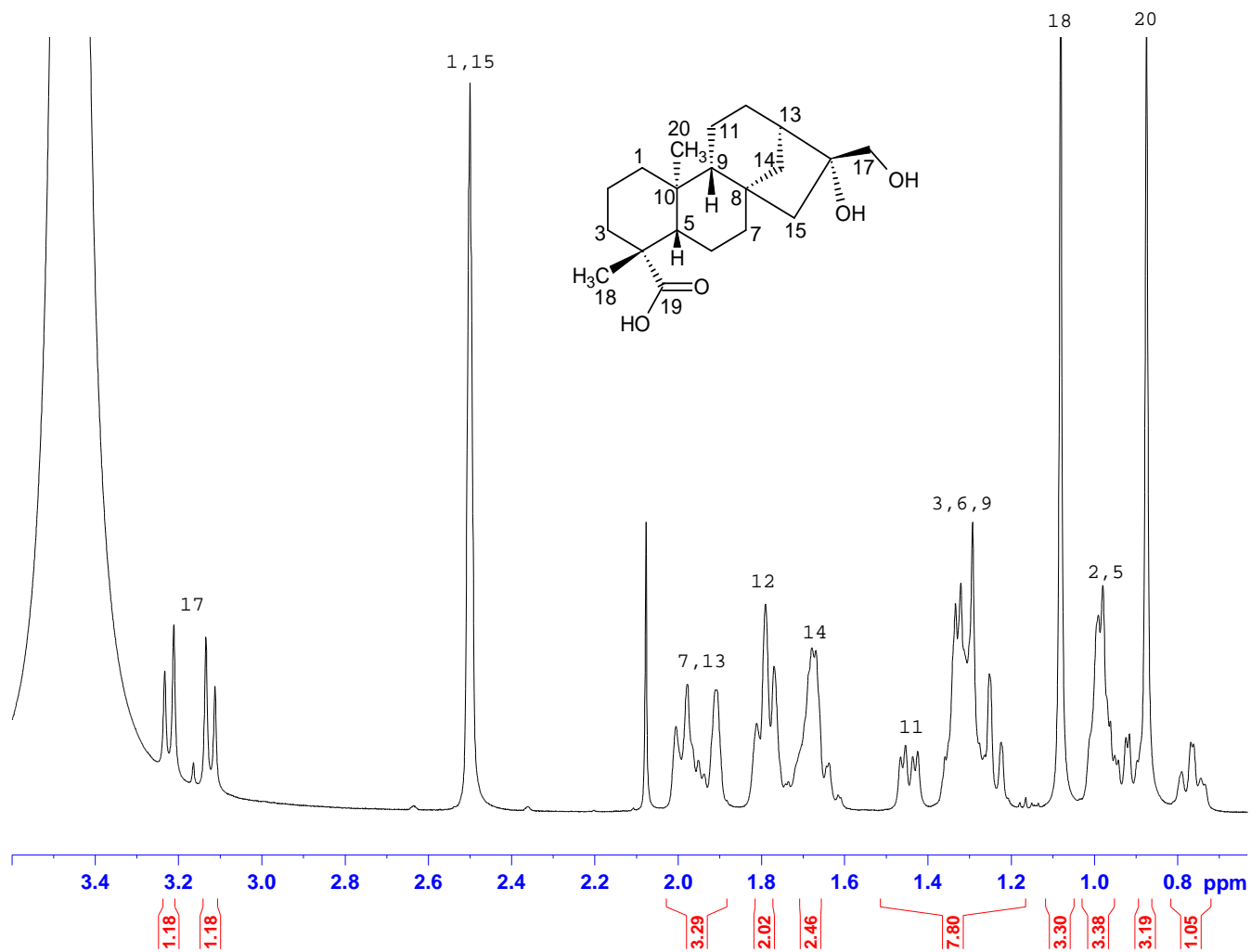
Plate 22b: COSY NMR Spectrum of 16α,17-dihydroxy-*ent*-kauran-19-oic acid (90) in (CD$_3$)$_2$SO
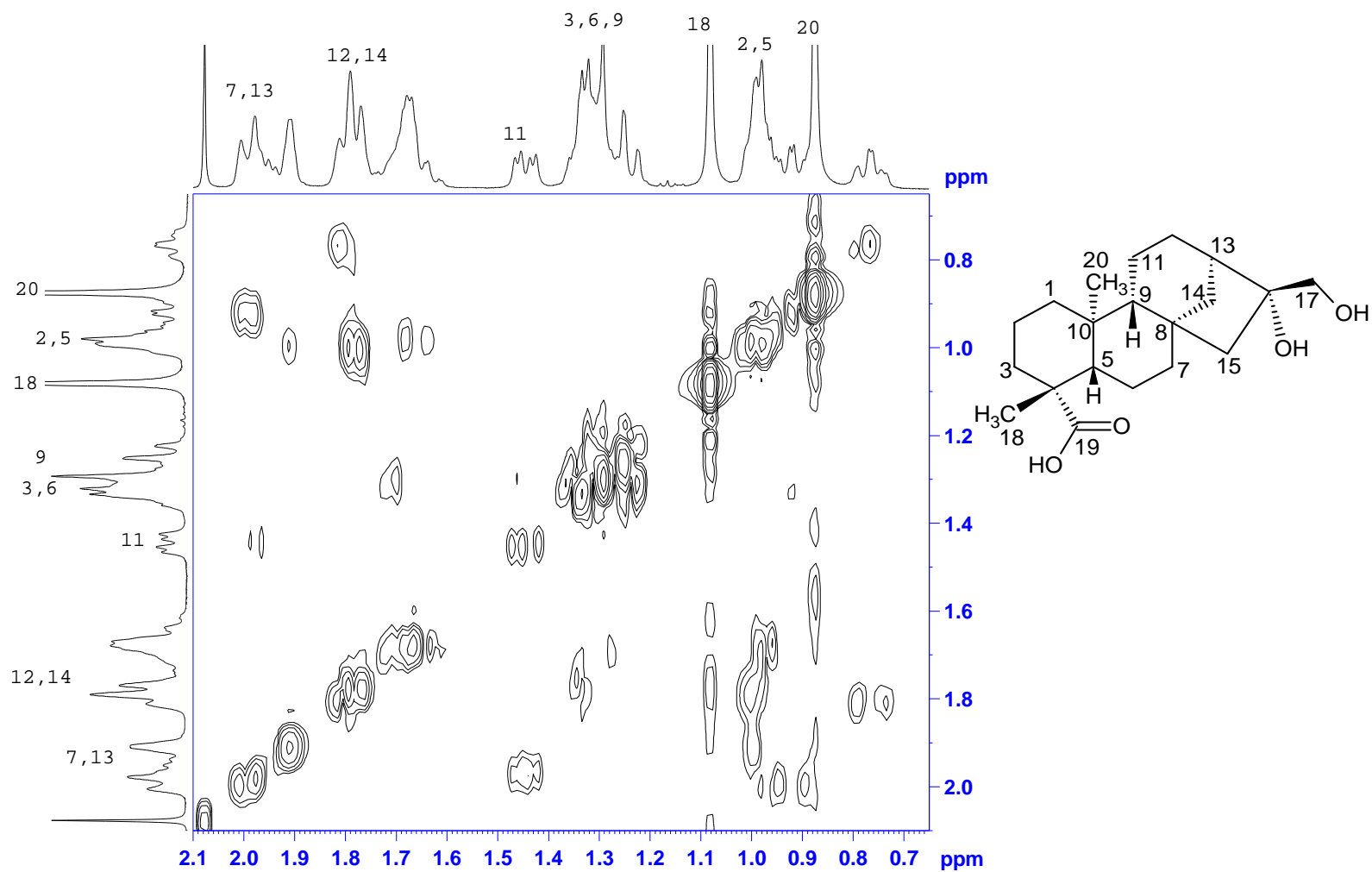
Plate 22c: ^{13}C NMR Spectrum of 16α,17-dihydroxy-*ent*-kauran-19-oic acid (90) in (CD$_3$)$_2$SO
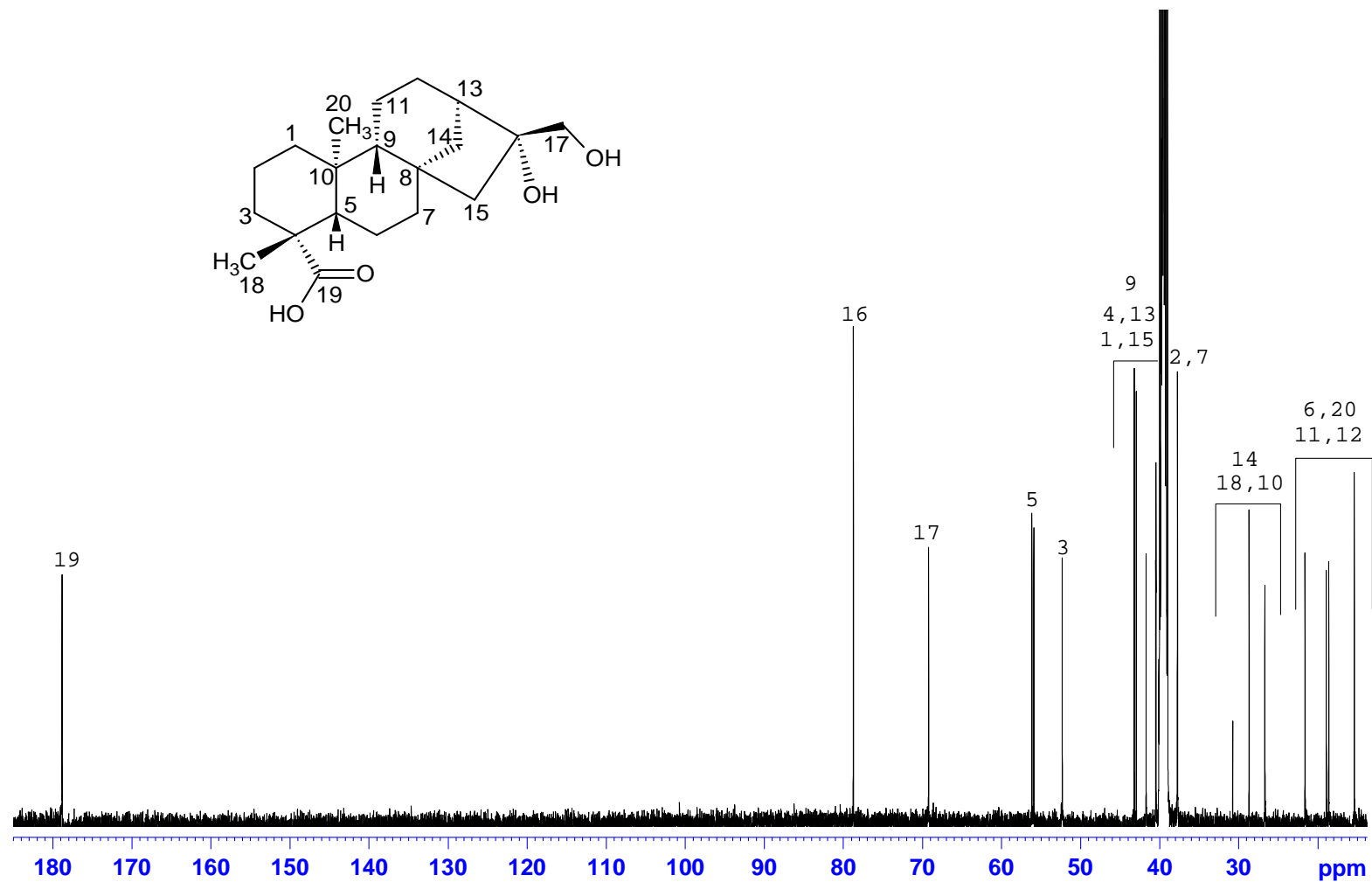
Plate 22d: HSQC NMR Spectrum of 16α,17-dihydroxy-*ent*-kauran-19-oic acid (90) in (CD₃)₂SO
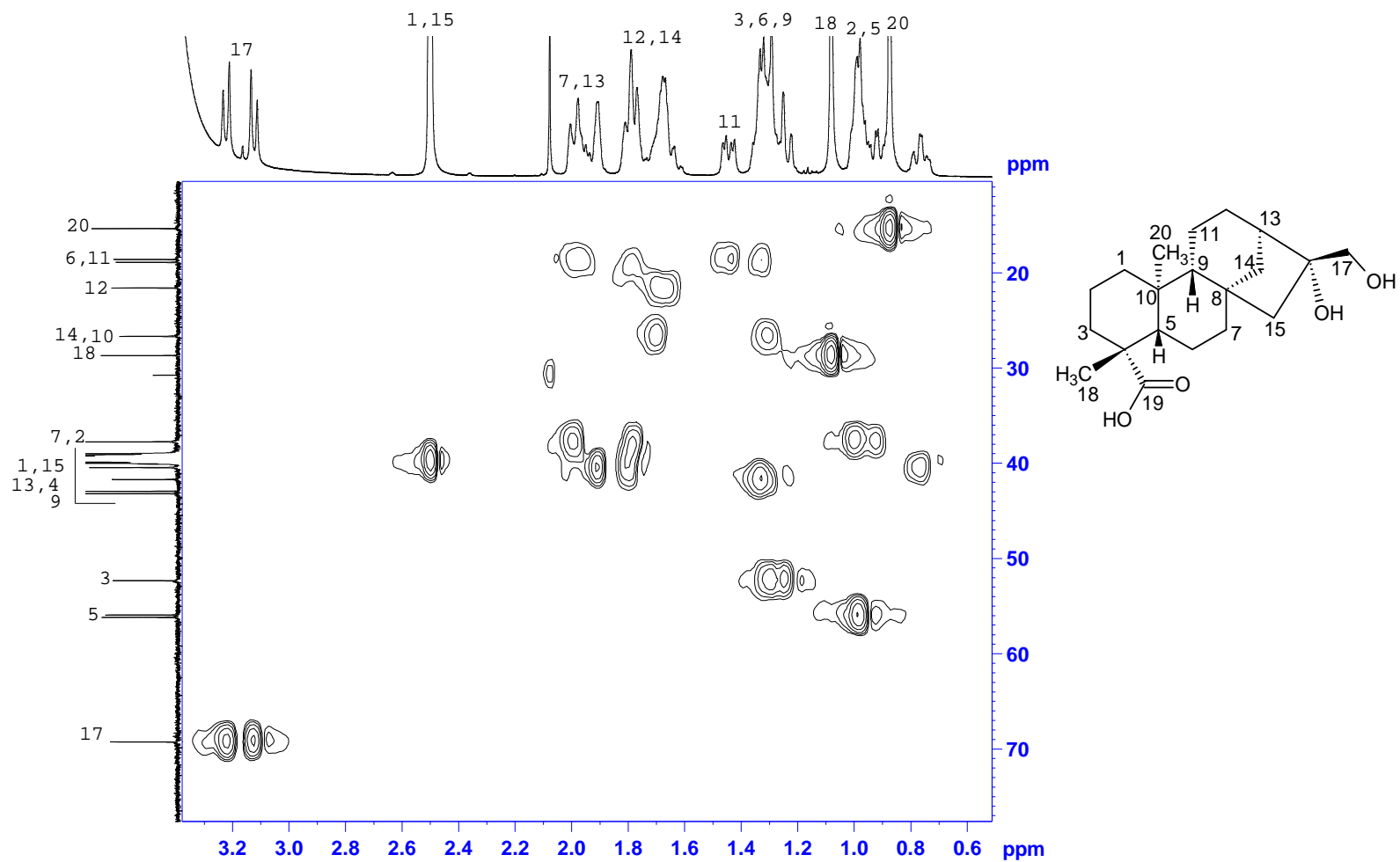
Plate 22e: HMBC NMR Spectrum of 16α,17-dihydroxy-*ent*-kauran-19-oic acid (90) in (CD₃)₂SO
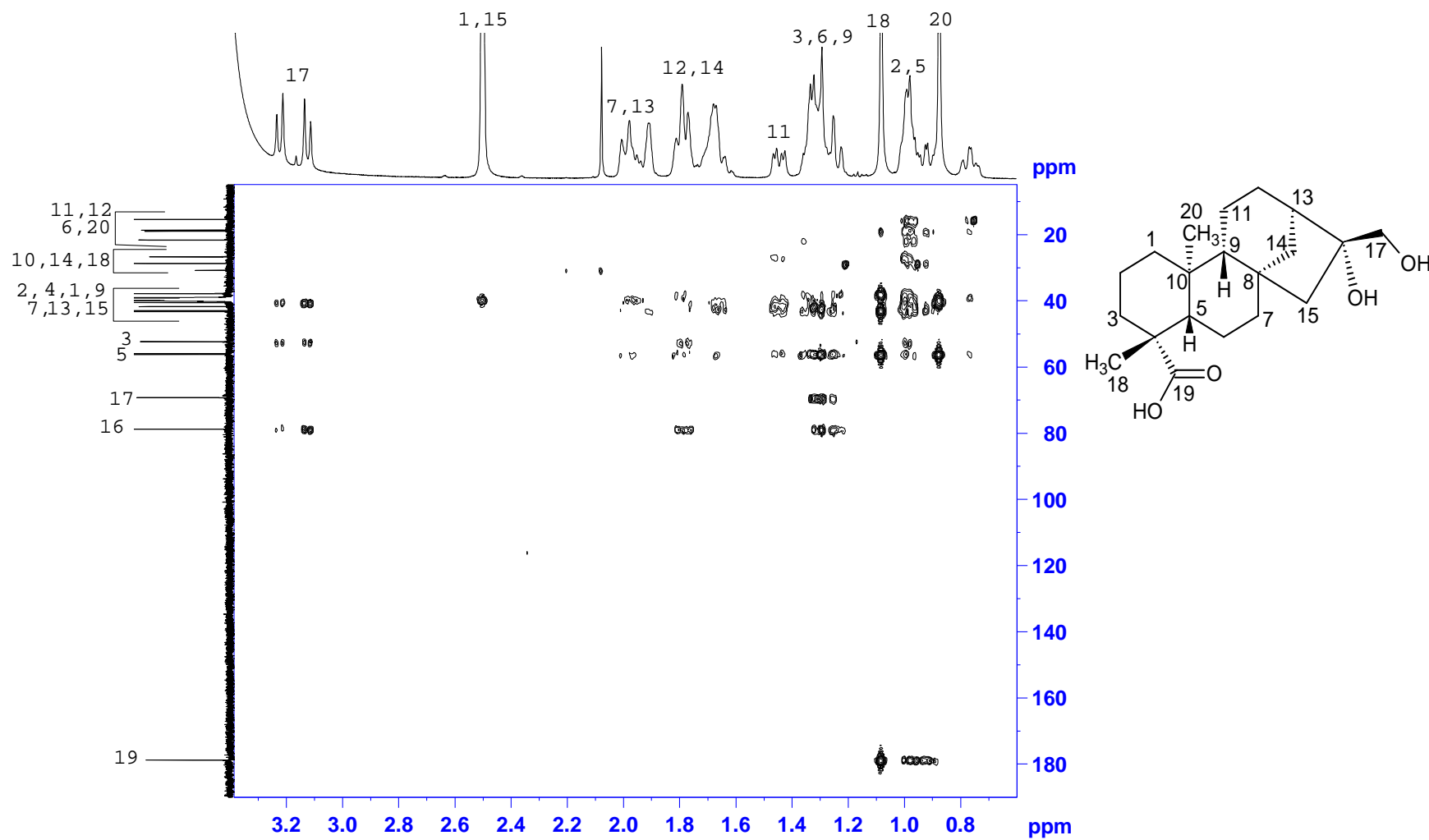
Plate 22f: DEPT-135 NMR Spectrum of 16α,17-dihydroxy-*ent*-kauran-19-oic acid (90) in (CD₃)₂SO
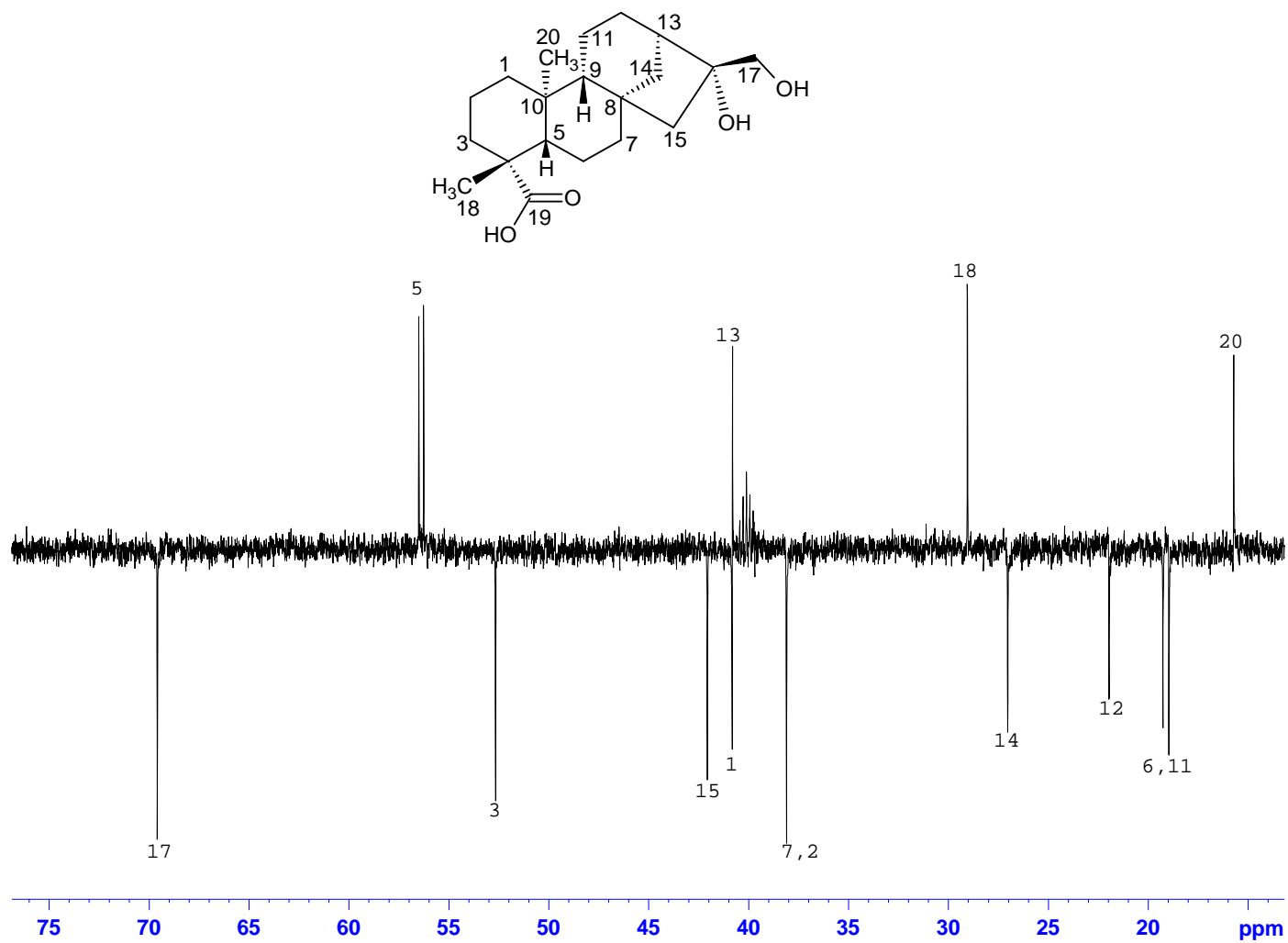
Plate 23a: ^{1}H NMR Spectrum of diaryether 132 in CDCl$_3$
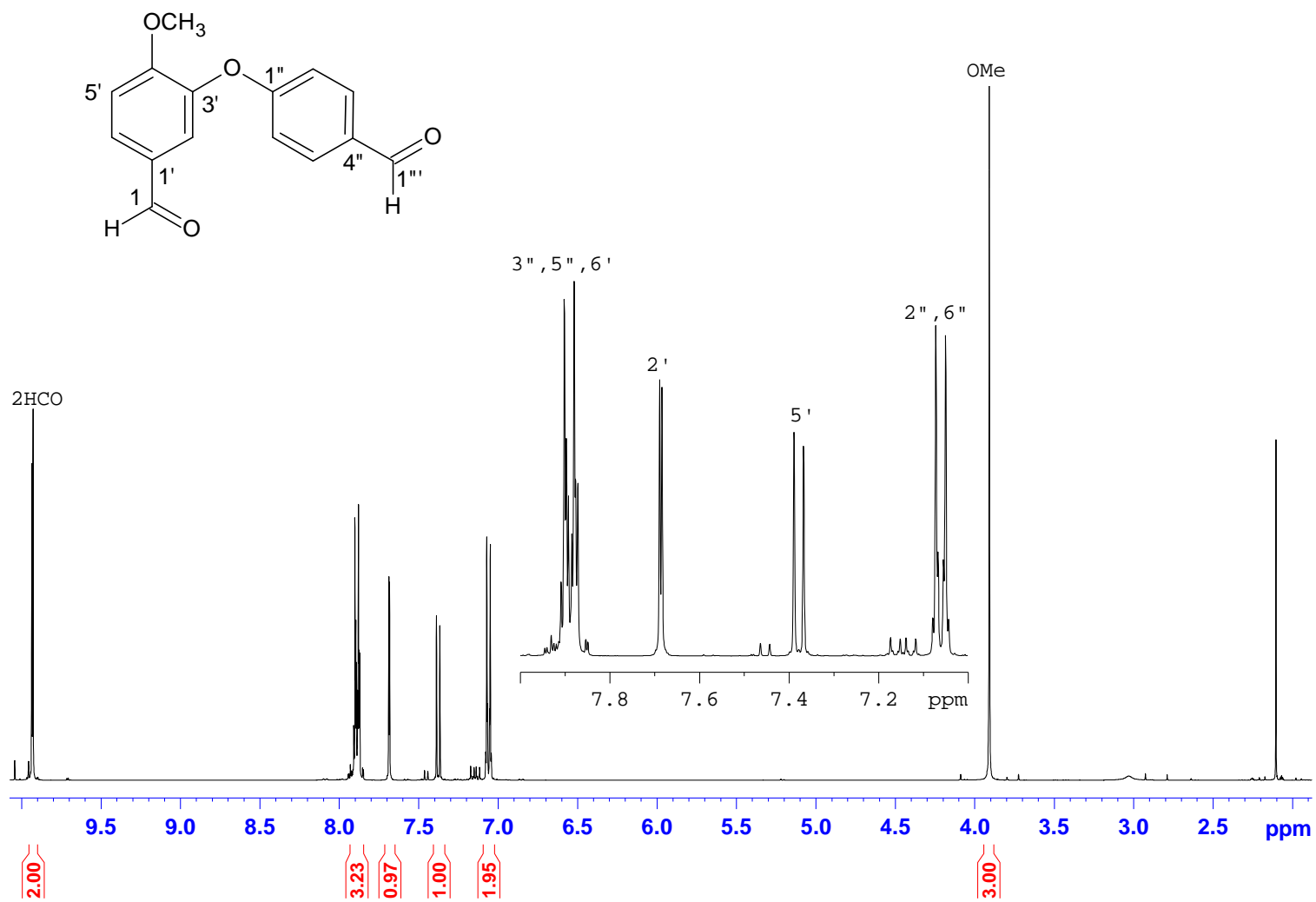
Plate 23b: ^{13}C NMR Spectrum of diaryether 132 in CDCl$_3$
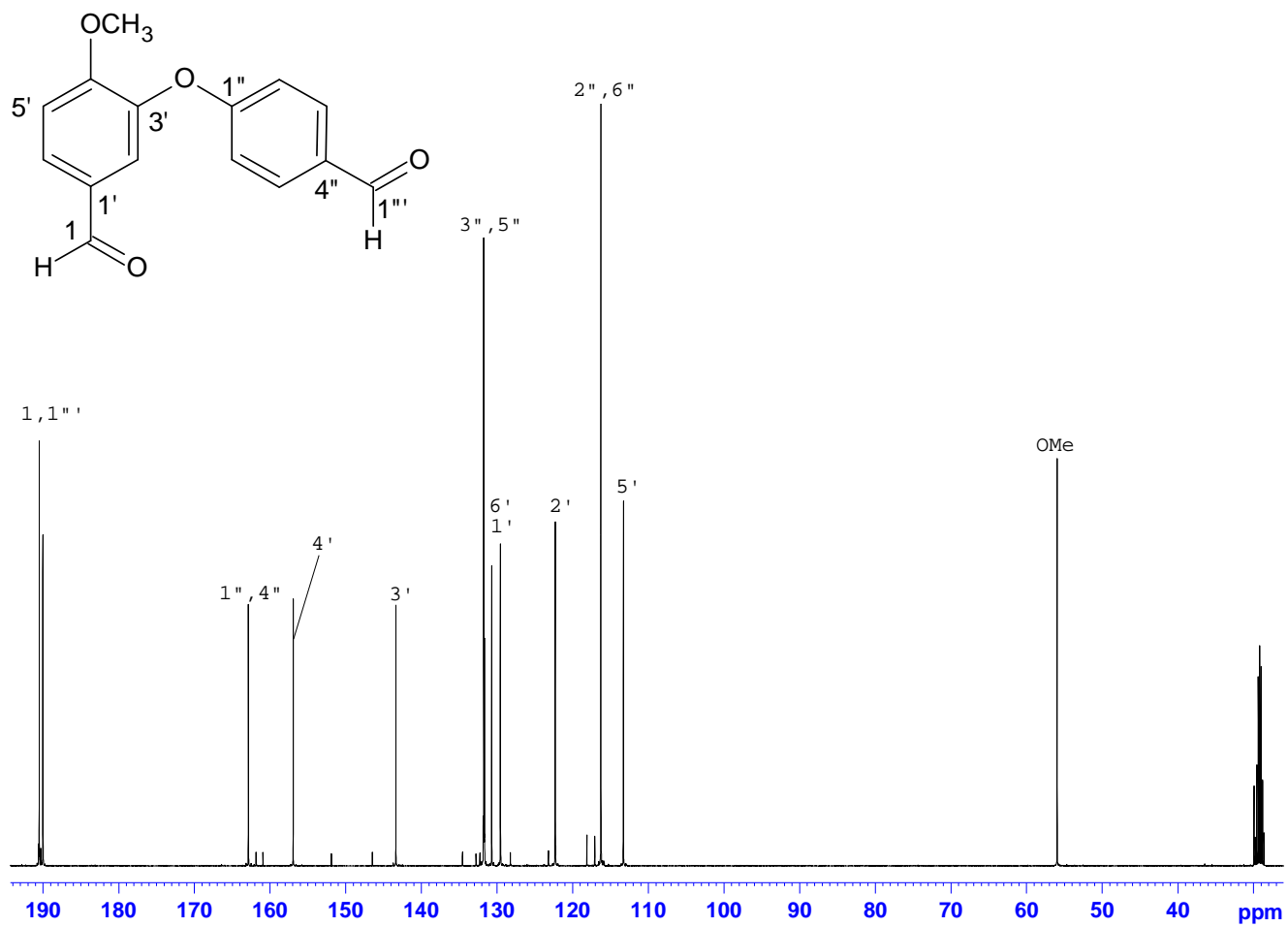
Plate 24a: ^{1}H NMR Spectrum of trimethoxybenzene (138) in CDCl$_3$

3OMe

2,4,6

7.5   7.0   6.5   6.0   5.5   5.0   4.5   4.0   3.5   3.0   2.5   2.0   ppm

3.00

9.28

298
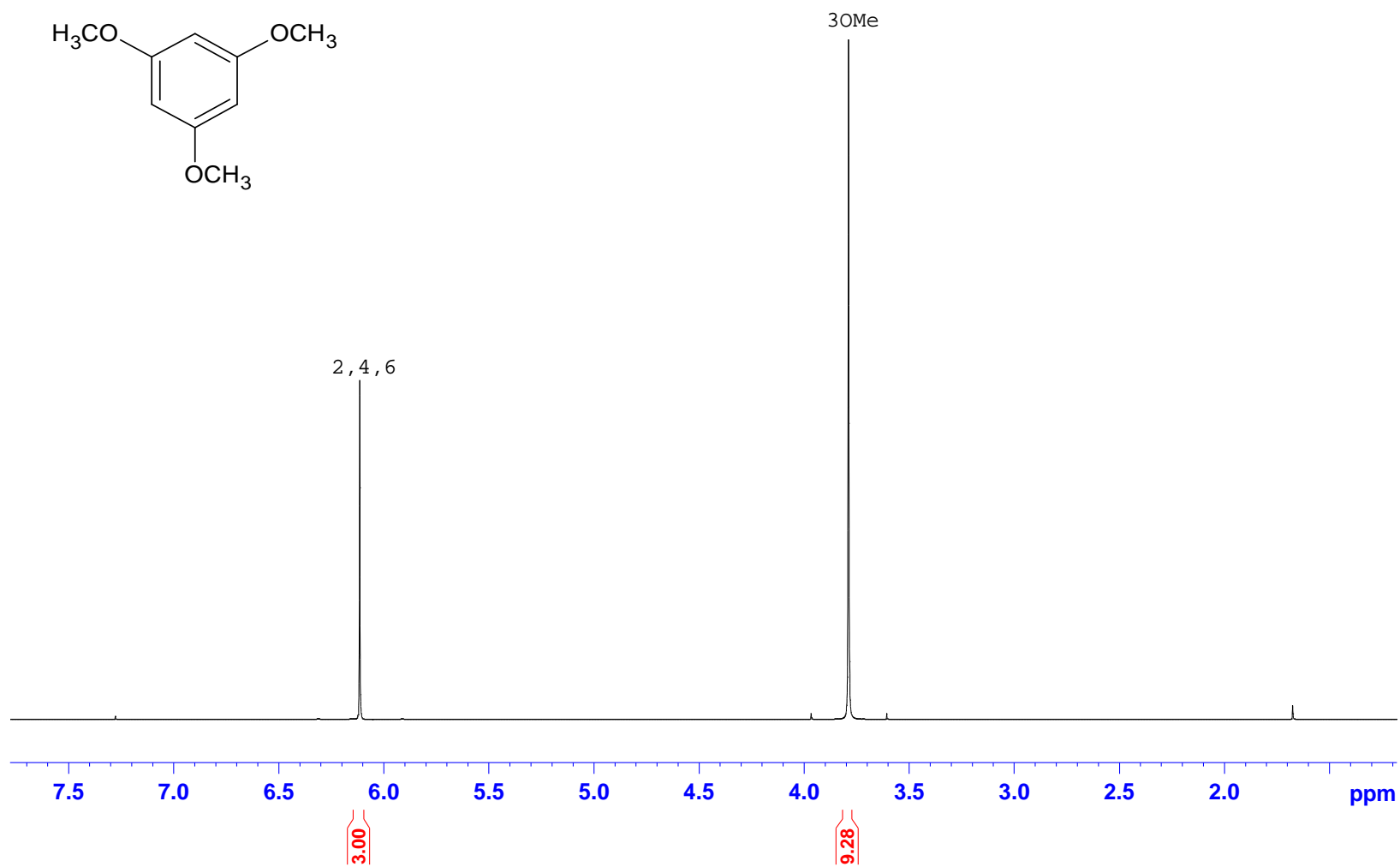
Plate 25a: ^{1}H NMR Spectrum of 2',4',6'-trimethoxyacetophenone (139) in CDCl$_3$

Plate 26a: ^{1}H NMR Spectrum of 2'-hydroxy-4,6-dimethoxyacetophenone (133) in CDCl$_3$
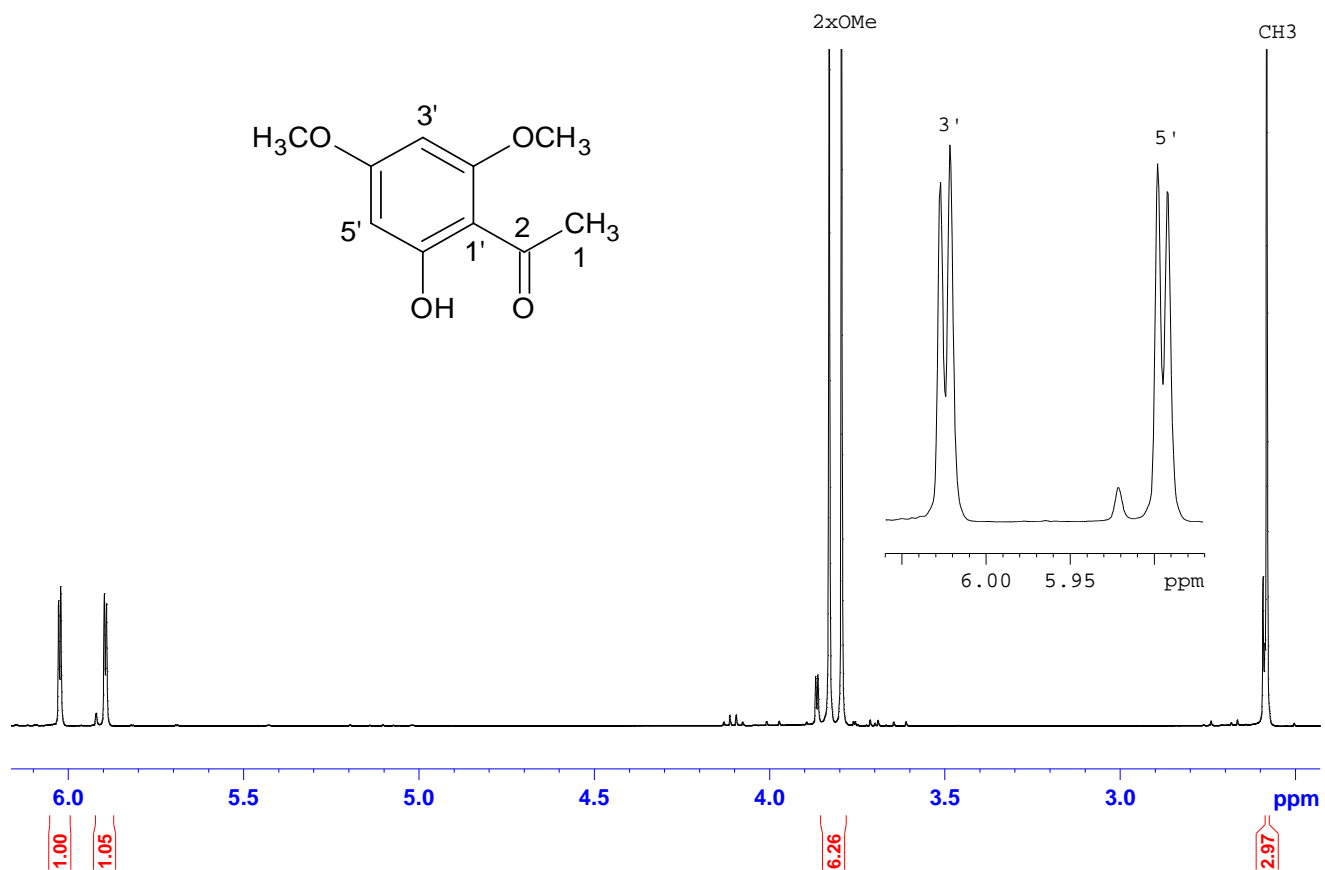
Plate 26b: ^{13}C NMR Spectrum of 2'-hydroxy-4,6-dimethoxyacetophenone (133) in CDCl$_3$
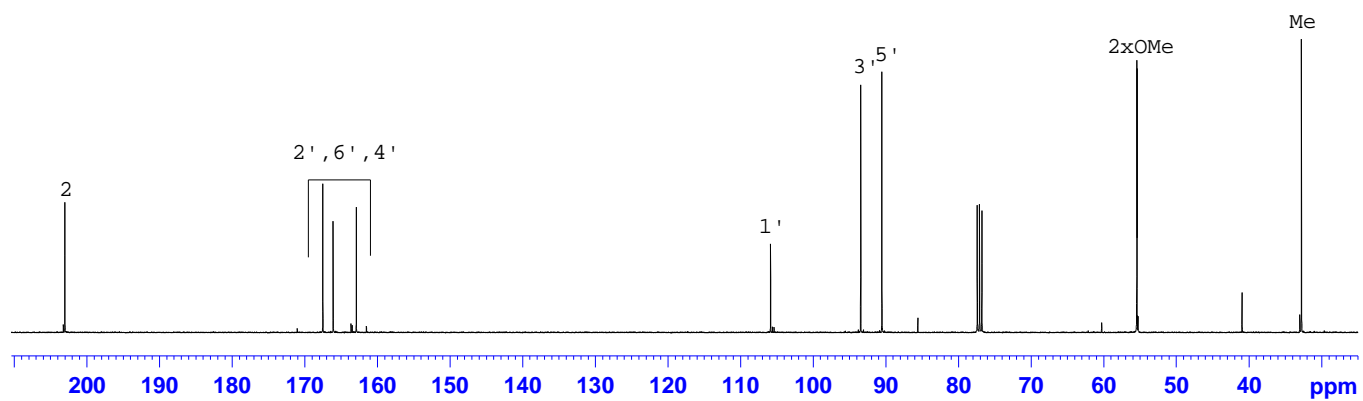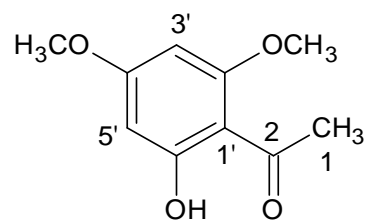
Plate 27a: ^1H NMR Spectrum of Dimeric chalcone 134 in CDCl$_3$

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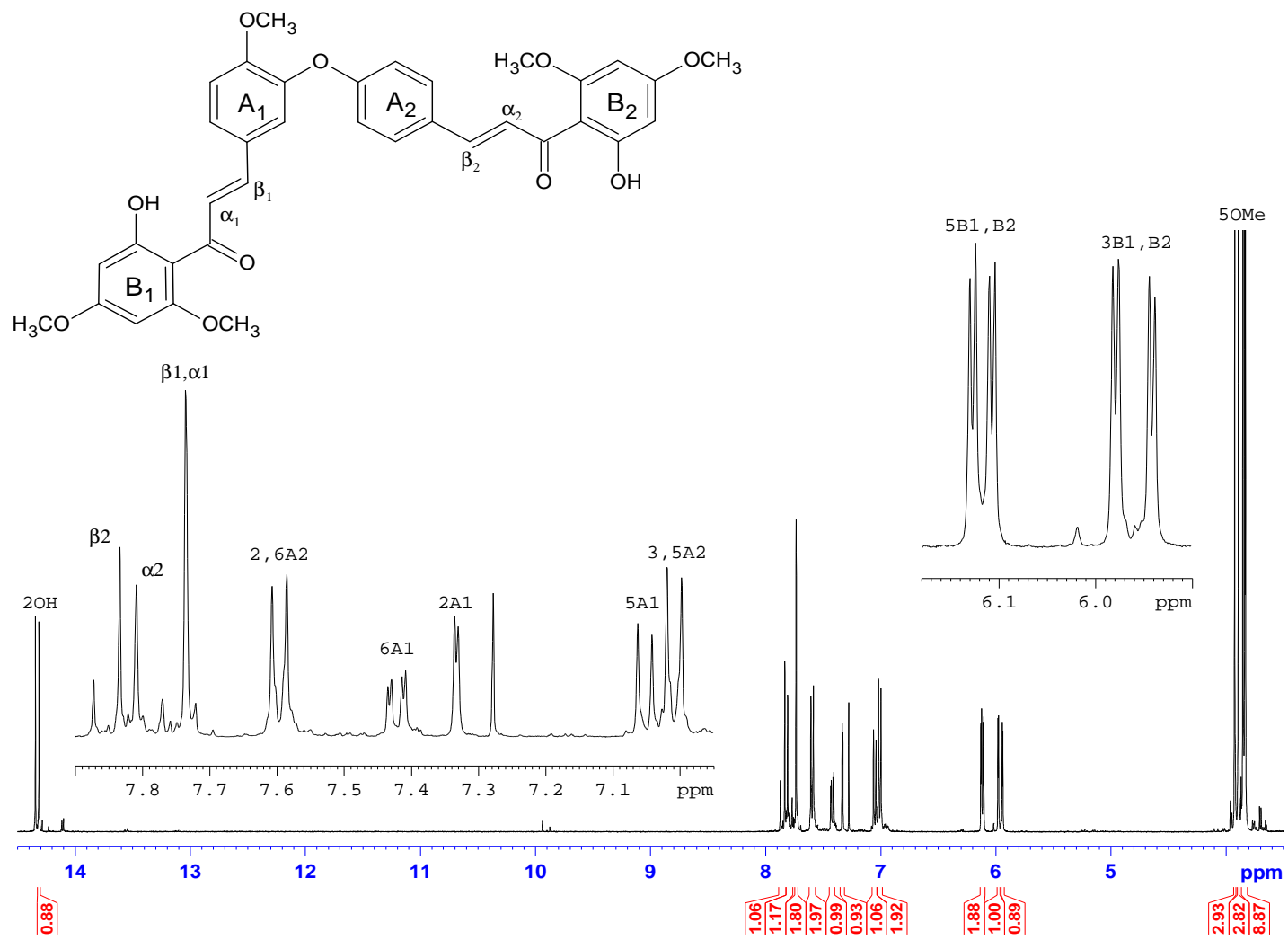
Plate 27b: ^{13}C NMR Spectrum of Dimeric chalcone 134 in CDCl$_3$
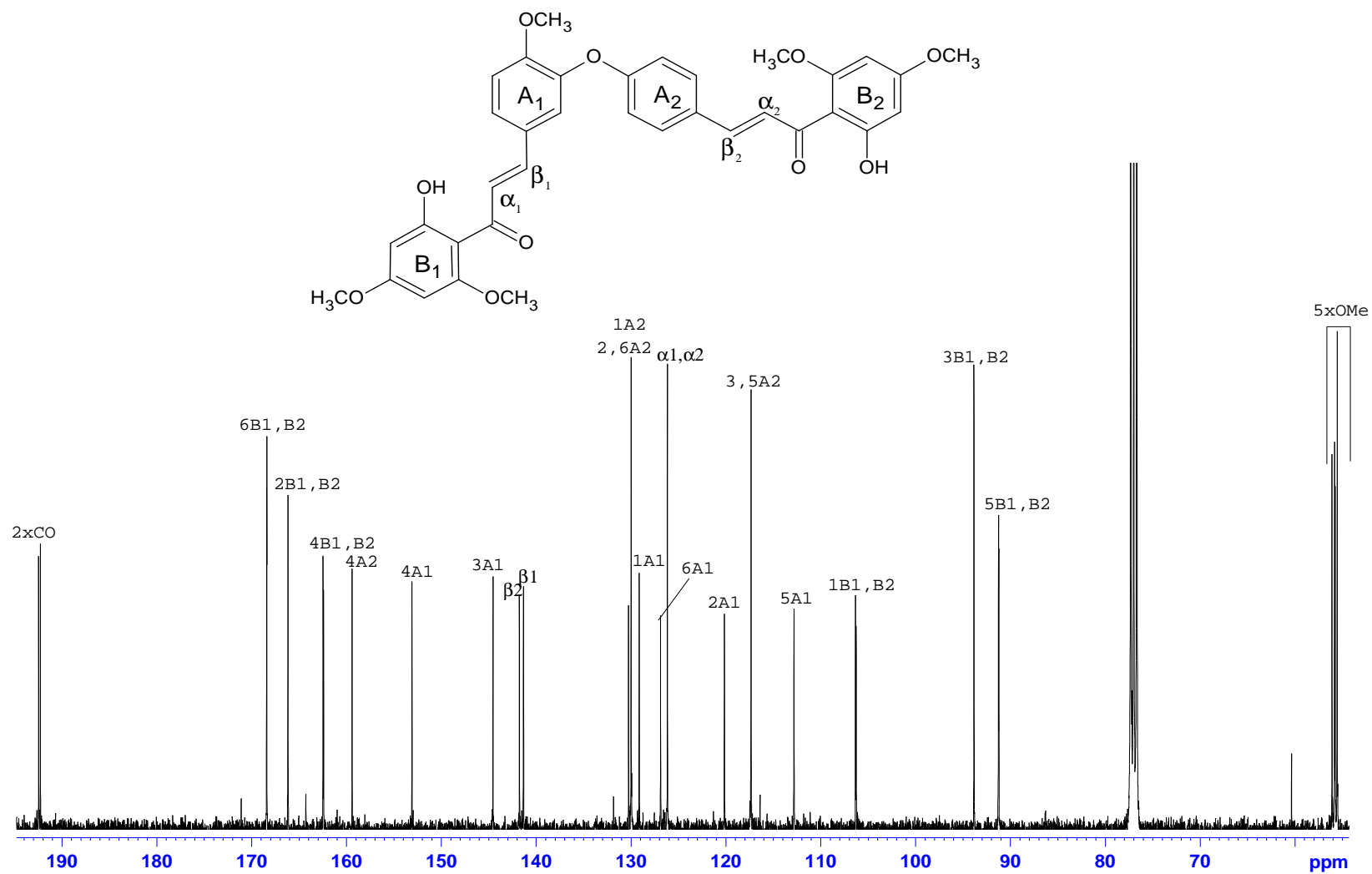
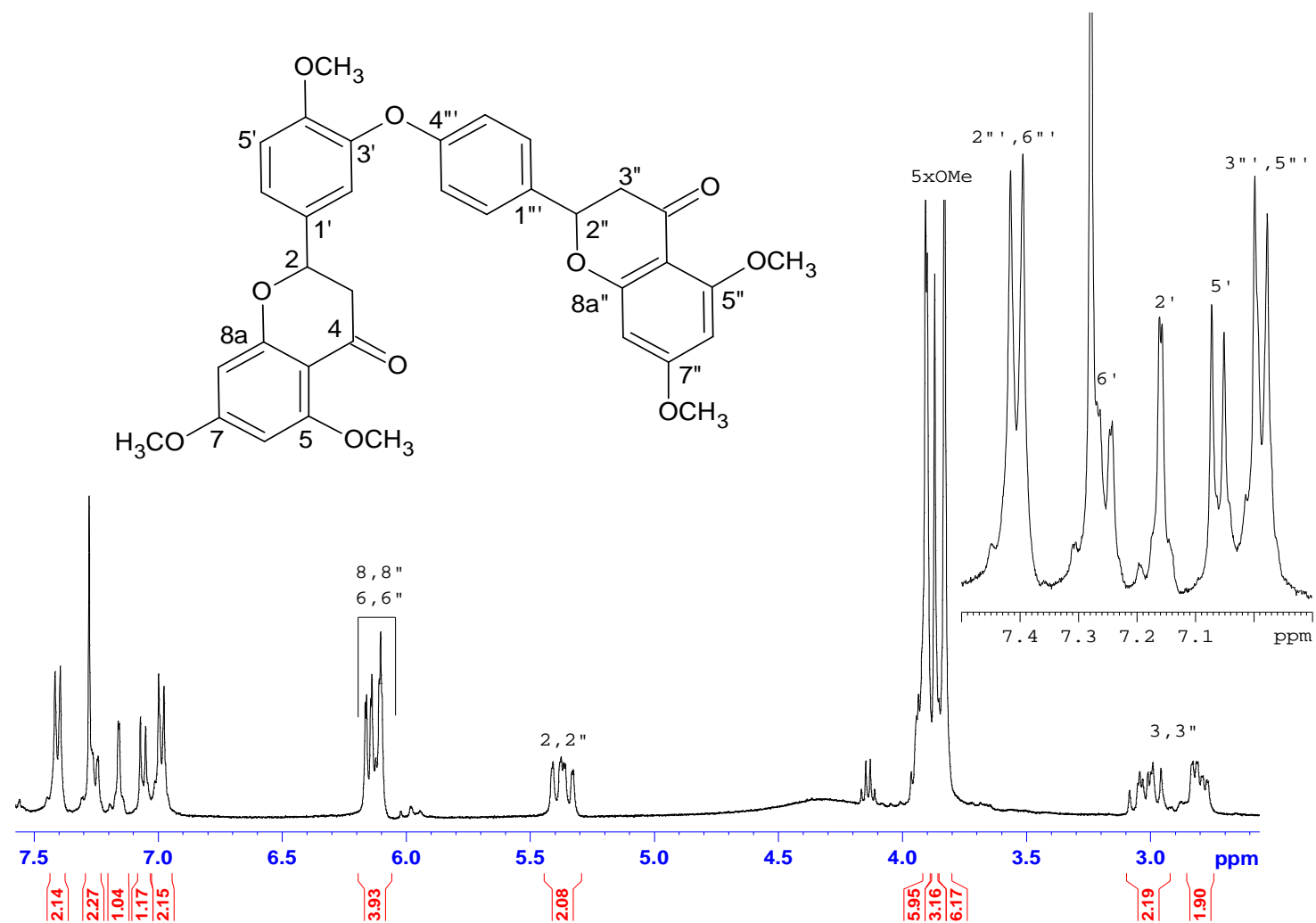# Plate 28a: ^{1}H NMR Spectrum of 5,7,4',5",7"-pentamethoxyochnaflavone (141) in CDCl$_3$

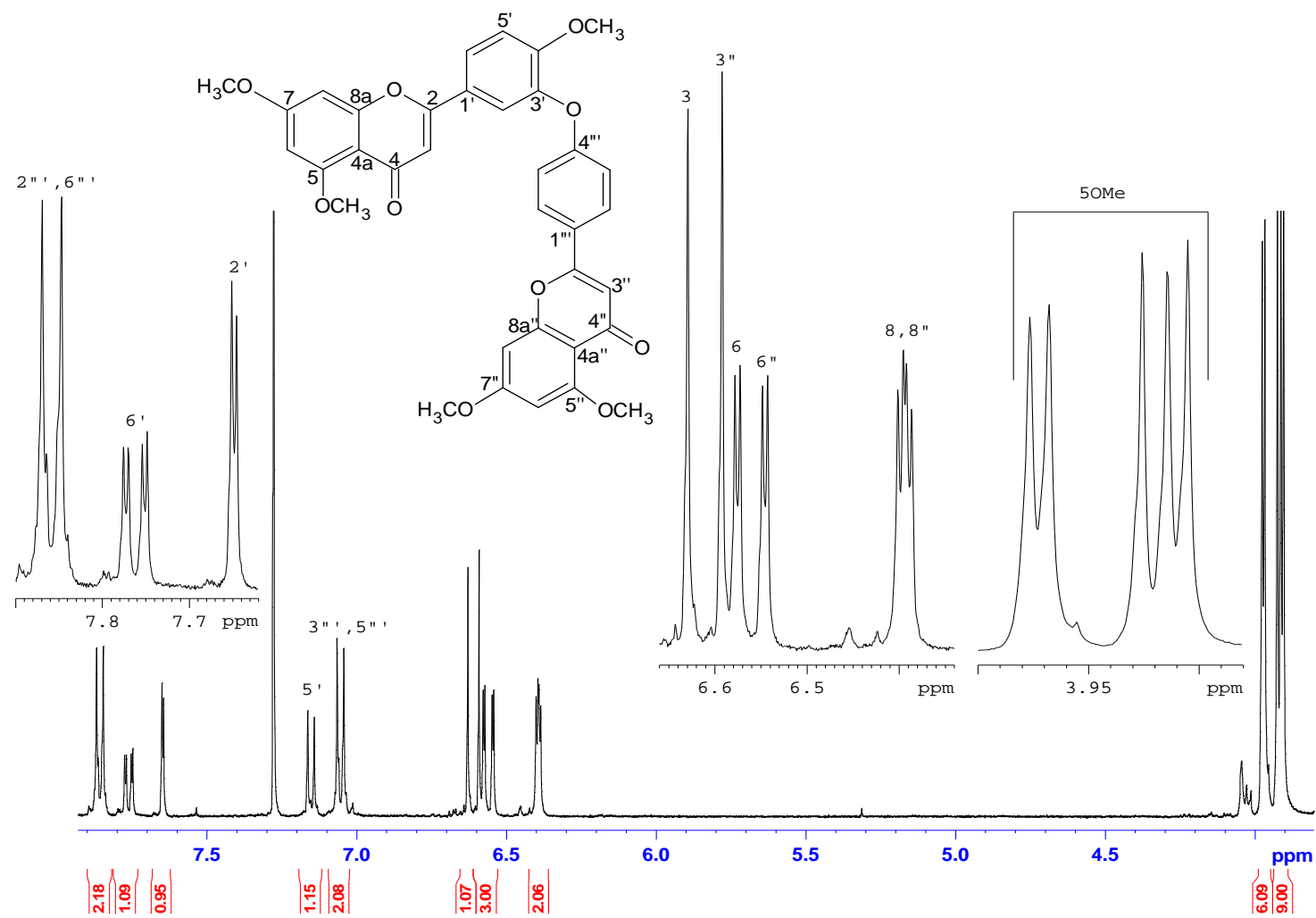# Plate 29a: ^{13}C NMR Spectrum of 5,7,4',5",7"-pentamethoxyochnaflavone (129) in CDCl$_3$

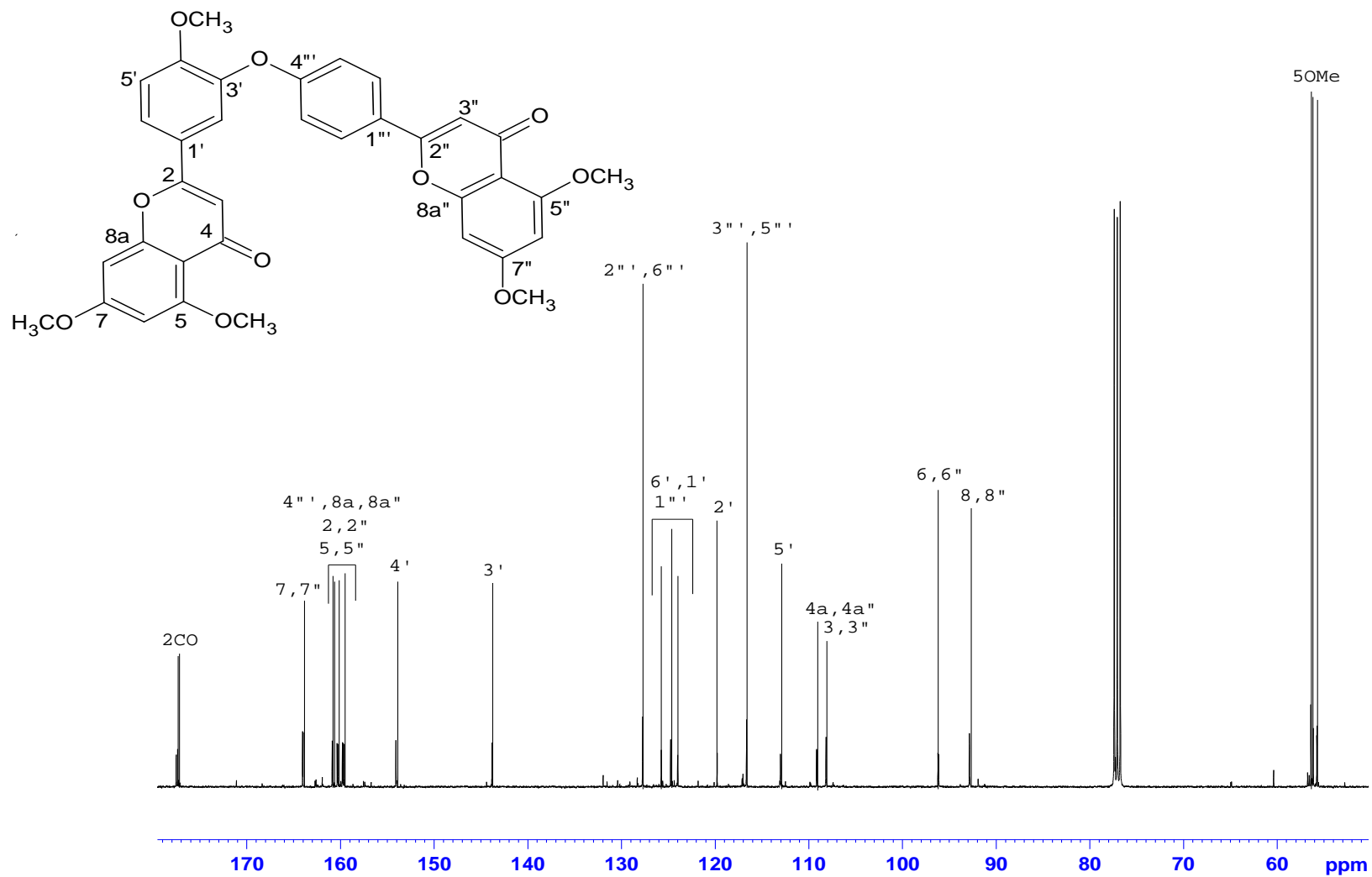# Plate 29b: ^{13}C NMR Spectrum of 5,7,4',5",7"-pentamethoxyochnaflavone (129) in CDCl$_3$

306

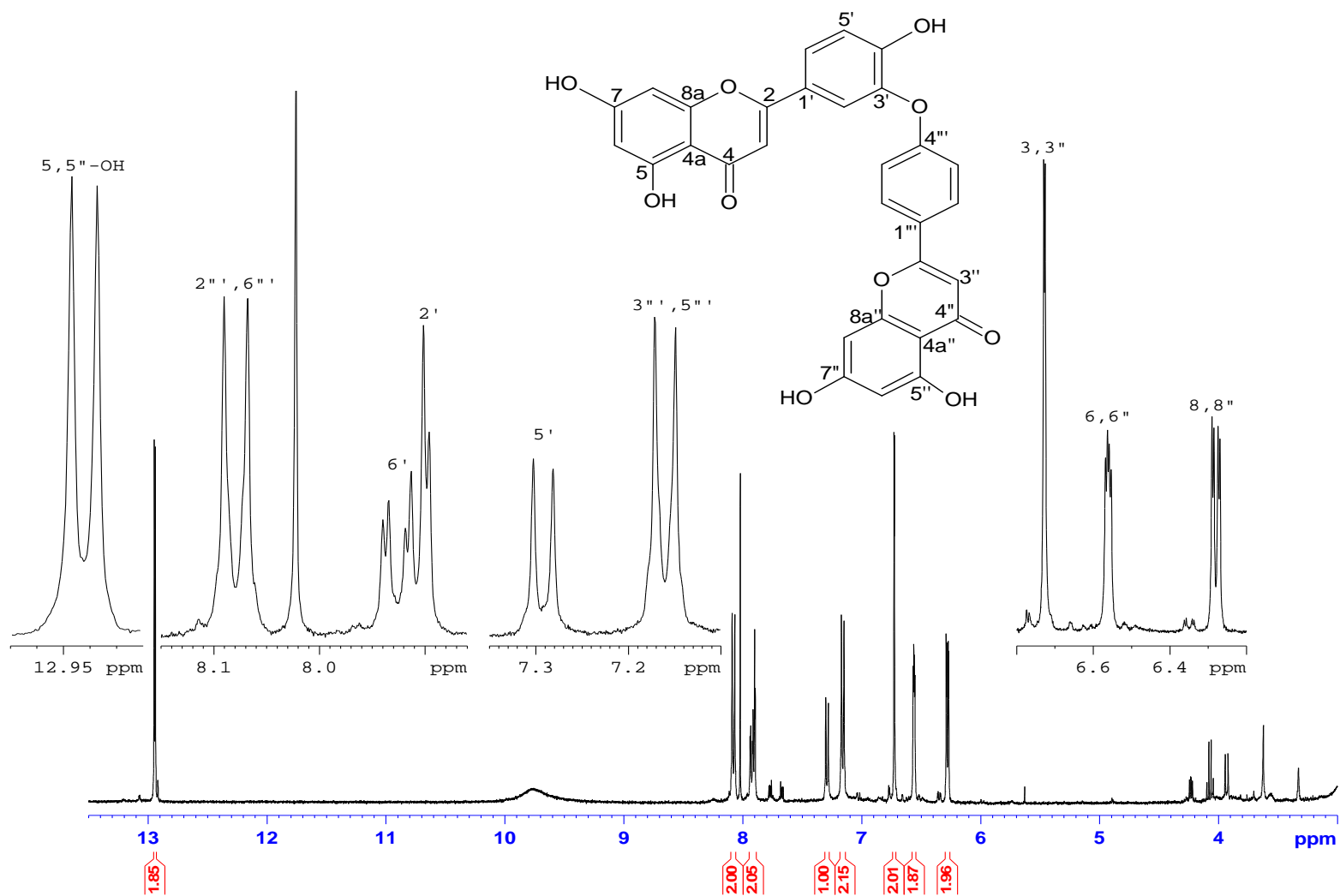# Plate 30a: ^{1}H NMR Spectrum of ochnaflavone (51) in (CD$_3$)$_2$CO

Plate 30b: ^{13}C NMR Spectrum of ochnaflavone (51) in $(CD_3)_2CO$