THE CHEMICAL INVESTIGATION OF LEDEBOURIA OVATIFOLIA, CLIVIA CAULESCENS AND HAEMANTHUS PAUCULIFOLIUS

By Tracy-Lee Pohl

Submitted in partial fulfilment of the requirements for the degree of

Master of Science

in the

Department of Chemistry,

University of Natal, Durban

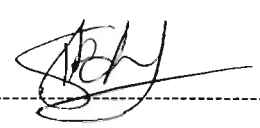
1999

PREFACE

The experimental work described in this thesis was carried out in the Department of Chemistry, University of Natal, Durban, from February 1998 to February 1999 under the supervision of Prof. D.A. Mulholland and Dr. N. Crouch.

This study represents original work by the author and has not been submitted in any other form to another university. Where use was made of the work of others, it has duly been acknowledged in the text.

Signed:



T.S. Pohl B.Sc.Hons (Natal)

I hereby certify that the above statement is correct.

Signed:

Professor D.A. Mulholland Ph.D. (Natal)

Signed:

Doctor N. Crouch Ph.D

ACKNOWLEDGEMENTS

I would like to thank my supervisor Prof. D.A. Mulholland for her support and guidance throughout the year. Her willingness to help with even the most basic problems and her constructive advice have been invaluable. I am also truly grateful for her financial assistance, without which this work would not have been possible.

I would also like to thank Dr. N. Crouch for his assistance throughout this work. His botanical input and attention to detail has been most appreciated. I would also like to thank him for collecting all my plant specimens and for obtaining the photographs of *C. caulescens* and *H. pauculifolius*.

Thanks must also be extended to all the technical staff, in particular, Mr. Bret Parel for all his help and guidance in the laboratory. His willingness to help at all times has been most appreciated. I would also like to extend my thanks to Mr. Dilip Jagjivan for running all my NMR spectra, especially the NOESY spectra. Thanks also to Mrs. Zarina Sayed-Ally for all her help with the running of my UV spectra.

I gratefully acknowledge Dr. P. Boschoff at the Cape Technicon for the running of the mass spectra and Dr, J.J. Nair and Prof. F. Viladomat form the Departament de Productes Naturals, Facultat de Parmacia, at the University of Barcelona for the running of the circular dichroism spectra.

Thank-you to Dr. J.P. Gerber and Dr. Hamdani Mahamed for all their advice in the laboratory and to all my colleagues and friends, Mr. Neil Koorbanally, Mr. Phil Coombs, and Mr. Vuyiswile Bangani, who have made this year so memorable.

Finally, I would like to thank my family and friends for all their love, support and encouragement throughout my university studies. I would especially like to thank my mother, Heather, for all the sacrifices she has made and for being the driving force behind all my achievements.

LIST OF ABBREVIATIONS

Acetyl CoA- acetyl coenzyme A

ADP- adenosine diphosphate

ATP- adenosine triphosphate

ax- axial

^{13}C NMR- carbon-13 nuclear magnetic resonance

CD- circular dichroism

CNS- central nervous system

COSY- correlated nuclear magnetic resonance spectrum

eq- equatorial

^{1}H NMR- proton nuclear magnetic resonance

HETCOR- heteronuclear shift correlation nuclear magnetic resonance

Hz- Hertz

IR- infra-red

Malonyl CoA- Malonyl coenzyme A

NIH- National Institute of Health

NOE- nuclear Overhauser effect

NOESY-nuclear Overhouser effect

Pi- inorganic phosphate

ppm- parts per million

TLC- thin layer chromatography

UV- ultra violet

LIST OF FIGURES

1.1 Homoisoflavonoids of the 3-benzyl-4-chromanone type.
1.2 Homoisoflavonoids of the 3-benzyl-3-hydroxy-4-chromanone type.
1.3 Homoisoflavonoids of the 3-benzylidene-4-chromanone type.
1.4 Homoisoflavonoids of the scillascillin, brazilin and hematoxylin type.
1.5 The numbering system for homoisoflavonoids.
1.6 The numbering system for brazilin type homoisoflavonoids.
1.7 The numbering system for chalcones.
1.8 Type 1 chalcones.
1.9 Type 2 chalcones.
1.10 Type 3 chalcones.
1.11 Type 4 chalcones.
1.12 The origin of the A and B rings of chalcones.
1.13 The NIH shift for the hydroxylation of cinnamic acid.

2.1 Compound I: 5, 7-dihydroxy-3-(4'-hydroxybenzyl)-4-chromanone.
2.2 Compound II: 2, 6'-dimethoxy-4, 4'-dihydroxychalcone.

3.1 An alkaloid of the lycorine type.
3.2 An alkaloid of the homolycorine type.
3.3 Alkaloids of the crinane type.
3.4 An alkaloid of the galanthamine type.
3.5 Alkaloids of the tazettine type.
3.6 An alkaloid of the montanine type.
3.7 An alkaloid of the narciclasine type.
3.8 An alkaloid of miscellaneous type.
3.9 Examples of alkaloid types to be discussed in section 3.2.3.
3.10 Examples of alkaloids derived via phenol oxidative coupling.

4.1 Alkaloids of the lycorine type.
4.2 Alkaloids of the homolycorine type.

4.3 Cliviamartine.
4.4 Clivonidine.
4.5 Alkaloids of the homolycorine type.
4.6 Miniatine and clivimine.
4.7 Alkaloid of the haemanthamine type.
4.8 Alkaloids of the miscellaneous type.
4.9 Compound III: hippeastrine.
4.10 Compound IV: haemanthamine.
4.11 The NOE correlations observed for compound IV.
4.12 Compound V: lycorine.
4.13 Compound VI: 11-(*S*)-hydroxyvittatine.
4.14 Compound VII: sternbergine.

5.1 Alkaloid of the lycorine type.
5.2 Alkaloids of the homolycorine type.
5.3 Alkaloids of the haemanthamine type.
5.4 Alkaloids of the montanine type.
5.5 Alkaloids of the galanthamine type.
5.6 Alkaloid of the tazettine type.
5.7 Alkaloids of the narciclasine type.
5.8 Compound VIII: haemanthustatin.
5.9 The initial structure proposed for compound VIII.
5.10 The NOE correlations observed for compound VIII.
5.11 Compound IX: montanine hydrochloride.
5.12 The NOE correlations observed for compound IX.
5.13 Two *N*-oxides isolated from the Amaryllidaceae family.
5.14 Compound: manthidine hydrochloride.
5.15 The NOE correlations observed for compound X.
5.16 Compound XI: sitosterol.

LIST OF TABLES

2.1 ^{1}H NMR data for compound I and literature data for 5, 7-dihydroxy-3-(4'-hydroxybenzyl)-4-chromanone.

2.2 ^{13}C NMR data for compound I and literature data for 5, 7-dihydroxy-3-(4'-hydroxybenzyl)-4-chromanone.

2.3 ^{1}H NMR data for compound I and literature data for 2',6'-dimethoxy-4,4'-dihydroxychalcone, 4,2',6'-trihydroxy-4'-methoxychalcone and 2',4',6'-trihydroxy-4-methoxychalcone.

2.4 ^{13}C NMR data for compound II.

3.1 Plant derived alkaloid drugs used in orthodox medicine.

4.1 The ^{1}H NMR data for compound III and the literature data for hippeastrine and 9-*O*-demethyl-5α-hydroxyhomolycorine.

4.2 ^{13}C NMR data for compound III and literature data for hippeastrine.

4.3 ^{1}H NMR data for compound IV and literature data for haemanthamine.

4.4 ^{13}C NMR data for compound IV and literature data for haemanthamine.

4.5 ^{1}H NMR data for compound V and literature data for lycorine.

4.6 ^{1}H NMR data for compound V and literature data for 9-*O*-methylpseudolycorine.

4.7 ^{13}C NMR data for compound V and literature data for lyorine.

4.8 ^{1}H NMR data for compounds IV and VI.

4.9 ^{1}H NMR data for compound VI and literature data for 11-(*S*)-hydroxyvittatine.

4.10 ^{13}C NMR data for compound VI and compound IV and literature data for hamayne.

4.11 ^{1}H NMR data for compounds VII and V and literature data for sternbergine.

4.12 ^{13}C NMR data for compound VII and V and literature data for sternbergine.

5.1 ^{1}H NMR data for compound VIII and literature data for narciclasine.

5.2 ^{13}C NMR data for compound VIII and literature data for narciclasine.

5.3 UV data for compound VIII and literature data for hippeastrine, narciclasine, pancratistatin and lycorine.

5.4 ^{1}H NMR data for compound IX and literature data for montanine.

5.5 ^{13}C NMR data for compound IX and literature data for pancracine.

5.6 ^{1}H NMR data for compound IX, base treated compound IX and literature data for montanine.

5.7 ^{13}C NMR data for compound IX, base treated compound IX and literature data for pancracine.

5.8 ^{1}H NMR data for compound X, compound IX and literature data for montanine.

5.9 ^{13}C NMR data for compound X, compound IX and literature data for pancracine.

5.10 ^{1}H NMR data for compound X, base treated compound X and literature data for montanine.

LIST OF SCHEMES

1.1 The biosynthesis of the intermediate *p*-coumaroyl CoA.

1.2 The biosynthesis of 2', 4', 6', 4-tetrahydroxychalcone.

1.3 The possible biosynthetic routes to basic homoisoflavonoids.

1.4 The possible biosynthetic route to scillascillin type homoisoflavonoids.

1.5 The possible biosynthetic route to brazilin type homoisoflavonoids.

2.1 Mass spectrometric fragmentation of compound I.

2.2 Mass spectrometric fragmentation of compound II

3.1 The biosynthesis of norbelladine

3.2 The biosynthesis of lycorine type alkaloids

3.3 The biosynthesis of homolycorine type alkaloids

3.4 The biosynthesis of 5,10b-ethanophenanthridine/crinane type alkaloids

3.5 Biosynthetic routes to montanine type alkaloids.

3.6 The biosynthetic relationship between haemanthamine, montanine and narciclasine type alkaloids.

4.1 The mass spectrometric fragmentation of compound III

4.2-4.9 The mass spectrometric fragmentation of compound IV

4.10-4.11 The mass spectrometric fragmentation of compound V

4.12-4.19 The mass spectrometric fragmentation of compound VI

4.20 The extraction protocol for *Clivia caulescens*

5.1 The mass spectrometric fragmentation of compound IX.

5.2 The mass spectrometric fragmentation of compound IX.

ABSTRACT

Ledebouria ovatifolia (Bak.) Jessop, *Clivia caulescens* R.A.Dyer and *Haemanthus pauculifolius* Snijman & Van Wyk were the three species investigated in this work.

Ledebouria ovatifolia belongs to the family Hyacinthaceae (Liliaceae *sensu lato*) and, to date, the chemical composition of this species has not been investigated. Members of this family are widely distributed, but are particularly well represented in southern Africa. The *Ledebouria* genus was formerly classified as part of the *Scilla* genus from which a large number of naturally occurring oxygen heterocycles known as homoisoflavonoids have been isolated. In this work the bulbs of *L. ovatifolia* were investigated and two compounds were isolated. These were the homoisoflavonoid, 5,7-dihydroxy-3-(4'-hydroxybenzyl)-4-chromanone and the chalcone, 6',2'-dimethoxy-4,4'-dihydroxychalcone, both of which are known naturally occurring compounds.

Clivia caulescens and *Haemanthus pauculifolius* are both members of the Amaryllidaceae family. The plants of the Amaryllidaceae family form a large group of over sixty genera, which are concentrated mainly in southern Africa. Plants from this family have been extensively used in traditional medicines and many have pharmacological properties. The compounds responsible for most of these effects are a group of isoquinoline alkaloids, which are found almost exclusively in plants belonging to this family. The alkaloids isolated from plants belonging to this group are known to cause poisoning in low doses and can cause excessive salivation and diarrhoea. Higher doses of the active compounds can cause CNS depression and large enough doses can prove fatal. Although many of these alkaloids are harmful to man, some of the unique Amaryllidaceae alkaloids exhibit anti-tumour and anti-viral activities, and are thus potentially beneficial to man.

The *Clivia* genus is endemic to South Africa and the most common species, *C. miniata,* is used by traditional healers to facilitate childbirth and as a snake bite remedy. In this chemical investigation both the bulbs and the leaves of *C. caulescens* were investigated. The ethanol extract of the bulbs yielded four alkaloids, hippeastrine, haemanthamine, lycorine and 11-(*S*)-hydroxyvittatine. The investigation of the leaf extract also yielded lycorine and hippeastrine as well as an additional alkaloid sternbergine.

Haemanthus pauculifilius is a recently described member of the *Haemanthus* genus, which consists of 27 taxa that are restricted to southern Africa and Namibia. In this chemical investigation the ethanol extract of the bulbs and leaves yielded the common triterpenoid sitosterol as well as two novel 5,11-methanomorphanthridine type alkaloids, montanine hydrochloride and manthidine hydrochloride and a novel alkaloid of the isocarbostyril type, haemanthustatin.

TABLE OF CONTENTS

	Page
Preface	ii
Acknowledgements	iii
List of abbreviations	iv
List of figures	v
List of tables	vii
List of schemes	viii
Abstract	x
Chapter 1 An Introduction to Homoisoflavonoids and Chalcones	
1.1 The Classification of Homoisoflavonoids.	1
1.2 The Classification of Chaclones	5
1.3 The biosynthesis of Homoisoflavonoids and Chalcones	8
1.4 The Biological Activity of Homoisoflavonoids and Chalcones	15
1.5 References	18
Chapter 2 Extractives from *Ledebouria ovatifolia*	
2.1 Introduction	22
2.2 Results and Discussion	24
2.2.1 The Structural Elucidation of Compound I	24
2.2.2 The Structural Elucidation of Compound II	30
2.3 Foreword to the Experimental Sections 2.4, 4.3 and 5.3	35
2.4 Experimental	37
2.4.1 Physical Data for Compound I	37
2.4.2 Physical Data for Compound II	38
2.5 References	39

Chapter 3 An Introduction to Amaryllidaceae Alkaloids

3.1 Introduction to the Alkaloids **41**

3.2 The Amaryllidaceae Alkaloids **44**

3.2.1 Introduction **44**

3.2.2 The Classification of the Amaryllidaceae Alkaloids **45**

3.2.3 The Biosynthesis of Amaryllidaceae Alkaloids **50**

3.3 References **59**

Chapter 4 Extractives from *Clivia caulescens*

4.1 A Review of Previous Work **63**

4.2 Results and Discussion **69**

4.2.1 The Structural Elucidation of Compound III **69**

4.2.2 The Structural Elucidation of Compound IV **75**

4.2.3 The Structural Elucidation of Compound V **87**

4.2.4 The Structural Elucidation of Compound VI **94**

4.2.5 The Structural Elucidation of Compound VII **104**

4.3 Experimental **108**

4.3.1 Physical Data for compound III **110**

4.3.2 Physical Data for Compound IV **111**

4.3.3 Physical Data for Compound V **112**

4.3.4 Physical Data for Compound VI **113**

4.3.5 Physical Data for Compound VII **114**

4.4 References **115**

Chapter 5 Extractives from *Haemanthus pauculifolius*

5.1 A Review of Previous Work **119**

5.2 Results and Discussion **124**

5.2.1 The Structural Elucidation of Compound VIII **124**

5.2.2 The Structural Elucidation of Compound IX **133**

5.2.3 The Structural Elucidation of Compound X **143**

5.2.4 The Structural Elucidation of Compound XI **149**

5.3 Experimental **150**

5.3.1 The Base Treatment of Compounds IX and X **150**

5.3.2 Physical Data for Compound VIII **150**

5.3.3 Physical Data for Compound IX **151**

5.3.4 Physical Data for Compound X **152**

5.4 References **154**

Appendix A

List of spectra **157**

CHAPTER 1

An Introduction to Homoisoflavonoids and Chalcones

1.1 THE CLASSIFICATION OF HOMOISOFLAVONOIDS

Homoisoflavonoids, which are sometimes referred to as homoisoflavanones, belong to a homogenous group of naturally occurring oxygen heterocycles [1]. The term "homoisoflavonoid" is actually a misnomer since these compounds are not biogenetically related to isoflavonoids and do not undergo the 2,3-aryl rearrangement of the C_6-C_3-C_6 moiety that is typical in the biosynthesis of isoflavonoids [2-5]. In addition, the homoisoflavonoid skeleton consists of sixteen carbon atoms as opposed to the fifteen carbons of the isoflavonoid skeleton [6]. Consequently, although this term is often used when reference is made to these compounds, the systematic name 3-benzyl-4-chromanones is more scientifically correct. These compounds are characterised by an additional benzyl carbon atom between the B and C rings [3]. The spectroscopic data for these compounds is definitive and is consistent with the sixteen carbon skeleton [3]. This skeleton includes a chromanone, chromone or chromane system with a benzyl or benzylidene group at position 3 [7]. Invariably these compounds exhibit extensive oxygenation which can include hydroxy, methoxy and acetate substitution [7]. The structural features of homoisoflavonoids can generally be classified into three basic structural types [1,3,8].

1. The 3-benzyl-4-chromanone or dihydroeucomin type.

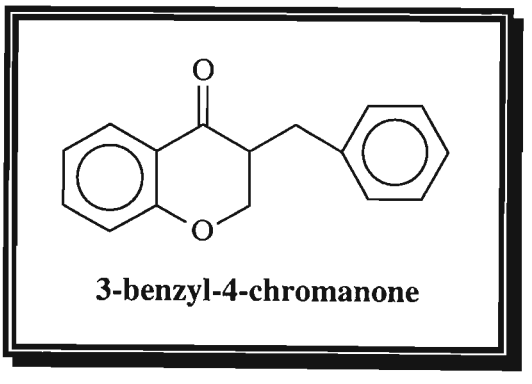


Figure 1.1 Homoisoflavonoid of the 3-benzyl-4-chromanone type.

2. The 3-benzyl-3-hydroxy-4-chromanone or eucomol type.

These compounds typically posses a hydroxy substituent at C-3.

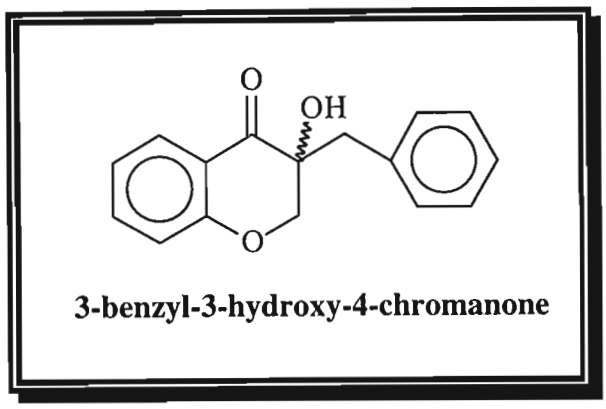


Figure 1.2 Homoisoflavonoid of the 3-benzyl-3-hydroxy-4-chromanone type.

3. The 3-benzylidene-4-chromanone or eucomin type.

These compounds are characterised by a 3,9-double bond and can be in the (Z) or the (E) configuration. They are also capable of undergoing various chemical transformations that include conversions to 3-benzyl-4-chromanones and 3-benzyl-3-hydroxy-4-chromanones [1].

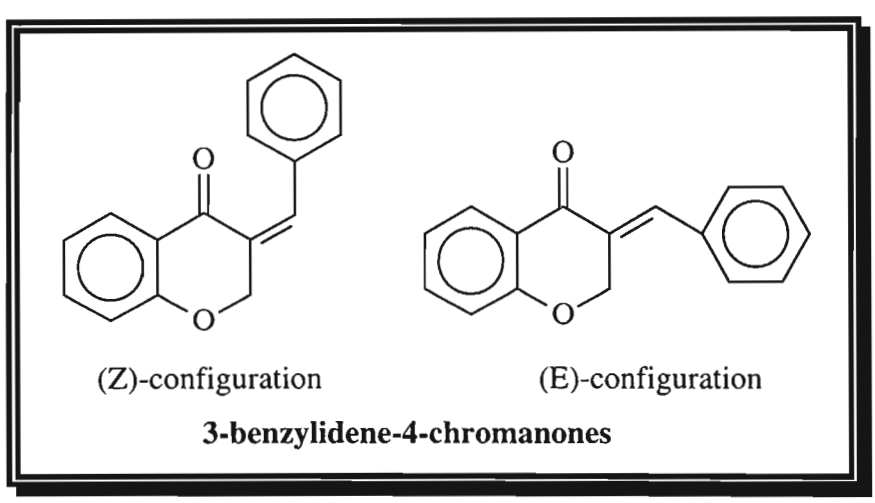


Figure 1.3 Homoisoflavonoid of the 3-benzylidene-4-chromanone type.

Groups 1-3 can be readily distinguished on the basis of their ^{13}C NMR spectra. The defining resonance is that of C-3. For group one, this carbon appears as an aliphatic methine carbon resonance whereas, for group two, it is an oxygenated, aliphatic quaternary carbon resonance.

Group three, on the other hand, displays a characteristic quaternary carbon resonance in the double bond region of the spectrum for C-3 [1].

In addition to these three basic structural types, a number of unusual compounds, which are characterised by a fourth ring, have been isolated [1,8]. These compounds are thought to be biogenetically related to homoisoflavonoids and are thus classified with this group [1,3,5]. These unusual compounds include scillascillins, brazilins and hematoxylin [1,3,7,8]. Scillascillin compounds typically posses a unique 3-*spiro*-cyclobutene system [3,8]. The C-4 carbonyl group is retained in these compounds but is absent in brazilin and hematoxylin compounds where a cyclopentene C-ring is observed [3]. The biosynthetic origin of these more complex homoisoflavonoids has been investigated and it has been postulated that they are derived directly from a 3-benzyl-3-hydroxy-4-chromanone precursor [5].

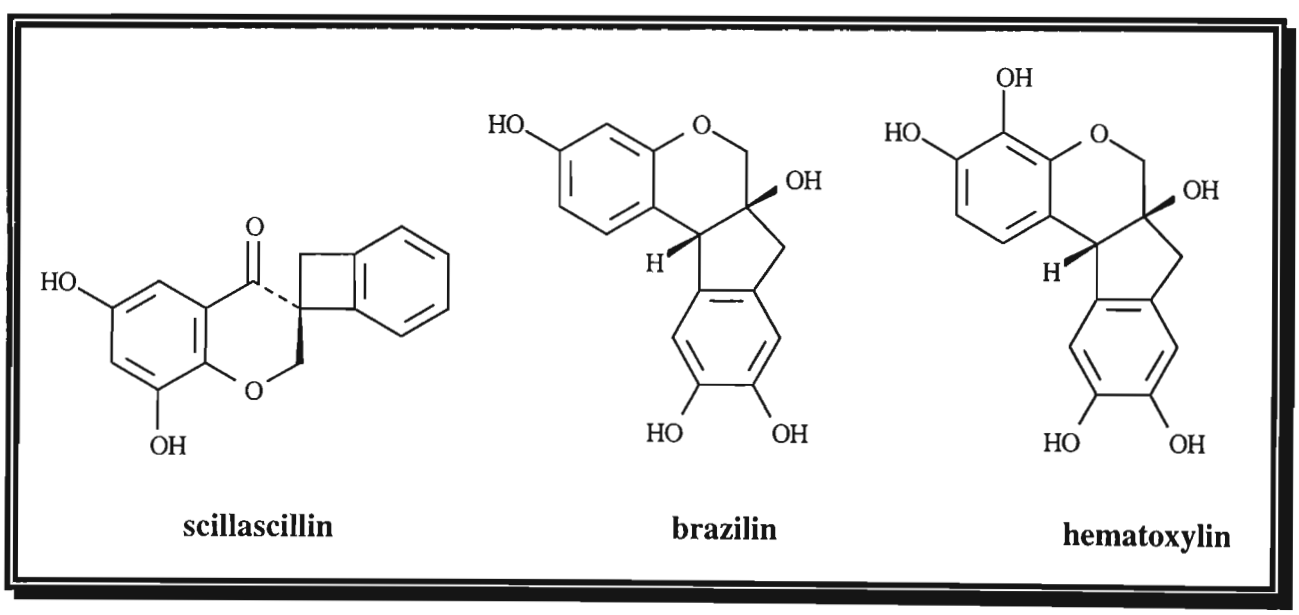


Figure 1.4 Homoisoflavonoids of the scillascillin, brazilin and hematoxylin types.

The numbering of the basic homoisoflavonoid skeleton is similar to that of all simple flavonoid compounds and is shown below using a 3-benzyl-4-chromanone type (**1**) and a scillascillin type (**2**) compound as examples [3].

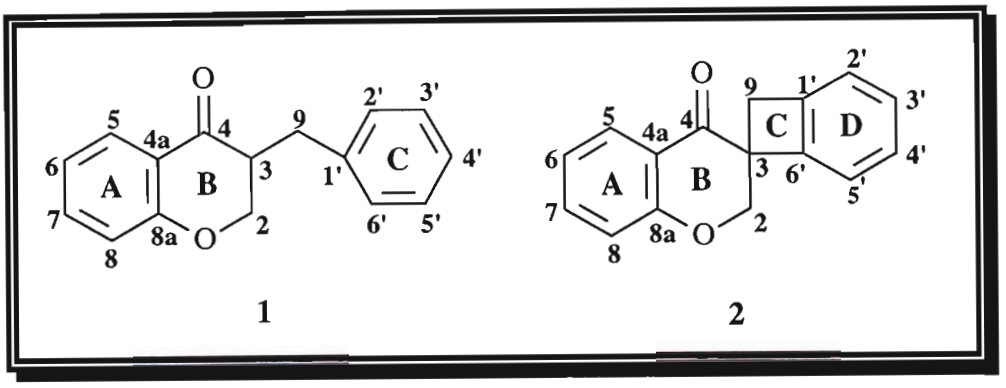


Figure 1.5 The numbering system for homoisoflavonoids.

The 4-chromanone part forms the basic unit and the additional carbon atom is designated as C-9 [3]. Brazilin type compounds and hematoxylin on the other hand, have a different numbering system that is generally used for polycycles and is shown below [3].

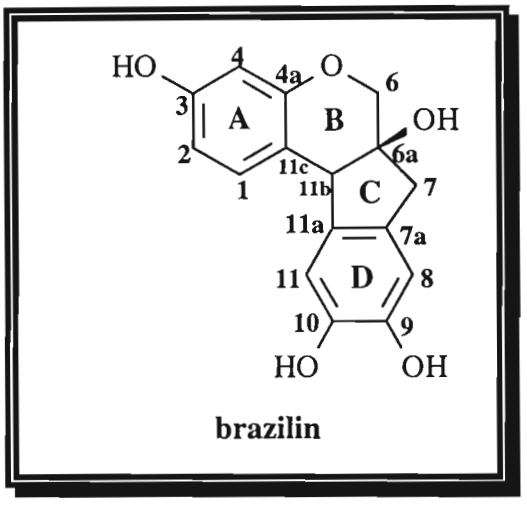


Figure 1.6 The numbering system for brazilin type homoisoflavonoids.

Eucomin and eucomol were isolated by Bohler and Tamm (1967) and classified as a new class of flavonoid compounds called Homoisoflavonoids [9]. Since then, numerous compounds of this class have been isolated and a review has been published [3]. Homoisoflavonoids have, to date, been isolated primarily from the Hyacinthaceae (Liliaceae *sensu lato*) family, particularly from the *Eucomis*, *Scilla* and *Muscari* genera [3,9-16]. In addition, numerous homoisoflavonoids have also been isolated from the Fabaceae family particularly from the *Caesalpinia* genus [6,8,17-20].

1.2 THE CLASSIFICATION OF CHALCONES

Chalcones and the closely related dihydrochalcones are unique in the flavonoid family as they are C_6-C_3-C_6 compounds characteristically lacking a central heterocyclic ring [22,23]. The numbering system used for these compounds is unique to this group [21-23]. The chalcones were initially recognized as being structurally related to acetophenone [22]. Consequently, the A-ring, which is, by convention, always written on the left, is identified by primed numbers and the B-ring by unprimed numbers [21-23]. This numbering system is an artifact of chemical nomenclature rules, and thus the two types of flavonoid compounds discussed in this chapter, homoisoflavonoids and chalcones, have two different numbering systems for the A and B rings [21-23] despite the fact that they are derived *via* the same biosynthetic route [24-26].

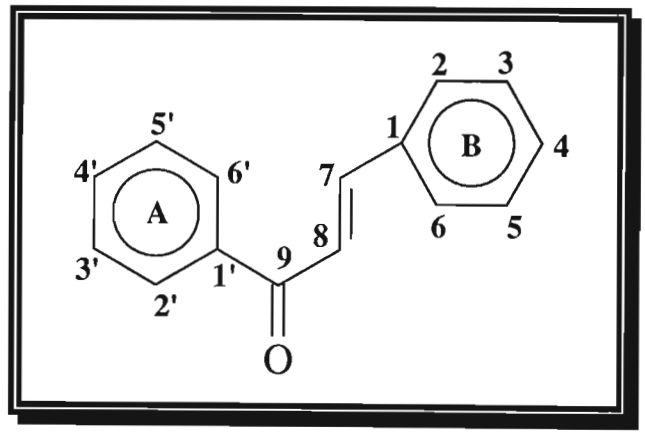


Figure 1.7 The numbering system for chalcones.

A large number of chalcones have been identified since the early 1970's ranging from extremely simple, to much more complex structures as seen in **figures 1.8-1.11** [21-23]. Unlike homoisoflavonoids, which appear to be restricted to two plant families, these compounds have been isolated from a wide variety of botanical sources [21-23]. The chalcones can be classified into four types according to the oxygenation pattern of the B ring.

1. Chalcones without B ring oxygenations.

For compounds in this group, the B ring characteristically has no oxygenation but the A ring can undergo extensive substitution.

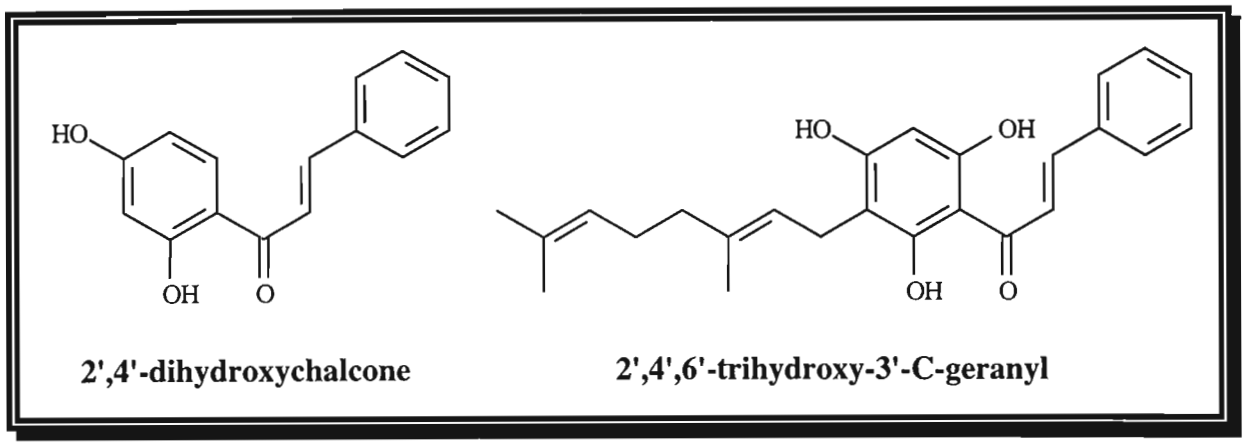


Figure 1.8 Type 1 chalcones.

2. Chalcones with one B ring oxygenation.

The second group contains those chalcones with one B ring oxygenation and, as before, the degree of A ring substitution and oxygenation can be extensive.

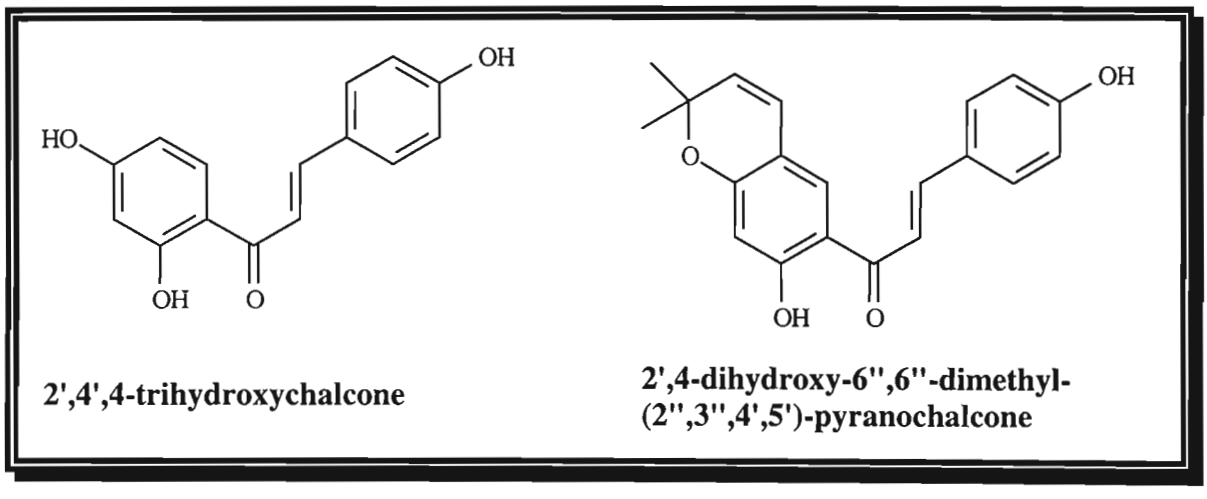
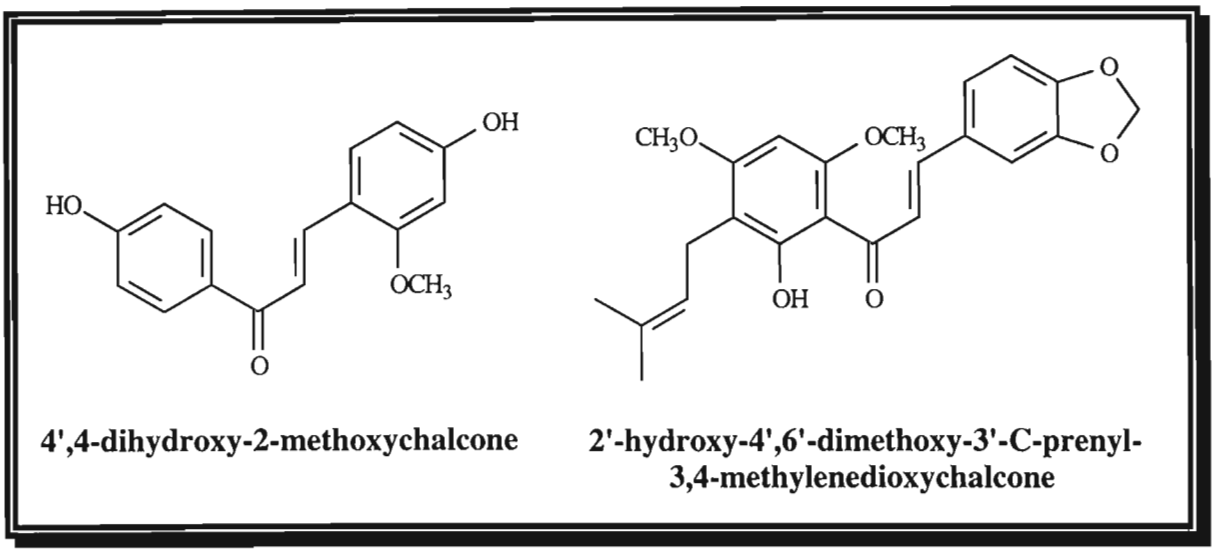


Figure 1.9 Type 2 chalcones.

3. Chalcones with two B ring oxygenations.

The third group contains those chalcones with two B ring oxygenations, but once again the A ring substitution can be varied and extensive.

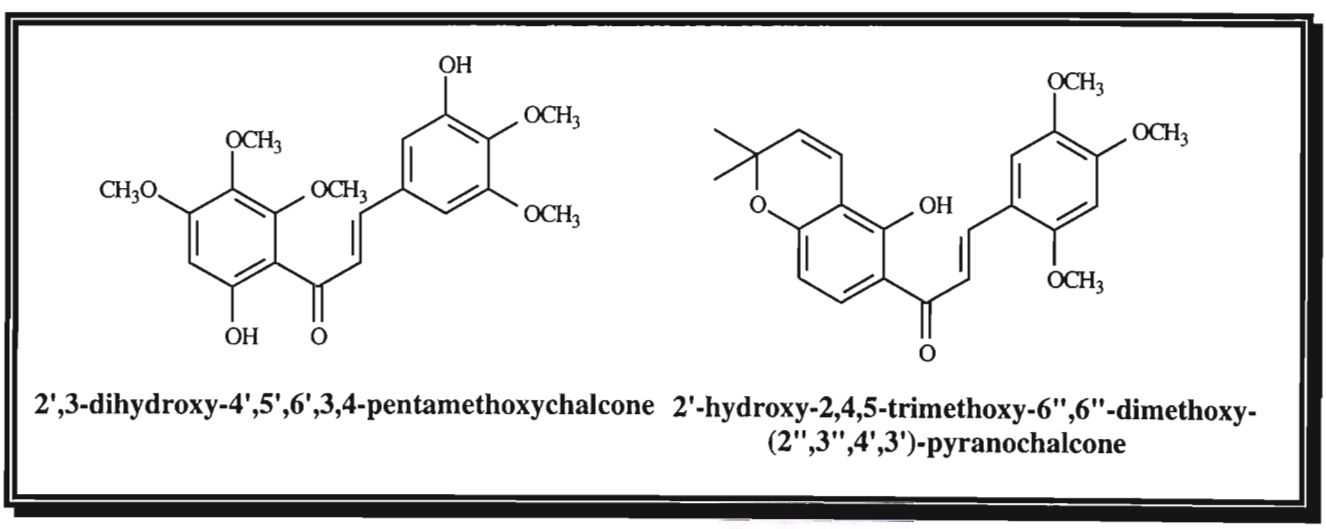


4',4-dihydroxy-2-methoxychalcone **2'-hydroxy-4',6'-dimethoxy-3'-C-prenyl-3,4-methylenedioxychalcone**

Figure 1.10 Type 3 chalcones.

4. Chalcones with three B ring oxygenations.

Chalcones which typically have three B ring oxygenations are classified in this group.



2',3-dihydroxy-4',5',6',3,4-pentamethoxychalcone **2'-hydroxy-2,4,5-trimethoxy-6'',6''-dimethoxy-(2'',3'',4',3')-pyranochalcone**

Figure 1.11 Type 4 chalcones.

1.3 THE BIOSYNTHESIS OF HOMOISOFLAVONOIDS AND CHALCONES

Plant derived aromatic compounds are generally biosynthesised *via* one of three biosynthetic pathways: the acetate-malonate, acetate-mevalonate or shikimic acid pathways [27]. However, a number of compounds have a mixed biogenesis and are derived from the products of two, or more, of these pathways [27]. Flavonoids are a class of compounds which fall into this category since their biosynthesis involves both the acetate-malonate, and the shikimic acid biosynthetic pathways [24-27]. The biogenesis of flavonoid compounds can be traced *in vivo* using radioactive tracer studies [24]. In addition, the recent advancement in the field of enzymology has also contributed significantly to the elucidation of the catalytic mechanisms in the biosynthetic routes to flavonoid compounds [25]. The key stage in the biosynthesis of all classes of flavonoids is the formation of a chalcone, which is the first common intermediate to all flavonoid compounds [24-26,28]. Since chalcones are known to be the direct precursors of homoisoflavonoids, the biosynthesis of chalcones is essentially a part of the biogenetic route to homoisoflavonoids [3-5].

The A ring of chalcones, and all flavonoid compounds, is derived from three acetate units condensed in a head to tail manner, while the B ring and the three carbons of the central ring are derived from cinnamic acid [27]. Consequently, the A ring of chalcones is acetate-malonate derived and the B ring, shikimic acid derived (**Fig. 1.12**). The precursors to chalcones are three malonyl-CoA units and *p*-coumaroyl CoA [24-26,28].

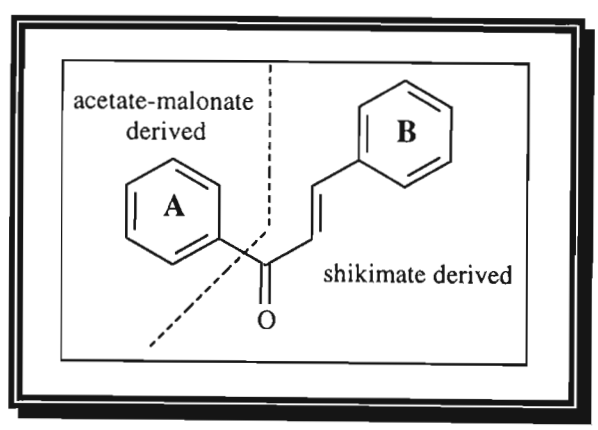


Figure 1.12 The origin of the A and B rings of chalcones

Malonyl-CoA, is derived *via* the acetate-malonate biosynthetic pathway from the glycolysis intermediate acetyl-CoA, and carbon dioxide [24-26,29]. This reaction is catalysed by the enxyme acetyl-CoA carboxylase (ACC) in the presence of adenosine-5'-triphosphate (ATP) and Mg^{2+} [24-26].

$$CH_3CO\text{-}SCoA + HCO_3^- + ATP \longrightarrow HOOC\text{-}CH_2\text{-}CO - SCoA + ADP + Pi$$

The shikimic acid biosynthetic pathway is responsible for the production of the second precursor, *p*-coumaroyl CoA and the related hydroxy cinnamic esters, from which the B ring is derived [24-26]. The precursors of the shikimic acid pathway, erythrose-4-phosphate and phosphoenolpyruvate, are primary metabolites derived from glucose-6-phosphate by the oxidative pentose phosphate cycle and glycolysis [30]. One of the end products of the shikimic acid pathway is the amino acid L-phenylalanine, the cinnamic acid derivatives of which are, as mentioned above, involved in the formation of the B ring. (**Scheme 1.1**). [25].

The initial reaction in the production of the chalcone precursor is the deamination of phenylalanine, which is catalysed by the enzyme phenylalanine ammonia-lyase (PAL) [24-26,30]. PAL catalyses the *anti* elimination of ammonia and the (pro-3*S*)-proton from L-phenylalanine to yield *trans*-cinnamic acid [24-26]. The *trans*-cinnamic acid is then hydroxylated to *p*-coumaric acid *via* the mono-oxygenase enzyme, cinnamate-4-hydroxylase (C4H) (**Scheme 1.1**) [24-26]. The reaction requires NADPH and molecular oxygen, and exhibits properties characteristic for plant cytochrome P450 enzymes [24-26]. This oxygenase enzyme is located at the endoplasmic membrane and consists essentially of cytochrome P-450 and NADPH-cytochrome-P-450 reductase [25]. The reaction mechanism involves a hydrogen shift, which is known as an NIH shift [24,25]. This phenomenon was first discovered at the National Institute of Health, hence its name, and involves the migration of the proton atom being replaced by the hydroxy group at C-4 [30]. Tritium labelling experiments have revealed that the proton shift in the formation of *p*-coumaric acid is from position four to position three [30] (**Fig. 1.13**).

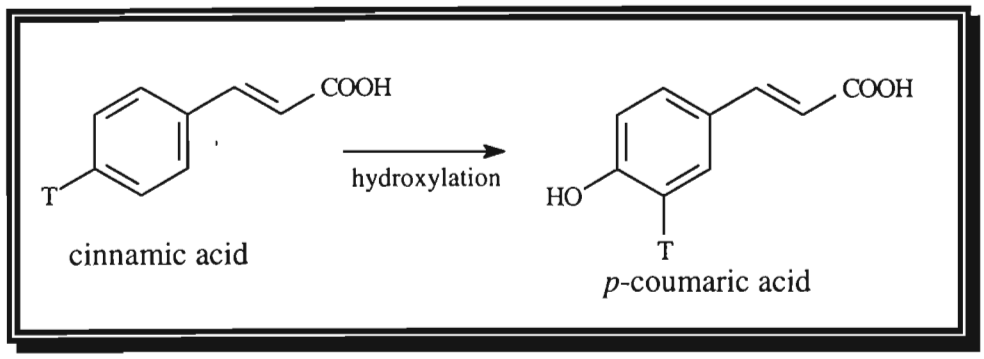
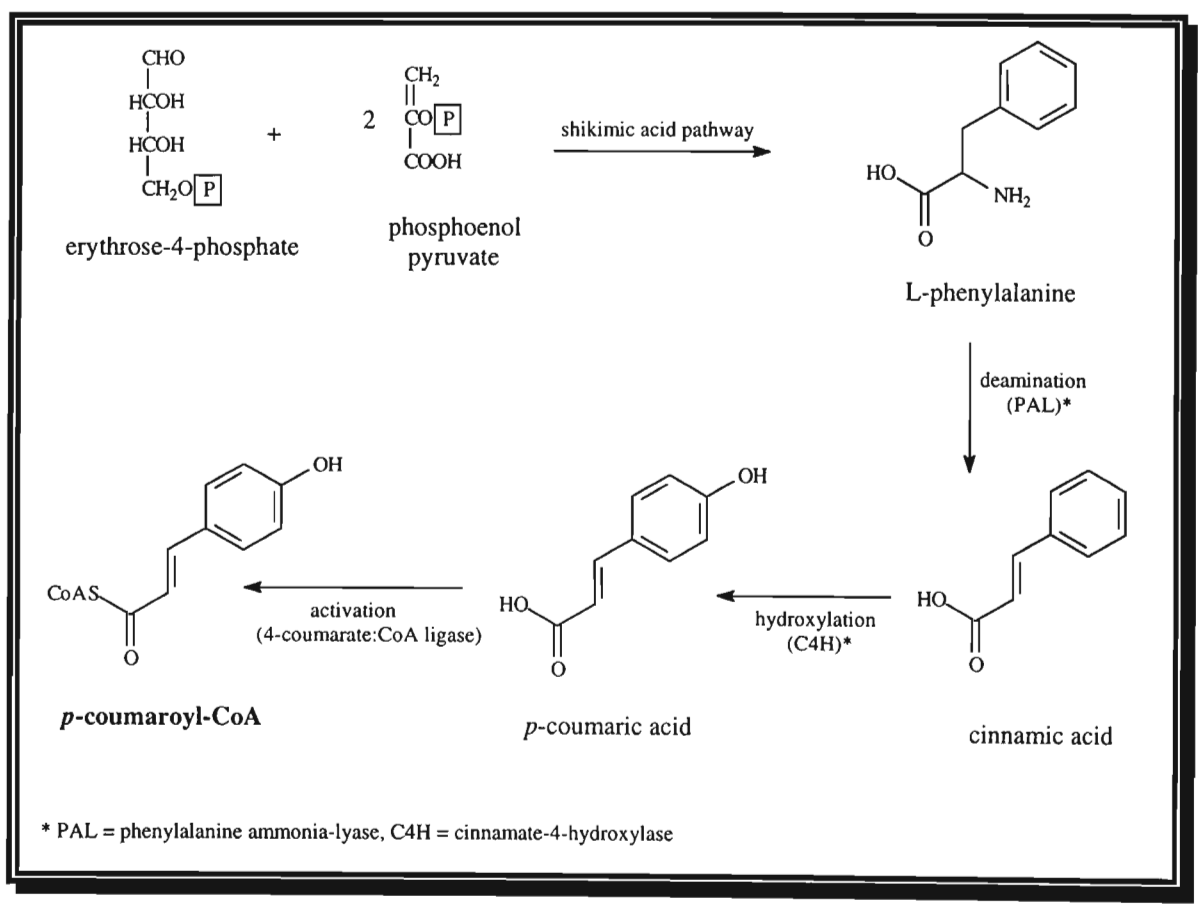


Figure 1.13 The NIH shift for the hydroxylation of cinnamic acid

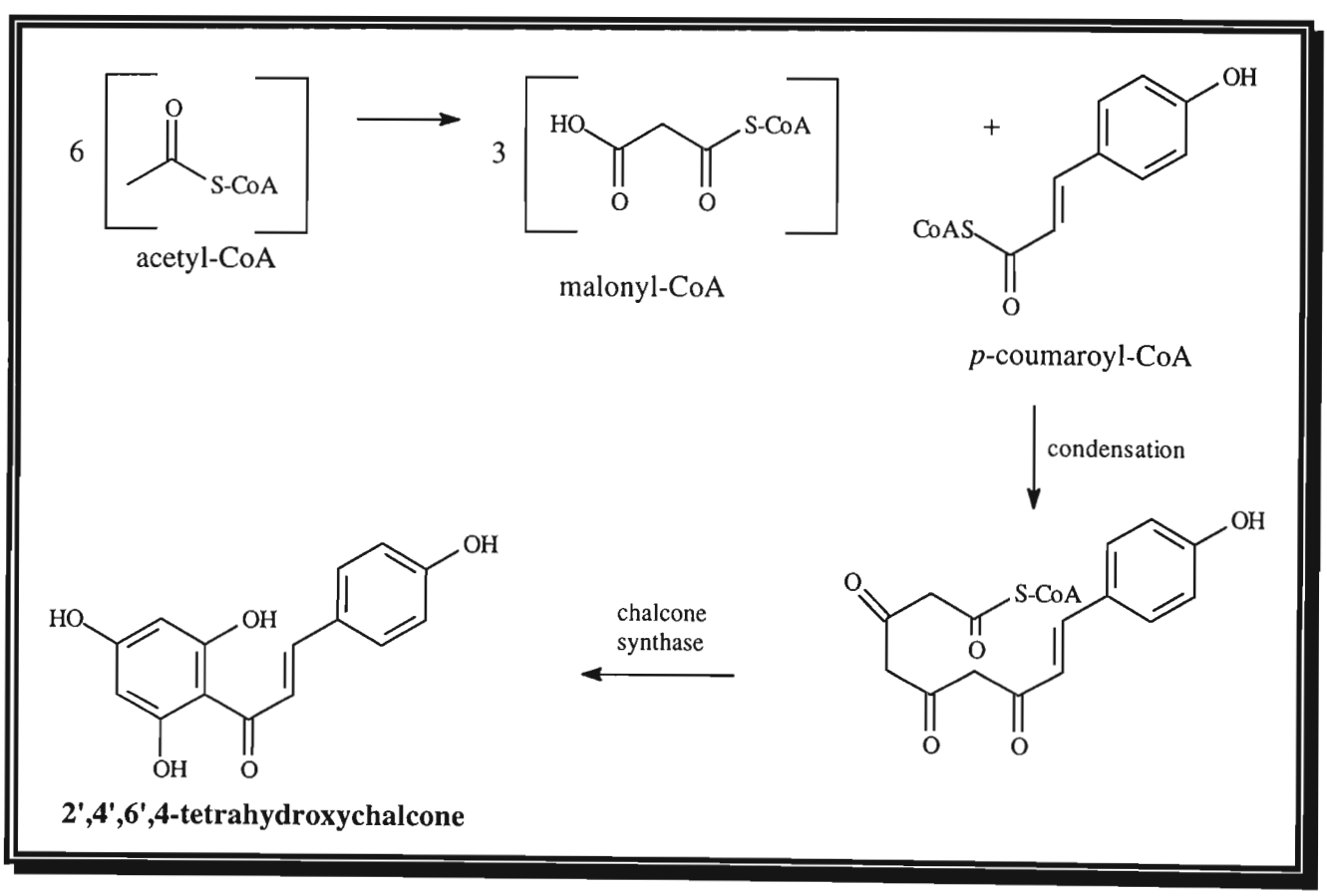
The *p*-coumaric acid is activated by the formation of the CoA ester, which is catalysed by 4-coumarate:CoA ligase [24-26]. The ligase reaction, which strictly requires ATP and Mg^{2+} as co-factors, proceeds *via* an acyl-AMP intermediate and thus characterises the enzyme as a synthetase [24-26].

Scheme 1.1 The biosynthesis of the intermediate *p*-coumaroyl CoA.



The formation of a chalcone is the most important step in flavonoid biosynthesis and is common to all flavonoid type compounds [24-26,28]. The enzyme catalysing this step, chalcone synthase, can therefore be regarded as the key enzyme in flavonoid biosynthesis [25,26]. *p*-Coumaroyl-CoA serves as one of the substrates for the synthase enzyme and has no co-factor requirements [24-26]. The overall reaction involves three successive condensation steps with acetate units derived from malonyl-CoA [26,28]. The result is the elongation of the aliphatic side chain of *p*-coumaric acid by six carbon atoms which then cyclise to give the aromatic A ring of the C_{15} intermediate, 2',4',6',4-tetrahydroxychalcone (**Scheme 1.2**) [24,28].

Scheme 1.2 The biosynthesis of 2',4',6',4-tetrahydroxychalcone.

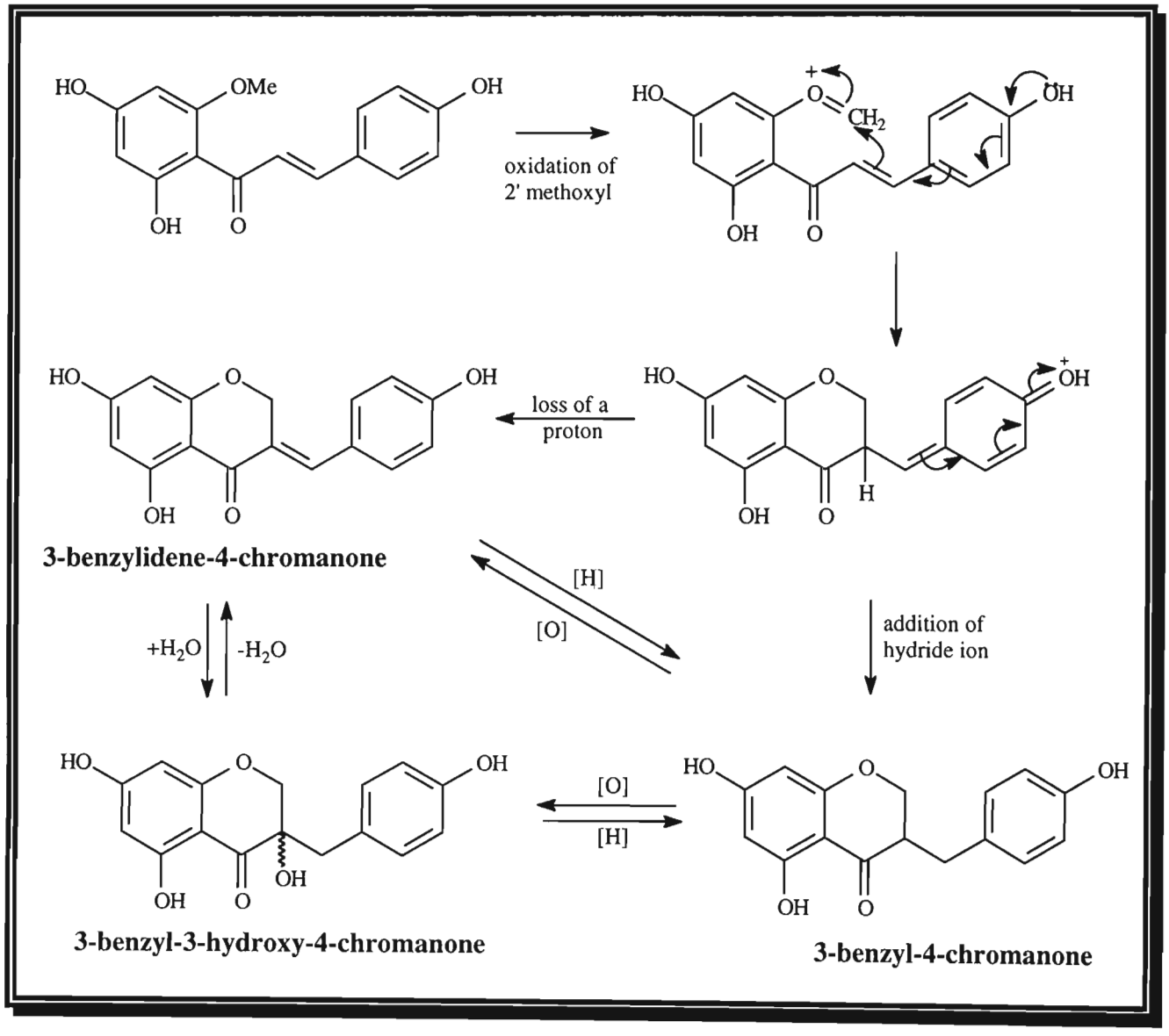


Radioactive labelling experimements have enabled researchers to determine how homoisoflavonoids are synthesised *in vivo* [4,5]. Investigations have revealed that the aromatic B ring in conjunction with C-3, C-4 and C-9 of homoisoflavonoids are derived from L-phenylalanine [4,5]. These studies further revealed that the A ring is acetate derived and that methionine is the

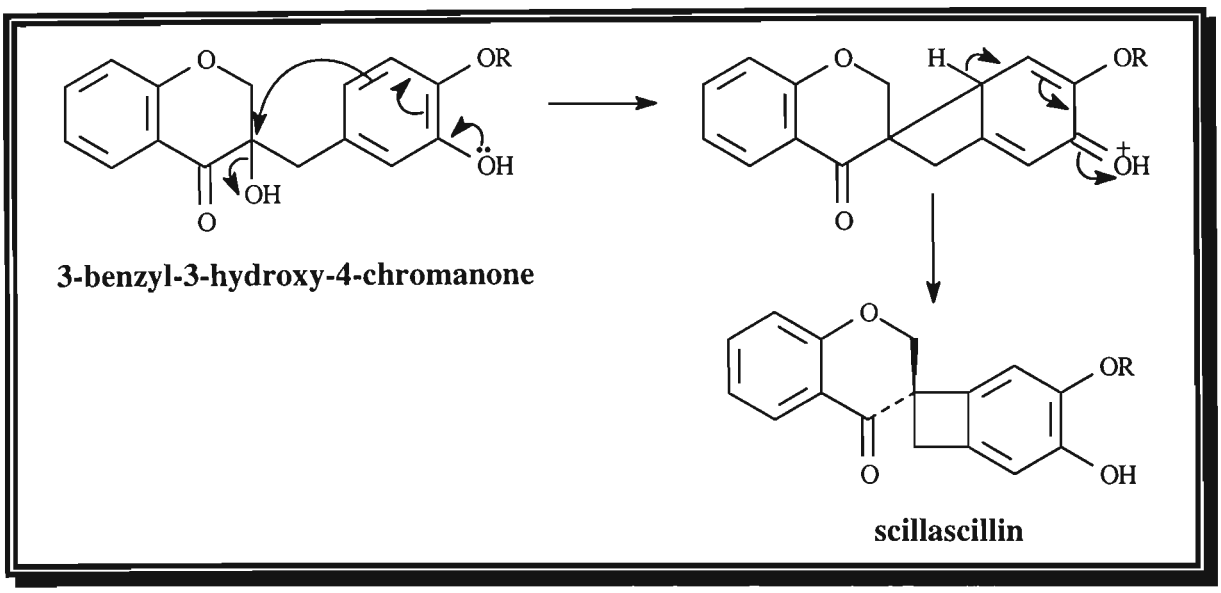
source of C-2 [4,5]. The radioactive labelling patterns observed from these precursors are consistent with the biosynthesis of flavonoid compounds [24-26] and differ only in the incorporation of an additional carbon atom (C-2), derived from L-methionine, which is added in order to produce the heterocyclic C-ring [5]. The biosynthetic hypothesis postulates that the basic C_{16} skeleton of homoisoflavonoids is derived from chalcones [4,5]. The biosynthetic scheme (**Scheme 1.3**) proposed for these compounds suggests that a 2'-methoxychalcone, with the methoxy group derived from L-methionine, is the ultimate precursor. The methoxy group is thought to be oxidised and the three basic homoisoflavonoids are produced by the subsequent cyclisation, which takes place with the addition of a hydride ion for 3-benzyl-4-chromanone homoisoflavonoids, and with the loss of a proton in the case of 3-benzylidene-4-chromanones [5]. 3-Benzyl-3-hydroxy-4-chromanones are formed by the addition of water to a 3-benzylidene-4-chromanone-type homoisoflavonoid or *via* the oxidation of the C-3 position of a 3-benzyl-4-chromanone-type homoisoflavonoid [5]. This biosynthetic scheme is outlined in **scheme 1.3**.

Scillascillin and brazilin compounds are formed *via* a slightly more complex mechanism and are thought to be derived from a suitably activated 3-benzyl-3-hydroxy-4-chromanone precursor [5]. Scillascillins, which are characterised by the 3-*spiro*-cyclobutene C ring, may be produced by the cyclisation of the precursor as shown in **scheme 1.4** [5].

Scheme 1.3 The possible biosynthetic routes to basic homoisoflavonoids.

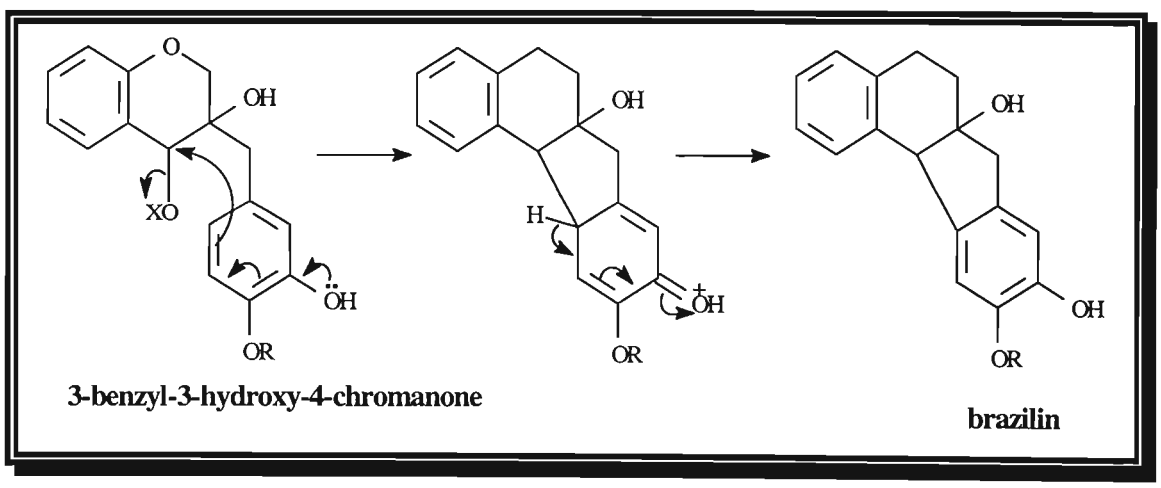


Scheme 1.4 The possible biosynthetic route to scillascillin type homoisoflavonoids.



The biosynthesis of brazilins is thought to occur in a similar manner to that of scillascillins except that the cyclisation of the activated precursor produces a cyclopentene C ring in this case (**Scheme 1.5**) [5].

Scheme 1.5 The Possible biosynthetic route to brazilin type homoisoflavonoids.



1.4 THE BIOLOGICAL ACTIVITY OF HOMOISOFLAVONOIDS AND CHALCONES

The biological activities of homoisoflavonoids and chalcones are numerous and diverse and a significant amount of further research is necessary to appreciate the full extent of their activities. Consequently only a few examples are outlined below.

Morphological studies of Hyacinthaceae (Liliaceae *sensu lato*) bulbs known to contain homoisoflavonoids have revealed that these compounds are located exclusively in a waxy layer between the fleshy storage leaves [3]. This highly specific distribution of homoisoflavonoids within the bulbs has prompted the investigation of their biological significance [3]. Biological investigations have revealed that plants subjected to conditions of stress exhibit marked biochemical changes including altered secondary metabolism [31]. In response to biological and environmental stress factors, which include mechanical wounding, micro-organism infection, UV irradiation and dehydration, the plant can be stimulated to produce stress metabolites. Several flavonoids have been identified as components of plants following fungal attack and have, in numerous cases, been identified as fungal inhibitors [32,33]. Consequently, the biological role of flavonoid compounds as phytoalexins has been extensively investigated [32,33]. The theory that homoisflavonoids may have an inhibitory effect on microorganisms has also been postulated. It has thus been suggested that they are not true plant metabolites but rather phytoalexins which are stimulated by pathogenic attack [3]. However this hypothesis is thought to be unlikely due to the high concentration of these compounds that can be found at the base of all storage leaves and in the vegetative apex [3].

A number of investigations have revealed that homoisoflavonoids have various biological activities. One such investigation reported the antimutagenic or toxic properties of two homoisoflavonoids isolated from the roots of *Hoffmanseggia intricata* (Fabaceae) [34]. The two compounds, identified as intricatin (8-hydroxy-7-methoxy-3-(4'-methoxybenzyl)-4-chromanone) and intricatinol (7,8-dihydroxy-3-(4'-methoxybenzyl)-4-chromanone) showed varying inhibition of the mutagenicity towards *Salmonella typhimurium* of 2-amino-anthracene, acetylaminofluorine and ethyl methanesulphonate [34]. The study reported that the dihydroxy anologue, intricatinol, was more broadly active and the theory that the increased potency could be associated with the presence of a dihydroxy moiety in ring A was postulated [34]. The anti-inflammatory effects of

homoisoflavonoids have also been investigated [35]. The anti-inflammatory properties of a crude extract from the bulbs of *Muscari comosum,* Hyacinthaceae (Liliaceae *sensu lato*) was investigated and the inhibition of croton oil-induced dermatitis in the mouse ear was used to assay this activity [35]. A positive response to the biological test was obtained only for the homoisoflavonoid-rich fractions and an inhibitory effect comparable to that of the potent anti-inflammatory drug, indomethacin, was observed [35]. In another investigation, the *in vitro* activity of a homoisoflavonoid isolated from *Veltheimia viridifolia,* Hyacinthaceae (Liliaceae *sensu lato*) against purified phosphodiesterase (PDE) isozymes was studied [36]. The compound, 5-hydroxy-6,7,8-trimethoxy-3-(4'-hydroxybenzyl)–4-chromanone, was reported to possess a weak selective profile for PDE IV and PDE V [36]. This study also investigated the bronchiospasmoloytic *in vivo* activity of the homoisoflavonoid on the respiration and the influence on the cardiac system of guinea pigs. The results indicated that the compound induced a slight increase in the expiratory flow and a pronounced increase in the respiratory rate in the test subjects [36]. In addition, a moderate bronchiospasmolysis was detected in conjunction with a decrease in heart rate [36]. The conclusions were that the homoisoflavonoid had a weak and especially short biological activity in the *in vivo* test system [36].

Various biological activities have also been reported for numerous chalcones. Chalcones have also been isolated as stress metabolites and their role as phytoalexins reported. For example, the parasitic grass *Imperata cylindrica* has been reported to stimulate the production of the chalcone 2,4-dihydroxy-4',6-dimethoxychalcone in the bulbs of *Pancratium biflorum* (Amaryllidaceae) [37]. An abundant growth of adventitious roots near the points of entry and emergence of the parasite from the bulb was observed. A large lesion along the course of the parasitic root was evident in the host and microscopic analysis of this region revealed that the host cells were necrotic and had formed a thick crust. The cells adjacent to this region were filled with a red substance. This zone was excised and the chemical content investigated [37]. The chalcone was one of a number of compounds isolated from this region and it was thought to be one of the compounds responsible for restricting the parasitic invasion by collapsing the cells nearest the parasite, thereby removing the components necessary for the nutrition and multiplication of the pathogen [37]. In a similar investigation the production of the retrochalcone echinatin, 2-methoxy-4,4'-dihydroxychalcone by *Glycyrrhiza echinata* (Fabaceae) (M-2) cultured cells was stimulated by the addition of yeast extract [31]. Consequently, the role of this compound as a stress metabolite was postulated [31]. In addition, the

chalcone, 4,4'-dihydroxy-2'-methoxychalcone has been reported as a stress metabolite in copper (II) treated *Pisum sativum* (Fabaceae) [38]. Numerous flavonoids have been isolated from grasses and the fact that many of these species are hosts to aphids has prompted the investigation of the feeding deterrent activity of a number of naturally occurring flavonoids [39]. A number of dihydrochalcones were shown to be highly active against the aphids *Schizaphis graminum* and *Myzus persicae* at concentractions that approximate naturally occurring levels in the host plants [39]. One of the more unusual activities reported for chalcones and dihydrochalcones is their uncoupling effect on isolated mitochondria from potato tubers and mung bean hypocotyls [40]. A number of the chalcones and dihydrochalcones tested in this study clearly uncoupled oxidative phosphorylation [40]. In addition, the uncoupling properties appeared to be linked to the structural characteristics of the compounds and it was suggested that the nature and position of the A ring had an effect on the uncoupling, with hydrogen or hydroxyl groups at C-2' and hydrogen, hydroxyl or nitrile groups at C-4' having the most potent uncoupling effect [40]. Chalcones have also been linked to the yellow colour of some flowers [41]. In general yellow to orange colours are due to the presence of carotenoids but in some cases the yellow colour can be ascribed to the anthochlor pigments (chalcones and aurones) as well as the yellow flavonols [41]. A number of families in the Asteraceae (Compositae *sensu lato*) are known to possess anthochlors as yellow flower pigments. For example, yellow ligules of *Pyrrhopappus* are essentially pigmented by the chalcone coreopsin (4'-*O*-glucose-3,4,2',4'-tetrahydroxychalcone) and carotenoids are absent in this genus [41]. In addition, some anthochlor pigments play a role in the UV honey patterns which guide insects, particularly bees, in their search for nectar. For example, in the genus *Potentilla* (Rosaceae), UV absorbing patterns are essentially given by the widespread chalcone isosalipurposide (2'-*O*-glucose-chalcone) [41].

1.5 REFERENCES

1. Kirkiacharian, B.S., Gomis, M., Tongo, H.G., Mahuteau, J. and Brion, J.D.; *Org. Mag. Res.*, (1984), **22**, 106.
2. Geiger, H., in *"The Flavonoids – Advances in Research Since 1980"*, Ed. by Harborne, J.B., (1988), Chapman and Hall Ltd, London, Chpt 10, 389.
3. Heller, W. and Tamm, C.; *Forstchr. Chem. Org. Natur.*, (1981), **40**, 105.
4. Dewick, P.M.; *J. Chem. Soc. Chem. Comm.*, (1973), 438.
5. Dewick, P.M.; *Phytochem.*, (1975), **14**, 983.
6. Namikoshi, M., Nakata, H., Nuno, M., Ozawa, T. and Saitoh, T.; *Chem. Pharm. Bull.*, (1987), **35**, 3568.
7. Adinolfi, M., Lanzetta, R., Laonigro, G. and Parrilli, M.; *Mag. Res. in Chem.*, (1986), **24**, 663.
8. Namikoshi, M., Nakata, H, Yamada, H., Nagai, M. and Saitoh, T.; *Chem Pharm. Bull.*, (1987), **35**, 2761.
9. Bohler, P. and Tamm, C.; *Tet. Lett.*, (1967), **36**, 3479.
10. Adinolfi, M., Barone, G., Belardini, M., Lanzetta, R., Laonigro, G., and Parrilli, M.; *Phytochem.*, (1984), **23**, 2091.
11. Adinolfi, M., Barone, G., Lanzetta, R., Laonigro, G., Mangoni, L., and Parrilli, M.; *Phytochem.*, (1985), **24**, 624.
12. Adolfini, M., Corsaro, M.M., Lanzetta, R., Laonigro, G., Mangoni, L. and Parrilli, M.; *Phytochem.*, (1987), **26**, 285.
13. Barone, G., Corsaro, M.M., Lanzetta, T. and Parrilli, M.; *Phytochem.*, (1988), **27**, 921.
14. Masterova, I., Suchy, V., Uhrin, D., Ubik, K., Grancaiova, Z. and Bobovnicky, B.; *Phytochem.*, (1991), **30**, 713.
15. Finckh, R.E. and Tamm, C.; *Experientia*, (1970), **26**, 472.
16. Sidwell, W.T.L. and Tamm, C.; *Tet. Lett.*, (1970), **7**, 475.
17. McPherson, D.D., Cordell, G.A., Soejarto, D.D, Pezzuto, J.M. and Fong, H.H.S.; *Phytochem.*, (1983), **22**, 2835.
18. Saitoh, T., Sakashita, S., Nakata, H., Shimokawa, T., Kinjo, J., Yamahara, J., Yamasaki, M. and Nohara, T.; *Chem. Pharm. Bull.*, (1986), **34**, 2506.
19. Namikoshi, M., Nakata, H. and Saitoh, T.; *Phytochem.*, (1987), **26**, 1831.
20. Namikoshi, M. and Saitoh, T.; *Chem. Pharm. Bull.*, (1987), **35**, 3597.

21. Bohm, B.; in "*The Flavonoids – Advances In Research*", Ed. Harborne, J.B. and Mabry, T.J.; (1982), Chapman and Hall Ltd., London, Chpt 6, 313.

22. Bohm, B.; in "*The Flavonoids – Advances in Research Since 1980*", Ed. Harborne, J.B., (1988), Chapman and Hall Ltd., London, Chpt 9, 329.

23. Bohm, B.; in "*The Flavonoids – Advances in Research Since 1986*", Ed. Harborne, J.B., (1993), Chapman and Hall Ltd., London, Chpt 9, 387.

24. Ebel, J. and Hahlbrock, K.; in "*The Flavonoids – Advances in Research*", Ed. Harborne, J.B. and Mabry, T.J., (1982), Chapman and Hall Ltd., London, Chpt. 11, 641.

25. Heller, W. and Forkmann, G.; in "*The Flavonoids – Advances in Research Since 1980*", Ed. Harborne, J.B. (1988), Chapman and Hall Ltd., London, Chpt 11, 399.

26. Heller W. and Forkman, G.; in "*The Flavonoids - Advances in Research Since 1986*", Ed. Harborne, J.B., (1993), Chapman and Hall Ltd., London, Chpt. 11, 499.

27. Vickery M.L. and Vickery, B.; "*Secondary Plant Metabolism*", (1981), The Macmillan Press Ltd., London, Chpt 7, 183.

28. Herbert R.B.; "*The Biosynthesis of Secondary Metabolites –2nd Edition*", (1989), Chapman and Hall Ltd., London, Chpt 5, 111.

29. Herbert R.B.; "*The Biosynthesis of Secondary Metabolites –2nd Edition*", (1989), Chapman and Hall Ltd., London, Chpt 3, 31.

30. Vickery M.L. and Vickery, B.; "*Secondary Plant Metabolism*", (1981), The Macmillan Press Ltd., London, Chpt 6, 157.

31. Ayabe, S., Iida, K. and Furuya, T.; *Phytochem.*, (1986), **25**, 2803.

32. Bohm, B.; in "*The Flavonoids – Advances in Research Since 1980*", Ed. Harborne, J.B., (1988), Chapman and Hall Ltd., London, Chpt 9, 382.

33. Bohm, B.; in "*The Flavonoids – Advances in Research Since 1986*", Ed. Harborne, J.B., (1993), Chapman and Hall Ltd., Chpt 9, 426.

34. Wall, M.E., Wani, M.C., Manikumar, G., Taylor, H. and McGivney R.; *J. Nat. Prod.*, (1989), **52**, 774.

35. Della Loggia, R., Del Negro, P., Tubaro, A., Barone, G. and Parrilli, M.; *Planta. Med.*, (1989), **55**, 587.

36. Amschler,G., Frahm, A.W., Hatzelmann, A., Kilian, U., Muller-Doblies, D. and Muller-Doblies, U.; *Planta. Med.*, (1996), **62**, 534.

37. Ghosal, S., Kumar, Y., Chakrabarti, D.K., Lal, J. and Singh, S.K.; *Phytochem.*, (1986), **25**, 1097.

38. Carlson, R.E. and Dolphin, D.H.; *Phytochem.*, (1982), **21**, 1733.

39. Dreyer, D.L. and Jones, K.C.; *Phytochem.*, (1981), **20**, 2489.

40. Ravanel, P.R., Tissut, M. and Douce, R.; *Phytochem.*, (1982), **21**, 2845.

41. Broullard, R.; in "*The Flavonoids – Advances in Research Since 1980*", Ed. Harborne, J.B., (1988), Chapman and Hall Ltd., London, Chpt 16, 525.

CHAPTER 2

Extractives from Ledebouria ovatifolia

2.1 INTRODUCTION

Ledebouria ovatifolia (Bak.) Jessop belongs to the family Hyacinthaceae (Liliaceae *sensu lato*). Members of this family are widely distributed, but are particularly well represented in southern Africa and in the region from the Mediterranean to south-western Asia [1]. There are approximately thirty species of *Ledebouria* in Africa and Asia, fifteen of which are common in South Africa [2]. *Ledebouria* was formerly included in the genus *Scilla,* but has now been independently classified [3]. The genera *Scilla*, *Schizocarphus* and *Ledebouria*, which were previously thought to be very closely related, were compared on the basis of a number of generic and botanical criteria including morphology, cytology, anatomy and chromatography [4]. It was concluded that, on the basis of physical characteristics, there is a much closer affinity between *Scilla* and *Schizocarphus* than between either of these groups and *Ledebouria* [4]. Jessop further suggested that *Ledebouria* be accepted as generically distinct to the extent that it possibly does not even belong to a particularly closely allied group within the Scilleae [4]. Due to the earlier classification system, members of the *Ledebouria* genus have many synonyms. Consequently, *Ledebouria ovatifolia* has previously been classified as: *Scilla ovatifolia* (Bak.), *Scilla lanceaefolia* (Jacq), *Scilla guttata* C.A. Sm., *Scilla climatocarpha* C.A. Sm., *Scilla cicatricosa* C.A Sm., *Scilla albomarginata* Van der Merwe, *Scilla elevans* Van der Merwe and *Scilla collina* Hutch. [3,4,5].

Lebebouria ovatifolia is widely distributed in South Africa throughout Mpumalanga, Swaziland, Free State, Lesotho, Kwazulu-Natal and the Transkei with a few scattered localities in the Eastern and the Northern Cape [5]. The bulbs grow in grassland or woodlands between trees and bush clumps. The bulbs themselves have very characteristic fleshy scales that produce copious adhesive, elastic threads when separated [5]. The leaves are occasionally grazed but the threads in the bulbs generally prevent them from being eaten by porcupines and Chacma baboons [5].

Plants of the *Ledebouria* genus are extensively used by traditional healers in Kwazulu-Natal, particularly in enemas and as purgatives for both humans and cattle [3]. The bulbs of *L. ovatifolia* are extensively used in enemas for the treatment of gastro-enteritis and in purgatives for adults [3]. The bulbs are also used to facilitate the relief of backache and as a treatment for influenza. However, the most unusual usage of this species must surely be its use to promote the growth of breasts in pubescent girls [3]. Other members of *Ledebouria* are also used by traditional healers [3].

L. revoluta is used by the Sotho people as a charm to drive away lightning and for the treatment of lumbago. *L. cooperi* is also used by the Sotho tribe as a soothing medicine for women in the fourth month of pregnancy and to inebriate boys during circumcision rites [3]. It is interesting to note that certain species of *Scilla* and *Eucomis* including *S. natalensis*, *S. nervosa*, *E. autumnalis*, *E. bicolor* and *E. comosa* are also reported to be extensively used by traditional healers as purgatives and in enemas [3], since chemical investigations (including this work) have shown that these three genera, *Scilla, Eucomis* and *Ledebouria*, are a source of homoisoflavonoids [6].

L. ovatifolia (syn. *S. ovatifolia* Bak.) is considered to be quite toxic and 1.5 kg of the bulb consumed by a sheep proved to be a fatal dose within a few hours [7]. However, two rabbits recovered after receiving 10 gm and 20 gm respectively of the bulb, leaf and flower [7]. Toxicity tests conducted on this species at Onderstepoort Research Station indicated that this species is indeed toxic to sheep and rabbits at a level of 2 g per kg of live mass [5].

2.2 RESULTS AND DISCUSSION

The methanol extract of the bulbs of *Ledebouria ovatifolia* Bak. Jessop yielded two compounds, 5,7-dihydroxy-3-(4'-hydroxybenzyl)-4-chromanone (**I**) and 2',6'-dimethoxy-4,4'-dihydroxy, chalcone (**II**).

2.2.1 THE STRUCTURAL ELUCIDATION OF COMPOUND I

The first compound was isolated as a pale yellow crystalline material and was identified as 5,7-dihydroxy-3-(4'-hydroxybenzyl)-4-chromanone.

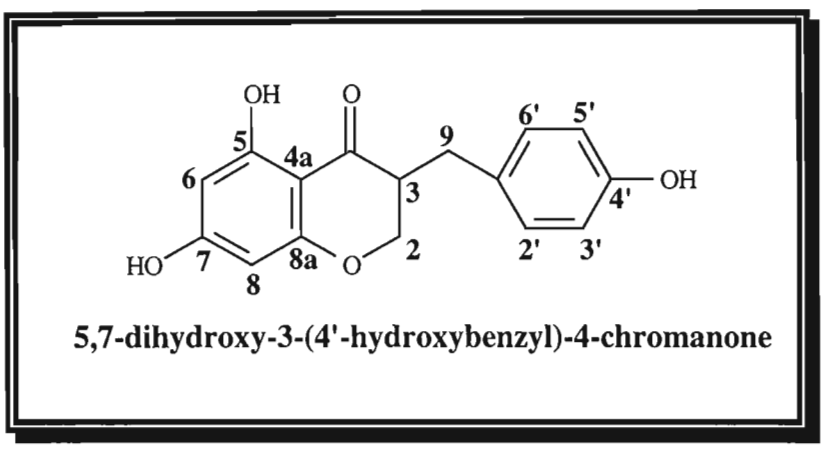


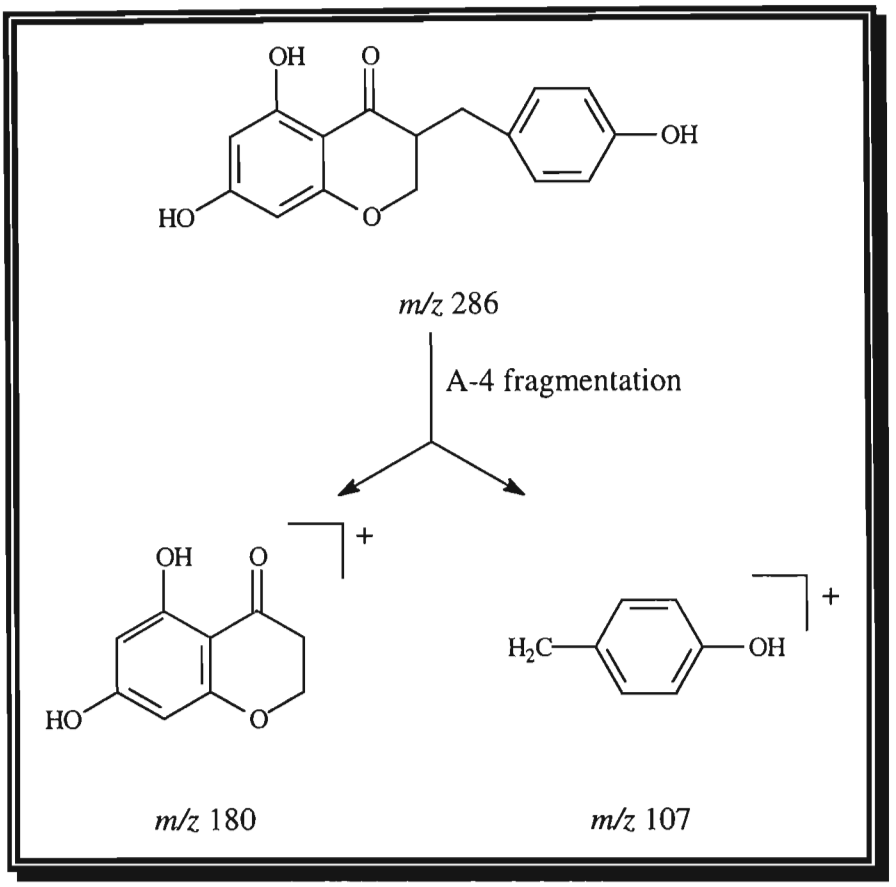
Figure 2.1. Compound I: 5,7-dihydroxy-3-(4'-hydroxybenzyl)-4-chromanone

This compound belongs to a class of naturally occurring compounds known as the homoisoflavonoids, and is of the 3-benzyl-4-chromanone type.

Inspection of the high resolution mass spectrum revealed a parent ion peak at, *m/z* 286.0830, which corresponded to a molecular formula of $C_{16}H_{14}O_5$, and was consistent with the proposed homoisoflavonoid structure. Intense signals were observed at *m/z* 180 and *m/z* 107 in the mass spectrum (**Spectrum 7, p 166**). This A-4 fragmentation pattern is common for saturated homoisoflavonoids of the 3-benzyl-4-chromanone type (**Scheme 2.1.**) [6]. The chromanone fragment at *m/z* 180 suggested that the A ring had two hydroxy substituents and the hydroxytropylium fragment at *m/z* 107 similarly suggested that the B ring had one hydroxy

substituent. The intense signal at *m/z* 153 is typically formed *via* the retro-Diels-Alder cleavage of the chromanone moeity [6].

Scheme 2.1 Mass spectrometric fragmentation of compound I.



The infra-red data (**Spectrum 5, p 164**) supported the postulated homoisoflavonoid structure for compound I, and corresponded to the literature data for 5,7-dihydroxy-3-(4'-hydroxybenzyl)-4-chromanone [10]. Peaks were observed at 3423 cm^{-1} (O-H stretching), 2926 cm^{-1} (aliphatic C-H stretching), 1637 cm^{-1} (C=O stretching), and 1513 cm^{-1} (aromatic C=C stretching).

The aromatic region of the ^1H NMR spectrum (**Spectrum 1, p 160**) confirmed the substitution patterns indicated by the mass spectrum. The aromatic AA'BB' system (δ7.09 and δ6.77, *J*=8.5Hz) confirmed the substitution pattern of the B ring and was indicative of *para* substitution and thus further confirmed the assignment of the two doublets, integrating to two protons each, as H-2',6' and H-3',5' respectively with an oxygenated substituent at C-4'. The remaining aromatic signals in

the spectrum were two singlet resonances (integrating to one proton each) at δ5.90 and δ5.88, which are typical of two non-equivalent aromatic protons. This confirmed the substitution pattern of the A ring, that had been suggested by the mass spectrum, and further implied that there were substituents at the C-5 and C-7 positions. Since no methoxy or acetate signals were observed, the implication was that the oxygenated substituents at C-5, C-7 and C-4' were, in fact, hydroxy groups. Finally, the five remaining alkyl resonances observed are characteristic for the 2H-2, H-3 and 2H-9 protons and were the primary evidence that suggested that compound I was indeed a member of the homoisoflavonoids [6]. The pair of double doublets at δ4.11 (J=7.1, 11.4 Hz) and δ4.28 (J=4.3, 11.5 Hz) are typical of two protons of the ABX system expected for each of the two non-equivalent H-2 protons and are shifted downfield due to their proximity to the oxygen at position one. A resonance which was ascribed to H-3 was observed, as expected, as a complex multiplet at δ2.81 and the two remaining double doublets at δ2.68 (J=10.3, 13.6 Hz) and δ3.13 (J=4.4, 13.7 Hz) were ascribed to the two non-equivalent H-9 protons.

This assignment was confirmed by the COSY spectrum (**Spectrum 2, p 161**) which clearly showed the coupling between the 2H-2 ABX system and the H-3 multiplet and the further coupling of this multiplet with the 2H-9 ABX system. The ^{1}H NMR data (**Table 2.1**.) was consistent with that observed in the literature [8,9] for 5,7-dihydroxy-3-(4'-hydroxybenzyl)-4-chromanone and confirmed the identification of compound I.

Table 2.1 ^{1}H NMR data for compound I and literature data for 5,7-dihydroxy-3-(4'-hydroxybenzyl)-4-chromanaone [8,9].

	^{1}H NMR data for compound I	^{1}H NMR literature data [8] for compound I	^{1}H NMR literature [9] data for compound I
Proton No.	#Shift / δppm	*Shift /δppm	**Shift /δppm
H-2	4.11 dd (7.1, 11.5)	4.08 dd (7.3, 11.4)	4.10 m
H-2	4.28 dd (4.3, 11.5)	4.25 dd (4.4, 11.4)	4.28 m
H-3	2.81 m	2.79 m	2.97 m
H-6	5.90 s	5.87 s	5.87 brs
H-8	5.88 s	5.85 s	5.87 brs
H-9	2.68 dd (10.3, 13.6)	2.65 dd (10.4, 13.4)	2.63 m
H-9	3.13 dd (4.4, 13.7)	3.04 dd (3.8, 13.4)	3.05 m
H-2',6'	7.09 d (8.5)	7.06 d (8.2)	7.07 d (7.9)
H-3',5'	6.77 d (8.5)	6.74 d (8.2)	6.74 d (7.9)

^{1}H NMR spectra measured in CD_3OD, 300 MHz.

* ^{1}H NMR spectra measured in CD_3OD, 400 MHz.

** ^{1}H NMR spectra measured in DMSO-d_6, 270 MHz.

The ^{13}C NMR spectrum (**Spectrum 3, p 162**) revealed eleven resonances in the downfield region of δ>90ppm and three in the aliphatic region. Since the sixteen resonances expected for compounds of the homoisoflavonoid type were not observed, the suggestion was that symmetry was involved. Indeed, this was confirmed by the two particularly intense methine resonances which were assigned to the aromatic carbons of the B ring, C-2'and C-6' and C-3' and C-5' respectively, which are identical and thus superimposed on the spectrum. The remaining aromatic methine resonances were assigned to C-6 and C-8.

The C-6 and C-8 chemical shifts can be used as a diagnostic feature for the identification of the C-7 oxygenated substituent [8]. Consequently, the C-6 shift at δ97.09 and the C-8 shift at δ95.82 suggest that C-7 carries a hydroxy group [8]. The six quaternary carbons were assigned to the five aromatic carbons C-1', C-4a, C-5, C-8 and C-8a and to the characteristic carbonyl C-4 resonance

observed at δ199.48. The two aliphatic methylene resonances were assigned to C-2 and C-9 and the remaining aliphatic methine carbon to C-3. This data compared favourably with the literature data [8,9] for 5,7-dihydroxy-3-(4'-hydroxybenzyl)-4-chromanone and thus confirmed the identification of this compound (**Table 2.2**).

5,7-Dihydroxy-3-(4'-hydroxybenzyl)-4-chromanone is a relatively common homoisoflavonoid and has previously been isolated from a number of sources such as *Muscari comosum* [9], *Ophiopogon jaburan* [10], *Muscari armeniacum* [11], *Muscari neglectum* [12] and *Chionodoxa luciliae* [13] to name but a few. Nevertheless, this compound has not been reported previously from *Ledebouria ovatifolia.*

Table 2.2 ^{13}C NMR data for compound I and literature data for 5,7-dihydroxy-3-(4'-hydroxybenzyl)-4-chromanone [8,9].

	^{13}C NMR data for compound I	^{13}C NMR literature data [8] for compound I	^{13}C NMR literature data [9] for compound I
Carbon No.	#Shift /δppm	*Shift /δppm	**Shift /δppm
C-2	70.18 t	70.1 t	68.8 t
C-3	48.11 d	48.2 d	45.5 d
C-4	199.48 s	199.4 s	197.7 s
C-4a	102.5 s	102.8 s	101.0 s
C-5	165.81 s	165.8 s	163.6 s
C-6	97.09 d	97.1 d	95.9 d
C-7	168.23 s	168.2 s	166.7 s
C-8	95.82 d	95.9 d	94.7 d
C-8a	164.74 s	164.7 s	162.6 s
C-9	33.04 t	32.9 t	31.2 t
C-1'	130.19 s	130.2 s	128.0 s
C-2'	131.15 d	131.2 d	129.8 d
C-3'	116.41 d	116.4 d	115.1 d
C-4'	157.23 s	157.2 s	155.5 s
C-5'	116.41 d	116.4 d	115.1 d
C-6'	131.15 d	131.2 d	129.8 d

^{13}C NMR spectrum measured in CD_3OD, 75 MHz.

* ^{13}C NMR spectrum measured in CD_3OD, 100 MHz.

** ^{13}C NMR spectrum measured in DMSO-d_6, 67.88 MHz.

2.2.2 THE STRUCTURAL ELUCIDATION OF COMPOUND II

The second compound which was isolated as a bright yellow crystalline material was identified as 2', 6'-dimethoxy-4, 4'-dihydroxychalcone.

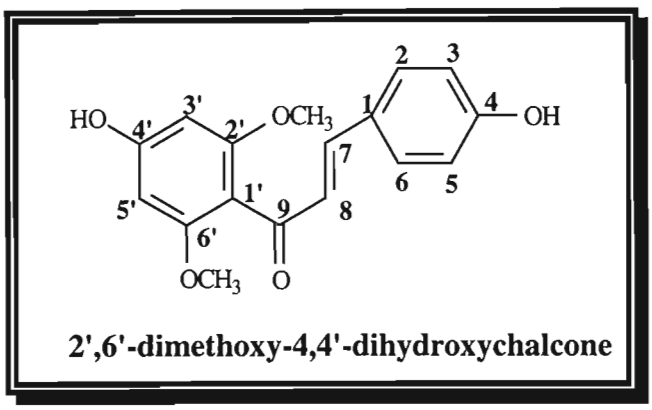
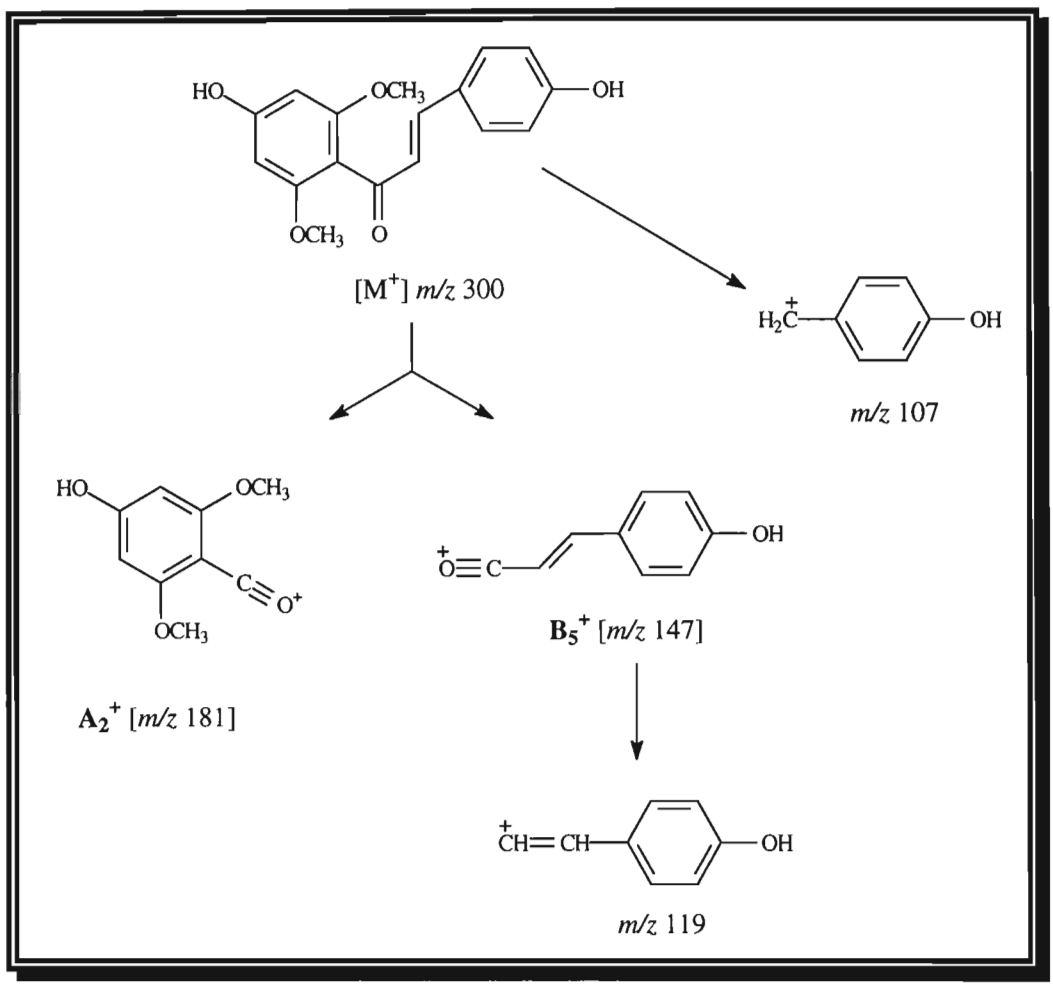


Figure 2.2 Compound II: 2', 6'-dimethoxy-4, 4'-dihydroxychalcone

The mass spectrometric data was in agreement with a substituted chalcone structure [14]. Analysis of the mass spectrum revealed a parent ion at *m/z* 300.1011, which corresponded to a molecular formula of $C_{17}H_6O_5$. The A_2^+ and B_5^+ fragments expected for a substituted chalcone [14] were indeed observed at *m/z* 181 and *m/z* 147 respectively (**Spectrum 14, p 173**). The intense A_2^+ (*m/z* 181) signal indicated that the A ring had a hydroxy and two methoxy substituents (**Scheme 2.2**). Similarly, the substitution pattern of the B ring could be deduced from the less intense B_5^+ fragment at *m/z* 147. The indication was that the B ring had one hydroxy substituent (**Scheme 2.2**). The substitution pattern of the B ring was further confirmed by the presence of the B_5^+ derived ethylene phenol fragment at *m/z* 119 and the hydroxytropylium fragment at *m/z* 107 (**Scheme 2.2**).

The infra-red data for compound II (**Spectrum 12, p 171**) was consistent with the proposed chalcone structure. Peaks were observed at 3421 cm^{-1} (O-H stretching), 2939 cm^{-1} (aliphatic C-H stretching), 1597 cm^{-1} (α-β unsaturated C=O stretching) and 1510 cm^{-1} (aromatic C=C stretching).

Scheme 2.2 Mass spectrometric fragmentation of compound II



The ^{1}H NMR data (**Spectrum 8, p 167**) revealed two doublets at δ7.46 and δ6.83 (J=8.7 Hz), which were indicative of *para*-substitution. The *para*-substitution confirmed the substitution pattern inferred by the mass spectrum, and further suggested that the hydroxy substituent was located at C-4. The A-ring protons in the ^{1}H NMR spectrum were observed as a two proton singlet δ6.19, which confirmed the substitution pattern of the A-ring. In addition, the singlet also indicated the presence of a plane of symmetry within the molecule. The aromatic singlet was thus assigned to H-3' and H-5' with the assumption that the C-2' and C-6' substituents were identical. The assignment of this singlet was confirmed by reference to the NMR data for similar compounds where a plane of symmetry is observed [15,16]. A six proton singlet was observed at δ3.76. This chemical shift is typical of aromatic methoxy substituents and the suggestion was that two identical methoxy signals

were superimposed upon each other. Consequently, the two methoxy substituents were assigned to C-2' and C-6' and the hydroxy substituent at C-4'.

A NOE NMR experiment (**Spectrum 9, p 168**) was conducted to confirm the position of the two methoxy groups. On irradiation of the singlet at δ3.76, a positive NOE effect for the two proton singlet at δ6.19 was seen confirming the placement of the methoxy groups at the C-2' and C-6' positions. The two doublets at δ7.29 and δ6.81, with a large coupling constant of 15.94 Hz, are characteristic of the trans α and β olefinic protons in the chalcone structure and were thus assigned to H-8 and H-7 respectively. The downfield resonance (δ7.29) was ascribed to H-7 due to its deshielded position directly adjacent to the C-9 carbonyl.

The ^{1}H NMR data compared relatively favourably to the literature data for 2', 6'-dimethoxy-4, 4'-dihydroxychalcone [17]. However, the literature NMR data failed to report which deuterated solvent was used and thus an accurate comparison could not be made.

Table 2.3 ^{1}H NMR data for compound I and literature data for 2', 6'-dimethoxy-4, 4'-dihydroxychalcone [17], 4, 2', 6'-trihydroxy-4'methoxychalcone (1) [15] and 2', 4', 6'-trihydroxy-4-methoxychalcone (2) [16].

	^{1}H NMR data for compound II	^{1}H NMR literature data [17] for compound II	^{1}H NMR literature data [15] for chalcone 1	^{1}H NMR literature data [16] for chalcone 2
Proton No.	**#Shift/ δppm**	***Shift/ δppm**	****Shift/ δppm**	*****Shift/ δppm**
H-2,6	7.46 d (8.7)	7.62 d (9)	7.56 d (9)	7.63 d (9)
H-3,5	6.83 d (8.7)	6.93 d (9)	6.90 d (9)	6.97 d (9)
H-5',3'	**6.19 s**	**6.09 d (2)** **6.13 d (2)**	**6.00 s**	**5.96 s**
H-7	7.29 d (16)	7.90 d (16)	8.10 d (16)	8.15 d (15)
H-8	6.81 d (16)	7.70 d (16)	7.76 d (16)	7.75 d (15)
2 OCH_3	3.76 s	3.88 s 4.01 s	3.80 s	3.87 s

^{1}H NMR spectra measured in CD_3OD, 300 MHz.

* Solvent not known.

** ^{1}H NMR spectra measured in aetone-d_6, 400 MHz.

*** ^{1}H NMR spectra measured in acetone-d_6, 90 MHz.

The ^{13}C NMR data (**Spectrum 10, p 169**) was consistent with the proposed structure for compound II. Twelve of the expected seventeen resonances were observed, but this was to be expected due to the symmetry element within the molecule. Only one aliphatic resonance was observed. This methyl signal was assigned to the protons of the two identical methoxy substituents, which were superimposed in the spectrum. The downfield region revealed eleven resonances. Of the five methine resonances observed, three were particularly intense. These signals were assigned to the identical aromatic carbons C-3' and C-5', C-2 and C-6 and C-3 and C-5. The remaining methine signals were assigned to the olefinic carbons C-7 and C-8. Five quaternary signals were present in the spectrum, one of which was more intense than the rest. Once again, the symmetry element was used to account for this phenomenon and this signal was ascribed to the identical aromatic carbons

of the B ring, C-2' and C-6'. Finally, the remaining resonances were assigned to the aromatic carbons, C-1', C-4', C-1and C-4 and the carbonyl carbon C-9.

Table 2.4 ^{13}C NMR data for compound II.

^{13}C NMR data for compound II	
Carbon No.	**#Shift/δppm**
C-1	127.49 s
C-2	131.55 d
C-3	116.93 d
C-4	161.55* s
C-5	116.94 d
C-6	131.55 d
C-7	127.00 d
C-8	147.33 d
C-9	197.86 s
C-1'	110.15 s
C-2'	160.32* s
C-3'	92.98 d
C-4'	162.17* s
C-5'	92.98 d
C-6'	160.32* s
2(OCH_3)	56.19 q

^{13}C NMR spectrum measured in CD_3OD, 300 MHz.

* assignments may be interchanged.

2.3 FOREWORD TO EXPERIMENTAL SECTIONS

Nuclear Magnetic Resonance Spectroscopy (NMR).

NMR spectrometry was recorded using a 300 MHz Varian Gemini spectrometer and a Varian Unity Inova 400 MHz spectrometer. The spectra were obtained using the solvents deuterated methanol or deuterated chloroform.

Infra Red Spectroscopy (IR).

Infra red spectra were recorded using a Nicolet Impact 400 D spectrometer. In all cases KBr discs were used, and the spectrometer was calibrated against air.

Ultraviolet Absorption Spectrometry (UV).

A Varian DMS 300 UV-visible spectrometer was used to obtain the UV spectra. Methanol was used as a solvent in all cases with the exception of compound IV where $CHCl_3$ was used.

High Resolution Mass Spectroscopy.

G.C/M.S spectra were recorded using a Finnigan 1020 GC/MS spectrometer using both injection and solid probe methods. High resolution mass spectra were recorded on a Kratos 9/50 HRMS instrument.

Melting points.

The melting points of all the crystalline compounds isolated in this work were recorded on a Kofler micro-hot stage melting point apparatus and are uncorrected.

Column Chromatography.

Different sized columns ranging from 1-3 cm in diameter were used in the isolation procedure. Merck 9385 silica gel was used as the solid phase and elution was allowed to proceed by gravity. Solvents used in the chromatography process included, hexane, dichloromethane, ethyl acetate and methanol.

Thin Layer Chromatography (TLC).

TLC was used to monitor the column chromatographic process using silica gel (0.2 mm thick) on aluminium backed plates (Merck Art.5554) which contained a fluorescent indicator (F254). The plates were developed with anisaldehyde spray reagent consisting of anisaldehyde: conc. H_2SO_4: methanol, in a ratio of 1:2:97, followed by heating.

Acetylations.

Compounds III, IV and V were acetylated. Approximately 20 mg of each compound was placed in a round bottomed flask with pyridine (1 cm^3) and acetic anhydride (1 cm^3). The flasks were left at room temperature for approximately 48 hrs. Thereafter, the excess acetic anhydride was removed by the addition of methanol (3 x 10 ml) after which the pyridine was removed by the addition of toluene (3 x 10 ml) and azeotropic distillation. The final traces of toluene were removed by further addition of methanol (3 x 190 ml).

Circular dichroism.

All the CD spectra were recorded using a Jasco J700 spectro-polarimeter. The concentration of all the samples was 1 mg/ml.

2.4 EXPERIMENTAL

Fresh *Ledebouria ovatifolia* (Bak.) Jessop bulbs (2.3kg) were purchased from the Warwick Triangle market in Durban, Kwazulu-Natal and a specimen voucher retained in the Natal Herbarium (*N.Crouch 761*). The bulbs were macerated and extracted, with continuous agitation, for approximately 65 hrs in methanol. Chromatographic separation was employed to afford the isolation of the compounds using a solvent system methylene chloride: ethyl acetate: hexane (7:4:2). The chromatographic process yielded two compounds, 5,7-dihydroxy-3-(4'-hydroxybenzyl)-4-chromanone (**Compound I**) as a pale yellow crystalline material, and 2',6'-dimethoxy-4,4'-methoxychalcone (**Compound II**) as a bright yellow crystalline material.

2.4.1 PHYSICAL DATA FOR COMPOUND I

Name: 5, 7-dihydroxy-3-(4'-hydroxybenzyl)-4-chromanone

Yield: 20 mg

Melting point: 95°C from MeOH (lit. 103-104°C from C_6H_6-EtOH [9])

Mass: [M^+] at *m/z* 286.0830, $C_{16}H_{14}O_5$ requires 286.0841.
EIMS: *m/z* 286 (97%), *m/z* 180 (84%), *m/z* 107 (100%).

Optical rotation: $[\alpha]_D$ = -25° (*c*, 0.004 gml^{-1} in MeOH), (lit. $[\alpha]_D$ = -34°, *c*, 0.4 in MeOH [9])

Infra-red: ν_{max} (KBr), 3423, 2926, 1637, 1513 cm^{-1}

UV: λ_{max} (MeOH), (log ε), 288 (4.22) nm.

^{1}H NMR: δ_H (ppm) CD_3OD

2.68 (1H, dd, *J*=13.6, 10.3 Hz, H-9β), 2.81 (1H, m, H-3), 3.13 (1H, dd, *J*=4.4, 13.7 Hz, H-9α), 4.11 (1H, dd, *J*=7.1, 11.5 Hz, H-2β), 4.28 (1H, dd, *J*=4.3, 11.5 Hz, H-2α), 5.88 (1H, s, H-8), 5.90 (1H, s, H-6), 6.77 (2H, d, *J*=8.54 Hz, H-3',5'), 7.09 (2H, d, *J*= 8.49 Hz, H-2',6').

^{13}C NMR: δ_C (ppm) CD_3OD

33.04 (t, C-9), 48.11 (d, C-3), 70.18 (t, C-2), 95.82 (d, C-8), 97.09 (d, C-6), 102.51 (s, C-4a), 116.41 (d, C-3', 5'), 130.19 (s, C-1'), 131.15 (d, C-2',6'), 157.23 (s, C-4'), 164.74 (s, C-8a), 165.81 (s, C-5), 168.23 (s, C-7), 199.48 (s, C-4).

2.4.2 PHYSICAL DATA FOR COMPOUND II

Name: 2', 6'-dimethoxy-4, 4'-methoxychalcone

Yield: 25 mg

Melting point: 215 ^{0}C [lit 192-193°C]

Mass: [M^+] at *m/z* 300.1011, $C_{17}H_{16}O_5$ requires 300.0998.
EIMS: *m/z* 300 (39%), *m/z* 181 (51%), 153 (22%), *m/z* 147 (), *m/z* 119 ().

Infra-red: ν_{max} (KBr), 3421, 2939, 1597, 1510 cm^{-1}.

UV: λ_{max} (MeOH), (log ε), 329 (4.09) nm.

^{1}H NMR: δ_H (ppm), CD_3OD

3.76 (6H, s, O-CH_3), 6.19 (2H, s, H-3',5'), 6.81 (1H, d, *J*=15.94 Hz, H-7), 6.83 (2H, d, *J*=8.67 Hz, H-3,5) 7.29 (1H, d, *J*=15.94 Hz, H-8) 7.46 (2H, d, *J*=8.67 Hz, H-2,6).

^{13}C NMR: δ_C (ppm), CD_3OD

56.19 (q, 2 x OCH_3), 92.98 (d, C-3',5'), 110.15 (s, C-1'), 116.93 (d, C-3,5), 127.00 (d, C-7), 127.49 (s, C-1), 131.55 (d, C-2,6), 147.33 (d, C-8), 160.32 (s, C-2',6'), 161.55 (s,C-4), 162.17 (s, C-4'), 197.86 (s, C-9).

2.5 REFERENCES

1. Du Plessis, N. and Duncan, G.; *"Bulbous Plants of Southern Africa"*, (1989), Tafelberg Publishers, Cape town, 71.
2. Dyer, R.A.; *"The Genera of Southern African Flowering Plants-Volume 2"*, (1976), Department of Agricultural Technical Services, Pretoria, 937.
3. Hutchings, A., Scott, A.H., Lewis, G. and Cunningham, A.B.; *"Zulu Medicinal Plants-An Inventory"*, (1996), University of Natal Press, Pietermaritzburg, 41.
4. Jessop, J.P.; *J. S. Afr. Bot.*, (1970), **36**, 233.
5. Fenter, S.; MSc dissertation, (1993), UNP, Pietermaritzburg, S.A., 204-343.
6. Heller, W. and Tamm, C.; *Forstchr. Chem. Org. Natur.*, (1981), **40**, 105.
7. Watt, J.M. and Breyer-Brandwijk, M.G.; *"The Medicinal and Poisonous Plants of Southern and Eastern Africa-2nd edition"*, (1962), E & S. Livingston Ltd, Edinburgh, 715.
8. Adolfini, M., Lanzetta, R., Laonigro, G., Parrilli, M. and Breitmaier, E.; *Mag. Res. in Chem.*, (1986), **24**, 663.
9. Adinolfi, M., Barone, G., Lanzetta, R., Laonigra, G., Mangoni, L., and Parrilli, M.; *Phytochem.*, (1985), **24**, 624.
10. Watanabe, Y., Sanada, S., Ida, Y. and Shoji, J.; *Chem. Pharm. Bull.*, (1985), **33**, 5358.
11. Adinolfi, M., Corsaro, M.M., Lanzetta, R., Laonigro, G., Mangoni, L. and Parrilli, M.; *Phytochem.*, (1987), **26**, 285.
12. Barone, G., Corsaro, M.M., Lanzetta, R. and Parrilli, M.; *Phytochem.*, (1988), **27**, 921.
13. Corsaro, M.M., Lanzetta, R., Mancino, A. and Parrilli, M.; *Phytochem.*, (1992), **31**, 1395.
14. Mabry, T. and Markham, K.R., in *"The Flavonoids"*, Ed. Harborne, J.B., Mabry, T. and Mabry, H., (1975), Chapman and Hall, London, Chpt 3, 105.
15. Messana, I., Ferrari, F., Alves De Moraes E Souza, M., and Gacs-Baitz, E.; *Phytochem.*, (1990), **29**, 329.
16. McCormick, S., Robson, K., and Bohm, B.; *Phytochem.*, (1985), **24**, 1614.
17. Song-San, S., Watanabe, S. and Saito, T.; *Phytochem.*, (1989), **28**, 1776.

CHAPTER 3

An Introduction to Amaryllidaceae Alkaloids

3.1 AN INTRODUCTION TO THE ALKALOIDS

Alkaloids are secondary metabolites that are isolated from plants belonging to a wide range of families, distributed throughout the world [1]. Due to the significant pharmacological activities and proven medicinal value of a large number of alkaloids, the isolation and chemical investigation of these compounds has received a great deal of attention, and has been closely associated with the development of potential therapeutic agents [1,2,3]. Most alkaloids exhibit some physiological action on the central nervous system, and thus many can be used for the benefit of man in alkaloid containing drugs [1]. Some examples of plant derived alkaloid drugs used in orthodox medicine are described below in **table 3.1** [4].

Table 3.1 Plant derived alkaloid drugs used in orthodox medicine [4].

Drugs	Plant Source	Medicinal Use
Cocaine	*Erythroxylum coca*	Local anaesthetic
Codeine	*Papaver* sp.	Analgesic
Colchicine	Hyacinthaceae	Treatment of gout
Emetine	*Cephaelis ipecacuanha*	Emetic
Ephedrine	*Ephedra* sp.	Bronchodilator
Hyoscine	Solanaceae	Sedative
Hyoscyamine	Solanaceae	Anti-spasmodic
Lobeline	*Lobelia* sp.	Respiratory stimulant
Morphine	*Papaver somniferum*	Narcotic
Papaverine	*Papaver* sp.	Heart disease
Physostigmine	*Physostigma venenosum*	Glaucoma
Quinine	*Cinchona* sp.	Malaria
Quinidine	*Cinchona* sp.	Heart disease
Reserpine	*Rauvolfia* sp.	Tranquilliser
Strychnine	*Strychnos* sp.	Stimulant
Tubocurarine	*Strychnos* sp. *Chondrodendron* sp.	Muscle relaxant
Vinblastine	*Catharanthus roseus*	Leukaemia, Hodgkin's disease
Vincristine	*Catharanthus roseus*	Leukaemia

Unfortunately, some of these drugs such as morphine and cocaine can have addictive properties and can thus, ultimately, be harmful to man [1]. In addition, many of the alkaloids are too toxic for medicinal purposes. For example the fatal dose of nicotine in man is 40 mg and that for strychnine much less [3]. Teratogenic properties have also been observed in some alkaloids resulting in foetuses with defects being born to mothers that have eaten plants containing the alkaloid. The most common foetal defect encountered is skeletal damage and there is evidence that suggests that *spina bifida* can in fact be induced by the alkaloid solanine which becomes concentrated in mothers that consume too many 'green' potatoes during pregnancy [1].

The alkaloids represent a very diverse and heterogeneous group of compounds whose only molecular similarity is the presence of nitrogen [1,2,3]. In addition, as their name indicates, they frequently have a basic nature [2,3,5]. Alkaloids can be sub-divided into three distinct groups according to their molecular structures and biosynthetic pathways. The **true alkaloids** are defined as those compounds, in which nitrogen forms part of a heterocyclic ring system, which are biosynthesised from amino acids. The **pseudoalkaloids** are also characterised by nitrogenous heterocyclic ring systems but these are not biosynthetically derived from amino acids. The third group contains physiologically active amines which are classified as **protoalkaloids** [1,3].

The nomenclature of the alkaloids has not been systematized, which makes classification problematic [1,2]. At present the two most commonly used systems classify alkaloids on the basis of either the plant source from which they were isolated, or the general similarity to a known molecular structure [2]. For example, clivonine is an alkaloid isolated from the plant *Clivia miniata*, whereas, 1-*O*-acetyllycorine is classified according to the similarity this structure has to the commonly isolated alkaloid lycorine.

The function of alkaloids in plants is not clearly defined, but is thought to be linked to the toxicity that is characteristic of many of these compounds [1,2]. Consequently, alkaloids are thought to play a major role in protecting the plant from animal and insect attack [2]. Alkaloid containing plants are usually bitter and thus generally unpalatable to predators. Therefore, these plants have a selective advantage over plants that do not contain alkaloids [1]. Toxic plants containing alkaloids are of great economic importance to farmers as the loss of livestock through the consumption of these plants can result in a loss of vital revenue. Further complicating the issue is the fact that animals moved to a strange location are often unable to differentiate between poisonous and edible plants, and are thus much more vulnerable to poisoning [4]. Unfortunately, an unpleasant and bitter taste is often not always a deterrent and it is well known that cattle can become addicted to buttercups

(*Ranunculus* sp.) and will continue to eat the plants even after recovering from poisoning [4]. Toxic alkaloid containing plants can also be dangerous to man as they can be mistaken for edible plants, particularly by children [4]. For example the deadly nightshade (*Atropa belladona*, Solanaceae), which has attractive fruits, and hemlock (*Conium maculatum*, Apiaceae), which can be mistaken for parsley, can pose a serious threat to humans [4]. The specific action of many alkaloids varies with species and thus the determination of the biological function of these compounds is difficult [1]. Further theories suggest that they may act as stimulators or regulators for plant activities, including growth, metabolism, and reproduction. It has also been postulated that they may even act as detoxifing agents that can render other compounds, which accumulate and cause damage to the plant, inactive [2].

Numerous biosynthetic schemes have been proposed for the biogenesis of many alkaloids, most of which have been based on the idea that alkaloids are derived from simple precursors such as phenylalanine, tryptophan, methionine, acetate units, terpene units, and a few other amino acids [2,5]. However, these theories have only been able to be experimentally tested since the advent of isotopically labelled compounds [1,2].

3.2 THE AMARYLLIDACEAE ALKALOIDS

3.2.1 INTRODUCTION

The plants of the monocotyledonous family, Amaryllidaceae, form a large group of over sixty genera and more than a thousand species [6,7,8,9]. This family is widely distributed with a particularly high density in Southern Africa and smaller centres of diversity in Andean South America and the Mediterranean [6,9]. The amaryllids are common in South Africa and are distributed throughout the three major areas of the country from the savanna in the north, to the tropical environment of the east and the winter rainfall areas in the south west [9]. Of the nine tribes of the Amaryllidaceae, the Amaryllideae and the Haemantheae are the most prominent in South Africa [9]. The Amaryllideae can be further subdivided into two monophyletic sub-tribes: The Crininae (*Boophane*, *Crinum*, *Ammocharis* and *Cybistetes*) and the Amaryllidinae (*Amaryllis*, *Nerine*, *Brunsvigia*, *Crossyne*, *Hessea*, *Strumaria* and *Carpolyza*) [9]. The tribe Haemantheae contains six genera, *Scadoxus*, *Cyrtanthus*, *Haemanthus*, *Clivia*, *Gethyllis* and *Apodolirion* [9]. Plants from the Amaryllidaceae family have been extensively used in traditional medicines and many have pharmacological uses [1,2,3,4,9]. The compounds responsible for most of these effects are a group of isoquinoline alkaloids, which are found exclusively in plants belonging to this family* [9,10]. The Amaryllidaceae family is one of the few monocotyledonous families from which alkaloids have been isolated. The alkaloids isolated from plants belonging to this group are known to cause poisoning in low doses and can cause excessive salivation and diarrhoea. Higher doses of the active compounds can cause CNS depression and large enough doses can, in fact, prove fatal [13]. Although some of these alkaloids are harmful to man, some of the unique Amaryllidaceae alkaloids exhibit anti-tumour activities, and are thus potentially beneficial to man. Anti-viral properties have also been reported for some of the alkaloids, in particular; lycorine and tazettine [13]. Other compounds commonly isolated from this family include chelidonic acid, organic acids, soluble nitrogen compounds and carbohydrates [13].

* There are three Amaryllidaceae alkaloids that have been isolated from plants outside of the Amaryllidaceae family. Lycorine and acetylcaranine have been isolated from *Urginea altissima* (Hyacinthaceae) [11], and the alkaloid crinamine has been isolated from *Dioscorea dregeana* (Discoreaceae) [12]

3.2.2 THE CLASSIFICATION OF THE AMARYLLIDACEAE ALKALOIDS

The alkaloids of this family are considered to be derived from two fragments: an aromatic C_6-C_1 unit and an aliphatic C_6-C_2 moiety [5,10]. The large number of different alkaloids that have been isolated from plants belonging to this family can be attributed to variations in the degree of oxygenation and aromatic substitution of the basic C_{15} alkaloid unit. The alkaloids of this group can be classified into eight distinct types [9,10]: the lycorine, homolycorine, galanthamine, crinane/ 5,10b-ethanophenanthridine, tazettine, montanine/ 5,11b-methanomorphanthridine, narciclasine/ isocarbostyril and the miscellaneous type. The alkaloids in all eight of these groups have a six membered aromatic A ring. The lycorine, homolycorine, 5,10b-ethanophenanthridine, narciclasine and galanthamine type alkaloids also exhibit some degree of similarity in that they have identical points of fusion for the A, B and C rings. The tazettine series also has six membered B and C rings; however, the points of fusion between these rings is significantly different. Alkaloids of the montanine series deviate from the above trend and typically display a seven membered B ring and a six membered C ring. Those alkaloids belonging to the miscellaneous class do not conform to any of the other classes and very little homogeneity is observed.

1. The lycorine type alkaloids.

The lycorine type alkaloids have a five membered heterogenous D ring and in many cases the C and/or D rings contain some degree of unsaturation and oxygenation.

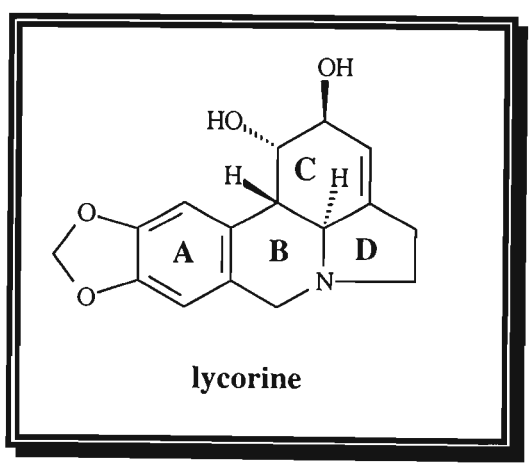


Figure 3.1 An alkaloid of the lycorine type.

2. The homolycorine type alkaloids.

Alkaloids of the homolycorine series are characterised by an oxygen-containing heterogeneous B ring and a five membered nitrogen containing D ring. In addition, the nitrogen is methylated and the C ring may exhibit some degree of unsaturation and oxygenation.

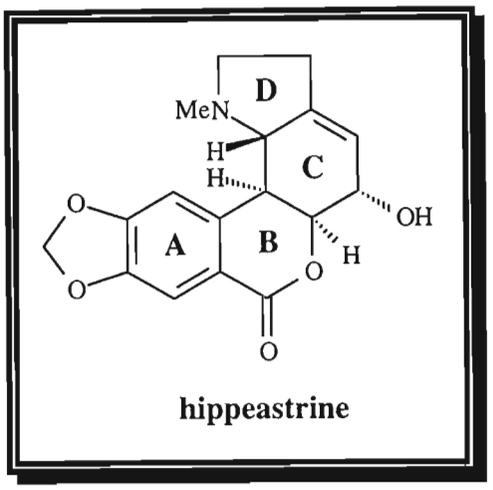


Figure 3.2 An alkaloid of the homolycorine type.

3. The 5,10b-ethanophenanthridine type alkaloids.

Alkaloids belonging to this group exhibit a typical C_{15} crinane skeleton with an ethane bridge between C-10b and the nitrogen in position 5 of the B ring. These crinane alkaloids can be further subdivided into haemanthamine, haemanthidine and crinine types. The haemanthamine and haemanthidine alkaloids have a nitrogen bridge which is characteristically α orientated with respect to the B-C ring juncture. Conversely, crinine alkaloids exhibit a β orientated bridge. Once again, a certain degree of unsaturation and oxygenation is observed in the C ring and oxygenation of the nitrogen bridge itself is also common.

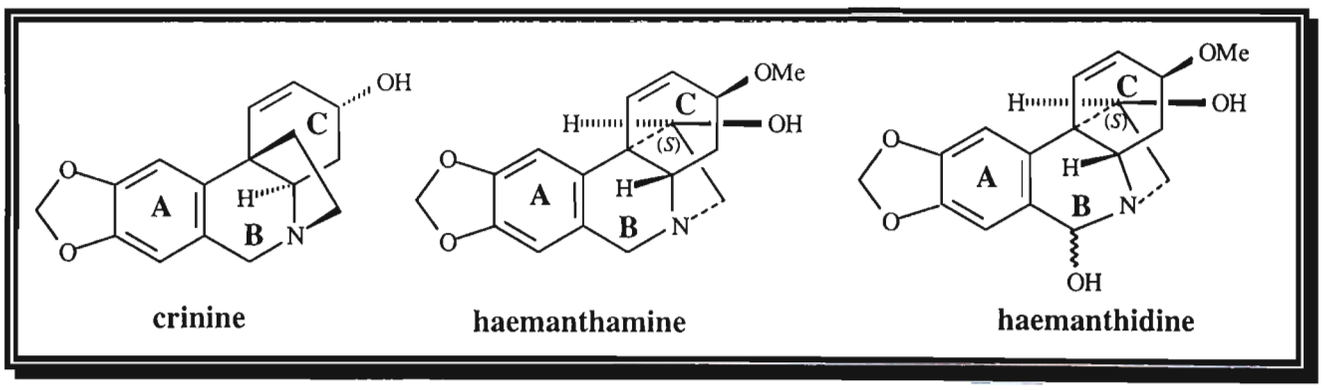
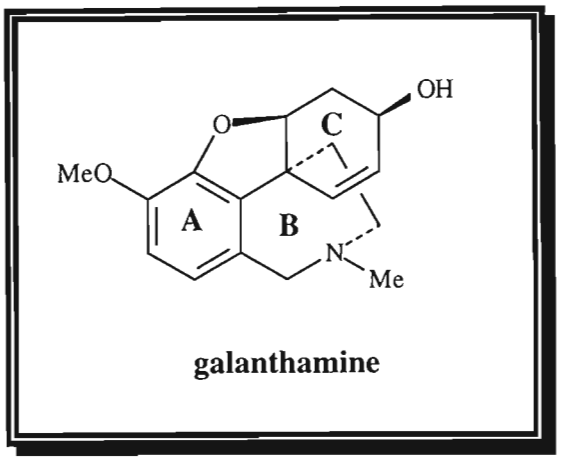


Figure 3.3 Alkaloids of the crinane type.

4. The galanthamine type alkaloids.

The galanthamine type alkaloids exhibit a unique oxygen bridge between the A and C rings in addition to an ethane nitrogen bridge that is α orientated with respect to the B-C ring juncture. Oxygenation and unsaturation of the C ring is also common in this group of alkaloids.

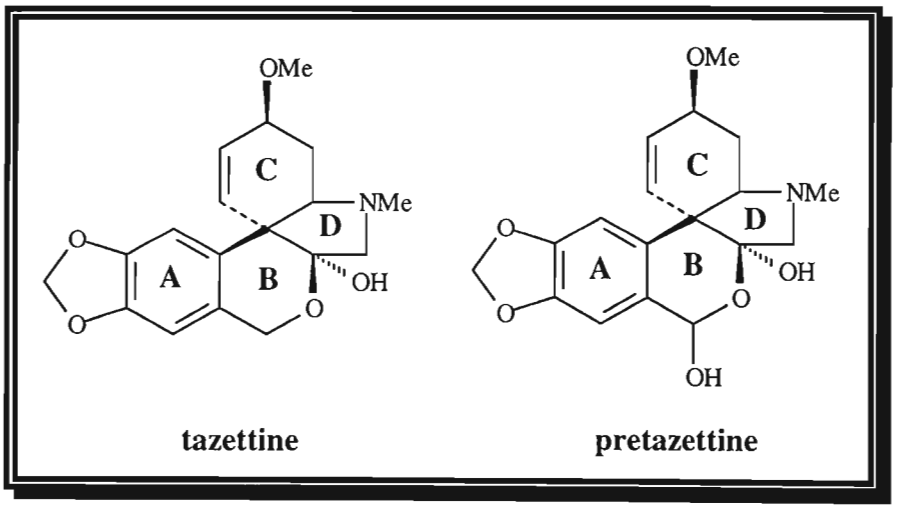


galanthamine

Figure 3.4 An alkaloid of the galanthamine type.

5. The tazettine type alkaloids.

This group can also be subdivided into tazettine and pretazettine types. These two types of alkaloids have an oxygen containing B ring in addition to a unique C and D ring conformation. The D ring is five membered and contains a methylated nitrogen heteroatom.



tazettine **pretazettine**

Figure 3.5 Alkaloids of the tazettine type.

6. The montanine type alkaloids.

As mentioned above, the alkaloids of this type have a typically seven membered nitrogen-containing B ring. The alkaloids are also characterised by an α nitrogen bridge across the B-ring, with the exception of montabuphine which has a β bridge. Oxygenation and unsaturation of the six membered C ring is also common.

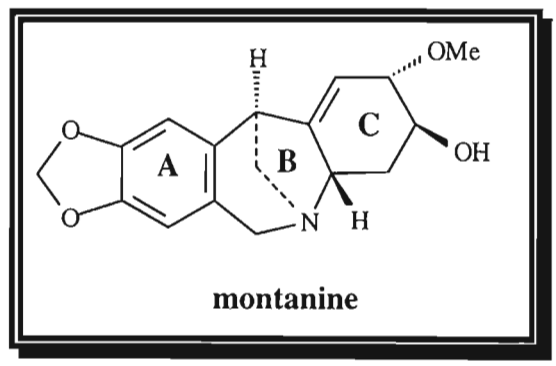


Figure 3.6 An alkaloid of the montanine type.

7. The isocarbostyril/narciclasine type alkaloids.

Alkaloids belonging to this group are characterised by the presence of an amide group in ring B. Unsaturation in the C-ring is also common and compounds of this type generally exhibit extensive oxygenation in the C ring.

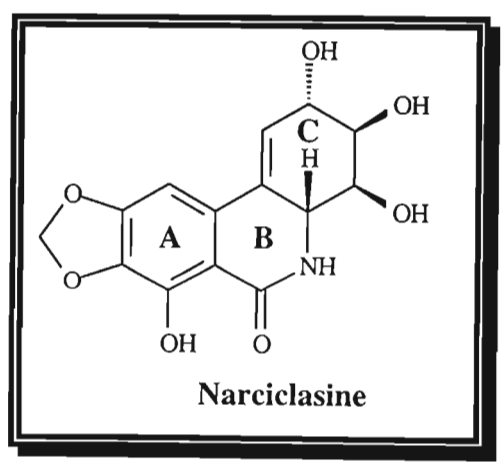


Figure 3.7 An alkaloid of the narciclasine type.

8. The miscellaneous type alkaloids.

This group contains those alkaloids which cannot be classified into any of the other seven groups, and can in many cases be considered as biosynthetic intermediates, rearrangement products or possibly as artifacts. An example of this type is belladine.

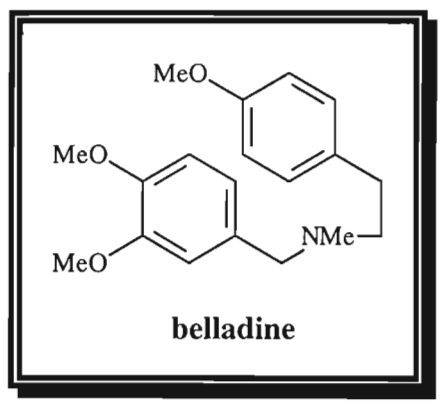


Figure 3.8 An alkaloid of the miscellaneous type.

3.2.3 THE BIOSYNTHESIS OF AMARYLLIDACEAE ALKALOIDS

The Amaryllidaceae family produces a class of true isoquinoline alkaloids that are biosynthetically derived from the amino acids tyrosine and phenylalanine [14,15]. Since only alkaloids of the lycorine **3**, homolycorine **4**, haemanthamine **5**, narciclasine **6** and montanine **7** type (**Figure 3.9**) were isolated in this work, only their biosynthesis will be discussed here.

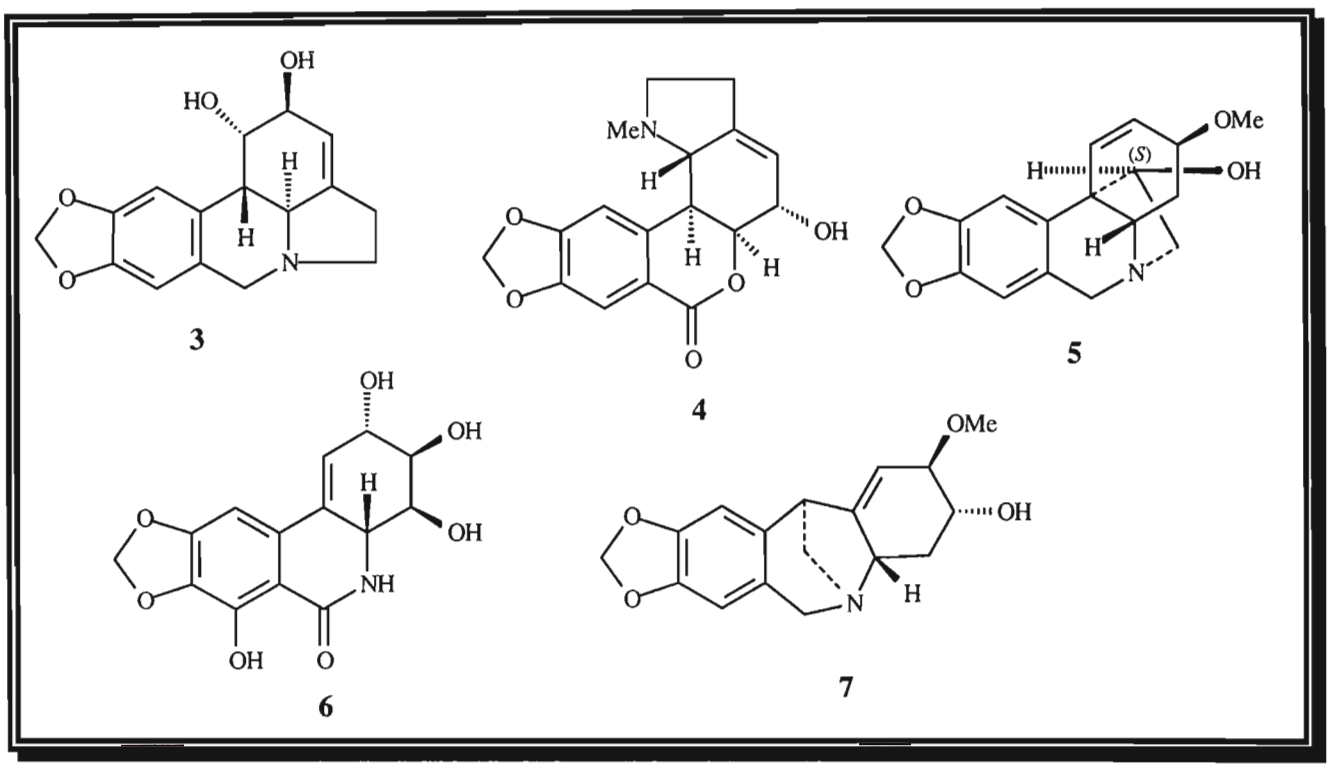


Figure 3.9 Examples of alkaloid types to be discussed in section 3.2.3.

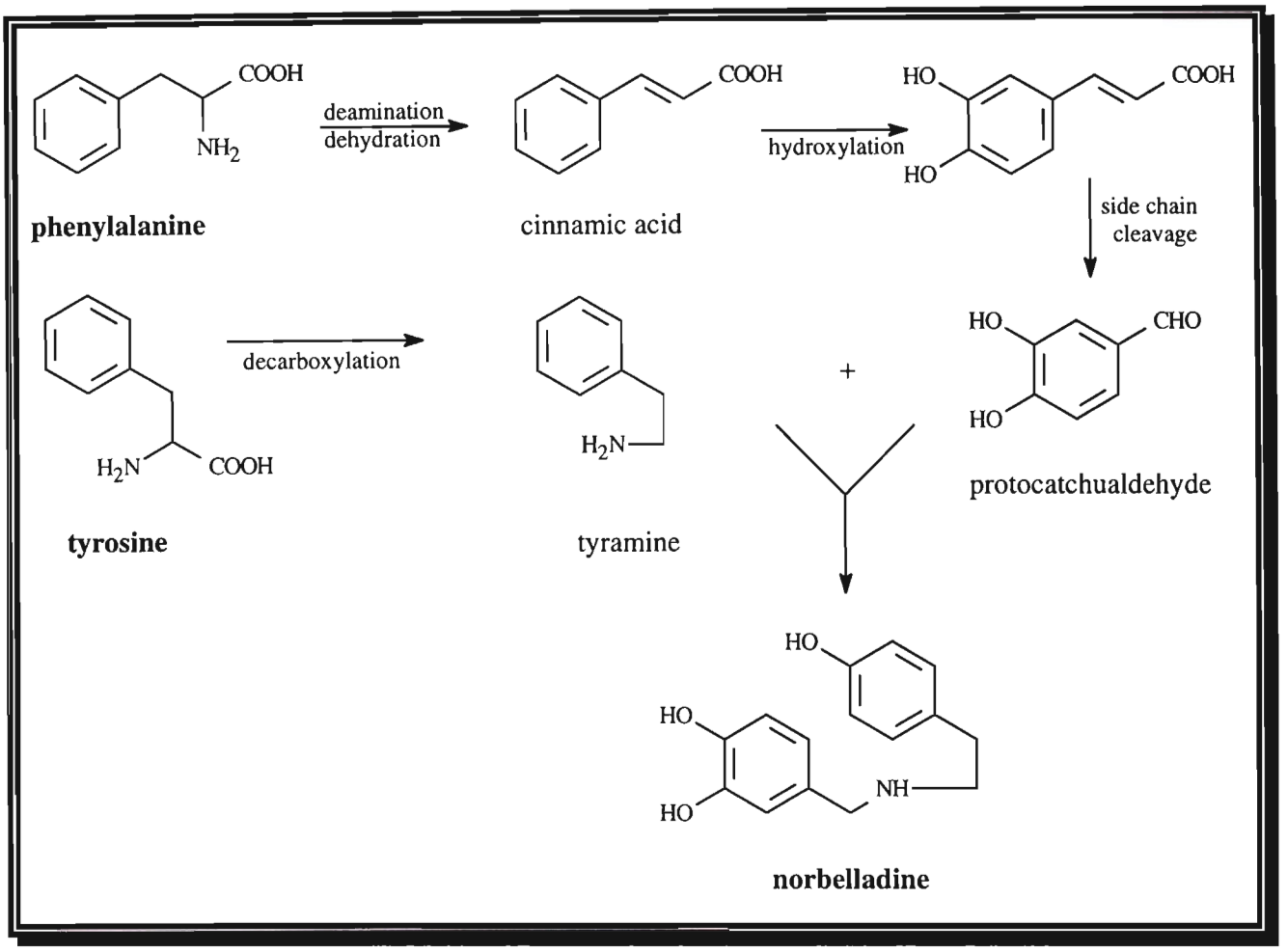
Phenylalanine gives rise to the C_6-C_1 unit from which the aromatic A ring and the benzylic carbon are derived [16,17,18]. Tyrosine, in turn, produces the C_6-C_2 moeity from which the aliphatic C ring and a two carbon side chain are derived [16,17,18]. In addition, the nitrogen atom is also derived from tyrosine [18]. Investigations have revealed that although tyrosine and phenylalanine are closely related, they are not interchangeable in the biosynthesis of Amaryllidaceae alkaloids. Consequently, phenylalanine cannot be incorporated into the C_6-C_2 unit and similarly, tyrosine is not a precursor for the C_6-C_1 fragment [17,18].

In 1957 two researchers, Barton and Cohen, suggested that alkaloids of the Amaryllidaceae were related to a common (at that time hypothetical) intermediate from which the alkaloids of the lycorine, 5,10b-ethanophenanthradine (crinane) and galanthamine type were derived [14]. They also postulated that the mechanism of biosynthesis was *via* intramolecular oxidative coupling of the two

phenolic units from tyrosine and phenylalanine [14]. Indeed, their hypothesis was sustained by tracer experiments that revealed that the ultimate precursor for alkaloids of these types is norbelladine and some of its methylated derivatives [15,16]. In these biosynthetic experiments isotopically labelled precursors are administered to the plant and after a period of growth the alkaloids are isolated. The alkaloids are then analysed to determine whether the radioactively labelled precursor has been incorporated into the structure. These techniques provided the basis for the biosynthetic mechanisms that have been suggested.

The precursor, norbelladine, is formed by the condensation of tyramine and protocatechualdehyde. Tyramine is derived from the decarboxylation of tyrosine *via* a decarboxylase enzyme (**Scheme 3.1**). Protocatechualdehyde is derived from phenylalanine *via* cinnamic acid (**Scheme 3.1**) [15,16,19]. The first step in the production of protocatechualdehyde involves the deamination of the phenylalanine. The enzyme phenylalanine ammonia lyase (PAL), which has been isolated from several Amaryllidaceae taxa, is thought to be responsible for this reaction [18]. The elimination of ammonia afforded by this enzyme is known to occur in an anti-*peri*planar manner with the loss of the β-*pro-S*-hydrogen and thus the product of the deamination reaction is thought to be *trans*-cinnamic acid [18].

Scheme 3.1 The biosynthesis of norbelladine [15].



One of the most significant developments in the biosynthetic studies of these alkaloids was the recognition that various groups of alkaloids can arise by different modes of phenol oxidative coupling of biosynthetic precursors such as norbelladine [16,19]. Hydroxy functional groups *ortho* and/or *para* to sites of new bond formation between aromatic rings are essential for biosynthetic schemes of this type [16]. For example, the tyrosine derived moeity of the alkaloid precursor, *O*-methylnorbelladine, can be twisted in different ways so that various modes of oxidative phenol coupling are possible [14,15,16,19]. Consequently, lycorine **3**, and homolycorine **4** type alkaloids are derived *via ortho-para'** oxidative coupling whereas crinane **5**, narciclasine **6** and montanine **7** type alkaloids are derived *via para-para'* coupling (**Figure 3.10**) [18].

**ortho-para'*- refers to positions of the phenol groups in the tyrosine and phenylalanine derived moieties respectively.

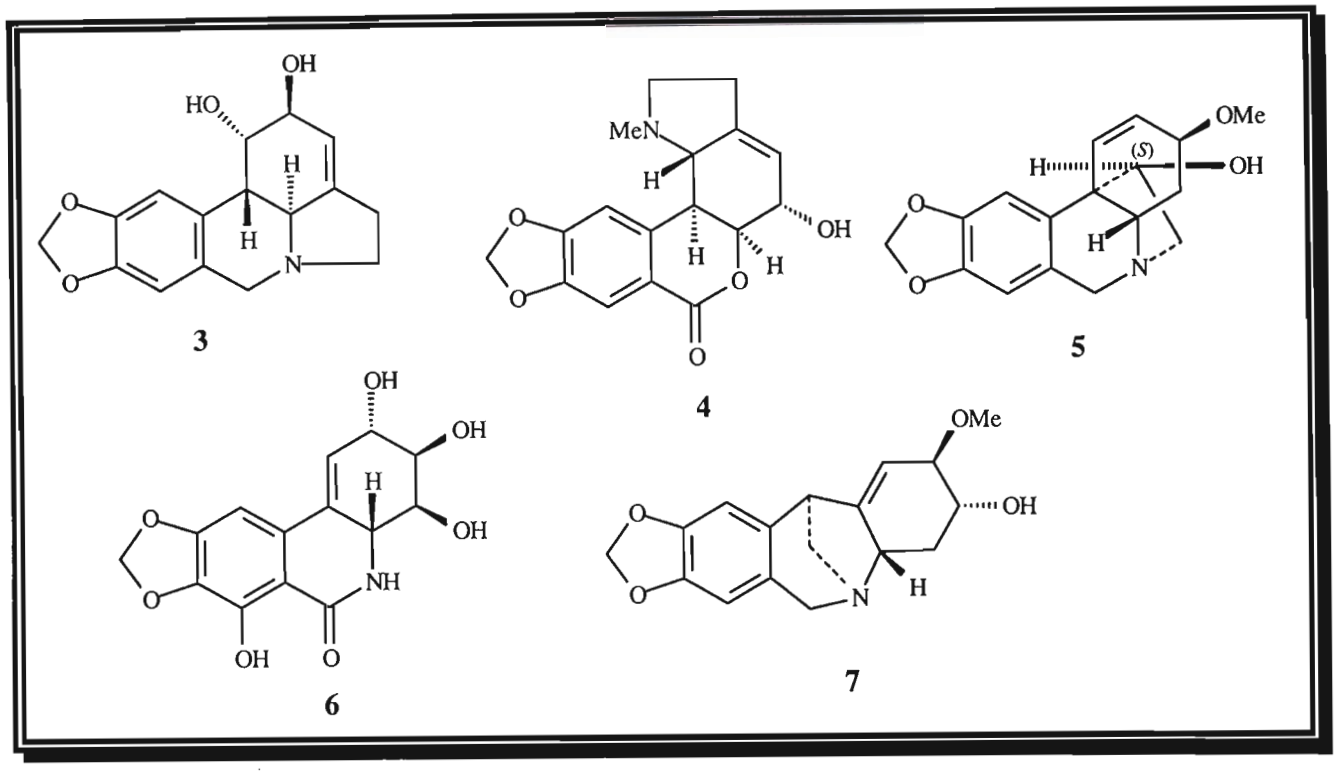


Figure 3.10 Examples of alkaloids derived *via* phenol oxidative coupling.

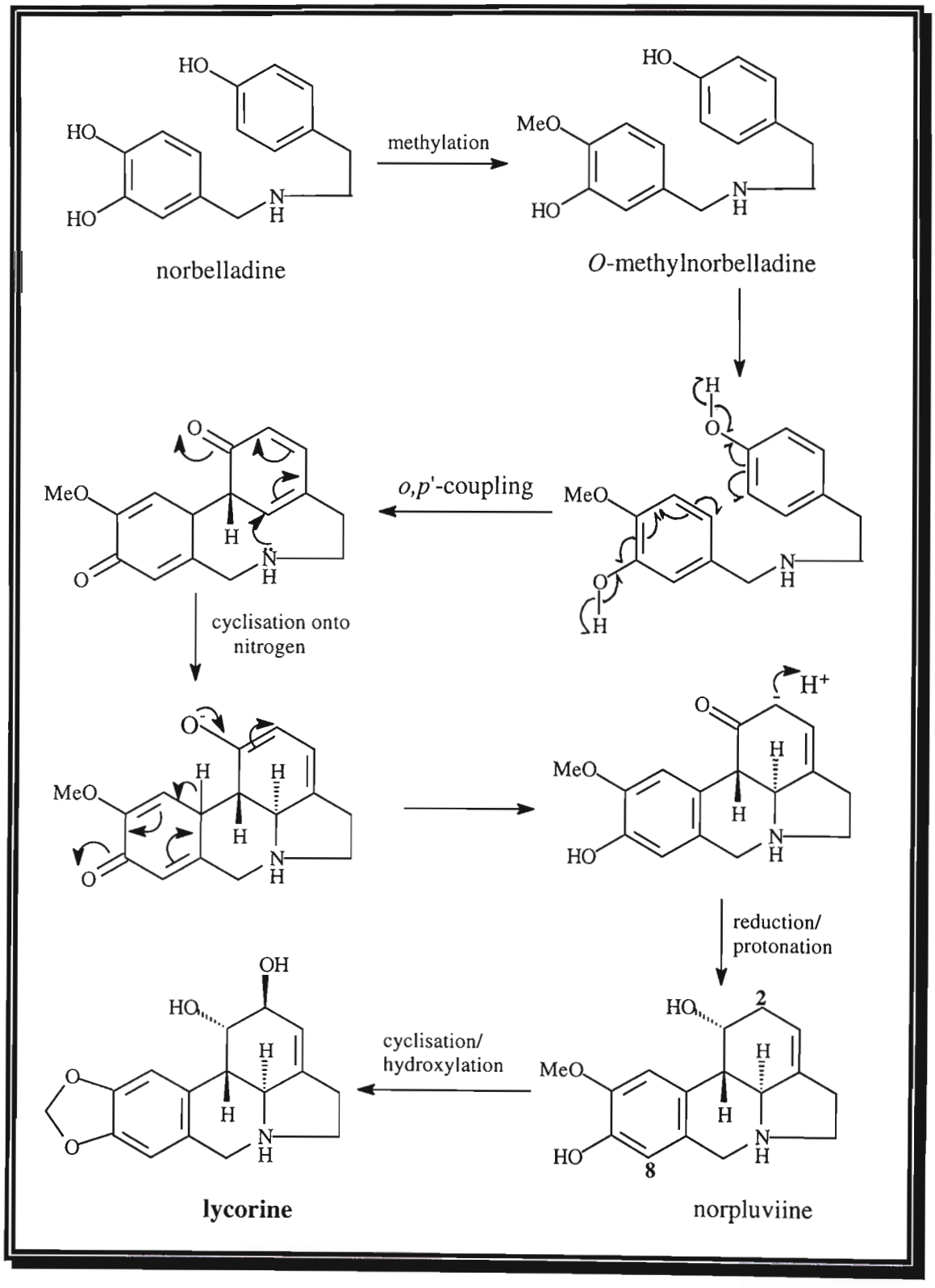
The biosynthetic scheme for lycorine type alkaloids postulates that the ultimate precursor is the molecule norbelladine [14,15,15,19]. This molecule is methylated to produce *O*-methylnorbelladine, followed by *o-p'* phenolic coupling and cyclisation onto nitrogen to produce norpluviine [15,19]. The methylenedioxy group is then formed by direct ring closure between the methoxy and adjacent hydroxy groups in the A ring [14,15,16]. The final step in this biosynthetic scheme is the formation of the hydroxy group at C-2 (**Scheme 3.2**) [19]. In an investigation of the mechanism of oxidative coupling taking place in the conversion of *O*-methylnorbelladine to lycorine, norbelladine was labelled with tritium *ortho* and *para* to the phenol hydroxy groups [20].

When the radioactive norpluviine was isolated, it was established that the tritium was retained at positions 8 and 2 [18,20]. In addition the tritium at position 2 was of the β configuration. Since the tritium label is retained in lycorine the implication is that the hydroxylation at C-2 proceeds with an inversion of configuration [18].

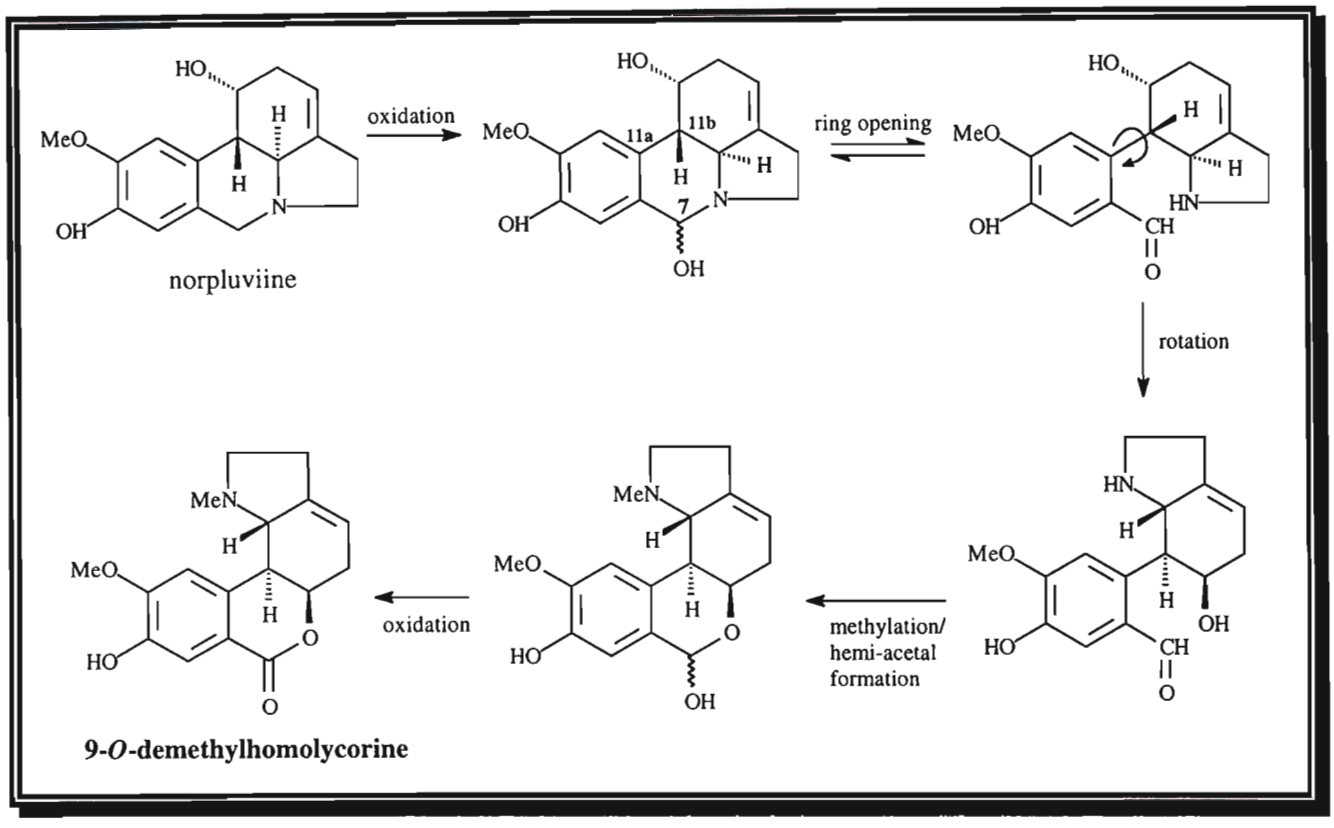
The biosynthesis of homolycorine type akaloids is also thought to proceed *via* *O*-methylnorbelladine and norpluviine, where an *o-p'* oxidative coupling is also implicated [18]. Norpluviine undergoes benzylic oxidation at position 7, which is followed by ring opening to produce an amino aldehyde [21]. Rotation about the C-11a and C-11b axis is then followed by

hemi-acetal formation and methylation of the nitrogen atom to produce 9-*O*-demethylhomolycorine (**Scheme 3.3**) [18,22].

Scheme 3.2 The biosynthesis of lycorine type alkaloids [14,15,16,19].

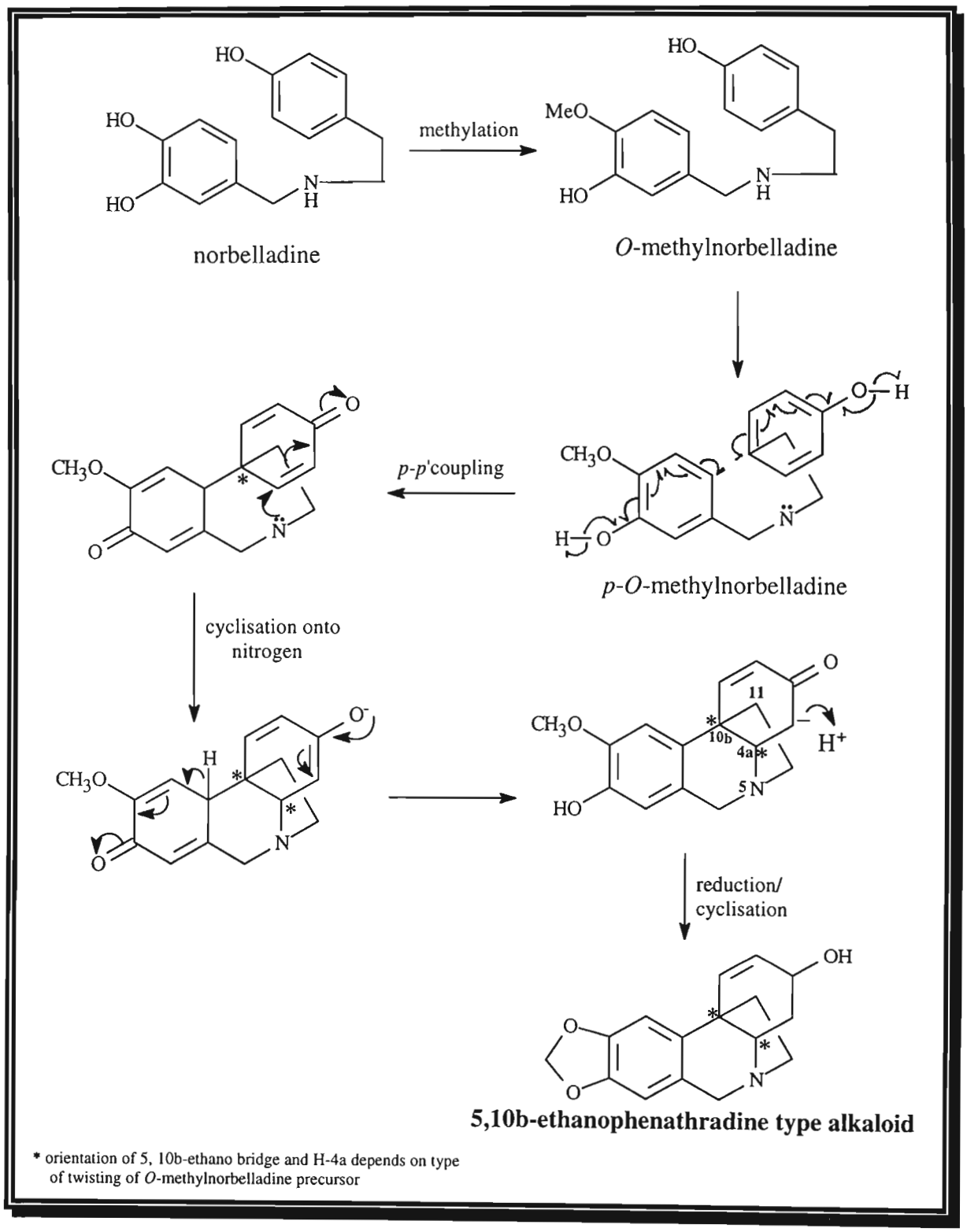


Scheme 3.3 The biosynthesis of homolycorine type alkaloids [18,21].



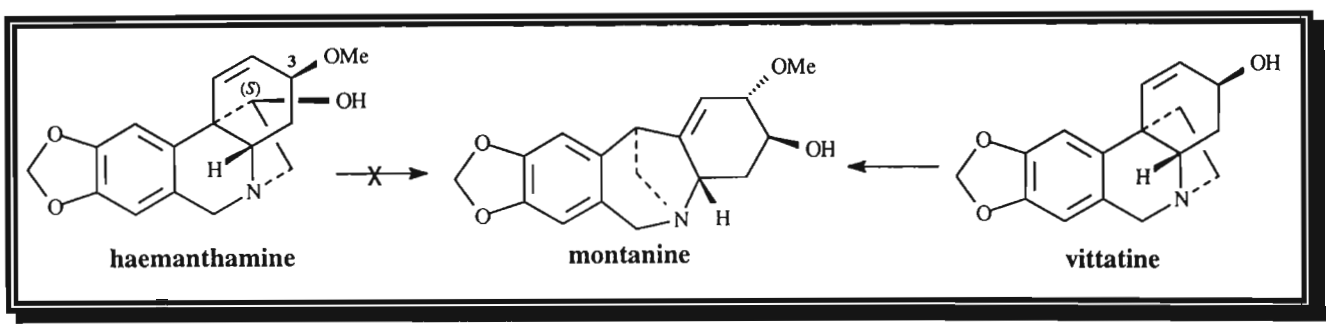
Crinane type alkaloids are also derived *via* phenol oxidative coupling. However, in this case, the tyrosine derived molecule of the *O*-methylnorbelladine precursor is twisted in such a way that *p-p'* oxidative coupling between the two aromatic rings takes place [14,15,19]. The resulting molecule has an ethanoic bridge between the nitrogen atom at position 5 and C-10b; hence the nomenclature 5,10b-ethanophenanthradine alkaloids. Depending on the manner in which the precusor is twisted, a β or an α bridge is possible giving rise to the two different series in this alkaloid group. (**Scheme 3.4**). The haemanthamine type alkaloids typically have an α 5,10b-ethanobridge and crinine type alkaloids, conversely, exhibit a β orientated bridge. Tracer studies have been carried out to determine if haemanthamine and crinine type alkaloids can be interconverted *in vivo* [22]. Tritium labelled crinine was fed to *Nerine bowdenii* and the subsequent results indicated that tritium label was not incorporated into any alkaloids of the haemanthamine type [22]. Accordingly, there is no evidence to suggest that the enantiomeric ring systems of the two series are interconvertible *in vivo* [22]. Haemanthamine itself, has a hydroxy group at C-11 which has been shown to arise by hydroxylation with normal retention of configuration [16].

Scheme 3.4 The biosynthesis of 5,10b-ethanophenanthridine/crinane type alkaloids [14,15,16,19].



Alkaloids of the montanine/5,11-methanomorphanthridine type are also biosynthetically derived *via* the *p-p'* oxidative coupling of the norpluviine precursor. In fact, these alkaloids are thought to be produced by the rearrangement of a haemanthamine-like intermediate [22,23]. Tracer studies have indicated that haemanthamine itself is not the precursor to montanine type alkaloids, since no radioactive montanine type alkaloids were isolated from *Heamanthus coccineus* which had been fed tritium labelled haemanthamine [22]. The researchers consequently speculated that the C-3 methoxy substituent is a point of interference in the *in vivo* rearrangement to the montanine nucleus [22]. Tritium labelled vittatine, on the other hand, was incorporated into both montanine and haemanthamine type alkaloids isolated from *Rhodophiala bifida*, which confirms the suggestion that a C-3 methoxy group hinders the rearrangement reaction (**Scheme 3.5**) [22].

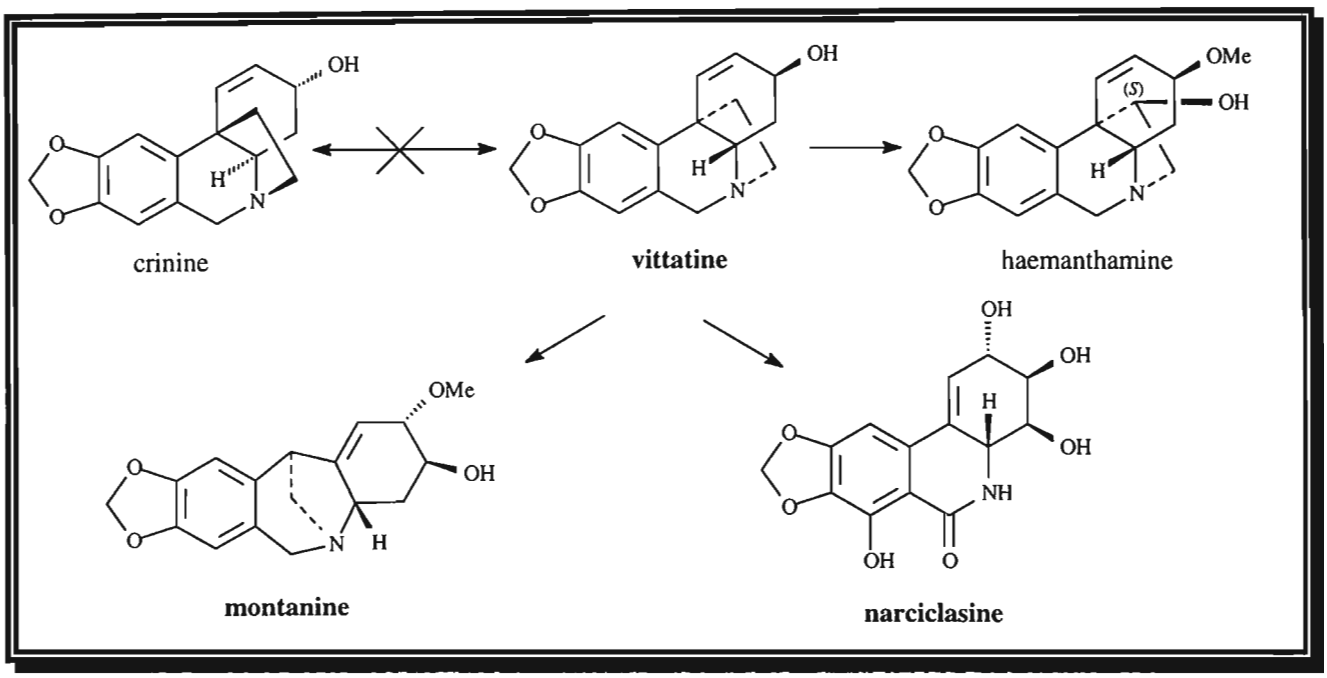
Scheme 3.5 Biosynthetic routes to montanine type alkaloids.



The conversion of 11-hydroxyvittatine to montanine requires a rearrangement, in addition to a methylation to produce the methoxy group at C-2 [22]. Evidence has also been obtained which suggests that a *pro-R*-hydrogen from the norbelladine precursor is retained at C-11 of the montanine skeleton [23].

The biosynthesis of narciclasine type alkaloids is also thought to proceed through *O*-methylnorbelladine and a haemanthamine type intermediate [18,24]. Once again *p-p'* oxidative coupling of the *O*-methylnorbelladine precursor is implicated [18,24]. The incorporation of tritium labelled vittatine into narciclasine has been demonstrated in *Pancratium maritimum,* which establishes this molecule as an intermediate in narciclasine biosynthesis [24]. Biosynthetic studies have also revealed that tritium labelled norpluviine has been incorporated directly into narciclasine by *Narcissus pseudonarcissus;* however this conversion was inefficient [21]. It has thus been suggested that since narciclasine type alkaloids are structurally similar to both haemanthamine and lycorine, these compounds could be derived *via* both biosynthetic routes. Despite this, the vittatine to narciclasine conversion is still significantly more efficient.

Scheme 3.6 The biosynthetic relationship between haemanthamine, montanine and narciclasine type alkaloids.



Norbelladine is also the ultimate biosynthetic precursor of galanthamine type alkaloids [14,15,19]. However, the biosynthesis of these alkaloids requires the *N*-methylation of the norbelladine precursor. It has been discovered that this methylation blocks the cyclisation onto nitrogen and thus prevents the entry of the precursor into the lycorine and crinane series [14,16]. Consequently, the methylation pattern of the norbelladine precursor controls its entry into the different biosynthetic pathways that give rise to the various alkaloid types.

3.3 REFERENCES

1. Vickery, M.L. and Vickery, B.; *"Secondary Plant Metabolism"*, (1981), The Macmillan Press Ltd, London, Chpt. 9, 255-257.
2. Pelletier, S.W.; in *"Chemistry of the Alkaloids"*, Ed. Pelletier, S.W., (1970), Van Nostrand Reinhold Company, New York, Chpt 1, 1-9.
3. Haslam, E.; *"Metabolites and Metabolism–A Commentary on Secondary Metabolism"*, (1985), Oxford University Press, England, Chpt 3, 52-54.
4. Vickery, M.L. and Vickery, B.; *"Secondary Plant Metabolism."*, (1981), The Macmillan Press Ltd, London, Chpt 1, 5-12.
5. Herbert, R.B.; *"The Biosynthesis of Secondary Metabolites-2nd Edition"*, (1989), Chapman and Hall Ltd, London, Chpt 6, 120-121.
6. Du Plessis, N. and Duncan, G.; *"Bulbous Plants of Southern Africa–A Guide to their Cultivation and Propagation"*, (1989), Tafelberg Publishers, Cape Town, 90.
7. Dyer, R.A.; *"The Genera of Southern African Flowering Plants–Volume 2"*, (1976), Department of Agricultural Technical Services, Pretoria, 947.
8. Hickey, M. and King, C.J.; *"100 Families of Flowering Plants"*, (1981), Cambridge University Press, London, 496-498.
9. Viladomat, F., Bastida, J., Codina, C., Nair, J.J. and Campbell, W.E.; *Recent Res. Dev. in Phytochem.*, (1997), **1**, 131.
10. Wildman, W.C.; in *"Chemistry of the Alkaloids"*, Ed. Pelletier, S.W., (1970), Van Nostrand Reinhold Company, New York, Chpt 6, 151.
11. Miyakado, M., Kato, T., Ohmo, N. and Koshimizu, K.; *Phytochem.*, (1975), **14**, 2717.
12. Page, B.A.; MSc dissertation, (1998), UND, Durban, S.A., 36-40.
13. Hutchings, A., Scott, A.H., Lewis, G. and Cunningham, A.B.; *"Zulu Medicinal Plants-An Inventory"*, (1996), University of Natal Press. Pietermaritzburg, 47-51.
14. .Spenser I.D.; in *"Chemistry of the Alkaloids"*, Ed. Pelletier, S.W., (1970), Van Nostrand Reinhold Company, New York, Chpt 21, 691.
15. Vickery, M.L. and Vickery, B.; *"Secondary Plant Metabolism."*, (1981), The Macmillan Press Ltd, London, Chpt 9, 273-275.
16. Herbert, R.B.; *"The Biosynthesis of Secondary Metabolites-2nd Edition"*, (1989), Chapman and Hall Ltd, London, Chpt 6, 156-160.
17. Wildman, W.C. and Heimer, N.E.; *J. Am. Chem. Soc.*, (1967), **89**, 5265.
18. Bastida, J., Viladomat, F. and Codina, C.; in *"Studies in Natural Product Chemistry"*, (1997), Ed. Atta-ur-Rahman, Elsevier Science Publishers, Amsterdam, Chpt 20, 232.

19. Haslam, E.; *"Metabolites and Metabolism–A Commentary on Secondary Metabolism."*, (1985), Oxford University Press, England, Chpt 3, 55-56.

20. Kirby, G.W. and Tiwari, H.P.; *J. Chem Soc. (C)*, (1966), 676.

21. Harken, R.D., Christensen, C.P. and Wildman, W.C.; *J. Org. Chem.*, (1976), **41**, 2450,

22. Feinstein, A.I. and Wildman, W.C.; *J. Org. Chem.*, (1976), **41**, 2447.

23. Fuganti, C., Ghiringhelli, D. and Grasselli, P.; J. *Chem. Soc. Chem. Comm.*, (1973), 430.

24. Fuganti, C. and Mazza, M.; *J. Chem. Soc. Chem. Comm.*, (1972), 239.

CHAPTER 4

Extractives from Clivia caulescens

***Clivia caulescens* in Buffelskloof Nature Reserve.**

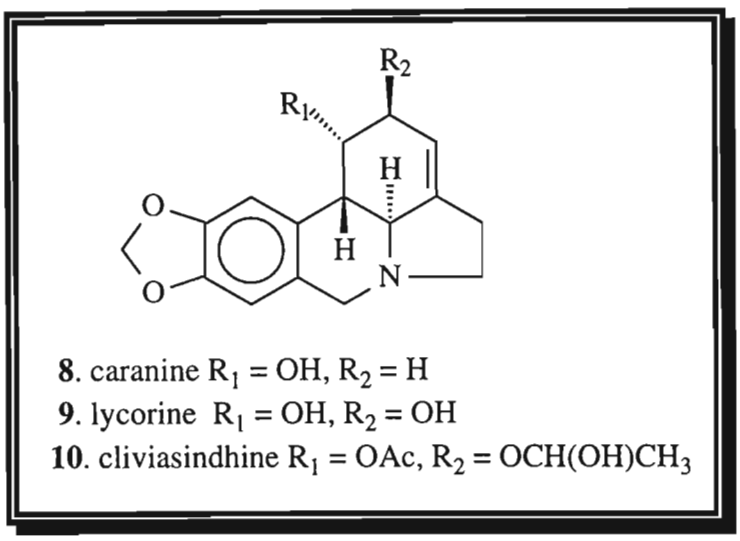
4.1 A REVIEW OF PREVIOUS WORK

The genus *Clivia* is endemic to South Africa and comprises four species: *C. caulescens, C. gardenii, C. nobilis* and *C. miniata* [1,2,3]. Their habitat is the subtropical region from the Eastern Cape to Mpumalanga [1,2]. The most attractive and variable species is *C. miniata,* thus of the four species *C. miniata* is most commonly found in cultivation [1,3]. The less common species, *C. caulescens,* can be found in Swaziland and Mpumalanga [1]. Plants belonging to the *Clivia* genus are generally evergreen, herb-like plants with a rhizomatous rootstock or pseudobulb [1,2,3]. Plants of the *Clivia* genus are used by traditional healers in South Africa in the treatment of a number of ailments. Root infusions of *C. miniata* are used as a traditional snakebite remedy by the Zulu and Xhosa tribes in South Africa [4,5]. In addition, the herb of this plant is used to facilitate and induce labour during childbirth [4,5]. Decoctions of the bulb are also used by the Xhosa people for the treatment of infertility and urinary complaints [4]. The use of *C. nobilis* is also common practice among traditional healers and this plant is often used to make protective charms [4]. *C. miniata* is reported to be highly toxic and is potentially lethal if eaten in large quantities [6]. Salivation, vomiting, diarrhoea and depression of the central nervous system have all been reported as symptoms of poisoning from the bulbs [4]. *C. nobilis* is apparently less toxic and a fairly large dose of a strong decoction of the bulbs is reported to produce a mild emetic effect [4]. The physiological effects are thought to be associated with the large number of alkaloids that have been isolated from these plants [2,4,5,6].

The most extensively studied species is *C. miniata* from which a relatively large number of alkaloids have been isolated [3]. The primary source of these alkaloids is the leaves and rhizomes. In addition, the species *C. nobilis* has also been investigated [7]. Eighteen alkaloids have, to date, been isolated from C. *miniata* and three alkaloids from C. *nobilis* including the alkaloid unique to this species, nobilisine **12** [3,7]. The alkaloids of the *Clivia* genus can be classified into four distinct types: The lycorine, the homolycorine, the haemanthamine/5,10b-ethanophenanthridine type and those alkaloids of miscellaneous type [3].

1. The lycorine type alkaloids

Three alkaloids of this type have been isolated from the two species of *Clivia* that have been previously investigated [3].



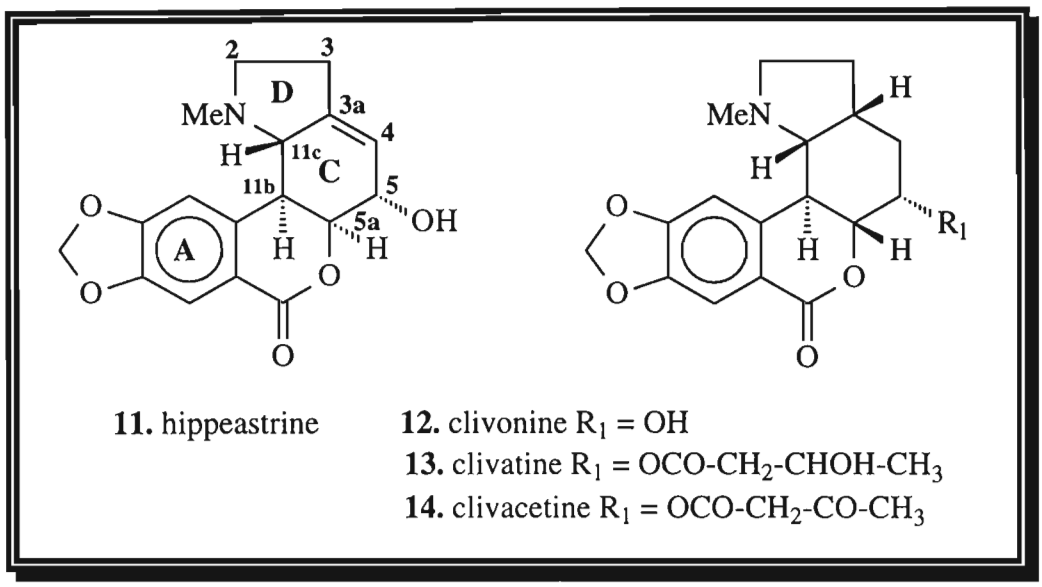
8. caranine R_1 = OH, R_2 = H
9. lycorine R_1 = OH, R_2 = OH
10. cliviasindhine R_1 = OAc, R_2 = $OCH(OH)CH_3$

Figure 4.1 Alkaloids of the lycorine type.

Lycorine **9** is an alkaloid common in the Amaryllidaceae family and its isolation is facilitated by the fact that it is insoluble in most organic solvents and tends to precipitate from crude extracts [8]. Caranine **8** is closely related to lycorine except that one of the hydroxy substituents of the glycol system is missing in caranine [3].

2. The homolycorine type alkaloids

There are twelve alkaloids of the homolycorine type that have been isolated from *Clivia,* eleven of which are unique to the *Clivia* genus [3]. These include: clivonine **12**, clivatine **13**, clivacetine **14**, cliviamartine **15**, clivonidine **16**, cliviasine **17**, clividine **18**, nobilisine **19**, cliviasyaline **20**, miniatine **21** and clivimine **22**. These homolycorine type alkaloids are structurally similar and variations are observed primarily in the C-ring. The orientation of the hydrogens at C-3a and C-5a vary according to the substituents at C-5 and the orientation of the B-C ring juncture. Two stereochemical modifications of the skeleton are exhibited by this series. The majority belong to the enantiomeric series containing a *cis* B-C ring juncture but there is also a much smaller group characterised by a *trans* B-C ring juncture [7,9]. The orientation of H-11c is characteristically β to the C-ring and hydrogen 11b is α to the C-ring [3].



11. hippeastrine

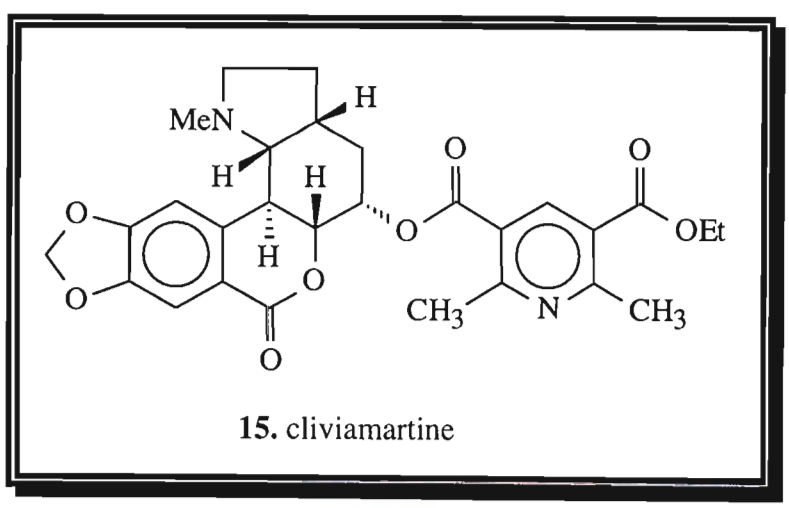
12. clivonine R_1 = OH

13. clivatine R_1 = OCO-CH_2-CHOH-CH_3

14. clivacetine R_1 = OCO-CH_2-CO-CH_3

Figure 4.2 Alkaloids of the homolycorine type.

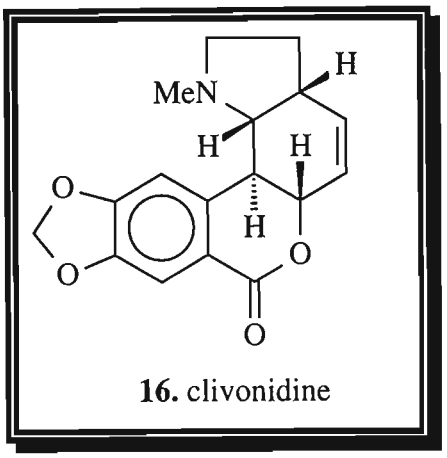
Hippeastrine **11** is commonly isolated from plants belonging to the Amaryllidaceae. This alkaloid has a characteristic 3a, 4-double bond and an α hydroxyl group at C-5. In addition, the orientation of the hydrogen at C-5a is α to the C ring [3]. The three alkaloids clivonine **12**, clivatine **13** and clivacetine **7** are very similar and differ only in the substituent at carbon 5. They represent a small sub-group of homolycorine alkaloids characterised by the absence of a 3a,4-double bond system. The orientation of H-3a and H-5a is characteristically β to the C ring to facilitate the bulky substituents at C-5 [3]. The alkaloid cliviamartine **15** also belongs to this sub-group and typically has extensive substitution at C-5 and is thus easily distinguishable.



15. cliviamartine

Figure 4.3 Cliviamartine.

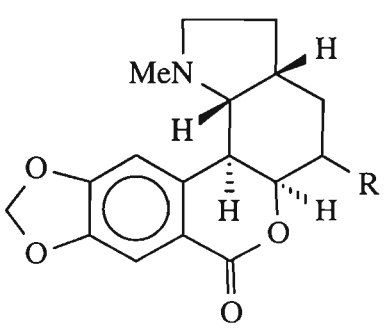
Clivonidine **16** is the only alkaloid of the homolycorine type isolated from *Clivia* species that exhibits a 4,5-double bond in the C ring [3].

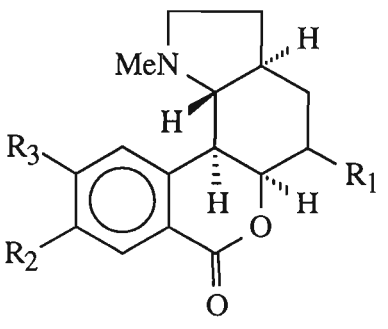


16. clivonidine

Figure 4.4 Clivonidine.

Cliviasine **17** and clividine **18** have an α orientation for the hydrogen atom at C-5a. Nobilisine **19** and clivisyaline **20** have characteristic orientations for hydrogen atoms 3a and 5a [3]. In addition, all four of these alkaloids also belong to the 3a,4-dihydrolactone-[2]-benzopyrano[3,4g]indole series.





17. cliviasine R = OHα
18. clividine R = OHβ

19. nobilisine R_1 = OHα, $R_2 + R_3$ = OCH_2O
20. clivisyaline R_1 = OHβ, R_2 = OH, R_3 = OCH_3

Figure 4.5 Alkaloids of the homolycorine type.

Miniatine **21** and clivimine **22** have complex substituents at C-5, but retain the β orientation of H-3a

and H-11c. In addition, a β orientation for H-5a is observed for these molecules [3].

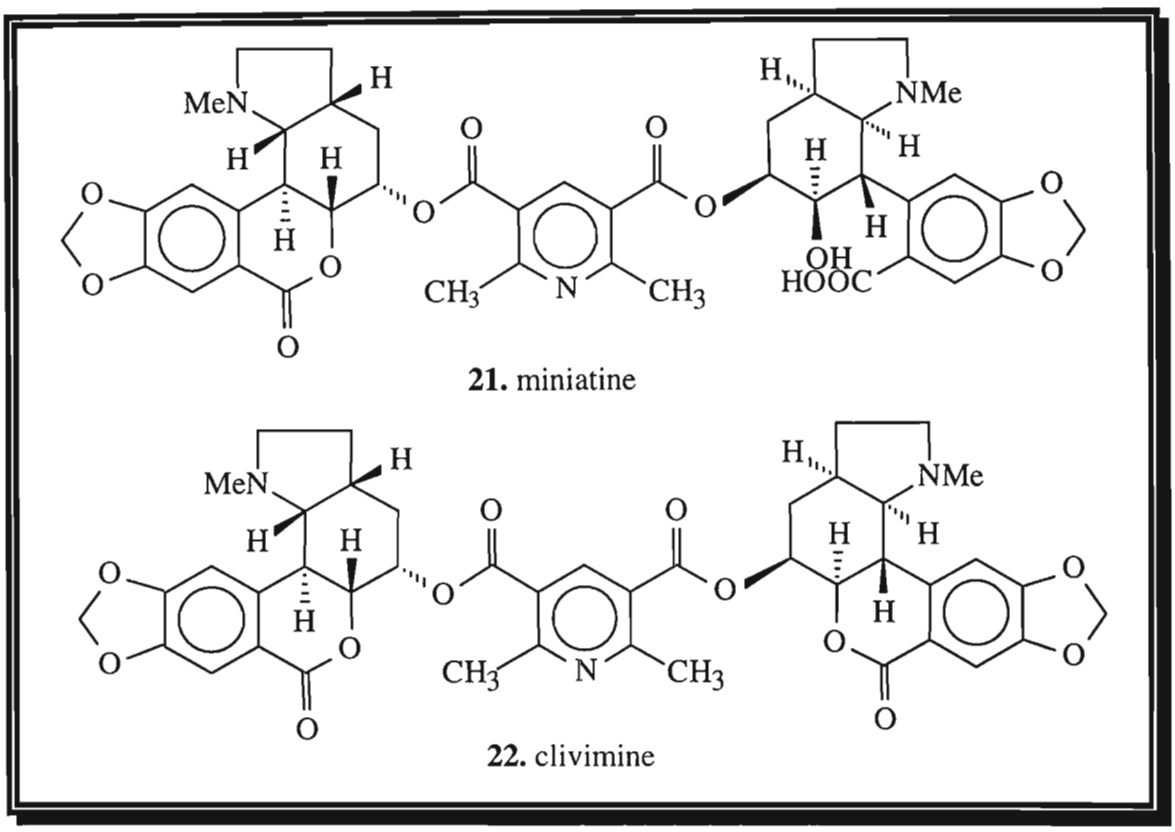
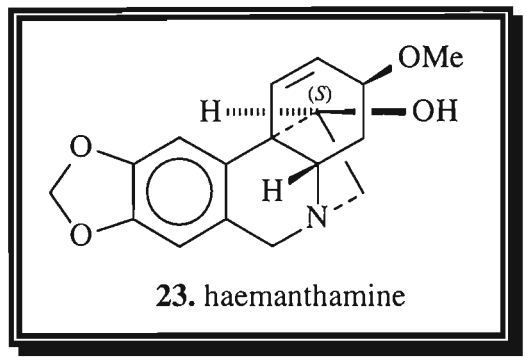


Figure 4.6 Miniatine and clivimine.

3. The haemanthamine type alkaloids

The only alkaloid of this type found in the *Clivia* genus is haemanthamine **23**, which is commonly isolated from other members of the Amaryllidaceae family [3]. This alkaloid has a characteristic 1,2-double bond as well as a β orientated methoxy functional group. Nevertheless, the most distinguishing feature of alkaloids of this type is the 5,10b-ethano nitrogen bridge, which has a typical α orientation.

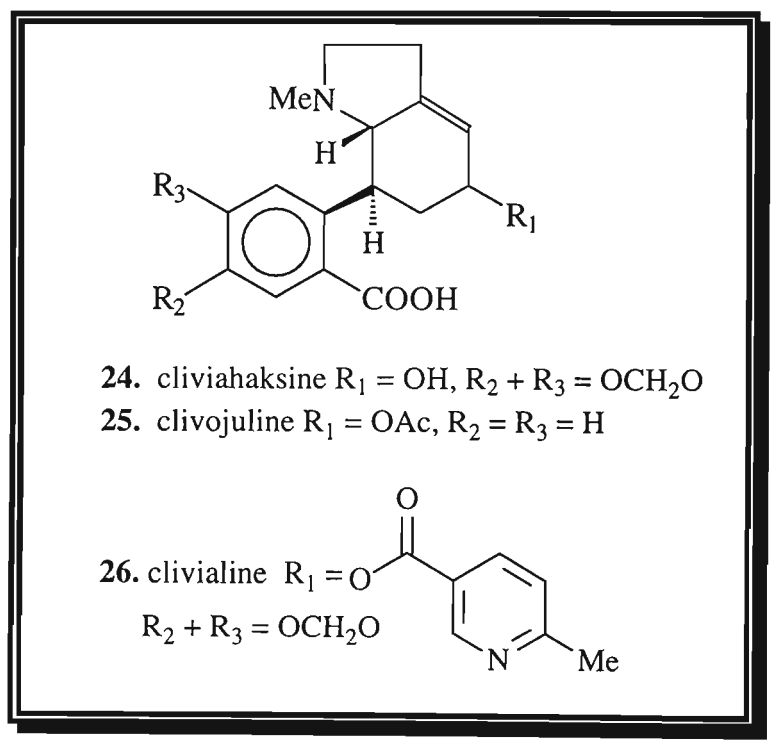


23. haemanthamine

Figure 4.7 Alkaloid of the haemanthamine type.

4. The miscellaneous type alkaloids

Three alkaloids of miscellaneous classification have been isolated from the *Clivia* genus, these include cliviahaksine **24**, clivojuline **25** and clivialine **26** [3].



24. cliviahaksine $R_1 = OH$, $R_2 + R_3 = OCH_2O$

25. clivojuline $R_1 = OAc$, $R_2 = R_3 = H$

26. clivialine $R_1 =$

$R_2 + R_3 = OCH_2O$

Figure 4.8 Alkaloids of the miscellaneous type.

4.2 RESULTS AND DISCUSSION

Both the rhizomes and the leaves of *Clivia caulescens* were investigated. The ethanol extract of the bulbs yielded four alkaloids, hippeastrine (**compound III**), haemanthamine (**compound IV**) lycorine, (**compound V**) and 11-(*S*)-hydroxyvittatine (**compound VI**). Compounds **III-V** are alkaloids commonly isolated from many members of the Amaryllidaceae family including *Clivia miniata* [3]. Although lycorine has been isolated from *Clivia nobilis*, hippeastrine and haemanthamine have not been isolated from this species [3,7]. The investigation of the leaf extract also yielded lycorine and hippeastrine as well as an additional alkaloid sternbergine (**compound VII**). Compound **VI** and **VII** have not been isolated previously from the *Clivia* genus and are infrequently isolated from the Amaryllidaceae family [3].

4.2.1 THE STRUCTURAL ELUCIDATION OF COMPOUND III

The first compound isolated from the bulb extract was a white crystalline material identified as hippeastrine (**compound III**). This compound was found in a particularly high concentration in the bulbs and was also isolated from the leaf extract. This alkaloid is reported to possess weak antifeedant activity against insects. This alkaloid has also been used in a cytotoxicity study where its selective cytotoxicity against tumour panel cell lines was recorded. In this study an ED_{50} of < 5 µg/ml was used as the criterion for significant activity. The authors reported that hippeastrine proved to be significantly active against the LNCaP (prostrate) and HT (sarcoma) cell lines [10].

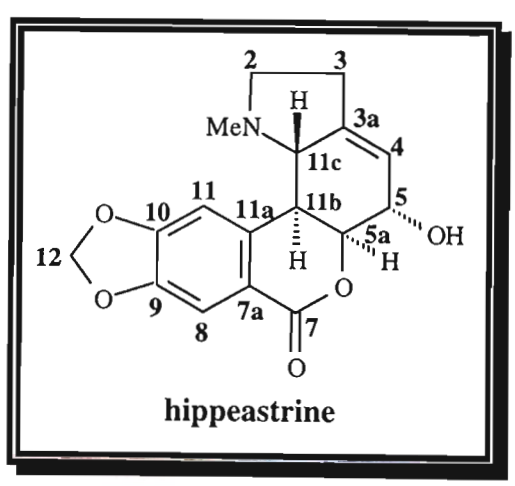
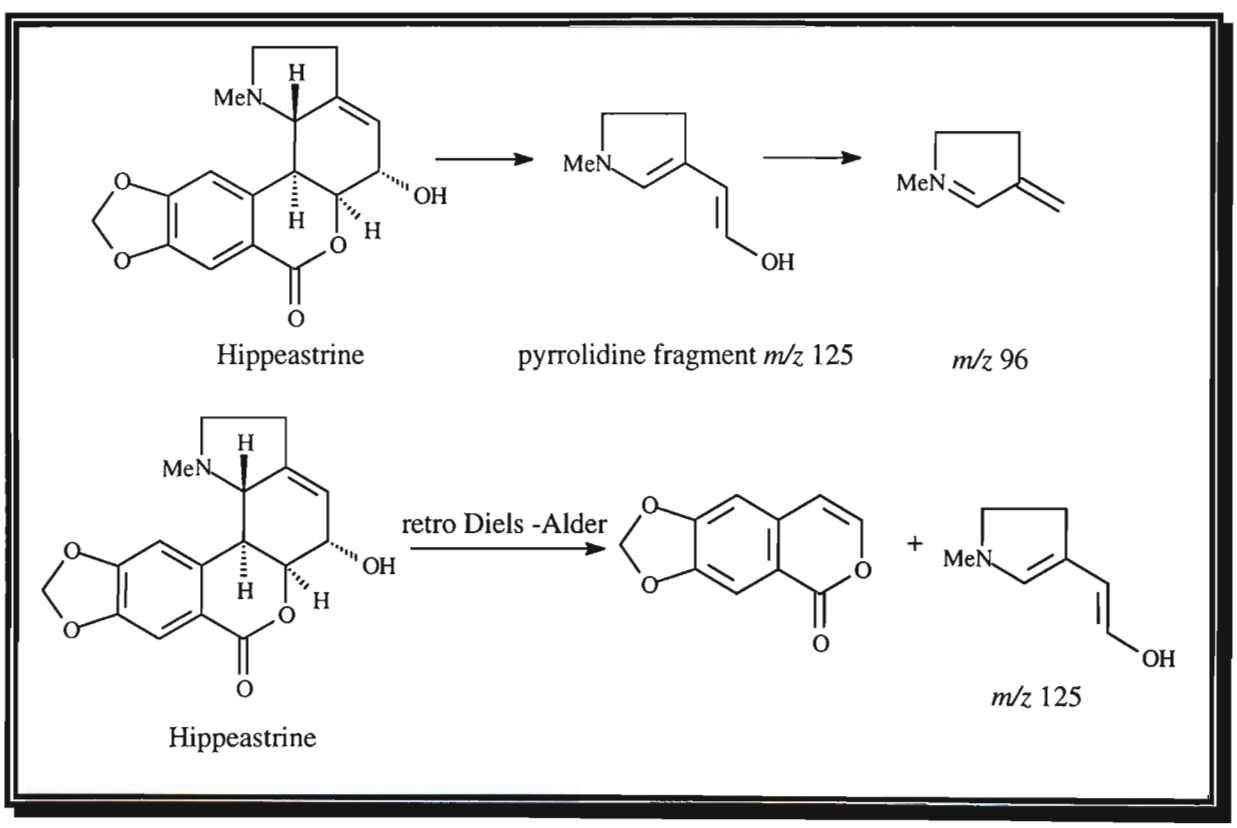


Figure 4.9 Compound III: hippeastrine

The mass spectrum (**Spectrum 23, p 182**) showed two very intense peaks at *m/z* 125 and *m/z* 96, however the parent ion at *m/z* 315 ($C_{17}H_{17}NO_5$) was not observed. This is in agreement with literature where the parent ion was observed at less than 1% [11]. This fragmentation pattern corresponds to lactone alkaloids of the benzopyrano[3,4-*g*]indole series with a hydroxyl group at C-5, which exhibit a retro-Diels–Alder cleavage of the C ring to produce the base ion at *m/z* 125 representing the pyrrolidine fragment (**Scheme 4.1**) [9,11]. The aromatic lactone fragment ion expected at *m/z* 191 was not observed.

Scheme 4.1 The mass spectrometric fragmentation of compound III.



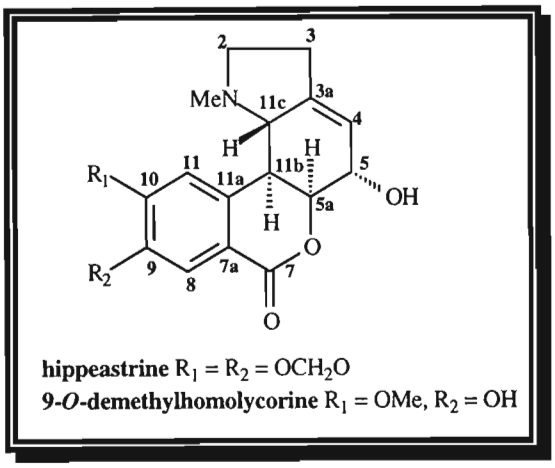
The infra-red data for compound III (**Spectrum 21, p 180**) closely matched that recorded in the literature for hippeastrine [11]. Peaks were observed at 3415 cm^{-1} (O-H stretching), 2939 cm^{-1} (aliphatic C-H stretching), 1720 cm^{-1} (C=O stretching), 1480 cm^{-1} (aromatic C=C stretching), 1038 cm^{-1} (C-N stretching) and at 938 cm^{-1}, which is indicative of the methylenedioxy group.

From the mass spectrum, infra-red data and ^1H NMR data it was evident that compound III was an

alkaloid of the homolycorine/benzopyrano[3,4g]indole type. The ^{1}H NMR spectrum (**Spectrum 15, p 174**) closely matched that of the literature data for hippeastrine (**Table 4.1**) [11]. Two singlets, observed in the aromatic region at δ7.10 and δ7.45, were ascribed to the aromatic hydrogens of the A ring, H-11 and H-8 respectively. The differentiation between these two protons was made on the basis of the deshielding effect that the lactone carbonyl group in ring B has on H-8, which would account for its more downfield assignment [9,12]. An intense singlet, corresponding to the two methylenedioxy protons was observed at δ6.14. A broad singlet was evident at δ5.70 and was assigned to the olefinic proton H-4. Two coupled alkyl proton resonances (δ4.60 and δ4.30) were observed at a downfield chemical shift, due to the deshielding effects of nearby oxygen groups and were assigned with reference to the COSY spectrum (**Spectrum 16, p 175**). The δ4.30 resonance was clearly coupled to the olefinic H-4 proton and was thus assigned as H-5β, which is directly adjacent to an α-hydroxy group at C-5. The broad singlet at δ4.60 was thus assigned as the H-5a proton which is directly adjacent to the lactone group in the B ring. The remaining five alkyl resonances were assigned with reference to literature [9,11] and the COSY spectrum. The multiplets at δ3.26 and δ2.37 were strongly coupled to one another and were ascribed to H-2α and H-2β respectively. Furthermore, the COSY spectrum indicated weak coupling between the two H-2 resonances and the multiplet, integrating to two hydrogens, at δ2.60, confirming its assignment as 2H-3. Similarly, based on the strong coupling observed in the COSY spectrum, the double doublet at δ2.90 (J=2.2, 9.7 Hz) and the multiplet at δ2.68 were identified as the H-11b and H-11c respectively. Finally, the intense singlet, integrating to three protons, observed at δ2.08 was assigned to the *N*-methyl protons. The proton spectrum of compound III was a reasonable match for the literature data for hippeastrine if solvent effects are taken into account. In addition, the assignment of the resonances was confirmed by comparison to a similar compound, 9-*O*-demethyl-5α-hydroxyhomolycorine [13], which differs structurally from hippeastrine in that it lacks a methylenedioxy group and has instead hydroxy and methoxy substituents at C-10 and C-9 respectively.

The ^{1}H NMR spectrum of the acetylated product of compound III showed the downfield shift of the broad singlet at δ4.30, assigned to H-5β, to δ5.34, and the appearance of sharp singlet integrating to three protons at δ2.03. This confirmed the fact that a hydroxy group was present at C-5.

Table 4.1 The ^{1}HNMR data for compound III and the literature data for hippeastrine [11] and 9-*O*-demethyl-5α-hydroxyhomolycorine [13].



hippeastrine $R_1 = R_2 = OCH_2O$
9-*O*-demethylhomolycorine R_1 = OMe, R_2 = OH

	^{1}H NMR data for compound III	**^{1}H NMR literature data [11] for hippeastrine**	**^{1}H NMR literature data [13] for 9-*O*-demethyl-2α-hydroxyhomolycorine**
Proton No.	**#Shift/ δ_H ppm**	***Shift/ δ_H ppm**	****Shift/ δ_H ppm**
H-2α	3.26 m	3.13 ddd (10, 8, 3)	3.17 ddd (9.7, 7.4, 2.5)
H-2β	2.37 dd (9.7, 19)	2.23 ddd (10, 9, 9)	2.31 dd (18.5,9.7)
2H-3	2.60 m	2.48 m	2.5-2.6 m
H-4	5.70 brs	5.63 brs	5.68 brs
H-5β	4.30 brs	4.38 brs	4.28 brt (1.0)
H-5a	4.60 brs	4.58 brs	4.59 brd (1.0)
H-8	7.45 s	7.45 s	7.47 s
H-11	7.10 s	6.92 s	6.95 s
H-11b	2.90 dd (9.6, 2.2)	2.85 dd (9.5, 2.5)	2.83 dd (9.7, 1.0)
H-11c	2.68 m	2.42 d (9.5)	2.66 brd (9.7)
OCH_2O	6.14 s	6.04 d (1.5)	
N-CH_3	2.08 s	2.03 s	2.08 s

^{1}H NMR spectrum measured in CD_3OD, 300 MHz.

*^{1}H NMR spectrum measured in $CDCl_3$, 500 MHz.

** ^{1}H NMR spectrum measured in $CD_3OD/CDCl_3$, 200 MHz.

The ^{13}C NMR data (**Spectrum 17, p 176**) agreed with that previously reported for hippeastrine (**Table 4.2**) [9,11] and confirmed the identity of the isolated compound. Seventeen carbon resonances were observed in the spectrum, ten of which were observed in the down field region δ>90ppm. Of the six quaternary resonances observed, four were assigned to the aromatic carbons C-7a, C-9, C-10, and C-11a. The remaining two resonances were ascribed to the lactone carbonyl C-7 and the olefinic carbon C-3a. The three methine signals were similarly assigned to the aromatic carbons C-8 and C-11, and the olefinic carbon C-3. The remaining methylene carbon, observed at δ103.9 is characteristic of the methylenedioxy functional group. The aliphatic region revealed five resonances corresponding to the oxygenated methine carbon C-5a, two methine carbons C-11a and C-11b, and two methylene carbons C-2 and C-3. The single remaining methyl carbon signal was ascribed to the N-methyl carbon atom

Table 4.2 ^{13}C NMR data for compound III and literature data for hippeastrine [9,11].

	^{13}C NMR data for compound III	^{13}C NMR literature data [11] for hippeastrine	^{13}C NMR literature data [9] for hippeastrine
Carbon No.	**#Shift/ δ_C ppm**	***Shift/ δ_C ppm**	****Shift/ δ_C ppm**
C-2	57.19 t	55.9 t	55.9 t
C-3	28.69 t	27.3 t	27.6 t
C-3a	**[a]140.79 s**	**142.4 s**	**138.9 s**
C-4	120.22 d	119.4 d	119.4 d
C-5	67.98 d	66.2 d	66.7d
C-5a	84.15 d	82.2 d	82.1 d
C-7	166.54 s	165.0 s	164.6 s
C-7a	119.47 s	118.0 s	118.5 s
C-8	110.21 d	109.8 d	109.9 d
C-9	149.68 s	148.0 s	148.1 s
C-10	153.82 s	151.9 s	152.0 s
C-11	109.80 d	108.5 d	108.8 d
C-11a	**[a]143.33 s**	**138.85 s**	**143.3 s**
C-11b	40.78 d	38.4 d	38.6 d
C-11c	68.32 d	67.1 d	67.3 d
OCH_2O	103.94 t	102.1 t	102.2 t
N-CH_3	43.66 q	42.9 q	43.2 q

^{13}C NMR spectrum measured in CD_3OD, 75 MHz.

* ^{13}C NMR spectrum measured in $CDCl_3$, 125 MHz.

** ^{13}C NMR spectrum measured in $CDCl_3$, 22.6 MHz.

[a] resonance assigned with reference to [11]

4.2.2 THE STRUCTURAL ELUCIDATION OF COMPOUND IV

The second compound isolated from the bulb extract was identified, on the basis of its physical and spectroscopic data, as the alkaloid haemanthamine (**IV**).

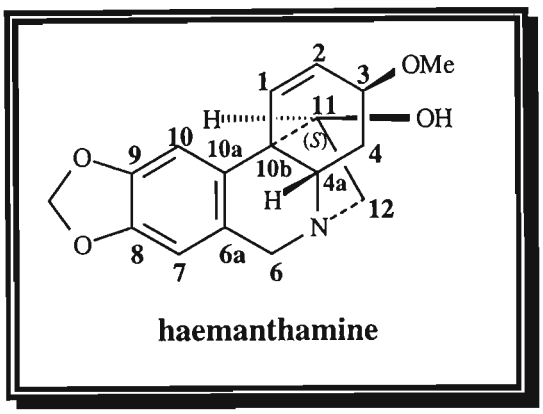


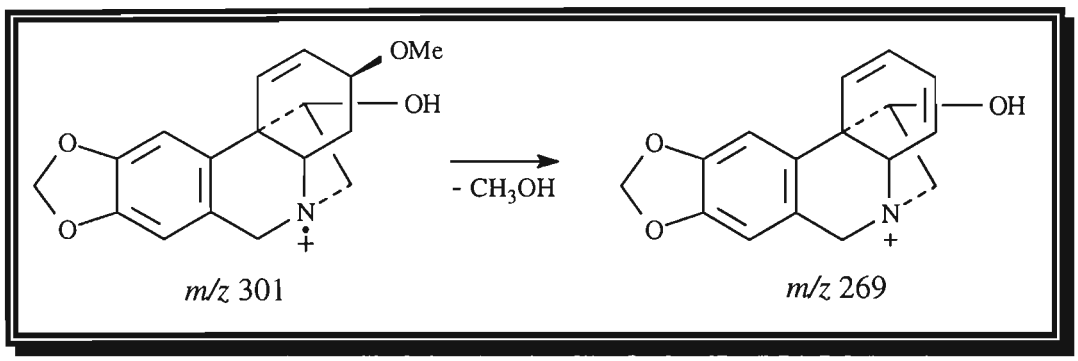
Figure 4.10 Compound IV: haemanthamine

High resolution mass spectrometry showed a molecular ion peak at *m/z* 301.1310 which corresponded to the molecular formula $C_{17}H_{19}NO_4$ and was in agreement with the proposed structure for compound IV. The fragmentation pattern observed in the mass spectrum (**Spectrum 32, p 191**) was typical of an alkaloid with a crinane skeleton [14]. An intense molecular ion peak excluded alkaloids from the crinamine series and was consistent with compounds of the haemanthamine series, characterised by a *trans* configuration between the C-3 substituent and the 5,10b-ethano bridge together with a subtituent in the bridge and a double bond in ring C [14,15].

Fragment ions were observed at *m/z* 272, *m/z* 269, *m/z* 257, *m/z* 240, *m/z* 227, *m/z* 225, *m/z* 211 and *m/z* 181. Literature data from two different sources was obtained, which postulated the mass spectrometric fragmentation pattern of haemanthamine [14,16]. Both sets of literature appear to agree on the origin of the fragments at *m/z* 240, *m/z* 211 and *m/z* 181. However, the origin of the remaining fragments appears to be a point of contention, as both sets of literature postulate different fragmentation routes.

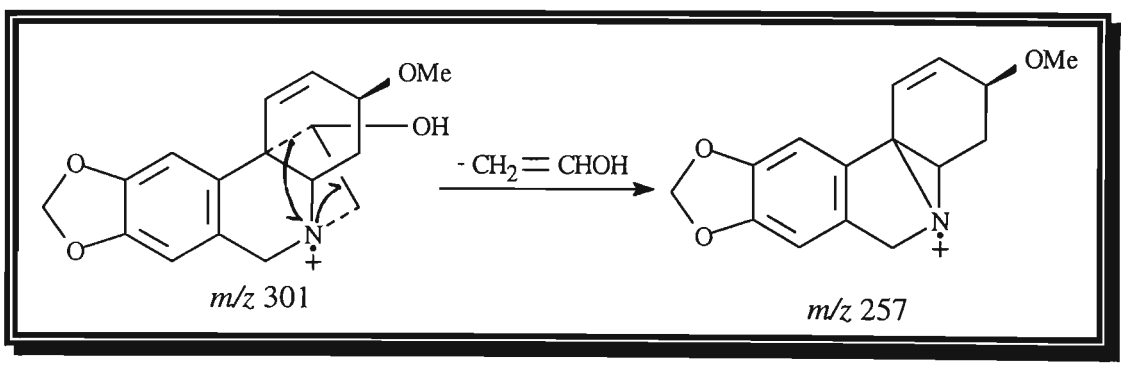
Duffield and his co-workers [14] suggest that the relatively intense peak at *m/z* 269 is produced *via* the elimination of methanol from the parent molecule (**Scheme 4.2**).

Scheme 4.2 The mass spectrometric fragmentation of compound IV [14].



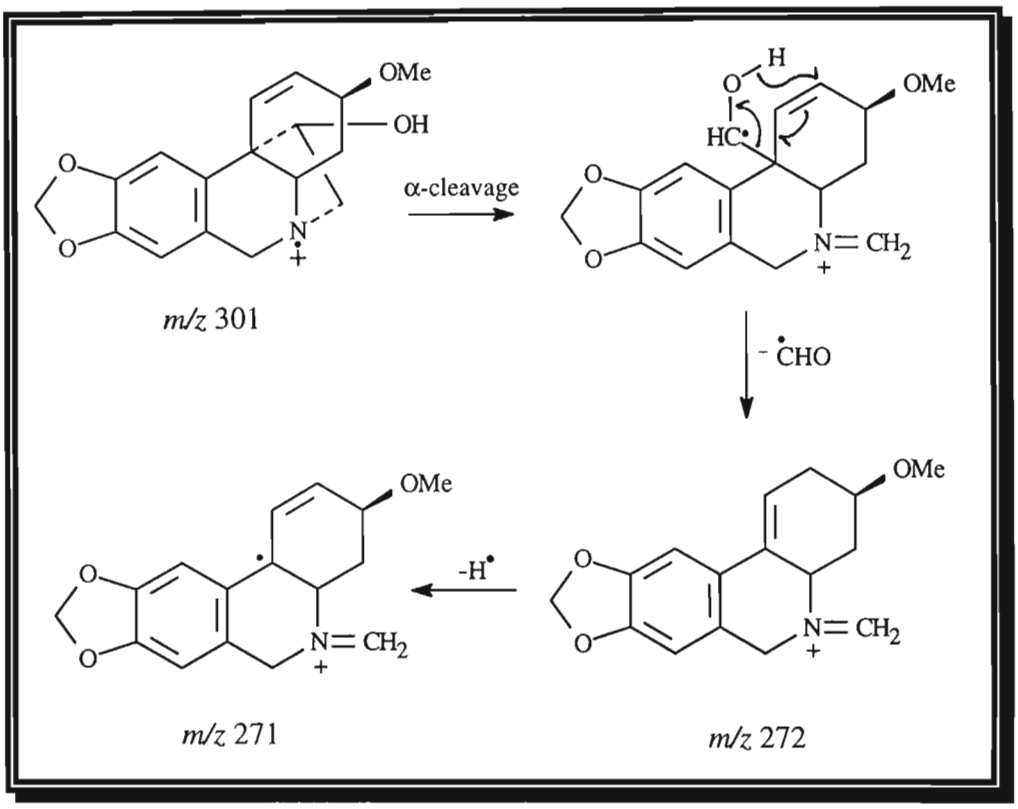
They further suggest that the signal at *m/z* 257 corresponds to the loss of the hydroxylated ethylene bridge (**Scheme 4.3**).

Scheme 4.3 The mass spectrometric fragmentation of compound IV [14].



Furthermore, the authors suggest that fragmentation peaks at *m/z* 272 and *m/z* 271 are afforded by the sequence shown below in **Scheme 4.4** [14].

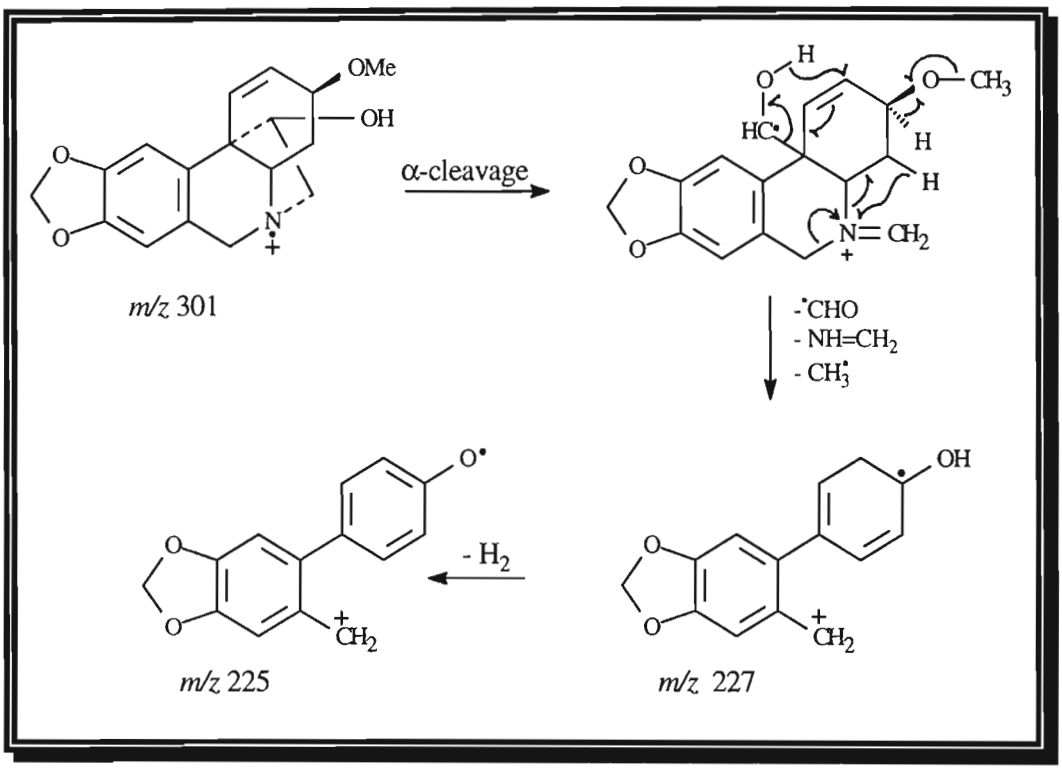
Scheme 4.4 The mass spectrometric fragmentation of compound IV [14].



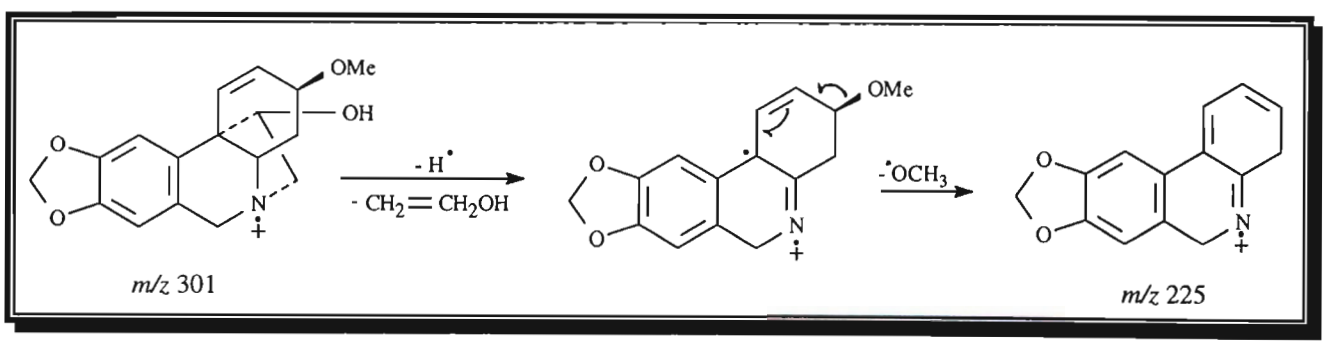
Duffield *et al.* [14] also postulate that the fragment at *m/z* 227 corresponds to $C_{14}H_{11}O_3^+$ and the postulated fragmentation occurs according to **Scheme 4.5**.

The Duffield theory [14] suggests that the second fragment at *m/z* 225 [M-76] corresponds to $C_{14}H_9O_3^+$ (67%) and $C_{14}H_{11}NO_2^+$ (33%). One hypothesis is that the first moeity could arise by the loss of H_2 from the fragment at *m/z* 227 (**Scheme 4.5**). However, the second moeity is thought to be produced by the loss of the 5,10b-ethano nitrogen bridge and the C-3 methoxy substituent [14] (**Scheme 4.6**).

Scheme 4.5 Mass spectrometric fragmentation of compound IV [14].

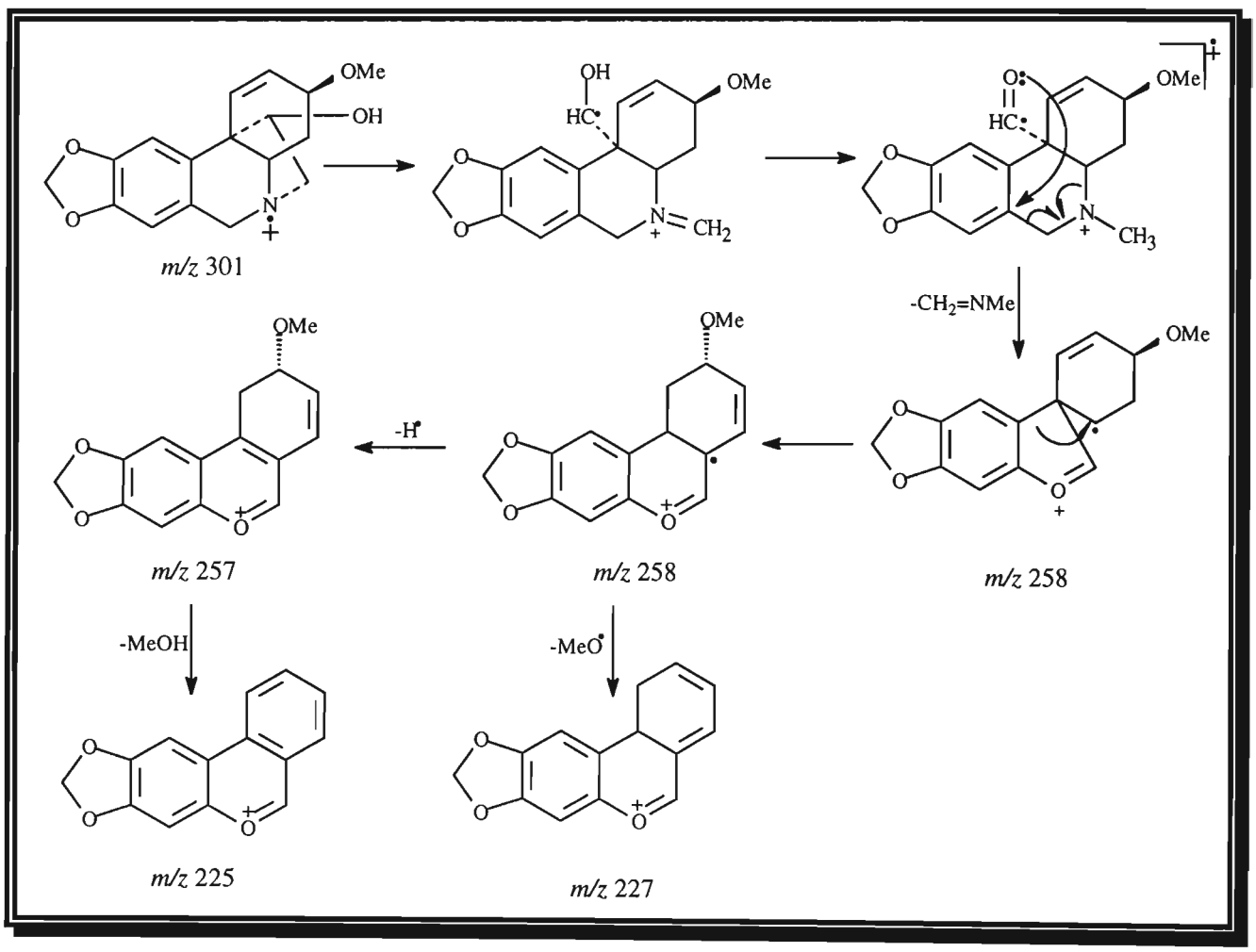


Scheme 4.6 The mass spectrometric fragmentation of compound IV [14].



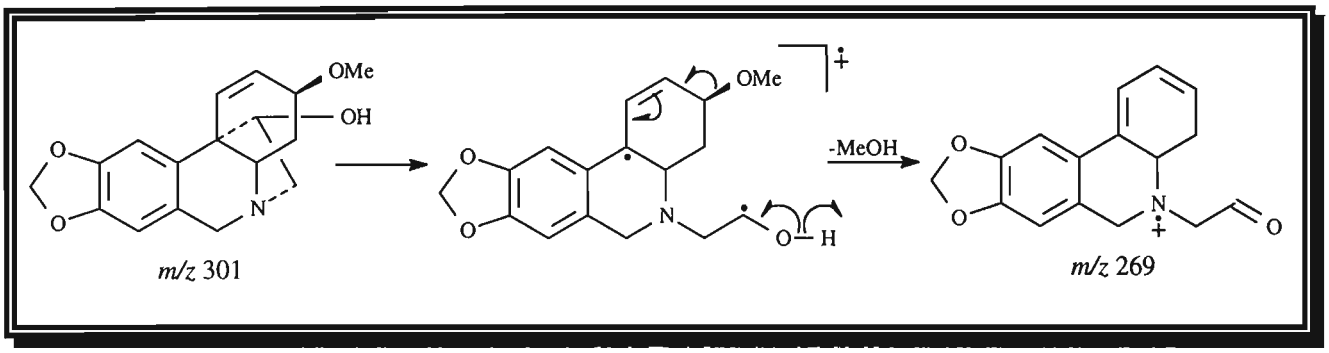
A completely different scheme for the origin of the peaks at *m/z* 269, *m/z* 257, *m/z* 227 and *m/z* 225 is suggested by the second literature source [16]. This source [16] suggest that the signals at *m/z* 225 and *m/z* 227 are derived from the ions *m/z* 257 and *m/z* 258 respectively (**Scheme 4.7**).

Scheme 4.7 The mass spectrometric fragmentation of compound IV [16].



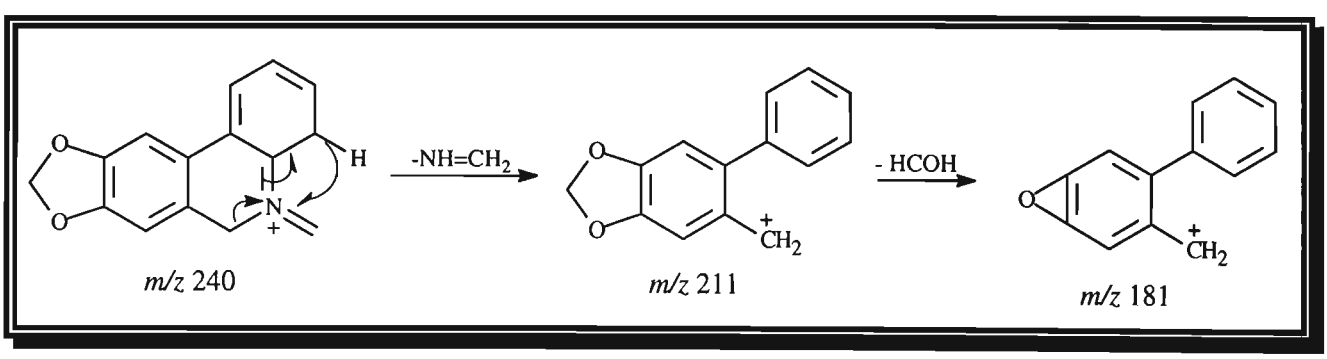
The fragmentation ion at *m/z* 269 is also postulated to be derived *via* a completely different manner [16] (**Scheme 4.8**).

Scheme 4.8 The mass spectrometric fragmentation of compound IV [16].



Both sets of literture [14,16] agree on the origin of the fragmentation ions at *m/z* 211, *m/z* 181 and *m/z* 240. The loss of 30 mass units from the fragment at *m/z* 211 produces an intense signal at *m/z* 181 which is characteristic for the ejection of formaldehyde from the methylenedioxy group and confirms the presence of this functional group (**Scheme 4.9**).

Scheme 4.9 The mass spectrometric fragmentation of compound IV [14,16].



The infra-red data for compound IV (**Spectrum 30, p 189**) closely matched the literature data for haemanthamine [18], and thus supported the proposed structure for this compound. Peaks were observed at 3448 cm^{-1} (O-H stretching), 2926 cm^{-1} (aliphatic C-H stretching), 1483 cm^{-1} (aromatic C=C stretching), 1029 cm^{-1} (C-N stretching) and at 929 cm^{-1} which is typical of the methylenedioxy group.

The NMR data supported the suggestion that compound IV was an alkaloid belonging to the haemanthamine series, and the ^{1}H NMR data (**Spectrum 24, p 183**) was consistent with that

previously reported for haemanthamine itself (**Table 4.3**) [17,18]. Two singlet resonances, observed at δ6.93 and δ6.57, were typical of aryl hydrogens adjacent to an oxygen function and were ascribed to the aromatic protons of the A ring, H-10 and H-7 respectively. The assignment of the more down field resonance to the H-10 proton was made on the basis of the deshielding effect of the 1,2-double bond which is spatially much closer to the H-10 proton. The more proximate H-10 is thus more susceptible to the effects of the double bond than the H-7 proton [19]. A prominant singlet, integrating to two hydrogens, was observed at δ5.92 and was ascribed to the methylenedioxy hydrogens. The doublet and the double doublet resonances at δ6.50 and δ6.28 were characteristic of the olefinic hydrogens H-1 and H-2. The doublet (J = 10.1 Hz) was associated with the olefinic hydrogen H-1 and the characteristic ABX splitting observed in the double doublet was typical of the coupling of H-2 with H-1 and H-3.

It is extremely difficult to differentiate between alkaloids of the haemanthamine and crinamine type since both series typically have an α orientated 5,10b-ethano bridge and the only significant difference in their basic structures is the orientation of the oxygenated substituent at C-3 in the C ring [14,19]. In the crinamine type alkaloids a *cis* relationship is observed between the C-3 substituent and the ethano bridge, whereas a *trans* relationship is observed for haemanthamine type alkaloids. The ABX splitting pattern of the olefinic hydrogen H-2 is characteristic for haemanthamine type alkaloids where a *trans* relationship is observed and was the primary NMR evidence which confirmed the mass spectrometric suggestion that compound IV was an alkaloid of the haemanthamine type as opposed to a crinamine type alkaloid where H-2 is normally split into a doublet [19].

The pair of doublets at δ4.31 and δ3.78 (J=16.9 and J=16.9 Hz) are typical of the non-equivalent H-6 protons in crinane type alkaloids [17] and were seen to be strongly coupled to each other in the COSY spectrum (**Spectrum 25, p 184**). These methylene protons are significantly deshielded due to the proximity to the nitrogen atom. The chemical shift of the multiplet at δ3.90 was indicative of a proton with an adjacent oxygen function. This resonance was coupled to the double doublet of H-2 and was thus assigned to the H-3 proton. The double doublet observed at δ3.99 was also typical of a proton adjacent to an oxygen function, and was assigned to H-11, which was split into a characteristic ABX pattern by the non-equivalent 2H-12 protons. Furthermore, the double doublets at δ3.16 (J=3.2, 13.7 Hz) and δ3.46 (J=7.08, 13.9 Hz) were coupled to each other and to H-11 in the

COSY spectrum which confirmed their assignment as H-12β and H-12α respectively. The remaining alkyl resonances were assigned with reference to literature [17,18] and the COSY spectrum. The COSY spectrum showed a distinct coupling between H-3 and the double double doublets at δ2.00 and δ2.20 which confirmed the literature assignments of these two resonances as H-4β and H-4α respectively. The H-4 resonances were also both coupled to the double doublet (*J*=4.5, 11.0 Hz) at δ3.32 which was consequently ascribed to H-4a.

The chemical shifts and multiplicities of all the resonances observed in the ^{1}H NMR spectrum of compound IV corresponded very closely to those recorded in the literature for haemanthamine, and suggested that the identification of the alkaloid was indeed correct [17,18].

Table 4.3 ^{1}H NMR data for compound IV and literature data for haemanthamine [17,18].

	^{1}H NMR data for compound IV	**^{1}H NMR literature data [18] for haemanthamine**	**^{1}H NMR literature data [17] for haemanthamine**
Proton No.	**#Shift/ δ_H ppm**	***Shift/ δ_H ppm**	****Shift/ δ_H ppm**
H-1	6.50 d (10.1)	6.40 d (10.1)	6.36 d (10)
H-2	6.28 dd (10.3, 5.0)	6.30 dd (10.1, 4.8)	6.25 dd (10,5)
H-3	3.90 m	3.88 m	3.82 m
H-4α	2.20 ddd (4.4, 9.2, 13.6)	2.12 ddd (13.5, 13.4, 3.6)	2.11 ddd (13.7,13.6)
H-4β	2.00 ddd (1.6, 12.2, 13.3)	2.06 ddd (13.5, 6.1, 1.9)	1.96 ddd (13.7, 13.6, 4.6)
H-4a	3.32 dd (11.0, 4.5)	3.32 dd (13.4, 6.1)	3.25 dd
H-6β	4.31 d (16.5)	4.30 d (16.8)	4.25 d (16.8)
H-6α	3.78 d (16.9)	3.68 d (16.8)	3.72 d (16.8)
H-7	6.57 s	6.48 s	6.41 s
H-10	6.93 s	6.84 s	6.74 s
H-11	3.99 dd (7.2, 2.8)	3.98 ddd (6.6, 3.5, 1.1)	3.96 dd
H-12α	3.46 dd (13.9, 7.1)	3.39 dd (14.0, 6.6)	3.30 dd
H-12β	3.16 dd (13.7, 3.2)	3.19 dd (14.0, 3.5)	3.19 dd
OCH_2O	5.91 s	5.90 s	5.81 d, 5.82 d (1.3)
OCH_3	3.39 s	3.36 s	Not given

^{1}H NMR spectrum measured in CD_3OD, 300 MHz.

* ^{1}H NMR spectrum measured in $CDCl_3$, 200 MHz.

** ^{1}H NMR spectrum measured in $CDCl_3$/1% C_5D_5N, 360 MHz.

The ^{1}H NMR spectrum of the acetylated product of compound IV (**Spectrum 29, p 188**) was slightly different to the spectrum of the non-acetylated compound. The most significant differences were the downfield shift of the multiplet ascribed to H-11 from δ3.99 to δ4.90, and the appearance of a sharp singlet integrating to three protons at δ1.95. This successful acetylation provided further proof that a hydroxy substituent was present at C-11.

The ^{13}C NMR data (**Spectrum 26, p 185**) indicated that the skeleton contained seventeen carbon atoms. Nine resonances were observed in the shift range δ>90ppm. Of these downfield resonances, four singlets were asssigned to the quaternary carbons (C-6a, C-8, C-9 and C-10a) and two doublets to the methine carbons (C-7 and C-10) of the aromatic A ring. The remaining two methine carbons were assigned to the carbon atoms of the 1,2-double bond and the methylene carbon to the methylenedioxy group. The aliphatic chemical shift range revealed eight resonances corresponding to the two oxygenated methine carbons C-3 and C-11, one methine C-4a, and three methylene carbons C-4, C-6 and C-12. The ^{13}C NMR data for compound IV was compared to two sets of literature data for haemanthamine (**Table 4.4**). The first set of data for haemanthamine was incomplete and ambiguous [20] but the second was more complete and corresponded to the data obtained for compound IV [21].

Table 4.4 ^{13}C NMR data for compound IV and literature data for haemanthamine [20,21].

	^{13}C NMR data for compound IV	^{13}C NMR literature data [21] for haemanthamine	^{13}C NMR literature data [20] for haemanthamine
Carbon No.	**#Shift/ δ_C ppm**	***Shift/ δ_C ppm**	****Shift/ δ_C ppm**
C-1	129.46 d	127.3 d	127.0 d
C-2	130.59 d	131.6 d	128.0 d
C-3	74.29 d	72.7 d	73.0 d
C-4	29.09 t	28.1 t	29.5 t
C-4a	64.03 d	62.6 d	62.7 d
C-6	61.71 t	61.2 t	61.5 t
C-6a	126.94 s	126.5 s	
C-7	107.86 d	106.7 d	
C-8	147.74 s	146.0 s	
C-9	148.20 s	146.3 s	
C-10	104.28 d	103.2 d	
C-10a	137.05 s	135.2 s	
C-10b	51.47 s	49.9 s	50.0 d
C-11	81.09 d	80.0 d	80.0 d
C-12	63.85 t	63.5 t	63.3 t
OCH_2O	102.25 t	100.7 t	
OCH_3	56.74 q	56.5 q	

^{13}C NMR spectrum measured in CD_3OD, 75 MHz.

* ^{13}C NMR spectrum measured in $CDCl_3$, 75 MHz.

** ^{13}C NMR spectrum measured in $CDCl_3$, 22.63 MHz.

There appears to be confusion in the literature regarding the stereochemistry at C-11 for haemanthamine. Bastida *et al.* [18] isolated a crinane alkaloid of the haemanthamine series from *Narcissus confusus,* which they described as the alkaloid heamanthamine. They reported a positive NOE effect between H-11 and H-4α and concluded that the stereochemistry at C-11 was (*R*). The optical rotation of the alkaloid was not reported. Pabuccuoglu *et al.* investigated similar compounds

from *Sternbergia sicula* and *Sternbergia lutea* [17]. They performed NOE studies on the various alkaloids obtained and concluded that the alkaloid haemanthamine has an (*S*) configuration at C-11 and a positive optical rotation. They also report the isolation of the alkaloid 11-*epi*-haemanthamine, which they illustrate as having an (*R*) configuration at C-11 and a negative optical rotation. The *Dictionary of Natural Products* [22] and the *Dictionary of Alkaloids* [23] support the latter designation and describes haemanthamine as having an (*S*) configuration at C-11 and a positive optical rotation, and 11-*epi*-haemanthamine as having an (*R*) configuration at C-11 and a negative optical rotation.

A NOESY NMR spectrum (**Spectrum 28, p 187**) was obtained for compound IV. The spectrum showed a positive NOE effect between H-11 and H-1. Furthermore, a positive NOE effect was observed between H-11 and H-12α, which in turn had a positive NOE correlation to H-6α. These correlations suggested that the C-11 hydroxy group was of the β configuration and that C-11 had an (*S*) stereochemistry. Compound IV also had a positive optical rotation which further confirmed the identification of this alkaloid as haemanthamine, according to *The Dictionary of Natural Products* [22]and Pabuccuoglu *et al.*

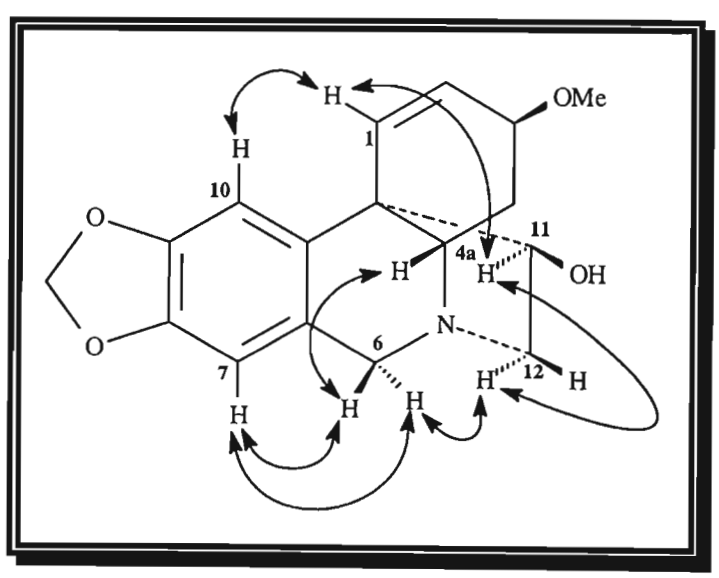


Figure 4.11 The NOE correlations observed for compound IV

The NOESY spectrum also confirmed the β orientation of H-4a, as a positive NOE effect was observed between this proton and H-6β. In addition, a positive NOE correlation was observed between the aromatic proton at δ6.93 and H-1, and between the aromatic proton at δ6.57 and both

H-6 protons, hereby confirming their assignment as H-10 and H-7 respectively.

Finally, the absolute configuration of the 5,10b-ethano bridge was determined using circular dichroism spectroscopy. In this technique, the shape, amplitude and sign of the CD spectrum can be used to determine configurational and conformational aspects of optically active compounds [24]. The sign of the CD bands, in particular, is generally related to the configuration of the molecule. Consequently, comparison to compounds of known stereochemistry can enable the researcher to determine the stereochemistry of the unknown compound. This technique is particularly useful in determining the stereochemistry of the ethano-bridge in crinane compounds since the configuration of the optically active benzyllic carbon C-10b dictates the shape and sign of the CD spectrum. Reference to literature [21,24,25] indicated that compound IV was of the α-5,10b-ethanophenanthridine series, since a minimum was observed at 244.5 nm and a maximum at 285.5 nm in the CD spectrum (**Spectrum 33, p 192**). This is in direct contrast to crinane alkaloids with a β-5,10b-ethano bridge where a maximum is generally observed at approximately 250nm and a minimum at approximately 290nm [24,25].

4.2.3 THE STRUCTURAL ELUCIDATION OF COMPOUND V

The third compound isolated from the bulb extract was also isolated from the leaf extract in high yield and was identified as the alkaloid lycorine (**compound V**) which is the most commonly isolated alkaloid from the Amaryllidaceae family [3].

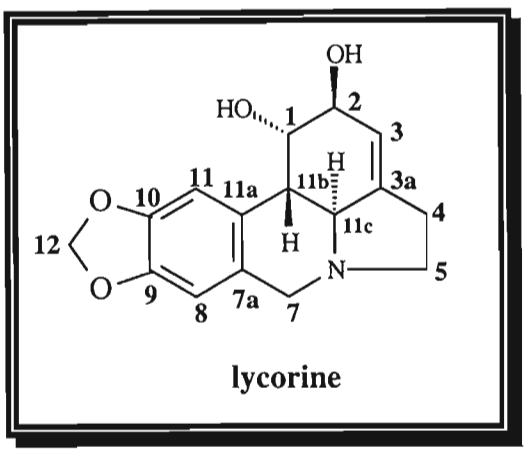


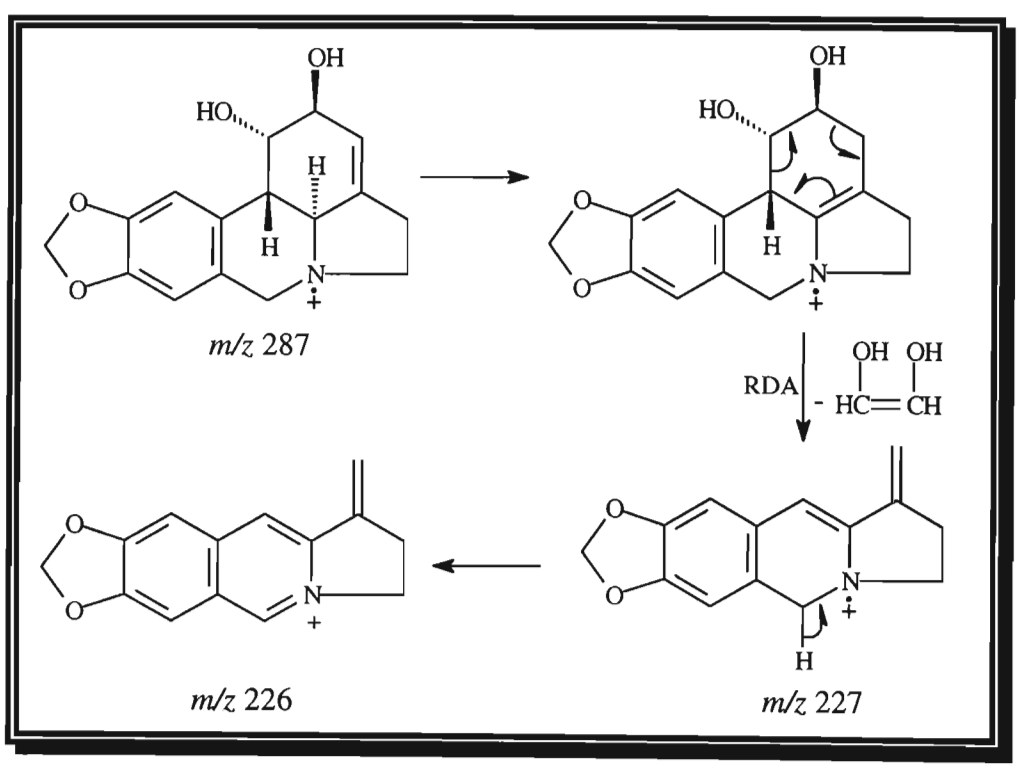
Figure 4.12 Compound V: lycorine

This compound was isolated as a white crystalline material that was characteristically insoluble in most organic solvents [7,26]. Investigations have shown that this alkaloid has distinct biological activities, which include inhibition of cell growth and cell division in higher plants, algae and yeasts [26,27]. This alkaloid also has the ability to inhibit ascorbic acid (AA) biosynthesis, and can thus affect certain physiological processes such as the biosynthetic control of hydroxyproline-containing proteins which require ascorbic acid [26]. In addition, studies have revealed that lycorine has the ability to inhibit cyanide-insensitive respiration and peroxidase enhancement [26,27]. Investigations have also shown that the ascorbic acid inhibitory effect of lycorine is related to the substitution of the C-ring and a free α- side of the molecule [27]. The dioxole ring and the A and B rings have little effect on the inhibitory effect of the lycorine molecule, whereas electron donor groups at C-1 and C-2 result in increased inhibition. In addition, a large decrease in activity is also associated with an α–configuration of the D ring [27]. Further studies have revealed that this alkaloid is capable of inhibiting the growth of the murine P-388 lymphocytic leukemia (PS system), sarcoma 37, Ehrlich ascites carcinoma and lymphoma-NK/LY [28]. This compound has also been tested for anti-malarial properties, however it exhibited very low selectivity in comparison to the values observed

for the anti-malarial control compounds [29]. The cytotoxic potential of lycorine was also recorded and this compound was found to be very cytotoxic against all the cancer cell lines tested which included BCA-1 (human breast cancer), HT-1080 (human fibrosarcoma), LUC-1 (human lung cancer),MEL-2 (human melanoma), COL-1 (human colon cancer), KB (human oral epidermoidal cancer), KB-V1 (vinblastine resistant KB), P-388 (murine lymphoid neoplasm), A-431 (human epidermoid carcinoma), LNCaP (hormone dependent human prostatic cancer), ZR-75-1 (hormone dependent breast cancer) and U-373 (human glioblastoma) [29].

High resolution mass spectrometry showed a molecular ion peak at *m/z* 287.1149 which corresponded to the molecular formula $C_{16}H_{17}NO_4$. Fragmentation peaks were also observed at *m/z* 268, *m/z* 226 and *m/z* 227 (**Spectrum 39, p 198**). The two intense signals at *m/z* 226 and *m/z* 227 are produced *via* the retro-Diels-Alder fragmentation of the parent molecule, where carbon atoms C-1 and C-2 and their substituents are eliminated from the parent molecule (**Scheme 4.10**) [30].

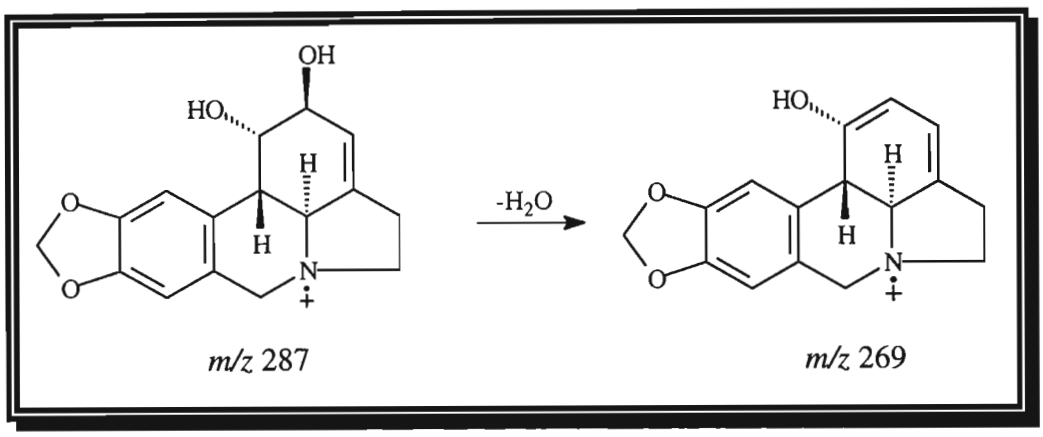
Scheme 4.10 The mass spectrometric fragmentation of compound V.



The signal at *m/z* 268 is thought to be produced *via* the elimination of water from the parent

molecule [16,30] (**Scheme 4.11**).

Scheme 4.11 The mass spectrometric fragmentation of compound V.



The infra-red data (**Spectrum 37, p 196**) supported the postulated structure for compound V and closely matched the literature data for lycorine [29]. Peaks were observed at 3335 cm^{-1} (O-H stretching), 2887 cm^{-1} (aliphatic C-H stretching), 1503 cm^{-1} (aromatic C=C stretching), 1039 cm^{-1} (C-N stretching) and at 939 cm^{-1}, which is characteristic for the methylenedioxy group.

The NMR data indicated that compound V was an alkaloid of the lycorine series. Two sets of ^1H NMR literature data for lycorine were obtained, [26,29] and compared to the ^1H NMR data for compound V (**Spectrum 34, p 193**). Comparison of the three sets of data (**Table 4.5**) revealed that compound V was not a close match for either set of literature data. Since the solvents used in the literature were 3:1 CD_3OD:CD_3OOD [26] and DMSO-d_6 [29] and the experimental data was obtained in deuterated methanol, solvent effects could account for the differences observed in the three sets of data. Consequently, in order to facilitate the assignment of the resonances for compound V, a third set of literature data [31] for a compound similar to lycorine, 9-*O*-methylpseudolycorine, which was measured in CD_3OD was obtained (**Table 4.6**). The aromatic hydrogens of the A ring, H-8 and H-11, were characteristically observed as singlet resonances at δ6.67 and δ6.92 respectively. In addition, the methylenedioxy singlet was also observed at δ5.95 hereby confirming the substitution of the A ring. The broad singlet in the double bond region of the spectrum, δ5.85, was assigned to H-3. Two additional broad singlets were observed in an upfield position at δ4.50 and δ4.20. These resonances are typical for hydrogens directly adjacent to oxygen

atoms and were thus assigned as H-1 and H-2 respectively. The doublet resonances at δ4.15 and δ3.59, with a large coupling constant of J=14.0 Hz and J=14.8 Hz respectively, are typically observed for the two protons H-7α and H-7β, and are shifted upfield due to their proximity to the nitrogen of the B-ring. Resonances ascribed to H-11c and H-11b were observed as a broad doublet at δ 2.89 (J=10.7Hz) and a multiplet at δ2.72 respectively and were assigned according to literature [29]. Finally, the multiplet at δ2.65 was assigned to the two H-4 protons, and the remaining broad quartet and the multiplet, at δ2.48 and δ3.37 respectively, were ascribed to the two non-equivalent H-5 protons.

Table 4.5 ^{1}H NMR data for compound V and literature data for lycorine [26,29].

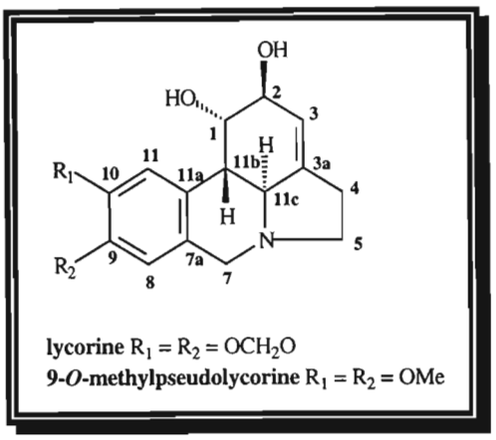
	^{1}H NMR data for compound V	**^{1}H NMR literature data [26] for lycorine**	**^{1}H NMR literature data [29] for lycorine**
Proton No.	**#Shift/ δ_H ppm**	***Shift/ δ_H ppm**	****Shift/ δ_H ppm**
H-1β	4.50 brs	4.58 dd (1.1,2.2)[a]	4.27 brs
H-2α	4.20 brs	4.26 brs	3.97 brs
H-3	5.58 brs	5.77 brs	5.37 brs
2H-4	2.65 m	2.88 m	2.44 m
H-5	2.48 brq (8.8)	3.75 m,	2.19 ddd (14.48.6,1.5)
H-5	3.37 m	3.49 m	3.19 dd (14.4, 7.5)
H-7β	4.15 d (14.0)	4.48 d (14.0)	4.02 d (14.4)
H-7α	3.59 d (14.8)	4.19 d (14.0)	3.32 d (14.4)
H-8	6.67 s	6.80 s	6.68 s
H-11	6.92 s	6.98 s	6.81 s
H-11b	2.72 m	2.99 dd (11.8, 2.2)	2.50 m
H-11c	2.89 d (10.7)	3.95 d (11.8)	2.60 d (10.6)
OCH_20	5.95 s	5.95 s	5.94 s 5.96 s

^{1}H NMR spectrum measured in CD_3OD, 300 MHz.

* ^{1}H NMR spectrum measured in CD_3OD-CD_3OOD (3:1), 270 MHz.

** ^{1}H NMR spectrum measured in DMSO-*d6*, 300 MHz.

Table 4.6 ^{1}H NMR data for compound V and literature data for 9-*O*-methylpseudolycorine [31].



lycorine $R_1 = R_2 = OCH_2O$

9-*O*-methylpseudolycorine $R_1 = R_2 = OMe$

	^{1}H NMR data for compound V	**^{1}H NMR literature data [31] for 9-*O*-methylpseudolycorine**
Proton No.	**#Shift/ δ_H ppm**	***Shift/ δ_H ppm**
H-1β	4.50 brs	4.53 dd (2.2, 2.2)
H-2α	4.20 brs	4.18 m
H-3	5.58 brs	5.55 m
2H-4	2.65 m	2.64 m
H-5	2.48 brq (8.8)	2.42 brq (8.8)
H-5	3.37 m	3.35 ddd (8.8)
H-7β	4.15 d (14.0)	4.16 d (14.0)
H-7α	3.59 d (14.8)	3.55 dd (14.0, 2.6)
H-8	6.67 s	6.75 s
H-11	6.92 s	6.96 s
H-11b	2.72 m	2.76 ddd (10.6, 2.2, 2.6)
H-11c	2.89 d (10.7)	2.87 dd (10.6,2.6)
OCH_20	5.95 s	
OCH_3		3.85 s
OCH_3		3.80 s

^{1}H NMR data measured in CD_3OD, 300 MHz.

* ^{1}H NMR data measured in CD_3OD, 270 MHz.

The ^{1}H NMR spectrum of the acetylated product (1,2-di-*O*-acetyllycorine) of compound V (**Spectrum 36, p 195**) revealed that both the C-1 and C-2 hydroxy groups were acetylated. This was confirmed by the downfield shift of the H-1 and H-2 proton resonances from δ4.50 and δ4.20 to δ5.73 and δ5.24 respectively and the appearance of two intense singlets, each integrating to three protons, at δ2.01 and δ1.90 corresponding to the methyl protons of the two acetate groups.

Sixteen signals were observed in the ^{13}C NMR spectrum (**Spectrum 35, p 194**), which was consistent with the proposed structure for compound V. Nine resonances were observed in the downfield region δ>90 ppm. These signals were assigned to the methylene carbon of the methylenedioxy group, the two methine aromatic carbons C-8 and C-11, the four quaternary aromatic carbons C-7a, C-9, C-10 and C-11a, and the olefinic carbons C-3 and C-3a. The seven resonances in the aliphatic region were similarly assigned to the three methylene carbons C-4, C-5 and C-7, the two oxygenated methine carbons C-1 and C-2, and to the two methine carbons 11b and 11c. The ^{13}C NMR data for compound V compared favourably to the literature data [27] for lycorine and is assigned in **Table 4.7**.

Table 4.7 ^{13}C NMR data for compound V and literature data for lyorine [29].

	^{13}C NMR data for compound V	^{13}C NMR literature data [29] for lycorine
Carbon No.	**#Shift/ δ_C ppm**	*** Shift/ δ_C ppm**
C-1	73.10 d	70.21 d
C-2	71.91 d	71.72 d
C-3	119.24 d	118.48 d
C-3a	143.57 s	141.68 s
C-4	29.33 t	28.13 t
C-5	54.71 t	53.31 t
C-7	57.74 t	56.73 t
C-7a	129.76 s	129.57 s
C-8	108.23 d	107.01 d
C-9	147.70 s	145.20 s
C-10	148.21 s	145.65 s
C-11	106.03 d	105.06 d
C-11a	130.10 s	129.57 s
C-11b	41.29 d	40.18 d
C-11c	62.47 d	60.83 d
OCH_2O	102.32 t	100.57 t

^{13}C NMR spectrum measured in CD_3OD, 75 MHz

* ^{13}C NMR spectrum measured in DMSO-*d6*, 75.6 MHz.

4.2.4 THE STRUCTURAL ELUCIDATION OF COMPOUND VI

Compound VI was isolated from the bulbs in an extremely low yield as an amorphous yellow material, and was identified as 11-(*S*)-hydroxyvittatine.

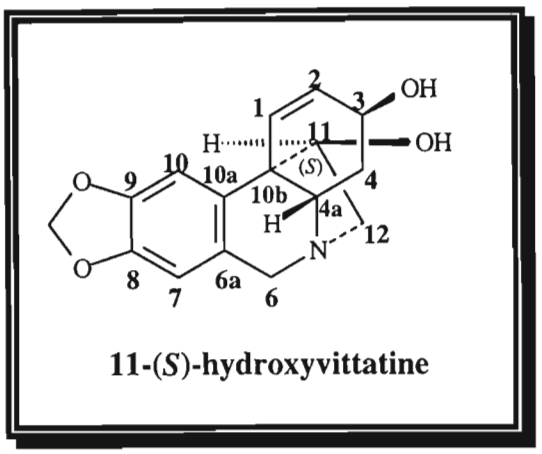


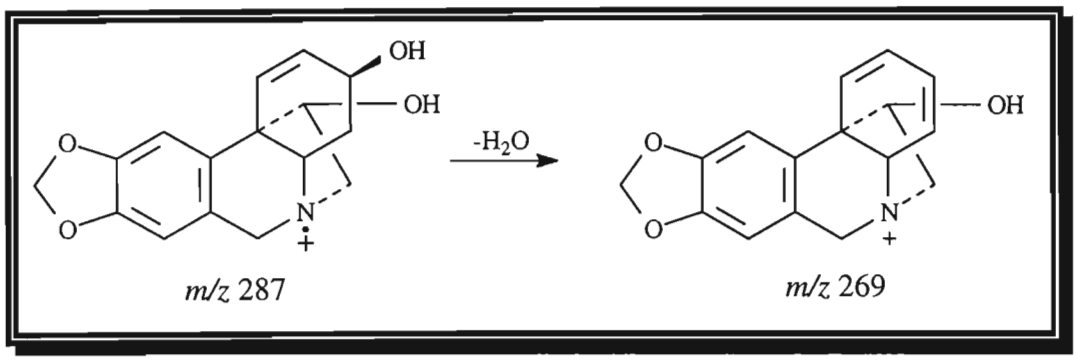
Figure 4.13 Compound VI: 11-(*S*)-hydroxyvittatine.

A molecular ion peak was observed at *m/z* 287.1163 in the high resolution mass spectrum, which corresponded to a molecular formula of $C_{16}H_{17}NO_4$. The fragmentation pattern observed in the spectrum (**Spectrum 46, p 205**) was characteristic of an alkaloid of the crinane type and further suggested that compound VI was an alkaloid of the haemanthamine series [14]. This conclusion was made on the basis of the intensity of the molecular ion peak which was consistent with compounds of the haemanthamine series with a *trans* configuration between the oxygenated C-3 substituent and the 5,10b-ethane bridge [14,15].

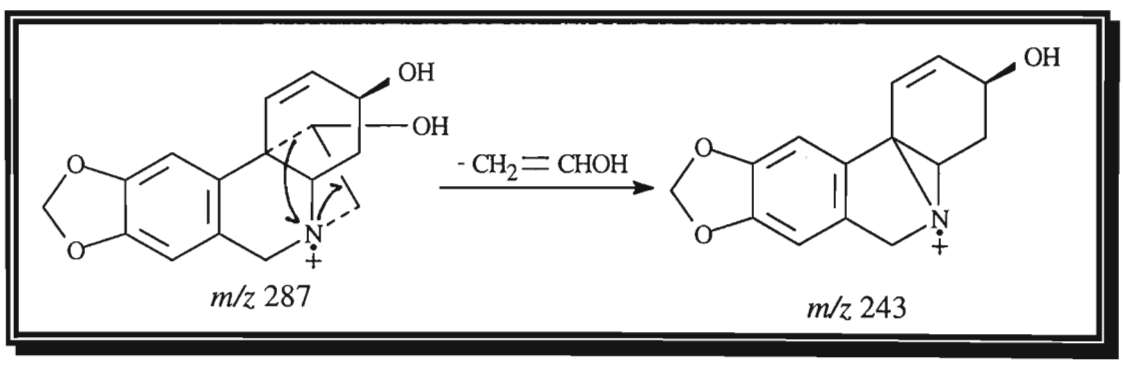
Since compound VI is an alkaloid of the haemanthamine series, the fragmentation ions are derived *via* the same routes as that for compound IV, haemanthamine. Consequently, two theories can be postulated for the origin of a number of the fragments.

An analogous theory to that postulated by Duffield *et al.* for haemanthamine can thus be used to describe the origin of the fragment at *m/z* 269 for compound VI. This fragment is produced *via* the elimination of water as seen in **scheme 4.12** [14]. Similarly, the peak at *m/z* 243 for compound VI is analogous with the peak at *m/z* 257 for haemanthamine and corresponds to the loss of the hydroxylated ethylene bridge (**Scheme 4.13**) [14].

Scheme 4.12 The mass spectrometric fragmentation of compound VI [14].

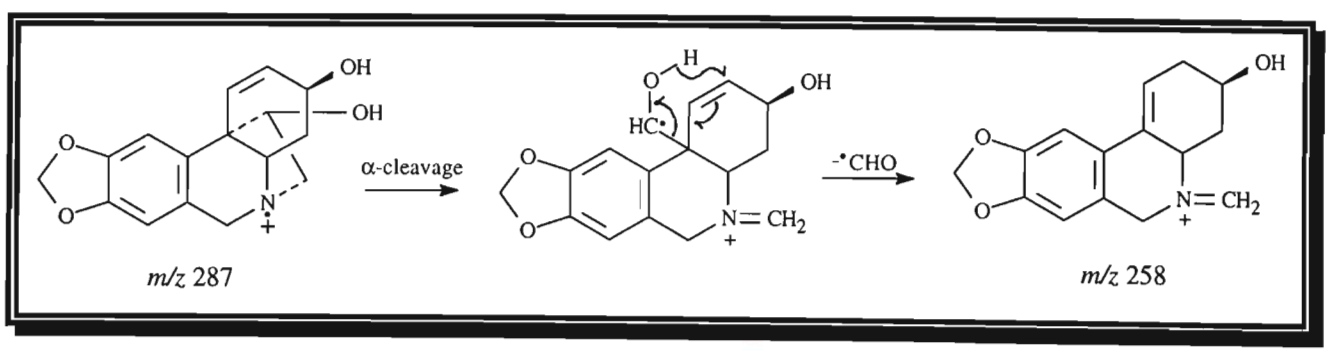


Scheme 4.13 The mass spectrometric fragmentation of compound VI [14].



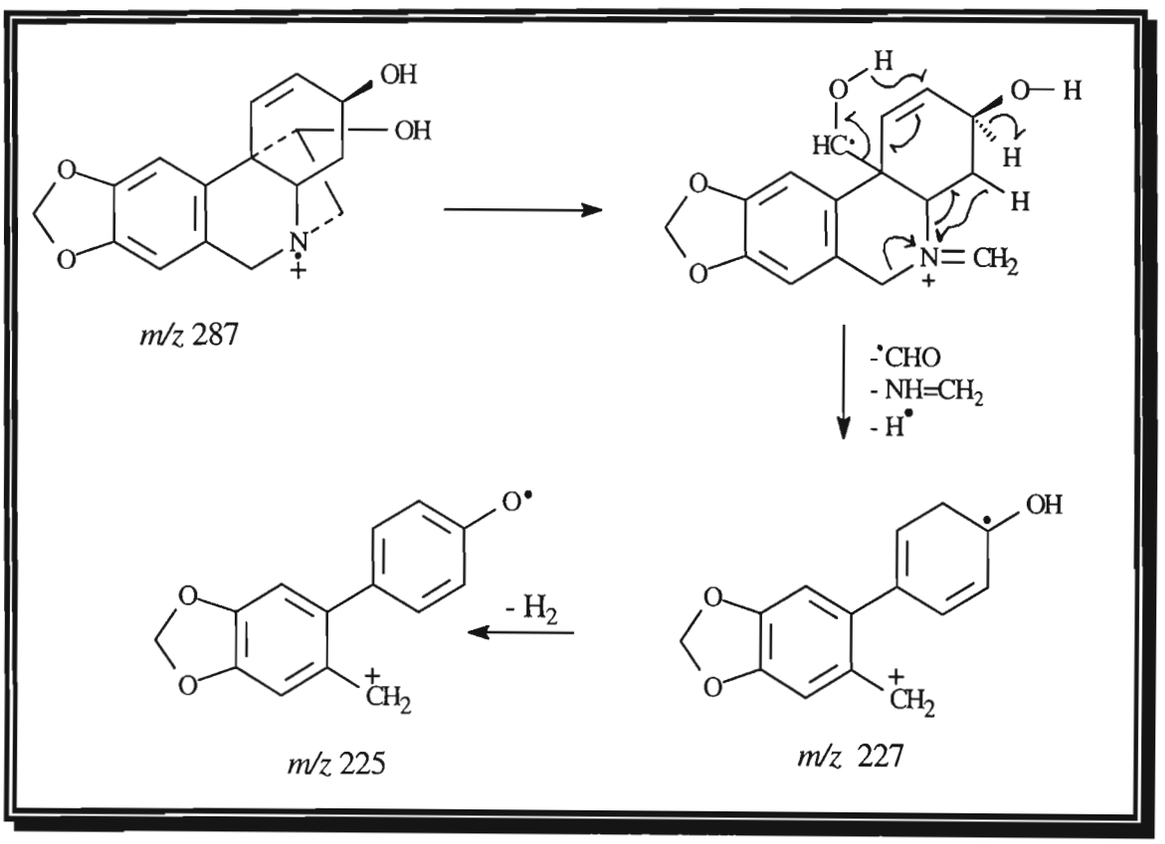
According to the Duffield *et al.* fragmentation theory, the fragment at *m/z* 258 is produced *via* the sequence shown in scheme 4.14 [14].

Scheme 4.14 The mass spectrometric fragmentation of compound VI [14].

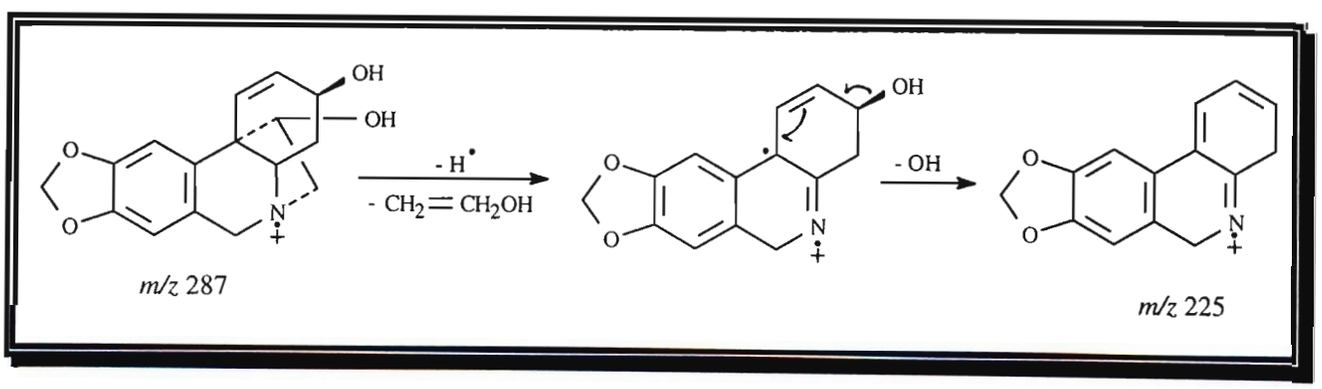


The fragments at *m/z* 227 and *m/z* 225 for compound VI are produced in a similar manner to the same molecular ion peaks for haemanthamine (**Scheme 4.15**) [14]. Once again, two hypotheses exist for the derivation of the *m/z* 225 moeity (**Scheme 4.15 and 4.16**) [14].

Scheme 4.15 The mass spectrometric fragmentation of compound VI [14].

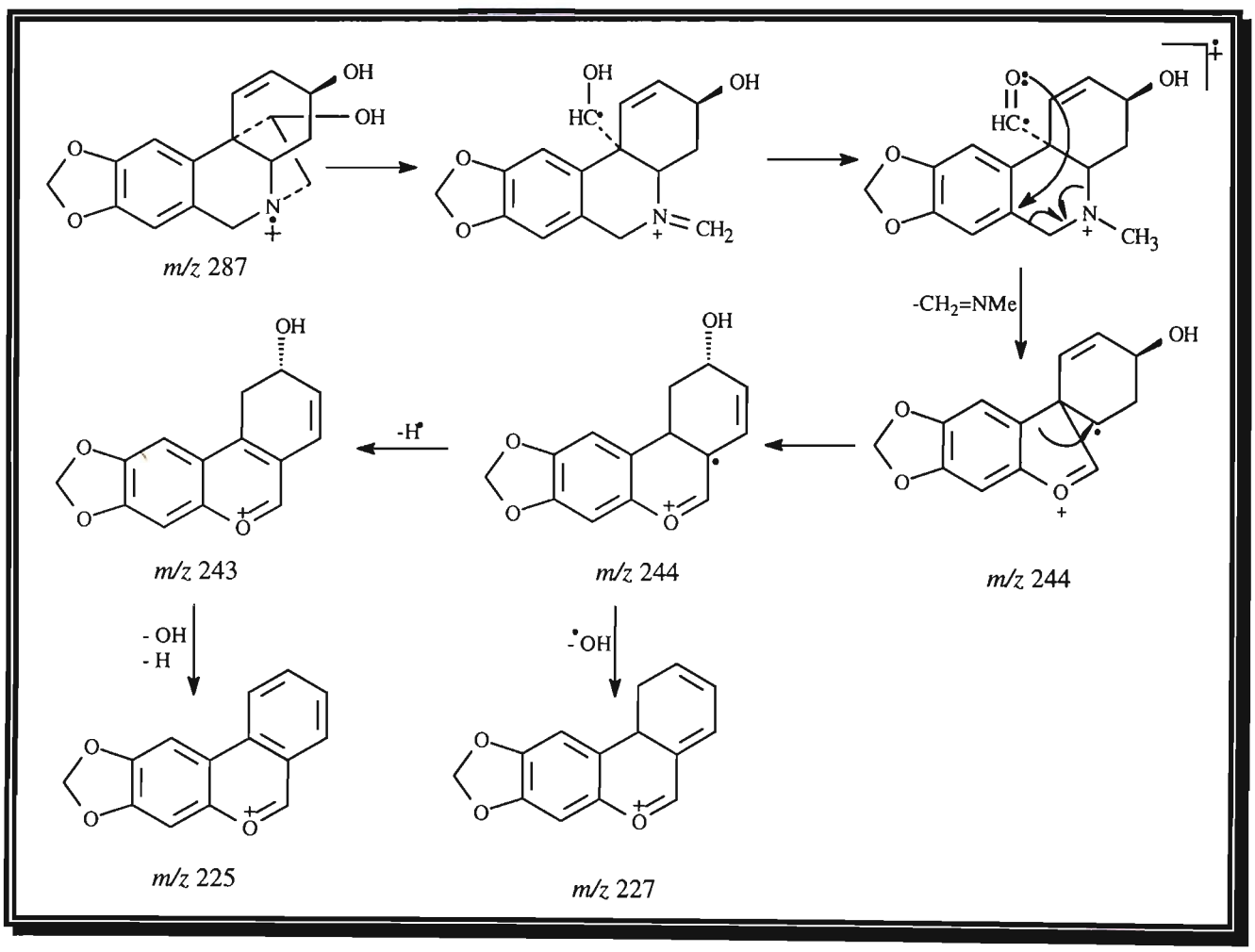


Scheme 4.16 The mass spectrometric fragmentation of compound VI [14].



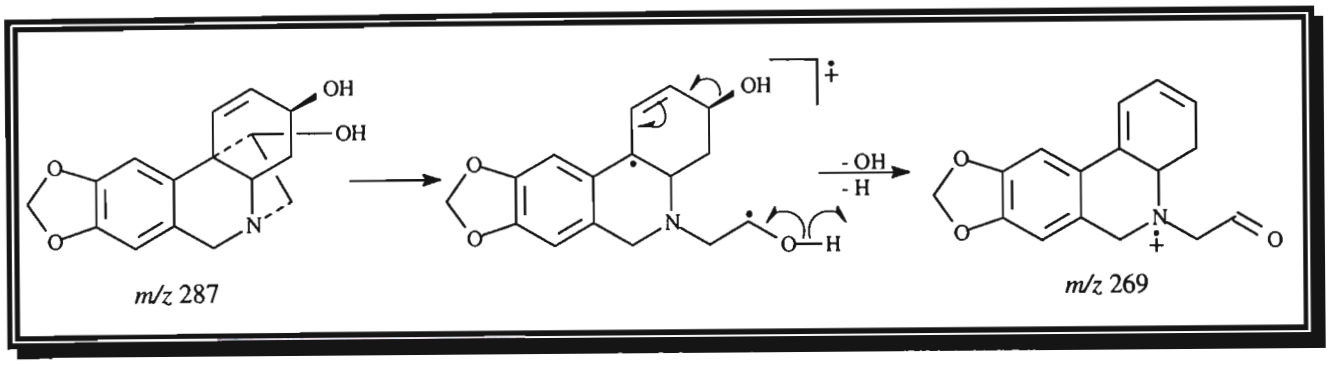
The second literature source [16], which postulates a different mechanism for the derivation of the peaks at *m/z* 243, *m/z* 244, *m/z* 225 and *m/z* 227, suggests that these peaks are derived according to **scheme 4.17** .

Scheme 4.17 The mass spectrometric fragmentation of compound VI [16].



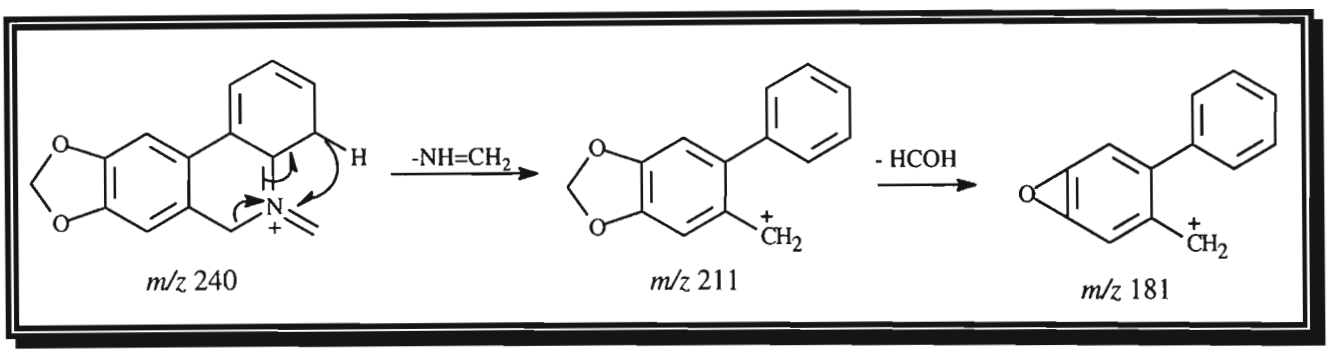
Once again, the second fragmentation theory [16] can be used to postulate a completely different derivation for the peak at *m/z* 269 (**Scheme 4.18**).

Scheme 4.18 The mass spectrometric fragmentation of compound VI [16].



Both fragmentation theories [14,16] agree on the origin of the fragmentation ions at *m/z* 211, *m/z* 181 and *m/z* 240 (**Scheme 4.19**).

Scheme 4.19 The mass spectrometric fragmentation of compound VI.



The infra-red data (**Spectrum 44, p 203**) supported the postulated structure for compound VI. Peaks were observed at 3426 cm^{-1} (O-H stretching), 2925 cm^{-1} (aliphatic C-H stretching), 1482 cm^{-1} (aromatic C=C stretching), 1038 cm^{-1} (C-N stretching) and the characteristic peak for the methylenedioxy group was observed at 936 cm^{-1}.

At first glance, the ^{1}H NMR spectrum of compound VI (**Spectrum 40, p 199**) looked remarkably like that of compound IV (**Spectrum 24, p 183**), haemanthamine (**Table 4.8**), and the implication was that this compound was also of the haemanthamine series.

Table 4.8 ^{1}H NMR data for compounds IV and VI.

	^{1}H NMR data for compound VI	^{1}H NMR data for compound IV
Proton No.	***Shift/ δ_H ppm**	***Shift/ δ_H ppm**
H-1	6.45 d (9.9)	6.50 d (10.1)
H-2	6.22 dd (10.1, 5.3)	6.28 dd (10.3, 5.0)
H-3	**4.34 m**	**3.90 m**
H-4α	2.29 ddd (13.2, 9.0, 4.4)	2.20 ddd (4.4, 9.2, 13.6)
H-4β	1.86 dd (13.2, 4.4)	2.00 ddd (1.6, 12.2, 13.3)
H-4a	3.46 m	3.32 dd (11.0, 4.5)
H-6β	4.36 d (17.0)	4.31 d (16.5)
H-6α	3.83 d (16.5)	3.78 d (16.9)
H-7	6.60 s	6.57 s
H-10	6.97 s	6.93 s
H-11	4.01 m	3.99 dd (7.2, 2.8)
H-12α	3.51 m	3.46 dd (13.9, 7.1)
H-12β	3.19 dd (14.0, 2.9)	3.16 dd (13.7, 3.2)
OCH_2O	5.92 s	5.91 s
OCH3		**3.39 s**

* ^{1}H NMR spectrum measured in CD_3OD, 300 MHz.

Indeed, once again the primary NMR evidence for this assumption was the doublet and double doublet splitting patterns of the olefinic hydrogens H-1 and H-2 which were observed at δ6.45 and δ6.22 respectively. The implication was that compound VI exhibited a *trans* relationship between the 5-10b, ethano bridge and the oxygenated sustituent at C-3 and consequently belonged to the haemanthamine series [19]. Three singlets were observed in the downfield region at δ6.97, δ6.60 and δ5.92 and were assigned to the aromatic protons of the A ring H-10 and H-7, and the methylenedioxy protons respectively. The assignment of the more downfield aromatic resonance as H-10 was, once again, made on the basis of the spatial proximity of H-10 to the 1,2-double bond which induces a deshielding effect [19].

The aliphatic region of the spectrum of compound VI looked very similar to that of haemanthamine, the only marked differences being the absence of a methoxy group proton singlet and the downfield

shift of H-3 (**Table 4.8**). The inference was that the substituent at C-3 for this compound was a hydroxy group as opposed to the methoxy group in haemanthamine. Two doublets with characteristically large coupling constants of approximately 17 Hz were observed at δ4.36 and δ3.83 for the non-equivalent H-6 protons. The expected strong coupling between these two resonances in the COSY spectrum was also evident.

The multiplet at δ4.31 was indicative of a proton adjacent to an oxygen atom. This resonance was coupled to the H-2 double doublet and was ascribed to H-3 even though it was significantly shifted compared to the H-3 proton for haemanthamine. Furthermore this multiplet was also coupled to the double doublet and triplet of doublets at δ1.86 and δ2.29 of H-4β and H-4α respectively, which confirmed the assignment of the multiplet as H-3. As for haemanthamine, a resonance was observed at δ4.01 and, as before, was assigned to H-11. The COSY spectrum (**Spectrum 41, p 200**) revealed strong coupling between this multiplet and the double doublet δ3.19 and the multiplet at δ3.51, which were themselves coupled to each other. The inference was that the double doublet and the multiplet were the H-12β and H-12α protons respectively. The remaining multiplet at δ3.46 was seen to be coupled in the COSY spectrum to the H-4 resonances and was assigned to H-4a.

This data corresponded closely to that of the literature values for 11-(*S*)-hydroxyvittatine (**Table 4.9**) [17] and confirmed the identification of the compound.

Table 4.9 ^{1}H NMR data for compound VI and literature data for 11-(*S*)-hydroxyvittatine [17].

	^{1}H NMR data for compound VI	^{1}H NMR literature data [17] for 11-hydroxyvittatine
Proton No.	**#Shift/ δ_H ppm**	***Shift/ δ_H ppm**
H-1	6.45 d (9.9)	6.23 d
H-2	6.22 dd (10.1, 5.3)	6.23 dd
H-3	4.34 m	4.35 m
H-4α	2.29 ddd (13.2, 9.0, 4.4)	2.30 ddd
H-4β	1.86 dd (13.2, 4.4)	1.99 ddd
H-4a	3.46 m	3.46 dd
H-6β	4.36 d (17.0)	4.35 d (16.6)
H-6α	3.83 d (16.5)	3.65 d (16.6)
H-7	6.60 s	6.34 s
H-10	6.97 s	6.69 s
H-11	4.01 m	4.00 dd
H-12α	3.51 m	3.30 m
H-12β	3.19 dd (14.0, 2.9)	3.30 m
OCH_2O	5.92 s	5.90 s

^{1}H NMR spectrum measured in CD_3OD, 300 MHz.

* ^{1}H NMR spectrum measured in $CDCl_3$/1%C_5D_5N, 360 MHz.

The ^{13}C NMR spectrum (**Spectrum 42, p 201**) furnished seventeen resonances corresponding to the necessary seventeen carbons required for the crinane skeleton. Nine resonances were observed in the down field region δ>90 and six resonances in the aliphatic region of the ^{13}C NMR spectrum of compound VI. The nine down field resonances included four quaternary carbons, C-6a, C-8, C-9 and C-10a, four methine carbons ascribed to the aromatic carbons C-7 and C-10 and the two olefinic carbons C-1 and C-2; and a methylene carbon ascribed to the methylenedioxy group. The aliphatic region revealed six resonances of which two were ascribed to the oxygenated carbons C-11 and C-3, two methine carbons C-10b and C-4a and two methylene carbons C-12 and C-4. This data was consistent with the proposed structure for 11-(*S*)-hydroxyvittatine.

There appears to be a discrepancy in the literature data regarding the structure of 11-(*S*)-hydroxyvittatine. *The Dictionary of Natural Products* [32] and *The Dictionary of Alkaloids* [33]

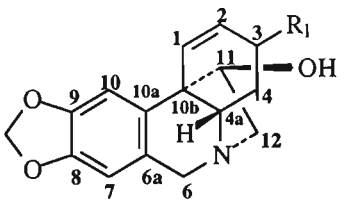
claim that vittatine and crinine are the same compound when they both, in fact, have different orientations of the 5,11b-ethano bridge, with crinine having a β configuration and vittatine having an α configuration [3]. Consequently, the reference in *The Dictionary of Natural Products* [32] and the *Dictionary of Alkaloids* [33] to 11-(*S*)-hydroxyvittatine actually refers to 11-(*S*)-hydroxycrinine.

No ^{13}C NMR literature data could be found for 11-(*S*)-hydroxyvittatine, therefore the ^{13}C NMR data for compound VI was compared to the literature data for hamayne [15], which differs from 11-(*S*)-hydroxyvittatine only in the orientation of the hydroxy group at C-3 which is α in the case of hamayne and β in the case of 11-(*S*)-hydroxyvittatine (**Table 4.10**).

As for compound IV the absolute configuration of compound VI was determined using circular dichroism. The CD spectrum obtained for compound VI (**Spectrum 47, p 206**) indicated that the 5,10b-ethano bridge was of the α orientation. A positive Cotton effect was observed at 294 nm followed by a negative cotton effect at 243 nm which was in agreement with the literature data for 11-(*S*)-hydroxyvittatine [24].

The optical rotation observed for compound VI was positive which corresponded to that recorded in the literature for 11-(*S*)-hydroxyvittatine [17] and confirmed the (*S*) configuration of C-11.

Table 4.10 ^{13}C NMR data for compound VI and compound IV and literature data for hamayne [15].



11-hydroxyvittatine R_1 = OHβ
hamayne R_1= OHα
haemanthamine R_1= OMeβ

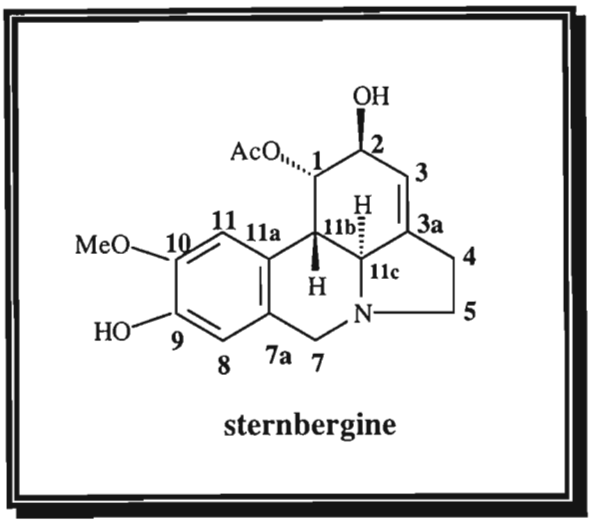
	^{13}C NMR data for compound VI	^{13}C NMR literature data [15] for hamayne	^{13}C NMR data for compound IV
Carbon No.	**#Shift/ δ_C ppm**	***Shift/ δ_C ppm**	**#Shift/ δ_C ppm**
C-1	127.84 d	122.9 d	129.46 d
C-2	132.97 d	137.4 d	130.59 d
C-3	64.65 d	67.0 d	74.29 d
C-4	32.97 t	33.2 t	29.09 t
C-4a	63.85 d	65.6 d	64.03 d
C-6	63.74 t	63.0 t	63.85t
C-6a	126.61 s	125.2 s	126.94 s
C-7	107.86 d	106.8 d	107.86 d
C-8	146.80 s	146.3 s	147.74 s
C-9	148.29 s	146.8 s	148.20 s
C-10	104.86 d	103.3 d	104.28 d
C-10a	136.99 s	135.4 s	137.05 s
C-10b	51.39 s	49.8 s	51.47 s
C-11	80.89 d	79.5 d	81.09 d
C-12	61.60 t	60.5 t	61.71 t
OCH_2O	102.29 t	101.0 t	102.25 t
OCH_3			56.74 q

^{13}C NMR spectrum measured in CD_3OD, 75 MHz.

* ^{13}C NMR spectrum measured in $CDCl_3$, 50 MHz.

4.2.5 THE STRUCTURAL ELUCIDATION OF COMPOUND VII

Compound VII was isolated from the leaf extract as a pale yellow amorphous substance and was identified as sternbergine.



sternbergine

Figure 4.14 Compound VII: sternbergine

The infra-red data for compound VII (**Spectrum 52, p 211**) was consistent with the proposed structure and closely matched the literature data for sternbergine [31]. Peaks were observed at 3427 cm^{-1} (O-H stretching), 2924 cm^{-1} (aliphatic C-H stretching), 1737 cm^{-1} (C=O stretching of acetyl group), 1514 cm^{-1} (aromatic C=C stretching) and 1041 cm^{-1} (C-N stretching). Furthermore, the absence of a peak between 930-940 cm^{-1} confirmed the fact that a methylenedioxy group was not present.

The spectral data indicated that this compound was an alkaloid of the lycorine type and the ^1H NMR for compound VII (**Spectrum 48, p 207**) was very similar to that of lycorine (compound V, **Spectrum 36, p 195**) (**Table 4.11**). The most pronounced difference observed in the spectrum of compound VII was the absence of the methylenedioxy resonance usually observed at approximately δ5.8. Nevertheless, the two singlet resonances for H-8 and H-11 at δ6.86 and δ6.63 were characteristic for aryl hydrogens adjacent to oxygen functions which implied that C-10 and C-9 were oxygenated. A singlet, integrating to three protons, was observed at δ3.85 which was ascribed to the protons of a methoxy group. The implication was thus, that one of the oxygen functions for

C-10 and C-9 was a methoxy group and one a hydroxy group. Furthermore, an additional three proton singlet at δ1.93 was observed in the proton spectrum of compound VII suggesting that an acetate group was present. Another pronounced difference between the spectra of compound V and compound VII was the absence of the H-1 singlet at δ4.50 and the appearance of a singlet at δ5.85 in the spectrum of compound VII. This further suggested that the acetate group was present at C-1. The H-1 singlet was coupled to the singlet at δ4.17 which was in turn coupled to the broad singlet at δ5.60 in the COSY spectrum (**Spectrum 49, p 208**). The two singlets were assigned to H-2 and the olefinic proton H-3 respectively. Furthermore, the fact that the olefinic proton was not coupled to the proton at δ5.58 confirmed that the acetate group was not at C-2. The two characteristic H-5 protons were observed as a broad quartet at δ2.50 and a multiplet and δ3.46 and were strongly coupled to one another in the COSY spectrum. The H-11b and H-11c protons were also coupled to one another in the COSY spectrum and were observed as doublets at δ2.92 and δ2.85 respectively. The remaining multiplet at δ2.68 was coupled to both the H-5 protons and was ascribed to the two H-4 protons. The ^{1}H NMR data was for compound VII was consistent with the literature data for sternbergine and was thus identified as such [31].

The ^{13}C NMR data (**Spectrum 50, p 209**) was consistent with the proposed structure (**Table 4.12**) and was similar to the literature data for sternbergine [31]. Eighteen resonances were observed in the ^{13}C spectrum. The most significant being the quaternary carbon signal at δ171.63, which is typical of the carbonyl of the acetate group, and the methyl signal at δ20.41, which is characteristic for the acetate group methyl carbon. In addition, the quaternary carbon signal at δ56.01 confirms the presence of a methoxy group.

Table 4.11 ^{1}H NMR data for Compounds VII and V and literature data for sternbergine [31].

	^{1}H NMR data for compound V, lycorine	**^{1}H NMR data for compound VII**	**^{1}H NMR literature data [31] for sternbergine.**
Proton NO.	**#Shift/ δ_H ppm**	**#Shift/ δ_H ppm**	***Shift/ δ_H ppm**
H-1β	4.50 brs	5.85 brs	5.76 dd (2.2, 3.3)[a]
H-2α	4.20 brs	4.17 brs	4.16 m
H-3	5.58 brs	5.60 brs	5.56 brs
2H-4	2.65 m	2.68 m	2.63 m
H-5	2.48 brq (8.8)	2.50 brq (8.9)	2.39 brq
H-5	3.37 m	3.46 d (1.9)	3.38 ddd
H-7β	4.15 d (14.0)	4.14 d (14.0)	4.14 d (14.0)
H-7α	3.49 d (14.8)	3.55 (14.3)	3.49 dd (14.0, 2.6)[a]
H-8	6.67 s	6.63 s	6.64 s
H-11	6.92 s	6.86 s	6.70 s
H-11b	2.72 m	2.92 d (10.5)	2.92 ddd[a]
H-11c	2.89 d (10.7)	2.85 d (10.7)	2.76 dd[a]
OCH_20	**5.95 s**		
OCH_3		**3.82 s**	**3.80 s**
OAc		**1.93 s**	**1.91 s**

^{1}H NMR spectrum measured in CD_3OD, 300 MHz

* ^{1}H NMR spectrum measured in $CDCl_3$, 270 MHz, [a] multiplicities observed in ^{1}H resolution enhanced spectrum

Table 4.12 ^{13}C NMR data for compound V and VII, and literature data for sternbergine [31].

Carbon No.	^{13}C NMR data for compound V, lycorine	^{13}C NMR data for compound VII	^{13}C NMR literature data [31] for sternbergine
	#Shift/ δ_C ppm	#Shift/ δ_C ppm	*Shift/ δ_C ppm
C-1	73.10 d	72.68	72.1 d
C-2	71.91 d	70.01	69.6 d
C-3	119.24 d	119.11	117.4 d
C-3a	143.57 s	146.08	144.1 s
C-4	29.33 t	28.86	28.4 t
C-5	57.74 t	56.54	56.1 t
C-7	54.71 t	54.33	53.7 t
C-7a	129.76 s	125.60	128.4 s
C-8	108.23 d	114.65	113.2 d
C-9	147.70 s	147.64	145.4 s
C-10	148.21 s	142.96	143.6 s
C-11	106.03 d	108.83	107.4 d
C-11a	130.10 s	129.10	124.9 s
C-11b	41.29 d	39.56	39.0 d
C-11c	62.47 d	62.71	61.7 d
OCH_3		56.01	55.9q
C=O		171.63	170.6 s
$C(O)CH_3$		20.41	21.0 q
OCH_2O	102.32 t		

^{13}C NMR spectrum measured in CD_3OD, MHz

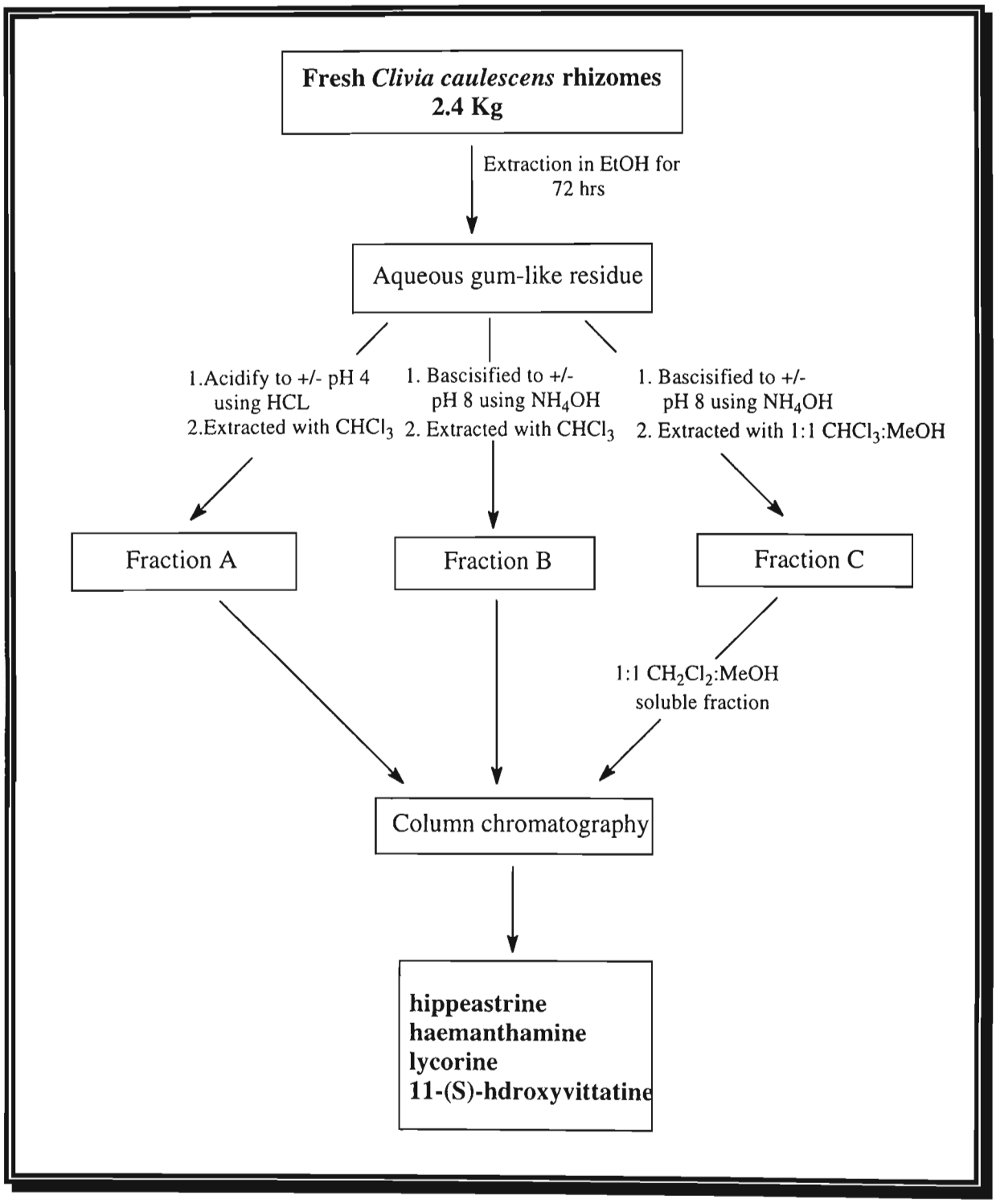
* ^{13}C NMR spectrum measured in $CDCl_3$, 50.305 MHz.

4.3 EXPERIMENTAL

Fresh *Clivia caulescens* R.A.Dyer rhizomes (2.4 kg) were collected from God's Window in Eastern Mpumalanga and a specimen voucher was retained in the Natal Herbarium (*Crouch 758*). The rhizomes were macerated and extracted with continuous agitation at room temperature for approximately 72 hrs in ethanol. A gum like residue was obtained and acidified to approximately pH 4 using concentrated hydrochloric acid. This aqueous mixture was then extracted with chloroform to produce fraction A (840mg). The remaining residue was then basicified to approximately pH 8 using concentrated ammonium hydroxide and extracted with chloroform to produce fraction B (3.95g). The remaining basic residue was then extracted with 1:1 chloroform:methanol to produce fraction C (20.26g). Thin layer chromatography revealed that fractions A, B and C were almost identical. Fraction A and B were combined but fraction C proved to be relatively insoluble and thus only that part that was soluble in 1:1 dichloromethane:methanol was added to fraction A and B. Column chromatography was then employed to afford the separation of the compounds. The polarity of the solvent was increased gradually until 100% methanol was used. Compound III, hippeastrine, was isolated from fractions 47-62; Compound IV, haemanthamine, was isolated from fractions 63-68 and compound V, lycorine, precipitated out of fractions 47-59.

The leaves of *Clivia caulescens* were also investigated. The same extraction procedure as above was used to produce the crude extract. Chromatographic techniques were, once again, employed to afford the isolation and purification of the alkaloid components. The alkaloids hippeastrine and lycorine were isolated in relatively high yield in addition to haemanthamine which was isolated in a much lower yield. Furthermore, an additional alkaloid of the lycorine type, compound VI, sternbergine, was isolated from the leaf extract.

Scheme 4.20 The extraction protocol for *Clivia caulescens*



4.3.1 PHYSICAL DATA FOR COMPOUND III

Names: Hippeastrine, Trispherene

Yield: 100 mg

Melting point: 215°C (lit. 214-216°C [11])

Mass: Molecular ion not observed.
EIMS: *m/z* 125 (100%), *m/z* 96 (38%).

Optical rotation: $[\alpha]_D = +96^o$ (*c*, 0.004 gml^{-1} in MeOH), (lit. $[\alpha]_D = +142^o$, *c*, 0.5 in EtOH [3])

Infra-red: ν_{max} (KBr), 3415, 2939, 1720, 1480, 1038, 938 cm^{-1}.

UV: λ_{max} (MeOH), (log ε),205 (3.74), 227 (3.87), 268 (3.18), 307 (3.22) nm.

^{1}H NMR: δ_H (ppm), CD_3OD

2.08 (3H, s, *N*-CH_3), 2.37 (1H, dd, J=9.7, 1.9 Hz, H-2β), 2.60 (2H, m, 2H-3), 2.68 (1H, m, H-11c), 2.90 (1H, dd, *J*=2.2, 9.6 Hz, H-11b), 3.26 (1H, m, H-2α), 4.30 (1H, brs, H-5β), 4.60 (1H, brs, H-5a), 5.70 (1H, brs, H-4), 6.14 (2H, s, OCH_2O), 7.10 (1H, s, H-11), 7.45 (1H, s, H-8).

^{13}C NMR: δ_C (ppm), CD_3OD

28.69 (t, C-3), 40.78 (d, C-11b), 43.66 (q, N-CH_3), 57.19 (t, C-2), 67.98 (d, C-5), 68.32 (d, C-11c), 84.15 (d, C-5a), 103.94 (t, OCH_2O), 109.80 (d, C-11), 110.21 (d, C-8), 119.47 (s, C-7a), 120.22 (d, C-4), 140.79 (s, C-3a), 143.33 (s, C-11a), 149.68 (s, C-9), 153.82 (s, C-10), 166.54 (s, C-7).

4.3.2 PHYSICAL DATA FOR COMPOUND IV

Names: Haemanthamine, natalensine

Yield: 40 mg

Melting point: 201°C (lit. 203°C [17])

Mass: $[M^+]$ at *m/z* 301.1310, $C_{17}H_{19}NO_4$ requires 301.1314.
EIMS: *m/z* 301 (100%), *m/z* 269 (57%), *m/z* 257 (57%), *m/z* 258 (17%), *m/z* 227 (93%), *m/z* 225 (70%), *m/z* 211 (35%), *m/z* 181 (59%).

Optical rotation: $[\alpha]_D = +32^\circ$ (*c*, 0.003 gml^{-1} in $CHCl_3$), (lit. $[\alpha]_D = +38^\circ$, *c*, 0.45 in $CHCl_3$ [3])

Infra-red: ν_{max} (KBr), 3448, 2926, 1483, 1029, 929 cm^{-1}.

UV: λ_{max} ($CDCl_3$), (log ε), 299 (4.24) nm.

Circular dichroism: λ_{max}, 245 nm [θ]= -6747, 295 nm [θ]= -937

^{1}H NMR: δ_H (ppm), CD_3OD

2.00 (1H, ddd, *J*=1.6, 12.2, 13.3 Hz, H-4β), 2.20 (1H, ddd, *J*=4.4, 9.2, 13.6 Hz, H-4α), 3.16 (1H, dd, *J*=3.2, 13.7 Hz, H-12β), 3.32 (1H, dd, *J*=4.5, 11.0 Hz, H-4a), 3.39 (3H, s, OCH_3), 3.46 (1H, dd, *J*=7.1, 13.9 Hz, H-12α), 3.78 (1H, d, *J*=16.9 Hz, H-6α), 3.90 (1H, m, H-3), 3.99 (1H, dd, *J*=2.8, 7.2 Hz, H-11), 4.31 (1H, d, *J*=16.5 Hz, H-6β), 5.91 (2H, s, OCH_2O), 6.28 (1H, dd, *J*=5.0, 10.3 Hz, H-2), 6.50 (1H, d, *J*=10.1 Hz, H-1), 6.57 (1H, s, H-7), 6.93 (1H, s, H-10).

^{13}C NMR: δ_C (ppm), CD_3OD

29.09 (t, C-4), 51.47 (s, H-10b), 56.74 (q, O-CH_3), 61.71 (t, C-6), 63.85 (t, C-12), 64.03 (d, C-4a), 74.29 (d, C-3), 81.09 (d, C-11), 102.25 (t, OCH_2O), 104.28 (d, C-10), 107.86 (d, C-7), 126.94 (s, C-6a), 129.46 (d, C-1), 130.59 (d, C-2), 137.05 (s, C-10a), 147.74 (s, C-8), 148.20 (s, C-9).

4.3.3 PHYSICAL DATA FOR COMPOUND V

Name: Lycorine

Yield: 150 mg

Melting point: 251°C (lit. 250°C [29])

Mass: $[M^+]$ at *m/z* 287.1149, $C_{16}H_{17}NO_4$ requires 287.1157.
EIMS: *m/z* 287 (36%), *m/z* 268 (26%), *m/z* 226 (100%), *m/z* 227 (87%).

Optical rotation: $[\alpha]_D = -43°$ (*c*, 0.004 gml^{-1} in MeOH), (lit. $[\alpha]_D = -62°$, *c*, 0.1 in EtOH [3])

Infra-red: ν_{max} (KBr), 3335, 2887, 1503, 1039, 939 cm^{-1}.

UV: λ_{max} (MeOH), (log ε), 204 (4.22), 237 (3.43), 292 (3.43) nm.

^{1}H NMR: δ_H (ppm), CD_3OD

2.48 (1H, brq, *J*=8.8 Hz, H-5), 2.65 (2H, m, 2H-4), 2.72 (1H, m, H-11b), 2.89 (1H, d, *J*=10.7 Hz, H-11c), 3.37 (1H, m, H-5), 3.59 (1H, d, *J*=14.8 Hz, H-7α), 4.15 (1H, d, *J*=14.0 Hz, H-7β), 4.20 (1H, brs, H-2), 4.50 (1H, brs, H-1), 5.58 (1H, brs, H-3), 5.95 (2H, s, OCH_2O), 6.67 (1H, s, H-8), 6.92 (1H, s, H-11)

^{13}C NMR: δ_C (ppm), CD_3OD

29.33 (t, C-4), 41.29 (d, C-11b), 54.71 (t, C-5), 57.74 (t, C-7), 62.47 (d, C-11c), 71.91 (d, C-2), 73.10 (d, C-1), 102.32 (t, OCH_2O), 106.03 (d, C-11), 108.23 (d, C-8), 119.24 (d, C-3), 129.76 (s, C-7a), 130.10 (s, C-11a), 143.57, (s, C-3a), 147.70 (s, C-9), 148.21 (s, C-10).

4.3.4 PHYSICAL DATA FOR COMPOUND VI

Name: 11-(*S*)-Hydroxyvittatine

Yield: 22 mg

Melting point: amorphous

Mass: $[M^+]$ at *m/z* 287.1163, $C_{16}H_{17}NO_4$ requires 287.1157.
EIMS: *m/z* 287 (96), *m/z* 270 (15%), *m/z* 269 (37%), *m/z* 268 (34%), *m/z* 258 (24%), *m/z* 243 (62%), *m/z* 227 (100%), *m/z* 225 (40%), *m/z* 211 (20%), *m/z* 181 (42%).

Optical rotation: $[\alpha]_D = +7^o$ (*c*, 0.004 gml^{-1} in MeOH).

Infra-red: ν_{max} (KBr), 3426, 2925, 1482, 1038, 936 cm^{-1}.

UV: λ_{max} (MeOH), (log ε), 206 (3.89), 229 (3.15), 293 (3.01) nm.

Circular dichroism: λ_{max}, 243 nm [θ]= -1506, 294 nm [θ]= +1441.

^{1}H NMR: δ_H (ppm), CD_3OD

1.86 (1H, dd, *J*=13.2, 4.4 Hz, H-4β), 2.29 (1H, ddd, *J*=13.2, 9.0, 4.4 Hz, H-4α), 3.19 (1H, dd, *J*=14.0, 2.9 Hz, H-12β), 3.46 (1H, m, H-4a), 3.51 (1H, m, H-12α), 3.83 (1H,d, *J*=16.5 Hz, H-6α) 4.01 (1H, m, H-11), 4.34 (1H, m, H-3), 4.36 (1H, d, *J*=17.0 Hz, H-6β), 5.92 (2H, s, OCH_2O), 6.22 (1H, dd, *J*=10.0, 5.3 Hz, H-2), 6.45 (1H, d, *J*=9.9 Hz, H-1), 6.60 (1H, s, H-7), 6.97 (1H, s, H-10).

^{13}C NMR: δ_C (ppm), CD_3OD

32.97 (t, C-4), 51.39 (s, C-10b), 61.60 (t, C-6), 63.74 (t, C-12), 63.85 (d, H-4a), 64.65 (d, H-3), 80.89 (d, H-11), 102.29 (t, OCH2O), 104.37 (d, C-10), 107.86 (d, C-7), 126.61 (s, C-6a), 127.84 (d, C-1), 132.97 (d, C-2), 136.99 (s, H-10a), 147.80 (s, C-8), 148.29 (s, C-9).

4.3.5 PHYSICAL DATA FOR COMPOUND VII

Name: Sternbergine

Yield: 20 mg

Melting point: amorphous (lit. 105-112°C form $CHCl_3$, 197-202°C from MeOH [31])

Mass: Not obtained due to decomposition of product.

Optical rotation: $[\alpha]_D = -42^\circ$ (*c*, 0.001 gml^{-1} in MeOH), (lit. $[\alpha]_D = -78.8^\circ$, *c*, 0.7 in $CHCl_3$ [3])

Infra-red: ν_{max} (KBr), 3427, 2924, 1737, 1514, 1041 cm^{-1}.

UV: λ_{max} (MeOH), (log ε), 205 (3.89), 227 (3.59), 283 (3.44) nm.

^{1}H NMR: δ_H (ppm), CD_3OD

1.93 (3H, s, O-Ac), 2.50 (1H, brq, *J*=8.8 Hz, H-5), 2.68 (2H, m, 2H-4), 2.85 (1H, d, *J*=10.7 Hz, H-11c), 2.92 (1H, d, *J*=10.5 Hz, H-11b), 3.46 (1H, d, *J*=1.9 Hz, H-5), 3.55 (1H, d, *J*=14.3 Hz, H-7α), 3.82 (3H, s, OCH_3), 4.14 (1H, d, *J*=14.0 Hz, H-7β), 4.17 (1H, brs, H-2), 5.60 (1H, brs, H-3), 5.85 (1H, brs, H-1), 6.63 (1H, s, H-8), 6.86 (1H, s, H-11).

^{13}C NMR: δ_C (ppm), CD_3OD

20.41 (q, $COCH_3$), 28.86 (t, C-4), 39.56 (d, C-11b), 54.33 (t, C-7), 56.01 (q, OCH3), 56.54 (t, C-5), 62.71 (d, C-11c), 70.01 (d, C-2), 72.68 (d C-1), 108.83 (d, C-11), 114.65 (d, C-8), 119.11 (d, C-3), 125.60 (s, C-7a), 129.10 (s, C-11a), 142.96 (s, C-10), 146.08 (s, C-3a), 147.64 (s, C-9), 171.63 (s, C=O).

4.4 REFERENCES

1. Du Plessis, N. and Duncan, G.; "*Bulbous Plants of Southern Africa–A Guide to their Cultivation and Propagation*", (1989), Tafelberg Publishers, Cape Town,
2. Dyer, R.A.; "*The Genera Of Southern African Flowering Plants-Volume 2*", (1976), Department of Agricultural Technical Services, Pretoria, 950.
3. Viladomat, F., Bastida, J., Codina, C., Nair, J.J. and Campbell, W.E.; *Recent Res. Devel. In Phytochem.*, (1997), **1**, 131.
4. Hutchings, A., Scott, A.H., Lewis, G. and Cunningham, A.B.; "*Zulu Medicinal Plant-An Inventory*", (1996), University of Natal Press, Pietermaritzburg, 50-51.
5. Watt, J.M. and Breyer-Brandwijk, M.G.; "*The Medicinal and Poisonous Plants of Southern and Eastern Africa-2nd edition*", (1962), E. & S. Livingston Ltd., Edinburgh, 28-30.
6. Munday, J.; "*Poisonous Plants in Southern African Gardens and Parks-A Field Guide*", (1988), Delta Books (PTY) Ltd., Johannesburg, 11-17.
7. Jeffs, P.W. and Mueller, L.; *J. Nat. Prod.*, (1988), **51**, 549.
8. Wildman, W.C.; in "*Chemistry of the Alkaloids*", Ed. Pelletier, S.W., (1970), Van Nostrand Reinhold Company, New York, Chpt 6, 151.
9. Jeffs, P.W., Abou-Donia, A. and Campau, D.; *J. Org. Chem.*, (1985), **50**, 1732.
10. Antoun, M.D., Mendoza, N.T. and Rios, Y.R.; *J. Nat. Prod.*, (1993), **56**, 1423.
11. Almaza, G., Fernandez, J.M., Wakori, E.W.T., Viladomat, F., Codina, C. and Bastida, J.; *Phytochem.*, (1996), **43**, 1375.
12. Hawksworth, W.A, Jeffs, P.W., Tidd, B.K. and Taube, T.P.; *J. Chem. Soc.*, (1965), 1991.
13. Bastida, J., Codina, C., Viladomat, F., Rubiralta, M., Quirion, J. and Husson, H.; *Phytochem.*, (1990), **29**, 2683.
14. Duffield, A.M., Aplin, R.T., Budzikiewicz, H., Djerassi, C., Murphy, C.F. and Wildman, W.C.; *J. Am. Chem. Soc.*, (1965), **87**, 4902.
15. Viladomat, F., Bastida, J., Codina, C., Campbell, W.E. and Mathee, S.; *Phytochem.*, (1994), **35**, 809.
16. Bastida, J., Viladomat, F., and Codina, C.; in "*Studies in Natural Product Chemistry*", (1997), Ed. Atta-ur-Rahman, Elsevier Science Publishers, Amsterdam, 20, 323.
17. Pabuccuoglu, V., Richomme, P., Gozler, T., Kivcak, B., Freyer, A. and Shamma, M.; *J. Nat. Prod.*, (1989), **52**, 785.

18. Bastida, J., Viladomat, F., Llabres, J., Codina, C., Feliz, M. and Rubiralta, M.; *Phytochem.*, (1987), **26**, 1519.

19. Haugwitz, R.D., Jeffs, P.W. and Wenkert, E.; *J. Chem. Soc.*, (1965), 2001.

20. Zetta, L., Gatti, G. and Fuganti, C.; *J. Chem. Soc. Perkin Trans II*, (1973), 1180.

21. Baudouin, G., Tillequin, F. and Koch, M.; *Heterocycles*, (1994), **38**, 965.

22. "*The Dictionary of Natural Products*", (1994), Chapman and Hall Ltd., London, H-00007.

23. Southon, I.W. and Buckingham, J.; "*Dictionary of Alkaloids*", (1989), Chapman and Hall Ltd., London, H-00002.

24. Wagner, J., Pham, H.L. and Dopke, W.; *Tetrahedron*, (1996), **52**, 6591.

25. Ali, A.A., Ramadan, M.A. and Frahm, A.W.; *Planta Med.*, (1984), **50**, 424.

26. Evidente, A., Cicala, M.R., Giudicianni, I., Randazzo, G. and Riccio, R.; *Phytochem.*, (1983), **22**, 581.

27. Evidente, A., Arrigoni, O., Liso, R., Calabrese, G. and Randazzo, G.; *Phytochem.*, (1986), **25**, 2739.

28. Pettit, G.R., Gaddamidi, V., Herald, D.L, Singh, S.B., Cragg, G.M. and Schmidt, J.M.; *J. Nat. Prod.*, (1986), **49**, 995.

29. Likhitwitayawuid, K., Angerhofer, C.K., Chai, H., Pezzuto, J.M. and Cordell, G.A.; *J. Nat. Prod.*, (1993), **56**, 1331.

30. Kinstle, T.H., Wildman, W.C. and Brown, C.L.; *Tet. Lett.*, (1966), **39**, 4659.

31. Evidente, A., Iasiello, I. and Randazzo, G.; *J. Nat. Prod.*, (1984), **47**, 1003.

32. "*The Dictionary of Natural Products*", (1994), Chapman and Hall Ltd., London, C-01995.

33. Southon, I.W. and Buckingham, J.; "*Dictionary of Alkaloids*", (1989), Chapman and Hall Ltd., London, C-00426.

CHAPTER 5

Extractives from Haemanthus pauculifolius

Haemanthus pauculifolius **in Pretoria National Botanic Gardens.**

Haemanthus pauculifolius **in flower.**

5.1. A REVIEW OF PREVIOUS WORK

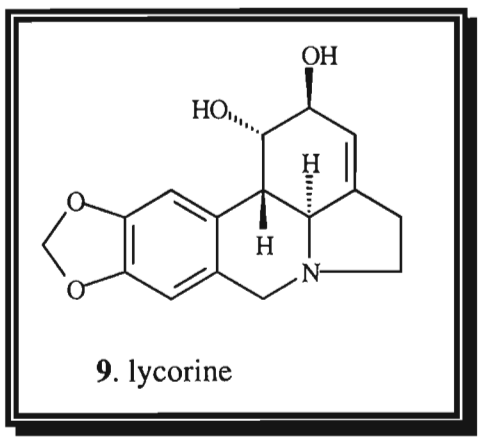
Haemanthus pauculifolius is a recently described member of the *Haemanthus* genus [1]. *Haemanthus* belongs to the Amaryllidaceae family and consists of twenty-seven taxa [2]. This genus is endemic to southern Africa and the distribution extends from central Namibia to South Africa where members of this genus are commonly found in the South Western Cape and the Transvaal [2,3]. However, the greatest concentration of species belonging to this genus is found in Namaqualand [3]. Plants belonging to the *Haemanthus* genus are generally classified as bulbous herbs with typically large fleshy bulbs [3,4]. *Haemanthus pauculifolius* is one of a group of three closely related species with white flowers and an evergreen habit [1]. In addition, these three species are shade loving and occur in summer rainfall regions [2,5]. The other two members of the group are *H. albiflos* (*H. albomaculatus*) and *H. deformis* [1].

Plants belonging to the *Haemanthus* genus have been used extensively by traditional healers in South Africa despite the fact that many of the plants are reported to be poisonous [6-8]. The bulbs of *H. albiflos* are used as an emetic and the Xhosa people have also been known to use infusions of the bulb to alleviate chronic coughs [6]. Despite the fact that this species is reported to be toxic, decoctions of the roots of *H. coccineus* are used as an emetic and the bulbs are used as diuretics in the treatment of dropsy [6,8]. The leaves of this species are also used as an antiseptic in the treatment of sores, ulcers and anthrax pustules [6,8]. *H. puniceus* is considered to be highly toxic and the bulbs and leaves have been incriminated in stock losses [7]. Furthermore, *H. amarylloides* is also toxic to animals and a 500g dose of the bulb and leaf has been known to result in the death of sheep within 24 hrs [8].

Historically, the genus *Scadoxus* has been included in *Haemanthus* [7]. Consequently, reference to chemical literature can be confusing, as plants ascribed to the *Haemanthus* genus in these papers are now, in fact, classified as part of the *Scadoxus* genus [9]. The phytochemical composition of a number of species in the *Haemanthus* genus has been investigated [9]. Several alkaloids have been isolated to date. The species that have been investigated include *H. albiflos, H. amarilloides, H. carneus, H. coccineus, H. crispus*, and *H. montanus* [9]. Alkaloids of the lycorine, homolycorine, haemanthamine/ 5,10b-ethanophenanthridine, montanine/ 5,11b-methanomorphanthridine, galanthamine and tazzetine types have been isolated.

1. The lycorine type alkaloids

The alkaloid lycorine **9** has been isolated previously from *H. coccineus* and appears to be the only alkaloid of this type isolated from this genus [9].

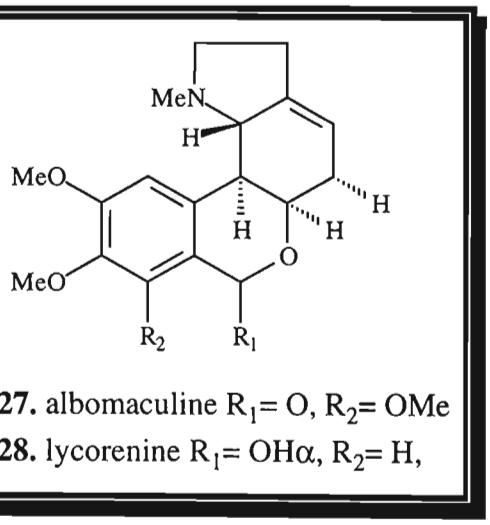


9. lycorine

Figure 5.1 Alkaloid of the lycorine type.

2. The homolycorine type alkaloids

Two alkaloids of this type, albomaculine **27** and lycorenine **28**, have both been isolated from *H. albiflos* [9]. Both of these alkaloids are characterised by a 3a,4-double bond and the presence of two methoxy groups instead of the more common methylenedioxy group. The only major difference between these two compounds is the absence of the ring C lactone for lycorenine **3**, which also has a hydroxy instead of a methoxy group at C-8.



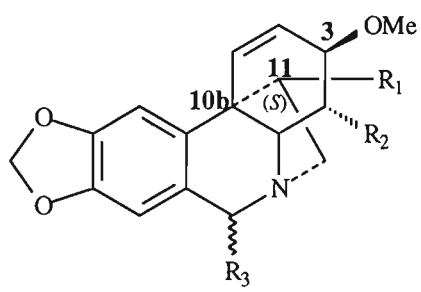
27. albomaculine R_1= O, R_2= OMe

28. lycorenine R_1= OHα, R_2= H,

Figure 5.2 Alkaloids of the homolycorine type.

3. The haemanthamine type alkaloids

Three crinane type alkaloids, all belonging to the haemanthamine series, have been isolated from *Haemanthus* [9]. All three alkaloids, haemanthamine **23**, haemanthadine **29** and albiflomanthine **30**, have a typically α orientated 5,10b-ethane bridge and a 1,2-double bond. In addition, all three alkaloids also posses a β orientated methoxy substituent at C-3 and a hydroxy substituent in the bridge at C-11. Haemanthadine **29** has an additional hydroxy substituent at C-6 and is commonly isolated as a mixture of two epimers. Albiflomanthine **30** differs from the other two in that it has a hydroxy substituent at C-4. These alkaloids have been isolated from *H. albiflos* [9].

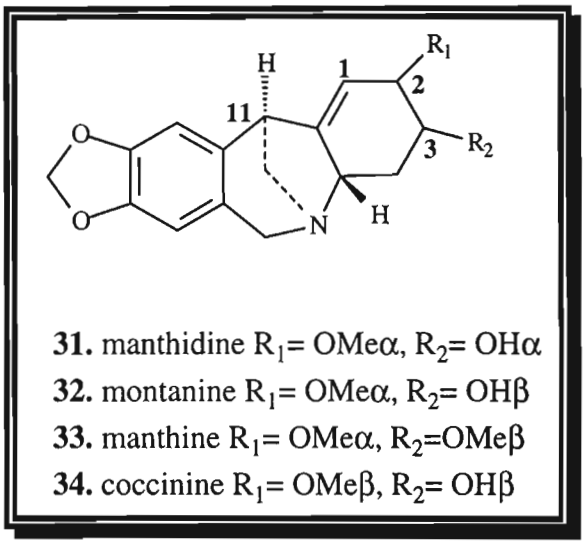


23. haemanthamine R_1= OH, R_2= H, R_3= H
29. haemanthadine* R_1= OH, R_2= H, R_3= OHα/β
30. albiflomanthine R_1= OH, R_2= OH, R_3= H
* mixture of two epimers

Figure 5.3 Alkaloids of the haemanthamine type.

4. The montanine type alkaloids.

Manthidine **31**, montanine **32**, manthine **33** and coccinine **34** are the four alkaloids of this type that have been isolated from *Haemanthus* [9]. These types of alkaloids are commonly isolated from the *Haemanthus* genus and have been isolated from four of the six species investigated [9]. All four of these compounds have an α orientated 5,11-methano bridge and a 1,11a-double bond and differ only in the nature and orientation of the oxygenated substituents at C-2 and C-3.

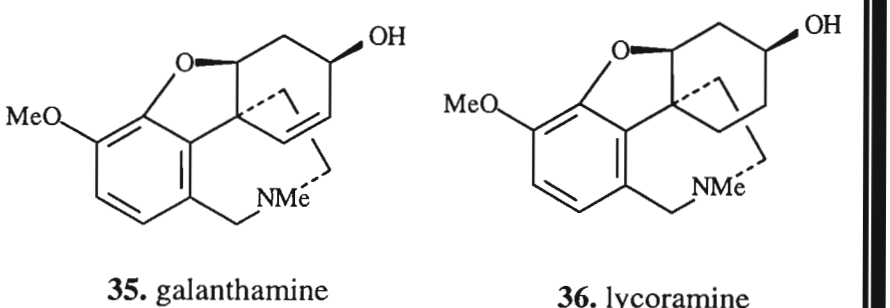


31. manthidine R_1= OMeα, R_2= OHα
32. montanine R_1= OMeα, R_2= OHβ
33. manthine R_1= OMeα, R_2=OMeβ
34. coccinine R_1= OMeβ, R_2= OHβ

Figure 5.4 Alkaloids of the montanine type.

5. The galanthamine type alakaloids

Galanthamine **35** and lycoramine **36** are the two alkaloids of this type isolated from *Haemanthus* [9]. Galanthamine **35** is commonly isolated from Amaryllidaceae plants. Both of these compounds are very similar and differ only in the absence of the double bond in lycoramine **36**.

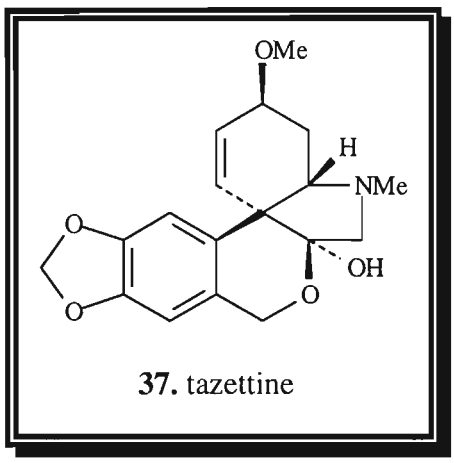


35. galanthamine **36.** lycoramine

Figure 5.5 Alkaloids of the galanthamine type.

6. The tazettine type alkaloids.

The only alkaloid of this type to be isolated from *Haemanthus* is tazettine **37** itself, which has been isolated from *H. albiflos* [9].



37. tazettine

Figure 5.6 Alkaloid of the tazettine type.

It is interesting to note that the alkaloids isolated from *H. albiflos*, which is the most closely related species to *H. pauculifolius* that has been phytochemically investigated, include albomaculine **27**, lycorenine **28**, haemanthamine **23**, haemanthidine **29**, albiflomanthine **30**, coccinine **34**, galanthamine **35**, lycoramine **36** and tazettine **37** [9].

5.2. RESULTS AND DISCUSSION

Both the bulbs and the leaves of *Haemanthus pauculifolius* were investigated. The ethanol extract of the bulbs yielded three alkaloids and a sterol. The standard acid/base extraction technique commonly used for Amaryllidaceae plants outlined in **Scheme 4.20** was not used in this case, since only a small amount of plant material was available. Compound VIII was isolated from the bulbs and identified as a novel alkaloid of the narciclasine type. Compounds IX and X were isolated from the bulbs and leaves and were originally thought to be the montanine type alkaloids, montanine and manthidine respectively. However, significant discrepancies between NMR data for both of these compounds and literature data for montanine and similar compounds indicated that these compounds were isolated as salts, montanine hydrochloride and manthidine hydrochloride respectively. Compound XI was also isolated from the bulbs and identified as sitosterol. It is interesting to note that although alkaloids of the montanine type have been isolated previously from other members of *Haemanthus*, alkaloids of the narciclasine type have not [9]. Narciclasine alkaloids have however been isolated from members of the *Scadoxus* genus [9], which is closely related to the *Haemanthus* genus.

5.2.1. THE STRUCTURAL ELUCIDATION OF COMPOUND VIII

The first compound isolated from the bulbous extract was a white crystalline material. Compound **VIII** proved to be a novel alkaloid of the isocarbostyril/narciclasine type and was called haemanthustatin.

Great interest has been shown in isocarbostyril/narciclasine alkaloids due to their particularly promising antineoplastic activity [10-14]. Although alkaloids of this type have not been previously isolated from *Haemanthus* they have been isolated from *Scadoxus*, in particular *Scadoxus multiflorus* (Martyn) Raf. subsp. multiflorus [9,10]. This species was once classified as *Haemanthus multiflorus* Martyn synonymous with *Haemanthus kalbreyeri* Baker [11]. The phenanthridone alkaloids isolated from this species include narciclasine, 7-deoxynarciclasine, kalbreclasine, pancratistatin, 7-deoxypancratistatin [9-11].

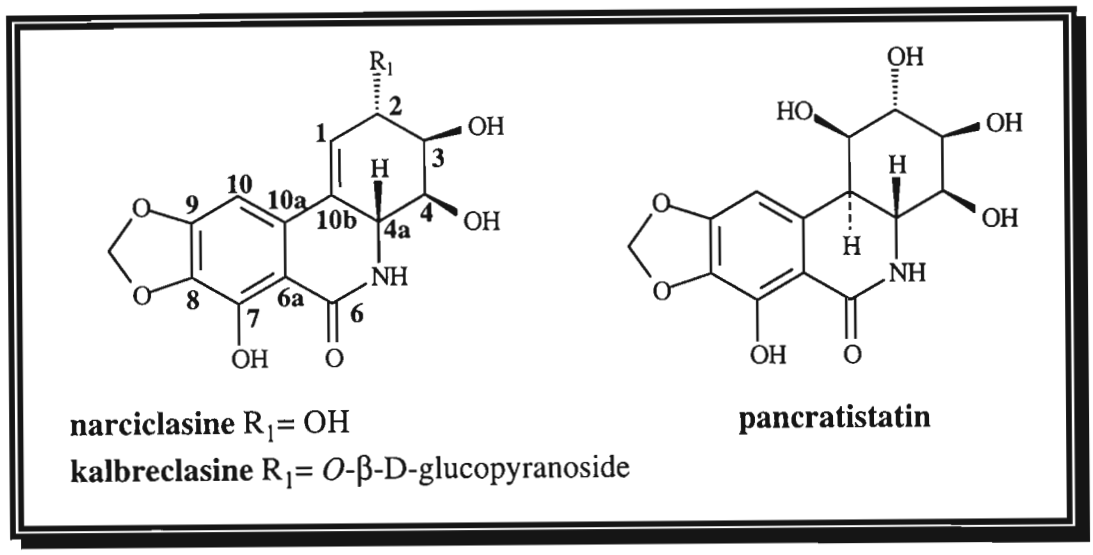


Figure 5.7 Alkaloids of the narciclasine type.

The antineoplastic properties of a number of these alkaloids has been reported in the literature [10-14]. Pettit *et al.* reported the isolation of the principal cytostatic constituent of *Zephyranthes candida*, as *trans*-1,10b-dihydronarciclasine [13]. This compound was found to strongly inhibit P-388 lymphocytic leukemia and an ED_{50} of 0.0032 µg/ml was reported [13]. Pancratistatin has reportedly been effective against the murine P-388 lymphocytic leukemia both *in vitro* and *in vivo*, and has also inhibited the growth of the *in vivo* murine M-5076 ovary sarcoma [14]. Consequently, this compound has been the subject of preclinical drug development in the United States and in Europe [12,14].

Furthermore, pancratistatin has been found to exhibit strong anti-RNA viral activity, particularly against Japanese encephalitis and has also shown activity against two other RNA flaviviruses (yellow fever and dengue) and against the bunyaviruses, Punta Tora and Rift Valley fever [14]. The compound *trans*-7-deoxy-dihydronarciclasine has also shown activity against a series of RNA viruses and is reported to be significantly cytotoxic [14]. Pettit *et al.* also report that *trans*-dihydronarciclasine exhibits substantial activity against the P-338 leukemia [14]. In their conclusion, Pettit and his co-workers postulate that narciclasine, pancratistatin and related *trans*-dihydrocarbostyrils are the most important antineoplastic components of Amaryllidaceae species that have been traditionally used in the treatment of cancer [14].

Narciclasine, pancratistatin and 7-deoxypancratistatine have been found to inhibit the germination of seeds and growth of roots in low concentrations of 1-5 x 10^{-4} M [11]. On the other hand, it has been reported that the 1-*O*-glucosides of pancratistatin and narciclasine have significantly promoted seed germination and root growth in similarly low concentrations in the same bioassay systems [11].

The mechanism of the antineoplastic action of pancratistatin is not known, however, its less potent congener, narciclasine, has been extensively researched [15]. The indication is that narciclasine inhibits the growth of eukaryotic cells by disrupting protein biosynthesis and the mechanism of inhibition has been observed in both cell free and intact cells [15].

Compound VIII was identified on the basis of spectral characteristics including, IR, NMR and UV.

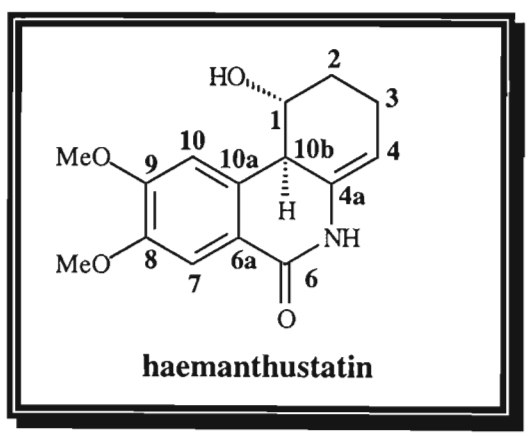


Figure 5.8 Compound VIII: haemanthustatin.

The infra-red data (**Spectrum 60, p 219**) for compound VIII was consistent with the narciclasine type structure postulated for this compound. Peaks were observed at 3434 cm^{-1} (O-H, N-H stretching), 2932 cm^{-1} (aliphatic C-H stretching), 1711 cm^{-1} (C=O stretching), 1512 cm^{-1} (aromatic C=C stretching) and 1084 cm^{-1} (C-N stretching). In addition, a peak was observed at 1601 cm^{-1} (N-H deformation), which confirmed that the nitrogen substitution for compound VIII was of the narciclasine type. The absence of a peak between 930-940 cm^{-1} further suggested that that the methylenedioxy group was not present. The carbonyl stretching of the amide group is at a higher wavenumber than would be expected but comparison with the carbonyl group of the amide for benzylamide showed that this is to be expected for an amide conjugated to an aromatic system [16].

The molecular formula could unfortunately not be obtained as the spectrum did not give a molecular ion (**Spectrum 62, p 221**). The only signal observed in the mass spectrum was at *m/z* 109. This type of fragmentation where a molecular ion peak is not obtainable has also been reported for another narciclasine type alkaloid, kalbreclasine [10].

The NMR data suggested that compound VIII was an alkaloid of the narciclasine type. However, the most significant difference between this compound and the other alkaloids of the narciclasine type that have been isolated previously, was that a 4,4a-double bond was present as opposed to the more common 1,10a-double bond found in other compounds of this type. In addition, there was no oxygenation at C-2 and C-3 in the C ring and at C-7 in the aromatic A ring. The methylenedioxy group was also absent, and is replaced by two methoxy substituents at C-8 and C-9. Consequently, the ^{1}H NMR data for compound VIII differs significantly from that for narciclasine (**Table 5.1**) [17].

Based on the ^{1}H NMR (**Spectrum 54, p 213**), COSY NMR (**Spectrum 55, p 214**), ^{13}C NMR (**Spectrum 56, p 215**) and HETCOR NMR (**Spectrum 58, p 217**) spectra, compound VIII was thought to be of the following structure (**Fig. 5.9**), with the more common 1,10b-double bond and a hydroxy substituent at C-4.

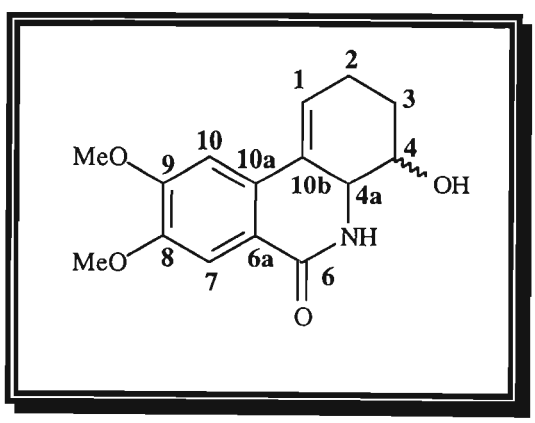


Figure 5.9 The initial structure proposed for compound VIII

Inspection of the NOESY NMR spectrum (**Spectrum 59, p 218**) showed that the assumed structure (**Figure 5.9**) was incorrect and that the actual structure for compound VIII was **Figure 5.8**.

Subsequent reappraisal of the ^{1}H NMR, COSY NMR, ^{13}C NMR and HETCOR NMR spectra showed that this data was also consistent with the **Figure 5.8** structure.

Two resonances were observed in the aromatic region of the ^{1}H NMR spectrum (**Spectrum 54, p 213**). The intense singlet at δ7.63 was ascribed to the aromatic proton of the A-ring, H-10. The remaining broad singlet at δ7.38 was similarly assigned to the H-7 proton. A methylenedioxy signal was not evident, but since only two aromatic resonances were observed, substitution at C-8 and C-9 was indicated. The broad singlet at δ5.92 is characteristic for a double bonded methine proton and was assigned to H-4. This signal was coupled to the aliphatic multiplet resonance integrating to two protons at δ2.66 in the COSY spectrum (**Spectrum 55, p 214**), which was consequently assigned to the 2H-3 protons. This multiplet was, in turn, coupled to the two proton multiplet at δ2.85 which was thus assigned to the 2H-2 protons. The doublet at a down field shift of δ5.00 was assigned to H-1. The HETCOR spectrum showed that this resonance was correlated to a ^{13}C NMR resonance at δ78.01 hereby confirming this assignment and a hydroxy group was placed at C-1. The unusual downfield shift of H-1 will be discussed later. Coupling between the multiplet at δ3.82 and the H-1 doublet was also evident and thus this resonance was ascribed to H-10b. Finally, the two remaining intense singlets, integrating to three protons each, at δ3.94 and δ4.03 are characteristic for aromatic methoxy substituents and were assigned as such.

The ^{13}C NMR spectrum (**Spectrum 56, p 215**) was in agreement with the postulated structure for compound VIII. Of the fifteen signals observed, nine were observed in the downfield region δ>90ppm and six in the aliphatic region. The aliphatic region contained two methylene carbon resonances, which were assigned to C-2 and C-3, as well as the two methine carbon signals ascribed to C-1 and C-10b. The two remaining methyl group carbon resonances were similarly ascribed to the two methoxy substituents at C-8 and C-9. The nine downfield resonances included three methine carbon signals assigned to the aromatic carbons C-7 and C-10 and the olefinic carbon C-4. The five remaining quaternary signals were similarly ascribed to the aromatic carbons C-6a, C-8, C-9, C-10a, the olefinic carbon C-4a and the carbonyl carbon C-6. The differences between the ^{13}C NMR data for compound VIII and the literature data for narciclasine [17] are significant (**Table 5.2**).

Table 5.1. ^{1}H NMR data for compound VIII and literature data for narciclasine [17].

	^{1}H NMR data for compound VIII.	**^{1}H NMR literature data [17] for narciclasine.**
Proton No.	**#Shift/ δ_H ppm**	***Shift/ δ_H ppm**
H-1	**5.00 d (4.4)**	**6.17 ddd (1.2, 2.4, 4.9)**
2H-2	2.85 m	4.23 ddd (1.2, 2.4)
2H-3	2.66 m	3.92[b] ddd (2.4)
H-4	**5.92 brs**	**3.90[b] dd (9.6)**
H-4a		4.35 ddd
H-7	7.38 brs	
H-10	7.63 s	6.75 s
H-10b	3.82 m	
OCH_2O		6.02[a] d (1.2) 6.00[a] d (1.2)
OCH_3 at C-9	3.94 s	
OCH_3 at C-8	4.03 s	

^{1}H NMR spectra measured in CD_3OD, 300 MHz.

* ^{1}H NMR spectra measured in CD_3OD, [a] This splitting only observed at 500 MHz [b] These signals partly overlap.

Table 5.2. ^{13}C NMR data for compound VIII and literature data for narciclasine [17].

	^{13}C NMR data for compound VIII.	**^{13}C NMR literature data [17] for narciclasine.**
Carbon No.	**#Shift/ δ_C ppm**	***Shift/ δ_C ppm**
C-1	78.01 d	124.7 d
C-2	31.77 t	69.1 d
C-3	27.49 t	72.3 d
C-4	121.91 d	68.8 d
C-4a	121.96 s	52.8 d
C-6	166.82 s	172.1 s
C-6a	136.16 s	129.2 s
C-7	111.94 d	168.9 s
C-8	151.22 s	152.3 s
C-9	155.59 s	144.8 s
C-10	113.69 d	95.7 d
C-10a	117.76 s	132.1 s
C-10b	69.44 d	133.3 s
OCH_2O		102.0 t
OCH_3 at (C-8)	57.15 q	
OCH_3 at (C-9)	56.65 q	

^{13}C NMR spectrum measured in CD_3OD, 300 MHz.

* ^{13}C NMR spectrum measured in CD_3SOCD_3,

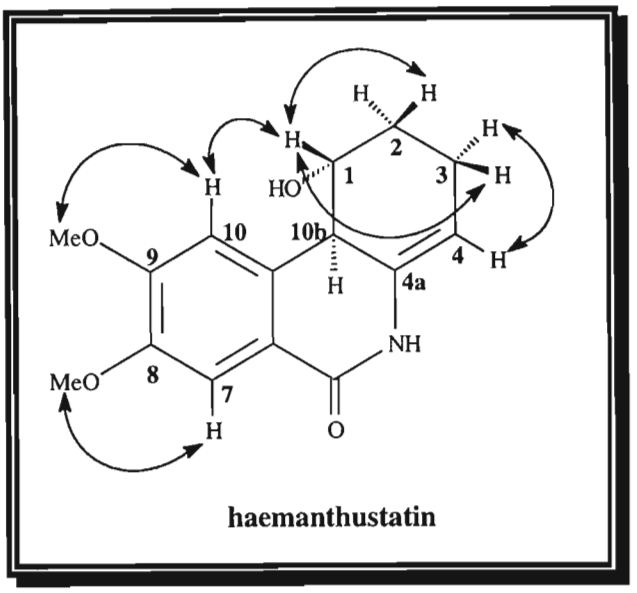


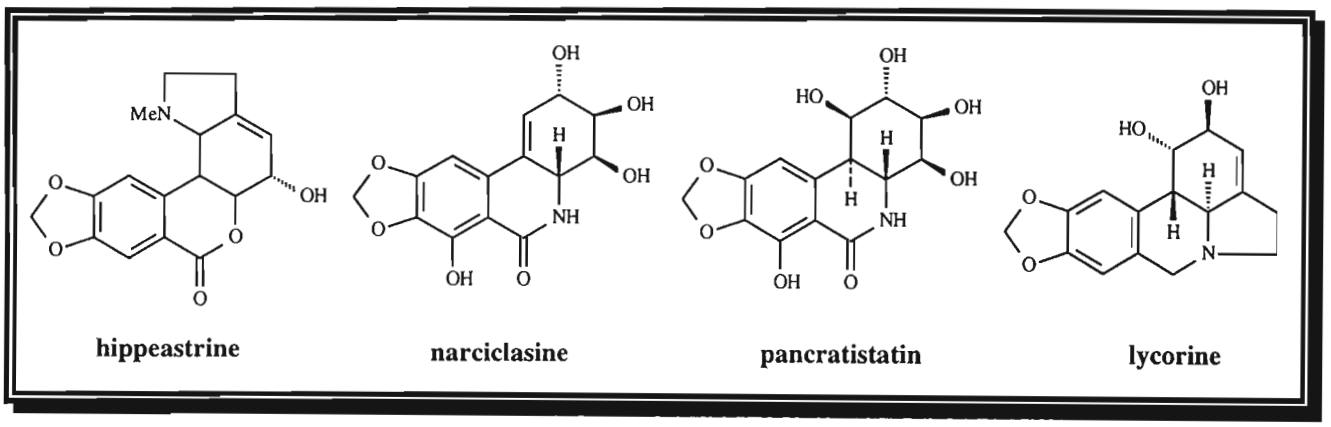
Figure 5.10 The NOE correlations observed for compound VIII

The orientations of the C-1 hydroxy group and H-10b proton were determined using the NOESY NMR spectrum (**Spectrum 59, p 218**). The spectrum revealed a positive NOE correlation between the doublet at δ5.00, which was assigned to H-1, and the aromatic proton H-10 at δ7.63. This was the primary evidence that suggested that the hydroxy group was situated at C-1 and that a 1,10b-double bond was not present, since the correlation between the H-10 proton and the olefinic proton that would be expected for a 1,10b-double bond, was not observed. Positive NOE effects were also observed between the H-1 proton at δ5.00 and the 2H-2 multiplet at δ2.85 and also between H-1 and the 2H-3 multiplet at δ2.66 protons. No NOE effect was seen between the H-10b proton mulitplet at δ3.82 and any of the other resonances. A model of the compound suggested that the only configuration that would explain the NOE correlations observed, was if both H-10b and the hydroxy group at C-1 were of the α orientation. On inspection of the model, the downfield shift of H-1β (δ5.00) could be explained based on the known anisotropic effects of benzene rings. The H-1β proton is in the deshielding region of the aromatic π-system and in this case is shifted approximately 1ppm downfield from its expected position [18].

A positive NOE effect was also observed between the methoxy group resonance at δ3.94 and the H-10 proton signal at δ7.63, which confirmed that this methoxy group is at C-9. Similarly, a positive correlation was observed between the methoxy group proton resonance at δ4.03 and the H-7 proton signal at δ7.38, which confirmed that this methoxy group was at C-8.

The UV spectrum (**Spectrum 61, p 220**) provided additional evidence for the 4,4a-double bond structure (**Fig 5.8**) as opposed to the 1,10b-double bond structure (**Fig 5.9**), which has a conjugated system through the C-6 carbonyl group, the aromatic A ring and the 1,10b-double bond. The UV spectrum obtained for compound VIII was compared to the literature data for narciclasine [12], pancratistatin [12], hippeastrine [19] (a homolycorine type compound), and lycorine [20] (**Table 5.3**). The data indicated that compound VIII was not of the narciclasine type. In fact, the UV data was most like that of the homolycorine alkaloid, which has a carbonyl group adjacent to the aromatic A ring, but lacks a conjugated double bond in the C ring. Further comparison of the UV spectrum obtained for compound VIII with the UV spectrum obtained for compound III (**Spectrum 22, p 181**), hippeastrine indicate that they were indeed similar.

Table 5.3 The UV data for compound VIII and literature data for hippeastrine [19], narciclasine [12], pancratistatin [12] and lycorine [20].



UV data for compound VIII	UV data for hippeastrine [19]	UV data for narciclasine [12]	UV data for pancratistatin [12]	UV data for lycorine [20]
λ_{max} (MeOH), (log ε)	λ_{max} (EtOH), (log ε)	λ_{max} (MeOH), (log ε)	λ_{max} (MeOH), (log ε)	λ_{max} (MeOH), (log ε)
204 (3.76)			209	207 (4.31)
			219	
226 (3.77)	227 (4.51)	233 (4.14)	233 (4.32)	236 (3.37)
268 (3.32)	268 (3.86)	248 (4.15)	278 (3.91)	
300 (3.10)	308 (3.89)	302 (3.75)	308	294 (3.56)

5.2.2. THE STRUCTURAL ELUCIDATION OF COMPOUND IX

Compound IX was isolated as a colourless amorphous substance and was identified as montanine hydrochloride.

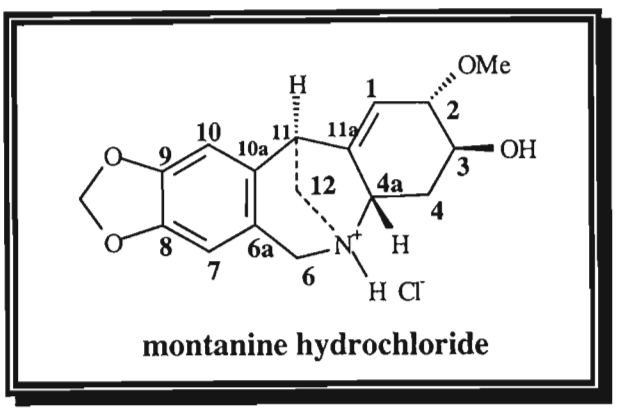
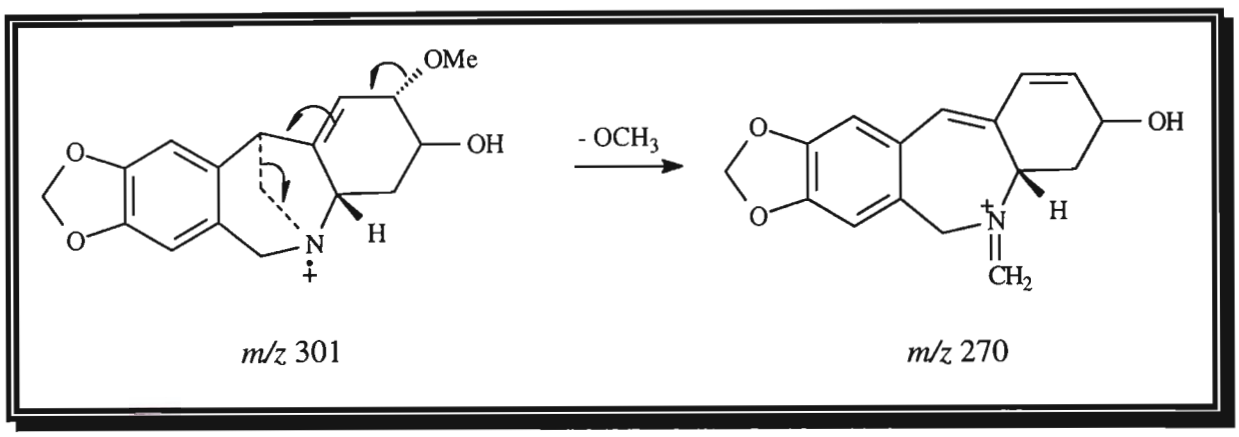


Figure 5.11 Compound IX: montanine hydrochloride

All the spectral data obtained indicated that this compound was an alkaloid of the 5,11-methanomorphanthridine or montanine type.

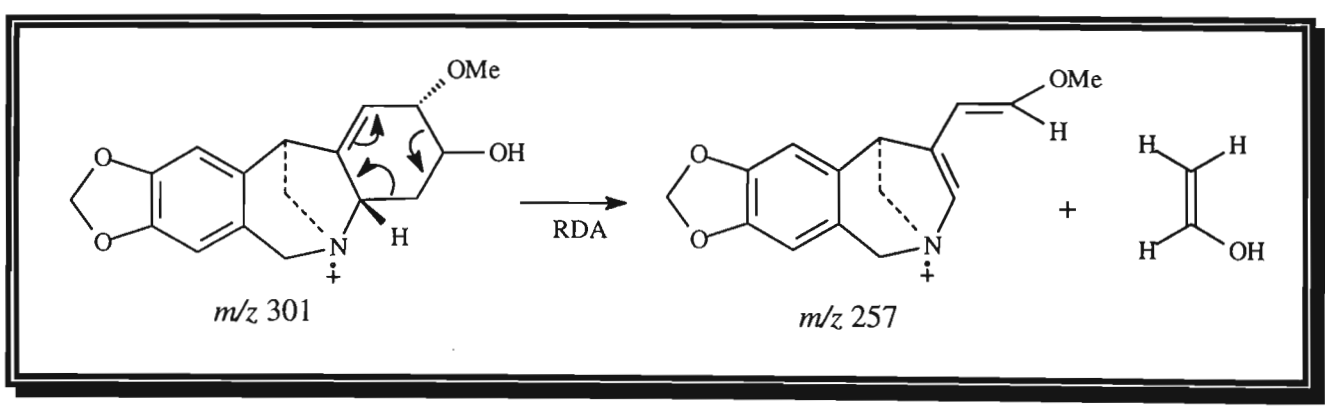
High resolution mass spectrometry showed that the highest peak in the mass spectrum of compound IX occurred at *m/z* 301.1321 and corresponded to a molecular formula of $C_{17}H_{19}NO_4$, which was consistent with the structure of montanine. Further fragments were observed at *m/z* 270, *m/z* 257, and *m/z* 223 (**Spectrum 71, p 230**). It has been suggested that the nature and stereochemical configuration of the substituents at C-2 and C-3 have a significant effect on the fragmentation observed in the mass spectra for these types of alkaloids [21]. Consequently, based on the fragmentation patterns observed the nature and stereochemical configuration of the substituents at these positions can be assigned [21]. For example, the signal at *m/z* 270 (M-31), which is thought to be derived via the elimination of the allylic methoxy group (**Scheme 5.1**) [22], can be used to determine the position of the methoxy group [21]. Although the relative intensity of the signal at *m/z* 270 is independent of the stereochemistry of the methoxy group, it has been observed that a C-2 methoxy group is cleaved in an abundance three to four times greater than a C-3 methoxy group [21]. Since the *m/z* 270 signal is particularly intense for compound IX the indication was that this compound possessed a C-2 methoxy group as opposed to a C-3 methoxy group.

Scheme 5.1. The mass spectrometric fragmentation of compound IX.



In addition, the fragment at *m/z* 257, which can be ascribed to the retro-Diels-Alder fragmentation of ring C (**Scheme 5.2**) [21,22], can be used as a diagnostic feature for the stereochemistry of the substituent at C-2 [21]. It has been shown that the stereochemisty of the C-2 substituent has a considerable effect on the extent to which the Diels-Alder fragmentation ion is observed, and there is a marked enhancement of this fragment when the C-2 substituent has an α configuration [21]. Consequently, since the *m/z* 257 fragment for compound IX was relatively intense, the indication was that the methoxy substituent at C-2 had an α configuration.

Scheme 5.2. The mass spectrometry fragmentation of compound IX.



The infra-red data obtained for compound IX (**Spectrum 69, p 228**) was consistent with the structure proposed for this compound. Peaks were observed at 3363 cm^{-1} (O-H stretching), 2947

cm^{-1} (aliphatic C-H stretching), 1486 cm^{-1} (aromatic C=C stretching), 1039 cm^{-1} (C-N stretching) and at 936 cm^{-1} which confirmed the presence of the methylenedioxy group.

The NMR data was also consistent with an alkaloid of the montanine type. The ^1H NMR spectrum (**Spectrum 63, p 222**) revealed two intense singlets in the aromatic region at δ6.83 and δ6.74 which were ascribed to the two aromatic protons of the A ring H-10 and H-7 respectively. Two intense doublets, integrating to one proton each, were observed at δ5.98 and δ5.99, and were assigned to the characteristic methylenedioxy group protons. The broad singlet in the double bond region of the spectrum at δ5.88 was assigned to the olefinic proton H-1.

The aliphatic region of the spectrum was much more complex and reference to the COSY spectrum (**Spectrum 64, p 223**) was necessary to facilitate the assignment of the signals in this region. Two doublets with a large coupling constant of 15.8Hz were observed at δ4.83 and δ4.51. These doublets were strongly coupled to each other in the COSY spectrum and are characteristic for the two non-equivalent methylene protons directly adjacent to a nitrogen atom. They were thus assigned to H-6α and H-6β respectively. The H-1 singlet was coupled to a broad singlet at δ3.57 (H-2), which was, in turn, coupled to a further broad singlet at δ4.18 (H-3). The COSY spectrum also revealed extensive coupling between the H-3 resonance and the two triplet of doublets at δ2.49 and δ1.75. Furthermore, these aliphatic resonances were strongly coupled to one another and were also coupled to a downfield double doublet at δ4.12. This coupling pattern is consistent with that expected for the two non-equivalent H-4 methylene protons which are coupled to the H-4a methine proton. The narrow doublet at δ3.97 (J=2.43Hz) was coupled to a broad doublet at δ3.70 and a narrow doublet at δ3.61 (J=2.75Hz). The two latter resonances were also strongly coupled to one another and the indication was that these three signals were due to the three protons of the bridge system, H-11 and the two non-equivalent H-12 protons respectively. The two H-12 methylene protons are significantly deshielded by the adjacent nitrogen atom. The remaining singlet, integrating to three protons, at δ3.48 was assigned to the protons of a methoxy group.

From **Table 5.4** it is obvious that there are discrepancies between the ^1H NMR literature data for montanine [23] and compound IX particularly for the H-6, H-12 and H-4a protons.

Table 5.4. ^{1}H NMR data for compound IX and literature data for montanine [23].

	^{1}H NMR data for compound IX	^{1}H NMR literature [23] data for montanine.
Proton No.	**#Shift/ δ_H ppm**	***Shift/ δ_H ppm**
H-1	5.88 brs	5.57 brs
H-2	3.57 brs	3.48 dd (2.5, 4)
H-3	4.18 brs	4.09 dd (2.5, 7.2)
H-4ax	1.75 ddd (2.4, 9.6, 12.0)	1.58 ddd (4, 12, 13)
H-4eq	2.49 ddd (4.3, 8.8, 11.5)	2.17 ddd (3, 5.5, 13)
H-4a	**4.12 dd (4.3, 12.1)**	**3.39-3.45 m**
H-6α	**4.51 d (15.8)**	**3.82 d (17)**
H-6β	**4.83 d (15.8)**	**4.35 d (17)**
H-7	6.74 s	6.46 s
H-10	6.83 s	6.55 s
H-11	3.97 d (2.4)	3.29 d (2.5)
H-12	**3.70 d (10.4)**	**3.04 d (12)**
H-12	**3.61 d (2.8)**	**3.09 dd (2.5, 12)**
OCH_2O	5.98 d (1.1) 5.99 d (1.1)	5.89 d (1.5) 5.87 d (1.5)
OCH_3	3.48 s	3.44 s

^{1}H NMR spectrum measured in CD_3OD, 300 MHz.

* ^{1}H NMR spectrum measured in $CDCl_3$, 500 MHz.

The ^{13}C NMR data for compound IX (**Spectrum 65, p 224**) was consistent with that expected for an alkaloid of the montanine type [24]. Seventeen resonances were observed with nine signals in the downfield region δ>90 ppm and eight signals in the aliphatic region. The only methylene carbon resonance at δ102.98 is typical of a methylenedioxy group carbon atom and was assigned as such. Three methine carbon signals were also observed in this region and were ascribed to the aromatic carbons of the A ring, C-7 and C-10, and to the olefinic carbon C-1. The remaining five quaternary carbon resonances in the downfield region were assigned to the aromatic carbons C-6a, C-8, C-9 and C-10a, and to the quaternary olefinic carbon C-11a. The C-11a resonance at δ149.33 is

considered to be a diagnostic feature for montanine type alkaloids [24] and played an important role in the identification of compound IX as a montanine type alkaloid. The aliphatic region contained four methine carbon resonances, which were ascribed to C-4a and C-11, and the two oxygenated carbons C-2 and C-3. The four remaining signals were assigned to the methylene carbon signals C-4, C-6 and C-12 and the methyl carbon of the C-2 methoxy group. ^{13}C NMR data for montanine was not available from the literature and thus the data for compound IX was compared to that of pancracine [24], which differs from montanine in that it has an α–hydroxy group as opposed to an α-methoxy group at C-2 (**Table 5.5**) .

The HETCOR spectrum (**Spectrum 67, p 226**) confirmed the ^{1}H and ^{13}C NMR assignments and aided in the assignment of H-11 and the two non-equivalent H-12 protons, which were difficult to assign using the COSY exclusively since all three resonances were coupled to one another.

The absolute configuration of the 5,11-methano bridge for compound IX was confirmed by the CD spectrum (**Spectrum 72, p 231**). Weak negative Cotton effects were observed at 297 nm and 283 nm followed by two strong negative cotton effects at 251 nm and 227 nm and the implication was that the bridge had an α configuration [24].

Table 5.5 ^{13}C NMR data for compound IX and literature data for pancracine [24].

	^{13}C NMR data for compound IX	^{13}C NMR literature data [24] for pancracine.
Carbon No.	#Shift/ δ_C ppm	*Shift/ δ_C ppm
C-1	118.16 d	115.89 d
C-2	78.97 d	68.95 d
C-3	67.07 d	70.99 d
C-4	29.69 t	31.33 t
C-4a	62.92 d	57.99 d
C-6	58.80 t	60.75 t
C-6a	130.23 s	132.98 s
C-7	108.06 d	$107.19 d
C-8	145.42 s	145.96 s
C-9	149.18 s	145.25 s
C-10	108.68 d	$106.76 d
C-10a	119.51 s	125.35 s
C-11	45.16 d	44.88 d
C-11a	149.33 s	152.54 s
C-12	56.11 t	55.12 t
OCH_2O	102.98 t	100.39 t
OCH_3	58.23 q	

^{13}C NMR spectrum measured in CD_3OD, 75 MHz.

* ^{13}C NMR spectrum measured in DMSO-d_6, 20 MHz.

$ Resonances may be interchanged.

The orientation of the oxygenated substituents was confirmed by the NOESY spectrum (**Spectrum 68, p 227**). A positive NOE effect was observed between the methoxy group protons and H-11, which confirmed the α configuration of the methoxy group. In addition, positive NOE correlations were observed between H-3 and the methoxy group, H-3 and the H-12 proton at δ3.70 and between

H-3 and the H-4α proton at δ1.75. These correlations all suggest that the hydroxy substituent is of the β configuration. A positive NOE effect was also observed between H-11 and the singlet at δ6.83 confirming its assignment as H-10. Similarly, the positive correlation between H-6α and the singlet at δ6.74 confirmed its assignment as H-7.

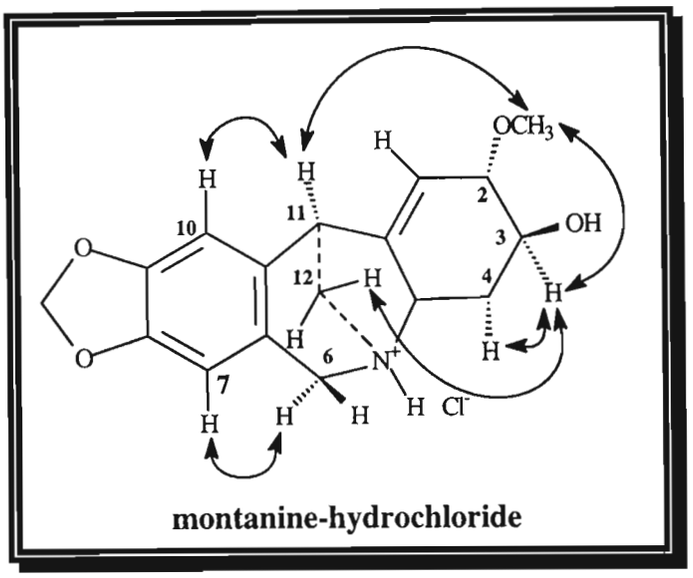


Figure 5.12 The NOE correlations observed for compound IX

Despite the fact that all the infra-red, UV, CD, mass spectrometry and NMR data indicated that compound IX was the alkaloid montanine, the chemical shifts of the H-6, H-12 and H-4a protons in the ^{1}H NMR spectrum differed from the literature data significantly [23].

Initially, solvent effects were thought to be responsible for the differences observed since the literature reported the use of $CDCl_3$ as opposed to the CD_3OD, which was used to obtain the spectrum for compound IX. However, when a ^{1}H NMR spectrum of compound IX was obtained in $CDCl_3$, the pronounced downfield shifts of the H-6, H-4a, and the H-12 protons were still observed. The downfield shifts of all the protons surrounding the nitrogen atom suggested that the nitrogen atom might be oxygenated.

Two *N*-oxides have been reported from the Amaryllidaceae family. The isolation of the homolycorine type alkaloid 8-*O*-demethylhomolycorine-*N*-oxide has been reported from *Narcissus papyraceus* [25], and the lycorine type alkaloid oxoassoanine-*N*-oxide has been reported from

Narcissus bicolor (**Figure 5.13**) [26]. The NMR data for oxoassoanine-*N*-oxide is difinitive [26]. The ^{1}H NMR data shows a downfield shift of approximately 1ppm for the two H-5 protons in the *N*-oxide. In addition, the ^{13}C NMR spectrum also shows that the C-5 carbon is shifted approximately 10ppm downfield in the *N*-oxide [26]. Although the H-6, H-4a and H-12 protons were shifted in the ^{1}H NMR spectrum of compound IX, the 10ppm downfield shift for C-6, C-4a and C-12 was not observed in the ^{13}C NMR spectrum. This indicated that the *N*-oxide was not present.

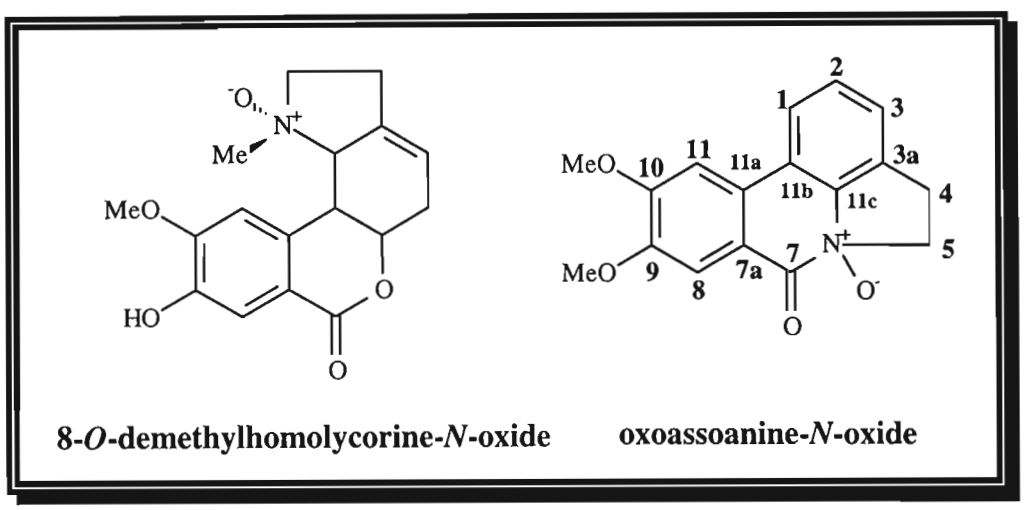


Fig 5.13 Two *N*-oxides isolated from the Amaryllidaceae family.

The possibility that there might be some other ionic interaction taking place at the nitrogen atom was considered and compound IX was treated with 4M NaOH. The ^{1}H NMR spectrum (**Spectrum 73, p 232**) of the base treated compound compared very favourably to the literature data for montanine (**Table 5.6**) [23] and the H-6, H-12 and H-4a proton resonances were at the expected chemical shifts. This suggested that the original compound was a salt. Re-examination of the mass spectrum indicated that the salt was a hydrochloride since two signals were observed at *m/z* 36 and *m/z* 38 in a ratio of 3:1 [27].

It is possible that the reason that this compound was isolated as its salt was that the usual acid /base extraction method employed for Amaryllidaceae plants was not used in this case, due to the small amount of crude residue obtained after ethanol extraction of the fresh plant material. Treatment of the crude extract with base in the extraction procedure would surely have converted the hydrochloride into the free amine. It is also interesting to note that crystals of sodium chloride

precipitated out of the crude extract in the early stages of this work. This all seems to suggest that compound IX, montanine-hydrochloride, is indeed a natural product.

Table 5.6 ^{1}H NMR data for compound IX, base treated compound IX and literature data for montanine [23].

	^{1}H NMR data for compound IX, montanine hydrochloride	**^{1}H NMR data for base treated compound IX, montanine**	**^{1}H NMR literature [23] data for montanine.**
Proton No.	**#Shift/ δ_H ppm**	***Shift/ δ_H ppm**	****Shift/ δ_H ppm**
H-1	5.88 brs	5.55 brs	5.57 brs
H-2	3.57 brs	3.44 d (2.6)	3.48 dd (2.5, 4)
H-3	4.18 brs	4.02 brs	4.09 dd (2.5, 7.2)
H-4ax	1.75 ddd (2.4, 9.6, 12.0)	1.45 ddd (3.0, 9.0, 12.5)	1.58 ddd (4, 12, 13)
H-4eq	2.49 ddd (4.3, 8.8, 11.5)	2.15 ddd (3.5, 4.9, 9.5)	2.17 ddd (3, 5.5, 13)
H-4a	**4.12 dd (4.3, 12.1)**	**3.38 m**	**3.39-3.45 m**
H-6α	**4.51 d (15.8)**	**3.78 d (16.7)**	**3.82 d (17)**
H-6β	**4.83 d (15.8)**	**4.28 d (16.5)**	**4.35 d (17)**
H-7	6.74 s	6.50 s	6.46 s
H-10	6.83 s	6.58 s	6.55 s
H-11	3.97 d (2.43)	3.35 d (3.2)	3.29 d (2.5)
H-12	**3.70 d (10.4)**	**3.00 d (2.6)**	**3.04 d (12)**
H-12	**3.61 d (2.8)**	**3.02 m**	**3.09 dd (2.5, 12)**
OCH_2O	5.98 d (1.1) 5.99 d (1.1)	5.85 d (1.1) 5.83 d (1.1)	5.89 d (1.5) 5.87 d (1.5)
OCH_3	3.48 s	3.40 s	3.44 s

^{1}H NMR spectrum measured in CD_3OD, 300 MHz.

* ^{1}H NMR spectrum measured in CD_3OD, 400 MHz.

** ^{1}H NMR spectrum measured in $CDCl_3$, 500 MHz.

The ^{13}C NMR data (**Spectrum 75, p 234**) for the base treated compound IX also corresponded more favourably to the literature data for pancracine [24] than the original compound and is assigned in **table 5.7**.

Table 5.7. ^{13}C NMR data for compound IX, base treated compound IX and literature data for pancracine [24].

	^{13} C NMR data for compound IX, montanine hydrochloride	13C NMR data for base treated compound IX, montanine	^{13}C NMR literature data [24] for pancracine.
Carbon No.	#Shift/ δ_C ppm	*Shift/ δ_C ppm	**Shift/ δ_C ppm
C-1	118.16 d	113.80	115.89 d
C-2	78.97 d	79.80	68.95 d
C-3	67.07 d	68.55	70.99 d
C-4	29.69 t	32.35	31.33 t
C-4a	62.92 d	[b]61.09	57.99 d
C-6	58.80 t	[b] 59.09	60.75 t
C-6a	130.23 s	133.17	132.98 s
C-7	108.06 d	107.91	[a]107.19 d
C-8	[a] 145.42 s	[a] 147.26	145.96 s
C-9	[a] 149.18 s	[a] 147.96	145.25 s
C-10	108.68 d	107.40	[a]106.76 d
C-10a	119.51 s	124.83	125.35 s
C-11	45.16 d	46.34	44.88 d
C-11a	149.33 s	154.29	152.54 s
C-12	56.11 t	55.76	55.12 t
OCH_2O	102.98 t	101.75	100.39 t
OCH_3	58.2.3 q	[b] 57.39	

^{13}C NMR spectrum measured in CD_3OD, 75 MHz.

* ^{13}C NMR spectrum measured in CD_3OD, 100 MHz.

** ^{13}C NMR spectrum measured in DMSO-d_6, 20 MHz.

[a/ b] Resonances may be interchanged.

5.2.3. THE STRUCTURAL ELUCIDATION OF COMPOUND X

Compound X was also isolated as an amorphous substance and was identified as manthidine hydrochloride.

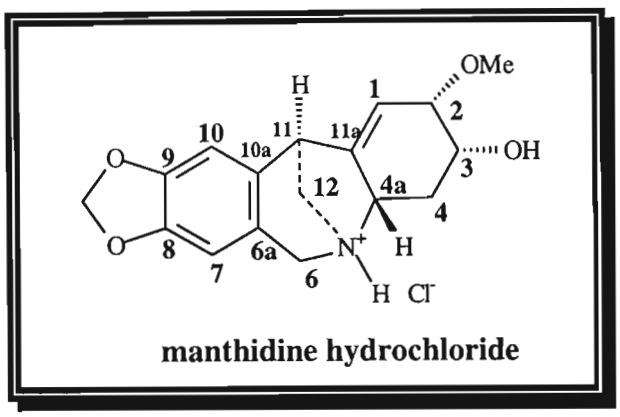


Figure 5.14 Compound X: manthidine hydrochloride

The spectral data obtained for this compound indicated that it too was an alkaloid of the 5,11-methanomorphanthridine or montanine type. In fact, the R_f values for both compound IX and X were very similar on TLC, and both compounds were observed as pink spots. The indication was that they were closely related compounds and this was confirmed by the spectral data.

High resolution mass spectrometry revealed that the highest peak in the mass spectrum, which occurred at *m/z* 301.1316 corresponded to a molecular formula of $C_{17}H_{19}NO_4$. The mass spectrum for compound X was almost identical to that of compound IX. Fragmentation peaks were also observed at *m/z* 270, *m/z* 257 *and m/z* 223 (**Spectrum 84, p 243**). Consequently, the same conclusions could be drawn from this data as that for compound IX. That is, that the intense signal at *m/z* 270 suggested that compound X exhibits a C-2 methoxy group [21]. Furthermore, the relatively intense peak at *m/z* 257 suggested that this C-2 methoxy group had an α configuration [21].

The infra-red data (**Spectrum 82, p 241**) also confirmed that compound XI was an alkaloid of the montanine type. The spectrum obtained was similar to that for compound IX and peaks were observed at 3426 cm^{-1} (O-H stretching), 2926 cm^{-1} (aliphatic C-H stretching), 1484 cm^{-1} (aromatic

C=C stretching), 1037 cm^{-1} (C-N stretching), and at 936 cm^{-1}, which is indicative of the methylenedioxy group.

The NMR data obtained for compound X was indicative of an alkaloid of the montanine type. In this case, the ^1H NMR data (**Spectrum 76, p 265**), although similar in many respects to that of compound IX, was not identical (**Table 5.8**). The downfield region of the spectrum was indeed similar to that of compound IX. Once again, two intense singlets were observed in the aromatic region of the spectrum at δ6.77 and δ6.68 and were assigned to the aromatic protons of the A ring, H-10 and H-7 respectively. The methylenedioxy protons were typically observed as two intense doublets with a small coupling constant of 1.1 Hz at δ5.97 and δ5.96. The remaining resonance in the downfield region was a broad singlet in the double bond region at δ5.79, which was ascribed to H-1. The aliphatic region of the spectrum for compound X was different to that of compound IX. One of the most pronounced differences was that the two doublets with a large coupling constant of 15.9 Hz, which are characteristic for the two H-6 protons, were shifted upfield to δ4.66 and δ4.28 compared to the shifts for these two protons in compound IX of δ4.83 and δ4.51. Coupling between H-1 and the doublet at δ3.54 was observed in the COSY spectrum (**Spectrum 77, p 236**) for compound X and this resonance was subsequently assigned to H-2. The H-2 proton was coupled to the broad singlet at δ4.13 in the COSY spectrum, and this resonance was ascribed to H-3. The H-3 singlet was also strongly coupled to the two triplet of doublets at δ1.65 and δ2.33 and the double doublet at δ3.91, which were all, in turn, coupled to one another. This coupling pattern was consistent with the coupling expected between H-3 and the two non-equivalent H-4 methylene protons and the H-4a methine proton. The two triplet of doublets were consequently assigned to the two H-4 protons and the double doublet to H-4a. The COSY spectrum also revealed coupling between the doublet at δ3.77 and the two resonances at δ3.49 and δ3.41, which were also coupled to one another. These signals were ascribed to H-11 and the two non-equivalent H-12 resonances respectively. The two H-12 resonances for compound X were significantly shifted compared to those for compound IX which were observed at δ3.70 and δ3.61. The remaining singlet at δ3.47, which integrated to three protons, was assigned to the protons of the aliphatic methoxy substituent.

No ^1H NMR literature data for manthidine was available, thus the ^1H NMR data for compound X was compared to the literature data for montanine [23], which differs only in the orientation of the C-3 hydroxy group which is β for montanine but α for manthidine. From **table 5.8** it is obvious that

there are discrepancies between the ^{1}H NMR data for compound X and the literature data for montanine [23].

Table 5.8. ^{1}H NMR data for compound X, compound IX and literature data for montanine [23].

	^{1}H NMR data for compound X	**^{1}H NMR data for compound IX, montanine hydrochloride**	**^{1}H NMR literature data [23] for montanine**
Proton No.	**#Shift/ δ_H ppm**	**#Shift/ δ_H ppm**	***Shift/ δ_H ppm**
H-1	5.79 brs	5.88 brs	5.57 brs
H-2	3.54 d (1.4)	3.57 brs	3.48 dd (2.5, 4)
H-3	4.13 brs	4.18 brs	4.09 dd (2.5, 7.2)
H-4ax	1.65 ddd (2.6, 9.5, 12.1)	1.75 ddd (2.4, 9.6, 12.0)	1.58 ddd (4, 12, 13)
H-4eq	2.33 ddd (3.8, 4.6, 7.5)	2.49 ddd (4.3, 8.8, 11.5)	2.17 ddd (3, 5.5, 13)
H-4a	**3.91 dd (2.6, 8.8)**	**4.12 dd (4.3, 12.1)**	**3.39-3.45 m**
H-6α	**4.28 dd (15.9)**	**4.51 d (15.8)**	**3.82 d (17)**
H-6β	**4.66 d (15.9)**	**4.83 d (15.8)**	**4.35 d (17)**
H-7	6.68 s	6.74 s	6.46 s
H-10	6.77 s	6.83 s	6.55 s
H-11	3.77 d (2.5)	3.97 d (2.4)	3.29 d (2.5)
H-12	**3.49 m**	**3.70 d (10.4)**	**3.04 d (12)**
H-12	**3.41 dd (2.1, 8.7)**	**3.61 d (2.8)**	**3.09 dd (2.5, 12)**
OCH_3	3.47 s	3.48 s	3.44 s
OCH_2O	5.97 d (1.1) 5.96 d (1.1)	5.98 s 5.99 s	5.89 d (1.5) 5.87 d (1.5)

^{1}H NMR spectrum measured in CD_3OD, 300 MHz.

* ^{1}H NMR spectrum measured in $CDCl_3$, 500 MHz.

The ^{13}C NMR data (**Spectrum 78, p 237**) was consistent with that expected for an alkaloid of the montanine type. The ^{13}C NMR spectrum for compound X was very similar to that obtained for compound IX and is assigned in **table 5.9**. No ^{13}C NMR literature data was available for

manthidine, thus the data for compound X was compared to that of pancracine [24] which differs in that it has an α–hydroxy group at C-2 and an α–hydroxy group at C-3 as opposed to an α-methoxy group at C-2 and an α-hydroxy group at C-3 in manthidine.

Table 5.9. ^{13}C NMR data for compound X, compound IX and literature data for pancracine [24].

	^{13}C NMR data for compound X	^{13}C NMR data for compound IX, montanine hydrochloride	^{13}C NMR literature data [24] for pancracine
Carbon No.	**#Shift/ δ_C ppm**	**#Shift/ δ_C ppm**	***Shift/ δ_C ppm**
C-1	116.83 d	118.16 d	115.89 d
C-2	79.46 d	78.97 d	68.95 d
C-3	67.75 d	67.07 d	70.99 d
C-4	30.71 t	29.69 t	31.33 t
C-4a	61.81 d	62.92 d	57.99 d
C-6	59.70 t	58.80 t	60.75 t
C-6a	131.34 s	130.23 s	132.98 s
C-7	107.90 d	108.06 d	$107.19 d
C-8	148.46 s	145.42 s	145.96 s
C-9	148.72 s	149.18 s	145.25 s
C-10	108.52 d	108.68 d	$106.76 d
C-10a	121.37 s	119.51 s	125.35 s
C-11	45.72 d	45.16 d	44.88 d
C-11a	149.05 s	149.33 s	152.54 s
C-12	56.13 t	56.11 t	55.12 t
OCH_2O	102.69 t	102.98 t	100.39 t
OCH_3	58.02 q	58.23 q	

^{13}C NMR spectrum measured in CD_3OD, 75 MHz.

* ^{13}C NMR spectrum measured in DMSO-*d6*, 20 MHz. $ Resonances may be interchanged.

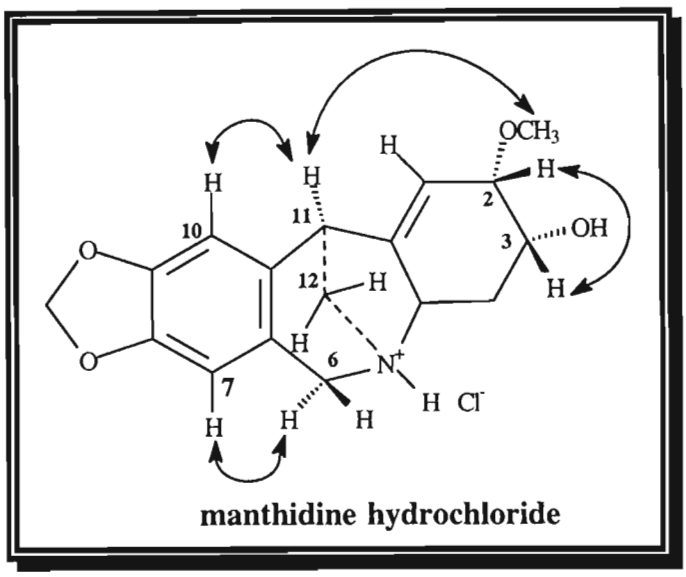


Figure 5.15 The NOE correlations observed for compound X

The orientation of the oxygenated substituents was confirmed by the NOESY spectrum (**Spectrum 81, p 240**). A positive NOE effect was observed between the methoxy group protons and H-11, which confirmed the α configuration of the methoxy group. In addition, positive NOE correlations were observed between H-3 and H-2, which suggested that the hydroxy substituent was of the α configuration. A positive NOE effect was also observed between H-11 and the singlet at δ6.77 confirming its assignment as H-10. Similarly, the positive correlation between H-6α and the singlet at δ6.68 confirmed its assignment as H-7.

The absolute configuration of the 5,11-methano bridge was determined from the CD spectrum (**Spectrum 85, p 244**). The spectrum obtained for compound X was similar to that obtained for compound IX. In this case, a weak negative Cotton effect was observed at 292 nm followed by two strong negative Cotton effects at 245 nm and 220 nm respectively. The implication was that the 5,11-methano bridge had an α configuration [24].

Once again, despite the fact that all the infra-red, UV, CD, mass spectrometry and NMR data indicated that compound X was the alkaloid manthidine, the chemical shifts of the H-6, H-12 and H-4a protons in the ^{1}H NMR suggested differed significantly from the literature data for montanine [23]. The possibility that compound X was an *N*-oxide was excluded on the basis of the ^{13}C NMR data which did not show the 10 ppm downfield shift for C-6, C-4a and C-12 that is typical if an *N*-

oxide is present [26]. Compound X was treated with 4M NaOH and as for compound IX the H-6, H-12 and H-4a protons were shifted upfield in the ^{1}H NMR spectrum (**Spectrum 86, p 245**) of the base treated compound (**Table 5.10**). These observations suggested that the original compound was a salt and indeed re-examination of the mass spectrum suggested that compound X was a hydrochloride since two signals were observed at *m/z* 36 and *m/z* 38 in a ratio of 3:1 [27]. This confirmed the identification of compound X as manthidine-hydrochloride.

Table 5.10 ^{1}H NMR data for compound X, base treated compound X and literature data for montanine [23].

	^{1}H NMR data for compound X, manthidine hydrochloride	**^{1}H NMR data for base treated compound X, manthidine**	**^{1}H NMR literature data [23] for montanine**
Proton No.	**#Shift/ δ_H ppm**	***Shift/ δ_H ppm**	****Shift/ δ_H ppm**
H-1	5.79 brs	5.55 brs	5.57 brs
H-2	3.54 d (1.4)	3.44 brs	3.48 dd (2.5, 4)
H-3	4.13 brs	4.02 brs	4.09 dd (2.5, 7.2)
H-4ax	1.65 ddd (2.6, 9.5, 12.1)	1.42 ddd (2.9, 9.5, 12.3)	1.58 ddd (4, 12, 13)
H-4eq	2.33 ddd (3.8, 4.6, 7.5)	2.09 ddd (4.0, 5.1, 9.0)	2.17 ddd (3, 5.5, 13)
H-4a	**3.91 dd (2.6, 8.8)**	**3.39 m**	**3.39-3.45 m**
H-6α	**4.28 dd (15.9)**	**3.80 d (16.7)**	**3.82 d (17)**
H-6β	**4.66 d (15.9)**	**4.30 d (16.5)**	**4.35 d (17)**
H-7	6.68 s	6.50 s	6.46 s
H-10	6.77 s	6.57 s	6.55 s
H-11	3.77 d (2.5)	3.35 d (2.0)	3.29 d (2.5)
H-12	**3.49 m**	**2.99 d (2.3)**	**3.04 d (12)**
H-12	**3.41 dd (2.1, 8.7)**	**3.02 m**	**3.09 dd (2.5, 12)**
OCH_3	3.47 s	3.40	3.44 s
OCH_2O	5.97 d (1.1) 5.96 d (1.1)	5.85 d (1.1) 5.83 d (1.1)	5.89 d (1.5) 5.87 d (1.5)

^{1}H NMR spectrum measured in CD_3OD, 300 MHz.

* ^{1}H NMR spectrum measured in CD_3OD, 400 MHz.

** ^{1}H NMR spectrum measured in $CDCl_3$, 500 MHz.

5.2.4. THE STRUCTURAL ELUCIDATION OF COMPOUND XI

Compound XI was isolated as a white crystalline compound and was identified as the common sterol, sitosterol. This sterol is commonly isolated from most plants and is easily identified on the basis of its ^{1}H NMR spectrum (**Spectrum 87, p 246**). Consequently, this compound will not be discussed further.

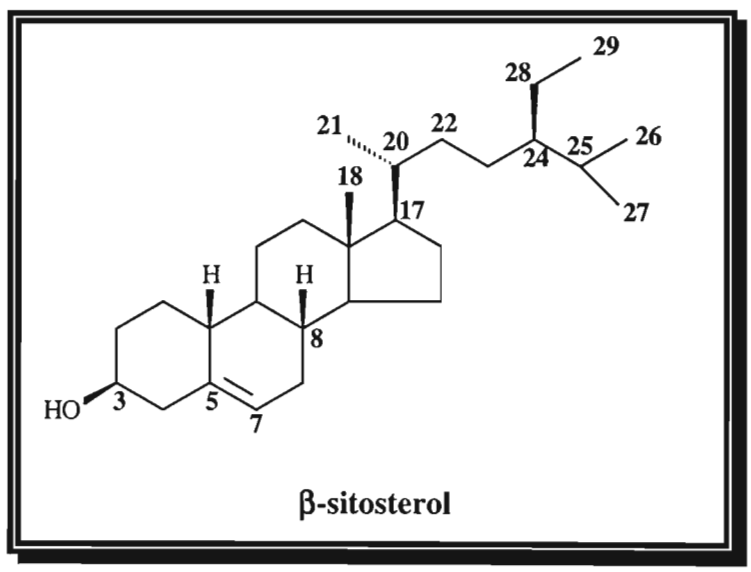


Figure 5.16 Compound XI: sitosterol

5.3 EXPERIMENTAL

Fresh *Haemanthus pauculifolius* Snijman & Van Wyk bulbs (1.2 kg) and leaves (167 g) were collected from Paris Dam and a specimen voucher retained at the Natal Herbarium (*Symmonds 3*). The bulbs and leaves were macerated and extracted separately in ethanol at room temperature with continuous agitation for approximately 4 and 2 days respectively. In both cases a gum like residue was obtained. Only small amounts of residue were obtained and the extraction procedure used for *Clivia caulescens* (**Scheme 4.20**) was not followed. Column chromatography was then used to afford the separation of the compounds. In addition, various ratios of methanol: dichloromethane were used in the chromatographic isolation procedures. Compound XI was the first compound isolated from the bulb and this sterol was found to be relatively non-polar. Compound VIII was the second compound isolated from the bulbs and was also found to be present in the leaves. Compounds IX and X were difficult to separate since they appeared to have very similar polarities.

5.3.1 THE BASE TREATMENT ON COMPOUNDS IX AND X.

4M NaOH (2 cm^3) was added to compound IX (20 mg) and X (20 mg) and the mixtures were allowed to stand at room temperature for about 5 minutes. The mixtures were then extracted with dichlomethane and NMR analysis showed that they contained the alkaloids montanine and manthidine respectively.

5.3.2. PHYSICAL DATA FOR COMPOUND VIII.

Name: Haemanthustatin

Yield: 20 mg

Melting point: melting point not observed, compound decomposes above 200°C.

Mass: Molecular ion not observed.

Optical rotation: $[\alpha]_D = +42^\circ$ (*c*, 0.001gml^{-1} in MeOH)

Infra-red: ν_{max} (KBr), 3434, 2932, 1711, 1601, 1512, 1084 cm^{-1}.

UV: λ_{max} (MeOH), (log ε), 204 (3.76), 226 (3.77), 268 (3.32), 300 (3.10) nm.

^{1}H NMR: δ_H (ppm), CD_3OD.

2.66 (2H, m, 2H-3), 2.85 (2H, m, 2H-2), 3.82 (1H, m, H-10b), 3.94 (3H, s, OCH_3), 4.03 (3H, s, OCH_3), 5.00 (1H, d, *J*=4.4 Hz,H-4), 5.92 (1H, brs, H-4), 7.38 (1H, brs, H-7), 7.63 (1H, s, H-10).

^{13}C NMR: δ_C (ppm), CD_3OD.

27.49 (t, C-3), 31.77 (t, C-2), 56.65 (q, OCH_3), 57.13 (q, OCH_3), 69.44 (d, C-10b), 78.01 (d, C-1), 111.94 (d, C-7), 113.69 (d, C-10), 117.76 (s, C-10a), 121.91 (d, C-4), 121.96 (s, C-4a), 136.16 (s, C-6a), 151.22 (s, C-8), 155.59 (s, C-9), 166.82 (s, C-6).

5.3.3. PHYSICAL DATA FOR COMPOUND IX.

Name: Montanine hydrochloride

Yield: 55 mg

Melting point: amorphous (lit. for montanine 201-202°C [9])

Mass: molecular ion not observed. *m/z* 301.1321, $C_{17}H_{19}NO_4$ requires 301.1314.
EIMS: *m/z* 301 (100%), *m/z* 270 (76%), *m/z* 257 (27%), *m/z* 36 (11%), *m/z* 38 (3%).

Optical rotation: $[\alpha]_D = -75^\circ$ (*c*, 0.008gml^{-1} in MeOH), (lit. for montanine $[\alpha]_D = -87.6^\circ$, *c* 0.57 in $CHCl_3$ [9])

Infra-red: ν_{max} (KBr), 3363, 2947, 1486, 1039, 936 cm^{-1}.

UV: λ_{max} (MeOH), (log ε), 204 (4.65), 240 (3.92), 294 (3.59) nm.

Circular dichroism: λ_{max}, 227 nm [θ]= -8946, 251 nm [θ]= -10728.91, 296 nm [θ]= -1754

^{1}H NMR: δ_H (ppm), CD_3OD.

1.75 (1H, ddd, *J*=2.4, 9.6, 12.0 Hz, H-4ax), 2.49 (1H, ddd, *J*=4.3, 8.8, 11.5 Hz, H-4eq), 3.48 (3H, s, OCH_3), 3.57 (1H, brs, H-2), 3.61 (1H, d, *J*=2.8, H-12 Hz), 3.70 (1H, d, *J*=10.4 Hz, H-12), 3.97 (1H, d, *J*=2.4 Hz, H-11), 4.12 (1H, dd, *J*=4.3, 12.1 Hz, H-4a), 4.18 (1H, brs, H-3), 4.51 (1H, d, *J*=15.8 Hz, H-6α), 4.83 (1H, d, *J*=15.8 Hz, H-6β), 5.88 (1H, brs, H-1), 5.98, 5.99 (2H, 2d, *J*=1.1 Hz, OCH_2O), 6.74 (1H, s, H-7), 6.83 (1H, s, H-10).

^{13}C NMR: δ_C (ppm), CD_3OD.

29.69 (t, C-4), 45.16 (d, C-11), 56.11 (t, C-12), 58.23 (q, OCH_3), 58.80 (t, C-6), 62.92 (d, C-4a), 67.07 (d, C-3), 78.97 (d, C-2), 102.98 (t, OCH_2O), 108.06 (d, C-7), 108.68 (d, C-10), 118.16 (d, C-1), 119.51 (s, C-10a), 130.23 (s, C-6a), 145.42 (s, C-8), 149.18 (s, C-9), 149.33 (s, H-11a).

^{1}H NMR: δ_H (ppm), CD_3OD for base treated compound IX, montanine.

1.45 (1H, ddd, *J*=3.0, 9.0, 12.5 Hz, H-4ax), 2.15 (1H, ddd, *J*=3.5, 4.9, 9.5 Hz, H-4eq), 3.00 (1H, d, *J*=2.6 Hz, H-12), 3.02 (1H, m, H-12), 3.35 (1H, *d*, *J*=3.2 Hz, H-11), 3.38 (1H, m, H-4a), 3.40 (3H, s, OCH_3), 3.44 (1H, d, *J*=2.6 Hz, H-2), 3.78 (1H, d, *J*=16.7 Hz, H-6α), 4.02 (1H, brs, H-3), 4.28 (1H, d, *J*=16.5 Hz, H-6β), 5.55 (1H, brs, H-1), 5.85, 5.83 (2H, 2d, *J*=1.1 Hz, OCH_2O), 6.50 (1H, s, H-7), 6.58 (1H, s, H-10).

^{13}C NMR: δ_C (ppm), CD_3OD for base treated compound IX, montanine.

32.35 (C-4), 46.34 (C-11), 55.76 (C-12), 57.39 (OCH3), 59.09 (C-6), 61.09 (C-4a), 68.55 (C-3), 79.80 (C-2), 101.75 (OCH2O), 107.40 (C-10), 107.90 (C-7), 113.80 (C-1), 124.83 (C-10a), 133.17 (C-6a), 147.26 (C-8), 147.96 (C-9), 154.29 (C-11a).

5.3.4. PHYSICAL DATA FOR COMPOUND X.

Name: Manthidine hydrochloride

Yield: 40 mg

Melting point: amorphous (lit. for manthidine 269-270°C [9])

Mass: Molecular ion not observed. *m/z* 301.1316, $C_{17}H_{19}NO_4$ requires 301.1314.
EIMS: *m/z* 301 (100%), *m/z* 270 (77%), *m/z* 257 (29%), *m/z* 36 (10 %), *m/z* 38 (3%).

Optical rotation: $[\alpha]_D$ = -14° (*c*, 0.004gml^{-1} in MeOH), (lit. for manthidine $[\alpha]_D$ = -26.6°, *c* 0.6 in $CHCl_3$ [9])

Infra-red: ν_{max} (KBr), 3426, 2926, 1484, 1037, 936 cm^{-1}.

UV: λ_{max} (MeOH), (log ε), 205 (3.89), 243 (3.13), 293 (3.01) nm.

Circular dichroism: λ_{max}, 220 nm [θ]= -2135, 245 nm [θ]= -1376, 292 nm [θ]= -147.

^{1}H NMR: δ_H (ppm), CD_3OD.
1.65 (1H, ddd, *J*=2.6, 9.5, 12.1 Hz, H-4ax), 2.33 (1H, ddd, *J*=3.8, 4.6, 7.5 Hz, H-4eq), 3.41 (1H, dd, *J*=2.1, 8.7 Hz, H-12), 3.49 (1H, m, H-12), 3.54 (1H, d, *J*=1.4 Hz, H-2), 3.77 (1H, d, *J*=2.5 Hz, H-11), 3.91 (1H, dd, *J*=2.6, 8.8 Hz, H-4a), 4.13 (1H, brs, H-3), 4.28 (1H, d, *J*=15.9 Hz, H-6α), 4.66 (1H, d, *J*=15.9 Hz, H-6β), 5.79 (1H, brs, H-1), 5.97, 5.96 (2H, 2d, *J*=1.1 Hz, OCH_2O), 6.68 (1H, s, H-7), 6.77 (1H, s, H-10).

^{13}C NMR: δ_C (ppm), CD_3OD.
30.71 (t, C-4), 45.72 (d, C-11), 56.13 (t, C-12), 58.02 (q, OCH_3), 59.70 (t, C-6), 61.81 (d, C-4a), 67.75 (d, C-3), 79.46 (d, C-2), 102.69 (t, OCH_2O), 107.90 (d, C-7), 108.52 (d, C-10), 116.82 (d, C-1), 121.37 (s, C-10a), 131.34 (s, C-6a), 148.46 (s, C-8), 148.72 (s, C-9), 149.05 (s, C-11a).

^{1}H NMR: δ_H (ppm), CD_3OD for base treated compound X, manthidine.
1.42 (1H, ddd, *J*=2.9, 9.5, 12.3 Hz, H-4ax), 2.09 (1H, ddd, *J*=4.0, 5.1, 9.0 Hz, H-4eq), 2.99 (1H d, *J*=2.3 Hz, H-12), 3.02 (1H, m, H-12), 3.35 (1H, d, *J*=2.0 Hz, H-11), 3.39 (1H, m, H-4a), 3.40 (3H, s, OCH_3), 3.44 (1H, brs, H-2), 3.80 (1H, d, *J*=16.7 Hz, H-6α), 4.02 (1H, brs, H-3), 4.30 (1H, d, *J*=16.5 Hz, H-6β), 5.55 (1H, brs, H-1), 5.85, 5.83 (2H, 2d, *J*=1.1 Hz, OCH_2O), 6.50 (1H, s, H-7), 6.57 (1H, s, H-10).

5.4 REFERENCES

1. Snijman, D.A. and Van Wyk, A.E.; *S.A. J. of Bot.*, (1993), **59**, 247.
2. Snijman, D.A.; *J. of S.A. Bot. Supp.*, (1984), **12**, 139.
3. Du Plessis, N and Duncan, G.; "*Bulbous Plants of Southern Africa–A Guide to their Cultivation and Propagation*", (1989), Tafelberg Publishers, Cape Town, 93.
4. Dyer, R.A.; "*The Genera Of Southern African Flowering Plants-Volume 2*", (1976), Department of Agricultural Technical Services, Pretoria, 949.
5. Craib, C.; *Herbertia*, (1997), **52**, 69.
6. Hutchings, A., Scott, A.H., Lewis, G. and Cunningham, A.B.; "*Zulu Medicinal Plants-An Inventory*", (1996), University of Natal Press, Pietermaritzburg, 48-49.
7. Munday, J.; "*Poisonous Plants in Southern African Gardens and Parks-A Field Guide*", (1988), Delta Books (Pty) Ltd., Johannesburg, 11-17.
8. Watt, J.M. and Breyer-Brandwijk, M.G.; "*Medicinal and Poisonous Plants of Southern and Eastern Africa-2nd Edition*", (1962), E & S Livingston Ltd., Edinburgh, 33-39.
9. Viladomat, F., Bastida, J., Codina, C., Nair, J.J. and Campbell, W.E.; *Recent Res. Dev. In Phytochem.*, (1997), **1,** 131.
10. Ghosal, S., Lochan, R., Ashutosh, Kumar, Y. and Srivastava, R.S.; *Phytochem.*, (1985), **24**, 1825.
11. Ghosal, S., Singh, S., Kumar, Y. and Srivastava, R.S.; *Phytochem.*, (1989), **28**, 611.
12. Pettit, G.R., Goddamidi, V., Herald, D.L., Singh, S.B., Cragg, G.M. and Schmidt, J.M.; *J. Nat. Prod.*, (1986), **49**, 995.
13. Pettit, G.R., Cragg, G.M., Singh, S.B., Duke, J.A. and Doubek, D.L.; *J. Nat. Prod.*, (1990), **53**, 176.
14. Pettit, G.R., Pettit, G. R. (III) and Buckhous, R.A.; *J. Nat. Prod.*, (1993), **56**, 1682.
15. Danishefsky, S. and Lee, J.Y.; *J. Am. Chem. Soc.*, (1989), **111**, 4829.
16. Keller, R.J.; "*The Sigma Library of FT-IR Spectra, 1st Ed.*", (1986), Sigma Chemical Company Inc., **2**, 301.
17. Evidente, A.; *Planta Med.*, (1991), **57**, 293.
18. Brown, D.W., Floyd, A.J. and Sainsbury, M.; "*Organic Spectroscopy*", (1988), John Wiley and Sons Ltd., Chichester, Chpt 4, 57.
19. Irie, H., Tsuda, Y and Uyeo, S.; *J. Chem. Soc.*, (1959), , 1446

20. Likhitwitayawuid, K., Angerhofer, C.K., Chai, H., Pezzuto, J.M. and Cordell, G.A.; *J. of Nat. Prod.*, (1993), **56**, 1331.

21. Wildman, W.C. and Brown, C.L.; *J. Am. Chem. Soc.*, (1968), **90**, 6439.

22. Duffield, A.M, Aplin, R.T., Budzikiewicz, H., Djerassi, C., Murphy, C.F. and Wildman, W.C.; *J. Am. Chem. Soc.*, (1965), **87**, 4902.

23. Ishizaki, M. and Hoshino, O.; *J. Org. Chem.*, (1992), **57**, 7285.

24. Ali, A.A, Mesbah, M.K. and Frahm, A.W.; *Planta Med.*, (1984), **50**, 188.

25. Bastida, J., Viladomat, F., and Codina, C.; in "*Studies in Natural Product Chemistry*", (1997), Ed. Atta-ur-Rahman, Elsevier Science Publishers, Amsterdam, Chpt. 20, 323.

26. Viladomat, F., Bastida, J., Tribo, G., Codina, C. and Rubiralta, M.; *Phytochem.*, (1990), **29**, 1310.

27. Brown, D.W., Floyd, A.J. and Sainsbury, M.; "*Organic Spectroscopy*", (1988), John Wiley and Sons Ltd., Chichester, Chpt 5, 177.

APPENDIX A

LIST OF SPECTRA

	Page
Spectrum 1: ^{1}H NMR spectrum of Compound I (300 MHz)	**160**
Spectrum 2: COSY NMR spectrum of Compound I (300 MHz)	**161**
Spectrum 3: ^{13}C NMR spectrum of Compound I (75 MHz)	**162**
Spectrum 4: ADEPT NMR spectrum of Compound I (75 MHz)	**163**
Spectrum 5: Infra-red spectrum of Compound I	**164**
Spectrum 6: UV spectrum of Compound I	**165**
Spectrum 7: Mass spectrum of Compound I	**166**
Spectrum 8: ^{1}H NMR spectrum of Compound II (300 MHz)	**167**
Spectrum 9: NOE NMR spectrum of Compound II (300 MHz)	**168**
Spectrum 10: ^{13}C NMR spectrum of Compound II (75 MHz)	**169**
Spectrum 11: ADEPT NMR spectrum of Compound II (75 MHz)	**170**
Spectrum 12: Infra-red spectrum of Compound II	**171**
Spectrum 13: UV spectrum of Compound II	**172**
Spectrum 14: Mass spectrum of Compound II	**173**
Spectrum 15: ^{1}H NMR spectrum of Compound III (300 MHz)	**174**
Spectrum 16: COSY NMR spectrum of Compound III (300 MHz)	**175**
Spectrum 17: ^{13}C NMR spectrum of compound III (75 MHz)	**176**
Spectrum 18: ADEPT NMR spectrum of Compound III (75 MHz)	**177**
Spectrum 19: HETCOR NMR spectrum of Compound III (300 MHz)	**178**
Spectrum 20: ^{1}H NMR spectrum of acetylated product of compound III (400 MHz)	**179**
Spectrum 21: Infra-red spectrum of Compound III	**180**
Spectrum 22: UV spectrum of Compound III	**181**
Spectrum 23: Mass spectrum of Compound III	**182**
Spectrum 24: ^{1}H NMR spectrum of Compound IV (300 MHz)	**183**
Spectrum 25: COSY NMR spectrum of Compound IV (300 MHz)	**184**
Spectrum 26: ^{13}C NMR spectrum of Compound IV (75 MHz)	**185**
Spectrum 27: ADEPT NMR spectrum of Compound IV (75 MHz)	**186**
Spectrum 28: NOESY NMR spectrum of Compound IV (400 MHz)	**187**
Spectrum 29: ^{1}H NMR spectrum of acetylated product of Compound IV (400 MHz)	**188**
Spectrum 30: Infra-red spectrum of Compound IV	**189**
Spectrum 31: UV spectrum of Compound IV	**190**
Spectrum 32: Mass spectrum of Compound IV	**191**

Spectrum 33: CD spectrum of Compound IV **192**
Spectrum 34: ^{1}H NMR spectrum of Compound V (300 MHz) **193**
Spectrum 35: ^{13}C NMR spectrum of Compound V (100 MHz) **194**
Spectrum 36: ^{1}H NMR spectrum of acetylated product of Compound V (400 MHz) **195**
Spectrum 37: Infra-red spectrum of Compound V **196**
Spectrum 38: UV spectrum of Compound V **197**
Spectrum 39: Mass Spectrum of Compound V **198**
Spectrum 40: ^{1}H NMR spectrum of Compound VI (300 MHz) **199**
Spectrum 41: COSY NMR spectrum of Compound VI (300 MHz) **200**
Spectrum 42: ^{13}C NMR spectrum of Compound VI (75 MHz) **201**
Spectrum 43: ADEPT NMR spectrum of Compound VI (75 MHz) **202**
Spectrum 44: Infra-red spectrum of Compound VI **203**
Spectrum 45: UV spectrum of Compound VI **204**
Spectrum 46: Mass spectrum of Compound VI **205**
Spectrum 47: CD spectrum of Compound VI **206**
Spectrum 48: ^{1}H NMR spectrum of Compound VII (300 MHz) **207**
Spectrum 49: COSY NMR spectrum of Compound VII (400 MHz) **208**
Spectrum 50: ^{13}C NMR spectrum of Compound VII (100 MHz) **209**
Spectrum 51: ADEPT NMR spectrum of Compound VII (100 MHz) **210**
Spectrum 52: Infra-red spectrum of Compound VII **211**
Spectrum 53: UV spectrum of Compound VII **212**
Spectrum 54: ^{1}H NMR spectrum of Compound VIII (300 MHz) **213**
Spectrum 55: COSY NMR spectrum of Compound VIII (300 MHz) **214**
Spectrum 56: ^{13}C NMR spectrum of Compound VIII (75 MHz) **215**
Spectrum 57: ADEPT NMR spectrum of Compound VIII (75 MHz) **216**
Spectrum 58: HETCOR NMR spectrum of Compound VIII (300 MHz) **217**
Spectrum 59: NOESY NMR spectrum of Compound VIII (400 MHz) **218**
Spectrum 60: Infra-red spectrum of Compound VIII **219**
Spectrum 61: UV spectrum of Compound VIII **220**
Spectrum 62: Mass spectrum of Compound VIII **221**
Spectrum 63: ^{1}H NMR spectrum of Compound IX (300 MHz) **222**
Spectrum 64: COSY NMR spectrum of Compound IX (300 MHz) **223**
Spectrum 65: ^{13}C NMR spectrum of Compound IX (75 MHz) **224**
Spectrum 66: ADEPT NMR spectrum of Compound IX (75 MHz) **225**
Spectrum 67: HETCOR NMR spectrum of Compound IX (300 MHz) **226**

Spectrum 68: NOESY NMR spectrum of Compound IX (400 MHz) **227**

Spectrum 69: Infra-red spectrum of Compound IX **228**

Spectrum 70: UV spectrum of Compound IX **229**

Spectrum 71: Mass spectrum of Compound IX **230**

Spectrum 72: CD spectrum of Compound IX **231**

Spectrum 73: ^{1}H NMR spectrum of base-treated compound IX (400 MHz) **232**

Spectrum 74: COSY NMR spectrum of base-treated Compound IX (400 MHz) **233**

Spectrum 75: ^{13}C NMR spectrum of base-treated Compound IX (100 MHz) **234**

Spectrum 76: ^{1}H NMR spectrum of Compound X (300 MHz) **235**

Spectrum 77: COSY NMR spectrum of Compound X (300 MHz) **236**

Spectrum 78: ^{13}C NMR spectrum of Compound X (75 MHz) **237**

Spectrum 79: ADEPT NMR spectrum of Compound X (100 MHz) **238**

Spectrum 80: HETCOR NMR spectrum of Compound X (400 MHz) **239**

Spectrum 81: NOESY NMR spectrum of Compound X (400 MHz) **240**

Spectrum 82: Infra-red spectrum of Compound X **241**

Spectrum 83: UV spectrum of Compound X **242**

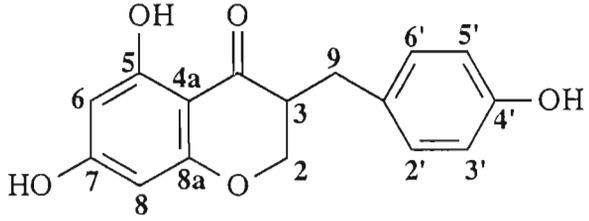
Spectrum 84:Mass spectrum of Compound X **243**

Spectrum 85: CD spectrum of Compound X **244**

Spectrum 86: ^{1}H NMR spectrum of base-treated Compound X (400 MHz) **245**

Spectrum 87: ^{1}H NMR spectrum of Compound XI (300 MHz) **246**

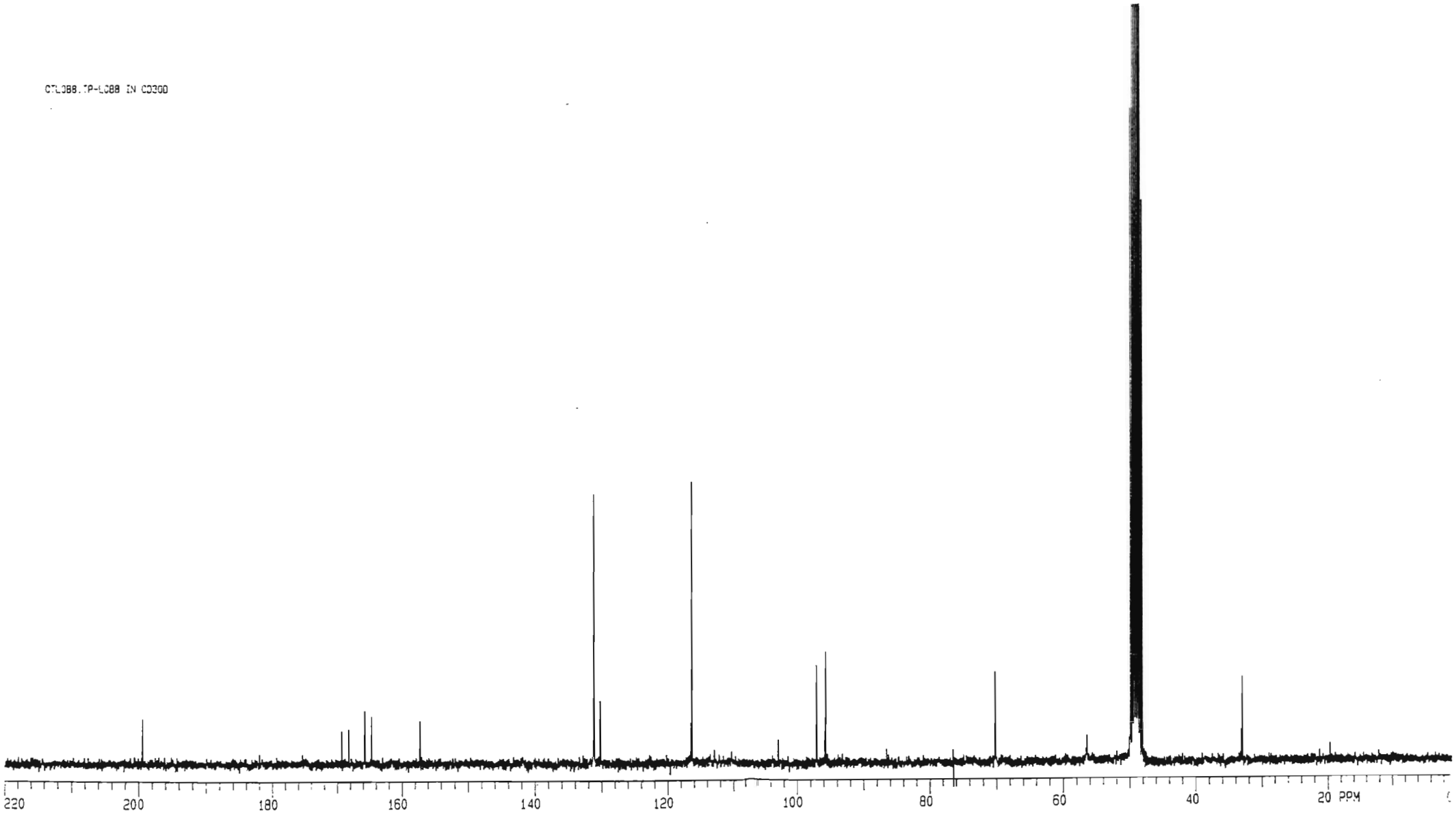
* All spectra measured in CD_3OD except for spectrum 87.



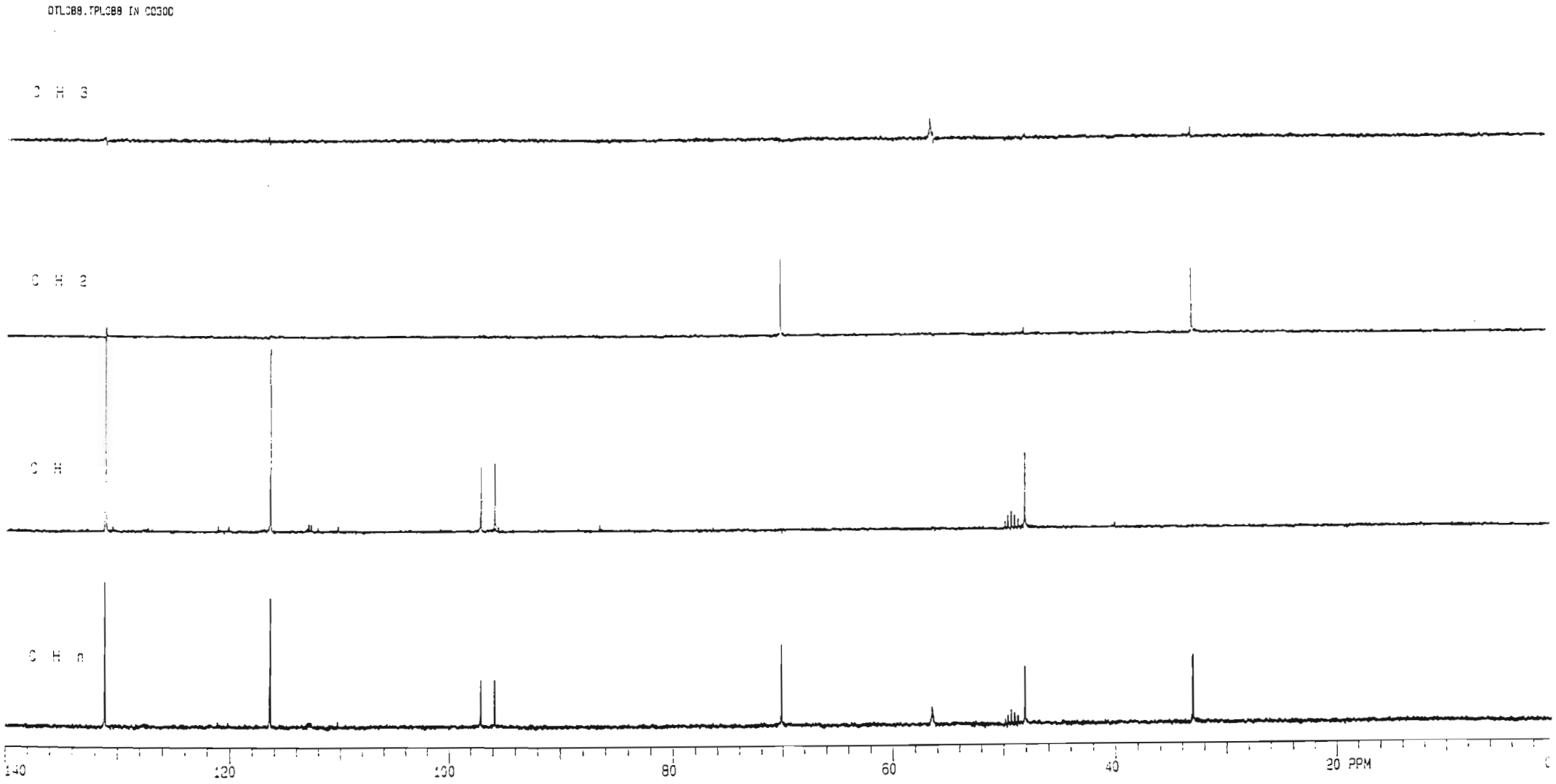
5,7-dihydroxy-3-(4'-hydroxybenzyl)-4-chromanone

^{1}H NMR spectrum of Compound I

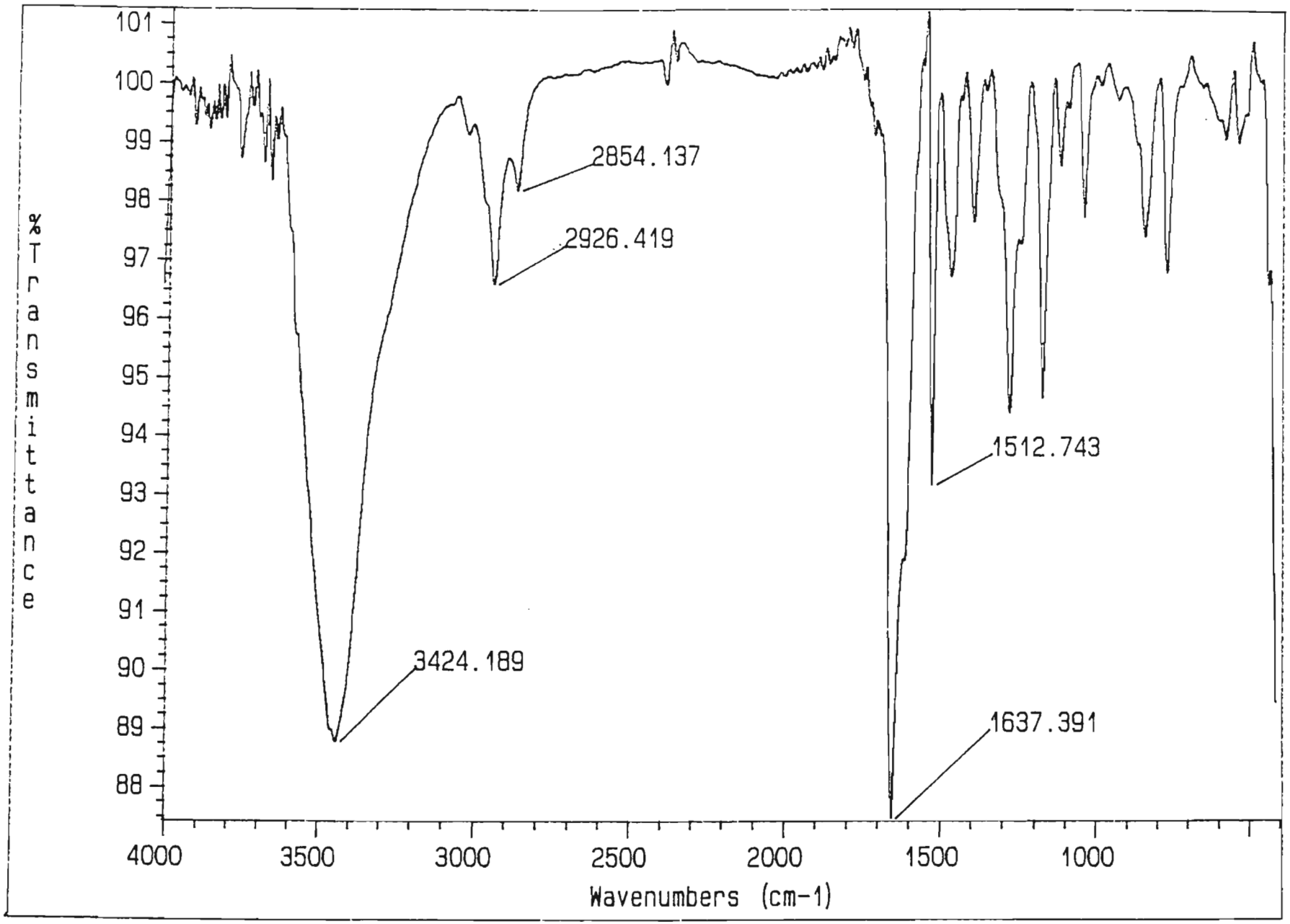
COSY NMR spectrum of Compound I



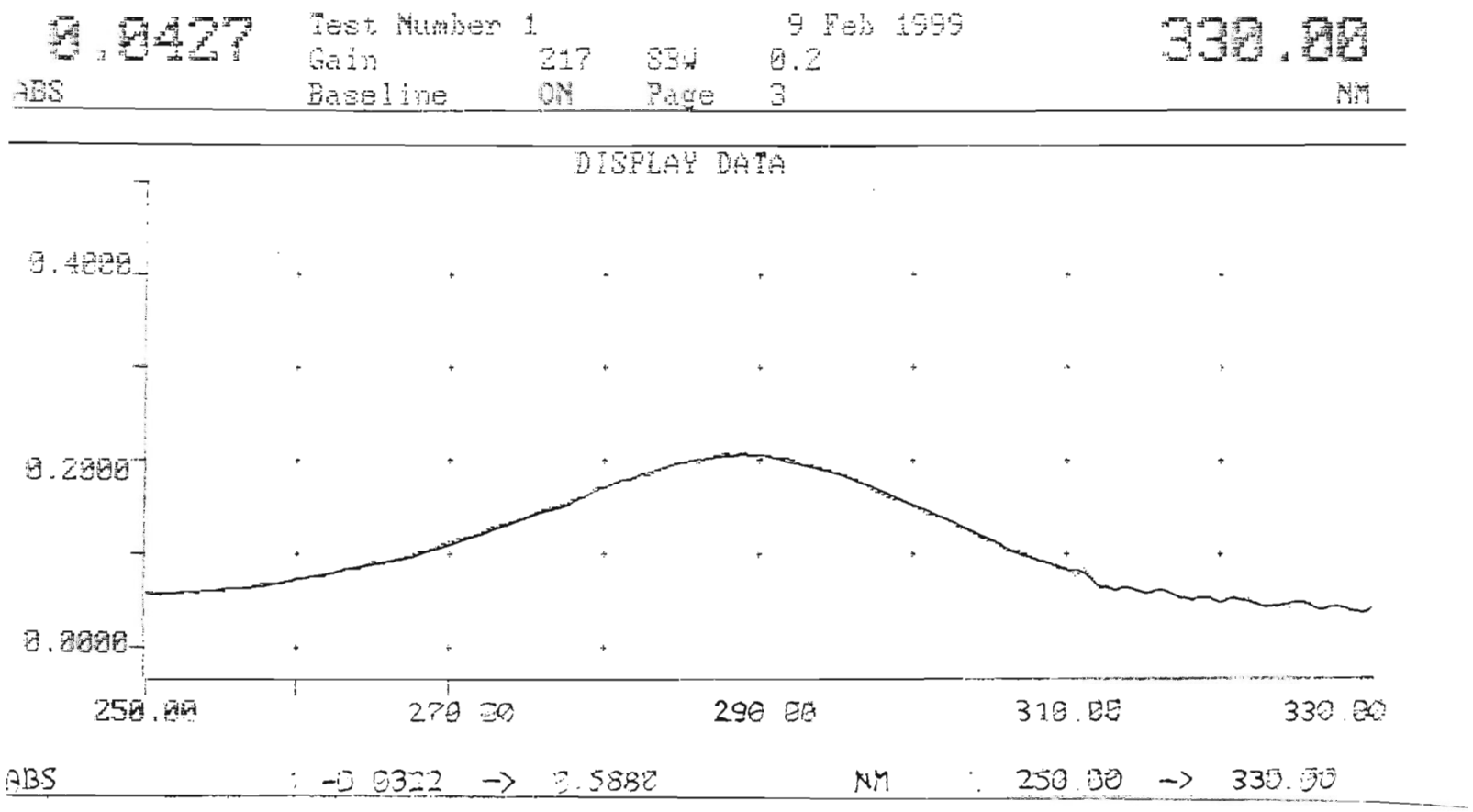
^{13}C NMR spectrum of Compound I



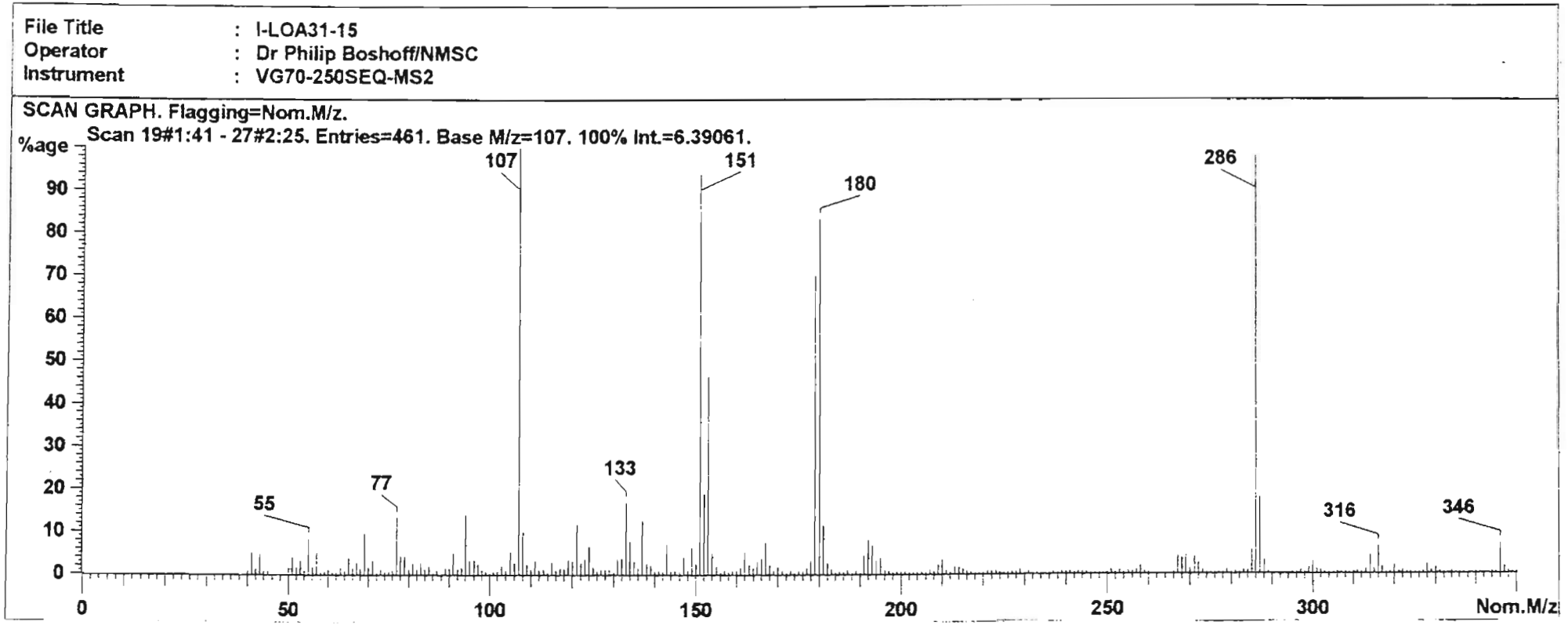
ADEPT NMR spectrum of Compound I



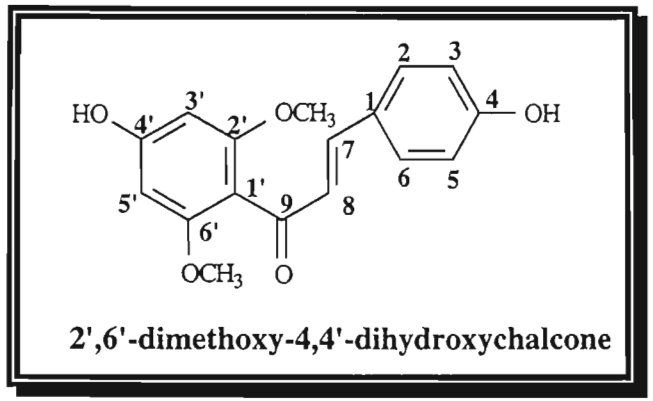
Infra-red spectrum of Compound I



UV spectrum of Compound I

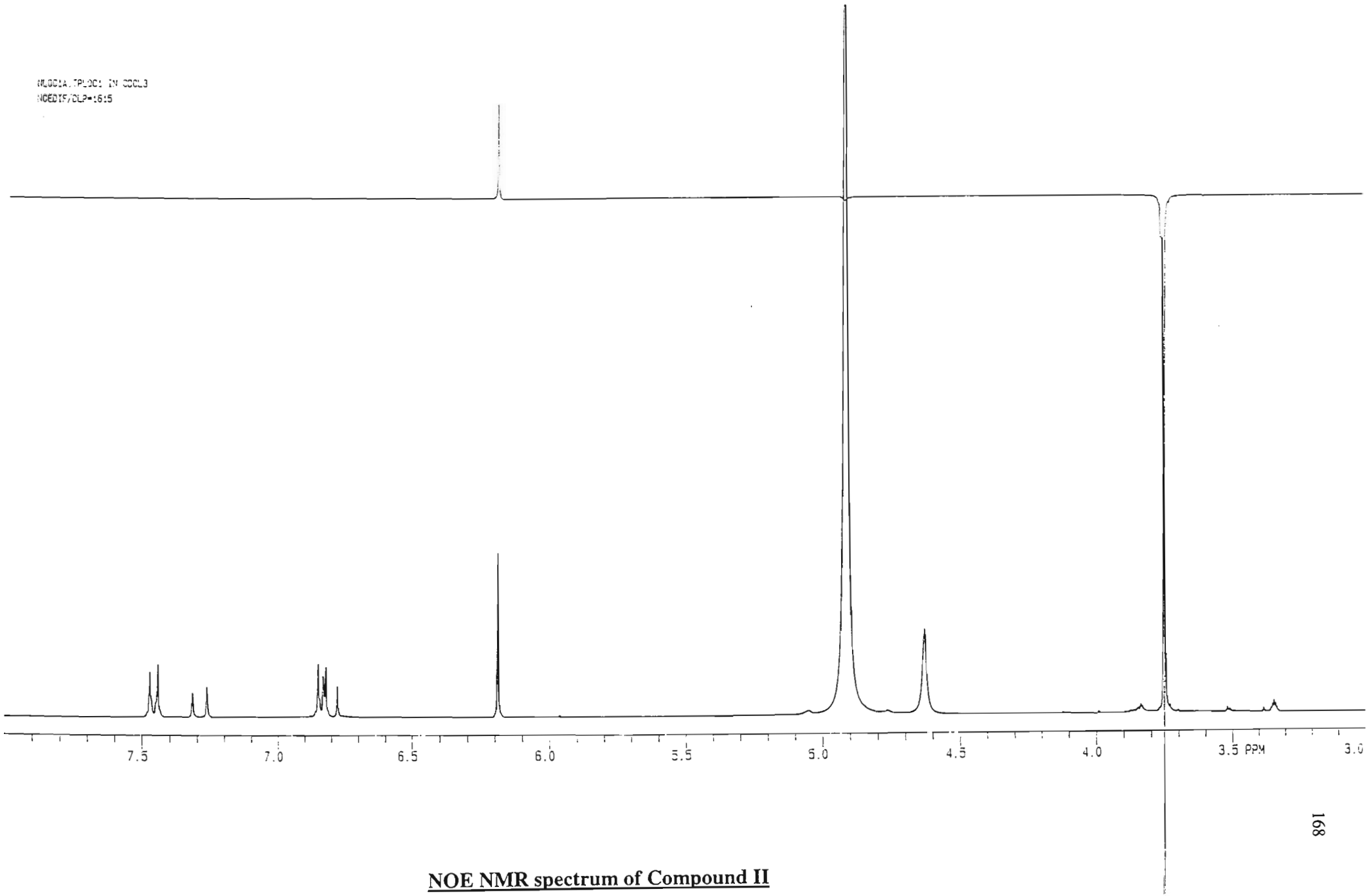


Mass spectrum of Compound I

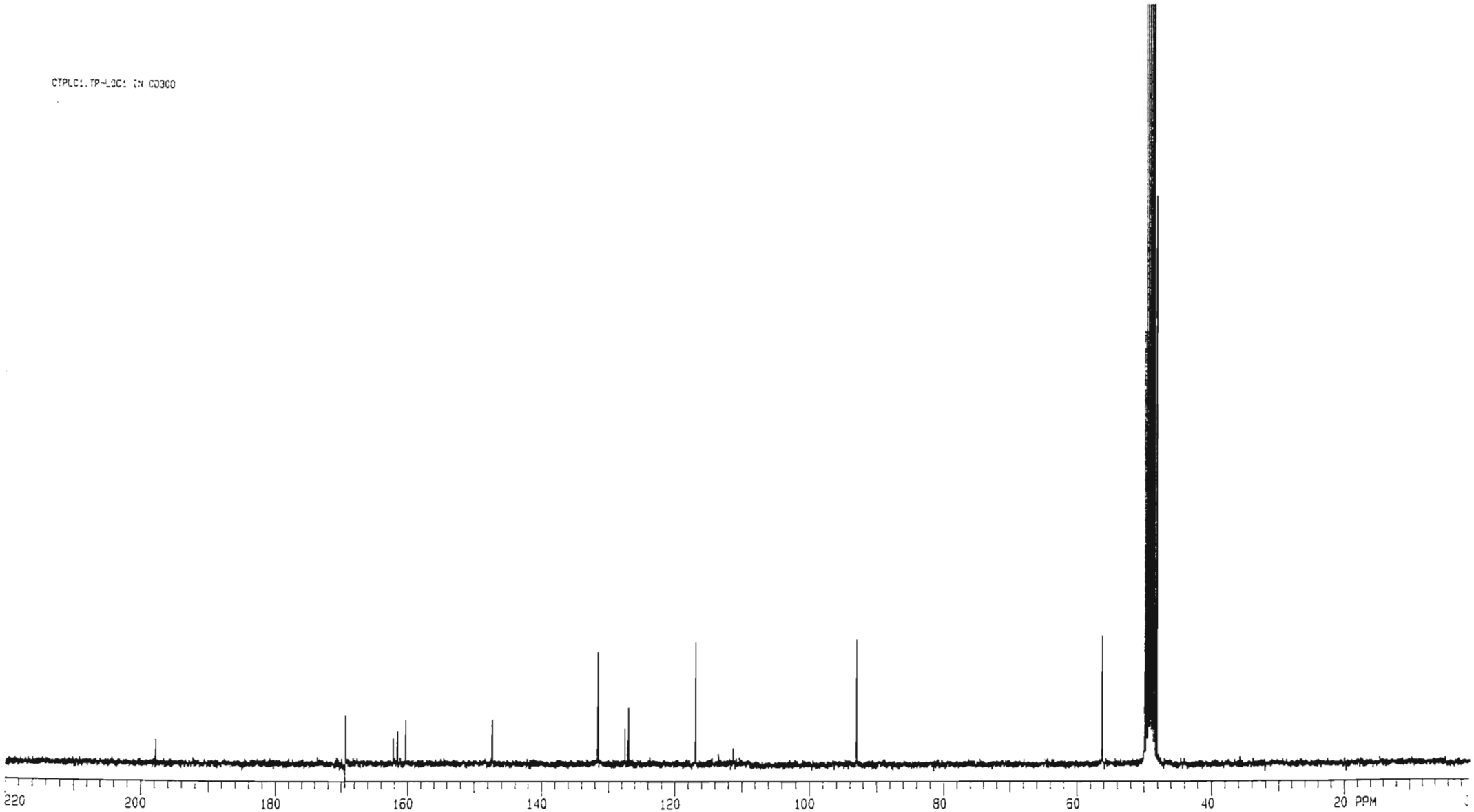


2',6'-dimethoxy-4,4'-dihydroxychalcone

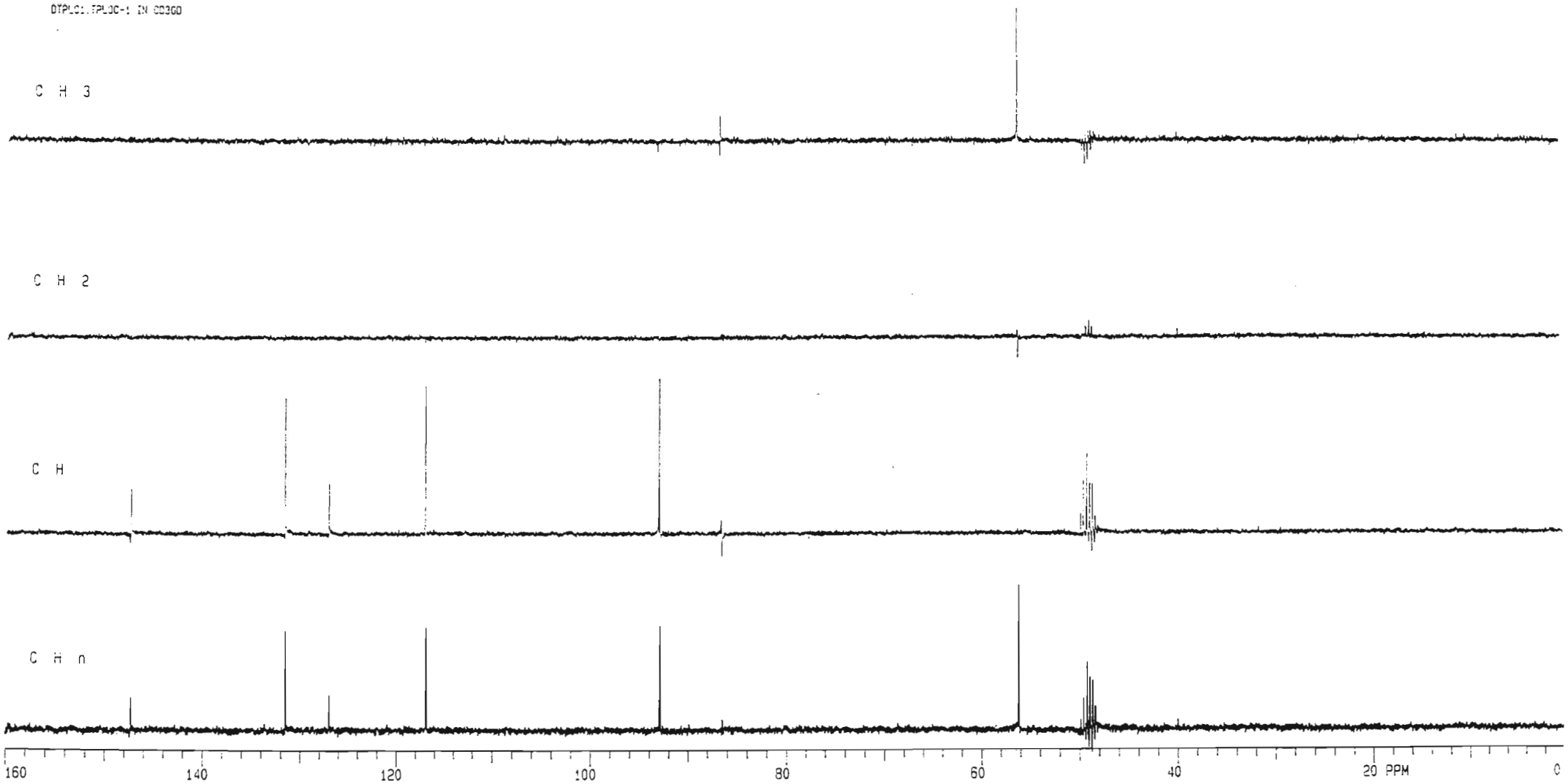
^{1}H NMR spectrum of Compound II



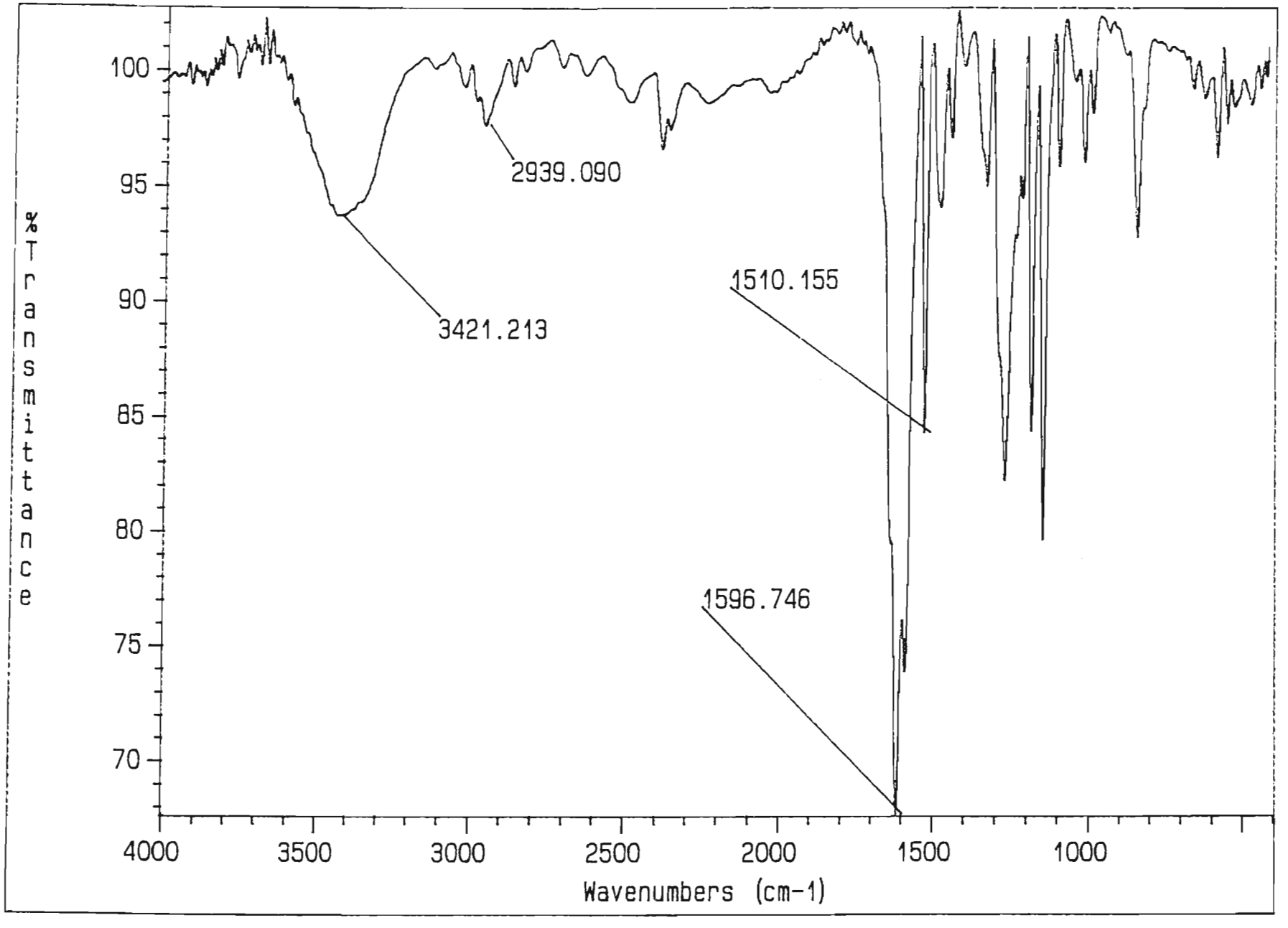
NOE NMR spectrum of Compound II



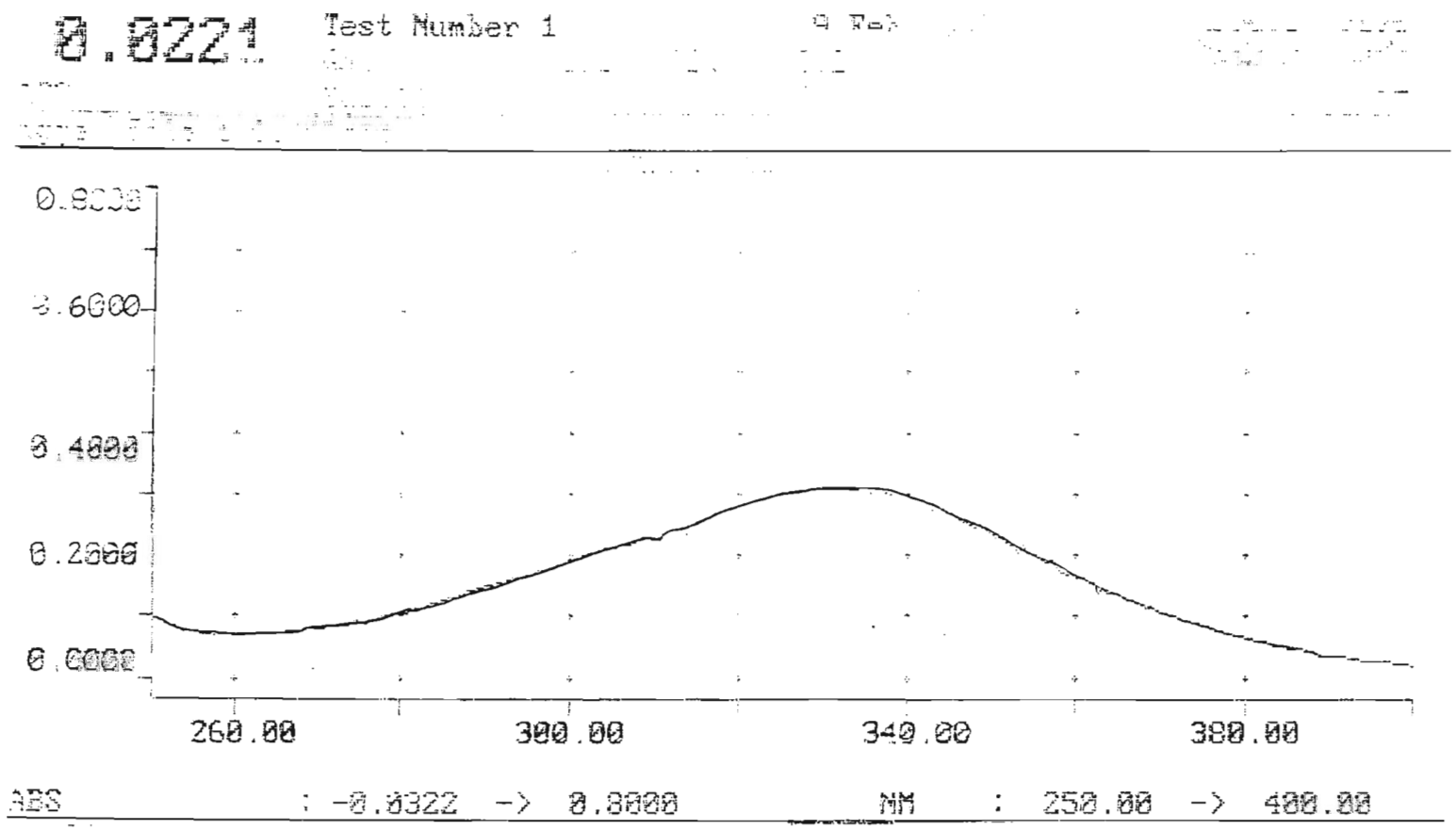
^{13}C NMR spectrum of Compound II



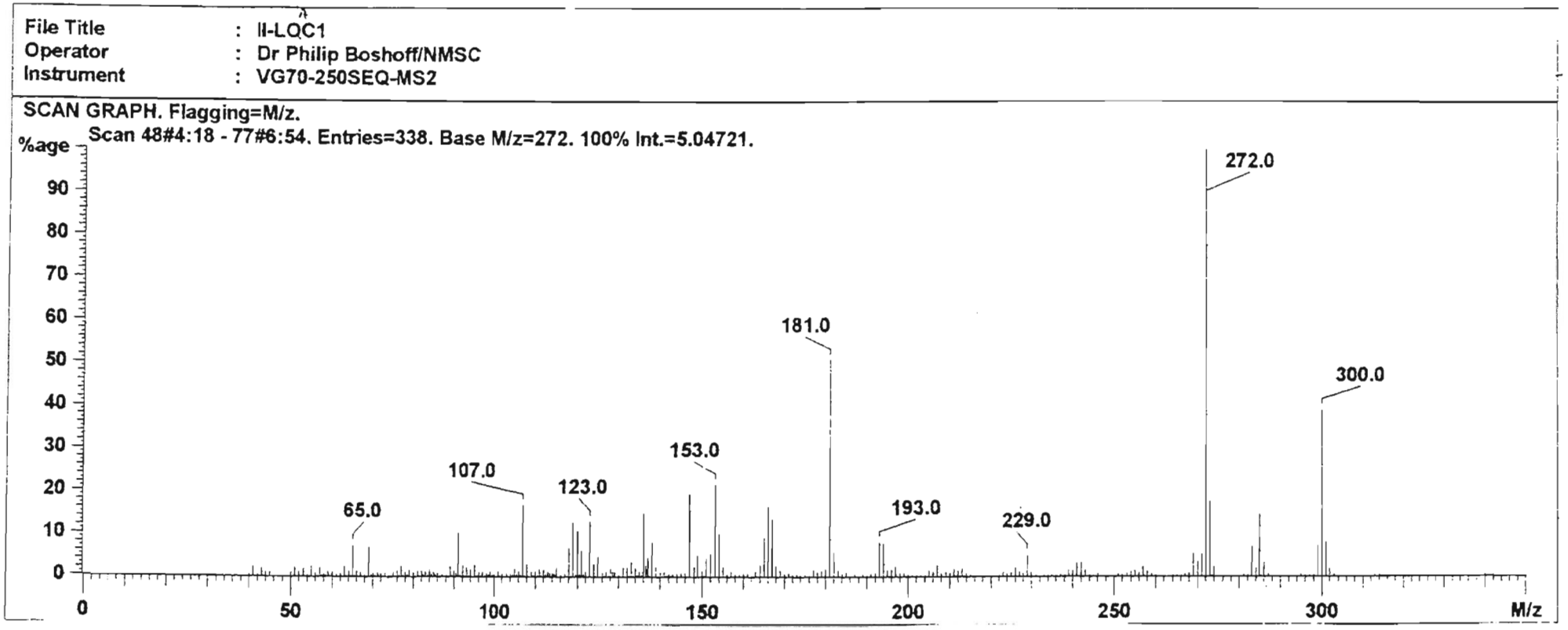
ADEPT NMR spectrum of Compound II



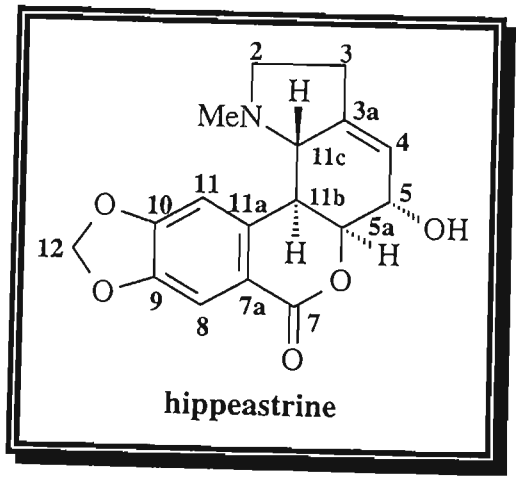
Infra-red spectrum of Compound II



UV spectrum of Compound II



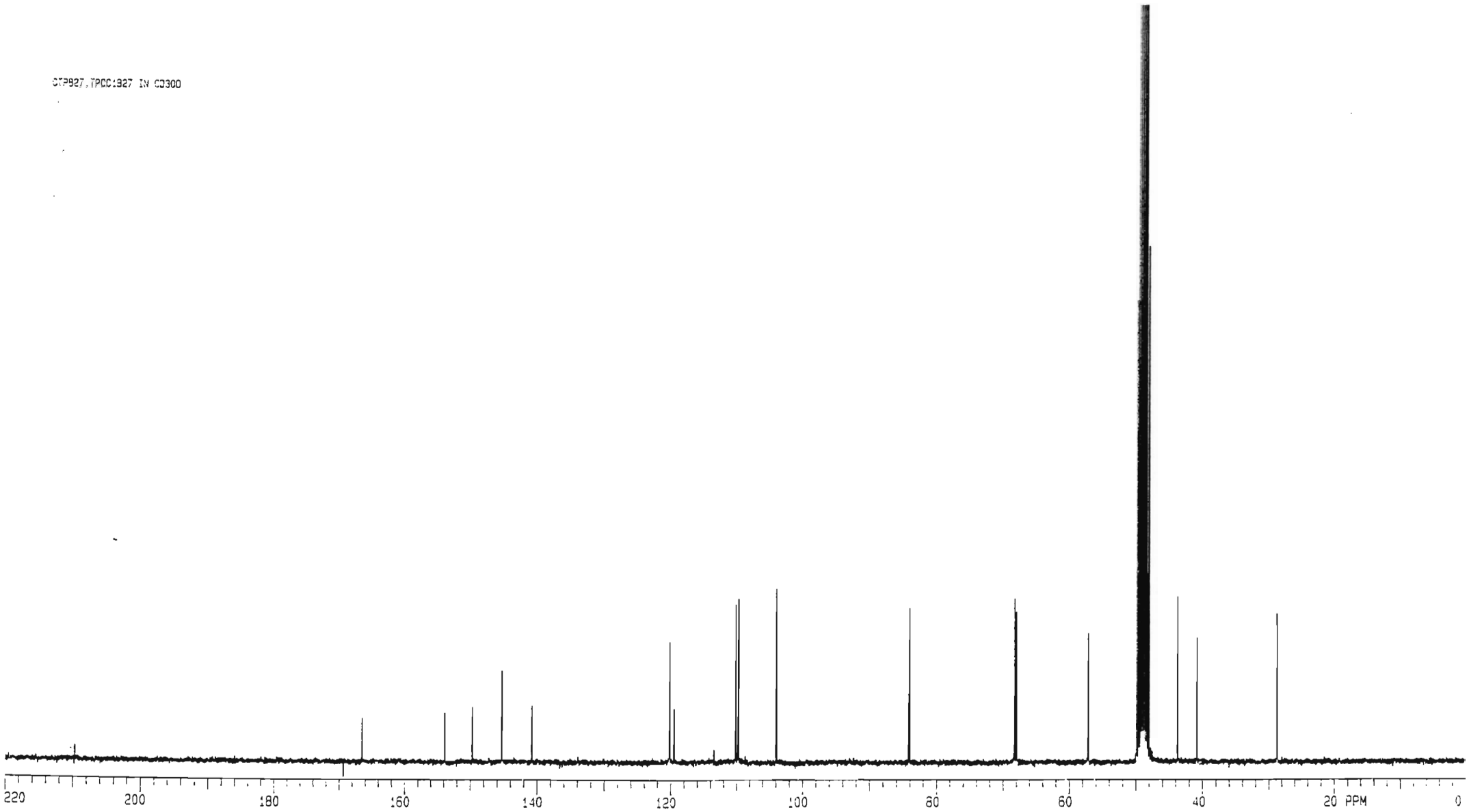
Mass spectrum of Compound II



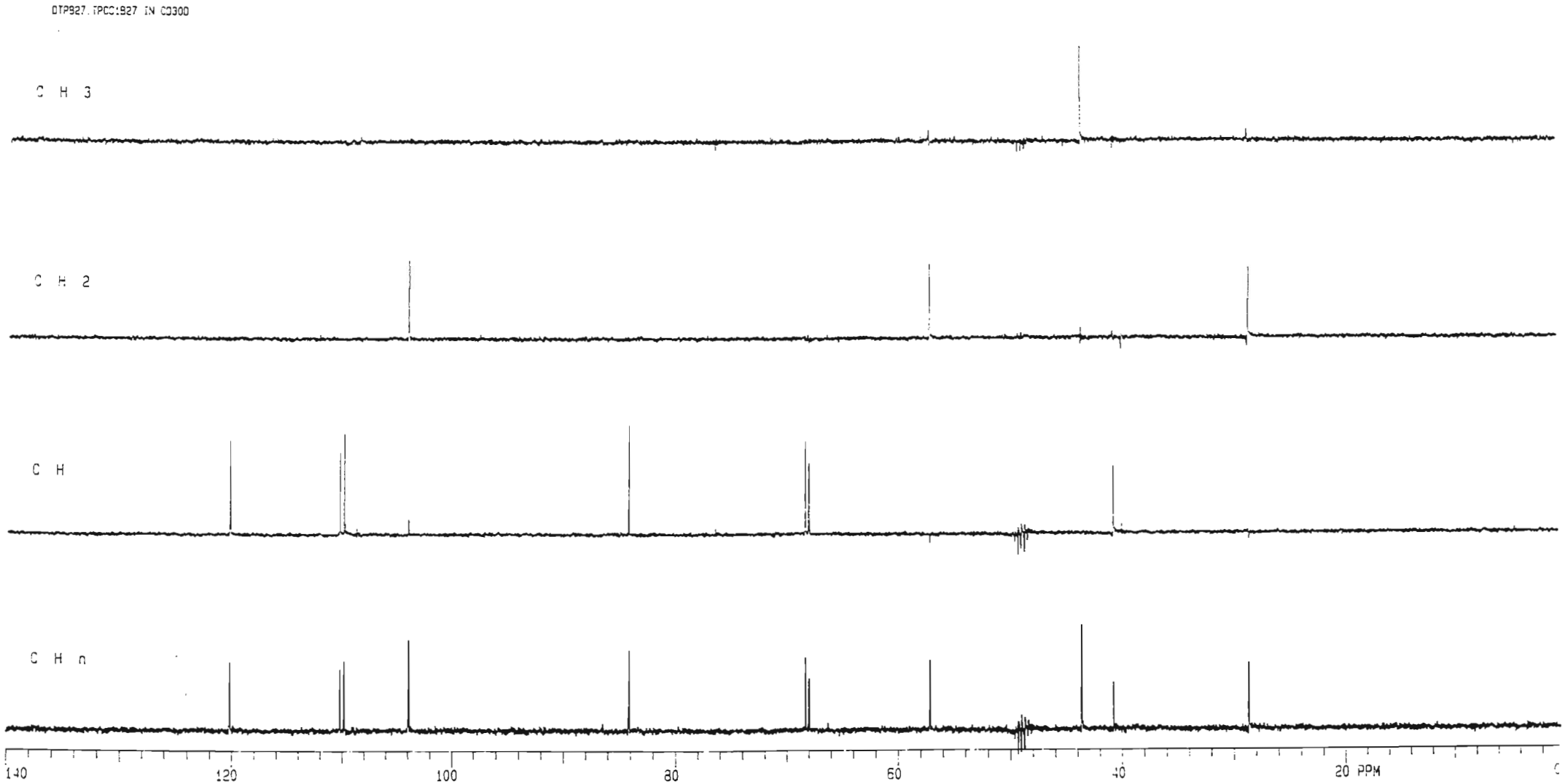
hippeastrine

^{1}H NMR spectrum of Compound III

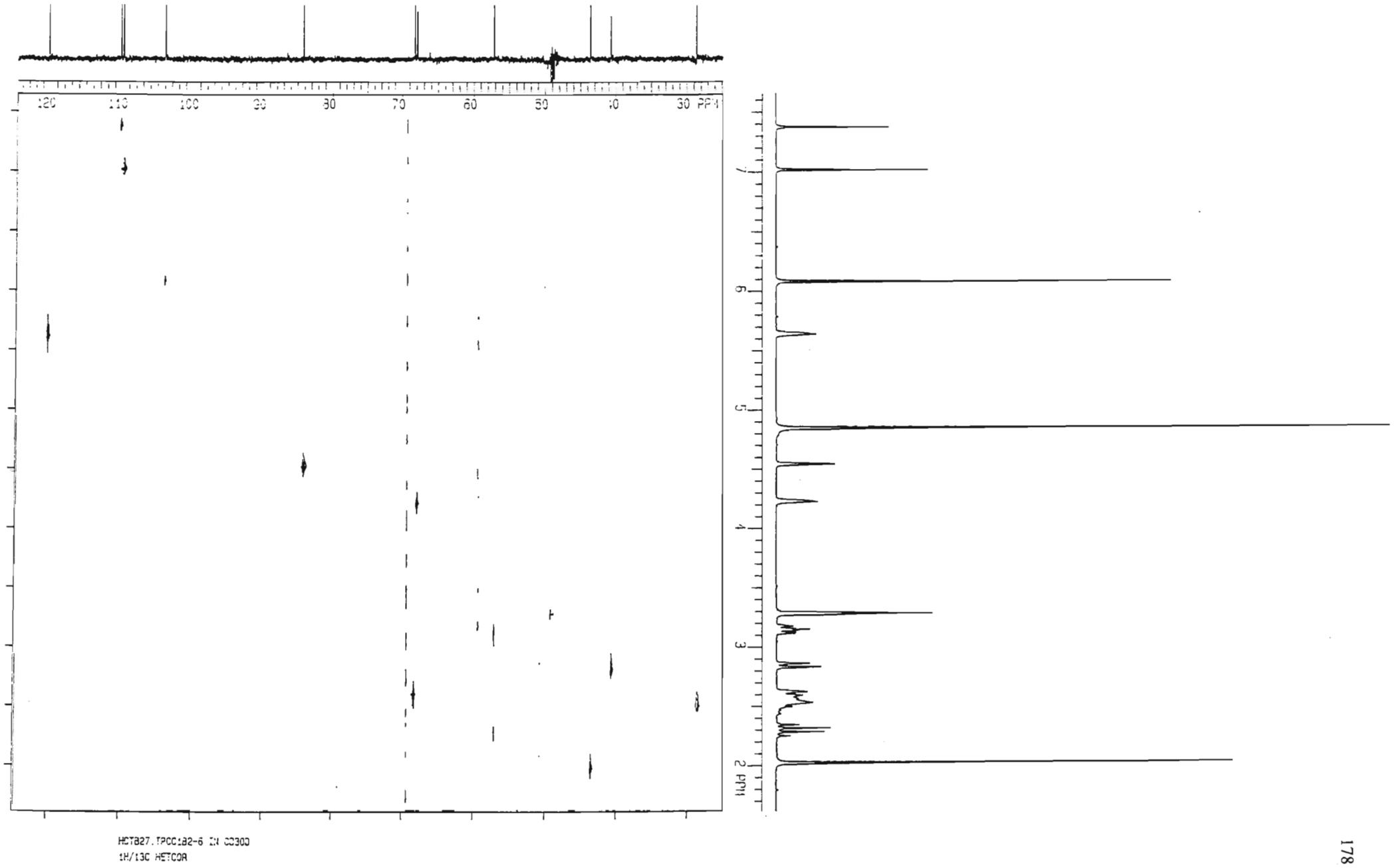
COSY NMR spectrum of Compound III



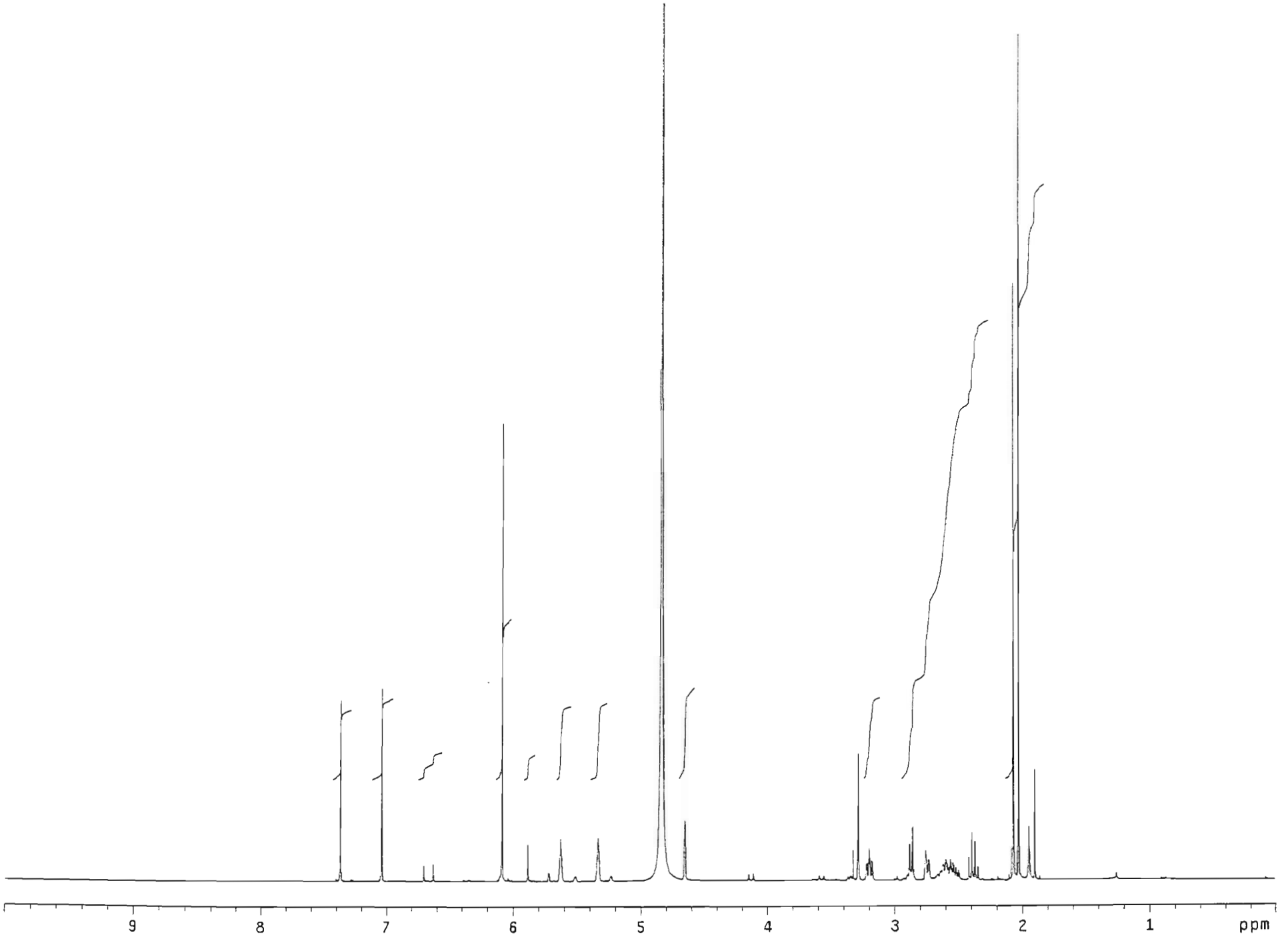
^{13}C NMR spectrum of compound III



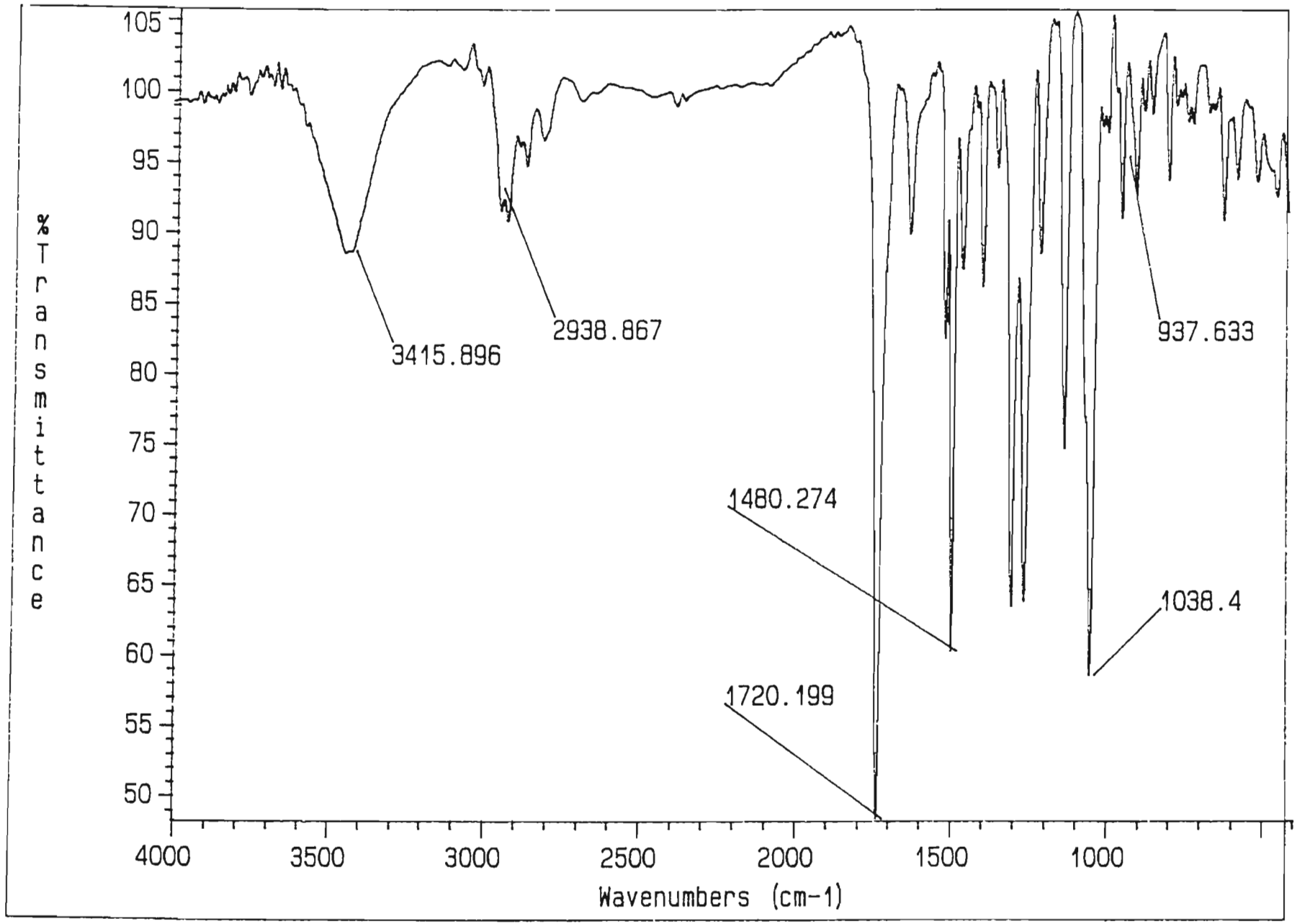
ADEPT NMR spectrum of Compound III



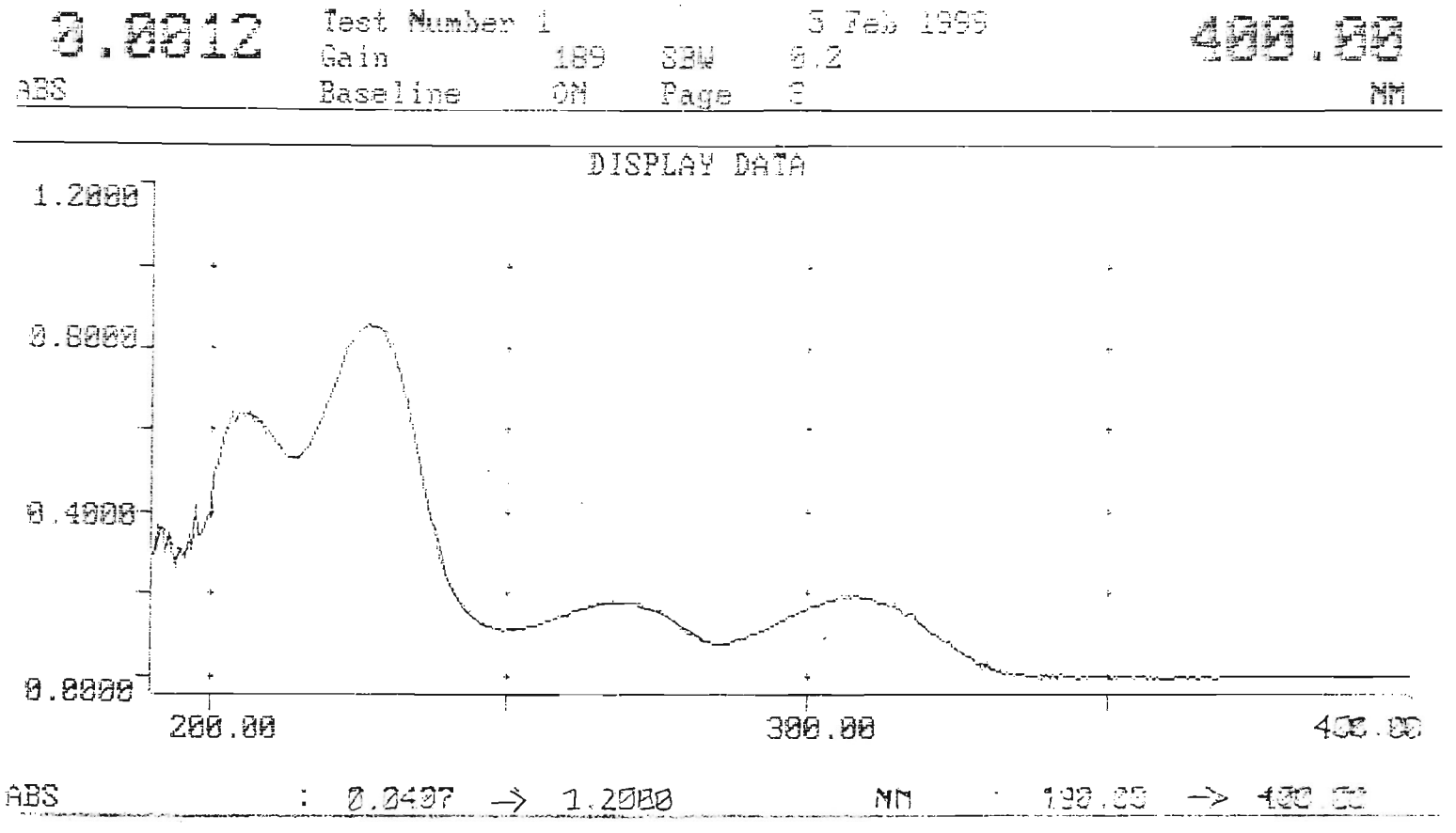
HETCOR NMR spectrum of Compound III



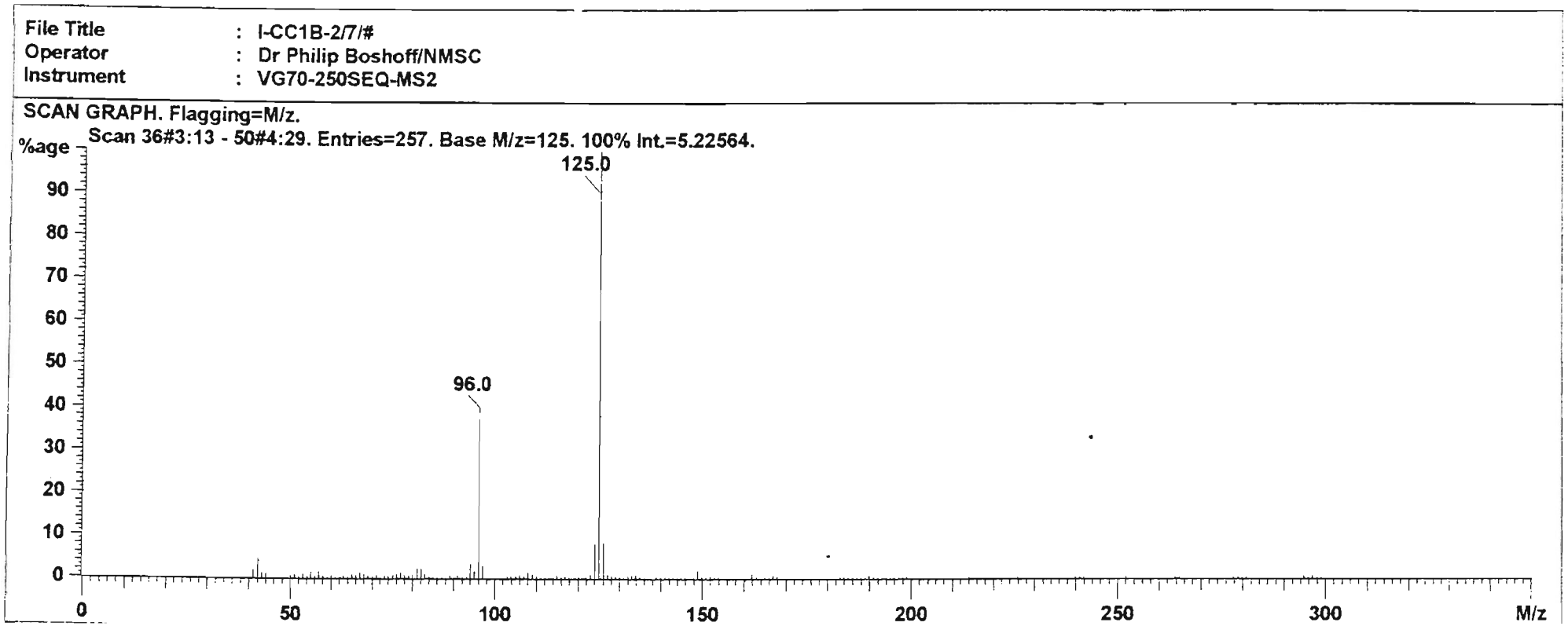
^{1}H NMR spectrum of acetylated product of compound III



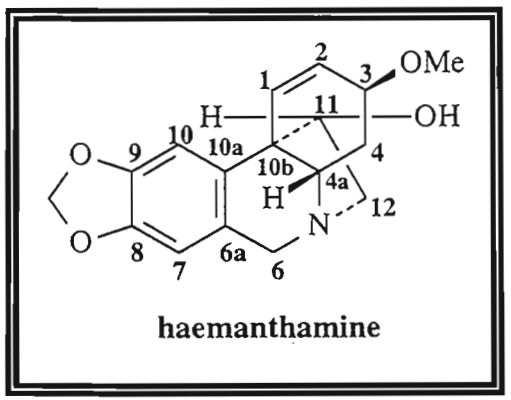
Infra-red spectrum of Compound III



UV spectrum of Compound III

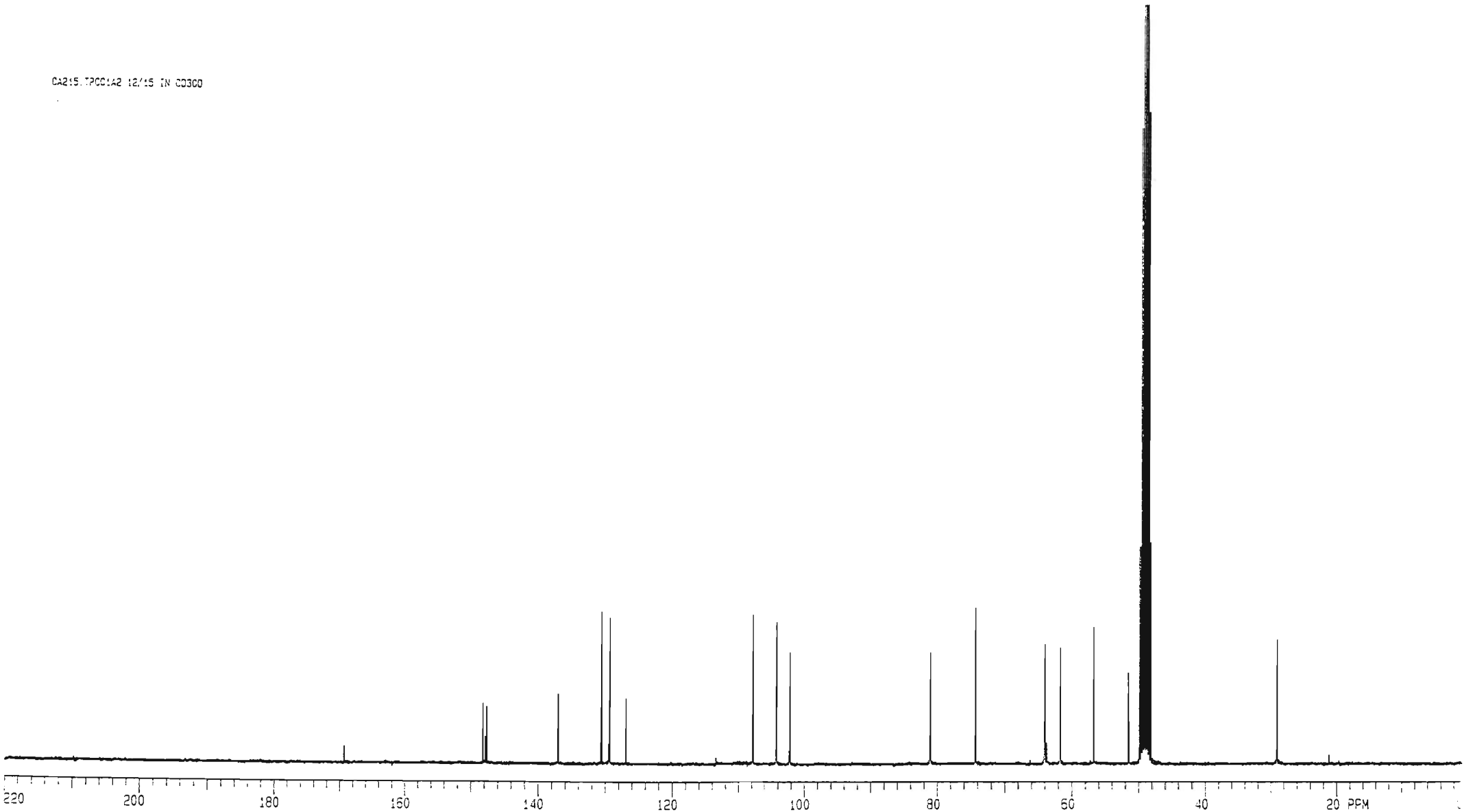


Mass spectrum of Compound III

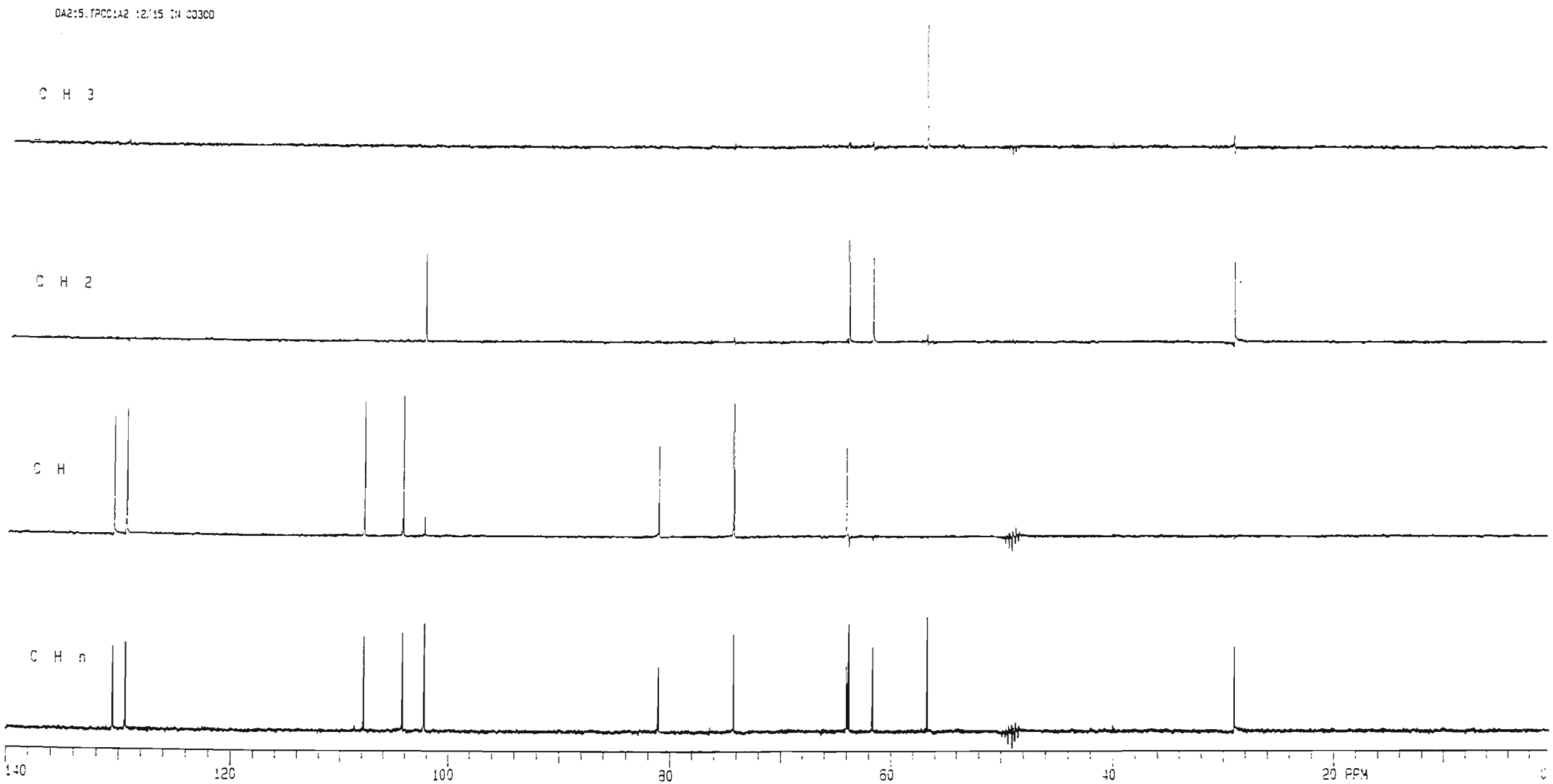


^{1}H NMR spectrum of Compound IV

COSY NMR spectrum of Compound IV



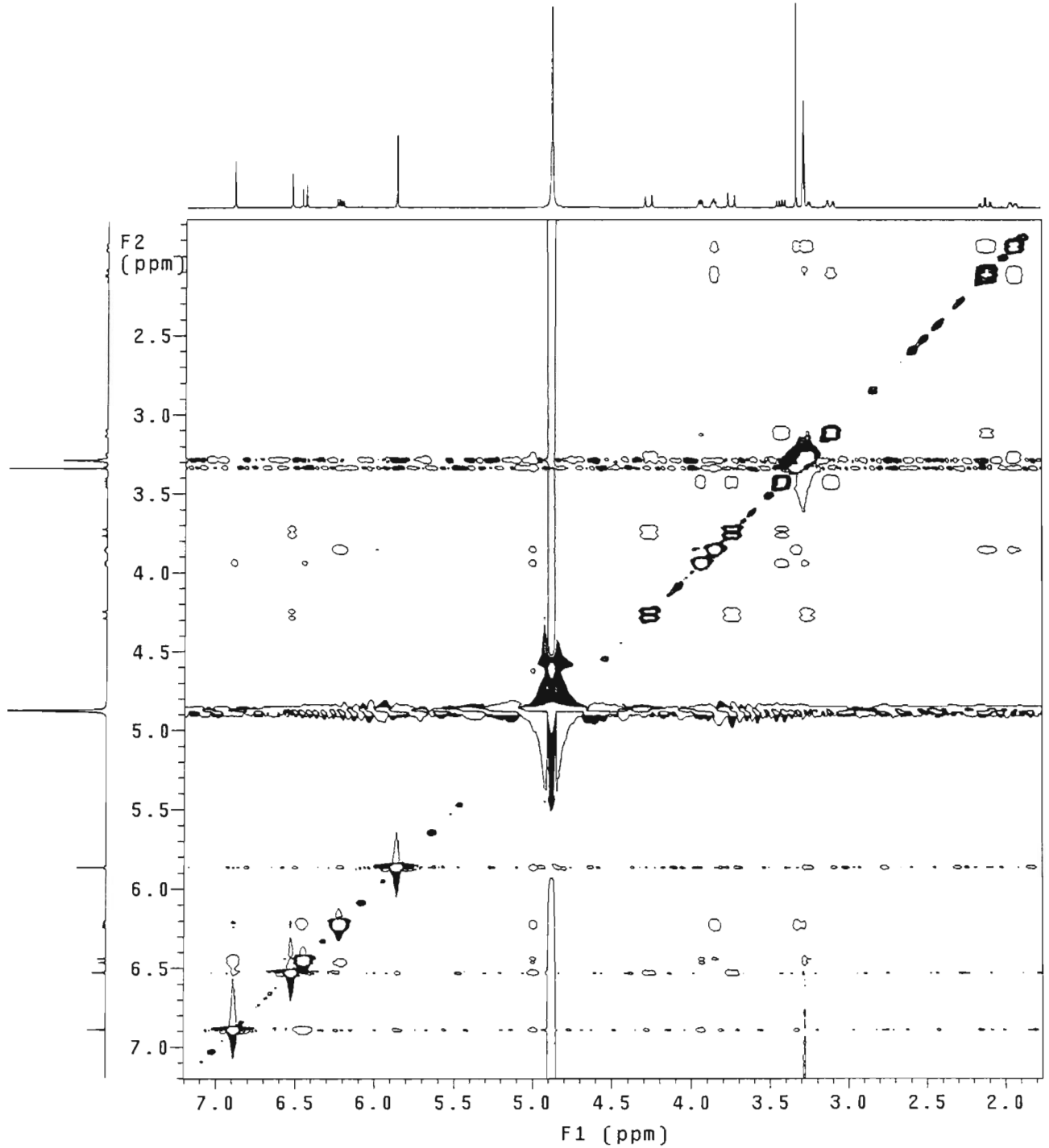
^{13}C NMR spectrum of Compound IV



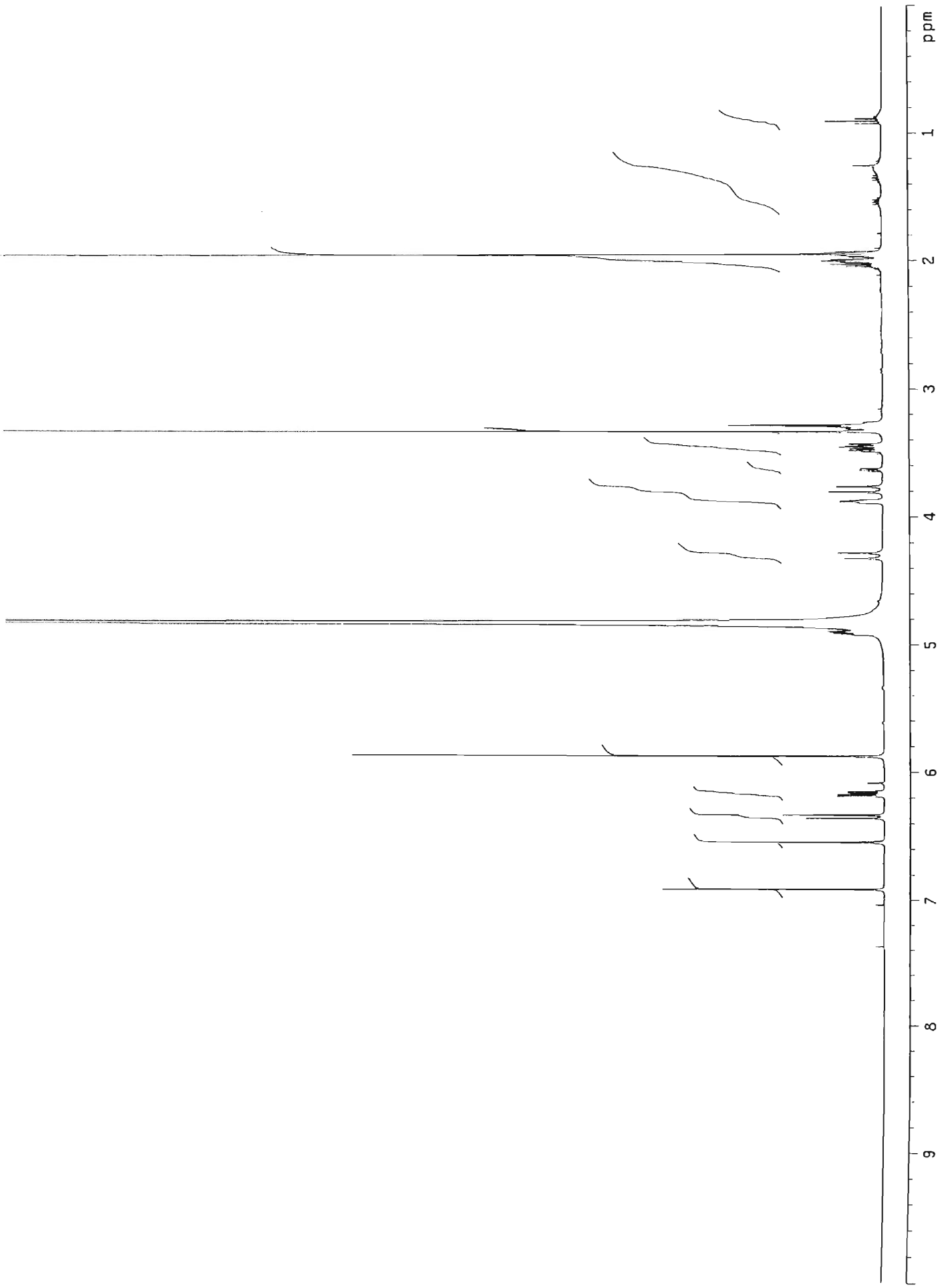
ADEPT NMR spectrum of Compound IV

Solvent: cd3od
Ambient temperature
INOVA-400 "undnmr400"

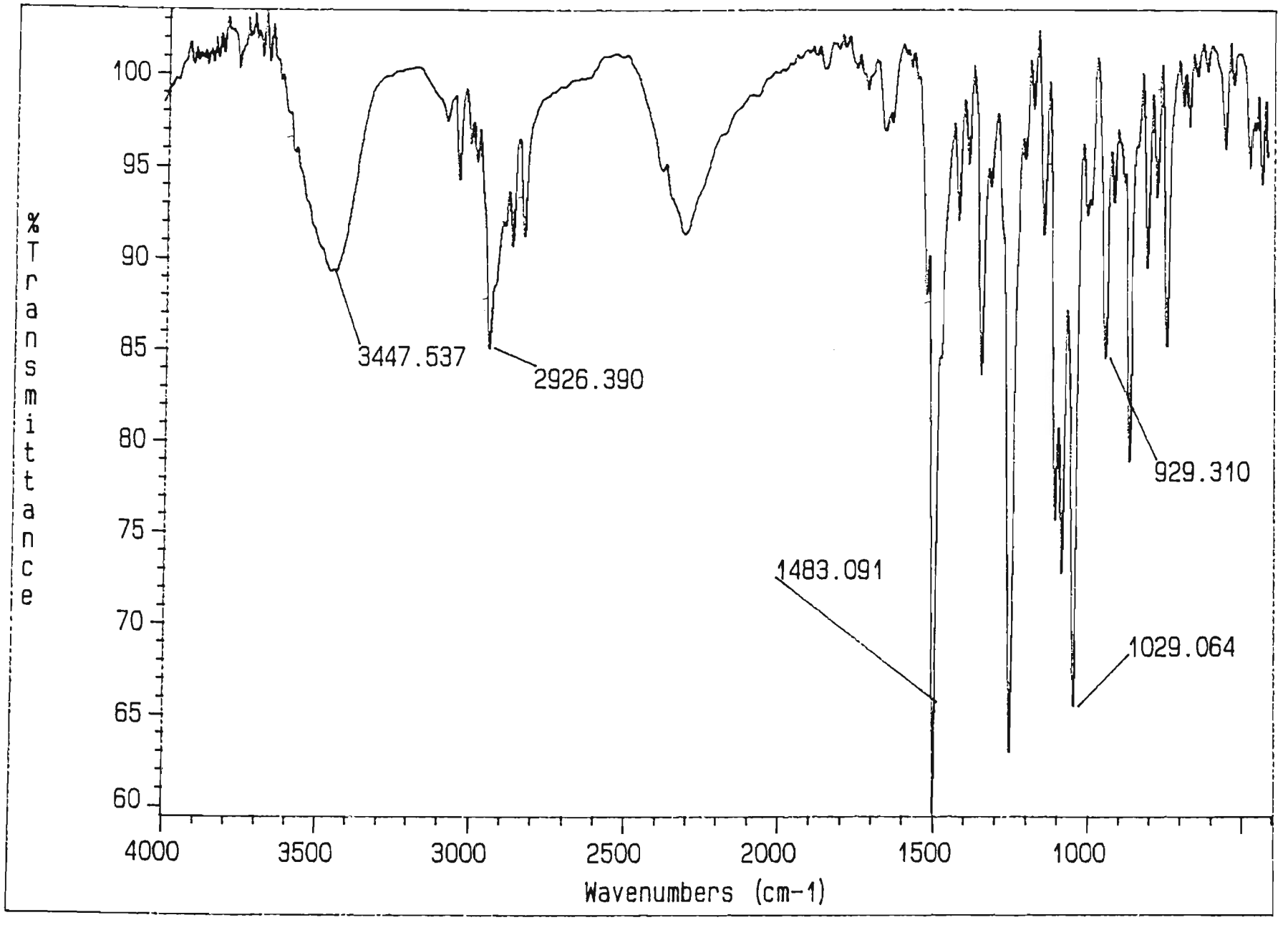
PULSE SEQUENCE: noesy
Relax. delay 4.416 sec
Mixing 1.104 sec
Acq. time 0.187 sec
Width 2736.4 Hz
2D Width 2736.4 Hz
16 repetitions
2 x 224 increments
OBSERVE H1, 399.9502544 MHz
DECOUPLE H1, 399.9522542 MHz
Power 30 dB
off during acquisition
DATA PROCESSING
Gauss apodization 0.086 sec
F1 DATA PROCESSING
Gauss apodization 0.043 sec
FT size 1024 x 1024
Total time 11.5 hours



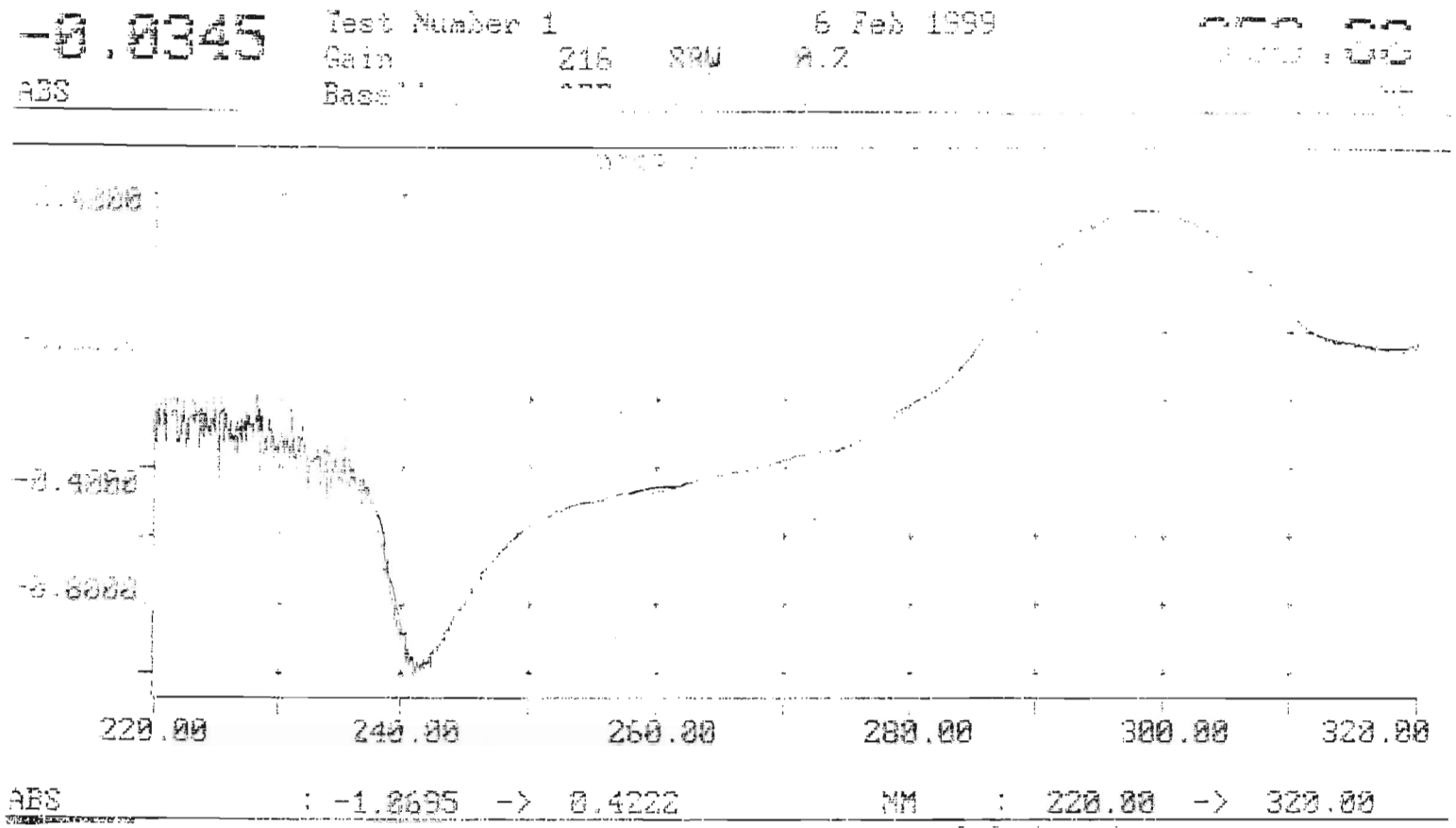
NOESY NMR spectrum of Compound IV



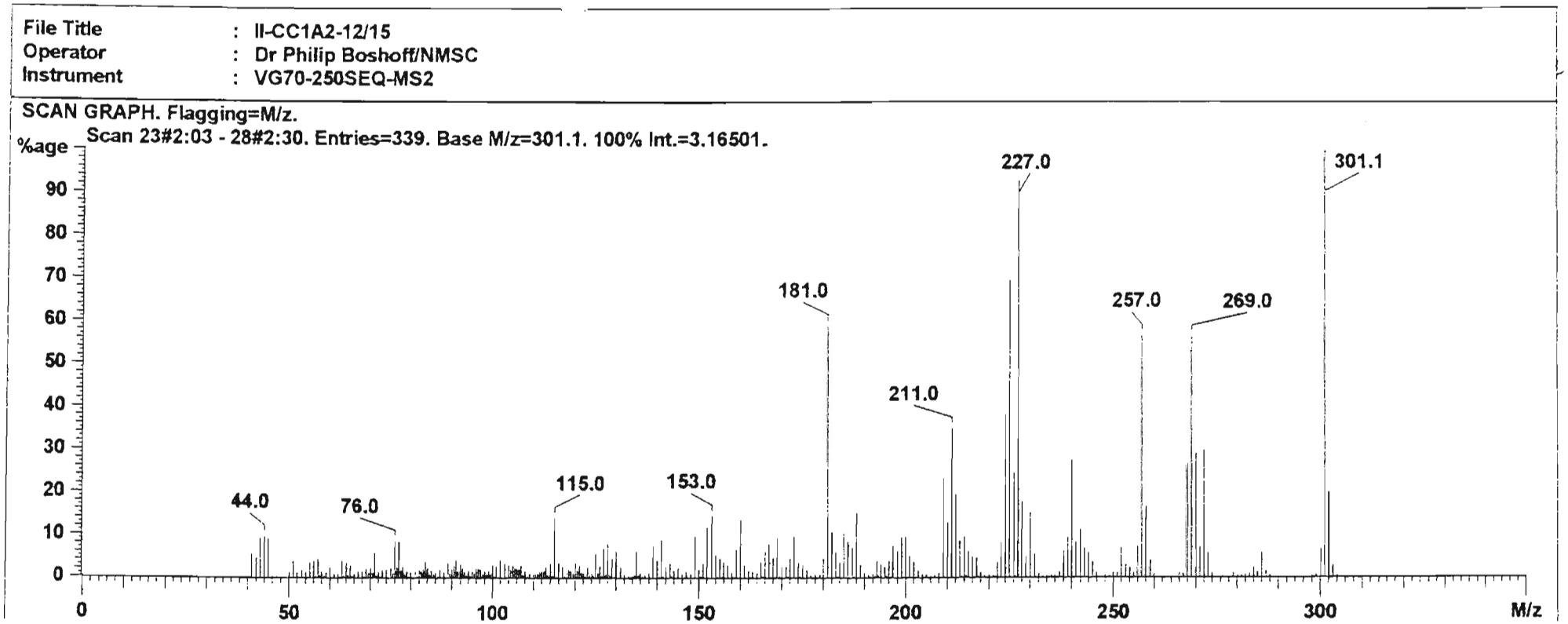
^{1}H NMR spectrum of acetylated product of Compound IV



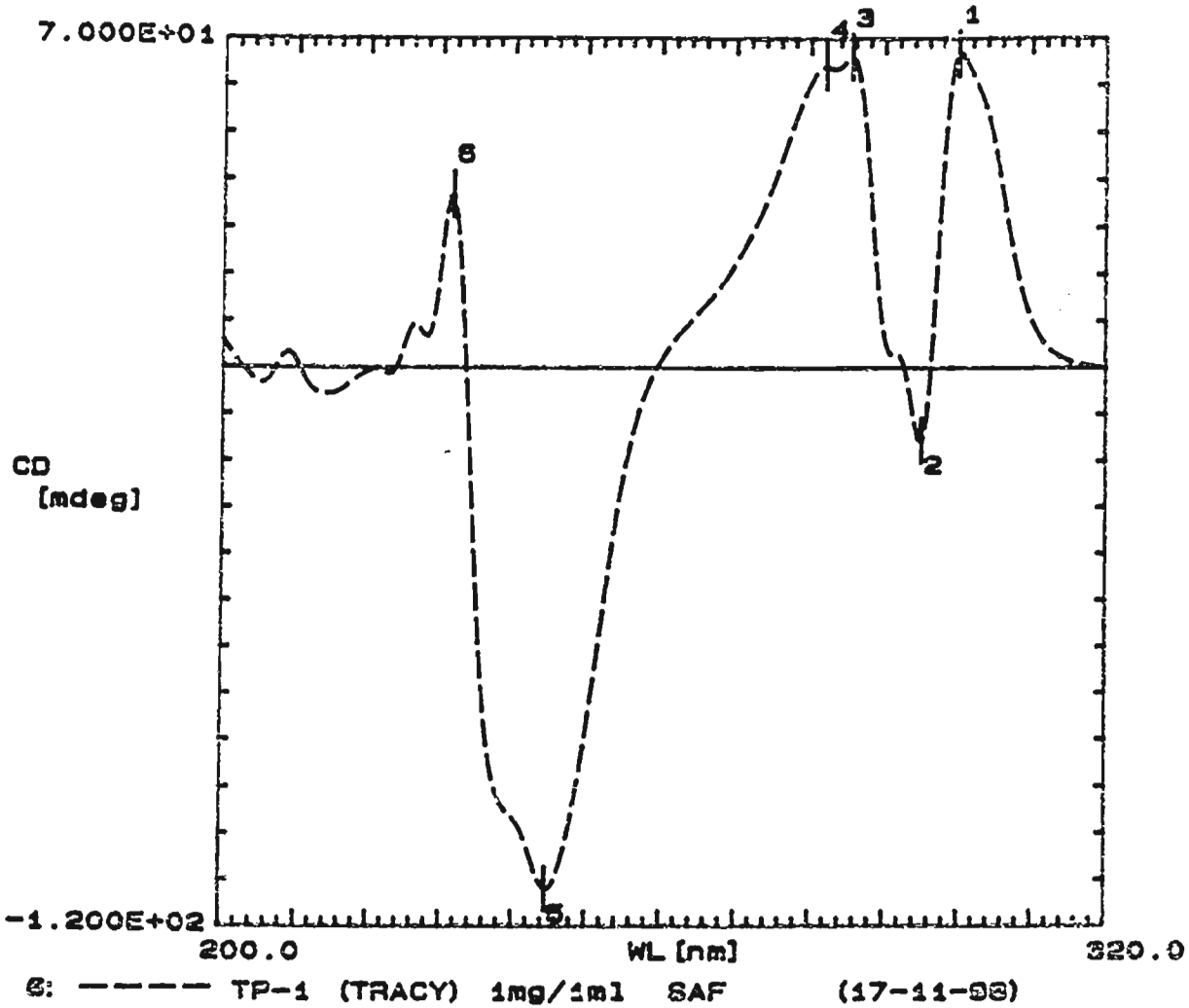
Infra-red spectrum of Compound IV



UV spectrum of Compound IV

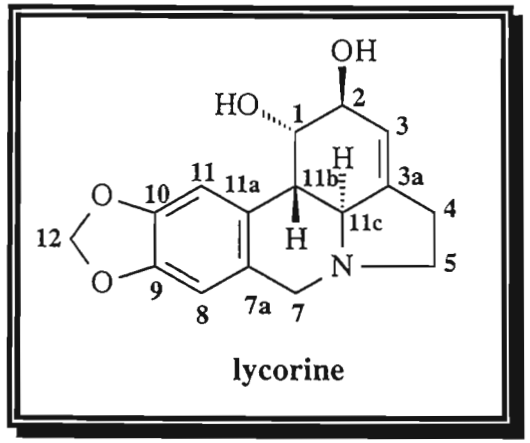


Mass spectrum of Compound IV



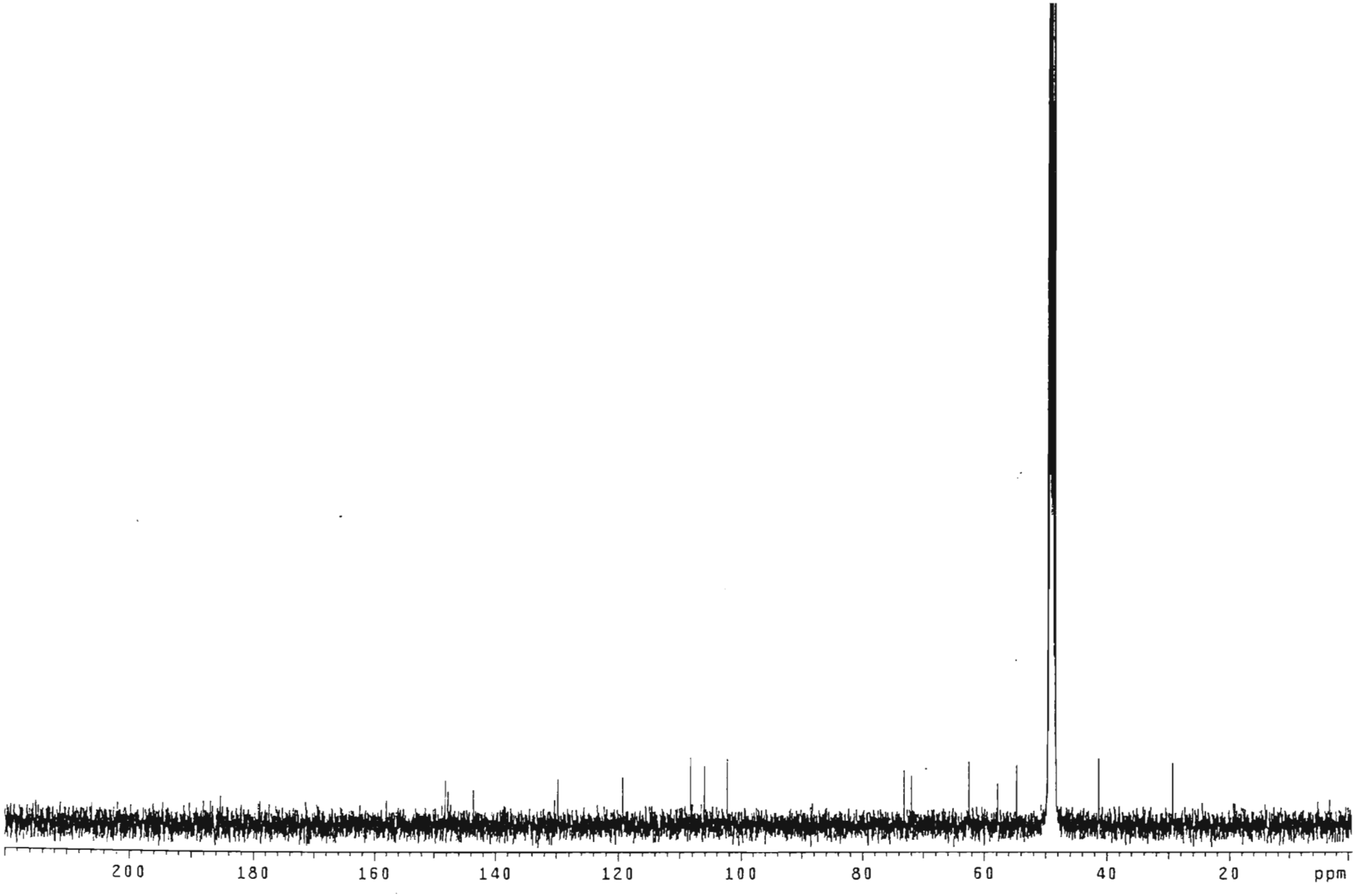
No.	Wavelength	Value
1	300.00 nm	6.689E+01
2	295.00 nm	-1.556E+01
3	285.50 nm	6.610E+01
4	282.00 nm	6.388E+01
5	244.50 nm	-1.120E+02
6	231.50 nm	3.696E+01

CD spectrum of Compound IV



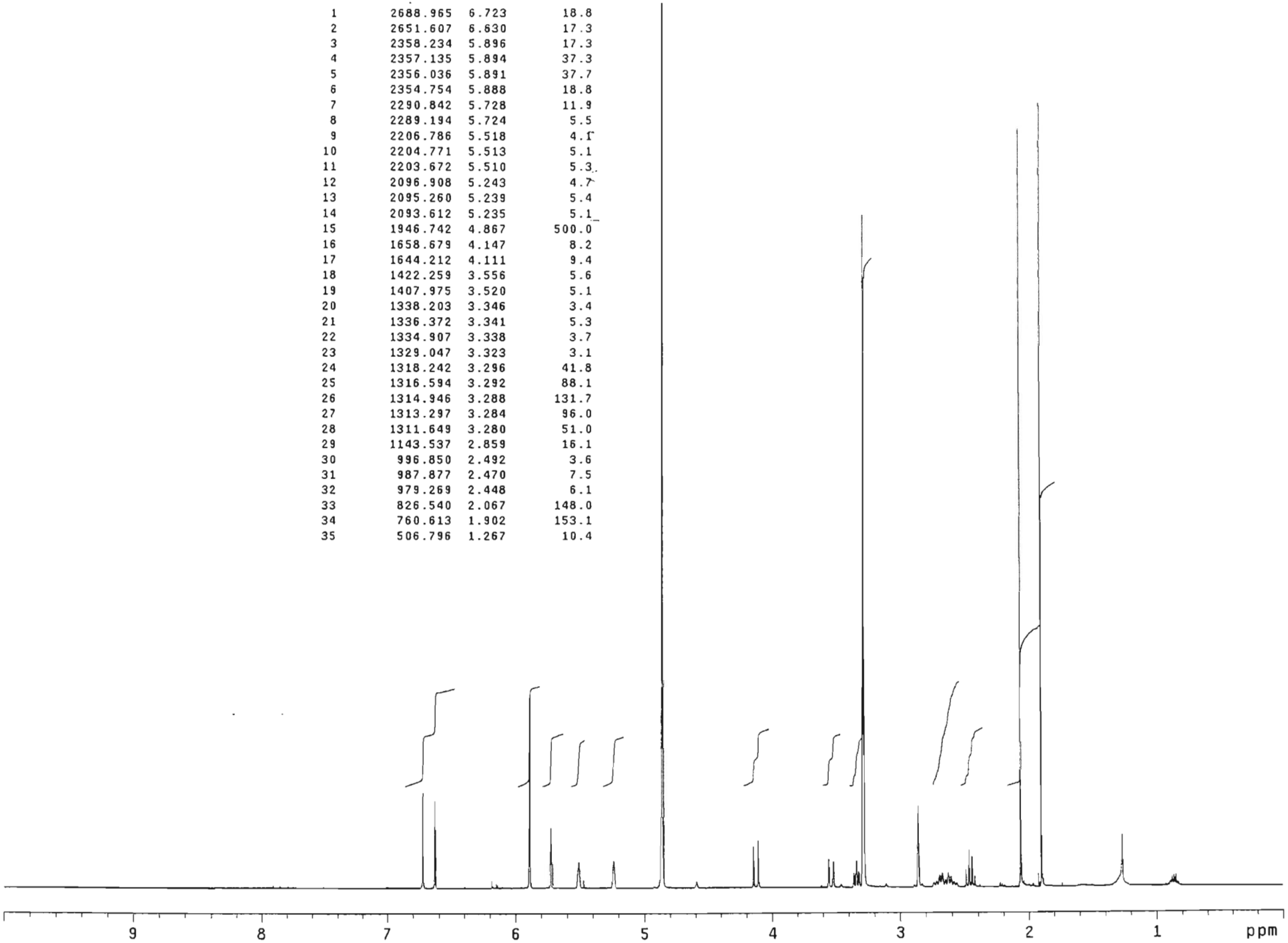
lycorine

^{1}H NMR spectrum of Compound V

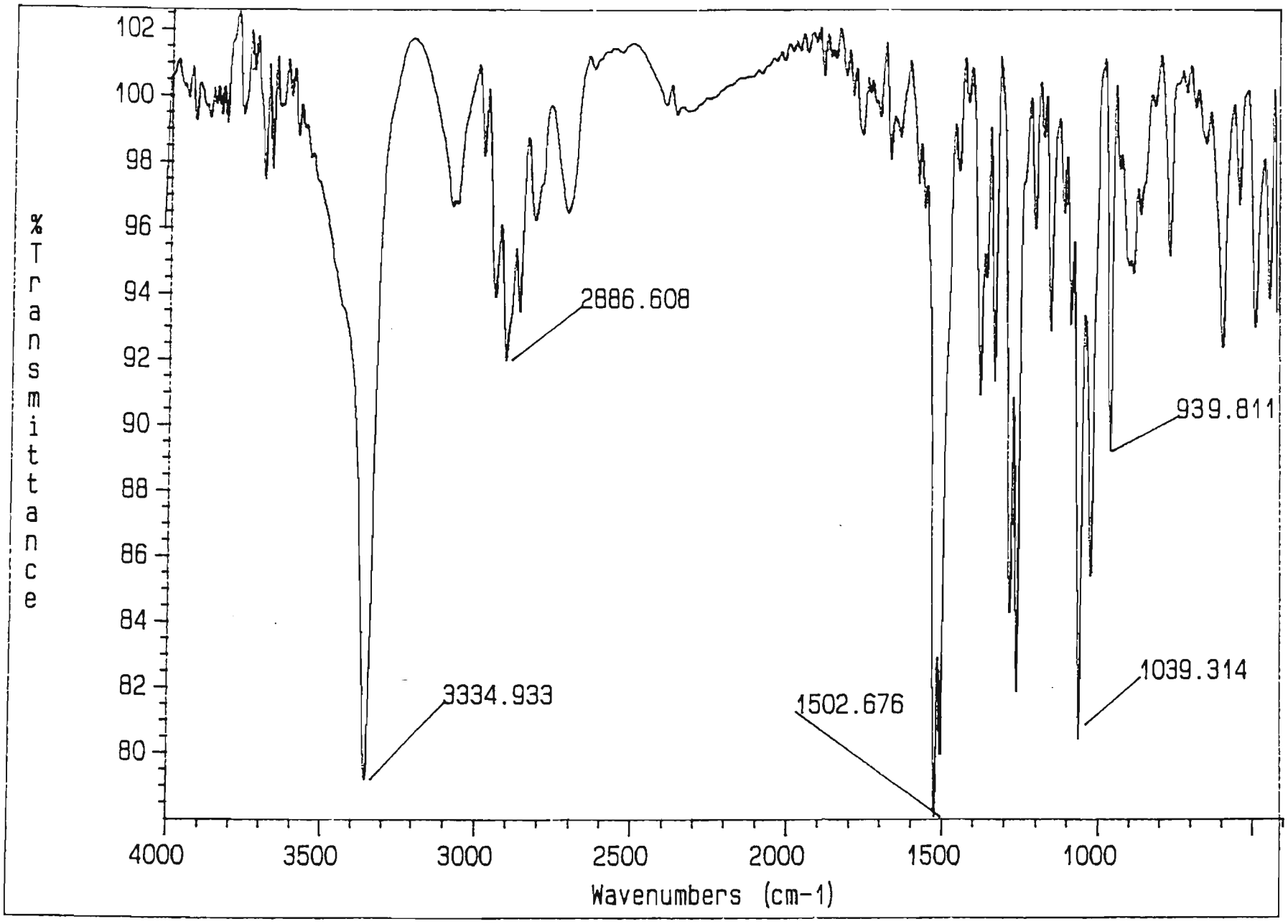


^{13}C NMR spectrum of Compound V

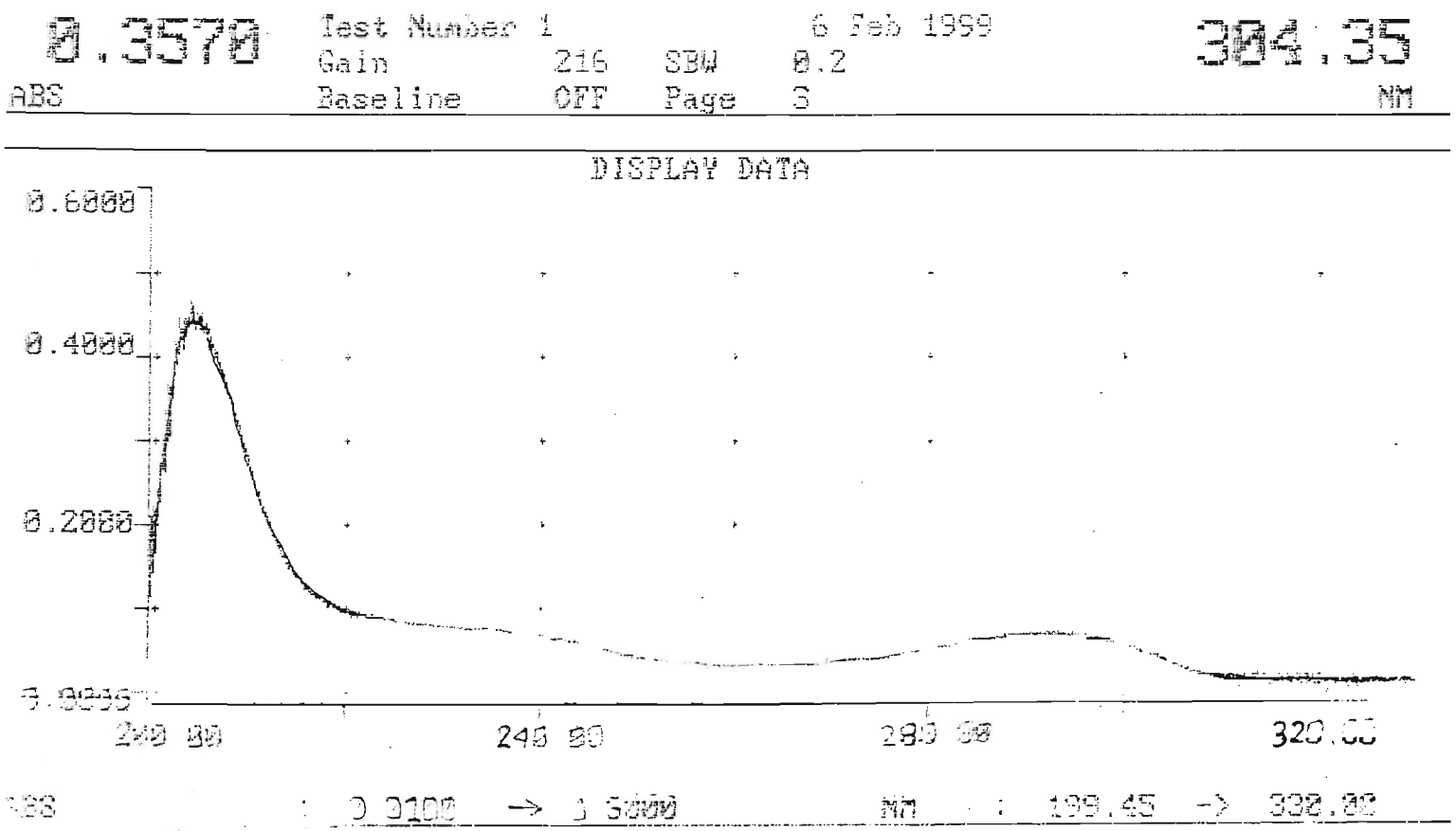
1	2688.965	6.723	18.8
2	2651.607	6.630	17.3
3	2358.234	5.896	17.3
4	2357.135	5.894	37.3
5	2356.036	5.891	37.7
6	2354.754	5.888	18.8
7	2290.842	5.728	11.9
8	2289.194	5.724	5.5
9	2206.786	5.518	4.1
10	2204.771	5.513	5.1
11	2203.672	5.510	5.3
12	2096.908	5.243	4.7
13	2095.260	5.239	5.4
14	2093.612	5.235	5.1
15	1946.742	4.867	500.0
16	1658.679	4.147	8.2
17	1644.212	4.111	9.4
18	1422.259	3.556	5.6
19	1407.975	3.520	5.1
20	1338.203	3.346	3.4
21	1336.372	3.341	5.3
22	1334.907	3.338	3.7
23	1329.047	3.323	3.1
24	1318.242	3.296	41.8
25	1316.594	3.292	88.1
26	1314.946	3.288	131.7
27	1313.297	3.284	96.0
28	1311.649	3.280	51.0
29	1143.537	2.859	16.1
30	996.850	2.492	3.6
31	987.877	2.470	7.5
32	979.269	2.448	6.1
33	826.540	2.067	148.0
34	760.613	1.902	153.1
35	506.796	1.267	10.4



^{1}H NMR spectrum of acetylated product of Compound V

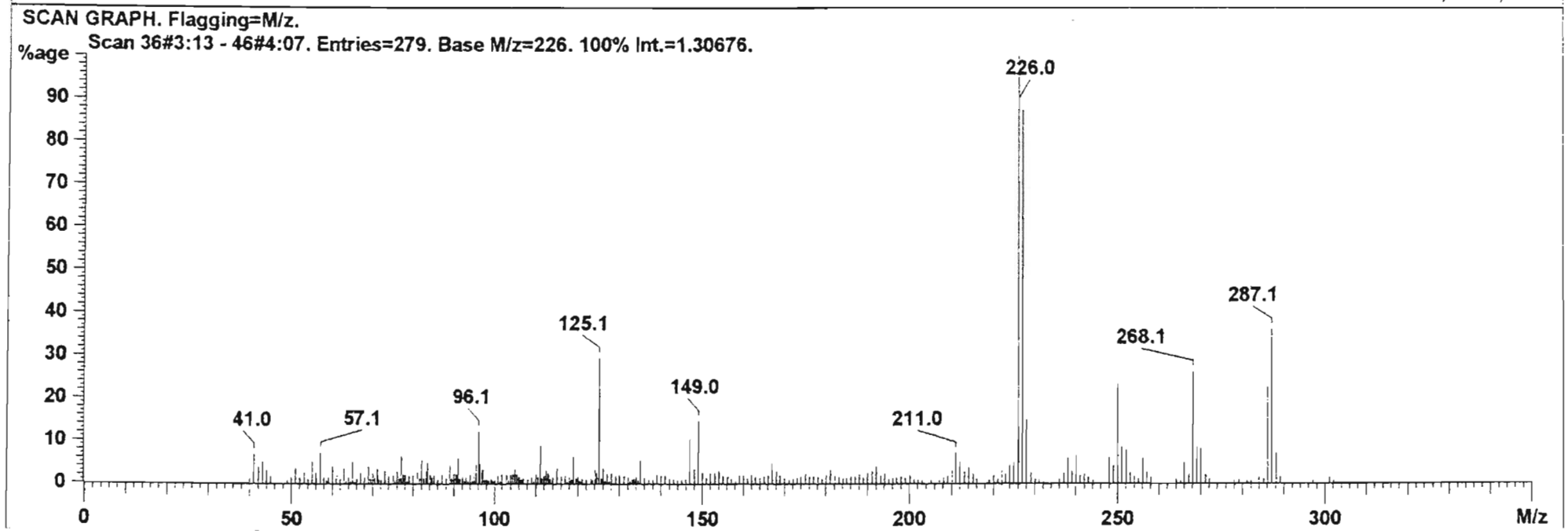


Infra-red spectrum of Compound V

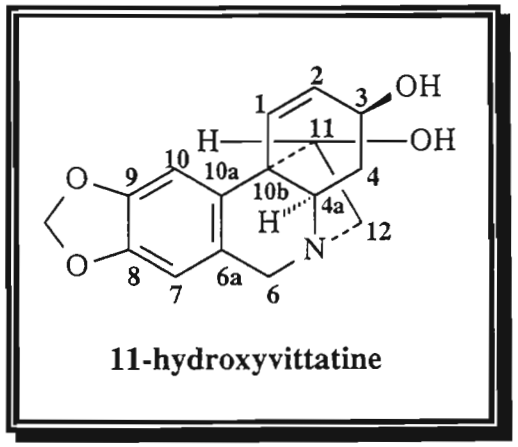


UV spectrum of Compound V

File Title : III-CC2-22-25
Operator : Dr Philip Boshoff/NMSC
Instrument : VG70-250SEQ-MS2



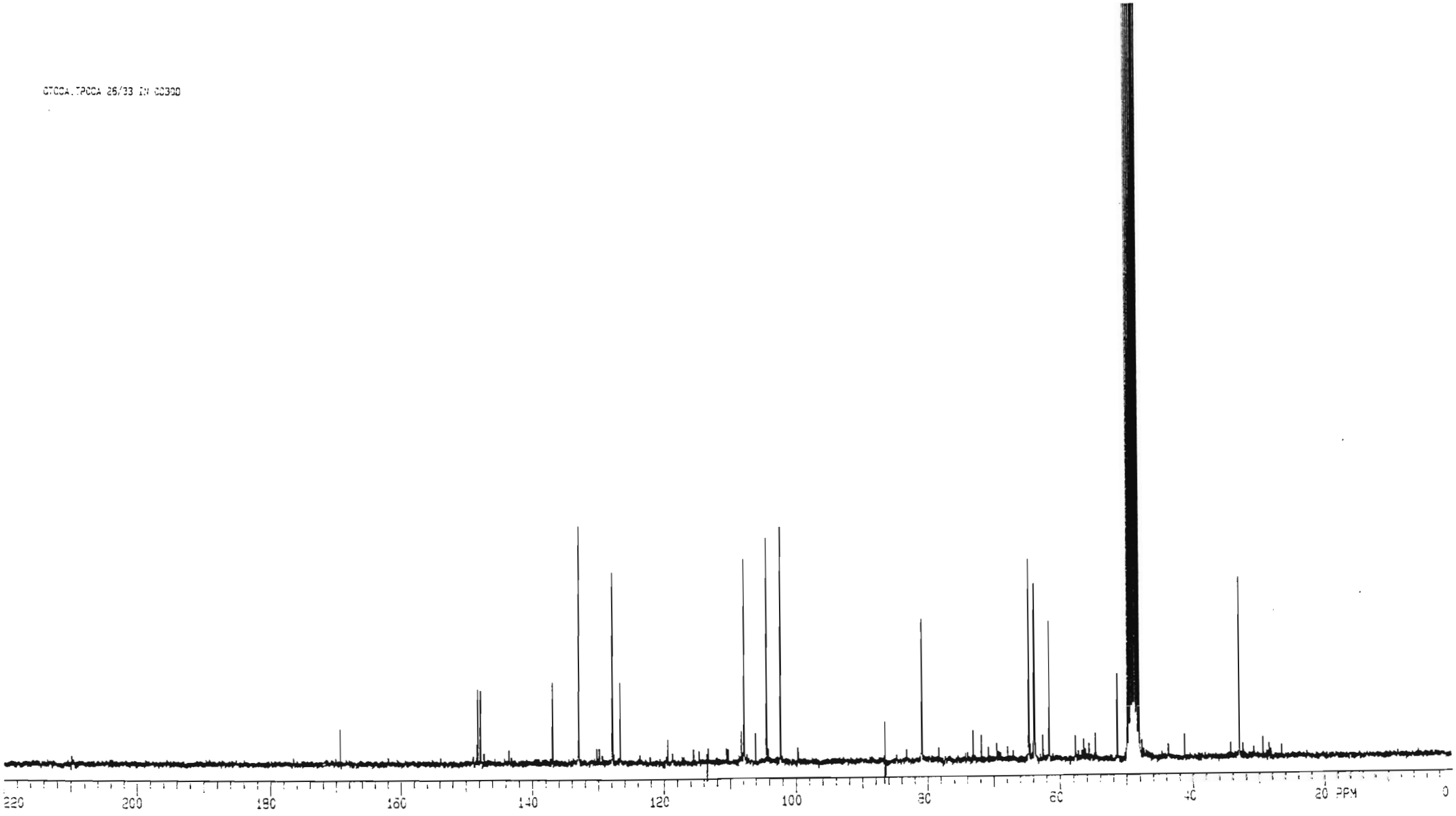
Mass Spectrum of Compound V



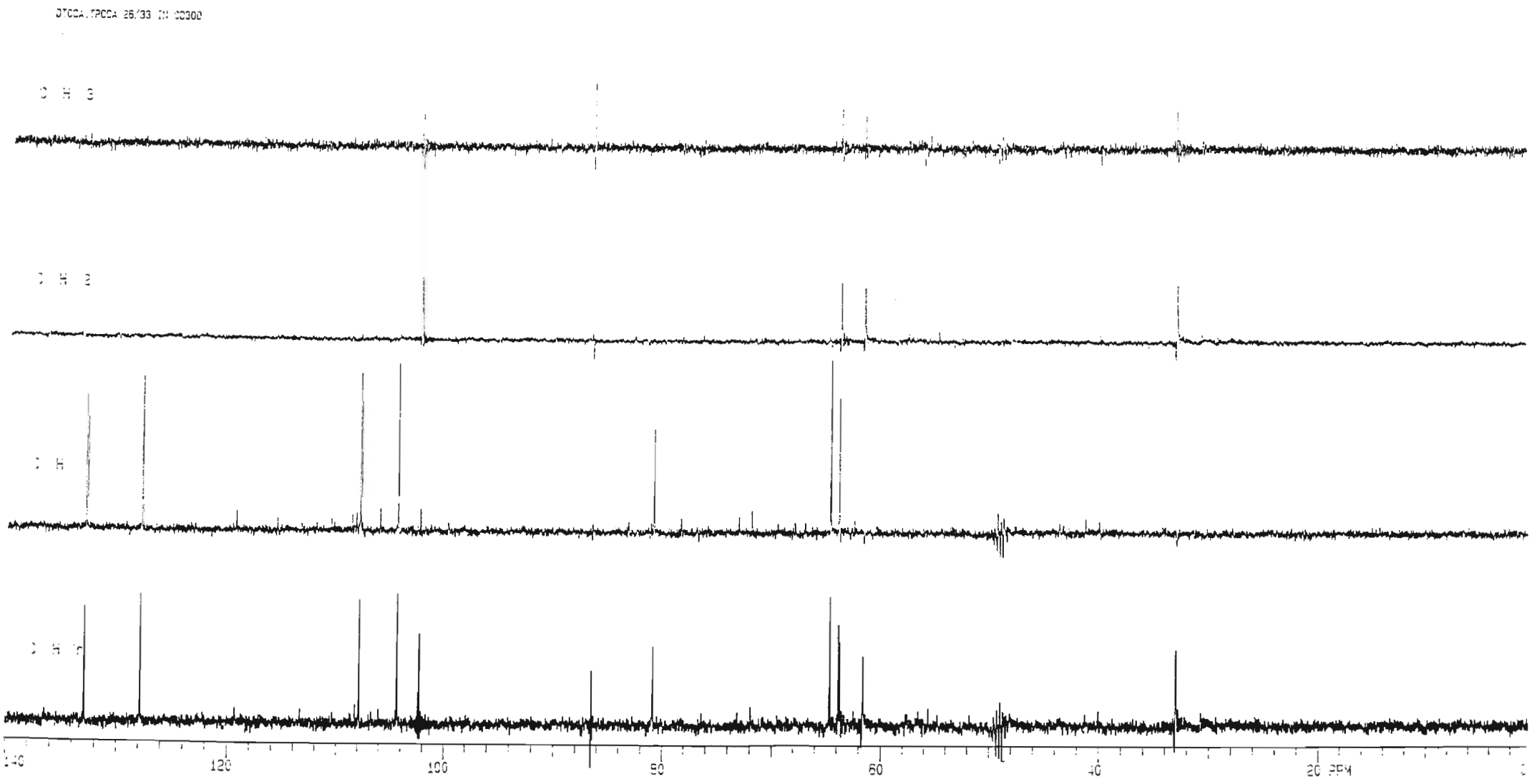
11-hydroxyvittatine

^{1}H NMR spectrum of Compound VI

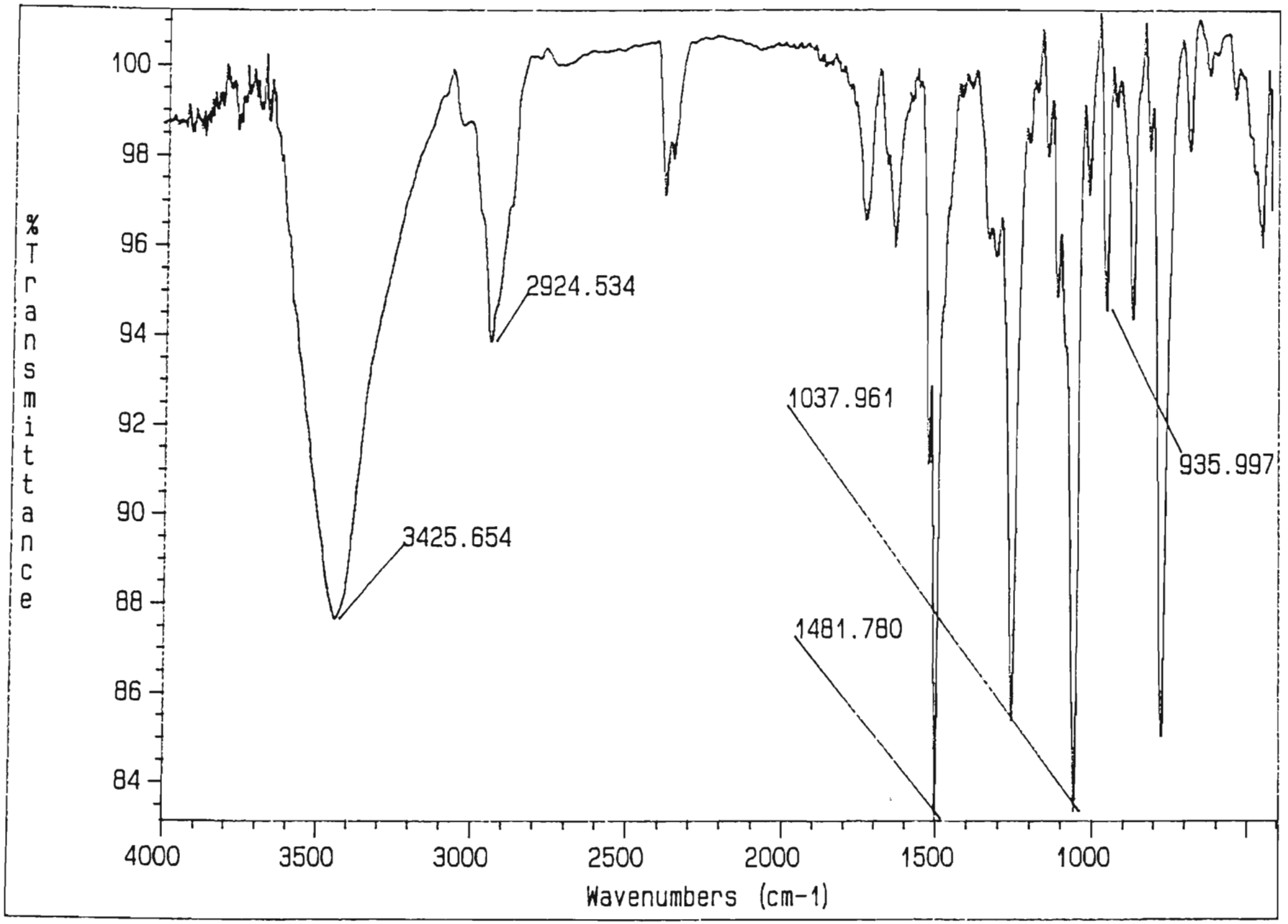
COSY NMR spectrum of Compound VI



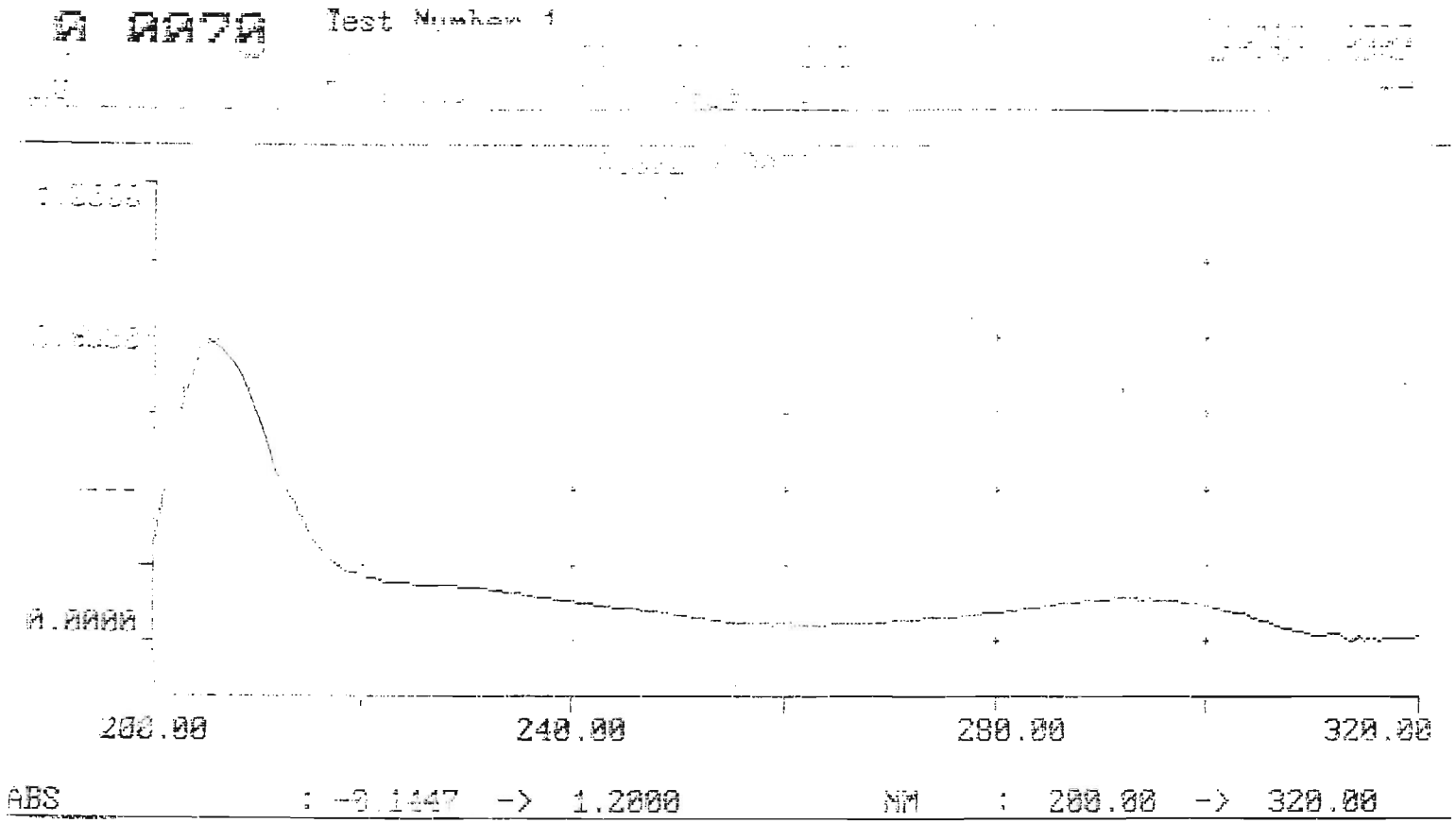
^{13}C NMR spectrum of Compound VI



ADEPT NMR spectrum of Compound VI

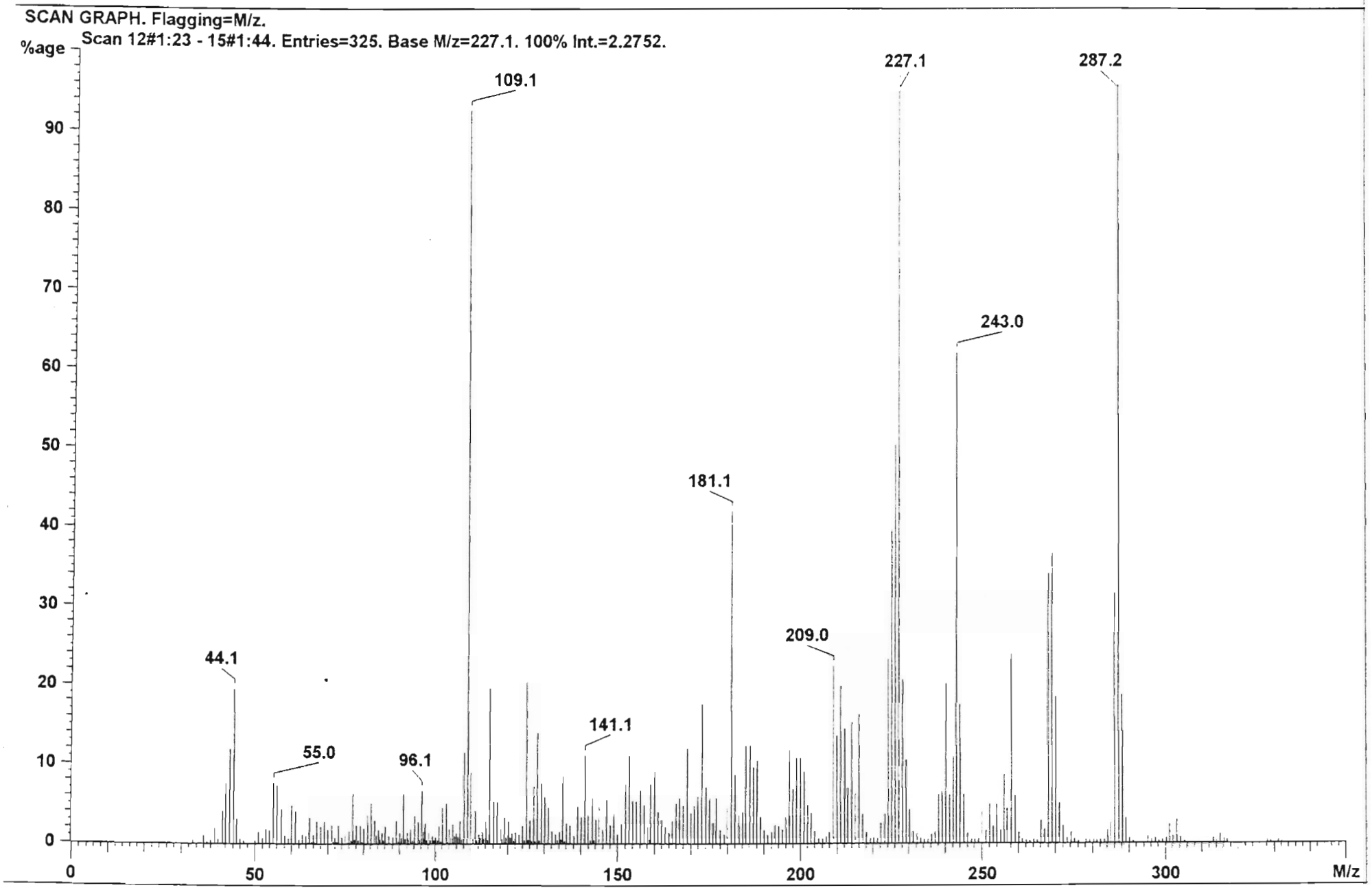


Infra-red spectrum of Compound VI

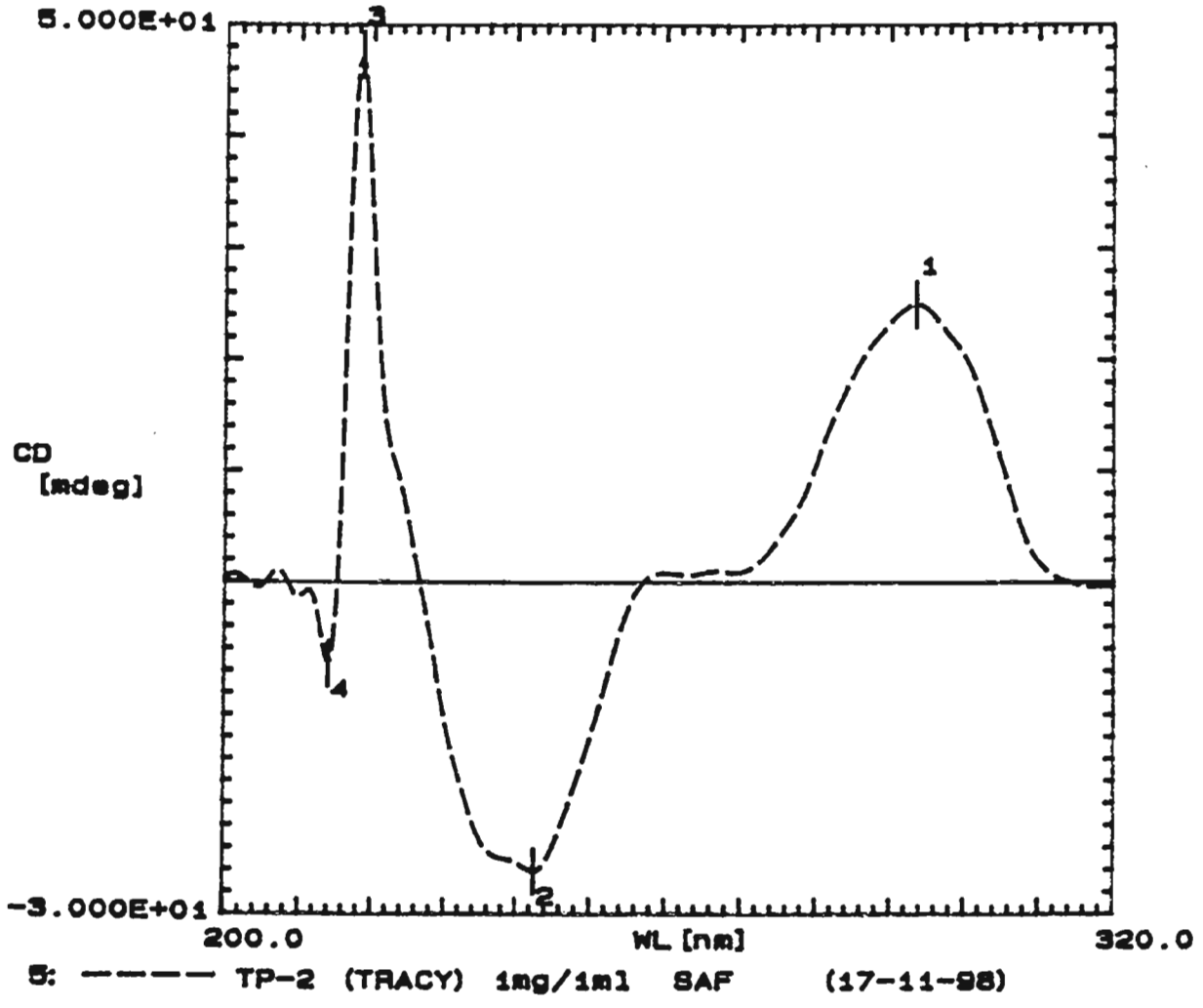


UV spectrum of Compound VI

Operator : Dr P.R.BOSHOFF NCMS
Instrument : VG70-SEQ
Notes : MSS Ltd - Maspec II Data System

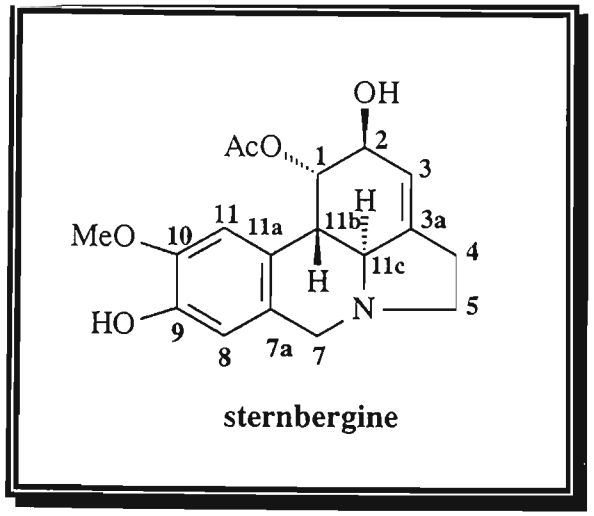


Mass spectrum of Compound VI



No.	Wavelength	Value
1	293.50 nm	2.508E+01
2	242.50 nm	-2.620E+01
3	218.50 nm	4.734E+01
4	214.00 nm	-7.413E+00

CD spectrum of Compound VI

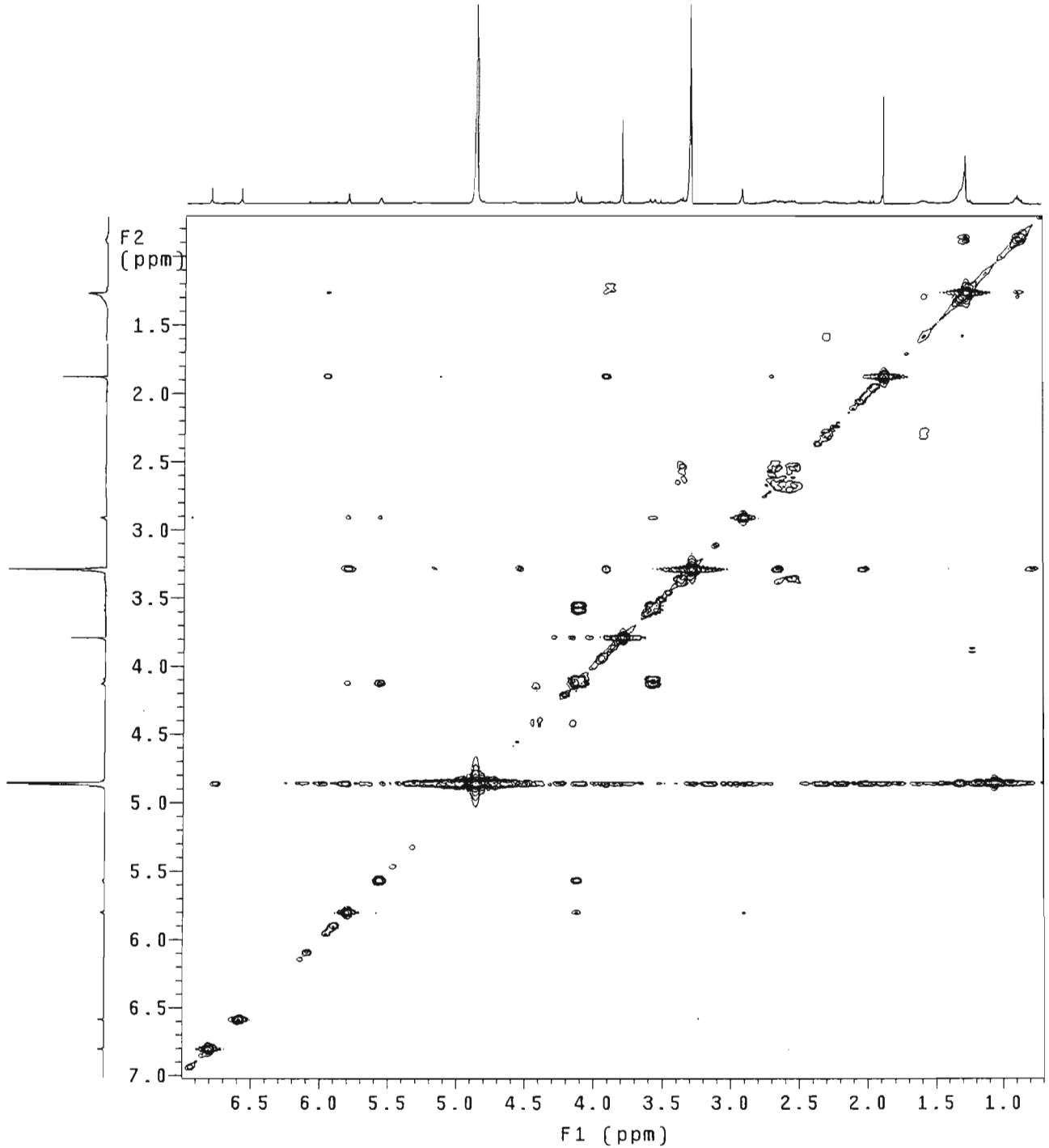


sternbergine

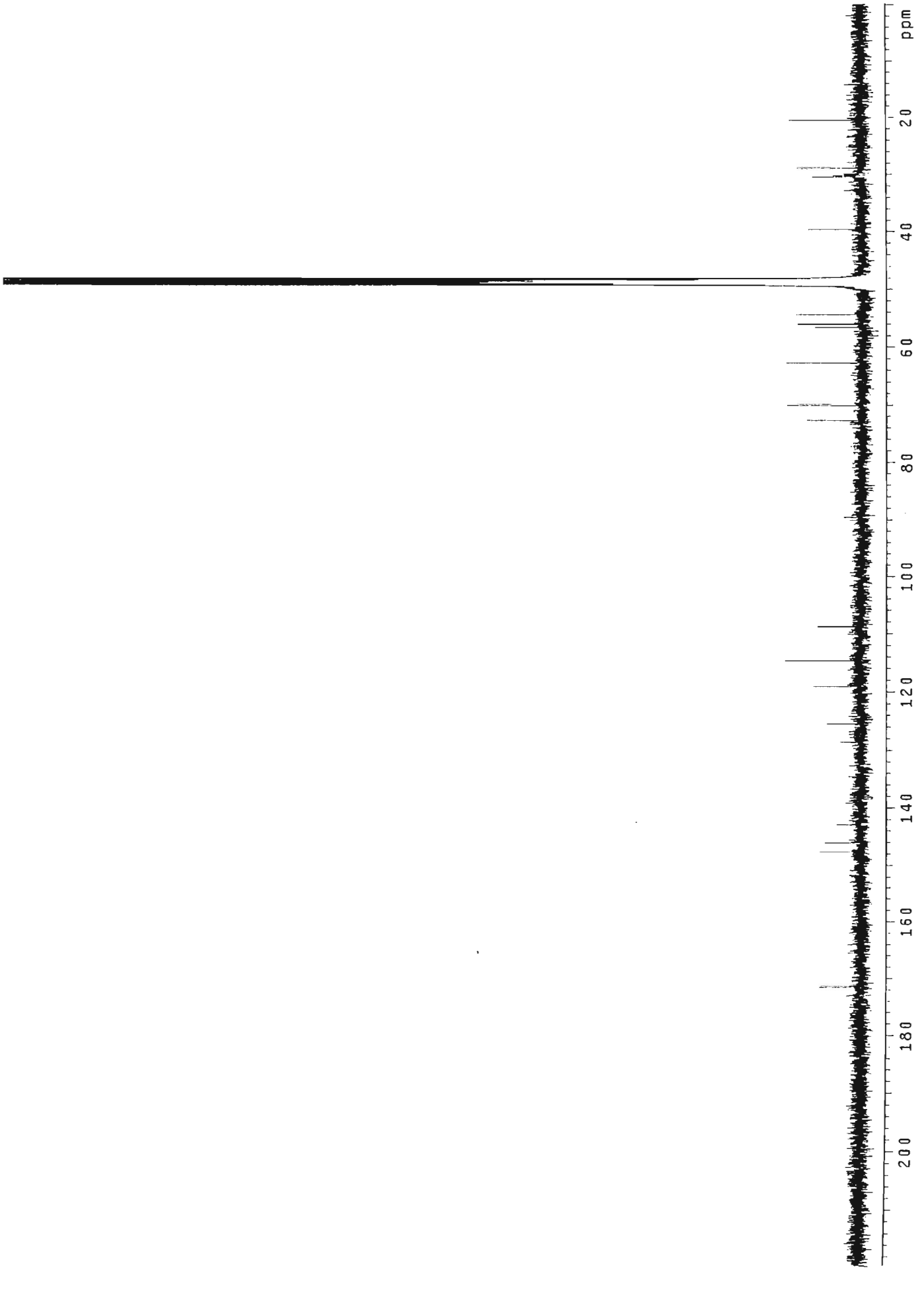
^{1}H NMR spectrum of Compound VII

Solvent: cd3od
Ambient temperature
INOVA-400 "undnmr400"

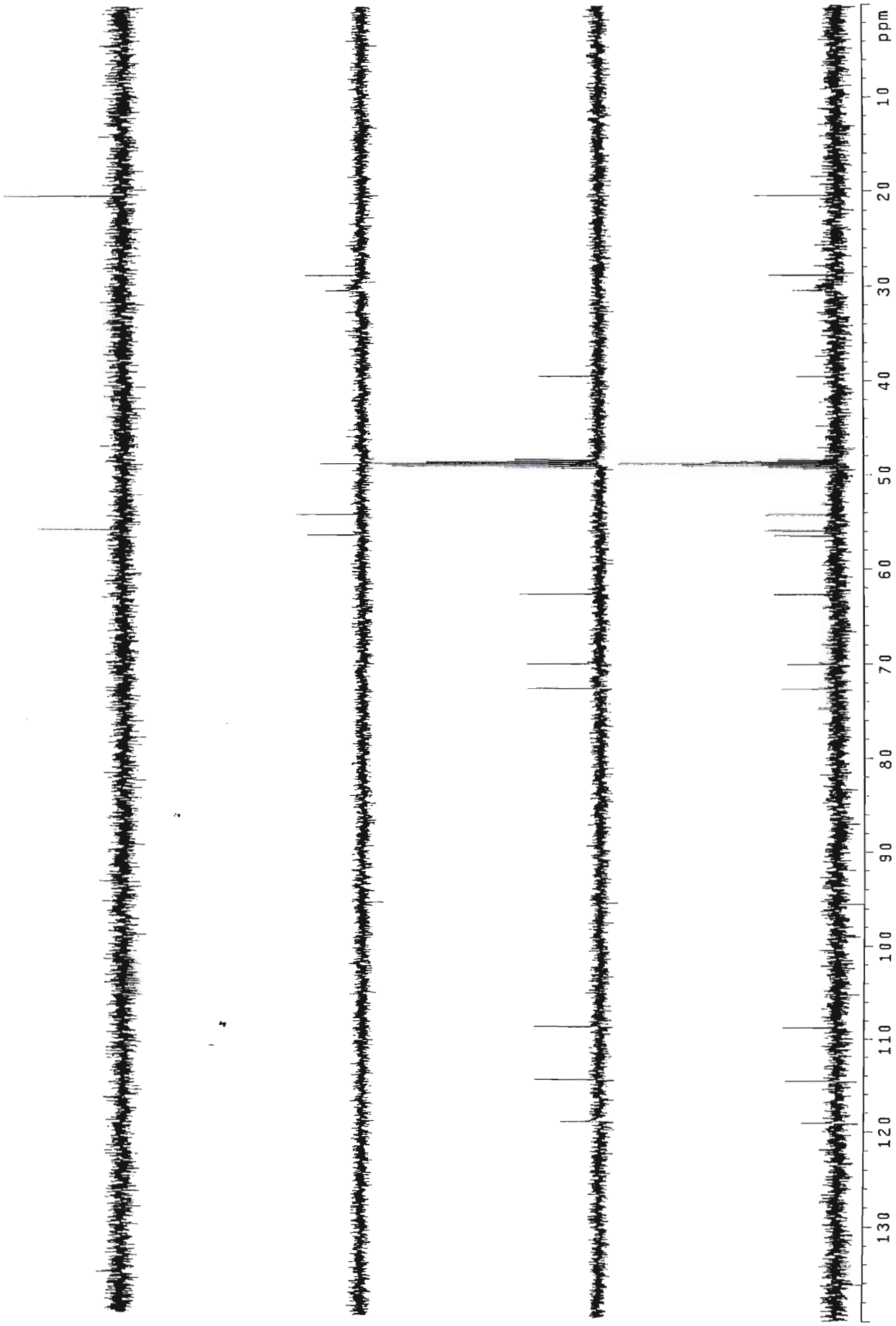
PULSE SEQUENCE: relayh
Relax. delay 1.000 sec
COSY 90-90
Acq. time 0.175 sec
Width 2930.1 Hz
2D Width 2930.1 Hz
32 repetitions
256 increments
OBSERVE H1, 399.9502544 MHz
DATA PROCESSING
Sine bell 0.087 sec
F1 DATA PROCESSING
Sine bell 0.044 sec
FT size 1024 x 1024
Total time 2.8 hours



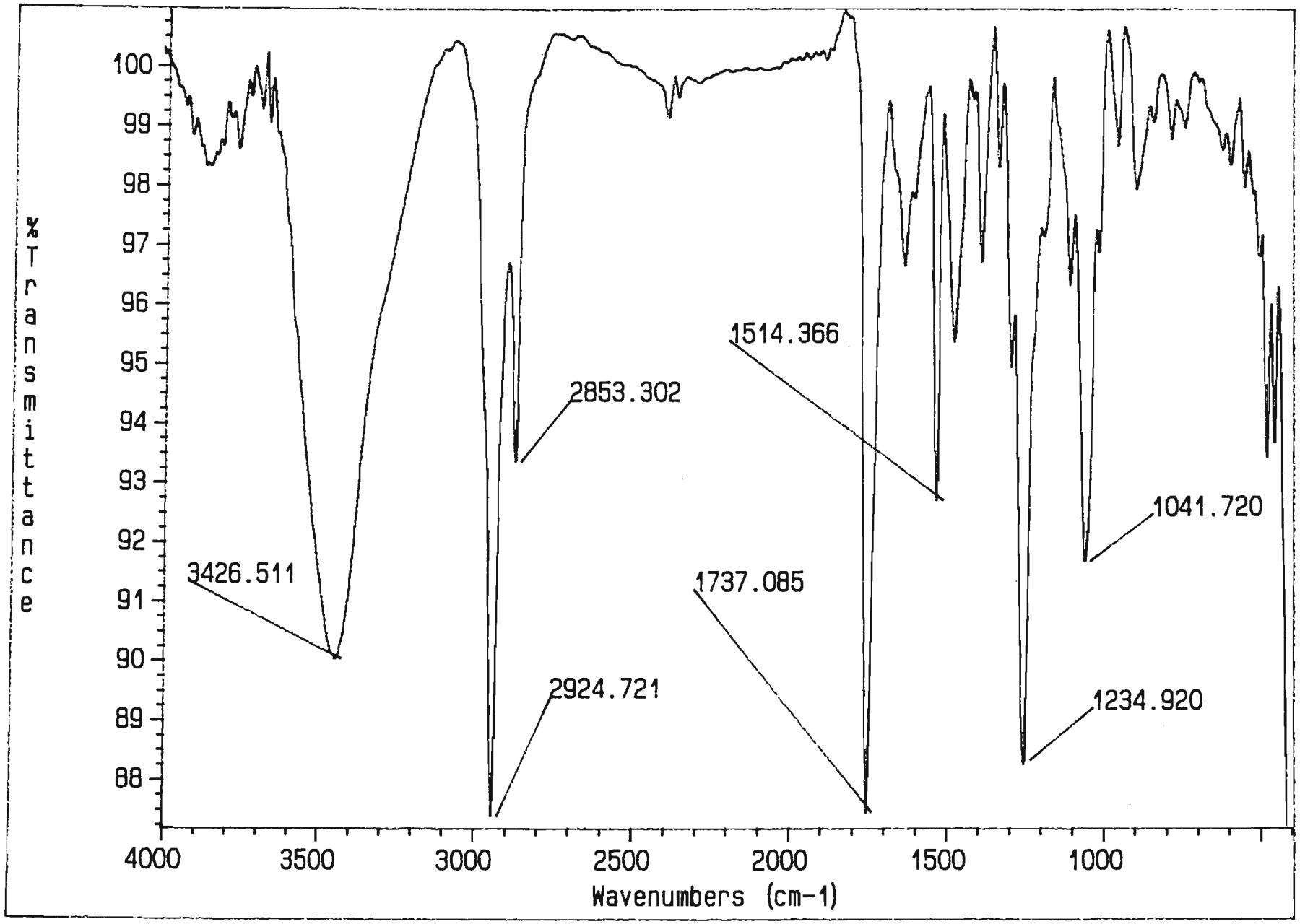
COSY NMR spectrum of Compound VII



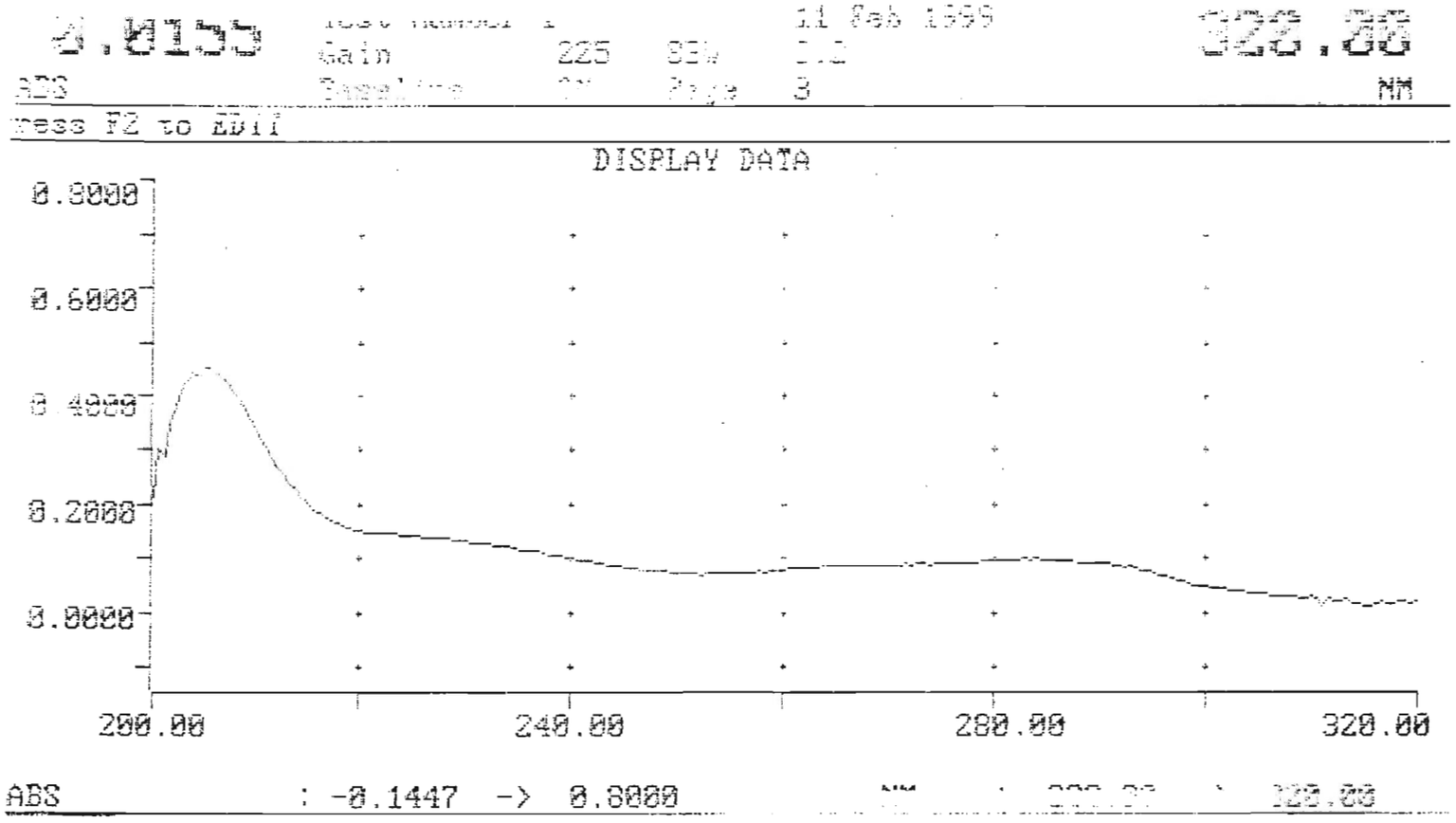
^{13}C NMR spectrum of Compound VII



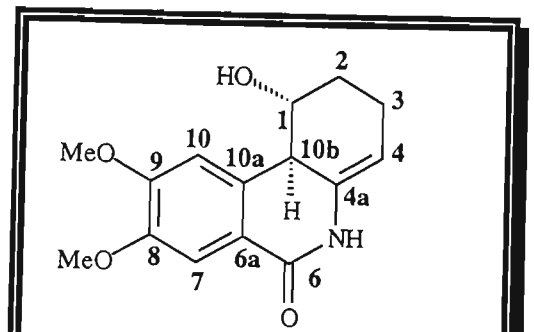
ADEPT NMR spectrum of Compound VII



Infra-red spectrum of Co...



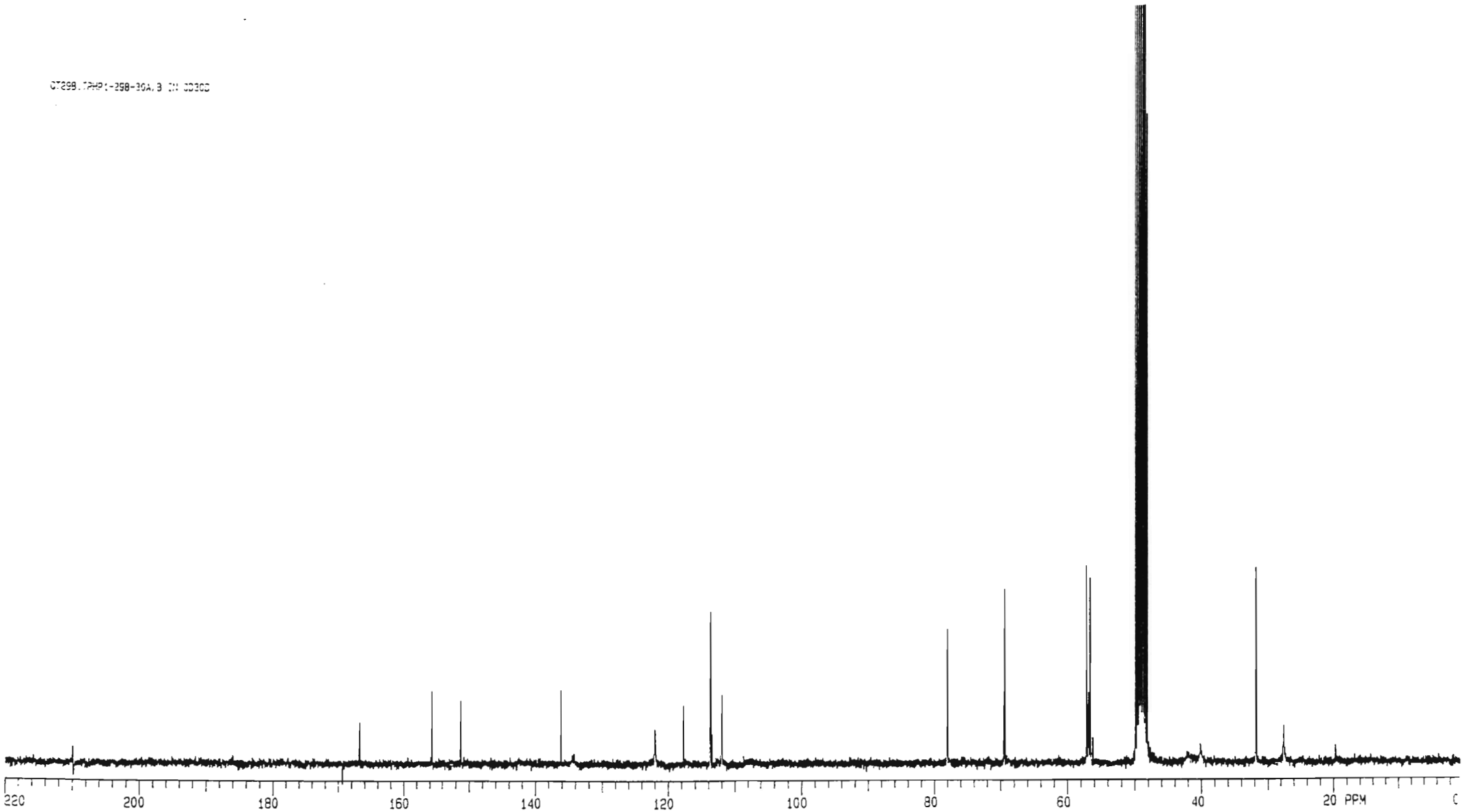
UV spectrum of Compound VII



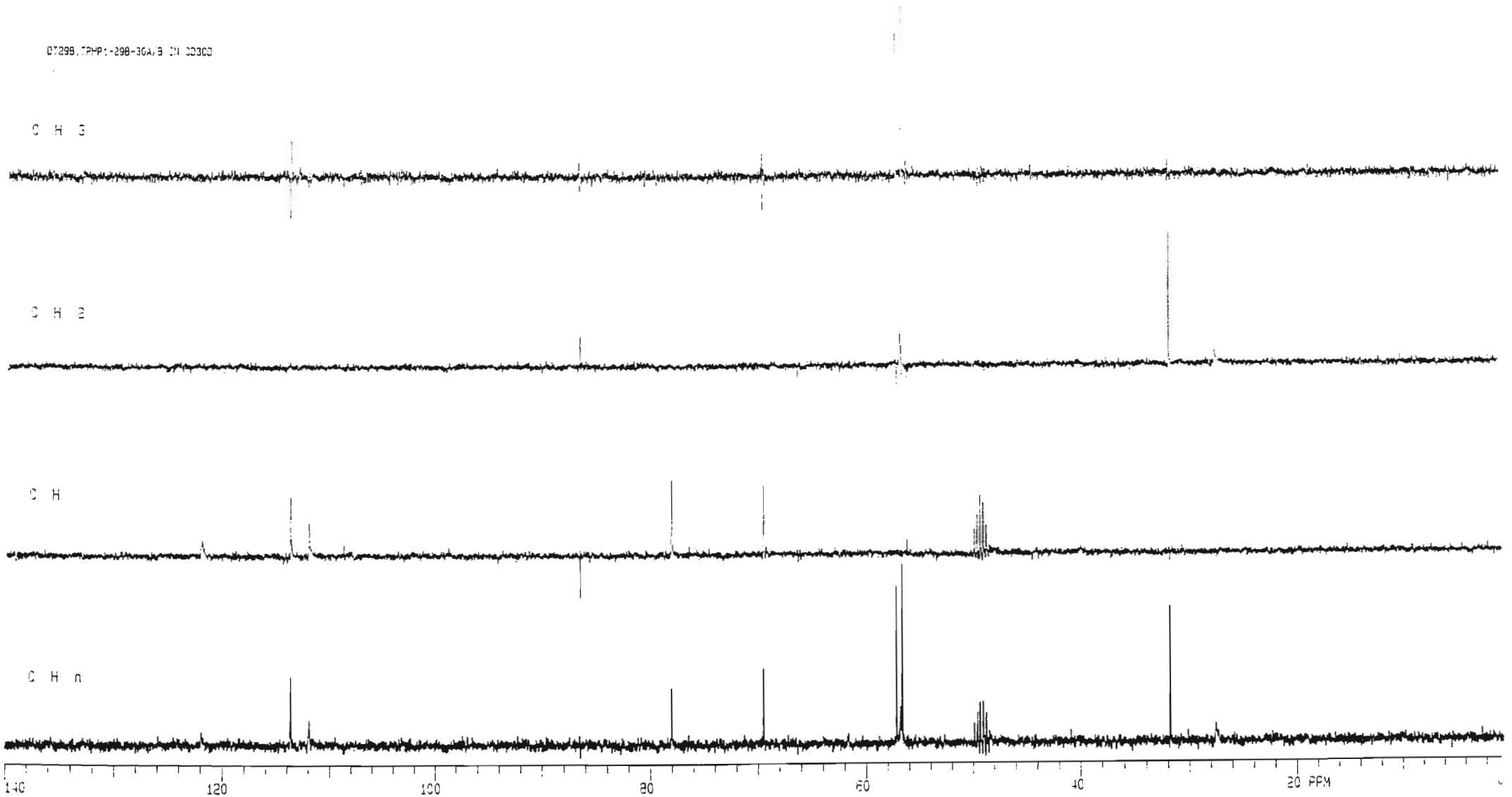
haemanthustatin

^{1}H NMR spectrum of Compound VIII

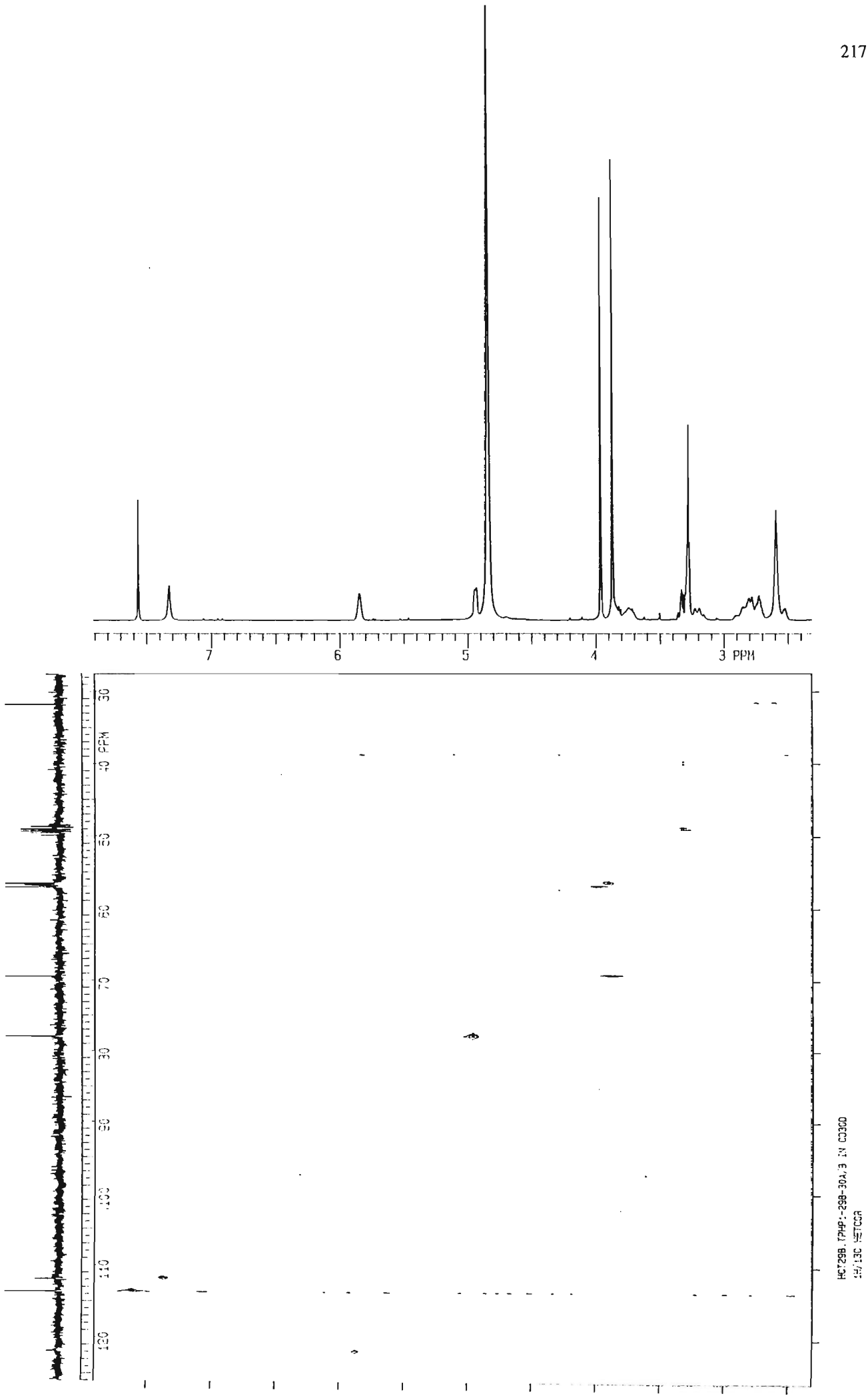
COSY NMR spectrum of Compound VIII



^{13}C NMR spectrum of Compound VIII



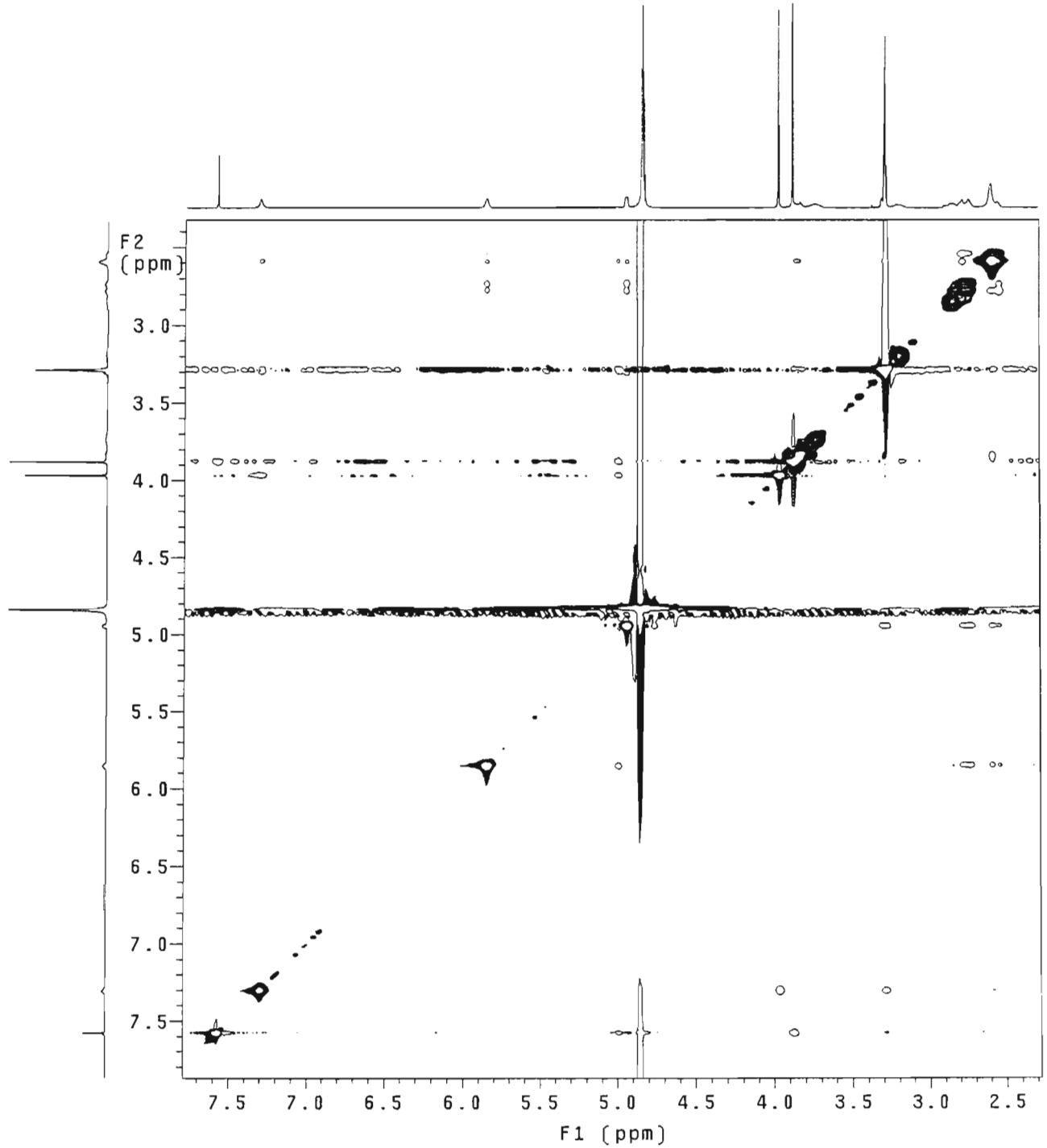
ADEPT NMR spectrum of Compound VIII



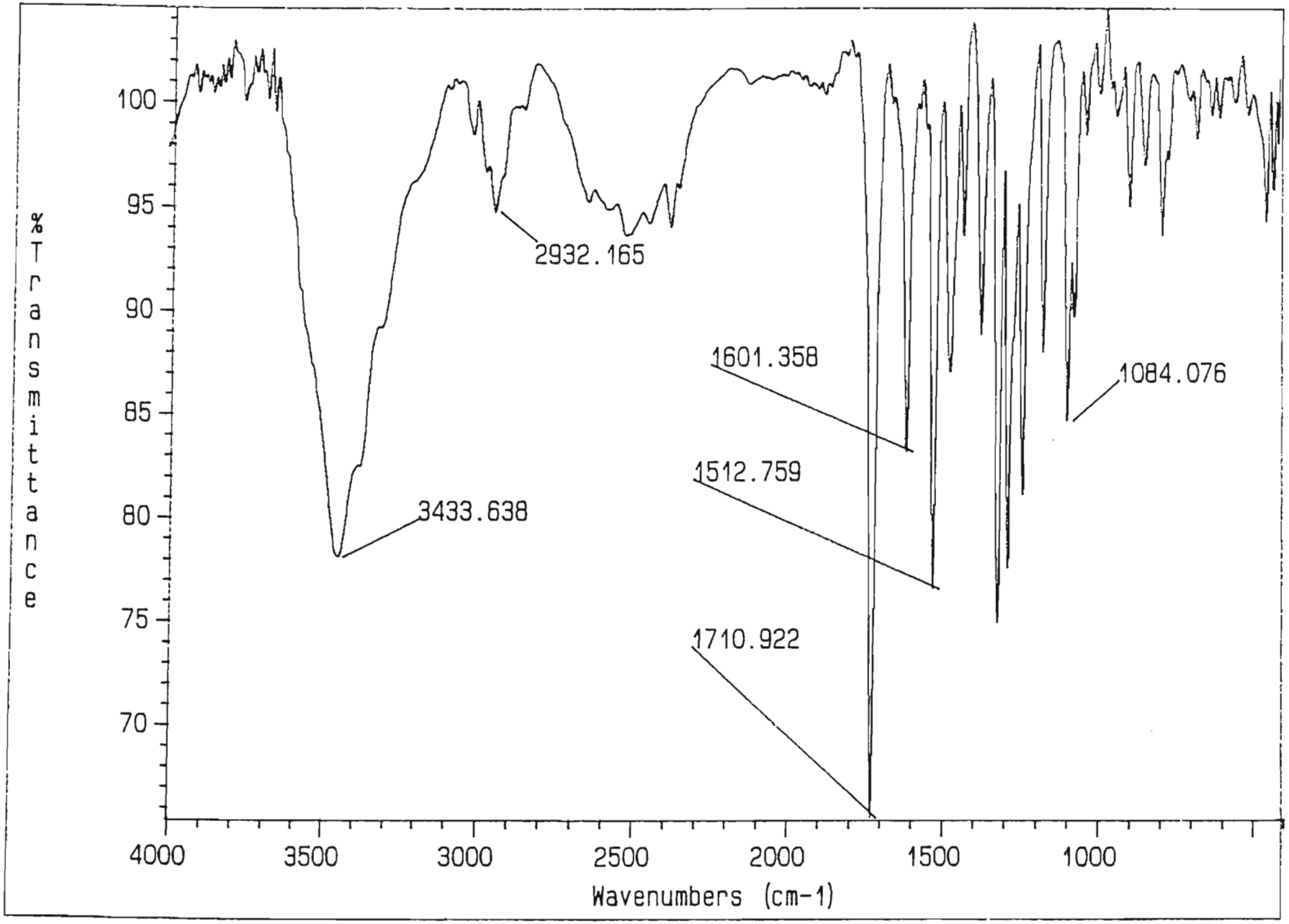
HETCOR NMR spectrum of Compound VIII

Solvent: cd3od
Ambient temperature
INOVA-400 "undnmr400"

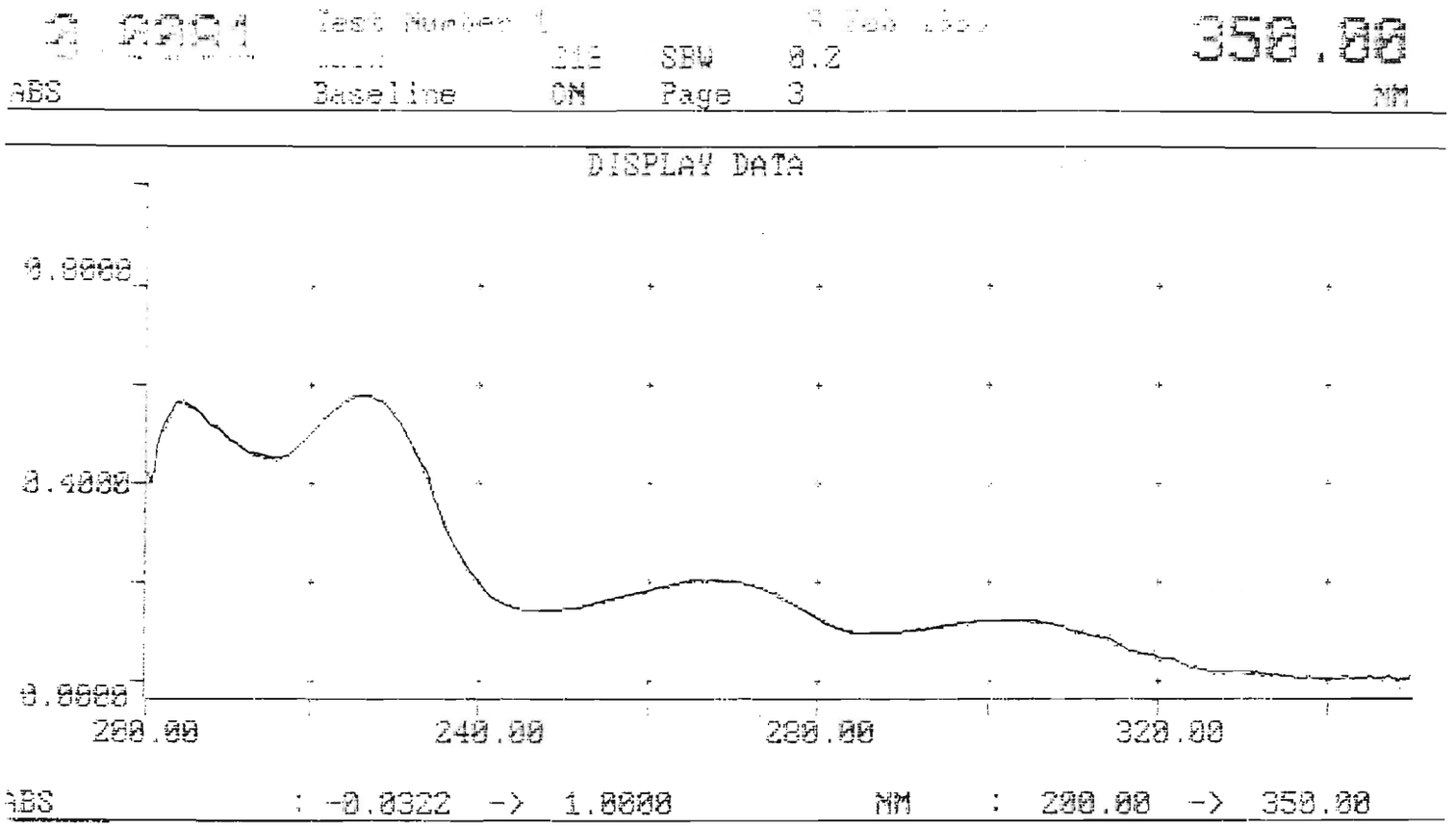
PULSE SEQUENCE: noesy
Relax. delay 4.854 sec
Mixing 1.214 sec
Acq. time 0.202 sec
Width 2534.4 Hz
2D Width 2534.4 Hz
16 repetitions
2 x 211 increments
OBSERVE H1, 399.9502544 MHz
DECOUPLE H1, 399.9522542 MHz
Power 30 dB
off during acquisition
DATA PROCESSING
Gauss apodization 0.093 sec
F1 DATA PROCESSING
Gauss apodization 0.046 sec
FT size 1024 x 1024
Total time 11.9 hours



NOESY NMR spectrum of Compound VIII

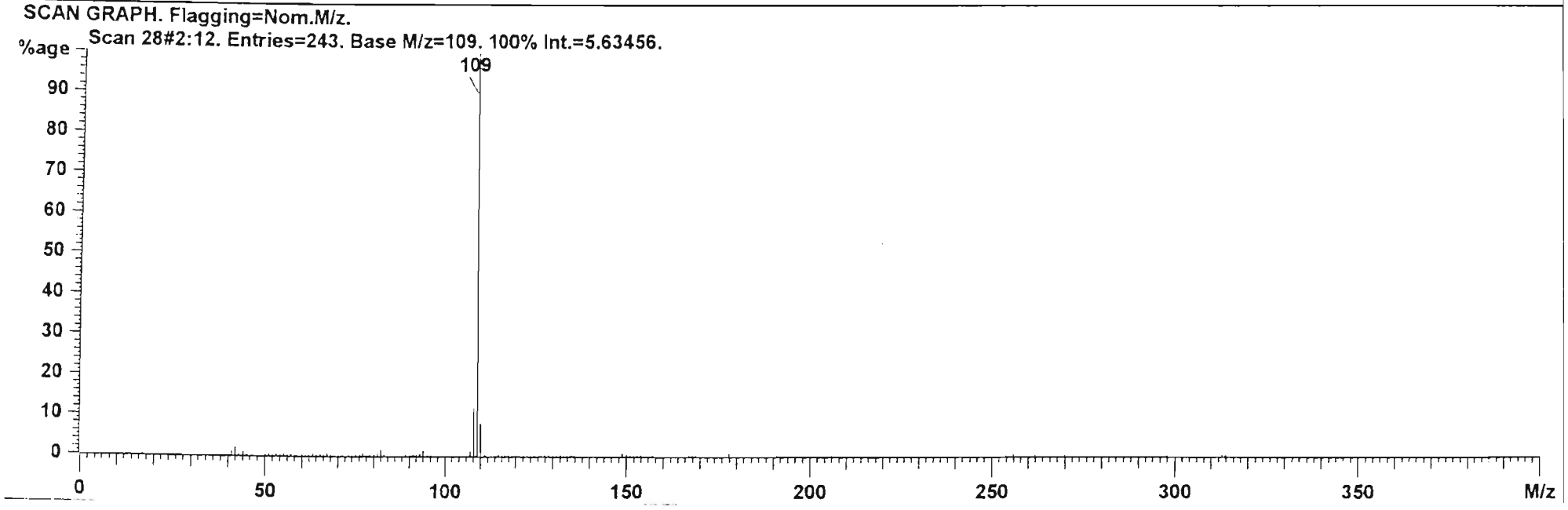


Infra-red spectrum of Compound VIII

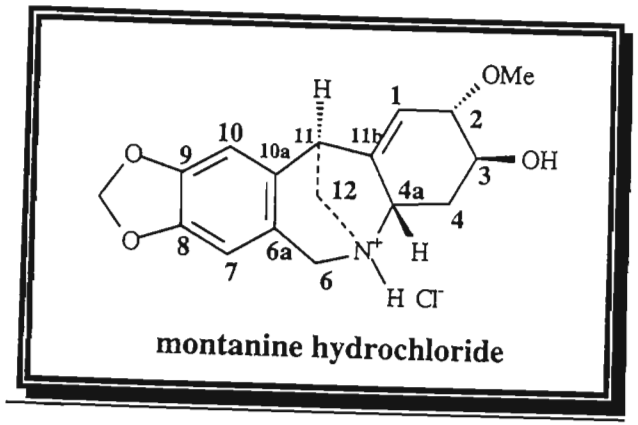


UV spectrum of Compound VIII

File Name	:	c:\maspec2\data\lc100517.ms2
File Title	:	T.POHE HP1 29A/30A/30B
Operator	:	Dr P.R.BOSHOFF NCMS
Instrument	:	VG70-SEQ/MSSMS2
Notes	:	MSS Ltd - Maspec II Data System



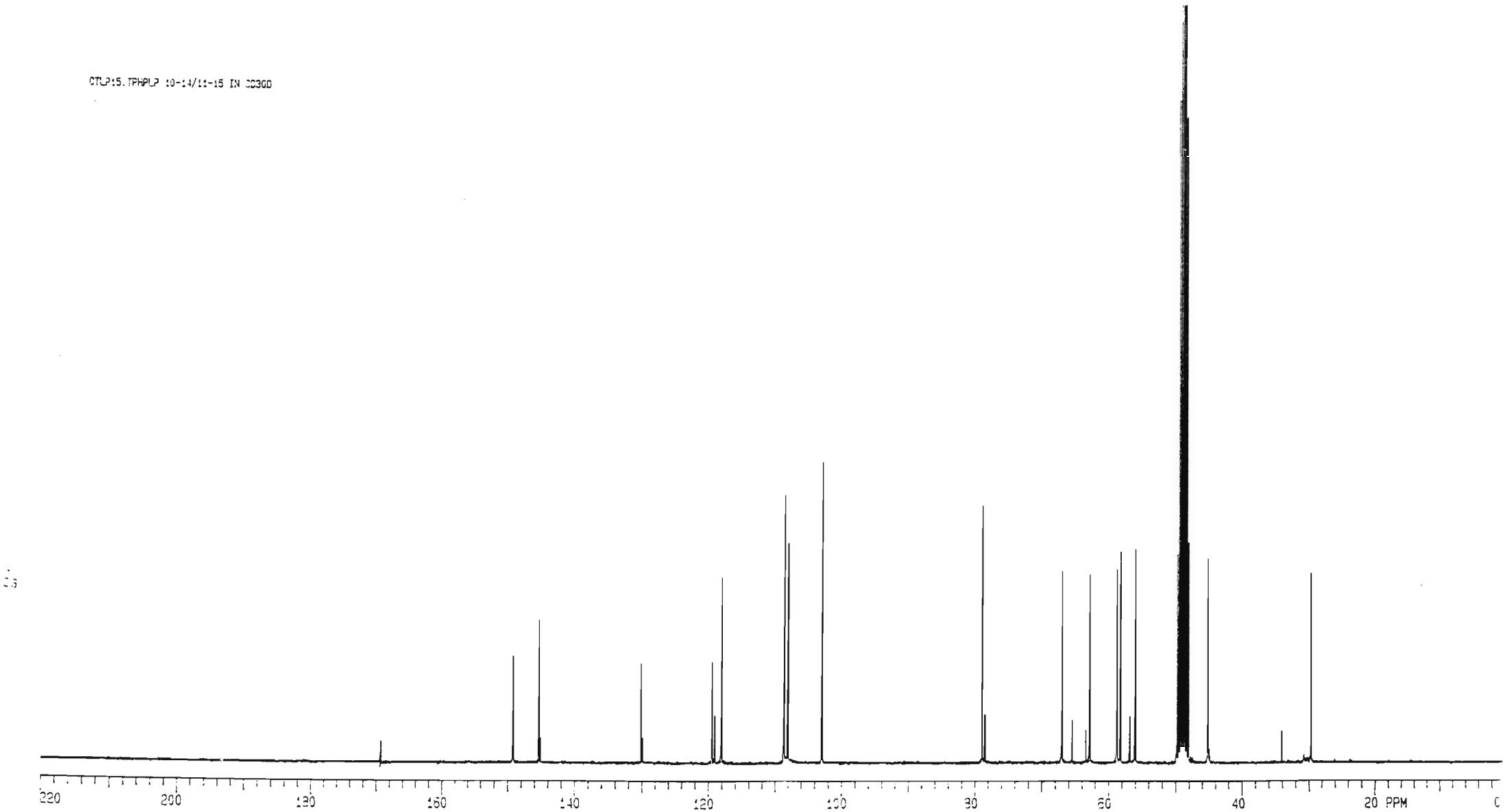
Mass spectrum of Compound VIII



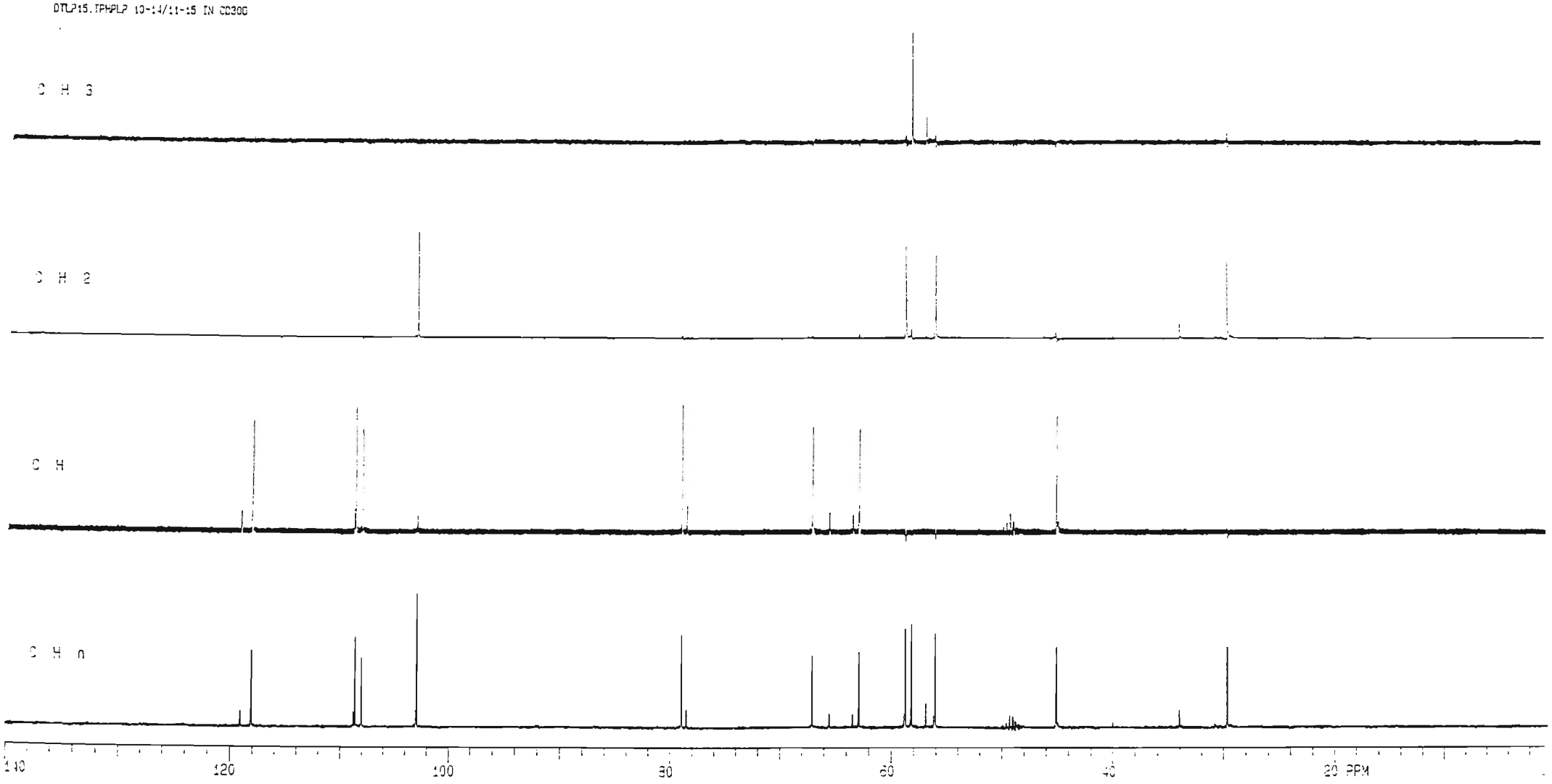
montanine hydrochloride

^{1}H NMR spectrum of Compound IX

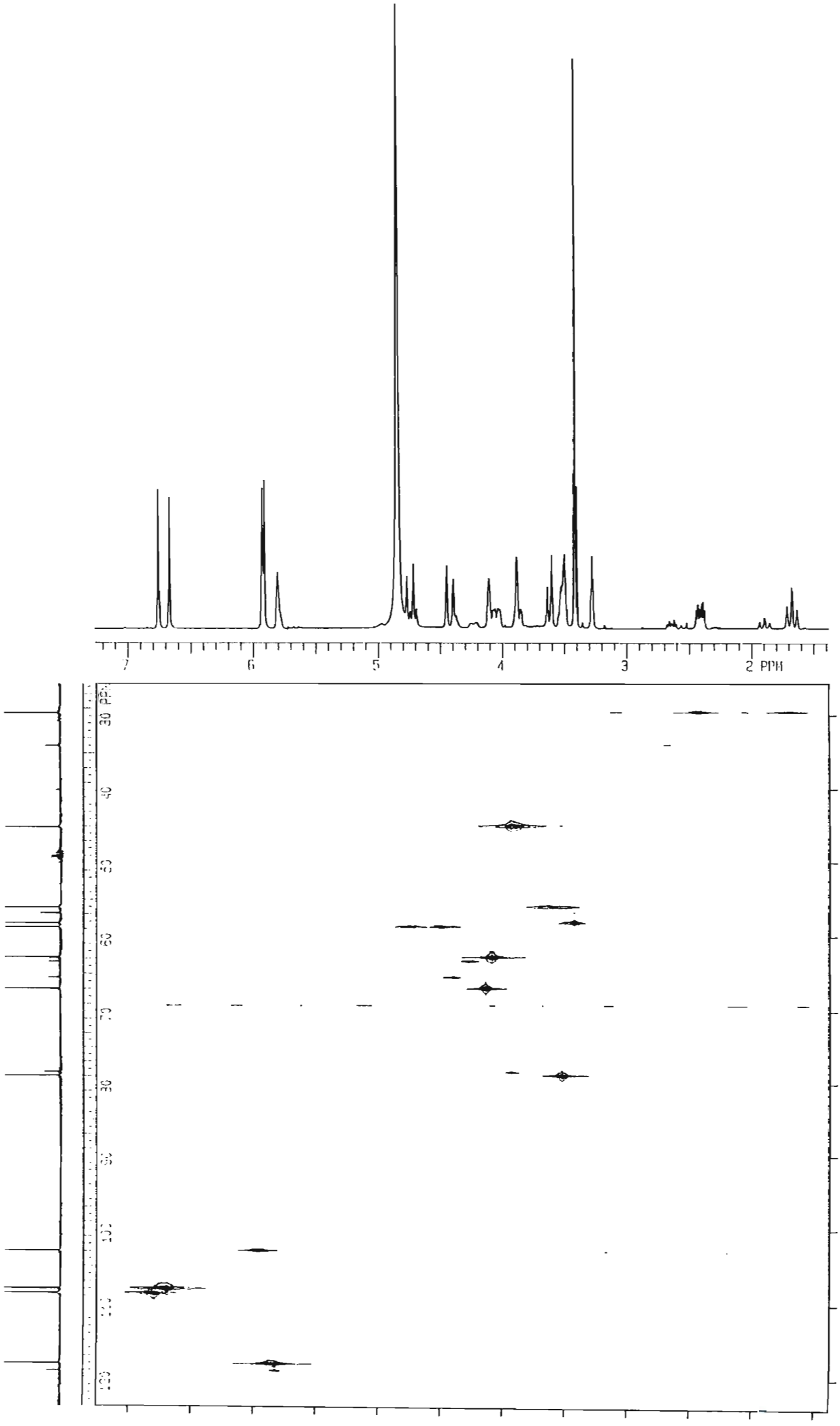
COSY NMR spectrum of Compound IX



^{13}C NMR spectrum of Compound IX



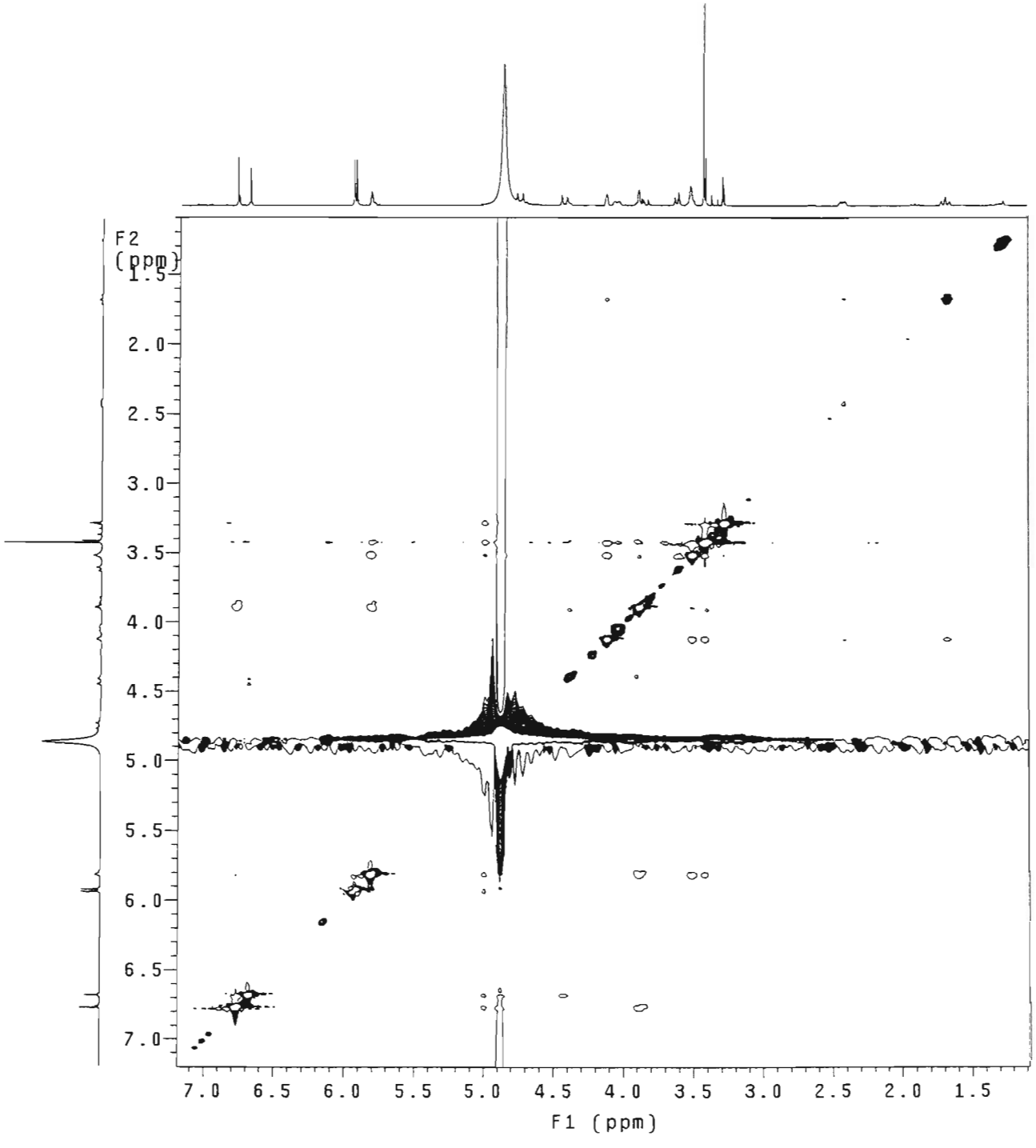
ADEPT NMR spectrum of Compound IX



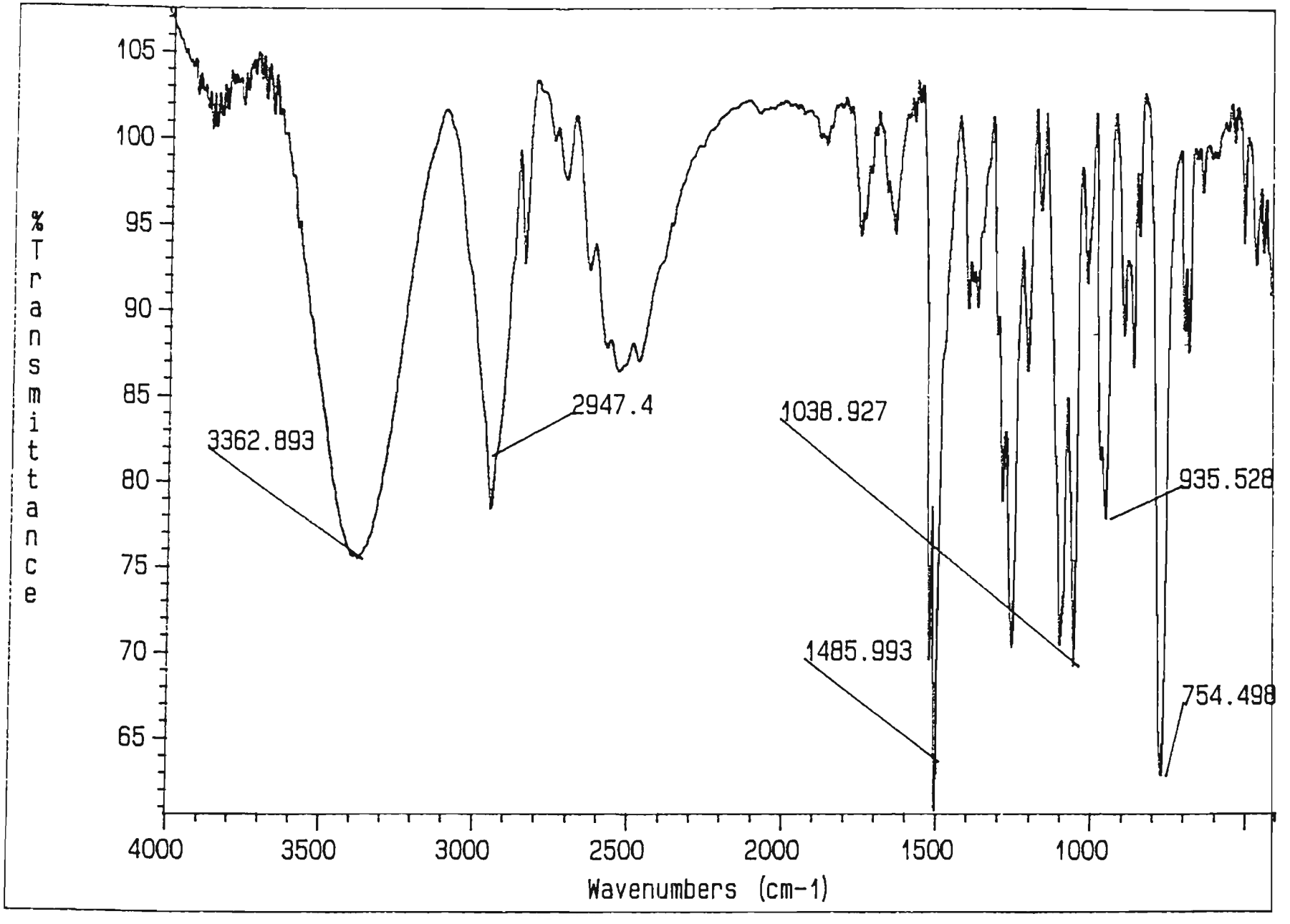
HETCOR NMR spectrum of Compound IX

Solvent: cd3od
Ambient temperature
File: notp15
INOVA-400 "undnmr400"

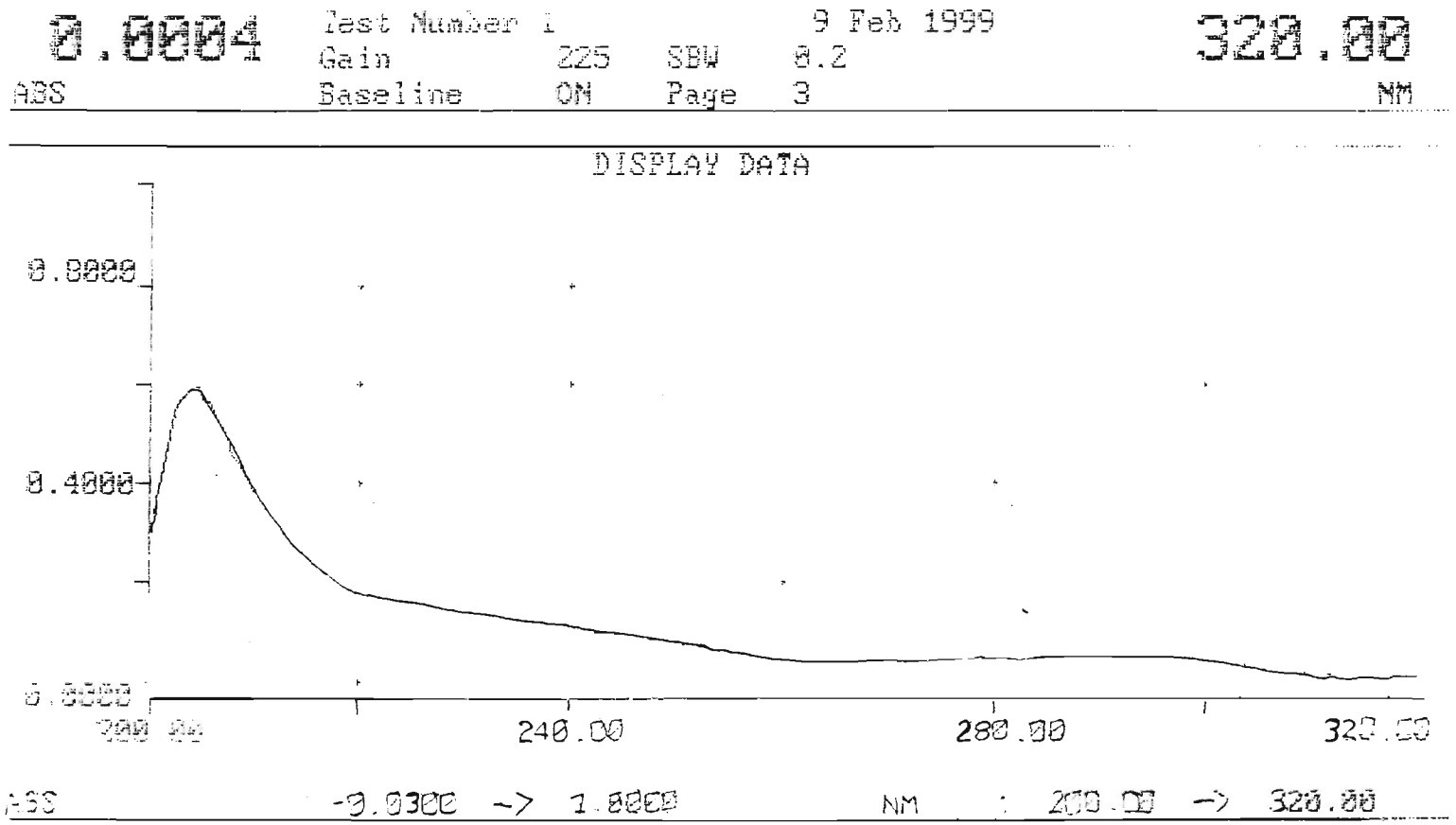
PULSE SEQUENCE: noesy
Relax. delay 7.756 sec
Mixing 1.939 sec
Acq. time 0.185 sec
Width 2770.1 Hz
2D Width 2770.1 Hz
32 repetitions
2 x 230 increments
OBSERVE H1, 399.9502544 MHz
DECOUPLE H1, 399.9522542 MHz
Power 30 dB
off during acquisition
DATA PROCESSING
Gauss apodization 0.085 sec
F1 DATA PROCESSING
Gauss apodization 0.043 sec
FT size 1024 x 1024
Total time 40.6 hours



NOESY NMR spectrum of Compound IX

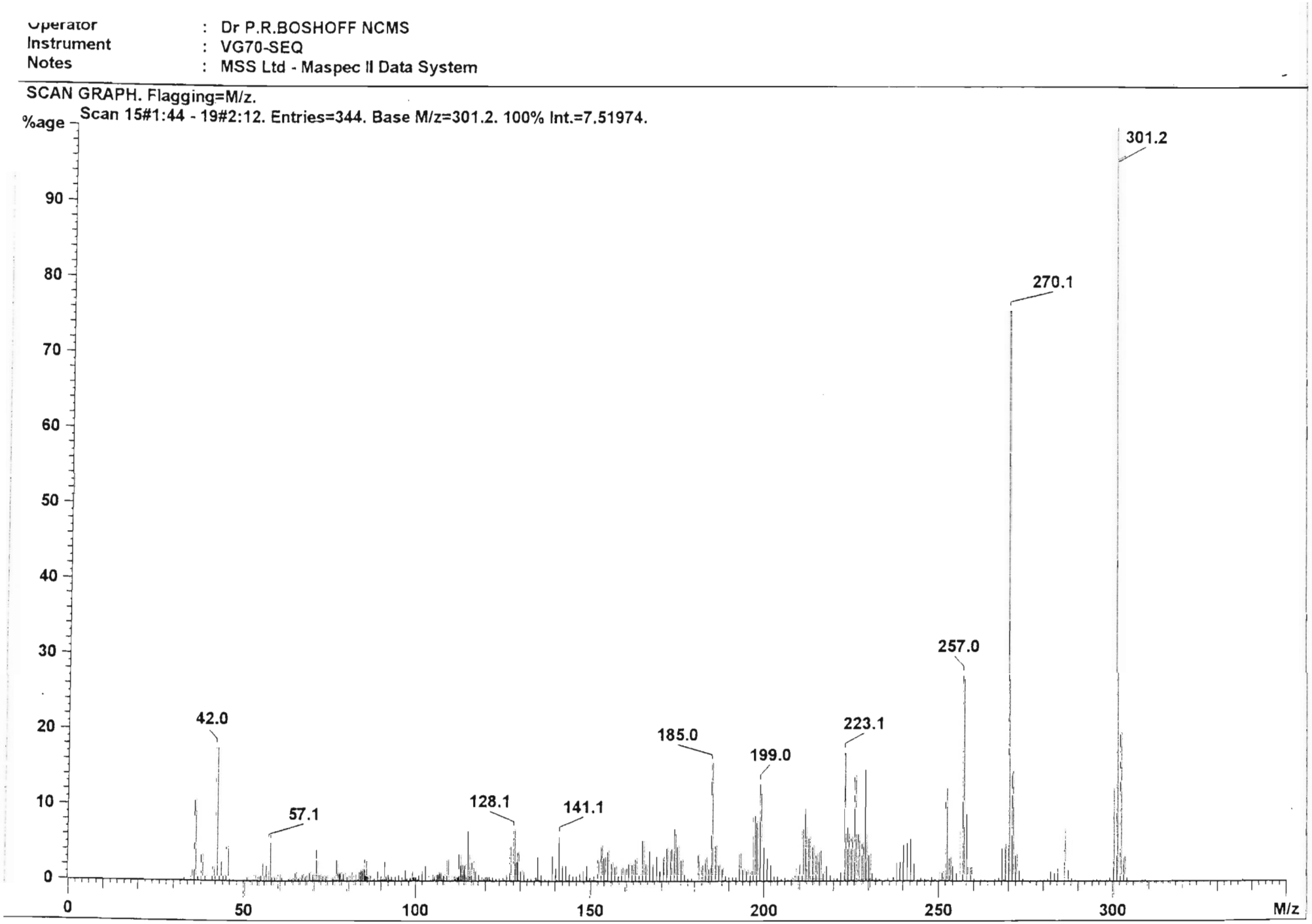


Infra-red spectrum of Compound IX

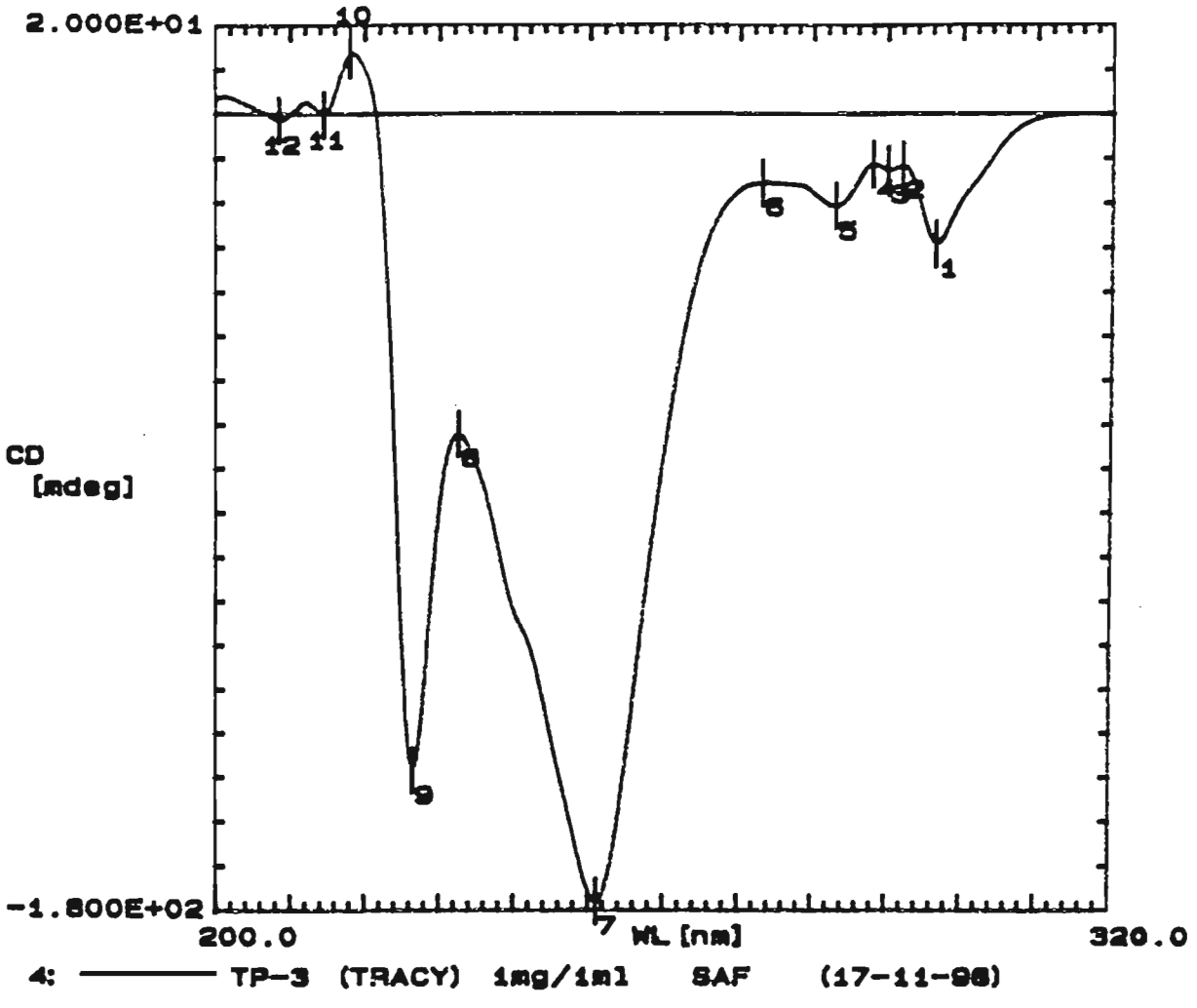


UV spectrum of Compound IX

Operator : Dr P.R.BOSHOFF NCMS
Instrument : VG70-SEQ
Notes : MSS Ltd - Maspec II Data System

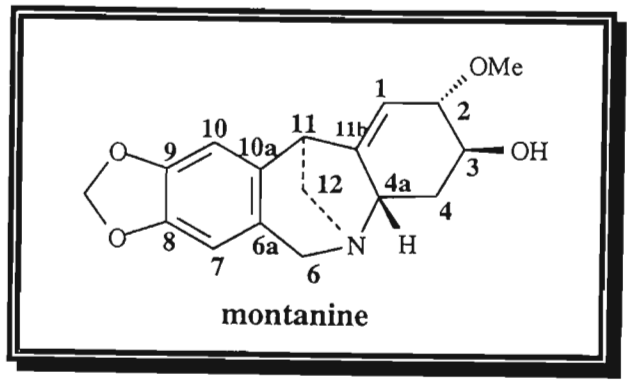


Mass spectrum of Compound IX



No.	Wavelength	Value
1	296.50 nm	-2.911E+01
2	292.00 nm	-1.147E+01
3	290.00 nm	-1.253E+01
4	288.00 nm	-1.124E+01
5	283.00 nm	-2.080E+01
6	273.00 nm	-1.554E+01
7	251.00 nm	-1.781E+02
8	232.50 nm	-7.204E+01
9	226.50 nm	-1.485E+02
10	218.00 nm	1.349E+01
11	214.50 nm	-2.502E-01
12	208.50 nm	-1.396E+00

CD spectrum of Compound IX



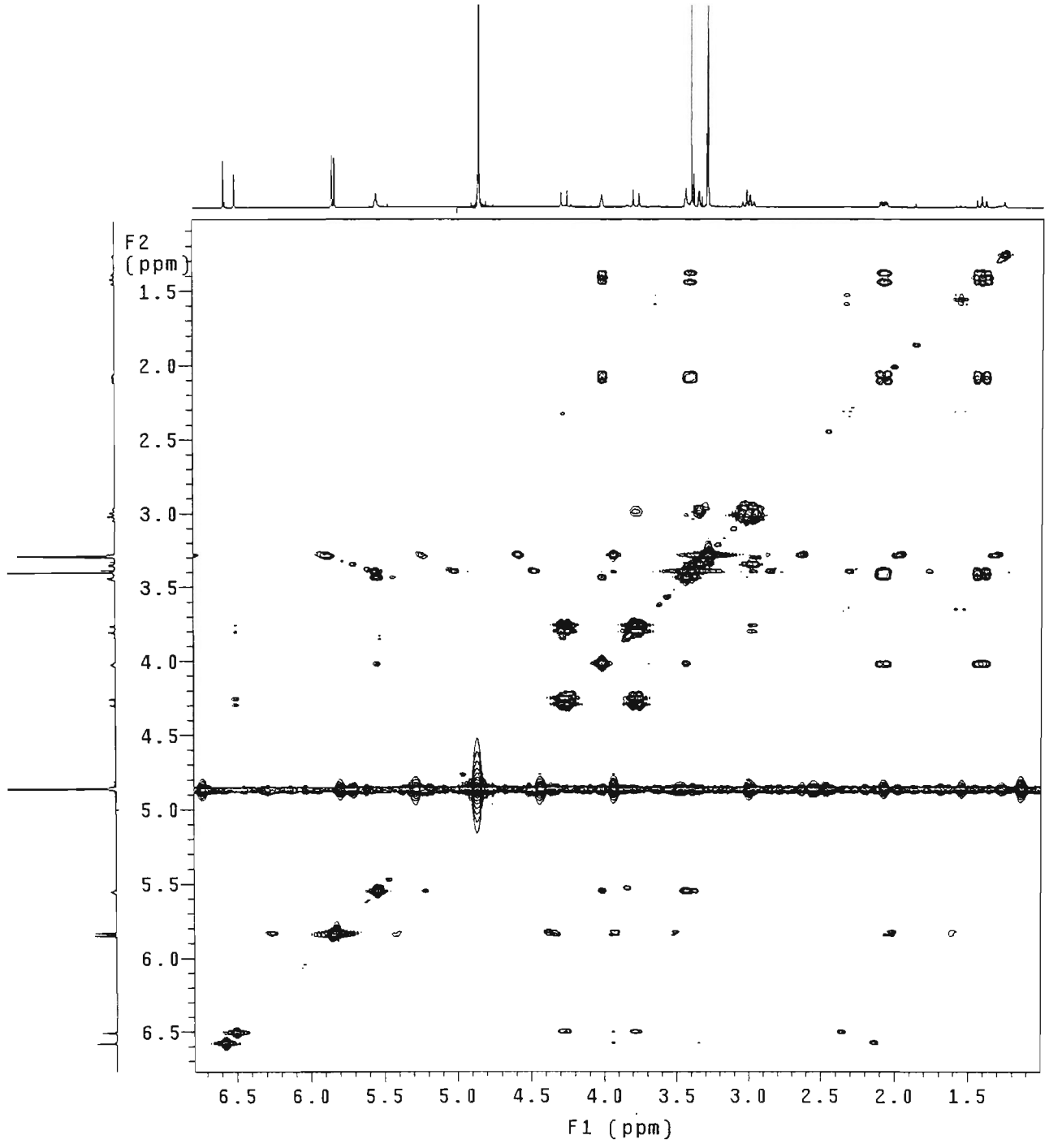
montanine

^{1}H NMR spectrum of base treated Compound IX

hcosy-90

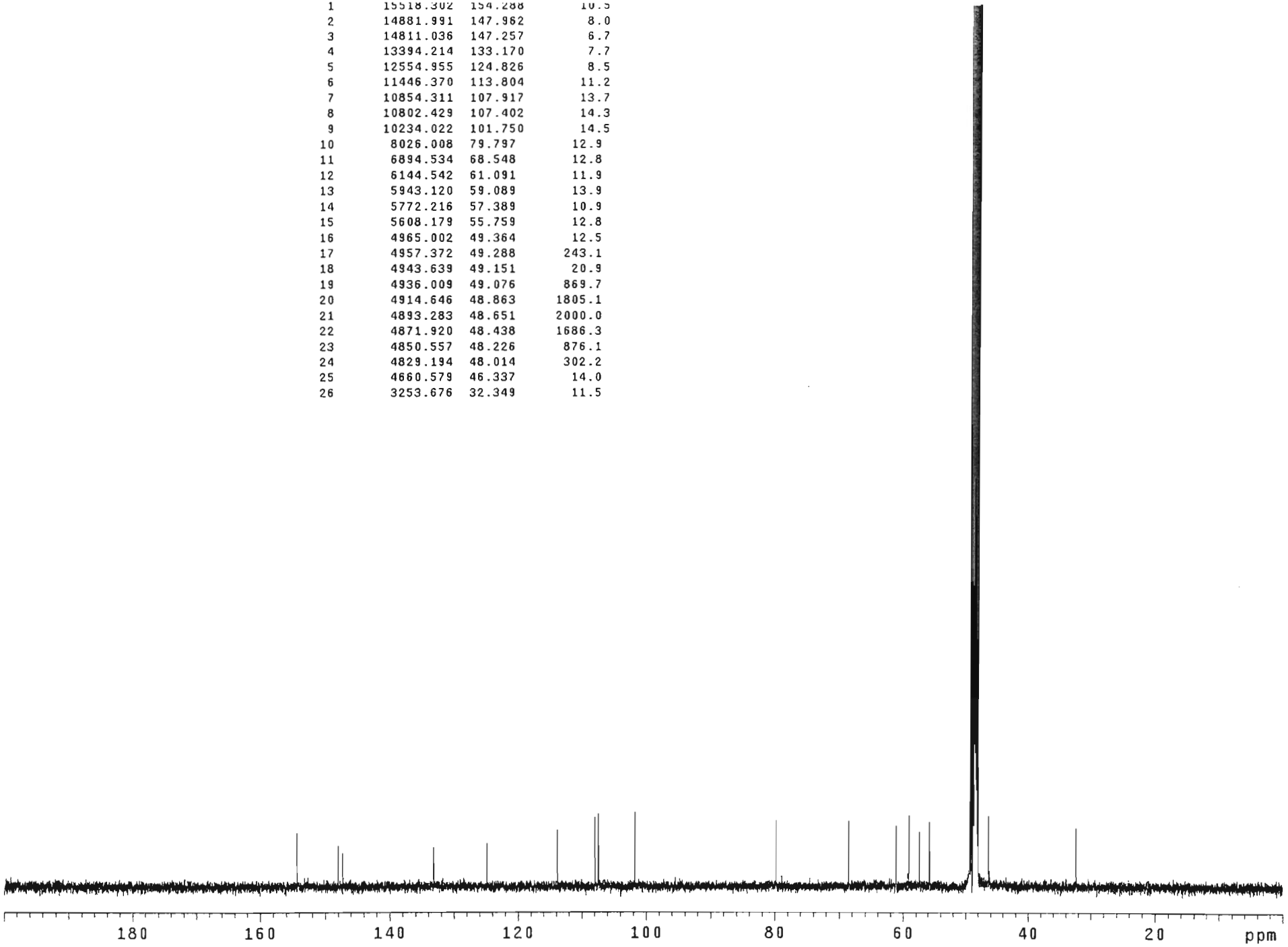
Solvent: cd3od
Ambient temperature
INOVA-400 "undnmr400"

PULSE SEQUENCE: relayh
Relax. delay 1.000 sec
COSY 90-90
Acq. time 0.209 sec
Width 2446.0 Hz
2D Width 2446.0 Hz
64 repetitions
256 increments
OBSERVE H1, 399.9502544 MHz
DATA PROCESSING
Sine bell 0.105 sec
F1 DATA PROCESSING
Sine bell 0.052 sec
FT size 1024 x 1024
Total time 5.7 hours

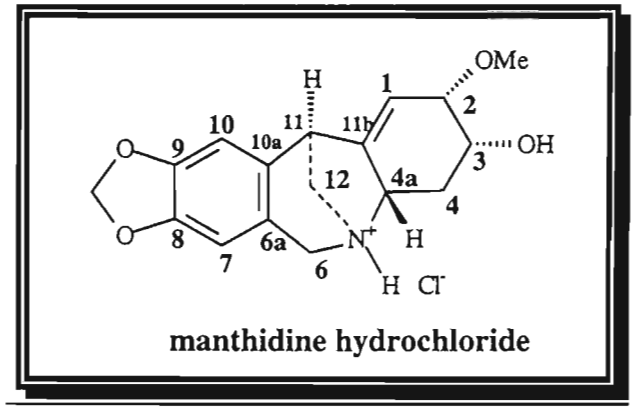


COSY NMR spectrum of base treated Compound IX

1	15518.302	154.288	10.5
2	14881.991	147.962	8.0
3	14811.036	147.257	6.7
4	13394.214	133.170	7.7
5	12554.955	124.826	8.5
6	11446.370	113.804	11.2
7	10854.311	107.917	13.7
8	10802.429	107.402	14.3
9	10234.022	101.750	14.5
10	8026.008	79.797	12.9
11	6894.534	68.548	12.8
12	6144.542	61.091	11.9
13	5943.120	59.089	13.9
14	5772.216	57.389	10.9
15	5608.179	55.759	12.8
16	4965.002	49.364	12.5
17	4957.372	49.288	243.1
18	4943.639	49.151	20.9
19	4936.009	49.076	869.7
20	4914.646	48.863	1805.1
21	4893.283	48.651	2000.0
22	4871.920	48.438	1686.3
23	4850.557	48.226	876.1
24	4829.194	48.014	302.2
25	4660.579	46.337	14.0
26	3253.676	32.349	11.5



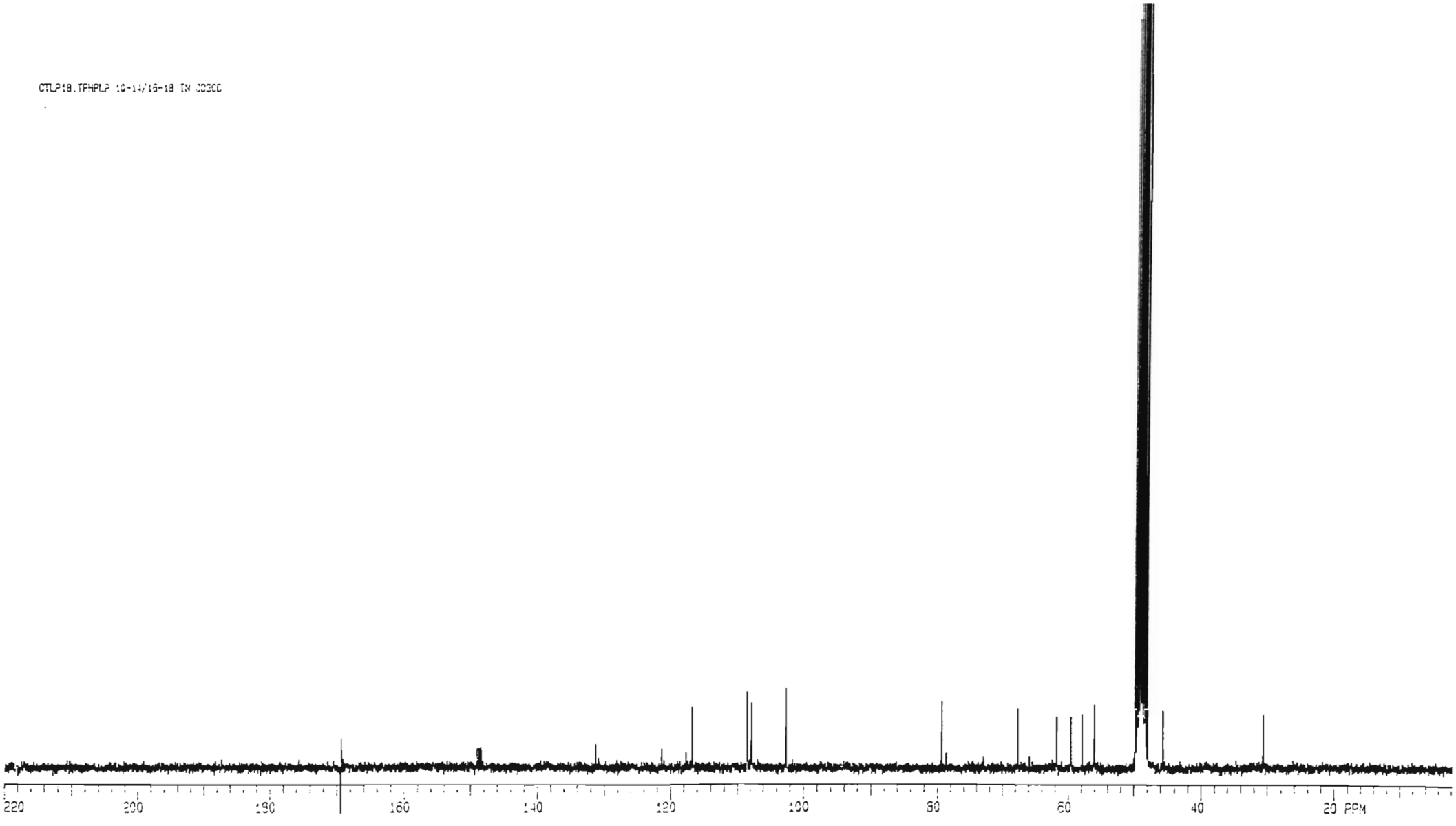
^{13}C NMR spectrumn of base treated Compound IX



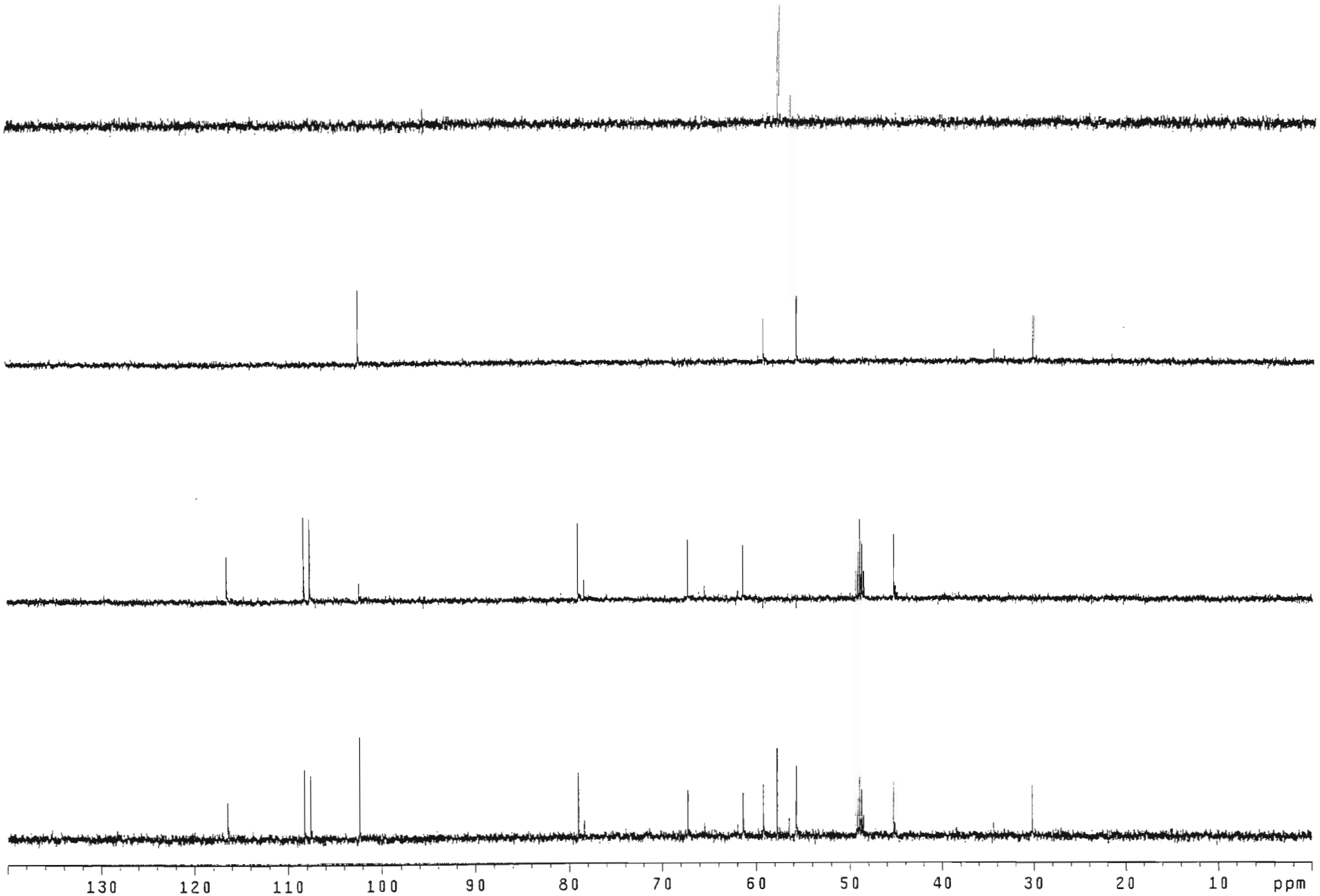
manthidine hydrochloride

^{1}H NMR spectrum of Compound X

COSY NMR spectrum of Compound X



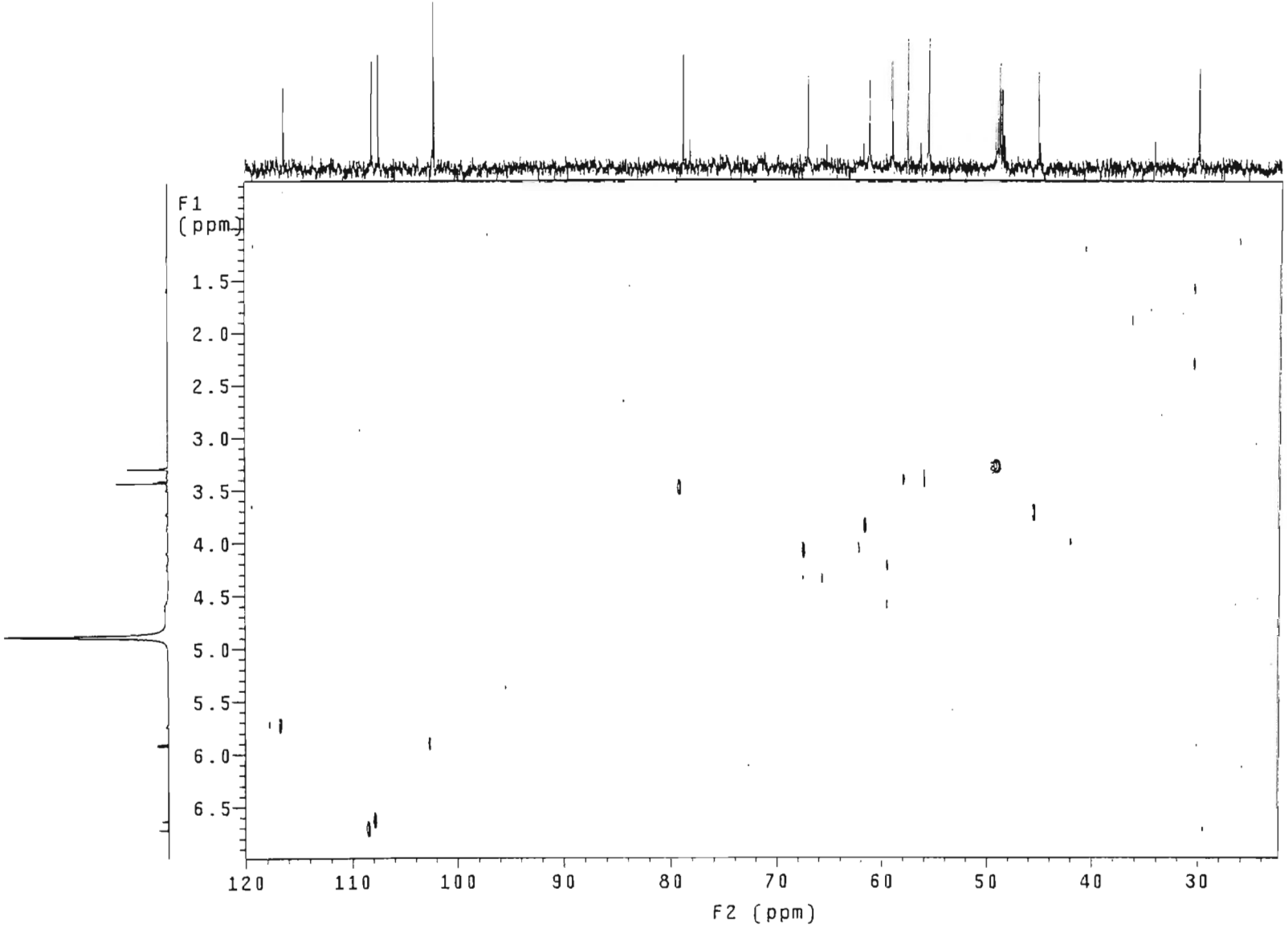
^{13}C NMR spectrum of Compound X



ADEPT NMR spectrum of Compound X

Solvent: cd3od
Ambient temperature
INOVA-400 "undnmr400"

PULSE SEQUENCE: hetcor
Relax. delay 1.000 sec
Acq. time 0.093 sec
Width 11013.2 Hz
2D Width 3183.4 Hz
512 repetitions
128 increments
OBSERVE C13, 100.5674257 MHz
DECOUPLE H1, 399.9518184 MHz
Power 38 dB
on during acquisition
off during delay
WALTZ-16 modulated
DATA PROCESSING
Sine bell 0.046 sec
F1 DATA PROCESSING
Sine bell 0.020 sec
FT size 2048 x 512
Total time 20.3 hours

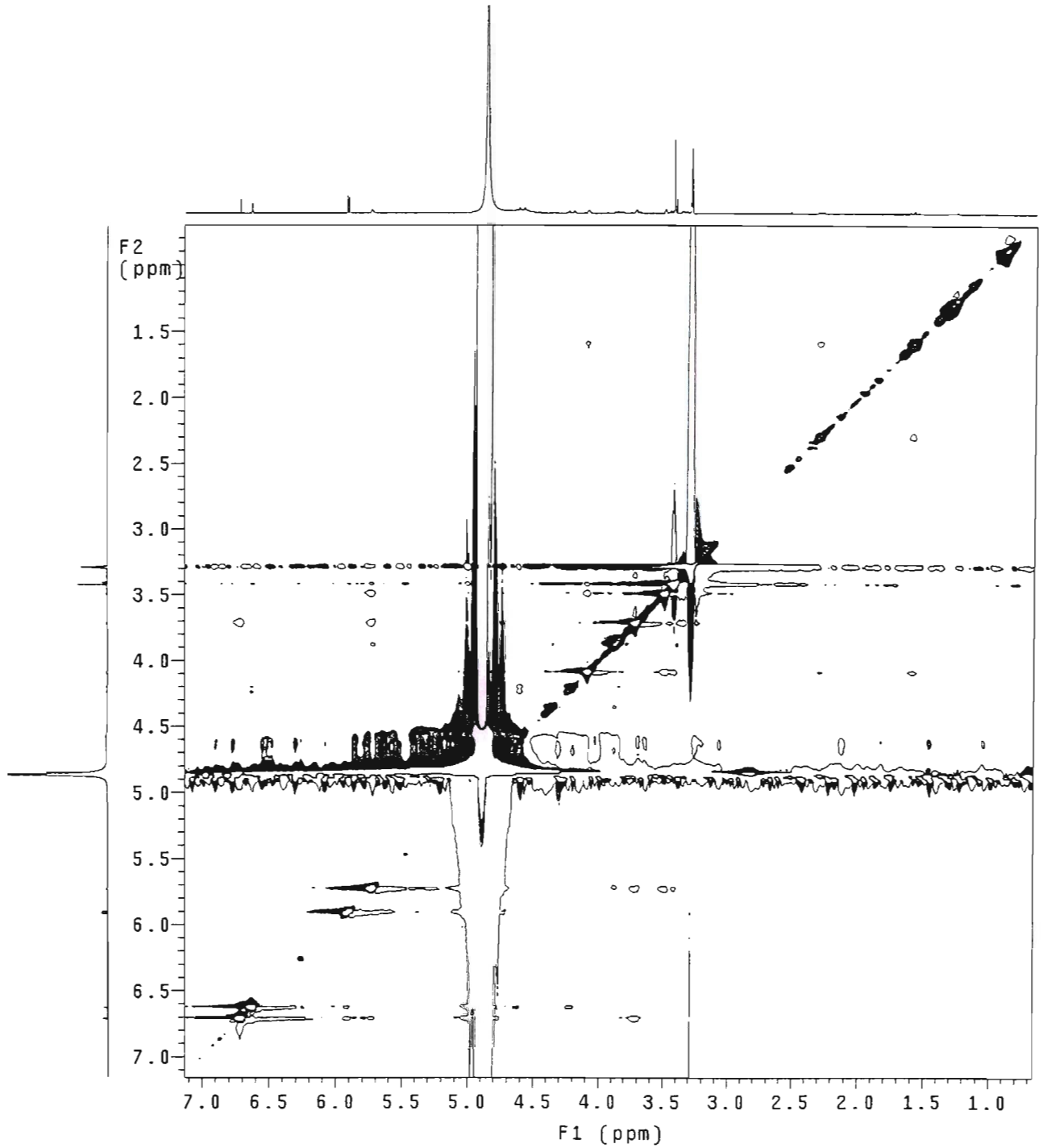


HETCOR NMR spectrum of Compound X

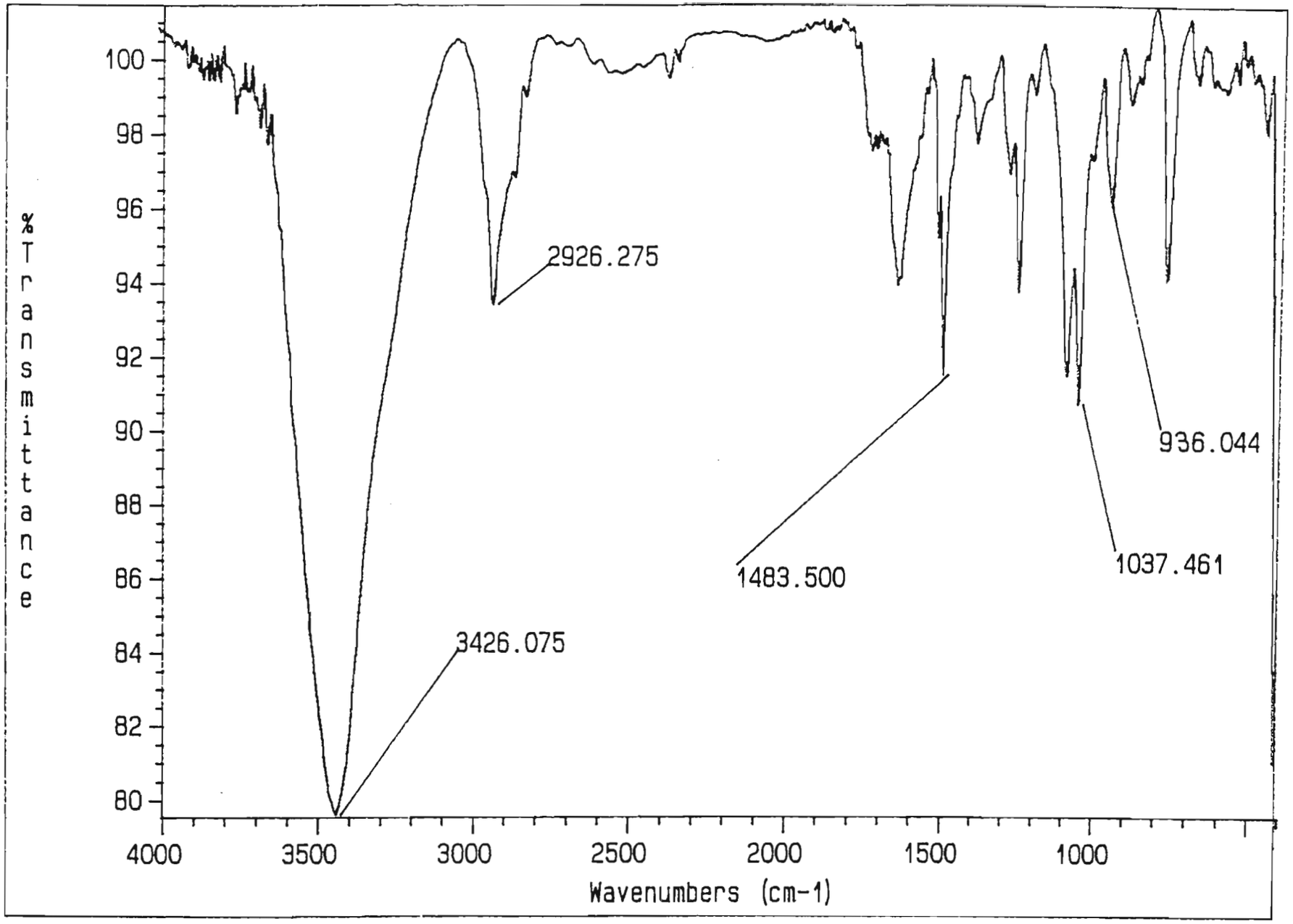
no1618.tphplp 10-14/16-18 in cd3od
ps-noesy

Solvent: cd3od
Ambient temperature
INOVA-400 "undnmr400"

PULSE SEQUENCE: noesy
Relax. delay 6.956 sec
Mixing 1.739 sec
Acq. time 0.176 sec
Width 2909.0 Hz
2D Width 2909.0 Hz
32 repetitions
2 x 240 increments
OBSERVE H1, 399.9502544 MHz
DECOUPLE H1, 399.9522542 MHz
Power 30 dB
off during acquisition
DATA PROCESSING
Gauss apodization 0.081 sec
F1 DATA PROCESSING
Gauss apodization 0.040 sec
FT size 1024 x 1024
Total time 38.0 hours



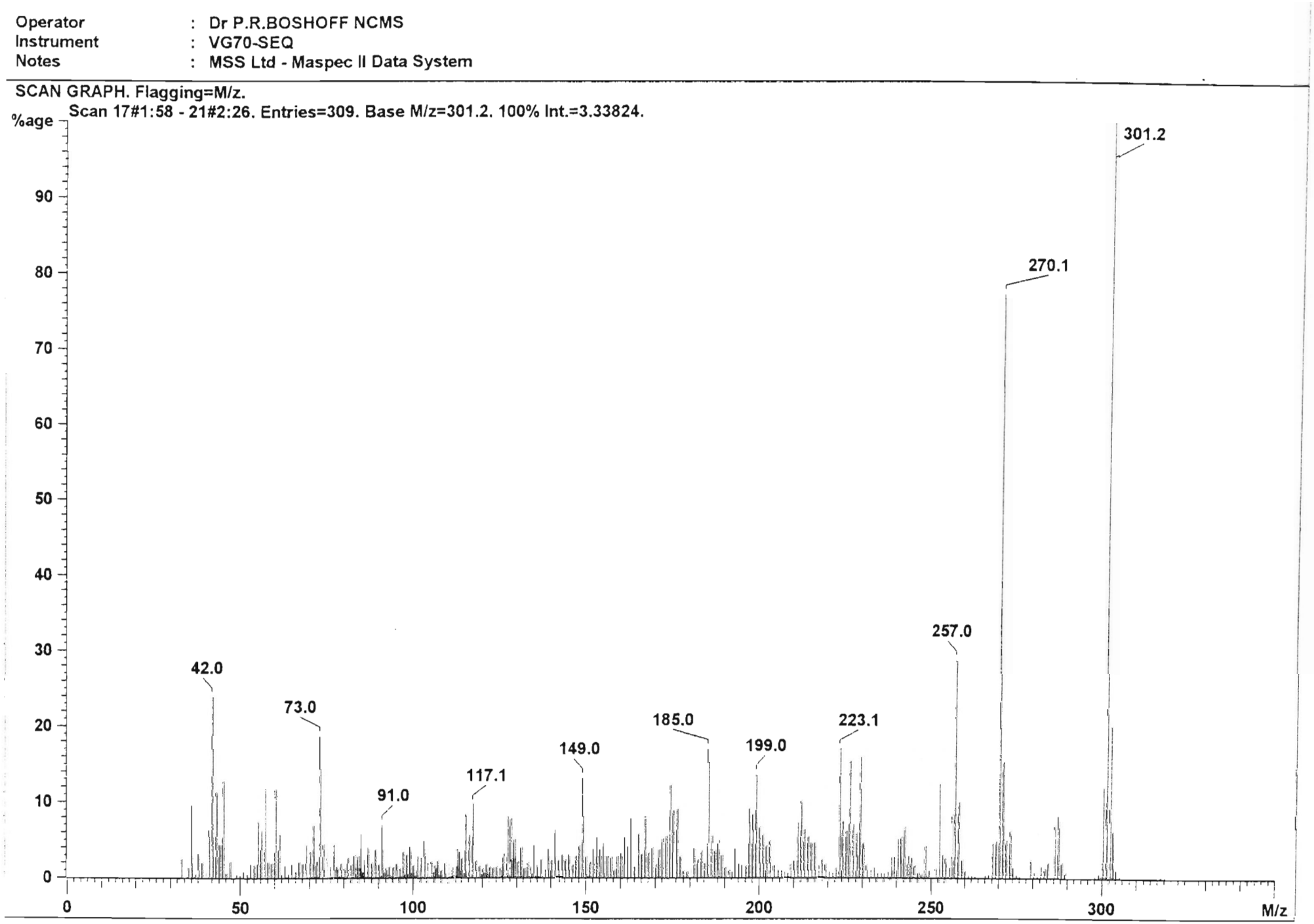
NOESY NMR spectrum of Compound X



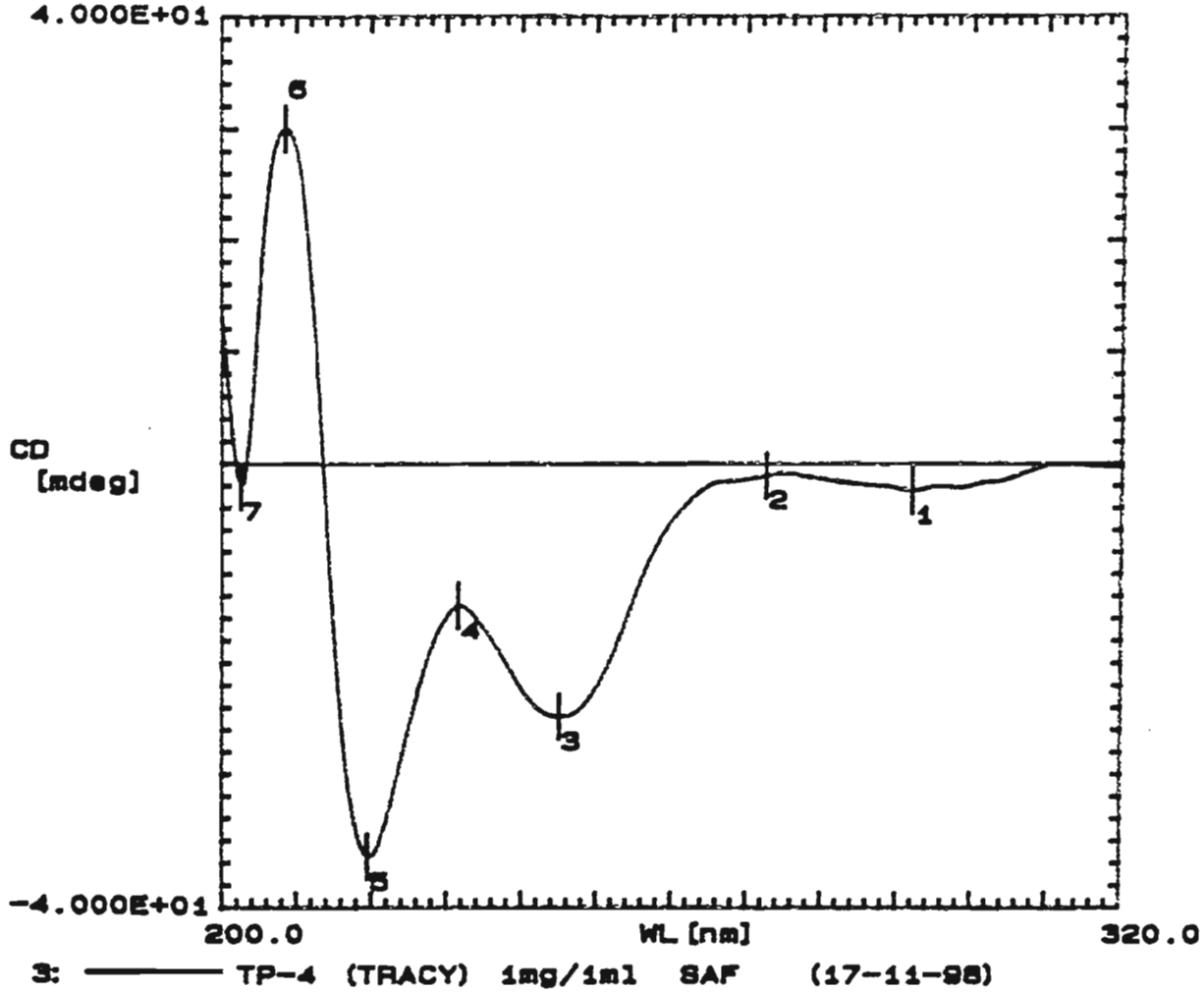
Infra-red spectrum of Compound X

UV spectrum of Compound X

Operator : Dr P.R.BOSHOFF NCMS
Instrument : VG70-SEQ
Notes : MSS Ltd - Maspec II Data System

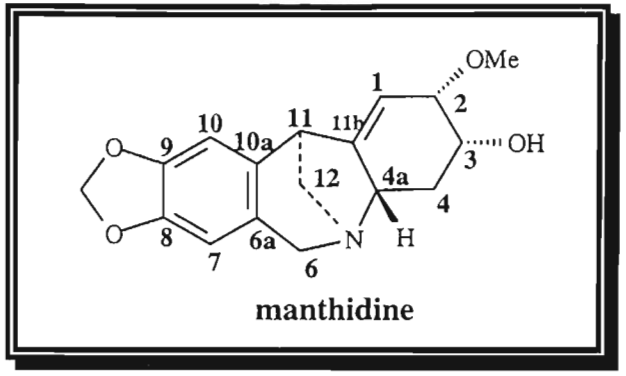


Mass spectrum of Compound X



No.	Wavelength	Value
1	292.00 nm	-2.440E+00
2	272.50 nm	-1.068E+00
3	245.00 nm	-2.284E+01
4	231.50 nm	-1.287E+01
5	219.50 nm	-3.544E+01
6	208.50 nm	3.006E+01
7	202.50 nm	-2.078E+00

CD spectrum of Compound X



manthidine

^{1}H NMR spectrum of base treated Compound X

^{1}H NMR spectrum of Compound XI