

# Homoisoflavonoids and stilbenoids from *Scilla* species

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Submitted in partial fulfilment of the requirements  
for the degree of Master of Science  
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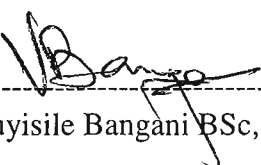
***TO MY DEAR MOTHER***

## PREFACE

The experimental work described in this thesis was carried out in the Department of Chemistry, University of Natal, Durban, from February 1997 to September 1998 under the supervision of Professor D.A. Mulholland.

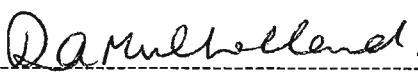
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Signed

  
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I hereby certify that the above statement is correct.

Signed

  
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Prof. D.A. Mulholland Ph.D

## ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to my supervisor, Professor Dulcie Mulholland for her advice, encouragement, enthusiasm and unwavering support during the course of this study.

I am also indebted to the following people for their part in the realisation of this effort :

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Kuni nonke bantakwethu ndithi : *Maz'enethole, ukwanda kwaliwa ngumthakathi.*

I acknowledge financial support from the FRD, the University of Natal and the Mazda Wildlife Fund. My thanks also go to the Natal and Mpumalanga Parks Boards, for issuing permits for the collection of some of the plant material used in this study and the

National Botanical Institute for allowing me access to their resources at the Natal Herbarium.

Last, but not least, I express my deepest appreciation to my mother, to whom I dedicate this work, for having been a source of inspiration to me despite harsh realities. To her I say: *Othembele kuYehova akasoze adaniswe.*

**ABBREVIATIONS**

Glc	Glucose or Glucopyranose
Rha	Rhamnose or Rhamnopyranose
Ac	Acetate
Ara	Arabinose or Arabinopyranose
<i>s</i>	singlet
<i>t</i>	triplet
<i>d</i>	doublet
<i>dd</i>	double doublet
<i>m</i>	multiplet
<i>q</i>	quartet
lit.	literature
Mp	melting point
<i>m/z</i>	mass to charge ratio
<i>c</i>	concentration
T.L.C	Thin Layer Chromatography
P.L.C	Preparative Layer Chromatography
TMS	Tetramethylsilane
HIF	Homoisoflavonoid
COSY	Correlated Spectroscopy
DEPT	Distortionless Enhancement by Polarisation Transfer
HETCOR	Heteronuclear chemical shift correlation
<sup>1</sup> H NMR	Proton Nuclear Magnetic Resonance
<sup>13</sup> C NMR	Carbon-13 Nuclear Magnetic Resonance

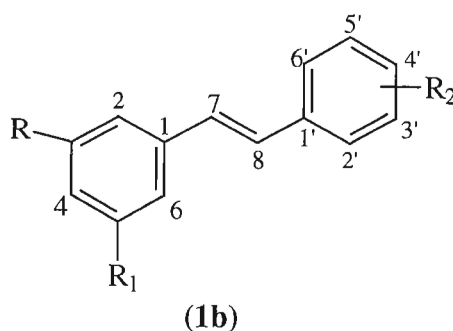
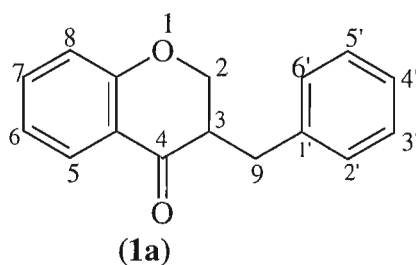
Hz	Hertz
cpd	compound
KZN	KwaZulu-Natal
Mpl	Mpumalanga
CC	Column chromatography
IR	Infra red
UV	Ultraviolet
spectr.	spectrum
NIH	National Institute of Health in Bethesda
hrms	high resolution mass spectrometry
RDA	<i>retro</i> Diels Ader
NOE	Nuclear Overhauser Effect

## NOMENCLATURE AND NUMBERING

The nomenclature that will be used when referring to homoisoflavonoid (**1a**) and stilbenoid (**1b**) structures in the discussion will be based on the numbering system illustrated below. Any numbering system assigned to any other structures mentioned in the text is the one derived from the primary literature.

Homoisoflavonoidal and stilbenoidal compounds mentioned in the text have been numbered using counting numbers while non-homoisoflavonoidal and non-stilbenoidal compounds have been numbered using counting numbers combined with the letter of the alphabet, e.g. 2a, 2b, 2c, etc. The compounds isolated in this work will be numbered using Roman numerals (**i**)-(**xii**)

Moreover the numbers allocated to homoisoflavonoids mentioned in the text are the ones assigned to them as per Table 2.1 in Chapter 2.





## ABSTRACT

Bulbs of *Scilla natalensis* Planch, *Scilla nervosa* (Burch.) Jessop, *Scilla dracomontana* Hilliard and Burt and *Scilla kraussii* Bak. (Hyacinthaceae) were investigated. The plants are widely used by the local African people for a variety of ailments that inflict them and their livestock.

Plant material was harvested in different localities i.e. KwaZulu-Natal (KZN) and Mpumalanga (Mpl). The bulbs have been found to contain homoisoflavonoids and stilbenoids. Ten homoisoflavonoids and two stilbenoids were isolated. Of the ten homoisoflavonoids isolated, nine were of the 3-benzyl-4-chromanone type while one was a 3-benzylidene-4-chromanone. Four of the 3-benzyl-4-chromanones were found to be novel compounds while others were recognised as having been reported before from other genera within the family Hyacinthaceae viz., *Eucomis* and *Muscari*. The 3-benzylidene-4-chromanone type compound isolated was also found to be a known compound. The stilbenoids, on the other hand, are reported for the first time in this genus although they seem to have a wide distribution in the plant kingdom.

The structures of the isolated compounds were elucidated using spectroscopic methods.

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# **CHAPTER 1**

## **INTRODUCTION**

## 1. INTRODUCTION

### 1.1 General

Humankind has harnessed the wonders and intrigues of nature since antiquity. The use by man of many products from nature to either improve his lifestyle, ward off disease or as a weapon in food gathering continues to this day. For instance, rotenoid containing plants belonging to the genera *Derris*, *Lonchocarpus*, *Piscidia* and *Tephrosia* have long been used by people in parts of South America, Tropical Africa and Asia as a fishing tool<sup>18</sup>, where roots or other plant parts are first crushed to release their active compounds before being thrown into ponds or slow running streams. The ichthyotoxic rotenoids, when released, rapidly stupefy any fish in the vicinity which subsequently float to the surface where they are collected.

Moreover the popularity of traditional medicine, despite the advances in modern medicine, among natives of different countries around the globe is a fact that no one can deny. *Cinchona* bark has long been used by people in the tropics to treat malaria and the chemical investigation into the active components of the extracts from these plants has provided man with what we today know as anti-malarials, e.g. quinine<sup>50</sup>.

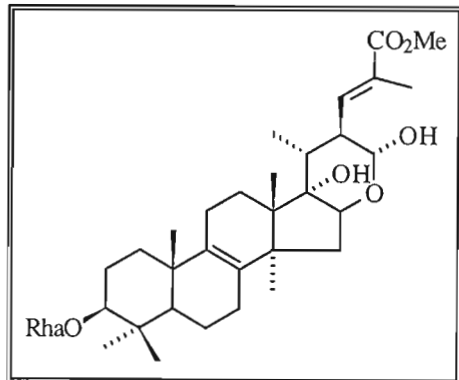
Many drugs on the pharmaceutical market today are of plant or animal origin<sup>50</sup>. Some have been manipulated somewhat to increase their efficacy while at the same time decreasing their toxicity and/or abuse potential. A case in point is that of lignocaine - a wholly synthetic local anaesthetic drug modelled on cocaine from cocoa leaves (*Erythroxylum coca*)<sup>50</sup>, which is less addictive than cocaine.

Measures to bring traditional healers into the mainstream of health care in this country need to be commended. This, it is hoped, will not only bring the secrecy under which most healers prefer to operate to an end as their traditional mixtures will be open to scientific scrutiny, but will also allow many people who rely on this type of health care easy access as, it is hoped, medical insurance will be incorporated within. Although there are success stories with regard to random screening e.g. taxol, much endeavour should nevertheless be directed towards plants which have been used over many decades

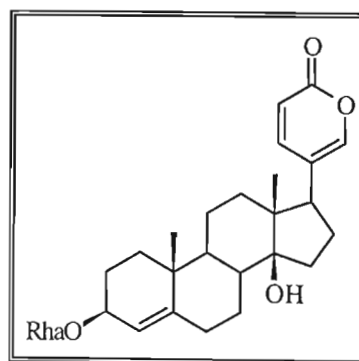
in folklore medicine, when searching for new drugs to fight many disorders that afflict man up to this day.

*Scilla natalensis* Planch., *S. nervosa* (Burch.) Jessop, *S. dracomontana* Hilliard and Burt and *S. kraussii* Bak. are four South African species in the family Hyacinthaceae (previously under the family Liliaceae). *Scilla nervosa* and *S. natalensis* (*sensu lato*) are two taxa which are widely used by the local African people for a variety of ailments that inflict them and their livestock<sup>31,66-69,72,78</sup>. It is for this reason that the chemistry of these particular species was investigated.

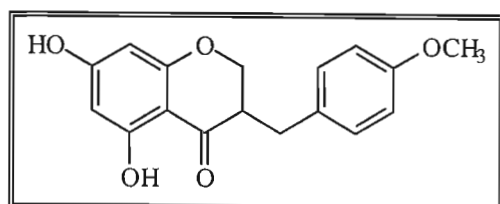
Previous investigations of the extracts from these species have revealed that they possess anti-microbial properties against a wide range of pus forming organisms *in vitro* and have anti-inflammatory properties<sup>31</sup>. Previous studies on other plants falling under this genus e.g. *S. maritima*, *S. scilloides*, *S. indica* and *S. peruviana* have revealed that this genus is a source of triterpenoid (**2a**)<sup>83</sup> and cardiac (**2b**)<sup>19</sup> glycosides as well as a small group of natural products commonly known as homoisoflavonoids e.g. 3,9-dihydroeucomin (**69**)<sup>17,23,58</sup>.



peruvianoside A (**2a**)

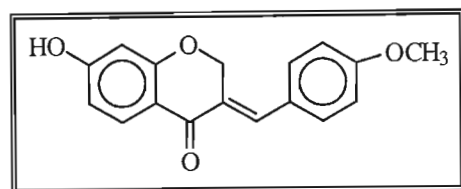


proscillaridin A (**2b**)

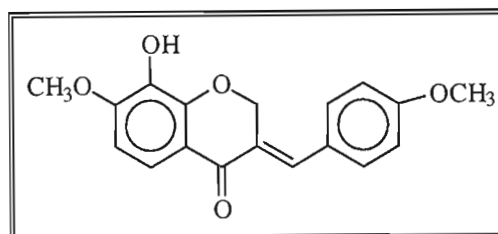


3,9-dihydroeucomin (**69**)

Homoisoflavonoids are common in the family Hyacinthaceae and this lends them as potential markers for taxonomy<sup>17</sup>. Despite the fact that they have also been reported outside the Hyacinthaceae, they are still a powerful taxonomic tool within this family in that those appearing outside the Hyacinthaceae are distinct as most lack oxygenation at the 5-position, e.g. bonducelin (**59**) and intricatin (**62**) from *Caesalpinia bonducella* (Fabaceae)<sup>21</sup> and *Hoffmannseggia intricata* (Fabaceae)<sup>33</sup> respectively.



bonducelin (**59**)



intricatin (**62**)

## 1.2 The genus *Scilla* (Hyacinthaceae)

Plants belonging to the genus *Scilla* L. have a wide distribution which extends throughout Europe, Africa and Asia. There are about forty *Scilla* species, most of which occur in Eurasia<sup>65</sup>. Six species are found in southern Africa while several others occur in tropical Africa<sup>65</sup>. In southern Africa they are found mostly in the eastern parts of the country, ranging from the Eastern Cape to Mpumalanga province including Lesotho and Swaziland. The only species which is not found in this eastern belt is *S. plumbea* Lindl. which is confined to the mountains of the South-Western Cape region<sup>65</sup>. In southern Africa, *Scilla* is an evergreen or summer growing genus. The species comprising this genus are glabrous or pubescent geophytes characterised by bulbs situated below or above the ground. The size and appearance of the bulbs varies considerably from species to species.

Bulb scales of these plants are imbricate and some bulbs have an oblong hard woody cortex with a fibrous neck. Roots are often hard and thick. Leaves may be smooth or hairy, and are generally lance shaped or broad and arranged in a rosette<sup>65</sup>. They bear small blue, white or green coloured flowers in terminal racemes<sup>31</sup>.



In addition to the species already mentioned *Scilla firmifolia* Bak. is also found in South Africa. Notably, some taxonomists have, on the basis of bulb morphology, sunk *S. kraussi* and *S. dracomontana* into *S. natalensis*<sup>64,107</sup>.

### 1.3 Ethnobotany

*Scilla* plants are widely used medicinally by the local African people, for their own health and that of their livestock. The same taxa have been reported to be toxic<sup>71,73,78</sup>. Traditional healers have used decoctions from these plants to treat rheumatism, venereal diseases, mental illness and a variety of other ailments<sup>17,31,68,69,72,78</sup>.

Of the four species forming part of this chemical investigation, only the use of *S. natalensis* and *S. nervosa* in traditional remedies has been well documented<sup>31,78</sup>. *Scilla kraussii* which has been considered synonymous with *S. natalensis*<sup>64</sup>, may have been incorrectly identified in previous ethnobotanical studies.

*Scilla natalensis*, commonly known as *inguduza*, is one of the most heavily exploited Zulu medicinal plants<sup>73,75</sup>, with people interviewed at the herbal market claiming that decoctions from this plant are also used to promote orgasm in men<sup>66</sup> in addition to some of the well documented traditional uses<sup>31,69,72,78</sup>. For example, the Zulu use decoctions from *S. natalensis*, as ingredients in infusions taken to facilitate labour at term<sup>31</sup>. Although *S. natalensis* has been reported as toxic to sheep (resulting in dyspnoea when ingested)<sup>71</sup> bulb preparations have been used by the locals to treat lung sickness in cattle<sup>31,78</sup>. The Sotho eat the cooked bulbs with food as an aperient, include bulb decoctions in enemas to treat 'internal tumours' and rub powdered bulbs into scarifications over sprains and fractures<sup>68,78</sup>. The Tswana rub the powdered bulbs into the back, joints and other body parts with the belief that it makes them strong and resilient to witchcraft<sup>69,78</sup>. The Swati apply a lotion prepared by boiling the macerated bulbs in water, to boils and veld sores<sup>78</sup>.

*Scilla nervosa* derives its name from one of its traditional medicinal applications, i.e. relief of conditions of nervousness in children<sup>78</sup>. In this regard, it is administered as an enema prepared by mixing lightly boiled decoctions with fresh lukewarm milk<sup>78</sup>. The

Xhosa use the bulbs as a remedy for dysentery<sup>78</sup>. Diluted bulb decoctions of *S. nervosa* are also used in small, once daily doses as analgesics in rheumatic fever<sup>31,78</sup>. The Sotho use *S. nervosa* bulbs to treat gall sickness in their livestock, while crushed bulbs are mixed with food and administered to children as an aperient<sup>68,78</sup>. The Tswana use the cooked bulbs, which are put into porridge and eaten three to four times a day for a month, for the treatment of infertility in women<sup>78</sup>.

Some *Scilla* spp. are used in Botswana for preventing witchcraft<sup>69</sup>, by sprinkling the whole yard with a mixture of crushed fresh bulbs and water while the Malawians living in Kaluluma's area (Kasangu district) use a gluey substance called *ulimo* from *Scilla* spp. as an insect trap<sup>67</sup>.

## 1.4 *Scilla* species in this study

### 1.4.1 *Scilla natalensis* Planch.

*Scilla natalensis* is found mainly in the province of KwaZulu-Natal (Fig.1.1). Its common name is slangkop<sup>31</sup>.

The bulb is about 60-65 cm in diameter, 8 cm in length and is covered with brown membranous leaf bases. Its leaves are uniformly green and hairy at times and are about 14 cm long and 4 cm broad at the base. The inflorescence, whose length reaches 35 cm, is erect and rigid and bears deep blue and occasionally white flowers<sup>71,108</sup>.

### 1.4.2 *Scilla nervosa* (Burch.) Jessop

This species is one of the most widely distributed *Scilla* species on the subcontinent, (Fig. 1.2). Synonyms include *Schizocarphus gerrardii*, *Schizocarphus nervosus*, *Schizocarphus rigidifolius* and *Schizocarphus acerosus*<sup>119-121</sup>. Locally, the Zulu people call it *ingcino*<sup>31</sup>.

The bulbs are oblong in shape and vary in size (about 2-5 cm in diameter and 3 cm in length) depending on the provenance. This was evidenced with the morphology of the plant material collected in Lydenburg District, Mpumalanga, and that purchased at the Warwick Triangle herbal market in Durban, KwaZulu-Natal. The bulbs are covered by

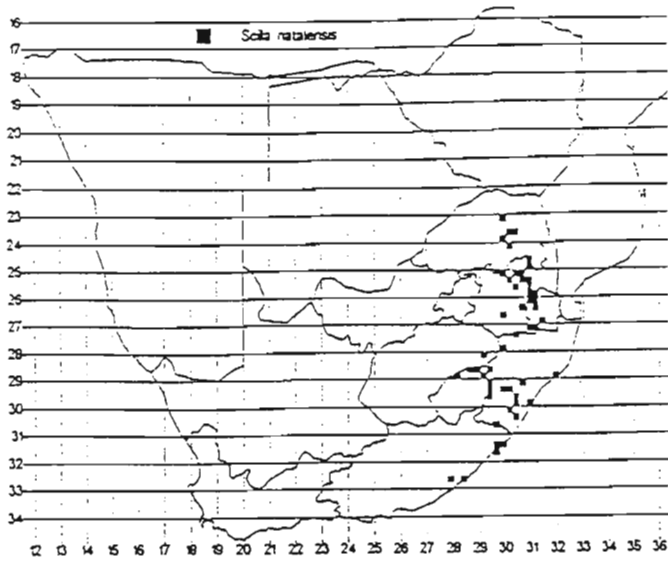
a dense coat of fibrous material, a feature distinct to this species. The slender leaves are about 10 cm long and 2-3 cm wide. The inflorescence is somewhat longer than the leaves and bears white flowers with bluish-green anthers<sup>31,52</sup>.

#### **1.4.3 *Scilla dracomontana* Hilliard and Burt.**

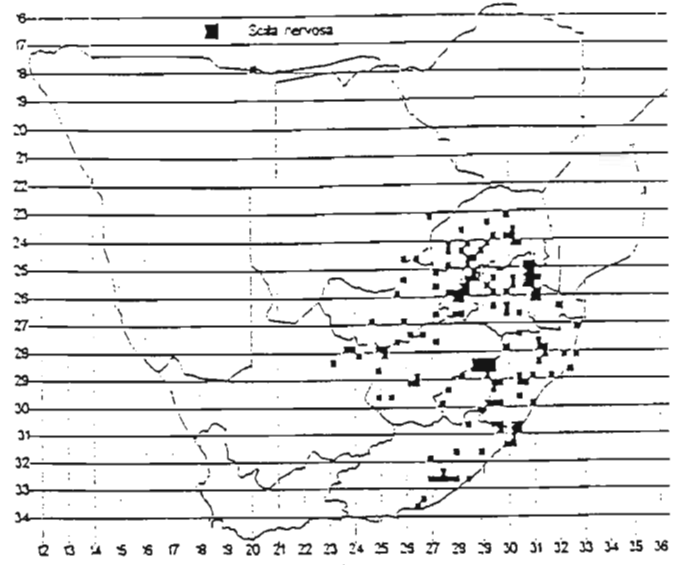
*Scilla dracomontana*, whose distribution is far more restricted than *S. natalensis* (Fig.1.3), is characterised by having small bulbs up to 3 cm in diameter and 5 cm in length. As its specific epithet implies, it occurs in the Drakensberg mountains. Its range in the Drakensberg extends from Giant's Castle (Estcourt District) to the upper Umzimouti Valley (Underberg District). It occurs at altitudes of between 1675 and 2100m above sea level<sup>74</sup>. It grows on sandstone cliffs, in rock crevices and on hardpacked earth, often forming extensive colonies. *Scilla dracomontana* is distinguished from both *S. natalensis* and *S. kraussii* in having its leaves fully developed and flat on the ground at flowering time, whilst its larger relatives possess erect immature leaves when the flowers open<sup>74</sup>.

#### **1.4.4 *Scilla kraussii* Bak.**

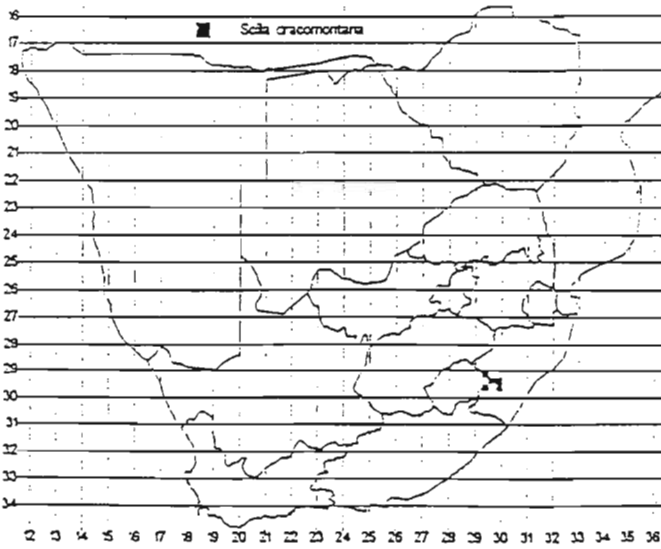
*Scilla kraussii* possesses bulbs which are about 2 cm in diameter and 4.5 cm long, covered by a thin layer of dead leaves. The plant has a solid butt below the bulb which is about 1 cm long and 2 cm in diameter<sup>51</sup>. Plants produce about four leaves; each of these are rigid and have deep green and blackish-purple upper and lower surfaces respectively. Leaves are generally about 7 cm in length and 1 cm wide. They are convex above with an acute longitudinal furrow, pilose on both surfaces, with hairs mainly along the nerves. *Scilla kraussii* is prevalent in Northeast Pondoland near Nkambati and Umtamvuna Rivers close to Port Edward. This species is also found in various parts of KwaZulu-Natal (Fig 1.4)<sup>51</sup>.



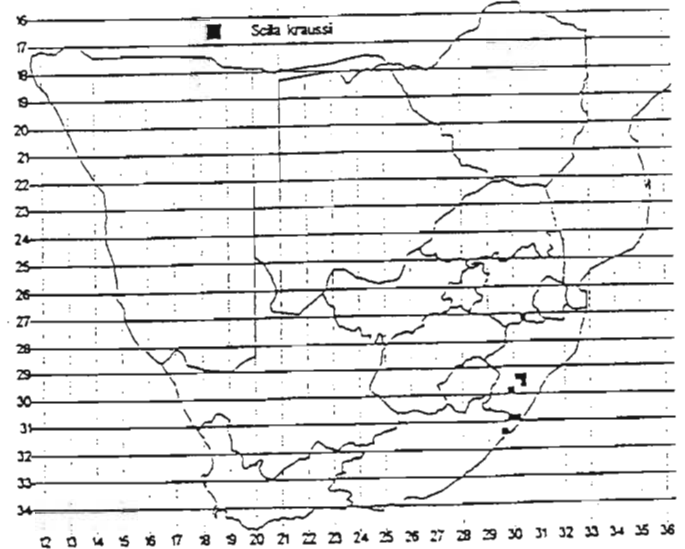
**Fig. 1.1.** Distribution of *Scilla natalensis*



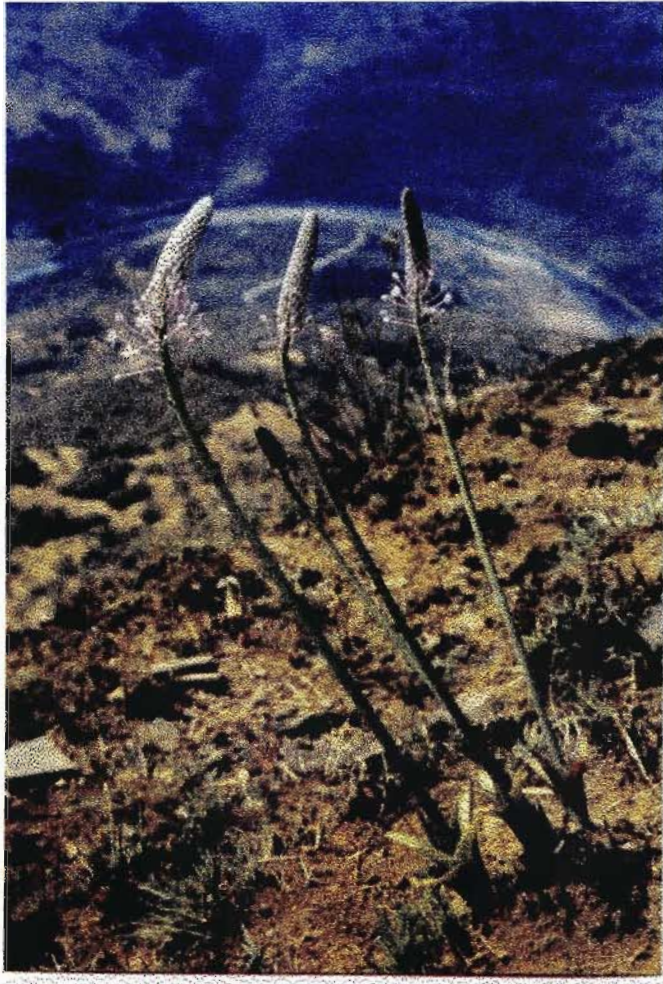
**Fig. 1.2.** Distribution of *Scilla nervosa*



**Fig. 1.3.** Distribution of *Scilla dracomontana*



**Fig. 1.4.** Distribution of *Scilla kraussii*



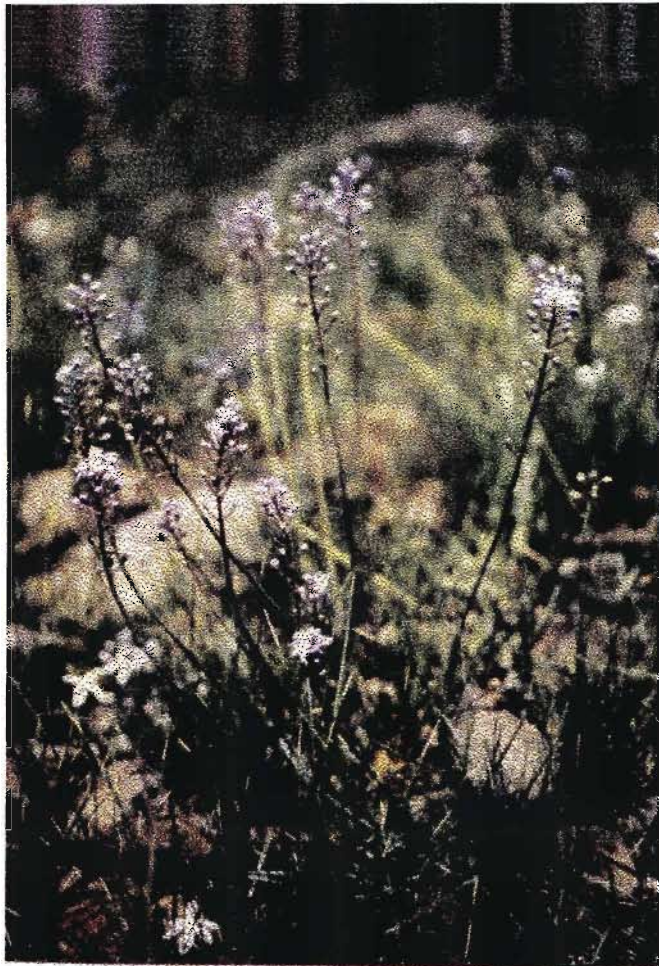
**Fig. 1.5.** *Scilla natalensis* growing on a hill in Hella Hella



**Fig. 1.6.** *Scilla nervosa* (harvested plant material)



**Fig. 1.7.** *Scilla dracomontana* growing on rock crevices in the Bamboo mountain



**Fig. 1.8.** *Scilla kraussii* growing in Mboma Estate, Karkloof



## **CHAPTER 2**

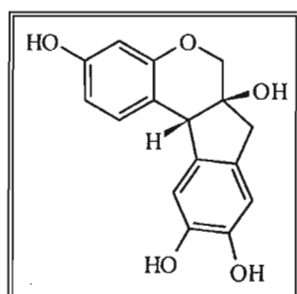
### **HOMOISOFLAVONOIDS AND STILBENOIDS: OCCURRENCE AND BIOSYNTHESIS**

## 2.1 Homoisoflavonoids

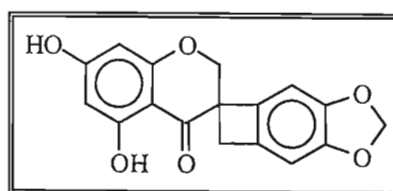
### 2.1.1 Occurrence

Homoisoflavonoids are a small group of natural compounds which are restricted in their distribution, having been previously reported to occur mainly in genera in the Hyacinthaceae<sup>1-9,17,31,56</sup>. However, some of these homoisoflavonoids have also been reported to occur outside this family, as reported earlier for example bonducellin (**59**) and intricatin (**62**)<sup>21,33</sup>. Within the Hyacinthaceae, members of the genera *Scilla*, *Eucomis* and *Muscari* have been the most dominant with regard to the production of homoisoflavonoids<sup>1-4,17,56,59-62</sup>. Other Hyacinthaceous genera from which these compounds have been reported are *Bellevallia*<sup>5</sup>, *Chionodoxa*<sup>7</sup>, *Ophiopogon*<sup>35,37</sup> and *Veltheimia*<sup>87</sup>. A number of representatives of the family Dracaenaceae, e.g. *Dracaena cinnabari*<sup>10</sup>, *Dracaena draco*<sup>22</sup> and *Dracaena loureri*<sup>32</sup> have also been reported to possess homoisoflavonoids.

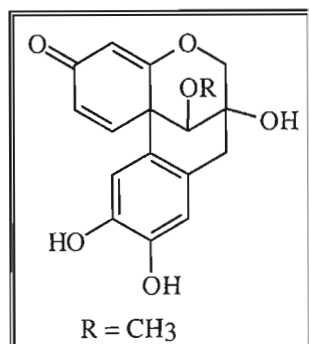
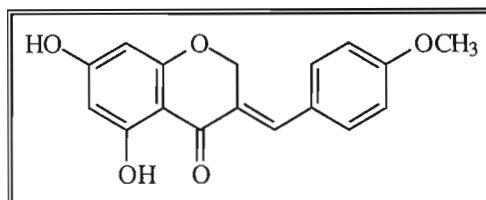
Homoisoflavonoids differ from the ubiquitous isoflavonoids by having a sixteen carbon skeleton instead of the fifteen characteristic of the isoflavonoids. This sixteen carbon skeleton bears either a chromane, chromone or chromanone moiety to which is attached, in most cases, a benzyl or benzylidene grouping at the 3-position<sup>15</sup>. A fourth ring is present in the brazillin (**6**)<sup>15,17</sup>, scillascillin (**13**)<sup>15,17</sup>, and dracaenone type compounds<sup>32</sup> e.g. caesalpin J (**29**). Substituents in the form of hydroxy, methoxy, acetoxy and in some cases methyl and aldehyde groups can occur<sup>14,15,47,56,58</sup>. These compounds were first reported to occur in *Eucomis* species in 1967, eucomin (**51**) being one of the first compounds to be isolated and characterised from *Eucomis bicolor*<sup>62</sup>. Within the plant these compounds have been found to be concentrated in the waxy layers between the storage leaves of the bulbs<sup>17</sup>.



brazillin (**6**)



scillascillin (**13**)

caesalpin J (**29**)eucomin (**51**)

Previous studies have revealed that these compounds possess anti-inflammatory, anti-mutagenic, analgesic and anti-bacterial properties<sup>17,32,33,88</sup>.

Table 2.1 represents a list of known, naturally occurring, homoisoflavanoids and biogenetically related compounds. Due to space constraints within the table, the list of names is provided in Appendix A. The nomenclature adopted is the one used in the primary literature. Where no such nomenclature is available, systematic nomenclature as used in Chemical Abstracts<sup>113</sup> has been adopted. The compilation of the list was aimed at investigating which of the homoisoflavanoids isolated in this work had been reported before.

**Table 2.1** List of known natural occurring homoisoflavonoids and biogenetically related compounds.

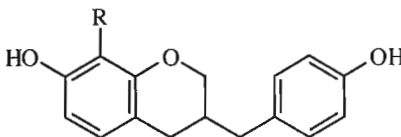
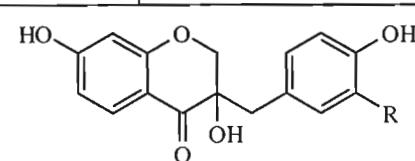
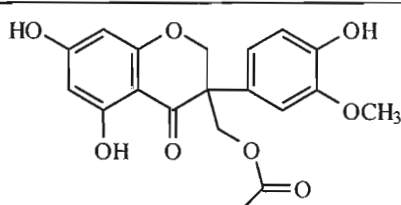
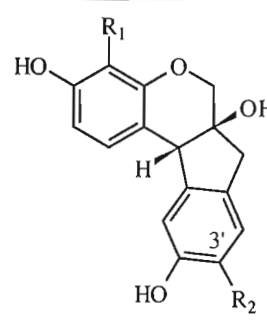
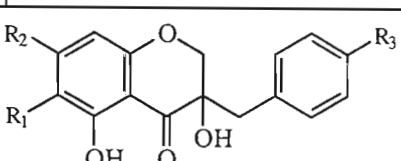
Compound(s)			Reference(s)
			
<b>R</b>			
H		(1)	10,22,56,58
OCH <sub>3</sub>		(2)	10,22,56,58
			
<b>R</b>			
H		(3)	11,46,56
OH		(4)	11,41,45,46,56
			
		(5)	3,15,56,58
			
<b>R<sub>1</sub></b>	<b>R<sub>2</sub></b>		
H	OH	(6)	11,17,44,56,58
H	OCH <sub>3</sub>	(7)	44,56,58
OH	OH	(8)	17,56,58
			
<b>R<sub>1</sub></b>	<b>R<sub>2</sub></b>	<b>R<sub>3</sub></b>	
H	OH	OH	(9)
H	OH	OCH <sub>3</sub>	(10)
H	OCH <sub>3</sub>	OCH <sub>3</sub>	(11)
CH <sub>3</sub>	OCH <sub>3</sub>	OH	(12)
			17,58
			17,56,58,62
			17,26,56,58
			47,56

Table 2.1 (continued)

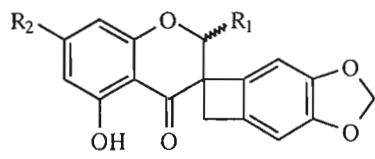
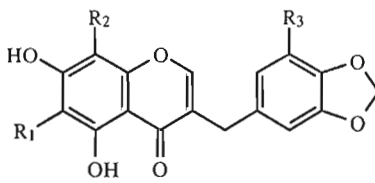
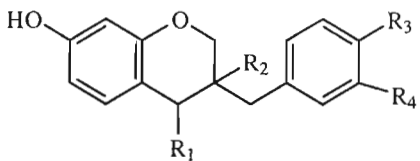
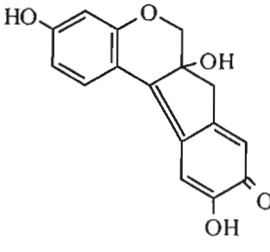
					
<b>R<sub>1</sub></b>	<b>R<sub>2</sub></b>				
H	OH			(13)	6,17,23,56,58
OH	OCH <sub>3</sub>			(14)	7,17,56,58
OH	OH			(15)	7,56
					
<b>R<sub>1</sub></b>	<b>R<sub>2</sub></b>	<b>R<sub>3</sub></b>			
CH <sub>3</sub>	H	H		(16)	37,48,56,58
H	CH <sub>3</sub>	H		(17)	37,38,56,58
CH <sub>3</sub>	CH <sub>3</sub>	H		(18)	35,37,47,56,58
CH <sub>3</sub>	CH <sub>3</sub>	OH		(19)	47,56
CHO	CH <sub>3</sub>	H		(20)	14,56
H	CH <sub>3</sub>	OH		(21)	48,56
CH <sub>3</sub>	H	OH		(22)	48,56
					
<b>R<sub>1</sub></b>	<b>R<sub>2</sub></b>	<b>R<sub>3</sub></b>	<b>R<sub>4</sub></b>		
OH	OH	OH	OCH <sub>3</sub>	(23)	44,56
OH	OH	OH	H	(24)	11,44,56
OCH <sub>3</sub>	OH	OH	OH	(25)	41,45,46,56
OCH <sub>3</sub>	OH	OH	H	(26)	45,56
OH	OH	OH	OH	(27)	11,41,44,56
					
				(28)	56,112

Table 2.1 (continued)

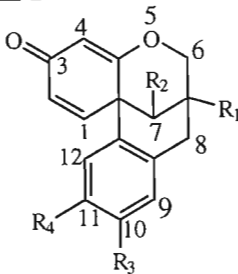
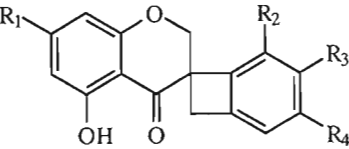
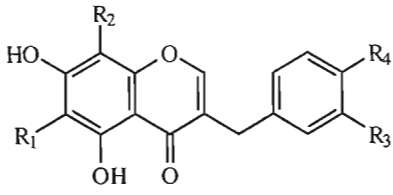
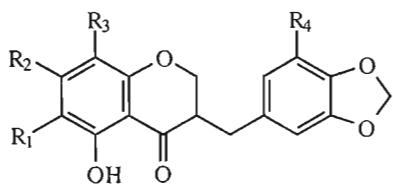
					
<b>R<sub>1</sub></b>	<b>R<sub>2</sub></b>	<b>R<sub>3</sub></b>	<b>R<sub>4</sub></b>		
OH	OCH <sub>3</sub>	OH	OH	(29)	32,39,56
H	H	OH	OCH <sub>3</sub>	(30)	32,56
OH	H	OH	OCH <sub>3</sub>	(31)	32,56
					
<b>R<sub>1</sub></b>	<b>R<sub>2</sub></b>	<b>R<sub>3</sub></b>	<b>R<sub>4</sub></b>		
OH	H	OH	OCH <sub>3</sub>	(32)	3,56,58
OCH <sub>3</sub>	H	OH	OH	(33)	4
OCH <sub>3</sub>	H	OCH <sub>3</sub>	OH	(34)	4
OH	H	OCH <sub>3</sub>	OH	(35)	4
OCH <sub>3</sub>	OH	OCH <sub>3</sub>	H	(36)	6
					
<b>R<sub>1</sub></b>	<b>R<sub>2</sub></b>	<b>R<sub>3</sub></b>	<b>R<sub>4</sub></b>		
CH <sub>3</sub>	H	H	OCH <sub>3</sub>	(37)	37,56,58
CH <sub>3</sub>	CH <sub>3</sub>	H	OCH <sub>3</sub>	(38)	35,56,58
H	H	H	OH	(39)	47,56
CH <sub>3</sub>	H	H	OH	(40)	47,56
CHO	CH <sub>3</sub>	H	OCH <sub>3</sub>	(41)	14,56
H	CH <sub>3</sub>	H	OH	(42)	37,56
					
<b>R<sub>1</sub></b>	<b>R<sub>2</sub></b>	<b>R<sub>3</sub></b>	<b>R<sub>4</sub></b>		
CH <sub>3</sub>	OH	H	H	(43)	37,38,48,56
CH <sub>3</sub>	OH	CH <sub>3</sub>	H	(44)	35,47,56,58
CHO	OH	CH <sub>3</sub>	H	(45)	47,56,58
CHO	OCH <sub>3</sub>	CH <sub>3</sub>	H	(46)	56,58
CH <sub>3</sub>	OH	CH <sub>3</sub>	OH	(47)	47,56

Table 2.1 (continued)

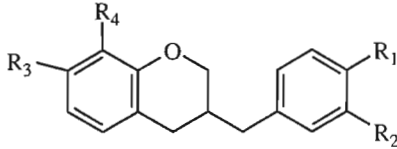
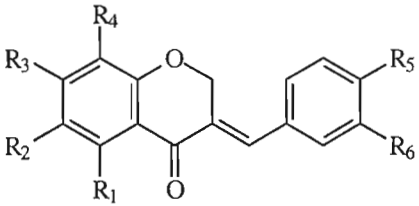
								
<b>R<sub>1</sub></b>	<b>R<sub>2</sub></b>	<b>R<sub>3</sub></b>	<b>R<sub>4</sub></b>					
OCH <sub>3</sub>	OH	OH	H			(48) 10,56		
OH	H	-OCH <sub>2</sub> O-				(49) 10,56		
								
<b>R<sub>1</sub></b>	<b>R<sub>2</sub></b>	<b>R<sub>3</sub></b>	<b>R<sub>4</sub></b>	<b>R<sub>5</sub></b>	<b>R<sub>6</sub></b>			
OH	H	OH	H	OH	H	(50) 56,58,59		
OH	H	OH	H	OCH <sub>3</sub>	H	(51) 56,58,62		
OH	H	OH	OCH <sub>3</sub>	OH	H	(52) 7,56,58,59		
OH	H	OH	OCH <sub>3</sub>	OCH <sub>3</sub>	H	(53) 56,58,59,60		
OH	OCH <sub>3</sub>	OH	H	OH	H	(54) 56,58,60		
OH	OCH <sub>3</sub>	OH	H	OH	OH	(55) 7,12,56		
OCH <sub>3</sub>	H	OH	H	OH	OH	(56) 12,56		
OH	H	OCH <sub>3</sub>	OH	OH	OH	(57) 5,56		
OH	H	OH	OCH <sub>3</sub>	OH	OH	(58) 7,56		
H	H	OH	H	OCH <sub>3</sub>	H	(59) 8,21,56		
H	H	OH	OCH <sub>3</sub>	OCH <sub>3</sub>	H	(60) 8,56		
H	H	OH	OH	OCH <sub>3</sub>	H	(61) 33,56		
H	H	OCH <sub>3</sub>	OH	OCH <sub>3</sub>	H	(62) 11,33,56		
H	H	OH	H	OH	H	(63) 11,56		
H	H	OH	H	OH	OH	(64) 11,41,45,56		
OH	H	OCH <sub>3</sub>	H	OCH <sub>3</sub>	H	(65) 26,58		

Table 2.1 (continued)

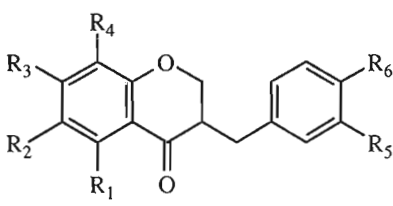
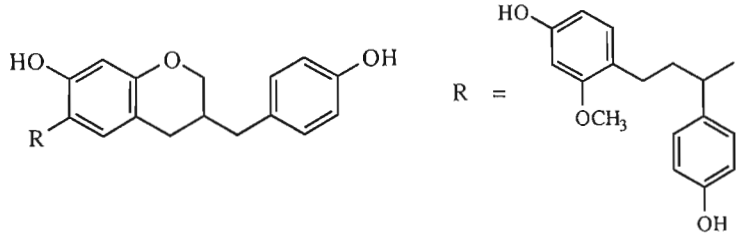
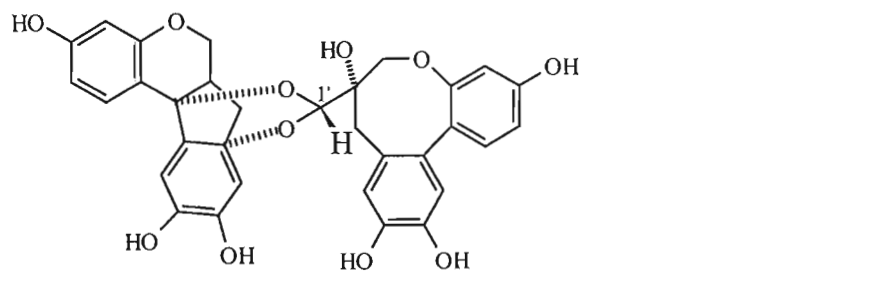
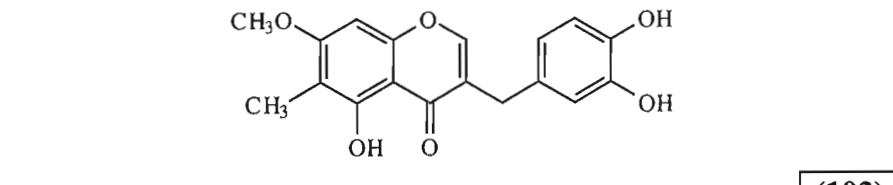
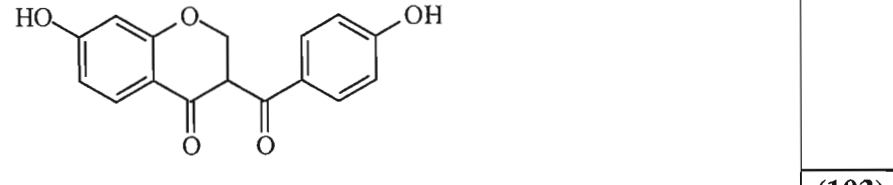
							
R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>		
H	H	OH	H	H	OH	(66)	22,56,58
H	H	OAc	H	H	OAc	(67)	22,56
OH	H	OH	H	H	OH	(68)	22,56
OCH <sub>3</sub>	H	OH	H	H	OH	(69)	56,59,60
OH	H	OH	H	H	OCH <sub>3</sub>	(70)	26,56,58
OH	H	OCH <sub>3</sub>	H	H	OCH <sub>3</sub>	(71)	26,56
OH	H	OH	H	OH	OCH <sub>3</sub>	(72)	2,4,56,58
OH	CH <sub>3</sub>	OH	H	H	OH	(73)	22,56
OH	CH <sub>3</sub>	OH	H	H	OCH <sub>3</sub>	(74)	56,58
OH	CH <sub>3</sub>	OH	CH <sub>3</sub>	H	OCH <sub>3</sub>	(75)	35,47,56,58
OH	OCH <sub>3</sub>	OH	H	H	OH	(76)	56,58,61
OH	H	OCH <sub>3</sub>	OH	H	OH	(77)	1,56,58
OH	H	OCH <sub>3</sub>	OAc	H	OH	(78)	3,56,58
OH	H	OH	OCH <sub>3</sub>	H	OH	(79)	1,3,56,58
OH	H	OCH <sub>3</sub>	OCH <sub>3</sub>	H	OH	(80)	1,3,56,58
OH	H	OH	OCH <sub>3</sub>	H	OCH <sub>3</sub>	(81)	56,58,60
OH	OCH <sub>3</sub>	OCH <sub>3</sub>	OH	H	OH	(82)	2,56,58
OH	CHO	OH	CH <sub>3</sub>	H	OCH <sub>3</sub>	(83)	47,56,58
OH	CHO	OCH <sub>3</sub>	CH <sub>3</sub>	H	OCH <sub>3</sub>	(84)	56,58
OH	OH	OCH <sub>3</sub>	OCH <sub>3</sub>	OH	OH	(85)	5,56
OH	OCH <sub>3</sub>	OH	H	OH	OH	(86)	4,56
OH	OCH <sub>3</sub>	OH	H	OCH <sub>3</sub>	OH	(87)	4,56
OH	OCH <sub>3</sub>	OH	H	OH	OCH <sub>3</sub>	(88)	4,56
OH	H	OCH <sub>3</sub>	OH	OH	OH	(89)	4,56
OH	H	OH	H	OH	OH	(90)	4,56
OCH <sub>3</sub>	H	OH	H	OH	OH	(91)	4,56
H	H	OH	H	OH	OH	(92)	41,56
OH	OCH <sub>3</sub>	OCH <sub>3</sub>	OCH <sub>3</sub>	H	OH	(93)	56,87
OCH <sub>3</sub>	H	OH	H	OH	OCH <sub>3</sub>	(94)	4,56
OH	H	OH	OCH <sub>3</sub>	OH	OCH <sub>3</sub>	(95)	7,56
OH	CH <sub>3</sub>	OCH <sub>3</sub>	OCH <sub>3</sub>	OH	OH	(96)	48,56
H	OH	OCH <sub>3</sub>	H	OH	OCH <sub>3</sub>	(97)	56, 110
OH	H	H	H	OH	OH	(98)	5,56
OH	CH <sub>3</sub>	OH	CH <sub>3</sub>	H	OH	(99)	56, 111



Table 2.1(continued)

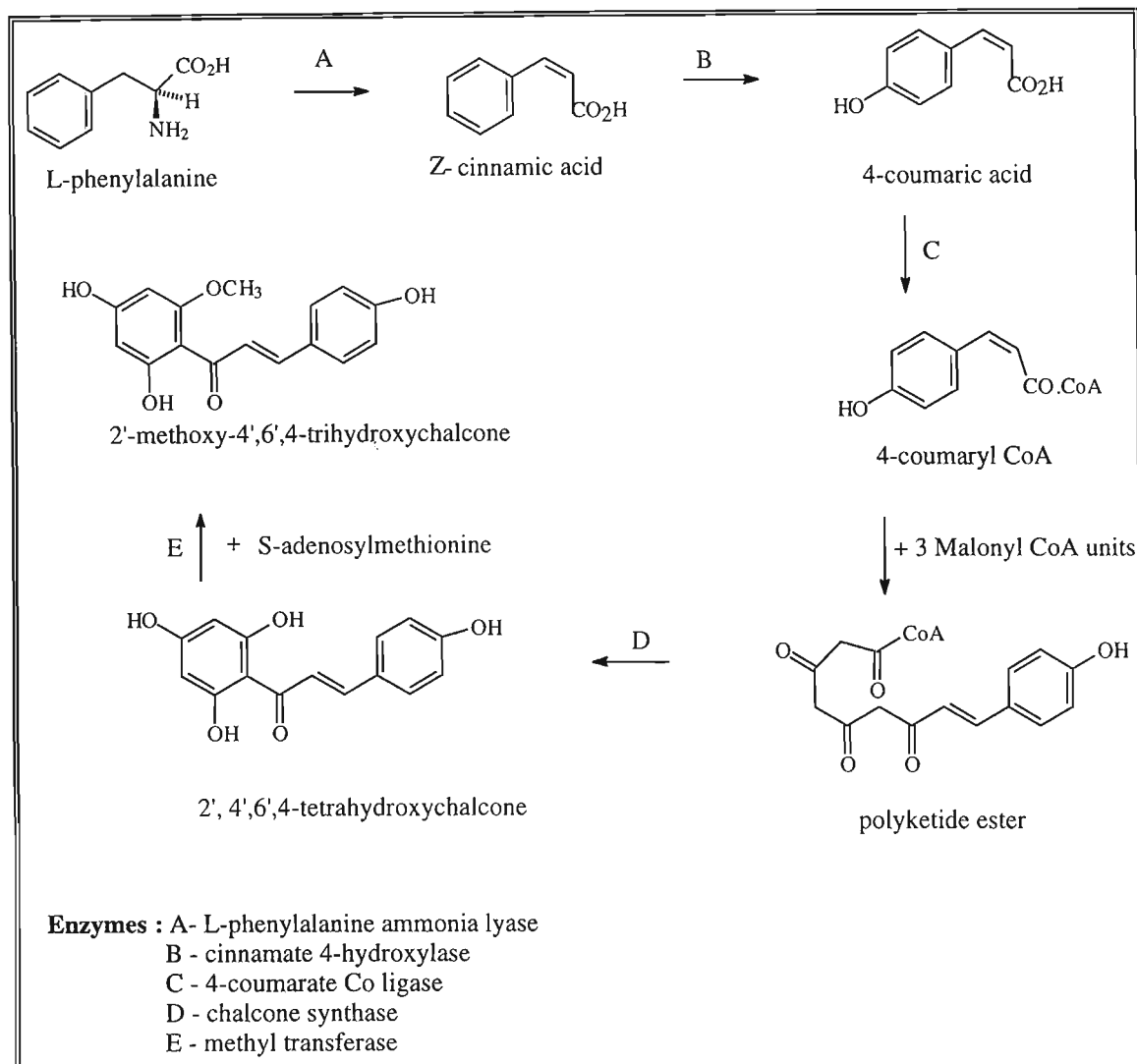
	
	
	
	

### 2.1.2 Biosynthesis of homoisoflavonoids

Homoisoflavonoids are phenolic compounds, hence they can be synthesised by either or both of two common biosynthetic routes leading to phenolic compounds: i.e. the shikimate pathway starting from carbohydrates, and the polyketide pathway from acetyl and malonyl CoA units<sup>27-29</sup>. Homoisoflavonoids are of mixed origin with ring B shikimate-derived and ring A polyketide-derived<sup>9</sup>. In this discussion the processes leading to homoisoflavonoid biosynthesis will be divided into two phases, viz.:

- (i) the chalcone formation phase and
- (ii) the 3-benzyl(idene)-4-chromanone formation phase.

## 2.1.2 (i) The chalcone formation phase (Scheme 2.1)

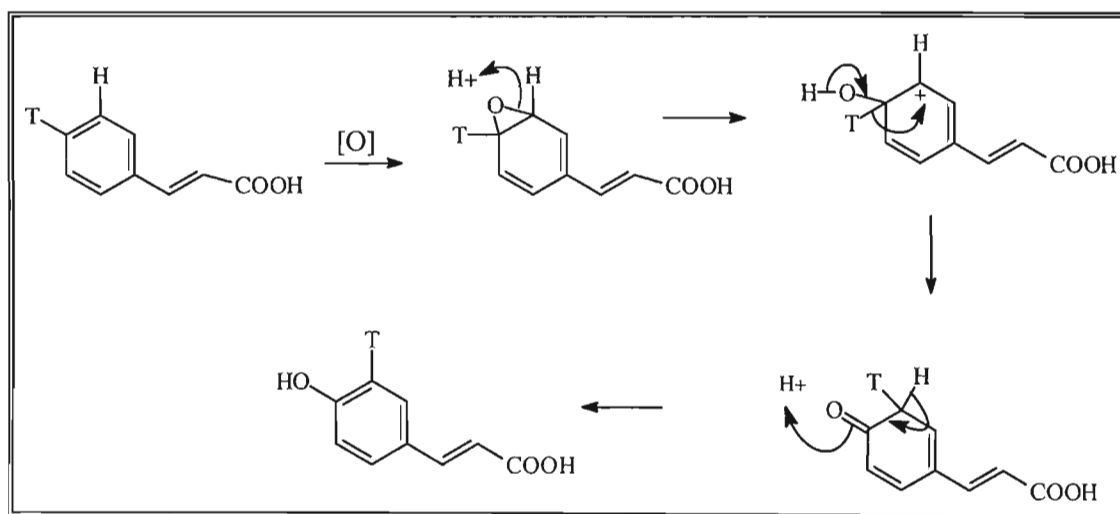


**Scheme 2.1.** The chalcone formation phase<sup>24,29</sup>.

Scheme 2.1 represents the events leading to 2'-methoxychalcone formation. The entry point for this pathway is the conversion of shikimate derived L-phenylalanine to cinnamic acid in a reaction catalysed by the enzyme L-phenylalanine ammonia lyase<sup>24</sup>. This involves the elimination of ammonia in an antiperiplanar fashion to yield (Z)-cinnamic acid. Subsequent hydroxylation of cinnamic acid, mediated by the enzyme cinnamate-4-hydroxylase results in the formation of 4-coumaric acid. Cinnamate hydroxylation occurs via a distinctive shift called the NIH shift (National Institute of Health in Bethesda, where it was discovered)<sup>29</sup>. According to this shift, the proton in the *para* position gives way to the OH group, being itself moved to the *meta* position. The NIH shift which was established by tritium (T) labelling experiments is depicted in

scheme 2.2 below. Further hydroxylation, without the NIH shift, leads to the 3,4-dihydroxy (catechol) pattern<sup>29</sup>.

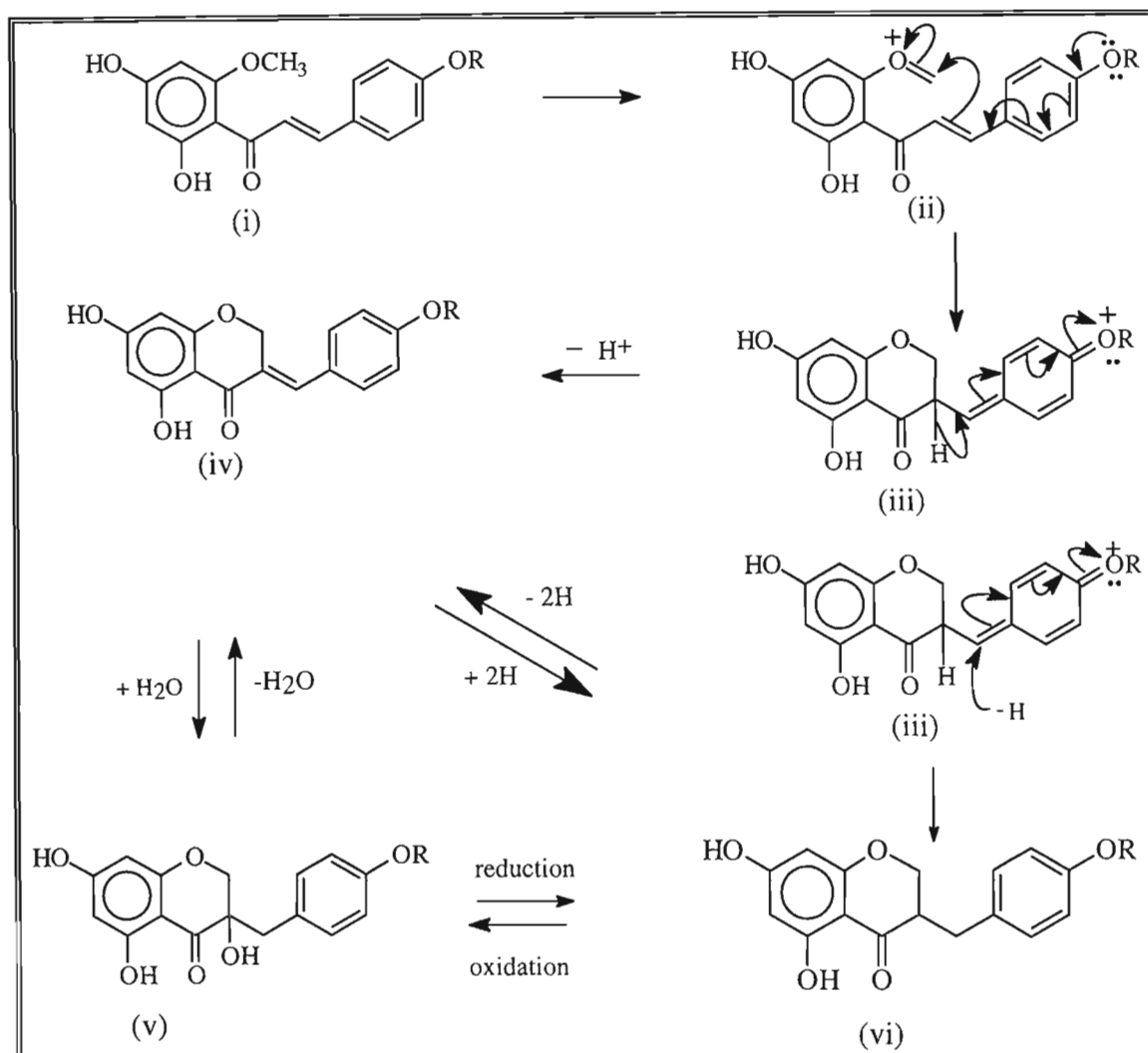
The 4-coumaric acid formed via the NIH shift is converted further to its CoA ester (4-coumaryl CoA) by the enzyme 4-coumarate: CoA ligase. The 4-coumaryl CoA molecule combines with three malonyl CoA derived acetate units. The CoA ester resulting from this combination cyclises, presumably in a Claisen type condensation to yield tetrahydrochalcone. The enzyme chalcone synthase acts as a catalyst in this transformation<sup>29</sup>. Methionine, probably as S-adenosylmethionine, a common methylating agent in living systems, provides the methyl group that gives 4,4',6'-trihydroxy-2'-methoxychalcone in a reaction that is mediated by a methyltransferase<sup>9,24</sup>.



**Scheme 2.2.** Hydroxylation of cinnamic acid to 4-coumaric acid via the NIH shift<sup>29</sup>.

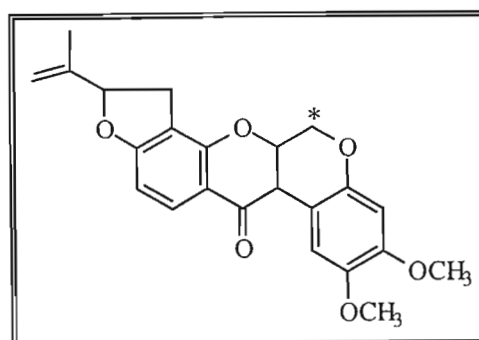
### 2.1.2 (ii) The 3-benzyl(idene)-4-chromanone formation phase (Scheme 2.3)

Scheme 2.3 is a summary of possible routes leading to 3-benzyl-4-chromanone and/or 3-benzylidene-4-chromanone derivatives, with 2'-methoxy-4,4',6'-trihydroxychalcone (i) from scheme 2.1 as the entry level intermediate.

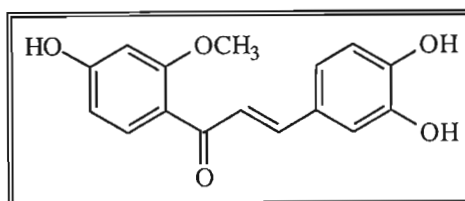


**Scheme 2.3.** Possible biosynthetic routes from 2'-methoxychalcone to simple homoisoflavonoids<sup>9</sup>.

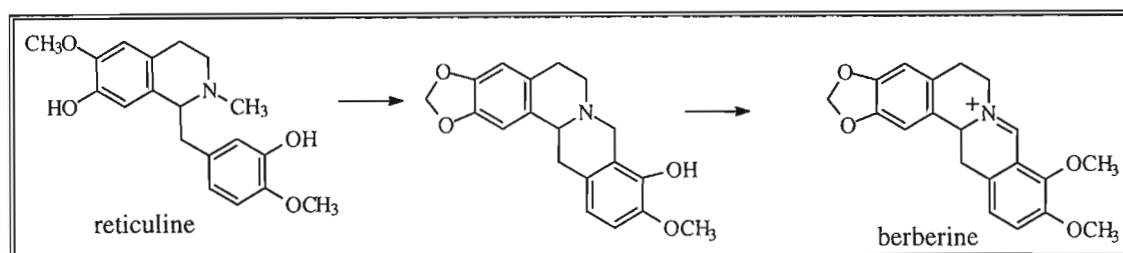
A close examination of this scheme proposed by P. Dewick<sup>9</sup> sees a methoxy group being used to create a carbon-carbon bond i.e. the C<sub>2</sub>, C<sub>3</sub> bond. This phenomenon is not rare in flavonoid chemistry as it has been demonstrated before in connection with the biosynthesis of rotenoids e.g. rotenone (**2c**) from 2'-methoxyflavones<sup>24</sup>. Moreover “berberine bridge” formation via cyclisation of N-methyl groups has been reported in alkaloid biosynthesis (scheme 2.4)<sup>30</sup>. Furthermore, the co-occurrence of the chalcone sappanchalcone (**2d**), together with a variety of homoisoflavonoids in *Caesalpinia sappan*<sup>11,41</sup>, gives credence to scheme 2.3. Although the chalcone oxonium and/or carbonium ion is suggested as the potential intermediate in this phase, a radical version has not been ruled out<sup>9,24</sup>.



rotenone (2c)



sappanchalcone (2d)

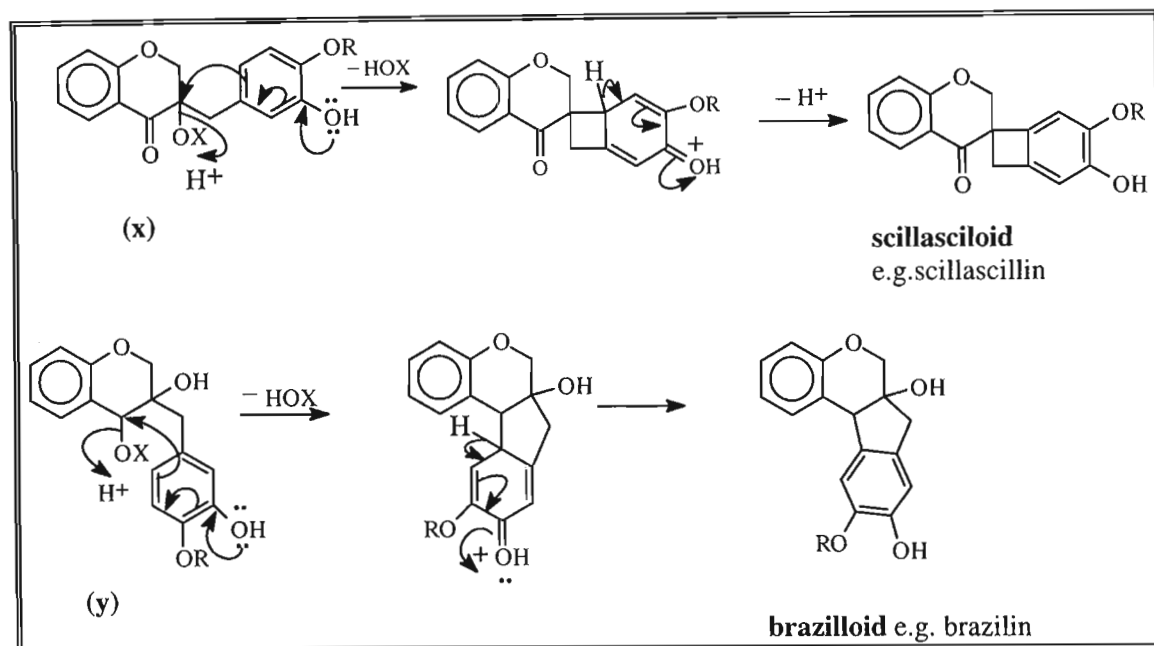


**Scheme 2.4.** Berberine from reticuline, in which the N-methyl furnishes the extra carbon atom of the “berberine bridge”<sup>30</sup>.

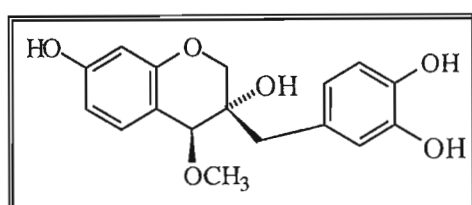
The relationship between 3-benzyl(idene)-type structures and their variants as suggested in scheme 2.3 has, nevertheless, not been fully established. Some workers maintain that the  $\Delta^{3,9}$  compounds arise from 3,9-dihydro relatives by way of their 3-hydroxylated derivatives: [scheme 2.3: (v)  $\rightarrow$  (vi)  $\rightarrow$  (iv)]<sup>9,17</sup>.

Another variation in the basic skeleton is to be seen in scillascillin, brazilin and dracaenone type compounds. Scillascillin carries an extra 3-spirocyclobutene ring, brazilin a cyclopentene ring whilst dracaenone-type structures like caesalpin J have an extra cyclohexene ring. Scillascilloid and brazilloid structures have been postulated to arise by way of activated intermediates (x) and (y) as represented in scheme 2.5<sup>9</sup>.

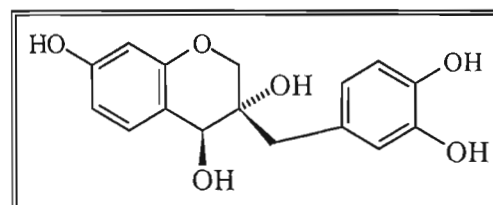
The co-occurrence of 4-O-methylsappanol (**25**) and sappanol (**27**) with brazilin (**6**) in *Caesalpinia sappan* as reported by Saitoh and co-workers<sup>41</sup> gives credence to scheme 2.5.



**Scheme 2.5.** Hypothetical biosynthetic routes to scillascilloid and brazilloid type compounds<sup>9,41</sup>.



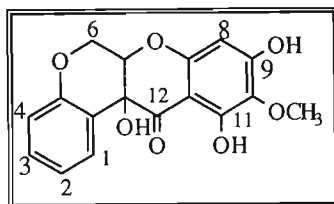
4-O-methylsappanol (**25**)



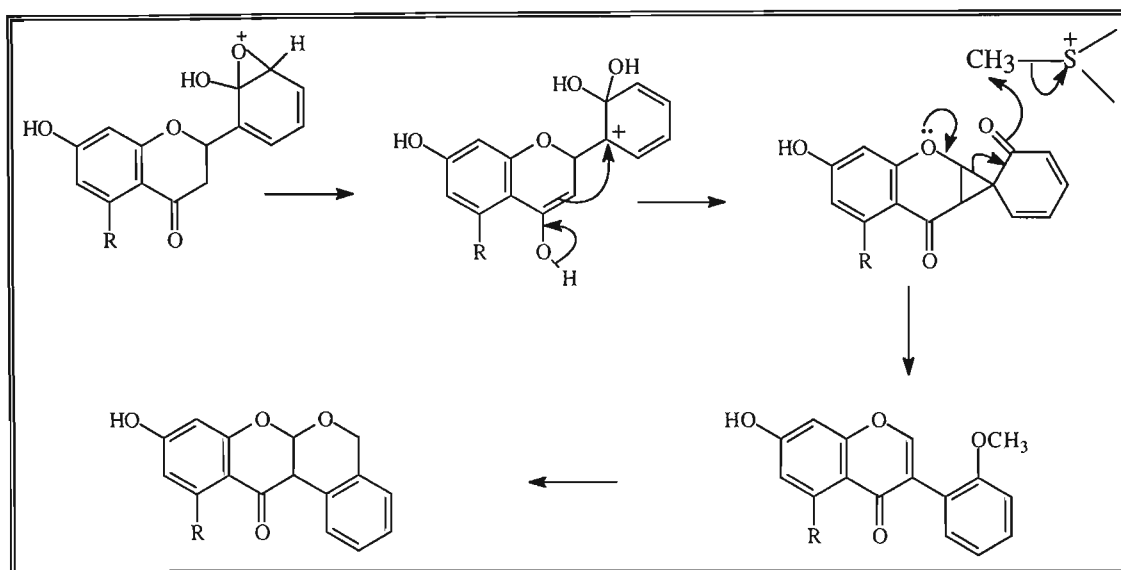
sappanol (**27**)

One other hypothesis for the formation of scillascilloid structures was suggested by Bhandari and co-workers<sup>24</sup>. Their study was on formation of rotenoids lacking the 3-oxygenation, e.g. Irispurinol (**2e**) from *Iris spuria* (Iridaceae). The 3-oxygenated rotenoids are characteristic of rotenoids emanating from the Papilionoideae division of the Fabaceae<sup>24</sup>. They hypothesised that these 3-deoxo rotenoids arise by way of the oxonium and carbocationic intermediates as depicted in scheme 2.6. From this scheme,

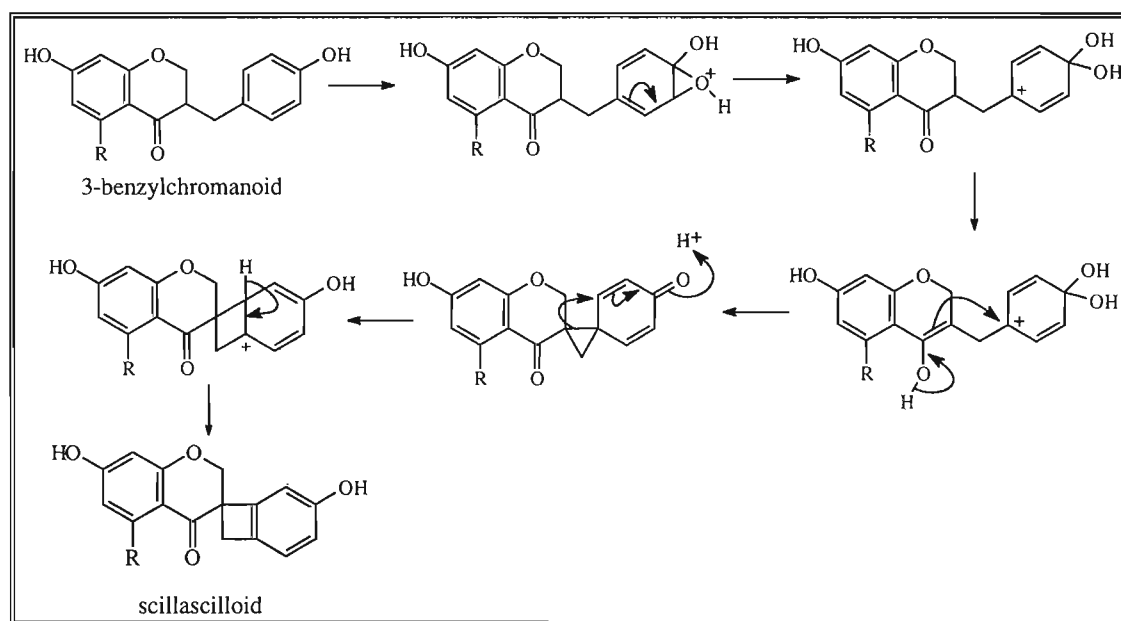
they extended the hypothesis to the formation of scillascilloid compounds from 3-benzyl-4-chromanones as represented in scheme 2.7<sup>24</sup>.



irispurinol (**2e**)

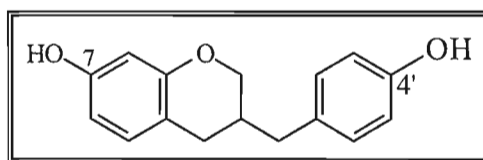
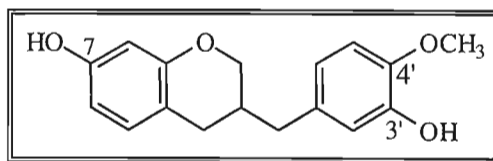
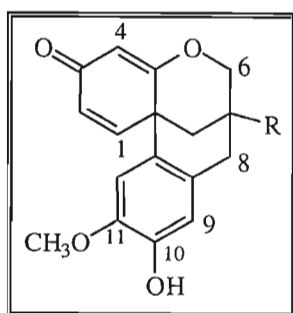


**Scheme 2.6.** Hypothesis for the formation of rotenoids lacking 3-hydroxylation<sup>24</sup>.

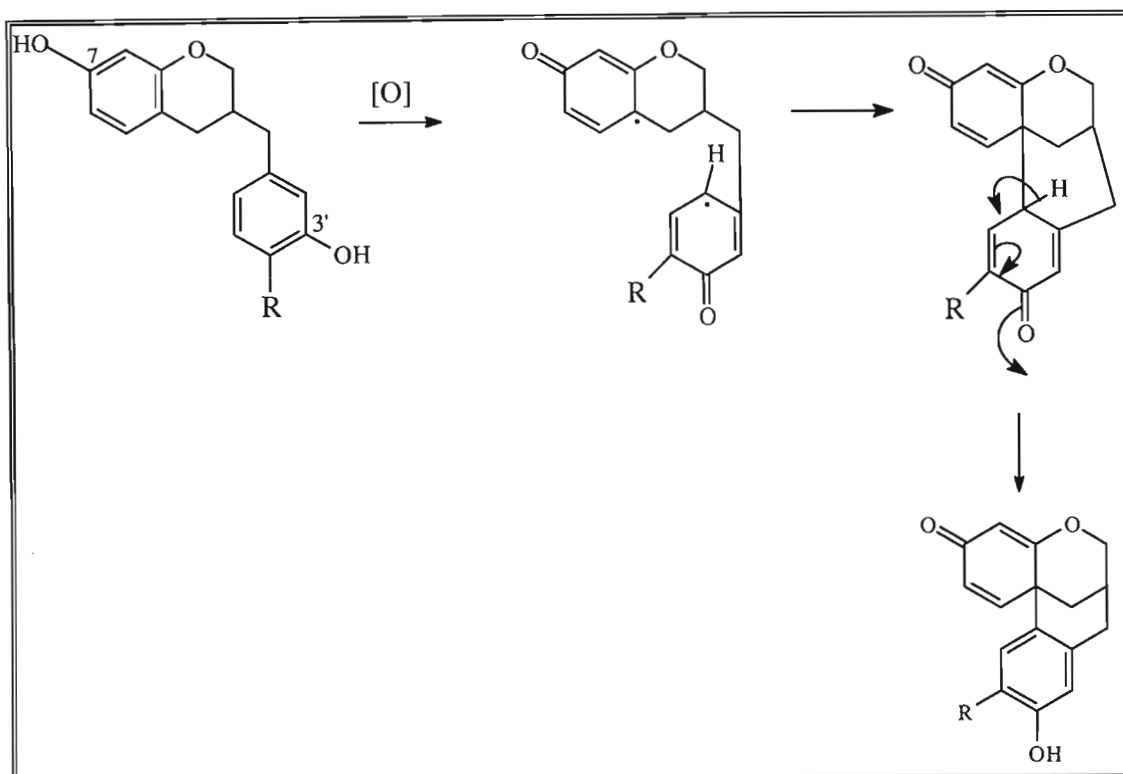


**Scheme 2.7.** Hypothesis for the formation of scillascilloids from 3-benzyl-4-chromanones<sup>24</sup>.

With regard to dracaenone type-compounds, they have been hypothesised to arise from 3-benzylchroman-type compounds by way of cyclisation via *para-para* phenolic oxidative coupling to a tetracyclic intermediate<sup>32</sup>. This intermediate then undergoes thermal suprafacial 1→5 sigmatropic hydrogen migration to the dracaenone skeleton as represented in scheme 2.8. The co-occurrence of 10-hydroxy-11-methoxydracaenone (**30**) and 7,10-dihydroxy-11-methoxydracaenone (**31**) with 7-hydroxy-3-(4-hydroxybenzyl)chroman (**1**) in *Dracaena loureiri*<sup>32</sup>, suggested that its close relative 7-hydroxy-3-(3-hydroxy-4-methoxybenzyl)chroman (**48**) isolated from a sister species *Dracaena cinnabari*<sup>10</sup>, may undergo the transformation presented in scheme 2.8.

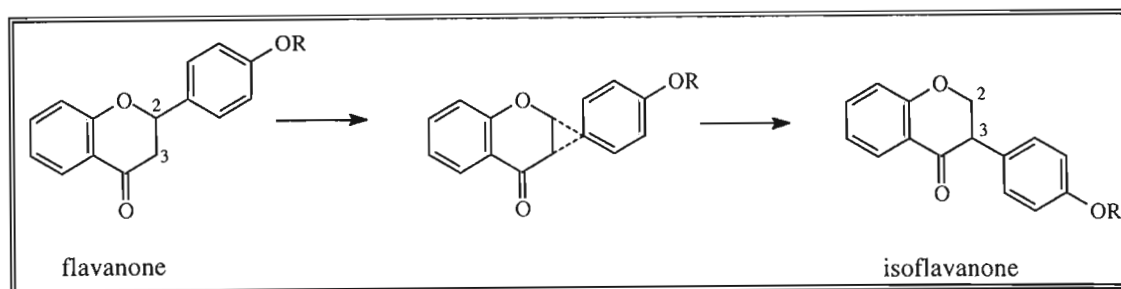
7-hydroxy-3-(4-hydroxybenzyl)chroman (**1**)7-hydroxy-3-(3-hydroxy-4-methoxybenzyl)chroman (**48**)10-hydroxy-11-methoxydracaenone (**30**) R = H7,10-dihydroxy-11-methoxydracaenone (**31**) R = OH



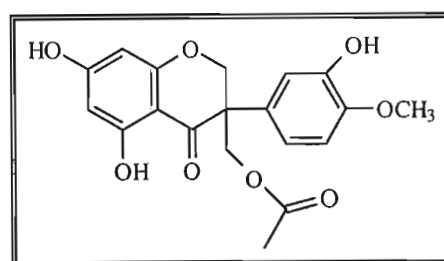


**Scheme 2.8.** Biogenesis of dracaenone-type homoisoflavonoids <sup>32</sup>

In summary, scheme 2.3 suggest that homoisoflavonoidal compounds are synthesized by modification of the C6.C3.C6 chalcone-flavanoid skeleton by insertion of an extra carbon atom from the 2'-methoxy group of 2'-methoxychalcone. In this scenario, a C6.C3 unit from phenylalanine is incorporated intact into the 3-benzyl-4-chromanone skeleton, becoming C-4,C-3,C-9 and the aromatic ring B, whilst another C6 unit comprising ring A is acetate/malonate (polyketide) derived. O-methylation as supplied by methionine provides the the C-2 of the heterocyclic ring C. Because of scheme 2.3, the name homoisoflavonoid has been dubbed a misnomer in that homoisoflavonoids are not formed by any step involving a 2,3-aryl shift (scheme 2.9) as is the case with isoflavonoids. The name benzylchromanoid which was subsequently coined is not correct either, especially in the light of dracaenone and comosin (**5**) type structures. Thus the term homoisoflavonoid, although “inappropriate and misleading” <sup>58</sup>, had been retained in this discussion because it encompasses every structural type within this series.



**Scheme 2.9.** The 2,3-aryl shift involved in isoflavanoid formation.



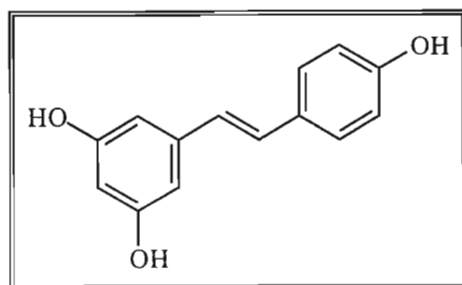
comosin (**5**)

## 2.2 Stilbenoids

### 2.2.1 Occurrence

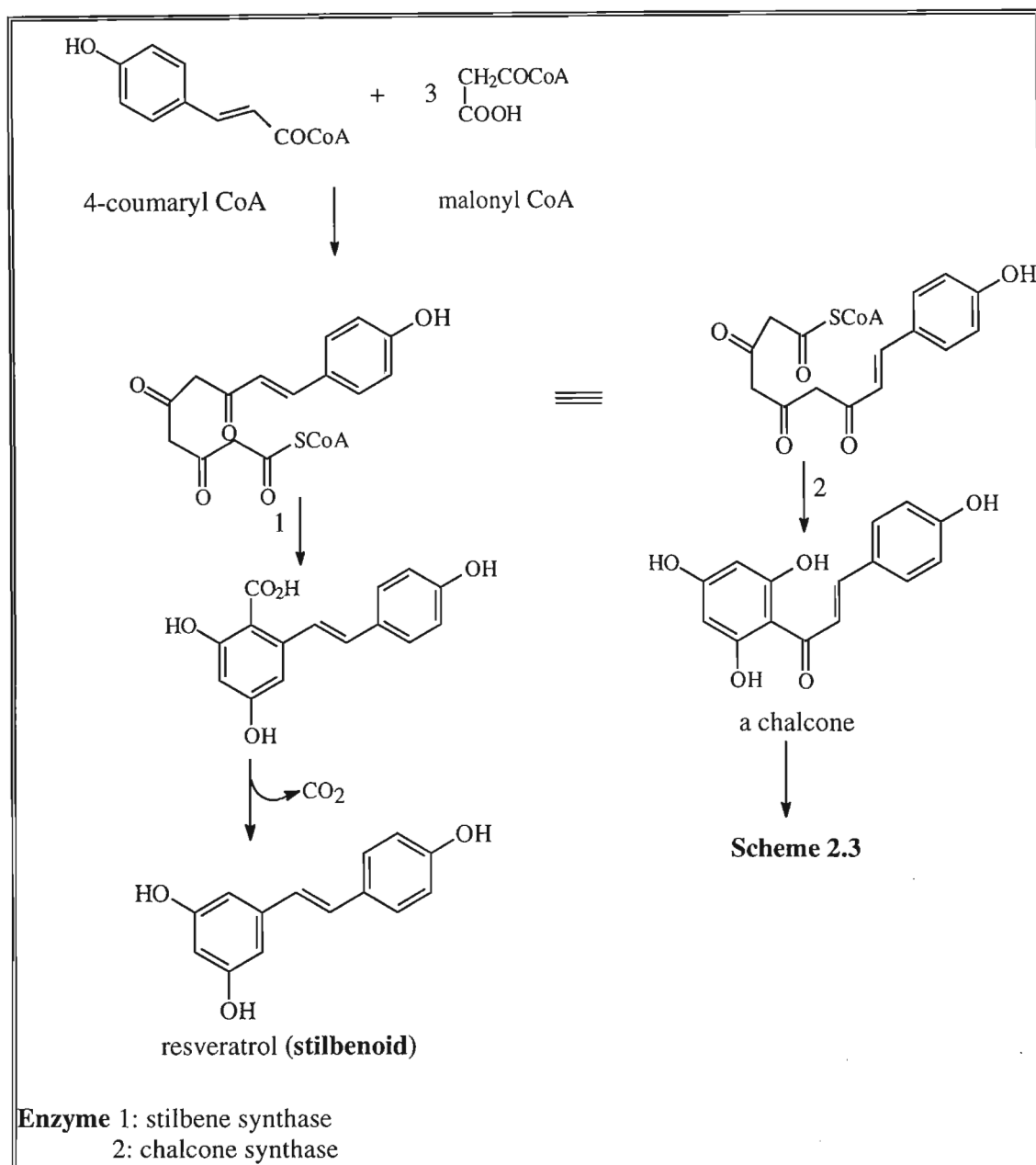
Stilbenoids have a wide distribution in the plant kingdom and have been previously reported from the following families: Poaceae<sup>95,102</sup>, Vitaceae<sup>55,97,103</sup>, Pinaceae<sup>98</sup>, Myristicaceae<sup>91</sup>, Polygonaceae<sup>89,91,93</sup>, Fabaceae<sup>96,98,101</sup>, Melanthiaceae<sup>94</sup> and Cyperaceae<sup>100</sup>. There have been no previous reports of stilbenoids in the genus *Scilla*. The isolation of stilbenoids from *Scilla* in this work represents the first report of these compounds not only in this genus but in the family Hyacinthaceae although they have been reported in the closely related family Melanthiaceae, previously included in the Liliaceae (*sensu lato*)<sup>94,107</sup>.

Stilbenoids have been isolated from wood, bark, leaves, seeds, stems and pods of the plants concerned and they occur either as free compounds or their glycosides. In some cases e.g. *Veratrum grandiflorum*<sup>94</sup> and *Arachis hypogea*<sup>96</sup> they have been found to be released in response to external stress stimuli. In such cases they act as phytoalexins, being a defence mechanism on the part of the plant. The formation of resveratrol dimers and trimers in grape (*Vitis vinifera*) has been linked to disease resistance as resveratrol (**104**) itself has been demonstrated to exhibit fungicidal and antioxidant properties<sup>55,95</sup>.

resveratrol (**104**)

### 2.2.2 Biosynthesis of stilbenoids

Scheme 2.10 below is a representation of a pathway leading to stilbenoid biosynthesis. It is evident from this scheme that stilbenoids are also synthesised from the same cinnamoyl CoA/malonyl CoA substrates that lead to homoisoflavonoid production via the same polyketide and shikimate pathways. The difference lies in the fact that, in stilbenoid synthesis, the enzyme-bound polyketide is folded differently and a decarboxylation occurs during cyclisation<sup>104,105</sup>. The enzyme involved is stilbene synthase. Since stilbenoids, like resveratrol (**104**), behave as phytoalexins that possess anti-fungal properties in some plants, the transfer of the gene coding for stilbene synthase to other plants lacking it should result in phytoalexin synthesis and subsequent enhanced disease resistance. This has been carried out by some workers where the gene from grape (*Vitis vinifera*) was transferred to tobacco plants which were later able to express the gene and showed increased resistance when challenged by the fungus *Botrytis cinerea*<sup>104,105,106</sup>.



**Scheme 2.10.** The pathway leading to the biosynthesis of stilbenoids <sup>104,105</sup>.

## **CHAPTER 3**

### **RESULTS AND DISCUSSION**

## 3.2 Structures of isolated compounds

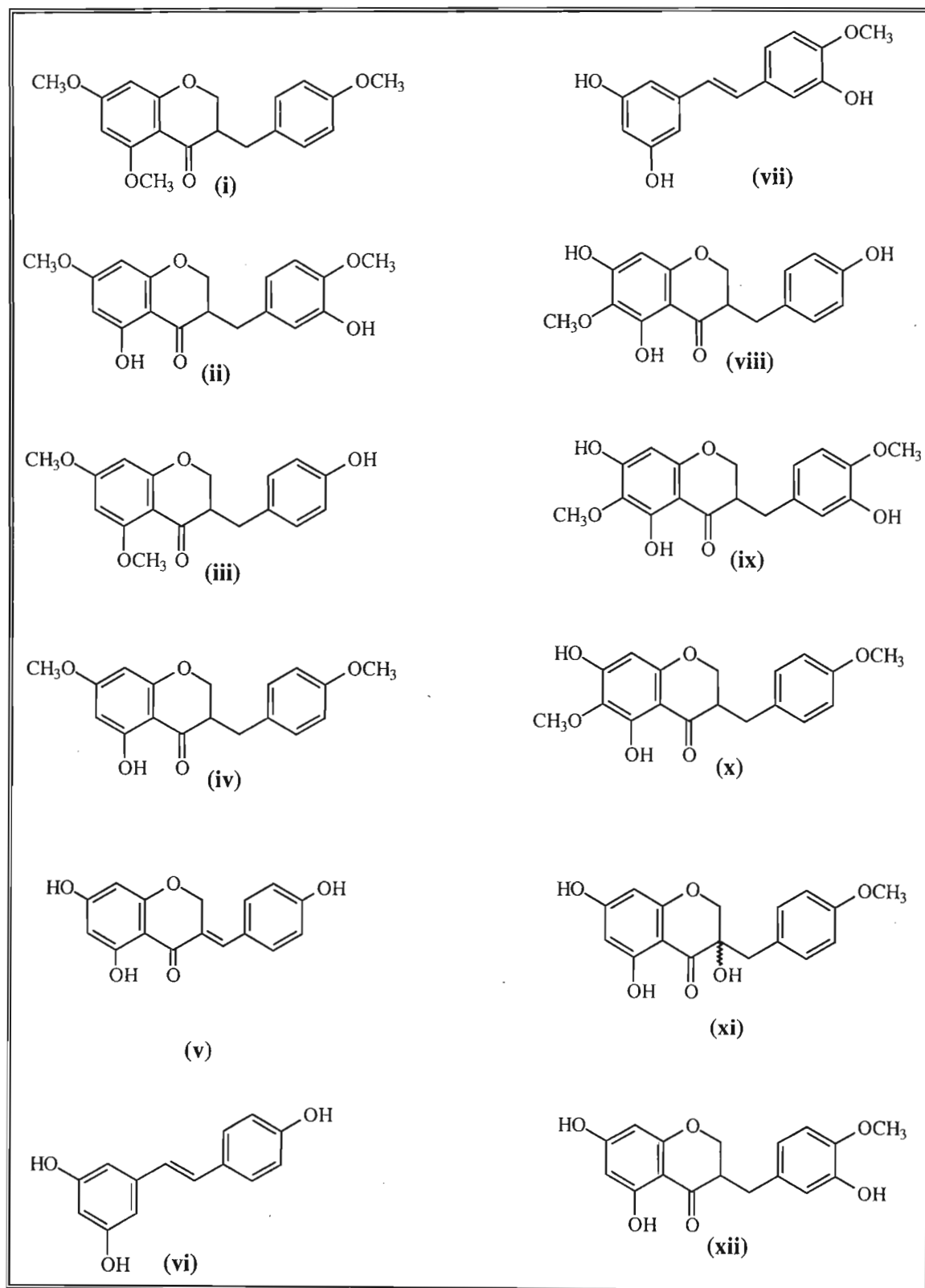


Chart 1

	i	ii	iii	iv	v	vi	vii	viii	ix	x	xi	xii
<i>S. nervosa</i> <sup>a</sup>	x	x	x		x	x	x					
<i>S. nervosa</i> <sup>b</sup>	x			x								
<i>S. natalensis</i> <sup>a</sup>								x				
<i>S. natalensis</i> <sup>b</sup>									x			
<i>S. d/montana</i>								x		x	x	
<i>S. kraussii</i>												x

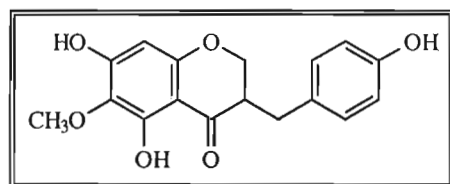
Chart 2

<sup>a</sup> - KwaZulu-Natal specimen

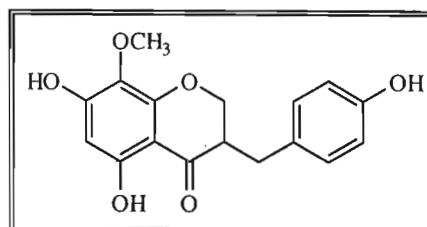
<sup>b</sup> - Mpumalanga specimen

Chart 1 is a representation of the compounds isolated from the *Scilla* species investigated in this work while Chart 2 gives an indication of which compounds have been isolated from the different species.

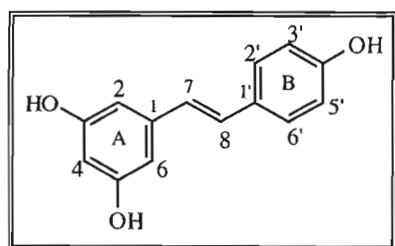
The following compounds are compounds which are going to be mentioned in the discussion and analysis of the results.



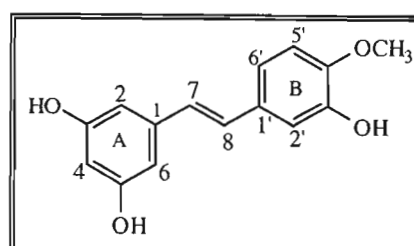
3,9-dihydroeucomnalin (76)



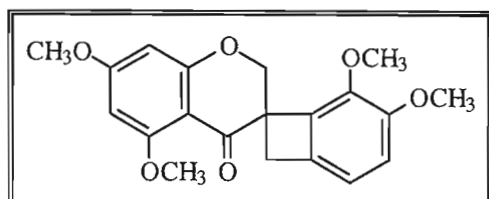
3,9-dihdropunctatin (79)



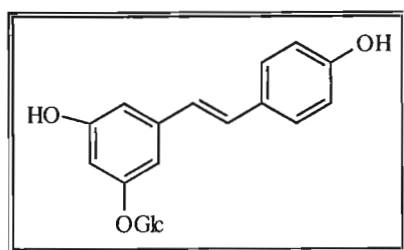
resveratrol (104)



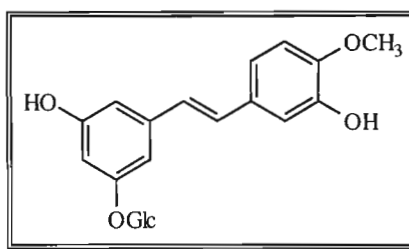
rhapontigenin (105)



compound (106)



piceid (107)



rhaponticin (108)

The 3-benzyl-4-chromanone structure (Fig. 3.1) of compounds (i), (ii), (iii), (iv), (viii), (ix), (x) and (xii) was deduced from the appearance in their  $^1\text{H}$  NMR spectra [Table 3.1] of the signals due to the  $-(2)\text{CH}_2-(3)\text{CH}-(9)\text{CH}_2-$  grouping. These were observed as a pair of double doublets within the chemical shift range : 4.10-4.32 ppm [AB of ABX,  $(2)\text{CH}_2$ ], one multiplet in the range : 2.72- 2.87 ppm [ $(3)\text{CH}$ ] and two double doublets in the ranges : 2.65-2.73 ppm and 3.10-3.26 ppm [ $(9)\text{CH}_2$ ] respectively. These chemical shifts agreed well with literature values <sup>4</sup> for 3-benzyl-4-chromanones. A COSY spectrum of compound (i) was run and it confirmed the expected coupling between these signals [spectrum 3]. Moreover, the long wave absorption maxima ( $\lambda_{\text{max}}$ ) recorded in the UV spectra for the 3-benzylchromanoids was in agreement with literature expectations as they ranged from 282 to 293nm while literature values lie between 285 and 290 nm <sup>17</sup>.

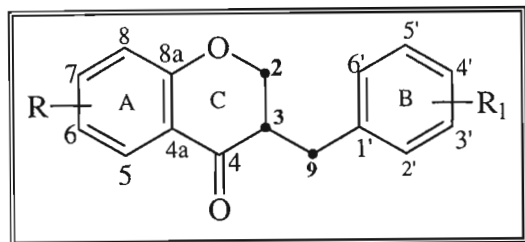
The 3-benzylidene-4-chromanone (i.e.  $\Delta^{3,9}$ ) structure (Fig. 3.2) of compound (v) was inferred from the appearance of the signals at  $\delta 5.35$  ppm and  $\delta 7.77$  ppm in the  $^1\text{H}$  NMR spectrum [spectrum 32a and 32b] corresponding to 2H-2 and H-9 respectively, and these were in agreement with the literature <sup>5,7,12,17</sup>. In addition  $\lambda_{\text{max}}$  was found to be 362 nm and this was also in agreement with what one would expect given the literature values of between 358 to 367 nm for this system <sup>17</sup>.

The stilbene nature (Fig 3.3) of compounds (vi) and (vii) was derived from comparison with literature data <sup>89,90,92,96,116</sup>.

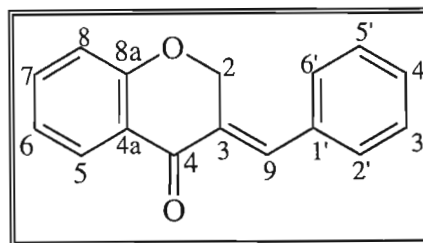
The molecular formulae of the compounds isolated were derived from molar masses determined by high resolution mass spectrometry (hrms). The formula :  $\text{db} = 2m + 2 - n/2$  was used to derive the double bond equivalence (db) based on the molecular



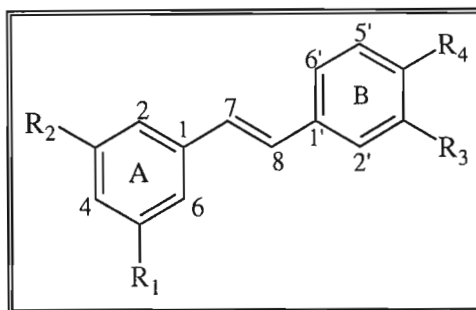
formula, where  $m$  stands for the number of carbon atoms and  $n$  for the number of hydrogen atoms in the molecular formula. The db formula is based on the notion that the presence of oxygen as the heteroatom in the molecules neither adds nor reduces the number of hydrogen atoms <sup>109</sup>.



**Fig. 3.1.** 3-benzyl-4-chromanone



**Fig. 3.2.** 3-benzylidene-4-chromanone



**Fig. 3.3.** stilbenoid structure

Tables 3.1 and 3.2 are a summary of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR data for isolated homoisoflavonoids while Tables 3.3 and 3.4 presents  $^1\text{H}$  and  $^{13}\text{C}$  NMR data for stilbenoids. Use was made of reported literature values <sup>1-7,12,15,16,17,90,100,116</sup> in the compilation of the tables. In addition, other tabular comparisons with literature values have been provided in the discussion where appropriate. All numerical values in parentheses in the  $^1\text{H}$  NMR tables in this section and subsequent sections are coupling constants in Hz.

**Table 3.1.**  $^1\text{H}$  NMR (300MHz,  $\text{CD}_3\text{OD}$ , TMS as internal standard) data of homoisoflavonoidal compounds. All chemical shift ( $\delta$ ) values are expressed in ppm.

Cpd	2H-2	H-3	H-6	H-8	2H-9	H-2'	H-6'	H-3'	H-5'	Other signals
(i)	4.11, 4.29 AB of ABX (3.8, 6.6, 11.4)	2.72 m	6.20d	6.15d	2.68dd (9.8, 12.6) 3.10dd (3.6, 12.6)	7.17d AA'BB' (8.7)		6.89d AA'BB' (8.7)		3.80s 5-OCH <sub>3</sub> # 3.86s 7-OCH <sub>3</sub> # 3.86s 4'-OCH <sub>3</sub> #
(ii)	4.14, 4.31 AB of ABX (4.2, 7.2, 11.4)	2.87 m	6.07d	6.03d	2.67dd (10.2, 13.8) 3.10dd (3.6, 13.8)	6.75d (2.1)	6.71dd (8.1)	-	6.89d (8.1)	3.84s 7-OCH <sub>3</sub> 3.86s 4'-OCH <sub>3</sub>
(iii) $\phi$	4.15, 4.32 AB of ABX (4.2, 7.2, 11.4)	2.82 m	6.23d	6.18d	2.72m 3.16m	7.31d AA'BB' (8.4)		7.07d AA'BB' (8.4)		2.30s 4'-OAc 3.86s 5-OMe 3.86s 7-OMe
(iv)	4.13, 4.31 AB of ABX (4.2, 7.2, 11.4)	2.88 m	6.07d	6.03d	2.72dd (9.9, 13.5) 3.17dd (4.5, 13.8)	7.19d AA'BB' (8.7)		6.90d AA'BB' (8.7)		3.84s 7-OCH <sub>3</sub> 3.81s 4'-OCH <sub>3</sub>
(v)	5.35d (1.8)	-	5.93d	5.87d	7.77t (1.8)	7.30d AA'BB' (8.7)		6.92d AA'BB' (8.4)		-
(viii)	4.10, 4.26 AB of ABX (4.2, 7.2, 11.4)	2.83 m	-	5.94s	2.67dd (10.2, 13.5) 3.13dd (4.2, 13.8)	7.09d AA'BB' (8.4)		6.78d AA'BB' (8.4)		3.81s 6-OCH <sub>3</sub>
(ix)	4.10, 4.2 AB of ABX (4.2, 7.2, 11.4)	2.84 m	-	5.95s	2.66dd (10.2, 13.5) 3.11dd (4.5, 13.5)	6.75d (2.1)	6.70dd (2.1) (8.1)	-	6.89d (8.1)	3.86s 4'-OCH <sub>3</sub> 3.81s 6-OCH <sub>3</sub>
(x)	4.10, 4.27 AB of ABX (4.5, 6.9, 11.4)	2.85 m	-	5.95s	2.73dd (9.9, 13.5) 3.26dd (4.5, 13.5)	7.19d AA'BB' (8.7)		6.90d AA'BB' (8.7)		3.81s 4'-OCH <sub>3</sub> 3.81s 6-OCH <sub>3</sub>
(xi)	4.08d 3.92d (11.4)	-	5.95d	5.92d	2.96d (3.3)	7.20 AA'BB' (8.7)		6.88 AA'BB' (8.7)		3.81s 4'-OCH <sub>3</sub>
(xii)	4.11, 4.28 AB of ABX (4.2, 7.2, 11.4)	2.82 m	5.91d	5.88d	2.65dd (10.2, 13.5) 3.11dd (4.5, 13.8)	6.75d (2.1)	6.71dd (1.8) (8.1)	-	6.89d (8.1)	3.87s 4'-OCH <sub>3</sub>

**Note:** This table has been compiled by comparison to the literature <sup>1-7, 12, 15</sup>

# - values interchangeable

(iii) $\phi$  - compound (iii) acetate

**Table 3.2.**  $^{13}\text{C}$  NMR data of homoisoflavonoidal compounds (75MHz,  $\text{CD}_3\text{OD}$ , TMS as internal standard). All chemical shift ( $\delta$ ) values are expressed in ppm.

C	(i)	(ii)	(iii) $\phi^*$	(iv)	(v)	(viii)	(ix)	(x)	(xi)*	(xii)*
2	70.1	70.4	70.1	70.4	68.6	70.3	70.3	70.3	72.9	70.4
3	$\xi$	$\xi$	$\xi$	$\xi$	127.0	$\xi$	$\xi$	$\xi$	73.5	$\xi$
4	193.9	199.8	193.7	199.8	186.5	200.2	200.1	200.1	199.8	199.4
4a	106.1	103.6	106.1	103.8	103.8	103.1	103.0	103.0	101.4	102.9
5	164.0	165.6	164.1	165.6	164.0	156.9	156.8	156.5	164.4	165.8
6	93.8	95.9	93.9	95.8	97.4	130.2	130.5	131.4	97.6	97.1
7	168.0	169.4	168.1	170.0	169.4	160.5	160.8	160.7	168.9	168.3
8	94.6	94.6	94.6	94.6	96.0	95.9	95.8	95.8	96.3	95.9
8a	166.6	164.6	166.7	164.6	160.7	160.1	160.1	160.1	160.3	164.7
9	33.3	33.1	33.4	32.9	138.0	33.0	33.2	32.9	40.7	33.1
1'	131.8	132.3	130.5	131.4	128.4	130.2	132.3	131.4	128.2	132.4
2'	131.1	117.1•	131.2	131.2	133.6	131.2	117.1•	131.2	132.8	117.1•
3'	115.0	147.9 $\phi$	122.9	115.1	116.8	116.4	147.9 $\phi$	115.1	114.5	147.9 $\phi$
4'	159.9	147.7 $\phi$	137.7	160.0	160.7	157.3	147.7 $\phi$	160.0	160.3	147.7 $\phi$
5'	115.0	113.0•	122.9	115.1	116.8	116.4	113.0•	115.1	114.5	113.0•
6'	131.1	121.4	131.2	131.2	133.6	131.2	121.4	131.2	132.8	121.4
5-OCH <sub>3</sub>	56.3	–	56.3	–	–	–	–	–	–	–
6-OCH <sub>3</sub>	–	–	–	–	–	61.0	61.0	61.0	–	–
7-OCH <sub>3</sub>	56.2	56.5	56.3	55.7	–	–	–	–	–	–
4'-OCH <sub>3</sub>	55.7	56.3	–	56.3	–	–	56.5	55.7	55.7	56.5
4'-OAc	–	–	171.4 <sup>a</sup> 20.9 <sup>b</sup>	–	–	–	–	–	–	–

**Note :** The chemical shift data in Table 3.2 has been compiled with reference to literature<sup>1-7, 13, 15, 16, 57</sup>

•,  $\phi$  - values are interchangeable;  $\xi$  - signal hidden under solvent peaks

<sup>a</sup> - resonance due to the carbonyl group; <sup>b</sup> - resonance due to the methyl group

\* - data collected at 125 MHz using a Varian Innova 500 MHz instrument

**Table 3.3.**  $^1\text{H}$  NMR data of stilbenoidal compounds (300MHz,  $\text{CD}_3\text{OD}$ , TMS as internal standard). All chemical shift ( $\delta$ ) values are expressed in ppm.

Cpd	H-2	H-4	H-6	H-7	H-8	H-2'	H-6'	H-3'	H-5'	Other
(vi)	6.48d (2.1)	6.19t (2.1)	6.48d (2.1)	6.86d (16.2)	6.97d (16.2)	7.39d AA'BB' (8.7)		6.80d (AA'BB') (8.7)		-
(vii)	6.48d (2.1)	6.20d (2.1)	6.48d (2.1)	6.95d (16.5)	6.82d (16.5)	7.04d (1.8)	6.97dd (1.8) (8.7)	-	6.93d (8.7)	3.90s 4'-OMe

**Table 3.4.**  $^{13}\text{C}$  NMR data of stilbenoidal compounds (75 MHz,  $\text{CD}_3\text{OD}$ , TMS as internal standard). All chemical shifts ( $\delta$ ) are expressed in ppm.

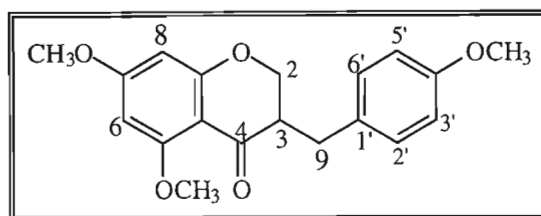
C	(vi)	(vii)
1	141.3	141.1
2	105.8	105.9
3	159.7	159.7
4	102.7	102.8
5	159.7	159.7
6	105.8	105.9
7	129.4	129.4
8	127.0	127.9
1'	130.4	132.2
2'	128.8	113.6
3'	116.5	149.0
4'	158.4	147.7
5'	116.5	112.7
6'	128.8	120.0
4'- OCH <sub>3</sub>	-	56.4

**Note :** data in Tables 3.3 and 3.4 collated with reference to the literature<sup>89-91, 97,116</sup>

### 3.2 Extractives from *Scilla nervosa* (Burch.) Jessop

From the hexane and chloroform extracts of *S. nervosa* bulbs (KZN) compounds (i) and (ii) were isolated while compounds (iii), (v), (vi) and (vii) were isolated from the ethyl acetate extract. Compounds (i) and (iv), were isolated from the hexane extract of *S. nervosa* (Mpl).

#### 3.2.1 Structural elucidation of compound (i)



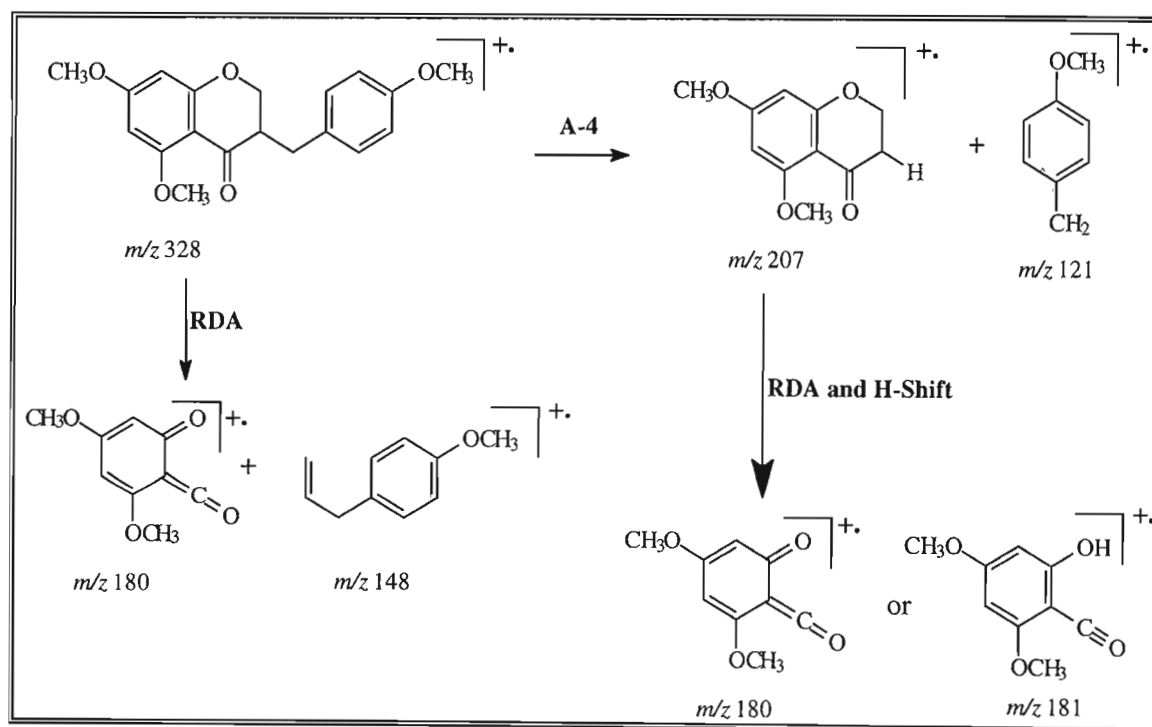
compound (i)

Compound (i) was found to have a molar mass of  $328.1311 \text{ g.mol}^{-1}$  (calculated  $328.1306 \text{ g.mol}^{-1}$ ). The molecular formula  $\text{C}_{19}\text{H}_{20}\text{O}_5$  was deduced. From the molecular formula a double bond equivalence of 10 was deduced using the formula mentioned in section 3.1 above.

The  $^1\text{H}$  NMR spectrum [spectrum 1] revealed that compound (i) had three methoxy groups. Substitution in ring A was deduced from mass,  $^1\text{H}$  NMR and UV spectra. The presence of two methoxy groups in ring A was deduced from the occurrence of peaks at  $m/z$  180 and 181 in the mass spectrum [spectrum 4] due to *retro*-Diels-Alder (RDA) cleavage and hydrogen shift of a chromanone fragment at  $m/z$  207, which was itself generated by an A-4 type cleavage of the molecular ion [Scheme 3.1]<sup>17</sup>. The hydride shift from C-2 to ring A which results in the peak at  $m/z$  181 is a well known behaviour of flavonoids in general<sup>17</sup>. The absence of free 5-hydroxy and 7-hydroxy groups was also deduced from the fact that no bathochromic shifts were observed in the UV spectra in the presence of  $\text{AlCl}_3$  and  $\text{NaOAc}$  respectively [spectra 7 and 8]<sup>1,2,29</sup>. The appearance of the H-6 and H-8 resonances downfield of 6.0 ppm [Tables 3.1], lends further support to the presence of a methoxy group C-7, as these protons absorb upfield of 6.0 ppm in the case of a free hydroxy group at C-7<sup>15</sup>. In the  $^{13}\text{C}$  NMR spectrum

[**spectrum 2**] the chemical shift  $\delta 193.9$  ppm due to the carbonyl carbon at C-4 was noticed to be significantly upfield of the norm (the norm being close to  $\delta 200$  ppm) relative to other homoisoflavonoids having a free 5-hydroxy group [**Table 3.2**]. The absence of the chelation between the 5-hydroxy group and the C-4 carbonyl group should explain the upfield shift of the C-4 resonance in this compound because the chelation imparts a deshielding effect on the carbonyl group. The carbon chemical shift value assigned to C-4 agreed well with those of other homoisoflavonoids having a methoxy substituent at C-5<sup>4,6</sup>.

With regard to substitution in ring B, the appearance of an AA'BB' system revealed a *para* disubstituted benzene ring [**spectrum 1 and Table 3.1**]. The presence of a methoxy group as the 4' substituent in this ring, was revealed by the presence of an intense peak at  $m/z$  121 in the mass spectrum [**spectrum 4**], corresponding to a methoxybenzyl/tropylium ion as represented in scheme 3.1.



**Scheme 3.1.** Fragmentation pattern of compound (i)<sup>17</sup>

[Scheme 3.1 serves as a representative scheme for related compounds]

The <sup>1</sup>H NMR data of compound (i) was compared to the literature data<sup>26</sup> for a synthetic analog [**Table 3.5**]. It became evident from this comparison that the resonances as well

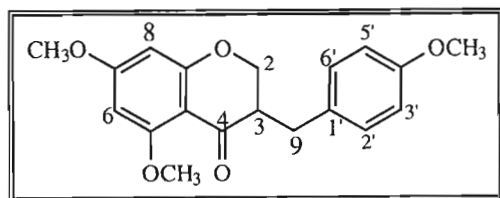
as respective coupling constants correlated well although data was recorded in  $\text{CDCl}_3$  in the case of the synthetic compound.

The carbon resonances presented in Table 3.2 were assigned by comparison with reported chemical shift values for other homoisoflavonoids <sup>1-7,15</sup>. The allocation of chemical shifts to ring A carbons in particular, was done in comparison with a synthetic scillascillin type compound (**104**) having a similar ring A substitution pattern to that suggested for compound (**i**) <sup>6</sup> [Table 3.6] because no <sup>13</sup>C NMR data for simple 3-benzyl-4-chromanones with the same substitution pattern could be found in the literature. From these results it was concluded that compound (**i**) was 5,7-dimethoxy-3-(4-methoxybenzyl)chroman-4-one, a novel compound.

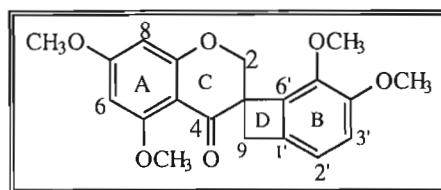
**Table 3.5.** <sup>1</sup>H NMR data of compound (**i**) compared to literature

Proton(s)	( <b>i</b> ) ( $\text{CD}_3\text{OD}$ )	( <b>i</b> ) ( $\text{CDCl}_3$ ) <sup>26</sup>
2H-2	4.11, 4.29 AB of ABX (3.8, 6.6, 11.4)	4.13, 4.31 AB of ABX (4.1, 6.5, 11.4)
H-3	2.72m	2.70m
H-6	6.20d AB (2.4)	6.07d AB (2.3)
H-8	6.15d	6.06d
2H-9	2.68dd, (9.8, 12.6) 3.10dd, (3.6, 12.6)	2.70m 3.20m
H-2'/H-6'	7.17d AA'BB' (8.7)	7.16 AA'BB' (8.8)
H-3'/H-5'	6.89d AA'BB' (8.7)	6.85 AA'BB' (8.8)
5-OCH <sub>3</sub>	3.81s#	3.89s
7-OCH <sub>3</sub>	3.86s#	3.82s
4'-OCH <sub>3</sub>	3.86s#	3.79s

# - values can be interchanged



compound (i)



compound (106)

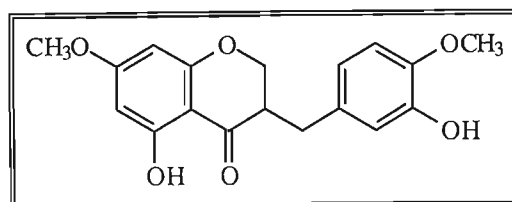
Table 3.6.  $^{13}\text{C}$  NMR data of compound (i) and (106)

C	(i) ( $\text{CD}_3\text{OD}$ )	(106) $^6(\text{CD}_3\text{OD})$
2	70.1	75.0
3	$\xi$	56.8
4	193.9	192.3
4a	<b>106.1</b>	<b>105.9</b>
5	<b>164.0</b>	<b>164.3</b>
6	<b>93.8</b>	<b>94.0</b>
7	<b>168.0</b>	<b>168.3</b>
8	<b>94.6</b>	<b>94.8</b>
8a	<b>166.6</b>	<b>166.9</b>
9	33.3	37.8
1'	131.8	136.5
2'	131.1	118.9
3'	115.0	116.1
4'	159.9	151.5
5'	115.0	146.0
6'	131.1	130.3
5-OCH <sub>3</sub>	56.3	56.3
7-OCH <sub>3</sub>	56.2	56.3
4'-OCH <sub>3</sub>	55.7	57.2
5'-OCH <sub>3</sub>	-	59.2

$\xi$  - hidden under solvent peaks



### 3.2.2 Structural elucidation of compound (ii)



compound (ii)

Compound (ii), was found to have a molar mass of  $330.1092 \text{ g.mol}^{-1}$  (calculated  $330.1093 \text{ g.mol}^{-1}$ ). This was consistent with the molecular formula  $\text{C}_{18}\text{H}_{18}\text{O}_6$ . A double bond equivalence of 10 was deduced.

The  $^1\text{H}$  NMR spectrum of compound (ii) was found to be very similar to that of compound (xii) isolated from *Scilla kraussii* [spectra 9 and 87 and Table 3.7]. The differences were that two methoxy groups appeared in the spectrum of compound (ii) in contrast to one for compound (xii). Moreover, the H-6 and H-8 proton signals appeared downfield of 6.00 ppm whereas those for (xii) appeared upfield of 6.00 ppm [Table 3.7]. Based on this comparison, it was presumed that compound (ii) was a methylated derivative of the known <sup>2,15</sup> compound (xii)[72]. Table 3.7 also lists the literature  $^1\text{H}$  NMR data of this known compound (xii) [72] <sup>2,15</sup>. To prove this assumption the mass, UV and proton spectra were analysed closely. The mass spectrum [spectrum 12] revealed peaks at  $m/z$  193, 166 and 167 corresponding to A-4, RDA and H-shift fragments. This gave an indication that ring A of compound (ii) carried one methoxy and one hydroxy group. A bathochromic shift (+24 nm) with  $\text{AlCl}_3$  [spectrum 15] and no shift with  $\text{NaOAc}$  [spectrum 16] in UV spectral measurements were indicative of a 5-hydroxy-7-methoxy substituted ring A. Furthermore, the appearance of the resonance due to H-6 and H-8 protons downfield of 6.00 ppm was also an indication of the presence of a methoxy group at C-7 <sup>15</sup>.

The  $^1\text{H}$  NMR spectrum revealed an aromatic ABX system [ $\delta 6.89 \text{ ppm}$  (d,  $J = 8.1 \text{ Hz}$ , H),  $\delta 6.71 \text{ ppm}$  (dd,  $J = 2.1, 8.1 \text{ Hz}$ , 1H),  $\delta 6.75 \text{ ppm}$  (d,  $J = 2.1 \text{ Hz}$ , 1H)] [Table 3.7] thus giving a clue that ring B was trisubstituted. One methoxy group and one hydroxy group were attached to this ring as revealed by the presence of a base peak at  $m/z$  137

corresponding to a hydroxy- methoxybenzyl/tropylium ion in the mass spectrum. The position of the methoxy group in this ring was deduced from NOE experiments. Upon irradiation of the doublet at  $\delta 6.89$  ppm [**spectra 11a** and **11b**], a positive NOE effect was observed for one of the methoxy group proton signals at  $\delta 3.86$  ppm and the double doublet signal at  $\delta 6.71$  ppm. From this result it was concluded that the signals at  $\delta 6.89$  ppm and  $\delta 3.86$  ppm were due to H-5' and protons of the methoxy group at 4' position respectively. The double doublet signal centred at  $\delta 6.71$  ppm was assigned to H-6'.

**Table 3.7.**  $^1\text{H}$  NMR data of compounds (ii) and (xii)[72]

Proton(s)	(ii) ( $\text{CD}_3\text{OD}$ )	(xii) ( $\text{CD}_3\text{OD}$ )	(72) [xii] $^{15}(\text{CD}_3\text{OD})$
2H-2	4.14, 4.31 AB of ABX (4.2, 7.2, 11.4)	4.11, 4.28 AB of ABX (4.2, 7.2, 11.4)	4.06, 4.23 AB of ABX (4.4, 7.3, 11.4)
H-3	2.87m	2.82m	2.78m
H-6	6.07d AB (2.4)	5.91d AB (2.1)	5.86d AB (2.2)
H-8	6.03d AB (2.4)	5.88d AB (2.1)	5.83d AB (2.2)
2H-9	2.67dd, (10.2, 13.8) 3.10dd, (3.6, 13.8)	2.65dd, (10.2, 13.5) 3.11dd, (3.6, 13.8)	2.60dd, (10.3, 13.6) 3.06dd, (4.0, 13.6)
H-2'	6.75d, (2.1)	6.75d, (2.1)	6.70d, (1.8)
H-5'	6.89d, (8.1)	6.89d, (8.1)	6.84d, (8.1)
H-6'	6.71dd, (1.8, 8.1)	6.71dd, (1.8, 8.1)	6.66d, (1.8, 8.1)
7-OCH <sub>3</sub>	3.84s	-	-
4'-OCH <sub>3</sub>	3.86s	3.87s	3.82s

The carbon shift assignments [Tables 3.2] were made with comparison to literature data especially relative to those reported for compound (xii) [72] <sup>4,15</sup>. Noteworthy in this comparison [Table 3.8], were the chemical shifts due to C-6 and C-8. In compound (ii) these carbons have been assigned shifts which are 1.2 ppm downfield to those of compound (72). This has to be expected if one bears in mind what Adinolfi and co-workers <sup>15</sup> found regarding the effect a methoxylated C-7 has on the chemical shifts of

the two *ortho* carbons, i.e. 'the resonances of the unsubstituted C-6 and C-8 should appear at 97.1-97.3 and 95.8-96 ppm respectively when C-7 carries a hydroxy group, and at higher field (-1,3 ppm), when C-7 carries a methoxy group'. For C-6, this means one subtracts 1.3 from 97.1 to get 95.8 ppm, and the value allocated to C-6 is equal to 95.9 ppm. The same applies to C-8 where a value of 94.6 ppm has been assigned and this is in good agreement with the expected value of 94.5 ppm if one takes Adinolfi and co-workers' substituents effects into consideration.

From this analysis it was concluded that compound **(ii)** was the 7-O-methyl derivative of the known compound **(72)**<sup>2,4</sup>. It was thus concluded that compound **(ii)** was 5-hydroxy-7-methoxy-3-(3-hydroxy-4-methoxybenzyl)chroman-4-one, a novel compound.

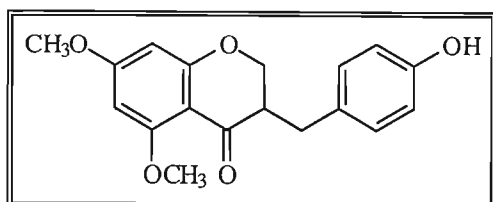
**Table 3.8.**  $^{13}\text{C}$  NMR data of compound (ii) and (72)

C	(ii) ( $\text{CD}_3\text{OD}$ )	(72) $^{15}\text{C}$ ( $\text{CD}_3\text{OD}$ )
2	70.4	70.2
3	$\xi$	47.9
4	199.8	199.3
4a	103.6	102.8
5	165.6	165.8
6	<b>95.9</b>	<b>97.1</b>
7	169.4	168.2
8	<b>94.6</b>	<b>95.8</b>
8a	164.6	164.7
9	33.1	33.1
1'	132.3	132.4
2'	117.1•	117.0
3'	147.9 $\phi$	147.9
4'	147.7 $\phi$	147.6
5'	113.0•	113.0
6'	121.4	121.4
7-OCH <sub>3</sub>	56.5	56.8
4'-OCH <sub>3</sub>	56.3	-

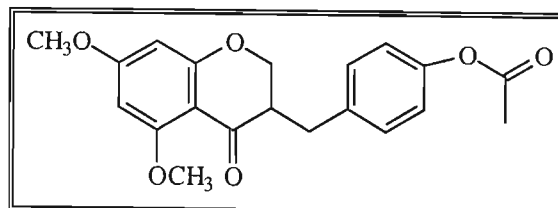
$\xi$  - hidden under solvent peaks

•,  $\phi$  - values can be interchanged

### 3.2.3 Structural elucidation of compound (iii)



compound (iii)



compound (iii) acetate

This compound was isolated after an acetylation step (Chapter 4) due to its co-elution with other compounds despite the use of different solvent systems. The discussion of its

structure is based on its acetyl derivative since there were only minute amounts available preventing deacetylation. The acetyl derivative had a molar mass of 356.1270 g.mol<sup>-1</sup> (calculated 356.1260 g.mol<sup>-1</sup>) corresponding to the molecular formula C<sub>20</sub>H<sub>20</sub>O<sub>6</sub>. A double bond equivalence of 11 was deduced.

The <sup>1</sup>H NMR spectrum [**spectrum 17**] revealed that compound (**iii**) acetate had two methoxy groups (s, 3.86 ppm, 6H) and one acetyl group (s, 2.30 ppm, 3H). The resonances due to H-6 and H-8 were observed to be downfield of 6.00 ppm [**Table 3.1**] implying that one of the methoxy groups was at C-7<sup>15</sup>. The absence of bathochromic shifts in the UV spectrum with NaOAc and AlCl<sub>3</sub> suggested the presence of either a methoxy or acetoxy group at the 7- or 5-position [**spectra 23** and **24**]. The nature of the substitution pattern in ring A was gathered from the mass spectrum [**spectrum 20**]. The appearance of relatively intense peaks at *m/z* 180 and 181 due to RDA and H-shift fragmentations similar to those for compound (**i**) as presented in scheme 3.1, gave evidence of the existence of a dimethoxy substituted ring A. The <sup>13</sup>C NMR spectrum also revealed a similar situation as in compound (**i**) with regard to the chemical shift of the carbonyl group at C-4 in that this shift occurred at δ193.7 ppm relative to those which are very close to δ200.0 ppm in cases where this group is under the influence of a deshielding chelation from the neighbouring 5-hydroxyl group [**Table 3.2**]. The resonances assigned to ring A carbons were assigned with comparison to those of compound (**i**) [**Table 3.9**]. In the <sup>13</sup>C NMR spectrum of compound (**iii**) acetate there were signals which were ascribed to the breakdown product of the acetate and these have been crossed in the spectrum [**spectrum 18**].

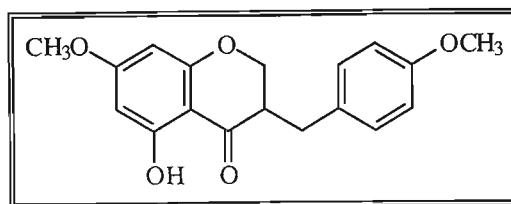
Substitution in ring B was deduced from the appearance of an AA'BB' system in the <sup>1</sup>H NMR spectrum. The downfield location of these signals when compared to other homoisoflavonoids having a 4'-hydroxy or 4'-methoxy group in this ring [**Table 3.1**] was an indication that the acetoxy group was attached at the 4'-position. A peak at *m/z* 152 in the mass spectrum corresponding to an acetoxybenzyl ion was also evidence for the 4' derivative. From these results it was concluded that the original compound (**iii**) was 5,7-dimethoxy-3-(4-hydroxybenzyl)chroman-4-one, a novel compound.

**Table 3.9.**  $^{13}\text{C}$  NMR data of compound (i) and (iii)

C	(i) ( $\text{CD}_3\text{OD}$ )	(iii) ( $\text{CD}_3\text{OD}$ )
2	70.1	70.1
3	$\xi$	$\xi$
4	193.9	193.7
4a	106.1	106.1
5	164.0	164.1
6	93.8	93.9
7	168.0	168.1
8	94.6	94.6
8a	166.6	166.7
9	33.3	33.4
1'	131.8	130.5
2'	131.1	131.2
3'	115.0	122.9
4'	159.9	137.7
5'	115.0	122.9
6'	131.1	131.2
5-OCH <sub>3</sub>	56.3	56.4
7-OCH <sub>3</sub>	56.2	56.4
4'-OCH <sub>3</sub>	55.7	
4'- <u>OCO</u> CH <sub>3</sub>		171.4
4'- <u>OCO</u> CH <sub>3</sub>		21.0

$\xi$  - hidden under solvent peaks

### 3.2.4 Structural elucidation of compound (iv)



compound (iv)[71]

Compound (iv), was found to have a molar mass of  $314.1154 \text{ g.mol}^{-1}$  (calculated  $314.1158 \text{ g.mol}^{-1}$ ) corresponding to the molecular formula  $\text{C}_{18}\text{H}_{18}\text{O}_5$ . A double bond equivalence of 10 was deduced.

This compound was found to be the known compound 7-O-methyl-3,9-dihydroeucomin (71), which was previously reported in *Eucomis bicolor*<sup>17,26</sup>. The long wave absorption maximum in the UV spectrum was located at 288 nm ( $\log \epsilon$  4.43) [spectrum 29] while the literature value was 288.5 (4.306)<sup>26</sup>.

The  $^1\text{H}$  NMR spectrum [spectrum 25] revealed that compound (iv) had two methoxy groups. Substitution in ring A was deduced from mass, proton and UV spectra. The existence of a hydroxy group at C-5 was revealed by a bathochromic shift (+23 nm) in the UV spectrum with  $\text{AlCl}_3$  [spectrum 30]. No shift was observed with  $\text{NaOAc}$  [spectrum 31] suggesting that a methoxy group occurred at C-7, an observation which was also corroborated by the appearance of resonances due to H-8 and H-6 downfield of 6.00 ppm [Table 3.1]<sup>15</sup>. In the mass spectrum [spectrum 27], the presence of low intensity peaks at  $m/z$  166 and 167 corresponding to RDA and H-shift fragments of the chromanone ion also confirmed this substitution in ring A.

Substitution in ring B was deduced from the appearance of the AA'BB' system signals [Table 3.1] in the  $^1\text{H}$  NMR spectrum indicating that it was *para* disubstituted. The methoxy group was the substituent in this ring as revealed by the presence of a base peak at  $m/z$  121 corresponding to a methoxybenzyl/tropylium ion.

The  $^1\text{H}$  NMR data of compound (iv) was finally compared to literature data [Table

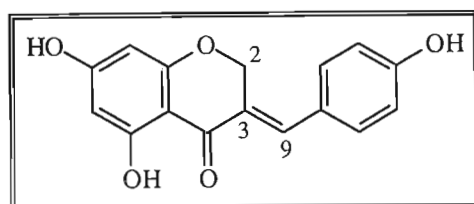
**3.10]** where it was found that the literature values correlated very well with the experimental values. Since there was no  $^{13}\text{C}$  NMR data available in the literature <sup>26</sup> for this compound the carbon resonances in Table 3.2 were assigned with comparison to data for other homoisoflavonoids. From these results and comparison it was concluded that compound **(iv)** was indeed the known 5-hydroxy-7-methoxy-3-(4-methoxybenzyl)chroman-4-one (7-O-methyl-3,9-dihydroeucomin) **(71)**.

**Table 3.10.**  $^1\text{H}$  NMR data of compounds **(iv)** and **(71)**

Proton(s)	<b>(iv)</b> ( $\text{CD}_3\text{OD}$ )	<b>(71)</b> ( $\text{CDCl}_3$ ) <sup>26</sup>
2H-2	4.13, 4.31 AB of ABX (4.2, 7.2, 11.5)	4.12, 4.28 AB of ABX (4.2, 6.5, 11.5)
H-3	2.88m	2.80m
H-6	6.07d, (2.1)	6.06, (2.3)
H-8	6.03d, (2.1)	5.97, (2.3)
2H-9	2.72dd, (9.9, 13.5) 3.17dd, (4.5, 13.8)	2.80m 3.20m
H-2'/H-6'	7.19d AA'BB' (8.7)	7.14 AA'BB' (8.8)
H-3'/H-5'	6.90d AA'BB' (8.7)	6.87 AA'BB' (8.8)
4'-OCH <sub>3</sub>	3.81s	3.80s
7-OCH <sub>3</sub>	3.84s	3.81s



### 3.2.5 Structural elucidation of compound (v)



compound (v)[50]

Compound (v) was assigned the molecular formula  $C_{16}H_{12}O_5$  based on the molar mass of 284.0678  $g \cdot mol^{-1}$  (calculated 284.0685  $g \cdot mol^{-1}$ ). A double bond equivalence of 11 was deduced.

The presence of a 3-benzylidene (i.e. a 3(9)-unsaturated) system was inferred from the appearance of the signals at  $\delta 5.35$  ppm, (d,  $J = 1.8$  Hz, 2H-2) and  $\delta 7.71$  ppm (t,  $J = 1.8$  Hz, H-9) in the  $^1H$  NMR spectrum [spectrum 32a, 32b and Table 3.1] as well as appearance of a resonance corresponding to an  $\alpha,\beta$ -unsaturated carbonyl group carbon atom at  $\delta 186.5$  ppm in the  $^{13}C$  NMR spectrum [spectrum 33, Table 3.2]<sup>5,7,16,17</sup>. The COSY spectrum [spectrum 34] confirmed the coupling between 2H-2 and H-9. Furthermore the UV spectrum exhibited an absorption maximum at 362 nm ( $\log \epsilon$  4.36) and this is typical of 3(9) unsaturated homoisoflavonoids<sup>17,26</sup>.

The mass spectrum [spectrum 35] revealed peaks at  $m/z$  153, 152, 132 and 107. The base peak at  $m/z$  107 which was thought to be a hydroxybenzyl ion was unexpected given previous reports on the fragmentation of these compounds as presented in scheme 3.2<sup>17</sup>. The mass spectral fragments at  $m/z$  152 and 153 indicated two hydroxy groups attached to ring A. These were at the 5 and 7 positions as confirmed by bathochromic shifts in the UV spectra with both  $AlCl_3$  (+40 nm) and  $NaOAc$  (+12 nm) respectively [spectra 39 and 40]. Moreover appearance of the H-6 and H-8 signals in the  $^1H$  NMR spectrum at chemical shifts of  $\delta 5.93$  ppm and  $\delta 5.87$  ppm was also an indication of a hydroxy group at C-7<sup>15</sup>.

The substitution pattern in ring B was deduced from the appearance of the fragment at  $m/z$  132 in the mass spectrum corresponding to the RDA fragment ion as depicted in

scheme 3.2, as well as the appearance in the  $^1\text{H}$  NMR spectrum of an aromatic AA'BB' system indicating the occurrence of a *para* disubstituted benzene ring [Table 3.1].

There have been conflicting reports in the literature regarding the configuration at C-9. Some reports claim the appearance of the 2H-2 and H-9 signals as a doublet and triplet respectively [spectrum 32b] are indicative of the (*Z*)-geometry at this double bond due to long range coupling<sup>5,12</sup> while others claim the opposite<sup>17,26</sup>. NOE experiments were performed to confirm which conformation was correct. Molecular models were constructed for both geometric isomers (*E* and *Z*) before the NOE experiments were performed. The model for the (*Z*)-isomer revealed that H-9 was close to 2H-2, H-2' and H-6' and this meant that one should get positive NOE effects for signals due to these protons if H-9 was irradiated [Fig. 3.4]. The model for the (*E*)-isomer revealed that only H-2' and H-6' were close to H-9 and this meant that one should get positive NOE effect for the H-2' and H-6' signals only if H-9 was irradiated [Fig. 3.5].

Irradiation of the triplet (appearing as broad singlet in spectrum 32a) at  $\delta 7.77$  ppm (H-9) led to a positive NOE effect of the doublet signal at  $\delta 7.30$  ppm only [spectrum 36a]. This supported the (*E*)-configuration of the 3(9)-double bond as the doublet at 7.30 ppm has been assigned to H-2' and H-6' [Table 3.1]. To provide further evidence for the (*E*)-geometry, the doublet at  $\delta 5.35$  ppm (2H-2), was irradiated and this led to a positive NOE effect of the doublet at  $\delta 7.30$  ppm (H-2'/H-6') only [spectrum 36b]. Finally, the doublet at  $\delta 7.30$  ppm (H-2'/H-6') was irradiated [spectrum 36c] and this resulted in a positive NOE effect of the doublet at  $\delta 6.92$  ppm (H-3' and H-5'), the triplet at  $\delta 7.77$  ppm (H-9) and the doublet at  $\delta 5.35$  ppm (2H-2). This last NOE result provided evidence that the geometry at the 3(9)-double bond was (*E*) as these results would not be expected in the case of a (*Z*)-isomer. The positive NOE response of H-2' and H-6' to the irradiation of H-9 can be attributed to the free rotation across the C-9, C-1' bond<sup>114</sup>.

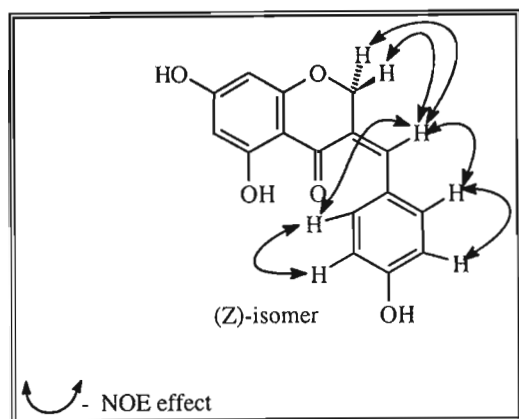


Fig. 3.4

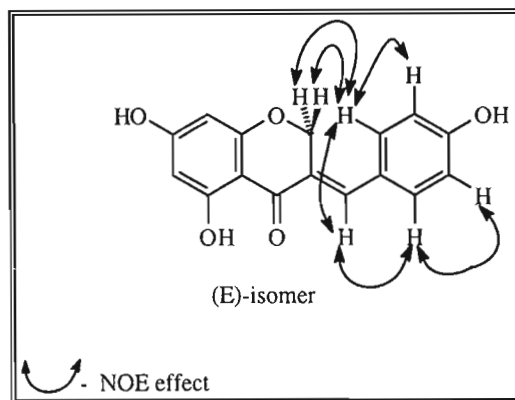
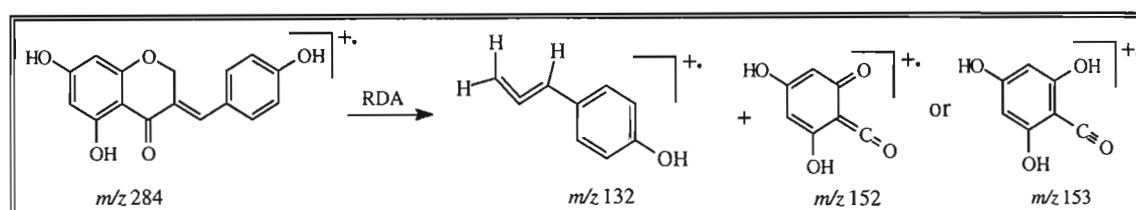


Fig. 3.5

Scheme 3.2. Fragmentation pattern of compound (v) <sup>17</sup>

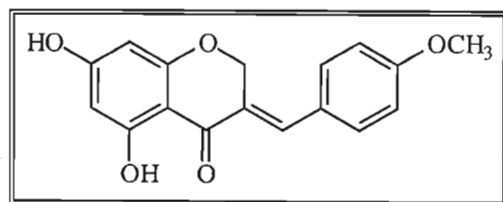
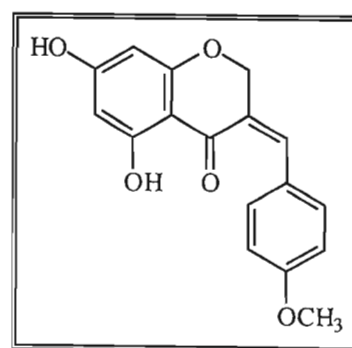
The <sup>1</sup>H NMR data of compound (v) was compared with literature data for (Z)- and (E)-eucomin as no data for (E)-4'-O-demethyl eucomin was available in the literature <sup>59</sup>. The literature referred to the data for eucomin as being comparable to that for demethyleucomin <sup>59</sup>. In Table 3.11, it may be observed that data for (E)-eucomin correlates well with that of compound (v). Finally, the <sup>13</sup>C NMR data of compound (v) was compared with that of eucomin (51) [Table 3.12] because no <sup>13</sup>C NMR data for demethyleucomin (50) could be found in the literature <sup>59</sup>. From this analysis and comparisons it was concluded that compound (v) was the known compound (E)-4'-O-demethyleucomin (50) which was isolated previously from *Eucomis punctata* <sup>59</sup>.

**Table 3.11.**  $^1\text{H}$  NMR data\* of compounds (**v**) and (**51**)

Proton(s)	( <b>v</b> ) ( $\text{CD}_3\text{OD}$ )	( <b>51</b> ) (E) ( $\text{CDCl}_3$ ) <sup>26</sup>	( <b>51</b> ) (Z) ( $\text{CDCl}_3$ ) <sup>26</sup>
2H-2	5.35d, (1.8)	5.31d, (1.7)	4.91s
H-6	5.93d AB (2.2)	6.00 AB (2.3)	5.99 AB (2.3)
H-8	5.87 AB (2.2)	5.90 AB (2.3)	5.93 AB (2.3)
H-9	7.77t, (1.8)	7.80t, (1.7)	6.87s
H-2'/H-6'	7.30d AA'BB' (8.7)	7.27 AA'BB' (8.8)	7.81 AA'BB' (8.8)
H-3'/H-5'	6.92d AA'BB' (8.7)	6.96 AA'BB' (8.8)	6.90 AA'BB' (8.8)
4'-OCH <sub>3</sub>	–	–	3.85s

\* - data for compound (**v**) recorded in a 300 MHz spectrometer

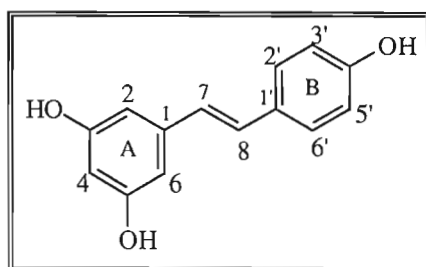
- data for compound (**51**) recorded in a 90 MHz spectrometer

(E)-eucomin (**51**)(Z)-eucomin (**51**)

**Table 3.12.**  $^{13}\text{C}$  NMR data for compound (v) and (51)

C	(v) ( $\text{CD}_3\text{OD}$ )	(51) ( $\text{CD}_3)_2\text{SO}^{17}$
2	68.6	74.1
3	127.0	125.5
4	186.5	186.6
4a	103.8	103.2
5	164.0	164.8
6	97.4	96.2
7	169.4	166.7
8	96.0	94.7
8a	160.7	162.5
9	138.0	140.5
1'	128.4	126.6
2'	133.6	133.1
3'	116.8	113.4
4'	160.7	160.6
5'	116.8	113.4
6'	133.6	133.1
4'-OCH <sub>3</sub>	–	55.2

### 3.2.6 Structural elucidation of compound (vi)



compound (vi)[104]

Compound (vi) was found to have a molar mass of  $228.0784 \text{ g}\cdot\text{mol}^{-1}$  (calculated

228.0786 g.mol<sup>-1</sup>) corresponding to the molecular formula C<sub>14</sub>H<sub>12</sub>O<sub>3</sub>. A double bond equivalence of 9 was deduced.

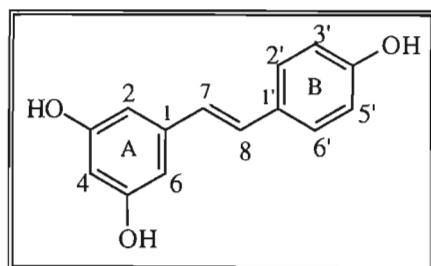
The <sup>13</sup>C NMR spectrum [spectrum 42] revealed 10 signals as one would expect given the chemical equivalence between carbons in the following pairs: C-3, C-5; C-2, C-6; C-2', C-6'; C-3', C-5'. The chemical shifts [Table 3.4] were assigned with comparison to literature values<sup>90,91,97</sup>.

The presence of an A<sub>2</sub>B system in the <sup>1</sup>H NMR spectrum [δ6.48 ppm (d, *J* = 2.1 Hz, 2H) and δ6.19 ppm (t, *J* = 2.1 Hz, 1H)] [spectra 41a and 41b] gave a clue to the occurrence of a 1,3,5-(resorcinol-type) trisubstituted ring A of a stilbenoid<sup>89,91</sup>. The protons were *meta* coupled to each other as the magnitude of the coupling constant (*J* = 2.1 Hz) suggested. This coupling was confirmed by the COSY spectrum [spectrum 43]. A *trans* olefinic bond within the molecule was revealed by the appearance in the <sup>1</sup>H NMR spectrum of two doublets coupled to each other at δ6.86ppm (*J* = 16.1 Hz) and δ6.97 ppm (*J* = 16.1 Hz) [spectra 41b and 44]. The magnitude of the coupling constant indicated a *trans* geometry<sup>90,97</sup>.

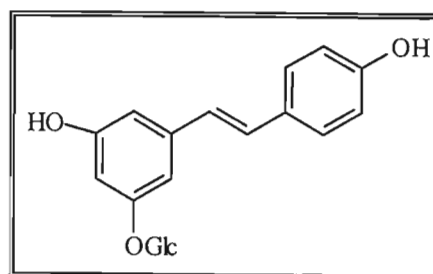
Ring B was found to be a *para* disubstituted benzene ring from the appearance in the proton spectrum of signals corresponding to an AA'BB' system [Table 3.4]. These signals were in agreement with literature values<sup>89</sup>. The <sup>1</sup>H NMR data of compound (vi) was finally compared with literature<sup>89</sup> values for resveratrol (104) [Table 3.13]. Since no <sup>13</sup>C NMR data for resveratrol was available in the literature<sup>89,94,95</sup>, the data of resveratrol-3-O-glucoside (piceid) (107)<sup>97</sup>, was compared with the data for resveratrol [Table 3.14]. Only the data due to the aglycone part of the molecule is quoted for piceid in Table 3.14. The close similarity between these values observed from the two tables led to the conclusion that compound (vi) was (E)-resveratrol (104) a known naturally occurring stilbenoid previously isolated from the roots of *Polygonum cuspidatum* (Polygonaceae)<sup>89</sup>, the dried rhizomes, stressed leaves of *Veratrum grandiflorum* (Melanthiaceae)<sup>94</sup>, and the seed of *Festuca versuta* (Poaceae)<sup>95</sup>, in grapes (*Vitis vinifera*) and wine<sup>55,97</sup>.

**Table 3.13.**  $^1\text{H}$  NMR data of compound (vi) and (E)-resveratrol (104)

Proton(s)	(vi) ( $\text{CD}_3\text{OD}$ )	(104) ( $\text{Me}_2\text{CO}-d_6$ ) <sup>89</sup>
H-2 / H-6	6.48d, (2.1)	6.53d, (2.0)
H-4	6.19t, (2.1)	6.25t, (2.0)
H-7	6.86d, (16.2)	6.87d, (16.5)
H-8	6.97d, (16.2)	7.01d, (16.5)
H-2' / H-6'	7.39d AA'BB' (8.7)	7.41d AA'BB' (8.5)
H-3' / H-5'	6.80d AA'BB' (8.7)	6.82d AA'BB' (8.5)



compound (vi)

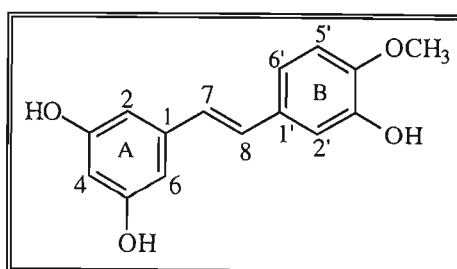


piceid (107)

**Table 3.14.**  $^{13}\text{C}$  NMR data of compounds (vi) and (107) aglycone

C	(vi) ( $\text{CD}_3\text{OD}$ )	(107) aglycone <sup>97</sup> ( $\text{CD}_3\text{OD}$ )
1	141.3	141.1
2	105.8	107.0
3	159.7	160.5
4	102.7	104.1
5	159.7	159.6
6	105.8	108.4
7	129.4	126.7
8	127.0	130.0
1'	130.4	130.3
2'	128.8	128.9
3'	116.5	116.5
4'	158.4	158.5
5'	116.5	116.5
6'	128.8	128.9

### 3.2.7 Structural elucidation of compound (vii)



compound (vii)[105]

Compound (vii) was found to have a molar mass of  $258.0903 \text{ g}\cdot\text{mol}^{-1}$  (calculated  $258.0892 \text{ g}\cdot\text{mol}^{-1}$ ) corresponding to the molecular formula  $\text{C}_{15}\text{H}_{14}\text{O}_4$ . A double bond equivalence of 9 was deduced. The substitution pattern in both ring A and ring B was inferred from NOE experiments, the proton and carbon spectra.

The  $^1\text{H}$  NMR spectra of compound (vii) was related to that of compound (vi) but

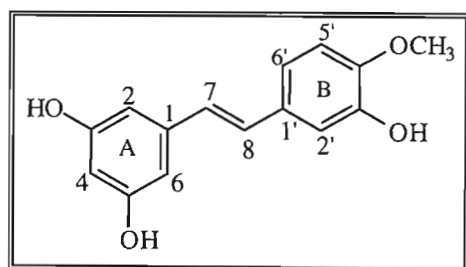


differed in the presence of one methoxy group and the appearance of a 1,3,4-(catechol-type) substitution in ring B [spectra 41a, 41b, 48a, 48b and Tables 3.3]. The resorcinol-type substitution pattern in ring A was still the same as in compound (vi). NOE experiments were carried out to determine the substitution pattern in ring B. Irradiation of the methoxy group proton signal at  $\delta$ 3.90 ppm resulted in a positive NOE effect of the doublet at  $\delta$ 6.93 ppm ( $J = 8.7$  Hz, H-5') [spectrum 51a]. This result meant that the methoxy group was in close proximity to H-5' and this could only happen if the methoxy group was at the 4' position. Moreover since there was no enhancement of any other proton resonance, this meant that the hydroxy group in this ring was adjacent to the methoxy group i.e. it was at the 3' position. Further NOE experiments were carried out to verify the substitution. Irradiation of the doublet at  $\delta$ 7.04 ppm ( $J = 1.8$  Hz) resulted in a positive NOE effect of the signals corresponding to the olefinic protons only [spectrum 51b]. This implied that the doublet at  $\delta$ 7.04 ppm ( $J = 1.8$  Hz) was due to H-2' and the two doublets centred at  $\delta$ 6.95 ppm and  $\delta$ 6.82 ppm ( $J = 16.2$  Hz) were due to H-7 and H-8 respectively <sup>114</sup>. Irradiation of the doublet centred at  $\delta$ 6.82 ppm resulted in a positive NOE effect of the signal at  $\delta$ 7.04 ppm (d,  $J = 1.8$  Hz) and  $\delta$ 6.48 ppm ( $J = 2.1$  Hz) [spectrum 51c]. The enhancement of the signal at 6.97 ppm, (dd,  $J = 1.8, 8.7$  Hz) due to H-6' could not be detected despite being expected as a result of the free rotation across the C-1', C-8 bond <sup>114</sup>. This may be due to the close proximity of 6.97 ppm to 6.82 ppm which led to the signals being affected in the NOE experiment [spectrum 51c]. The results of the latter NOE experiment indicated that the doublet at  $\delta$ 6.82 ppm was due to H-8 while those at  $\delta$ 7.04 ppm and  $\delta$ 6.48 ppm were due to H-2' and H-2/H-6 respectively <sup>114</sup>. A molecular model of compound (vii) revealed that the olefinic H-8 was in close proximity with H-2', H-6' (free rotation across C-1', C-8 bond), H-2 and H-6 (the proximity of H-2 to H-8 is due to the free rotation of the C-1, C-7 bond) <sup>114</sup>. Comparison of the <sup>1</sup>H NMR data of compound (vii) with literature data of the known rhapontigenin (105) <sup>116</sup> showed a close correlation in chemical shift values as well as coupling constants [Table 3.15], with minor differences attributable to solvent effects. Hence it was concluded that compound (vii) was rhapontigenin (105). However the <sup>13</sup>C NMR spectrum [spectrum 49] showed 15 'major' signals two of which were unaccounted for, as one would expect to get 13 signals for rhapontigenin (vii) [105] as some of its carbons are chemically equivalent e.g. C-2 and C-6 as well as

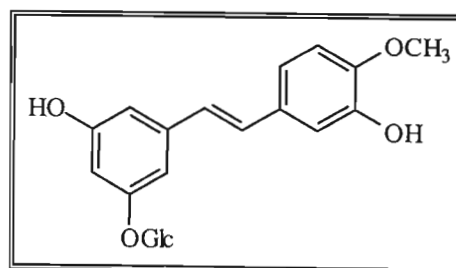
C-3 and C-5. The peaks which could not be accounted for are marked with a cross in spectrum 49. The literature  $^{13}\text{C}$  NMR data of an aglycone part of rhaponticin (**108**) - a rhapontigenin glycoside - was used to compare with the assignments made for compound (**vii**) since complete  $^{13}\text{C}$  NMR data of rhapontigenin was not found in the literature <sup>116</sup>. From this comparison, a close correlation in the respective assignments emerged thus indicating that compound (**vii**) was rhapontigenin (**105**). From these observations it was concluded that compound (**vii**) was indeed rhapontigenin (**105**) previously isolated from *Rheum undulatum* <sup>56</sup> and *Rhei rhizoma* <sup>116</sup>, both species being members of the family Polygonaceae.

**Table 3.15.**  $^1\text{H}$  NMR data of compounds (**vii**) and rhapontigenin (**105**)

Proton(s)	( <b>vii</b> ) ( $\text{CD}_3\text{OD}$ )	( <b>105</b> ) ( $\text{Me}_2\text{CO}-d_6$ ) <sup>116</sup>
H-2	6.48d, (2.1)	6.56d, (2.0)
H-4	6.20t, (2.1)	6.29t, (2.0)
H-6	6.48d, (2.1)	6.56d, (2.0)
H-7	6.95d, (16.2)	7.02d, (16.0)
H-8	6.82d, (16.2)	6.84d, (16.0)
H-2'	7.04d, (1.8)	7.09d, (2.0)
H-5'	6.93d, (8.7)	6.88d, (8.0)
H-6'	6.97dd, (1.8, 8.7)	7.02dd, (2.0, 8.0)
4'-OMe	3.90s	3.84s



compound (**vii**)



rhaponticin (**108**)

**Table 3.16.**  $^{13}\text{C}$  NMR data of compounds (vii) and (108) aglycone

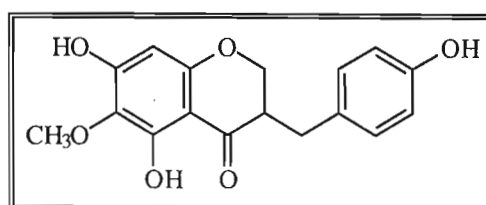
C	(vii) ( $\text{CD}_3\text{OD}$ )	(108) aglycone ( $\text{DMSO-d}_6 + \text{D}_2\text{O}$ ) <sup>116</sup>
1	141.1	139.1
2	105.9	105.0
3	159.7	158.4
4	102.8	102.8
5	159.7	158.0
6	105.9	107.0
7	127.9	126.0
8	129.4	128.5
1'	132.2	129.8
2'	113.6	112.7
3'	149.0#	146.3
4'	147.7#	147.6
5'	112.7	112.0
6'	120.0	118.6
4'- $\text{OCH}_3$	56.4	55.5

# - values are interchangeable

### 3.3 Extractives from *Scilla natalensis* Planch.

Compound (viii) was isolated from the methanol extract of *S. natalensis* (KZN) while compound (ix) was isolated from the hexane and ether extracts of *S. natalensis* (Mpl).

#### 3.3.1 Structural elucidation of compound (viii)



compound (viii)[76]

Compound (viii), was found to have a molar mass of  $316.0945 \text{ g}\cdot\text{mol}^{-1}$  (calcd.  $316.0947$

$\text{g}\cdot\text{mol}^{-1}$ ) corresponding to the molecular formula  $\text{C}_{17}\text{H}_{16}\text{O}_6$ . A double bond equivalence of 10 was deduced.

This compound was found to be the known compound 3,9-dihydroeucomnalin [5,7-dihydroxy-6-methoxy-3-(4-hydroxybenzyl)chroman-4-one] (**76**) which was previously isolated from *Eucomis autumnalin*<sup>61</sup>. The UV spectrum [**spectrum 59**] exhibited an absorption maximum at 290 nm ( $\log \epsilon$  4.63) while the literature value was reported to be 292 nm ( $\log \epsilon$  4.61)<sup>61</sup>.

The  $^1\text{H}$  NMR spectrum showed the presence of one methoxy group (s,  $\delta$ 3.81 ppm, 3H). The appearance in the mass spectrum [**spectrum 57**] of peaks at  $m/z$  209, 183 and 182 corresponding to A-4 and RDA fragments gave an indication that two hydroxy groups and one methoxy group were attached to ring A while the base peak at  $m/z$  107 was indicative of a hydroxybenzyl moiety and hence a hydroxylated ring B. The arrangement of substituents on ring A was deduced from UV experiments and by comparison of carbon chemical shift data [**spectrum 56** and **Table 3.18**] to literature<sup>15</sup> values.

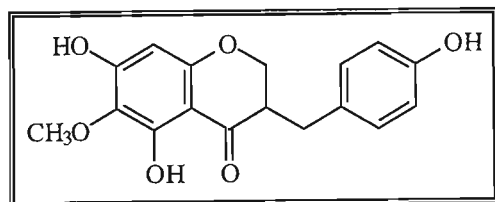
Bathochromic shifts in UV spectral measurements with  $\text{AlCl}_3$  (+22 nm) [**spectrum 60**] and  $\text{NaOAc}$  (+37 nm) [**spectrum 61**] were indicative of hydroxy groups at C-5 and C-7 respectively. The methoxy group in ring A could be placed at either C-6 or C-8 since both C-5 and C-7 were shown to be carrying hydroxy groups. In order to locate the position of the methoxy group in this ring, the carbon chemical shift data of the known 3,9-dihydroeucomnalin (**76**) and 3,9-dihydropunctatin (**79**)<sup>15</sup> were compared with that of compound (**viii**) [**Table 3.18**]. In this comparison particular emphasis was placed on resonances due to C-5, C-6 and C-8 (highlighted in bold in the Table 3.18). The chemical shift assigned to C-5 of compound (**viii**) was 156.9 ppm and this was to be expected given Adinolfi and co-workers'<sup>15</sup> assertion that this carbon should resonate at about 9.0 ppm upfield from the norm (the norm being 165.8 ppm in case of a 5,7 dihydroxy substituted ring A) when there is a methoxy group at C-6 [ $165.8 - 156.9 = 8.9$  ppm]. Moreover, the norm regarding C-6 and C-8 is appearance of their shifts in the ranges 97.1-97.3 and 95.8-96.0 respectively in cases where there is a hydroxy group at C-7<sup>15</sup>. Since  $\delta$ 95.9 ppm, a value assigned to C-8 in compound (**viii**), was outside the

range 97.1-97.3 (a range for unsubstituted C-6) this implied that the unsubstituted position in this ring should be C-8. It was concluded from this comparison that the methoxy group was at C-6.

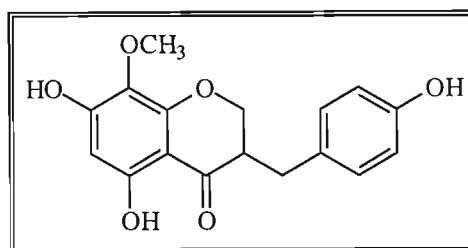
Substitution in ring B was inferred from the appearance of an intense peak at  $m/z$  107 in the mass spectrum corresponding to a hydroxybenzyl/tropylium ion as well as the aromatic AA'BB' system signals in the  $^1\text{H}$  NMR spectrum [spectrum 55 and Table 3.17]. From this information it was concluded that ring B was a *para* disubstituted benzene ring. Finally the  $^1\text{H}$  NMR data of compound (viii) was compared to the literature data of 3,9-dihydroeucommalin (76) [Table 3.17]. The close correlation observed in Table 3.17 together with other results in this discussion led to the conclusion that compound (viii) was indeed 3,9-dihydroeucommalin (76).

**Table 3.17.**  $^1\text{H}$  NMR data of compound (viii)[76]

Proton(s)	(viii) ( $\text{CD}_3\text{OD}$ )	(76) $^{15}\text{C}$ ( $\text{CD}_3\text{OD}$ )
2H-2	4.10, 4.20 AB of ABX (4.2, 7.2, 11.4)	4.00, 4.17 AB of ABX (4.3, 7.7, 11.4)
H-3	2.83m	2.75m
H-8	5.94s	5.89s
2H-9	2.67dd, (10.2, 13.5) 3.13dd, (4.2,13.5)	2.59dd, (10.6, 13.0) 3.07dd, (4.0, 13.0)
H-2'/H-6'	7.09d AA'BB' (8.4)	7.03d AA'BB' (8.0)
H-3'/H-5'	6.78d AA'BB' (8.4)	6.72d AA'BB' (8.0)
6-OCH <sub>3</sub>	3.81s	3.76s



compound (viii)[76]



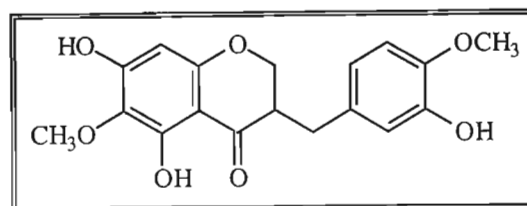
3,9-dihdropunctatin (79)

**Table 3.18.**  $^{13}\text{C}$  NMR data of compounds (viii)[76] and (79)

C	(76) $^{15}(\text{CD}_3\text{OD})$	(viii) ( $\text{CD}_3\text{OD}$ )	(79) $^{15}(\text{CD}_3\text{OD})$
2	70.5	70.3	70.2
3	49.1	ξ	48.0
4	199.9	200.2	199.3
4a	102.7	103.1	102.8
<b>5</b>	<b>156.7</b>	<b>156.9</b>	<b>161.0</b>
<b>6</b>	<b>130.4</b>	<b>130.2</b>	<b>97.2</b>
7	161.1	160.5	161.6
<b>8</b>	<b>95.9</b>	<b>95.9</b>	<b>130.1</b>
8a	160.0	160.1	155.6
9	32.9	33.0	33.1
1'	130.0	130.2	129.8
2'	131.0	131.2	131.1
3'	116.3	116.4	116.4
4'	157.1	157.3	157.1
5'	116.3	116.4	116.4
6'	131.0	131.2	131.1
6-OCH <sub>3</sub>	60.9	61.0	
8-OCH <sub>3</sub>			61.5

ξ - hidden under solvent peaks

### 3.3.2 Structural elucidation of compound (ix)



compound (ix)[88]

Compound (ix) was found to have a molar mass of  $346.1044 \text{ g}\cdot\text{mol}^{-1}$  (calculated  $346.1052 \text{ g}\cdot\text{mol}^{-1}$ ) corresponding to the molecular formula  $\text{C}_{18}\text{H}_{18}\text{O}_7$ . A double bond equivalence of 10 was deduced.

The  $^1\text{H}$  NMR spectrum showed that compound (ix) had two methoxy groups (s,  $\delta 3.81$  ppm, 3H and s,  $\delta 3.86$  ppm, 3H) [spectrum 62]. Substitution in ring A was deduced from UV experiments, the mass and  $^1\text{H}$  NMR spectra. Although a number of expected fragments due to RDA fragmentation<sup>4</sup> could not be detected in the mass spectrum [spectrum 64], the presence of a low intensity peak at  $m/z$  209 corresponding to a dihydroxy-methoxychromanone fragment ion was testimony to the existence of a ring A with two hydroxy and one methoxy groups. The appearance of a singlet integrating for one proton at  $\delta 5.95$  ppm (normally due to H-8/H-6) in the  $^1\text{H}$  NMR spectrum was also an indication that there was only one unsubstituted position in ring A. The fact that this chemical shift was upfield of 6.0 ppm provided evidence that a hydroxy group occurred at C-7<sup>15</sup>. This was confirmed by a bathochromic shift (+36 nm) with NaOAc [spectrum 69]. A hydroxy group also occurred at C-5 because a bathochromic shift (+23 nm) was recorded with  $\text{AlCl}_3$  [spectrum 68]. The position of the methoxy group was determined by comparing carbon chemical shift data of compound (ix) with literature data of known compound (88) [Table 3.20]<sup>4</sup>. The comparison followed the same trend as in compound (viii) in terms of comparing carbon shift data for C-5, C-6 and C-8. It was concluded from this comparison that the methoxy group was at C-6 due to the close similarity between carbon shifts due to compound (ix) and those of known compound (88)<sup>4</sup>.

Ring B was found to be trisubstituted as revealed by the appearance of an aromatic ABX

system in the proton spectrum. A base peak at  $m/z$  137 in the mass spectrum, corresponding to a hydroxymethoxybenzyl ion indicated that ring B contained one hydroxy and one methoxy substituent. Despite the similarity in chemical shifts for this ABX system with those of compound (ii) [Table 3.1] and other related 3',4'-oxygenated compounds<sup>4,13,15,57</sup> thus suggesting a similar substitution pattern, a NOE experiment was performed for confirmation. Irradiation of the singlet at  $\delta$ 3.86 ppm (OMe) led to the positive NOE effect of the doublet at  $\delta$ 6.89 ppm,  $J = 8.1$  Hz [spectrum 65a]. This was an indication that the methoxy group was at the 4'-position because the signal at  $\delta$ 6.89 ppm corresponded to H-5' [Table 3.19]. To provide an unambiguous proof of this substitution, the doublet at  $\delta$ 6.89 ppm was then irradiated. This irradiation led to the positive NOE effect of the singlet at  $\delta$ 3.86 ppm (OMe) and the double doublet at  $\delta$ 6.70 ppm,  $J = 1.8, 8.1$  Hz [spectrum 65b] thus providing enough evidence that the methoxy group and H-6' were adjacent to H-5'. Irradiation of the doublet at  $\delta$ 6.75 ppm ( $J = 2.1$  Hz, H-2') led to the positive NOE effect of the signals corresponding to H-3 and H-9 [spectrum 65c]. The double doublet signal for H-6' was also affected in this latter NOE experiment due to its close proximity to the H-2' signal and this resulted in the positive enhancement of the doublet at  $\delta$ 6.89 ppm as well. The NOE experiment supported the mass spectral results that only one methoxy group was attached to ring B.

The  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of compound (ix) was finally compared to the literature data of the known compound (88) and they correlated very well [Tables 3.19 and 3.20]. From this analysis it was concluded that compound (ix) was 5,7-dihydroxy-6-methoxy-3-(3-hydroxy-4-methoxybenzyl)chroman-4-one (88), a known compound having been previously isolated from *Muscari armeniacum*<sup>4</sup>.



**Table 3.19.**  $^1\text{H}$  NMR data of compound (ix)[88] compared to literature

Proton(s)	(ix) ( $\text{CD}_3\text{OD}$ )	(88) <sup>4</sup> ( $\text{CD}_3\text{OD}$ )
2H-2	4.10, 4.26 AB of ABX (4.2, 7.2, 11.4)	4.06, 4.24 AB of ABX (4.1, 7.3, 11.4)
H-3	2.85m	2.81m
H-8	5.95s	5.91s
2H-9	2.66dd, (10.2, 13.5) 3.11dd, (4.5, 13.5)	2.62dd, (9.9, 13.6) 3.06dd, (4.8, 13.6)
H-2'	6.75d, (2.1)	6.71d, (1.8)
H-5'	6.89d, (8.1)	6.85d, (8.1)
H-6'	6.70dd, (2.1, 8.1)	6.66dd, (1.8, 8.1)
6-OCH <sub>3</sub>	3.81s	3.77s
4'-OCH <sub>3</sub>	3.86s	3.82s

**Table 3.20.**  $^{13}\text{C}$  NMR data for compound **(ix)**[**88**] compared to literature

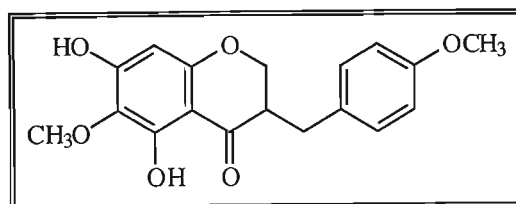
C	<b>(ix)</b> (CD <sub>3</sub> OD)	<b>(88)</b> <sup>4</sup> (CD <sub>3</sub> OD)
2	70.3	70.3
3	#	#
4	200.1	200.0
4a	103.0	103.0
5	<b>156.8</b>	<b>156.8</b>
6	<b>130.5</b>	<b>129.2</b>
7	160.8	160.9
8	<b>95.8</b>	<b>95.8</b>
8a	160.1	160.1
9	33.2	33.1
1'	132.3	132.3
2'	117.1	117.0
3'	147.9	147.8
4'	147.7	147.8
5'	113.0	112.9
6'	121.4	121.4
3'-OCH <sub>3</sub>	61.0	61.0
4'-OCH <sub>3</sub>	56.5	56.4

# - hidden under solvent peaks

### 3.4 Extractives from *Scilla dracomontana* Hilliard and Burt.

Compound **(x)** was isolated from the hexane fraction while compounds **(viii)** and **(xi)** were isolated from the ether fraction of the fresh bulbs extracts from this plant.

### 3.4.1 Structural elucidation of compound (x)



compound (x)

Compound (x) was found to have a molar mass of  $330.1100 \text{ g.mol}^{-1}$  (calculated  $330.1103 \text{ g.mol}^{-1}$ ) corresponding to a molecular formula  $\text{C}_{18}\text{H}_{18}\text{O}_6$ . A double bond equivalence of 10 was deduced.

The  $^1\text{H}$  NMR spectrum [spectrum 70] showed that compound (x) had two methoxy groups (s,  $\delta 3.81 \text{ ppm}$ , 6H). Substitution in ring A was deduced from UV experiments,  $^{13}\text{C}$  and  $^1\text{H}$  NMR spectra. The expected mass spectral fragments at  $m/z$  209, 182, 183 could not be detected in the mass spectrum [spectrum 73] despite the appearance of the base peak at  $m/z$  121 which confirmed the occurrence of the A-4 fragmentation pattern as depicted in scheme 3.1. This also meant that the other methoxy group must be attached to ring A despite the non-appearance of the expected fragment ions. In the  $^1\text{H}$  NMR spectrum, the appearance of a singlet integrating for one proton at  $\delta 5.95 \text{ ppm}$  (normally due to H-8/H-6) was proof that there was only one unsubstituted position in ring A. The fact that this was upfield of 6.0 ppm provided evidence that a free hydroxy group occurred at C-7. This was confirmed by a bathochromic shift (+36 nm) with NaOAc [spectrum 77]. A bathochromic shift (+23 nm) with  $\text{AlCl}_3$  [spectrum 76] gave an indication that a hydroxy group occurred at C-5. The location of the methoxy group in this ring was assigned with comparison to literature values<sup>15</sup> and with reference to the chemical shift data of compound (viii) [Table 3.22]. The comparison followed the same trend as in compound (viii) above. From this comparison it was concluded that the methoxy group in this ring was at position 6.

NOE experiments were performed to locate the position of the other methoxy group on the molecule. Since the two methoxy groups resonated at the same chemical shift i.e.  $\delta 3.81 \text{ ppm}$  in  $\text{CD}_3\text{OD}$ , the NOE spectra were recorded in  $\text{CDCl}_3$  as the methoxy proton

resonances resolved into two separate peaks in this solvent. Irradiation of the methoxy group proton signal at  $\delta 3.89$  ppm did not cause enhancement of any signal [**spectrum 72a**] while irradiation of the one at  $\delta 3.77$  ppm caused the enhancement of the doublet at  $\delta 6.84$  ppm corresponding to H-3' and H-5' [**spectrum 72b**]. This meant that the resonance at  $\delta 3.77$  ppm was due to the methoxy group at the 4' position and this was supported by the appearance of the base peak at  $m/z$  121 in the mass spectrum corresponding to the methoxybenzyl/tropylium ion. The appearance of signals due to an AA'BB' system in the  $^1\text{H}$  NMR spectrum [**Table 3.1**] was consistent with the presence of a *para* disubstituted benzene ring B. From these results it was concluded that compound (**x**) was a methylated derivative of 3,9-dihydroeucomnaline (**76**), i.e. 4'-O-methyl-3,9-dihydroeucomnaline which has hitherto not been reported from nature.

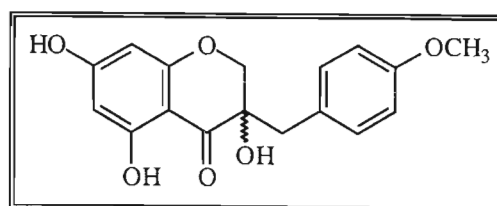
**Table 3.21.**  $^1\text{H}$  NMR data of compound (**x**) and (**viii**)

Proton(s)	( <b>x</b> ) ( $\text{CD}_3\text{OD}$ )	( <b>viii</b> ) ( $\text{CD}_3\text{OD}$ )
2H-2	4.10, 4.27 AB of ABX (4.5, 6.9, 11.4)	4.10, 4.20 AB of ABX (4.2, 7.2, 11.4)
H-3	2.83m	2.83m
H-8	5.95s	5.94s
2H-9	2.73dd, (9.9, 13.5) 3.26dd, (4.5, 13.5)	2.67dd, (10.2, 13.5) 3.13dd, (4.2, 13.8)
H-2'/H-6'	7.19d AA'BB' (8.7)	7.09d AA'BB' (8.4)
H-3'/H-5'	6.90d AA'BB' (8.7)	6.78d AA'BB' (8.4)
6-OCH <sub>3</sub>	3.81s	3.81s
4'-OCH <sub>3</sub>	3.81s	-

**Table 3.22.**  $^{13}\text{C}$  NMR data of compounds (x), (viii)[76] and 3,9-dihydropunctatin (79)

C	(76) $^{15}(\text{CD}_3\text{OD})$	(viii) $(\text{CD}_3\text{OD})$	(x) $(\text{CD}_3\text{OD})$	(79) $^{15}(\text{CD}_3\text{OD})$
2	70.2	70.3	70.3	70.5
3	49.1	$\xi$	$\xi$	48.0
4	199.9	200.2	200.1	199.3
4a	102.7	103.1	103.0	102.8
<b>5</b>	<b>156.7</b>	<b>156.9</b>	<b>156.5</b>	<b>161.0</b>
<b>6</b>	<b>130.4</b>	<b>130.2</b>	<b>131.4</b>	<b>97.2</b>
7	161.1	160.5	160.7	161.6
<b>8</b>	<b>95.9</b>	<b>95.9</b>	<b>95.8</b>	<b>130.1</b>
8a	160.0	160.1	160.1	155.6
9	32.9	33.0	32.9	33.1
1'	130.0	130.2	131.4	129.8
2'	131.0	131.2	131.2	131.1
3'	116.3	116.4	115.1	116.4
4'	157.1	157.3	160.0	157.1
5'	116.3	116.4	115.1	116.4
6'	131.0	131.2	131.2	131.1
6-OCH <sub>3</sub>	60.9	61.0	61.0	–
8-OCH <sub>3</sub>	–	–	–	61.5
4'-OCH <sub>3</sub>	–	–	55.7	–

### 3.4.2 Structural elucidation of compound (xi)



compound (xi)[10]

Compound (xi) was found to have a molar mass of  $316.0940 \text{ g}\cdot\text{mol}^{-1}$  (calculated  $316.0947 \text{ g}\cdot\text{mol}^{-1}$ ) corresponding to the molecular formula  $\text{C}_{17}\text{H}_{16}\text{O}_6$ . A double bond

equivalence of 10 was deduced. A quaternary C-3 was inferred from the non-appearance in the proton spectrum of the resonances attributed to the -(2)CH<sub>2</sub>-(3)CH-(9)CH<sub>2</sub>- grouping. Furthermore the <sup>13</sup>C NMR spectrum revealed an extra C-O signal (apart from methoxy group carbon resonance at δ55.7 ppm and the C-2 resonance at δ72.9 ppm ) at δ73.5 ppm which, when compared to literature values, agreed with the placement of a hydroxy group at C-3 <sup>16</sup>.

Substitution in ring A was deduced from <sup>1</sup>H NMR, UV and mass spectra. The appearance of an aromatic AB system corresponding to H-6 (δ5.92 ppm, d, *J* = 2.1 Hz ) and H-8 (δ5.92 ppm, d, *J* = 2.1 Hz) in the <sup>1</sup>H NMR spectrum [**spectrum 78**] meant this ring was substituted at the 5 and 7 positions. Bathochromic shifts with AlCl<sub>3</sub> (+22 nm) [**spectrum 85**] and NaOAc (+38 nm) [**spectrum 86**] in the UV spectrum were indicative of hydroxy groups at C-5 and C-7 respectively. The appearance of a fragment at *m/z* 195 in the mass spectrum [**spectrum 82**], albeit at very low intensity <sup>17</sup>, corresponding to a 3,5,7-trihydroxy-4-chromanone fragment gave further evidence for the presence of a 5,7-dihydroxy substituted ring A. A fragment expected to appear at *m/z* 298 - corresponding to loss of one water molecule from the molecular ion - was, however, not detected in the spectrum <sup>17</sup>.

Substitution in ring B was deduced from the appearance in the <sup>1</sup>H NMR spectrum of an AA'BB' system [**Table 3.1**]. A methoxy group was placed at the 4' position due to the presence of a fragment at *m/z* 121 (base peak) in the mass spectrum corresponding to a methoxybenzyl/tropylium ion. Finally the <sup>1</sup>H and <sup>13</sup>C NMR data of compound (xi) were compared to the literature data of eucomol (10) [**Tables 3.23 and 3.24**] <sup>17,62</sup>. From these results and comparison it was concluded that compound (xi) was eucomol (10), a known compound which was isolated previously from *Eucomis bicolor* <sup>62</sup>.

**Table 3.23.**  $^1\text{H}$  NMR data of compound **(xi)** and eucomol **(10)**

Proton(s)	<b>(xi)</b> ( $\text{CD}_3\text{OD}$ )	<b>(10)</b> ( $\text{CDCl}_3$ ) <sup>62</sup>
2H-2	3.92d, 4.08d AB (11.4)	4.02, 4.16 AB (11.0)
H-6	5.95d AB (2.1)	5.98d AB (2.0)
H-8	5.92d AB (2.1)	5.94d AB (2.0)
2H-9	2.96d, (3.3)	2.90
H-2'/H-6'	7.20d AA'BB' (8.7)	7.08d AA'BB' (8.0)
H-3'/H-5'	6.88d AA'BB' (8.7)	6.80d AA'BB' (8.0)
4'-OCH <sub>3</sub>	3.81s	3.73s

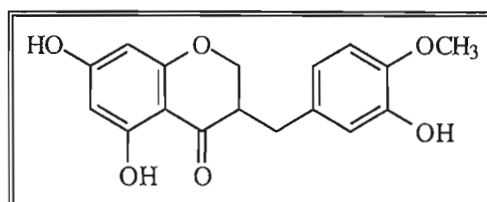
**Table 3.24.**  $^{13}\text{C}$  NMR data for compound (xi) and eucomol (10)

C	(xi) ( $\text{CD}_3\text{OD}$ )	(10) ( $(\text{CD}_3)_2\text{SO}$ ) <sup>17</sup>
2	72.9	71.8
3	73.5	71.6
4	199.8	198.0
4a	101.4	100.1
5	164.4	164.0
6	97.6	96.3
7	168.9	166.8
8	96.3	95.0
8a	160.3	162.5
9	40.7	38.7
1'	128.2	127.0
2'	132.8	131.4
3'	114.5	113.3
4'	160.3	158.2
5'	114.6	113.3
6'	132.8	131.4
4'-OCH <sub>3</sub>	55.7	54.9

### 3.5 Extractives from *S.kraussii* Bak.

Compound (xii) was isolated from the ether extract of the bulbs of this species.

#### 3.5.1 Structural elucidation of compound (xii)



compound (xii)[72]

Compound (xii) was assigned the molecular formula  $\text{C}_{17}\text{H}_{16}\text{O}_6$  based on the molar



mass of 316.0952 g.mol<sup>-1</sup> (calculated 316.0947 g.mol<sup>-1</sup>). A double bond equivalence of 10 was deduced

Comparison of the <sup>1</sup>H and <sup>13</sup>C NMR spectra of compounds (xii) and (ii) [spectra 9 and 87 and Table 3.1] revealed a close similarity between the two except that (ii) had two methoxy groups to one in (xii). The missing methoxy group in (xii) was found to be the one at C-7 as revealed by the peak at *m/z* 179 in the mass spectrum [spectrum 90] corresponding to a 5,7-dihydroxychromanone fragment generated via A-4 cleavage of the molecular ion. Further evidence that a hydroxy group was present at C-7 was revealed by the appearance of chemical shifts due to H-6 and H-8 downfield of 6.0 ppm i.e. at δ5.92 ppm and δ5.96 ppm. Bathochromic shifts with both AlCl<sub>3</sub> (+23 nm) [spectrum 93] and NaOAc (+35 nm) [spectrum 94] were also indicative of a 5,7-dihydroxy substitution in ring A.

The <sup>1</sup>H NMR spectrum also revealed the presence of an aromatic ABX system [spectrum 87 and Table 3.1] which indicated a trisubstituted ring B much the same as in compound (ii). A NOE experiment [spectrum 89] which involved irradiation of the singlet at δ3.87 ppm (OMe) resulted in a positive NOE effect of the doublet signal at δ6.89 ppm (H-5'). From these results, it was concluded that the methoxy group was at the 4'-position. Since no other signal enhancement was observed, this meant the hydroxy group in ring B was adjacent to the methoxy group i.e. it was at the 3' position.

The <sup>1</sup>H NMR and <sup>13</sup>C NMR data for compound (xii) were compared to those of the known compound 5,7-dihydroxy-3-(3-hydroxy-4-methoxybenzyl)chroman-4-one (72)<sup>15</sup> [Tables 3.7 and 3.25]. It was found from this comparison that the resonances and respective coupling constants correlated very well. From this analysis and comparison it was concluded that compound (xii) was 5,7-dihydroxy-3-(3-hydroxy-4-methoxybenzyl)chroman-4-one (72), a compound previously isolated from *Muscari comosum*<sup>2</sup>.

**Table 3.25.**  $^{13}\text{C}$  NMR data of compound (xii)[72] compared to literature

C	(xii) ( $\text{CD}_3\text{OD}$ )	(72) $^{15}$ ( $\text{CD}_3\text{OD}$ )
2	70.4	70.2
3	$\xi$	47.9
4	199.4	199.3
4a	102.9	102.8
5	165.8	165.8
6	97.1	97.1
7	168.3	168.2
8	95.9	95.8
8a	164.7	164.7
9	33.1	33.1
1'	132.4	132.4
2'	117.1•	117.0
3'	147.9 $\phi$	147.9
4'	147.7 $\phi$	147.6
5'	113.0•	113.0
6'	121.4	121.4
4'- $\text{OCH}_3$	56.5	56.8

$\xi$  - hidden under solvent peaks

•,  $\phi$  - values are interchangeable

### 3.6 Conclusion

The presence of homoisoflavonoids in all *Scilla* species in this study was to be expected given the documented occurrence<sup>1-7,12,15,17,61,62</sup> of these compounds in the Hyacinthaceae. One notable feature distinguishing these from other homoisoflavonoids isolated outside this family, is the 5,7-dioxy substitution pattern which has been found lacking in most homoisoflavonoids emanating from other families<sup>10,32</sup>.

Some of the homoisoflavonoids isolated from the Hyacinthaceae are of the eucomnalin type i.e. they carry an extra methoxy group at C-6. Some workers have found the presence of the extra oxygenated group at either C-6 (eucomnalin type) or C-8 (punctatin type) useful in the grouping of the species within a genus<sup>17</sup>. Notable in this work for the production of the eucomnalin type are *S. natalensis* and *S. dracomontana*. This may provide a clue on the relationship between these two species especially in view of the fact they were once sunk into one species i.e. *S. natalensis*<sup>64,107</sup>. The appearance of homoisoflavonoids in the sister genus *Ledebouria*<sup>122</sup>, which has been undergoing chemical investigation by one member of our group confirms that homoisoflavonoids are widespread within the family Hyacinthaceae.

Stilbenoids, on the other hand, have been isolated for the first time from this genus. Previous reports have revealed that purple-blue spots appeared on chromatograms of extracts from other *Scilla*, *Chionodoxa* and *Muscari* species after they had been treated with a number of spray reagents but these had not been characterised<sup>17</sup>. Although stilbenoids have been isolated from only *S. nervosa* in this work, it needs to be mentioned that these purple-blue spots appeared in the chromatograms of all the species studied but they were in much higher concentration in *S. nervosa*. Their significance in this genus and the family cannot be a taxonomic one because stilbenoids have been found in a vast array of genera which are not part of the Hyacinthaceae as alluded to in section 2.2 (chapter 2). Their significance may be attributed to the ability of these plants to colonise a variety of habitats as stilbenoids have been documented to possess antifungal properties<sup>94,96</sup>. For instance, the wide distribution of *S. nervosa* i.e. from near-tropical KwaZulu-Natal extending to near-desert Botswana, could be attributed to the production of these compounds in quantities enough to enable this species to thrive

where others fail to grow. In other plants, stilbenoids have been reported to act as phytoalexins<sup>94,96</sup> i.e. produced by the plant in response to stress (fungal attack or physical damage to leaves, etc.).

Coming to potential ethnomedical benefits of the plants to their users, the appearance of both homoisoflavonoids and stilbenoids in these *Scilla* species could provide support for their ethnomedical usage. Homoisoflavonoids have been shown by some workers<sup>88</sup> to possess anti-inflammatory properties by inhibiting croton oil induced dermatitis in mouse ear and this inhibition was comparable to that of indomethacin, a potent non steroidal anti-inflammatory drug. This could explain why the locals find these plants beneficial in treating conditions that have an inflammation aspect e.g. fractures, boils and veld sores<sup>78</sup>. Furthermore, they have been found to possess anti-allergic, anti-histaminic, anti-mutagenic and angioprotective properties in addition to being potent phosphodiesterase inhibitors<sup>87</sup>.

Stilbenoids, on the other hand, could also play a part in anti-inflammatory action by virtue of being anti-oxidants. Inflammation processes are accompanied by the release of highly reactive and toxic oxygen radicals. By acting as scavengers of these radicals, stilbenoids could play a vital role in the anti-inflammation process. Moreover, recent research<sup>55,97</sup> into the presence of resveratrol (**104**) and its glycosides in grapes (*Vitis vinifera*) and red wines has been suggested to provide an explanation for the 'French paradox' i.e. the epidemiological evidence that moderate consumption of red wine by the French may counteract the effects of saturated fats prevalent in dairy and meat products thus reducing predisposition to coronary heart disease (CHD). This is because of their anti-oxidant properties. Reports<sup>97</sup> have appeared recently linking the aglycone in resveratrol glycosides in the inhibition of the copper ( $\text{Cu}^{2+}$ ) catalysed oxidation of human low density lipoprotein (LDL) and the aggregation of platelets, the two processes which are a major cause of CHD in affluent societies and this has generated much interest in resveratrol and its glycosides.

# **CHAPTER 4**

## **EXPERIMENTAL**

## 4.1 General

### 4.1.1 Melting points

Melting points for crystalline compounds isolated were determined on a an Ernst Leitz Weltzlar melting point apparatus and are uncorrected.

### 4.1.2 Optical rotations

Optical rotations were recorded at room temperature in either methanol or chloroform (depending on solubility) on an Optical Activity Ltd AA-5 automatic polarimeter. The concentrations of the solutions are expressed in g /100 ml.

### 4.1.3 Infrared spectroscopy

The samples were run on NaCl discs and spectra recorded on a Mattison FTIR spectrophotometer. Either chloroform or methanol was used as solvent depending on the solubility of the sample. The sample was dissolved in the appropriate solvent and a few drops applied to the disc. Discs were allowed to dry before spectra were recorded.

### 4.1.4 Ultraviolet spectroscopy

Ultraviolet absorption spectra were recorded on a Varian DMS 300 UV-visible spectrophotometer with methanol used as solvent. The NaOAc and AlCl<sub>3</sub> solutions used for bathochromic shift tests were prepared by dissolving 0.5 g of each salt (anhydrous) in 100 ml redistilled methanol.

### 4.1.5 Mass spectrometry

High resolution (HRMS) and electron impact (EIMS) mass spectra were recorded by Dr. P. Boschoff at the Cape Technikon on Kratos High Resolution MS 9/50 and a Finnigan 1020 GC MS instruments.

### 4.1.6 NMR Spectroscopy

NMR spectra were recorded at room temperature on a Varian Gemini 300 MHz spectrometer. For <sup>13</sup>C NMR, spectra were recorded at 75.4 MHz. The less concentrated samples were recorded on a Varian Innova 500 MHz spectrometer. The solvent used was deuteriomethanol (CD<sub>3</sub>OD) with deuteriochloroform (CDCl<sub>3</sub>) used where

necessary, e.g. NOE experiments involving peaks that appeared superimposed in the  $^1\text{H}$  NMR spectrum when  $\text{CD}_3\text{OD}$  was used as solvent. All  $\delta$  values are expressed in ppm relative to TMS. Peaks due to solvent impurities are marked with a cross (X) on the respective spectra in which they appear.

#### **4.1.7 Chromatography**

##### **4.1.7 a) Column chromatography**

For column chromatography, use was made of glass columns (1-4 cm in diameter) packed with silica gel 60 (particle size 0,040 - 0,063 mm, 230 - 400 mesh ASTM, Merck Art 9385) and where necessary Sephadex LH-20. In the initial stages of purification, coarser silica gel (Merck Art 7734) and/or silica gel S 31608 (particle size 0.063-0.1 mm) were used under gravity. Repeated chromatography, either as flash or under gravity, was performed in order to separate the compounds in the extracts. A variety of solvent systems were used as eluants. The solvents most used, albeit in different proportions - depending on the polarity of the compounds to be separated, were hexane, dichloromethane and ethyl acetate. Mixtures of dichloromethane and methanol were also used especially for elution of very polar compounds. Flash chromatography involved the use of pressure to elute the compounds from the column. Final separation and purification involved use of mini-columns made from pasteur pipettes.

##### **4.1.7 b) Thin and Preparative Layer chromatography**

Precoated, aluminium backed silica gel 60 plates (thickness 0,2 mm ; Merck Art 5533) were used to follow the separation process as well as to detect the composition of the different fractions collected from the column. After developing the plates in an appropriate solvent, they were sprayed with anisaldehyde spray reagent. The anisaldehyde reagent was made by mixing anisaldehyde, conc. sulphuric acid and methanol in the ratio 1: 2: 80. Spots were observed after heating the developed and sprayed plate with a heating gun. Spots due to homoisoflavonoids were visualised as bright yellow to orange in colour while those due to stilbenoids appeared as bright purple-blue in colour.

Compounds which were difficult to separate by column chromatography were separated using preparative TLC. The plates used were commercially available Merck F-254 silica gel plates with indicator. A UV lamp set at 254 nm was used to detect the spots of the separated compounds.

## 4.2 Preparation and extraction of plant material

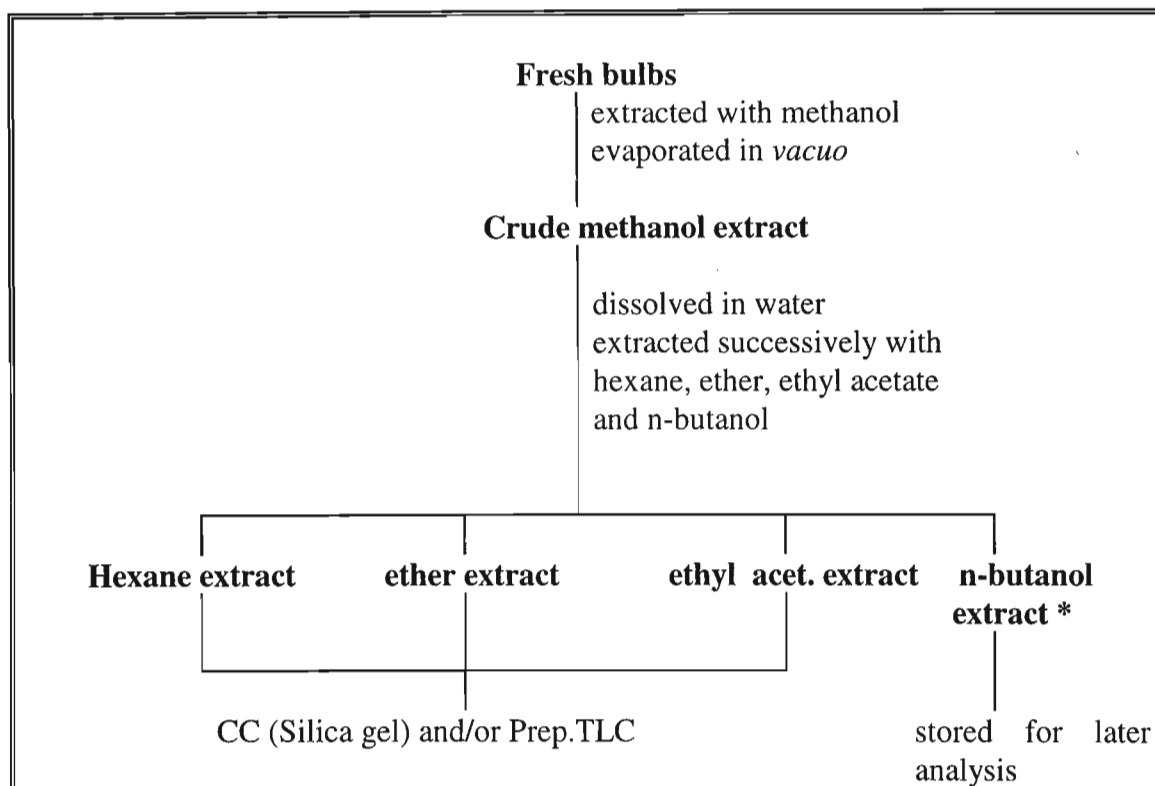
### 4.2.1 General extraction procedures

Plant material for this project was collected from KwaZulu-Natal and Mpumalanga and was authenticated by Dr. Neil Crouch of the National Botanical Institute. Voucher specimens were deposited at the Natal Herbarium. The acronyms KZN for KwaZulu-Natal and Mpl for Mpumalanga will be used when reference is made to the locality of collection where appropriate.

Fresh bulbs of *Scilla nervosa* (Burch.) Jessop [KZN] (1.3 kg) and *Scilla natalensis* Planch. [KZN] (1.2 kg) were cut into small chunks, air dried overnight and extracted for twenty four hours using a Soxhlet apparatus with hexane, ethyl acetate and methanol successively. A portion of the *S. nervosa* bulbs (200 g) was soaked in chloroform for 12 hrs. The fractions recovered after solvent evaporation were subjected to column chromatography.

Fresh bulbs of *Scilla nervosa* [Mpl], *S. natalensis* [Mpl], *S. dracomontana* Hilliard and Burt and *S. kraussii* Bak. were subjected to the extraction procedure presented in scheme 4.1. The bulbs were chopped into small pieces and extracted with methanol (2.5 L) in 5 L conical flasks. The flasks were agitated on a Labcon mechanical shaker at 140 rpm for 24 hrs. This was done twice with the first solvent substituted with fresh solvent after 24 hrs.





**Scheme 4.1.** General extraction procedure for *Scilla* species

\* - After extraction with the three solvents, i.e. hexane, ether and ethyl acetate, the aqueous mother liquors of each extract were further extracted with n-butanol (500 ml). However this polar fraction was put aside for future analysis and does not form part of the work presented here. The ether and ethyl acetate extracts had, in certain cases, similar TLC profiles and the researcher used his judgement to choose which one to investigate between the two.

### 4.3 Extractives from *Scilla nervosa* (Burch.) Jessop

Fresh bulbs of *Scilla nervosa* [KZN] were purchased at the Warwick Triangle herbal market, Berea, Durban. The voucher specimen number is **V.Bangani and N.Crouch 2**. The bulbs were subjected to the procedure as described in 4.2.1 above. The masses of the different extracts obtained after evaporation of solvent were:

Hexane - 1.5 g

Chloroform - 18 g

Ethyl acetate - 34 g

Methanol - 46 g

Fresh bulbs of *S. nervosa* [Mpl] were collected in Lydenburg, Mpumalanga. The voucher specimen number is **Crouch 752**. A mass of 950 g of the bulbs was extracted as presented in scheme 4.1 and section 4.2 above. The masses of the extracts obtained after evaporation of solvent were:

Crude methanol extract - 260 g : a portion (62 g) of this crude extract was subjected to the procedure outlined in scheme 4.1 to yield the following extracts:

Hexane extract - 0.94 g

Ether extract - 13 g

Ethyl acetate extract - 24 g

Separation by column chromatography of the hexane extract from *S. nervosa* [KZN] yielded compounds **(i)** and **(ii)**, while the ethyl acetate fraction yielded compounds **(iii)**, **(iv)**, **(v)**, **(vi)** and **(vii)**. The chloroform extract also yielded compound **(i)** and **(ii)**.

From the hexane extract of *S. nervosa* [Mpl] compounds **(i)** and **(iv)** were isolated by column chromatography.

Compound **(iii)** was isolated from the ethyl acetate extract via an acetylation step. Acetylation was performed on a mixture (87 mg) of compound **(iii)** with another unknown compound after persistent co-elution despite use of different solvent systems. A  $^1\text{H}$  NMR spectrum was run prior to acetylation to make sure that none of the constituents of the mixture have acetyl groups as previous reports have revealed that acetyl groups do occur in some homoisoflavonoids<sup>15</sup>.

The acetylation was carried out - after the  $^1\text{H}$  NMR spectrum revealed no such acetyl groups.

#### **Acetylation:**

The mixture (87 mg) was dissolved in pyridine (5 ml). Acetic anhydride (3 ml) was added, the mixture was warmed on a steam bath and left to stand overnight under anhydrous conditions. The excess acetic anhydride was removed by the addition and *in vacuo* removal of methanol (10 ml), after which pyridine was removed by addition of toluene (3 x 10 ml) and azeotropic distillation. The final traces of toluene were removed by further addition and removal of methanol (3 x 10ml). After all the above steps, the

acetylated mixture was separated using a mini column to provide 12 mg of acetylated compound **(iii)**.

#### 4.3.1 Physical data for compound (i)

Name : 5,7-dimethoxy-3-(4-methoxybenzyl)chroman-4-one

Yield : 75 mg, 30 mg from the KZN specimen and 45 mg from the Mpl specimen

Physical description : off white vitreous solid

$[\alpha]_D = -50^\circ$  ( $c = 0.3$  g/ 100 ml, CH<sub>3</sub>OH), lit.value<sup>9</sup> [synthetic compound] =  $+12 \pm 2^\circ$  ( $c$  0.227, CHCl<sub>3</sub>)<sup>26</sup>

Mass spectrum : HRMS  $m/z$ : 328.1306 [M<sup>+</sup>] (C<sub>19</sub>H<sub>20</sub>O<sub>5</sub> requires 328.1311), EIMS  $m/z$  (rel.int.): 328 [M<sup>+</sup>] (100), 207 (37), 181 (35), 180 (61) 148 (26), 121 (90) [**spectrum 4**]

Data for <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy is presented in **Tables 3.1** and **3.2** in Chapter 3 and [**spectra 1** and **2**]

IR data :  $\nu_{\max}$ [NaCl](cm<sup>-1</sup>) : 2932 (C-H aromatic and aliphatic stretching), 1686 (C=O stretching), 1608 (C=C stretching), 1524 (CH<sub>2</sub>, CH<sub>3</sub> bending), 1259, 1041 (C-O stretching) [**spectrum 5**]

UV data :  $\lambda_{\max}$  nm (log  $\epsilon$ ) : 283 (4.47) [**spectrum 6**], lit.value : 283.5 (4.281)<sup>26</sup>

Bathochromic shifts : no shift (AlCl<sub>3</sub>) [**spectrum 7**]

no shift (NaOAc) [**spectrum 8**]

<sup>9</sup> - negative optical rotation have been reported for most naturally occurring 3-benzyl-4-chromanones with one exception being 3,9-dihydroeucomin<sup>17</sup>

### 4.3.2 Physical data for compound (ii)

Name : 5-hydroxy-7-methoxy-3-(3-hydroxy-4-methoxybenzyl)chroman-4-one

Yield : 62 mg

Physical description : light yellow crystals

Mp : 115-117 °C

$[\alpha]_D = -36^0$  ( $c = 0.55$  g / 100 ml,  $\text{CHCl}_3$ )

Mass spectrum : HRMS  $m/z$ : 330.1092 [ $\text{M}^+$ ] ( $\text{C}_{18}\text{H}_{18}\text{O}_6$  requires 330.1093), EIMS  $m/z$  (rel.int.): 330 [ $\text{M}^+$ ] (37), 193 (7), 167 (21), 166 (3), 149 (15), 137 (100) [**spectrum 12**]

Data for  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopy is presented in **Tables 3.1** and **3.2** in Chapter 3 [**spectra 9** and **10**]

IR data :  $\nu_{\text{max}}[\text{NaCl}](\text{cm}^{-1})$  : 3461 (O-H stretching, H-bonded), 2953, 2835 (C-H aromatic and aliphatic stretching), 1645 (C=O), 1581 (C=C stretching), 1515, 1279 ( $\text{CH}_2$  and  $\text{CH}_3$  bending), 1168 (C-O stretching) [**spectrum 13**]

UV data :  $\lambda_{\text{max}}$  nm (log  $\epsilon$ ) : 288 (4.34) [**spectrum 14**]

Bathochromic shift : 312 (+ $\text{AlCl}_3$ ) [**spectrum 15**]

no shift (+ $\text{NaOAc}$ ) [**spectrum 16**]

### 4.3.3 Physical data for compound (iii) (as its acetyl derivative)

Name : 5,7-dimethoxy-3-(4-acetoxybenzyl)chroman-4-one

Yield : 12 mg

Physical description : vitreous off white solid

$[\alpha]_D = 0^\circ$  (0,0076g / 100ml), a zero optical activity could be due the low concentration of sample or the compound may have been a racemate.

Mass spectrum : HRMS  $m/z$ : 356.1270 [ $M^+$ ] ( $C_{20}H_{20}O_6$  requires 356.1260), EIMS  $m/z$  (rel.int.): 356 [ $M^+$ ] (75), 314 (41), 297 (10), 221 (11), 207 (67), 181 (63), 180 (100), 152 (25), 107 (19), 77 (7) [**spectrum 20**]

Data for  $^1H$  and  $^{13}C$  NMR spectroscopy is presented in **Tables 3.1** and **3.2** in Chapter 3 and [**spectra 17** and **18**]

IR data :  $\nu_{max}$ [NaCl] ( $cm^{-1}$ ) : 2930, 2857 (C-H aromatic and aliphatic stretching), 1729 (ester group stretching), 1671 (C=O stretching), 1613 (C=C stretching), 1466, 1228 ( $CH_2$  and  $CH_3$  bending), 1165 (C-O stretching) [**spectrum 21**]

UV data :  $\lambda_{max}$  nm (log  $\epsilon$ ) : 282 (4.17) [**spectrum 22**]

Bathochromic shift : no shift (+AlCl<sub>3</sub>) [**spectrum 23**]

no shift (+NaOAc) [**spectrum 24**]

#### 4.3.4 Physical data for compound (iv)

Name : 5-hydroxy-7-methoxy-3-(4-methoxybenzyl)chroman-4-one

Yield : 28 mg

Physical description : off white needle shaped crystals

Mp : 81-84 °C, lit. values : 85-87 °C<sup>26</sup>, 87-88 °C<sup>56</sup>

$[\alpha]_D = -38^\circ$  ( $c = 0.024$  g / 100 ml, MeOH), lit.value [synthetic compound] :  $+23 \pm 2^\circ$   
( $c$  0.218, CHCl<sub>3</sub>)<sup>26</sup>

Mass spectrum : HRMS  $m/z$ : 314.1154 [M<sup>+</sup>] (C<sub>18</sub>H<sub>18</sub>O<sub>5</sub> requires 314.1158), EIMS  $m/z$   
(rel.int.): 314 [M<sup>+</sup>] (36), 167 (8), 166 (5), 149 (15), 129 (27), 121 (100) [spectrum 27]

Data for <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy are presented in **Tables 3.1** and **3.2** in Chapter 3  
[spectra 25 and 26]

IR data :  $\nu_{\max}$ [NaCl] (cm<sup>-1</sup>) : 2929, 2848 (C-H aromatic and aliphatic stretching), 1655  
(C=O), 1578 (C=C stretching), 1518 (CH<sub>2</sub> and CH<sub>3</sub> bending), 1164 (C-O stretching)  
[spectrum 28]

UV data :  $\lambda_{\max}$  nm (log  $\epsilon$ ) : 288 (4.43) [spectrum 29], lit. value : 288.5 (4.306)<sup>26</sup>

Bathochromic shift : 311 (+AlCl<sub>3</sub>) [spectrum 30]

no shift (+NaOAc) [spectrum 31]

#### 4.3.5 Physical data for compound (v)

Name : (E)-5,7-dihydroxy-3-(4-hydroxybenzylidene)chroman-4-one or (E)-4'-O-demethyleucomin)

Yield : 35 mg

Physical description : fine bright yellow crystals

Mp : 197-201 °C, lit.value = 209-213 °C<sup>59</sup>

Mass spectrum : HRMS  $m/z$ : 284.0678 [ $M^+$ ] ( $C_{16}H_{12}O_5$  requires 284.0685), EIMS  $m/z$  (rel.int.): 284 [ $M^+$ ] (99), 207 (51), 180 (78), 167 (88), 153 (100), 152 (43), 137 (89), 132 (49), 131 (51), 107 (96), 57 (56), 41 (53) [spectrum 35]

Data for  $^1H$  and  $^{13}C$  NMR spectroscopy is presented in **Tables 3.1** and **3.2** in Chapter 3 [spectra 32, 32a, 32b and 33]

IR data  $\nu_{max}[NaCl](cm^{-1})$  : 3384 (O-H stretching, H-bonded), 1642, (C=O stretching), 1605 (C=C stretching), 1514, 1294 ( $CH_2$  bending), 1166 (C-O stretching) [spectr. 37]

UV data :  $\lambda_{max}$  nm (log  $\epsilon$ ) : 362 (4.36) [spectrum 38], lit. value = 364 (4.47)<sup>59</sup>

Bathochromic shift : 402 (+ $AlCl_3$ ) [spectrum 39]

374 (+ $NaOAc$ ) [spectrum 40]

### 4.3.6 Physical data for compound (vi)

Name : (E)-3,4',5-trihydroxystilbene or (E)-resveratrol

Yield : 14 mg

Physical description : off white crystals turning to brown on standing

Mp : 263-265 °C, lit.values : 265-267 °C <sup>56</sup>, 260 °C <sup>89</sup>, 253-255 <sup>95</sup>

Mass spectrum : HRMS  $m/z$ : 228.0784 [ $M^+$ ] ( $C_{14}H_{12}O_3$  requires 228.0786), EIMS  $m/z$  (rel.int.): 228 [ $M^+$ ] (100), 227 (24), 211 (16), 181 (23), 157 (13), 137 (25), 129 (10), 115 (12), 83 (14), 69 (22), 57 (30) [**spectrum 45**]

Data for  $^1H$  and  $^{13}C$  NMR spectroscopy is presented in **Tables 3.3** and **3.4** in Chapter 3 [**spectra 41a, 41b** and **42**]

IR data :  $\nu_{max}$ [NaCl]( $cm^{-1}$ ) : 3307 (O-H stretching, H-bonded), 2927 (C-H aromatic and olefinic stretching), 1599, 1516 (C=C stretching), [**spectrum 46**]

UV data :  $\lambda_{max}$  nm ( $\log \epsilon$ ) : 306 (4.12), 320sh (3.83) [**spectrum 47**], lit.value : 305 (4.02), 320 (3.34) <sup>89</sup>



#### 4.3.7 Physical data for compound (vii)

Name : (E)-3,3',5-trihydroxy-4'-methoxystilbene or (E)-rhapontigenin

Yield : 12 mg

Physical description : off white crystals turning dark brown on standing

Mp : 189-192 °C, lit.values : 186-187 °C<sup>56</sup>, 196-197 °C<sup>116</sup>

Mass spectrum : HRMS  $m/z$ : 258.0903 [ $M^+$ ] ( $C_{15}H_{14}O_4$  requires 258.0892), EIMS  $m/z$  (rel.int.): 258 [ $M^+$ ] (100), 225 (13), 197 (20), 169 (8), 129 (5) [**spectrum 52**]

Data for  $^1H$  and  $^{13}C$  NMR spectroscopy is presented in **Tables 3.3** and **3.4** in Chapter 3 [**spectra 48a, 48b and 49**]

IR data :  $\nu_{max}[NaCl](cm^{-1})$  : 3396 (O-H stretching, H-bonded), 2932 (C-H aromatic, olefinic and aliphatic stretching), 1612, 1523 (C=C stretching) [**spectrum 53**]

UV data :  $\lambda_{max}$  nm (log  $\epsilon$ ) : 323 (4.57) [**spectr. 54**], lit.value : 302 (4.49), 322 (4.53)<sup>116</sup>

#### 4.4 Extractives from *S. natalensis* Planch

Fresh bulbs of *Scilla natalensis*[KZN] (voucher specimen no. **V.Bangani and N.Crouch 1**) were also bought at Warwick Triangle herbal market. A mass of 1.2kg of fresh bulbs was extracted as described in section 4.2.1. The masses of the extracts obtained were:

Hexane - 2.5g

Ethyl acetate - 12.8g

Methanol - 64g

Fresh bulbs of *S. natalensis* [Mpl] (1.3kg) (voucher specimen no. **Crouch 753**) collected in Lydenburg, Mpumalanga were also subjected to the procedure outlined in Scheme 4.1 The masses of the extracts obtained were:

Crude methanolic extract - 140g : a portion (28g) of this crude extract was subjected to the procedure outlined Scheme 4.1 to give the following extracts:

Hexane - 170 mg

Ether - 750 mg

Ethyl acetate - 450 mg

Column chromatography of a portion (5 g) of the methanol fraction of *S. natalensis* [KZN] yielded compound (**viii**) while the ether and the hexane extract from the Mpumalanga specimen yielded compound (**ix**).

#### 4.4.1 Physical data for compound (viii)

Name : 5,7-dihydroxy-6-methoxy-3-(4-hydroxybenzyl)chroman-4-one (3,9-dihydroeucomnaline)

Yield : 34 mg, 19 mg from *Scilla natalensis*\* (KZN) and 15 mg from *Scilla dracomontana*

Physical description : yellowish green crystals

Mp: 205-207 °C, lit.value : 207-209 °C<sup>56,61</sup>

$[\alpha]_D = 0^\circ$ , (0.0087g / 100ml, MeOH), a zero optical activity could be due the low concentration of sample or the compound may have been a racemate, lit.value :  $-10^\circ$  (dioxan)<sup>56,61</sup>

Mass spectrum : HRMS  $m/z$ : 316.0945 [ $M^+$ ] ( $C_{17}H_{16}O_6$  requires 316.0947), EIMS  $m/z$  (rel.int.): 316 [ $M^+$ ] (100), 210 (60), 209 (23), 183 (10), 182 (7), 167 (13), 149 (7), 137 (48), 121 (6), 108 (9), 107 (84), 69 (13) [spectrum 57]

Data for  $^1H$  and  $^{13}C$  NMR spectroscopy is presented in **Tables 3.1** and **3.2** in Chapter 3 [spectra 55 and 56]

IR data :  $\nu_{max}[\text{NaCl}](\text{cm}^{-1})$  : 3390 (O-H stretching, H-bonded), 2925, 2856 (C-H aromatic and aliphatic stretching), 1653 (C=O and C=C stretching, signal overlap), 1521, 1451, 1269 ( $\text{CH}_2$  and  $\text{CH}_3$  bending), 1172 (C-O stretching) [spectrum 58]

UV data :  $\lambda_{max}$  nm (log  $\epsilon$ ) : 290 (4.63) [spectrum 59], lit. value : 292 (4.61)<sup>61</sup>

Bathochromic shift : 312 (+ $\text{AlCl}_3$ ) [spectrum 60]

327 (+ $\text{NaOAc}$ ) [spectrum 61]

\* - sample lost, only have *S. dracomontana* sample.

#### 4.4.2 Physical data for compound (ix)

Name : 5,7-dihydroxy-6-methoxy-3-(3-hydroxy-4-methoxybenzyl)chroman-4-one

Yield : 23 mg

Physical description : vitreous orange-yellow solid

$[\alpha]_D = -62.5^0$  ( $c = 0.08\text{g}/100\text{ml}$ ,  $\text{CH}_3\text{OH}$ ), lit. value : not available <sup>4</sup>

Mass spectrum : HRMS  $m/z$ : 346.1044 [ $\text{M}^+$ ] ( $\text{C}_{18}\text{H}_{18}\text{O}_7$  requires 346.1052), EIMS  $m/z$  (rel.int.): 346 [ $\text{M}^+$ ] (39), 209 (2), 137 (100), 122 (5) [**spectrum 64**]

Data for  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopy is presented in **Tables 3.1** and **3.2** in Chapter 3 [**spectra 62** and **63**]

IR data :  $\nu_{\text{max}}[\text{NaCl}](\text{cm}^{-1})$  : 3422 (O-H stretching, H-bonded), 2938, 2849 (C-H aromatic and aliphatic stretching), 1653 (C=O stretching), 1595 (C=C stretching), 1519, 1277 ( $\text{CH}_2$  and  $\text{CH}_3$  bending), 1169 (C-O stretching) [**spectrum 66**]

UV data :  $\lambda_{\text{max}}$  nm ( $\log \epsilon$ ) : 292 (4.69) [**spectrum 67**], lit. value : not available <sup>4</sup>

Bathochromic shift : 315 (+ $\text{AlCl}_3$ ) [**spectrum 68**]

328 (+ $\text{NaOAc}$ ) [**spectrum 69**]

#### 4.5 Extractives from *Scilla dracomontana* Hilliard and Burtt.

Fresh bulbs of *Scilla dracomontana* (1.2 kg) (voucher specimen no. **N.Crouch and V.Bangani 754**). collected from the Bamboo mountain district near Underberg in the KwaZulu-Natal Drakensberg were subjected to the same procedure as outlined in scheme 4.1. The masses of the extracts obtained were:

Hexane - 420 mg

Ether - 4.5 g

Ethyl acetate - 6.8 g

Column chromatophic separation of the hexane extract yielded compound (**x**) while preparative TLC of the ether extract yielded compounds (**viii**) and (**xi**)

#### 4.5.1 Physical data for compound (x)

Name : 5,7-dihydroxy-6-methoxy-3-(4-methoxybenzyl)chroman-4-one

Yield : 28 mg

Physical description : vitreous yellow solid

$[\alpha]_D = -100^0$  ( $c = 0.025$  g / 100 ml, CH<sub>3</sub>OH)

Mass spectrum : HRMS  $m/z$ : 330.1100 [ $M^+$ ] (C<sub>17</sub>H<sub>18</sub>O<sub>6</sub> requires 330.1103), EIMS  $m/z$  (rel.int.): 330 [ $M^+$ ] (32), 167 (6), 149 (6), 121 (100), 77 (7) [**spectrum 73**]

Data for <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy is presented in **Tables 3.1** and **3.2** in Chapter 3 [**spectra 70** and **71**]

IR data :  $\nu_{\max}$ [NaCl](cm<sup>-1</sup>) : 3375 (O-H stretching, H-bonded), 2940, 2850 (C-H aromatic and aliphatic stretching), 1655 (C=O stretching), 1591 (C=C stretching), 1527, 1464, 1306 (CH<sub>2</sub> and CH<sub>3</sub> bending), 1171 (C-O stretching) [**spectrum 74**]

UV data :  $\lambda_{\max}$  nm (log  $\epsilon$ ) : 293 (4.58) [**spectrum 75**]

Bathochromic shift : 312 (+AlCl<sub>3</sub>) [**spectrum 76**]

327 (+NaOAc) [**spectrum 77**]

#### 4.5.2 Physical data for compound (xi)

Name : 3,5,7-trihydroxy-3-(4-methoxybenzyl)chroman-4-one (eucomol)

Yield : 12 mg

Physical description : greenish yellow crystals

Mp : 131-133 °C, lit.value = 134.5-135 °C <sup>56,62</sup>

$[\alpha]_D$  = sample too dilute, could not obtain angle of rotation, lit.value = -32 ° (CHCl<sub>3</sub>)  
56,62

Mass spectrum : HRMS  $m/z$ : 316.0940 [M<sup>+</sup>] (C<sub>17</sub>H<sub>16</sub>O<sub>6</sub> requires 316.0947), EIMS  $m/z$  (rel.int.): 316 [M<sup>+</sup>] (7), 195 (4), 137 (7), 121 (100) [**spectrum 82**]

Data for <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy is presented in **Tables 3.1** and **3.2** in Chapter 3 and [**spectra 78** and **79**]

IR data :  $\nu_{\max}$ [NaCl](cm<sup>-1</sup>) : 3489 (O-H stretching, H-bonded), 2929 (C-H aromatic and aliphatic stretching), 1653 (C=O stretching), 1590 (C=C stretching), 1516 (CH<sub>2</sub> and CH<sub>3</sub> bending), 1169 (C-O stretching) [**spectrum 83**]

UV data :  $\lambda_{\max}$  nm (log  $\epsilon$ ) : 291 (4.45) [**spectrum 84**], lit. value: 293 (3.29)\* <sup>62</sup>

Bathochromic shift : 312 (+AlCl<sub>3</sub>) [**spectrum 85**]

327 (+NaOAc) [**spectrum 86**]

\* - probably recorded erroneously as literature log  $\epsilon$  values lie in the range 4.2 to 4.7 <sup>17</sup>.

#### 4.6 Extractives from *Scilla kraussii* Bak.

*Scilla kraussii* was collected near Umtamvuna close to Port Edward on the KwaZulu-Natal south coast. Fresh bulbs (1.3 kg) were also subjected to the procedure as presented in scheme 4.1. The voucher specimen number is **N. Crouch and D. Mulholland 770**. The masses of the extracts were:

Crude methanolic extract - 130 g : a portion (48 g) of the crude methanolic extract was subjected to the procedure outlined scheme 4.1 to give the following extracts:

Hexane - 18 mg

Ether - 90 mg

Ethyl acetate - 150 mg

The hexane extract was noticed to be rich in fatty acids and thus not investigated. The ethyl acetate extract had a similar TLC profile as the ether extract. The ether extract was chosen for investigation. On chromatographic separation the ether extract yielded compound **(xii)**.



#### 4.6.1 Physical data for compound (xii)

Name : 5,7-dihydroxy-3-(3-hydroxy-4-methoxybenzyl)chroman-4-one

Yield : 24 mg

Physical description : vitreous light yellow solid

$[\alpha]_D = -58^\circ$  ( $c = 0.065$  g / 100 ml in MeOH), lit. value :  $-34^\circ$  ( $c$  0.4, MeOH)<sup>2</sup>

Mass spectrum : HRMS  $m/z$ : 316.0952 [ $M^+$ ] ( $C_{17}H_{16}O_6$  requires 316.0947), EIMS  $m/z$  (rel.int.): 316 [ $M^+$ ] (31), 137 (100), 122 (6) [**spectrum 90**]

Data for  $^1H$  and  $^{13}C$  NMR spectroscopy is presented in **Tables 3.1** and **3.2** in Chapter 3 [**spectra 87** and **88**]

IR data :  $\nu_{max}$  [NaCl]( $cm^{-1}$ ) : 3403 (O-H stretching, H-bonded), 2923, 2847 (C-H aromatic and aliphatic stretching), 1638 (C=O stretching), 1600 (C=C stretching), 1517, 1274 ( $CH_2$  and  $CH_3$  bending), 1165 (C-O stretching) [**spectrum 91**]

UV data :  $\lambda_{max}$  nm ( $\log \epsilon$ ) : 290 (4.37) [**spectrum 92**], lit. value : 287 (4.22)<sup>2</sup>

Bathochromic shift : 313 (+ $AlCl_3$ ) [**spectrum 93**]

325 (+NaOAc) [**spectrum 94**]

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## REFERENCES

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Cpd.	Compound name
1	7-hydroxy-3-(4-hydroxybenzyl)chroman
2	7-hydroxy-3-(4-hydroxybenzyl)-8-methoxychroman
3	3'-deoxysappanone B
4	sappanone B
5	comosin
6	brazilin
7	3'-O-methylbrazilin
8	haematoxylin
9	4'-demethyleucomol
10	eucomol (R and S forms)
11	7-O-methyleucomol
12	3,5-dihydroxy-3-(4-hydroxybenzyl)-7-methoxy-6-methylchroman-4-one
13	scillascillin
14	2-hydroxy-7-O-methylscillascillin
15	2-hydroxyscillascillin
16	ophiopogonone A
17	isoophiopogonone A
18	8-methylophiopogonone A
19	5,7-dihydroxy-3-(2-hydroxy-3,4-methylenedioxybenzyl)-6,8-dimethylchromone
20	6-aldehydoisoophiopogonone A
21	isoophiopogonone A
22	2'-hydroxyophiopogonone A
23	3'-O-methylsappanol and/or 3'-O-methylepisappanol
24	3'-deoxysappanol
25	4-O-methylsappanol and/or 4-O-methylepisappanol
26	3'-deoxy-4-O-methylsappanol
27	sappanol (3R,4S) and /or episappanol (3R,4R)
28	brazilein
29	caesalpin J

30	10-hydroxy-11-methoxydracaenone
31	7,10-dihydroxy-11-methoxydracaenone
32	muscomosin
33	3',4',5-trihydroxy-7-methoxyspiro[2H-1-benzopyran-3(4H),7']- bicyclo[4.2.0]octa[1,3,5]-trien]-4-one
34	3',5-dihydroxy-4',7-dimethoxyspiro[2H-1-benzopyran-3(4H),7']- bicyclo[4.2.0]octa[1,3,5]-trien]-4-one
35	3',5,7-trihydroxy-4'-methoxyspiro[2H-1-benzopyran-3(4H),7']- bicyclo[4.2.0]octa[1,3,5]-trien]-4-one
36	2',5-dihydroxy-4',7-dimethoxyspiro[2H-1-benzopyran-3(4H),7']- bicyclo[4.2.0]octa[1,3,5]-trien]-4-one
37	ophiopogonone B
38	8-methylphiopogonone B
39	5,7-dihydroxy-3-(4-hydroxybenzyl)chromone
40	5,7-dihydroxy-3-(4-hydroxybenzyl)-6-methylchromone
41	6-aldehydoisoophiopogonone B
42	4'-demethylisoophiopogonone B
43	ophiopogonanone A
44	8-methylphiopogonanone A
45	6-aldehydoisoophiopogonanone A
46	7-O-methyl-6-formylisoophiopogonanone A
47	5,7-dihydroxy-3-(5-hydroxy-3,4-methylenedioxybenzyl)-6,8-dimethyl-4- chromanone
48	7-hydroxy-3-(3-hydroxy-4-methoxybenzyl)chroman
49	3-(4-hydroxybenzyl)-7,8-methylenedioxychroman
50	4'-demethyleucomin
51	eucomin
52	punctatin
53	4'-O-methylpunctatin
54	eucomnalin
55	5,7-dihydroxy-6-methoxy-3-(3,4-dihydroxybenzylidene)chroman-4-one

56	7-hydroxy-5-methoxy-3-(3,4-dihydroxybenzylidene)chroman-4-one
57	5,8-dihydroxy-7-methoxy-3-(3,4-dihydroxybenzylidene)chroman-4-one
58	5,7-hydroxy-8-methoxy-3-(3,4-dihydroxybenzylidene)chroman-4-one
59	bonducellin
60	8-methoxybonducellin
61	intricatinol
62	intricatin
63	7-hydroxy-3-(4-hydroxybenzylidene)chroman-4-one
64	sappanone A
65	7-O-methyleucomin
66	7-hydroxy-3-(4-hydroxybenzyl)chroman-4-one
67	4',7-diacetoxy-3-benzylchroman-4-one
68	4'-demethyl-3,9-dihydroeucomin
69	4'-demethyl-3,9-dihydro-5-O-methyleucomin
70	3,9-dihydroeucomin
71	7-O-methyl-3,9-dihydroeucomin
72	3'-hydroxy-3,9-dihydroeucomin
73	5,7-dihydroxy-6-methyl-3-(4-hydroxybenzyl)chroman-4-one
74	ophipogonanone B
75	8-methylhipogonanone B
76	3,9-dihydroeucominalin
77	5,8-dihydroxy-7-methoxy-3-(4-hydroxybenzyl)chroman-4-one
78	8-acetoxy-7-methoxy-3-(4-hydroxybenzyl)chroman-4-one
79	3,9-dihydropunctatin
80	7-O-methyl-3,9-dihydropunctatin
81	4'-O-methyl-3,9-dihydropunctatin
82	muscomin
83	6-formylisophiopogonanone B
84	7-O-methyl-6-formylisophiopogonanone B
85	5,6-dihydroxy-7,8-dimethoxy-3-(3,4-dihydroxybenzyl)chroman-4-one
86	5,7-dihydroxy-6-methoxy-3-(3,4-dihydroxybenzyl)chroman-4-one

<b>87</b>	5,7-dihydroxy-6-methoxy-3-(4-hydroxy-3-methoxybenzyl)chroman-4-one
<b>88</b>	5,7-dihydroxy-6-methoxy-3-(3-hydroxy-4-methoxybenzyl)chroman-4-one
<b>89</b>	5,8-dihydroxy-7-methoxy-3-(3,4-dihydroxybenzyl)chroman-4-one
<b>90</b>	5,7-dihydroxy-3-(3,4-dihydroxybenzyl)chroman-4-one
<b>91</b>	7-hydroxy-5-methoxy-3-(3,4-dihydroxybenzyl)chroman-4-one
<b>92</b>	3-deoxysappanone B
<b>93</b>	5-hydroxy-6,7,8-trimethoxy-3-(4-hydroxybenzyl)chroman-4-one
<b>94</b>	7-hydroxy-5-methoxy-3-(3-hydroxy-4-methoxybenzyl)chroman-4-one
<b>95</b>	5,7-dihydroxy-8-methoxy-3-(3-hydroxy-4-methoxybenzyl)chroman-4-one
<b>96</b>	5-hydroxy-7,8-dimethoxy-6-methyl-3-(4-hydroxybenzyl)chroman-4-one
<b>97</b>	6-hydroxy-7-methoxy-3-(3-hydroxy-4-methoxybenzyl)chroman-4-one
<b>98</b>	5-hydroxy-3-(3,4-dihydroxybenzyl)chroman-4-one
<b>99</b>	5,7-dihydroxy-6,8-dimethyl-3-(4-hydroxybenzyl)chroman-4-one
<b>100</b>	homoisosocotrin-4'-ol
<b>101</b>	protosappanin E1 and / or its 1'-epimer
<b>102</b>	5-hydroxy-7-methoxy-6-methyl-3-(3,4-dihydroxybenzyl)chromone
<b>103</b>	gancaonin K

# **APPENDIX A**

## **LIST OF NAMES OF COMPOUNDS IN TABLE 2.1**

Cpd.	Compound name
1	7-hydroxy-3-(4-hydroxybenzyl)chroman
2	7-hydroxy-3-(4-hydroxybenzyl)-8-methoxychroman
3	3'-deoxysappanone B
4	sappanone B
5	comosin
6	brazilin
7	3'-O-methylbrazilin
8	haematoxylin
9	4'-demethyleucomol
10	eucomol (R and S forms)
11	7-O-methyleucomol
12	3,5-dihydroxy-3-(4-hydroxybenzyl)-7-methoxy-6-methylchroman-4-one
13	scillascillin
14	2-hydroxy-7-O-methylscillascillin
15	2-hydroxyscillascillin
16	ophiopogonone A
17	isoophiopogonone A
18	8-methylphiopogonone A
19	5,7-dihydroxy-3-(2-hydroxy-3,4-methylenedioxybenzyl)-6,8-dimethylchromone
20	6-aldehydoisoophiopogonone A
21	isoophiopogonone A
22	2'-hydroxyophiopogonone A
23	3'-O-methylsappanol and/or 3'-O-methylepisappanol
24	3'-deoxysappanol
25	4-O-methylsappanol and/or 4-O-methylepisappanol
26	3'-deoxy-4-O-methylsappanol
27	sappanol (3R,4S) and /or episappanol (3R,4R)
28	brazilein
29	caesalpin J

30	10-hydroxy-11-methoxydracaenone
31	7,10-dihydroxy-11-methoxydracaenone
32	muscomosin
33	3',4',5-trihydroxy-7-methoxyspiro[2H-1-benzopyran-3(4H),7'- bicyclo[4.2.0]octa[1,3,5]-trien]-4-one
34	3',5-dihydroxy-4',7-dimethoxyspiro[2H-1-benzopyran-3(4H),7'- bicyclo[4.2.0]octa[1,3,5]-trien]-4-one
35	3',5,7-trihydroxy-4'-methoxyspiro[2H-1-benzopyran-3(4H),7'- bicyclo[4.2.0]octa[1,3,5]-trien]-4-one
36	2',5-dihydroxy-4',7-dimethoxyspiro[2H-1-benzopyran-3(4H),7'- bicyclo[4.2.0]octa[1,3,5]-trien]-4-one
37	ophiopogonone B
38	8-methylphiopogonone B
39	5,7-dihydroxy-3-(4-hydroxybenzyl)chromone
40	5,7-dihydroxy-3-(4-hydroxybenzyl)-6-methylchromone
41	6-aldehydoisoophiopogonone B
42	4'-demethylisoophiopogonone B
43	ophiopogonanone A
44	8-methylphiopogonanone A
45	6-aldehydoisoophiopogonanone A
46	7-O-methyl-6-formylisoophiopogonanone A
47	5,7-dihydroxy-3-(5-hydroxy-3,4-methylenedioxybenzyl)-6,8-dimethyl-4- chromanone
48	7-hydroxy-3-(3-hydroxy-4-methoxybenzyl)chroman
49	3-(4-hydroxybenzyl)-7,8-methylenedioxychroman
50	4'-demethyleucomin
51	eucomin
52	punctatin
53	4'-O-methylpunctatin
54	eucomnalin
55	5,7-dihydroxy-6-methoxy-3-(3,4-dihydroxybenzylidene)chroman-4-one



56	7-hydroxy-5-methoxy-3-(3,4-dihydroxybenzylidene)chroman-4-one
57	5,8-dihydroxy-7-methoxy-3-(3,4-dihydroxybenzylidene)chroman-4-one
58	5,7-hydroxy-8-methoxy-3-(3,4-dihydroxybenzylidene)chroman-4-one
59	bonducellin
60	8-methoxybonducellin
61	intricatinal
62	intricatin
63	7-hydroxy-3-(4-hydroxybenzylidene)chroman-4-one
64	sappanone A
65	7-O-methyleucomin
66	7-hydroxy-3-(4-hydroxybenzyl)chroman-4-one
67	4',7-diacetoxy-3-benzylchroman-4-one
68	4'-demethyl-3,9-dihydroeucomin
69	4'-demethyl-3,9-dihydro-5-O-methyleucomin
70	3,9-dihydroeucomin
71	7-O-methyl-3,9-dihydroeucomin
72	3'-hydroxy-3,9-dihydroeucomin
73	5,7-dihydroxy-6-methyl-3-(4-hydroxybenzyl)chroman-4-one
74	ophiopogonanone B
75	8-methylphiopogonanone B
76	3,9-dihydroeucomnalin
77	5,8-dihydroxy-7-methoxy-3-(4-hydroxybenzyl)chroman-4-one
78	8-acetoxy-7-methoxy-3-(4-hydroxybenzyl)chroman-4-one
79	3,9-dihydropunctatin
80	7-O-methyl-3,9-dihydropunctatin
81	4'-O-methyl-3,9-dihydropunctatin
82	muscomin
83	6-formylisophiopogonanone B
84	7-O-methyl-6-formylisophiopogonanone B
85	5,6-dihydroxy-7,8-dimethoxy-3-(3,4-dihydroxybenzyl)chroman-4-one
86	5,7-dihydroxy-6-methoxy-3-(3,4-dihydroxybenzyl)chroman-4-one

<b>87</b>	5,7-dihydroxy-6-methoxy-3-(4-hydroxy-3-methoxybenzyl)chroman-4-one
<b>88</b>	5,7-dihydroxy-6-methoxy-3-(3-hydroxy-4-methoxybenzyl)chroman-4-one
<b>89</b>	5,8-dihydroxy-7-methoxy-3-(3,4-dihydroxybenzyl)chroman-4-one
<b>90</b>	5,7-dihydroxy-3-(3,4-dihydroxybenzyl)chroman-4-one
<b>91</b>	7-hydroxy-5-methoxy-3-(3,4-dihydroxybenzyl)chroman-4-one
<b>92</b>	3-deoxysappanone B
<b>93</b>	5-hydroxy-6,7,8-trimethoxy-3-(4-hydroxybenzyl)chroman-4-one
<b>94</b>	7-hydroxy-5-methoxy-3-(3-hydroxy-4-methoxybenzyl)chroman-4-one
<b>95</b>	5,7-dihydroxy-8-methoxy-3-(3-hydroxy-4-methoxybenzyl)chroman-4-one
<b>96</b>	5-hydroxy-7,8-dimethoxy-6-methyl-3-(4-hydroxybenzyl)chroman-4-one
<b>97</b>	6-hydroxy-7-methoxy-3-(3-hydroxy-4-methoxybenzyl)chroman-4-one
<b>98</b>	5-hydroxy-3-(3,4-dihydroxybenzyl)chroman-4-one
<b>99</b>	5,7-dihydroxy-6,8-dimethyl-3-(4-hydroxybenzyl)chroman-4-one
<b>100</b>	homoisosocotrin-4'-ol
<b>101</b>	protosappanin E1 and / or its 1'-epimer
<b>102</b>	5-hydroxy-7-methoxy-6-methyl-3-(3,4-dihydroxybenzyl)chromone
<b>103</b>	gancaonin K

**APPENDIX B**  
**LIST OF SPECTRA**

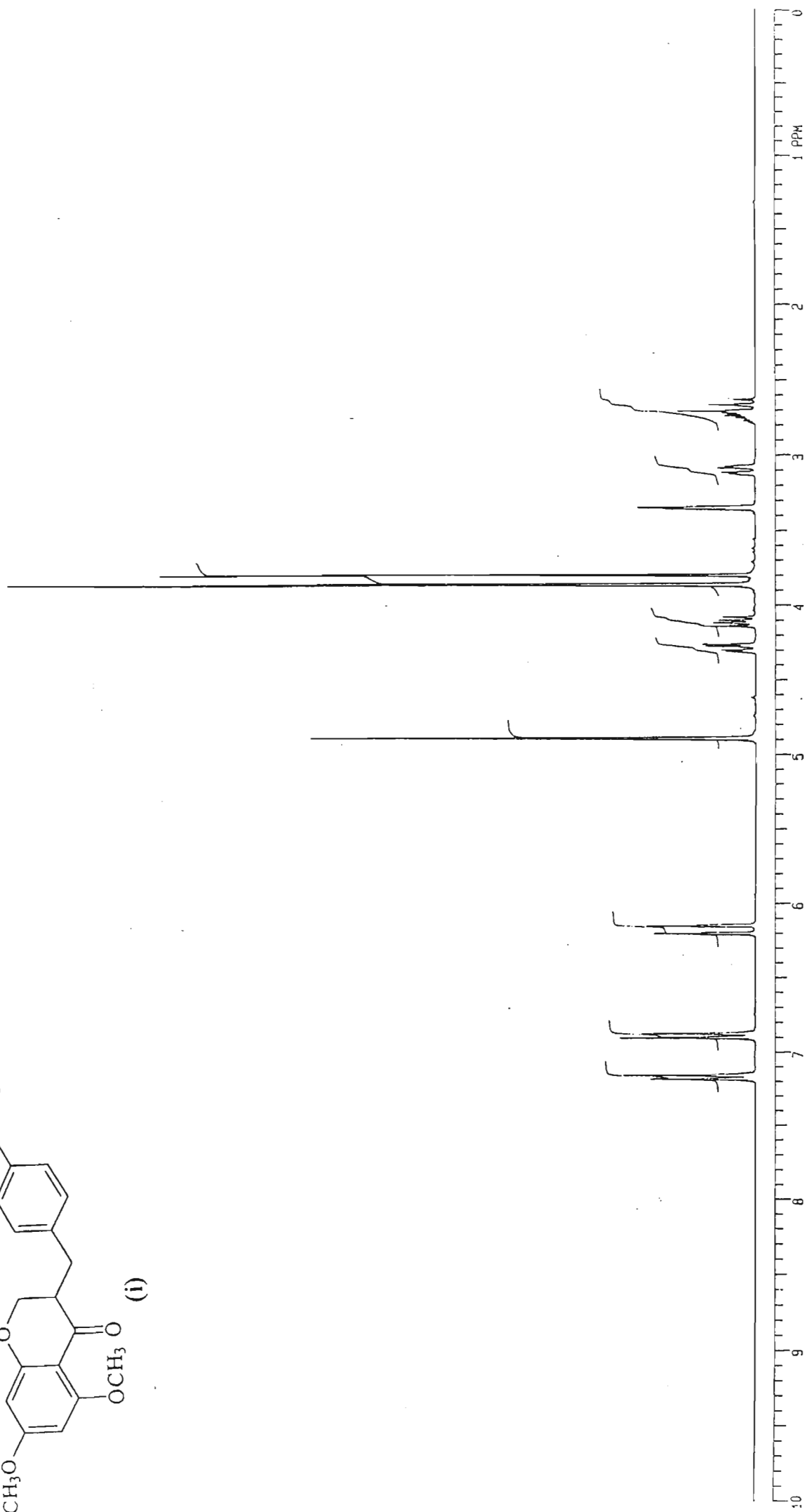
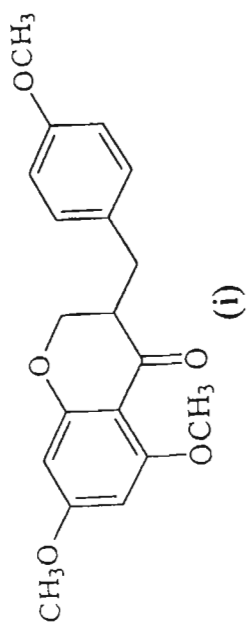
## LIST OF SPECTRA

Cpd	Spectrum no.	Type of spectrum	Page
(i)	1	$^1\text{H}$ NMR	S1
	2	$^{13}\text{C}$ NMR	S2
	3	COSY	S3
	4	Mass	S3
	5	IR	S4
	6	UV	S4
	7	UV (+ $\text{AlCl}_3$ )*	S5
	8	UV (+ $\text{NaOAc}$ )*	S5
(ii)	9	$^1\text{H}$ NMR	S6
	10	$^{13}\text{C}$ NMR	S7
	11a	NOE	S8
	11b	NOE (expanded)	S9
	12	Mass	S9
	13	IR	S10
	14	UV	S10
	15	UV (+ $\text{AlCl}_3$ )*	S11
16	UV (+ $\text{NaOAc}$ )*	S11	
(iii)	17	$^1\text{H}$ NMR	S12
	18	$^{13}\text{C}$ NMR	S13
	19	ADEPT	S14
	20	Mass	S14
	21	IR	S15
	22	UV	S15
	23	UV (+ $\text{AlCl}_3$ )*	S16
	24	UV (+ $\text{NaOAc}$ )*	S16
(iv)	25	$^1\text{H}$ NMR	S17
	26	$^{13}\text{C}$ NMR	S18
	27	Mass	S19
	28	IR	S20
	29	UV	S20
	30	UV (+ $\text{AlCl}_3$ )*	S21
	31	UV (+ $\text{NaOAc}$ )*	S21
(v)	32a	$^1\text{H}$ NMR	S22
	32b	$^1\text{H}$ NMR (expanded)	S23
	33	$^{13}\text{C}$ NMR	S24
	34	COSY	S25
	35	Mass	S25
	36a	NOE	S26

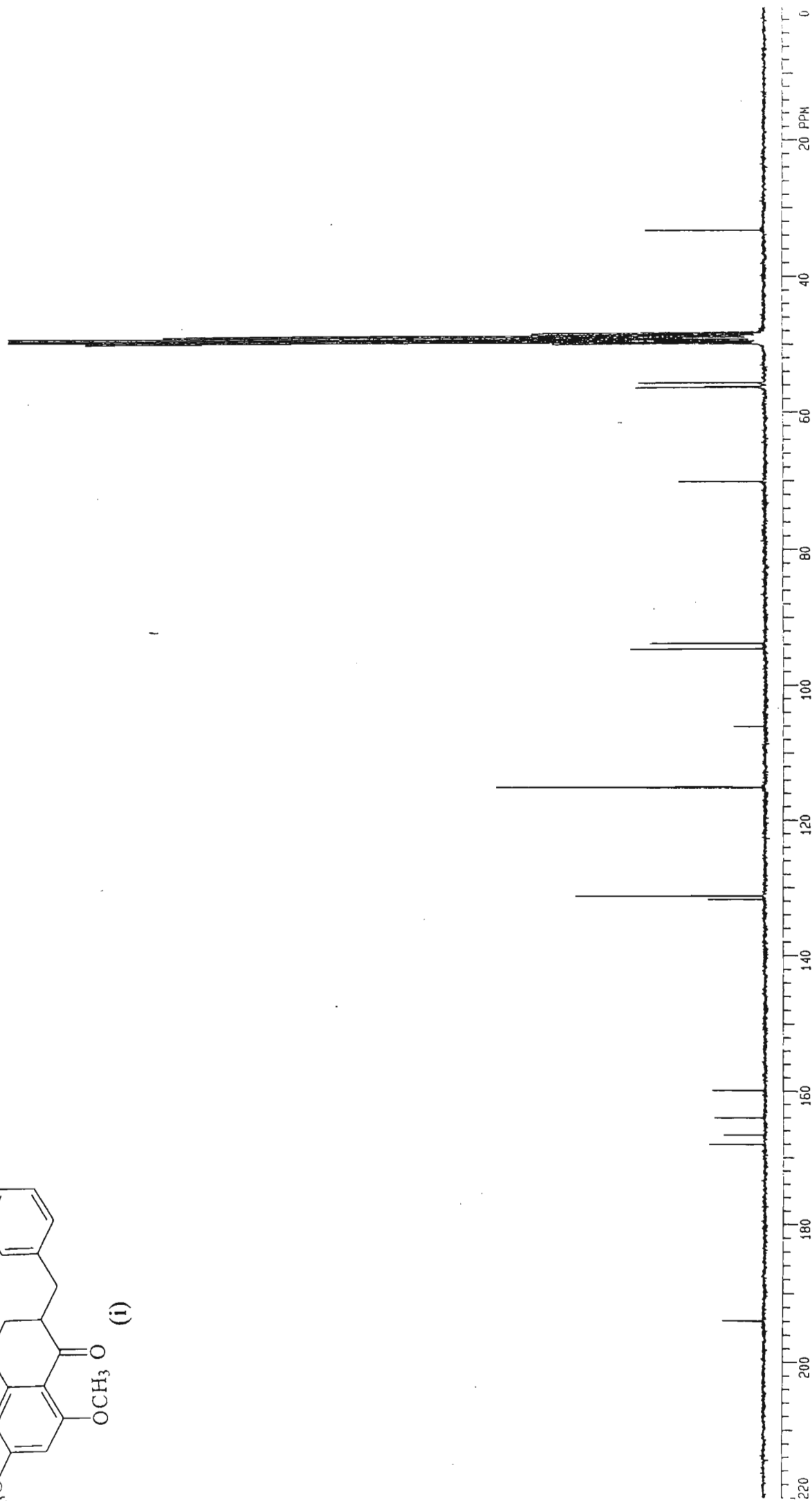
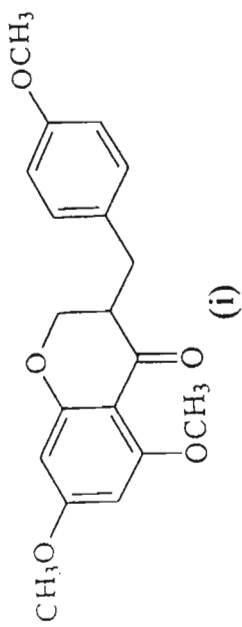
	36b	NOE	S26
	36c	NOE	S27
	37	IR	S28
	38	UV	S28
	39	UV (+ AlCl <sub>3</sub> )*	S29
	40	UV (+ NaOAc)*	S29
<b>(vi)</b>	41a	<sup>1</sup> H NMR	S30
	41b	<sup>1</sup> H NMR (expanded)	S31
	42	<sup>13</sup> C NMR	S32
	43	ADEPT	S33
	44	COSY	S34
	45	Mass	S34
	46	IR	S35
	47	UV	S35
<b>(vii)</b>	48a	<sup>1</sup> H NMR	S36
	48b	<sup>1</sup> H NMR (expanded)	S37
	49	<sup>13</sup> C NMR	S38
	50	ADEPT	S39
	51a	NOE	S39
	51b	NOE	S40
	51c	NOE	S40
	52	Mass	S41
	53	IR	S42
	54	UV	S42
<b>(viii)</b>	55	<sup>1</sup> H NMR	S43
	56	<sup>13</sup> C NMR	S44
	57	Mass	S45
	58	IR	S46
	59	UV	S46
	60	UV (+ AlCl <sub>3</sub> )*	S47
	61	UV (+ NaOAc)*	S47
<b>(ix)</b>	62	<sup>1</sup> H NMR	S48
	63	<sup>13</sup> C NMR	S49
	64	Mass	S50
	65a	NOE	S50
	65b	NOE	S51
	65c	NOE	S51
	66	IR	S52
	67	UV	S52
	68	UV (+ AlCl <sub>3</sub> )*	S53
	69	UV (+ NaOAc)*	S53
<b>(x)</b>	70	<sup>1</sup> H NMR	S54

	71	$^{13}\text{C}$ NMR	S55
	72a	NOE	S56
	72b	NOE	S56
	73	Mass	S57
	74	IR	S58
	75	UV	S58
	76	UV (+ $\text{AlCl}_3$ )*	S59
	77	UV (+ $\text{NaOAc}$ )*	S59
(xi)	78	$^1\text{H}$ NMR	S60
	79	$^{13}\text{C}$ NMR	S61
	80	ADEPT	S62
	81	HETCOR	S63
	82	Mass	S63
	83	IR	S64
	84	UV	S64
	85	UV (+ $\text{AlCl}_3$ )*	S65
	86	UV (+ $\text{NaOAc}$ )*	S65
(xii)	87	$^1\text{H}$ NMR	S66
	88	$^{13}\text{C}$ NMR	S67
	89	NOE	S68
	90	Mass	S68
	91	IR	S69
	92	UV	S69
	93	UV (+ $\text{AlCl}_3$ )*	S70
	94	UV (+ $\text{NaOAc}$ )*	S70

\* - Where there is bathochromic shift, the spectrum on the right in each profile is the one indicating the shift while the one on the left is the normal spectrum of the compound without addition of the bathochromic shift inducing agent.

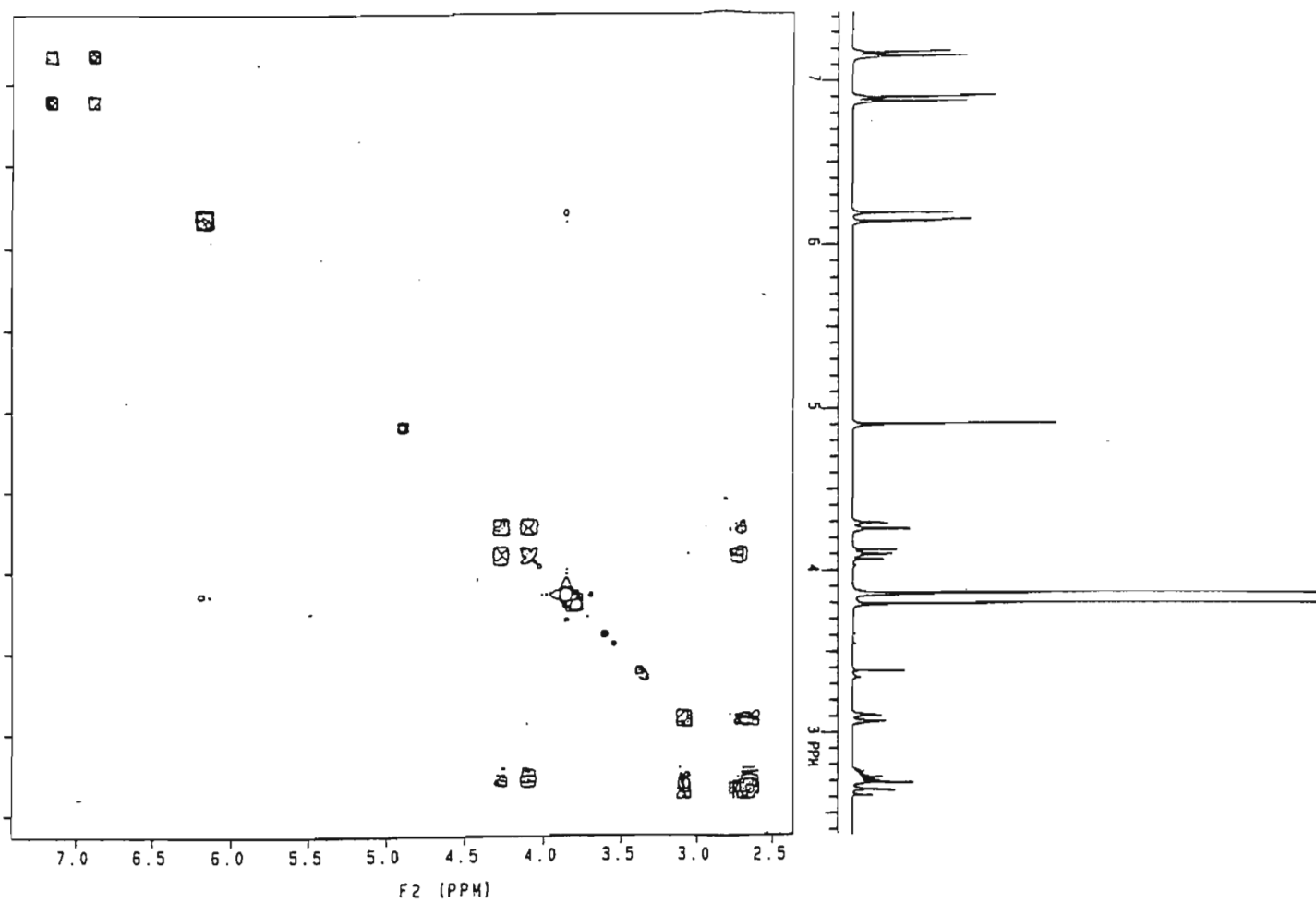


Spectrum 1 : <sup>1</sup>H NMR spectrum of compound (i)

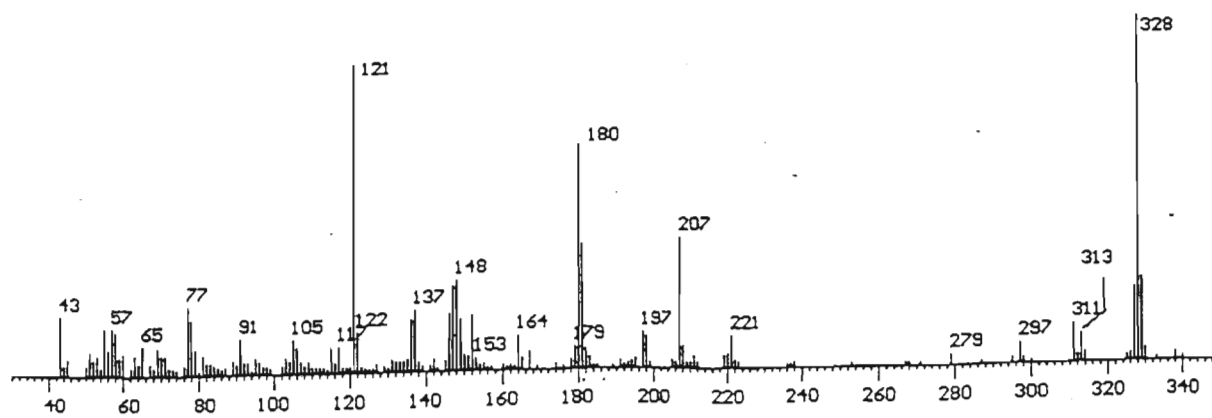


Spectrum 2 :  $^{13}\text{C}$  NMR spectrum of compound (i)

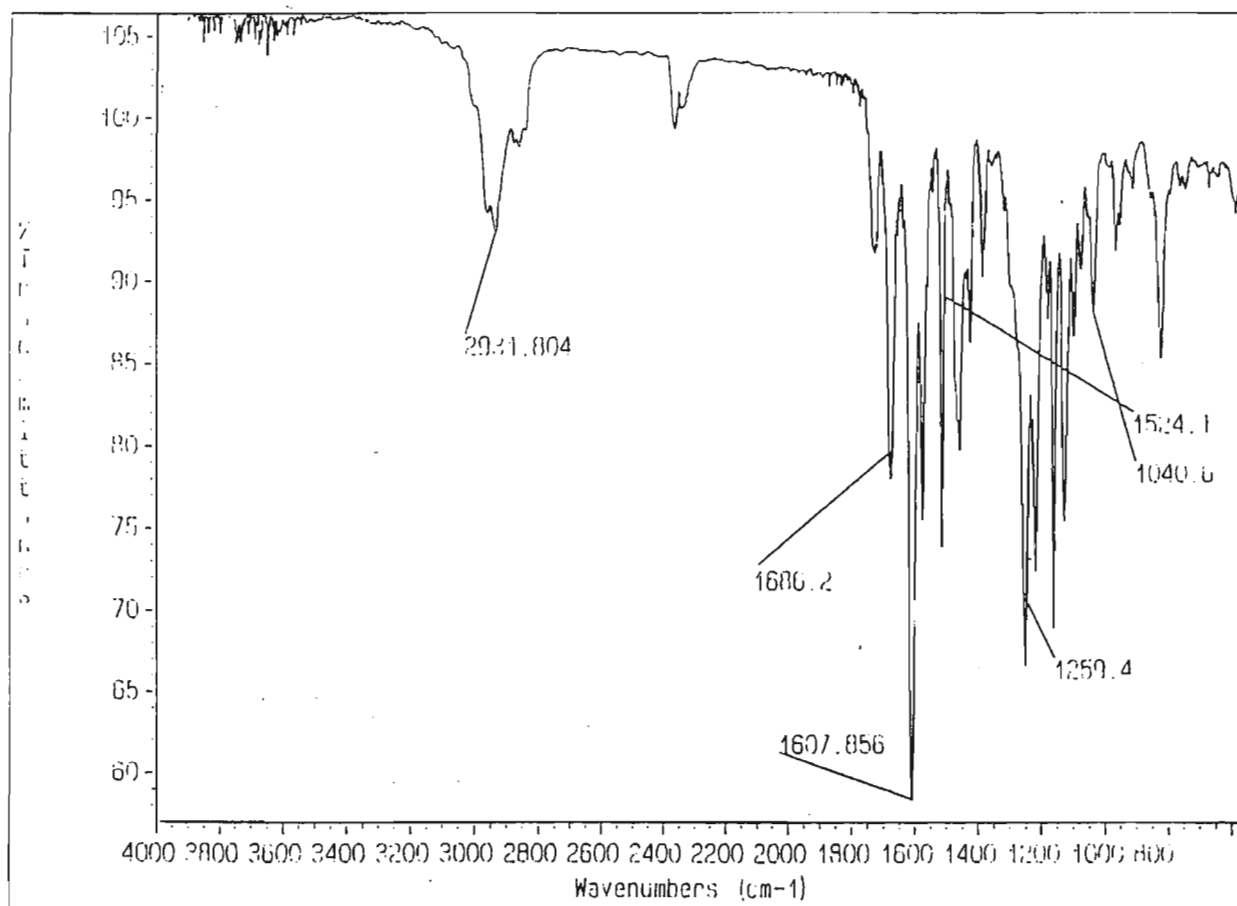




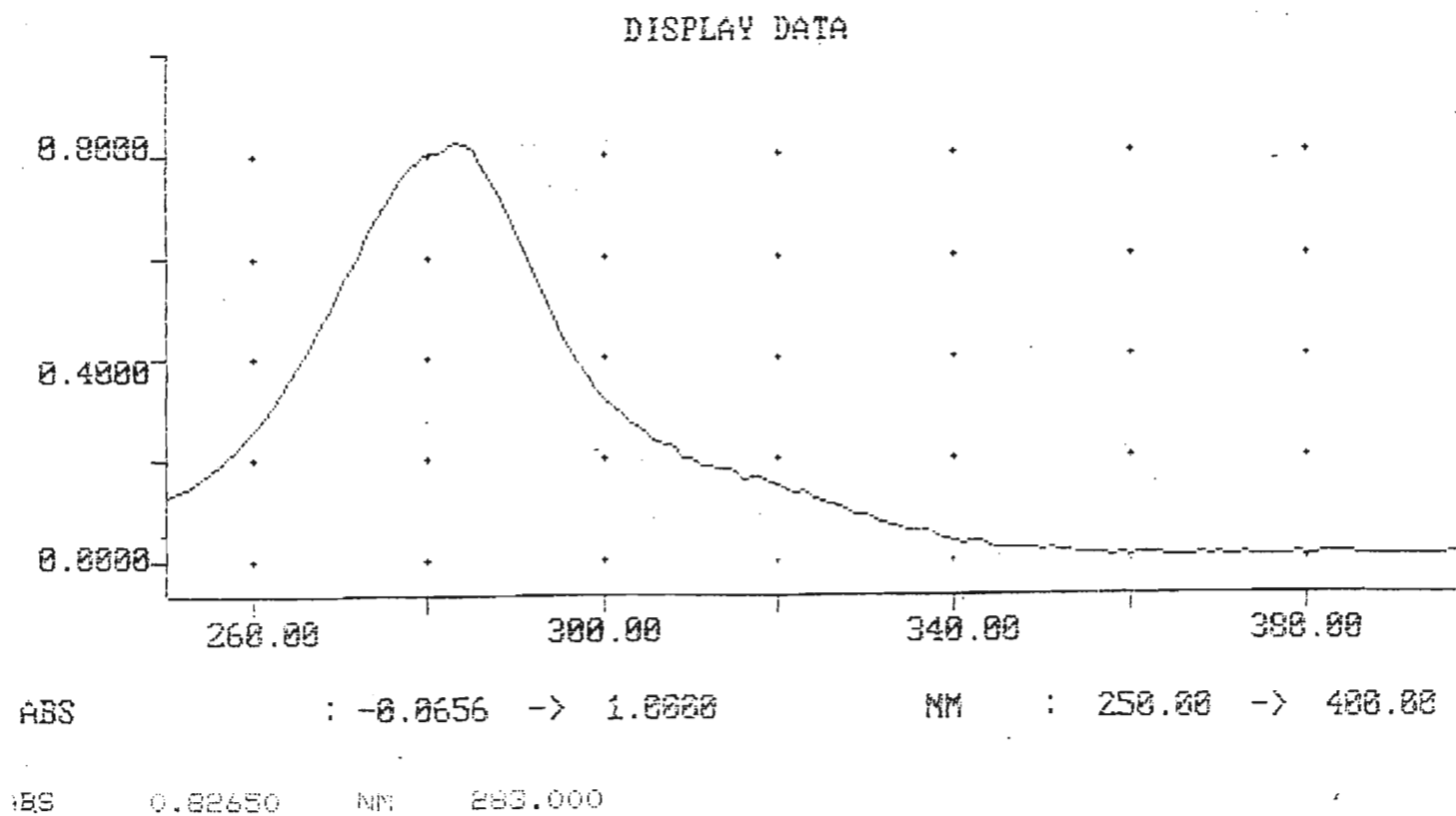
Spectrum 3 : COSY spectrum of compound (i)



Spectrum 4 : Mass spectrum of compound (i)

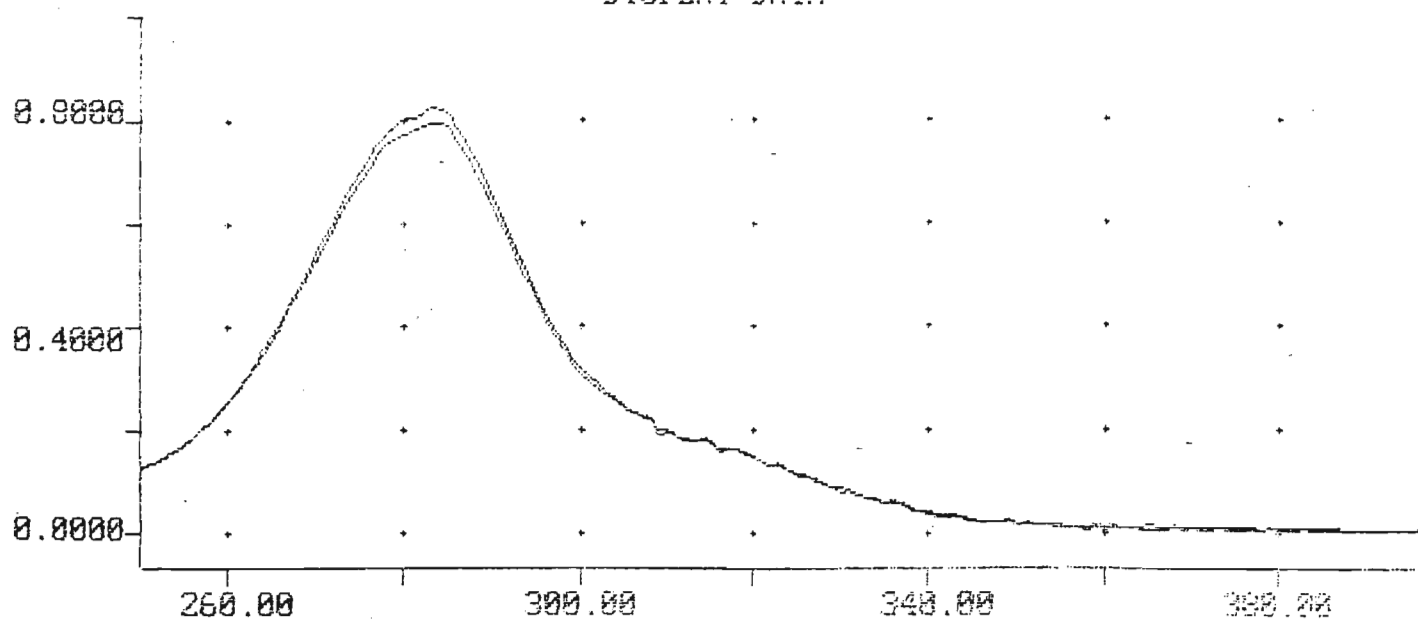


**Spectrum 5 : IR spectrum of compound (i)**



**Spectrum 6 : UV spectrum of compound (i)**

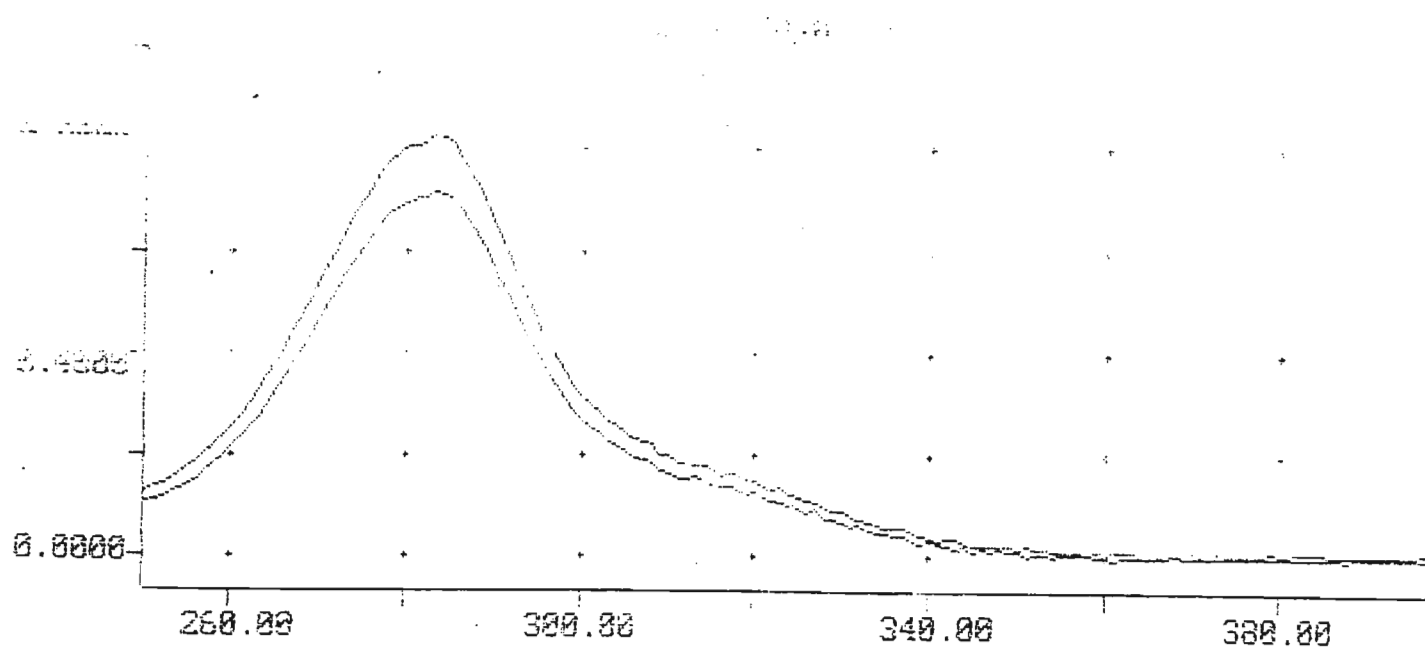
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 ABS 0.79802 NM 283.000

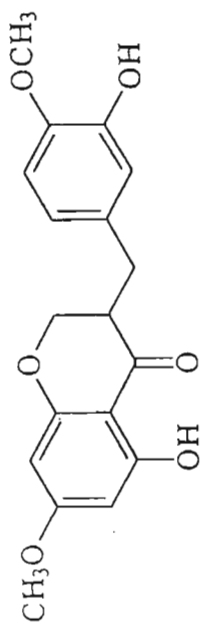
**Spectrum 7 : UV and UV (AlCl<sub>3</sub>) spectra of compound (i)**



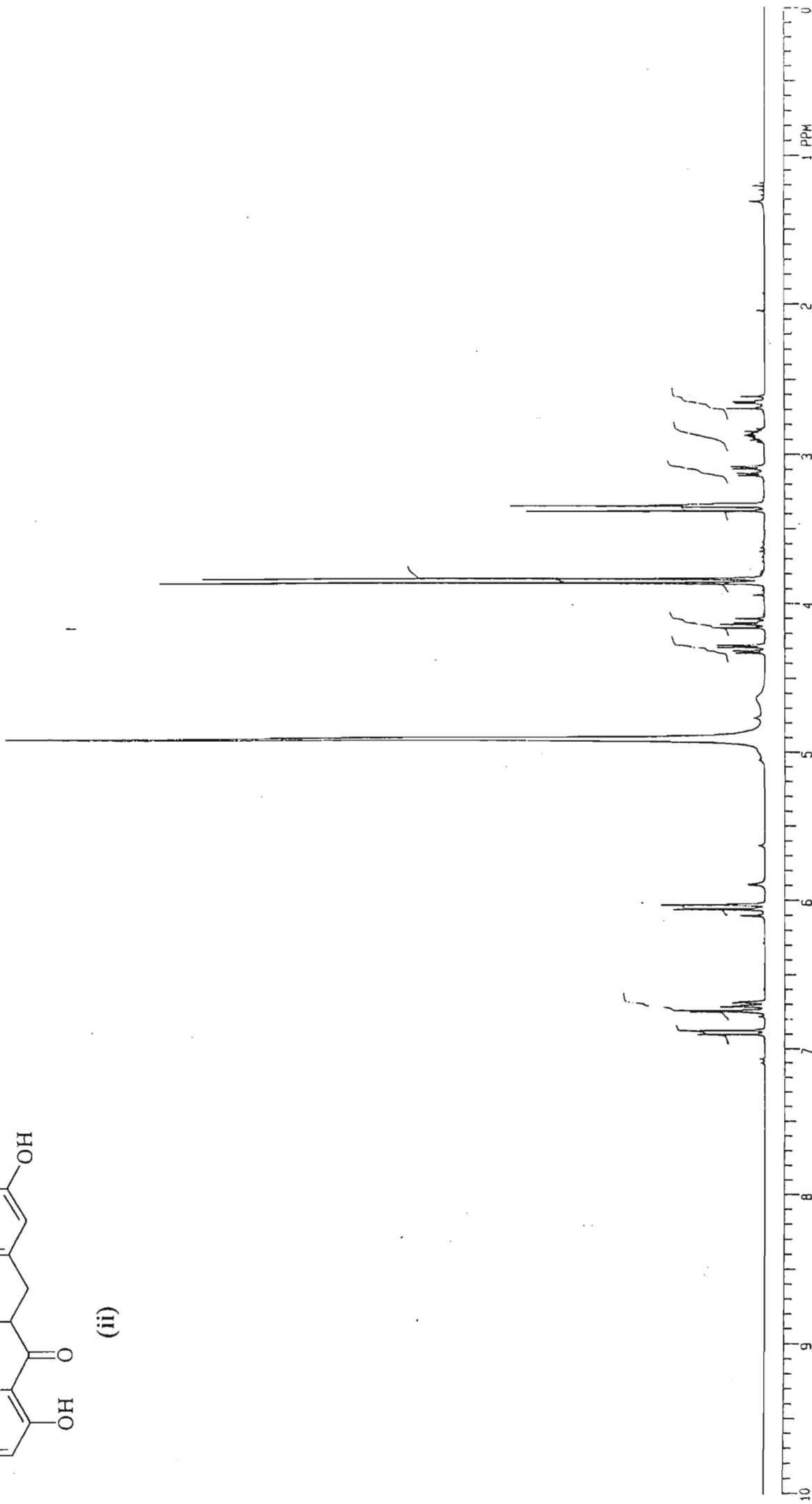
ABS : -0.0656 -> 1.0000 NM : 250.00 -> 400.00

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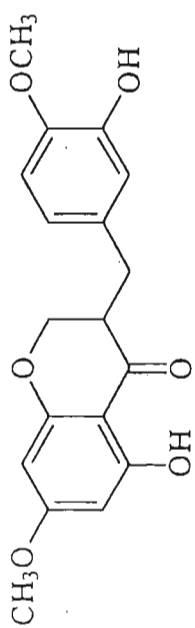
**Spectrum 8 : UV and UV (NaOAc) spectra of compound (i)**



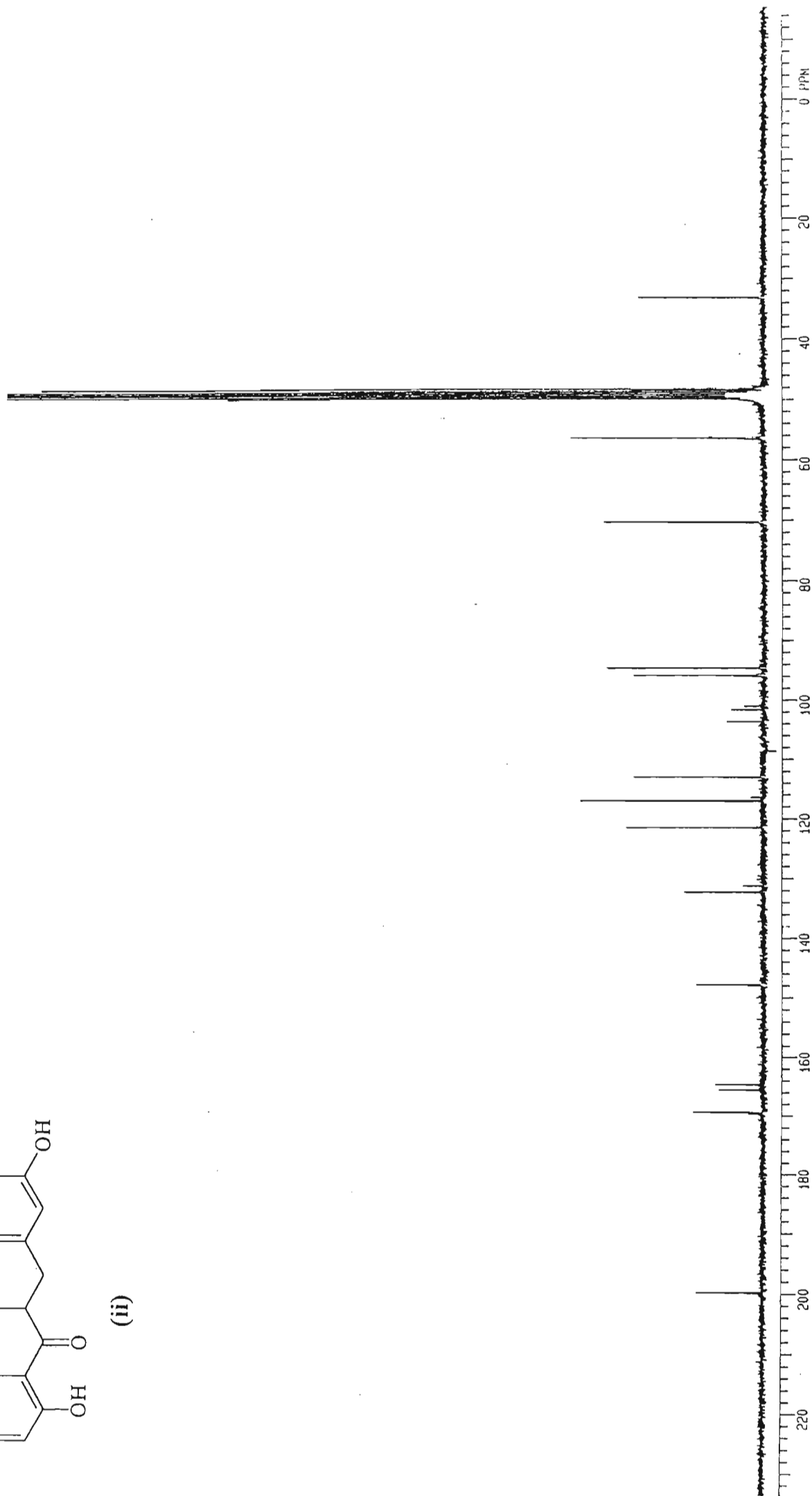
(ii)



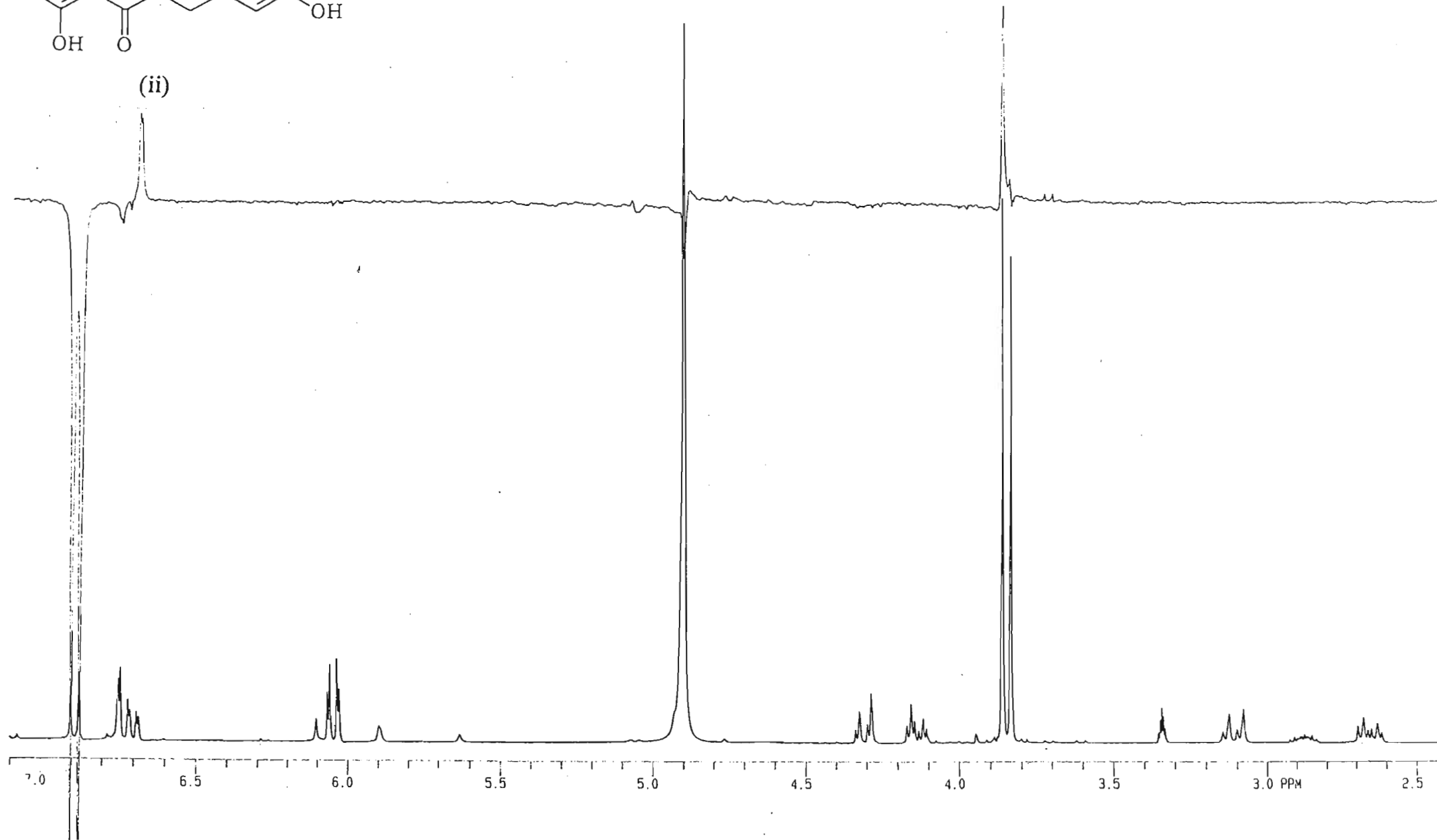
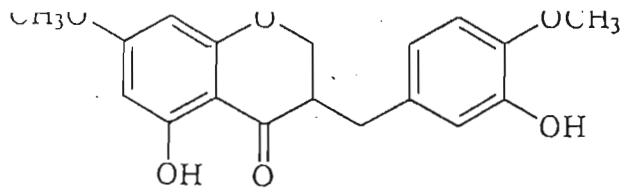
Spectrum 9 : <sup>1</sup>H NMR spectrum of compound (ii)



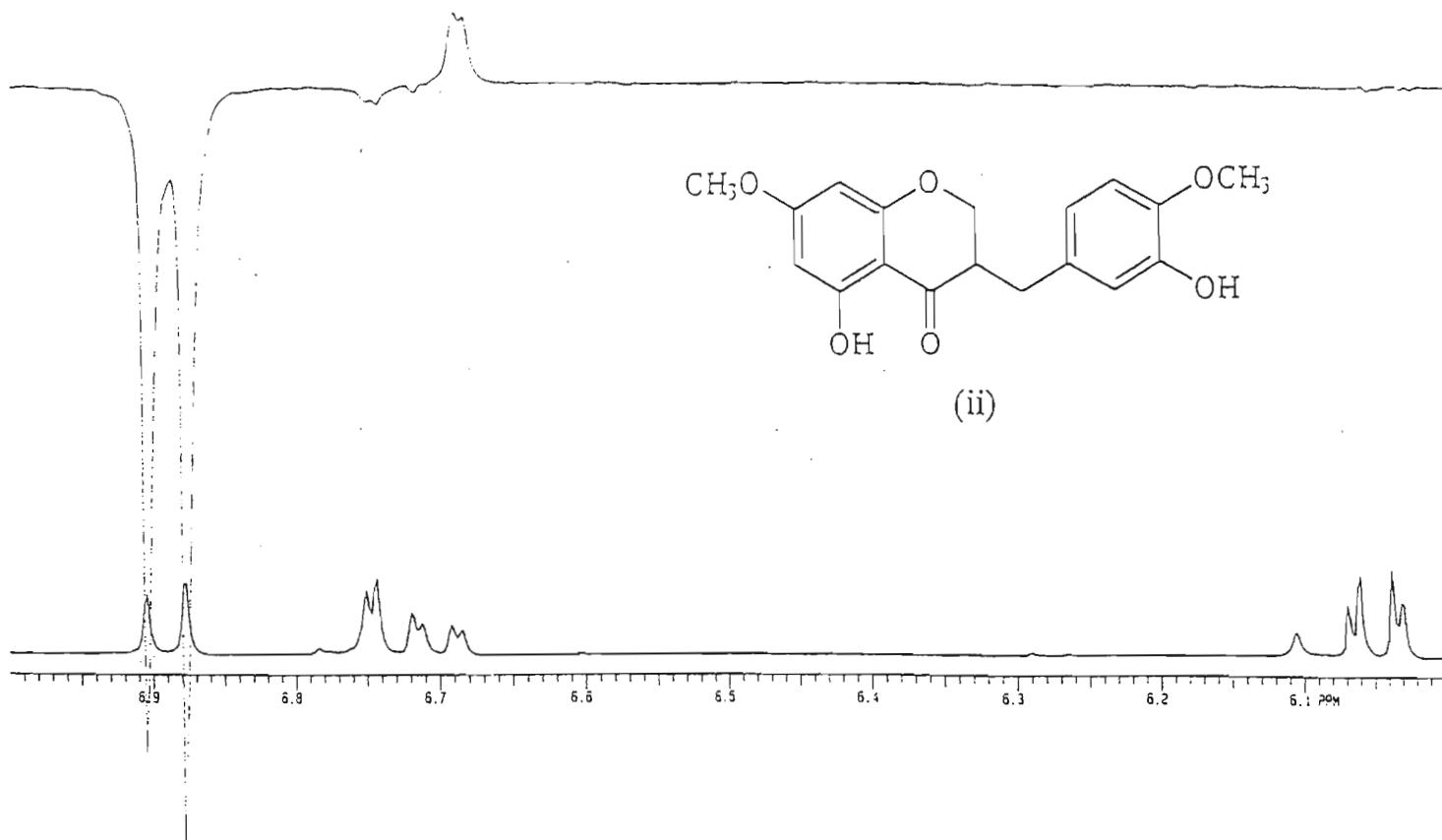
(ii)



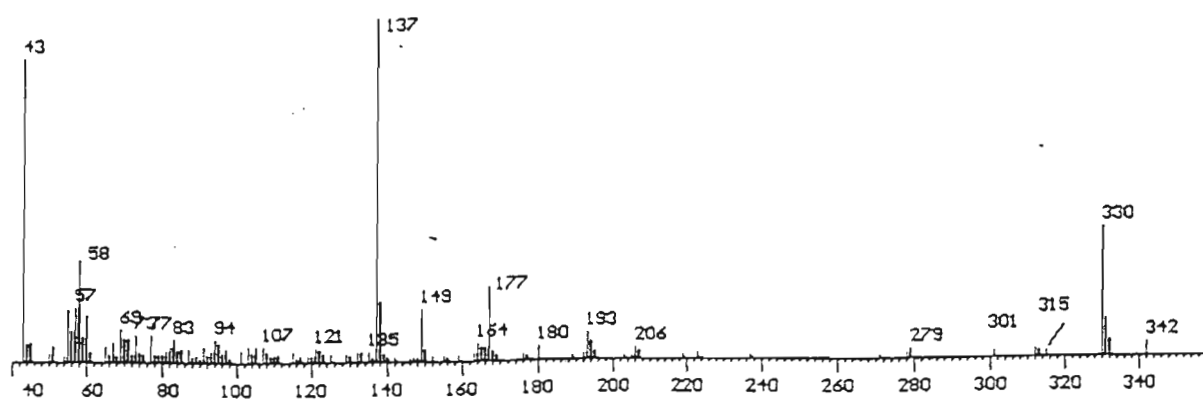
Spectrum 10:  $^{13}\text{C}$  NMR spectrum of compound (ii)



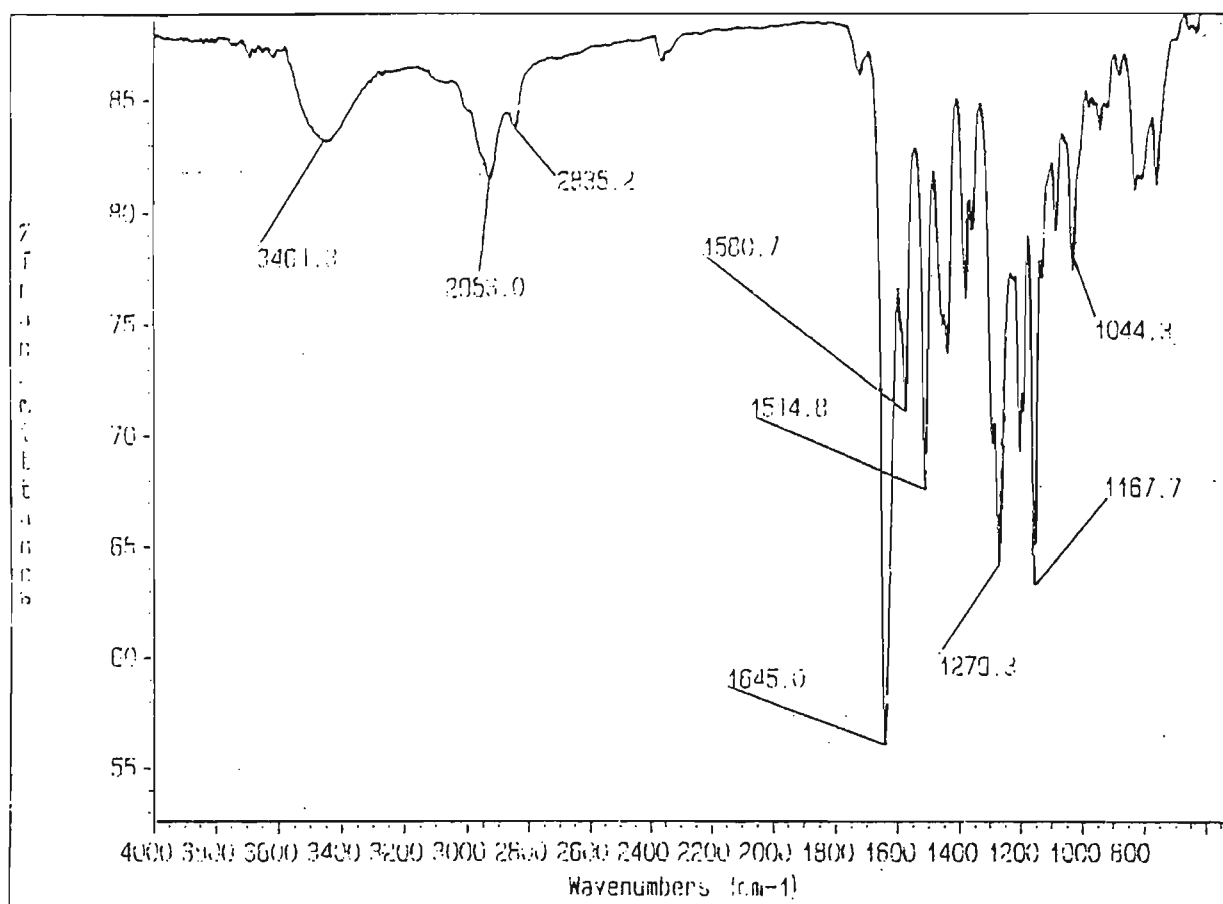
**Spectrum 11a : NOE spectrum of compound (ii)**



Spectrum 11b : NOE spectrum (expanded) of compound (ii)

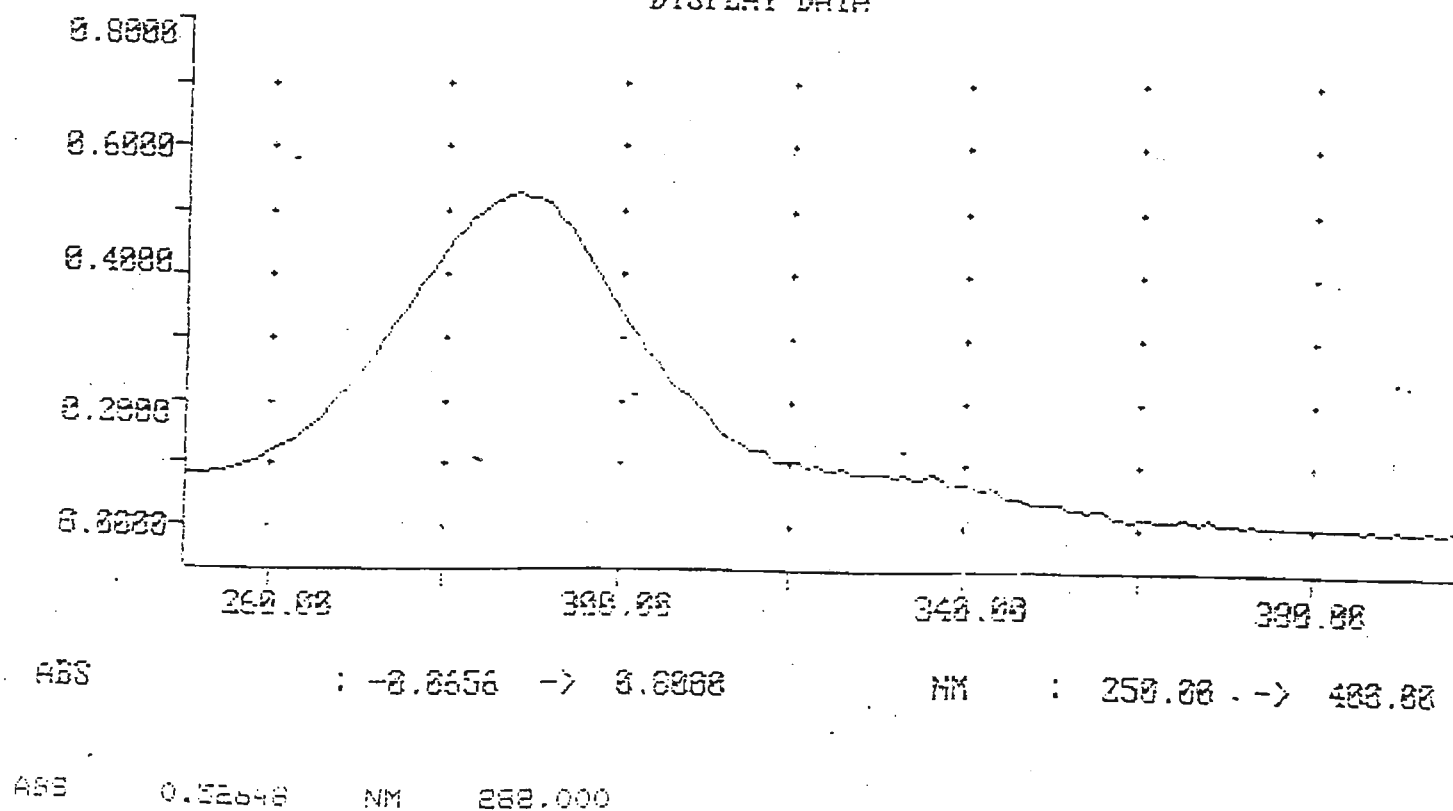


Spectrum 12 : Mass spectrum of compound (ii)



**Spectrum 13 : IR spectrum of compound (ii)**

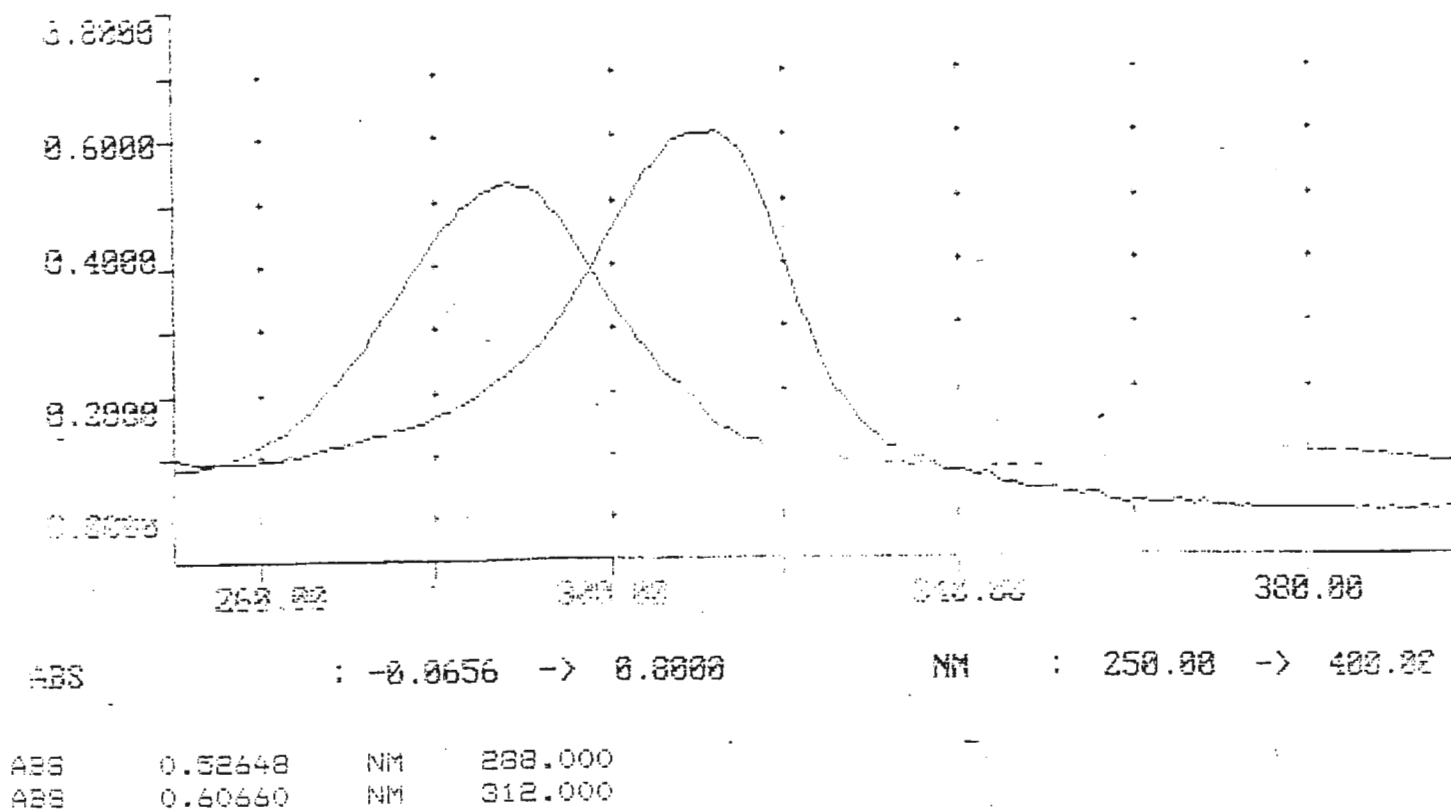
DISPLAY DATA



**Spectrum 14 : UV spectrum of compound (ii)**

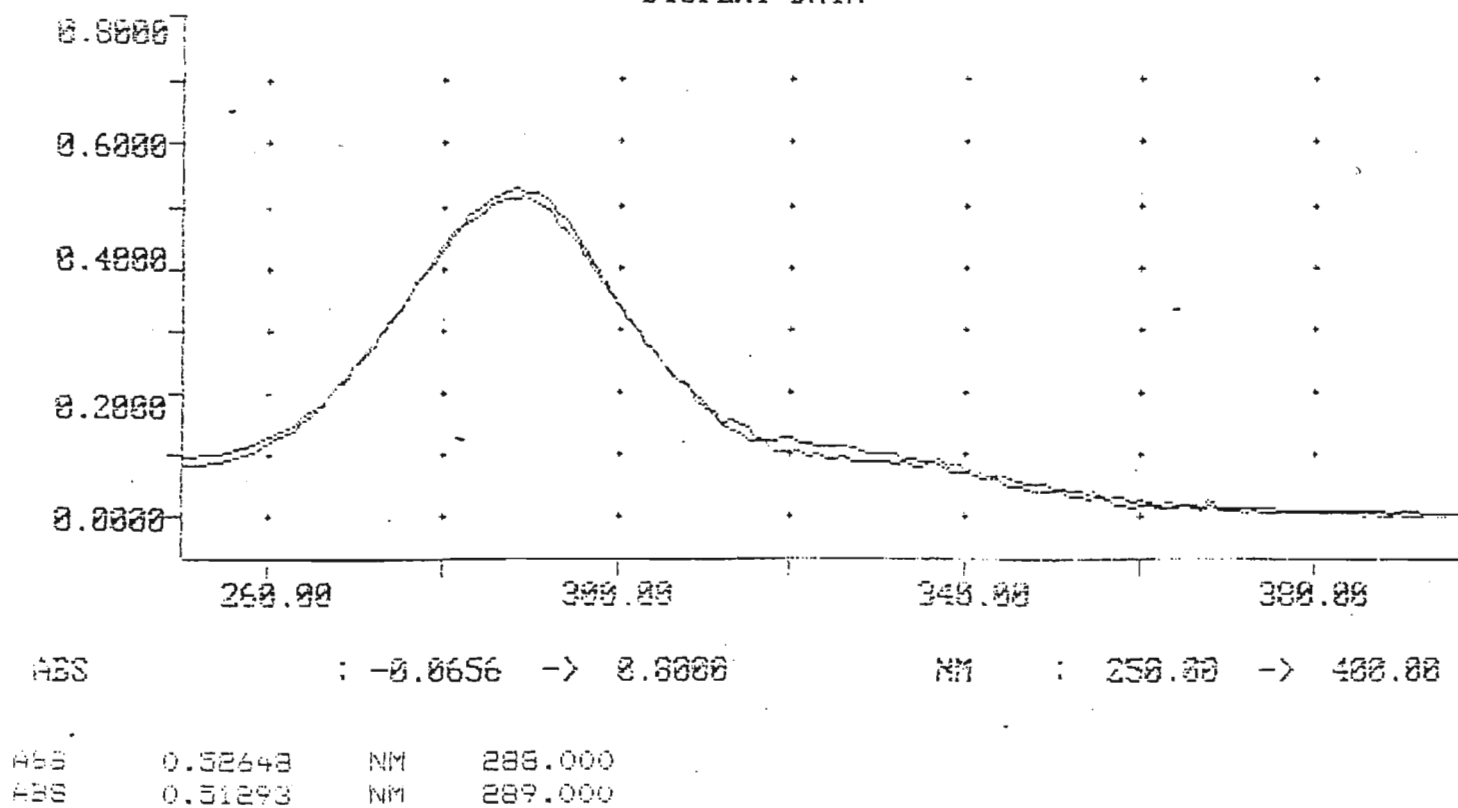


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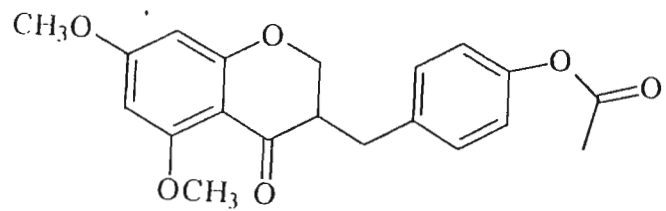


**Spectrum 15 : UV and UV (+ AlCl<sub>3</sub>) spectra of compound (ii)**

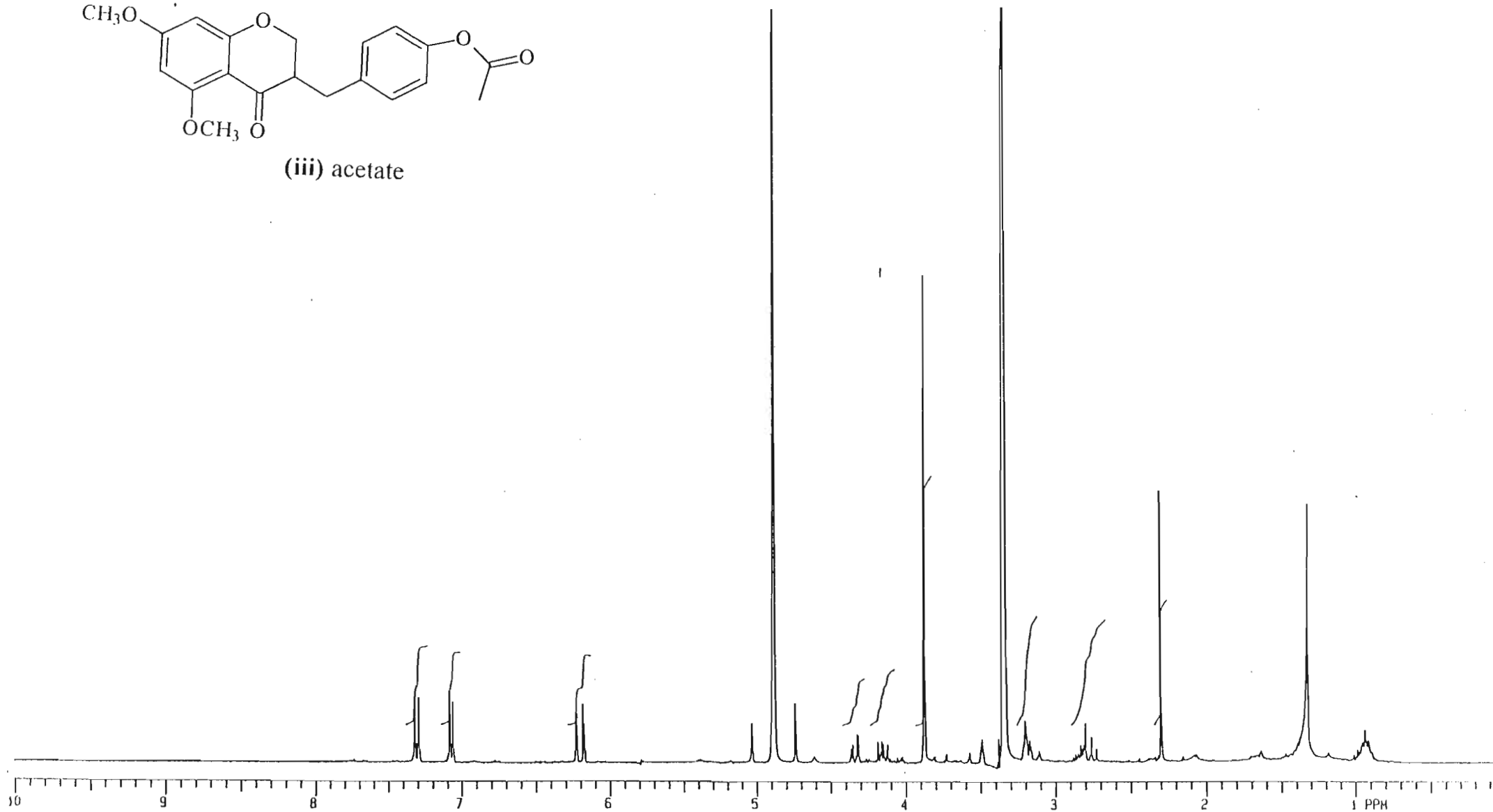
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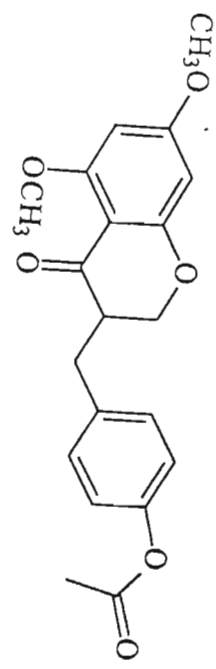
**Spectrum 16 : UV and UV (+ NaOAc) spectra of compound (ii)**



(iii) acetate

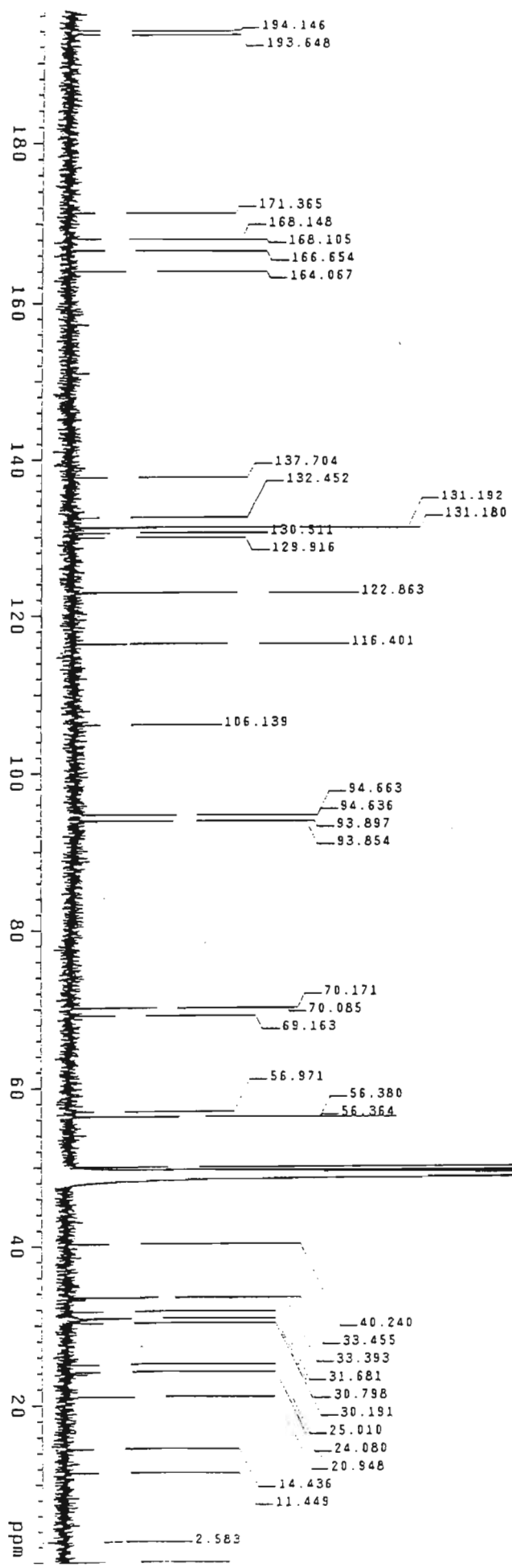


Spectrum 17 : <sup>1</sup>H NMR spectrum of compound (iii) acetate

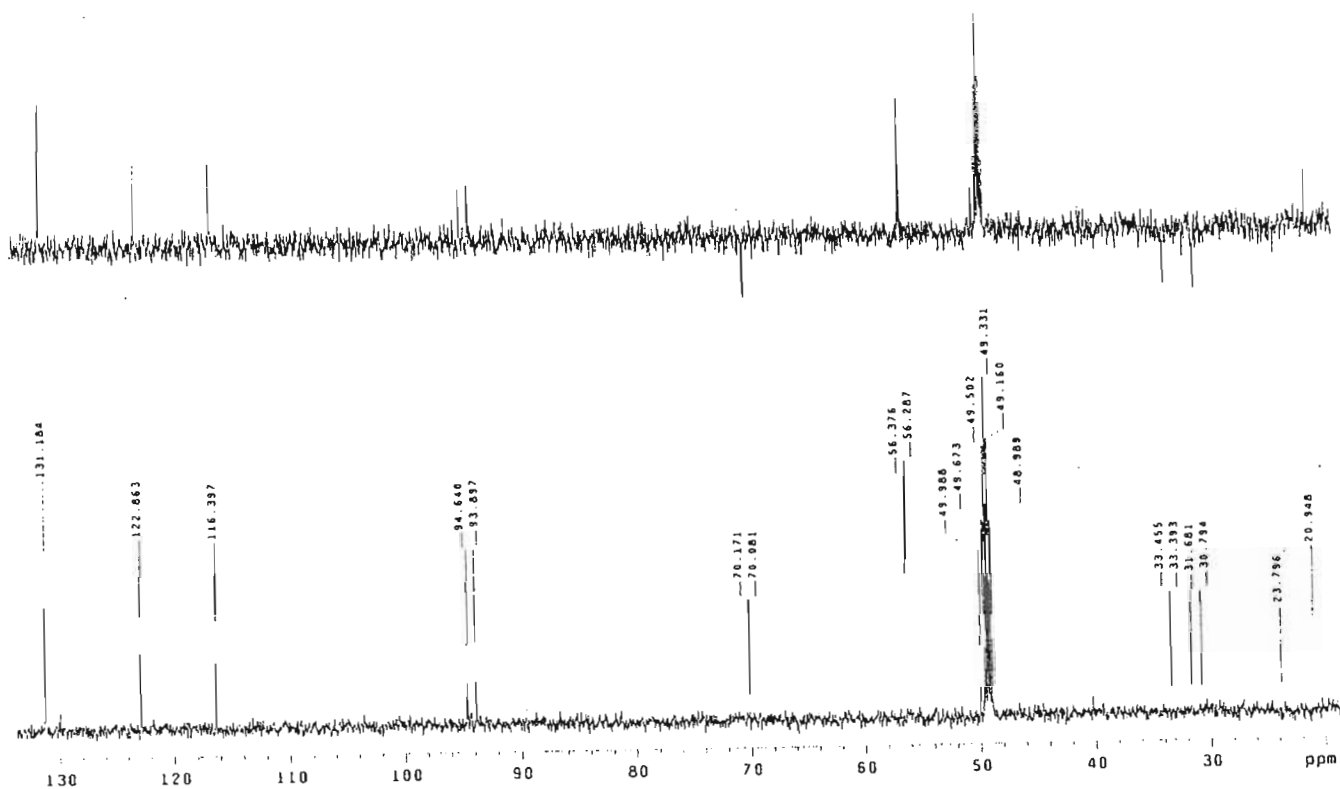


(iii) acetate

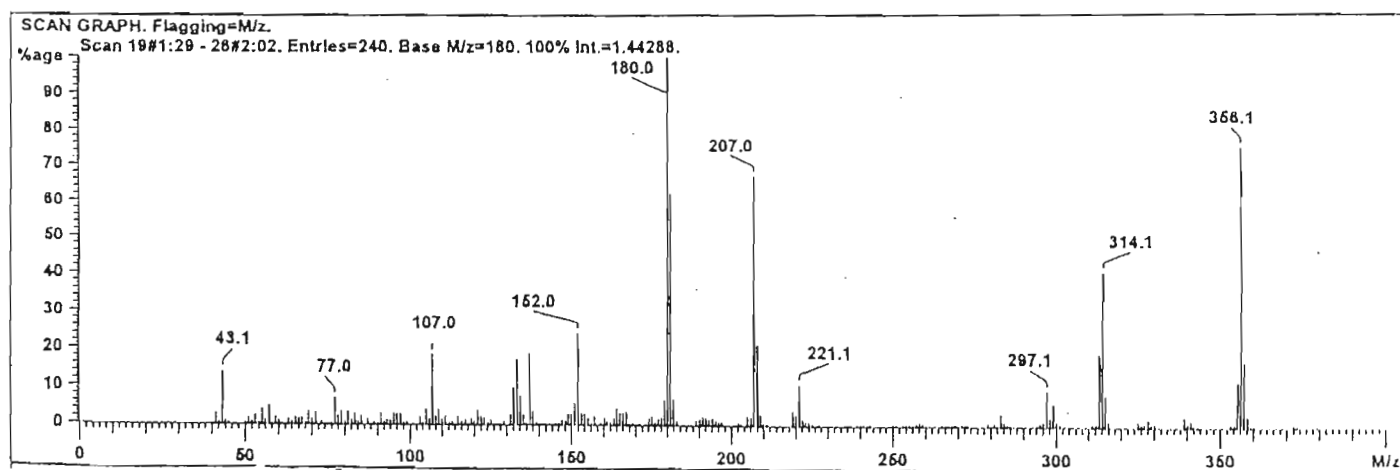
Spectrum 18 :  $^{13}\text{C}$  NMR spectrum of compound (iii) acetate



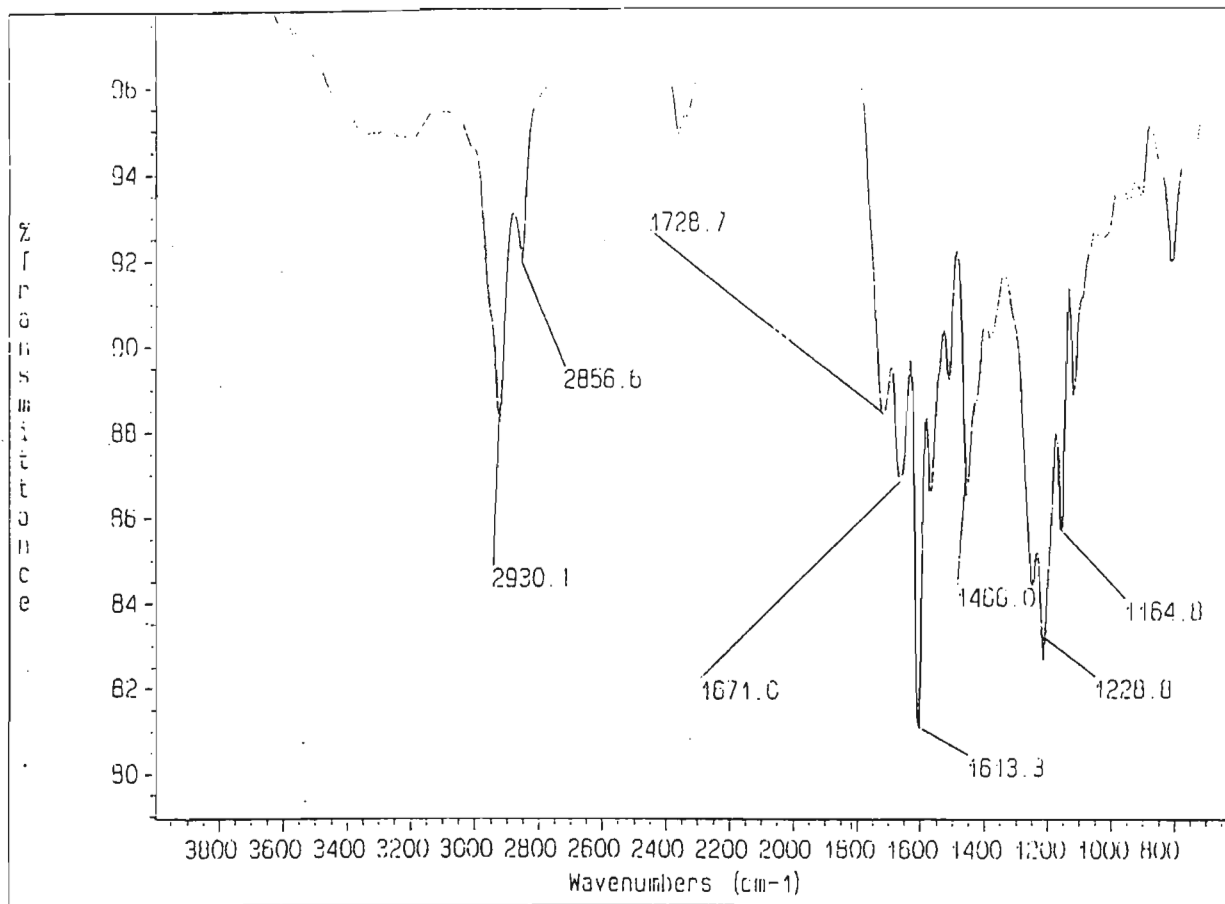
Upper trace -Cl<sub>1</sub>,Cl<sub>3</sub> up,Cl<sub>2</sub> down  
 Lower trace-Cl<sub>1</sub> only



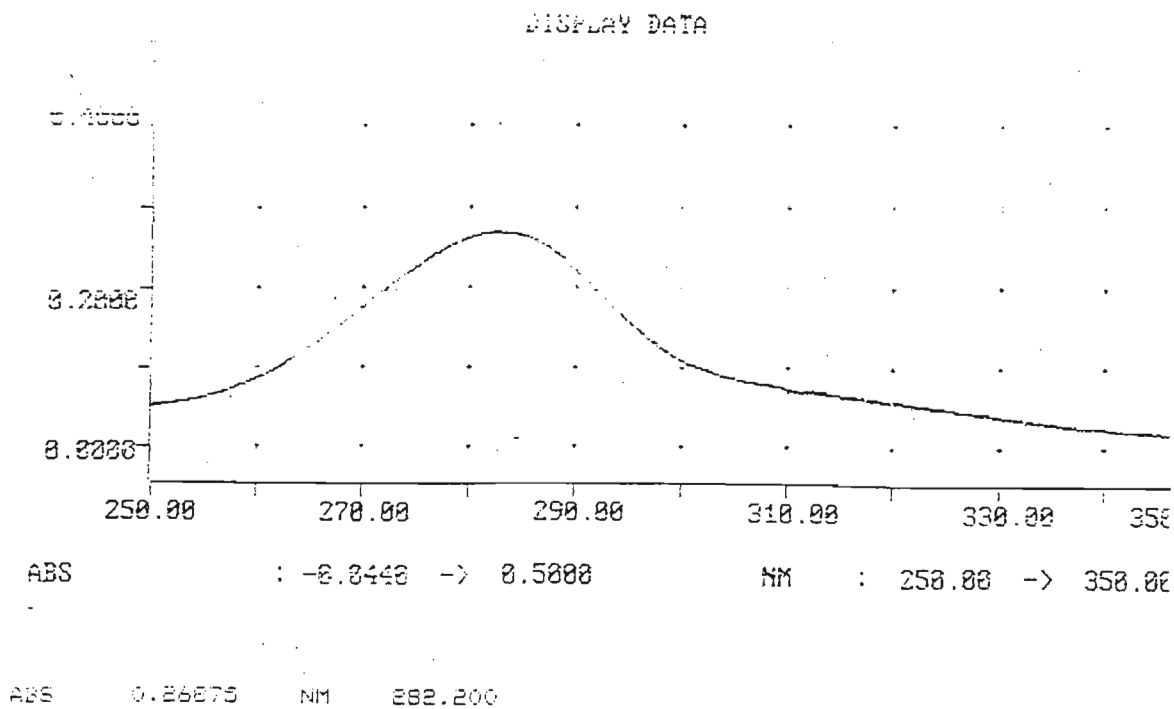
**Spectrum 19 : ADEPT spectrum of compound (iii) acetate**



**Spectrum 20 : Mass spectrum of compound (iii) acetate**

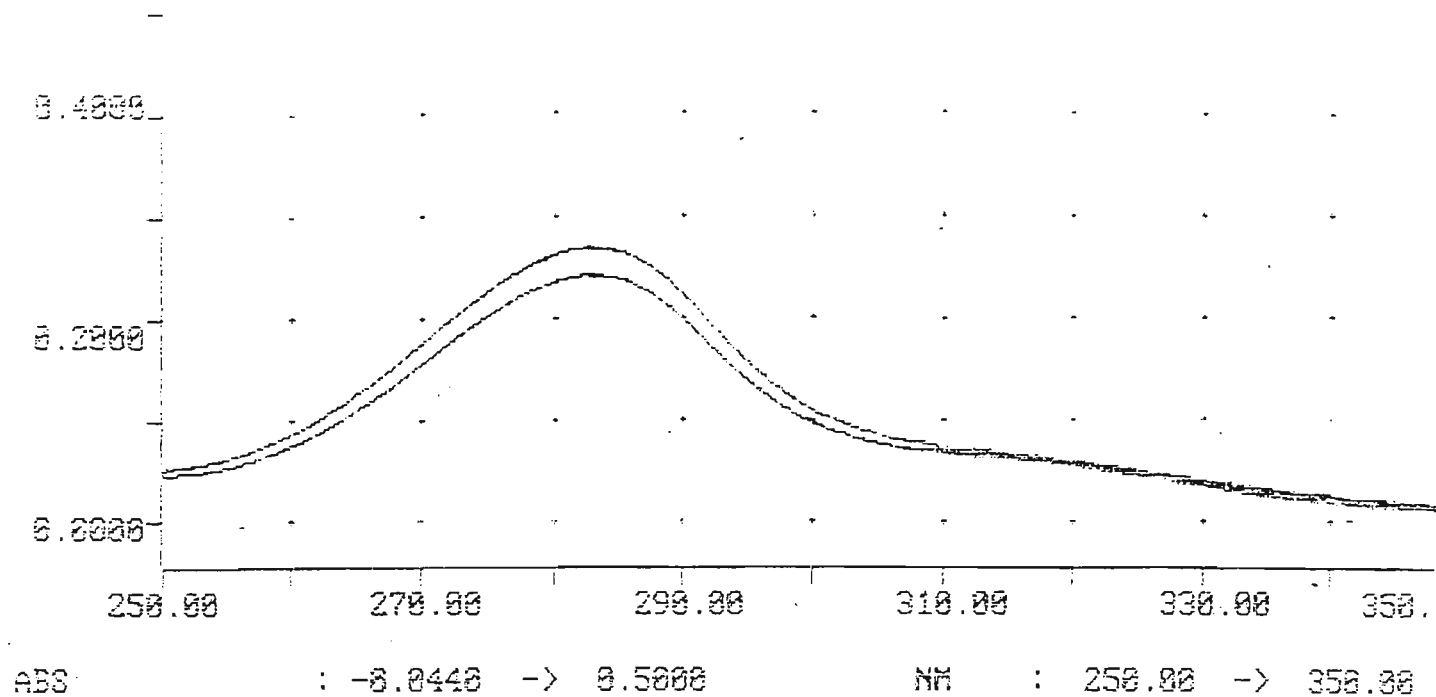


**Spectrum 21 : IR spectrum of compound (iii) acetate**



**Spectrum 22 : UV spectrum of compound (iii) acetate**

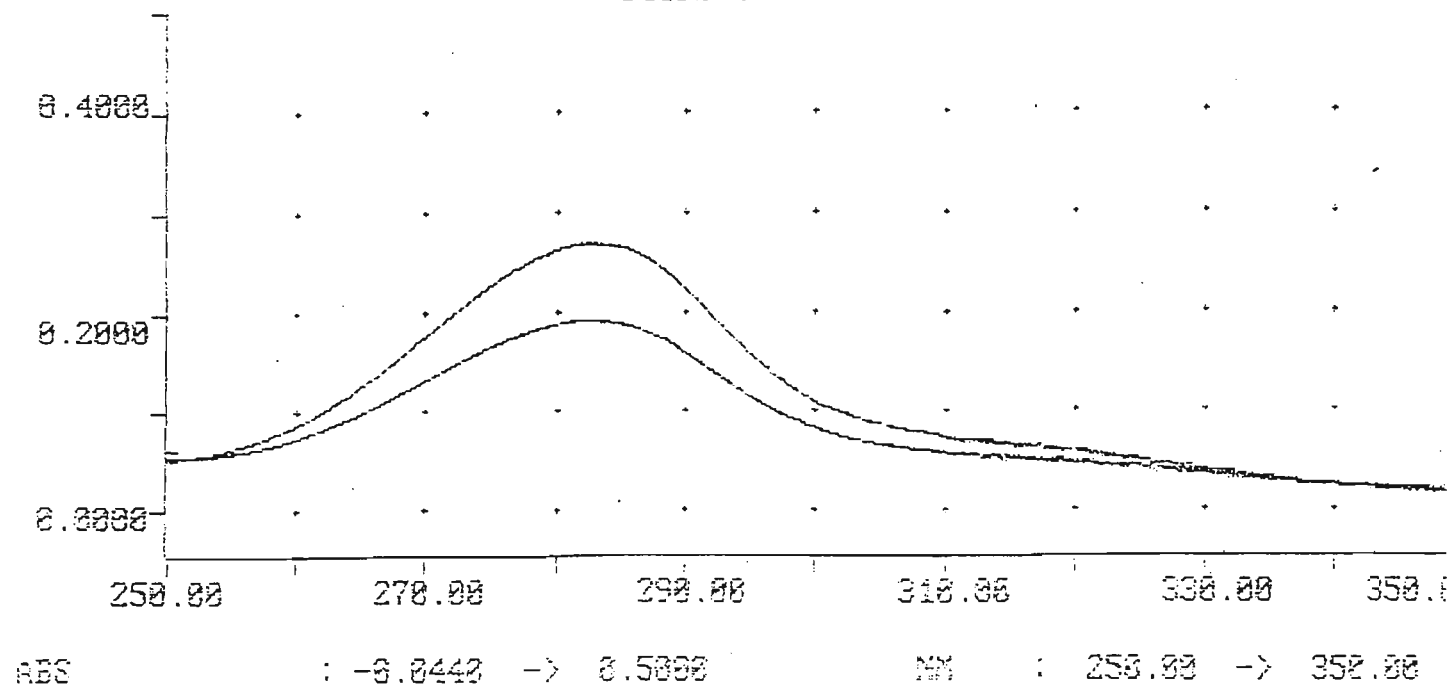
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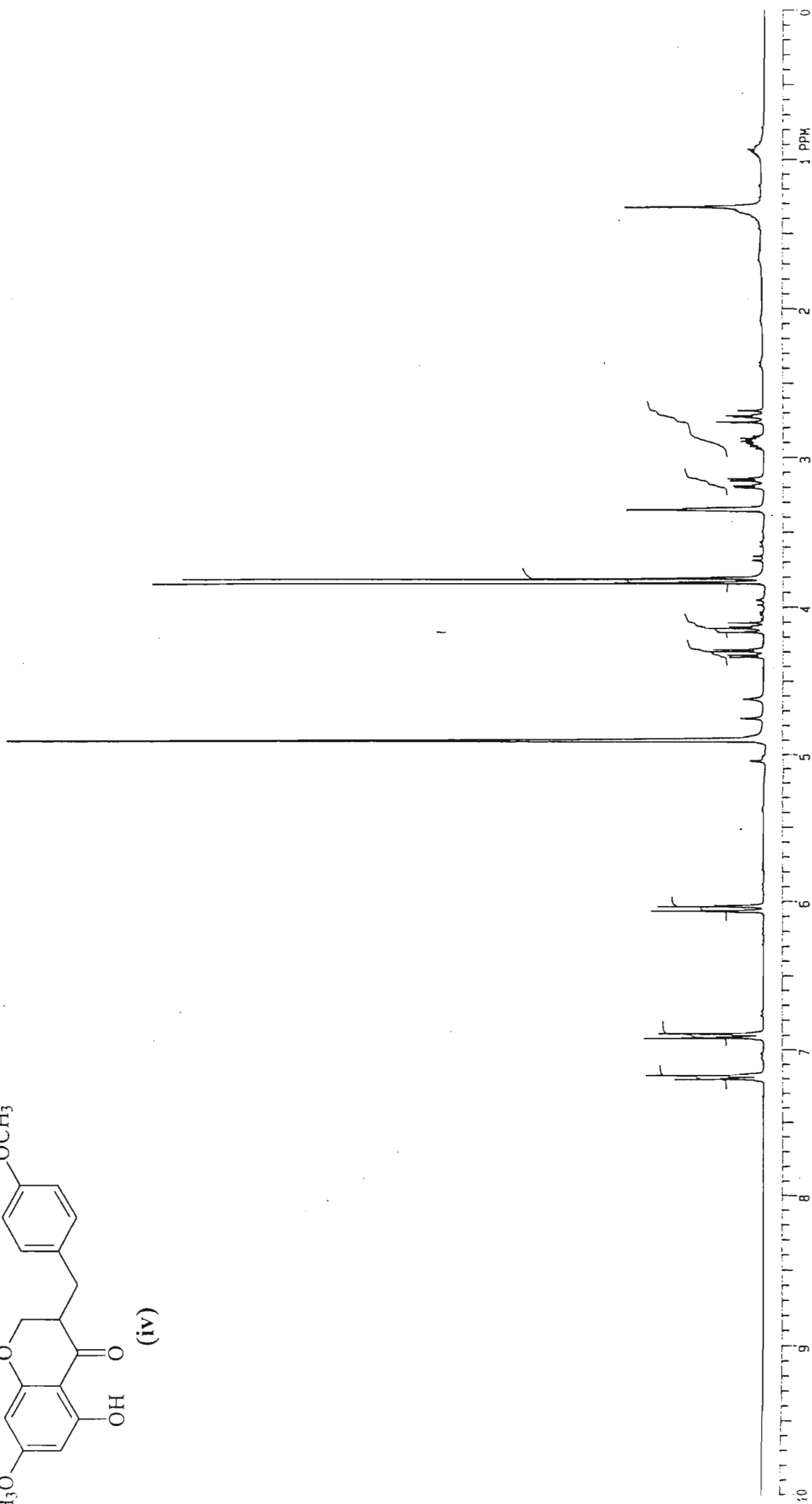
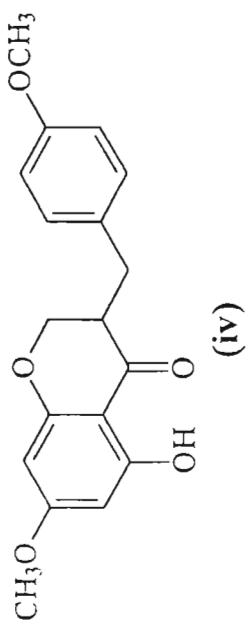
**Spectrum 23 : UV and UV (+ AlCl<sub>3</sub>) spectrum of compound (iii) acetate**

## DISPLAY DATA

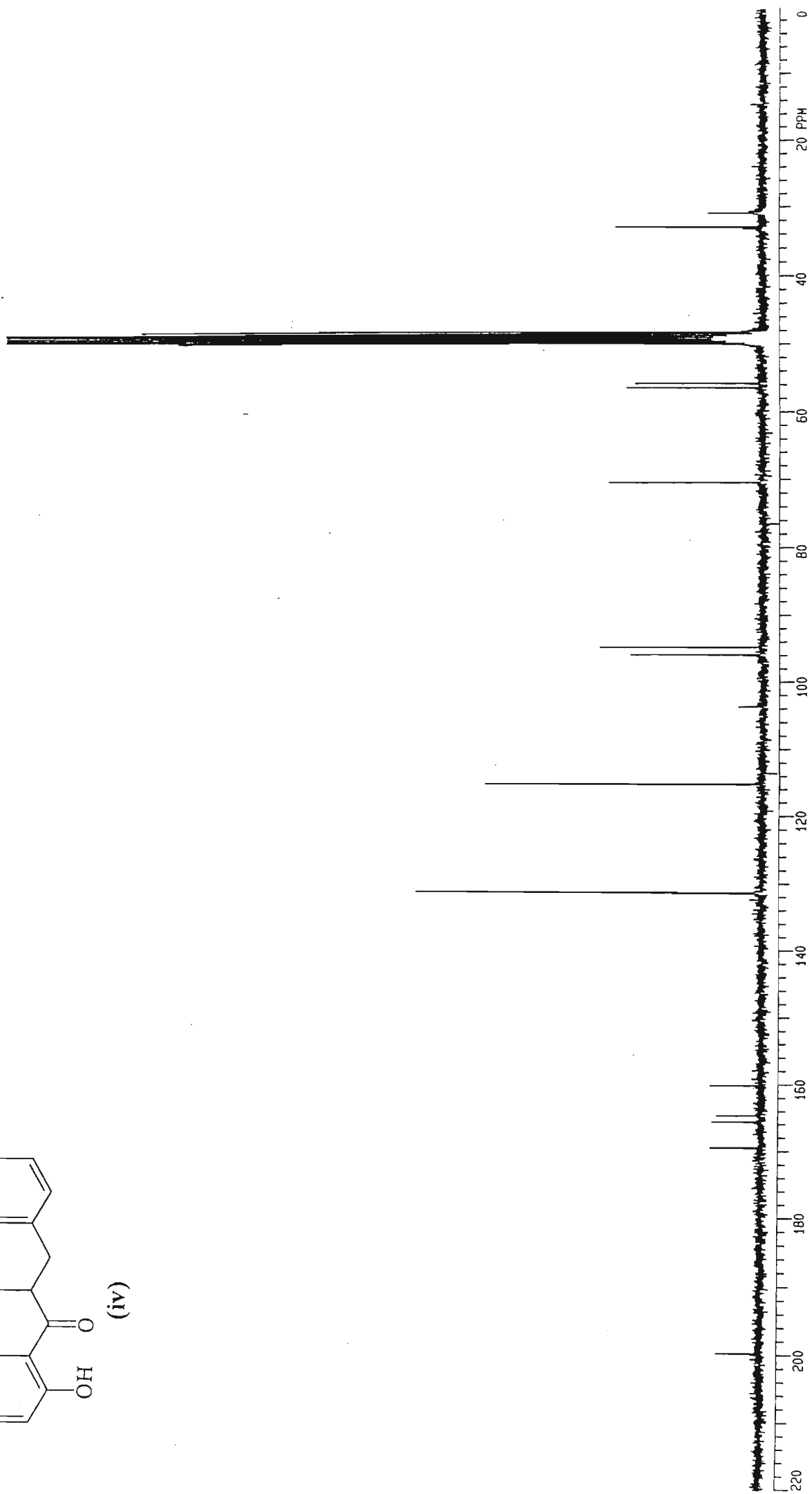
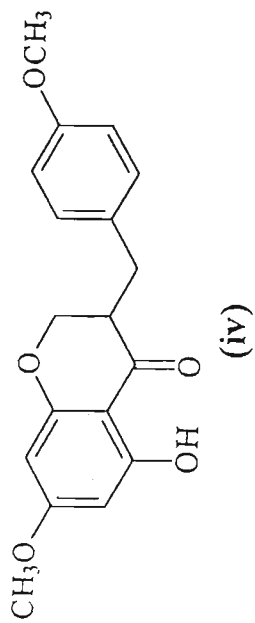


ABS      0.26575      NM      282.300  
 ABS      0.19210      NM      282.500

**Spectrum 24 : UV and UV (+ NaOAc) spectrum of compound (iii) acetate**

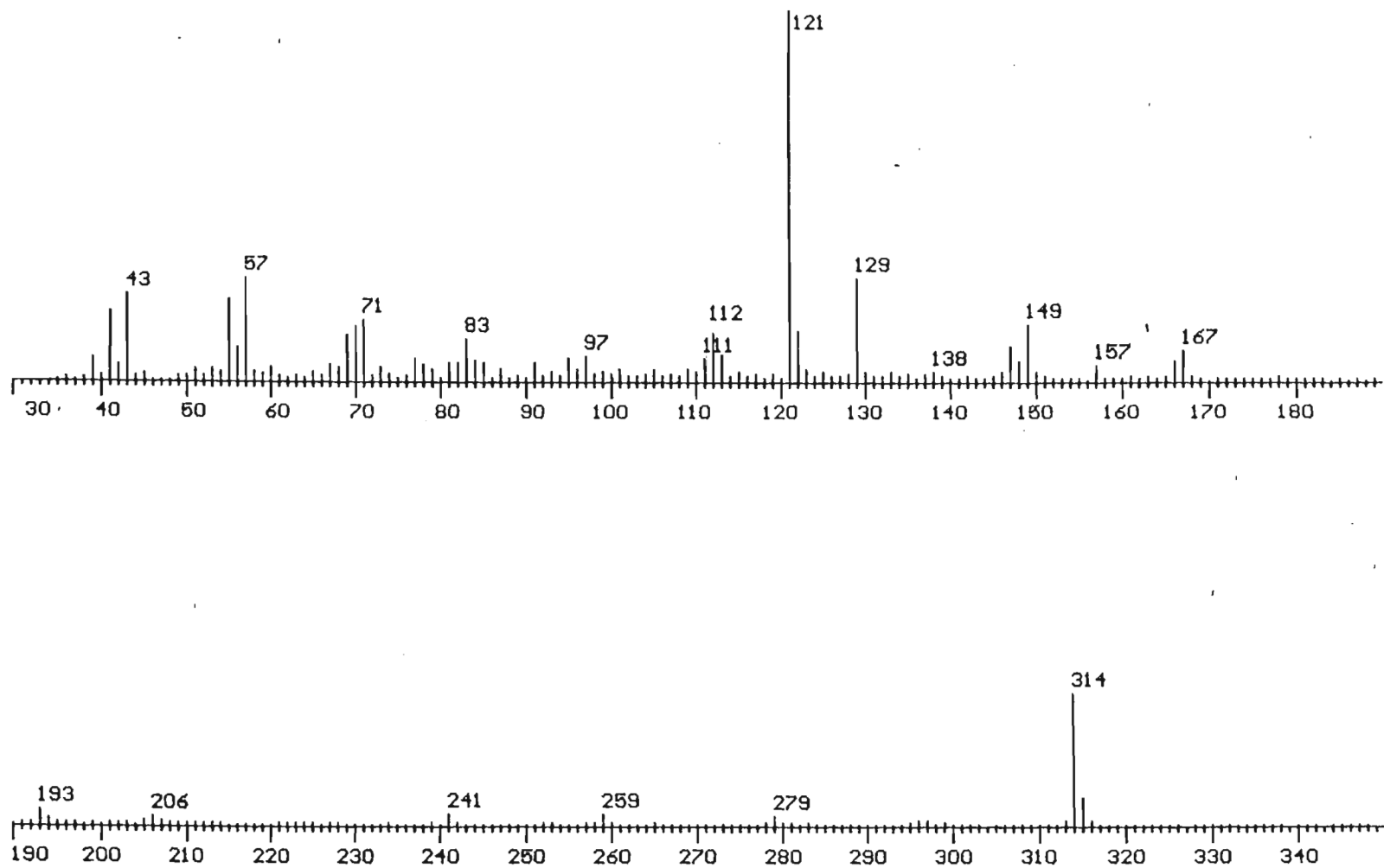


Spectrum 25: <sup>1</sup>H NMR spectrum of compound (iv)

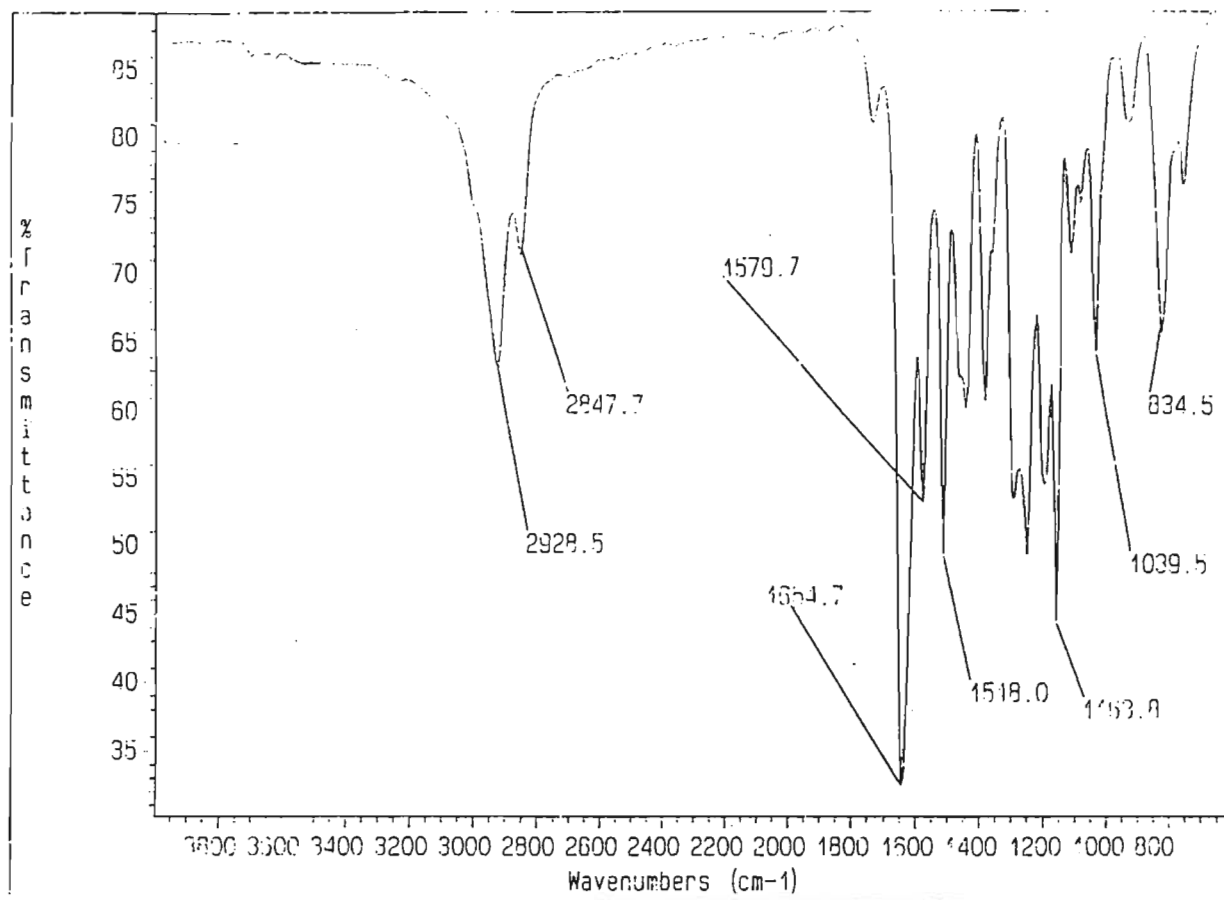


Spectrum 26 :  $^{13}\text{C}$  NMR spectrum of compound (iv)

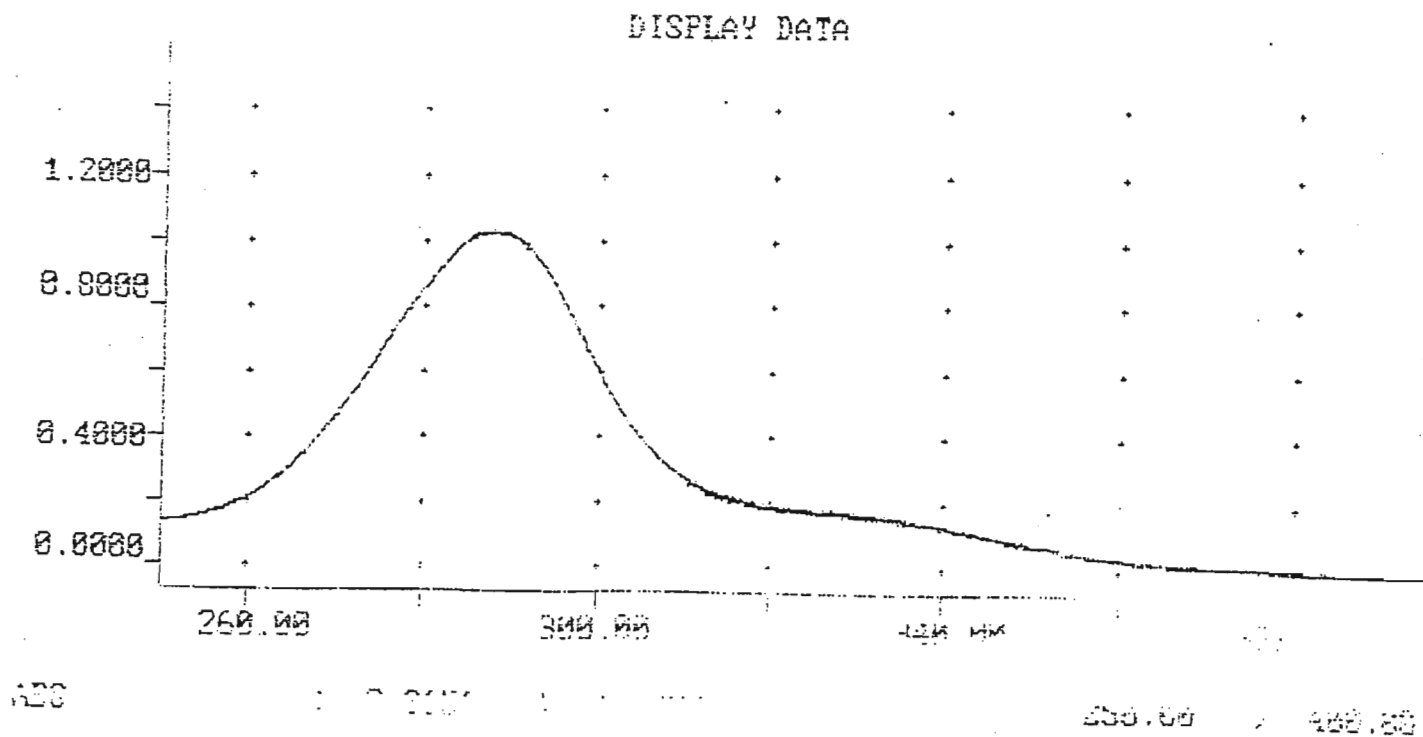




Spectrum 27 : Mass spectrum of compound (iv)

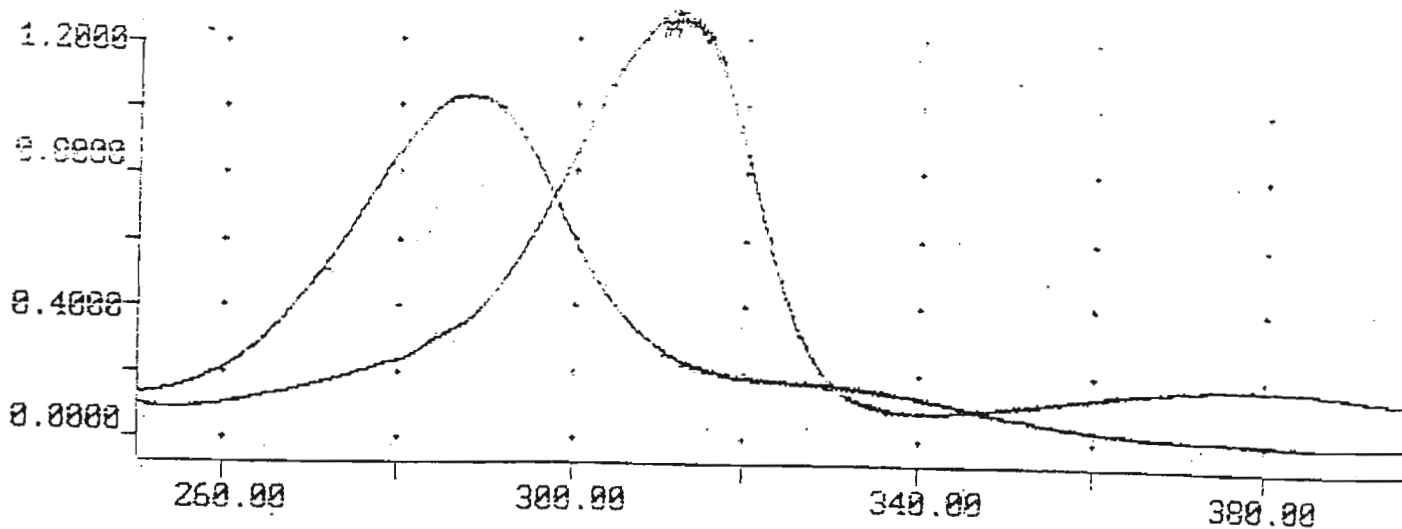


Spectrum 28 : IR spectrum of compound (iv)



Spectrum 29 : UV spectrum of compound (iv)

DISPLAY DATA

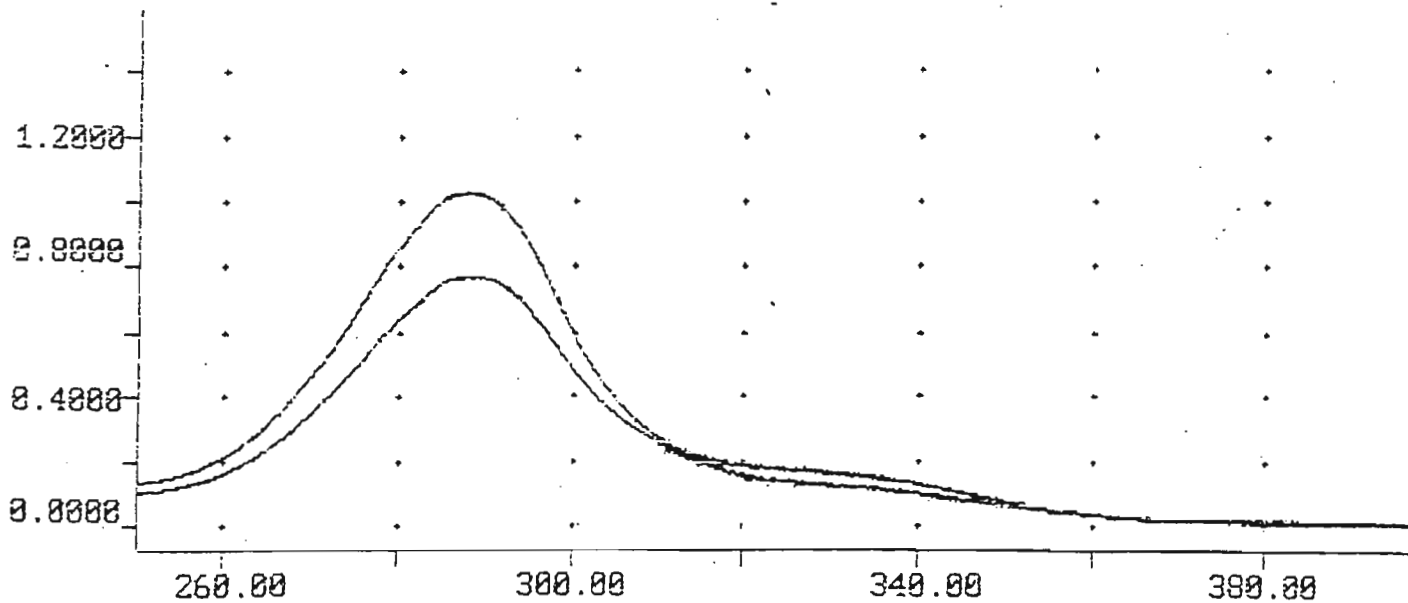


ABS : -0.8656 -> 1.5839 NM : 250.00 -> 400.00

ABS	1.02914	NM	287.450
ABS	1.28449	NM	311.100

Spectrum 30 : UV and UV (AlCl<sub>3</sub>) spectra of compound (iv)

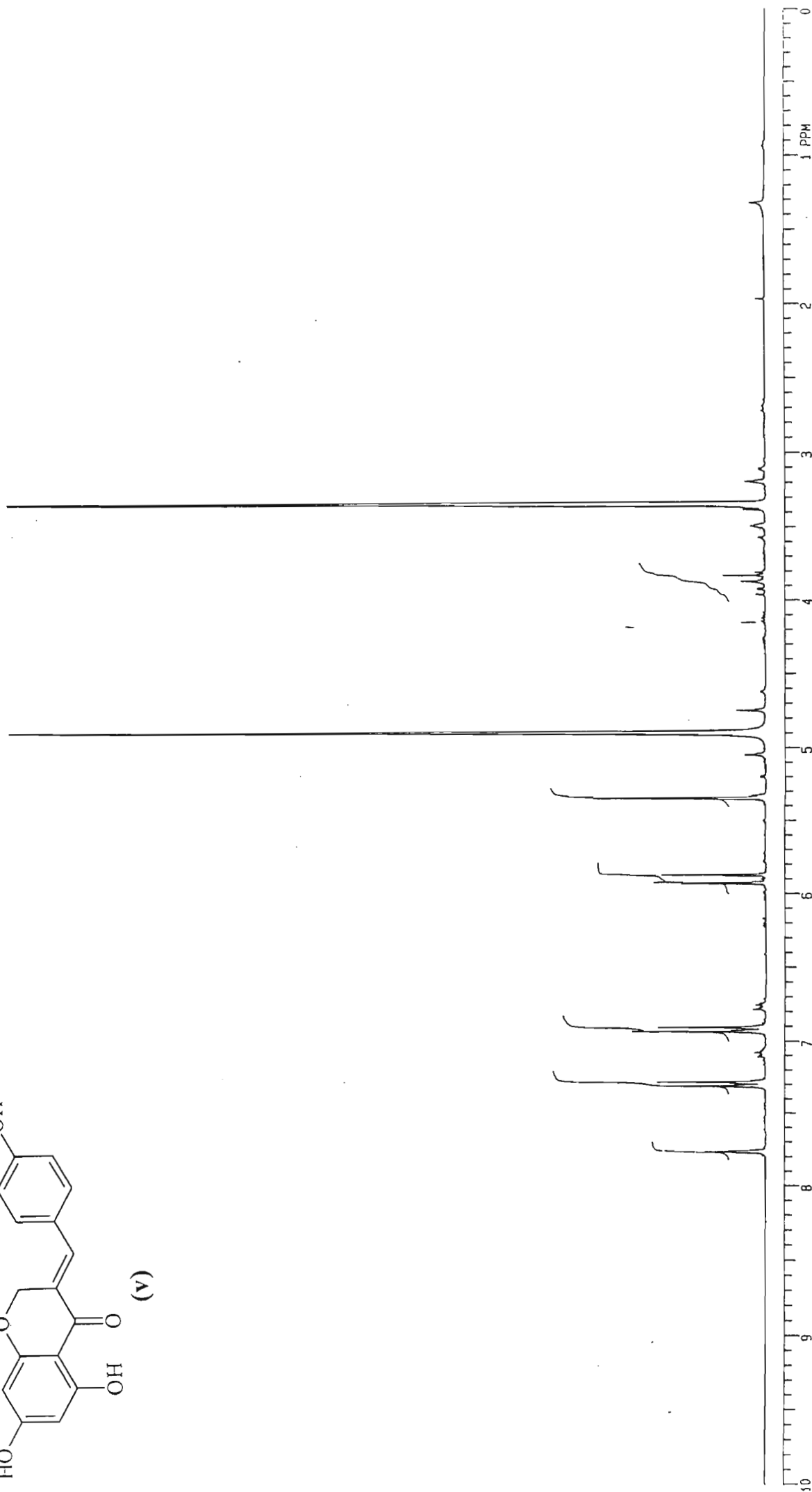
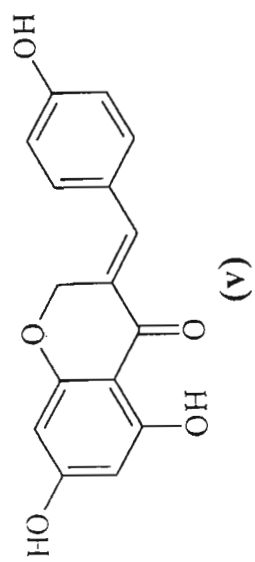
DISPLAY DATA



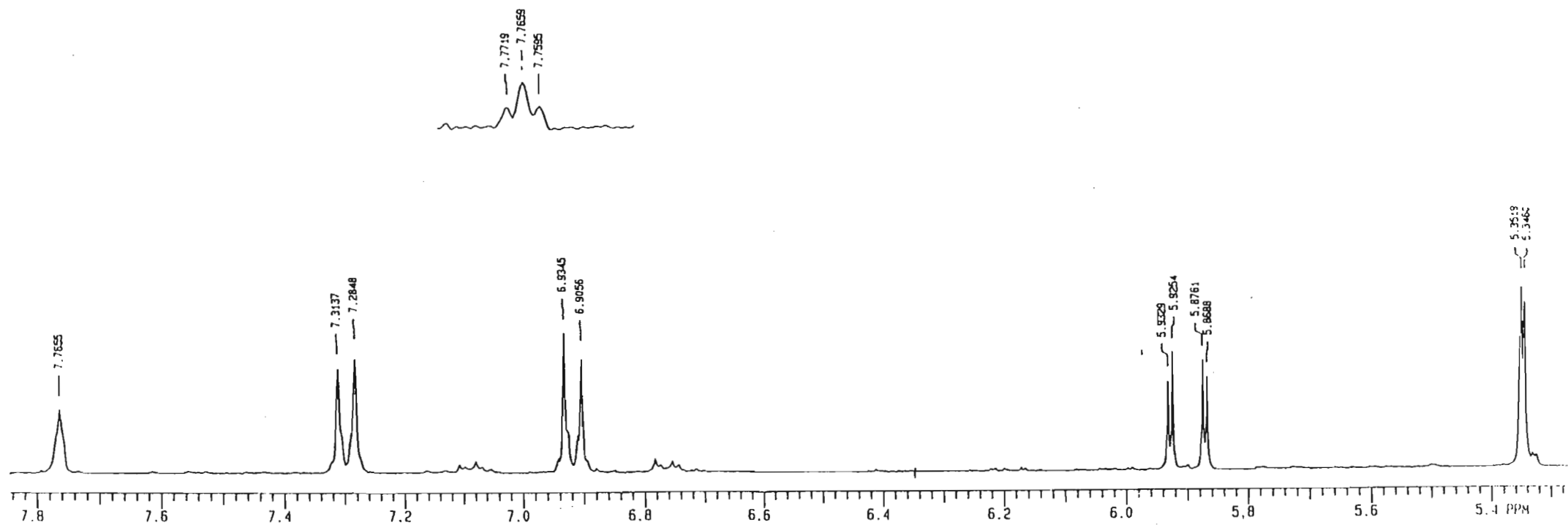
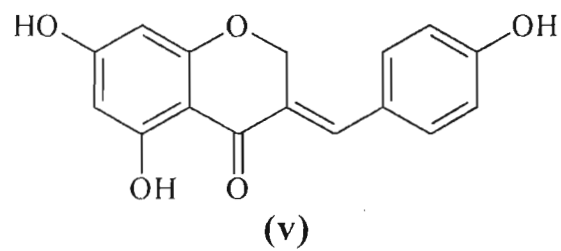
ABS : -0.8656 -> 1.5839 NM : 250.00 -> 400.00

ABS	1.02914	NM	287.450
ABS	0.77419	NM	287.450

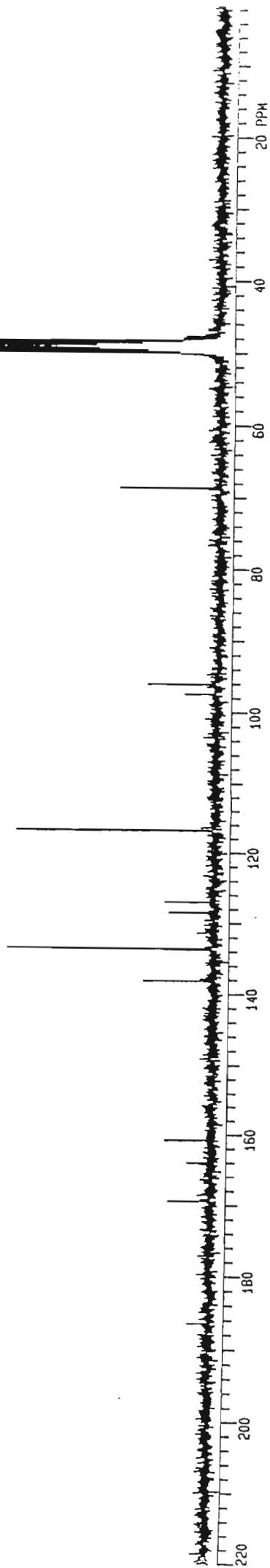
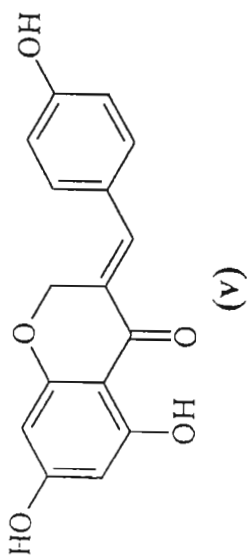
Spectrum 31 : UV and UV (NaOAc) spectra of compound (iv)



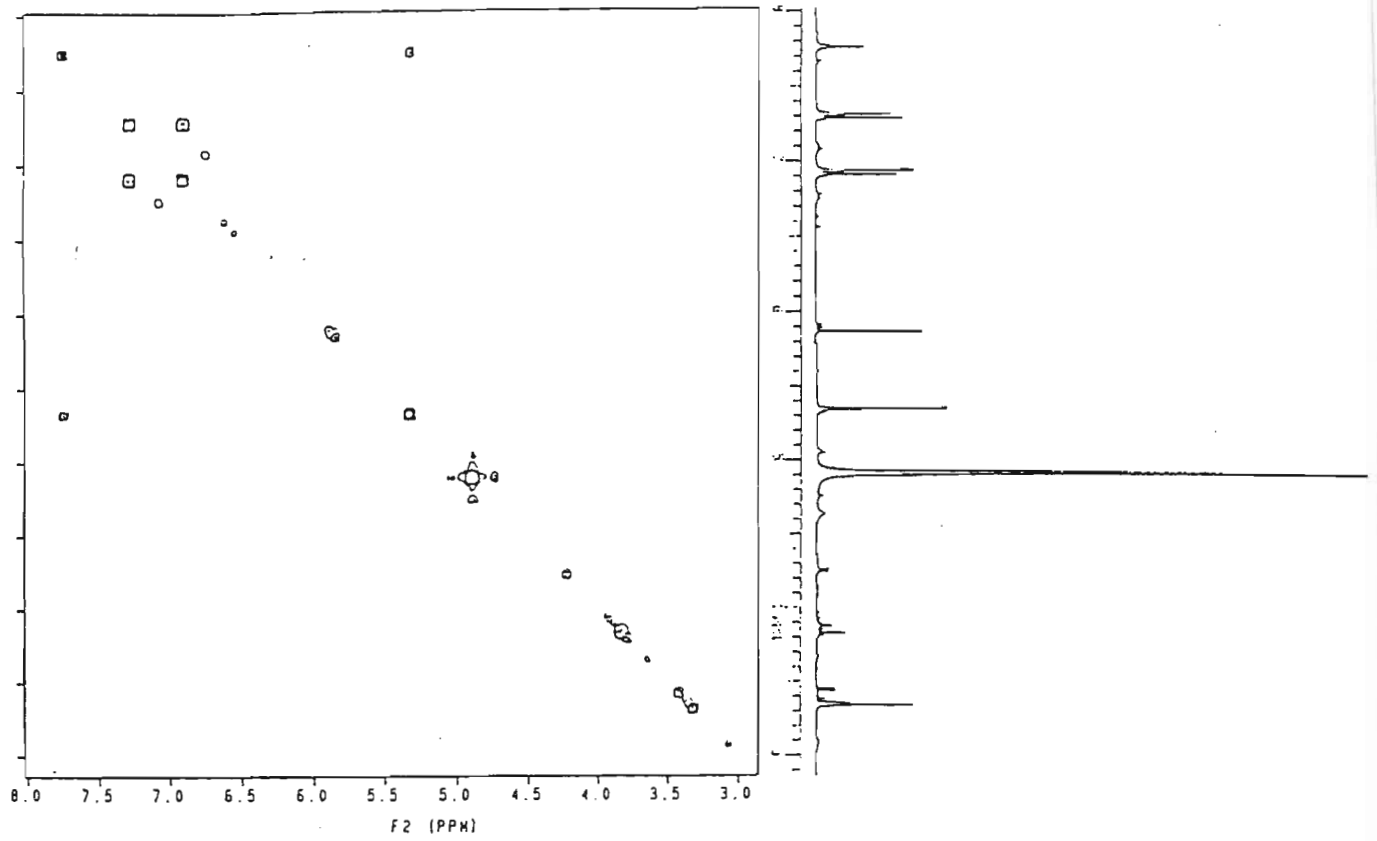
Spectrum 32a :  $^1\text{H}$  NMR spectrum of compound (v)



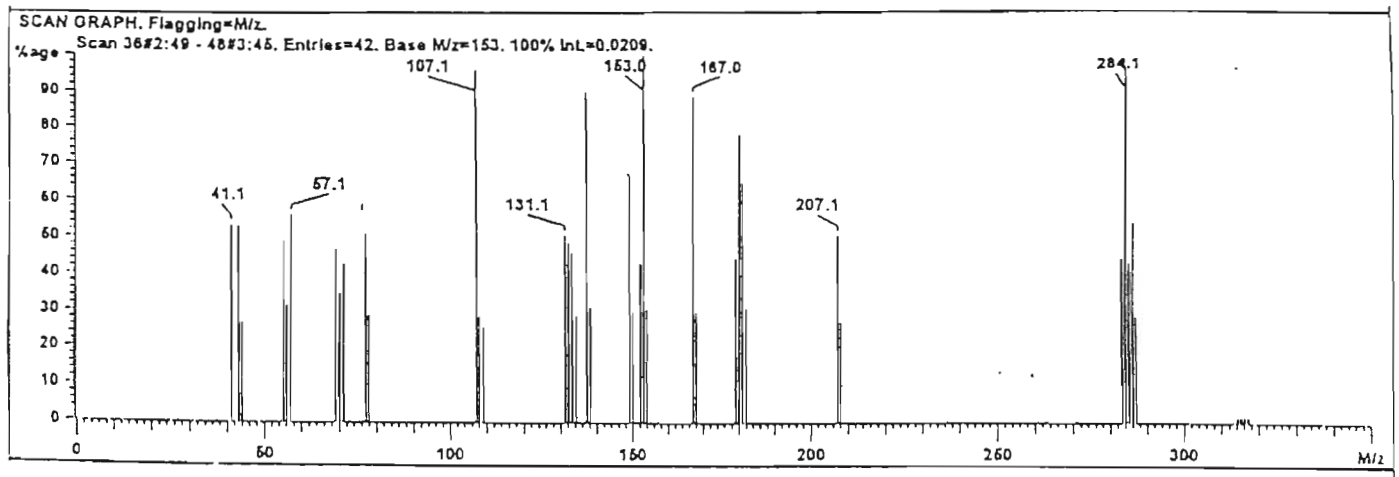
Spectrum 32b : <sup>1</sup>H NMR spectrum (expanded) of compound (v)



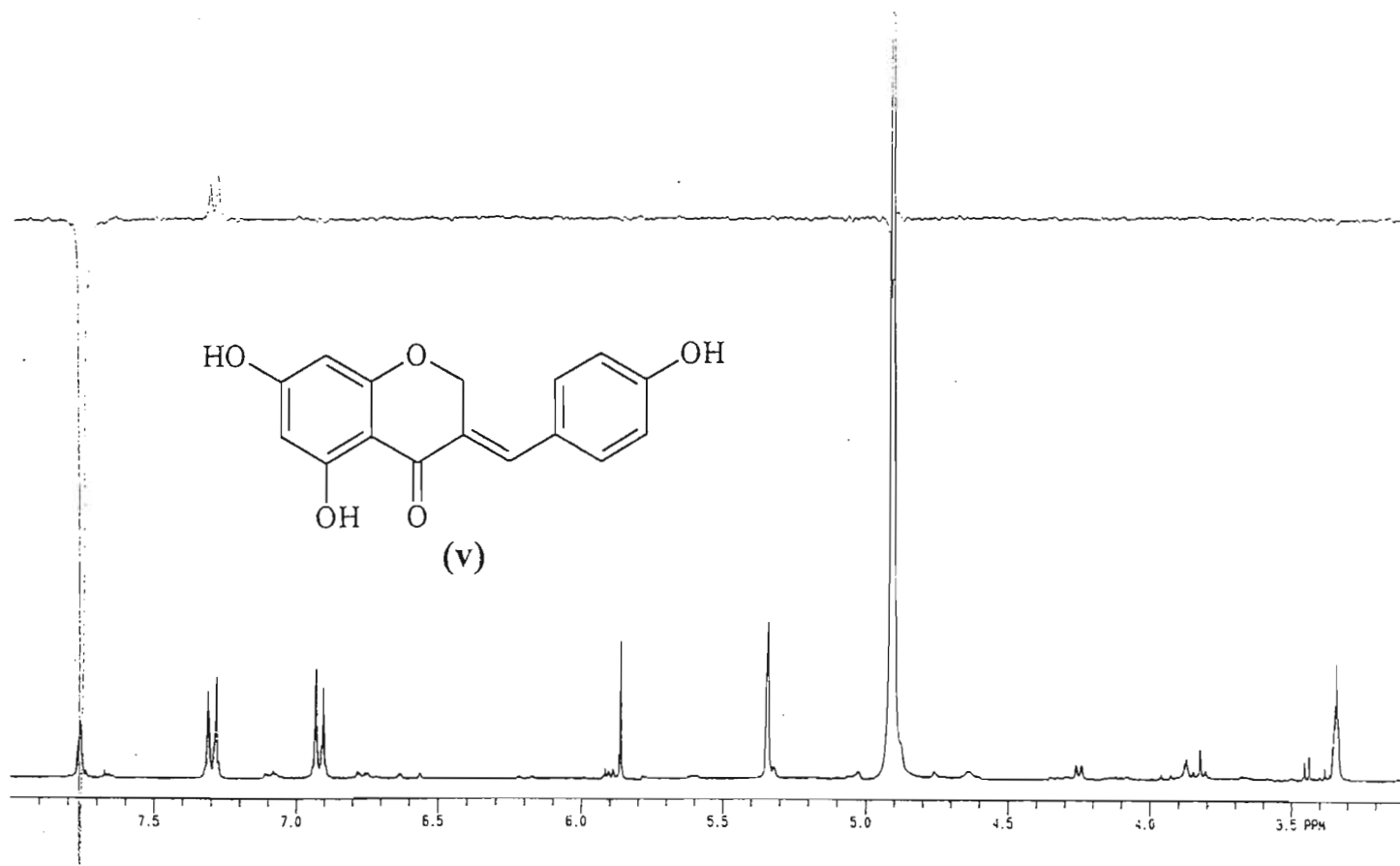
Spectrum 33 :  $^{13}\text{C}$  NMR spectrum of compound (v)



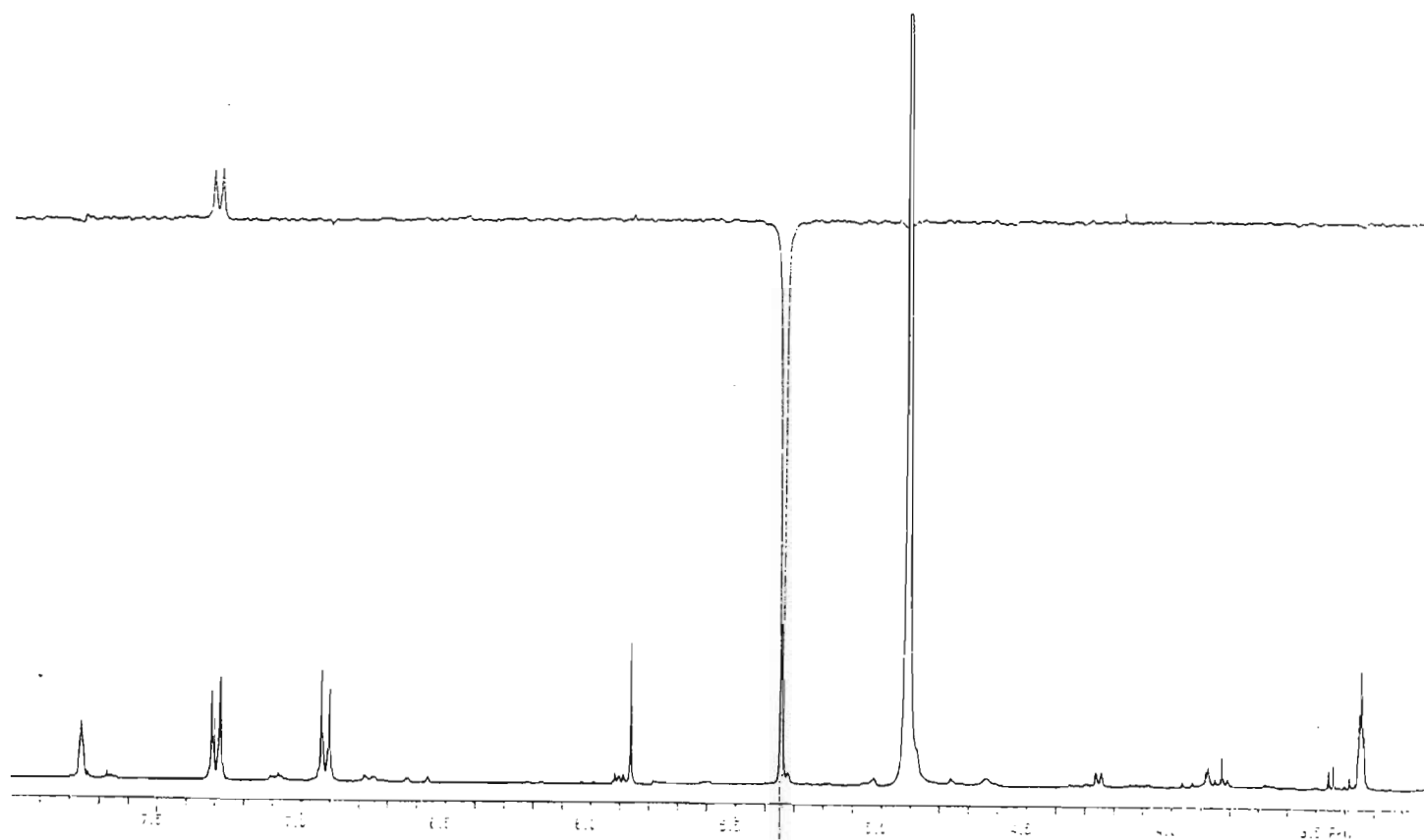
Spectrum 34 : COSY spectrum of compound (v)



Spectrum 35 : Mass spectrum of compound (v)

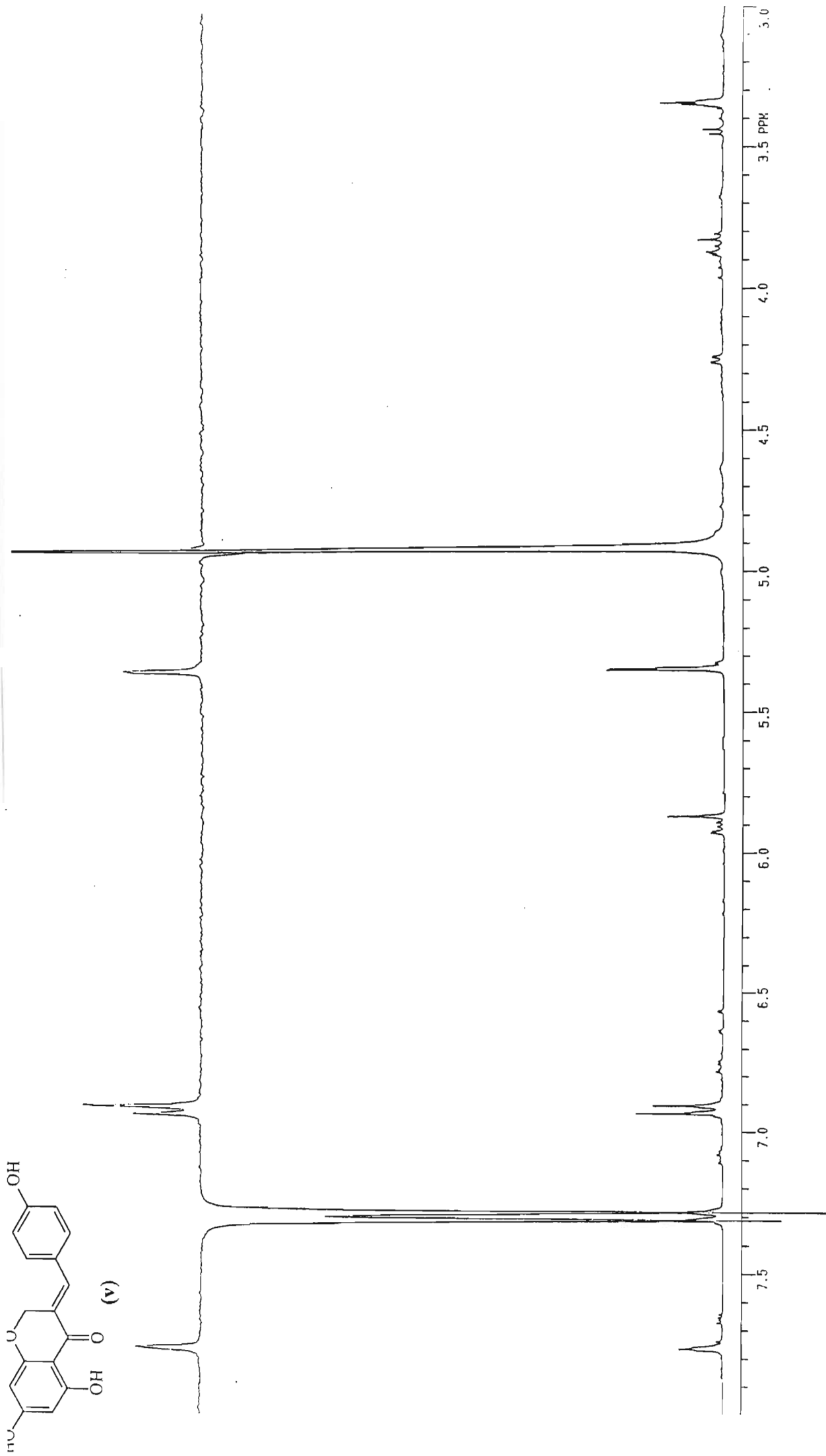


**Spectrum 36a : NOE spectrum of compound (v)**

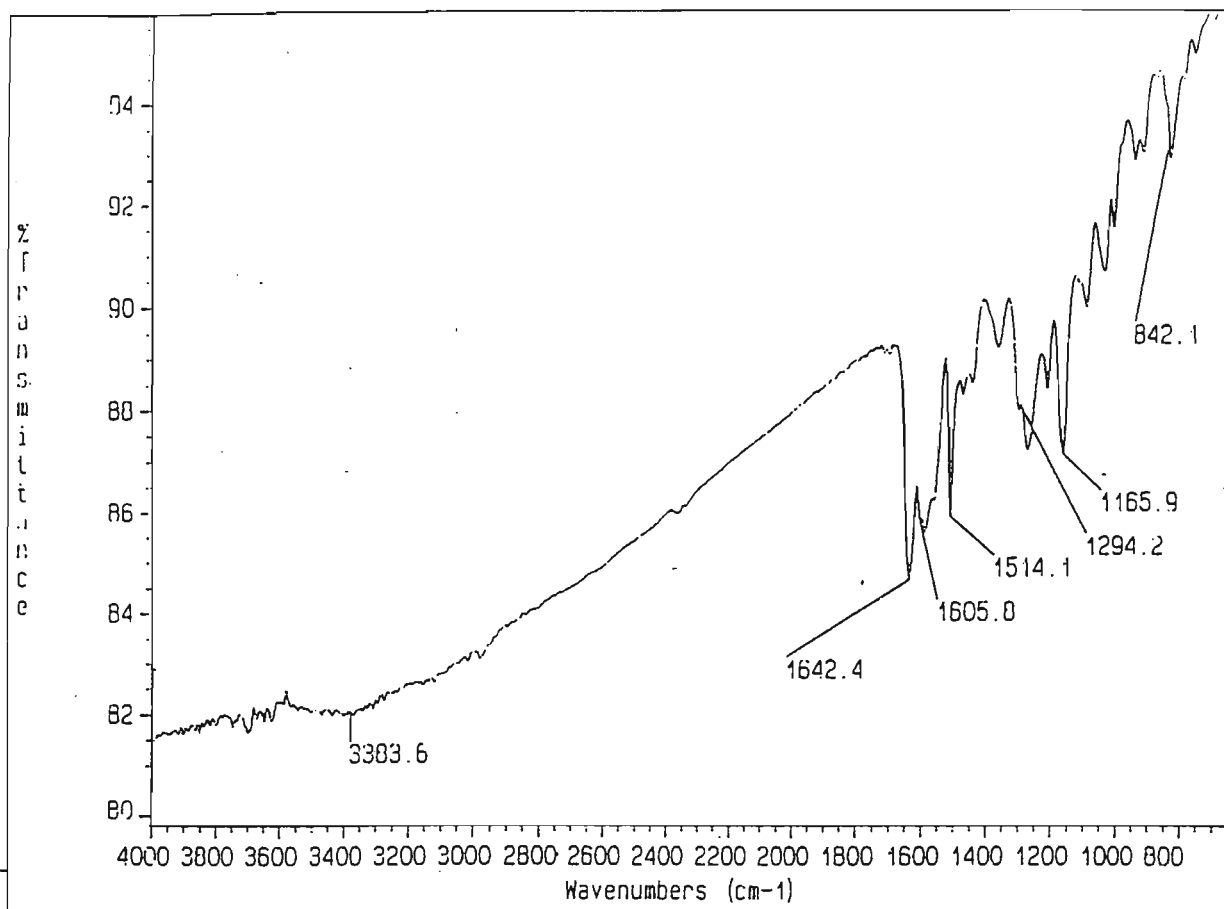


**Spectrum 36b : NOE spectrum of compound (v)**

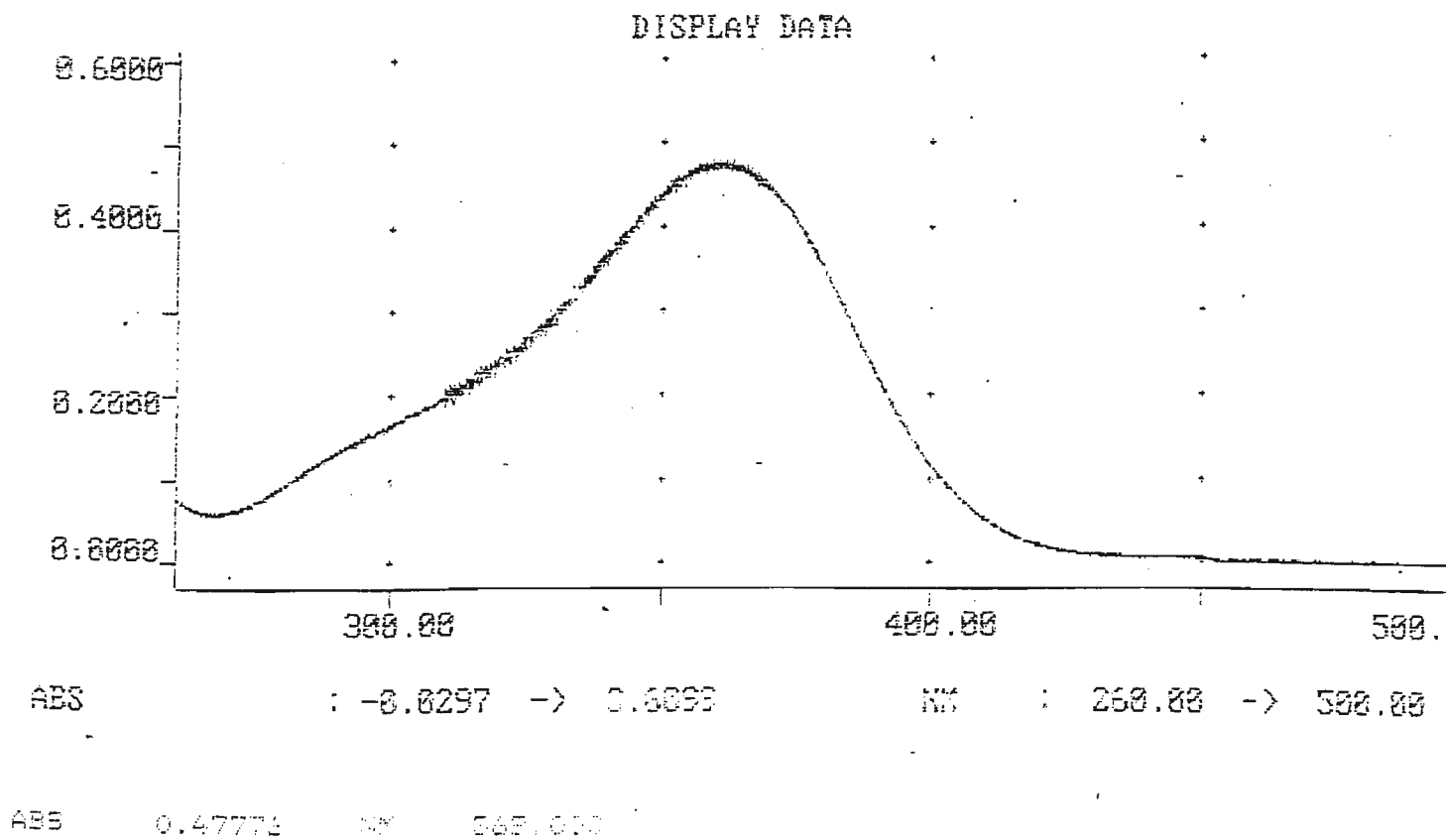




Spectrum 36c : NOE spectrum of compound (v)

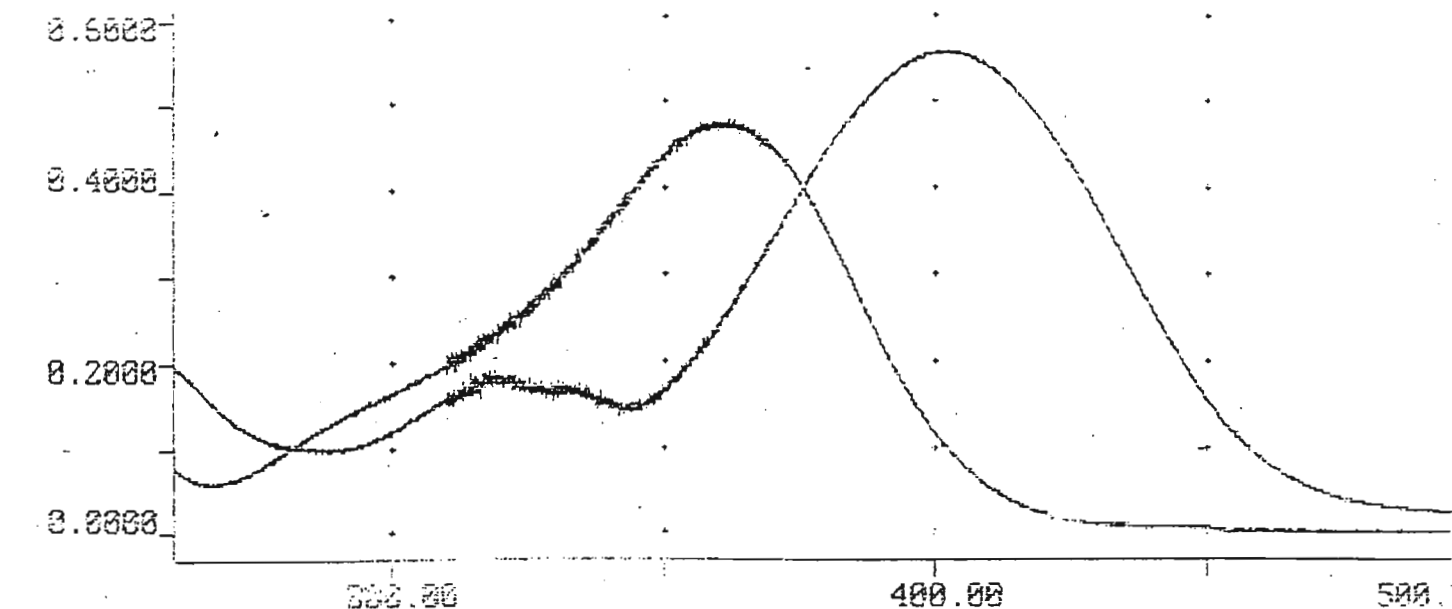


**Spectrum 37 : IR spectrum of compound (v)**



**Spectrum 38 : UV spectrum of compound (v)**

## DISPLAY DATA



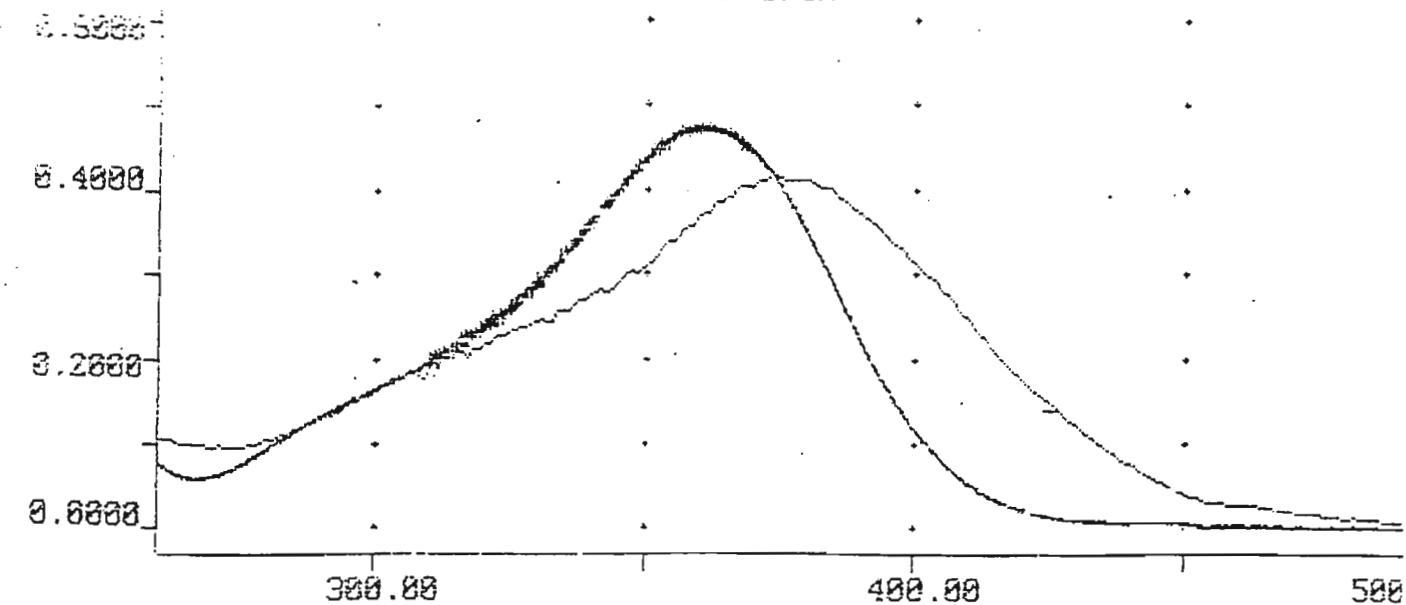
ABS : -0.0297 → 0.6299 NM : 260.00 → 500.00

ABS 0.47776 NM 362.000

ABS 0.50888 NM 401.950

**Spectrum 39 : UV and UV (+ AlCl<sub>3</sub>) spectra of compound (v)**

## DISPLAY DATA

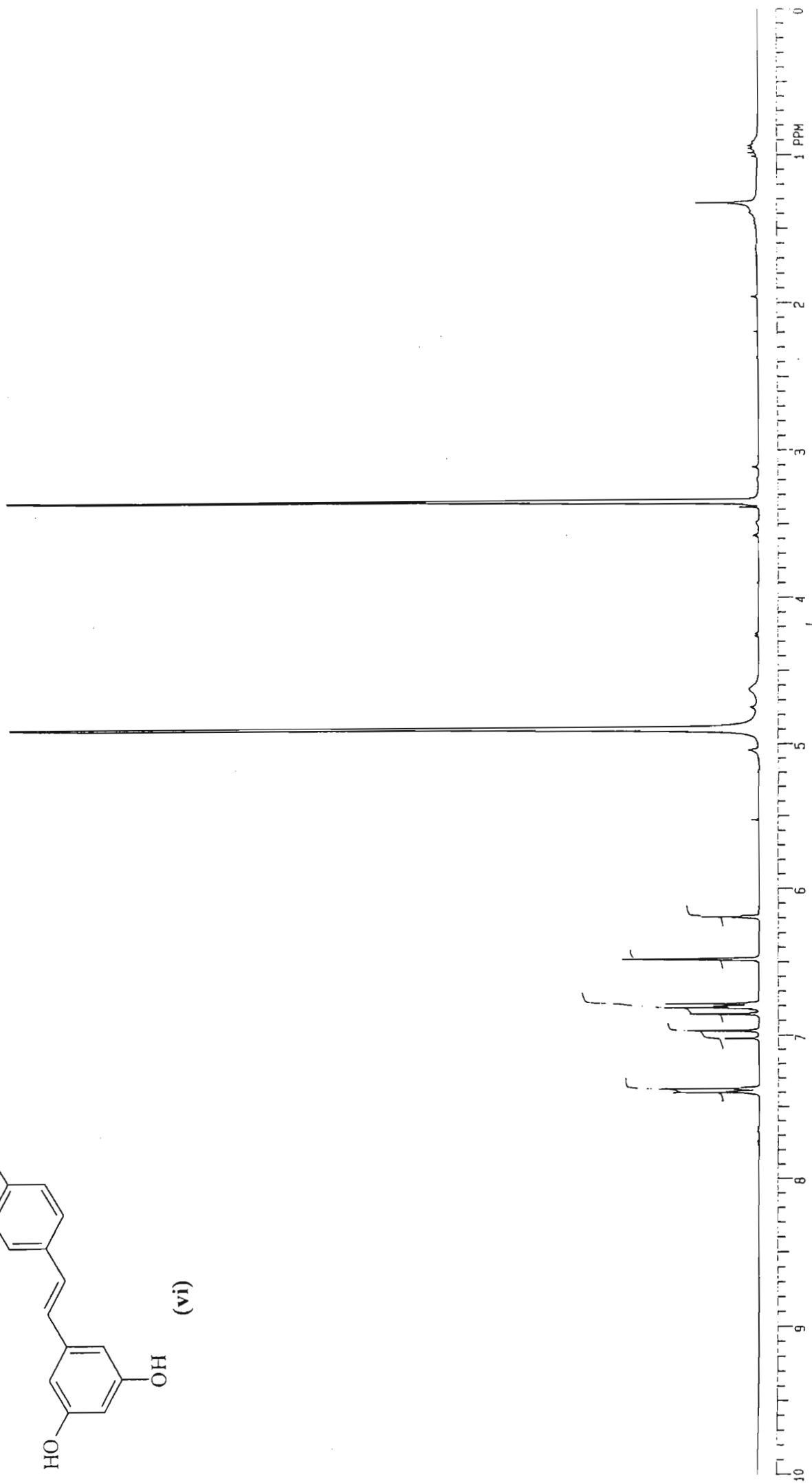
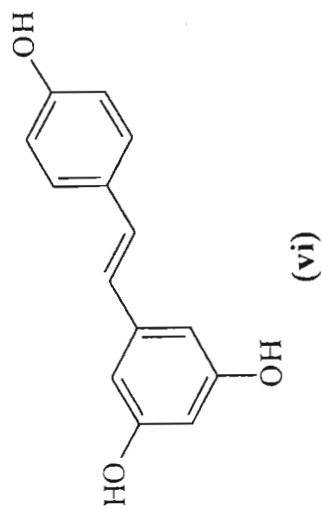


ABS : -0.0297 → 0.6099 NM : 260.00 → 500.00

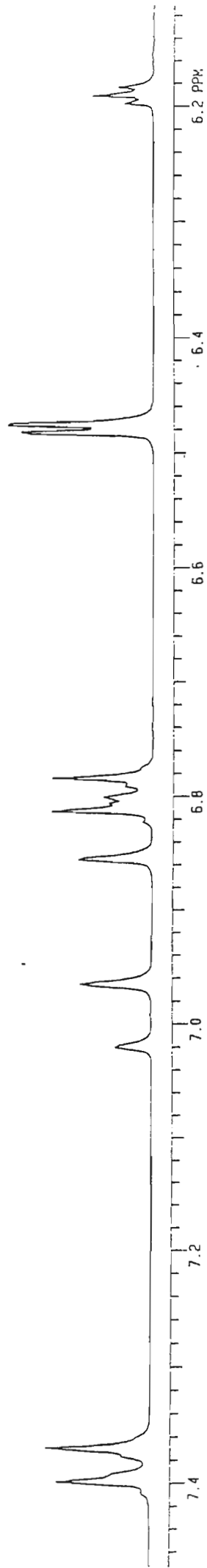
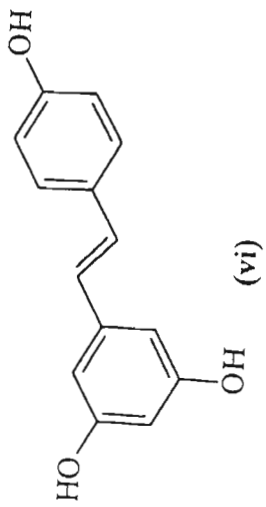
ABS 0.47776 NM 362.000

ABS 0.41805 NM 374.000

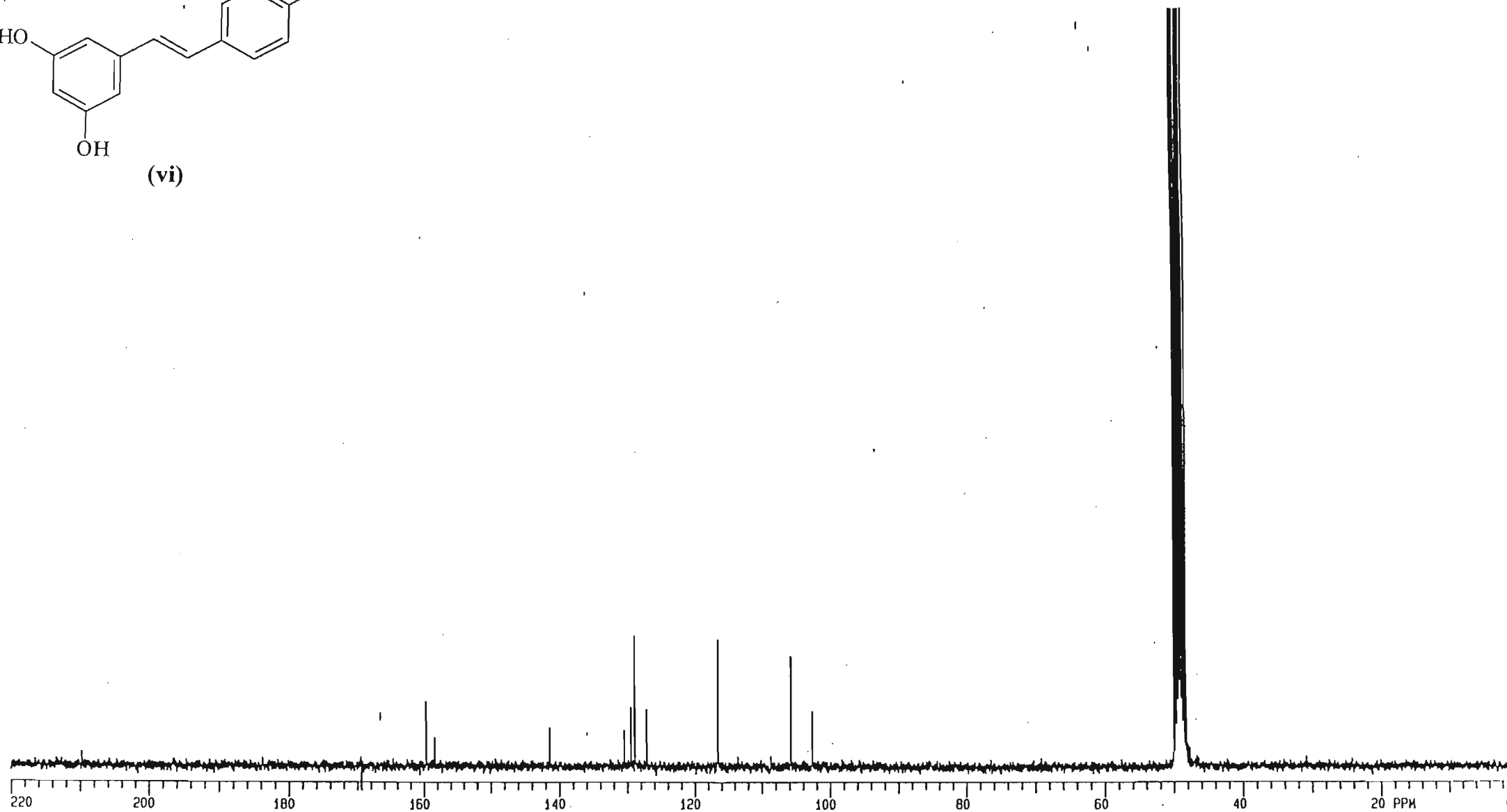
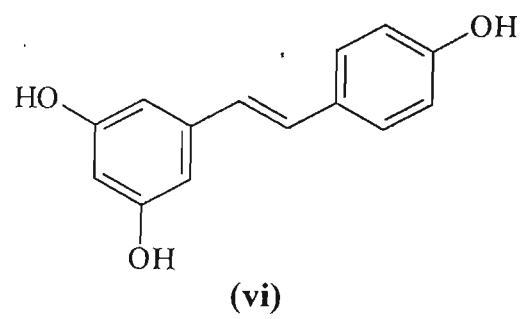
**Spectrum 40 : UV and UV (+ NaOAc) spectra of compound (v)**



Spectrum 41a:  $^1\text{H}$  NMR spectrum of compound (vi)



Spectrum 41b :  $^1\text{H}$  NMR spectrum (expanded) of compound (vi)



Spectrum 42 :  $^{13}\text{C}$  NMR spectrum of compound (vi)

C H 3



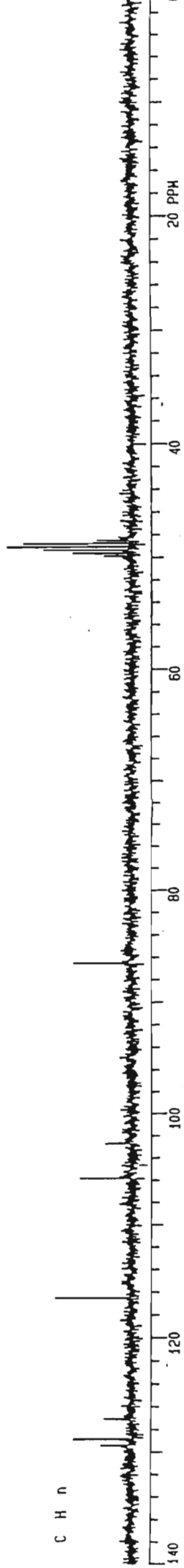
C H 2



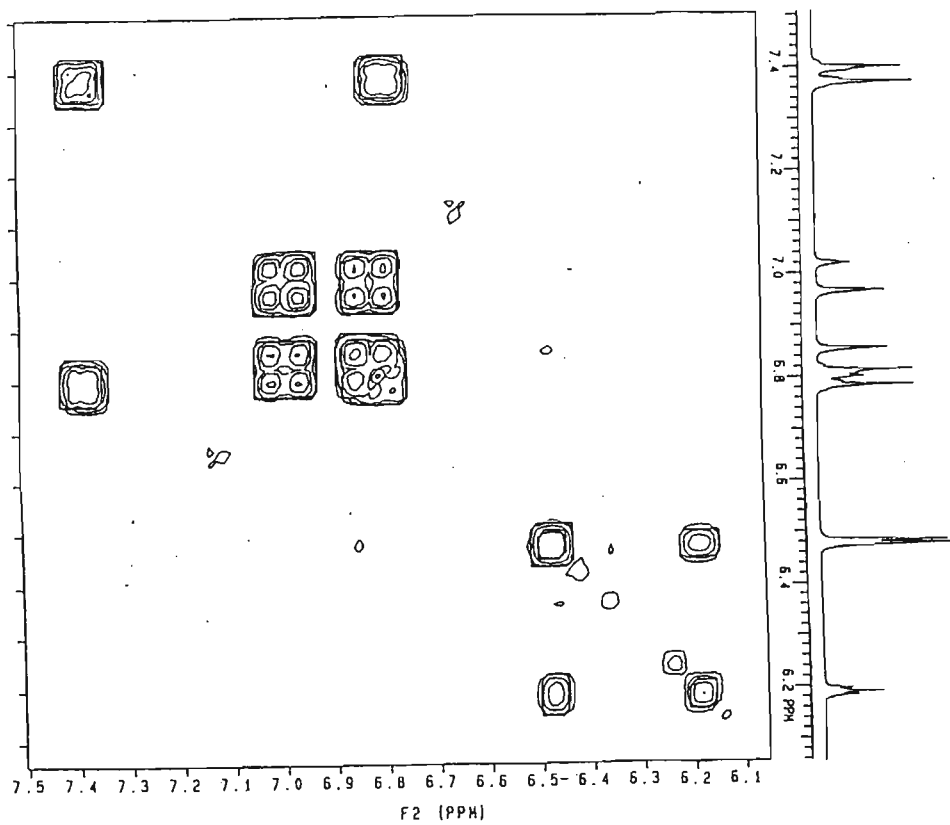
C H



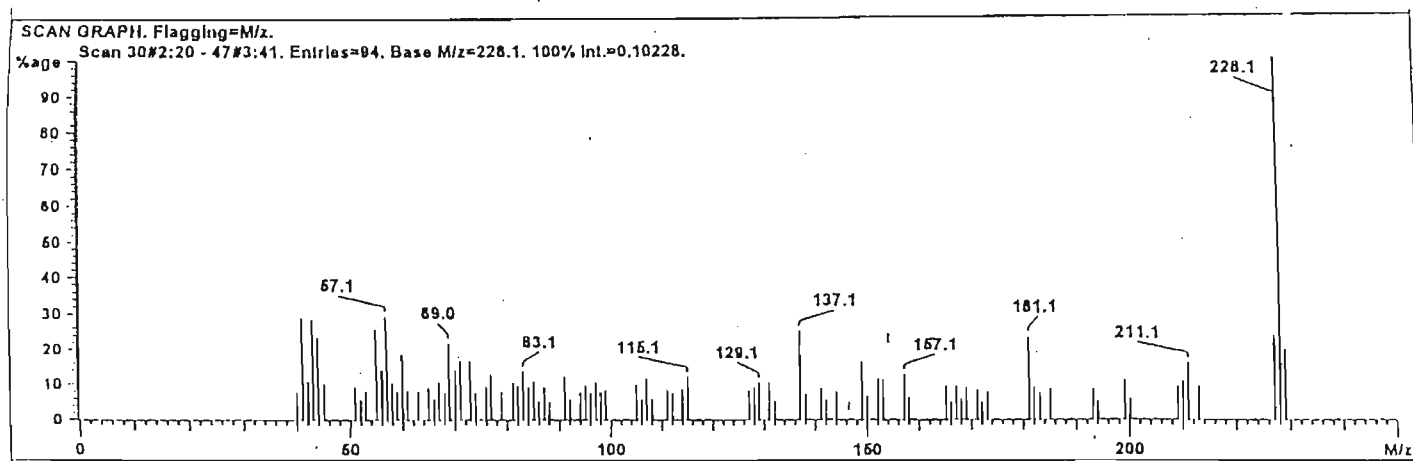
C H n



Spectrum 43 : ADEPT spectrum of compound (vi)

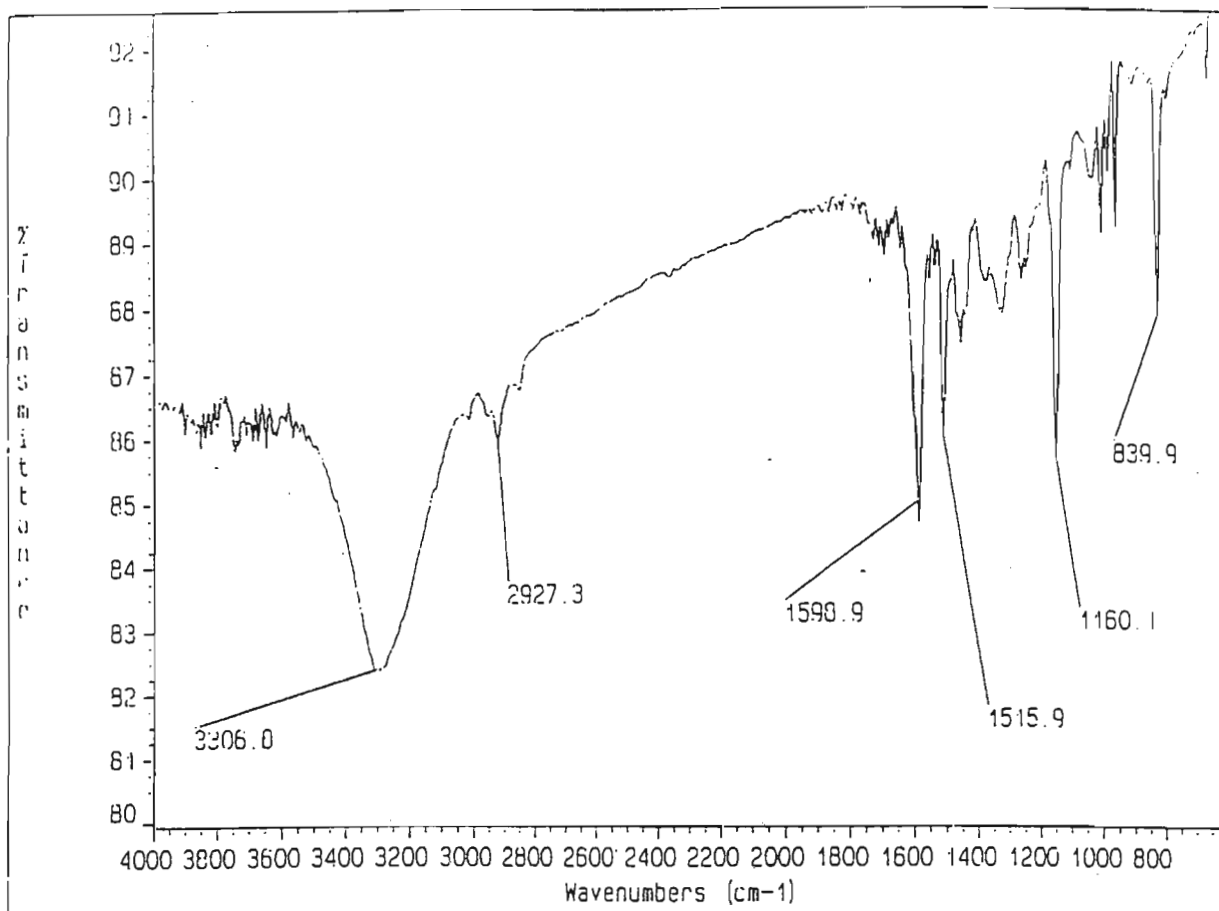


Spectrum 44 : COSY spectrum of compound (vi)



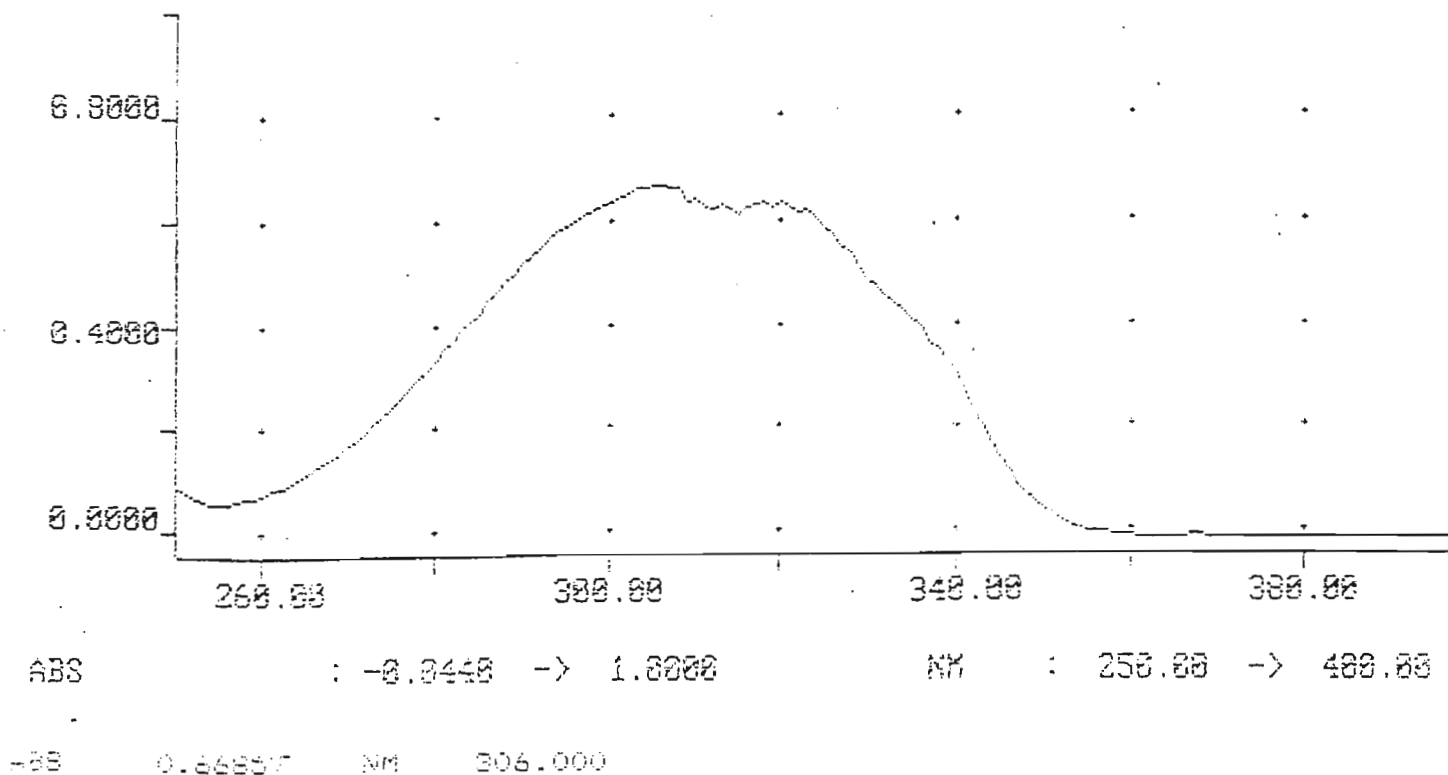
Spectrum 45 : Mass spectrum of compound (vi)



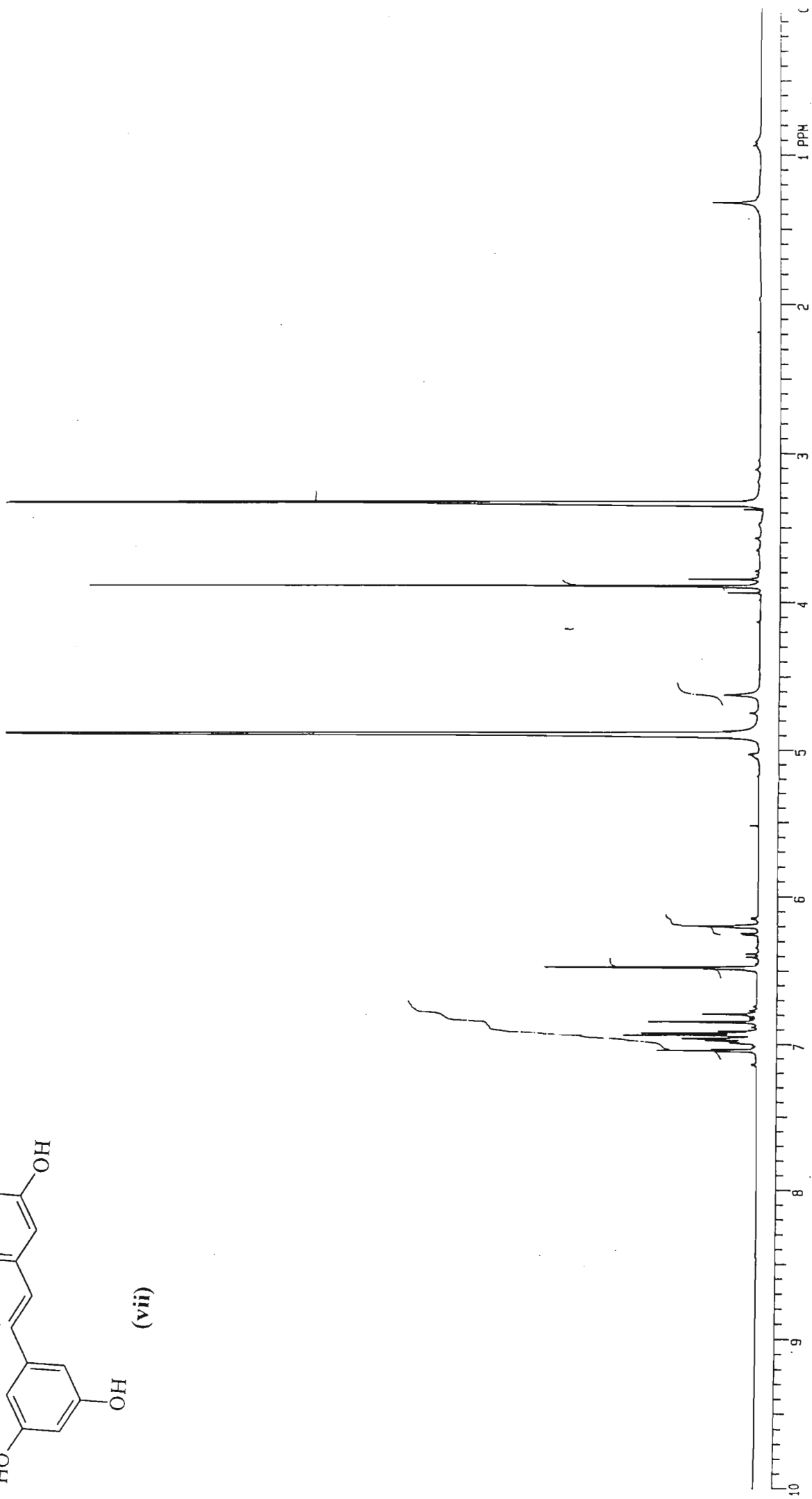
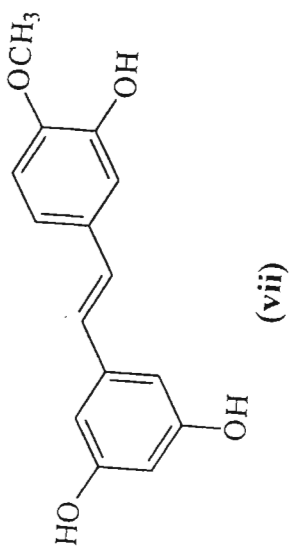


**Spectrum 46 : IR spectrum of compound (vi)**

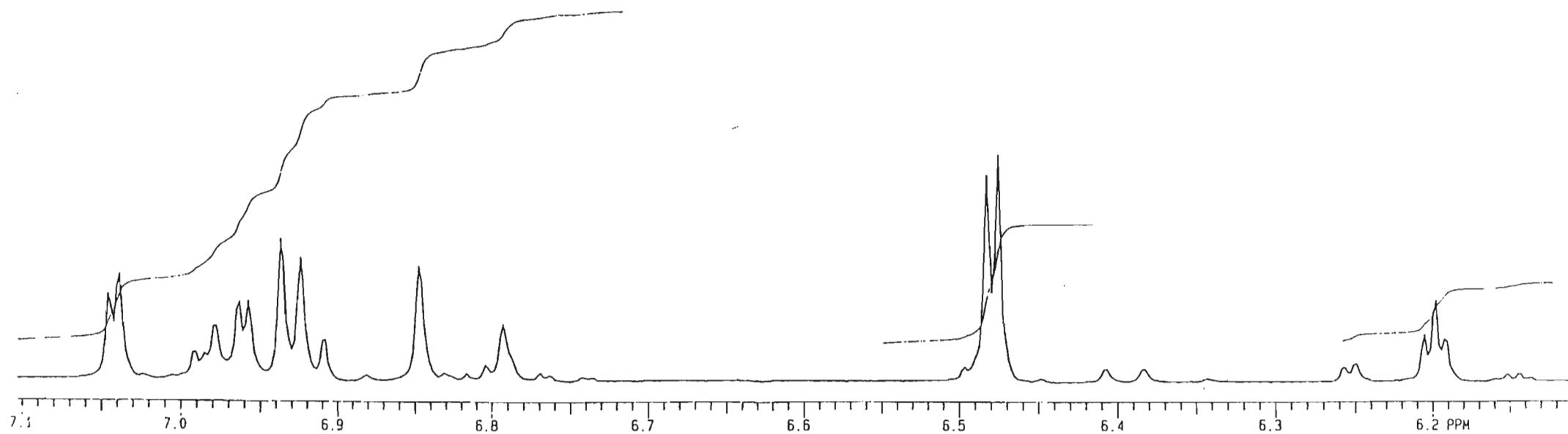
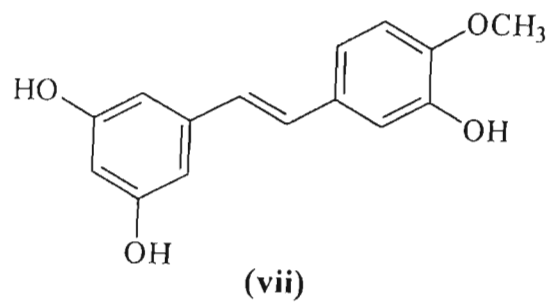
DISPLAY DATA



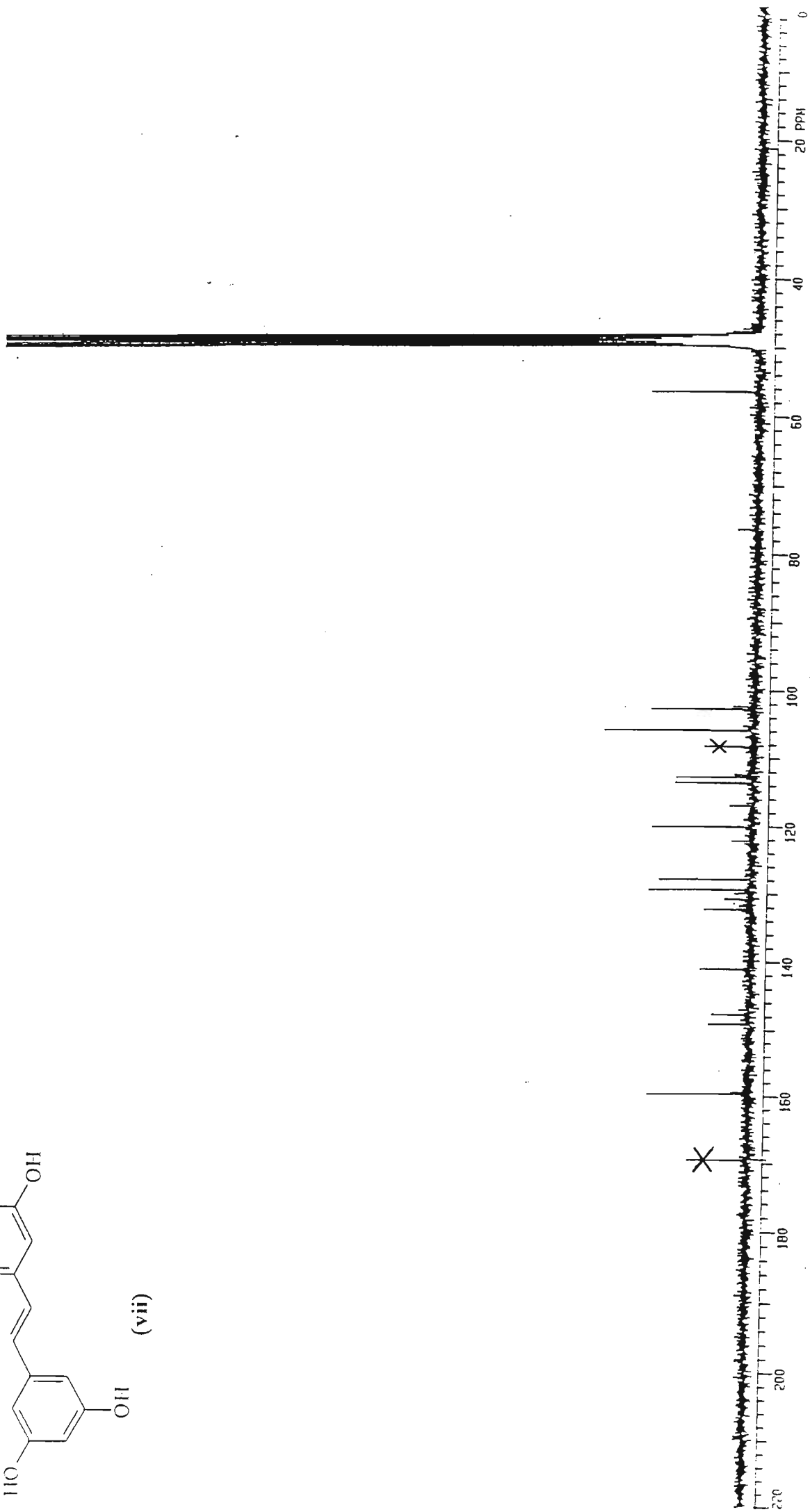
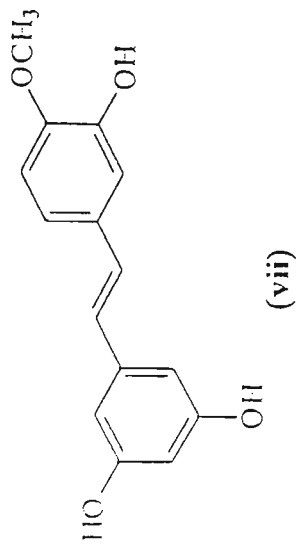
**Spectrum 47 : UV spectrum of compound (vi)**



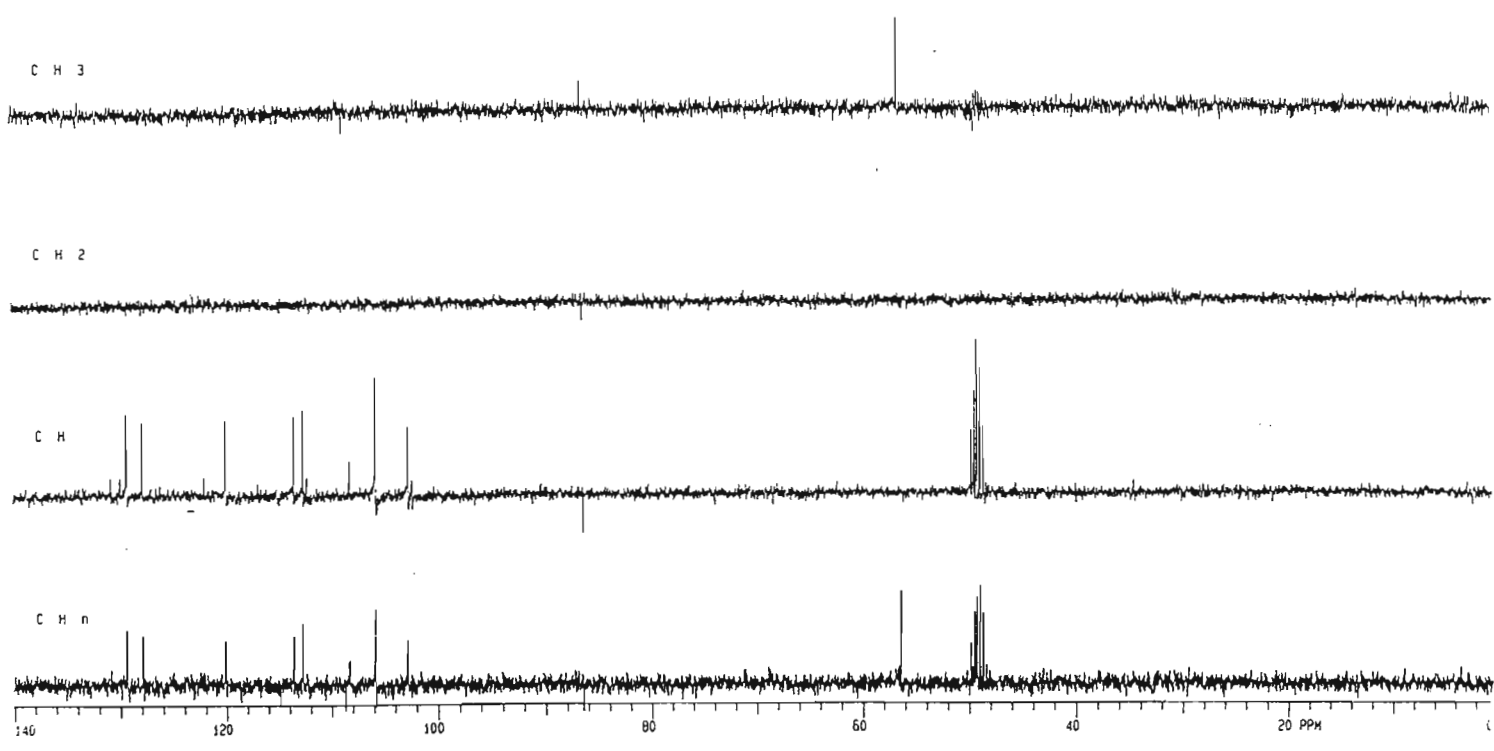
Spectrum 48a : <sup>1</sup>H NMR spectrum of compound (vii)



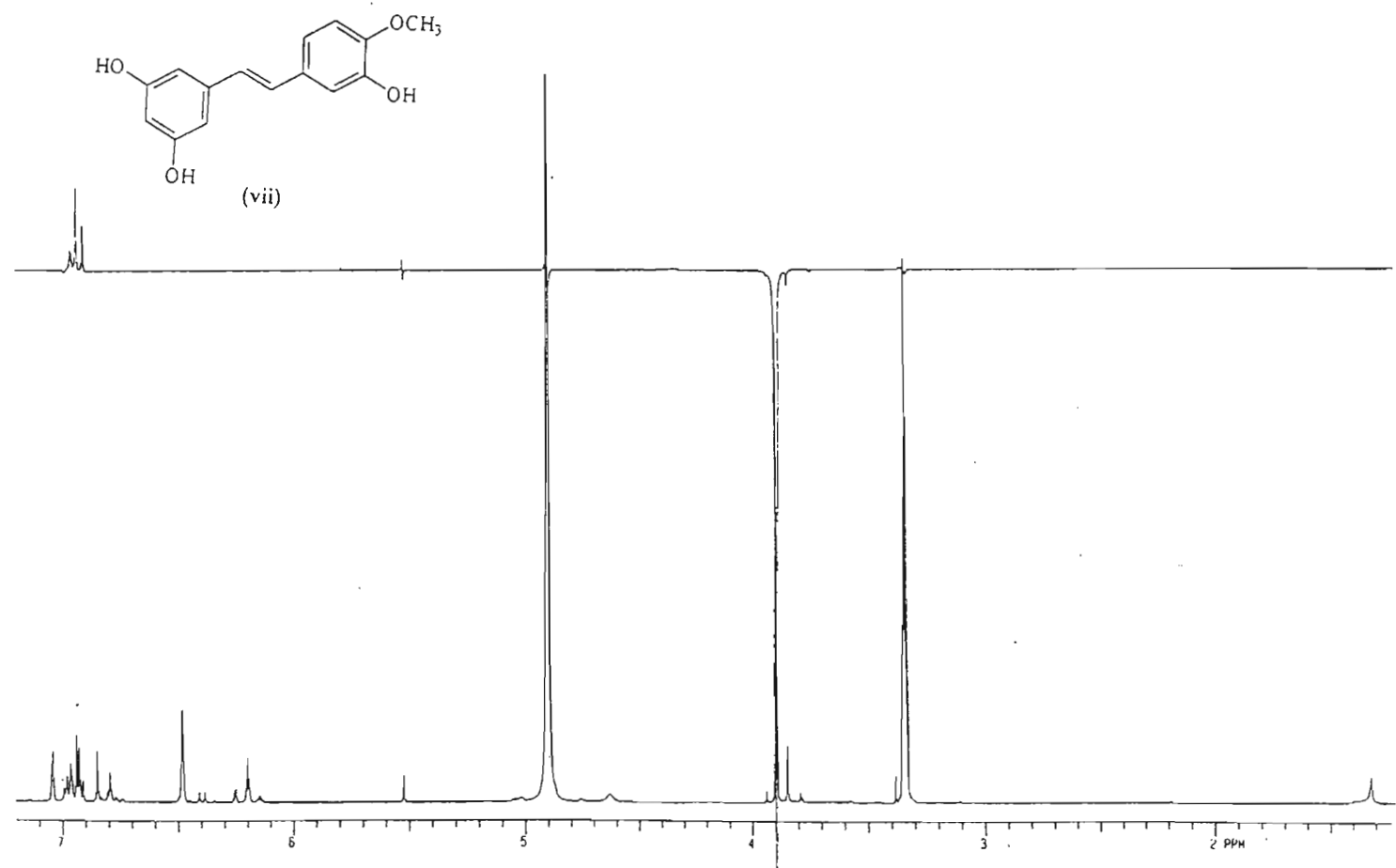
**Spectrum 48b : <sup>1</sup>H NMR spectrum (olefinic and aromatic region expansion) of compound (vii)**



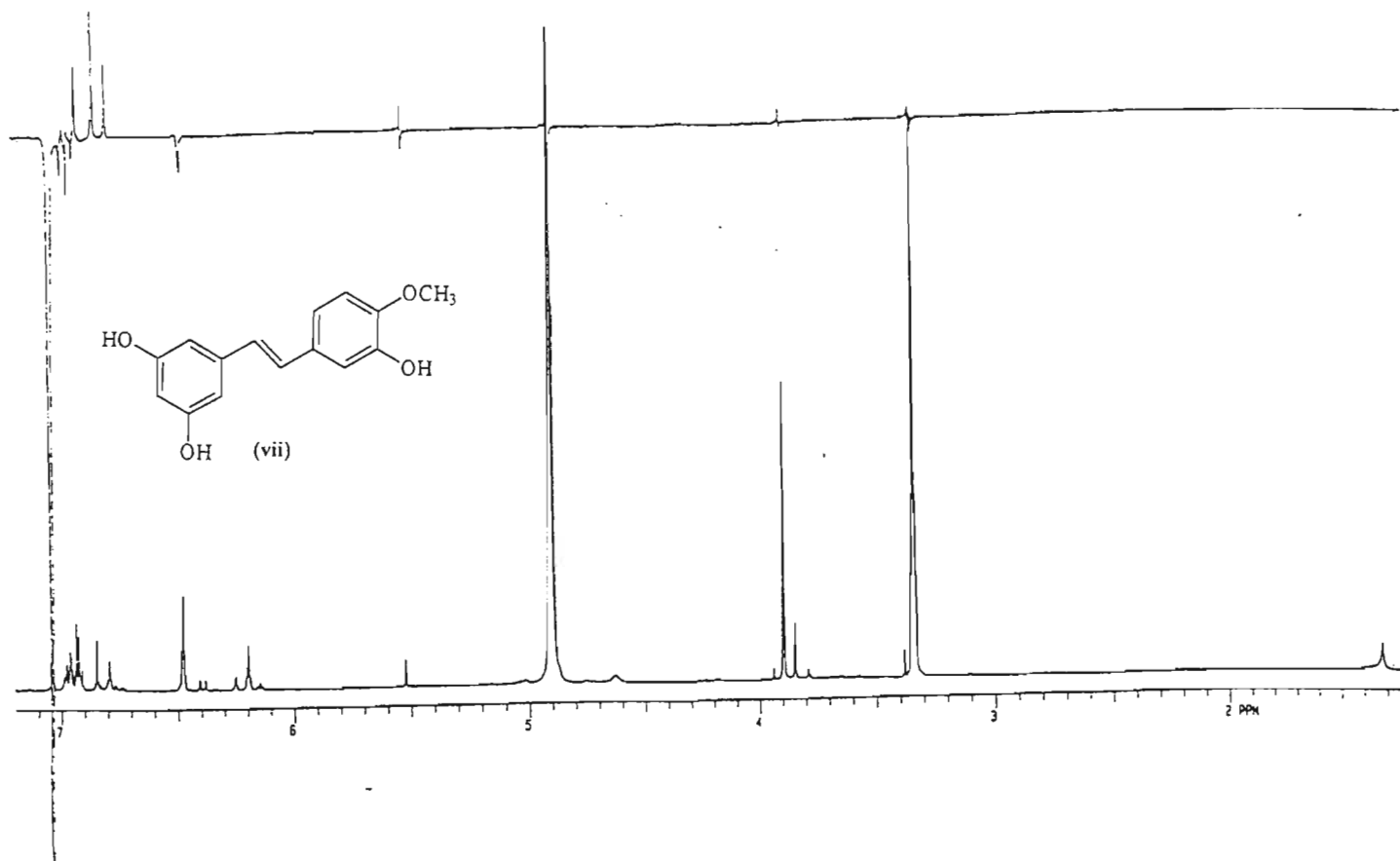
Spectrum 49 :  $^{13}\text{C}$  NMR spectrum of compound (vii)



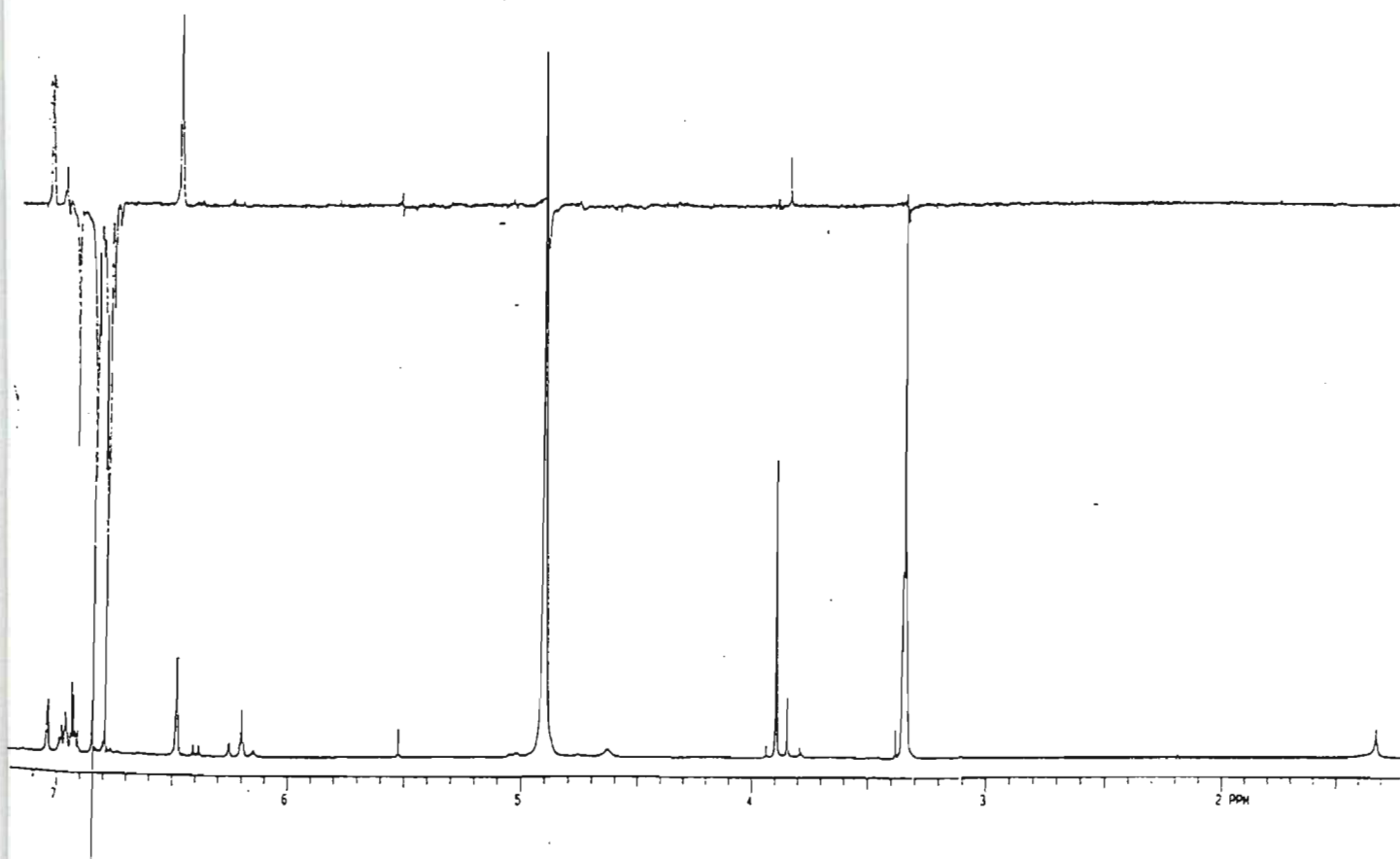
Spectrum 50 : ADEPT spectrum of compound (vii)



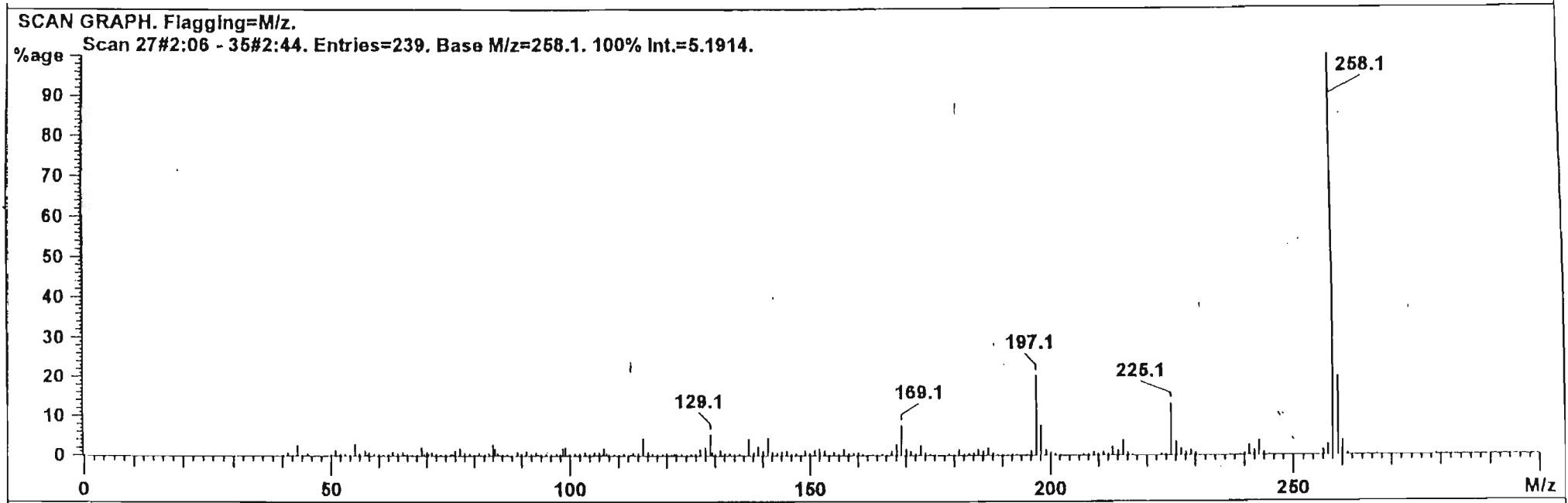
Spectrum 51a : NOE spectrum of compound (vii)



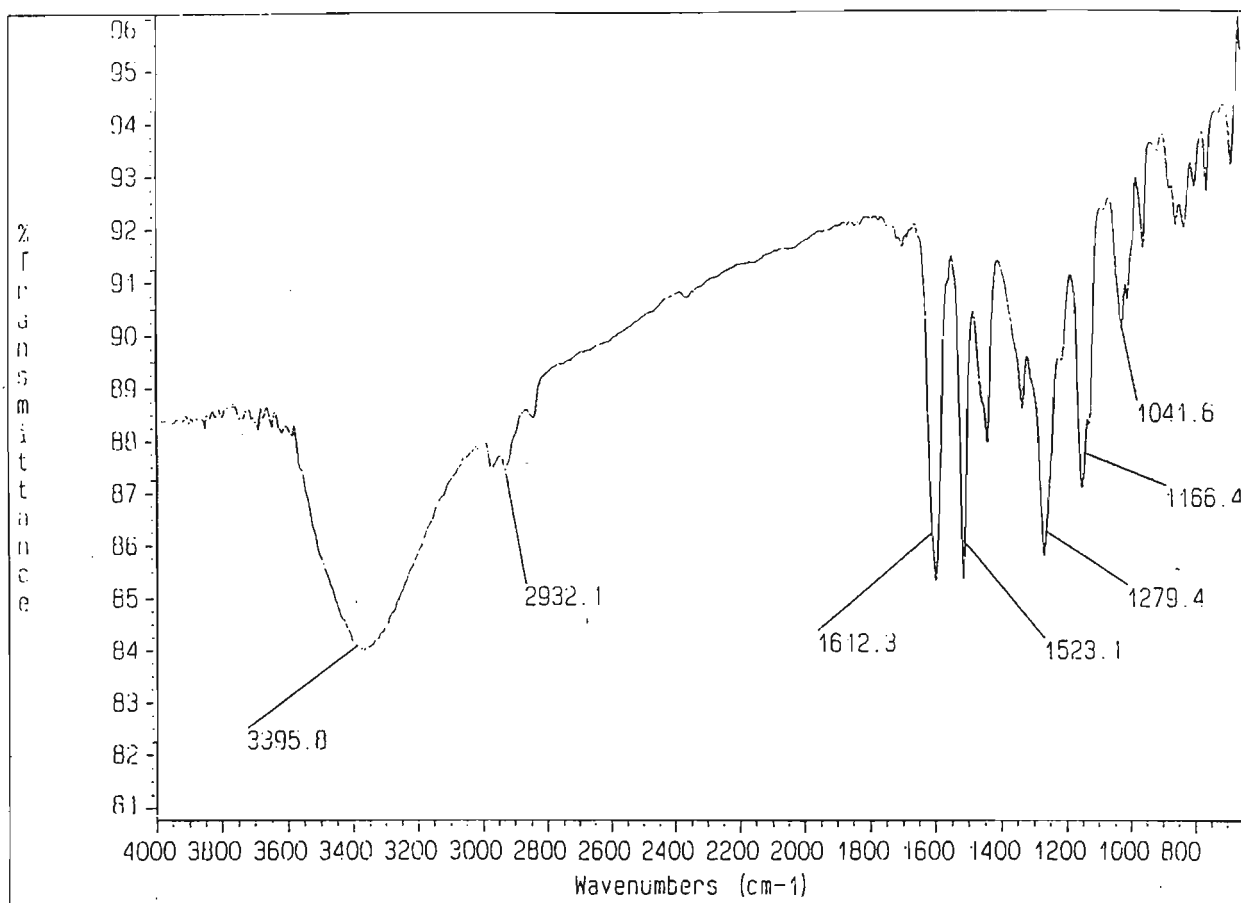
Spectrum 51b : NOE spectrum of compound (vii)



Spectrum 51c : NOE spectrum of compound (vii)

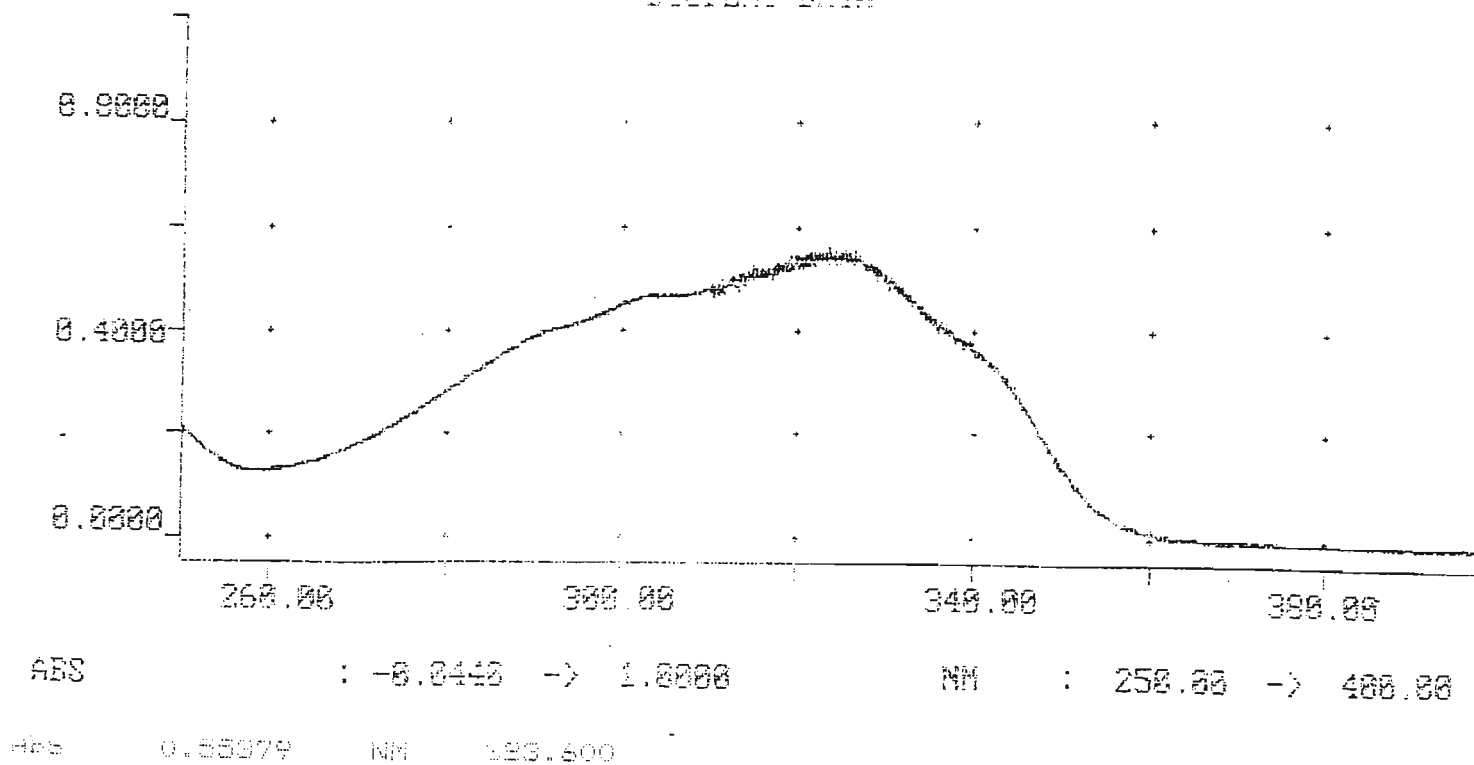


Spectrum 52 : Mass spectrum of compound (vii)



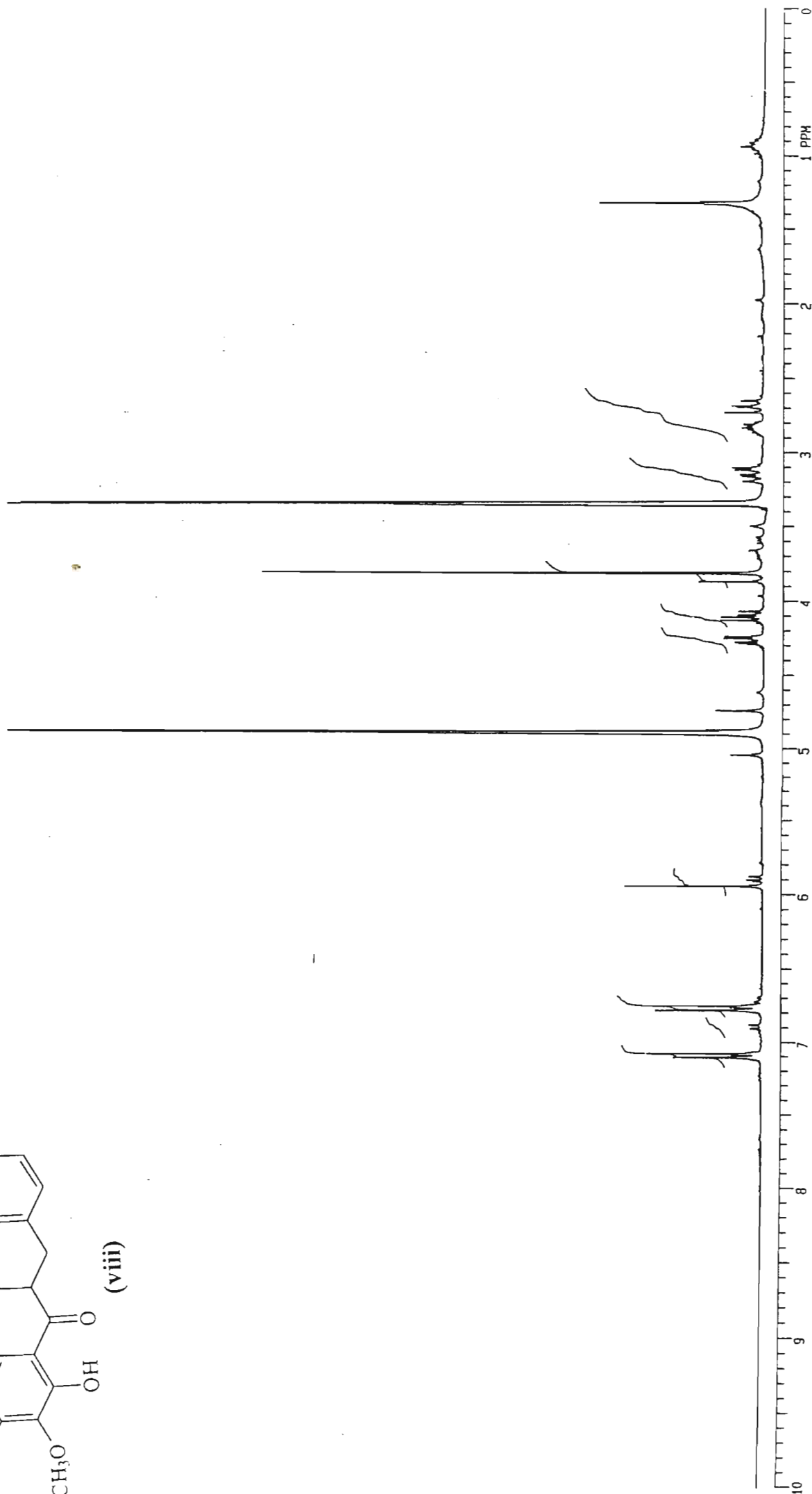
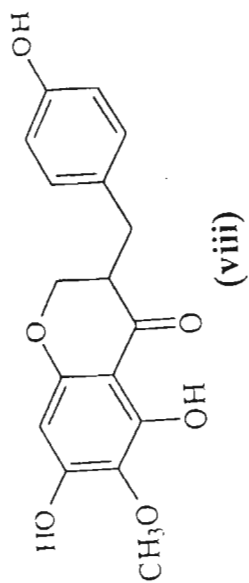
**Spectrum 53 : IR spectrum of compound (vii)**

DISPLAY DATA

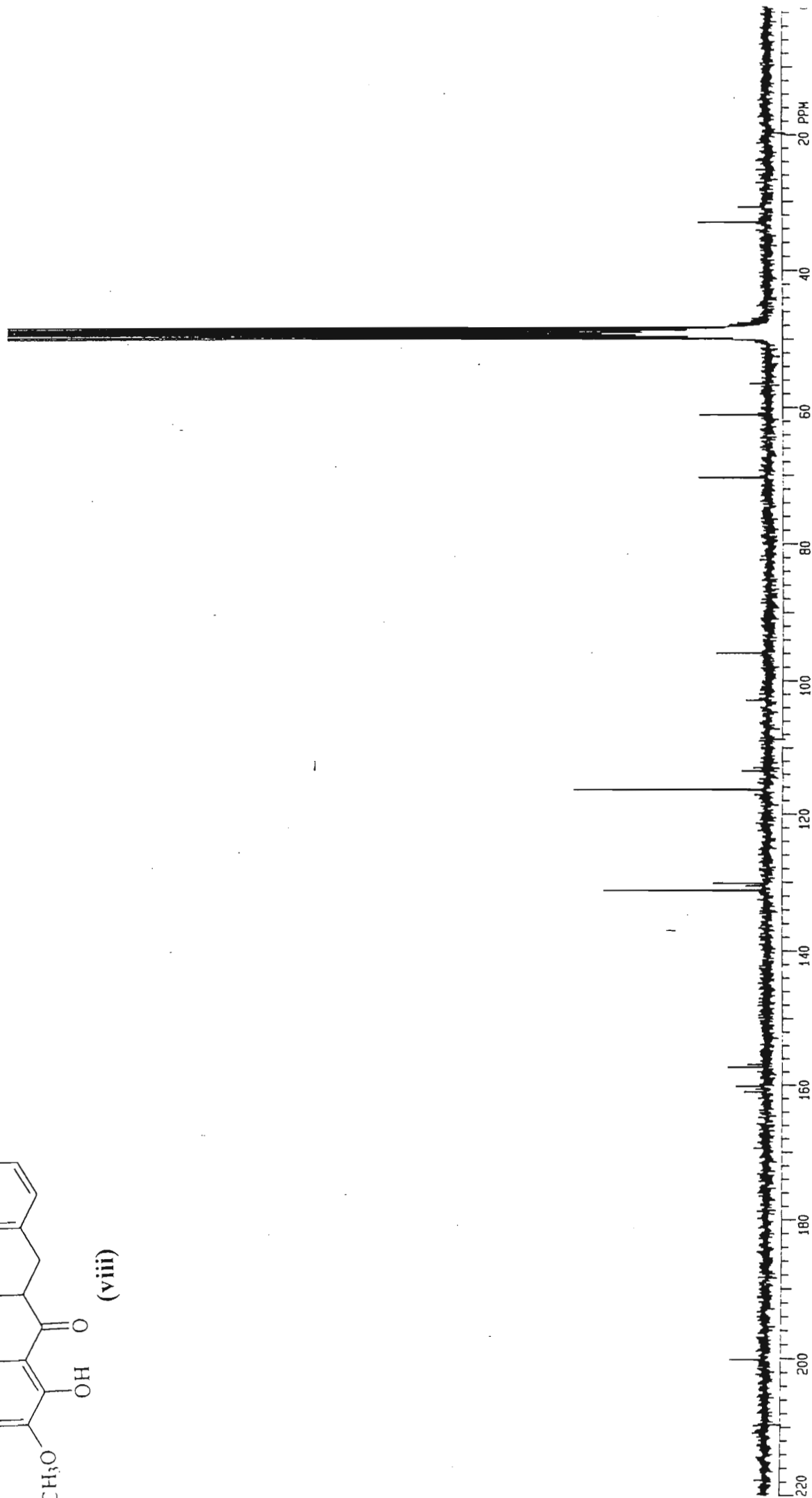
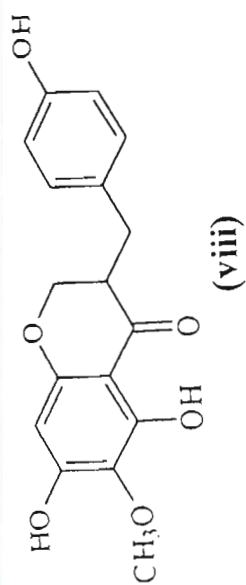


**Spectrum 54 : UV spectrum of compound (vii)**

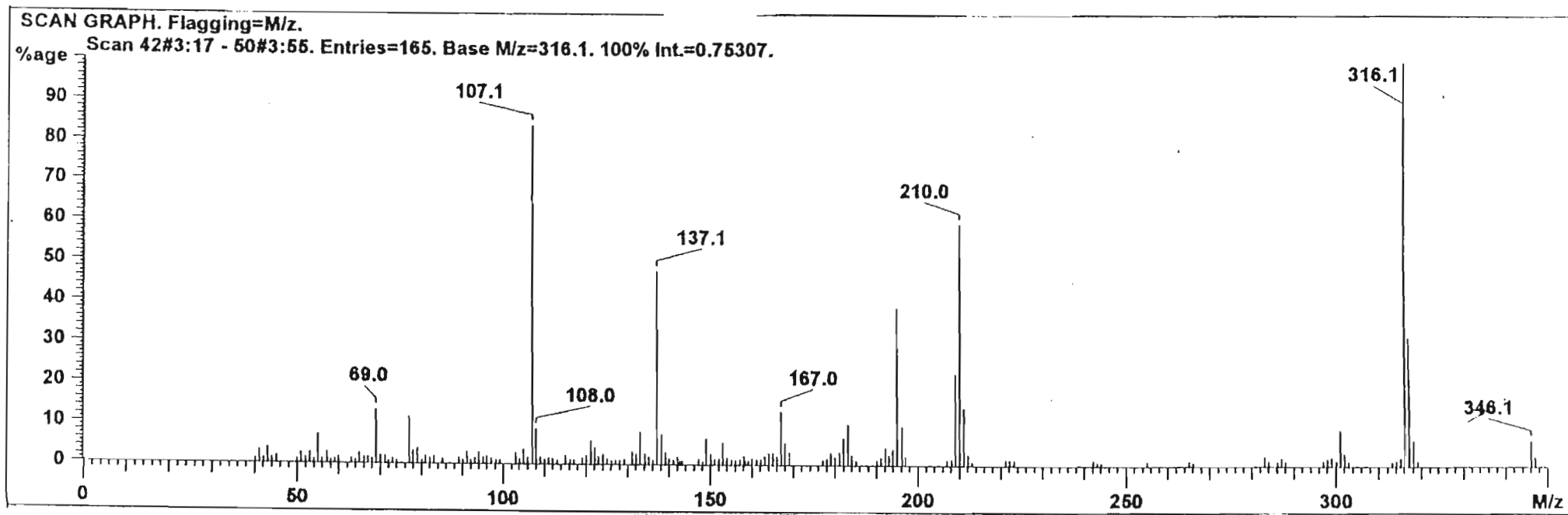




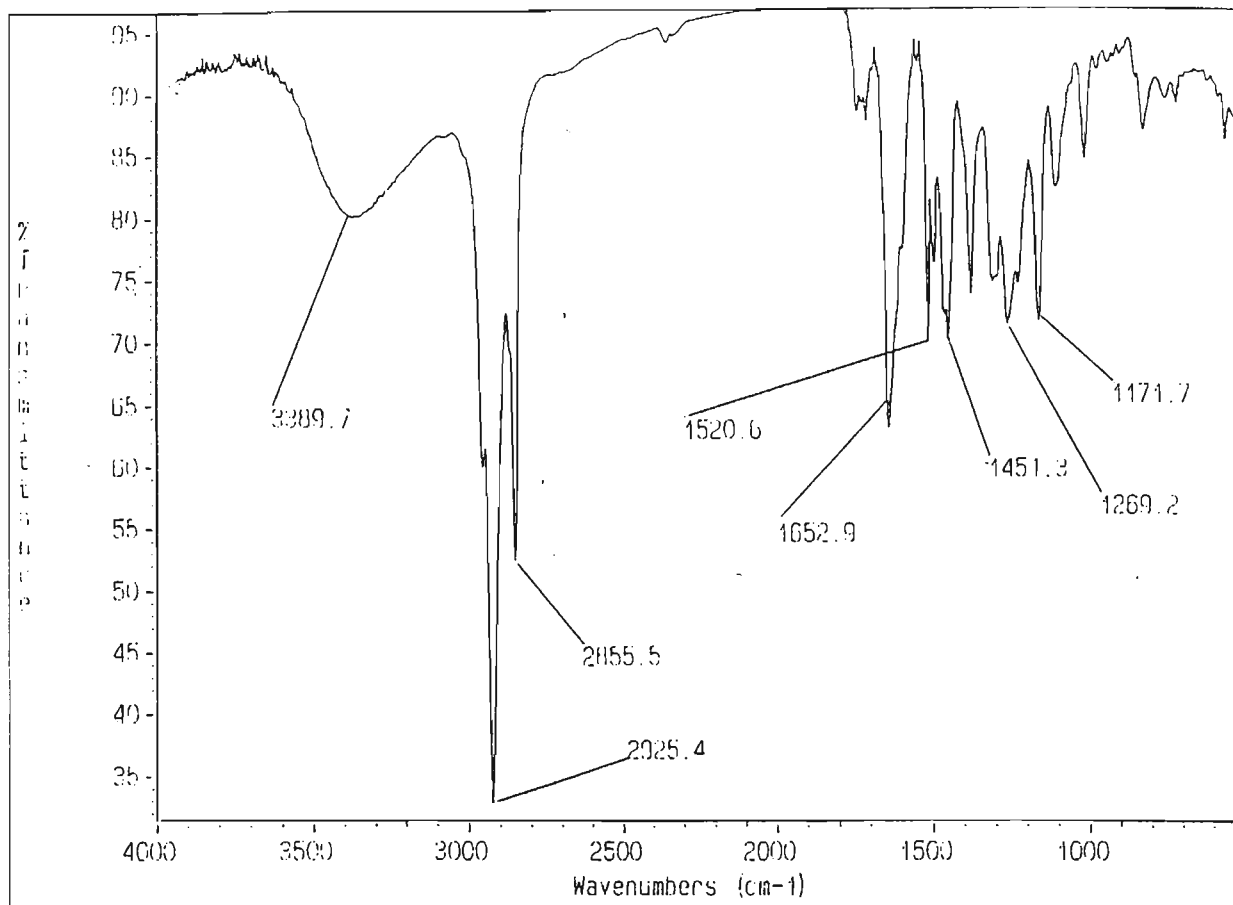
Spectrum 55: <sup>1</sup>H NMR spectrum of compound (viii)



Spectrum 56:  $^{13}\text{C}$  NMR spectrum of compound (viii)

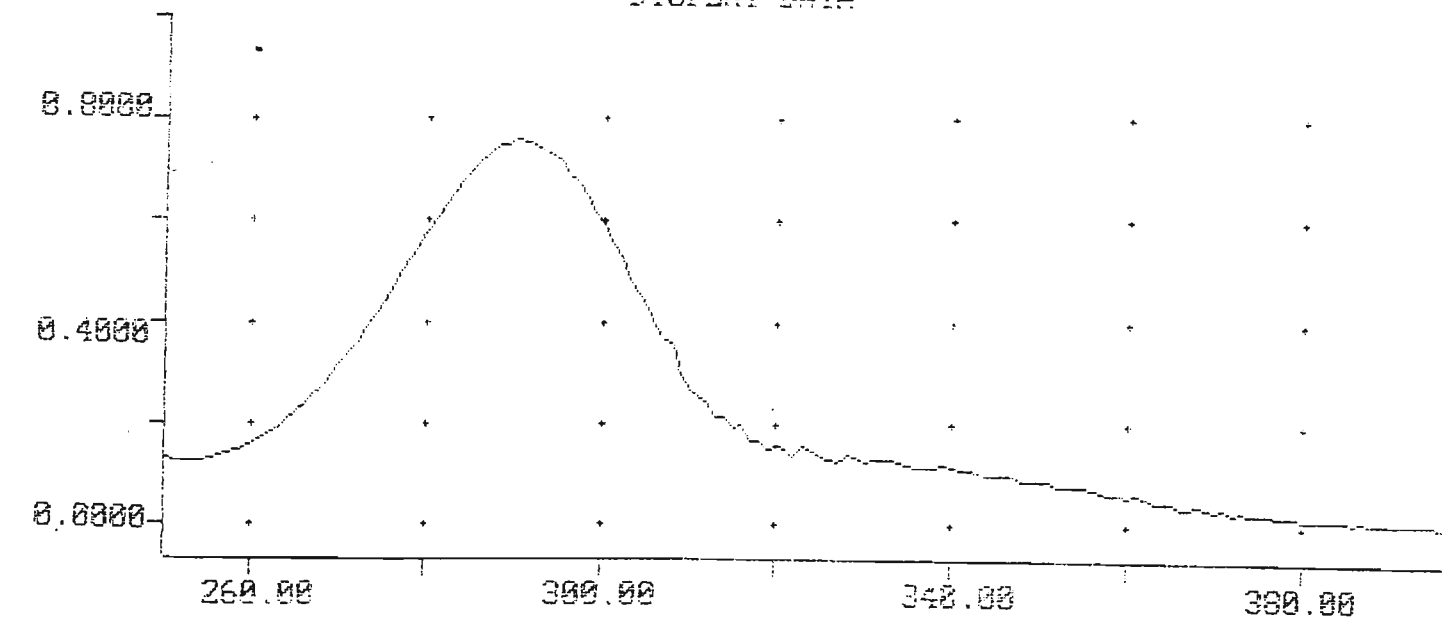


Spectrum 57 : Mass spectrum of compound (viii)



**Spectrum 58 : IR spectrum of compound (viii)**

DISPLAY DATA

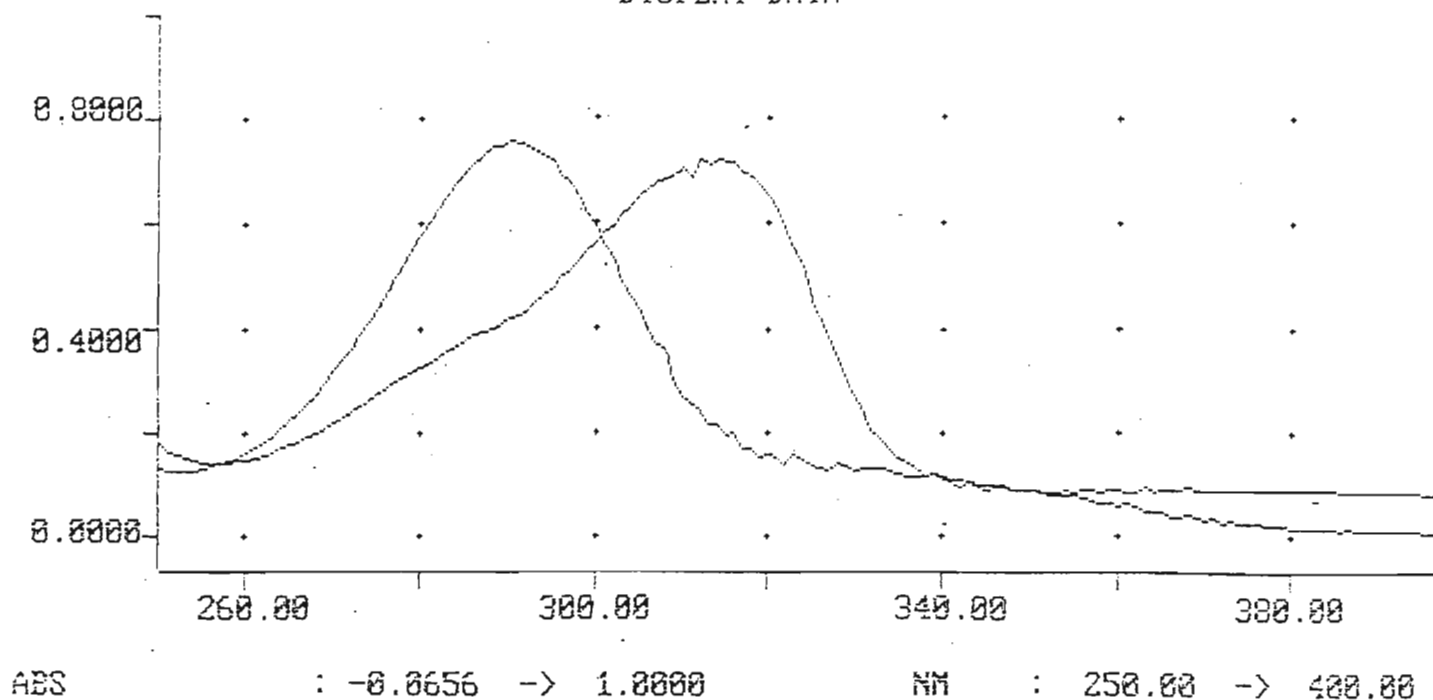


ABS : -0.0656 -> 1.0000      NM : 250.00 -> 400.00

ABS 0.75934      NM 290.000

**Spectrum 59 : UV spectrum of compound (viii)**

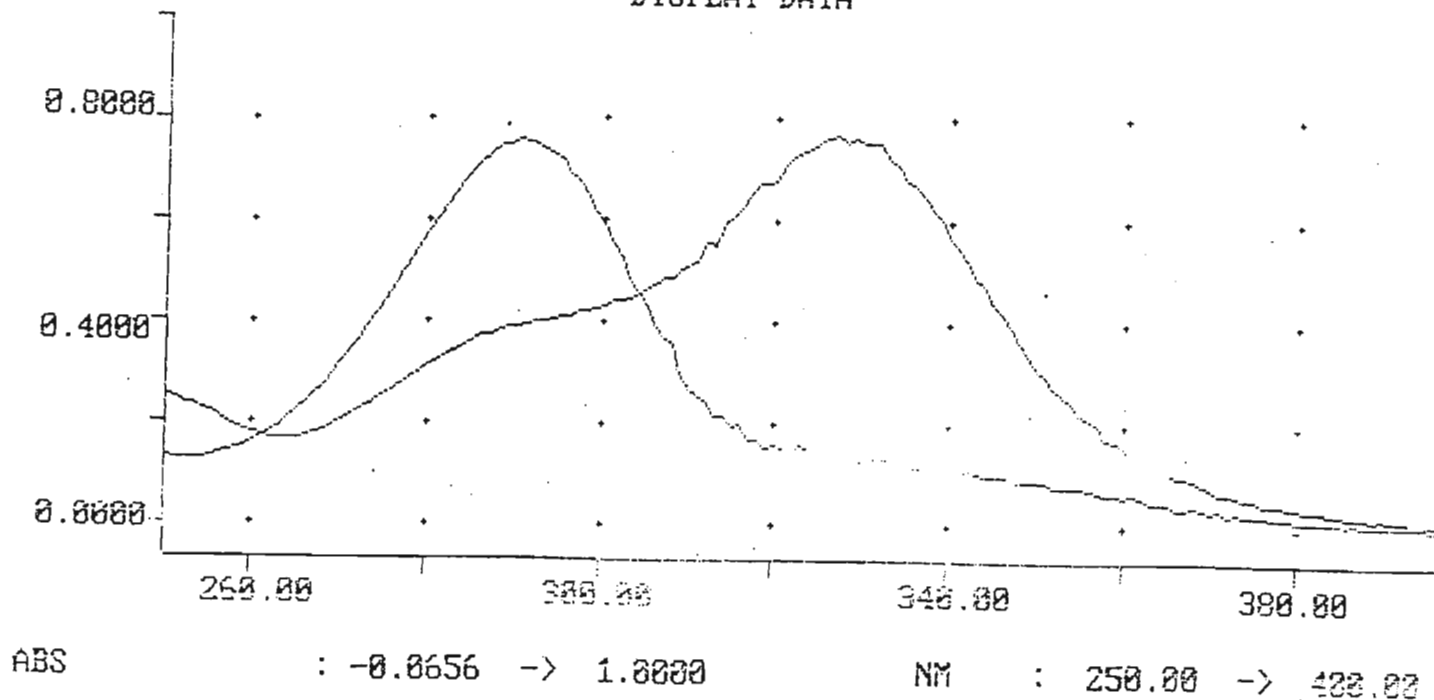
## DISPLAY DATA



ABS      0.75934      NM      290.000      -  
 ABS      0.72091      NM      312.000

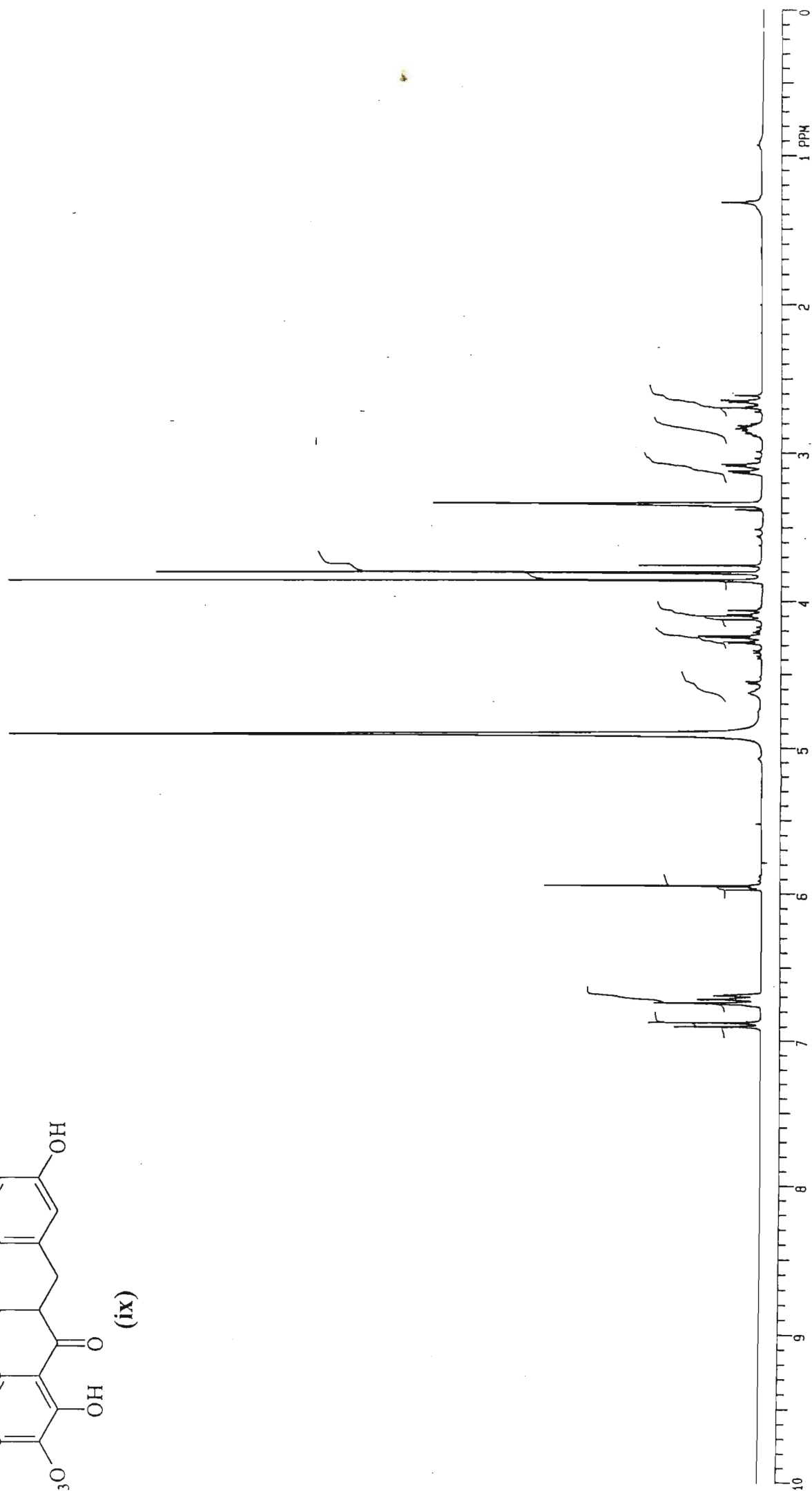
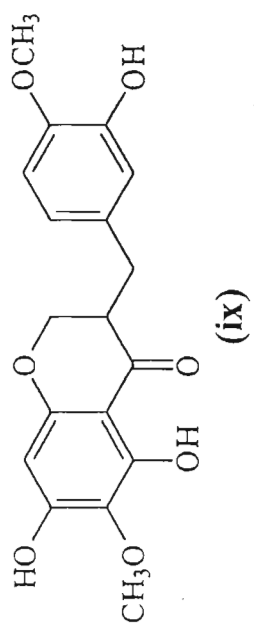
Spectrum 60 : UV and UV (AlCl<sub>3</sub>) spectra of compound (viii)

## DISPLAY DATA

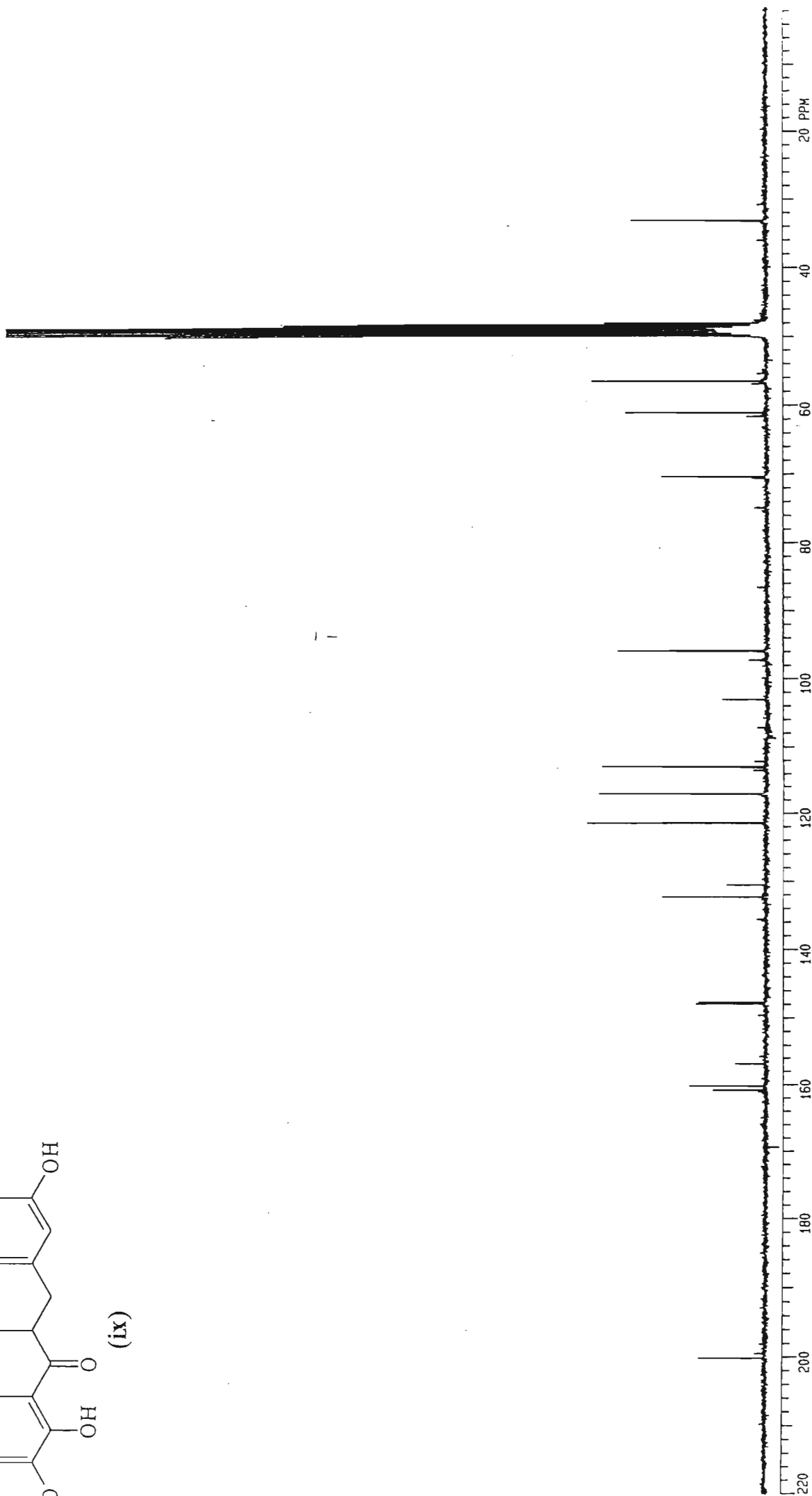
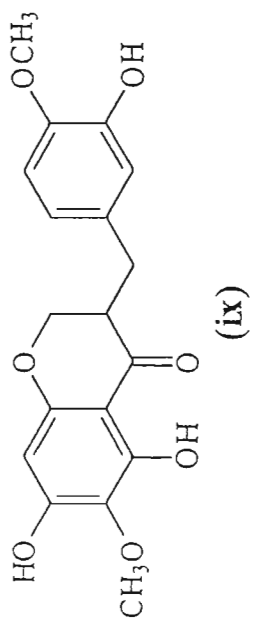


ABS      0.75934      NM      290.000  
 ABS      0.76989      NM      327.000

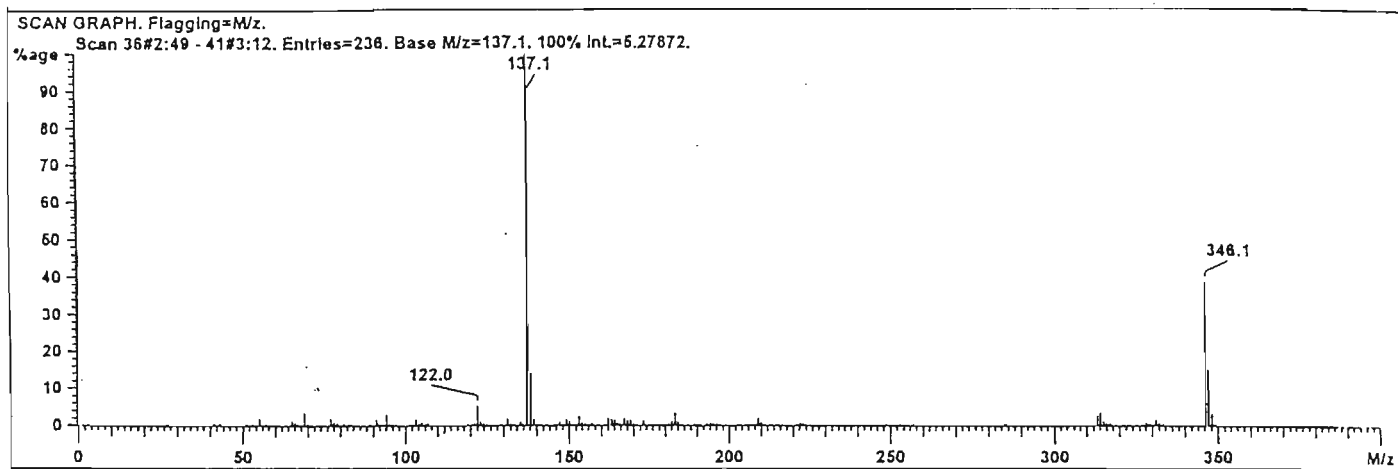
Spectrum 61 : UV and UV (NaOAc) spectra of compound (viii)



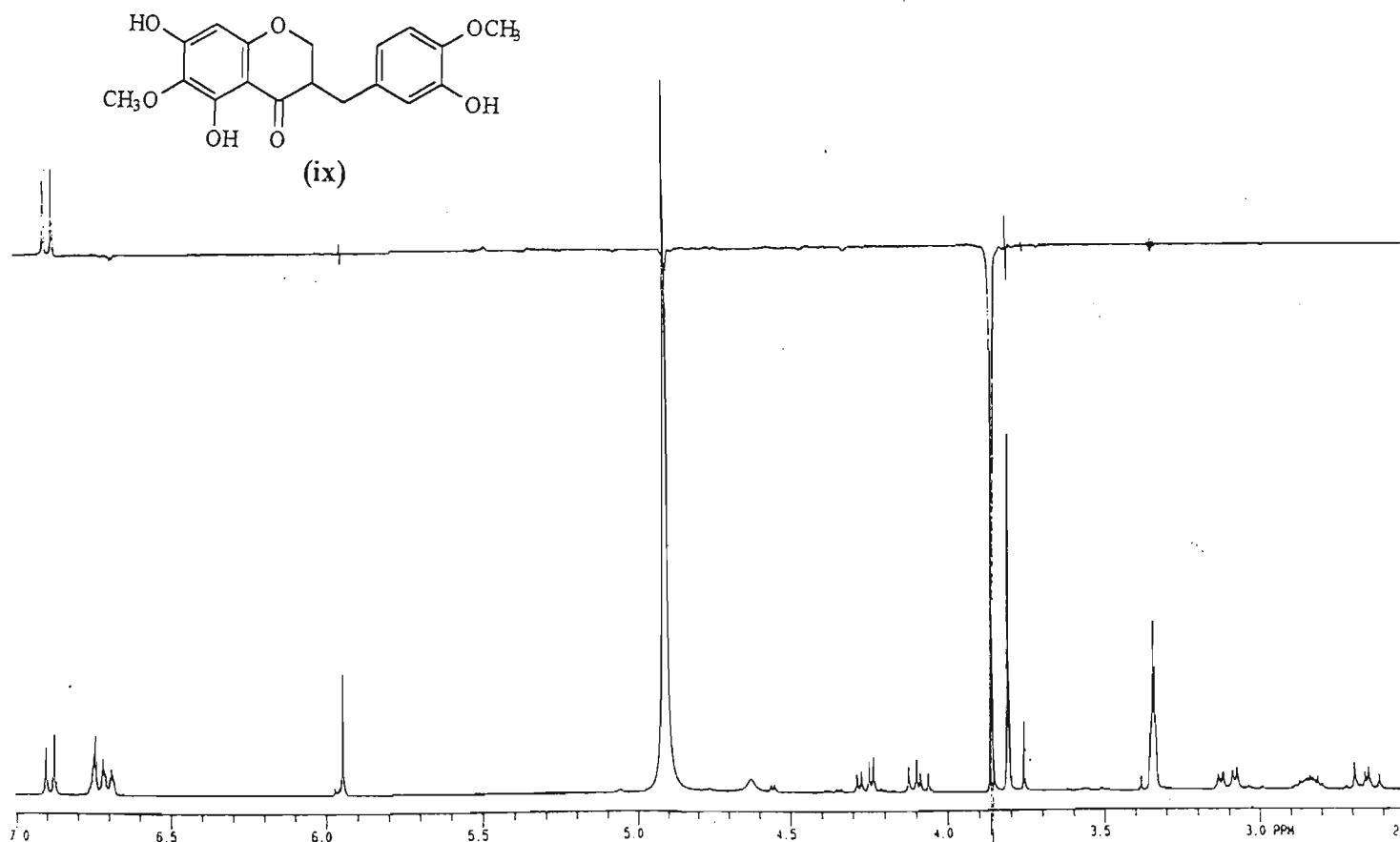
Spectrum 62 : <sup>1</sup>H NMR spectrum of compound (ix)



Spectrum 63 : <sup>13</sup>C NMR spectrum of compound (ix)

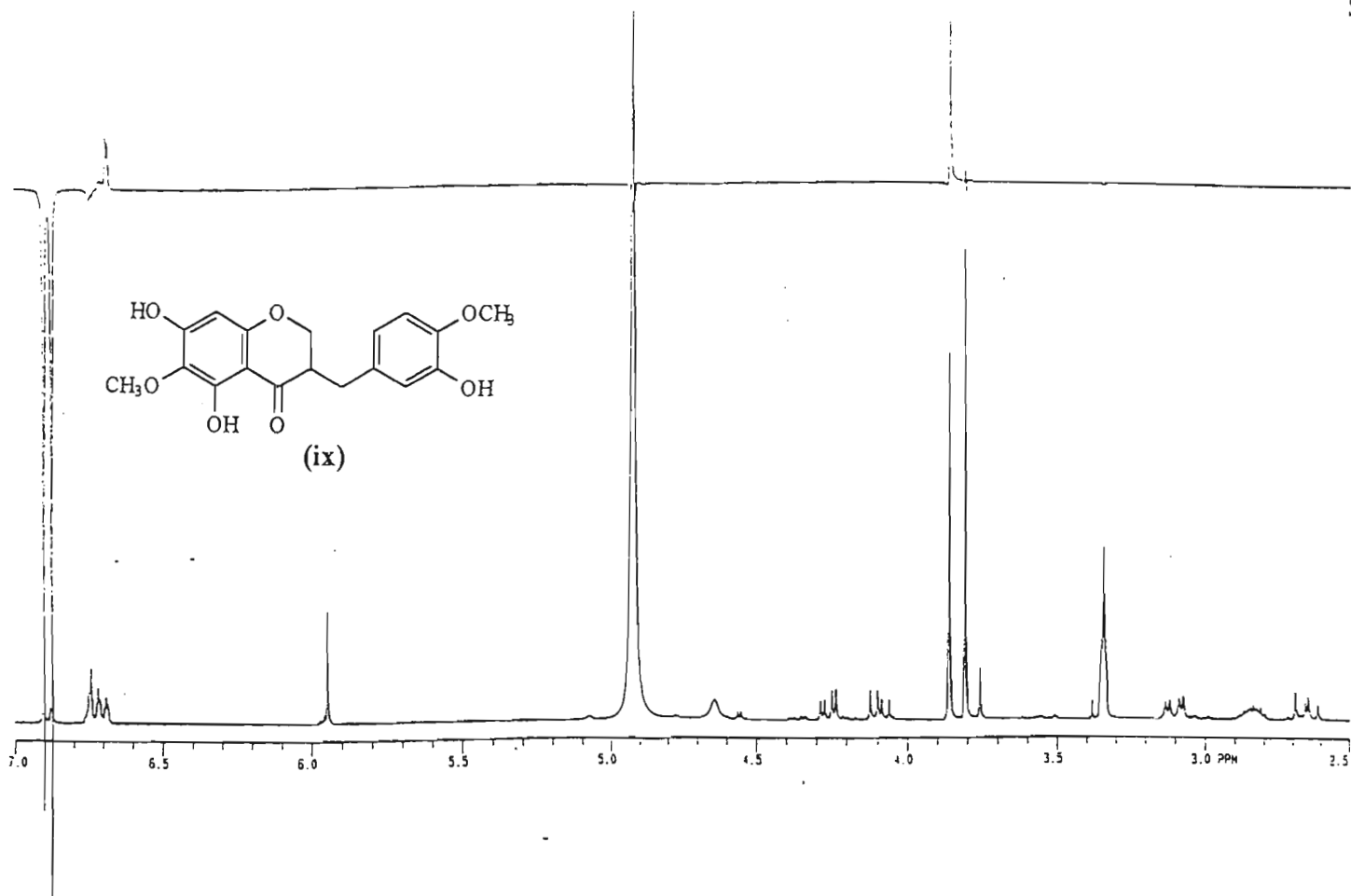


Spectrum 64 : Mass spectrum of compound (ix)

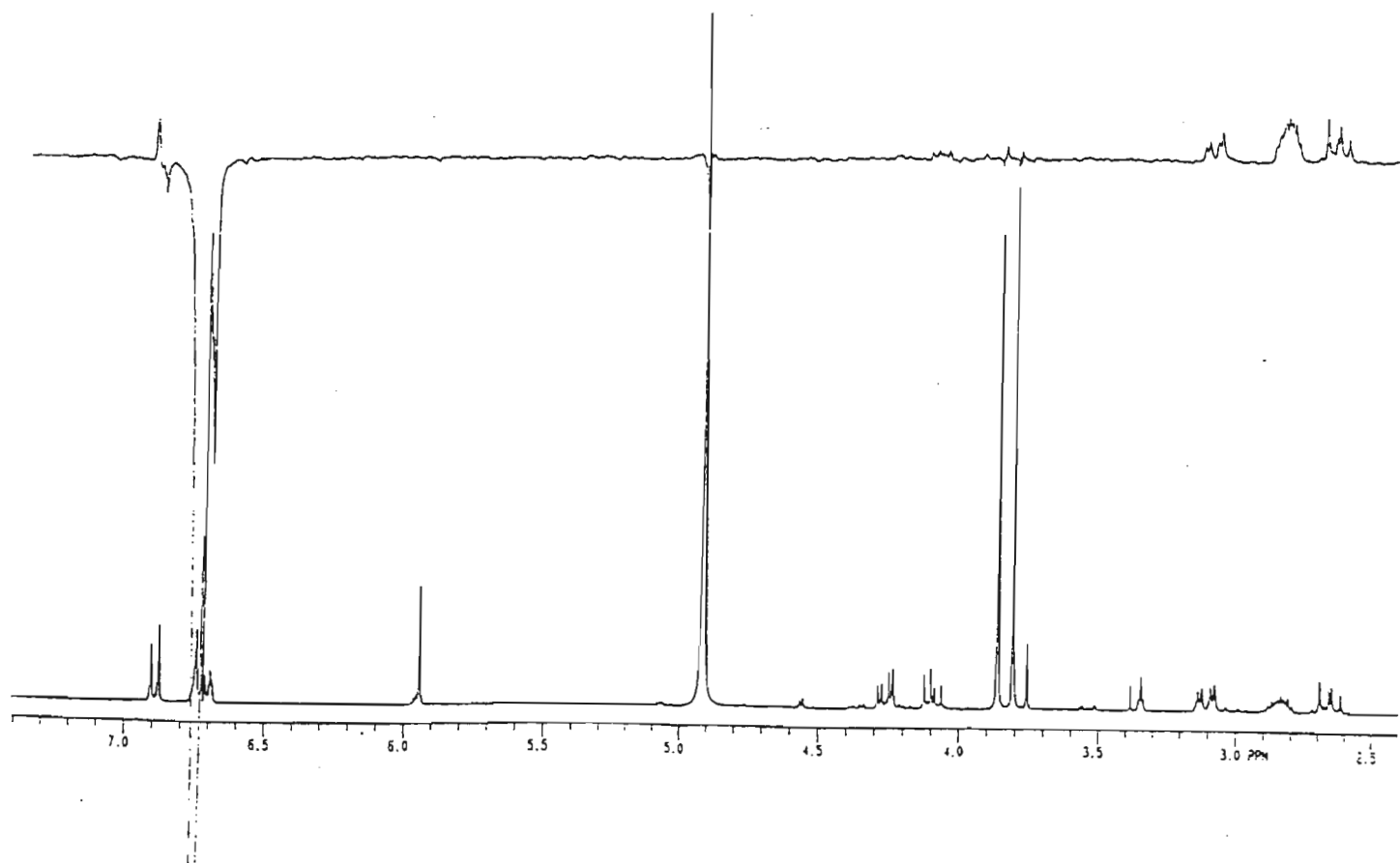


Spectrum 65a : NOE spectrum of compound (ix)

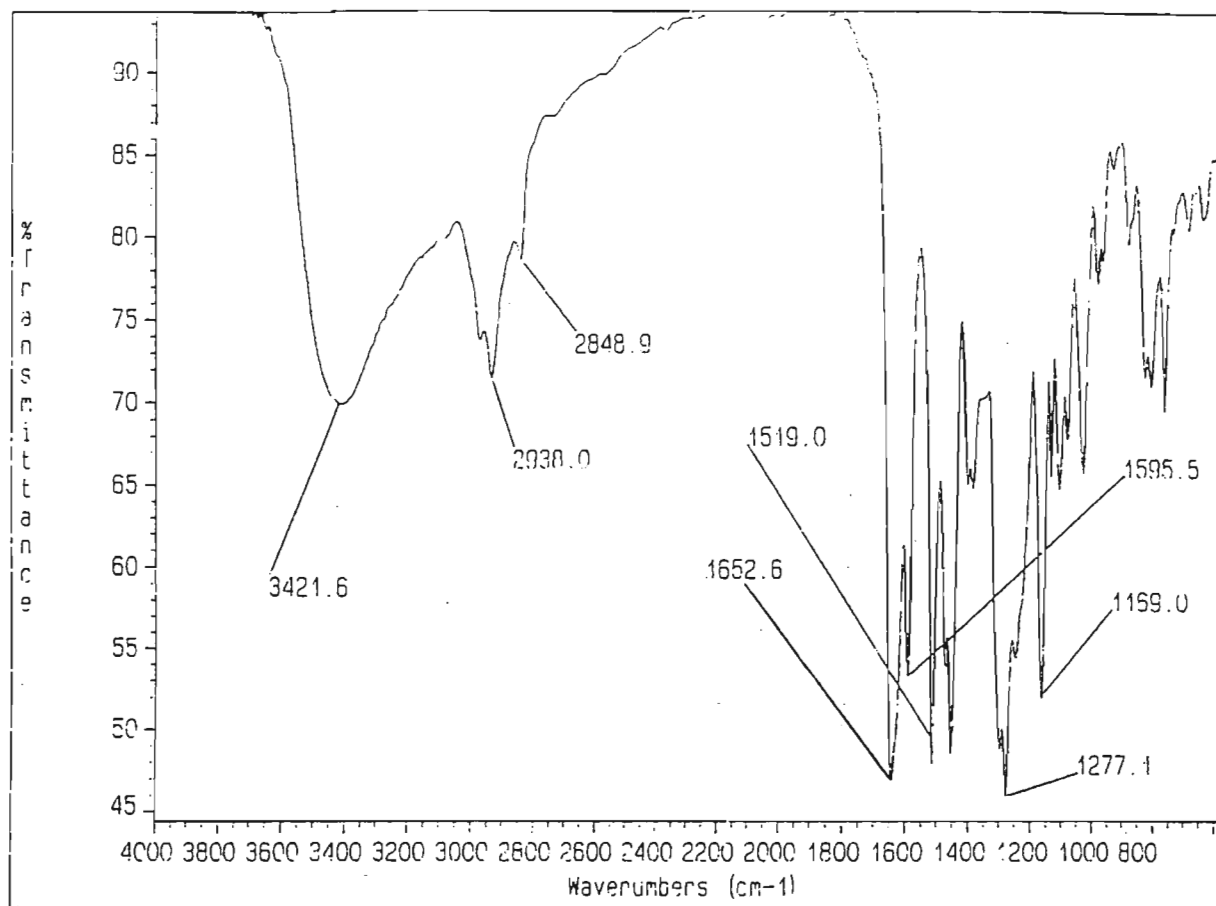




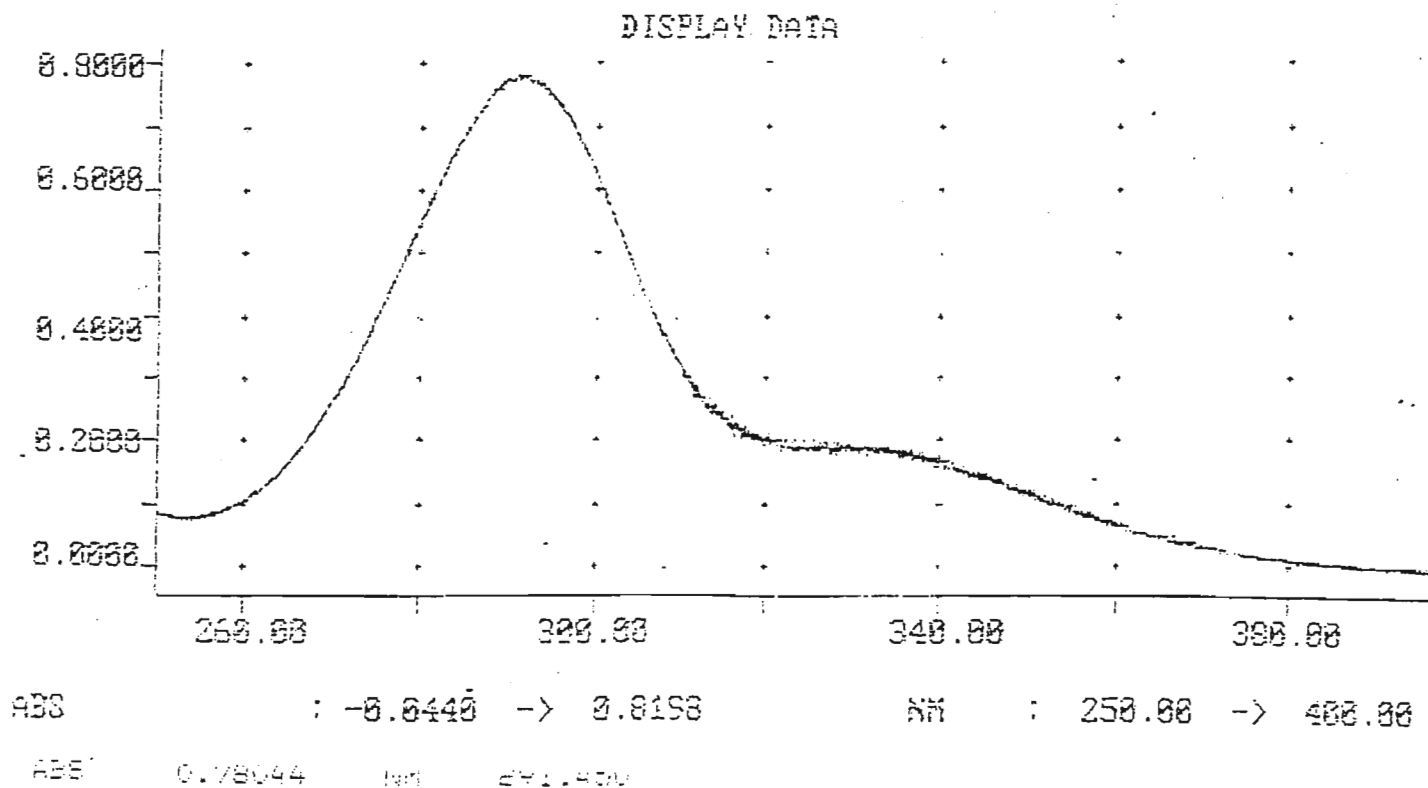
Spectrum 65b : NOE spectrum of compound (ix)



Spectrum 65c : NOE spectrum of compound (ix)

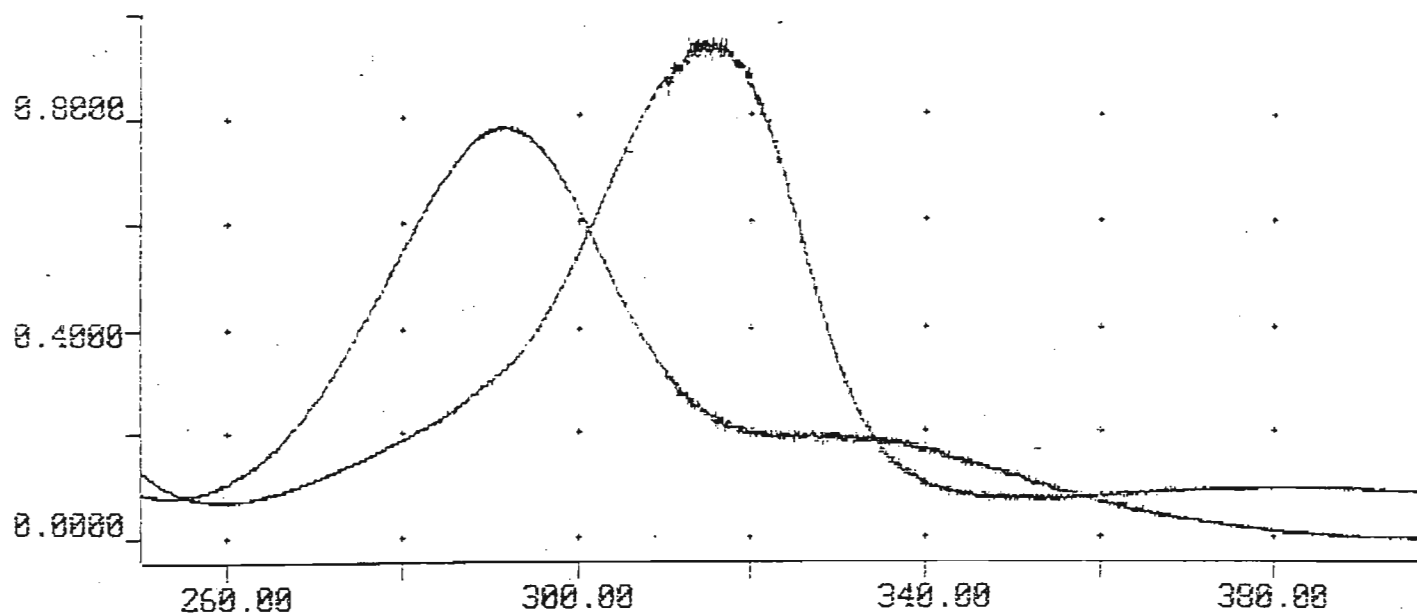


**Spectrum 66 : IR spectrum of compound (ix)**



**Spectrum 67 : UV spectrum of compound (ix)**

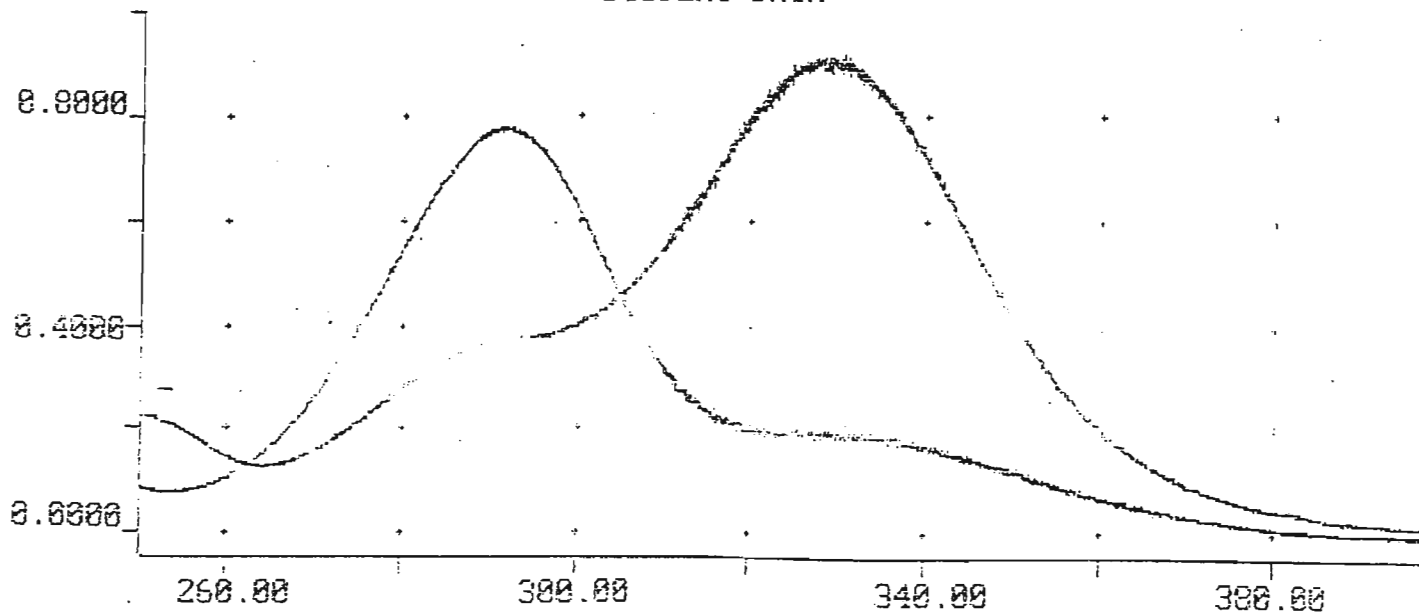
## DISPLAY DATA



ABS : -0.0440 -> 1.0000 NM : 250.00 -> 400.00  
 ABS 0.78044 NM 291.450  
 ABS 0.93033 NM 315.150

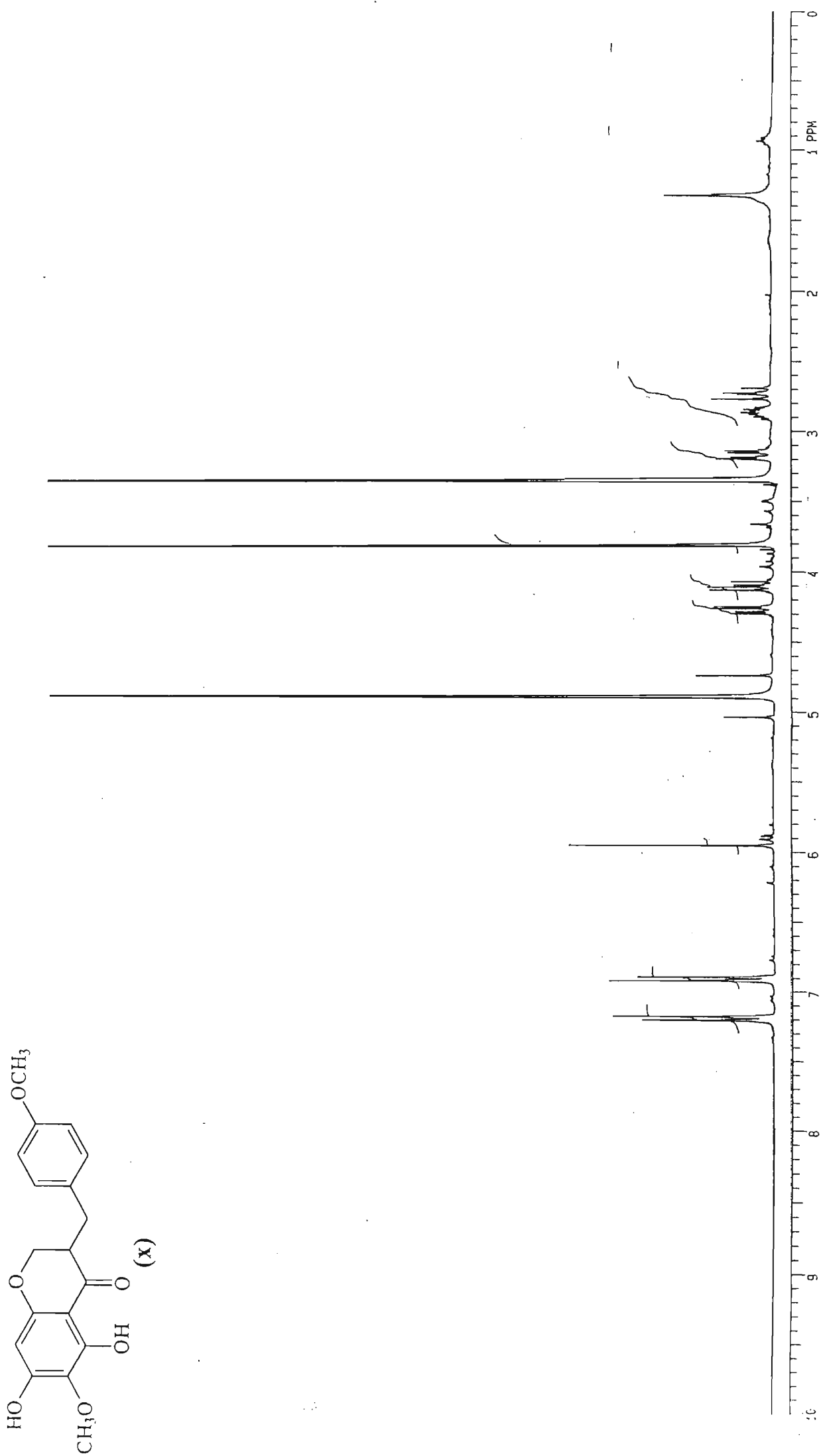
Spectrum 68 : UV and UV (+ AlCl<sub>3</sub>) spectra of compound (ix)

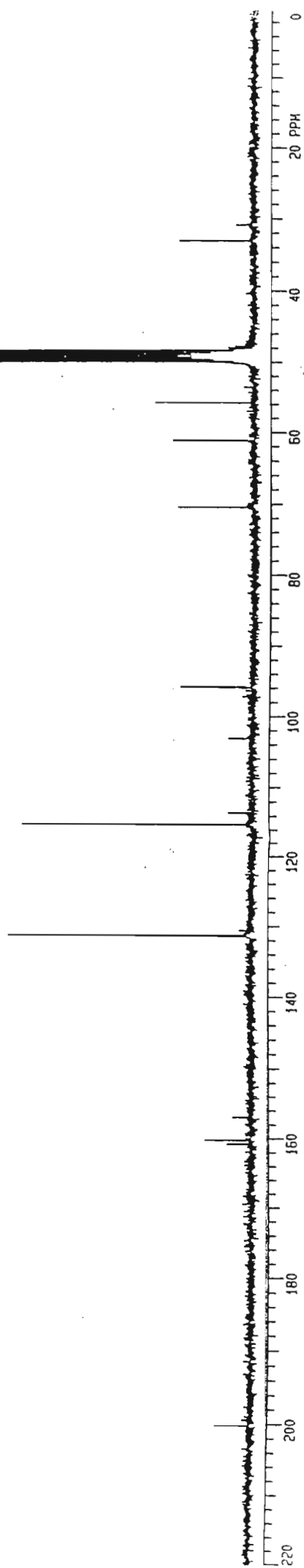
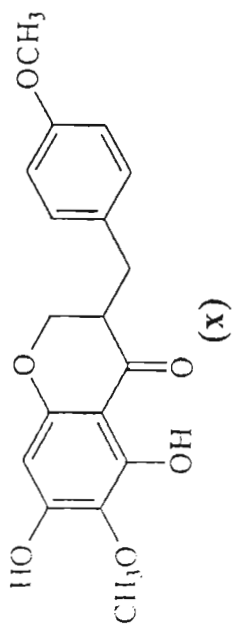
## DISPLAY DATA



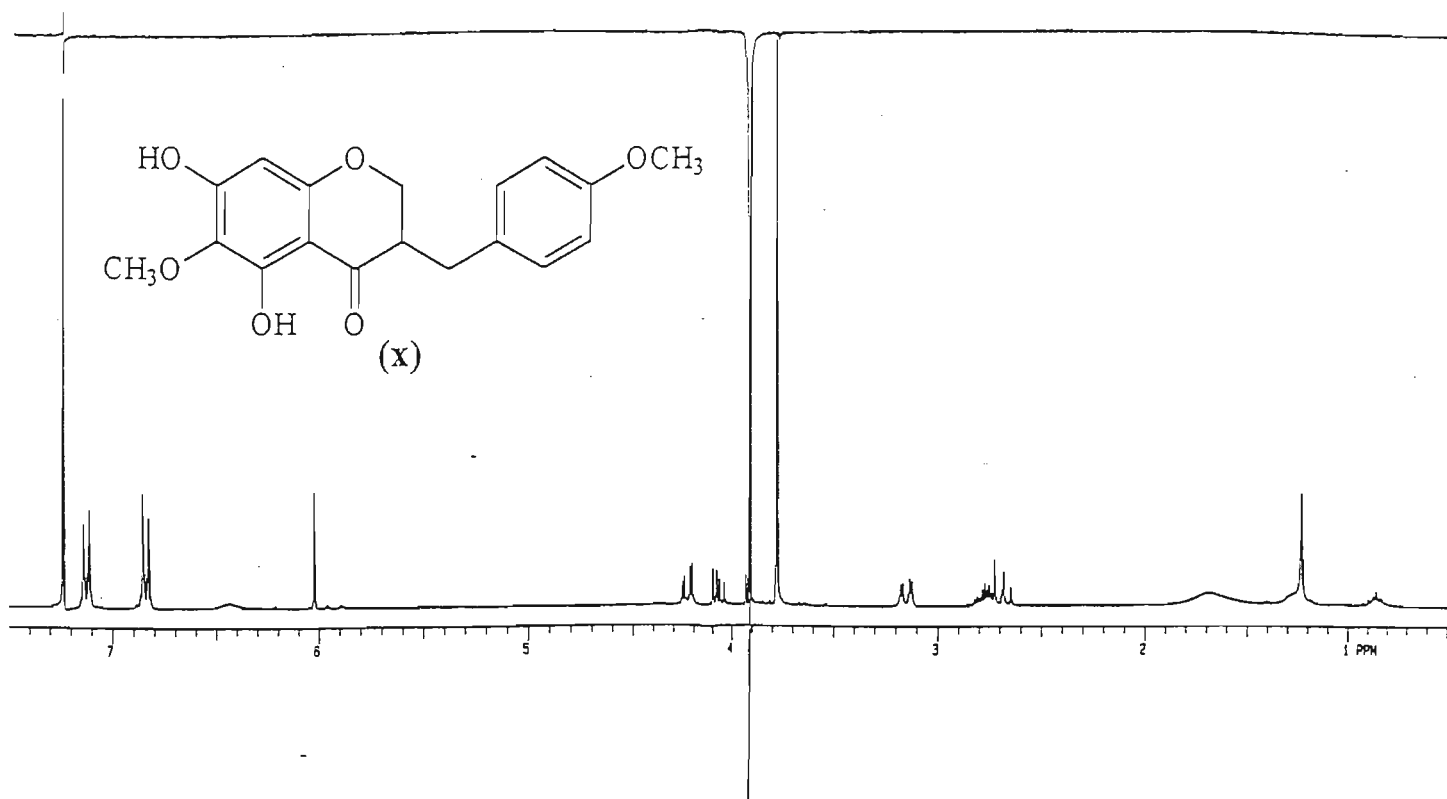
ABS : -0.0440 -> 1.0000 NM : 250.00 -> 400.00  
 ABS 0.78044 NM 291.450  
 ABS 0.91715 NM 328.350

Spectrum 69 : UV and UV (+ NaOAc) spectra of compound (ix)

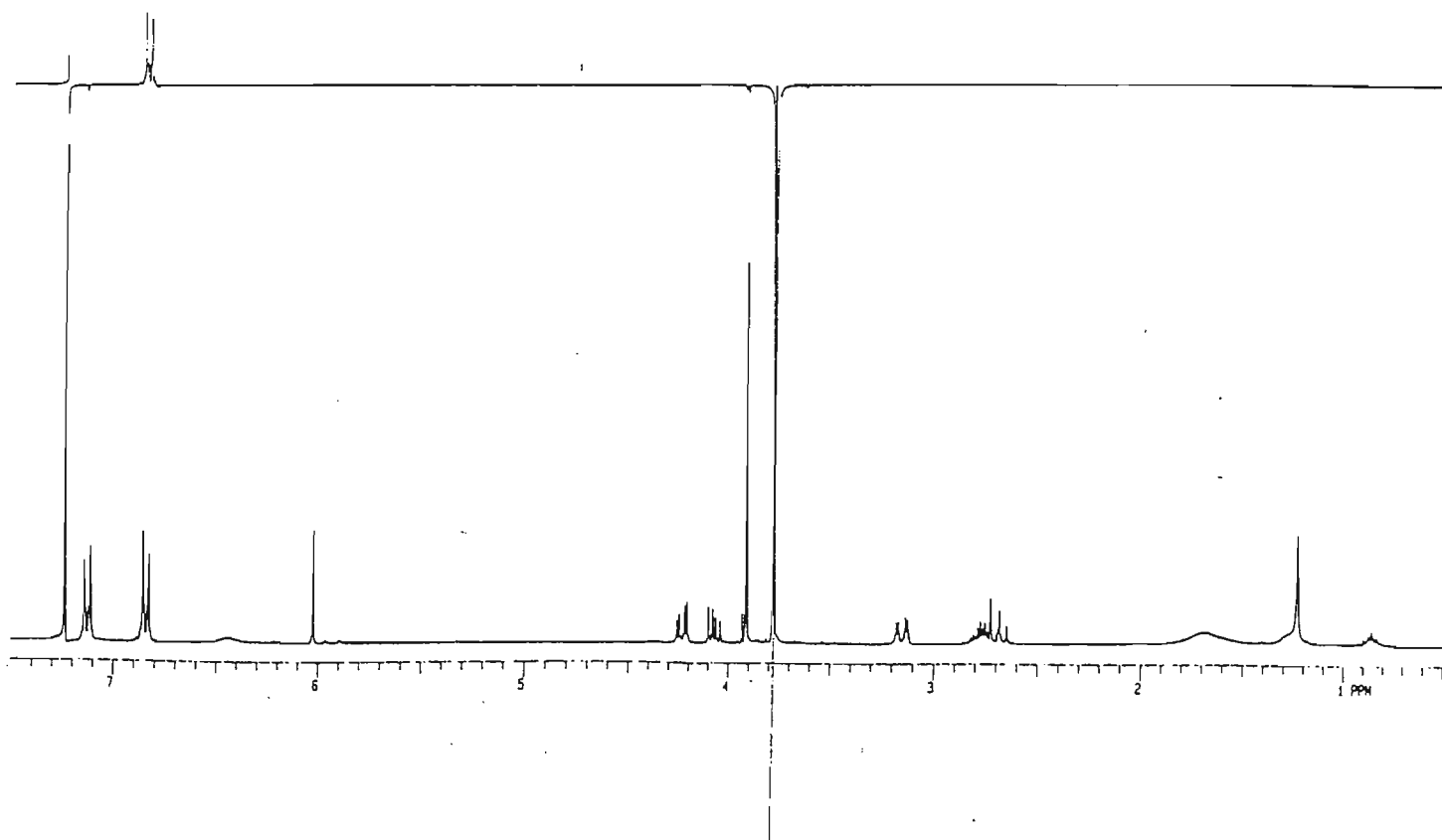
Spectrum 70 : <sup>1</sup>H NMR spectrum of compound (x)



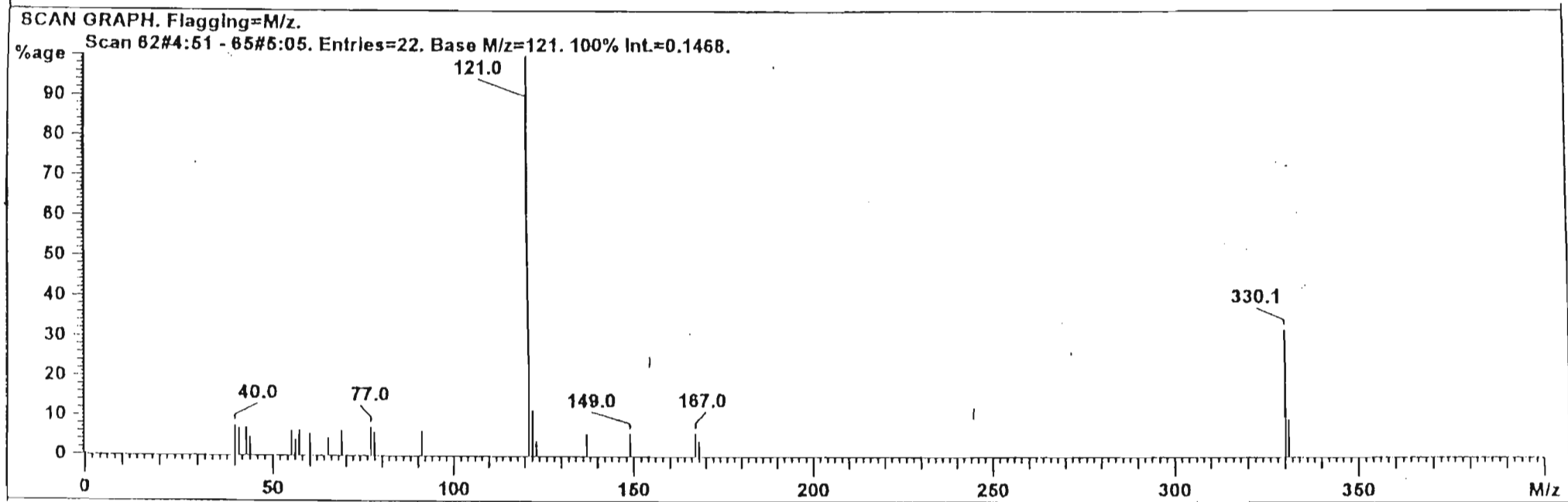
Spectrum 71 : <sup>13</sup>C NMR spectrum of compound (x)



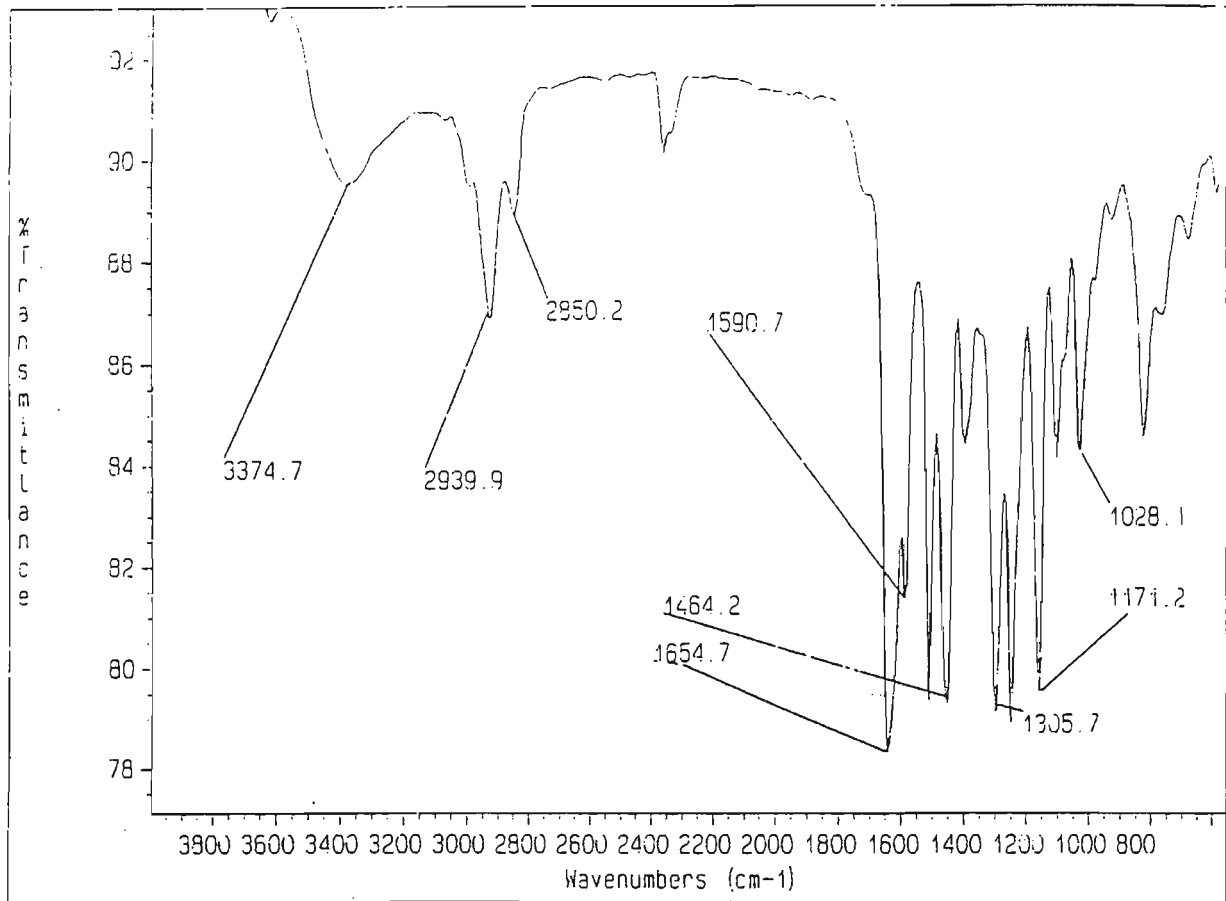
**Spectrum 72a : NOE spectrum of compound (x)**



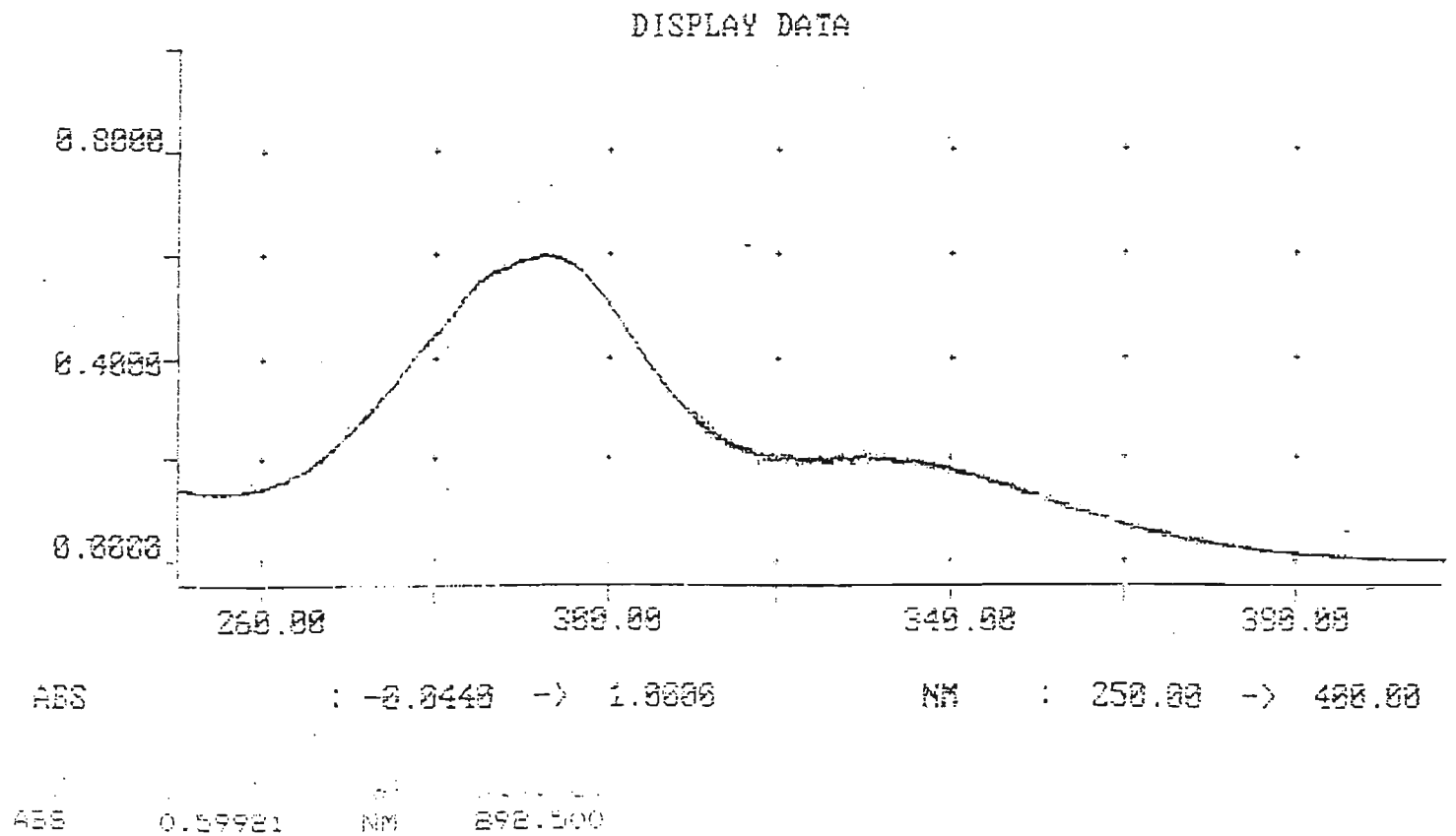
**Spectrum 72b : NOE spectrum of compound (x)**



Spectrum 73 : Mass spectrum of compound (x)



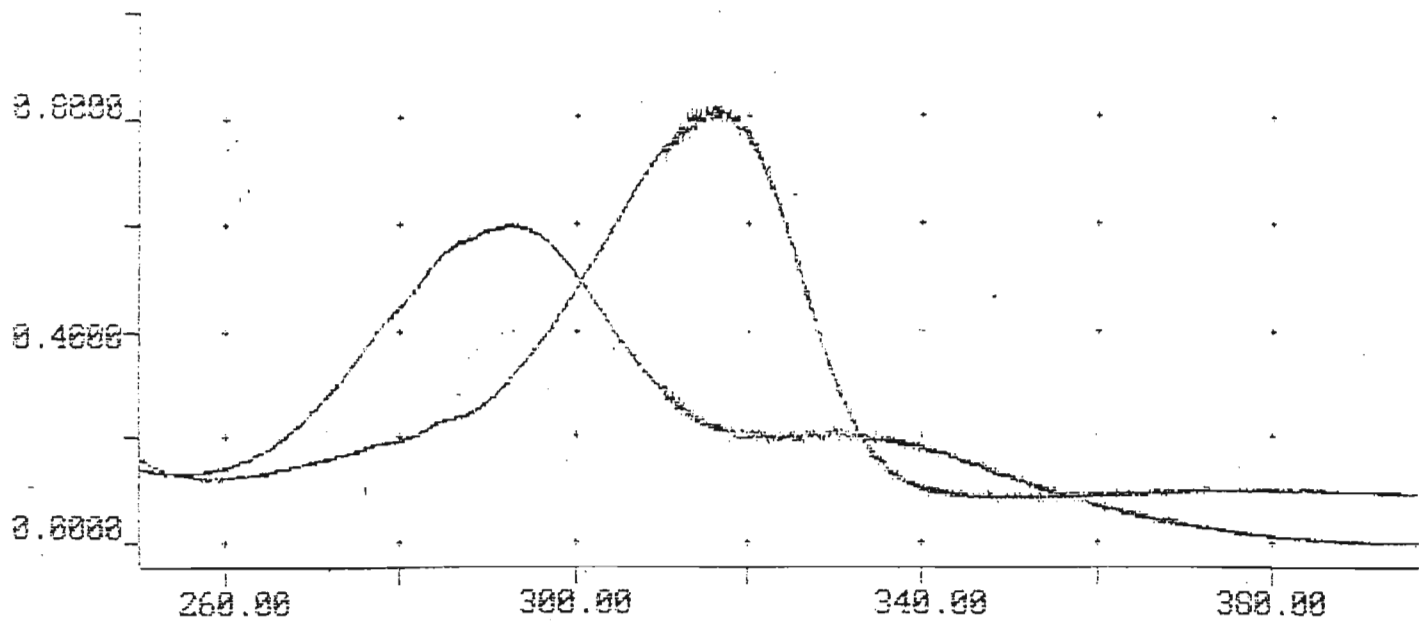
**Spectrum 74 : IR spectrum of compound (x)**



**Spectrum 75 : UV spectrum of compound (x)**



DISPLAY DATA



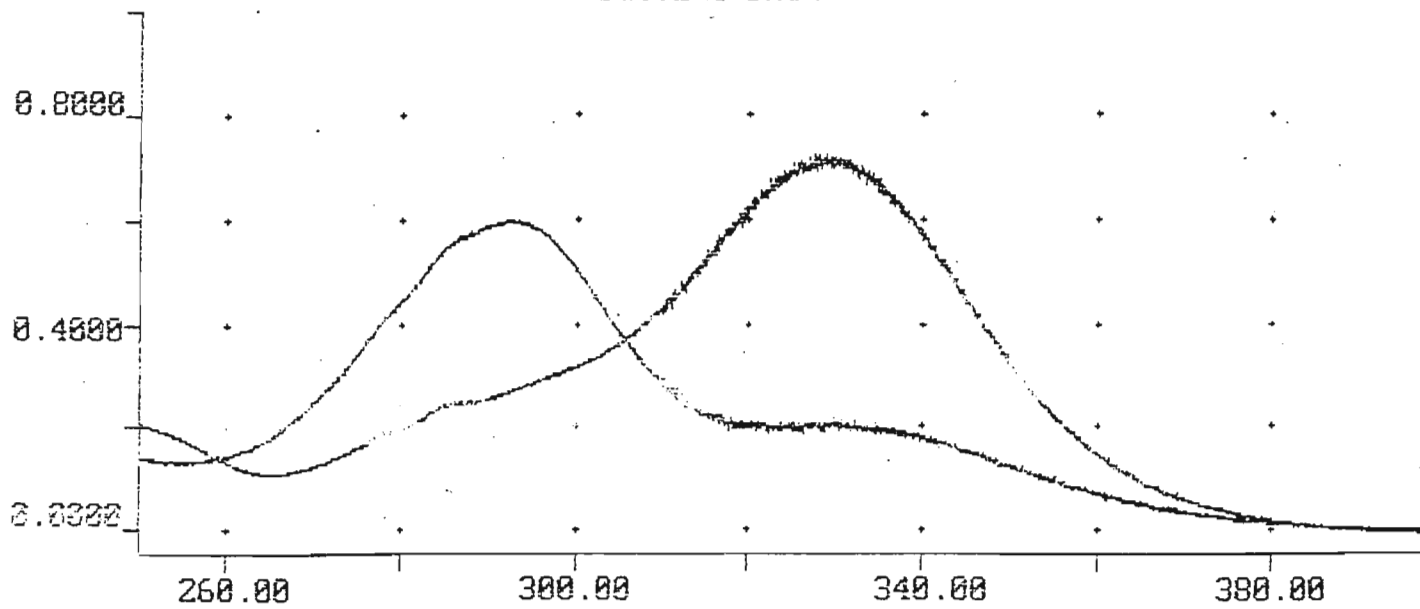
ABS : -0.0440 -> 1.0000 NM : 250.00 -> 400.00

ABS 0.59921 NM 292.500

ABS 0.80621 NM 315.450

Spectrum 76 : UV and UV (+ AlCl<sub>3</sub>) spectra of compound (x)

DISPLAY DATA

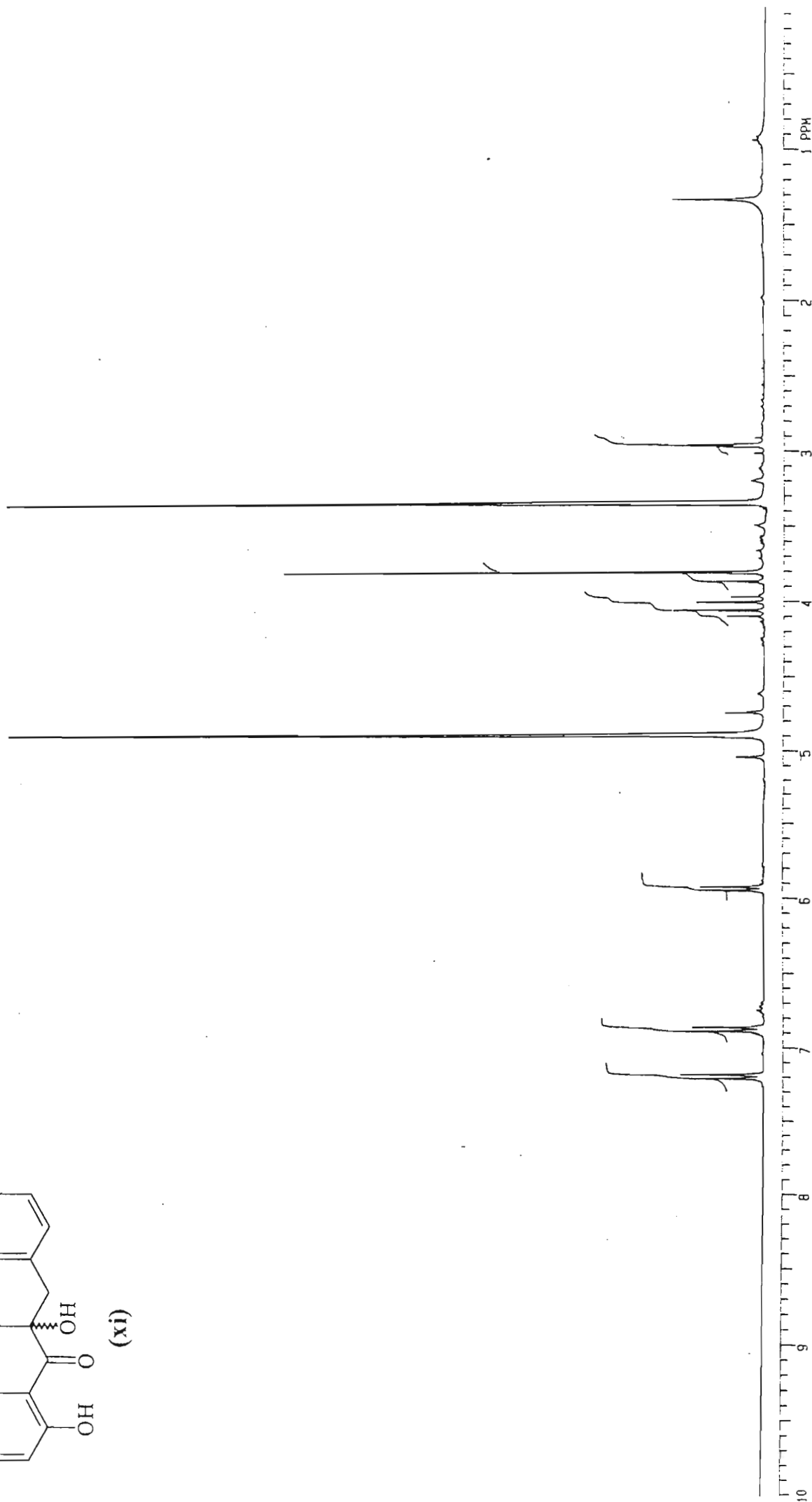
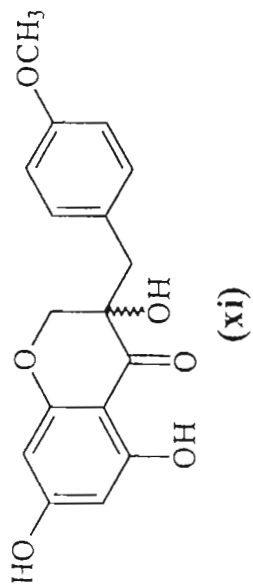


ABS : -0.0440 -> 1.0000 NM : 250.00 -> 400.00

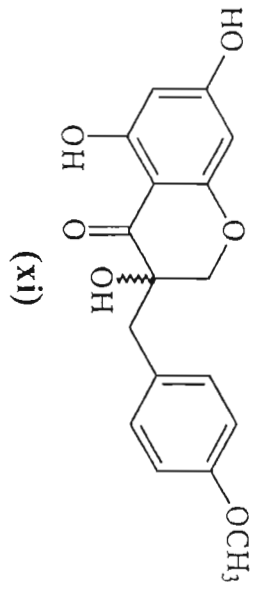
ABS 0.59921 NM 292.500

ABS 0.71697 NM 328.700

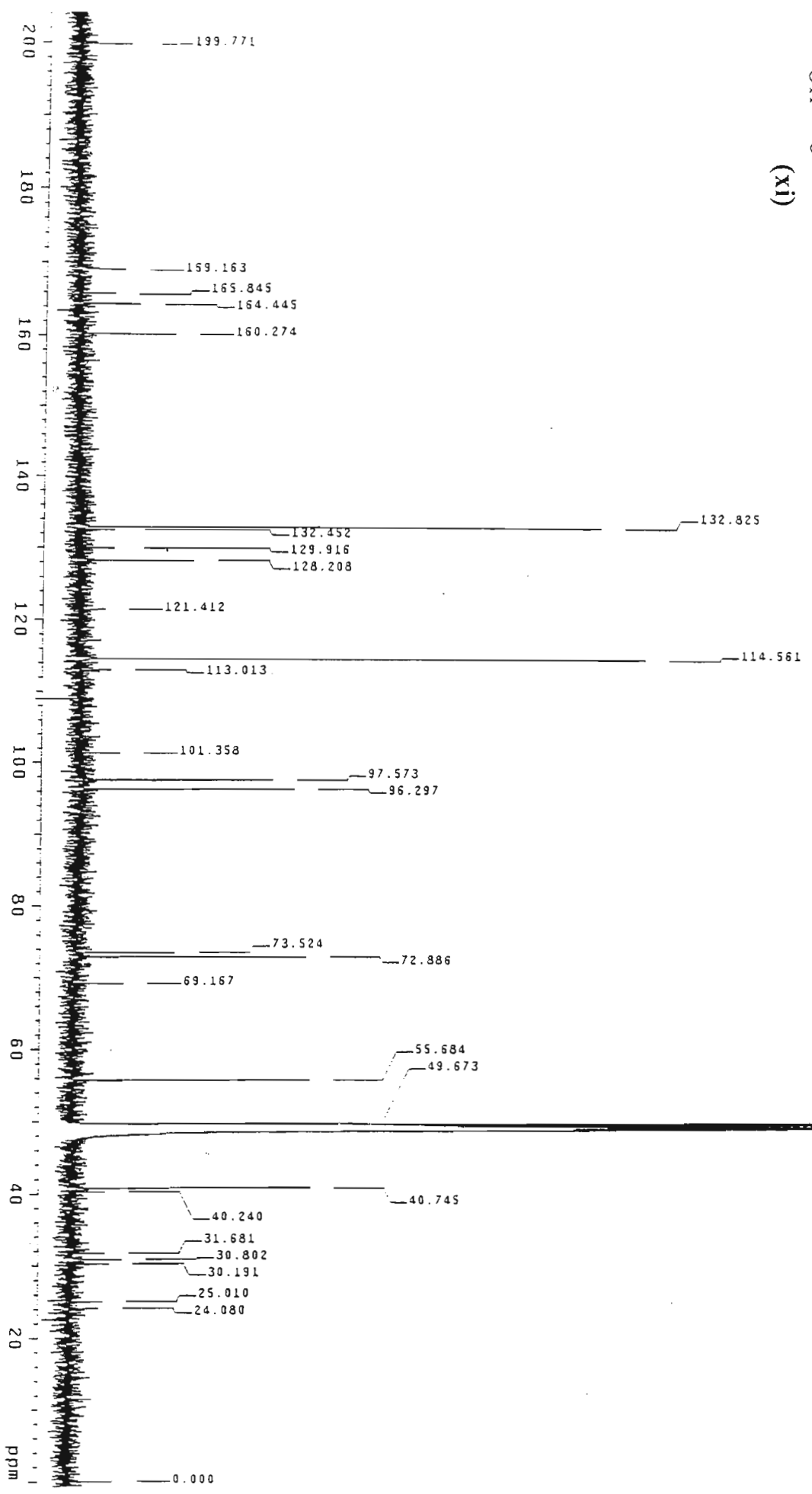
Spectrum 77 : UV and UV (+ NaOAc) spectra of compound (x)



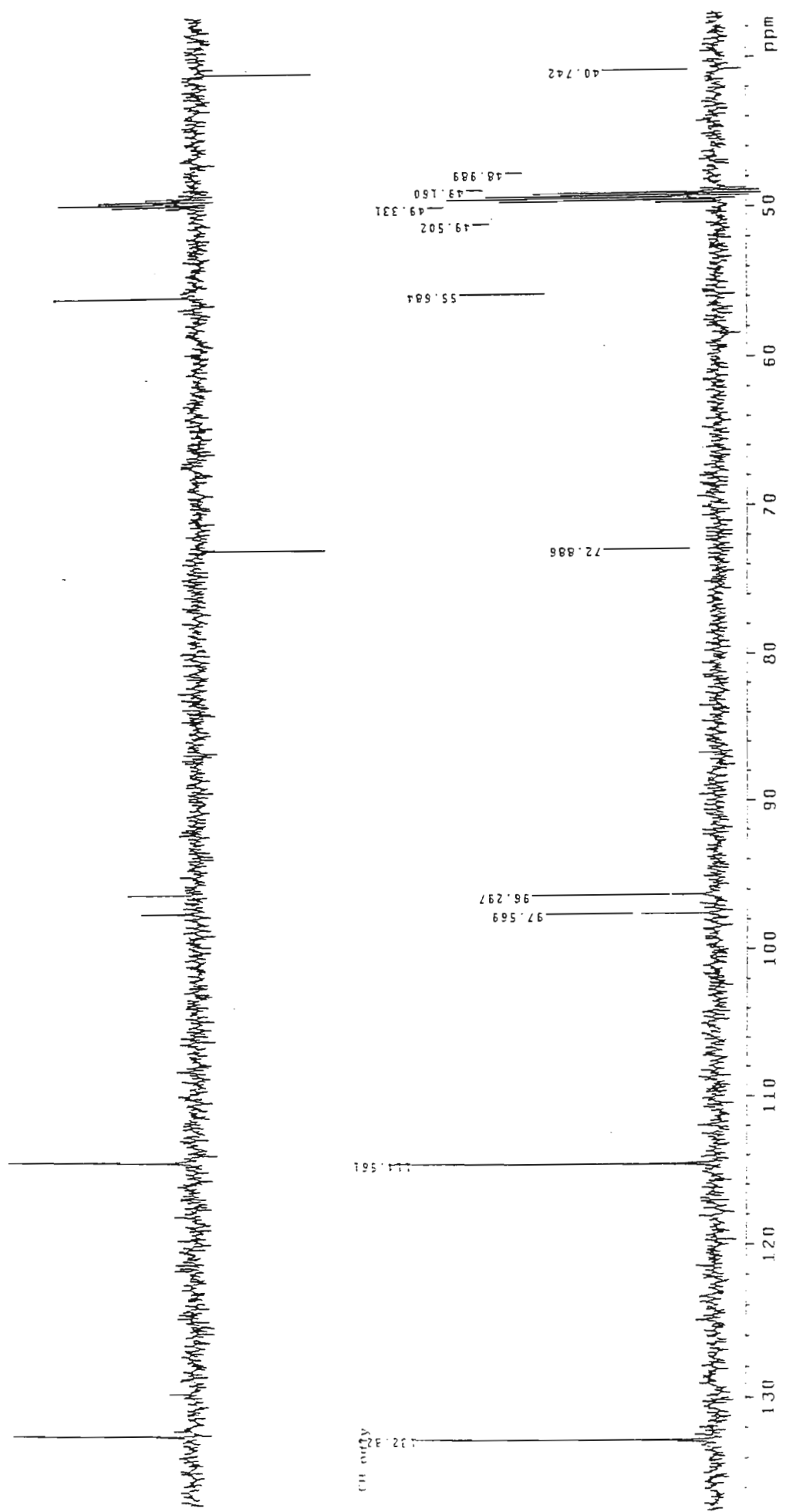
Spectrum 78:  $^1\text{H}$  NMR spectrum of compound (xi)



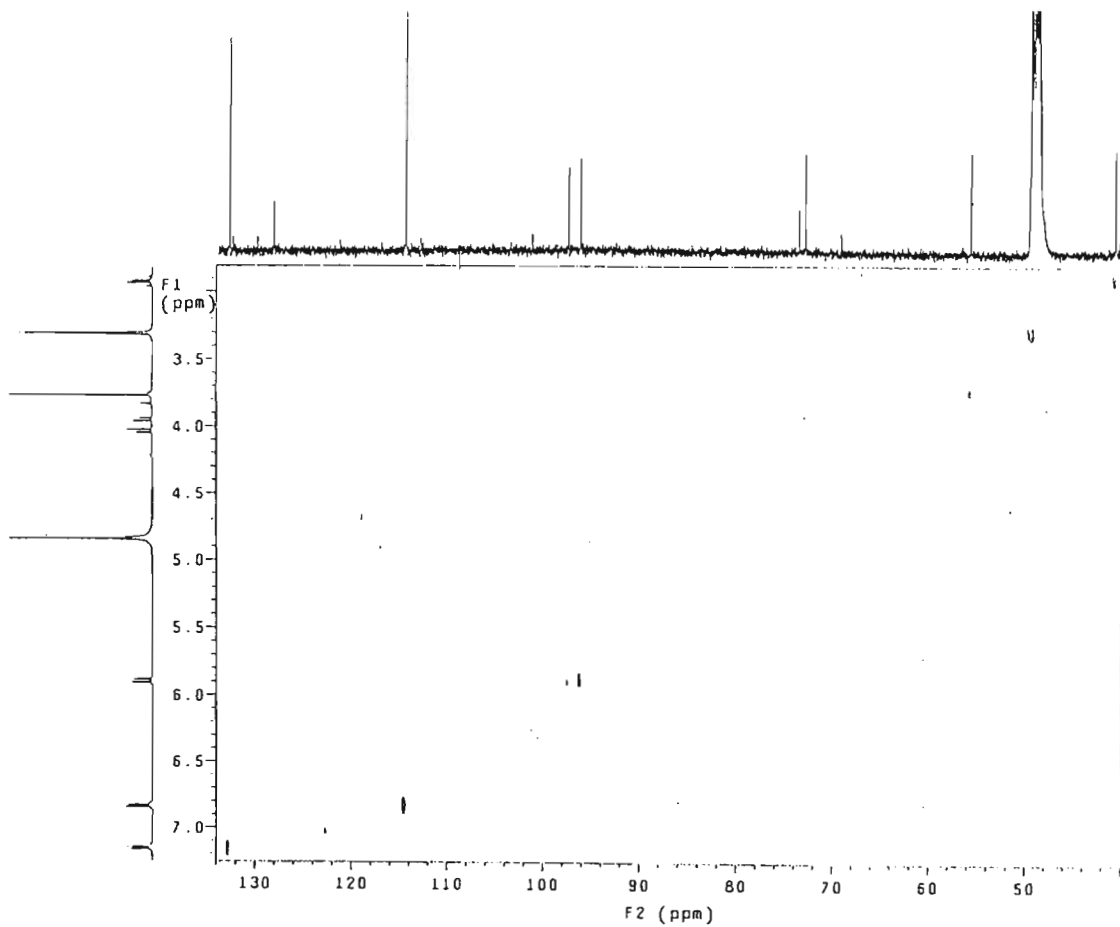
Spectrum 79 :  $^{13}\text{C}$  NMR spectrum of compound (xi)



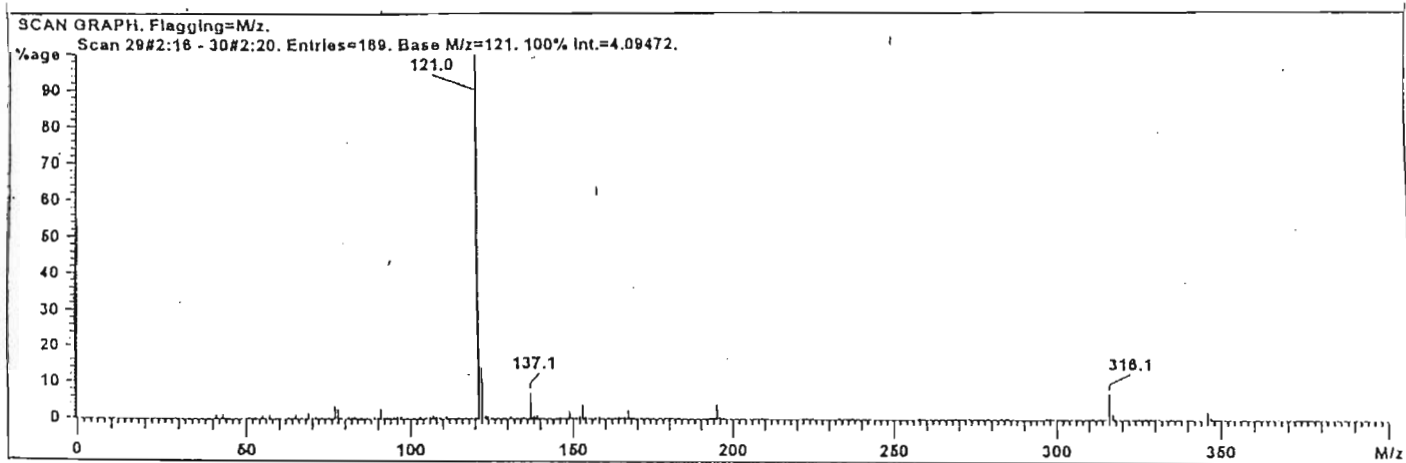
CH<sub>2</sub> up; CH<sub>2</sub> down



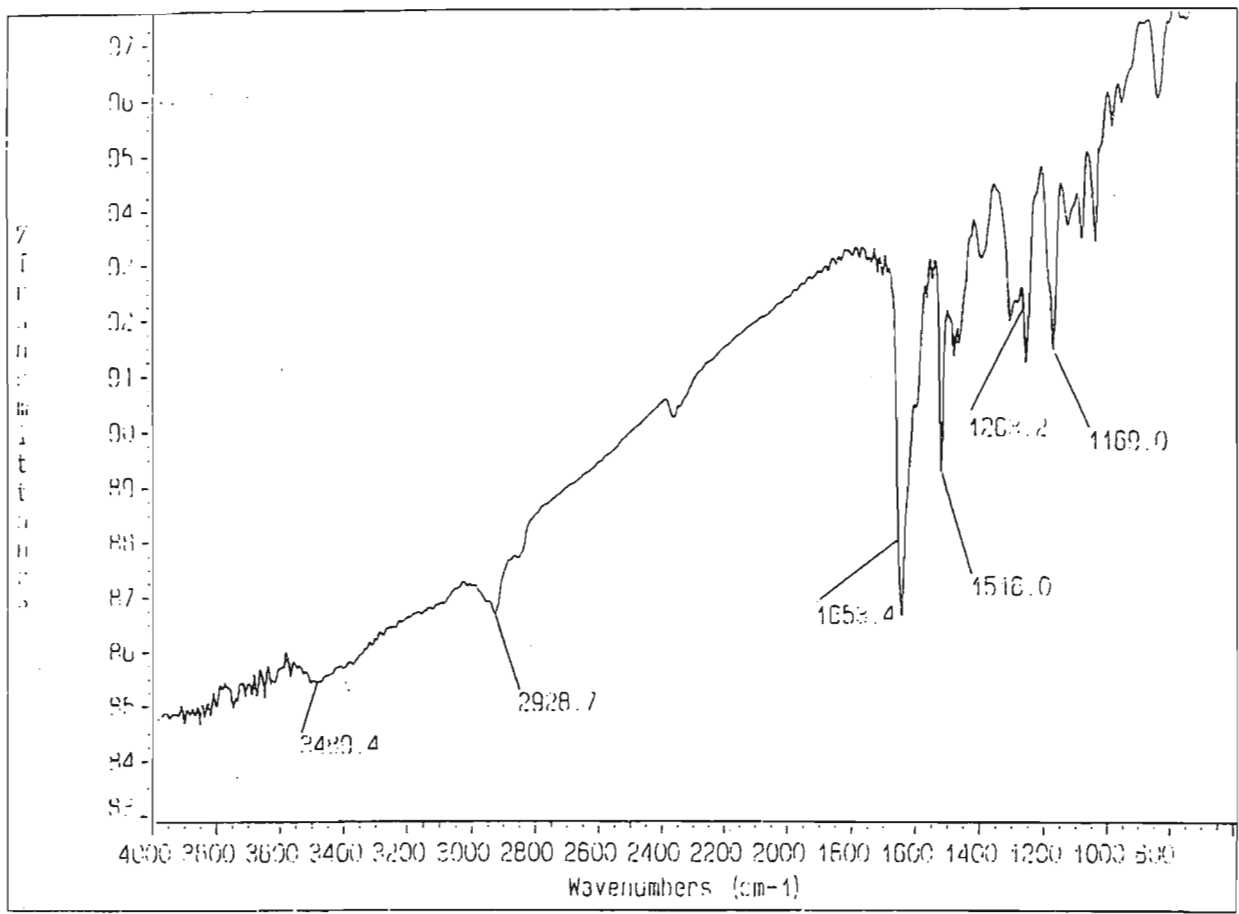
Spectrum 80 : ADEPT spectrum of compound (xi)



**Spectrum 81 : HETCOR spectrum of compound (xi)**

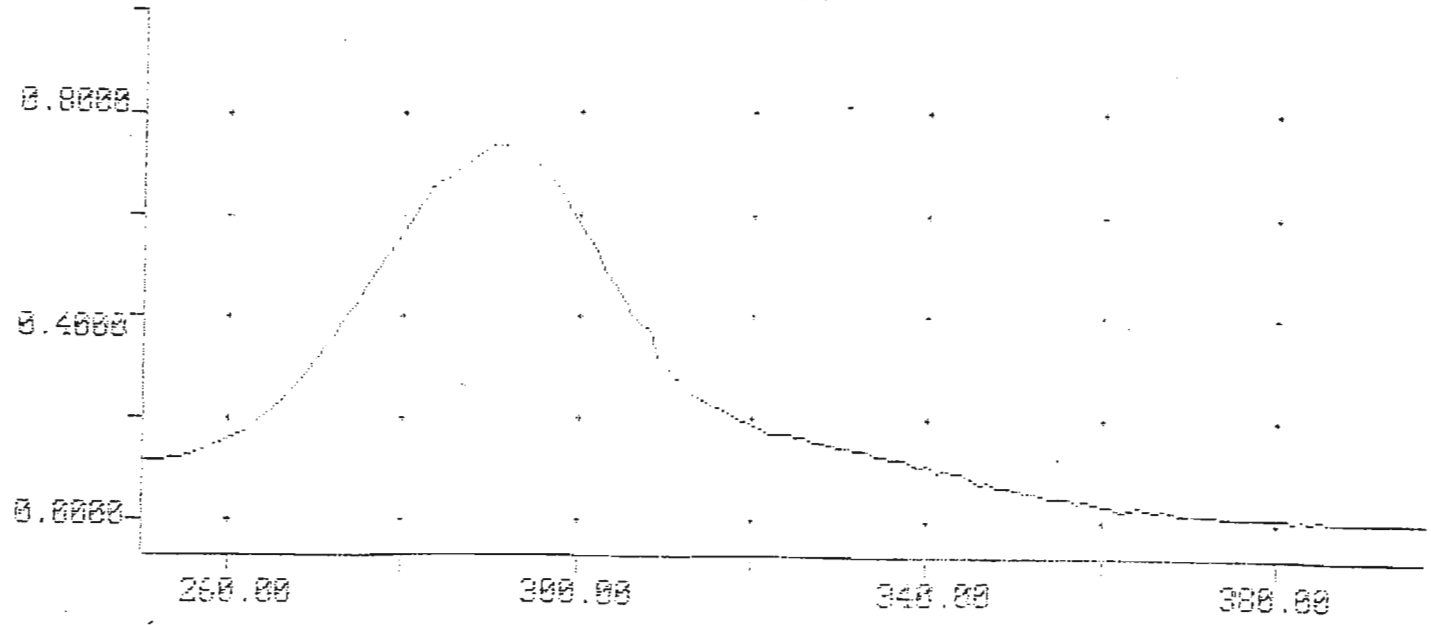


**Spectrum 82 : Mass spectrum of compound (xi)**



**Spectrum 83 : IR spectrum of compound (xi)**

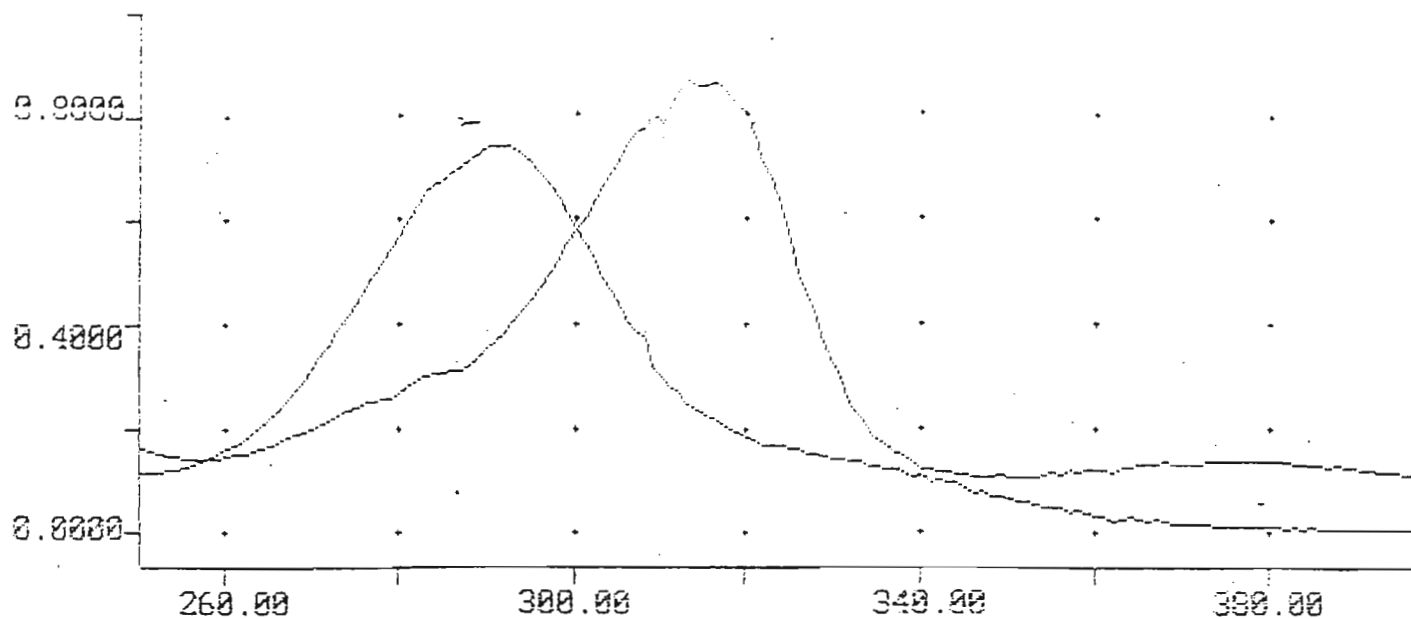
DISPLAY DATA



Abs : 0.0000 -> 1.0000      NM : 250.00 -> 400.00  
Abs 0.7555      NM 281.000

**Spectrum 84 : UV spectrum of compound (xi)**

## DISPLAY DATA



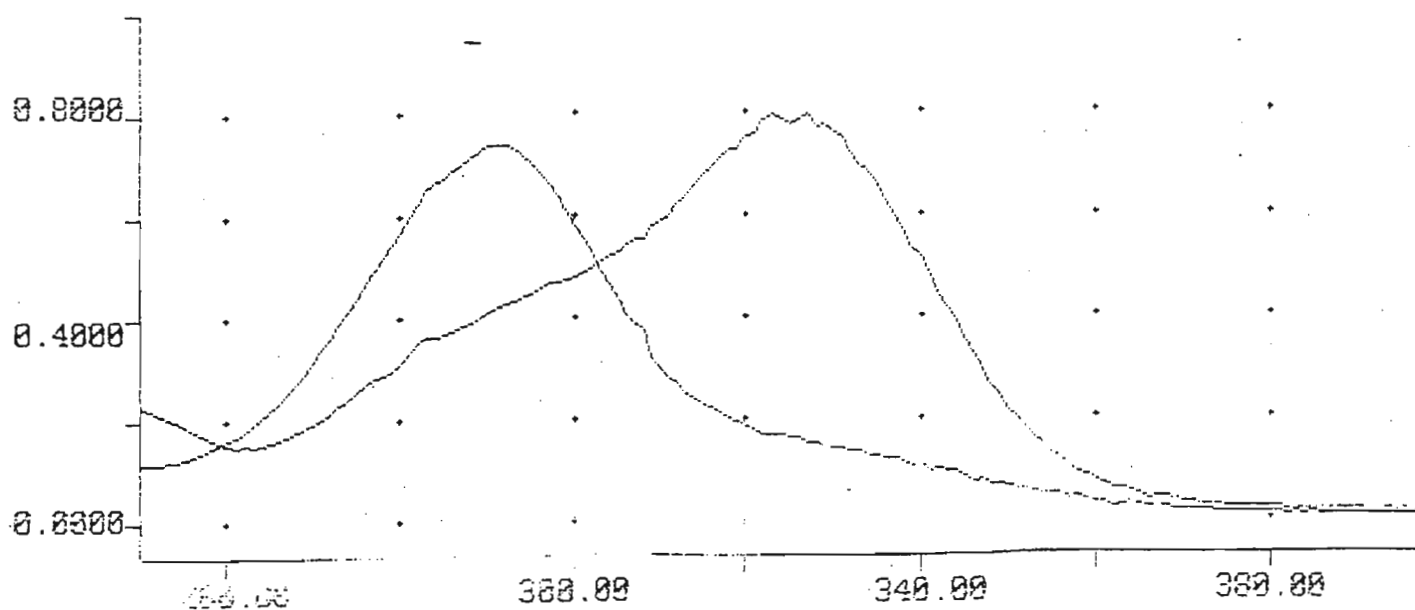
ABS : -0.8656 -> 1.0000 NM : 250.00 -> 400.00

ABS 0.73850 NM 291.000

ABS 0.86310 NM 313.000

**Spectrum 85 : UV and UV (+ AlCl<sub>3</sub>) spectra of compound (xi)**

## DISPLAY DATA

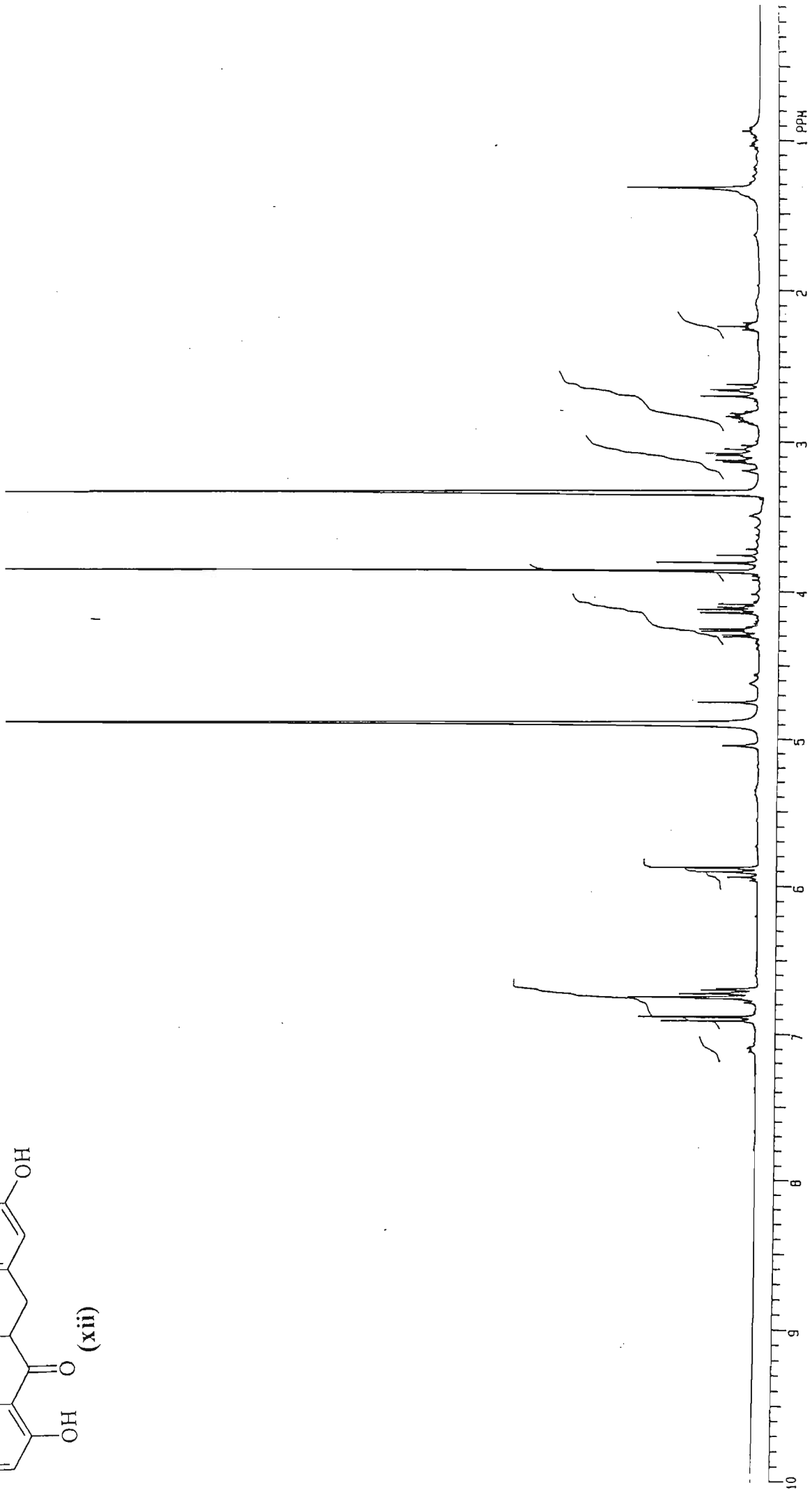
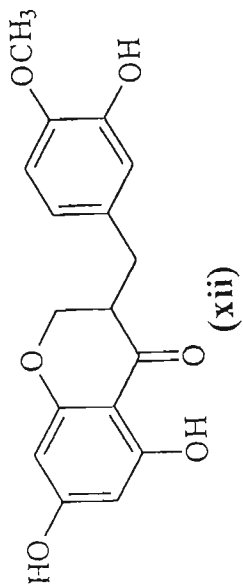


ABS : -0.8656 -> 1.0000 NM : 250.00 -> 400.00

ABS 0.73850 NM 291.000

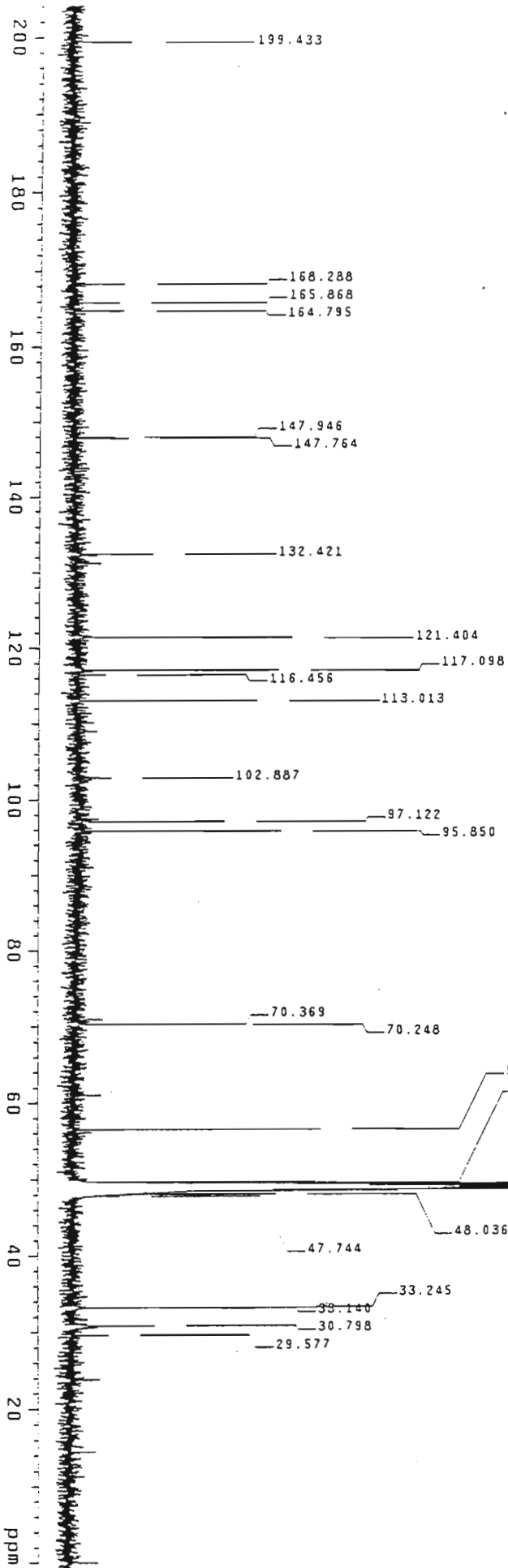
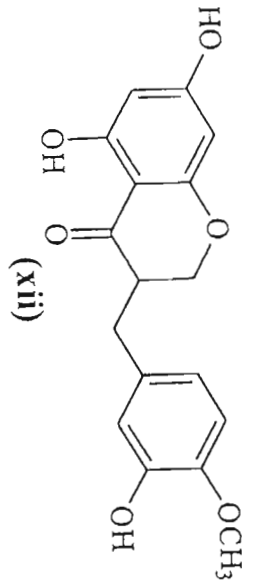
ABS 0.79926 NM 327.000

**Spectrum 86 : UV and UV (+ NaOAc) spectra of compound (xi)**



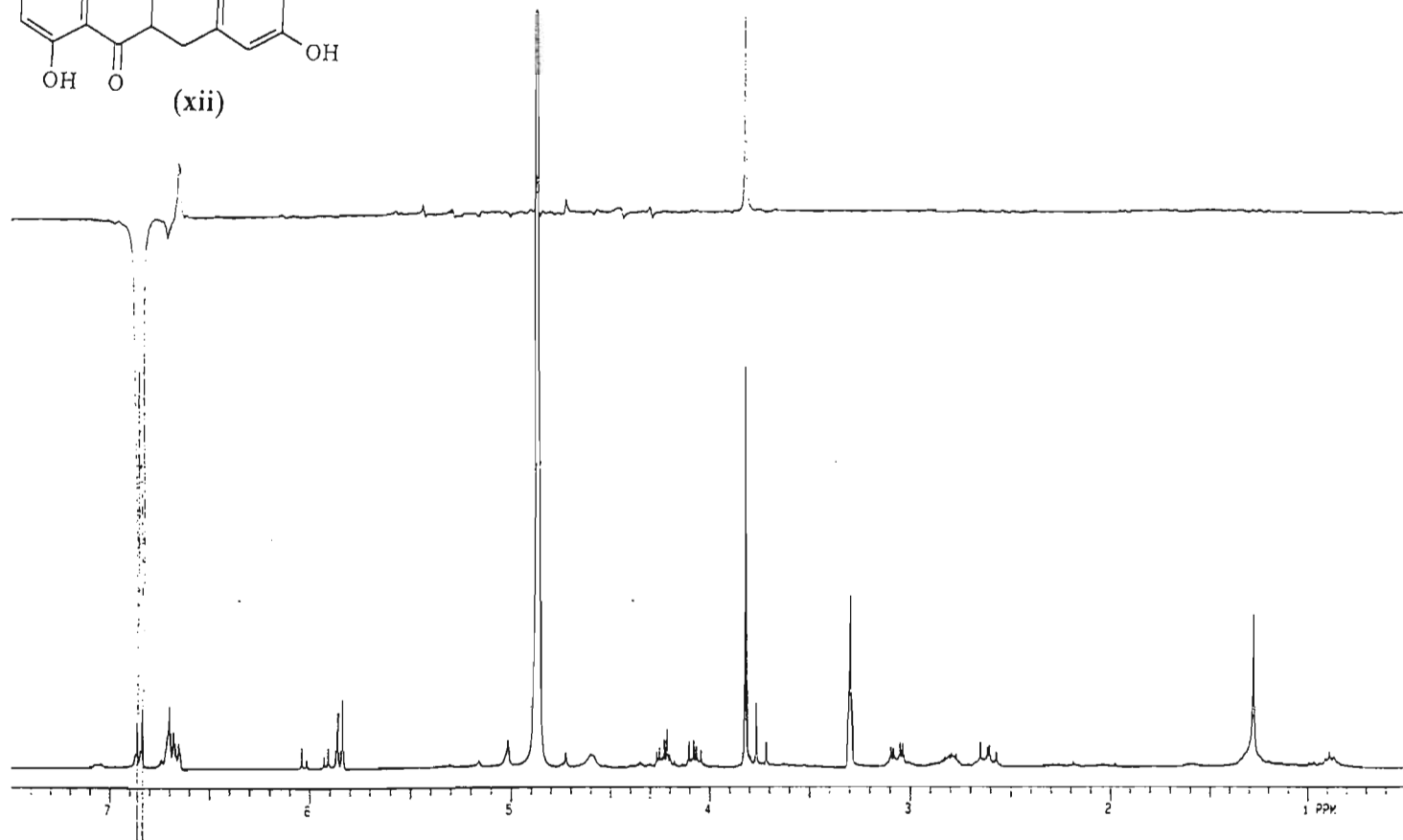
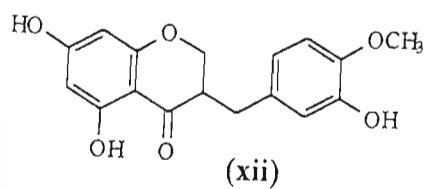
Spectrum 87: <sup>1</sup>H NMR spectrum of compound (xii)



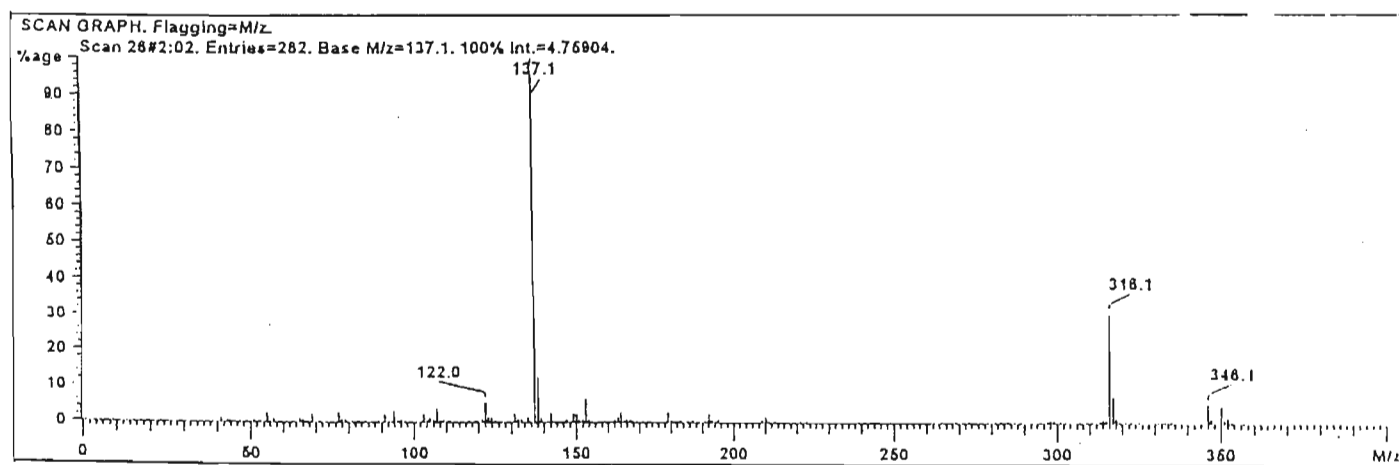


49.8 49.0 48.2 ppm

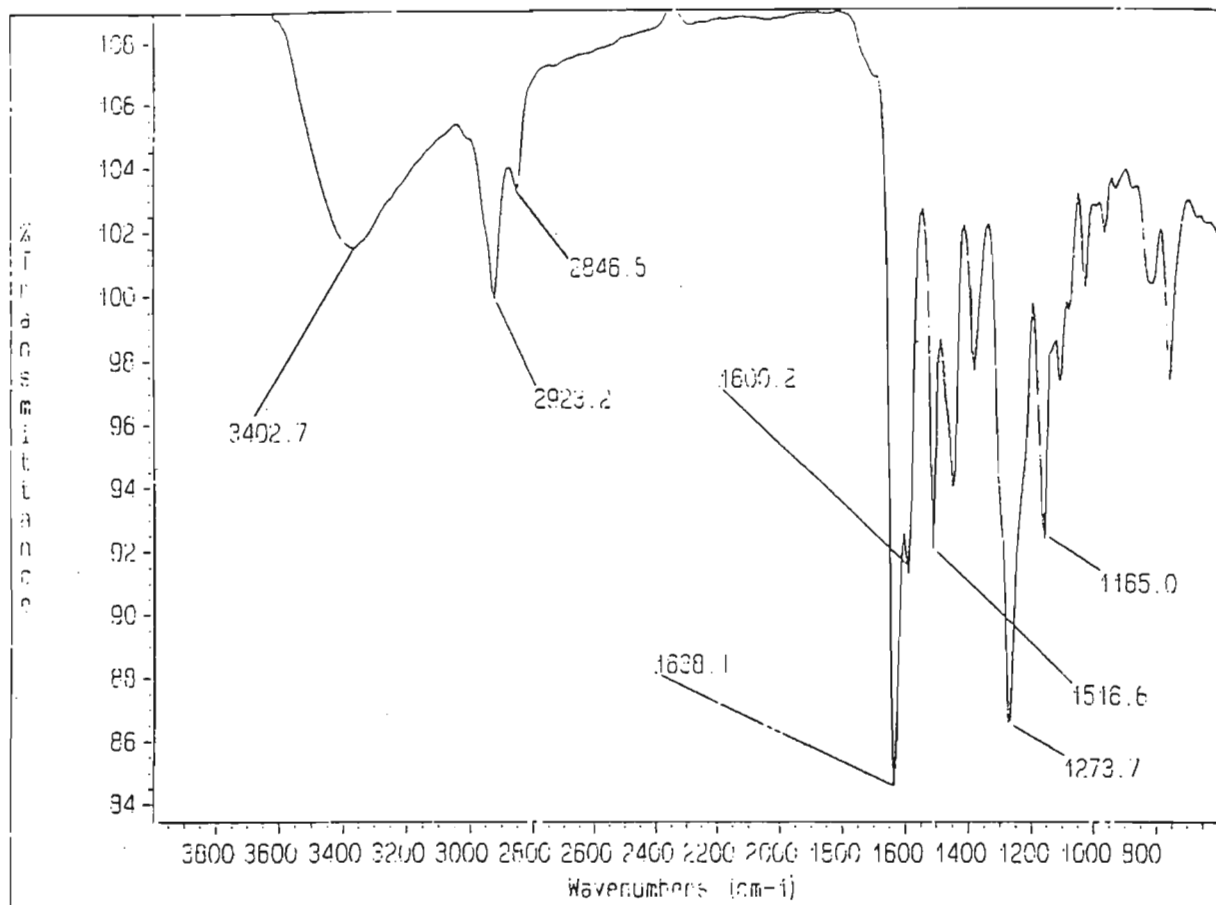
Spectrum 88 :  $^{13}\text{C}$  NMR spectrum of compound (xii)



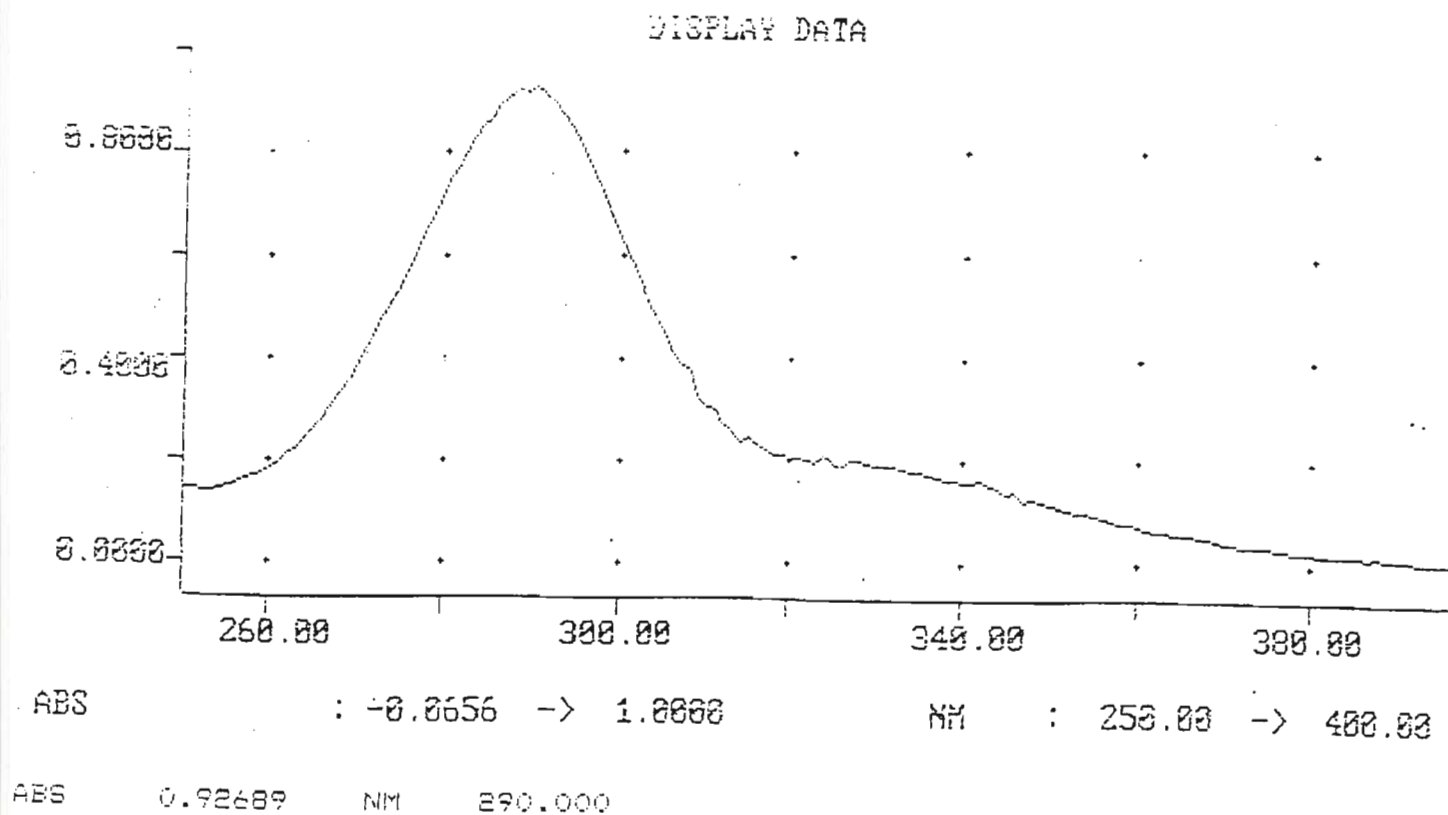
Spectrum 89 : NOE spectrum of compound (xii)



Spectrum 90 : Mass spectrum of compound (xii)

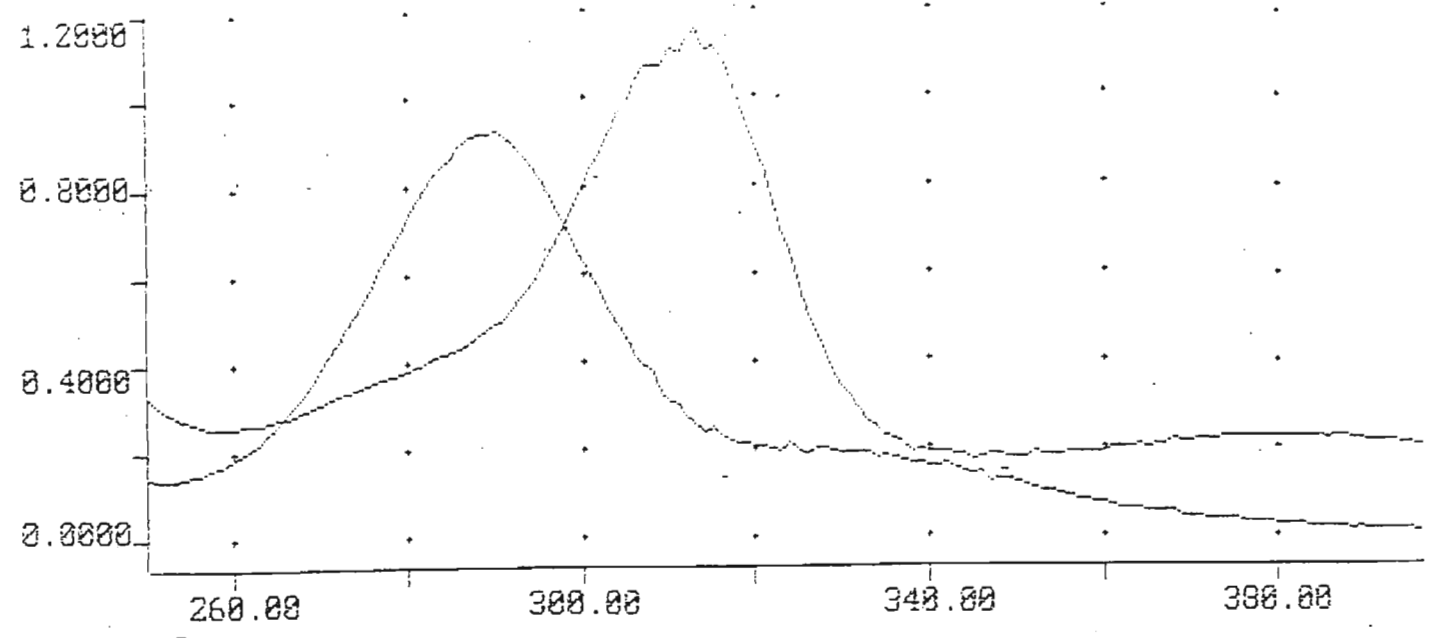


**Spectrum 91 : IR spectrum of compound (xii)**



**Spectrum 92 : UV spectrum of compound (xii)**

DISPLAY DATA

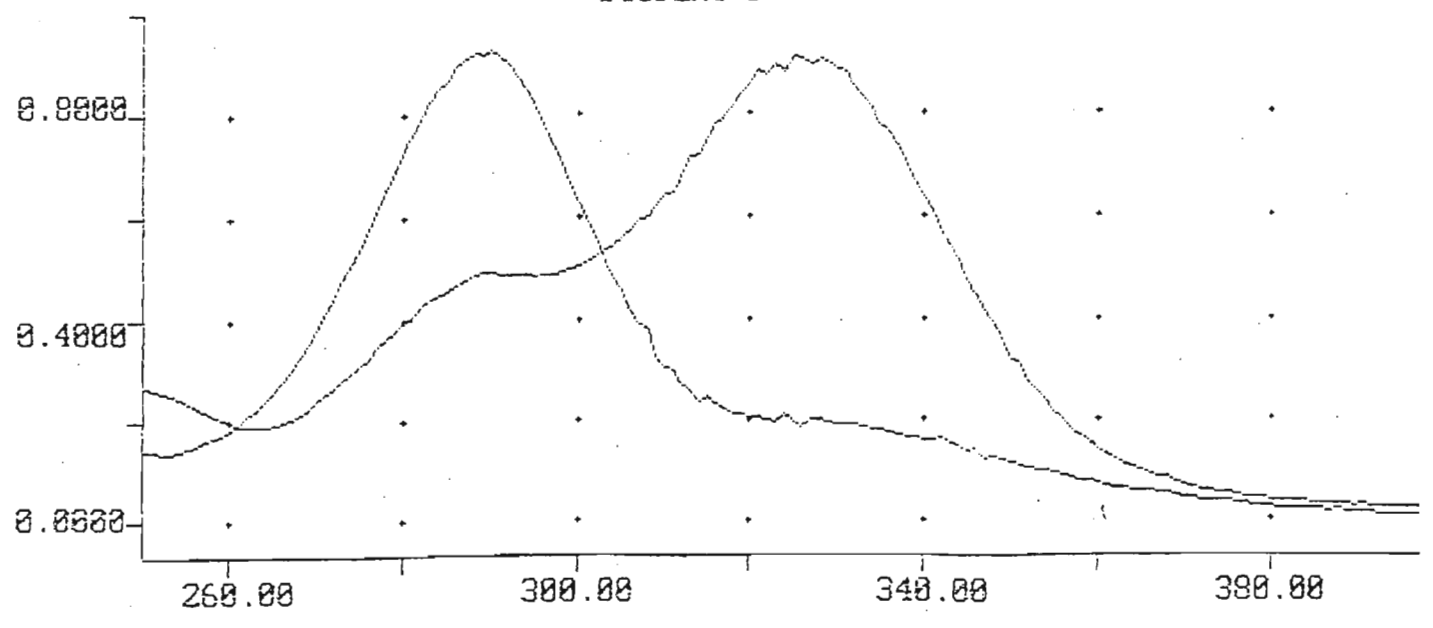


ABS : -0.8656 -> 1.2000 NM : 250.00 -> 400.00

ABS	0.92689	NM	290.000
ABS	1.15114	NM	313.000

Spectrum 93 : UV and UV (+ AlCl<sub>3</sub>) spectra of compound (xii)

DISPLAY DATA



ABS : -0.8656 -> 1.0000 NM : 250.00 -> 400.00

ABS	0.92689	NM	290.000
ABS	0.91078	NM	325.000

Spectrum 94 : UV and UV (+ NaOAc) spectra of compound (xii)