STUDIES ON *DESMODIUM* SPECIES FOR THE ALLELOCHEMICALS INVOLVED IN *STRIGA* SUPPRESSION

BY

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A thesis submitted in fulfillment of the requirement for the degree of Doctor of Philosophy in Chemistry of the University of Nairobi

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DECLARATION

This thesis is my original work and has not been presented for any degree in any university.

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DEDICATION

"To my son, Ernest Mwaniki and my husband, Sam Mwaniki, you keep my spirit alive."

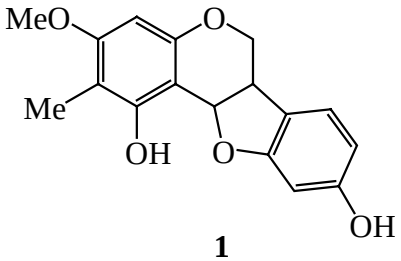
ABSTRACT

The fodder legumes *Desmodium uncinatum* and *D. intortum* suppress the growth of *Striga hermonthica* (witch-weed) via an allelopathic mechanism that involves *Striga* germination stimulants and post-germination growth inhibitors. In this study, the root extracts (dichloromethane, acetone and methanol) from the two *Desmodium* species were investigated for *Striga* germination stimulation and post-germination radicle growth inhibition activities. The less polar extract (dichloromethane) showed *Striga* germination stimulation activity of over 40% at 10 ppm, but did not show any post-germination radicle growth inhibition at the same concentration. On the other hand, the polar extracts (acetone and methanol) were found to exhibit post-germination radicle growth inhibition activities of over 45% at 10 ppm.

Chromatographic separation of the dichloromethane and acetone extracts of *D. uncinatum* roots yielded eleven compounds. These include, two pterocarpans [1,9-dihydroxy-3-methoxy-2-methylpterocarpan (uncinacarpan, **1**) and 3,9-dihydroxy-1-methoxy-2-(3-methylbut-2-enyl)pterocarpan (edudiol, **2**)], two isoflavanones [5,7-dihydroxy-2',3',4'-trimethoxy-6-(3-methylbut-2-enyl)isoflavanone (uncinanone D, **3**) and 5,4'-dihydroxy-7,2'-dimethoxy-6-methylisoflavanone (uncinanone E, **4**)], three abietane diterpenes [7-oxo-15-hydroxydehydroabietic acid (**5**), 7α-hydroxycallitrisic acid (**6**) and 7,15-dihydroxy-8,11,13-abietatrien-18-oic acid (**7**)], a phytosterol [sitosterol (**8**)], a pentacyclic triterpene [lupeol (**9**)], a long chain fatty acid [hexadecanoic acid (**10**)] and a flavone-*C*-glycoside [vitexin (**11**)]. Among these compounds, uncinacarpan (**1**), uncinanone D (**3**) and uncinanone E (**4**) are novel.

The dichloromethane and acetone extracts of *D. intortum* roots afforded five compounds, four isoflavanones [7,2',4'-trihydroxy-2",2"-dimethylpyrano[5,6:6,7]isoflavanone (intortunone, **12**), 5,7,2',4'-tetrahydroxyisoflavanone (dalbergioidin, **13**), 5,7,2',4'-tetrahydroxy-6-(3-methylbut-2-enyl)isoflavanone (uncinanone A, **14**) and 4",5"-dihydro-5',2',4'-trihydroxy-5"-isopropenylfurano-(2",3":7,6)isoflavanone (uncinanone B, **15**)] and an abietane diterpene [7-oxodehydroabietic acid (**16**)]. Intortunone (**12**) is a novel compound. The isolated compounds were characterized by use of a combination of spectroscopic techniques (UV, IR, MS, 1D- and 2D-NMR) and by chemical derivatization.

The isoflavanones, intortunone, dalbergioidin and uncinanone B from *D. intortum* were found to exhibit weak *Striga* germination stimulation activities (below 25% at 10 ppm) whereas the flavone-*C*-glycoside, vitexin was found to mildly inhibit the post-germination radicle growth of *Striga* by 37 % at 100 ppm.



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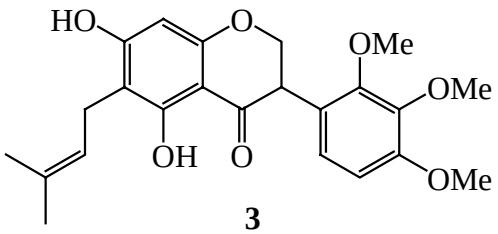

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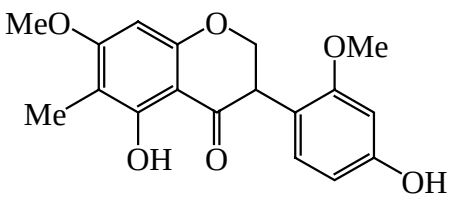

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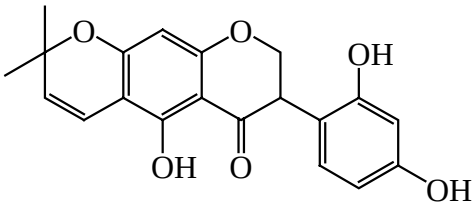

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ABBREVIATIONS

$CDCl_3$: Deuterated chloroform

CH_2Cl_2: Dichloromethane

^{13}C NMR: Carbon-13 Nuclear Magnetic Resonance

COSY: Correlation Spectroscopy

DMSO-d_6: Deuterated Dimethyl Sulfoxide

EIMS: Electron Ionization Mass Spectroscopy

EtOAc: Ethyl acetate

GOESY: Gradient Enhanced Nuclear Overhauser Effect

HMBC: Heteronuclear Multiple Bond Correlation

HMQC: Heteronuclear Multiple Quantum Correlation

^{1}H NMR: Proton Nuclear Magnetic Resonance

HPLC: High Performance Liquid Chromatography

MeCN: Acetonitrile

MeOH: Methanol

MeOH-d_4: Deuterated Methanol

NADPH: Nicotinamide adenine dinucleotide phosphate, reduced form

NaOCl: Sodium hypochlorite

RDA: r*etro*-Diels Alder

SAM: S-adenosyl-L-methionine

CHAPTER 1

INTRODUCTION

1.1 General

Plants produce many compounds that affect the growth and development of other organisms. These secondary metabolites protect the plant from microorganisms, herbivores, competing plant species and from the stress exerted by the environment.

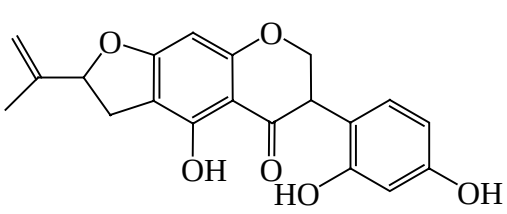
Secondary metabolites produced from living systems (higher plants, algae, bacteria or fungi) may influence the growth and development of other living systems *via* a mechanism referred to as allelopathy (Macias et al., 1998). The effects of allelopathic interactions depend on the secondary metabolites (allelochemicals) released from the donor living systems to the environment and not on the physical contact between the two interactive parties (Tang et al., 1995). Allelochemicals are released through processes such as volatilization, root exudation, leaching and decomposition of plant residues (Dilday et al., 1998).

Since weeds are one of the major constraints to crop production worldwide, the application of allelochemicals for their control has become an area of scientific interest. Strategies capitalizing on allelopathy are suggested to serve as a way towards obtaining new solutions in agriculture (Einhellig & Leather, 1988). These strategies include the use of plants with allelopathic effects in crop rotation, use of cover crop and residue management especially in no tillage systems, genetic transfer of allelopathic traits into

commercial crop cultivars in order to increase their capability for weed control and finally development of allelochemicals as biorational herbicides (Einhellig & Leather, 1988).

During investigations into control of insect damage to maize crops in Kenya, Khan et al., (2000) found that inter-cropping maize crops with plants producing repellant secondary metabolites, the fodder legumes, *Desmodium uncinatum* (silver leaf) and *D. intortum* (green leaf) significantly reduced infestation by *Striga hermonthica* (witch-weed) and caused gradual elimination of the *S. hermonthica* seed bank.

Further studies on *D. uncinatum* showed that the suppression of the parasitic weed, *S. hermonthica*, was due to an allelopathic effect of *D. uncinatum* root exudates, which involve *Striga* germination stimulant and post-germination growth inhibitor (Khan et al., 2002). Tsanuo et al. (2003) reported two bioactive compounds from *D. uncinatum* root exudates, namely; uncinanone B (**15**), a *Striga* germination stimulant, and uncinanone C (**17**), a moderate inhibitor of *Striga* radicle growth.



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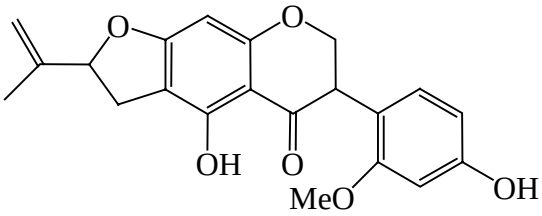

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High performance Liquid Chromatography (HPLC) analysis of the aqueous root exudates from *D. uncinatum*, however, showed the presence of a series of other compounds, and

bioassays of different HPLC fractions obtained from the root exudates showed different *Striga* response on germination stimulation and post germination inhibition activities (Tsanuo, 2002). Thus, indicating the presence of other compounds contributing to this allelopathic effect. In this study, extraction, isolation and identification of a series of other compounds from *D. uncinatum* and *D. intortum* roots have been conducted. In addition, the *Striga* germination stimulation and post-germination growth inhibition activities of the extracts and some of the isolated compounds were performed.

1.2 Hypothesis of the study

Germination stimulation of *Striga hermonthica* seeds and inhibition of post germination growth of *Striga* seedlings are attributed to individual and/or blend of compounds from the roots of *Desmodium uncinatum* and *D. intortum*.

1.3 Overall Objective of the study

To isolate and characterize the allelochemicals from organic extracts and aqueous exudates of *D. uncinatum* and *D. intortum* roots and evaluate their bio-activities against the parasitic weed *Striga hermonthica*.

1.4 Specific objectives of the study

(i) Obtain organic extracts and aqueous exudates from the roots of *Desmodium uncinatum* and *D. intortum*.

(ii) Isolate bioactive compounds from the crude organic extracts and aqueous exudates.

(iii) Characterize the isolated compounds.

(iv) Establish the *Striga* germination stimulation and post-germination inhibition activities of the crude organic extracts, aqueous exudates, isolated pure compounds and their blends on the parasitic weed, *Striga hermonthica*.

(v) Establish whether *Striga* germination stimulants also induce chemotropic growth on germinated *Striga* seeds.

CHAPTER 2

LITERATURE REVIEW

2.1 Allelopathy

Allelopathy refers to any process involving secondary metabolites produced by higher plants, algae, bacteria or fungi that influence the growth and development of agricultural and biological systems (Macias et al., 1998). Chemicals that impose allelopathic influences are called allelochemicals and are released into the environment by means of four ecological processes: volatilisation, leaching, root exudation, and decomposition of plant residues (Macias et al., 1998). In plants, the commonly visible effects of allelochemicals include: inhibited or retarded germination rate; seeds darkened and swollen, reduced root or radicle, shoot or coleoptile extension, swelling or necrosis of root tips, curling of the root axis, discolouration, lack of root hairs, increased number of seminal roots, reduced dry weight accumulation, and lowered reproductive capacity (An et al., 1998).

Allelopathy plays an important role in regulating plant biodiversity and in agriculture (Chou, 1999). In agriculture, utilization of allelopathic potential in development of weed management strategies is receiving increased attention with some of the key areas of interest being: to selectively enhance allelopathic traits of crop cultivars in breeding programs, to transfer allelopathic genes into commercial cultivars through biotechnology, and to identify and characterize those substances involved in strong allelopathic activity

and to use them either directly as natural herbicides or as templates for developing new and environmentally friendly synthetic herbicides (An et al., 1998).

2.2 Biological information on *Striga*

The genus *Striga* belongs to the tribe Rhinantheae in the family Scrophulariaceae. This family is known to consist of both free-living and parasitic species with the genus *Striga* being among the most important parasitic members of this family (Parker & Riches, 1993). Members of the genus *Striga* are characterized by opposite leaves, irregular flowers with corolla divided into a tube and spreading lobes, herbaceous habit, small seeds, and parasitism (Musselman, 1987). *Striga* species have a complex life cycle that is adapted to that of their host plants. They produce large number of seeds that can remain viable for many years (Doggett, 1984; Parker & Riches, 1993). The seeds require a dormant after-ripening period hence they cannot germinate at the end of crop season they are produced. Further, the seeds need a pre-conditioning in moist conditions after which they are ready for germination in the presence of appropriate organic stimulants exuded by the roots of potential host plants or certain non-host plants (Doggett, 1984; Parker & Riches, 1993).

Striga is mainly distributed in tropical and subtropical regions having its greatest diversity in sub-Saharan Africa (Doggett, 1984). Of all known *Striga* species, three are known to cause the most economically significant damage to crops. These are the obligate parasitic species namely, *S. hermonthica*, *S. asiatica* and *S. gesnerioides*. *S. hermonthica* and *S. asiatica* parasitize cereals such as maize, sorghum, upland rice and

millet, whereas *S. gesnerioides* attacks dicotyledonous species such as cowpea, tobacco and sweet potato (Doggett, 1984; Musselman & Ayensu, 1984; Butler, 1995).

S. hermonthica is the most common species in Kenya, occurring in the region along the lake Victoria basin and western Kenya (Doggett, 1984). It has purple flowers (Figure 2.1), which are the largest and most showy of the agronomically important species of the genus (Safa et al., 1984).

Figure 2.1: *Striga hermonthica* parasitizing on maize

2.3 Influence of *Striga* development by allelochemicals exuded into the soil

2.3.1 Germination

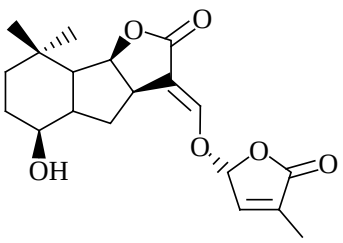
Striga seeds require a period of after-ripening in dry conditions, which lowers the moisture content of the seeds. To germinate, the after-ripened seeds need to undergo pre-conditioning in warm and moist conditions for two to three weeks. Once the after-ripening and pre-conditioning requirements are met, *Striga* seeds germinate upon

exposure to certain compounds, referred to as germination stimulants, produced by the roots of the host or non-host plants. The germination stimulants have been shown to promote germination by initiating ethene formation within the *Striga* seeds (Logan & Stewart, 1991; 1995). Endogenous ethene is necessary for natural germination and its role in the stimulation of *Striga* germination has been reported (Logan & Stewart, 1991; 1995).

Various compounds with germination stimulant activity on *Striga* species have been isolated and characterized from host and non-host plants. The first natural germination stimulant to be isolated was strigol (**18**) from the root exudates of cotton (*Gossypium hirsutum* L.), a non-host plant of *Striga* (Cook et al., 1966, 1972). Strigol was later isolated from the root exudates of maize (*Zea mays* L.) and proso-millet (*Panicum miliaceum* L.), which are host plants to *Striga asiatica* (Siame et al., 1993). Other close analogues of strigol, including alectrol (**19**) and sorgolactone (**20**), have been identified as *Striga* germination stimulants from host plants (Hauck et al., 1992; Muller et al., 1992). In addition, structurally related synthetic analogues of strigol, commonly referred to as germination releaser (GR) compounds, have also been identified as germination stimulants. One of the most potent synthetic germination stimulant is 2-methyl-4-((2-oxo-2,3,3a,8b-tetrahydro-4H-indeno[1,2-b]furan-3-ylidene)-methoxy)-but-2-en-4-olide (**21**), commonly referred to as GR-24 (Johnson et al., 1981).

Other naturally occurring sesquiterpene lactones with similar structural features (lactone rings) to strigol have been identified as *Striga* germination stimulants. These include:

confertiflorin (**22**) from *Ambrosia confertiflora* DC, parthenin (**23**) from *Parthenium hysterophorus*, costunolide (**24**) from *Magnolia grandiflora*, 11β,13-dihydroparthenolide (**25**) from *Ambrosia artemisiifolia*, santamarin (**26**) from *Magnolia grandiflora*, and reynosin (**27**) from *Magnolia grandiflora*. (Fischer *et al*., 1989; Fischer *et al*., 1990; Rugutt & Rugutt, 1997).



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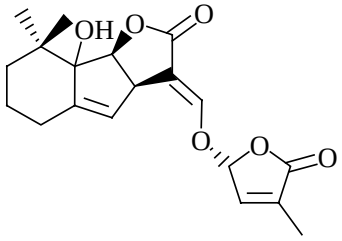

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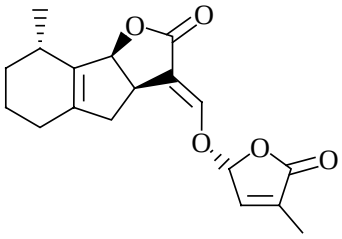

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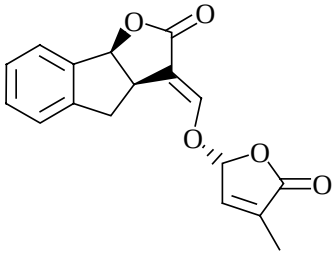

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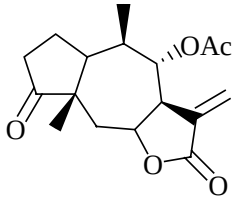

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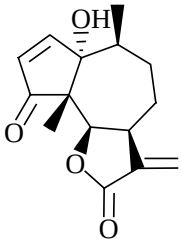

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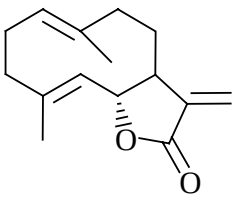

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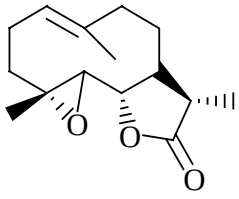

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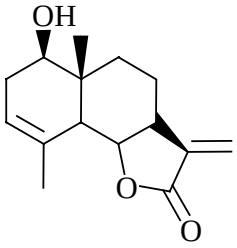

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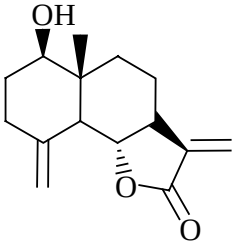

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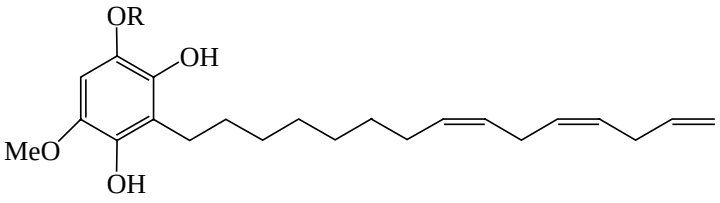
Another group of compounds reported to have germination stimulant activity on *Striga* seeds are the phenolics such as the hydroquinone, dihydrosorgoleone (**28**) from sorghum (Chang et al., 1986; Netzly et al., 1988). The hydroquinone is readily oxidized to the

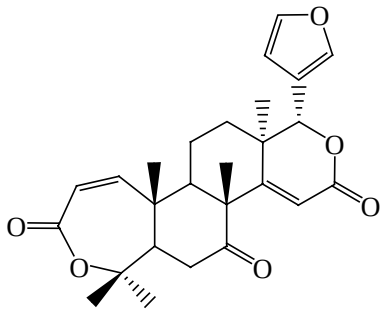
more stable benzoquinone analogue that does not stimulate germination (Chang et al., 1986). However, a related compound, 4,6-dimethoxy-2-[(8'Z,11'Z)–8',11',14'-penta decatriene]resorcinol (**29**) was found to retard the oxidation process hence enhancing the activity of the hydroquinone and extending its lifetime in the exudate (Fate & Lynn, 1996).

Limonoids such as deoxyobacunone (**30**), obacunone (**31**), harrisonin (**32**), 12β-acetoxyharrisonin (**33**) and pedonin (**34**) from *Harrisonia abyssinica* have also been found to stimulate germination of *Striga* seeds (Ruggut et al., 2001).



28: R = H

29: R = CH_3



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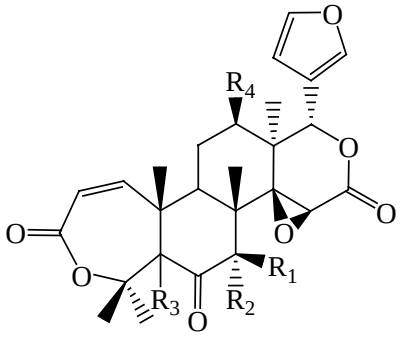
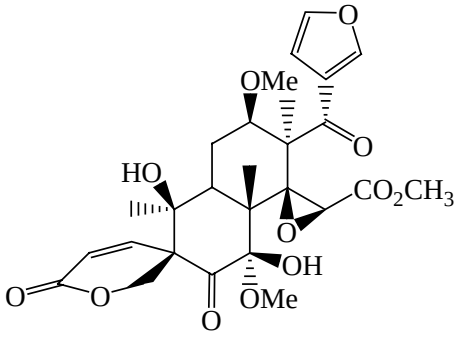


31: $R_1 = R_2 = O$, $R_3 = R_4 = H$

32: $R_1 = R_3 = OH$, $R_2 = OCH_3$; $R_4 = H$

33: $R_1 = R_3 = OH$, $R_2 = OCH_3$; $R_4 = OAc$



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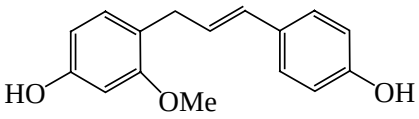
2.3.2 Upper haustorial initiation

Soon after germination, the radicle of the *Striga* seedling grows towards the host root and this has been suggested to be due to a weak chemotropic influence (Doggett, 1984; Parker & Riches, 1993). On contact with the host root the elongation of the radicle stops and the upper haustorium, a specialized organ of absorption of a parasitic plant develops, suggesting that some chemosignals are released by the host plant to initiate the development of a haustorium (Nickrent et al., 1979). The first upper haustorial induction factor, xenognosin A (**35**), was reported for the parasitic plant, *Agalinis purpurea*, (Scrophulariaceae) (Lynn et al., 1981). Several phenolic compounds and cytokinins have so far been identified to be upper haustorial initiation factors. Examples are xenognosin B (**36**) (Steffens et al., 1982), 2,6-dimethoxy-*p*-benzoquinone (**37**) (Chang & Lynn, 1986), 6-benzylaminopurine (**38**), kinetin (**39**), and zeatin (**40**) (Riopel & Timko, 1995).



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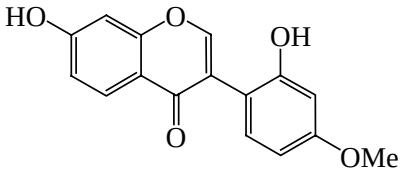

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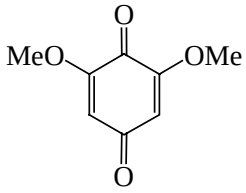

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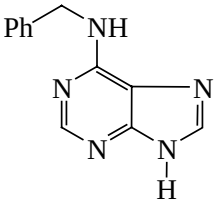

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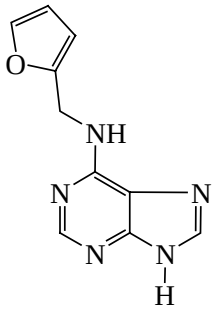

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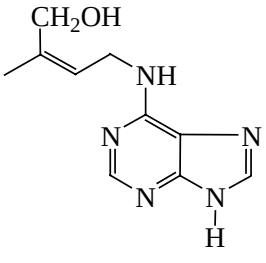

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2.4 Host defense against *Striga*

Due to the devastation caused by *Striga* agricultural process, investigations to identify and exploit host defence mechanisms have been carried out. Some of the defence mechanisms identified include:

i. Avoidance mechanisms. Some host plant genotypes have been found to reduce *Striga* infestation by diminishing root length density in the upper soil profile where most *Striga* seeds are found (Cherif-Ari et al., 1990; Olivier & Leroux, 1992). Since most of the germination stimulants are released by the plant root hairs, then the fewer roots in the upper 10 cm of soil have been implicated as an avoidance mechanism of resistance.

ii. Resistance *via* low production of germination stimulant. Host plants that produce low amounts of *Striga* germination stimulants cause germination of fewer seeds hence less attack. Some sorghum cultivars have been found to be low stimulant producers thus resisting severe attack by *Striga* (Hess et al., 1992; Weerasuriya et al., 1993).

iii. Resistance due to the metabolic chemical protection like antibiosis. Host plants may produce chemicals that reduce further development of germinated *Striga*. For example, the attachment of *Striga gesneroides* to cowpeas (*Vigna unguiculata*) roots and further development on the roots are delayed or the attached *Striga* seedlings may die (Lane et al., 1993). Also, *Striga hermonthica* seedlings attached on the roots of *Sorghum versicolor* have been reported to have poor development and sometimes die (Lane et al., 1994). Arnaud et al.

(1999) found that such mechanism prevents *Striga* penetration to the stele thus causing poor development and death.

2.5 Role of legume intercrop in the control of *Striga* infestation in cereal crops

Several control methods are employed to combat *Striga* damage on cereal crops. These include: hand pulling, use of trap and catch crops, fertilizers, seed treatments, chemical stimulants, development of tolerant lines, inter-cropping with legumes and biological control methods (Doggett, 1984; Parker & Riches, 1993; Manyong et al., 2002).

In Africa, intercropping of legume crops with cereal crops has been found to cause remarkable reduction of *Striga* infestation and improved production of the cereal crops (Carson, 1989; Kunjo & Jobe, 2002). A number of *Striga* control mechanisms by legume intercrop have been proposed. These include: intercrop acting as a trap-crop and hence stimulating germination of *Striga* ahead of the cereal crops, increasing available nitrogen, offering shade thus reducing soil temperature, interfering with the stimulant exudation of the host and exuding inhibitor to reduce *Striga* seed germination (Parker & Riches, 1993). *Desmodium uncinatum* controls *Striga* infestation in intercrop with cereals through an allelopathic mechanism, which involves post-germination growth inhibitors exuded from its roots (Khan et al., 2002).

2.6 The Genus *Desmodium*

Desmodium belongs to the family Fabaceae and subfamily Papilionoideae. The genus is widely distributed over the temperate and tropical regions with many species occurring

in North and South America (Rotar & Urata, 1967). There are some 350 species of *Desmodium* in the tropics (Skerman et al., 1988).

The species, *Desmodium uncinatum* (silver leaf) and *D. intortum* (green leaf) are native to Central and South America, however they are now widespread as fodder crops in the tropical and sub-tropical regions of the world (Skerman et al., 1988).

D. uncinatum (Figure 2.2a) is a large perennial legume with stems that grow several metres long and trail over surrounding vegetation. The stems are covered with short, hooked hairs that stick to hair or clothing. The stems can root at the nodes if they touch moist soil. Leaves are trifoliate with pointed leaflets; the upper side is dark green with an irregular generally pyriform silvering about the midrib, the lower side lighter green and uniform in colour; both sides are covered with whitish hairs. The flowers are pink to bluish colour and the seeds are mainly light brown with mixture of olive-green to cream (Hacker, 1992a).

Desmodium intortum (Figure 2.2b) is a large trailing and scrambling perennial legume. It has a strong taproot and the stems can root at the nodes if in contact with moist soil. It is finer and less hairy than *D. uncinatum*. The leaves are trifoliate without silvering at the midrib and leaflets are not as pointed as those of *D. uncinatum*. Flowers are deep pink (Hacker, 1992b).

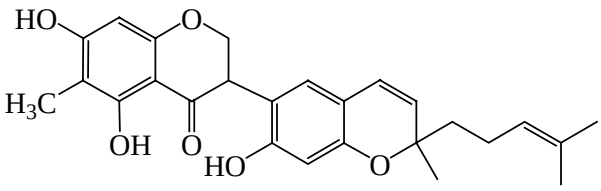
(a) (b)

Figure 2.2: (a) Desmodium uncinatum (b) Desmodium intortum

2.7 Economic importance, ethno-medical information and biological activities of *Desmodium*

The genus *Desmodium* is used in erosion control, ground cover and wildlife protection in lands cleared of vegetation (Trout, 2004). Many *Desmodium* species including *D. uncinatum* and *D. intortum* are also highly valued as fodder. In traditional medicine, various *Desmodium* species are used for treatment of different ailments. For example, the leaves of *Desmodium repandum* and *D. salicifolium* are used in East Africa for treatment of abdominal pains and eye trouble respectively (Kokwaro, 1993); *D. gangeticum* is of great therapeutic value in treating typhoid, piles, inflammation, asthma, bronchitis and dysentery in India (Mishra et al., 2005); and *D. styracifolium* is a renowned Chinese medicine for its heat-clearing and diuretic properties (Zhao et al., 2007).

Various biological activities have been reported from extracts of some *Desmodium* species. These include; antileishmanial activity (Iwu et al., 1992), cardio-cerebrovascular effects (Xu et al., 1980), antiwrithing and central nervous system depressant activities (Jabbar et al., 2001) and antidiabetic activity (Govindarajan et al., 2007). Bioactive compounds have also been reported from some *Desmodium* species, which include: anti-microbial agents desmodianone A (**41**), B (**42**) and C (**43**) from *D. canum* (Delle Monache et al., 1996); a pterocarpan, gangetin (**44**) with anti-inflammatory and analgesic activities from *D. gangeticum* (Ghosh et al., 1983) and glycolipids with antileishmanial and immunomodulatory activities from *D. gangeticum* (Mishra et al., 2005).



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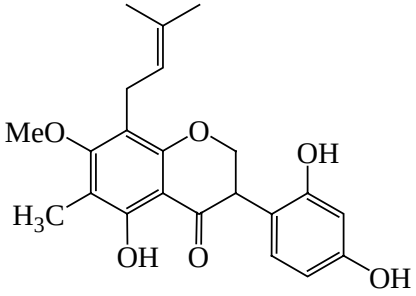

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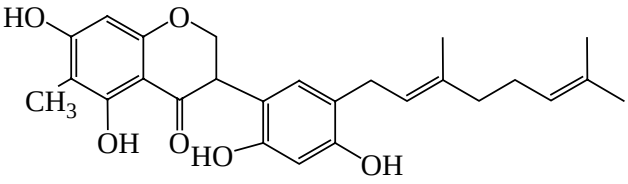

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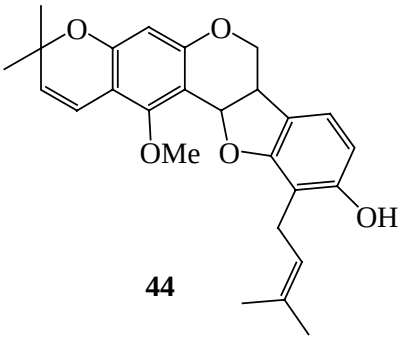

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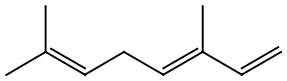
When fodder legumes, *D. uncinatum* and *D. intortum* are deployed as maize intercrops, they have been found to reduce damage to maize by stem borers such as *Busseola fusca* (Noctuidae) and *Chilo partellus* (Pyralidae) (Khan et al., 2000). Volatile repellents such as (E)-β-ocimene (**45**) and (E)-4,8-dimethyl-1,3,7-nonatriene (**46**) emitted from these

legumes have been shown to be responsible (Khan et al., 2000). When this maize-desmodium intercropping is done in areas infested with parasitic witch-weed, *Striga hermonthica*, dramatic reduction in the infestation of maize is observed (Khan et al., 2002; 2006).



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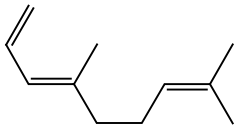

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2.8 Phytochemical information on *Desmodium*

Early interest in the phytochemistry of *Desmodium* species was on their alkaloids (Ghosal & Mukherjee, 1966; Banerjee & Ghosal., 1969; Ghosal & Srivastava, 1973; Ghosal & Mehta, 1974). Alkaloids reported from *Desmodium* have been found to be mainly β-phenethylamines and indole alkaloids. Other types of alkaloids reported from this genus include some tetrahydroisoquinoline, β-carboline, pyrimidine and pyrrole alkaloids.

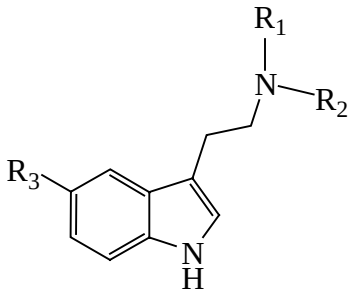
Summary of the alkaloids so far reported from various species in the genus, *Desmodium* is presented in table 1.

Table 1: Alkaloids from the genus *Desmodium*

Compound	Source	Part used	Reference
i. Indole alkaloids			
Tryptamine (**47**)	*D. tiliaefolium*	Roots	Ghosal & Srivastava, 1973
N,*N*-dimethyltryptamine (**48**)	*D. pulchellum*	Whole plant	Ghosal & Mukherjee, 1966
5-methoxy-*N*-methyltryptamine (**49**)	*D. pulchellum*	Whole plant	Ghosal & Mukherjee, 1966
5-methoxy-*N*,*N*-dimethyltryptamine (**50**)	*D. gangeticum*	Aerial parts	Banerjee & Ghosal., 1969
N,*N*-dimethyltryptamine-*N*-oxide (**51**)	*D. pulchellum*	Whole plant	Ghosal & Mukherjee, 1966
Bufotenine-*N*-oxide (**52**)	"	"	"
5-methoxy-*N*,*N*-dimethyl tryptamine-N_b-oxide (**53**)	*D. gangeticum*	Aerial parts	Banerjee & Ghosal., 1969
Abrine (**54**)	*D. tiliaefolium*	Roots	Ghosal & Srivastava, 1973
Hypaphorine (**55**)	"	"	"
3-(Dimethylaminomethyl) indole (**56**)	*D. pulchellum*	Whole plant	Ghosal & Mukherjee, 1966
ii. β-Phenethylamines			
β-phenethylamine (**57**)	*D. cephalotes*	Leaves	Ghosal & Mehta, 1974
Tyramine (**58**)	*D. tiliaefolium*	Roots	Ghosal & Srivastava, 1973
Hordenine (**59**)	"	"	"
Candicine (**60**)	*D. cephalotes*	"	Ghosal & Mehta, 1974
Coryneine (**61**)	*D. triflorum*	Leaves	Ghosal et al., 1971
3,4-dimethoxyphenethyl amine (**62**)	*D. tiliaefolium*	Roots	Ghosal & Srivastava, 1973
N,*N*-dimethyl-3,4- dimethoxy phenethylamine (**63**)	"	"	"
N-methyl-3,4-dimethoxy-β-hydroxyphenethylamine (**64**)	"	"	"

Table 1 continued.

Compound	Source	Part used	Reference
iii. β-Carbolines			
N_b-methyltetrahydroharman (**65**)	*D. gangeticum*	Aerial parts	Banerjee & Ghosal., 1969
1,2,3,4-tetrahydro-6-methoxy-β-carboline (**66**)	*D. pulchellum*	Whole plant	Ghosal & Mukherjee, 1966
1,2,3,4-tetrahydro-6-methoxy-2-methyl-β- carboline (**67**)	"	"	"
iv. Tetrahydroisoquinoline			
Salsoline (**68**)	*D. tiliaefolium*	Roots	Ghosal & Srivastava, 1973
Salsolidine (**69**)	"	"	"
v. Pyrrole			
Desmodimine (**70**)	*D. styracifolium*	Aerial parts	Yang et al., 1993
vi. Pyrimidine			
Uridine triacetate (**71**)	*D. gangeticum*	Roots	Mishra et al., 2005

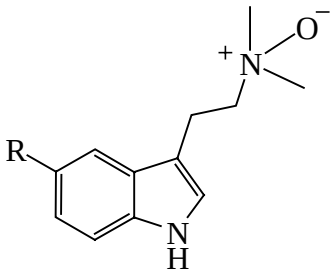


47: $R_1 = R_2 = R_3 = H$

48: $R_1 = R_2 = CH_3$, $R_3 = H$

49: $R_1 = CH_3$, $R_2 = H$, $R_3 = OCH_3$

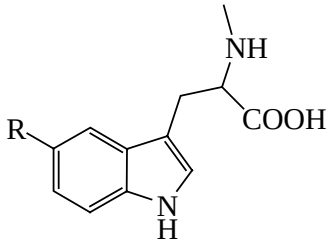
50: $R_1 = R_2 = CH_3$, $R_3 = OCH_3$



51: R = H

52: R = OH

53: R = OCH_3



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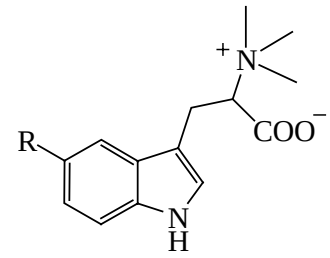

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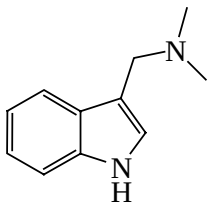

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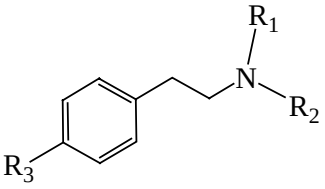
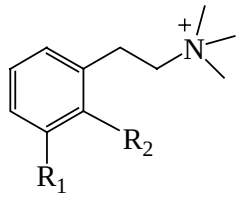


57: $R_1 = R_2 = R_3 = H$

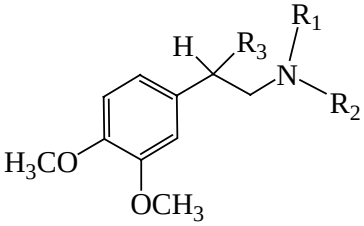
58: $R_1 = R_2 = H$, $R_3 = OH$

59: $R_1 = R_2 = CH_3$, $R_3 = OH$



60: $R_1 = OH$, $R_2 = H$

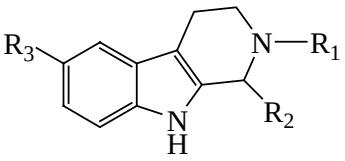
61: $R_1 = R_2 = OH$



62: $R_1 = R_2 = R_3 = H$

63: $R_1 = R_2 = CH_3$, $R_3 = H$

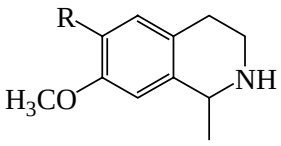
64: $R_1 = CH_3$, $R_2 = H$, $R_3 = OH$



65: $R_1 = R_2 = CH_3$, $R_3 = H$

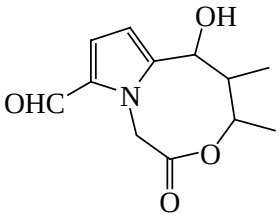
66: $R_1 = R_2 = H$, $R_3 = OCH_3$

67: $R_1 = H$, $R_2 = CH_3$, $R_3 = OCH_3$



68: R = OH

69: R = OCH_3



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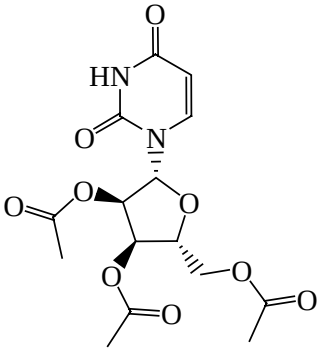
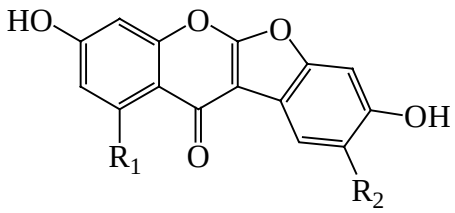


71

Purushothaman et al (1971) reported the presence of the first non-basic component from *Desmodium*, gangetin (**44**), a pterocarpan from *D. gangeticum*. To date several flavonoids, which include flavones, flavonols, isoflavones, isoflavans, isoflavanones and pterocarpans have been reported from the genus (Table 2). Three coumaronochromones, 5,7,4'-trihydroxycoumaronochromone (**72**) from *D. styracifolium* (Zhao et al., 2007), desmoxyphyllin A (**73**) from *D. oxyphyllum* (Mizuno et al., 1992) and *D. styracifolium* (Zhao et al., 2007) and desmoxyphyllin B (**74**) from *D. oxyphyllum* (Mizuno et al., 1992) have also been reported. Other compounds isolated from the genus include sterols, triterpenoids and glycolipids (Zhao et al., 2007; Mishra et al., 2005).



72: R_1 = OH, R_2 = H

73: R_1 = OH, R_2 = OCH_3

74: R_1 = H, R_2 = OCH_3

Presented next (Table 2) are the different classes of flavonoids so far reported from the genus, *Desmodium*.

Table 2: Flavonoids from the genus *Desmodium*

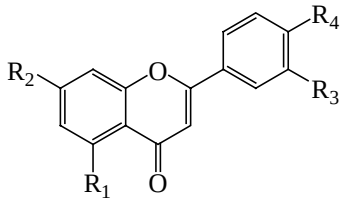
Compound	Source	Part Used	Reference
i. Flavones and their glycosides			
Luteolin (**75**)	*D. styracifolium*	Aerial parts	Zhao, et al., 2007
Chrysoeriol (**76**)	"	"	"
Hydnocarpin D (**77**)	"	"	"
Desmodol (**78**)	*D. caudatum*	Roots	Ueno et al., 1978
Lanceolatin B (**79**)	*D. sequax*	"	Siddiqui & Zaman, 1998
5'-methoxypongaglabrone (**80**)	"	"	"
2"-*O*-β-D-xylopyranosyl isoorientin (**81**)	*D. canadense*	Aerial parts	Chernobrovaya, 1975
2"-*O*-β-D-xylopyranosyl isovitexin (**82**)	"	"	"
8-*C*-prenyl-5,7,5'-trimethoxy-3',4'-methylenedioxyflavone (**83**)	*D. gangeticum*	Whole plant	Mishra et al., 2005
5,7-dihydroxy-8-prenylflavone-4'-*O*-α-L-rhamnopyranosyl-(1-6)-β-D-glucopyranoside (**84**)	*D. gangeticum*	Stem	Yadava & Tripathi, 1998
ii. Flavonols and their glycosides			
Quercetin (**85**)	*D. styracifolium*	Aerial parts	Zhao, et al., 2007
Karanjin (**86**)	*D. sequax*	Roots	Siddiqui & Zaman, 1998
Pongapin (**87**)	"	"	"
5'-methoxypongapin (**88**)	"	"	"
Kaempferol-7-*O*-β-D-glucopyranoside (**89**)	*D. gangeticum*	Whole plant	Mishra et al., 2005
Quercetin-7-*O*-β-D-glucopyranoside (**90**)	"	"	"
Rutin (**91**)	"	"	"

Table 2 continued.

Compound	Source	Part used	Reference
iii. Isoflavones			
Genistein (**92**)	*D. uncinatum*	Roots	Tsanuo, et al., 2003
Formononetin (**93**)	*D. styracifolium*	Aerial parts	Zhao, et al., 2007
Orobol (**94**)	"	"	"
iv. Isoflavans			
6-Desmethyldesmodian A (**95**)	*D. canum*	Roots	Lima et al., 2006
Desmodian A (**96**)	"	"	"
6-Desmethyldesmodian B (**97**)	"	"	"
Desmodian B (**98**)	"	"	"
Desmodian C (**99**)	"	"	"
3'-hydroxydesmodian B (**100**)	"	"	"
v. Isoflavanones and their Glycosides			
Uncinanone A (**101**)	*D. uncinatum*	Roots	Tsanuo et al., 2003
Uncinanone B (**15**)	"	"	"
Uncinanone C (**17**)	"	"	"
Desmodianone A (**41**)	*D. canum*	"	Delle Monache et al, 1996
Desmodianone B (**42**)	"	"	"
Desmodianone C (**43**)	"	"	"
Desmodianone D (**102**)	"	"	Botta et al., 2003
Desmodianone E (**103**)	"	"	"
6-methyltetrapterol A (**104**)	"	"	"

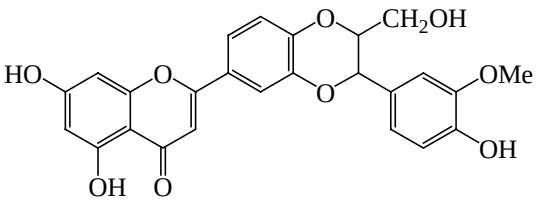
Table 2 continued.

Compound	Source	Part used	Reference
Secundiflorol H (**105**)	*D. styracifolium*	Aerial parts	Zhao et al., 2007
Homoferreirin (**106**)	"	"	"
Isoferreirin (**107**)	"	"	"
5,7-dihydroxy-2',3',4'-trimethoxyisoflavanone (**108**)	"	"	"
5,7-dihydroxy-2'-methoxy-3',4'-methylenedioxyisoflavanone (**109**)	"	"	"
5,7-dihydroxy-2',3',4'-trimethoxyisoflavanone-7-*O*-β-glucopyranoside (**110**)	"	"	"
5,7-dihydroxy-2'-methoxy-3',4'-methylenedioxyisoflavanone-7-*O*-β-glucopyranoside (**111**)	"	"	"
5,7-dihydroxy-2',4'-dimethoxyiso-flavanone-7-*O*-β-glucopyranoside (**112**)	"	"	"
5,7,4'-trihydroxy-2',3'-dimethoxyisoflavanone-7-*O*-β-glucopyranoside (**113**)	"	"	"
vi. Pterocarpans			
Gangetin (**44**)	*D. gangeticum*	Roots	Purushothaman et al., 1971
Desmodin (**114**)	"	"	Purushothaman et al., 1975
Gangetinin (**115**)	"	"	"
Desmocarpin (**116**)	"	"	Ingham & Dewick, 1984
3,9-dihydroxypterocarpan (**117**)	*D. styracifolium*	Aerial parts	Zhao et al., 2007



75: $R_1 = R_2 = R_3 = R_4 = OH$

76: $R_1 = R_2 = R_4 = OH$, $R_3 = OMe$



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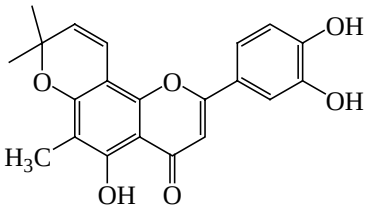

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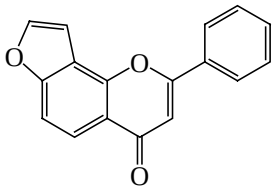

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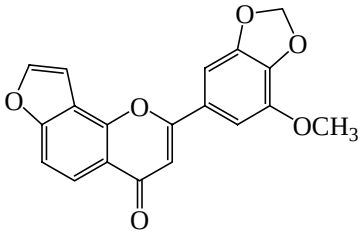

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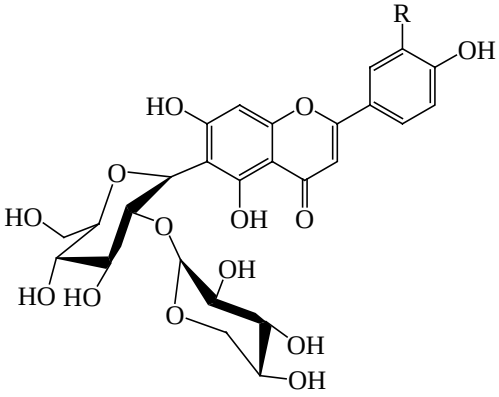
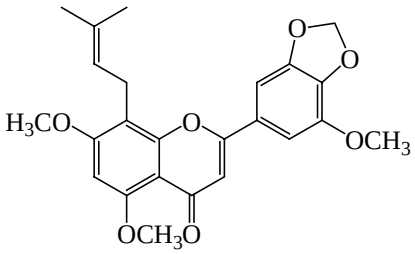


81: R = OH

82: R = H



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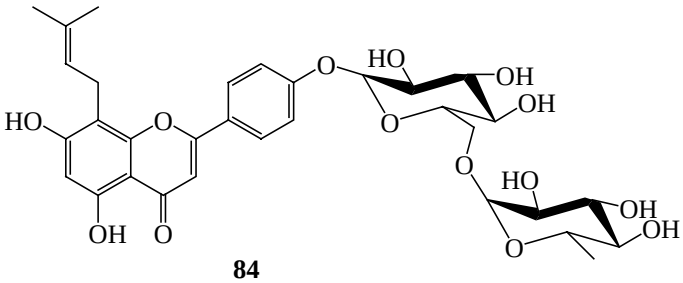

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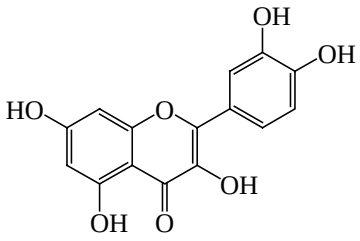

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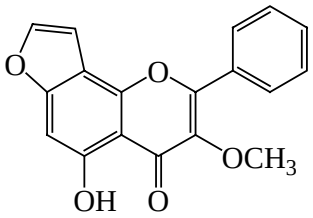

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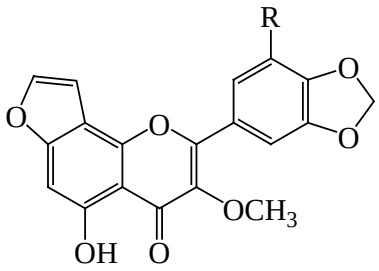
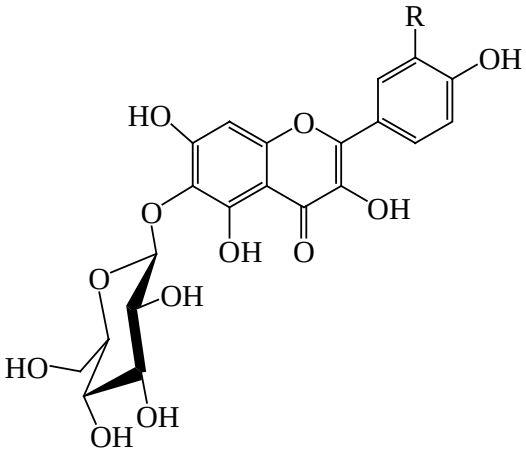


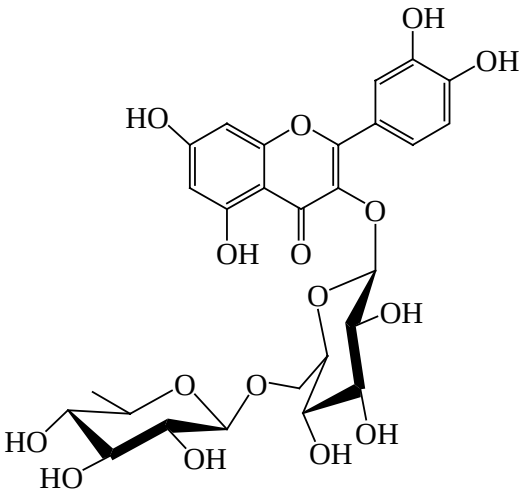
87: R = H

88: R = OCH_3



89: R = H

90: R = OH



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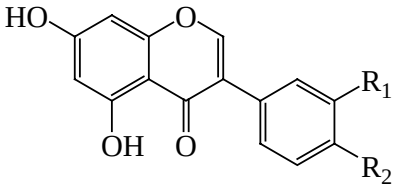
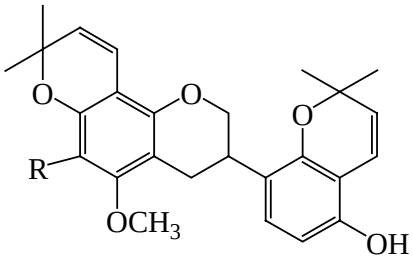


92: R_1 = H, R_2 = OH

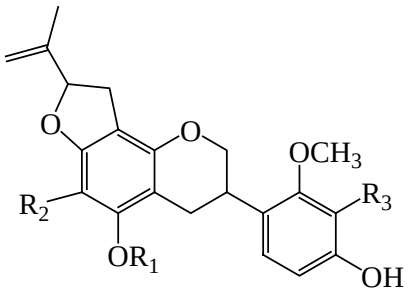
93: R_1 = H, R_2 = OCH_3

94: R_1 = R_2 = OH



95: R = H

96: R = CH_3

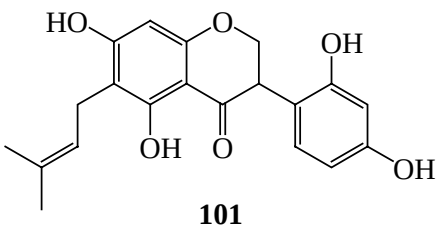


97: $R_1 = CH_3$, $R_2 = R_3 = H$

98: $R_1 = R_2 = CH_3$, $R_3 = H$

99: $R_1 = R_3 = H$, $R_2 = CH_3$

100: $R_1 = R_2 = CH_3$, $R_3 = OH$



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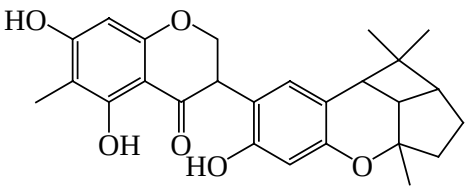

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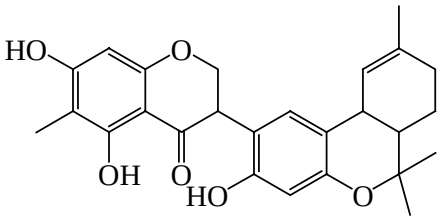

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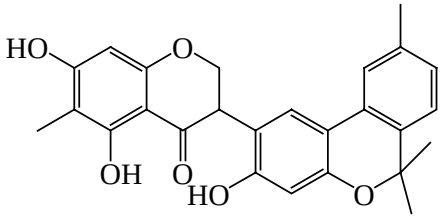

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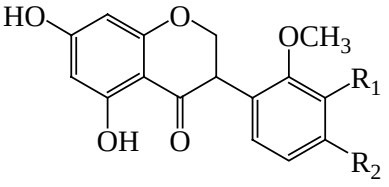
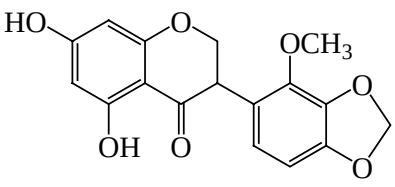


105: $R_1 = OH$, $R_2 = OCH_3$

106: $R_1 = H$, $R_2 = OCH_3$

107: $R_1 = H$, $R_2 = OH$

108: $R_1 = R_2 = OCH_3$



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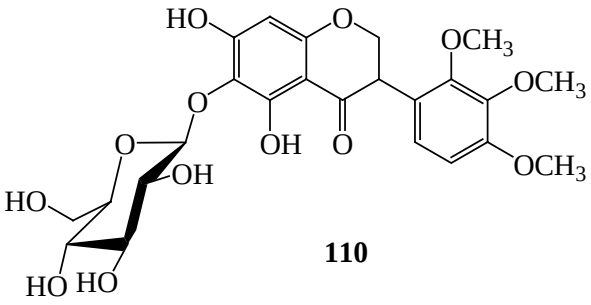

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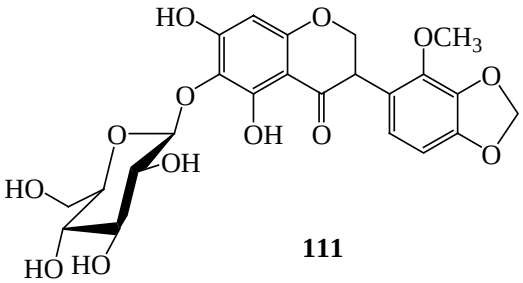

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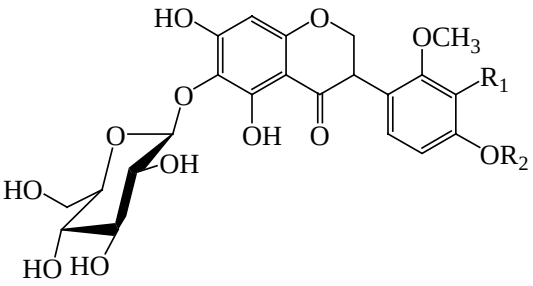
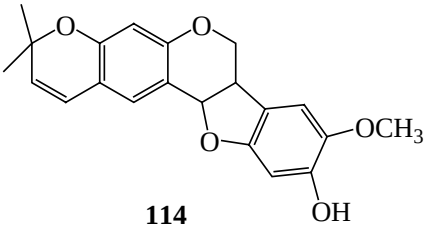


112: R_1 = H, R_2 = CH_3

113: R_1 = OCH_3, R_2 = H



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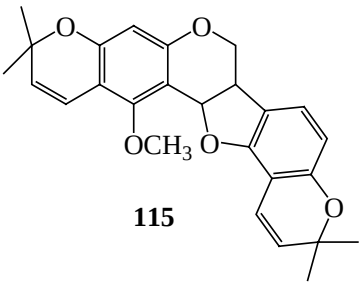

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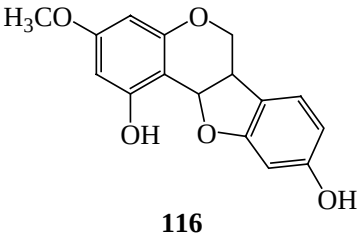

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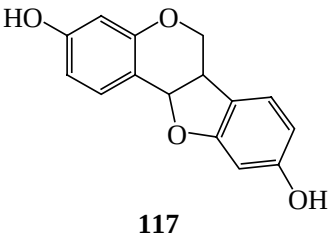

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CHAPTER 3

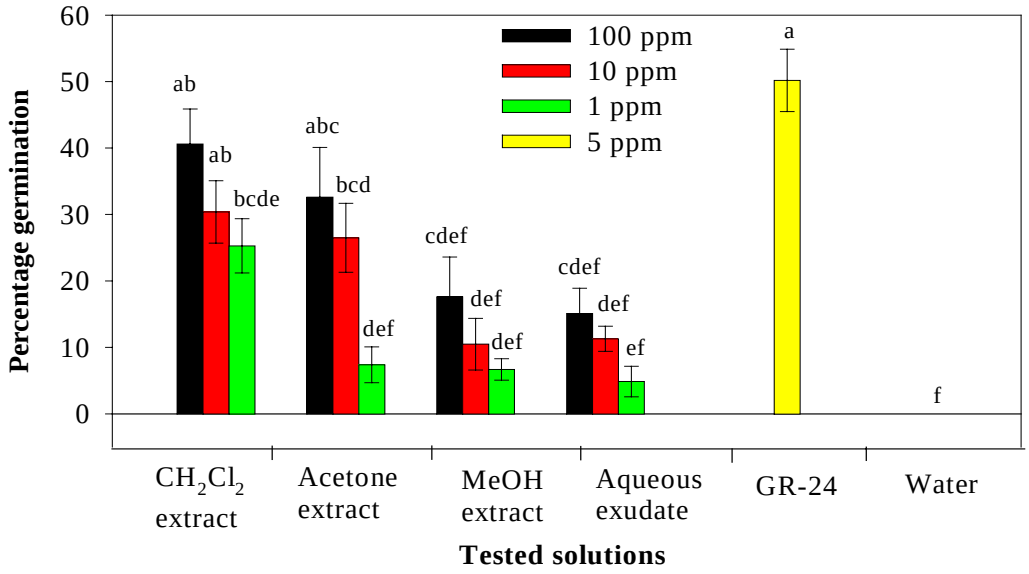
RESULTS AND DISCUSSION

3.1 Studies on *Desmodium uncinatum*

3.1.1 Biological activities of *D. uncinatum* on *Striga hermonthica*

Fresh roots of *D. uncinatum* were sequentially extracted with dichloromethane (CH_2Cl_2), acetone (Me_2CO) and methanol (MeOH). The extracts were tested for germination stimulation and post-germination growth inhibition activities on *Striga hermonthica*. The activities were compared with GR-24, a synthetic sesquiterpene known to stimulate *Striga* germination (Johnson et al., 1981) as positive control. Since *Striga* seeds do not germinate in absence of a germination stimulant, distilled water was used as a negative control

At 10 ppm, the CH_2Cl_2 extract exhibited high *Striga* germination stimulation activity, which was not significantly different from that due to GR-24 at 5 ppm; whereas the germination responses due to the polar MeOH extract and aqueous root exudates were not significantly different from the inactive distilled water (Figure 3.1).



(Means with the same letter are not significantly different (P<0.05) by Tukey's studentized range test)

Figure 3.1: Germination response of *S. hermonthica* seeds to *D. uncinatum* root extracts at varying concentrations

In contrast to the high *Striga* germination stimulation activity observed for the CH_2Cl_2 extract, this extract did not show any radicle growth inhibition activity on germinated *S. hermonthica* seeds; where as the polar aqueous root exudates, methanol and acetone extracts showed high radicle growth inhibition activities (Table 3). These findings show that the less polar components of *D. uncinatum* root extract mainly stimulate germination of *S. hermonthica* seeds whereas the highly polar components mainly inhibit the post-germination growth.

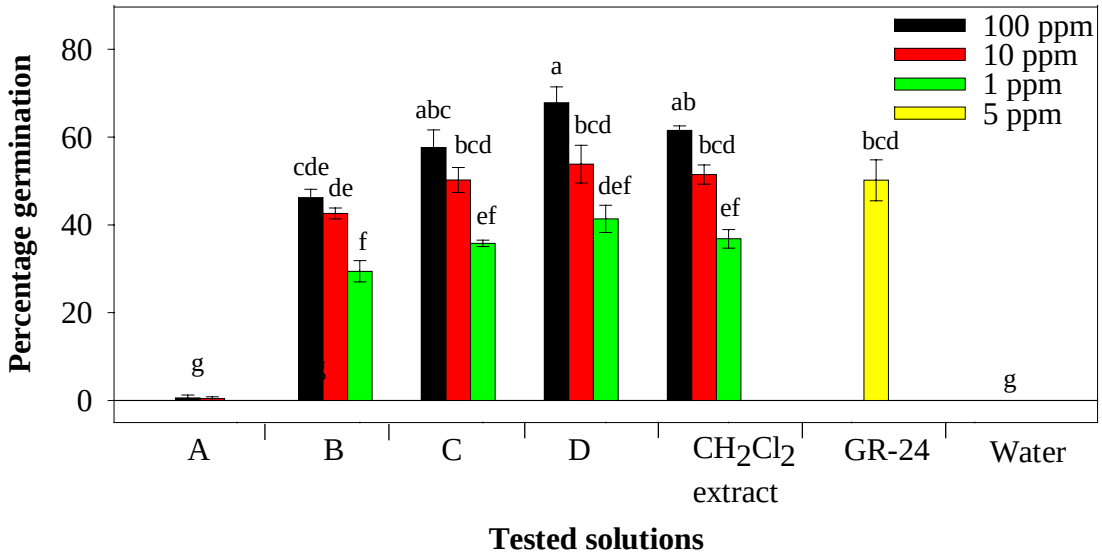
Table 3: Radicle growth inhibition of germinated *S. hermonthica* seeds exposed to root extracts of *D. uncinatum* at varying concentrations.

Tested samples	Concentration (ppm)	Radicle length, mm ± s. e. (n = 10)	Percentage Radicle length reduction
GR-24	5	0.60 ± 0.03 [a]	0.0
CH_2Cl_2 extract	1	0.59 ± 0.04 [a]	1.7
	10	0. 62 ± 0.05 [a]	-3.3
	100	0.67 ± 0.05 [a]	-11.7
Acetone extract	1	0.31 ± 0.02 [b]	48.3
	10	0.28 ± 0.02 [b]	53.3
	100	0.27 ± 0.01 [b]	55.0
Methanol extract	1	0.26 ± 0.04 [b]	56.7
	10	0.24 ± 0.02 [b]	60.0
	100	0.23 ± 0.02 [b]	61.7
Aqueous exudate	1	0.30 ± 0.03 [b]	50.0
	10	0.26 ± 0.03 [b]	56.7
	100	0.22 ± 0.02 [b]	63.3

(Means with the same letter are not significantly different ($P<0.05$) by Tukey's studentized range test).

Fractionation of the CH_2Cl_2 extract on silica gel by vacuum liquid chromatography (Experimental section 4) gave four major fractions (A, B, C and D). Germination tests with *S. hermonthica* seeds showed that *Striga* germination induction caused by the crude CH_2Cl_2 extract and fractions B, C and D at 100 and 10 μg/ml was not significantly different to that observed for GR-24 at 5 μg/ml; whereas fraction A was inactive as a germination stimulant even at a concentration of 100 ppm (Figure 3.2). These findings indicate that sets of constituents in the CH_2Cl_2 extract that are associated with the

germination stimulation of *S. hermonthica* seeds are of medium polarity, whereas the least polar components in this extract have no effect on the germination of *Striga*.



(Means with the same letter are not significantly different (P<0.05) by Tukey's studentized range test)

Figure 3.2: Germination response of *S. hermonthica* seeds to fractions of CH_2Cl_2 extract of *D. uncinatum* roots at varying concentrations.

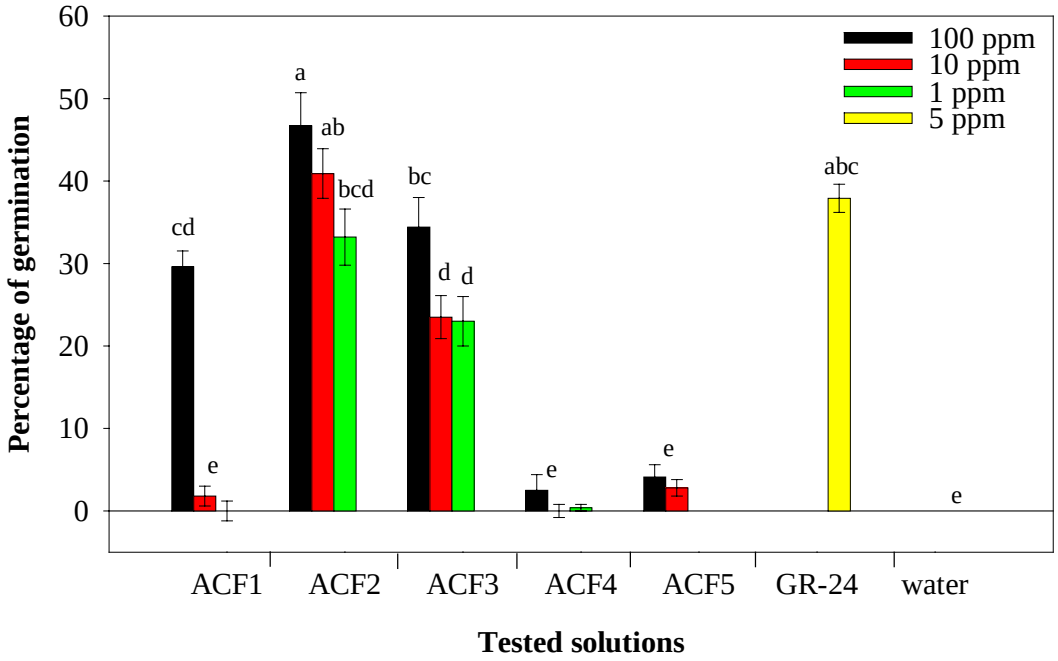
Similarly, fractionation of the acetone extract on a reverse-phase silica gel column yielded five fractions (ACF1 – ACF5). The most polar fraction (ACF1) in this extract had the highest radicle length reduction whereas the less polar fractions (ACF4 and ACF5) did not have any effect (Table 4). With respect to *Striga* germination stimulation, fractions ACF2 and ACF3 were found to have higher activities compared to other fractions (Figure 3.3). These findings indicate that the polar components (ACF1) in this extract are responsible for the radicle growth inhibition whereas the constituents in

the medium polar fractions (ACF2 & ACF3) are responsible for the germination stimulation of *Striga*. This is in agreement with the pattern observed with the crude extracts of different polarities.

Table 4: Radicle growth inhibition of germinated *S. hermonthica* seeds exposed to fractions of acetone extract of *D. uncinatum* roots at varying concentrations.

Tested solution	Concentration (ppm)	Radicle length, mm ± s. e. (n = 10)	Percentage Radicle length reduction
ACF1	1	0.36 ± 0.03 $^{d e}$	50.68
	10	0.27 ± 0.02 ^{e}	63.01
	100	0.20 ± 0.01 ^{e}	72.60
ACF2	1	0.76 ±0.03 $^{a b}$	-3.42
	10	0.58 ± 0.03 $^{a b c}$	21.23
	100	0.31 ± 0.03 ^{e}	57.53
ACF3	1	0.71 ± 0.05 $^{a b}$	2.74
	10	0.56 ± 0.03 $^{b c d}$	23.97
	100	0.38 ± 0.03 $^{c d e}$	47.95
ACF4	1	0.74 ± 0.05 $^{a b}$	-0.68
	10	0.75 ± 0.06 $^{a b}$	-2.05
	100	0.67 ± 0.05 $^{a b}$	8.22
ACF5	1	0.76 ± 0.06 $^{a b}$	-3.42
	10	0.77 ± 0.04 ^{a}	-4.79
	100	0.74 ± 0.04 $^{a b}$	-0.68
GR-24	5	0.73 ± 0.06 $^{a b}$	0.00

(Means with the same letter are not significantly different ($P < 0.05$) by Tukey's studentized range test.)



(Means with the same letter are not significantly different ($P < 0.05$) by Tukey's studentized range test)

Figure 3.3: Germination response of *S. hermonthica* seeds to fractions of acetone extract of *D. uncinatum* roots at varying concentrations.

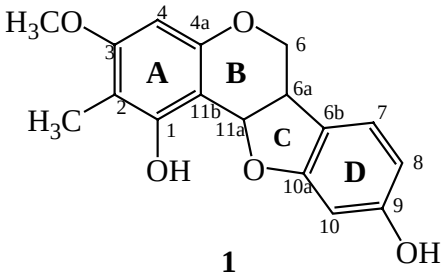
3.1.2 Isolation and characterization of compounds from *D. uncinatum* root extracts

Chromatographic separation of the CH_2Cl_2 extract on silica gel eluting with *n*-hexane containing increasing amounts of acetone gave four fractions (labelled A-D). Further fractionation of fraction D on silica gel column eluting with *n*-hexane containing increasing amounts of ethylacetate and Purification of the sub-fractions on a semi-preparative HPLC using reverse phase octadecyl silane column eluting with a decreasing polarity of acetonitrile/ water mixture from 50% to 90% acetonitrile in water gave

compounds **1** to **6**. Chromatographic separation of the combined fractions B and C on silica gel column eluting with *n*-hexane containing increasing amounts of ethylacetate gave compounds **8** to **10** (Section 4.4.1). Fractionation of the acetone extract on reverse phase octadecyl silane column eluting with 50 % methanol in water decreasing polarity to 100% methanol, gave five fractions (labelled ACF1-ACF5). Purification of fraction ACF1 and ACF2 led to isolation of compounds **11** and **7** respectively (Section 4.4.1).

Compound **1** was isolated as a white paste. The HR-EIMS gave a molecular ion peak at *m/z* 300.0993 corresponding to the molecular formula of $C_{17}H_{16}O_5$. The UV spectrum (λ_{max} 234 nm and 283 nm) is typical of pterocarpans (Tanaka et al., 2003). The ^{1}H-NMR and ^{1}H,^{1}H-COSY spectra (Appendix III and IV respectively) showed four aliphatic protons in a single spin system at δ 4.22 (dd, *J* = 4.4, -10.4 Hz), 3.50 (dd, *J* = -10.4,11.0 Hz), 3.43 (ddd, *J* = 4.4, 6.6, 11.0 Hz) and 5.65 (d, *J* = 6.6 Hz), attributed to CH_2-6, H-6a and H-11a of a pterocarpan skeleton, respectively (Table 5). The presence of a pterocarpan skeleton was supported by the ^{13}C-NMR spectrum (Appendix II), which showed the corresponding carbons at δ 66.5 (C-6), 39.5 (C-6a) and δ 76.3 (C-11a). In the ^{1}H-NMR spectrum further signals were observed which showed the presence of a methyl group (δ 2.07, s), a methoxyl group (δ 3.80, s), an aromatic singlet at δ 6.13 and aromatic protons with an ABX spin system (δ 6.33, d, *J* = 2.2 Hz; δ 6.36, dd, *J* = 2.2, 8.2 Hz and δ 7.12, d, *J* = 8.2 Hz). The molecular formula $C_{17}H_{16}O_5$ and the presence of five oxygenated aromatic carbon atoms (δ 155.2, 155.8, 158.8, 159.8 and 160.8) in the ^{13}C-NMR spectrum is consistent with two hydroxyl substituents, in addition to the methyl and methoxyl groups on pterocarpan skeleton. In the EIMS (Appendix I), the fragment ions

resulting from fragmentation of the pterocarpans (Tòth et al., 2000) were also consistent with the methyl, methoxyl and two hydroxyl substituents in pterocarpan skeleton (Scheme 1). Acetylation of compound **1** formed a diacetate (**1a**), whose EIMS (Appendix VIII) gave a molecular ion peak at *m/z* 384 and two fragment ions (*m/z* 341 and *m/z* 299) attributed to loss of two acetyl groups, thus confirming the presence of the two hydroxyl groups in compound **1**. In the HMBC spectrum (Appendix VI), correlation of H-11a (δ 5.65) with the signal at δ 155.8 and also correlation of the methyl protons (δ 2.07) with the signals at δ 155.8, 105.6 and 159.8 is consistent with tri-substituted A-ring with the methyl being at C-2 and oxygenations at C-1 and C-3. The methoxyl group was placed at C-3 and hydroxyl at C-1, due to HMBC correlation of the methyl protons with C-3. The substitution pattern in A-ring was confirmed by 1D-GOESY spectrum (Appendix VIII), which showed NOE interaction between the methyl protons (δ 2.07) and the methoxyl group (δ 3.80); and between the methoxyl group (δ 3.80) and an aromatic singlet at δ 6.13 (H-4). Then the ABX spin system corresponds to D-ring protons with the biogenetically expected oxygenation at C-9, and this was confirmed by the HMBC data (Table 5). Natural pterocarpans are known to occur in *cis* configuration (Dewick, 1988; 1994). In agreement with this, the coupling constant between H-6a and H-11a (J = 6.6 Hz) and the strong NOE between H-11a (δ 5.65) and H-6a (δ 3.43) are consistent with *cis*-geometry at the ring junction (Van Aardt et al., 1999; 2001). This compound showed high negative optical rotation ($[\alpha]_D$ = -250°) consistent with 6a*R*:11a*R* absolute configuration. Thus compound **1** was characterized as (6a*R*: 11a*R*)-1,9-dihydroxy-3-methoxy-2-methylpterocarpan for which the trivial name uncinacarpan is suggested.



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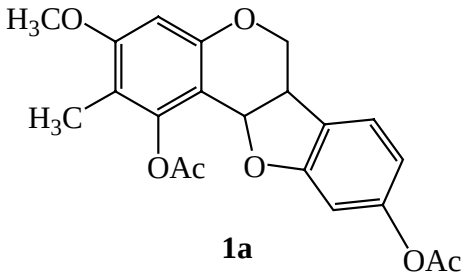
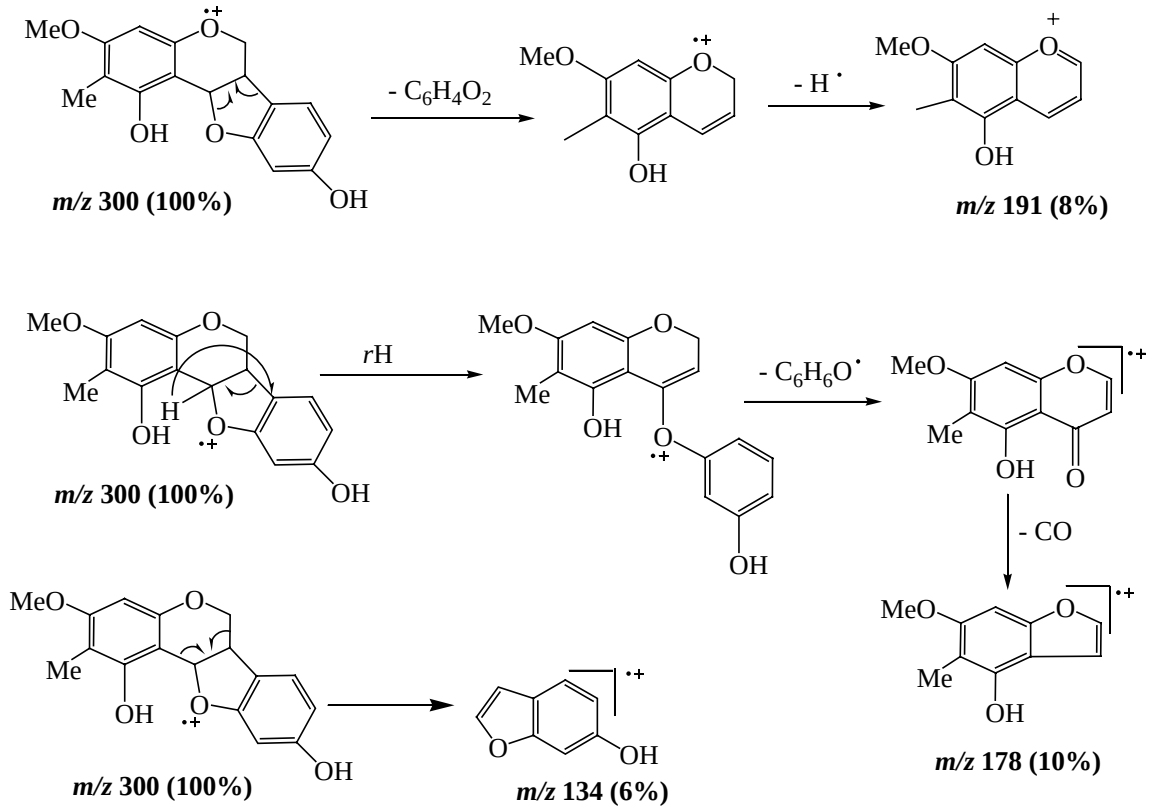


1a

Ac = $COCH_3$

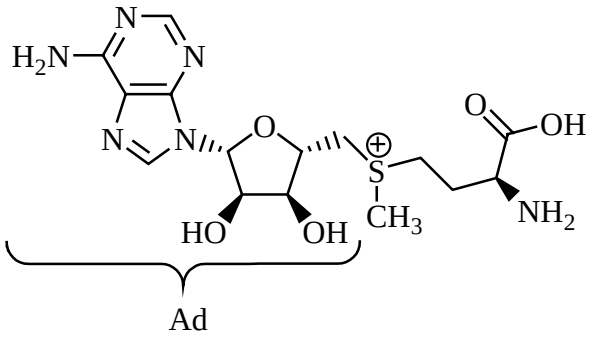
Table 5: ^{1}H- (500 MHz), ^{13}C-NMR (125 MHz) spectral data (in MeOH-d_4) and HMBC correlations of uncinacarpan (**1**).

Position	**δ_C**	**δ_H (*J* in Hz)**	**HMBC**
1	155.8		
2	105.6		
3	159.8		
4	91.6	6.13, s	C-2, 3, 4a, 11b
4a	155.2		
6	66.5	3.50, dd (-10.4, 11.0)	C-4a, 6a, 6b, 11a
		4.22, dd (4.4, -10.4)	C-4a, 6a, 6b, 11a
6a	39.5	3.43, ddd (4.4, 6.6, 11.0)	C-6, 6b, 10a
6b	118.4		
7	124.9	7.12, d (8.2)	C-9, 6a, 10a
8	107.6	6.36, dd (8.2, 2.2)	C-9, 10, 6b
9	158.8		
10	97.9	6.33, d (2.2)	C-8, 9, 6b, 10a
10a	160.8		
11a	76.3	5.65, d (6.6)	C-1, 6, 6a, 11b
11b	101.8		
2-CH_3	7.2	2.07, s	C-1, 2, 3
3-OCH_3	54.9	3.80, s	C-3



Scheme 1: Proposed EIMS fragmentation for uncinacarpan (**1**) in accordance with Tòth et al. (2000)

Like most flavonoids, the oxygenation pattern in uncinacarpan (**1**) reflects its biogenetic origin from shikimate and acetate pathways (Winkel-Shirley, 2001). In addition, it has both *O*-methyl and *C*-methyl groups on ring A. Methylation in most plant metabolites is attributed to the transfer of methyl groups from a methyl donor, S-adenosyl-L-methionine (SAM) (**118**), *via* the enzymes, methyltransferases (Roje, 2006).



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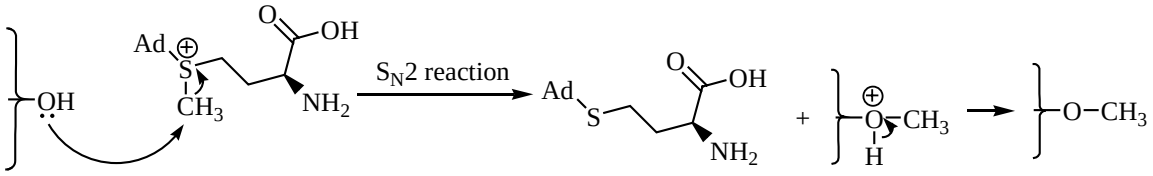
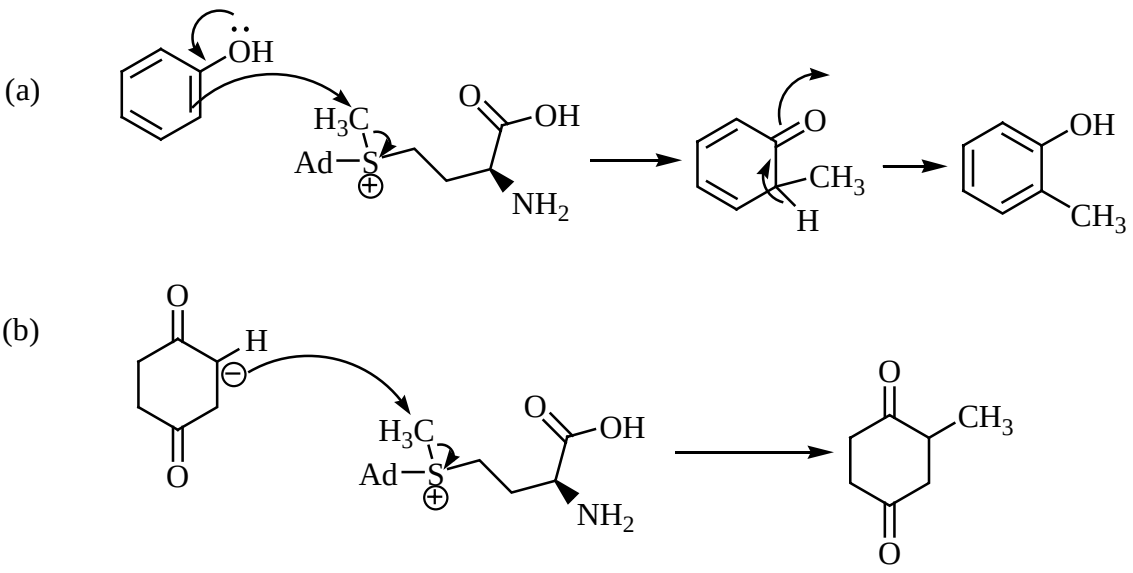
O-methylation is a common feature in the flavonoids and this has been attributed to action of SAM-dependent enzymes, *O*-methyltransferases, which catalyze methylation of hydroxyl moieties of flavonoids (Roje, 2006). In chemical language, this transfer of methyl group is a nucleophilic substitution reaction (S_N2) facilitated by the positively charged sulphur in SAM with the hydroxyl group as the nucleophile and S-adenosylhomocysteine, a neutral molecule, as a good leaving group (Scheme 2) (Dewick, 2002).



Scheme 2: *O*-methylation using SAM

Therefore, the fact that SAM provides methyl groups in many biological methylations (Roje, 2006) including flavonoids (Wu et al., 1997; Rakwal et al., 2000; Roje, 2006), it is suggested here that the biogenesis of uncinacarpan (**1**) also utilizes a methyl group from L-methionine *via* a SAM-dependent enzyme for the *O*-methylation at C-3 position.

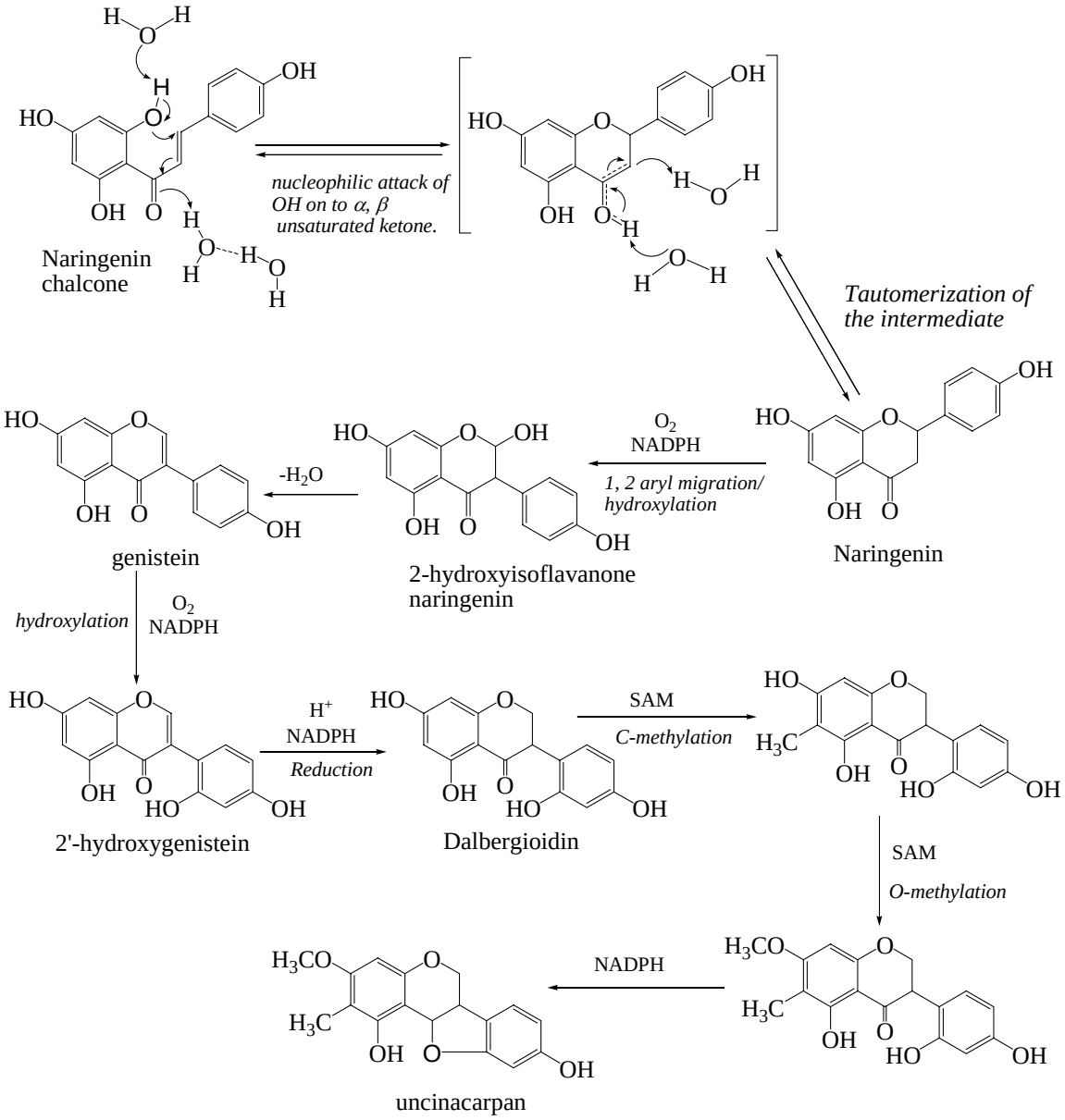
Naturally, *C*-methylation also occurs *via* SAM and some SAM-dependant *C*-methyltransferases have been identified from plants (Roje, 2006). Chemically, *C*-methylation using SAM may occur also *via* nucleophilic substitution mechanism (Scheme 3) that involves a nucleophilic carbon that is either *ortho* or *para*-positions to a phenol hydroxyl group (Scheme 3a) or adjacent to one or more carbonyl groups (Scheme 3b) (Dewick, 2002).



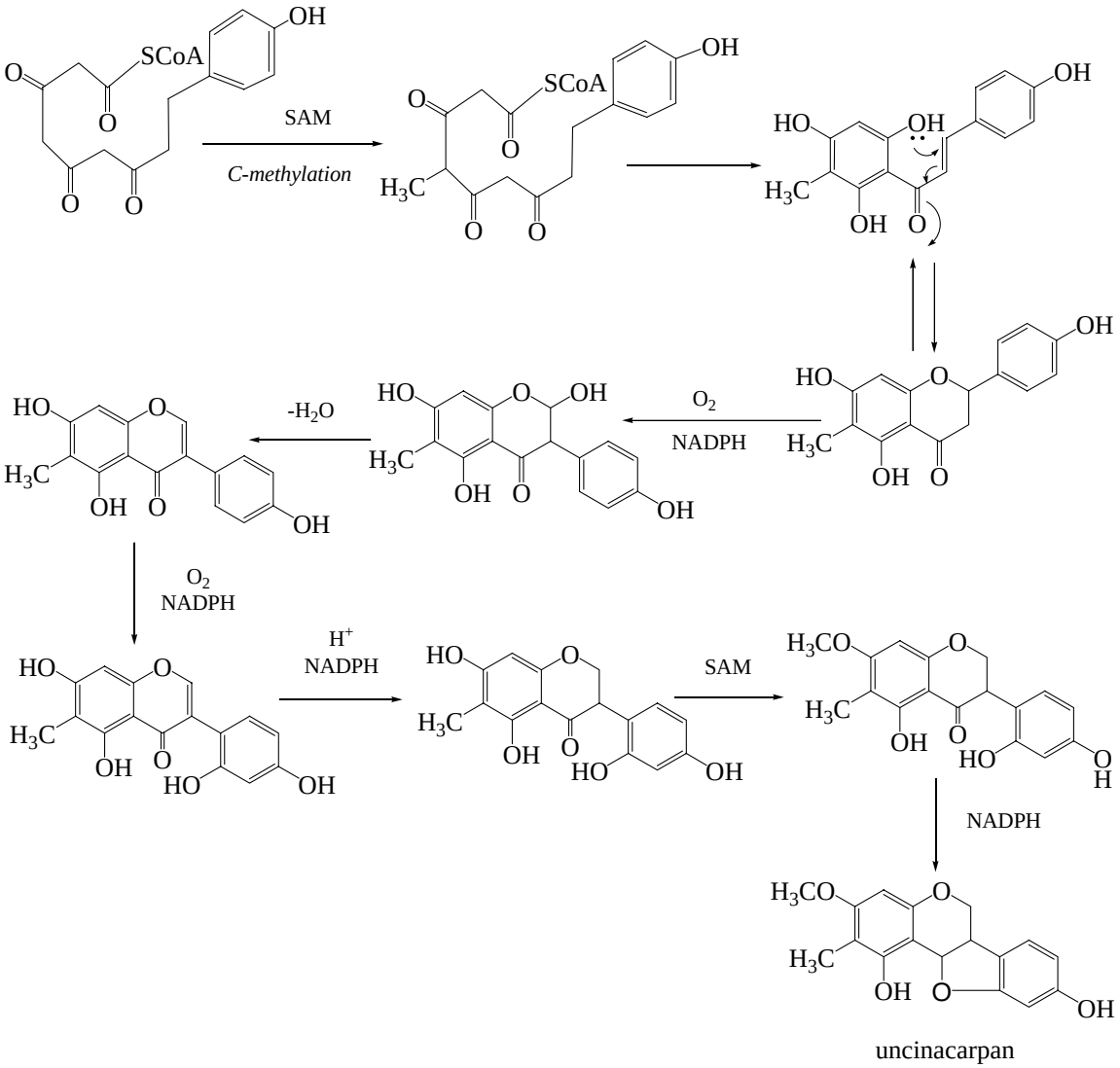
Scheme 3: *C*-methylation using SAM via nucleophilic substitution mechanism.

The 2-*C*-methyl group in uncinacarpan (**1**) may therefore be attributed to a SAM-dependant *C*-methyltransferases in its biogenesis and the analogous chemical reaction being a nucleophilic substitution. However, there are two possible stages this substitution can occur in the biogenesis of uncinacarpan (**1**): (i) as a later step after cyclization and aromatization of the acetate pathway derivative whereby the carbon at C-2 position is *ortho* to phenolic groups, hence activated and become susceptible to methylation (Scheme 4a); or (ii) as a step before cyclization of the acetate pathway derivative

whereby the β-diketo structure of the carbonyl groups increases the acidity of the C-2 carbon and facilitate the *C*-methylation process (Scheme 4b).



Scheme 4a: Proposed biogenetic route for uncinacarpan (**1**): *C*-methylation occurring after the cyclization and aromatization of the acetate derived part

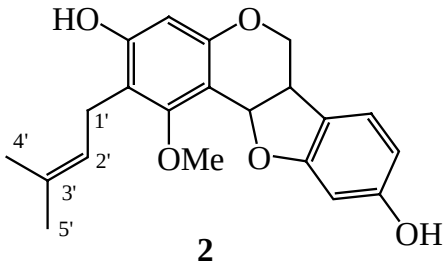


Scheme 4b: Proposed biogenetic route for uncinacarpan (**1**): *C*-methylation occurring before cyclization of the acetate pathway derivative

Unlike *O*-methylation, *C*-methylation is not common in flavonoids. Most of the *C*-methylated flavonoids reported are from the family Myrtaceae and this has been suggested to be a distinctive feature of the family (Mustafa et al., 2005; Wollenweber et al., 2000).

Compound **2** was assigned a molecular formula of $C_{21}H_{22}O_5$ (*m/z* 354.1467) from HR-EIMS. The ^{13}C- and ^{1}H-NMR spectra (Appendix X & XI respectively) showed this compound to be a pterocarpan derivative with a 3-methylbut-2-enyl, a methoxyl and two hydroxyl substituents. Comparison of the NMR data of compound **2** (Table 6) with those of compound **1** (Table 5) showed that **2** differs from **1** by the presence of a 3-methylbut-2-enyl group in place of a methyl group at C-2. The fragment ions in the EIMS (Appendix IX) also revealed the presence of a 3-methylbut-2-enyl group on a pterocarpan skeleton (Scheme 5). The placement of the 3-methylbut-2-enyl group at the C-2 position was confirmed by the HMBC spectrum (Appendix XIII), showing correlations between the methylene protons of the 3-methylbut-2-enyl group (δ 3.39, d, J = 6.3 Hz) with C-1 (δ 159.9), C-2 (δ 114.3) and C-3 (δ 157.8). The ^{13}C-NMR chemical shift position of the methoxyl group in **2** (δ 63.5) is substantially downfield shifted compared to what has been observed in **1** (δ 54.9) suggesting that it is di-*ortho*-substituted (Panichpol and Waterman, 1978); and could only be located at C-1. This was confirmed from HMBC correlation between the methoxyl protons with C-1 and also from NOE interaction (in a GOESY spectrum, Appendix XIV) of the methoxyl protons with H-11a (δ 5.63) and with methylene protons (δ 3.39) of the 3-methylbut-2-enyl group at C-2. Thus compound **2** was characterized as 3,9-dihydroxy-1-methoxy-2-(3-methylbut-2-enyl)pterocarpan. (**2**). This compound under the name of edudiol has been reported from *Neorautanenia edulis* and the structure proposed based on physical methods and by partial synthesis (Brink et al., 1977). However the first NMR evidence for the identity of this compound is reported here. The relative configuration (at the ring junction, 6a:11a) of edudiol is suggested to be *cis* from the consideration that natural pterocarpans are known to occur in *cis* geometry

(Dewick, 1988, 1994) and the presence of a small coupling constant ($J_{H\text{-}6a,\ 11a}$ = 6.6 Hz) and strong NOE interaction between H-6a (δ 3.38) and H-11a (δ 5.63) are consistent with *cis* configuration (van Aardt et al., 1999; 2001).



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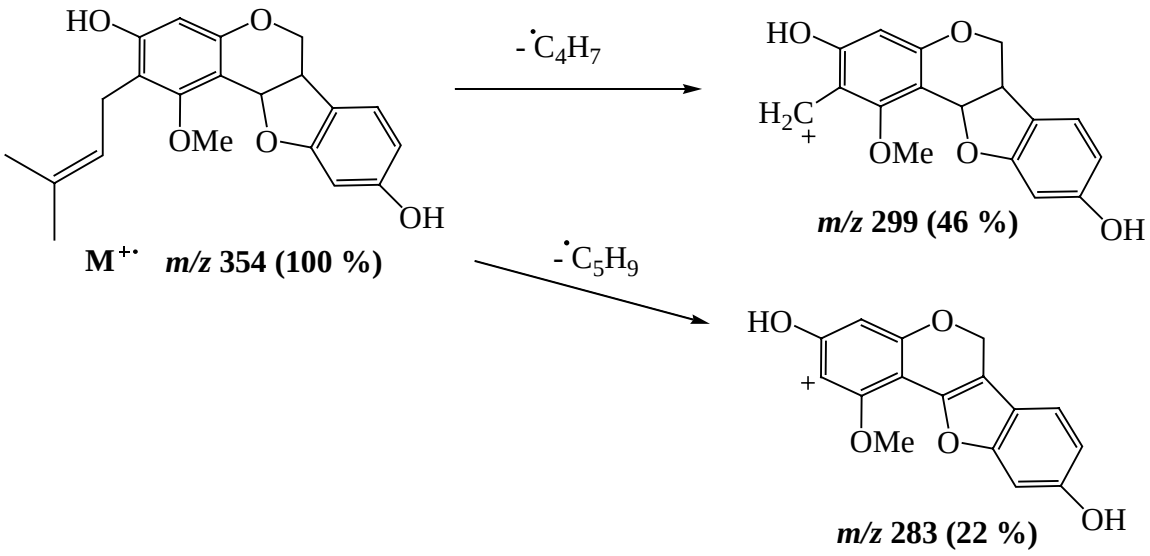


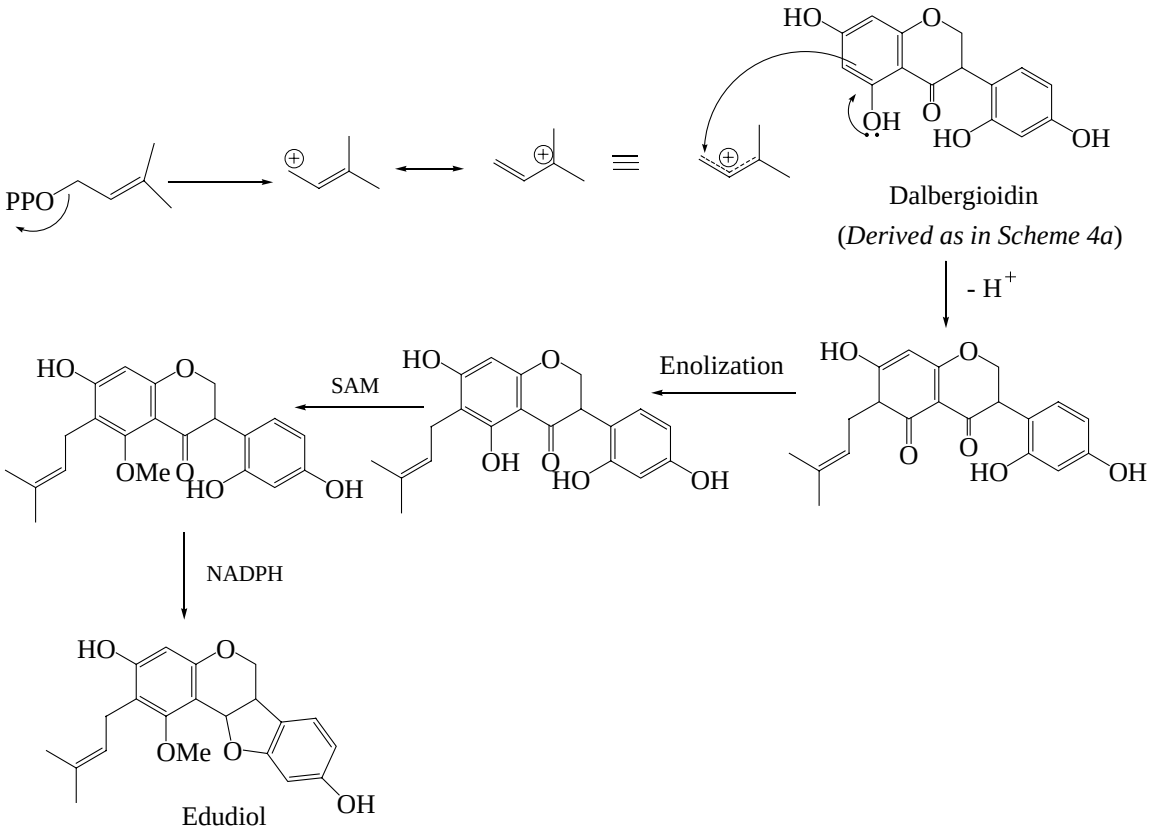
M$^{+\cdot}$ ***m/z* 354 (100 %)**

Scheme 5: Proposed EIMS fragment ions for edudiol (**2**)

Table 6: ^{1}H- (500 MHz), ^{13}C-NMR (125 MHz) spectral data (in $CDCl_3$) and HMBC correlations of edudiol (**2**).

Position	δ_C	δ_H (*J* **in Hz**)	**HMBC**
1	159.9		
2	114.3		
3	157.8		
4	100.9	6.26, s	C-2, 3, 4a, 11b
4a	155.8		
6	66.7	3.59, dd (-11.0, 11.4)	C-4a, 6b, 11a
		4.16, dd (-11.0, 5.0)	C-4a, 6a, 6b, 11a
6a	39.3	3.38, ddd (5.0, 6.0, 11.4)	C-6, 6b, 10a
6b	119.9		
7	125.2	7.07, d (8.0)	C-9, 6a, 10a
8	107.8	6.35, dd (8.1, 2.1)	C-10, 6b
9	157.2		
10	98.9	6.38, d (2.0)	C-8, 6b
10a	161.3		
11a	76.3	5.63, d (6.6)	C-1, 6, 6a, 11b, 4a
11b	107.3		
1’	23.4	3.39, d (6.3)	C-1, 2, 3, 2’, 3’
2’	122.4	5.24, br t (6.8)	C-2, 4’, 5’
3’	135.9		
4’	18.4	1.83, s	C-2’, 3’, 5’
5’	26.2	1.76, s	C-2’, 3’, 4’
1-OCH_3	63.5	3.91, s	C-1

Like in the case of uncinacarpan (**1**), a tetrahydroxychalcone appear to be the precursor of edudiol (**2**). The *O*-methylation at C-1 position is attributed to SAM-dependent enzymes whereas a dimethylallyl derivative at C-2 position is from a C_5 natural alkylating agent, dimethylallyl diphosphate (Scheme 6).

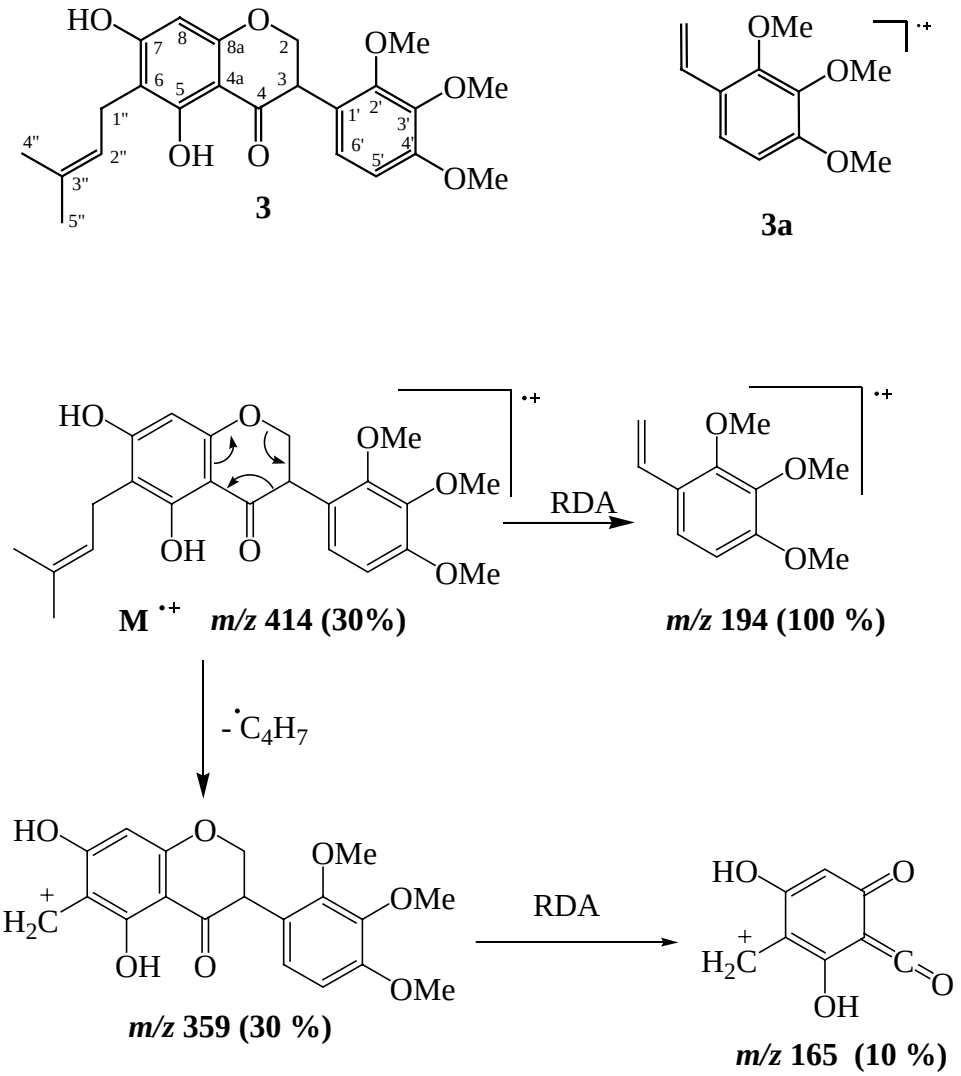


Scheme 6: Proposed biogenesis of edudiol (**2**)

Compound **3** ($C_{23}H_{26}O_7$) was isolated as a colourless amorphous solid. The ^{1}H- (δ 4.40, dd, *J* = 5.4, -10.8 Hz for H-2eq; δ 4.51, dd, *J* = -10.8, 11.0 Hz for H-2ax and δ 4.23, dd, *J*= 5.5, 11.1 Hz for H-3) and ^{13}C- (δ 70.8 for C-2; δ 47.8 for C-3 and δ 197.9 for C-4) NMR spectra (Appendix XVII & XVI respectively) are consistent with compound **3** being an isoflavanone derivative (Tsanuo et al., 2003). The ^{1}H- and ^{13}C-NMR data (Table 7) further revealed the presence of a 3-methylbut-2-enyl, three methoxyl and two hydroxyl substituents on the isoflavanone skeleton. The presence of an aromatic singlet (δ 5.96) and two *ortho*-coupled aromatic protons (δ 6.91, d, *J* = 8.6 Hz and δ 6.78, d, *J* = 8.6 Hz) was also evident from ^{1}H-NMR spectrum. In the EIMS (Appendix XV) the fragment ion at *m/z* 194 (**3a**) resulting in *retro*-Diels Alder (RDA) cleavage of C-ring (Scheme 7) indicated the placement of the three methoxyl groups in B-ring and the two hydroxyl and the 3-methylbut-2-enyl groups in A-ring. With the biogenetically expected oxygenations at C-5 and C-7, the 3-methylbut-2-enyl group could either be at C-6 or C-8. In the HMBC spectrum, the singlet at δ 5.97 and the methylene protons at C-2 showed correlation with C-8a (δ 161.8) allowing the assignment of the singlet at δ 5.97 to H-8; consequently the 3-methylbut-2-enyl group could only be placed at C-6. This was confirmed by the HMBC experiments (Appendix XX), showing the correlations between the methylene protons at C-1" (δ 3.25) with C-5 (δ 161.8), C-6 (δ 108.7) and C-7 (δ 164.9).

In the B-ring, the two *ortho*-coupled doublets at δ 6.78 and 6.91 (*J* = 8.6 Hz) can be assigned to H-5' and H-6' with the three methoxyl groups being at C-2', C-3' and C-4'. The ^{13}C NMR chemical shift positions for B-ring carbon atoms (Table 7) and the

methoxyl groups is consistent with the placement of the methoxyl groups at C-2', C-3' and C-4'. Hence compound **3** was characterized as 5,7-dihydroxy-2',3',4'-trimethoxy-6-(3-methylbut-2-enyl)isoflavanone (**3**) for which the trivial name uncinanone D is suggested. Its biogenesis is related to that of isoflavanone intermediate in the formation of edudiol (**2**) (Scheme 6) but with some additional hydroxylation followed by *O*-methylation steps.

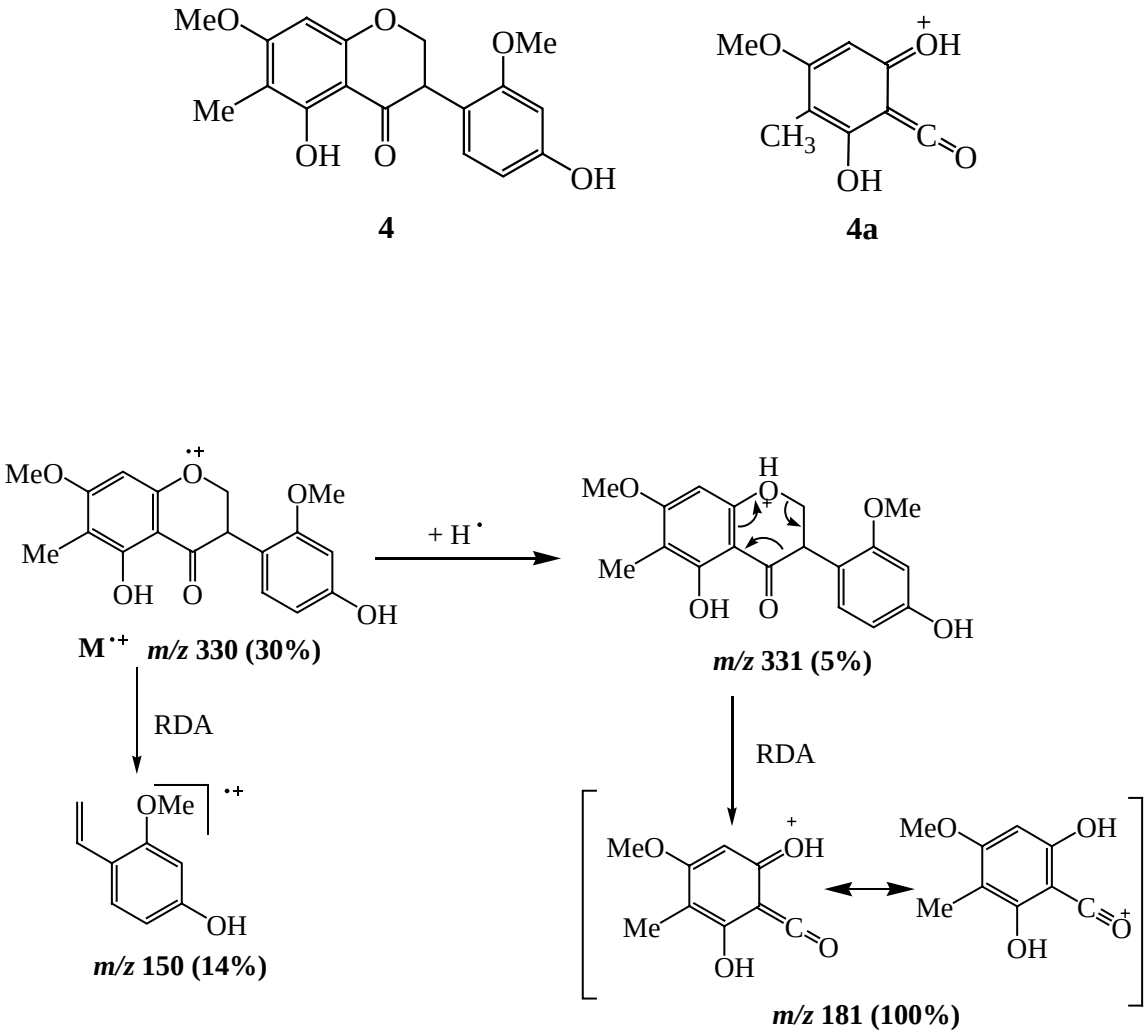


Scheme 7: Proposed EIMS fragmentation for uncinanone D (**3**)

Table 7: ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) spectral data (in MeOH-d_4) along with HMBC correlations of uncinanone D (**3**)

Position	δ_C	δ_H (***J* in Hz**)	**HMBC**
2	70.8	4.40 dd, (-10.8, 5.4)	C-4, 8a, 1’
		4.51 dd (-10.8, 11.0)	C-4, 8a
3	47.8	4.23 dd (11.1, 5.5)	C-2, 4, 1’, 2’, 6’
4	197.9		
4a	102.4		
5	161.8		
6	108.7		
7	164.9		
8	94.2	5.97 s	C-4a, 6, 7, 8a
8a	161.8		
1’	121.6		
2’	152.2		
3’	142.5		
4’	154.1		
5’	107.7	6.78 d (8.6)	C-1’, 3’, 4’
6’	125.1	6.91 d (8.6)	C-3, 2’, 4’
1”	20.9	3.25 d (7.1)	C-5, 6, 7, 2”, 3”
2”	122.9	5.24 br t (7.3)	
3”	130.6		
4”	24.9	1.79 s	C-2”, 3”, 4”
5”	16.8	1.70 s	C-2”, 3”, 5”
2’-OCH_3	60.3	3.87 s	C-2’
3’-OCH_3	60.0	3.85 s	C-3’
4’-OCH_3	55.5	3.88 s	C-4’

Compound **4** ($C_{18}H_{18}O_6$) is also an isoflavanone derivative having two methoxyl, two hydroxyl and a methyl substituents from the ^{1}H- and ^{13}C-NMR data (Appendix XXIV & XXIII respectively; Table 8). In the EIMS (Appendix XXII) the fragment ion at *m/z* 181 (**4a**) resulting from RDA cleavage of C-ring (Scheme 8) is consistent with the placement of one hydroxyl (δ 12.33 for 5-OH), methoxyl (at C-7) and methyl (at C-6 or C-8) in A-ring. HMBC spectrum (Appendix XXVII) showed correlation of the methyl protons (δ 2.04) with C-5 (δ 161.1), C-6 (δ 106.2) and C-7 (δ 165.8), and correlation of the aromatic singlet (δ 6.05) with C-8a (δ 161.9) and C-4a (δ 103.6), which allows the assignment of the singlet to H-8 and locating the methyl at C-6. The placement of latter was confirmed by a GOESY experiment (Appendix XXVIII), which showed NOE interaction between the methyl protons (δ 2.04) with both 5-OH (δ 12.33) and 7-OMe (δ 3.88). An AXY spin system at δ 6.97 (d, *J* = 8.1 Hz), δ 6.41 (dd, *J* = 2.1, 8.2 Hz) and δ 6.47, d, *J* = 2.1 Hz) can be assigned to H-6', H-5' and H-3' of B-ring respectively, with the remaining methoxyl and hydroxyl groups being at C-2' and C-4'. A GOESY experiment showed interaction of the methoxyl group (δ 3.80) with H-3' (δ 6.47), CH_2-2 (δ 4.45 and δ 4.55) and H-3 (δ 4.31) allowing the placement of the methoxyl group at C-2'. This was confirmed from the HMBC spectrum which showed correlations between the methoxyl protons (δ 3.80) and C-2' (Table 8) demonstrating this new compound to be 5,4'-dihydroxy-7,2'-dimethoxy-6-methylisoflavanone (**4**) for which the trivial name uncinanone E is assigned. The biosynthetic route of this compound is similar to that of formation of isoflavanone intermediate in uncinacarpan (**1**) (Scheme 4a & b) with an additional step of *O*-methylation.



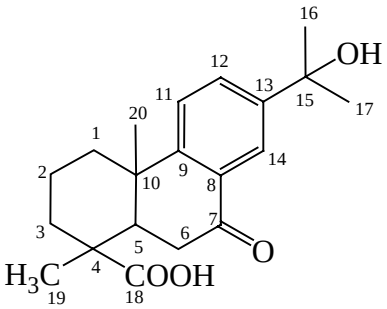
Scheme 8: Proposed EIMS fragmentation for uncinanone E (**4**)

Table 8: ^{1}H- (500 MHz), ^{13}C-NMR spectral data (in $CDCl_3$) and HMBC correlations of uncinanone E (**4**)

Position	δ_C	δ_H (***J*** **in Hz)**	**HMBC**
2	71.0	4.45 dd (-10.9, 5.5)	C-3, 4, 8a, 1’
		4.55 dd (-10.9, 11.0)	C-3, 4, 8a
3	47.1	4.31 dd (11.0, 5.4)	C-2, 4, 1’, 2’, 6’
4	197.9		
4a	103.6		
5	161.1		
6	106.2		
7	165.8		
8	90.8	6.05 s	C-6, 7, 8a, 4a
8a	161.9		
1’	115.7		
2’	158.9		
3’	100.0	6.47 d (2.1)	C-1’, 2’, 4’, 5’
4’	157.0		
5’	107.7	6.41 dd (8.1, 2.1)	C-1’, 3’, 4’
6’	131.3	6.97 d (8.1)	C-3, 2’, 4’
5-OH		12.33 s	C-4a, 5, 6
7-OCH_3	56.2	3.89 s	C-7
2’-OCH_3	56.0	3.80 s	C-2’
6-CH_3	7.2	2.04, s	C-5, 6, 7

Compound **5** was isolated as a yellow amorphous solid. Its EIMS (Appendix XXIX) showed a molecular ion peak at *m/z* 330 consistent with the molecular formula $C_{20}H_{26}O_4$. The ^{13}C-NMR spectrum (Appendix XXX) showed signals for twenty-carbon atoms characteristic of a C_{20} polyisoprenoid (diterpene). The ^{1}H-NMR spectral data (Appendix XXXI; Table 9) showed the presence of a 1,2,4-trisubstituted benzene ring (δ 7.48, d, *J* = 8.3 Hz; 7.78, dd, *J* = 1.9, 8.3 Hz; 8.11, d, *J* = 1.8 Hz) and four methyl groups (δ 1.34, s, 3H; 1.39, s, 3H; 1.57, s, 6H). Further revealed by the ^{1}H-NMR spectrum is an aliphatic broad doublet at δ 2.4 (*J* = 12.7 Hz, 1H) characteristic of H-1β of a dehydroabietane diterpene (Kuo et al., 2004). The ^{13}C-NMR data (Table 10) revealed the presence of two carbonyl carbons (δ 180.4 and 199.8, a carboxylic acid and a ketone respectively). The placement of the keto group (δ 199.8) at C-7 was established by HMBC (Appendix XXXIV) correlation of aromatic proton H-14 (δ 8.11) with C-7 (δ 199.8), C-9 (δ 154.6) and C-12 (δ 131.3). The location of the carboxylic acid group (C-18) was evident from the HMBC correlations of CH_3-19 (δ 1.34, s) with C-5 (δ 44.5) and C-18 (δ 180.4) and that of CH_3-20 (δ 1.39, s) with C-5 (δ 44.5). A GOESY experiment (Appendix XXXV) showed NOE interaction of H-1β (δ 2.49, d) with CH_3-20 (δ 1.34, s) and CH_3-19 (δ 1.39, s) consistent with the assignment of β-configuration to the two methyl groups and hence α-configuration to the carboxylic acid. The placement of the hydroxyl group at C-15 was evident from the downfield shift of the geminal methyl protons (δ 1.57, s, 6H) signal for the CH_3-16 and -17. The presence of a tertiary hydroxyl group was confirmed by the HMBC correlation of methyl protons (δ 1.57, s, 6H) with the quaternary carbon (δ 71.6) and methyl carbon (δ 30.7). In the EIMS, the fragment ion at *m/z* 315 (base peak)

resulting from loss of a methyl radical is attributed to α-cleavage of C-C bond next to oxygen (Scheme 9) thus consistent with the presence of a tertiary hydroxyl and methyl groups. Based on the above evidence and comparison of the spectral data of compound **5** with those reported in literature (Ayer & Macaulay, 1987), this compound was identified as the abietane diterpene, 7-oxo-15-hydroxydehydroabietic acid. This is the first report of this compound from the genus *Desmodium*.



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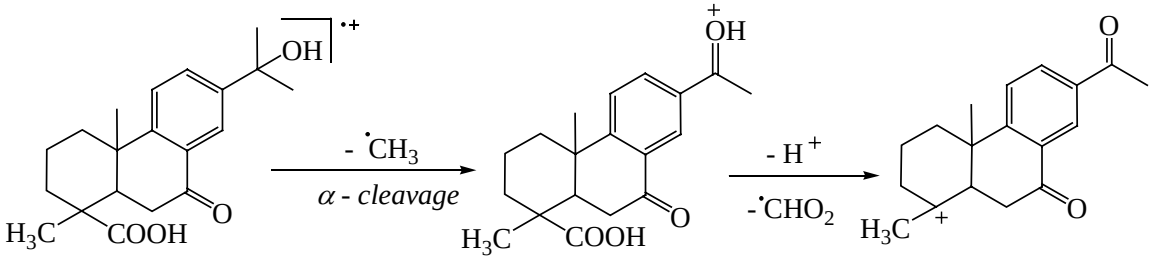
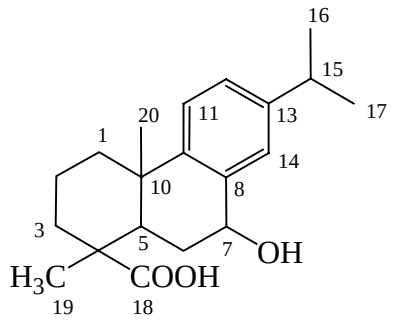


M $^{\bullet+}$ ***m/z* 330 (5 %)** ***m/z* 315 (100 %)** ***m/z* 269 (15 %)**

Scheme 9: Proposed EIMS fragmentation for 7-oxo-15-hydroxydehydroabietic acid (**5**)

The EIMS (Appendix XXXVI) of compound **6** showed a molecular ion peak at *m/z* 316 corresponding to the molecular formula $C_{20}H_{28}O_3$. The ^{1}H- and ^{13}C-NMR spectra (Appendix XXXVIII & XXXVII respectively) were characteristic of an abietane diterpene skeleton. Comparison of the ^{1}H- (Table 9) and ^{13}C- (Table 10) NMR data of compounds **5** with **6** showed that the carbonyl at C-7 of **5** is replaced by a secondary hydroxyl substituent (δ 4.68, br). Also observed from the ^{1}H-NMR data of compound **6** is a typical H-1β signal (δ 2.26, d, *J* = 12.3 Hz) of dehydroabietanes (Kuo et al., 2004) and an isopropyl group (δ 2.86, sept., *J* = 6.9 Hz and δ 1.23, d, *J* = 6.9 Hz *J* = 6.7 Hz). The ^{13}C-NMR spectrum showed the presence of a carboxylic acid (δ 183.0) assigned to C-18 from HMBC (Appendix XLI) correlation of the carbonyl carbon with CH_3-19 (δ 1.22, s). As in compound **5**, the NOESY spectrum (Appendix XLII) showed NOE interaction of H-1β (δ 2.26, d) with CH_3-20 (δ 1.12, s) and H-11 (δ 7.16, d) and that of CH_3-19 (δ 1.22, s) with CH_3-20 (δ 1.12, s) consistent with assigning β-configuration to the methyl groups (C-19 and C-20) and α-configuration for the carboxylic acid. The placement of the hydroxyl group at C-7 was confirmed from the HMBC correlation of H-14 (δ 7.15, d) with C-7 (δ 68.1), C-9 (δ 146.7), C-12 (δ 126.5) and C-15 (δ 33.5). The NOE interaction of H-7 (δ 4.67, br) with CH_3-19 (δ 1.22, s) and CH_3-20 (δ 1.12, s) is consistent with β-orientation for H-7 and hence the hydroxyl group is α-oriented. These spectral data are consistent with compound **6** being 7α-hydroxy-8,11,13-abietatrien-18-oic acid (trivial name, 7α-hydroxycallitrisic acid) (Lee et al., 1994). This is the first report of this compound from the genus *Desmodium*.



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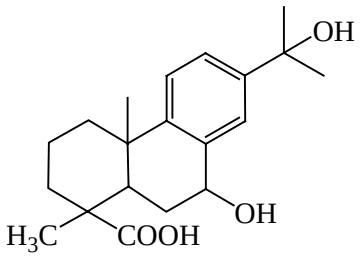
The EIMS (Appendix XLIII) of compound **7** showed a molecular ion peak at *m/z* 332 consistent with the molecular formula $C_{20}H_{28}O_4$. Like compounds **5** and **6**, the ^{1}H- (Table 9) and ^{13}C-NMR (Table 10) data were characteristic of an abietane diterpene skeleton. The presence of a secondary hydroxyl group (at C-7) and a carboxylic acid group (at C-18) as in compound **6** were evident from the ^{1}H- and ^{13}C-NMR spectra (Appendix XLV & XLIV respectively). Similar to compound **5**, downfield shifted methyl protons (δ 1.55, s, 6H) and a quaternary carbon at δ 71.8 attributed to the presence of a tertiary hydroxyl group at C-15 were observed. The HMBC correlation of H-14 (δ 7.47, d, J = 2.0 Hz) with C-7 (δ 67.6), C-9 (δ 147.8), C-12 (δ 124.5) and C-15 (δ 71.8) and that of CH_3-16 and –17 with C-13 (δ 147.1) and C-15 (δ 71.8) confirmed the placement of a secondary hydroxyl group at C-7 (δ 67.6) and the tertiary hydroxyl group at C-15 (δ 71.8). The NOE interaction of H-1β (δ 2.41, d, 12.8 Hz) with CH_3-20 (δ 1.19, s) and H-11 (δ 7.29, d) and that of CH_3-20 (δ 1.19, s) with CH_3-19 (δ 1.30, s) was consistent with assigning β-configuration to the methyl groups (C-19 and C-20). The hydroxyl at C-7 was assigned to have α-orientation (H-7 is then β-oriented) from the NOE interaction of H-7 (δ 4.77, br) with CH_3-19 (δ 1.30, s) and CH_3-20 (δ 1.19, s). Therefore, compound **7** was identified as

7α,15-dihydroxy-8,11,13-abietatrien-18-oic acid. This is the first report of this compound from the genus *Desmodium*.



7

Table 9: ^{1}H-NMR (500 MHz) spectral data of compounds **5**, **6** and **7**.

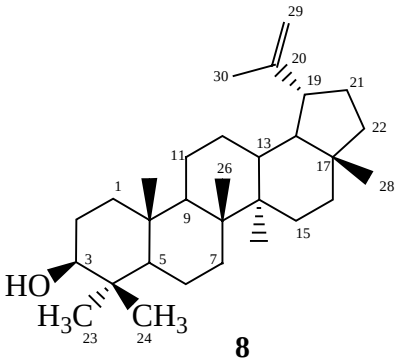
	δ_H (*J* in Hz)		
Position	**5 (MeOH-d_4)**	**6 ($CDCl_3$)**	**7 (MeOH-d_4)**
1	1.64, m	1.45, m	1.52, m
	2.49, d (12.7)	2.26, d (12.3)	2.41, d (12.8)
2	1.86, m	1.67, m	1.77, m
		1.76, m	1.89, m
3	1.86, m	1.67, m	1.71, m
		1.76, m	1.97, m
5	2.70, d (14.2)	2.45, d (11.9)	2.58, d (12.8)
6	2.43, d (17.6)	2.00, m	1.71, m
	2.85, m		2.17, m
7		4.67, br	4.77, br
11	7.48, d (8.3)	7.16, d (8.3)	7.29, d (8.4)
12	7.78, dd (1.9, 8.3)	7.10, dd (2.0, 8.3)	7.39, dd (2.0, 8.4)
14	8.11, d (1.8)	7.15, d (2.0)	7.47, d (2.0)
15		2.86,sept (6.9)	
16	1.57, s	1.23, d (6.7)	1.55, s
17	1.57, s	1.23, d (6.7)	1.55, s
19	1.39, s	1.22, s	1.30, s
20	1.34, s	1.12, s	1.19, s

Table 10: ^{13}C-NMR (125 MHz) spectral data of compounds **5**, **6** and **7**.

	δ_C		
Position	**5 (MeOH-d_4)**	**6 ($CDCl_3$)**	**7 (MeOH-d_4)**
1	37.5	37.7	38.0
2	18.2	18.6	18.7
3	36.9	36.2	36.7
4	46.5	46.9	37.5
5	44.5	39.7	39.9
6	37.6	30.7	31.3
7	199.8	68.1	67.6
8	130.3	135.6	135.8
9	154.6	146.7	147.8
10	37.9	37.3	37.5
11	123.9	124.2	123.8
12	131.3	126.5	124.5
13	148.2	146.4	147.1
14	123.1	128.3	126.6
15	71.6	33.5	71.8
16	30.7	24.1	30.8
17	30.7	24.0	30.8
18	180.4	183.0	181.6
19	15.8	16.4	16.2
20	22.8	23.8	23.7

The EIMS (Appendix XLVII) of compound **8** gave a molecular ion peak at *m/z* 426, which is consistent with the formula $C_{30}H_{50}O$. The ^{1}H- and ^{13}C-NMR spectra (Appendix L & XLVIII respectively; Table 11) together with H-C direct and long range correlation (Appendix LI & LII) and the comparison with literature values (Mahato & Kundu, 1994;

Reynolds et al., 1986) lead to identification of compound **8** as the pentacyclic triterpene, lupeol.

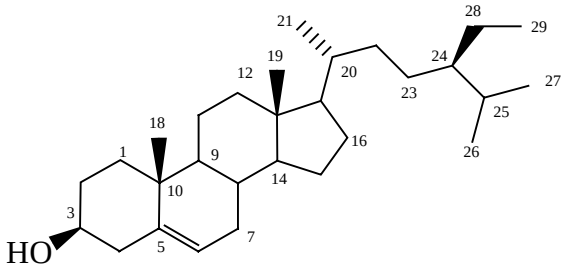


8

Table 11: ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) spectral data ($CDCl_3$) of lupeol (**8)**

Position	**δ_H (*J* in Hz)**	**δ_C**	**Position**	**δ_H (*J* in Hz)**	**δ_C**
1	0.88, m; 1.64, m	38.7	16	1.47, m; 1.35, m	35.5
2	1.64, m	27.4	17		42.9
3	3.18, m	78.9	18	1.35, m	48.2
4		38.8	19	2.35, m	47.9
5	0.67, d (9.3)	55.2	20		150.9
6	1.52, m; 1.37, m	18.3	21	1.30, m; 1.90, m	29.8
7	1.39, m	34.2	22	1.20, m; 1.39, m	39.9
8		40.8	23	0.95, s	27.9
9	1.28, m	50.4	24	0.75, s	15.3
10		37.1	25	1.01, s	16.1
11	1.40, m; 1.25, m	20.9	26	0.81, s	15.9
12	1.65, m; 1.15, m	25.1	27	0.93, s	14.5
13	1.66, m	38.0	28	0.77, s	17.9
14		42.8	29	4.55, d; 4.67, d	109.3
15	1.68, m; 1.01, m	27.4	30	1.67, s	19.3

The EIMS (Appendix LIII) of compound **9** showed a molecular ion peak at *m/z* 414 consistent with molecular formula, $C_{29}H_{50}O$. By comparison of the ^{13}C-NMR spectrum (Appendix LIV; Table 12) and characteristic peaks in the ^{1}H-NMR spectrum (Appendix LV) with literature values (McCarthy et al., 2005; Saxena & Albert, 2005), compound **9** was identified as the phytosterol, β-sitosterol.

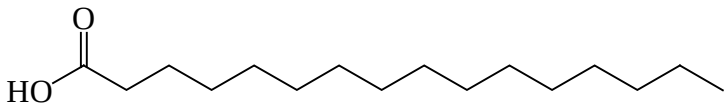


9

Table 12: ^{13}C-NMR (125 MHz, $CDCl_3$) spectral data of β-sitosterol (**9)**

Position	**δ_C**	**Position**	**δ_C**
1	37.7	16	28.7
2	32.1	17	56.4
3	72.2	18	12.3
4	40.2	19	19.4
5	141.1	20	36.5
6	121.2	21	19.2
7	32.3	22	34.3
8	32.3	23	26.4
9	50.5	24	46.2
10	36.9	25	29.5
11	21.5	26	20.2
12	42.7	27	19.8
13	42.7	28	23.4
14	57.1	29	12.4
15	24.7		

The EIMS (Appendix LVI) of compound **10** showed a molecular ion peak at *m/z* 256 consistent with molecular formula $C_{16}H_{32}O_2$. EIMS further showed characteristic peaks of $(M\text{-}C_nH_{2n+1})^+$ fragment ions at *m/z* 227, 213, 199, 185, 171, 157, 143, 129, 115 and 73. The ^{1}H-NMR spectral data (δ 2.34, t, *J* = 7.5 Hz, 2H; δ 1.62, m, 2H; 0.87, t, *J* = 7.0 Hz, 3H) was characteristic of a straight chain hydrocarbon portion of a fatty acid. The ^{13}C-NMR (Appendix LVII; Table 13) revealed the presence of sixteen carbon atoms with one being a carbonyl carbon (δ 179.9) of a carboxylyic acid. Using ^{1}H-, ^{13}C-NMR and EIMS data compound **10** was identified as the long-chain fatty acid hexadecanoic acid commonly known as palmitic acid.



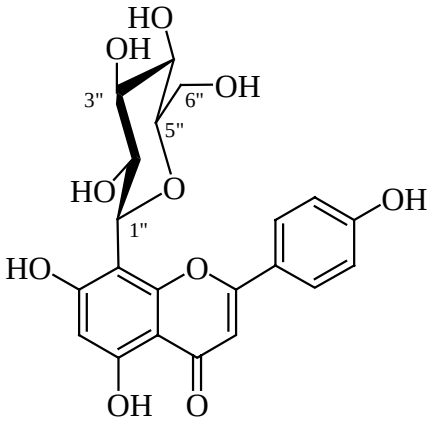
10

Table 13: ^{13}C-NMR (125 MHz, $CDCl_3$) spectral data of hexadecanoic acid (**10**)

Position	δ_C	**Position**	δ_C
1	179.91	9	29.68
2	33.99	10	29.66
3	24.65	11	29.65
4	29.06	12	29.60
5	29.25	13	29.37
6	29.37	14	31.91
7	29.44	15	22.68
8	29.69	16	14.12

Compound **11** was isolated as yellow amorphous solid. The ^{1}H- (δ 6.62, s for H-3) and ^{13}C- (δ 162.1 for C-2, δ 103.0 for C-3 and δ 182.0 for C-4) NMR spectra (Appendix LIX; Table 14) are consistent with compound **11** being a flavone derivative. Further revealed by the ^{1}H- and ^{13}C-NMR spectra were a set of seven aliphatic protons (δ 4.78, d, *J* = 9.8 Hz, 1H; 3.89, t, *J* = 9.0 Hz, 1H; 3.76, d, *J* = 11.4 Hz, 1H; 3.56, dd, *J* = 5.7, 11.7 Hz, 1H; 3.38, m, 1H; 3.31, m, 1H; 3.29, m, 1H) and six oxygenated aliphatic carbon atoms (δ 74.8, 72.2, 62.4, 71.6, 79.8, 82.6) characteristic of a glucopyranosyl substituent (Zhang and Xu, 2003) on the flavone skeleton. The presence of a *C*-linkage of the sugar unit to the aglycone was evident from the upfield shift of the anomeric proton (δ 4.78, d, *J* = 9.8 Hz). The presence of aromatic protons with AA'XX' spin system (δ 7.97,d, *J* = 8.3 Hz, 2H; 6.90, d, *J* =8.3 Hz, 2H) and an aromatic singlet (δ 6.15) in the ^{1}H-NMR; and five oxygenated unsaturated carbon signals (δ 162.1, 160.9, 160.0, 158.0, 156.3) is consistent with three hydroxyl substituents on the flavone skeleton, in addition to the glucopyranosyl unit. The hydroxyl groups were assigned to C-5, C-7 and C-4' positions from the biogenetic considerations. In the ^{1}H-NMR spectrum, the presence of a deshielded proton signal (δ 13.1, s) is attributed to a hydrogen-bonded hydroxyl group at C-5. The aromatic protons with AA'XX' spin system were due to B-ring protons with a hydroxyl group at C-4'. This was confirmed by HMBC (Appendix LXII) correlations of H-2' (δ 7.97, d) with C-4' (δ 160.9) and C-6' (δ 129.3) and that of H-3' (δ 6.90, d) with C-1' (δ 121.7), C-4' (δ 160.9) and C-5' (δ 116.7). In A-ring, the glucopyranosyl unit could either be placed at C-6 or C-8 position. Its placement at C-8 rather than C-6 was established by the HMBC correlations of the anomeric proton, H-1" (δ 4.78, d) with C-8 (δ 101.8) and C-8a (δ 156.3) and of H-6 (δ 6.15, s) with C-5 (δ 160.0) and C-8 (δ 101.8).

The glucopyranosyl unit was characterized to be of β-configured from the large 1,2-diaxial coupling constant ($J_{1",2"}$ = 9.8 Hz) (Xie et al., 2003). From the above evidence and comparison of the spectral data of compound **11** with those reported in literature (Zhang and Xu, 2003), this compound was therefore characterized as 8-*C*-β-glucopyranosyl-5,7,4'-trihydroxyflavone (trivial name vitexin). This is the first report on the occurrence of this compound in the genus *Desmodium*.



11

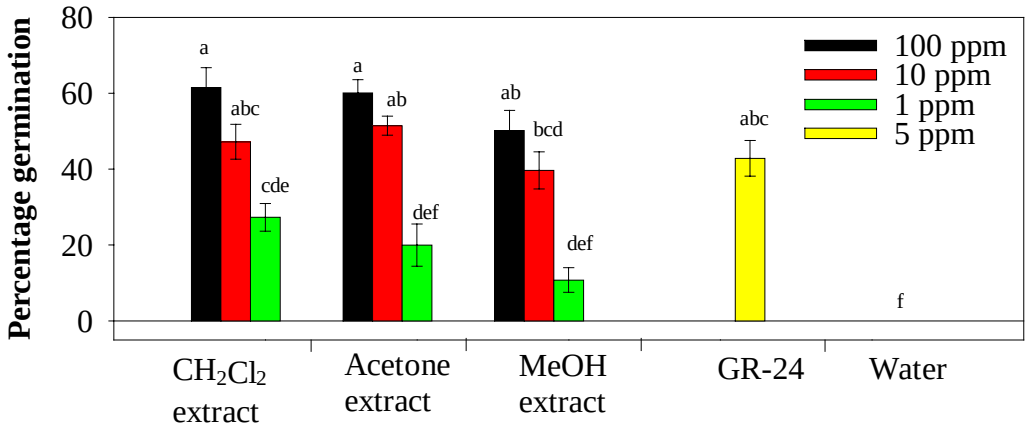
Table 14: ^{1}H- (500 MHz), ^{13}C-NMR (125 MHz) spectral data (in DMSO-d_6) and HMBC correlations of vitexin (**11**)

Position	**δ_C**	**δ_H (*J* in Hz)**	**HMBC**
2	162.1		
3	103.0	6.62, s	C-2, C-4, C-4a, C-1’
4	182.0		
4a	101.6		
5	160.0		
6	100.1	6.15, s	C-5, C-8
7	158.0		
8	101.8		
8a	156.3		
1’	121.7		
2’	129.3	7.97, d (8.3)	C-2, C-4’, C-6’
3’	116.7	6.90 d (8.3)	C-1’, C-4’, C-5’
4’	160.9		
5’	116.7	6.90 d (8.3)	C-1’, C-3’, C-4’
6’	129.3	7.97, d (8.3)	C-2, C-2’, C-4’
1”	74.8	4.78, d (9.8)	C-2”, C-8, C-8a
2”	72.2	3.89, t (9.0)	
3”	79.8	3.31, m	C-5”
4”	71.6	3.38, m	
5”	82.6	3.29, m	C-4”
6”a	62.4	3.76, d (11.4)	
b		3.56, dd (5.7, 11.7)	
5-OH		δ 13.1, s	

3.2 Studies on *Desmodium intortum*

3.2.1 Biological activities of *Desmodium intortum* on *Striga hermonthica*

Fresh roots of *D. intortum* were sequentially extracted with CH_2Cl_2, Me_2CO and MeOH at room temperature. The extracts were tested for germination stimulation and post-germination growth inhibition activities on *Striga hermonthica*. *Striga* germination stimulation effect was not statistically different for the three extracts at the same concentrations (Figure 3.4).



(Means with the same letter are not significantly different (P<0.05) by Tukey's studentized range test)

Figure 3.4: Germination response of *S. hermonthica* seeds to *D. intortum* roots extracts at varying concentrations.

Unlike the high *Striga* germination stimulation effect observed for the three extracts of *D. intortum* roots, the post-germination radicle growth inhibition was high for the Me_2CO and MeOH extracts and insignificant for CH_2Cl_2 extract (Table 15). Like in the case of

D. uncinatum roots extracts, these findings show that the highly polar components of *D. intortum* roots extracts mainly inhibit the post-germination growth.

Table 15: Radicle growth inhibition of germinated *S. hermonthica* seeds exposed to root extracts of *D. intortum* at varying concentrations.

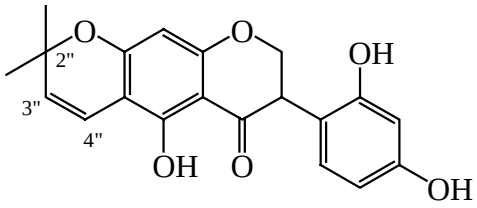
Tested solution	Concentration (ppm)	Radicle length, mm ± s. e. (n = 10)	Percentage Radicle length reduction
CH_2Cl_2 extract	1	1.02 ± 0.04 [a]	-4.1
	10	0.92 ± 0.03 [ab]	6.1
	100	0.86 ± 0.03 [bc]	12.2
Acetone extract	1	0.65 ± 0.03 [de]	33.7
	10	0.52 ± 0.01 [ef]	46.9
	100	0.44 ± 0.03 [f]	55.1
Methanol extract	1	0.75 ± 0.03 [cd]	23.5
	10	0.52 ± 0.02 [ef]	46.9
	100	0.53 ± 0.02 [ef]	45.9
GR-24	5	0.98 ± 0.04 [ab]	0.0

3.2.2 Isolation and characterization of compounds from *D. intortum* root extracts

The CH_2Cl_2 extract of *D. intortum* roots was subjected to chromatographic separation (Section 4.4.2) to give compounds **12** and **16**; similar treatment of the acetone extract (Section 4.4.2) yielded compound **13** to **15**.

The EIMS (Appendix LXIII) of compound **12** showed a molecular ion peak at *m/z* 354, which is consistent with molecular formula of $C_{20}H_{18}O_6$. The ^{1}H-NMR (Appendix LXIV) and ^{13}C-NMR (Appendix LXVI) spectra showed ^{1}H signals (δ 4.46, dd, *J* = 10.8, -11.0 Hz for H-2ax; δ 4.31, dd, *J* = -11.0, 5.5 Hz for H-2eq and δ 4.08, dd, *J* = 10.4, 5.5 Hz for H-3) and the corresponding ^{13}C signals (δ 69.8 for C-2, δ 46.0 for C-3 and δ 197.8 for C-4) (Table 16) characteristic of an isoflavanone skeleton (Tsanuo et al., 2003). The ^{1}H-NMR further revealed the presence of an aromatic singlet (δ 5.77), aromatic protons with an ABX spin system (δ 6.76, d, *J* = 8.0 Hz; 6.20, d, *J* = 2.0 Hz; 6.19, dd, *J* = 8.0, 2.0 Hz) and a 2,2-dimethylpyran ring (δ 6.46, d, *J* = 10 Hz; 5. 36, d, *J* = 11.1 Hz; 1.29, s; 1.30, s). The molecular formula $C_{20}H_{18}O_6$ and the presence of five oxygenated aromatic carbon atoms (δ 162.5, 161.5, 158.3, 156.5, 155.6) in the ^{13}C-NMR (Appendix LXVI) are consistent with three hydroxyl substituents in addition to the 2,2-dimethylpyran moiety on an isoflavanone skeleton. The aromatic protons with an ABX spin system were assigned to ring B (H-3', -5' and –6') from the HMBC (Appendix LXVII) correlations between the aromatic proton at C-6' (δ 6.76, d, *J* = 8.0 Hz) and C-3 (δ 46.0). This was confirmed by NOESY spectrum (Appendix LXVIII) which showed NOE interaction between H-6' and CH_2-2 (δ 4.46, t, *J* = 10.8 Hz) and H-3 (δ 4.08, dd, *J* = 10.4). This data further indicated that C-2' and C-4' are substituted with hydroxyl groups; and hence the third hydroxyl group (at C-5) and the 2,2-dimethylpyran substituents are located in ring A. The placement of the 2,2-dimethylpyran ring fused to the C-6 and C-7 positions was confirmed by the HMBC correlation of the olefinic proton, H-4" (δ 6.46, d, *J* = 10 Hz) with C-7 (δ 158.3) and C-5 (δ 161.5) and that of the other olefinic proton, H-3" (δ 5.36, d, *J* = 11.1 Hz) with C-6 (δ 102.4). The location of two hydroxyl substituents (at C-2' and

C-4') on ring B, one hydroxyl (at C-5) and 2,2-dimethylpyran ring on ring A is consistent with the EIMS spectrum, which showed fragment ion *m/z* 219 (base peak) attributed to RDA cleavage of ring C (Scheme 10). Thus compound **12** was characterized as 5,2',4'-trihydroxy-2",2"-dimethylpyrano[5",6":6,7] isoflavanone. This new isoflavanone is given the trivial name intortunone.



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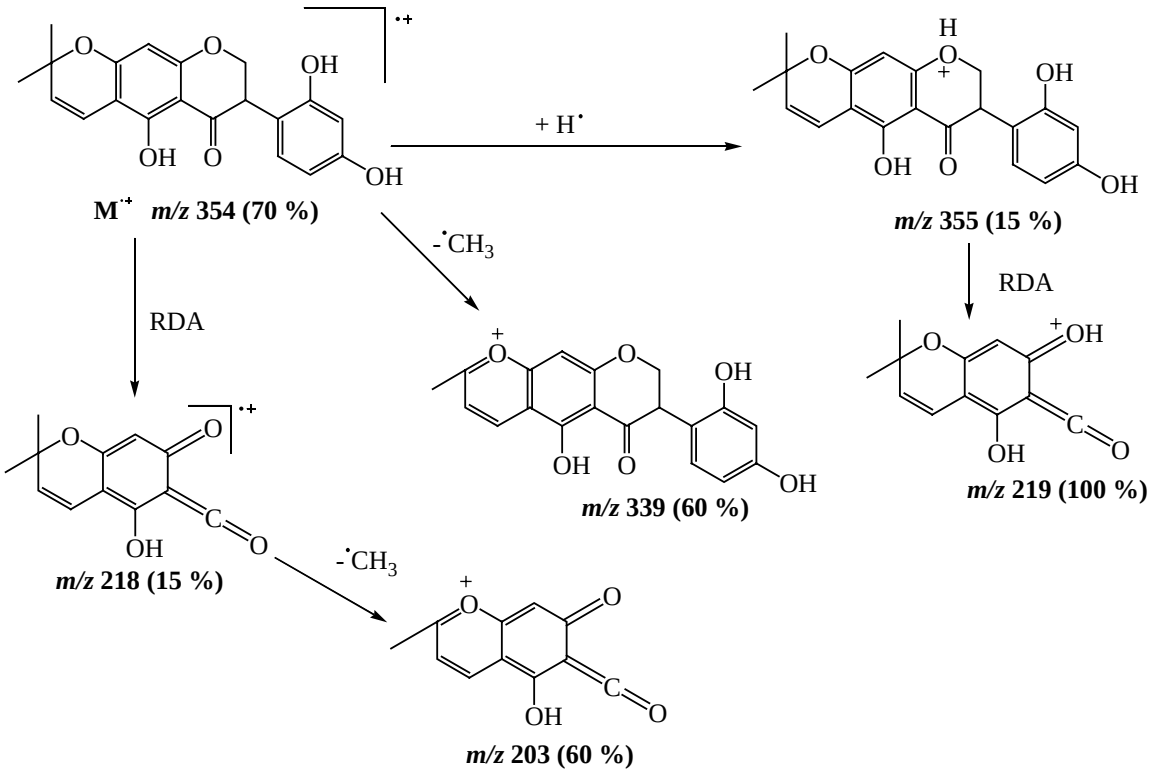
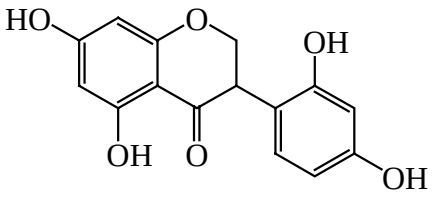


Scheme 10: Proposed EIMS fragmentation for intortunone (**12**)

Table 16: ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) spectral data (in MeOH-d_4) along with HMBC correlations of intortunone (**12**)

Position	δ_C	δ_H (***J*** **in Hz)**	**HMBC**
2	69.8	4.31 dd, (-11.0, 5.5)	C-4, 8a, 1’
		4.46 dd (-11.0, 10.8)	C-4, 8a, 2
3	46.0	4.08 dd (10.4, 5.5)	C-2, 4, 1’, 2’, 6’
4	197.8		
4a	102.4		
5	161.5		
6	102.4		
7	158.3		
8	95.4	5.77, s	C-4a, 8a
8a	162.5		
1’	112.0		
2’	155.6		
3’	102.4	6.20, d (2.0)	C-1’, 4’, 5’
4’	156.5		
5’	106.7	6.19 dd (8.6, 2.0)	C-1’, 3’, 4’
6’	130.0	6.76 d (8.0)	C-3, 2’, 4’
2”	77.8		
3”	125.7	5.36, d (11.1)	C-6, 2”, 3”, 5”
4”	114.9	6.46, d (10.0)	C-5, 7, 2”
2”-CH_3	27.7	1.30, s	C-2”, 3”
	27.7	1.29, s	C-2”, 3”

The EIMS (Appendix LXIX) of compound **13** gave molecular ion peak at *m/z* 288, which is consistent with the molecular formula $C_{15}H_{12}O_6$. The ^{1}H- (Appendix LXXI) and ^{13}C- (Appendix LXX) NMR spectrum showed ^{1}H signals (δ 4.55, dd, *J* = 10.8, -10.9 Hz for H-2ax; 4.38, dd, *J* = 5.5, -10.9 Hz for H-2eq and 4.19, dd, J = 5.5, 10.5 Hz for H-3) and corresponding ^{13}C signals (δ 70.5 for C-2, δ 47.0 for C-3 and δ 198.3 for C-4) (Table 17) consistent with this being an isoflavanone derivative (Tsanuo et al., 2003). The ^{1}H-NMR spectrum further showed the presence of three aromatic protons with an ABX spin system (δ 6.86, d, *J* = 8.3 Hz; 6.34, *J* = 2.4 Hz; 6.28, dd, *J* = 2.4, 8.3 Hz) and two *meta*-coupled aromatic protons (δ 5.89, d, *J* = 2.1 Hz and 5.87, d, *J* = 2.1 Hz). The protons with the ABX spin system were assigned to aromatic protons in ring B (oxygenation at C-2' and C-4') by the HMBC, which showed correlation of H-6 (δ 6.86, d, *J* = 8.3 Hz) with C-3 (δ 47.0), C-2' (δ 156.6) and C-4' (δ 158.2). Then the *meta*-coupled protons were located at C-6 and C-8 positions of ring A with the biogenetically expected oxygenation at C-5 and C-7. The ^{13}C-NMR spectrum showed the presence of five oxygenated aromatic carbon signals (δ 164.8, 164.8, 164.2, 158.2, 156.6) supporting a tetrahydroxyisoflavanone skeleton. The EIMS fragmentation showed fragment ions at *m/z* 153 (base peak) and *m/z* 136 (40%) attributed to RDA fragmentation (Scheme 11) is in agreement with the placement of two hydroxyl groups in ring A and two hydroxyl groups in ring B. Therefore, compound **13** was characterized as 5,7,2',4'-tetrahydroxyisoflavanone. This compound (trivial name, dalbergioidin) was previously isolated from other legumes including *Phaseolus vulgaris* (Woodward, 1979) and *Vigna angularis* (Abe et al., 1987). However this is the first report from this genus.



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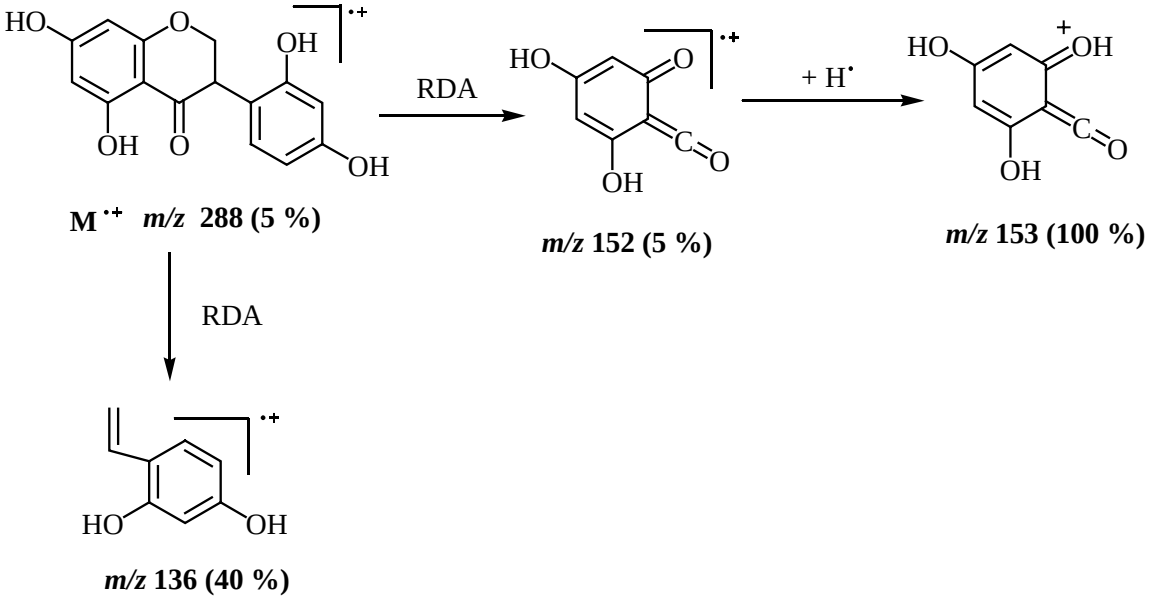


Scheme 11: Proposed EIMS fragmentation for dalbergioidin (**13**)

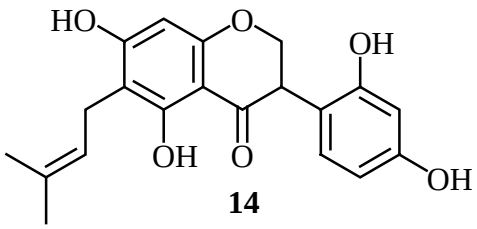
Table 17: ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) spectral data (in MeOH-d_4) along with HMBC correlations of dalbergioidin (**13**)

Position	δ_C	δ_H (***J*** **in Hz)**	**HMBC**
2	70.5	4.38, dd (-10.9, 5.5)	C-3, 4, 8a
		4. 55, dd (-10.9, 10.8)	C-3, 4, 8a
3	47.0	4.19, dd (10.5, 5.5)	C-2, 4, 1', 2', 6'
4	198.3		
4a	102.7		
5	164.2		
6	96.2*	5.89*, d (2.1)	C-7, 8, 4a
7	164.8		
8	95.1*	5.87*, d (2.1)	C-6, 7, 4a, 8a
8a	164.2		
1'	113.0		
2'	156.6		
3'	102.7	6. 34, d (2.4)	C-1', 4', 5'
4'	158.2		
5'	106.8	6. 28, dd (8.3, 2.4)	C-1', 3'
6'	130.9	6. 86, d (8.3)	C-3, 2', 4'

*Values in the same column are interchangeable

The molecular ion peak (*m/z* 356, by EIMS) of compound **14** is consistent with the molecular formula of $C_{20}H_{20}O_6$. The ^{1}H and ^{13}C-NMR spectral data (Table 18) revealed the characteristic features for an isoflavanone skeleton. In addition, the ^{1}H-NMR spectrum (Appendix LXXVI) showed the presence of a 3-methylbut-2-enyl substituent (δ 5.26, t, *J* = 7.2 Hz; 3.26, d, *J* = 7.2 Hz; 1.76, s; 1.65, s), three aromatic protons with an ABX spin system (δ 6.94, d, *J* = 8.3 Hz; 6.46, d, *J* = 2.4 Hz; 6.34, dd, *J* = 2.3, 8.3 Hz), an aromatic singlet (δ 6.05), and a down field shifted signal (δ 12.69, s) attributed to a hydrogen bonded hydroxyl proton. The ^{13}C-NMR further revealed the presence of five oxygenated aromatic carbon atoms (δ 164.1, 162.0, 161.7, 157.8, 156.6) attributed to presence of four hydroxyl groups on the isoflavanone skeleton. The HMBC (Appendix LXXIX) correlation of the aromatic doublet (δ 6.94) for H-6 with C-3 (δ 46.7), C-2' (δ 156.6) and C-4' (δ 157.8) allowed the assignment of the protons with the ABX spin system to ring B at C-3', C-5' and C-6' with oxygenations at C-2' and C-4'. This was in agreement with the NOESY spectrum (Appendix LXXX) which showed NOE interaction of H-6' (δ 6.94, d) with H-2 (δ 4.58, t) and H-3 (δ 4.23, dd). The 3-methylbut-2-enyl group was located at C-6 from the HMBC correlations of H-1" (δ 3.26, d) with C-5 (δ 162.0), C-6 (δ 108.3) and C-7 (δ 164.1). Furthermore, the HMBC correlations of the aromatic singlet (δ 6.05) with C-4a (δ 103.0), C-7 (δ 108.3) and C-8a (δ 161.7) and that of the hydroxyl proton (δ 12.69) with C-4a (δ 103.0), C-5 (δ 162.0) and C-6 (δ 108.3) allowed the location of the aromatic singlet and the hydroxyl group to C-8 and C-5 respectively. In agreement with the above data, the EIMS showed a fragment ion *m/z* 221 (50%) resulting from RDA cleavage of ring C and *m/z* 165 (base peak) as a result of RDA cleavage of ring C with a loss of a C_4H_7 radical from the 3-methylbut-2-enyl

substituent (Scheme 12). Thus compound **14** was identified as 5,7,2',4'-tetrahydroxy-6-(3-methylbut-2-enyl)isoflavanone. This compound (trivial name uncinanone A) was earlier reported from *Desmodium uncinatum* (Tsanuo et al., 2003). However this is the first report of this compound from *D. intortum*.



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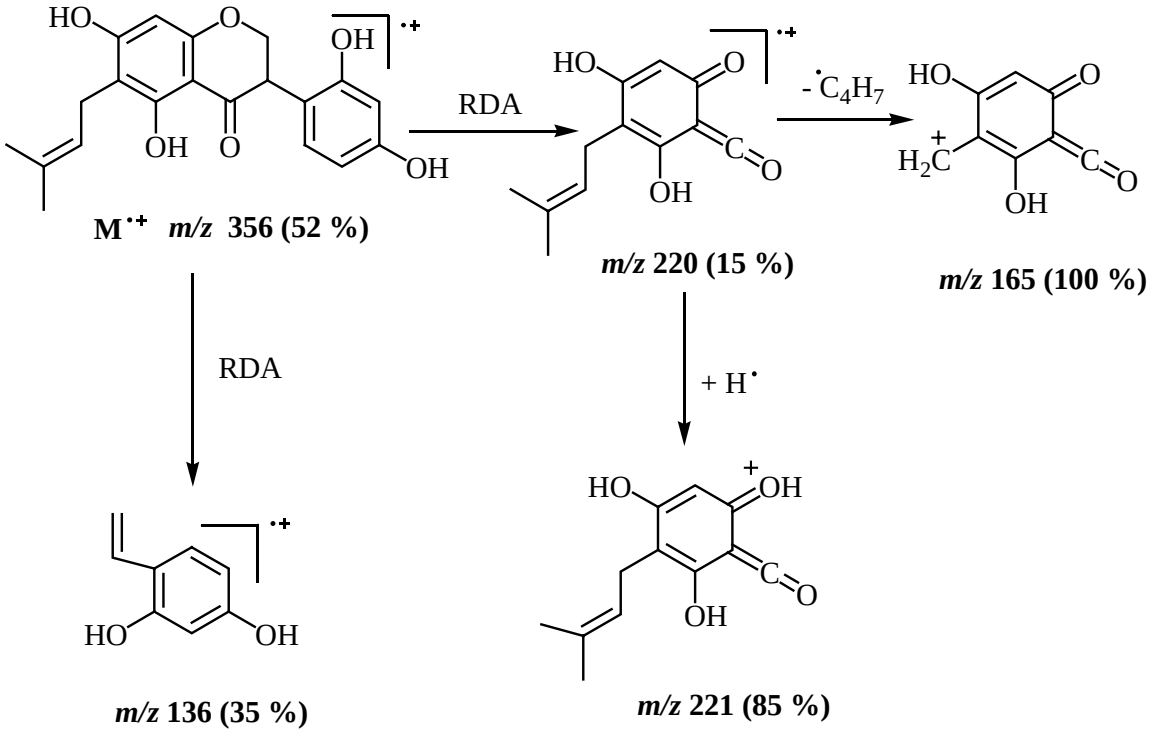


M$^{\bullet+}$ ***m/z*** **356 (52 %)**

m/z **220 (15 %)**

m/z **165 (100 %)**

m/z **136 (35 %)**

m/z **221 (85 %)**

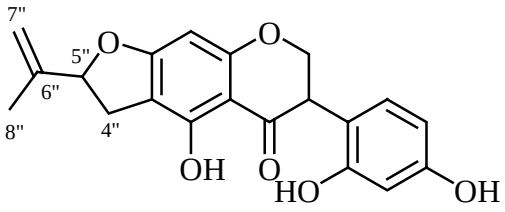
Scheme 12: Proposed EIMS fragmentation for uncinanone A (**14**)

Table 18: ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) spectral data (in acetone-d_6) along with HMBC correlations of uncinanone A (**14**)

Position	δ_C	δ_H (*J* **in Hz)**	**HMBC**
2	70.7	4.44 dd, (-10.9, 5.4)	C-3
		4.58 dd (-10.9, 10.5)	C-4, 8a
3	46.7	4.23, dd (10.1, 5.2)	C-2, 1', 6'
4	198.0		
4a	103.0		
5	162.0		
6	108.3		
7	164.1		
8	94.5	6.05, s	C-6, 4a, 8a
8a	161.7		
1'	113.3		
2'	156.6		
3'	103.0		
4'	157.8	6.46, d (2.4)	C-1', 4', 5'
5'	107.2	6. 34, dd (8.3, 2.3)	C-1', 3'
6'	130.6	6.94, d (8.3)	C-3, 2', 4'
1"	21.1	3.26, d (7.2)	C-5, 6, 7, 2", 3"
2"	123.1	5.26, t (7.2)	C-4", 5"
3"	130.6		
4"	25.3	1.65, s	C-2", 3", 5"
5"	17.2	1.76, s	C-2", 3", 4"
5-OH		12.69, s	C-5, 6, 4a

Compound **15** ($C_{20}H_{18}O_{6,}$ M^+, *m/z* 354) also exhibited characteristic signals for an isoflavanone skeleton in the ^{1}H- and ^{13}C-NMR spectral data (Table 19). The ^{1}H-NMR spectrum (Appendix LXXXII) further revealed the presence of an aromatic singlet (δ 5.97), aromatic protons with an ABX spin system (δ 6.95 and 6.94, each d, *J* = 8.2 Hz; δ 6.47, d, *J* = 1.2 Hz; δ 6.35 and δ 6.34, each dd, *J* = 1.2, 8.2 Hz) and an isopropenylfurano substituent (δ 5.39, t, *J* = 8.3 Hz; δ 5.10 & 4.95, each singlet; δ 3.30 & 3.29, each dd, *J* = 9.7, 15.2 Hz; δ 2.88 & 2.86 each d, *J* = 15.2 Hz). The ^{13}C-NMR spectrum showed five oxygenated aromatic carbons (δ 170.2, 164.4, 159.5, 156.6, 156.6) indicating the presence of three hydroxyl substituents on the isoflavanone skeleton in addition to the isopropenylfurano substituent. The protons with an ABX spin system were assigned to ring B (H-3', 5', and 6') from the HMBC correlations of H-6' (δ 6.95 and δ 6.94, each d, *J* = 8.2 Hz) with C-3 (45.0). This indicated that two hydroxyl groups are at C-2' and C-4' with the third hydroxyl and the isopropenylfurano substituent in ring A. The placement of isopropenylfurano substituent fused to C-6 and C-7 was confirmed by HMBC correlations of methylene protons at C-4" with C-5 (δ 159.5), C-6 (δ 106.5), C-7 (δ 170.2), C-5" (δ 88.8) and C-6" (δ 143.2). The location of the aromatic singlet (δ 5.97) to C-8 position was confirmed by HMBC correlations of H-8 with C-6 (δ 106.5), C-7 (δ 170.2), C-4a (δ 101.6) and C-8a (δ 164.4). Therefore, the third hydroxyl group was located at C-5, which is in agreement with biogenetic oxygenation pattern and with the presence of a deshielded proton signal (δ 12.59, s). In the EIMS the fragment ion *m/z* 218 (base peak) attributed to RDA cleavage of ring C (Scheme 13) is consistent with one hydroxyl group and the isopropenylfurano substituents on the A ring. From the above data and comparison with literature (Tsanuo et al., 2003), compound **15** was identified as

5',2',4'-trihydroxy-5"-isopropenylfurano-(2",3":7,6)isoflavanone (trivial name uncinanone B), which was previously isolated from *Desmodium uncinatum* (Tsanuo et al., 2003). Isoflavanones with free OH group at C-4' racemize at C-3 during the extraction and isolation process. The presence of a second stereogenic center in compound **15** allows it to occur as a diastereomeric mixture, which is manifested here with signal duplication in the ^{1}H-NMR spectrum.



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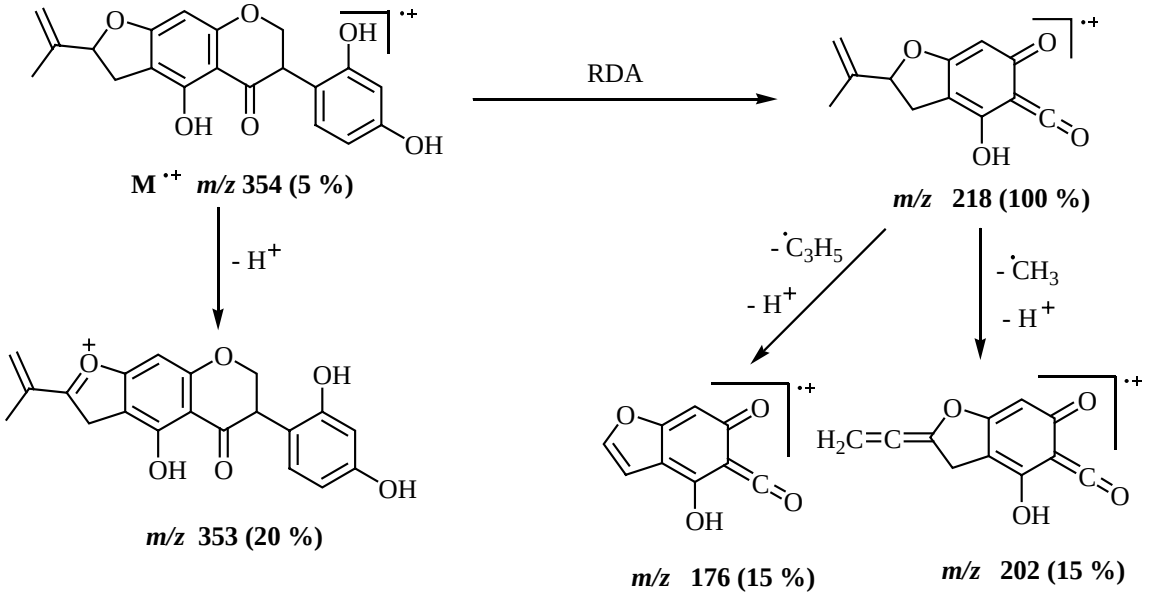

Scheme 13: Proposed EIMS fragment ions for uncinanone B (**15**)

Table 19: ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) spectral data (in acetone-d_6) along with HMBC correlations of uncinanone B (**15**)

Position	δ_C	δ_H (***J*** **in Hz)**	**HMBC**
2	69.7	4.63; 4.62, dd (-10.8, 10.7)	1', 8a, 4
		4.48; 4.47, dd (-10.8, 5.4)	1', 8a, 4
3	45.0	4.25; 4.24, dd (-10.8, 5.4)	6'
4	196.7		
4a	101.6		
5	159.5		
6	106.5		
7	170.2		
8	91.0	5.97, s	6, 7, 4a, 8a
8a	164.4		
1'	115.5		
2'	156.9		
3'	105.7	6.47, d (1.2)	1', 2', 4', 5'
4'	156.9		
5'	108.8	6.35; 6.34, dd (1.2, 8.2)	1', 2', 3', 4'
6'	128.9	6.95; 6.94, d (8.2)	3, 2', 4'
4"	30.3	3.30; 3.29, dd (9.7, 15.2)	5, 6, 7, 5", 6"
		2.88; 2.86, d (15.2)	5, 6, 7, 6"
5"	88.8	5.39, t (8.3)	7"
6"	143.2		
7"	113.5	5.10; 4.95, s	5", 6", 8"
8"	17.1	1.78, s	5", 6", 7"
5-OH		12.59, br s	5, 4a, 6

Compound **16** gave a molecular ion peak at *m/z* 314 (from EIMS), which is consistent with the molecular formula $C_{20}H_{26}O_3$. The ^{13}C-NMR spectrum (Table 20) showed the presence of twenty-carbon atoms characteristic of a diterpene skeleton. The ^{1}H-NMR spectrum (Table 20) showed the presence of four methyl groups (δ 1.37, s; 1.32, s and 1.25, d, *J* = 6.9 Hz for 6H), a methine proton (δ 2.96, sept, *J* = 6.9 Hz) and aromatic protons with an AXY spin system (δ 7.82, d, *J* = 2.0 Hz; 7.52, dd, *J* = 2.0, 8.2 Hz; 7.44, d, *J* = 8.2 Hz) suggesting a dehydroabietane diterpene skeleton. Further revealed by the ^{1}H-NMR spectrum is an aliphatic broad doublet (δ 2.47, *J* = 13.6 Hz) characteristic of H-1β of a dehydroabietane diterpenes (Kuo et al., 2004). The ^{13}C-NMR spectrum showed the presence of two carbonyl carbons, δ 179.9 and δ 198.4, for a carboxylic acid and a keto group respectively. The location of a keto group at C-7 was confirmed by the HMBC correlation of H-14 (δ 7.82) with C-7 (δ 198.4), C-9 (δ 153.0) and C-12 (δ 133.6). The HMBC correlations of CH_3-19 (δ 1.37) with C-5 (δ 43.8), C-3 (δ 36.8) and C-18 (δ 179.9) allowed the placement of the carboxylic acid group at C-18. The NOE interaction of H-1β (δ 2.47, *J* = 13.6 Hz) with CH_3-19 (δ 1.37) and CH_3-20 (δ 1.32) was consistent with the assignment of β-configuration to the two methyl groups and hence α-configuration to the carboxylic acid. Based on the above evidence and comparison of the spectral data of compound **16** with those reported in literature (Ayer and Macaulay, 1987), this compound was characterized as 7-oxodehydroabietic acid. This compound was reported earlier from the honey mushroom, *Armillaria mellea* (Ayer and Macaulay, 1987). However, this is the first report of this compound from the genus *Desmodium*.

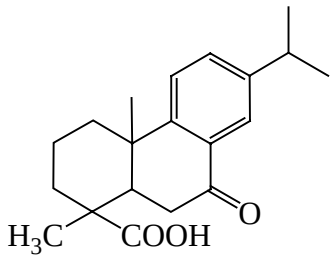

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Table 20: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectral data (in acetone-d_6) of 7-oxo-dehydroabietic acid (**16**)

Position	δ_C	δ_H (***J*** **in Hz)**
1	37.3	1.87, m
		2.47, d, J = 13.6 Hz
2	18.3	1.62, m; 1.86, m
3	36.8	1.62, m; 2.46, m
4	46.4	
5	43.8	2.68, dd (2.9, 14.2)
6	38.0	2.81, t (14.3)
		2.37, dd (2.8, 14.3)
7	198.4	
8	130.9	
9	153.0	
10	37.9	
11	123.9	7.44, d (8.2)
12	133.6	7.52, dd (2.0, 8.2)
13	147.0	
14	125.4	7.82, d (2.0)
15	34.0	2.96, sept, (6.9)
16	24.2	1.25, d (6.9)
17	24.2	1.25, d (6.9)
18	179.9	
19	16.4	1.37, s
20	24.1	1.32, s

3.3 Biological activity of the isolated compounds from *Desmodium uncinatum* and *D. intortum* root extracts

The CH_2Cl_2 extract of *D. uncinatum* roots exhibited significant activity on germination stimulation of *Striga hermonthica* seeds (Figure 3.1) but none of the nine compounds isolated from the extract showed any significant response. From the acetone extract of *D. uncinatum* roots, two compounds (7α,15-dihydroxy-8,11,13-abietatrien-18-oic acid, **7** and vitexin, **11**) were isolated and found inactive as *Striga* germination stimulants whereas mild radicle growth inhibition was observed at 100 ppm of vitexin (**11**) (Table 21).

Table 21: Radicle growth reduction of germinated *S. hermonthica* seeds exposed to Vitexin at varying concentrations.

Tested sample	Concentration (ppm)	Radicle length (mm)	Percentage radicle length reduction
Vitexin	1	1.04 [a] ± 0.06	-6.12
	10	0.95 [a] ± 0.06	2.72
	100	0.61 [b] ± 0.03	37.42
GR-24	5	0.98 [a] ± 0.06	0.00

(Means with the same letter are not significantly different (P <0.05) by Tukey's studentized range test.)

From the roots of *D. intortum*, mild germination response (19.4 ± 1.6 %, 21.6 ± 1.4 % and 25.2 ± 1.9 %) were observed for compounds **12**, **13** and **15** respectively at 10 ppm with reference compound GR-24 showing germination stimulation activity of 52.2 ± 1.53 % in the same bioassay.

The lack of significant *Striga* germination stimulation and post-germination inhibition activities for the isolated compounds as compared to the crude *Desmodium* roots extracts was attributed to synergistic effect of the complex composition of root components.

3.4 Chemotropic response of germinated *Striga hermonthica* seeds

The test was carried out in agar plate consisting of three wells of equal dimension that were 5 mm apart (Section 4.7). *S. hermonthica* seeds were found to germinate in response to the germination stimulant (GR-24) diffusing through the agar (Table 22) and did not germinate in the agar when no stimulant was placed in the test well. To determine any directed growth pattern towards the host source, the orientation of the radicle from the germinated *Striga* seeds was noted. The radicles growing directly towards the source of stimulant and those forming a curvature towards the source were counted as positive directed growth where as those growing away from the source or in parallel directions without any curvature towards the source were considered to be negative. The results (Table 22) showed that *S. hermonthica* seeds tend to grow towards the germination stimulant source thus showed positive chemotropic effect of *Striga* germination stimulant. This is the first report on chemotropic effect using GR-24.

Table 22: Germination of *S. hermonthica* and chemotropic effect on germination stimulants.

Region	% Germination ± s.e n = 8	% Towards the stimulant (S.E)
Well 1 (5 mm from source)	28.5 [a] ± 2.9	64.8 [b] ± 7.1
Well 2 (10 mm from source)	22.8 [a] ± 3.3	84.9 [a] ± 4.8

(In each column means with the same letter are not significantly different ($P < 0.05$) by Tukey's studentized range test.)

CHAPTER 4

CONCLUSION AND RECOMMENDATION

4.1 Conclusion

- Eleven compounds were isolated from the roots of *D. uncinatum* and characterized as four isoflavonoids (two pterocarpans and two isoflavanones), a *C*-glycosylated flavone, three abietane diterpenes, a phytosterol, a pentacyclic triterpene and a long chain fatty acid.
- Of the four isoflavonoids, three were new compounds named as uncinacarpan (a pterocarpan), uncinanone D and uncinanone E (isoflavanones).
- *C*-alkylation is a common feature in the isolated isoflavonoids.
- The dichloromethane extract of *D. uncinatum* roots was found to be active in *Striga* germination stimulation activity whereas the acetone and methanol extracts were highly active in *Striga* post-germination radicle growth inhibition. Therefore, a set of polar components from the roots of *D. uncinatum* is attributed to post-germination radicle growth inhibition of *Striga* whereas a set of less polar components is attributed to the *Striga* germination stimulation effect.
- The roots extracts of *D. uncinatum* and their fractions are significantly active on *Striga* as compared to the isolated compounds. These effects are attributed to synergistic effect of the different compounds in the root extracts or due to an active compound, which may not have been isolated or may have degenerated during the chromatographic process.

- Four isoflavanones including a new compound (intortunone) and an abietane diterpene were isolated and characterized from the roots extracts of *D. intortum*.
- The root extracts (dichloromethane, acetone and methanol) of *D. intortum* exhibited high *Striga* germination stimulation activity and only the polar extracts (acetone and methanol) exhibited *Striga* post-germination growth inhibition activity. Therefore, like in *D. uncinatum*, the *Striga* post-germination growth inhibition is mediated by a set of polar components in this species.
- Isoflavanones (dalbergioidin, intortunone and uncinanone B) showed low *Striga* germination stimulation activities thus are some of the components responsible for the germination stimulation properties exhibited by the *D. intortum* root extracts.
- Synergistic effect of the compounds from *D. intortum* roots is attributed to the effects of the root extracts on *Striga*.
- Isoflavonoids, especially isoflavanones are among the major components present in the roots of both *D. uncinatum* and *D. intortum*.
- This is the first report of abietane diterpenes from the genus *Desmodium*.
- The cause of *Striga* suppression by the two *Desmodium* species is attributed to the overall effect of different sets of secondary metabolites exuded from their roots.

4.2 Recommendations

(1) Test of different combinations of characterized compounds for *Striga* seed germination and/ or radicle growth inhibition is recommended in order to demonstrate the synergistic effects beyond doubt.

(2) Examine the mechanism of inhibition of the *Striga* radicle growth by the roots extracts and compounds of *Desmodium* by studying their biological interaction. This would help in development of compounds for use to control *Striga* weed problems.

(3) Biochemical studies on the *Desmodium* species to identify the key precursors of *Striga* radical inhibitors, the associated enzymes and the genes involved in transforming these precursors to target inhibitors are recommended. This would set a stage for use of genetic engineering techniques to develop a food legume with capability for weed control.

CHAPTER 5

EXPERIMENTAL

5.1 General

The UV spectra were generated from a 168-diode array detector module installed in a Beckman System gold 126 HPLC using a reverse phase octadecyl bonded silica (C-18) as the stationary phase and water/acetonitrile mixture as the mobile phase. Column chromatography was performed on silica gel 60 Merck (70–230 mesh). Mass spectra were obtained using a VG Autospec spectrometer at 70 eV. The ^{1}H- and ^{13}C-NMR spectra were acquired using Bruker Avance 500 spectrometer at 500 MHz and 125 MHz respectively. Melting points were determined using a Gallenkamp apparatus and were uncorrected.

5.2 Plant material

The seeds of *D. uncinatum* (Jacq.) DC were purchased from Western Seed Company, Kitale, Kenya, and planted in June, 2004 at Thomas Odhiambo Mbita Campus (TOMC) of the International Centre of Insect Physiology and Ecology (ICIPE), Mbita, Kenya. The seeds of *D. intortum* were purchased from Simlaw Seed Company in Nairobi, Kenya and planted in June, 2004 at TOMC. Both plants were uprooted after four months, in October, 2004. The seeds of *Striga hermonthica* were collected from *Striga* plants parasitizing maize in January, 2004 at TOMC.

5.3 Extraction

Wet roots of *D. uncinatum* (7.8 kg) were ground and successively extracted with dichloromethane, acetone and methanol. The solvents were then removed *in vacuo* at 40^0C to give 12.3 g, 56.4 g and 71.2 g of dichloromethane, acetone and methanol extracts respectively.

Similarly, wet ground roots of *D. intortum* (7.3 kg) were successively extracted to give 16.9 g, 38.9 g and 67.6 g of dichloromethane, acetone and methanol extracts respectively.

5.4 Fractionation and isolation

5.4.1 Fractions and pure compounds from *D. uncinatum* root extracts

The dichloromethane extract (9 g) of *D. uncinatum* roots was subjected to vacuum liquid chromatography on silica gel eluting with *n*-hexane containing increasing amounts of acetone. Four major fractions were collected each ca.500 ml (labelled as A–D): A (eluted with *n*-hexane, 1.5 g), B (eluted with 5% acetone in hexane, 3 g), C (20% acetone in hexane, 490 mg) and D (eluted with 50% acetone, 4.5 g). Fraction D was loaded onto a silica gel column and eluted with hexane containing increasing amounts (5%, 20%, 50%, 75%, 100%) of EtOAc to give five sub-fractions each ca. 200 ml. The sub-fractions that was eluted with 20–75% EtOAc in hexane were combined and part of this was further purified on a semi-prep HPLC (Ultrasphere C-18 column, 10 mm x 250 mm, 5 μm, gradient elution with MeCN and H_2O mixtures: 50% MeCN in H_2O (4 min), gradual increase to 90% MeCN (19 min), gradual decrease to 50% MeCN (4 min) and maintained at this level (3 min) before the next cycle of injection. The flow rate was 3 ml/min and

elution of the compounds was monitored at 215 nm). This yielded four isoflavonoids, **1** (4.7 mg, Rt = 7.7 min), **2** (0.7 mg, Rt = 10.9 min), **3** (1.9 mg, Rt = 16.2 min) and **4** (0.9 mg, Rt = 12.3 min) and two abietane diterpenes, **5** (17.8 mg, Rt = 4.8 min) and **6** (10.5 mg, Rt = 14.5 min). Fractions B and C were combined and loaded onto a silica gel column and eluted with hexane with increasing amounts (up to 50%) of EtOAc to give the major compounds **8** (54.9 mg), **9** (30.3 mg) and **10** (20.8 mg).

The acetone extract (56.4 g) was partitioned between water and EtOAc to give 24.7 g of EtOAc fraction. The EtOAc fraction (20 g) was subjected to vacuum liquid chromatography on reverse phase octadecyl silane (C-18) column eluting with a mixture of water and MeOH at a decreasing polarity from 50% MeOH in water to 100% MeOH. The components remaining in the column were washed out with acetone. This afforded five major fractions each ca.500 ml (labelled as ACF1-ACF5): ACF1 (eluted with 50 % MeOH in H_2O, 1.1 g), ACF2 (eluted with 75% MeOH in H_2O, 1.7 g), ACF3 (eluted with 90% MeOH in H_2O, 4.6 g), ACF4 (eluted with MeOH, 8.2 g) and ACF5 (eluted with acetone, 2.4 g). Fraction ACF2 was further purified on an analytical HPLC (Nucleosil C-18 column, 4.6 mm x 250 mm, 5 μm, gradient elution with MeCN and H_2O mixtures: 50% MeCN in H_2O (1 min), gradual increase to 75% MeCN (10 min), maintained at this level (1 min), gradual decrease to 50% MeCN (1 min) and maintained at this level (1 min) before the next cycle of injection. The flow rate was 1 ml/min and elution of the compounds was monitored at 215 nm). This led to isolation of another abietane diterpene, compound **7** (16.7 mg, Rt = 5.3 min) and more of the earlier isolated compound **5** (25.9 mg, Rt = 5.8 min). Fraction ACF1 was further separated on an analytical HPLC

(Kromasil C-4 column, 4.6 mm x 250 mm, 5 μm, gradient elution with MeCN and H_2O mixtures: 20% MeCN in H_2O (2 min), gradual increase to 40% MeCN (13 min), gradual decrease to 20% MeCN (3 min) and maintained at this level (2 min) before the next cycle of injection. The flow rate was 1 ml/min and elution of the compounds was monitored at 215 nm). This led to isolation of the glycosylated flavone, compound **11** (1 mg, Rt =7.2 min).

5.4.2 Fractions and pure compounds from *D. intortum* root extracts

The dichloromethane extract (15 g) of *D. intortum* roots was subjected to vacuum liquid chromatography on silica gel eluting with *n*-hexane containing increasing amounts of EtOAc. Four major fractions were collected, each ca.500 ml (labelled as 1–4): 1 (eluted with 5% EtOAc in hexane, 0.75 g), 2 (eluted with 20% EtOAc in hexane, 5.7 g), 3 (50% EtOAc in hexane, 2.5 g) and 4 (eluted with 100% EtOAc, 1.7 g). Fraction 3 was loaded onto a silica gel column and eluted with hexane containing increasing amounts (5%, 20%, 50%, 75%, 100%) of EtOAc to give five sub-fractions each ca. 200 ml. The sub-fractions that was eluted with 20–75% EtOAc-hexane were combined and part of this was further purified on an analytical HPLC [Ultrasphere C-18 column, 4.6 mm x 250 mm, 5μm, gradient elution with MeCN and H_2O mixtures: 50% MeCN in H_2O (4 min), gradual increase to 90% MeCN (19 min), gradual decrease to 50% MeCN (4 min) and maintained at this level (3 min) before the next cycle of injection. The flow rate was 1 ml/min and elution of the compounds was monitored at 215 nm]. This yielded two compounds, the isoflavanone **12** (4.5 mg, Rt = 12.9 min) and the abietane diterpene **16** (21.9 mg, Rt = 15.0 min).

The acetone extract (38.9 g) was partitioned between water and EtOAc to give 11.2 g of EtOAc fraction. The EtOAc fraction (9 g) was subjected to vacuum liquid chromatography on reverse phase octadecyl silica (C-18) column eluting with a mixture of water and MeOH with decreasing polarity from 50% MeOH in water to 100% MeOH. The column was finally washed out with acetone. This gave five major fractions each ca.500 ml (labelled as INTAC1-INTAC5): INTAC1 (eluted with 50 % MeOH in H_2O, 142 mg), INTAC2 (eluted with 75% MeOH in H_2O, 770 mg), INTAC3 (eluted with 90% MeOH in H_2O, 980 mg), INTAC4 (eluted with MeOH, 1.4 g) and INTAC5 (eluted with acetone, 945 mg). Fraction INTAC1 was further purified on a HPLC (Nucleosil C-18 column, 4.6 mm x 250 mm, 5 μm, isocratic solvent system of 45% MeCN in H_2O for 15 min. The flow rate was 1 ml/min and elution of the compounds was monitored at 215 nm). This led to isolation of compound **13** (11.8 mg, Rt = 4.75 min). Fraction INTAC2 was further separated on a HPLC (Nucleosil C-18 column, 4.6 mm x 250 mm, 5 μm, isocratic solvent system of 65% MeCN in H_2O for 15 min. The flow rate was 1 ml/min and elution of the compounds was monitored at 215 nm). This led to isolation of more of compound **12** (6.0 mg, Rt = 6.87 min) together with compounds **14** (5.2 mg, Rt = 5.07 min) and **15** (5.5 mg, Rt = 6.28 min).

5.5 Trapping of root exudates released by *Desmodium* plants

Desmodium root exudates were trapped as described by Tsanuo et al. (2003). *D. uncinatum* seeds were first surface sterilized by soaking in 1% NaOCl solution for 10 minutes, washing thoroughly with tap water and then rinsing severally with distilled

water. Perspex tray were covered with a thin sheet of cotton wool and made wet with distilled water. Filter papers, Nigel 27-cm diameter were placed on the wet cotton and the surface sterilized seeds were placed on the wet filter paper in the tray. The trays were then wrapped in black polythene sheet and kept at room temperature for the seeds to germinate. The germinated *D. uncinatum* seedlings were transferred on a perforated aluminium sheet placed on a Perspex tray containing distilled water. The roots of the seedlings emerged from the bottom side of the tray into the water. Water from the tray was continuously pumped at a low flow rate of about 4ml/min for 12-16 hrs through cartridges containing octadecyl-bonded silica (C-18) adsorbent to trap the root exudates. After every three days of trapping exudates on the C-18 adsorbent, the seedlings were transferred to a nutrient solution for three days to relieve the seedlings from mineral deficiency. From the nutrient solution the seedlings were then placed in Perspex trays containing fresh distilled water and the trapping continued.

5.6 Bioassay

5.6.1 Surface sterilization and pre-conditioning of *S. hermonthica* seeds

Striga hermonthica seeds were surface sterilized and pre-conditioned in accordance with Thuring et al. (1997) and Fischer et al. (1989). *Striga hermonthica* seeds were soaked in a 1% NaOCl solution for 6-8 min with continuous shaking. Square disks of 1 cm length were cut from glass micro-fibre filter paper. The *Striga* seeds were then rinsed with distilled water five times. Some 30-40 rinsed seeds were placed on to each glass micro-fibre filter paper disk. Ten disks were placed in a clean 9 cm petri dish and wetted with 50 μl distilled water. Each petri-dish was covered and wrapped with Parafilm to prevent

them from drying up. All the petri-dishes were then sealed in a polythene bag and incubated at 25^0C for 2 weeks.

5.6.2 Germination assay

The *Striga* seed germination tests were performed as described by Thuring et al. (1997). After incubation, the glass micro-fibre disks with the conditioned seeds were removed from petri-dishes and placed on Whatman N^{o} 1 filter paper to remove excess moisture. The disks were then returned into the petri dishes and 50 µl of the test solution was applied on to each disk. The petri-dishes were then sealed, placed in polythene bag and incubated at 35^0C or 24 hrs. The percentage germination response [(number of germinated *Striga* seeds per disk /total number of *Striga* seeds on the disk) x 100] was computed.

5.6.3 Post germination assay

Crude extracts and pure compounds from *Desmodium* were tested for inhibition of radicle growth in accordance with Tsanuo et al. (2003). The conditioned seeds were exposed to the test solution and GR-24 and incubated for 24 hrs at 35^0C and then for another 24 hrs at 25^0C. Radicle length was measured after 48 hrs using a graticule mounted on a dissecting microscope. Percentage inhibition [(1-lt/lc) x 100, where; lt = radicle length of a germinated seed exposed to treatment, and lc = radicle length of the control germinated seed] was computed.

5.7 Studies on chemotropic effects of germination stimulants on germinated *Striga* seeds

Aqueous 1.5% of bacto-agar solution was prepared and autoclaved for 20 min. After cooling for 20 min, the agar solution was poured directly into the petri-dishes to give a height level of 9 mm. The agar solution was then allowed to cool until it starts to gel. Three depressions of equal dimensions were made in the agar, 5 mm apart from each other (Figure 3.5). A germination stimulant (50 μl) was placed in one of the outer chambers and the pre-conditioned *Striga* seeds were placed into the other two chambers, which were 5 mm apart parallel to the stimulants chamber. The direction in which the radicle grew was observed after 72 hrs.

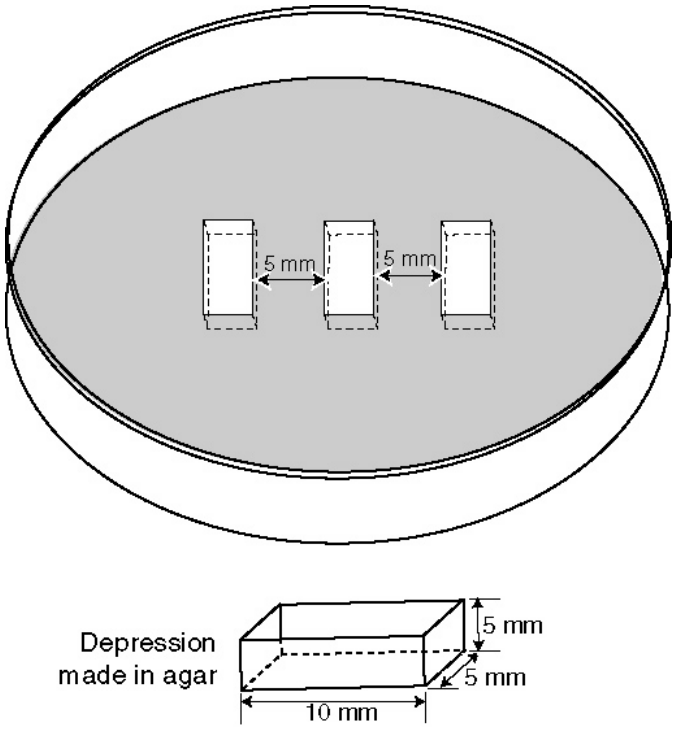

Figure 3.5: Diagram of the petridish setup for the assay of chemotropism.

5.8 Data analysis

Differences in means were analyzed using one-way analysis of variance (ANOVA) and means compared using Tukey's Studentised Range Test (SAS program version 8.2).

5.9 Physical and spectroscopic data of the isolated compounds

Uncinacarpan (**1**)

White paste. $[\alpha]_D$ = -250° (*c* 0.001, MeOH). UV λ_{max} (MeOH)nm: 283, 234 . ^{1}H-NMR (Table 5). ^{13}C-NMR (Table 5). EIMS *m/z* (rel. int.): 300 $[M]^{+\cdot}$ (100), 299 (40), 285 (10), 191 (8), 178 (10), 167 (10), 150 (8), 134 (6). HREIMS *m/z* 300.0993 $[M]^{+\cdot}$ (Calc. for $C_{17}H_{16}O_5$: 300.0998)

Edudiol (**2**)

Amorphous powder. UV λ_{max} (MeOH)nm: 286, 234. ^{1}H-NMR (Table 6). ^{13}C-NMR (Table 6). EIMS *m/z* (rel. int.): 354 $[M]^{+\cdot}$ (100), 299 (46), 283 (22), 147 (8), 123 (8). HREIMS *m/z* 354.1467 $[M]^+$ (Calc. for $C_{21}H_{22}O_5$: 354.1467).

Uncinanone D (**3**)

Amorphous powder. UV λ_{max} (MeOH)nm: 295, 235. ^{1}H-NMR (Table 7). ^{13}C-NMR (Table 7). EIMS *m/z* (rel. int.): 414 $[M]^{+\cdot}$ (30), 359 $[M\text{-}C_4H_7]^+$ (30), 194 $[C_{11}H_{14}O_3]^+$ (100), 181 (85), 179 (30), 165 (10), 151 (10). HREIMS *m/z* 414.1678 $[M^+]$ (Calc. for $C_{23}H_{26}O_7$: 414.1679).

Uncinanone E (**4**)

Amorphous powder. UV λ_{max} (MeOH)nm: 291, 236. ^{1}H-NMR (Table 8). ^{13}C-NMR (Table 8). EIMS *m/z* (rel. int.): 330 [M^{+}] (30), 181 [$C_9H_9O_4$]$^{+}$ (100), 150 (14), 135 (10), 107 (10). HREIMS *m/z* 330.1103 [M^{+}] (Calc. for $C_{18}H_{18}O_6$: 330.1103).

7-Oxo-15-hydroxydehydroabietic acid (**5**)

Yellow crystals. M.p.: 114-116^{o}C. UV λ_{max} (MeOH)nm: 212, 152. ^{1}H-NMR (Table 9). ^{13}C-NMR (Table 10) EIMS *m/z* (rel. int.): 330 [M]$^{+\cdot}$ (5), 316 (20), 315 (100), 269 (15), 187 (13), 145 (10), 43 (48).

7-Hydroxy-8,11,13-abieatrien-18-oic acid (**6**)

Yellow crystals. M.p.: 111-113^{o}C. UV λ_{max} (MeOH)nm: 214. ^{1}H-NMR (Table 9). ^{13}C-NMR (Table 10). EIMS *m/z* (rel. int.): 317 [M+1]$^{+}$ (6), 316 [M]$^{+\cdot}$ (30), 299 (10), 298 (20), 283 (15), 273 (15), 253 (5), 237 (100), 209 (8), 195 (90), 162 (60).

7,15-Dihydroxy-8,11,13-abietatrien-18-oic acid (**7**)

Amorphous powder. UV λ_{max} (MeOH)nm: 214. ^{1}H-NMR (Table 9). ^{13}C-NMR (Table 10). EIMS *m/z* (rel. int.): 332 [M]$^{+\cdot}$ (5), 317 (60), 314 (35), 296 (56), 281 (15), 253 (30), 235 (100), 195 (85), 181 (25), 165 (28), 59 (38), 43 (75).

Lupeol (**8**)

White crystals. M.p.: 212–214 ^{o}C. ^{1}H-NMR (Table 11). ^{13}C-NMR (Table 11). EIMS *m/z* (rel. int.): 426 [M]$^{+\cdot}$ (45), 411 (20), 315 (15), 218 (65), 207 (75), 189 (85), 135 (70), 109 (80), 95 (100), 81 (100), 69 (90).

β-Sitosterol (**9**)

White crystals. M.p.: 136-137°C. ^{13}C-NMR (Table 12). EIMS *m/z* (rel. int.): 414 $[M]^{+\cdot}$ (45), 396 (20), 381 (15), 329 (20), 303 (20), 271 (18), 255 (30), 213 (25), 159 (30), 145 (35), 107 (42), 95 (40), 81 (48), 69 (44), 55 (95), 43 (100).

Hexadecanoic acid (**10**)

White crystals. M.p.: 60-62 °C. ^{13}C-NMR (Table 13). EIMS *m/z* (rel. int.): 256 $[M]^{+\cdot}$ (45), 213 (20), 185 (15), 171 (15), 157 (15), 129 (40), 115 (15), 97 (20), 85 (23), 73 (85), 60 (85), 43 (100).

Vitexin (**11**)

Amorphous powder. ^{1}H-NMR (Table 14). ^{13}C-NMR (Table 14).

Intortunone (**12**)

Amorphous powder. ^{1}H-NMR (Table 16). ^{13}C-NMR (Table 16). EIMS *m/z* (rel. int.): 355 $[M+1]^{+}$ (15), 354 $[M]^{+\cdot}$ (70), 339 (60), 321 (80), 219 (100), 217 (52), 203 (60), 177 (15), 136 (25).

Dalbergioidin (**13**)

Amorphous powder. ^{1}H-NMR (Table 17). ^{13}C-NMR (Table 17). EIMS *m/z* (rel. int.): 288 $[M]^{+\cdot}$ (5), 153 (100), 136 (40), 126 (25), 110 (20).

Uncinanone A (**14**)

Amorphous powder. ^{1}H-NMR (Table 18). ^{13}C-NMR (Table 18). EIMS *m/z* (rel. int.): 356 $[M]^{+\cdot}$ (52), 301 (12), 220 (15), 221 (85), 194 (55), 179 (25), 165 (100), 139 (88), 136 (35).

Uncinanone B (**15**)

Amorphous powder. ^{1}H-NMR (Table 19). ^{13}C-NMR (Table 19). EIMS *m/z* (rel. int.): 354 $[M]^{+\cdot}$ (5), 353 (20), 218 (5), 219 (100), 203 (10), 177 (10), 136 (18).

7-Oxodehydroabietic acid (**16**)

Amorphous powder. ^{1}H-NMR (Table 20). ^{13}C-NMR (Table 20). EIMS *m/z* (rel. int.): 314 $[M]^{+\cdot}$ (15), 313 (45), 298 (22), 274 (10), 268 (6), 254 (22), 253 (100), 211 (32).

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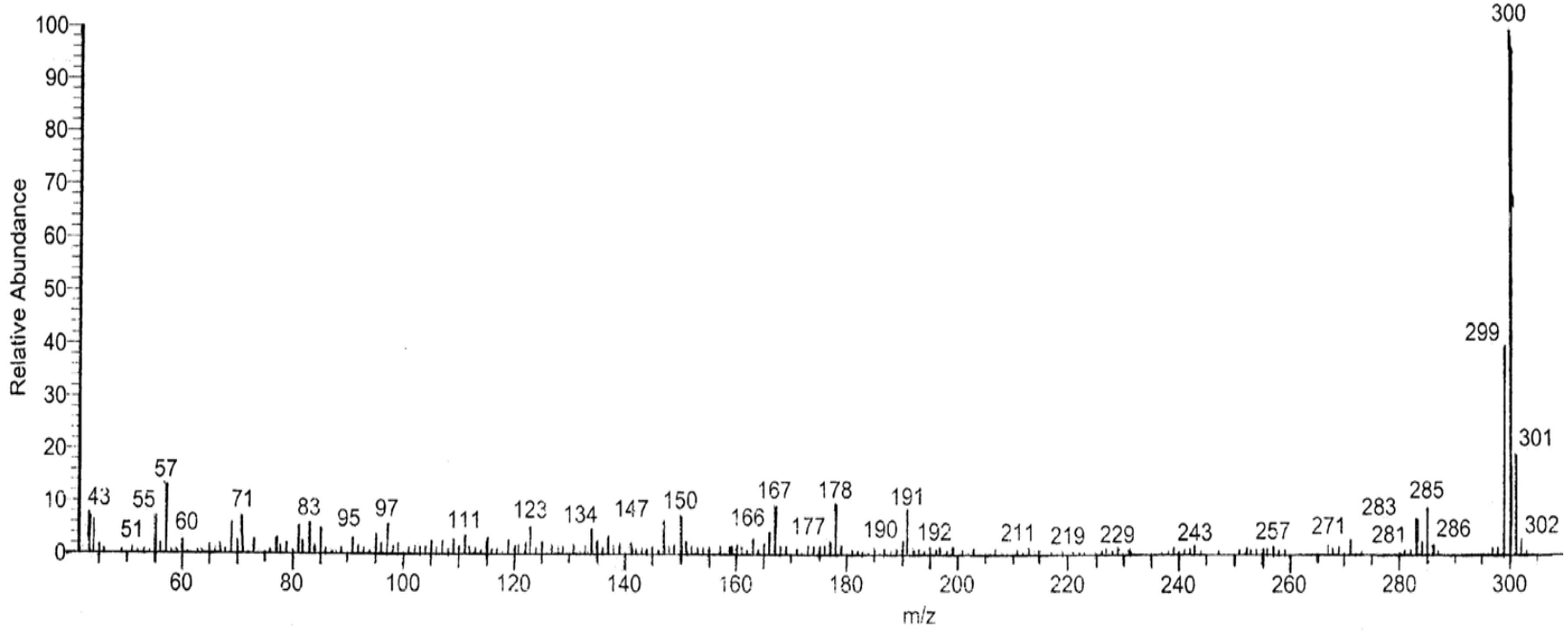
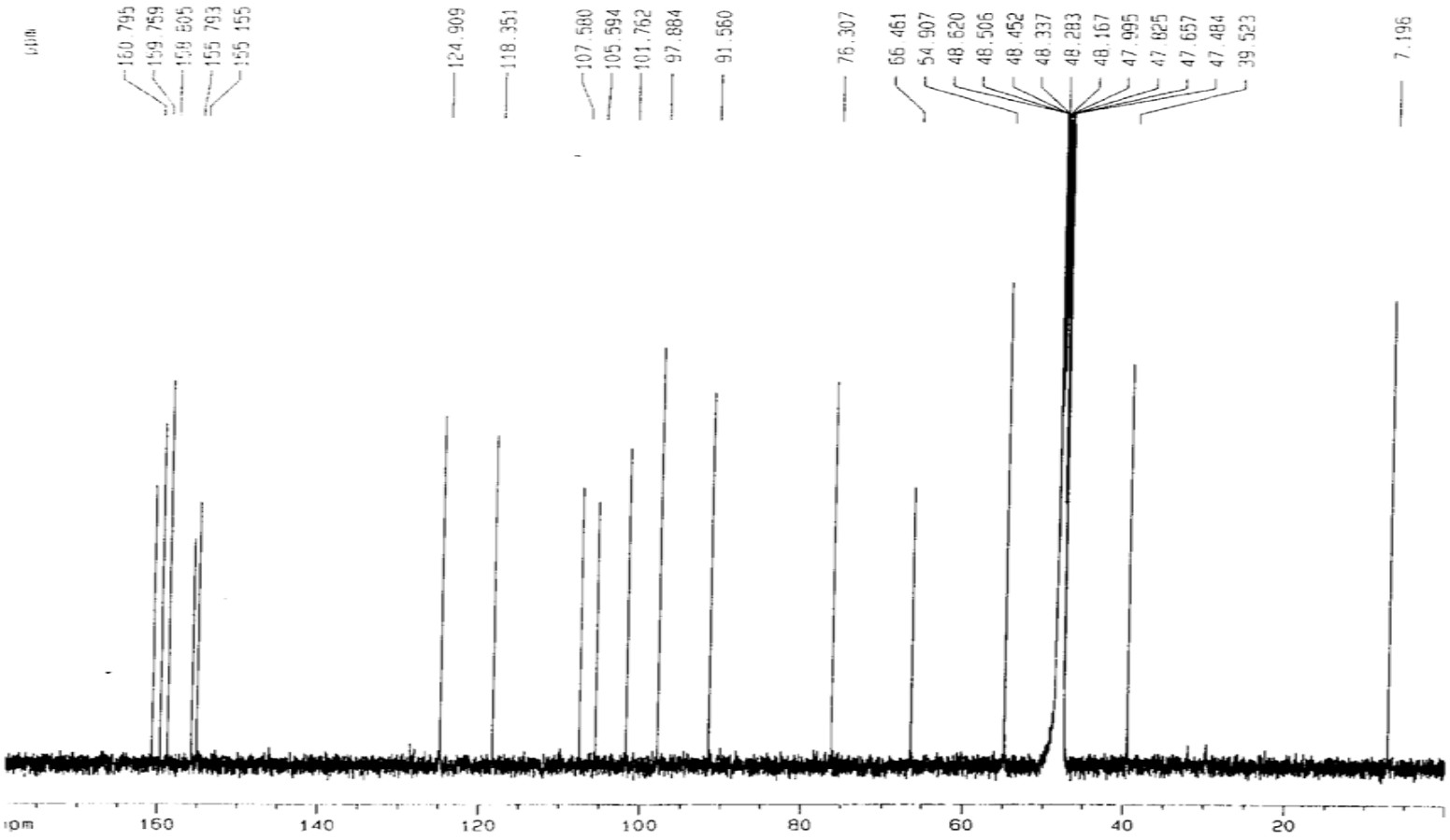
APPENDIX

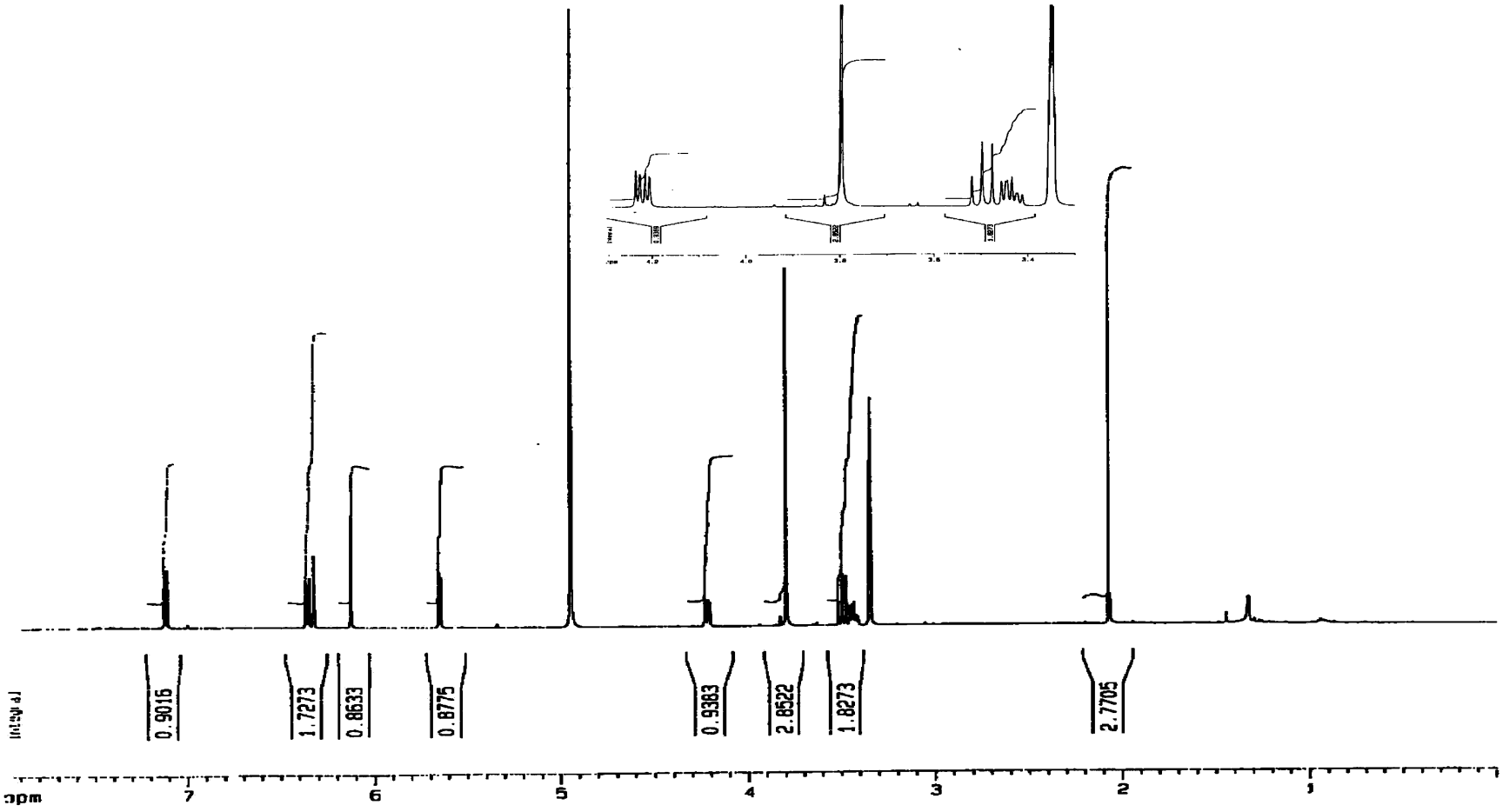
I. Mass spectrum of uncinacarpan (**1**)



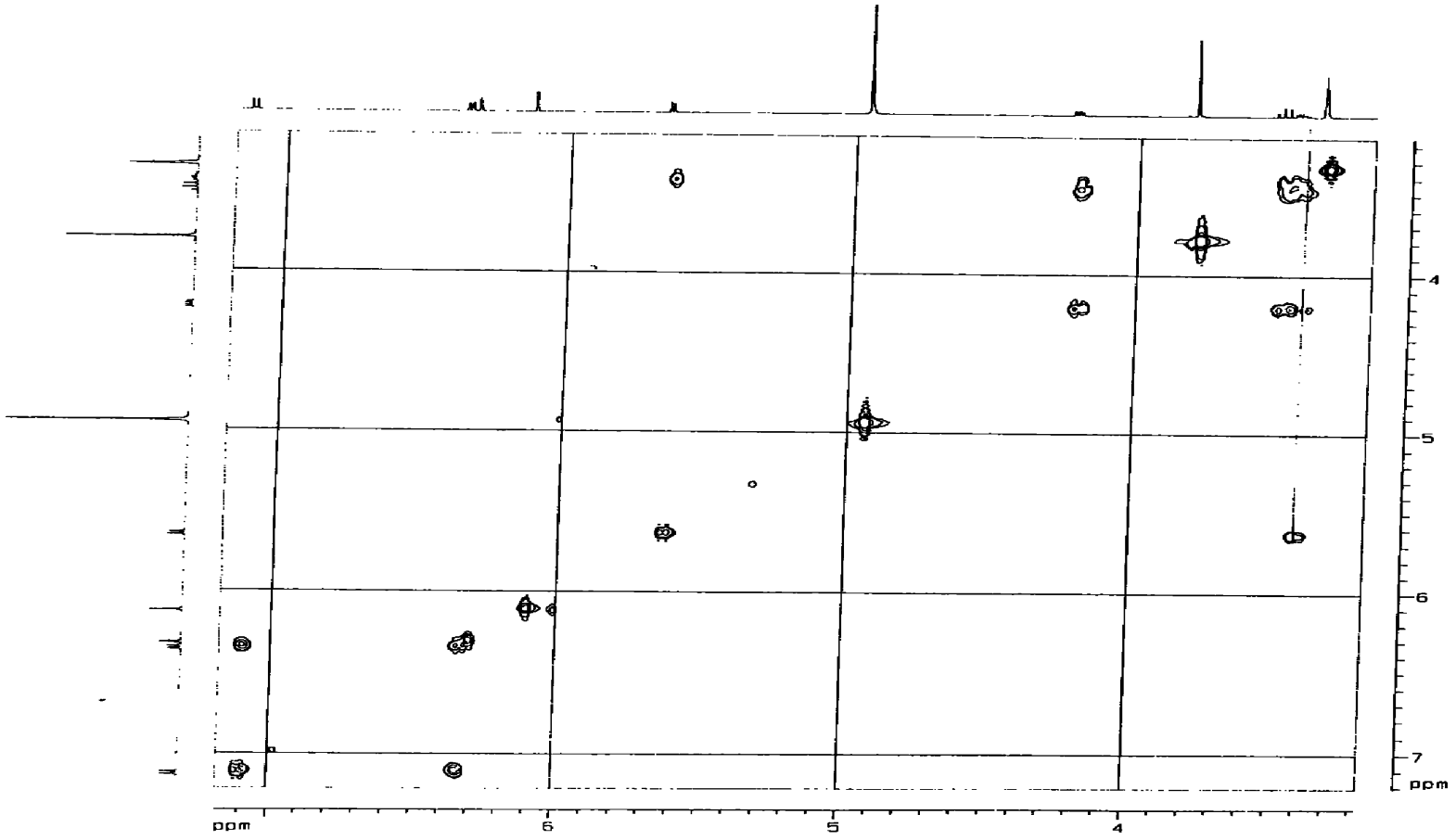
II. ^{13}C-NMR (125 MHz, MeOH-d_4) spectrum of uncinacarpan (**1**)



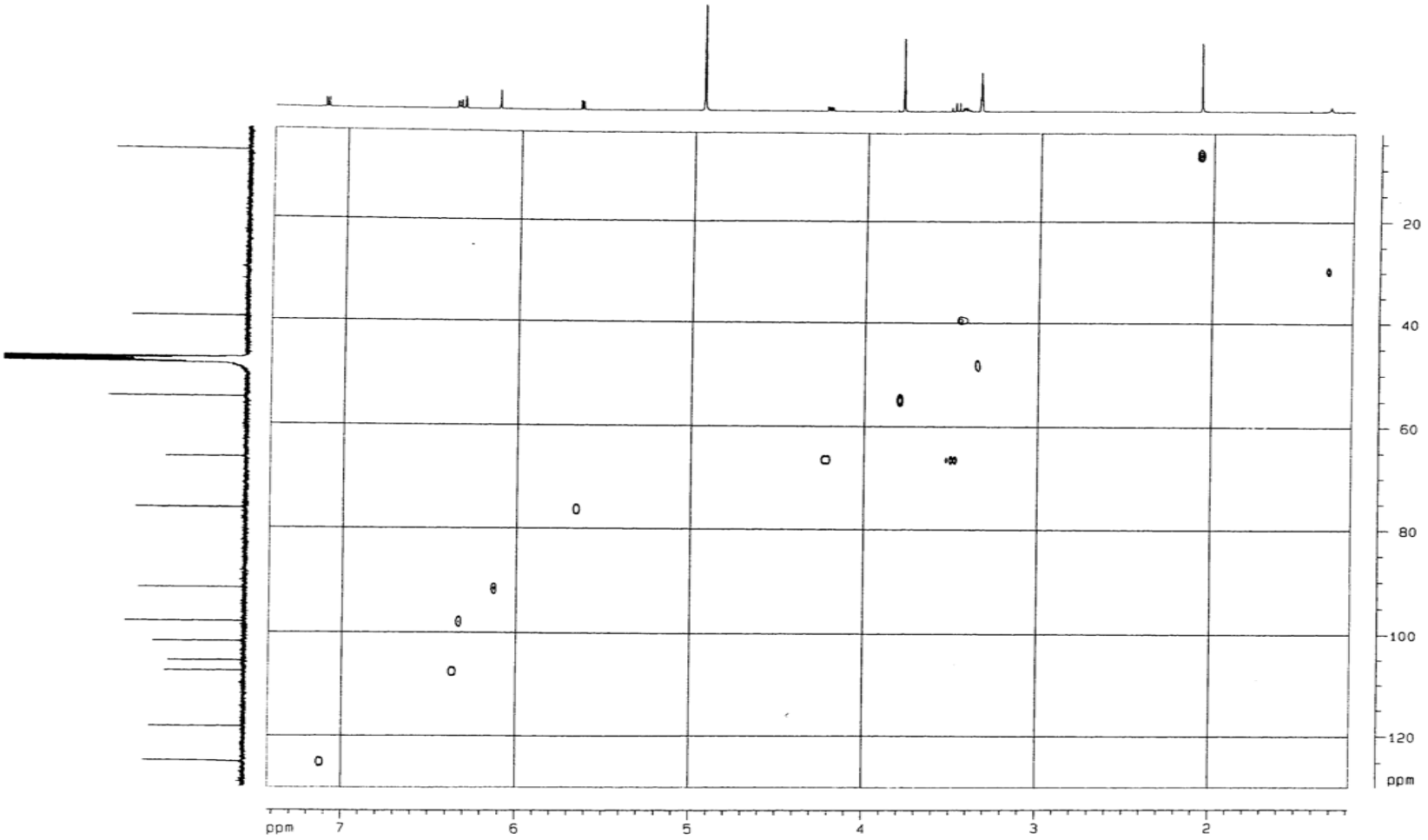
III. ^{1}H-NMR (500 MHz, MeOH-d_4) spectra of uncinacarpan (**1**)



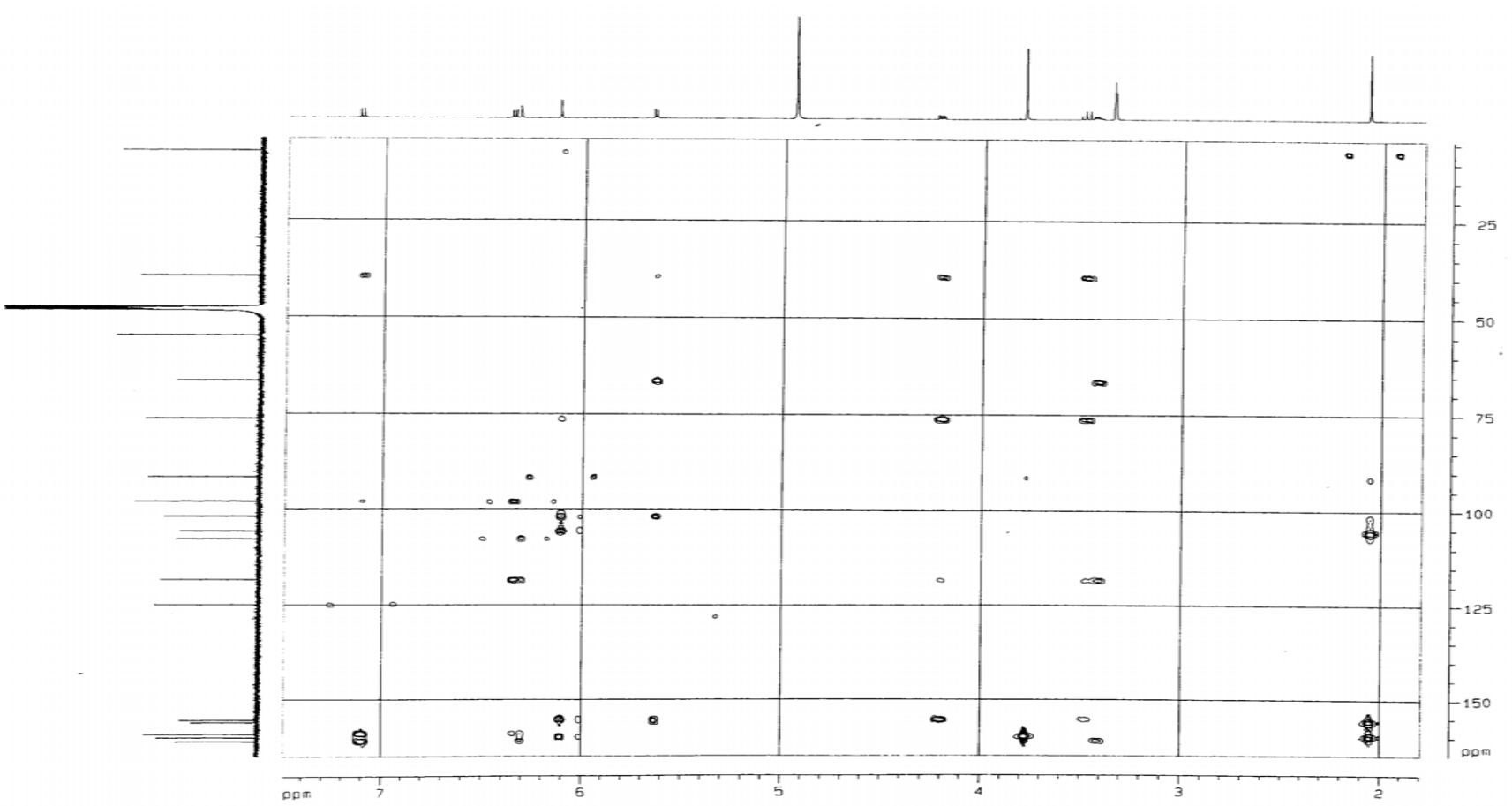
IV. ^{1}H, ^{1}H-COSY spectrum of uncinacarpan (**1**)



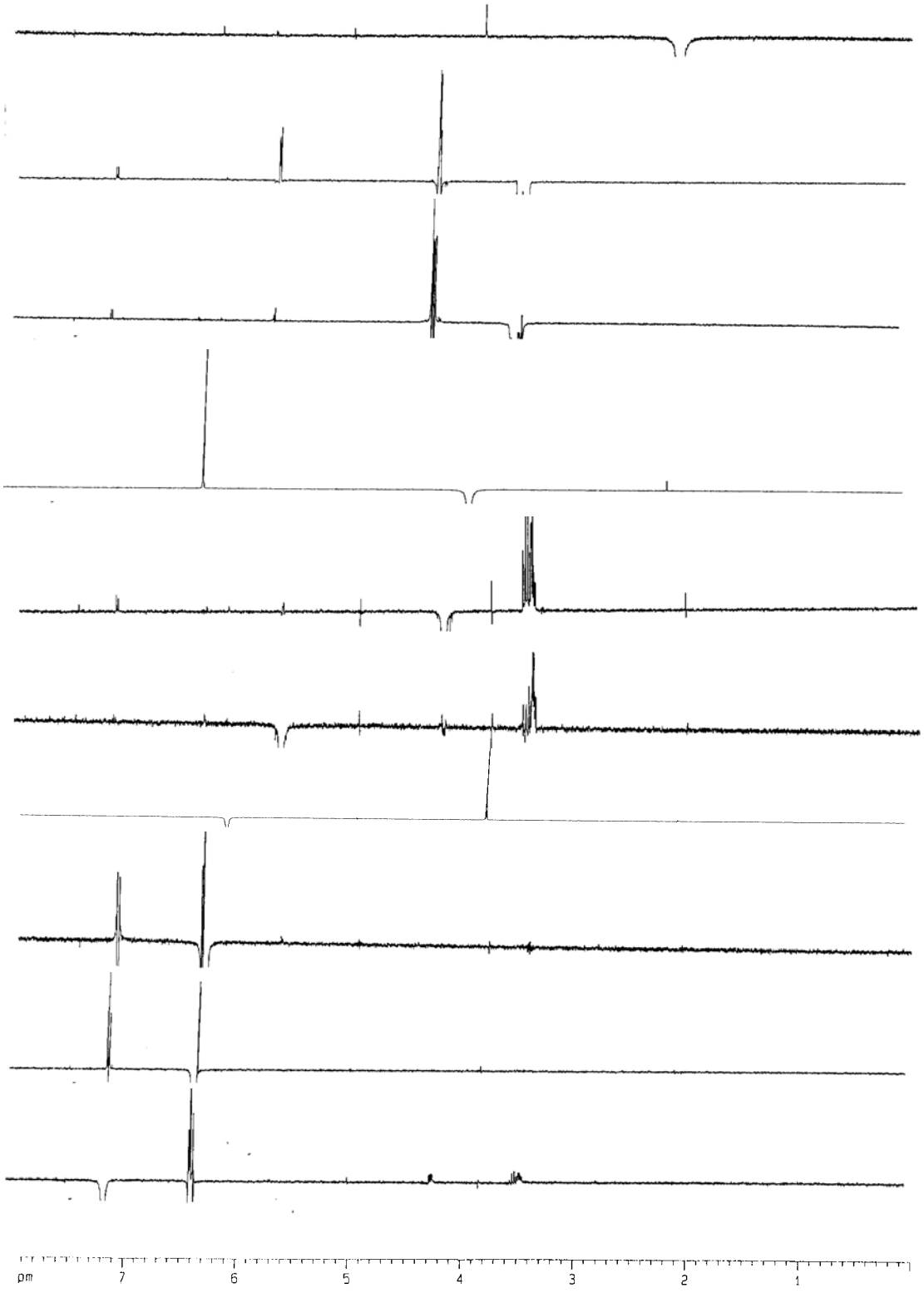
V. HMQC spectrum of uncinacarpan (**1**)



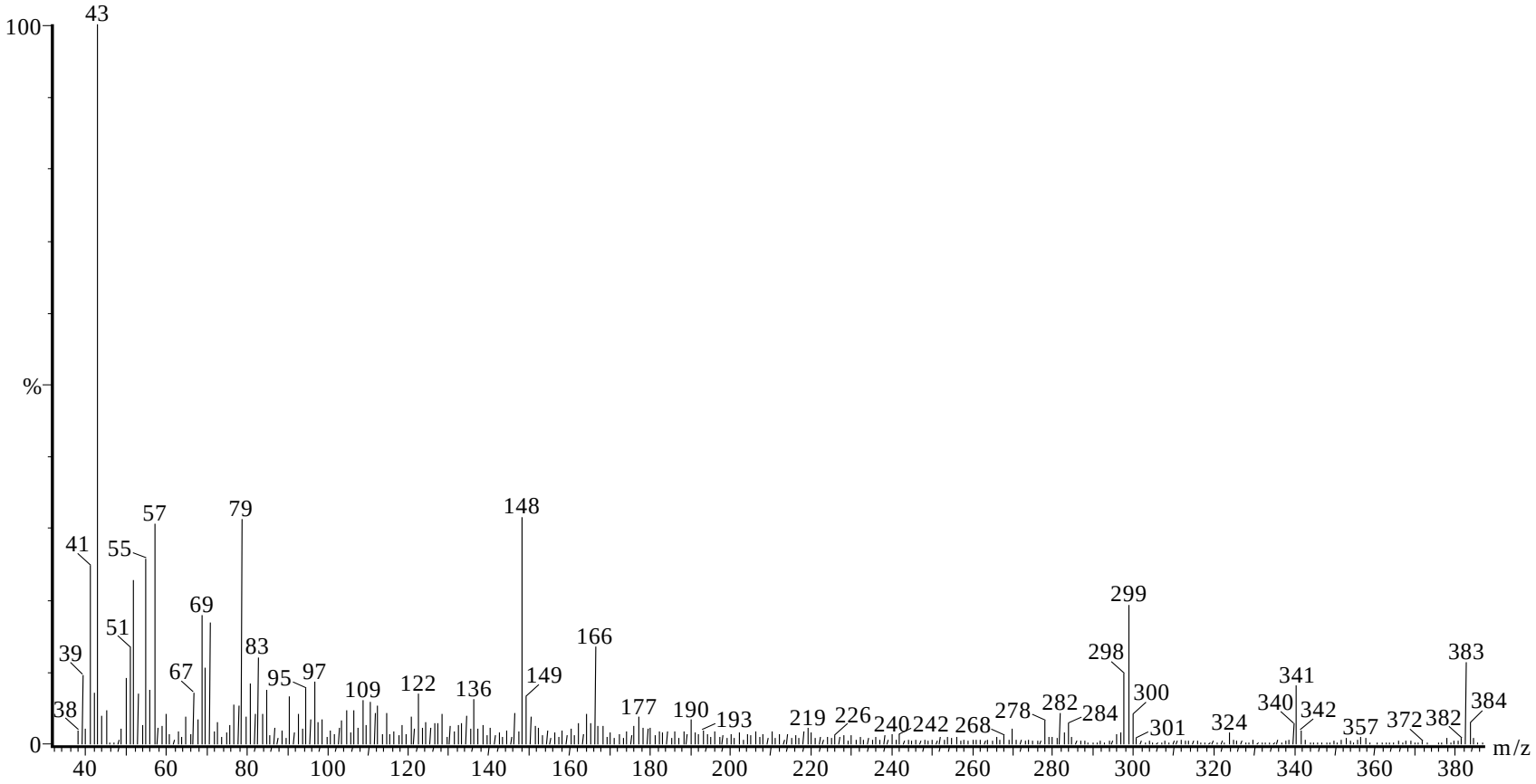
VI. HMBC spectrum of uncinacarpan (**1**)



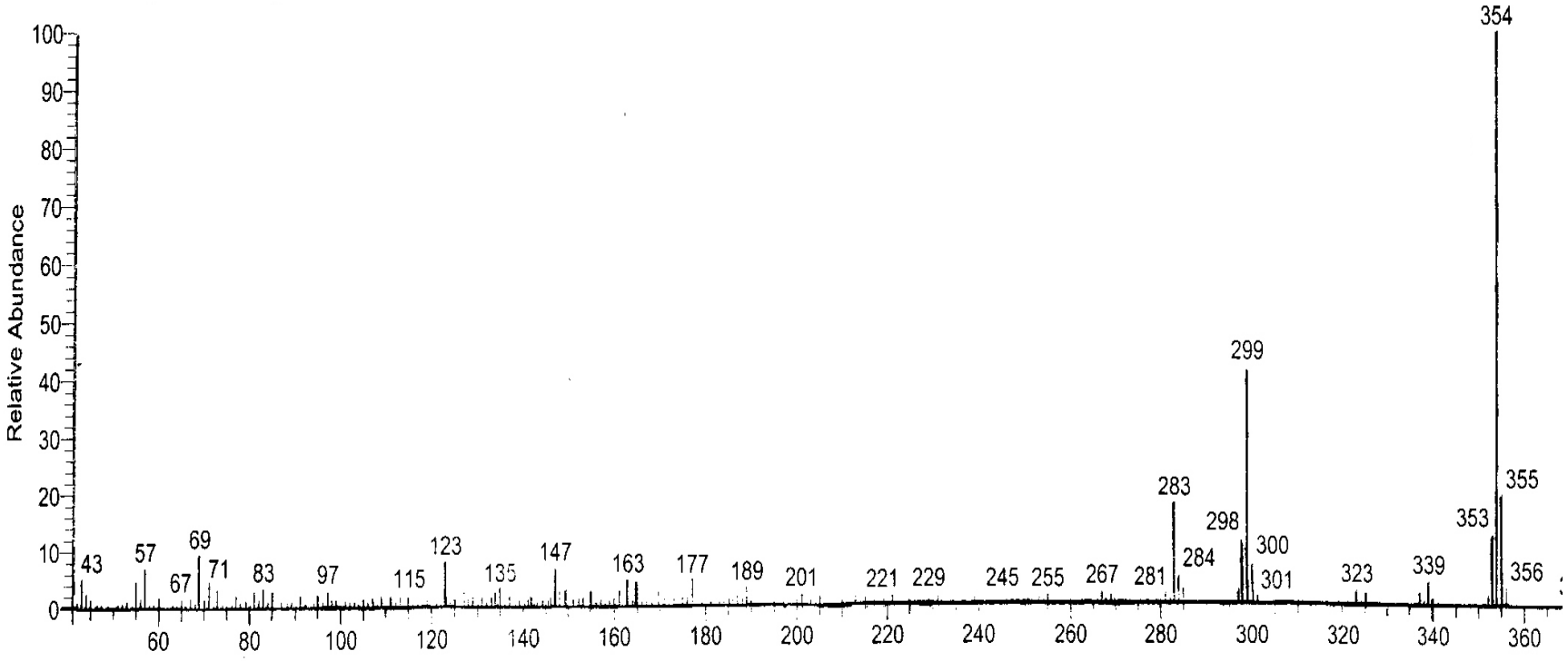
VII. GOESY spectra of uncinacarpan (**1**)



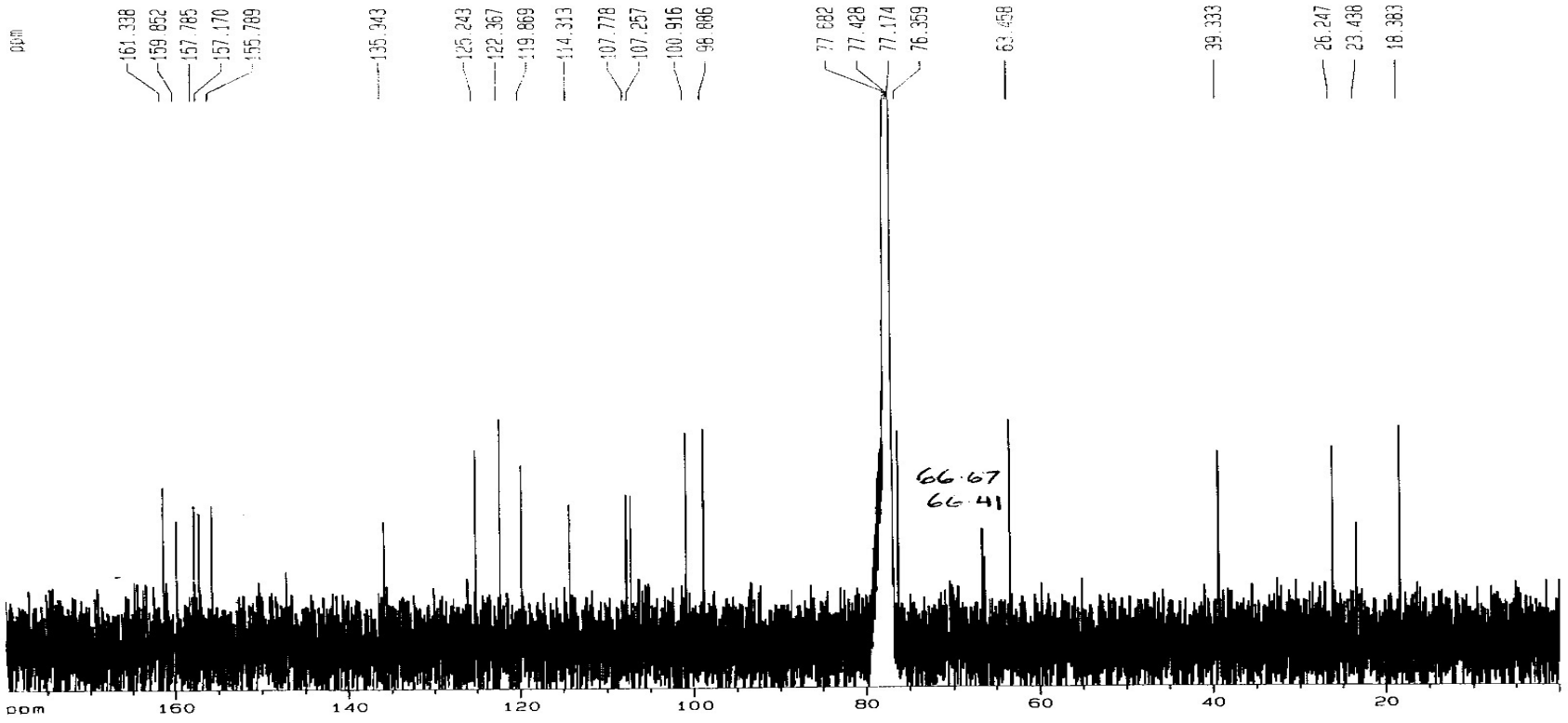
VIII. Mass spectrum of uncinacarpan diacetate (**1a**)



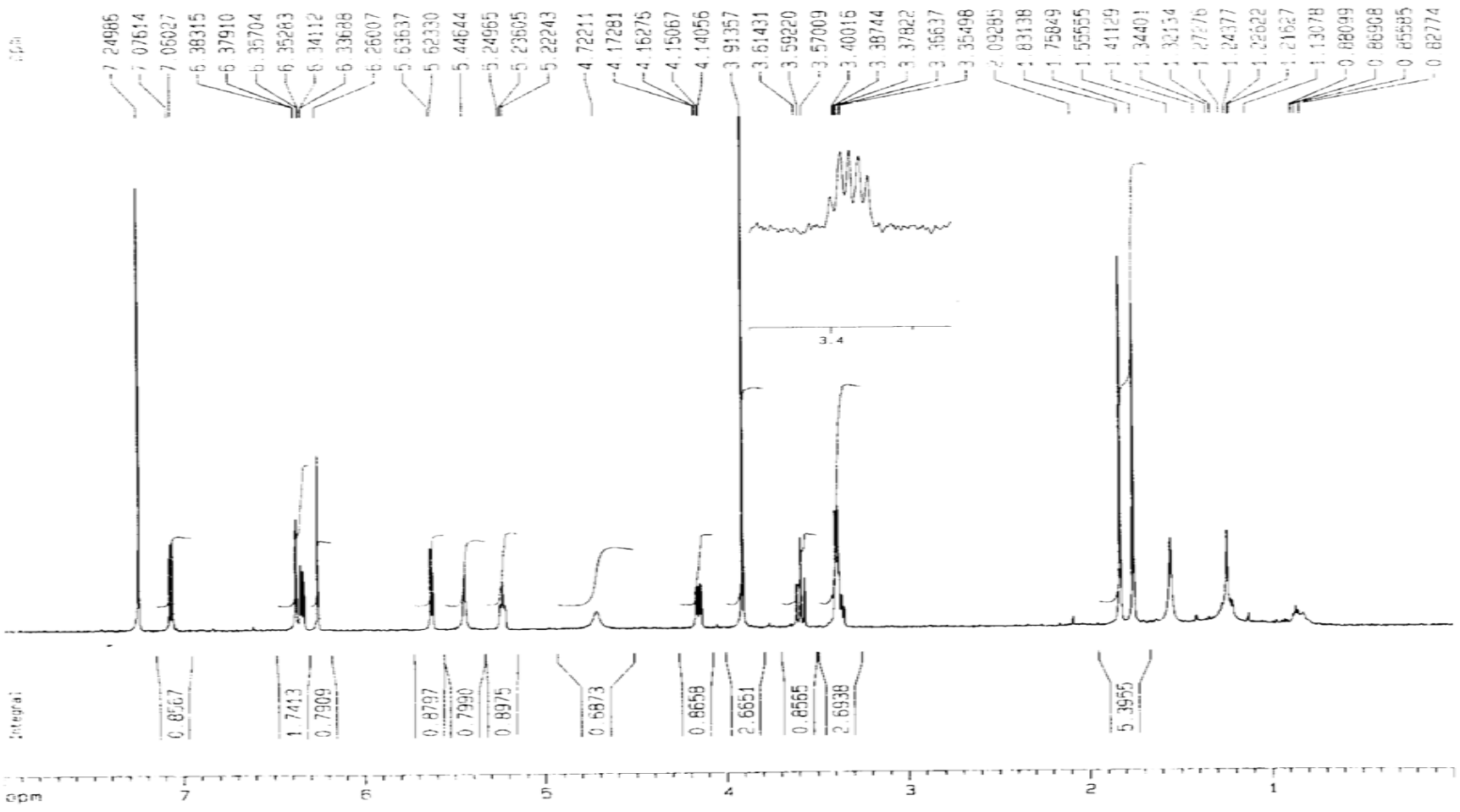
IX. Mass spectrum of edudiol (**2**)



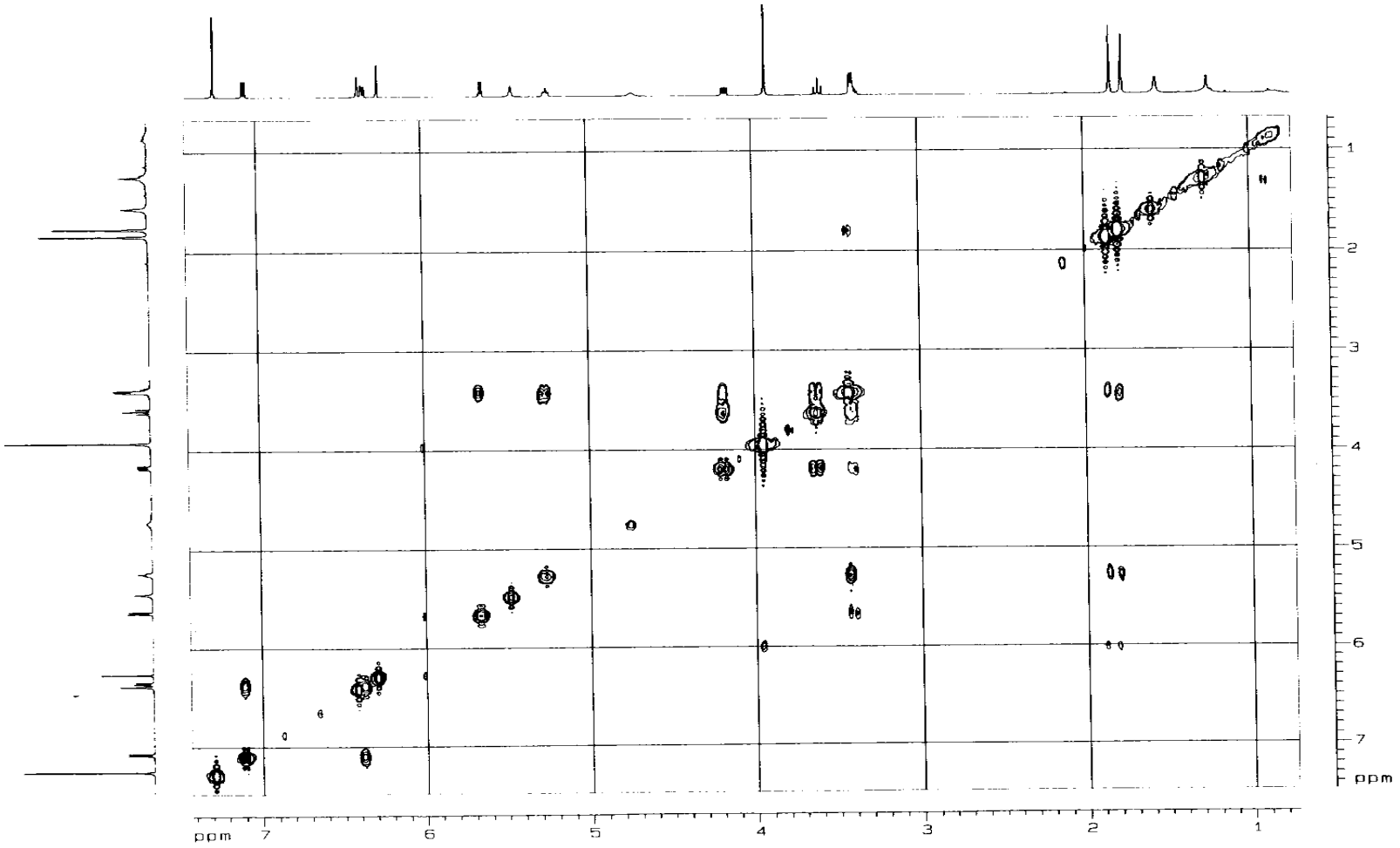
X. ^{13}C-NMR (125 MHz, $CDCl_3$) spectrum of edudiol (**2**)



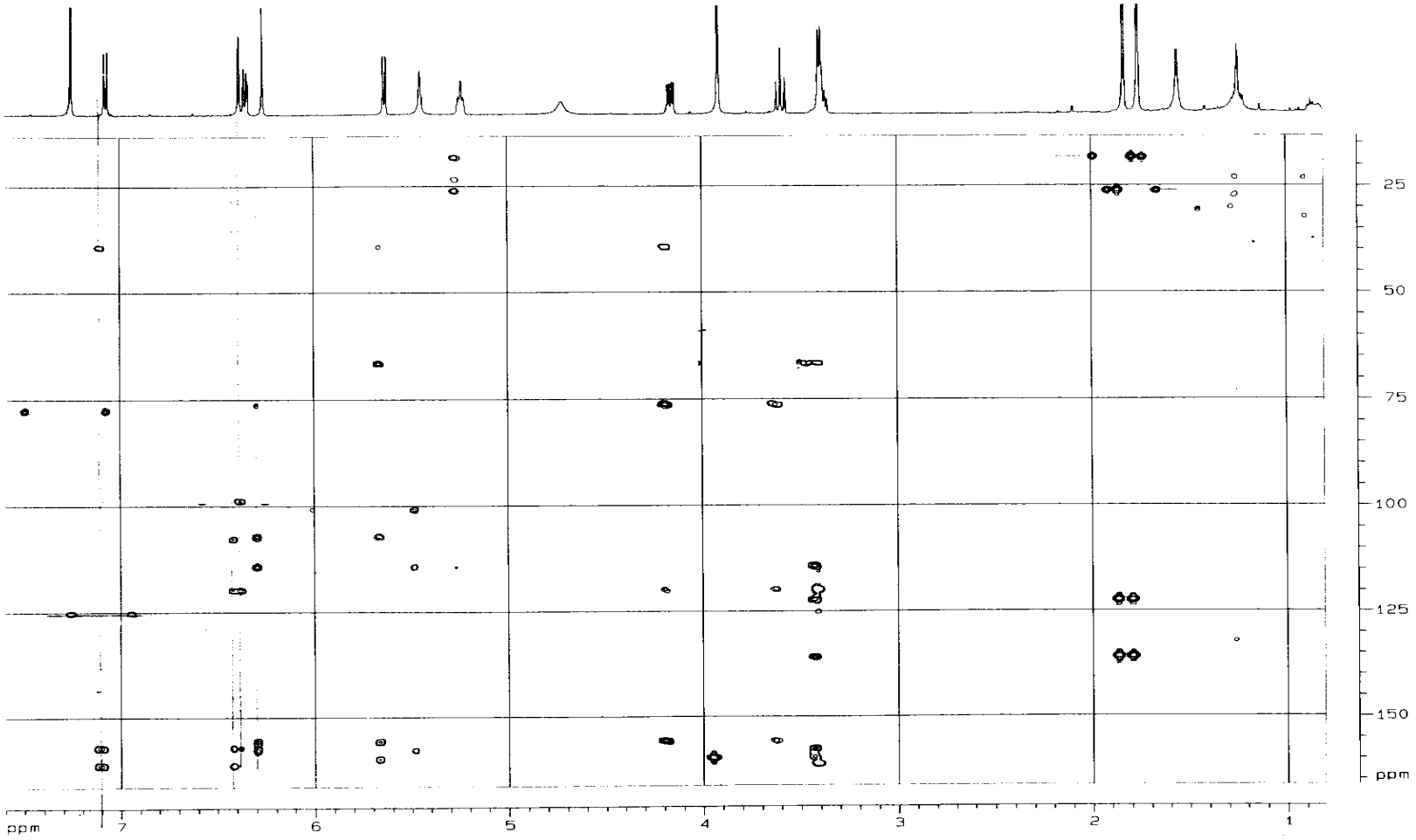
XI. ^{1}H-NMR (500 MHz, $CDCl_3$) spectrum of edudiol (**2**)



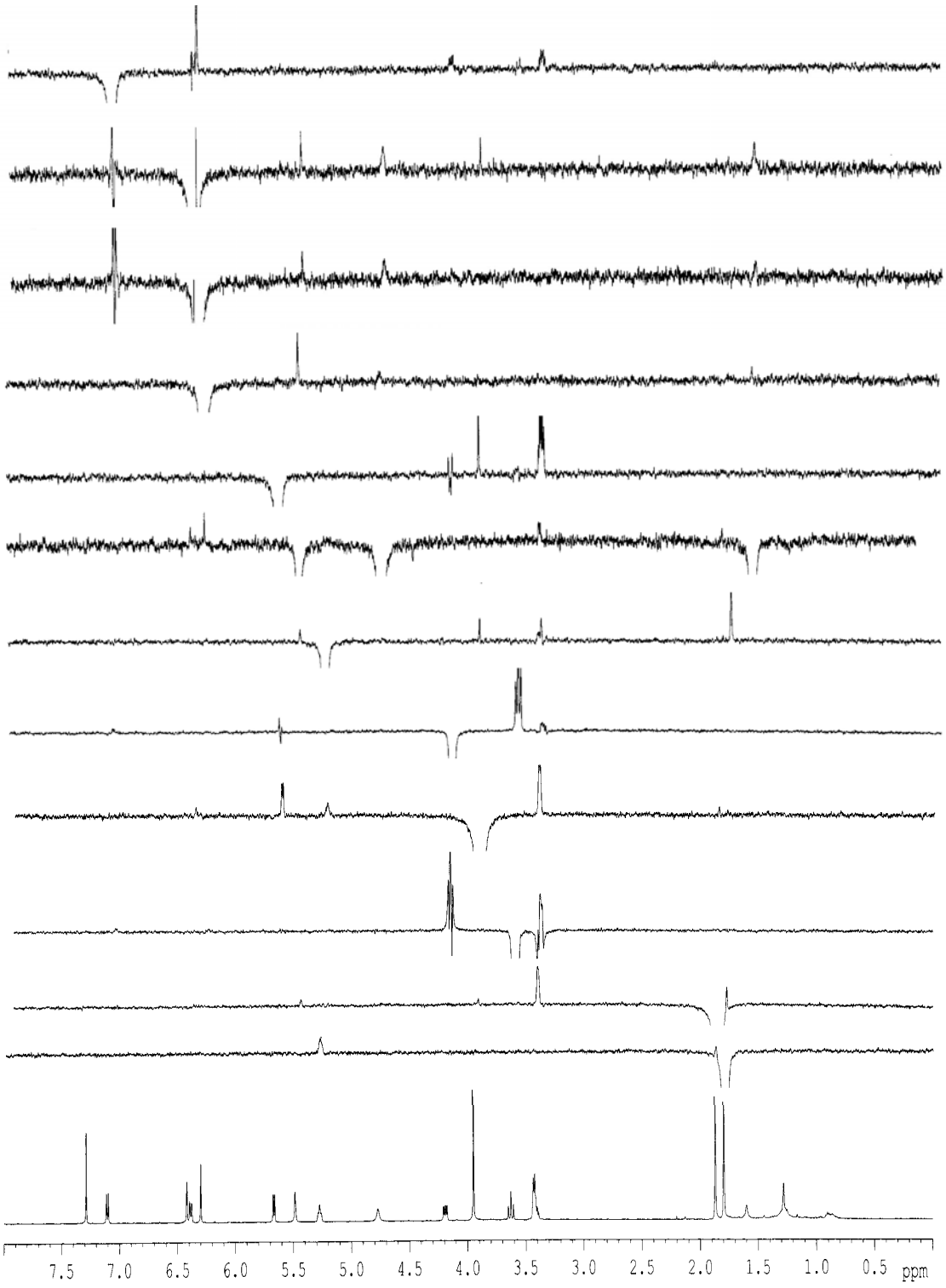
XII. ^{1}H, ^{1}H-COSY spectrum of edudiol (**2**)



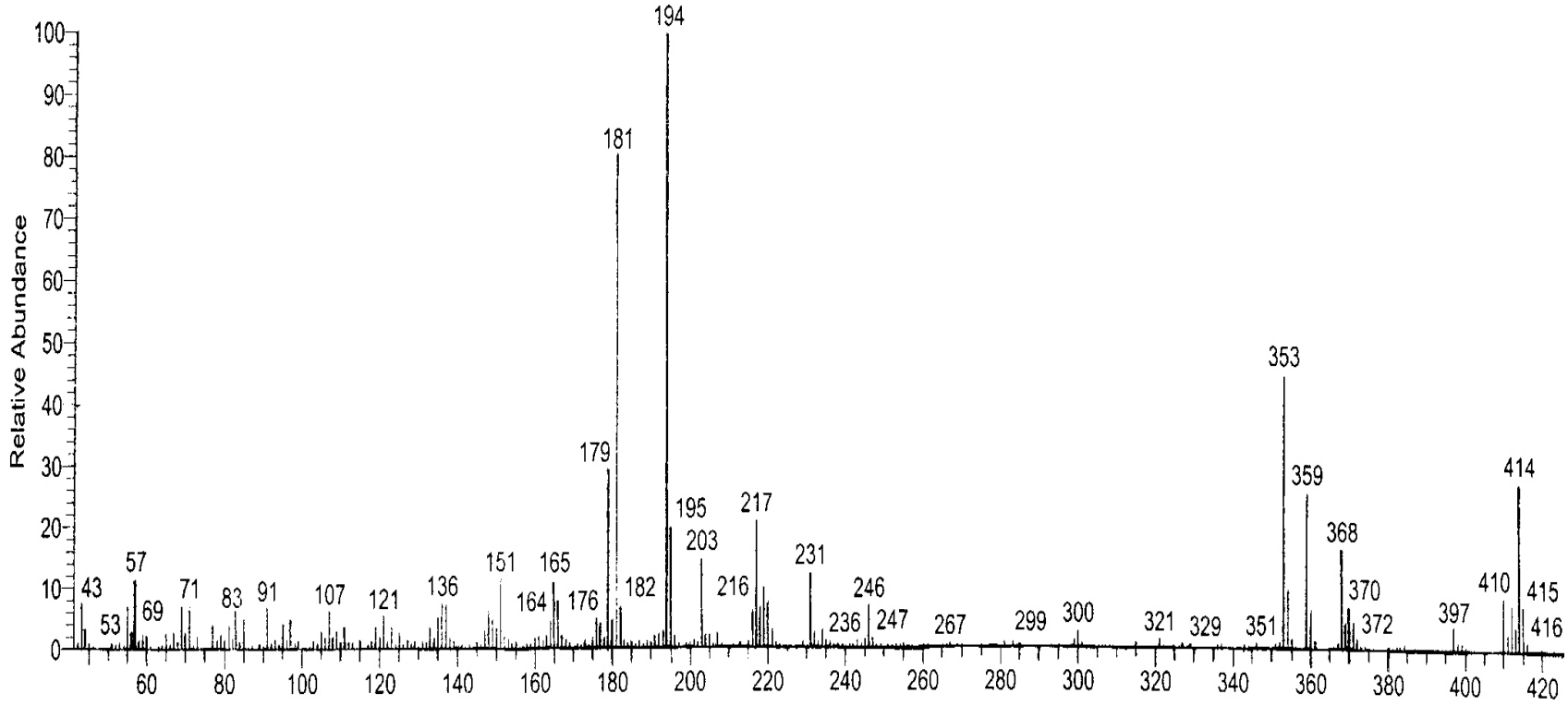
XIII. HMBC spectra of edudiol (**2**)



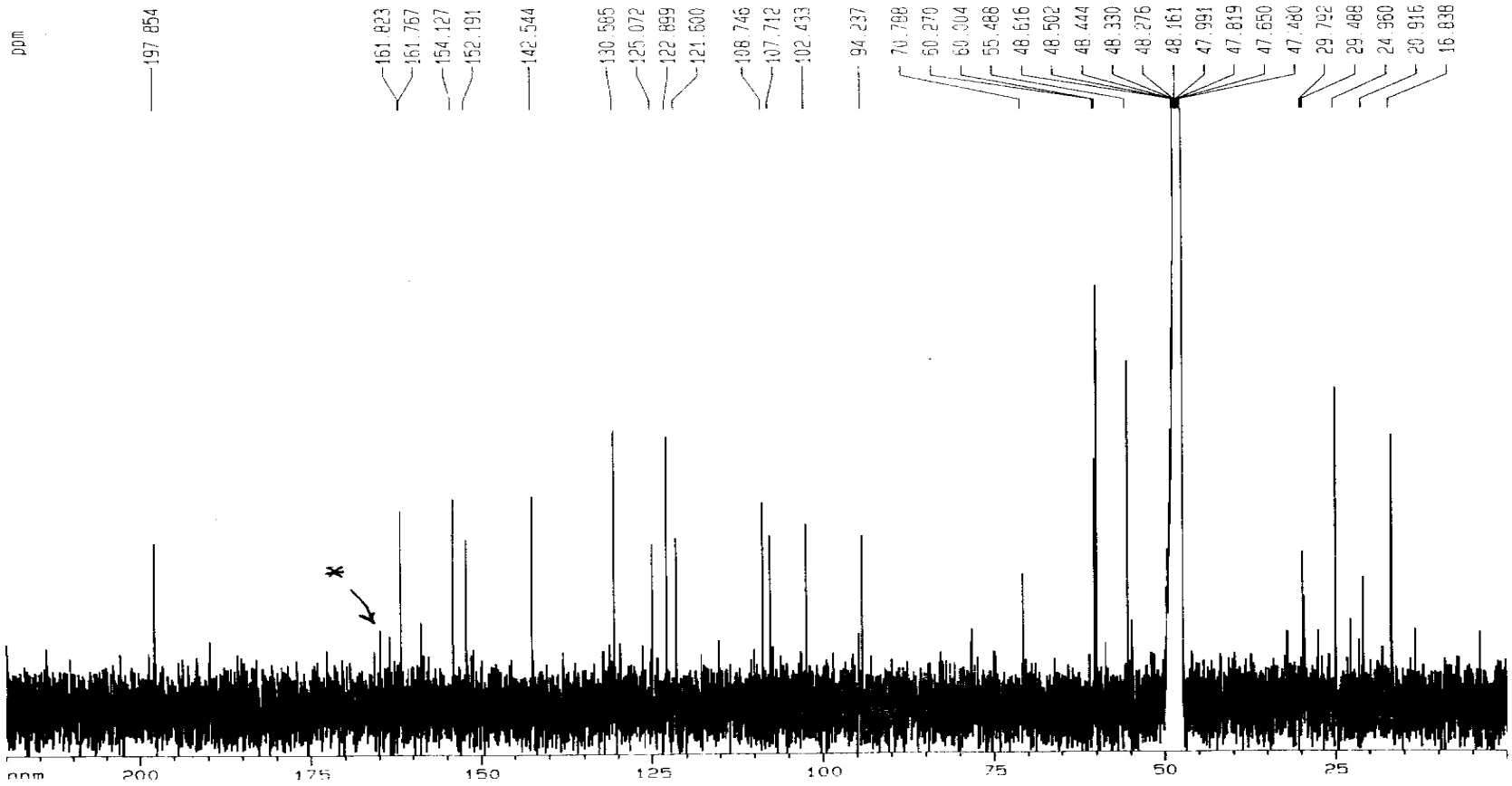
XIV. GOESY spectra of edudiol (**2**)



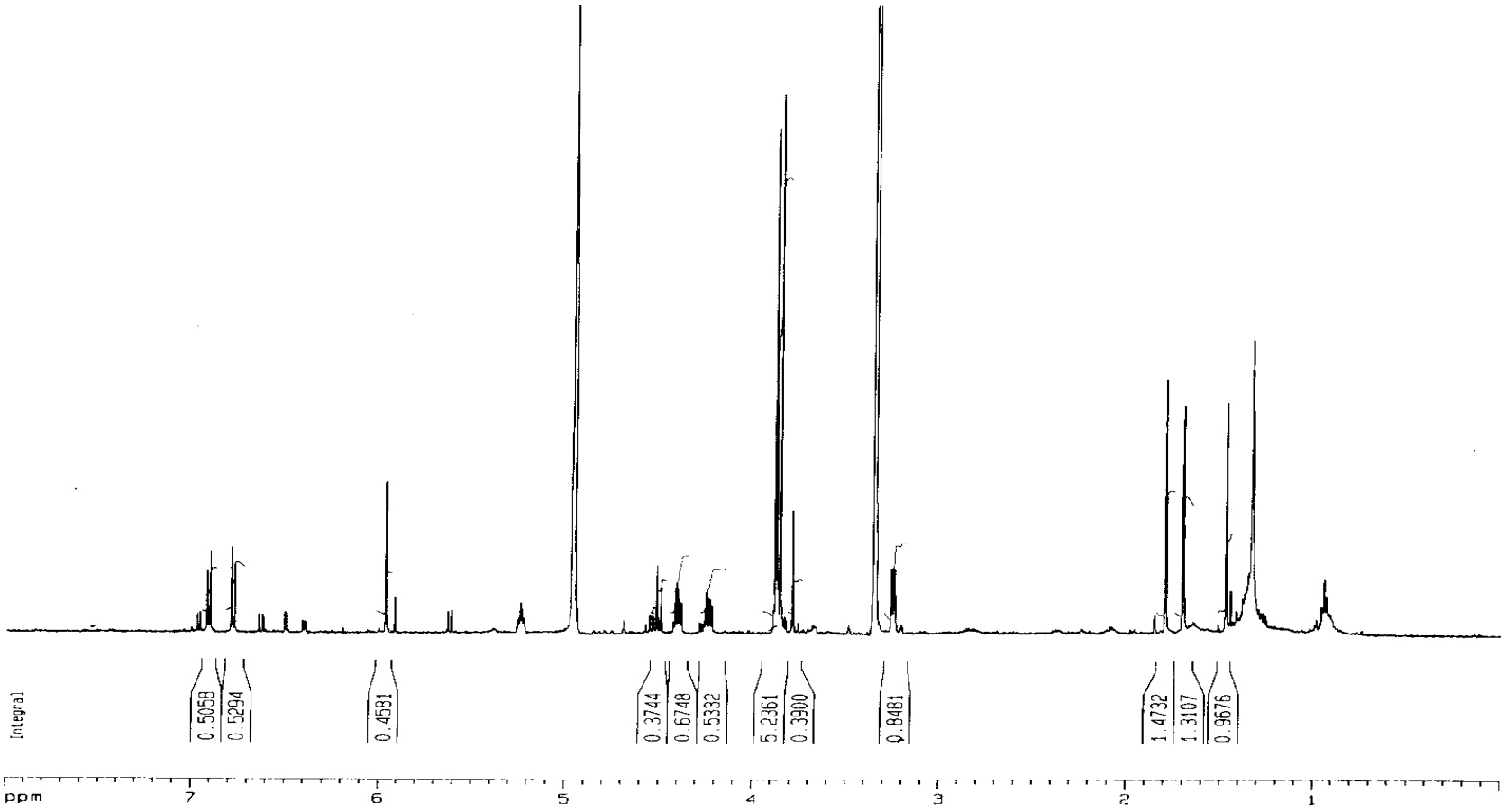
XV. Mass spectrum of uncinanone D (**3**)



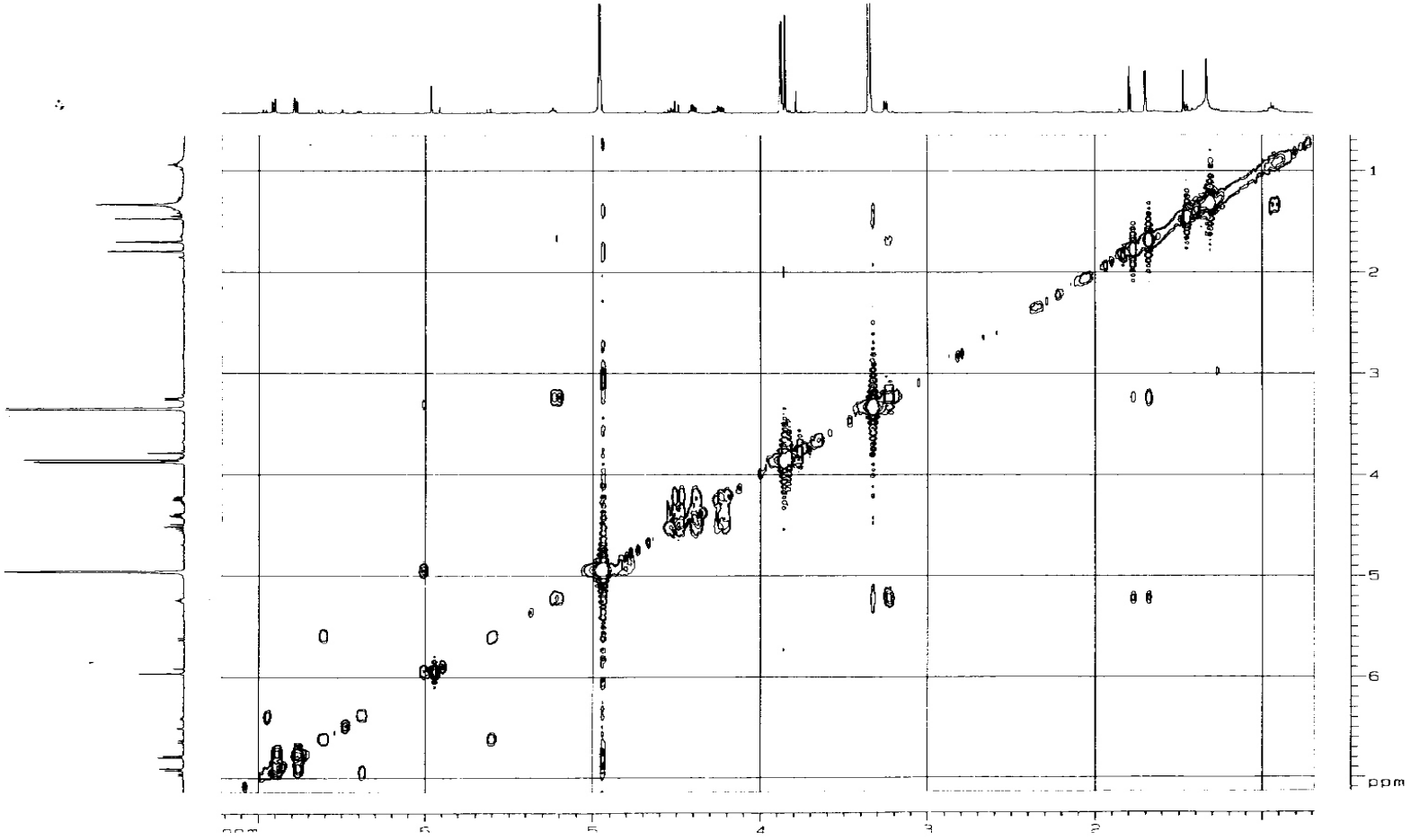
XVI. ^{13}C-NMR (125 MHz, MeOH-d_4) spectrum of uncinanone D (**3**)



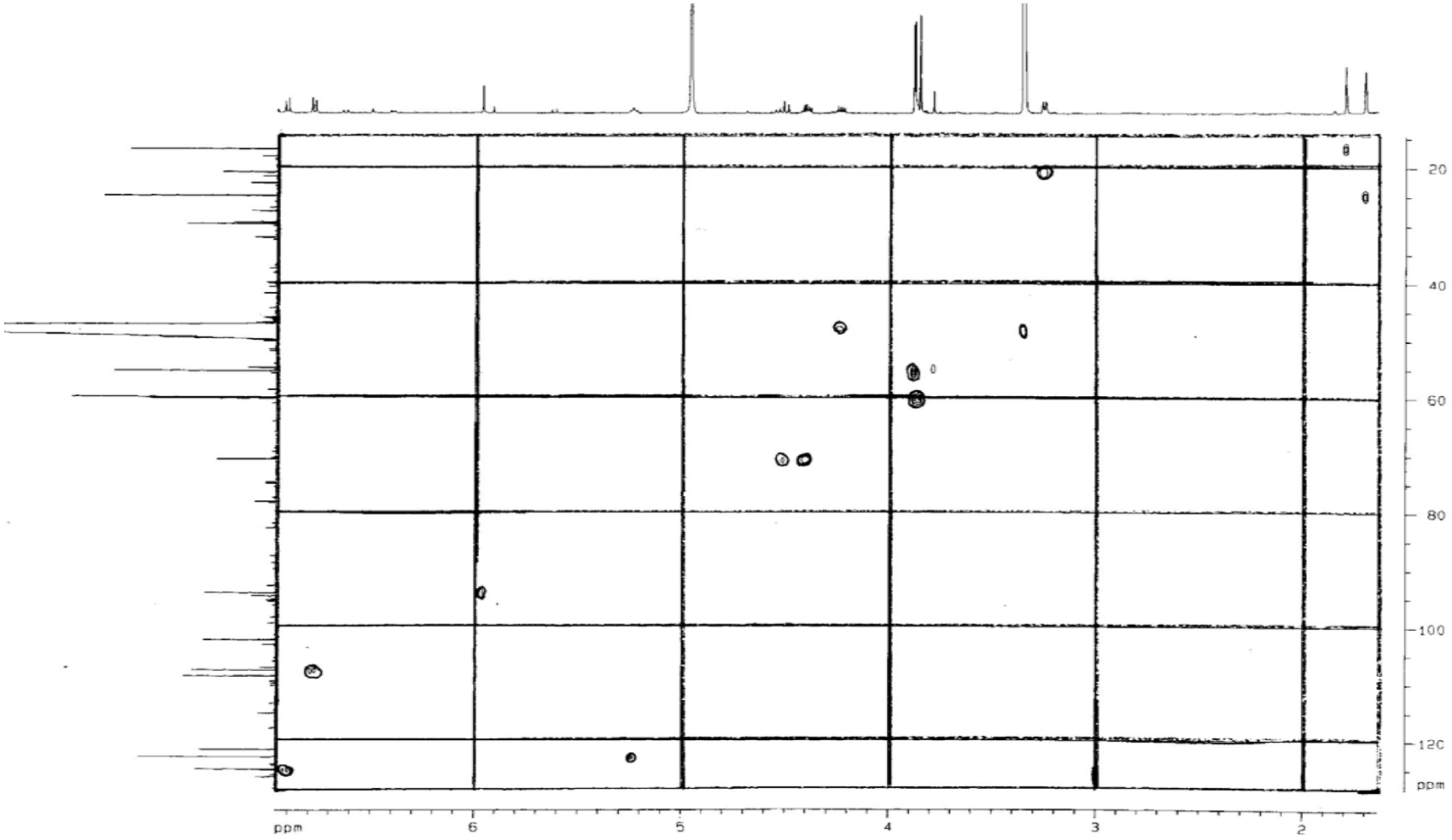
XVII. ^{1}H-NMR (500 MHz, MeOH-d_4) spectrum of uncinanone D (**3**)



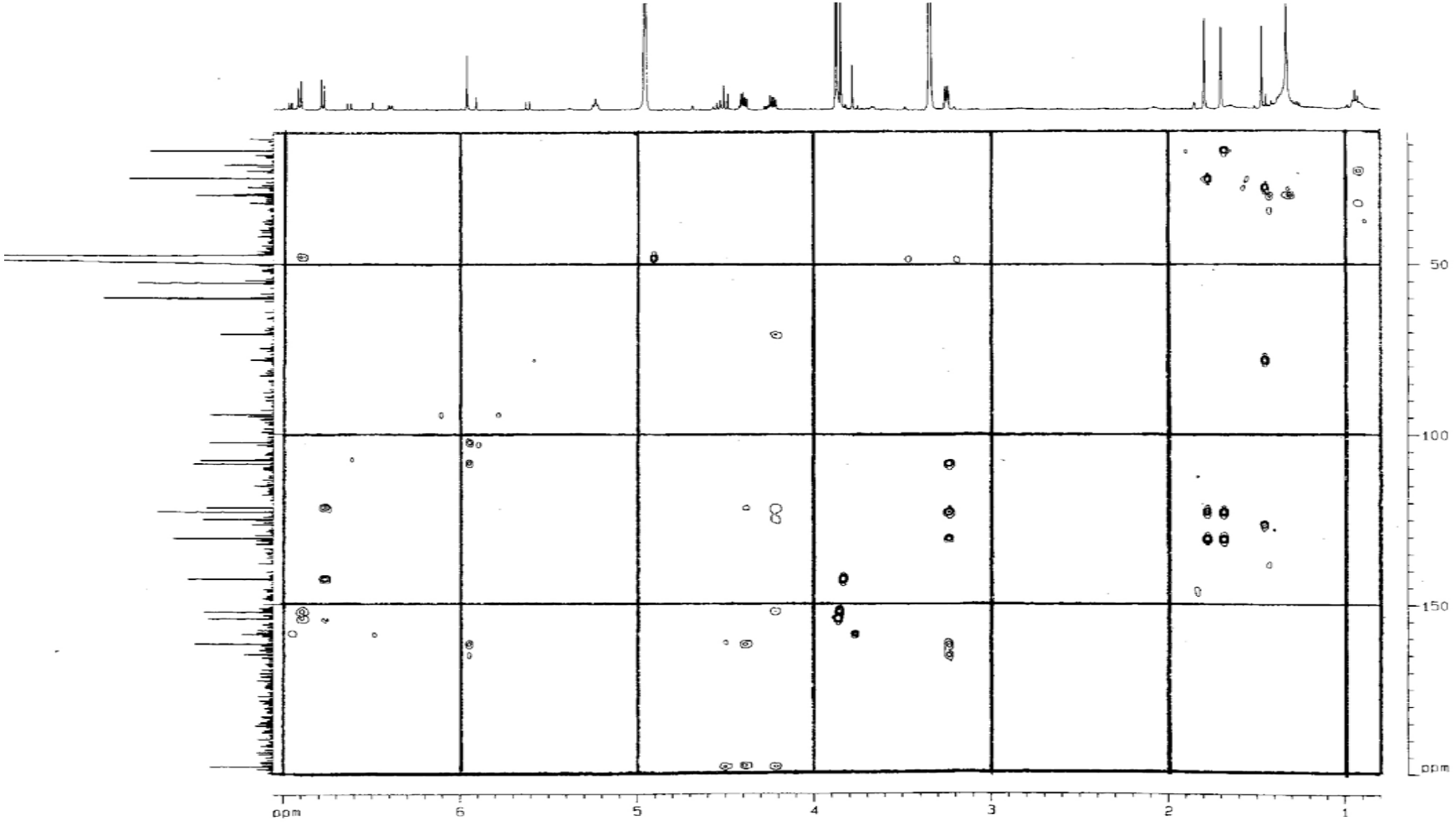
XVIII. ^{1}H, ^{1}H-COSY spectrum of uncinanone D (**3**)



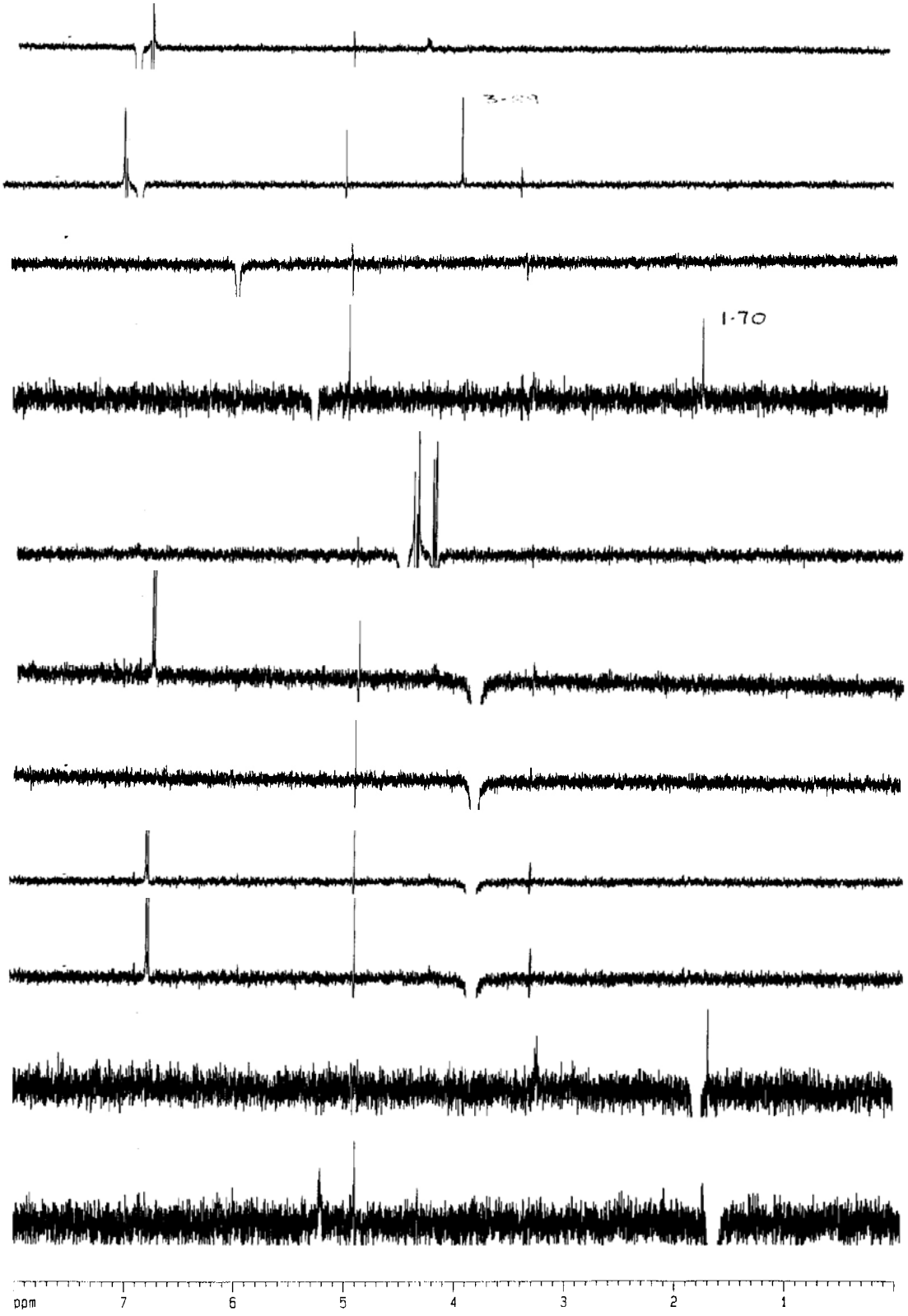
XIX. HMQC spectrum of uncinanone D (**3**)



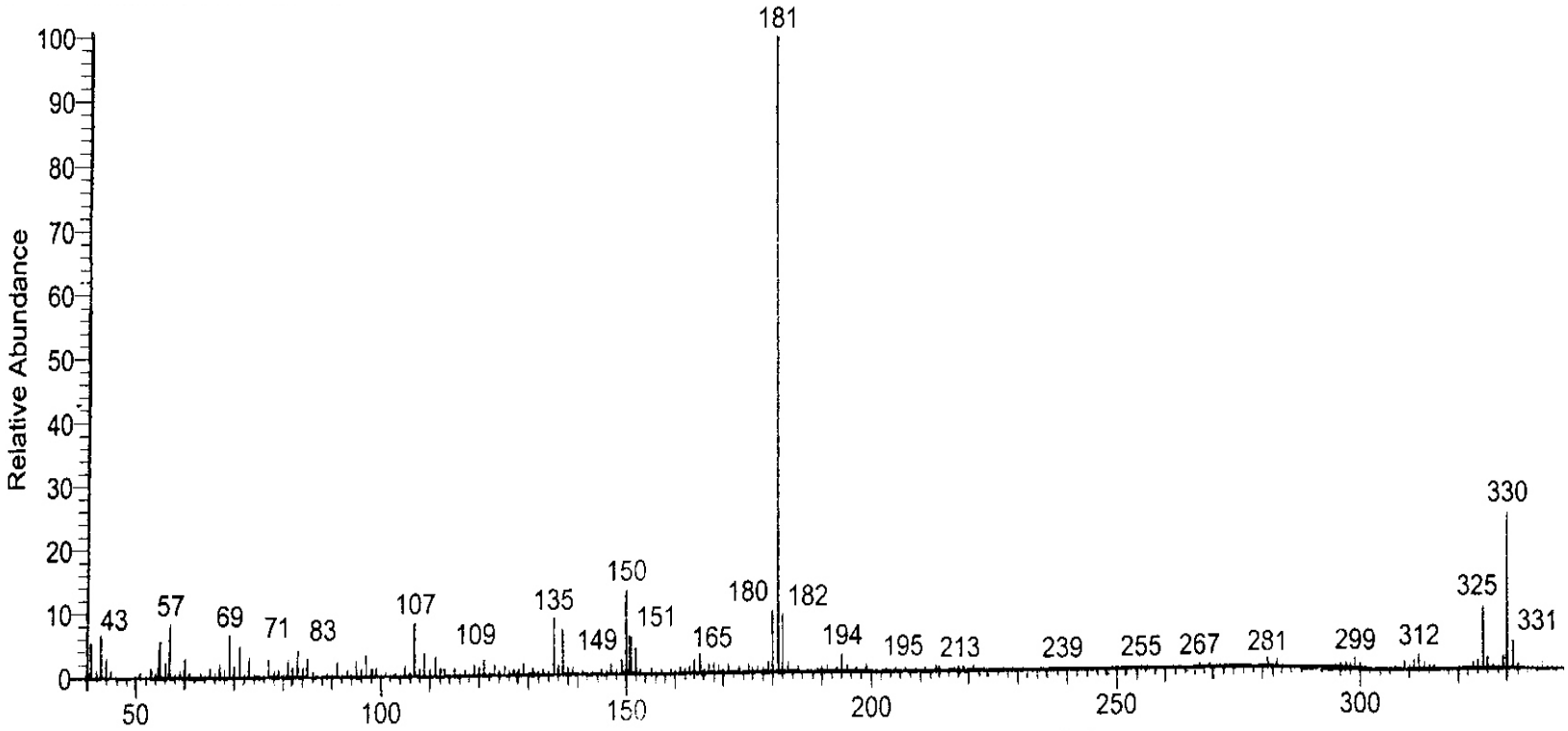
XX. HMBC spectrum of uncinanone D (**3**)



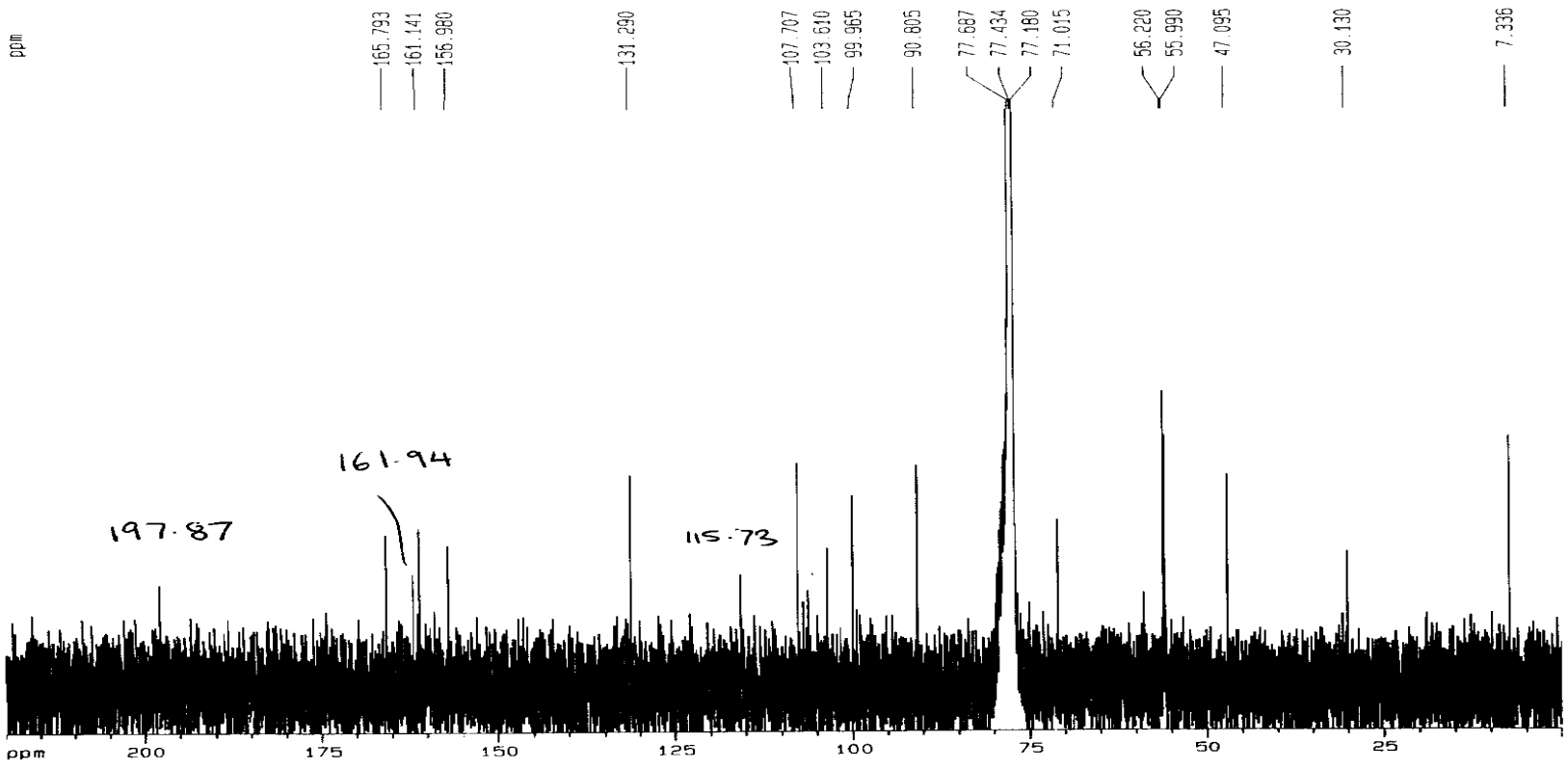
XXI. GOESY spectra of uncinanone D (**3**)



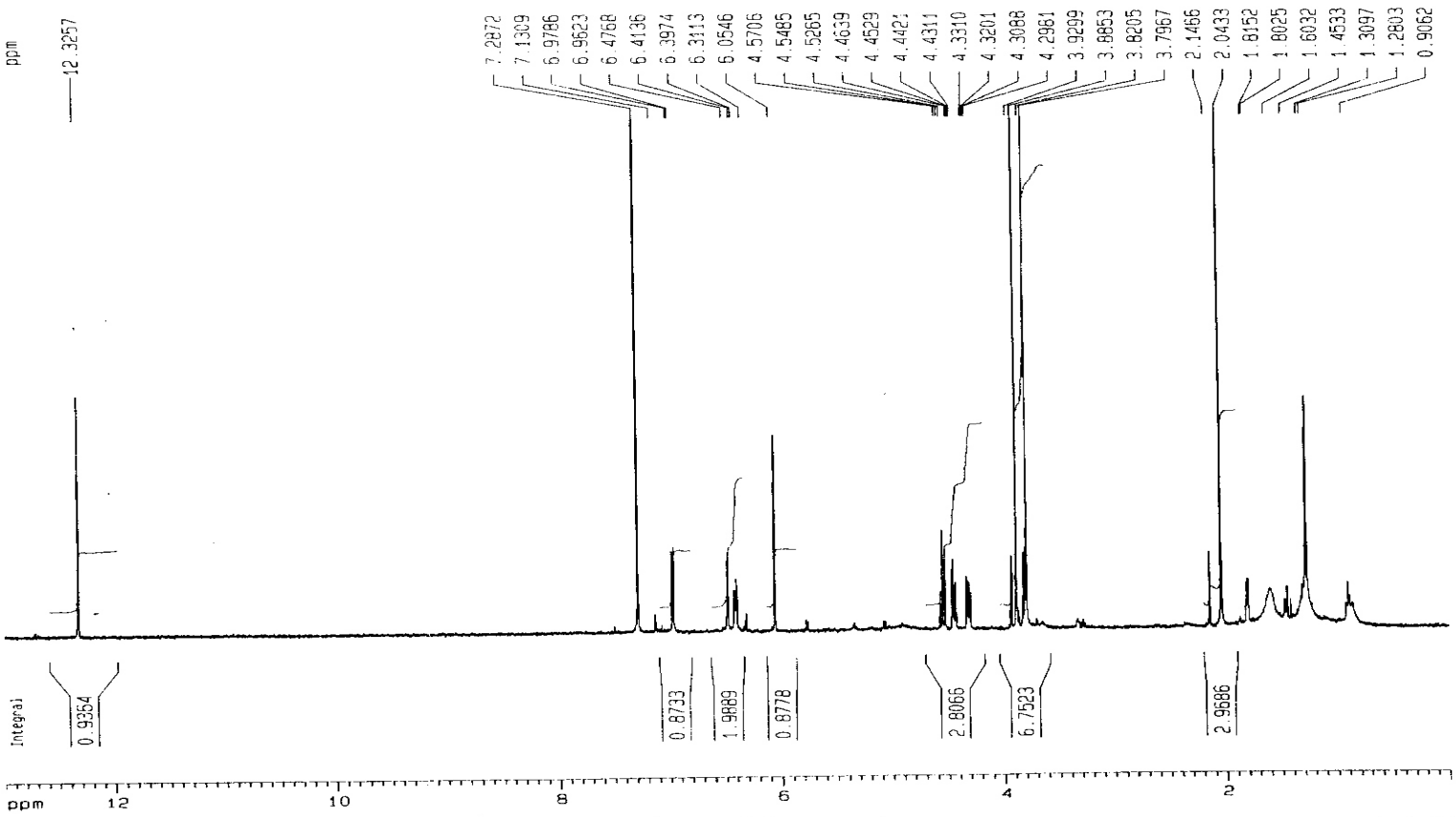
XXII. Mass spectrum of uncinanone E (**4**)



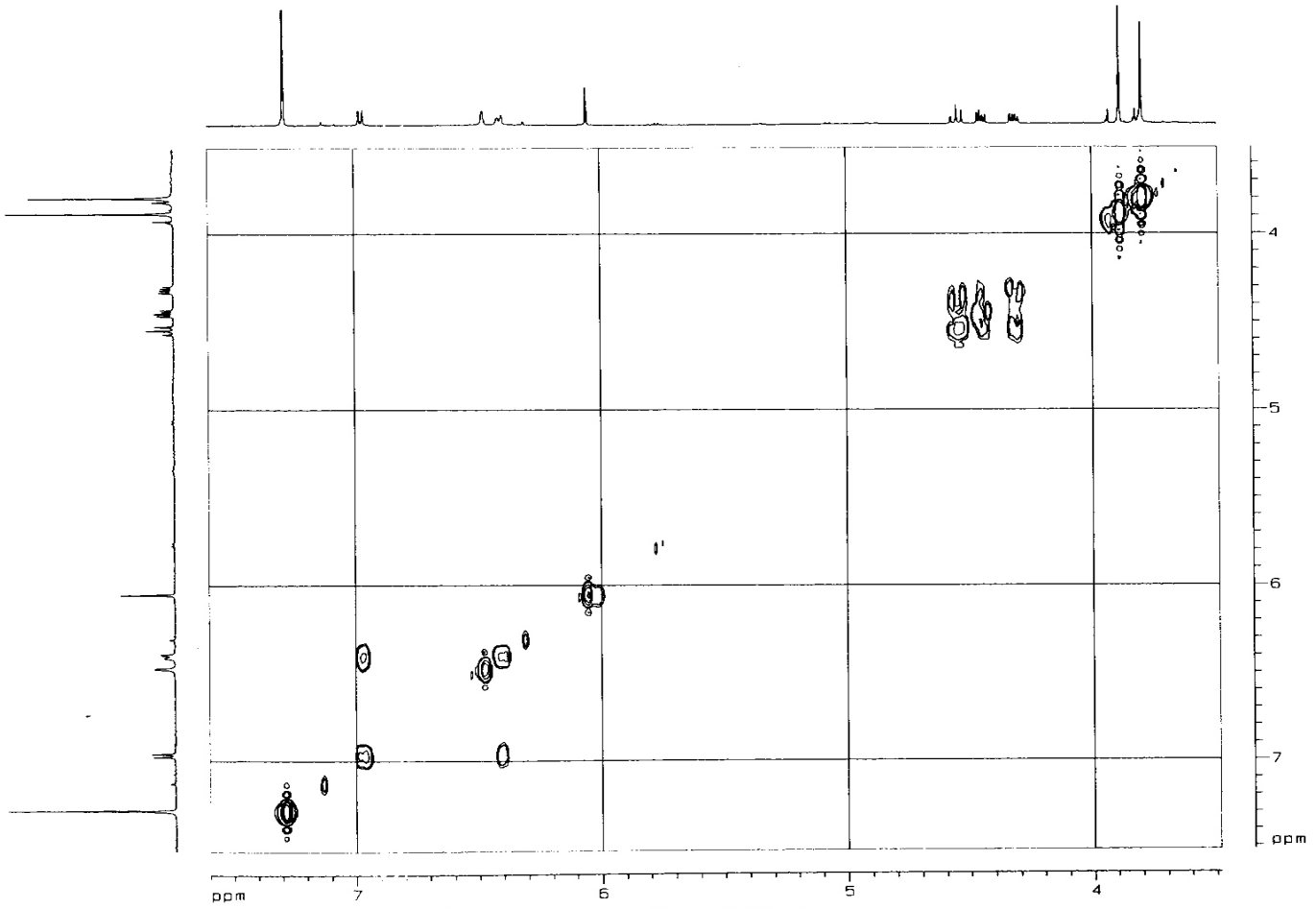
XXIII. ^{13}C-NMR (125 MHz, $CDCl_3$) spectrum of uncinanone E (**4**)



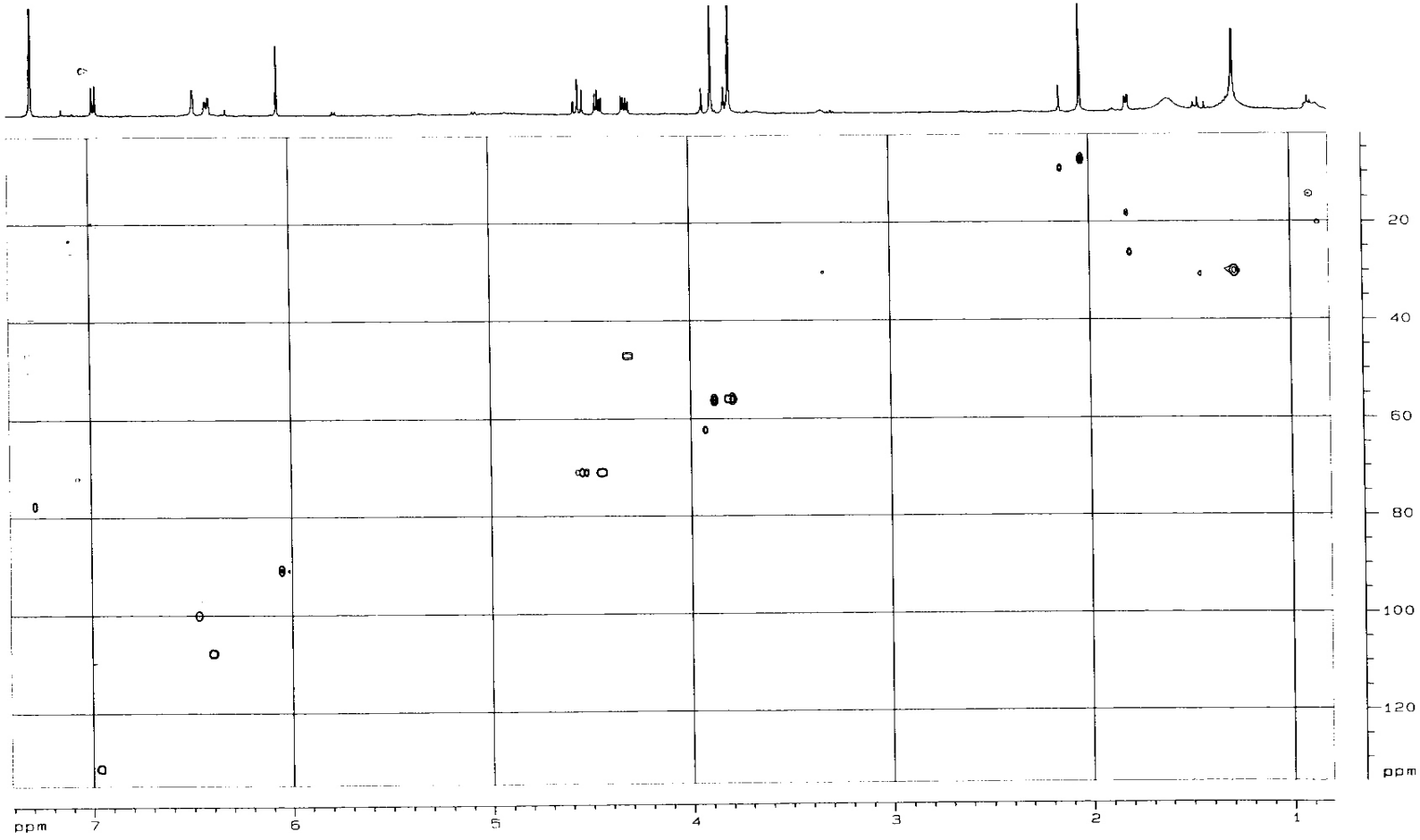
XXIV. ^{1}H-NMR (500 MHz, $CDCl_3$) spectrum of uncinanone E (**4**)



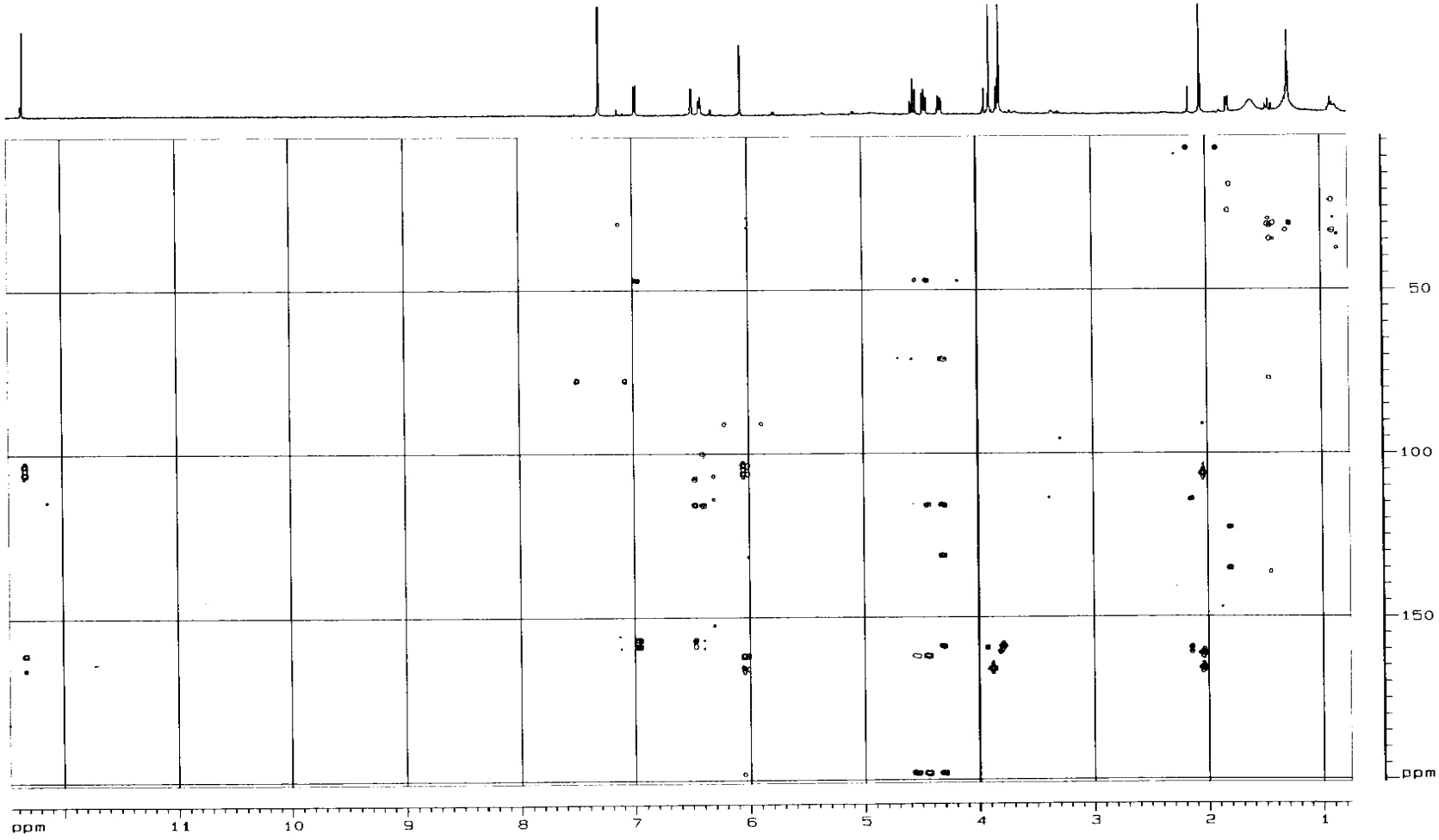
XXV. ^{1}H, ^{1}H-COSY spectrum of uncinanone E (**4**)



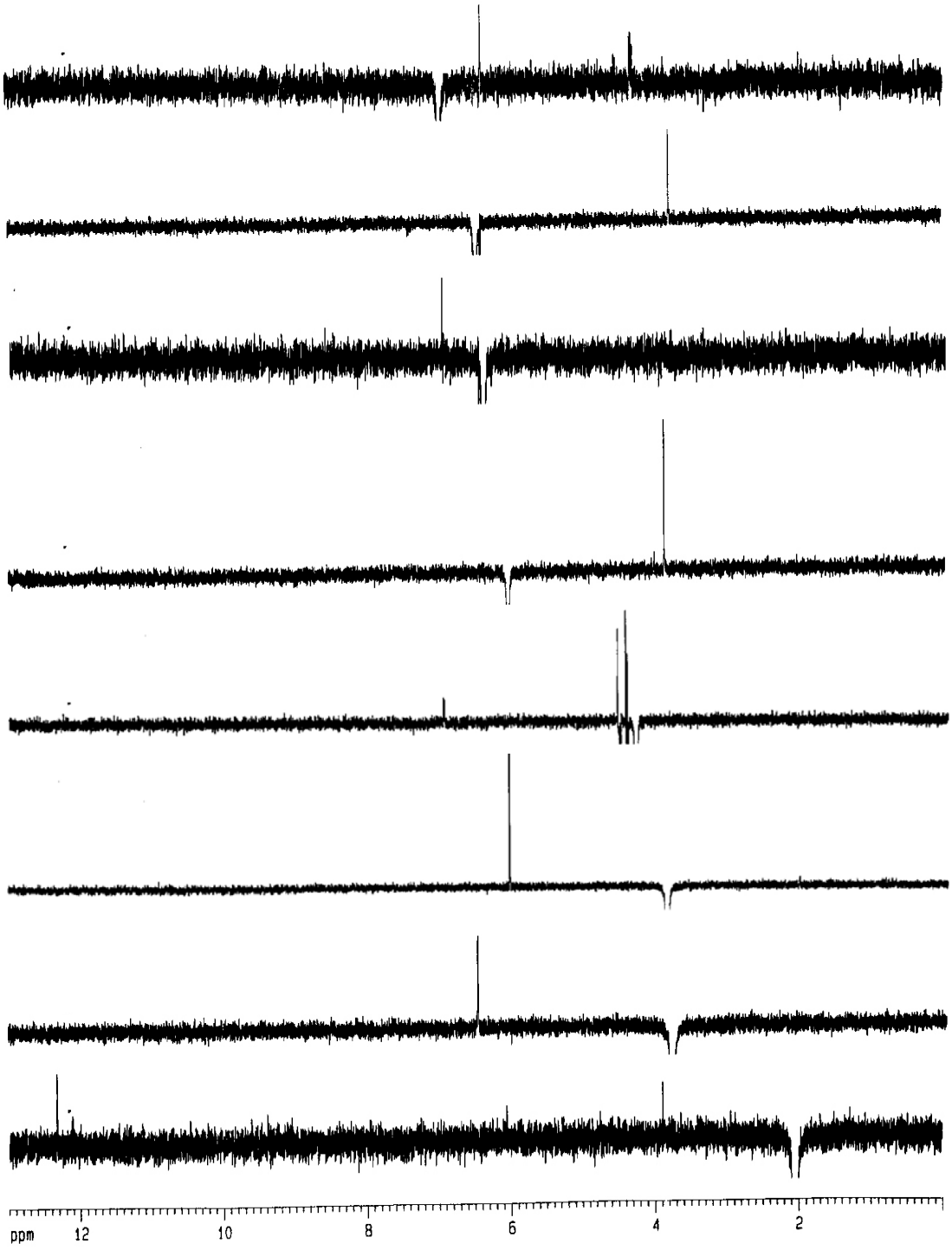
XXVI. HMQC spectrum of uncinanone E (**4**)



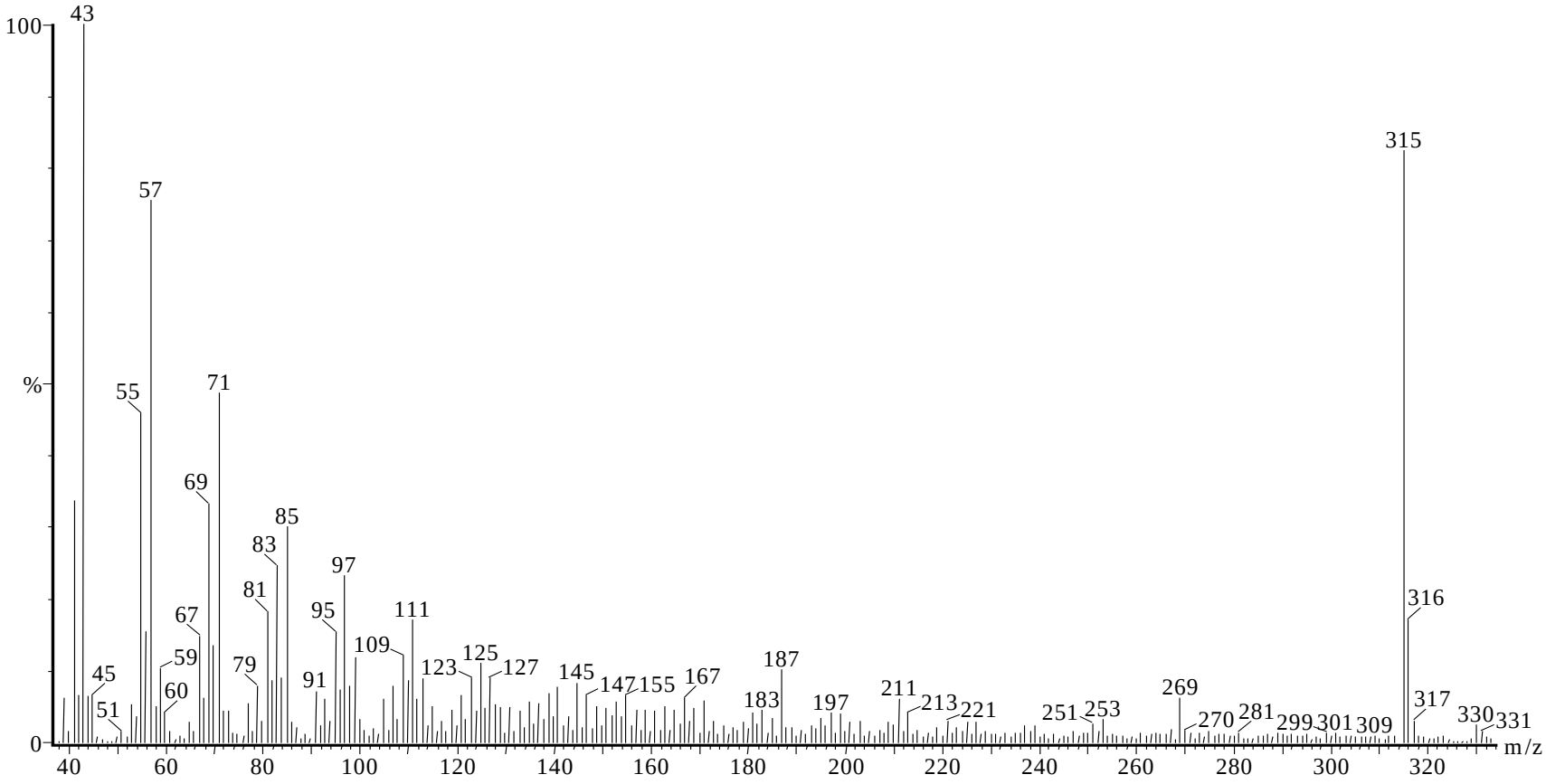
XXVII. HMBC spectrum of uncinanone E (**4**)



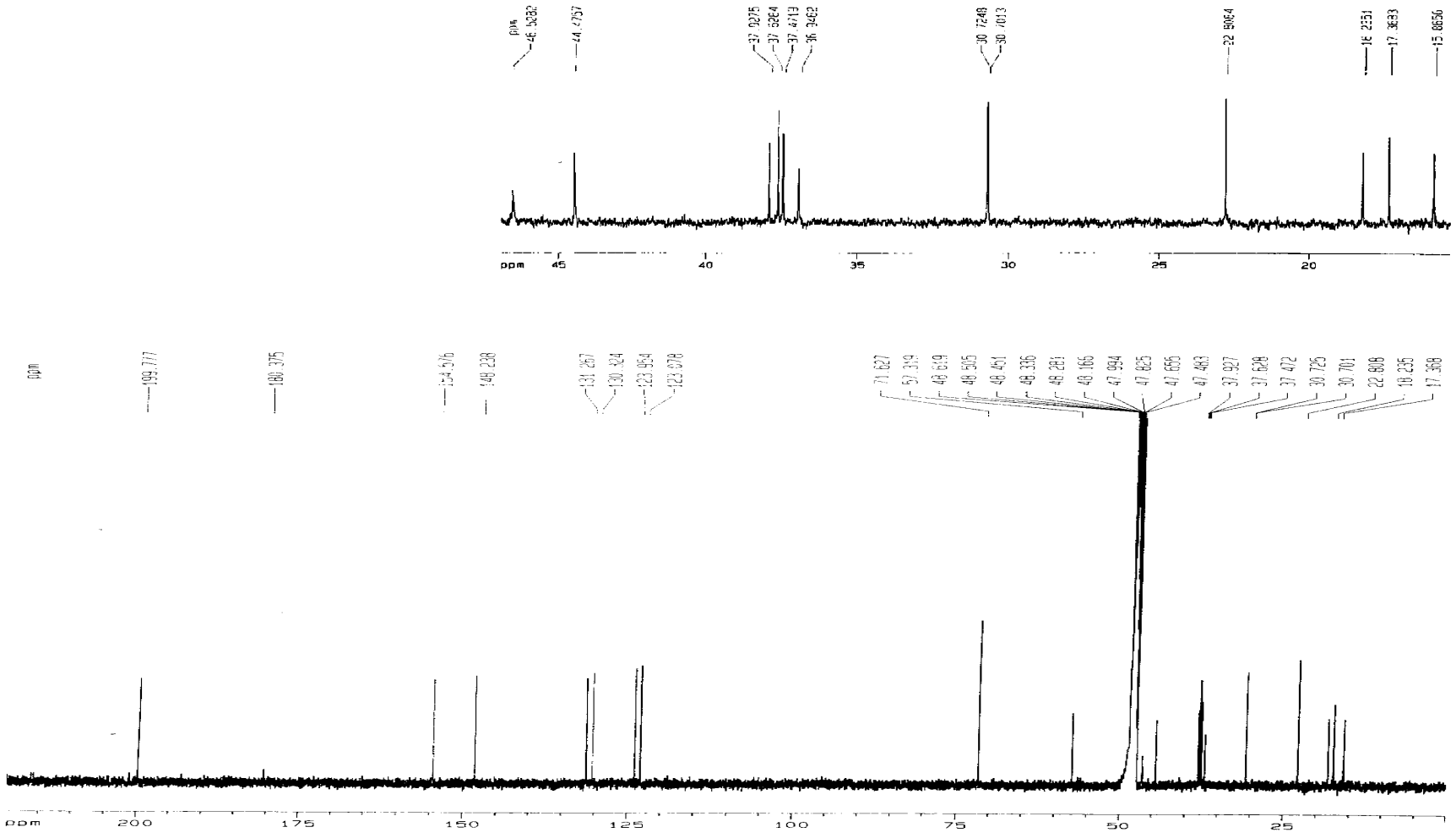
XXVIII. GOESY spectra of uncinanone E (**4**)



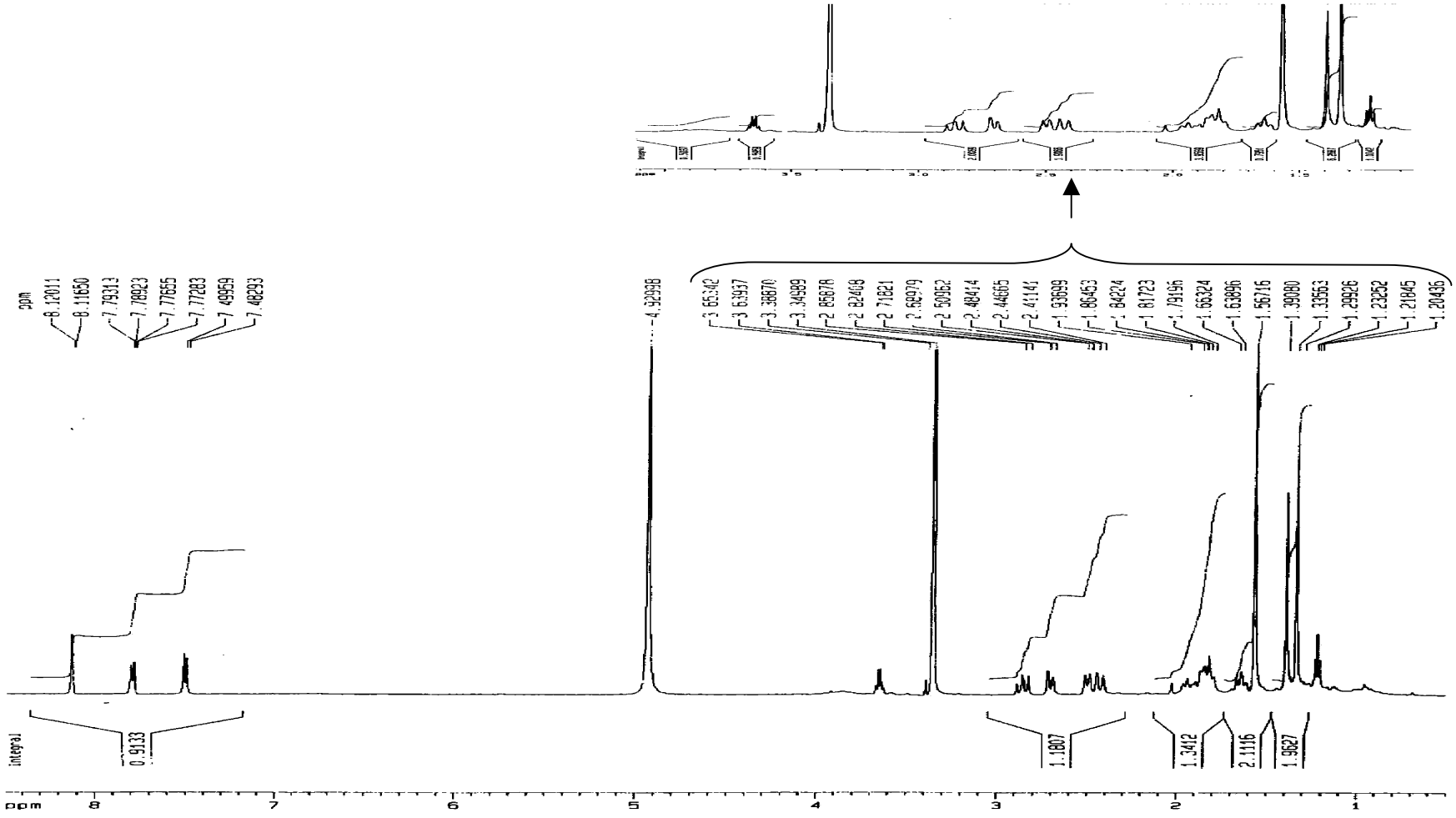
XXIX. Mass spectrum of 7-oxo-15-hydroxydehydroabietic acid (**5**)



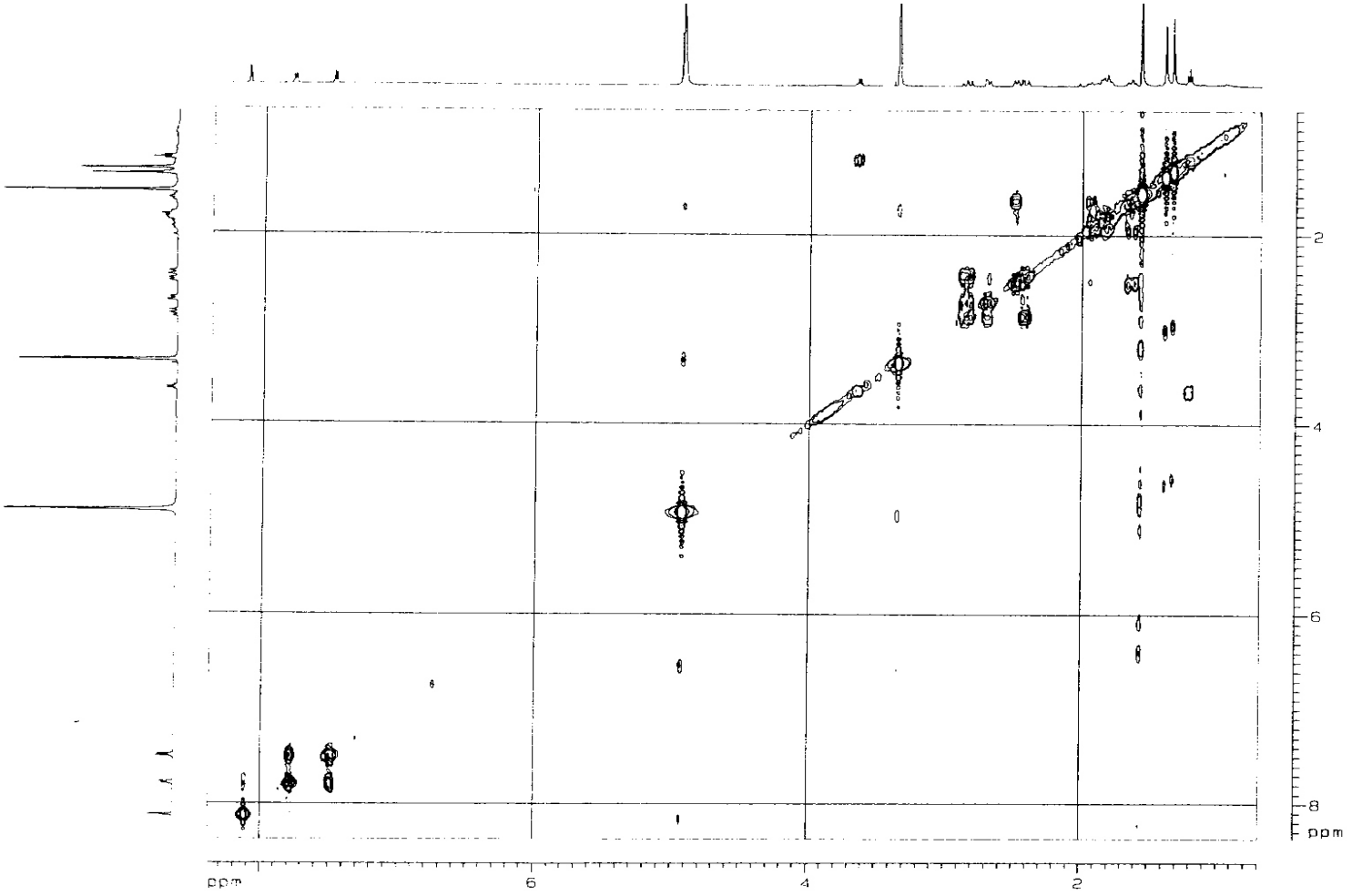
XXX. ^{13}C-NMR (125 MHz, MeOH-d_4) spectrum of 7-oxo-15-hydroxydehydroabietic acid (**5**)



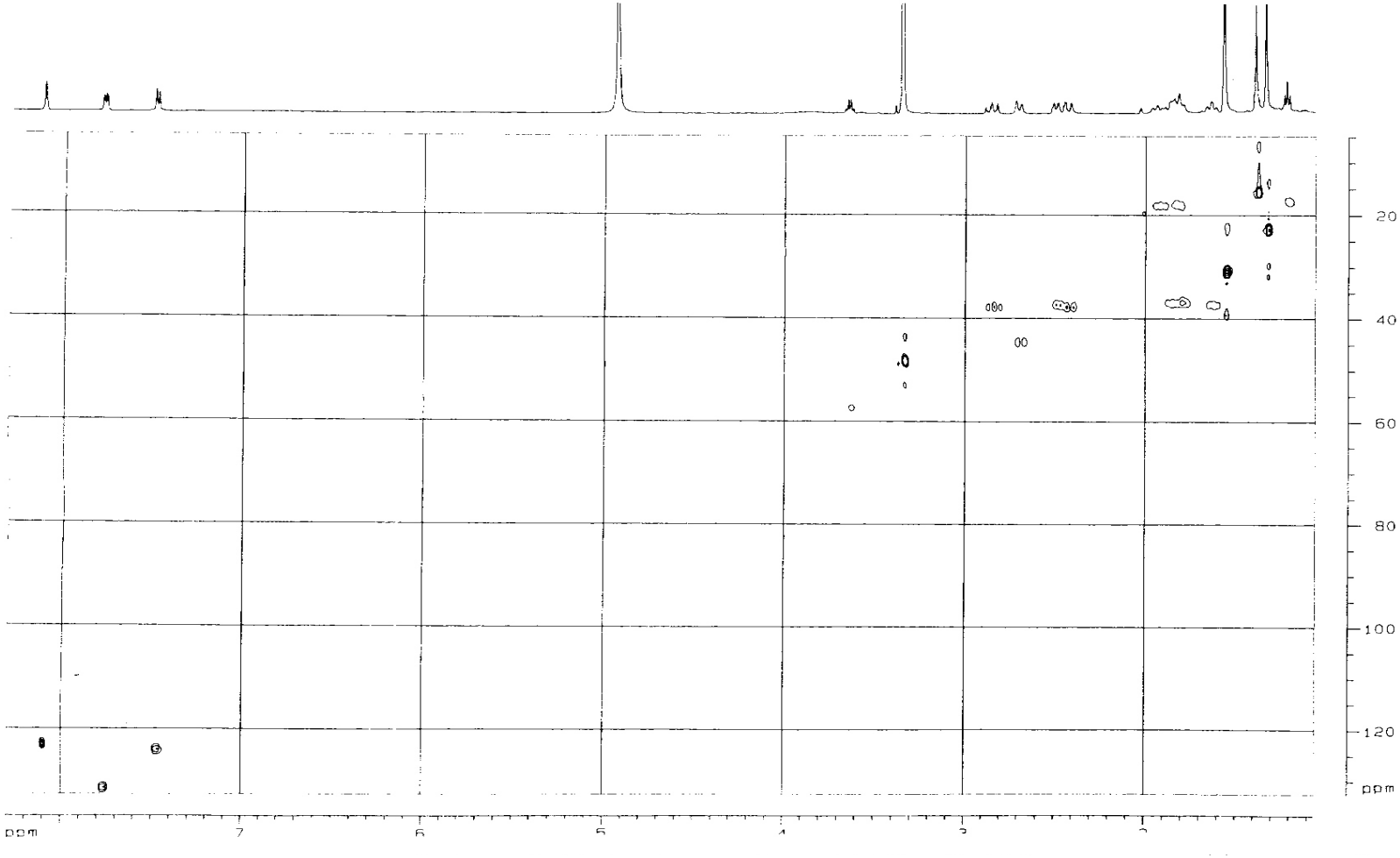
XXXI. ^{1}H-NMR (500 MHz, MeOH-d_4) spectrum of 7-oxo-15-hydroxydehydroabietic acid (**5**)



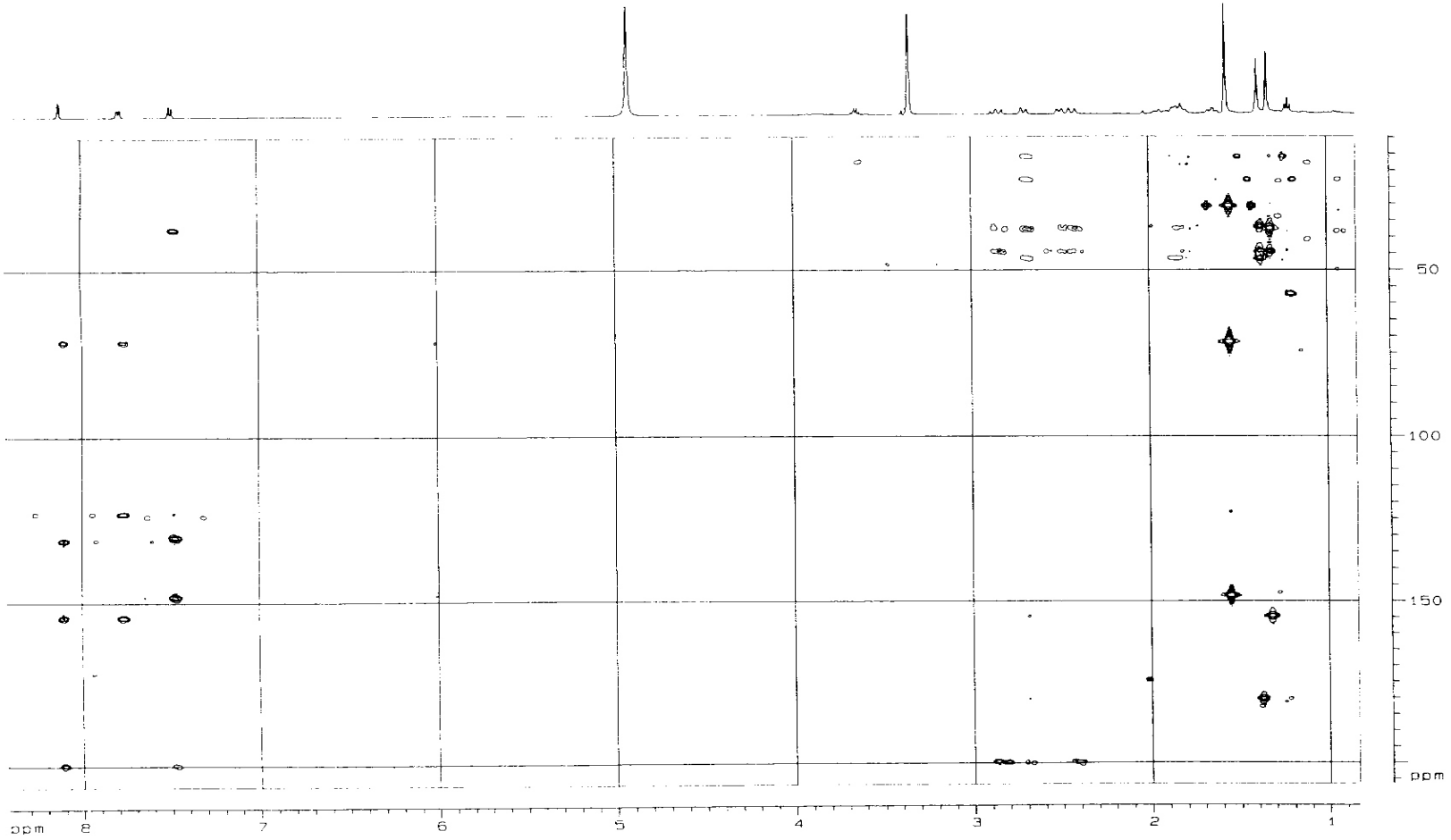
XXXII. ^{1}H, ^{1}H-COSY spectrum of 7-oxo-15-hydroxydehydroabietic acid (**5**)



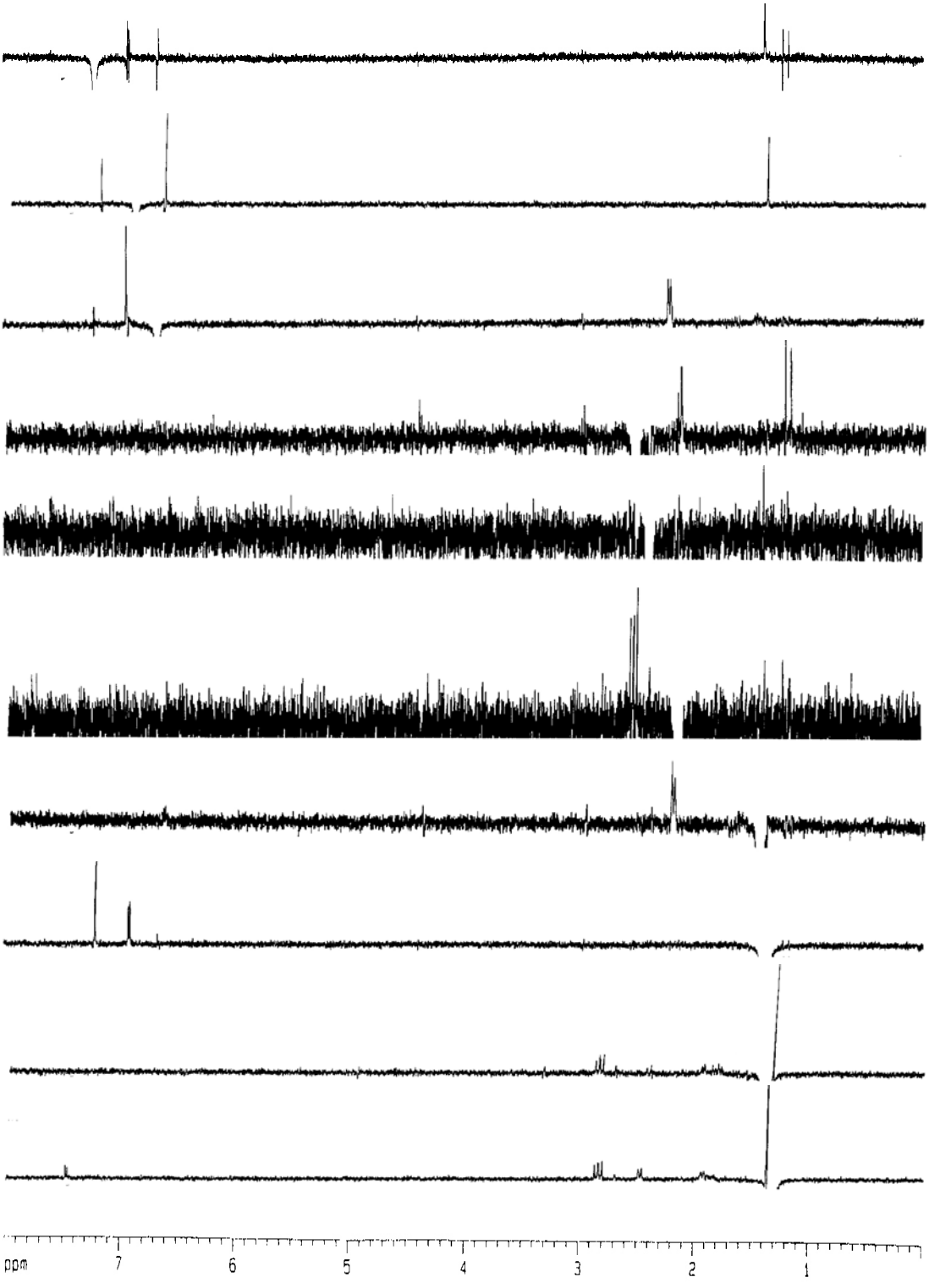
XXXIII. HMQC spectrum of 7-oxo-15-hydroxydehydroabietic acid (**5**)



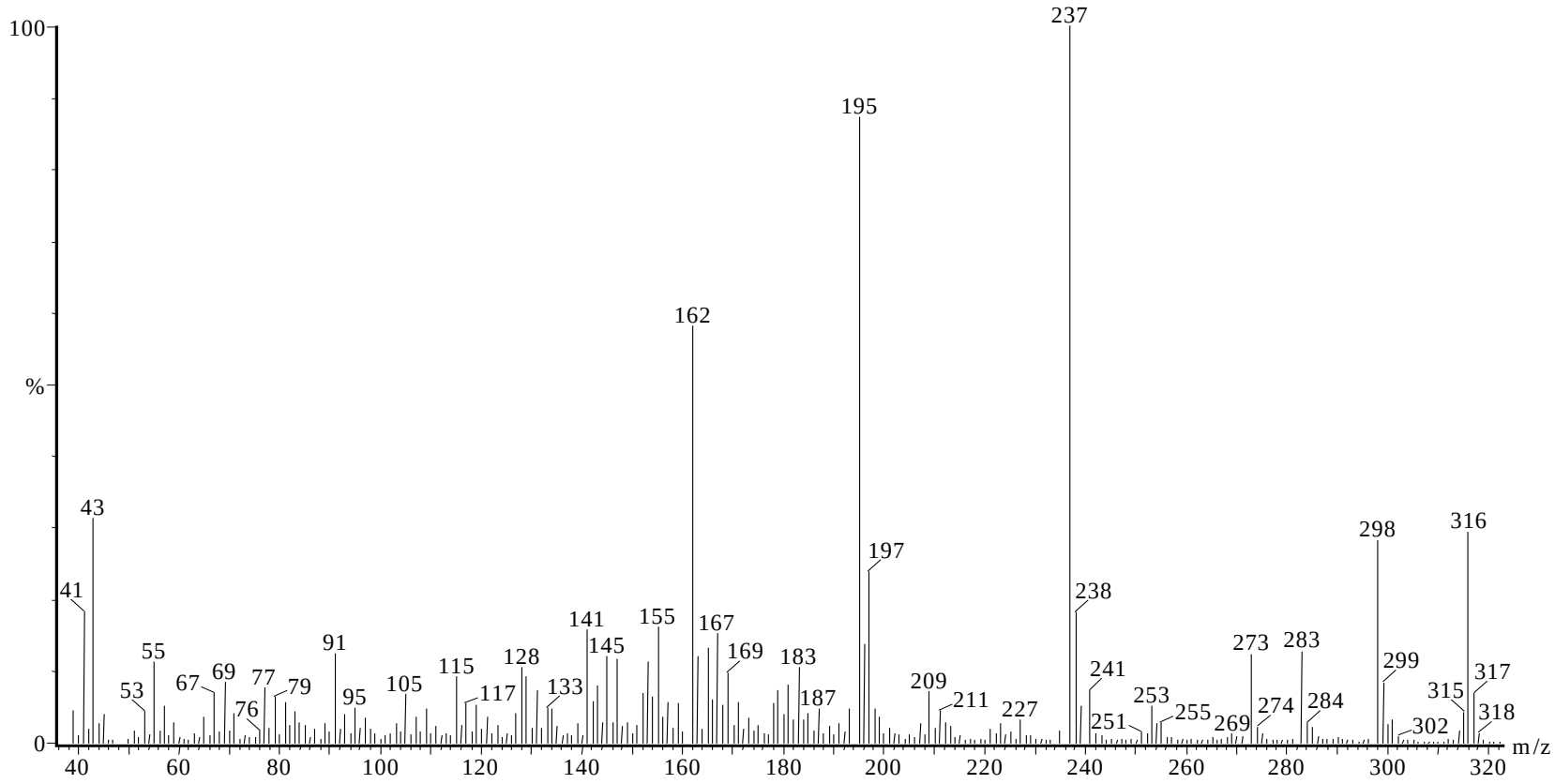
XXXIV. HMBC spectrum of 7-oxo-15-hydroxydehydroabietic acid (**5**)



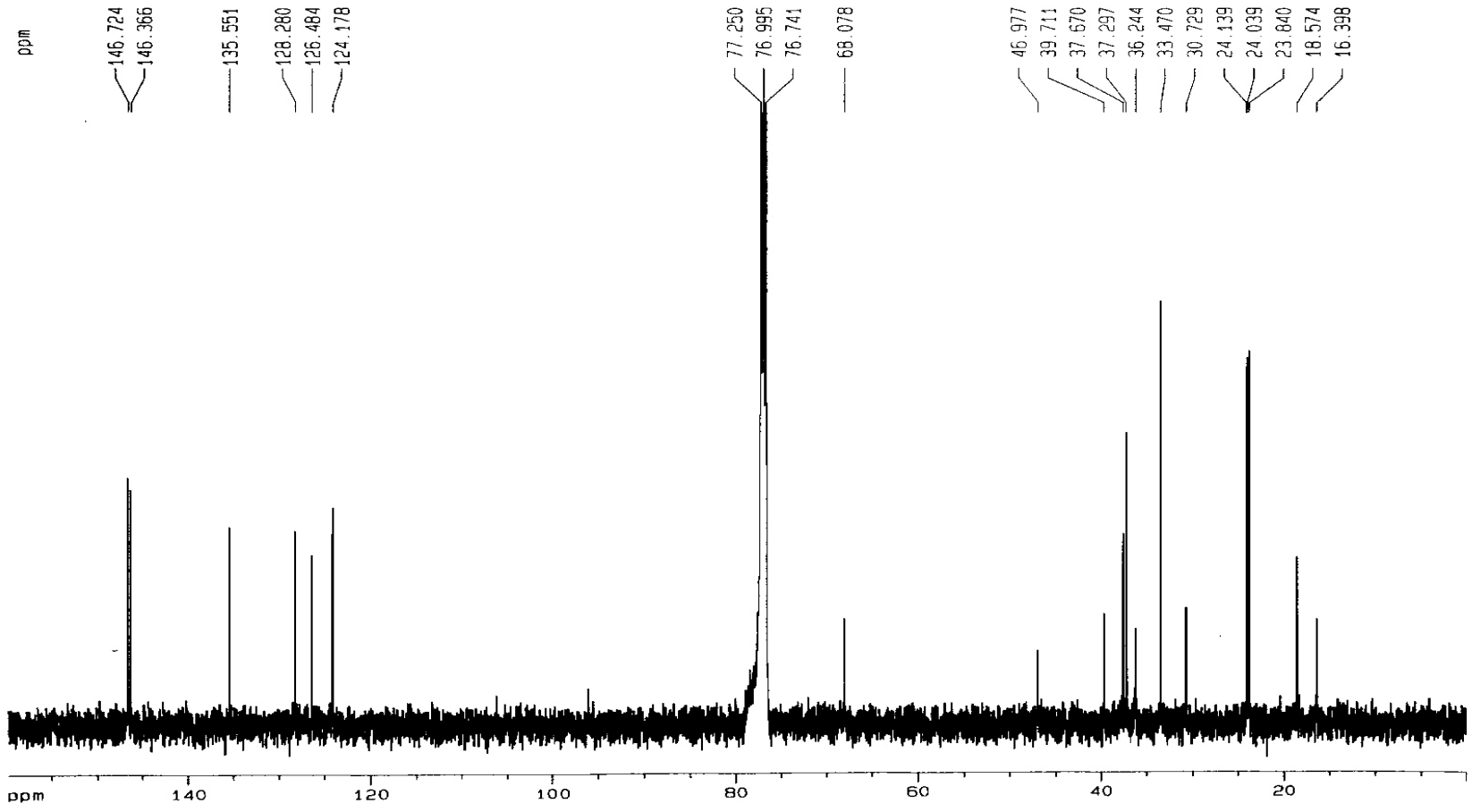
XXXV. GOESY spectra of 7-oxo-15-hydroxydehydroabietic acid (**5**)



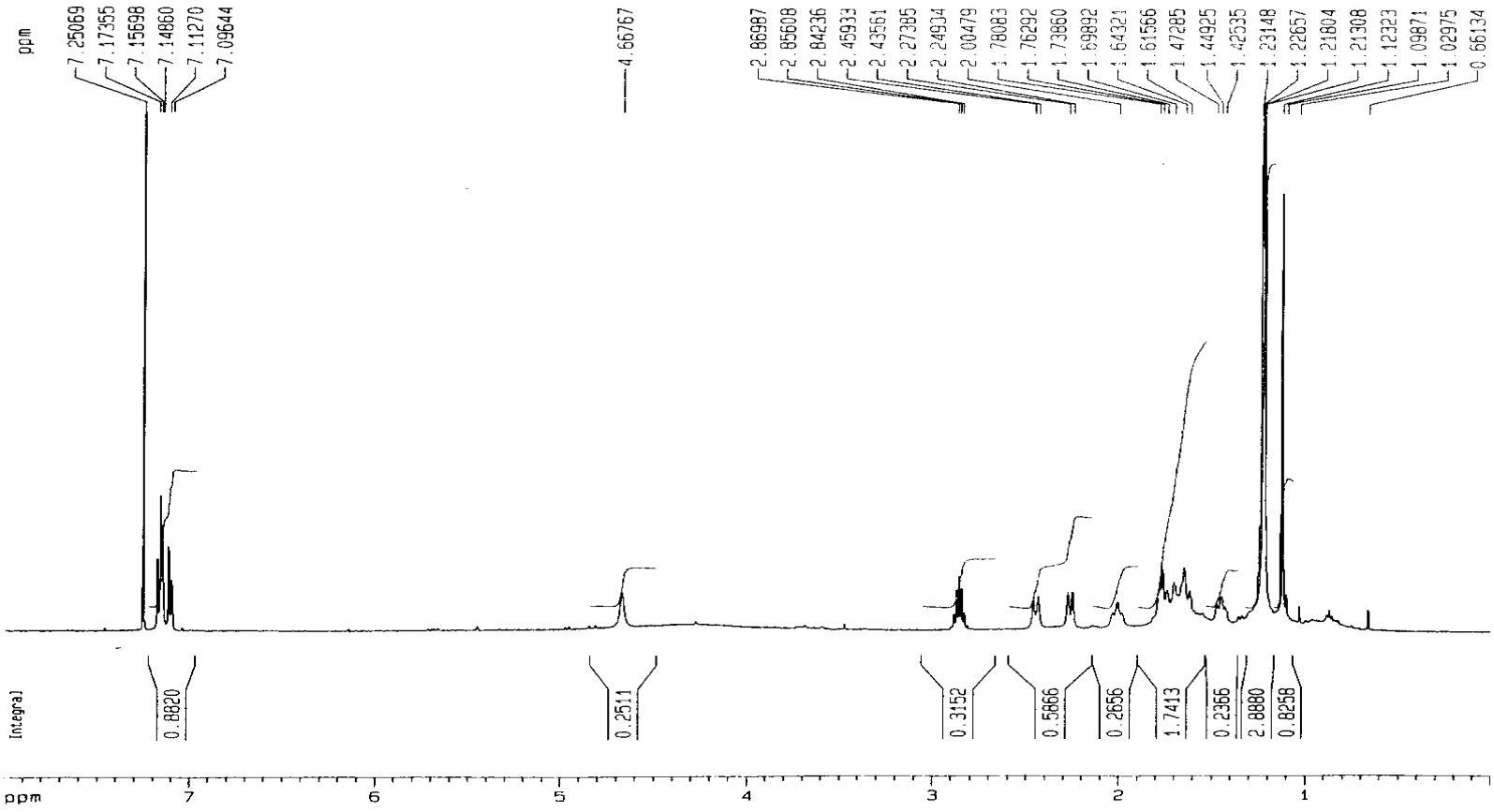
XXXVI. Mass spectrum of 7-hydroxy-8,11,13-abietatrien-18-oic acid (**6**)



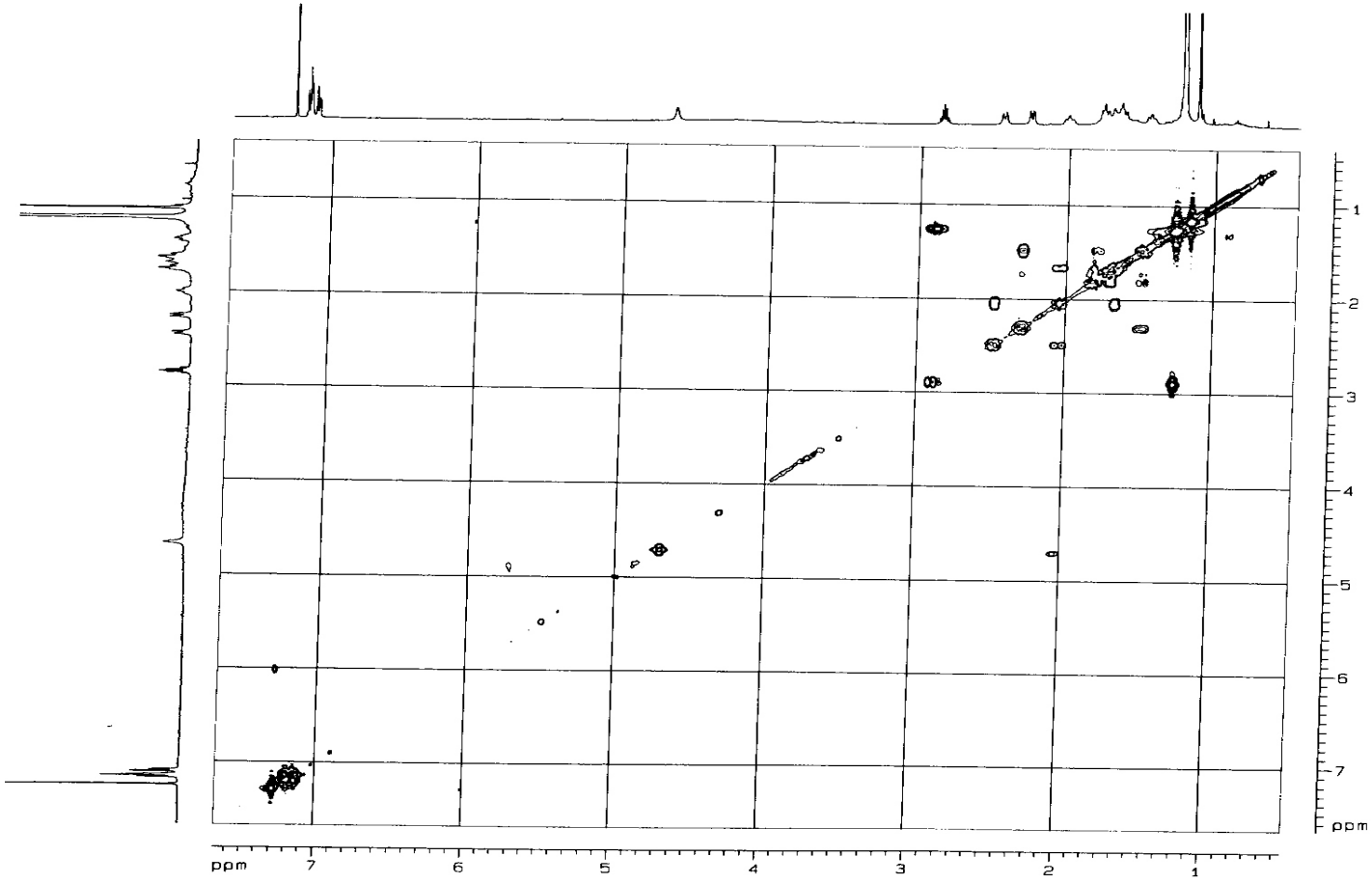
XXXVII. ^{13}C-NMR ($CDCl_3$, 125MHz) spectrum of 7-hydroxy-8,11,13-abietatrien-18-oic acid (**6**)



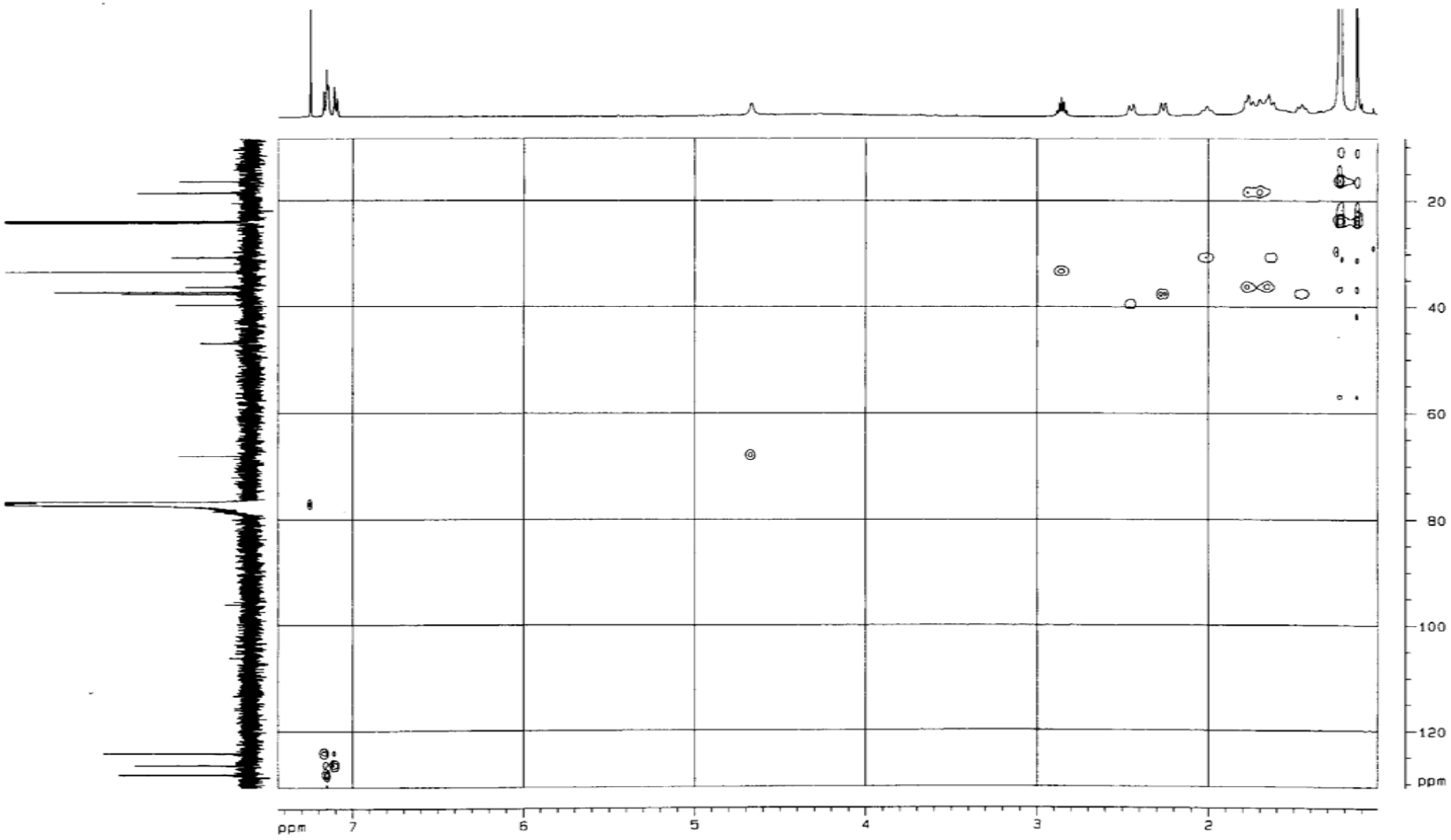
XXXVIII. ^{1}H-NMR ($CDCl_3$, 500MHz) spectrum of 7-hydroxy-8,11,13-abietatrien-18-oic acid (**6**)



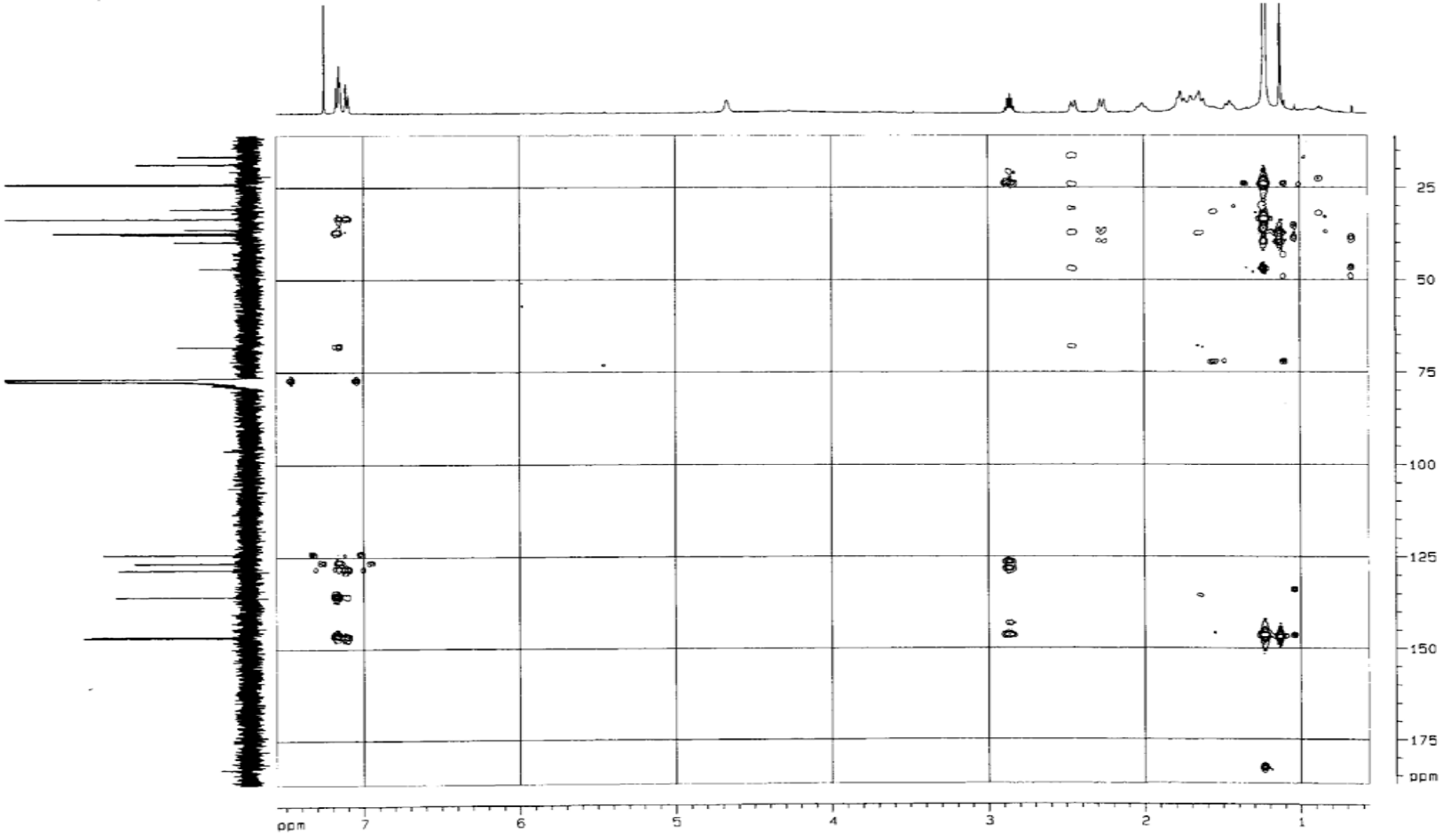
XXXIX. ^{1}H, ^{1}H-COSY spectrum of 7-hydroxy-8,11,13-abietatrien-18-oic acid (**6**)



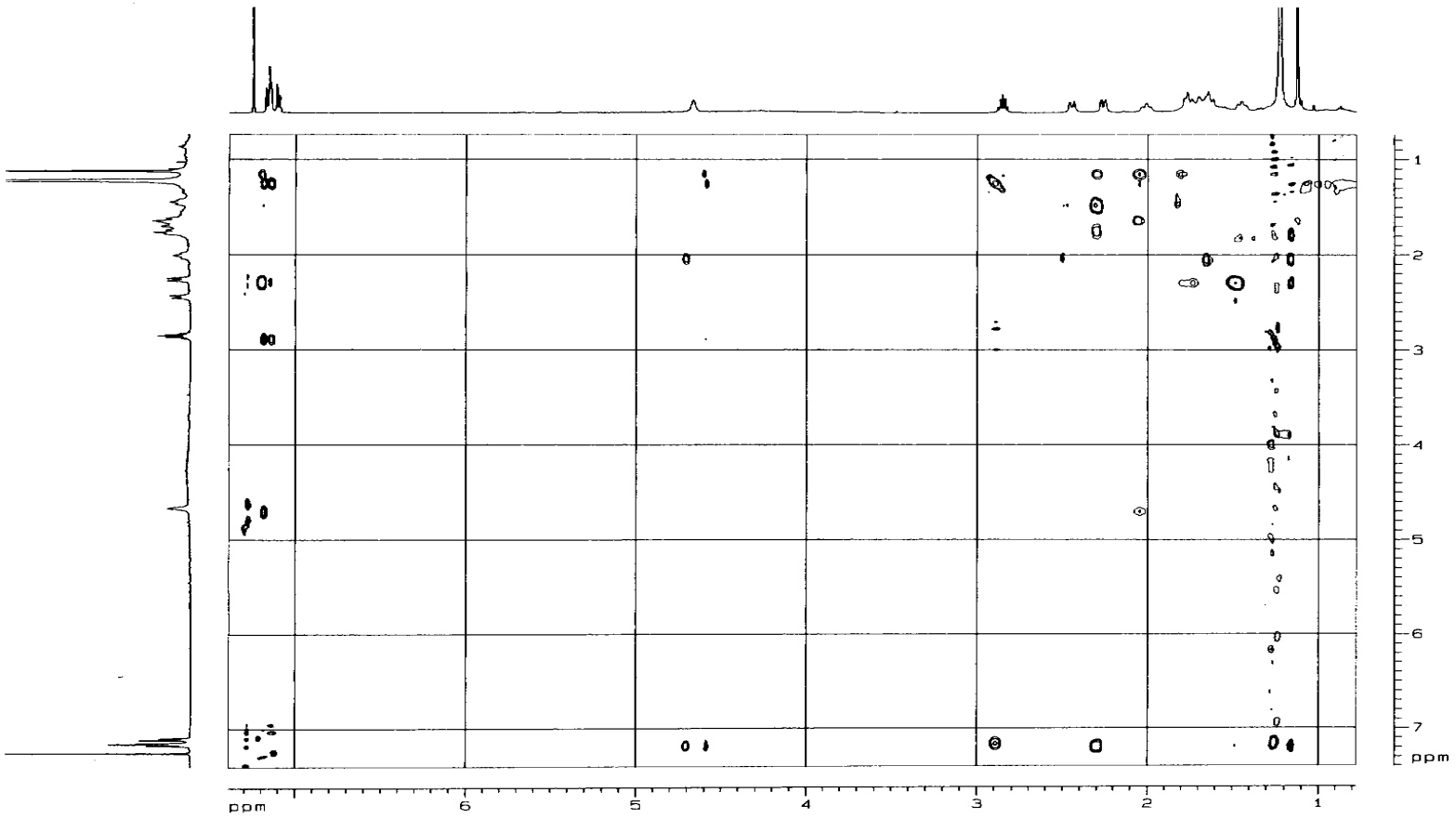
XL. HMQC spectrum of 7-hydroxy-8,11,13-abietatrien-18-oic acid (**6**)



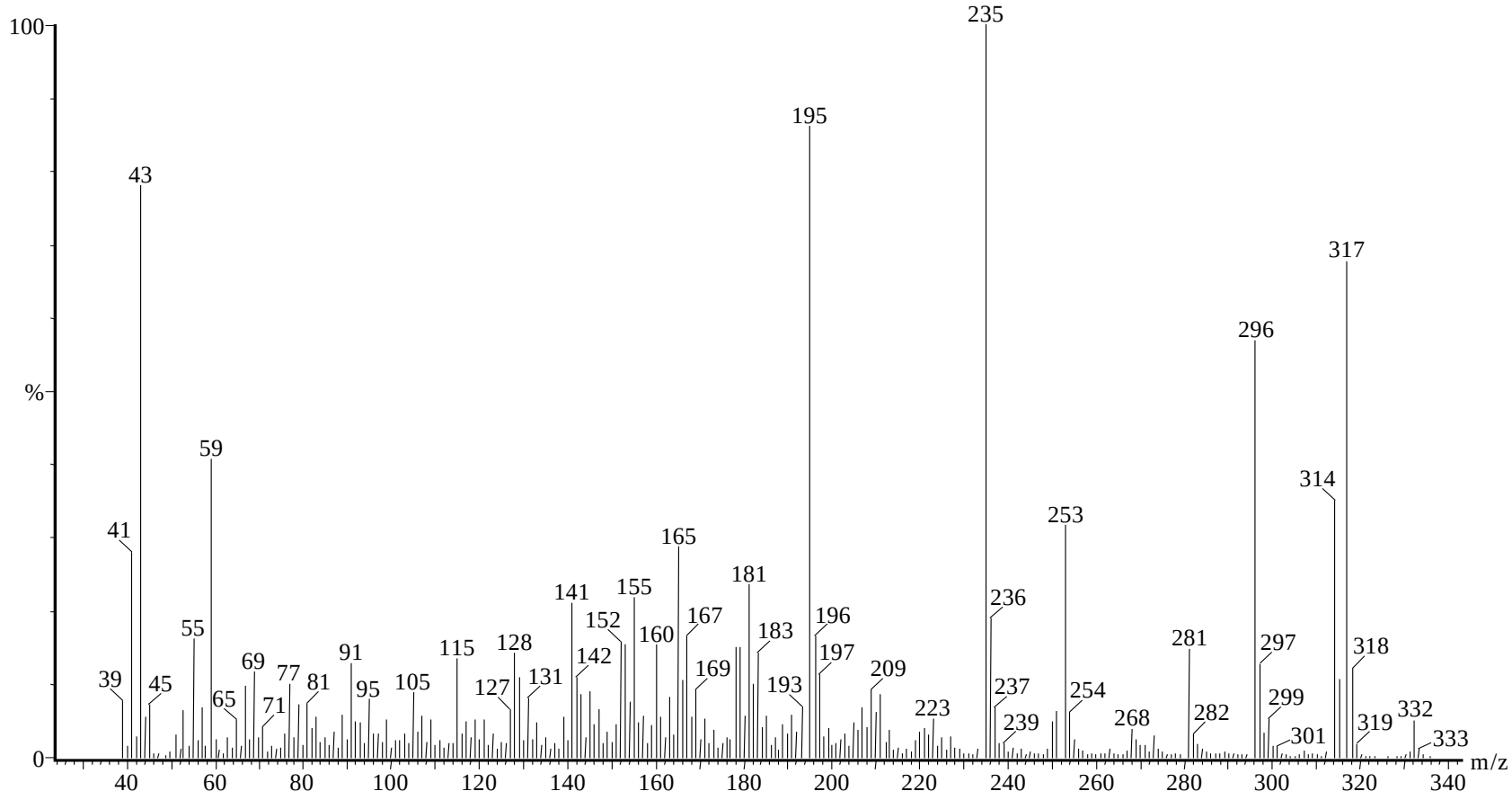
XLI. HMBC spectrum of 7-hydroxy-8,11,13-abietatrien-18-oic acid (**6**)



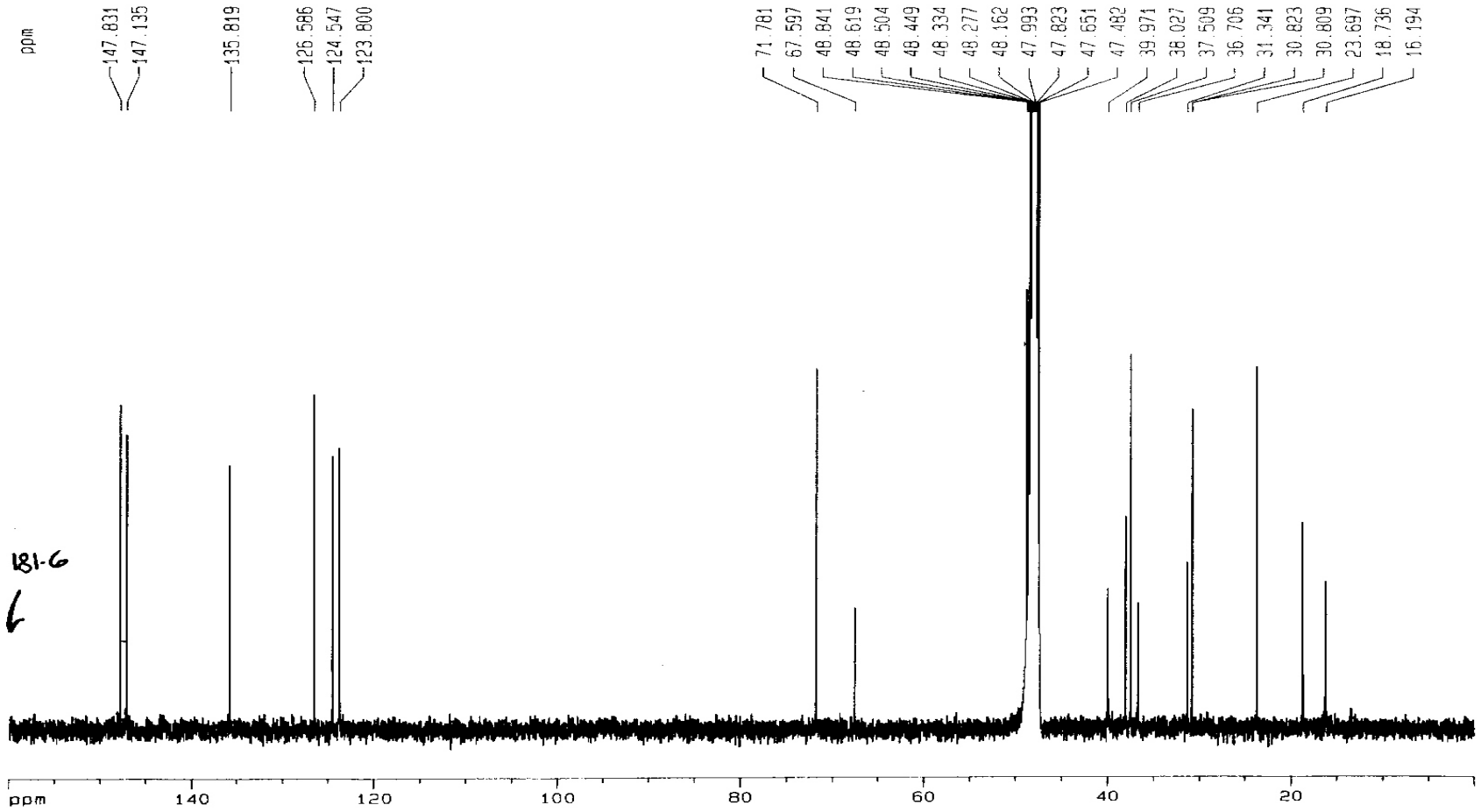
XLII. NOESY spectrum of 7-hydroxy-8,11,13-abietatrien-18-oic acid (**6**)



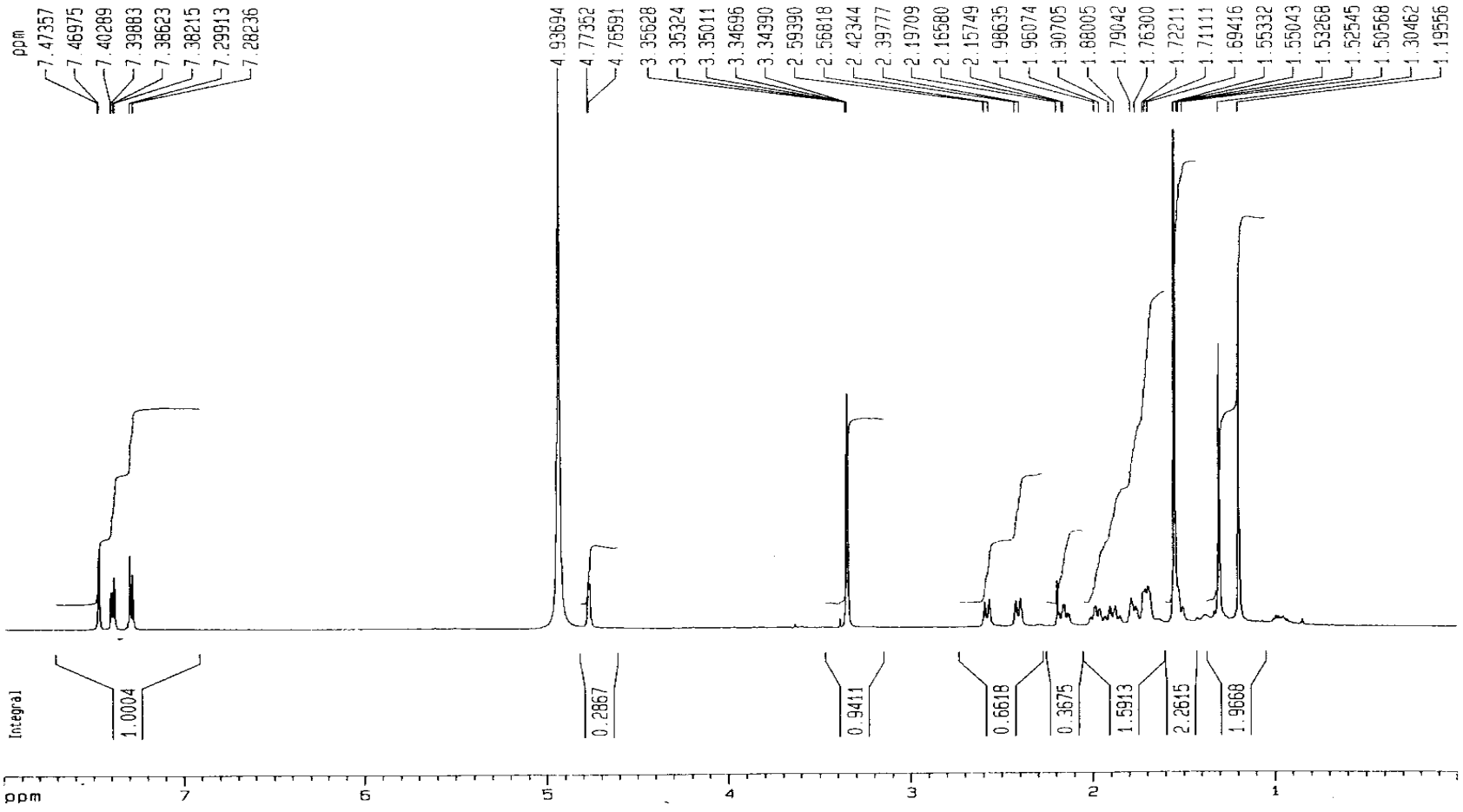
XLIII. Mass spectrum of 7,15-dihydroxy-8,11,13-abietatrien-18-oic acid (**7**)



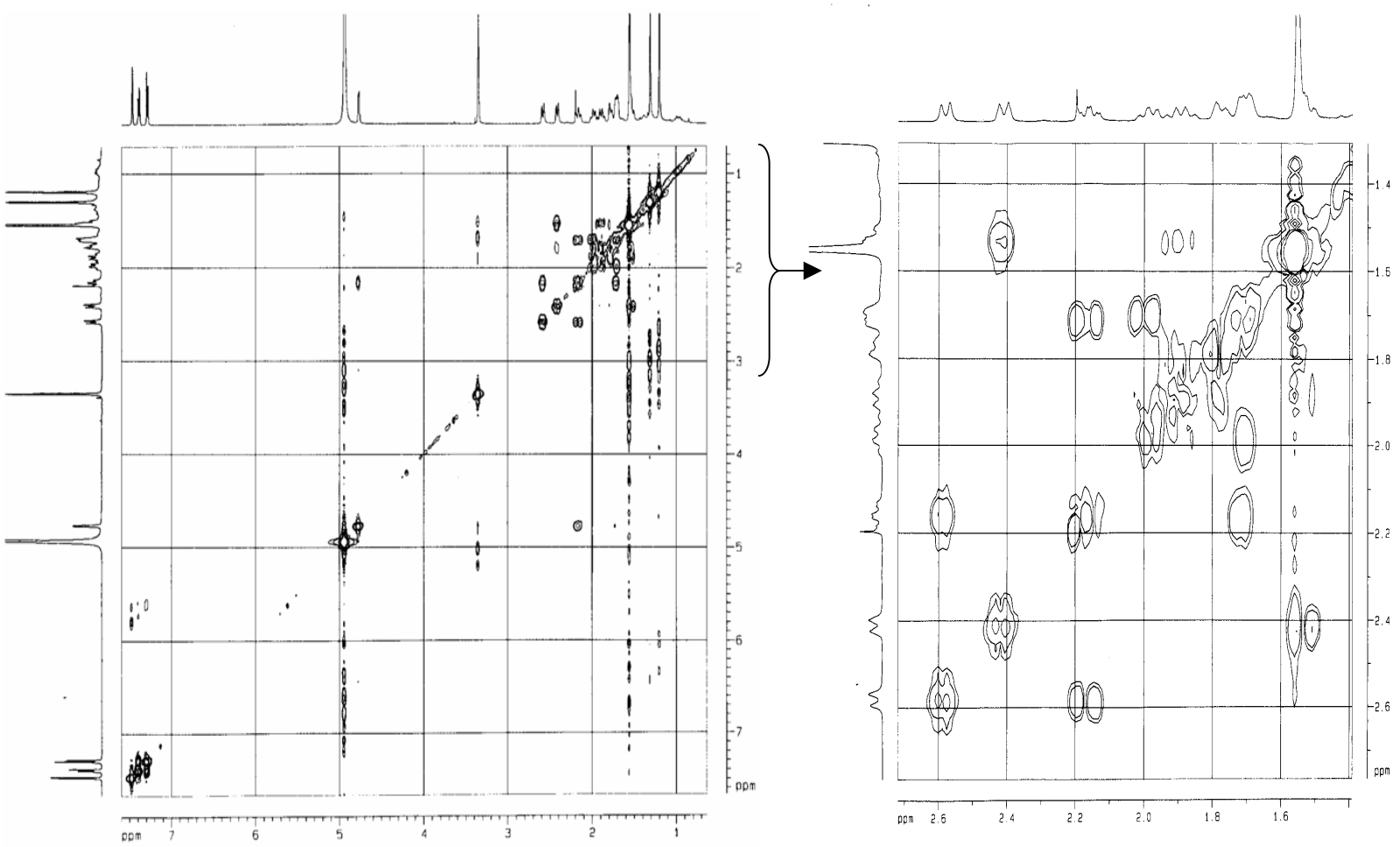
XLIV. ^{13}C-NMR (MeOH-d_4, 125 MHz) spectrum of 7,15-dihydroxy-8,11,13-abietatrien-18-oic acid (**7**)



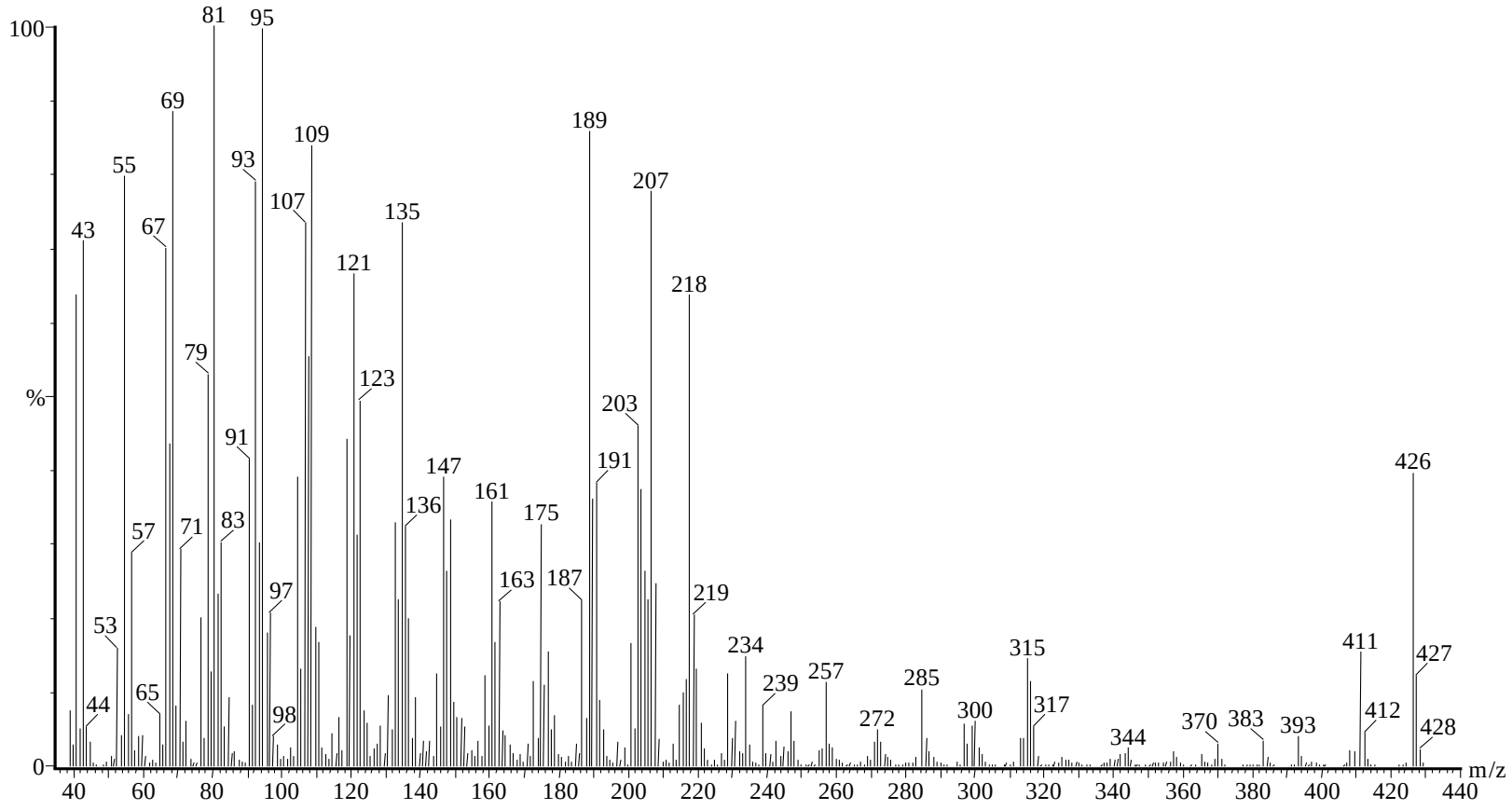
XLV. ^{1}H-NMR (MeOH-d_4, 500 MHz) spectrum of 7,15-dihydroxy-8,11,13-abietatrien-18-oic acid (**7**)



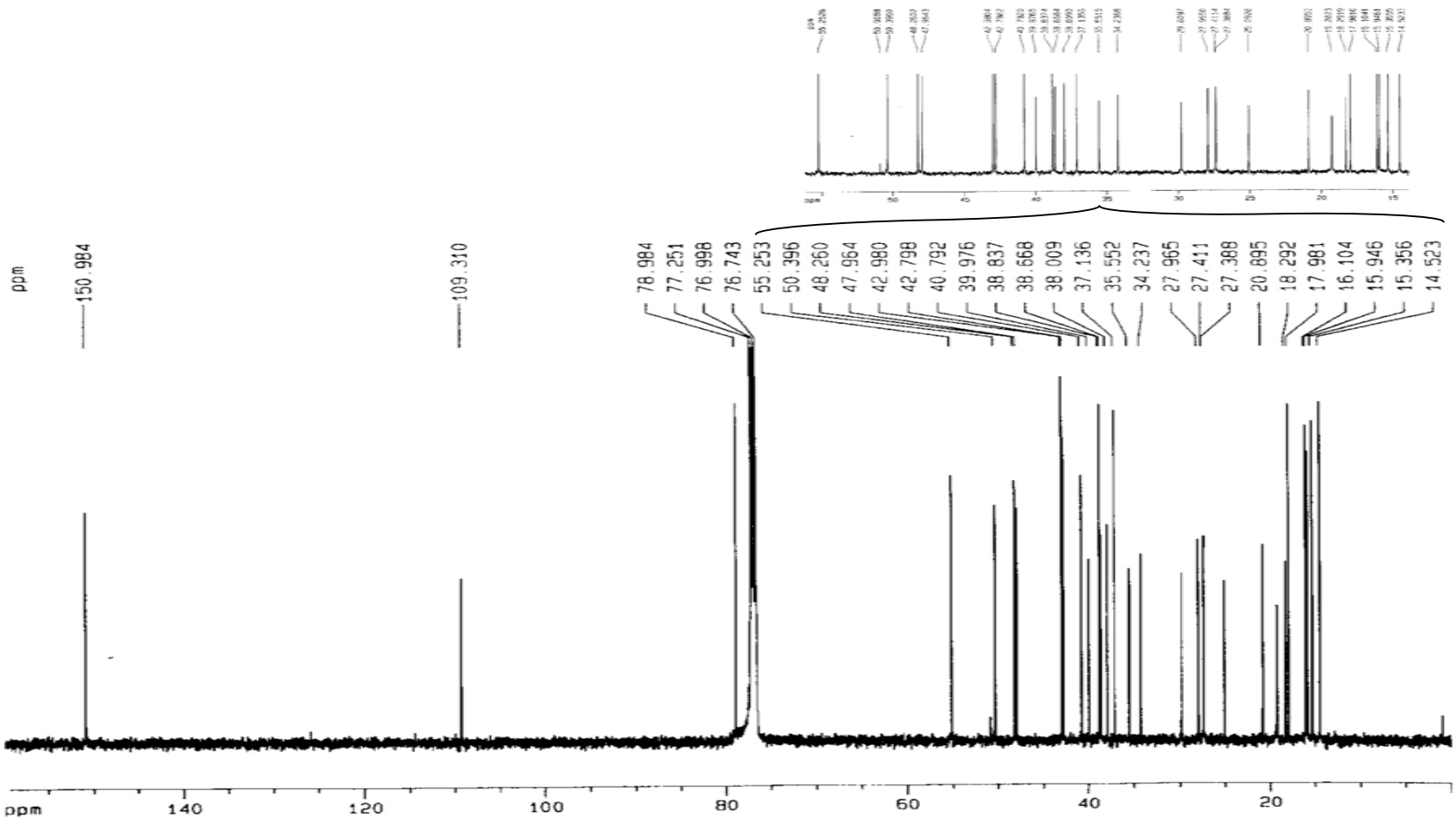
XLVI. $^{1}H,^{1}H$-COSY spectrum of 7,15-dihydroxy-8,11,13-abietatrien-18-oic acid (**7**)



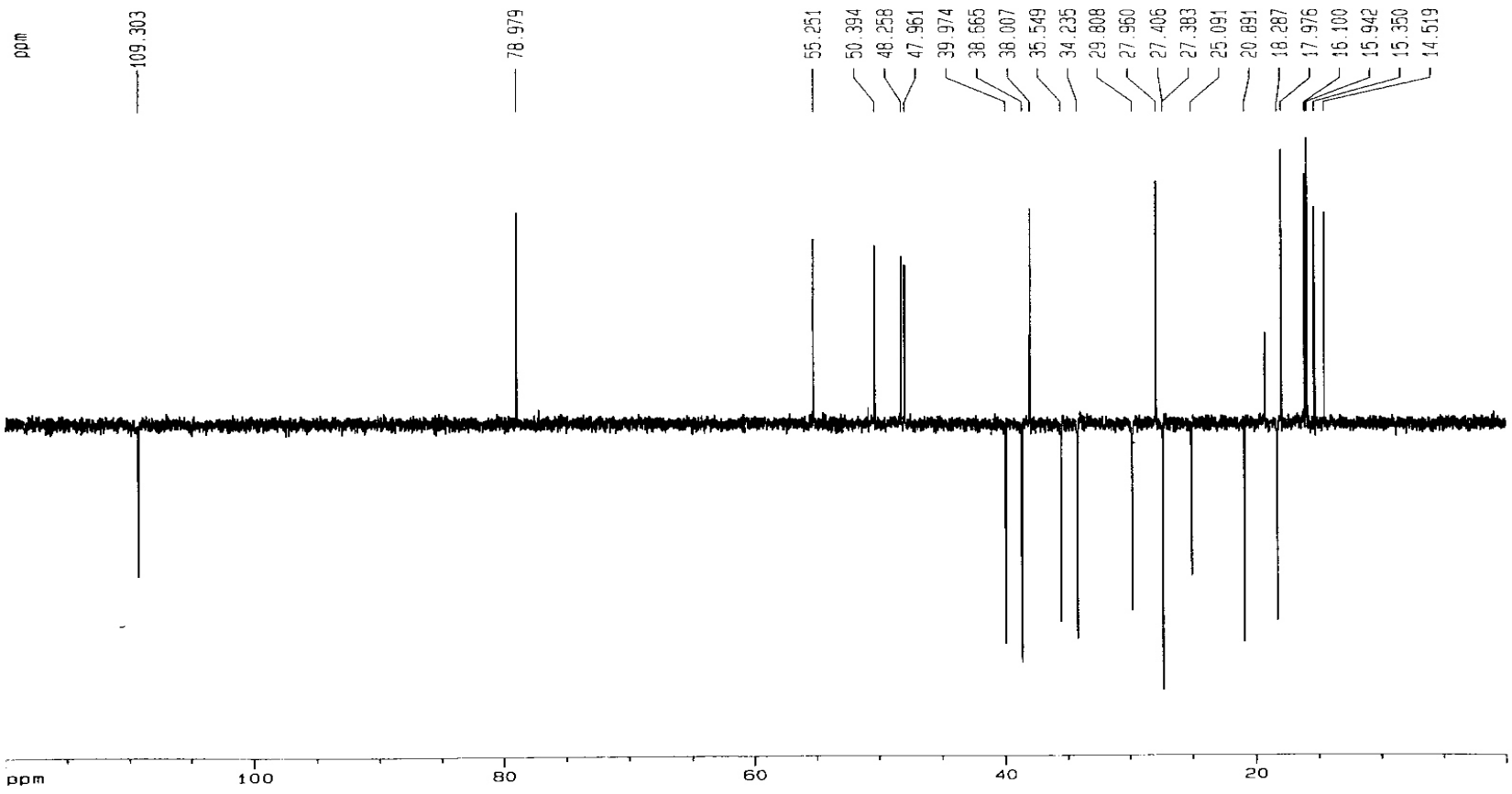
XLVII. Mass spectrum of lupeol (**8**)



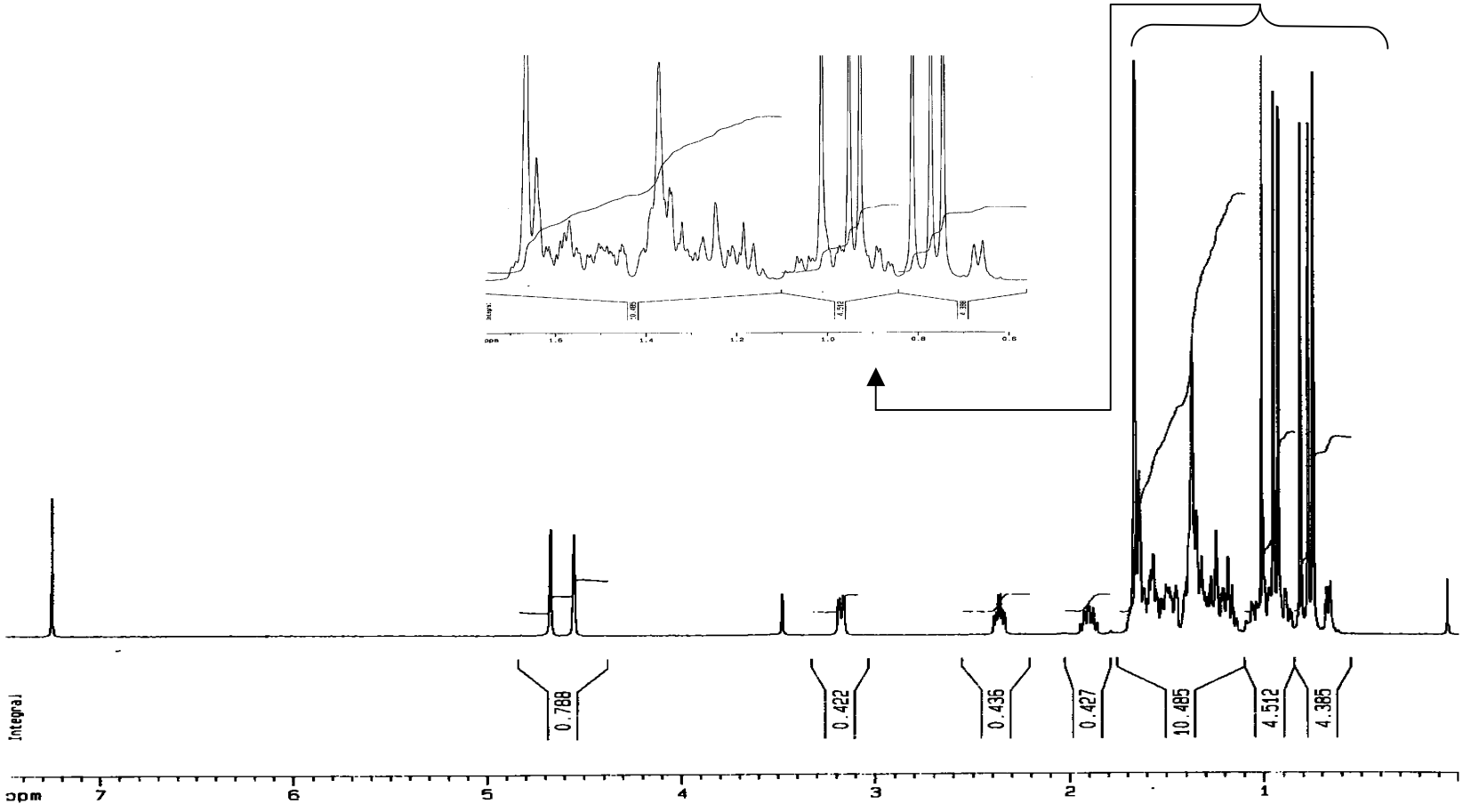
XLVIII. ^{13}C-NMR (125 MHz, $CDCl_3$) spectrum of lupeol (**8**)



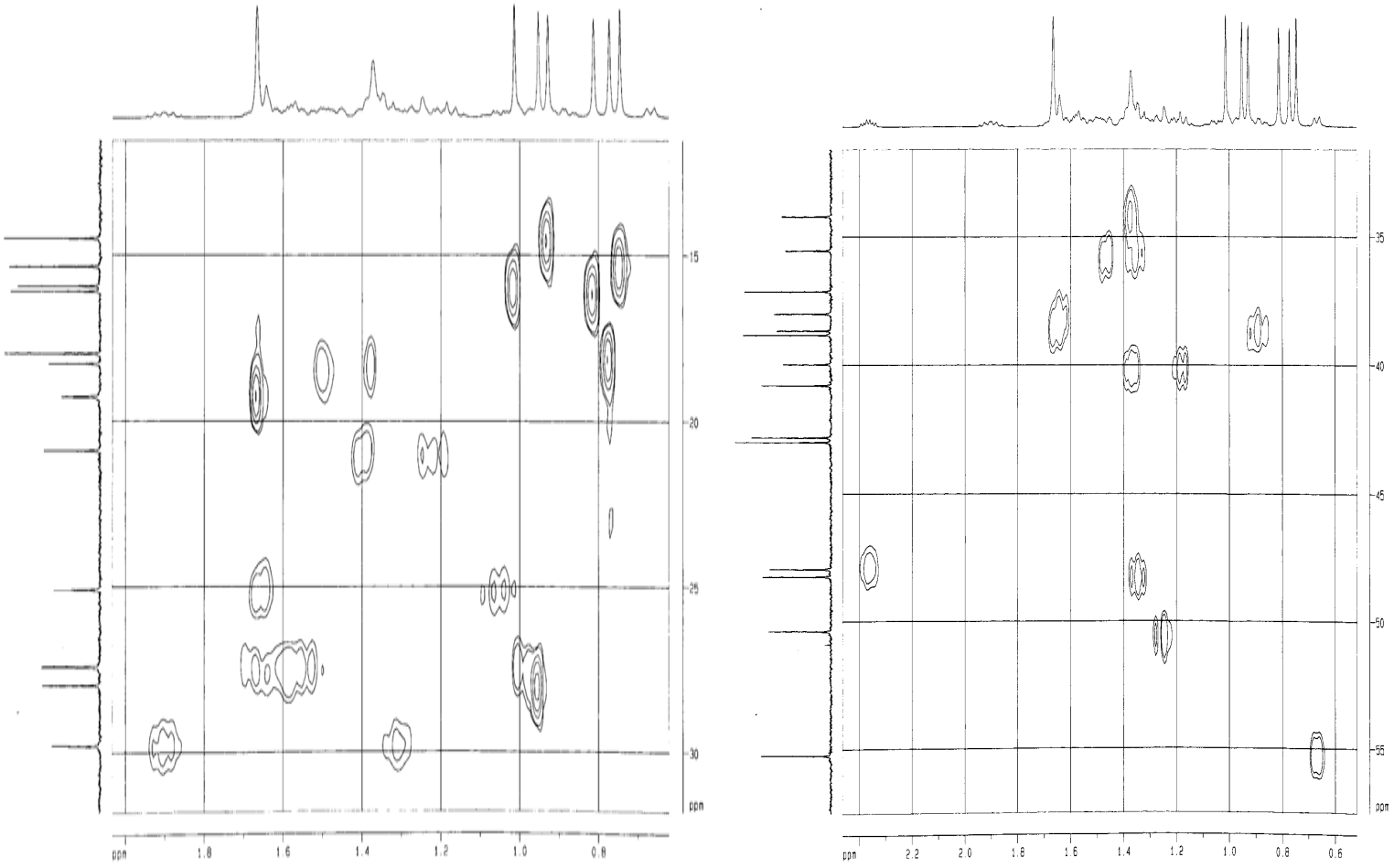
XLIX. DEPT spectrum of lupeol (**8**)



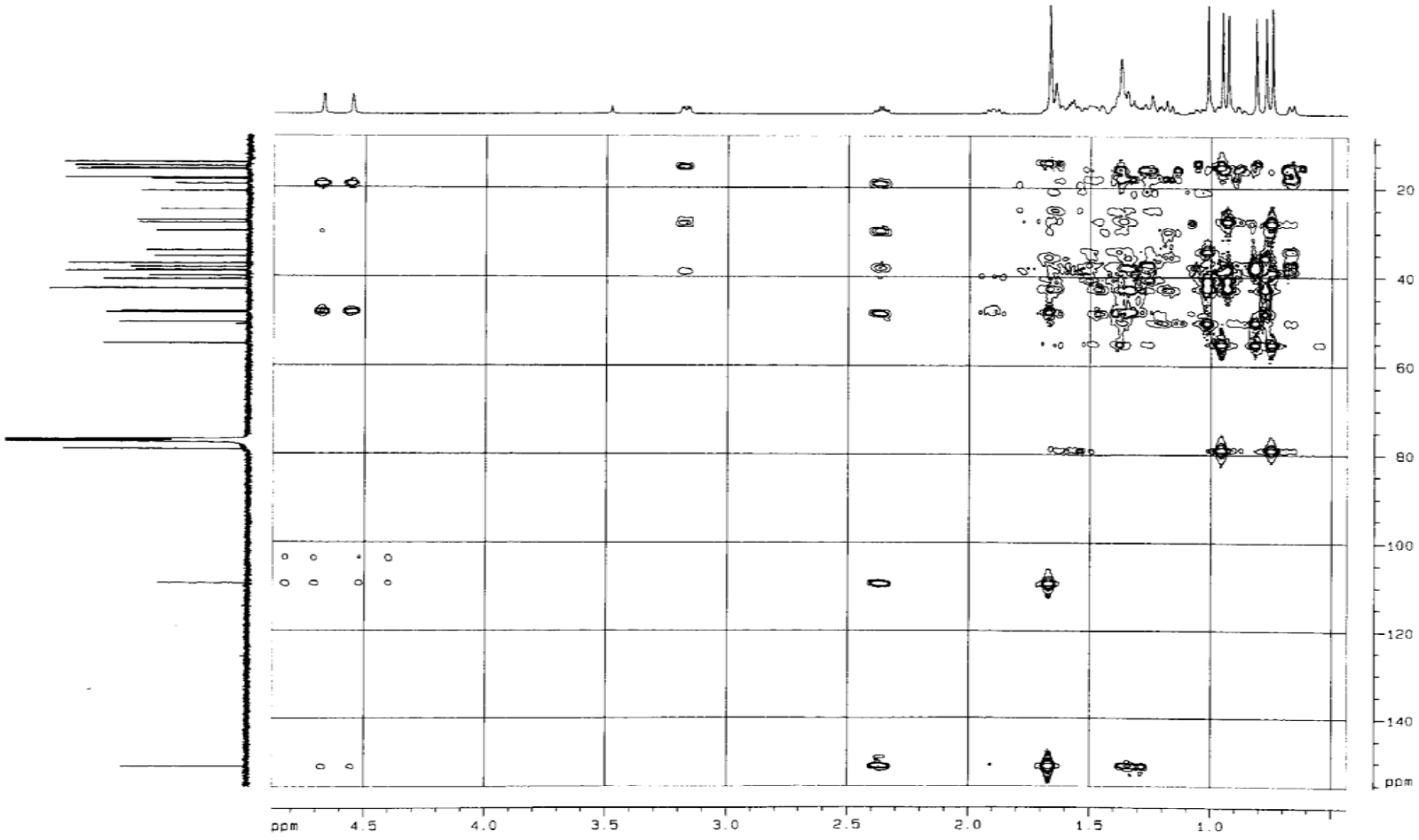
L. ^{1}H-NMR (500 MHz, $CDCl_3$) spectrum of lupeol (**8**)



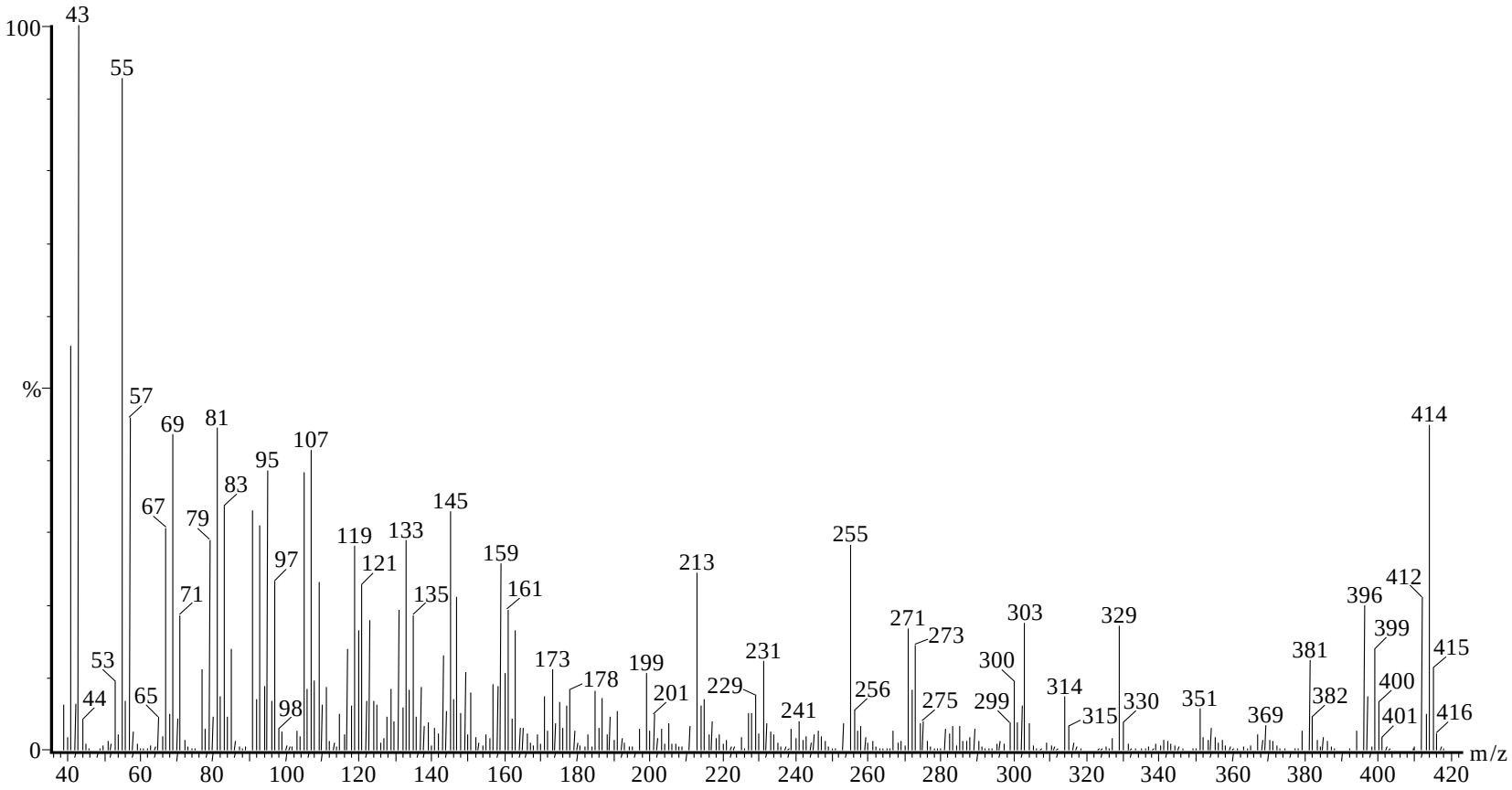
LI. HMQC spectra of lupeol (**8**)



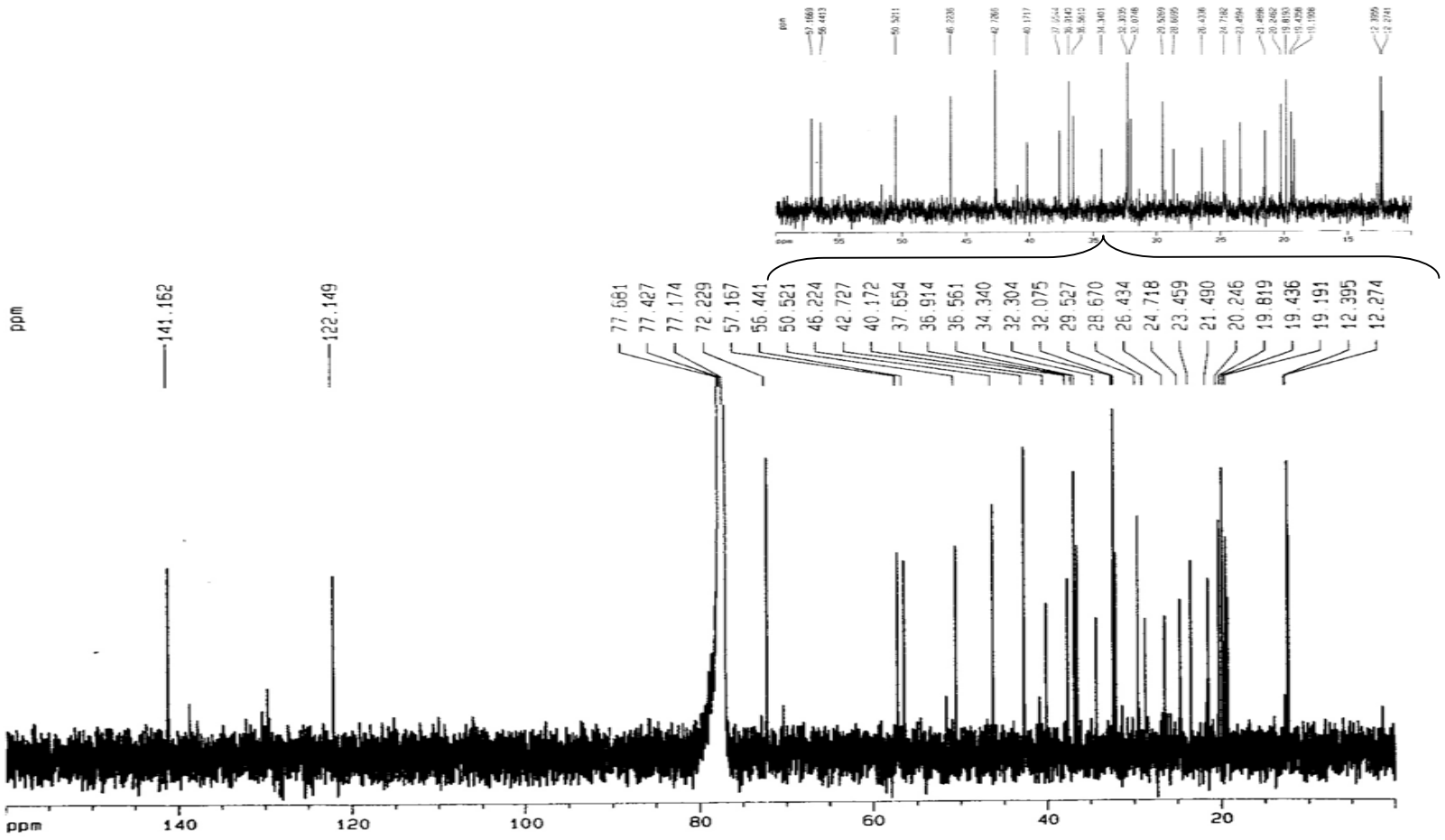
LII. HMBC spectrum of lupeol (**8**)



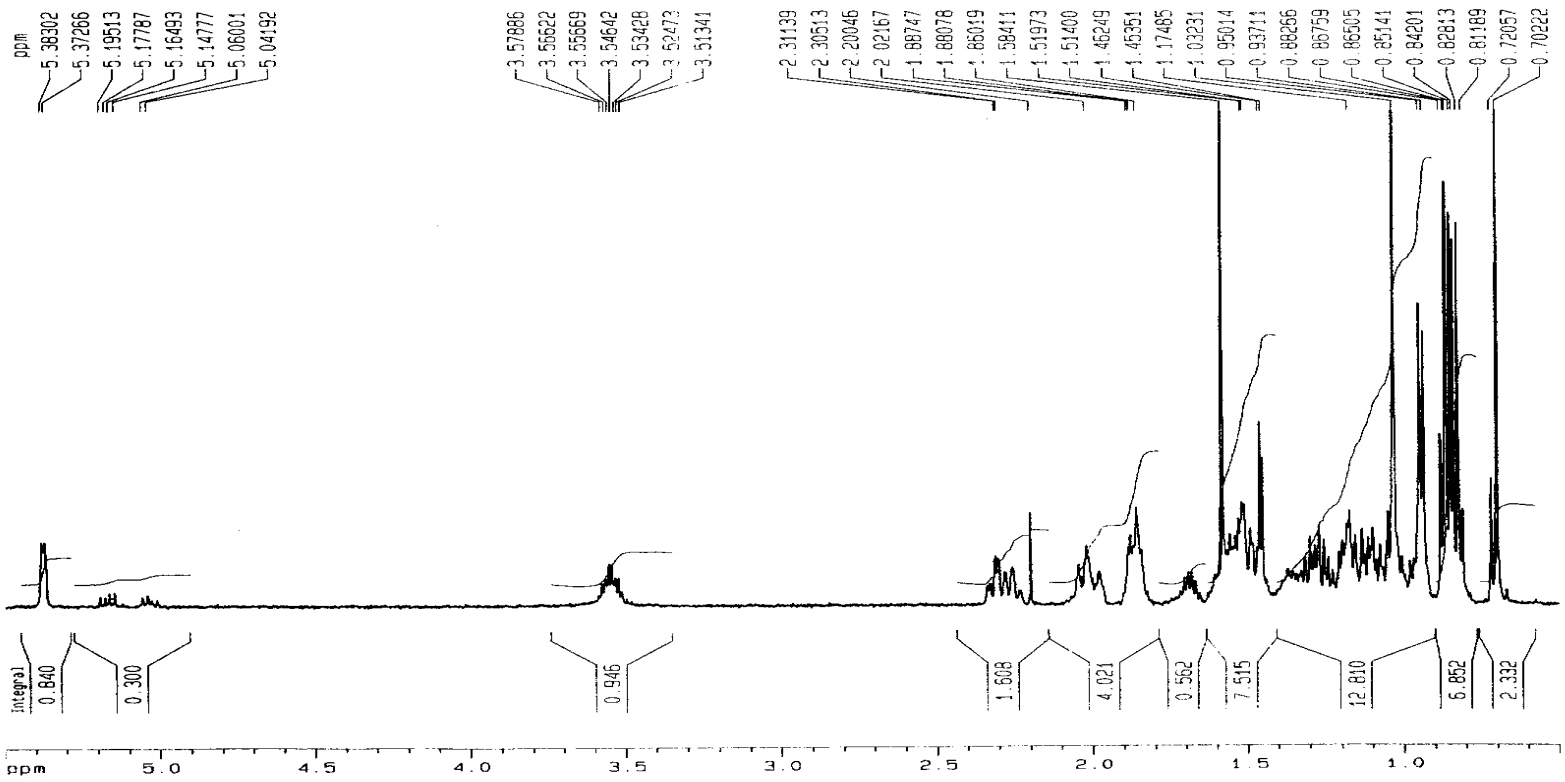
LIII. Mass spectrum of β-sitosterol (**9**)



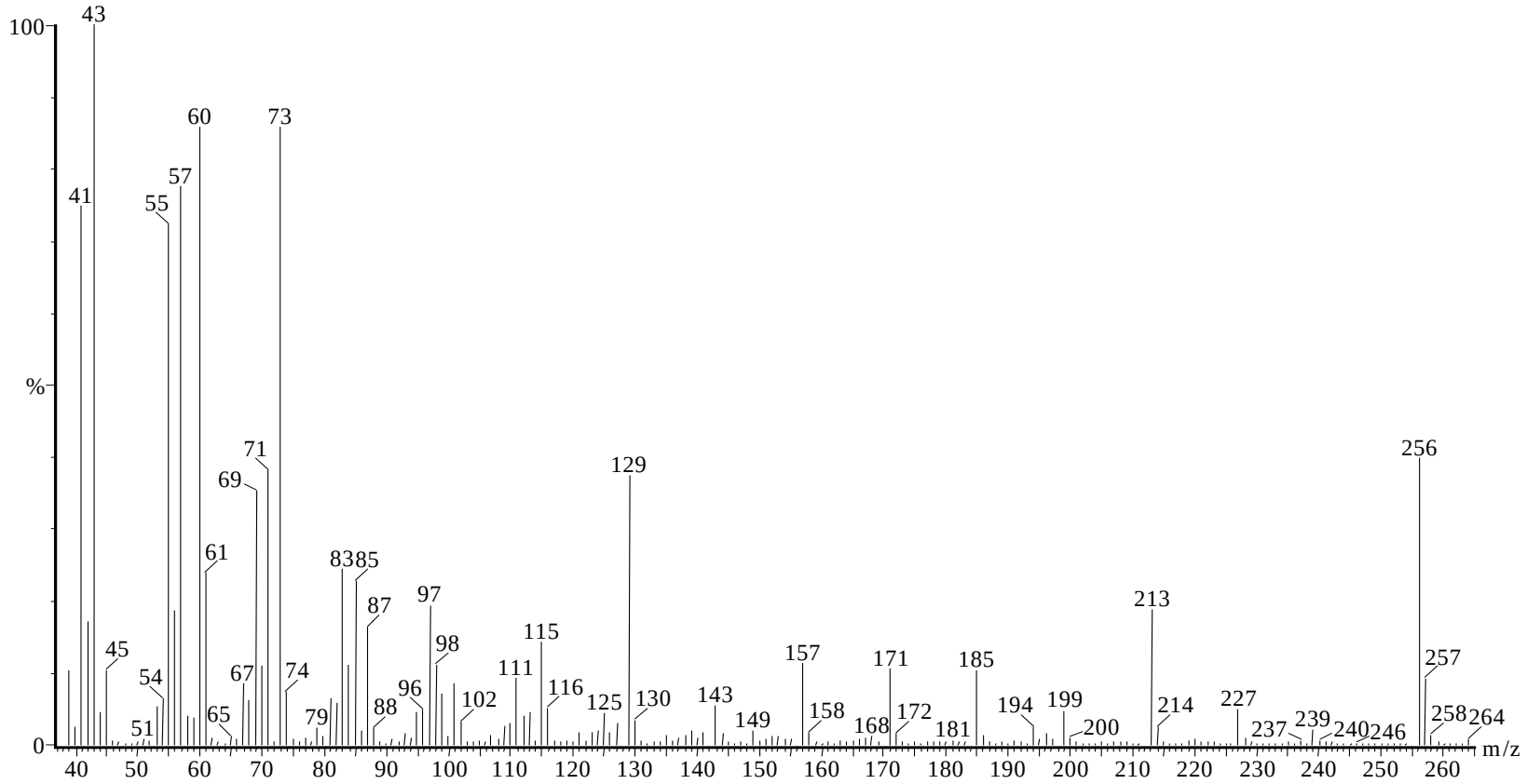
LIV. ^{13}C-NMR (125MHz, $CDCl_3$) spectrum of β-sitosterol (**9**)



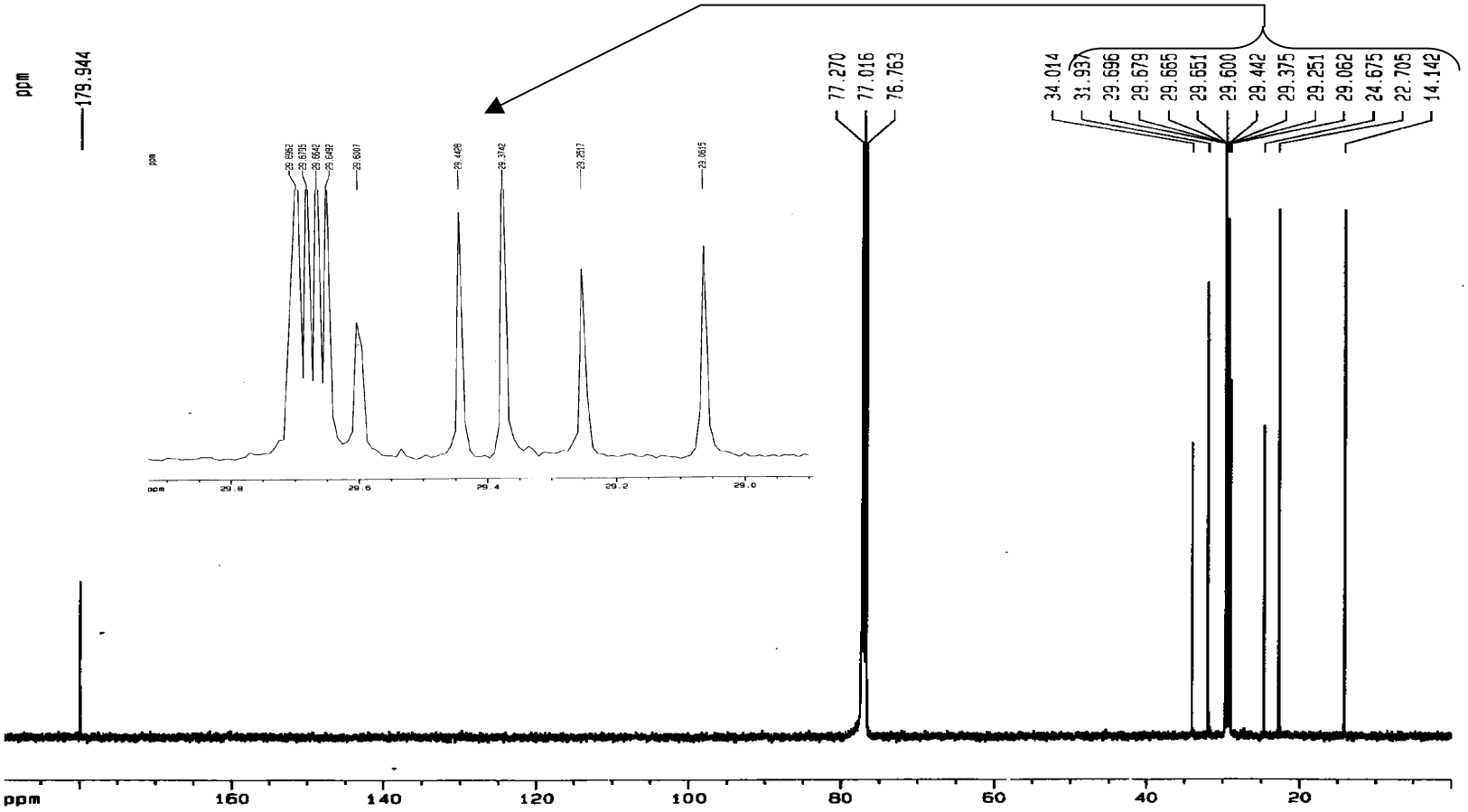
LV. ^{1}H-NMR (500 MHz, $CDCl_3$) spectrum of β-sitosterol (**9**)



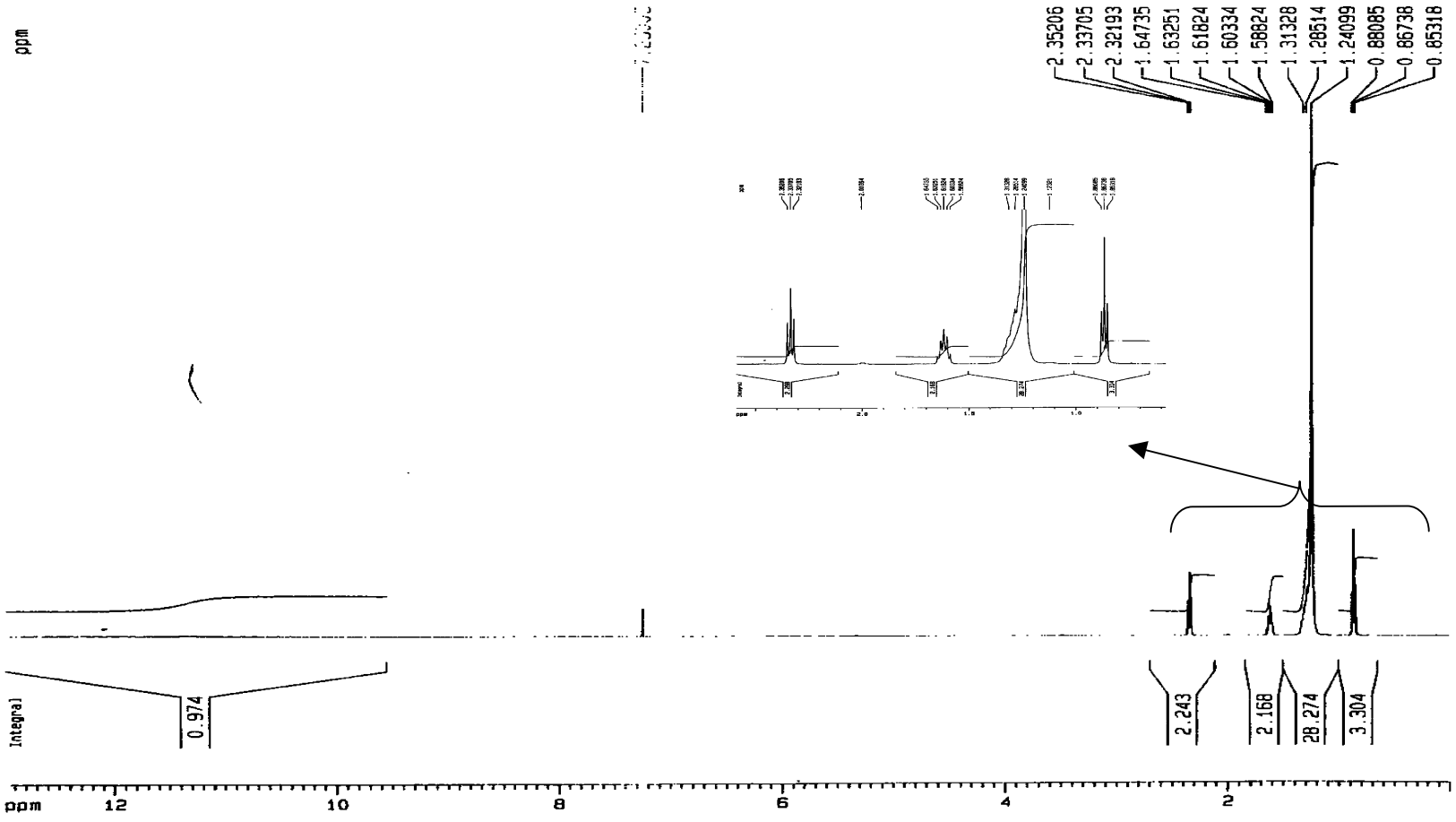
LVI. Mass spectrum of hexadecanoic acid (**10**)



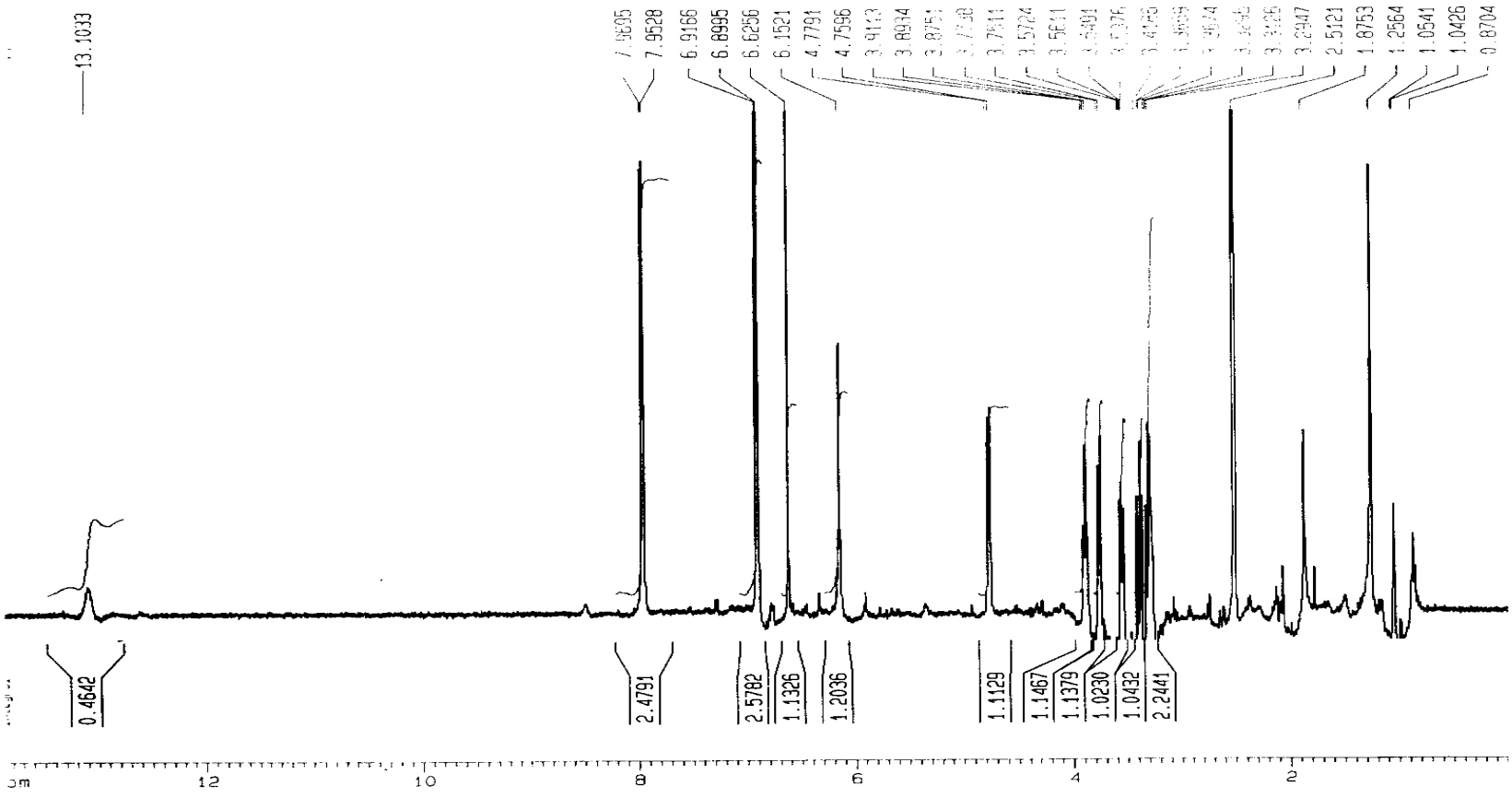
LVII. ^{13}C-NMR (125MHz, $CDCl_3$) of hexadecanoic acid (**10**)



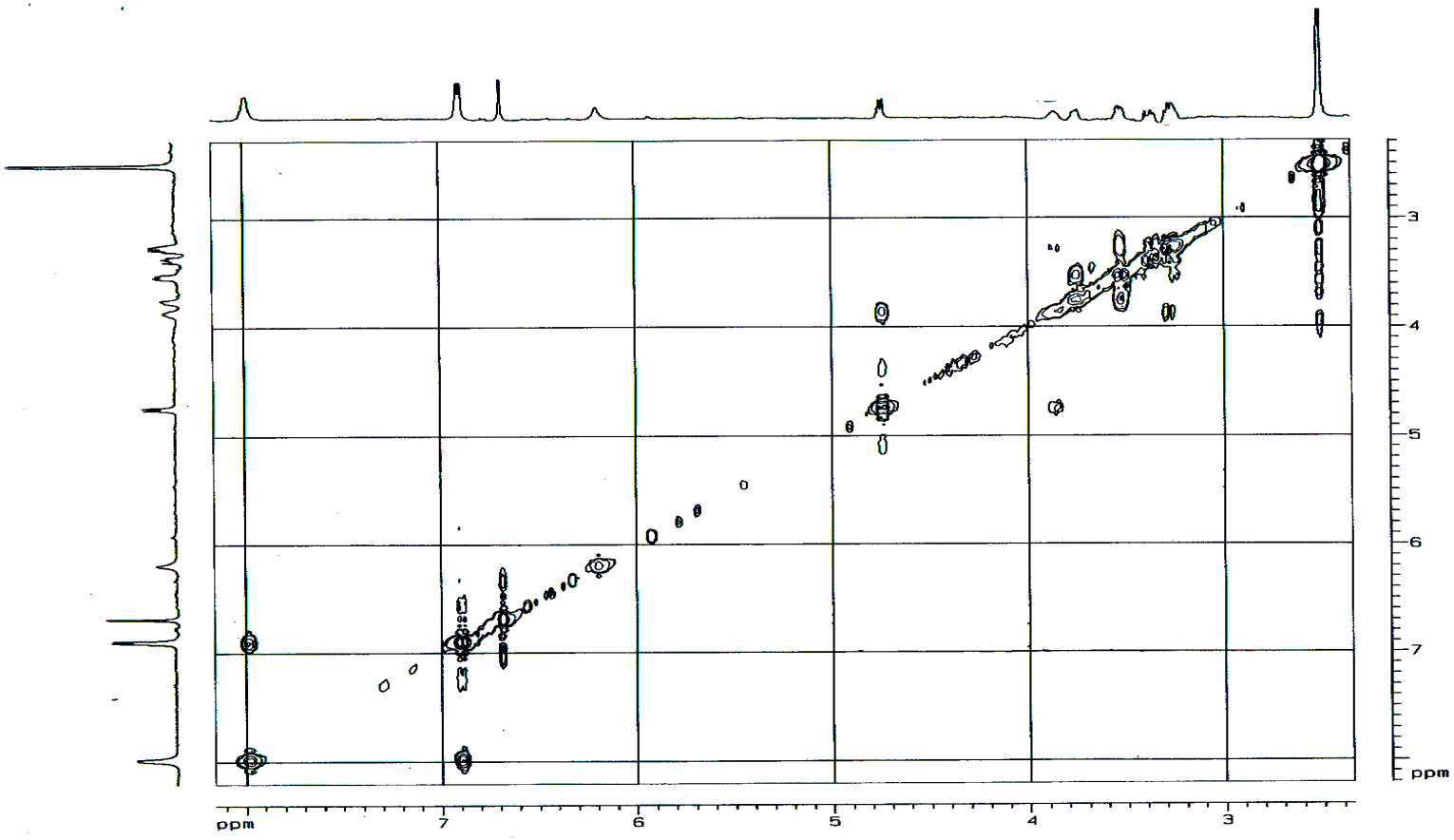
LVIII. ^{1}H-NMR (500MHz, $CDCl_3$) spectrum of hexadecanoic acid (**10**)



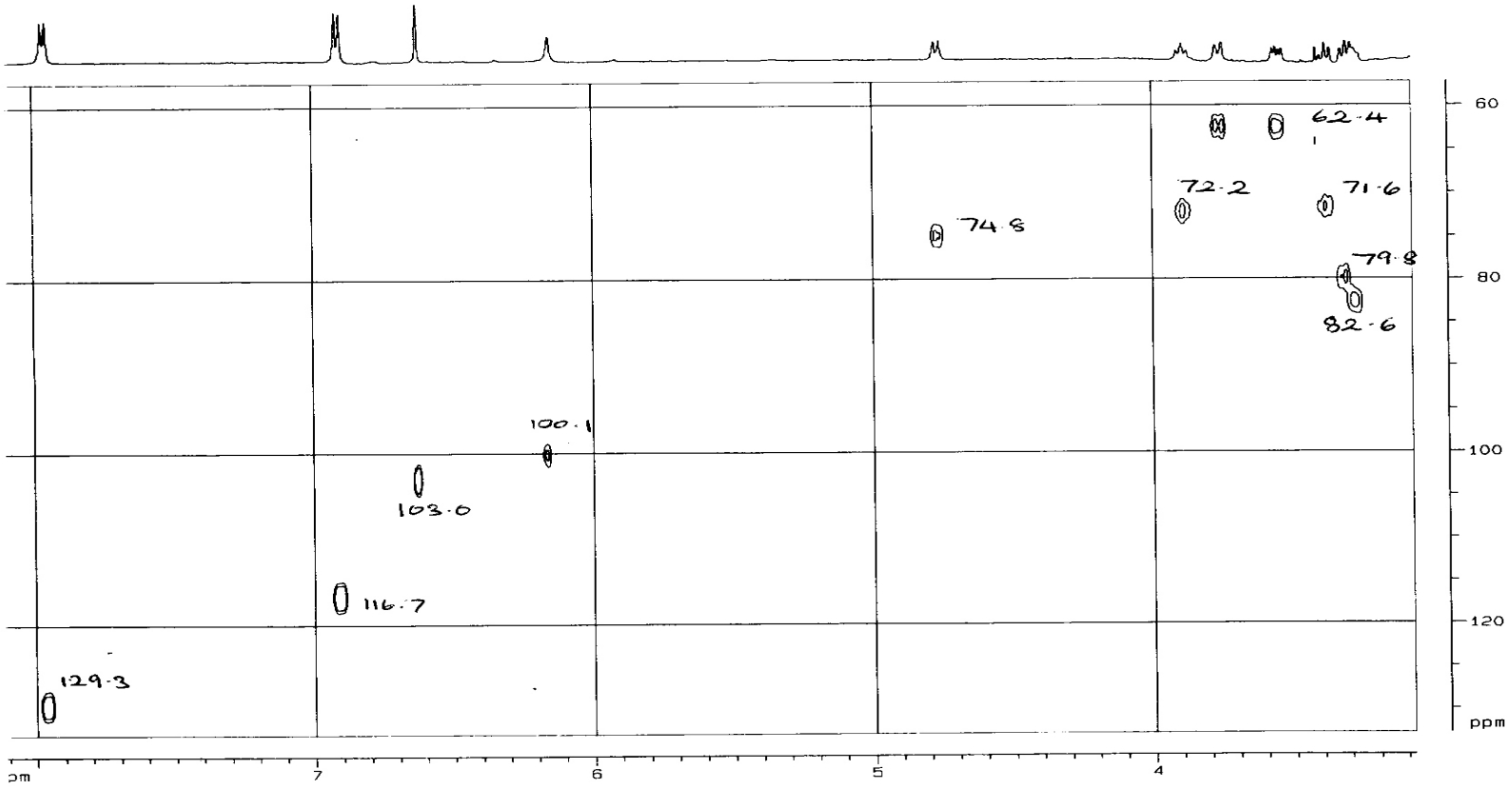
LIX. ^{1}H-NMR (500 MHz, DMSO-d_6) spectrum of vitexin (**11**)



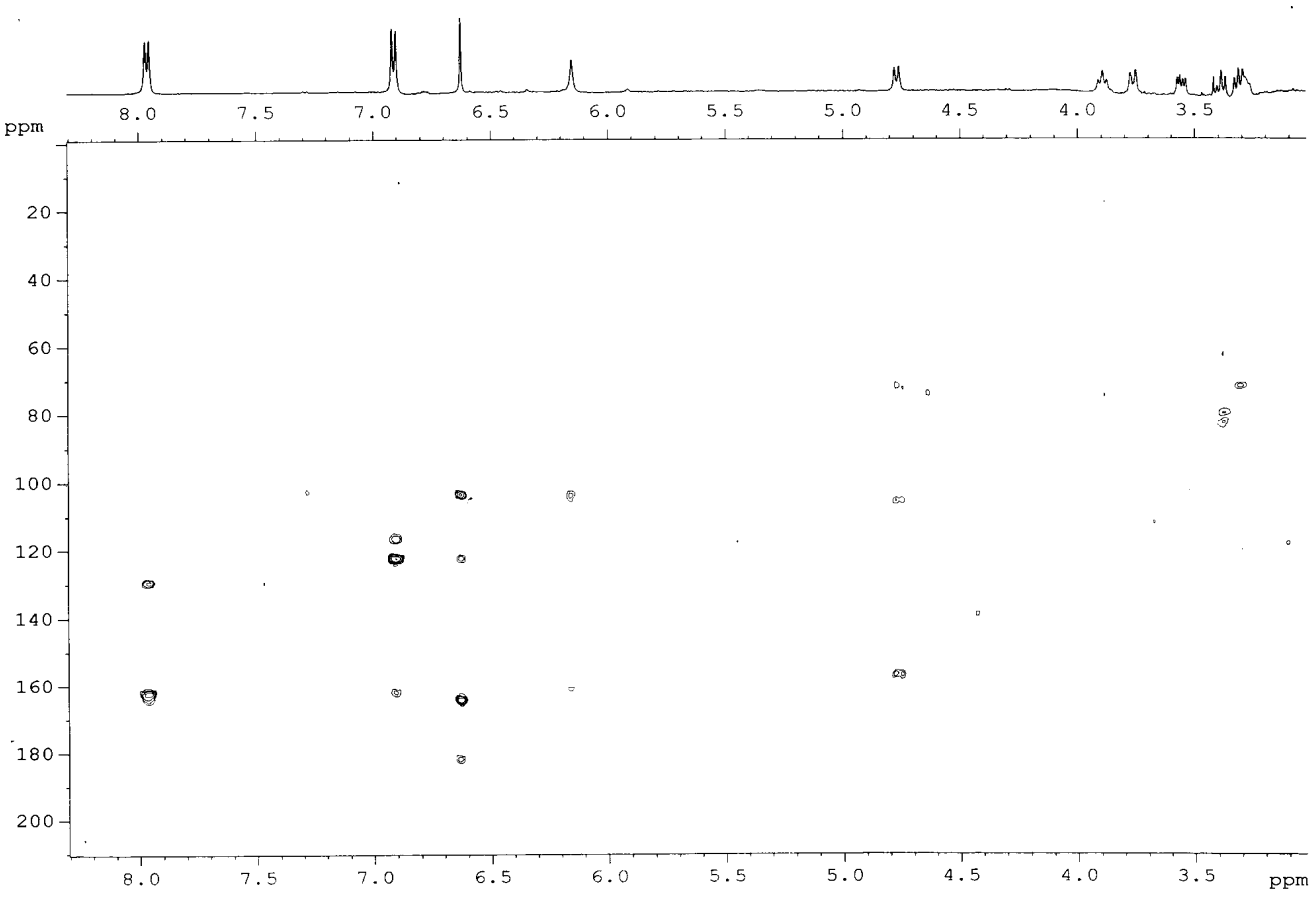
LX. ^{1}H, ^{1}H-COSY spectrum of vitexin (**11**)



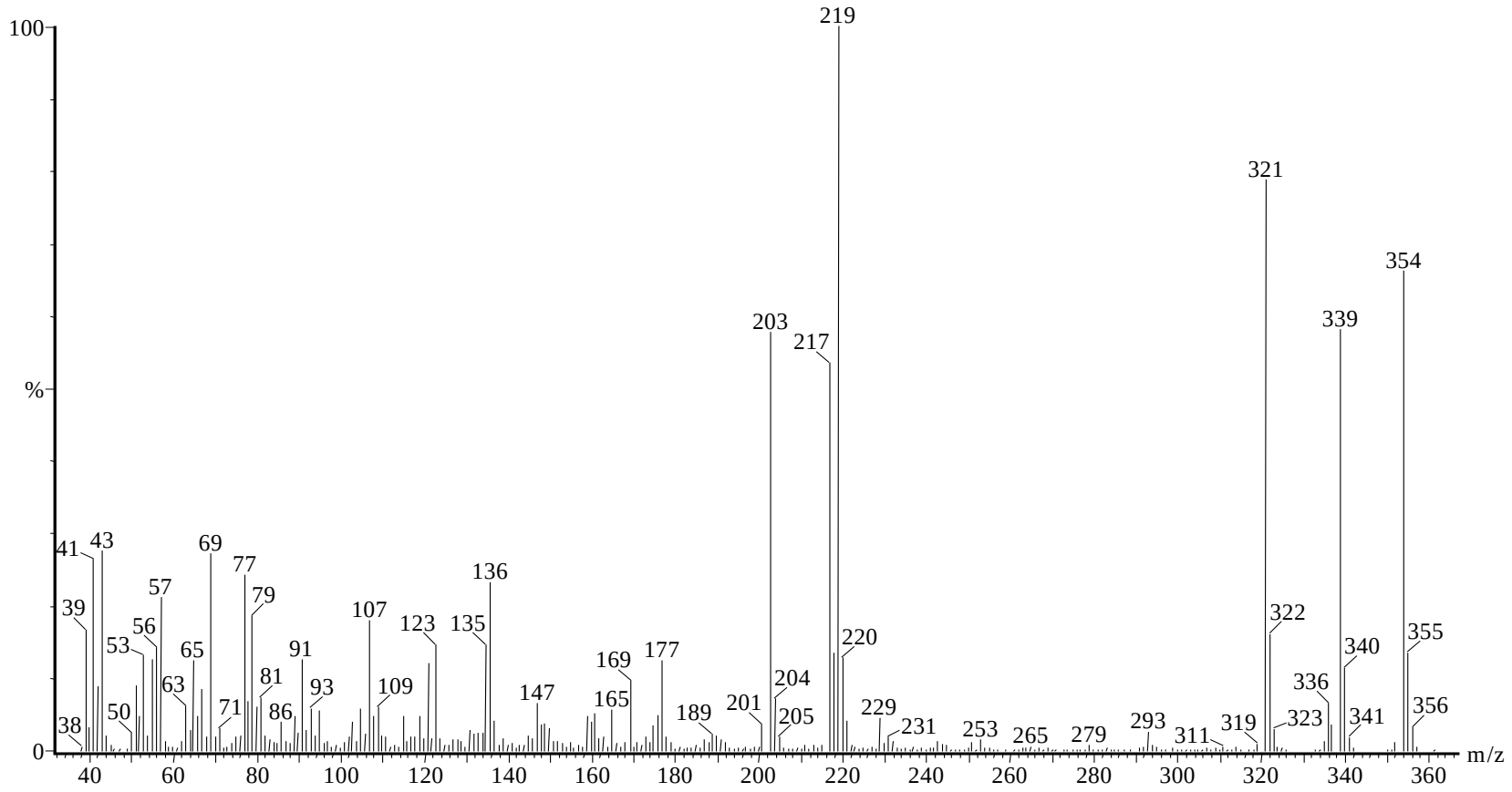
LXI. HMQC spectrum of vitexin (**11**)



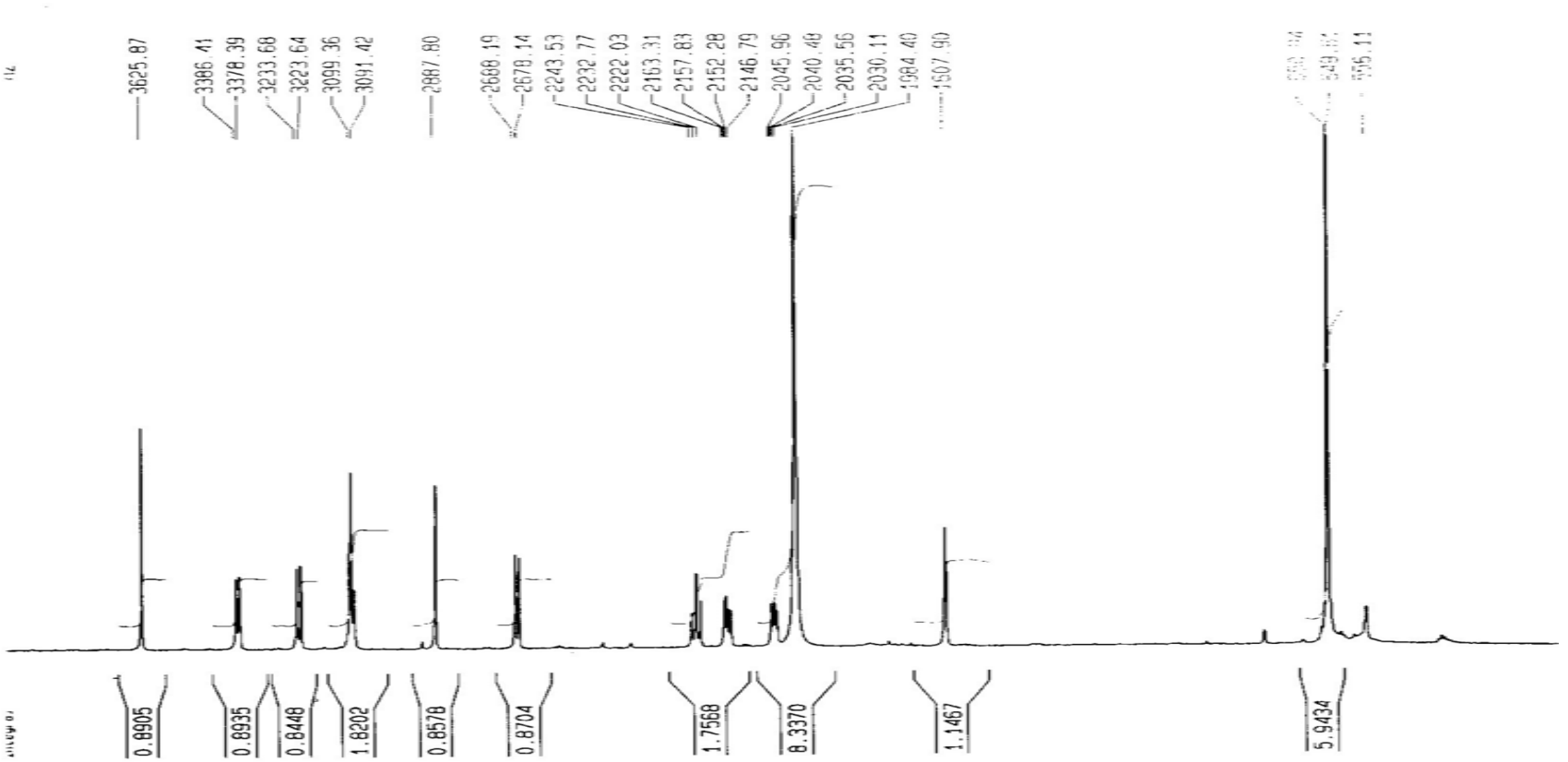
LXII. HMBC spectrum of vitexin (**11**)



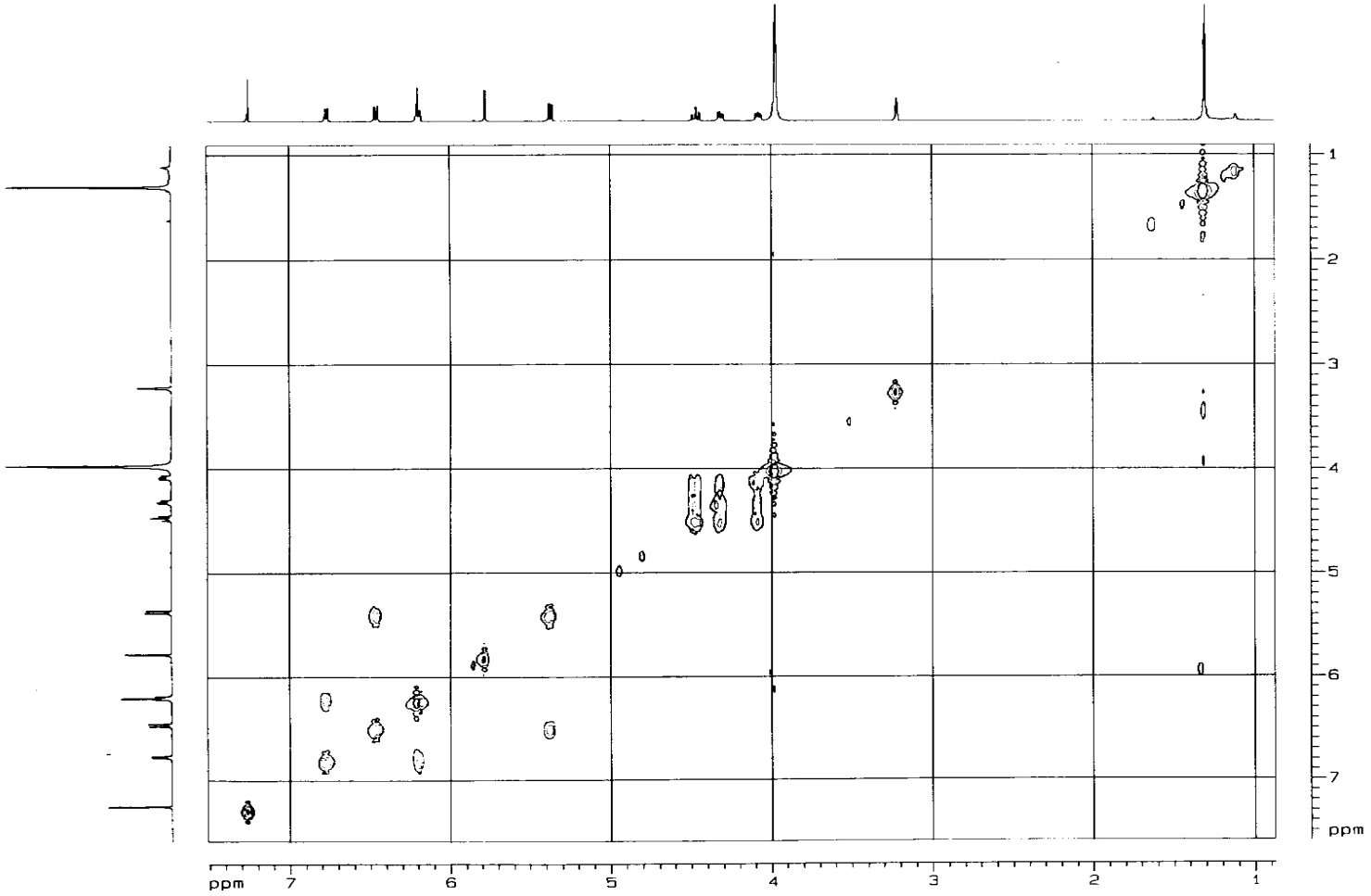
LXIII. Mass spectrum of intortunone (**12**)



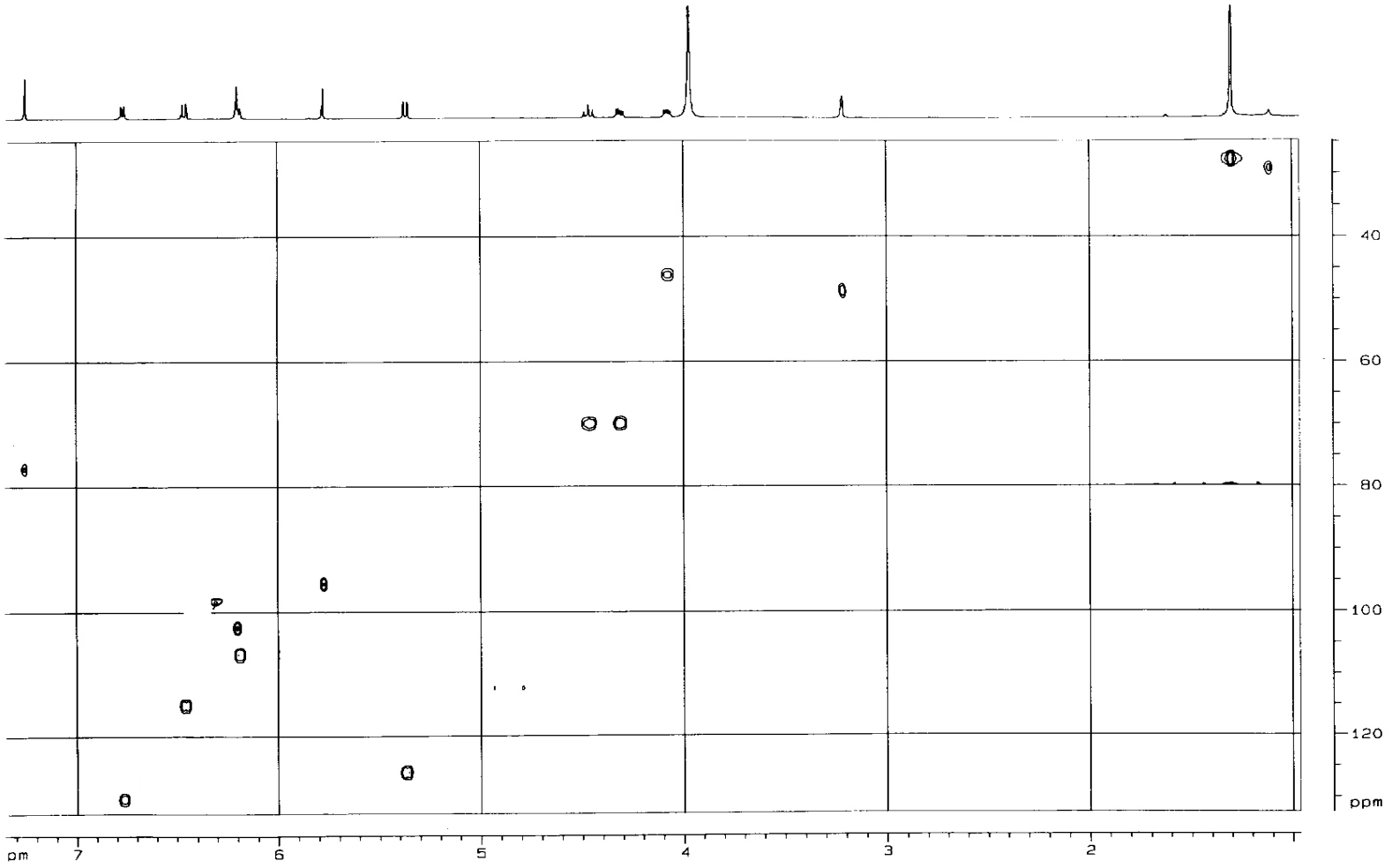
LXIV. ^{1}H-NMR (500 MHz, MeOH-d_4) spectrum of intortunone (**12**)



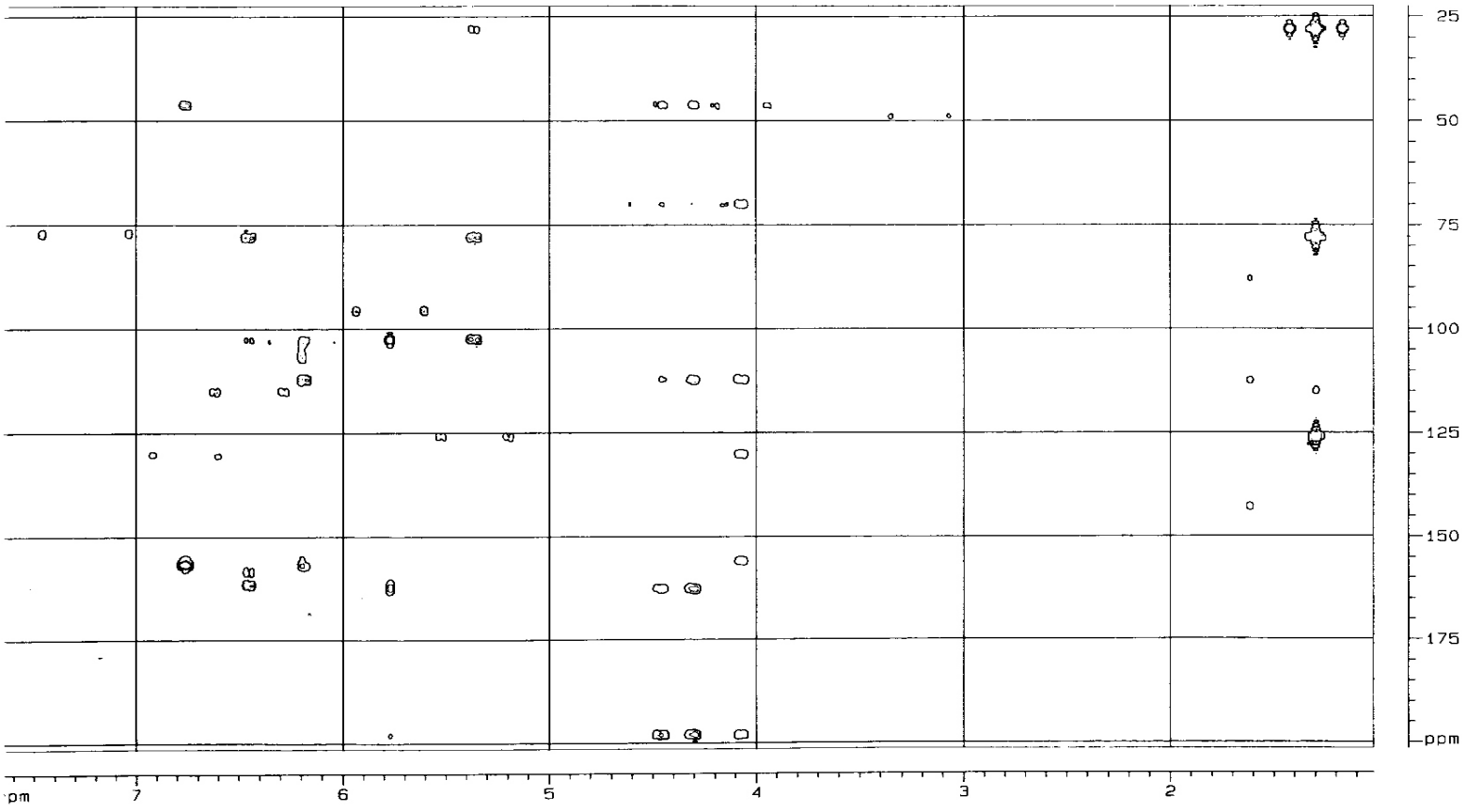
LXV. ^{1}H, ^{1}H-COSY spectrum of intortunone (**12**)



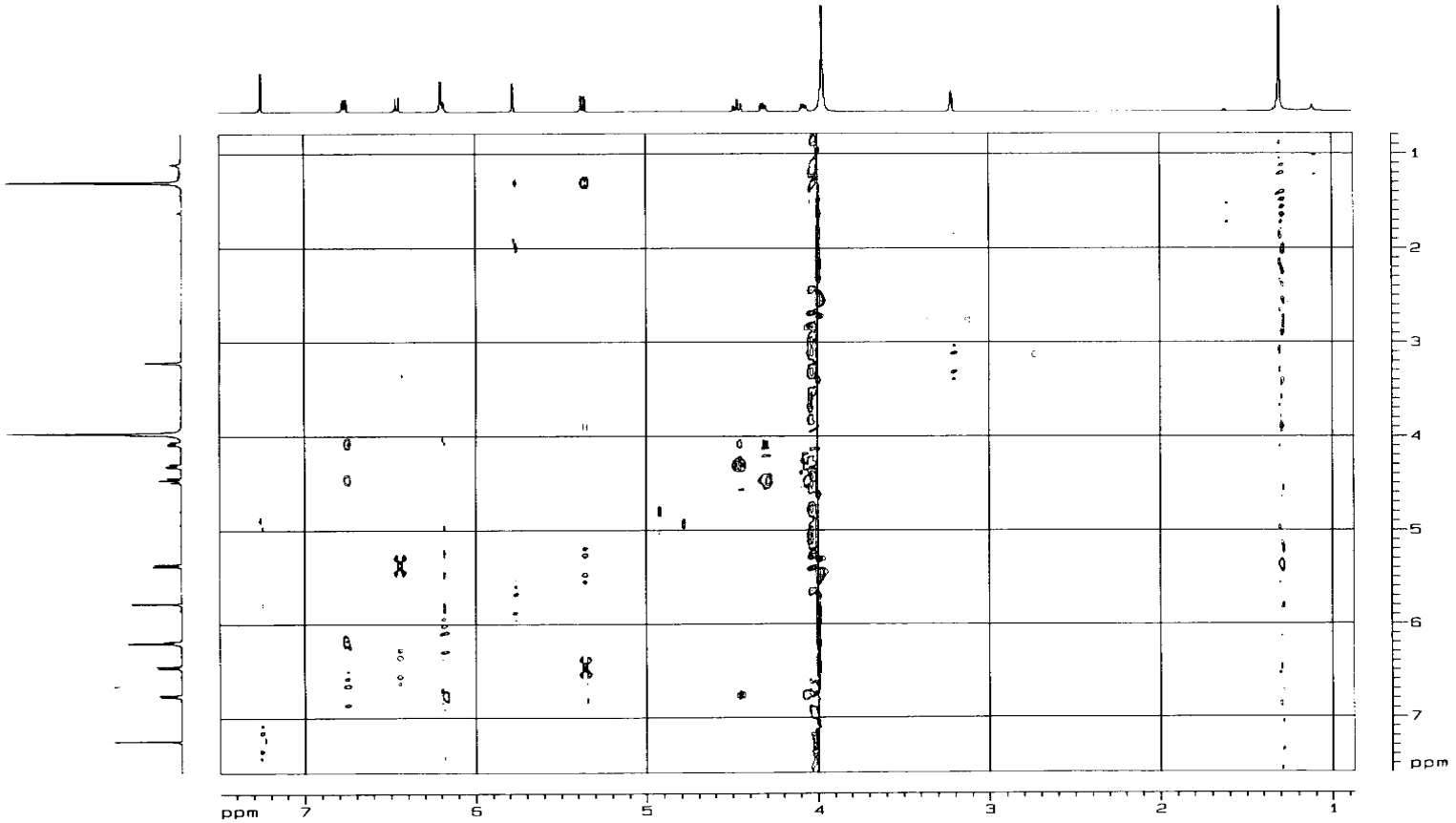
LXVI. HMQC spectrum of intortunone (**12**)



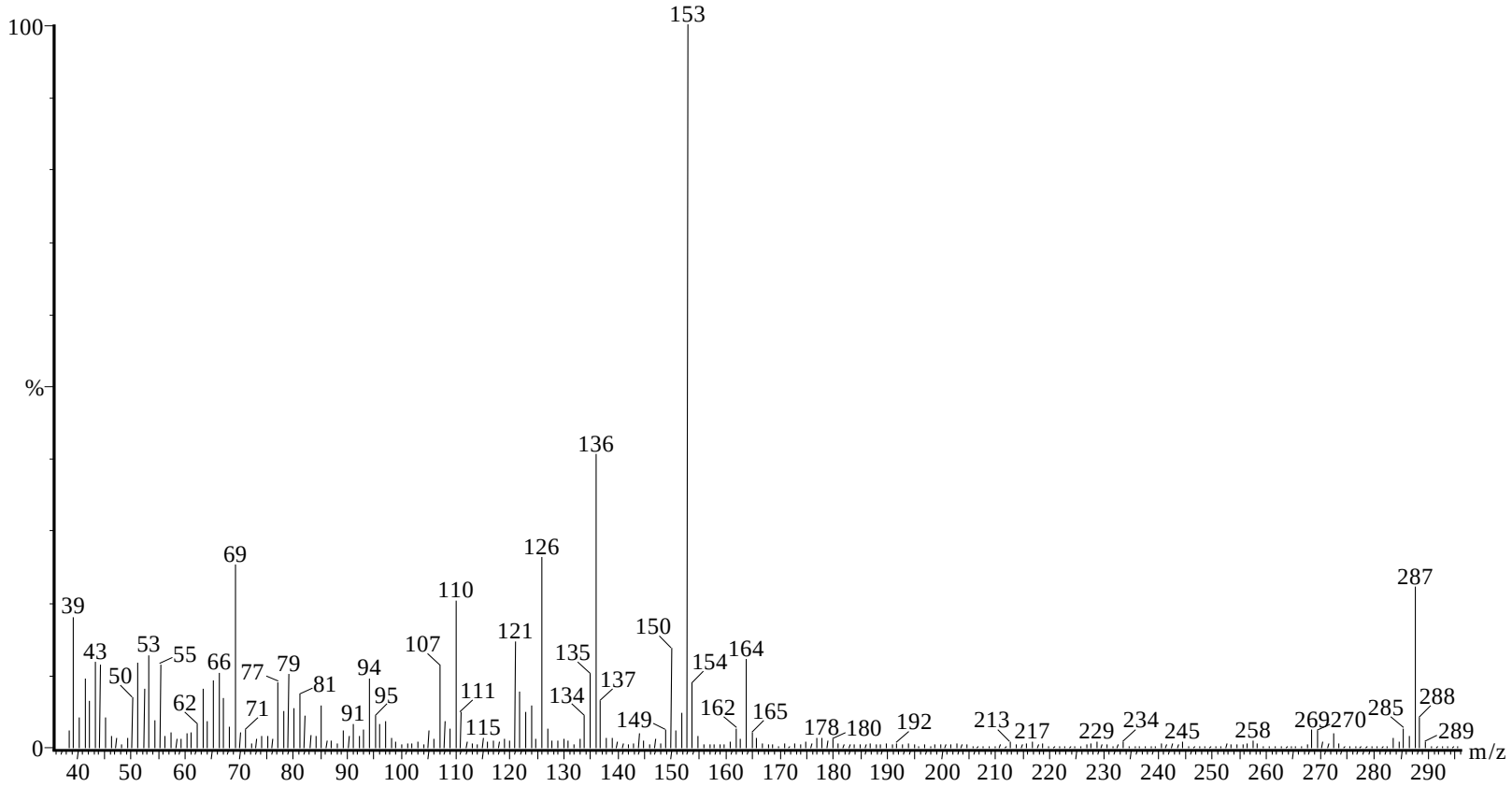
LXVII. HMBC spectrum of intortunone (**12**)



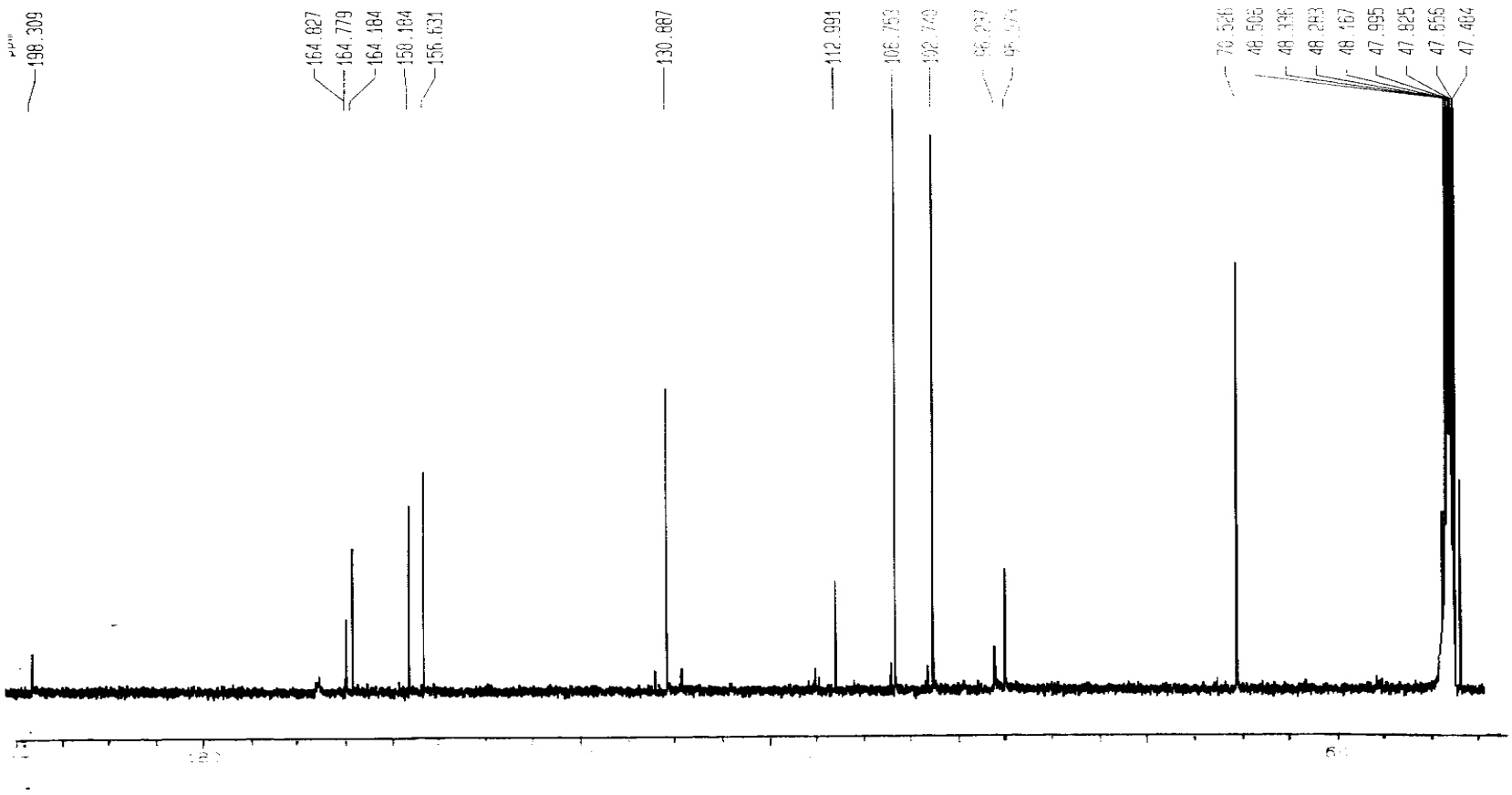
LXVIII. NOESY spectrum of intortunone (**12**)



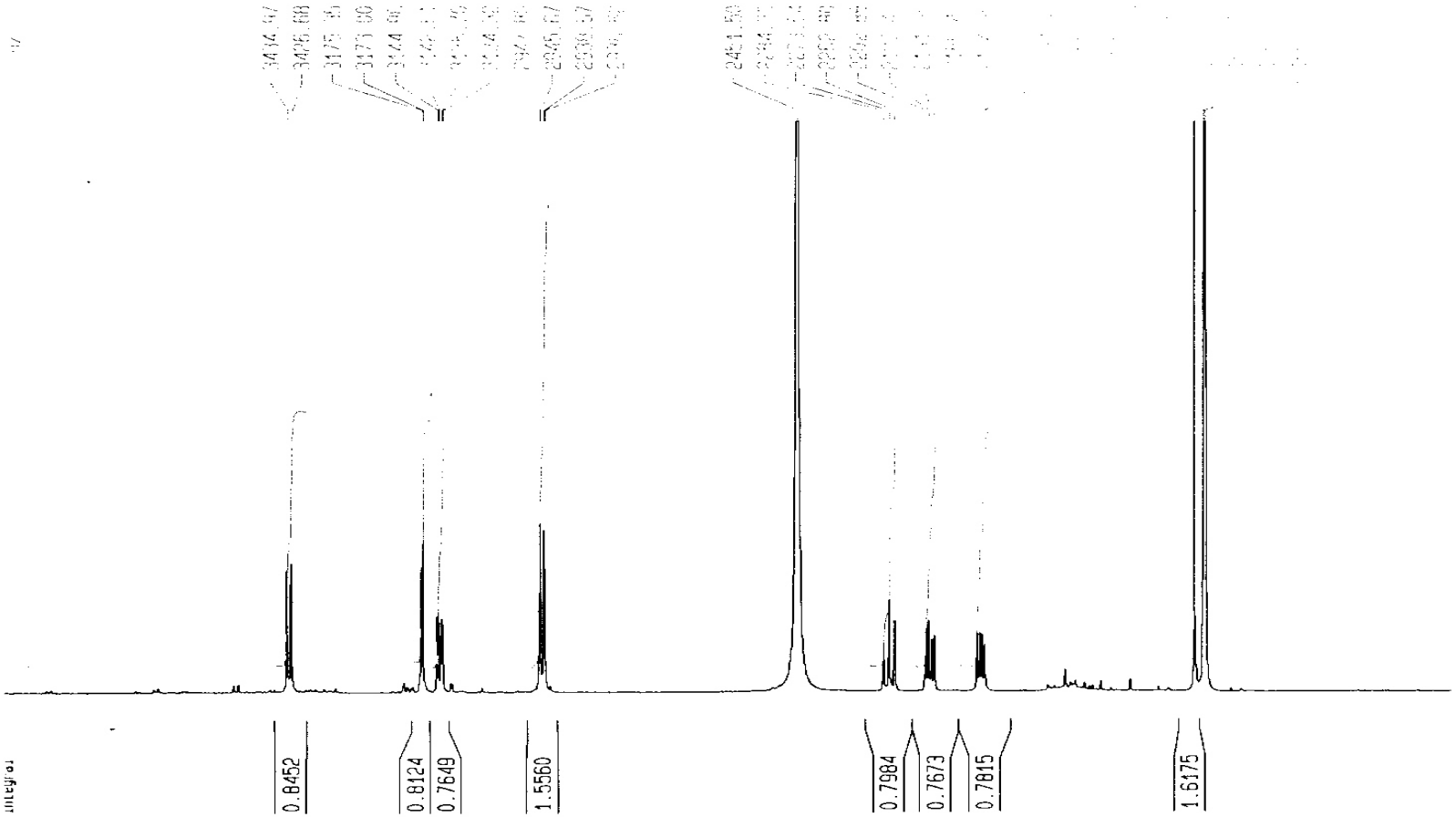
LXIX. Mass spectrum of dalbergioidin (**13**)



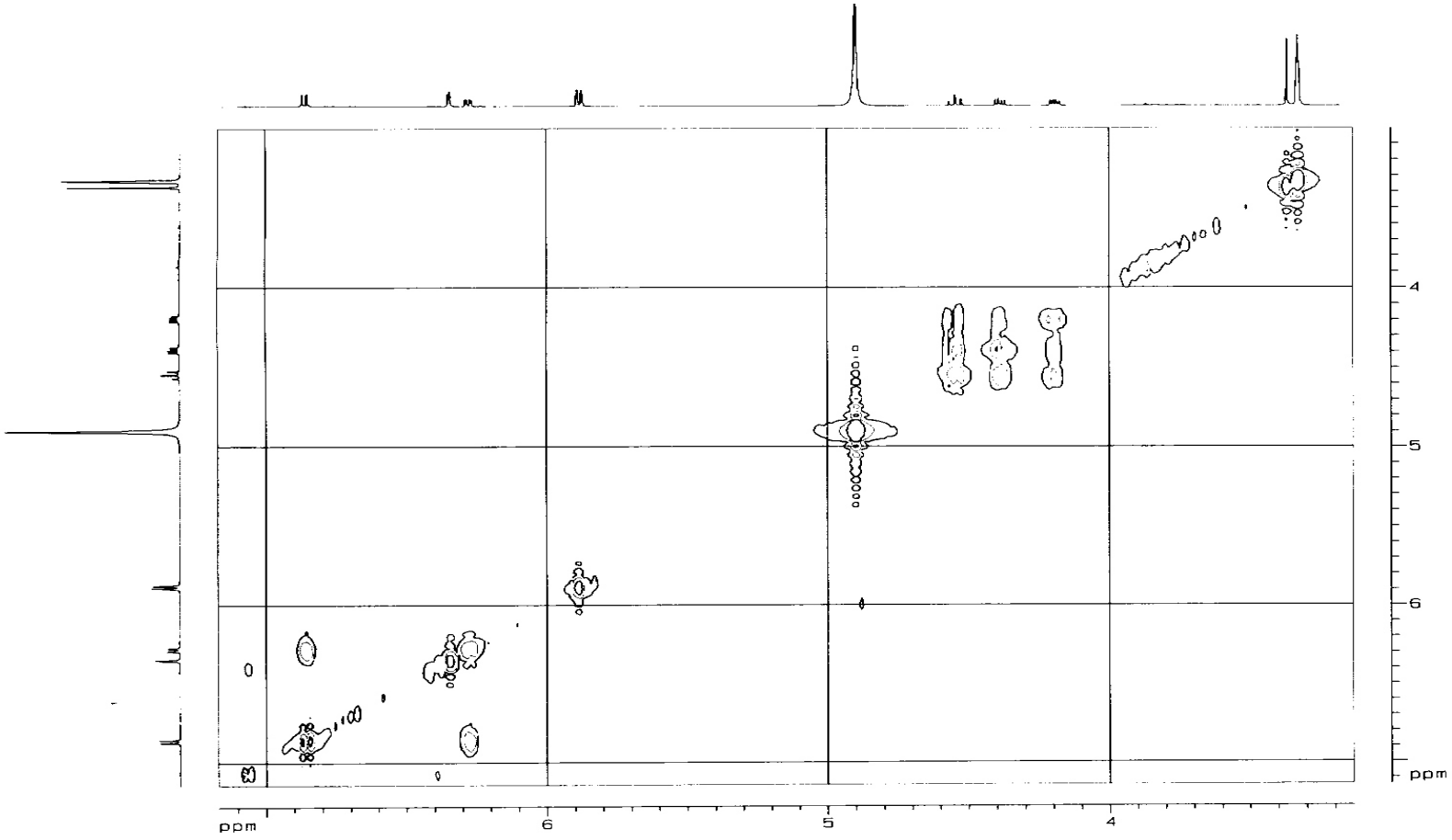
LXX. ^{13}C-NMR (125 MHz, MeOH-d_4) spectrum of dalbergioidin (**13**)



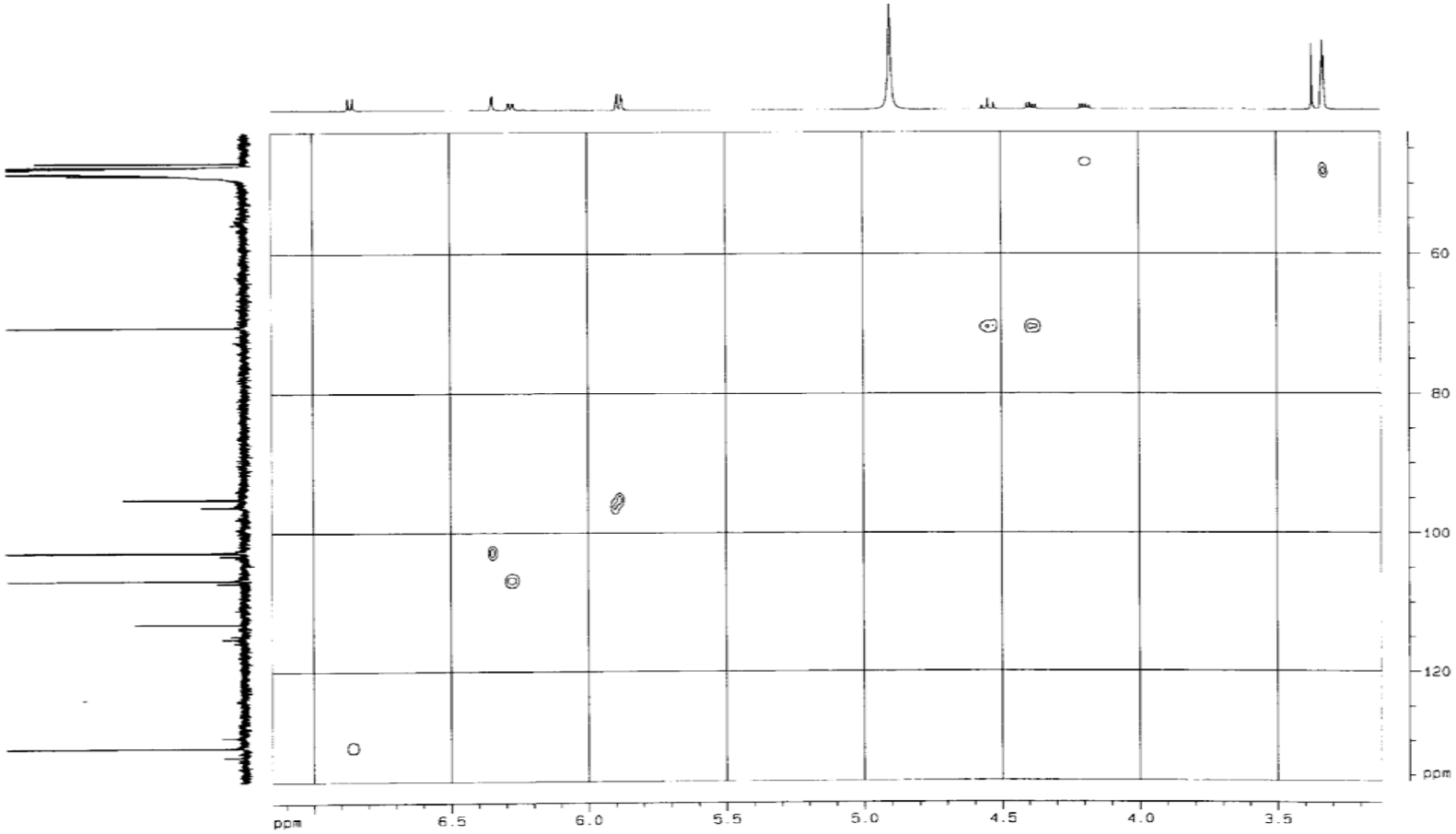
LXXI. ^{1}H-NMR (500MHz, MeOH-d_4) spectrum of dalbergioidin (**13**)



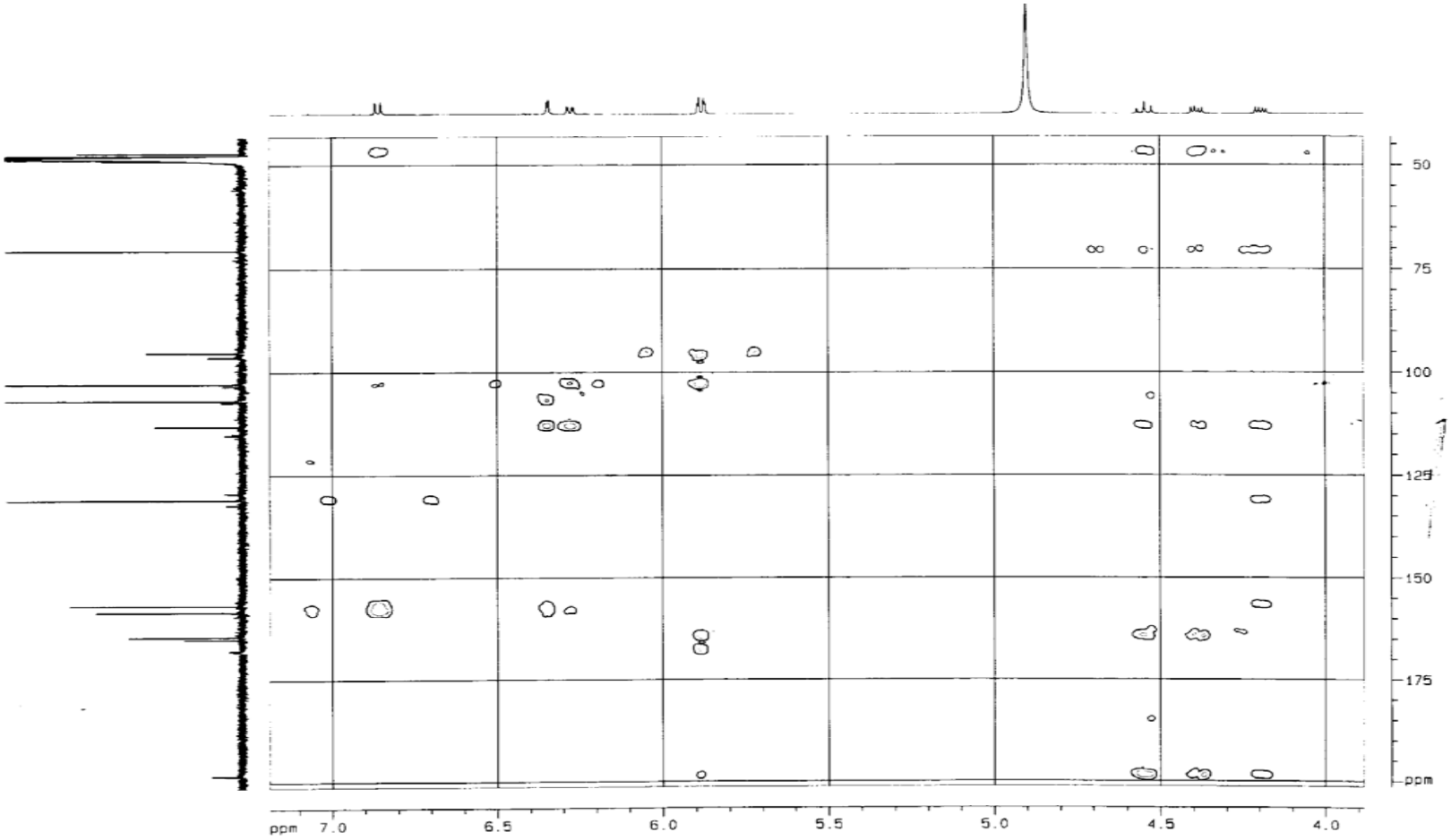
LXXII. ^{1}H, ^{1}H-COSY spectrum of dalbergioidin (**13**)



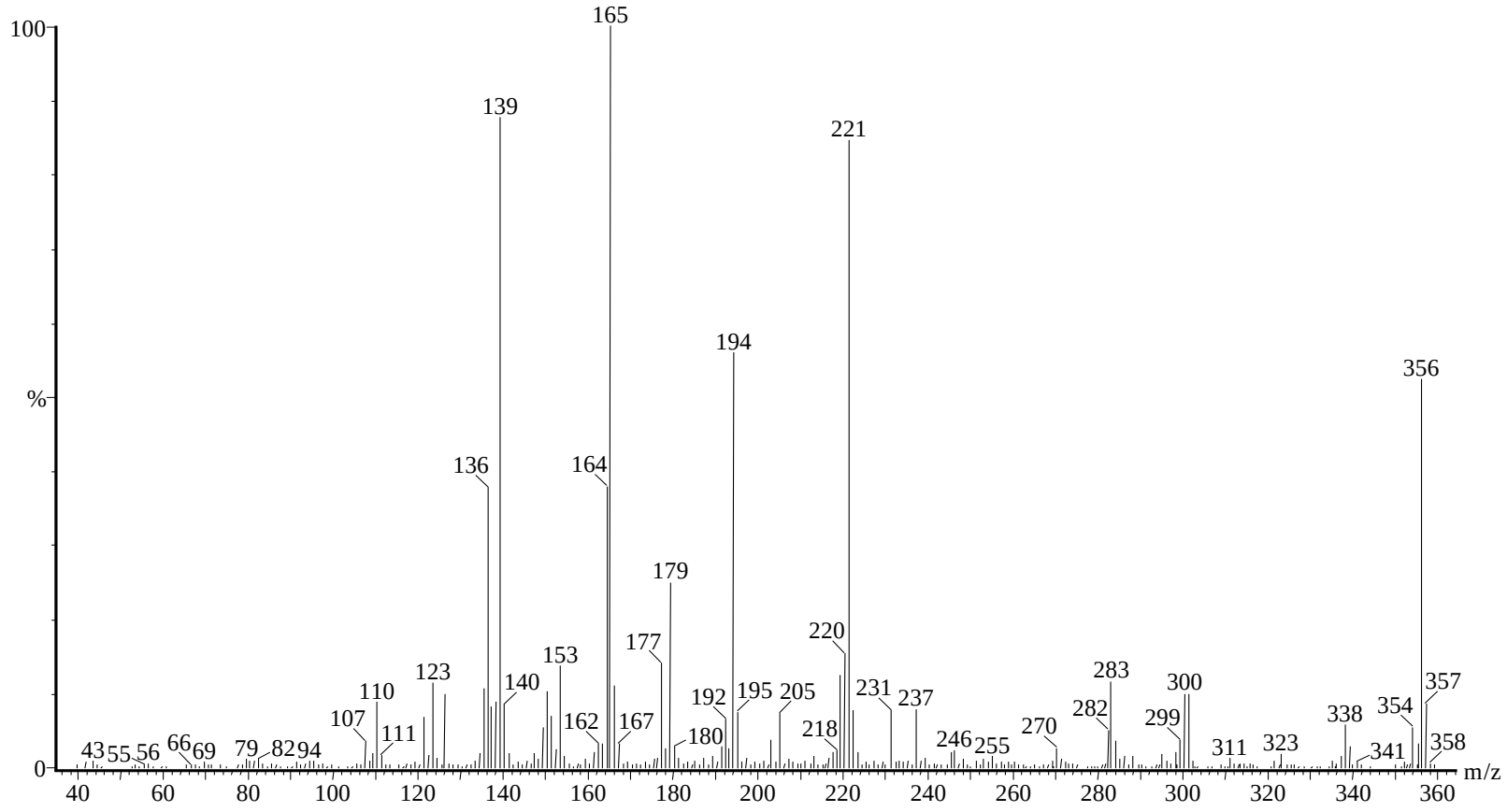
LXXIII. HMQC spectrum of dalbergioidin (**13**)



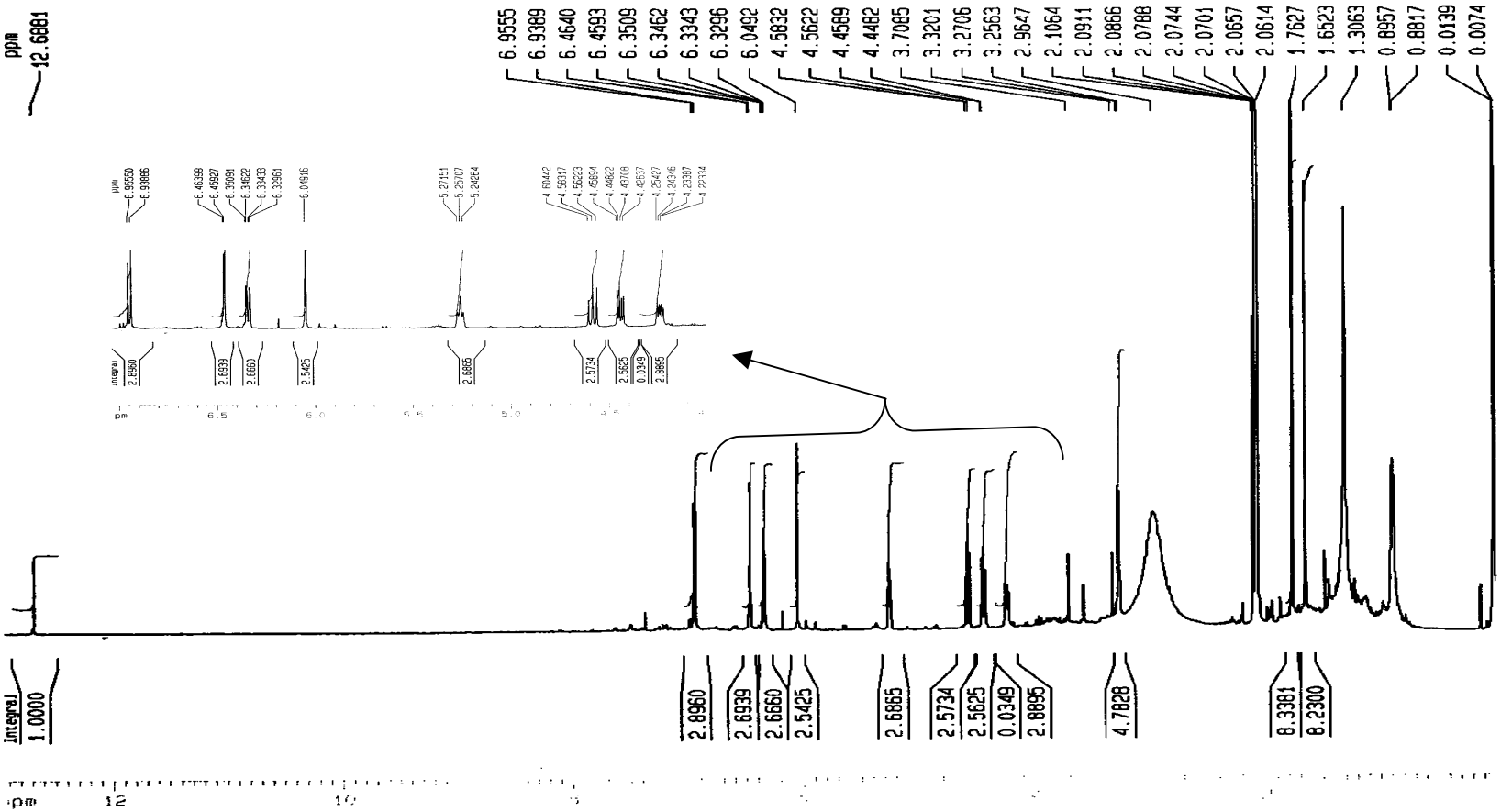
LXXIV. HMBC spectrum of dalbergioidin (**13**)



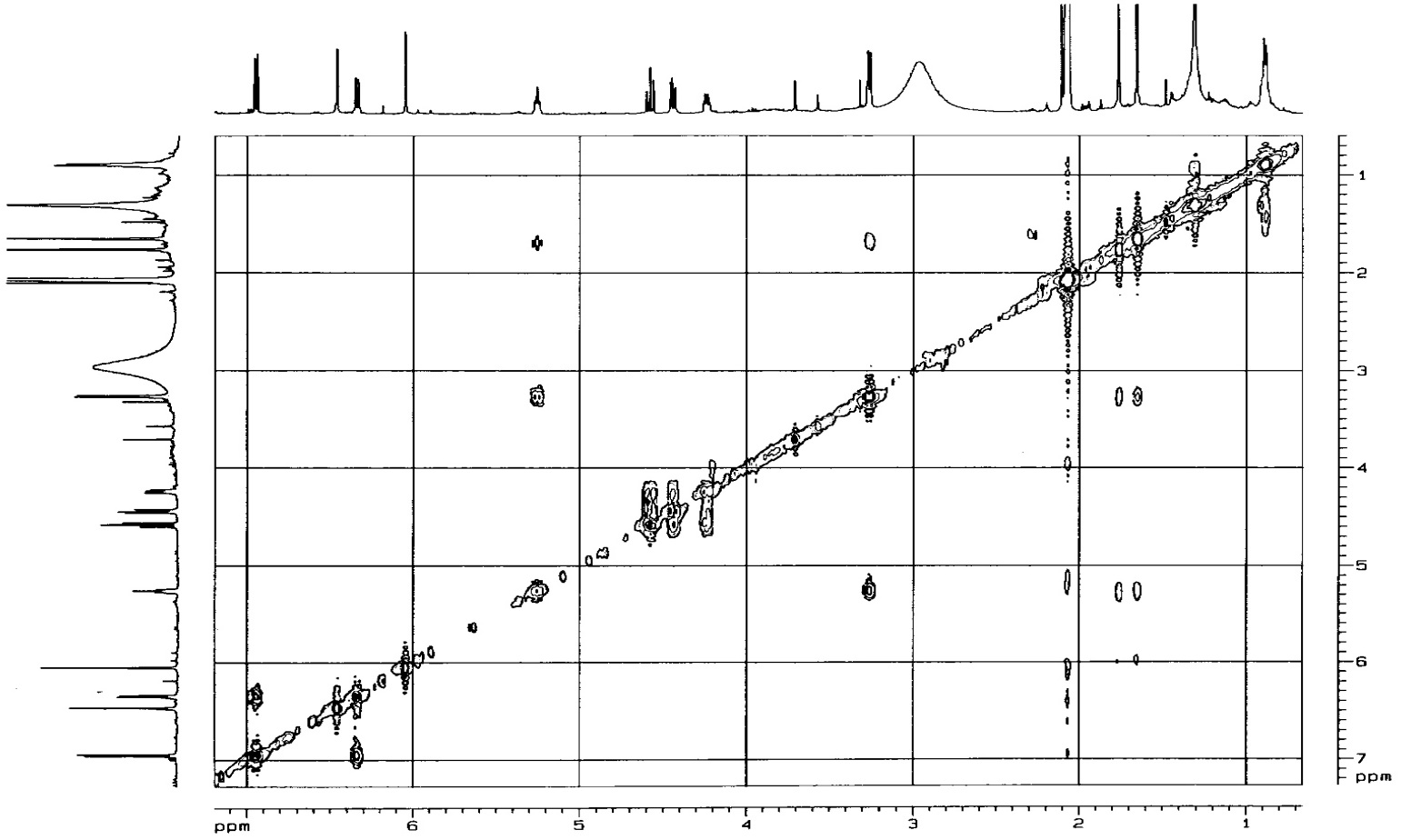
LXXV. Mass spectrum of uncinanone A (**14**)



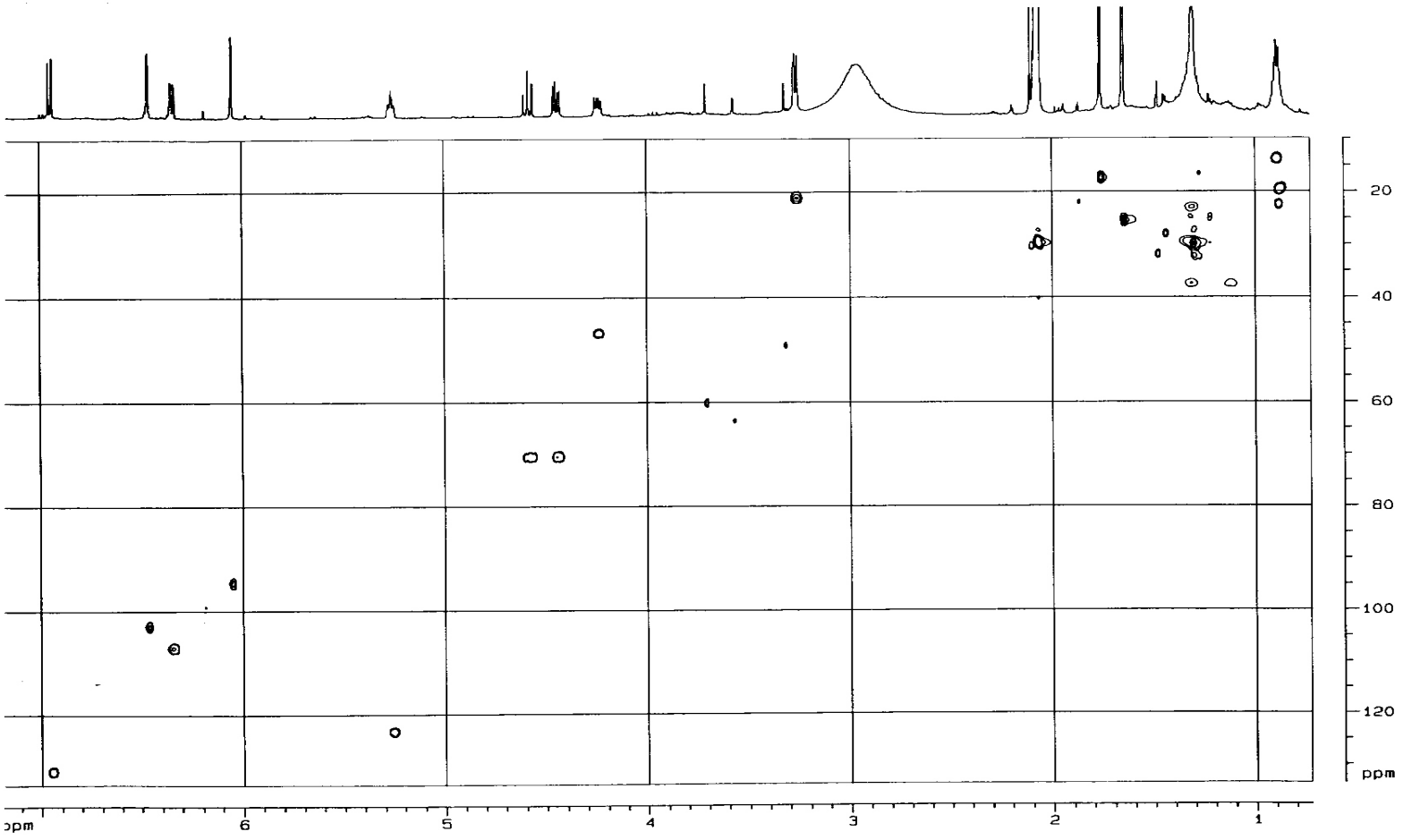
LXXVI. ^{1}H-NMR (500 MHz, acetone-d_6) spectrum of uncinanone A (**14**)



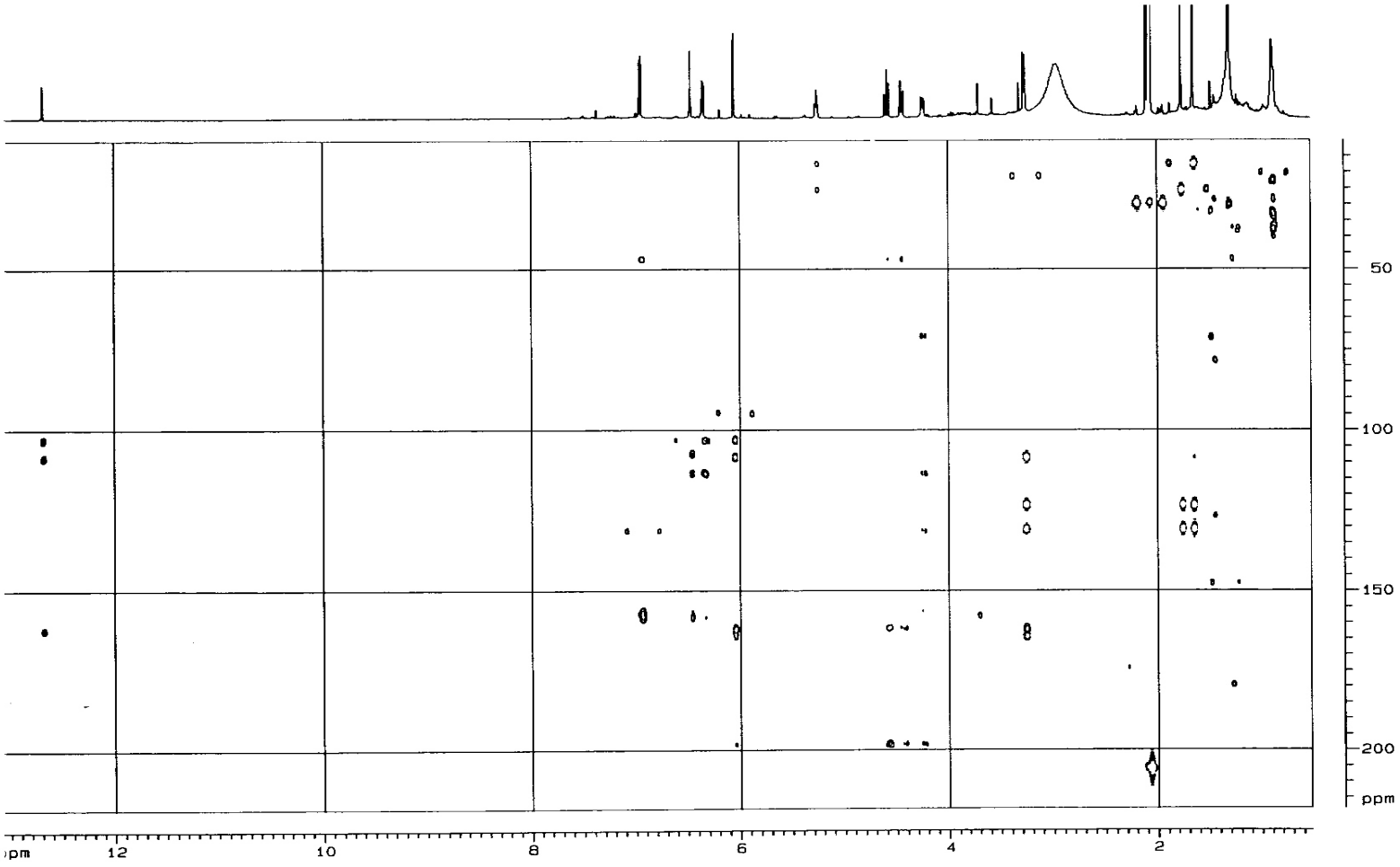
LXXVII. ^{1}H, ^{1}H-COSY spectrum of uncinanone A (**14**)



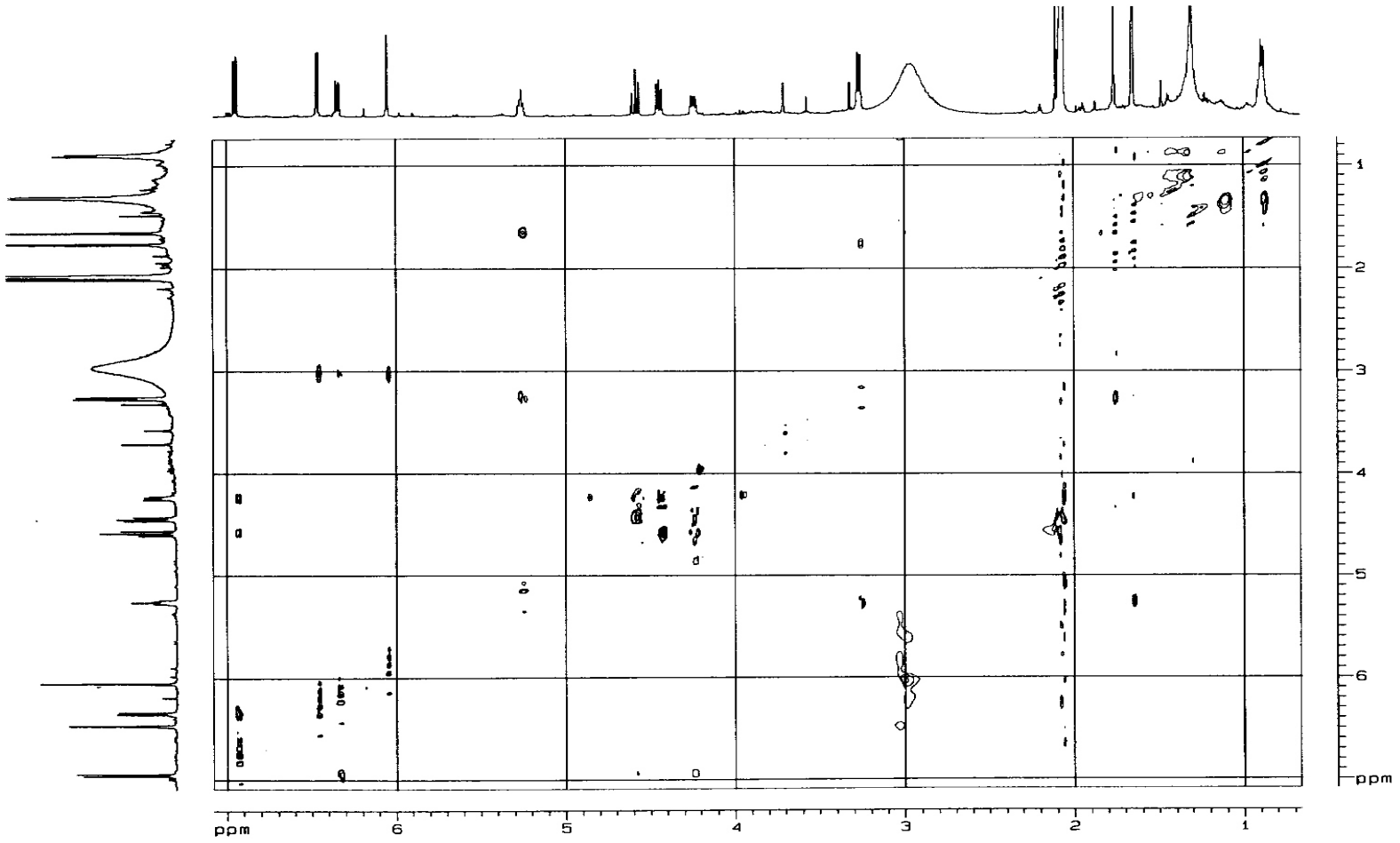
LXXVIII. HMQC spectrum of uncinanone A (**14**)



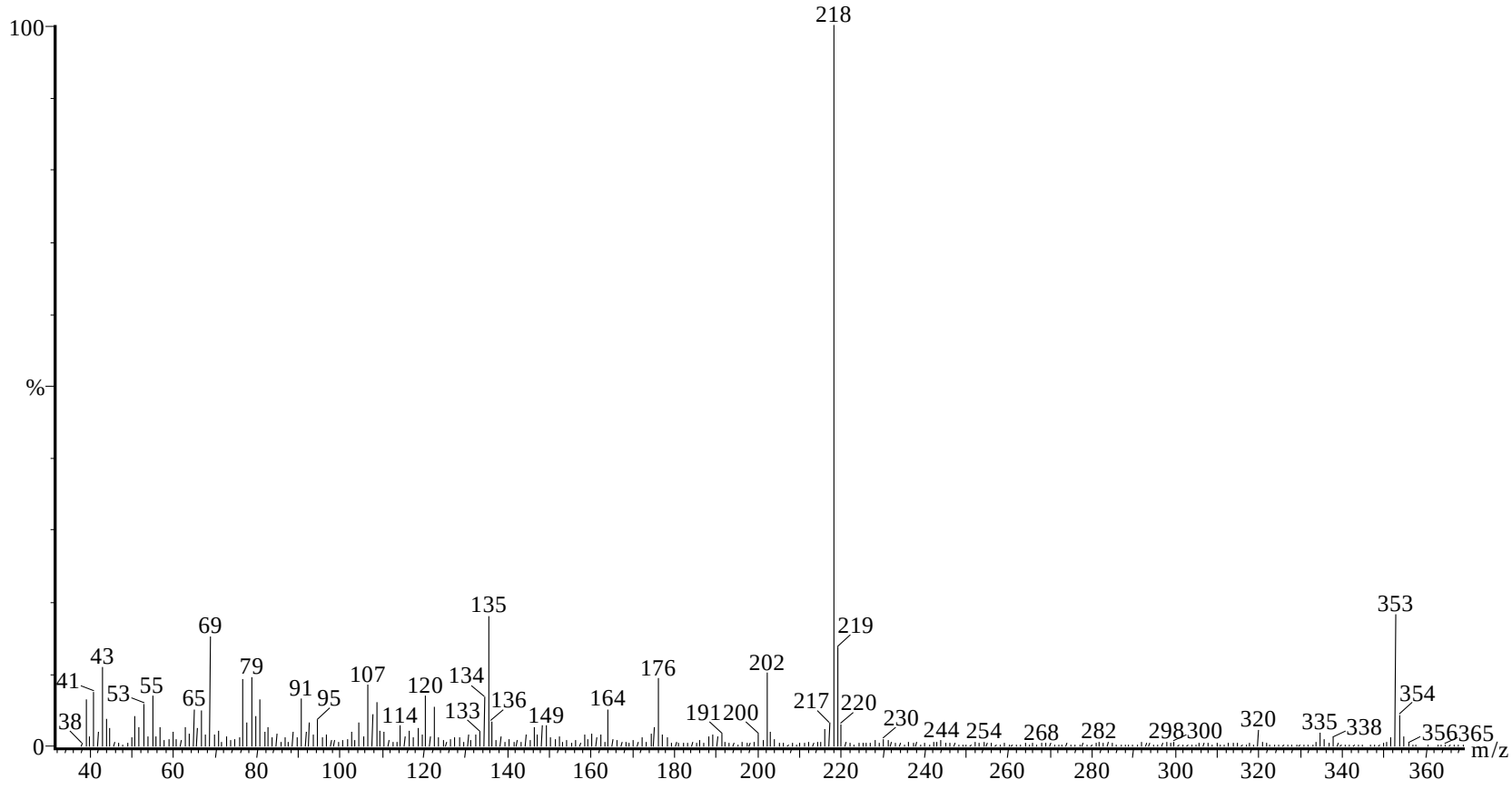
LXXIX. HMBC spectrum of uncinanone A (**14**)



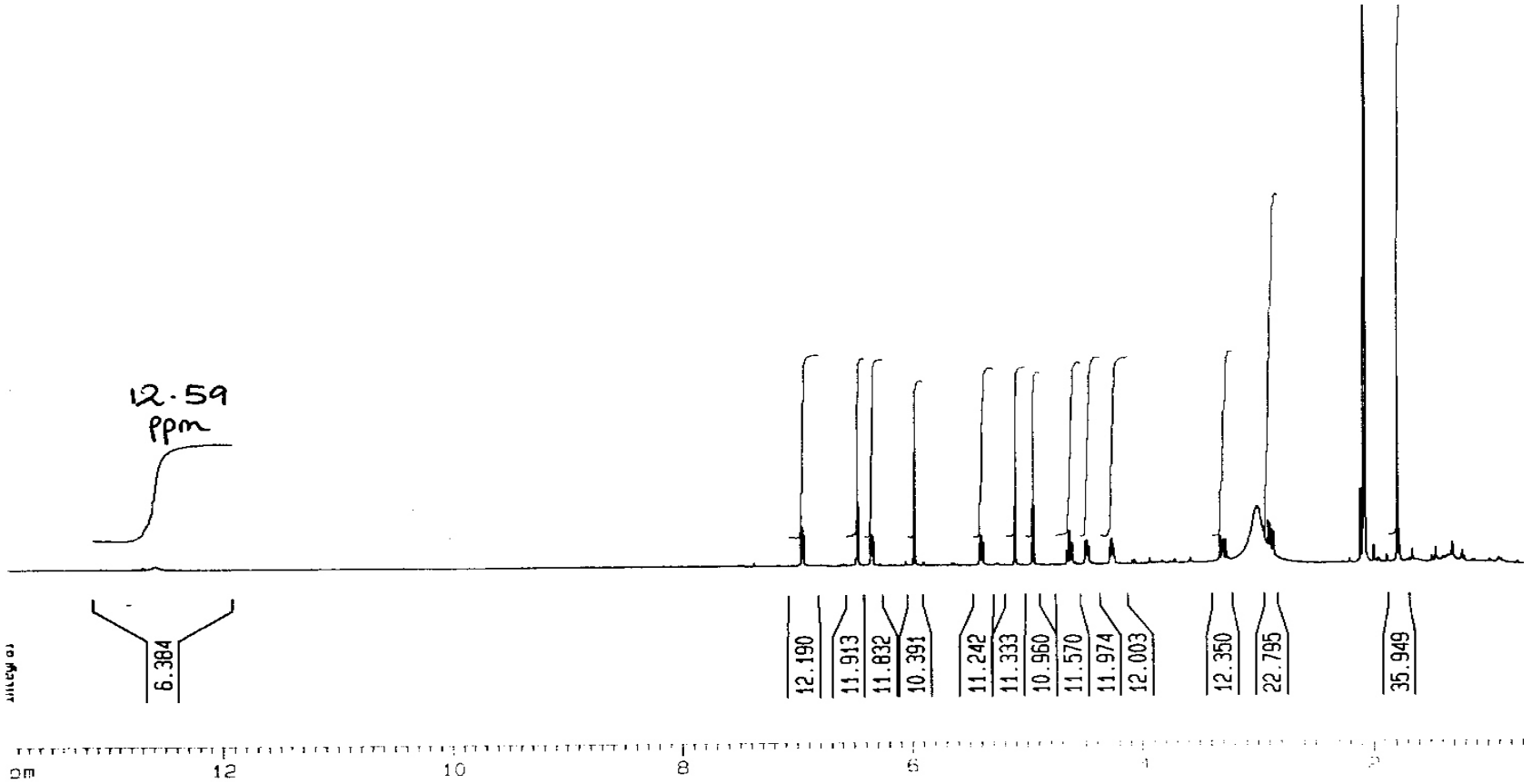
LXXX. NOESY spectrum of uncinanone A (**14**)



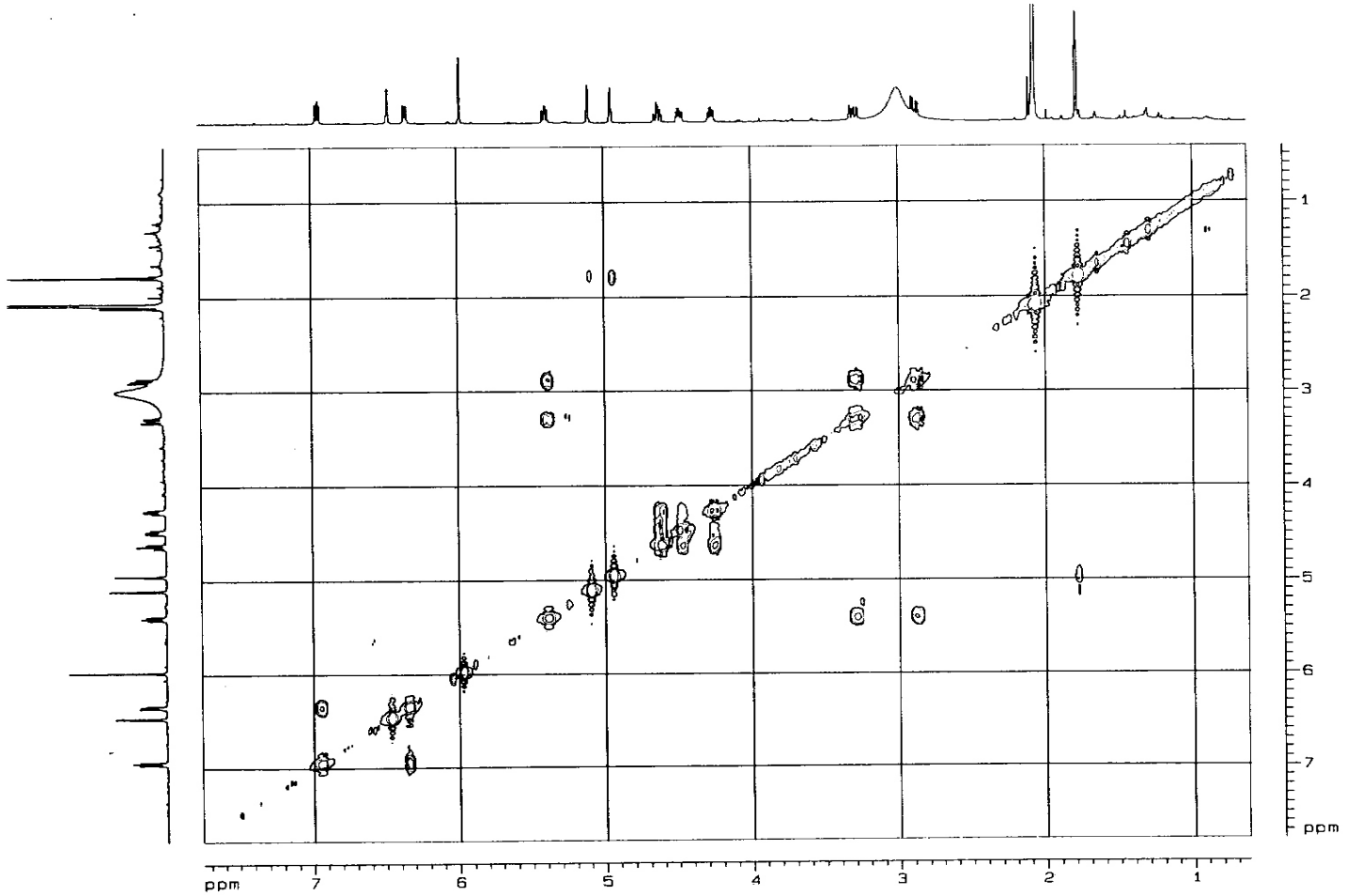
LXXXI. Mass spectrum of uncinanone B (**15**)



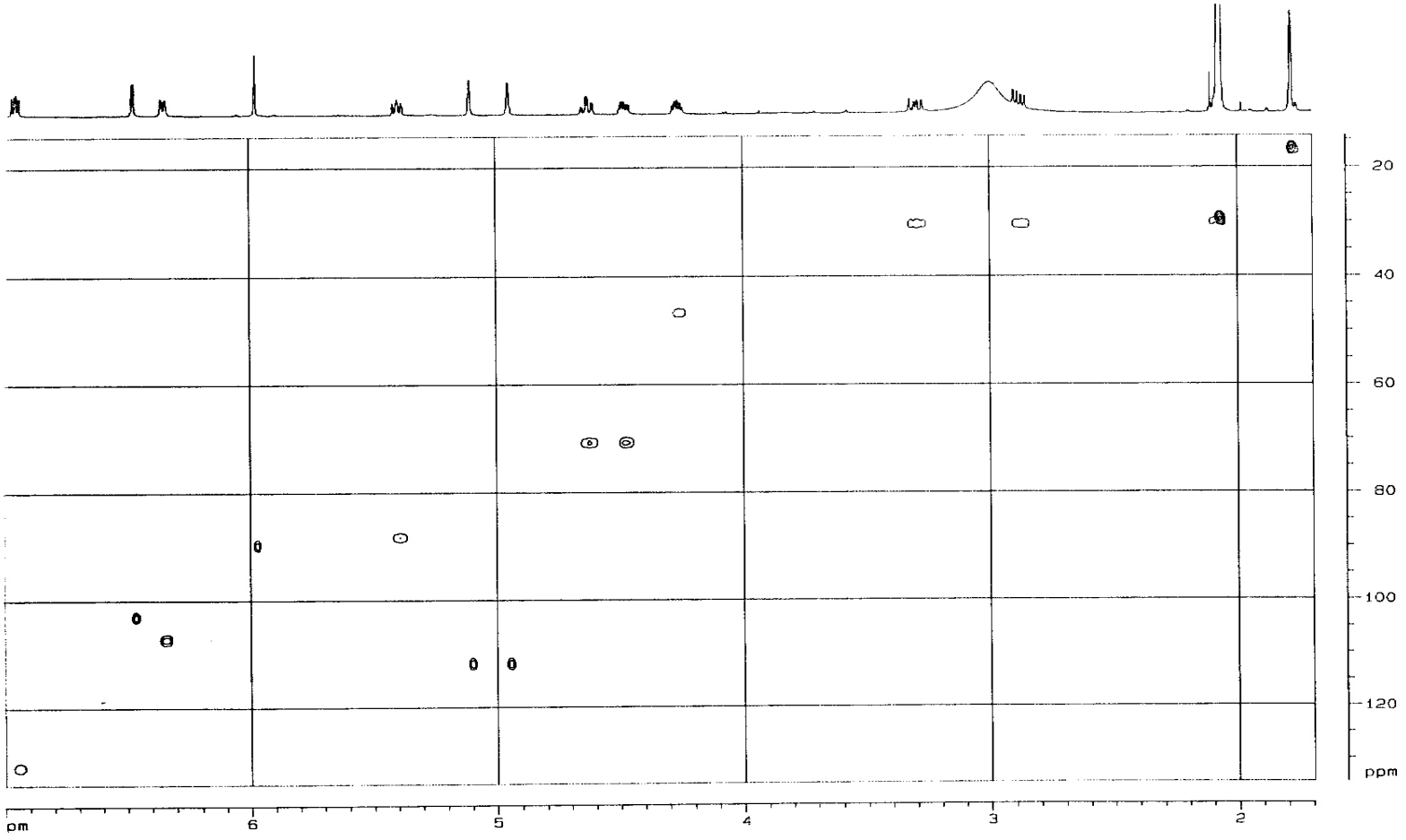
LXXXII. ^{1}H-NMR (500 MHz, acetone-d_6) spectrum of uncinanone B (**15**)



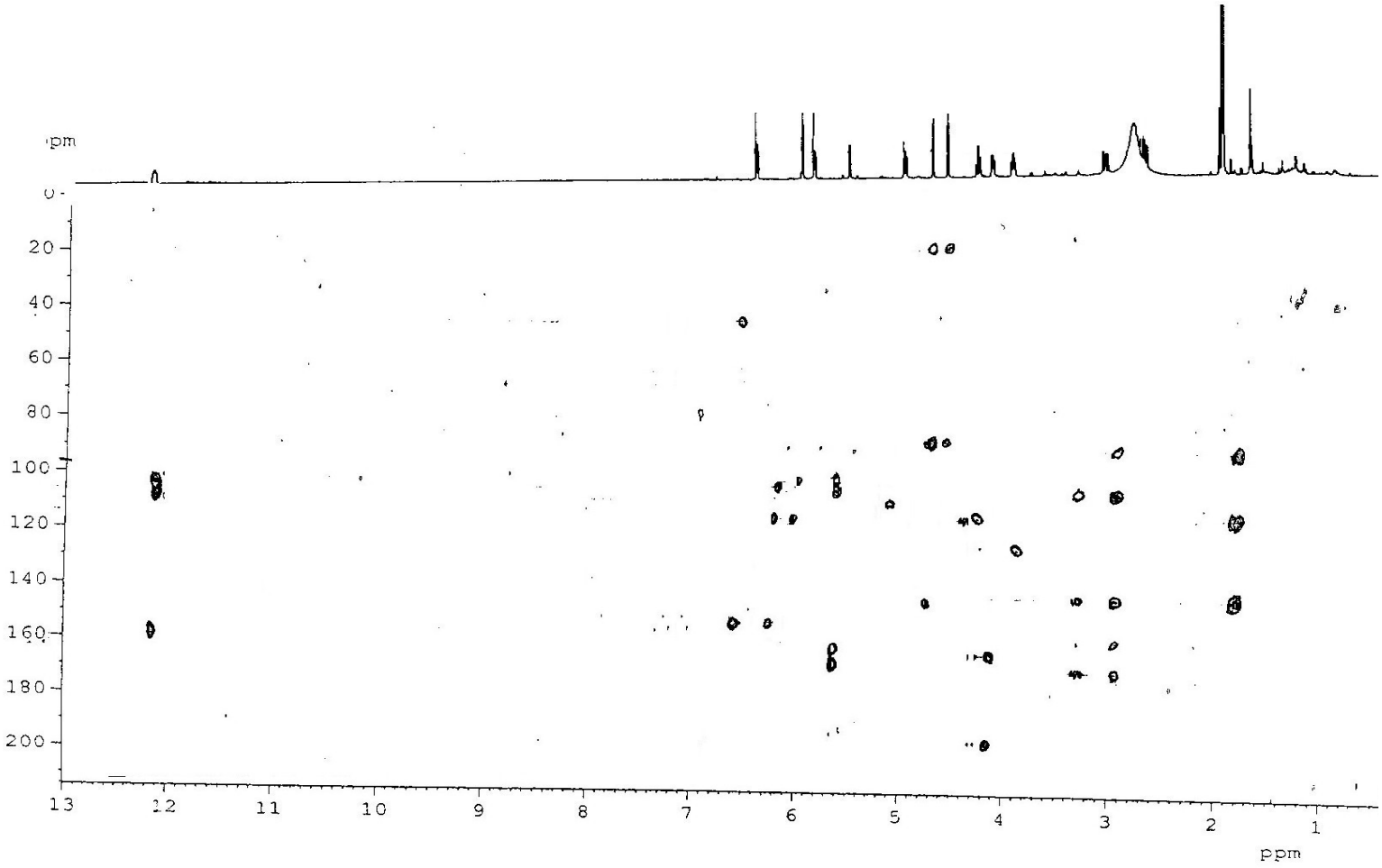
LXXXIII. ^{1}H, ^{1}H-COSY spectrum of uncinanone B (**15**)



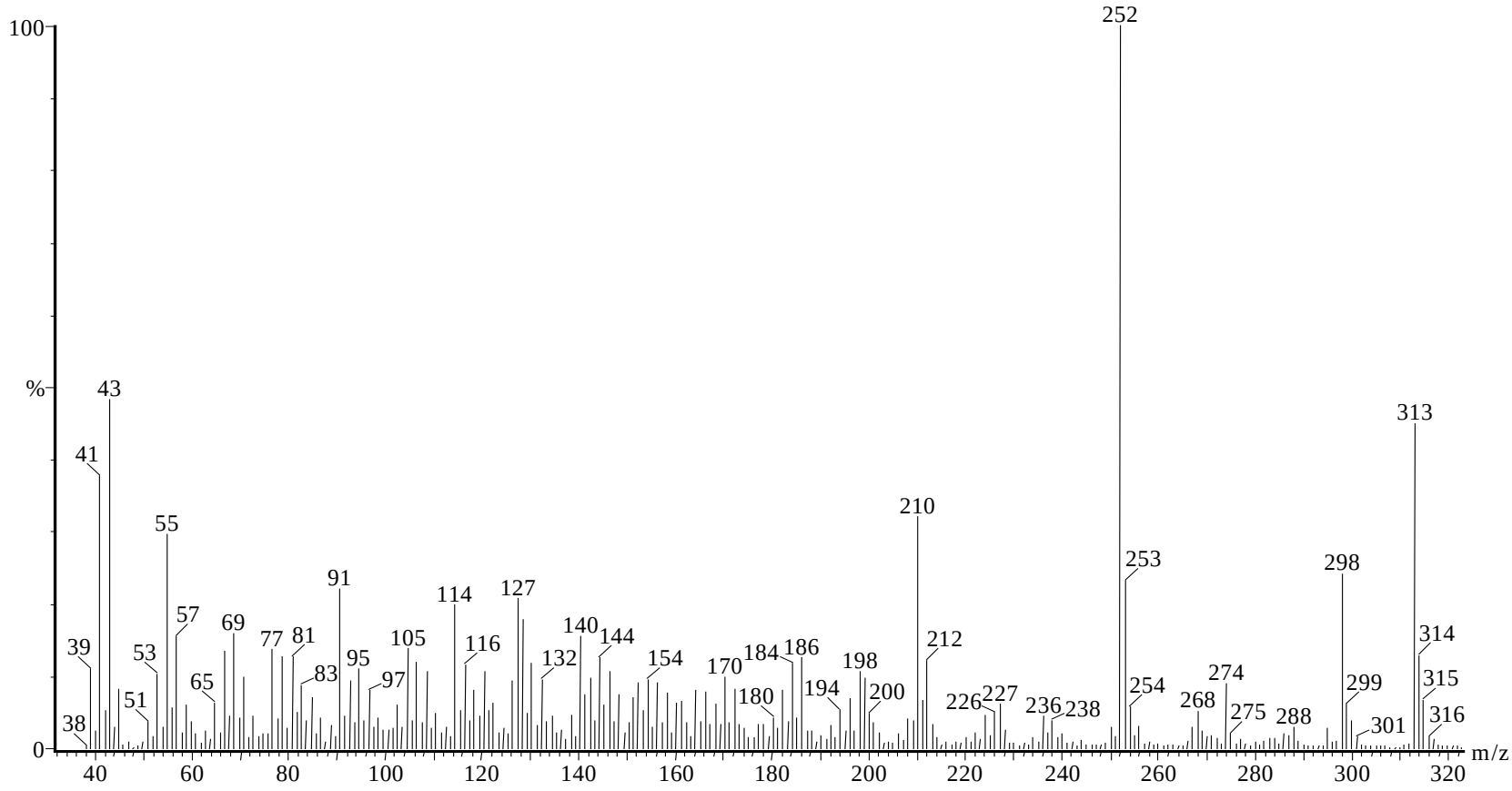
LXXXIV. HMQC spectrum of uncinanone B (**15**)



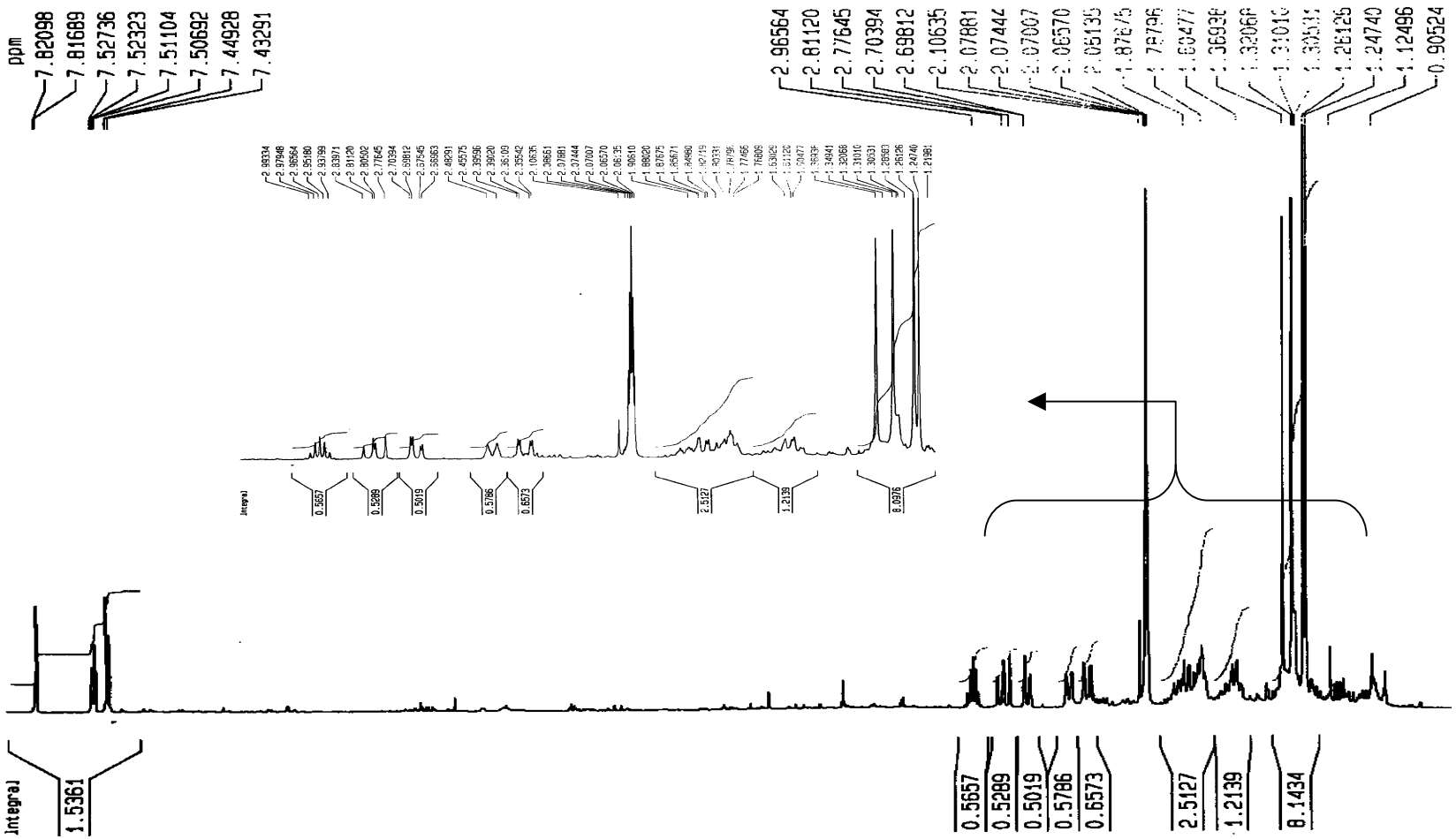
LXXXV. HMBC spectrum of uncinanone B (**15**)



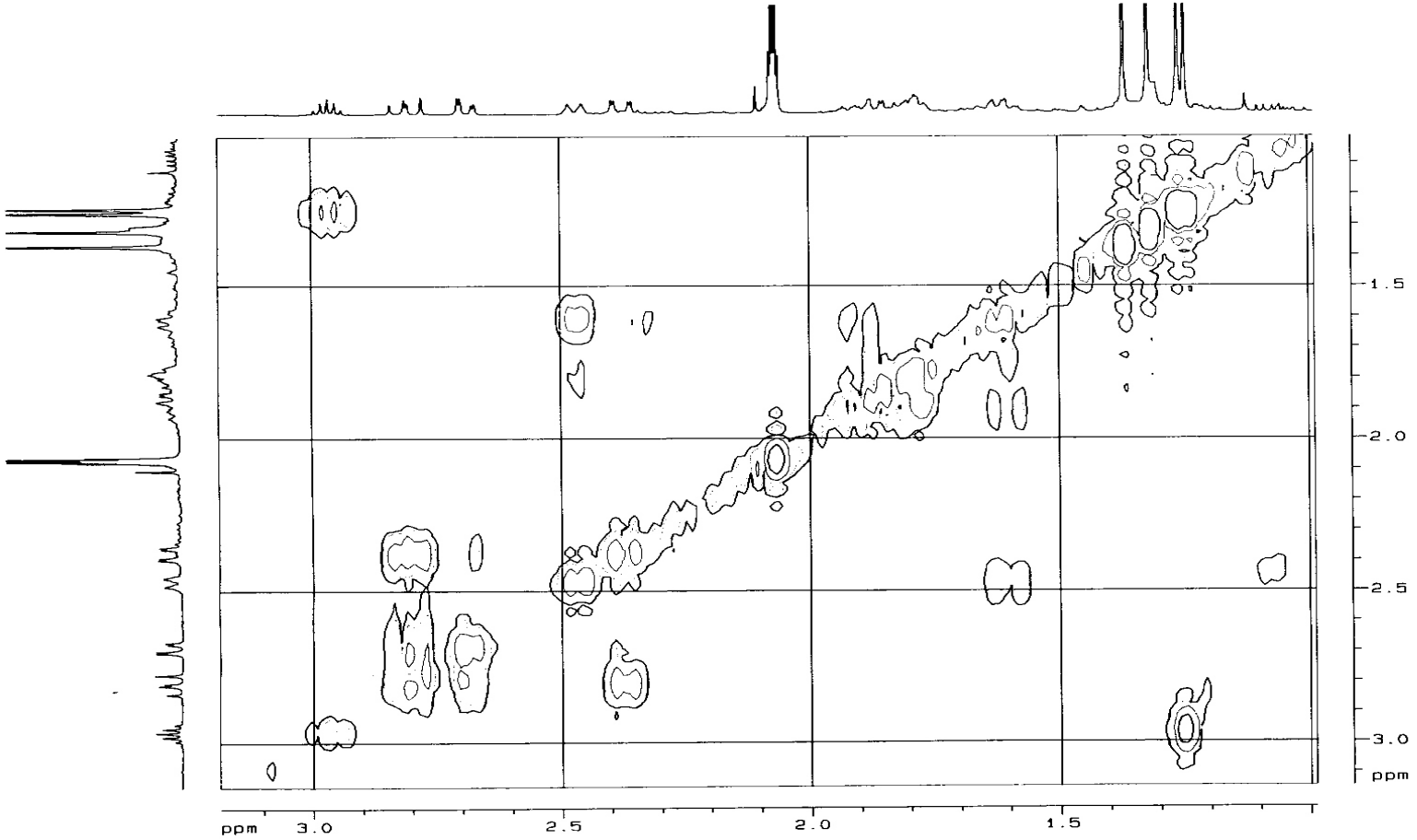
LXXXVI. Mass spectrum of 7-oxo-dehydroabietic acid (**16**)



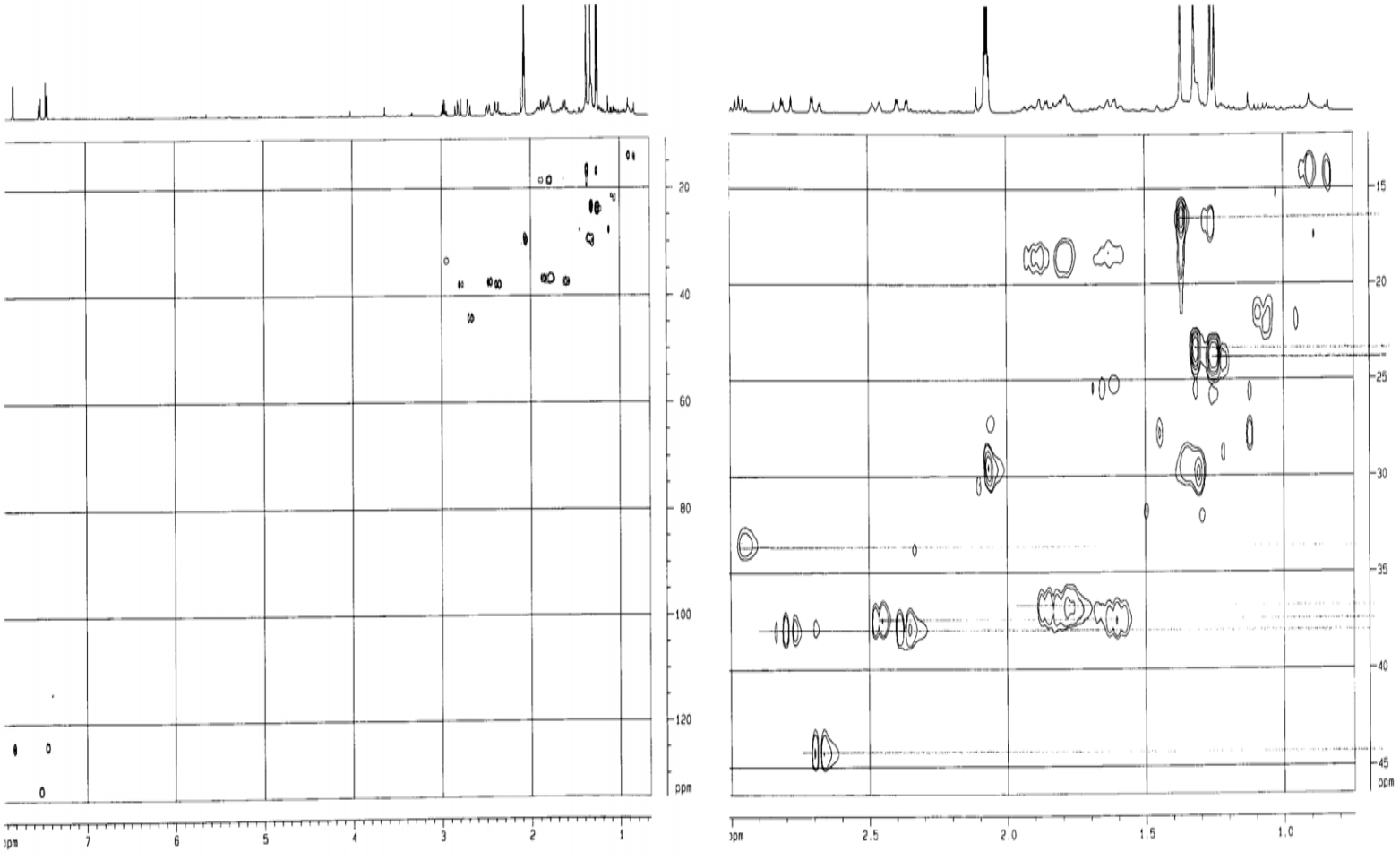
LXXXVII. ^{1}H-NMR (500 MHz, acetone-d_6) spectrum of 7-oxo-dehydroabietic acid (**16**)



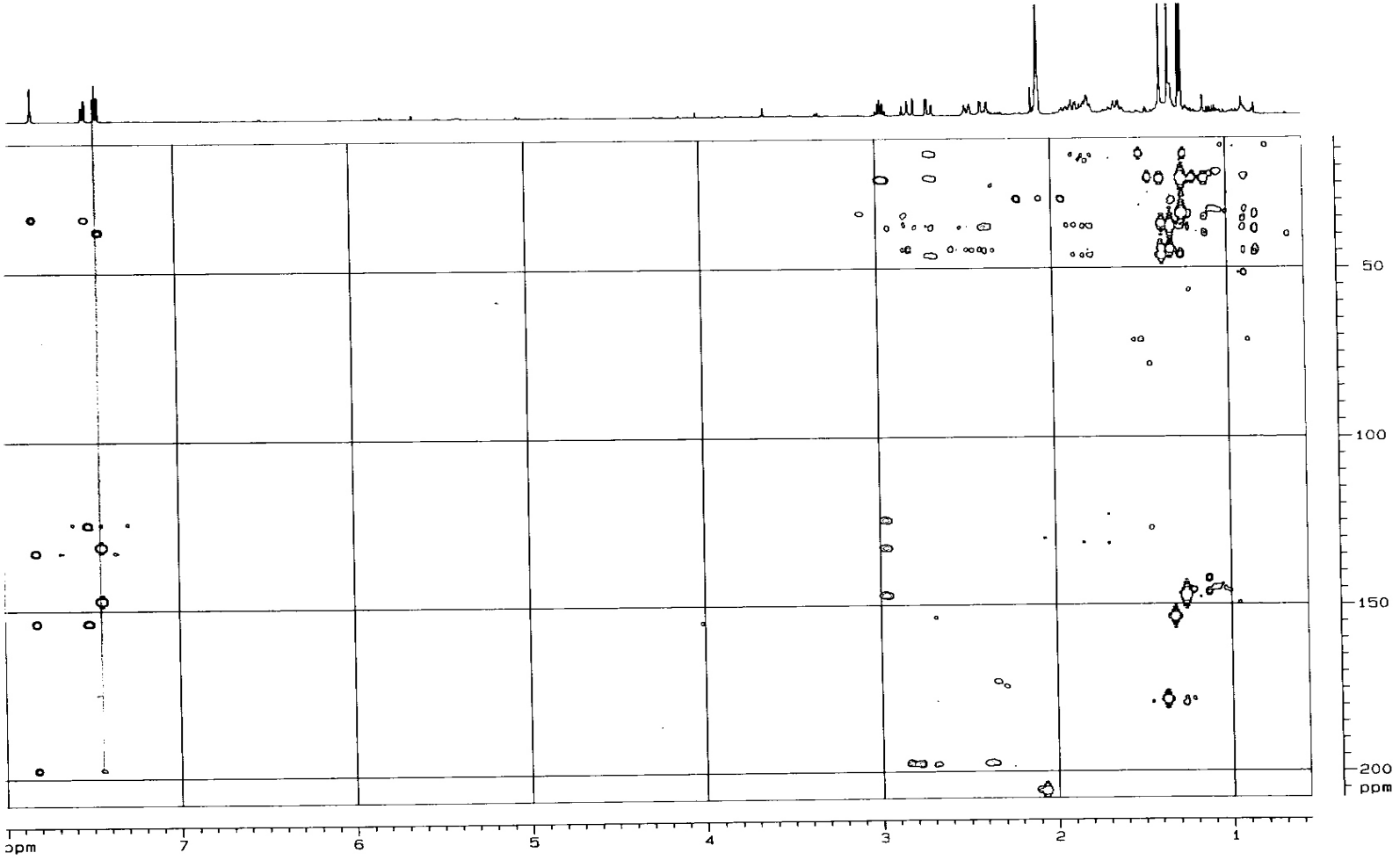
LXXXVIII. ^{1}H, ^{1}H-COSY spectrum of 7-oxo-dehydroabietic acid (**16**)



LXXXIX. HMQC spectrum of 7-oxo-dehydroabietic acid (**16**)



XC. HMBC spectrum of 7-oxo-dehydroabietic acid (**16**)



XCI. NOESY spectrum of 7-oxo-dehydroabietic acid (**16**)