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Isolation, Structural Elucidation and Structure-Activity Studies of Natural Products From Regional Plants of the Asteraceae.

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ISOLATION, STRUCTURAL ELUCIDATION AND STRUCTURE-ACTIVITY STUDIES OF NATURAL PRODUCTS FROM REGIONAL PLANTS OF THE ASTERACEAE

A Dissertation

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in

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by
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To My Father

and

the Memory of My Mother

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ABSTRACT

In a biochemical systematic study of the plant family Asteraceae combined with a search for bioactive natural products, the isolation and structural elucidation of secondary metabolites from seven plant species is reported. Investigation of the roots of *Solidago canadensis* provided six known and one new clerodane diterpenes related to kolavenic acid and five known matricaria ester-type polyacetylenes. The roots and aerial parts of *Solidago rugosa* afforded the known diterpenes kolavenol, hardwickiic acid, (-)-kaur-16-en-19-oic acid, (+)-manool, (+)-3β-hydroxymanool, manoyl oxide and *ent*-abietic acid and the new labdane diterpene, (+)-18-tigloyloxymanool, plus four new *ent*-abietane diterpenes. The isolation of two new and eight known eudesmane-type sesquiterpene cinnamates, and benzyl 2,6-dimethoxybenzoate from the roots of *Brintonia discoidea* (syn. *Solidago discoidea*) is reported. The roots of *Chrysoma pauciflosculosa* (syn. *Solidago pauciflosculosa*) provided the known polyacetylenes *cis,cis*- and *cis,trans*-matricaria esters, a mixture of two isomeric epoxides of matricaria ester, and two known triterpenes, epifriedelinol and friedelin. In addition, the ubiquitous 2,6-dimethoxybenzoquinone and a new benzotropolone were found. The roots of *Erigeron philadelphicus* afforded, besides the above four polyacetylenes, two isomeric matricaria lactones. The roots of giant ragweed (*Ambrosia trifida*) afforded thiarubrine B and its related thiophene, as well as a new 1,2-dithia-3,5-cyclohexadiene derivative, 3-(pent-3-yn-1-ynyl)-6-(3,4-epoxy-but-1-ynyl)-1,2-dithiacyclohexa-3,5-diene. The stems of *A. trifida* gave, besides β-cubebene and α-farnesene, two carotane-type sesquiterpenes, the known lasidiol angelate (1α-angeloyloxycarotol) and the new 1α-(2'-methylbutyroyloxy)-carotol, plus 2,6-dimethoxybenzoquinone and hexadecanal. The aerial parts of *Liatris ohlingerae* afforded five known and two new heliangolide-type sesquiterpene lactones related to

liscunditrin and four known triterpenes, taraxasterol, pseudo-taraxasterol and their acetates. The detailed structure elucidation using spectroscopic methods and chemical transformations is described.

In order to demonstrate structure-activity relationships, a series of chemical transformations on sesquiterpene lactones were performed. Epoxidation of costunolide yielded 1,10-epoxycostunolide, parthenolide, and the cyclization products santamarin, reynosin, magnolialide and a 1,4-epoxyeudesmenolide. Reduction of santamarin afforded 11,13-dihydrosantamarin and an eudesmen-triol, while reduction of reynosin provided 11,13-dihydroreynosin and two eudesman-triols. The acid-catalyzed transformation of dihydroparthenolide in methanol afforded two guaianolide derivatives. These sesquiterpenes were tested against *Mycobacterium tuberculosis* and *M. avium* and the structure-activity relationships among sesquiterpene lactones are discussed. Polyacetylenes from *S. canadensis*, *C. pauciflosculosa* and *E. philadelphicus* also exhibited significant activity against these two strains of mycobacteria.

CHAPTER 1

INTRODUCTION

The genus *Solidago*, commonly known as goldenrod, belongs to the tribe Asterae in the Asteraceae family. It consists of *ca.* 120 species, mostly found in North America and a few in Eurasia and South America. In many countries members of this genus have long been used in folk medicine as anti-bacterial and anti-inflammatory agents, for the treatment of chronic nephritis, kidney and bladder stones, rheumatism as well as diuretics [1-5]. So far about 30 species of this genus have been chemically investigated. Their major constituents are diterpenes, polyacetylenes, hydroxybenzoates, flavonoids, polysaccharides and saponins. Two reviews dealing with the distribution of clerodane diterpenes [6] and polyacetylenes [7] in plants including *Solidago* have previously appeared. However, constituents in this genus other than the diterpenes and polyacetylenes have not yet been reviewed. The main purpose of this introduction is to give a detailed review of the occurrence of the variety of compounds found in the genus *Solidago*, with the exception of the polysaccharides and saponins, which will only be briefly discussed here. The biological activities of major constituents and the biogenetic or biosynthetic aspects of some compounds will also be discussed.

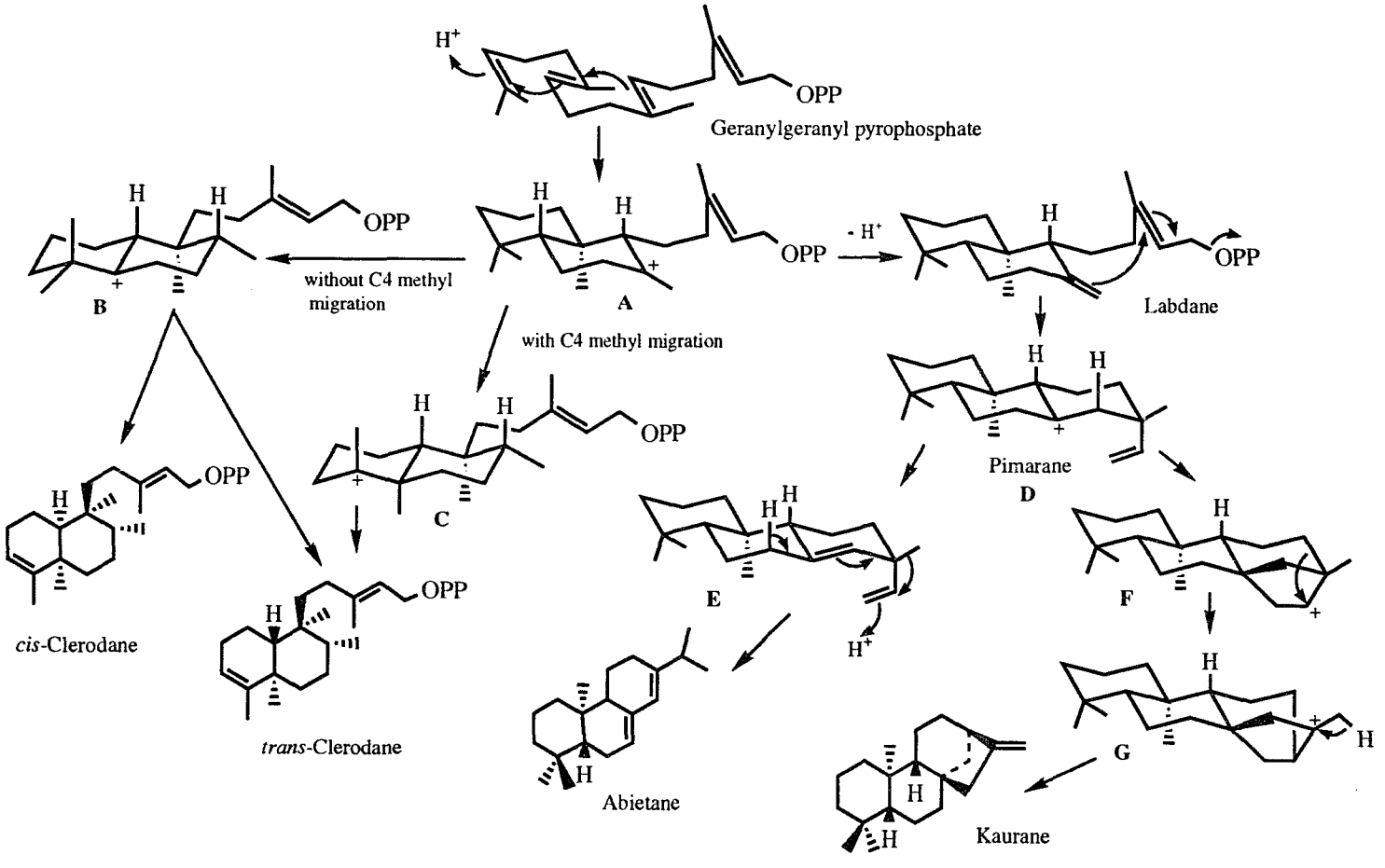
Diterpenes

Solidago species have a remarkable ability to produce diterpenes. Only a few linear diterpenes have been reported in this genus. Clerodanes, labdanes, abietanes and kauranes are the four major skeletal types of diterpenes found in the genus. Among those the clerodanes are the most common group. The biogenesis of the four cyclic skeletal types of diterpenes is outlined in Scheme 1.1. The clerodanes are thought to arise by successive methyl and hydride shifts from the labdane precursors **A** or **B**, which are cyclization products of geranylgeranyl pyrophosphate. Methyl migration from C4 to C5 in **A** *via* intermediate **C** leads to the *trans*- clerodanes, while

the *cis*- clerodanes require a stepwise process, with a "pause" at intermediate **B**, which can then lead to either *cis*- or *trans*- compounds, depending on which of the C4 methyl groups migrate [8].

The abietanes and kauranes must arise from the same precursor **A**, *via* the labdane and pimarane intermediate **D**, *via* enzymatic cyclization processes. A series of hydride, double bond and methyl shifts lead to the abietanes, while the double bond addition followed by alkyl and hydride shifts give kaurane type compounds. These biosynthetic rearrangements have been confirmed by biosynthetic studies [9, 10].

The distribution of diterpenes isolated from 23 *Solidago* species is given in Table 1.1. Among them *S. altissima* has been extensively investigated. Although *S. altissima* is native to North America, most of the chemical studies have been done by Japanese scientists. *Solidago altissima* was introduced to Japan in the 1950's and it is now found through out the country due to the favorable climatic conditions and possibly due to its aggressive allelopathic effects [11]. Diterpenes isolated from this species are exclusively clerodanes with *cis*- or *trans*- ring junction. Most of them are related to kolavenic acid (**1**) [12] and solidagolactone I (**14**) [13] with variations only in substitutional groups, which in most cases are angelate, tiglate or acetate at the C6 or C7 position. In addition, some represent 3,4-epoxide derivatives (**12**, **13**, **21**, **23**-**25**) [12-17]. Dehydrokolavenic acid (**9**) [18] could be biogenetically derived by enzymatic allylic oxidation at C2 of kolavenic acid followed by extended elimination resulting in a double bond shift shown in **9**. Kolavenic acid is also the precursor of solidagonal acid (**22**), which may be formed *via* oxidative cleavage of the C3-C4 double bond followed by an aldol condensation [16, 17]. The hydroperoxide **27** seems to arise from the kolavenic acid *via* an ene reaction [16]. An unusual diterpene skeleton is found in *S. altissima*, the tricyclosolidagolactone (**26**), which must be derived from a *cis*-clerodane precursor, most likely by intramolecular aldol reaction of compound **11** [19, 20]. The diterpenes isolated from *S. elongata* are very similar to those found in *S.*



Scheme 1.1. The possible biogenetic pathway of different skeletal diterpenes from *Solidago spp.*

Table 1.1. Distribution of diterpenes in the genus *Solidago*

Species	Diterpenes	References
Solidago altissima L.	**1-4, 6, 8-29**	12-20, 90, 91, 110-114
S. arguta Ait.	**30-38**	22-24
S. canadensis L.	**1-4, 6-7, 14-15, 40-46**	25-28
S. chilensis Meyen	**39-40, 44**	30, 115
S. drummondi Torr & Gray	**115-123**	2
S. elongata Nutt.	**1-2, 4-5, 15, 17-18, 23-24, 47-48**	21
S. flexicaulis L.	**115, 123**	2
S. gigantea Ait.	**49-51, 62**	32, 116
S. gigantea Ait. var. *serotina*	**49-50, 52-61**	33-35
S. juncea Ait.	**44, 63-64, 83, 100-102**	47
S. microglossa DC.	**40-41, 62, 65, 82**	31
S. missouriensis Nutt.	**83-85, 93-99, 110-111**	44-46
S. multiradiata Ait.	**83**	*
S. multiradiata var. *mutiradiata*	**89**	*
S. nemoralis Ait.	**83, 86-88, 103**	27, 48
S. petradoria Blake	**105, 124, 125**	43, *
S. racemosa Greene	**115, 123**	2
S. rigida L.	**83**	39, 48
S. rugosa Mill.	**2, 15, 43-44, 63, 66, 83, 89-92, 104, 106-110, 112-114**	27, 49
S. sempervirens L.	**40, 63, 74**	40-42
S. serotina Ait.	**32, 52-55, 57-60, 67-71**	36, 37
S. shortii Torr & Gray	**72, 73**	22, 39
S. virgaurea L.	**11, 24-25, 28-29, 75-81**	38

* Unpublished data cited in reference 117.

altissima except for kolavelool (**47**) and its 6-angelate (**48**) [21]. It seems that they are biogenetically very closely related species. The nomenclature of elongatolides (**10**, **23**, **24**, **28**) also called solidagolactones, which are named after the plant source, is somewhat confusing.

cis-Clerodane furan diterpenes (**30**, **32-38**) seem to be characteristic for *S. arguta*[22-24]. The furan compounds might be the precursors for α,β-unsaturated γ-lactones. Alternatively, the lactols derived reductively from the lactones could give the furan ring upon extended elimination. The very common Canadian goldenrod *S. canadensis* afforded both labdane and *trans*-clerodane diterpenes [25-29]. Biogenetically, the clerodanes appear to be related to the labdanes, *via* a series of methyl and hydride shifts [8]. Solidagenone (**40**) and kolavenic acid (**1**) are representatives of these two skeletal types. The two isomeric spiro ethers (**41**, **42**) isolated from *S. canadensis* can be readily converted into solidagenone (**40**) [25], indicating that they could be the direct precursors for solidagenone. Compound **40** and its related γ-lactone (**39**) have also been isolated from *S. chilensis*, a South American species which is used as a medicinal plant by the local people [30]. The labdanes, solidagenone and its congeners (**41**, **62**, **82**), along with a furan-containing *trans*-clerodane (**65**) have been found in *S. microglossa*, the only *Solidago* species occurring in Brazil [31].

Solidago gigantea, *S. gigantea* var. *serotina* and *S. serotina* are three closely related taxa. However, the diterpenes isolated from these three species have shown some structural differences. *cis*-Clerodane diterpenebutenolides, 6-deoxy-solidagolactone IV-18,19-olides (**49**, **50**), have been found in both *S. gigantea* [32] and *S. gigantea* var. *serotina* [33, 34], while furan-containing *cis*-clerodanes, solidagoic acid A (**52**) and its derivatives (**53-61**), seem to be characteristic for *S. gigantea* var. *serotina* [34, 35] and *S. serotina* [36]. Four *trans*-clerodane aldehydes (**68-71**) have been

isolated from *S. serotina*[37] which are absent in *S. gigantea* and *S. gigantea* var. *serotina*.

The diterpenes found in *S. virguarea* [38] are exclusively *cis*-clerodane lactones including solidagolactone II, III, V and VII (**28**, **29**, **11**, **24**) and their related compounds (**25**, **75-81**). Two further *cis*-clerodanes, **72** and **73**, have been isolated from *S. shortii* [22, 39]. The *trans*-clerodane hardwickiic acid (**63**) along with two labdane diterpenes, sempervirenic acid (**74**) and solidagenone (**40**) have been isolated from *S. sempervirens* [40-42]. From *S. petrodoria* two labdane spiro ethers (**124**, **125**) and an abietene succinate (**105**) have been obtained [43].

There are two labdanes, more specifically *ent*-13-epimanoyl oxide (**110b**) and *ent*-3-oxo-13-epimanoyl oxide (**111**), and three oxidized kaurene derivatives (**83-85**) isolated from *S. missouriensis* [44-46]. However, the abietanes (**93-99**) seem to be more frequently found in this species [44, 46]. The 7,13-abietadienes (**93-96**) are presumably directly derived *via* dehydration from other naturally occurring diterpenes, namely the 8(14)-abieten-13-ols (**97-99**) found in the same plant [46]. The *trans*-clerodanes hardwickiic acid (**63**), junceic acid (**44**) and its epoxide (**64**), kaur-16-en-19-oic acid (**83**) and three abietanes (**100-102**) have been isolated from *S. juncea* [47]. The compound **83** has also been found in *S. rigida* [39, 45].

The kaur-16-en-19-oic acid (**83**) and its further oxidized derivatives (**86-88**) along with an abietane acetate (**103**) are the major constituents from *S. nemoralis* [27, 48], while *trans*-clerodanes, labdanes, abietanes and kauranes co-exist in the same plant, *S. rugosa* [27, 49]. The clerodanes include kolavenol (**2**), three γ-lactones (**15**, **43**, **66**) and two furan-containing diterpene acids (**44**, **63**). Labdanes are (+)-manool (**112**) and its derivatives (**110a**, **113**, **114**). Abietanes include abietic acid (**104**) and some further oxidized derivatives (**106-109**), while the kauranes are represented by kaur-16-en-19-oic acid (**83**) and its related compounds (**89-92**).

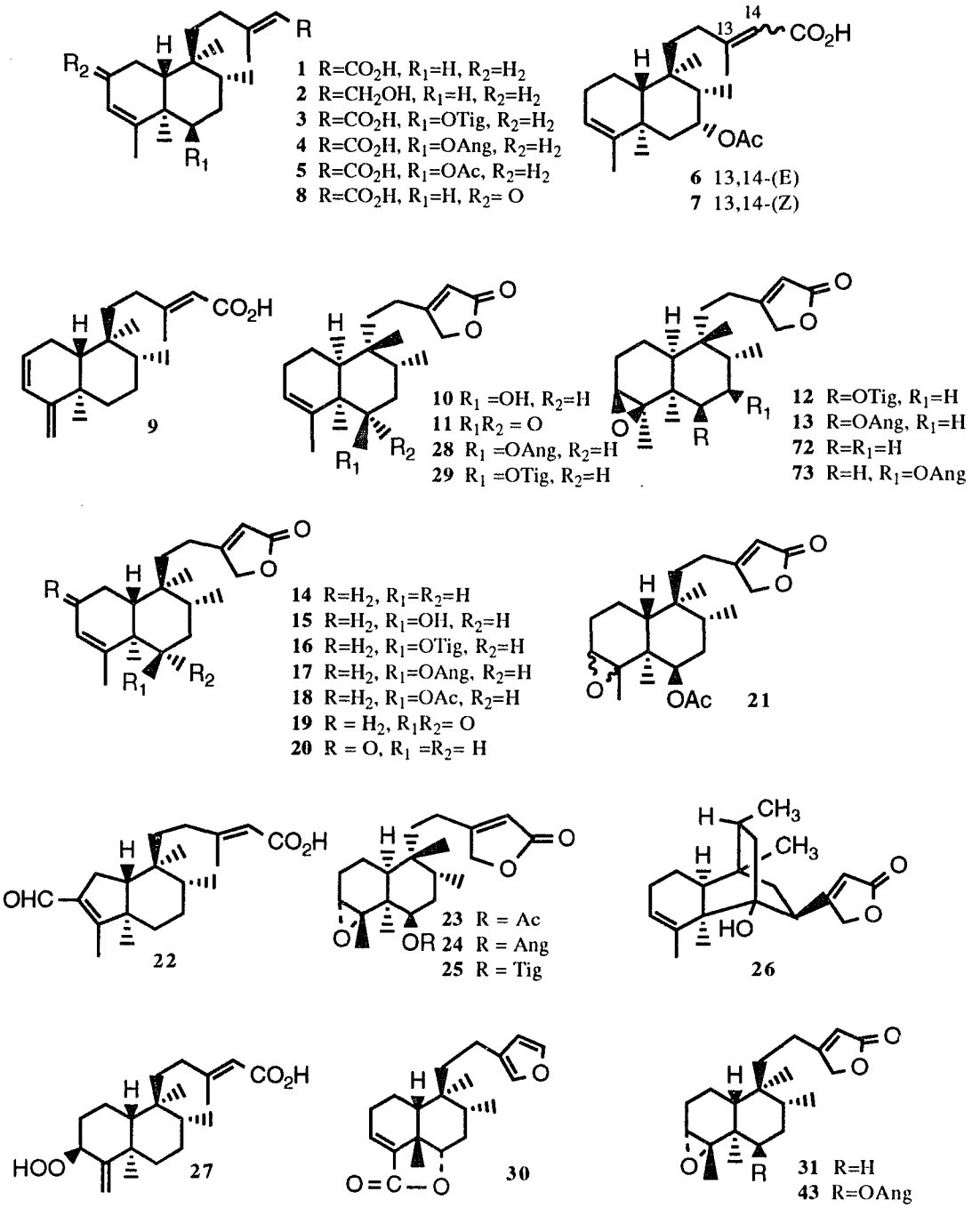
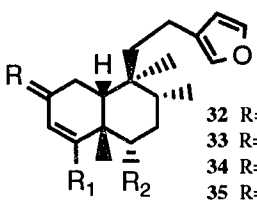
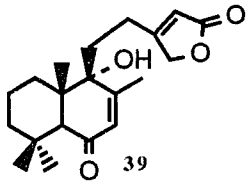


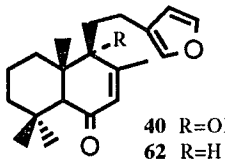
Figure 1.1. Diterpenes from *Solidago species* (continued)



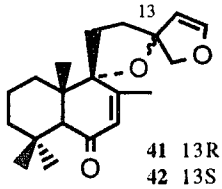
32 R=H_2, R_1=Me, R_2=H
33 R=H_2, R_1=CH_2OAc, R_2=H
34 R=H_2, R_1=CH_2OH, R_2=H
35 R=H_2, R_1=CH_2OAc, R_2=OH
36 R=H_2, R_1=CH_2OH, R_2=OH
37 R= O, R_1=Me, R_2=H
38 R=H_2, R_1=Me, R_2=OH



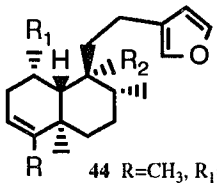
39



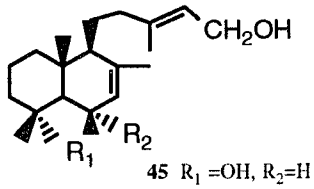
40 R=OH
62 R=H



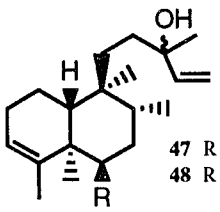
41 13R
42 13S



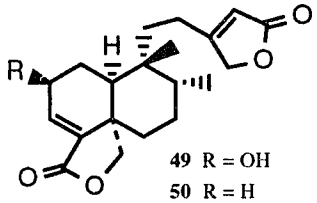
44 R=CH_3, R_1=H, R_2=CO_2H
63 R=CO_2H, R_1=H, R_2=CH_3
65 R=CH_3, R'=OH, R_2=CH_3



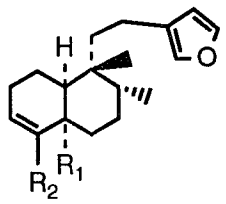
45 R_1 =OH, R_2=H
46 R_1R_2 = O



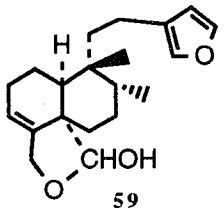
47 R = H
48 R = OAng



49 R = OH
50 R = H
51 R = O-β-glucopyranosyl



52 R_1 =CO_2H, R_2 =CH_3
53 R_1 =CO_2H, R_2 =CH_2OAng
54 R_1 =CHO, R_2 =CH_3
55 R_1 =R_2 =CHO
56 R_1 =CH_2OH, R_2 =CH_3
57 R_1 =CH_3, R_2 =CH_2OH
58 R_1 =R_2 =CH_2OH



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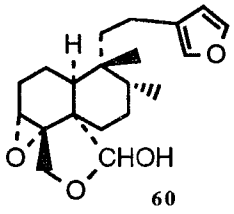

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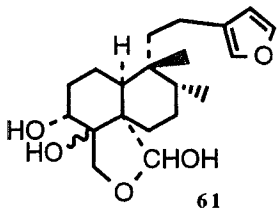

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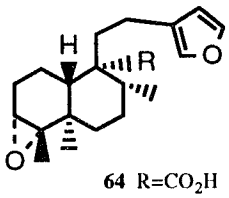


64 R=CO_2H
67 R=CH_3

Figure 1.1. (continued)

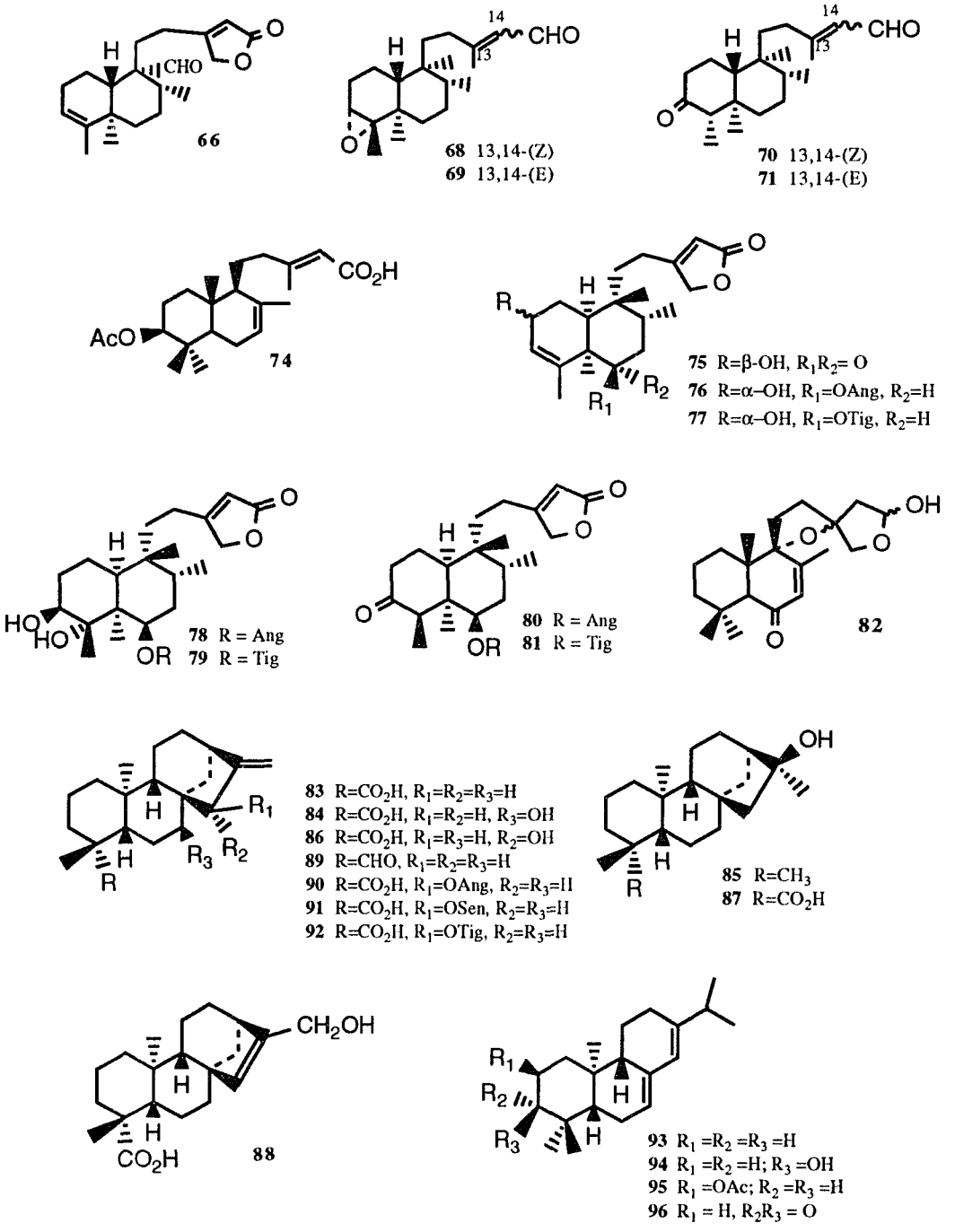


Figure 1.1. (continued)

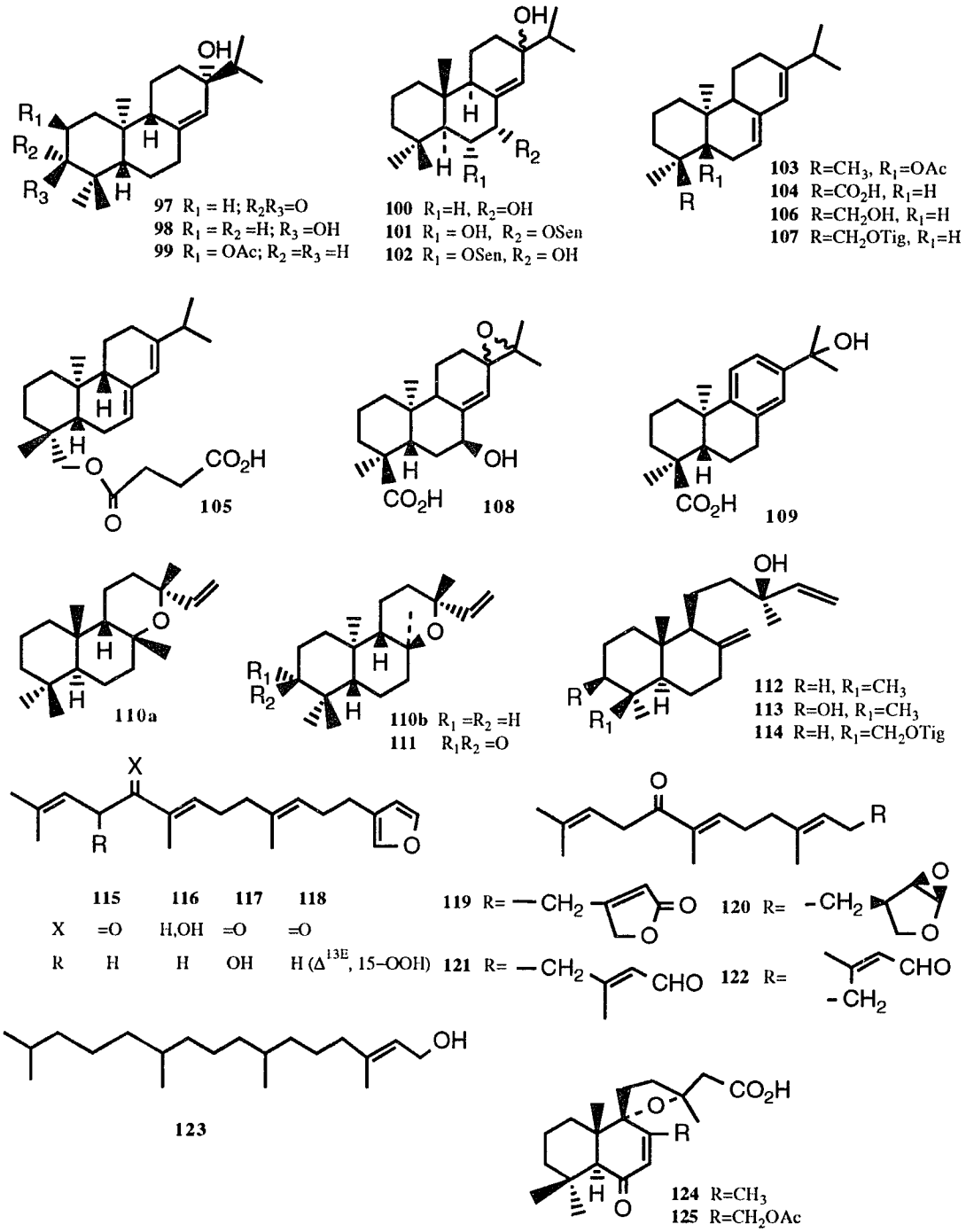

97 R_1 = H; R_2R_3=O
98 R_1 = R_2 =H; R_3 =OH
99 R_1 = OAc; R_2 =R_3 =H
100 R_1=H, R_2=OH
101 R_1 = OH, R_2 = OSen
102 R_1 = OSen, R_2 = OH
103 R=CH_3, R_1=OAc
104 R=CO_2H, R_1=H
106 R=CH_2OH, R_1=H
107 R=CH_2OTig, R_1=H
105
108
109
110a
110b R_1 =R_2 =H
111 R_1R_2 =O
112 R=H, R_1=CH_3
113 R=OH, R_1=CH_3
114 R=H, R_1=CH_2OTig
115 116 117 118
X =O H,OH =O =O
R H H OH H (Δ^{13E}, 15-OOH)
119 R=
120 R=
121 R=
122 R=
123
124 R=CH_3
125 R=CH_2OAc

Figure 1.1.

Only three *Solidago* species, namely *S. drummondii*, *S. racemosa* and *S. flexicaulis* [2], have been reported to contain alicyclic diterpenes (**115-123**), all derived from geranylgeraniol.

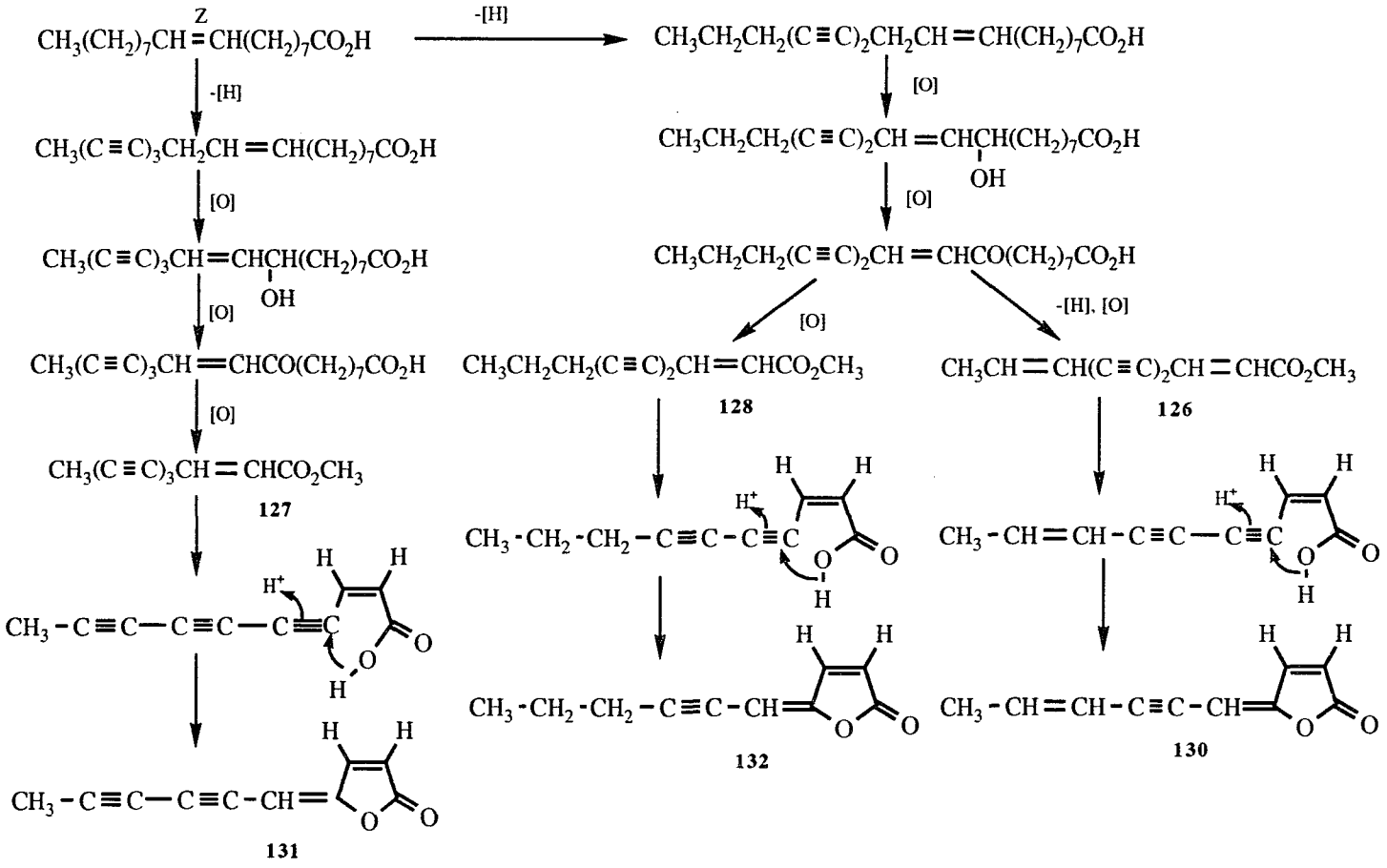
The biological activities of diterpenes will be discussed in the later section.

Polyacetylenes

Polyacetylenes are another major group of compounds found in *Solidago* species[7, 50]. The acetylenes found in this genus are mostly C_{10}- and C_{17}- straight chain and lactonic compounds. Biogenetically, polyacetylenes are assumed to arise from oleic acid *via* a series of oxidation and dehydrogenation steps. The C_{17}-acetylenes (**137-142**) from *Solidago* species are most likely derived from oleic acid by a β-oxidation followed by decarboxylation and further dehydrogenation steps. This has been verified by incorporation studies [51, 52].

The C_{10}-acetylenes from the genus *Solidago* are also thought to be derived from C_{18}-acetylenes, either by four times β-oxidation or by direct oxidation involving a Bayer-Villiger like reaction [53]. However, incorporation studies[53, 54] have shown that dehydromatricaria ester (**127**) and matricaria ester (**126**) are biosynthesized by the direct oxidation pathway. Therefore, it is reasonable to assume that all of the straight chain C_{10}-acetylenes isolated from *Solidago* species follow the same biosynthetic route as **126** and **127** do. The C_{10}-acetylenic lactones (**130-132**) could be easily derived from the straight chain C_{10}-acetylenes. A reasonable biosynthetic route for both straight chain and lactonic C_{10}-acetylenes is outlined in Scheme 1.2.

The distribution of acetylenic compounds in 15 *Solidago* species is listed in Table 1.2. C_{10}-Acetylenes (**126-134**) seem to be typical constituents in this genus. Matricaria esters (**126a-c**), dehydromatricaria esters (**127a, b**), matricaria lactones (**130a, b**) and dehydromatricaria lactones (**131a, b**) are widespread among *Solidago* species, including *S. altissima* [11, 55-57], *S. canadensis* [26], *S. decurrens* [3], *S. flexicaulis*

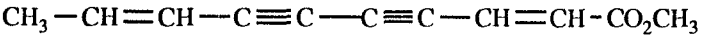


Scheme 1.2. The possible biosynthesis of C_{10}-acetylenes and lactones from *Solidago spp.*

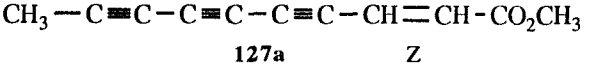
Table 1.2. Distribution of acetylenes in the genus *Solidago*

Species	Acetylenes	References
Solidago altissima L.	**126a, 127a, 131a, 133a**	11, 55-57, 60, 77, 118
S. canadensis L.	**126a, 127a, 131a, 133a, 133b, 134**	26, 118, *
S. chinensis Spr.	**127a**	50
S. chilensis Meyen	**126a**	30
S. decurrens Lour.	**126a, 126b**	3
S. elongata Nutt.	**126a, 127a, 127b, 131a, 131b**	118, *
S. flexicaulis L.	**126a, 126b, 127a, 127b**	39
S. graminifolia Salisb.	**126a, 127a, 127b, 135**	118, *
S. multiradiata Ait. var. *multiradiata*	**141, 142**	61
S. nemoralis Ait.	**127a, 131a, 133c, 136**	27, 118
S. odora Ait.	**126a, 126b, 126c, 127a, 127b**	27
S. petiolaris Ait.	**126a, 127a, 127b, 131a, 131b**	118, *
S. sempervirens L.	**127b, 129**	50
S. spathulata DC	**139, 140**	62
S. virgaurea L.	**126a, 127a, 127b, 128, 130a, 130b, 132, 137 138, 141, 142**	50, 58, 59, 96

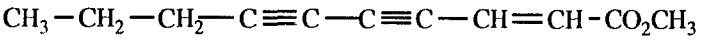
* Unpublished data cited in reference 7.



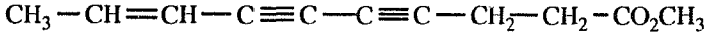
126a 2,3-(Z), 8,9-(Z)
126b 2,3-(E), 8,9-(Z)
126c 2,3-(E), 8,9-(E)



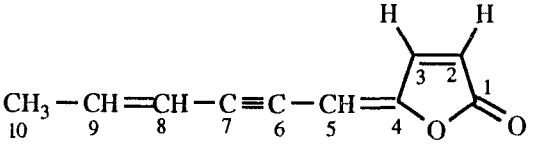
127a Z
127b E



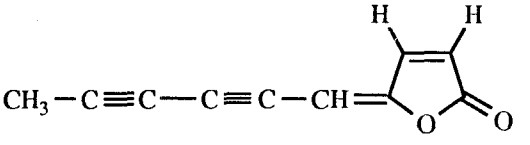
128 Z



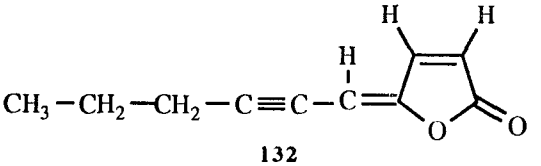
129



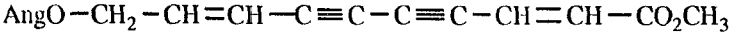
130a 4,5-(Z), 8,9-(Z)
130b 4,5-(E), 8,9-(Z)



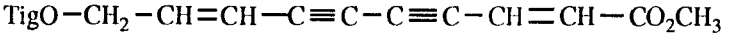
131a 4,5-(Z)
131b 4,5-(E)



132

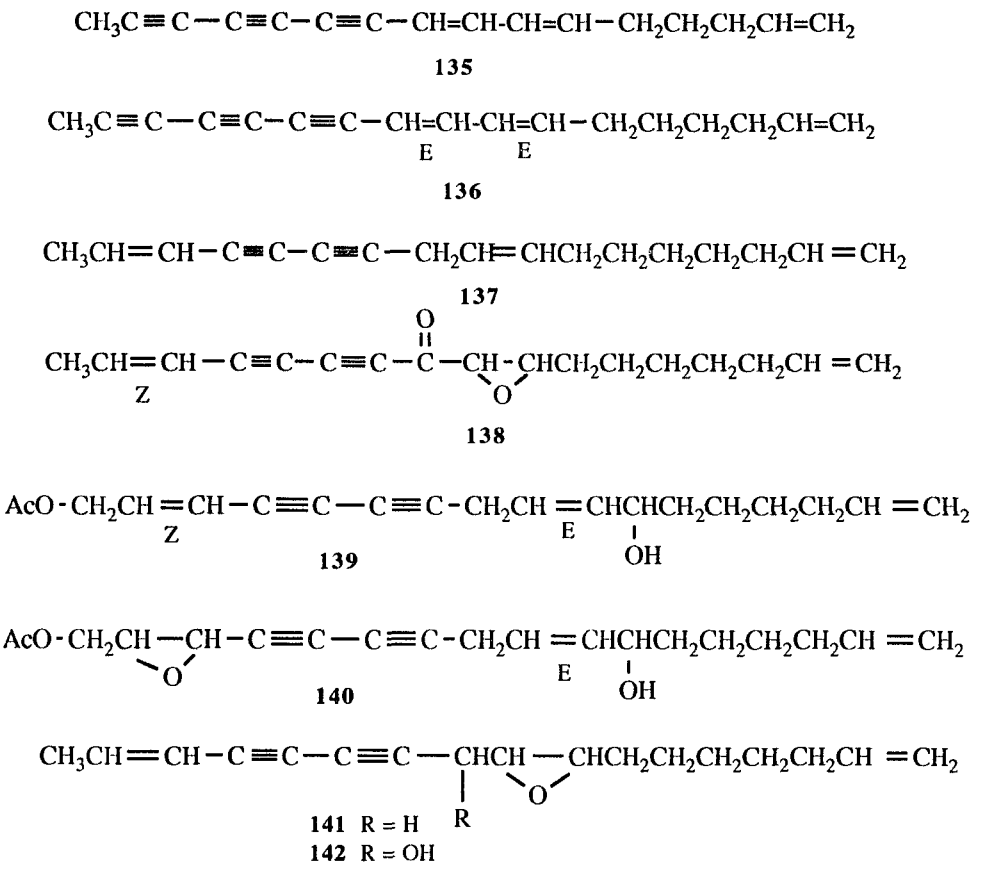


133a 2,3-(Z), 8,9-(Z)
133b 2,3-(E), 8,9-(Z)
133c 2,3-(E), 8,9-(E)



134 2,3-(Z), 8,9-(Z)

Figure 1.2. Polyacetylenes from *Solidago species* (continued)



135

136

137

138

139

140

141 R = H
142 R = OH

Figure 1.2.

[39], *S. odora* [27] and *S. virguarea* [58, 59] as well as other species. Some C_{10}-acetylene angelates (**133a-c**) and one tiglate (**134**) have also been isolated from *S. altissima* [55, 56, 60], *S. canadensis* [26], and *S. nemoralis* [27] . Only one C_{16}-acetylene (**135**) has been found in the genus *Solidago,* that is *S. gramilifolia* [7]. C_{17}-Acetylenes with one vinyl end-group (**136-142**) have also been isolated from *Solidago* species. From *S. virgaurea* L [58] four biogenetically related C_{17}-acetylenes (**137**, **138**, **141**, **142**) have been found. Three of them are epoxides (**138**, **141**, **142**). The epoxides **141** and **142** have also been isolated from *S. multiradiata* Ait. var. *multiradiata* [61]. Two C_{17}-acetylenes (**139**, **140**) closely related to the epoxides **141** and **142** have been found in *S. spathulata* DC [62] and another C_{17}-acetylene (**136**) was isolated from *S. nemoralis* Ait. [27].

The biological activity of acetylenes will be discussed later.

Miscellaneous Compounds

Besides diterpenes and acetylenes, which are widespread in the genus *Solidago*, a number of other constituents, such as sesquiterpenes, triterpenes, coumarins, benzyl benzoates, flavonoids, polysaccharides and saponins, have also been found in this genus, but they vary from species to species. The distribution of compounds other than diterpenes and polyacetylenes from 16 *Solidago* species is listed in Table 1.3. However, polysaccharides and saponins are not included in this list. These compounds, which were mainly obtained from *S. virgaurea* and *S. canadensis*, have been studied extensively by Hiller and coworkers [63-74].

Among the sesquiterpenes, germacrene D (**154**) is the most frequently found constituent in *Solidago* species, including *S. altissima* [12, 19, 75], *S. canadensis* [27], *S. drummondii* [2], *S. nemoralis* [27], *S. odora* [27], *S. petradria* [43], *S. rugosa* [27] and *S. spathulata* [62]. Other germacrenes are 1β-hydroxygermacra-4(15),5,10(14)-triene (**152**) from *S. altissima* [17], germacra-1(10),4,6-triene (**155**) from *S. nemoralis* [27] and

Table 1.3. Miscellaneous constituents found in the genus *Solidago*

Species	Compounds isolated	References
Solidago altissima L.	**150, 152, 154, 182**	12, 17, 19, 70, 75
S. arguta Ait.	**145, 146**	22
S. canadensis L.	**154, 156, 157, 194-201**	27, 78-80
S. chilensis Meyen.	**151, 157**	30
S. decurrens Lour.	**169-173, 185-187**	3
S. drummodii Torr & Gray	**154, 158, 188, 189**	2
S. microglossa DC.	**147, 149, 194**	31
S. multiradiata var. *multiradiata*	**183, 184, 192, 193**	61
S. nemoralis Ait.	**143, 144, 148, 153-155, 158-164, 168**	27
S. odora Ait.	**153, 154, 167, 174-181**	27
S. petradoria Blake	**154**	43
S. rigida L.	**170**	39
S. rugosa Mill.	**154**	27
S. spathulata DC.	**154, 156, 190, 191**	62
S. virgaurea L.	**169, 196, 197, 199-206**	39, 80, 81
S. wrightii Gray var. *adenophora*	**165, 166**	76

the bicyclogermacrene (**156**) from *S. spathulata* [62]. Caryophyllene (**158**) and its benzoates (**159-162**) seem to be very typical sesquiterpenes found in *S. nemoralis* [27]. Caryophyllene (**158**) has also been isolated from *S. drummondii* [2] and its 1,10-epoxide (**157**) was obtained from *S. canadensis* [27] and *S. chilensis* [30]. Two eudesmene cinnamates (**163, 164**) have been found in *S. nemoralis* [27] and from aerial parts of *S. wrightii* another eudesmene cinnamate (**165**) and its isomeric eremophilene cinnamate (**166**) was isolated[76]. There are few triterpenoids (**143-151**) reported from *Solidago* species. Oleanolic acid (**145**) is the most abundant terpenoid constituent in *S. arguta* roots while oleanonic acid (**146**) is slightly less abundant [22]. Three triterpenes (**143, 144, 148**) have been isolated from the aerial parts of *S. nemoralis* [27]. Two triterpenes, one of which was identified as bauerenol (**150**), were obtained from the roots of *S. altissima* [77]. α-Amyrin (**151**) has been found in *S. chilensis* [30], baurenol (**149**) was isolated from *S. microglossa*[31] and spinasterol (**147**) was obtained from both *S. microglossa* [31] and *S. wrightii* [76].

Solidago odora is devoid of diterpenoids, however, the phenylpropane derivatives (**174-181**) seem to be characteristic for this species [27]. Another constituent of this plant, *p*-methoxybenzaldehyde (**167**), might also be derived from the phenylpropane precursor. A new anethole angelate (**182**), another phenylpropane derivative, has been isolated from the roots of *S. altissima* [12, 17]. Two cinnamyl angelates (**183, 184**) have been obtained from *S. multiradiata* var. *multiradiata* [61]. Three further phenylpropane derivatives, the 3-methoxy-4-acetoxycinnamyl angelate (**185**), 3,5-dimethoxy-4-acetoxycinnamyl angelate (**186**) and 3,5-dimethoxy-4-hydroxycinnamic alcohol (**187**), were isolated from the Chinese medicinal plant *S. decurrens* Lour. (named Yi-Zhi-Huang-Hua in Chinese)[3]. Diterpenes are absent in *S. decurrens* Lour., while the benzyl benzoates (**169-173**) seem to be the major constituents in this species [3]. Benzyl 2,6-dimethoxybenzoate (**169**) has also been

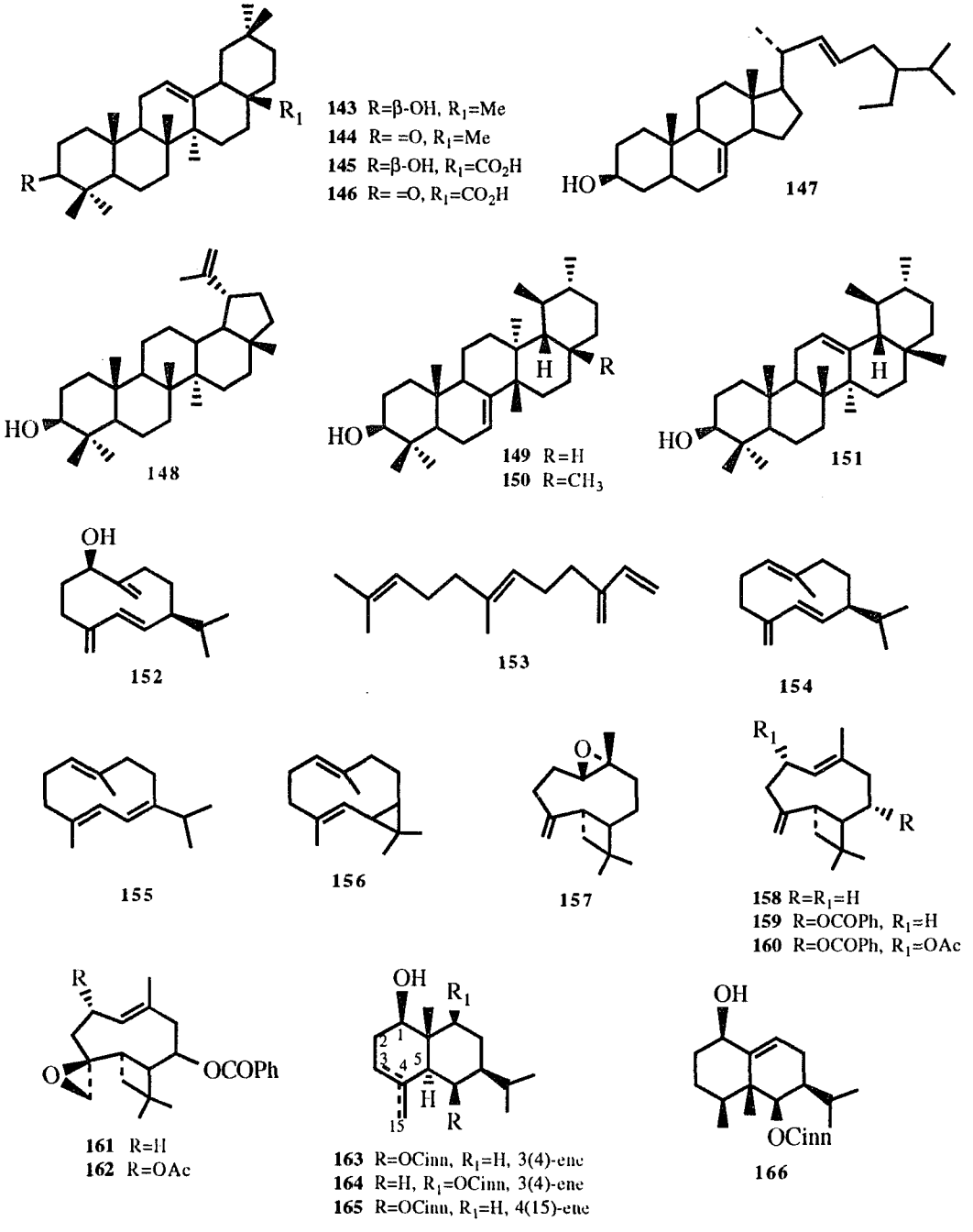
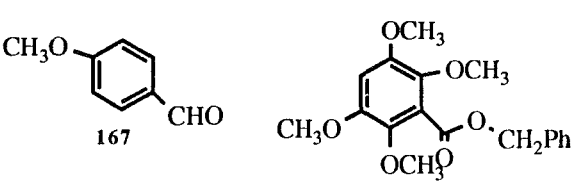


Figure 1.3. Miscellaneous compounds from *Solidsgo species* (continued)



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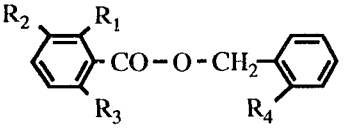
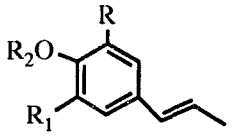
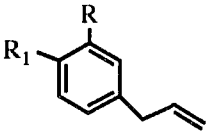
168



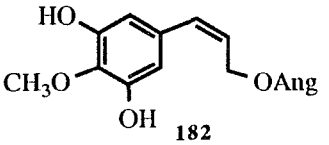
169 $R_1=R_3=OCH_3$, $R_2=R_4=H$
170 $R_1=OH$, $R_2=H$, $R_3=R_4=OCH_3$
171 $R_1=R_3=R_4=OCH_3$, $R_2=H$
172 $R_1=R_2=R_3=R_4=OCH_3$
173 $R_1=OH$, $R_2=R_4=H$, $R_3=OCH_3$



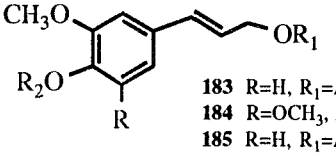
174 $R=R_1=H$, $R_2=CH_3$
175 $R=R_1=OCH_3$, $R_2=CH_3$
176 $R=H$, $R_1=OCH_3$, $R_2=CH_3$
177 $R=R_1=R_2=H$
178 $R=R_1=OCH_3$, $R_2=Ang$



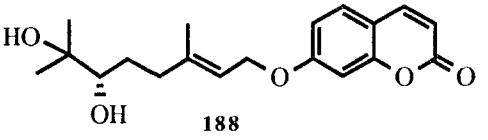
179 $R=H$, $R_1=OCH_3$
180 $R=R_1=OCH_3$
181 $R=H$, $R_1=OH$



182



183 $R=H$, $R_1=Ang$, $R_2=H$
184 $R=OCH_3$, $R_1=Ang$, $R_2=H$
185 $R=H$, $R_1=Ang$, $R_2=Ac$
186 $R=OCH_3$, $R_1=Ang$, $R_2=Ac$
187 $R=OCH_3$, $R_1=H$, $R_2=H$



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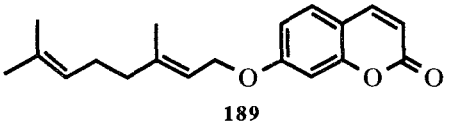

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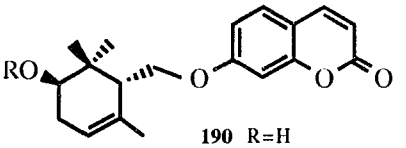
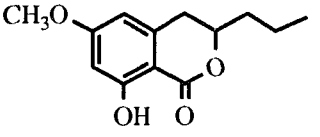


190 R=H
191 R=Ac



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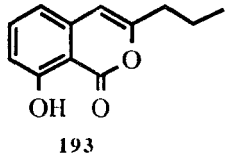
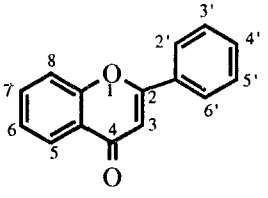


193



194 3,5,7,3',4'-pentaOH
195 3,5,7,4'-tetraOH
196 5,7,3'4'-tetraOH-3-rutinoside
197 3,5,7,4'-tetraOH-3'-OMe
198 5,7,4'-triOH-3'-OMe-3-gluco-rhamnoside
199 5,7,3',4'-tetraOH-3-glucoside
200 5,7,4'-triOH-3-gluco-rhamnoside
201 5,7,3',4'-tetraOH-3-gluco-rhamnoside
202 5,7,3',4'-tetraOH-3-rhamnoside
203 5,7,4'-triOH-3-glucoside
204 5,7,4'-triOH-3-rutinoside
205 5,7,4'-triOH-3-robinobioside
206 5,7,4'-triOH-3'-OMe-3-rutinoside

Figure 1.3.

found in *S. virgaurea* [39], and benzyl 2,3,5,6-tetramethoxybenzoate (**168**) has been isolated from *S. nemoralis* [27].

Two coumarins, marmin (**188**) and aurapten (**189**), both being umbelliferonyl geranyl ethers, have been isolated from the aerial parts of *S. drummondii* [2]. Two other umbelliferone derivatives (**190, 191**), which are closely related to the geranyl ethers, have been obtained from *S. spathulata* DC [62]. One isocoumarin (**193**) and its derivative, 3-propyl-8-hydroxy-6-methoxyisocumarin (**192**) were isolated from *S. multiradiata* var. *multiradiata* [61].

Only *S. canadensis* and *S. virguarea* have been extensively investigated for flavonoids [78-81]. The flavonoids found in these two species are mainly quercetin (**194**), kaempferol (**195**) and their corresponding glycosides (**196-206**).

Biological Activities

Solidago species have long been used as folk medicinal herbs in many countries. For instance, *S. virgaurea* has been used in European countries for its diuretic and antiphlogistic properties [81, 82] and *S. decurrens* is used in China as anti-bacterial and anti-inflammatory agent [3]. However, only a few compounds listed in Table 1.1-1.3 have been tested for biological activities, thus leaving the majority of *Solidago* constituents untested and/or their bioactivities unreported.

The clerodanes are best known for their insect antifeedant properties and related insecticidal properties [83]. The role of diterpenes from *Solidago* species as insect antifeedants and growth inhibitors has been reviewed by Cooper-Driver and Le Quesne *et al*.[84, 85]. Kolavenol (**2**), which could be obtained from many higher plants including several *Solidago* species, has been reported to have activity against leafcutting ants (*Atta cephalotes*) [86]. The hardwickiic acid (**63**), often found in *Solidago* species, is described as insecticidal against *Aphis craccivora* [87]. More than

two dozens of clerodane diterpenes have been found in *S. altissima*, which exhibited defense properties on the cabbage looper, *Trichoplusia ni* [88].

Kolavenic acid (**1**), a common constituent in *Solidago* species, has exhibited strong inhibition of crown gall tumors on potato disc and cytotoxicity against human tumor cells A-547 (human lung carcinoma), MCF-7 (human breast carcinoma) and HT-29 (human colon adenocarcinoma), while its methyl ester is less active, suggesting that carboxyl group is essential for its cytotoxicity [89]. Kolavenic acid has also been reported to be strongly active against Gram-positive bacteria, such as *Bacillus subtilis* and *Staphylococcus aurens*. Structure-activity studies demonstrated that the α,β-unsaturated carboxyl group and the ring skeleton appeared to be responsible for the antimicrobial activity [90, 91].

Solidagolactones (**10**, **11**, **28**, **29**) from various *Solidago* species have been reported with piscicidal activity [14, 15].

The biological activities and therapeutic uses of abietane acids have been recently reviewed by San Feliciano *et al.* [92]. Such compounds often showed antimicrobial, antiulcer and cardiovascular activities. Abietic acid (**104**) has exhibited inhibitory effect on both (Na^++K^+)-and (H^++K^+)-ATPases, typical membrane-bound enzymes. It also inhibited gastric acid secretion caused by (H^++K^+)-ATPase [93]. The antithrombotic action of abietic acid (**104**) has also been reported [94].

Polyacetylenes are highly active antifungal and nematicidal agents and are phototoxic against certain viruses [95]. Both *cis*- and *trans*-dehydromatricaria esters (**127a** and **b**), which are common constituents of *Solidago* species, showed anti-tumor activities[57, 96]. *cis*-Dehydromatricaria ester (**127a**) exhibited *in vitro* inhibitory effect on both tumor and normal mammalian cells, such as MK-1 (human gastric adenocarcinoma), L-929 (mouse fibroblast-derived tumor), B-16 (mouse melanoma) and MRC-5 (human fibroblast) [57]. The *cis*- and *trans*-dehydromatricaria esters have also been reported to exhibit cytotoxicity, antiviral and antifungal activities, which,

could be enhanced by ultraviolet absorbance [97]. *cis*-Dehydromatricaria ester has been reported to have ovicidal effect on the fruit-fly (*Drosophila melanogaster*) and the house-fly (*Musca domestica*) [98].

C_{10}-Polyacetylenes have been described as allelochemical substances both against competitive plants and plant-parasite nematodes [11, 99]. cis-Dehydromatricaria ester (**127a**), *cis*- and *trans*-matricaria esters (**126a** and **b**) and *cis*-lachnophyllum ester (**128**) showed strong growth inhibitory effects on plants. The *cis*- and *trans*-dehydromatricaria esters (**127a** and **b**) found in soil surrounding *S. altissima* communities were inhibitory to test plants [100]. *cis*-Dehydromatricaria ester (**127a**), 10-angeloyoxy-*cis*-matricaria ester (**133a**) and dehydromatricaria lactone (**130a**) showed strong inhibition to the seedlings of barnyard millet (*Panicum crus-galli* L. var. *frumentaceum* Trin.) [55, 56]. The *cis*- and *trans*-matricaria esters (**126a** and **b**) have effects on germination and radicle growth of two Florida sandhill grasses *Shizachyrium scoparium* and *Leptochloa dubia*, as well as commercial lettuce and radish. They were highly active against three of four test species, completely inhibiting germination at 106 and 92 ppm [101].

Rather than diterpenes and acetylenes, benzyl benzoates (**169-173**) are the major constituents of *S. decurrens* which has long been used as antibacterial and anti-inflammatory agent in traditional Chinese medicine [3]. Caryophyllene (**158**) and its epoxide (**157**) have also shown the activity against the leafcutting ant *A. cephalotes* and its mutualistic fungus [86]. Finally, the pharmacological properties of the main saponins, polysaccharides and flavonoids from *Solidago* species have been extensively investigated [82, 102-109]. The phenolic glucosides were described to show antiphlogistic, analgesic and diuretic activities [82, 103, 105, 107]. Finally, triterpene saponins have been reported to exhibit antimycotic and antiexudative activities [104, 108] and polysaccharides have been described to have antitumor activity [103, 106].

The isolation of natural products from a number of the Asteraceae plant species is showing renewed interest recently because of the continued discovery of biologically active compounds and an increased demand for more natural, non-classical medicinal therapies. In Chapter 2 and 3 of this dissertation, the isolation and structure elucidation of secondary metabolites from seven plant species of the family Asteraceae is described. Chapter 2 involves the isolation and biological activity study of polyacetylenes and terpenoids from the genus *Solidago* and other closely related species. The isolation of thiarubrines from *Ambrosia trifida* (tribe Heliantheae, Asteraceae) and sesquiterpene lactones from *Liatris ohlingerae* (tribe Eupatorieae, Asteraceae) is described in Chapter 3. The structure-activity study of sesquiterpene lactones is demonstrated in Chapter 4.

CHAPTER 2

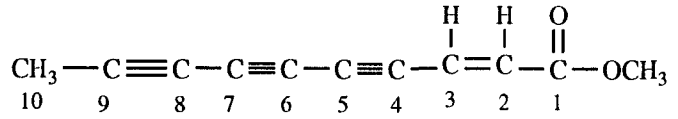
ISOLATION AND BIOLOGICAL ACTIVITY STUDY OF CONSTITUENTS FROM THE GENUS *SOLIDAGO* AND OTHER RELATED SPECIES

2.1

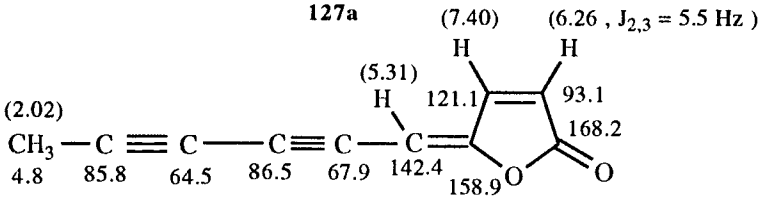
Polyacetylenes and Diterpenes from *Solidago canadensis*

Introduction

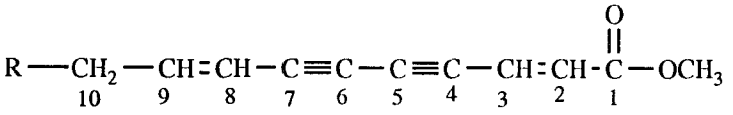
Polyacetylenes and diterpenes are common constituents in members of the tribe Astereae [7, 50, 117]. In recent years a large number of reports have appeared dealing with the biological activities of these compounds. Polyacetylenes usually possess high antifungal and nematicidal activities and are phototoxic against certain viruses [95]. Diterpenes exhibit insect antifeedant, antifungal, antibacterial and antiviral activities [117]. In continuation of our search for biologically active compounds from the Asteraceae family, we have investigated roots of *Solidago canadensis*. Previous chemical investigations of the aerial parts of *S. canadensis* resulted in the isolation of mono and sesquiterpenes [5, 119], diterpenes [25, 27], flavonoids [80, 102] and saponins [120]. Our study provided five known polyacetylenes related to matricaria esters [7, 50], as well as six known and one new clerodane type diterpenoids (**1-4**, **6**, **7**, **14**), including kolavenic acid (**1**) [121, 122]. Since the ^{13}C NMR spectral data for the diterpenes **2-4**, **6**, **7** and **14** have not been reported previously, they were assigned by COSY, $^{1}H,^{13}C$-correlation, DEPT and Insensitive Nuclei Enhancement by Polarization Transfer (INEPT) [123, 124].



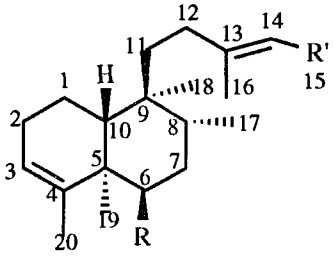
127a



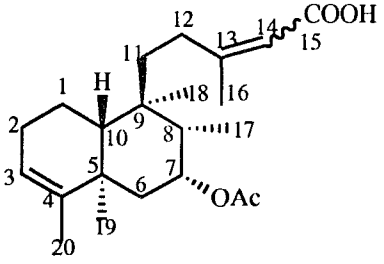
131a



133a 2Z, 8Z, R=OAngelate
133b 2E, 8Z, R=OAngelate
134 2Z, 8Z, R=OTiglate



	R	R'
1	H	-COOH
2	H	$-CH_2OH$
3	OTig	-COOH
4	OAng	-COOH



6 13E
7 13Z

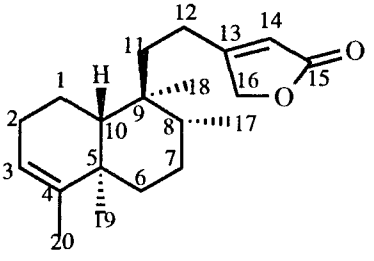


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Figure. 2.1.1. Polyacetylenes and diterpenes from *Solidago canadensis*

Results and Discussion

cis-Dehydromatricaria ester (*cis*-DME, **127a**) had been obtained from roots of *S. altissima* [100, 125]. The ^{1}H NMR data of *cis*-DME are in agreement with those reported in the literature [100, 125]. The X-ray structure of **127a** has been previously described [26].

cis-Dehydromatricaria lactone (**131a**) was previously isolated from *Anthenais* species[125]. Its ^{1}H and ^{13}C NMR data has been reported in our previous publication [26].

The three isomeric matricaria ester derivatives, the tiglate **134** and the angelates **133a** and **133b**, are known compounds [50, 126] and their ^{1}H NMR data are in full agreement with previously reported values [127].

Kolavenic acid (**1**) was previously found in S. *altissima* [12] and other taxa [121, 122]. Its ^{1}H NMR data had been described before [122]. The IR spectrum showed a very strong and broad absorption from 3500 cm^{-1} to 2500 cm^{-1}, indicating a carboxylic acid. The mass spectrum of **1** exhibited a prominent molecular peak at *m/z* 304, which is in agreement with the structure. The ^{13}C NMR spectrum of **5** was essentially identical with reported data [121]. The quaternary carbons C-5 and C-9 were assigned by INAPT, since previous assignments [121] were tentative. Polarization of the proton absorption at δ 5.19 (H-3), using a coupling parameter of 8 Hz ($^3J_{C,H}$), transferred to a methyl-carbon at δ 18.0 (C-20) and a quaternary carbon at δ 38.1 which had to be due to C-5. Therefore, the other quaternary carbon at δ 38.7 must be C-9. Irradiation of the methyl signal (H-19) using a coupling of 8 Hz showed a strong signal at δ 144.4 which confirmed the assignment of C-4, while another olefinic quaternary carbon at δ 163.8 was assigned to C-13 (Table 2.1.2).

Kolavenol (**2**) was previously described as a constituent of *Hardwickia pinnata*[122] and *Solidago elongata* [21]. Its ^{1}H NMR spectrum showed a two-proton doublet at δ 4.14 (H-15, J=6.8 Hz), which was coupled to a oxygen-bearing carbon at δ 59.4 shown by the 2D carbon-proton correlation experiment, indicating an alcohol.

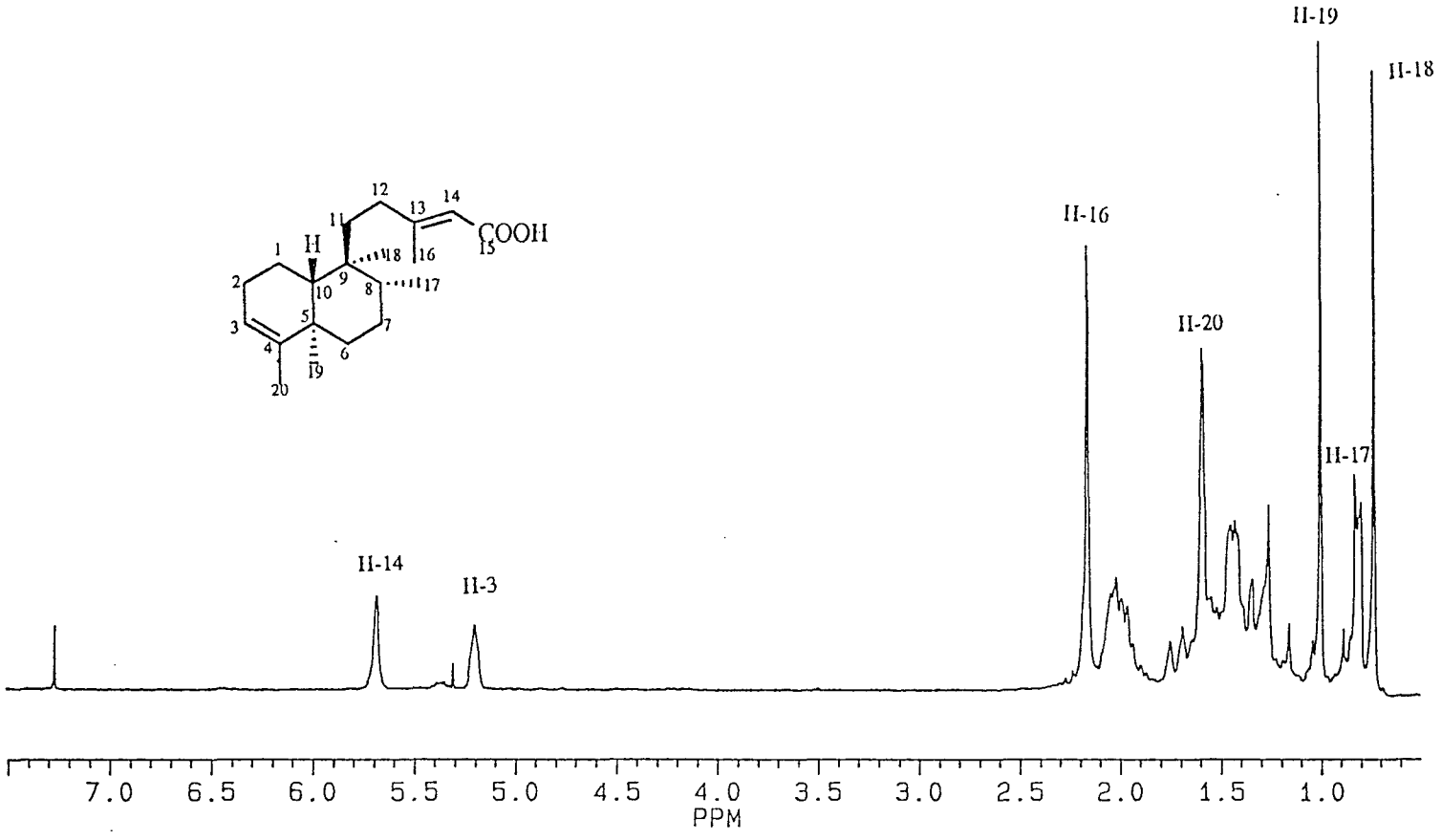


Figure 2.1.2. 200 MHz ^{1}H NMR spectrum of kolavenic acid (**1**)

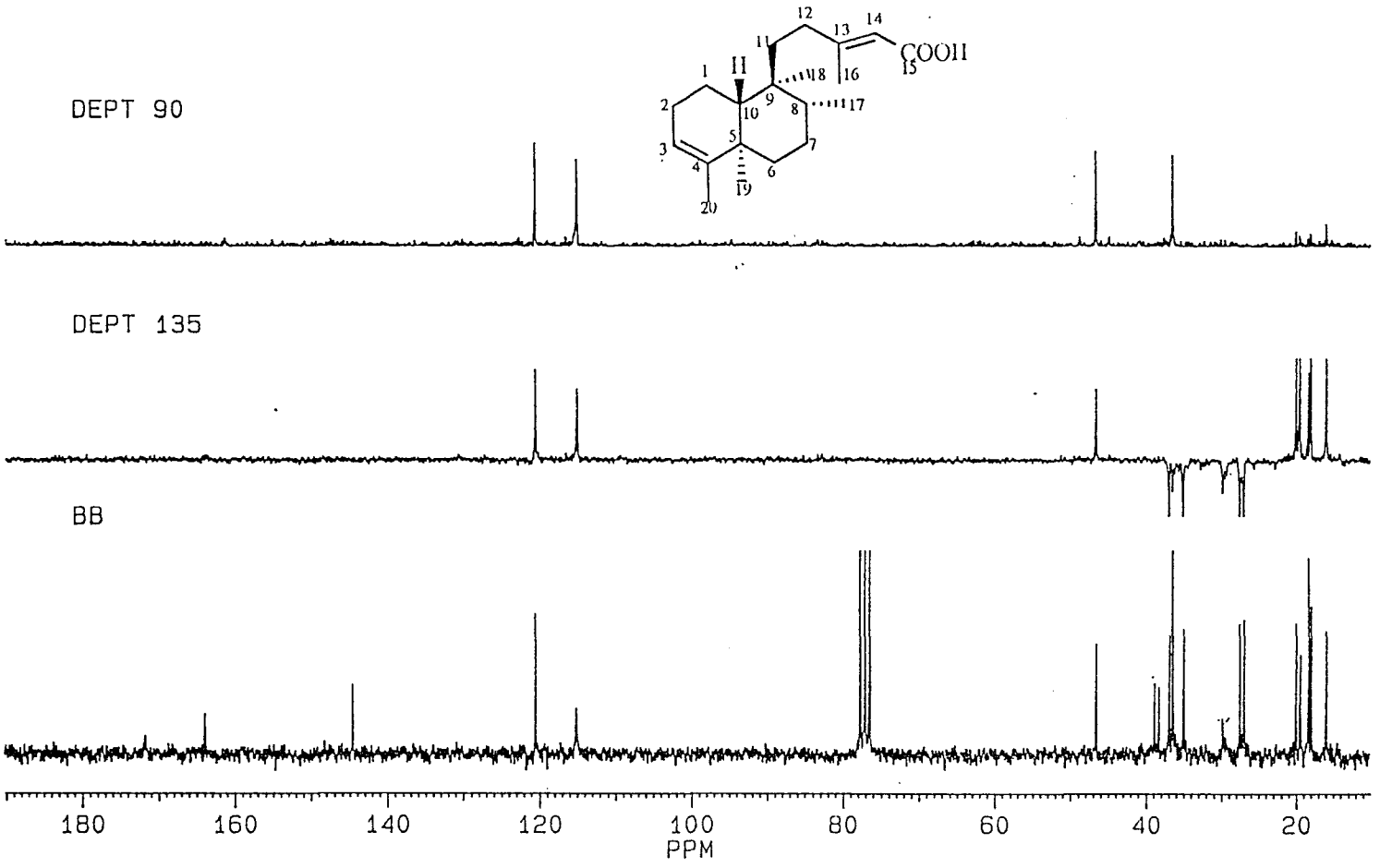


Figure 2.1.3. DEPT 90°, DEPT 135° and Broad Band ^{13}C NMR spectra of kolavenic acid (1)

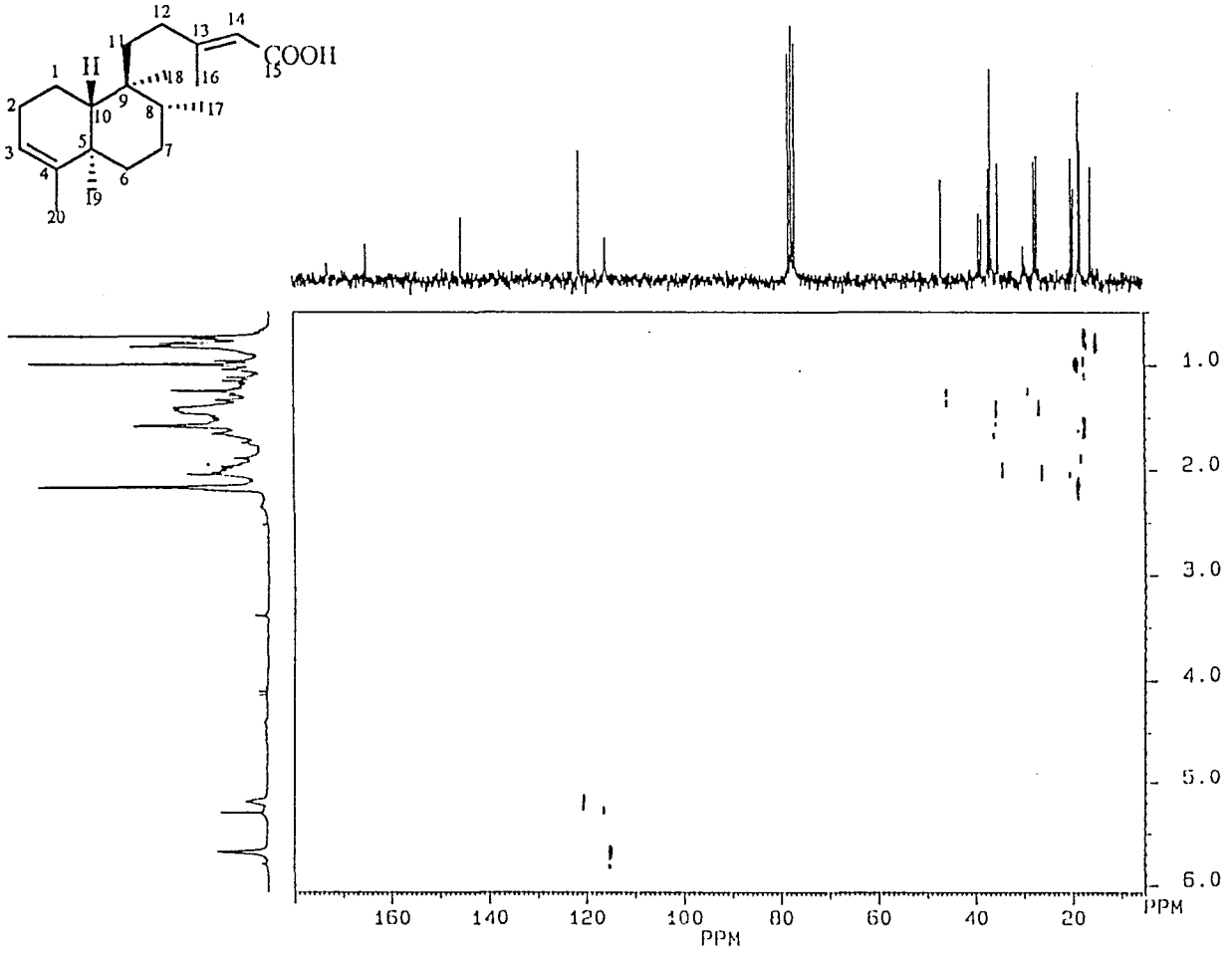


Figure 2.1.4. 2D ^{13}C-^{1}H Heteronuclear correlation spectrum of kolavenic acid (**1**)

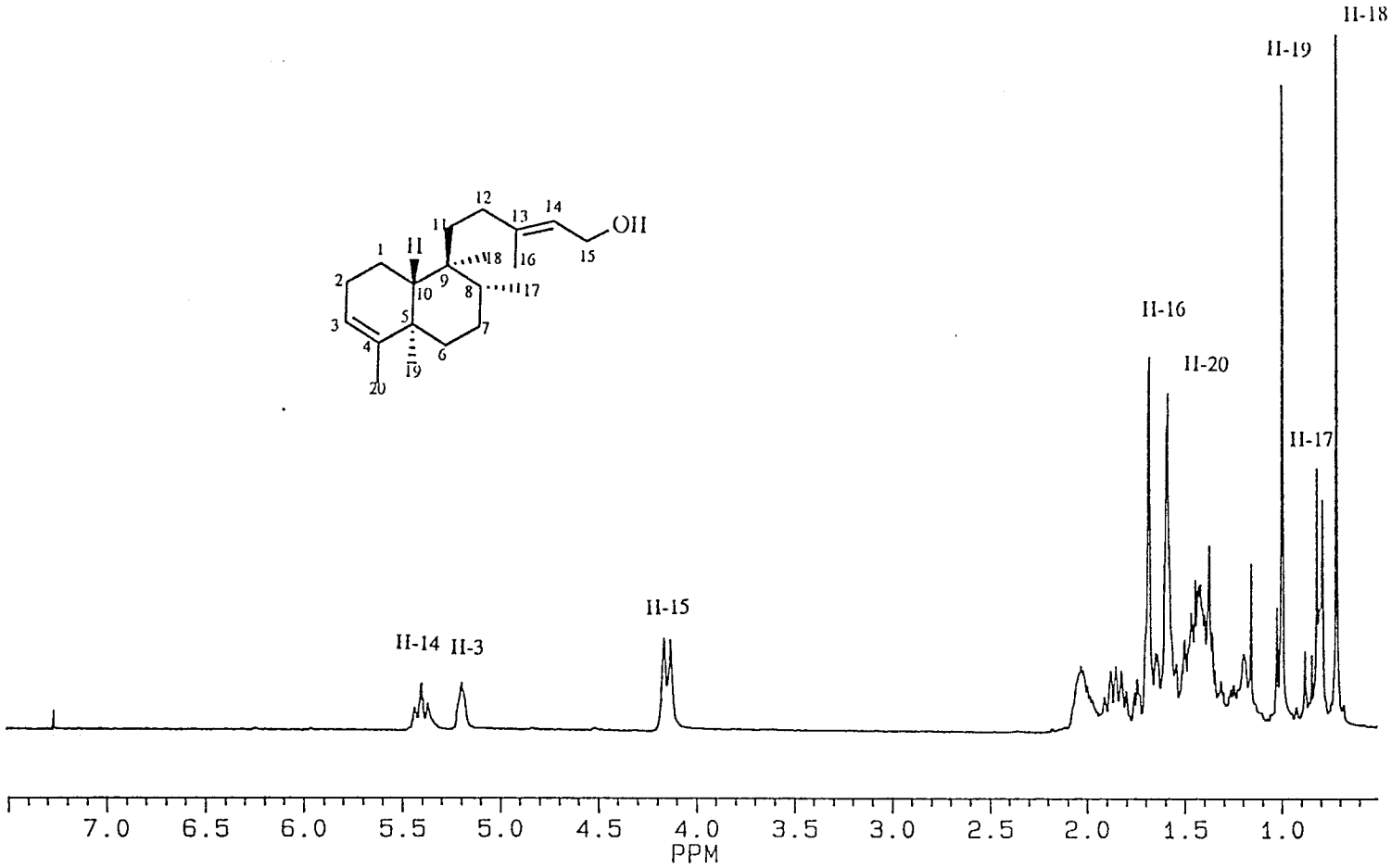


Figure 2.1.5. 200 MHz ^{1}H NMR spectrum of kolavenol (2)

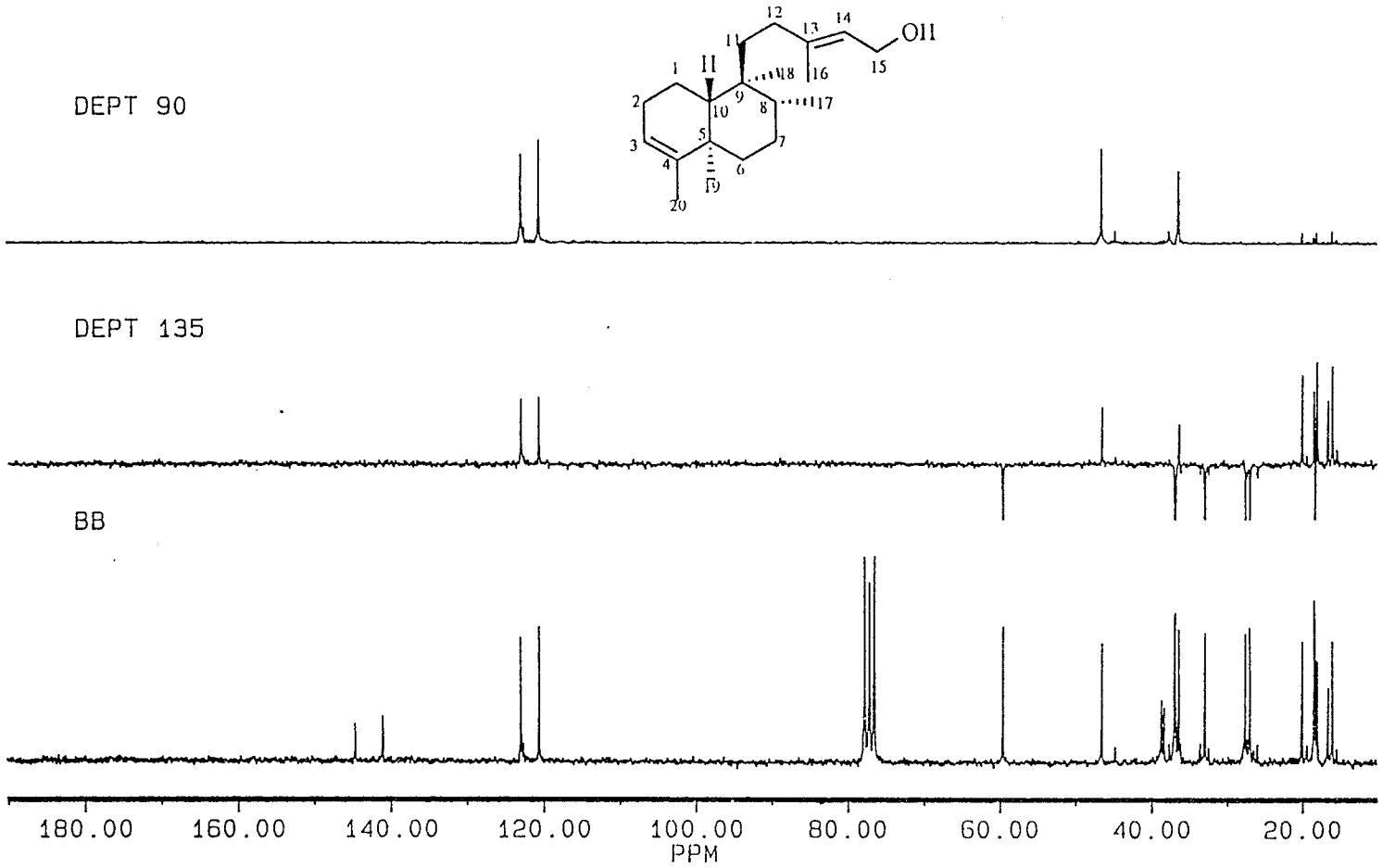


Figure 2.1.6. DEPT 90°, DEPT 135° and Broad Band ^{13}C NMR spectra of kolavenol (**2**)

Table 2.1.1. ^{1}H NMR spectral data of compounds **1-4**, **6**, **7** and **14** (200 MHz, $CDCl_3$ as internal standard)

H	**1**	**2**	**3**	**4**	**6***	**7***	**14**
3	5.19 *br s*	5.18 *br s*	5.49 *br s*	5.49 *br s*	5.14 (5.12) *br s*	5.14 (5.14) *br s*	5.19 *br s*
6			4.99 *br s*	5.02 *br s*			
7					5.11 (5.19) *br s*	5.11 (5.20) *br s*	
14	5.68 *br s*	5.38 *br tt*	5.69 *br s*	5.70 *br s*	5.66 (5.87) *br s*	5.64 (5.94) *br s*	5.83 *br tt*
15		4.14 *d*					
16	2.17 *d*	1.64 *br s*	2.16 *br s*	2.17 *br s*	2.13 (2.16) *br s*	2.08 (2.23) *br s*	4.73 *d*
17	0.81 *d*	0.78 *d*	0.83 *d*	0.85 *d*	0.90 (0.78) *d*	0.89 (0.82) *d*	0.81 *d*
18	0.74 *s*	0.71 *s*	1.03 *s*	1.03 *s*	0.97 (0.93) *s*	0.96 (0.97) *s*	0.77 *s*
19	1.00 *s*	0.99 *s*	1.24 *s*	1.25 *s*	1.17 (1.21) *s*	1.17 (1.22) *s*	1.00 *s*
20	1.58 *d*	1.58 *d*	1.53 *br s*	1.56 *br s*	1.57 (1.53) *br s*	1.58 (1.54) *br s*	1.58 *d*
3'			6.78 *qq*	5.99 *qq*			
4'			1.77 *br d*	1.98 *dq*			
5'			1.78 *br d*	1.83 *dq*			
OAc					2.04 (1.72) *s*	2.05 (1.72) *s*	

* Data obtained from 400 MHz NMR spectrometer; numbers in parentheses indicate chemical shifts obtained in C_6D_6.
J (Hz): **1**: 17,8 = 5.2; 20,3 = 1.3; 16,14 = 0.8; **2**: 17,8 = 6.0; 20,3 = 1.4; 14,15 = 6.8; 14,16 = 1.1; **3**: 17,8 = 6.3; 3',4' = 5.4; 3',5' = 1.8; **4**: 17,8 = 6.3; 3',4' = 7.3; 3',5' =1.2; 4',5' = 1.2; **6**: 17,8 = 6.7 (in $CDCl_3$); 17,8 = 7.0 (in C_6D_6); **7**: 17,8 = 7.0 (in both $CDCl_3$ and C_6D_6); **14**: 17,8 = 6.0; 20,3 =1.7; 14,16 = 4.5; 12,14 = 1.4.

Table 2.1.2. ^{13}C NMR spectral data of compounds **1-4, 6, 7** and **14** (100 MHz, in $CDCl_3$)*

C	1	2	3	4	6	7	14
1	36.3 *t*	36.7 *t*	34.6 *t*	34.2 *t*	37.3 *t*	37.3 *t*	35.5 *t*
2	26.8 *t*	26.9 *t*	26.5 *t*	26.5 *t*	26.6 *t*	26.7 *t*	26.8 *t*
3	120.4 *d*	120.4 *d*	124.2 *d*	124.5 *d*	120.1 *d*	120.0 *d*	120.2 *d*
4	144.4 *s*	144.5 *s*	138.2 *s*	138.0 *s*	144.4 *s*	144.4 *s*	144.4 *s*
5	38.1 *s*	38.2 *s*	42.9 *s*	42.9 *s*	38.4 *s*	38.2 *s*	38.1 *s*
6	36.8 *t*	36.8 *t*	74.4 *d*	74.0 *d*	39.9 *t*	39.9 *t*	36.7 *t*
7	27.4 *t*	27.5 *t*	31.5 *t*	31.6 *t*	75.2 *d*	75.2 *d*	27.3 *t*
8	36.3 *d*	36.2 *d*	31.9 *d*	31.9 *d*	38.2 *d*	38.3 *d*	36.3 *d*
9	38.7 *s*	38.6 *s*	38.5 *s*	38.4 *s*	37.3 *s*	37.4 *s*	38.7 *s*
10	46.4 *d*	46.4 *d*	44.4 *d*	44.3 *d*	46.3 *d*	46.3 *d*	46.5 *d*
11	18.2 *t*	18.2 *t*	21.8 *t*	21.6 *t*	17.9 *t*	17.9 *t*	18.1 *t*
12	34.8 *t*	32.8 *t*	35.5 *t*	35.5 *t*	34.9 *t*	34.7 *t*	22.3 *t*
13	163.8 *s*	140.9 *s*	162.9 *s*	162.9 *s*	161.4 *s*	158.9 *s*	171.1 *s*
14	115.0 *d*	122.8 *d*	116.2 *d*	115.6 *d*	116.2 *d*	117.5 *d*	115.0 *d*
15	171.6 *s*	59.4 *t*	172.2 *s*	172.1 *s*	172.2 *s*	173.0 *s*	174.0 *s*
16	19.4 *q*	16.5 *q*	19.4 *q*	19.4 *q*	19.3 *q*	19.0 *q*	73.0 *t*
17	15.9 *q*	16.0 *q*	15.3 *q*	15.5 *q*	12.0 *q*	12.0 *q*	16.0 q
18	18.3 *q*	18.3 *q*	28.5 *q*	28.5 *q*	19.6 *q*	19.6 *q*	18.1*q*
19	19.9 *q*	19.9 *q*	24.7 *q*	24.7 *q*	21.4 *q*	21.4 *q*	19.9 *q*
20	18.0 *q*	18.0 *q*	18.2 *q*	18.3 *q*	18.0 *q*	18.0 *q*	17.9 *q*
1'			167.5 *s*	167.0 *s*	170.8 *s*	170.6 *s*	
2'			129.1 *s*	128.3 *s*	21.4 *q*	19.0 *q*	
3'			136.7 *d*	137.5 *d*			
4'			14.3 *q*	15.3 *q*			
5'			12.1 *q*	20.7 *q*			

*Peak multiplicities were obtained by heteronuclear multipulse programs (DEPT), *s*=singlet, *d*=doublet, *t*= triplet, *q*=quartet.

This was further confirmed by a broad OH absorption at 3340 cm^{-1} of its IR spectrum. The mass spectrum of **2** showed a prominent molecular peak at *m/z* 290, which is in agreement with its structure. The ^{13}C NMR spectrum of **2** indicated the presence of 20 carbons: five CH_3, seven CH_2, four CH with two olefinic carbons, and four quaternary carbons which included two olefinic signals. The DEPT, 2D COSY and 2D $^{1}H,^{13}C$-correlation methods, coupled with the comparison of spectral data of kolavenic acid, gave the total assignment of ^{13}C NMR spectrum of **2** (Table 2.1.2).

6β-Tigloyloxykolavenic acid (**3**) and 6β-angeloyloxykolavenic acid (**4**) had been previously isolated from the roots of *S. altissima* [113]. Their ^{1}H-NMR data were very close to those reported previously [113]. The angelate moiety in compound **4** and tiglate moiety in compound **3** were derived by their diagnostic ^{1}H NMR signals, especially the chemical shifts of H-3', which in **4** was a quartet of a quartet at δ 5.99 (J=7.3, 1.2 Hz), while in **3** it was shifted downfield to δ 6.78 (J=5.4, 1.8 Hz) due to the deshielding effect of the carbonyl group (C-1'). The two methyl groups of the side chain, Me-4' and Me-5', in compound **4** appeared as two doublets of quatets at δ 1.98 (Me-4', J=7.3, 1.2 Hz) and 1.83 (Me-5', J=1.2, 1.2 Hz), while in compound **3** they absorbed as two broad doublets at δ 1.77 (Me-4', J=5.4 Hz) and 1.78 (Me-5', J=1.8 Hz). The mass spectral analysis of both compounds showed two strong peaks at *m/z* 83 = $[CH_3CH{=}CCH_3CO]^+$ and 55 = $[83\text{-}CO]^+$ which are characteristic for angelate and tiglate moieties. The presence of a prominent peak at *m/z* 302 = $[M\text{-}100]^+$ in both spectra, resulted from McLafferty rearrangement by the loss of 2-methylbutyric acid from the parent mass [M^+, *m/z*=402], further confirmed the presence of angelate or tiglate moieties. The ^{13}C NMR spectra of both **3** and **4** showed presence of 25 carbons. Their assignments were made by COSY, $^{1}H,^{13}C$-correlation and DEPT methods. The INAPT method was applied for the assignments of quaternary carbons. In compound **4**, the INAPT spectrum of H-3 at δ 5.49, using a coupling of 8 Hz ($^{3}J_{C,H}$), generated a strong signal at δ 42.9 which confirmed the assignment of C-5. Therefore,

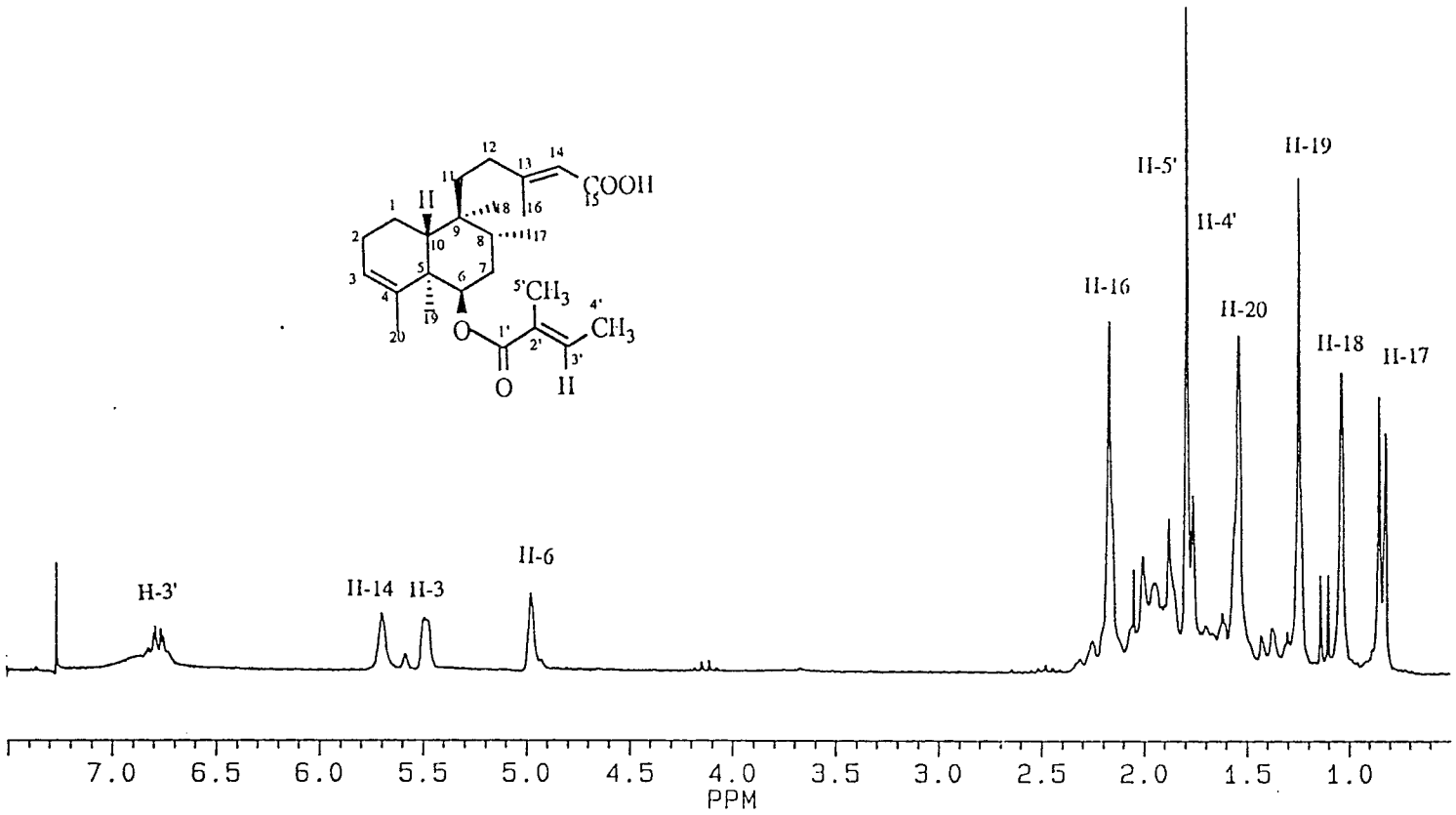


Figure 2.1.7. 200 MHz ^{1}H NMR spectrum of 6β-tigloyloxykolavenic acid (3)

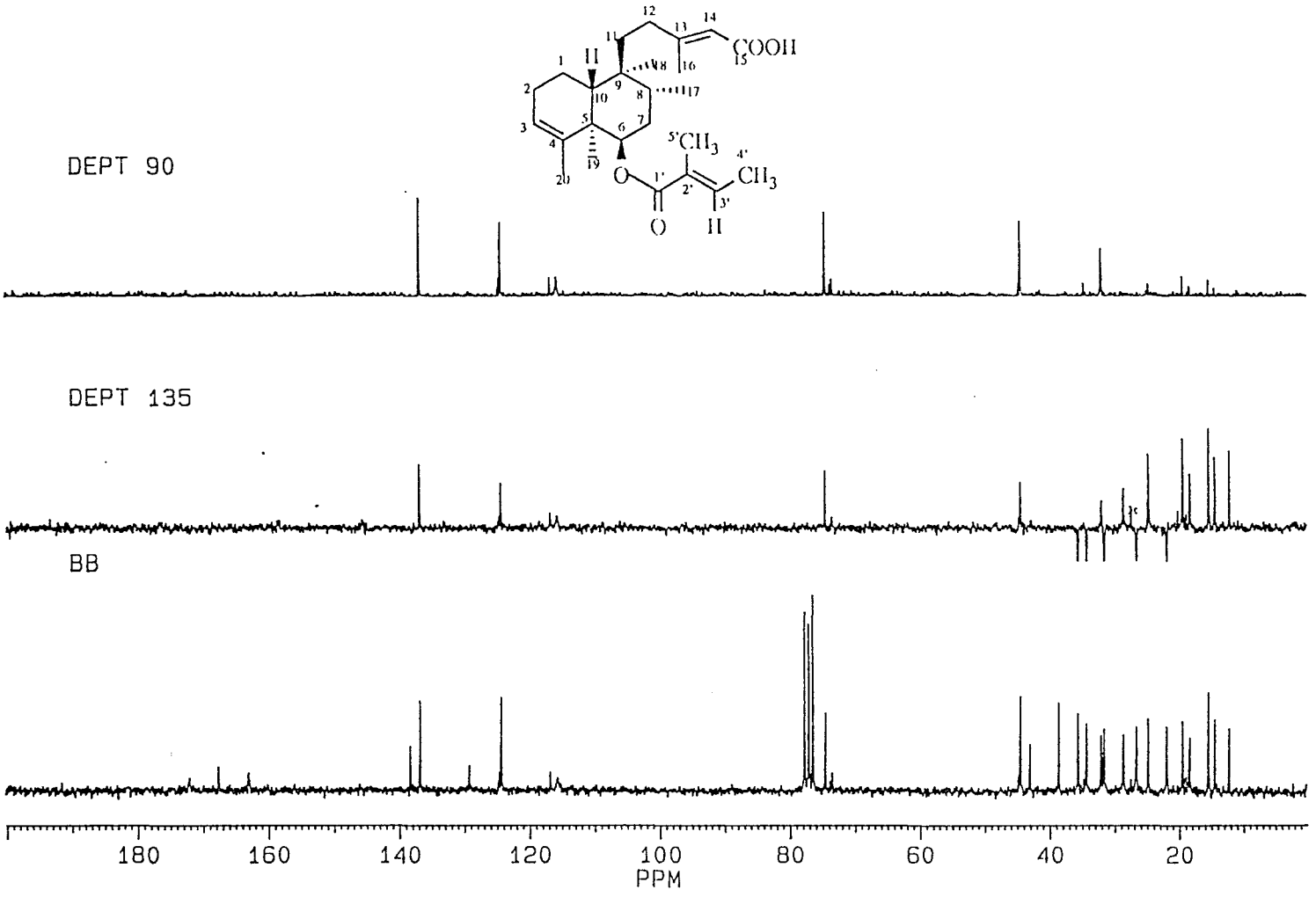


Figure 2.1.8. DEPT 90°, DEPT 135° and and Broad Band ^{13}C NMR spectra of 6β-tigloyloxykolavenic acid (3)

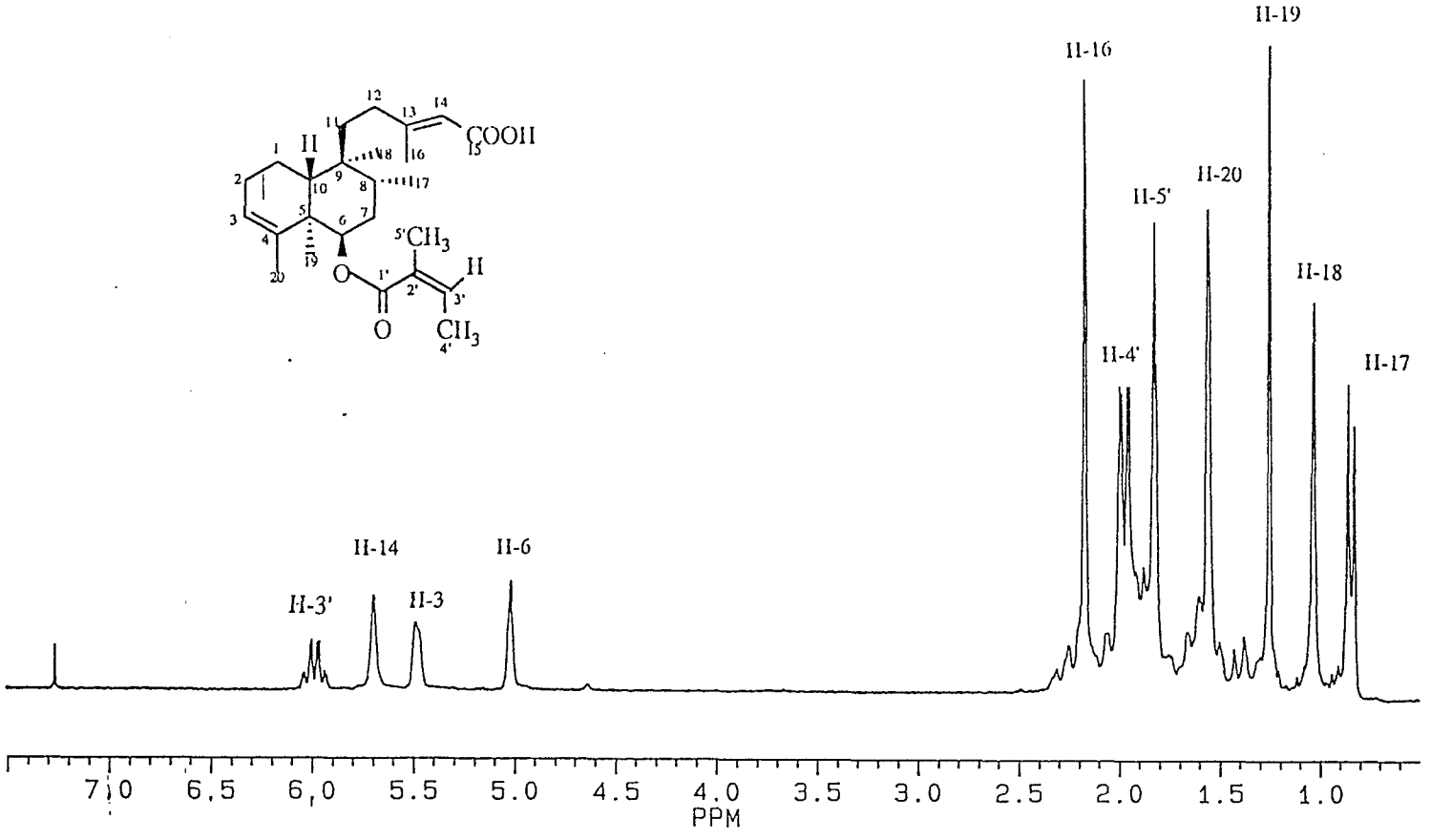


Figure 2.1.9. 200 MHz ^{1}H NMR spectrum of 6β-angeloyloxykolavenic acid (**4**)

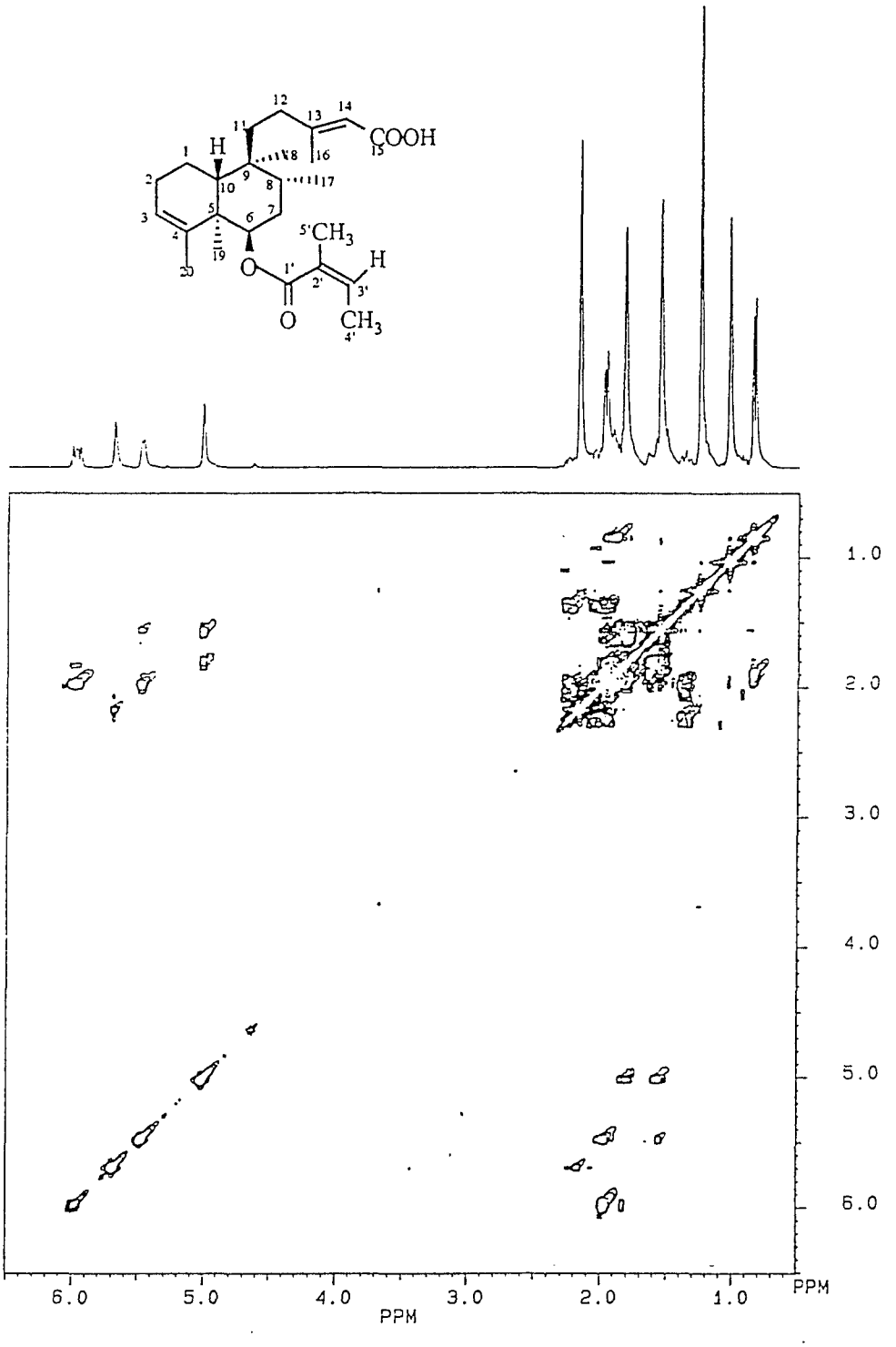


Figure 2.1.10. 400 MHz 2D ^{1}H NMR COSY spectrum of 6β-angeloyloxykolavenic acid (**4**)

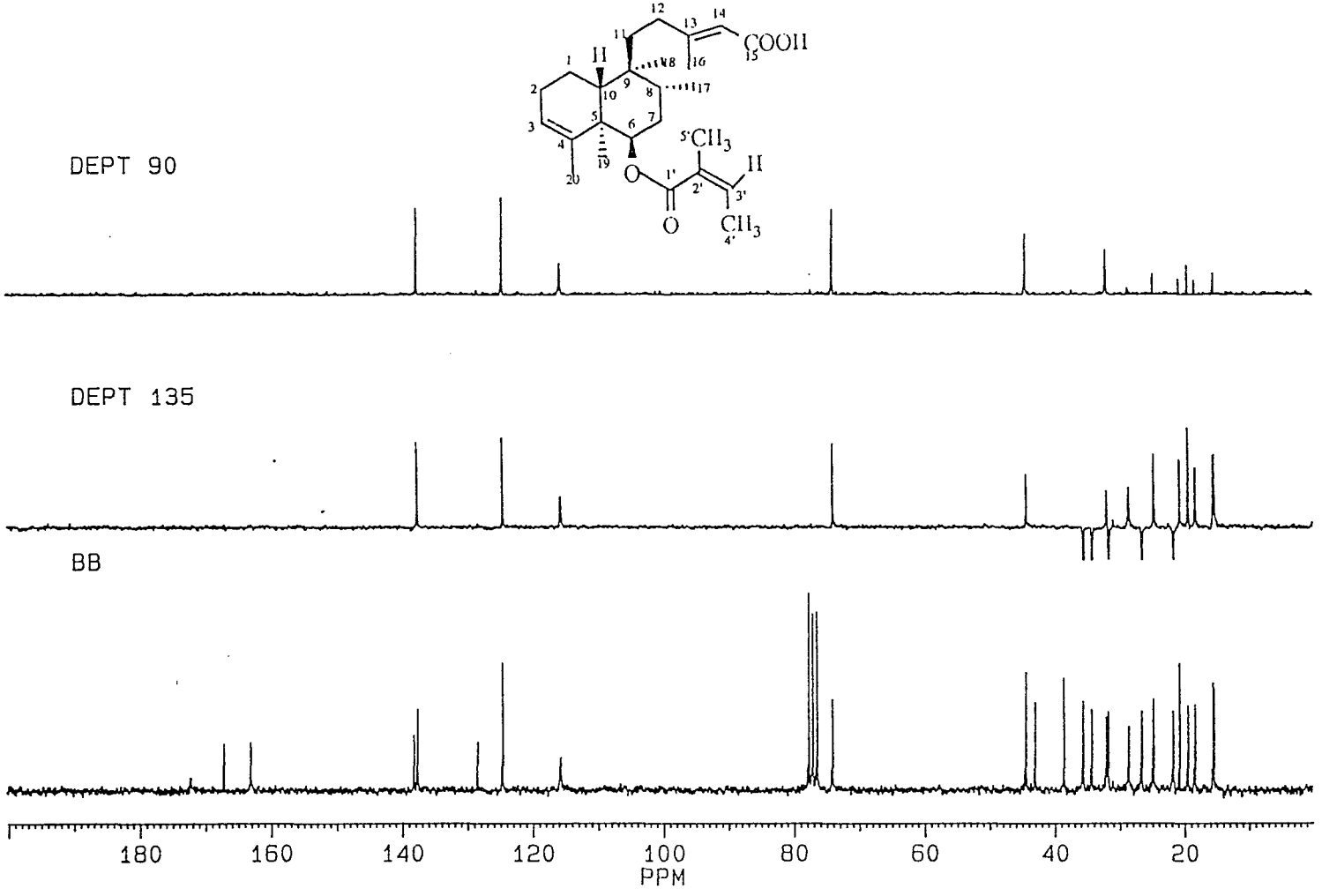


Figure 2.1.11. DEPT 90°, DEPT 135° and Broad Band ^{13}C NMR spectra of 6β-angeloyloxykolavenic acid (**4**)

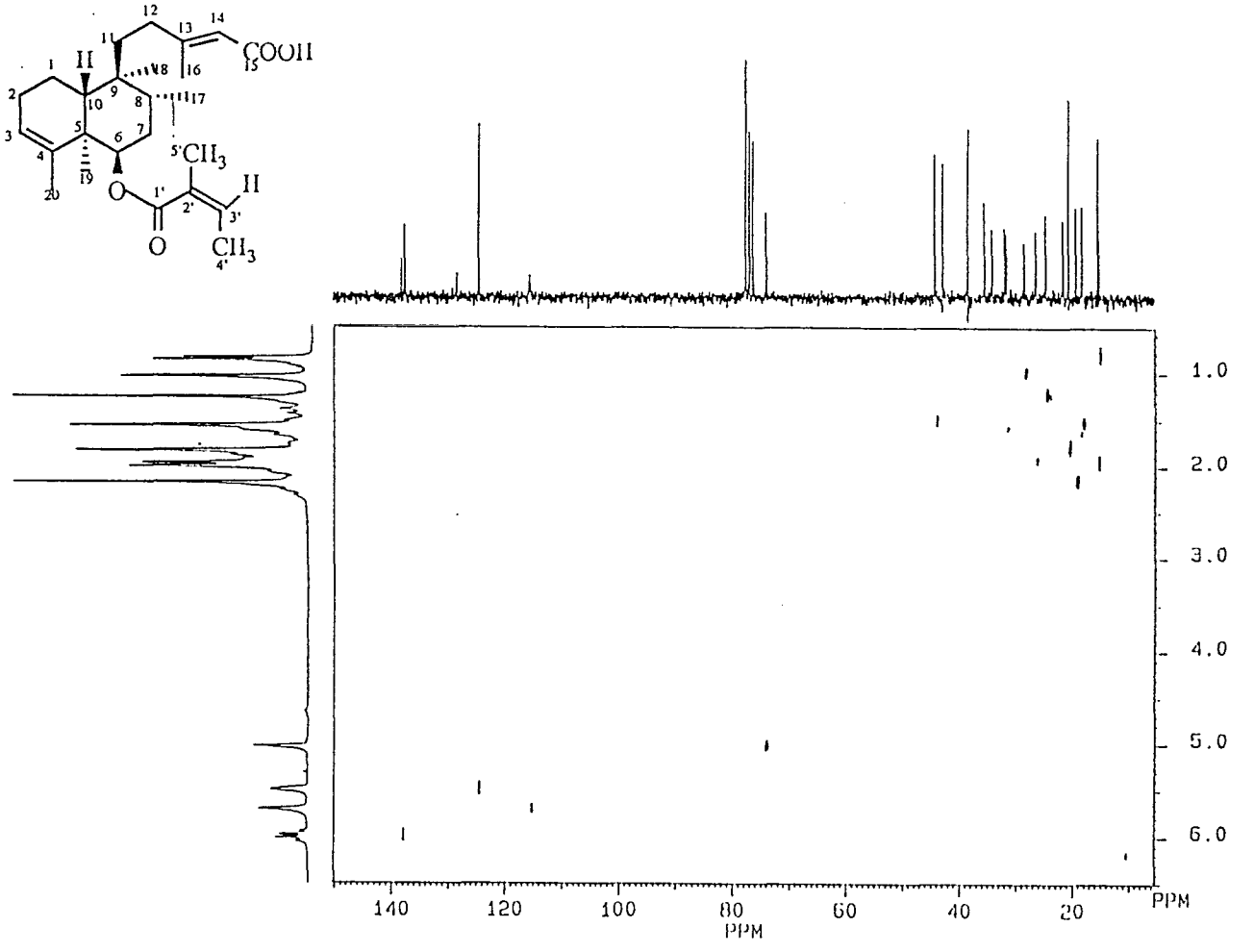


Figure 2.1.12. 2D ^{13}C-^{1}H Heteronuclear correlation spectrum of 6β-angeloyloxykolavenic acid (4)

the other quaternary carbon signal at δ 38.4 could be assigned to C-9. Upon irradiation of H-19, the appearance of a signal at δ 138.0 with a coupling of 8 Hz, confirmed the assignment of C-4. Then the other olefinic quaternary carbon signal at δ 162.9 was assigned to C-13. The quaternary carbons in compound **3** were assigned by direct comparison with **4** (Table 2.1.2).

Solidagolactone (**14**) was previously found in *S. altissima* [13] and *Acritopappus longifolius* [128] and it showed ^{1}H NMR spectral signals essentially identical with reported values [128]. The mass spectrum of **14** gave a molecular ion at *m/z* 302, along with a prominent peak at *m/z* 111 belonging to the side chain of the molecule. Its IR spectrum showed strong absorptions at 1748 and 1780 cm^{-1} which further confirmed the presence of an α,β-unsaturated lactone moiety. The ^{13}C NMR assignments made use of 2D COSY, 2D ^{1}H,^{13}C-correlation , DEPT and comparison with the spectral data of kolavenic acid and other closely related compounds listed in Table 2.1.2.

13*E*-7α-Acetoxylkolavenic acid (**6**) and 13*Z*-7α-acetoxylkolavenic acid (**7**) showed very similar spectroscopic features. The ^{1}H NMR spectrum of **6** was identical with that of a known diterpene previously isolated from *S. altissima* [113]. Compound **7** was identified as a new natural product. The mass spectra of both compounds showed no parent peaks but prominent peaks at *m/z* 302, which must be formed from the parent ion (M^+, *m/z*=362) by McLafferty rearrangement with the loss of acetic acid. Strong peaks at *m/z* 43 $[Ac]^+$ confirmed the presence of acetate groups in both compounds. Major differences between the ^{1}H NMR spectra of **6** and **7** were found in the absorptions of H-14 and H-16 (Table 2.1.1), suggesting that they only differed in the configuration of the double bond at C-13. The stereochemistry of this double bond in both **6** and **7** was determined by NOE difference experiments. Irradiation of H-14 of **7** showed NOE enhancement of H-16, indicating H-14 and Me-16 were *cis*-oriented. In contrast, **6** did not exhibit such an effect, suggesting a *trans*-orientation of

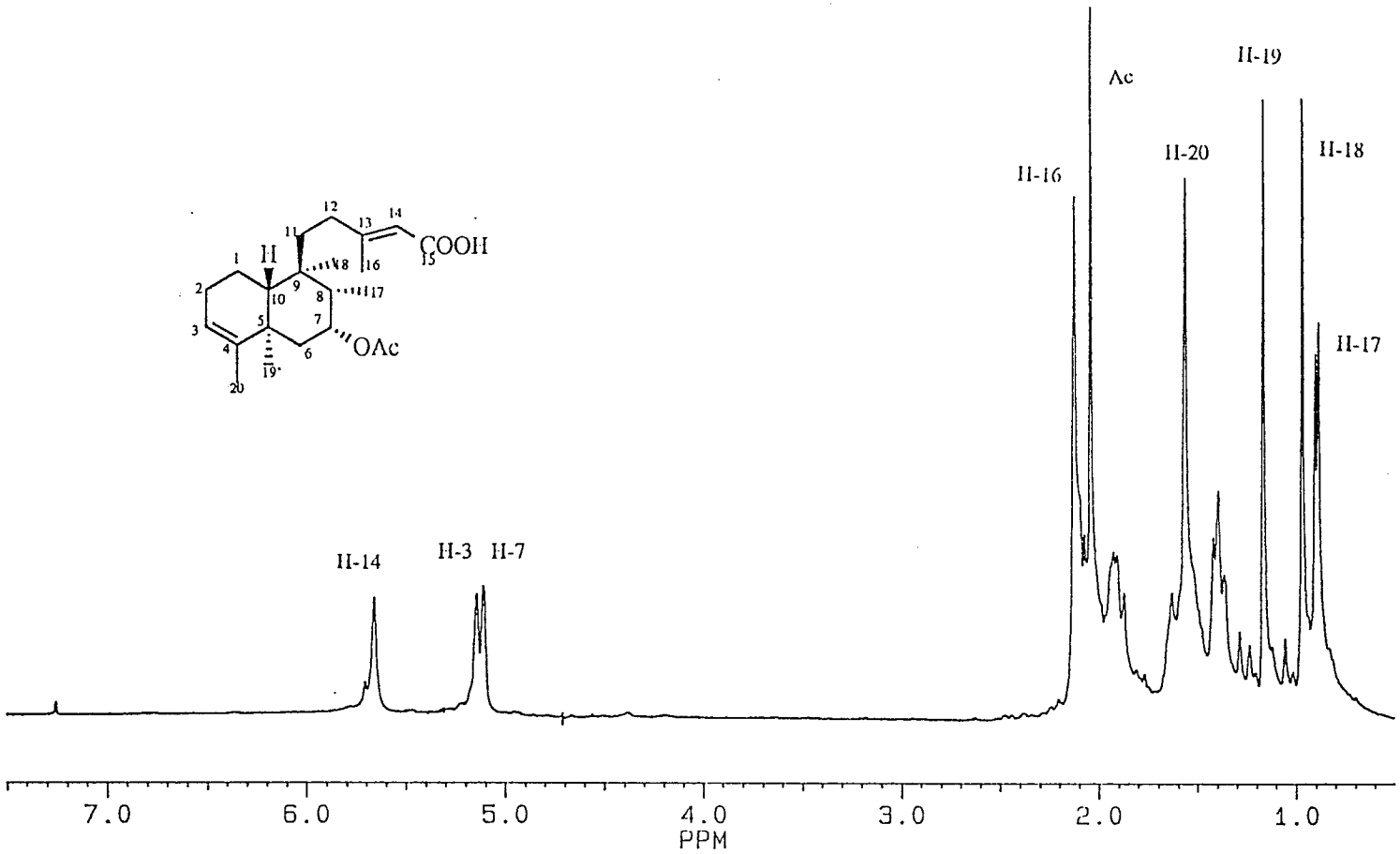


Figure 2.1.13. 400 MHz ^{1}H NMR spectrum of 13*E*-7α-acetoxylkolavenic acid (**6**)

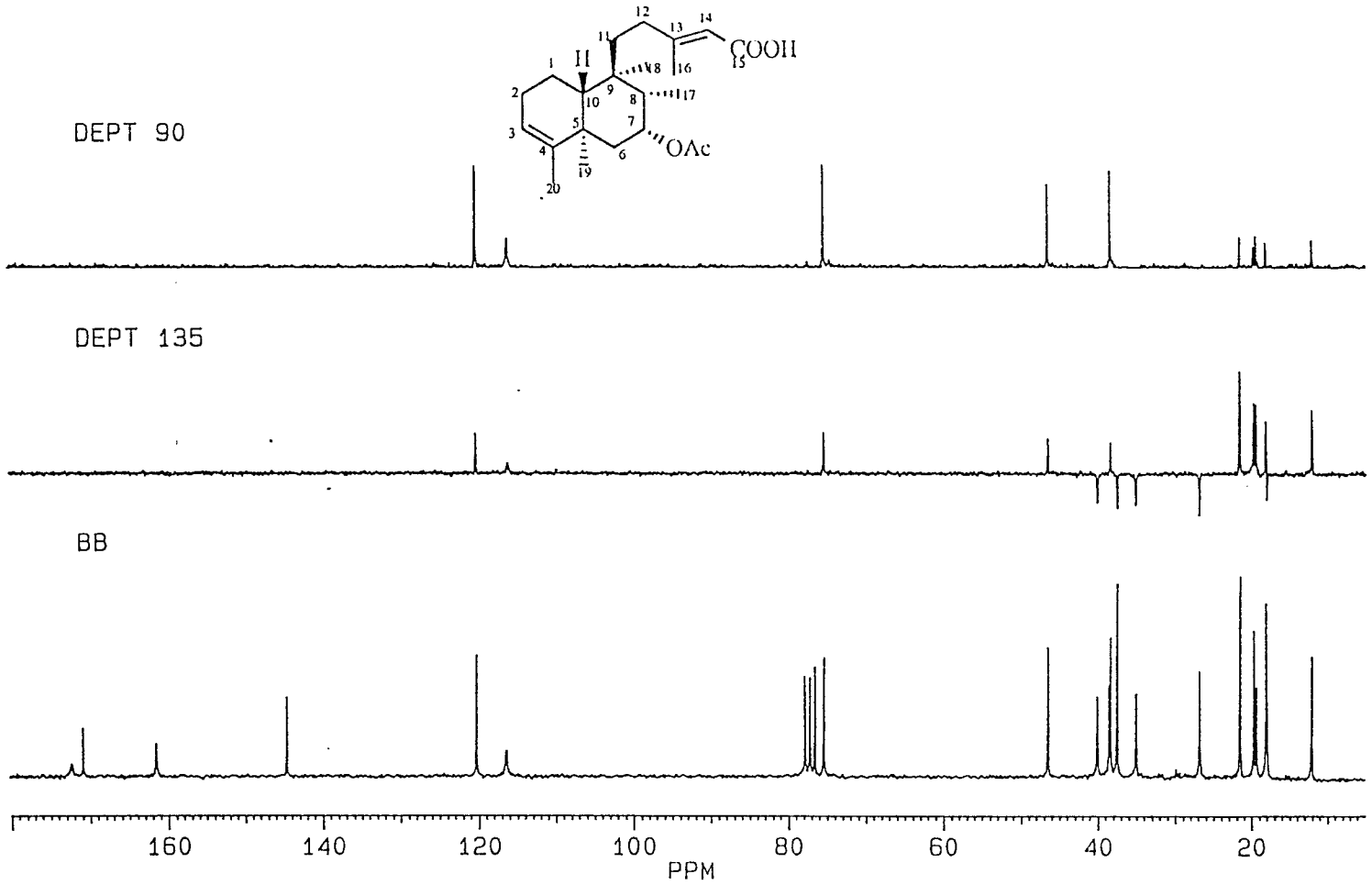


Figure 2.1.14. DEPT 90°, DEPT 135° and Broad Band ^{13}C NMR spectra of 13*E*-7α-acetoxylkolavenic acid (**6**)

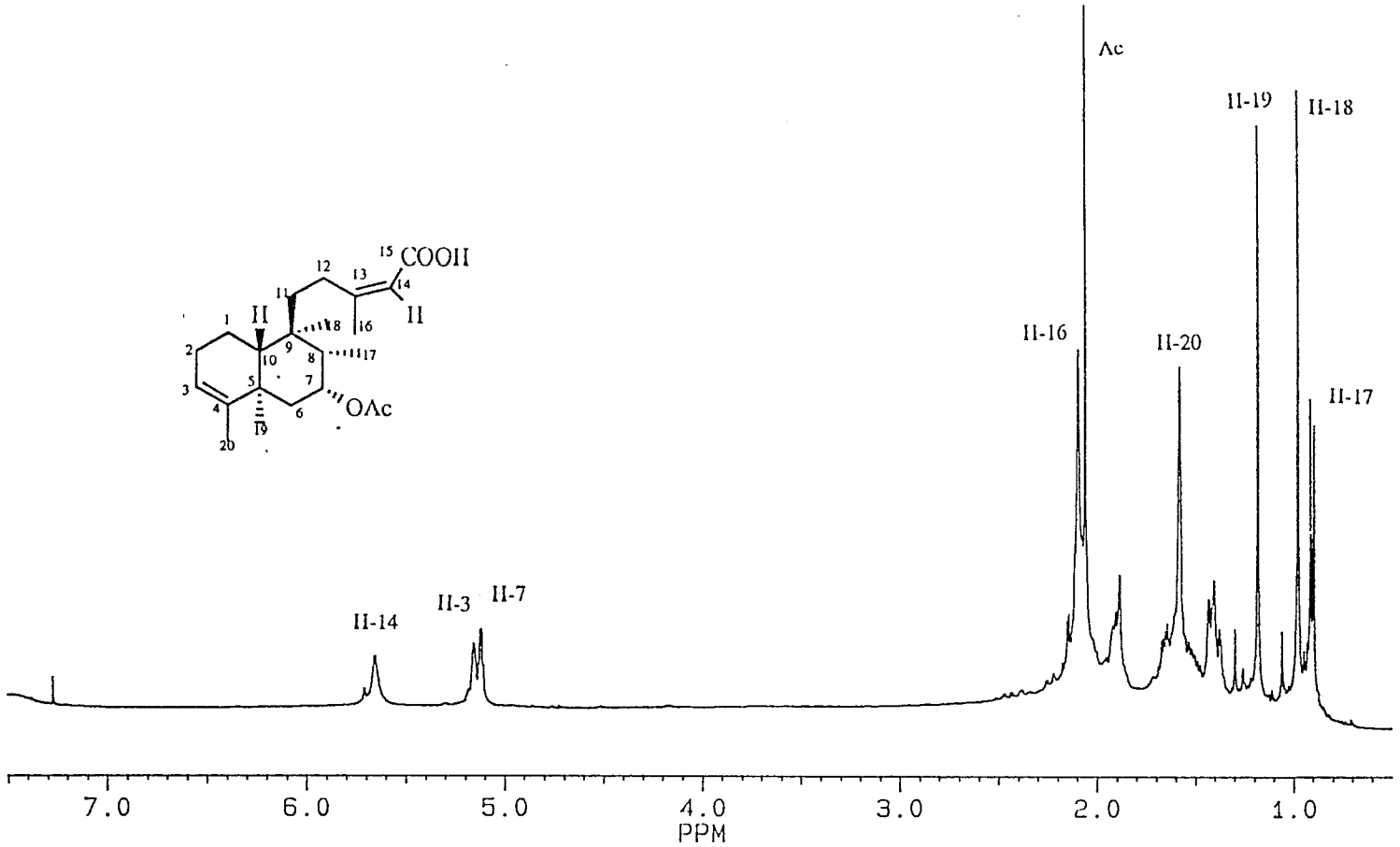


Figure 2.1.15. 400 MHz ^{1}H NMR spectrum of 13Z-7α-acetoxylkolavenic acid (7)

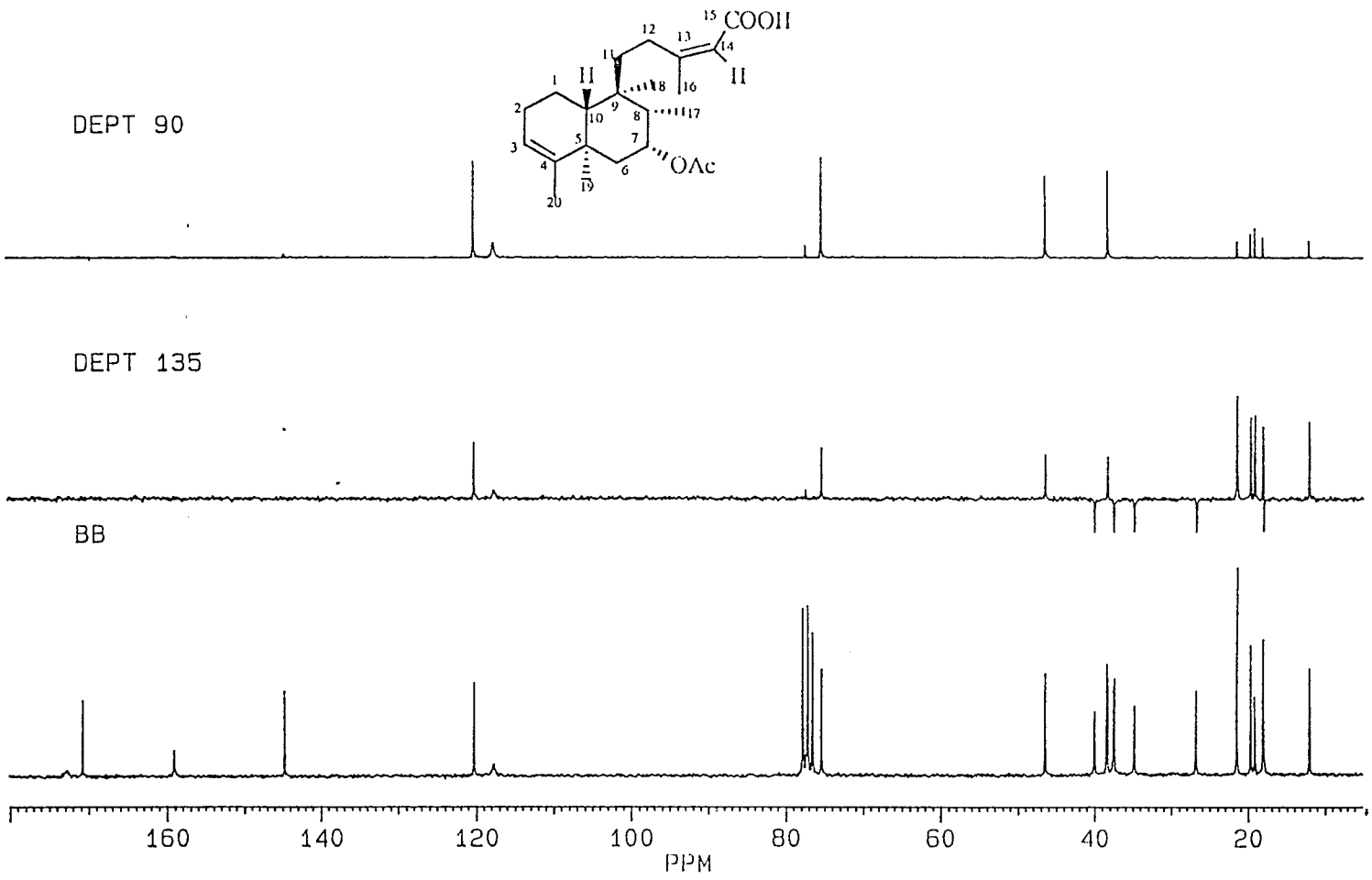


Figure 2.1.16. DEPT 90°, DEPT 135° and Broad Band ^{13}C NMR spectra of 13Z-7α-acetoxylkolavenic acid (7)

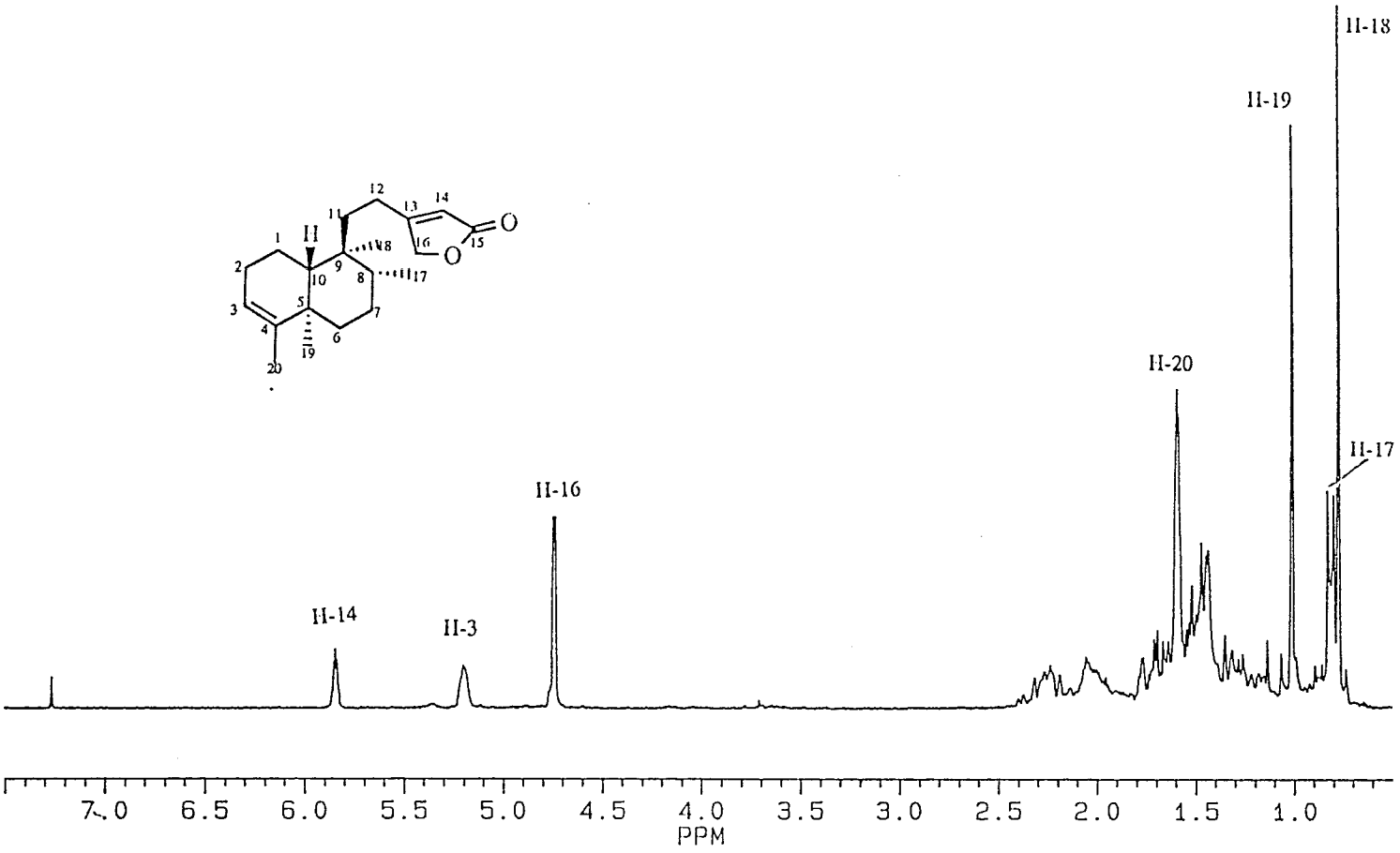


Figure 2.1.17. 200 MHz ^{1}H NMR spectrum of solidagolactone (**14**)

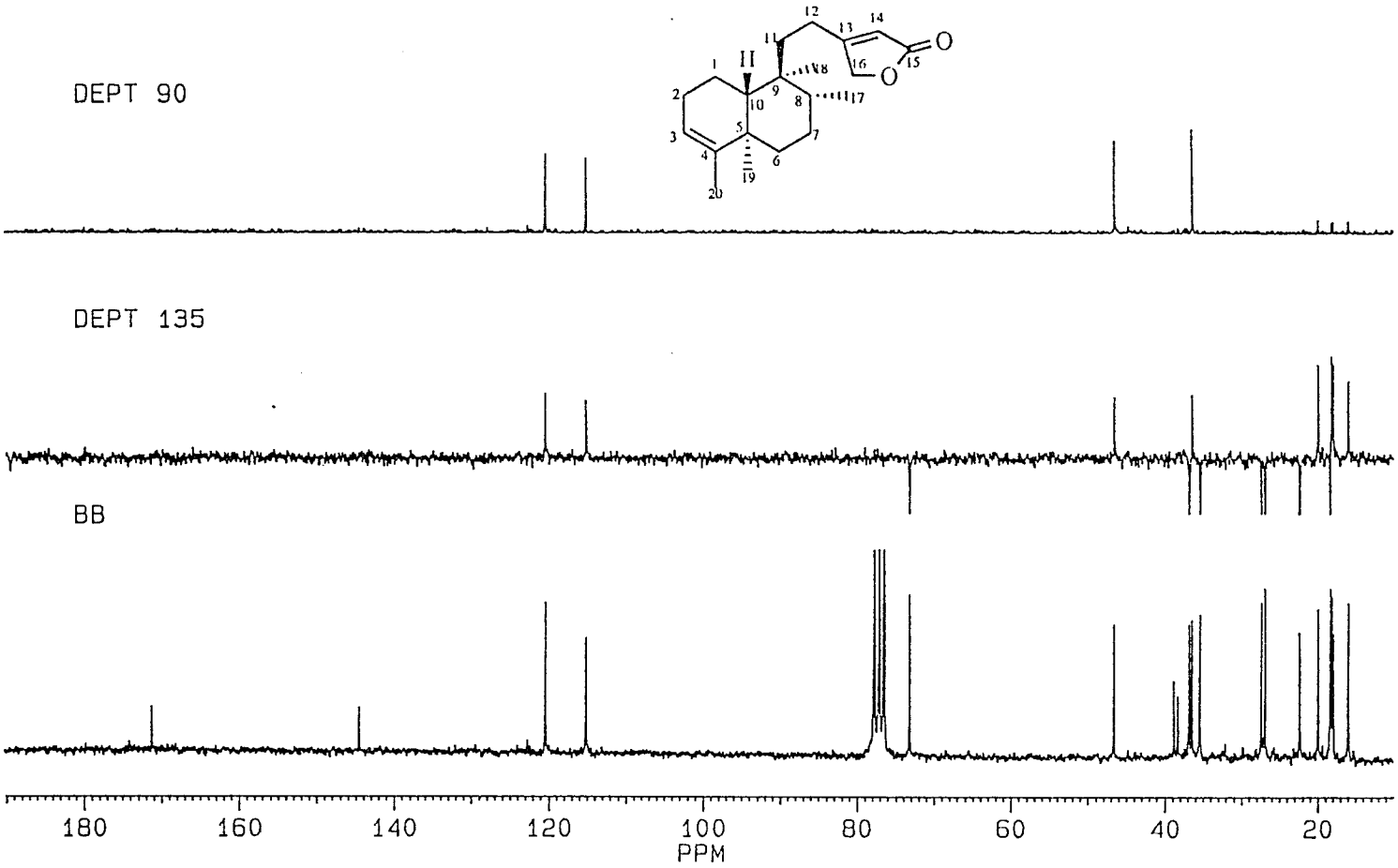


Figure 2.1.18. DEPT 90°, DEPT 135° and Broad Band ^{13}C NMR spectra of solidagolactone (**14**)

H-14 and 16-methyl group. The ^{13}C NMR spectra of both compounds showed 22 carbon signals. Their assignments were performed by COSY, $^{1}H,^{13}C$-correlation and DEPT, as well as comparison with spectral data of related compounds listed in Table 2.1.2.

Experimental

^{1}H and ^{13}C NMR spectra were recorded on either a Bruker-AC 200 or an AM 400 spectrometer in $CDCl_3$. IR spectra were obtained on a Perkin-Elmer 1760X FT-IR spectrometer as a film on NaCl plates. The mass spectra were run on a Hewlett-Packard 5971A GC-MS spectrometer. Column chromatography (CC) separations were made on silica gel (60-200 mesh, J. T. Baker). Vacuum liquid chromatographic (VLC) separations [129] were made on silica gel (MN Kieselgel G). Semi-preparative HPLC separations were performed on a 10 μ C18 column (250x10 mm) coupled to a Hewlett-Packard 1090 HPLC system with diode array detection at 230 nm.

Plant material. Roots of *Solidago canadensis* were collected on October 25, 1990 in East Baton Rouge Parish, Louisiana, U. S. A. (Voucher No. T. Lu-4; voucher deposited at the Louisiana State University Herbarium, U.S.A.).

Extraction and isolation. Fresh roots of *S. canadensis* (1,500 g) were soaked in CH_2Cl_2 for 24 hours yielding 14.1 g of crude extract. VLC separation (hexane-EtOAc, by increasing polarity) yielded 13x120 ml fractions. Dry CC (hexane-EtOAc, 7:1) of fraction 3 gave after recrystallization from CH_2Cl_2, 54 mg of *cis*-DME (**127a**); from a more polar band 673 mg of kolavenic acid (**1**) was obtained. VLC separation (hexane-Me_2CO, 4:1) of fraction 4, followed by Lobar CC (hexane-Me_2CO, 4:1, silica gel 60) and prep. TLC (hexane-Et_2O, 2:1) provided acetylenes **131a**, **133a, 133b** and **134**. Further prep. HPLC (MeCN-H_2O, 7:3) yielded 10 mg **2**, 12 mg of **3,** 24 mg of **4** and 5 mg of **14**. Dry CC (CH_2Cl_2-Me_2CO, 19:1) of fraction 5, followed by prep.

HPLC (MeOH-H_2O, 4:1) afforded 30 mg of **6**, and prep. HPLC (MeOH-H_2O, 17:3) of fraction 7 gave 50 mg of **7**.

6β-Tigloyloxykolavenic acid (**3**). $C_{25}H_{38}O_4$, colorless oil; IR ν_{max}^{NaCl} cm^{-1}: 3500-2500 (br COOH); 1698 (C=O, ester and acid); 1645 (C=C); 1259, 1152 (CC(=O)OC, ester). EIMS *m/z* (rel. int.): 384 $[M-H_2O]^+$ (0.35); 357 $[M-COOH]^+$ (0.32); 302 $[M-100]^+$ (4.1); 287 $[302-CH_3]^+$ (2.3); 189 $[302-CH_2CH_2C(CH_3)=CHCOOH]^+$ (19.7); 187 $[189-2H]^+$ (22.8); 119 (32.2); 83 $[O=CC(CH_3)=CHCH_3]^+$ (100); 55 $[83-CO]^+$ (52). ^1H NMR data in Table 2.1.1 and ^{13}C NMR data in Table 2.1.2.

6β-Angeloyloxykolavenic acid (**4**). $C_{25}H_{38}O_4$, colorless oil; IR ν_{max}^{NaCl} cm^{-1}: 3500-2500 (br COOH); 1707, 1695 (C=O, ester and acid); 1643 (C=C); 1237, 1161 (CC(=O)OC, ester). EIMS *m/z* (rel. int.): 402 $[M]^+$ (0.20); 384 $[M-H_2O]^+$ (0.38); 302 $[M-100]^+$ (3.8); 287 $[302-CH_3]^+$ (1.8); 189 $[302-CH_2CH_2C(CH_3)=CHCOOH]^+$ (18.3); 187 $[189-2H]^+$ (25.2); 119(33.0); 83 $[O=CC(CH_3)=CHCH_3]^+$ (100); 55 $[83-CO]^+$ (56). ^1H NMR data in Table 2.1.1 and ^{13}C NMR data in Table 2.1.2.

13E-7α-Acetoxylkolavenic acid (**6**). $C_{22}C_{34}O_4$, colorless oil; IR ν_{max}^{NaCl} cm^{-1}: 3500-2400 (br. COOH); 1735, 1691 (C=O, ester and acid); 1645 (C=C); 1244, 1167 (CC(=O)OC, ester). EIMS *m/z* (rel. int.): 344 $[M-H_2O]^+$ (7.8); 302 $[M-CH_3COOH]^+$ (9.3); 287 $[302-CH_3]^+$ (16.6); 189 $[302-CH_2CH_2C(CH_3)=CHCOOH]^+$ (33); 187 $[189-2H]^+$ (100); 119 (84); 95 (61); 43 $[CH_3CO]^+$ (80). ^1H NMR data in Table 2.1.1 and ^{13}C NMR data in Table 2.1.2.

13Z-7α-Acetoxylkolavenic acid (**7**). $C_{22}H_{34}O_4$, colorless oil; IR ν_{max}^{NaCl} cm^{-1}: 3500-2400 (br. COOH); 1734, 1692 (C=O, ester and acid); 1644 (C=C); 1245, 1167 (CC(=O)OC, ester). EIMS *m/z* (rel. int.): 302 $[M-CH_3COOH]^+$ (4.2); 189 $[302-CH_2CH_2C(CH_3)=CHCOOH]^+$ (78); 187 $[189-2H]^+$ (100); 119 (85); 95 (35); 43 $[CH_3CO]^+$ (73). ^1H NMR data in Table 2.1.1 and ^{13}C NMR data in Table 2.1.2.

2.2

Diterpenes from *Solidago rugosa*

Introduction

In continuation of our search for biologically active compounds from the Asteraceae family, we have investigated the roots and aerial parts of *Solidago rugosa* Mill., a member of the large North American genus *Solidago* [130]. Previous studies of this species of unreported collection site had resulted in the isolation of several diterpene acids and aldehydes [27]. Our investigation of this species provided two known clerodane diterpenes, kolavenol (**2**) [26] and hardwickiic acid (**63**) [122], four labdane diterpenes (**110a, 112-114**), one of which (**114**) is new. In addition, the five abietenes **104** and **106-109**, four of which are new, and the known kaurenic acid (**83**) [27] were obtained. The structures of the known and the new compounds were elucidated by spectroscopic methods, especially by mass spectral analysis and high field ^{1}H and ^{13}C NMR including COSY, NOESY, inverse $^{1}H,^{13}C$-correlation methods as well as chemical transformations.

Results and Discussion

The structure of kolavenol (**2**) which had been previously isolated from *Solidago canadensis* [26] and related taxa [21, 122], was derived by direct comparison with an authentic sample. Hardwickiic acid (**63**) was previously found in *Hardwickia pinnata* [122] and *Solidago juncea* [47]. Although the ^{13}C NMR data of the methyl ester of **63** was reported before [131], we performed unambiguous assignments of its ^{13}C

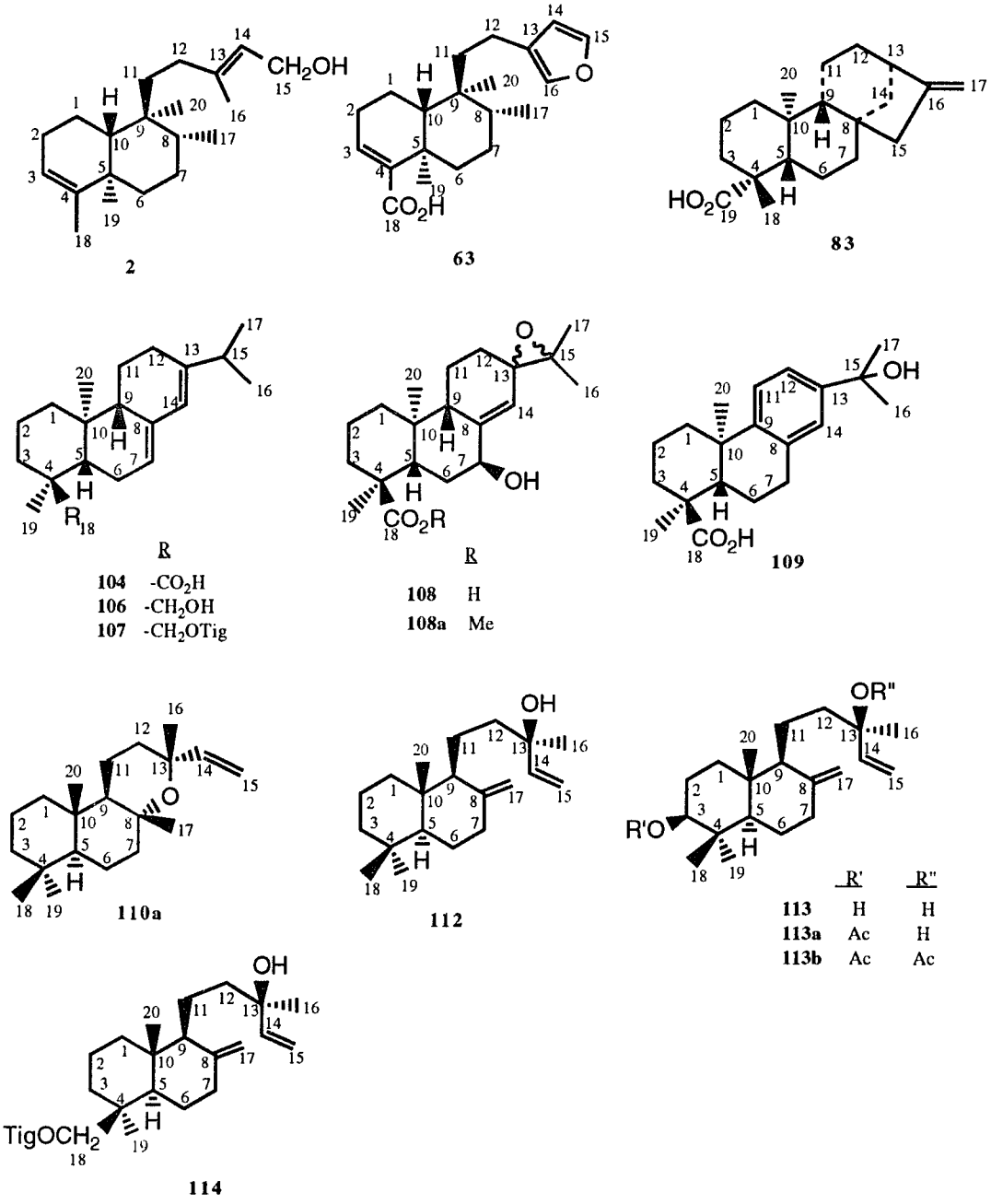


Figure. 2.2.1. Diterpenes from *Solidago rugosa*

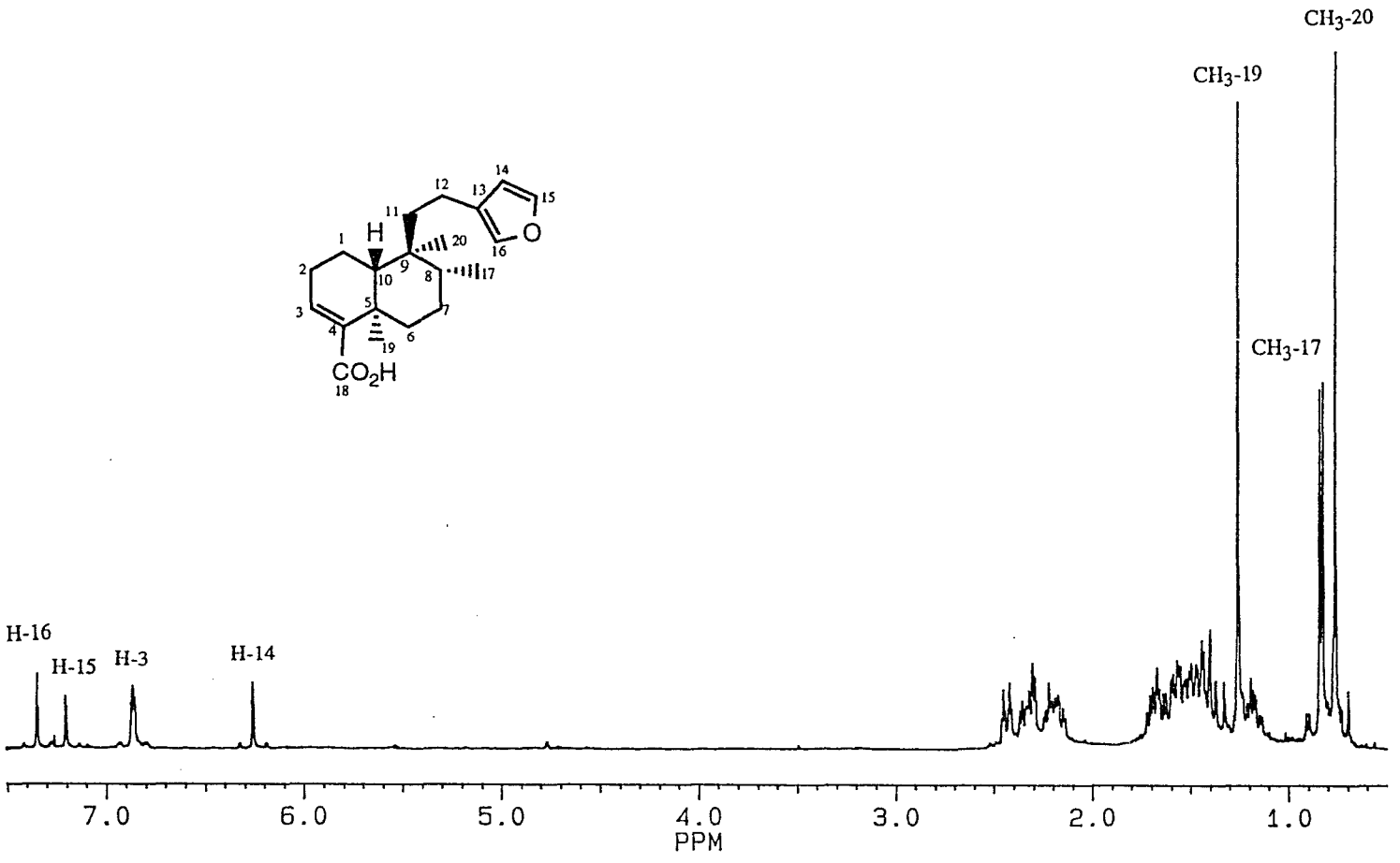


Figure 2.2.2. 400 MHz ^{1}H NMR spectrum of hardwickiic acid (**63**)

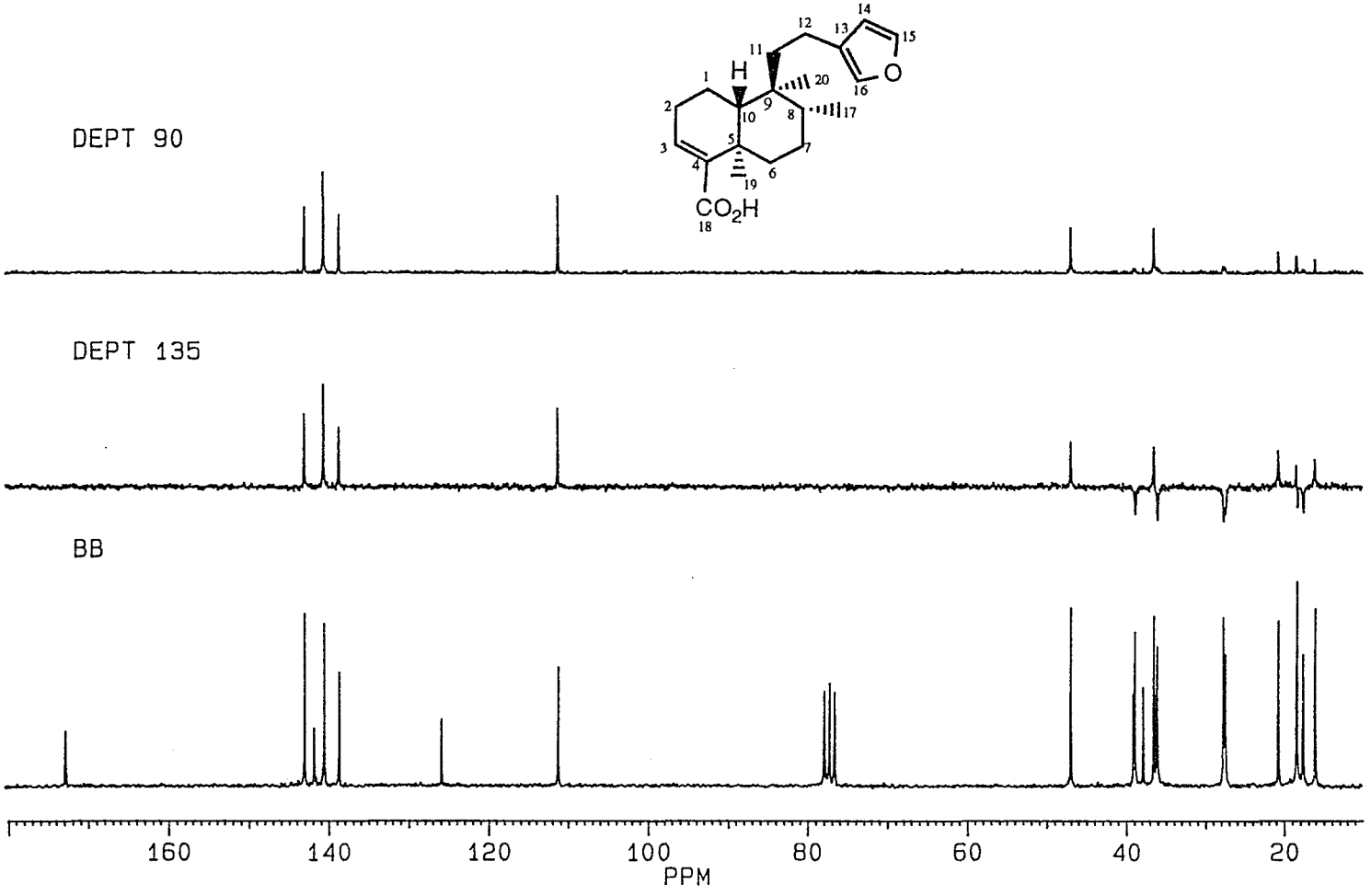


Figure 2.2.3. DEPT 90°, DEPT 135° and Broad Band ^{13}C NMR spectra of hardwickiic acid (**63**)

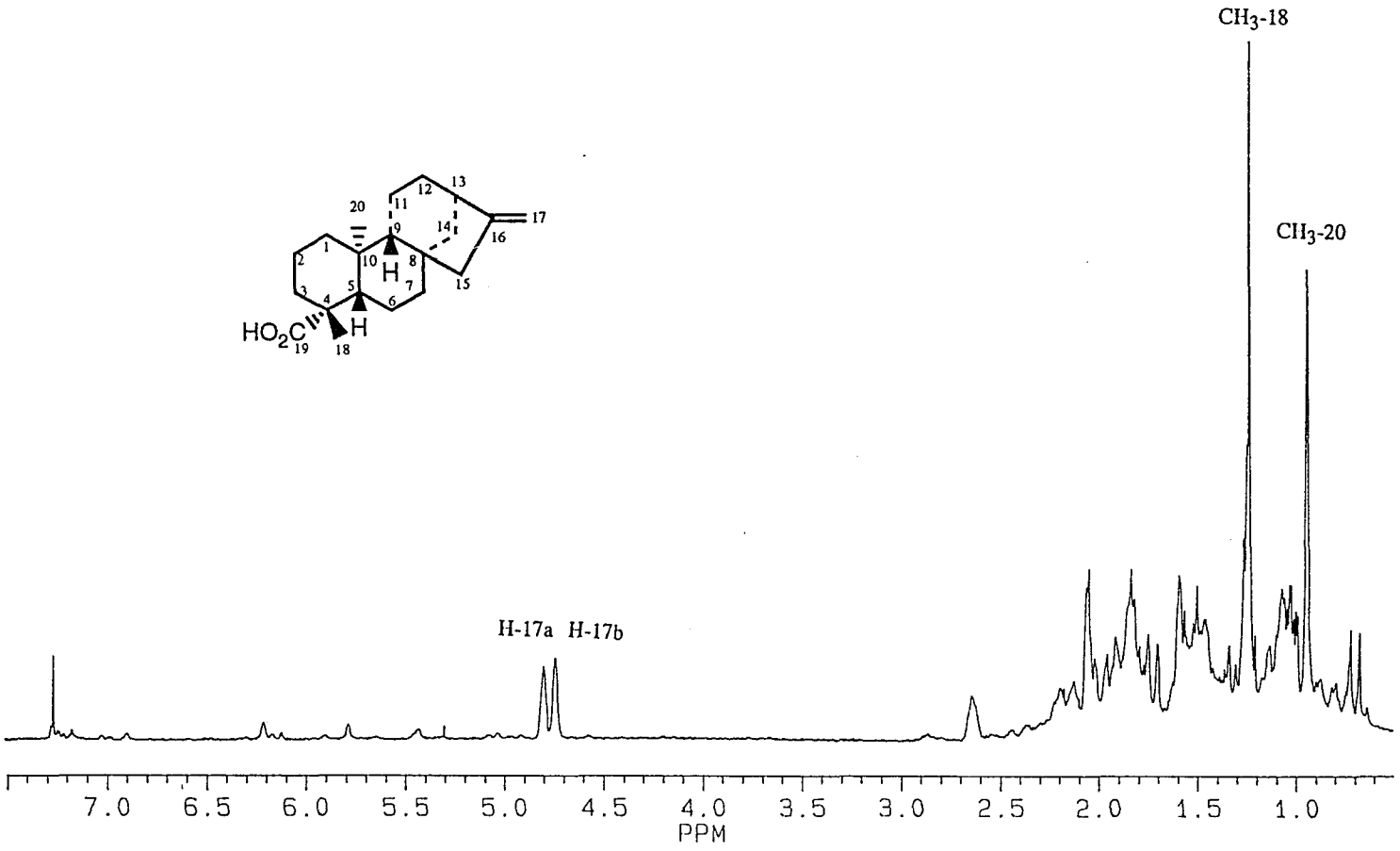


Figure 2.2.4. 200 MHz ^{1}H NMR spectrum of (-)-kaur-16-en-19-oic acid (**83**)

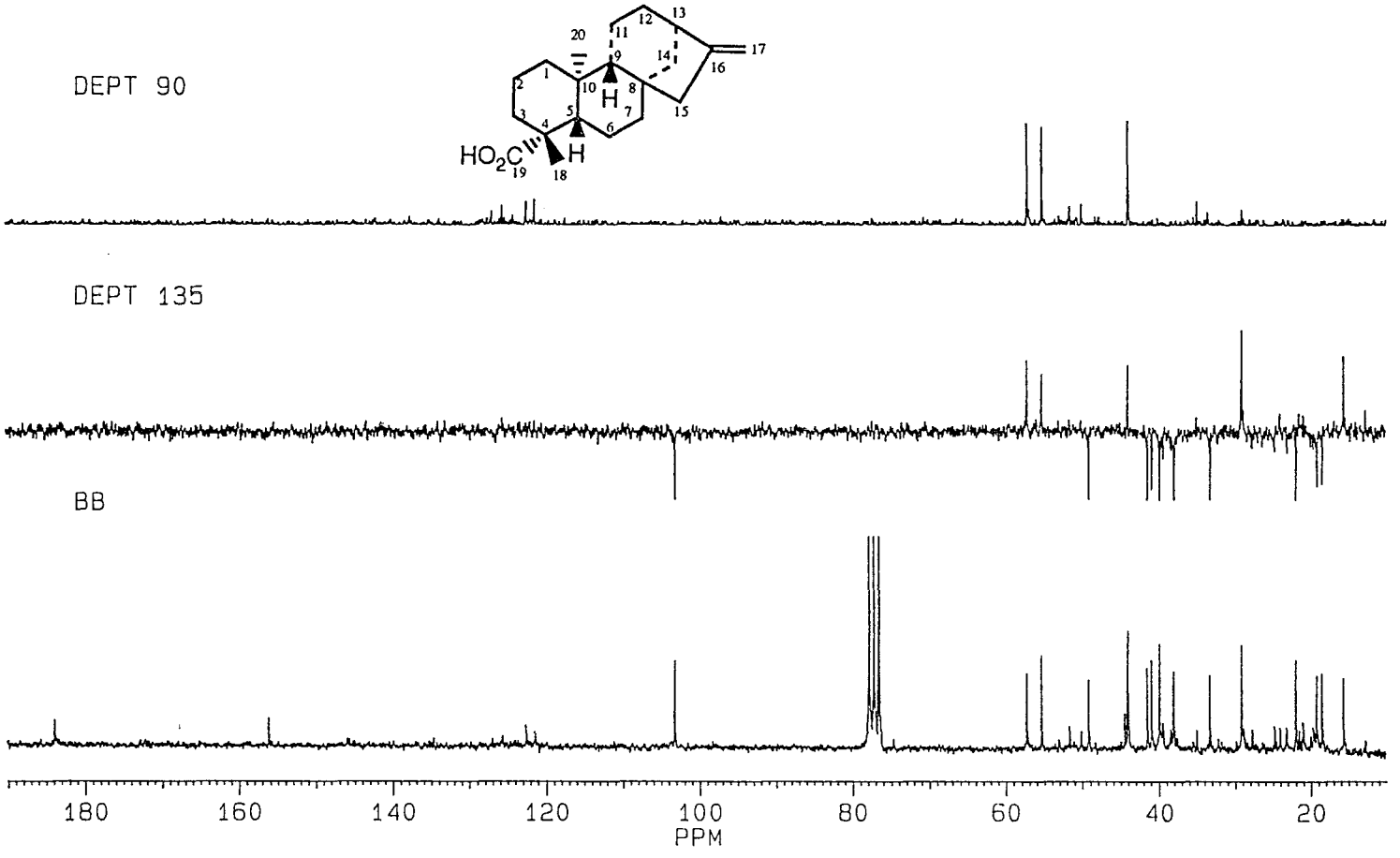


Figure 2.2.5. DEPT 90°, DEPT 135° and Broad Band ^{13}C NMR spectra of (-)-kaur-16-en-19-oic acid (**83**)

Table 2.2.1. ^{1}H NMR data of compounds **63, 112, 113, 113a-b and 114** (400 MHz, $CDCl_3$ as internal standard)

H	**63**	**112**	**113**	**113a**	**113b**	**114**
3	6.86 dd		3.24 dd	4.52 dd	4.50 dd	
7α		1.96 ddd	1.95 ddd	1.97 ddd	1.96 (overlap)	1.94 ddd
7β		2.37 ddd	2.39 ddd	2.39 ddd	2.39 ddd	2.39 ddd
14	6.26 br d	5.91 dd	5.90 dd	5.90 dd	5.94 dd	5.91 dd
15	7.35 t	5.05 dd 5.20 dd	5.06 dd 5.20 dd	5.06 dd 5.20 dd	5.11 br d 5.13 br d	5.05 dd 5.20 dd
16	7.20 br s	1.27 s	1.27 s	1.27 s	1.53 s	1.27 s
17	0.83 d	4.51 d 4.81 d	4.49 d 4.82 d	4.50 d 4.83 d	4.51 d 4.83 d	4.53 d 4.82 d
18	------	0.86 s	0.98 s	0.87 s	0.87 s	3.88 d 4.29 d
19	1.26 s	0.78 s	0.76 s	0.84 s	0.84 s	0.99 s
20	0.76 s	0.67 s	0.67 s	0.70 s	0.70 s	0.70 s
3'	------	------	------	------	------	6.82 qq
4'	------	------	------	------	------	1.78 br d
5'	------	------	------	------	------	1.82 br d
OAc	------	------	------	2.05 s	2.05 s	------
OAc'	------	------	------	------	2.01 s	------

J (Hz): **63**: 3, 2a = 4.3; 3, 2b = 3.1; 14, 15 = 14, 16 =15, 16 = 1.7; 17, 8 = 6.4; **112**: 7α, 7β = 12.0; 7α, 6α = 5.1; 7α, 6β = 12.8; 7β, 6α = 2.5; 7β, 6β = 4.1; 14, 15a = 10.8; 14, 15b = 17.5; 15a, 15b = 1.1; 17a, 17b =1.0; **113**: 3, 2α =4.5; 3, 2β = 11.7; 7α, 7β = 12.8; 7α, 6α = 5.0; 7α, 6β =12.7; 7β, 6α = 2.5; 7β, 6β = 4.1; 14, 15a = 10.6; 14, 15b = 17.6; 15a, 15b = 1.0; 17a, 17b = 1.0; **113a**: 3, 2α = 4.9; 3, 2β = 11.2; 7α, 7β = 12.8; 7α, 6α = 5.0; 7α, 6β = 12.8; 7β, 6α = 2.5; 7β, 6β =4.1; 14, 15a = 10.7; 14, 15b = 17.3; 15a, 15b = 1.1; 17a, 17b = 1.1; **113b**: 3, 2α = 4.2; 3, 2β = 11.8; 7α, 7β = 12.8; 7β, 6α = 2.4; 7β, 6β = 4.1; 14, 15a = 10.9; 14, 15b = 17.5; 17a, 17b = 1.0; **114**: 7α, 7β = 12.7, 7α, 6α = 5.0; 7α, 6β = 12.6; 7β, 6α = 2.4; 7β, 6β = 3.7; 14, 15a = 10.6; 14, 15b = 17.2; 15a, 15b = 1.1; 17a, 17b = 1.0; 18a, 18b = 11.0; 3', 4' = 6.8; 3', 5' = 1.6.

Table 2.2.2. ^{1}H NMR spectral data of compounds **104, 106-108, 108a** and **109** (400 MHz, *200 MHz, $CDCl_3$ as internal standard)

H	**104**	**106***	**107***	**108**	**108a**	**109***
5				1.87 dd	1.86 overlap	
6α				1.16 br dd	1.16 overlap	
6β				2.27 ddd	2.28 ddd	
7	5.37 br d	5.40 br s	5.41 br d	4.90 br dd	4.90 br dd	2.89 m
11						}7.23 (2H) d
12						
14	5.77 br s	5.78 br s	5.77 br s	6.12 d	6.11 d	7.16 br s
15	2.22 m	2.22 m	2.23 m	------	------	------
16	1.01 d	1.01 d	1.01 d	1.42 s	1.42 s	1.56 s
17	1.00 d	1.00 d	1.00 d	1.40 s	1.39 s	1.56 s
18	------	3.15 d	4.02 d	------	------	------
18'	------	3.37 d	4.42 d	------	------	------
19	1.25 s	0.88 s	0.99 s	1.28 s	1.21 s	1.38 s
20	0.83 s	0.83 s	0.81 s	0.46 s	0.36 s	1.13 s
3'	------	------	6.84 qq	------	------	------
4'	------	------	1.80 br d	------	------	------
5'	------	------	1.84 br d	------	------	------
MeO	------	------	------	------	3.64 s	------

J (Hz): **104**: 7, 6α = 2.6; 16, 15 = 17, 15 =6.8; **106**: 16, 15 =17, 15 =6.8; 18, 18' =10.9; **107**: 7, 6α = 2.4; 16, 15 = 17, 15 = 6.7; 18, 18' = 11.0; 3', 4' = 7.0; 3', 5' = 1.2; **108**: 5, 6β = 9.5; 5, 6α = 5.4; 6α, 6β = 13.5; 6α, 7 = 2.1, 6β, 7 = 4.2; 14, 7 = 1.4; **108a**: 6β, 6α =13.4; 6β, 5 =9.2; 6α, 7 = 2.1; 6β, 7 = 4.2; 14, 7 =1.4; **109**: 11, 14 = 12, 14 = 1.0.

Table 2.2.3. ^{13}C NMR spectral data of compounds **63**, **83**, **104**, **108**, **112**, **113**, **113a**, **113b** and **114** (100 MHz, $CDCl_3$ as int. std.)*

C	**63**	**83**	**104**	**108**	**112**	**113**	**113a**†	**113b**†	**114**†
1	35.8 t	41.3 t	38.3 t	37.7 t	39.1 t	37.1 t	36.7 t	36.6 t	38.5 t
2	18.2 t	19.1 t	18.1 t	18.5 t	19.4 t	27.9 t	24.3 t	24.3 t	19.0 t
3	140.3 d	39.7 t	37.2 t	37.9 t	38.4 t	78.9 d	80.8 d	80.7 d	36.5 t
4	141.5 s	43.7 s	46.4 s	43.6 s	39.5 s	39.1 s	38.0 s	38.0 s	37.8 s
5	37.6 s	55.1 d	44.9 d	49.6 d	55.6 d	54.6 d	54.7 d	54.7 d	56.3 d
6	38.7 t	21.8 t	25.6 t	25.5 t	24.4 t	24.0 t	23.8 t	23.8 t	24.5 t
7	27.3 t	40.7 t	120.5 d	72.5 d	41.4 t	38.1 t	38.1 t	38.0 t	38.9 t
8	36.3 d	44.2 s	145.2 s	149.5 s	145.1 s	148.1 s	147.9 s	147.7 s	147.9 s
9	38.8 s	57.1 d	51.0 d	55.3 d	57.3 d	56.9 d	56.8 d	56.8 d	57.4 d
10	46.7 d	39.7 s	34.5 s	37.4 s	40.0 s	39.5 s	39.4 s	39.3 s	40.1 s
11	17.5 t	18.4 t	22.5 t	20.4 t	17.7 t	17.8 t	17.8 t	17.6 t	17.8 t
12	27.5 t	33.11 t	27.5 t	32.5 t	42.2 t	41.2 t	41.2 t	39.2 t	41.3 t
13	125.6 s	43.9 d	135.6 s	70.8 s	77.2 s	73.6 s	73.6 s	83.3 s	73.8 s
14	111.0 d	37.8 t	122.5 d	124.5 d	145.3 d	145.0 d	145.1 d	141.9 d	145.3 d
15	142.7 d	49.0 t	34.9 d	76.7 s	111.5 t	111.7 t	111.7 t	113.8 t	111.6 t
16	138.4 d	155.9 s	20.9 q	28.8 q	27.7 q	28.1 q	28.0 q	23.5 q	27.7 q
17	15.9 q	103.0 t	21.4 q	28.3 q	106.5 t	106.7 t	106.9 t	107.0 t	107.0 t
18	172.6 s	29.0 q	185.3 s	182.4 s	33.6 q	28.3 q	28.2 q	28.2 q	66.7 t
19	20.5 q	183.8 s	16.7 q	28.0 q	21.7 q	15.3 q	16.5 q	16.5 q	27.7 q
20	18.3 q	15.6 q	14.0 q	13.2 q	14.4 q	14.4 q	14.5 q	14.5 q	15.2 q

* Peak multiplicities were determined by heteronuclear multipulse programs (DEPT).

† Ester side chains: **113a**: 170.9 s, 21.3 q (Ac); **113b**: 171.0 s, 169.9 s, 21.3 q, 22.2 q; **114**: 168.6 s (C-1'), 129.0 s (C-2'), 136.8 d (C-3'), 14.3 q (C-4'), 12.1 q (C-5').

NMR spectrum by combined DEPT, COSY and $^{1}H,^{13}C$-correlation methods, the results being summarized in Table 2.2.3. The (-)-kaur-16-en-19-oic acid (**83**) has been described from several *Solidago* species including *S. rugosa* [27, 45, 48].

Other diterpenes isolated from this plant were *ent*-abietic acid (**104**) [27] and its related compounds (**106-109**). Compound **104**, which had been previously isolated form *Solidago rugosa*[27], exhibited ^{1}H NMR and mass spectral data that were nearly identical with those previously reported [27]. The ^{13}C NMR data of **104** showed the presence of 20 carbons, which were assigned by DEPT and ^{1}H, ^{13}C-correlation methods. Unambiguous assignments of four quaternary carbons (C-4, C-10, C-8 and C-13) required the application of the selective INEPT methodology [123, 124]. Irradiation of the proton signal at δ 1.25 (H-19) using a coupling parameter of 8 Hz, transferred the polarization to two carbon signals, the carbonyl signal at δ 185.3 (C-18) and a quaternary carbon signal at δ 46.3 which was assigned to C-4. Polarization transfer from the proton signal at δ 0.83 (H-20), with a coupling parameter of 8 Hz, gave rise to a strong signal at δ 34.5 which was assigned to C-10. Polarization (8 Hz) of the olefinic H-14 (δ 5.77) enhanced two carbon signals, one methine carbon at δ 34.8 (C-15) and a quaternary carbon at δ 135.5 (C-13). The weak signal at δ 145.2 was assigned to C-8.

Compound **106** was separated from **104** by HPLC and was identified as an isomer of the abietinol [138]. Comparison of ^{1}H NMR data of **106** with those of **104** showed that **106** contained two mutually coupled proton doublets at δ 3.15 and 3.37, which were absent in **104**. Furthermore, the upfield shift of the methyl singlet (H-19) from δ 1.25 in **104** to δ 0.88 in **106**, indicated a CH_2OH group in **106** instead of a CO_2H group in **104**. This was further confirmed a strong IR OH-absorption at 3402 cm^{-1}. The mass spectrum of **106** showed a strong molecular ion peak at m/z 288 which is in agreement with the empirical formula $C_{20}H_{32}O$. On the basis of

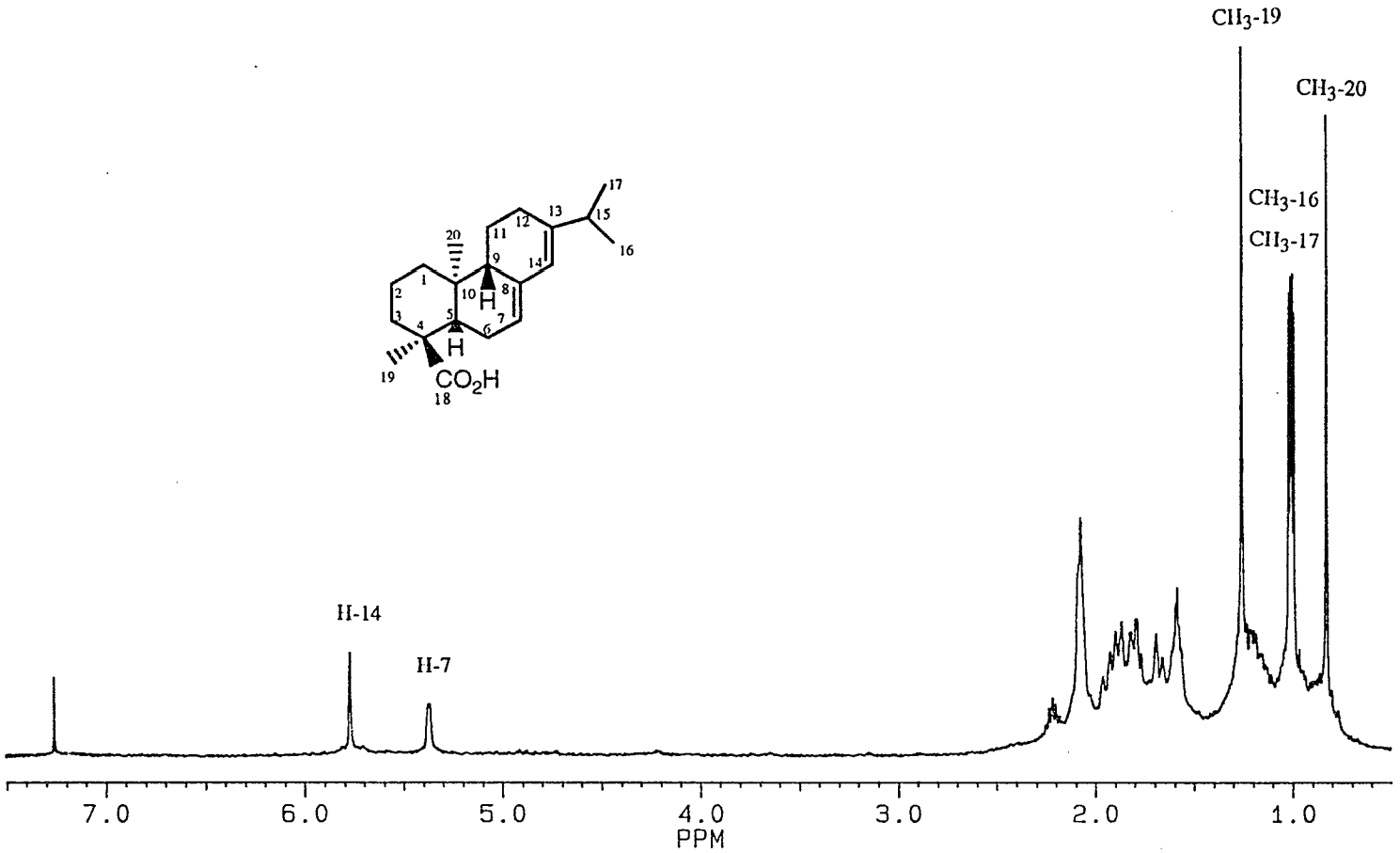


Figure 2.2.6. 400 MHz ^{1}H NMR spectrum of abieta-7,13(14)-dien-18-oic acid (**104**)

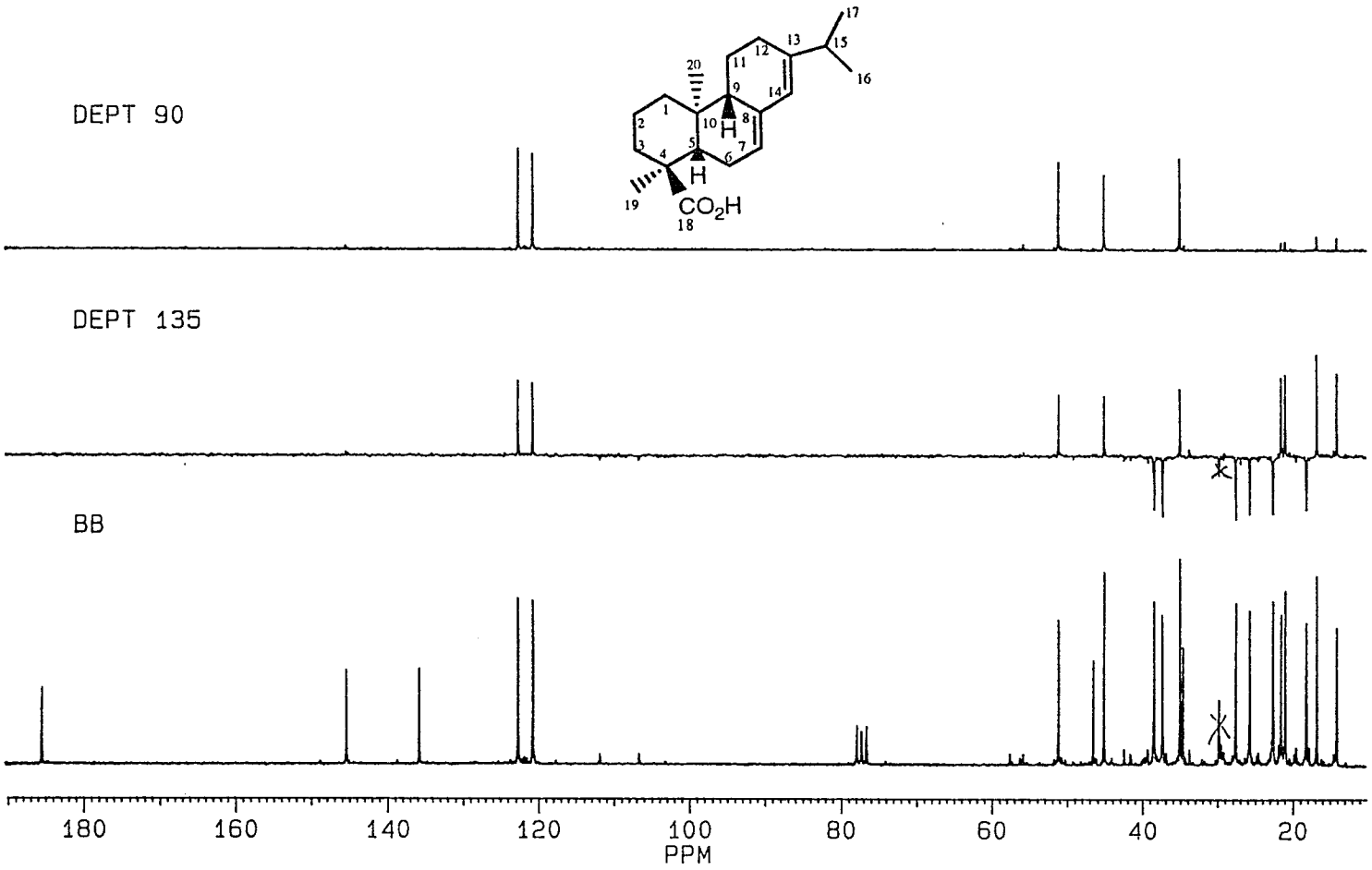


Figure 2.2.7. DEPT 90°, DEPT 135° and ^{13}C NMR spectra of abieta-7,13(14)-dien-18-oic acid (**104**)

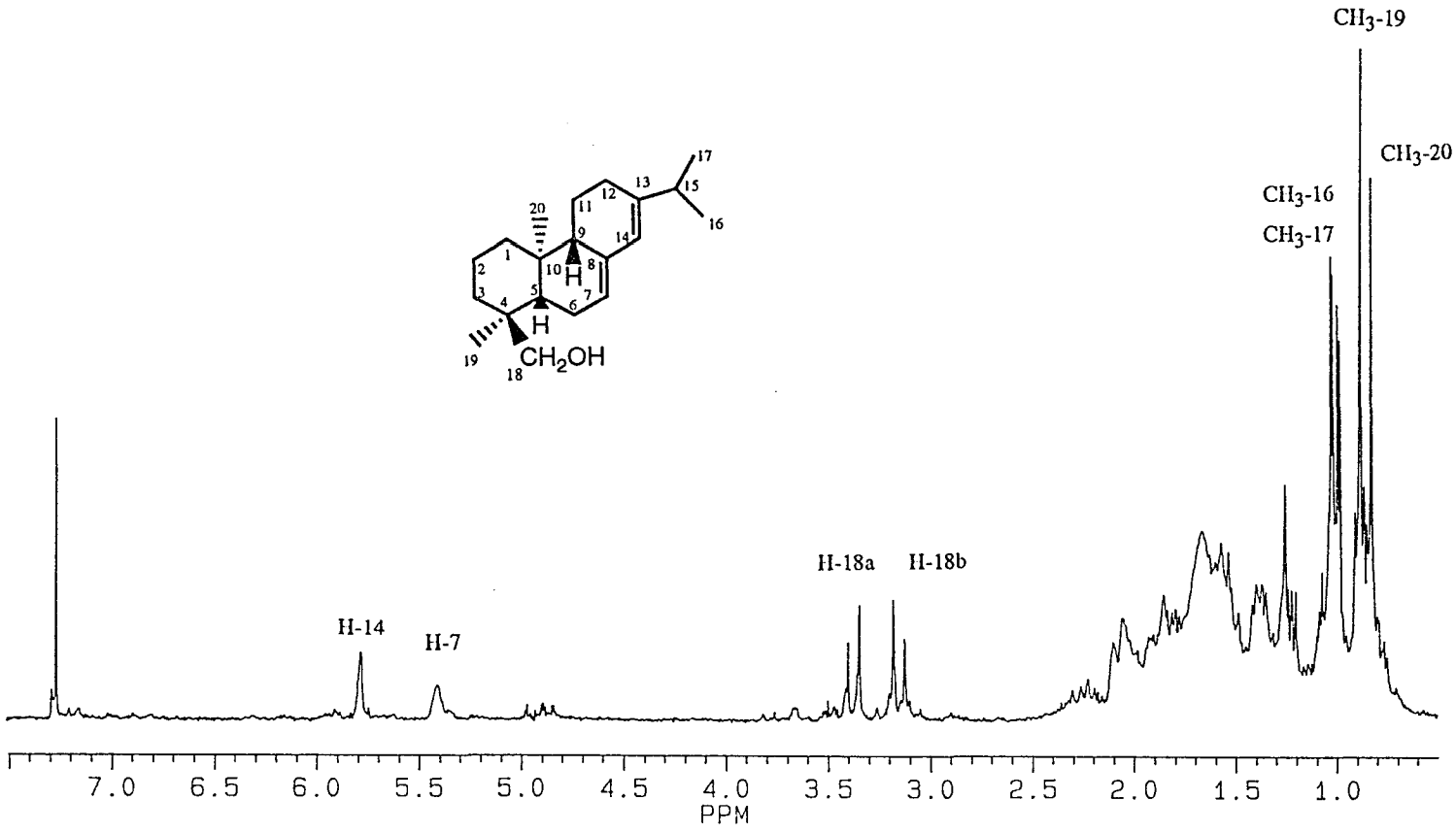


Figure 2.2.8. 200 MHz ^{1}H NMR spectrum of 18-hydroxyabieta-7,13(14)-diene (**106**)

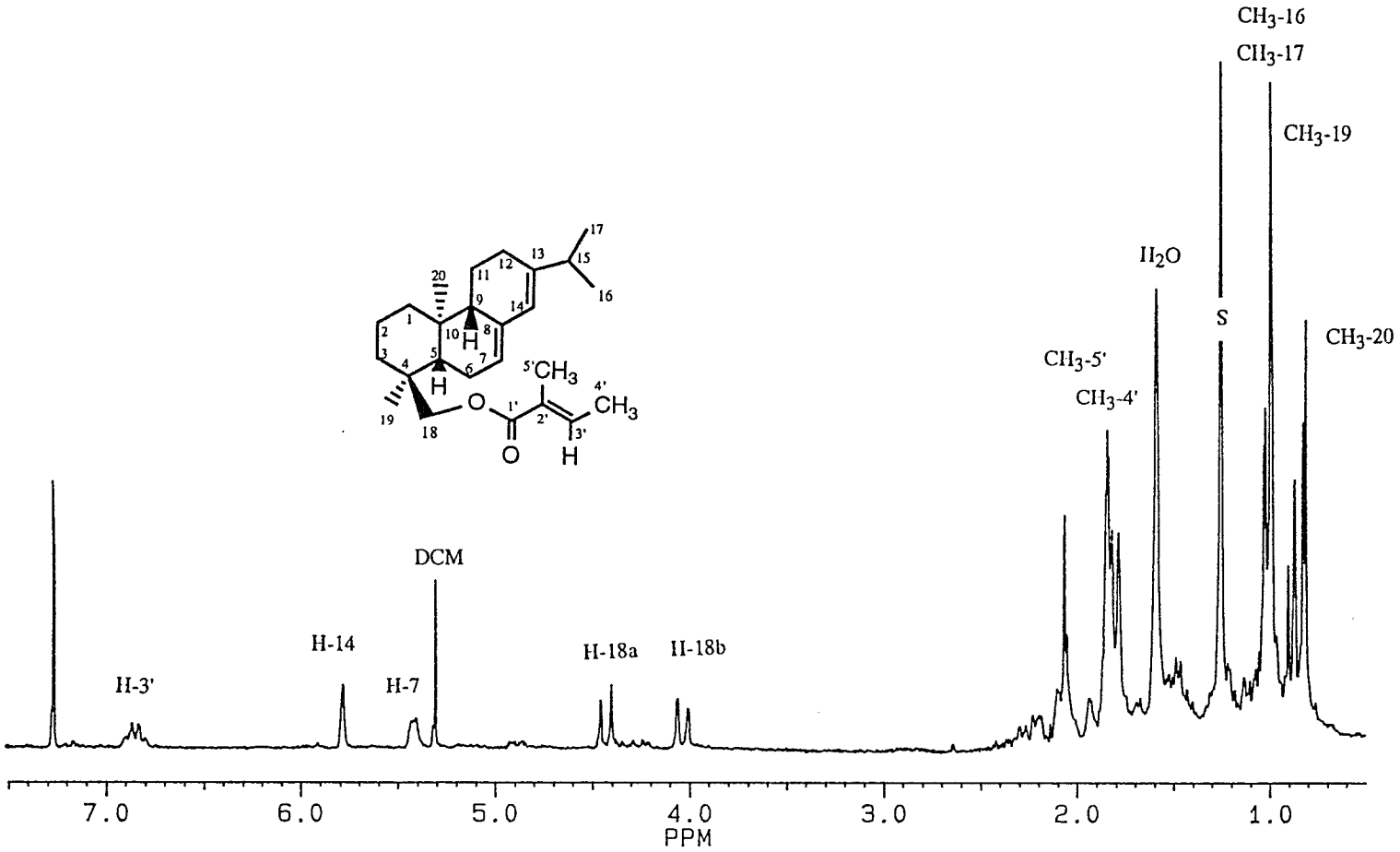


Figure 2.2.9. 200 MHz ^{1}H NMR spectrum of 18-tigloyloxyabieta-7,13(14)-diene (**107**)

biogenetic considerations, **106** must belong to the *ent*-abietinol series since only *ent*-abietanes have been isolated from *Solidago* species, including *S. rugosa* [27, 46].

Compound **107** was identified as the tiglate of **106** since its ^{1}H NMR spectrum clearly showed a proton quartet of a quartet at δ 6.84 coupled to two broad methyl doublets at δ 1.80 and 1.84, which are diagnostic for the tiglate moiety [139]. The only other differences between these two compounds resided in a downfield shifts of the two H-18 protons and the methyl group at C-4 (Me-19) due to the deshielding effect of the tiglate carbonyl group. The two geminally coupled C-18 protons absorbed at δ 4.02 and 4.42 in **107** compared to δ 3.15 and 3.37 in **106**. The Me-19 singlet appeared at δ 0.99 in **107** and at δ 0.88 in **106**. The mass spectral data of **107** further supported its structure with a prominent molecular ion at m/z 370 and strong peaks at m/z 287 $[M-83]^+$, m/z 270 $[M-100]^+$, being derived from the loss of the tiglic acid. Further strong peaks m/z 83 and 55 were also characteristic for the tiglate moiety.

Compound **108** is the most polar of the diterpenes from *S. rugosa* (TLC, R_f = 0.09, hexane-acetone, 4:1). Its IR spectrum showed a strong broad absorption ranging from 3500-2500 cm^{-1}, which indicated a carboxylic acid group. After methylation, its methyl ester **108a** still showed an IR absorbance at 3440 cm^{-1}, suggesting the presence of hydroxyl(s). This was further supported by the ^{13}C NMR data of **108** which showed the presence of three oxygenated carbons with one CH absorption at δ 72.5 and two quaternary carbon signals at δ 70.8 and 76.7. The ^{13}C NMR of **108** indicated the presence of 20 carbons with only two olefinic carbon signals, one methine at δ 124.5 and another quaternary carbon signal at δ 149.5, indicating that only one carbon-carbon double bond was present in the molecule. Inspection of the COSY spectrum of **108** revealed that the olefinic proton at δ 6.12 (d, J=1.4 Hz) only showed allylic coupling to the proton signal at δ 4.90 which was under an oxygen function. The latter was further coupled to two geminally coupled protons at δ 1.16 and 2.27 which were further coupled to a proton signal at δ 1.87. All of the above

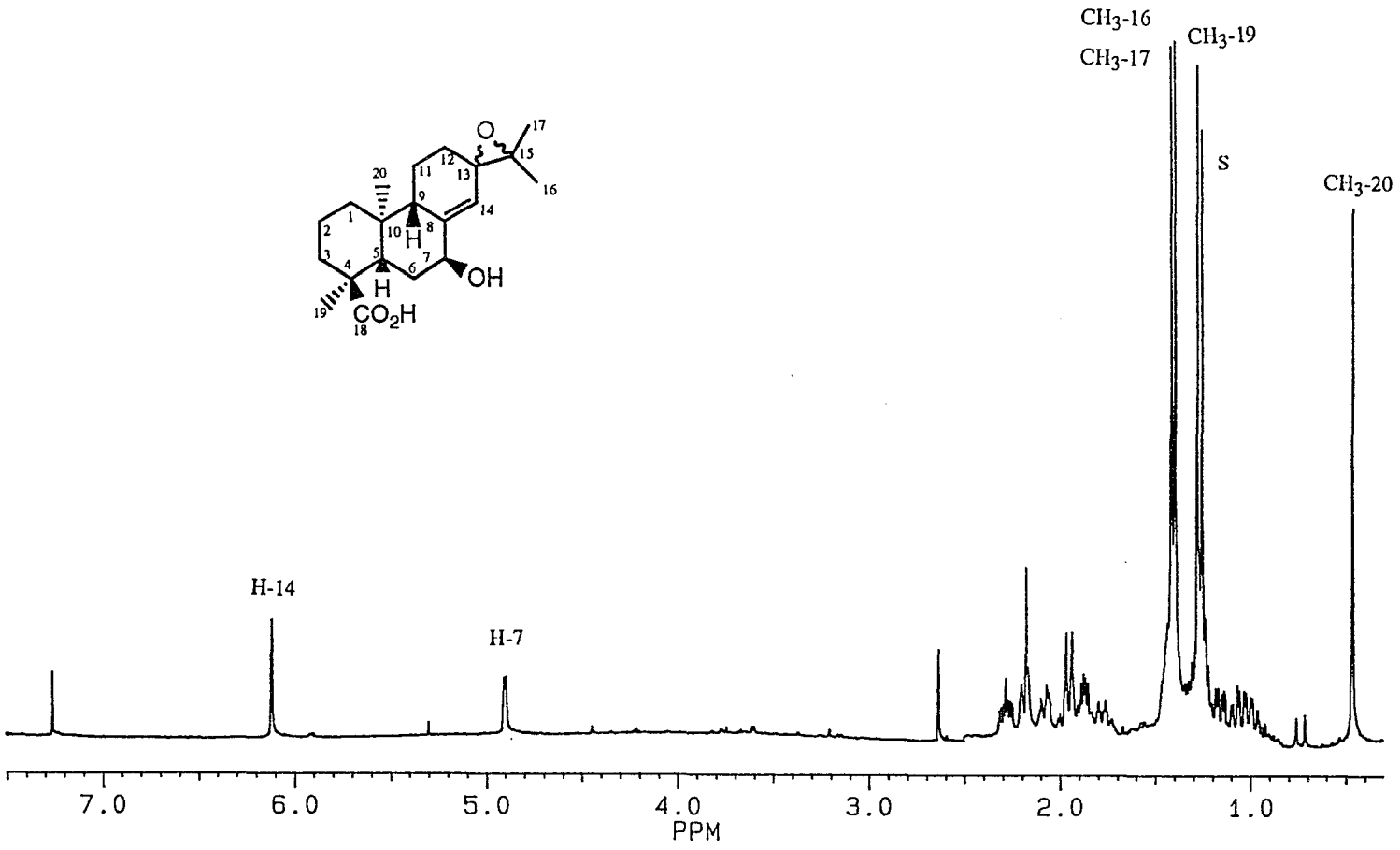


Figure 2.2.10. 400 MHz ^{1}H NMR spectrum of 7-hydroxy-13,15-epoxyabieta-8(14)-en-18-oic acid (**108**)

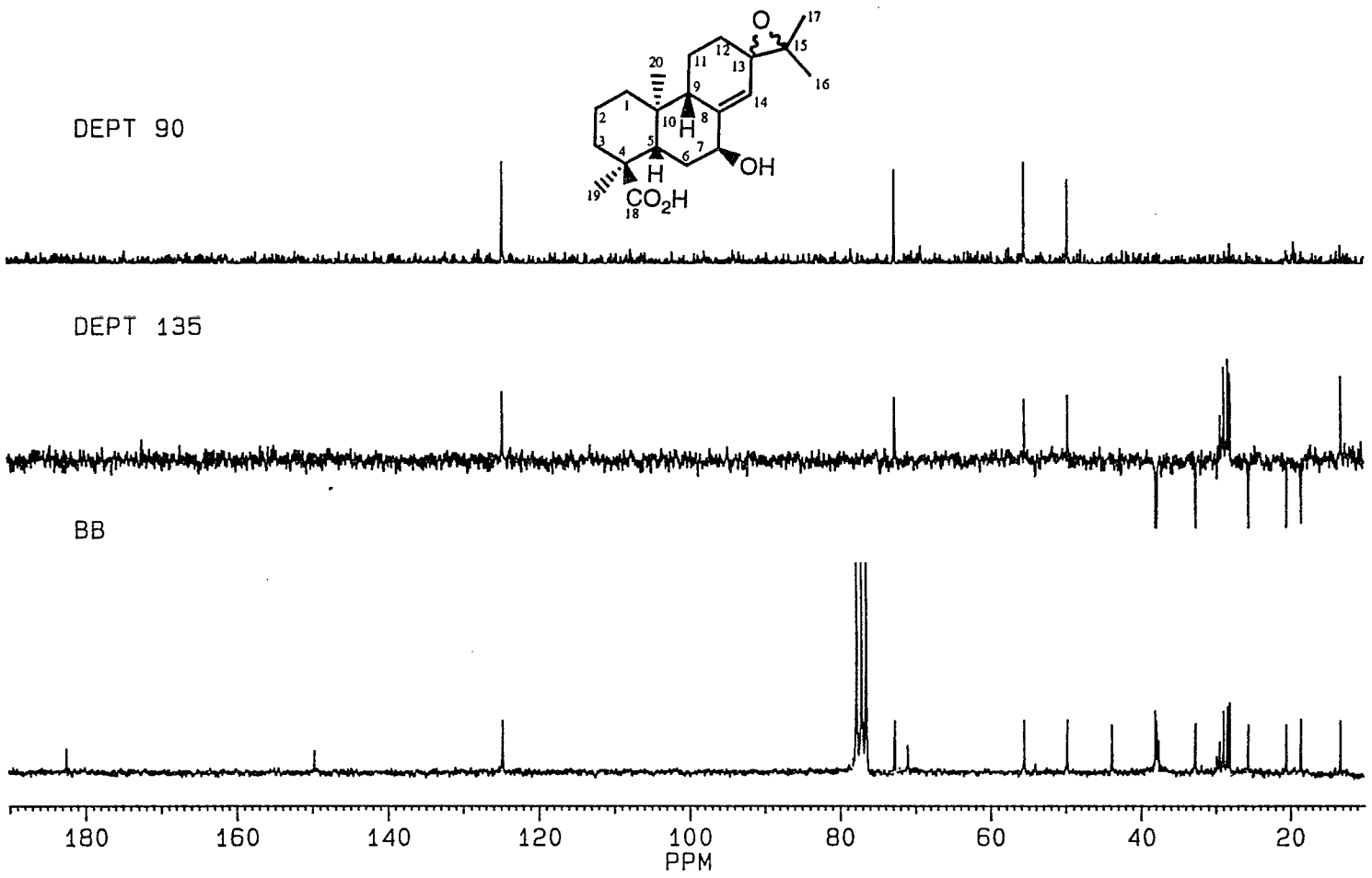


Figure 2.2.11. DEPT 90°, DEPT 135° and Broad Band ^{13}C NMR spectra of 7-hydroxy-13,15-epoxyabieta-8(14)-en-18-oic acid (**108**)

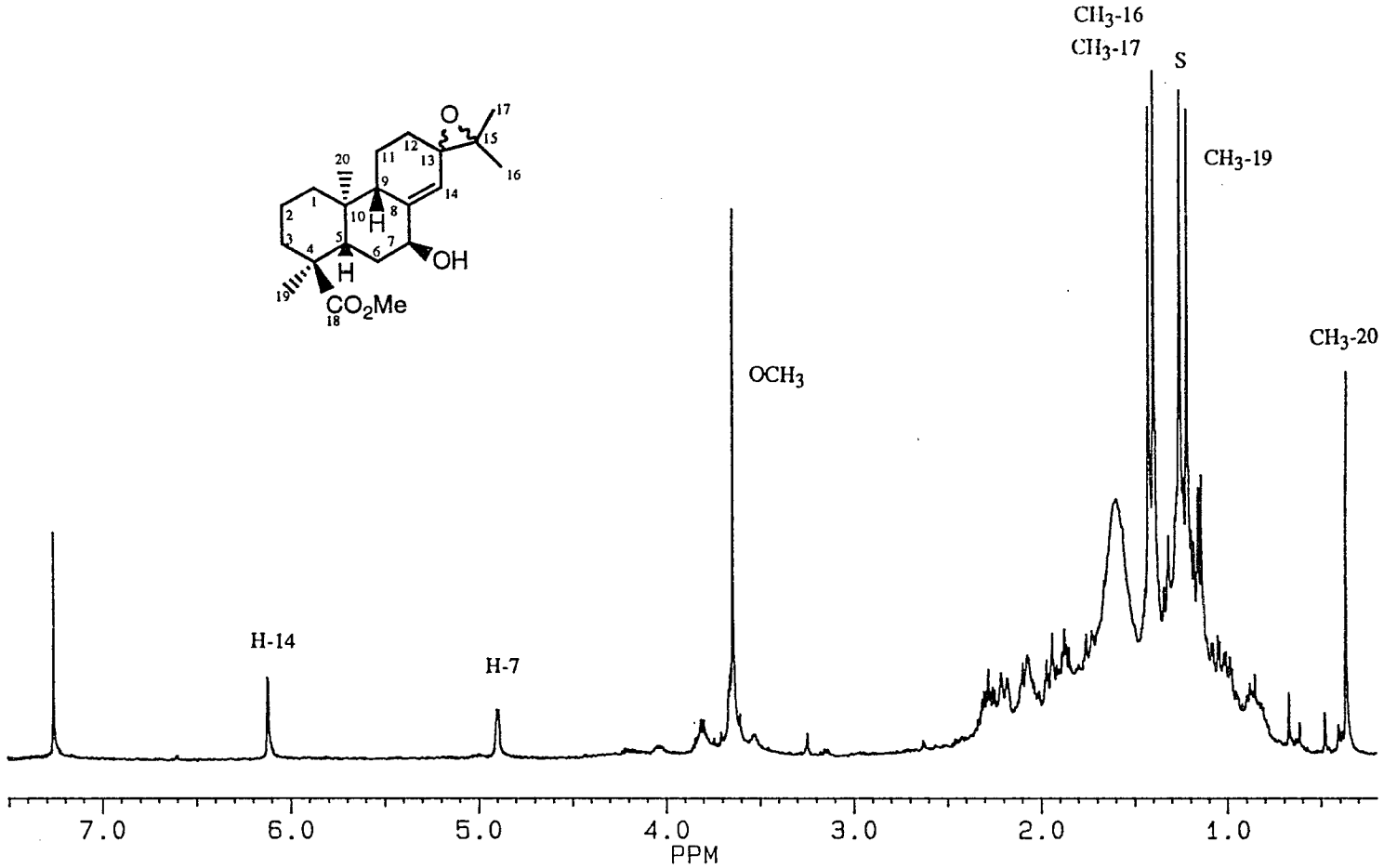


Figure 2.2.12. 400 MHz ^{1}H NMR spectrum of 7-hydroxy-13,15-epoxyabieta-8(14)-en-18-oic acid methyl ester (**108a**)

information together with comparison to other analogs from *Solidago* species [46, 47] suggested that **108** was a 7-hydroxy-8(14)-ene abietic acid with additional functional groups. Instead of the isopropyl group in most other abietanes, the two down field methyl singlets at δ 1.40 and 1.42 (Me-16, Me-17), along with two oxygenated quaternary carbon signals at δ 70.8 and δ 76.7, indicated that either an epoxide or two hydroxyl groups could be at positions 13 and 15. The FAB mass spectrum of the methyl ester **108a** showed prominent peaks at m/z 347 $[M-H]^+$, 331 $[MH-H_2O]^+$ and 329 $[347-H_2O]^+$ indicating that it was an epoxide rather than a diol. The stereochemistry at C-13 still remains open. Inspection of a stereo-model of **108** suggested that the OH-7 should be β-oriented (axial position) for H-7 only showed small couplings (J = 2.1, 4.2 Hz) to the two neighboring protons at C-6. The ^{13}C NMR spectrum of **108** was assigned by DEPT, COSY and $^1H,^{13}C$-correlation methods (Table 2.2.3).

The ^1H NMR and mass spectral data of **109** were very similar to the reported data of 15-hydroxydehydroabietic acid previously isolated from *Cedrus deodara* [140]. However, based on biogenetic consideration, **109** should also belong to the same enantiomeric series as the other abietanes from *Soligago rugosa* and related *Solidago* species [27, 46]. Therefore, the stereostructure of **109** was assigned as *ent*-15-hydroxydehydroabietic acid.

Based on the mass spectral analysis as well as IR, ^1H and ^{13}C NMR data, especially COSY and $^1H,^{13}C$-correlations, compound **112** was identified as (+)-manool[132]. It had been previously found in *Juniperus pseudosabina* [133] and *Denekia capensis* [134]. (+)-3β-Hydroxymanool (**113**), previously isolated from *J. pseudosabina* [133] and *Gleichenia japonica* [135], exhibited ^1H NMR data essentially identical with those reported before [133]. Acetylation products monoacetate **113a** and diacetate **113b** confirmed the presence of two hydroxyl groups in **113**. The ^{13}C NMR spectra of **113** and its acetates **113a** and **113b** were assigned by the DEPT, $^1H,^{13}C$-correlation

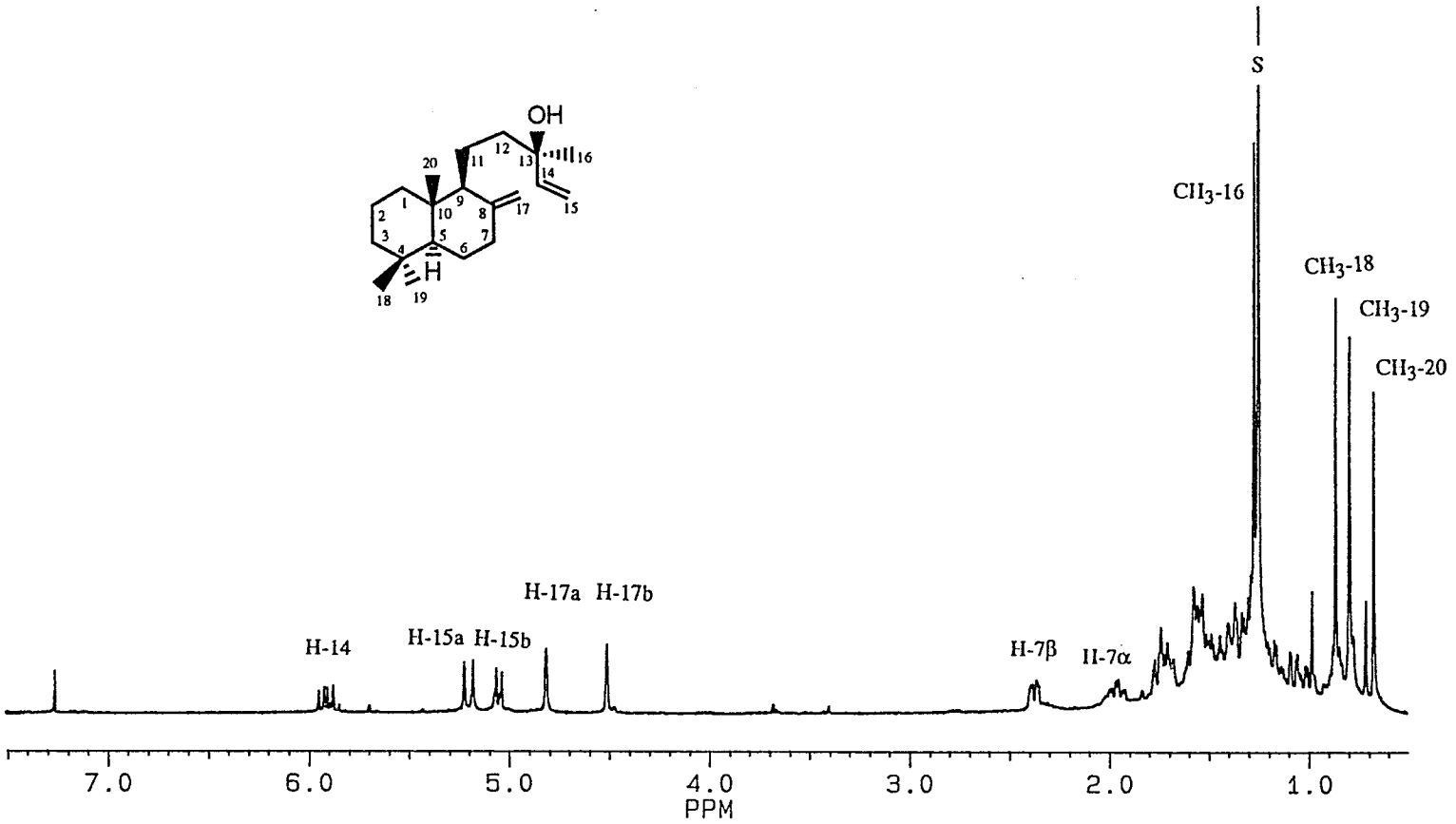


Figure 2.2.13. 400 MHz ^{1}H NMR spectrum of (+)-manool (**112**)

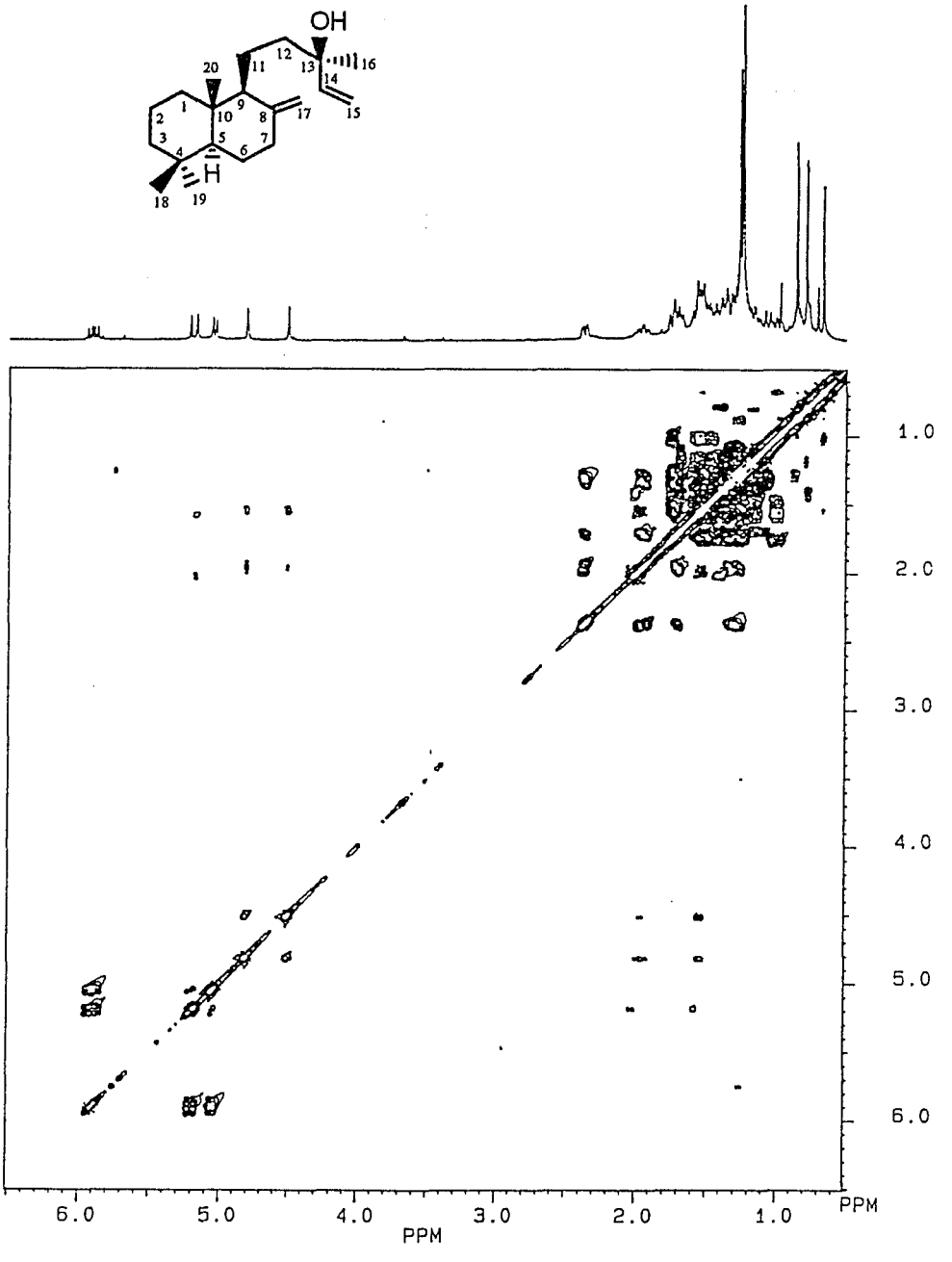


Figure 2.2.14. 400 MHz 2D ^{1}H NMR COSY spectrum of (+)-manool (**112**)

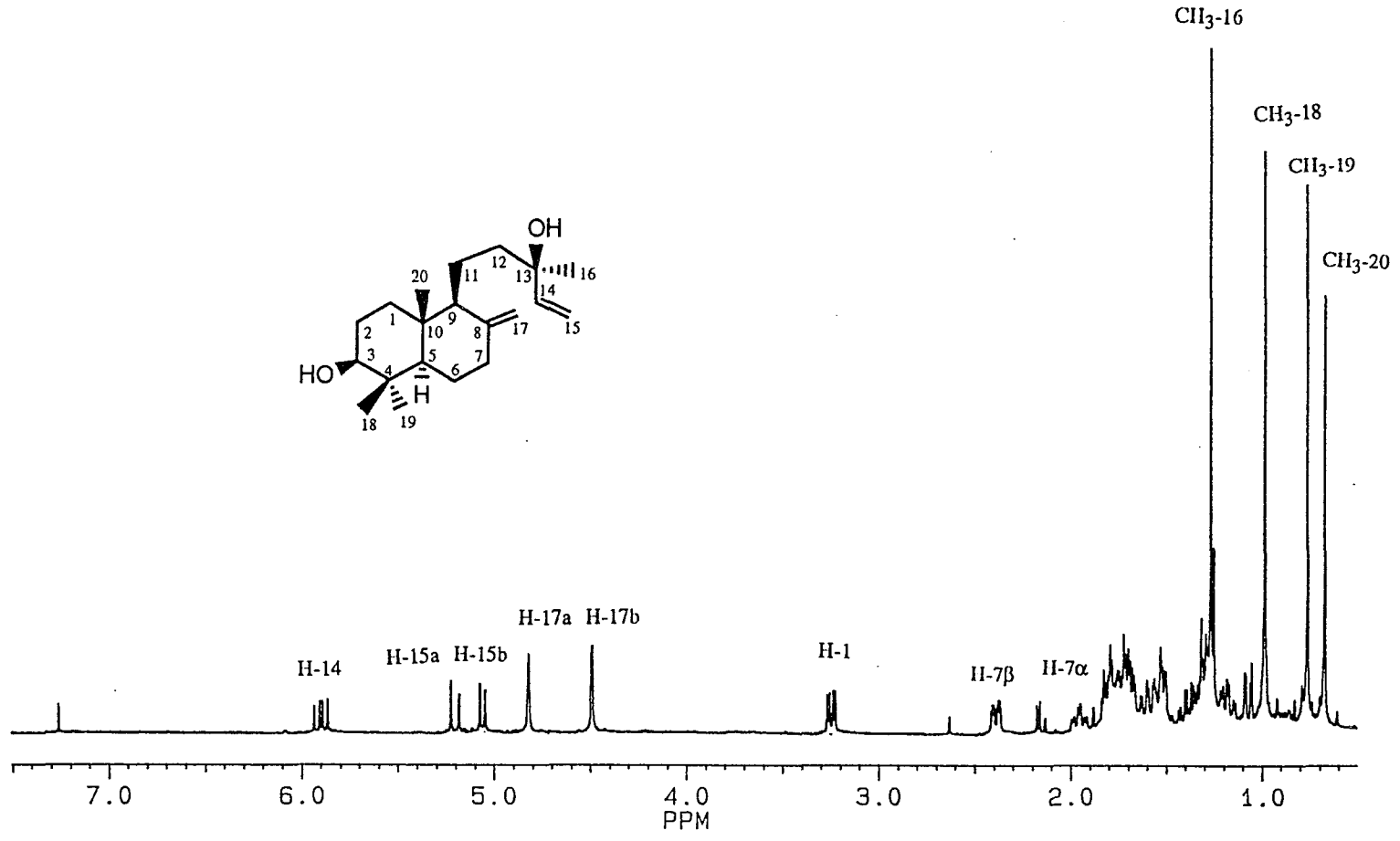


Figure 2.2.15. 400 MHz ^{1}H NMR spectrum of (+)-3β-hydroxymanool (**113**)

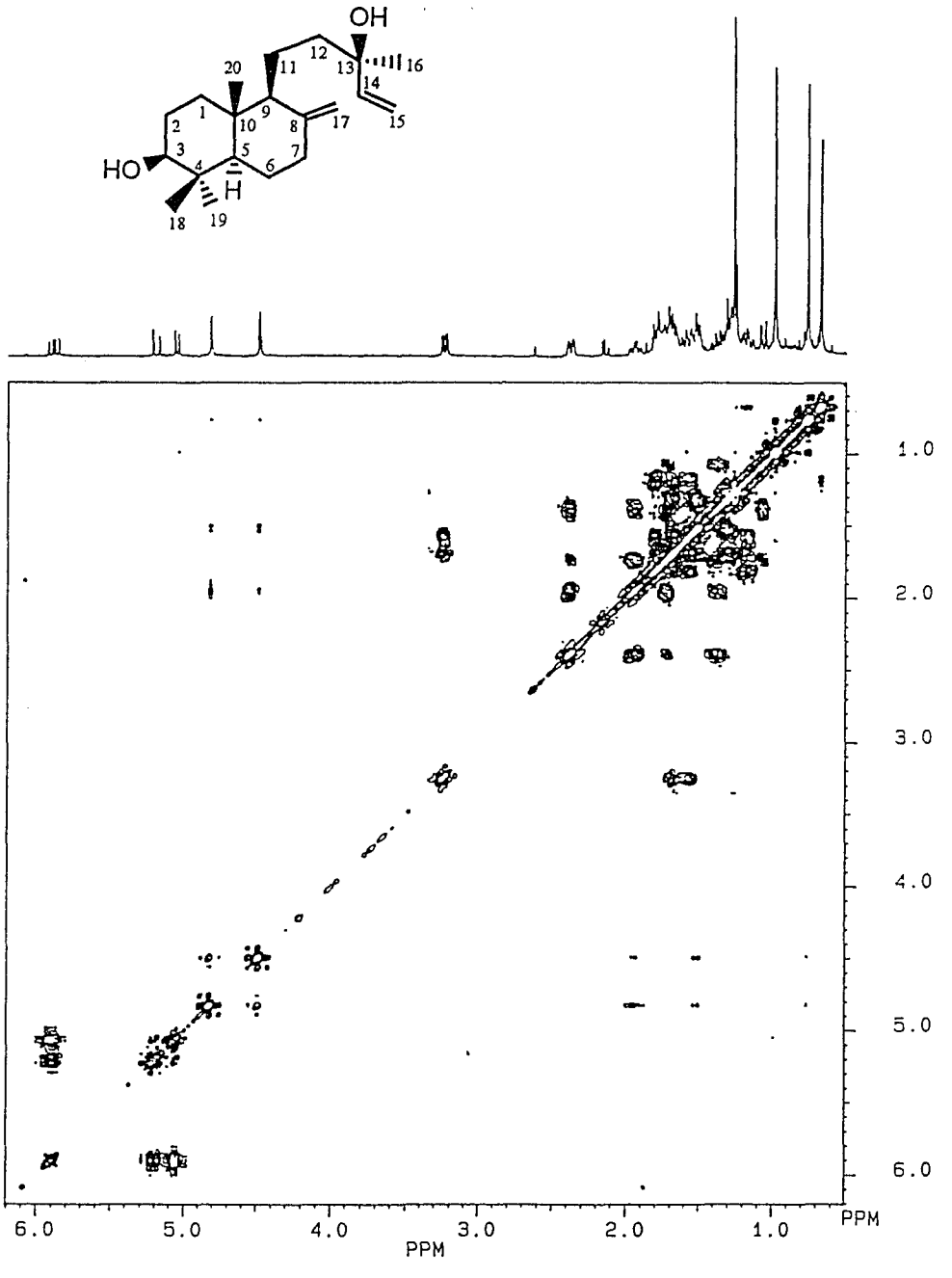


Figure 2.2.16. 400 MHz 2D ^{1}H NMR COSY spectrum of (+)-3β-hydroxymanool (**113**)

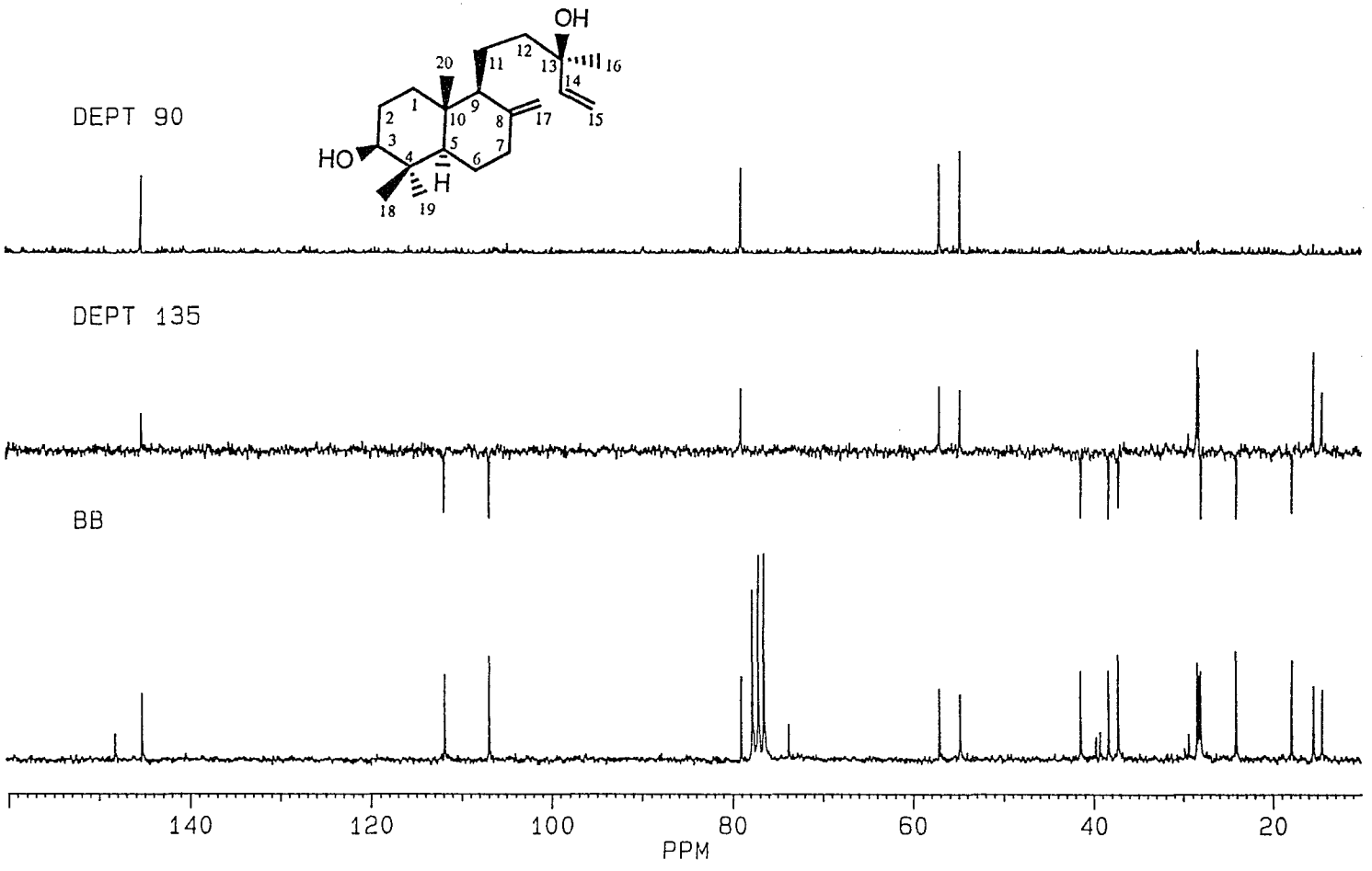


Figure 2.2.17. DEPT 90°, DEPT 135° and Broad Band ^{13}C NMR spectra of (+)-3β-hydroxymanool (**113**)

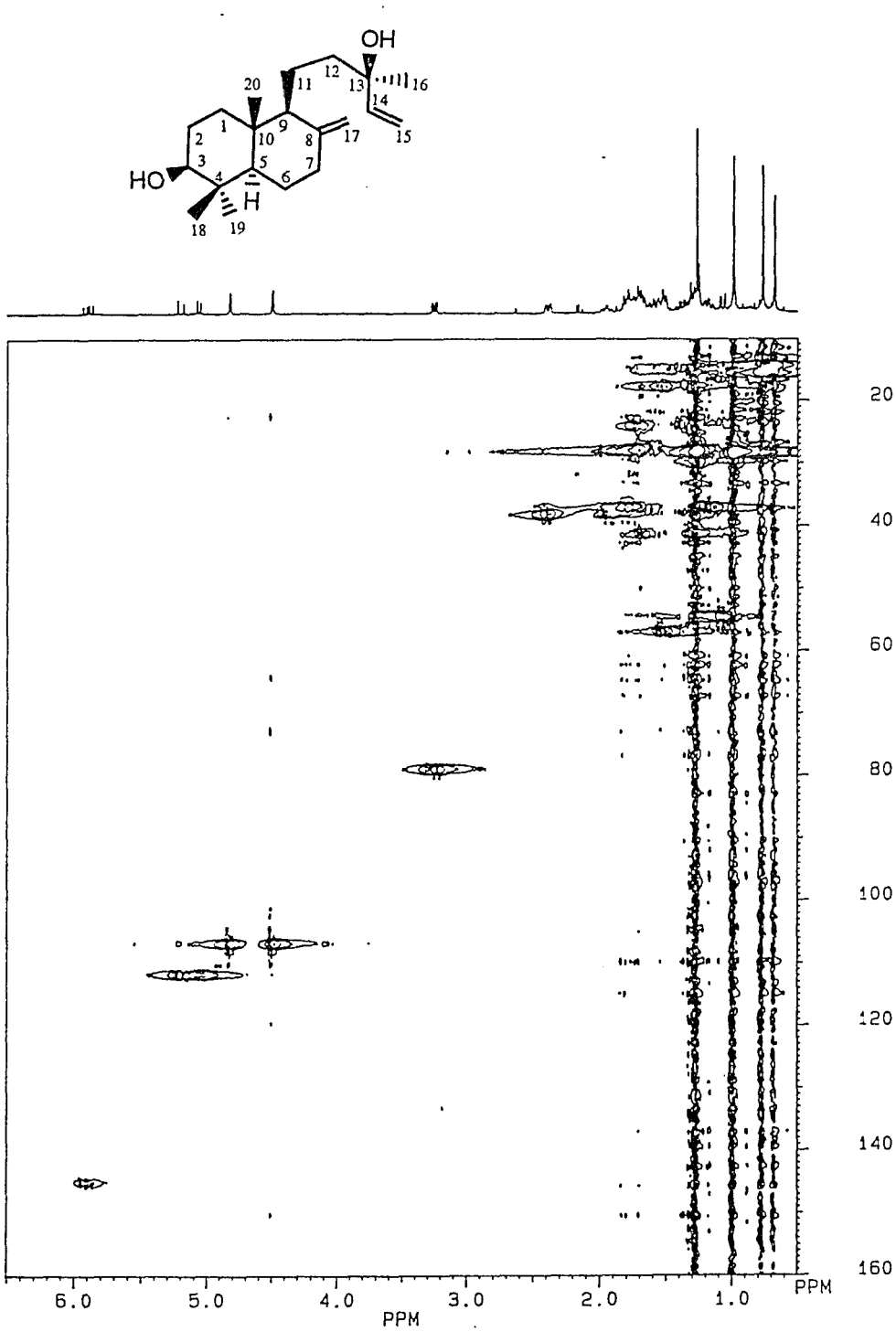


Figure 2.2.18. 2D Inverse ^{1}H-^{13}C heteronuclear correlation spectrum of (+)-3β-hydroxymanool (**113**)

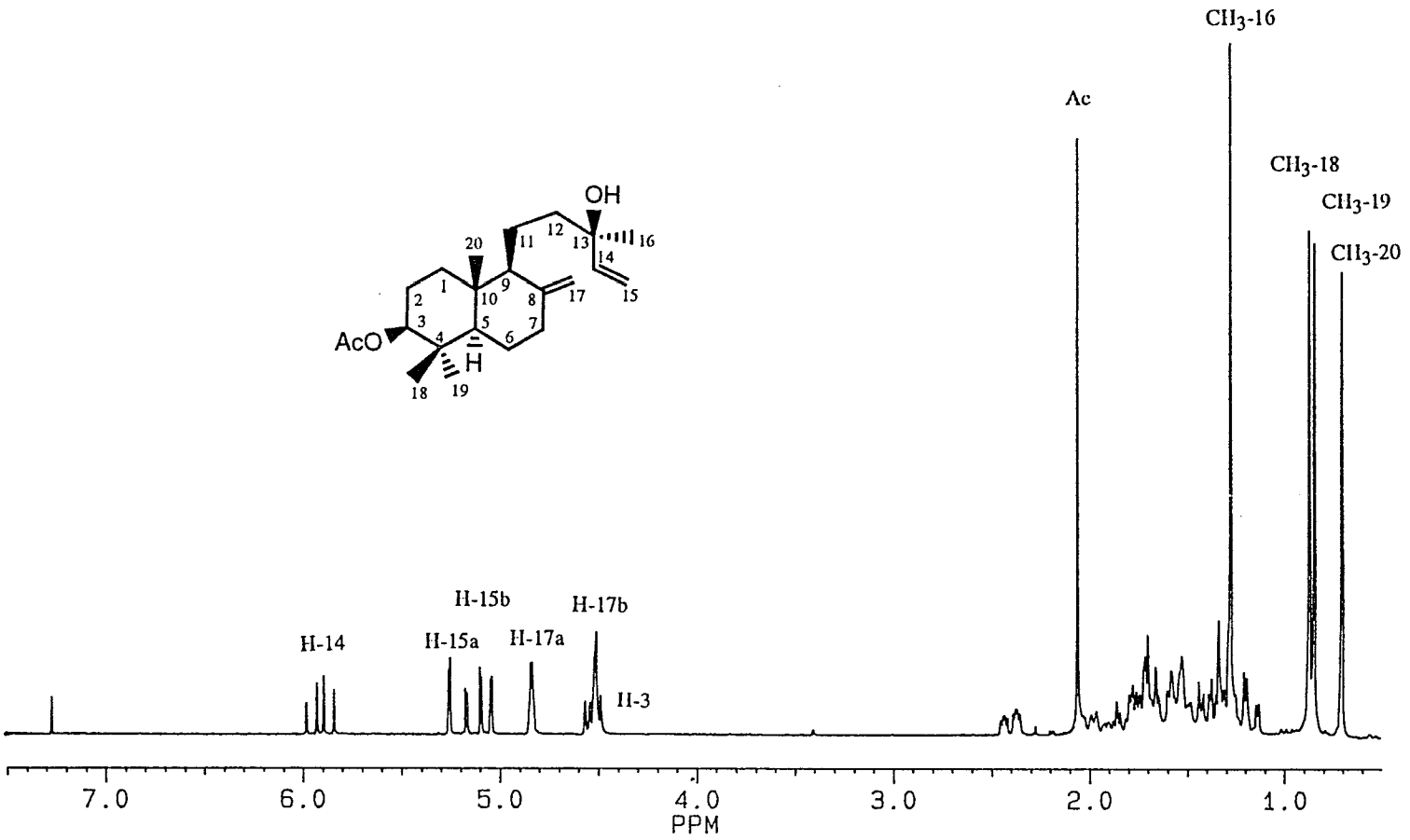


Figure 2.2.19. 400 MHz ^{1}H NMR spectrum of (+)-3β-acetoxymanool (**113a**)

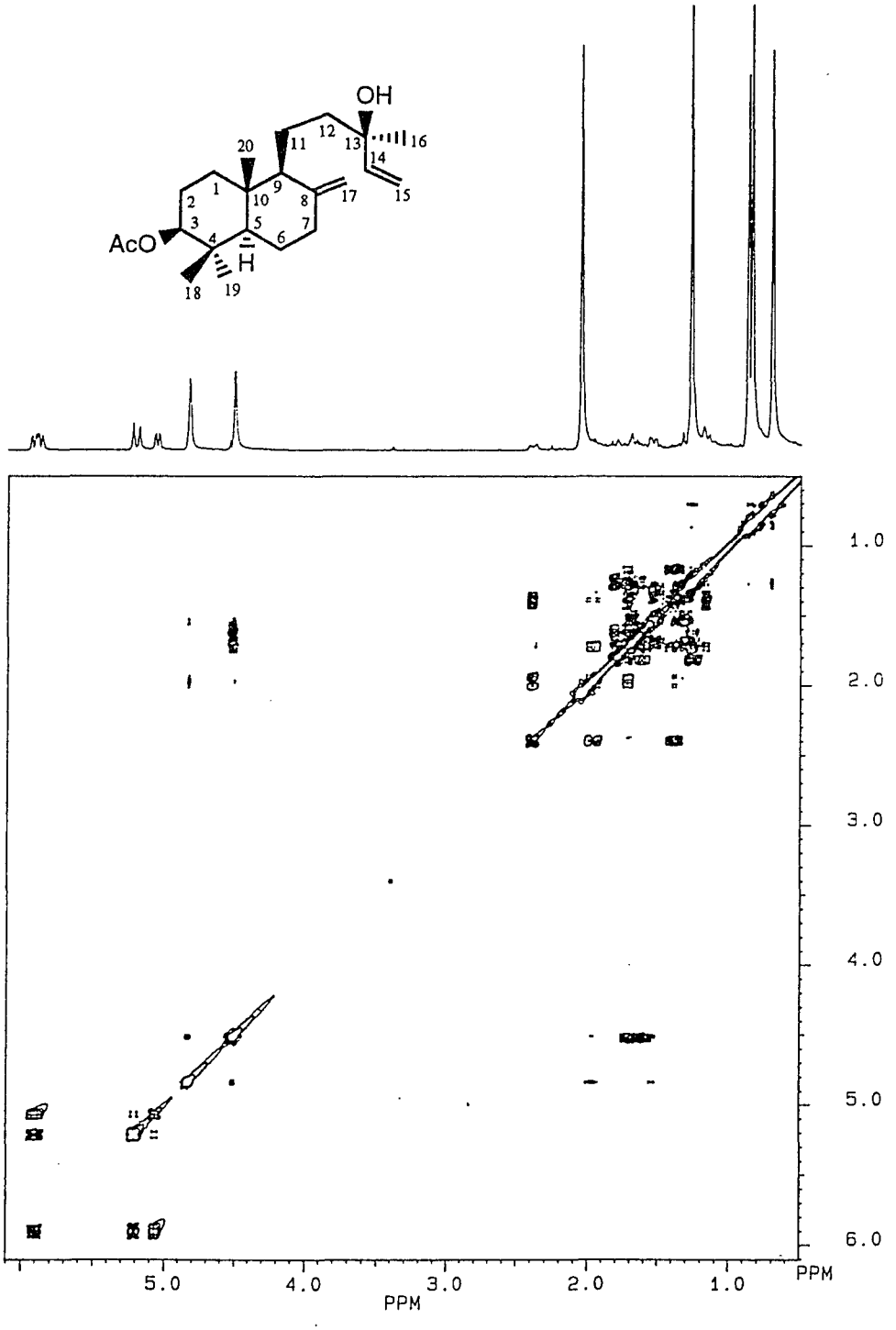


Figure 2.2.20. 400 MHz 2D ^{1}H NMR COSY spectrum of (+)-3β-acetoxymanool (**113a**)

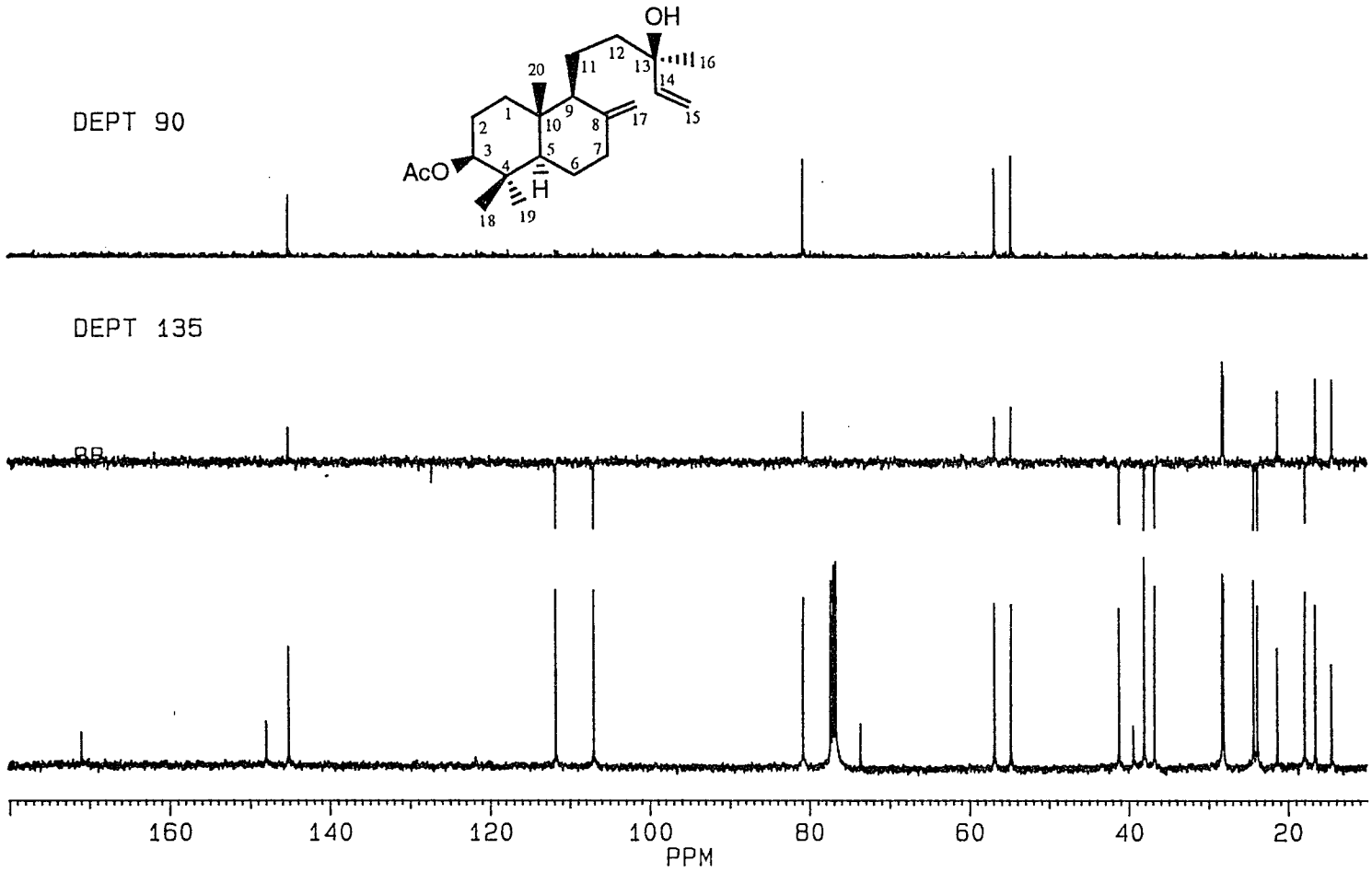


Figure 2.2.21. DEPT 90°, DEPT 135° and Broad Band ^{13}C NMR spectra of (+)-3β-acetoxymanool (**113a**)

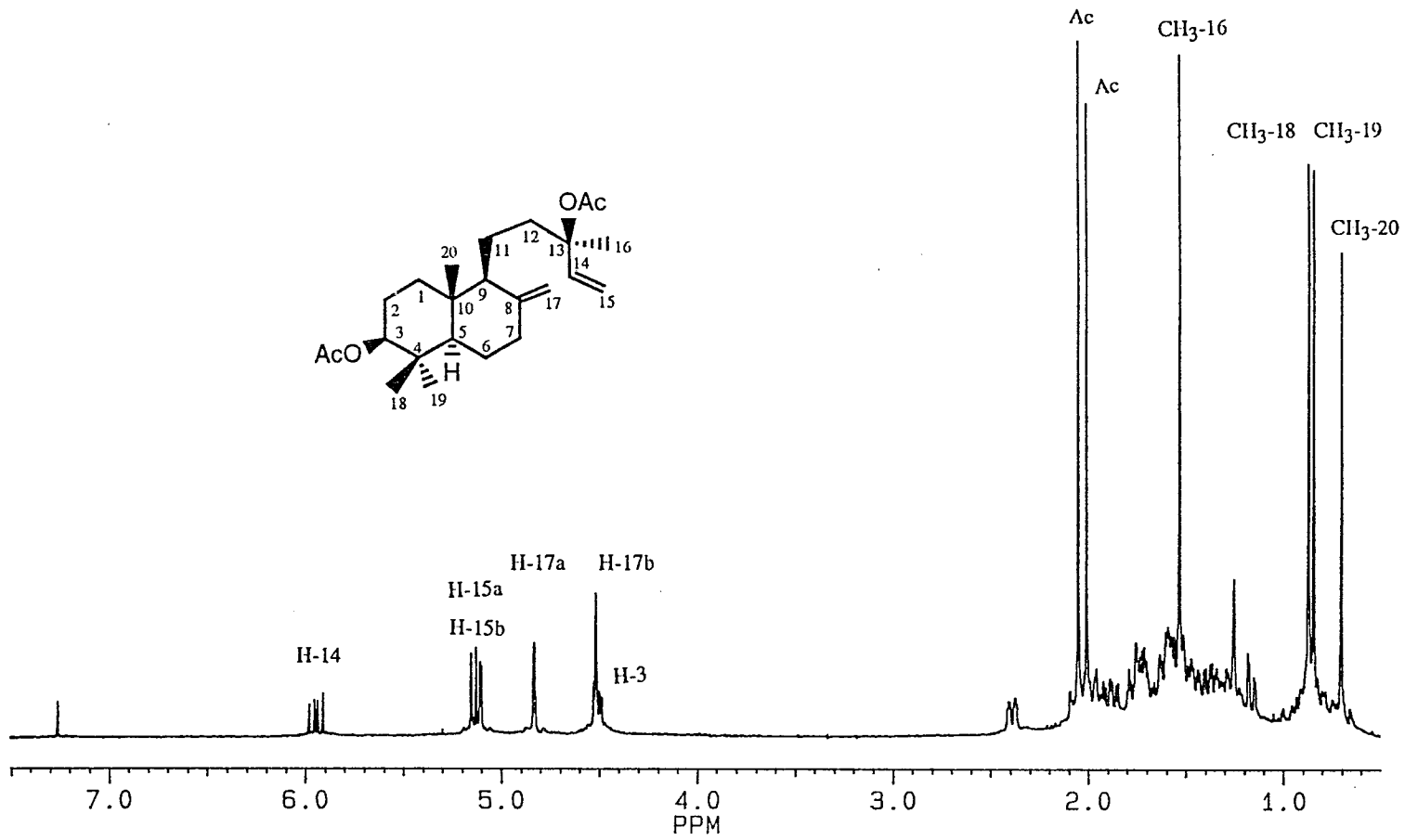


Figure 2.2.22. 400 MHz ^{1}H NMR spectrum of (+)-3β,13-diacetoxymanool (**113b**)

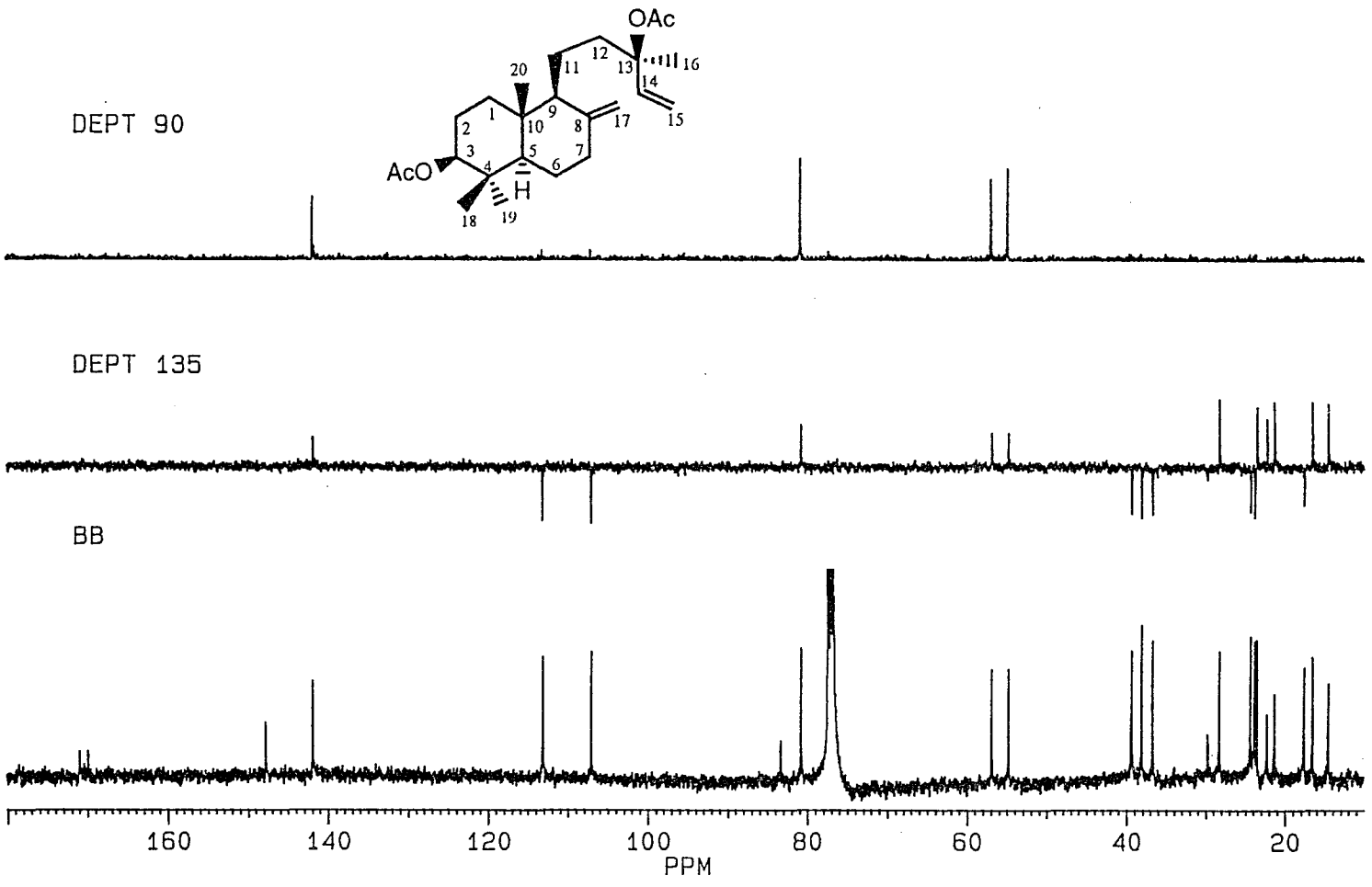


Figure 2.2.23. DEPT 90°, DEPT 135° and Boad Band ^{13}C NMR spectra of (+)-3β,13-diacetoxymanool (**113b**)

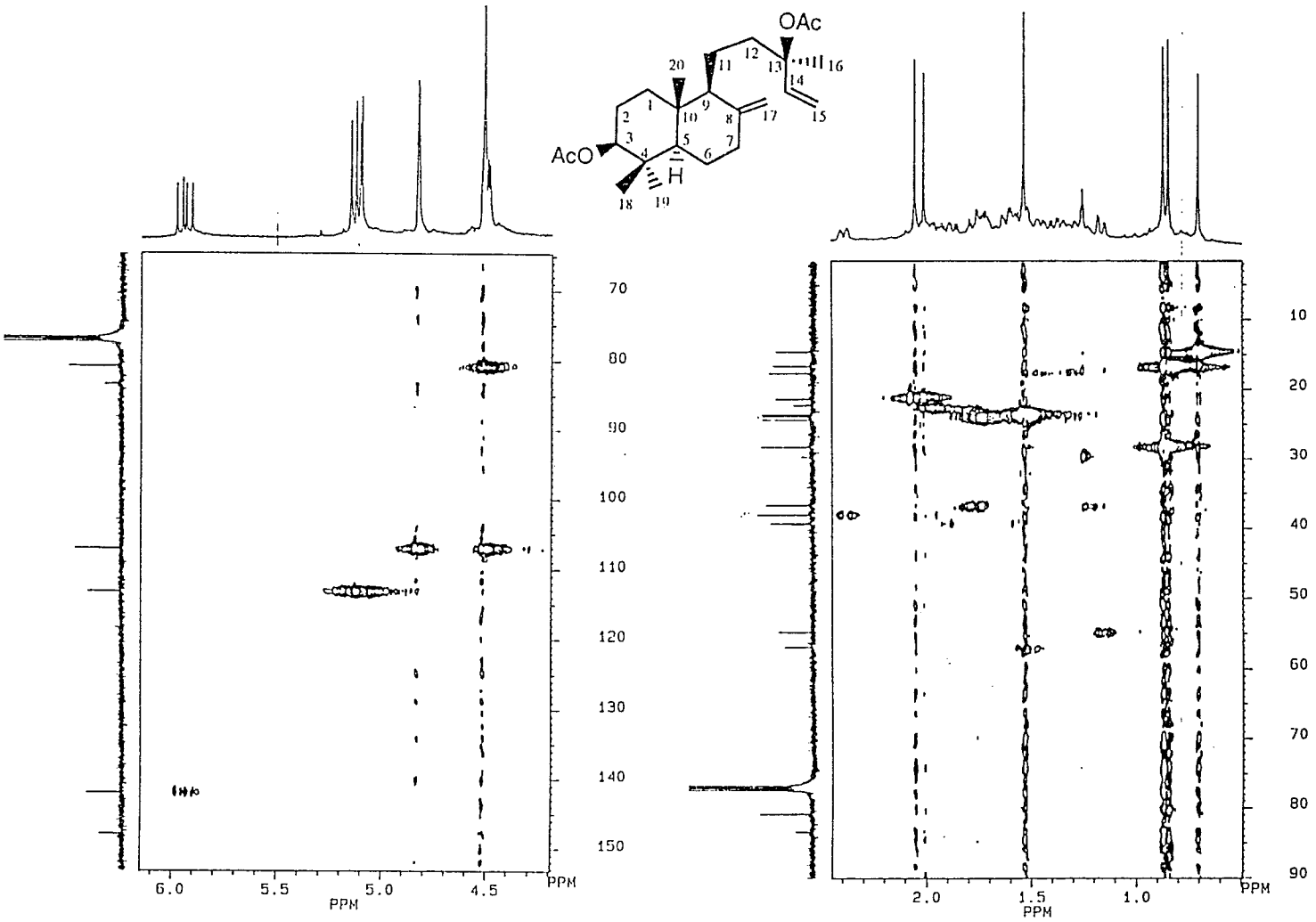


Figure 2.2.24. 2D Inverse ^{1}H-^{13}C heteronuclear correlation spectrum of (+)-3β,13-diacetoxymanool (**113b**)

methods (Table 2.2.3). The structure of manoyl oxide (**110a**) [136] was derived by mass spectral analysis and direct comparison with previously reported ^{1}H NMR data[46].

Compound **114** is a new labdane diterpene. Compared with the ^{1}H NMR data of **112**, the spectrum of **114** clearly indicated the presence of a tiglate moiety, as indicated by a diagnostic one-proton quartet of a quartet at δ 6.82 which was coupled to two broad methyl doublets at δ 1.78 and 1.82. This was further confirmed by its mass spectral data with a prominent peak at m/z 370 $[M-H_2O]^+$, the peaks at m/z 288 and 270, being derived from the loss of tiglic acid $[M-100]^+$ from the parent ion (m/z 388) and m/z 370, respectively. Strong peaks at m/z 83 and 55 further supported the presence of a tiglate side chain. Close inspection of the ^{1}H NMR spectrum of **114** revealed that the tiglate moiety was either attached to C-18 or C-19 (both at C-4) for it only showed five methyl signals in the molecule, two of which belonging to the tiglate group and other two being due to Me-20 (δ 0.70) and Me-16 (δ 1.27). There were two geminally coupled proton doublets at δ 3.88 and 4.28, as shown by COSY and ^{1}H,^{13}C-correlation experiments. Their association with the tiglate ester group was further confirmed by 2D inverse long range ^{1}H,^{13}C-correlation experiment [137], which showed that the two proton signals at δ 3.88 and 4.28 clearly coupled to the tiglate carbonyl signal at δ 168.6. NOESY experiments revealed that these two proton doublets showed NOE's with Me-20 (δ 0.70) while the methyl signal at δ 0.99 (C-19) exhibited no NOE with Me-20, indicating that these two methyls were of opposite orientation on the *trans*-decaline ring. Thus the tiglate side chain must be attached to C-18. The ^{13}C NMR of **114** indicated the presence of 25 carbons with five CH_3, ten CH_2 including two olefinic and one oxygenated carbon, four CH including two olefinic carbons, and six quaternary carbons including one oxygenated, one carbonyl and two olefinic carbons. The total assignments of these carbons were made by DEPT, HOMO- and HETERO-nuclear correlation and inverse long-range ^{1}H,^{13}C-correlation methods.

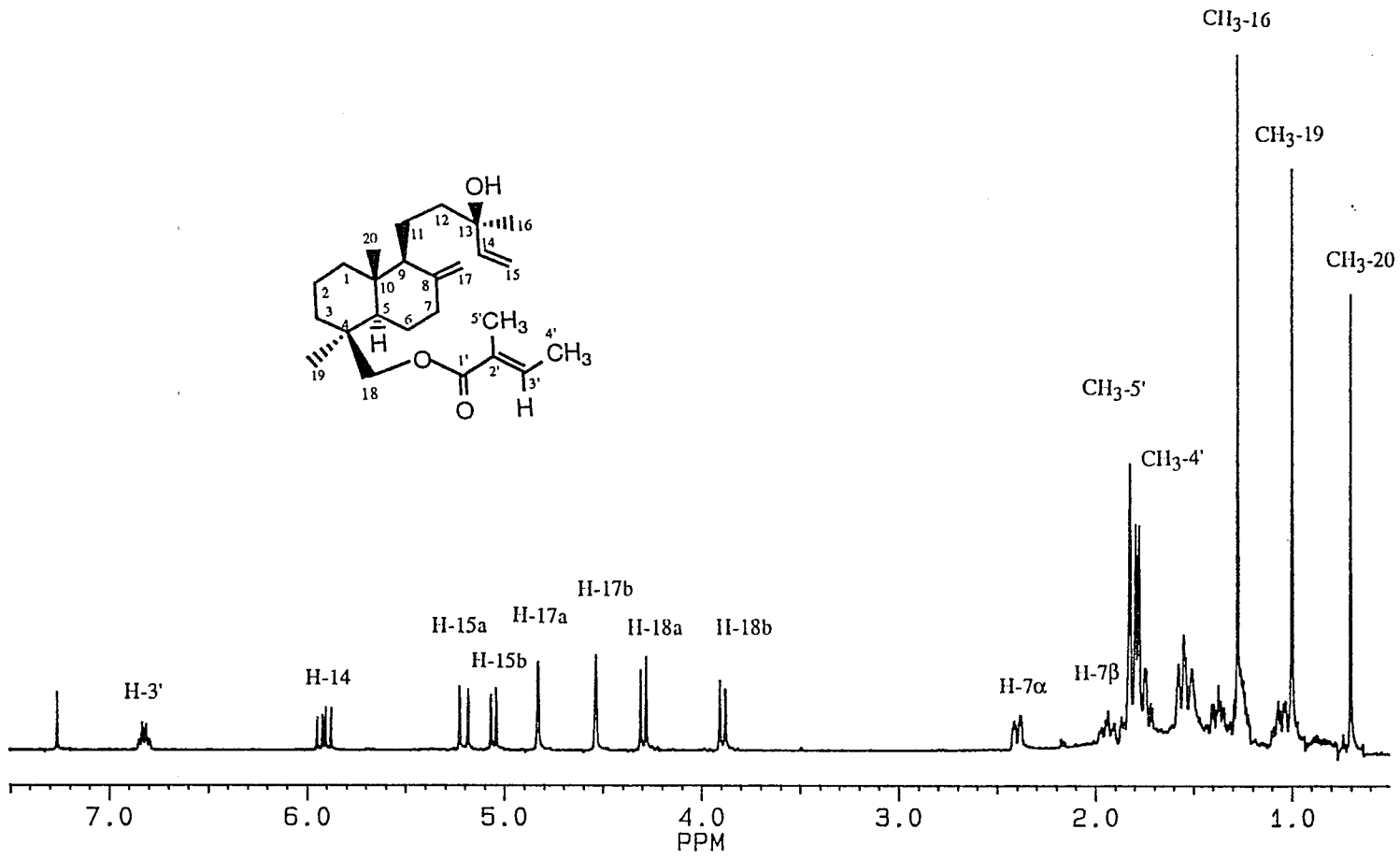


Figure 2.2.25. 400 MHz ^{1}H NMR spectrum of (+)-18-tigloyloxymanool (**114**)

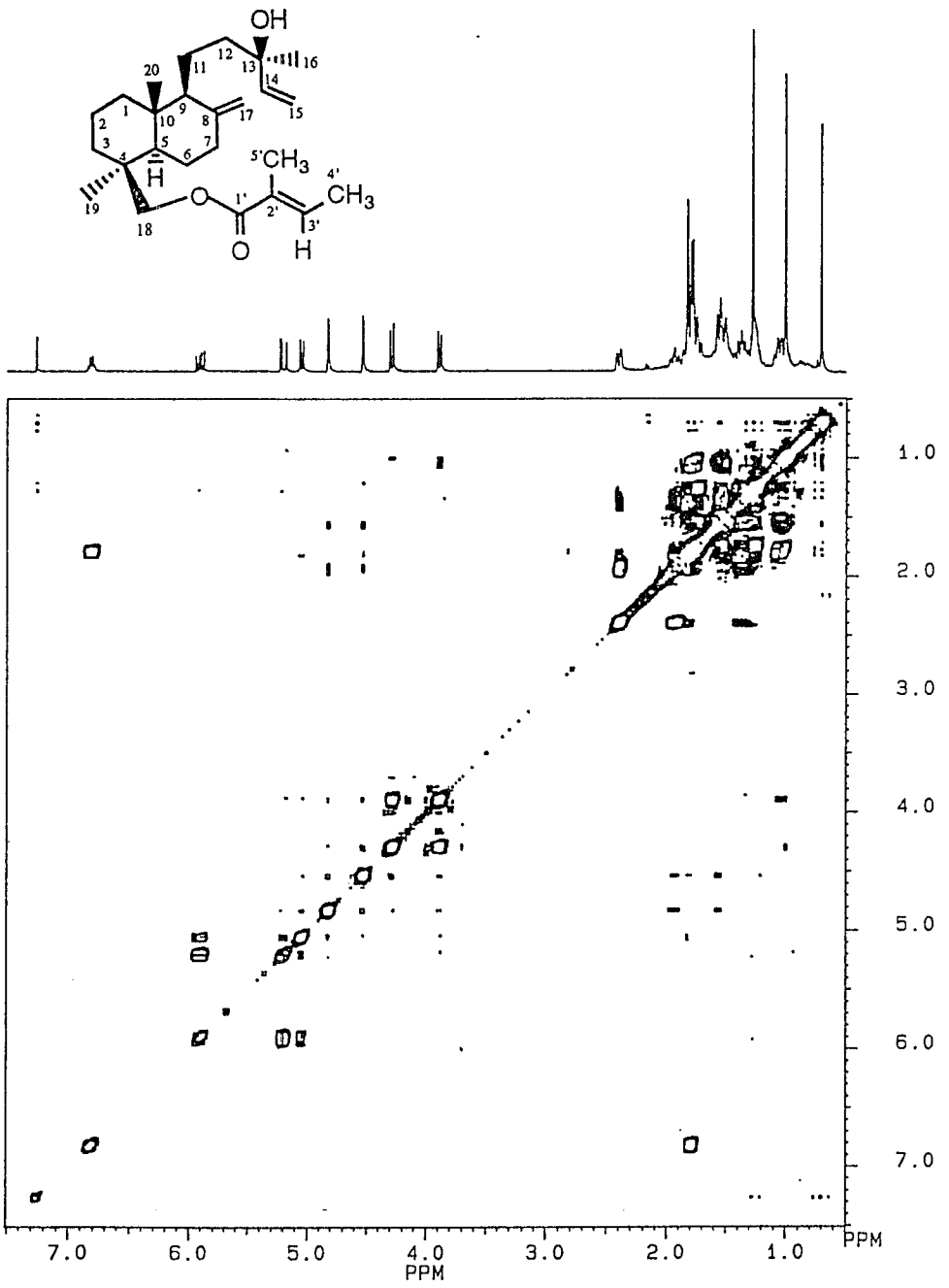


Figure 2.2.26. 400 MHz 2D ^{1}H NMR COSY spectrum of (+)-18-tigloyloxymanool (**114**)

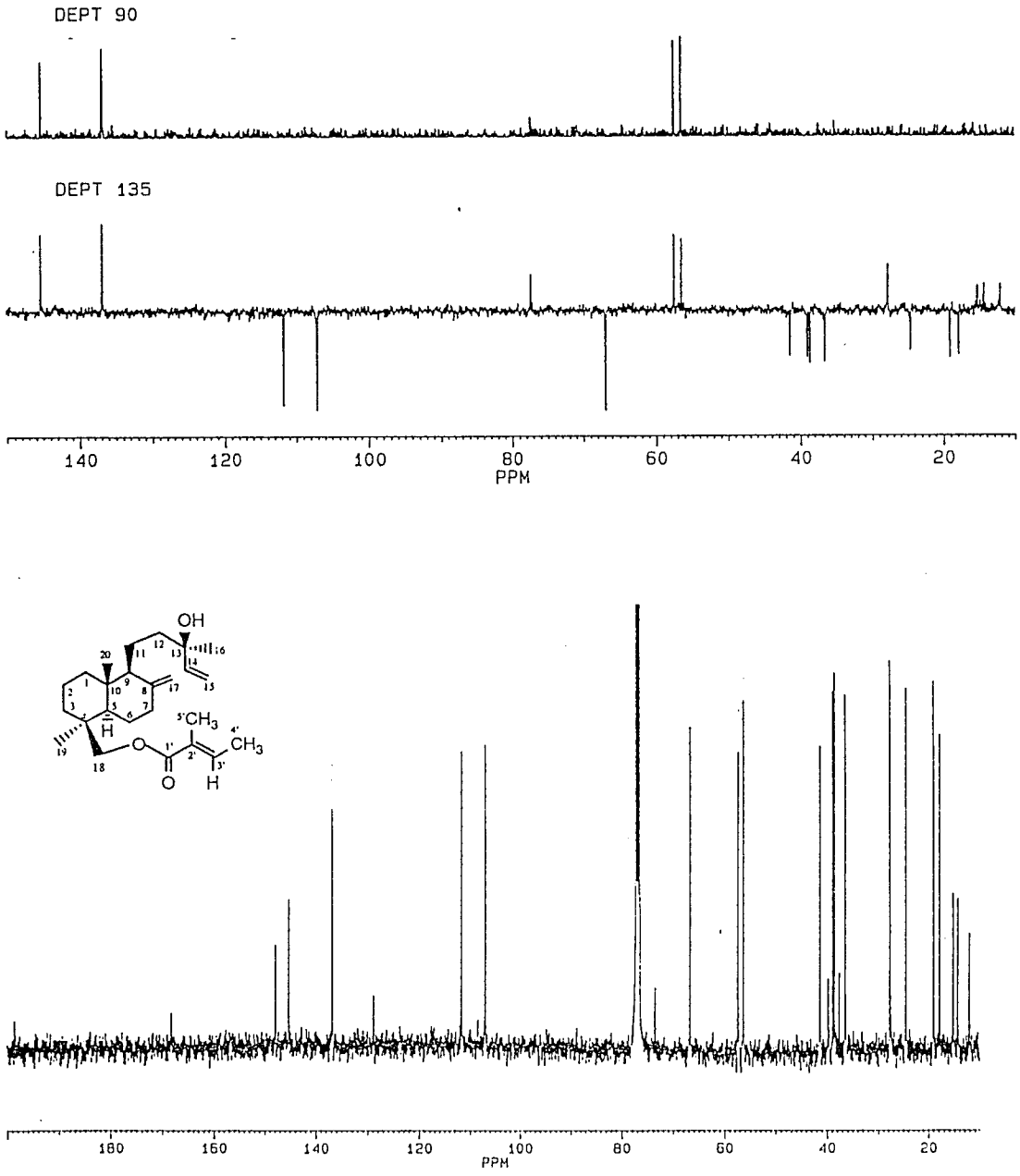


Figure 2.2.27. DEPT 90°, DEPT 135° and ^{13}C NMR spectra of (+)-18-tigloyloxymanool (**114**)

Experimental

General. ^1H and ^{13}C NMR: $CDCl_3$, Bruker AM 400 or Bruker AC 200 spectrometer; IR: film on KBr plates; MS: Hewlett-Packard 5971A GC-MS or TSQ70 FAB mass spectrometer; VLC: silica gel (MN Kieselgel G); prep. TLC: precoated MN Sil-G 25 UV_{254} plates (thickness 0.25 mm); semiprep. HPLC: 10μ C18 reversed-phase column (250x10 mm, AllTech) coupled to a LDC/Milton Roy CM 4000 multi-solvent delivery system and an ISCO UV detector using a detection wavelength at 230 nm. *Plant material.* Roots and aerial parts of *Solidago rugosa* Mill. were collected on 19 October, 1991, in Washington Parish, Louisiana, U.S.A. (voucher No. Fischer-Lu 432; voucher deposited at the Louisiana State University Herbarium).

Extraction and isolation. Freshly dried roots (1.1 kg), flowers (380 g) and stems and leaves (900 g) were soaked separately at r.t. in CH_2Cl_2 for 24 hr, yielding 19 g, 14 g and 18 g crude extracts, respectively. About 7 g of the crude root extract was chromatographed by VLC yielding 10x150 ml frs. Further separation of fr. 5 by semiprep. HPLC using CH_3CN-H_2O (17:3) as mobile phase afforded 4 mg of kolavenol (**2**), 27 mg of hardwickiic acid (**63**), 5 mg of **114** and 3 mg of **106**. VLC separation of the crude flower extract (7 g) yielded 20x100 ml fractions. Repeated prep. TLC (hexane-CH_2Cl_2, 9:1) of fr. 2 afforded 2 mg of manoyl oxide (**110a**) and 1 mg of **107**. Prep. TLC (petroleum ether-CH_2Cl_2, 5:1) of fr. 3 gave further 2 mg of **107**. Further separation of fr. 4 by prep. HPLC (methanol-H_2O, 17:3) provided 4 mg of abietic acid (**104**) and 9 mg of **83**. Fr. 6 gave 1.65 g of **104** and fr. 7, after prep. HPLC (methanol-H_2O, 9:1), yielded 8 mg of **104** and 1 mg of **106**. Fr. 12 provided 370 mg of **113** while fr. 13 gave, after further repeated prep. TLC separation (hexan-acetone, 3:1), 2 mg of **108** and 2 mg of **109** Comparison by TLC (hexane-EtOAc, 4:1) and ^1H NMR of the crude extract of leaves and stems with the flower and root extracts showed that the leaves and stems contained the same terpenoid constituents as the flower and root extracts with minor quantitative differences.

18-Hydroxyabieta-7,13(14)-diene (**106**). $C_{20}H_{32}O$, yellow gum; IR ν_{max}^{KBr} cm^{-1}: 3402 (OH, br); EIMS m/z (rel. int.): 288 $[M]^+$ (100), 273 $[M-CH_3]^+$ (11.7), 257 $[M-CH_2OH]^+$ (28.5), 255 $[273-H_2O]^+$ (33.7), 105 (81.1); ^1H NMR data in Table 2.2.2.

18-Tigloyloxyabieta-7,13(14)-diene (**107**). $C_{25}H_{38}O_2$, yellow gum; IR ν_{max}^{KBr} cm^{-1}: 1717 (C=O), 1265 (conj. ester); EIMS m/z (rel. int.): 370 $[M]^+$ (26.5), 287 $[M-83]^+$ (36.4), 270 $[M-100]^+$ (74.4), 255 $[270-CH_3]^+$ (58.3), 227 $[270-C_3H_7]^+$ (23.0), 187 (100), 131 (58.2), 83 $[CH_3CH=C(CH_3)CO]^+$ (41.8), 55 $[83-CO]^+$ (46.8); ^1H NMR data in Table 2.2.2.

7-Hydroxy-13,15-epoxyabieta-8(14)-ene-18-oic acid (**108**). $C_{20}H_{30}O_4$, colorless powder; IR ν_{max}^{KBr} cm^{-1}: 3500-2500 (COOH, br.), 1696 (C=O); ^1H NMR data in Table 2.2.2 and ^{13}C NMR data in Table 2.2.3. Methylation reaction: *ca.* 3 mg of **108** was dissolved in 2 ml anhydrous ethyl ether. Freshly prepared CH_2N_2 from the reaction of 1-methyl-3-nitro-1-nitrosoguanidine (MNNG) with 5 M KOH solution was collected in ethyl ether and was added to the stirred solution of **108** over 0.5 hr and then left for 6 hr in the hood. After removal of the solvent *ca.* 3 mg of **108a** was obtained.

7-Hydroxy-13,15-epoxyabieta-8(14)-ene-18-oic acid methyl ester (**108a**). $C_{21}H_{32}O_4$, colorless powder; IR ν_{max}^{KBr} cm^{-1}: 3440 (OH, br), 1724 (C=O); FABMS m/z: 347 $[M-H]^+$, 331 $[MH-H_2O]^+$, 329 $[347-H_2O]^{+\cdot}$; ^1H NMR data in Table 2.2.2.

15-Hydroxydehydroabietic acid (**109**). $C_{20}H_{28}O_3$, colorless powder; IR ν_{max}^{KBr} cm^{-1}: 3500-2500 (CO_2H, br), 1699 (C=O); EIMS m/z (rel int.): 316 $[M]^+$ (12.2), 301 $[M-CH_3]^+$ (100), 298 $[M-H_2O]^+$ (15.0), 283 $[301-H_2O]^+$ (45.7), 237 $[283-CO_2H-H]^+$ (64.0), 197 (31.4), 181 (36.4), 43 (63.8); ^1H NMR data in Table 2.2.2.

(+)-Manool (**112**). $C_{20}H_{34}O$, colorless gum; IR ν_{max}^{KBr} cm^{-1}: 3431 (OH, br.), 1633, 1461 (C=C); EIMS m/z (rel. int.): 290 $[M]^+$ (0.2), 272 $[M-H_2O]^+$ (14.0), 257 $[272-CH_3]^+$ (49.0), 137(100), 95(77), 81(80); ^1H NMR data in Table 2.2.1 and ^{13}C NMR data in Table 2.2.3.

(+)-3β-Hydroxymanool (**113**). $C_{20}H_{34}O_2$, colorless oil; IR ν_{max}^{KBr} cm^{-1}: 3402 (OH, br), 1645, 1457 (C=C); EIMS m/z (rel int.): 306 $[M]^+$ (0.1), 288 $[M-H_2O]^+$ (2.8), 273 $[288-CH_3]^+$ (10.6), 255 $[273-H_2O]^+$ (13.1), 135 (100), 43 (68.7); ^1H NMR data in Table 2.2.1 and ^{13}C NMR data in Table 2.2.3. Acetylation of **113**: compound **113** (41 mg) was stirred with Ac_2O/4-dimethylaminopyridine for 16 hr. Work up with 5 ml distilled water, acidified with 2N HCl, extracted with CH_2Cl_2, gave 46 mg of crude extract. Further separation by VLC using hexane/Me_2CO by increasing polarity afforded 14 mg of monoacetate **113a** and 5 mg of diacetate **113b**; R_f on TLC (hexane-Me_2CO, 4:1): **113a**: 0.57, **113b**: 0.78.

(+)-3β-Acetoxymanool (**113a**). $C_{22}H_{36}O_3$, colorless oil; IR ν_{max}^{KBr} cm^{-1}: 3485 (OH, br), 1734 (C=O), 1242 (CC(=O)OC, ester); EIMS m/z (rel. int.): 348 $[M]^+$ (0.1), 330 $[M-H_2O]^+$ (5.1), 315 $[330-CH_3]^+$ (7.2), 288 $[M-HOAc]^+$ (3.1), 270 $[330-HOAc]^+$ (6.3), 255 $[270-CH_3]^+$ (28.4), 135 (88.8), 43 $[Ac]^+$ (100); ^1H NMR data in Table 2.2.1 and ^{13}C NMR data in Table 2.2.3.

(+)-3β,13-Diacetoxymanool (**113b**). $C_{24}H_{38}O_4$, colorless oil; IR ν_{max}^{KBr} cm^{-1}: 1735 (C=O), 1241 (CC(=O)OC, ester); EIMS m/z (rel. int.): 330 $[M-HOAc]^+$ (0.4), 315 $[330-CH_3]^+$ (1.5), 270 $[330-HOAc]^+$ (15.5), 255 $[270-CH_3]^+$ (66.5), 135 (100), 43 $[Ac]^+$ (99.3); ^1H NMR data in Table 2.2.1 and ^{13}C NMR data in Table 2.2.3.

(+)-18-Tigloyloxymanool (**114**). $C_{25}H_{40}O_3$, colorless oil; IR ν_{max}^{KBr} cm^{-1}: 3496 (OH, br), 1708 (C=O), 1648, 1452 (C=C, conj.), 1263, 1144 (ester); EIMS m/z (rel. int.): 370 $[M-H_2O]^+$ (1.5), 355 $[370-CH_3]^+$ (1.3), 288 $[M-CH_3CH=C(CH_3)CO_2H]^+$ (1.0), 270 $[370-CH_3CH=C(CH_3)CO_2H]^+$ (12.9), 257 (21.5), 255 $[270-CH_3]^+$ (14.3), 135 (43.1), 83 $[CH_3CH=C(CH_3)CO]^+$ (100), 55 $[83-CO]^+$ (40.1); ^1H NMR data inTable 2.2.1 and ^{13}C NMR data in Table 2.2.3.

2.3

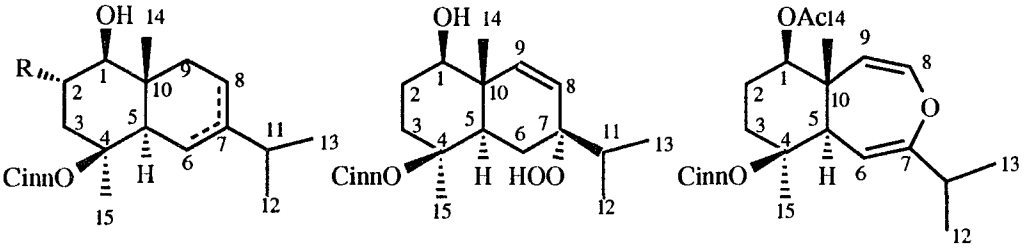
Sesquiterpenoids from *Brintonia discoidea*

Introduction

In continuation of our biochemical systematic study of the family Asteraceae combined with a search for bioactive natural products, we have investigated *Brintonia discoidea* (syn. *Solidago discoidea*), a monotypic genus of the tribe Astereae found in the Gulf Coastal Plain of the U. S. A. [130]. Members of the subtribe *Solidaginiae* of the tribe Astereae frequently produce diterpenes and acetylenic compounds [50, 117]. These were absent in the roots of *B. discoidea*, and instead cinnamate esters of eudesmane-type sesquiterpenes and a benzyl benzoate were found. All known *Brintonia* eudesmanes had been previously found in members of the genus *Verbesina* of the subtribe Verbesiniae in the tribe Heliantheae [141-144] and only one report described the presence of a eudesmane cinnamate in *S. wrightii* [76]. The benzyl 2,6-dimethoxybenzoate had been previously isolated from *S. virgaurea* [39] and *S. decurrens* [3]. The structures of the new and known compounds were elucidated by spectroscopic methods, especially by mass spectral analysis and high field ^{1}H and ^{13}C NMR, COSY and ^{1}H-^{13}C correlation methods as well as chemical transformations.

Results and Discussion

The structures of the known sesquiterpene cinnamates (**163, 165, 207-210, 213** and **216**) were established by spectral comparison with published data (^{1}H and ^{13}C NMR, MS, IR). Compounds **207-210** had been previously isolated from *V.*

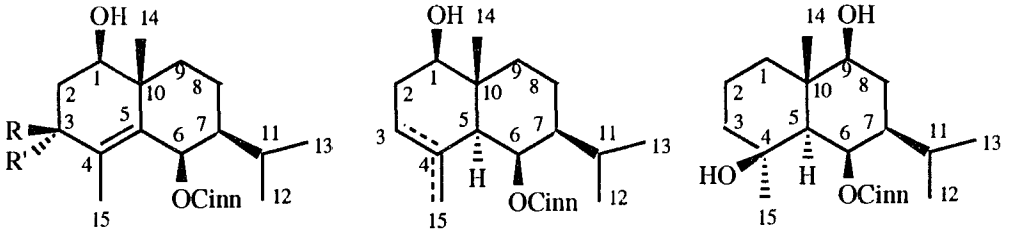


R

207 OH, 7(8)-ene
208 OH, 6(7)-ene
209 H, 7(8)-ene
210 H, 6(7)-ene

211

212

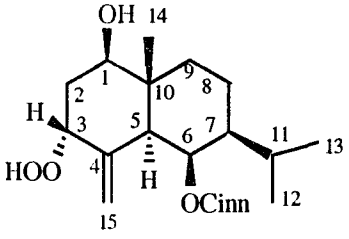


R R'

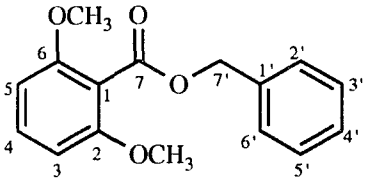
213 H OOH
214 OOH H
215 =O

163 3(4)-ene
165 4(15)-ene

216



217



169

Cinn =

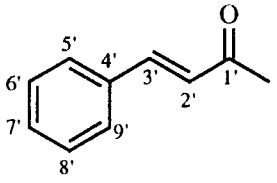


Figure 2.3.1. Sesquiterpenes from *Brintonia discoidea*

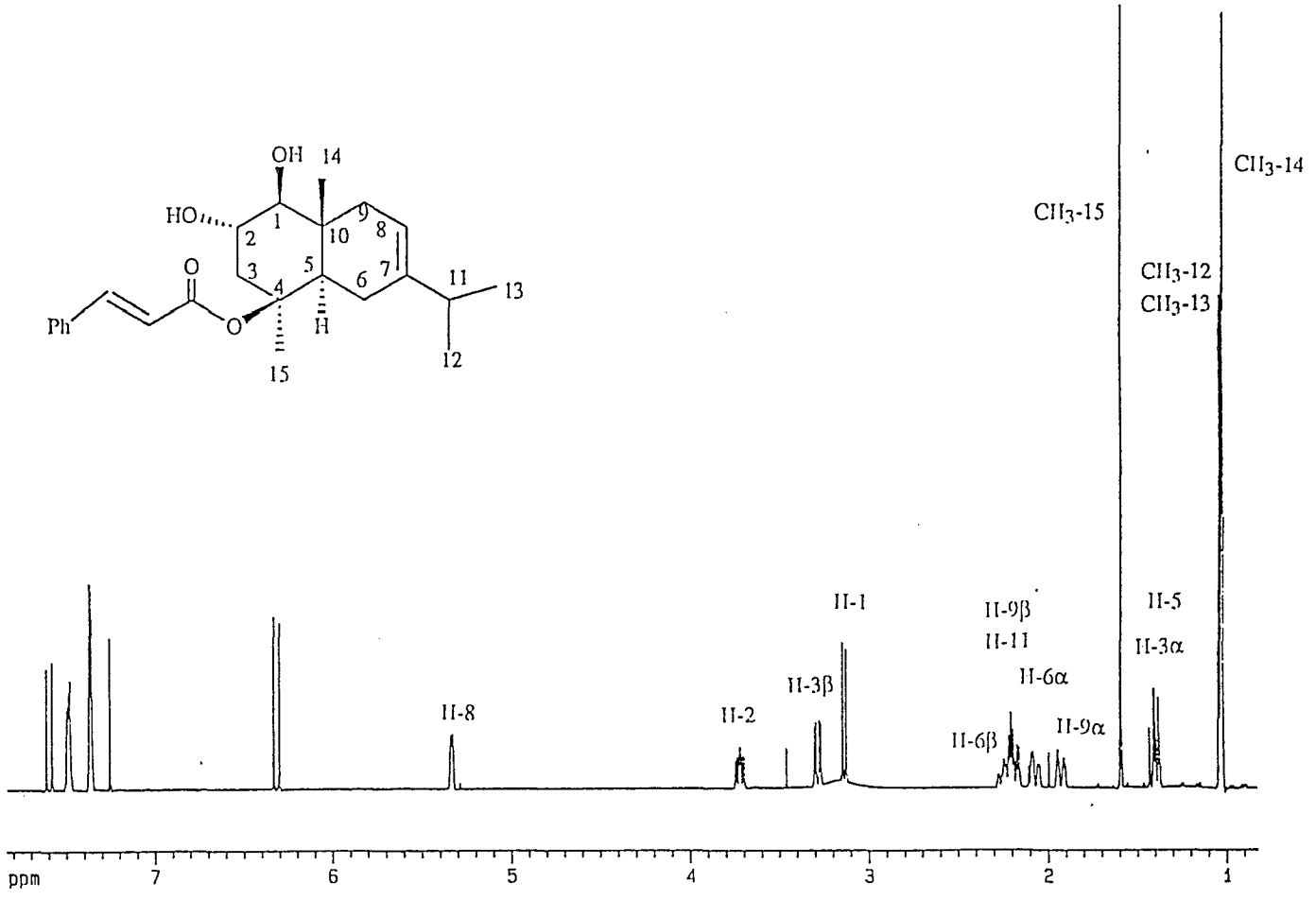


Figure 2.3.2. 500 MHz ^{1}H NMR spectrum of 4β-cinnamoyloxy-1β,2α-dihydroxyeudesm-7-ene (**207**)

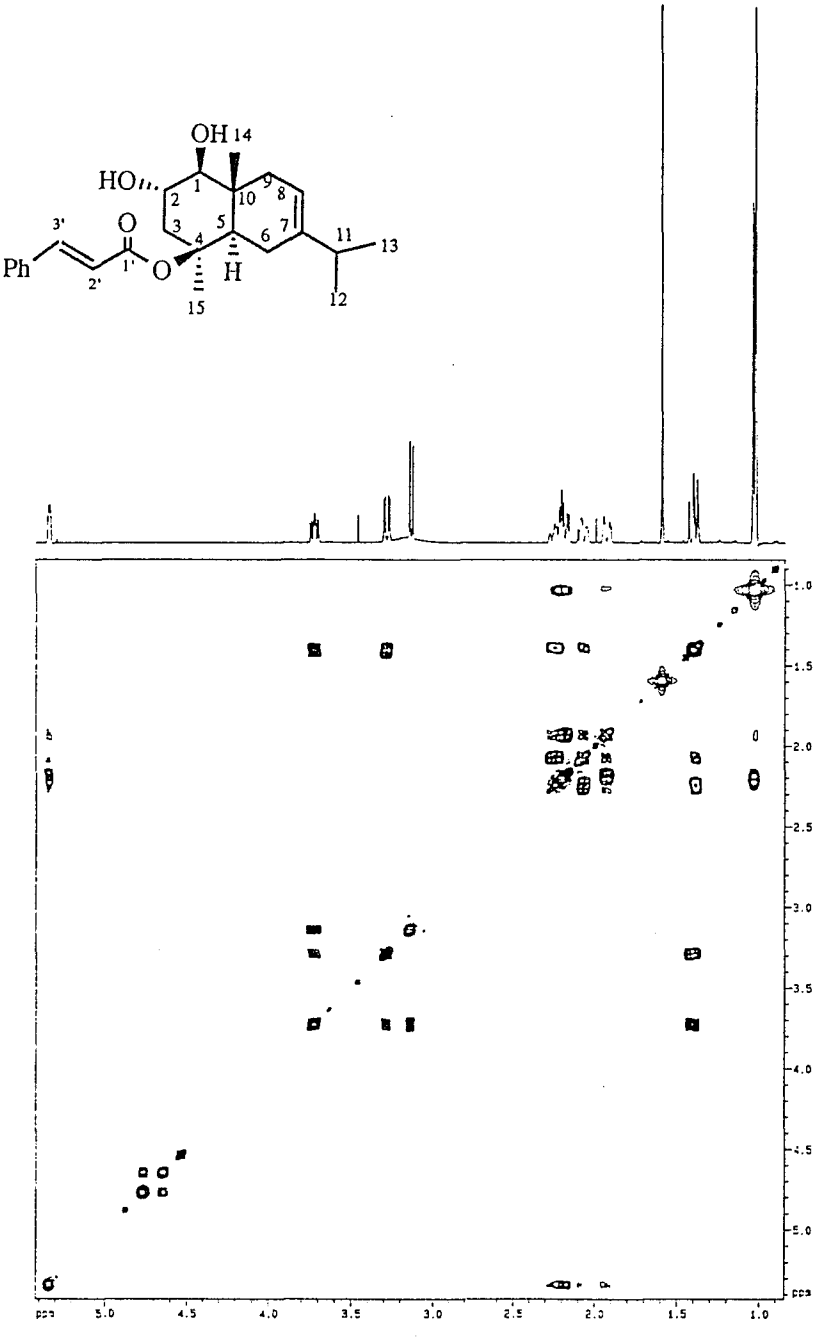


Figure 2.3.3. 500 MHz ^{1}H NMR COSY spectrum of 4β-cinnamoyloxy-1β,2α-dihydroxyeudesm-7-ene (**207**)

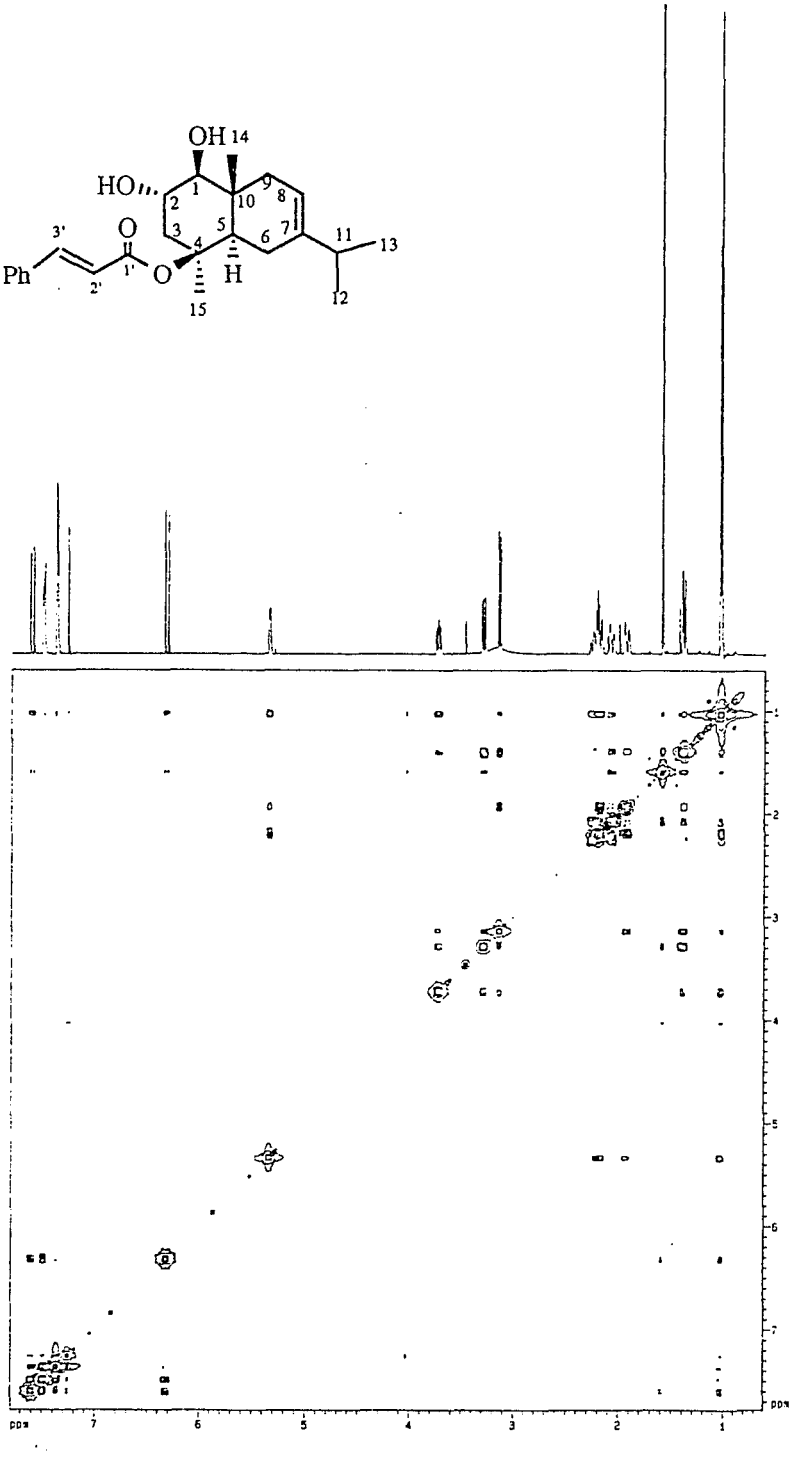


Figure 2.3.4. 500 MHz 2D NOE spectrum of 4β-cinnamoyloxy-1β,2α-dihydroxyeudesm-7-ene (**207**)

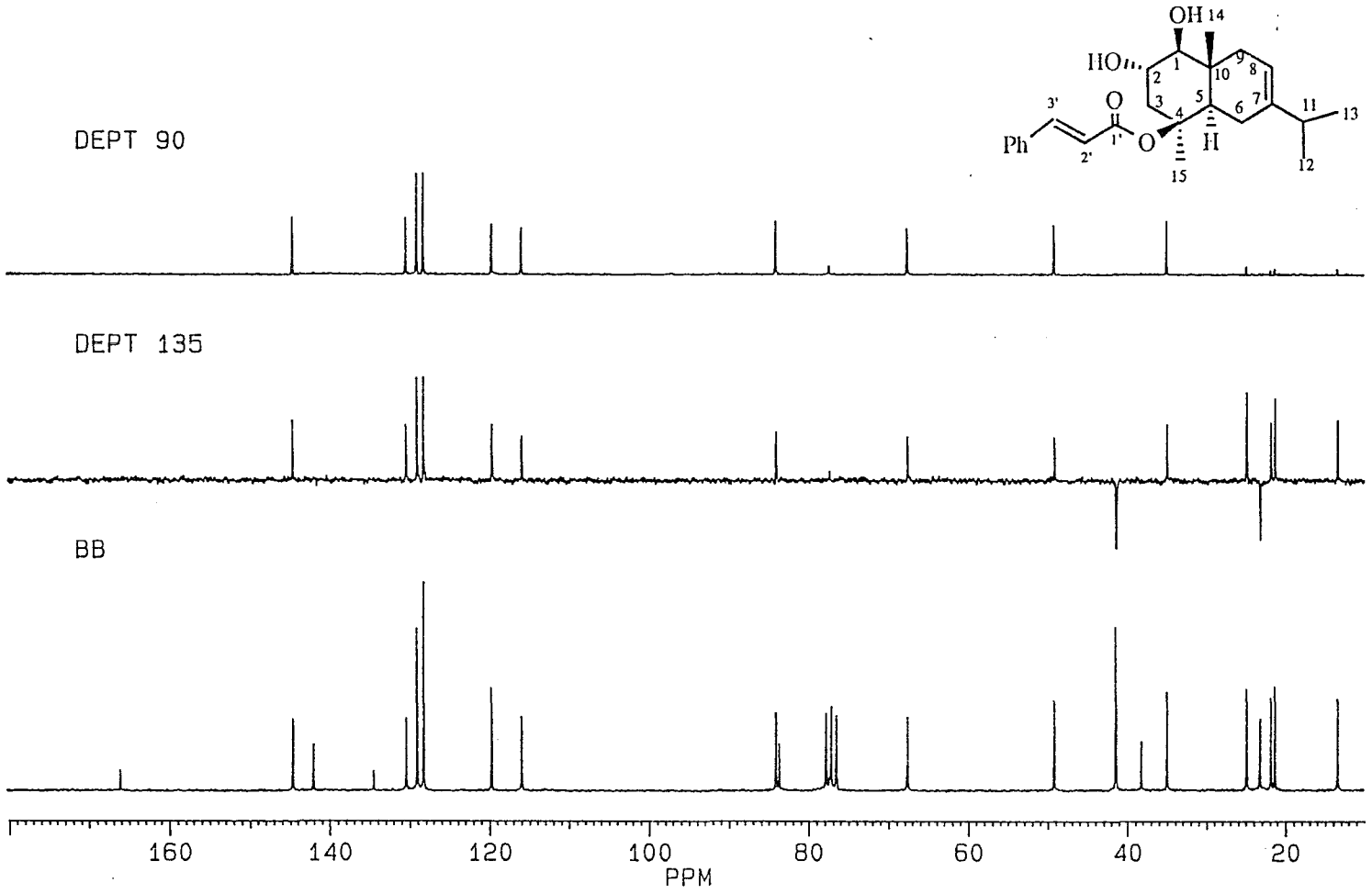


Figure 2.3.5. DEPT 90°, DEPT 135° and Broad Band ^{13}C NMR spectra of 4β-cinnamoyloxy-1β,2α-dihydroxyeudesm-7-ene (**207**)

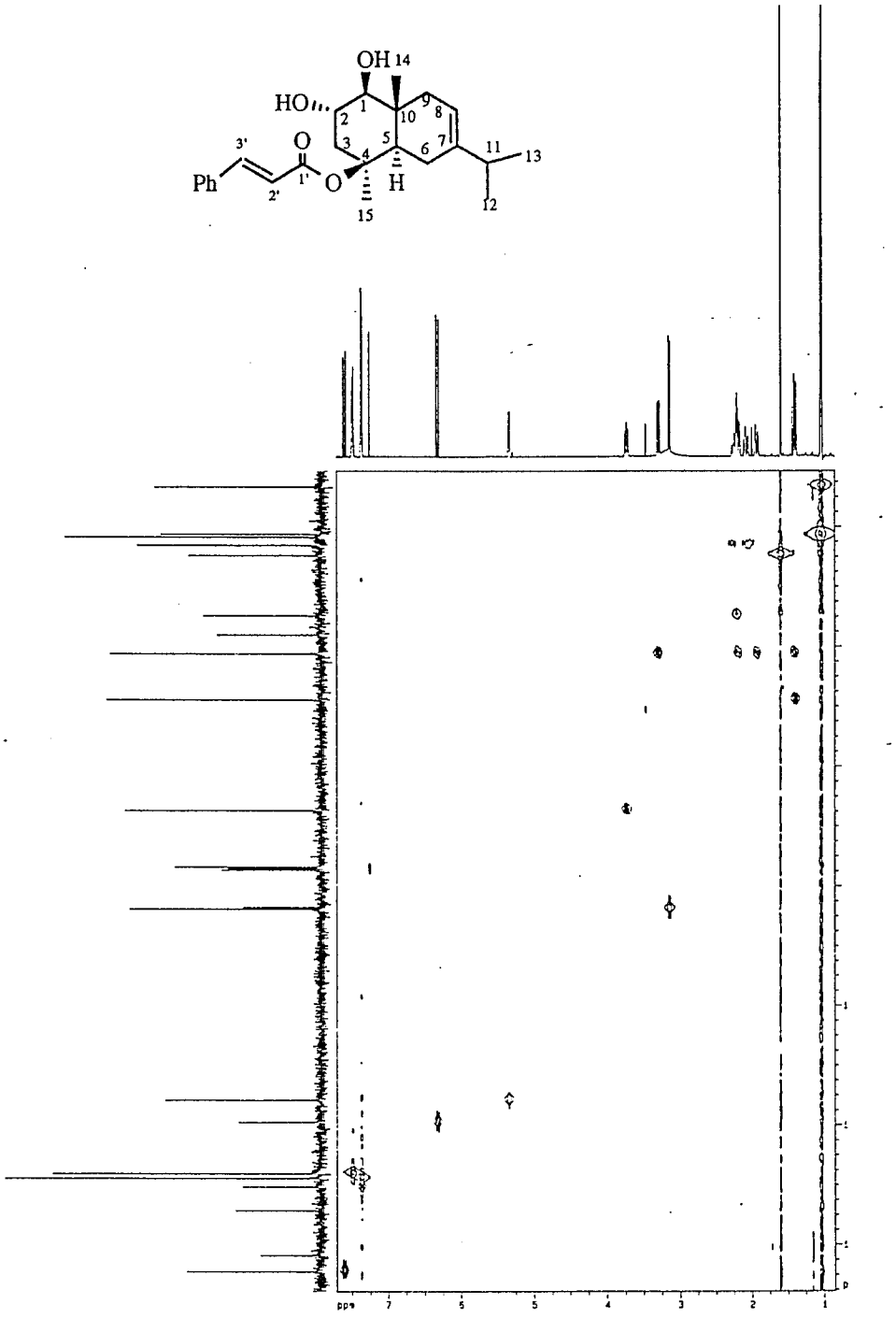


Figure 2.3.6. 2D Inverse ^{1}H-^{13}C heteronuclear correlation spectrum of 4β-cinnamoyloxy-1β,2α-dihydroxyeudesm-7-ene (**207**)

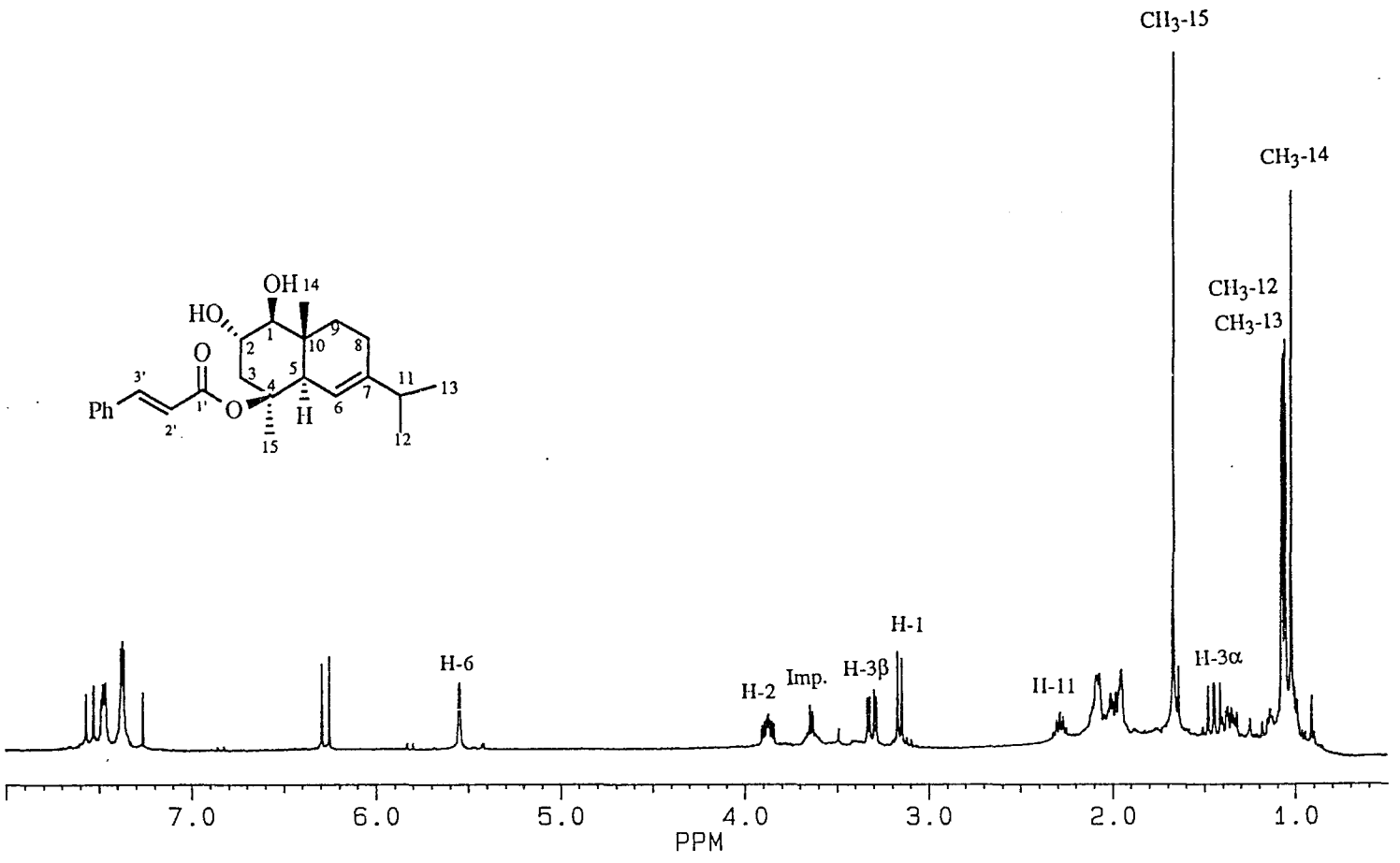


Figure 2.3.7. 400 MHz ^{1}H NMR spectrum of 4β-cinnamoyloxy-1β,2α-dihydroxyeudesm-6-ene (**208**)

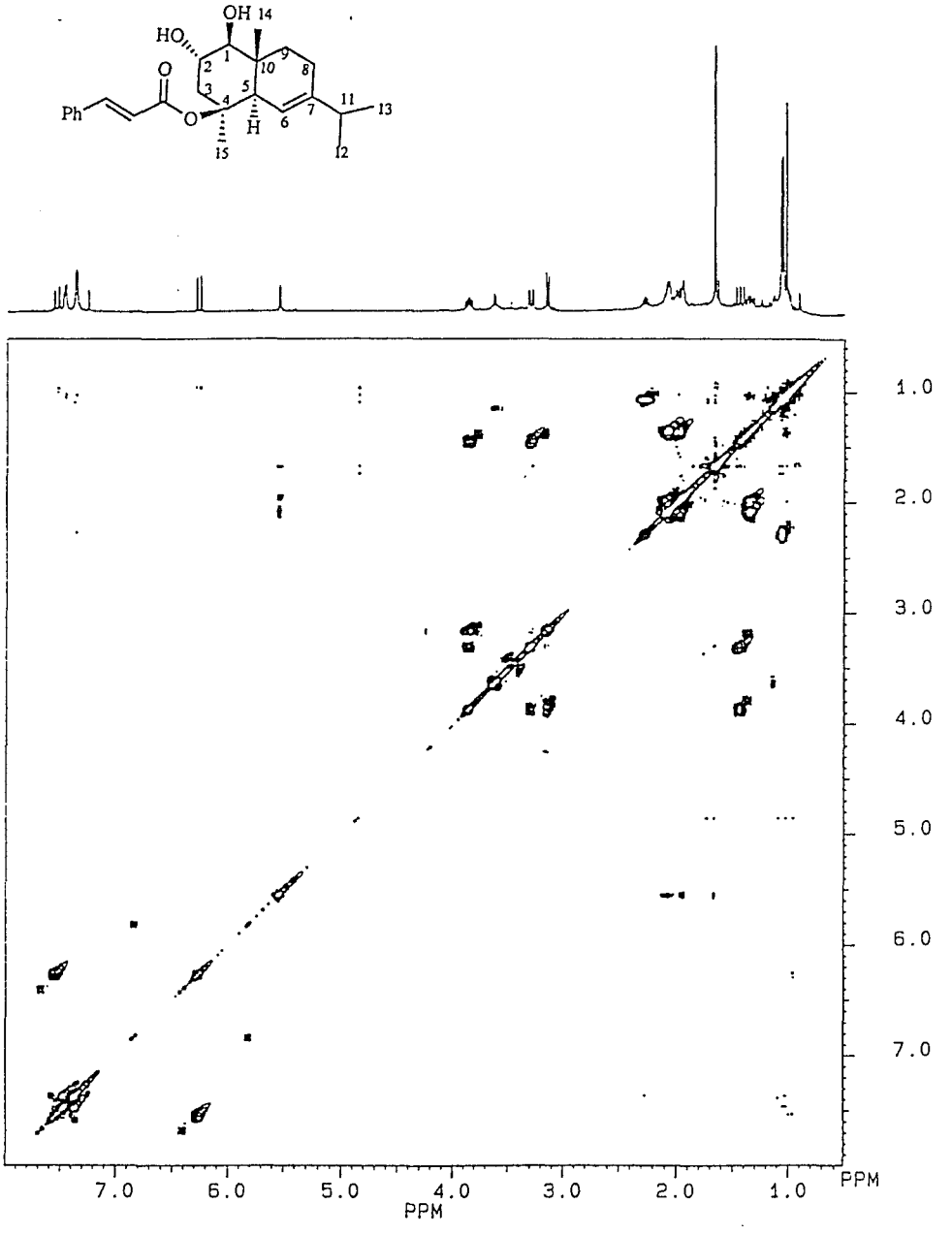


Figure 2.3.8. 400 MHz 2D ^{1}H NMR COSY spectrum of 4β-cinnamoyloxy-1β,2α-dihydroxyeudesm-6-ene (**208**)

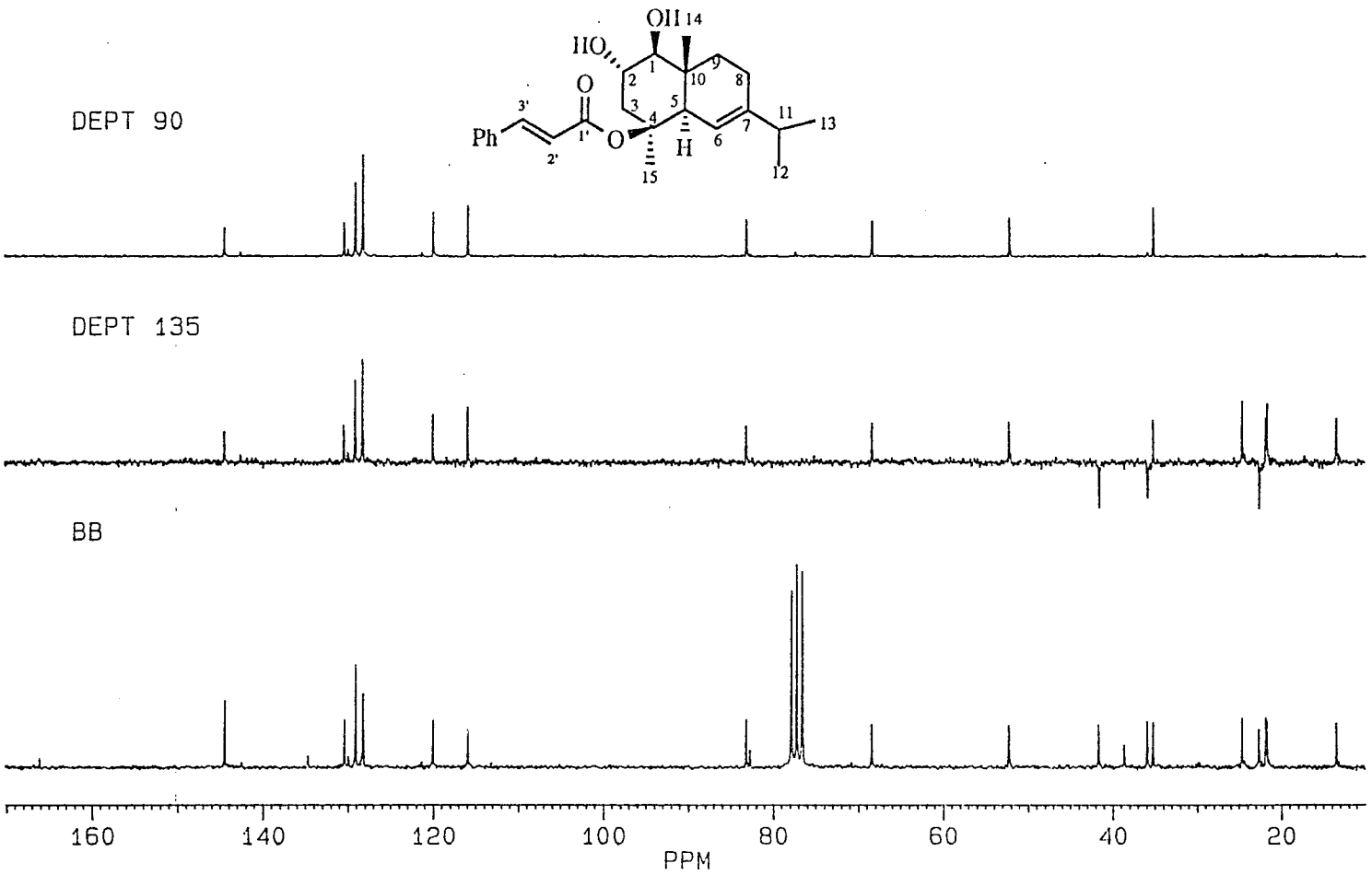


Figure 2.3.9. DEPT 90°, DEPT 135° and Broad Band ^{13}C NMR spectra of 4β-cinnamoyloxy-1β,2α-dihydroxyeudesm-6-enc (**208**)

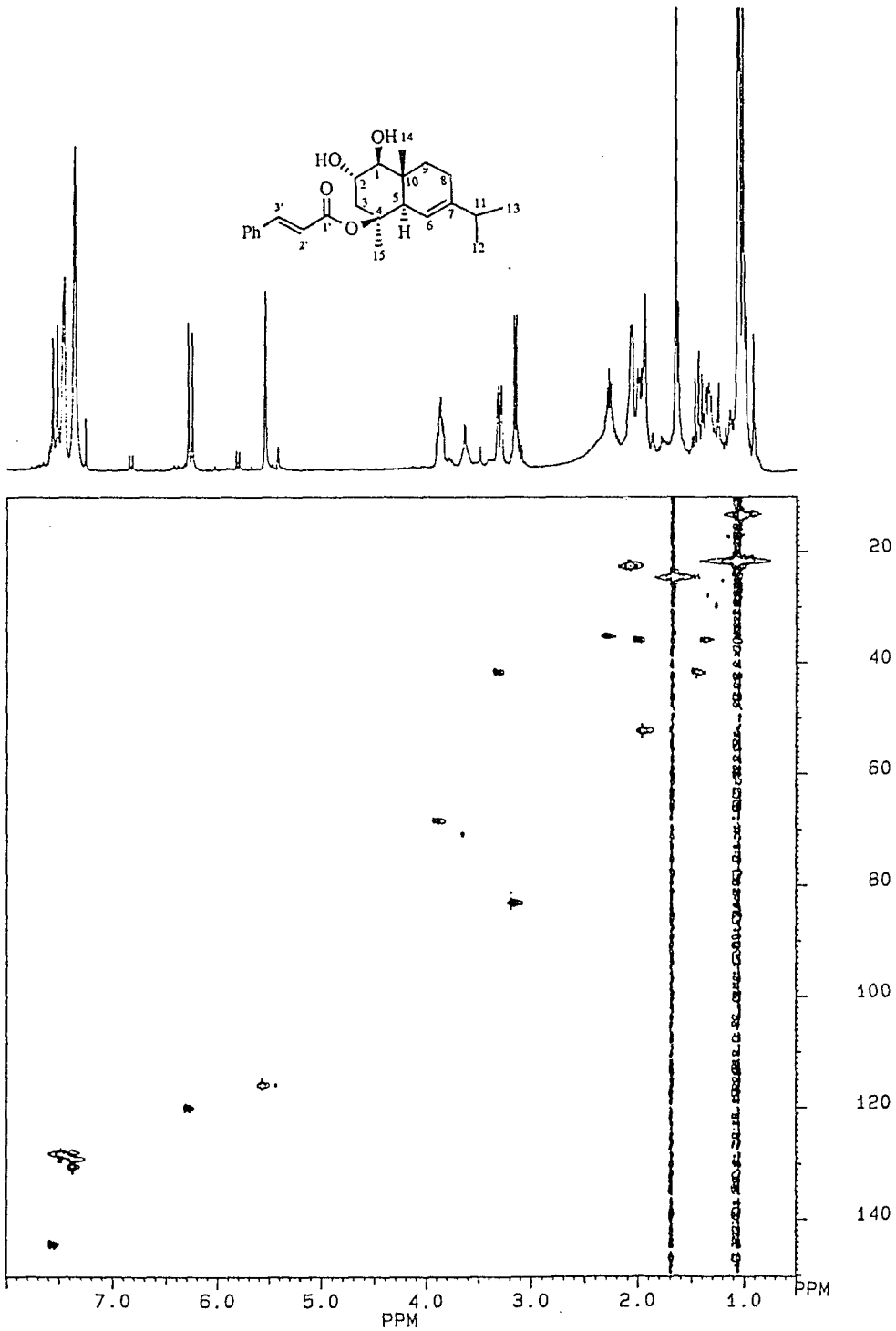


Figure 2.3.10. 2D Inverse ^{1}H-^{13}C heteronuclear correlation spectrum of 4β-cinnamoyloxy-1β,2α-dihydroxyeudesm-6-ene (**208**)

Table 2.3.1. ^{1}H NMR spectral data of compounds **207-212** (400 MHz, $CDCl_3$ as internal standard)

H	**207***	**208**	**209**	**210**	**211**	**212**
1	3.14 *d*	3.16 *d*	3.37 *dd*	3.38 *dd*	3.47 *dd*	4.67 *dd*
2	3.73 *ddd*	3.87 *ddd*	1.62 *m*	1.71 *m*	1.70 *m*	1.68 *m*
3α	1.41 *dd*	1.45 *dd*	1.41 *ddd*	1.44 *ddd*	1.47 *m*	1.48 *m*
3β	3.29 *dd*	3.31 *dd*	3.02 *ddd*	2.99 *ddd*	2.98 *ddd*	3.02 *ddd*
5	1.39 *dd*	1.95 *br s*	1.34 *dd*	1.84 *br d*	1.94 *br d*	2.63 *d*
6α	2.07 *br dd*	}5.55 *br s*	2.09 *br dd*	}5.55 *br s*	}1.63 *m*	}5.10 *d*
6β	2.26 *br dd*		2.29 *br dd*			
8	5.34 *br d*	2.08 *m*	5.37 *br d*	2.07 *m*	6.31 *d*	6.12 *d*
9α	1.93 *br d*	1.36 *ddd*	1.92 *br d*	1.32 *ddd*	}5.52 *d*	}4.54 *d*
9β	2.19 *br dd*	1.99 *ddd*	2.16 *br dd*	1.95 *ddd*		
11	2.21 *m*	2.29 *m*	2.23 *m*	2.28 *m*	2.20 *m*	2.37 *m*
12	1.04 *d*	1.07 *d*	1.06 *d*	1.07 *d*	0.99 *d*	1.10 *d*
13	1.03 *d*	1.06 *d*	1.05 *d*	1.06 *d*	0.97 *d*	1.10 *d*
14	1.02 *s*	1.03 *s*	1.03 *s*	1.01 *s*	1.08 *s*	1.27 *s*
15	1.60 *s*	1.67 *s*	1.58 *s*	1.62 *s*	1.60 *s*	1.58 *s*
2'	6.32 *d*	6.27 *d*	6.37 *d*	6.30 *d*	6.35 *d*	6.38 *d*
3'	7.60 *d*	7.55 *d*	7.63 *d*	7.56 *d*	7.61 *d*	7.65 *d*
5', 7', 9'	7.37 *m*	7.37 *m*	7.38 *m*	7.37 *m*	7.38 *m*	7.38 *m*
6', 8'	7.49 *m*	7.47 *m*	7.51 *m*	7.48 *m*	7.51 *m*	7.54 *m*
OAc	-------	-------	-------	-------	-------	2.07 *s*

* Data obtained from 500 MHz NMR spectrometer.

J (Hz): **207**: 1,2=9.5, 2,3α=11.8, 2,3β=4.3, 3α,3β=14.2, 5,6α=4.2, 5,6β=11.9, 6α,6β=17.9, 8,9β=5.5, 9α,9β=16.5, 12,11=13,11=6.8, 2',3'=16.0; **208**: 1,2=9.2, 2,3α=11.8, 2,3β=4.8, 3α,3β=14.5, 9α,8α=3.9, 9β,8α=1.9, 9α,8β=7.9, 9β,8β=6.0, 9α,9β=12.8, 12,11=13,11=6.9, 2',3'=16.0; **209**: 1,2α=5.2, 1,2β=10.5, 3α,2α=5.0, 3α,2β=13.7, 3β,2α=3β,2β=3.2, 3α,3β=14.7, 5,6α=5.0, 5,6β=11.8, 6α,6β=17.1, 8,9β=5.9, 9α,9β=17.1, 12,11=13,11=7.0, 2',3'=16.0; **210**: 1,2α=4.7, 1,2β=11.1, 3α,2α=4.6, 3α,2β=14.1, 3β,2α=3β,2β=3.3, 5,6=1.4, 9α,8α=4.0, 9α,8β=7.5, 9β,8α=1.7, 9β,8β=6.0, 9α,9β=12.7, 12,11=13,11=6.9, 2',3'=16.0; **211**: 1, 2α=6.8; 1, 2β=8.7; 3β, 2α=3β, 2β=3.3; 3β, 3α=14.9; 5, 6β=8.6; 8, 9=10.1; 11, 12=11, 13=6.7; 2', 3'=16.0; **212**: 1, 2α=4.8; 1, 2β=10.6; 3β, 2α=3β, 2β=2.8; 3α, 3β=13.6; 5, 6=6.0; 8, 9=7.9; 11, 12=11, 13=6.8; 2', 3'=16.0.

Table 2.3.2. ^{1}H NMR spectral data of compounds **163, 165** and **213-217** (400 MHz in $CDCl_3$)

H	**163**	**165**	**213**	**214**	**215**	**216**	**217**
1α	3.52 *dd*	3.38 *dd*	3.68 *dd*	3.34 *dd*	3.77 *dd*	1.39 *m*	3.62 dd
1β	-----	-----	-----	-----	-----	1.66 *m*	-----
2α	1.98 *m*	}1.64 *m*	1.71 *ddd*	}2.20 *m*	}2.60 *dd* (distorted)	}1.65 *m*	1.80 m
2β	2.27 *br d*		2.29 *br dd*				2.09 *m*
3α	}5.33 *br d*	2.14 *ddd*	-----	4.45 *dd*	-----	1.09 *ddd*	-----
3β		2.32 *ddd*	4.37 *dd*	-----	-----	1.98 *ddd*	4.44 *dd*
5	2.09 *br s*	1.92 *br s*	-----	-----	-----	1.07 *br s*	2.29 *br s*
6	5.74 *br s*	5.77 *br s*	6.08 *d*	6.00 *d*	6.31 *d*	5.83 *br s*	5.78 *br s*
7	1.19 *m*	1.12 *m*	1.06 *m*	1.06 *m*	1.17 *m*	1.06 *m*	1.15 *m*
8α	}1.66 *m*	}1.58 *m*	}1.74 *m*	}1.74 *m*	}1.80 *m*	1.56 *ddd*	
8β						1.87 *ddd*	
9α	1.17 *m*	1.24 *ddd*	1.19 *m*	1.19 *m*	1.30 *m*	3.19 *dd*	
9β	1.99 *ddd*	2.05 *ddd*	2.05 *m*	2.05 *m*	2.27 *ddd*	-----	
11	1.45 *m*	1.37 *m*	1.58 *m*	1.59 *m*	1.62 *m*	1.48 *m*	1.30 *m*
12	1.07 *d*	1.04 *d*	0.94 *d*	0.96 *d*	0.99 *d*	0.95 *d*	1.04 *d*
13	0.88 *d*	0.87 *d*	0.94 *d*	0.96 *d*	0.98 *d*	0.88 *d*	0.88 *d*
14	1.09 *s*	1.03 *s*	1.11 *s*	1.16 *s*	1.30 *s*	1.34 *s*	1.02 *s*
15	1.68 *br s*	4.68 *br s*	2.06 *s*	2.00 *s*	2.03 *s*	1.40 *s*	5.14 *d*
		4.79 *br s*					5.17 *d*
2'	6.38 *d*	6.42 *d*	6.41 *d*	6.40 *d*	6.41 *d*	6.42 *d*	6.41 *d*
3'	7.67 *d*	7.70 *d*	7.66 *d*	7.63 *d*	7.67 *d*	7.68 *d*	7.72 *d*
5', 7', 9'	7.37 *m*	7.38 *m*	7.39 *m*	7.38 *m*	7.39 *m*	7.36 *m*	7.39 *m*
6', 8'	7.52 *m*	7.54 *m*	7.53 *m*	7.53 *m*	7.53 *m*	7.51 *m*	7.53 *m*

J (Hz): **163**: 1, 2α=6.3; 1, 2β=10.3; 2α, 2β=17.8; 3, 15=1.4; 9α, 9β=12.8; 9β, 8α=9β, 8β=3.1; 12, 11=6.5; 13, 11=6.7; 2', 3'=16.0; **165**: 1, 2α=4.3; 1, 2β=11.6; 3α, 2α=5.2; 3α, 2β=13.8; 3α, 3β=14.0; 3β, 2α=3β, 2β=4.7; 9α, 8α=4.2; 9α, 8β=13.0; 9α, 9β=13.0; 9β, 8α=9β, 8β=3.3; 12, 11=13, 11=6.7; 2', 3'=16.0; **213**: 1, 2α=3.5; 1, 2β=12.8; 2α, 2β=16.0; 2α, 3=4.1; 2β, 3=1.2, 6,7=2.2; 11, 12=11, 13=6.7; 2', 3'=16.0; **214**: 1, 2α=4.0; 1, 2β=12.4; 3, 2α=3, 2β=8.0; 6, 7=2.4; 11, 12=11, 13=6.6; 2', 3'=16.0; **215**: 1, 2α=7.3; 1, 2β=10.9; 6, 7=2.3; 9β, 8α=9β, 8β=3.3; 9α, 9β=13.2; 11, 12=11, 13=6.7; 2', 3'=16.0; **216**: 3α, 3β=13.0; 3β, 2α=3β, 2β=3.0; 8α, 8β=12.7; 8α, 9α=3.5; 8β, 9α=11.5; 8β, 7=2.8; 12, 11=13, 11=6.7; 2', 3'=16.0; **217**: 1,2α=4.5; 1,2β=12.4; 2α, 3=4.1; 2β, 3=2.2; 5,15=5,15'=1.8; 11,12=11,13=6.5; 2',3'=16.0.

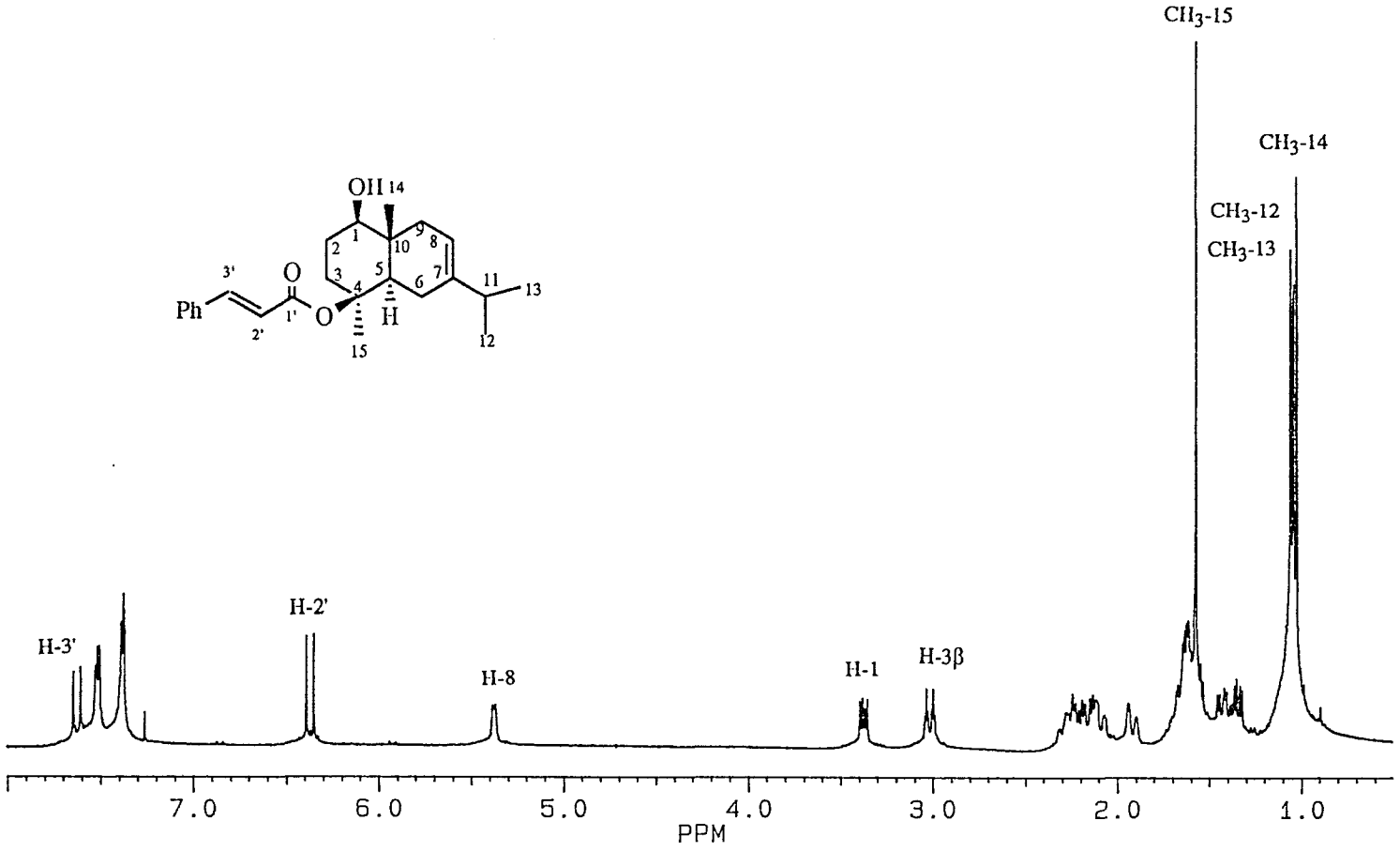


Figure 2.3.11. 400 MHz ^{1}H NMR spectrum of 4β-cinnamoyloxy-1β-hydroxyeudesm-7-ene (**209**)

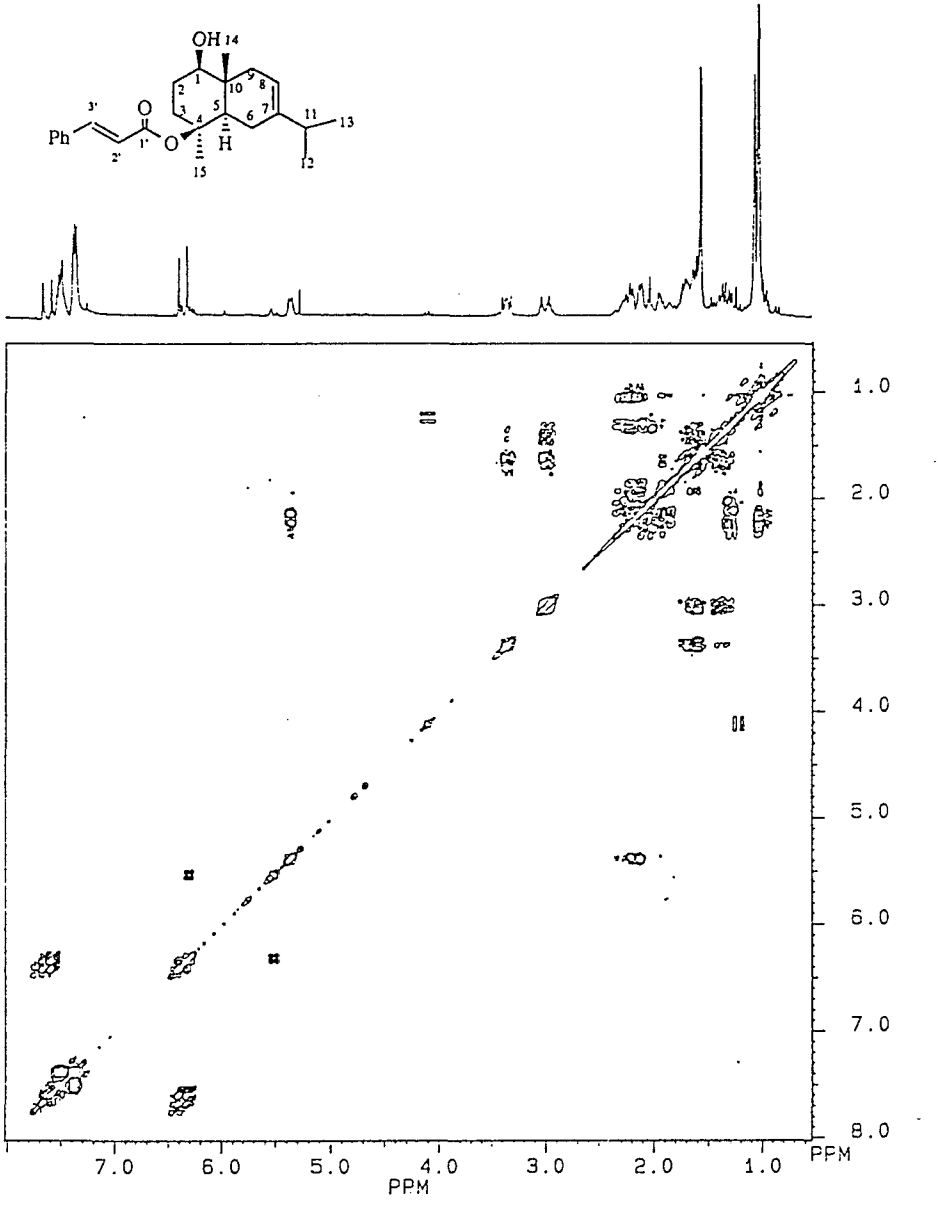


Figure 2.3.12. 400 MHz 2D ^{1}H NMR COSY spectrum of 4β-cinnamoyloxy-1β-hydroxyeudesm-7-ene (**209**)

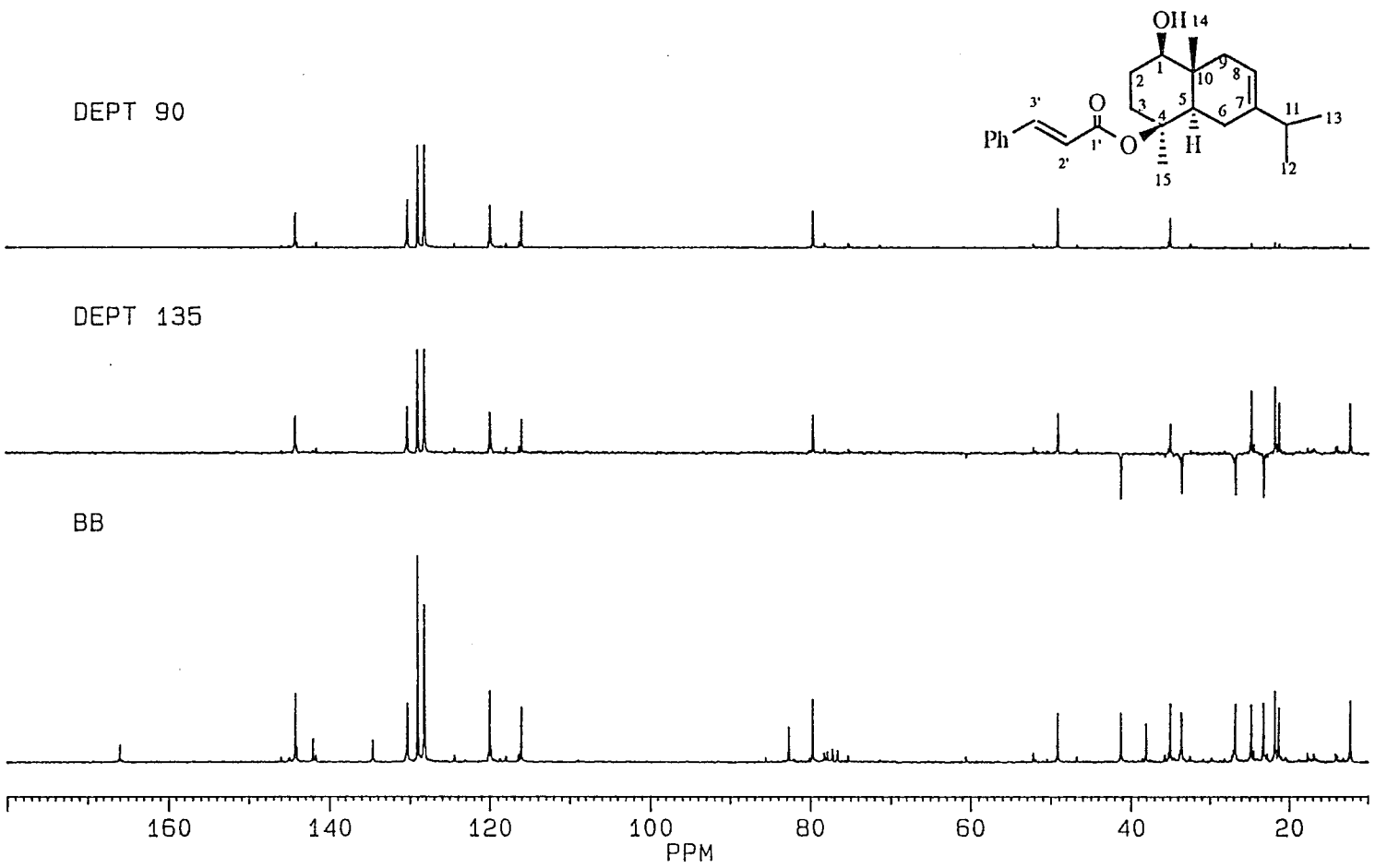


Figure 2.3.13. DEPT 90°, DEPT 135° and Broad Band ^{13}C NMR spectra of 4β-cinnamoyloxy-1β-hydroxyeudesm-7-ene (**209**)

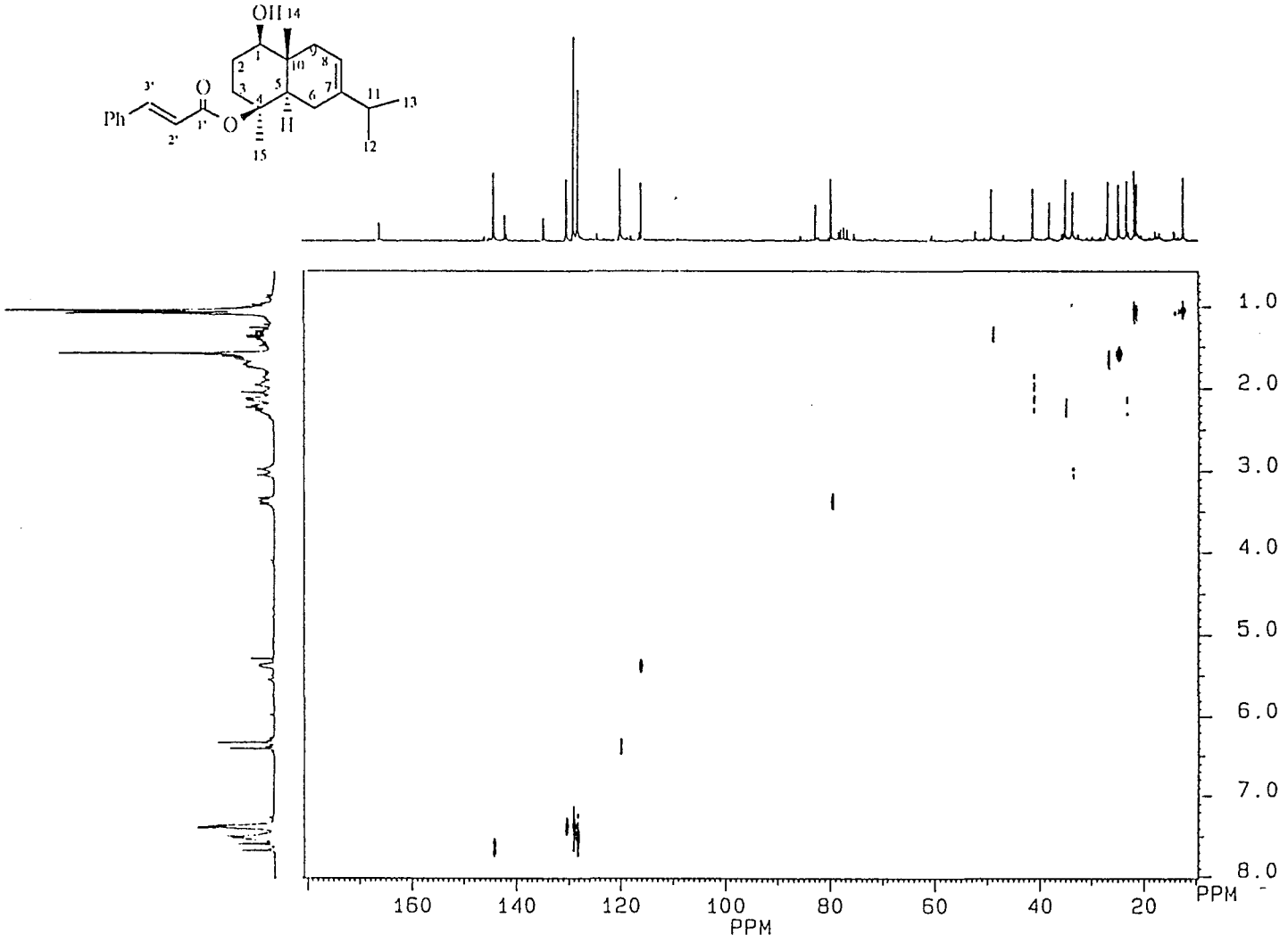


Figure 2.3.14. 2D ^{13}C-^{1}H heteronuclear correlation spectrum of 4β-cinnamoyloxy-1β-hydroxyeudesm-7-ene (**209**)

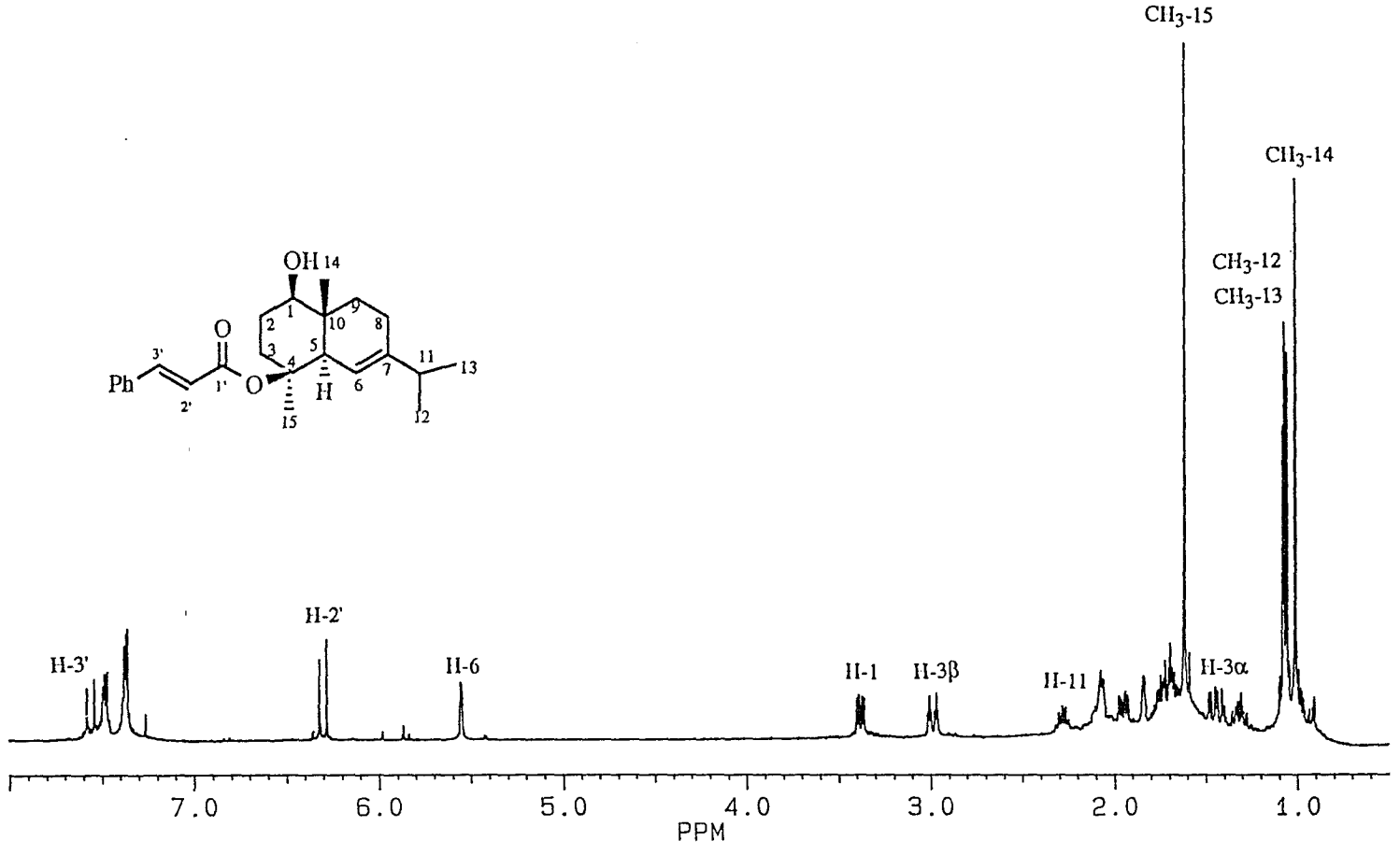


Figure 2.3.15. 400 MHz ^{1}H NMR spectrum of 4β-cinnamoyloxy-1β-hydroxyeudesm-6-ene (**210**)

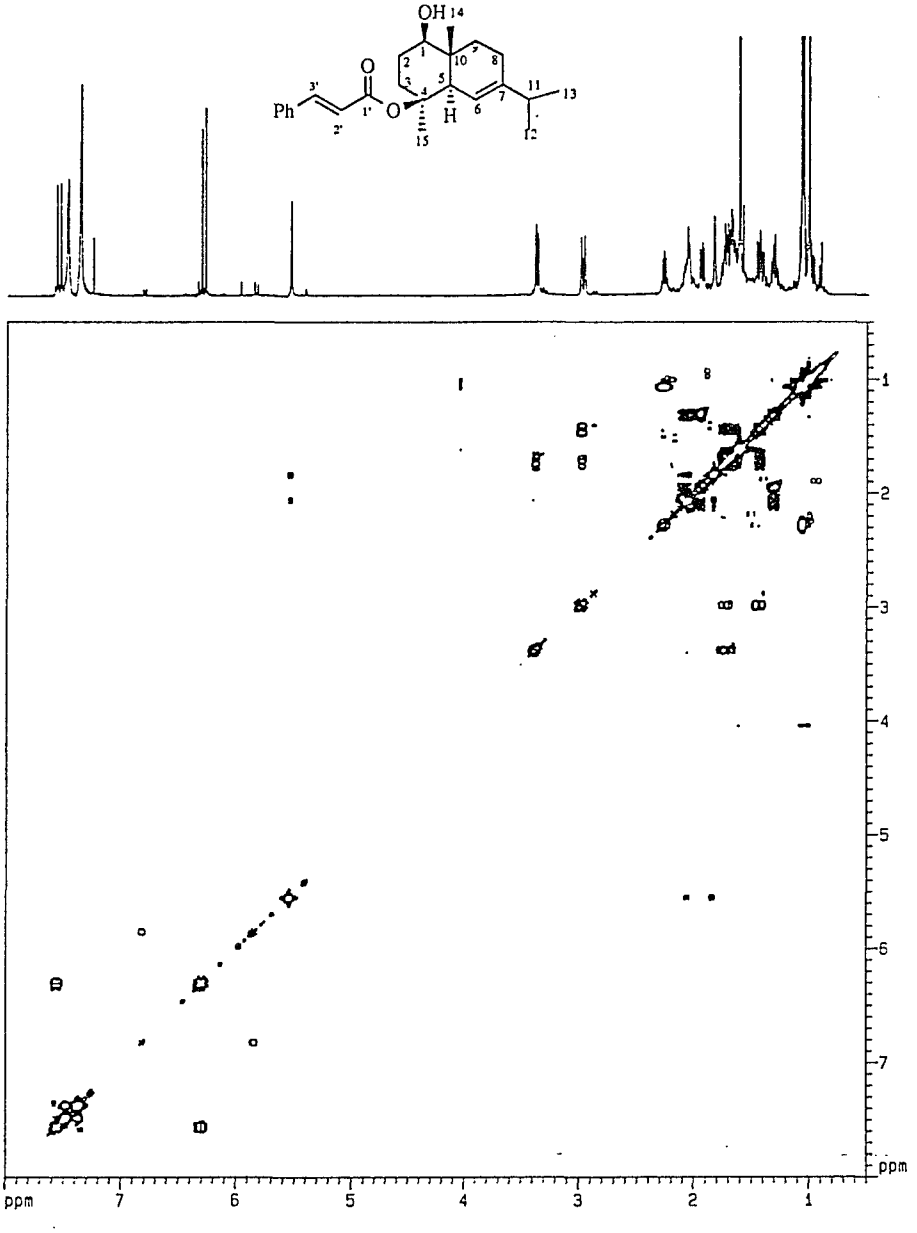


Figure 2.3.16. 500 MHz 2D ^{1}H NMR COSY spectrum of 4β-cinnamoyloxy-1β-hydroxyeudesm-6-ene (**210**)

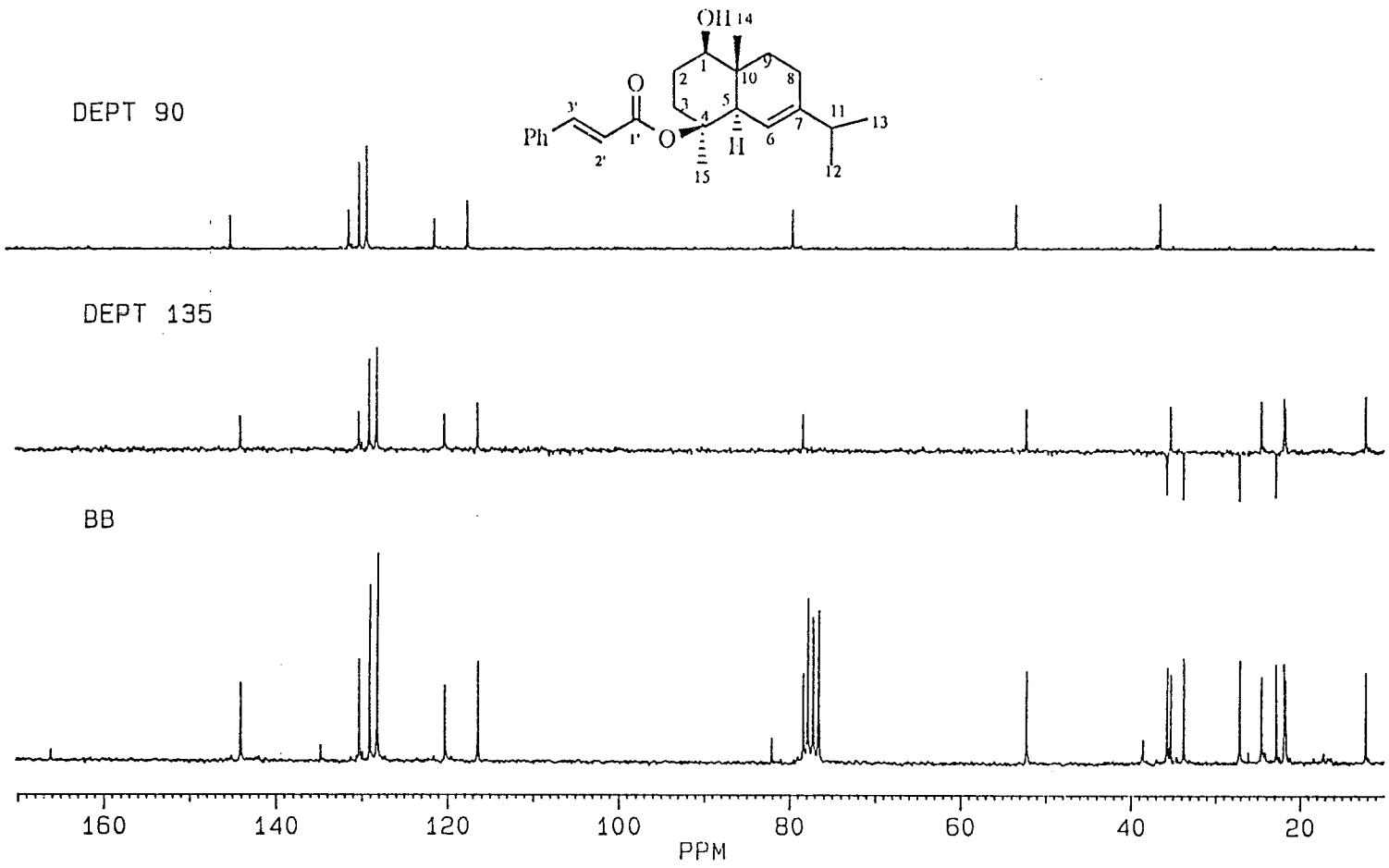


Figure 2.3.17. DEPT 90°, DEPT 135° and Broad Band ^{13}C NMR spectra of 4β-cinnamoyloxy-1β-hydroxyeudesm-6-ene (**210**)

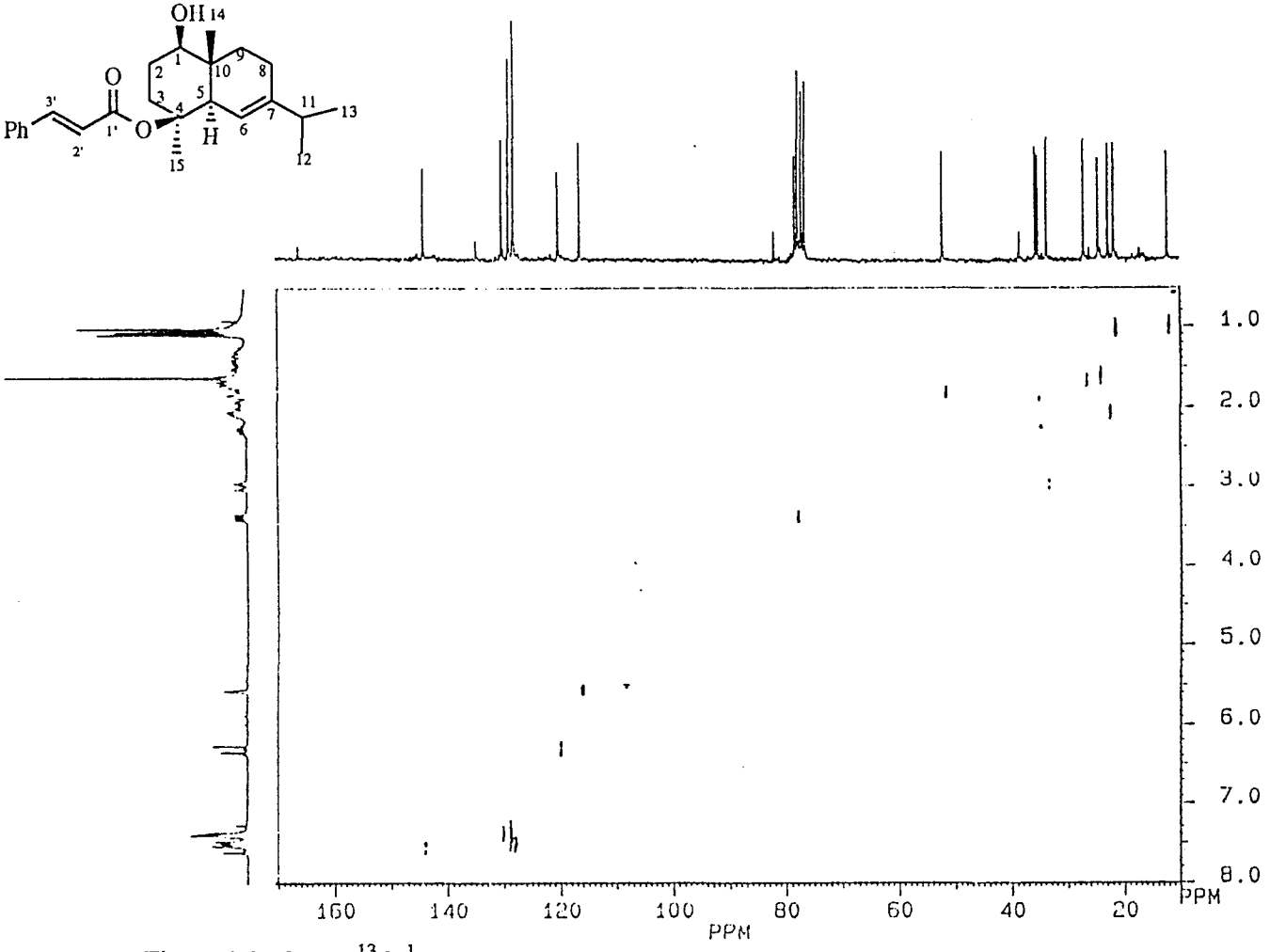


Figure 2.3.18. 2D ^{13}C-^{1}H Heteronuclear correlation spectrum of 4β-cinnamoyloxy-1β-hydroxyeudesm-6-ene (**210**)

Table 2.3.3. ^{13}C NMR spectral data of compounds **163, 165, 207-211, 213** and **216** (100 MHz in $CDCl_3$).*

C	**163**	**165**	**207**	**208**	**209**	**210**	**211**	**213**	**216**
1	76.74 *d*	80.09 *d*	83.79 *d*	82.96 *d*	79.49 *d*	78.17 *d*	75.25 *d*	73.77 *d*	40.46 *t*
2	32.06 *t*	34.51 *t*	67.35 *d*	68.21 *d*	26.69 *t*	26.98 *t*	27.23 *t*	30.65 *t*	20.81 *t*
3	121.22 *d*	30.76 *t*	41.19 *t*	41.50 *t*	33.40 *t*	33.56 *t*	33.71 *t*	85.12 *d*	39.52 *t*
4	133.46 *s*	147.82 *s*	83.49 *s*	82.54 *d*	82.44 *s*	81.85 *s*	82.42 *s*	138.34 *s*	71.75 *s*
5	50.77 *d*	51. 75 *d*	48.91 *d*	52.05 *d*	51.97 *d*	52.01 *d*	46.61 *d*	140.26 *s*	53.02 *d*
6	71.35 *d*	71.15 *d*	23.10 *t*	115.69 *d*	23.16 *t*	116.22 *d*	22.97 *t*	70.69 *d*	70.86 *d*
7	49.25 *d*	50.36 *d*	141.74 *s*	142.27 *s*	141.80 *s*	143.74 *s*	85.75 *s*	48.62 *d*	49.83 *d*
8	20.29 *t*	20.43 *t*	115.76 *d*	22.56 *t*	115.82 *d*	22.68 *t*	141.87 *d*	20.30 *t*	26.73 *t*
9	35.42 *t*	37.38 *t*	41.19 *t*	35.73 *t*	40.99 *t*	35.54 *t*	124.00 *d*	38.18 *t*	80.45 *d*
10	37.73 *s*	40.35 *s*	38.02 *s*	38.45 *s*	37.86 *s*	38.34 *s*	41.12 *s*	39.61 *s*	39.37 *s*
11	28.59 *d*	28.08 *d*	34.80 *d*	35.07 *d*	34.81 *d*	35.10 *d*	32.35 *d*	29.04 *d*	28.69 *d*
12	22.15 *q*	21.95 *q*	21.73 *q*	21.72 *q*	21.72 *q*	21.72 *q*	17.68 *q*	20.81 *q*	21.33 *q*
13	20.11 *q*	20.33 *q*	21.25 *q*	21.61 *q*	21.22 *q*	21.58 *q*	16.84 *q*	20.81 *q*	20.41 *q*
14	12.18 *q*	13.20 *q*	13.35 *q*	13.41 *q*	12.32 *q*	12.17 *q*	13.45 *q*	16.87 *q*	13.71 *q*
15	20.67 *q*	108.93 *t*	24.79 *q*	24.55 *q*	24.69 *q*	24.41 *q*	24.85 *q*	17.59 *q*	29.70 *q*
1'	166.25 *s*	166.83 *s*	165.97 *s*	166.01 *s*	165.86 *s*	166.04 *s*	165.90 *s*	165.98 *s*	167.08 *s*
2'	118.57 *d*	118.60 *d*	119.45 *d*	119.78 *d*	119.78 *d*	120.07 *d*	119.70 *d*	118.36 *d*	118.42 *d*
3'	144.74 *d*	144.85 *d*	144.43 *d*	144.26 *d*	144.00 *d*	143.89 *d*	144.21 *d*	144.86 *d*	145.14 *d*
4'	134.37 *s*	134.45 *s*	134.24 *s*	134.48 *s*	134.37 *s*	134.56 *s*	134.44 *s*	134.41 *s*	134.27 *s*
5', 9'	128.86 *d*	128.86 *d*	128.84 *d*	128.86 *d*	128.75 *d*	128.82 *d*	128.89 *d*	128.88 *d*	128.79 *d*
6', 8'	128.12 *d*	128.12 *d*	128.05 *d*	128.00 *d*	127.93 *d*	127.94 *d*	128.05 *d*	128.11 *d*	128.12 *d*
7'	130.27 *d*	130.26 *d*	130.26 *d*	130.18 *d*	130.04 *d*	130.04 *d*	130.22 *d*	130.30 *d*	130.28 *d*

* Peak multiplicities were determined by heteronuclear multipulse programs (DEPT); *s*=singlet; *d*=doublet; *t*= triplet; *q*=quartet.

oerstediana [144] and **207** was also obtained from *V. virgata* [145]. A sesquiterpene hydroperoxide was previously found in the roots of *V. subcordata* and its stereochemistry at C-3 had been assigned H-3α (**214**) [146]. The isomeric eudesmene cinnamates **163** and **165** were previously obtained from aerial parts of *V. glabrata* and *V. luetzelburgii* [141], **165** from *S. wrightii* [76] and compound **216** was found in roots of *V. sordescens* [142].

The ^{13}C NMR spectra of the eight eudesmane cinnamates (**163, 165, 207-210, 213** and **216**) exhibited 24 carbon signals which were unambiguously assigned by DEPT, selective INEPT and ^{1}H-^{13}C correlations. The ^{1}H NMR spectral data for compounds **163, 165, 213** and **216** are included in Table 2.3.2 since COSY experiments allowed for more complete proton assignments. Since the ^{13}C NMR spectra of compounds **208-210, 213** as well as **163** and **216** were not previously reported, they are included in Table 2.3.3, which also lists the data of compounds **165** and **207** with unambiguous assignments of previously reported values [76, 145].

The structure of the new sesquiterpene **211** was established by MS, ^{1}H and ^{13}C NMR spectral analysis as well as a chemical transformation. The mass spectrum of **211** exhibited strong peaks at m/z 147 $[C_6H_5CH{=}CHCO_2]^+$, 131 and 103 that were diagnostic of the cinnamate moiety. The ^{13}C NMR of **5** showed 24 carbon signals, of which nine carbons were due to the cinnamate moiety. The DEPT experiment indicated that the other 15 carbons included four methyl, three methylene, five methine with two olefinic and one oxygenated carbon, and three quaternary carbons including two oxygenated ones. The carbon assignments were made by DEPT and ^{1}H-^{13}C correlation methods in combination with COSY experiments, as well as by direct comparison with the spectral data of closely related compounds (Table 2.3.3). Comparison of the ^{1}H NMR spectrum of **211** with those of the known terpenoids **209** and **210** suggested, that in **211,** a 4β-cinnamate moiety and a 1β-hydroxyl groups were present. Two mutually coupled olefinic doublets at δ 5.52 (J=10.1 Hz) and 6.31

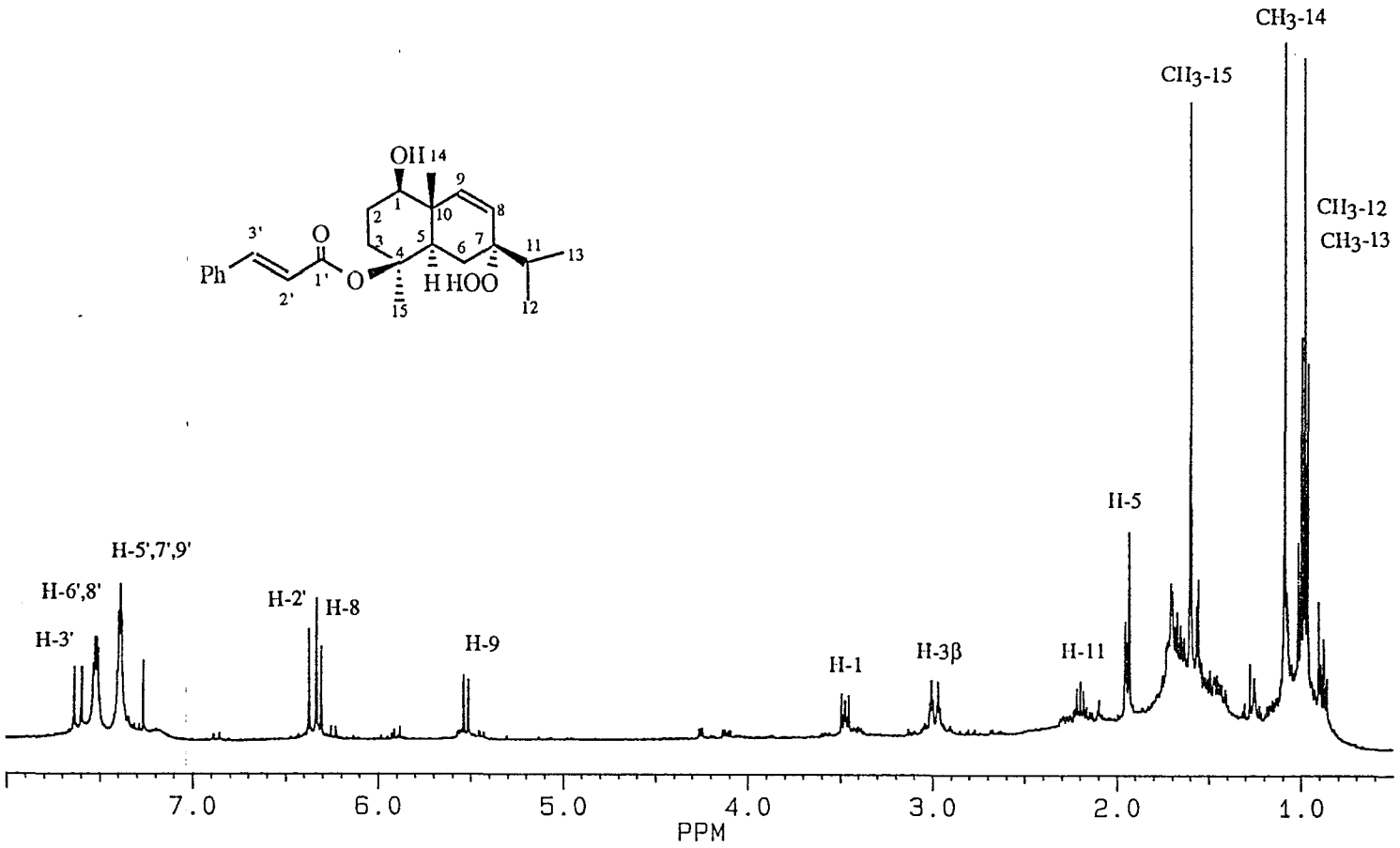


Figure 2.3.19. 400 MHz ^{1}H NMR spectrum of 4β-cinnamoyloxy-7α-hydroperoxy-1β-hydroxyeudesm-8-ene (211)

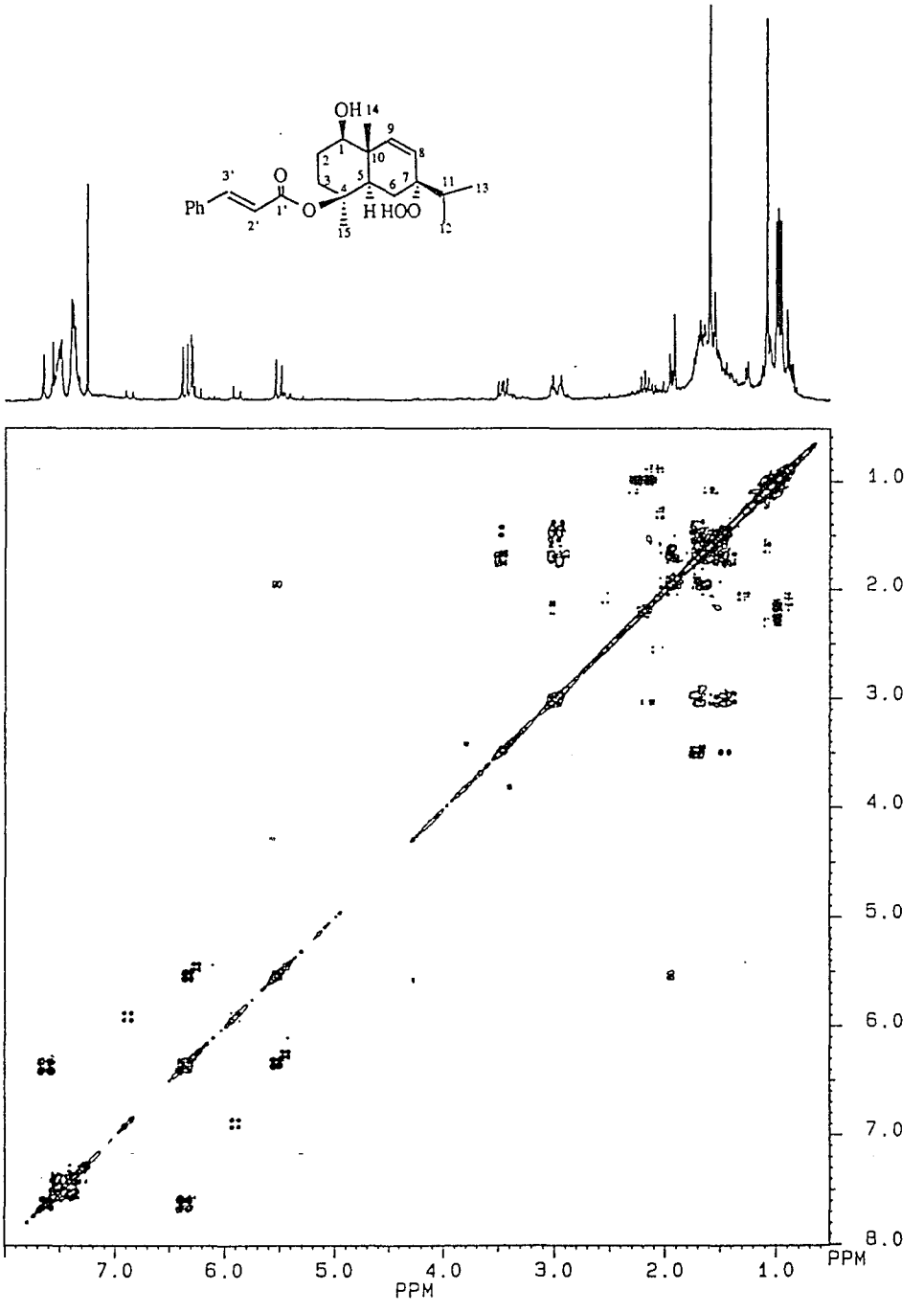


Figure 2.3.20. 400 MHz 2D ^{1}H NMR COSY spectrum of 4β-cinnamoyloxy-7α-hydroperoxy-1β-hydroxyeudesm-8-ene (**211**)

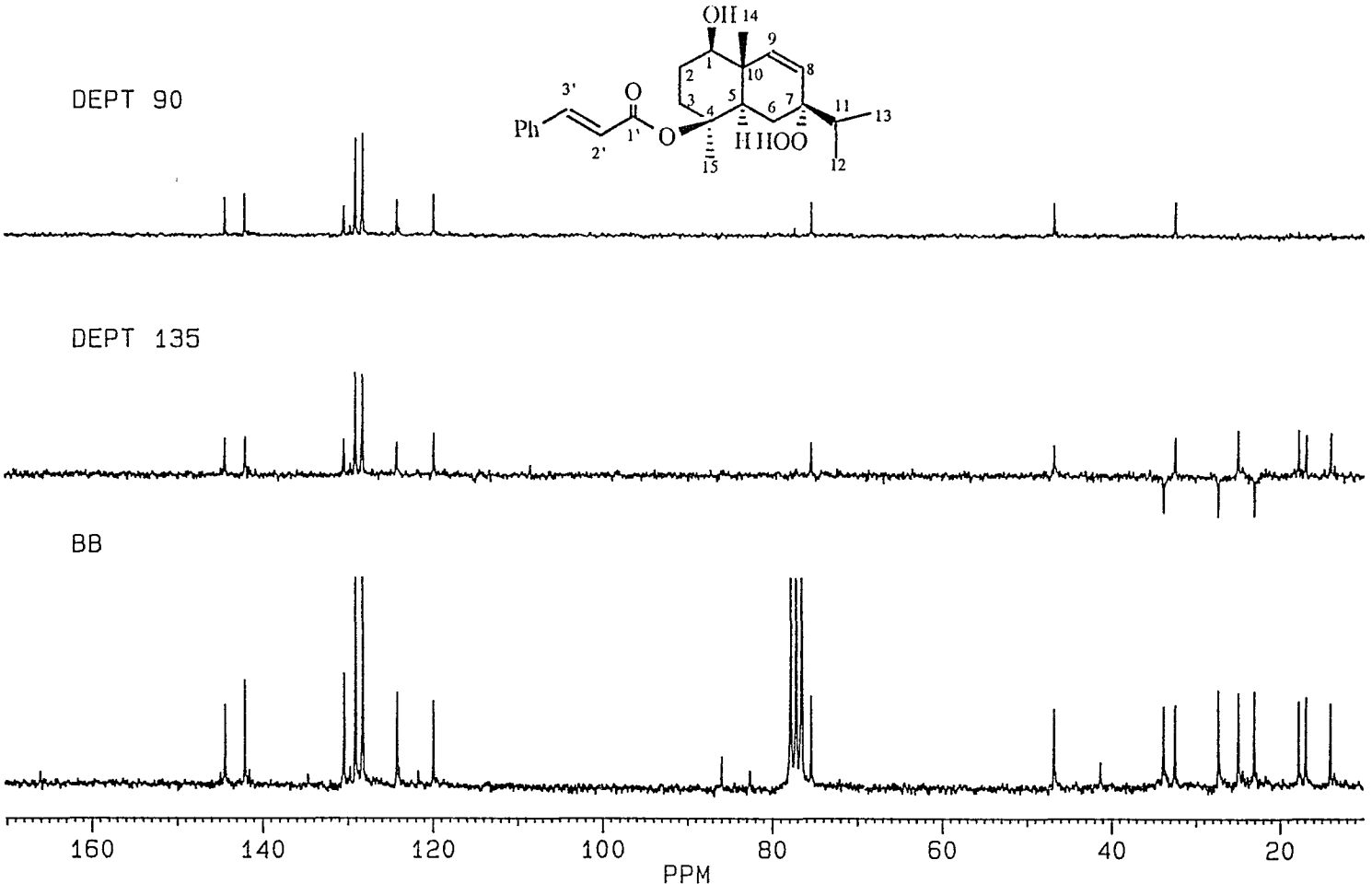
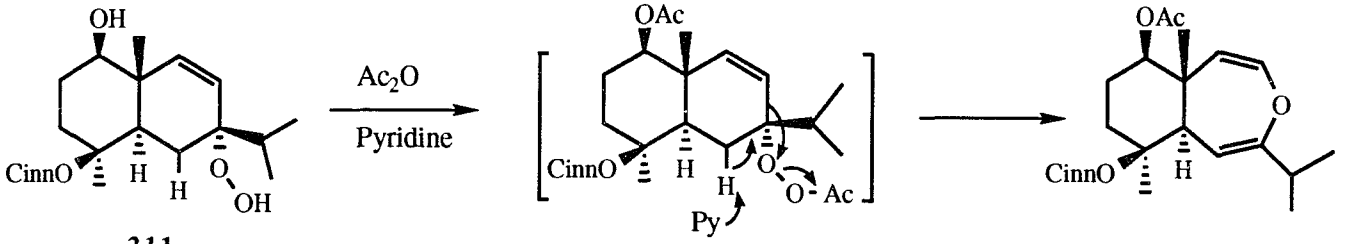


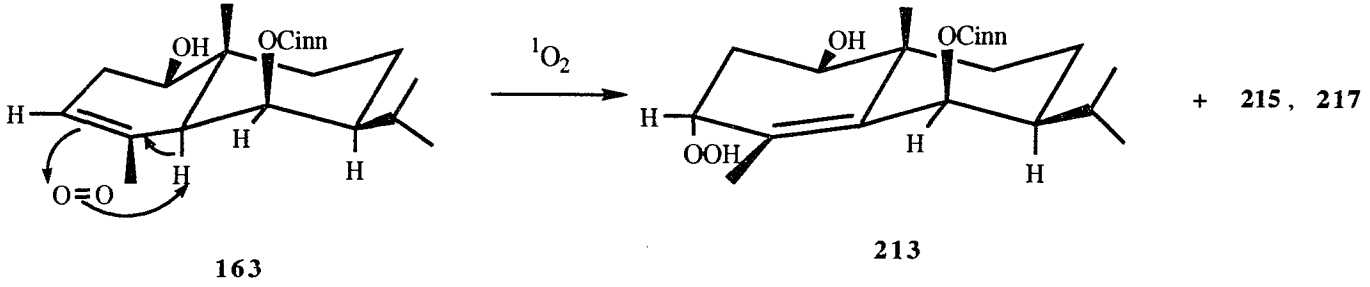
Figure 2.3.21. DEPT 90°, DEPT 135° and Broad Band ^{13}C NMR spectra of 4β-cinnamoyloxy-7α-hydroperoxy-1β-hydroxyeudesm-8-ene (**211**)

indicated a double bond at C-8. The DEPT experiment showed one CH (δ 75.25) and two quaternary carbon signals at δ 82.42 and 85.75 indicating the presence of three oxygenated carbons. This required that besides the secondary hydroxyl group at C-1, either a tertiary hydroxyl or a tertiary hydroperoxide group had to be present in the molecule, a structural arrangement which could not be easily determined on the basis of available NMR data alone. Acetylation of **211** with acetic anhydride in pyridine afforded the vinyl ether monoacetate **212** (Scheme 2.3.1), a rearrangement product which provided evidence that compound **211** must bear a tertiary hydroperoxide moiety rather than a tertiary hydroxyl group. Attachment of the hydroperoxide group to C-7 in **211** was further confirmed by its COSY experiment which showed a pair of methyl doublets at δ 0.97 (H-13) and 0.99 (H-12) that were coupled to the same multiplet at δ 2.20 (H-11) which showed no further coupling. Deshielding of H-5α (δ 1.94, br d) in **211** by the 7-hydroperoxide group supported its α-orientation. The structure of **212** was derived by the analysis of its ^{1}H NMR and mass spectral data. Comparison with the ^{1}H NMR data of **211**, the newly introduced olefinic proton doublet at δ 5.12 (H-6, J=6.0 Hz) and the significant upfield shift of one of the other two olefinic proton doublets at δ 4.54 (H-9) together with a reduced coupling constant (**211**, $J_{8,9}$= 10.1 Hz; **212**, $J_{8,9}$= 7.9 Hz) indicated a vinyl ether moiety in **212**. This was strongly supported by its FAB mass spectral data in a nitrobenzyl alcohol matrix, which showed significant peaks at m/z 577 $[M+NO_2C_6H_4CH_2OH]^+$, 425 $[M+1]^+$, 424 $[M]^+$, 423 $[M-1]^+$ and 277 $[M-C_6H_5CH{=}CHCO_2]^+$.

The other two isomeric eudesmane cinnamates **213** and **214** were isolated as a mixture (by ^{1}H NMR) which could not be completely separated by HPLC. Their spectral data (^{1}H and ^{13}C NMR, IR and MS) clearly indicated the presence of a cinnamate moiety. The ^{1}H NMR spectrum of **213** was essentially identical with data previously reported for a hydroperoxide the stereochemistry of which had been formulated as the H-3α epimer [146]. The C-3- isomer **214** showed spectral features



Scheme 2.3.1. Rearrangement of compound **211** under acylation conditions.



Scheme 2.3.2. Singlet oxygen reaction of compound **163**.

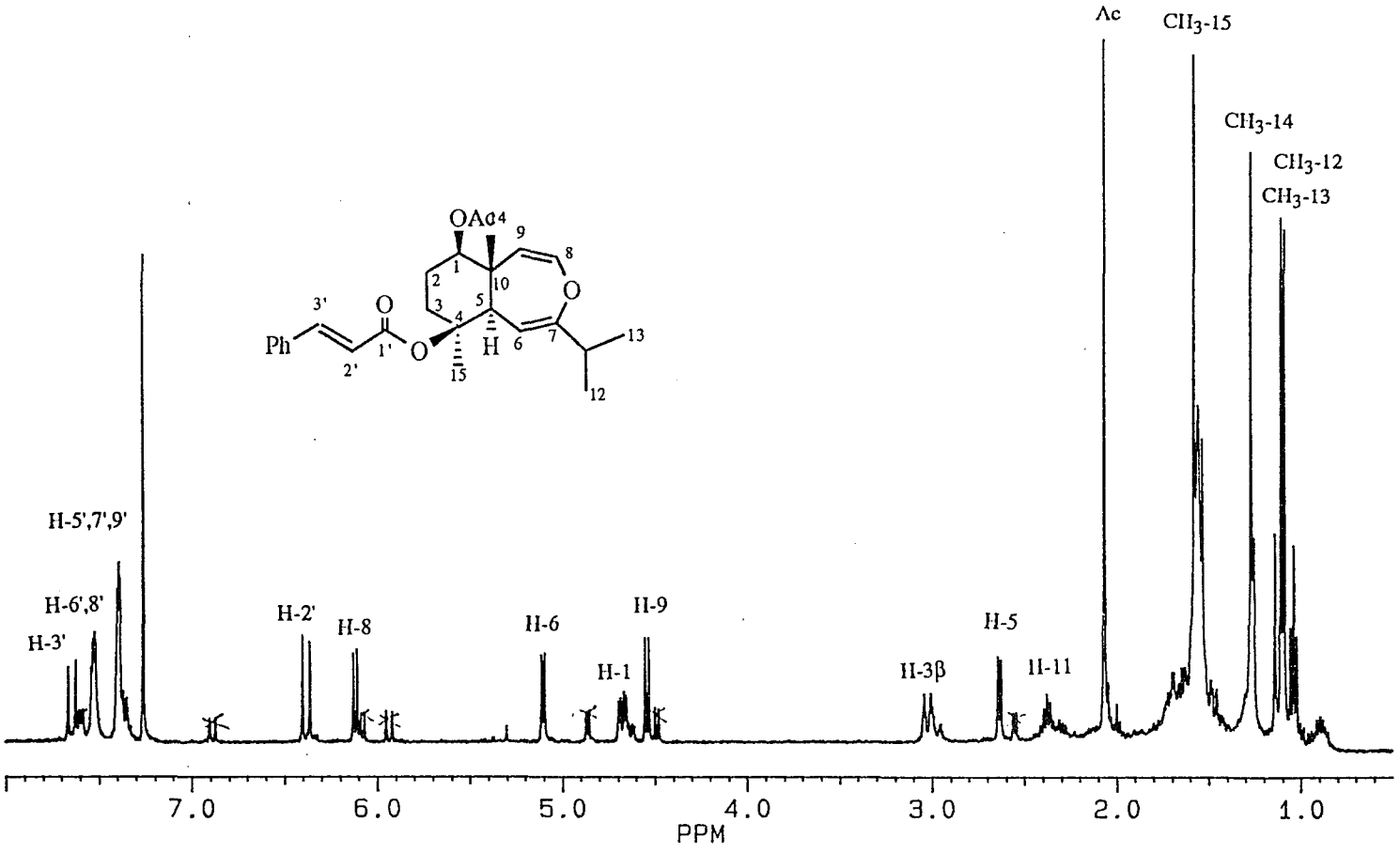


Figure 2.3.22. 400 MHz ^{1}H NMR spectrum of oxepin derivative (**212**)

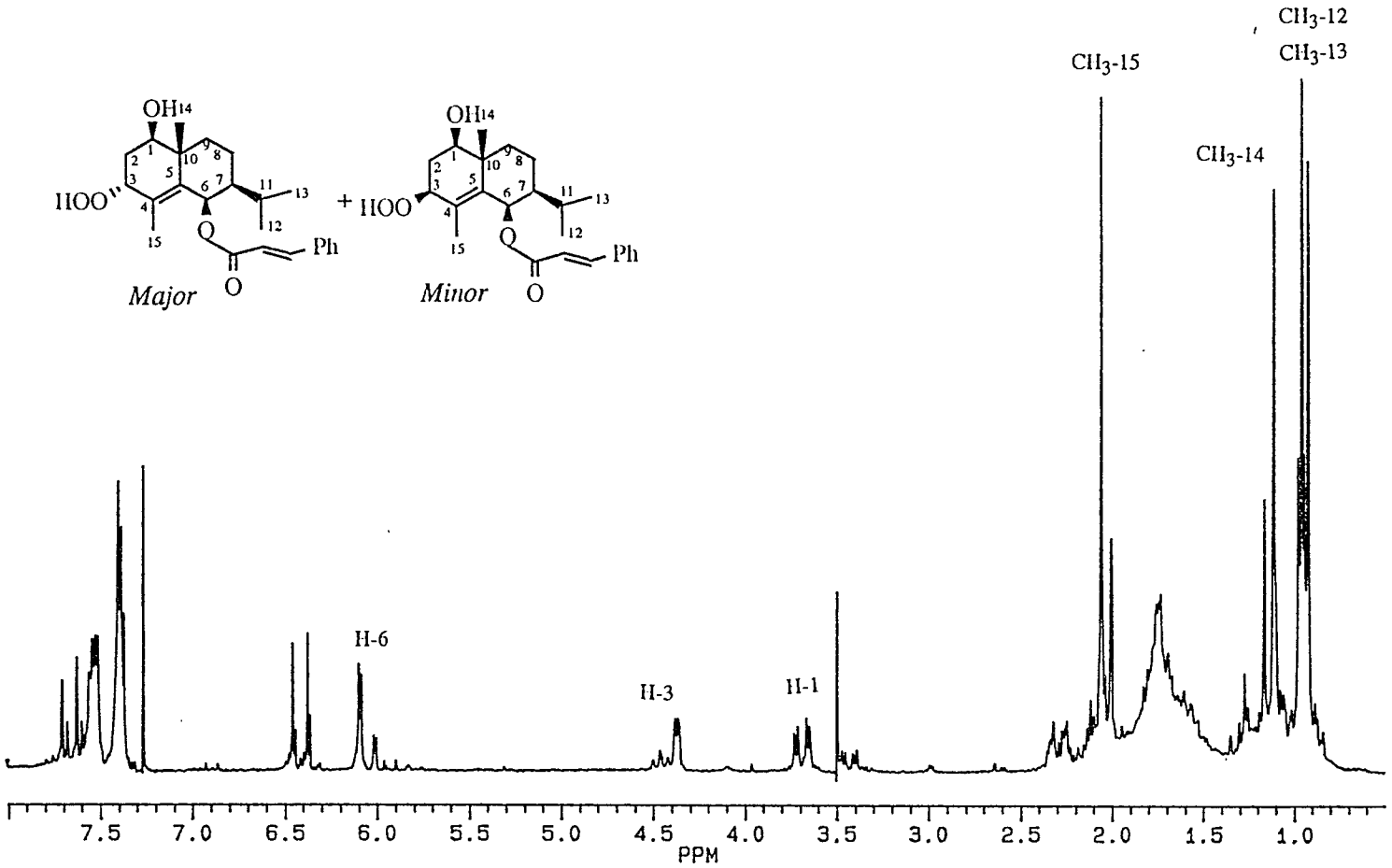


Figure 2.3.23. 200 MHz ^{1}H NMR spectrum of isomeric mixture of hydroperoxides **213** (major) and **214** (minor)

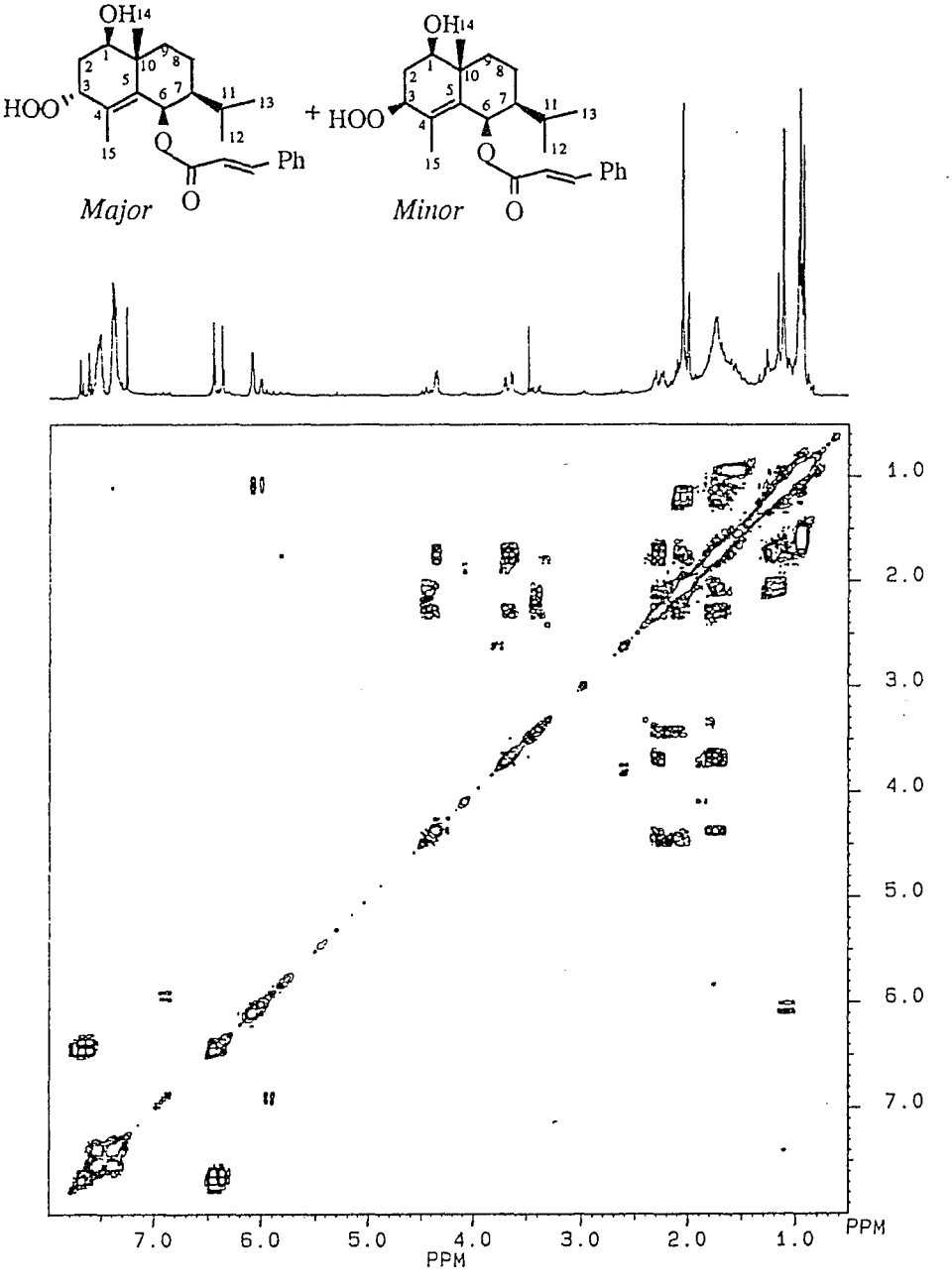


Figure 2.3.24. 200 MHz ^{1}H NMR COSY spectrum of isomeric mixture of hydroperoxides **213** (major) and **214** (minor)

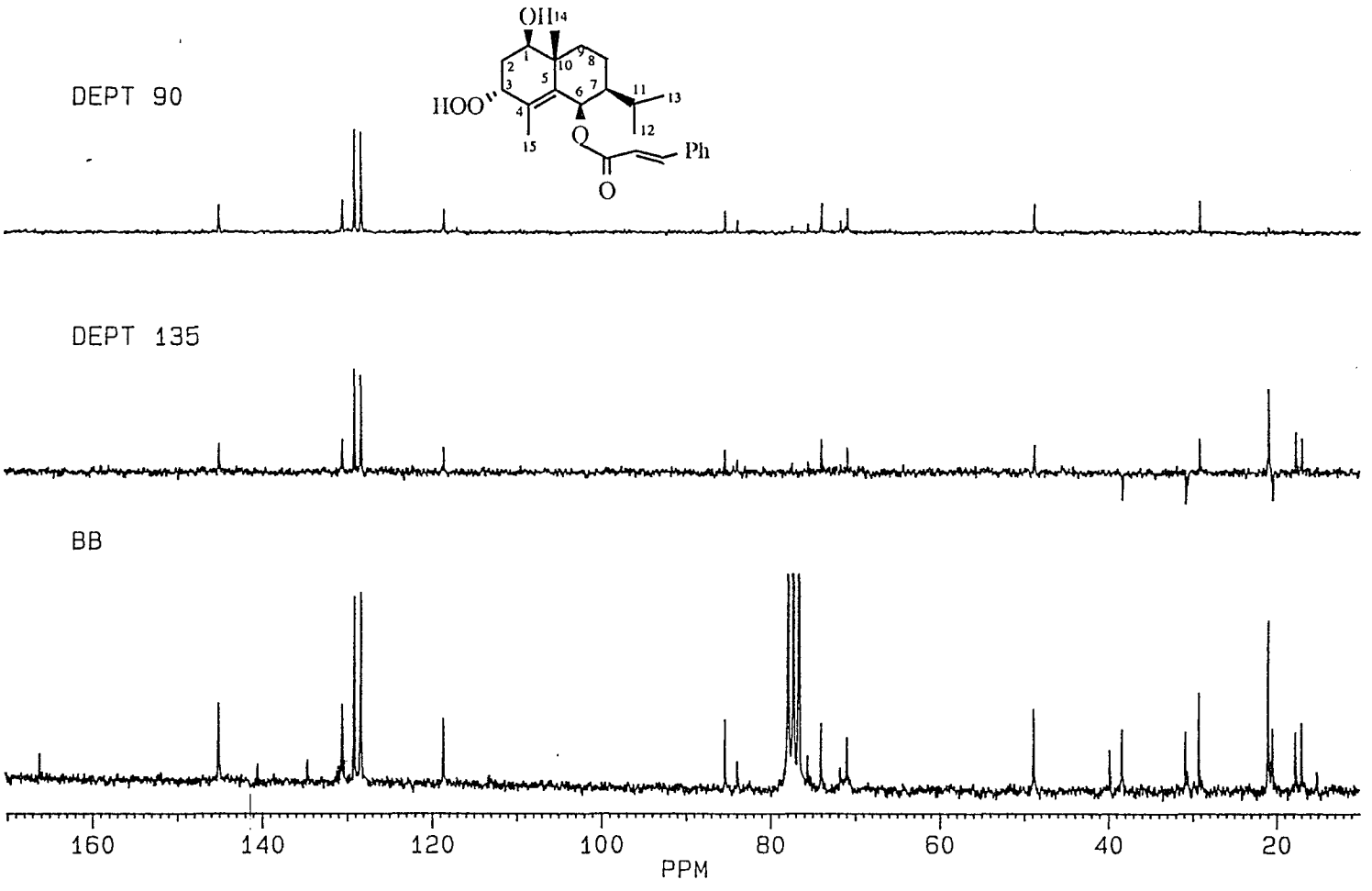


Figure 2.3.25. DEPT 90°, DEPT 135° and Broad Band ^{13}C NMR spectra of hydroperoxide **213**

similar to **213** with the exception of differences in chemical shifts due to configurational differences at C-3 (Table 2.3.2). Oxidation of a mixture of **213** and **214** with activated MnO_2 gave the conjugated ketone **215**, the ^1H NMR data of which were nearly identical to those previously reported [142, 146] except for the chemical shift of H-1 which we found to absorb as a doublet of doublet at δ 3.77. Compounds **213** and **214** probably represent artefacts formed by air-oxidation of **163** with molecular oxygen via a free radical process [147]. In order to resolve the stereochemistry at C-3 in hydroperoxides **213** and **214,** a singlet oxygen reaction of **163** was carried out in the presence of methylene blue as a photosensitizer under irradiation with a tungsten lamp, affording hydroperoxides **213**, **215** and **217**. The absence of **214** as a product of the singlet oxygen reaction supports the notion that formation of **213** and **214** most likely followed a stereochemically nonspecific free radical process rather than a concerted ene-reaction. Mechanistic and steric arguments require that the hydroperoxide group at C-3 in **213** must be α since H-5 in its precursor **163** is also α-oriented (Scheme 2.3.2). Thus, the C-3 isomer **214** must have a 3β-hydroperoxide group. These analyses are further supported by the ^1H NMR data of **217**. The pair of exocyclic methylene doublets at δ 5.14 and 5.17 (H-15, J=1.8 Hz) showed allylic couplings only to the proton signal at δ 2.29 (H-5) and no allylic coupling to the proton doublet of doublet at δ 4.44 (H-3) was observed in the COSY spectrum. Inspection of molecular models indicated that the coupling data were only in agreement with OOH-3α group in **217.** This is consistent with the proposed configuration at C-3 for compound **213** and the fact that in this concerted process, molecular oxygen has to approach the double bond from the α-face to abstract the α-oriented H-5. This data therefore suggests that the previously described eudesmene hydroperoxide, which had been assigned structure **214** [146], should be revised to structure **213.** The ^{13}C NMR spectrum of **213** exhibited 24 carbon signals which were assigned by the use of DEPT and $^1H,^{13}C$-correlations together with data obtained from

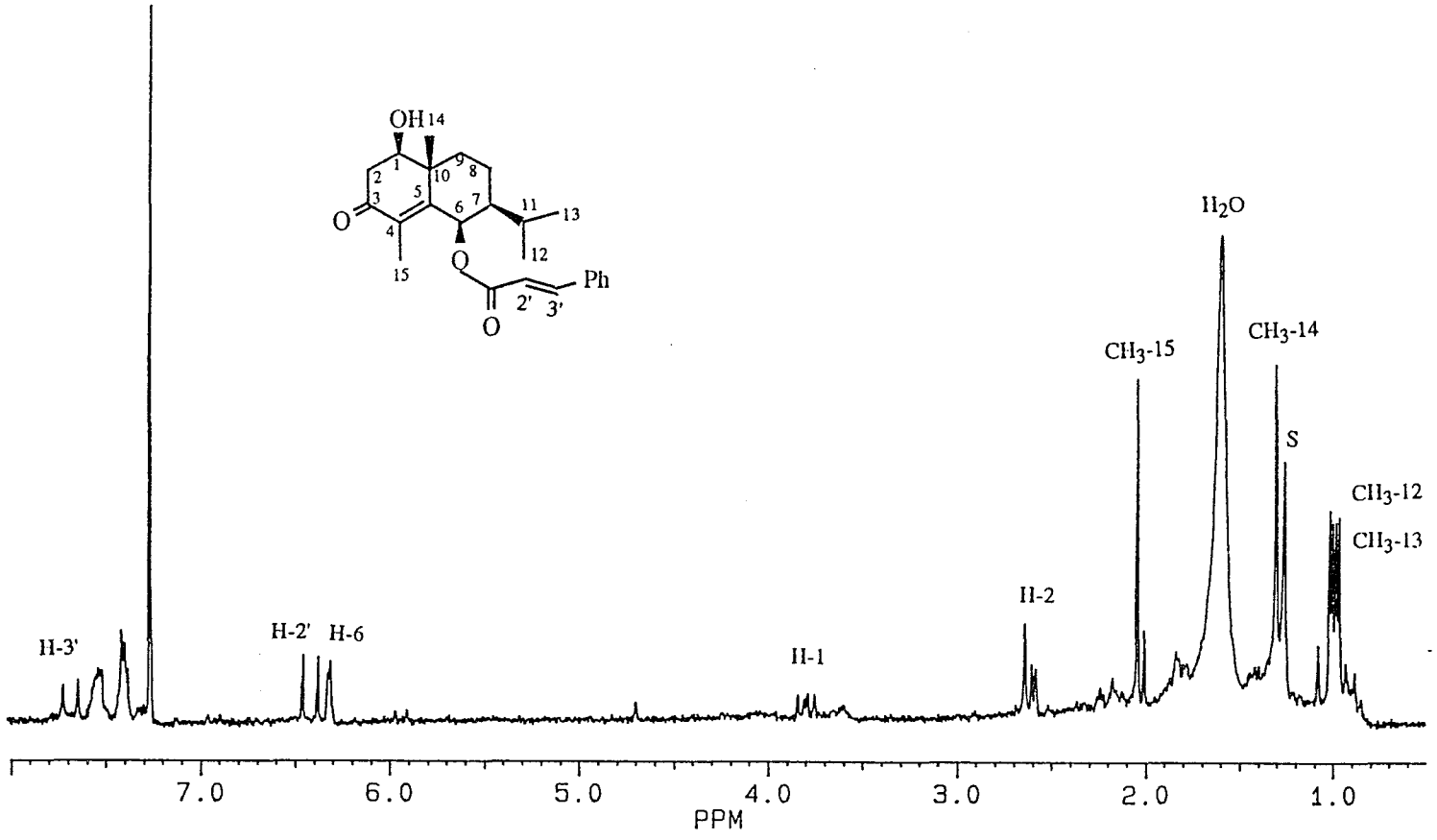


Figure 2.3.26. 200 MHz ^{1}H NMR spectrum of 6β-cinnamoyloxy-1β-hydroxy-3-one-4-ene (**215**)

COSY experiments. The unambiguous assignments of the two olefinic quaternary carbons 4 and 5 was carried out using the selective INEPT methodology [123, 124]. Selective polarization transfer from the proton signal at δ 3.68 (H-1), using a coupling parameter of 8 Hz ($^3J_{C,H}$),gave rise to the signal at δ 140.26 which was assigned to C-5. Therefore, the other quaternary carbon signal at δ 138.3 could be assigned to C-4.

Benzyl 2,6-dimethoxybenzoate (**169**) was previously isolated from *S. decurrens* [3] and *S. virgaurea* [39] and spectral data were in agreement with published values. The previously unreported ^{13}C NMR data of **169** showed only 11 signals due to its symmetrical structure. The combined application of COSY, ^1H-^{13}C correlation and DEPT experiments allowed for most of the carbon assignments. Quaternary carbons were assigned using the selective INEPT method. Polarization of the methoxy singlet at δ 3.80, using a coupling parameter of 8 Hz ($^3J_{C,H}$), transferred to the quaternary carbon signal at δ 157.5, which gave the assignments of C-2 and C-6. Irradiation of the methylene singlet at δ 5.38 (H-7'), using a coupling parameter of 8 Hz ($^3J_{C,H}$), transferred the polarization to two quaternary carbons: a strong signal at δ 166.4 (C-7) and a weaker two-bond coupled signal at δ 136.2 (C-1'). The enhancement of the signal at δ 113.1, upon polarization ($^3J_{C,H}$=8 Hz) of the two-proton doublet at δ 6.55 (H-3, H-5), confirmed its assignment as C-1 (see Experimental).

Taxonomically, the monotypic genus *Brintonia* is often included in the genus *Solidago* of the subtribe Solidaginiae (Asteraceae) [130]. Based on our chemical data, its closer association with the genus *Verbesina* of the subtribe Verbesiniae could be suggested. However, a eudesmane cinnamate had been previously isolated from *S. wrightii* [76] and benzyl 2,6-dimethoxybenzoate was found in *S. virgaurea* [39] and *S.*

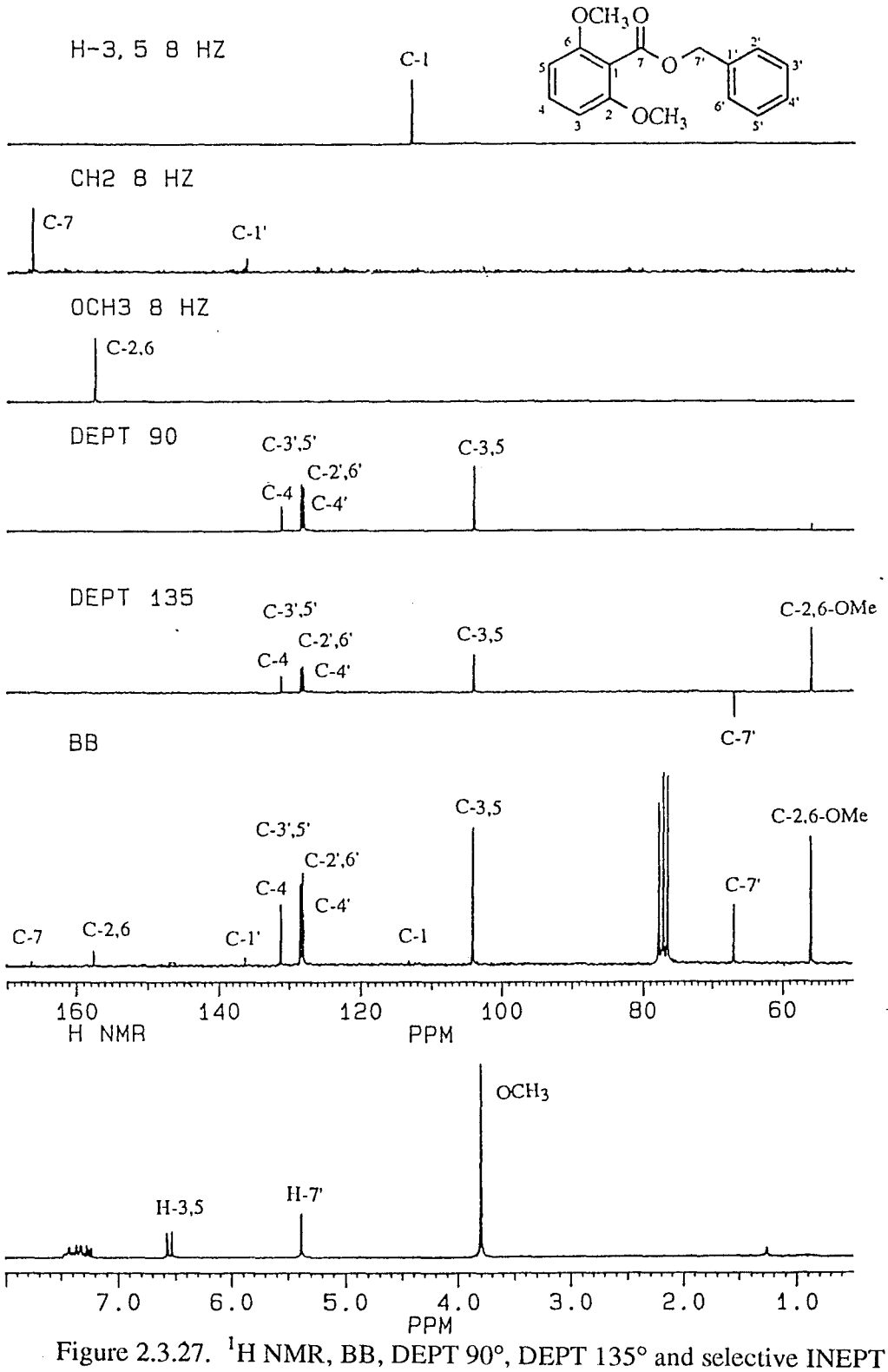


Figure 2.3.27. ^{1}H NMR, BB, DEPT 90°, DEPT 135° and selective INEPT spectra of benzyl 2,6-dimethoxybenzoate (**169**)

decurrens [3]. Therefore, further biochemical data within the subtribe Solidaginiae will be necessary, before final conclusions on the taxonomic association of *Brintonia* can be drawn.

Experimental

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM 400 spectrometer in $CDCl_3$. IR spectra were obtained from a Perkin-Elmer 1760X spectrometer in film on KBr plates. The mass spectra were run on a Hewlett-Packard 5971A GC-MS spectrometer. Vacuum liquid chromatographic (VLC) [129] separations were made on silica gel (MN Kieselgel G). Prep. thin layer chromatographic (TLC) separations were carried out on precoated MN Sil-G 25 UV_{254} plates (thickness 0.25 mm). Semipreparative HPLC separations were performed on a 10μ C-18 reversed-phase column (250x10mm, Phenomenex) coupled to a LDC/Milton Roy CM 4000 multi-solvent delivery system and an ISCO UV detector using a detecction wavelength at 264 nm.

Plant materials. Roots and aerial parts of *Brintonia discoidea* were collected on October 19, 1991 in Washington Parish, Louisiana, U.S.A. (Voucher No. Fischer-Lu 430; voucher deposited at the Louisiana State University Herbarium).

Extraction and isolation of constituents. Fresh roots (244 g) were soaked in CH_2Cl_2 for 24 hr to give 2.6 g of crude extract, which was chromatographed by VLC yielding 11x150 ml fractions. Fraction 5 provided 3 mg of **169** after further prep. TLC separation (hexane-EtOAc, 19:1). Reversed-phase semipreparative HPLC separation of fraction 6 (MeOH-H_2O, 3:1) provided 13 mg **163**, 5 mg **165** and 10 mg **213**. Fraction 7 afforded 641 mg of compound **209** and fraction 9 gave 61 mg of **216**. Fraction 8 after further semipreparative HPLC (MeOH-H_2O, 4:1) afforded 70 mg of **209**, 3 mg of **210** and 3 mg **211**. Fraction 10 provided 59 mg of **207** and fraction 11 afforded 13 mg of **208** upon further purification by semipreparative HPLC (MeOH-

H2O, 7:3). Comparison by TLC (hexane-EtOAc, 9:1) and ^{1}H NMR of the crude extract of leaves and stems with the above root extract showed that the aerial parts had the same terpenoid patterns as the root extract with minor quantitative differences.

6β-Cinnamoyloxy-1β-hydroxyeudesm-3-ene (**163**). $C_{24}H_{32}O_3$, colorless powder; IR ν_{max}^{KBr} cm^{-1}: 3427 (OH); 1707 (C=O); 1636, 1450 (C=C); 1176 (CC(=O)OC, ester). EIMS *m/z* (rel. int.): 368 $[M]^+$ (6.1); 350 $[M-H_2O]^+$ (1.4); 220 $[M-C_6H_5CH=CHCO_2H]^+$ (23.6); 202 $[220-H_2O]^+$ (29.2); 177 $[220-(CH_3)_2CH]^+$ (18.6); 159 $[202-(CH_3)_2CH]^+$ (34.2); 148 $[C_6H_5CH=CHCO_2H]^+$ (2.0); 147 $[148-H]^+$ (4.4); 131 $[C_6H_5CH=CHCO]^+$ (100); 103 $[131-CO]^+$ (32.1); 77 $[C_6H_5]^+$ (15.0); 43 $[(CH_3)_2CH]^+$ (14.3). ^{1}H NMR data in Table 2.3.2 and ^{13}C NMR data in Table 2.3.3.

Singlet oxygen reaction of compound **163**. Methylene blue (1 mg) and **163** (6 mg) were dissolved in 20 ml CH_2Cl_2 and irradiated using a tungsten filament lamp, while oxygen was bubbled through the solution for 6 hr at 0°-5°C. Removal of solvent and prep. TLC (hexane-EtOAc, 4:1) afforded ketone **215** (1 mg) and 2 mg of a 1:1 mixture of hydroperoxides **213** and **217.**

6β-Cinnamoyloxy-3α-hydroperoxy-1β-hydroxyeudesm-4(15)-ene(**217**). $C_{24}H_{32}O_5$, oil; IR ν_{max}^{KBr} cm^{-1}: 3402 (OH); 1706 (C=O), 1635, 1450 (C=C), 1167 (conj. ester); CIMS *m/z* (rel. int.): 383 $[M-17]^+$ (8), 235 $[383-C_6H_5CH=CHCO_2H]^+$ (42), 149 $[C_6H_5CH=CHCO_2H + 1]^+$ (100), 131 $[C_6H_5CH=CHCO]^+$ (58); ^{1}H NMR data of **217** is in Table 2.3.2.

6β-Cinnamoyloxy-1β-hydroxyeudesm-4(15)-ene (**165**). $C_{24}H_{32}O_3$, powder; IR ν_{max}^{KBr} cm^{-1}: 3426 (OH); 1706 (C=O), 1637, 1451 (C=C), 1175, 1033 (CC(=O)OC, ester); EIMS *m/z* (rel. int.): 368 $[M]^+$ (2.8), 350 $[M-H_2O]^+$ (1.0), 220 $[M-C_6H_5CH=CHCO_2H]^+$ (8.8), 202 $[220-H_2O]^+$ (7.8), 177 $[220-(CH_3)_2CH]^+$ (9.0), 159 $[202-(CH_3)_2CH]^+$ (12.7), 148 $[C_6H_5CH=CHCO_2H]^+$ (0.9), 147 $[148-H]^+$ (2.2), 131 $[C_6H_5CH=CHCO]^+$ (100), 103 $[131-CO]^+$ (20.2), 77 $[C_6H_5]^+$ (8.8), 43 $[(CH_3)_2CH]^+$ (5.2); ^{1}H NMR data in Table 2.3.2 and ^{13}C NMR data in Table 2.3.3.

Benzyl 2,6-dimethoxybenzoate (**169**). $C_{16}H_{16}O_4$, colorless oil; UV λ_{max}^{EtOH} nm: 212; 278; IR ν_{max}^{KBr} cm^{-1}: 1732 (C=O), 1594, 1472 (aromatic C=C), 1256, 1111 (conj. ester); EIMS *m/z* (rel. int.): 272 $[M]^+$ (52.8), 241 $[M\text{-}OMe]^+$ (4.2), 181 $[M\text{-}C_7H_7]^+$ (4.9), 165 $[M\text{-}C_7H_7O]^+$ (100), 150 $[165\text{-}Me]^+$ (18.8), 138 (67.5), 122 $[150\text{-}CO]^+$ (9.8), 107 $[C_7H_7O]^+$ (29.8), 91 $[C_7H_7]^+$ (85.6), 77 $[C_6H_5]^+$ (20.0); ^1H NMR (400 MHz, $CDCl_3$): δ 3.80 (6H, s, OMe), 5.38 (2H, s, H-7'), 6.55 (2H, d, J = 8.3 Hz, H-3,5), 7.27 (1H, t, J= 8.3 Hz, H-4), 7.34 (1H, m, H-4'), 7.35 (2H, m, H- 3', 5'), 7.44 (2H, br. d, J= 7.0Hz, H-2', 6'); ^{13}C NMR (100 MHz, $CDCl_3$): δ 113.1 (s, C-1), 157.5 (s, C-2, C-6), 104.0 (d, C-3, C-5), 131.1 (d, C-4), 166.4 (s, C-7), 136.2 (s, C-1'), 128.1 (d, C-2', C-6'), 128.3 (d, C-3', C-5'), 127.9 (d, C-4'), 66.8 (t, C-7'), 56.0 (q, C-2, 6- OMe); Peak multiplicities (s=singlet, d=doublet, t=triplet, q=quartet) were determined by DEPT experiments.

4β-Cinnamoyloxy-1β,2α-dihydroxyeudesm-7-ene (**207**). $C_{24}H_{32}O_4$, powder; IR ν_{max}^{KBr} cm^{-1}: 3416 (OH), 1706 (C=O), 1632, 1455 (C=C), 1161, 1042 (CC(=O)OC, ester); EIMS (probe) *m/z* (rel. int.): 236 $[M\text{-}C_6H_5CH{=}CHCO_2H]^+$ (56.8), 218 $[M\text{-}H_2O]^+$ (8.0), 203 $[218\text{-}CH_3]^+$ (12.5), 193 $[236\text{-}(CH_3)_2CH]^+$ (4.5), 148 $[C_6H_5CH{=}CHCO_2H]^+$ (60.2), 147 $[148\text{-}H]^+$ (17.0), 131 $[C_6H_5CH{=}CHCO]^+$ (100), 103 $[131\text{-}CO]^+$ (62.5), 77 $[C_6H_5]^+$ (30.7), 43 $[(CH_3)_2CH]^+$ (47.7); ^1H NMR data in Table 2.3.1 and ^{13}C NMR data in Table 2.3.3.

4β-Cinnamoyloxy-1β,2α-dihydroxyeudesm-6-ene (**208**). $C_{24}H_{32}O_4$, powder; IR ν_{max}^{KBr} cm^{-1}: 3396 (OH), 1707 (C=O), 1635, 1455 (C=C), 1162 (CC(=O)OC, ester); EIMS (probe) *m/z* (rel. int.): 384 $[M]^+$ (0.4), 253 $[M\text{-}C_6H_5CH{=}CHCO]^+$ (1.8), 236 $[M\text{-}C_6H_5CH{=}CHCO_2H]^+$ (14.1), 221 $[236\text{-}CH_3]^+$ (4.8), 218 $[236\text{-}H_2O]^+$ (5.3), 203 $[208\text{-}CH_3]^+$ (18.5), 193 $[236\text{-}(CH_3)_2CH]^+$ (5.3), 148 $[C_6H_5CH{=}CHCO_2H]^+$ (12.8), 147 $[148\text{-}H]^+$ (4.4), 131 $[C_6H_5CH{=}CHCO]^+$ (100), 103 $[131\text{-}CO]^+$ (43.2), 77 $[C_6H_5]^+$ (47.1), 43 $[(CH_3)_2CH]^+$ (37.2); ^1H NMR spectral data in Table 2.3.1 and ^{13}C NMR data in Table 2.3.3.

4β-Cinnamoyloxy-1β-hydroxyeudesm-7-ene (**209**). $C_{24}H_{32}O_3$, powder; IR ν_{max}^{KBr} cm^{-1}: 3436 (OH), 1705 (C=O), 1636, 1452 (C=C), 1163, 1030 (CC(=O)OC, ester); EIMS *m/z* (rel. int.): 368 $[M]^+$ (0.7), 350 $[M-H_2O]^+$ (0.3), 220 $[M-C_6H_5CH=CHCO_2H]^+$ (12.8), 202 $[220-H_2O]^+$ (18.6), 187 $[202-CH_3]^+$ (5.8), 177 $[220-(CH_3)_2CH]^+$ (15.1), 159 $[202-(CH_3)_2CH]^+$ (28.1), 148 $[C_6H_5CH=CHCO_2H]^+$ (1.5), 147 $[148-H]^+$ (3.6), 131 $[C_6H_5CH=CHCO]^+$ (100), 103 $[131-CO]^+$ (27), 77 $[C_6H_5]^+$ (11.7), 43 $[(CH_3)_2CH]^+$ (9.3); ^1H NMR data in Table 2.3.1 and ^{13}C NMR data in Table 2.3.3.

4β-Cinnamoyloxy-1β-hydroxyeudesm-6-ene (**210**). $C_{24}H_{32}O_3$, powder; IR ν_{max}^{KBr} cm^{-1}: 3413 (OH), 1703 (C=O), 1635, 1453 (C=C), 1161 (CC(=O)OC); EIMS (probe) *m/z* (rel.int.): 368 $[M]^+$ (0.3), 220 $[M-C_6H_5CH=CHCO_2H]^+$ (14.5), 202 $[220-H_2O]^+$ (10.9), 187 $[202-CH_3]^+$ (15.8), 177 $[220-(CH_3)_2CH]^+$ (9.1), 159 $[202-(CH_3)_2CH]^+$ (1.6), 148 $[C_6H_5CH=CHCO_2H]^+$ (7.5), 147 $[148-H]^+$ (8.3), 131 $[C_6H_5CH=CHCO]^+$ (100), 103 $[131-CO]^+$ (35.3), 77 $[C_6H_5]^+$ (33.7), 43 $[(CH_3)_2CH]^+$ (30.5); ^1H NMR data in Table 2.3.1 and ^{13}C NMR data in Table 2.3.3.

4β-Cinnamoyloxy-7α-hydroperoxy-1β-hydroxyeudesm-8-ene (**211**). $C_{24}H_{32}O_5$, powder; IR ν_{max}^{KBr} cm^{-1}: 3413 (OH), 1704 (C=O), 1635, 1453 (C=C), 1178, 1029 (conj. ester); PDMS *m/z* (rel. int.): 383 $[M-OH]^+$ (5.5), 235 $[383-C_6H_5CH=CHCO_2H]^+$ (22.6), 147 $[C_6H_5CH=CHCO_2]^+$ (12.4), 131 $[C_6H_5CH=CHCO]^+$ (97.8), 103 $[131-CO]^+$ (22.0), 43 $[(C_3H_7]^+$ (100); ^1H NMR spectral data in Table 2.3.1 and ^{13}C NMR data in Table 2.3.3.

Oxepin Derivative **212**. Acetylation of **211** (3mg) with Ac_2O in pyridine for 12 hr at room temp., after prep. TLC (hexane-EtOAc, 4:1), afforded 1 mg of the oxepin derivative **212**, $C_{26}H_{32}O_5$, colorless powder; IR ν_{max}^{KBr} cm^{-1}: 1733, 1712 (C=O), 1640, 1455 (C=C), 1244, 1027 (conj.ester); EIMS (probe) *m/z* (rel. int.): 381 $[M-Ac]^+$ (7.8), 233 $[M-148-43]^+$ (60.6), 217 (5.8), 148 $[C_6H_5CH=CHCO_2H]^+$ (55.2), 131 $[C_6H_5CH=CHCO]^+$ (100), 103 $[131-CO]^+$ (5.2), 97 (16.7); 83 (17.6), 81 (40.0), 77

$[C_6H_5]^+$ (16.7), 69 (68.2), 60 $[MeCO_2H]^+$ (20.4), 55 (29.9), 43 $[C_3H_7]^+$ and $[MeCO]^+$(63.2); FAB MS (nitrobenzyl alcohol, MW 153, as matrix): 577 $[M+153]^+$ (6), 425 $[M+1]^+$ (2), 424 $[M]^+$ (2), 423 $[M-1]^+$ (3), 277 $[M-147]^+$ (20); ^1H NMR data in Table 2.3.1.

6β-Cinnamoyloxy-3α-hydroperoxy-1β-hydroxyeudesm-4-ene (**213**) *and the 3β-isomer* (**214**). $C_{24}H_{32}O_4$, powder; IR ν_{max}^{KBr} cm^{-1}: 3417 (OH), 1707 (C=O), 1636, 1454 (C=C), 1171 (conj. ester); EIMS *m/z* (rel. int.): 382 $[M-H_2O]^+$ (2.9), 339 $[382-C_3H_7]^+$ (0.8), 234 $[382-C_6H_5CH=CHCO_2H]^+$ (14.7), 191 $[234-C_3H_7]^+$ (27.0), 148 $[C_6H_5CH=CHCO_2H]^+$ (2.7), 147 $[148-H]^+$ (6.9), 131 $[C_6H_5CH=CHCO]^+$ (100), 103 $[131-CO]^+$ (16.1), 77 $[C_6H_5]^+$ (6.7), 43 $[C_3H_7]^+$ (6.7); ^1H NMR data in Table 2.3.2 and ^{13}C NMR data in Table 2.3.3.

6β-Cinnamoyloxy-1β-hydroxyeudesm-3-one-4-ene (**215**). A mixture of **213** and **214** (3 mg) was stirred with activated MnO_2 in 10 ml CH_2Cl_2 for 1 hr at room temp., affording 2 mg of **215**, $C_{24}H_{30}O_4$, powder; IR ν_{max}^{KBr} cm^{-1}: 3441 (OH), 1712, 1676 (C=O), 1639, 1459 (C=C), 1261, 1075 (conj. ester); EIMS *m/z* (rel. int.): 382 $[M]^+$ (3.2), 339 $[M-C_3H_7]^+$ (1.1), 234 $[M-C_6H_5CH=CHCO_2H]^+$ (12.1), 191 $[234-C_3H_7]^+$ (23.5), 148 $[C_6H_5CH=CHCO_2H]^+$ (2.8), 147 $[148-H]^+$ (6.8), 131 $[C_6H_5CH=CHCO]^+$ (100), 103 $[131-CO]^+$ (18.4), 77 $[C_6H_5]^+$ (10.2), 43 $[C_3H_7]^+$ (8.0); ^1H NMR data in Table 2.3.2.

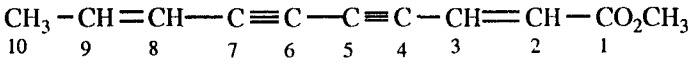
6β-Cinnamoyloxy-4β,9β-dihydroxyeudesmane (**216**). $C_{24}H_{34}O_4$, gum; IR ν_{max}^{KBr} cm^{-1}: 3458 (OH), 1702 (C=O), 1637, 1454 (C=C), 1170 (CC(=O)OC, ester); EIMS (probe) *m/z* (rel. int.): 384 $[M-2]^+$ (0.1), 220 $[M-H_2O-C_6H_5CH=CHCO_2H]^+$ (1.4), 177 $[220-(CH_3)_2CH]^+$ (1.1), 148 $[C_6H_5CH=CHCO_2H]^+$ (17.5), 147 $[148-H]^+$ (2.4), 131 $[C_6H_5CH=CHCO]^+$ (46.7), 103 $[131-CO]^+$ (19.5), 101 (100), 77 $[C_6H_5]^+$ (11.3), 43 $[(CH_3)_2CH]^+$ (13.9); ^1H NMR data in Table 2.3.2 and ^{13}C NMR data in Table 2.3.3.

2.4

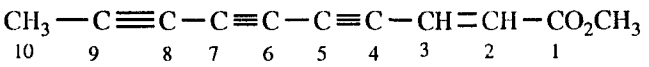
Anti-mycobacterial Polyacetylenes and Other Constituents from *Chrysoma pauciflosculosa* and *Erigeron philadelphicus*

Introduction

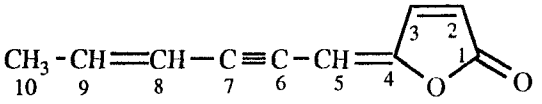
Polyacetylenes are common constituents in the members of the tribe Astereae (Asteraceae) [7, 50]. In recent years a large number of reports have appeared dealing with the biological activities of these compounds. Polyacetylenes often exhibit antifungal and nematicidal activities and are phototoxic against certain viruses [95, 97]. They has also been reported to possess anti-tumor activities [57]. In continuation of our search for biologically active compounds from the Asteraceae family, we have investigated roots of *Chrysoma pauciflosculosa* (syn. *Solidago pauciflosculosa*) and *Erigeron philadelphicus*. *Chrysoma pauciflosculosa*, the woody goldenrod is a shrub in the Asteraceae family found in the Florida scrub. Previous investigation of this plant provided *cis,cis*- and *cis,trans*-matricaria esters (ME) which showed strong inhibitory effects on sandhill grasses [101]. *Erigeron philadelphicus*, commonly referred to fleabane, has also been reported to contain matricaria esters [148]. A reinvestigation of *C. pauciflosculosa* resulted in the isolation of the previously found acetylenes *cis,cis*- and *cis,trans*-matricaria esters (**126a** and **b**) and the epoxide derivatives **218a** and **218b** [101]. In addition, the two triterpenes epifriedelinol (**219**) and friedelin (**220**) as well as the known 2,6-dimethoxybenzoquinone (**221**) [149] and a new benzotropolone (**222**) were isolated. The structure of **222** was determined by mass spectral analysis and ^{1}H and ^{13}C NMR methods. Investigation of *E. philadelphicus* also afforded acetylenes **126a-b, 218a-b**



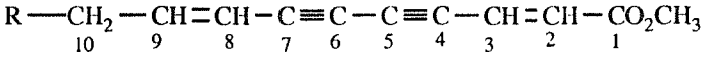
126a 2,3-(Z), 8,9-(Z)
126b 2,3-(E), 8,9-(Z)



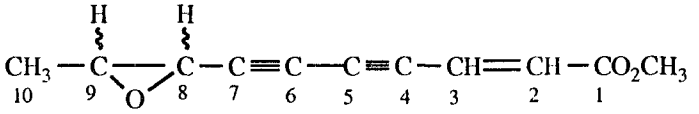
127a 2,3-(Z)



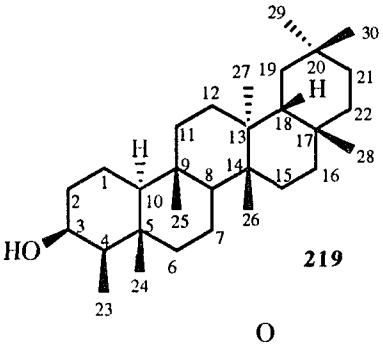
130a 4,5-(Z), 8,9-(Z)
130b 4,5-(E), 8,9-(Z)



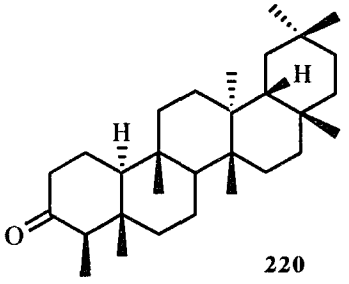
133a 2,3-(Z), 8,9-(Z), R=OAngelate
133b 2,3-(E), 8,9-(Z), R=OAngelate
134 2,3-(Z), 8,9-(Z), R=OTiglate



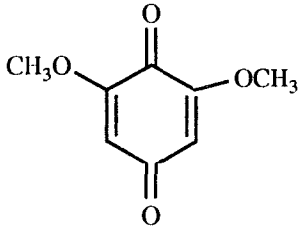
218a 2,3-(Z), 8,9-cis
218b 2,3-(Z), 8,9-trans



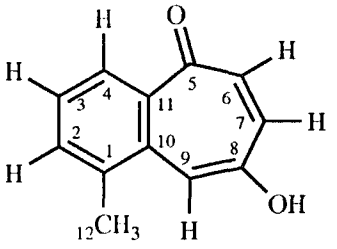
219



220



221



222

Figure 2.4.1. Polyacetylenes and other constituents from *Chrysoma pauciflosculosa* and *Erigeron philadelphicus*

and two other known matricaria lactones **130a** and **130b**. Several polyacetylenes and compounds **219-222** were tested for their activity against *Mycobacterium tuberculosis* and *M. avium*. The results will be presented in the later section.

Results and Dicussion

The matricaria esters **126a** and **126b** and their epoxides **218a** and **218b** had been previously found in *Chrysoma pauciflosculosa* [101] and *Erigeron philadelphicus* [148]. Compounds **126a** and **126b** are among the most wide-spread acetylenic compounds in the Asteraceae [50] and they are abundant in the roots of *Solidago canadensis* [26]. The ^{1}H NMR spectral data of matricaria esters **126a** and **126b** and the epoxy derivatives of matricaria ester **218a** and **218b** were identical to those of previously reported data [50, 148]. The structures of isomeric matricaria lactones **130a** and **130b** were derived by high field ^{1}H NMR analysis and by direct comparison with those of previously reported data [58].

The molecular structure of epifriedelinol (**219**) was previously described [101]. Oxidation of epifriedelinol with Jones' reagent afforded friedelin (**220**) which was identical to the naturally occurring triterpene isolated from *C. pauciflosculosa* as indicated by their ^{1}H and ^{13}C NMR and mass spectral data. Both epifriedelinol (**219**) and friedelin (**220**) had been previously isolated from *Palicourea rigida* [150].

The ^{1}H NMR of compound **222** showed that except for one methyl singlet at δ 2.69, all of the proton signals were in the δ 7.0-8.5 region, indicating that it represented a conjugated aromatic compound. Strong UV absorbance at λ_{max} 240 and 282 further suggested the conjugation with aromatic ring. The ^{13}C NMR data of **222** indicated the presence of 12 carbons with one methyl, one carbonyl, six CH and four quaternary carbons in olefinic or aromatic absorption region. A broad proton singlet at δ 8.17 and the IR absorbance at 3289 cm^{-1} indicated the presence of an OH group which could be either on the aromatic ring or part of a conjugated double bond. Close

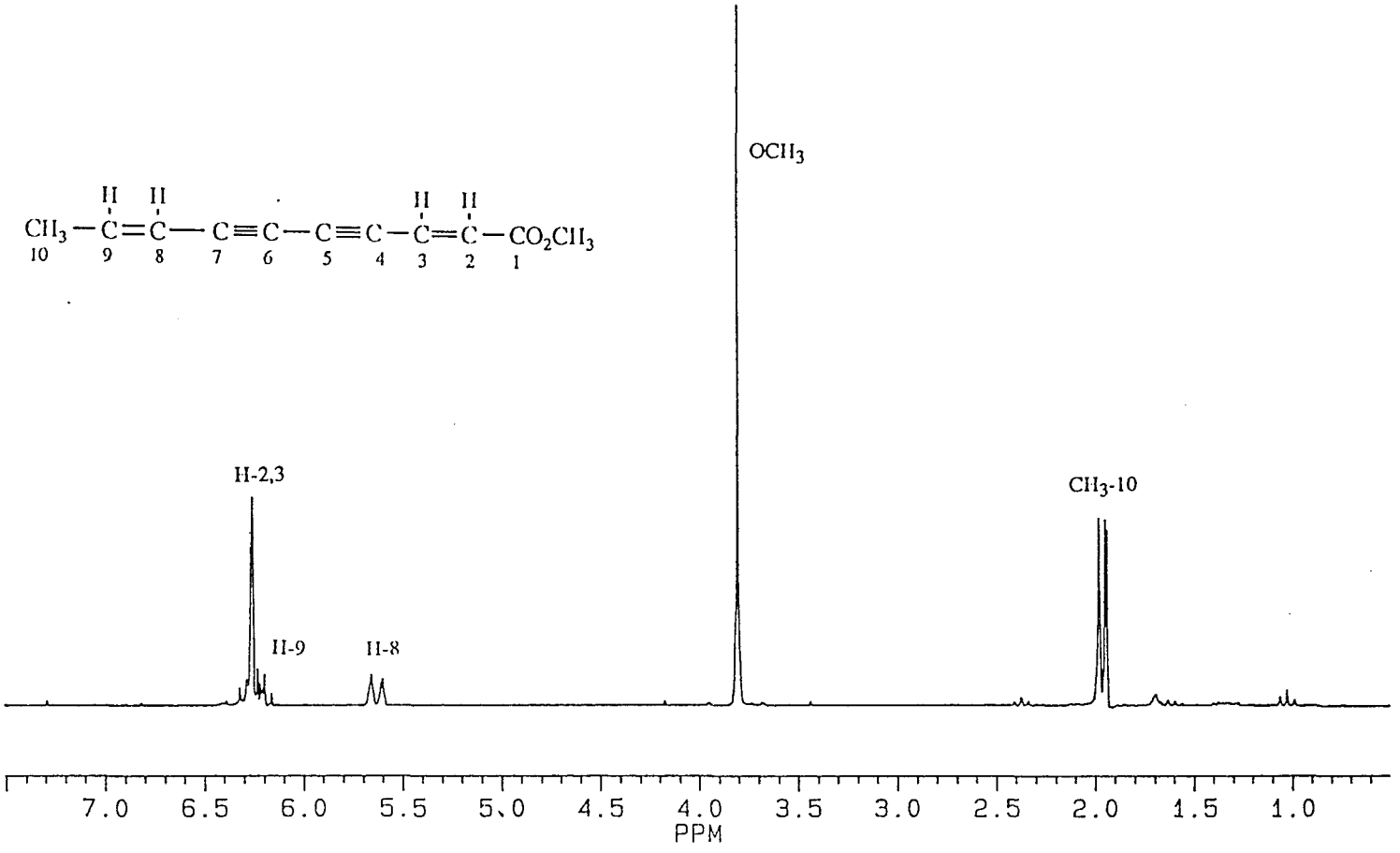


Figure 2.4.2. 200 MHz ^{1}H NMR spectrum of *cis,cis*-matricaria ester (**126a**)

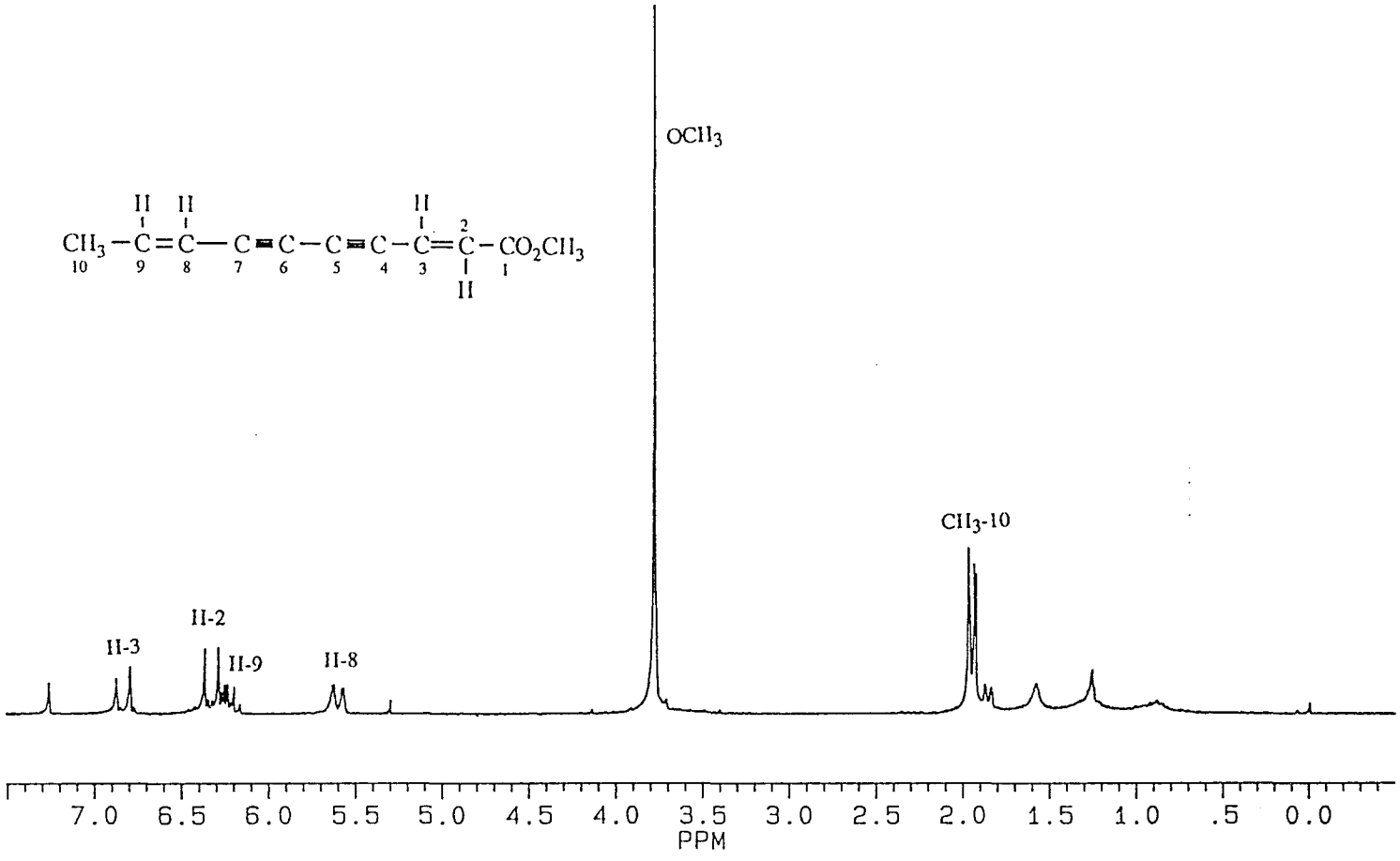


Figure 2.4.3. 200 MHz ^{1}H NMR spectrum of *cis,trans*-matricaria ester (**126b**)

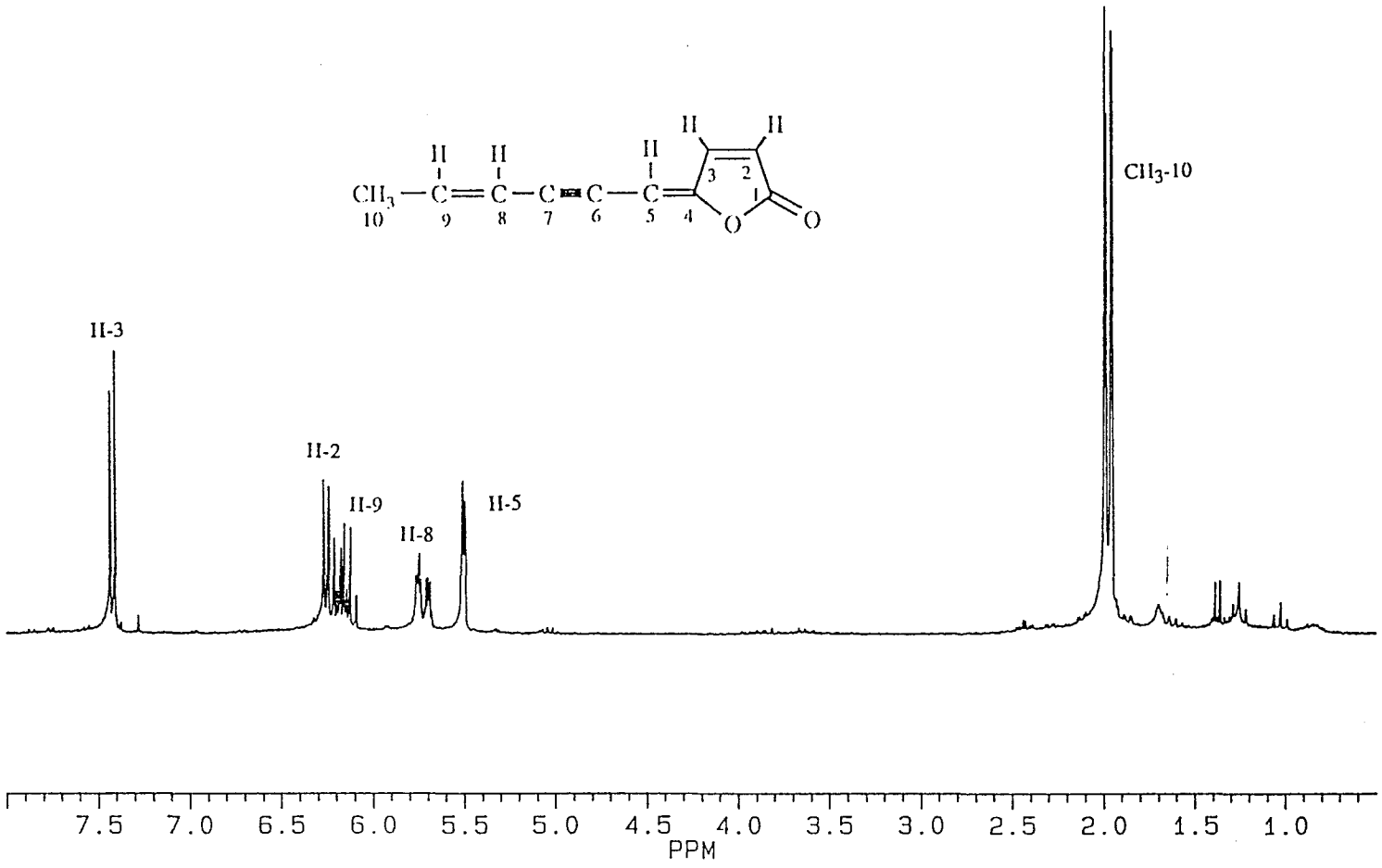


Figure 2.4.4. 200 MHz ^{1}H NMR spectrum of 4Z,8Z-matricaria lactone (**130a**)

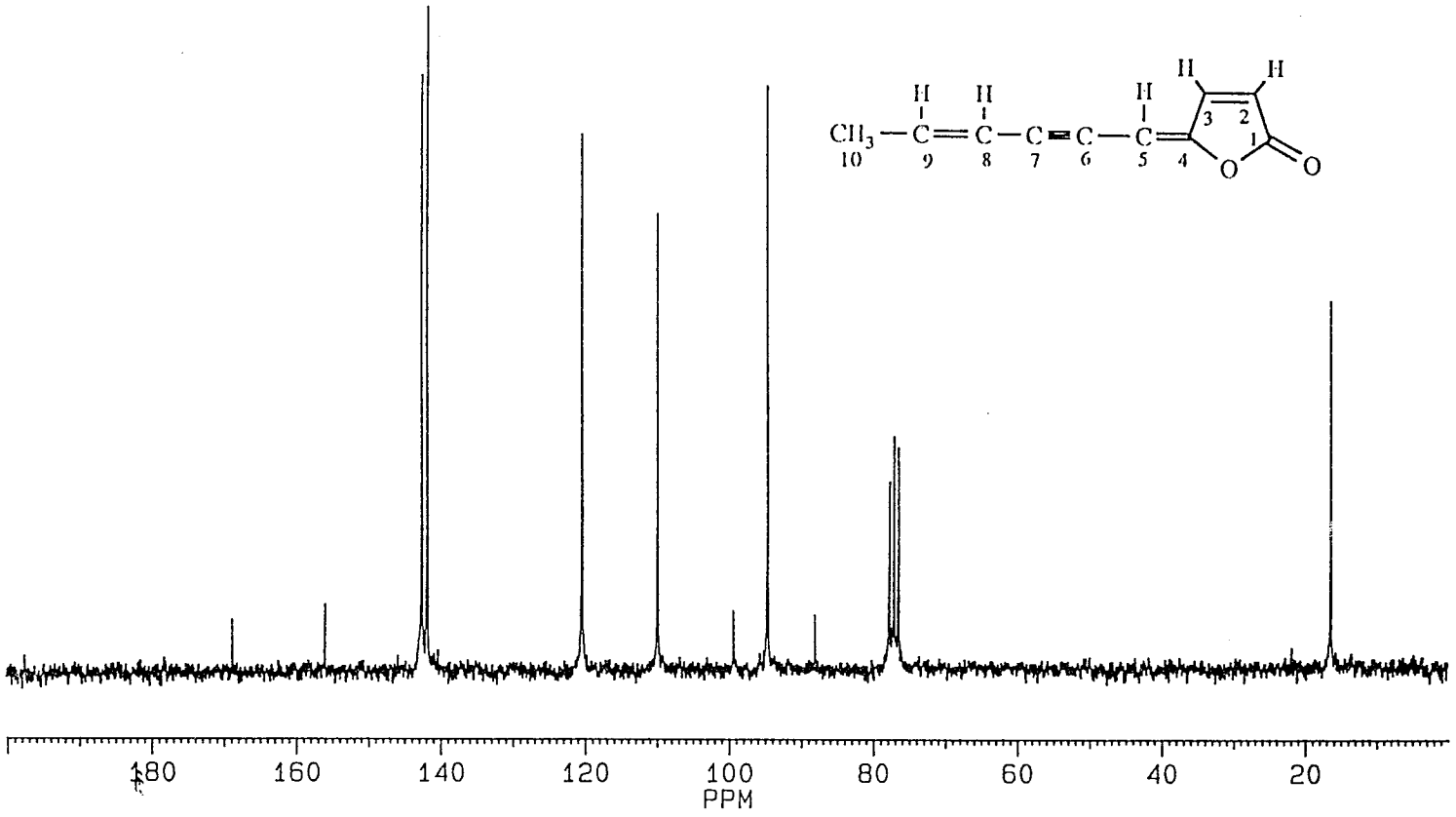


Figure 2.4.5. 50 MHz ^{13}C NMR spectrum of 4Z,8Z-matricaria lactone (**130a**)

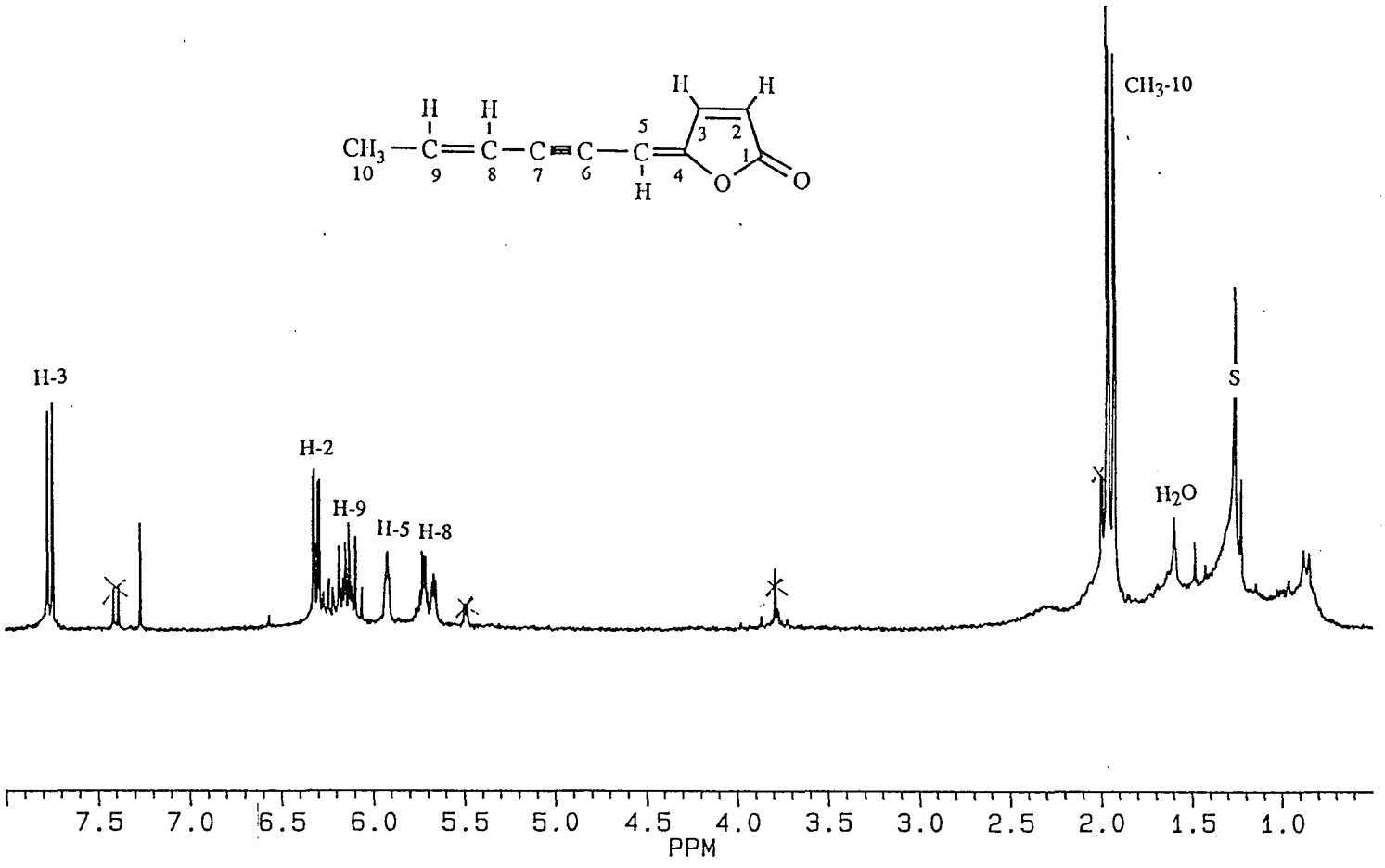


Figure 2.4.6. 200 MHz ^{1}H NMR spectrum of 4*E*,8*Z*-matricaria lactone (**130b**)

Table 2.4.1. ^{1}H NMR data of compounds **130a** and **130b** (400 MHz, $CDCl_3$ as int. std.)

H	**130a**	**130b**
2	6.24 d	6.30 dd
3	7.40 d	7.75 d
5	5.48 br d	5.91 dd
8	5.71 dq	5.69 dq
9	6.16 dq	6.13 dq
10	1.96 dd	1.93 dd

J (Hz): **130a**: 2,3=5.4, 3,5=2.6, 8,9=10.5, 8,10=1.7, 9,10=7.0; **130b**: 2,3=5.4, 3,5=2.1, 2,5=1.8, 8,9=11, 8,10=1.6, 9,10=7.0.

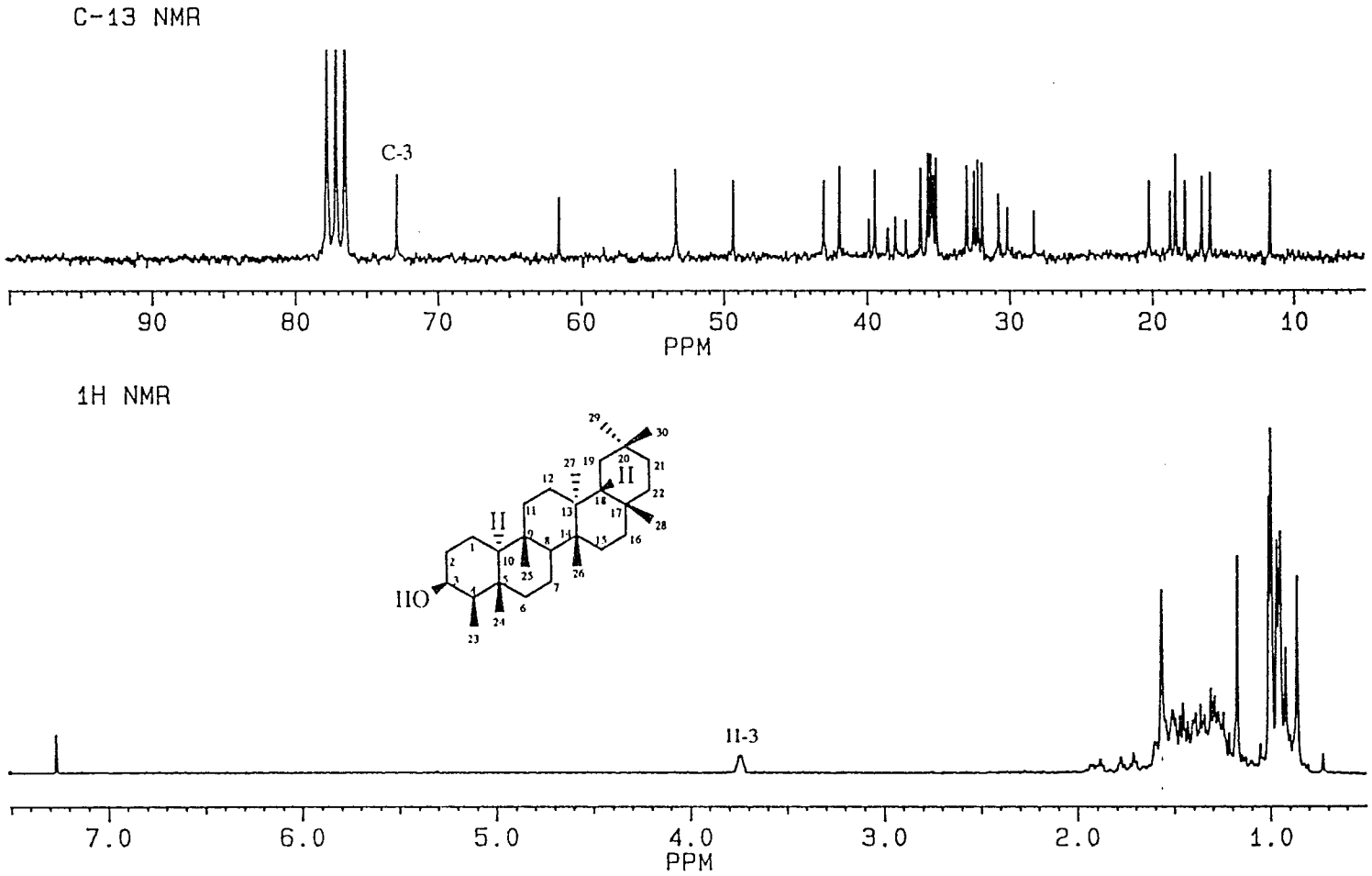


Figure 2.4.7. 200 MHz ^{1}H and 50 MHz ^{13}C NMR spectra of epifriedelinol (**219**)

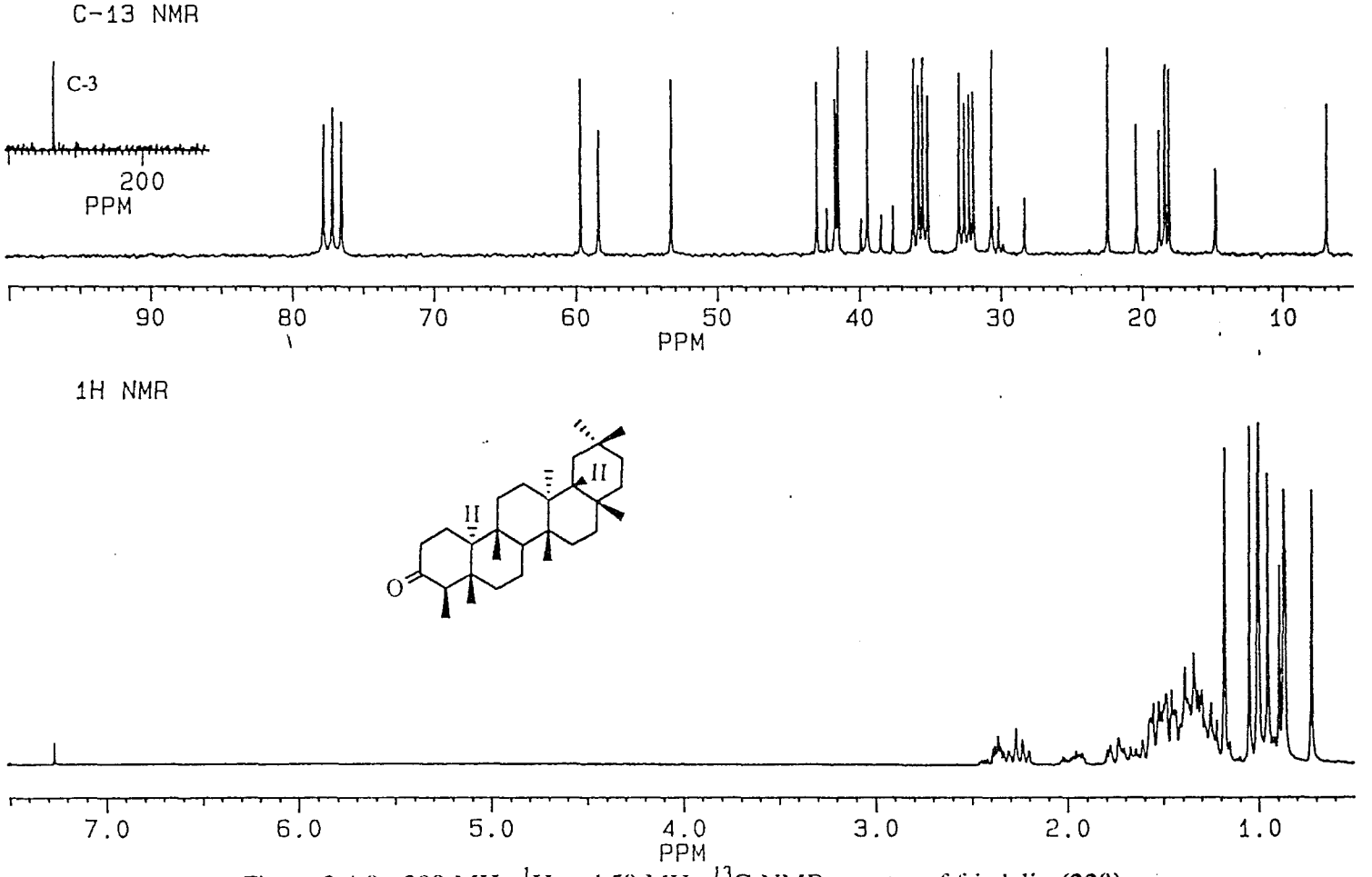


Figure 2.4.8. 200 MHz ^{1}H and 50 MHz ^{13}C NMR spectra of friedelin (**220**)

Table 2.4.2. ^{13}C NMR spectral data of epifriedelinol (**219**) and friedelin (**220**) (100 MHz, $CDCl_3$ as internal standard)

C	**219**	**220**
1	41.7	41.3
2	35.2	41.5
3	72.2	213.1
4	49.2	59.5
5	37.1	42.1
6	35.6	35.6
7	30.6	30.5
8	53.2	53.1
9	37.1	37.4
10	61.4	58.2
11	32.3	32.4
12	36.1	36.0
13	38.4	38.3
14	39.7	39.7
15	31.8	31.8
16	32.8	32.8
17	30.0	30.0
18	42.9	42.8
19	39.3	39.2
20	28.2	28.1
21	35.0	35.0
22	35.4	35.4
23	11.6	6.8
24	16.4	22.3
25	15.8	14.7
26	17.6	17.9
27	18.2	18.2
28	20.1	20.2
29	32.1	32.1
30	18.6	18.6

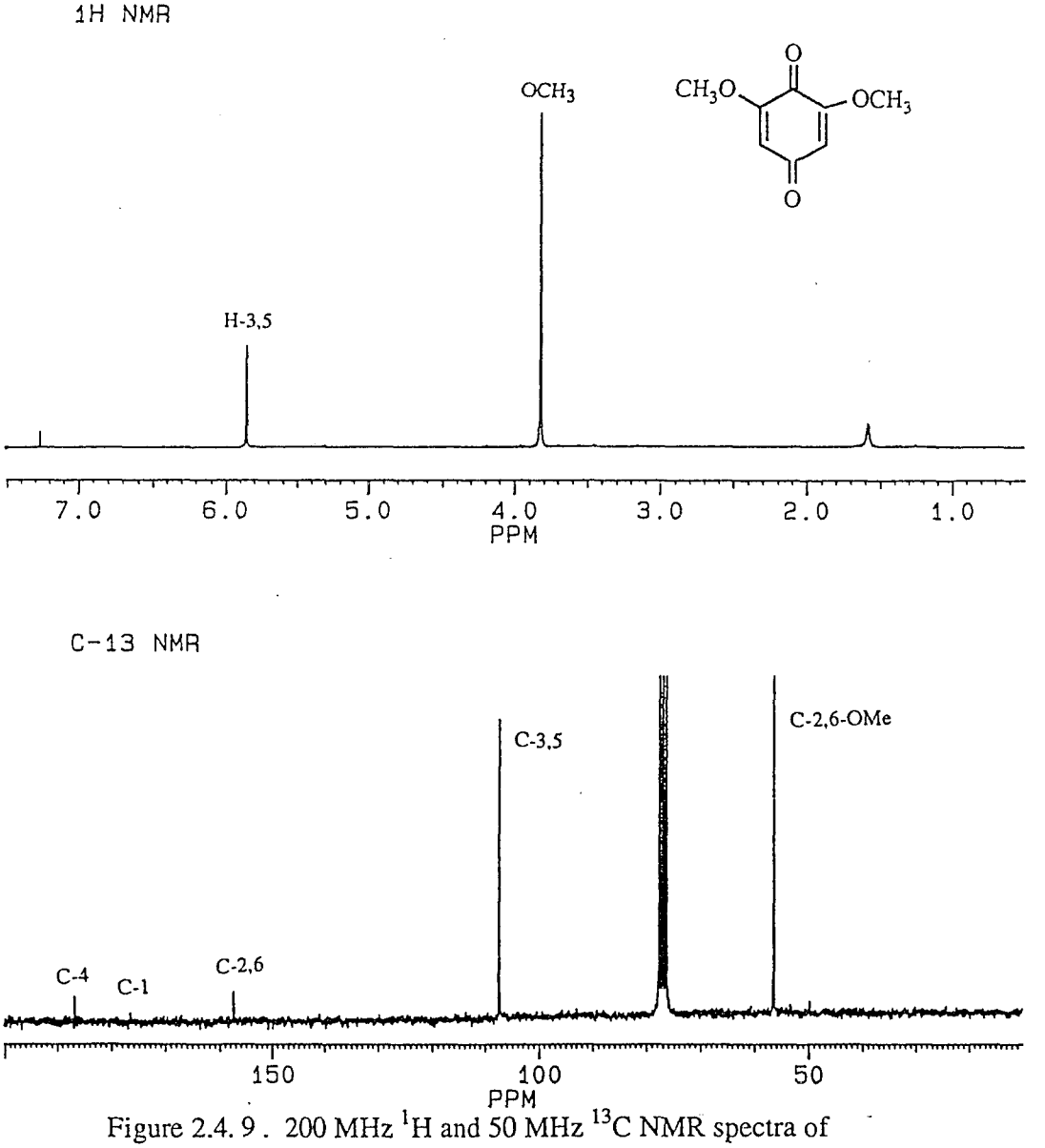


Figure 2.4. 9 . 200 MHz ^{1}H and 50 MHz ^{13}C NMR spectra of 2,6-dimethoxybenzoquinone (**221**)

inspection of the 2D COSY spectrum of **222** revealed that three aromatic proton signals at δ 7.41 (t, J=7.7, 7.3 Hz), δ 7.53 (br d, J=7.3 Hz) and δ 7.64 (br d, J=7.7 Hz) were coupled to each other and all further showed small couplings with the methyl singlet at δ 2.69, indicating the aromatic ring was 1,2,3-trisubstituted including a methyl group. A proton singlet at δ 7.90 and two mutually coupled olefinic proton doublets at δ 7.16 and δ 7.83 (J=12.7 Hz) indicated strong deshielding effect or extended conjugation. The combined information suggested that **222** was a benzotropolone or benzocycloheptatrienone with one methyl and one hydroxyl substituents. The positions of these substituents were determined by NOEDIFF experiment. Irradiation of the methyl signal at δ 2.69 showed NOEs with the proton singlet at δ 7.90 (2.4%), with the OH at δ 8.17 (2.0%) and with the aromatic proton doublet at δ 7.53 (1%), while irradiation of the OH signal at δ 8.17 also showed the NOEs with the proton singlet at δ 7.90 (2%) and the methyl signal at δ 2.69, as well as the proton doublet at δ 7.83 (1%), suggesting that the only possible substitutional pattern in agreement with the above data of **222** is 1-methyl-8-hydroxy-benzocycloheptatrien-5-one. The mass spectrum of **222** showed a strong molecular ion peak at m/z 186 (100%) which was in agreement with the empirical formula $C_{12}H_{10}O_2$. The ^{13}C assignments of **222** were made by a ^{1}H-^{13}C HETCOR experiment. For the assignments of quaternary carbons the selective INEPT method was applied [123, 124]. Irradiation of the OH signal at d 8.17, using a coupling parameter of 8 Hz, transferred the polarization to three carbon signals, one strong CH carbon at δ 113.6 (C-9), another weak CH signal at δ 144.8 (C-7) and one weak quaternary carbon signal at δ 154.6 which gave the assignment to C-8. Irradiation of the methyl signal at δ 2.69 using a coupling parameter of 6 Hz, transferred the enhancement to a CH carbon at δ 133.2 (C-2) and to two quaternary carbons, a strong signal at δ 137.5 assigned to C-10 and a weaker one at δ 134.4 (C-1). Therefore, the other non-oxygenated quaternary carbon signal at δ 133.5 was assigned to C-11.

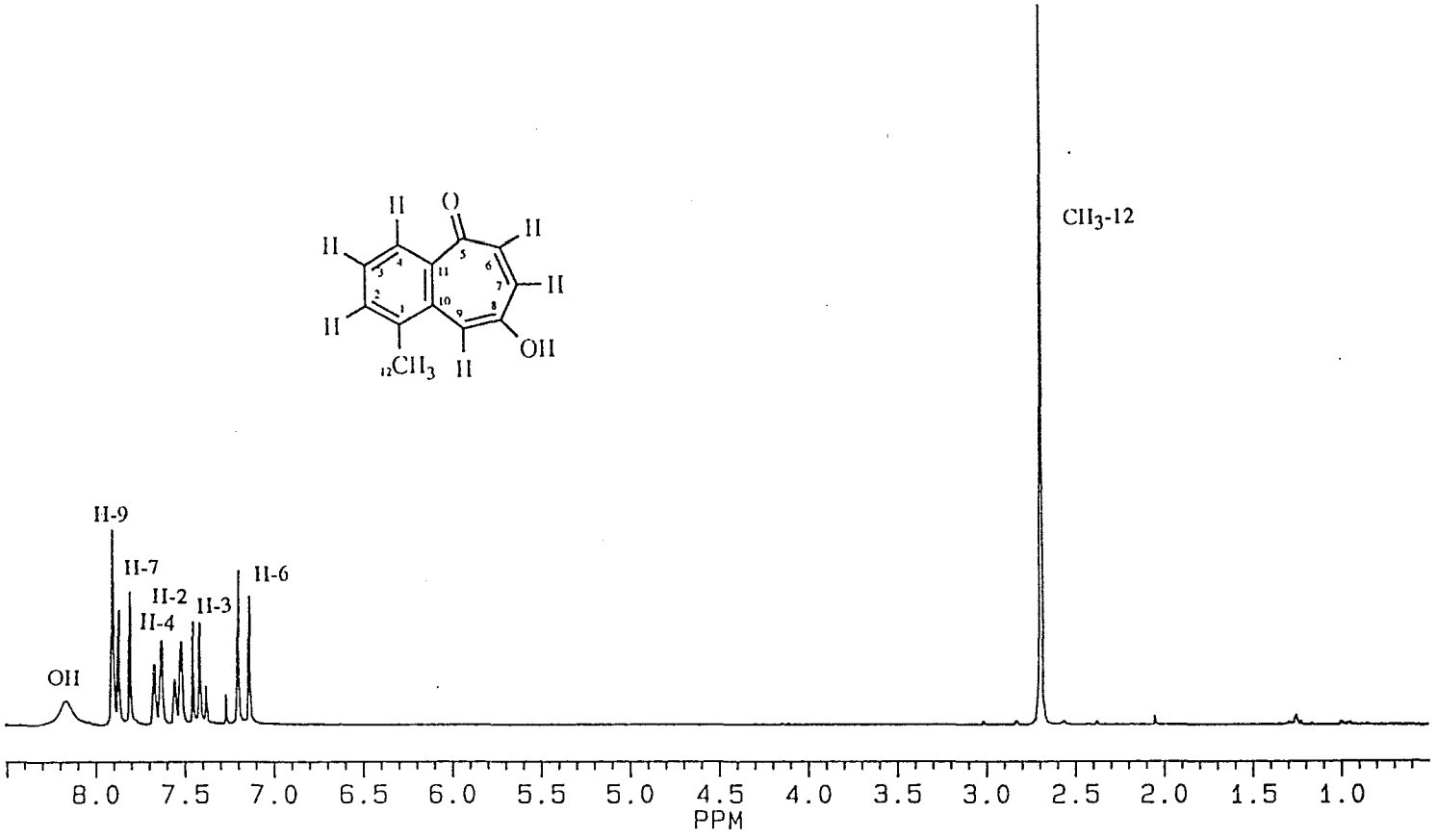


Figure 2.4.10. 200 MHz ^{1}H NMR spectrum of 1-methyl-8-hydroxybenzotropolone (**222**)

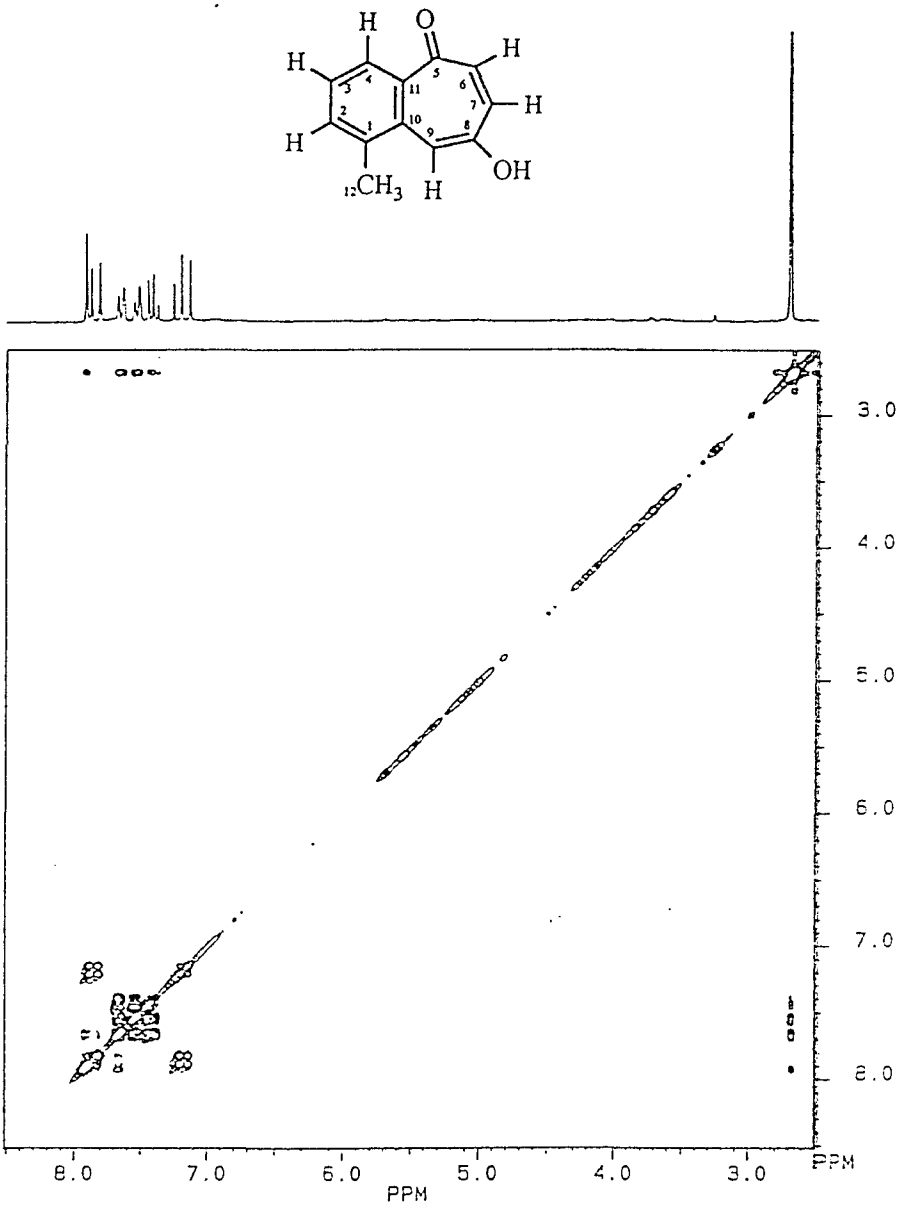


Figure 2.4.11. 2D ^{1}H-COSY spectrum of 1-methyl-8-hydroxybenzotropolone (222)

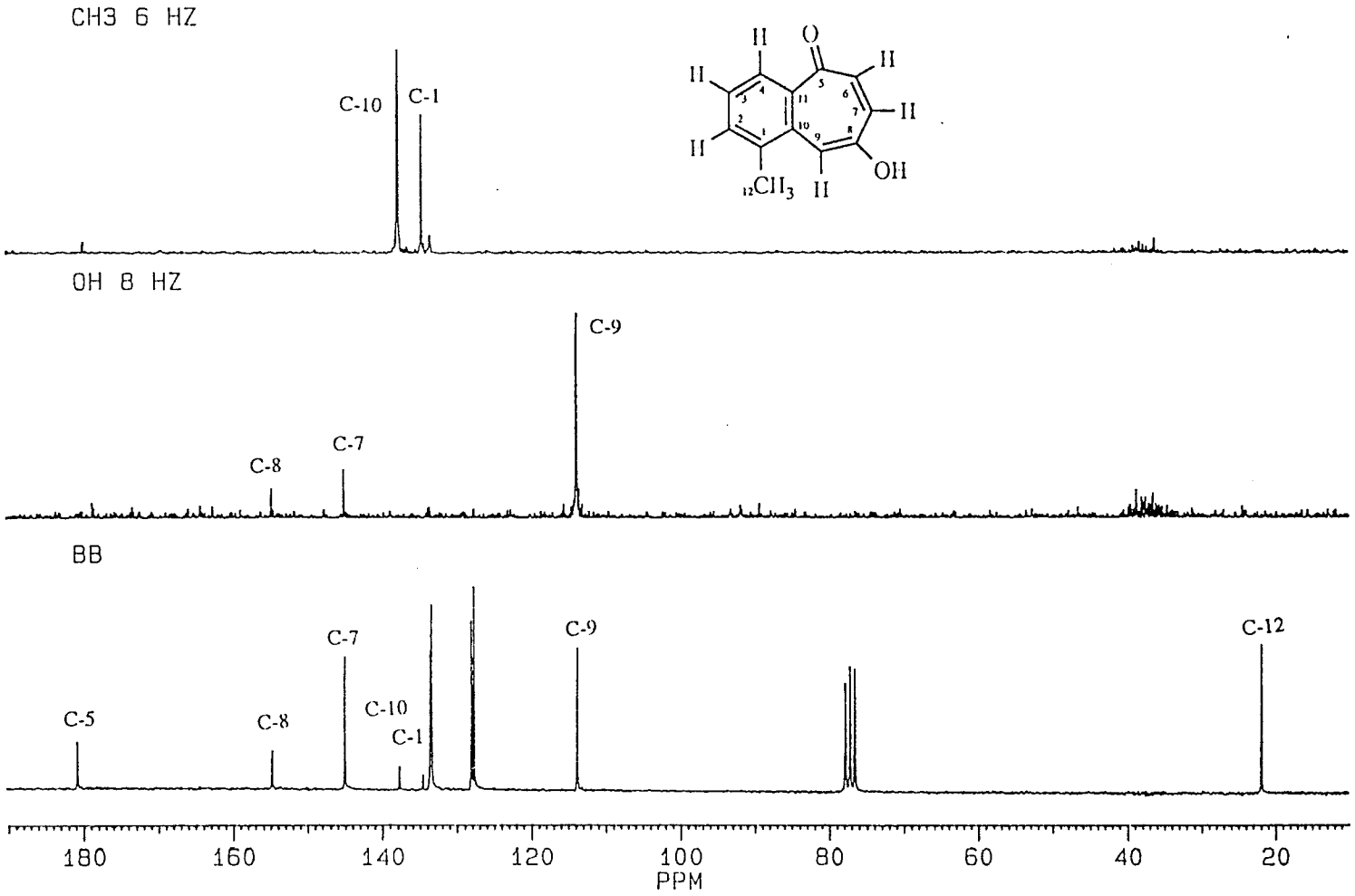


Figure 2.4.12. ^{13}C NMR and selective INEPT spectra of 1-methyl-8-hydroxybenzotropolone (**222**)

Anti-mycobacterial Activities

The polyacetylenes **126a-b**, **127a**, **130a-b**, **133a-b** and **134** and compounds **219-222** were tested against the pathogenic *Mycobacterium tuberculosis* H37Rv and *M. avium*. The biological activities of these compounds are listed in Table 2.4.3. All tested polyacetylenes showed activities against both Mycobacteria species. *cis,cis*-Matricaria lactone (**130a**) which exhibited inhibitory effect against *M. tuberculosis* and *M. avium* with minimum inhibitory concentrations (MICs) of 12.5 μg ml^{-1} and 50 μg ml^{-1}, respectively. Compounds **127a** and **133a**, which were obtained from *Solidago canadensis* along with **133b** and **134**[26], exhibited significant activity against *M. tuberculosis* and *M. avium* with MICs of 25 μg ml^{-1}. The linear structure of polyacetylenes may be responsible for their activities for they can penetrate the outer membranes of the Mycobacteria. A stereochemical dependence seems to play a role since compound **130b**, which is the 4,5-(*E*)-isomer of **130a**, is considerablly less active than its congener **130a**. Similarly, the 10-tigloyloxy-*cis*-matricaria ester (**134**) is less active than 10-angeloyloxy-*cis*-matricaria ester (**133a**). The non-acetylenic compounds **219-222** showed no activity against *M. tuberculosis* and *M. avium* at the concentrations below 100 μg ml^{-1}.

Experimental

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on either Bruker AM 400 or Bruker AC 200 spectrometer. IR spectra were obtained on a Perkin-Elmer 1760X FT-IR spectrometer as a film on KBr plates. The UV-Vis spectra were run in CH_3OH on an Aviv 14DS spectrophotometer and the mass spectra were recorded on a Hewlett-Packard 5971A GC-mass spectrometer. CC separations were made on silica gel (60-200 mesh, J. T. Baker) and VLC separations were carried out on silica gel (MN Kieselgel G).

Table 2.4.3. The Minimum Inhibitory Concentrations (μg ml^{-1}) of polyacetylenes and other constituents against pathogenic Mycobateria*

Compound	*M. tuberculosis* H37Rv	*M. avium*
126a	50	25
126b	50	50
127a	25	25
130a	12.5	50
130b	>50	>100
133a	25	25
133b	>50	50
134	>100	>100
219	>100	>100
220	>100	>100
221	>50	>100
222	>100	>100

*Data obtained from GWL Hansen's Disease Laboratory, U.S. Department of Health and Human Services.

Plant material. Roots of *Chrysoma pauciflosculosa* were collected in March, 1991 by Dr. Bruce Williamson in Pensacola, Florida, U.S.A.

Extraction and isolation. Fresh roots (946 g) were extracted with CH_2Cl_2 providing 5.13 g of crude extract. Separation by VLC on silica gel using hexane-EtOAc by increasing polarity provided 18x150 ml fractions. Fr. 6 afforded 97 mg of crystalline friedelin (**220**). Frs 7-10 formed a colorless precipitate which after filtration and recrystallization in hexane gave 778 mg of epifriedelinol (**219**). Further separation of fr. 7 by column chromatography (CC) using hexane-Et_2O (9:1) afforded 69 mg of *cis,cis*-matricaria ester (**126a**), 4 mg of *cis,trans*-matricaria ester (**126b**) and 4 mg of epimeric mixture of 8,9-epoxy-2,3-*cis*-matricaria esters (**218a** and **218b**). Combined frs 8 and 9, after filtration removal of **219**, provided 950 mg of an oily triterpene, the structure of which was unidentified. Frs 10 and 11 afforded 135 mg of **222** and fr. 14 yielded 40 mg of 2,6-dimethoxybenzoquinone (**221**).

Fresh roots (100 g) of *Erigeron philadelphicus* collected in 4 March, 1990 in East Baton Rouge Parish, Louisiana, U.S.A. (voucher Lu-No. 1 deposited at LSU Herbarium, U.S.A.) were soaked in CH_2Cl_2 overnight provided 738 mg of crude extract. VLC separation of the crude using hexane or mixtures of hexane and EtOAc as solvents by increasing polarity gave 13x75 ml fractions. Fr. 3 gave, after further CC (hexane-ethyl ether, 9:1), 195 mg of **126a**, 25 mg of **126b** and 5 mg of a mixture of **218a** and **218b**. Combined frs. 5 and 6 afforded, after further CC (petroleum ether-CH_2Cl_2, 1:7), 21 mg of **130a** and 11 mg of **130b**.

Oxidation of epifriedelinol (**219**). Epifriedelinol (31 mg) and 0.2 g of pyridinium chlorochromate were stirred in 10 ml of CH_2Cl_2 for 2 hr. After filtration the reaction solution was washed trice with water and dried over anhydrous Na_2SO_4 and filtered over a small column of anhydrous $MgSO_4$. Evaporation of the solvent gave 23 mg of a white powder (**220**), which was identical with the natural product as indicated by the ^1H and ^{13}C NMR data.

1-Methyl-8-hydroxy-benzocycloheptatrien-5-one (**222**). $C_{12}H_{10}O_2$, yellow crystal; IR ν_{max}^{KBr} cm^{-1}: 3289 (OH), 1710 (C=O), 1623, 1574, 1465, 1233, 886, 854, 748, 717; UV λ_{max}^{MeOH} nm: 282, 240; EIMS m/z (rel. int.): 186 $[M]^+$ (100), 158 $[M-CO]^+$ (90.5), 157 $[158-H]^+$ (67.1), 141 $[158-OH]^+$ (45.3), 128 (21.5), 115 $[141-C_2H_2]^+$ (9.8); ^1H NMR (200 MHz, $CDCl_3$): δ 2.69 (3H, s, CH_3), 7.16 (1H, d, J=12.7 Hz, H-6), 7.41 (1H, t, J=7.7, 7.3 Hz, H-3), 7.53 (1H, br d, J=7.3 Hz, H-2), 7.64 (1H, br d, J=7.7 Hz, H-4), 7.83 (1H, br d, J=12.7 Hz, H-7), 7.90 (1H, br s, H-9), 8.17 (1H, br s, OH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 134.4 (s, C-1), 133.2 (d, C-2), 127.5 (d, C-3), 133.4 (d, C-4), 180.6 (s, C-5), 127.9 (d, C-6), 144.8 (d, C-7), 154.6 (s, C-8), 113.6 (d, C-9), 137.5 (s, C-10), 133.5 (s, C-11), 21.8 (q, C-12); peak multiplicities (s=singlet, d=doublet, t=triplet, q=quartet) were determined by DEPT experiments.

CHAPTER 3

ISOLATION OF CONSTITUENTS FROM THE TRIBE HELIANTHEAE AND EUPATORIEAE

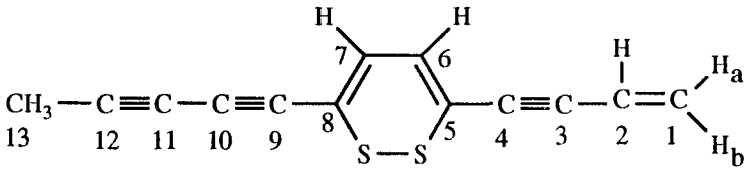
3.1

Sesquiterpenes and Thiarubrines from *Ambrosia trifida*

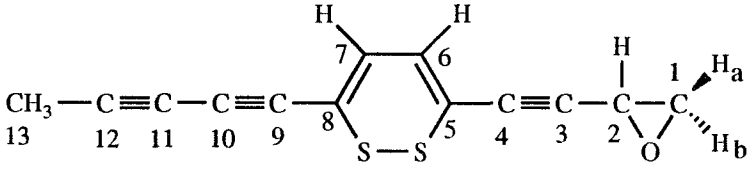
Introduction

Polyacetylenes and their sulfur derivatives are common constituents in members of the Asteraceae family [7, 151]. Recently, these compounds have attracted attention due to their broad spectrum of biological activities [7, 95, 151]. In continuation of our chemical study of the sulfur-containing polyacetylenes in wild plants and hairy root cultures of the Asteraceae [152], we have investigated stems and roots of local giant ragweed (*Ambrosia trifida*) of the tribe Heliantheae. Roots of *A. trifida* provided the sesquiterpenes β-bisabolene and β-farnesene as well as squalene, stigmasterol and sitosterol. In addition thiarubrine B (**223**) and its thiophene analogue (**225**) were obtained as the major sulfur-containing polyacetylenes with traces of thiarubine A and thiophene A [7, 95, 151, 152] and a new dithiacyclohexa-3,5-diene. Investigation of the stems of *A. trifida* also led to the isolation and characterization of two carotane-type sesquiterpenes which are common constituents in the members of the Umbelliferae family [153-55]. Other species in the Asteraceae family in which sesquiterpenes with a carotane skeleton have been previously reported include *Blainvillea acmellea* [156] and *Lasiantheae fruticosa* [157] of the tribe Heliantheae. The known 2,6-dimethoxybenzoquinone and hexadecanal were also found in the stems of *A. trifida*.

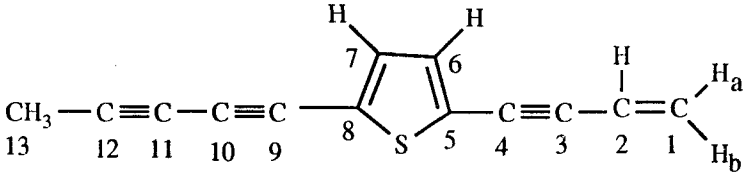
The high field ^{1}H and ^{13}C NMR and mass spectral analysis, in particular, the inverse long-range $^{1}H,^{13}C$-correlation method, provided the basis for the structure elucidation of compounds **224**, **226** and **227**.



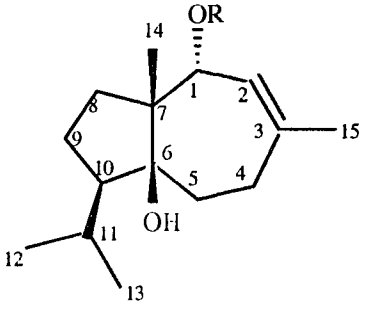
223



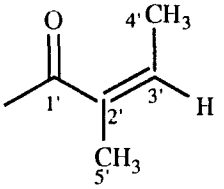
224



225



226 R=



227 R=

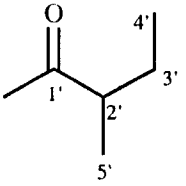


Figure 3.1.1. Thiarubrines and Sesquiterpenes from *Ambrosia trifida*

Results and Discussion

Thiarubrine B (**223**) and thiophene (**225**) are known compounds which were previously found in several species of the Asteraceae family [7, 151, 152, 158] as well as hairy roots cultures of *A. artemisiifolia* [152]. The ^{1}H and ^{13}C NMR spectral data of **223** were previously reported [152, 158]. Compound **224** is a new 1,2-dithia-3,5-cyclohexadiene derivative. Its structure was elucidated by mass spectral analysis and ^{1}H NMR spectroscopic comparison with thiarubrine B (**223**). The UV-Vis spectrum of **224** exhibited an absorption maximum at 482 nm which is characteristic for the conjugated 1,2-dithia-3,5-cyclohexadiene moiety [151, 158]. The ^{1}H NMR spectrum of **224** showed an acetylenic methyl singlet at δ 2.05 (H-13) and an AB system at δ 6.60 (H-6, J=6.8 Hz) and δ 6.64 (H-7, J=6.8 Hz), values which are very similar to those of thiarubrine B (**223**)[158]. An ABX pattern with signals at δ 2.97 (H-1a, J=5.8, 2.6 Hz), δ 3.20 (H-1b, J=5.8, 4.1 Hz) and δ 3.55 (H-2, J=4.1, 2.6 Hz) was the only significant difference between the ^{1}H NMR absorptions of **223** and **224** (Table 3.1.1). Mass spectral analysis of **224** showed a molecular ion at *m/z* 244, strongly suggesting the presence of an additional oxygen atom when compared with thiarubrine B (**223**) ($[M]^{+}$ at *m/z* 228). Based on these data we have assigned structure **2** to the new 1,2-epoxy-thiarubrine B. The chirality at C-2 in **224** remains open.

The ^{1}H and ^{13}C NMR spectral data of compounds **226** and **227** suggested that they represent sesquiterpenoid esters. The presence of an angelate moiety in compound **226** was derived from its 400 MHz ^{1}H NMR spectral data. A quartet of a quartet at δ 6.06 (H-3', J=7.2, 1.2 Hz), a three-proton doublet of a quartet at δ 2.01 (H-4', J=7.2, 1.2 Hz), and another three-proton doublet of a quartet at δ 1.90 (H-5', J=1.2, 1.2 Hz) were indicative of the angelate moiety. Mass spectral analysis of **226** showed strong diagnostic peaks at *m/z* 83 and 55 which further confirmed the presence of the angelate group. The COSY spectrum of **226** showed that two methyl doublets at δ 0.93 (J=6.8 Hz) and δ 0.99 (J=6.8 Hz) were coupled to the same proton multiplet at δ

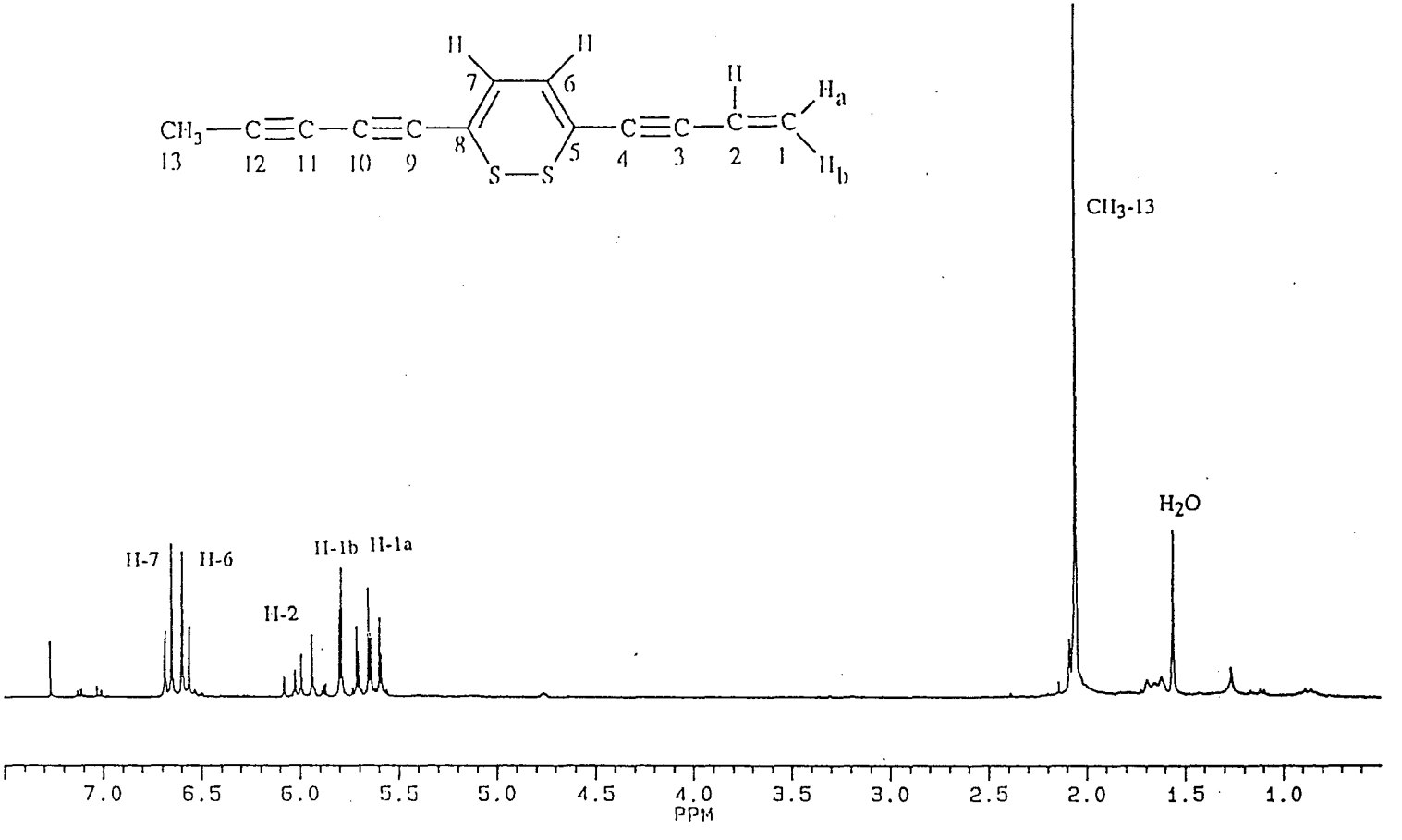


Figure 3.1.2. 200 MHz ^{1}H NMR spectrum of thiarubrine B (**223**)

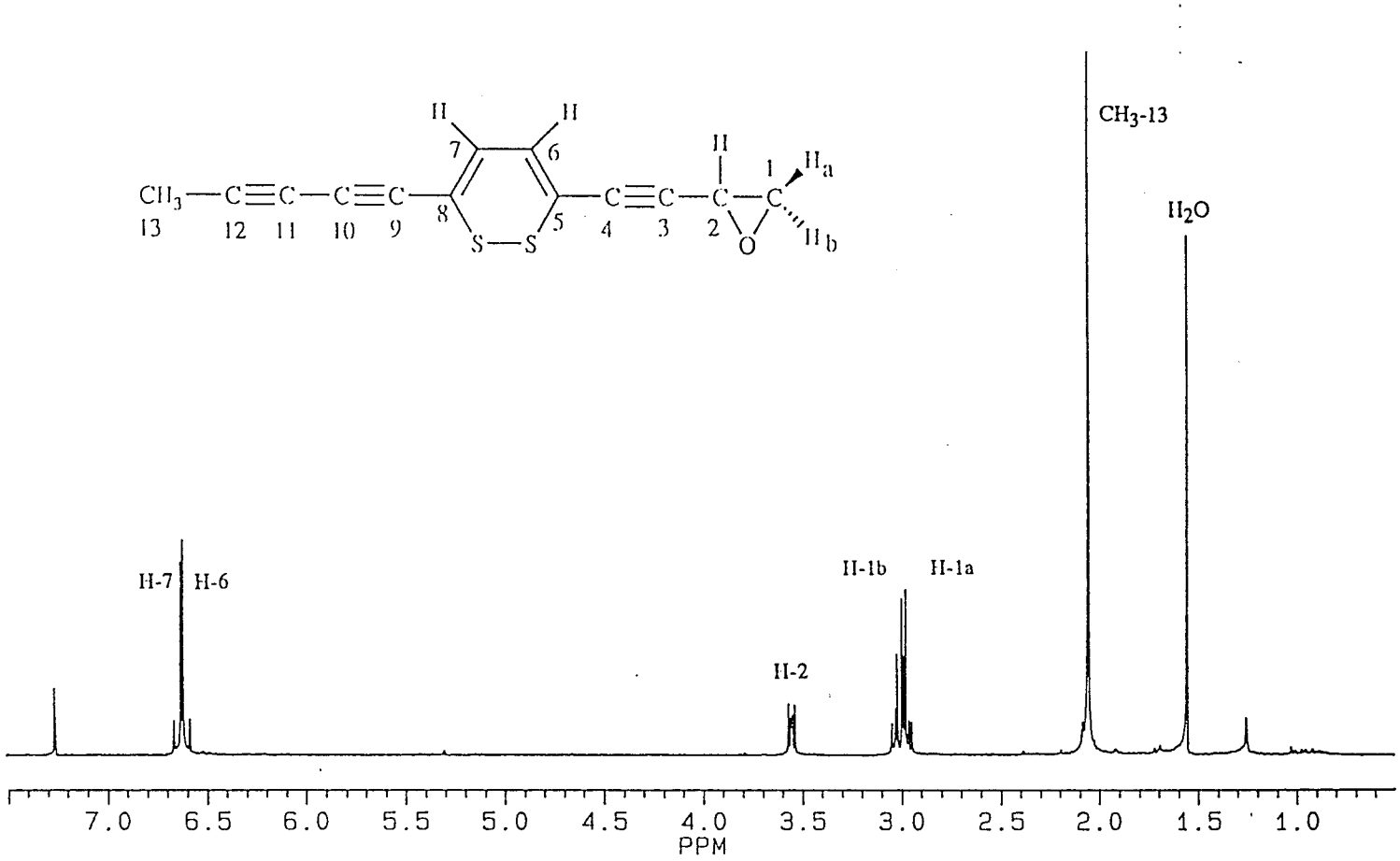


Figure 3.1.3. 200 MHz ^{1}H NMR spectrum of 1,2-epoxythiarubrine B (**224**)

Table 3.1.1. ^{1}H NMR spectral data of compounds **223** to **225** (200 MHz, $CDCl_3$ as intl. std.)

H	**223**	**224**	**225**
1*a*	5.61 *dd*	2.97 *dd*	5.58 *dd*
1*b*	5.74 *dd*	3.02 *dd*	5.74 *dd*
2	6.00 *dd*	3.55 *dd*	5.93 *dd*
6	6.57 *d*	6.60 *d*	7.02 *d*
7	6.66 *d*	6.64 *d*	7.12 *d*
13	2.05 *s*	2.05 *s*	2.04 *s*

J (Hz): **223**: 1*a*, 1*b* = 2.3; 1*a*, 2 = 10.9; 1*b*, 2 = 17.5; 6, 7 = 6.8. **224**: 1*a*, 1*b* = 5.8; 1*a*, 2 = 2.6; 1*b*, 2 = 4.1; 6, 7 = 6.8. **225**: 1*a*, 1*b* = 2.1; 1*a*, 2 = 11.0; 1*b*, 2 =17.6; 6, 7 = 4.0.

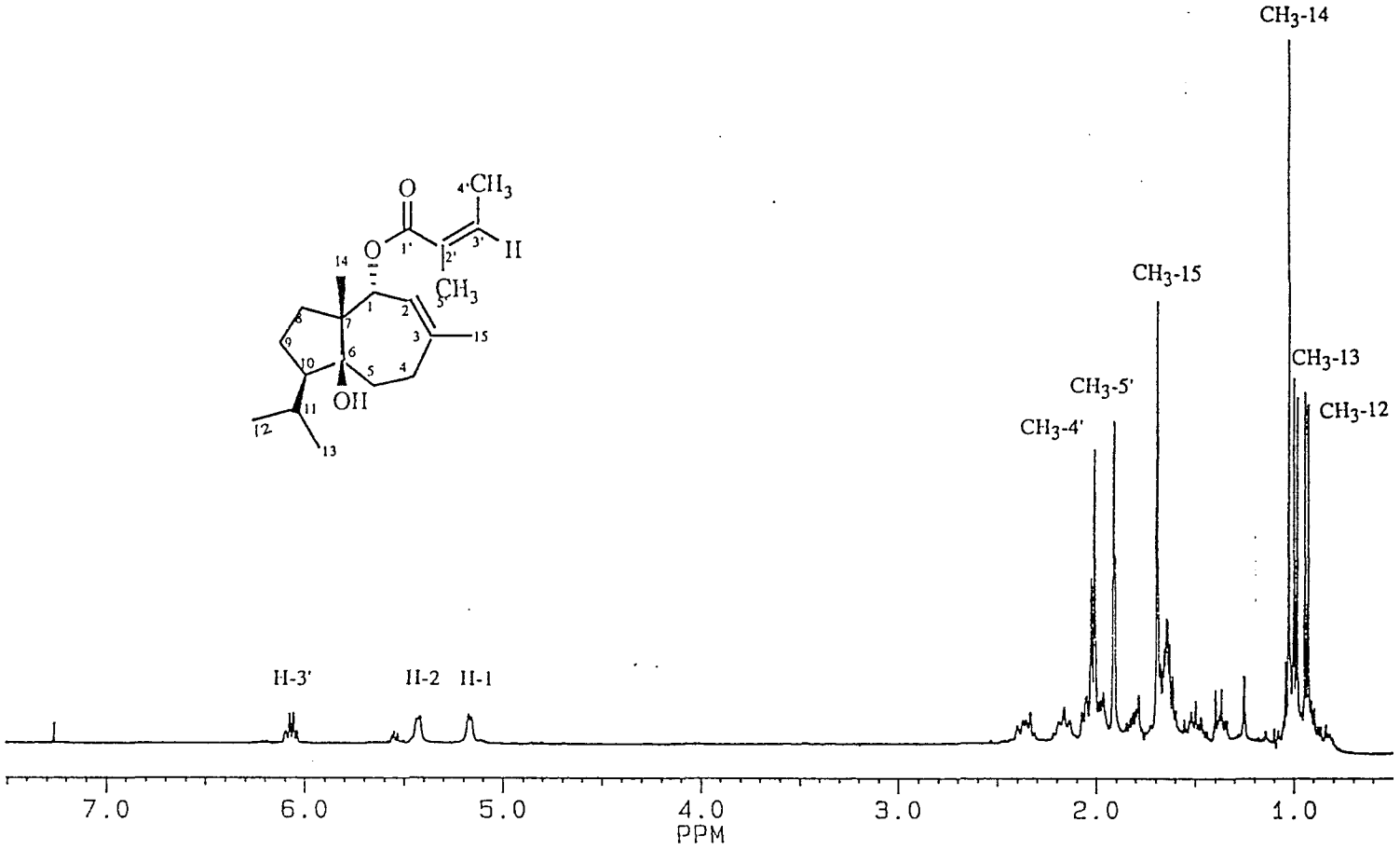


Figure 3.1.4. 400 MHz ^{1}H NMR spectrum of 1α-angeloyloxycarptol (**226**)

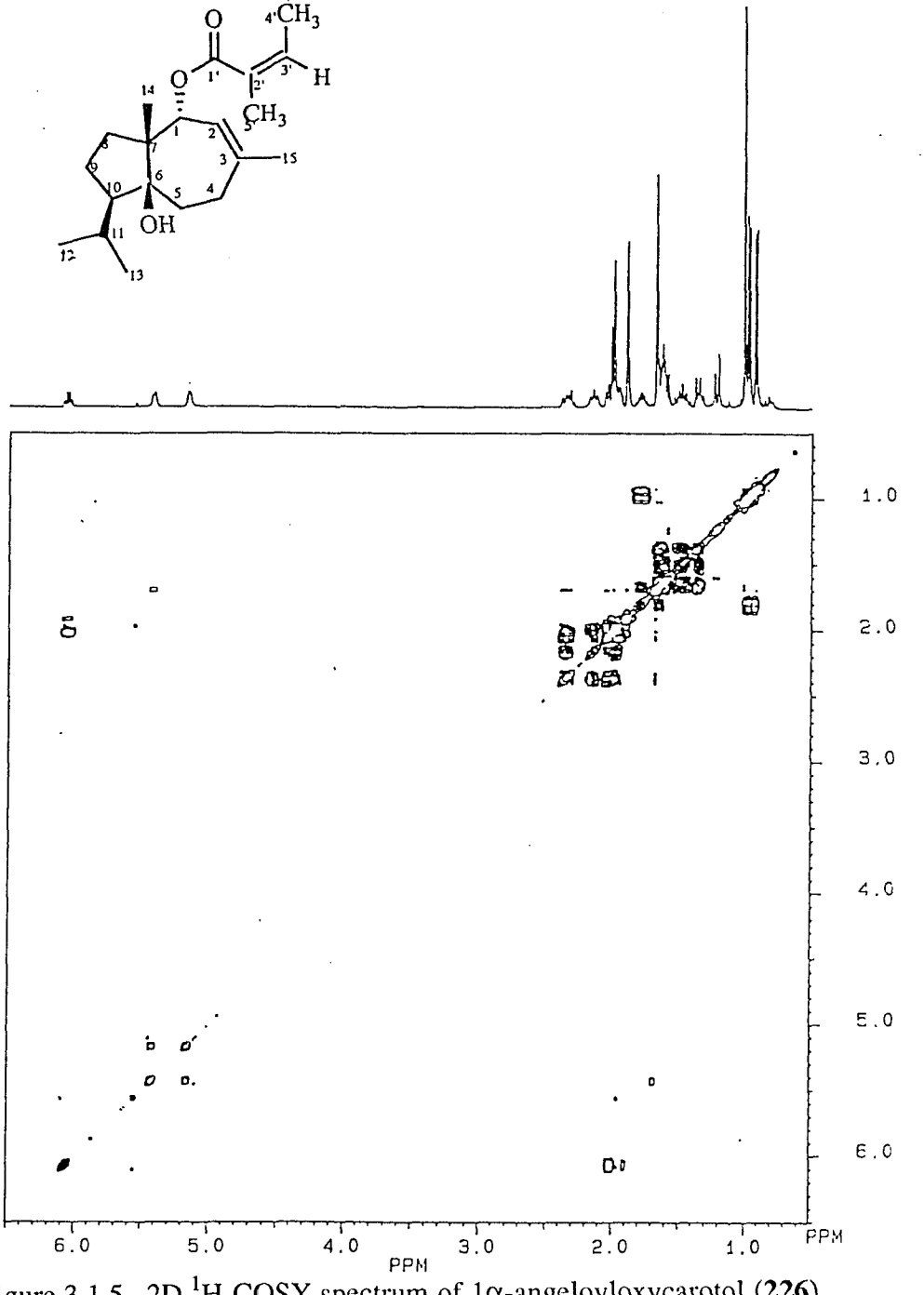


Figure 3.1.5. 2D ^{1}H-COSY spectrum of 1α-angeloyloxycarotol (**226**)

1.80, indicating the presence of an isopropyl group. This was further confirmed by a prominent peak at *m/z* 43 = $[CH(CH_3)_2]^+$ in its mass spectrum. The isopropyl proton was further coupled to another methine proton which appeared as a multiplet at δ 1.65, the latter being coupled to two methylene proton multiplets at δ 1.49 and 1.65, while these two methylene showed further coupling to two additional methylene protons which appeared as multiplets at δ 1.68 and 1.38. The above data suggested the presence of partial structure **A** (Figure 3.1.6) in compound **226**. A more detailed inspection of the 2D COSY spectrum of **226** revealed that an olefinic proton broad doublet at δ 5.42 (J=5.7 Hz) was allylically coupled to a methyl broad singlet at δ 1.69 and vicinally coupled to a proton doublet at δ 5.16 (J= 5.7 Hz) which itself showed no further coupling. The inverse long-range $^1H,^{13}C$-correlation method [137], generally optimized for three-bond ^1H-^{13}C couplings, showed that the olefinic proton correlated with a methylene carbon at δ 30.3 with proton multiplets at δ 2.35 and 2.00, and the methylene protons were further vicinally coupled to another set of methylene multiplets at δ 2.16 and 1.97 as shown by its ^1H-COSY spectrum. The above data suggested a structural arrangement represented by substructure **B** (Figure 3.1.6). Furthermore, a broad IR absorption at 3543 cm^{-1} combined with mass spectral peaks at *m/z* 302 $[M-H_2O]^+$ and 202 $[220-H_2O]^+$ in **226**, indicated the presence of a hydroxyl group attached to a quaternary carbon at δ 83.2. Additionally, the ^1H NMR spectrum indicated the presence of an angular methyl group, as suggested by a three-proton singlet at δ 1.02, which showed long range correlation with the quaternary carbon at δ 83.2. These gave the connectivity of substructure **C** (Figure 3.1.6). All of the above arguments are in agreement with the ^{13}C NMR data which showed the presence of 20 carbons with six CH_3, four CH_2, five CH including two olefinic and one oxygenated carbons, one ester carbonyl, and four quaternary including two olefinic and one oxygenated carbons (Table 3.1.3).

Table 3.1.2. ^{1}H NMR spectral data of compounds **226** and **227** (400 MHz, $CDCl_3$ as int. std.)

H	**226**	**227**
1	5.16 *d*	5.08 *d*
2	5.42 *br d*	5.38 *br d*
4*a*, 4*b*	2.35 *m*, 2.00 *m*	2.35 *m*, 2.01 *m*
5*a*, 5*b*	2.16 *m*, 1.97 *m*	2.16 *m*, 1.99 *m*
8*a*, 8*b*	1.68 *m*, 1.38 *m*	1.67 *m*, 1.38 *m*
9*a*, 9*b*	1.65 *m*, 1.49 *m*	1.65 *m*, 1.50 *m*
10	1.65 *m*	1.64 *m*
11	1.80 *m*	1.80 *m*
12	0.93 *d*	0.94 *d*
13	0.99 *d*	1.01 *d*
14	1.02 *s*	1.00 *s*
15	1.69 *br s*	1.68 *br s*
2'	---	2.08 *m*
3'	6.06 *qq*	2.18 *m*
4'	2.01 *dq*	0.94 *t*
5'	1.90 *dq*	0.99 *d*

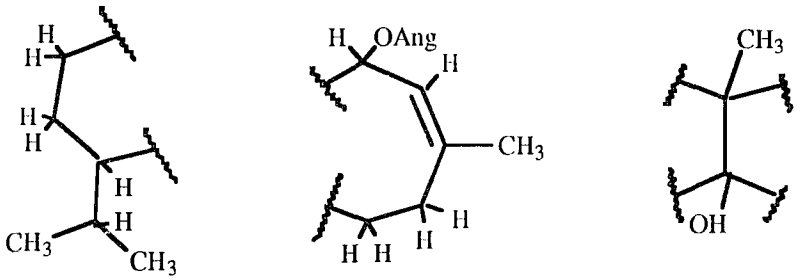
J (Hz): **226**: 1, 2 = 5.7; 11, 12 = 6.8; 11, 13 = 6.8; 3', 4' = 7.2; 3', 5' = 1.2; 4', 5' = 1.2. **227**: 1, 2 = 5.8; 11, 12 = 6.7; 11,13 = 6.7; 3', 4' =6.4; 2', 5' = 6.2.

Table 3.1.3. ^{13}C NMR spectral data of compounds **226** and **227** (100 MHz, $CDCl_3$ as int. std.)*

C	**226**	**227**
1	77.3 *d*	77.4 *d*
2	122.2 *d*	121.8 *d*
3	142.3 *s*	142.6 *s*
4	30.3 *t*	30.2 *t*
5	35.3 *t*	35.2 *t*
6	83.2 *s*	83.2 *s*
7	53.5 *s*	53.4 *s*
8	35.9 *t*	35.9 *t*
9	24.8 *t*	24.8 *t*
10	56.2 *d*	56.6 *d*
11	26.7 *d*	26.8 *d*
12	21.3 *q*	21.3 *q*
13	24.3 *q*	24.4 *q*
14	22.8 *q*	22.7 *q*
15	25.7 *q*	25.7 *q*
1'	167.4 *s*	172.6 *s*
2'	127.8 *s*	25.8 *d*
3'	138.4 *d*	44.1 *t*
4'	15.7 *q*	22.4 *q*
5'	20.9 *q*	22.4 *q*

*Peak multiplicities were obtained by heteronuclear multipulse programs (DEPT); *s*=singlet, *d*=doublet, *t*=triplet, *q*=quartet.

In order to assemble substructures **A**, **B**, and **C**, the inverse long-range $^{1}H,^{13}C$-correlation NMR technique was applied. The proton doublet at δ 5.16 (J=5.7 Hz) was correlated with a carbonyl signal at δ 167.4, a quaternary olefinic carbon at δ 142.3, a methylene carbon at δ 35.9 and an oxygenated quaternary carbon at δ 83.2. The angular methyl singlet at δ 1.02 also showed correlations with the methylene at δ 35.9



A **B** **C**

Figure. 3.1.6. Partial Structures **A**, **B** and **C** in Compound **226**

and the oxygenated quaternary carbon at δ 83.2, as well as a methine signal at δ 77.3 whose proton signal was assigned as the doublet at δ 5.16 (J=5.7 Hz), as shown by the 2D $^{1}H,^{13}C$-correlation. The olefinic proton broad doublet at δ 5.42 (J=5.7 Hz) was correlated with a methyl at δ 25.7, a methylene at δ 30.3 and a quaternary carbon at δ 53.5. Based on the above data, the combination of the connectivities for substructures **A**, **B**, and **C** gives structure **226**. The ^{1}H and ^{13}C NMR data of **226** were assigned without ambiguity by combined application of COSY, NOESY, $^{1}H,^{13}C$-correlation, inverse long-range $^{1}H,^{13}C$-correlation [137]and DEPT methods (Tables 3.1.2 and 3.1.3). Comparison of our NMR spectral data of **226** with the data reported for lasidiol angelate [157] revealed their structural identity. The relative stereochemistry of **226** was established by 2D NOESY experiment in which the angular methyl (H-14) showed NOEs with the proton signal at δ 5.16 (H-1), indicating that H-1 was β-oriented and the angelate substituent was in the α-orientation. The NOEs observed between the

angelate protons and the isopropyl protons indicated that the hydroxyl group on C-6 had to be β-oriented since this is the only arrangement where the two groups could exhibit through space interaction (Figure 3.1.7). Further NOEs between protons in compound **226** are indicated by double-headed arrows in Figure 3.1.7.

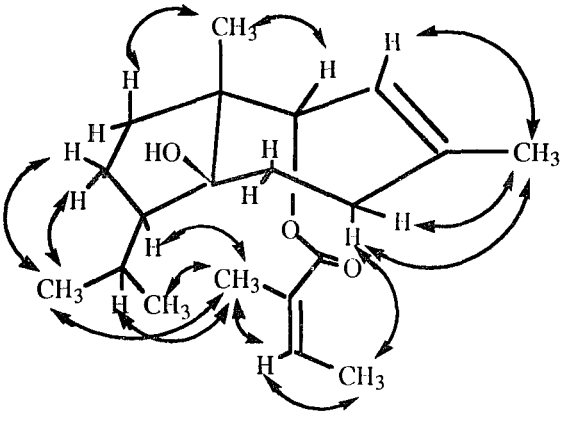


Figure. 3.1.7. Selective NOEs observed in Compound **226**

The ^{1}H and ^{13}C NMR spectra of compound **227** were very similar to those of **226**, but differed from **226** by the presence of signals typical for the 2',3'-dihydroangelate moiety. This was further confirmed by its mass spectral data with a molecular peak at *m/z* 322, two more mass units than that observed for compound **226** ($[M]^+$ at *m/z* 320). A prominent peak at *m/z* 220 derived from the loss of 2-methylbutyric acid $[M-102]^+$ together with diagnostic 2-methylbutyrate fragments at *m/z* 85 and 57 further supported the NMR spectral data.

The isolation of the two carotane sesquiterpenes **226** and **227** from *A. trifida* represents further evidence that this skeletal type occurs more frequently in the tribe Heliantheae of the family Asteraceae than previously expected. Other carotol cinnamates were isolated from the aerial parts of *Blainvillea acmellae* [156] and *L.*

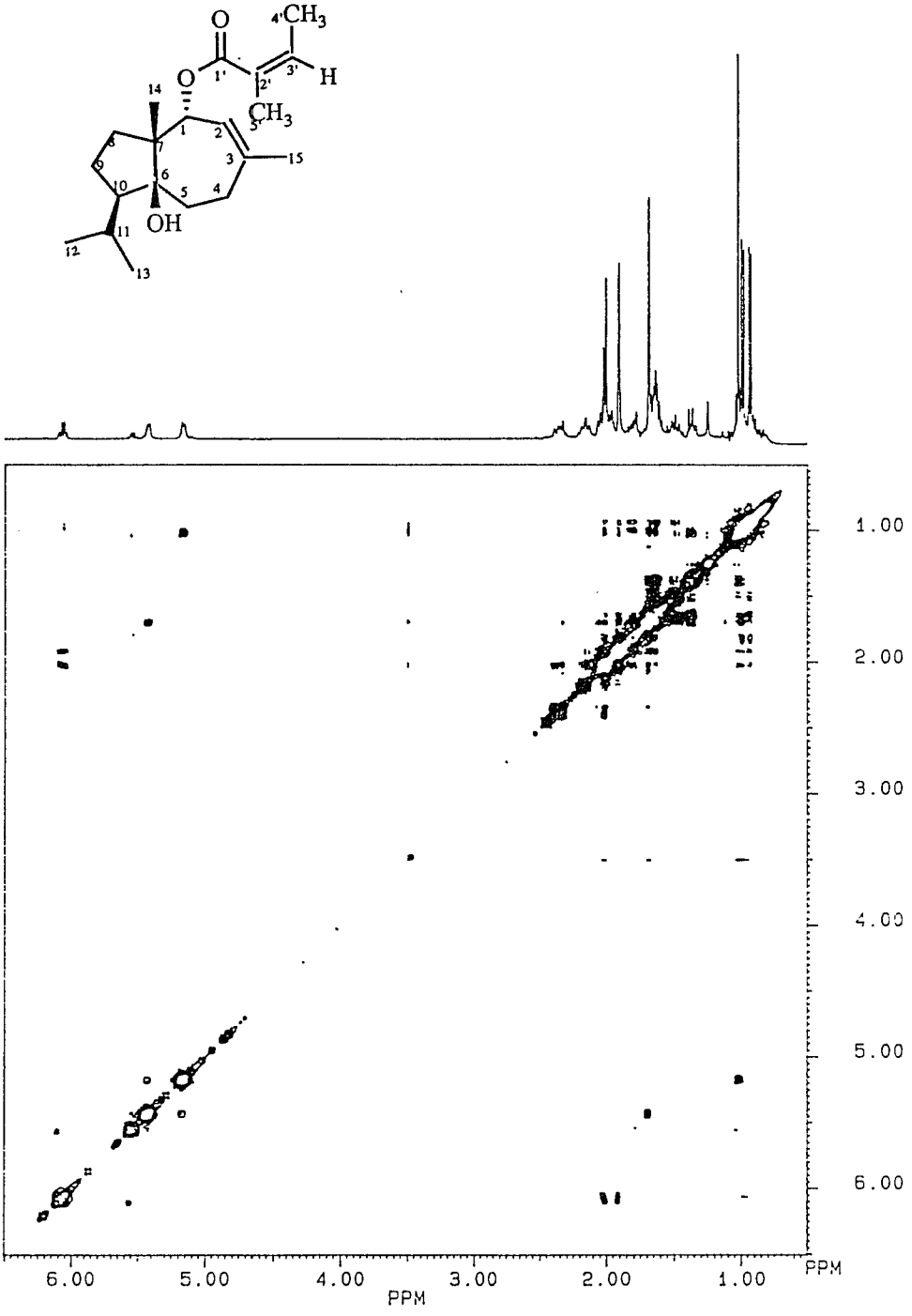


Figure 3.1.8. 2D NOESY spectrum of 1α-angeloyloxycarotol (**226**)

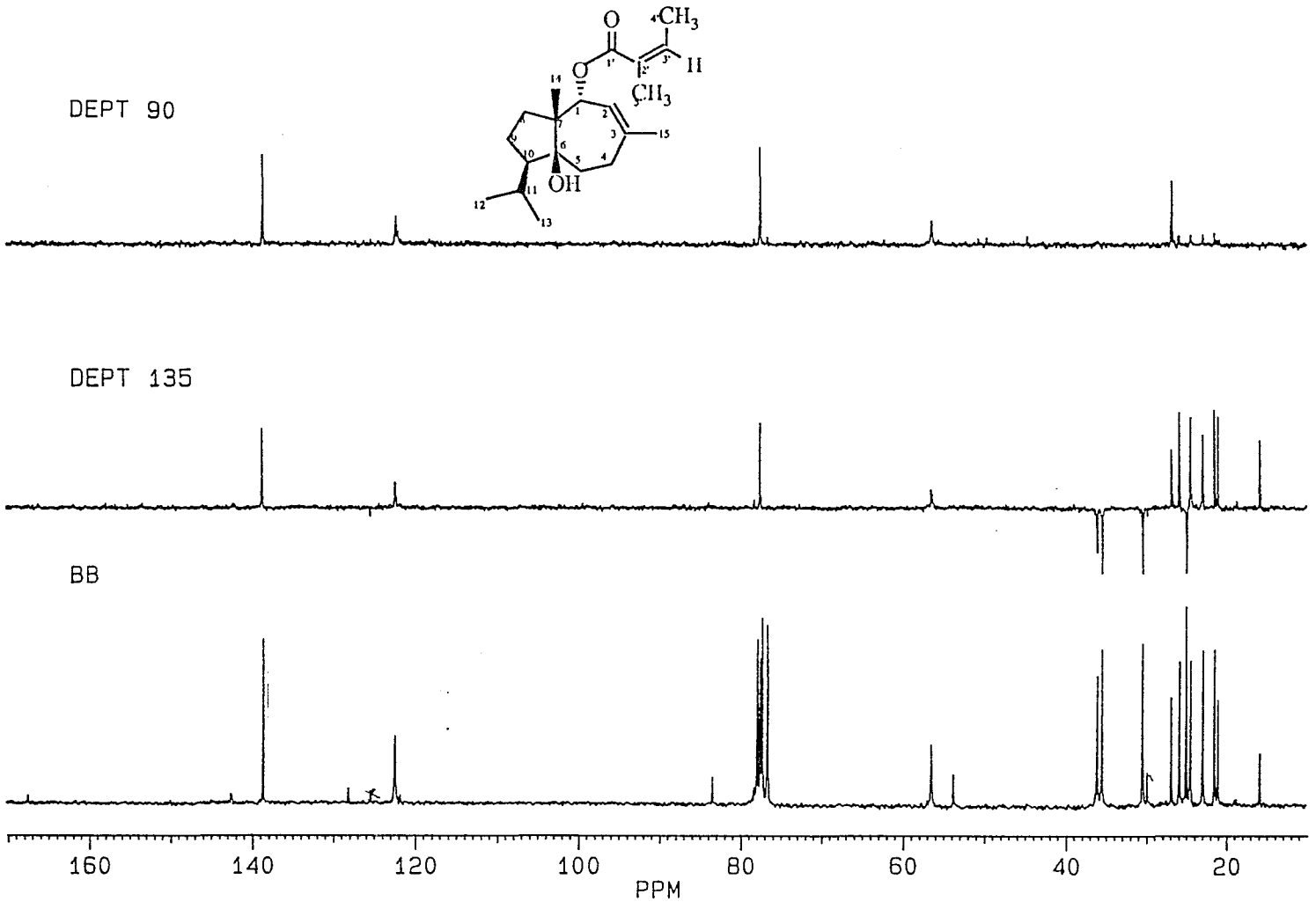


Figure 3.1.9. DEPT 90°, DEPT 135° and BB ^{13}C NMR spectra of 1α-angeloyloxycarotol (**226**)

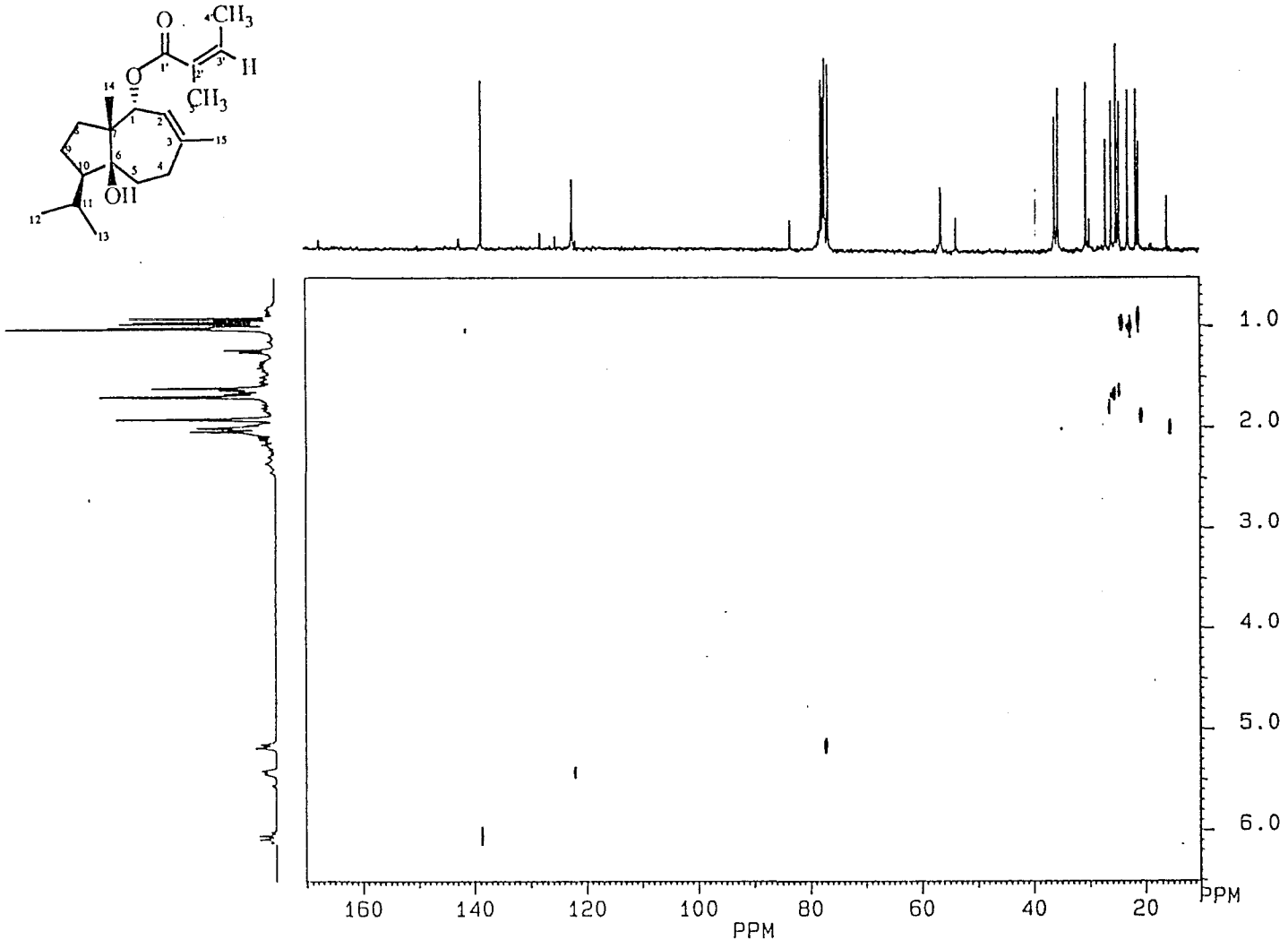


Figure 3.1.10. 2D ^{13}C-^{1}H Heteronuclear correlation spectrum of 1α-angeloyloxycarotol (**226**)

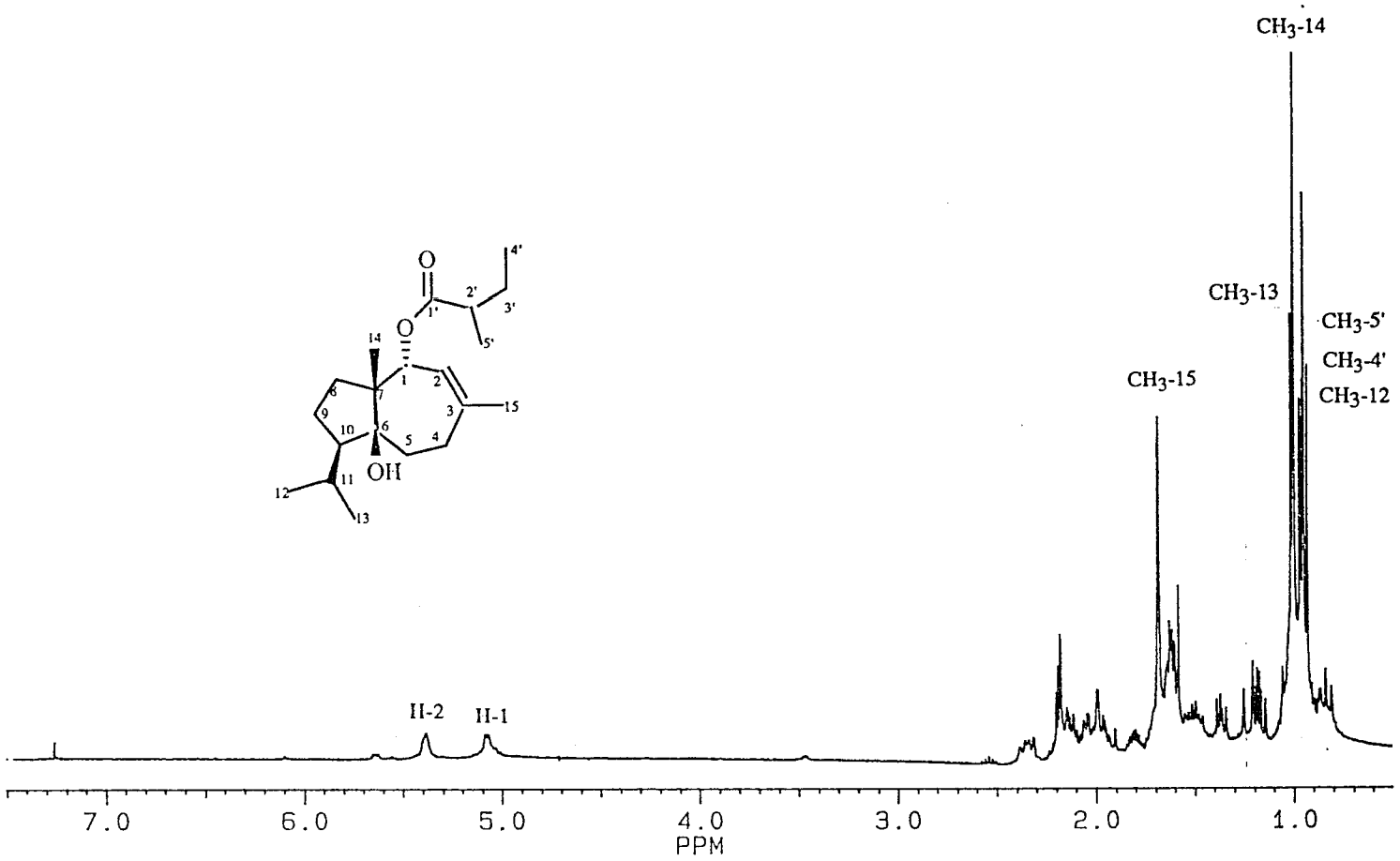


Figure 3.1.11. 400 MHz ^{1}H NMR spectrum of 1α-(2'-methylbutyroyloxy)-carotol (**227**)

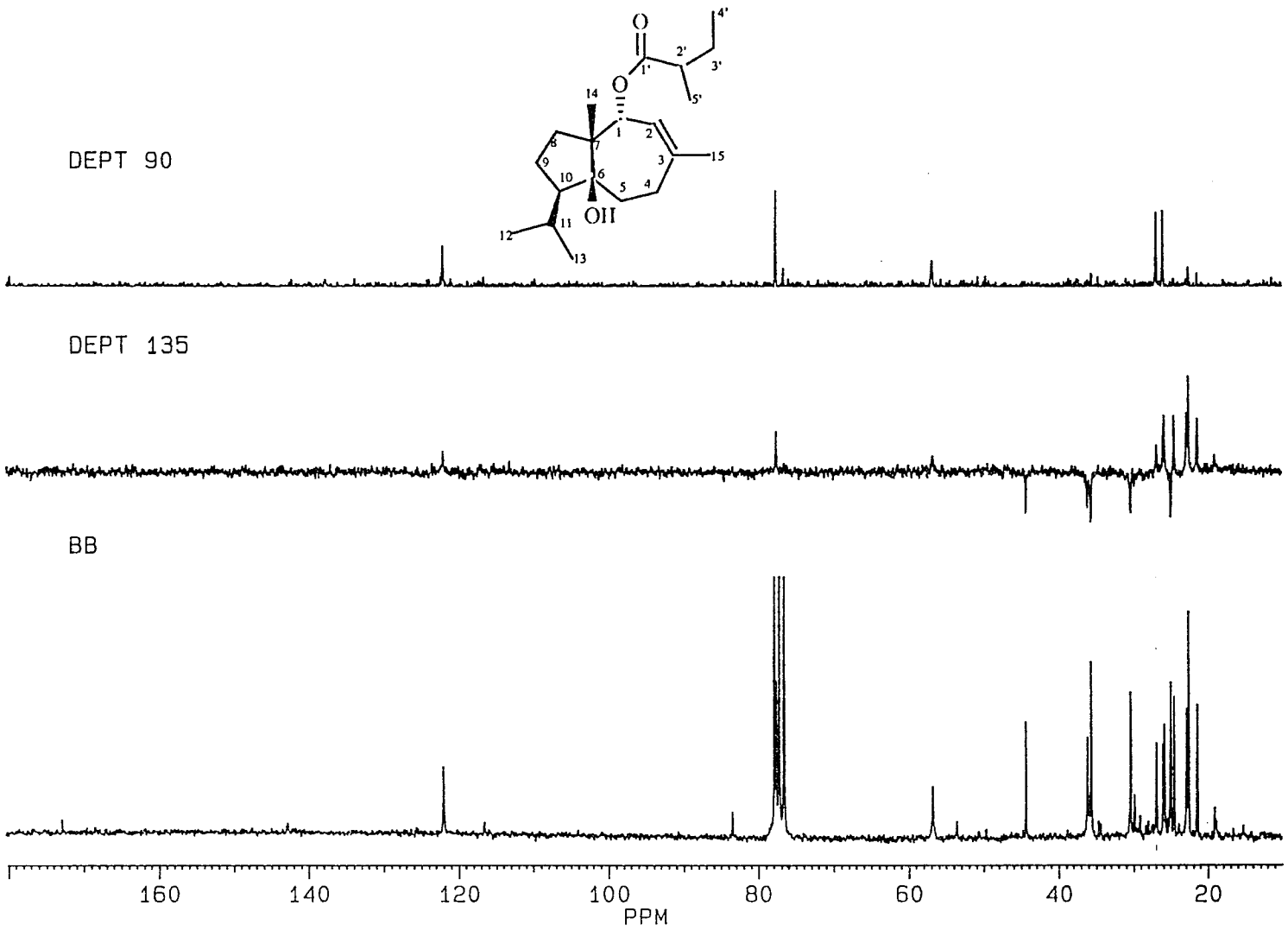


Figure 3.1.12. DEPT 90°, DEPT 135° and BB ^{13}C NMR spectra of 1α-(2'-methylbutyroyloxy)-carotol (**227**)

fruticosa [157] of the tribe Heliantheae (Asteraceae). Similar carotane-type sesquiterpenes are commonly found in the Umbelliferae family [153-155, 159, 160].

Other compounds obtained from the stems and roots of *A. trifida* were 2,6-dimethoxybenzoquinone [149] and hexadecanal, the structures of which were established by ^{1}H and ^{13}C NMR and mass spectral analysis.

Extraction of fresh leaves of *A. trifida* with methylene dichloride provided an oil which mainly consisted of volatile terpenoids. GC-MS analysis of the leaf volatiles showed as major constituents β-cubebene and β-caryophyllene and GC-MS data of the non-polar fractions of the stem extract gave β-cubebene and α-farnesene as major constituents. GC-MS identifications of the non-polar fractions of the extract of roots supported the ^{1}H NMR data for β-bisabolene and *trans*-β-farnesene, while more polar fractions contained mixtures of triglycerides, fatty acids, stigmasterol and sistosterol. It is of interest to note that sesquiterpene lactones, which are common constituents in aerial parts of other members of the genus *Ambrosia* [161], were not found in *A. trifida*.

Experimental

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on either a Bruker-AC 200 or a Bruker AM 400 spectrometer. IR spectra were obtained on a Perkin-Elmer 1760X FT-IR spectrometer as a film on NaCl plates. The UV-Vis spectra were run in CH_3CN on an Aviv 14DS spectrophotometer and the mass spectra were recorded on a Hewlett-Packard 5985 GC-MS spectrometer. Column chromatographic (CC) separations were made on silica gel (60-200 mesh, J. T. Baker) and vacuum liquid chromatographic (VLC) separations [129] were carried out on silica gel (MN Kieselgel G). Semi-preparative HPLC separations were performed on a 10 μ silica gel column (250x10 mm, AllTech) coupled to a LDC/Milton Roy CM 4000 multi-solvent delivery system and an ISCO UV detector using an absorption wavelength at 230 nm.

Plant material. Roots, stems and leaves of *Ambrosia trifida L.* were collected on 4 May, 1991 in East Baton Rouge Parish , Louisiana, U.S.A. (Voucher: T. Lu No.6; deposited at the Louisiana State University Herbarium, U.S.A.).

Extractions and isolations of constituents. Fresh roots (1.1 kg) and stems (3 kg) were soaked separately in CH_2Cl_2 for 24 hr yielding 1.21 g and 2.15 g of crude extract, respectively. VLC separation of the root extract yielded 14x100 ml fractions, of which fraction 1, after prep. TLC (hexane), gave traces of **225** and fractions 2 and 3 contained a red oily. Upon further separation by dry CC, fraction 2 gave 17 mg of thiarubrine B (**223**) plus traces of thiarubrine A, while fraction 3 yielded 20 mg of 1,2-epoxythiarubrine B (**224**). The deep red dithiacyclohexadienes **223** and **224** decomposed rapidly when exposed to light, while the yellow thiophene **225** is relatively stable. The R_f values in hexane-EtOAc (9:1) of **223**-**225** are: **225** (R_f 0.81), **223** (R_f 0.72) and **224** (R_f 0.35). Since both **223** and **224** are very sensitive to light, all experiments were carried out in strongly reduced light. Fraction 11 provided yellow crystal which upon recrystallization from hexane/EtOAc (1:1) yielded 20 mg of 2,6-dimethoxybenzoquinone [149].

VLC separation of the crude extract of stems yielded 22x100 ml fractions. Similar to the isolation of roots constituents, prep. TLC (hexane) of fraction 1 yielded thiophene **225** (2 mg), fraction 2 gave 82 mg of thiarubrine B (**223**) and fraction 3, after further dry CC, afforded 7 mg of epoxide **224**. Upon treatment with Me_2CO, fraction 5 gave a precipitate which after further CC yielded 46 mg of hexadecanal. VLC separation of fractions 7 and 8, followed by normal phase semiprep. HPLC (hexane-Et_2O, 19;1), provided the sesquiterpenes **226** (102 mg) and **227** (10 mg). Recrystallization from hexane-EtOAc (1:1) of fraction 15 afforded 1,6-dimethoxybenzoquinone (35 mg).

3-(Pent-3-yn-1-ynyl)-6-(3,4-epoxy-but-1-ynyl)-1,2-dithiacyclohexa-3,5-diene (**224**). $C_{13}H_8OS_2$, deep red oil; UV $\lambda_{max}^{CH_3CN}$ nm: 482, 341, 323; probe EIMS *m/z* (rel. int.): 244 $[M]^+$ (100); 214 (54.9); 148 (25.5); 86 (16.7); 74 (14.7); 62 (19.6). ^1H NMR data in Table 3.1.1.

1α-Angeloyloxycarotol (**226**). $C_{20}H_{32}O_3$, colorless oil; IR ν_{max}^{NaCl} cm^{-1}: 3543 (OH); 1709 (C=O); 1648 (C=C); 1234, 1157 (CC(=O)OC, ester); EIMS *m/z* (rel. int.): 320 $[M]^+$ (0.8); 302 $[M-H_2O]^+$ (0.2); 277 $[M-CH(CH_3)_2]^+$ (0.1); 259 $[302-CH(CH_3)_2]^+$ (1.2); 220 $[M-100]^+$ (4.7); 202 $[220-H_2O]^+$ (20.3); 177 $[220-CH(CH_3)_2]^+$ (12.1); 159 $[202-CH(CH_3)_2]^+$ (39.4); 83 $[O=CC(CH_3)=CHCH_3]^+$ (100); 55 $[83-CO]^+$ (73.2); 43 $[CH(CH_3)_2]^+$ (26.7); ^1H NMR data in Table 3.1.2 and ^{13}C NMR data in Table 3.1.3.

1α-(2'-Methylbutyroyloxy)-carotol (**227**). $C_{20}H_{34}O_3$, colorless oil; IR ν_{max}^{NaCl} cm^{-1}: 3543 (OH); 1721 (C=O); 1291, 1191 (CC(=O)OC, ester); EIMS *m/z* (rel. int.): 322 $[M]^+$ (1.4); 304 $[M-H_2O]^+$ (0.5); 279 $[M-CH(CH_3)_2]^+$ (0.1); 261 $[304-CH(CH_3)_2]^+$ (0.8); 220 $[M-102]^+$ (21.0); 202 $[220-H_2O]^+$ (36.0); 177 $[220-CH(CH_3)_2]^+$ (46.1); 159 $[202-CH(CH_3)_2]^+$ (100); 85 $[O=CCH(CH_3)CH_2CH_3]^+$ (55.7); 57 $[85-CO]^+$ (55.7); 43 $[CH(CH_3)_2]^+$ (36.9); ^1H NMR data in Table 3.1.2 and ^{13}C NMR data in Table 3.1.3.

3.2

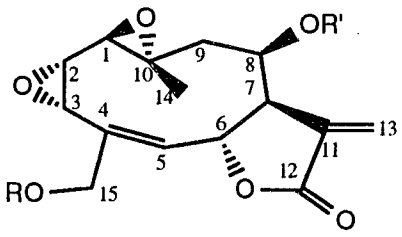
Terpenoids from *Liatris ohlingerae*

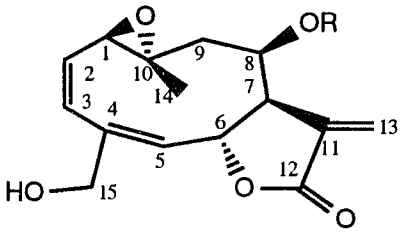
Introduction

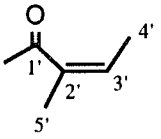
Liatris, a genus in the tribe Eupatorieae of the Asteraceae family, had been previously reported to produce a variety of cytotoxic and antitumor sesquiterpene lactones and diterpenes [162-169]. In continuation of our search for biologically active compounds from the Asteraceae family, we have investigated the aerial parts of the Scrub Blazing Star [*Liatris ohlingerae* (Blake) Robinson], a federally protected endemic species the white sand scrub of central Florida [170]. In spite of the limited amounts of plant material (43 g) of this rare species, five known and two new 15-hydroxylated heliangolide-type sesquiterpene lactones were isolated. The known lactones included liscundin (**228a**) [163], liscunditrin (**228b**) [163] and eleganin (**228c**) [163] and the triterpenoids were a mixture of taraxasterol (**230a**) [171], pseudo-taraxasterol (**230c**) [171] and their acetates **230b** and **230d**, repectively. The structures of all known and new compounds were determined by spectroscopic methods, especially by mass spectral analysis and high field ^{1}H and ^{13}C NMR methods. The molecular structure of liscunditrin (**228b**) was established by single crystal X-ray diffraction analysis.

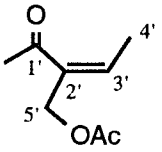
Results and Discussion

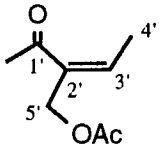
Vacuum liquid chromatographic separation [129] of the CH_2Cl_2 extract of *Liatris ohlingerae* provided two pair of isomeric triterpenes, taraxasterol (**230a**) and pseudo-taraxasterol (**230c**) as well as taraxasteryl acetate (**230b**) and pseudo-taraxasterylacetate (**230d**). Taraxasterol (**230a**) had been previously isolated from

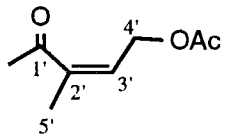


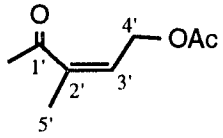


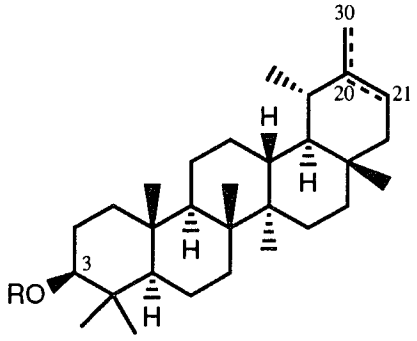
228a R = H, R' = 

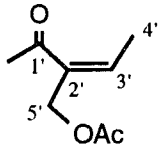
229a R = 

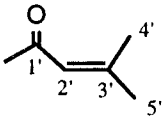
228b R = H, R' = 

229b R = 

228c R = H, R' = 



228d R = Ac, R' = 

228e R = H, R' = 

230a R = H, 20,30-ene
230b R = Ac, 20,30-ene
230c R = H, 20,21-ene
230d R = Ac, 20,21-ene

Figure 3.2.1. Terpenes from *Liatris ohlingerae*

Launaea nudicaulis [171] and **230a** and its acetate (**230b**) were also found in other *Liatris* species [167]. *pseudo*-Taraxasterol (**230c**) and its acetate (**230d**) had been prepared from taraxasterol (**230a**) by acid-induced isomerization and acetylation [171]. The structures of these four triterpenes were established by mass spectral and ^{1}H NMR analysis, as well as direct comparison of the ^{13}C NMR data with those reported in the literature [171, 172].

Liscundin (**228a**) and liscunditrin (**228b**) had been previously isolated from *Liatris secunda* [163] and *L. mucronata* [167] and compound **229b** was obtained from *L. mucronata* [167]. The ^{1}H and ^{13}C NMR data of **228a**, **228b** and **229b** were essentially identical with those of previously reported data [167]. The molecular structure of liscunditrin (**228b**) was determined by single crystal X-ray diffraction (Fig. 3.2.2); details will be presented at the end of this section. Eleganin (**228c**) was previously found in *Liatris elegans* [163] and *L. scabra* [166]. Its ^{1}H NMR data were in agreement with reported values [163]. Since the ^{13}C NMR data of **228c** had not been previously described, the ^{13}C NMR spectrum of **228c** was assigned by combined application of COSY, ^{1}H,^{13}C HETCOR and the DEPT methods, the data being listed in Table 3.2.2.

Acetylliscunditrin (**228d**) had been previously prepared from punctaliatrin [163], but to our best knowledge, this is the first report of this compound as a natural product. Comparison of the 400 MHz ^{1}H NMR spectra of **228b** and **228d** revealed that their major differences were due to downfield shifts of 15-H signals at δ 4.27 (H-15a) and 4.31 (H-15b) in **228b** to δ 4.64 and 4.71 in **228d**, and the presence of an additional acetate methyl singlet at δ 2.11 in compound **228d**. The mass spectrum of **228d** showed the presence of an ion peak at m/z 416 $[M\text{-}HOAc]^{+}$ and a base peak at m/z 43 $[Ac]^{+}$, which further supported the presence of an acetate group at C-15 in **228d**.

A new heliangolide-type sesquiterpene lactone, which was named ohlingerin, was assigned the structure **228e** on the basis of following data. Strong IR absorptions

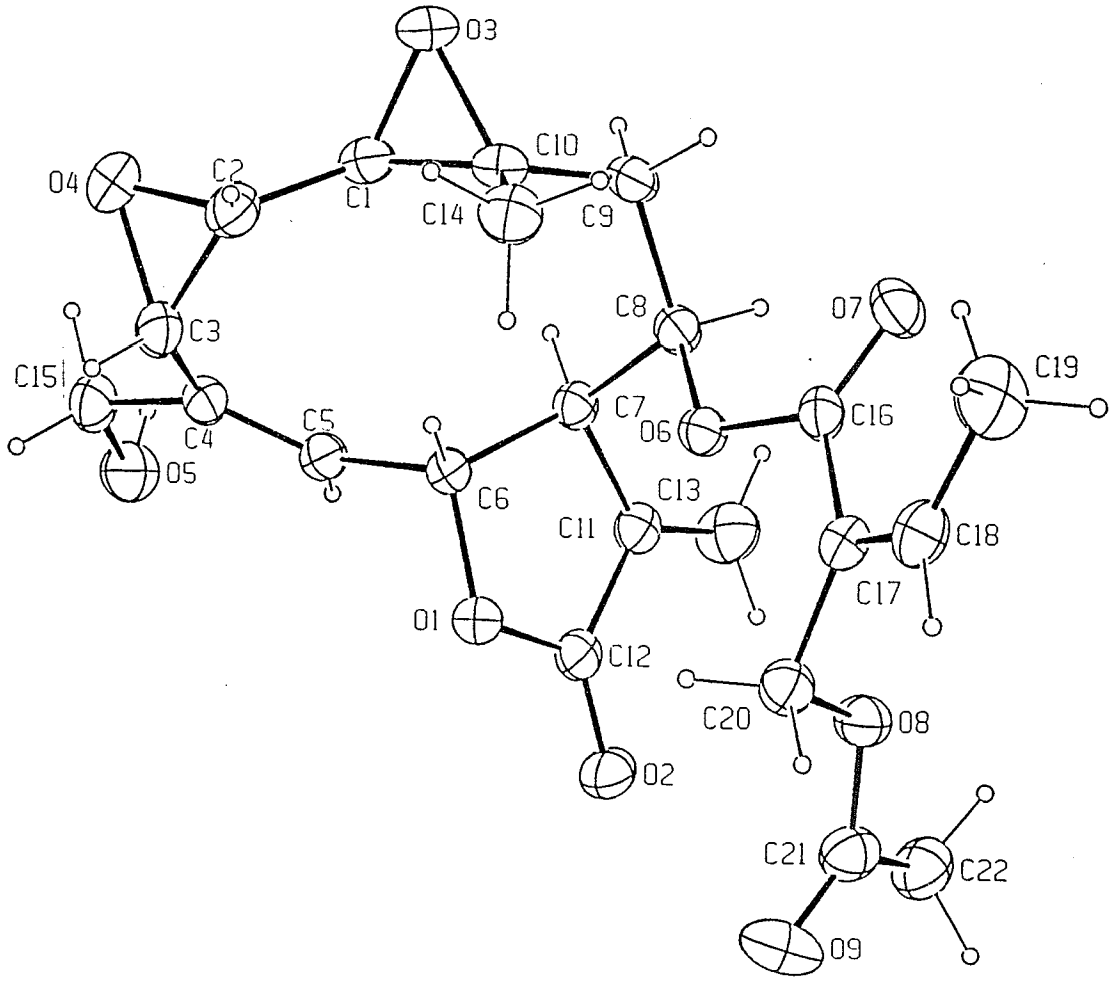


Figure 3.2.2. X-ray structure of liscunditrin (**228b**)

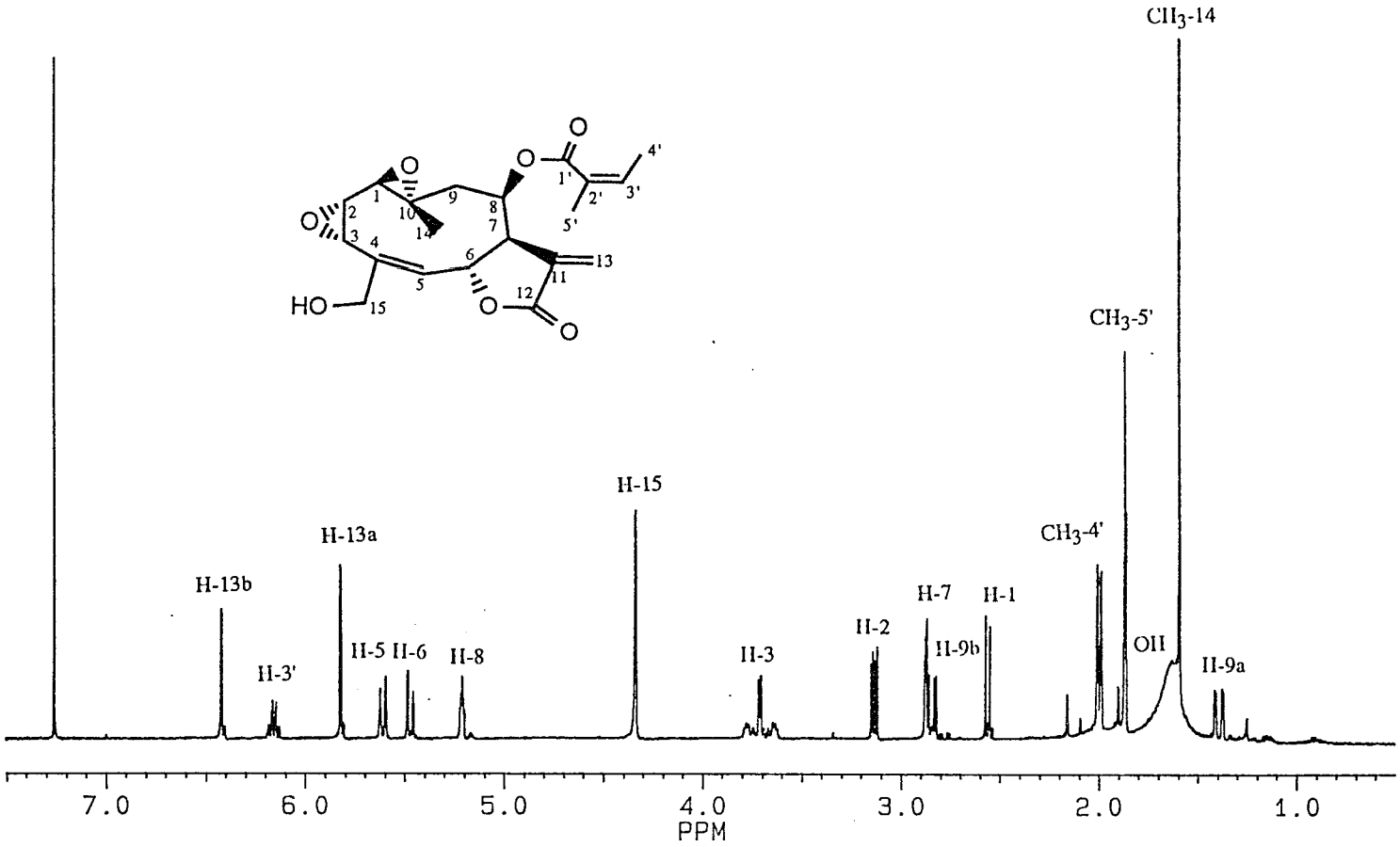


Figure 3.2.3. 400 MHz ^{1}H NMR spectrum of liscundin (**228a**)

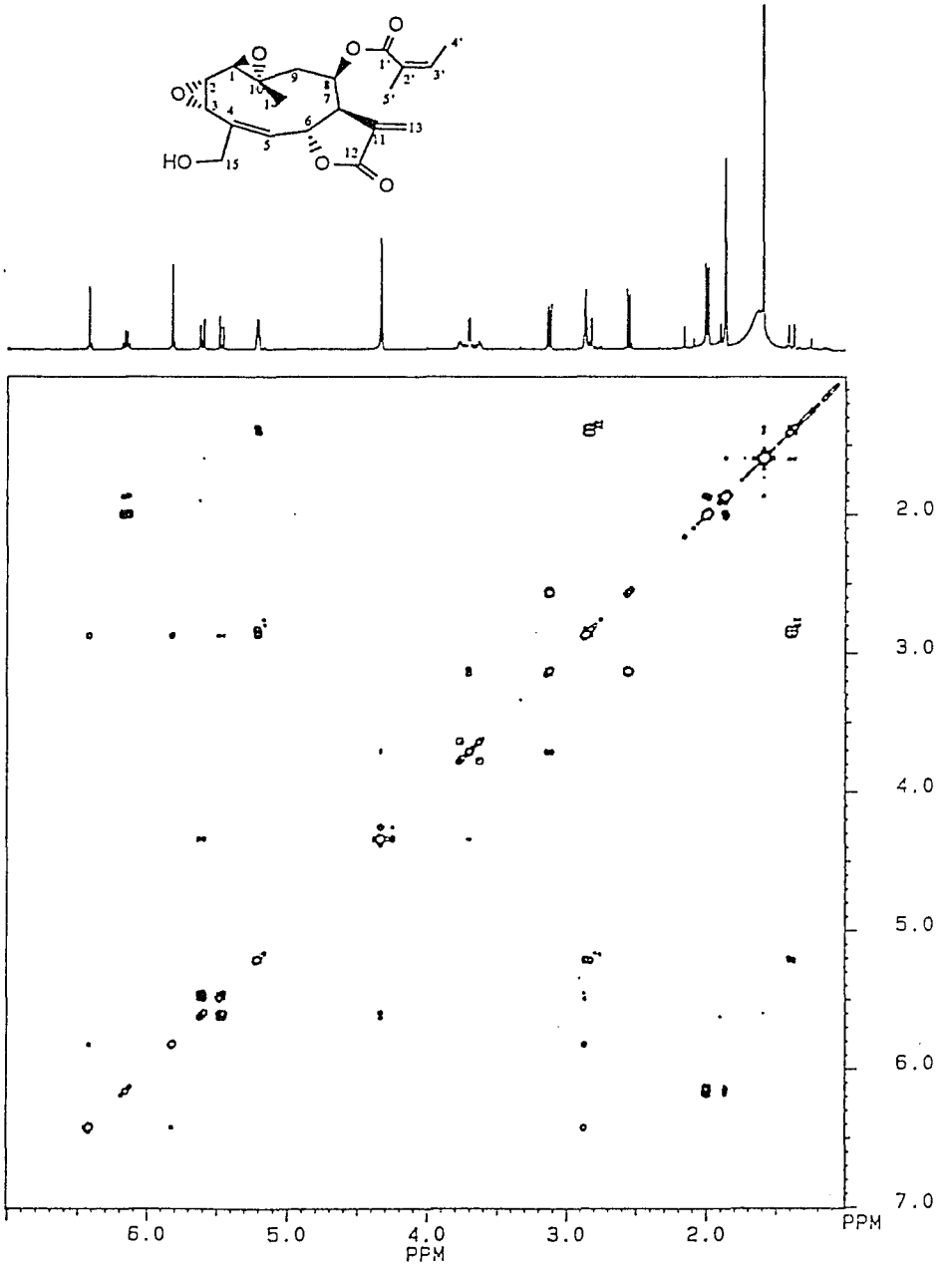


Figure 3.2.4. 400 MHz ^{1}H-COSY spectrum of liscundin (228a)

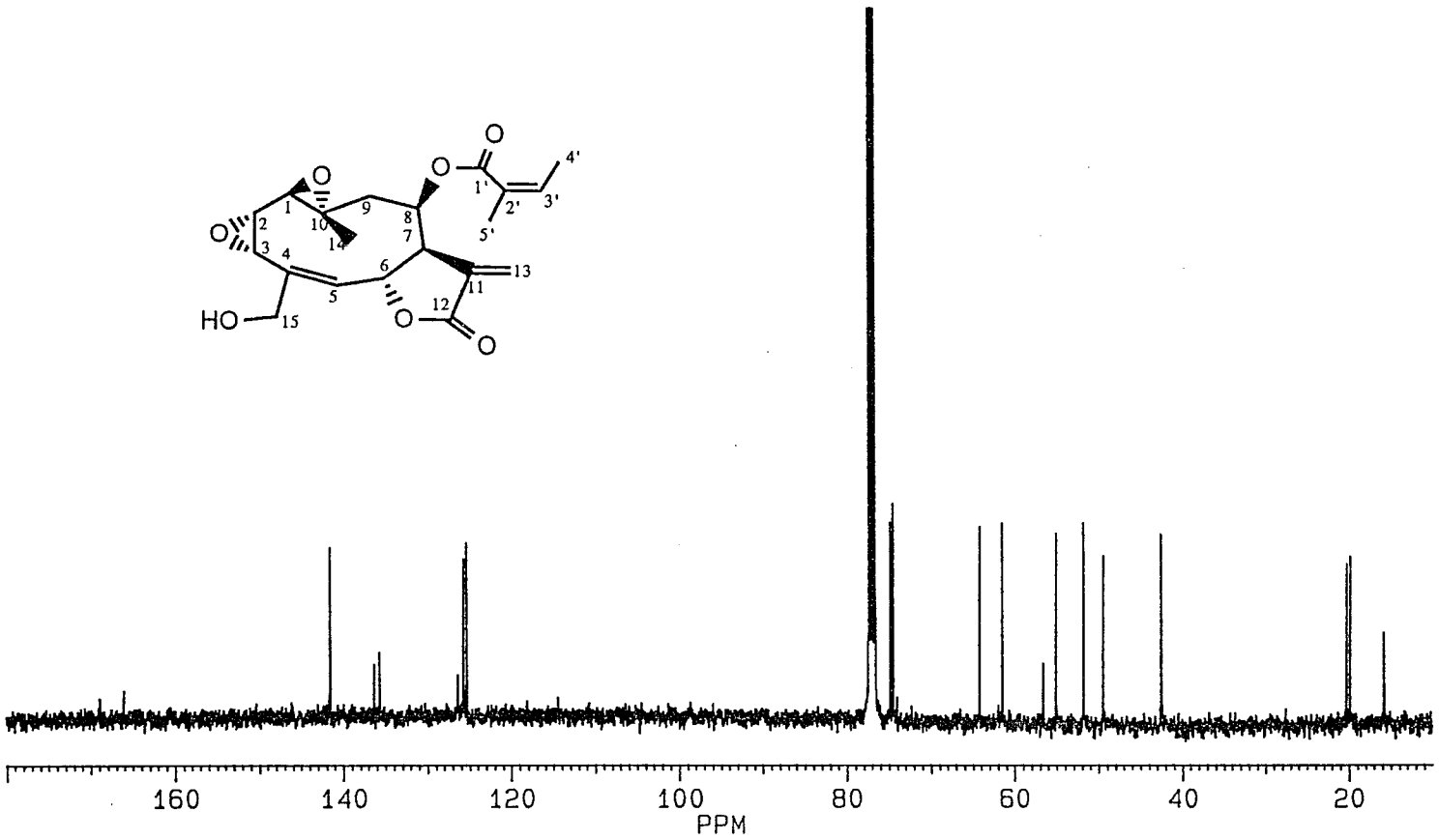


Figure 3.2.5. 100 MHz ^{13}C NMR spectrum of liscundin (**228a**)

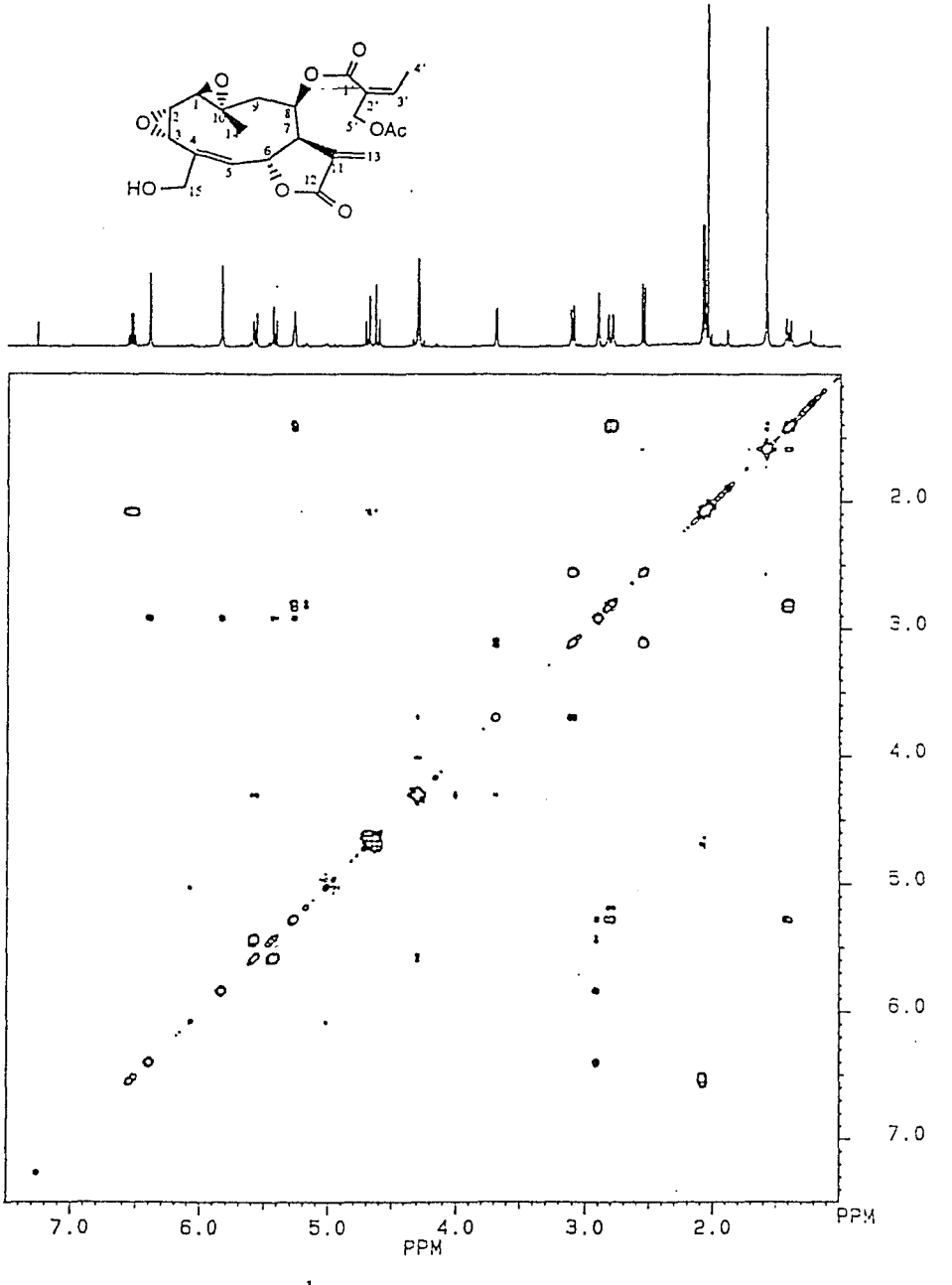


Figure 3.2.6. 400 MHz ^{1}H-COSY spectrum of liscunditrin (**228b**)

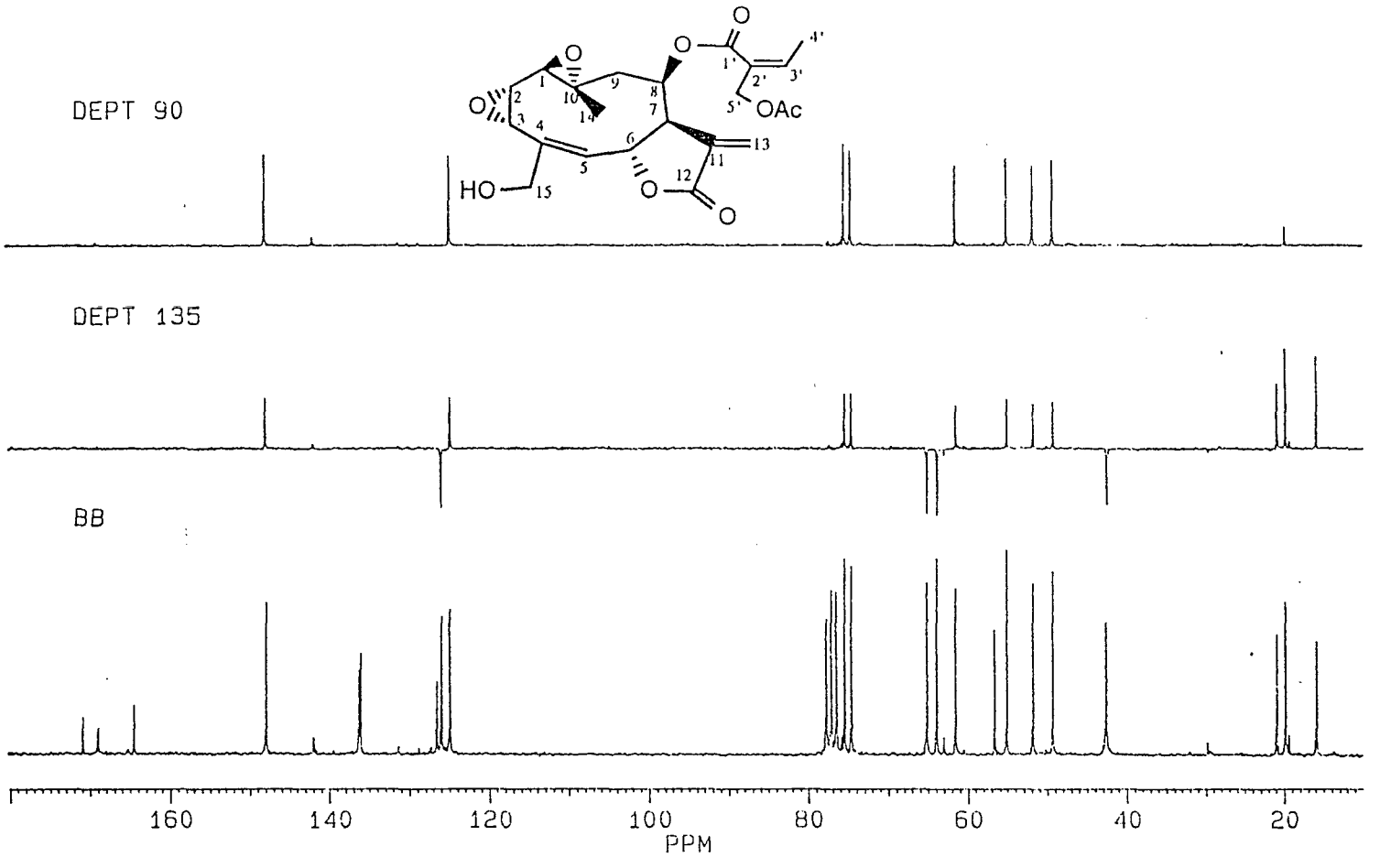


Figure 3.2.7. DEPT 90°, DEPT 135°and BB ^{13}C NMR spectra of liscunditrin (**228b**)

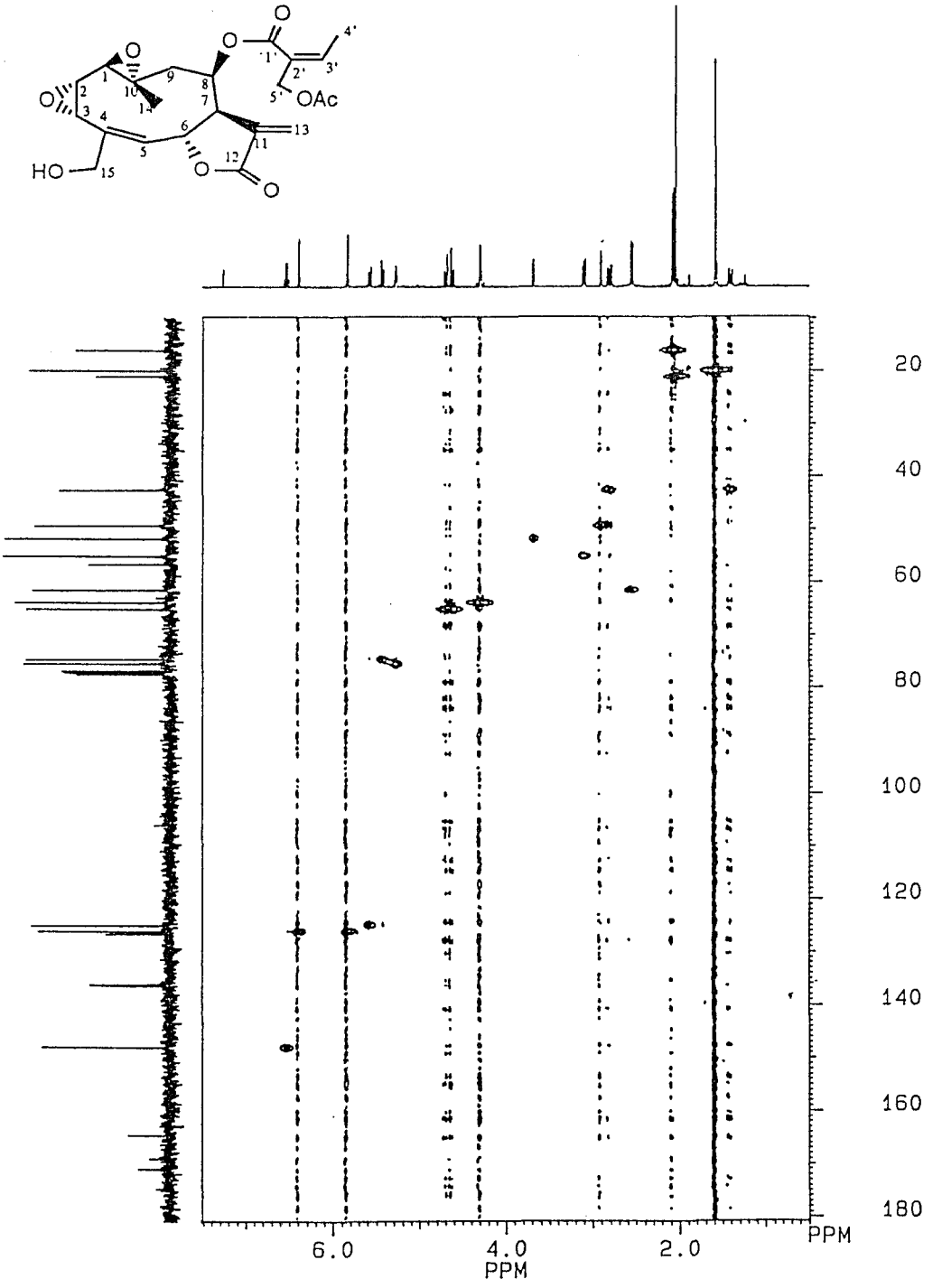


Figure 3.2.8. 2D Inverse ^{1}H-^{13}C heteronuclear correlation spectrum of liscunditrin (**228b**)

Table 3.2.1. ^{1}H NMR spectral data of compounds **228a-e**, **229a** and **229b** (400 MHz, $CDCl_3$ as internal standard)

H	**228a**	**228b**	**228c**	**228d**	**228e**	**229a**	**229b**
1	2.56 d	2.55 d	2.56 d	2.53 d	2.54 d	3.29 d	3.28 dd
2	3.13 dd	3.11 dd	3.13 dd	3.13 dd	3.13 dd	5.71 dd	5.72 dd
3	3.71 br d	3.69 br d	3.71 br d	3.71 br d	3.69 br d	6.18 br d	6.18 br d
5	5.61 br dd	5.57 br dd	5.61 br dd	5.50 br d	5.60 br dd	5.60 br d	5.62 br d
6	5.47 dd	5.43 br d	5.47 br d	5.43 br d	5.45 br d	5.13 br d	5.17 dd
7	2.87 br t	2.91 br t	2.88 br t	2.87 br s	2.84 br t	3.04 br s	3.03 br t
8	5.21 m	5.27 m	5.19 m	5.30 m	5.16 m	5.33 br s	5.24 m
9	1.39 dd 2.84 dd	1.41 dd 2.81 dd	1.40 dd 2.83 dd	1.42 dd 2.84 dd	1.36 dd 2.77 dd	1.44 dd 2.84 dd	1.43 dd 2.81 dd
13	5.82 d 6.42 d	5.84 d 6.40 d	5.82 d 6.42 d	5.85 d 6.44 d	5.81 d 6.40 d	5.85 d 6.42 d	5.84 d 6.42 d
14	1.59 s	1.58 s	1.59 s	1.60 s	1.59 s	1.43 s	1.41 s
15	4.34 s	4.27 d 4.31 d	4.34 br s	4.64 d 4.71 d	4.33 br s	4.16 d 4.20 d	4.18 d 4.22 d
2'	----	----	----	----	5.62 m	----	----
3'	6.16 qq	6.54 q	6.09 m	6.56 br q	----	6.53 q	6.06 m
4'	1.99 dq	2.08 d	4.95 dm 5.07 dm	2.10 d	2.16 br s	2.08 d	4.95 dm 5.06 dm
5'	1.87 dq	4.62 d 4.70 d	1.91 br d	4.68 br d 4.76 br d	1.90 br s	4.60 br d 4.68 br d	1.88 m
Ac	----	2.04 s	2.08 s	2.06 s	----	2.04 s	2.08 s
Ac'	----	----	----	2.11 s	----	----	----

J (Hz): **228a**: 1, 2=8.5, 2, 3=4.2, 5, 6=11.0, 5, 15=1.2, 6, 7=1.0, 7, 8=1.6, 8, 9a=2.3, 8, 9b=4.4, 9a, 9b=15.0, 13a, 13b=1.8, 3', 4'=7.3, 3', 5'=4', 5'=1.6; **228b**: 1, 2=8.5, 2, 3=4.1, 5, 6=11.0, 5, 15=1.1, 6, 7=1.2, 7, 8=1.4, 8, 9a=2.1, 8, 9b=4.4, 9a, 9b=15.0, 13a, 13b=1.6, 15a, 15b=16.0, 3', 4'=7.3, 5'a, 5'b=12.5; **228c**: 1, 2=8.5, 2, 3=4.1, 5, 6=11.0, 5, 15=1.1, 6, 7=1.2, 7, 8=1.5, 8, 9a=2.0, 8, 9b=4.3, 9a, 9b=15.0, 13a, 13b=1.6, 3', 4'a=4.6, 3', 4'b=5.5, 4'a, 4'b=16.5, 3', 5'=4'a, 5'=4'b, 5'=1.7; **228d**: 1, 2=8.5, 2, 3=4.1, 5, 6=11.1, 8, 9a=2.2, 8, 9b=4.3, 9a, 9b=15.1, 13a, 13b=1.6, 15a, 15b=15.7, 3', 4'=7.3, 5'a, 5'b=14.2; **228e**: 1, 2=8.6, 2, 3=3.8, 5, 6=11.0, 5, 15=1.2, 6, 7=1.0, 7, 8=1.5, 8, 9a=1.8, 8, 9b=4.0, 9a, 9b=14.8, 13a, 13b=1.5; **229a**: 1, 2=7.5, 2,3 =11.5, 5, 6=10.6, 8, 9a=2.6, 8, 9b=4.0, 9a, 9b=15.1, 13a, 13b=1.4, 15a, 15b=15.0, 3', 4'=7.2, 5'a, 5'b=12.2; **229b**: 1, 2=7.4, 1, 3=1.0, 2, 3=11.6, 5, 6=10.6, 6, 7=1.1, 7, 8=1.5, 8, 9a=2.9, 8, 9b=4.2, 9a, 9b=14.9, 13a, 13b=1.6, 15a, 15b=15.0, 3', 4'a=4.7, 3' 4'b=5.5, 4'a, 4'b=16.5, 3', 5'=1.7, 4'a, 5'=4'b, 5'=1.8.

Table 3.2.2. ^{13}C NMR data of compounds **228a-c**, **228e** and **229a** (100 MHz, $CDCl_3$ as internal standard)*

C	**228a**	**228b**	**228c**	**228e**	**229a**
1	61.5 d	61.4 d	61.3d	61.5 d	60.3 d
2	55.0 d	54.9 d	55.0d	55.0 d	131.4 d
3	51.7 d	51.7 d	51.7d	51.7 d	128.5 d
4	135.6 s	135.9 s	135.8s	135.6 s	139.1 s
5	125.3 d	124.8 d	125.0d	125.3 d	124.9 d
6	74.5 d	74.5 d	74.4 d	74.5 d	75.2 d
7	49.4 d	49.2 d	49.3 d	49.4 d	50.0 d
8	74.8 d	75.3 d	75.6 d	74.0 d	77.4 d
9	42.5 t	42.5 t	42.4 t	42.6 t	42.9 t
10	56.6 s	56.5 s	56.4 s	56.8 s	61.5 s
11	136.3 s	136.1 s	136.1 s	136.3 s	136.9 s
12	168.9 s	168.8 s	168.8 s	169.0 s	169.0 s
13	125.7 t	125.8 t	125.9 t	125.5 t	125.3 d
14	19.9 q	19.8 q	19.4 q	20.0 q	19.6 q
15	64.2 t	63.8 t	62.9 t	64.1 t	65.3 t
1'	166.0 s	164.4 s	165.1 s	164.8s	164.5 s
2'	126.3 s	126.4 s	127.1 s	114.4 d	126.5 s
3'	141.5 d	147.7 d	142.0 d	160.5 s	147.9 d
4'	15.8 q	15.9 q	64.1 t	20.4 q	15.9 q
5'	20.3 q	65.0 t	19.9 q	27.6 q	65.1 t
Ac{	------	170.8 s	170.8 s	------	170.7 s
	------	20.9 q	20.9 q	------	20.9 q

* Peak multiplicities were determined by heteronuclear multipulse programs (DEPT); s=singlet; d=doublet; t= triplet; q=quartet.

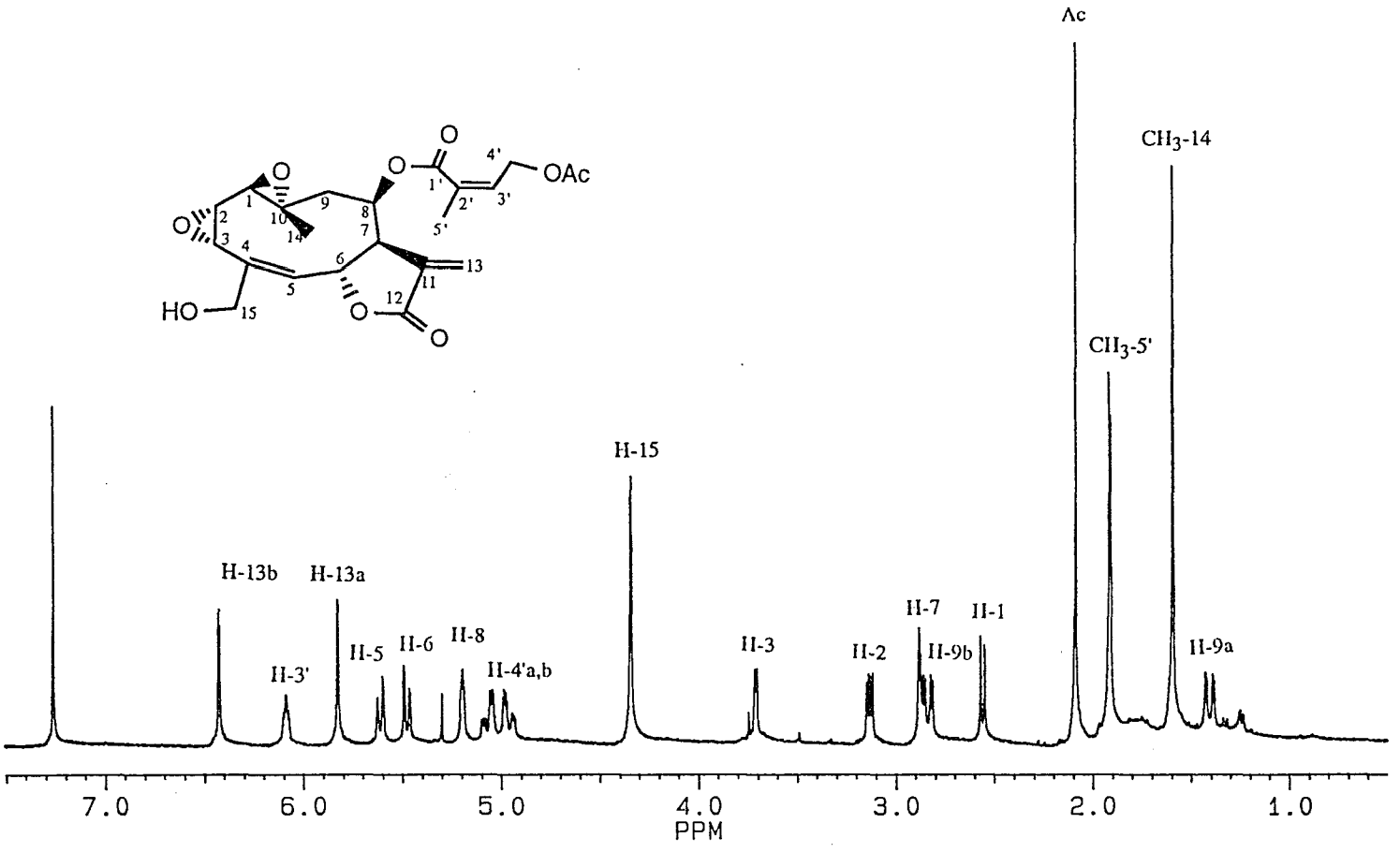


Figure 3.2.9. 400 MHz ^{1}H NMR spectrum of eleganin (**228c**)

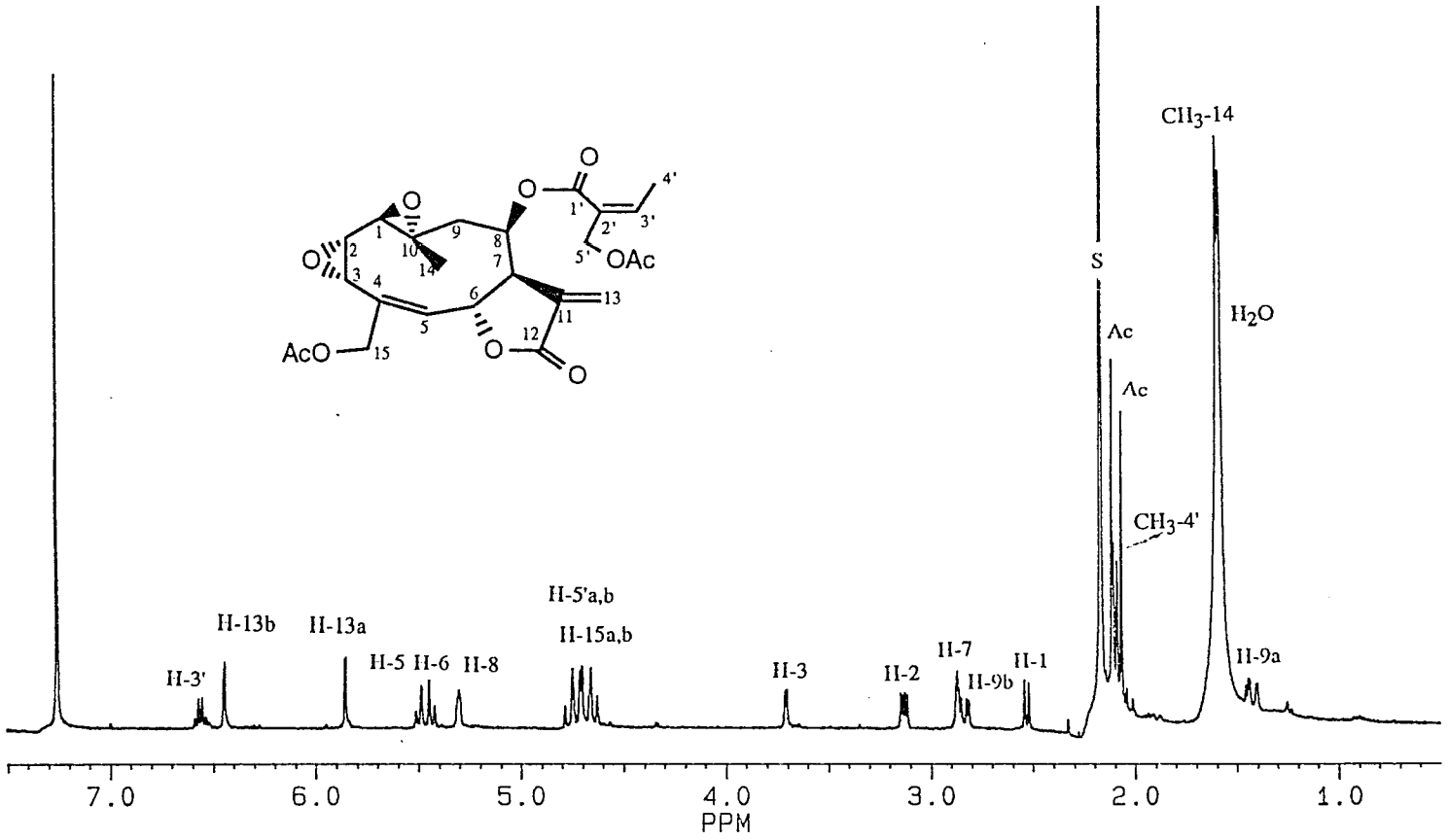


Figure 3.2.10. 400 MHz ^{1}H NMR spectrum of acetylliscunditrin (**228d**)

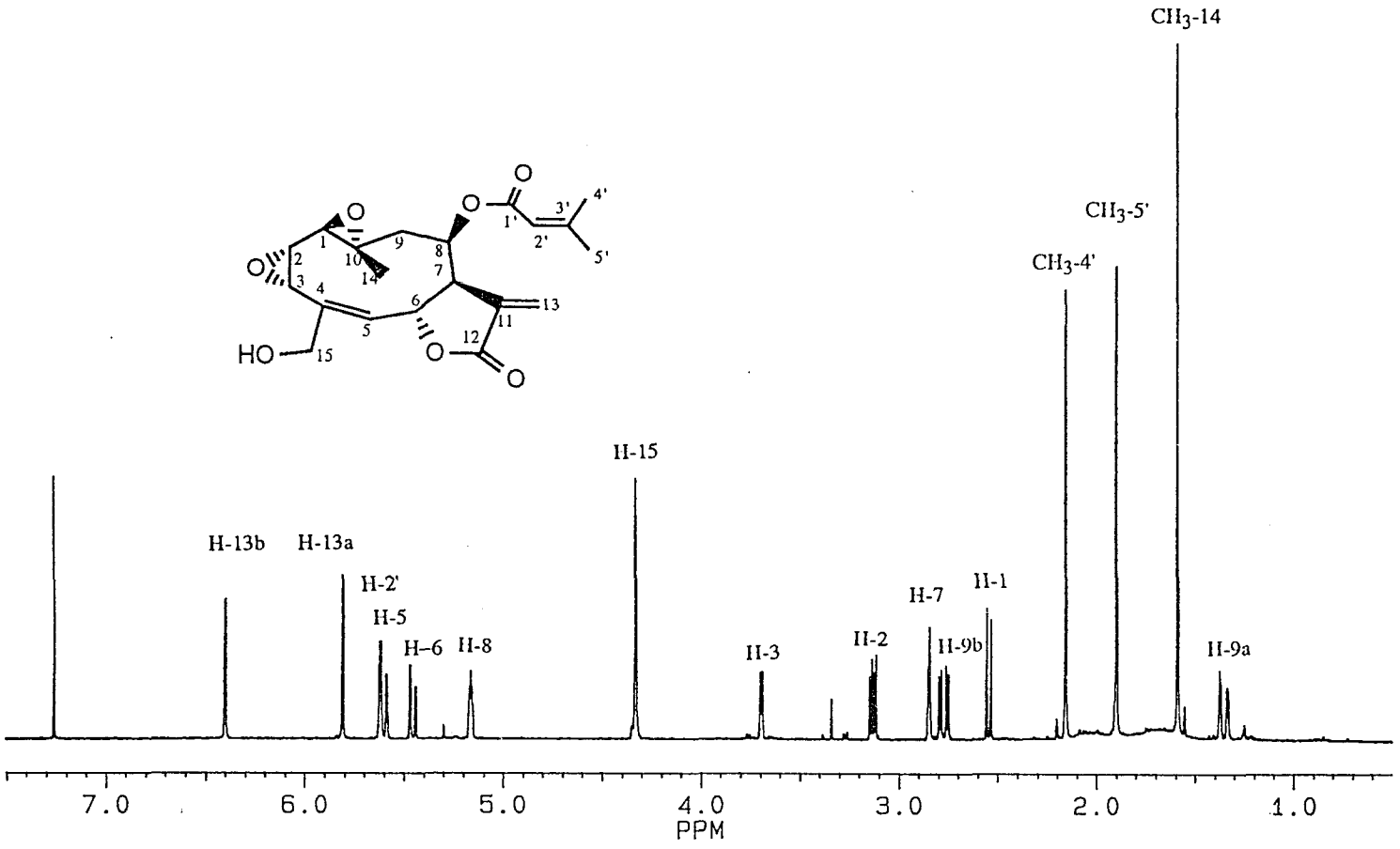


Figure 3.2.11. 400 MHz ^{1}H NMR spectrum of ohlingerin (**228e**)

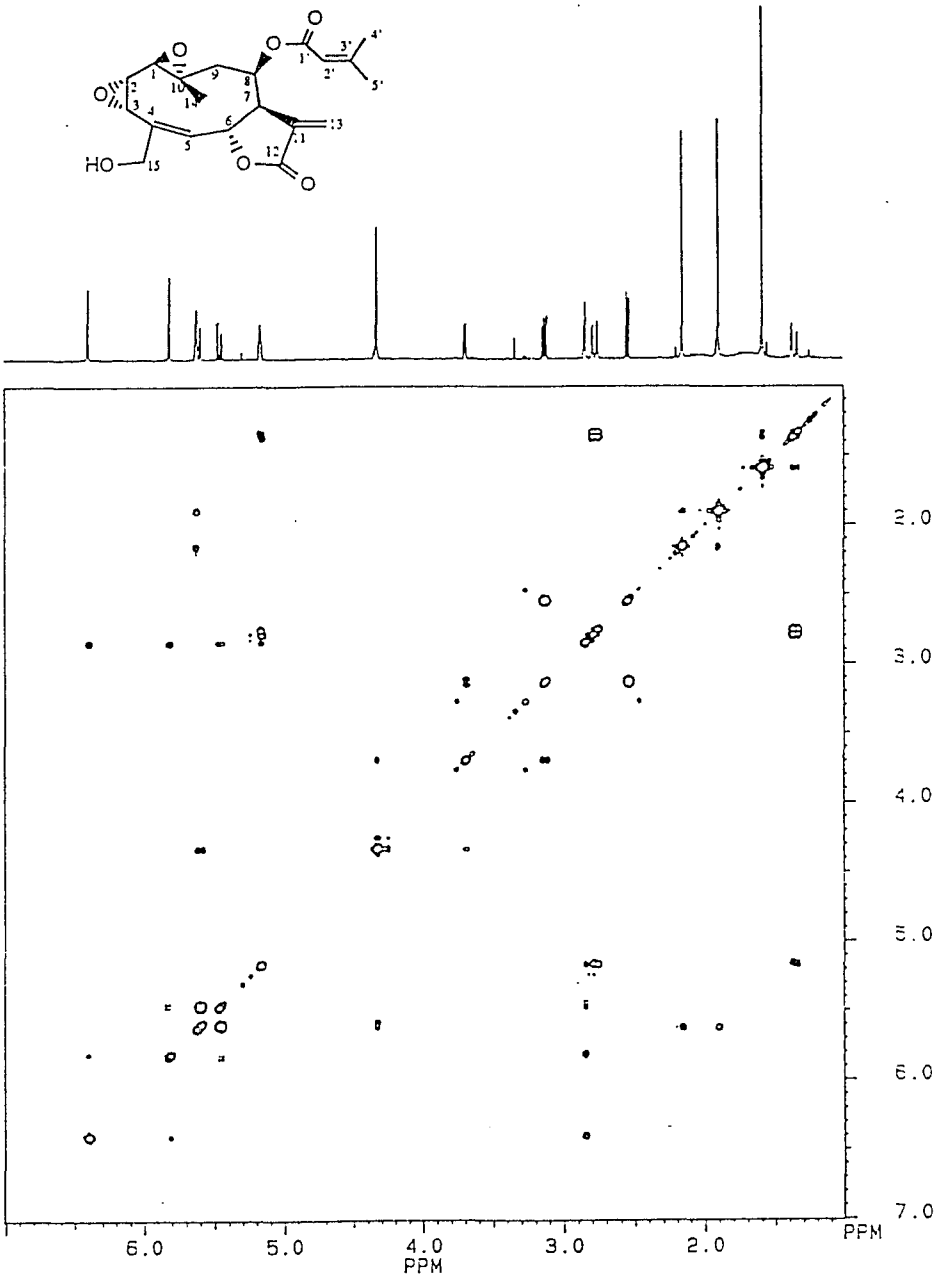


Figure 3.2.12. 400 MHz ^{1}H-COSY of ohlingerin (**228e**)

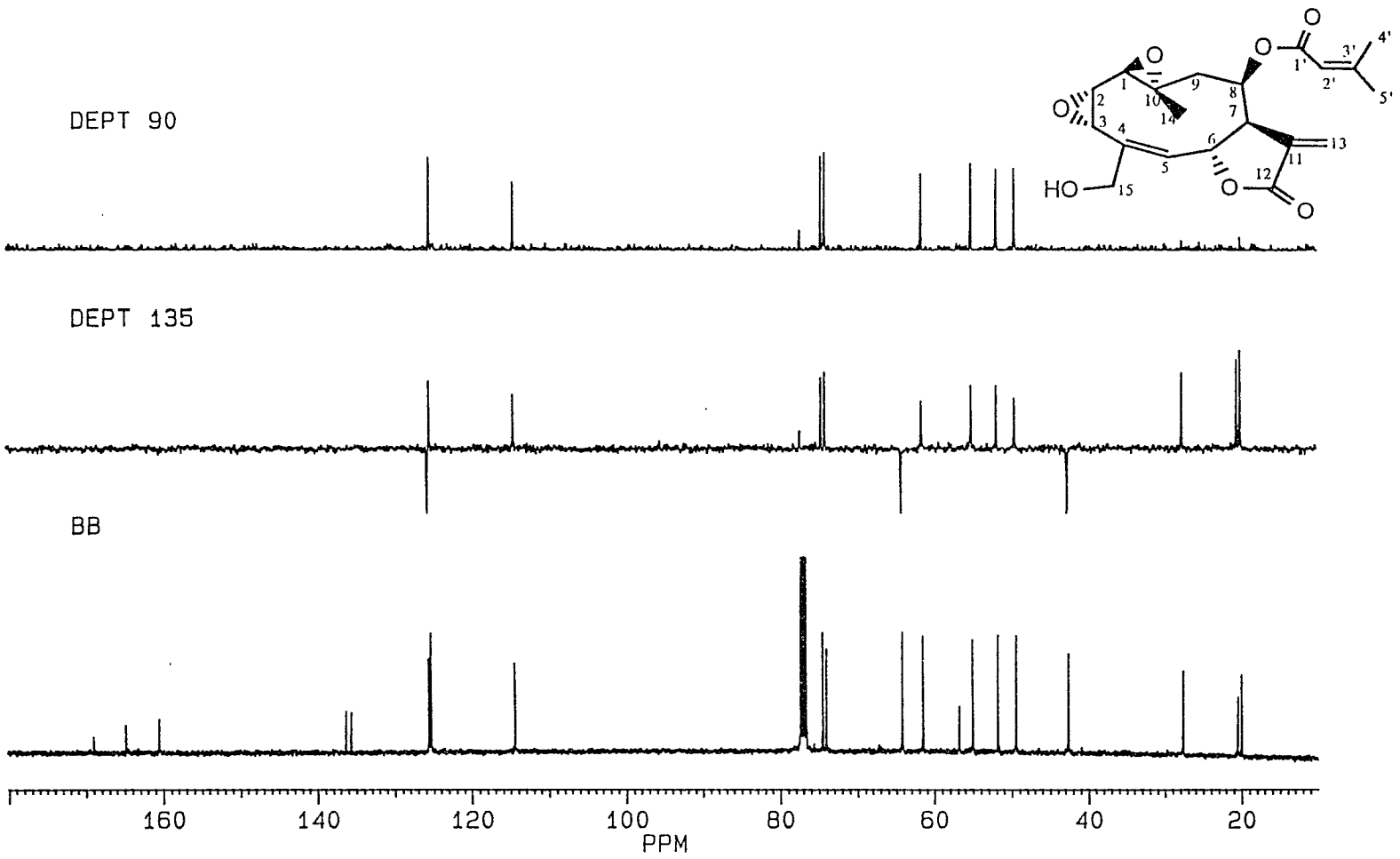


Figure 3.2.13. DEPT 90°, DEPT 135° and BB ^{13}C NMR spectra of ohlingerin (**228e**)

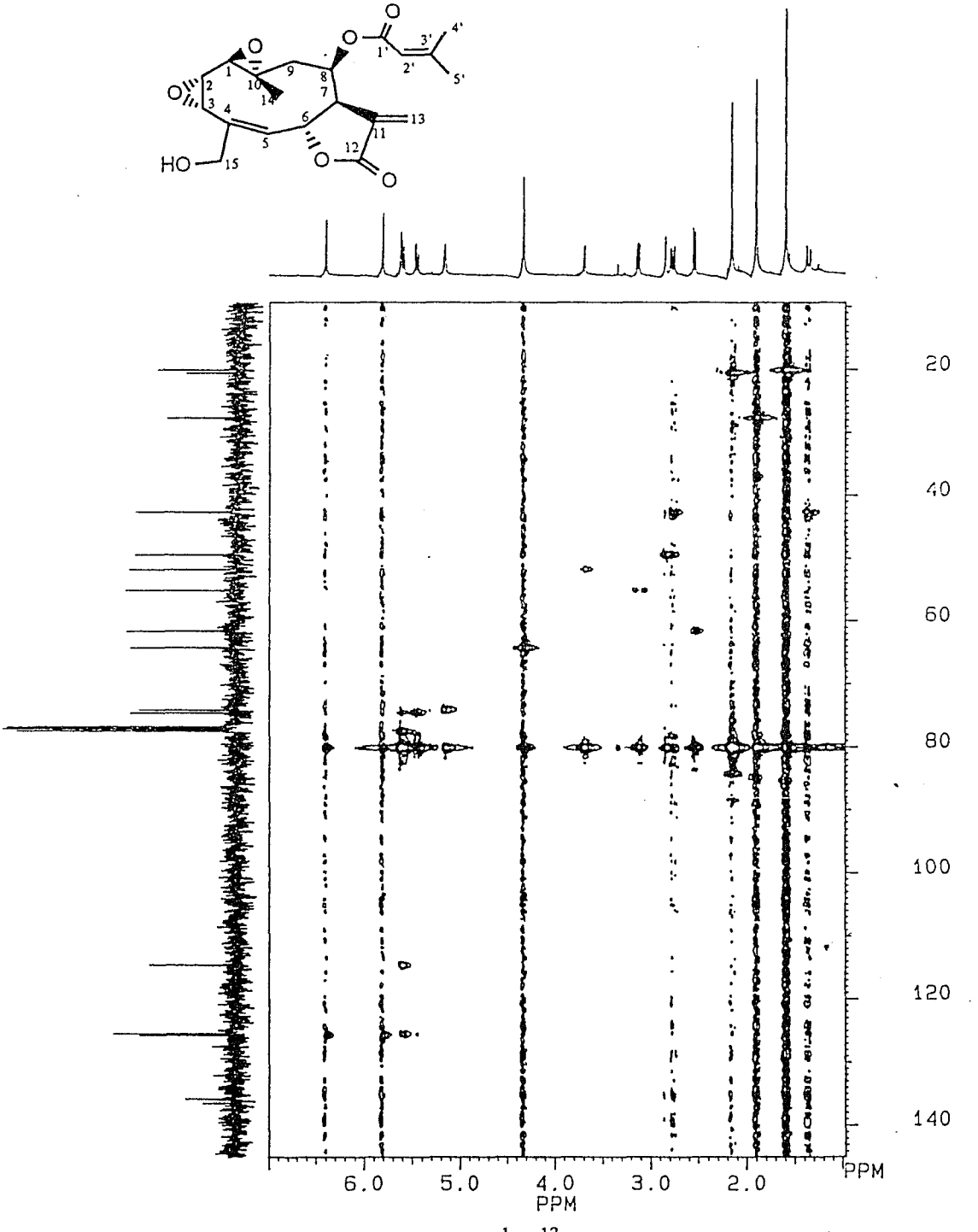


Figure 3.2.14. 2D Inverse ^{1}H-^{13}C heteronuclear correlation spectrum of ohlingerin (**228e**)

at 1763 and 1647 cm^{-1} indicated a conjugated γ-lactone. The ^1H NMR values of **228e** were very similar to those of the medium ring portion of **228b** but showed different side chain signals. A proton multiplet at δ 5.62 was coupled to two broad methyl singlets at δ 2.16 and 1.90, as shown by the ^1H-COSY spectrum, indicating the presence of a senecioate moiety. The EI mass spectrum of **228e** gave a weak molecular ion at m/z 376 and two very strong peaks at m/z 83 $[Me_2C=CHCO]^+$ and 55 $[83\text{-}CO]^+$ which were in agreement with the presence of a senecioate group at C-8. The ^{13}C NMR data of **228e** indicated the presence of 20 carbons, which were unambigeously assigned by ^1H-COSY, ^1H, ^{13}C-correlation and DEPT experiments (Table 3.2.2). The FAB MS of **228e** gave prominent peaks at m/z 377 $[MH]^+$, 277 $[MH\text{-}100]^+$ which supported its empirical formula $C_{20}H_{24}O_7$.

Another new sesquiterpene lactone, **229a**, exhibited ^1H NMR data which were very similar to those of the medium ring signals of **229b** but gave different side chain signals, which were diagnostic of the acetylsarracinoyl moiety, a side chain also present in compounds **228b** and **228d**. A proton quartet at δ 6.53, which was coupled to a methyl doublet at δ 2.08 and two geminal proton doublets at δ 4.60 and 4.68 along with the acetyl methyl singlet at δ 2.04 were characteristic for acetylsarracinate moiety. The ^{13}C NMR spectrum of **229a** indicated the presence of 22 carbons which were unambiguously assigned by DEPT, COSY and the inverse $^1H,^{13}C$ correlation NMR methods. The FAB mass spectrum of **229a** showed a prominent M+1 ion peak at m/z 419 which was in agreement with the empirical formula $C_{22}H_{26}O_8$. Other strong peaks at m/z 243, derived by the loss of acetylsarracinic acid and H_2O from $[MH]^+$, *m/z* 141 $[CH_3CH(CH_2OAc)CO]^+$, 81 $[141\text{-}AcOH]^+$ and 43 $[Ac]^+$ further confirmed the presence of an acetylsarracinate moiety.

The crystal structure of liscunditrin (**228b**)

The crystal structure of liscunditrin (**228b**) is illustrated in Figure 3.2.2, in which the heliangolide skeleton is evident. Its conformation is detailed by the torsion

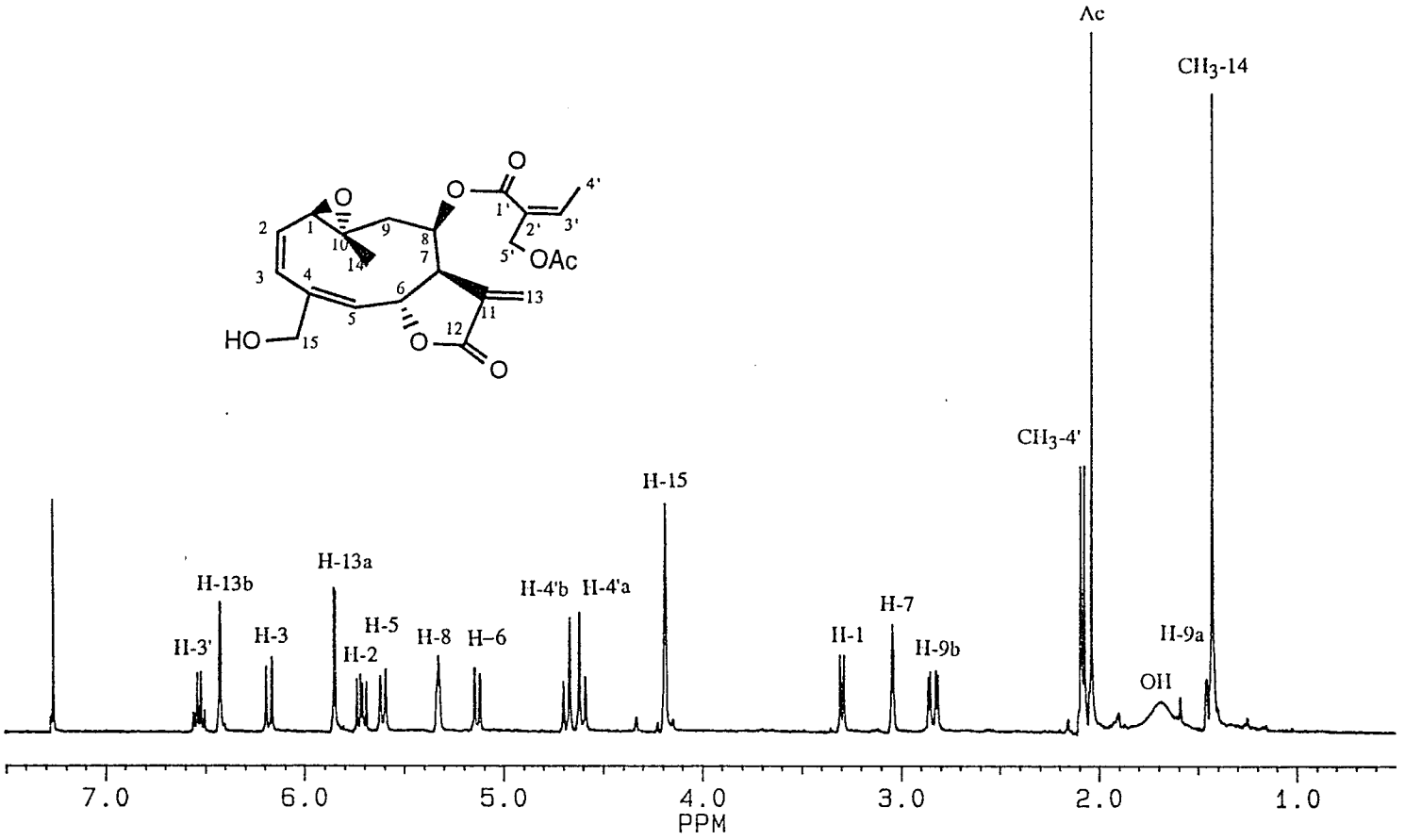


Figure 3.2.15. 400 MHz ^{1}H NMR spectrum of punctaliatrin-5'-acetate (**229a**)

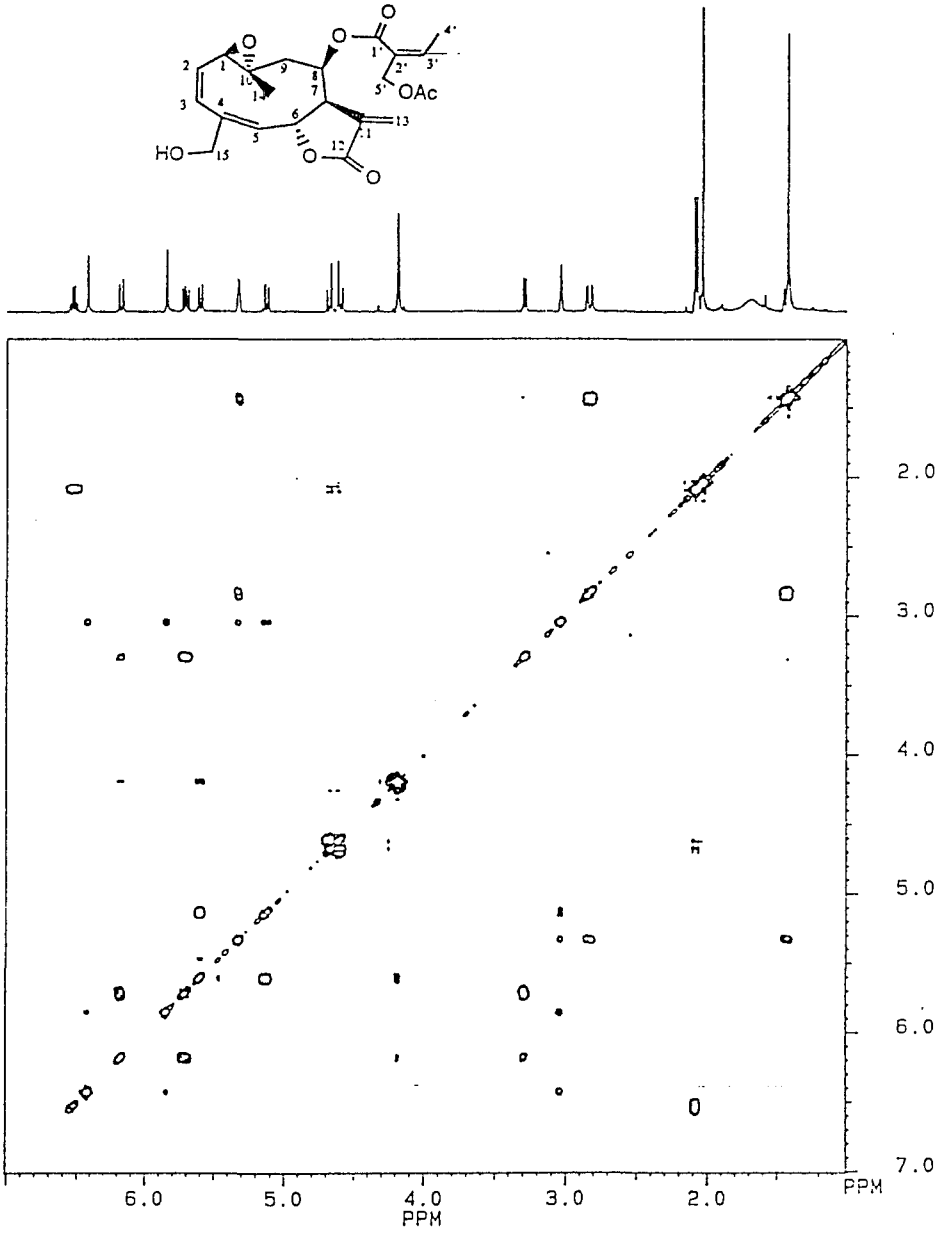


Figure 3.2.16. 400 MHz ^{1}H-COSY spectrum of punctaliatrin-5'-acetate (**229a**)

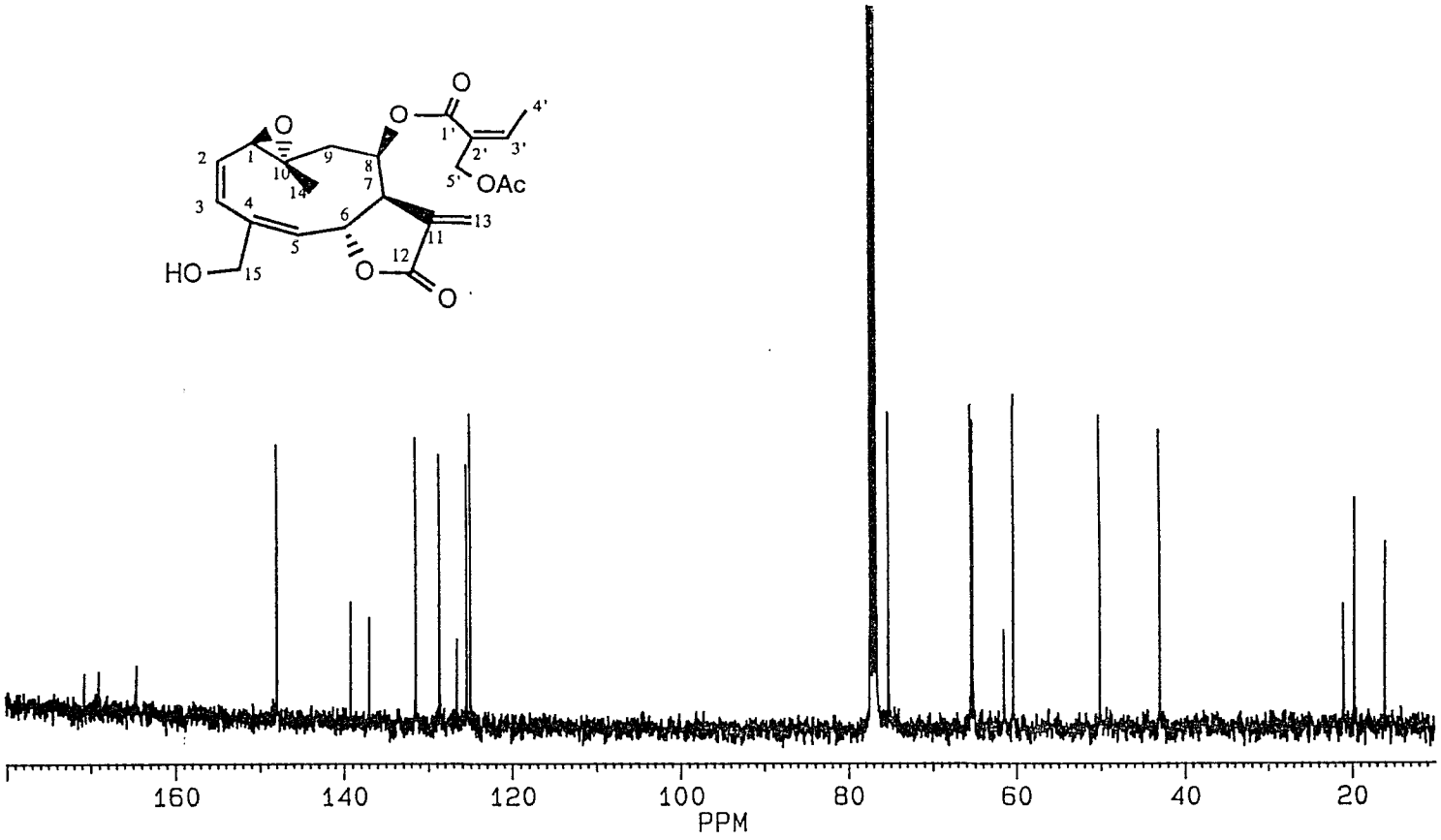


Figure 3.2.17. 100 MHz ^{13}C NMR spectrum of punctaliatrin-5'-acetate (**229a**)

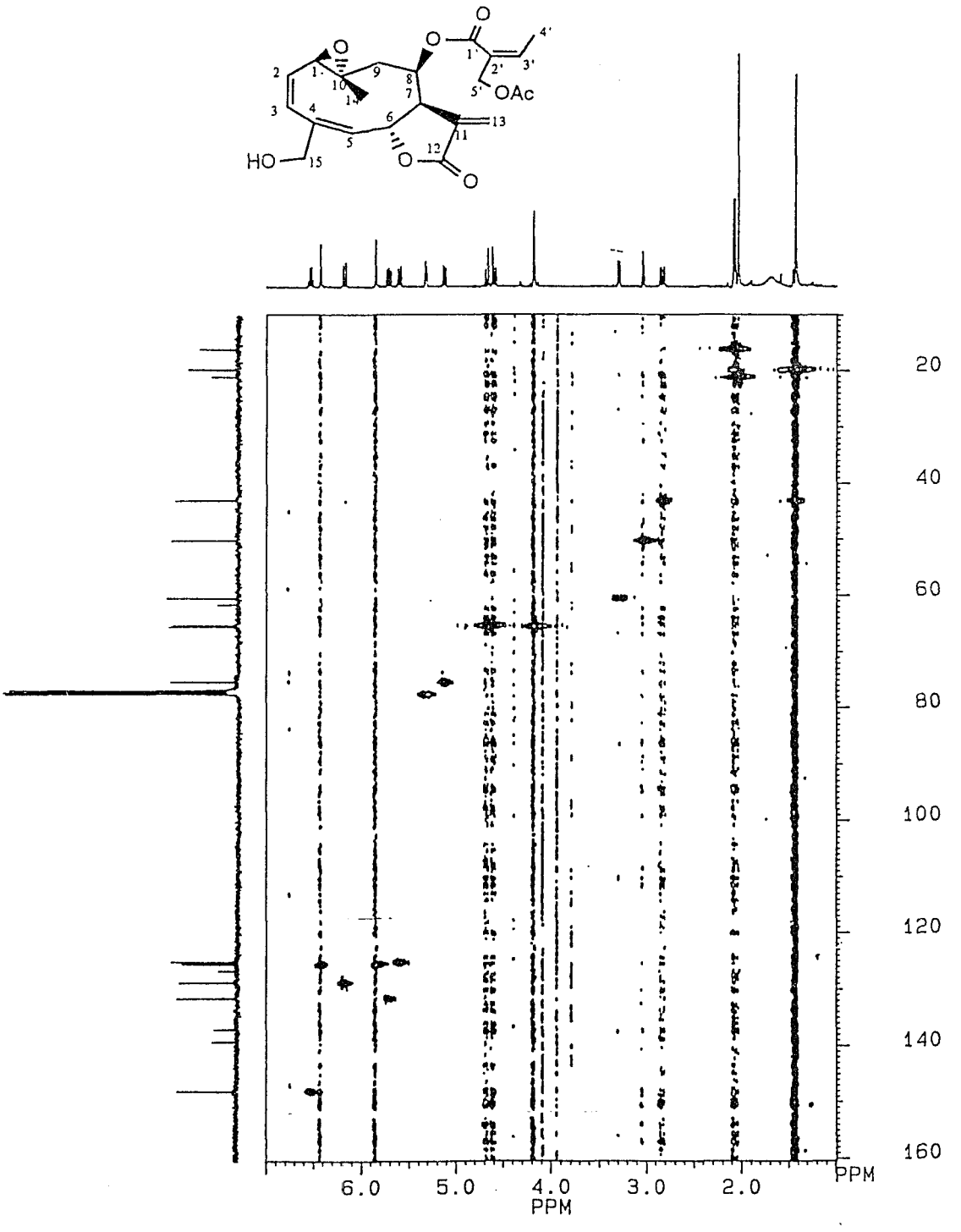


Figure 3.2.18. 2D Inverse ^{1}H-^{13}C heteronuclear correlation spectrum of punctaliatrin-5'-acetate (**229a**)

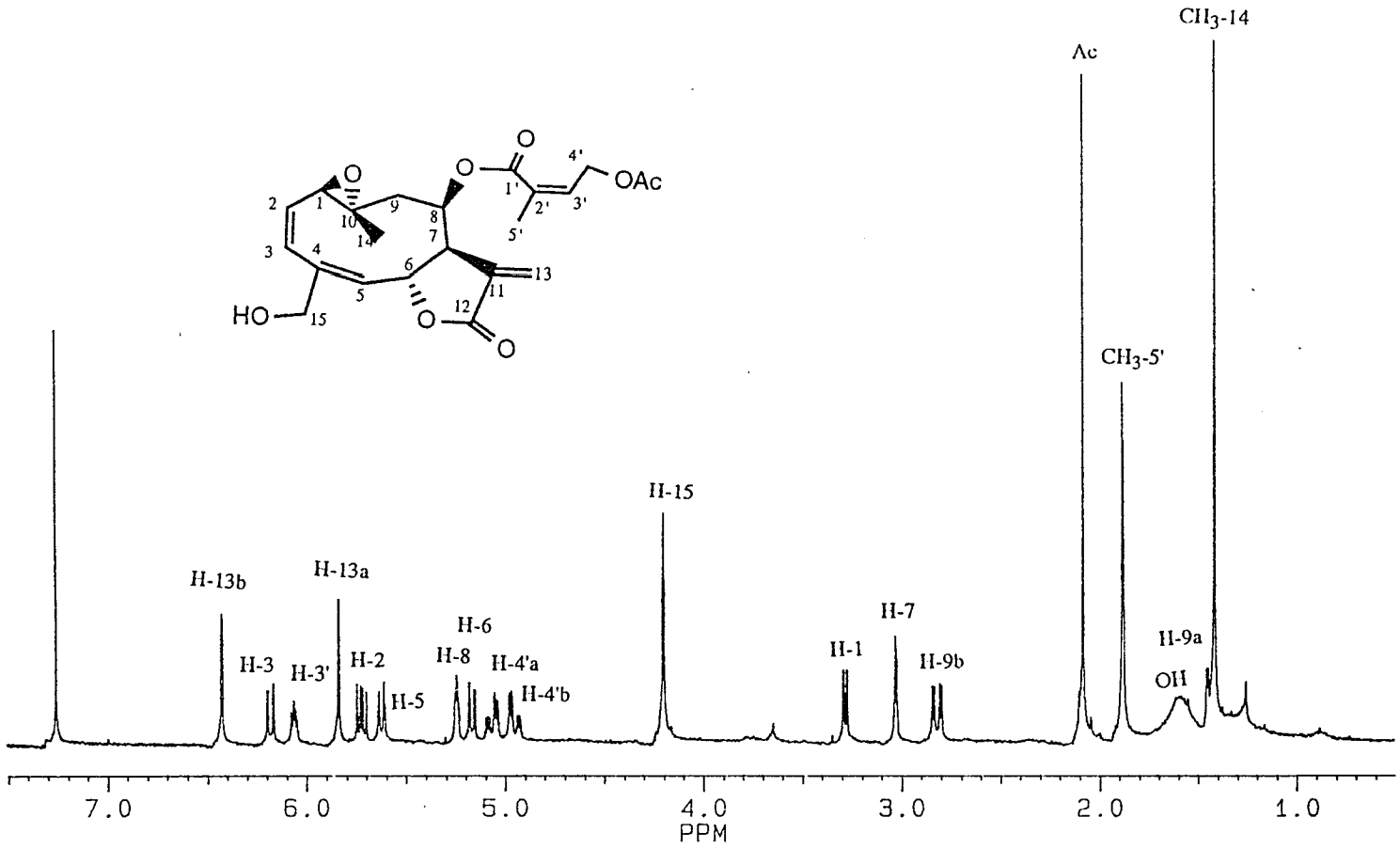


Figure 3.2.19. 400 MHz ^{1}H NMR spectrum of 4'-acetoxy-5'-deoxypunctaliatrin (**229b**)

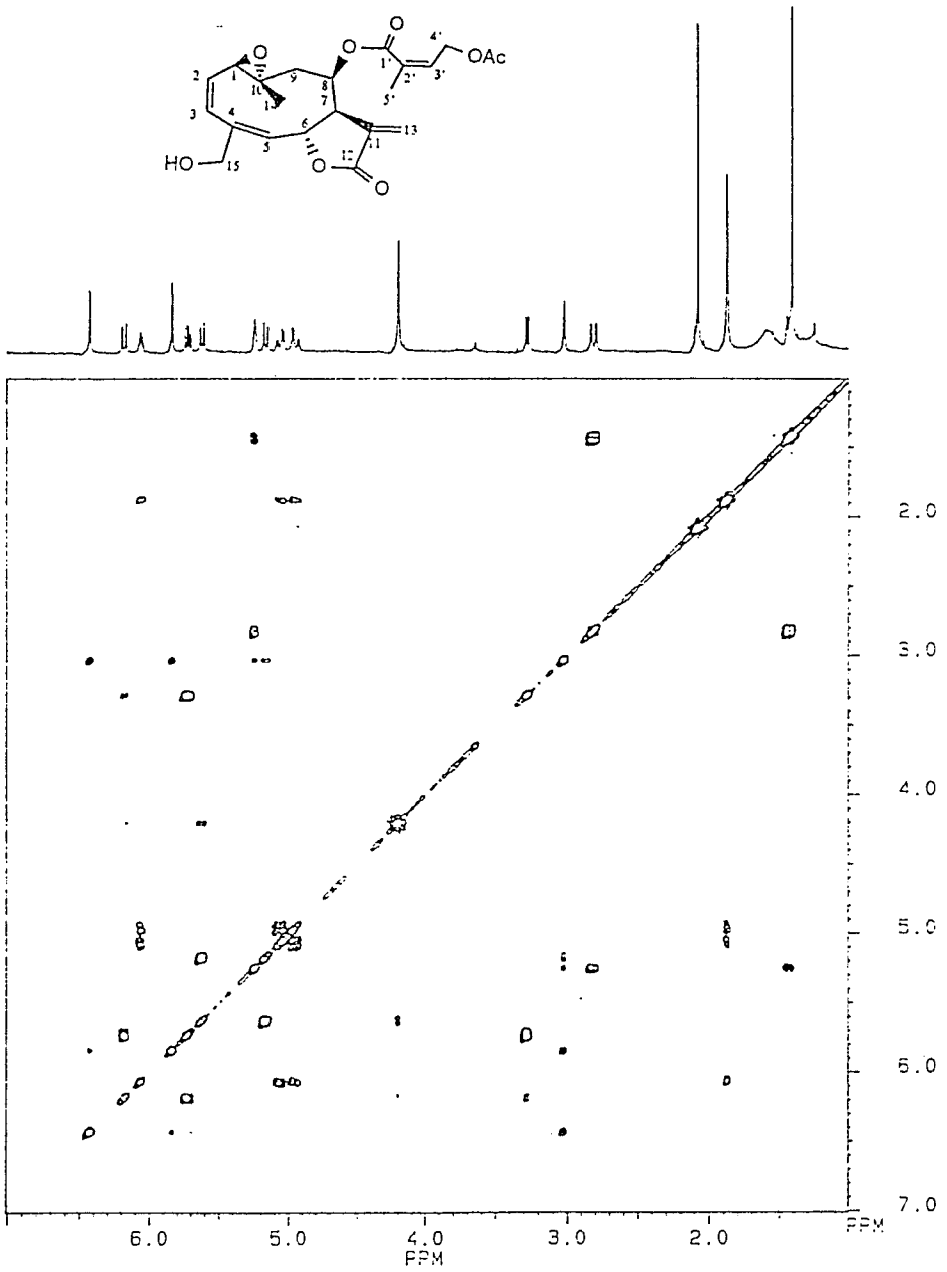


Figure 3.2.20. 400 MHz ^{1}H-COSY spectrum of
4'-acetoxy-5'-deoxypunctaliatrin (**229b**)

angles listed in Table 3.2.3. A search of the Cambridge Crystallographic Database [173] for the acetylsarracinate (hereafter *sarac*) fragment reveals that the crystal structures of only two other compounds containing this ester side chain, both from *Liatris* species, have been determined [174, 175]. The structure of isochapliatrin [174] contains two independent molecules, both of which exhibit disorder in the acetyl group, and thus the precision of the determination of the *sarac* group is limited. The other [175], a 3-germacrenolide, has an ordered acetyl group, and the precision of the determination is much higher. The distances within the *sarac* group in that structure agree well with those of liscunditrin. The conformation of the *sarac* group is somewhat variable over the three structure determinations. All have the C17=C18 double bond *S-cis* to the C16=O7 ester carbonyl, with torsion angles about the C16-C17 bond less than 30°; it is largest in liscunditrin, at 27.5(2)°. The orientation of the acetyl group with respect to the remainder of the *sarac* substituent exhibits no pattern. The torsion angle about the C20-O8 bond in liscunditrin is essentially *anti*, at 167.1(1)°, but the other compounds exhibit values ranging from -145° to +97°. The OH group of liscunditrin is involved in an intermolecular hydrogen bond with O9 of the *sarac* acetyl group, and this interaction doubtless controls the conformation of the *sarac* substituent. The O···O distance of the hydrogen bond is 2.827(2)Å, and the angle about H is 170(2)°.

Experimental

General. Melting point was measured on a Thomas Hoover apparatus and is uncorrected. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker AM 400 spectrometer. IR spectra were obtained on a Perkin-Elmer 1760X spectrometer as a film on KBr plates. Mass spectra were recorded on a Hewlett-Packard 5971A GC-MS or a TSQ70 FAB mass spectrometer. VLC separations were carried out on silica gel (MN Kieselgel G) and semi-prep. HPLC separations were performed on a 10μ C18 reversed-phase column (250x10 mm, AllTech) coupled to a LDC/Milton Roy

Table 3.2.3. Torsion angles in liscunditrin (**228b**) (°)

Atoms				Angles
C12	O1	C6	C7	-17.5 (2)
C6	O1	C12	C11	7.8 (2)
H5O	O5	C15	C4	-102 (2)
C16	O6	C8	C9	-71.5 (2)
C8	O6	C16	O7	8.0 (2)
C21	O8	C20	C17	167.1 (2)
C20	O8	C21	O9	-2.6 (2)
C10	C1	C2	C3	-117.9 (2)
C2	C1	C10	C9	153.0 (2)
C1	C2	C3	C4	-4.1 (2)
C2	C3	C4	C5	54.3 (2)
C3	C4	C5	C6	0.4 (2)
C5	C4	C15	O5	2.0 (2)
C4	C5	C6	C7	-124.5 (2)
O1	C6	C7	C11	19.3 (2)
C5	C6	C7	C8	143.1 (2)
C6	C7	C8	O6	49.1 (2)
C6	C7	C8	C9	-76.9 (2)
C6	C7	C11	C12	-15.4 (2)
C7	C8	C9	C10	62.7 (2)
C8	C9	C10	C1	-86.1 (2)
C7	C11	C12	O1	5.6 (2)
C13	C11	C12	O2	5.6 (2)
O7	C16	C17	C18	27.5 (2)
C16	C17	C18	C19	0.1 (3)
C16	C17	C20	O8	64.4 (2)

CM4000 multi-solvent delivery system and an ISCO UV detector using a detection wavelength at 230 nm.

Plant material. The aerial parts of *Liatris ohlingerae* (Blake) Robinson were collected on 12 August, 1992 in Highland County, Florida, U.S.A. (N. H. Fischer and H. D. Fischer No. 452; voucher deposited at LSU Herbarium, U.S.A.). Plant material was obtained by selective harvesting of leaves from several plants so that the health of each individual plant was not adversely affected. Air-dried aerial parts (43 g) were extracted with CH_2Cl_2 twice providing 4.4 g of crude extract. VLC separation of the crude using hexane followed by mixtures of hexane and EtOAc of increasing polarity provided 17x150 ml fractions. The less polar frs 3 and 4 formed a white precipitate which upon further VLC separation afforded 250 mg of a mixture of triterpenes **230a** and **230c**. Frs. 7 and 8 also formed white precipitate which provided 300 mg of a mixture of triterpenes **230b** and **230d**. Later fractions contained sesquiterpene lactones as determined by ^{1}H NMR. Further purification of fr. 13 by prep. HPLC using MeOH-H_2O (1:1) as a mobile phase afforded 2 mg of **228d**. Prep. HPLC separation (MeOH-H_2O, 9:11) of the combined frs. 14 and 15 yielded 4 mg of **228a**, 17 mg of **228b**, 2 mg of **228c** and 14 mg of **228e**. Fr. 16 provided a colorless crystal which upon recrystallization in hexane afforded 200 mg of **228b**. Further prep. HPLC separation of fr. 16 using MeOH-H_2O (1:1) provided 8 mg of **229a** and 2 mg of **229b**.

Liscundin (**228a**). $C_{20}H_{24}O_7$, powder; IR ν_{max}^{KBr} cm^{-1}: 3431 (OH), 1763 (γ-lactone), 1720 (C=O), 1647, 1457 (C=C), 1224, 1151 and 1119 (CC(=O)OC, ester); EIMS m/z (rel. int.): 376 $[M]^+$ (0.1), 358 $[M-H_2O]^+$ (0.4), 259 [M-100-OH]$^+$ (1.5), 111 (25.9), 83 $[CH_3CH=C(CH_3)CO]^+$ (100), 55 [83-CO]$^+$ (58.2); ^{1}H NMR data in Table 3.2.1 and ^{13}C NMR data in Table 3.2.2.

Liscunditrin (**228b**). $C_{22}H_{26}O_9$, crystal, mp 168-170°; IR ν_{max}^{KBr} cm^{-1}: 3489 (OH), 1766 (γ-lactone), 1724 (C=O), 1653, 1457 (C=C), 1235 and 1156 (CC(=O)OC, ester); EIMS m/z (rel. int.): 375 [M-OAc]$^+$ (0.4), 259 [M-157-H_2O]$^+$ (6.1), 241 (8.3),

157 [$CH_3CH=C(CH_2OAc)CO_2$]$^+$ (1.1), 147 (21.1), 129 (100), 112 (31.8), 57 (56.2), 43 [Ac]$^+$ (26.4); ^{1}H NMR data in Table 3.2.1 and ^{13}C NMR data in Table 3.2.2.

Eleganin (**228c**). $C_{22}H_{26}O_9$, powder; IR ν_{max}^{KBr} cm^{-1}: 3481 (OH), 1763 (γ-lactone), 1740 and 1718 (C=O), 1653, 1457 (C=C), 1216 and 1143 (CC(=O)OC, ester); EIMS m/z (rel. int.): 417 [M-OH]$^+$ (0.3), 375 [M-OAc]$^+$ (4.9), 374 [M-HOAc]$^+$ (0.4), 276 [M-158]$^+$ (0.4), 259 [M-158-OH]$^+$ (1.2), 157 [$CH_3CH=C(CH_2OAc)CO_2$]$^+$ (2.9), 111 (64.3), 99 [$CH_2CH=C(CH_3)CO_2H$]$^+$ (97.6), 82 [99-OH]$^+$ (19.9), 43 [Ac]$^+$ (100); ^{1}H NMR data in Table 3.2.1 and ^{13}C NMR data in Table 3.2.2.

Acetylliscunditrin (**228d**). $C_{24}H_{28}O_{10}$, powder; IR ν_{max}^{KBr} cm^{-1}: 1768 (γ-lactone), 1740, 1735 and 1730 (C=O), 1663, 1457 (C=C), 1227 and 1155 (CC(=O)OC, ester); EIMS m/z (rel int.): 416 [M-HOAc]$^+$ (0.2), 259 [M-158-OAc]$^+$ (1.4), 153 (34.8), 141 [$CH_3CH=C(CH_2OAc)CO$]$^+$ (33.0), 99 [$CH_3CH=C(CH_2)CO_2H$]$^+$ (19.0), 81 [141-HOAc]$^+$ (62.6), 43 [Ac]$^+$ (100); ^{1}H NMR data in Table 3.2.1.

Ohlingerin (**228e**). $C_{20}H_{24}O_7$, powder; IR ν_{max}^{KBr} cm^{-1}: 3504 (OH), 1763 (γ-lactone), 1720 (C=O), 1647 and 1444 (C=C), 1220 and 1141 (CC(=O)OC, ester); EIMS m/z (rel. int.): 376 [M]$^+$ (0.2), 259 [M-100-OH]$^+$ (0.3), 111(8.2), 83 [$Me_2C=CHCO$]$^+$ (100), 55 [83-CO]$^+$ (22.7); FAB MS m/z: 377 [MH]$^+$, 277 [MH-100]$^+$, 213, 147; ^{1}H NMR data in Table 3.2.1 and ^{13}C NMR data in Table 3.2.2.

Punctaliatrin-5'-acetate (**229a**). $C_{22}H_{26}O_8$, powder; IR ν_{max}^{KBr} cm^{-1}: 3461 (OH), 1762 (γ-lactone), 1723 (C=O), 1653, 1443 (C=C), 1234 and 1156 (CC(=O)OC, ester); EIMS m/z (rel. int.): 388 [MH-CH_2OH]$^+$ (1.9), 375 [M-Ac]$^+$ (0.2), 346 [MH-CH_2OAc]$^+$ (3.9), 328 [346-H_2O]$^+$ (4.6), 230 [M-157-CH_2OH]$^+$ (34.6), 202 (27.1), 159 (24.7), 141 [$CH_3CH=C(CH_2OAc)CO$]$^+$ (100), 99 [$CH_3CH=C(CH_2)CO_2H$]$^+$ (27.3), 81 [141-HOAc]$^+$ (73.8), 43 [AC]$^+$ (92.9); FAB MS m/z: 419 [MH]$^+$, 404 [MH-Me]$^+$, 359 [MH-HOAc]$^+$, 243 [MH-158-H_2O]$^+$, 141, 99, 81; ^{1}H NMR data in Table 3.2.1 and ^{13}C NMR data in Table 3.2.2.

4''-acetoxy-5'-deoxypunctaliatrin (**229b**). $C_{22}H_{26}O_8$, powder; IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1762 (γ-lactone), 1742 and 1726 (C=O), 1653, 1440 (C=C), 1217 and 1143 (CC(=O)OC, ester); EIMS m/z (rel. int.): 388 $[MH-CH_2OH]^+$ (0.4), 375 $[M-Ac]^+$ (0.4), 346 $[MH-CH_2OAc]^+$ (11.0), 329 $[346-OH]^+$ (2.1), 231 (15.7), 202 (8.3), 158 $[AcOCH_2CH=C(CH_3)CO_2H]^+$ (15.6), 99 $[CH_2CH=C(CH_3)CO_2H]^+$ (100), 82 $[99-OH]^+$ (15.3), 43 $[Ac]^+$ (68.6); ^1H NMR data in Table 3.2.1.

X-Ray data of liscunditrin (**228b**). Recrystallization of **228b** in hexane afforded colorless crystals, mp 168-170°. A colorless plate of dimensions 0.18x0.40x0.55mm was used for data collection on an Enraf-Nonius CAD4 diffractometer equipped with CuK_α radiation (λ=1.54184 Å), and a graphite monochromator. Crystal data are: $C_{22}H_{26}O_9$, M_r=434.4, orthorhombic space group $P2_12_12_1$, a=8.9665(7), b=9.9812(4), c=23.712(2)Å, V=2122.1(4)Å^3, Z=4, d_c=1.359 g cm^{-3}, T=24°. Intensity data were measured by ω-2θ scans of variable rate, designed to yield I=25σ(I) for all significant reflections. Two octants of data were collected within the limits 2<θ<75°. Data reduction included corrections for background, Lorentz, polarization, and absorption effects. Absorption corrections (μ=8.5 cm^{-1}) were based on ψ scans, with minimum relative transmission coefficient 85.7%. Of 4197 unique data, 4034 had I>3σ(I) and were used in the refinement.

The structure was solved by direct methods using RANTAN [176] and refined by full-matrix least squares, treating nonhydrogen atoms anisotropically, using the Enraf-Nonius MolEN programs [177]. Hydrogen atoms were located using difference maps and refined isotropically, except for those of the methyl groups, which were placed in calculated positions. Convergence was achieved with R=0.0321, R_w=0.0421, and GOF=2.565. The absolute configuration was determined by refinement of the mirror-image structure under identical circumstances, yielding R=0.0323, R_w=0.0424, and GOF=2.582. The crystal structure is illustrated in Figure 3.2.2, and its coordinates are tabulated in Table 3.2.4.

Table 3.2.4. Positional parameters and their estimated s.d.s. for liscunditrin(**228b**)

Atoms	x	y	z	$B_{eq}(Å^2)$
O1	0.1550(1)	0.46276(9)	0.72223(4)	3.26(2)
O2	-0.0176(1)	0.3914(1)	0.66205(5)	4.41(2)
O3	0.4657(1)	0.9248(1)	0.79913(5)	4.01(2)
O4	0.3904(1)	0.7062(1)	0.90706(5)	4.53(2)
O5	-0.0445(1)	0.5221(1)	0.90577(5)	4.60(2)
O6	0.3072(1)	0.67973(9)	0.64996(4)	2.92(2)
O7	0.3935(1)	0.8309(1)	0.58754(4)	4.21(2)
O8	0.2083(1)	0.4857(1)	0.55582(4)	3.80(2)
O9	0.1727(2)	0.2658(1)	0.55773(7)	5.92(3)
C1	0.3776(2)	0.8074(1)	0.81223(6)	3.30(3)
C2	0.4454(2)	0.7056(2)	0.84988(6)	3.72(3)
C3	0.3563(2)	0.5928(1)	0.87078(6)	3.57(3)
C4	0.1934(2)	0.5734(1)	0.86021(6)	3.20(2)
C5	0.1262(2)	0.5712(1)	0.81045(5)	3.12(2)
C6	0.1923(2)	0.5864(1)	0.75284(5)	2.84(2)
C7	0.1187(2)	0.7013(1)	0.71847(5)	2.79(2)
C8	0.2246(2)	0.7817(1)	0.68069(5)	2.89(2)
C9	0.3223(2)	0.8838(1)	0.71167(6)	3.27(2)
C10	0.4317(2)	0.8296(1)	0.75445(6)	3.20(2)
C11	0.0048(2)	0.6274(1)	0.68456(6)	3.13(2)
C12	0.0406(2)	0.4830(1)	0.68659(5)	3.15(2)
C13	-0.1119(2)	0.6745(2)	0.65644(8)	4.96(4)
C14	0.5669(2)	0.7595(2)	0.73129(7)	4.15(3)
C15	0.1077(2)	0.5499(2)	0.91429(6)	4.05(3)
C16	0.3801(2)	0.7155(1)	0.60264(5)	2.98(2)
C17	0.4377(2)	0.5956(1)	0.57254(5)	3.18(2)
C18	0.5579(2)	0.6027(2)	0.53907(6)	4.17(3)
C19	0.6520(2)	0.7202(2)	0.52573(8)	5.81(4)
C20	0.3563(2)	0.4667(2)	0.58002(6)	3.56(3)
C21	0.1259(2)	0.3758(2)	0.54763(7)	4.07(3)
C22	-0.0245(2)	0.4080(2)	0.52518(8)	4.90(4)

CHAPTER 4

ANTI-MYCOBACTERIAL ACTIVITIES OF SESQUITERPENE LACTONES: A STRUCTURE-ACTIVITY STUDY

Introduction

Costunolide (**231**) and dehydrocostuslactone (**232**) are the major sesquiterpene lactones from costus oil (*Saussurea lappa*). In recent years, sesquiterpenes related to these two compounds have drawn considerable attention due to their wide spectrum of biological activities, which include anti-tumor and cytotoxicity [178, 179], antibacterial [179], molluscicidal [180] and insect anti-feedant activity [181]. They have also been reported to display significant plant growth regulatory activity [182, 183] Although the biological activity of these compounds is generally attributed to the alkylating properties of the α-methylene-γ-lactone moiety [184, 185], the functions of other structural and conformational variations in the molecule is still unclear. In order to further study the structure-activity relationships among these compounds, we carried out a series of chemical transformations on costunolide (**231**) and tested their activity against *Mycobacterium tuberculosis* and *M. avium*. The results of both chemical and biological aspects will be presented here.

Results and Discussion

Costunolide (**231**) is assumed to be a key intermediate in the formation of eudesmane-type sesquiterpenes [186]. Although epoxidation and cyclization of costunolide (**231**) with peracetic acid and *m*-chloroperbenzoic acid have been performed before [178, 187-189], previous studies only reported the epoxidation at the 1,10-position to form 1,10-epoxycostunolide (**234**) [187] and subsequent cyclization of **234** produced santamarin (**235**) [188, 189], reynosin (**236**) [188, 189] and magnolialide (**237**) [189], while 4,5-epoxidation product parthenolide (**233**) and the cyclization product **238** were previously not found as reaction products. Costunolide (**231**) was allowed to react at room temperature with *m*-chloroperbenzoic acid in $CHCl_3$ for 1 hr. in the presence of NaOAc as a buffer. Vacuum liquid chromatographic (VLC) separation of

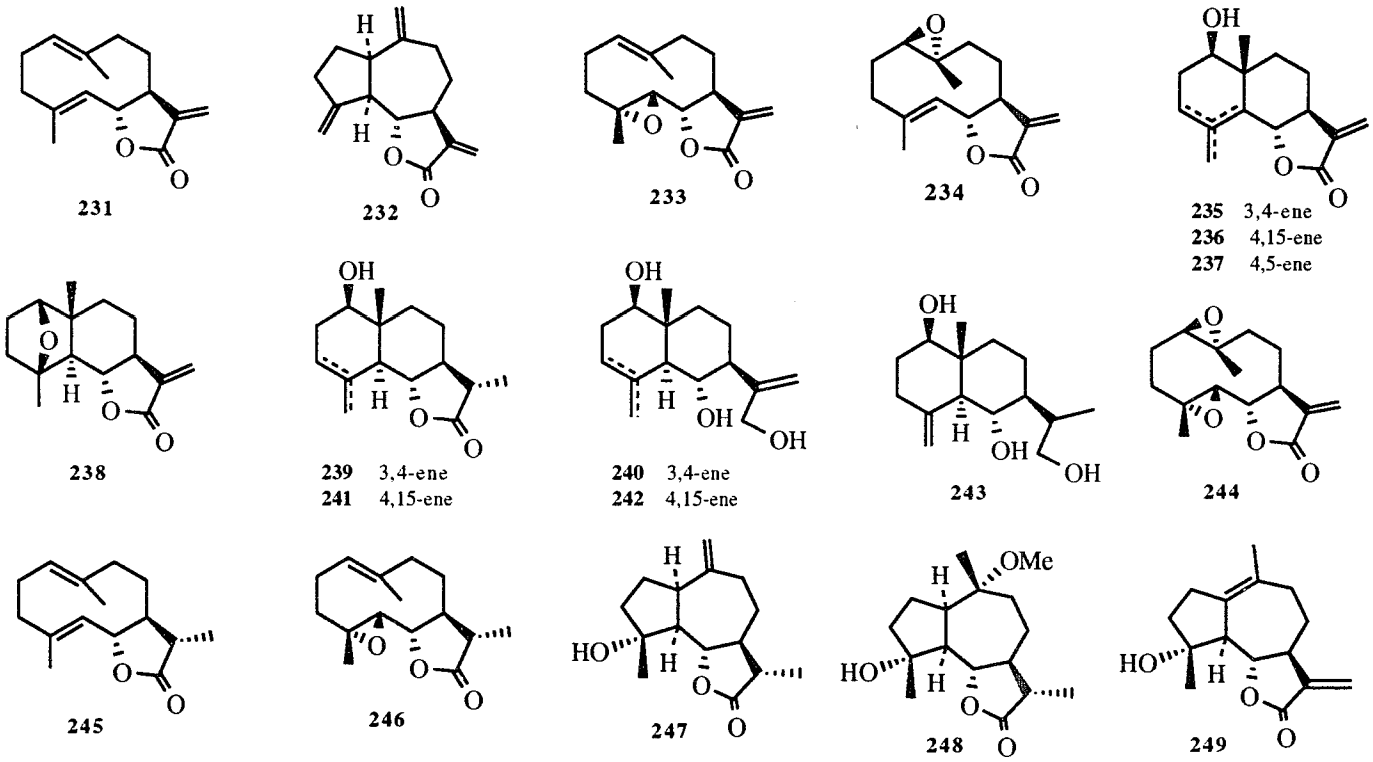


Figure 4.1. Costunolide and Its Chemical Transformation Products

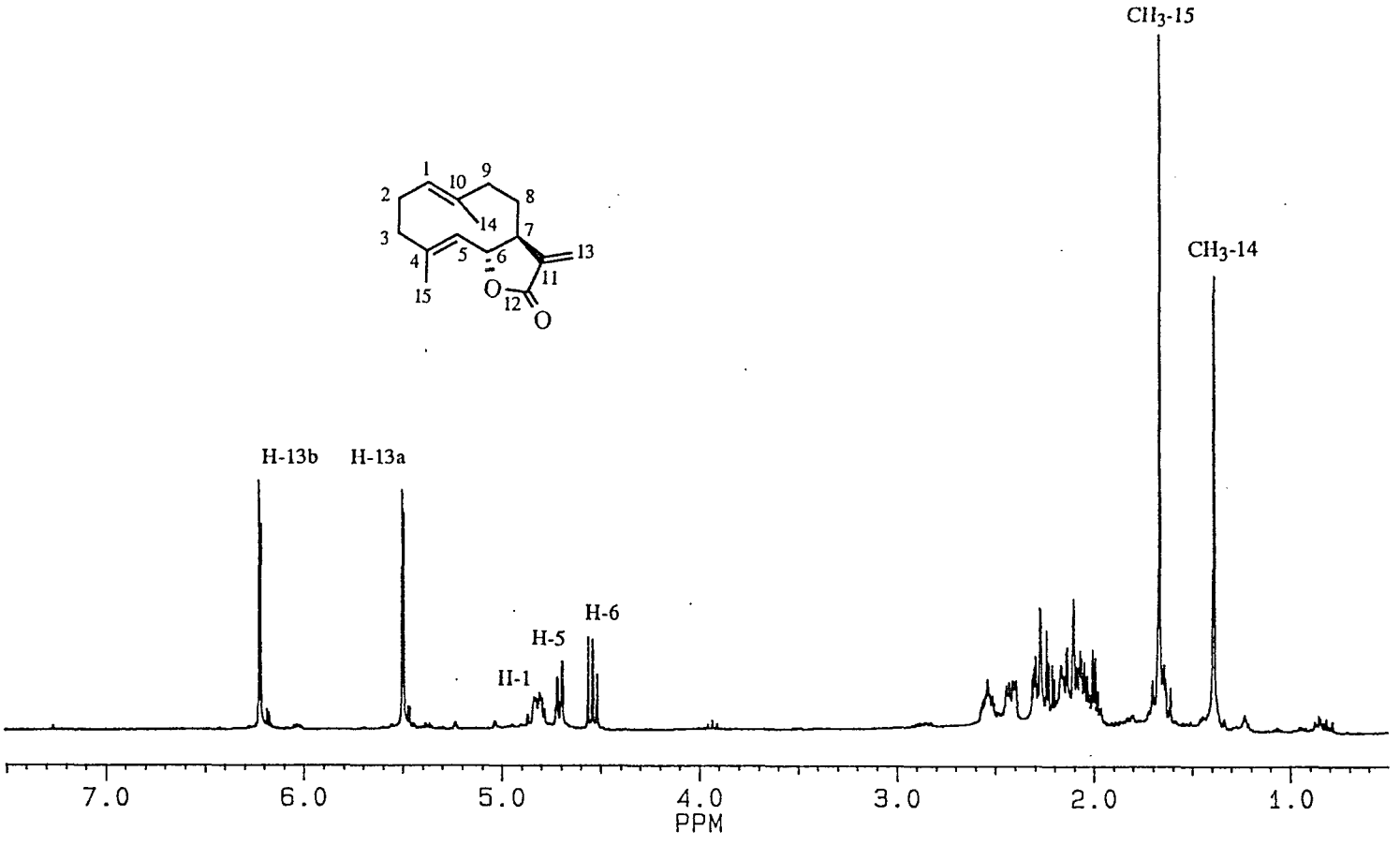


Figure 4.2. 400 MHz ^{1}H NMR spectrum of costunolide (**231**)

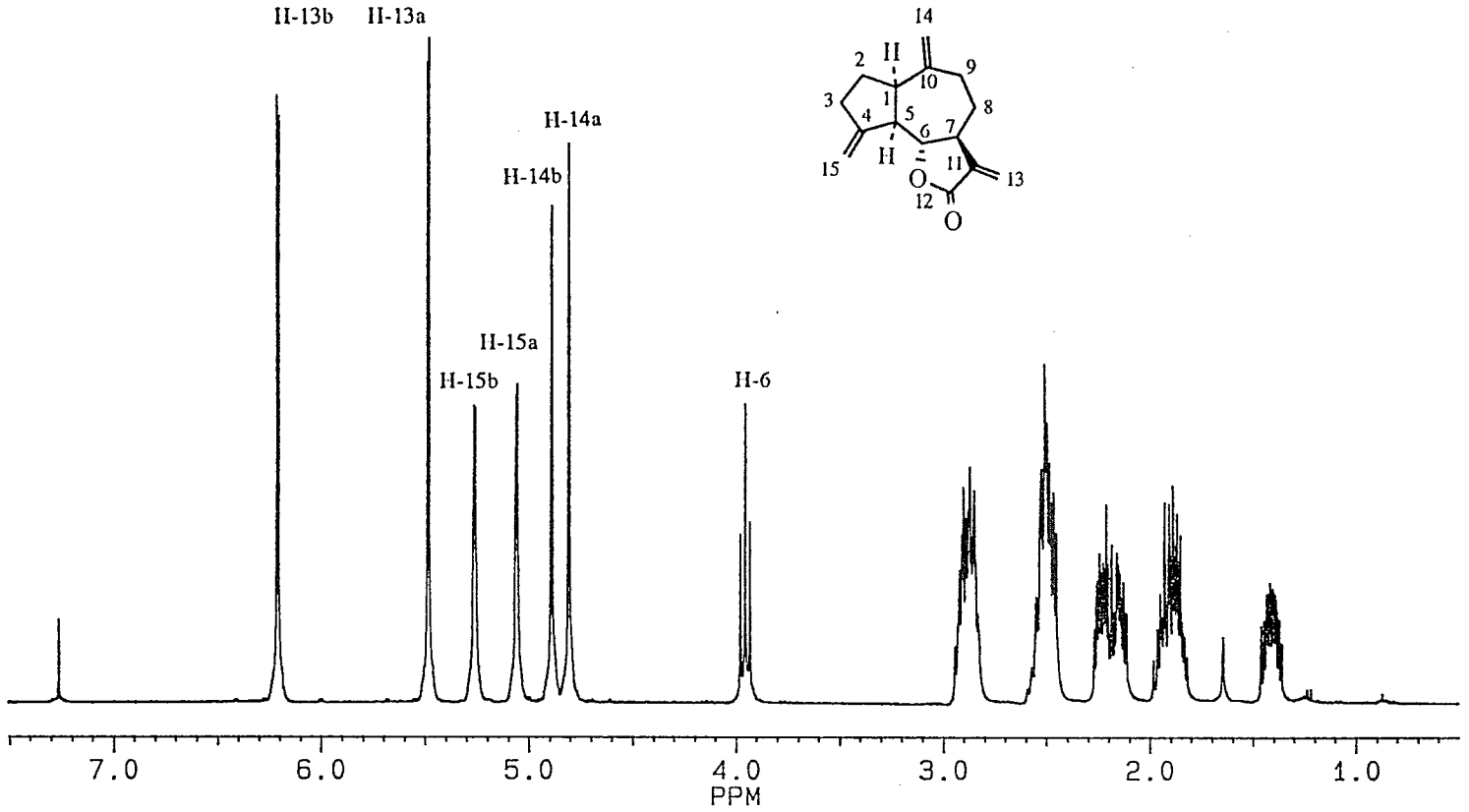
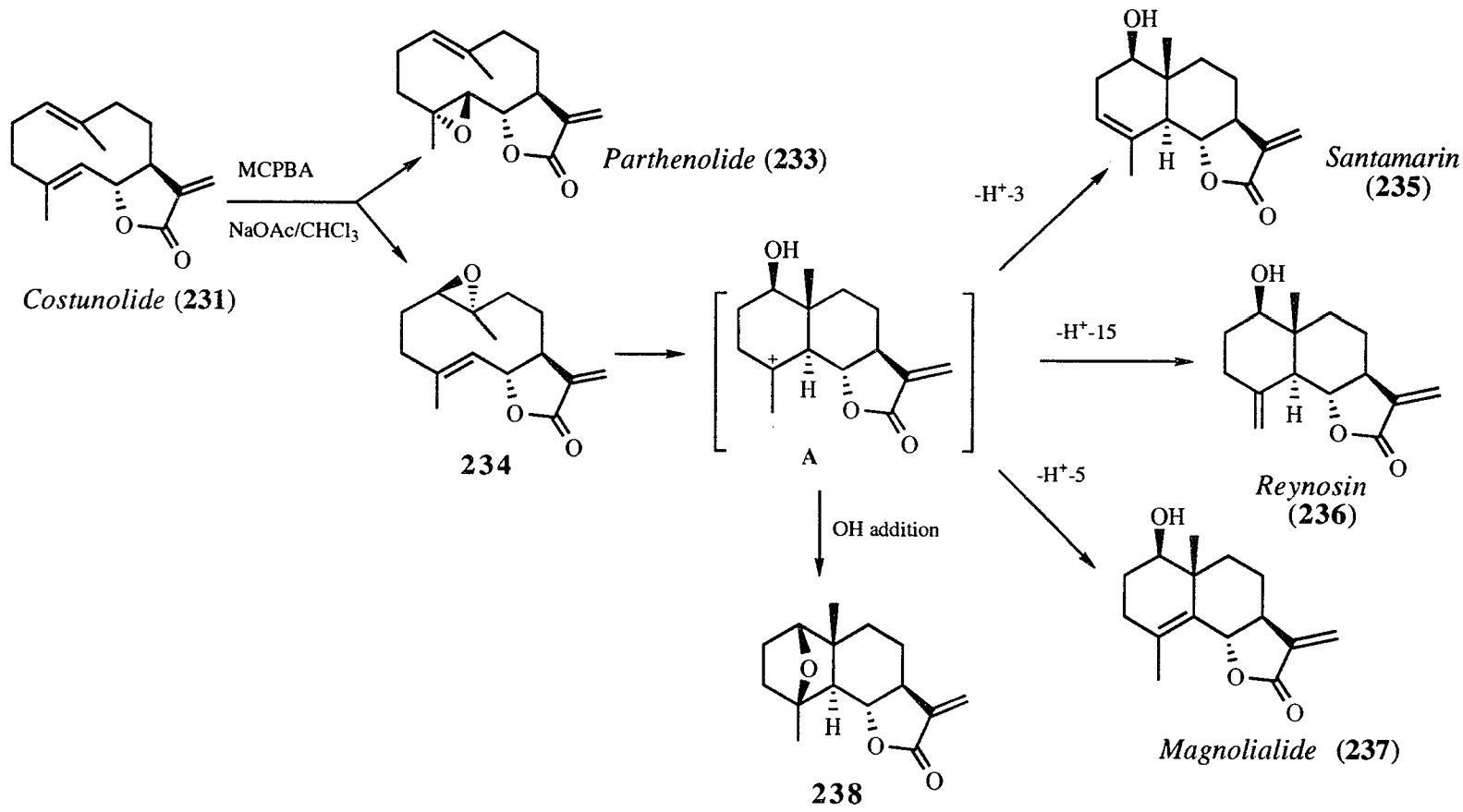


Figure 4.3. 400 MHz ^{1}H NMR spectrum of dehydrocostuslactone (**232**)

the reaction mixture on silica gel[129] provided, besides 1,10-epoxycostunolide (**234**) and parthenolide (**233**), the known eudesmanolides santamarin (**235**), reynosin (**236**) and magnolialide (**237**). Plus the new 1,4-epoxy-11(13)-eudesmen-12,6-olide (**238**). 1,10-Epoxycostunolide (**234**), santamarin (**235**) and reynosin (**236**) were identified by direct comparison with the spectroscopic data (^{1}H NMR and MS) of authentic samples and the data reported in the literature [178]. Magnolialide (**237**), which had been previously isolated from the root bark of *Magnolia grandiflora* [189], exhibited ^{1}H NMR and mass spectral data which were essentially identical with those of previously reported [189].

Parthenolide (**233**) had been previously isolated from local Grand Magnolia (*M. grandiflora*) [190] and ts structure was established by direct comparison with the mass spectral and ^{1}H NMR data of an authentic sample. This is the first report of obtaining **233** as an epoxidation product from costunolide (**231**) supporting the proposal that costunolide (**231**) is the biogenetic precursor for parthenolide (**233**) [186].

The spectral data of compound **238** suggested an eudesmane skeleton very similar to those of other cyclization products, santamarin (**235**) and reynosin (**236**). The strong IR absorption at 1772 cm^{-1} indicated the presence of an α,β-unsaturated-γ-lactone. This was further supported by its ^{1}H NMR spectrum which showed the typical lactonic α-methylene doublets at δ 5.34 and 6.02. The ^{13}C NMR spectrum of **238** supported the presence of three oxygenated carbons with two methine signals at δ 80.3 and 84.1, and one quaternary carbon signal at δ 83.4, suggesting either 1,4-diol or 1,4-epoxide moiety in the molecule. Since no hydroxyl absorption was observed in the IR spectrum of **238**, a 1,4-epoxide structure was proposed. This was further confirmed by a prominent mass spectral molecular ion at m/z 248, which was in agreement with the empirical formula $C_{15}H_{20}O_3$. The ^{13}C NMR of **238** showed the presence of 15 carbons including one carbonyl, two CH_3, five CH_2 including one exocyclic-CH_2, four CH two being oxygenated, and three quaternary including one



Scheme 4.1. Epoxidation and cyclization of costunolide (**231**)

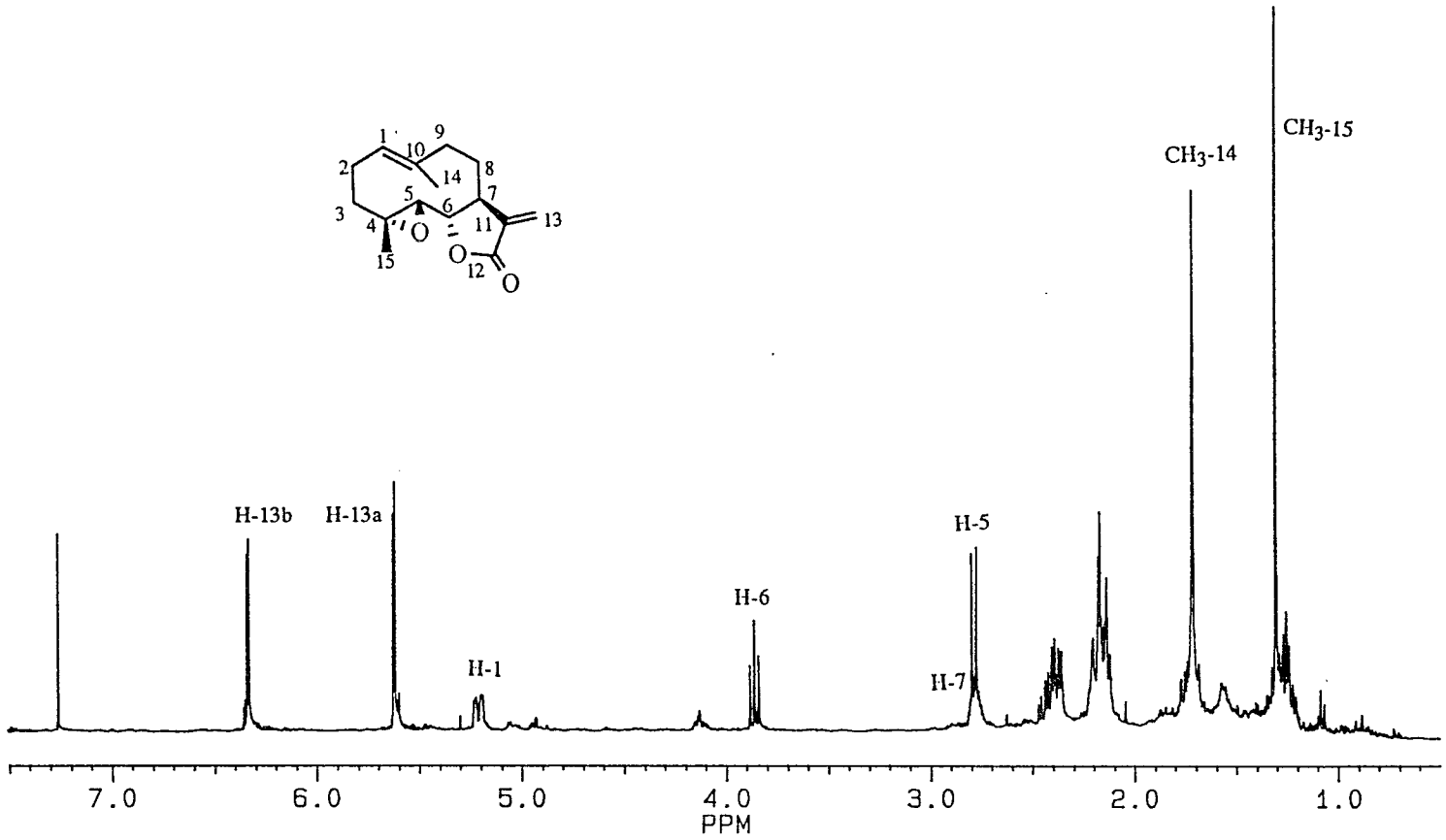


Figure 4.4. 400 MHz ^{1}H NMR spectrum of parthenolide (**233**)

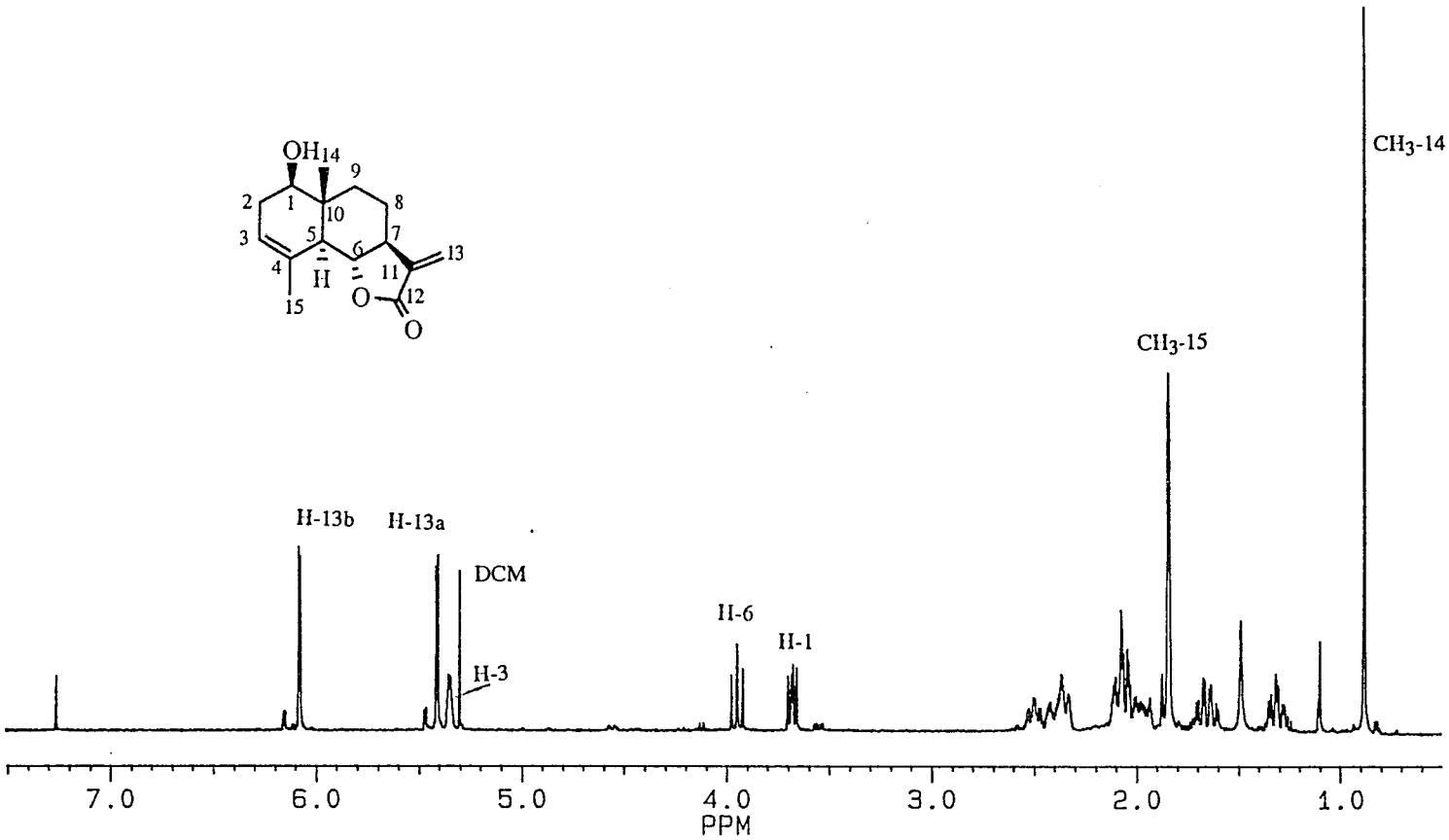


Figure 4.5. 400 MHz ^{1}H NMR spectrum of santamarin (**235**)

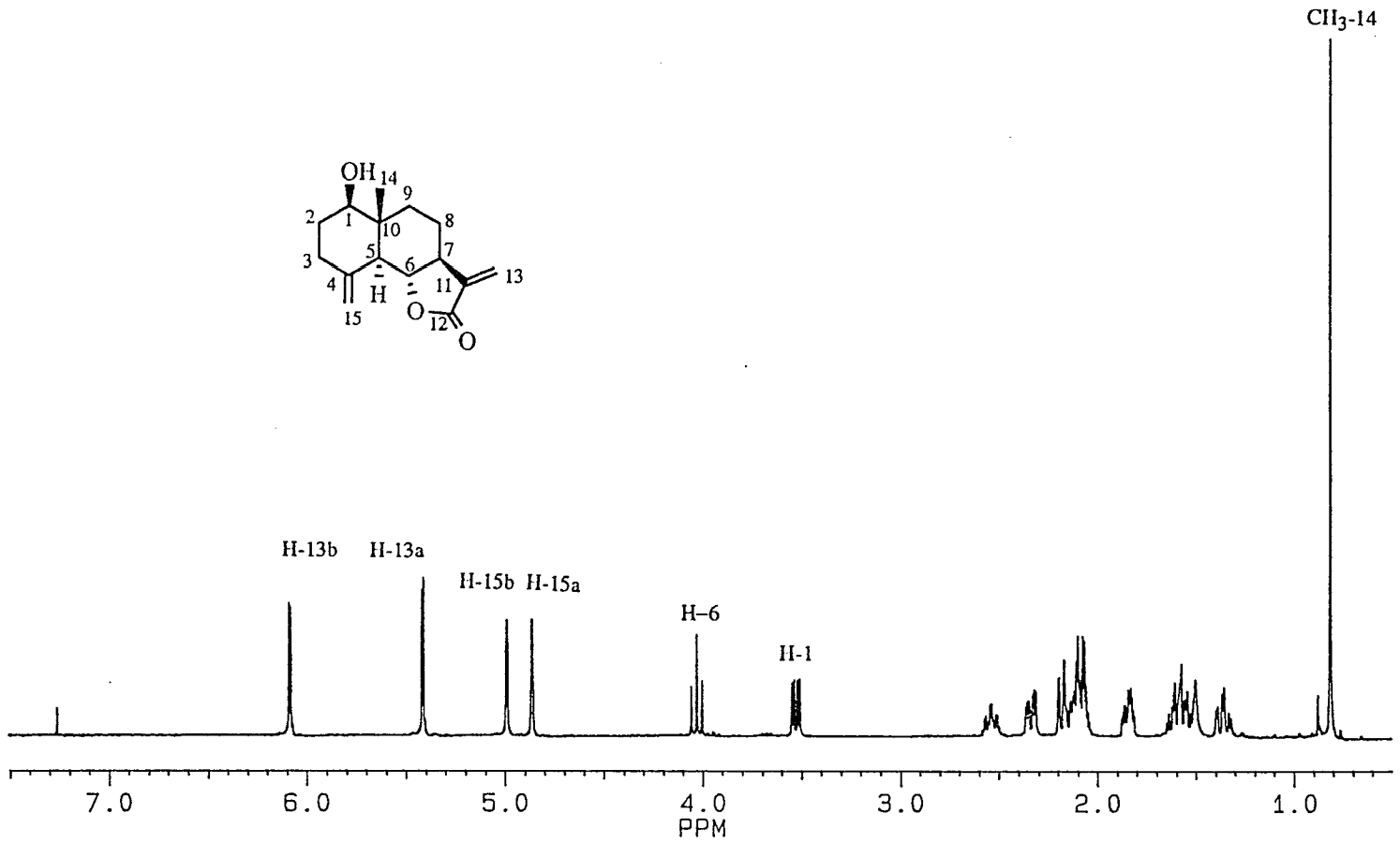


Figure 4.6. 400 MHz ^{1}H NMR spectrum of reynosin (**236**)

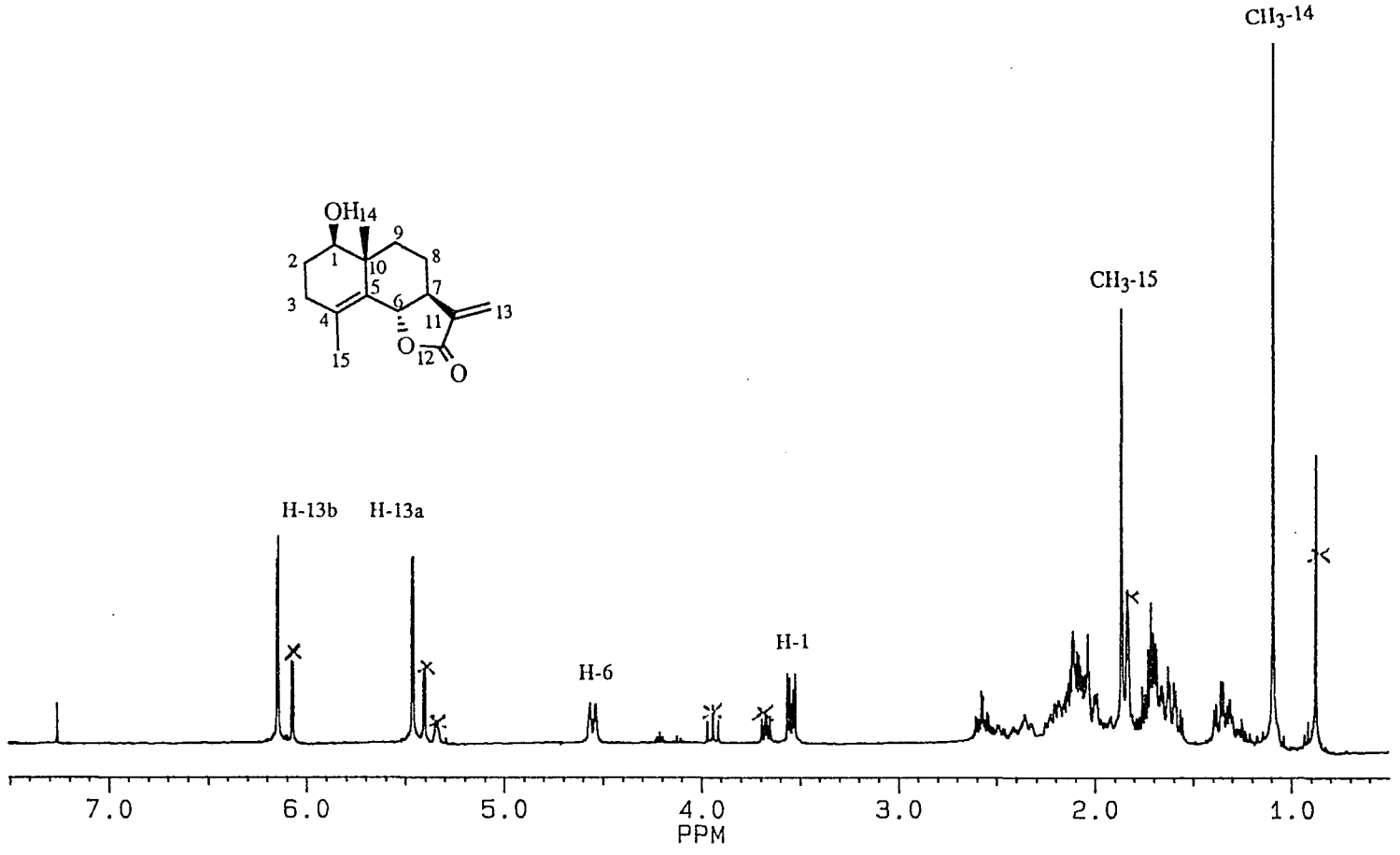


Figure 4.7. 400 MHz ^{1}H NMR spectrum of magnolialide (**237**)

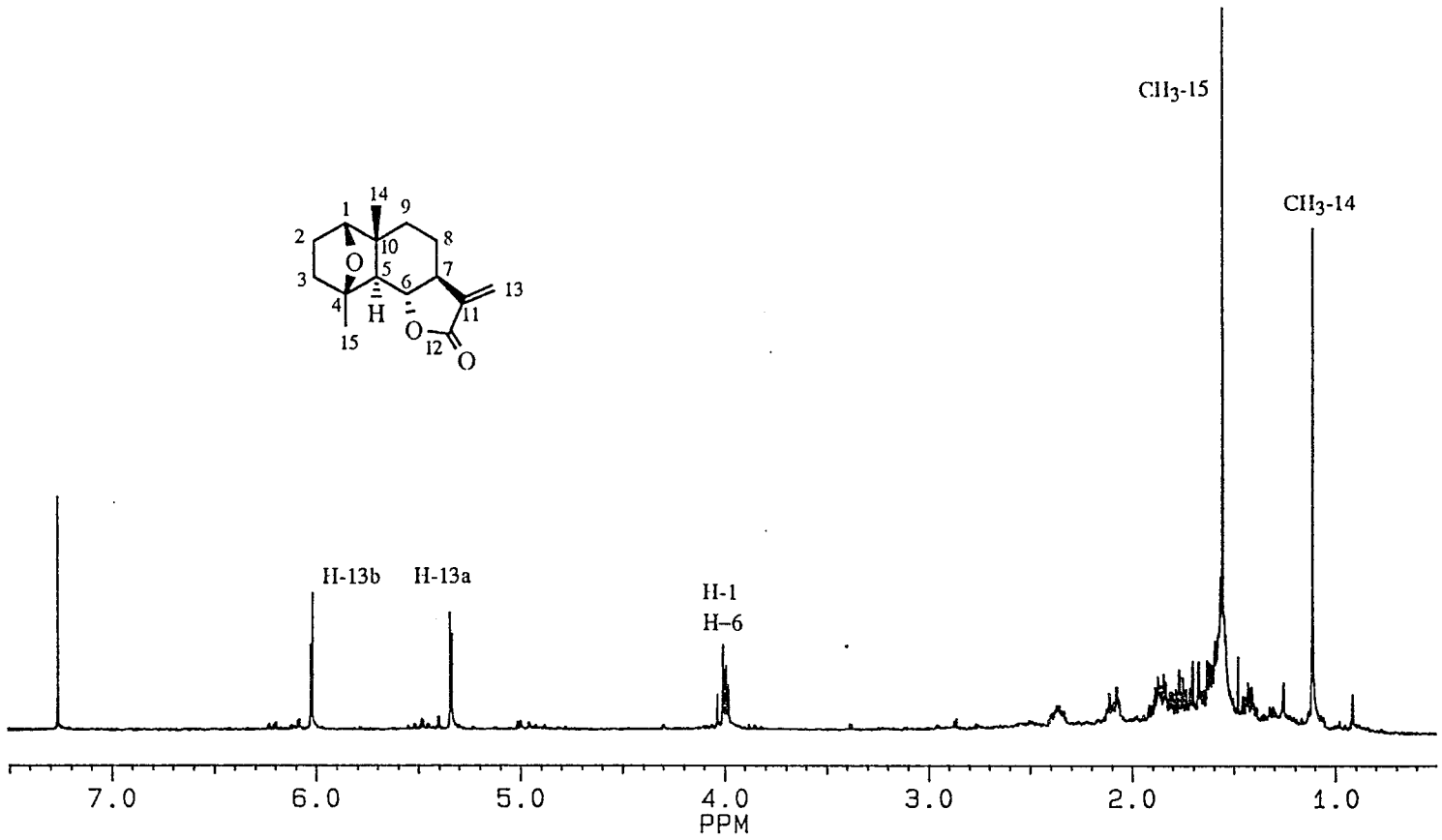


Figure 4.8. 400 MHz ^{1}H NMR spectrum of 1,4-epoxy-11(13)-eudesmen-12,6-olide (**238**)

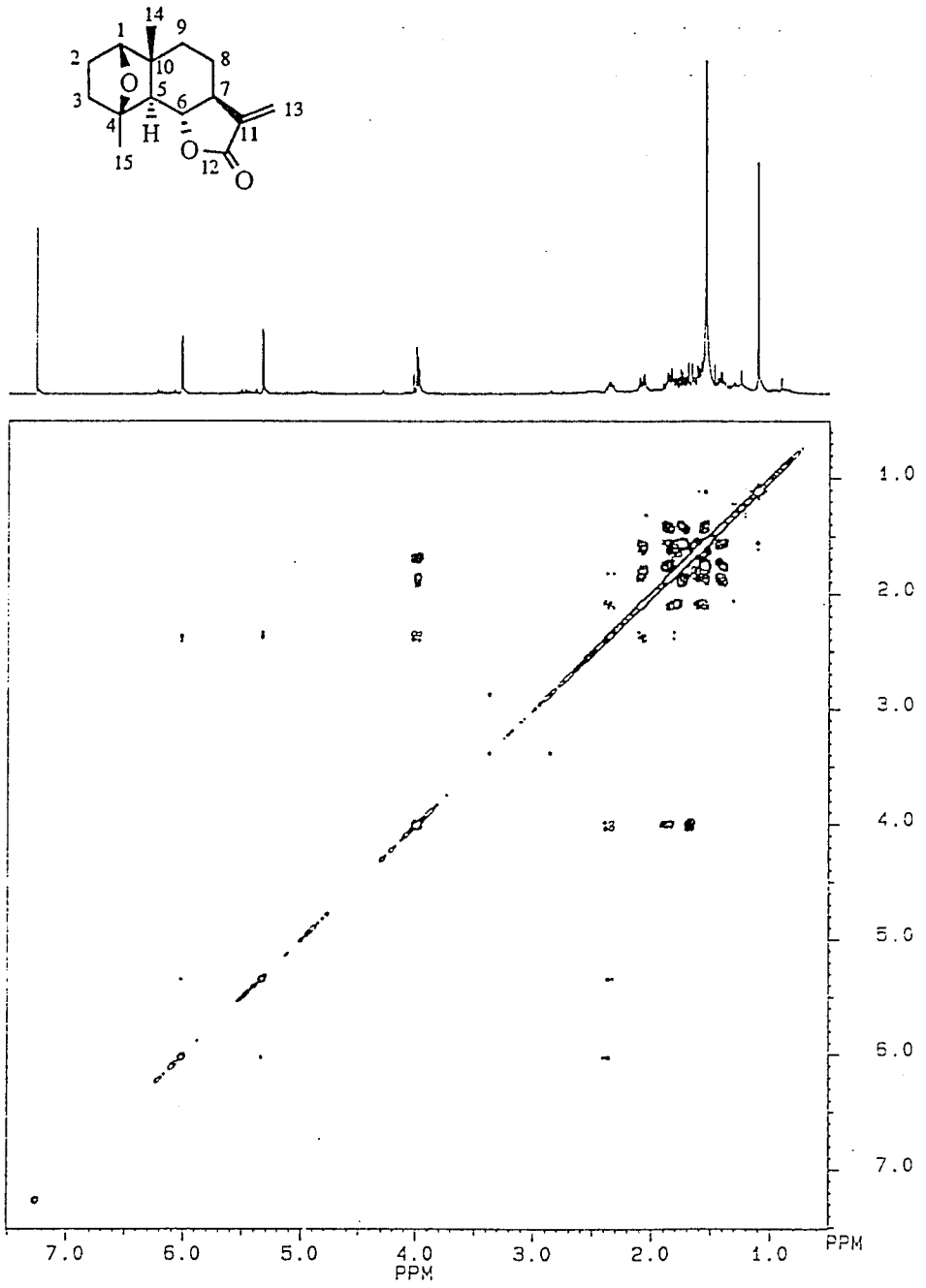


Figure 4.9. 400 MHz ^{1}H-COSY spectrum of 1,4-epoxy-11(13)-eudesmen-12,6-olide (**238**)

Table 4.1. ^{1}H NMR spectral data of compounds **231-233, 235-238** (400 MHz, $CDCl_3$ as internal standard)

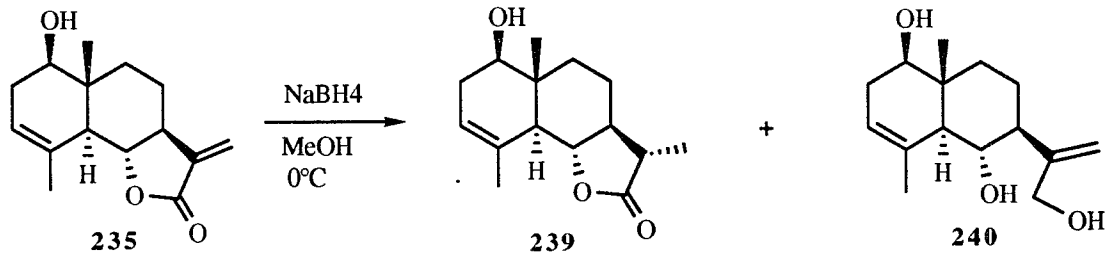
H	**231**	**232**	**233**	**235**	**236**	**237**	**238**
1	4.81 m		5.21 br d	3.67 dd	3.53 dd	3.55 dd	4.00 dd
2α			2.39 m				
2β			2.19 m				
3			1.27 m	5.34 m			
5	4.70 br d		2.78 d	2.03 d	2.18 d		1.69 d
6	4.54 dd	3.95 dd	3.86 dd	3.94 dd	4.03 dd	4.55 br d	4.00 dd
7			2.77 m				2.37 m
8			1.73 m				
9			2.14 m				
13	5.50 d 6.22 d	5.48 d 6.21 d	5.62 d 6.33 d	5.40 d 6.07 d	5.41 d 6.08 d	5.47 d 6.15 d	5.34 d 6.02 d
14	1.39 br s	4.80 br s 4.89 br s	1.71 br s	0.87 s	0.81 s	1.10 s	1.11 s
15	1.67 d	5.06 d 5.26 d	1.30 s	1.84 d	4.86 br s 4.99 br s	1.87 br s	1.55 s

J (Hz): **231**: 5,6 = 10, 6,7 = 9.0, 13a,13b = 3.5, 5,15 = 1.2, **232**: 5,6 = 6,7 = 9.3, 13a,13b = 3.3, 15a,15b = 1.8; **233**: 1,2β = 16.8, 5,6 = 6,7 = 8.6, 13a,13b = 3.4; **235**: 1,2α = 6.6, 1,2β = 9.8, 5,6 = 6,7 = 11.2, 13a,13b = 3.1, 15,3 = 1.0; **236**: 1,2α = 4.6, 1,2β = 11.5, 5,6 = 6,7 = 10.8, 13a,13b = 3.2; **237**: 1,2α = 4.6, 1,2β = 11.2, 6,7 = 11.4, 13a,13b = 3.2; **238**: 1,2α = 6.0, 1,2β = 5.3, 5,6 = 11.2, 6,7 = 10.4, 13a,13b = 3.4.

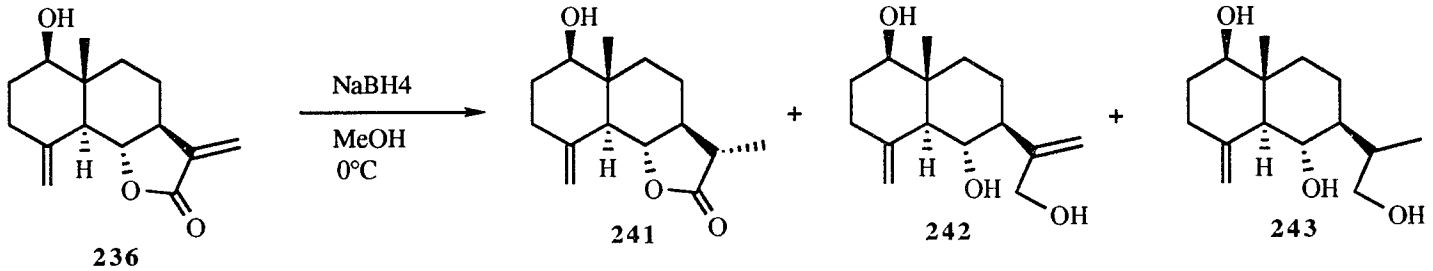
olefinic and one oxygenated carbon. The assignments of the carbon signals were made by combined application of COSY and DEPT methods and direct comparison with those related compounds [191] (Table 4.3). Mechanistically, compound **238** , like the cyclization products **235**, **236** and **237**, is formed through a carbocationic intermediate **A**, by intramolecular nucleophilic attack of the C-1 hydroxyl group at the cationic center C-4 giving the epoxy bridge (Scheme 4.1).

Reduction of santamarin (**235**) with $NaBH_4$ in MeOH at 0°C afforded dihydrosantamarin (**239**) [192] as the major and the triol **240** as a minor product (Scheme 4.2). The structure of dihydrosantamarin (**239**) was established by direct comparison with the spectroscopic data of an authentic sample. Inspection of the ^{1}H-COSY spectrum of **240** revealed two geminally coupled proton doublets at δ 4.13 and 4.17 (J=12.6 Hz) which showed allylic couplings with the methylene signals at δ 5.10 and 5.24, indicating a reductive opening of lactonic ring of santamarin. The ^{13}C NMR data of **240** showed the presence of three oxygenated carbons with two methine signals at δ 76.0 and 71.2 and one methylene carbon absorption at δ 65.7. This suggested the presence of three hydroxyl groups which was further supported by a very strong IR absorption at 3361 cm^{-1}. The mass spectrum of **240** showed a prominent molecular ion at m/z 252 and the peaks at m/z 234 $[M-H_2O]^+$ and m/z 216 $[M-2H_2O]^+$ further supported its structure. The ^{13}C NMR spectrum exhibited four olefinic signals at δ 113.0, 121.8, 135.2 and 151.0 due to one methylene (C-13), one methine (C-3) and two quaternary carbons (C-4 and C-11), respectively. Complete ^{13}C assignments were obtained by DEPT, 2D ^{1}H-COSY and inverse ^{1}H,^{13}C-correlation experiments (Table 4.3).

Reduction of reynosin (**236**) using the same condition as described above for **235** provided, after VLC separation, the major dihydroreynosin (**241**) and the two minor lactonic ring opening products **242** and **243** (Scheme 4.3). Dihydroreynosin



Scheme 4.2. Reduction of santamarin (**235**)



Scheme 4.3. Reduction of reynosin (**236**)

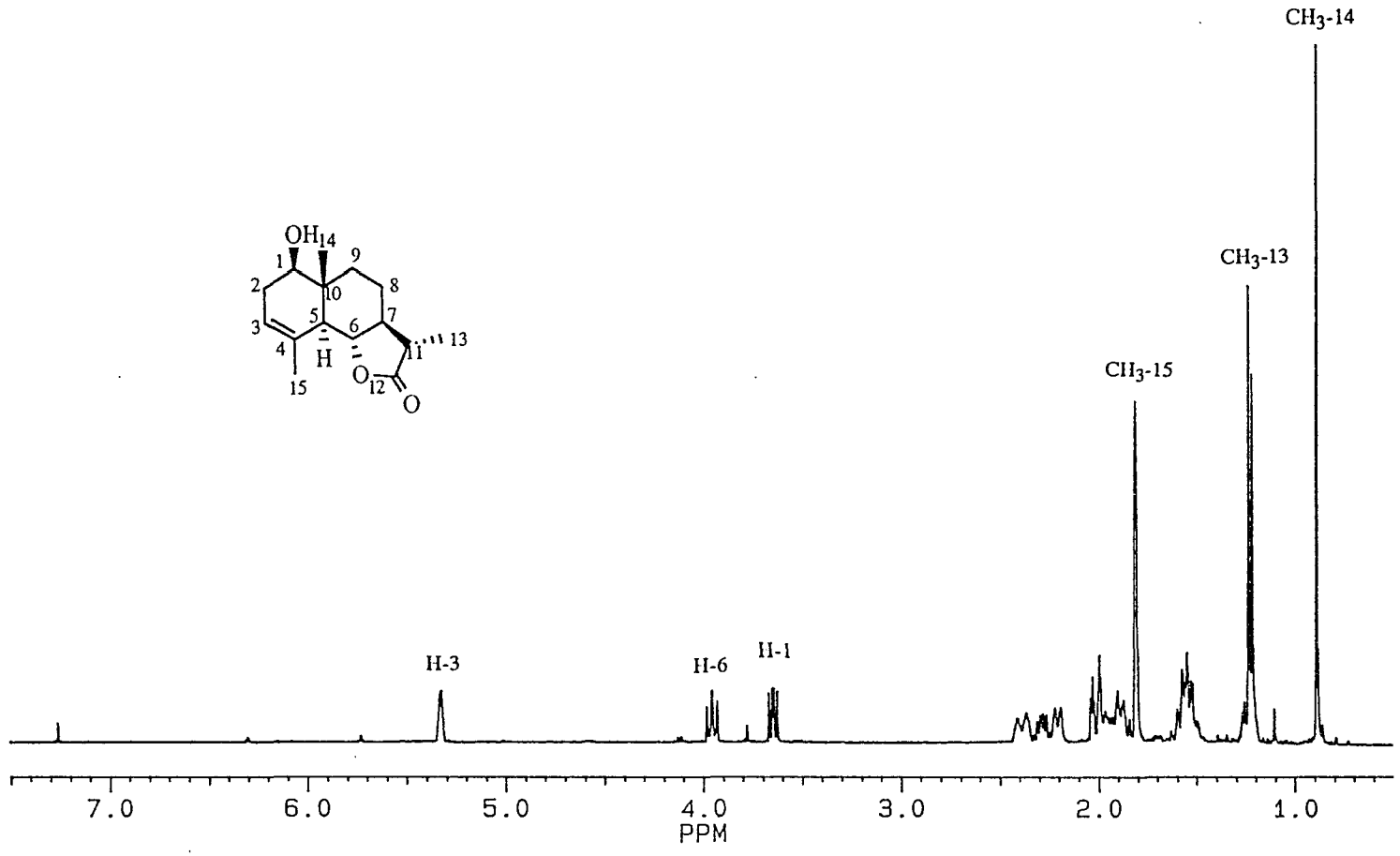


Figure 4.10. 400 MHz ^{1}H NMR spectrum of dihydrosantamarin (**239**)

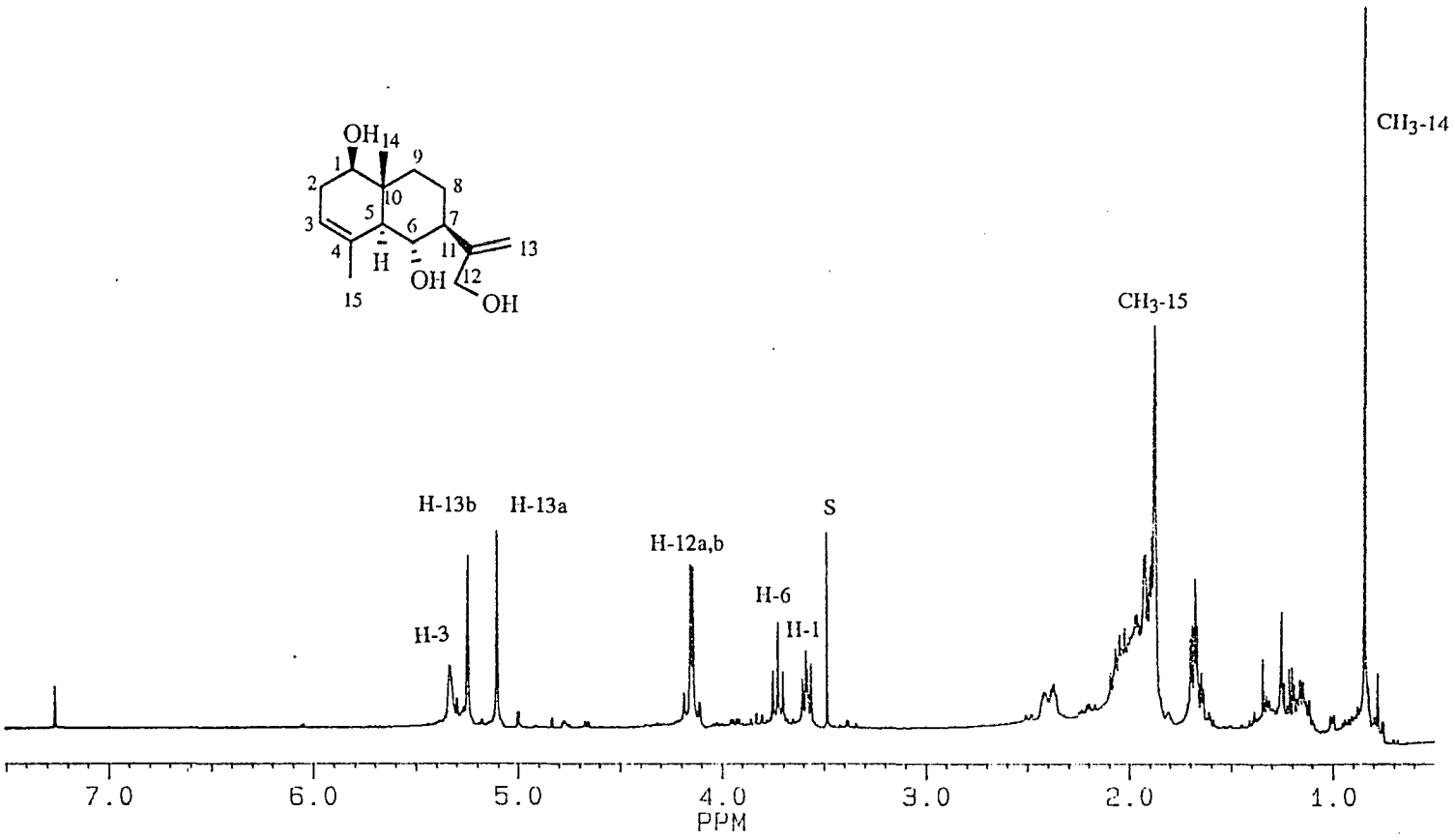


Figure 4.11. 400 MHz ^{1}H NMR spectrum of 1β,6α,12-trihydroxy-3,11(13)-eudesmadiene (**240**)

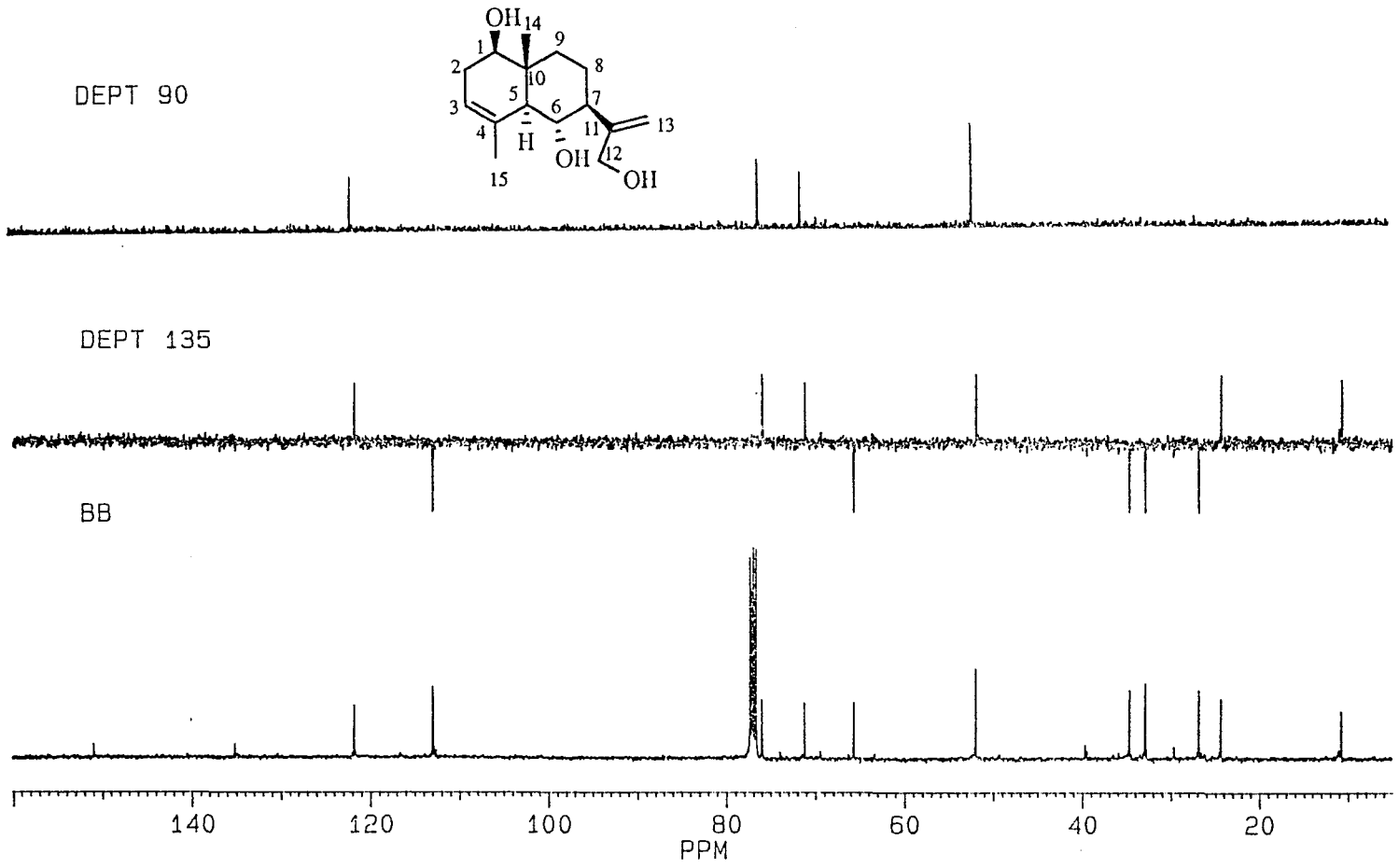


Figure 4.12. DEPT 90°, DEPT 135° and BB ^{13}C NMR spectra of 1β,6α,12-trihydroxy-3,11(13)-eudesmadiene (**240**)

(**241**) was identified by spectral comparison with an authentic sample and data reported in the literature [178].

The ^{1}H NMR data of **242** was similar to that of compound **240** as shown by inspection of its ^{1}H-COSY spectrum. Two geminally coupled proton doublets which appeared as an AB system at δ 4.11 and 4.16 (J=12.6 Hz) were allylically coupled to a pair of broad methylene singlets at δ 5.09 (H-13a) and 5.22 (H-13b), indicating that the lactone was reductively opened and the carbonyl group (C-12) in reynosin (**236**) was reduced to CH_2OH. The IR spectrum of **242** had a very strong absorption at 3364 cm^{-1} but no carbonyl absorption, which further supported the above argument. The mass spectrum of **242** showed a parent peak at m/z 252 and exhibited further prominent peaks at m/z 234 $[M-H_2O]^+$, 216 $[M-2H_2O]^+$ and 201 $[M-2H_2O-Me]^+$ which were in agreement with the proposed structure **242**. The ^{13}C NMR of **242** showed three oxygenated carbon signals at δ 78.8, 70.1 and 66.5 assigned to carbons 1, 6 and 12 bearing the respective hydroxyl groups. It also exhibited four olefinic signals with two methylene carbons at δ 108.3 (C-15) and 112.5 (C-13) and two quaternary carbons at δ 145.3 (C-4) and 151.3 (C-11). Combined application of ^{1}H-COSY, inverse ^{1}H,^{13}C-correlation and DEPT methods allowed for all ^{13}C assignments of **242** (Table 4.3).

The structure of compound **243** was unambiguously established by comparison of its spectroscopic data with those of **242**. Instead of the 13-methylene signals at δ 5.09 and 5.22 in compound **242**, the ^{1}H NMR spectrum of **243** showed a methyl doublet at δ 0.93 coupled with a proton multiplet at δ 2.07, indicating that it was a 11,13-dihydro derivative of **242**. This was further confirmed by its mass spectral data with a molecular ion at m/z 254, which was two mass units higher than the one observed for **242**. Other prominent peaks at m/z 236 $[M-H_2O]^+$, 218 $[M-2H_2O]^+$ and 203 $[M-2H_2O-Me]^+$ were also two mass units higher than the corresponding peaks of compound **242**. Its ^{13}C NMR spectrum showed three oxygenated carbon signals at δ

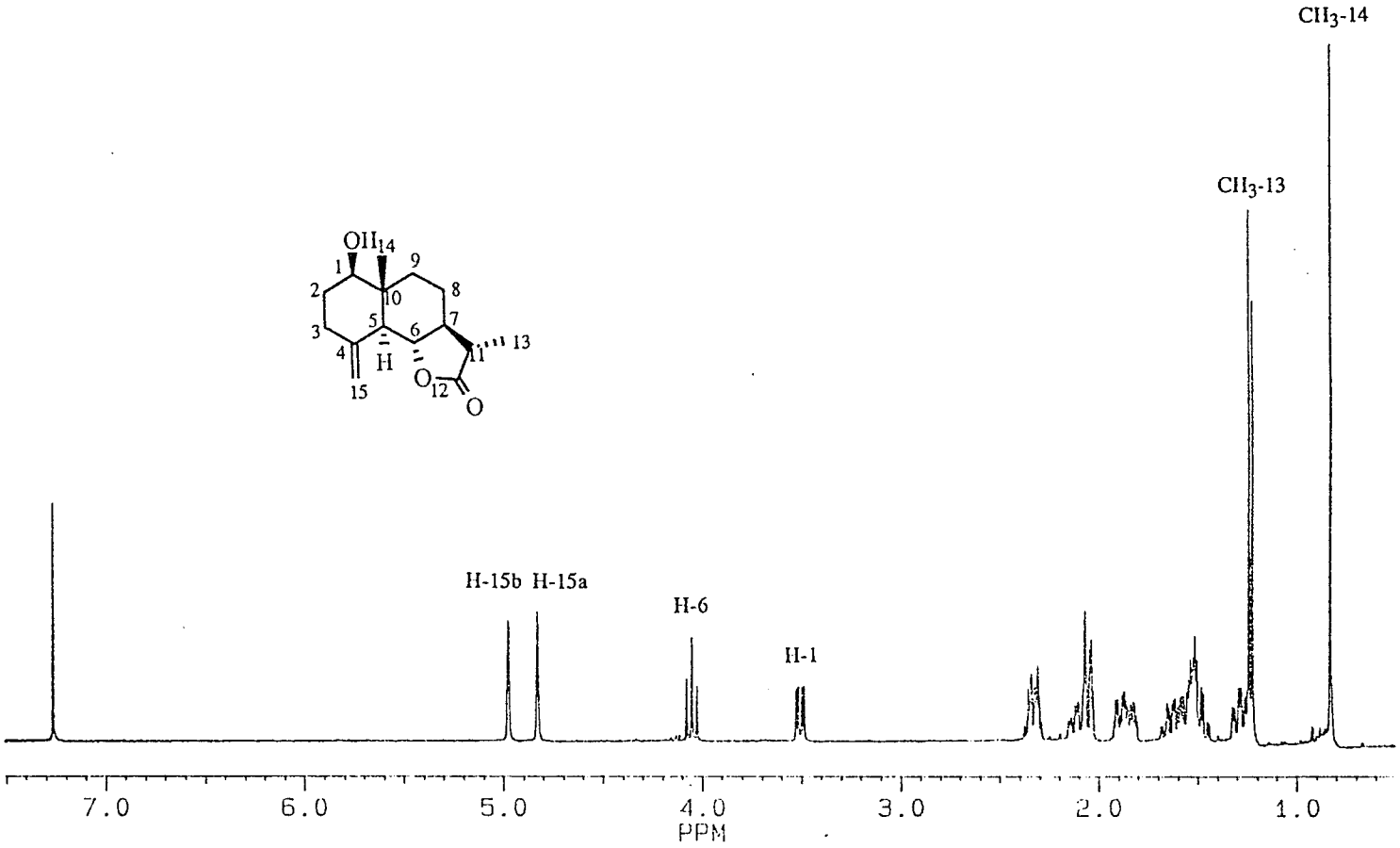


Figure 4.13. 400 MHz ^{1}H NMR spectrum of dihydroreynosin (**241**)

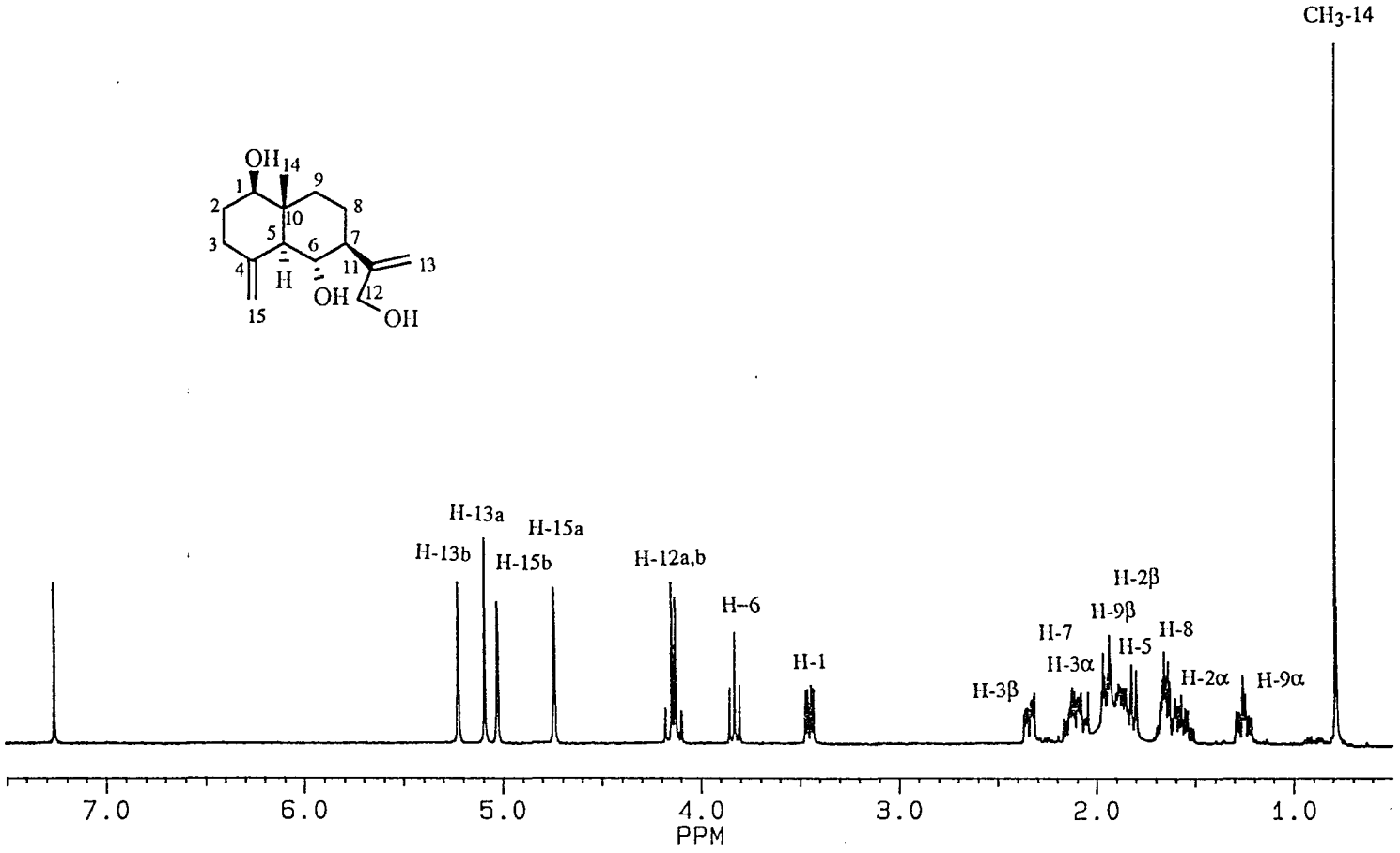


Figure 4.14. 400 MHz ^{1}H NMR spectrum of 1β,6α,12-trihydroxy-4(15),11(13)-eudesmadiene (**242**)

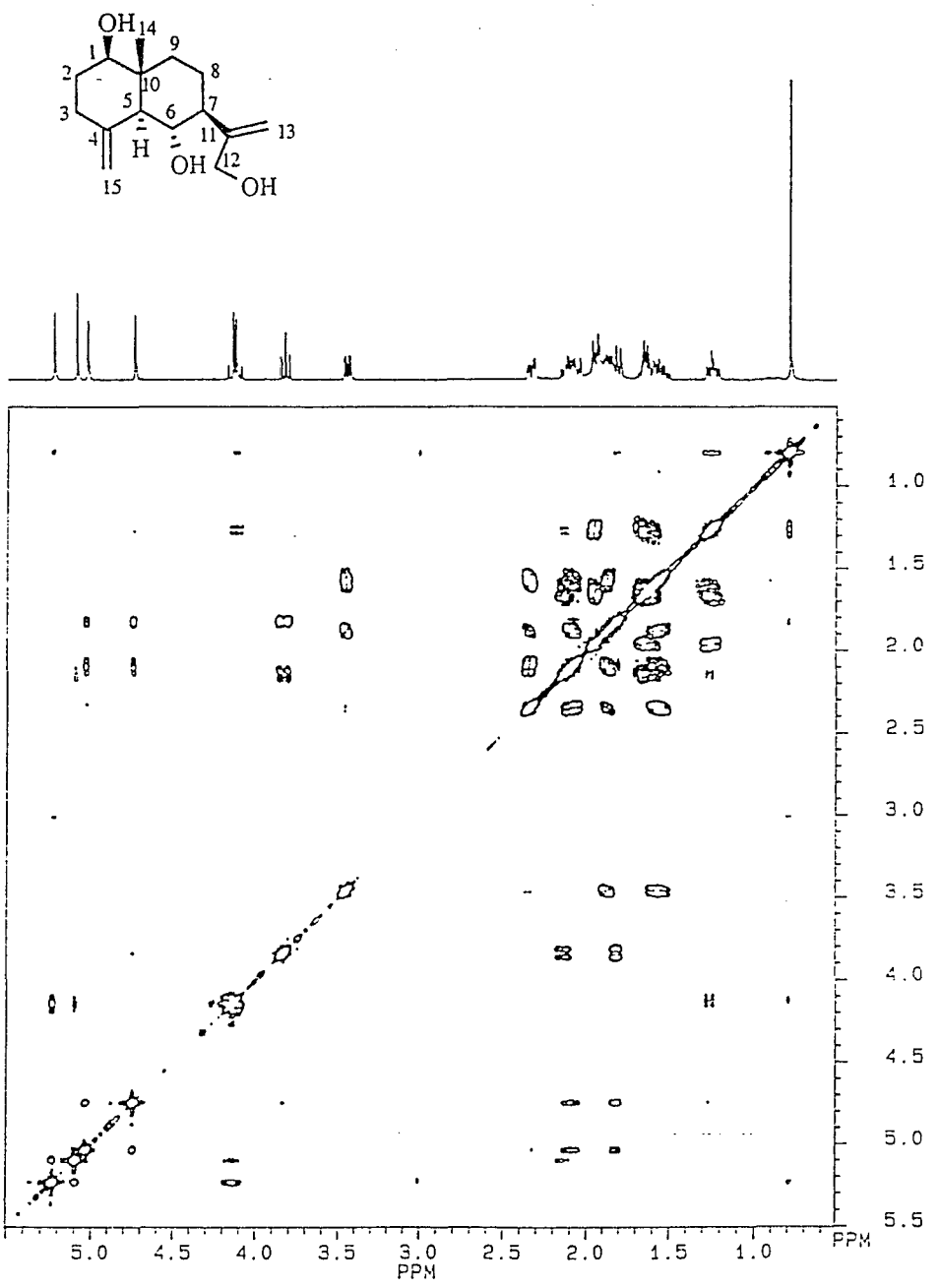


Figure 4.15. 400 MHz ^{1}H-COSY spectrum of 1β,6α,12-trihydroxy-4(15),11(13)-eudesmadiene(**242**)

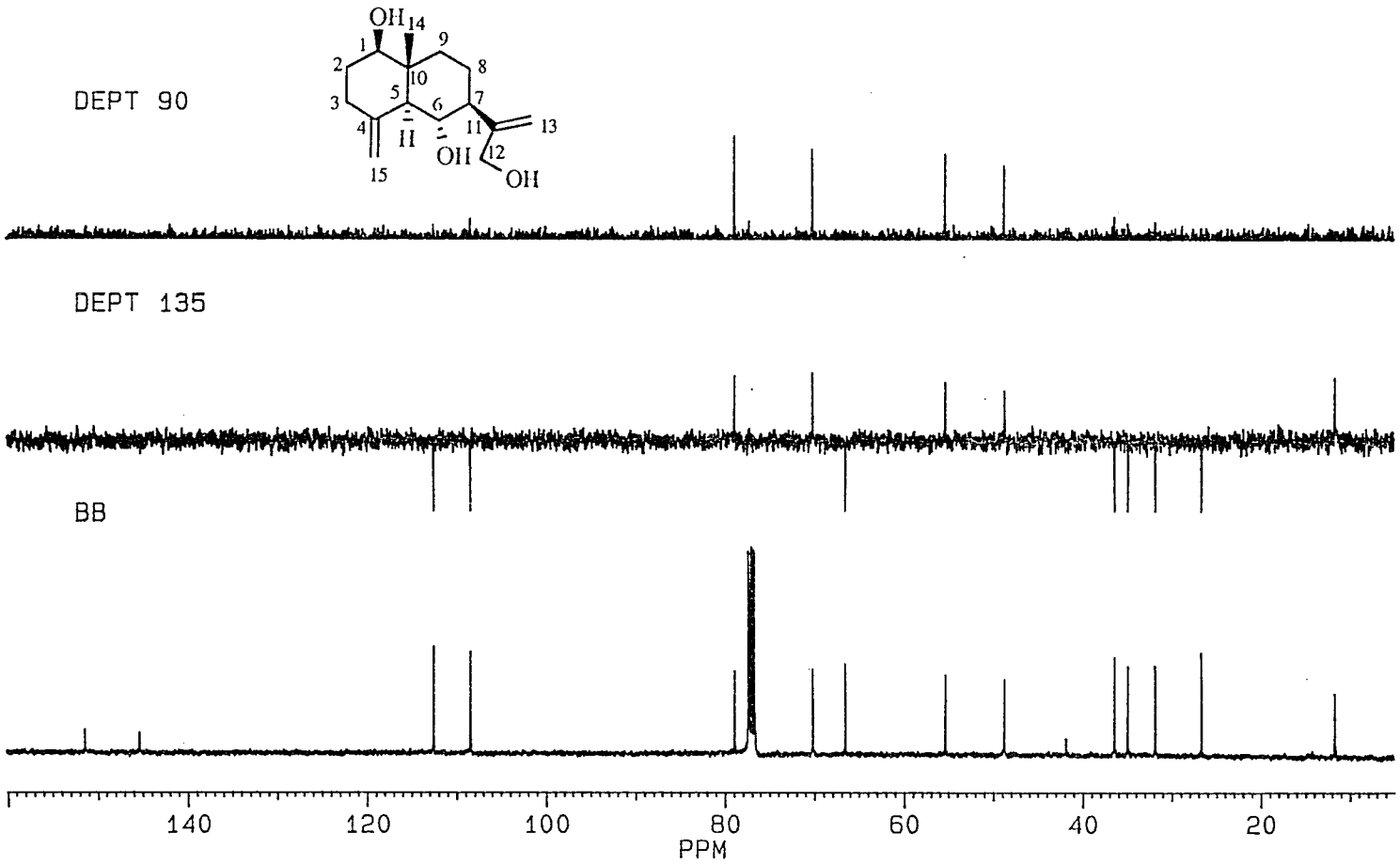


Figure 4.16. DEPT 90°, DEPT 135° and BB ^{13}C NMR spectra of 1β,6α,12-trihydroxy-4(15),11(13)-eudesmadiene (**242**)

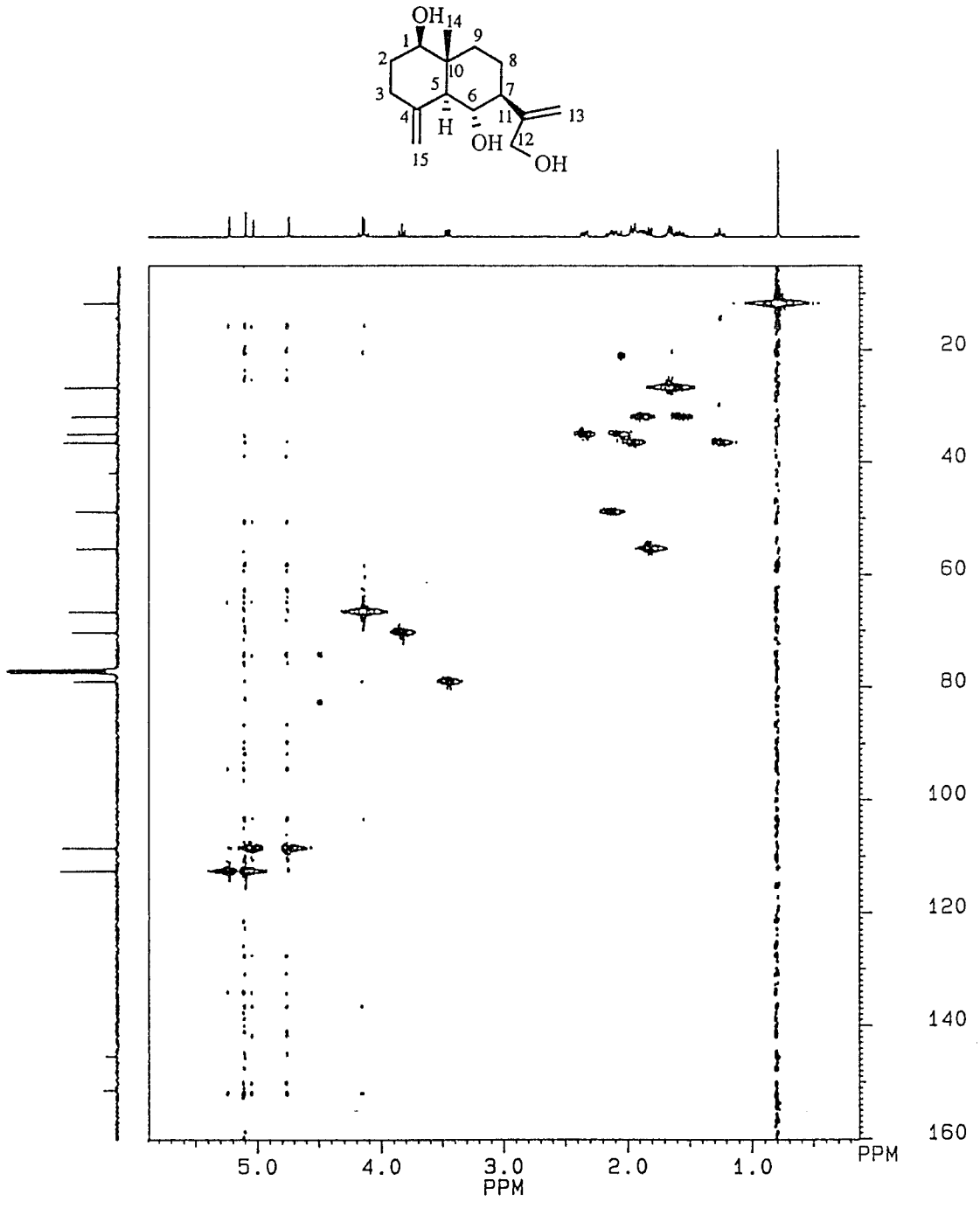


Figure 4.17. 2D Inverse ^{1}H-^{13}C heteronuclear spectrum of 1β,6α,12-trihydroxy-4(15),11(13)-eudesmadiene(**242**)

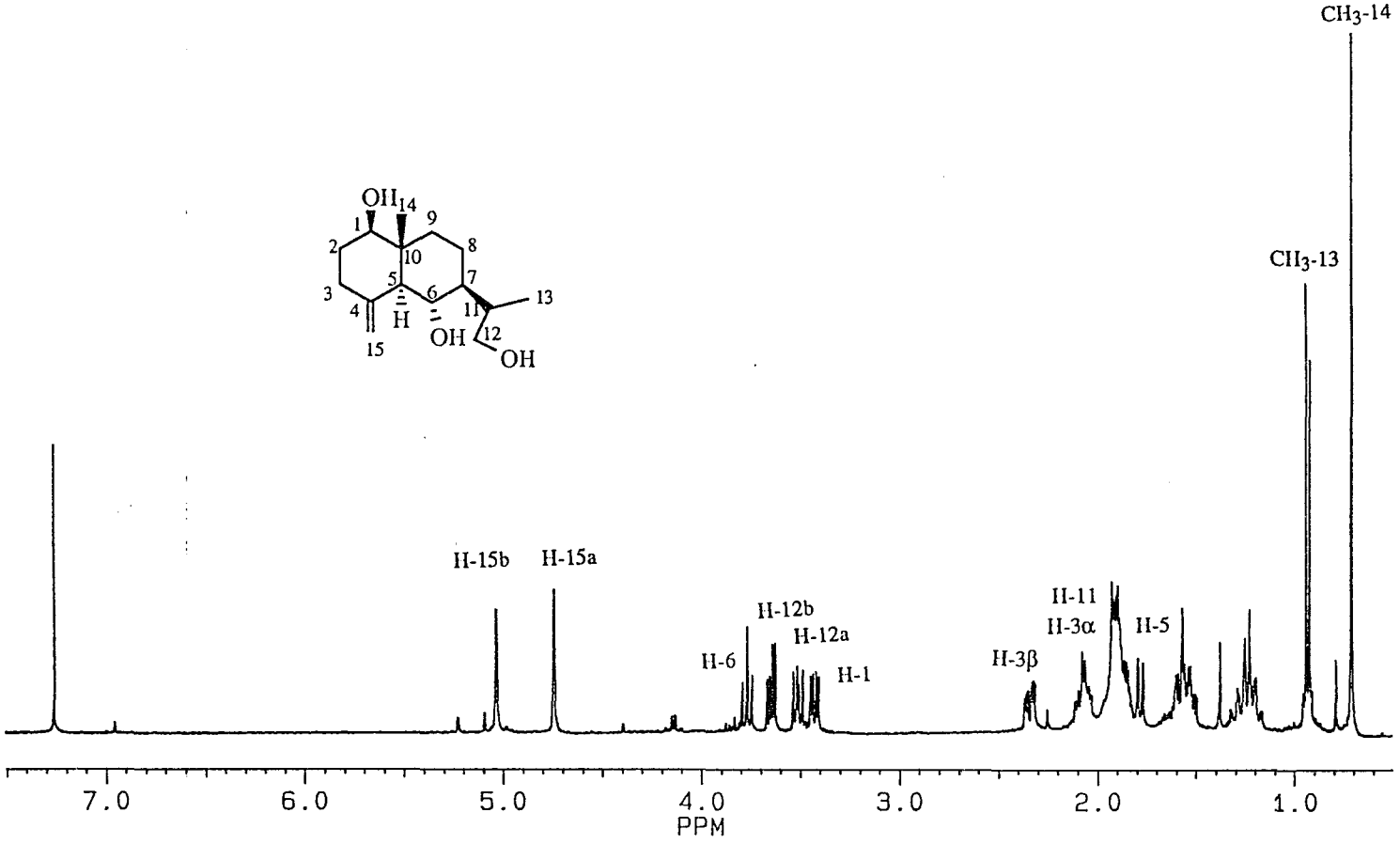


Figure 4.18. 400 MHz ^{1}H NMR spectrum of 1β,6α,12-trihydroxy-4(15)-cudesmene (**243**)

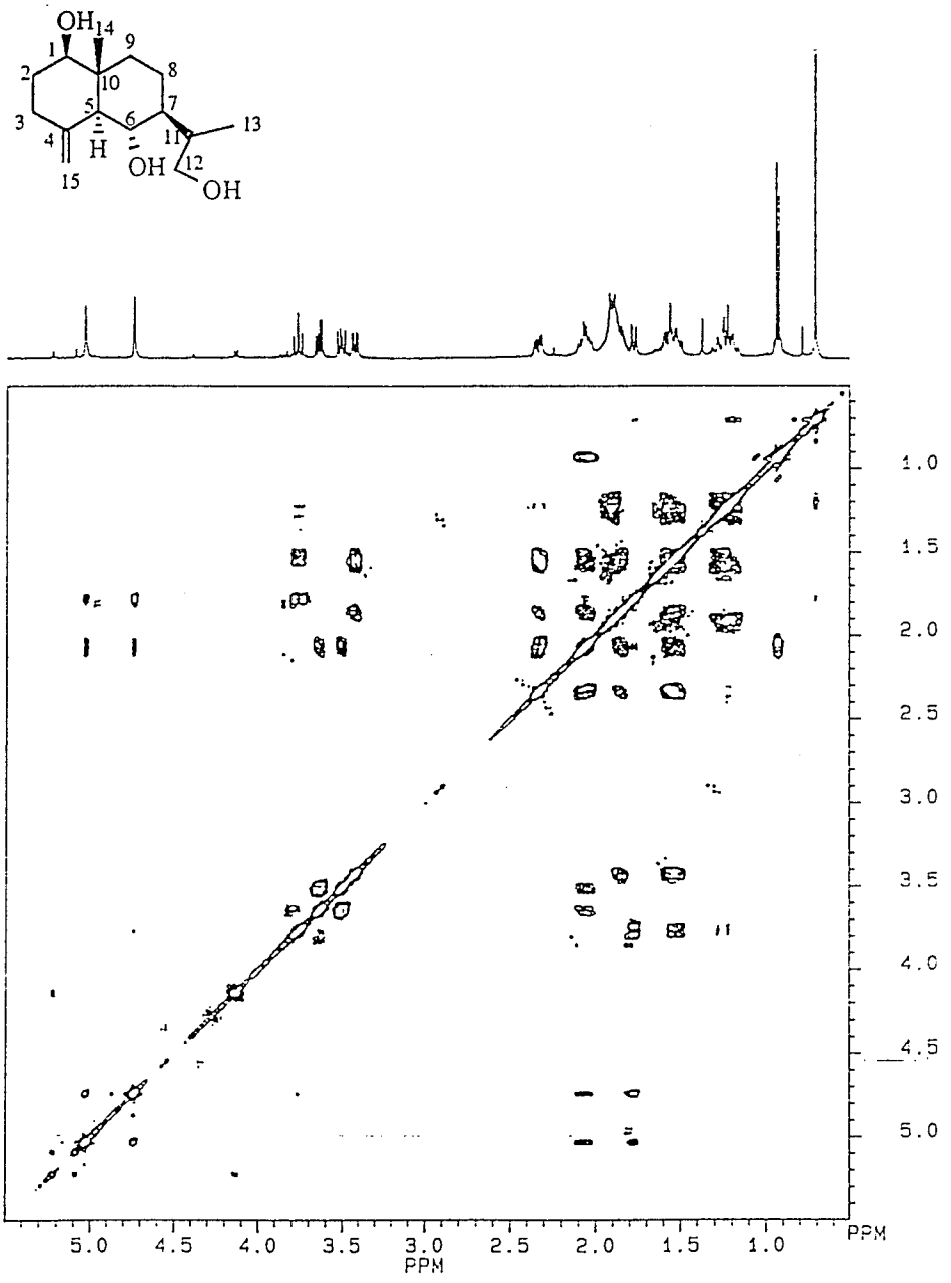


Figure 4.19. 400 MHz ^{1}H-COSY spectrum of 1β,6α,12-trihydroxy-4(15)-eudesmene(**243**)

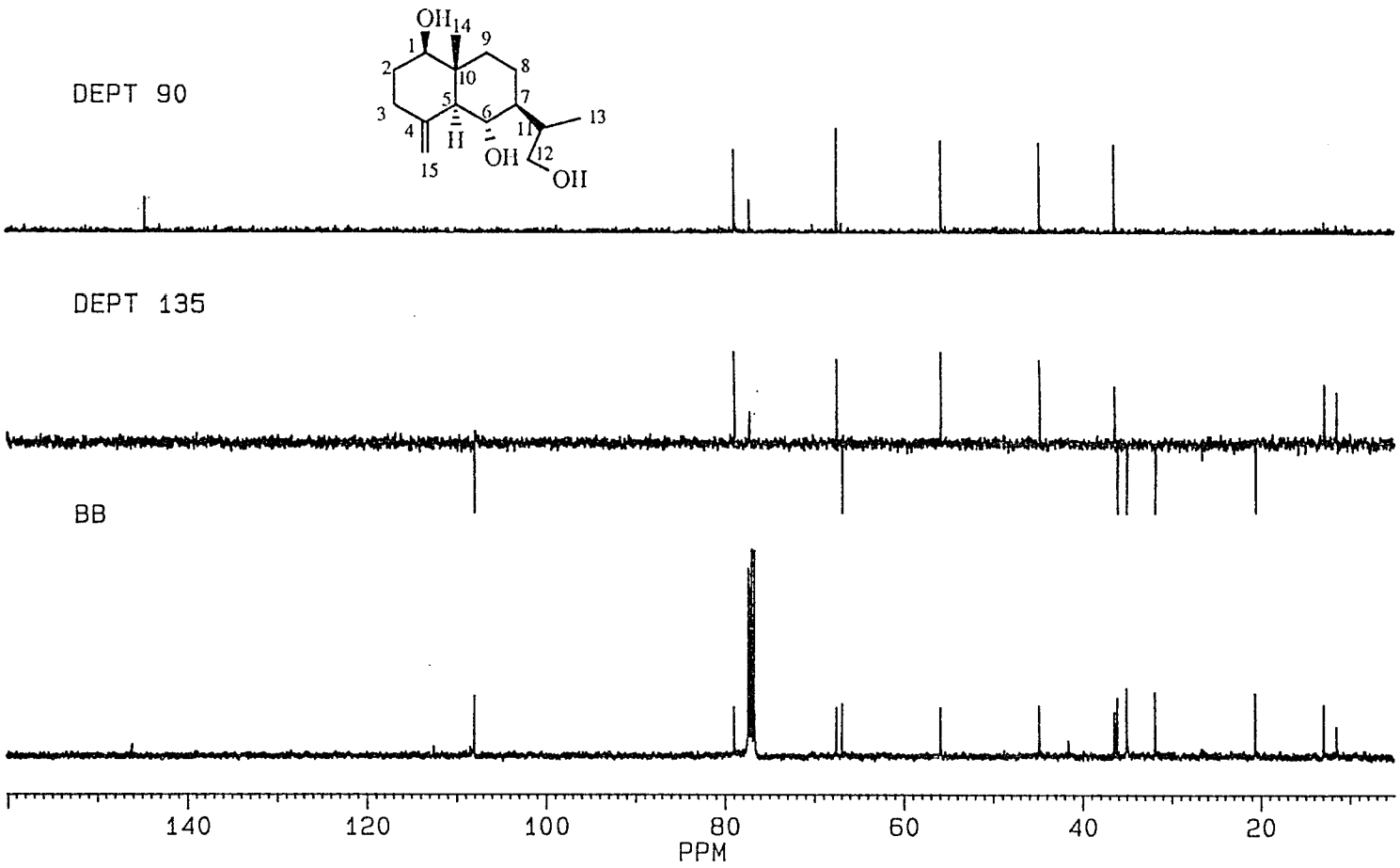


Figure 4.20. DEPT 90°, DEPT 135° and BB ^{13}C NMR spectrum of 1β,6α,12-trihydroxy-4(15)-eudesmene (**243**)

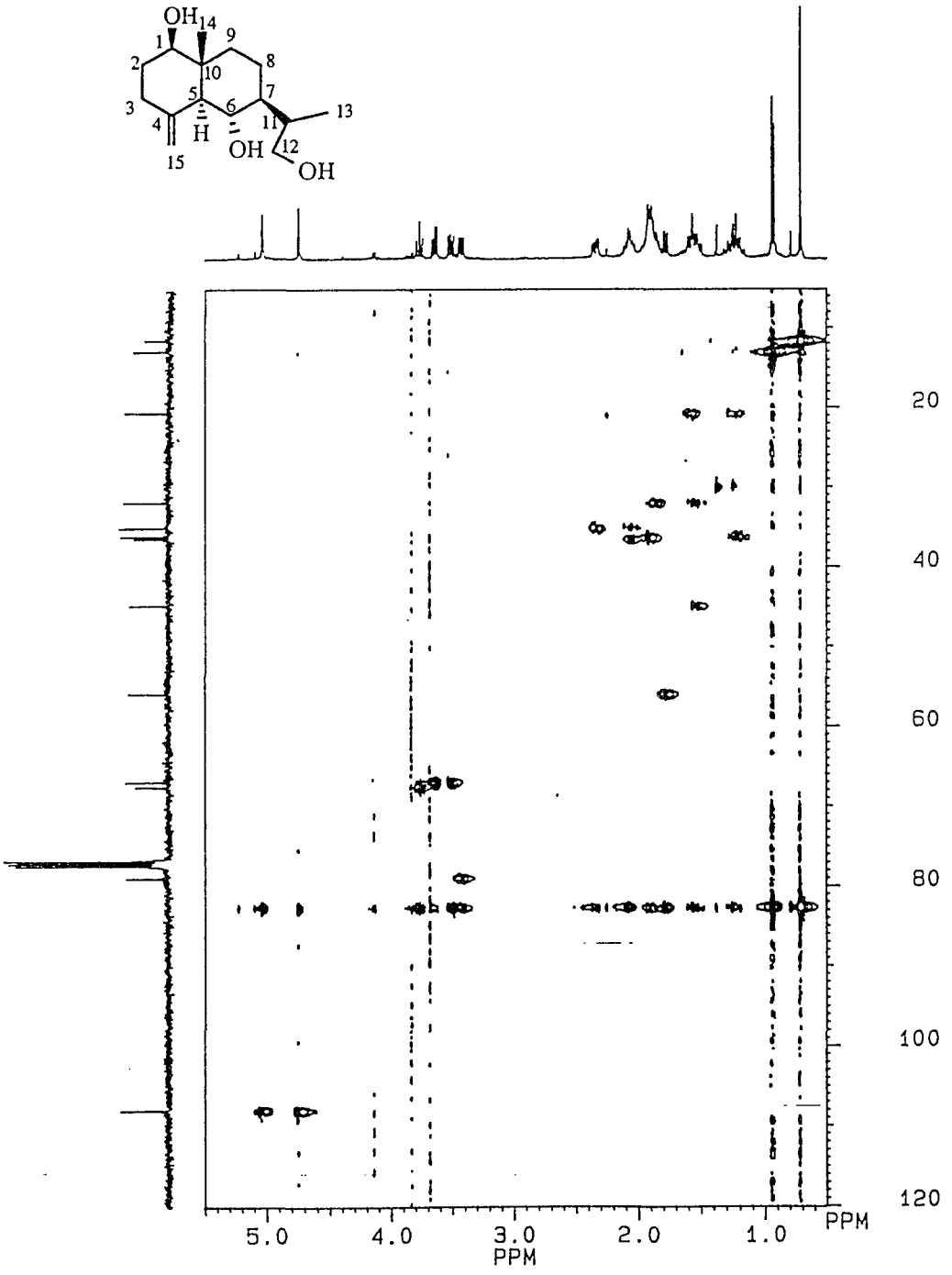


Figure 4.21. 2D Inverse ^{1}H-^{13}C heteronuclear correlation spectrum of 1β,6α,12-trihydroxy-4(15)-eudesmene(**243**)

Table 4.2. ^{1}H NMR spectral data of compounds **239-243, 247** and **248** (400 MHz, $CDCl_3$ as internal standard)

H	**239**	**240**	**241**	**242**	**243**	**247**	**248**
1	3.64 dd	3.58 dd	3.50 dd	3.45 dd	3.43 dd	2.98 ddd	2.78 ddd
2α		1.92 m		1.57 m	1.58 m	1.89 m	1.83 m
2β		2.40 m		1.87 m	1.85 m	1.75 m	1.53 m
3	5.32 m	5.33 m		2.07 br dd 2.34 ddd	2.06 m 2.34 ddd	1.85 m	1.82 m
5	2.01 d	1.92 d	2.04 d	1.81 br d	1.78 br d	2.27 dd	2.25 dd
6	3.95 dd	3.72 dd	4.05 dd	3.83 dd	3.77 dd	4.04 dd	4.25 dd
7		2.08 m		2.14 m	1.56 m	1.78 m	1.78 m
8		1.69 m		1.58 m 1.67 m	1.28 m 1.62 m	2.12 m 1.28 m	2.00 m 1.42 m
9α		1.16 m		1.25 ddd	1.23 m	2.64 ddd	1.93 m
9β		1.95 m		1.95 ddd	1.90 m	1.86 m	1.63 m
11		------		-------	2.07 m	2.21 m	2.22 m
12		4.13 d 4.17 d		4.11 d 4.16 d	3.51 dd 3.65 dd	-------	-------
13	1.22 d	5.10 br s 5.24 br s	1.23 d	5.09 br s 5.33 br s	0.93 d	1.24 d	1.23 d
14	0.89 s	0.84 s	0.82 s	0.79 s	0.71 s	4.94 br s 4.97 br s	1.16 s
15	1.81 br s	1.87 br s	4.82 d 4.97 d	4.74 br s 5.03 br s	4.74 br s 5.03 br s	1.30 s	1.34 s
OMe	--------	--------	--------	--------	--------	--------	3.17 s

J (Hz): **239**: 1,2α = 6.7, 1,2β = 9.9, 5,6 = 11.2, 6,7 = 9.8, 13,11 = 7.0; **240**: 1,2α = 7.0, 1,2β = 9.5, 5,6 = 6,7 = 10.0, 12a,12b = 12.6; **241**: 1,2α = 4.5, 1,2β = 11.5, 5,6 = 6,7 = 10.5, 15a,15b = 1.1; **242**: 1,2α = 4.7, 1,2β = 11.5, 2α,3α = 5.6, 2α,3β = 4.9, 2β,3β = 2.1, 3α,3β = 13.5, 5,6 = 6,7 = 10.0, 9α,8α = 5.5, 9α,8β = 12.5, 9β,8α = 9β,8β = 3.2, 9α,9β = 13.0, 12a,12b = 12.6; **243**: 1,2α = 4.7, 1,2β = 11.5, 3β, 2α = 4.9, 3β,2β = .2.2, 3α,3β = 13.2, 5,6 = 6,7 = 9.8, 12a,11 = 7.5, 12b,11 = 5.4, 12a,12b = 11.2; **247**: 1,5 = 11.8, 5,6 = 11.4, 6,7 = 9.0, 9α,8α = 9α,8β = 3.7, 9α,9β = 13.1, 13,11 = 7.1; **248**: 1,2α = 3.2, 1,2β = 7.8, 1,5 = 12.1, 5,6 = 11.7, 6,7 = 10.3, 13,11 = 7.1.

Table 4.3. ^{13}C NMR spectral data of compounds **238**, **240**, **242**, **243** and **248** (100 MHz, $CDCl_3$ as internal standard)*

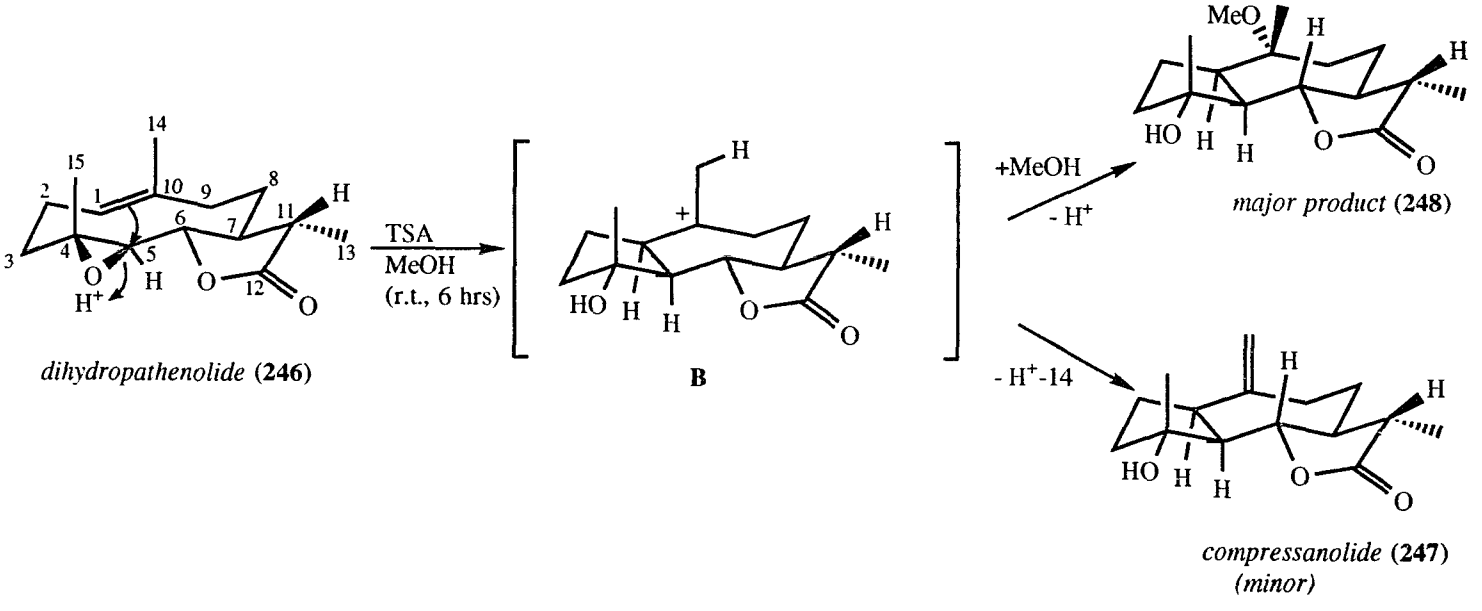
C	**238**	**240**	**242**	**243**	**248**
1	84.1 d	76.0 d	78.8 d	78.9 d	45.9 d
2	42.3 t	32.9 t	31.8 t	31.8 t	25.5 t
3	23.5 t	121.8 d	34.9 t	35.0 t	39.2 t
4	83.4 s	135.2 s	145.3 s	146.0 s	80.2 s
5	59.0 d	52.0 d	55.3 d	55.8 d	54.9 d
6	80.3 d	71.2 d	70.1 d	67.4 d	82.9 d
7	54.0 d	52.0 d	48.7 d	44.8 d	51.1 d
8	30.3 t	26.9 t	26.6 t	20.6 t	25.8 t
9	22.4 t	34.7 t	36.4 t	36.1 t	37.4 t
10	41.8 s	39.7 s	41.8 s	41.5 s	78.0 s
11	138.8 s	151.0 s	151.3 s	36.4 d	41.3 d
12	170.6 s	65.7 t	66.5 t	66.8 t	177.9 s
13	115.8 t	113.0 t	112.5 t	12.9 q	12.9 q
14	17.4 q	10.8 q	11.6 q	11.5 q	21.9 q
15	21.0 q	24.4 q	108.3 t	107.9 t	23.8 q
OMe	-------	------	------	------	48.1 s

* Peak multiplicities were determined by heteronuclear multipulse programs (DEPT); s=singlet; d=doublet; t= triplet; q=quartet.

78.9 (C-1), 67.4 (C-6) and 66.8 (C-12) due to the three hydroxyl groups. However, it only exhibited two olefinic signals, one due to a CH_2 at δ 107.9 (C-15) and a quaternary at δ 146.0 (C-4). This was in agreement with the fact of only one exocyclic double bond was present in the molecule. The ^{13}C NMR data assigned on the basis of its ^{1}H-COSY and $^{1}H,^{13}C$-HETCOR analyses are listed in Table 4.3.

Acid-catalyzed cyclization of dihydroparthenolide (**246**) in MeOH at room temperature afforded a guaianolide derivative **248** as the major product along with compressanolide (**247**) as a minor one (Scheme 4.4). Compounds **247** and **248** were obviously derived from a carbocationic intermediate **B**, by losing a H^+-14 to give compressanolide (**247**) and by nucleophilic addition of MeOH to provide **248**. Compressanolide (**247**) was identified by comparison of the spectral data (^{1}H NMR and MS) with those reported in literature [178].

The ^{1}H NMR spectrum of **248** showed the presence of four methyl signals with one singlet at δ 3.17 which is typical for a methoxy group. The Me-14 singlet at δ 1.16 indicates that it is β-oriented while the methoxy group is α-oriented comparing with those spectral data of other guaianolide analogues [193, 194]. Inspection of the molecular model of dihydroparthenolide (**246**) revealed that nucleophiles only could approach the molecule from the less hindered outside of the medium ring of **246** to give 10α-substituted compounds since inside the ring of **246** was too crowded to accommodate any nucleophiles. The mass spectrum of **248** showed a molecular ion at m/z 282 and further peaks at m/z 264 $[M-H_2O]^+$, 250 $[M-MeOH]^+$, 232 $[M-H_2O-MeOH]$ and 217 $[232-Me]^+$ which were in agreement with the proposed structure.
The ^{13}C NMR of **248** exhibited the presence of 16 carbons with one methoxy signal at δ 48.1. Combined ^{1}H-COSY, DEPT and inverse ^{1}H-^{13}C heteronuclear correlation methods provided the complete assignments of both ^{1}H and ^{13}C NMR spectra of **248**, which were listed in Table 4.2 and Table 4.3, respectively.



Scheme 4.4. The acid-catalyzed transformation of dihydroparthenolide into guaianolide derivatives

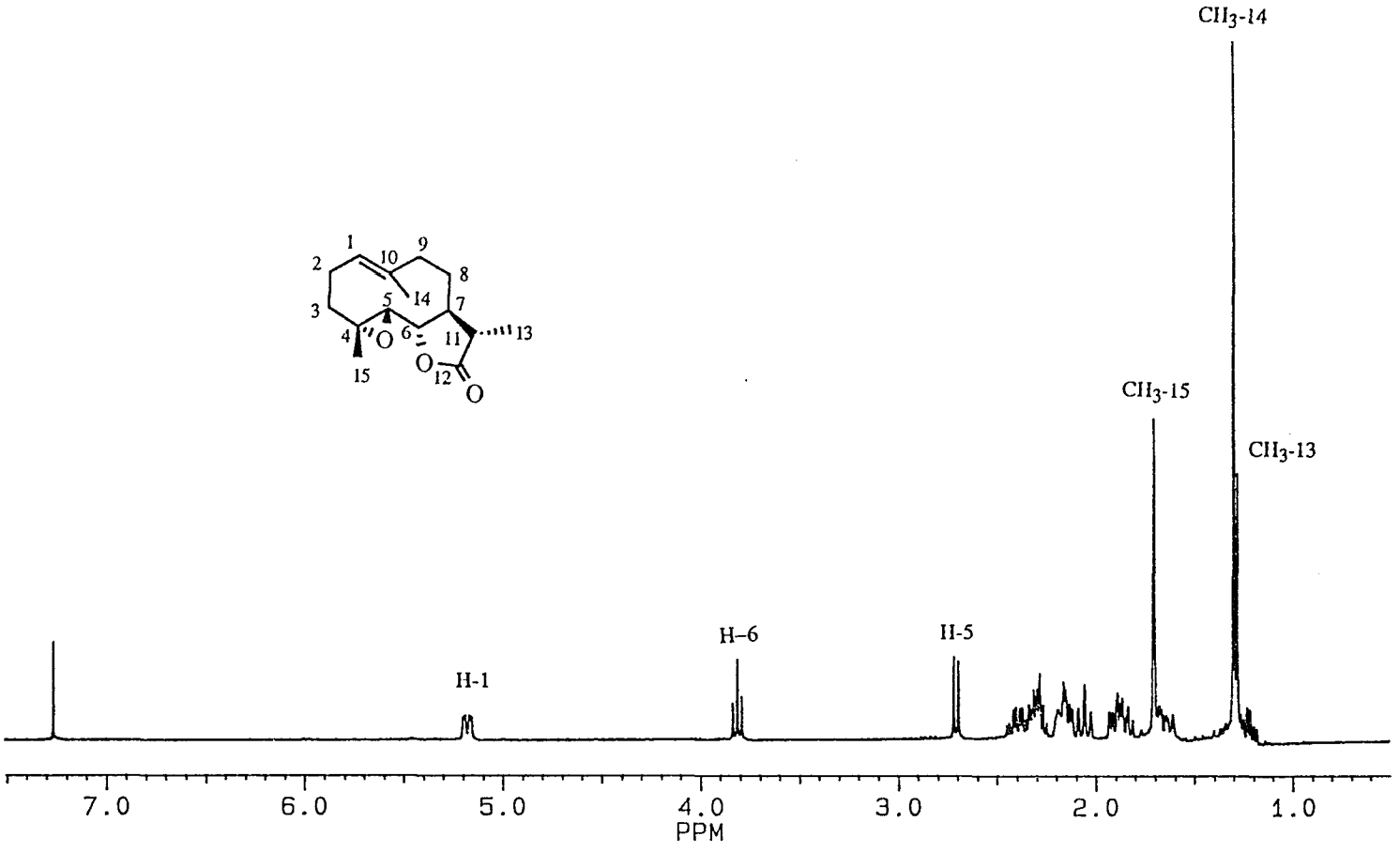


Figure 4.22. 400 MHz ^{1}H NMR spectrum of dihydroparthenolide (**246**)

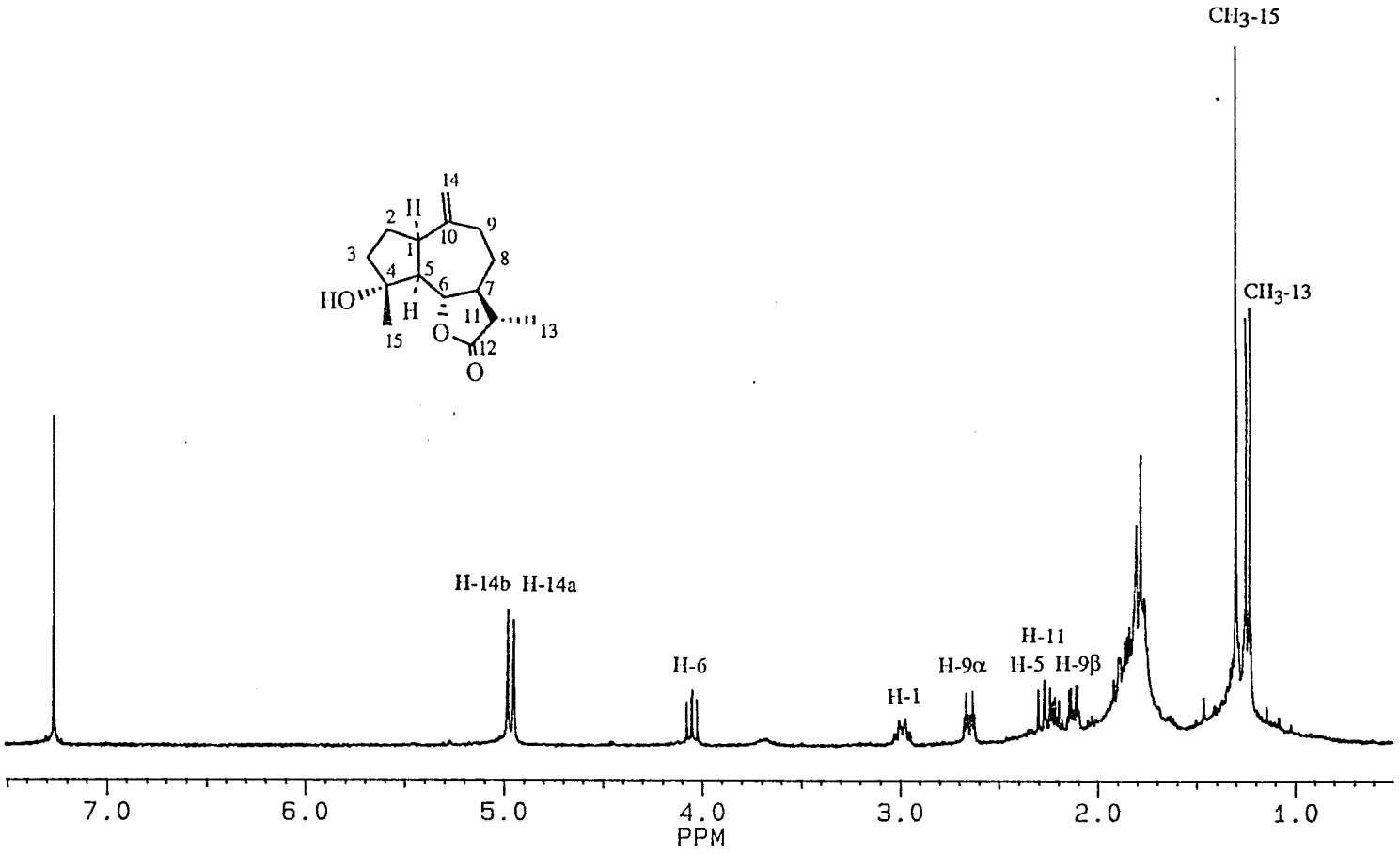


Figure 4.23. 400 MHz ^{1}H NMR spectrum of compressanolide (**247**)

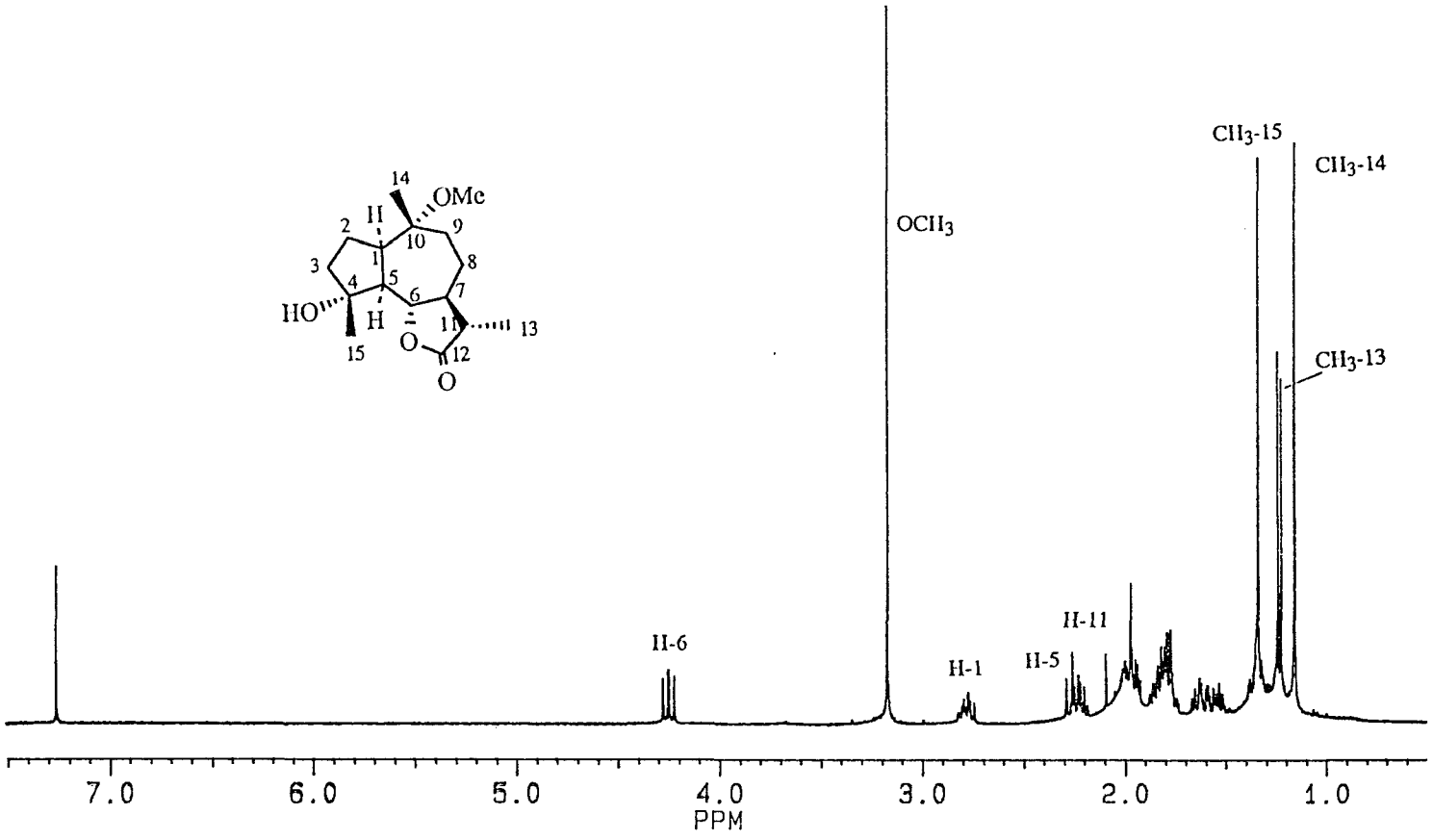


Figure 4.24. 400 MHz ^{1}H NMR spectrum of 4α-hydroxy-10α-methoxyguaian-12,6-olide (**248**)

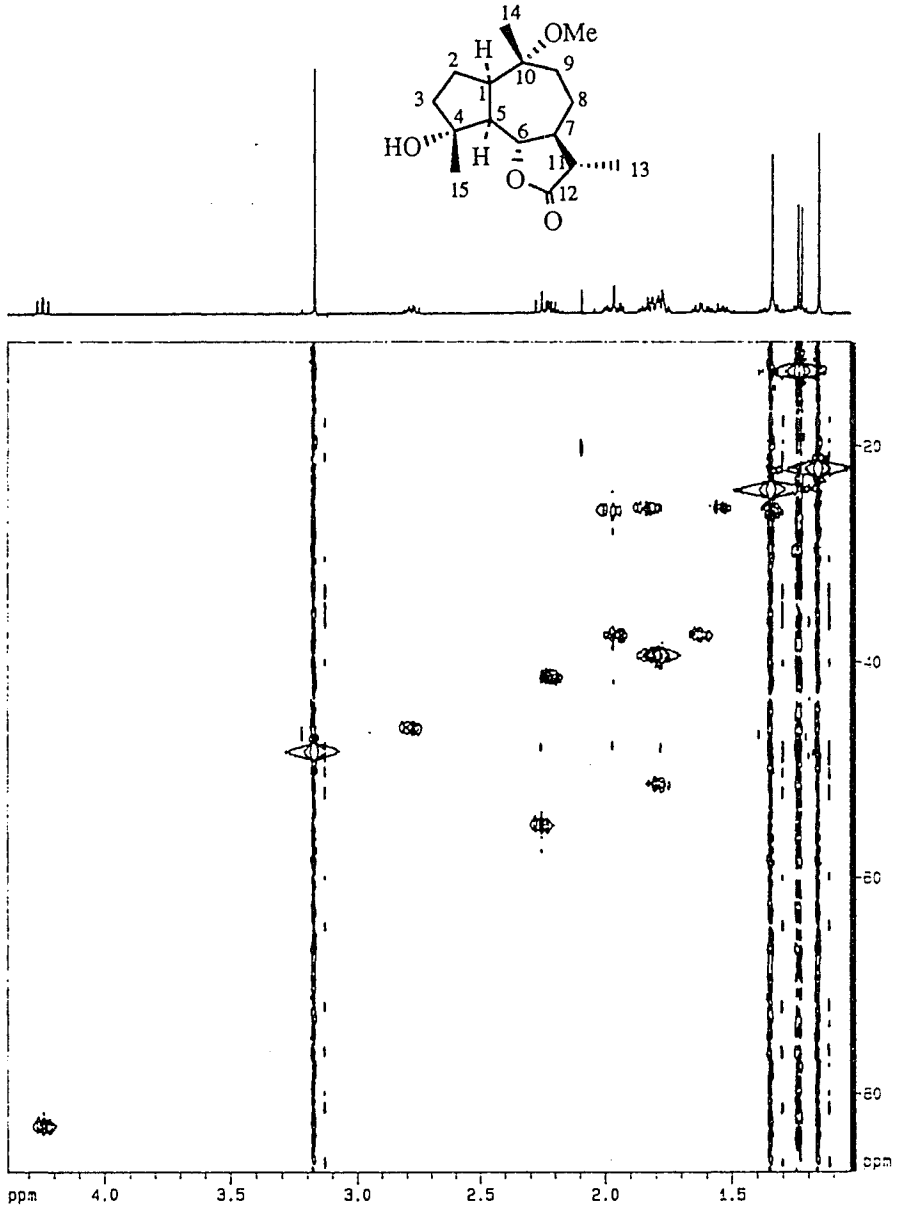


Figure 4.25. 2D Inverse ^{1}H-^{13}C heteronuclear correlation spectrum of 4α-hydroxy-10α-methoxyguaian-12,6-olide(**248**)

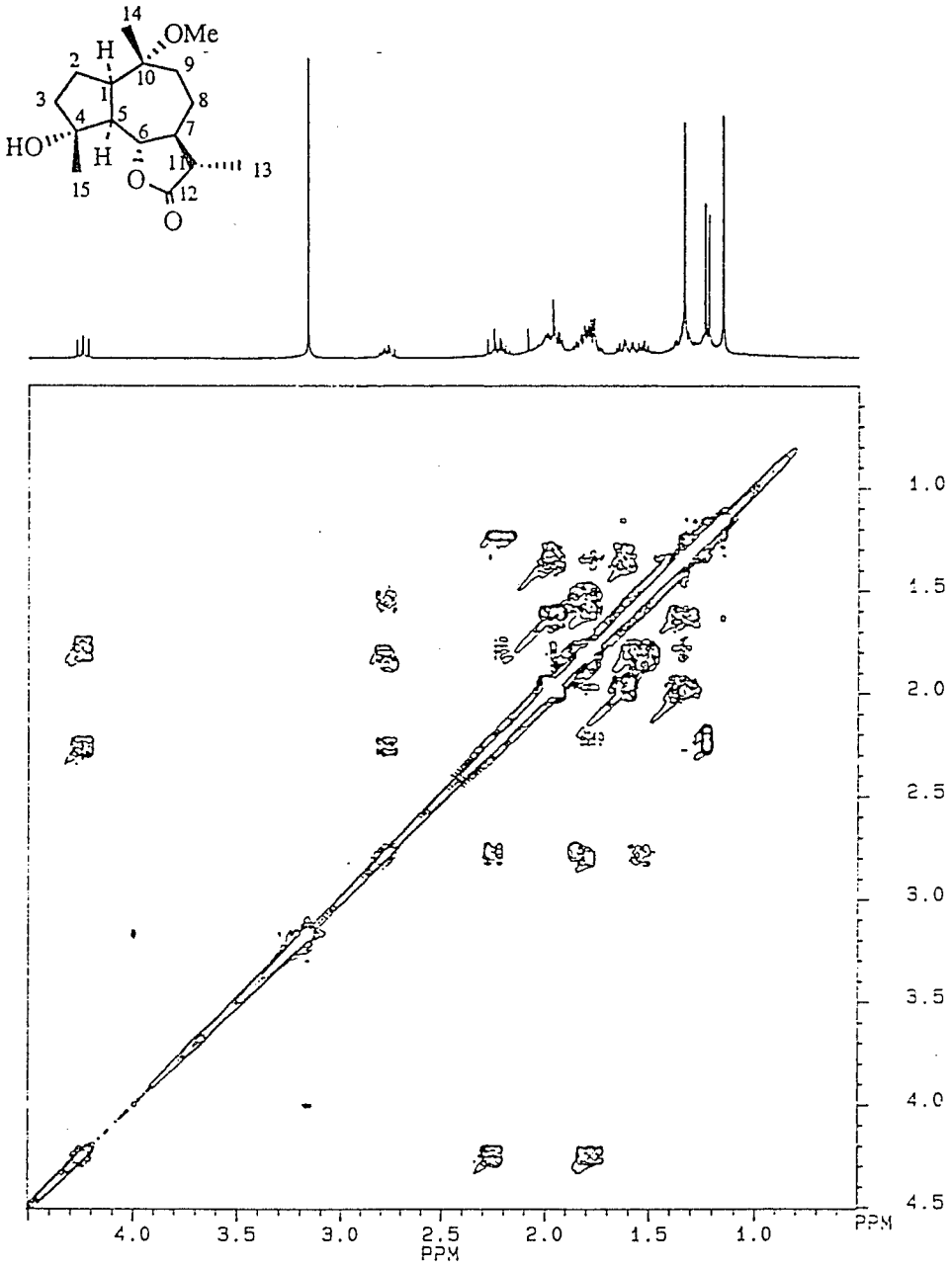


Figure 4.26. 400 MHz 1H-COSY spectrum of 4α-hydroxy-10α-methoxyguaian-12,6-olide(**248**)

Anti-mycobacterial Activities:

The biological activities of 14 tested sesquiterpenes are listed in Table 4.4. Dehydrocostuslactone (**232**) was the most active against both *Mycobacterium tuberculosis* an *M. avium* with minimum inhibitory concentrations (MICs) at 2 μg ml^{-1} and 16 μg ml^{-1}, respectively. The results in Table 4.4 indicated distinct structure-activity relationships. Only those lactones (**231-236**) bearing the alkylating α,β-unsaturated-γ-lactone moiety were active against *M. tuberculosis* and/or *M. avium* at the concentration lower than 128 μg ml^{-1}. In contrast the 11,13-dihydrosesquiterpene lactones **239**, **241**, **245** [195], **246** [195]) as well as the sesquiterpenes obtained by reductive opening the lactonic ring (**240**, **242**, **243**) showed no activity against *M. tuberculosis* and *M. avium* at concentrations below 128 μg ml^{-1}. However, the α,β-unsaturated-γ-lactone moiety appears to be essential but not exclusive requirement for the anti-mycobacterial activity of sesquiterpene lactones. The 1,10-, and 4,5-double bonds and/or their epoxides in the germacranolide skeleton seem to be also involved in the biological activities. For instance, costunolide (**231**) showed MICs against *M. tuberculosis* and *M. avium* at 32 μg ml^{-1} and 128 μg ml^{-1}, respectively. Its 4,5-epoxidderivative, parthenolide (**233**) was more active with MICs 16 μg ml^{-1} and 64 μg ml^{-1} against *M. tuberculosis* and *M. avium*, respectively. However, 1,10-epoxycostunolide (**234**) was less active with MICs 64 μg ml^{-1} against *M. tuberculosis* and greater than 128 μg ml^{-1} against *M. avium*. In contrast, the diepoxide michelenolide (**244**) [178, 196] was essentially inactive with MICs higher than 128 μg ml^{-1} against both *M. tuberculosis* and *M. avium*, in spite of the presence of the alkylating α-methylene-γ-lactone moiety. This suggest that in the germacranolide series the epoxides **233** and **234** are possibly first cyclized by the microorganisms to the guaianolide **249** and the eudesmanolides **235** and/or **236** which represent the actual toxin for the organism. Alternatively, enhanced activity of parthenolide (**233**) may be due to transannular cyclization to give the quaianolide-type intermediate **B** with a

Table 4.4. The Minimum Inhibitory Concentrations (μg ml^{-1}) of Sesdquiterpene Lactones against Pathogenic Mycobateria*

Compound	*M. tuberculosis* H37Rv	*M. avium*
231	32	128
232	2	16
233	16	64
234	64	>128
235	64	>128
236	64	>128
239	>128	>128
240	>128	>128
241	>128	>128
242	>128	>128
243	>128	>128
244	>128	>128
245	>128	>128
246	>128	>128

*Data obtained from GWL Hansen's Disease Laboratory, U.S. Department of Health and Human Services.

carbocationic center at C-10, which could in addition to the α-methylene-γ-lactone, acts as a center for selective alkylation of nucleophilic group (e.g. sulphydryl or amino group) at the active center of an enzyme (Scheme 4.4).

Experimental

General. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker AM 400 spectrometer. IR spectra were obtained on a Perkin-Elmer 1760X FT-IR spectrometer as a film on KBr plates. Mass spectra were recorded on a Hewlett-Packard 5971A GC-mass spectrometer. Vacuum liquid chromatographic separations were carried out on TLC grade silica gel (MN Kieselgel G).

Costunolide (**231**) and dehydrocostuslactone (**232**) were obtained from costus resin (*Saussurea Lappa* Clark) obtained commercially as Costus Resinoid (Pierre Chauvet, S. A., France). About 11 g of costus oil was chromatographed by VLC using hexane followed by mixtures of hexane and CH_2Cl_2 as solvent by increasing polarity, providing 18x200 ml fractions. The less polar fractions contained mainly dehydrocostuslactone (**232**) which upon recrystallization from hexane gave colorless crystals. More polar fractions provided, after recrystallization from hexane, costunolide (**231**) as colorless crystals.

Costunolide (**231**). $C_{15}H_{20}O_2$, colorless crystal; IR ν_{max}^{KBr} cm^{-1}: 1762 (γ-lactone), 1664 and 1438 (C=C), 1290, 1246 and 1140 (CC(=O)OC), 996, 944; EIMS m/z (rel. int.): 232 $[M]^+$ (15.3), 217 $[M\text{-}Me]^+$ (22.1), 123 (44.1), 109 (61.2), 81 (100), 53 (47.9); ^{1}H NMR data in Table 4.1.

Dehydrocostuslactone (**232**). $C_{15}H_{18}O_2$, colorless crystal; IR ν_{max}^{KBr} cm^{-1}: 1767 (γ-lactone), 1641 and 1438 (C=C), 1259 and 1146 (CC(=O)OC), 1000, 892, 814; EIMS m/z (rel. int.): 230 $[M]^+$ (47.9), 215 $[M\text{-}Me]^+$ (17.8), 201 (31.7), 172 (22.1), 150 (93.2), 117 (52.5), 105 (53.1), 91 (100), 79 (74.2), 53 (53.8); ^{1}H NMR data in Table 4.1.

Epoxidation and cyclization of costunolide (**231**). 450 mg of costunolide (1.94 m mol) was dissolved in $CHCl_3$ (20 ml). 220 mg of NaOAc and 446 mg of *m*-chloroperbenzoic acid (2.58 m mol) were added. The resulting solution was stirred for 1 hr. and then extracted with 10% Na_2CO_3 (4x50 ml), washed with water (4x50 ml), dried over anhydrous Na_2SO_4. After removal of $CHCl_3$, 584 mg of oily crude was obtained. VLC separation of the crude using hexane-EtOAc as solvent by increasing polarity provided 4 mg of parthenolide (**233**), 2 mg of **234**, 189 mg of santamarin (**235**), 88 mg of reynosin (**236**), 2 mg of **238**, 78 mg of a mixture of santamarin and magnolialide (**237**) and 50 mg of unreacted starting material costunolide (**231**).

Parthenolide (**233**). $C_{15}H_{20}O_3$, powder; IR ν_{max}^{KBr} cm^{-1}: 1766 (γ-lactone), 1654 and 1458 (C=C), 1289 and 1141 (CC(=O)OC), 986; EIMS m/z (rel. int.): 248 $[M]^+$ (0.3), 233 $[M\text{-}Me]^+$ (2.3), 230 $[M\text{-}H_2O]^+$ (1.8), 215 $[230\text{-}Me]^+$ (3.8), 190 (35.6), 175 (20.2), 163 (27.8), 145 (29.8), 123 (27.2), 95 (62.9), 81 (68.0), 67 (38.5), 53 (61.4), 43 (100); ^1H NMR data in Table 4.1.

Santamarin (**235**). $C_{15}H_{20}O_3$, powder; IR ν_{max}^{KBr} cm^{-1}: 3475 (OH), 1768 (γ-lactone), 1674 and 1438 (C=C), 1264 and 1136 (CC(=O)OC), 978; EIMS m/z (rel. int.): 248 $[M]^+$ (96.2), 230 $[M\text{-}H_2O]^+$ (14.6), 215 $[230\text{-}Me]^+$ (12.1), 191 (12.5), 175 (19.1), 163 (27.8), 152 (76.8), 133 (29.0), 119 (31.3), 107 (100), 91 (55.7), 81 (43.2), 53 (46.8); ^1H NMR data in Table 4.1.

Reynosin (**236**). $C_{15}H_{20}O_3$, powder; IR ν_{max}^{KBr} cm^{-1}: 3453 (OH), 1763 (γ-lactone), 1652 and 1457 (C=C), 1257 and 1133 (CC(=O)OC), 996, 943: EIMS m/z (rel. int.): 248 $[M]^+$ (3.6), 230 $[M\text{-}H_2O]^+$ (100), 215 $[230\text{-}Me]^+$ (14.7), 201 (10.4), 187 (10.5), 175 (11.0), 163 (76.4), 149 (23.4), 133 (21.2), 119 (24.6), 105 (31.0), 91 (40.1), 79 (31.6), 67 (20.3), 53 (31.4), 41 (28.2); ^1H NMR data in Table 4.1.

Magnolialide (**237**). $C_{15}H_{20}O_3$, powder; EIMS (rel. int.): 248 $[M]^+$ (31.2), 230 $[M\text{-}H_2O]^+$ (82.3), 215 $[230\text{-}Me]^+$ (29.8), 204 (100), 191 (51.1), 163 (67.1), 145

(35.8), 119 (32.8), 105 (49.1), 91 (61.1), 79 (43.0), 67 (32.8), 53 (50.8), 41 (48.1); ^{1}H NMR data in Table 4.1.

1,4-Epoxy-11(13)-eudesmen-12,6-olide (**238**). $C_{15}H_{20}O_3$, colorless gum; IR ν_{max}^{KBr} cm^{-1}: 1772 (γ-lactone), 1464 (C=C), 1251, 1127 (CC(=O)OC), 1011, 968; EIMS (rel. int.): 248 [M]$^+$ (6.8), 233 [M-Me]$^+$ (24.7), 220 [M-CO]$^+$ (16.1), 215 (15.9), 204 (27.0), 190 (65), 175 (32.3), 163 (79.9), 145 (53.5), 135 (32.6), 123 (33.6), 119 (33.5), 107, 105 (45.6), 95 (53.6), 93, 91 (69.0), 79 (53.9), 67 (37.7), 53 (68.1), 43 (100); ^{1}H NMR data in Table 4.2 and ^{13}C NMR data in Table 4.3.

Reduction of santamarin (**236**). Santamarin (103 mg, 0.41 m mol) was dissolved in 25 ml CH_3OH, treated with $NaBH_4$ (250 mg, 6.6 m mol) at 0°C, stirred for 5 hr. The resulting solution was then acidified with 2N HCl to PH=1, diluted with water, extracted with CH_2Cl_2 (4x20 ml). After removal of solvent, about 87 mg of crude was obtained in a white powder form. VLC separation of the crude using hexane or mixtures of hexane and EtOAc as solvent by increasing polarity afforded 47 mg of dihydrosantamarine (**239**), 7 mg of the triol **240** and 20 mg of a mixture of **239** and **236**.

Dihydrosantamarin (**239**). $C_{15}H_{22}O_3$, powder; EIMS (rel. int.): 250 [M]$^+$ (100), 235 [M-Me]$^+$ (3.8), 232 [M-H_2O]$^+$ (8.5), 222 [M-CO]$^+$ (9.3), 217 [232-Me]$^+$ (6.2), 193 (22.5), 177 (16.0), 165, 159 (22.7), 137 (34.6), 123, 121 (26.7), 119, 107 (48.9), 97, 95, 93, 91, 81 (96.5), 55 (52.6), 43, 41 (48.5); ^{1}H NMR data in Table 4.2.

1β,6α,12-Trihydroxy-3,11(13)-eudesmadiene (**240**). $C_{15}H_{24}O_3$, colorless gum; IR ν_{max}^{KBr} cm^{-1}: 3361 (OH), 1652, 1440 (C=C), 1093, 1035; EIMS (rel. int.): 252 [M]$^+$ (5.2), 234 [M-H_2O]+ (9.0), 216 [234-H_2O]$^+$ (9.9), 201 [216-Me]$^+$ (13.1), 183 [201-H_2O]$^+$ (12.1), 173 (11.8), 159 (16.1), 145 (24.7), 135 (28.3), 121 (52.3), 107 (100), 97 (96.6), 95, 93, 91 (52.7), 81, 79 (50.2), 77, 69, 67 (37.4), 55 (52.8), 43 (55.5), 41 (61.1); ^{1}H NMR data in Table 4.2 and ^{13}C NMR data in Table 4.3.

Reduction of reynosin (**237**). Using similar condition as above, reynosin (75.5 mg, 0.30 m mol) was dissolved in 10 ml CH_3OH, treated with $NaBH_4$ (240 mg, 6.3 m mol) at 0°C, stirred for 2 hr. The reaction solution was neutralized with 2N HCl and diluted with water. The resulting mixture was extracted with CH_2Cl_2 (4x20 ml) and evaporated in vacuum providing 70 mg of crude . VLC separation of the crude using hexane-EtOAc as solvent by increasing polarity afforded 46 mg of dihydroreynosin (**241**), 12 mg of triol **242** and 9 mg of **243**.

Dihydroreynosin (**241**). $C_{15}H_{22}O_3$, powder; EIMS (rel. int.): 250 $[M]^+$ (3.3), 232 $[M-H_2O]^+$ (100), 217 $[232-Me]^+$ (8.7), 206 (7.6), 191 (12.6), 177 (13.1), 165 (86.4), 159 (44.3), 147 (25.5), 133 (36.5), 121 (46.0), 107 (40.3), 105 (36.4), 91 (38.4), 81 (31.8), 79 (31.2), 67 (22.2), 55 (43.3), 43 (22.2), 41 (28.6); ^1H NMR data in Table 4.2.

1β,6α,12-Trihydroxy-4(15), 11(13)-eudesmadiene (**242**). $C_{15}H_{24}O_3$, colorless crystal; IR ν_{max}^{KBr} cm^{-1}: 3364 (OH), 1653, 1457 (C=C), 1007, 867; EIMS (rel. int.): 252 $[M]^+$ (3.4), 234 $[M-H_2O]^+$ (23.2), 219 $[234-Me]^+$ (6.1), 216 $[234-H_2O]^+$ (10.4), 201 $[216-Me]^+$ (18.7), 187, 185 (30.9), 173 (18.2), 159 (27.3), 145 (39.8), 135 (35.1), 133, 131, 121 (63.6), 109, 107 (100), 105, 95, 93 (72.6), 91 (70.5), 81, 79 (83.0), 67 (52.3), 55 (63.6), 43 (55.6), 41 (62.8); ^1H NMR data in Table 4.2 and ^{13}C NMR data in Table 4.3.

1β,6α,12-Trihydroxy-4(15)-eudesmene (**243**). $C_{15}H_{26}O_3$, colorless gum; IR ν_{max}^{KBr} cm^{-1}: 3381 (OH), 1655, 1455 (C=C), 1005, 731; EIMS (rel. int.): 254 $[M]^+$ (0.2), 236 $[M-H_2O]^+$ (10.2), 221 $[236-Me]^+$ (5.0), 218 $[236-H_2O]^+$ (14.3), 203 $[218-Me]^+$ (16.1), 187 (26.9), 177 (14.3), 159 (58.1), 145 (28.1), 134 (80.0), 121 (100), 109, 107 (83.9), 93 (56.2), 91, 81 (97.9), 69 (33.3), 67, 55 (49.0), 43 (40.1), 41 (39.1); ^1H NMR data in Table 4.2 and ^{13}C NMR data in Table 4.3.

Acid-catalyzed transformation of dihydroparthenolide (**246**). About 52 mg (0.208 moles) of dihydroparthenolide (**246**) was dissolved in 10 ml MeOH and *ca.* 120

mg of *p*-toluenesulfonic acid (TSA) was added. The mixture was stirred at room temperature for 6 hrs and checked regularly by TLC. After removal of solvent in *vacuo*, a purple crude oil (150 mg) was obtained. VLC separation of the crude using hexane or mixtures of hexane and EtOAc as solvent by increasing polarity provided 10 mg of compressanolide (**247**) and 25 mg of **248**.

Compressanolide (**247**). $C_{15}H_{22}O_3$, yellow oil; EIMS (rel. int.): 250 $[M]^+$ (3.6), 232 $[M-H_2O]^+$ (16.9), 217 $[232-Me]^+$ (4.5), 192 (10.5), 159 (15.5), 152 (19.6), 119 (41.9), 107 (24.0), 91 (31.0), 71 (43.1), 55 (36.6), 43 (100); ^1H NMR data in Table 4.2.

4α-Hydroxy-10α-methoxyguaian-12,6-olide (**248**). $C_{16}H_{26}O_4$, yellow oil; IR ν_{max}^{KBr} cm^{-1}: 3467 (OH), 1769 (C=O), 1458, 1381, 1131, 1072, 994; EIMS (rel. int.): 282 $[M]^+$ (1.0), 264 $[M-H_2O]^+$ (5.8), 250 $[M-MeOH]^+$ (3.6), 232 $[264-MeOH]^+$ (9.1), 217 $[232-Me]^+$ (5.3), 192 (11.2), 177 (6.9), 85 (100), 72 (22.4), 55 (38.4), 43 (81.2); ^1H NMR data in Table 4.2 and ^{13}C NMR data in Table 4.3.

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VITA

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DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Tiansheng Lu

Major Field: Chemistry

Title of Dissertation: Isolation, Structural Elucidation and Structure-Activity Studies of Natural Products from Regional Plants of the Asteraceae

Approved:

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Date of Examination:

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