CHEMICAL CONSTITUENTS OF PLANTS NATIVE TO VENDA

By

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PIETERMARITZBURG

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To my son Ntshuxeko who was born in my absence during the course of this project.

DECLARATION

I hereby certify that this research is a result of my own investigation which has not already been accepted in substance for any degree and is not being submitted in candidature for any other degree.

Signed:.....

M.J. MASHIMBYE

I hereby certify that this statement is correct.

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ABBREVIATIONS

^{13}C NMR - carbon nuclear magnetic resonance

COSY - correlated spectroscopy

DABCO - 1,4-diazabicyclo[2.2.2]octane

DEPT - distortionless enhancement over polarization transfer

d - doublet

dd - doublet of doublet

DIBAH - diisobutylaluminium hydride

DMSO - dimethyl sulphoxide

eV - electron volt

GC - Gas chromatography

^{1}H NMR - proton nuclear magnetic resonance

HETCOR - heteronuclear correlation

Hz - hertz

IR - infrared

MHz - mega hertz

MS - mass spectrum

NOE - Nuclear Overhauser effects

PCC - pyridinium chlorochromate

ppm - parts per million

QDL - 3-quinuclidinol

q - quartet

R_f - retention value

Red-al - Sodium bis(2-methoxyethoxy)aluminium hydride

s - singlet

t - triplet

THF - tetrahydrofuran

tlc - thin layer chromatography

TMS - tetramethylsilane

ABSTRACT

An investigation into the chemical constituents of plants indigenous to Venda forms the main thrust of this thesis. From the root bark of *Cassine transvaalensis* **(Burtt Davy) Codd** (CELASTRACEAE) (+)-11,11-dimethyl-1,3,8,10-tetrahydroxy-9-methoxy peltogynan, pristimerin, (-)6-hydroxy-20(29)-lupen-3-one, 3-oxofriedelan-28-al, 3-oxofriedelan-28-ol and galactitol were isolated whilst chemical investigations of the stem bark of *C. papillosa* **(Hoechst.) Kuntze** (CELASTRACEAE) indicated the presence of a peltogynoid (+)6R,13R-11,11-dimethyl-1,3,8,10-tetrahydroxy-9-methoxy peltogynan and (-)4'-O-methoxy epigallocatechin. They were accompanied by ouratea proanthocyanidin, galactitol, tingenone, tingenin B, and three pentacyclic triterpenes, 3-oxofriedelan-28-ol, 30-hydroxylupeol and 30-hydroxylup-20(29)-en-3-one. The absolute configuration of the peltogynan was established as 6R,13R. Evidence is presented for the structure of the lower unit in ouratea proanthocyanidin using NOE experiments. The 4,8-linkage of ouratea proanthocyanidin is confirmed by spectroscopic evidence of the acetate derivative. The biogenetic relationship of (-)4'-O-methoxy epigallocatechin and 6R,13R-11,11-dimethyl-1,3,8,10-tetrahydroxy-9-methoxy peltogynan is also established. Assignments of the ^{1}H NMR and ^{13}C NMR of (-)4'-O-methoxyepigallocatechin were also undertaken and revealed previous incorrect assignments of H-6 and H-8.

Chemical investigation of the hot-tasting bark of *Warburgia*

salutaris **(Bertol. f.) Chiov.** led to the isolation of two compounds, (-)-warburganal and polygodial, with antifeedant and antifungal activities. Iditol was also found to be the constituent of the plant. The anti-ulcer activities of warburganal were established and preliminary results obtained on its anti-bilharzia activity.

Synthetic work aimed at the preparation of warburganal analogues was initiated.

Rapanone, an anthelmintic agent, was isolated from *Rapanea melanophloeos* **(L.) Mez** together with betulinic acid. Chemical investigation of *Spirostachys africana* **Sonder** led to the isolation of lupeol and 3α-acetyl-taraxer-14-en-28-ß-oic acid.

Conclusions are drawn regarding the taxanomic significance of the isolated compounds.

PUBLICATIONS

1. "Anti-ulcer agent, use and pharmaceutical compositions thereof."
   Fourie T.G., Mashimbye M.J. and Snyckers F.O.(1991), *South African Patent Application Number* 91/9839.

2. "11,11-Dimethyl-1,3,8,10-tetrahydroxy-9-methoxypeltogynan and three pentacyclic triterpenes from *Cassine transvaalensis* **(Burtt Davy) Codd**(CELASTRACEAE)".
   Drewes, S.E., Mashimbye, M.J., Field, J. and Ramesar, N.(1991), *Phytochemistry*, 30(10), 3490.

3. "Flavonoids and triterpenes from *Cassine papillosa* **(Hoechst.) Kuntze** (CELASTRACEAE) and the absolute configuration of 11,11-dimethyl-1,3,8,10-tetrahydroxy-9-methoxypeltogynan".
   Drewes, S.E. and Mashimbye, M.J.(1993), *Phytochemistry*, 32(4), 1041.

4. "Observations of the molluscicidal properties of (-)-warburganal on South African *Bulinus africanus*(PLANORBIDAE)".
   Drewes S.E., Mashimbye M.J., Appleton C.C. and Cunningham A.B. *J. Med. Appl. Malac.* accepted for publication(1993).

CHAPTER 1

INTRODUCTION

Many researchers throughout the world are actively involved in research on traditional medicine in search of new biologically active compounds[1]. Our choice of Venda plants arose because of the availability of the documented material and as part of our continuing interest in the medicinal plants of Southern Africa[2,3,4]. Plants investigated under this project include *Cassine transvaalensis*, *C. papillosa* (CELASTRACEAE), *Warburgia salutaris* (CANELACEAE), *Rapanea melanophloeos* (MYRSINACEAE), and *Spirostachys africana* (EUPHORBIACEAE).

Cassine transvaalensis **(Burtt Davy) Codd** is a small bushy tree which grows along the eastern seaboard of the province of Natal and extends northwards through Venda to Zimbabwe. Two historic trees near Bulawayo, Mzilikazi's tree and Baden-Powell's tree, are both specimens of *C. transvaalensis*. The Venda and Zulu people drink large quantities of the extract of the bark as a general stomach conditioner. It is also used as an enema to relieve stomach aches and fevers. The bark, which has a faint aromatic scent, contains 13.34% tannin which could explain its medicinal use. The fruit is edible[5].

Cassine papillosa **(Hoechst.) Kuntze** is a small to medium-sized tree, 7-15 m high, occasionally taller and attaining 20 m.It grows throughout the coastal forest belt, found especially in the medium-moist and moist forest types, from the south-western and southern Cape Province, through to Cape Midlands, the eastern Cape Province, the Transkei and Natal, to north-eastern Transvaal and Venda. The tree is reputed among the Zulus to have powerful magic qualities. They believe it has the ability to blunt evil influences, and that it is a species liked both by girls and by ancestor spirits. The wood is white to yellowish, hard, and generally useful, especially in wagon-making. The bark is used for tanning[5,6]. A comparative study between this plant and that of

C. transvaalensis was essential. Moreover, quinonoid triterpenes with antitumour activities were previously isolated from plants belonging to the same family[7].

Warburgia salutaris **(Bertol. f.) Chiov.**, Pepper-bark tree (English), Peperbasboom (Afrikaans), Mulanga (Venda), Xibaha (Tsonga) is a slender tree usually 5 to 10 m in height, but reaching 20 m in some areas; occurring in evergreen forests and wooded ravines. In Southern Africa the plant is found in North-Eastern Natal, Swaziland, South-Eastern and Northern Transvaal. This tree has been used medicinally from early times; the specific name means 'salutary' or 'health giving'[8]. Among the Vendas and Tsongas the bark is used for cold, chest complaints, stomach ulcer, purgative, malaria, sore throat, skin sores, backache as well as an aphrodisiac[2,3]. Because of overusage of the plant by the traditional healers the species is becoming rare in the wild [8,9]. In Venda two trees are known to be in existence[10]. The Institute of Natural Resources (University of Natal, Pietermarizburg) is presently engaged in the programme of avoiding this predicament by encouraging herbalists, local institutions such as Silverglen Nursery, Durban and private individuals to cultivate endangered species in various parts of the country.

Spirostachys africana **Sonder** is a medium sized tree with a rounded crown, usually about 10 m in height, but occasionally reaching 15 m; occurring in low altitude bush, often along rivers and streams. It is a tree of widespread occurrence in Southern Africa.

The copius milky latex is very poisonous and causes extreme irritation to sensitive skin and severe pain and even damage to the eyes. Africans use it as a fish poison and to tip arrow heads. The wood is hard, heavy, durable, close-grained and not touched by termites or borers; the sapwood is creamy-white and the heartwood rich brown with a satin-like lustre. This wood is not suitable for fuel; it burns with a strong, sweetish odour,

initially pleasant and aromatic, but later becoming sickening and causing headache and nausea, while the smoke itself is said to taint food. It does not make satisfactory ox-yokes either as it causes burn-like sores on the animal's neck and its effects on insects are so unpleasant to them that pieces of wood are placed among clothing as a repellant. Splendid furniture has been made from the timber though large pieces are difficult to come by as the trunks are often hollow[11].

Rapanea melanophloeos **(L.) Mez** is a medium to tall tree usually 4 to 10 m in height, but sometimes reaching 20 m; occurring in evergreen forest, in the riverine fringes and sometimes in the drier coastal and mountain forests.

The Zulus use the decoction of the bark as an expectorant and an emetic. In Venda the bark is chewed or stamped and soaked or powdered for sore throats as well as for wounds. The tree is hardy and attractive in the garden, but so easily cultivated that, given ideal conditions, it is apt to take over and become difficult to eradicate[12].

1.1 Review of the family CELASTRACEAE.

The family CELASTRACEAE consists of about 60 to 70 genera and 850 species, composed of trees, shrubs and climbers, which are sometimes spiny. Members all have simple leaves and bisexual or unisexual flowers, the calyx with 3-5 segments, the petals and stamens usually 3-5, a fleshy disc present, and the ovary superior and 1-5-chambered with usually 2 ovules to each chamber. The fruit is a capsule, berry or drupe. In South Africa it is represented by 12 genera- *Maytenus*, *Putterlickia*, *Catha*, *Pterocelastrus*, *Cassine*, *Allocassine*, *Maurocenia*, *Hartogia*, *Pleurostylia*, *Salacia*, *Hippocratea*, and *Pseudosalacia*[6].

The genera *Cassine* occur in Africa, Madagascar, Australia and tropical America. It has 40 species in Africa and about 16 in South Africa mainly in the eastern area from Transvaal to Natal

and to the south-western coastal districts of the Cape. All the species of *Cassine* are trees or shrubs with leathery leaves, opposite or alternate, flowers in cymes or panicles, bisexual, or male or female, sometimes borne on separate trees, the sepals and petals 4-5, the stamens 5, the ovary usually 3-5 celled, and the fruit a hard or fleshy drupe which does not burst open. The seeds have no aril. The generic name *Cassine* is based on a Florida Indian name[6].

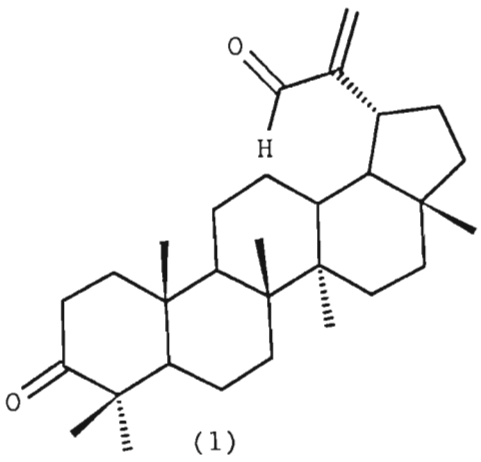
The number of the genera, however, differs according to the views of different botanists. Dr. N.K.B. Robson- who dealt with the family in *Flora Zambesiaca* - splits the genus *Cassine* into several different genera, such as *Mystroxylon*, *Elaedendron*, and *Crocoxylon*, while one of his new genera, *Lydenburgia*, included in *Catha* by Botanical Research Institute (Pretoria). Dr. N.K.B. Robson's view is not at present upheld by the Botanical Research Institute in Pretoria which considers *Cassine* as a large variable genus including the genera *Elaeodendron*, *Lauridia*, *Crocoxylon* and *Pseudocassine*[6].

1.1.1 Isolated compounds.

Most species of the family contain triterpenes[13]. Structure elucidations of these triterpenes are based on their mass fragmentation patterns[14] coupled with their methyl group resonances and other spectroscopic evidence[15].

3-Oxo-lup-20(29)-en-30-al(1), a cytotoxic triterpene, was isolated from *Maytenus nemerosa*[16] and *Gymnosporia emarginata*[17]. Cytotoxicity of this triterpene was found to be based on the presence of the carbonyl group at C-3 and α,ß-unsaturated aldehyde at C-29[16]. This finding was consistent with observations in other structural series of compounds regarding the contribution of two electrophilic groups in a single molecule towards the expression of antineoplastic activity[18,19]. 30-Hydroxy-lup-20(29)-en-3-one(2) can be used as a source of the cytotoxic triterpenes because it can be converted into the

cytotoxic agent by CrO_3-pyridine or pyridinium chlorochromate oxidation[17].



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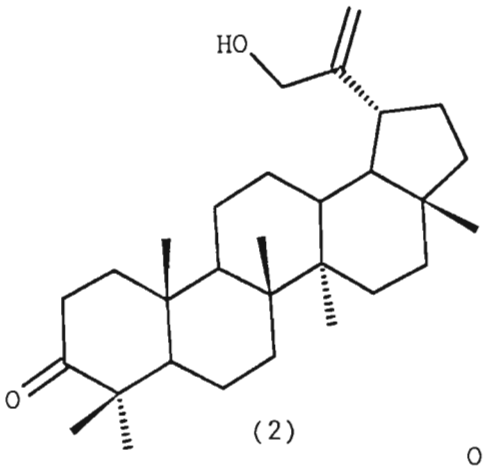

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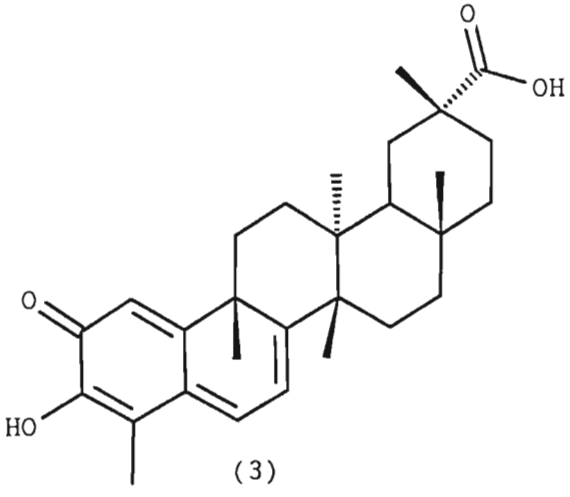

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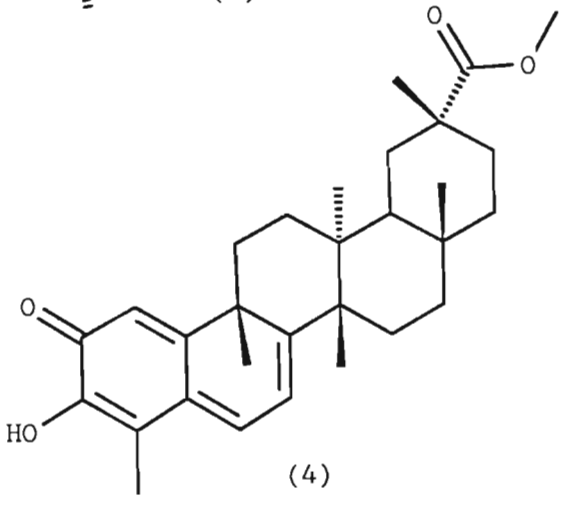

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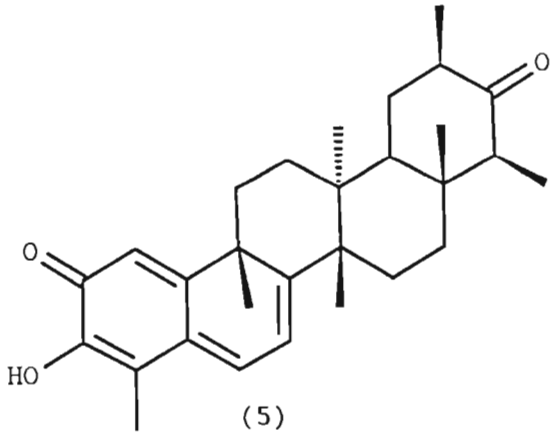

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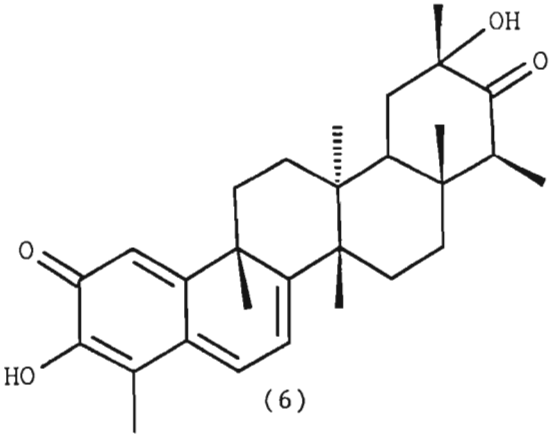

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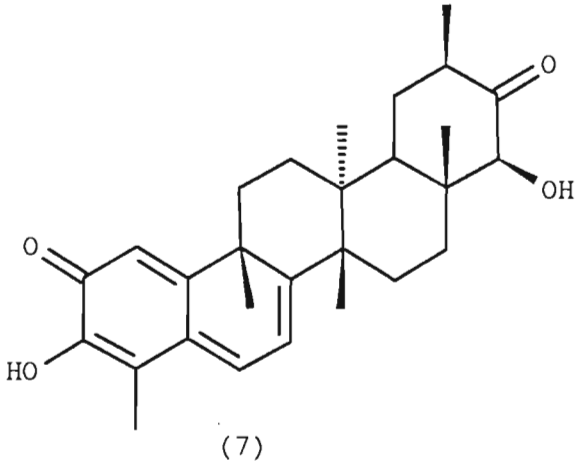
Quinonoid triterpenes have been identified so far from the family Celastraceae. These are celastrol(3), pristimerin(4), tingenone(5), 20-hydroxytingenone(6), 22-hydroxytingenone(7),

dispermoquinone(8), iquesterin(9) and a quinone-methide(10) from *Salacia macrosperma*[20]. Several of these compounds show antitumour activity, and tingenone(5) is used clinically in Brazil for the treatment of skin cancer[20]. Many plants belonging to the family Celastraceae, which are indigenous to Venda, have not been previously investigated. The present investigations include *Cassine transvaalensis* and *C. Papillosa* which belong to this family.



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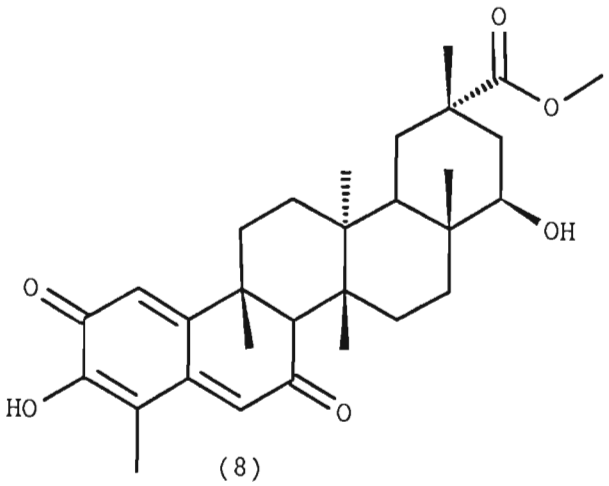

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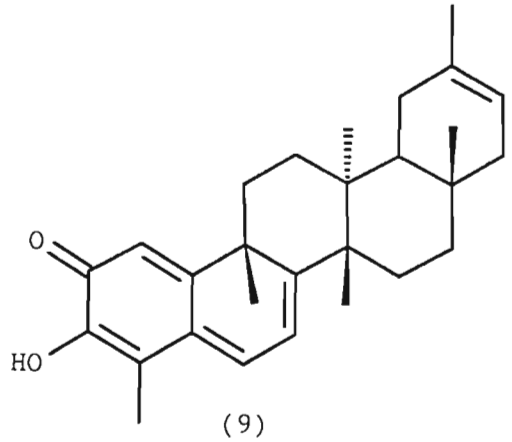

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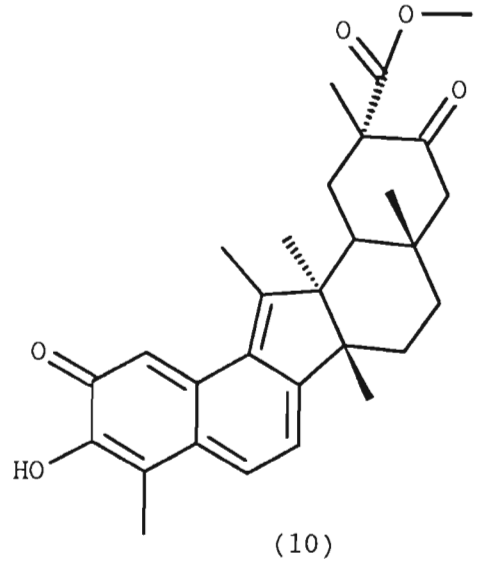

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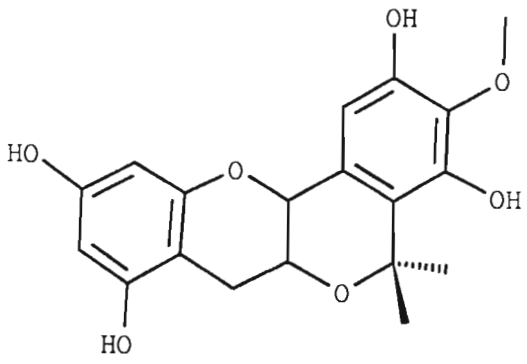
Quinonoid triterpenes were previously isolated from plants using hexane and dichloromethane as extracting solvents. The compounds were separated on a silica gel column using a combination of benzene and hexane as eluting solvents. The pure compounds were then analyzed using spectroscopic methods such as MS, ^{1}H NMR, ^{13}C NMR, UV and Infra Red[7,21].

Several genera of the Leguminosae and the Mimosoideae are known to be sources of peltogynoids[22,23,24,25,26]. However, only one example of a peltogynoid described as a leucoanthocyanidin has been isolated from the CELASTRACEAE. The compound, named elaeocyanidin(11), was isolated from the root bark of *Elaedendron balae*[27] and its absolute configuration was not established. 4′-O-Methyl-(-)-epigalocatechin(12) was isolated from the root bark of *E. balae* and seems to have a biogenetic relationship with Elaecyanidin(11).



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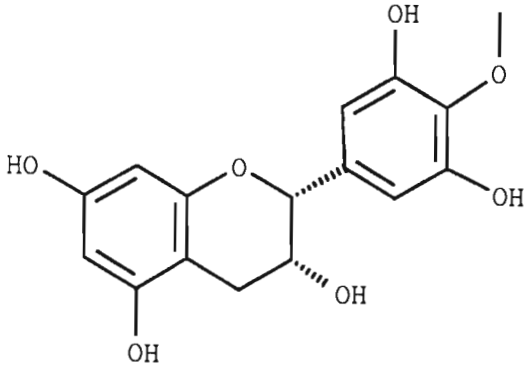

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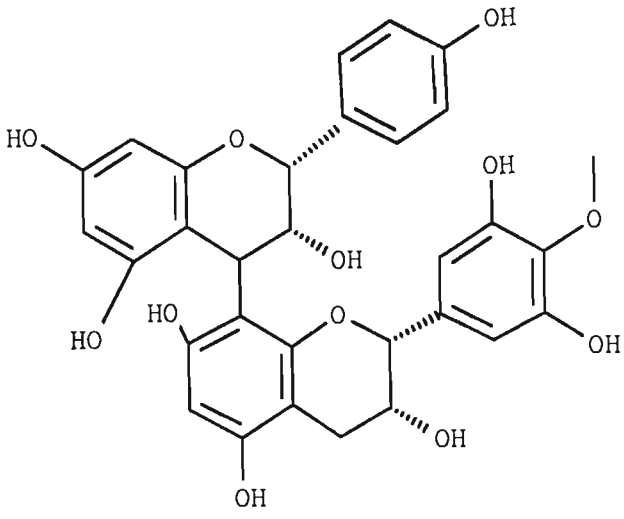
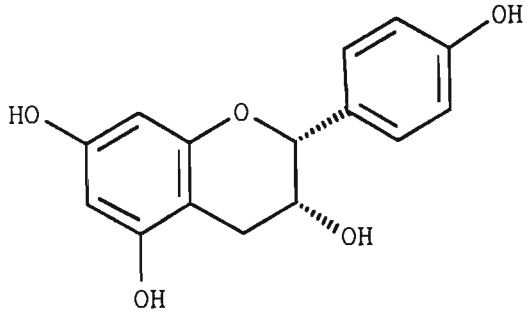
Proanthocyanidins, ouratea proanthocyanidin A(13) and ouratea proanthocyanidin B(14) were isolated from the root bark of *Prionostemma aspera* and *Maytenus rigida* together with 4′-O-methyl-(-)-epigallocatechin(12)[28]. These proanthocyanidins are formed by the linkage of one molecule of (-)-epiafzelechin(15) C_4-C_8 to one of (-)-4′-O-methylepigallocatechin(12). Their occurrence in the Celastraceae with (-)-4′-O-methylepigallocatechin(12) is of particular interest both on taxonomic and biogenetic grounds, since they are the only currently known methylated proanthocyanidins. Their presence could throw light on the mechanism of the formation of proanthocyanidins in plants.





(15)

(13) Nona-O-acetyl derivative mp = 134-135°C

(14) Nona-O-acetyl derivative mp = 171-174°C

Alkaloids were also found to be constituents of the family Celastraceae. The genera in which alkaloids were found include *Cassine*, *Catha*, *Celastratrus*, *Euonymus*, *Maytenus* and *Tripterygium*[13].

1.1.2 Phytochemical screening.

Phytochemical work done on the family Celastraceae is tabulated in the review by Wagner *et al.*[13]. Phytochemical screening of *C. papillosa* and *C. transvaalensis* indicated flavonoids and other phenolic compounds to be constituents of the two species[13,5,29].

1.2 Review of the family CANELLACEAE.

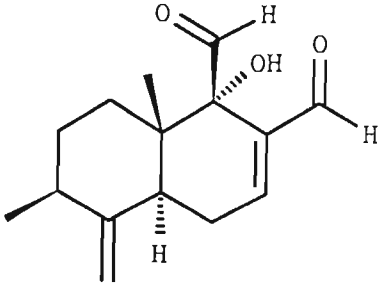
1.2.1 Species and geographical distribution.

Canellaceae is a small family of plants consisting of nine species, grouped into four genera[30]. Of these *Winterana* and *Cinnamodendron* are endemic to South America, *Warburgia* to Africa and *Cinnamosma* to Madagascar. The East African genus consists of

two species, *Warburgia ugandensis* **Spraque** And *Warburgia stuhlmannii*. In South Africa only one species is known, *W. salutaris* **(Bertol. f.) Chiov**[31].

1.2.2 Chemical constituents.

Previous chemical investigations of plants belonging to the Canellaceae revealed sesquiterpenoids to be constituents of these plants. From the genus *Winterana*, muzigadial(canellal)(16), 9-deoxymuzigadial(17), 9-deoxyisomuzigadial(18), 3ß-acetoxypolygodial(19) and 4,13-α-epoxymuzigadial(20) were isolated from *C. winterana*[32,33]. Chemical investigation of the African genus *Warburgia*, revealed warburganal(21), ugandensidial(22), muzigadial(16), warburgin(23), ugandensalide(24), and warburgiadione(25) to be constituents of *W. ugandensis* **Sprague**[34,35,36,37,38] whilst polygodial(26), cinnamolide(27) and bemadienolide(28) were found to be constituents of *W. stuhlmannii*[36]. Chemical investigation of the Madagascaran *Cinnamosma fragans* **Baillon** indicated cinnamolide(27), cinnamosmolide(29), and cinnamodial(22) to be constituents of the plant[39].



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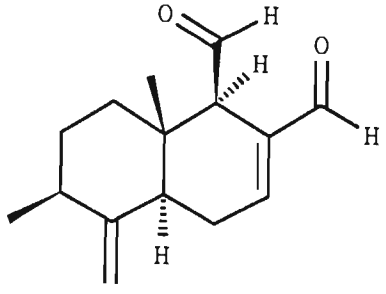

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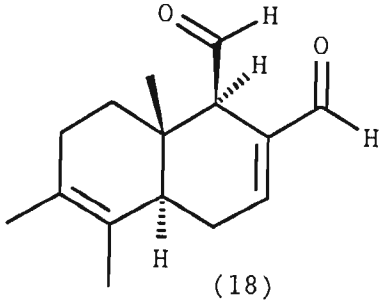

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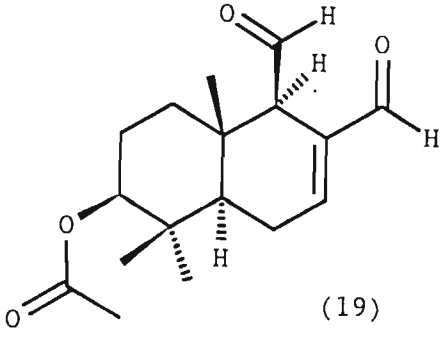

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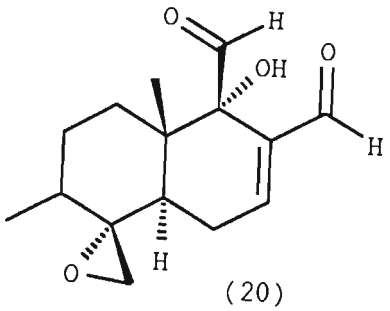

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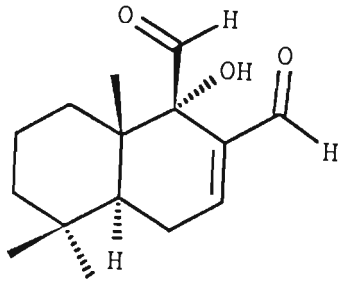

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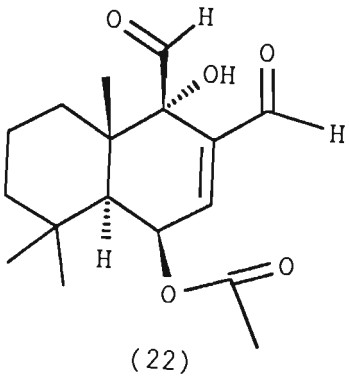

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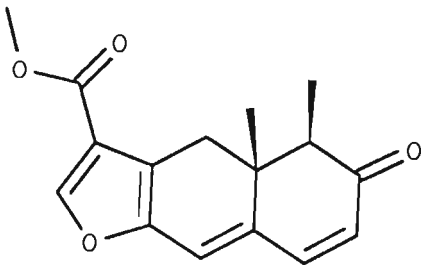

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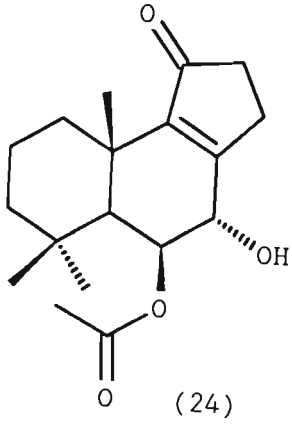

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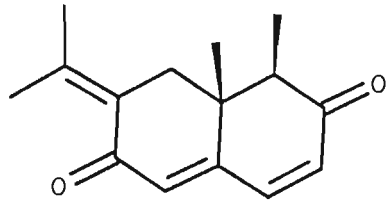

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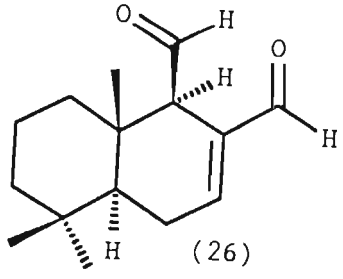

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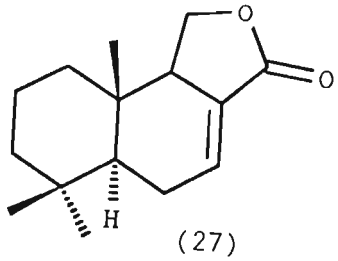

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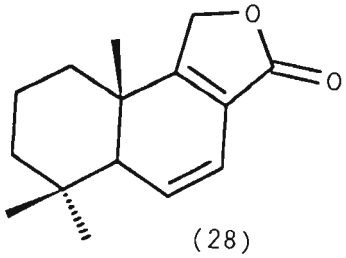

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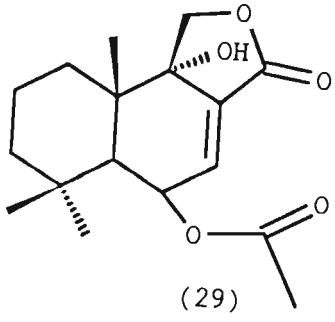

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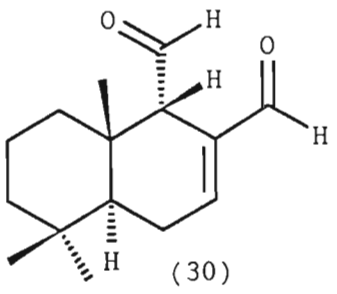

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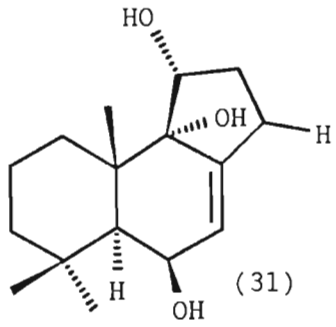

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1.2.3 Biological Activity of Drimane sesquiterpenoids.

Drimane sesquiterpenoids possess a wide range of biological activities including antibacterial, antifungal, anticomplemental, antifeedant, plant growth regulatory, cytotoxic, phytotoxic, piscicidal and mulluscicidal properties. The very hot taste of several biological drimanes, and their skin irritant properties have attracted much attention[30]. Jansen *et al.*[30] associated the mode of action of biological active drimanes to the ß-orientation of the aldehyde at C-9 (Scheme 1). The alcohol and acid derivatives at C-9 were also found to be inactive. From Scheme 1 it can be concluded that an enal and a 9ß-aldehyde group are required for the activity.

1.2.3.1 Antifungal and Antibacterial Activity.

Taniguchi *et al.*[40] found the genus *Warburgia* to have a broad range of biological activity. Fractionation and bioassaying of the extracts led to the isolation of the antimicrobial principles which were identified as the sesquiterpene dialdehydes polygodial(26), warburganal(21), muzigadial(16), and isotadeonal(30). The results of several bioassays are shown in Table 1[30]. Polygodial(26) proved to be the most potent antifungal compound tested[41]. It killed the cells of *Saccharomyces cerevisiae* within ten minutes when treated with a fungicidal concentration of 50 μg/ml.

Table 1.

Antimicrobial activity of drimanic dialdehydes

	*MIC(μg/ml)			
Microorganisms tested	(26)	(21)	(16)	(30)
Staphylococcus aurens	>100	>100	>100	>100
Escherichia coli	>100	>100	>100	>100
Pseudomonus aeruginosa	>100	>100	>100	>100
Saccharomyces cerevisiae	0.78	3.13	1.56	>100
Hansenula anomala	1.56	12.5	25	>100
Candida utilis	1.56	3.13	3.13	>100
Sclerotinia libertiana	1.56	3.13	3.13	>100
Mucor muceda	6.25	25	25	>100
Rhizopus chinensis	12.5	100	100	>100
Aspergillus niger	25	50	50	>100
Penicillium crustosum	25	50	50	>100
Trichophyton mentagrophytes		2	3	>100
Bacillus subtilis	>100	>100	>100	>100

*MIC = minimum inhibitory concentration.

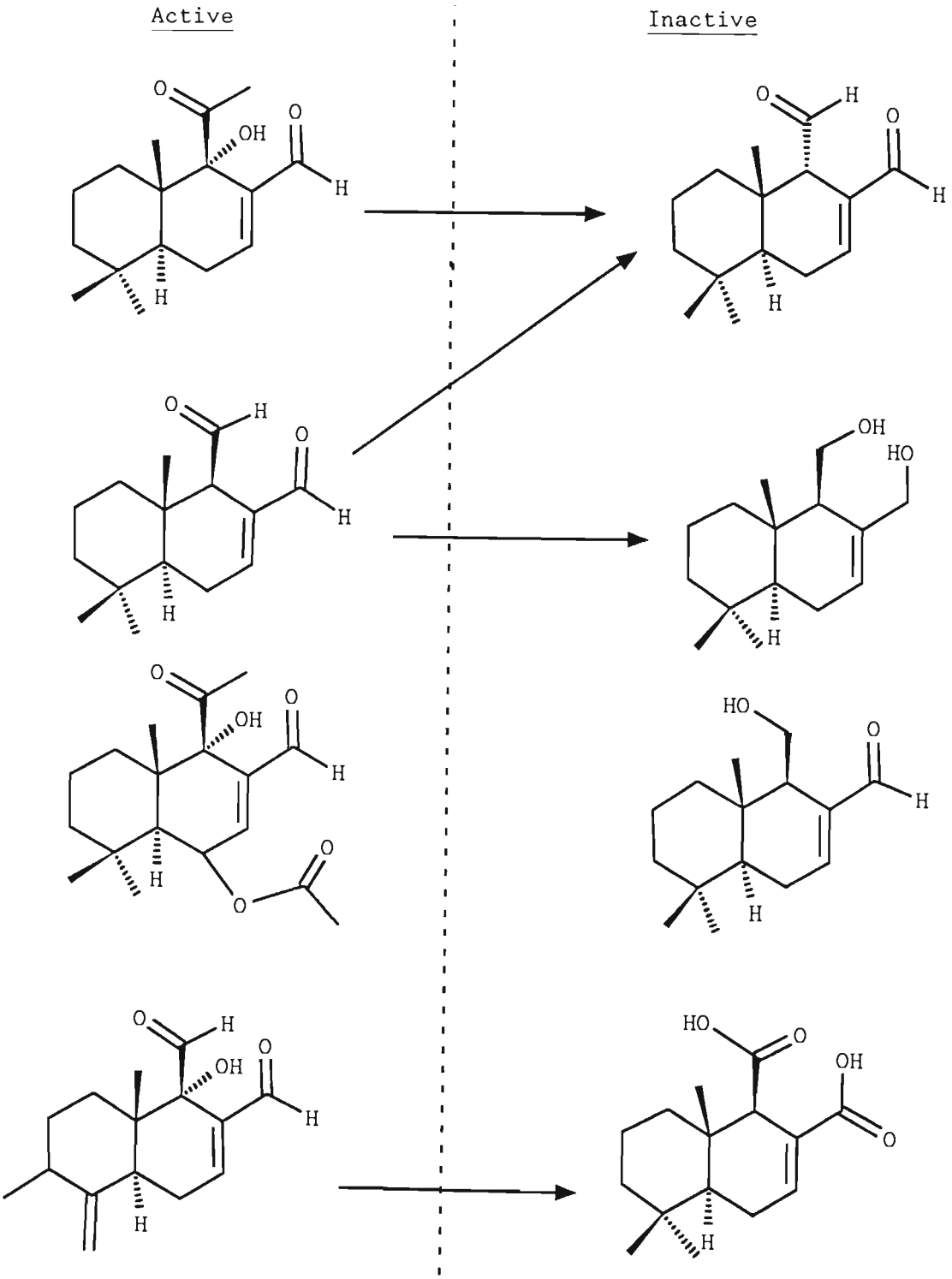
Periniporin A(31), a metabolite of *Perenniporia medullaepanis*, showed a remarkable effect on the growth of *Bacillus subtilis* but it was inactive against Gram-negative bacteria[42]. Cinnamolide(27) was active against *Trichophyton rubrum*, *T. menthagrophytes*, and *Microsporum gypseum*[43].

1.2.3.2 Plant Growth Regulatory Activity.

Polygodial(26) completely inhibited the germination of rice in husk at a concentration of ca. 100 ppm[44]. Rice seed (*Oryza sativa*) germination was also inhibited by cryptoporic acid A(32) which produces the characteristic bitterness of the fungus *Cryptoporous volvatus* at 200 ppm concentration. Polygonal(33) is also active

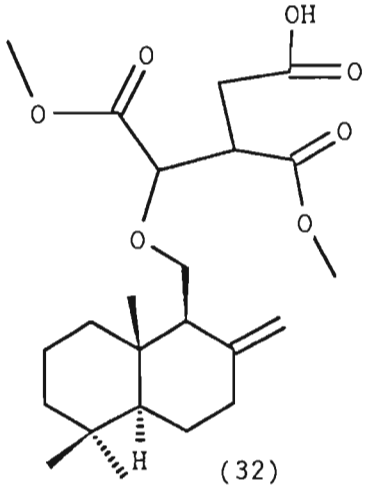
but at a much higher concentration[45].

Comparison of biologically active and inactive sesquiterpenoids.



SCHEME 1.

Root elongation of lettuce was completely inhibited by pereniporin A(31) at 100 ppm[42]. Altiloxin A(34) and B(35) also had little effect on the root elongation of lettuce[46]. The root production of asparagus on the other hand was diminished by 50% at a concentration of 10 ppm[46]. The germination of wheat seed (*Triticum aestirum* **var. Norman**, Graminaceae) was only slightly reduced by polygodial(26) and warburganal(21) at a concentration of 1%. A higher concentration improved the inhibition but the germinated seeds had twisted leaves instead of normal ones[47].



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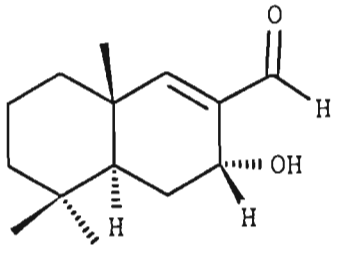

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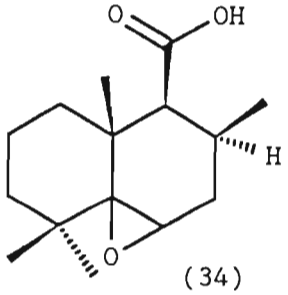

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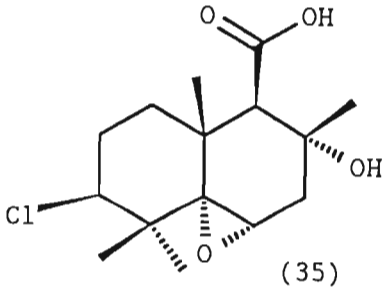

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1.2.3.3 Taste, Skin-irritant Properties and Anticomplemental Activity.

In East Africa the leaves of *Warburgia* species are sometimes used as spices[48,49]. In Japan the pungent hot tasting tade-juri is made from squeezed *Polygonum hydropier* **L.** leaves[50]. It turned out that

the drimanic aldehydes polygodial(26), warburganal(21), muzigadial(16), cinnamodial(22) and polygonal(33), a nordrimane, were responsible for this phenomenon[51,52]. The nordrimane is fairly weak in comparison with other compounds[44]. The bitterness of the fungus *Crytoporous volvatus* is caused by crytoporic acid A, an albicanyl-ether of iso-citric acid[45].

Polygodial(26) has been reported on several occasions to display skin irritant properties[53,54].

When guinea pigs were sensitized to polygodial(26) by using intradermal injections in Freund's[55] complete adjuvant they showed a high response when the skin was treated with polygodial(26), the primary sensitizer. Related compounds, eg. warburganal(21), having the same configuration also showed an allergic contact dermatitis. It was halved when the racemic mixture of warburganal(21) was used, so the allergic response was stereospecific to a particular enantiomer[56].

Several constituents of *P. hydropier* **L.** leaves and seeds were tested for their anticomplemental properties. Polygodial(26) and polygonic acid(36) gave positive tests, while warburganal(21) and muzigadial(16) showed no activity[53].

1.2.3.4 Piscicidal and Molluscicidal Activity.

Muzigadial(16) and warburganal(21) were tested as potential helicocides (snail killers) because the extract of the bark of *Warburgia ugandensis* had been known for some time to have molluscicidal activity. A simple snail test was chosen because it could give a lead to agents useful for controlling the dangerous schistosomes and bilharzia[54].

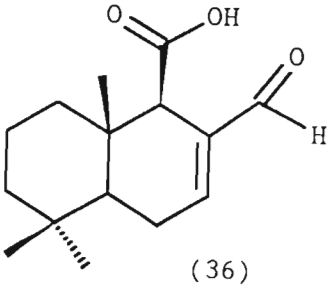
Treatment of killie fish, *Oryzia latipes*, with polygodial(26) at 0.4 ppm killed them within 30 minutes[47,57]. After an injection of 2 mg of polygodial(26) into the hepatopancreas of the nudibranch *Dendrodoris limbata*, suffering of the animal was

evident and death occurred between 3 and 16 hours[58].

1.2.3.5 Antifeedant Activity.

The insect antifeedant properties of the drimanes has been reviewed[59]. Polygodial(26) was used in laboratory and field tests to control slugs(*Deroceras reticulum*) and wheat bulb flies(*Delia coartata*) in winter wheat[60,61], where its effect was marginal on clay loam soil but obvious on peaty loam soil though still inferior to commercial pesticides. It showed no toxicity towards slugs.

Olepupuane(37) and polygodial(26) inhibited feeding of the Pacific damsel fish[62]. Polygodial(26) also inhibited feeding of the marine fish *Chromis chromis* and the fresh water fish *Carassius carassius*[63]. Drim-7-enyl glyceride(38) found in some British Columbia nudibranchs was active against tide pool sculpin *Oligocottus maculosus* at a level of 18 μg/mg of pellet[64]. Albicanyl acetate(39) and 6ß-acetoxyolepupuane(40) showed antifeedant properties in a standard goldfish(*Carassius auratus*) bioassay[63].



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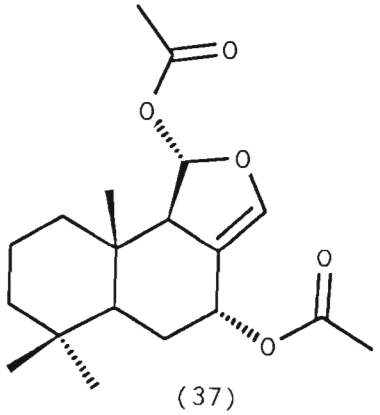

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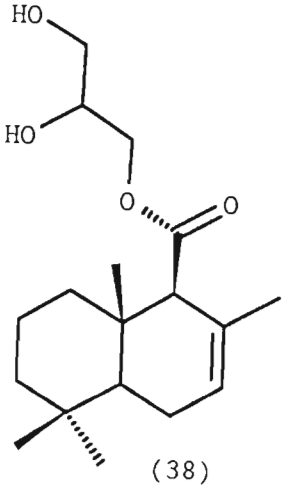

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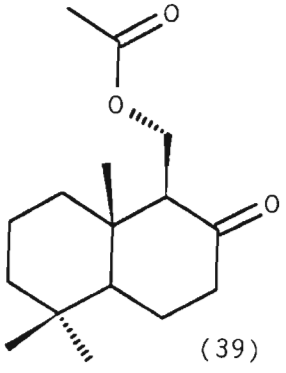

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Warburganal(21) and muzigadial(16) inhibited the feeding of larvae of two species of African armyworm, the polyphagous *Spodoptera exempta* and the polyphagous *S. littoralis* at a concentration of 0.1 ppm in a regular leaf disk method[65]. Polygodial(26) and ugandensidial(22) were also antifeedants for these insects but less reactive[66]. Polygodial(26) was also active against diamond moth larvae down to 0.1% and it inhibited intake by fifth-instar larvae of *Pieries brassicae* at the concentration of 200 ppm[67].

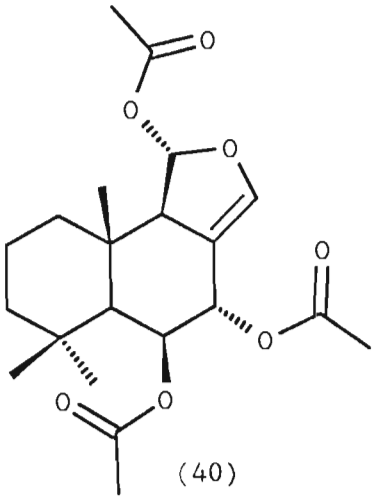
1.2.3.6 Mode of Action of Biologically-Active Drimanes.

Investigations revealed that the active antifeedants were all hot and spicy to the human tongue whereas all inactive derivatives are devoid of hot taste (Scheme 1).

From Scheme 1 it can be concluded that an enal and a 9ß-aldehyde group are required for activity. Mild treatment with base inverted the 9ß-aldehyde into the 9α-aldehyde group with concomitant loss of activity and hotness. The enhanced activity of the 9α-hydroxy compounds suggested an involvement of this functionality with the best fit of the molecule on the sensilla. Similar conditions were found by Sterner *et al.* in a structure-activity relationship study with regard to the mutagenicity of unsaturated dialdehydes[68].

Taniguchi *et al.*[41,69] studied the antifungal activity of polygodial(26). A variety of physiological effects due to polygodial(26), e.g. inhibition of growth, alcohol fermentation and papain activity appeared to result from its irreversible reaction with sulfhydryl groups. However, in a biomimetic reaction, the inactive isopolygodial(30) also had a high reactivity with the sulfhydryl group of L-cysteine(41).

Based on kinetic data, Sodano *et al.* proposed that the biological activity of the enal-aldehydes was primarily related to their ability to form adducts with amino groups rather than sulfhydryl



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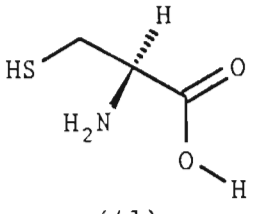
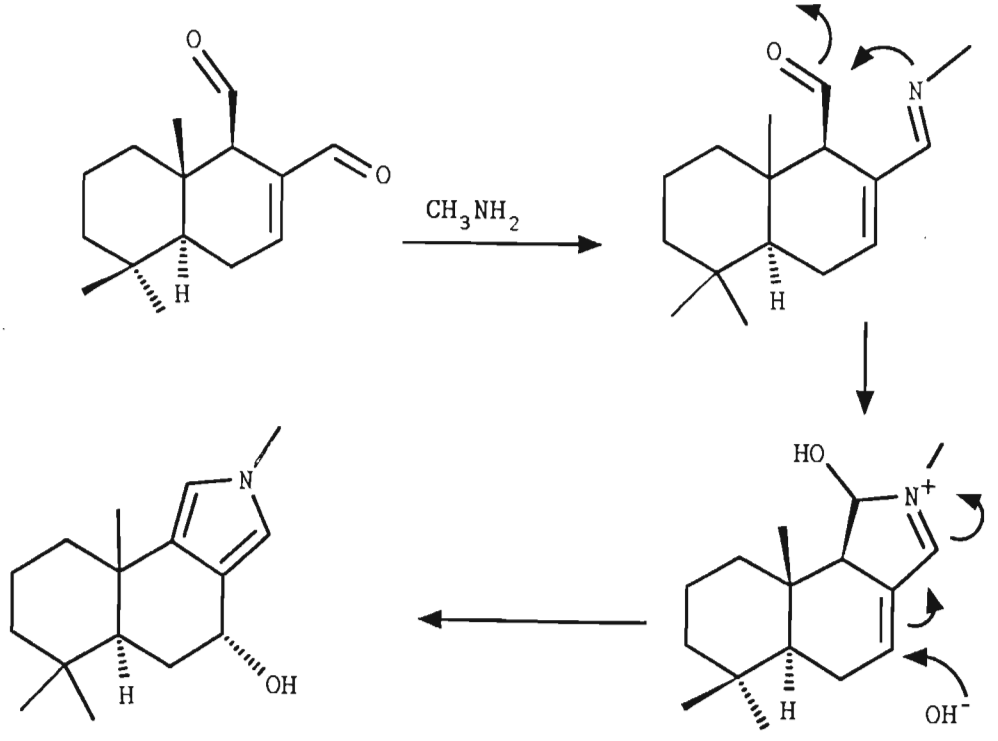


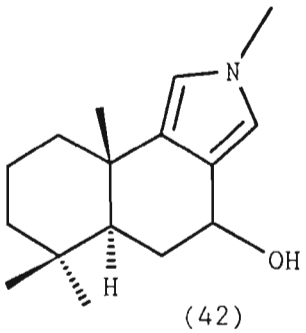
(41)

groups on the receptors[70]. Similar reactivity was observed for both polygodial(26) and isopolygodial(30) in a reaction with thiols, while the reaction with substrates possessing both amino and sulfhydryl groups was dependent upon the stereochemistry of the 9-aldehyde group, the 9ß-isomer exhibiting the higher reactivity. With amines or amino group acids a remarkable difference in reactivity was observed, the 9α-isomer being practically unreactive. They were able, under biomimetic conditions, to obtain ^{1}H NMR evidence for their proposed mechanism (Scheme 2)[71].



SCHEME 2.

After reaction with model amine, i.e. methylamine, one single product, the pyrrole(42), was observed which, because of its instability, was only examined by ^1H NMR spectroscopy. The inactive 9α-isomer cannot form intermediates of the type(42) due to the greater distance between the C-9 axial aldehyde and the enal moeity. Several other suitable enal aldehydes were also investigated and gave rise to the same observations[72].



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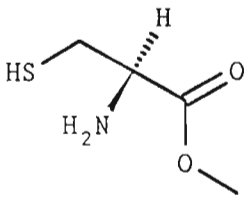

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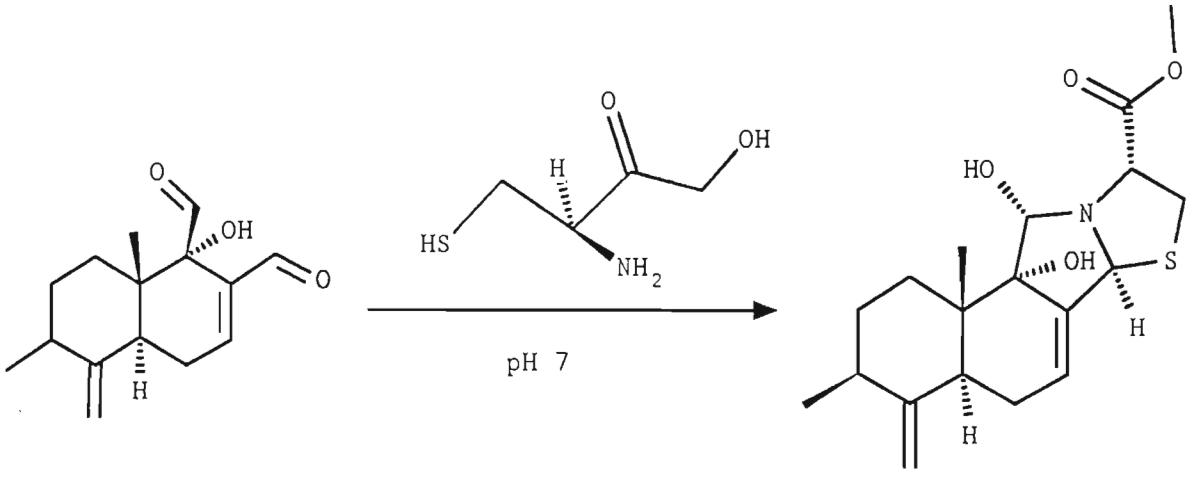
The biological mechanism of hot tasting and antifeedant activity of 1,4-dialdehydes may also result from covalent binding to primary amino groups of the chemoreceptive sites[73] rather than from Michael addition of membrane sulfhydryl groups[74], even though both are available at the receptor site[75]. A model study of the reaction of muzigadial(16) with L-cysteine methyl ester(43) in vitro is in agreement with this[76] (Scheme 3).

Cell permeability studies revealed that polygodial(26) preferentially damaged the cell membrane and caused an appreciable amount of leakage of cellular constituents, *e.g.* proteins and saccharides. A decrease in cellular dry weight was also observed. The permeability changes were supported by microscopic evidence; the structural integrity of the cell membrane was markedly disrupted by polygodial(26)[50,77,78]. However the polygodial(26) binding site in the cell membrane is not yet established.

Reaction of muzigadial with L-cysteine methyl ester.



SCHEME 3.

1.3 Review of the Biological Activity of some members of the family EUPHORBIACEAE.

The family Euphorbiaceae is comprised of over 300 genera and over 5000 species, mostly natives of the tropics of both hemispheres[79]. The family has been divided into a number of tribes, the largest being the genus *Euphorbia* which comprises over 2000 species. There are close to 200 *Euphorbia* species in South Africa with a wide distribution[80].

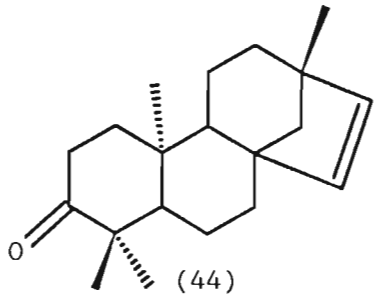
Plants of the family Euphorbiaceae are used in traditional medicine for different purposes: against fever, as diuretic agents, mouth-wash, treatment of jaundice gonorrhoea, as chewing sticks and as purgatives[81,82]. Some are poisonous and others are used as analgesics and antihypertensive agents[80,81,83,84].

1.3.1 Compounds isolated from wood of *Spirostachys africana*.

Chemical investigation of the wood of *Spirostachys africana*

Sonder revealed stachenone(44), 3α-hydroxystach-15-en-2-one(45) and stach-1,15-dien-3-one(46) to be constituents of the plant[85]. These tetracyclic diterpenes with the stachane(47) nucleus were also isolated in unrelated families. These are listed below:

The trunkwood of East Indian *Erythroxylon monogynum* **Roxb.**(ERYTHROCEAE) was found to contain stachene(48), erythroxylol A(49), erythroxylol B(50), erythroxyldiol A(51), 4ß-hydroxy-18-norhibaene(52) and 4α-hydroxy-18-norhibaene(53)[86,87] and chemical investigation of the roct wood of Australian *Erythroxylon australe* showed stach-15-en-1-one(54), 2ß-hydroxystach-15-en-1-one(55), stach-15-epoxy-1-one(56), 19-hydroxystach-15-en-1-one(57),2-hydroxystach-2,15-dien-1-one(58) and stachene(48) to be constituents of the plant[88]. Lloyd *et al.*, in their chemical investigation of the leaves the Australian Compositae, *Helichrysum dendroideum* **N.H. Wakefield**, found (+)stach-15-en-3α,19-diol(59), (+)stach-15-en-17,19-diol(60) and (+)stach-15-en-3α,17-diol(61)[89].



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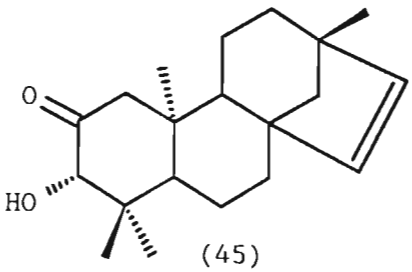

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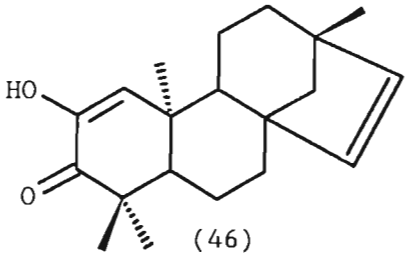

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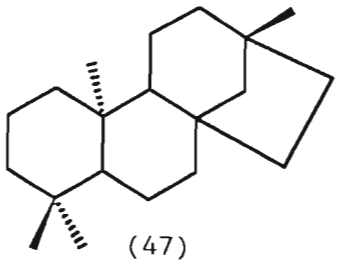

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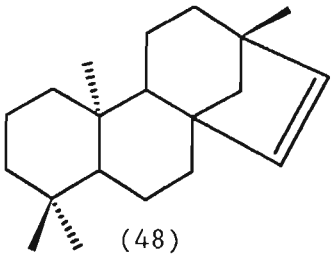

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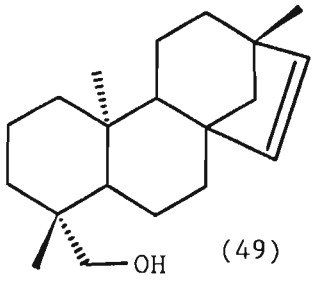

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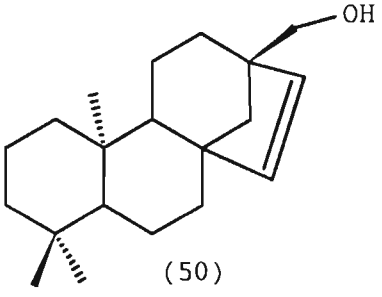

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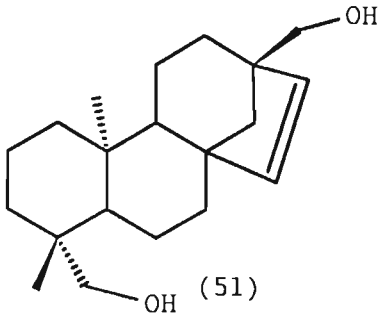

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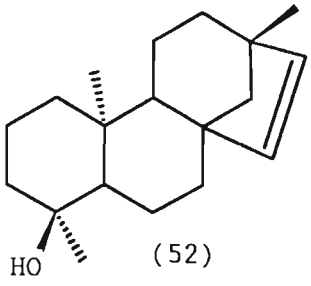

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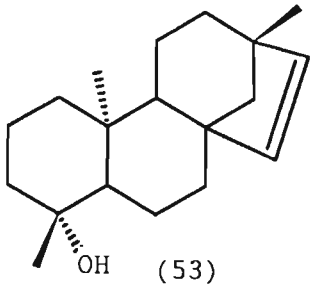

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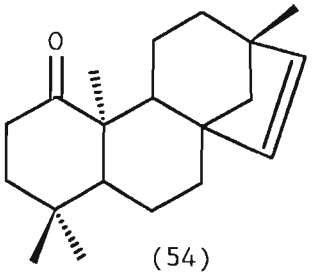

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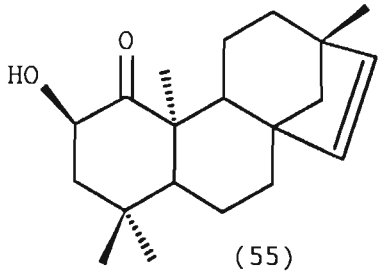

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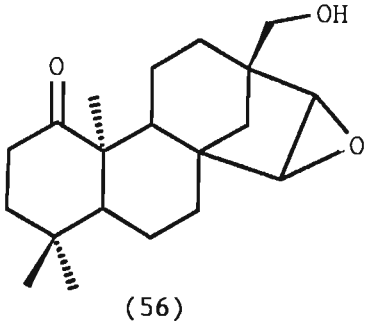

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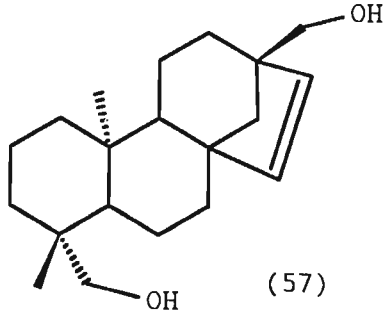

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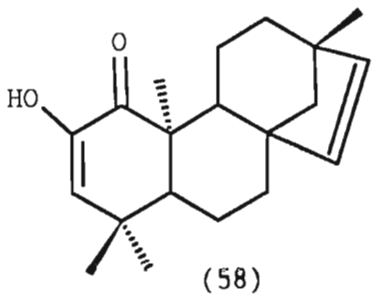

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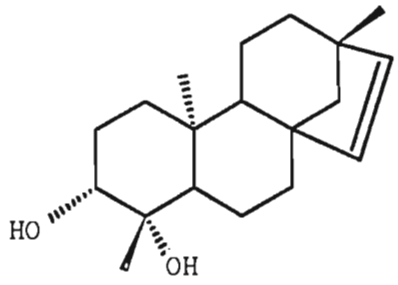

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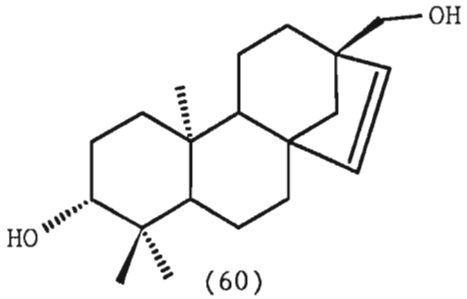

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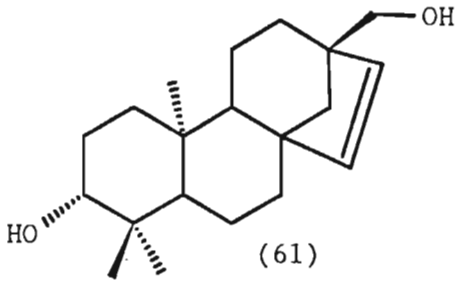


(61)

1.3.2 Phytochemical screening.

Phytochemical studies of this plant are unknown. However, the toxicity and the skin irritating effects of the milky latex prompted an investigation of the chemical components of the bark[90].

1.4 Review of the family MYRSINACEAE.

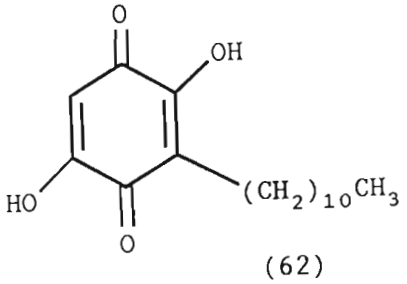
The family MYRSINACEAE comprises about 35 genera and 1000 species, worldwide, mainly in the tropics and subtropics[91]. Some of the species of the genera *Maesa*, *Embelia*, *Myrisine* and *Rapanea* are endemic to Southern Africa[92].

Two species, *Rapanea gilliana* **(Sonder) Mez** and *R. melanophloeos* **(L.) Mez** are found in South Africa[92].

1.4.1 Chemical constituents.

Chemical investigations of the MYRSINACEAE revealed benzoquinones to be major constituents of the family.

From MYRSINACEAE species 3,6-dihydroxy-2-alkylbenzoquinones, such as embelin(62), rapanone(63) and maesaquinone(64), ardisiaquinone A(65), ardisiaquinone B(66), ardisiaquinone C(67), 2-hydroxy-5-methoxy-3-pentadecenyl-(tridecenyl-tridecyl-)benzoquinone(68), acetylmaesaquinone(69) and the methylene dimer, vilangin(70), have been isolated[93].



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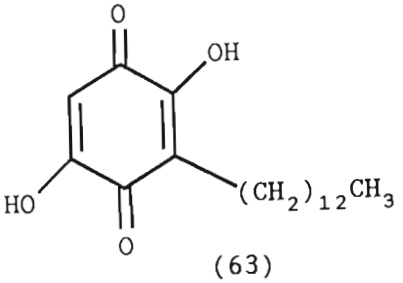

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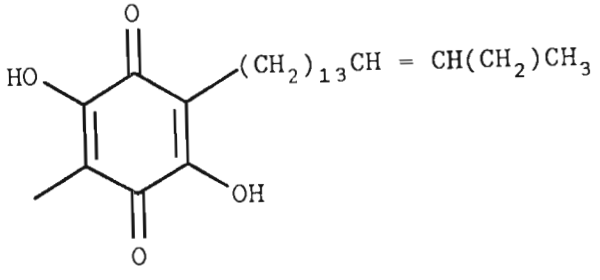

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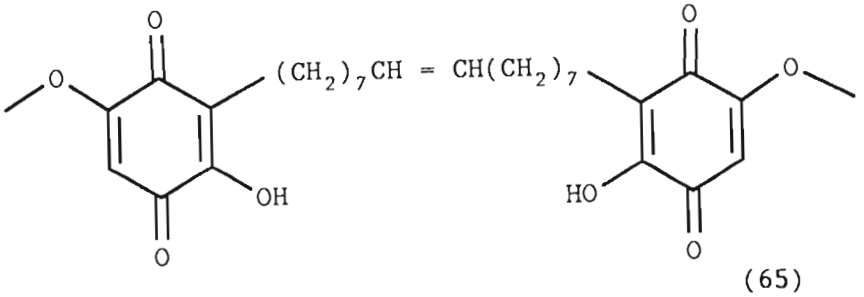
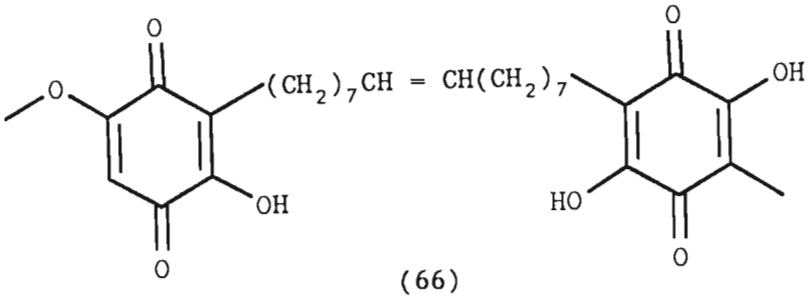


(65)

Although rapanone(63) has been isolated from the bulbs of *Oxalis purpurea* **Linn. var.** *jacquinii* **Sonder** (Geraniaceae)[94] and from the roots of *Connarus monocarpus* **Linn.** (Connaraceae)[95] and polygonaquinone(71), an alkyl homologue of maesaquinone(64) has been isolated from *Polygonatum falcatum* **A. Gray** (Liliaceae)[96], the distribution of these hydroxybenzoquinone derivatives is assumed to be a chemotaxonomical characteristic of the MYRSINACEAE.



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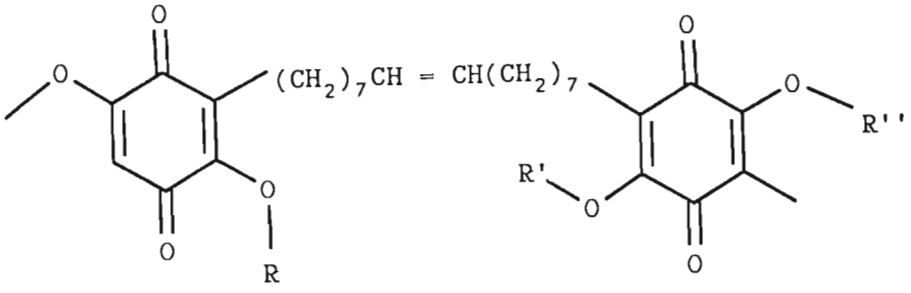
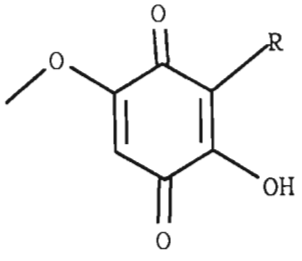


(67) R : CH_3CO, R',R'': H

R': CH_3CO, R, R'': H or
R'': CH_3CO, R, R': H

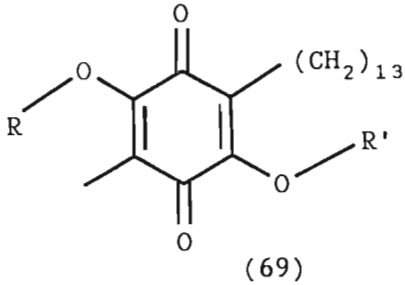


R : $C_{15}H_{29}$

$C_{13}H_{25}$

$C_{13}H_{27}$

(68)



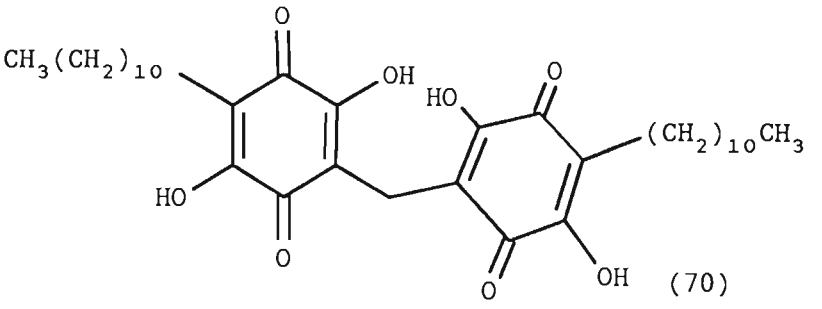
(69)

R: H, R'': CH_3CO or

R: CH_3CO, R': H

Embelin(62) and rapanone(63) are reputed to possess anthelmintic activity.

Alkyl and alkenylresorcinols were found to be constituents of *Rapanea laetevirens*[97]. A suggestion that 1,4-benzoquinones are probably derived from resorcinol type precursors by oxidative processes was partly established by Croft *et al.*[98] in their conversion of grevillol[5-tridecylresorcinol](72) into rapanone(63) and 5,5′-(hexadec-8-yne-1,16-diyl)diresorcinol(73) into ardisiaquinone A(65).



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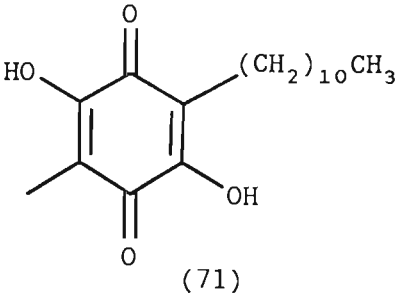

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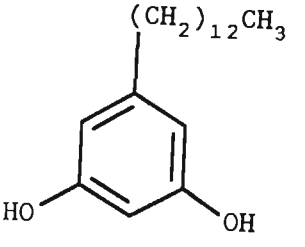

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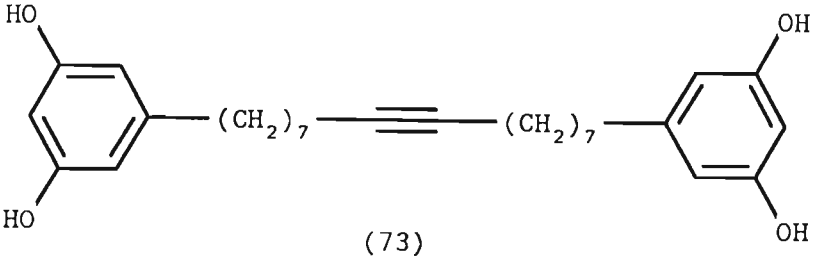
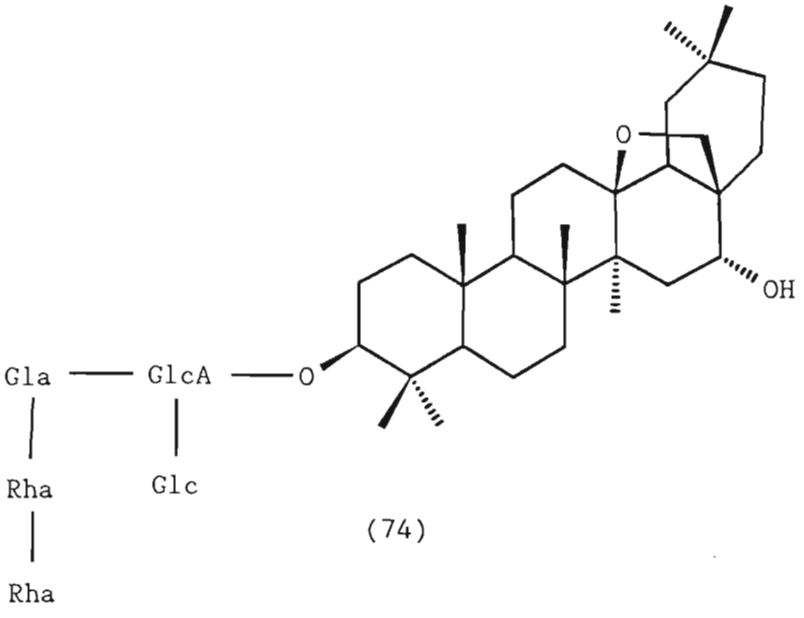


(73)

Mavi *et al.*[99] recently isolated a molluscicidal and antifungal triterpenoid saponin, sakurasosaponin(74), from the leaves of *Rapanea melanophloeos*.

1.4.2 Phytochemical screening.

The methanol extract from the leaves of *R. melanophloeos* showed molluscicidal activity(50 ppm) against the schistosomiasis-transmitting snail *Biomphalaria glabrata* and displayed antifungal activity against the plant pathogenic fungus *Cladosporium cucumerinum* in a TLC assay[99].



(74)

CHAPTER 2

DISCUSSION

2.1 Extractives from *Cassine transvaalensis*.

Cassine transvaalensis **(Burtt Davy) Codd** was collected from Nzhelele (Venda). It was identified by Mr E. Netshiungani of the Venda herbarium.

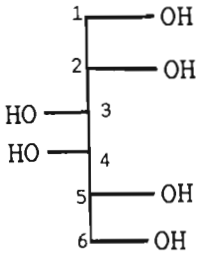
The air dried root bark(4.6 kg) was milled to a fine powder. This powdered root bark could be extracted in two ways: firstly the differential solvent extraction technique[100] could be used. This extraction technique employs the exhaustive extraction of the plant by a series of solvents of increasing polarity. This gives a differential fractionation of the extractives on the basis of their differing polarities. Alternatively, the material can be extracted with a single powerful solvent which extracts practically all the constituents. The constituents are then separated according to some specific procedure which depends on the extractives present[101].

As this investigation was carried out without any phytochemical screening the former technique was employed. The milled root bark was therefore differentially extracted in a Soxhlet using petroleum ether(40-60°C), benzene, acetone and ethanol as the extracting solvents. The average extraction period for each solvent was 24 hours, by which time the extraction appeared to be complete. The petroleum ether(40-60°C) extract(45.3 g) was yellow, the benzene extract(82.2 g) red, the acetone extract(300 g) brown and ethanol extract(380.0 g) separated out as golden brown amorphous material.

2.1.1 Galactitol(75).

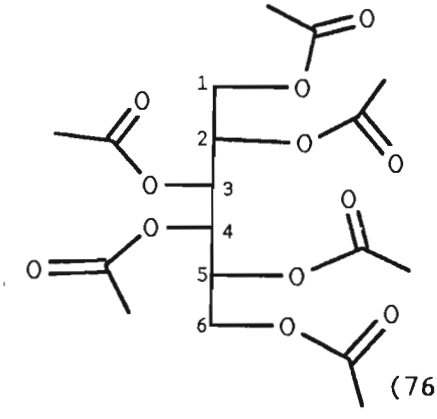
The ethanol extraction of the root bark(24 hours, Soxhlet) afforded a brown solution from which a precipitate settled on

cooling. This precipitate was filtered off and recrystallized from water-methanol mixture. In all 35.4 g of needles mp 187-188°C) were obtained. It was characterized by physical spectroscopic evidence. The hexa-acetate derivative(76) was prepared to support the results.



(75)

IR spectrum indicated the presence of the hydroxyl group which disappeared on acetylation. ^{13}C NMR(p. 161, 162) revealed the presence of two oxygen bonded methine(δ69.44 and 68.68) and one oxygen bonded methylene group(δ62.5). This evidence suggested a symmetrical six carbon straightchain structure with six hydroxyl groups. In ^{1}H NMR(p. 160) the triplet at δ3.85(1H, t, J = 6.22 Hz) is due to a methine bonded to the hydroxyl group. The three peaks at δ3.56 and δ3.54 are due to overlapping signals of methine and methylene bonded to O-H groups. The hydroxyl group which is observed in the IR spectrum is not evident since D_2O is used as a solvent. The above information suggests that the compound is a hexitol[102].



(76)

Table 2.

Physical properties of galactitol hexaacetate compared with acetate of the isolated compound.

Physical Properties	Present investigation	Galactitol hexaacetate[102].
mp	168°C	168-169°C
Optical rotation	$[\alpha]_D^{20°C}$ = 0.00	$[\alpha]_D$ = 0.00

Synthesis of the acetyl derivative and comparison of its physical properties with the hexaacetates(Table 2) of the hexitols, proved the compound to be galactitol. ^{13}C NMR of the isolated compound(p. 161, 162) and galactitol[103] are also compared in Table 3.

Table 3.

^{13}C NMR of galactitol compared with the isolated compound.

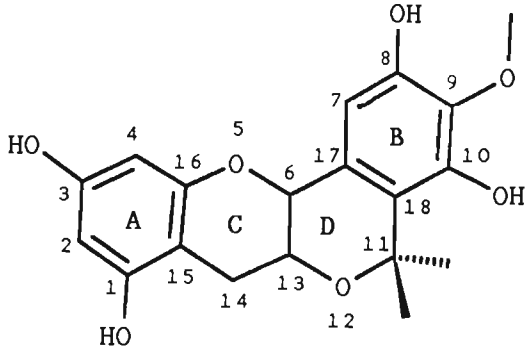
Carbon	Present Investigation	Galactitol[103].
C-1	62.5(t)	63.25(t)
C-2	68.68(d)	69.25(d)
C-3	69.44(d)	70.15(d)
C-4	69.44(d)	70.15(d)
C-5	68.68(d)	69.25(d)
C-6	62.5(t)	63.25(t)

2.1.2 (+)-11,11-Dimethyl-1,3,8,10-tetrahydroxy-9-methoxypeltogynan(11).

The(+)-11,11-dimethyl-1,3,8,10-tetrahydroxy-9-methoxypeltogynan

(11) was obtained from the ethanol extract of the root bark of *C. transvaalensis*.

Several genera of the Leguminosae are known to be sources of peltogynoids[22,23,24,26,104,105,106,107,108]. However only one example of a peltogynoid, described[27] as a leucoanthocyanidin, has been isolated from the family Celastraceae. The compound, named elaeocyanidin, was isolated from the root bark of *Elaeodendron balae*. The isolated compound(11) appears to be identical to elaeocyanidin apart from the fact that it has a slightly higher mp, 154°C, (as opposed to 149°C) and an optical rotation of +174° (as opposed to +205°). It is perhaps of taxonomic significance that two plants from the Celastraceae should both possess a peltogynoid with a unique pattern of substitution in the form of a *gem*-dimethyl group in the D ring.



(11)

The ^{1}H NMR spectrum(p. 166) clearly indicates the presence of a flavonoid-type compound possessing A and C rings very akin to those of catechin[109]. Particularly characteristic was the pair of meta coupled doublets at δ5.85 and δ6.02, the H-6 signal at δ4.42(d, J = 0.97 Hz), the H-13 signal at δ4.24(ddd) and two nonequivalent protons on C-14 giving rise to signals at δ2.77(dd) and 2.88(dd). Further examination of the data indicated an unsplit aromatic proton, one aromatic methoxy group, four phenolic groups, and, surprisingly, two non-equivalent methyl groups. All these protons are readily accommodated in the proposed structure.

Unusual features of the compound are (i) the hydroxy group at C-1 (not common, but also found in, for example, crombeone[25], (ii) the absence of a hydroxyl or keto-group at C-14{a feature it shares with the peltogynan (+)-pubeschin[110]}, (iii) the 9-methoxy group and (iv) the geminal dimethyl group at C-11. The latter two features are unique to the present compound.

Placement of the dimethyl group on ring D was supported by evidence from long range HETCOR(heteronuclear correlation) correlation programme(Figure 1, JCH = 10 Hz) which revealed that the protons on the methyl groups are strongly coupled with C-11 and also to a lesser extent to C-18. The mass fragmentation pattern(Scheme 8) also supports placement of the methyl groups on C-11.

From the normal HETCOR spectrum(Figure 2) all protonated carbons(i.e. the two methyl groups, C-6, C-13, C-14, C-2, C-4, C-7 and the methoxy group) could be positively identified. Allocation of the methoxy group to C-9 'between' the two phenolic groups at C-8 and C-10 was again based on long range HETCOR correlations(Figure 1). It shows the methoxy protons unambiguously coupled to C-9 and, in addition, it is seen that C-9 also couples(long range) to phenolic protons on C-8 and C-10 and also to the unsplit aromatic at C-7. In agreement with this placement was the observation that the hydroxy proton on C-10 was not only coupled to C-10 but also to with C-9 and C-18. The localisation of OH-8 and OMe-9 and OH-10 depends on correctness of the assignment of ^{13}C NMR data(p. 167, 168) for C-17 and C-18 and needs independent confirmation. Finally, the compound does not reduce ammoniacal $AgNO_3$ solution, a good qualitative test for the presence of ortho-hydroxyphenols.

Mass fragmentation of (11) indicated a strong molecular ion peak at *m/z* 360. Retro Diels-Alder fragmentation leads to key fragments at *m/z* 139(66%) and 222(18%). Loss of a methyl group from the latter fragment gives rise to the base peak at *m/z* 207(Scheme 4). The stereochemistry around C-6 and C-13 is readily

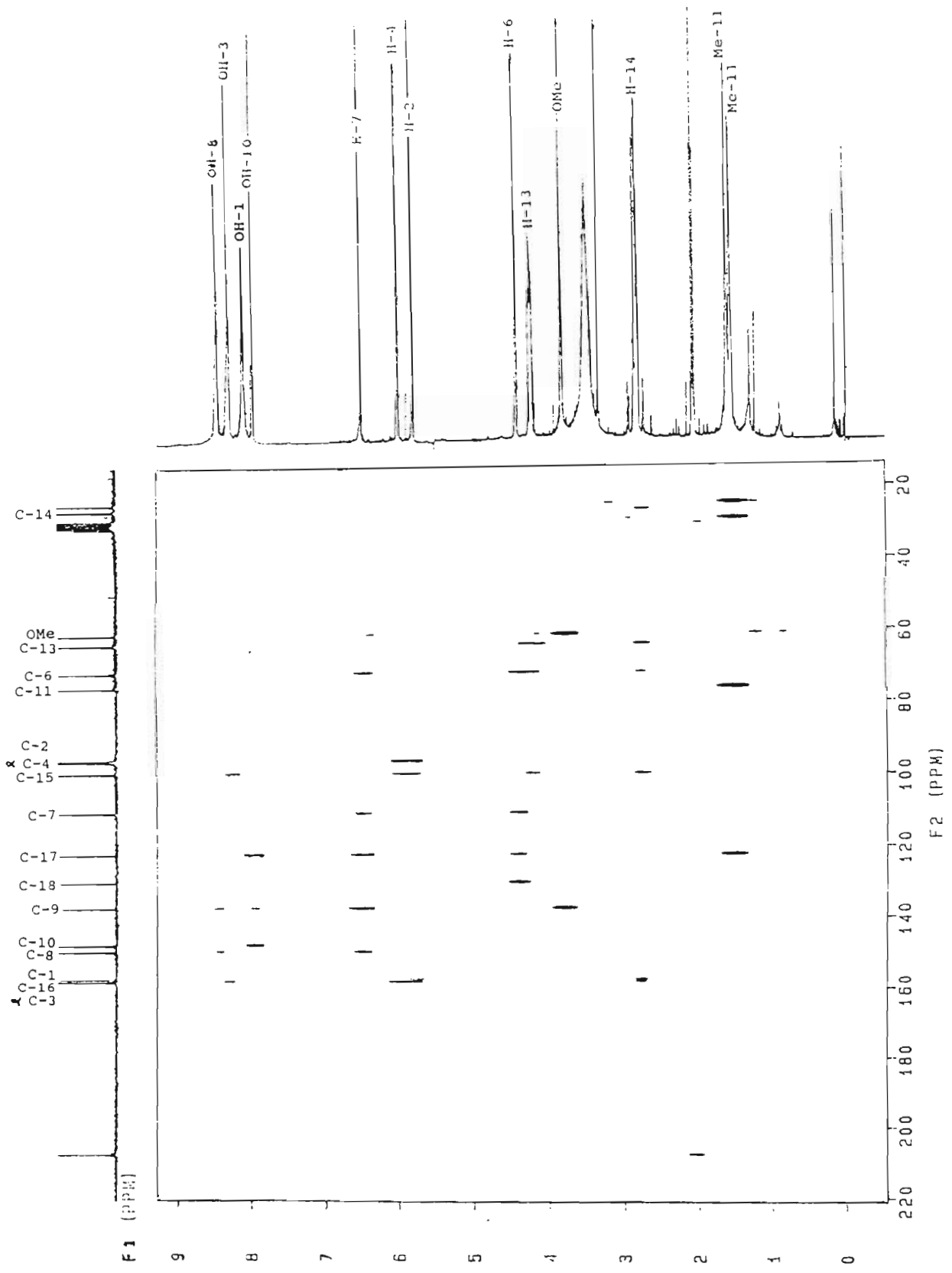


FIGURE 1. Long range HETCOR specturm of the isolated peltogynan(11).

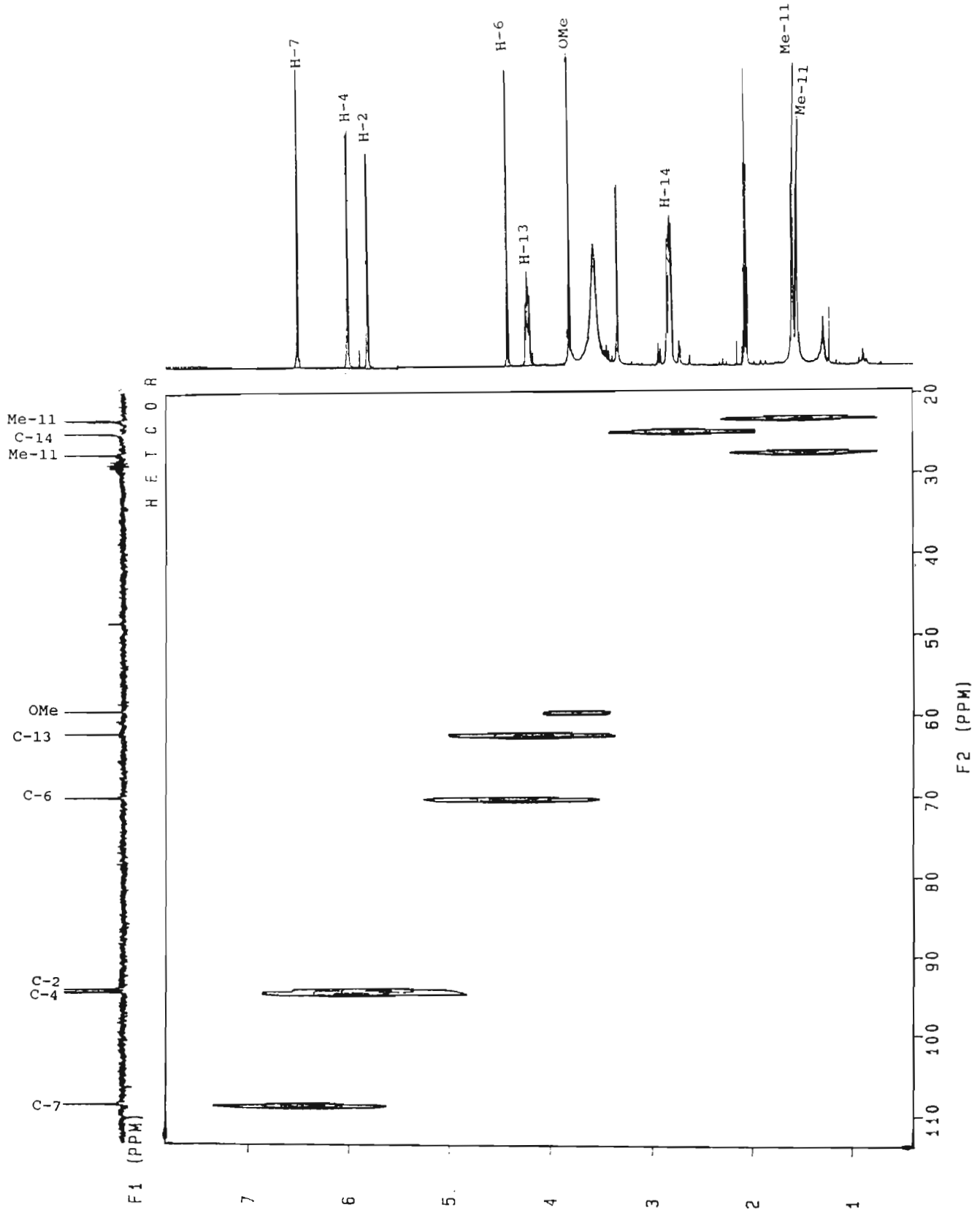
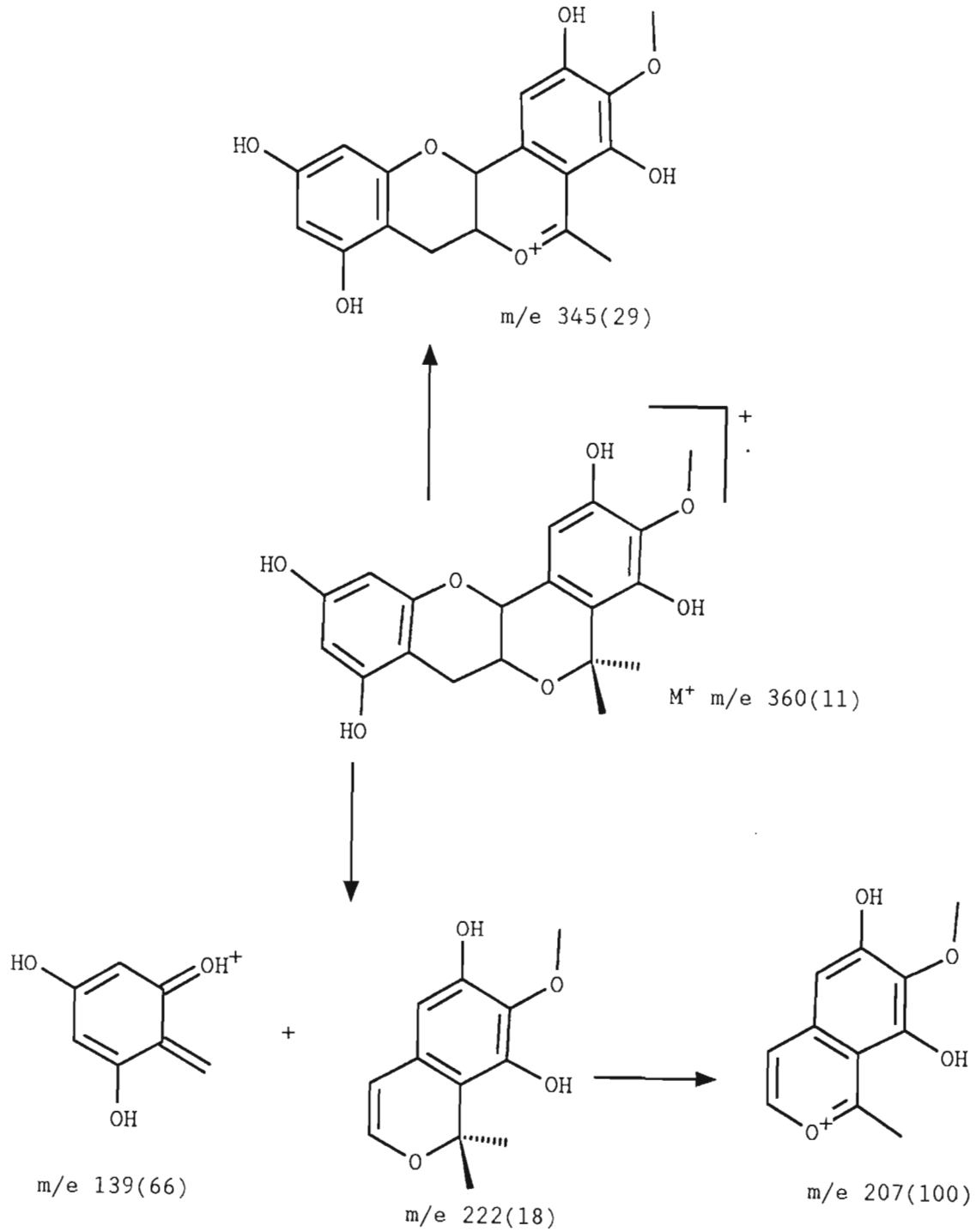


FIGURE 2. HETCOR spectrum of the isolated peltogynan(11).

deduced from accurate measurement of the relevant coupling constants($J_{a/b}$ = 0.97 Hz, $J_{b/c}$ = 2.24 Hz, $J_{b/d}$ = 4.64 Hz and $J_{c/d}$ = 17.3 Hz). In this regard comparison with the data given by



SCHEME 4. Mass fragmentation pattern of (+)-11,11-dimethyl-9-methoxypeltogynan(11).

Malan[111] for (2R, 3R, 4R)-2,3-cis-3,4-cis-3',4',7'-tri-O-methyl-4-O-acetylmopanol proved helpful. By analogy to related peltogynans the absolute stereochemistry is likely to be (6R, 13R).

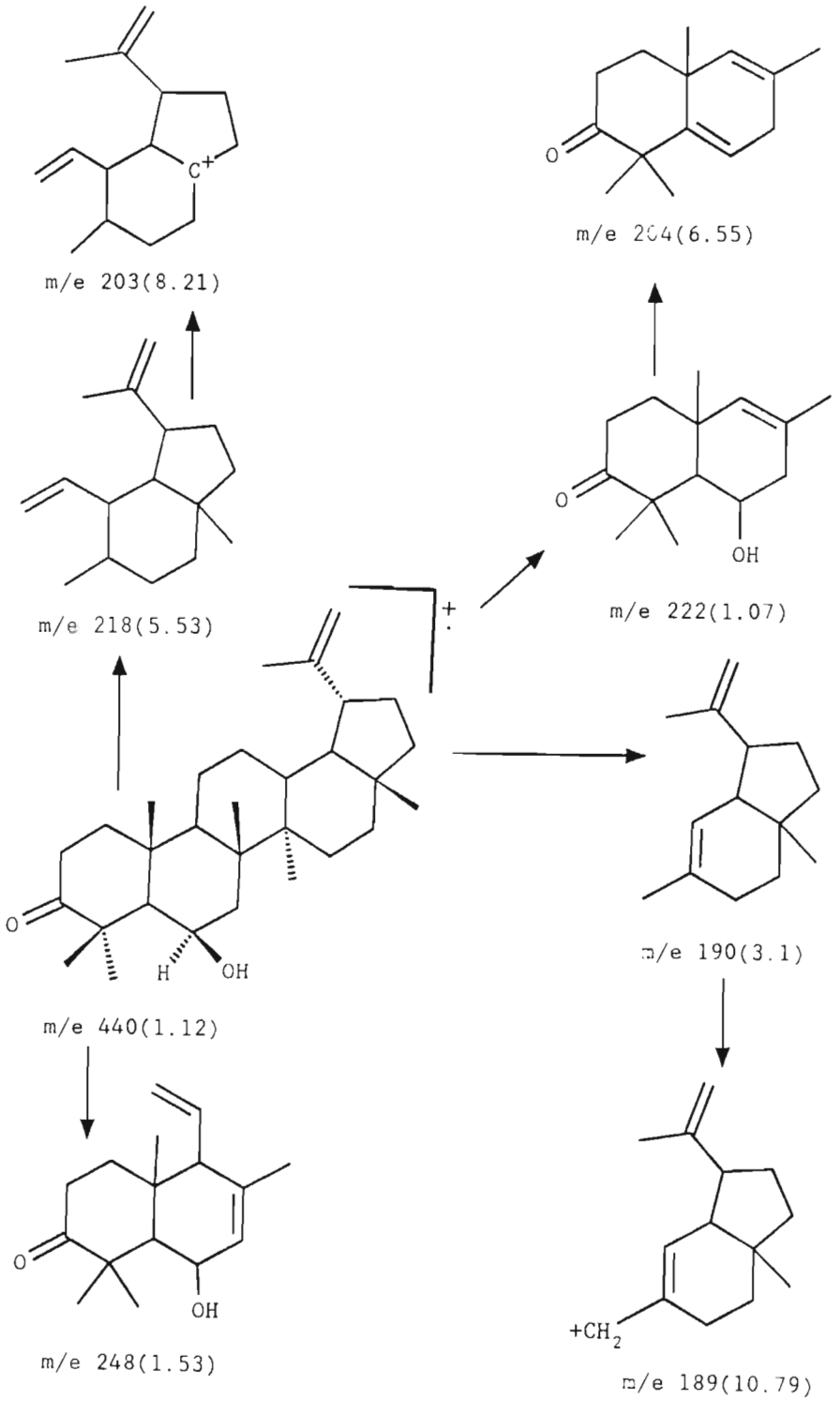
2.1.3 6ß-Hydroxy-lup-20(29)-en-3-one(77).

The petroleum ether(40-60°C) extract(45.3 g) was partitioned between *n*-hexane and 95% aqueous methanol. The hexane fraction(23.0 g) was adsorbed onto silica gel 60(Merck Art. 9385), transferred into the column and separated by flash chromatography[112] to give two components of R_f0.44 and 0.69(hexane-ethyl acetate, 4:1). The lower R_f fraction(3.1 g) was repeatedly recrystallized from hexane-ethyl acetate to give 6ß-hydroxy-lup-20(29)en-3-one(77). The characterization of (77) was based on physical and spectroscopic evidence.

Elemental analysis and mass spectroscopy(M^+ at *m/z* 440) established the molecular formula of (77) as $C_{30}H_{48}O_2$. Its IR spectrum indicated a hydroxyl group(3480 cm^{-1}), a carbonyl group(1690 cm^{-1}), geminal dimethyl groups(1380 and 1450 cm^{-1}) and an exocyclic methylene(1640 and 880 cm^{-1})[113].

The fragmentation pattern observed in the mass spectrum strongly suggest the compound to be a lup-20(29)-ene type triterpene (Scheme 5)[114]. The mass spectrum of (77) showed significant ions at *m/z* 316(f_2), 218(b), 209(a), 122(f_1), 175(e), arising by bond cleavages of ring C as shown in Scheme 5. The fragmentation pattern suggests the assignment of the hydroxy and carbonyl groups to A/B ring parts. The g-type ion(*m/z* 125, $C_8H_{13}O$) confirms the assignment of the carbonyl group to ring A and the hydroxy group to ring B. The fragment ion *m/z* 209 results from the a-type cleavage followed by loss of water. 6ß-Hydroxy-lup-20(29)-en-3-one(77) has previously been isolated from *Pleurostylia capensis*(Celastraceae) by Dantanarayana *et al.*[115]. Previously, Marini-Bettòlo had reported the isolation of rigidenol from *Maytenus rigida*[116] indicating its structure as a diastereomer of (77). The structure of rigidenol was subsequently revised by

Marini-Bettòlo to 11α-hydroxy-lup-20(29)-en-3-one[117].



SCHEME 5. Mass fragmentation pattern of 6ß-hydroxylup-20(29)-en-3-one(77).

Dantanarayana *et al*. base the allocation of a 6ß-hydroxy in (77) on (i) the observed deshielding of the C(24)-C(25) tertiary methyl resonances normally regarded as resulting from a 1,3-diaxial interaction with a hydroxy group and (ii) the fact that the chemical shift of the carbinol methine proton was identical to that of the corresponding proton at C-6 in 6ß-hydroxy lupane reported earlier. Our own investigations, based on single crystal X-ray analysis{ Tables with interatomic distances and interatomic angles are in the Appendix 1, p. 153}, coupled with ^{1}H and ^{13}C NMR (p. 169 and 170 respectively) at 200 MHz, show unambiguously the relative configuration of (77) and confirm the presence of an axial 6ß-hydroxy group(Figure 3). In particular the proton

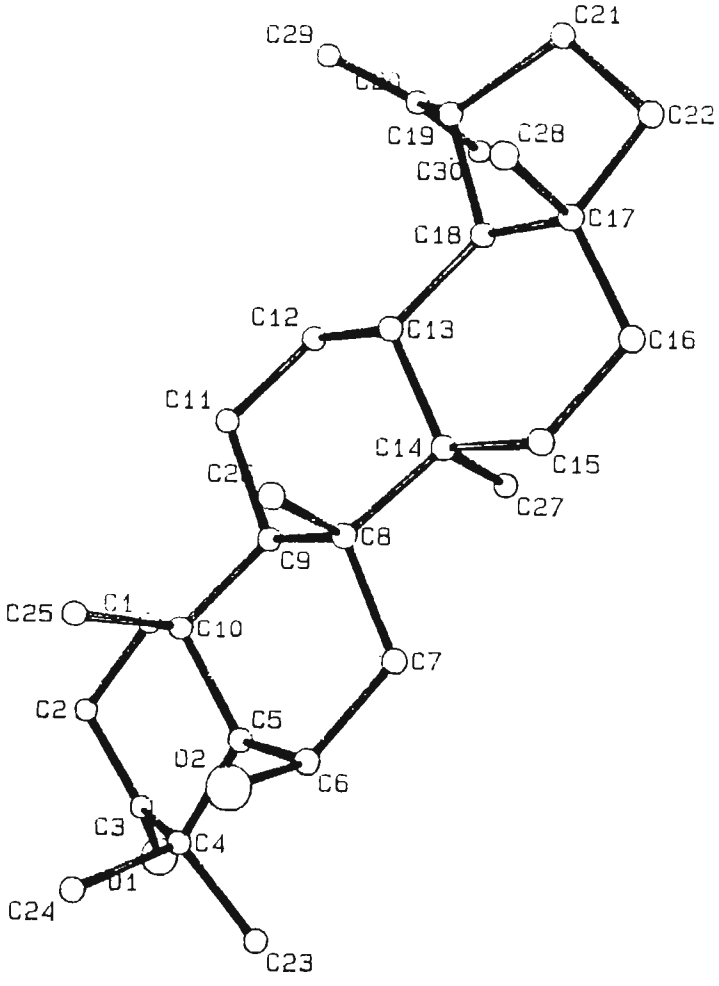


FIGURE 3. X-Ray structure of 6ß-hydroxy-20(29)-en-3-one(77).

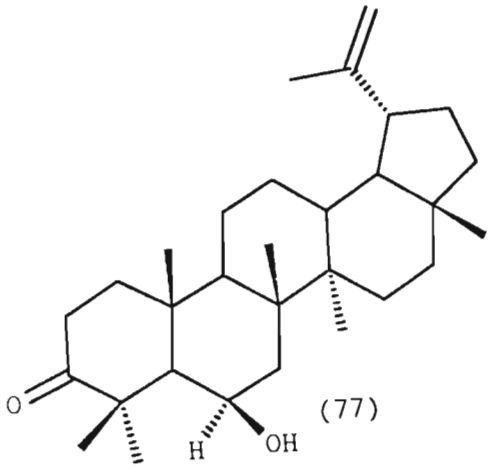
spectrum reveals chemical shift details not previously reported: (i) H-2 is made up of two non-equivalent methylene protons each resonating as a double doublet of doublets at δ2.82 and 2.26(J = 2.85, 6.48 and 15.01 Hz). (ii) H-19 appears as a double doublet of doublets at δ2.43(J = 5.35, 5.59 and 11.07 Hz) and long range HETCOR examination reveals additional coupling at H-18 and H-21. Long range HETCOR(Coupling constant = 16 Hz), p. 172, also showed that C-3 is coupled to the protons of the methyl groups at C-23 and C-24. A comparison of the C resonances(Table 4) recorded by

Table 4.

Comparison of ^{13}C NMR data for 6ß-hydroxy-lup-20(29)-en-3-one(77).

Carbon	Dantanarayana *et al.*[115]	Present investigation
1	43.0	42.90
2	34.5	34.58
3	216.7	217.39
4	49.0	49.11
5	56.6	56.75
6	69.7	69.90
7	42.2	42.29
8	40.0	40.15
9	50.7	50.84
10	36.8	36.87
11	21.3	21.34
12	25.2	25.25
13	37.2	37.25
14	42.2	42.24
15	27.5	27.58
16	35.5	35.28
17	43.2	43.24
18	48.3	48.48
19	48.0	48.09
20	150.8	151.25
21	29.8	29.89
22	39.9	40.05
23	25.0	25.06
24	23.7	23.80
25	17.0	17.02
26	17.1	17.14
27	14.8	14.85
28	18.0	18.05
29	109.2	109.77
30	19.3	19.38

us and those found by Dantanarayana *et al.*[115] confirms the identity of the compounds. In addition the melting points are identical. Our compound does, however, show a lower specific rotation $[\alpha]_D$= -7.85°(lit[115] $[\alpha]_D$= -14°).



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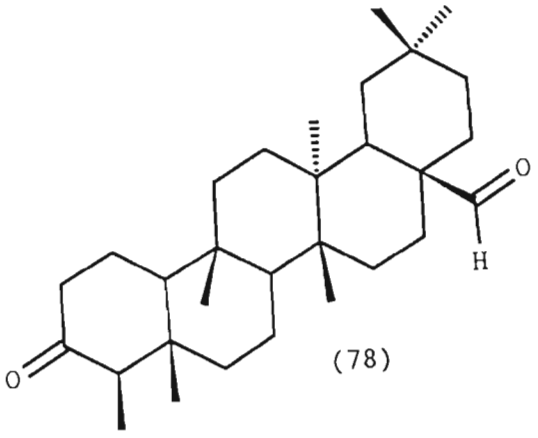
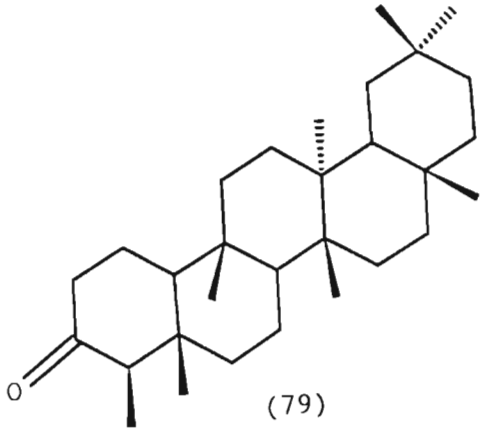
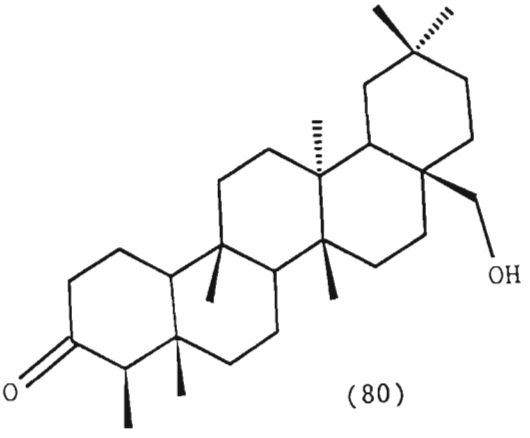


(78)



(79)



(80)

2.1.4 3-Oxo-friedelan-28-al(78).

The higher R_f component(2.3 g) from the hexane fraction was further purified by flash chromatography[112] to give 62 mg of 3-oxo-friedelan-28-al(78). Characterization of the compound as 3-oxo-friedelan-28-al(78) rests chiefly on ^1H NMR, ^{13}C NMR, MS and physical properties.

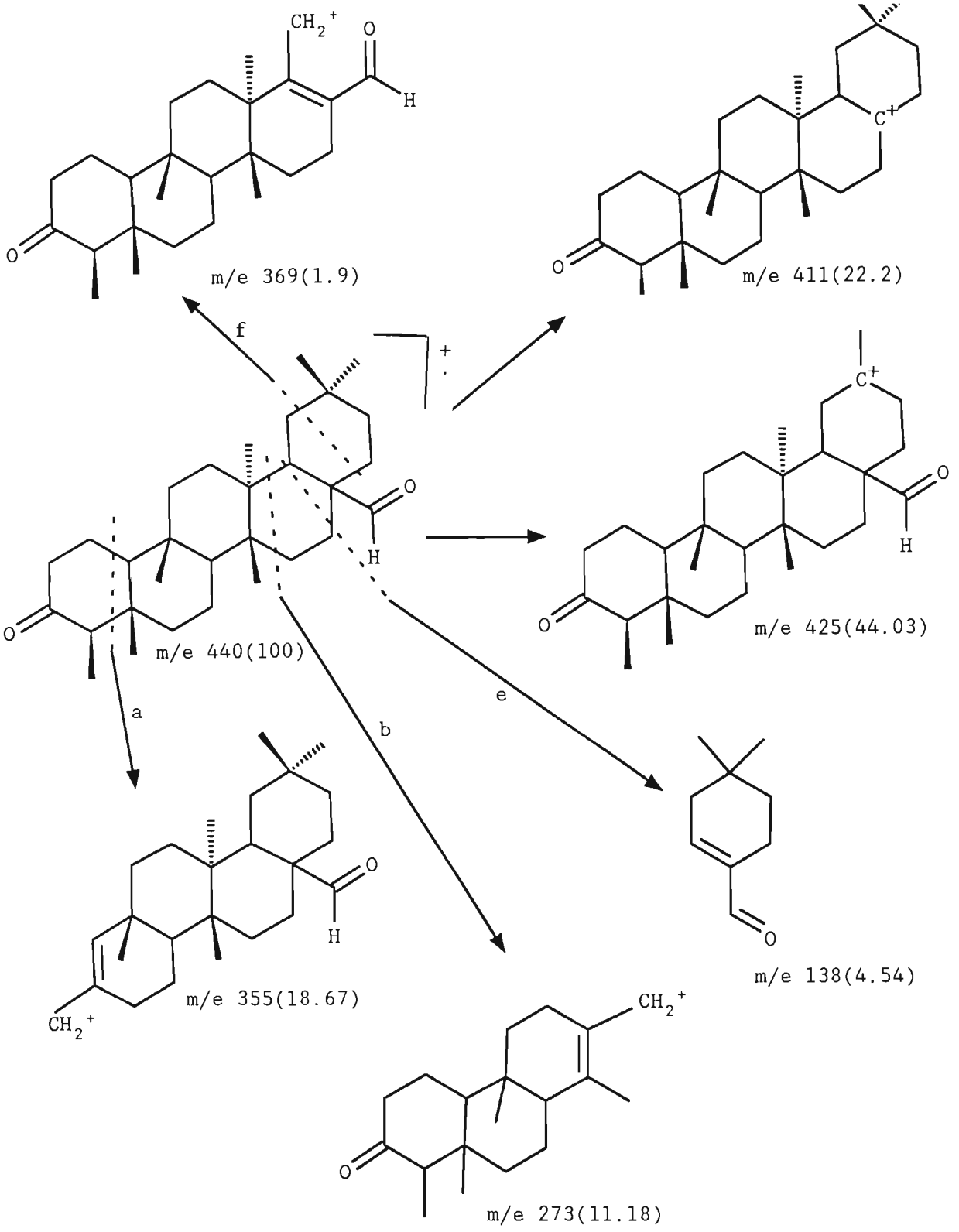
Elemental analysis and mass spectroscopy(M^+ at m/z 440) established the molecular formula of (78) as $C_{30}H_{48}O_2$. The IR spectrum of the compound showed peaks at 2710 and 1718 cm^{-1} (aldehyde), 1380 and 1440 cm^{-1} (geminal dimethyl groups) and 1710 cm^{-1}(six-membered ring ketone)[118].

Table 5.

^1H NMR data of 3-oxo-friedelane(79) and the isolated compounds.

Proton	3-oxo-friedelane[119]	78	80
H-1	1.6(1H, m) 1.94(1H, m)		1.60(1H, m) 1.93(1H, m)
H-2	2.36(1H, m) 2.35(1H, m)	2.36(1H, m) 2.35(1H, m)	2.37(1H, m) 2.37(1H, m)
H-4	2.24(1H, q)	2.24(1H, q)	2.26(1H, q)
H-23	0.87(3H, d)	0.87(3H, d)	0.87(3H, d)
H-24	0.72(3H, s)	0.71(3H, s)	0.72(3H,s)
H-25	0.85(3H, s)	0.87(3H, s)	0.87(3H, s)
H-26	0.99(3H, s)	0.88(3H, s)	1.03(3H, s)
H-27	1.03(3H, s)	1.09(3H, s)	1.05(3H, s)
H-28	1.16(3H, s)	9.41(1H, s)	3.26(2H, d)
H-29	0.99(3H, s)	0.87(3H, s)	1.03(3H, s)
H-30	0.94(3H, s)	0.98(3H, s)	1.22(3H, s)

The fragmentation pattern observed in the mass spectrum strongly indicates the compound to be a dioxygenated friedelane derivative[120]. The mass spectrum of the compound showed significant peaks at m/z 369(f), 355(a), 273(d) and 138(e), arising from ring cleavages indicated in Scheme 6. The prominent



SCHEME 6. Mass fragmentation pattern of 3-oxo-friedelan-28 -al(78).

Table 6.

^{13}C NMR of 3-oxofriedelane(79) and the isolated compounds compared.

Carbon	3-oxo-friedelane(79)[119]	78	80
C-1	22.1	22.32	22.34
C-2	41.4	41.55	41.76
C-3	213.0	213.75	213.61
C-4	58.0	58.28	58.43
C-5	42.0	42.14	42.40
C-6	41.0	41.25	41.48
C-7	18.0	18.20	18.46
C-8	52.9	50.51	53.60
C-9	37.3	39.49	37.63
C-10	59.3	59.60	59.64
C-11	35.3	36.32	35.84
C-12	30.3	28.59	30.81
C-13	39.5	39.51	40.16
C-14	38.1	37.57	38.44
C-15	32.3	30.15	32.94
C-16	35.9	36.39	36.07
C-17	29.9	44.70	30.74
C-18	42.6	44.05	42.03
C-19	35.2	35.20	33.34
C-20	28.0	26.26	27.99
C-21	32.6	29.01	29.97
C-22	39.1	41.55	39.73
C-23	6.7	6.82	6.86
C-24	14.5	14.70	14.68
C-25	17.8	16.12	17.92
C-26	20.1	19.00	20.82
C-27	18.5	18.66	18.53
C-28	32.0	206.08	74.95
C-29	31.7	26.93	25.86
C-30	34.9	31.84	32.15

peak at *m/z* 411 indicates the loss of the angular formyl group whilst the peak at *m/z* 425 shows the loss of the methyl group. The a-type ion(*m/z* 355) suggests the assignment of the carbonyl group to ring A and the e-type ion(*m/z* 138) showed the assignment of the other carbonyl to ring E or on its methyl substituents.

The ^{1}H NMR data(p. 173) showed a sharp singlet(1H) at δ9.4 due to the aldehyde proton, seven methyl groups and no olefinic protons. ^{1}H NMR data comparison of the compound and friedelin(Table 5)[119] indicate the absence of C-28 methyl group and suggest the formyl group to be at C-28.

The DEPT technique in ^{13}C NMR(p. 174, 175) indicated the presence of seven methyl groups, one aldehyde carbonyl(δ206.08), one ketonic carbonyl(δ213.75), five methine groups and eleven methylene groups. Furthermore the ^{13}C NMR of the compound (78) and 3-oxo-friedelane(79) are compared in Table 6.

Comparison of the mp and optical rotation indicate the compound to be canophyllal, a compound initially isolated from *Calophyllum inophyllum* **Linn** by Govindachari *et al.*[118]. A review on friedelin derivatives by Chandler *et al.*[121] shows canophyllal to have a hydroxyl group at C-3 and possessing only one carbonyl group and naming our compound canophyllal at this stage might further increase confusion about the correct structure of canophyllal.

2.1.5 3-Oxofriedelan-28-ol(80).

The 95% aqueous methanol extract, from the petroleum ether extract, afforded a brown solid(22.3 g) which was subjected to column chromatography to give 148 mg of 3-oxo-friedelan-28-ol(80). Characterization of the compound as 3-oxo-friedelan-28-ol(80) is presented below.

The compound was isolated as white crystals, mp 278-279°C, *m/z* 442 for $C_{30}H_{50}O_2$. The IR spectrum indicates a strong absorption at 3400 cm^{-1}(O-H stretching vibrations), a strong absorption at 1680 cm^{-1} due to C=O stretching vibrations and absorption at 1380 cm^{-1} and 1440 cm^{-1} due to geminal dimethyl groups.

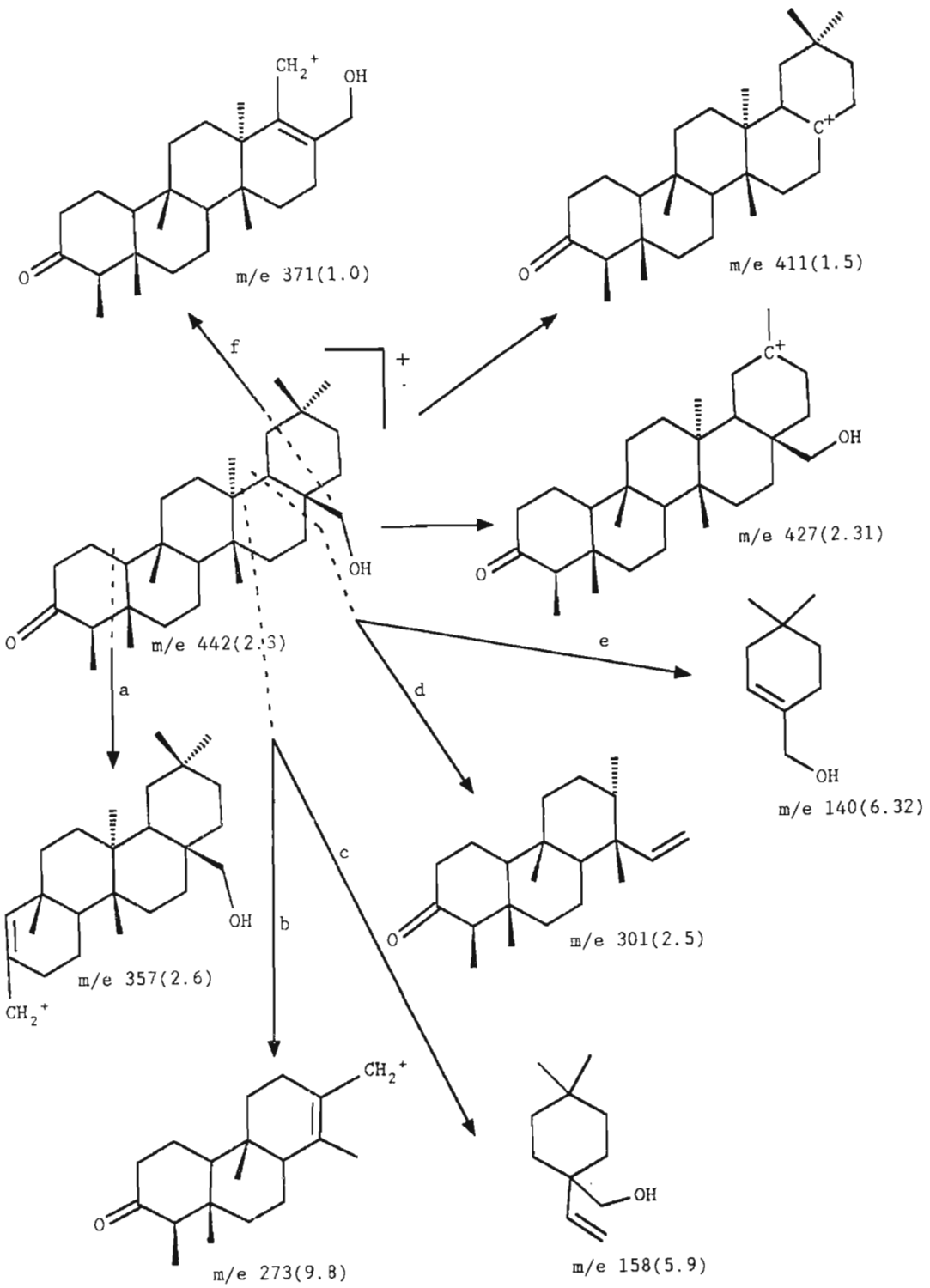
Mass spectral signals showed the compoumd to be a friedelin derivative with a carbonyl group at C-3 and a hydroxyl group at C-28. Cleavage of ring D between C(13)-C(18) and C(14)-C(15)

bonds results in the formation of an ion *m/z* 273. Alternate cleavage of ring D between C(13)-C(18) and C(16)-C(17) bonds results in the fragments *m/z* 140 and *m/z* 301 which limits the hydroxyl group to C-28, C-29 and C-30 and limits the assignment of a carbonyl group to rings A, B or C. The a-type cleavage (Scheme 7) leads to fragment ion *m/z* 357 and strongly suggests the carbonyl group to be in ring A.

The ^{1}H NMR(p. 176) indicates the presence of a methylene group bonded to oxygen{δ3.26(2H, d, J = 1.65 Hz, CH_2OH)}, one methine adjacent to a methyl group{δ2.24(1H, q, J = 6.23 Hz}, a methyl group adjacent to one proton {δ0.88(3H, d, J = 6.69 Hz)}, one hydroxyl group{δ1.95(1H, s, broad)} and 6 tertiary methyl groups{δ1.37; 1.22; 1.05; 1.03; 0.87 and 0.73(3H each, s)}.

From the ^{13}C NMR results(p. 177, 178), the compound has 7 methyl groups, one methylene group bonded to oxygen(δ74.98, triplet), one carbonyl group(δ213.9, singlet), 4 methine groups and 11 other methylene groups.

The above data and physical properties confirm the compound to be canophyllol a compound first isolated from *Calophyllum inophyllum* **Linn** by Govindachari *et al.*[118]. However, in a review by Chandler *et al.*[121] canophyllol is shown to have a hydroxyl group at C-3 and no carbonyl group which indicates confusion about the structure of canophyllal. Gamlath *et al.*[122], in their isolation of three 19(10-9)*abeo*-8α,9ß,10α-Euphane triterpernoids from *Reissantia indica*(Celastraceae) claim to have isolated canophyllol. Their characterization of the isolated compound as canophyllol is based on physical properties which are coincidentally the same as those of Govindachari *et al.*[118]. The canophyllal isolated by Gamlath *et al.*[122] had the following signals on a 400 MHz NMR: (i) δ3.29 and δ3.24(1H each, dd, J = 10 and 4.8 Hz, CH_2OH), (ii) δ2.24(1H, q, J = 6.4 Hz, 4-H), (iii) δ0.88(3H, d, J = 6.4 Hz, C(4)-CH_3), (iv) δ1.22; 1.05; 1.03, 1.02; 0.87 and 0.73(3H each, s, CH_3 groups on quaternary carbons). These values are identical with our results. According to the



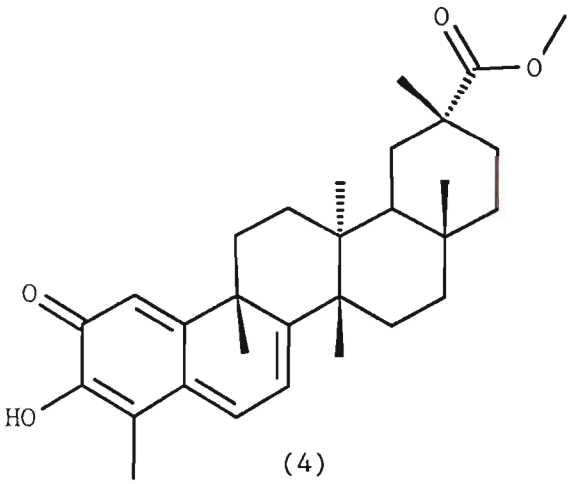
SCHEME 7. Mass fragmentation pattern of 3-oxo-friedelan-28 -ol(80).

formula indicated in their publication the molecular mass of their compound could have been *m/z* 444. A compound isolated by Gamlath *et al.*[122], according to the indicated structure, is 3,30-dihydroxyfriedelane(81). A closer look at the mass spectrum could have avoided the confusion as the peak at *m/z* 411 could have indicated the loss of angular CH_2-OH indicating the hydroxyl group to be at C-28.

2.1.6 Pristimerin(4).

Pristimerin was isolated from the benzene extract as a mixture with 3-oxofriedelan-28-ol(80). No further attempts were made to separate the two compounds. However peaks characteristic of pristimerin(4) and 3-oxofriedelan-28-ol(80) in the DEPT technique could be identified(Figure 4, p. 48). Peaks identified as characteristic of pristimerin[7] are: (i) δ134.42(d, C-6), (ii) δ120.1(d, C-1), (iii) δ118.5(d, C-7) and δ51.8(q, C-31) and peaks identified as typical of 3-oxofriedelan-28-ol are: δ74.98(t, C-28), (ii) δ59.64(d, C-10), (iii) δ58.43(d, C-4) and δ53.60(d, C-8).

From the above information it was concluded that pristimerin(4) is a constituent of *C. transvaalensis*.



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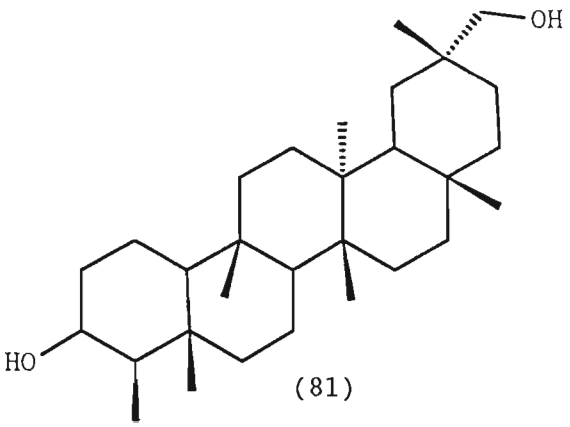

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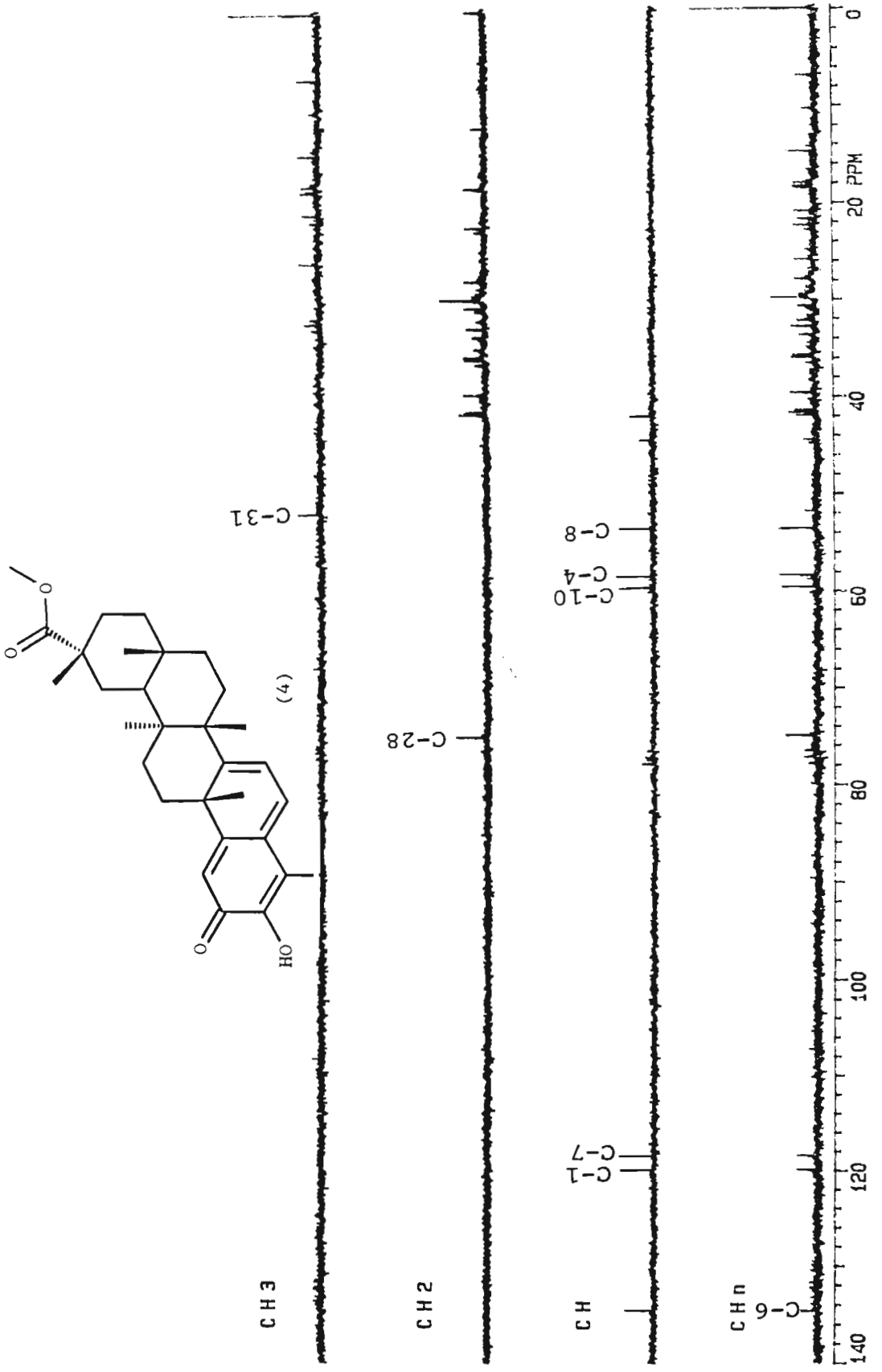


FIGURE 4. DEPT spectrum of pristimerin

2.2 Extractives from *Cassine papillosa* (Hoechst) Kuntze.

Air dried powdered stem bark of *Cassine papillosa* **(Hoechst) Kuntze** were collected from Ingwavuma (Natal), 2.3 kg, was extracted in a Soxhlet extraction apparatus with hexane followed by successive extractions with benzene, ethyl acetate and ethanol. The average extraction period for each solvent was 24 hours, by which time extraction appeared to be complete as the solvent was no longer coloured.

C. papillosa could have been collected from Phiphidi (Venda) but in this intance the bark was available from Ingwavuma(Natal).

2.2.1 Galactitol(75).

Galactitol separated out as a white crystalline material when the ethanol extract was allowed to cool to room temperature. Recrystallization using methanol-ethanol-water mixture afforded pure galactitol which had physical properties identical to those reported in the literature[102] and the ones obtained for *C. transvaalensis*.

2.2.2 (-)4'-O-methoxyepigallocatechin(12).

(-)4'-O-methoxyepigallocatechin(12) was isolated from the ethyl acetate extract as a whitish amorphous material(1.3 g), R_f0.26(dichloromethane: ethyl acetate, 1:1), after repeated column chromatography. Characterization of the compound as (-)4'-O-methoxyepigallocatechin was based on its failure to reduce ammoniacal $AgNO_3$ (which is a good qualitative test for the presence of orthohydroxyphenols), physical and spectroscopic evidence.

The IR spectrum of the compound indicated the presence of hydroxyl groups(3400 cm^{-1}, broad). Its ^1H NMR(p. 179) closely agrees with that obtained from ouratea-catechin, (-)4'-O-methoxyepigallocatechin previously isolated from *Ouratea*[123]

species and *Maytenus rigida*[28]. This was fully characterized by ^{1}H NMR by Anjaneyulu *et al.* after finding the third source *Elaeodendron glaucum*[124]. Incorrect assignments of H-6 and H-8 by the previous authors[28,123] were observed.

^{13}C NMR studies(p. 180) which were not previously undertaken show interesting results. HETCOR(Figure 5) reveals the correct assignments of ^{1}H NMR as fully described by Anjaneyulu *et al.*[124] except for H-6 and H-8. The methylene group carbon(δ28.78, t), as revealed by DEPT(p. 181), is associated with two nonequivalent protons at δ2.87. The methoxy carbon(δ60.51, q) is linked to the methyl protons at δ3.78(3H, s). Incorrect assignments of H-6 and H-8 was also observed based on HETCOR(Figure 5) and calculations of ^{13}C NMR chemical shifts of oxysubstituted benzene ring using equation 1.

$$\delta_c = 128.5 + \Sigma z_i \quad \text{[125]} \qquad \text{equation 1}$$

Calculated results are as follows: $\delta_{C\text{-}6}$ = 95.0 and $\delta_{C\text{-}8}$ = 93.8. From the calculated results, the chemical shift of C-8 should be upfield to that of C-6. This conclusion is further supported by the ^{13}C NMR of (-)-epicatechin(Table 8)[126]. ^{13}C NMR(p. 180) as shown on 200 MHz show doublet peaks at δ96.14 and 95.59 and correlation of this results with the proton spectra link C-6 to δ6.02 and C-8 to δ5.93. COSY(correlation spectroscopy) show H-2(δ4.84, 1H, d, J = 0.78 Hz) to be coupling to H-3(δ4.23, 1H, d, J = 3.89 Hz), H-4(δ2.87, 2H) and B-ring aromatic protons(δ6.59, 2H, s). COSY results(Figure 6) confirm the correct assignment to H-2 oxymethine proton as opposed to H-3 oxymethine proton which cannot couple to H-2′ and H-6′. This result further confirms the assignment of aromatic protons in the B-ring to C-2′ and C-6′. Tabulated ^{13}C NMR are provided in Table 7 and are compared with those of (-)-epicatechin(82).

The mass spectrum shows the molecular ion *m/z* 320 which, when coupled with elemental analysis, suggests the molecular formula $C_{16}H_{16}O_7$(calculated% C,60.00; H4.85, found C,60.19; H,4.28).

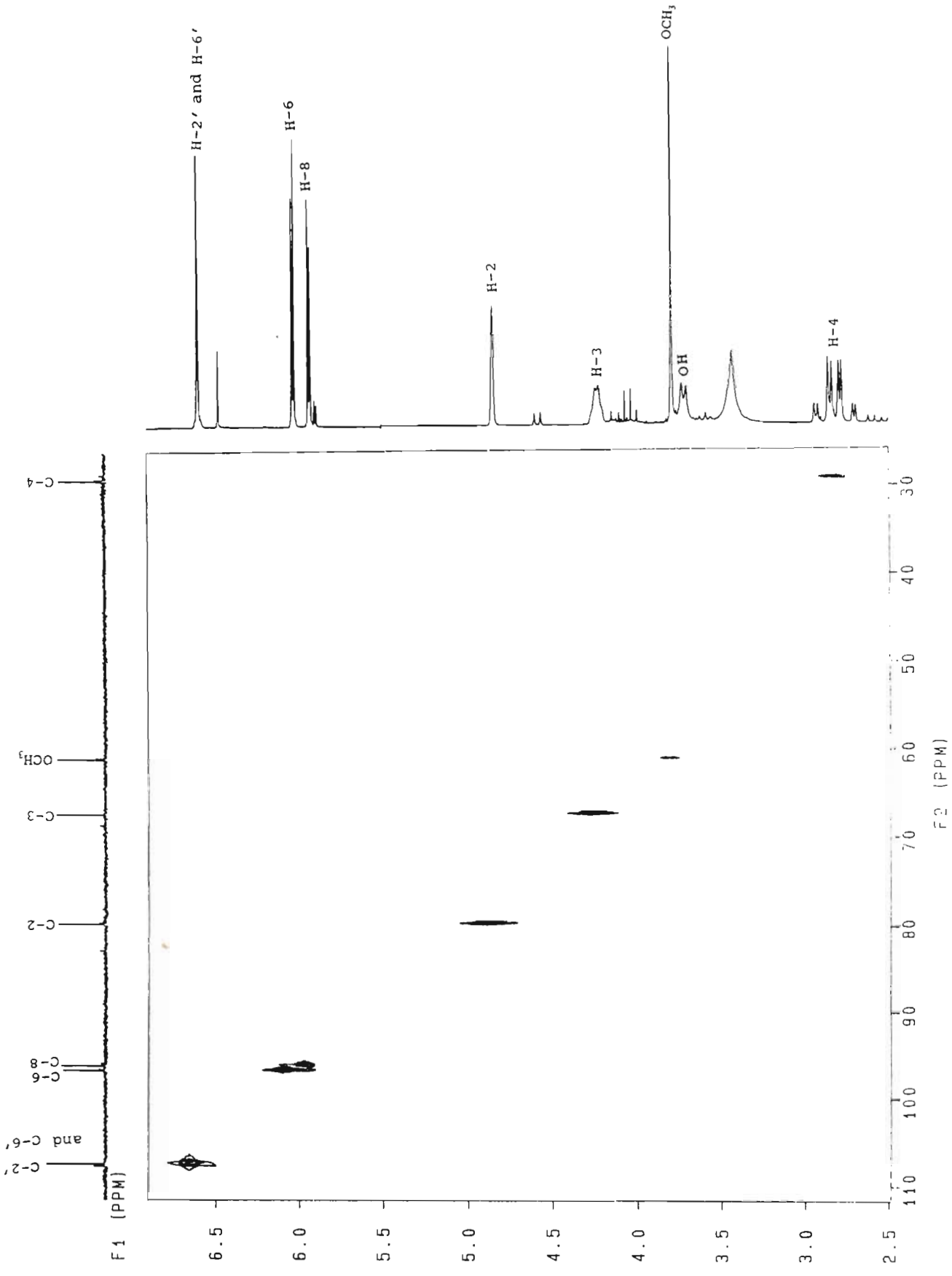


FIGURE 5. HETCOR spectrum of (-)4′-O-methoxyepigallocatechin.

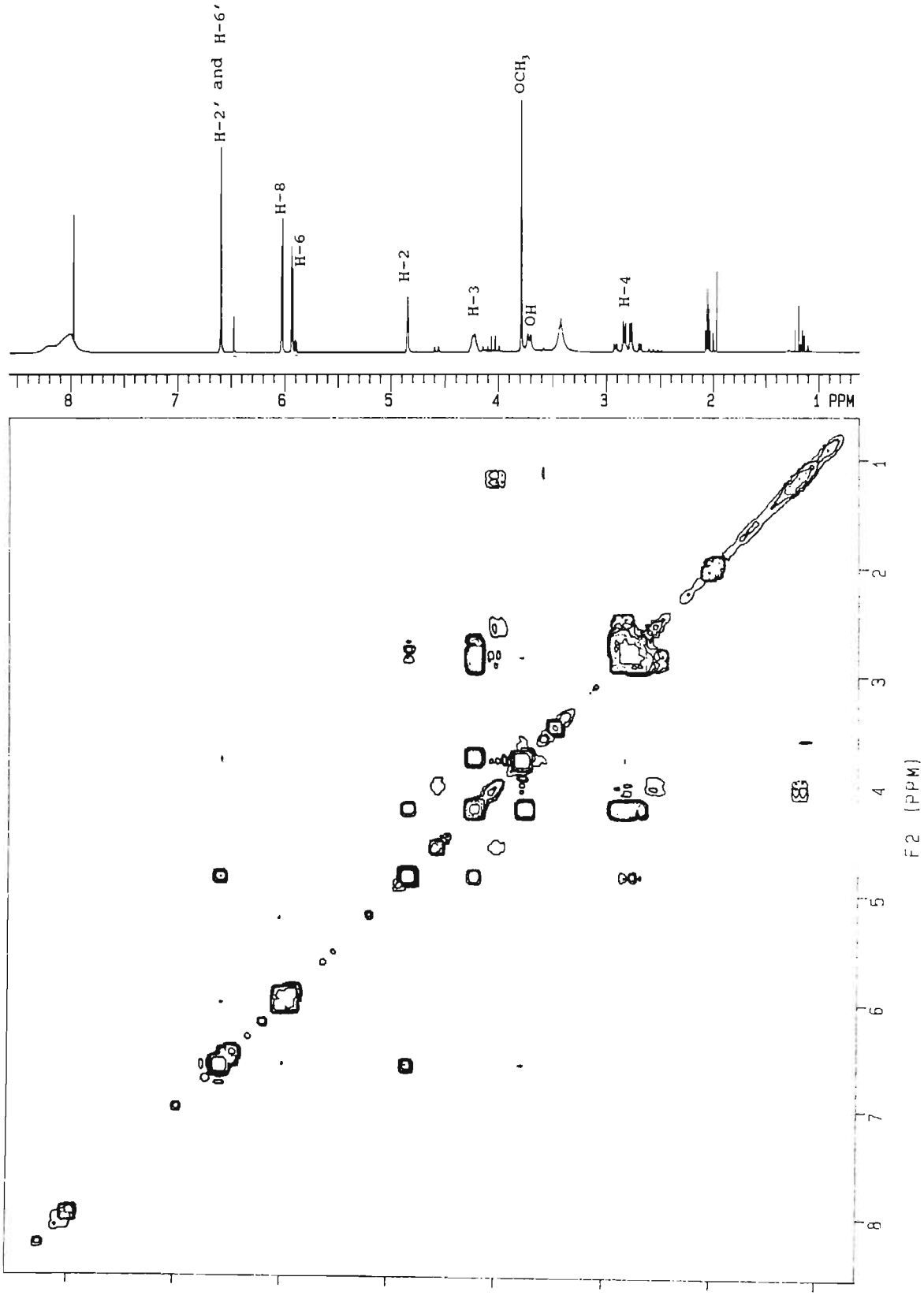
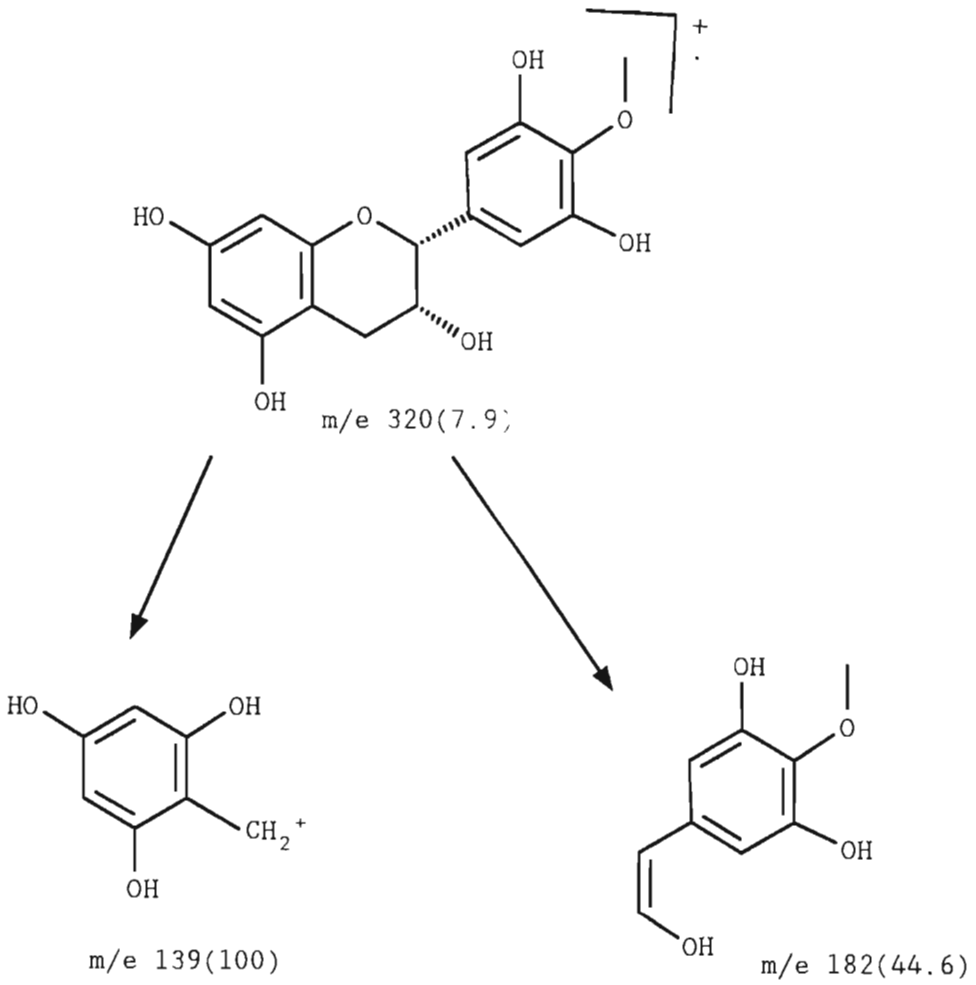


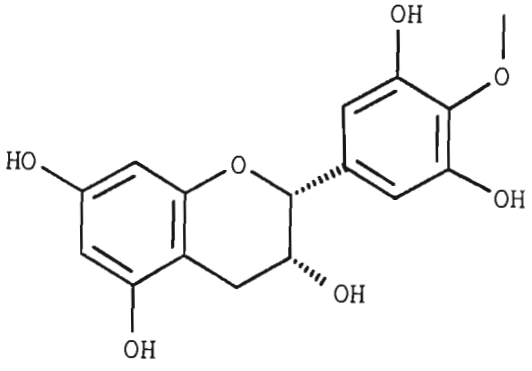
FIGURE 6. COSY spectrum of (-)4′-O-methoxyepigallocatechin.

Cleavage patterns observed(Scheme 8) confine the methoxy group to the B-ring and the A-ring to be meta dihydroxysubstituted. Cleavage of ring C between C(1)-C(2) and C(3)-C(4) results in the formation of a base peak, *m/z* 139 and the fragment ion *m/z* 182 which limits the methoxy group to the B-ring and the hydroxyl group to C-3.



SCHEME 8 Mass fragmentation pattern of (-)-4'-O -methoxyepigallocatechin(12).

The compound has mp 141°C and $[\alpha]_D^{22°C}$ = -50.1 which is in agreement with the values obtained by Anjaneyulu *et al.*[124].



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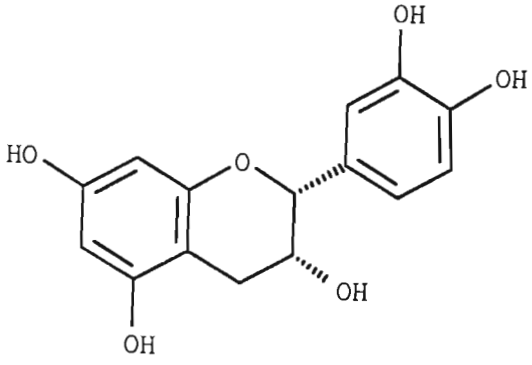


(82)

Table 7.

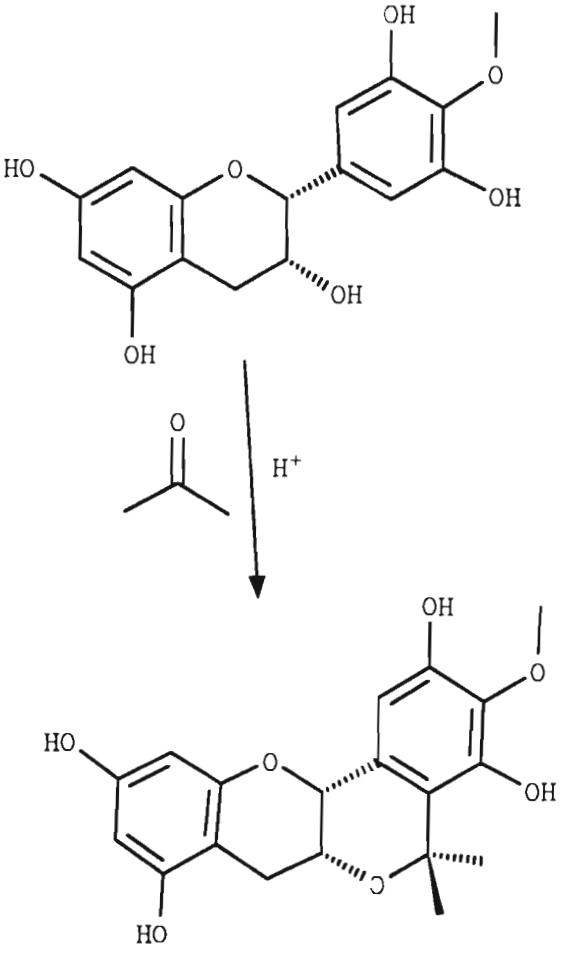
^{13}C NMR of the isolated compound and (-)-epichatechin compared.

Carbon	Present investigation	(-)-epicatechin (82)[126]
2	79.14d	78.1d
3	66.78d	65.1d
4	28.78t	28.0t
5	156.81s	156.0s
6	96.14d	95.6d
7	157.44s	156.3s
8	95.59d	94.5d
9	157.40s	155.7s
10	99.72s	98.8s
1′	136.16s	130.7s
2′	106.97d	118.1d
3′	150.74s	144.4s
4′	135.38s	144.5s
5′	150.74s	115.0d
6′	106.97d	118.1d
$O\underline{C}H_3$	60.51q	

2.2.3 (+)6R,13R-11,11-Dimethyl-1,3,8,10-tetrahydroxy-9-methoxypeltogynan(83).

Removal of the ethanol under reduced pressure, after the decantation separation of galactitol, followed by dissolution of

the brown extract and extraction with chloroform afforded a brown solid. Repeated column chromatography followed by recrystallization using hexane-ethyl acetate solvent mixture afforded 378 mg of (+)-6R,13R-11,11-dimethyl-1,3,8,10-tetrahydoxypeltogynan which had physical and spectral properties identical to the compound previously isolated[27].



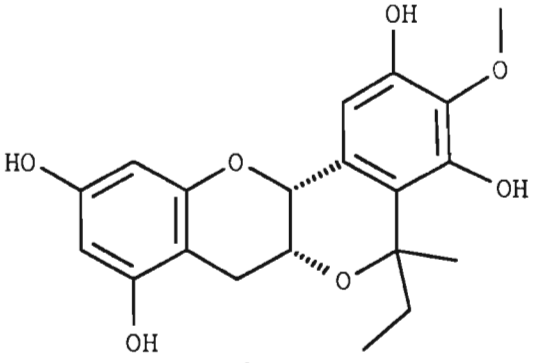
SCHEME 9 Synthesis of (+)6R,13R-11,11-Dimethyl-1,3,8,10-tetrahydroxy-9-methoxypeltogynan(83) from epigallocatechin.

The absolute configuration of the peltogynan previously isolated from *C. transvaalensis* and *Elaedendron balae*[27] was established by a reaction between acetone and (-)4'-O-methoxy epigallocatechin (12). The reaction which proceeds with retention of configuration

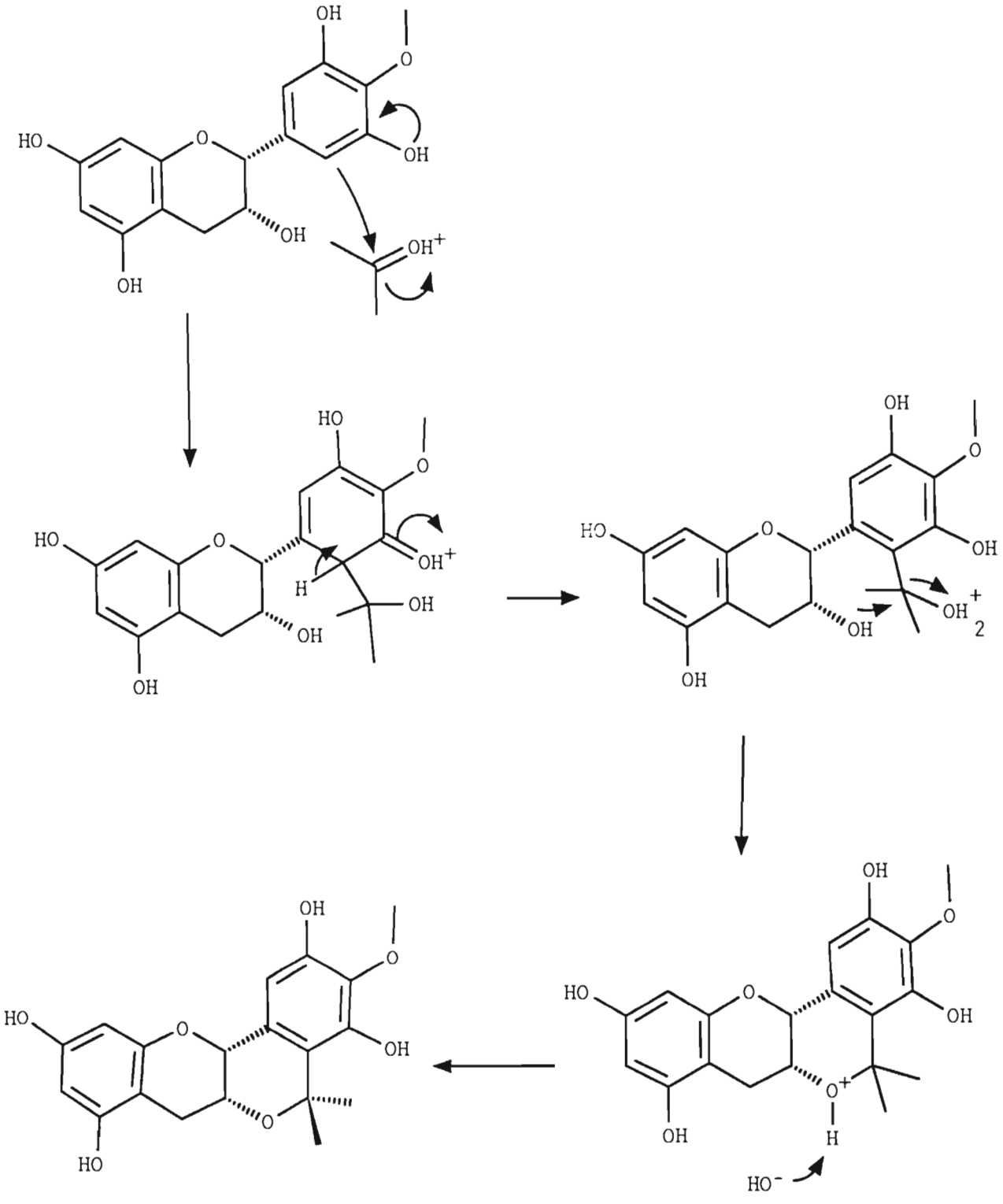
at C-2 and C-3(Scheme 9) leads to a tetracyclic peltogynan (83), identical in all respects to that previously isolated[27].

Treatment of flavan-3-ols in acetone at ambient temperature with a variety of acids such as sulphuric acid (98%), concentrated hydrobromic - and hydrochloric acid, and p-toluene sulphonic acid to give a tetracyclic ring system in peltogynoids[127] strongly suggest a biogenetic relationship between (-)4'-O-methoxy epigallocatechin and (+)6R,13R-11,11-dimethyl-1,3,8,10-tetrahydroxy-9-methoxypeltogynan(83), both isolated from the stem bark of *C. papillosa*.

The successful reaction between (-)-4'-O-methylepigallocatechin (12) and acetone in p-toluene sulphonic acid indicates a route towards the synthesis of 11,11-dimethyl substituted peltogynans (Scheme 9). Attempted preparation of similar peltogynans using formaldehyde, paraformaldehyde and acetaldehyde as reagents were unsuccesful. However, the reaction with butanone gave a mixture 11-methyl-11-ethyl substituted peltogynans(84) {^{1}H and ^{13}C NMR data on p. 182 - 184}. A conclusion drawn from this observation is that the reaction proceeds with ketones and not aldehydes. In contrast to our observation is the reaction between aldehydes and ketones with phenols in the presence of acid catalysts to give bisphenol acetals[128,129].



(84)



SCHEME 10 Proposed mechanism for the reaction of (-)-4'-o-methylepigallocatechin(12) and acetone.

The most appropriate mechanism(Scheme 10) for this reaction is nucleophilic attack of the protonated carbonyl carbon of acetone by the phenolic B ring to form the intermediate dimethylmethylol. The presence of water presumably leads to C-alkylation[127]. This derivative could then serve as intermediate for the tetracyclic peltogynan. Such a sequence closely parallels the course of the reactions usually employed for the synthesis of the peltogynoids[130,131,132].

Notable in the formation of this peltogynan is the higher yield obtained by us as compared to yields obtained by Ferreira *et al.*[127]. This is presumably attributed to the methyl substituted pyrogallol-type B-ring in (12) acting as a more potent nucleophile as compared to the pyrocatechol-type B-rings and the unsubstituted pyrogallol-type B-ring, an observation previously made by Ferreira *et al.*[127].

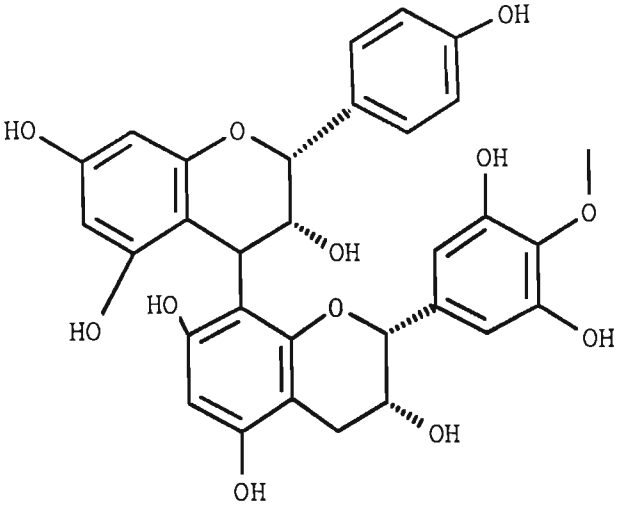
2.2.4 Ouratea proanthocyanidin(13).

The structure of the terminal unit of ouratea proanthocyanidin (13) previously isolated from *E. balae*[27] can be established using NOE experiments[133]. Irradiation at 862.22 Hz {which is the resonance frequency of the 3t (t = terminal unit) proton} enhances the intensities of the 2t, 4t, 2't and 6't protons chemical shift peaks(Figure 7). Taking into consideration that the 4t protons have an integral equivalent to 2H and that its chemical shift appears at δ2.85 one can infer that it is the terminal unit which bears the methylene group. Furthermore the DEPT and HETCOR experiments(p. 187 and 188 respectively) confirm the peak at δ2.85 to be a methylene peak. Irradiation at the 2t resonance frequency (978.97 Hz) enhances the intensities of the 3t, 2't and 6't proton chemical shift peaks(Figure 8) whilst irradiation at the 4t protons' resonance frequency only enhances the 3t proton chemical shift peak(Figure 9).

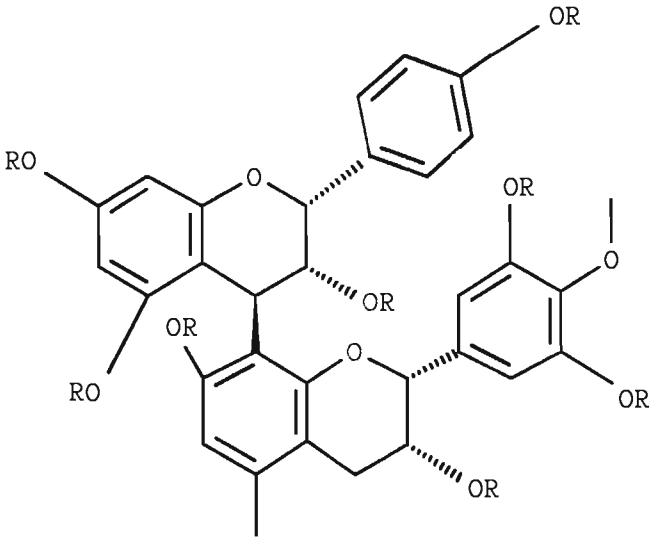
The 4,8-linkage of ouratea proanthocyanidin(13) was confirmed by ^{1}H NMR studies of the nona-O-acetyl derivative(Table 8). The

rotamer populations(p. 189) for the nona-O-acetyl derivative(85) are present in the ratio of 2:1{δ7.47[b]; δ7.39[a], J = 8.54 Hz, for H-6'u and H-2'u}; {δ7.09[b]; δ7.05[a], J = 8.72 Hz for H-3'u and H-5'u}; { δ6.27[b]; δ6.98[a], J = 2.29 Hz, for H-6u}; δ6.60[a]; δ6.08[b], J = 2.29 Hz, for H-8u (u = upper unit)}. Furthermore the signals for H-4u appear at δ4.49; δ4.47 and δ4.46. The above information is in agreement with C(4)-C(8) linked proanthocyanidins[134] and also confirms a 4R configuration of the upper unit[135].

a = minor rotamer.
b = major rotamer.



(13)



(85) R: CH_3CO

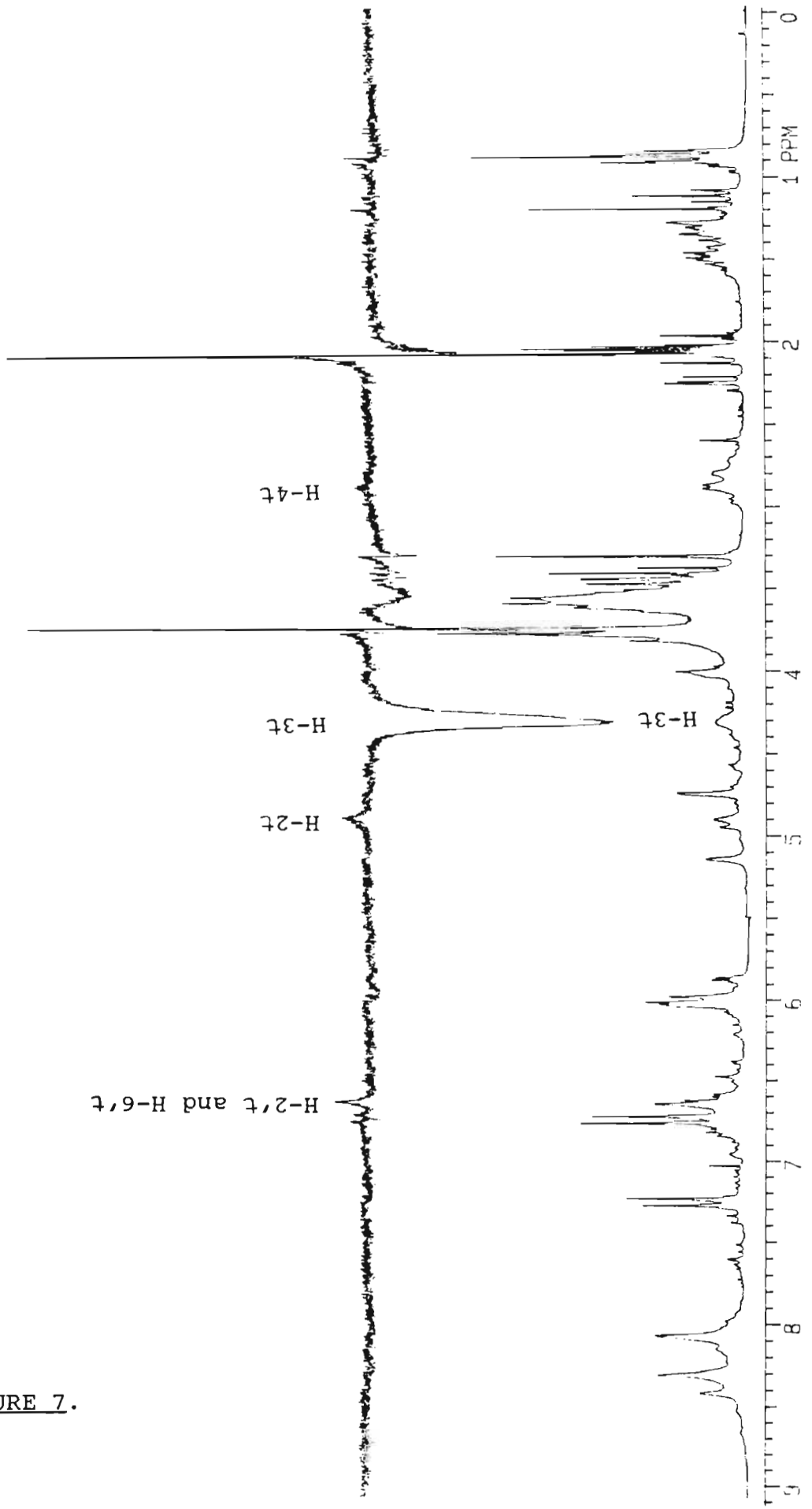


FIGURE 7.

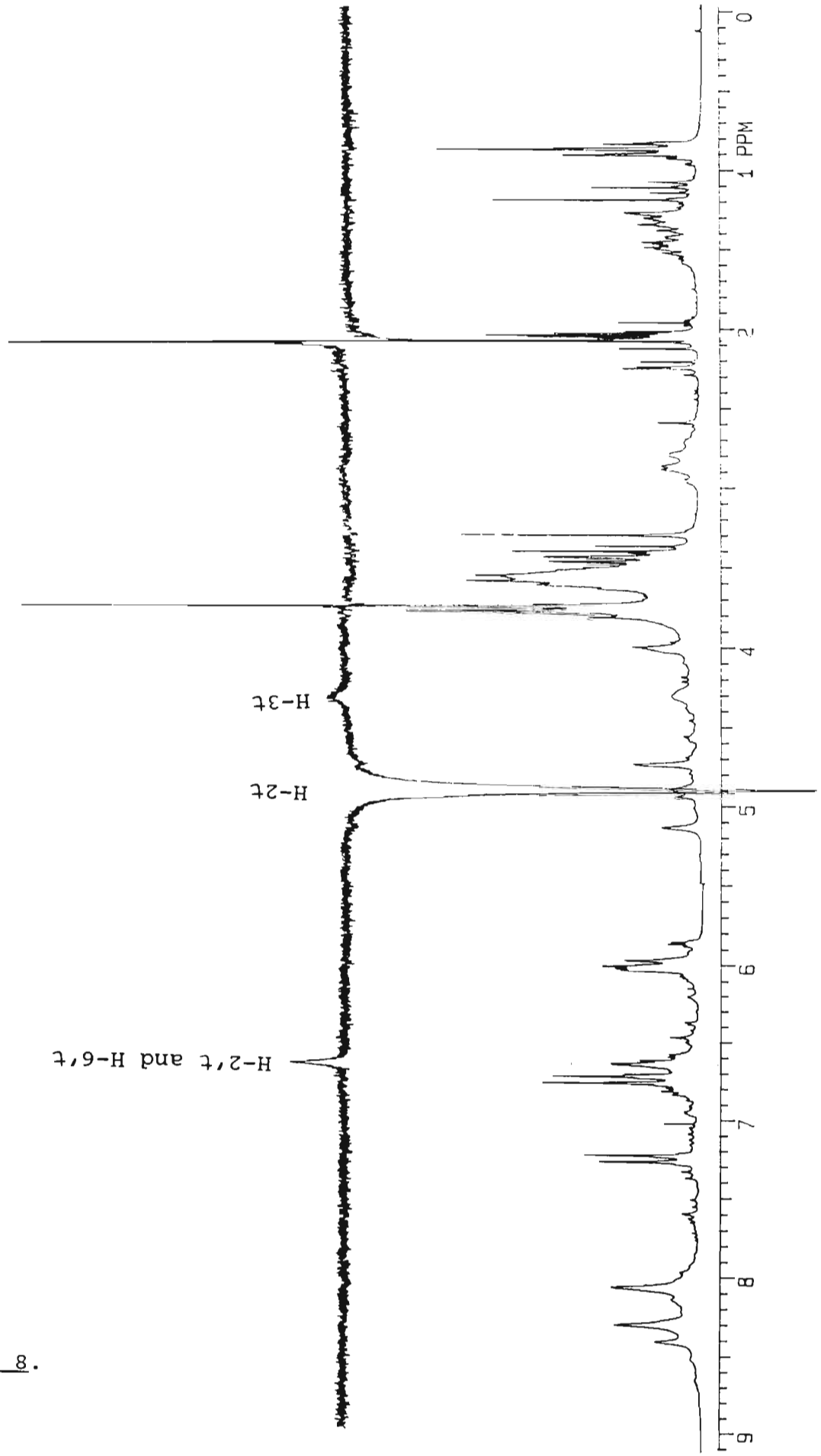


FIGURE 8.

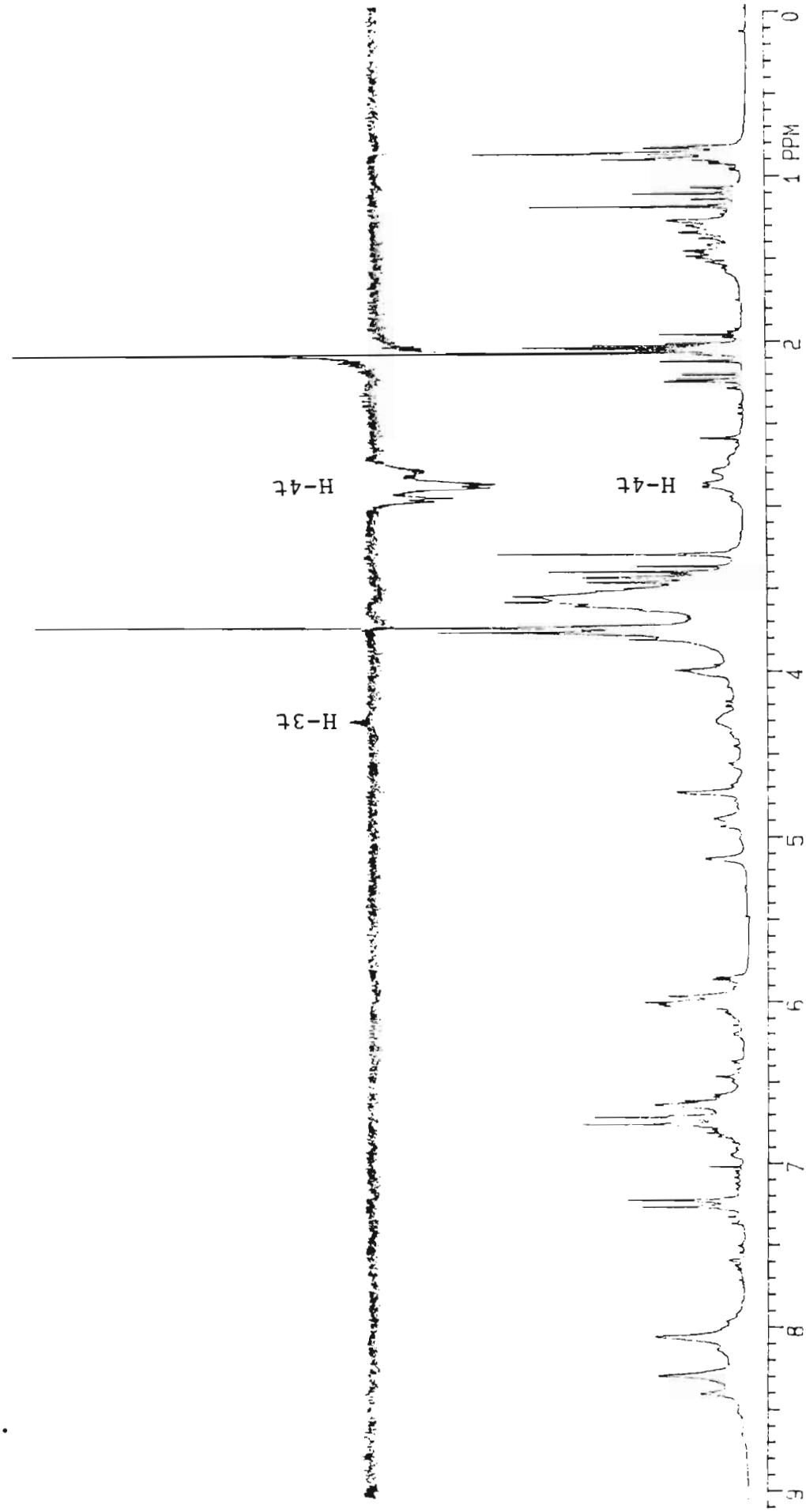


FIGURE 9.

Table 8.

Chemical shift peaks of H-6u, H-6t and H-8u in ouratea proanthocyanidin nona-O-acetate(85).

Chemical Shifts(δ)	Coupling Constant(Hz)	Proton
7.47	8.54	H-2'u and H-6'u major rotamer
7.73	8.54	H-2'u and H-6'u minor rotamer
7.09	8.72	H-3'u and H-5'u major rotamer
7.05	8.72	H-3'u and H-5'u minor rotamer
6.27	2.29	H-6u major rotamer
6.98	2.29	H-6u minor rotamer
6.60	2.29	H-8u minor rotamer
6.08	2.29	H-8u major rotamer

2.2.5 30-Hydroxylup-20(29)-en-3-one(2).

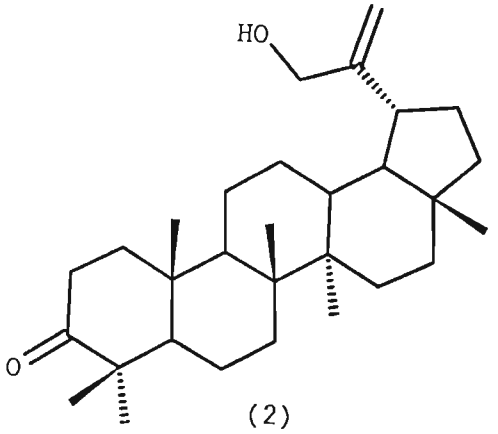
30-Hydroxylup-20(29)-en-3-one(2) was isolated as a white crystalline material(1.2 g) from the hexane extract of the plant material. Characterization of the compound as 30-hydroxylup-20(29)-en-3-one is based on physical and spectroscopic evidence.

The molecular formula of the isolated compound was shown to be

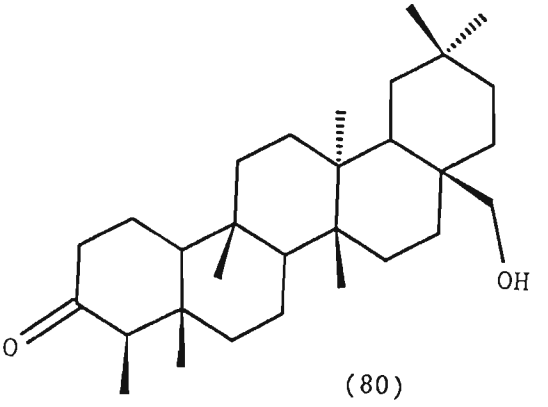
$C_{30}H_{48}O_2$ (*m/z* calc. 440.3654, found 440.3725). The IR spectrum of the compound showed the presence of a vinylidene group(1640 and 880 cm^{-1}), the hydroxyl group(3540 cm^{-1}), geminal methyl groups(1460 and 1390 cm^{-1}) and a carbonyl group(1700 cm^{-1}).

Mass spectral fragmentation patterns strongly suggest the compound to be a lupene-type triterpene(Scheme 11) with a carbonyl at C-3 and a hydroxyl group at C-30. The mass spectrum show significant peaks at *m/z* 206(a and e) and 234(b). Loss of angular methyl group from fragment ion *m/z* 206 results in the formation of fragment *m/z* 191 which on loss of proton gives rise to a fragment at *m/z* 190. The b-type fragment, *m/z* 234, on loosing the angular methyl group gives rise to a fragment at *m/z* 219. The above information confines the carbonyl group to rings A and B and the secondary alcohol to C-27 and C-30.

In addition to resonances assigned as six methyl groups, the ^1H NMR spectrum(p. 192) of the compound contained signals corresponding to one hydroxymethyl group(δ4.11, 2H, doublet, J = 1.13 Hz) and two olefinic protons(δ4.94) which are readily accommodated in the structure of 30-Hydroxylup-20(29)en-3-one(2).

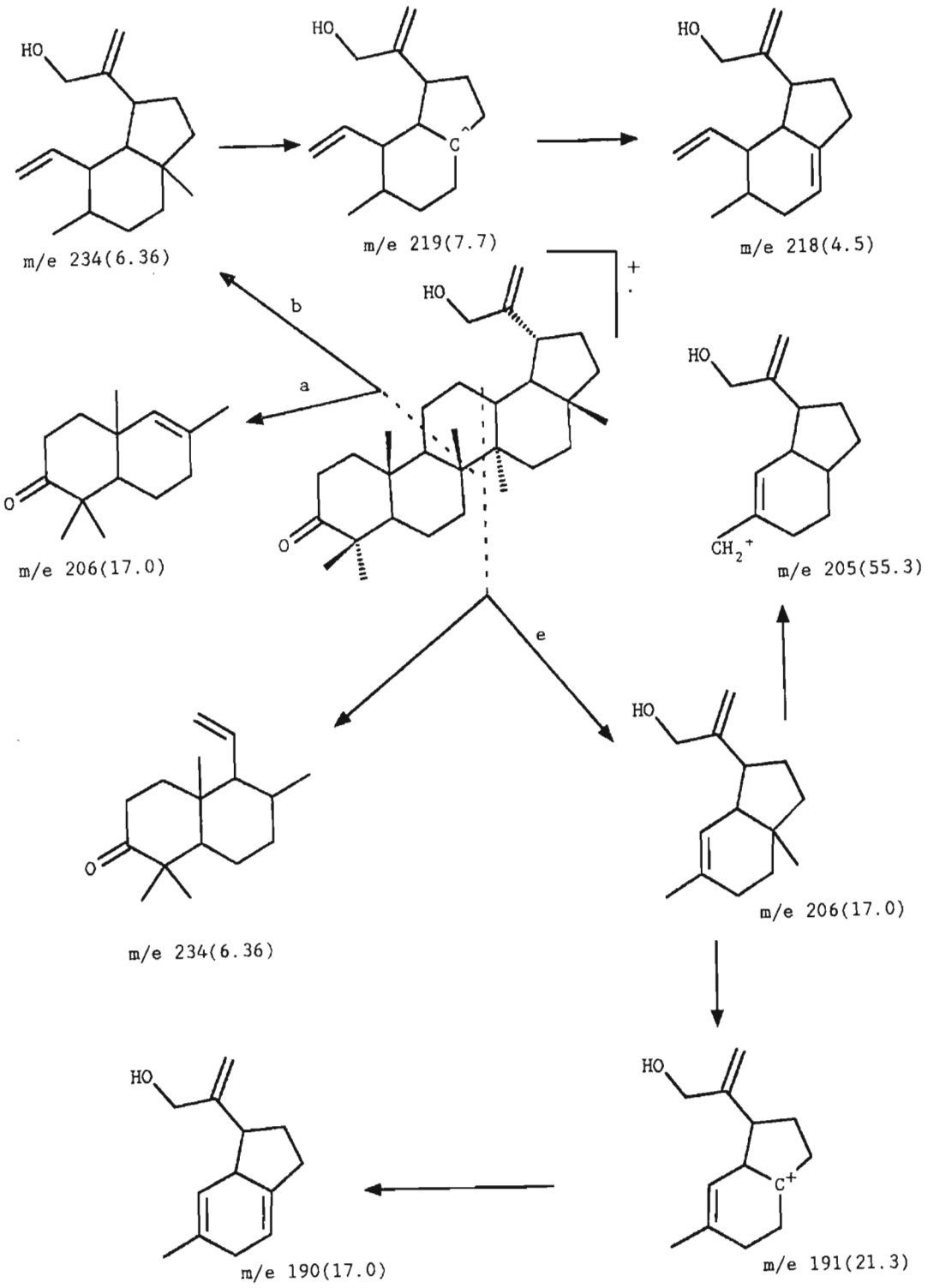


(2)



(80)

The ^{13}C NMR(p. 193, 194) showed the presence of a vinylidene group {δ106.70(C=$\underline{C}H_2$), δ154.63($\underline{C}$=CH_2)}, one oxymethine(δ64.87), one



SCHEME 11 Mass fragmentation pattern of 30-Hydroxylup-20(29)-en -3-one(2)

carbonyl group(δ218.35) and six methyl groups{δ14.43, δ15.76, δ15.97, δ17.69, δ21.01 and δ26.63 } which is in agreement with the structure of 30-hydroxylup-20(29)-en-3-one(2). The full ^{13}C NMR spectrum of the compound is presented in table 9.

Table 9.

The ^{13}C NMR Chemical Shifts of Lupene-type triterpenes.

Number of		Substance.			
Carbon	Lupeol[136]	2	86	93	88
C-1	38.7	39.78	40.93	36.69	38.80
C-2	27.5	27.34	28.65	27.33	27.48
C-3	79.0	218.35	79.63	77.33	78.96
C-4	38.9	38.04	39.24	40.19	38.84
C-5	55.3	54.79	57.65	55.07	55.43
C-6	18.3	19.63	20.44	17.84	18.36
C-7	34.3	43.69	36.48	31.96	34.36
C-8	40.9	39.54	40.93	40.19	40.79
C-9	50.5	49.67	52.67	50.09	50.55
C-10	37.2	36.81	36.48	38.61	37.13
C-11	21.0	21.51	23.06	20.26	20.97
C-12	25.2	26.63	29.66	25.15	25.18
C-13	38.1	38.04	37.60	37.67	38.13
C-14	42.9	42.79	42.97	41.94	42.80
C-15	27.5	31.70	29.74	29.35	27.50
C-16	35.6	35.58	37.60	33.92	35.67
C-17	43.0	42.97	44.81	55.75	42.98
C-18	48.0	48.72	50.63	46.86	48.12
C-19	48.3	47.29	45.00	48.85	48.41
C-20	150.9	154.63	157.71	150.69	150.94
C-21	29.9	33.51	33.73	30.26	29.91
C-22	40.0	40.73	41.86	38.40	40.11
C-23	28.0	26.63	29.58	27.76	28.07
C-24	15.3	15.76	17.28	15.42	15.42
C-25	16.1	17.69	17.89	15.45	16.17
C-26	16.0	15.97	17.67	15.45	16.01
C-27	14.6	14.43	16.14	13.96	14.58
C-28	18.0	21.01	19.26	178.35	18.05
C-29	109.3	106.70	107.40	109.32	109.66
C-30	19.2	64.87	65.95	18.56	19.35

Oxidation of 30-hydroxylup-20(29)-en-3-one(2) using CrO_3 in pyridine lead to the formation of 3-oxolup-20(29)-en-30-al(1),

a toxic principle isolated from *Maytenus nemerosa*[16]. Thus the compound under investigation can be used as the source of this cytotoxic compound.

2.2.6 3-Oxofriedelan-28-ol(80).

3-Oxofriedelan-28-ol(80), page 64, was isolated from the 95% aqueous methanol extract as white crystals, mp 278°C. Its IR spectrum indicated the presence of a hydroxyl group(3600 cm^{-1}), a carbonyl in a six membered ring(1710 cm^{-1}), and geminal dimethyl groups(1390 and 1450 cm^{-1}).

The ^{13}C NMR(p. 177) showed the presence of four methine groups, eleven methylene groups, one oxymethylene group(δ74.98, t), seven methyl groups, and one six-membered ring carbonyl(δ213.90, s). The ^{1}H NMR(p. 176) indicated the presence of seven methyl groups. A two-proton singlet at δ3.26 is due to the methylene of the primary alcoholic group.

The mass spectrum suggested a substituted friedelane derivative (Scheme 7) with a carbonyl group at C-3 and the hydroxyl group at C-28. Fragment ions *m/z* 140 and *m/z* 302 seem to arise from D-ring cleavage between C(13)-C(18) and C(16)-C(17) bonds and strongly suggest the hydroxyl group to be at C-28 and the carbonyl to be either at ring A, B or C. Fragment ion *m/z* 273 arises from an alternative cleavage of the D-ring between C(13)-C(18) and C(16)-C(17).

The above data and physical properties confirm the compound to be 3-oxofriedelan-28-ol(80) which is identical to canophyllol, a compound first isolated from *Calophyllum inophyllum* Linn by Govindachari *et al.*[118].

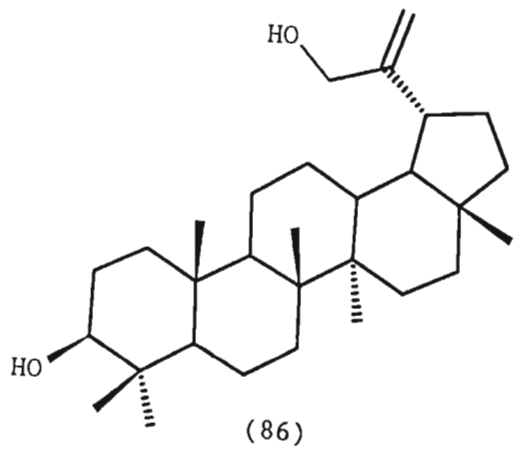
2.2.7 Lup-20(29)-en-3ß,30-diol(86).

Lup-20(29)-en-3ß,30-diol(86) was also isolated from the hexane extract of the plant after the removal of 30-hydroxylup-20(29)-

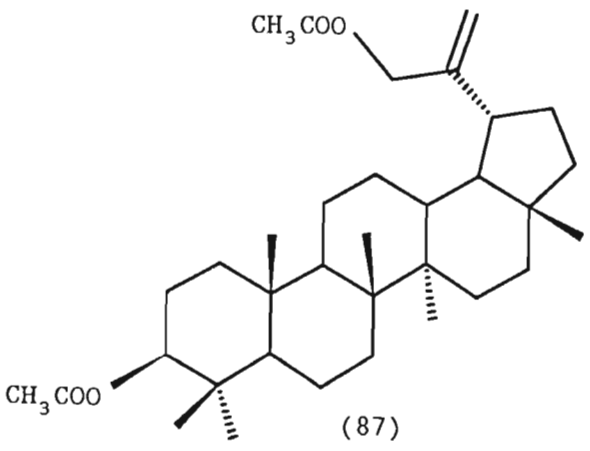
en-3-one(2) and 3-oxofriedelan-28-ol(80) from the silica gel column. It crystallized as a white amorphous material from hexane-ethyl acetate solvent mixture. The compound had mp 234-245°C, $[\alpha]_D^{23°C}$-13.905°. Based on elemental analysis and mass spectrum, the molecular formula of the compound was found to be $C_{30}H_{50}O_2$.

The IR spectrum indicated the presence of a hydroxyl groups(3350 cm^{-1}) which was easily acetylated, and geminal methyl groups(1380 and 1460 cm^{-1}).

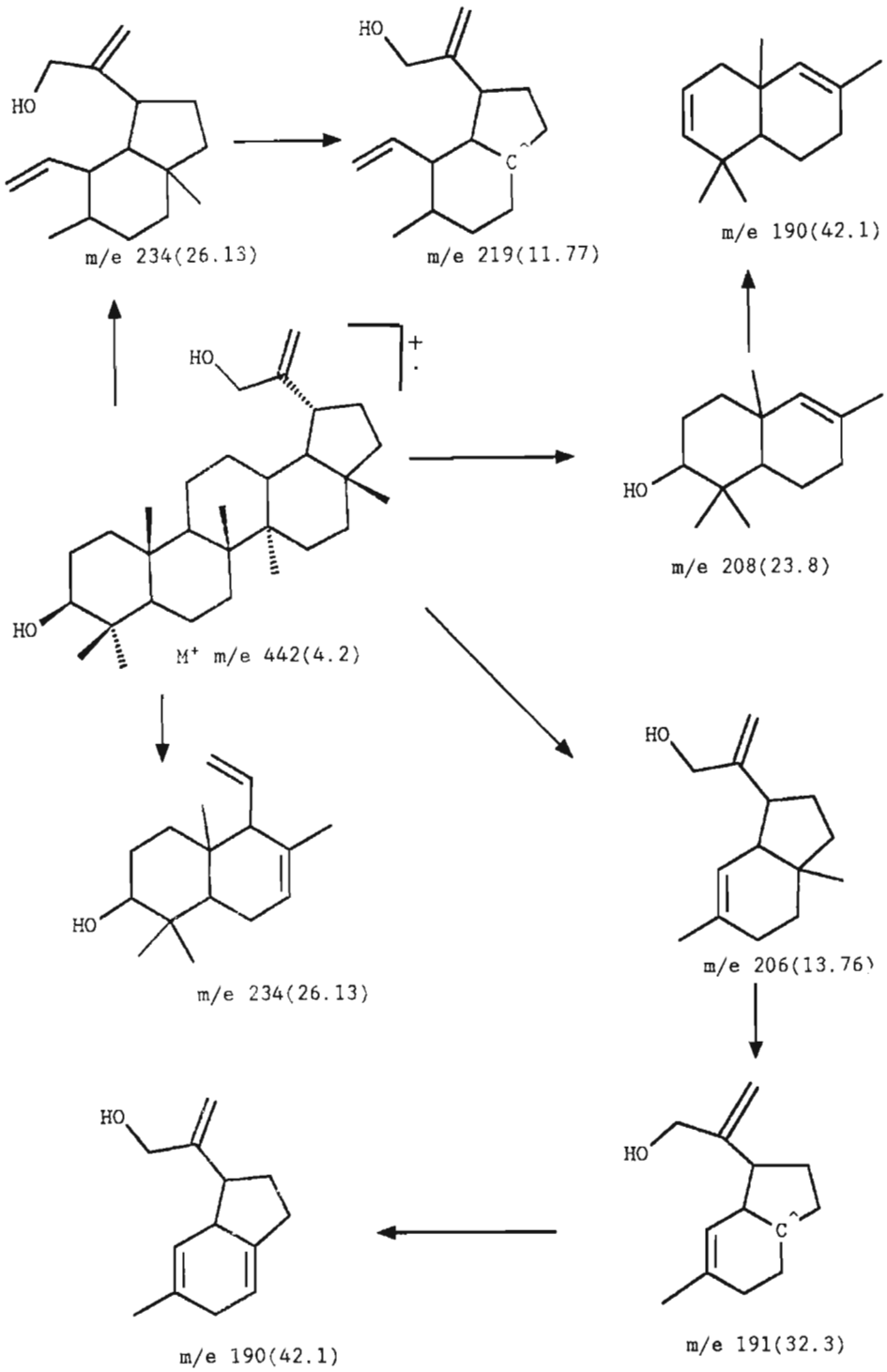
The fragmentation pattern observed in the mass spectrum strongly suggests the compound to be lup-20(29)-ene type and allowed the allocation of the hydroxyl at C-3 and the other hydroxyl group at C-30(Scheme 12). Cleavage of ring C between C(9)-C(11) and C(8)-C(14) bonds results in the formation of an ion *m/z* 208 which on loss of H_2O gave a peak at *m/z* 190. The above information confines the secondary hydroxyl group to ring A and the primary hydroxyl group to C-27, C-28 and C-30. Alternative cleavage of ring C between C(12)-C(13) and C(8)-C(14) bonds results in the formation of two fragments *m/z* 234 and *m/z* 206. Loss of the angular methyl group from fragment *m/z* 206 results in the formation of ion *m/z* 191 which on loss of proton give rise to a fragment at *m/z* 190. The above information limits the primary hydroxyl group to C-27 and C-30. Fragment *m/z* 234 also arises due to cleavage between C(9)-C(11) and C(8)-C(14) bonds which on loss of the angular methyl group give rise to a fragment *m/z* 218.



(86)



(87)



SCHEME 12 Mass fragmentation pattern of lup-20(29)-en-3ß,30-diol(86).

The DEPT technique(p. 197) indicates the presence of six methyl groups, one oxygen bonded methylene group(δ65.96), one methine bonded to oxygen(δ79.69) and a vinylidene group {δ157.7(C=CH_2), δ107.56(C=CH_2)}. ^{13}C NMR comparison of the compound(93) and related lupene-type triterpenes(table 9) suggest assignment of the primary hydroxyl group to C-30 and the secondary hydroxyl group to C-3. The ^{13}C NMR(p. 196) also reveals the absence of an isopropenyl group.

^{1}H NMR and ^{13}C NMR at 200 MHz reveals chemical shifts details not previously reported: (i) COSY spectra(correlation spectroscopy), p. 198, show coupling between the hydroxyl proton on carbon 3 and the methine bonded to oxygen(δ3.15, 1H, m) and the hydroxyl proton resonates as a doublet at δ3.53(J = 5.31 Hz). (ii) H-30 appears as a doublet at δ4.05(J = 5.13 Hz) and COSY reveals coupling between this methylene group and a hydroxyl group at δ3.88(1H, triplet, J = 6.04 Hz). (iii) H-19 appears as a doublet of doublets at δ2.36(1H, J = 5.13 and 10.98 Hz). (iv) HETCOR(heteronuclear correlation), p. 199 confirms the assignments of the hydroxyl groups, C-3(δ79.69), C-30(δ65.96) and C-19(δ45.90). ^{13}C NMR data of the compound are listed in Table 9.

Table 10.

Physical properties of the isolated acetate derivative and lup-20(29)-en-3ß,30-diacetate(87).

Physical Properties	Present investigation	Lup-20(29)-en-3ß 30-diacetate[15]
mp	163-164°C	163-164°C
Optical Rotation	$[\alpha]_D^{23°C}$ = +7.33° (c 0.15, chloroform)	[α] = +11.0°

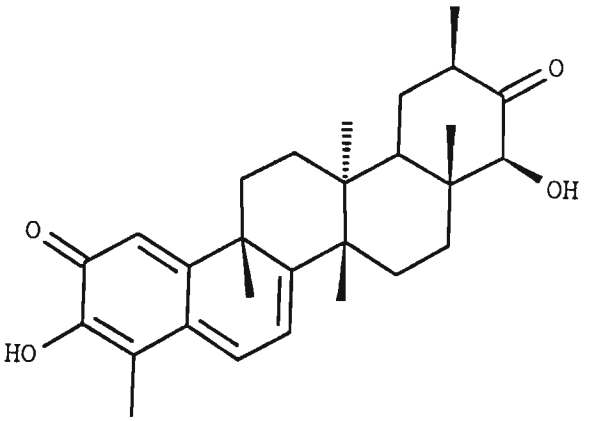
Comparison of physical and spectral data obtained for compound

(86) with those recorded for lup-20(29)-en-3ß,30-diol[15,137] indicated that the two were identical. The acetate derivative had similar physical properties with lup-20(29)-en-3ß,30-diacetate(87)[15](Table 10).

The compound (86) can be used as a source of a toxic principle, 3-Oxo-20(29)-lupen-30-al(1), by oxidation using CrO_3 in pyridine[16]. 3-Oxo-20(29)-lupen-30-al's toxicity is based on the carbonyl moiety at C-3 and the α,ß-unsaturated aldehyde moiety at C-19[16].

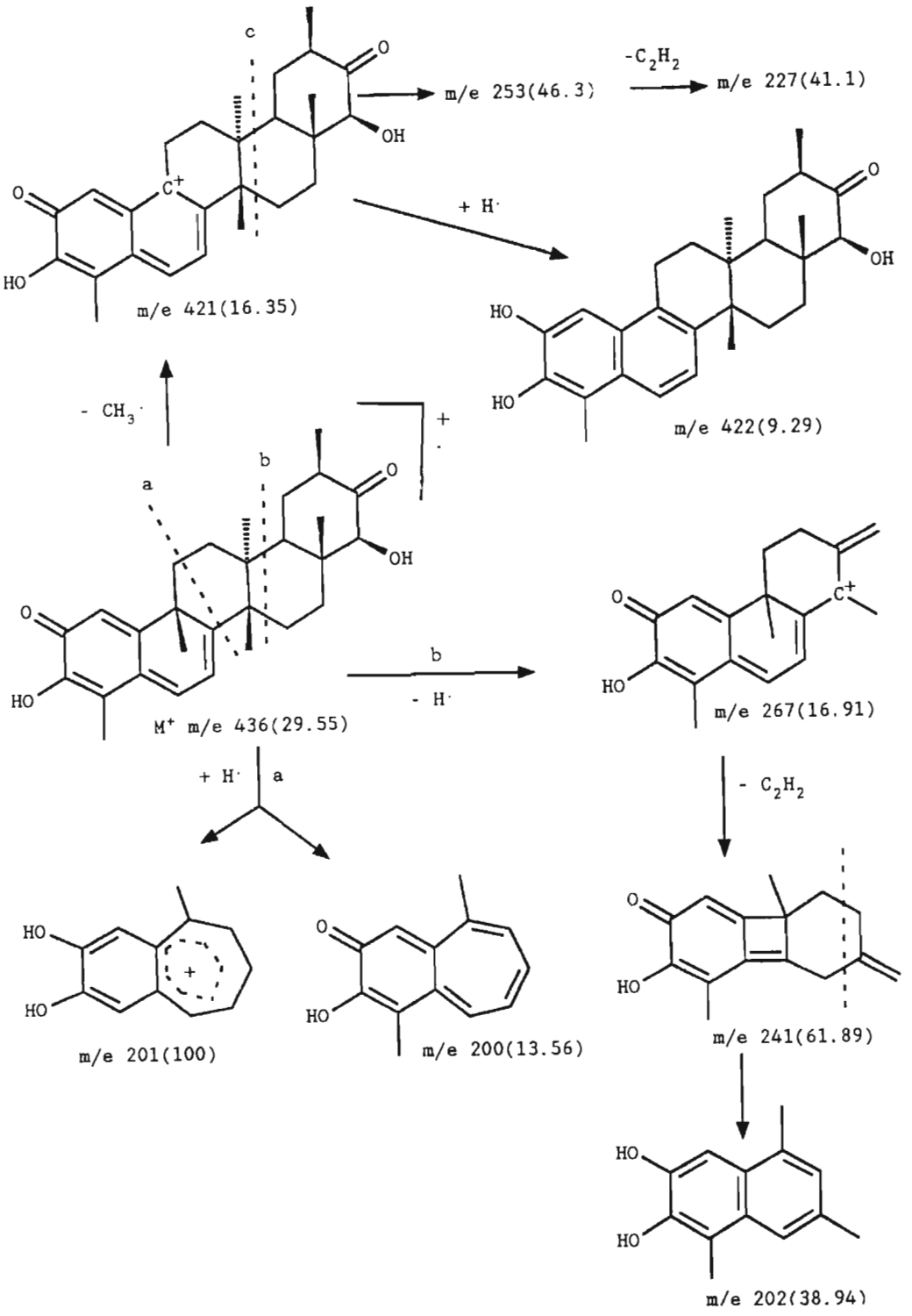
2.2.8 Tingenin B(22-hydroxytingenone)(7).

22-Hydroxytingenone(7) was isolated from the hexane extract. The hexane extract was partitioned between hexane and 95% aqeuous methanol. Repeated column chromatography of the 95% aqeuous methanol partitioned extract lead to the separation of a orange-red material which contained a mixture of 30-hydroxylupeol(86) and 22-hydroxytingenone(7). Recrystallization using hexane-ethyl acetate mixture lead to the separation of 30-hydroxylupeol(86) which recrystallized as white material. Removal of the mother liquor, after recrystallization of hydroxylupeol, afforded orange-red material which was recrystallized from tetrahydrofuran to give red crystals, mp 204-205°C.



(7)

Physical properties and spectral examination{^{1}H NMR(Table 11 and



SCHEME 13 Mass fragmentation pattern of 22-hydroxytingenone(7).

p. 203), ^{13}C NMR(p. 204), DEPT(p. 205) and mass(Scheme 13[138,139])}showed that the compound was 22-hydroxytingenone(7)[7].

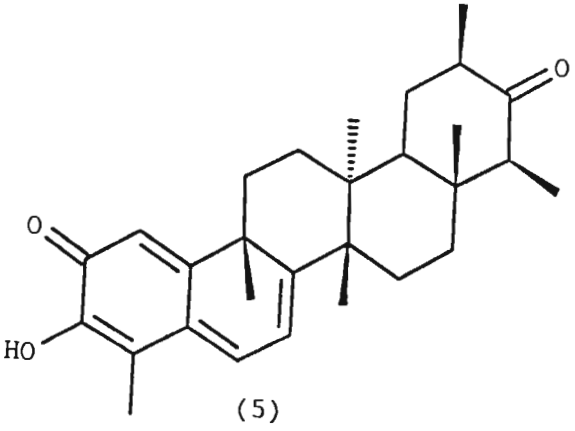
Table 11.

^{1}H NMR comparison of the isolated compound and 22-hydroxytingenone(7).

Proton	Present investigation	tingenin B[7]
H-23	δ2.23(3H, s)	δ2.22(3H, s)
H-1	δ6.54(1H, d, J = 1.37 Hz)	δ6.53(1H, d, J = 1.6 Hz)
H-6	δ7.05(1H, dd, J = 7.12 and 1.47 Hz)	δ7.05(1H, dd, J = 8.0 and 1.6 Hz)
H-7	δ6.39(1H, d, J = 7.18 Hz)	δ6.38(1H, d, J = 8.0 Hz)

2.2.9 Tingenone(5).

Tingenone(5) separated from the benzene extract as orange-red crystals(51 mg) contaminated with 22-hydroxytingenone(7). ^{1}H NMR data indicated the orange-red material to be a 1:1 mixture of 22-hydroxytingenone and tingenone(Figure 10). Furthermore ^{13}C NMR(Figure 11) showed distinct peaks at δ76.41(doublet) and δ52.53(triplet) typical of C-22 peaks of 22-hydroxytingenone(7) and tingenone(5) respectively[7].



(5)

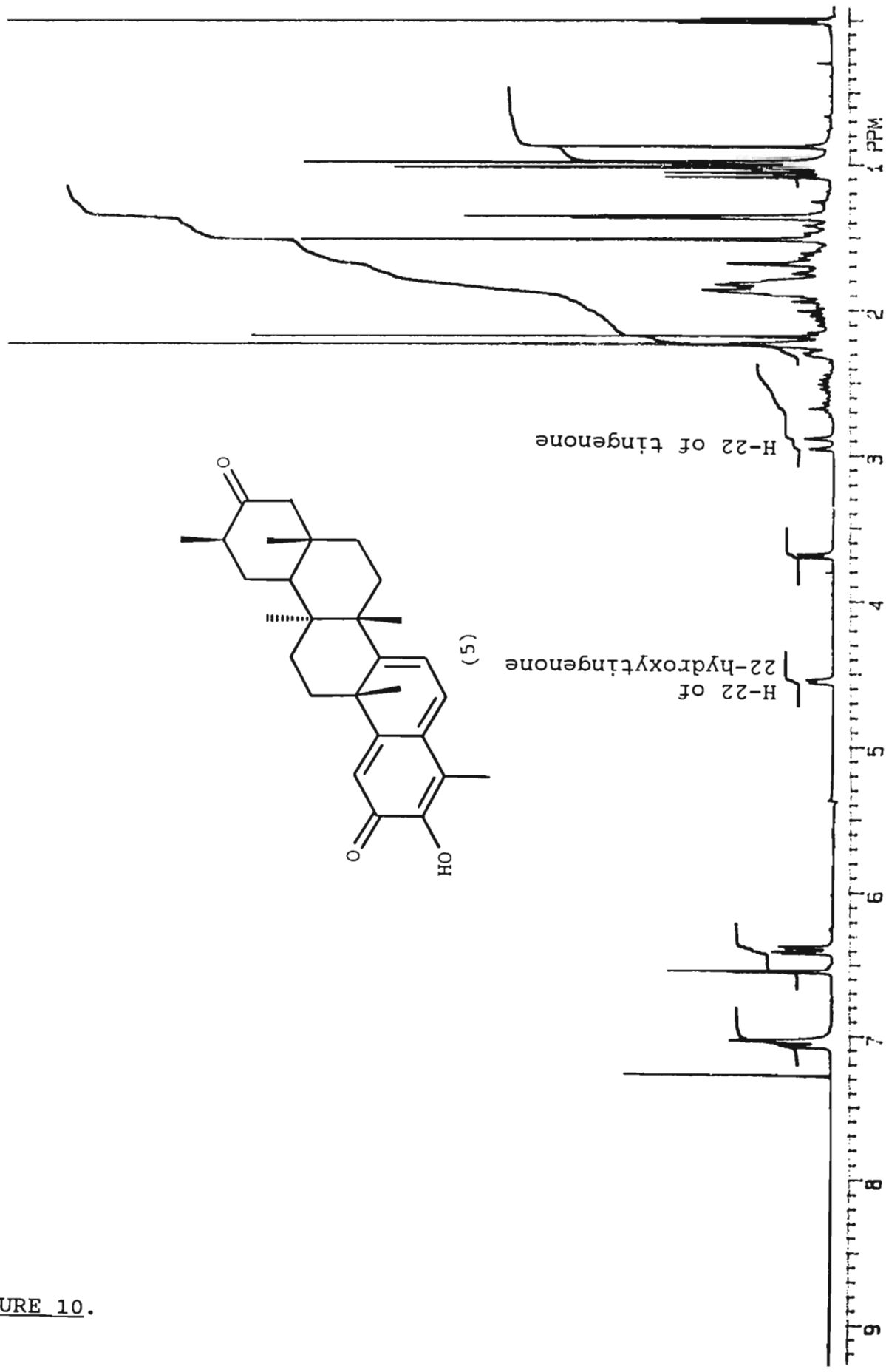

H-22 of tingenone
H-22 of
22-hydroxytingenone
(5)
HO
O
O
PPM
9
8
7
6
5
4
3
2
1

FIGURE 10.

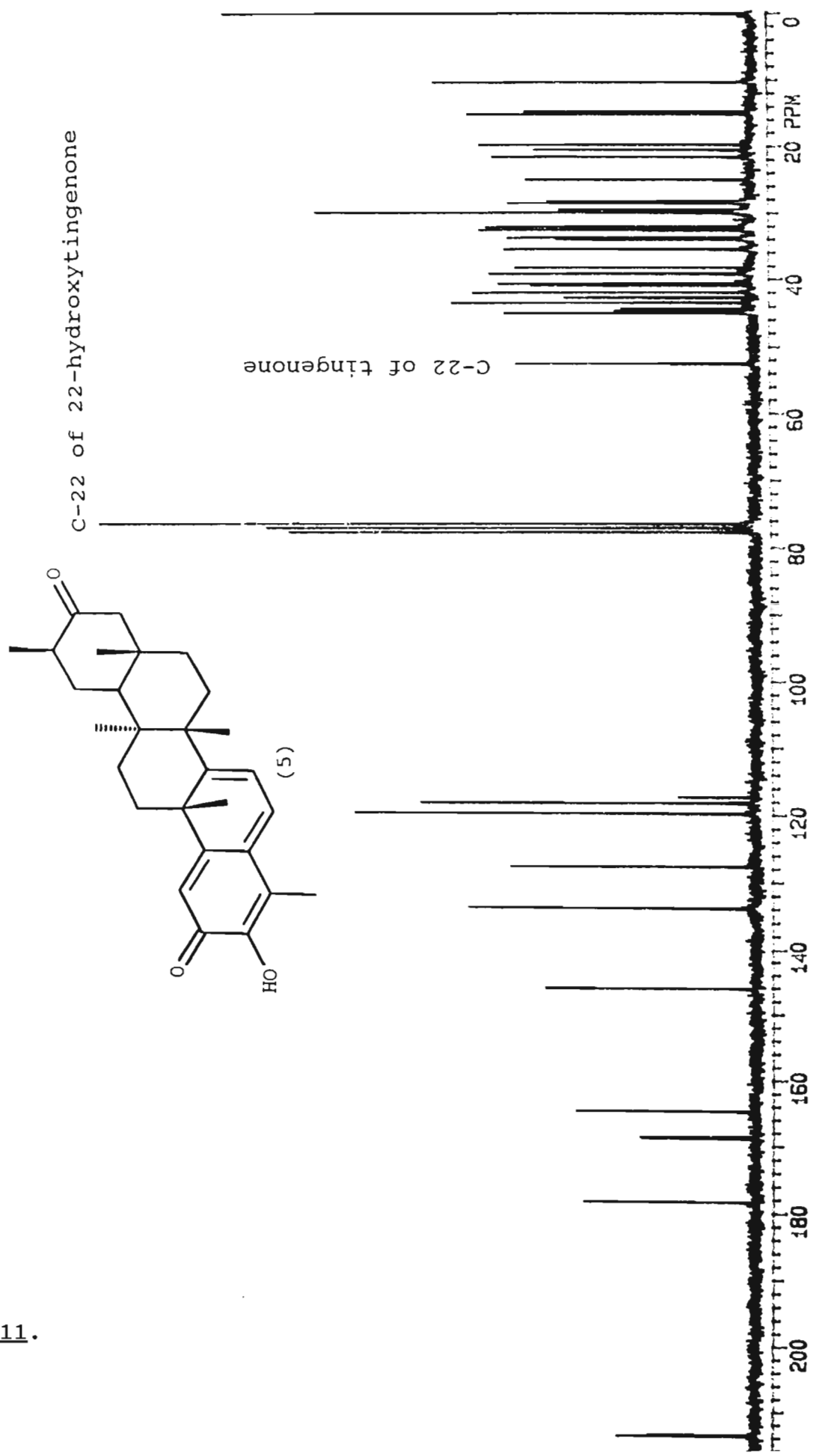

C-22 of 22-hydroxytingenone
C-22 of tingenone
0
20 PPM
40
60
80
100
120
140
160
180
200
O
O
HO
(5)

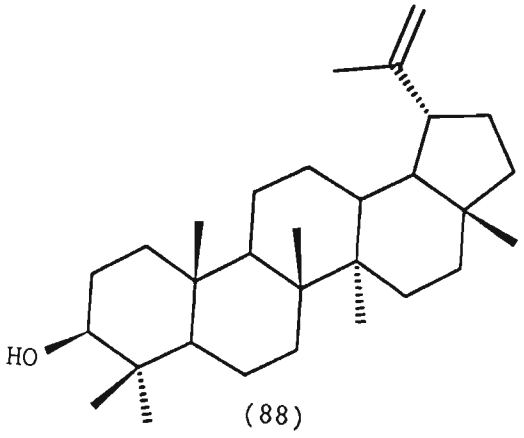
FIGURE 11.

2.3 Extractives from *Spirostachys africana* **Sonder**.

Powdered stem bark of *Spirostachys africana* **Sonder** was continuously extracted in acetone solvent using a Soxhlet extraction apparatus for 24 hours. The solid extract was dissolved in aqueous medium and extracted with hexane. This material was then subjected to separation using column chromatography.

2.3.1 Lupeol(88)

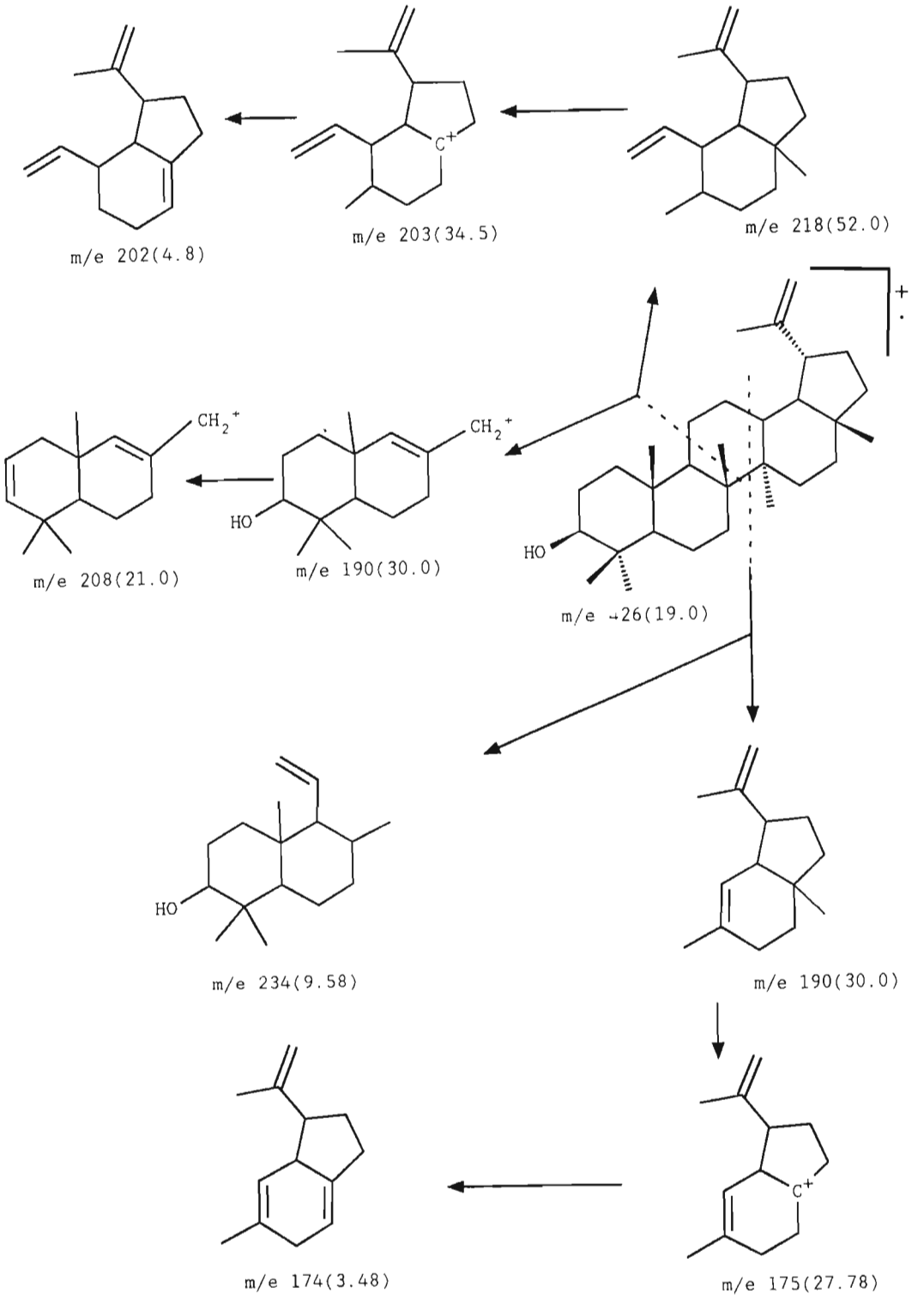
Lupeol(88) was isolated from the hexane partitioned fraction of *Spirostachys africana* after repeated column chromatography as white crystals(442.3 mg), mp 206°C, $[\alpha]_D^{22°C}$ +25.6°(c in $CHCl_3$). The IR spectrum of the isolated compound show the presence of a hydroxyl group(3300 cm^{-1}), geminal dimethyl groups(1450 and 1380 cm^{-1}) and an isopropenyl group(1640 and 880 cm^{-1}).



(88)

The fragmentation pattern observed in the mass spectrum strongly suggests the compound to be lup-20(29)-ene type triterpene and allowed the allocation of the hydroxyl at C-3(Scheme 14).

In ^{13}C NMR the DEPT technique(p. 208) indicated the presence of five methine{δ55.43(d), δ50.56(d), δ48.41(d), δ48.12(d) and δ38.13(d)}, one oxymethine(δ77.54), ten methylene groups{δ40.11(t), δ38.80(t), δ35.67(t), δ34.36(t), δ29.91(t), δ27.50(t), δ27.48(t), δ25.18(t), δ20.97(t) and δ18.36(t)}, seven



SCHEME 14 Mass fragmentation pattern of lupeol(88).

methyl groups {δ28.07(q), δ19.35(q), δ18.05(q), δ15.17(q), δ16.01(q), δ15.42(q) and δ14.58(q)} and an isopropylene group{δ109.66(t) and δ150.94(s)}. Full ^{13}C NMR data are listed in Table 9. The ^{1}H NMR(p. 206) shows the presence of seven methyl groups{δ1.03(3H, s), δ0.96(3H, s), δ0.94(3H, s), δ0.82(3H, s), δ78(3H, s), and δ0.76(3H, s)}, an oxymethine{δ3.17(1H, m)} and an isopropenyl group{δ1.68(3H, s), δ4.56(1H, dd, J = 1.37 and 2.3 Hz and δ4.68(1H, d, J = 2.02 Hz} .

The spectroscopic evidence, coupled with physical properties, confirms the compound to be lupeol(88)[140].

2.3.2 <u>3α-Acetyl-taraxer-14-en-28ß-oic acid(89)</u>.

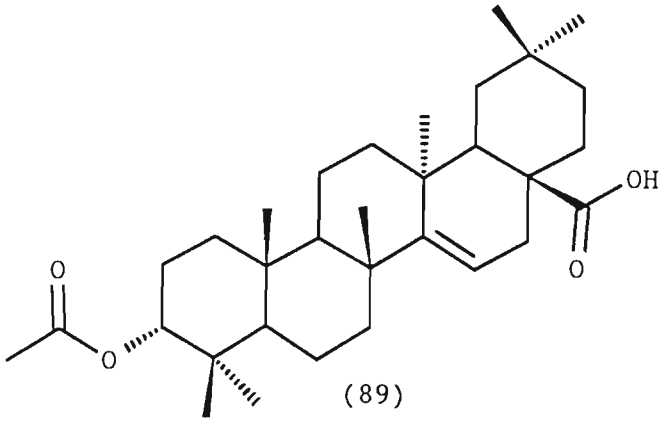
3α-Acetyl-taraxer-14-en-28ß-oic acid(89) separated as white crystals(2.01 g) after repeated column chromatography from the hexane partitioned fraction. Characterization of this compound as 3α-acetyl-taraxer-14-en-28ß-oic acid(89) rests chiefly on physical properties, spectroscopic evidence and failure of the compound to undergo acetylation.

The compound, $C_{32}H_{50}O_4$, has mp 297°C and $[\alpha]_D^{22°C}$+21.3°(c 0.51 in $CHCl_3$. Its IR spectrum showed absorption typical of a hydroxyl(3500 cm^{-1}), carbonyl (1680 cm^{-1}), acetoxyl(1720 cm^{-1}), a trisubstituted double bond(830 cm^{-1}) and geminal methyl groups(1370 and 1480 cm^{-1}). Its ^{1}H NMR spectrum(p. 209) indicated the presence of seven tertiary methyl groups and confirms the presence of an acetoxyl group(δ2.207, 3H, s), carboxylic proton(δ5.938, 1H, br s) and a vinylic function(δ4.78, 1H, dd, J = 6.67 and 10.55 Hz). Treatment of the compound with acetic anhydride in pyridine afforded unreacted starting material indicating the absence of a hydroxyl group.

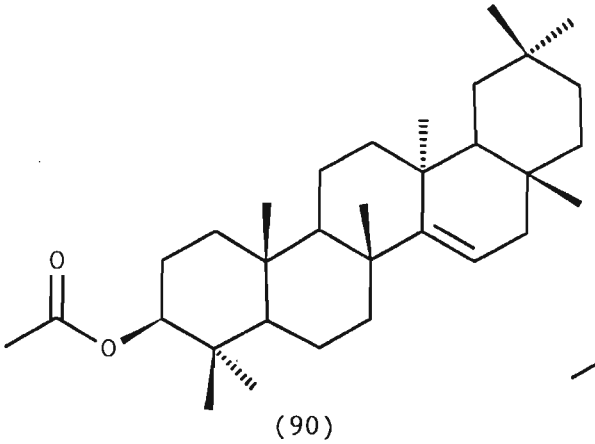
The mass spectrum of the compound revealed a fragment ion at *m/z* 189, confirming the taraxe-14-ene skeleton(Scheme 15)[141,142]. A prominent peak arising out of retro-Diels-Alder fragmentation involving C(14)-C(15) double D-ring is observed at *m/z* 344 which

can lead to the ion fragments appearing at *m/z* 329, 284 and 269 due to loss of CH_3, CH_3COOH and both groups, respectively. This fragment ions assigns the acetoxyl group to rings A/B and it must be at the usual C-3 position. The mass spectrum also suggested the presence of a carboxyl group in rings D/E.

The ^{13}C NMR(p. 210, 211) indicates the presence of an oxymethine (δ80.01, d), an ester carbonyl(δ169.82, s), a carboxylic carbonyl(δ179.46, s), a trisubstituted double bond(δ116.49, d), a tetrasubstituted double bond(δ159.75, s), an acetoxy methyl group(δ20.45, q) and seven methyl groups(δ27.32, 14.86, 25.53, 28.51, 31.68 and 21.8).



(89)



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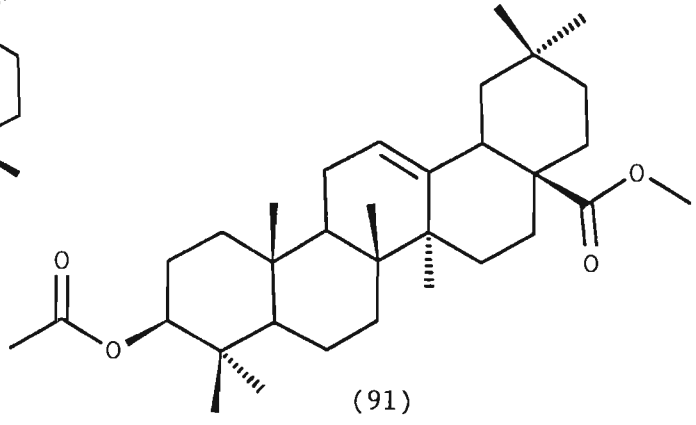
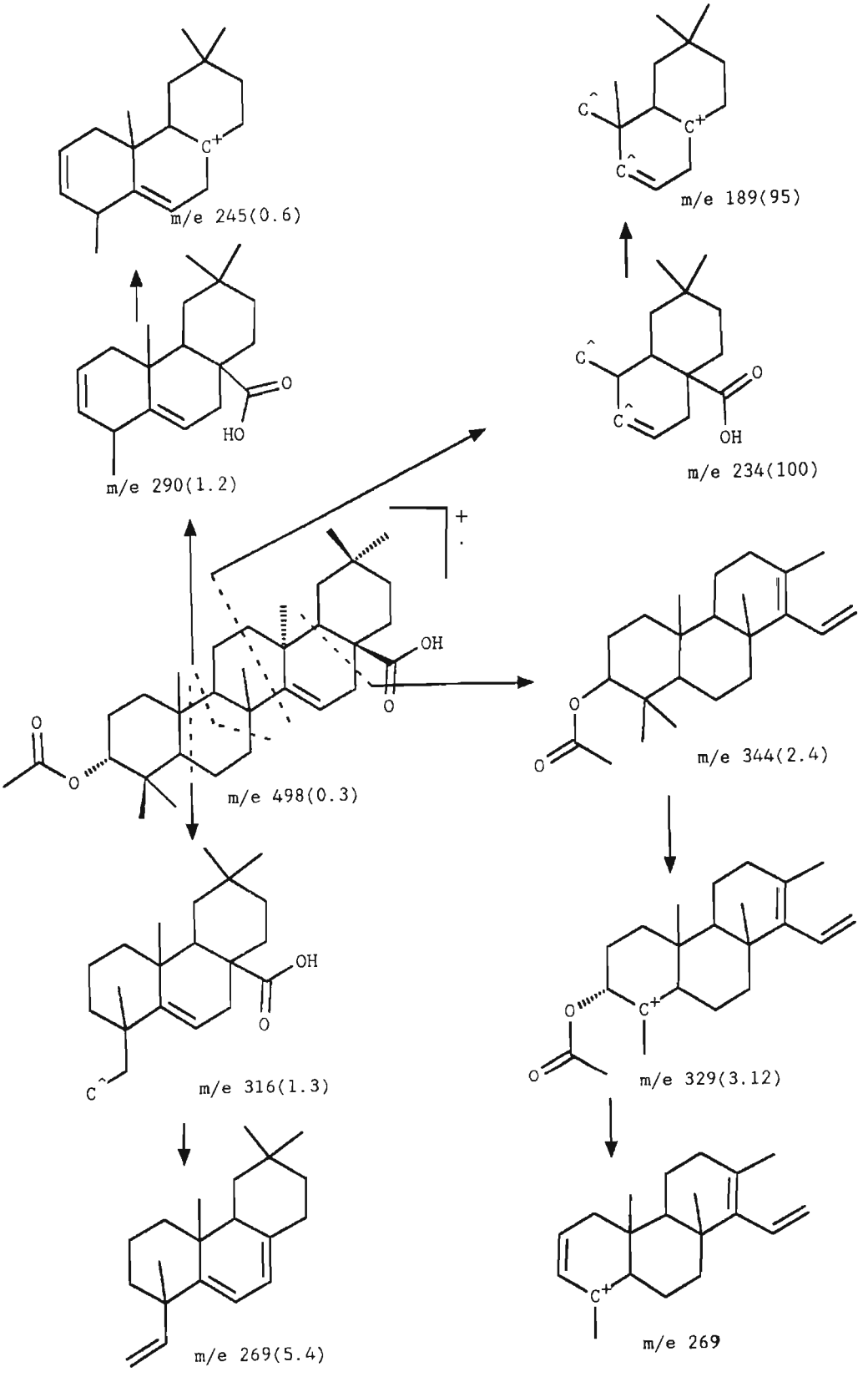


(91)

The above data are all accommodated in the structure of 3α-acetyl-taraxer-14-en-28ß-oic acid(89) previously isolated by Woo *et al.*[143]. The published ^{13}C NMR spectra of taraxeryl



SCHEME 15 Mass fragmentation pattern of 3α-acetyltaraxer-14-en-28ß-oic acid(89).

acetate(90)[144] and O-acetylmethyloleanoate(91)[145] are shown in table 12 and compared with the isolated compound.

Table 12.

Comparison of ^{13}C NMR of the isolated compound, taraxeryl acetate(90) and O-Acetylmethyloleanoate(91).

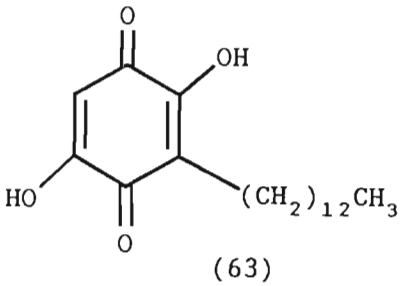
Carbon	Present investigation	(90)[144]	(91)[145]
1	37.15	37.7	38.0
2	23.11	23.5	23.4
3	80.01	81.0	80.6
4	37.33	38.0	37.5
5	54.96	55.7	55.2
6	18.12	18.7	18.1
7	33.65	33.2	32.8
8	38.49	39.0	39.2
9	48.59	49.3	47.4
10	36.75	37.6	36.8
11	17.05	17.6	22.9
12	33.08	36.7	122.2
13	37.00	37.7	143.6
14	159.75	158.0	41.5
15	116.49	117.0	27.6
16	23.12	33.8	23.2
17	50.47	35.8	46.6
18	41.46	48.9	41.2
19	41.30	41.3	45.8
20	28.92	28.9	30.5
21	35.22	35.2	33.8
22	31.80	37.5	32.2
23	27.32	28.0	27.9
24	16.12	16.6	16.6
25	14.86	15.5	15.2
26	25.53	25.9	16.9
27	28.51	29.9	25.8
28	179.46	29.9	177.9
29	31.68	33.4	32.9
30	21.80	21.3	23.4
$\underline{C}H_3COO$	20.45	21.3	21.0
$CH_3\underline{C}OO$	169.82	170.8	170.5

2.4 Extractives from *Rapanea melanophloeos* (L.) Mez.

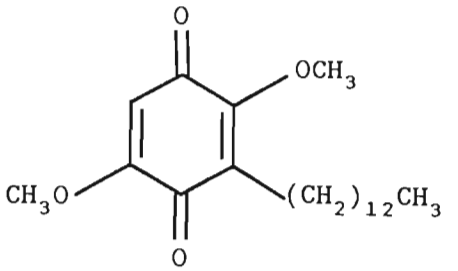
The powdered stem bark of *Rapanea melanophloeos* **(L) Mez** was differentially extracted in a Soxhlet extraction apparatus using hexane, ethyl acetate and methanol as extracting solvents. Hexane was removed and the residue dissolved in petroleum ether(40-60°C). Part of the extract, a yellow precipitate, did not dissolve in petroleum ether. It was then filtered off and recrystallized from chloroform to give crystalline orange material.

2.4.1 Rapanone(63).

Rapanone(63) separated out as an orange crystalline material mp 140°C after repeated column chromatography. Characterization of the isolated material as rapanone(63) rests chiefly on spectroscopic evidence and physical properties. The compound dissolved readily in KOH solution forming a violet solution which regenerated the original compound on acidification using dilute solution of HCl, a reaction initially observed in vilangin(70)[146]. The infrared spectrum shows a characteristic carbonyl absorption at 1620 cm^{-1} and the hydroxyl peak at 3320 cm^{-1} indicates the compound to be a 2,5-dihydroxybenzoquinone derivative[147]. The hydroxyl groups at 3320 cm^{-1} disappeared on methylation. Accurate mass measurements of the dimethyl ether derivative(92) suggested the molecular formula $C_{21}H_{34}O_4$, for the dimethyl ether derivative and $C_{19}H_{30}O_4$ for the isolated compound.



(63)



(92)

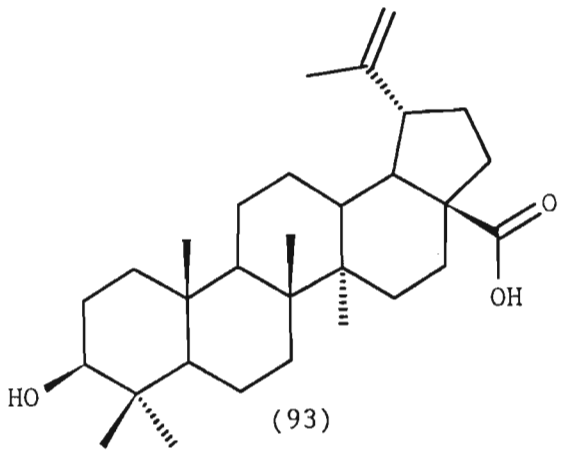
The ^{1}H NMR spectrum(p. 212) showed the compound to posses two phenolic protons{δ9.67(2H, s)}, one aromatic proton{δ6.17(1H, s)}, one methyl group{δ0.86(3H, t, J = 6.15 Hz)} and a methylene group neighbouring a double bond{δ2.76(2H, t, J = 7.33 Hz)} whilst ^{13}C NMR spectrum(p. 213, 214) indicated the presence of two carbonyls{δ173.37(s, superimposable)}, two oxyalkenyl carbons{δ170.95(s, superimposable)}, a trisubstituted double bond{δ104.41(d)}, a substituted alkenyl carbon{δ118.24(s)}, twelve methylene groups{δ32.41-23.19(triplets)} and one methyl group{δ14.32(q)}.

The above data, coupled with physical properties, confirm the compound to be rapanone(63)[148]. Diazomethylation of the compound gave a dimethyl ether derivative(92) which confirms the presence of only two phenolic hydrogens(^{1}H and ^{13}C NMR data on p. 215 - 217).

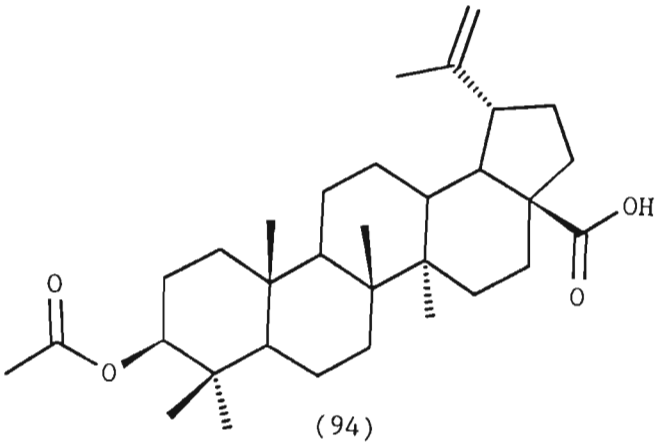
2.4.2 Betulinic acid(93).

Betulinic acid(93) was separated from the ethyl acetate extract after repeated column chromatography using hexane/ethyl acetate(1:1) solvent mixture. The compound swells in solvents such as ethyl acetate, acetone and methanol. The compound was however isolated after recrystallization in methanol to give 308 mg, mp 296°C, $[\alpha]_D^{23°C}$ +7.88(c 1.02 in pyridine). The IR spectrum revealed the presence of a hydroxyl group(3400 cm^{-1}), a carbonyl group (1700 cm^{-1}) and a vinyl group(1650 cm^{-1})[149]. Absorption at 880 cm^{-1} is due to out-of-plane bending of an olefin. Preparation of the acetate confirmed the presence of an OH group and a carboxylic group, since the IR spectrum of the acetate no longer showed the O-H stretch at 3400 cm^{-1} but still showed C=O stretch at 1700 cm^{-1}. The acetate(94) also showed an additional peak at 1750 cm^{-1} due to the acetoxy carbonyl stretch.

The fragmentation pattern observed in the mass spectrum strongly suggested the compound to be a lup-20(29)-ene type triterpene and allowed allocation of the carboxyl group at C-17 and the hydroxyl



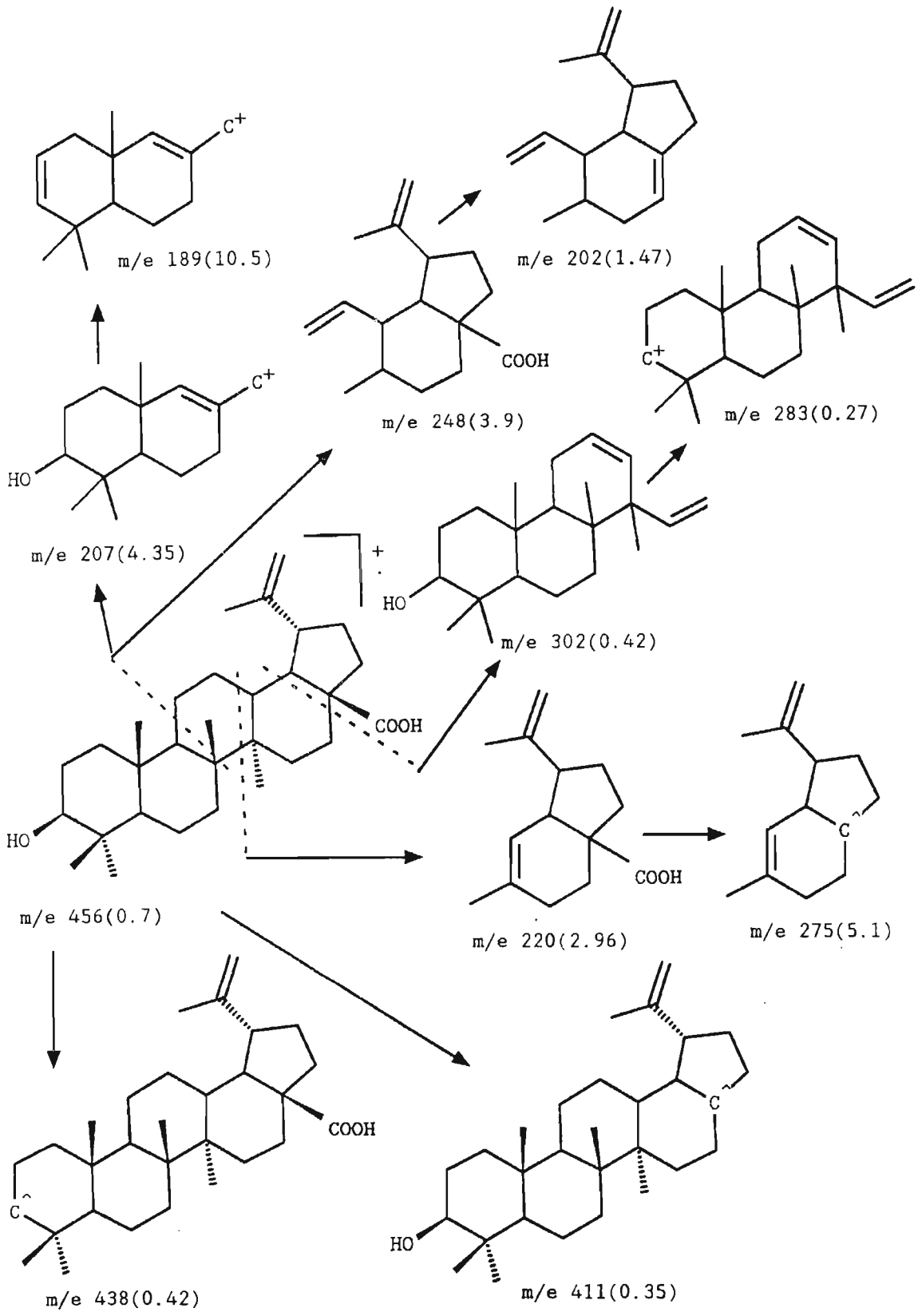
(93)



(94)

group in A-ring(Scheme 16)[149]. The mass data of our compound were in fact very similar to those of betulinic acid(93)[149]. The fragment ions *m/z* 438 and *m/z* 411 are due to the loss of H_2O and angular COOH, respectively. Cleavage of C-ring between C(12)-C(13) and C(8)-C(14) bonds results in the formation of an ion *m/z* 220 which on loss of COOH gave rise to a peak at *m/z* 175. Alternative cleavage of C-ring between C(8)-C(14) and C(9)-C(11) bonds followed by loss of a proton resulted in the fragment ion *m/z* 207, which on losing H_2O gave rise to a peak at *m/z* 189. The fragment ion *m/z* 248 also results from C-ring cleavage and then loses a carboxyl group to form a fragment *m/z* 203 which rearranges and loses a proton to give rise to a peak at *m/z* 202. Fragment ions resulting from D-ring cleavage are also observed. Cleavage of the C(13)-C(18) and C(16)-C(17) bonds give rise to a peak at *m/z* 302 which on losing a hydroxyl group forms an ion at *m/z* 283.

In ^{13}C NMR(p. 219, 220), the proton noise decoupled spectrum shows the compound to be having six methyl groups{δ13.96, δ15.42, δ15.45($2CH_3$), δ18.56 and δ27.76}, oxymethine(δ77.33, d), a vinylidene group {δ150.69, ($\underline{C}=CH_2$) and δ109.32, ($C=\underline{C}H_2$)} and an acidic carbonyl (δ178.35, s)[150] whilst the ^1H NMR(p. 218) displays six tertiary methyl groups{δ1.65, δ1.07, δ0.93, δ0.90, δ0.85 and δ0.68} and two vinylidene protons{δ4.79(1H, d, J = 2.14 Hz) and δ4.62(1H, d, J = 2.11 Hz)} long range coupled to a vinylic methyl group(δ1.65, 3H, s)[149]. The peak at δ3.34(2H, m) is due to



SCHEME 16 Mass fragmentation pattern of betulinic acid(93).

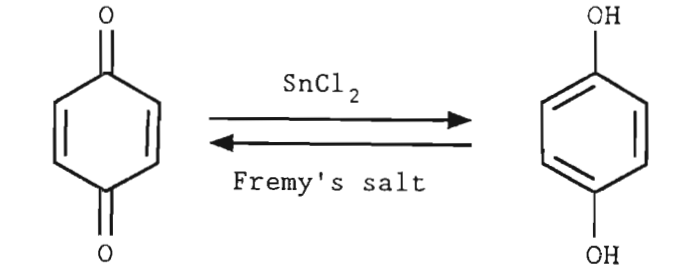
overlapping H-3 and H-19 methine peaks and this assignment is strongly confirmed by HETCOR results(p. 221) where C-3(δ77.32, d) and C-19(δ46.85, d) are coupled to a multiplet at δ3.34(2H). The ^{13}C NMR results of the compound are tabulated together with other isolated lupene derivatives in Table 9.

Physical properties further confirm the compound to be betulinic acid[113,151].

2.4.3 The chemistry of Rapanone(63).

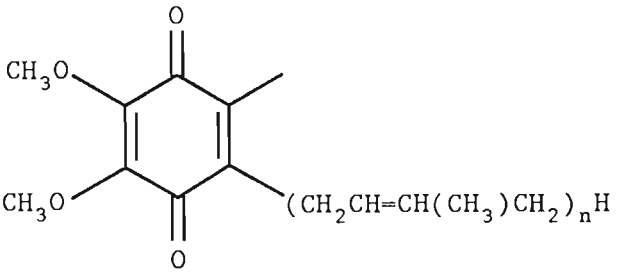
Rapanone belongs to the class of compounds known as quinones. Quinones are an interesting and valuable class of compounds because of their oxidation-reduction properties[152,153]. They can easily be reduced to hydroquinones(Scheme 17) by reagents like $NaBH_4$ and $SnCl_2$[153], and hydroquinones can be easily be reoxidized back to quinones by Fremy's salt, silver oxide or chromic acid[152,153].

These redox properties of quinones are important to the functioning of living cells, where compounds called ubiquinones act as biochemical oxidizing agents to mediate the electron-transfer processes involved in energy production[153]. Ubiquinones, also called *coenzymes Q*(95), are components of the cells of all aerobic organisms, from the simplest bacterium to humans.



Benzoquinone Hydroquinone

SCHEME 17

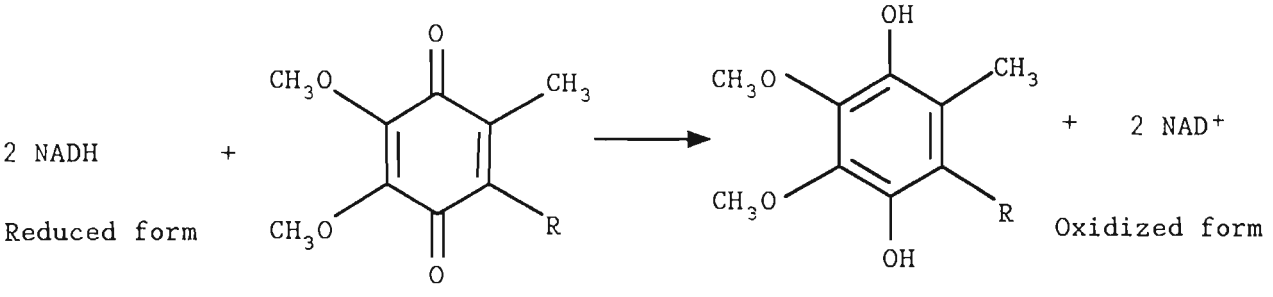


(95)

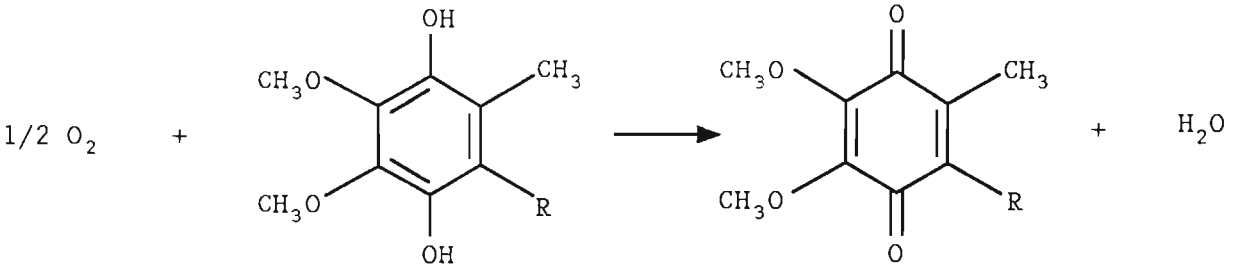
Ubiquinones (n = 1 - 10)

Ubiquinones(95) function within the mitochondria of cells as mobile electron carriers(oxidizing agents) to mediate the

Step 1:



Step 2:



Net Change: 2 NADH + 1/2 O_2 ⟶ 2 NAD^+ + H_2O

SCHEME 18

respiration process whereby electrons are transported from the biological reducing agent NADH(reduced form of nicotinamide adenine dinucleotide) to molecular oxygen. Although a complex series of steps is involved in the overall process, the ultimate result is a cycle whereby NADH is oxidized to NAD, oxygen is reduced to water, and energy is produced[153]. Ubiquinone acts only as an intermediary and is itself unchanged(Scheme 18).

Rapanone(63), due to its acidic properties forms several metallic salts and exhibits properties of dihydroxybenzoquinones in the formation of a yellow diacetate, a dibenzoate and a violet dianilino derivative[148].

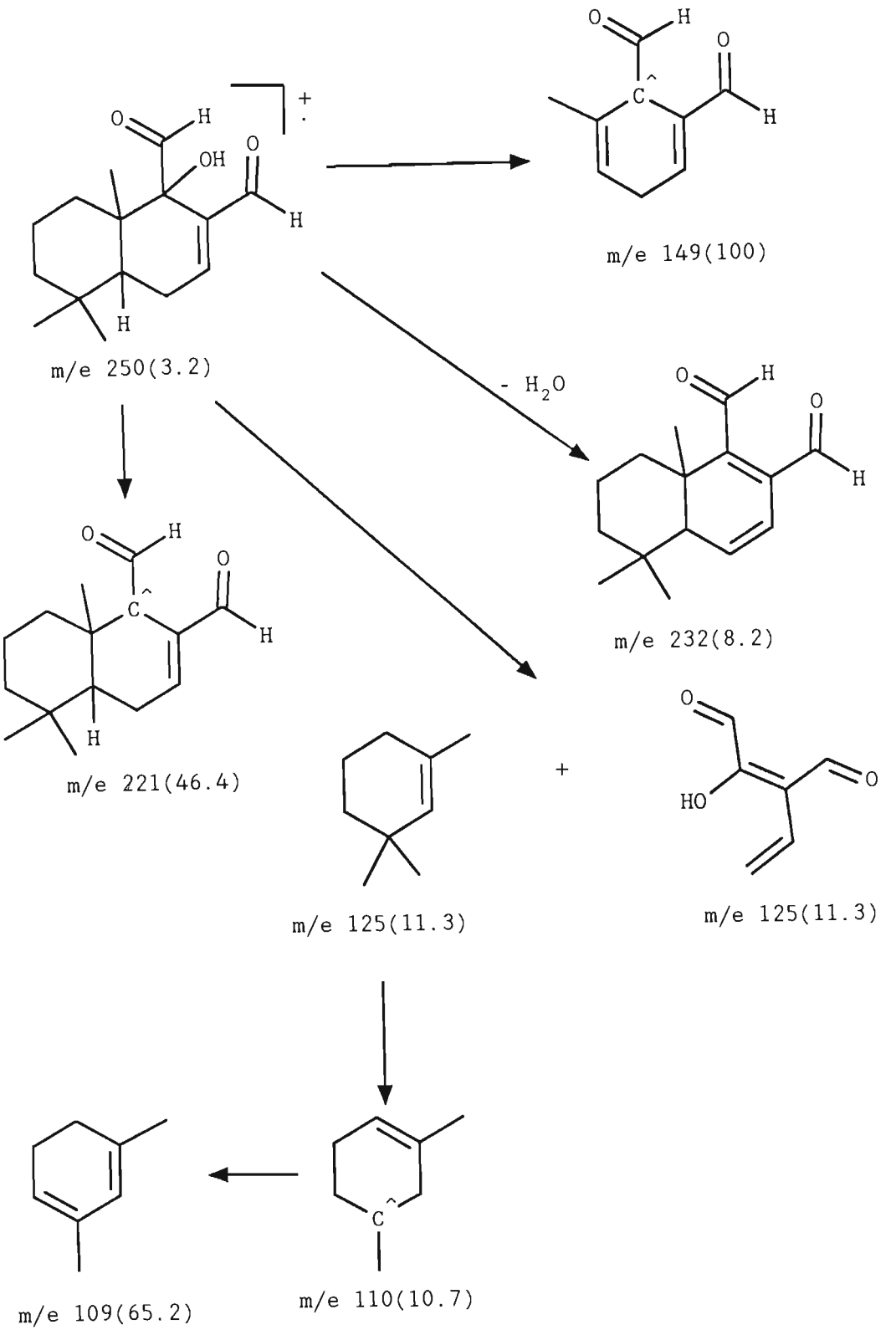
2.5 Extractives from *Warburgia salutaris* **(Bertol. f.) Chiov**.

The plant material was collected from Maungani mountains (Venda). It was left for a month in a shade to dry. A cold differential extraction was carried out on the dried powdered stem bark of *Warburgia salutaris* **(Bertol. f) Chiov** using petroleum ether (40-60°C), dichloromethane, ethyl acetate and finally ethanol as extracting solvents. Tlc analysis of the dichloromethane and petroleum extracts gave products of the same R_f values. The two extracts were combined and the solvents removed to give a yellow extract.

2.5.1 (-)-Warburganal(21).

(-)-Warburganal(21) was isolated from the combined petroleum ether(40-60°C) and dichloromethane extracts(132.05 g) after repeated column chromatography on silica gel 60 (Merck Art no. 9385) as white needles(3.0 g) with the following physical properties: mp 105-106°C, $[\alpha] = -234.28$ (c 1.32 in $CHCl_3$).

Physical properties and spectral examination {^{1}H NMR(p. 224), ^{13}C NMR(p. 225, 226), mass(Scheme 19)} showed that the compound was warburganal(21)[36], a compound previously isolated from *W. ugandensis* which exhibited very strong antifeedant properties



SCHEME 19 Mass fragmentation pattern of warburganal(21).

against African army worm[36].

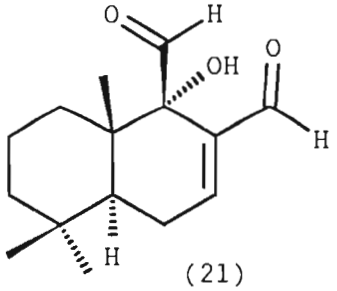
In our investigation concerning the biological activity of (-)-warburganal(21), which was done jointly with Noristan Laboratories, Pretoria (South Africa), the compound was found to have anti-ulcer activities which compared favourably with the present leading pharmaceutical product on the market, retinidine(96). Results of such a comparison are provided in Table 13.

Table 13.

Anti-ulcer activities of warburganal and retinidine(96) compared.

Compound	% Inhibition	P*	Dose(mg/kg)
Warburganal	86	<0.05	20
Retinidine	73	<0.01	15

P* = Probability.



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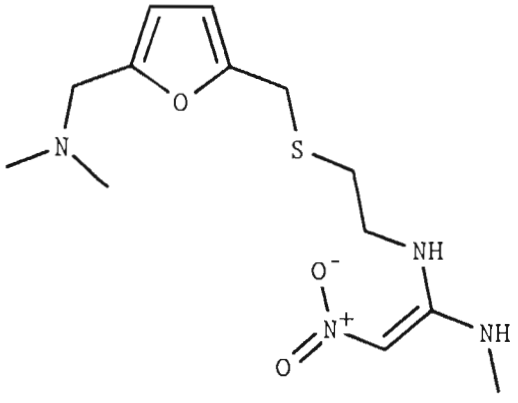


(96)

Molluscicidal and ovicidal activities of (-)-warburganal(21) on South African snails *Bulinus africanus*(PLANORBIDAE) were also undertaken jointly with the Department of Zoology and Entomology, University of Natal, Pietermaritzburg. Data of such results are in Tables 14-16.

Table 14.

Survival of *Bulinus africanus* for the 24 hours exposure period and additional 48 hours post-exposure recovery period in different (-)-warburganal concentrations(N = 10 in each case). No mortality occurred in the controls.

Concentration (ppm)	After 24 hours (no. alive)		After 72 hours (no. alive)	
	juveniles	adults	juveniles	adults
0.1	10	9	9	9
0.5	4	9	4	9
1.0	3	8	2	5
1.5	2	5	0	4
2.0	0	3	0	2
4.0	0	1	0	0
5.0	0	1	0	0
6.0	0	0	0	0
8.0	0	0	0	0
10.0	0	0	0	0
15.0	0	0	0	0
20.0	0	0	0	0
25.0	0	0	0	0

Table 15

Survival of *B. africanus* for the 24 hours exposure period and additional 48 hours post-exposure recovery period in different endo-S concentrations (N = 10 in each case). No mortality occurred in the controls.

Concentration (ppm)	After 24 hours (no. alive)		After 72 hours (no. alive)	
	juveniles	adults	juveniles	adults
2.0	10	10	4	9
4.0	9	8	1	1
6.0	2	4	0	0
8.0	2	3	0	0
10.0	0	0	0	0

From the results the following observations can be made: (i) Juvenile *B. africanus* was more susceptible to warburganal than adults. (ii) The LC_{90} for juveniles lay between 1.5 and 2.0 ppm after exposure but dropped to 1.0-1.5 ppm after the post-exposure period. (iii) For adults, the LC_{90} lay at 4.0 ppm after exposure and approximately 3.0 ppm after the post-exposure period. (iv) All controls survived. (v) Probit transformation of the results ≤ 6.0 ppm after 72 hours gave the following linear regression equations for mortality on concentration: juveniles $Y = 5.860 + 2.132X$ ($r = 0.9997$, $p = 0/0.015$), adults $Y = 4.801 + 3.375X$ ($r = 0.9853$, $p = 0.015$). (vi) The 72 hours LC_{90} dosages calculated from these equations were 2.0 and 2.7 ppm for juveniles and adults respectively. (vii) The trials with Endod-S (Table 16) showed that after the exposure period, the LC_{90} was approximately 9.0 ppm for juveniles and 9.0-10 ppm for adult *B. africanus*. After the post-exposure period this fell to 4.0 ppm for both. (viii) Warburganal is ovicidal with 90% egg mortality at approximately 1.0 ppm after the 72 hours screening period and

there is also a slight increase in the incubation period with increasing molluscicide concentration. These trials were carried out with the kind collaboration of Professor C.C. Appleton, Department of Zoology and Entomology, University of Natal, Pietermaritzburg.

Table 16.

Mortality of *B. africanus* eggs following the 72 hours screening procedure using (-)-warburganal at concentrations of 2.0 - 0.1 ppm. Incubation periods and the proportion showing initial development are also given.

Concentration (ppm)	No. eggs	No. showing signs of development	No. hatched (mortality in brackets)	Incubation period (days)
Control	74	68(91.9%)	68(8.1%)	13-14
0.1	74	41(55.4%)	41(44.6%)	13-14
0.5	78	23(29.5%)	17(78.2%)	15
1.0	90	33(36.7%)	9(90.0%)	15
1.5	78	20(25.6%)	5(93.6%)	15
2.0	81	19(23.5%)	3(96.3%)	15

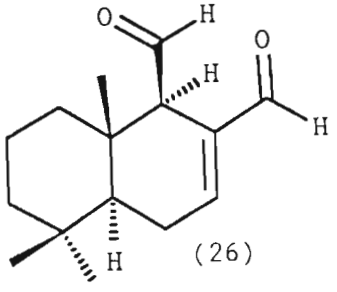
2.5.2 Polygodial(26).

Polygodial(26) was isolated from the dichlomethane extract as a white crystalline material, mp 54-55°C, $[\alpha]_D^{20°C}$ -122°(c 0.74 in EtOH).

Characterization of the compound as polygodial(26) comes from a closer look of its spectral properties followed by comparison of

its physical properties with those of polygodial(26)[154].

The IR spectrum shows aldehydic C-H stretch(2860 and 2750 cm^{-1}), carbonyl(1770 cm^{-1}) and an enal functionality(1690 and 1650 cm^{-1}). Furthermore infrared absorption in the Methyl ''breathing'' region shows bands at 1400 and 1380 cm^{-1} characteristic of geminal dimethyl groups[154].



(26)

The spectroscopic data {^{1}H NMR(p. 127), ^{13}C NMR(p. 128, 229)} is well accommodated in the structure of polygodial(26)[154].
Further evidence, which shows correct assignments by the previous authors, is revealed by COSY and long range HETCOR results. From COSY(correlation spectroscopy) results(Figure 12), the following couplings are observed: (i) the doublet aldehyde proton(δ9.5) is coupled to H-9(δ2.8), (ii) H-9 in turn is coupled to H-7(δ7.17), (iii) H-7(δ7.17) is coupled to H-6 protons(δ2.5), (iv) H-6(δ2.5) is further coupled to H-5(δ1.3). The correctness of the assignments of the C-9 and C-5 methine protons is shown in long range HETCOR(J = 10 Hz) results(Figure 13) which indicate C-9 to be strongly coupled to H-9 and weakly coupled to H-11 whilst C-5 is indicated to be strongly coupled to H-5 and weakly coupled to H-13 and H-14. The methylene group at C-6 is differentiated from

the C-1, C-2 and C-3 methylene groups by its coupling with C-7. The two aldehydic carbonyls can also be differentiated by long range HETCOR as C-11 is coupled to H-9 whilst C-12 is seen to be coupled to H-7.

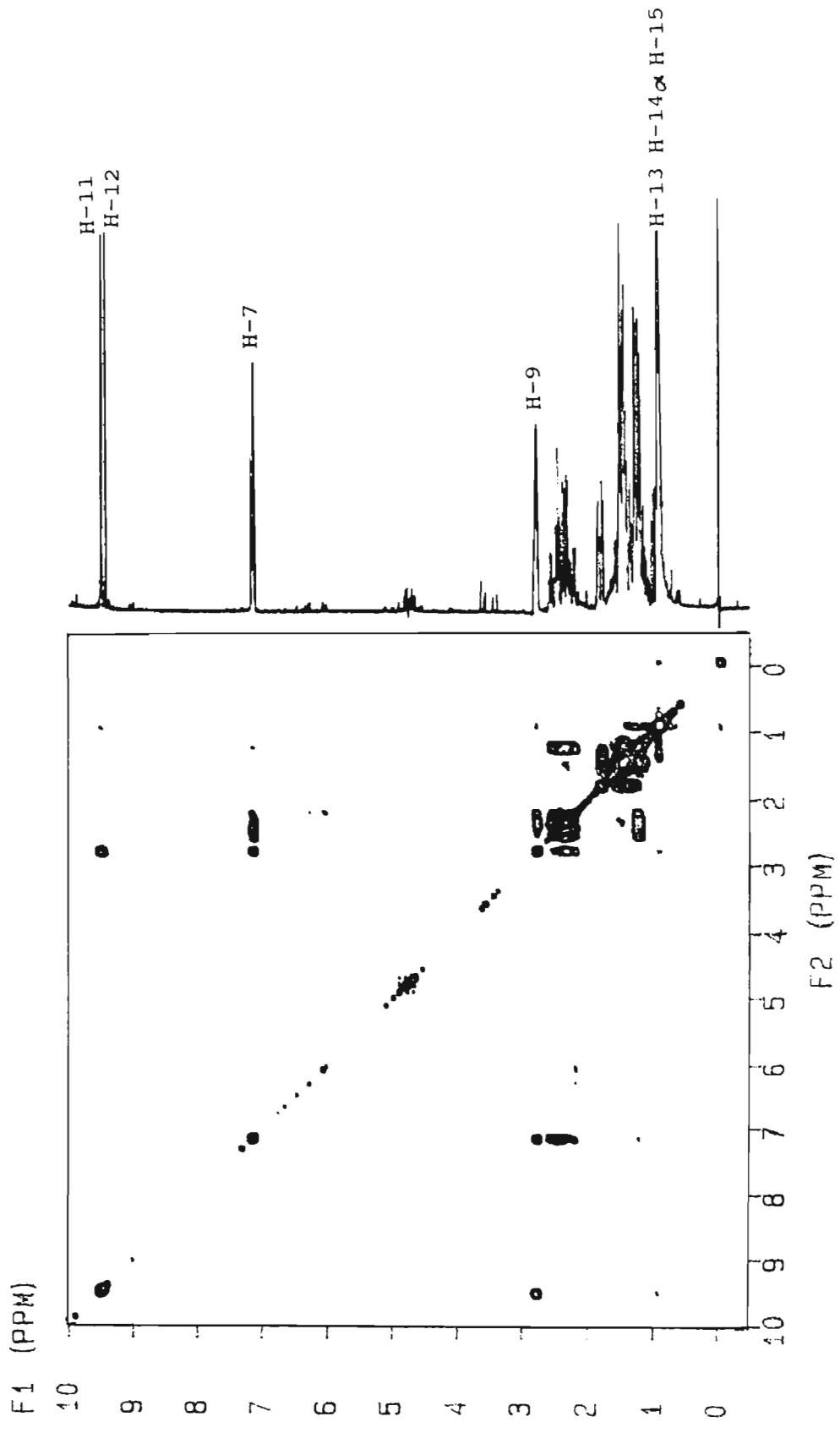


FIGURE 12. COSY spectrum of polygodial.

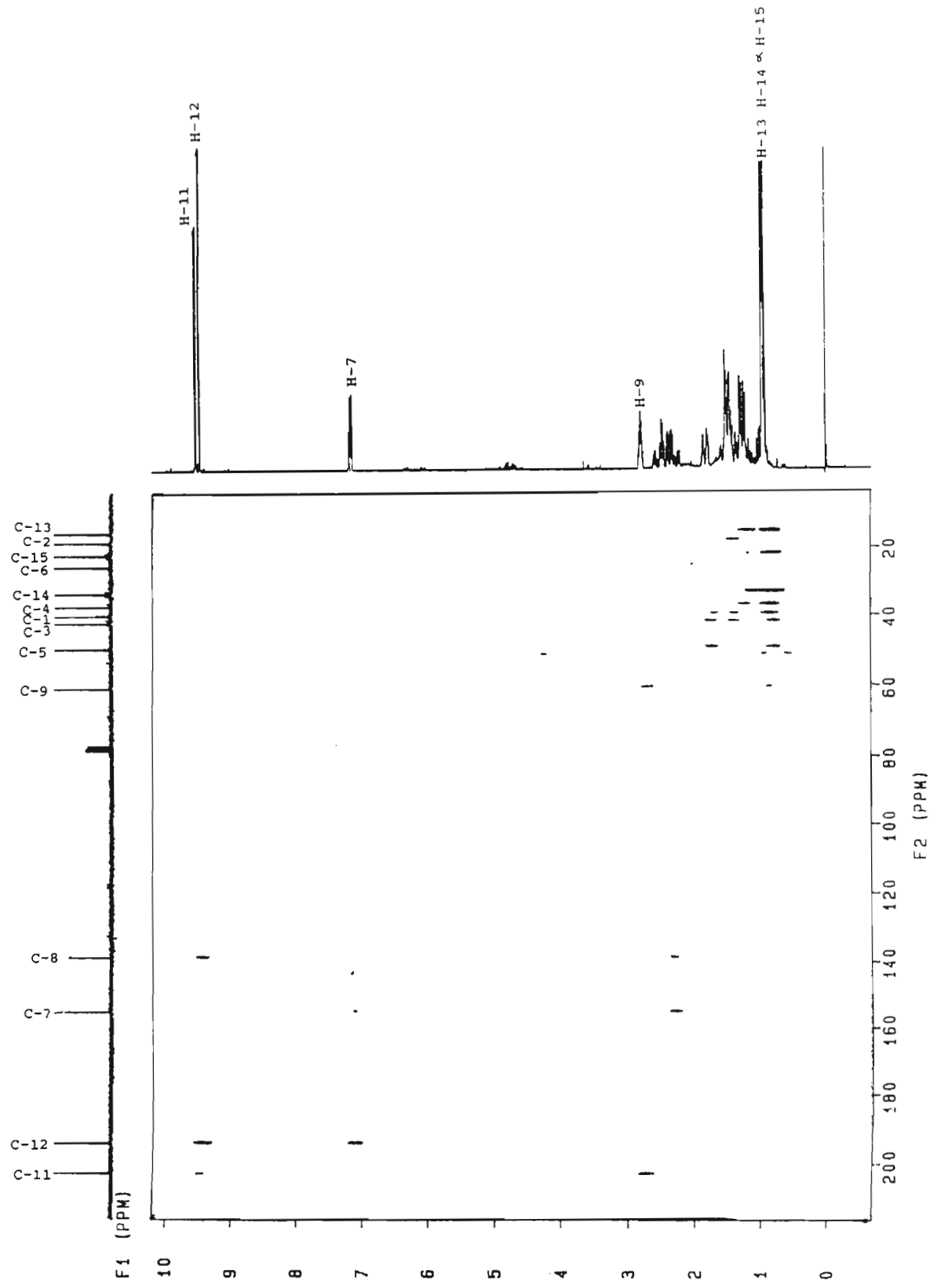
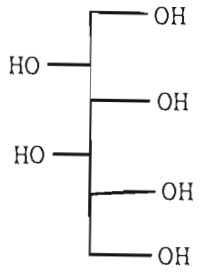


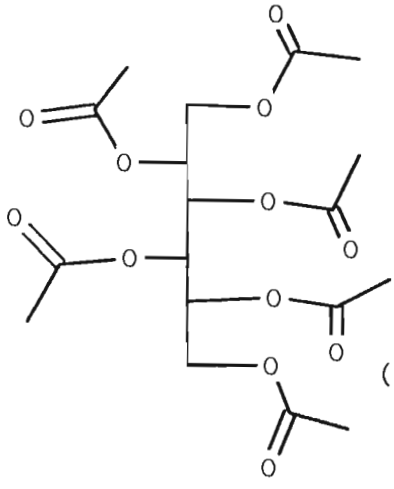
FIGURE 13. Long range HETCOR spectrum of polygodial(J = 10 Hz).

2.5.3 Iditol(97).

Iditol(97) was isolated from the ethanol extract by a derivatization method. The crude ethanol extract was acetylated using the pyridine-acetic anhydride method[155]. Recrystallization of the product in hexane-ethyl acetate solvent mixture afforded a hexaacetate derivative(259 mg) which had physical and spectral properties(^{1}H NMR and ^{13}C NMR data on p. 230 - 232) identical to those of iditol hexaacetate(98)[102]. Comparison of the physical properties of the compound and iditol hexaacetate are given in Table 17.



(97)



(98)

Table 17.

Physical properties of iditol and the isolated compound compared.

Physical properties	Present investigation	Iditol hexaacetate(105)[102]
mp	121°C	121-122°C
Optical rotation	$[\alpha]_D^{20°C}$= +25.7°	$[\alpha]_D$= +25.3°

2.6 Taxonomic significance of the isolated compounds.

The isolated compounds have a strong taxonomic significance when compared with compounds isolated from the same families in other

continents. It is in this context that the review which follows was undertaken.

2.6.1 Canellaceae.

In our chemical investigation of the South African *Warburgia salutaris*, two drimane sesquiterpenoids, (-)-warburganal(21) and polygodial(26), were isolated. Previously many drimane sesquiterpenoids had been isolated from the family Canellaceae. A comparative review of plants belonging to the family Canellaceae with polygodial(26) and (-)-warburganal(21) as constituents is given in table 18. Of the plants already investigated in the family Canellaceae polygodial(26) and warburganal(21) are only found in the African genus *Warburgia*. The presence of polygodial(26) and warburganal(21) is of taxonomic significance at genus level.

Table 18.

Warburganal(21) and polygodial(26) isolated from Canellaceae.

Species. Reference.	Polygodial(26).	Warburganal(21).
1. *W.salutaris*	+	+
2. *W. ugandensis*	+	+
3. *W. stuhlmannii*	+	+
4. *C. fragrans*	-	-
5. *C. winterana*	-	-

Warburganal(21) and polygodial(26) were also isolated from other taxonomically unrelated plants(table 19), *Polygonum hydropiper*

(Polygonaceae) and *Porella vernicosa* complex(Porellaceae). Polygodial was found to be constituent of *Pseudowinterana axillaris*, *P. traversii*, *P. colorata*(Winteraceae), *Tasmannia aromatica*, *Drimys lanceolata* and *D. winterii*(Winteraceae).

Table 19.

The distribution of warburganal(21) and polygodial(26) in *Warburgia* species and other unrelated plants.

Species.	Compounds		
	Warburganal.	Polygodial.	Reference.
Polygonum hydropiper	+	+	51
Warburgia salutaris	+	+	present investigation
W. ugandensis	+	+	36
W. Stuhlmannii	+	+	36
Porella vernicosa	-	+	1
Pseudowintera colorata	-	+	156
P. axillaris	-	+	156
P. traversii	-	+	156
Drimys lanceolata	-	+	59
P. winterii	-	+	156

2.6.2 Celastraceae.

Chemical investigation of *Cassine transvaalensis* and *C. papillosa* showed interesting results. It revealed the peltogynan 6R,13R-

11,11-dimethyl-1,3,8,10-tetrahydroxy-9-methoxypeltogynan(83), previously isolated from *Elaeodendron balae*[27], to be a constituent of the two plant species. This unusual peltogynan with geminal dimethyl groups at C-11 and a methoxy group at C-9 is unique to the family Celastraceae. However it is of taxonomic significance that the three plants from Celastraceae occurring as far apart as Sri Lanka and South Africa should both possess a peltogynan with this unusual pattern of substitution. The family Leguminosae is known to be a source of peltogynoids[22,23] but not one of these has this unique pattern of substitution. According to Dyer[157] *Elaeodendron* is synonymous to *Cassine*. In table 20 four species are presented which show *Elaeodendron* to be synonymous with *Cassine*. According to F. von Breitenbach[158] *Elaeodendron* is synonymous to *Cassine* and *Mystroxylon*. Furthermore according to Palmer *et al*.[6] *C. papillosa* **(Hoechst.) Kuntze** can also be referred to as *Elaeodendron papillosum* **(Hoechst)** or *Elaeodendron capensis* **Eckl. & Zeyh.** From the above information it can be concluded that the isolated peltogynan is of taxonomic importance at genus level. Further chemical investigations of plants belonging to the genus *Cassine* might strengthen this conclusion.

Table 20.

Synonymous relationship of *Elaeodendron* and *Cassine* compared.

Botanical Name	Synonym	Reference
Cassine velutinum **Loes**	*Elaeodendron velutinum* **Harv**	158
C. aethiopica **Thumb**	*E. aethiopicum* **Oliv.**	158
C. croceum **Thumb O. Ktze**	*E. croceum* **DC**	158
C. laurifolia **Harv.**	*E. laurifolium* **Harv.**	6
C. metabolica **Loes**	*E. metabolicum* **Loes**	5
C. sphaerophyllum **O. Ktze**	*E. sphaerophyllum* **Presl.**	158
C. papillosa	*E. papillosum* **(Hoechst.)** or	6
	E. capensis **Eckl. & Zeyh.**	5

(-)4'-O-Methoxyepigallocatechin(12) and ouratea proanthocyanidin(13) were isolated from *C. papillosa*. Although (-)4'-O-methoxyepigallocatechin(12) and ouratea proanthocyanidin(13) have been isolated from *Ouratea sp.*[123] (Ochnaceae) the distribution of this catechin derivative(12) and a proanthocyanidin(13) is assumed to be a chemotaxonomic characteristic of the Celastraceae. These compounds were previously isolated from Indian *Elaeodendron glaucum*[124], Sri Lankan *Elaeodendron balae*[27] and Brazilian *Maytenus rigida*[28] (Celastraceae). 4'-O-Methoxyepigallocatechin(12) seems to be a secondary metabolite of most plants belonging to the family Celastraceae. In our investigation we also established the link between the peltogynan and this flavonoid(12). A biogenetical relationship between 4'-O-methoxyepigallocatechin(12) and the isolated ouratea proanthocyanidin(13) cannot be ruled out as this flavonoid(12) forms the lower unit of ouratea proanthocyanidin(13). 4'-O-Methoxyepigallocatechin(12) can be used to establish further chemotaxonomic characteristics in the family Celastraceae.

Chemical investigation of *C. transvaalensis* revealed the quinonoid triterpene pristimerin(4) to be a constituent of the plant whilst similar investigation of *C. papillosa* showed quinonoid triterpenes tingenone(5) and 22-hydroxytingenone(7) to be constituents. Quinonoid triterpenes are unique to the Celastraceae and Hippocrateaceae families. However Dyer[159] refers to the Hippocrateaceae as synonymous to Celastraceae and in this context quinonoid tritepenes are unique to the family Celastraceae. Quinonoid triterpenes already isolated, including their source, are listed in Table 21.

A hexitol, galactitol(75), was isolated from both *C. papillosa* and *C. transvaalensis* in large amounts. Galactitol was previously isolated from *Maytenus nemerosa*[16]. In previous investigations the hexitol dulcitol was isolated from *Euonymus tingens*[7], *Gymnosporia emarginata*[160] and *Pleurostylia opposita*[161], and was found to be a characteristic constituent of the Celastraceae. The conclusion

is that both hexitols, galactitol and dulcitol, may be of taxonomic importance in family Celastraceae.

Table 21.

Quinonoid triterpenes from family Celastraceae.

Compound.	Species.	Reference.
Pristimerin	*Cassine matabelica*	162
	C. Transvaalensis	Present investigation
	Catha cassinoides	21
	Celastrus paniculatus	163
	Denhamla pittosporoides	164
	Gymnosporia emarginata	160
	Hippocratea aspera	165
	Maytenus canariensis	21
	M. chuchuhuasca	166
	M. disperma	164
	M. ilicifolia	165
	Pachystimia canbyi	13
	Pleurostylia opposita	115
	Pristimera grahamii	167
	P. indica	122
	Salacia crassifolia	168
	S. macrosperma	20
	S. reticulata	169
Celastrol	*Celastrus scandens*	170
	C. strigillosa	171
	Catha cassinoides	21
	Tripterygium forrestii	172
	T. regelii	171
	T. wilfordii	173
Tingenone	*Cassine papillosa*	Present investigation

table 21 continue

	Catha cassinoides	21
	Euonymus tingens	7
	Hippocratea indica	122
	Maytenus chuchuhuasca	166
	M. ilicifolia	165
	M. rigida	115
	Peritassa campestris	168
	Plenckia populnea	168
22-Hydroxytingenone	*Cassine papillosa*	Present investigation
	Euonymus tingens	7
	Maytenus nemerosa	174
	Salacia macrosperma	20
20α-Hydroxytingenone	*Euonymus tingens*	139
	Maytenus rigida	116
	M. nemerosa	16
Iquesterin	*Gymnosporia emarginata*	160
	Catha cassinoides	21
	S. reticulata	169
Dispermochinon	*Maytenus disperma*	175
Salacia-chinomethide	*Salacia macrosperma*	166

The distribution of triterpenes in the Celastraceae has been reviewed[13]. Dantanarayana *et al.*[161], in their review, found seven genera of the Celastraceae to consist of lupanes. Further chemical investigations of the family Celastraceae together with our results {(-)6ß-hydroxylup-20(29)-en-3-one(77) was isolated from *C. transvaalensis* whilst lup-20(29)-en-3ß,30-diol(86) and 30-hydroxylup-20(29)-en-3-one(2) were isolated from *C. papillosa*} show lupane-type triterpenes to be characteristic of the Celastraceae. 11α-Hydroxylup-20(29)-en-3-one was isolated from *Maytenus rigida* by Marini-Bettolo *et al.*[117] and chemical

investigation of *Maytenus nemerosa*[16] revealed 3-oxo-lup-20(29)-en-30-al, lup-20(29)-en-3ß,30-diol and 30-hydroxylup-20(29)-en-3-one to be constituents of the plant.

2.6.3 Myrsinaceae.

Rapanone(63), an anthelmintic agent isolated from *Rapanea melanophloeos*, belongs to the class of compounds known as hydroxybenzoquinone derivatives. A review of these compounds has already been undertaken by Ogawa *et al.*[93] and this class of compounds was found to be chemotaxonomically characteristic of the Myrsinaceae.

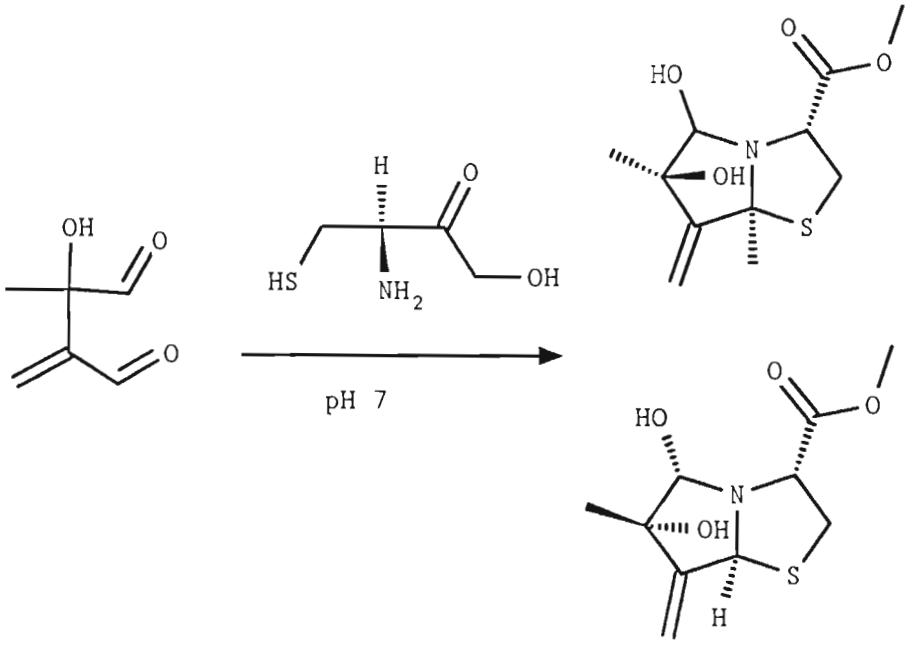
2.7 Towards the synthesis of warbuganal analogues.

The use of (-)-warburganal(21) to control the Bilharzia parasite was envisaged. However the plant source is presently very scarce because of overusage of the plant by traditional healers[9]. In Venda two plants are known to be still in existence[10]. Attempts to synthesize straight chain (-)-warburganal analogues were considered. Synthetic routes which can lead to the synthesis of monocyclic (-)-warburganal(21) and polygodial(26) analogues respectively, were also considered. It is envisaged that the synthetic analoques of (-)-warburganal(21) and polygodial(26) will be sent to Noristan Laboritories, Pretoria, for antiulcer activity tests and to Dr. C. Appleton, Department of Zoology, University of Natal, for molluscicidal tests.

2.7.1 Straight chain (-)-warburganal analogues.

Synthesis of straight chain (-)-warburganal analogues was envisaged as this will have a free rotation between the two carbons joining the two aldehydes and reactions with sulfhydryl groups and formation of adducts with amino groups (Scheme 20) would proceed without any difficulty. The schemes proposed are based on the retrosynthetic approaches of the straight chain warburganal analogues which include the Baylis-Hillman

reaction[176]. In the first scheme (Scheme 21), methyl acrylate(99) is allowed to react with ethyl pyruvate(100) in the presence of DABCO(1,4-diazabicyclo[2.2.2]octane)[177]. The hydroxyl group of 1-ethyl-2,4-dimethyl-2-hydroxy-3-methylenesuccinoate(101) is then protected by chloromethyl methyl ether[178]. Reduction of the methyl methyl ether-protected diester(102) using DIBAH(diisobutylaluminium hydride)[179] is expected to give the methyl methyl ether-protected product(103). Deprotection will then yield 2-hydroxy-2-methyl-3-methylene butan-1,4-dial(104).

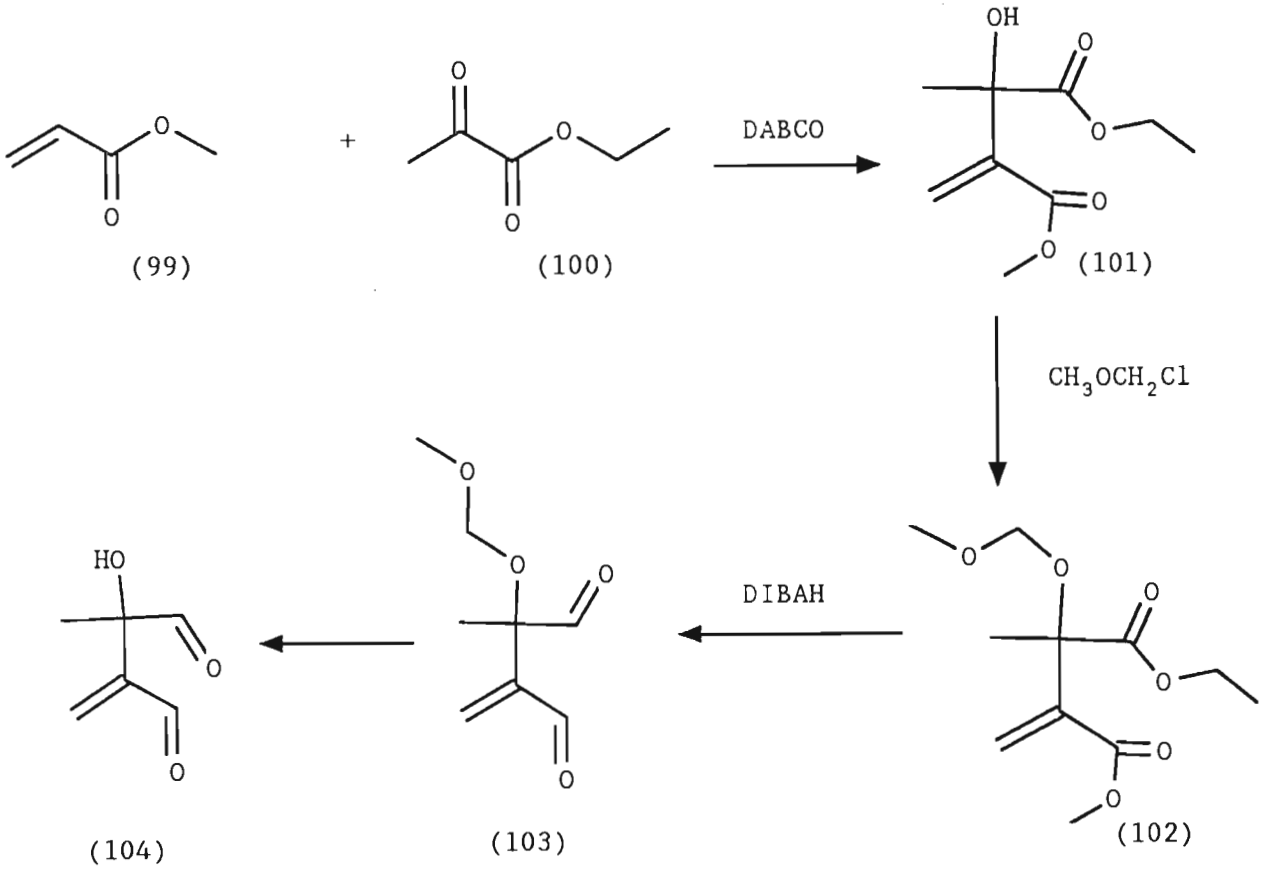


SCHEME 20.

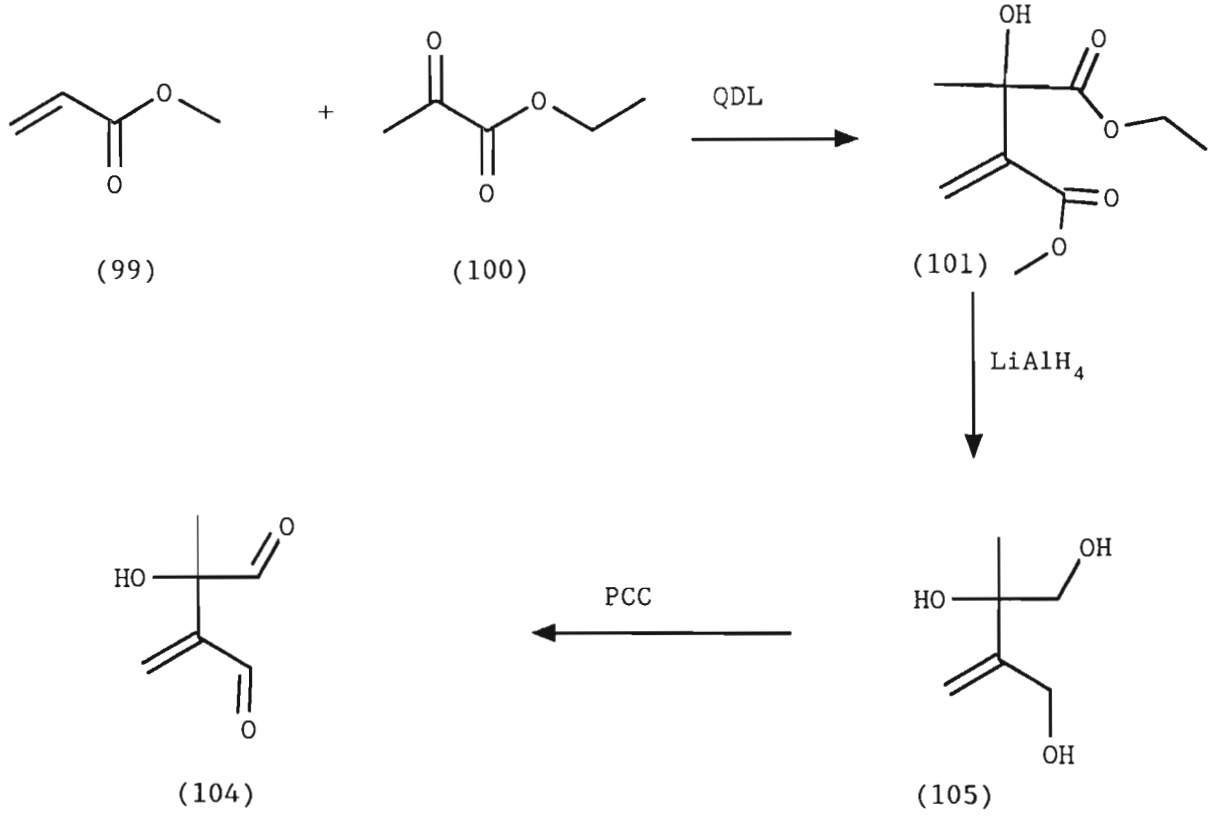
In the second scheme (Scheme 22), methyl acrylate(99) is allowed to react with ethyl pyruvate(100) in the presence of QDL(3-quinuclidinol)[176]. The diester product(101) is reduced by $LiAlH_4$ to give 2-methyl-3-methylene butan-1,2,4-triol(105)[178] which is then oxidized using pyridinium chlorochromate[180] to give the desired product(104).

The third scheme (Scheme 23) involves the reduction of the diester(101) by Red-Al[sodium bis(2-methoxyethoxy)aluminium hydride][181] to give 2-methyl-3-methylene butan-1,2,4-triol(105) which is then subjected to oxidation using pyridinium

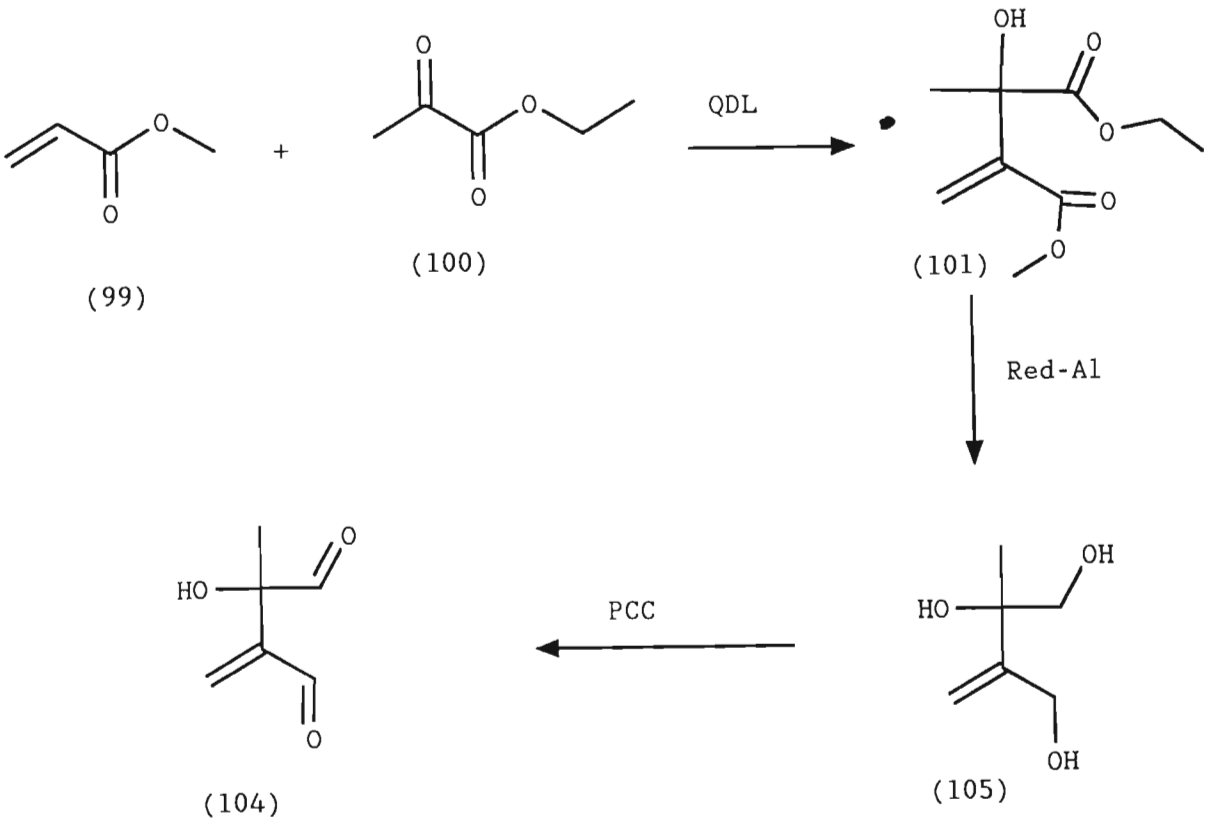
chlorochromate[180] to give the expected product(104).



SCHEME 21.



SCHEME 22.



SCHEME 23.

2.7.2 1-Ethyl-2-hydroxy-2,4-dimethyl-3-methylene succinoate(101).

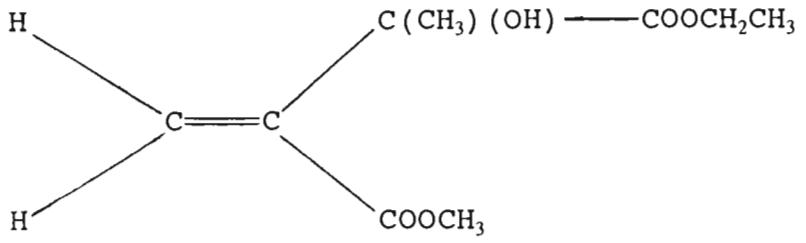
The synthesis of 1-ethyl-2-hydroxy-2,4-dimethyl-3-methylene succinoate(101) from ethyl pyruvate and methyl acrylate using DABCO and QDL catalysts(Schemes 21 and 22 respectively) proceeded in very low yields.

In isolating the product, the unreacted methyl acrylate was removed under vacuum and the product purified by column chromatography(dichloromethane).

Characterization of the expected product by Spectroscopy

The structure of the alcohol(101) was confirmed by ^{1}H NMR, ^{13}C NMR and mass spectrometry.

The ^{1}H NMR spectrum(p. 233) is typical of the α-substituted acrylate systems, the allylic portion of each molecule being an AB system. The vinylic protons are not chemically equivalent and each appears as a doublet due to both geminal couplings(Figure 14).



(101)

FIGURE 14.

The vinylic protons of the alcohol(p. 233) are centred at δ6.363 and δ5.99 while the hydroxyl group is centred at δ3.98. The oxymethylene protons resonate at δ4.22 as a doublet and the three methyl groups resonate at δ1.25, δ1.59 and δ3.76 as a triplet, singlet and oxymethyl singlet respectively.

^{13}C NMR(p. 234) showed the presence of nine carbons, which is consistent with the expected product. The ^{13}C NMR showed the presence of two ester carbonyls{δ174.77 and δ166.51}, a vinylidene group{δ141.87, (C=CH_2) and δ125.63(C=CH_2)}, a tertiary oxygen bonded carbon{δ73.72}, an oxymethylene{δ61.98}, an oxymethyl group{δ52.27} and two methyl groups{δ23.88 and δ14.02}. The above are consistent with the structure of the expected product.

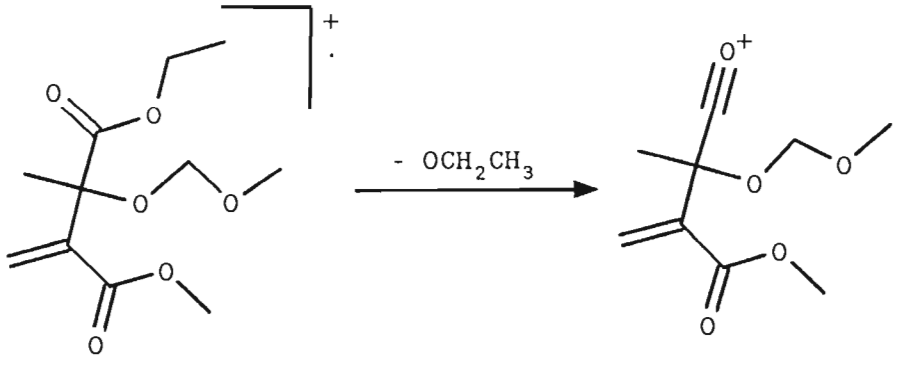
2.7.3 1-Ethyl-2-(methoxymethoxy)-2,4-dimethyl-3-methylenesuccinoate(102).

Formation of the methoxymethyl ether-protected product(102) proceeded with a low yield. Characterization of the product is based on IR, ^{1}H NMR, ^{13}C NMR and mass spectrometry.

The IR spectrum showed the disappearance of the hydroxyl group and the presence of ester carbonyls(1710-1770 cm^{-1}, broad), a carbon-carbon double bond(1630 cm^{-1}) and a vinylidene group{930, 860 and 830 cm^{-1}(out of plane bending of olefins)}.

The ^{1}H NMR spectrum(p. 236) bears resemblance to that of 1-ethyl-3-hydroxy-2,4-dimethyl-3-methylene succinoate except for the disappearance of the hydroxyl group and the emergence of oxymethylene and oxymethyl groups at δ3.33 and δ4.78 which fits very well into the structure of the succinoate ester derivative(102). The ^{13}C NMR(p. 237) is differentiated from that of 1-ethyl-2-hydroxy-2,4-dimethyl-3- methylene succinoate by the emergence of the oxymethylene peak at δ91.91 and oxymethyl peak at δ55.79. The tertiary oxygen-bonded carbon is also moved downfield to δ78.53 by the introduction of the methyl methylene ether moiety into the structure of 1-ethyl-2-hydroxy-2,4-dimethyl-3-methylene succinoate(101).

The base peak in the mass spectrum is *m/z* 45 which results from the loss of the ethoxy fragment and a fragment with *m/z* 185(Scheme 24). The above data are consistent with the structure of 1-ethyl-2-(methoxymethoxy)-2,4-dimethyl-3-methylene succinoate(102).

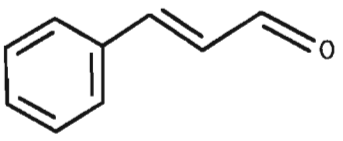


m/e 230(0.3) m/e 185(17.7)

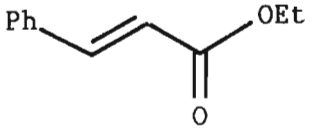
SCHEME 24 Mass fragmention pattern of 1-ethyl-2-(methoxymethoxy)-2,4-dimethyl-3-methylenesuccinoate(47).

2.7.4 3-Methylene-2-methyl-2-(methoxymethoxy) butan-1,4-dial.

The DIBAH reduction(Scheme 21) of the diester(102) to give the dialdehyde(103) was unsuccesful. The DIBAH reduction is known to proceed without difficulty, however, Baeckstrom *et al.*[179] in their preparation of cinnamaldehyde(106) from DIBAH reduction of ethyl



(106)



(107)

cinnamate(107) only found trace amounts of cinnamaldehyde(106). In the present case reduction appears to have proceeded beyond the aldehyde stage.

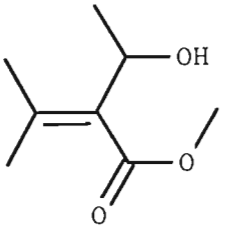
2.7.5 3-Methylene-2-methyl butan-1,2,4-triol(105).

An alternative scheme(Scheme 22) involves the conversion of 1-ethyl-2-hydroxy-2,4-dimethyl-3-methylene succinoate(101) using lithium aluminium hydride to 3-methylene-2-methyl *n*-butan-1,2,4-triol(105). On oxidation with pyridinium chlorochromate this should give the corresponding dial(104). Reduction of the diester(101) using lithium aluminium aldehyde was unsuccessful.

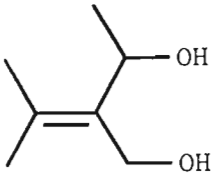
2.7.6 Red-al reduction.

Another method used in the reduction of α,ß-unsaturated esters is the use of Red-Al(Scheme 23). The use of this method in the conversion of the diester(101) to 3-methylene-2-methyl n-butan-1,2,4-triol(105) was unsuccessful. The failure of the reaction at this stage cannot be expained. G. Loizou[182] used the same procedure for the conversion of methyl-3-hydroxy-2-

methylenebutanoate(108) to 3-hydroxy-2-methylenebutan-1-ol(109).



(108)



(109)

CHAPTER 3

EXPERIMENTAL

Mass Spectra were obtained with a Hewlett Packard 5890 GC/MS spectrometer operating at 70 eV. C, H, N analysis was done on a Perkin Elmer 240 Elemental Analyser. ^{1}H NMR and ^{13}C NMR were obtained on a Varian Gemini 200 instrument. All spectra were recorded in $CDCl_3$ as solvent unless otherwise stated and TMS was used as internal standard. Optical rotations were measured on a Perkin Elmer 241 Polarimeter. Mps: uncorrected were determined on a Kofler microheating stage. IR spectra were recorded on a Perkin Elmer 1420 Ratio Recording Infrared Spectrophotometer. Precoated Silica gel 60 F_{254}(Merck Art. 5735) plastic sheets were used for thin layer chromatography. Preparative column chromatography was performed, using the flash technique of Still *et al*[112]., on Silica gel 60(Merck Art. 9385).

3.1 Chemical investigation of *C. transvaalensis* (Burtt Davy) Codd

3.1.1 Plant material.

The plant material of *C. transvaalensis* was collected in Nzhelele (Venda, South Africa).

3.1.2 Extraction.

Dry, powdered root bark(4. 6 kg) of *C.transvaalensis* was differentially extracted in a Soxhlet extraction apparatus for 24 hours. The extraction was carried out first with petroleum ether(40-60°C), then benzene, acetone and finally with ethanol. The average extraction period for each solvent was 24 hours, by which time the extraction appeared to be complete. A precipitate developed when the ethanol extract was allowed to cool to room temperature. The precipitate was separated by decantation. The petroleum ether(40-60°C) extract(45.3 g) was yellow, the benzene extract(82.2 g) red, the acetone extract(300.0 g) brown and ethanol extract(380.0 g) separated out as golden brown amorphous material.

3.1.3 Isolation of galactitol(75)

The separated precipitate(47.4 g) was washed with hot acetone (200 ml) and further washed with hot methanol(200 ml). Recrystallization from water-methanol mixture afforded galactitol(35.4 g) as white crystals mp 187-188°C, lit.[102] 188.5°C, $[\alpha]_D^{20°C}$0.0(1.0 in H_2O), , lit.[102] $[\alpha]_D$0.0(H_2O). (Found: C, 38.89; H, 7.72 $C_6H_{14}O_6$ requires C, 39.56; H, 7.74). IR $\nu_{max}cm^{-1}$: 3340, 2960, 1480, 1460, 1380, 1210, 1120, 1080, 1050, 1030, 930, 870 and 720; MS *m/z*(rel. int.): 146(1.4), 134(1.3), 133(25.0), 115(5.4), 104(2.6), 103(30.1), 97(2.5), 91(8.8), 85(9.1), 74(50.1), 73(100), 71(10.7), 61(63.7), 60(17.4), 57(22.4), 56(30.3), 45(29.7), 44(25.2), 43(51.7), 42 (11.7); ^1H NMR(D_2O): δ = 3.87(2H, t, J = 6.23 Hz, H-2 and H-5), 3.54(2H, d, J = 3.82

Hz, H-3 and H-4), 3.55(4H, d, J = 6.27 Hz, H-1 and H-6); ^{13}C NMR(D_2O): δ = 65.91(t, C-1 and C-6), 72.01(d, C-2 and C-5), 72.84(d, C-3 and C-4).

3.1.4 Galactitol hexaacetate(76).

Isolated galactitol(1.2 g) was dissolved in pyridine(3.0 ml). Acetic anhydride(8.0 ml) was added to the mixture and refluxed overnight(12 hours). It was left to cool to room temperature and then transferred into a beaker with ice. Crystals developed. They were filtered and recrystallized from ethyl acetate to give galactitol hexaacetate(1.9 g) mp 169°C, lit.[102] 168-169°C, $[\alpha]_D^{24°C}$0.00(c 1.107 in $CHCl_3$), lit.[102] $[\alpha]_D$0.00($CHCl_3$). (Found C, 49.85; H, 6.01 $C_{26}H_{26}O_{12}$ requires C, 49.76; H, 6.03). IR ν_{max}(cm^{-1}): 2980, 1740, 1440, 1380, 1240, 1080, 1050, 970; MS *m/z*(rel. int.): 362(1.31), 361(7.34), 290(6.33), 289(44.04), 259(23.25), 216(49.03), 211(9.29), 187(67.50), 170(28.78), 158(15.31), 157(46.20), 145(57.58), 140(8.01), 139(52.50), 128(38.79), 127(37.65), 115(100), 110(17.93), 103(43.67); ^1H NMR($CDCl_3$): δ = 5.35(2H, d, J = 0.66 Hz, H-3 and H-4), 5.31(2H, ddd, J = 4.99, 2.11 and 0.89 Hz), 4.83(2H, dd, J = 7.51 and 4.03 Hz, H-1 and H-6), 4.28(2H, dd, J = 4.76 and 6.87 Hz, the other H-1 and H-6 protons), 2.11(6H, s, acetate methyl groups at C-3 and C-4), 2.09(6H, s, acetate methyl groups at C-2 and C-5), 2.02(6H, s, acetate methyl groups at C-1 and C-6); ^{13}C NMR($CDCl_3$): δ = 170.44(s, O$\underline{C}CH_3$ at C-3 and C-4), 170.28(s, O$\underline{C}CH_3$ at C-2 and C-5), 169.77(s, O$\underline{C}CH_3$ at C-1 and C-6), 67.49(d, C-3 and C-4), 67.40(d, C-2 and C-5), 62.18(t, C-1 and C-6), 20.73(q, CO$\underline{C}H_3$ at C-3 and C-4), 20.66(q, CO$\underline{C}H_3$ at C-2 and C-5), 20.58(q, CO$\underline{C}H_3$ at C-1 and C-6).

3.1.5 Isolation of (-)6ß-hydroxy-lup-20(29)-en-3-one(77).

The petroleum ether(40-60°C) extract was partitioned between *n*-hexane and 95% aqueous methanol. The *n*-hexane fraction was adsorbed on 23.0 g silica gel 60 and the adsorbed material transfered into the column and eluted with hexane/ethyl

acetate(95:5). Fractions collected were as follows:-

Fraction.	Amount.	R_f values (4:1 hexane/ethyl acetate)
1	2.3g	0.69
2	3.1g	0.44

Fraction 2 was recrystallized from hexane:ethyl acetate mixture to give (-)6ß-hydroxy-20(29)-lup-3-one(219 mg) mp 235°C; lit.[115] mp 233-234°C, $[\alpha]_D^{20°C}$-7.85°(c 0.80 in $CHCl_3$), lit.[115] $[\alpha]_D$-14.0°($CHCl_3$). (Found C, 81.69; H, 11.01 $C_{30}H_{48}O_2$ requires C, 81.74; H, 10.98). IR $\nu_{max}cm^{-1}$: 3430, 2930, 2880, 1690, 1640, 1450, 1380 and 880; MS *m/z*(rel. int.): 440(1.12), 248(1.53), 218(5.53), 205(10.67) 204 (6.55), 203(8.21), 190(3.1), 189(10.79), 175(8.93), 174(1.4); ^{1}H NMR($CDCl_3$): δ = 4.69 and 4.58(2H, d, J = 2.42 Hz, vinylic protons), 4.56(1H, J = 1.3 Hz, H-6), 2.82(1H, td, J = 5.75 Hz), 2.26(1H, m), 1.73(1H, m), 1.68(3H, s), 1.43(3H, s), 1.42(3H, s), 1.41(3H, s), 1.14(3H, s), 0.90(3H, s), 0.80(3H, s); ^{13}C NMR($CDCl_3$): δ = 217.30(s, C-3), 151.20(s, C-20), 109.70(t, C-29), 69.92(d, C-6), 56.76(d, C-5), 50.8(d, C-9), 49.11(s, C-4), 48.48(d, C-18), 48.09(d, C-19), 43.24(s, C-17), 42.28(t, C-7), 42.28(t, C-1), 42.24(s, C-14), 40.15(s, C-8), 40.05(t, C-22), 37.25(d, C-13), 36.87(s, C-10), 35.58(t, C-16), 35.54(t, C-2), 29.89(t, C-21), 27.58(t, C-15), 25.25(t, C-12), 25.06(q, C-30), 23.80(q, C-24), 21.34(t, C-11), 19.38(q, C-30), 18.05(q, C-28), 17.14(q, C-26), 17.02(q, C-25), 14.85(q, C-27).

3.1.6 Isolation of 3-oxo-friedelan-28-al(78).

Fraction 1(2.3 g), from the *n*-hexane partitioned fraction, was further chromatographed on a column using 9:1 hexane:ethyl acetate solvent mixture. Fractions with R_f values = 0.44 were combined and the solvent removed to give 1.5 g of pure material. The material was recrystallized from hexane-ethyl acetate solvent mixture to give 62 mg of 3-oxo-friedelan-28-al(78) mp 262-263°C, lit.[118] mp 263-265°C $[\alpha]_D^{20°C}$-21.87°(c 0.178 in $CHCl_3$), lit.[118] $[\alpha]_D$-

16°($CHCl_3$). (Found C, 81.48; H, 10.85 $C_{30}H_{48}O_2$ requires C, 81.76; H, 10.98). IR ν_{max}(cm^{-1}): 2960, 2840, 1710, 1705, 1450 and 1380; MS *m/z*(rel. int.): 440(100), 425(44.03), 411(22.2), 397(7.36), 369(1.9), 355(18.67), 273(11.18) and 138(4.54); ^1H NMR($CDCl_3$): δ = 9.4(1H, s, -COH), 1.09 (3H, s), 0.98(3H, H), 0.88(6H, s), 0.87(6H, s), 0.71(3H, s) and 1.2-2.4 (protons in the methylene group region); ^{13}C NMR($CDCl_3$): δ = 213.75(s, C-3), 206.0(d, C-28), 59.59(d), 58.27(d), 50.50(d), 44.70(s), 44.05(d), 42.14(s), 41.55(t), 41.25(t), 39.50(s), 39.49(s), 37.57(s), 36.39(t), 36.32(t), 35.19(t), 31.84(q), 30.16(t), 29.01(t), 28.59(t), 26.93(q), 26.26(t), 22.32(t), 19.0(q), 18.66(q), 18.2(t), 16.11(q), 14.70(q), and 6.82(q).

3.1.7 Isolation of 3-oxo-friedelan-28-ol(80).

The 95% aqueous methanol partitioned fraction(22.31 g) was dissolved in chloroform and adsorbed on silica gel(23 g). The adsorbed material was chromatographed on a column using 9:1 hexane: ethyl acetate solvent mixture. Fractions collected were as follows:-

Fraction.	Amount.	R_f values (1:1 hexane/ethyl acetate).
1	3.0g	0.99
2	3.2g	0.88

Further purification of fraction 2 by repeated column chromatography followed by recrystallization in hexane-ethyl acetate afforded 3-oxo-friedelan-28-ol(80), 148 mg, mp 278-279°C, lit.[122] 277-280°C, $[\alpha]_D^{20°C}$-18.88°(c 0.43 in $CHCl_3$), lit.[122] $[\alpha]_D$-21.0°(c 1.02 in $CHCl_3$). (Found C, 81.52; H, 11.16 $C_{30}H_{50}O_2$ requires C, 81.39; H, 11.38). IR ν_{max}(cm^{-1}): 3400, 1680, 1440, 1380, 1040; ^1H NMR($CDCl_3$): δ = 3.26(2H, s, broad, $-CH_2-$), 2.36 and 2.25(2H, m, nonequivalent methylene protons), 1.95(1H, broad, OH), 1.22(3H, s), 1.05(3H, s), 1.03(3H, s), 0.89(3H, s), 0.87(3H, s), 0.86(3H, s), 0.72(3H, s) and 1.3-δ1.8(signals in the methylene group

region); ^{13}C NMR($CDCl_3$): δ = 213.90(s, C-3), 74.98(t, C-28), {δ = 32.15. 25.87, 20.82, 18.51, 17.92, 14.69, 6.85 (seven methyl groups)}; MS *m/z*(rel. int.): 442(2.33), 302(3.29), 273(9.87), 159(5.95) and 140(6.32).

3.1.8 Isolation of (+)-11,11-dimethyl-1,3,8,10-tetrahydroxy-9-methoxy peltogynan(11).

The acidic chloroform extract (10.0 g) indicated the presence of one major component (R_f0.51, chloroform/ethyl acetate(2:3). The material was further purified by repeated flash chromatography in the same solvent. The major component was collected and recrystallized(chloroform/ethyl acetate) giving pure material(2.3 g) mp 154°C, $[\alpha]_D^{20°C}$+173.92° (c 1.02 in acetone). (Found C, 63.27; H, 5.56 $C_{19}H_{20}O_7$ requires C, 63.32; H, 5.59). IR ν_{max}(cm^{-1}): 3400, 2900, 1615, 1515, 1470, 1440, 1355, 1270, 1145, 1085, 1070, 1010, 840, 820 and 755; ^1H NMR(CD_3COCD_3): δ = 8.45(1H, s, C-8 phenolic hydrogen), 8.30(1H, s, C-3 phenolic hydrogen), 8.10(1H, s, C-1 phenolic hydrogen), 7.95(1H, s, C-10 phenolic hydrogen), 6.15(1H, H-7), δ6.01(1H, d, J = 2.3 Hz, H-4), 5.82(1H, d, J = 2.36 Hz, H-2), 4.42(1H, s-broad, H-6), 4.23(1H, ddd, H-13), 3.82(3H, s, C-9 methoxy group), 2.83(2H, ddd, nonequivalent C-14 hydrogens), 1.65(3H, s, C-11 methyl group), 1.55(3H, s, C-11 methyl group); $_{13}C$ NMR(CD_3COCD_3): δ = 156.33(s, C-3), 156.29(s, C-16), 155.58(s, C-1), 148.07(s, C-8), 146.24(s, C-10), 135.85(s, C-9), 128.53(s, C-17), 120.68(s, C-18), 109.04(d, C-7), 94.77(d, C-4), 94.41(d, C-2), 74.39(s, C-11), 70.22(d, C-6), 62.31(d, C-13), 59.54(q, methoxy group at C-9), 27.46(q, methyl group at C-11), 24.77(t, C-14), 23.11(q, methyl group at C-11).

3.1.9 Isolation of pristimerin(4).

The benzene extract was separated by column chromatography (hexane/dichloromethane 1:1). Of the collected 15 ml fractions, fractions 27-51 were combined and the solvent removed to give orange material(1.3 g) which recrystallized from ethyl acetate as orange crystals, 201 mg. ^{13}C NMR results revealed the

recrystallized fraction to be a mixture of pristimerin and 3-oxofriedelan-28-ol(Figure 4). Peaks identified as characteristic of pristimerin[7] are: (i) δ128.22(d, C-6), (ii) δ120.1(d, C-1), (iii) δ118.5(d, C-7) and δ51.8(q, C-31) and peaks identified as typical of 3-oxofriedelan-28-ol are: (i) δ74.98(t, C-28), (ii) δ59.64(d, C-10), (iii) δ58.43(d, C-4) and δ53.60(d, C-8).

3.2 Chemical investigation of *Cassine papillosa* (Hoechst.) Kuntze.

3.2.1 Extraction

The powdered stem bark(2.3 kg) of *C. papillosa* was extracted in a Soxhlet extraction apparatus with hexane followed by successive extractions with benzene, ethyl acetate and ethanol. Removal of the hexane from the first extract gave a brown gum(336.2 g) which was partitioned between *n*-hexane and 95% aqueous methanol. From the aqueous methanol a brown solid(35.1 g) was obtained and this was separated by column chromatography using hexane/ethyl acetate(9:1).

3.2.2 Isolation of 30-Hydroxylup-20(29)-en-3-one(2).

From the collected 15 ml fractions, fractions 65-100 were combined, the solvent removed and recrystallized in hexane-ethyl acetate solvent mixture to give 1.2 g colourless crystals, mp 184-185°C, lit.[17] mp 186-187°C, $[\alpha]_D^{23°C}$+22.23°(c 1.021 in $CHCl_3$), lit.[17] $[\alpha]_D$+21.2°($CHCl_3$). (Found C, 82.01; H, 11.22 $C_{30}H_{48}O_2$ requires C, 81.76; H, 10.97). IR $\nu_{max}cm^{-1}$: 3500, 2960, 2880, 1700, 1460, 1390, 1040, 900; MS *m/z*(rel. int.): 440[[M^+](4.2), 355(1.1), 313(14.9), 257(2.1), 234(6.36), 232(55.5), 221(21.3), 220(11.4), 219(7.7), 206(17.0), 205(55.3), 121(59.5), 55(100); ^{1}H NMR($CDCl_3$): δ = 4.95(1H, d, J = 1.65 Hz, H-29), 4.93(1H, d, J = 1.64 Hz, H-29), 4.11(2H, d, J = 1.13 Hz, H-30), 2.45(1H, m, H-19), 1.07(3H, s, H-26), 1.06(3H, s, H-23), 1.02(3H, s, H-28), 0.95(3H, s, H-27), 0.92(3H, s, H-24), 0.78(3H, s, H-25); ^{13}C NMR($CDCl_3$: δ = 218.35(s, C-3), 154.63(s, C-20), 106.7(t, C-29), 64.87(t, C-30),

54.79(d, C-5), 49.67(d, C-9), 48.72(d, C-18), 47.29(d, C-19), 43.69(t, C-7), 42.97(s, C-17), 42.79(s, C-15), 40.73(t, C-22), 39.78(t, C-1), 39.54(s, C-8), 38.04(d, C-13), 38.04(s, C-4), 36.81(s, C-10), 35.58(t, C-16), 33.51(t, C-21), 31.7(t, C-15), 27.34(t, C-2), 26.63(t, C-12), 26.63(q, C-23), 21.51(t, C-11), 21.01(q, C-28), 19.63(t, C-6), 17.69(q, C-25), 15.97(q, C-26), 15.76(q, C-24), 14.43(q, C-27).

3.2.3 Isolation of 3-oxo-friedelan-28-ol(80).

Combining fractions 155-210 and removing the solvent gave 2.03 g of crystalline material. Recrystallization using hexane-ethyl acetate solvent mixture afforded white crystals(365 mg) mp 278°C, lit.[122] mp 277-278°C, $[\alpha]_D^{21°C}$-19.94(c 1.01 in $CHCl_3$), lit.[122] $[\alpha]_D$-21.0(c 1.02 in $CHCl_3$). (Found C, 81.39; H, 11.16 $C_{30}H_{50}O_2$ requires C, 81.39; H,11.38). IR V_{max}(cm^{-1}): 3400, 2960, 1680, 1440, 1380; MS *m/z*(rel. int.): 442[M^+](2.33), 302(3.29), 159(5.95), 140(6.32); ^1H NMR($CDCl_3$): δ = 3.62(2H, s, broad, -CH_2-O), 2.36 and 2.25(2H, m, nonequivalent methylene protons), 1.95(1H, broad, OH), 1.22(3H, s), 1.05(3H, s), 1.03(3H, s), 0.89(3H, s), 0.87(3H, s), 0.86(3H, s), 72(3H, s), 1.3-1.8(signals in the methylene group region); ^{13}C NMR($CDCl_3$): δ = 213.90(s, C-3), 74.98(t, C-28), {δ = 32.15, 25.87, 25.87, 18.51, 17.92, 14.69, 6.85(seven methyl groups)}.

3.2.4 Isolation of 30-Hydroxylupeol(86).

Removal of the solvent from the collected fractions 230-300 gave orange-red material(1.5 g). Recrystallization of the material using hexane-ethyl acetate solvent mixture afforded 383 mg of orange amorphous material. Further recrystallization afforded 156 mg of white material mp 234-235°C, lit.[15] mp 235-236°C, $[\alpha]_D^{23°C}$-13.90°(c 0.34 in THF), lit.[15] [α] -7.8°. (Found C, 81.02; H, 11.05 $C_{30}H_{50}O_2$ requires C, 81.39; H, 11.38). IR $\nu_{max}cm^{-1}$: 3340, 2970, 2930, 1460, 1400, 1050, 930; MS *m/z*(rel. int.): 442[M^+](6.43), 424(3.93), 381(1.02), 316(6.27), 315(13.16), 297(3.56), 273(3.17), 257(4.97), 247(6.59), 234(26.13), 220(19.42),

219(11.77), 208(23.81), 207(74.71), 206(13.76), 205(17.79), 191(32.29), 190(42.10), 189(81.19), 107(100); ^{1}H NMR(THF-d_8): δ = 5.04(1H, d, J = 1.71 Hz, H-29), 4.90(1H, d, J = 1.61 Hz, H-29), 4.09(2H, d, J = 5.13 Hz, H-30), 3.87(1H, t, J = 5.31 Hz, C-30 hydroxyl group), 3.53(1H, d, J = 6.04 Hz, C-3 hydroxyl group), 3.14(1H, m, H-3), 2.36(1H, dd,J = 5.13 Hz and 5.85, H-19), 1.18(3H, s, H-26), 1.10(3H, s, H-23), 1.05(3H, s, H-27), 0.97(3H, s, H-25), 0.92(3H, s, H-28), 0.84(3H, s, H-24); ^{13}C NMR(THF-d_8): δ = 1557.71(s, C-20), 107.40(t, C-29), 79.63(d, C-3), 65.95(t, C-30), 57.65(d, C-5), 52.67(d, C-9), 50.63(d, C-18), 45.00(d, C-19), 44.81(s, C-17), 42.97(s, C-14), 41.86(t, C-22), 40.93(s, C-8), 40.93(t, C-1), 39.24(s, C-4), 37.60(d, C-13), 37.60(t, C-16), 36.48(s, C-10), 36.48(t, C-7), 33.73(t, C-21), 29.74(t, C-15), 29.66(t, C-12), 29.58(q, C-23), 28.65(t, C-2), 23.06(t, C-11), 20.44(t, C-6), 19.26(q, C-28), δ17.89(q, C-25), 17.67(q, C-26), 17.28(q, C-24), 16.14(q, C-27).

3.2.5 Isolation of tingenin B(7).

Removal of the mother liquor, after recrystallization of 30-hydroxylupeol(86), afforded orange-red material(1.3 g) which was recrystallized from tetrahydrofuran to give red crystals(60 mg), mp 204-205°C lit.[7] mp 210-211°C. (Found C, 76.84; H, 8.43 $C_{28}H_{36}O_4$ requires C, 77.0; H, 8.3). IR ν_{max}cm^{-1}: 3360, 2960, 1680, 1580, 1520, 1430, 1220, 880; MS *m/z*(rel. int.): 436[M$^+$](29.55), 434(11.89), 420(20.55), 274(16.54), 267(16.91), 257(12.45), 254(13.75), 253(46.28), 242(19.6), 241(61.89), 237(16.82), 236(11.7), 228(14.59), 227(41.07), 225(25.0), 215(21.18), 214(22.86), 213(29.74), 211(22.4), 201(100), 191(18.58), 189(15.52), 187(19.88), 167(48.69), 165(38.19), 163(19.42), 157(21.74), 149(99.07), 137(34.94), 135(12.54), 129(62.64); ^{1}H NMR($CDCl_3$): δ = 7.04(1H, dd, J = 1.47 and 7.12 Hz, H-6), 7.0(1H, s, hydroxyl group at C-3), 6.54(1H, d, J = 1.37 Hz, H-1), 6.40(1H, d, J = 18.0 Hz, H-7), 4.5(1H, d, J = 2.6 Hz, H-22), 3.6(1H, d, J = 4.29 Hz, hydroxyl group at C-22), 2.23(3H, s, H-23), 1.51(3H, s, H-25), 1.35(3H, d, J = 6.3 Hz, H-28), 1.01(3H, s, H-30), 0.98(3H, s, H-27), 0.87(3H, s, H-28); ^{13}C NMR($CDCl_3$): δ

= 213.60(s, C-21), 178.38(s, C-2), 168.56(s, C-8), 164.05(s, C-4), 146.05(s, C-3), 133.81(d, C-6), 127.68(s, C-10), 119.78(d, C-1), 118.18(d, C-7), 76.41(d, C-22), 44.98(d, C-18), 40.86(d, C-20), 39.17(q, C-25), 33.99(t, C-19), 32.02(t, C-16), 29.91(t, C-11), 29.49(t, C-15), 28.24(t, C-12), 25.00(q, C-28), 21.61(q, C-26), 20.52(q, C-27), 14.76(q, C-30), 10.30(q, C-23).

3.2.6 Isolation of (-)4'-O-methyl epigallocatechin(12).

Removal of the solvent from the ethyl acetate extract gave 110.8 g of solid material. A purple precipitate developed when dichloromethane was added to the extract. The precipitate was separated from the dichloromethane by filtration to give 47.75 g of solid. The precipitate(10.1 g) was adsorbed to 10 g of silica gel, transfered into the column and eluted with dichloromethane/ethyl acetate(1:1). The first fractions collected indicated the presence of one compound R_f0.26 (dichloromethane/ethyl acetate 1:1).The fractions were combined and the solvent was removed to give 3.5 g of pure material. The material was recrystallized from dichloromethane/ethyl acetate solvent mixture to give 1.39 g of pure (-)4'-O-methyl epigallocatechin(12) mp 141°C, lit.[123] mp 141-143°C, $[\alpha]_D^{25°C}$-50.02(c 1.1 in acetone), lit.[123] [α]-53°(c 1.5 in acetone), $[\alpha]_D^{22°C}$-60(ethanol). (Found C, 60.19; H, 4.28 $C_{16}H_{16}O_7$ requires C, 60.00; H, 4.85). IR ν_{max}(cm^{-1}): 3400, 2940, 1620, 1590, 1510, 1460, 1430, 810; MS *m/z*(rel. int.): 320[M^+](7.94), 302(5.75), 275(0.92), 241(0.98), 217(2.55), 215(1.08), 213(1.14), 193(0.95), 182(44.64), 168(11.34), 167(97.72), 154(13.99), 153(27.16), 139(100), 125(15.81), 110(51.09), 97(29.84); ^1H NMR(CD_3COCD_3): δ = 6.59(2H, d, J = 0.64 Hz, H-2' and H-6'), 6.02(1H, d, J = 2.36 Hz, H-6), 5.93(1H, d, J = 2.29 Hz, H-8), 4.84(1H, d, J = 0.78 Hz, H-2), 4.23(1H, d, J = 3.89 Hz, H-3), 3.78(3H, s, OCH_3), 3.77(1H, d, J = 10.49 Hz, C-3 hydroxyl group), 2.87(1H, ddd, J = 0.78, 4.39 and 16.76 Hz, H-4), 2.73(1H, dd, J = 3.23 and 16.72 Hz, H-4), 8.00(4H, s, broad, phenolic hydroxyl groups); ^{13}C NMR(CD_3COCD_3): δ = 157.44(s, C-7), 157.40(s, C-9), 156.81(s, C-5), 150.74(s, C-3' and C-5'), 136.16(s, C-1'), 135.38(s, C-4'),

106.97(d, C-2' and C-6'), 99.72(s, C-10), 96.14(d, C-6), 95.59(d, C-8), 79.14(d, C-2), 66.78(d, C-3), 60.51(q, OCH_3), 28.78(t, C-4).

3.2.7 Isolation of ouratea proanthocyanidin(13).

After complete removal of (-)4'-O-methyl epigallocatechin(12) from the column, ethyl acetate was used as a solvent. This resulted in the collection of 1.9 g of pure material. Repeated column chromatography afforded whitish amorphous material(721 mg). Further purification using Toyopearl in ethanol as solvent gave pure material(334 mg) which exhibited the following physical and spectral properties: $[\alpha]_D^{27°C}$+49.05°(c 1.038 in acetone), lit.[123] $[\alpha]_D$+54°(c 1.6 in acetone). IR ν_{max}(cm^{-1}): 3400, 2980, 1630, 1610, 1560, 1525, 860; MS *m/z*(rel. int.): 592.158[M^+](0.0), 360(0.65), 345(0.96), 320(10.78), 302(6.1), 245(1.46), 244(7.15), 227(1.79), 217(2.34), 213(3.9), 207(3.65), 201(2.15), 199(1.07), 194(2.32), 192(2.41), 185(2.47), 182(44.32), 171(2.35), 168(7.8), 167(53.88), 154(13.97), 153(24.36), 149(24.07), 139(100), 126(68.27); ^{1}H NMR(CD_3COCD_3): δ = 8.00-8.55(7H, phenolic hydroxyl groups), 7.25(2H, d, J = 8.53 Hz, H-2'u and H-6'u), 6.74(2H, d, J = 8.59 Hz, H-3'u and H-5'u), 6.64(2H, s, H-2't and H-6't), 6.02(2H, d, J = 1.28 Hz, H-6u and H-8u), 5.97(1H, s, H-6t), 5.13(1H, s, broad, H-2u), 4.89(1H, d, J = 0.87 Hz, H-2t), 4.73(1H, s, broad, H-4u), 4.30(1H, s, broad, H-3t), 3.99(1H, s, broad, H-3u), 3.74(3H, s, OCH_3), 2.83(2H, ddd, J = 0.78, 4.56 and 17.45 Hz, H-4t); ^{13}C NMR(CD_3COCD_3): δ = 158.28(s, C-5t), 158.22(s, C-5u), 157.44(s, C-7u), 157.53(s, C-7t), 155.81(s, C-9t), 155.70(s, C-9u), 154.2(s, C-4'u), 150.77(s, C-3't and C-5't), 135.84(s, C-1't), 135.13(s, C-4't), 131.312(s, C-1'u), 129.03(d, C-2'u and C-6'u), 115.37(d, C-3'u and C-5'u), 106.91(s, C-8t), 106.58(d, C-2't and C-6't), 100.43(s, C-10u and C-10t), 97.0(d, C-8u), 96.35(d, C-6t), 95.78(d, C-6u), 79.01(d, C-2t), 76.77(d, C-2u), 72.67(d, C-3u), 66.27(d, C-3t), 60.38(q, O$\underline{C}H_3$), 36.87(d, C-4u), 30.57(t, C-4t).

3.2.8 Isolation of galactitol(75).

The ethanol extract was allowed to cool down to room temperature and crystals developed. The crystals were separated by decantation and washed with acetone. The yield(20.6 g) was recrystallized was recrystallized using methanol-ethanol-water mixture to give 7.6 g of pure galactitol with had the following physical and spectral properties identical to material isolated previously(section 3.1.3).

3.2.9 Isolation of (+)6R,13R-11,11-dimethyl-1,3,8,10-tetrahydroxy-9-methoxypeltogynan(83).

Ethanol was removed from the ectract, after the decantation separation of galactitol, and the residue dissolved in 1500 ml of 1.0 mol.l^{-1}HCl and extracted with chloroform. Ethanol was removed from the extract, after the decantation separation of galactitol, and the residue dissolved in 1500 ml of 1.0 mol.l^{-1}HCl and extracted with chloroform. The acidic chloroform extract(18.6 g) was adsorbed onto 20.0 g silica gel, tranfered into the column and eluted with chloroform/ethyl acetate(2:3). The collected material(2.1 g) were further purified by flash chromatography[112]. The pure material was recrystallized in hexane/ethyl acetate solvent mixture to give (+)-6R,13R-11,11-dimethyl-1,3,8,10-tetrahydroxy-9-methoxypeltogynan(378 mg) which had the same physical and spectral properties as the compound previously isolated(Section 3.1.8).

3.2.10 Isolation of tingenone(5).

10.0 g of the benzene extract(82.1 g) was adsorbed onto 10.0 g of silica gel, transferred into the column and eluted with hexane/dichloromethane(1:1). The first compound to be collected(765 mg) was recrystallized from tetrahydrofuran to give orange-red crystals(51 mg). ^{1}H NMR data indicated the material to be a 1:1 mixture of tingenin B and tingenone. Furthermore ^{13}C NMR showed distinct peaks at δ76.41(doublet) and δ52.53(triplet)

typical of C-22 peaks of tingenin B and tingenone respectively[7].

3.2.11 Synthesis of (+)-6R,13R-11,11-dimethyl-1,3,8,10-tetrahydroxy-9-methoxypeltogynan(83) from (-)4'-O-methyl epigallocatechin(12).

(-)4'-O-Methyl epigallocatechin(300 mg, 0.93 mmol.) was dissolved in acetone(10 ml) containing 2% H_2O v/v. Toluene-p-sulphonic acid(200 mg, 1.05 mmol.) was added and the solution stirred at room temperature for four days. The remaining acetone was then removed under vacuum and the product purified by flash chromatography using chloroform/ethyl acetate(2:3) to give 281 mg(0.78 mmol., yield 83.3%), of pure (+)6R,13R-11,11-dimethyl-1,3,8,10-tetrahydroxypeltogynan(83). Physical and spectral properties were the same as that of the isolated peltogynan[27].

3.2.12 Synthesis of 11-methyl,11-ethyl-1,3,8,10-tetrahydroxy-9-methoxypeltogynan(84) from (-)4'-O-methyl epigallocatechin(12)

(-)4'-O-Methyl epigallocatechin(300 mg, 0.93 mmol.) was dissolved in butanone(10 ml) containing 2% H_2O v/v. Toluene-p-sulphonic acid(150 mg, 1.06 mmoles) was added and the solution stirred at room temperature for four days. The remaining butanone was removed under vacuum and the product purified by flash chromatography using chloroform/ethyl acetate(2:3) to give 28 mg of 11-methyl,11-ethyl-1,3,8,10-tetrahydroxy-9-methoxypeltogynan(84) exhibiting the following physical and spectral properties: mp 165°C, IR ν_{max}(cm^{-1}): 3400, 2900, 1610, 1515, 1470, 1440, 1355, 1275, 1150, 1085, 1070, 1010, 840, 820 and 755; ^1H NMR(CD_3COCD_3) δ: 8.31(1H, s, OH), 8.19(1H, s, OH), 8.16(1H, s, OH), 7.84(1H, s, OH), 6.48(1H, s, H-7), 5.99(1H, d, J = 2.28 Hz, H-4), 5.79(1H, d, J = 2.39 Hz, H-2), 4.42(1H, s, broad, H-6), 4.17(1H, ddd, H-13), 3.8(3H, s, OMe), 2.82(2H, s, broad, H-14), 1.79(2H, s), 1.55(3H, s), 0.88(3H, t, J = 2.89 Hz). ^{13}C NMR(CD_3COCD_3) δ: 157.2(s, C-1), 157.1(s, C-3), 156.4(s, C-16), 148.8(s, C-8), 147.1(s, C-10), 136.7(s, C-9), 129.4(s, C-17),

122.6(s, C-18), 109.5(d, C-7), 99.2(s, C-15), 95.2(d, C-4), 94.9(d, C-2), 77.3(s, C-11), 70.7(d, C-6), 63.2(d, C-13), 60.2(q, C-9 OMe), 27.3(t, C-14), 25.1(t), 23.6(q), 7.5(q).

3.2.13 Synthesis of galactitol hexaacetate(76).

1.091 g of the isolated galactitol was dissolved in 3.0 ml pyridine. 8.0 ml of acetic anhydride was added to the mixture and refluxed overnight(12 hours). It was left to cool to room temperature and transferred into a beaker with ice. Crystals developed. They were filtered and recrystallized from ethyl acetate to give galactitol hexaacetate(1.78 g) which had physical and spectral properties in agreement with literature values[102].

3.2.14 Ouratea proanthocyanidin-nona-O-acetate(85).

Ouratea proanthocyanidin(103.8 mg, 0.17 mmol.) was dissolved in pyridine(1.0 ml) and acetic anhydride(3.0 ml) added. The mixture was refluxed overnight with stirring, dissolved in 25 ml of water and transferred into a separating funnel. The mixture was extracted three times with chloroform. The combined chloroform layers were dried using anhydrous magnesium sulphate. Filtration and removal of the solvent under reduced pressure afforded 98 mg of impure material which was purified using the chromatotron in a chloroform/ethyl acetate(9:1) solvent mixture to afford pure ouratea proanthocyanidin-nona-O-acetate(49 mg, 0.05 mmol, 30,7% yield) mp 133°C, lit.[123] mp 134-135°C, $[\alpha]_D^{22°C}$+19.18(c 0.37 in $CHCl_3$), lit.[123] $[\alpha]_D^{22°C}$+22.8(c 2.0 in $CHCl_3$). (Found C, 60.45; H, 4.69 $C_{49}H_{46}O_{21}$ requires C, 60.62; H, 4.77). IR ν_{max}(cm^{-1}): 2980, 1780, 1620, 1600, 1510, 1440, 1380, 1200, 1130, 1060, 900; MS *m/z*(rel. int.): 970[M^+](0.0), 602(2.12), 571(2.15), 477(4.25), 433(8.51), 391(8.93), 298(14.89), 297(17.02), 257(55.32), 182(100), 107(93.62); ^{1}H NMR($CDCl_3$): δ = 7.47(2H, d, J = 8.54 Hz, H-2'u and H-6' majar rotamers), 7.39(d, J = 8.7 Hz, H-2' and H-6'u minor rotamers), 7.09(2H, d, J = 8.72 Hz, H-3'u and H-5'u major rotamers), 7.05(d, J = 7.21 Hz, H-3'u and H-5'u minor rotamers), 6.98(d, J = 2.29 Hz, H-6u minor rotamer), 6.73(2H, s, H-2't and

H-6't), 6.65(1H, s, H-6t), 6.602(d, J = 2.29 Hz, H-8u minor rotamer), 6.27(2H, d, J = 2.29 Hz, H-6u major rotamer), 6.08(2H, d, J = 2.29 Hz, H-8u major rotamer), 5.00-5.65(4H, H-2t, H-2u, H-3t, H-3u), 4.48(1H, d, J = 4.78 Hz ,H-4u), 3.79(3H, s, OCH_3), δ2.88(2H, m, J = 0.51 and 2.42 Hz, H-4t), 1.8-2.4(27H, s, acetate methyl groups).

3.2.15 Lup-20(29)-en-3ß,30-diacetate(87).

Lup-20(29)-en-ß,30-diol(46 mg, 0.10 mmol.), pyridine(1.0 ml) and acetic anhydride(3.0 ml) were refluxed for 12 hours. The material was then transfered into a beaker with ice. A precipitate developed. Filtration and washing with water afforded 50 mg of white crystals. Recrystallization from methanol afforded 32.2 mg(yield 61.3%) mp 164°C, lit.[15] mp 165-166°C, $[\alpha]_D^{22°C}$+10.2(c 0.21 in $CHCl_3$) lit.[15] $[\alpha]_D$+11.0. (Found C, 77.49; H, 10.32 $C_{34}H_{54}O_4$ requires C, 77.52; H, 10.33). ^{1}H NMR($CDCl_3$): δ = 4.95 and 4.92(2H, d, H-29), δ4.55(2H, s, H-30), 4.47(1H, m, H-3), 2.29(1H, dd, H-19), 2.11(3H, CH_3), 2.04(3H, s, CH_3), 1.03(3H, s, H-26), 0.94(3H, s, H-27), 0.84(3H, s, H-23), 0.834(6H, s, H-24 and H-25), 0.78(3H, s, H-28); ^{13}C NMR($CDCl_3$) δ = 38.36(t, C-1), 26.45(t, C-2), 80.93(d, C-3), 40.84(s, C-4), 55.32(d, C-5), 21.06(t, C-6), 38.36(t, C-7), 42.77(s, C-8), 50.26(d, C-9), 37.05(s, C-10), 23.68(t, C-11), 31.29(t, C-12), 37.99(d, C-13), 43.02(s, C-14), 27.36(t, C-15), 35.40(t, C-16), 44.32(s, C-17), 48.89(d, C-18), 37.99(d, C-19), 149.17(s, C-20), 34.19(t, C-21), 39.19(t, C-22), 27.93(q, C-23), 15.97(q, C-24), 16.50(q, C-25), 16.18(q, C-26), 14.52(q, C-27), 17.70(q, C-28), 110.05(t, C-29), 65.97(t, C-30), 21.34(q, $\underline{C}H_3COO$), 171.03(q, $CH_3\underline{C}OO$), 21.07(q, $\underline{C}H_3COO$), 170.82(s, $CH_3\underline{C}OO$).

3.3 Chemical investigation of *Warburgia salutaris*(Bertol. f.) Chiov

3.3.1 Plant material

The plant material were collected in Nzhelele, Venda. The bark was air dried in a shade for twelve weeks. The dried material,

6.2 kg, was milled to a fine powder before extracting.

3.3.2 Extraction

A cold differential extraction was carried out on the dried powdered stem bark (6.2 kg) of *W. salutaris* using petroleum ether(40-60°C), dichloromethane, ethyl acetate and finally ethanol as extracting solvents. The stem bark was soaked in petroleum ether(10 litres) for 14 days, filtered and the mother liquor removed under vacuum to give 56.68 g. The residue was then left in 10 litres of dichloromethane for two weeks. Removal of the solvent under vacuum afforded 75.3 g of yellow extract. The same procedure was carried out but using ethyl acetate as the solvent to give 53.6 g of brown extract. Ten litres of ethanol were then transferred into the container with the residue and the mixture left for two weeks. Filtration and removal of the solvent afforded 82.0 g of brown material.

3.3.3 Isolation of (-)-warburganal(21)

Tlc analysis of the petroleum ether(40-60°C) and dichloromethane extracts showed the two extracts to be identical. The two extracts were combined, dissolved in 600 ml ethyl acetate and adsorbed onto silica gel 60(140 g). The mixture was applied to the column of silica gel 60(400 g) in petroleum ether. The column was eluted with hexane/ethyl acetate(9:1) solvent mixture. 250 ml fractions were collected. Removal of solvent from fraction 4 afforded 13.8 g which indicated the presence of one major component, R_f0.57 (hexane/ethyl acetate 1:1). The material was further adsorbed onto silica gel 60 and eluted with hexane/ethyl acetate(9:1) solvent mixture. Pure fractions were collected and the solvent removed to give yellow crystals(5.3 g) which were recrystallized in hexane/ethyl acetate solvent mixture to give of white needles(3.3 g) mp 105-106°C, lit.[36,183,184] mp 107-108°C, $[\alpha]_D^{20°C}$-234.28° (c, 1.3249 in $CHCl_3$), lit.[36,183,184] $[\alpha]_D$-263.0° (c, 0.38 in $CHCl_3$). m/e Calculated for $C_{15}H_{22}O_3$ (M^+), 250.1563, found 250.1487. MS *m/z*(rel. int.): 250(3.25), 232(8.24), 221(46.47),

204(6.90), 201(2.59), 189(22.48), 167(30.34), 149(100), 125(11.3), 110(10.7), 109(65.20); IR V_{max}(cm_{-1}): 3480, 1682, 1638, 2818, 1719, 1390, 1380; ^{1}H NMR($CDCl_3$): δ = 9.728(1H, s, H-11), 9.413(1H, s, H-12), 7.287 (1H, dd, J = 4.994 and 2.56 Hz, 7-H), 4.1(s, intramolecular bonded OH), 2.59(1H, dd, J = 12 and 5.5 Hz, H-5), 1.095(3H, s, CH_3), 1.093(3H, s, CH_3), 0.946(3H, s, CH_3); ^{13}C NMR($CDCl_3$): δ = 202.66(d, C-12), 192.5(d, C-11), 157.2(d, C-7), 140.5(s, C-8), 77.1(s, C-9), 41.7(d, C-5), 41.4(s, C-10), 41.3(t, C-3), 33.0(s, C-4), 33.0(q, C-13), 31.2(t, C-1), 25.9(t, C-6), 22.0(q, C-14), 17.7(t, C-2), 17.0(q, C-15).

3.3.4 Isolation of polygodial(26)

Removal of solvent from fraction 2 afforded a yellow oil(30.3 g) which was further rechromatographed on silica gel 60 using hexane/ethyl acetate(9:1) solvent mixture. 50 ml fractions were collected. Fractions 1 - 5 were combined and the solvent removed to give 2.1 g which were further purified using the chromatotron. Pure fractions were combined and the solvent removed under vacuum to give 0.9 g material which was recrystallized in hexane to give white crystals(0.66 g), mp 54-55°C, lit.[154,185] mp 56.9-57.3°C, $[\alpha]_D^{20°C}$-122°(c 0.74 in EtOH), lit.[154,185] $[\alpha]_D$-133°(c 0.88 in EtOH). m/e Calculated for $C_{15}H_{22}O_2(M^+)$ 234.3391, found 234.1635. MS *m/z*(rel. int.): 234(1.59), 216(77.16), 202(6.80), 201(45.90), 183(53.94), 174(2.84), 173(23.89), 160(8.55), 159(17.99), 155(21.07), 147(26.67), 146(23.31), 145(91.11), 135(10.15), 134(100), 133(84.17), 132(68.09), 131(59.73), 128(28.80), 119(23.93), 115(42.25), 105(25.65), 95(2.09), 91(33.18), 77(21.82); IR v_{max}(cm^{-1}): 2950, 2860, 2750, 1770, 1715, 1690, 1650, 1400, 1380, 840; ^{1}H NMR($CDCl_3$): δ = 9.48 (d, J 4.4 Hz, 9-CHO), 9.41 (s, 8-CHO), 7.1 (m, 7-H), 2.769(s, broad, H-9), 0.914(3H, CH_3), 0.901(3H, CH_3) and 0.878 (3H, CH_3); ^{13}C NMR($CDCl_3$): δ = 202.59(d, C-12), 193(d, C-11), 155.01(d, C-7), 138.5(s, C-8), 60.41(d, C-9), 49.03(d, C-5), 41.79(t, C-3), 39.62(t, C-1), 36.92(s, C-10), 33.17(s, C-4), 33.17(q, C-13), 25.26(t, C-6), 21.98(q, C-14), 18.04(t, C-2), 15.27(q, C-15).

3.3.5 Isolation of iditol(97)

1.0 g of the crude ethanol extract(82.05 g) was acetylated using the pyridine-acetic anhydride method[155]. Recrystallization of the product in hexane-ethyl acetate solvent mixture afforded a hexaacetate derivative(98), 259 mg, which had the following physical and spectral properties: mp 121°C, lit.[102] mp 121-122°C, $[\alpha]_D^{20°C}$+25.7°(c 1.23 $CHCl_3$), lit.[102] $[\alpha]_D$+25.3°($CHCl_3$). (Found C, 49.78; H, 6.27 $C_{18}H_{26}O_{12}$ requires C, 49.76; H, 6.03); IR ν_{max}(cm^{-1}): 2980, 1750, 1380, 1220, 1040, 860, 620; ^1H NMR($CDCl_3$): δ = 5.46(1H, dd, J = 1.42 and 10.34 Hz), 5.08(1H, m), 4.23(1H, dd, J = 2.76 and 12.45 Hz), 4.07(1H, dd, J = 5.01 and 12.52 Hz); ^{13}C NMR($CDCl_3$): δ = 171.07(s, O<u>C</u>OCH_3), 170.41(s, O<u>C</u>OCH_3), 170.19(s, O<u>C</u>OCH_3), 68.03(d, -CHO-), 67.58(d, -CHO-), 62.03(t, -CH_2O-).

3.4 Chemical investigation of *Spirostachys africana* **Sonder**

3.4.1 Plant material

The stem bark of *Spirostachys africana*(3.4 kg) was collected in Nzhelele, Venda. It was left in the shade for four weeks to dry then ground to a fine powder.

3.4.2 Extraction

The ground stem bark was continuously extracted in a Soxhlet extractor for 48 hours using acetone as the solvent. Acetone was removed under vacuum to give 450.7 g of brown material. The brown material was dissolved in 1500 ml 80% aqueous methanol and extracted with hexane. Removal of the hexane under reduced pressure afforded 70.1 g of orange oil.

3.4.3 Isolation of lupeol(88)

70.1 g of the plant material was adsorbed onto 80 g of silica gel 60, transferred into the column and eluted with hexane/ethyl acetate(9:1). The 15 ml fractions were collected. Of the

collected fractions, 20 - 50 had the same material. They were combined and the solvent removed to give of yellow material(2.3 g) which was further purified by flash chromatography to give white material(1.5 g). Recrystallization using hexane-ethyl acetate solvent mixture afforded pure material(442.3 mg) mp 206°C, lit.[186] mp 215-216°C, $[\alpha]_D^{22°C}$+25.6(c 0.62 in $CHCl_3$), lit.[186] $[\alpha]_D$+26.4°($CHCl_3$). (Found: C,84.39; H,11.82 $C_{30}H_{50}O$ requires C,84.44; H,11.80); MS *m/z*(rel. int.): 426(19.1), 411(6.9), 408(0.6), 302(0.8), 218(52.0), 208(21.0), 203(34.5), 202(4.8), 191(28.0), 190(30.0), 175(27.7), 174(3.4), 173(8.3),109(93.6), 95(99.52), 81.2(100); IR: 3300, 2960, 2920, 1640, 1450, 1380, 1035, 880; ^{1}H NMR($CDCl_3$): δ = 1.03(3H, s, CH_3), 0.96(3H, s, CH_3), 0.94(3H, s, CH_3), 0.82(3H, s,CH_3), 78(3H, s, CH_3), 0.76(3H, s, CH_3), 3.17(1H, m, H-3), 1.68(3H, s, H-30), 4.56(1H, dd, J = 1.37 and 2.3 Hz and 4.68(1H, d, J = 2.02 Hz; ^{13}C NMR($CDCl_3$): δ = 38.80(t, C-1), 27.48(t, C-2), 78.96(d, C-3), 38.84(s, C-4), 55.43(d, C-5), 18.36(t, C-6), 34.36(t, C-7), 40.79(s, C-8), 50.55(d, C-9), 37.13(s, C-10), 20.97(t, C-11), 25.18(t, C-12), 38.13(d, C-13), 42.80(s, C-14), 27.50(t, C-15), 35.67(t, C-16), 42.98(s, C-17), 48.12(d, C-18), 48.41(d, C-19), 150.94(s, C-20), 29.91(t, C-21), 40.11(t, C-22), 28.07(q, C-23), 15.42(q, C-24), 16.17(q, C-25), 16.01(q, C-26), 14.58(q, C-27), 18.05(q, C-28), 109.66(t, C-29), 19.35(q, C-30).

3.4.4 Isolation of 3α-acetyl-taraxer-14-en-28ß-oic acid(89)

After the elution of lupeol, the column was eluted with hexane/ethyl acetate(4:1). The 15 ml fractions were collected. Fractions 15 - 61 were combined and the solvent removed to give yellow material(7.14 g). The yellow material was adsorbed onto silica gel 60(8.0 g), transferred into the column and eluted with hexane:ethyl acetate(7:3). Fractions 10 -35 were collected and the solvent removed to give of crystalline material(3.2 g) which was recrystallized in hexane/ethyl acetate solvent mixture to give white material(2.01 g), mp 297°C, lit.[143] mp 301-302°C, $[\alpha]_D^{22°C}$+21.3°(c, 0.51 in $CHCl_3$), lit.[143], $[\alpha]_D$+23.1°(c, 0.6 in $CHCl_3$). (Found C, 77.58; H, 10.13 $C_{32}H_{50}O_4$ requires C, 77.06; H,

10.10). IR ν_{max}(cm^{-1}): 2924, 2840, 1720, 1680, 1485, 1380, 1225, 1015; ^{1}H NMR($CDCl_3$): δ = 5.93(1H, s, broad, H-15), 4.78(1H, dd, J = 6.67 and 10.55 Hz, H-3), 2.21(3H, s, $OCOCH_3$), 1.27(3H, H-27), 0.97(3H, H-30), 0.94(3H, H-29), 0.90(3H, H-25), 0.87(6H, H-23, H-24); ^{13}C NMR($CDCl_3$): δ = 37.15(t, C-1), 23.11(t, C-2), 80.01(d, C-3), 37.33(s, C-4), 54.96(d, C-5), 18.12(t, C-6), 33.65(t, C-7), 38.49(s, C-8), 48.59(d, C-9), 36.75(s, C-10), 17.05(t, C-11), 33.08(t, C-12), 37.00(s, C-13), 159.75(s, C-14), 116.49(d, C-15), 23.12(t, C-16), 50.47(s, C-17), 41.46(d, C-18), 41.30(t, C-19), 28.92(s, C-20), 35.22(t, C-21), 31.80(t, C-22), 27.32(q, C-23), 16.12(q, C-24), 14.86(q, C-25), 25.53(q, C-26), 28.51(q, C-27), 179.46(s, C-28), 31.68(q, C-29), 21.54(q, C-30), 20.45(q, $\underline{C}H_3COO$), 169.82(s, $CH_3\underline{C}OO$).

3.5 Towards the synthesis of straight chain (-)-warburganal analogues

3.5.1 1-Ethyl-2-hydroxy-2,4-dimethyl-3-methylenesuccinoate(101)

Ethyl pyruvate(10 ml, 91.2 mmoles) and methyl acrylate(15 ml, 166.5 mmoles) were stirred together at room temperature for five days in a sealed container containing 1,4-diazabicyclo[2.2.2]octane(10 g, 88.2 mmoles). The remaining methyl acrylate was removed under vacuum and the product was purified using flash chromatography to give of colourless oil(8.7 g, 47.26% yield) exhibiting the following spectral properties:- MS *m/z*(rel. int.): 185(13.35), 173(83.27), 157(25.09), 143(30.06), 142(22.06), 141(54.51), 140(24.2), 139(18.82), 129(50.9), 125(14.49), 118(29.42), 113(10.7), 111(17.08), 93(34.39), 84(35.83), 82(67.92), 70(5.76), 59(11.75), 54(12.62) and 45(100); ^{1}H NMR($CDCl_3$): δ = 1.254(3H, t, J = 7.14 Hz, CH_3), 1.598(3H, s, CH_3), 3.765(3H, s, OCH_3), 3.984(1H, s, OH), 4.224(2H, q, J = 7.12 Hz, OCH_2), 5.991 and 6.363(2H, doublets, $=CH_2$); ^{13}C NMR($CDCl_3$): δ = 174.77(s, CO), 166.52(s, CO), 141.87(s, $\underline{C}=CH_2$), 125.63(t, $C=\underline{C}H_2$), 73.72(s, $\underline{C}$-O), 61.98(t, $O\underline{C}H_2CH_3$), 52.27(q, OCH_3), 23.88(q, CH_3) and 14.02(q, $OCH_2\underline{C}H_3$).

3.5.2 1-Ethyl-2-(methoxymethoxy)-2,4-dimethyl-3-methylenesuccinoate(102)

4-Ethyl-3-hydroxy-1,3-dimethyl-2-methylene succinoate(8.64 g, 42.0 mmoles) in anhydrous CH_2Cl_2(100 ml) was treated with at 0°C with freshly distilled chloromethyl methyl ether(5.5 ml, 65.0 mmoles) and diisopropylethylamine(16.5 ml, 95 mmoles), stirred overnight, and then quenched with dilute HCl to pH 1-2. After extraction with CH_2Cl_2 and washing with water to neutrality, the organic phase was dried over anhydrous magnesium sulphate, filtered and the solvent removed under vacuum. Flash chromatography using CH_2Cl_2 as solvent afforded the product(5.41 g, 54.76%) which exhibited the following spectral properties:- MS *m/z*(rel. int.): 231(0.61, M + 1), 216(2.64), 215(22.97), 201(8.87), 187(9.44), 185(17.75), 173(83.55), 157(21.04), 143(23.39), 141(43.03), 140(16.56), 139(19.16), 129(44.48), 126(7.42), 125(11.52), 118(19.65), 113(8.86), 111(12.24), 109(5.56), 97(27.91), 85(7.7), 83(11.49), 45(100), 43(12.8); IR V_{max}(cm^{-1}): 2950, 1730, 1625, 1450, 900, 860, 760; ^1H NMR($CDCl_3$): δ = 1.25(3H, t, J = 7.15 Hz, CH_3), 1.64(3H, s, CH_3), 3.34(3H, s, OCH_3), 3.75(3H, s, OCH_3), 4.17(2H, m, OCH_2), 4.78(2H, OCH_2), 6.07 and 6.40(2H, doublets, J = 0.59 and 0.59 Hz, $C=CH_2$); ^{13}C NMR($CDCl_3$): δ = 171.28(s), 165.79(s), 140.85(s, $\underline{C}=CH_2$), 125.68(t, $C=\underline{C}H_2$), 91.91(t, O-CH_2-O), 78.54(s, tertiary oxycarbon), 61.36(t, OCH_2), 55.79(q, OCH_3), 52.06(q, OCH_3), 22.82(q, CH_3), 13.99(q, CH_3).

3.5.3 Attempted synthesis of 3-methylene-2-methyl-2-(methoxymethoxy) butan-1,4-dial(103)

A solution of 4-ethyl-3-(methoxymethoxy)-1,3-dimethyl-2-methylene succinoate(5.2 g, 22.0 mmoles) in anhydrous *n*-hexane(30 ml) was treated at -80°C with a solution of 20% diisobutylaluminium hydride in *n*-hexane(12.1 ml, 12.0 mmoles). After 15 minutes the reaction was quenched with saturated aqueous NH_4Cl, diluted with ether and filtered through Celite cake. The organic phase was separated and evaporated under reduced pressure to give a crude

product which was purified by flash chromatography(*n*-hexane/ethyl acetate 9:1) to give colourless oil(1.3 g) which bears no resemblance to the expected product spectrometrically.

3.5.4 Synthesis of 1-ethyl-2-hydroxy-2,4-dimethyl-3-methylenesuccinoate(101) using QDL as a catalyst

Ethyl pyruvate(12 ml, 109 mmoles), methyl acrylate(16 ml, 177 mmoles) and (±)-3-quinuclidinol(QDL)(1.67 g, 13.0 mmoles) were transferred into a sealed container and the mixture stirred continuously for three days. The remaining methyl acrylate was removed under vacuum and product purified by flash chromatography[112] to give a colourless oil (12.3 g, 55.8%) which had the same spectral properties as the DABCO-catalysed reaction(section 3.6.1).

3.5.5 Attempted synthesis of 3-methylene-2-methyl *n*-butan-1,2,4-triol(105).

To a suspension of $LiAlH_4$(3.8 g, 100.0 mmoles) in anhydrous tetrahydrofuran(100 ml) was added a solution of 4-ethyl-3-hydroxy-1,3-dimethyl-2-methylenesuccinoate(12.1 g, 59.0 mmoles) in the same solvent(100 ml). After 10 minutes the reaction was complete. The mixture was treated sequentially with ethyl acetate(7.7 ml, 100 mmoles) and 10% aqueous KOH(22.5 ml) and then stirred for 30 minutes. The precipitate was filtered off and washed with ether, and the filtrate evaporated under reduced pressure to give a colourless oil which could not be identified by spectrometric methods.

3.6 Chemical investigation of *Rapanea melanophloeos*

3.6.1 Plant material

The stem bark(4.7 kg) was collected in Thathe Vondo, Venda. It was left in the shade for six weeks to dry and then milled to a fine powder.

3.6.2 Extraction

The plant material was differentially extracted in a Soxhlet extractor using hexane, ethyl acetate and methanol as extracting solvents. Hexane was removed and the residue(78.5 g) dissolved in petroleum ether(40-60°C). Part of the extract, a yellow precipitate, did not dissolve in petroleum ether. It was then filtered to give 16.5 g which was further recrystallized from chloroform to give an orange material(10.05 g).

3.6.3 Isolation of rapanone(63)

The orange precipitate(10.05 g) was purified by column chromatography(ethyl acetate/methanol, 1:1). 15 ml fractions were collected and those with same R_f values were combined. Removal of the solvent under reduced pressure afforded 9.2 g of material. The procedure was repeated and the solvent removed to give 8.4 g of orange material which was recrystallized from chloroform to give of orange crystals(7.6 g), mp 140°C, lit.[148] mp 140-141°C. (Found: C, 70.84; H, 9.41, $C_{19}H_{30}O_4$ requires C, 70.87; H, 9.39). MS *m/z*(rel. int.): 256(11.96), 213(9.88), 185(7.27), 171(7.49), 157(9.98), 138(17.7), 137(18.27), 129(33.74), 121(51.38), 115(13.01), 111(7.8), 98(11.38), 97(21.18), 96(8.67), 93(15.75), 87(17.95), 85(18.18), 84(12.53), 81(7.87), 73(100), 71(24.94), 69(29.02), 67(7.97), 65(14.51), 61(12.44), 59(52.69), 57(29.82), 54(31.06), 53(8.99); IR: 3320, 1620, 1235, 1100; ^{1}H NMR(pyridine - d_6): δ = 9.76(2H, s), 6.17(1H, s), 2.76(2H, t, J = 7.33 Hz), 1.73(2H, m), 1.23(20H, s), 0.86(3H, t, J = 6.15 Hz); ^{13}C NMR(pyridine - d_6): δ = 170.9(s), 118.2(s), 104.4(d), 32.4(t), 30.4(t), 30.1(t), 29.8(t), 29.1(t), 23.4(t), 23.1(t), 14.5(q).

3.6.4 Rapanone dimethyl ether(92)

Rapanone(265.6 mg) was methylated with diazomethane by standard procedures. The methylated material was purified by chromatotron separation using dichloromethane. Removal of the solvent under reduced pressure afforded a yellow powder(114 mg) mp 60°C. (Found

C, 71.83; H, 9.79 $C_{21}H_{34}O_4$ requires C, 71.96; H, 9.77). ^1H NMR($CDCl_3$): δ = 5.73(1H, s), 4.05(3H, s), 3.81(3H, s), 2.42(2H, t, J = 6.85 Hz), 1.25(20H, s), 0.87(3H, t, J = 6.21 Hz); ^{13}C NMR($CDCl_3$): δ = 183.6(s). 182.4(s), 158.7(s), 155.8(s), 130.7(s), 105.3(d), 61.3(q), 56.5(q), 32.0(t), 29.7(t), 29.6(t), 29.4(t), 28.7(t), 23.1(t), 22.7(t), 14.1(q).

3.6.5 Isolation of betulinic acid(93)

22.1 g of the ethyl acetate extract was adsorbed onto 22 g silica gel 60, transferred into the column and eluted with hexane/ethyl acetate(1:1) solvent mixture. 15 ml fractions were collected. Fractions 12 - 48 were combined and the solvent removed to give impure material(15.42 g). The above procedure was repeated and the solvent removed to give pure material(1.23 g). It was discovered that the material swells in solvents such as ethyl acetate, acetone and methanol. Recrystallization in methanol afforded white powder(308 mg) mp 277°C, lit.[113,151] mp 275-278°C, $[\alpha]_D^{21°C}$+7.21°(c 1.04 in pyridine), lit.[113,151] $[\alpha]_D$+7.9(pyridine). (Found C, 78.65; H, 10.61. Calculated for $C_{30}H_{48}O_3$: C, 78.90; H, 10.59). MS *m/z*(rel. int.): 456(0.72), 438(0.42), 411(0.35), 302(0.41), 283(0.27), 248(2.98), 220(2.96), 207(4.35), 203(4.22), 202(1.47), 190(4.27), 189(10.47), 175(5.10), 174(1.20), 109(22.98); IR: 3400, 2960, 1700, 1650, 1460, 1380, 880; ^1H NMR(pyridine - d_6): δ = 1.65(3H, s), 1.07(3H, s), 0.93(3H, s), 0.905(3H, s), 0.85(3H, s), 0.68(3H, s), 4.79(1H, d, J = 2.14 Hz), 4.63(1H, d J = 2.11 Hz), 3.35(2H, m); ^{13}C NMR(pyridine - d_6): δ = 36.69(t, C-1), 27.33(t, C-2), 77.33(d, C-3), 40.19(, C-4), 55.07(, C-5), 17.84(, C-6), 31.96(, C-7), 40.19(, C-8), 50.09(, C-9), 38.61(, C-10), 20.26(, C-11), 25.15(, C-12), 37.67(, C-13), 41.94(, C-14), 29.35(, C-15), 33.92(, C-16), 55.75(, C-17), 46.86(, C-18), 48.85(, C-19), 150.69(s, C-20), 30.26(, C-21), 38.40(, C-22), 27.76(q, C-23), 15.42(q, C-24), 15.45(q, C-25), 14.45(q, C-26), 13.96(q, C-27), 178.35(s, C-28), 109.32(d, C-29), 18.56(q, C-30).

3.6.6 Betulinic acid acetate(94)

Betulinic acid(104 mg) was acethylated by pyridine-acetic anhydride method[155] to give the acetate(120 mg) which was recrystallized from acetone/hexane mixture to pure product(37 mg) mp 270°C, lit.[113] 269-271°C, $[\alpha]_D^{21°C}$+16.8°(c, 0.37 in $CHCl_3$), lit.[113] $[\alpha]_D$+17.13(c, 0.12 in $CHCl_3$). (Found: C, 76.68; H, 10.35. Calc. for $C_{32}H_{50}O_4$: C, 77.06; H, 10.10). IR ν_{max}(cm^{-1}):2960, 1750, 1690, 1640, 1460, 1380, 1250, 1050, 770; ^1H NMR($CDCl_3$): δ = 0.83(3H, s), 0.84(3H, s), 0.85(3H, s), 0.95(3H, s), 0.96(3H, s), 1.68(H, s), 1.69(3H, s), 2.04(, s), 2.23(3H, s), 2.96(1H, m), 4.47(1H, m), 4.62(1H, d, J = 2.06 Hz), 6.74(1H, d, J = 2.1 Hz); ^{13}C NMR($CDCl_3$): δ = 171.94(s), 171.56(s), 150.30(s), 110.34(t), 81.15(d), 57.89(s), 55.59(d), 50.18(d), 49.30(d), 46.62(d), 42.56(s), 40.85(s), 38.51(s), 38.03(d), 37.89(s), 37.20(s), 36.14(t), 34.31(t), 31.59(t), 30.27(t), 29.82(t), 28.00(q), 25.48(t), 23.74(t), 22.47(q), 21.39(q), 20.90(t), 19.36(q), 18.19(t), 16.52(q), 16.25(q), 15.99(q), 14.62(q).

REFERENCES.

1. Asakawa, Y. and Heidelberger, M.(1982), *Progress in the Chemistry of Organic Natural Products*, 42, p. 4, Springer-Verlag, Berlin.

2. Hans-Joachim Arnold and Mairam Gulumian(1984), *Journal of Ethnopharmacology*, 12, 35.

3. Mabogo, D.E.N.(1990), *M.Sc. Thesis*, University of Pretoria.

4. Drewes, S.E., Hogan, C.J., Kaye, P.T. and Roos, G.H.P. (1989), *Journal of the Chemical Society, Perkin Trans. 1*, 1585.

5. Palgrave, K.C.(1990), *Trees of Southern Africa*, 2nd edition, p. 493, Struik Publishers(Cape Town).

6. Palmer, E. and Pitman, N.(1972), *Trees of Southern Africa, Vol. II*, p. 1271, A.A. Balkema, Cape Town.

7. Nakanishi, K., Gullo, V.P., Miura, I., Govindachari, T.R. and Viswanathan, N.(1973), *Journal of the American Chemical Society*, 95, 6473.

8. Ref. 5, p. 618.

9. Cunningham, A.B.(1990), *Mitteilungen aus dem Institut fur Allegemeine Botanik, Hamburg*, 23b, 979.

10. E. Netshiungani, Venda Herbarium.

11. Ref. 5, p. 435.

12. Ref. 5, p. 721.

13. Bruning, R. and Wagner, H.(1978), *Phytochemistry*, 17,

1821.

14. Budzikiewicz, H., Wilson, J.M. and Djerassi, C.(1963), *Journal of the American Chemical Society*, 85, 3688.

15. Wai-Haan Hui and Man-Mooi Li(1977), *Journal of the Chemical Society, Perkin Trans. 1*, 897.

16. Fang, S., Berry, D.E., Lynn, D.G., Hecht, S.M., Campbell, J. and Lynn W.S.(1984), *Phytochemistry*, 23(3), 631.

17. Wijeratne, D.B., Kumar, V. and Sultanbawa, M.U.S.(1981), *Journal of the Chemical Society, Perkin Trans. 1*, 2724.

18. Kupchan, S.M., Eakin, M.A. and Thomas, A.M.(1971), *Journal of Medicinal Chemistry*, 14, 1147.

19. Kupchan, S.M., Atland, H.W.(1973), *Journal of Medicinal Chemistry*, 16, 913.

20. Monache, F.D., Marini-Bettòlo, G.B., Pomponi, M., de Mêllo J.F., de Lima, O.G., and Thomson, R.H.(1979), *Journal of the Chemical Society*, 3127.

21. Gonzalez, A.G., Franscisco, C.G., Freire, R., Hernandez, R., Salazar, J.A. and Suarez, E.(1975), *Phytochemistry*, 14, 1067.

22. Drewes, S.E. and Roux, D.G.(1966), *Journal of the Chemical Society*, C, 1644.

23. Gottlieb O.R. and de Sausa, J.R.(1974), *Phytochemistry*, 13, 1229.

24. Tindale, E.V. and Roux, D.G.(1974), *Phytochemistry*, 13, 829.

25. Brand, E.V., Ferreira, D. and Roux, D.G.(1981), *Journal of the Chemical Society, Perkin Trans 1*, 514.

26. Chan, W.R., Forsyth, W.G.C. and Hassal, C.H.(1958), *Journal of the Chemical Society*, 3174.

27. Weeratunga, G., Bohlin. L., Verpoorte, R. and Kumar, V.(1985), *Phytochemistry*, 24, 2093.

28. Monache, F.D., Pomponi, M., Marini-Bettòlo, G.B., D'Albuquerque I.L. and de Lima O.G.(1976), *Phytochemistry*, 15, 573.

29. Watt, J.M. and Breyer-Brandwyk, M.G.(1962), *Medicinal and Poisonous Plants of Southern and Eastern Africa*, 2nd ed., p. 177, E. and S. Livingstone, Edinburgh and London.

30. Jansen, J.M. and de Groot, A.(1991), *Natural Product Reports*, 310.

31. Ref. 5, p. 618.

32. Al-Said, M.S., El-Khawaja, S.M., El-Feraly, S and Hufford, C.D.(1990), *Phytochemistry*, 29(3), 975.

33. Al-Said, M.S., Khalifa, S.I. and El-Feraly, S.(1989) *Phytochemistry*, 28(1), 297.

34. Brooks, C.J.W. and Draffan, G.H.(1966), *Chemical Communications*, No. 19, 701.

35. Kubo, I., Miura, I., Pettei, M.J., Lee, Y.-W., Pilkiewicz, F. and Nakanishi, K.(1977), *Tetrahedron Letters*, No. 52,4553.

36. Kubo, I., Miura, I., Pettei, M.J., Lee, Y.-W., Pilkiewicz, F. and Nakanishi, K.(1976), *Journal of the Chemical*

Society, Chemical Communications, 1013.

37. Brooks, C.J.W. and Draffan, G.H.(1969), *Tetrahedron*, 25, 2865.

38. Brooks, C.J.W. and Draffan, G.H.(1969), *Tetrahedron*, 25, 2887.

39. Canonica, L., Corbella, A., Jommi, G., Krepinsky', J. Ferrari, G. and Casagrande C.(1967), *Tetrahedron Letters*, No. 23, 2137.

40. Taniguchi, M., Chapya, A., Kubo, I. and Nakanishi, K.(1978), *Chemical Pharmacy Bulletin*, 26, 2910.

41. Taniguchi, M., Adachi, T., Oi, S., Kimura, A., Katsumura, S., Isoe, S. and Kubo, I.(1984), *Agri. Bio. Chem.*, 48(1), 73.

42. Kida, T., Shibai, H. and Seto, H.(1986), *Journal of Antibiotics*, 39, 613.

43. Canonica, L., Corbella, A., Gariboldi, P., Jommi, G., Krepinsky, J., Ferrari, G. and Casagrande, C.(1969), *Tetrahedron*, 25, 3895.

44. Asakawa, Y. and Takemoto, T.(1979), *Experientia*, 35, 1420.

45. Hashimoto, T., Tori, M., Mizuno, Y. and Asakawa, Y.(1987), *Tetrahedron Letters*, 28, 6303.

46. Ichihara, A., Sawamura, S. and Sakamura, S.(1984), *Tetrahedron Letters*,25, 3209.

47. Asakawa, Y., Dawson, G.W., Griffiths, D.C., Lalemand, J. -J., Ley, S.V., Mori, K., Mudd, A., Pezechk-Leclaire, M., Watanabe, H., Woodcock, C.M. and Zhong-ning, Z.(1988),

Journal of Chemical Ecology, 14(10), 1845.

48. Kokwaro, J.O.(1979), *Medicinal Plants of East Africa*, p. 45, East African Literature Bureau.

49. Ref. 29, p. 120.

50. Kubo, I.(1989), *Drug News Persp.*, 2, 292.

51. Fukuyama, Y., Sato, T., Asakawa, Y. and Takemoto, T.(1982), *Phytochemistry*, 21, 2895.

52. Kubo, I. and Ganjian, I.(1981), *Experientia*, 37, 1063.

53. Fukuyama, Y., Sato, T., Miura, I. and Asakawa, Y.(1985), *Phytochemistry*, 24,1521.

54. Dossaji, S.F., Kairu, M.K., Gondwe, A.T. and Ouma, J.H. (1977), *Lloydia*, 40, 290.

55. Klecak, G., Geleick, H. and Frei, J.K.(1977), *Journal of Soc. Cosmet. Chem.*, 28, 53.

56. Stampf, J., Benezra, C. and Asakawa, Y.(1982), *Arch. Dermatol. Res.*,272, 277.

57. Asakawa, Y.(1984), *Rev. Latinoamer. Quim.*,14, 109.

58. Cinimo, G., De rosa, S., De Stefano, S. and Sodand, G.(1985), *Experientia*, 41, 1335.

59. van Beek T.A. and de Groot A.(1986), *Recl. Trav. Chim. Pays-Bas*, 105, 513.

60. Scott, G.C., Pickett, J.A., Smith, M.C., Woodcock, C.M., Harris, P.G.W., Hammon, R.P. and Koetecha, H.D.(1984), '*Proceedings 1984 British Crop Protection Conference-Pest*

and Disease', 133.

61. Scott, G.C.(1981), *'Proceedings 1981 British Crop Protection Conference-Pest and Disease'*, 441.

62. Okuda, R.K., Schueuer, P.J., Hochlowski, J.E., Walker, R.P. and Faulkner D.J.(1981), *Journal of Organic Chemistry*, 48, 1866.

63. Cinimo, G., De Rosa, S., De Stefano, S. and Sodano, G.(1982), *Comp. Biochem. Physiol.*,73, 471.

64. Gustafson, K. and Anderson, R.J.(1985), *Tetrahedron*, 41, 1101.

65. Nakanishi, K. and Kubo, I.(1977), *Israel Journal of Chemistry*, 16, 28.

66. Meiwald, J., Pretwich, G.D. Nakanishi, K. and Kubo, I.(1978), *Science*, 199, 1167.

67. Schoonhoven, L.M. and Fu-Shun, Y.(1989), *Journal of Insect Physiology*, 35, 725.

68. Sterner, O., Carter, R.E. and Nilsson, L.M.(1987), *Mutat. Res.*, 188, 169.

69. Taniguchi, M., Adachi, T., Haraguchi, H., Oi, S. and Kubo, I.(1983), *Journal of Biochemistry*, 94, 149.

70. D'Ischia, M., Prota, G. and Sodano, G.(1982), *Tetrahedron Letters*, 23, 3295.

71. Cinimo, G., Sodano, G. and Spinella, A.(1984), *Tetrahedron Letters*, 25, 4151.

72. Cinimo, G., Sodano, G. and Spinella, A.(1987),

Tetrahedron, 43, 5401.

73. Caprioli, V., Cinimo, G., Colle, R., Gavagnim, M., Sodano, G. and Spinella A.(1987), *Journal of Natural Products*, 50, 146.

74. Ma, W.-C.(1977), *Physiol. Entomol.*, 2, 199.

75. Frazier, J.L. and Lam, P.Y.-S.(1986), *Chem. Sences*, 11,600.

76. Frazier, J.L. and Lam, P.Y.-S.(1987), *Tetrahedron Letters*, 28, 5477.

77. Taniguchi, M., Yano, Y. Tada, E., Ikenishi, K., Oi, S., Haraguchi, H., Hashimoto, K. and Kubo, I.(1988), *Agric. Bio. Chem.*, 52, 1409.

78. Kubo, I. and Taniguchi, M.(1988), *Journal of Natural Products*, 51, 22.

79. Dyer, R.A.(1975), *The Genera of South African Flowering Plants*, Volume 1, p. 306, Department of Agricultural Technical Services, Pretoria.

80. Ulyate, G.(1966), *M.Sc. Thesis*, Wits University.

81. Odebiyi, O.O.(1980), *Planta Medica*, 30, 144.

82. Stuart, K.L. and Haynes, L.T.(1963), *Journal of Chemical Society*,(C), 1784.

83. Stuart, K.L. and Woo-Ming, R.B.(1975), *Phytochemistry*, 14, 594.

84. Bhakani, D.S., Satish, S. and Dhar, M.M.(1970), *Phytochemistry*, 9, 2573.

Phytochemistry, 9, 2573.

85. Baarschers, W.H., Horn, D.H.S. and Johnson, L.R.F.(1962), *Journal of Chemical Society*, 4046.

86. McCrindle, R., Martin, A. and Murray, R.D.H.(1968), *Journal of Chemical Society*,(C), 2349.

87. Martin, A. and Murray, R.D.H.(1968), *Journal of Chemical Society*,(C), 2529.

88. Connoly, J.D. and Harding, A.E.(1972), *Journal of Chemical Society*, Perkin 1, 1996.

89. Lloyd, H.A. and Fales, H.M.(1967), *Tetrahedron Letters*, No. 48, 4891.

90. Ref. 5, p. 435.

91. Ref. 79, p. 439.

92. Ref. 5, p. 717.

93. Ogawa, H. and Natori, S.(1968), *Phytochemistry*, 7, 773.

94. Fernandez, O. and Pizzarroso, A.(1948), *Chemical Abstracts*, 42, 8888.

95. Aiyan, S.N.(1964), *Phytochemistry*, 3, 335.

96. Nakata, H., Sasaki, K. and Morimoto, I. and Hirata, Y. (1964), *Tetrahedron*, 20, 2319.

97. Madrigal, R.V., Spencer, G.F., Plattner, R.D. and Smith C.R.(1977), *Lipids*, 12(4), 402.

98. Croft, J.A., Ritchie, E. and Taylor, W.C.(1976),

Australian Journal of Chemistry, 29, 1979.

99. Ohtani, K., Mavi, S. and Hostettmann, K.(1993), *Phytochemistry*, 33(1), 83.

100. King, F.E., Clarke-Lewis, J.W. and Forbes, W.F.(1955), *Journal of the Chemical Society*, 2948.

101. King, F.E. and King, T.J.(1951), *Journal of the Chemical Society*, 5691.

102. Pigman, W. and Horton, D.(Editors)(1972), *The Carbohydrates, Chemistry and Biochemistry*, Volume 1A, 2nd ed. p. 485, Academic Press(New York).

103. Voelter, W., Breitmaier, E., Rathbone, E.B. and Stephen A.M.(1973), *Tetrahedron*, 29, 3845.

104. De Almeida, M.E., Gottlieb, O.R., de Sousa, J.R. and Teixeira, M.A.(1974), *Phytochemistry*, 13, 1225.

105. Robinson, G.M. and Robinson, R.(1935), *Journal of the Chemical Society*, 744.

106. Drewes, S.E. and Roux, D.G.(1967), *Journal of the Chemical Society*, (C), 1407.

107. (a) Gottlieb, O.R. and de Sousa, J.R.(1972), *Phytochemistry*, 11, 2841. (b) McPherson, D.D., Cordell, G.A., Soejarto, D.D., Pezzuto, J.M. and Fong, H.H.S. (1983), *Phytochemistry*, 22(12), 2835.

108. Malan, J.C.S., Young, D.A., Steinberg, J.P., and Ferreira, D.(1990), *Journal of the Chemical Society, Perkin Trans 1*, 219.

109. Batterham, T.J. and Highet, R.J.(1964), *Australian*

Journal of Chemistry, 17, 428.

110. Malan, E. and Roux, D.G.(1974), *Phytochemistry*, 13, 1575.

111. Malan, J.C.S.(1989), *Ph.D. Thesis*, University of Orange Free State, p. 121.

112. Still, W.C., Kahn, M. and Mitra, A.(1978), *Journal of Organic Chemistry*, 43, 2923.

113. Robinson, F.P. and Martel, H.(1970), *Phytochemistry*, 9, 907.

114. Adam, G., Lischewski, M., Pheit, H.V., Preiss, A., Schmidt, J. and Sung, T.V.(1982), *Phytochemistry*, 21(6), 1385.

115. Dantanarayana, A.P., Kumar, N.S., Muthukuda, P.M. and Wazeer, M.I.M.(1982), *Phytochemistry*, 21(8), 2065.

116. Marta, M., Delle Monache, F., Marini-Bettòlo, G.B., De Mêllo, J.F., and De Lima, O.G.(1979), *Gaz. Chim. Ital.*, 109, 61.

117. Gonzalez, A.G., Fraga, B.M., Gonzalez, P., Marta, M., Delle Monache, F., Marini-Bettòlo, G.B., De Mêllo, J.F., and Goncalves, O.(1982), *Phytochemistry*, 21, 470.

118. Govindachari, T.R., Viswanathan, N., Pai, B.R., Rao, U.R. and Srinivasan, M.(1967), *Tetrahedron*, 23, 1901.

119. Patra, A., Chaudhuri, S.K. and Ruegger, H.(1990), *Journal of the Indian Chemical Society*, 67, 394.

120. Courtney, J.L. and Shannon, J.S.(1963), *Tetrahedron Letters*, No. 1, 13.

121. Chandler, R.F., and Hooper, S.N.(1979), *Phytochemistry*, 18, 711.

122. Gamlath, C.B., Gunatilaka, A.A.L. and Subramaniam, S.(1989), *Journal of the Chemical Society, Perkin Trans. 1*, 2259.

123. Monache, D.E., d'Albuquerque, I.L., Ferrari, F. and Marini-Bettòlo, G.B.(1967), *Tetrahedron Letters*, No. 43, 4211.

124. Anjaneyulu, A.S.R. and Rao, M.N.(1979), *Indian Journal of Chemistry*, 18B, 292.

125. Williams, D. H. and Fleming, I.(1987), *Spectroscopic methods in Organic Chemistry*, 4th ed., p. 131, McGraw-Hill Book Company (United Kingdom) Limited.

126. Breitmaier, E. and, Voelter W.(1987), *Carbon-13 NMR Spectroscopy*, 3rd ed. p. 455, VCH Verlagsgesellschaft, Weinheim (Federal Republic of Germany).

127. Van der Westhuizen, J.H., Steenkamp, J.A. and Ferreira, D.(1990), *Tetrahedron*, 46(23), 7849.

128. Wang, Z.Y. and Hay, A.S.(1989), *Synthesis*, 471.

129. Schnell, H., Krimm H.(1963), *Angew. Chem.*, 75, 662; *Angew. Chem. Int. Ed. Engl.*(1963), 2, 373.

130. Brown, B.R. and MacBride, J.A.H.(1964), *Journal of the Chemical Society*, 3822.

131. Clark-Lewis, J.W. and Skingle, D.C.(1967), *Australian Journal of Chemistry*, 20, 2169.

132. Bryant, R., Hassal, C.H. and Weatherston, J.(1964),

Journal of the Chemical Society, 4941.

133. Saunders, J.K.M. and Hunter, B.K.(1987), *Modern NMR Spectroscopy*, p. 292, Oxford University Press, Great Britain.

134. Hemingway, R.W., Foo, L.Y. and Porter, L.J.(1982), *Journal of Chemical Society, Perkin Trans. 1*, 1209.

135. Fletcher, A.C., Porter, L.J., Haslam, E., Gupta, R.K. (1977), *Journal of Chemical Society, Perkin Trans. 1*, 1628.

136. Kikuchi, H., Tensho, A., Shimizu, I., Shiokawa, H., Kuno, A., Yamada, S., Fujiwara, T. and Tomita, K.(1983), *Chemistry Letters*, 603.

137. Bohlmann, F. and Jakupovic, J.(1979), *Phytochemistry*, 18, 1189.

138. Monache, F.D., Marini-Bettòlo, G.B., de Lima O.G., d'Albuquerque I.L. and de Barros Coêlho, J.S.(1973), *Journal of Chemical Society, Perkin Trans. 1*, 2725.

139. Brown, P.M., Michael, M., Thomson, R.H., King, T.J., Krishnamoorthy, V. and Seshadri, T.R.(1973), *Journal of Chemical Society, Perkin Trans. 1*, 2721.

140. Ghisalberti, E.L., Jeffries, P.R. and Sefton, M.A. (1973), *Phytochemistry*, 12, 1125.

141. Ageta, H. and Arai, Y.(1983), *Phytochemistry*, 22(8), 1801.

142. Rasool, N., Khan, A.Q. and Malik, A.(1989), *Phytochemistry*, 28(4), 1193.

143. Woo, W.S. and Wagner, H.(1977), *Phytochemistry*, 16, 1845.

144. Ogihara, K., Higa, M. Hokama, K. and Suga, T.(1987), *Phytochemistry*, 26(3), 783.

145. Chakravarty, A.K., Mukhopadhyay, S. and Das, B.(1991), *Phytochemistry*, 30(12), 4087.

146. Rao, CH.B. and Venkateswarlu, V.(1961), *Journal of Organic Chemistry*, 26, 4529.

147. Ogawa, H., Sakaki, S., Yoshihira, K. and Natori, S. (1968), *Tetrahedron Letters*, No. 11, 1387.

148. Murthy, V.K. Rao, T.V.P. and Venkateswarlu, V.(1965), *Tetrahedron*, 21, 1445.

149. Herz, W., Santhanam, P.S. and Wahlberg, I.(1972), *Phytochemistry*, 11, 3061.

150. Sholichin, M. Yamasaki, K., Kasai, R. and Tanaka, O. (1980), *Chemical Pharmacy Bulletin*, 28(3), 1006.

151. Buckingham, J.(editor)(1982), *Dictionary of Organic Compounds*, 5th ed. p. 635, Chapman and Hall, London.

152. Carey, F.A.(1987), *Organic Chemistry*, p. 988, McGraw-Hill Book Company, New York.

153. McMurry, J.(1988), *Organic Chemistry*, 2nd ed., p. 954, Brooks/Cole Publishing Company, California.

154. Barnes, C.S. and Loder, J.W.(1962), *Australian Journal of Chemistry*, 15, 322.

155. Geissman, T.A. and Dittmar, H.F.K.(1965), *Phytochemistry*, 4, 359.

156. McCallion, R.F., Cole, A.L.J., Walker, J.R.L., Blunt, J.W. and Munro, M.H.G.(1982), *Planta Medica*, 44, 134.

157. Ref. 79, p. 334.

158. von Breitenbach, F.(1965), *The Indigenous Trees of Southern Africa*, Vol. IV, part 3, 632.

159. Ref. 79, p. 744.

160. Wijeratne, D.B.T., Kumar, V., Sultanbawa, M.U.S. and Balasubramaniam, S.(1982), *Phytochemistry*, 21(9), 2422.

161. Dantanarayana, A.P., Kumar, N.S., Muthukuda, P.M. and Balasubramaniam, S.(1983), *Phytochemistry*, 22(2), 473.

162. Gonzales, A.G., Franscisco, C.G., Freire, R., Hernandes, R., Salazar, J.A. and Suarez, E.(1975), *Anais Ass. Brasil. Quim.*, 70, 376.

163. Jain, M.K.(1963), *Indian Journal of Chemistry*, 1, 500.

164. Grant, P.K. and Johnson, A.W.(1957), *Journal of the Chemical Society*, 4079, 4669.

165. Gonvales de Lima, O., D'Albuquerque, I.L., De Barros Coêlho, J.S., Martins D.G., Lacerda, A. and Maciel, G.M. (1969), *Rev. Inst. Antibioticos (Recife)*, 9, 17.

166. Martinod, P., Paredes, A., Delle Monache, F. and Marini-Bettòlo, G.B.(1976), *Phytochemistry*, 15, 562.

167. Edwards, J.M. and Schwarting, A.E.(1973), *Phytochemistry*, 12, 945.

168. Goncalves de Lima, O., D'Albuquerque, I.L., De Méllo, J.F., Maciel,l G.M., De Maraes, M.A. and Souza, E.(1972),

Rev. Inst. Antibioticos (Recife), 12, 19.

169. Kumar, V., Wazeer, M.I.M., and Wijeratne, D.B.T.(1985), *Phytochemistry*, 24(9), 2067.

170. Gisvold, O.(1942), *Journal of the American Pharmaceutical Association*, 31, 529.

171. Nakanishi, K., Takahashi, Y., and Budzikiewicz, H.(1965), *Journal of Organic Chemistry*, 30, 1729.

172. Turner, A.B.(1966), *Quinomethides in nature:* Zechmeister: *Fortschritte der Chemie organischer Naturstoffe*, Vol. XXIV, S. 289 ff.

173. Chou, T.Q. and Mei, P.F.(1963), *Chinese Journal of Physiology*, 10, 529.

174. Nakanishi, K., Gullo, V.P., Govindachari, T.R. and Viswanathan, N.(1973), *Journal of the American Chemical Society*, 95, 6473.

175. Martin, J.D.(1973), *Tetrahedron*, 29, 2997.

176. Drewes, S.E. and Roos, G.H.P.(1988), *Tetrahedron*, 44(15), 4653.

177. Hoffman, H.M.R. and Grundke, C.(1987), *Chem. Ber.*, 120, 1461.

178. Banfi, L., Bernardi, A., Colombo, L., Gennari, C. and Scolastico, C.(1984), *Journal of Organic Chemistry*, 49, 3784.

179. Baeckstrom, P., Li, L., Wickramaratne, M. and Norin, T.(1990), *Synthetic Communications*, 20(3), 423.

180. Corey, E.J. and Suggs, J.W.(1975), *Tetrahedron Letters*, No. 31, 2647.

181. Cerny, M., Malek, J., Capka, M. and Chvalovsky, V.(1969), *Journal of the Chemical Society, Chemical Communications*, 34, 1025.

182. Loizou, G.(1987) *M.Sc. Thesis*, University of Natal, Pietermaritzburg.

183. Ayer, W.A. and Talamas, F.X.(1988), *Canadian Journal of Chemistry*, 66, 1675.

184. Okawara, H., Nakai, H., and Ohno, M.(1982), *Tetrahedron Letters*, 23(10), 1087.

185. Mori, K. and Watanabe, H.(1986), *Tetrahedron*, 42(1), 273.

186. (a) Hui, W.H. and Li, M.M.(1976), *Journal of the Chemical Society, Perkin Trans. 1*, 23. (b) Ref. 151, p. 3597.

187. Sheldrick, G.M.(1986), *Program for Crystal Structure Determination*, University of Göttingen.

188. Sheldrick, G.M.(1976), *Program for Crystal Structure Determination and Refinement*, University of Cambridge.

189. *International Tables for X-ray Crystallography*, (1974) Vol. 4, pp. 99, 149. Kynock Press, Birmingham.

APPENDIX 1.

CRYSTAL DATA.

6ß-Hydroxy-lup-20(29)-en-3-one Crystallizes in the monoclinic space group $P2_1$ with a = 8.035(2), b = 9.778(3), c = 16.534(4) Å; ß = 90.40(2)°; V = 1299.0(6) Å. Calculated density = 1.19 gcm^{-3} for Z = 2. Using graphite monochromated MoK_α radiation (λ = 0.71069Å) 3042 unique reflection intensities were measured in the range $2 \leq 2\theta \leq 60°$ on a CAD-4 diffractometer. Of these, 1688 reflections with $I \geq 3\sigma(I)$ were used in the solution(direct methods, SHELX 86[187] and refinement (full-matrix least-squares, SHELX 76[188]) of the structure. The R value converged to 0.093(319 parameters, R_w = 0.103 with $w = 1.39/\sigma^2(F) + 0.007F^2$). Anomalous scattering factors were taken from reference[189]. Inverting the coordinates of all the atoms($x, y, z \rightarrow x, y, z$) and refinement of the structure did not change the final R factors and thus it was not possible to establish the absolute structure. Tables of coordinates, temperature factors, interatomic distances and angles are deposited at the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, U.K.

Table 23

6ß-Hydroxy-lup-20(29)-en-3-one.

INTERATOMIC DISTANCES(Å) FOR $C_{30}H_{48}O_2$.

O(1) - C(3)	1.204(9)	O(2) - C(6)	1.401(10)
H(1) - H(36)	1.150(11)	C(1) - H(2)	1.080(0)
C(1) - H(3)	1.080(0)	C(1) - C(2)	1.516(12)
C(1) - C(10)	1.597(9)	C(2) - H(4)	1.080(0)
C(2) - H(5)	1.080(0)	C(2) - C(3)	1.474(10)
C(3) - C(4)	1.552(10)	C(4) - C(5)	1.541(11)
C(4) - C(23)	1.535(13)	C(4) - C(24)	1.557(13)
C(5) - H(6)	1.080(0)	C(5) - C(6)	1.548(9)
C(5) - C(10)	1.576(10)	C(6) - H(7)	1.080(0)
C(6) - C(7)	1.525(12)	C(7) - H(8)	1.080(0)
C(7) - H(9)	1.080(0)	C(7) - C(8)	1.575(10)
C(8) - C(9)	1.582(8)	C(8) - C(14)	1.597(11)
C(8) - C(26)	1.515(11)	C(9) - H(10)	1.080(0)
C(9) - C(10)	1.562(10)	C(9) - C(11)	1.570(9)
C(10) - C(25)	1.513(12)	C(11) - H(11)	1.080(0)
C(11) - H(12)	1.080(0)	C(11) - C(12)	1.504(13)
C(12) - H(13)	1.080(0)	C(12) - H(14)	1.080(0)
C(12) - C(13)	1.496(10)	C(13) - H(15)	1.080(0)
C(13) - C(14)	1.532(10)	C(13) - C(18)	1.567(11)
C(14) - C(15)	1.572(10)	C(14) - C(27)	1.554(12)
C(15) - H(16)	1.080(0)	C(15) - H(17)	1.080(0)
C(15) - C(16)	1.512(14)	C(16) - H(18)	1.080(0)
C(16) - H(19)	1.080(0)	C(16) - C(17)	1.51(2)
C(17) - C(18)	1.532(11)	C(17) - C(22)	1.565(15)
C(17) - C(28)	1.610(15)	C(18) - H(20)	1.080(0)
C(18) - C(19)	1.523(11)	C(19) - H(21)	1.080(0)
C(19) - C(20)	1.503(12)	C(19) - C(21)	1.591(15)
C(20) - C(29)	1.350(15)	C(20) - C(30)	1.509(13)
C(21) - H(22)	1.080(0)	C(21) - H(23)	1.080(0)
C(21) - C(22)	1.52(2)	C(22) - H(24)	1.080(0)
C(22) - H(25)	1.080(0)	C(23) - H(26)	1.080(0)

C(23) - H(27)	1.080(0)	C(23) - H(28)	1.080(0)
C(24) - H(29)	1.080(0)	C(24) - H(30)	1.080(0)
C(24) - H(31)	1.080(0)	C(25) - H(32)	1.080(0)
C(25) - H(33)	1.080(0)	C(25) - H(34)	1.080(0)
C(26) - H(35)	1.080(0)	C(26) - H(36)	1.080(0)
C(26) - H(37)	1.080(0)	C(27) - H(38)	1.080(0)
C(27) - H(39)	1.080(0)	C(27) - H(40)	1.080(0)
C(27) - H(41)	1.080(0)	C(28) - H(42)	1.080(0)
C(28) - H(43)	1.080(0)	C(29) - H(44)	1.47(11)
C(29) - H(45)	1.080(0)	C(30) - H(46)	1.080(0)
C(30) - H(47)	1.080(0)	C(30) - H(48)	1.080(0)

Table 24

6ß-Hydroxy-lup-20(29)-en-3-one.

INTERATOMIC ANGLES(°) FOR $C_{30}H_{48}O_2$.

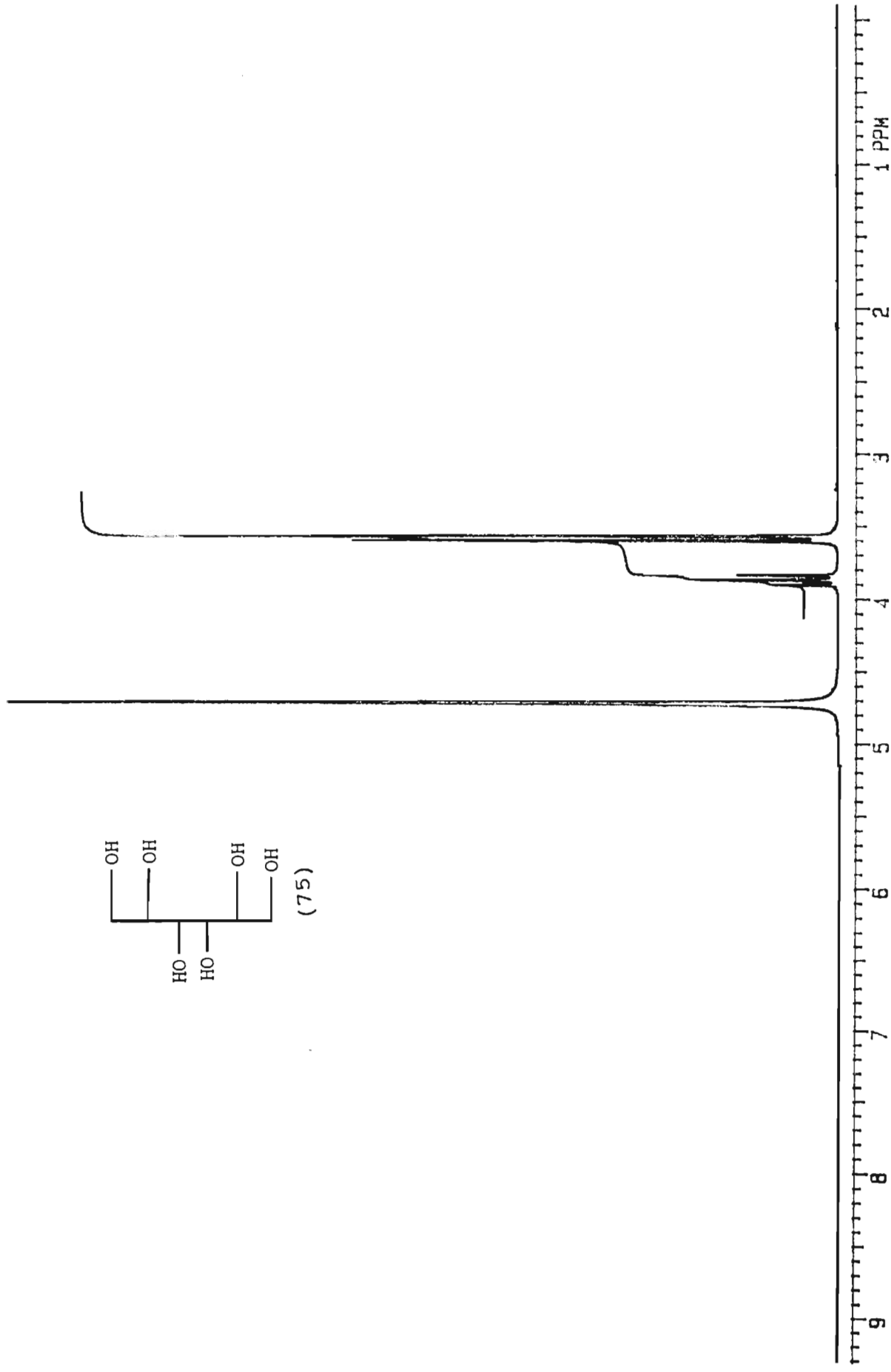
H(2) - C(1) - H(3)	109.5(0)	H(2) - C(1) - C(2)	109.2(4)
H(3) - C(1) - C(2)	108.0(5)	H(2) - C(1) - C(10)	109.5(4)
H(3) - C(1) - C(10)	108.7(5)	C(2) - C(1) - C(10)	111.9(6)
C(1) - C(2) - H(4)	109.5(5)	C(1) - C(2) - H(5)	108.9(4)
H(4) - C(2) - H(5)	109.5(0)	C(1) - C(2) - C(3)	110.5(7)
H(4) - C(2) - C(3)	108.1(5)	H(5) - C(2) - C(3)	110.7(4)
O(1) - C(3) - C(2)	123.0(7)	O(1) - C(3) - C(4)	121.1(7)
C(2) - C(3) - C(4)	115.9(6)	C(3) - C(4) - C(5)	106.2(5)
C(3) - C(4) - C(23)	107.0(7)	C(5) - C(4) - C(23)	110.7(8)
C(3) - C(4) - C(24)	105.8(7)	C(5) - C(4) - C(24)	117.1(6)
C(23) - C(4) - C(24)	109.5(7)	C(4) - C(5) - H(6)	101.1(4)
C(4) - C(5) - C(6)	113.5(6)	H(6) - C(5) - C(6)	110.7(4)
C(4) - C(5) - C(10)	118.4(6)	H(6) - C(5) - C(10)	99.5(4)
C(6) - C(5) - C(10)	111.9(6)	O(2) - C(6) - C(5)	110.9(6)
O(2) - C(6) - H(7)	105.5(4)	C(5) - C(6) - H(7)	109.5(4)
O(2) - C(6) - C(7)	115.5(7)	C(5) - C(6) - C(7)	110.0(6)
H(7) - C(6) - C(7)	105.1(4)	C(6) - C(7) - H(8)	109.8(5)
C(6) - C(7) - H(9)	106.8(4)	H(8) - C(7) - H(9)	109.5(0)
C(6) - C(7) - C(8)	116.9(7)	H(8) - C(7) - C(8)	107.8(4)
H(9) - C(7) - C(8)	105.9(4)	C(7) - C(8) - C(9)	106.0(5)
C(7) - C(8) - C(14)	109.5(6)	C(9) - C(8) - C(14)	105.9(5)
C(7) - C(8) - C(26)	111.2(6)	C(9) - C(8) - C(26)	111.7(6)
C(14) - C(8) - C(26)	112.2(6)	C(8) - C(9) - H(10)	108.8(4)
C(8) - C(9) - C(10)	115.6(5)	H(10) - C(9) - C(10)	100.2(3)
C(8) - C(9) - C(11)	108.6(5)	H(10) - C(9) - C(11)	108.7(4)
C(10) - C(9) - C(11)	114.5(5)	C(1) - C(10) - C(5)	103.6(5)
C(1) - C(10) - C(9)	105.0(5)	C(5) - C(10) - C(9)	106.3(5)
C(1) - C(10) - C(25)	108.5(7)	C(5) - C(10) - C(25)	116.7(6)
C(9) - C(10) - C(25)	115.5(6)	C(9) - C(11) - H(11)	110.4(3)
C(9) - C(11) - H(12)	106.4(4)	H(11) - C(11) - H(12)	109.5(0)
C(9) - C(11) - C(12)	113.4(6)	H(11) - C(11) - C(12)	109.3(4)

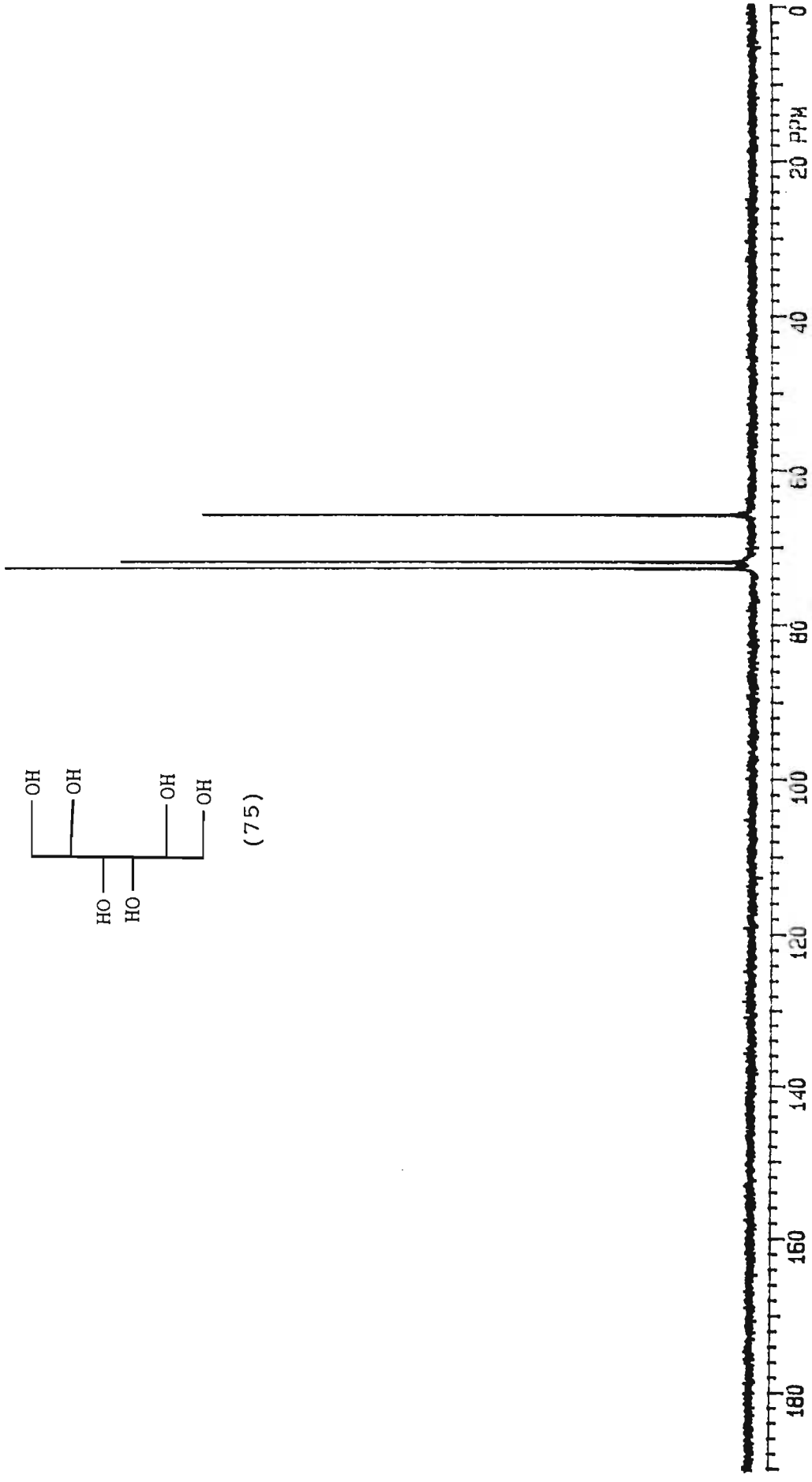
H(12) - C(11) - C(12 107.8(5)	C(11) - C(12) - H(13) 108.6(5)
C(11) - C(12) - H(14) 109.6(4)	H(13) - C(12) - H(14) 109.5(0)
C(11) - C(12) - C(13) 111.2(6)	H(13) - C(12) - C(13) 109.1(4)
H(14) - C(12) - C(13) 108.9(4)	C(12) - C(13) - H(15) 104.3(5)
C(12) - C(13) - C(14) 111.6(6)	H(15) - C(13) - C(14) 107.9(4)
C(12) - C(13) - C(18) 114.7(6)	H(15) - C(13) - C(18) 107.0(4)
C(14) - C(13) - C(18) 110.8(6)	C(8) - C(14) - C(13) 107.7(6)
C(8) - C(14) - C(15) 107.7(6)	C(13) - C(14) - C(15) 110.7(7)
C(8) - C(14) - C(27) 114.5(6)	C(13) - C(14) - C(27) 110.0(6)
C(15) - C(14) - C(27) 106.2(8)	C(14) - C(15) - H(16) 109.9(5)
C(14) - C(15) - H(17) 106.9(6)	H(16) - C(15) - H(17) 109.5(0)
C(14) - C(15) - C(16) 113.5(7)	H(16) - C(15) - C(16) 109.8(6)
H(17) - C(15) - C(16) 107.2(7)	C(15) - C(16) - H(18) 111.9(5)
C(15) - C(16) - H(19) 107.1(7)	H(18) - C(16) - H(19) 109.5(0)
C(15) - C(16) - C(17) 111.3(8)	H(18) - C(16) - C(17) 110.7(5)
H(19) - C(16) - C(17) 106.1(6)	C(16) - C(17) - C(18) 109.1(8)
C(16) - C(17) - C(22) 115.5(9)	C(18) - C(17) - C(22) 100.6(8)
C(16) - C(17) - C(28) 113.3(9)	C(18) - C(17) - C(28) 110.9(9)
C(22) - C(17) - C(28) 106.8(9)	C(13) - C(18) - C(17) 112.1(7)
C(13) - C(18) - H(20) 98.4(4)	C(17) - C(18) - H(20) 115.0(5)
C(13) - C(18) - C(19) 120.6(7)	C(17) - C(18) - C(19) 104.4(7)
H(20) - C(18) - C(19) 106.6(4)	C(18) - C(19) - H(21) 107.0(4)
C(18) - C(19) - C(20) 118.3(6)	H(21) - C(19) - C(20) 102.4(5)
C(18) - C(19) - C(21) 102.9(7)	H(21) - C(19) - C(21) 116.3(5)
C(20) - C(19) - C(21) 110.5(8)	C(19) - C(20) - C(29) 121.3(9)
C(19) - C(20) - C(30) 119.5(8)	C(29) - C(20) - C(30) 119(10)
C(19) - C(21) - H(22) 108.6(5)	C(19) - C(21) - H(23) 110.3(5)
H(22) - C(21) - H(23) 109.5(0)	C(19) - C(21) - C(22) 107.1(8)
H(22) - C(21) - C(22) 111.7(7)	H(23) - C(21) - C(22) 109.6(6)
C(17) - C(22) - C(21) 103.2(9)	C(17) - C(22) - H(24) 111.5(7)
C(21) - C(22) - H(24) 110.3(7)	C(17) - C(22) - H(25) 110.3(5)
C(21) - C(22) - H(25) 112.0(6)	H(24) - C(22) - H(25) 109.5(0)
C(4) - C(23) - H(26) 100.6(5)	C(4) - C(23) - H(27) 113.4(6)
H(26) - C(23) - H(27) 109.5(0)	C(4) - C(23) - H(28) 114.0(6)
H(26) - C(23) - H(28) 109.5(0)	H(27) - C(23) - H(28) 109.5(0)
C(4) - C(24) - H(29) 95.5(4)	C(4) - C(24) - H(30) 94.4(5)
H(29) - C(24) - H(30) 109.5(0)	C(4) - C(24) - H(31) 136.1(5)

H(29) - C(24) - H(31) 109.5(0)	H(30) - C(24) - H(31) 109.5(0)
C(10) - C(25) - H(32) 105.5(4)	C(10) - C(25) - H(33) 108.8(4)
H(32) - C(25) - H(33) 109.5(0)	C(10) - C(25) - H(34) 114.0(4)
H(32) - C(25) - H(34) 109.5(0)	H(33) - C(25) - H(34) 109.5(0)
C(8) - C(26) - H(35) 117.2(4)	C(8) - C(26) - H(36) 114.9(4)
H(35) - C(26) - H(36) 109.5(0)	C(8) - C(26) - H(37) 95.2(4)
H(35) - C(26) - H(37) 109.5(0)	H(36) - C(26) - H(37) 109.5(0)
H(1) - H(36) - C(26) 148.0(5)	C(14) - C(27) - H(38) 96.3(5)
C(14) - C(27) - H(39) 110.2(5)	H(38) - C(27) - H(39) 109.5(0)
C(14) - C(27) - H(40) 120.9(4)	H(38) - C(27) - H(40) 109.5(0)
H(39) - C(27) - H(40) 109.5(0)	C(17) - C(28) - H(41) 120.7(5)
C(17) - C(28) - H(42) 108.8(6)	H(41) - C(28) - H(42) 109.5(0)
C(17) - C(28) - H(43) 98.1(6)	H(41) - C(28) - H(43) 109.5(0)
H(42) - C(28) - H(43) 109.5(0)	C(20) - C(29) - H(44) 86.0(4)
C(20) - C(29) - H(45) 110.0(6)	H(44) - C(29) - H(45) 83.0(7)
C(20) - C(30) - H(46) 120.2(6)	C(20) - C(30) - H(47) 89.0(6)
H(46) - C(30) - H(47) 109.5(0)	C(20) - C(30) - H(48) 116.8(6)
H(46) - C(30) - H(48) 109.5(0)	H(47) - C(30) - H(48) 109.5(0)

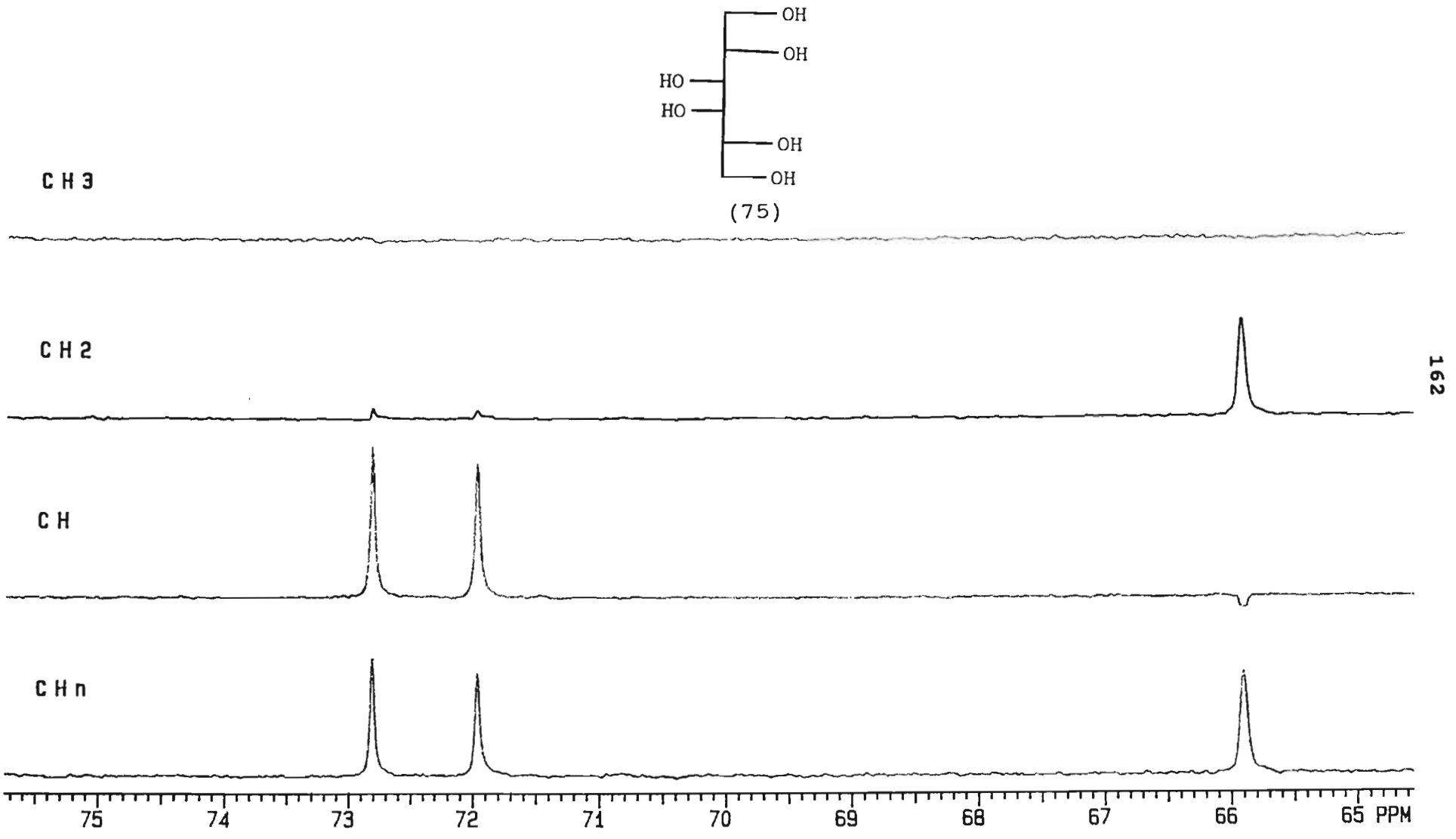
APPENDIX 2.

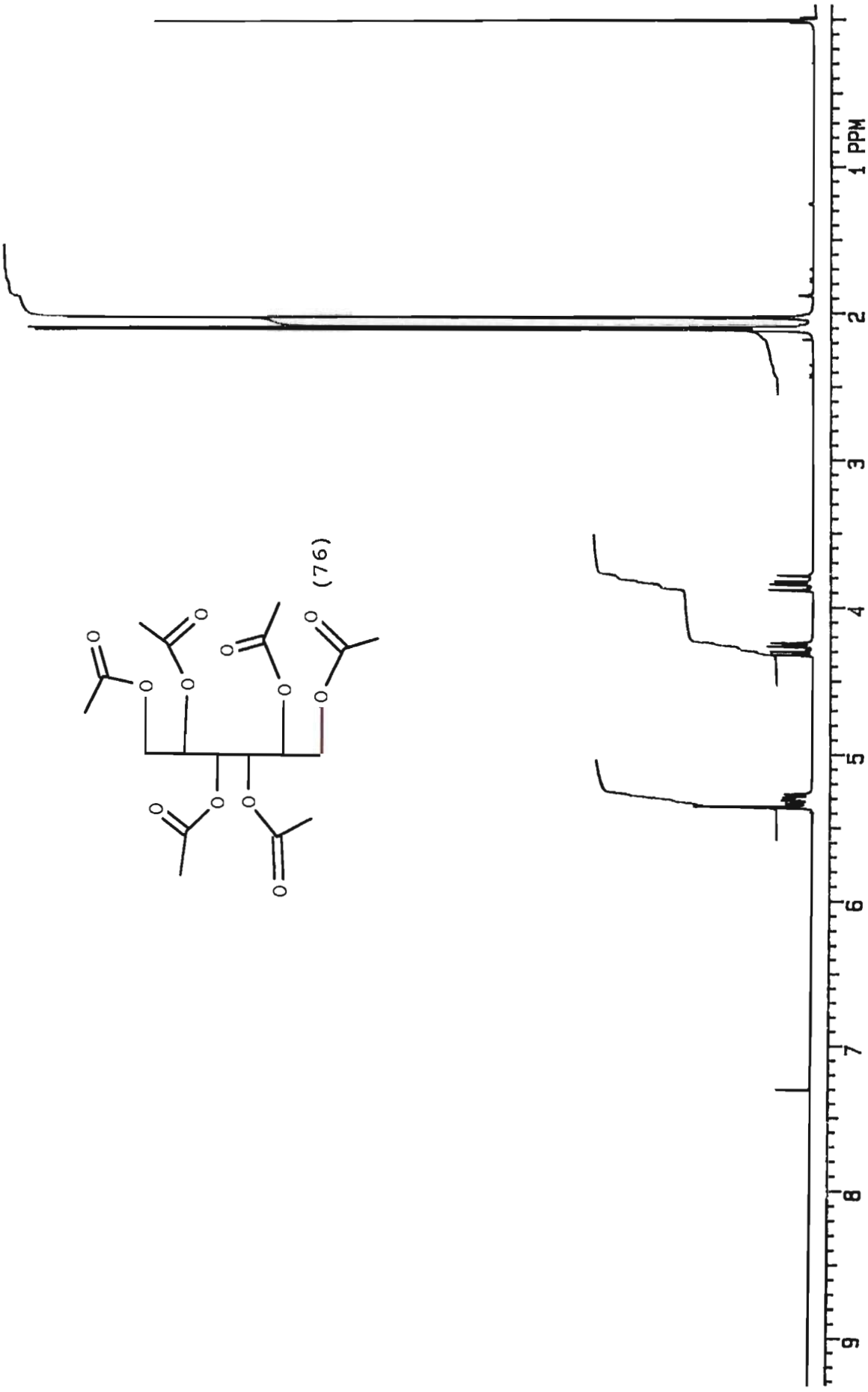
^{1}H NMR and ^{13}C NMR.


OH
OH
OH
OH
HO
HO
(75)
9
8
7
6
5
4
3
2
1 PPM

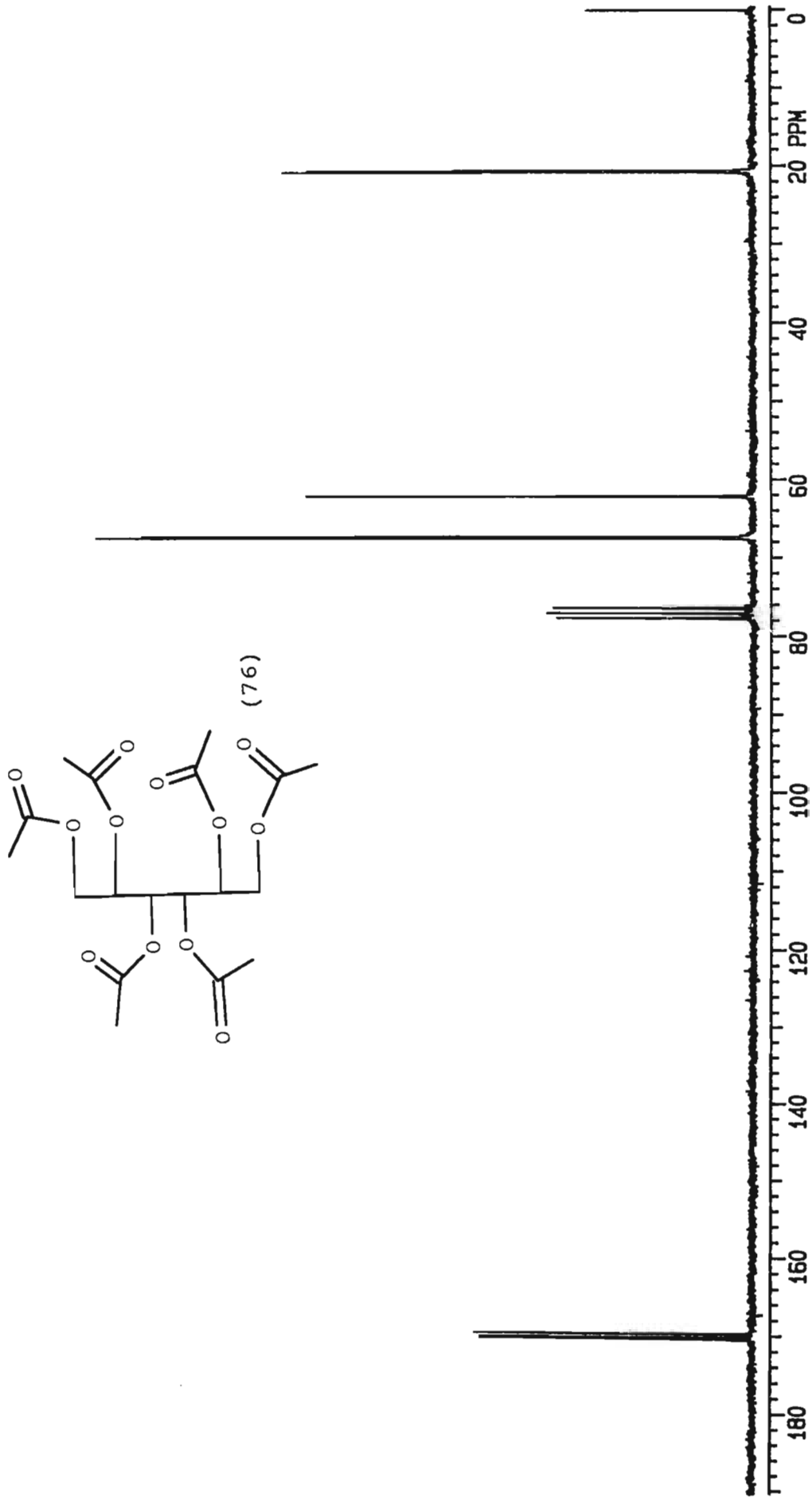


(75)

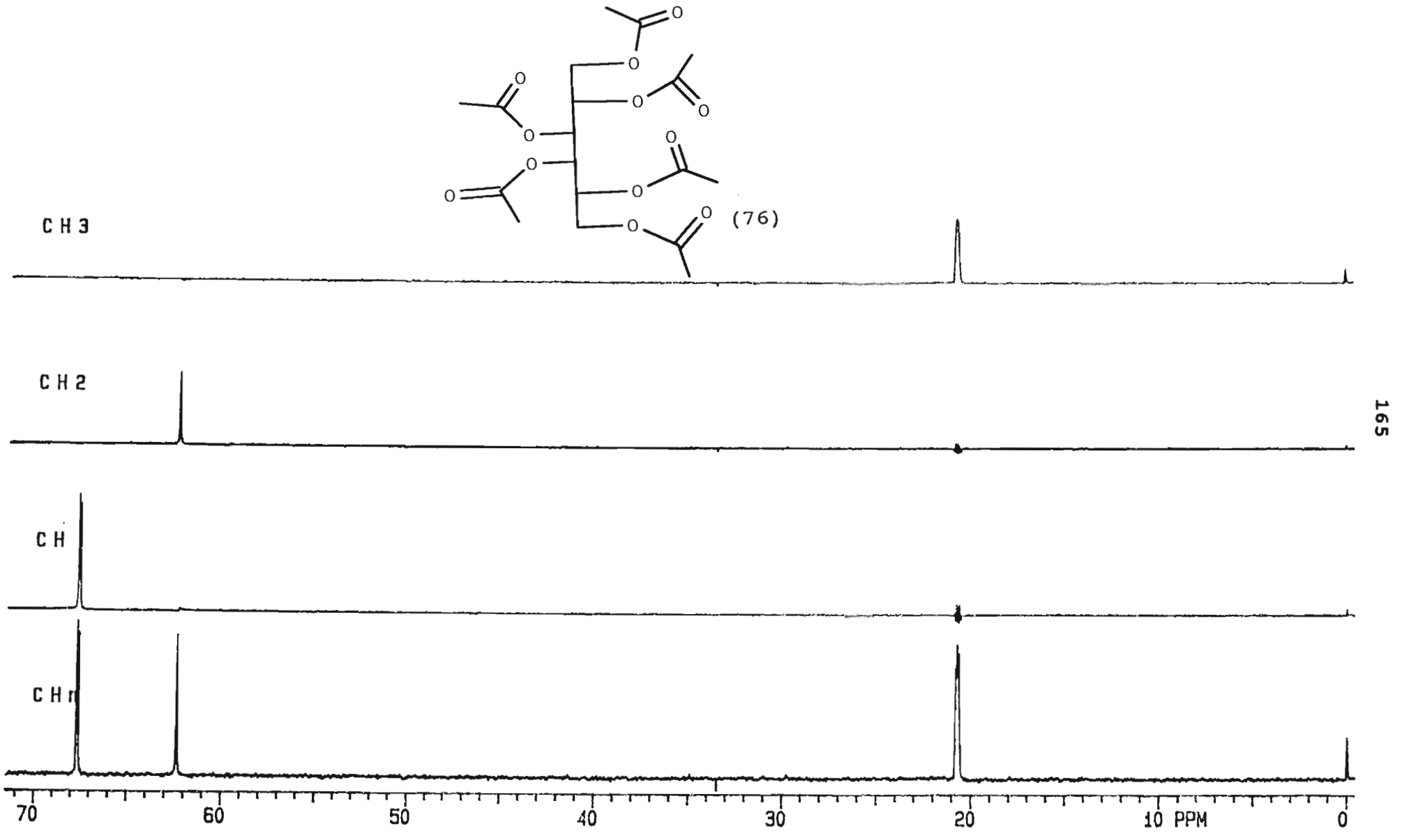

OH
OH
HO
HO
OH
OH
(75)
CH3
CH2
CH
CHn
162
75
74
73
72
71
70
69
68
67
66
65 PPM

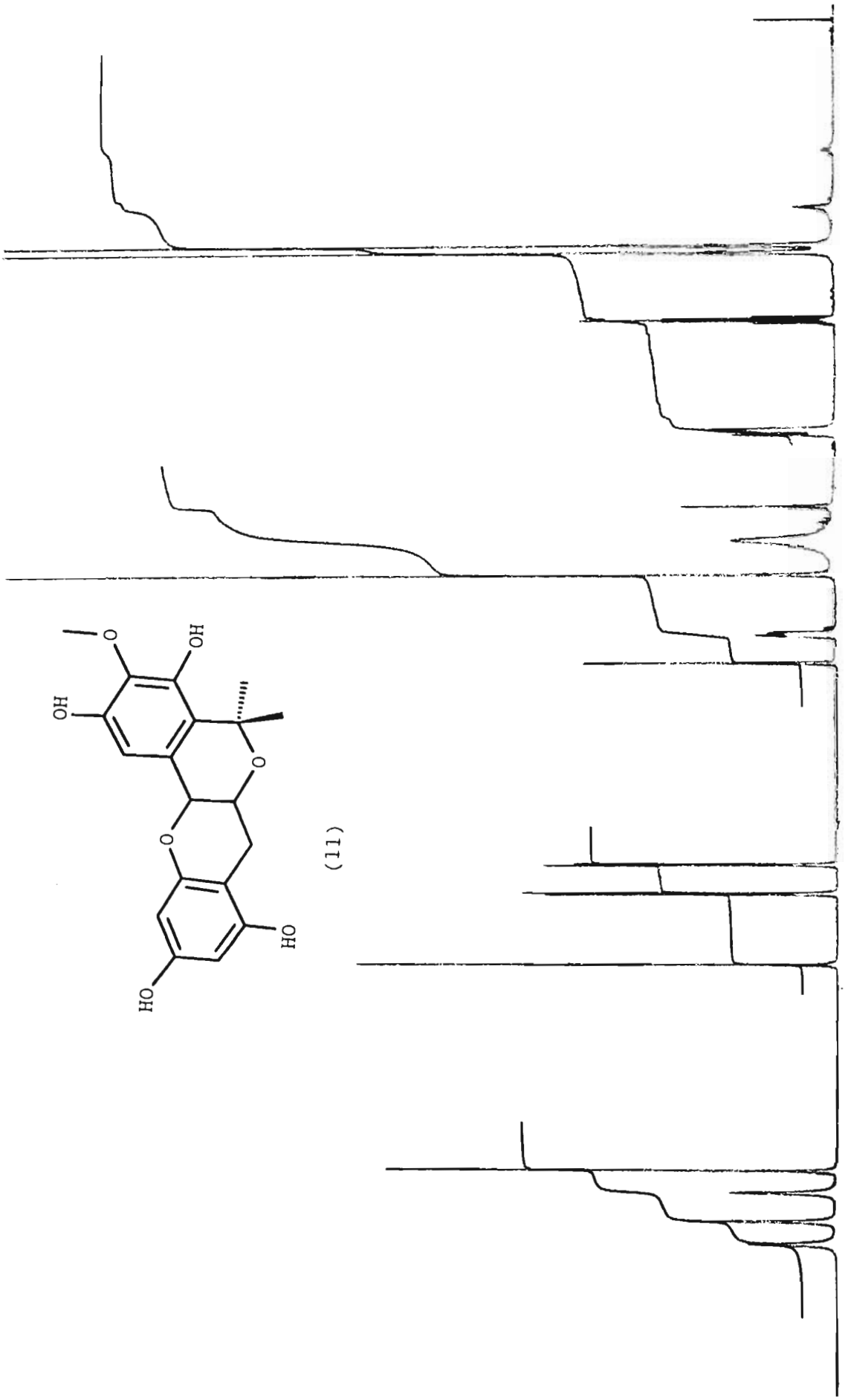


(76)

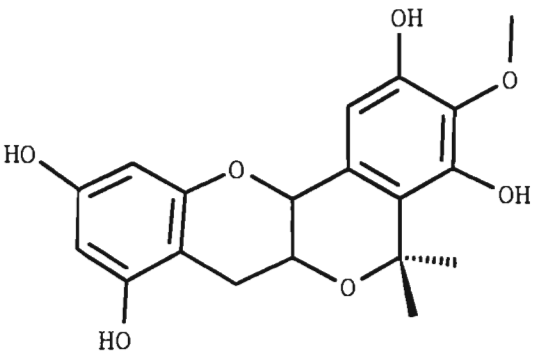


(76)


(76)
CH3
CH2
CH
CHn
70
60
50
40
30
20
10 PPM
0

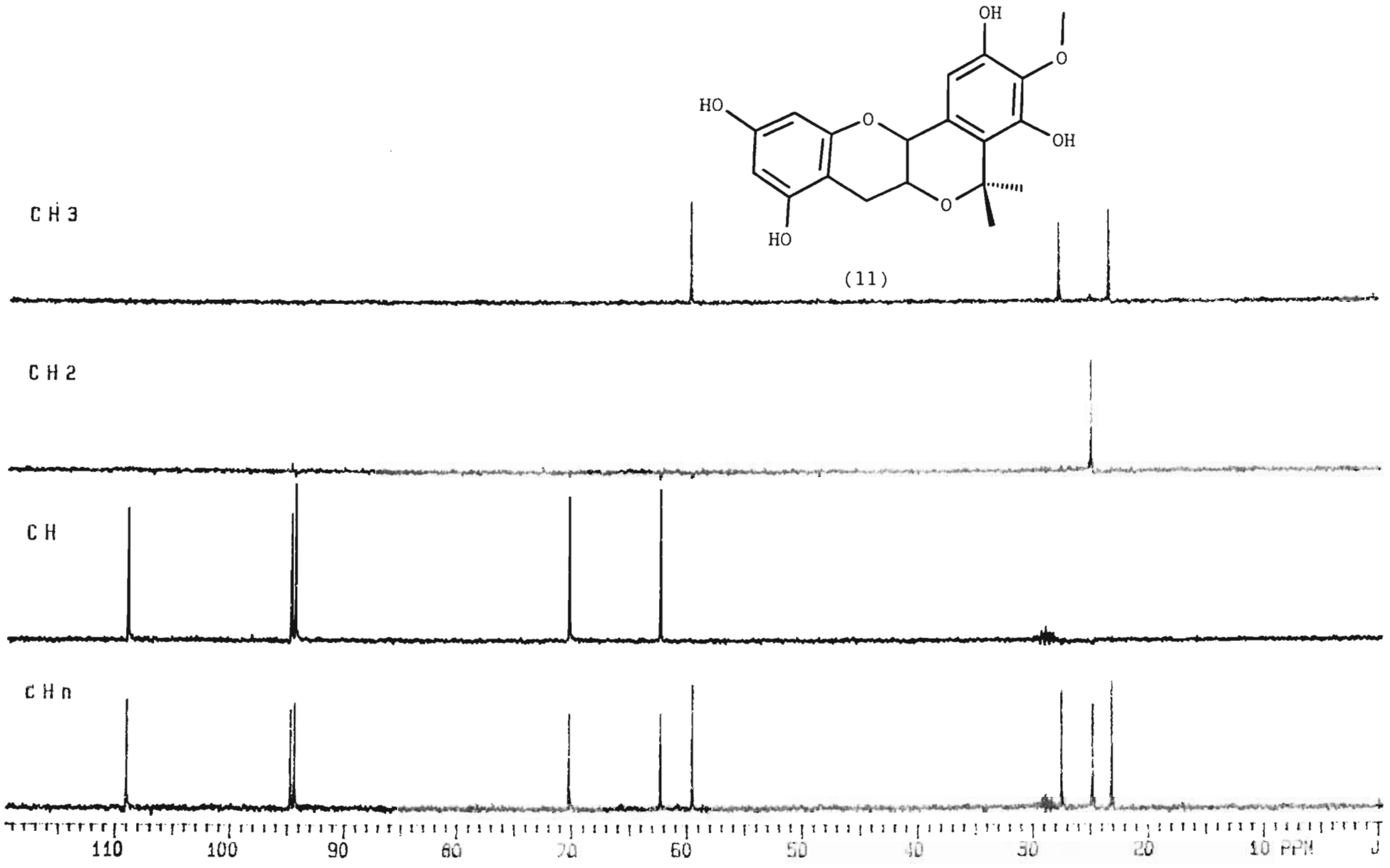


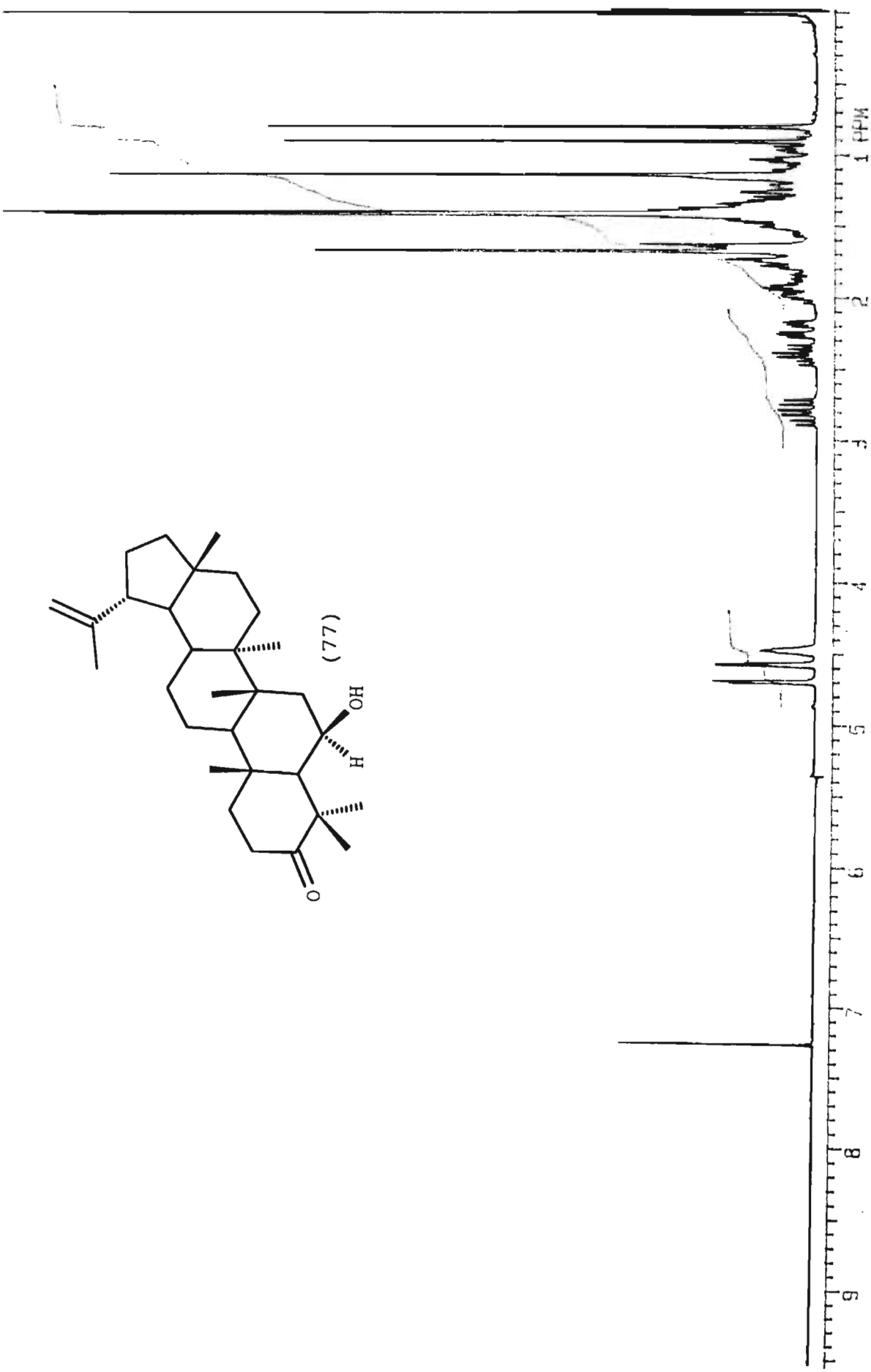
(11)


OH
O
HO
O
OH
O
HO

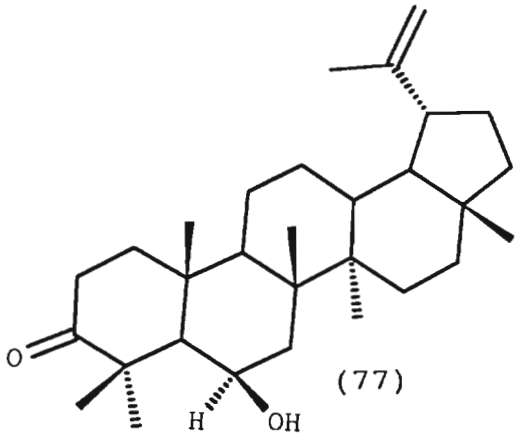
(11)

31
30
29
28
27 PPM 26
180
160
140
120
100
80
60
40
20 PPM
0

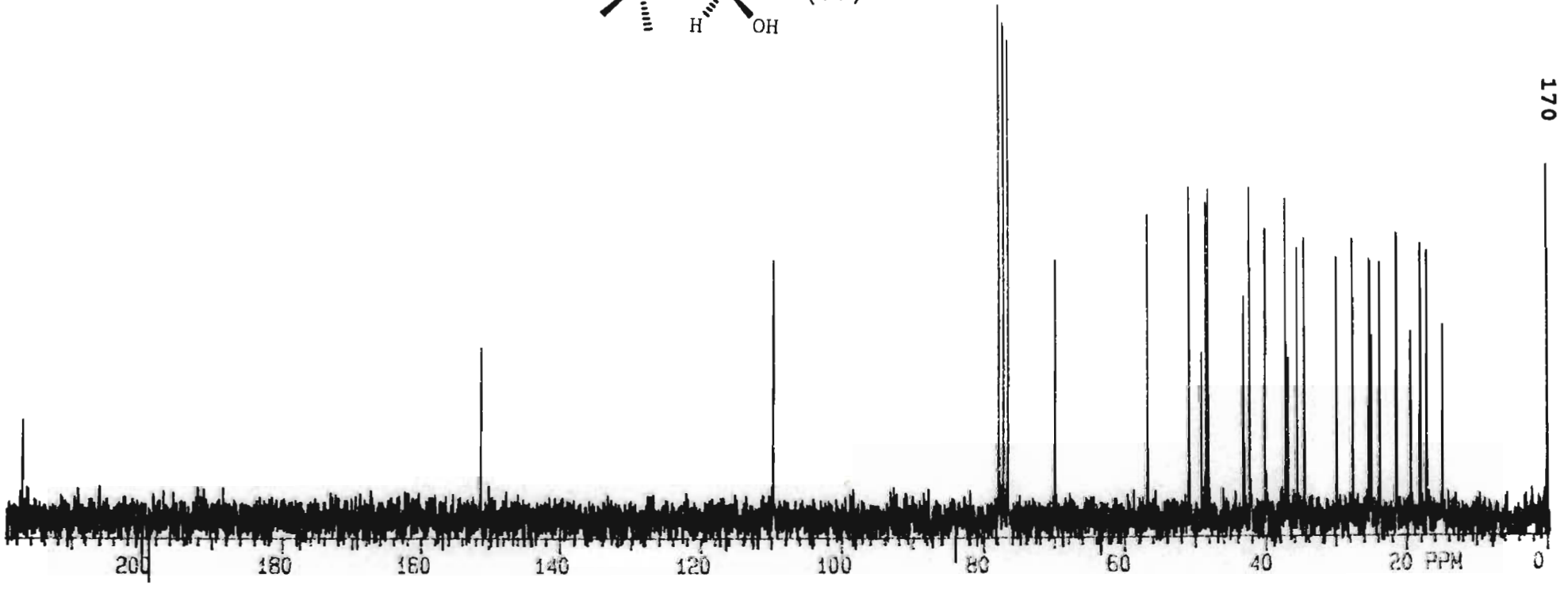

OH
O
HO
O
OH
O
HO
CH3
(11)
CH2
CH
CHn
110
100
90
80
70
60
50
40
30
20
10 PPM

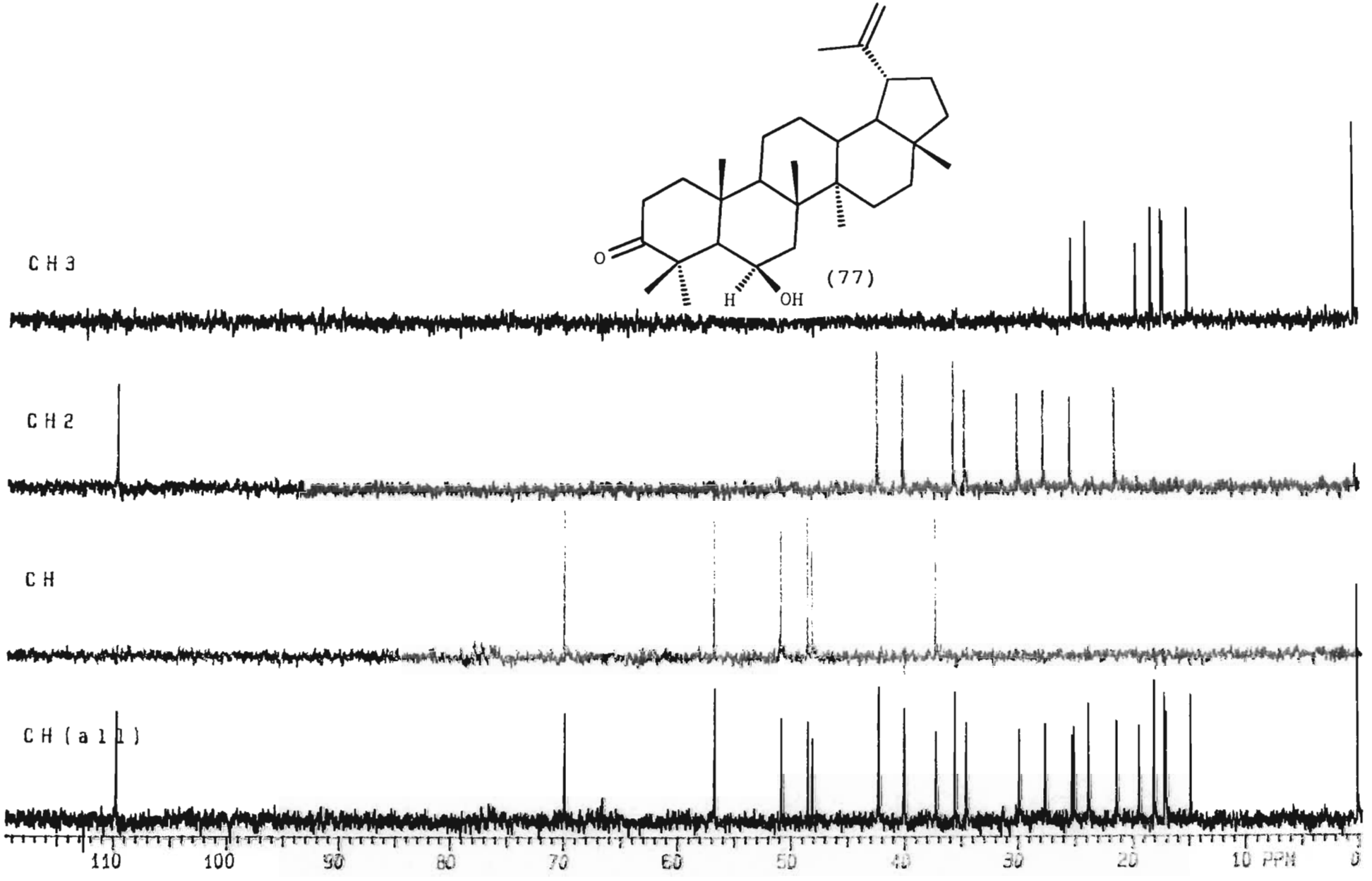


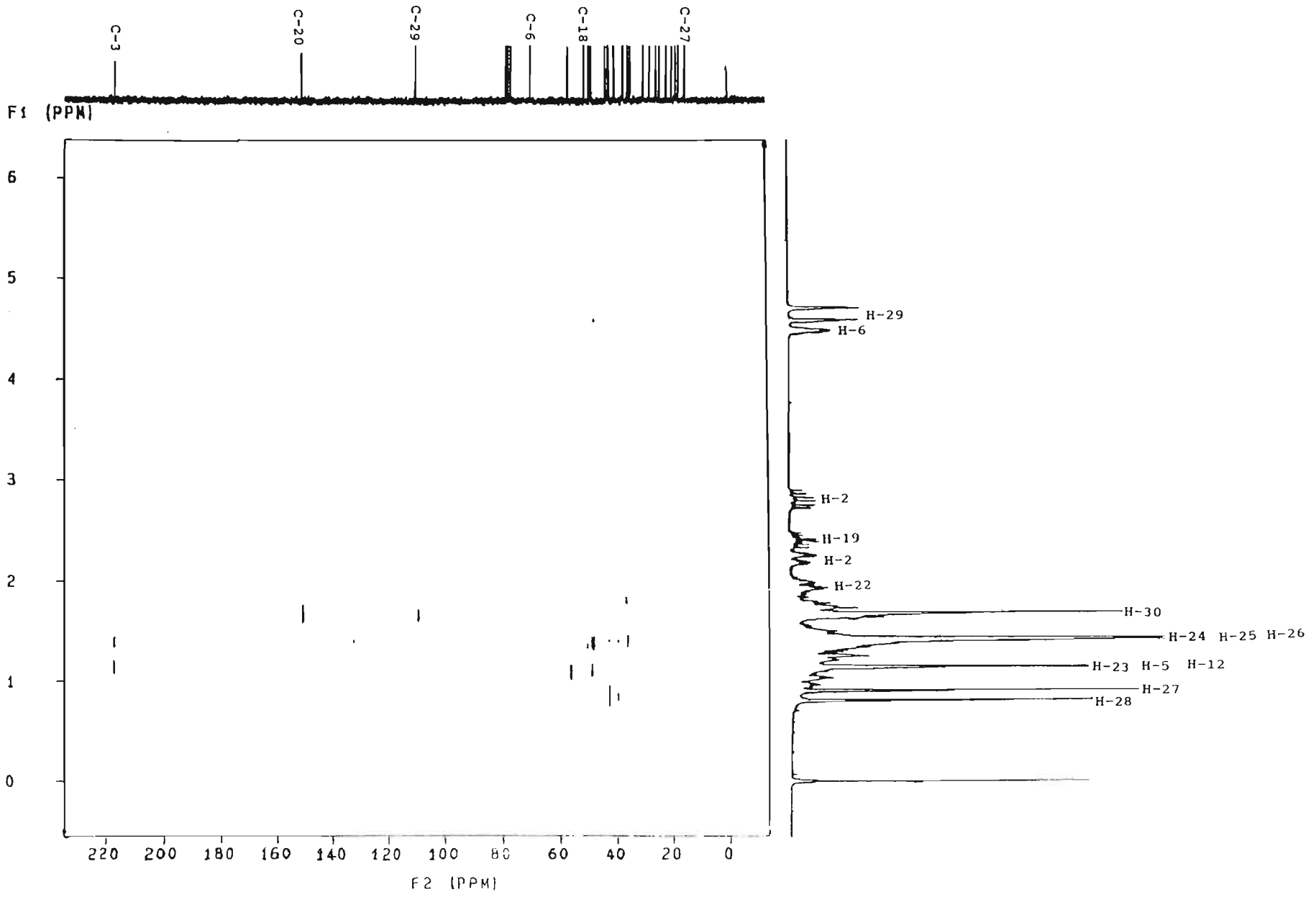
(77)

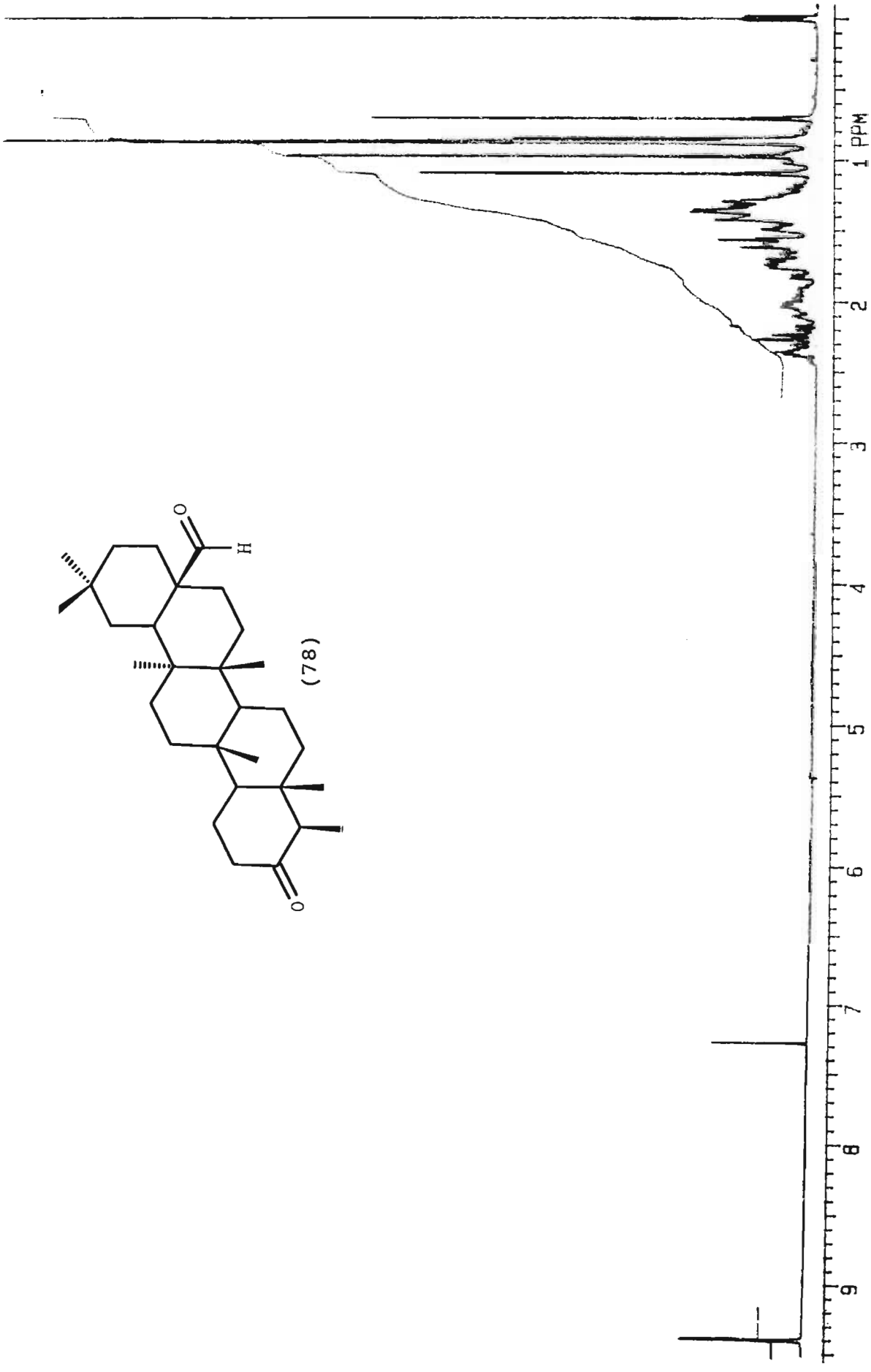


(77)

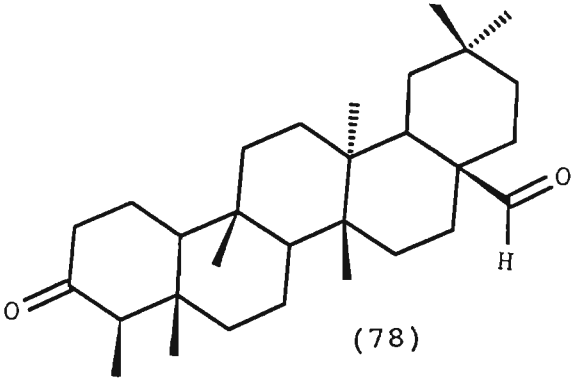



O
H
OH
(77)
CH3
CH2
CH
CH(all)
110
100
90
80
70
60
50
40
30
20
10 PPM
0

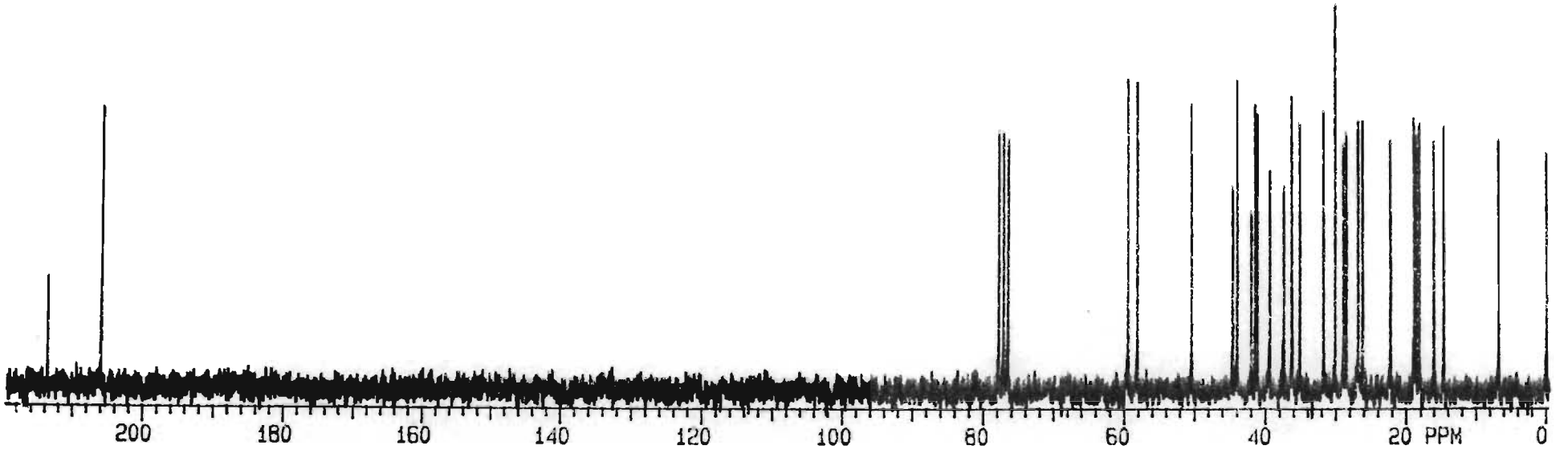

C-3
C-20
C-29
C-6
C-18
C-27
F1 (PPM)
6
5
4
3
2
1
0
H-29
H-6
H-2
H-19
H-2
H-22
H-30
H-24 H-25 H-26
H-23 H-5 H-12
H-27
H-28
220 200 180 160 140 120 100 80 60 40 20 0
F2 (PPM)

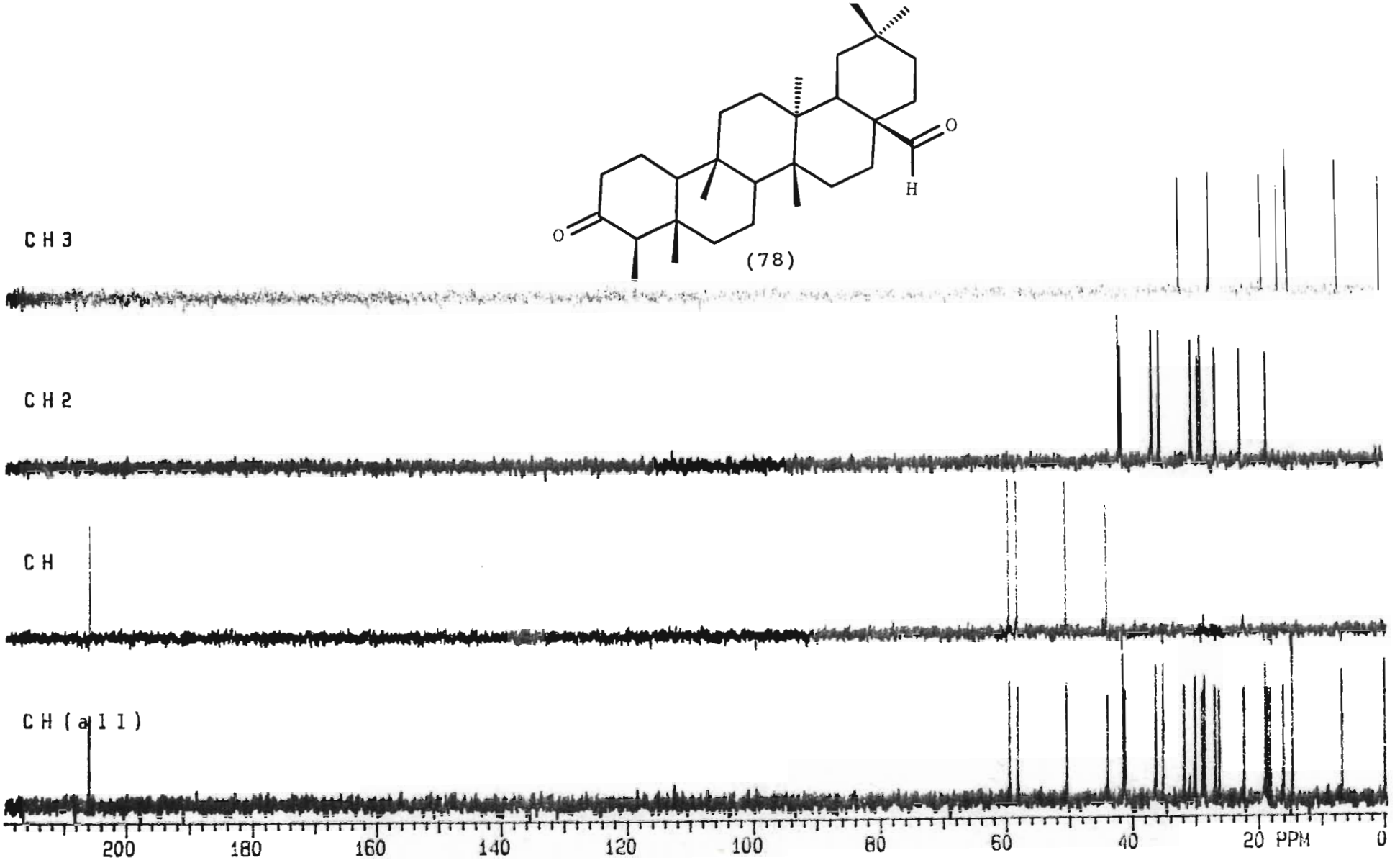


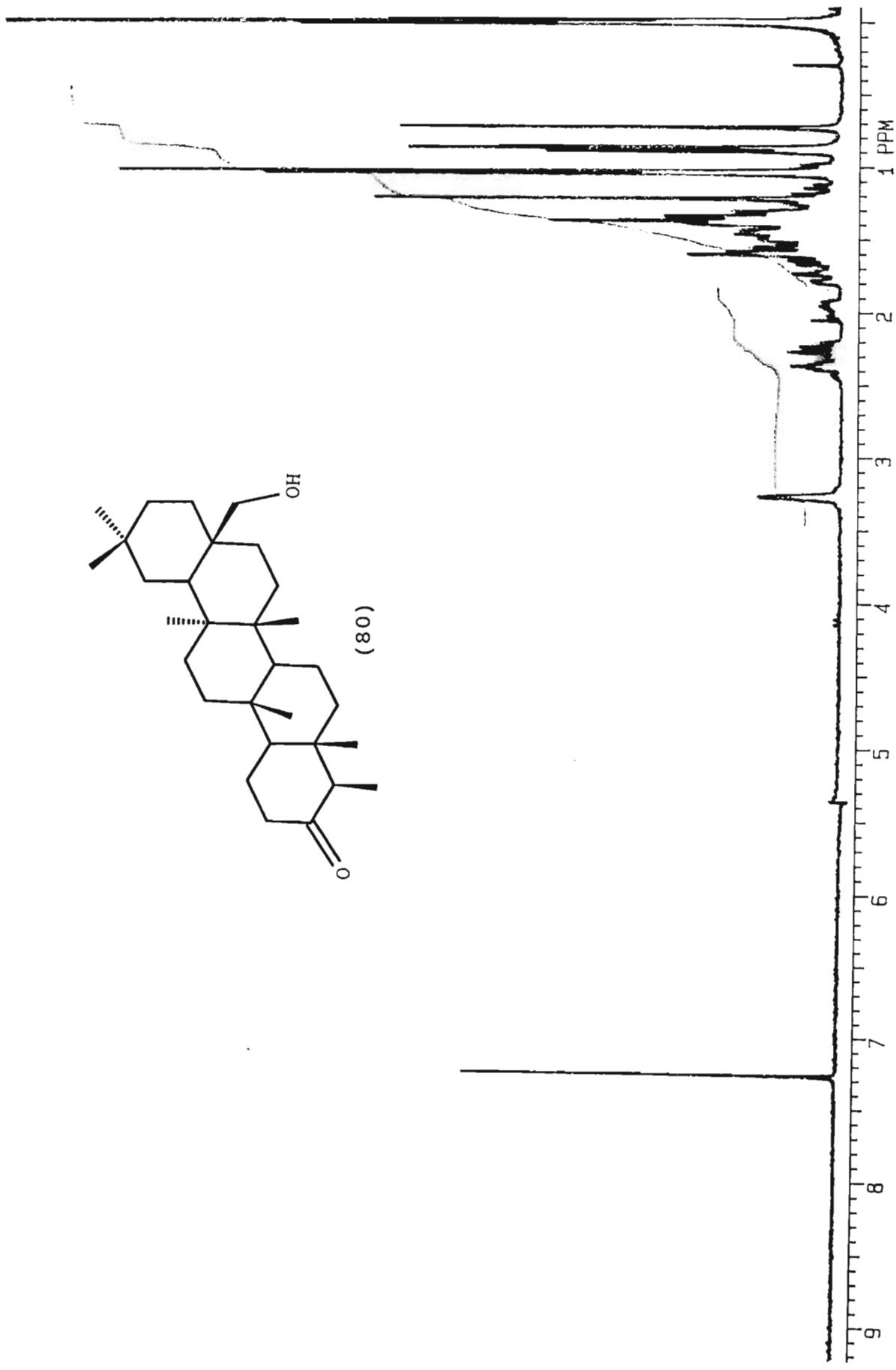
(78)

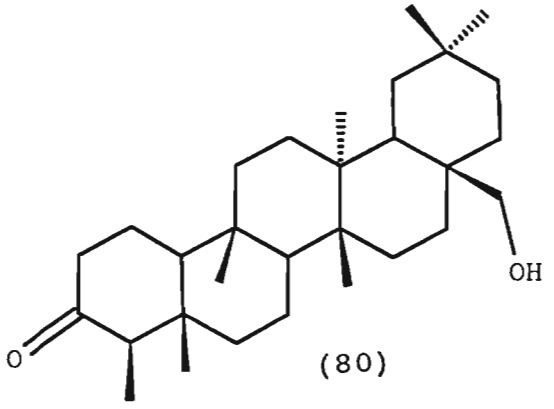


(78)

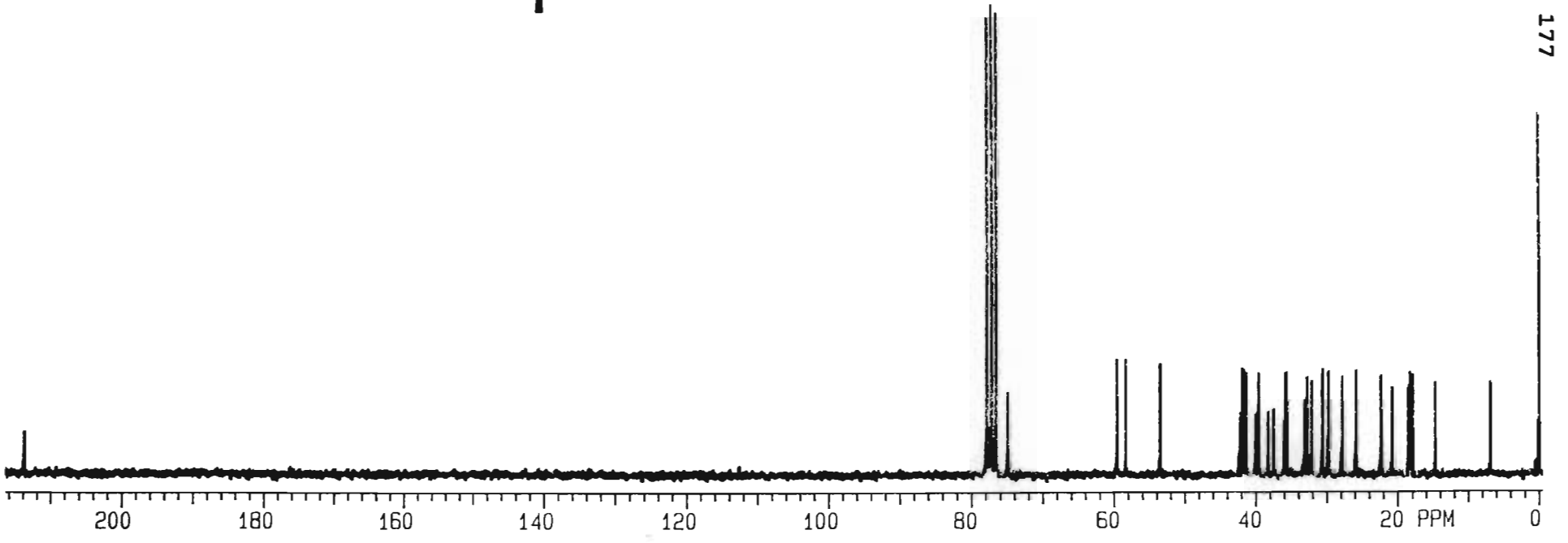


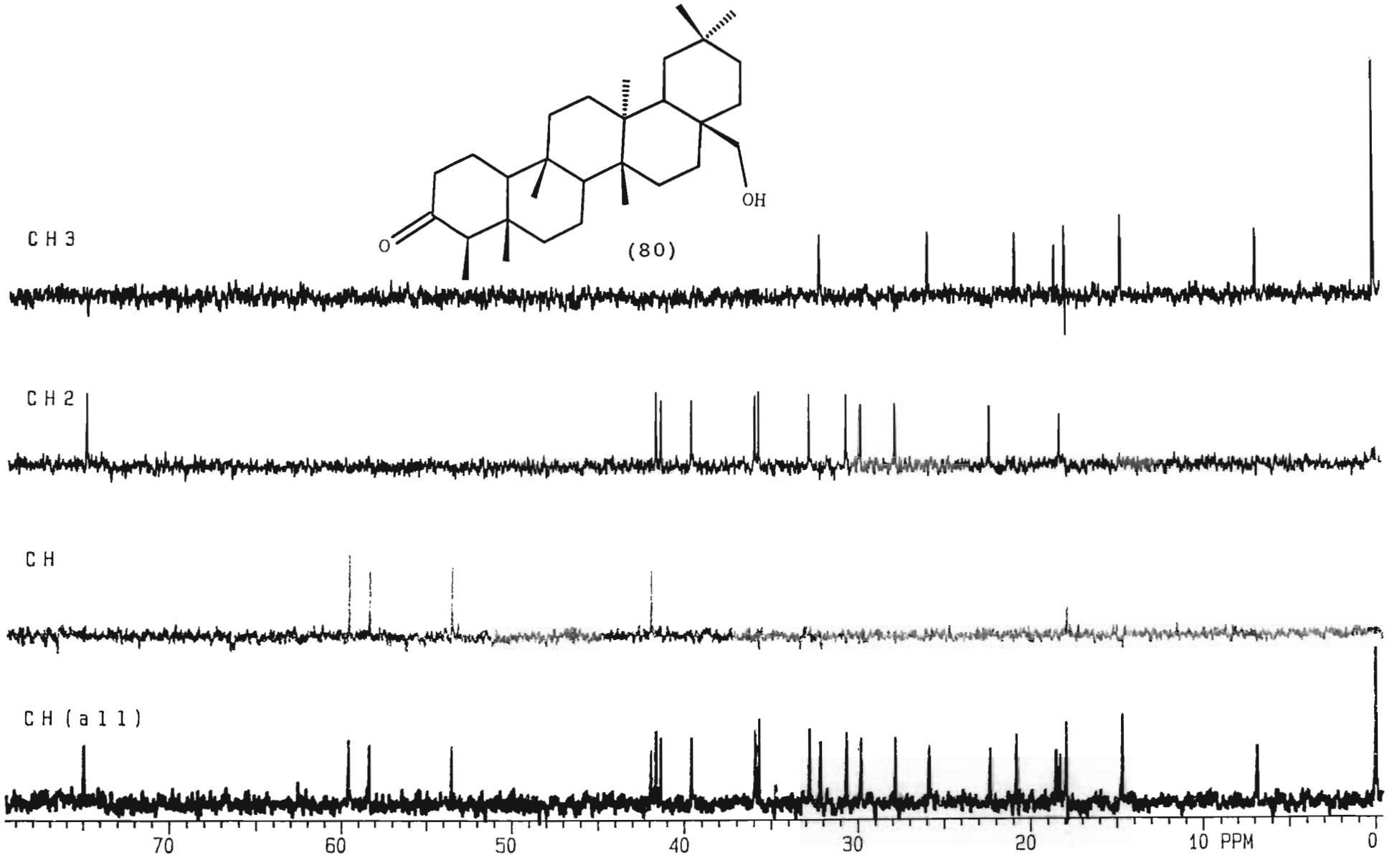

O
O
H
(78)
CH3
CH2
CH
CH(all)
200
180
160
140
120
100
80
60
40
20
PPM
0

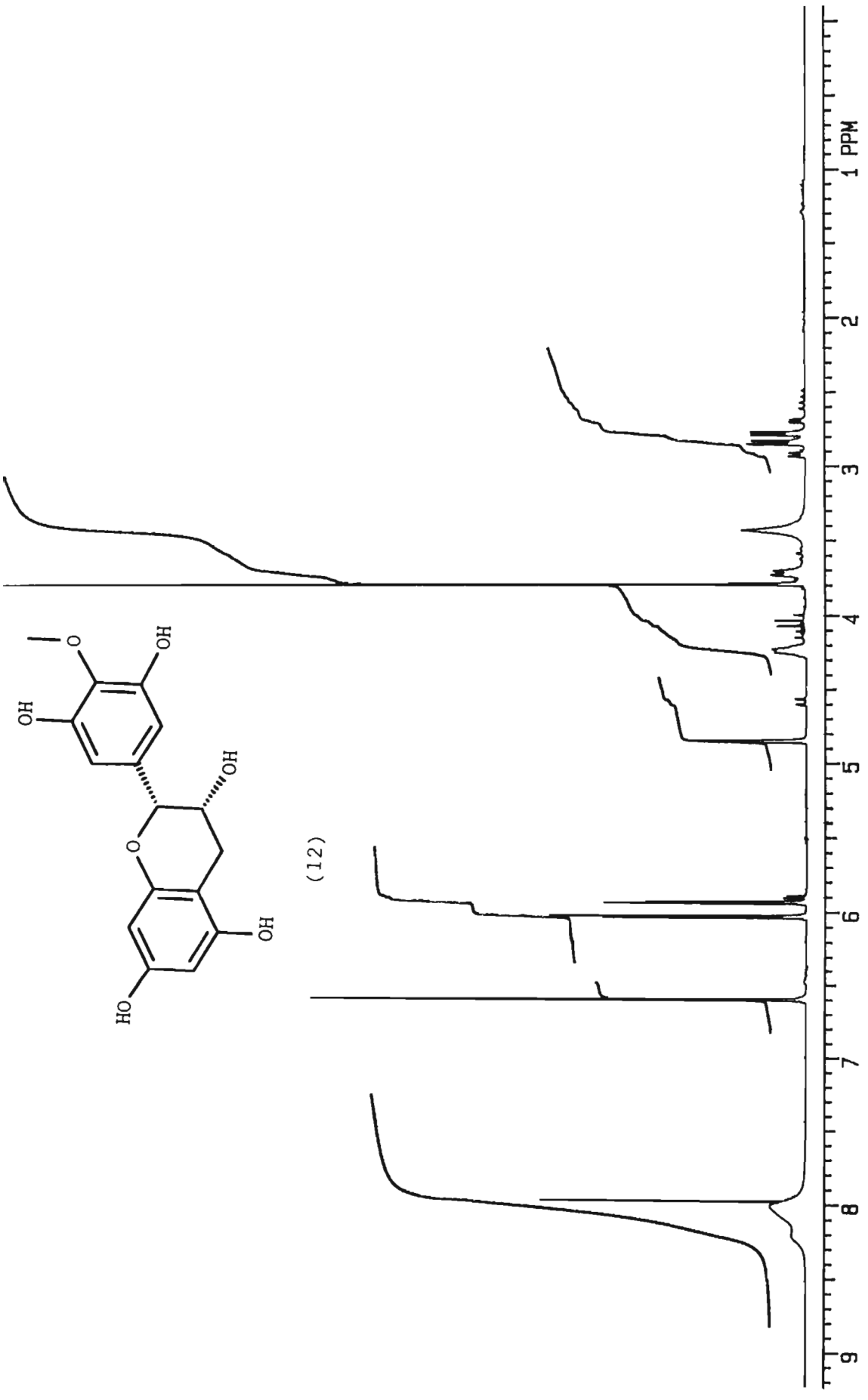

OH
O
(80)
1 PPM
2
3
4
5
6
7
8
9

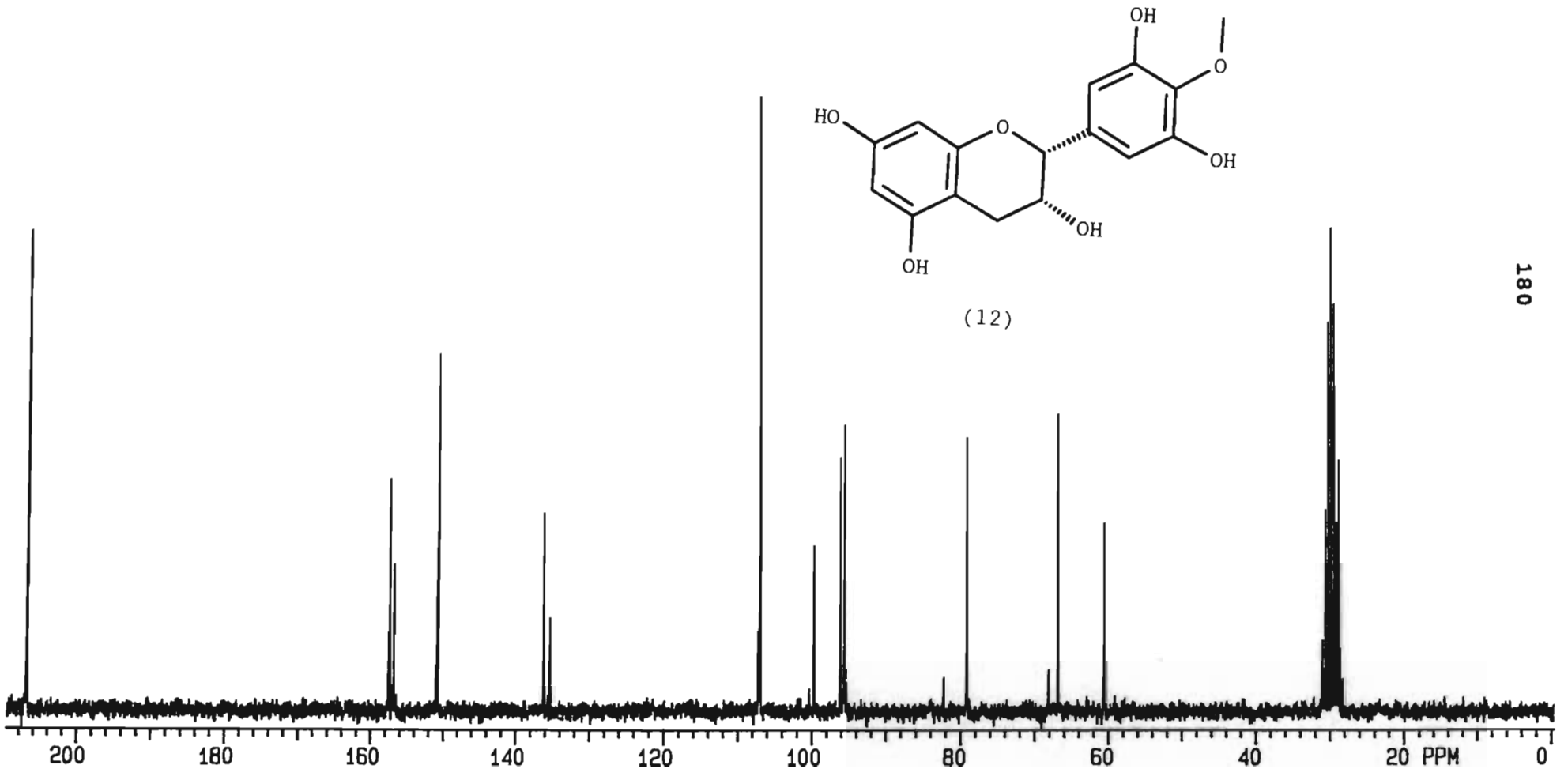


(80)

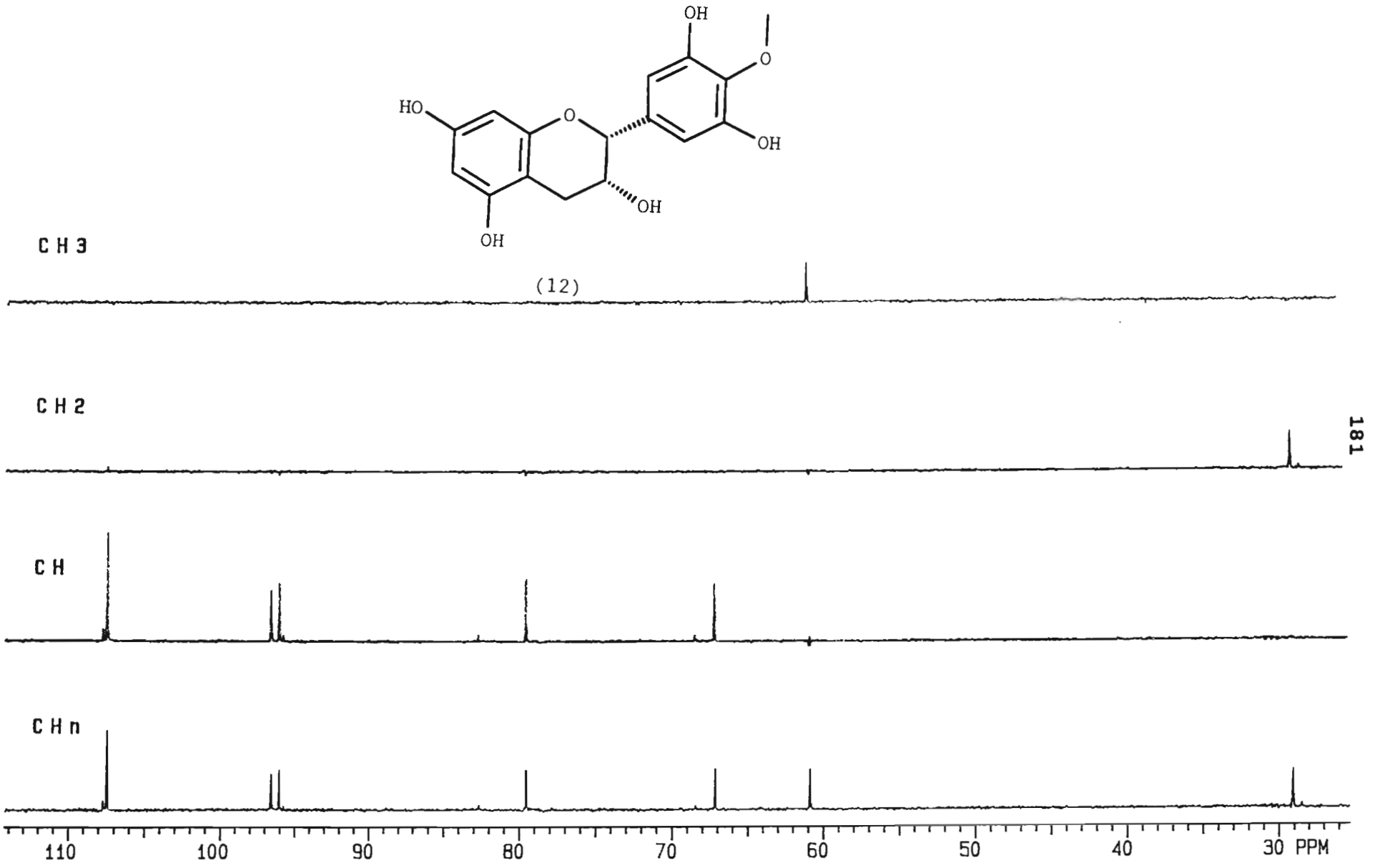


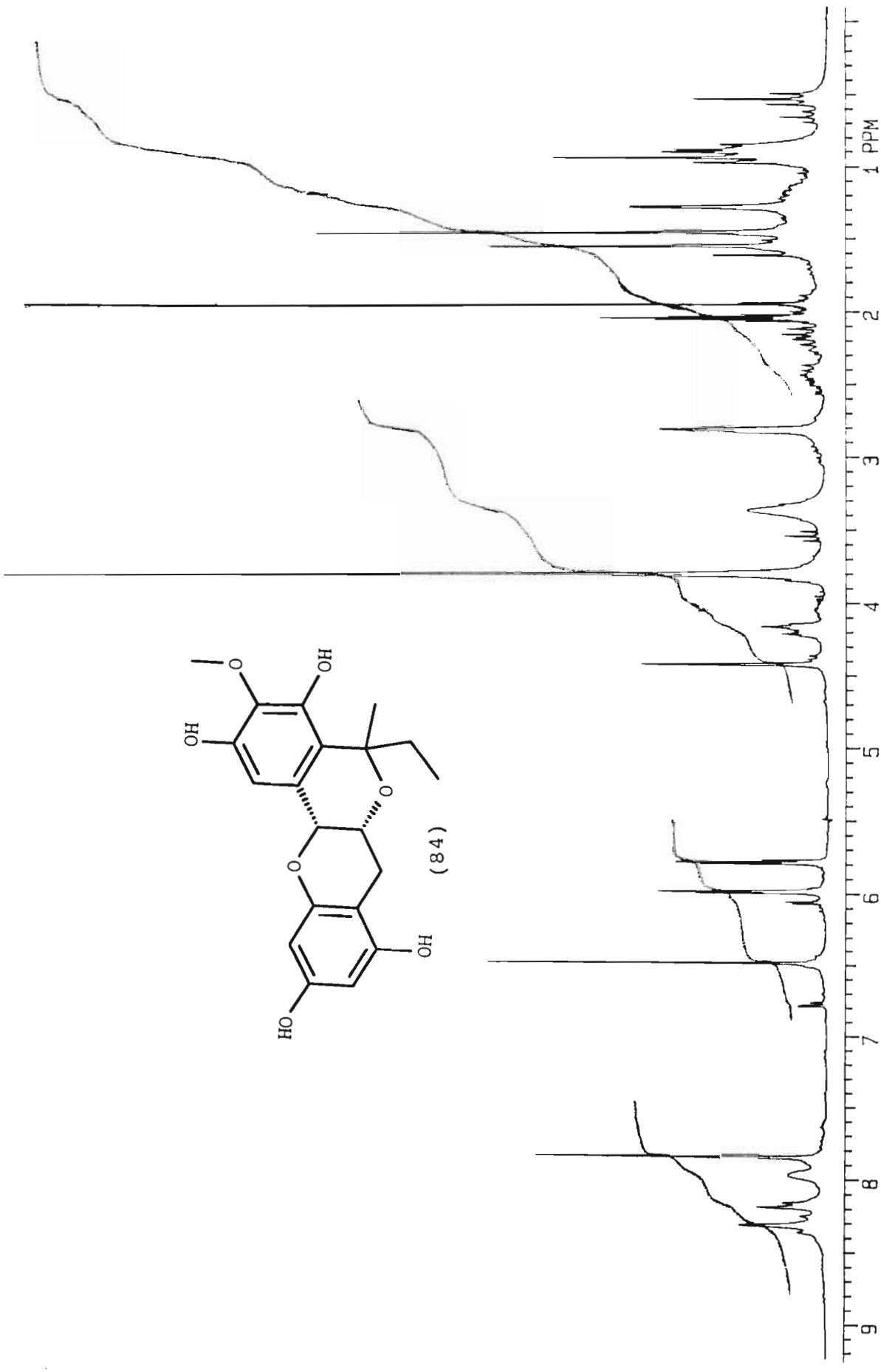

OH
O
(80)
CH3
CH2
CH
CH(all)
70
60
50
40
30
20
10 PPM
0


OH
O
OH
OH
O
OH
HO
(12)
9
8
7
6
5
4
3
2
1 PPM

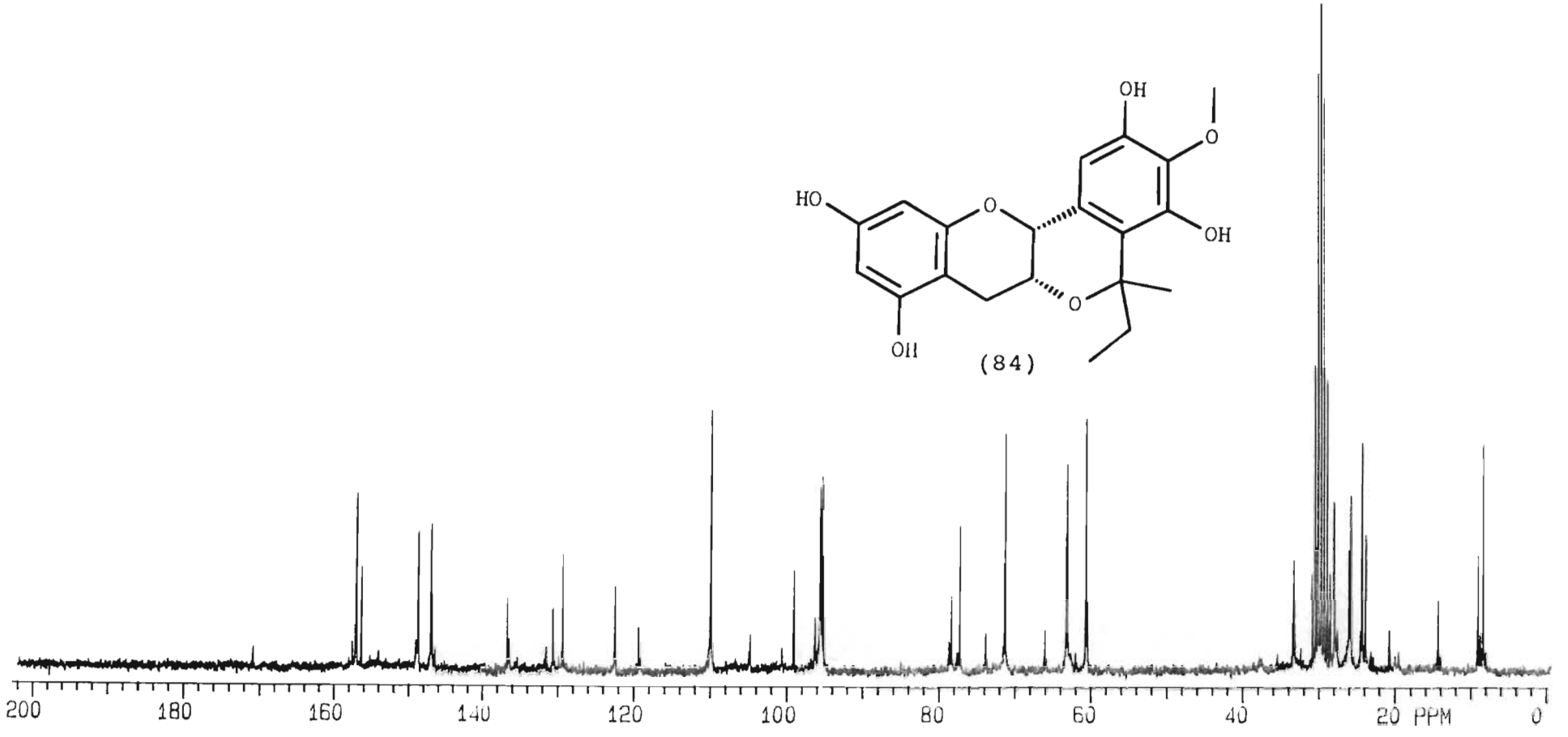


(12)

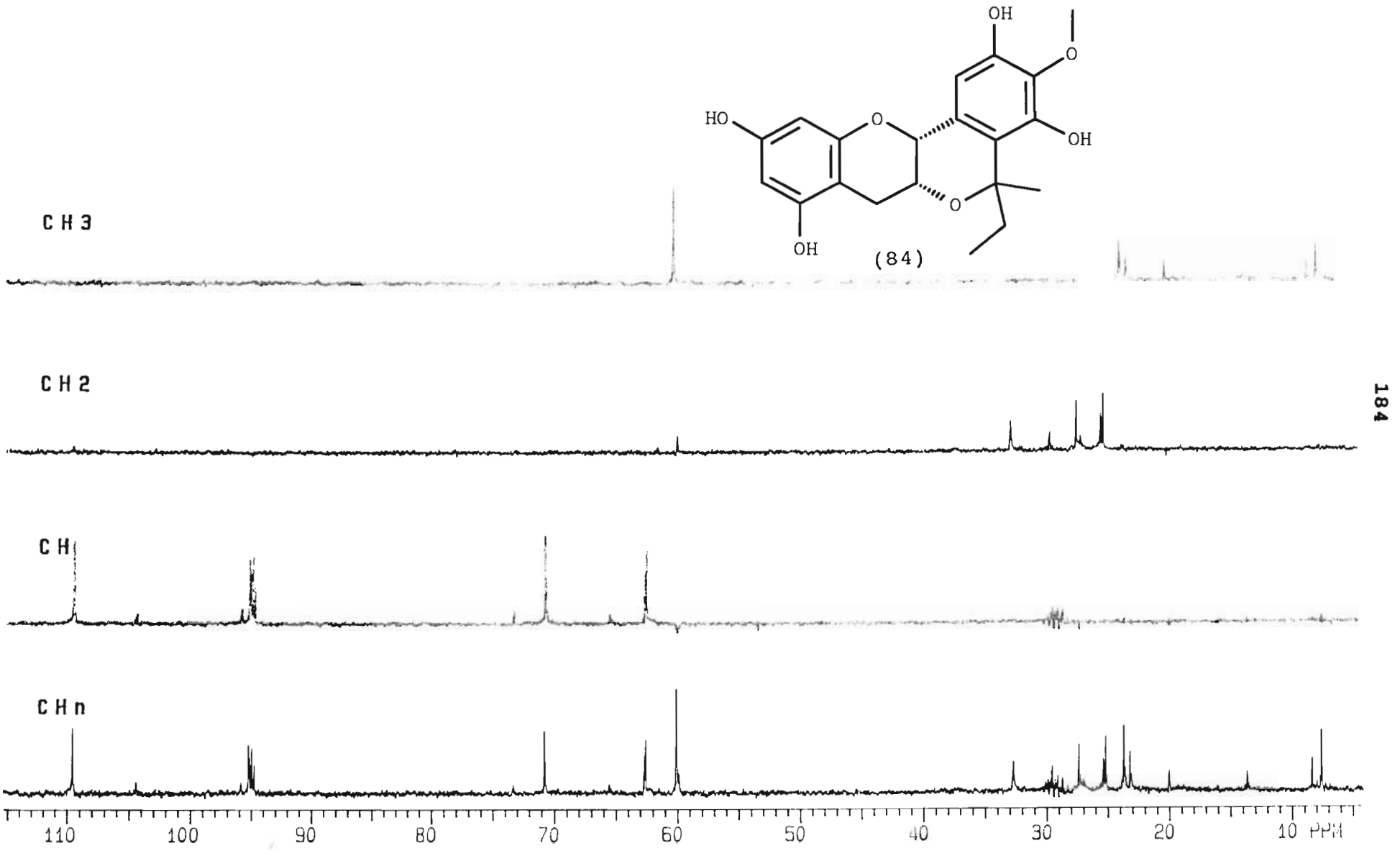

OH
O
HO
O
OH
OH
OH
CH3
(12)
CH2
181
CH
CHn
110
100
90
80
70
60
50
40
30 PPM

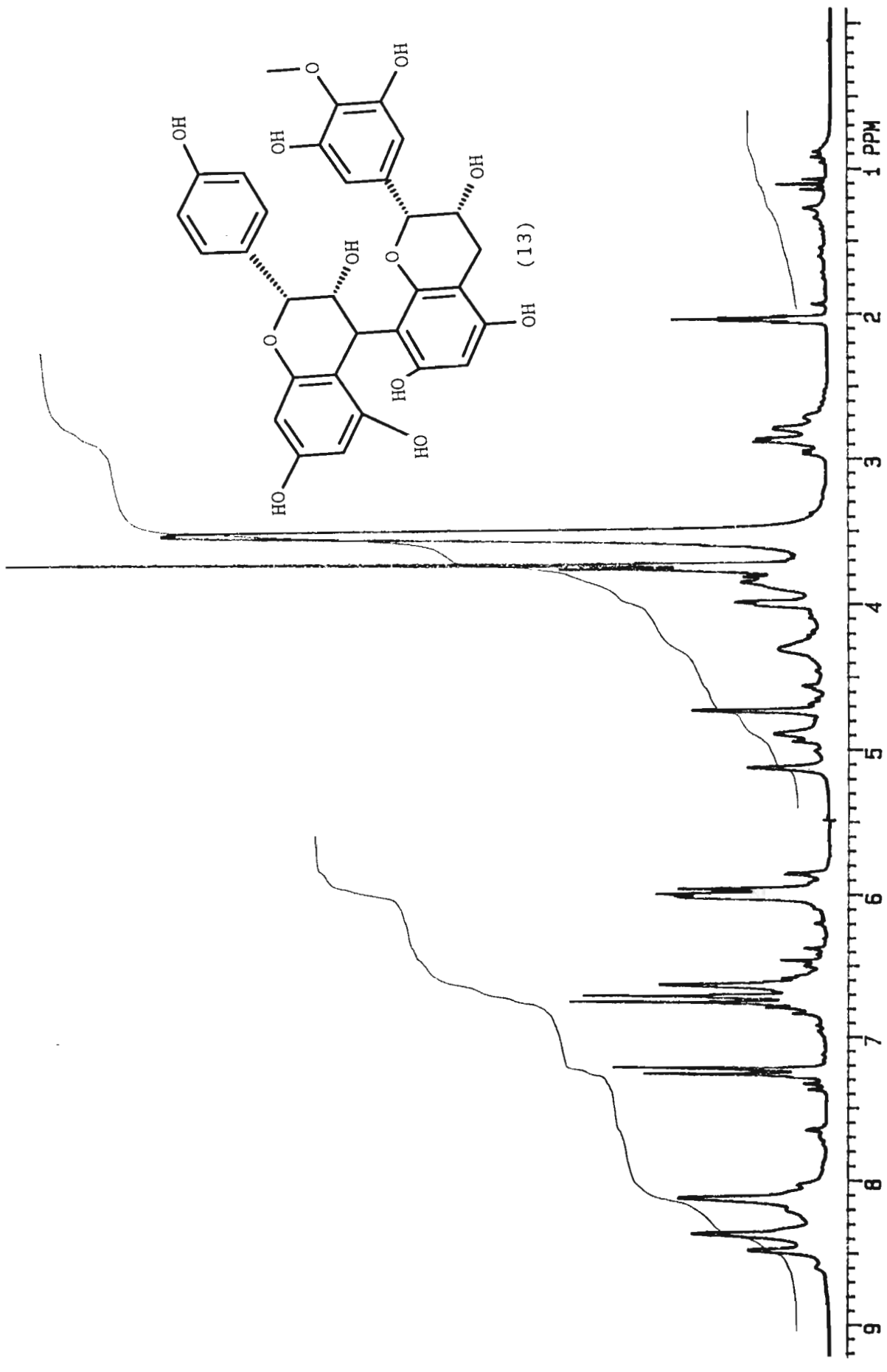


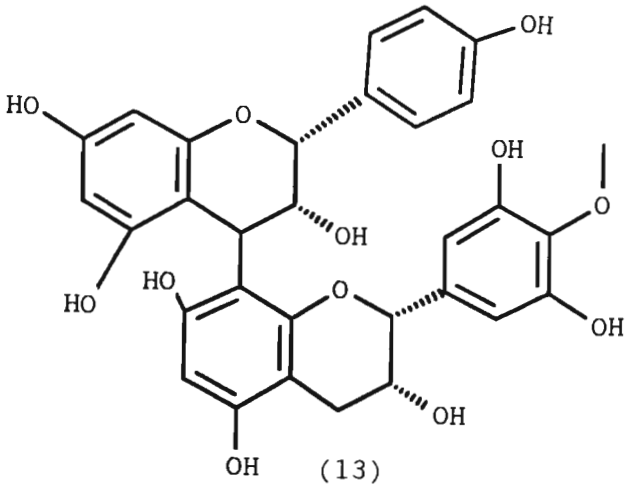
(84)



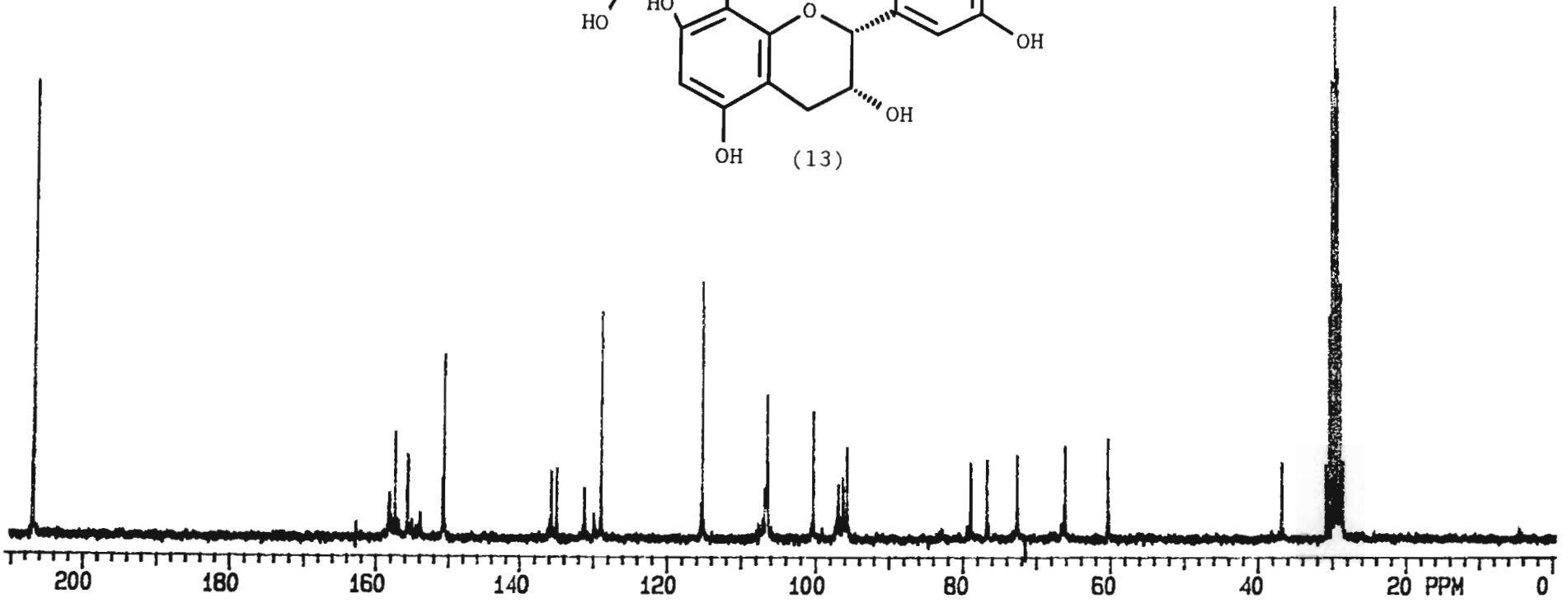
(84)

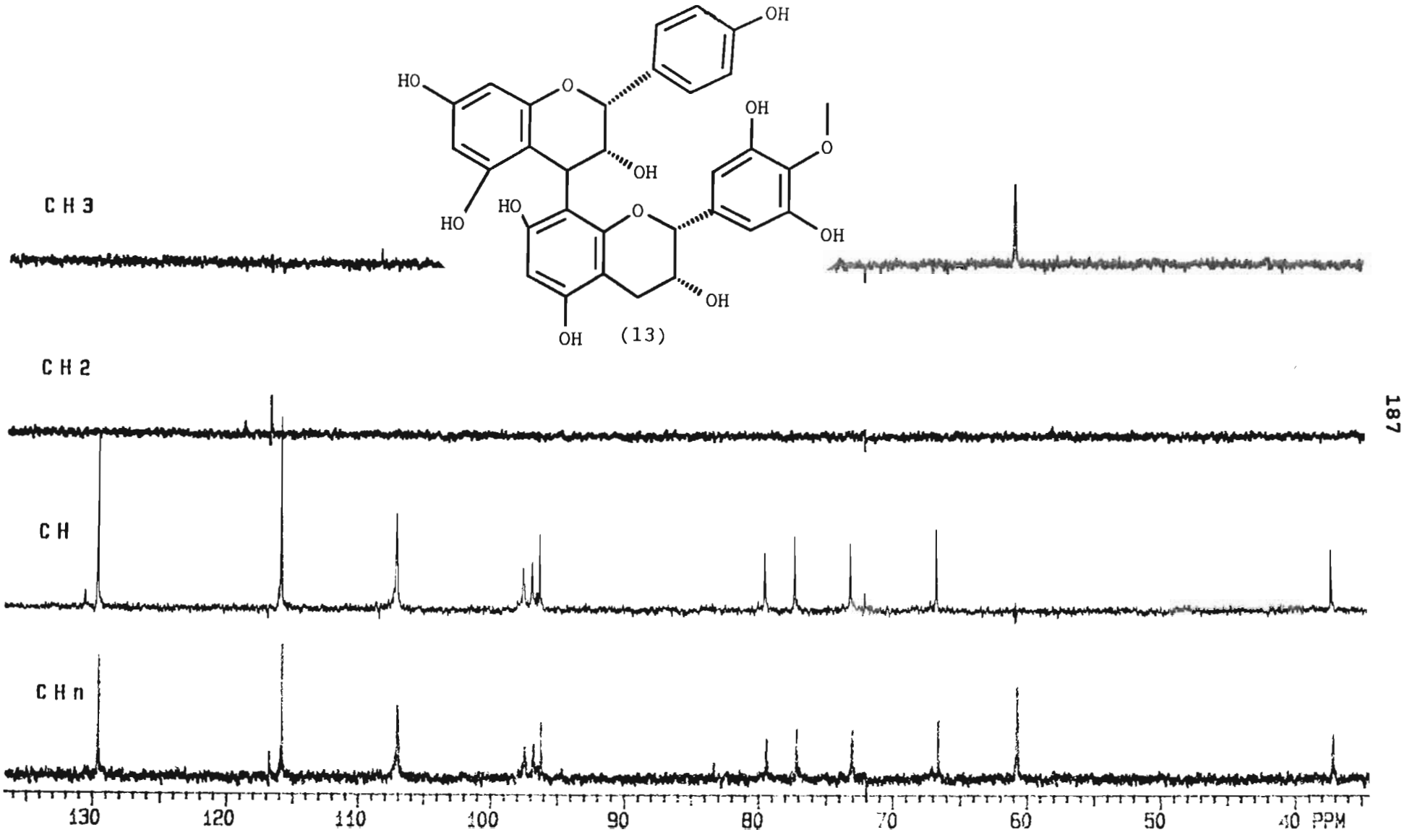

OH
O
HO
O
OH
O
OH
(84)
CH3
CH2
CH
CHn
110
100
90
80
70
60
50
40
30
20
10 PPM


OH
OH
O
OH
OH
OH
O
OH
HO
HO
OH
(13)
9
8
7
6
5
4
3
2
1 PPM

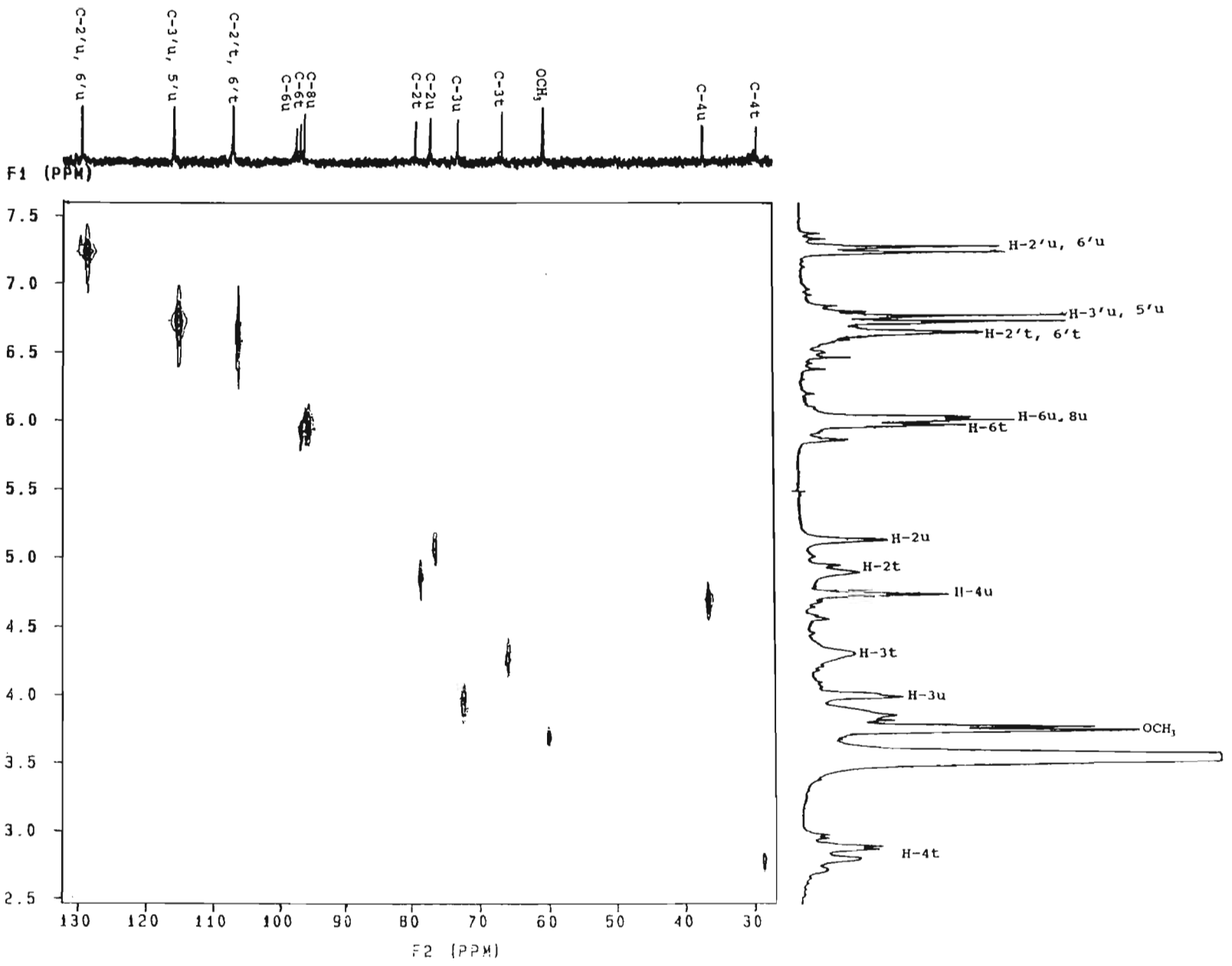

HO
O
OH
OH
HO
HO
O
OH
O
OH
OH
OH

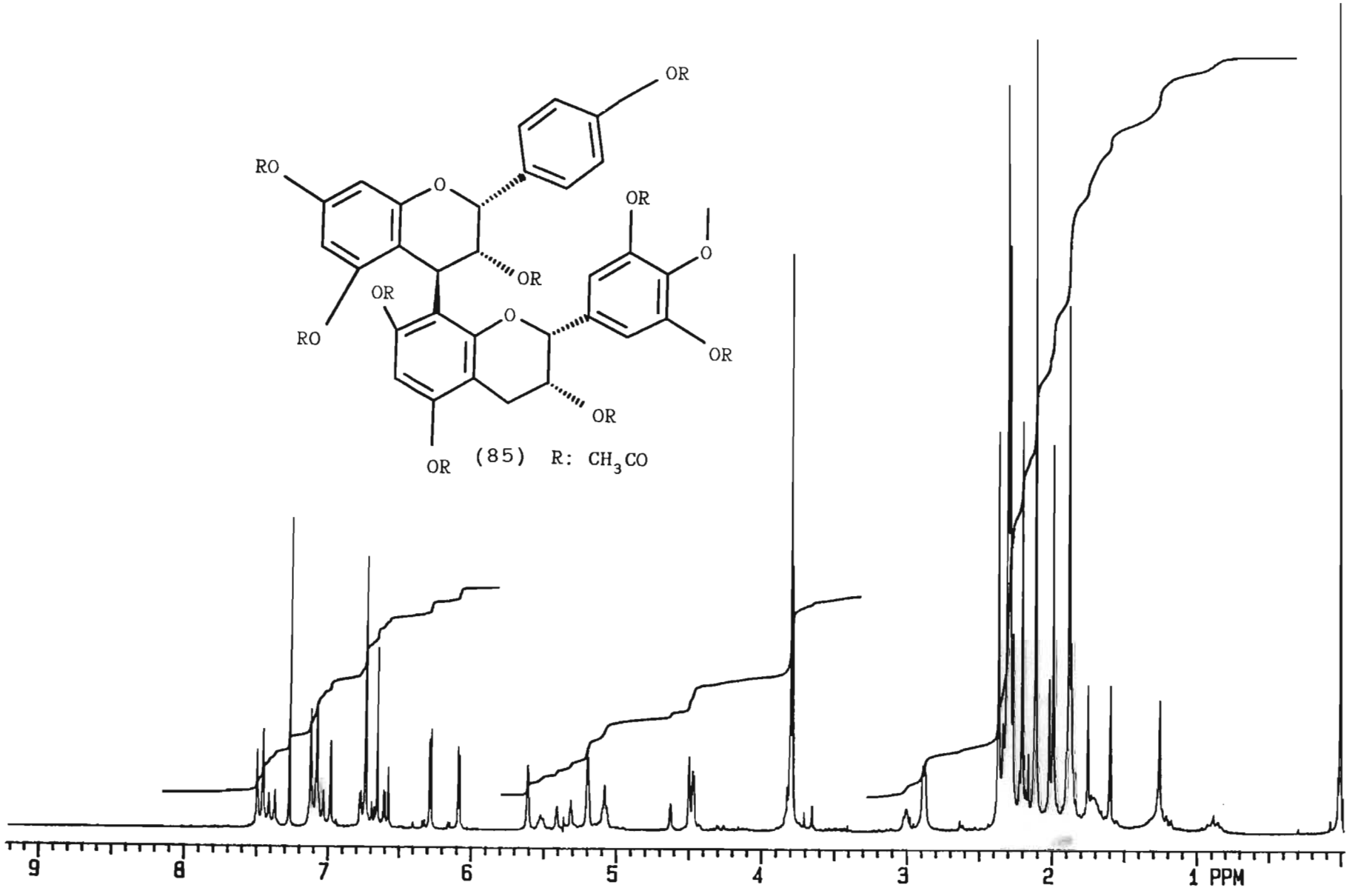
(13)


200
180
160
140
120
100
80
60
40
20 PPM
0

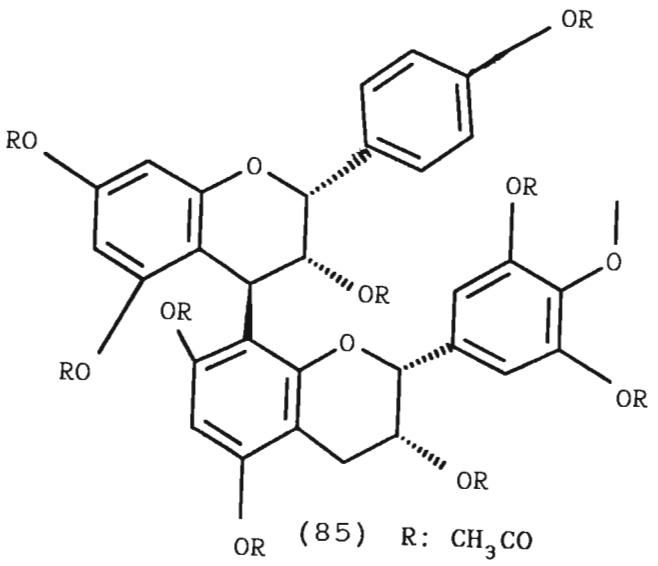


(13)

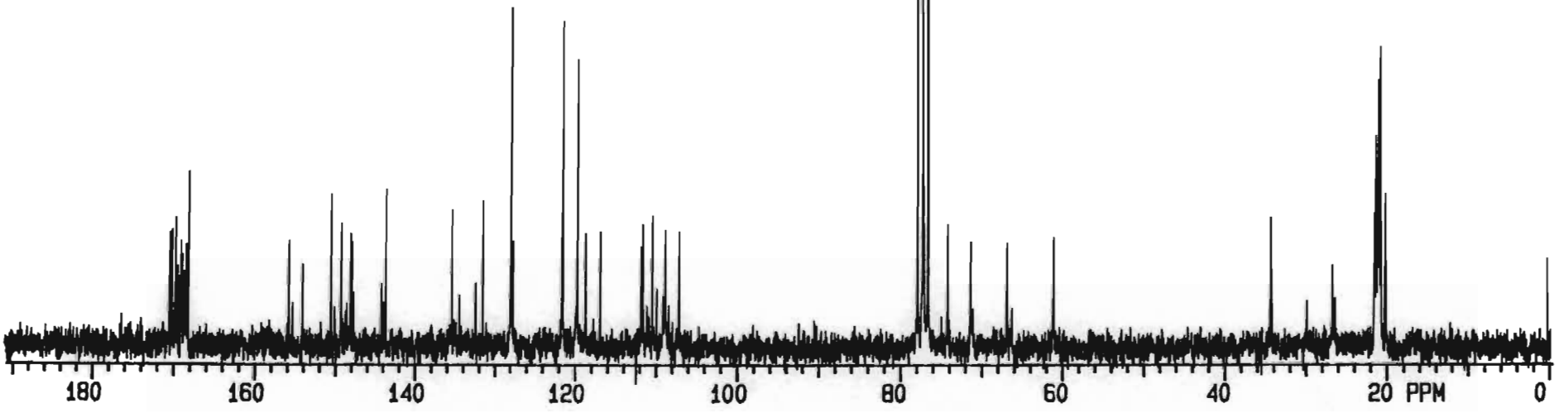

C-2'u, 6'u
C-3'u, 5'u
C-2't, 6't
C-6u
C-6t
C-8u
C-2t
C-2u
C-3u
C-3t
OCH3
C-4u
C-4t
F1 (PPM)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
130
120
110
100
90
80
70
60
50
40
30
F2 (PPM)
H-2'u, 6'u
H-3'u, 5'u
H-2't, 6't
H-6u, 8u
H-6t
H-2u
H-2t
H-4u
H-3t
H-3u
OCH3
H-4t

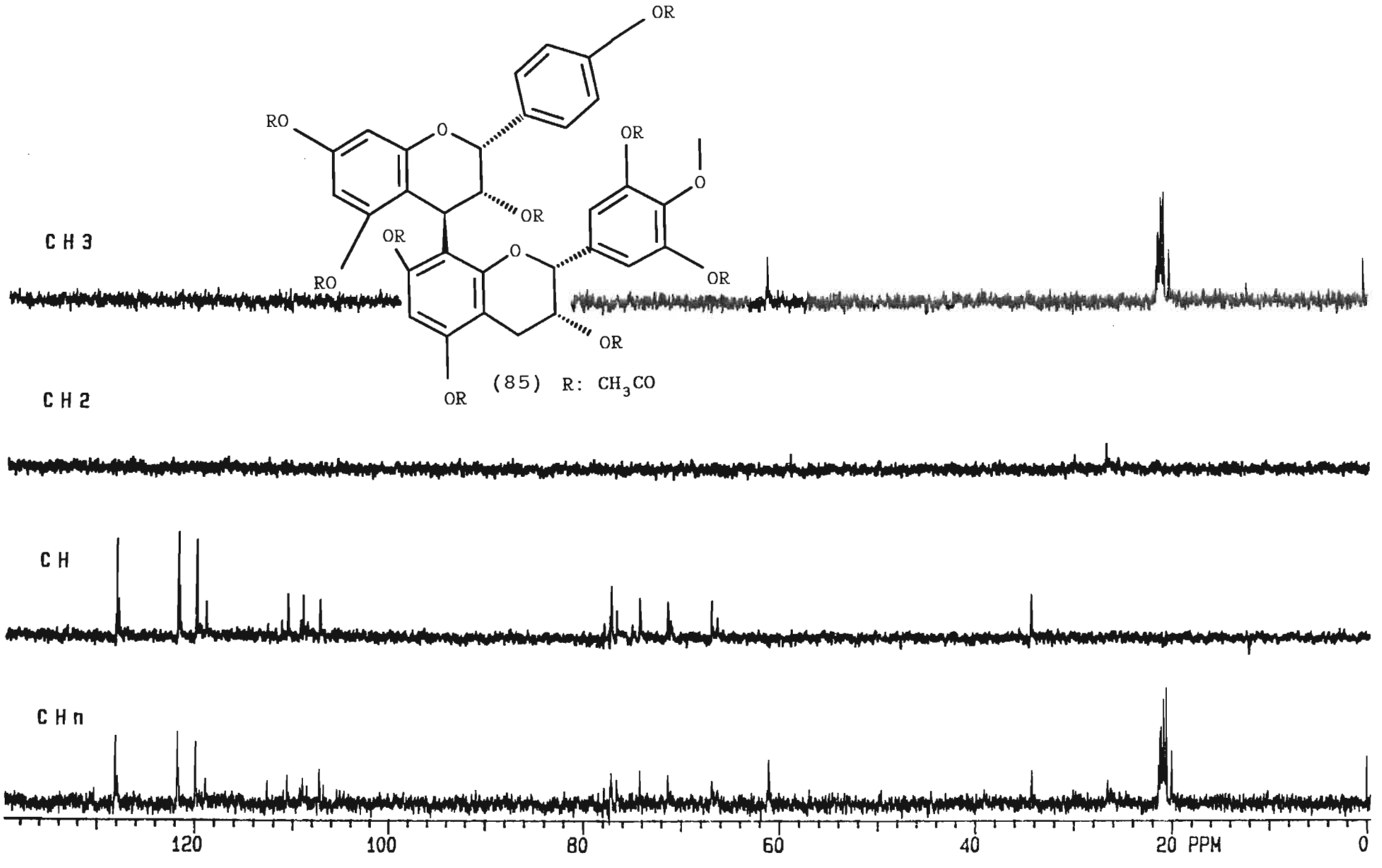


(85) R: CH_3CO

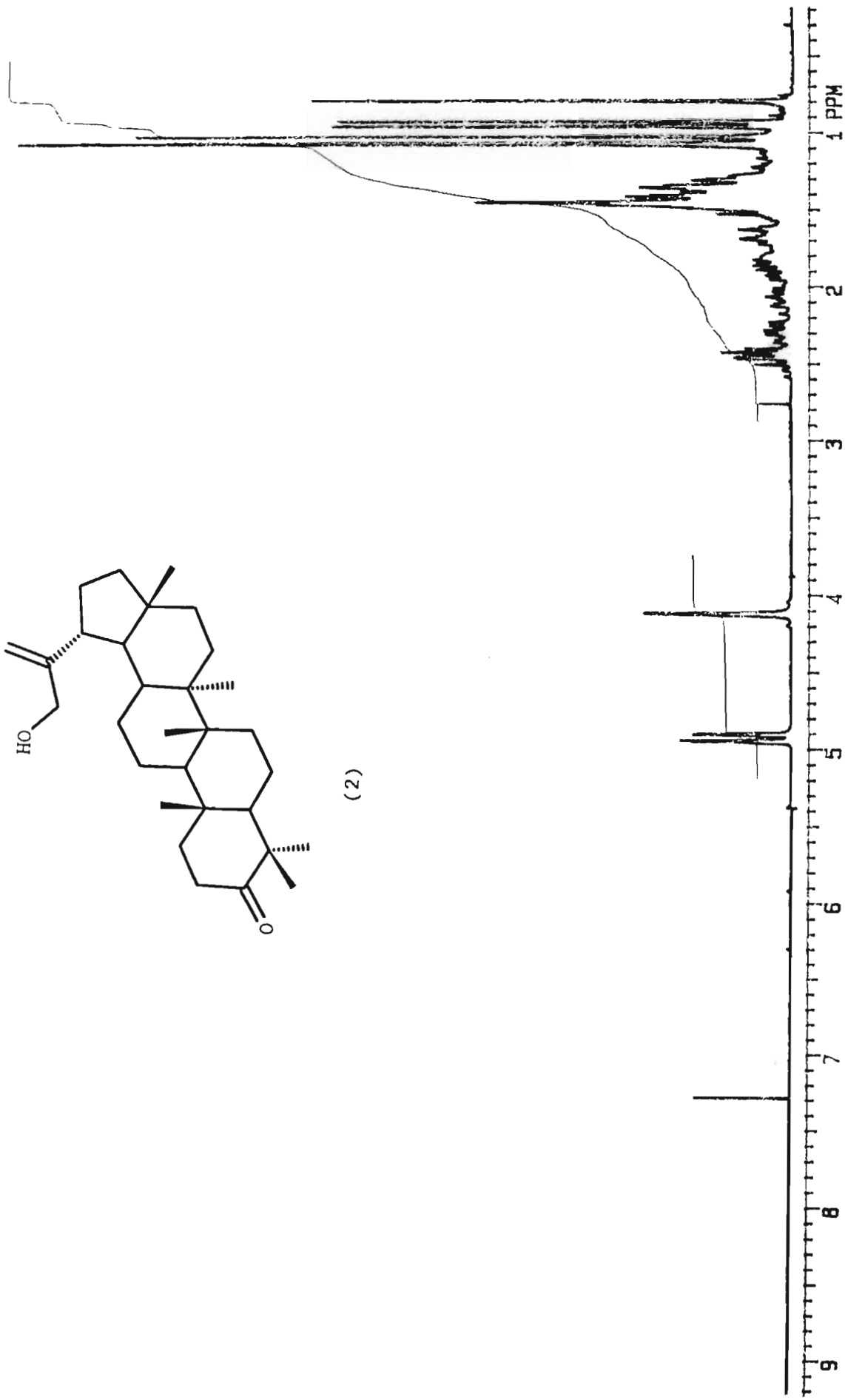

OR
RO
O
OR
OR
O
OR
RO
OR
O
OR
OR
OR

(85) R: CH_3CO

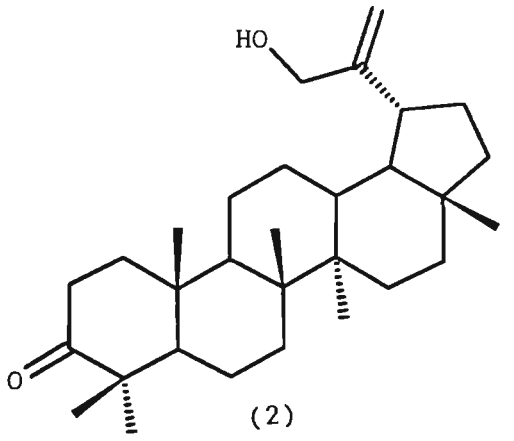

180
160
140
120
100
80
60
40
20 PPM
0


OR
RO
O
OR
OR
O
OR
OR
O
RO
OR
OR
OR
CH3
CH2
CH
CHn
120
100
80
60
40
20 PPM
0

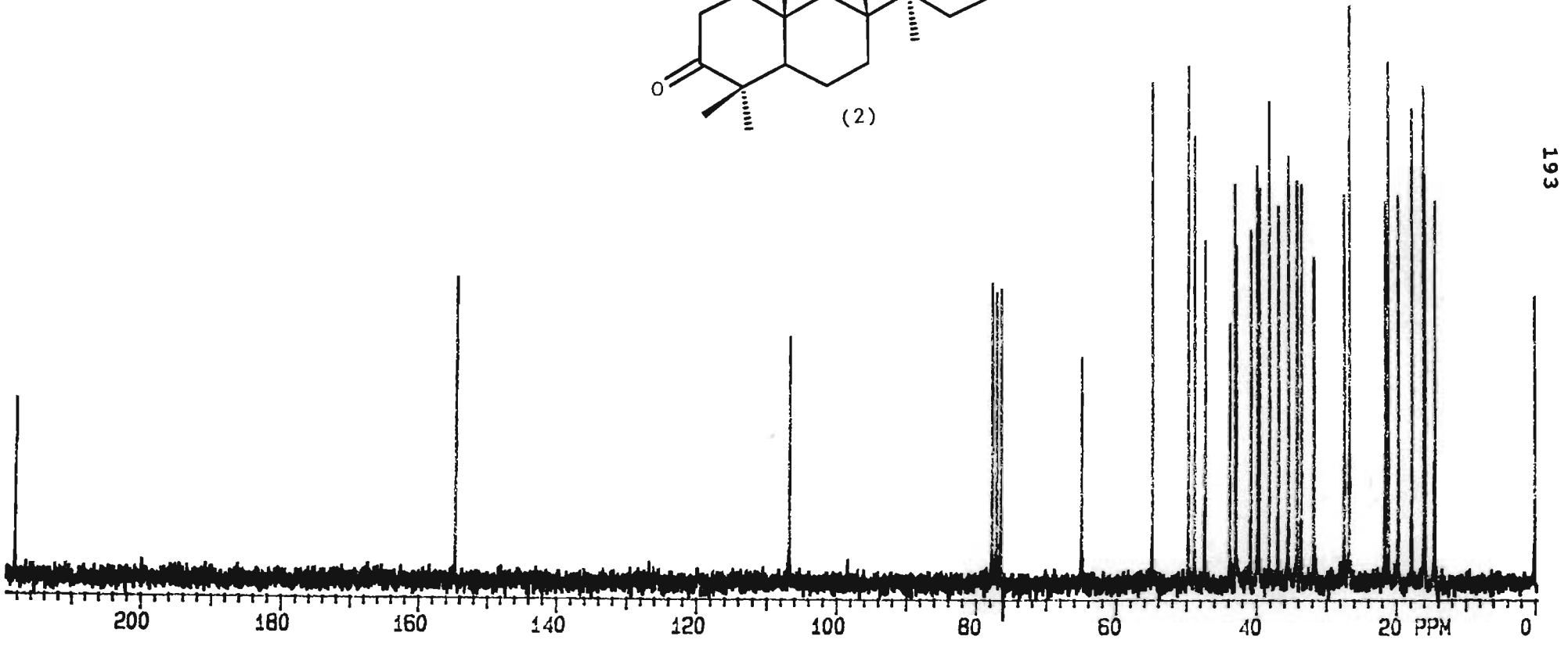
(85) R: CH_3CO

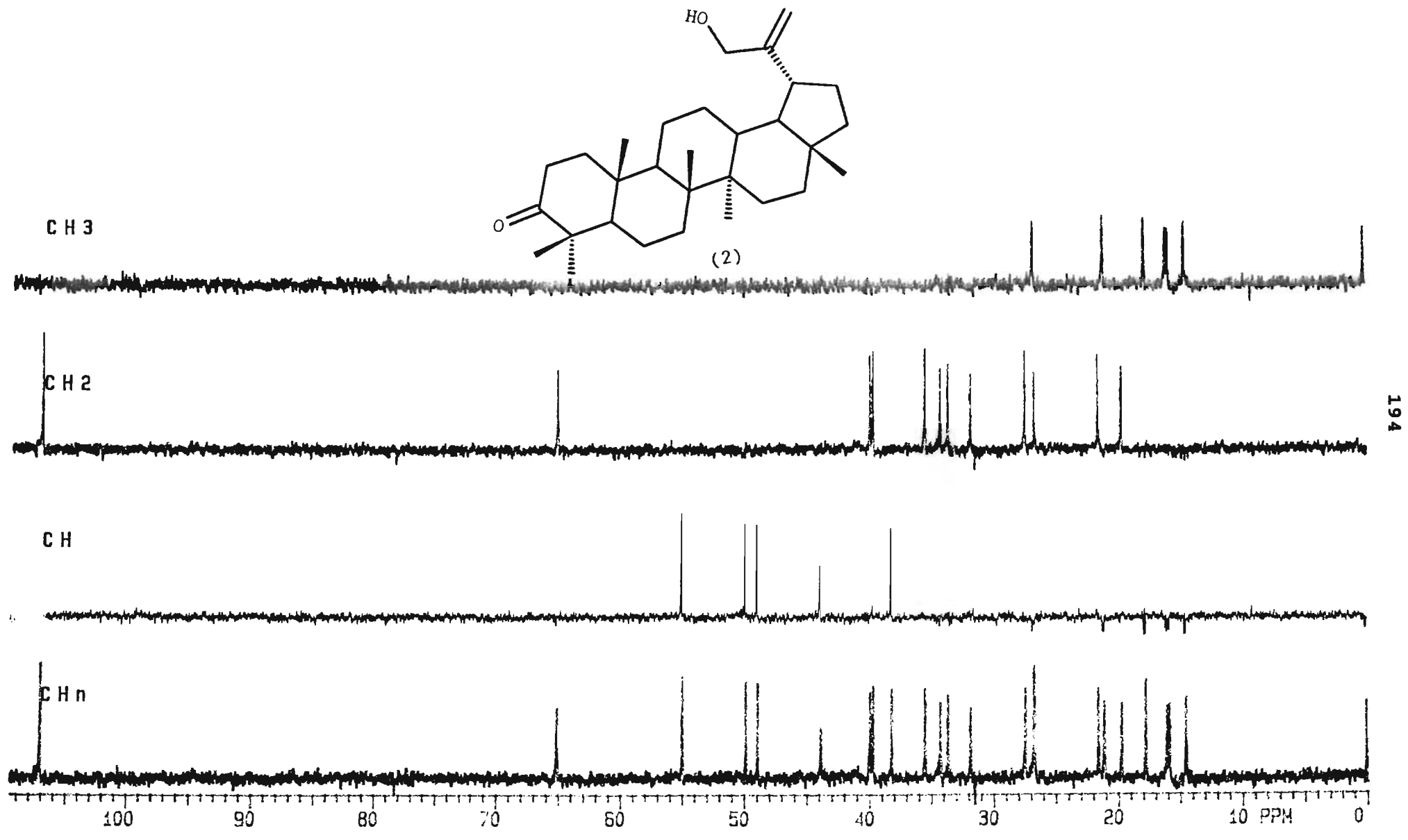


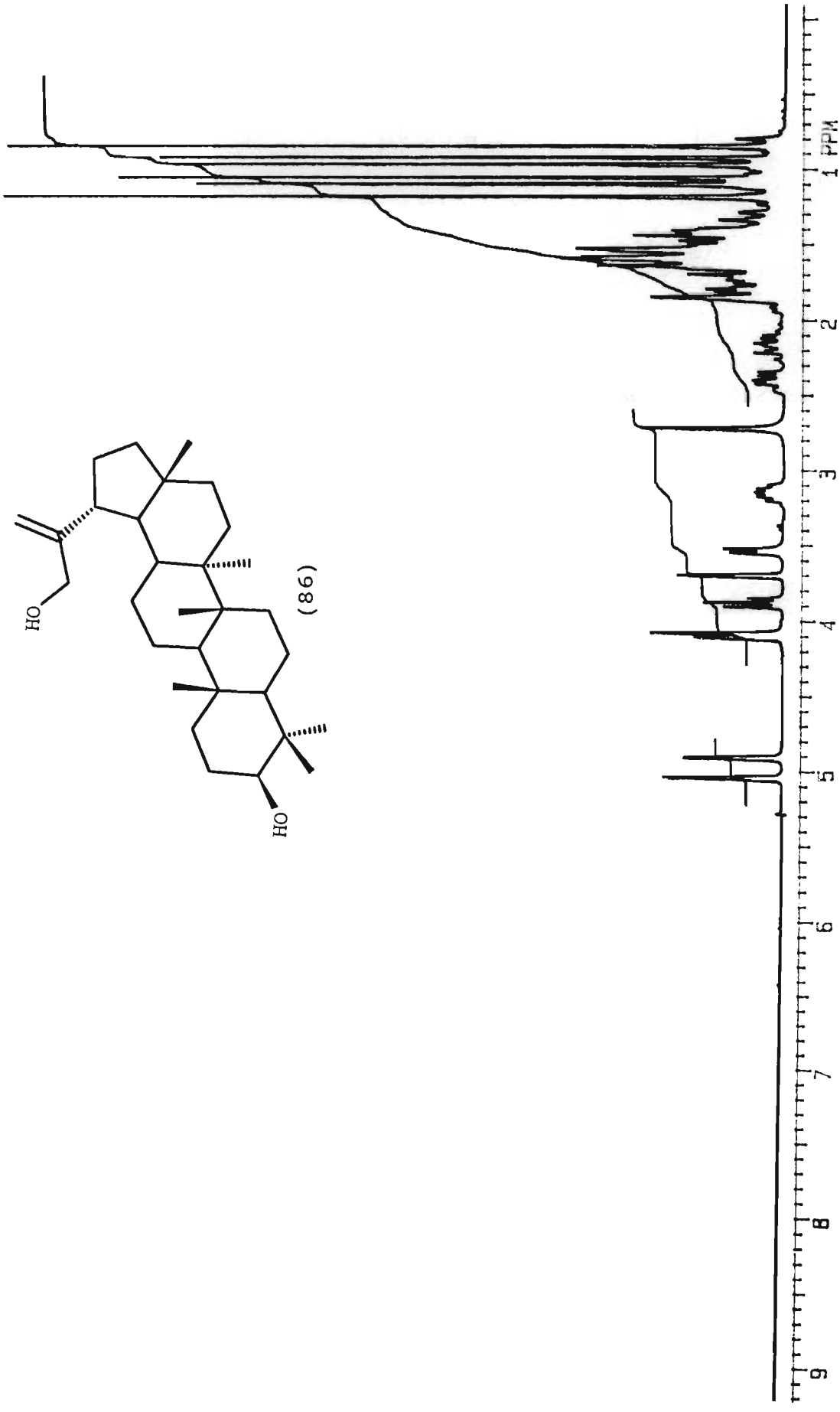
(2)

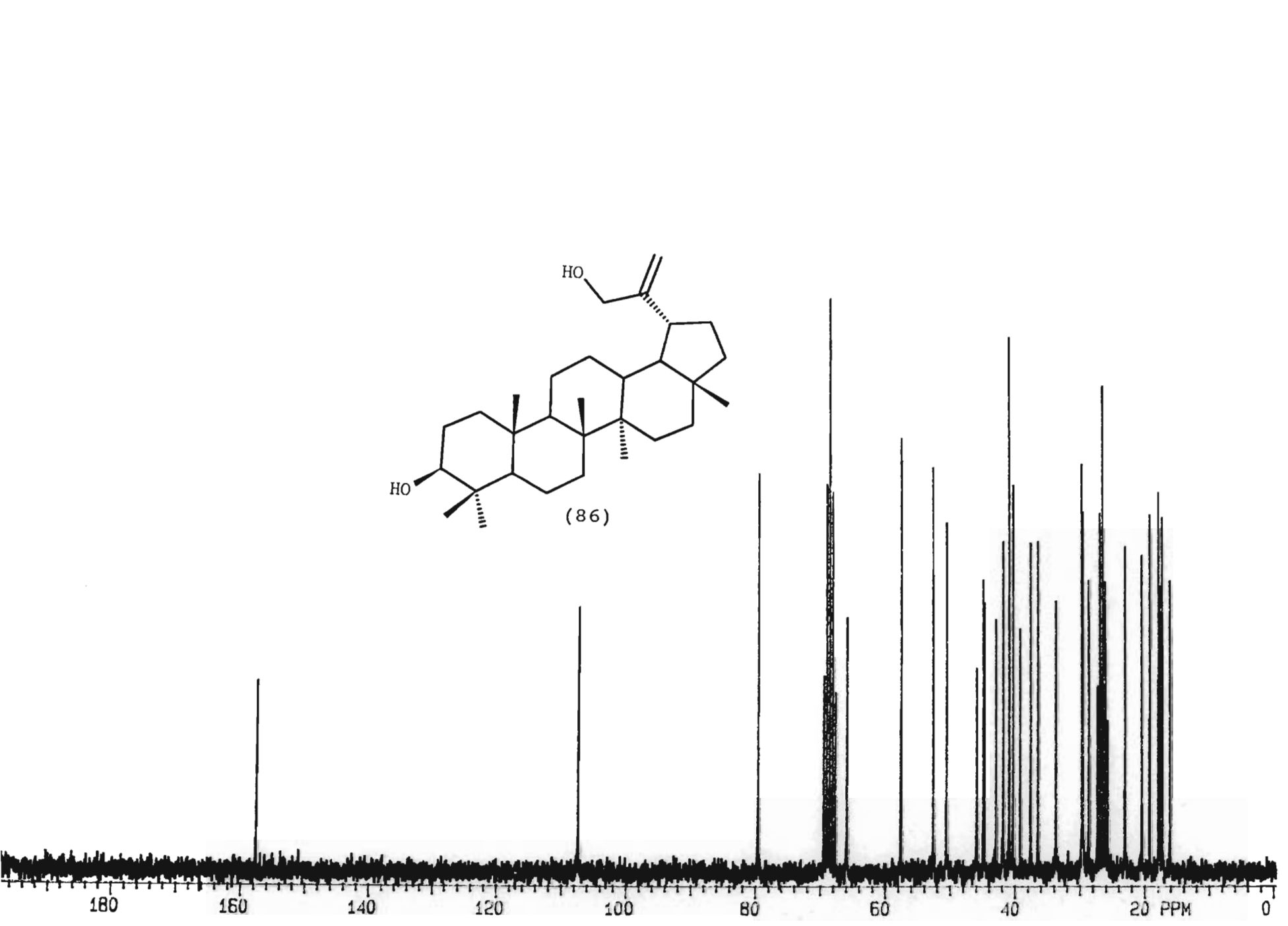


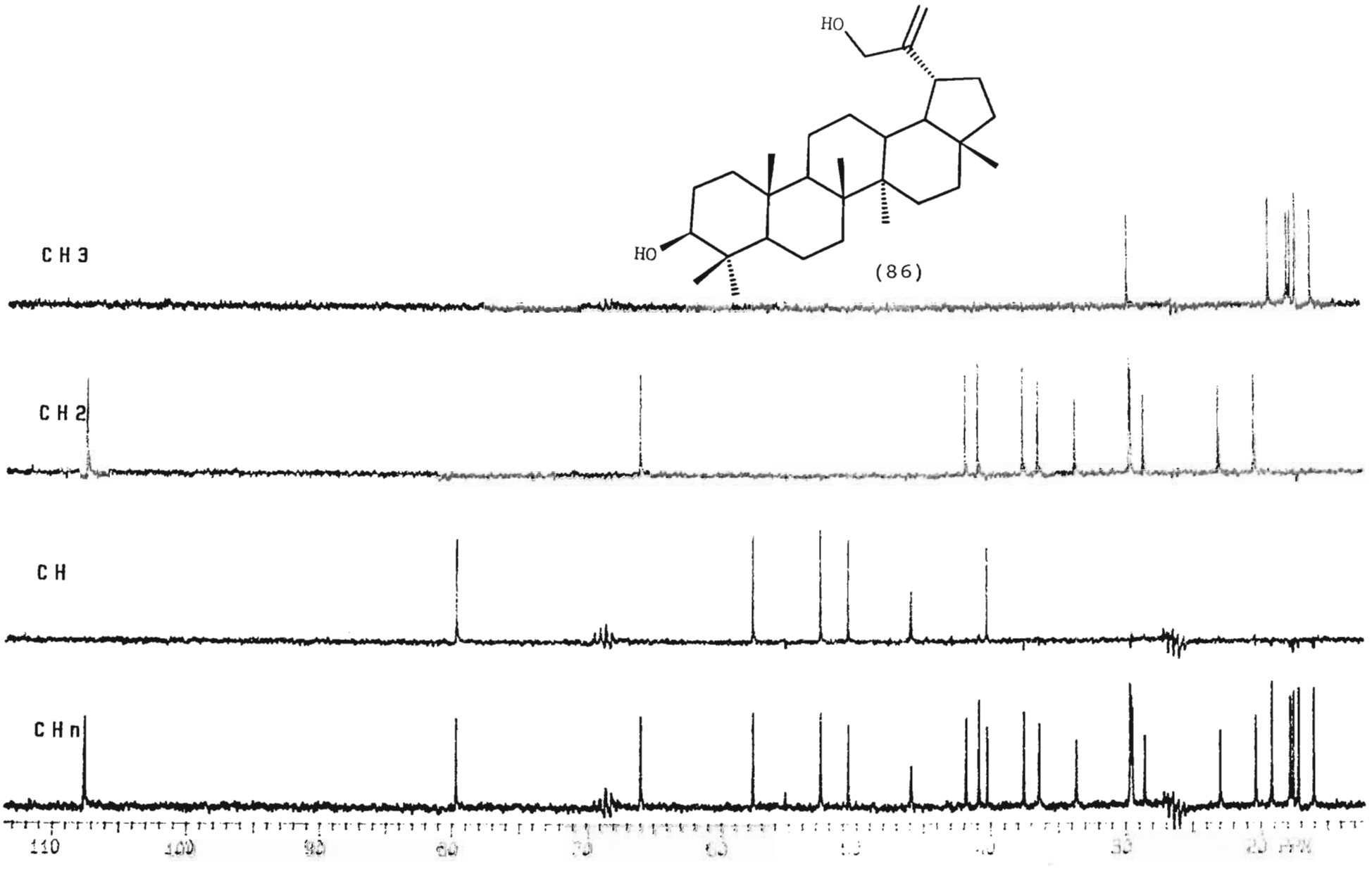
(2)

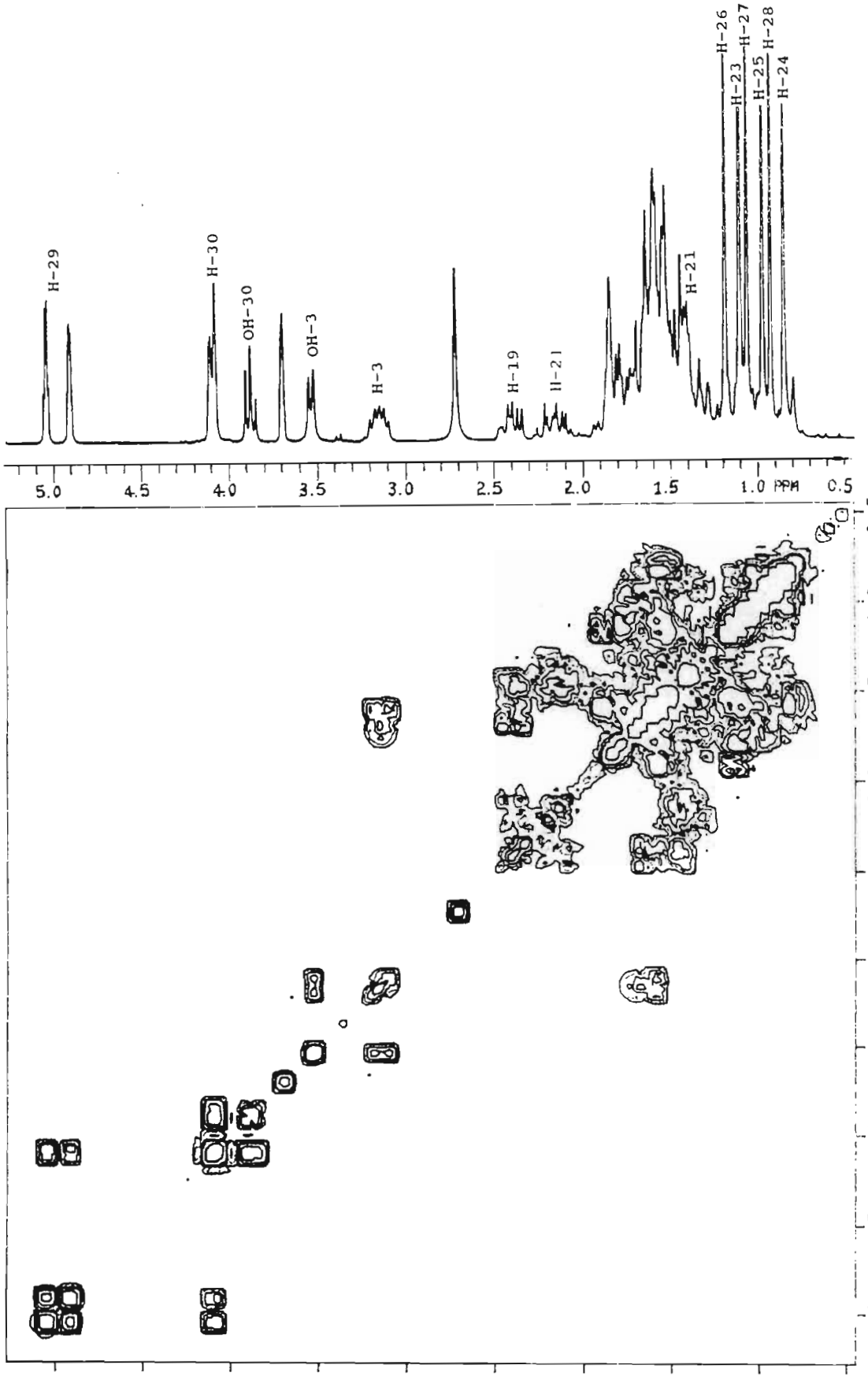


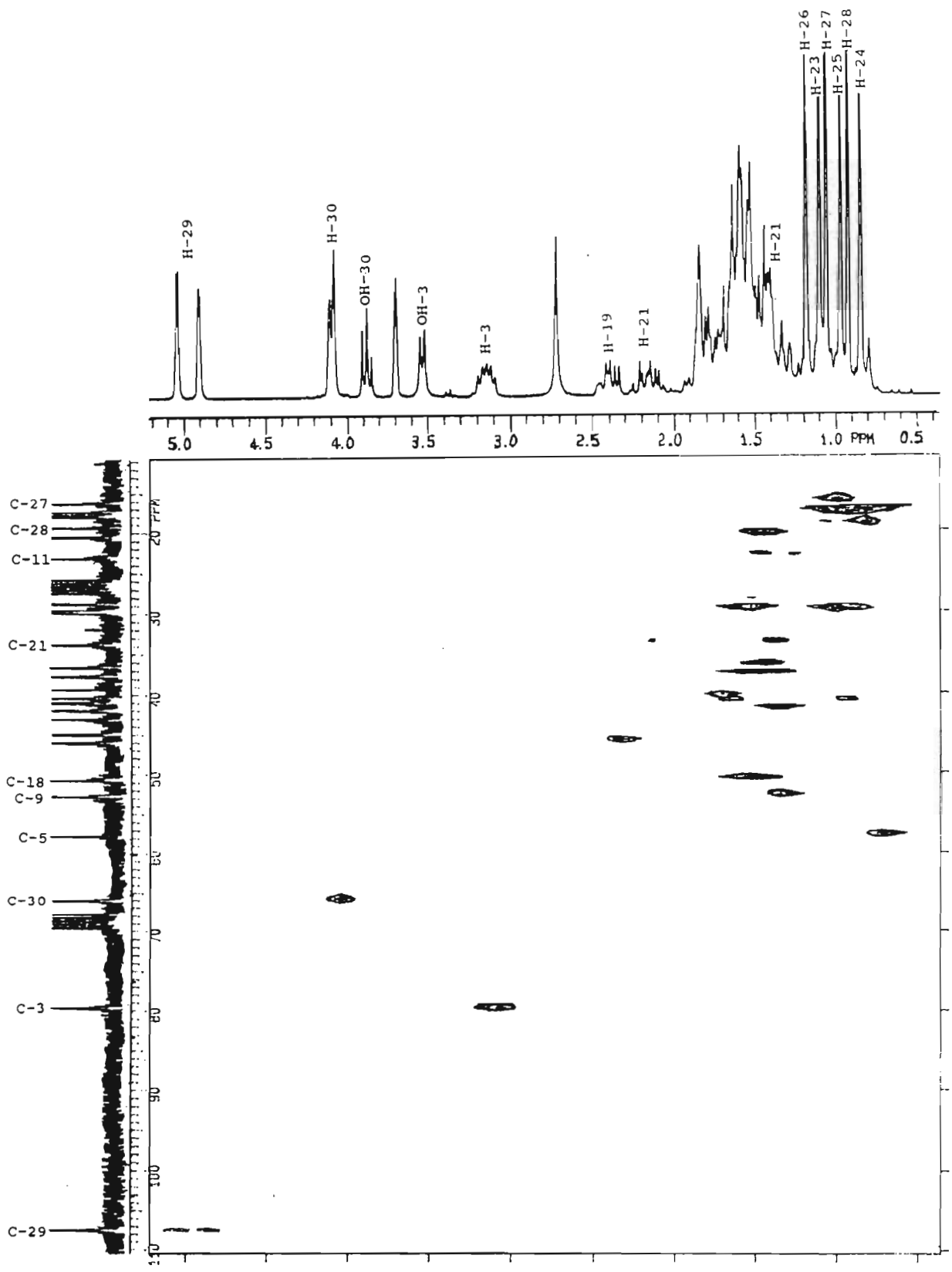

HO
O
(2)
CH3
CH2
CH
CHn
100
90
80
70
60
50
40
30
20
10 PPM
0

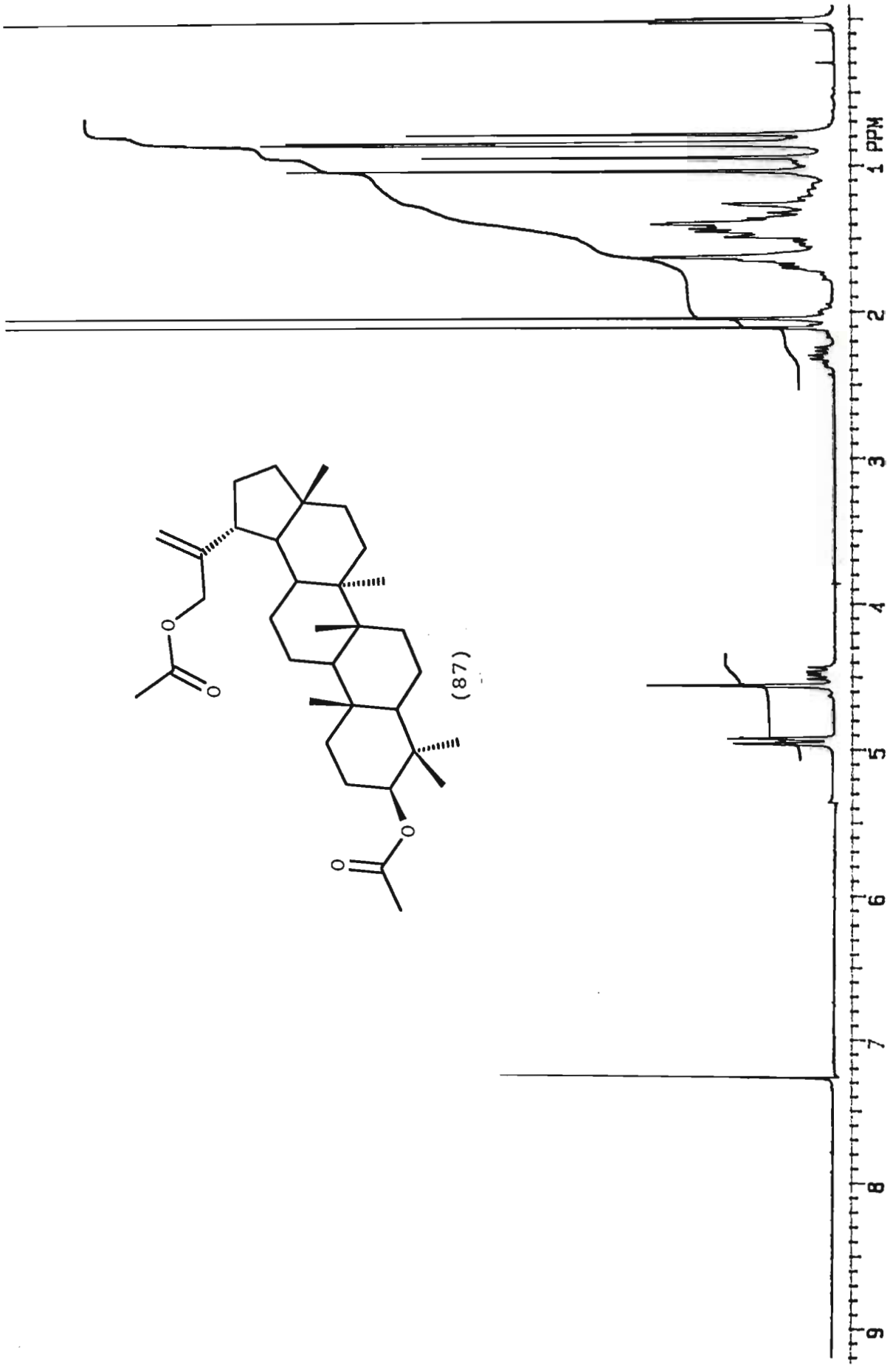

HO
HO
(86)
1 PPM
2
3
4
5
6
7
8
9


HO
HO
(86)
180
160
140
120
100
80
60
40
20 PPM
0

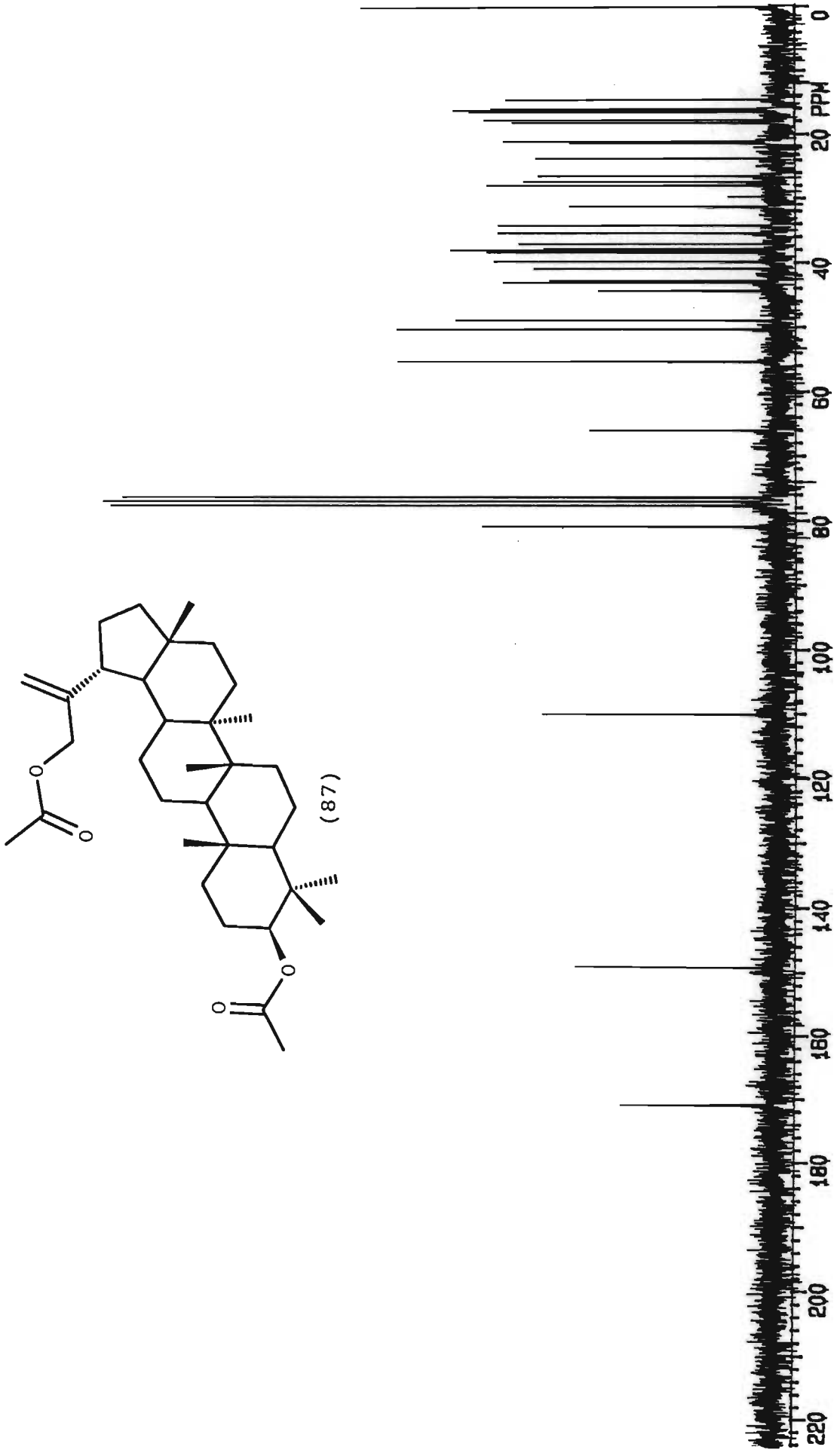

HO
HO
(86)
CH3
CH2
CH
CHn
110
100
90
80
70
60
50
40
30
20 PPM


H-29
H-30
OH-30
OH-3
H-3
H-19
H-21
H-21
H-26
H-23
H-27
H-25
H-28
H-24
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0 PPM
0.5
F2 (PPM)

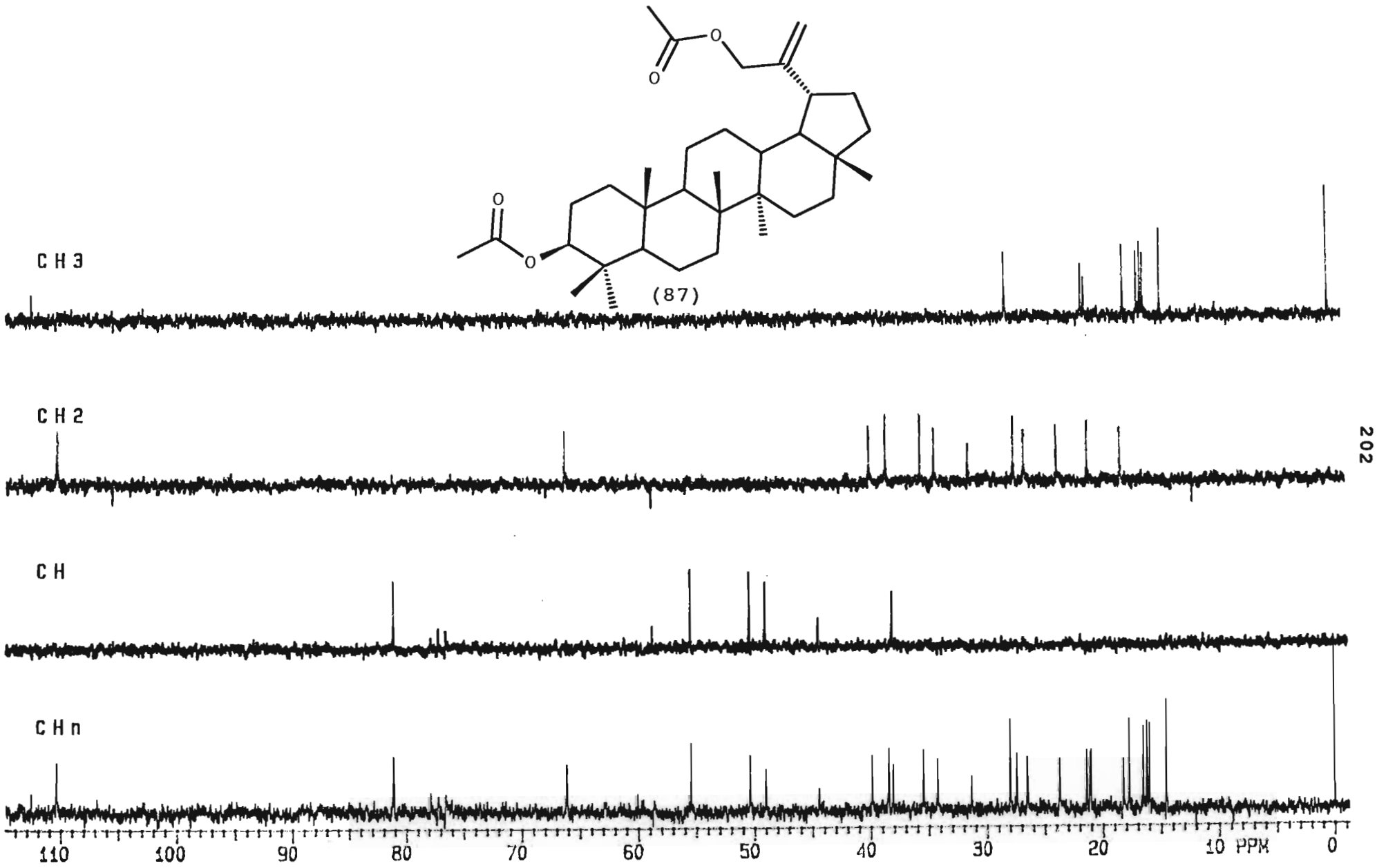

H-29
H-30
OH-30
OH-3
H-3
H-19
H-21
H-21
H-26
H-23
H-27
H-25
H-28
H-24
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0 PPM
0.5
C-27
C-28
C-11
C-21
C-18
C-9
C-5
C-30
C-3
C-29
20 PPM
30
40
50
60
70
80
90
100
110

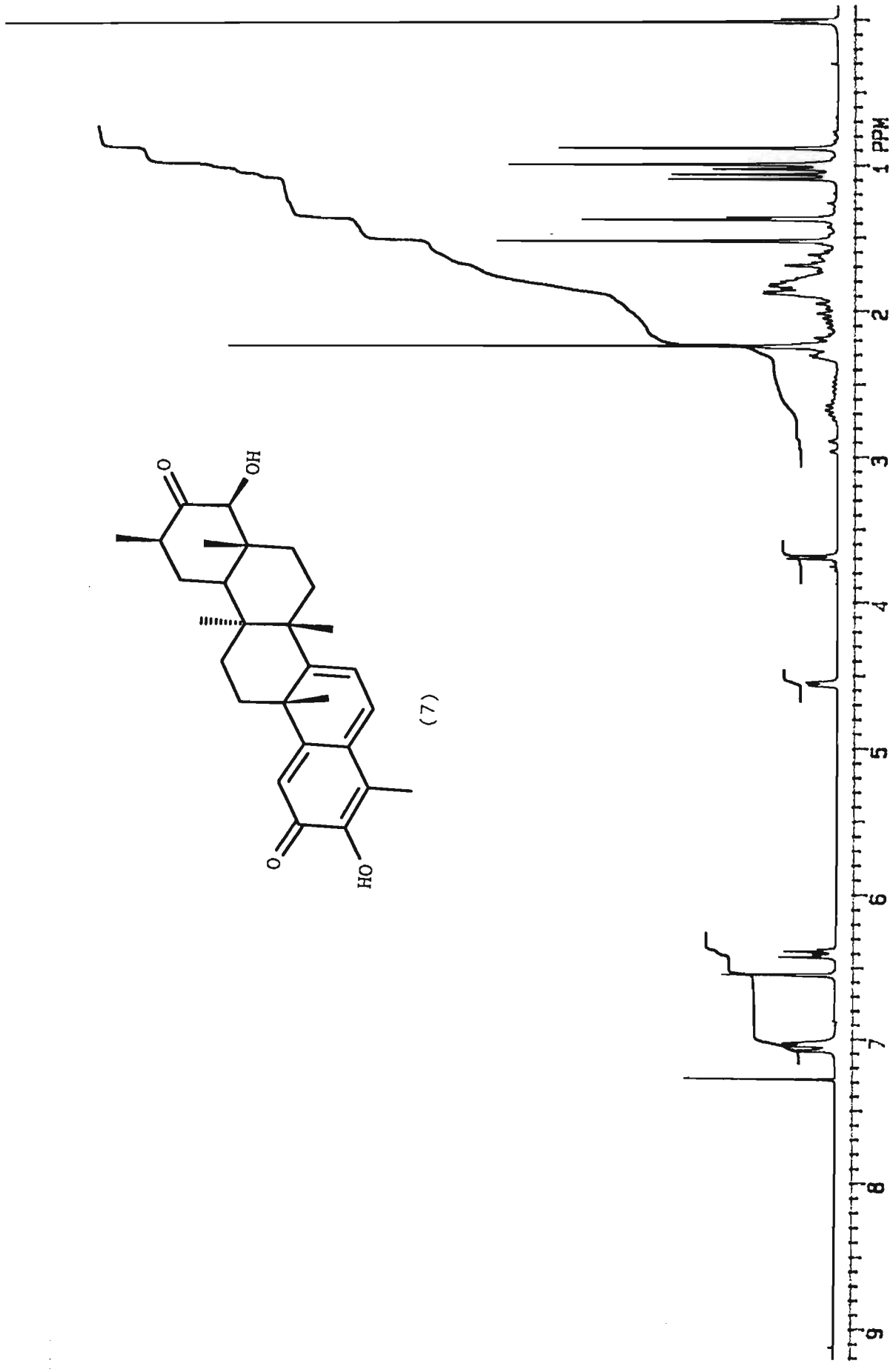


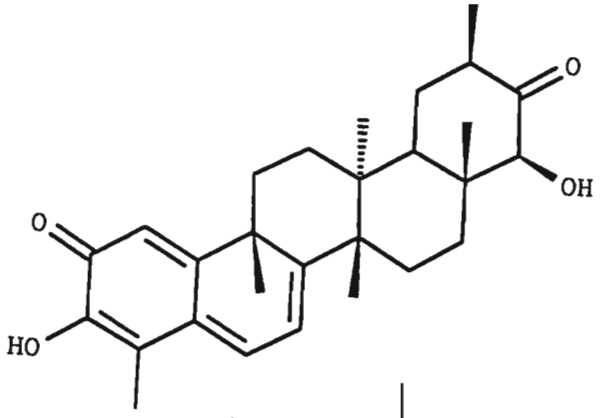
(87)



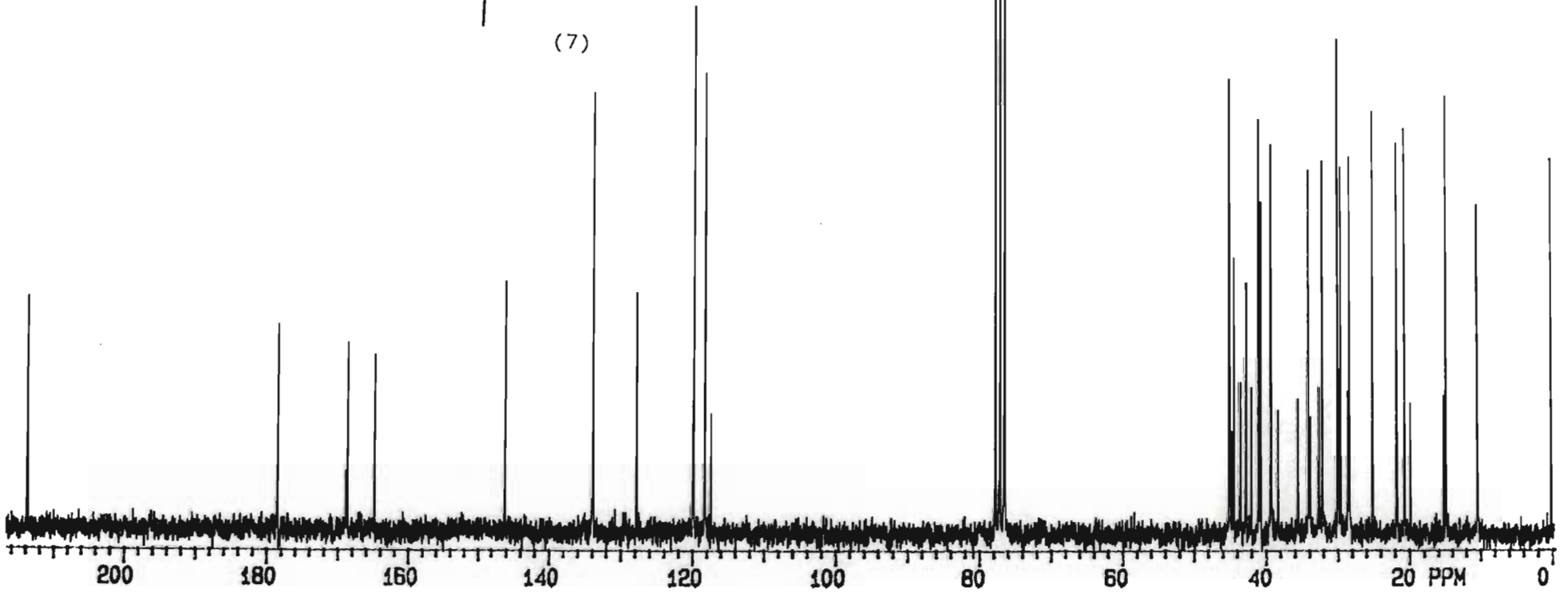
(87)

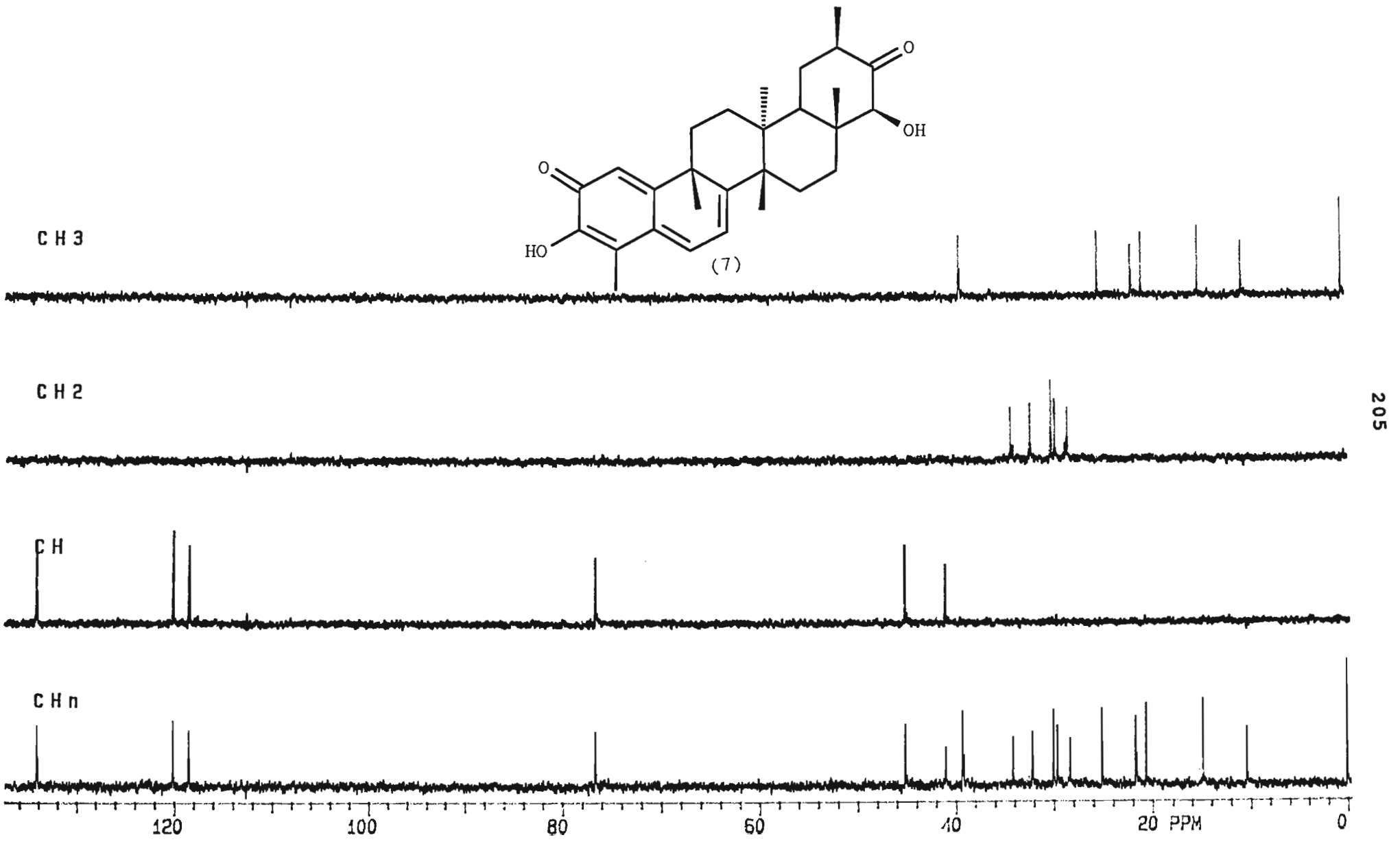

CH3
CH2
CH
CHn
(87)
202
110
100
90
80
70
60
50
40
30
20
10 PPM
0

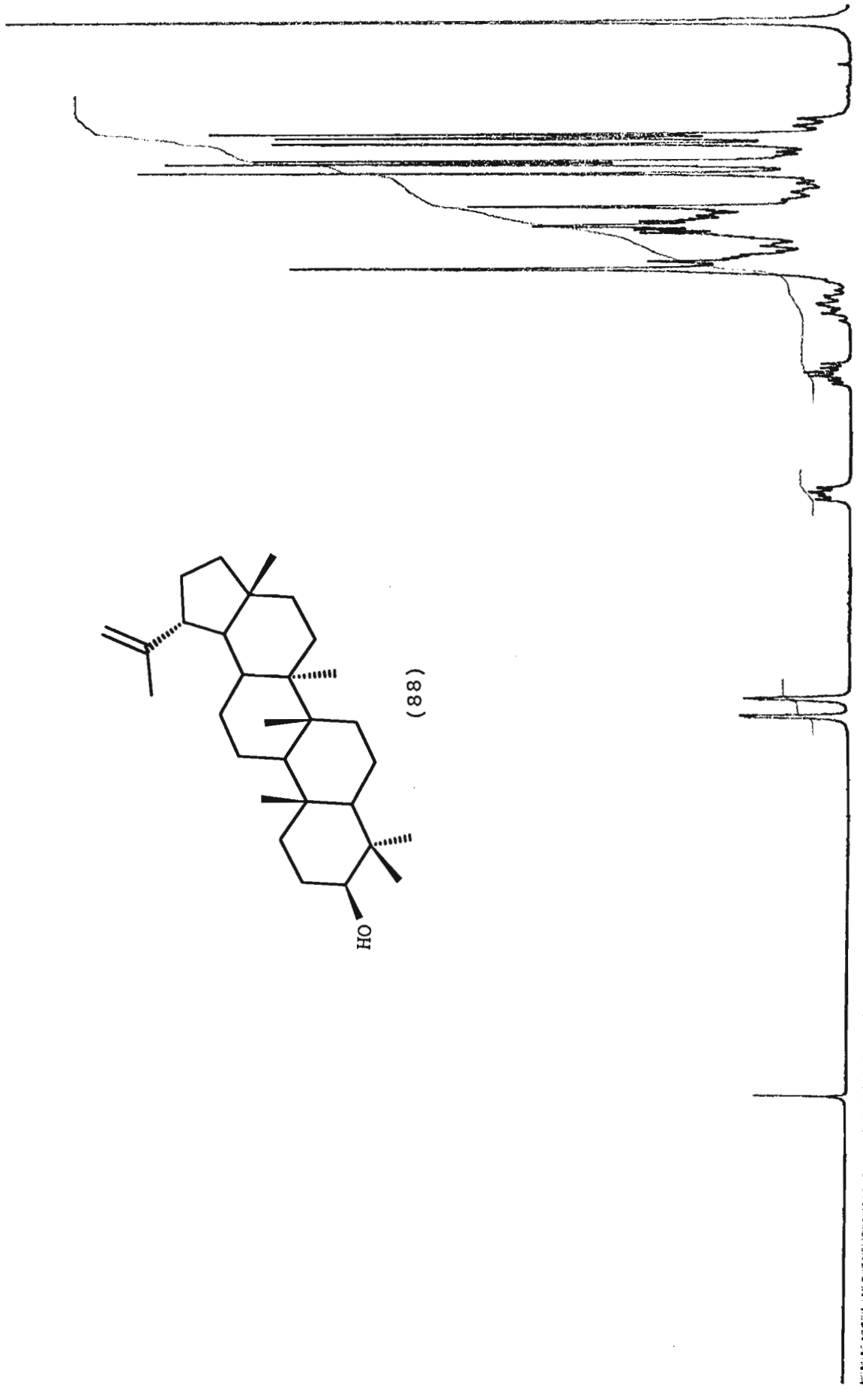

O
OH
O
HO
(7)
9
8
7
6
5
4
3
2
1 PPM



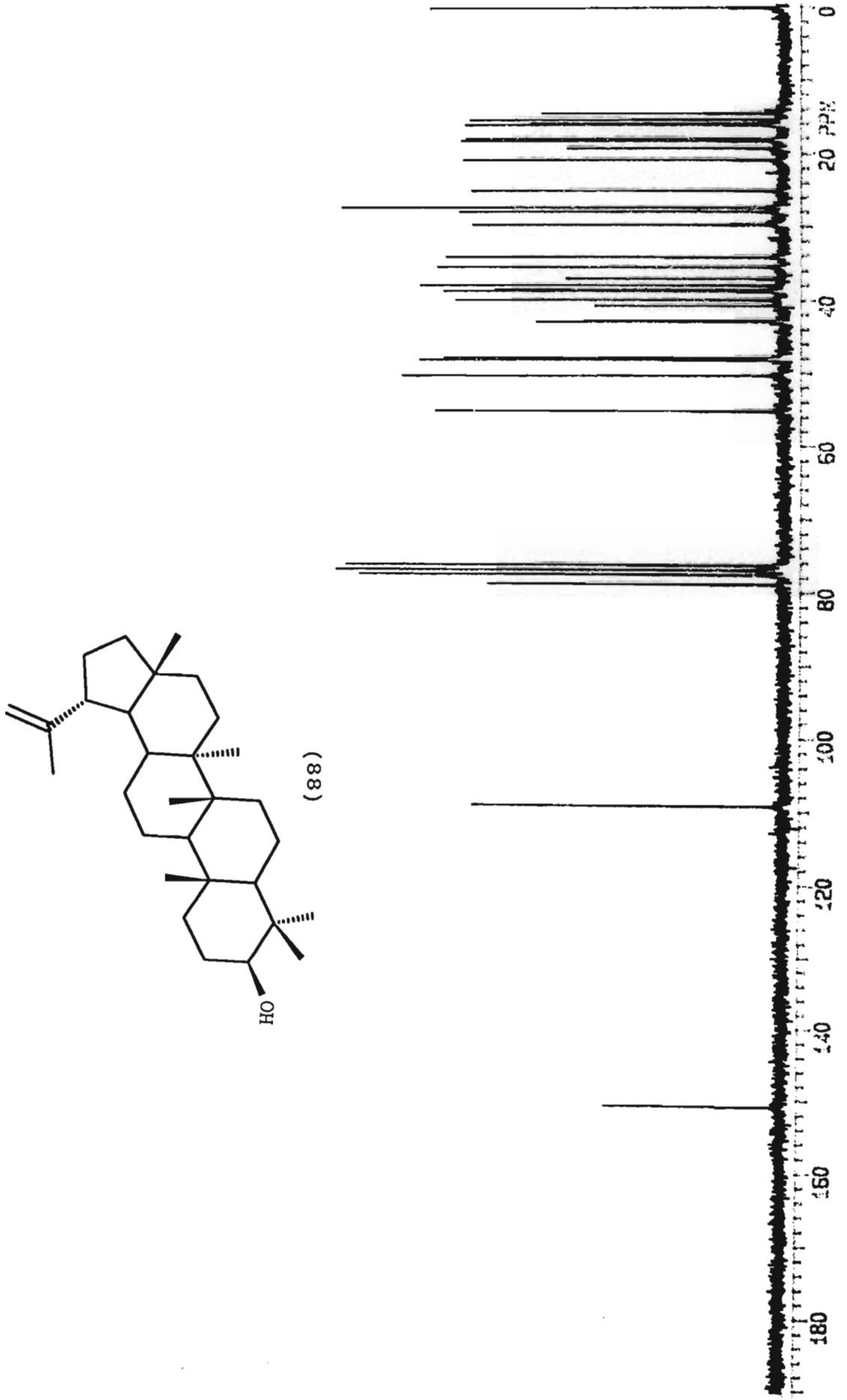
(7)

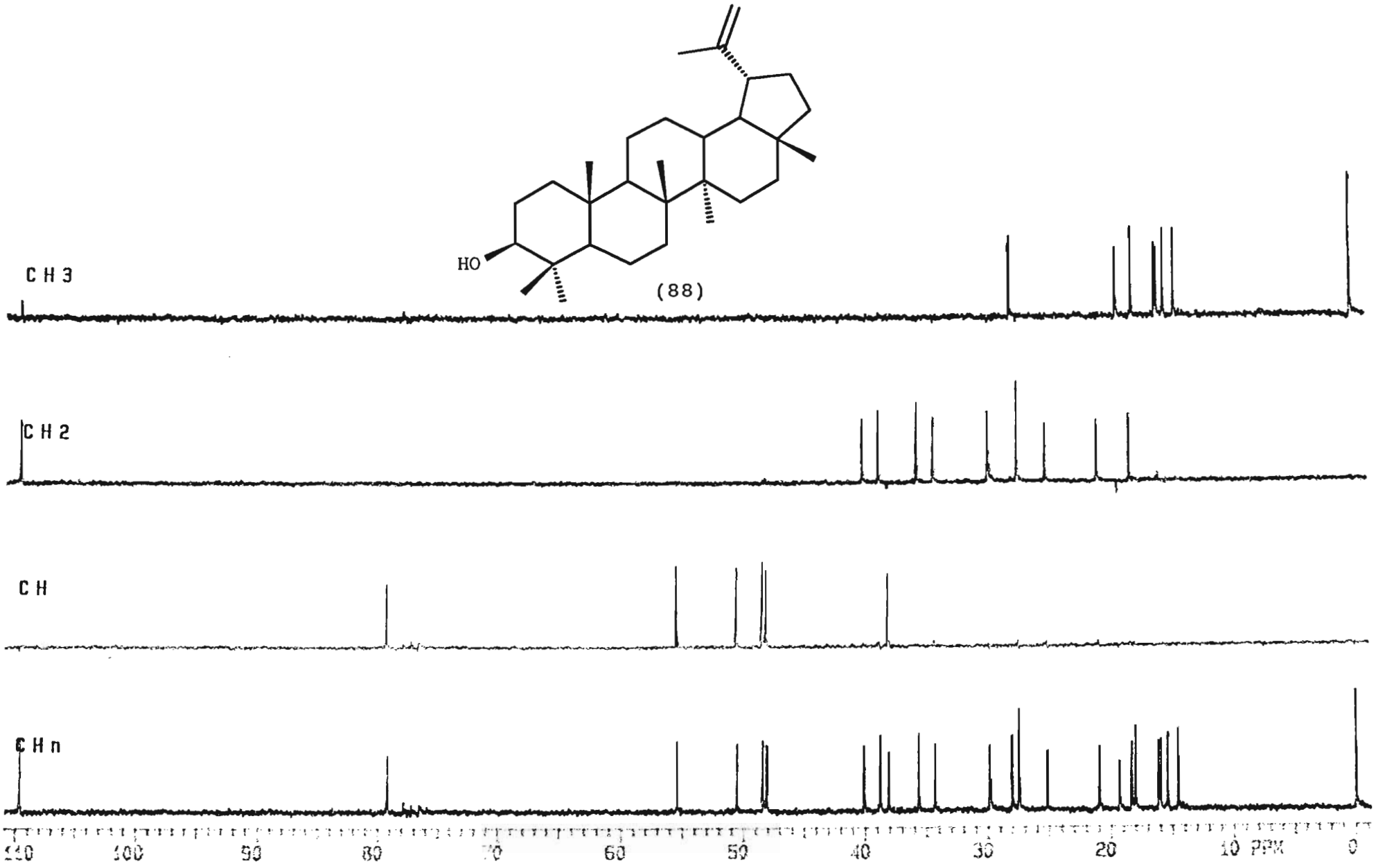


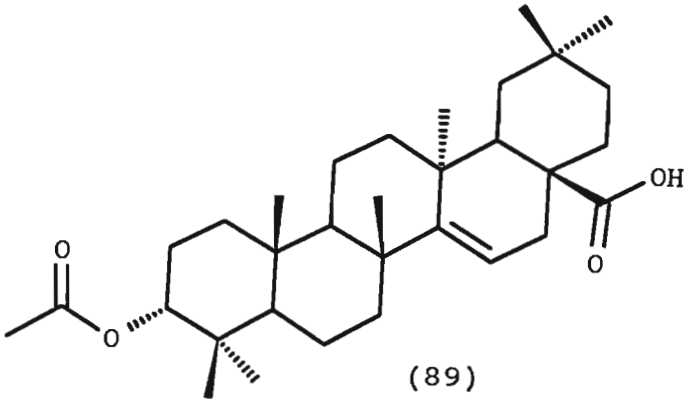

O
OH
O
HO
(7)
CH3
CH2
CH
CHn
120
100
80
60
40
20 PPM
0



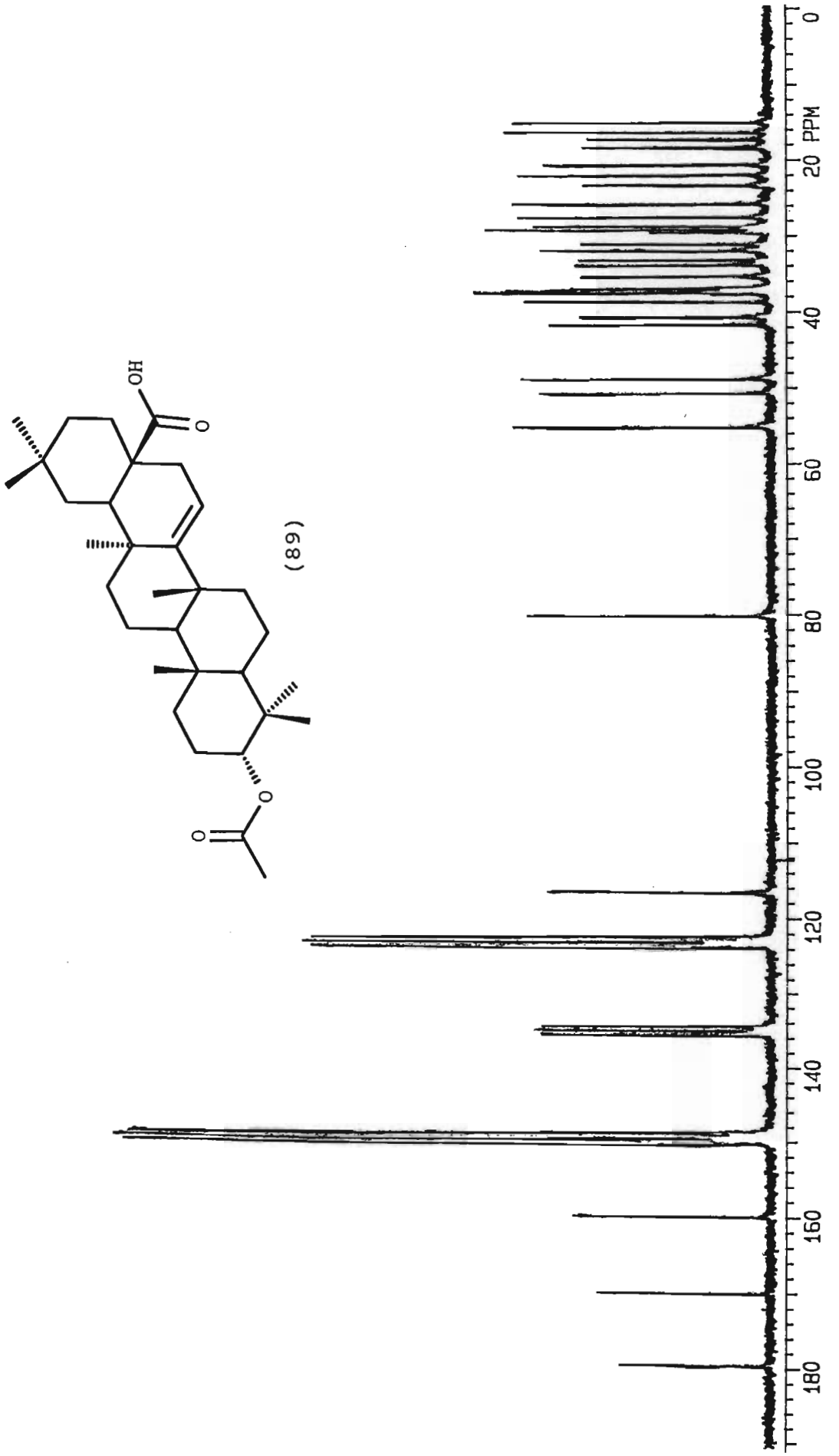
(88)

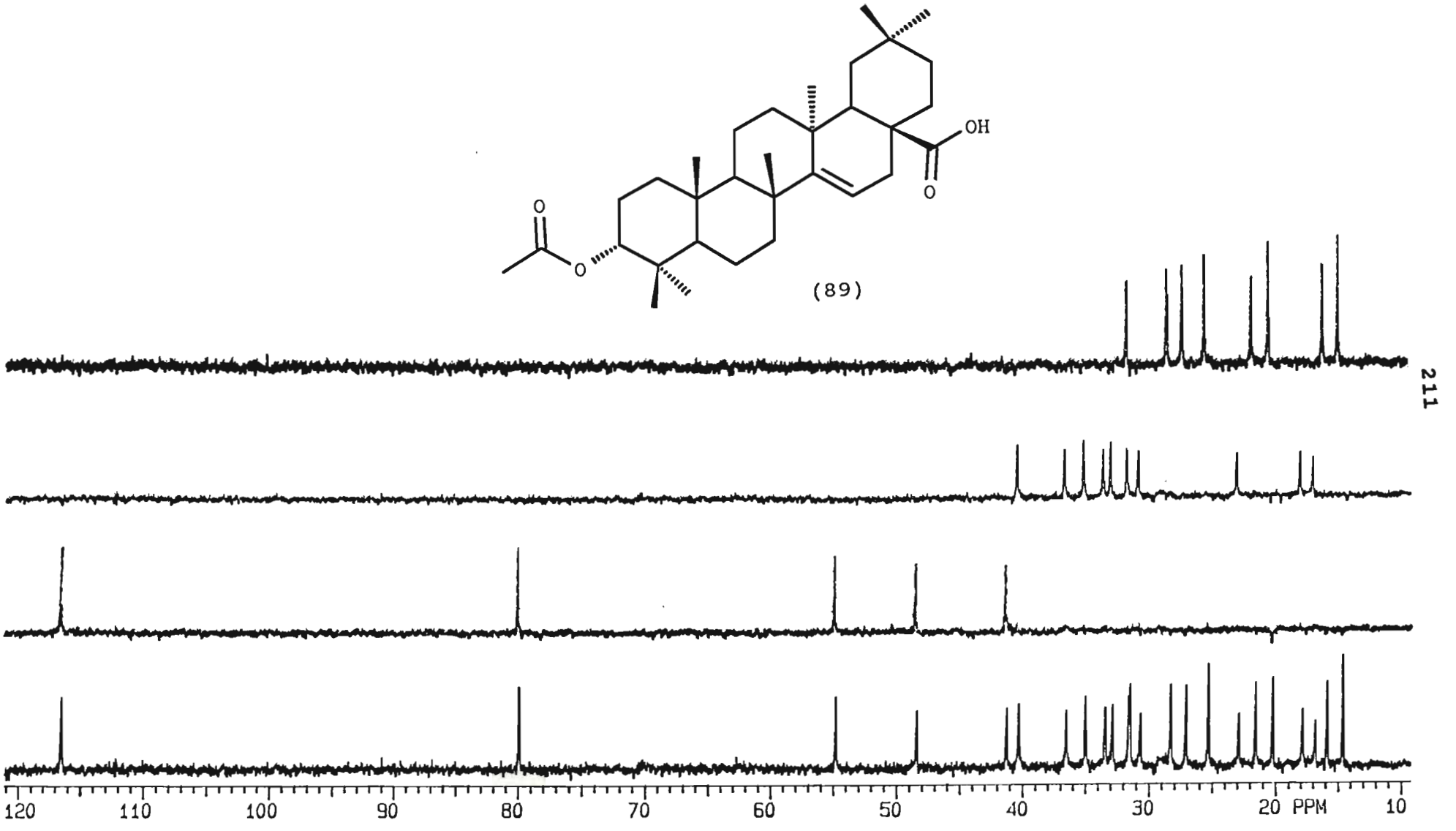

HO
(88)
0
20
40
60
80
100
120
140
160
180
PPM

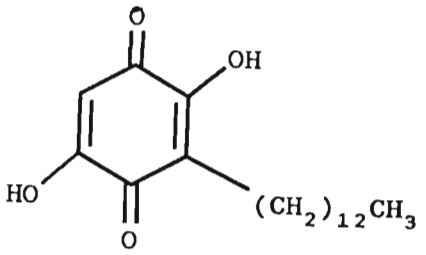

HO
(88)
CH3
CH2
CH
CHn
110
100
90
80
70
60
50
40
30
20
10 PPM
0



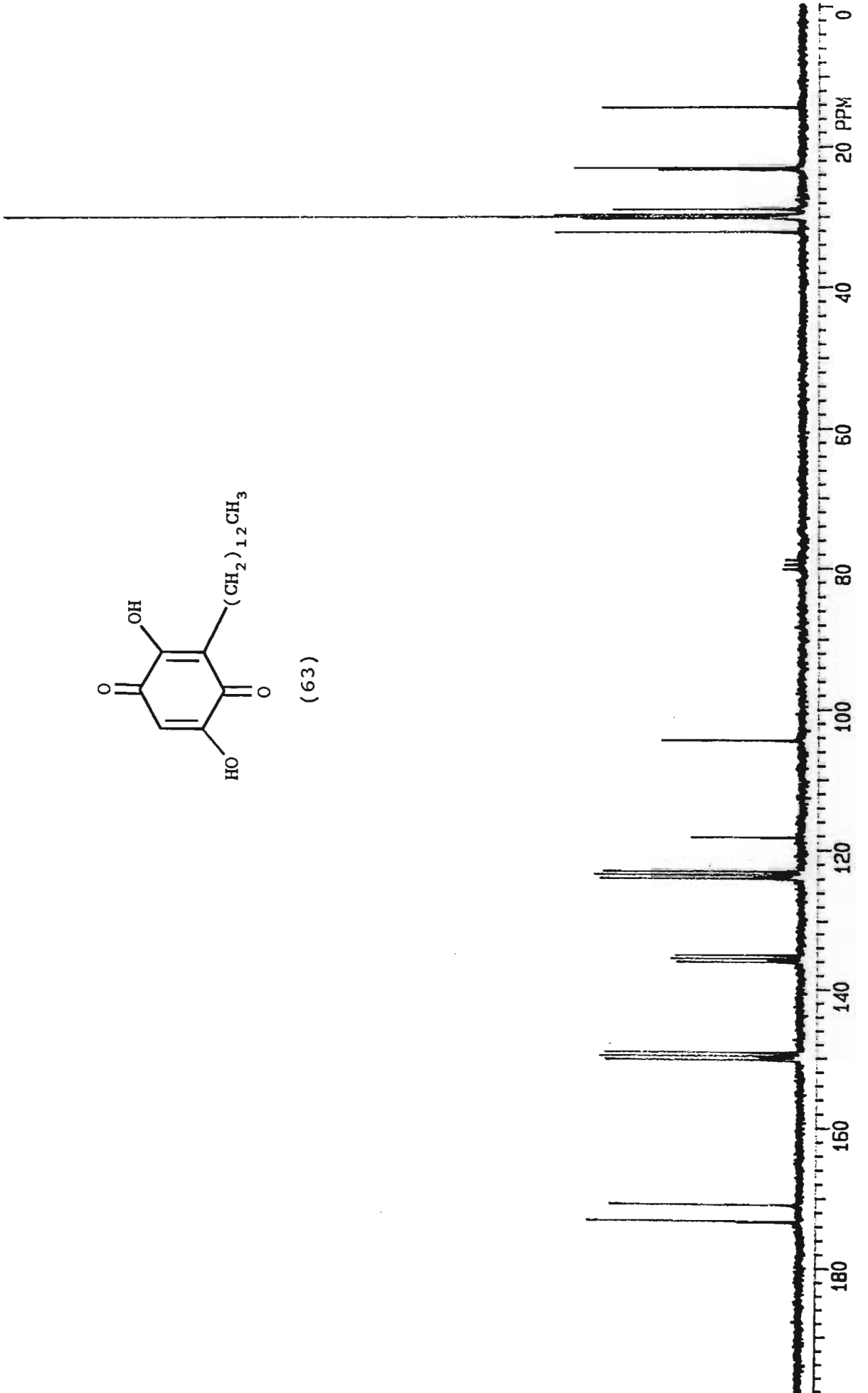
(89)


OH
O
O
O
(89)
0
20 PPM
40
60
80
100
120
140
160
180

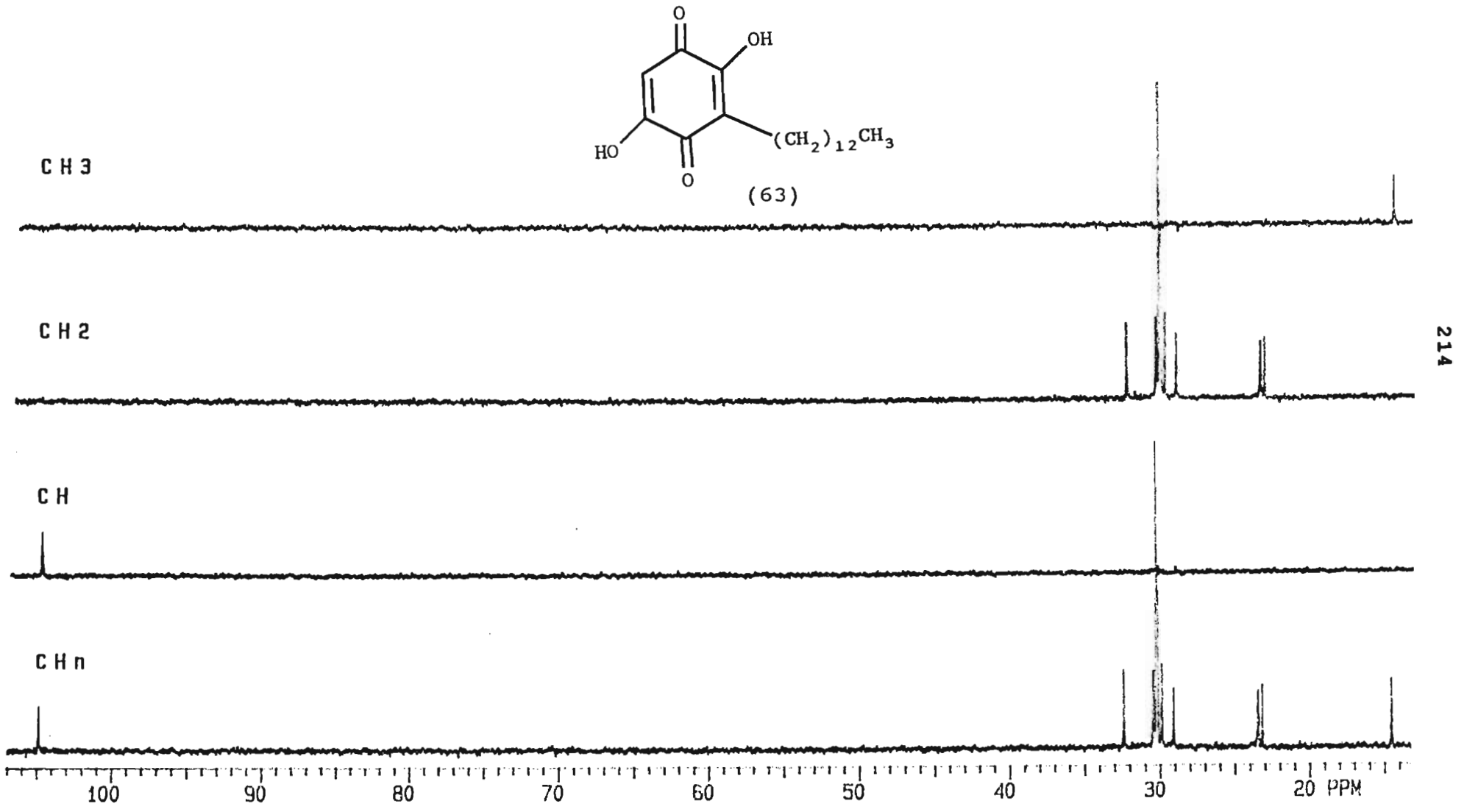

OH
O
O
O
(89)
120
110
100
90
80
70
60
50
40
30
20
PPM
10

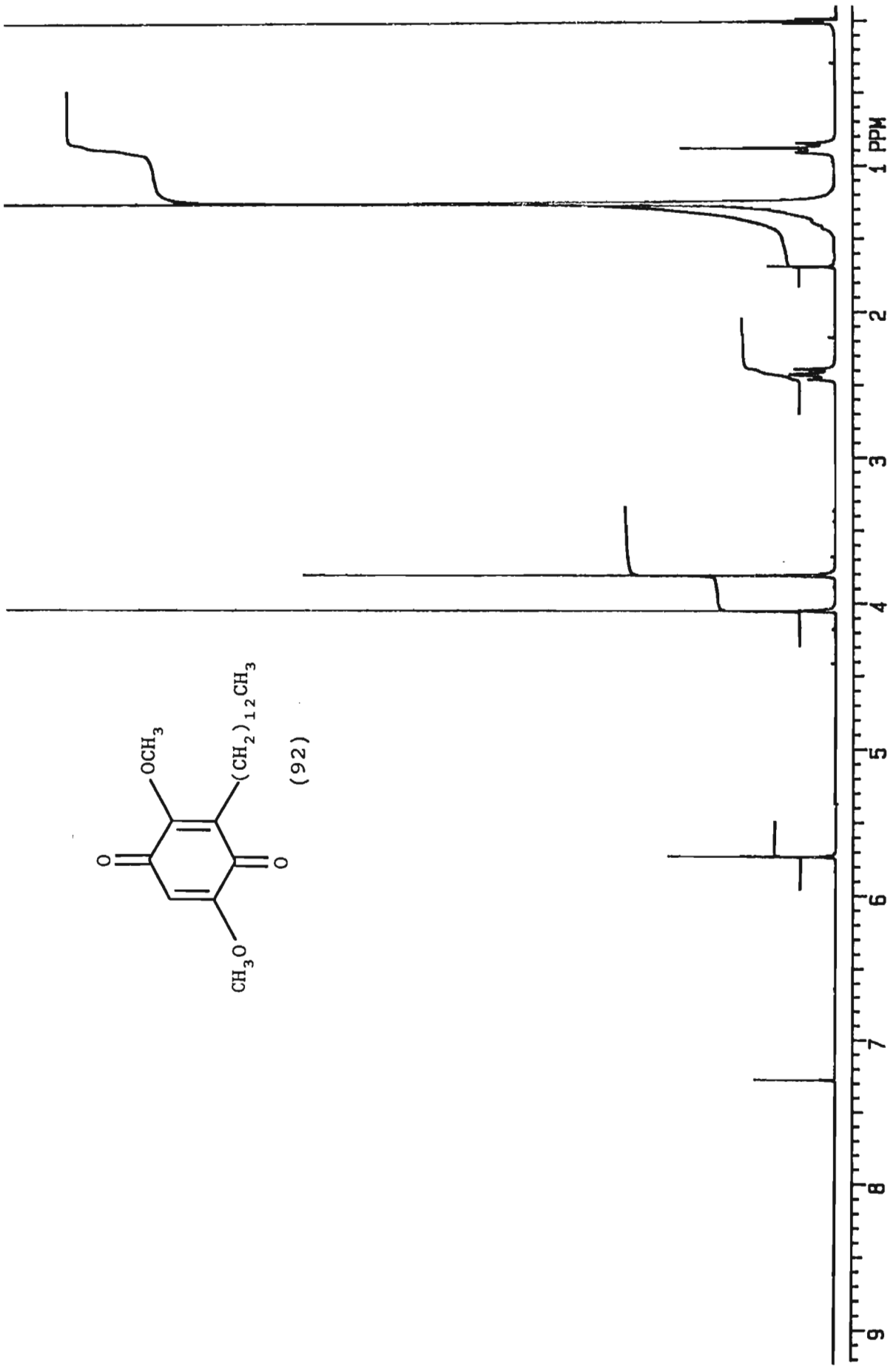


(63)

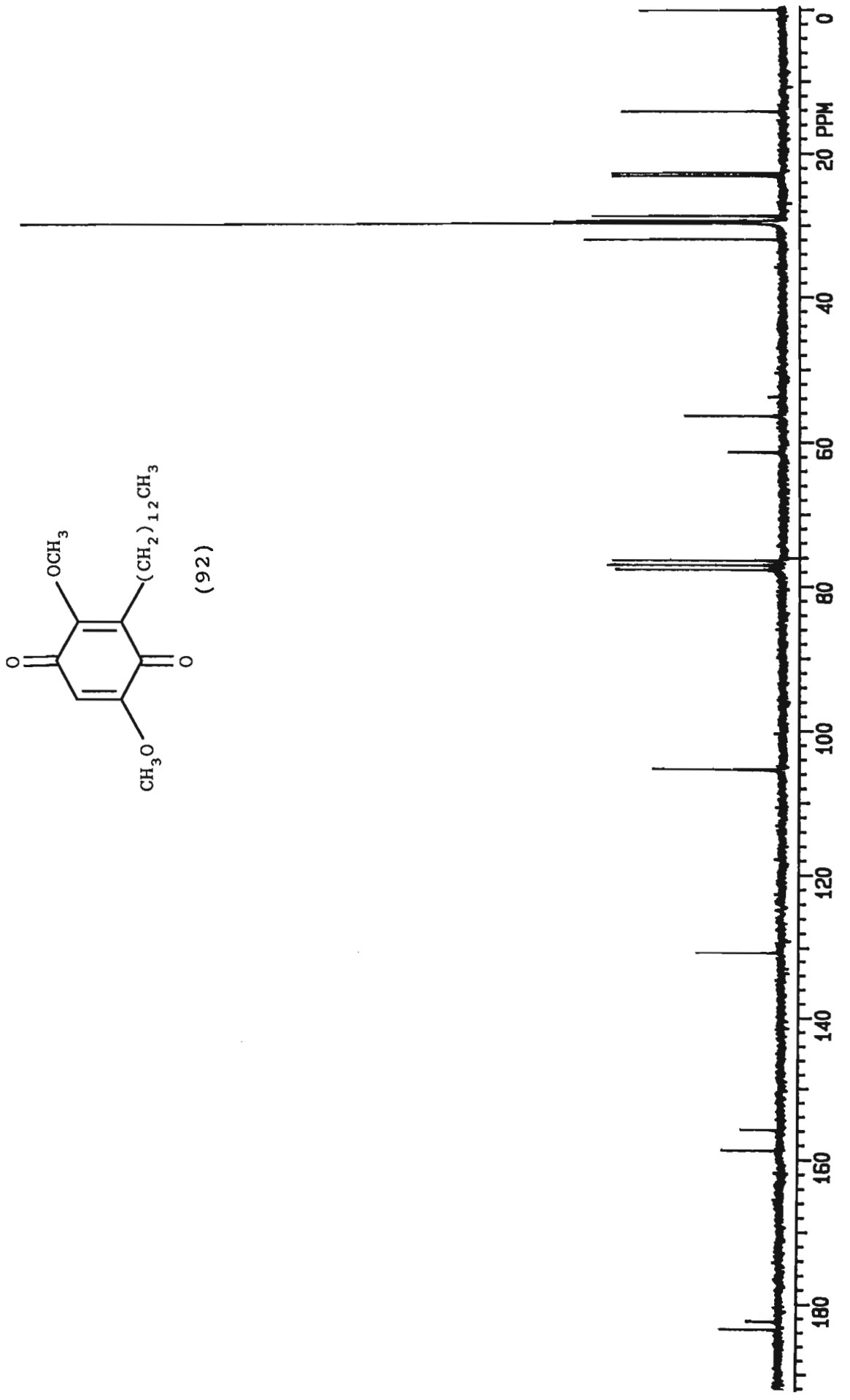


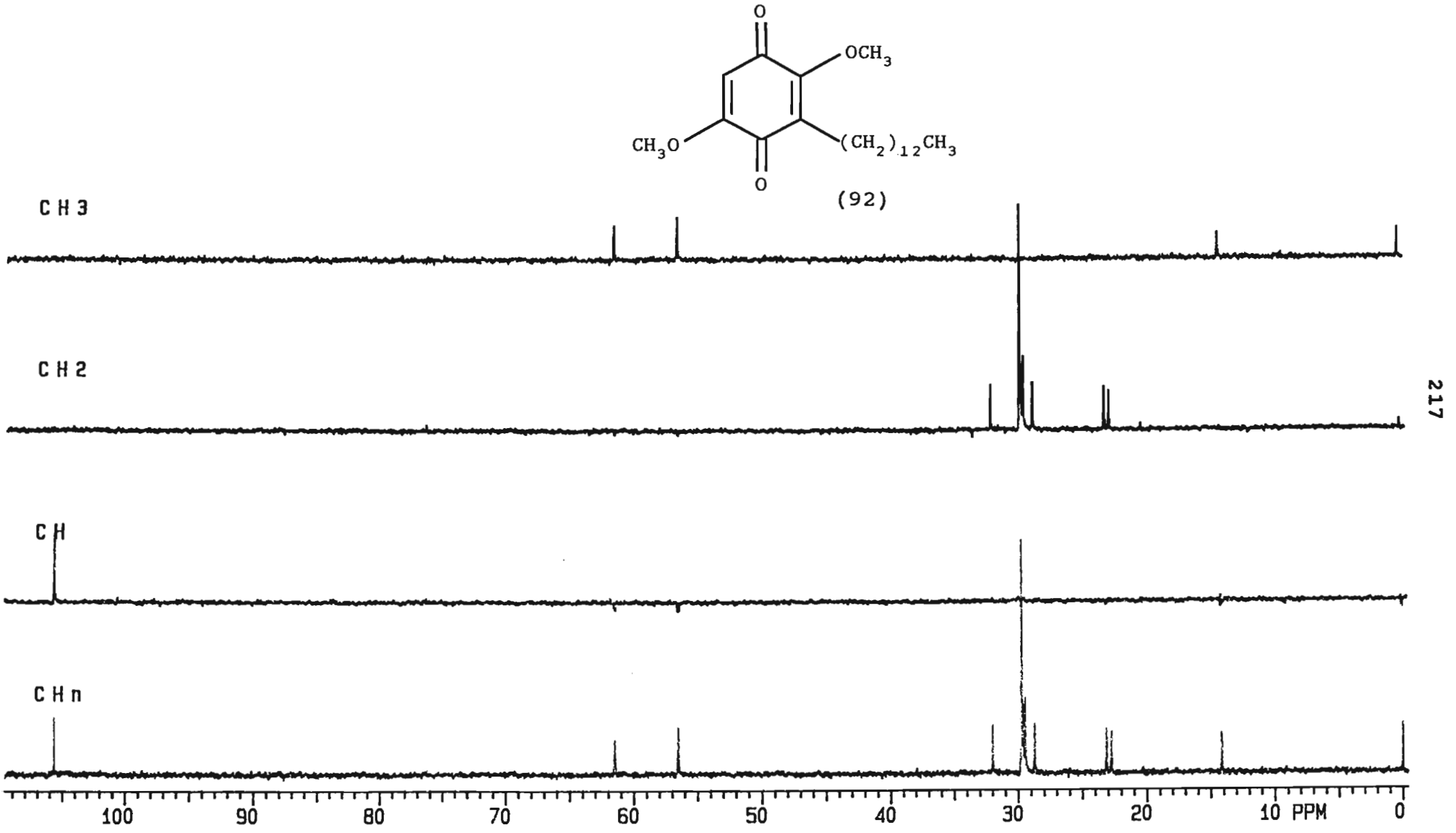
(63)

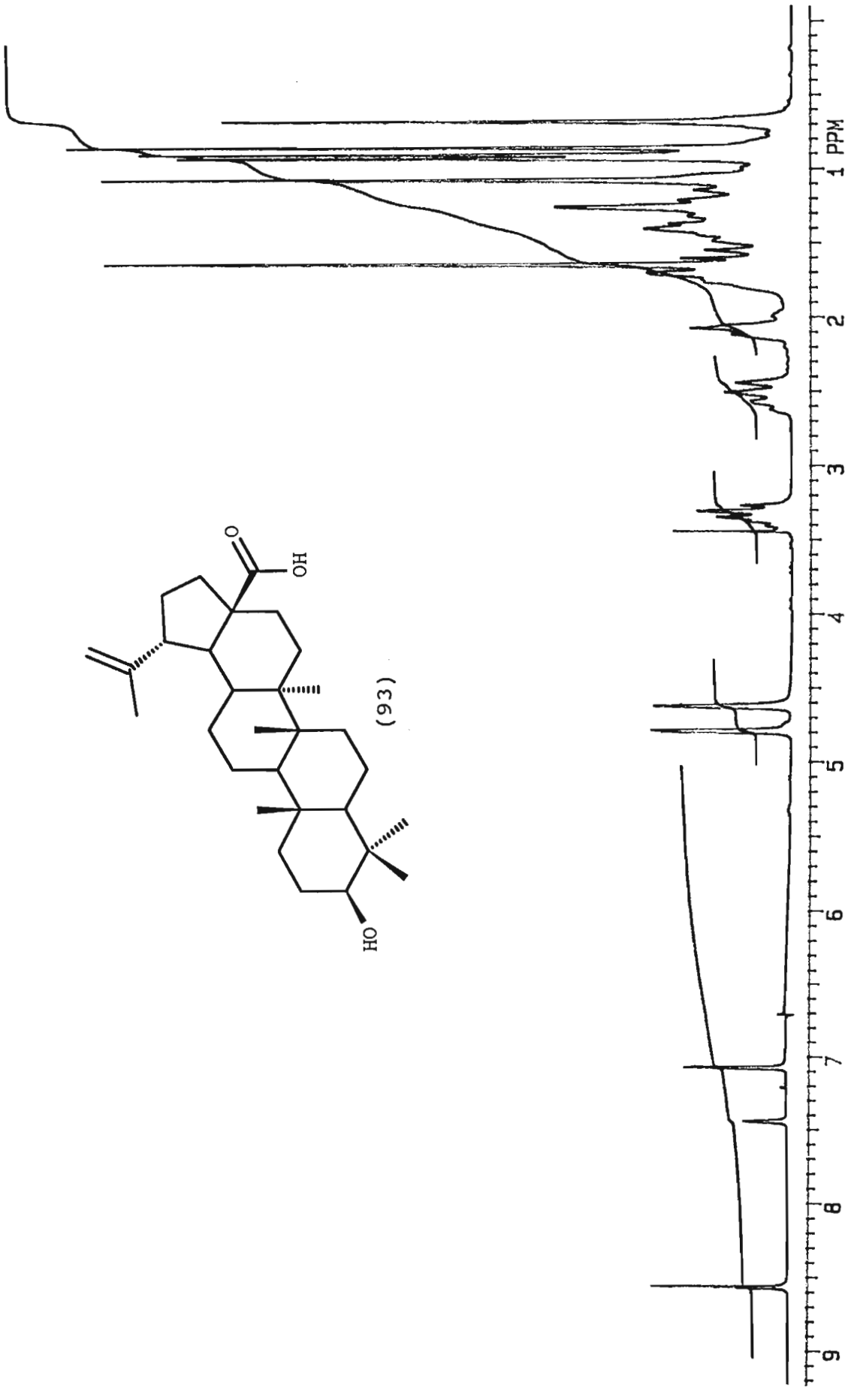

O
OH
HO
O
$(CH_2)_{12}CH_3$
(63)
CH3
CH2
CH
CHn
100
90
80
70
60
50
40
30
20 PPM



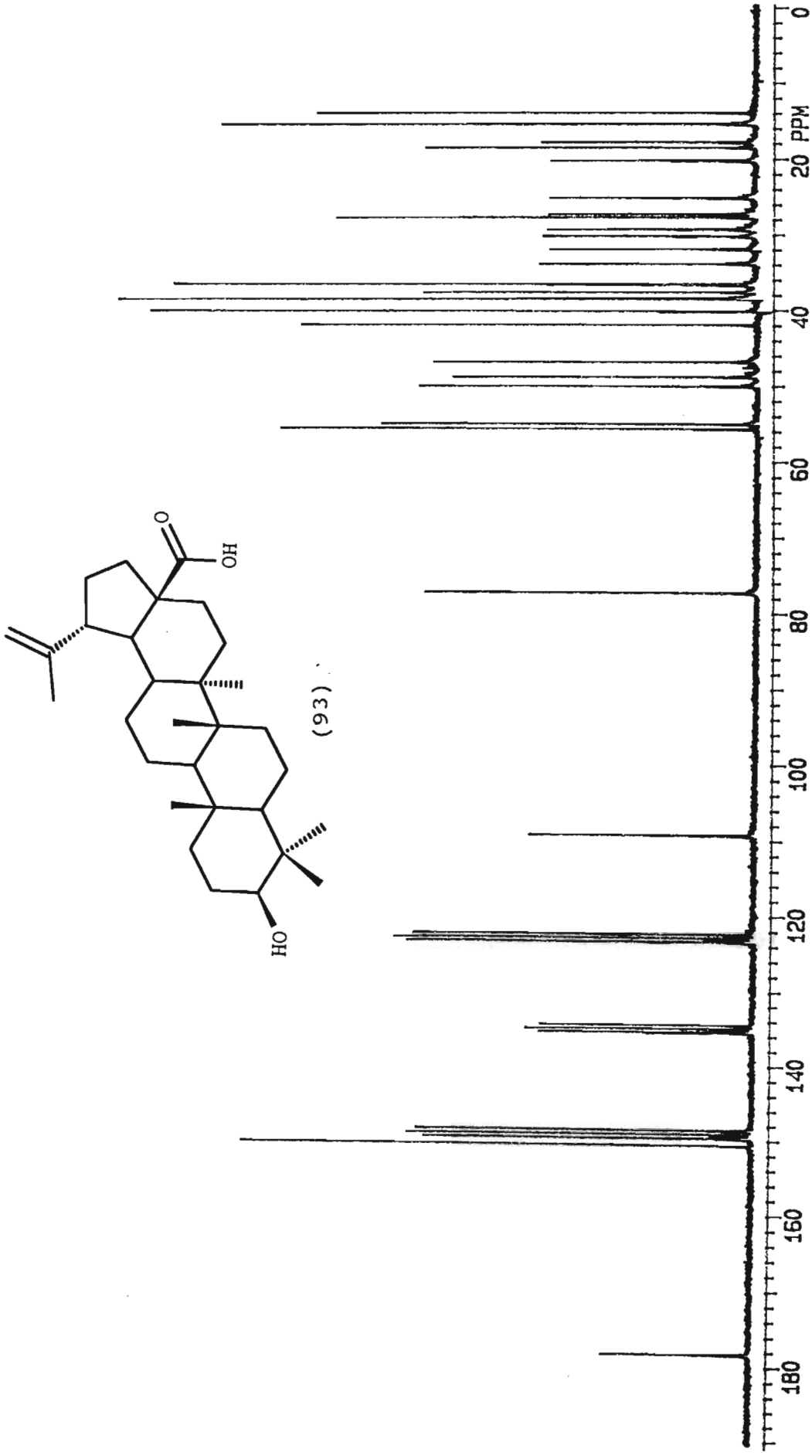
(92)

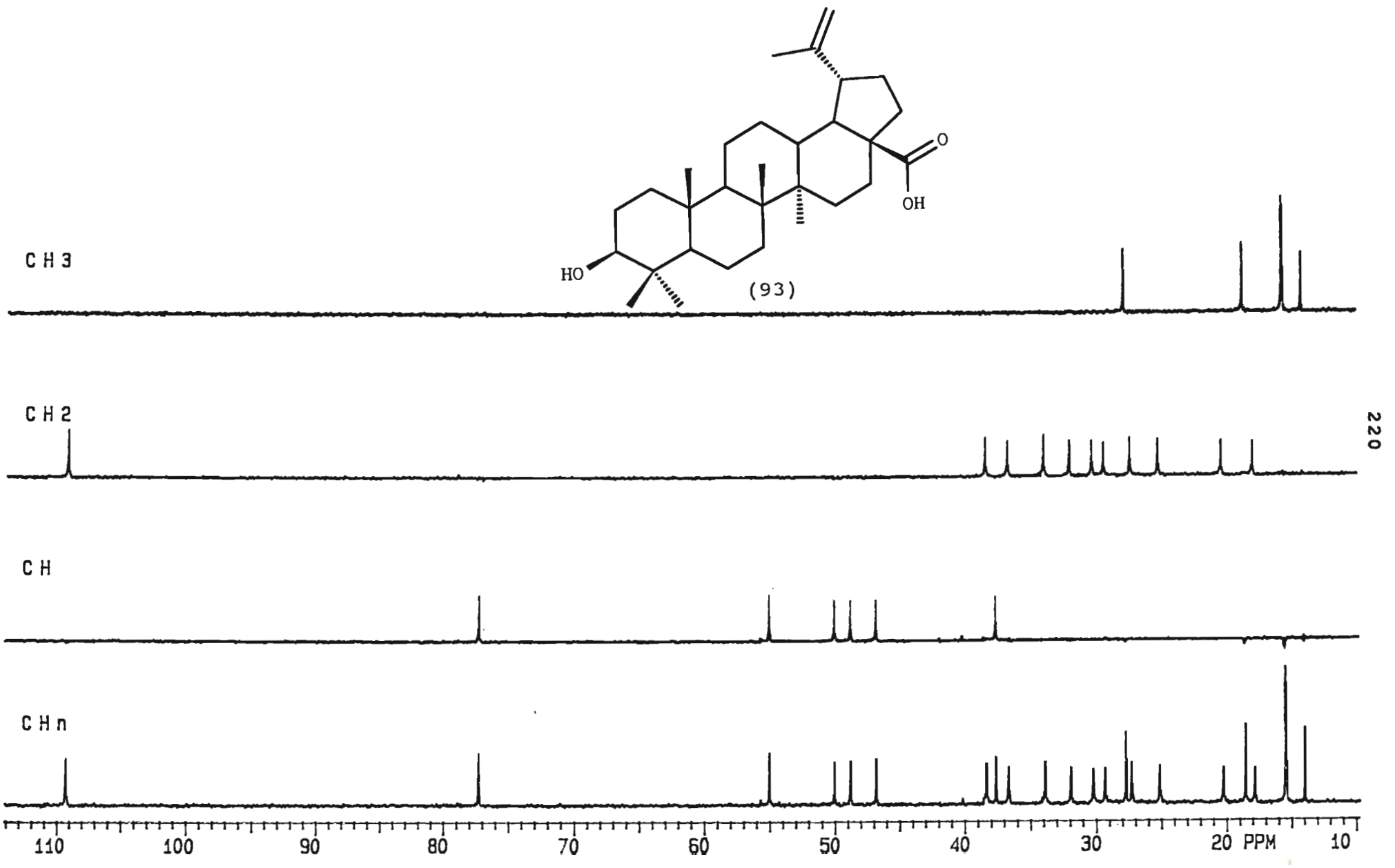

OCH3
(CH2)12CH3
O
O
CH3O
(92)
0
20 PPM
40
60
80
100
120
140
160
180

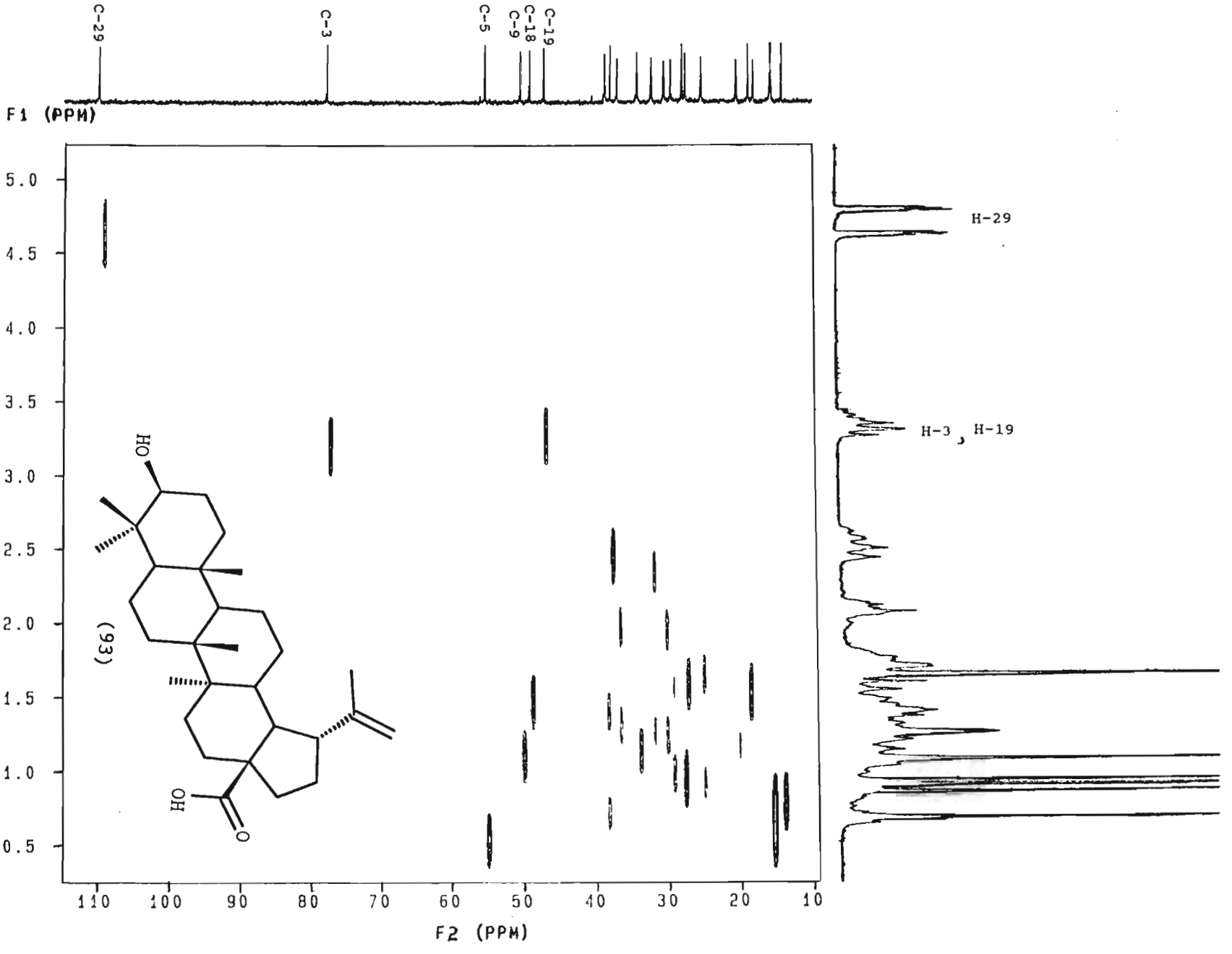

O
OCH3
CH3O
(CH2)12CH3
O
(92)
CH3
CH2
CH
CHn
100
90
80
70
60
50
40
30
20
10 PPM
0

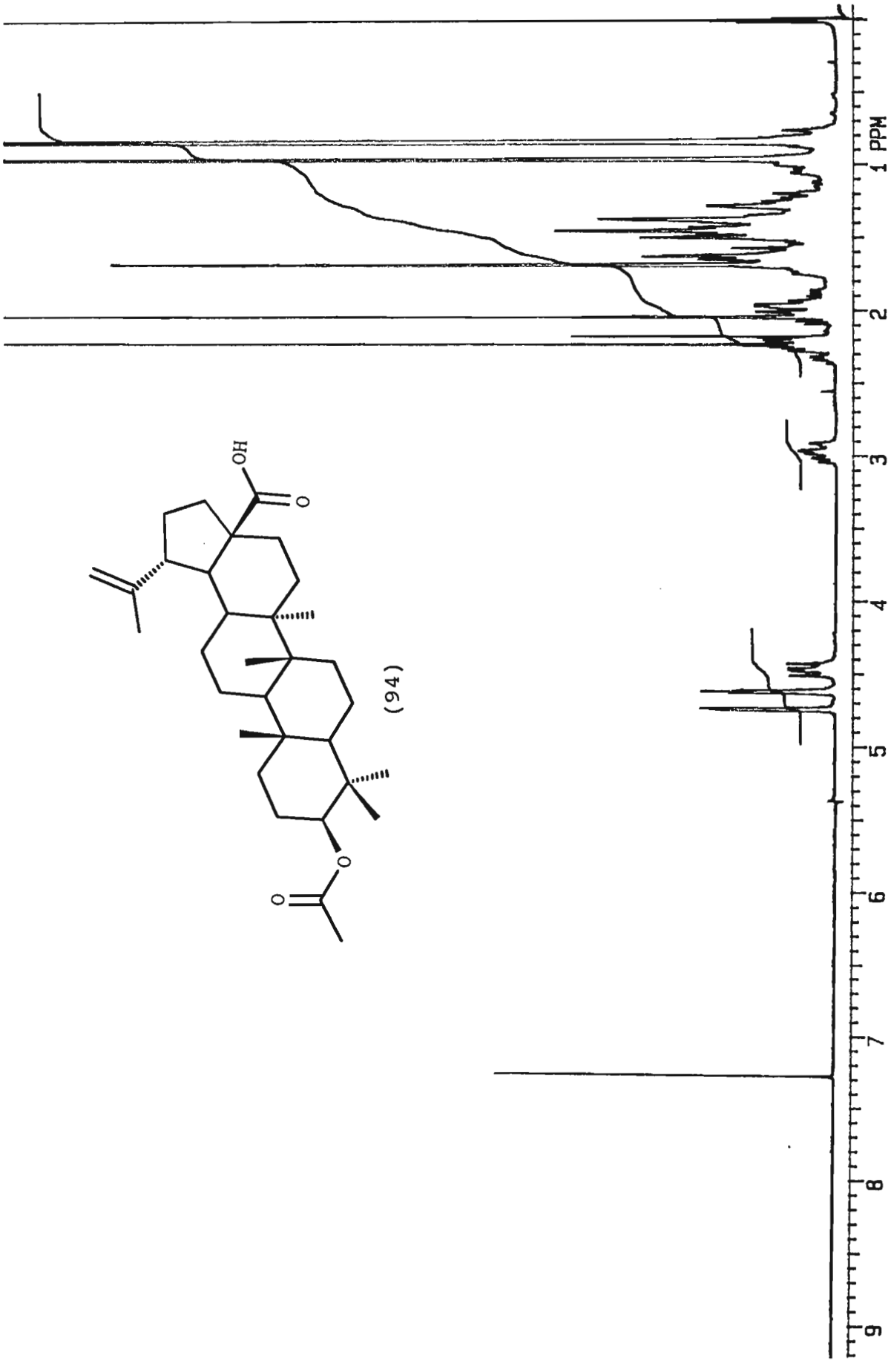


(93)

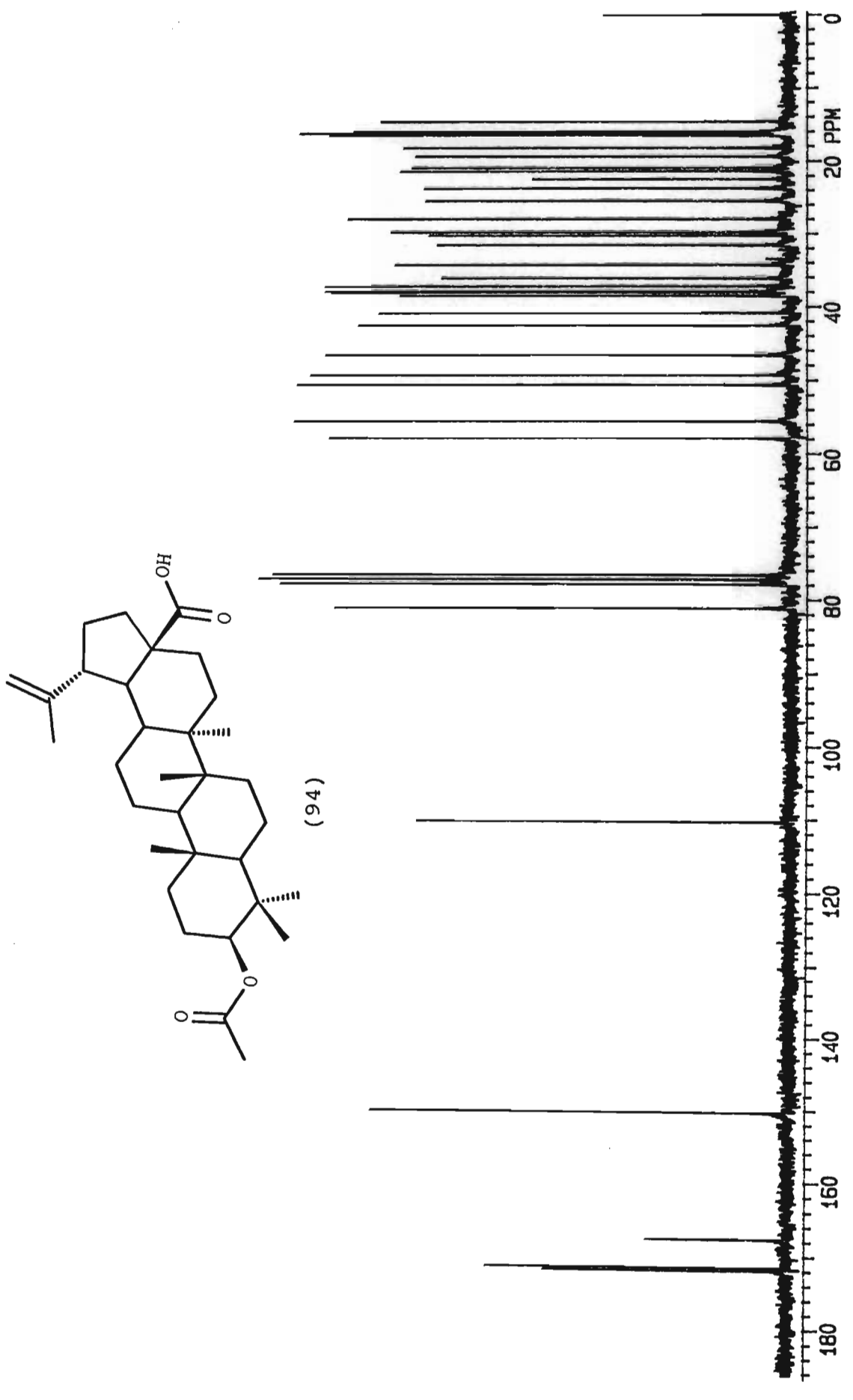

O
OH
HO
(93)
0
20 PPM
40
60
80
100
120
140
160
180

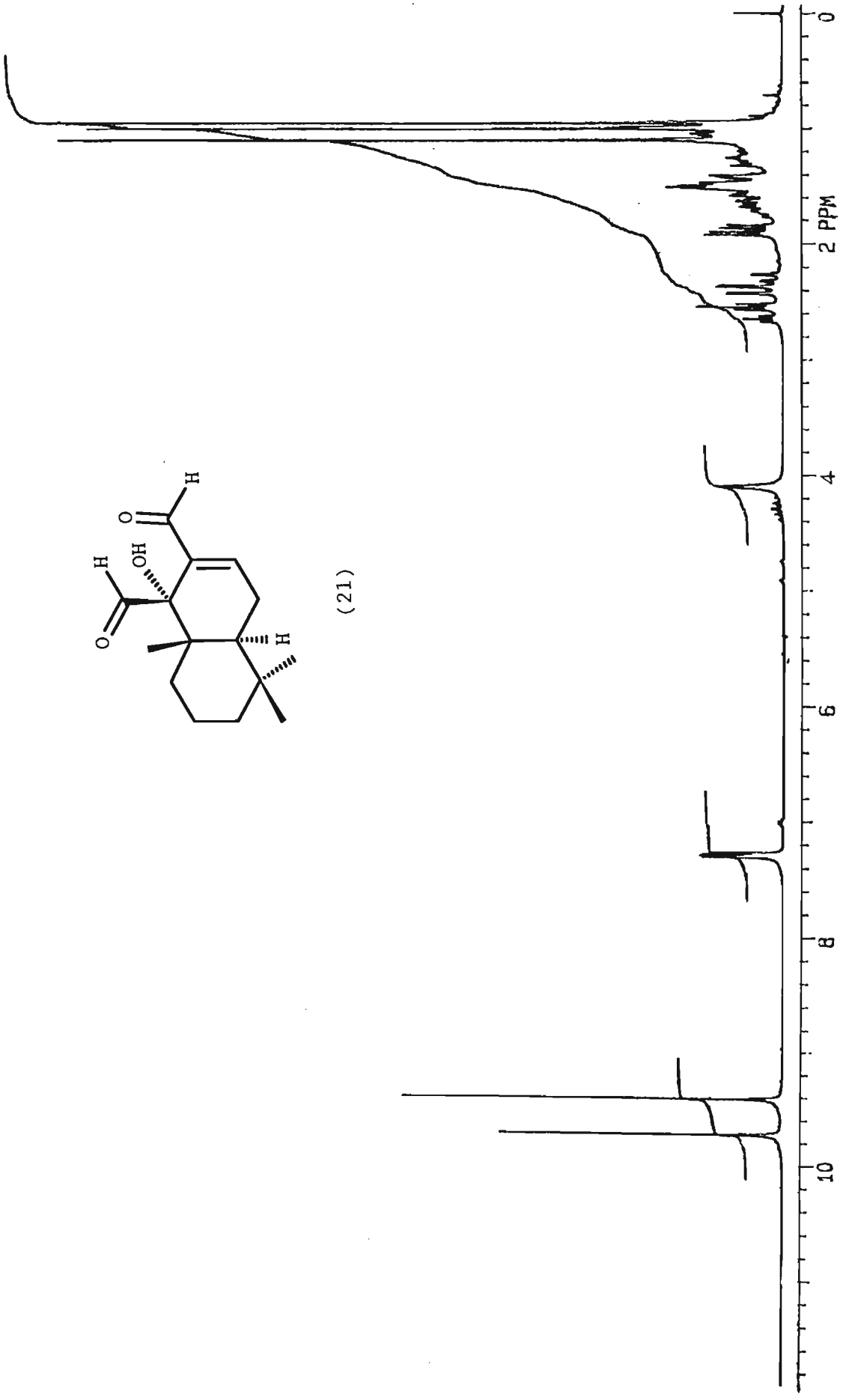

HO
O
OH
(93)
CH3
CH2
CH
CHn
220
110
100
90
80
70
60
50
40
30
20
PPM
10


C-29
C-3
C-5
C-9
C-18
C-19
F1 (PPM)
H-29
H-3
H-19
HO
OH
O
(93)
F2 (PPM)

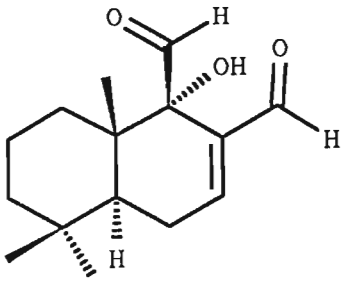


(94)

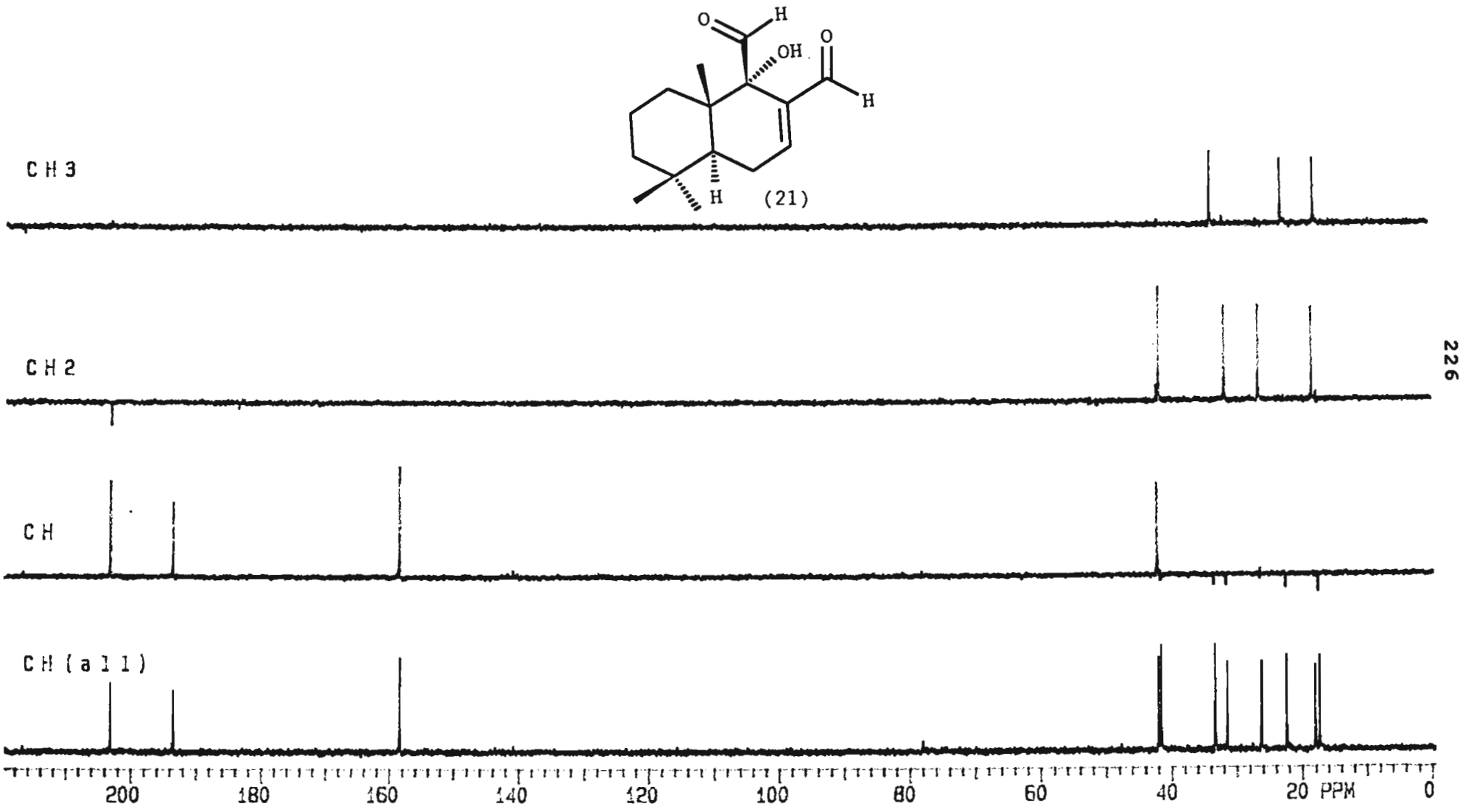

OH
O
O
O
(94)
0
20
40
60
80
100
120
140
160
180
PPM

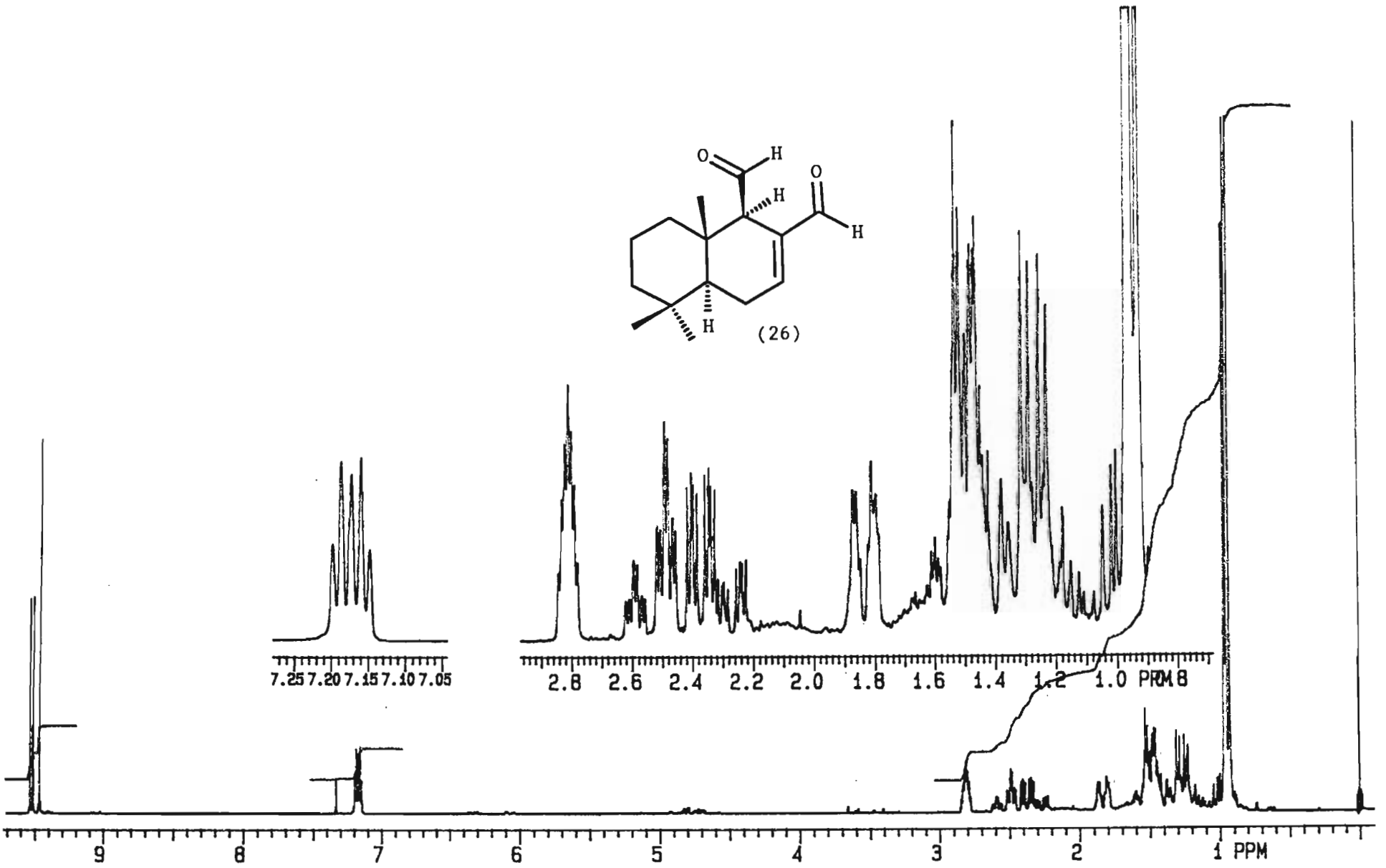


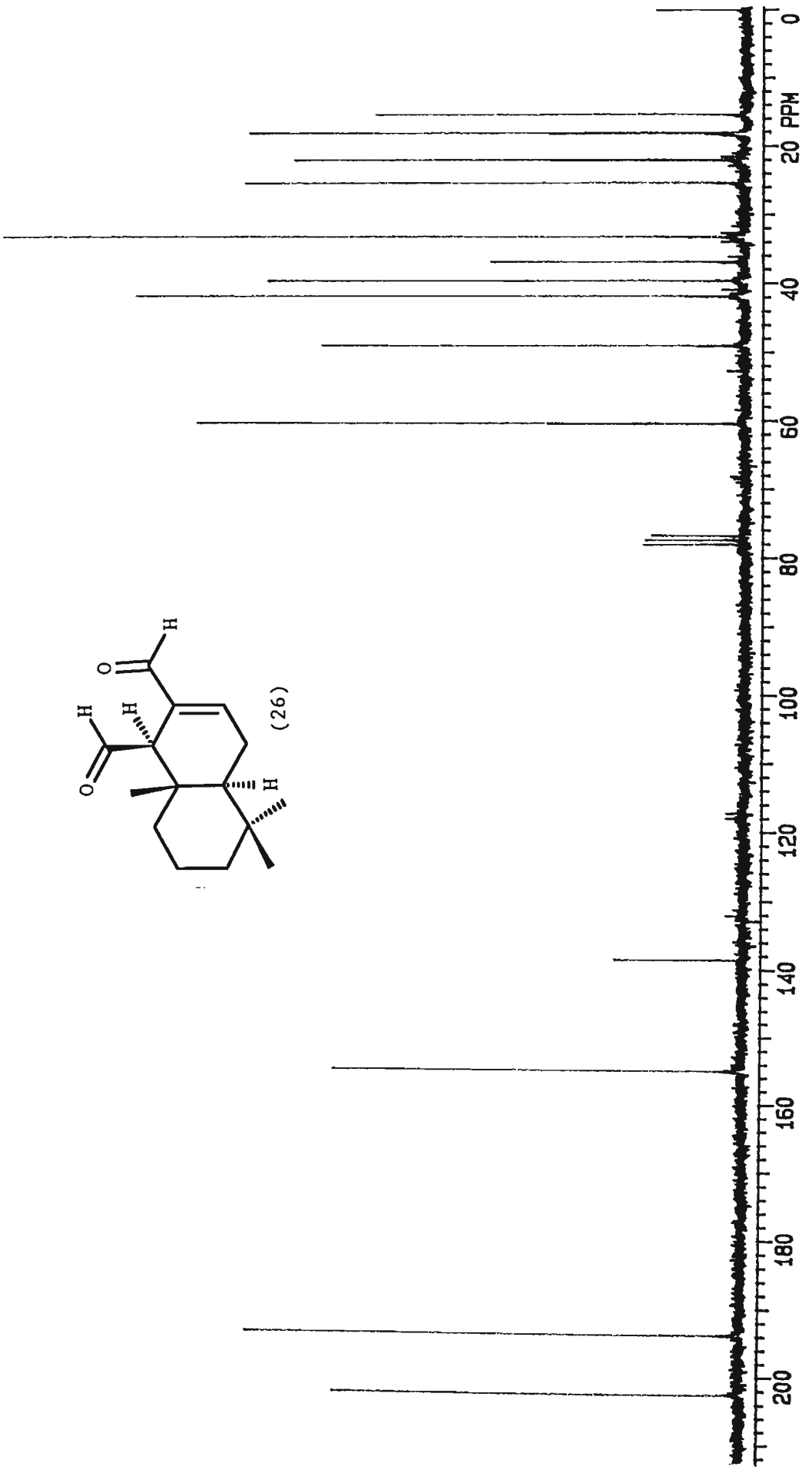
(21)

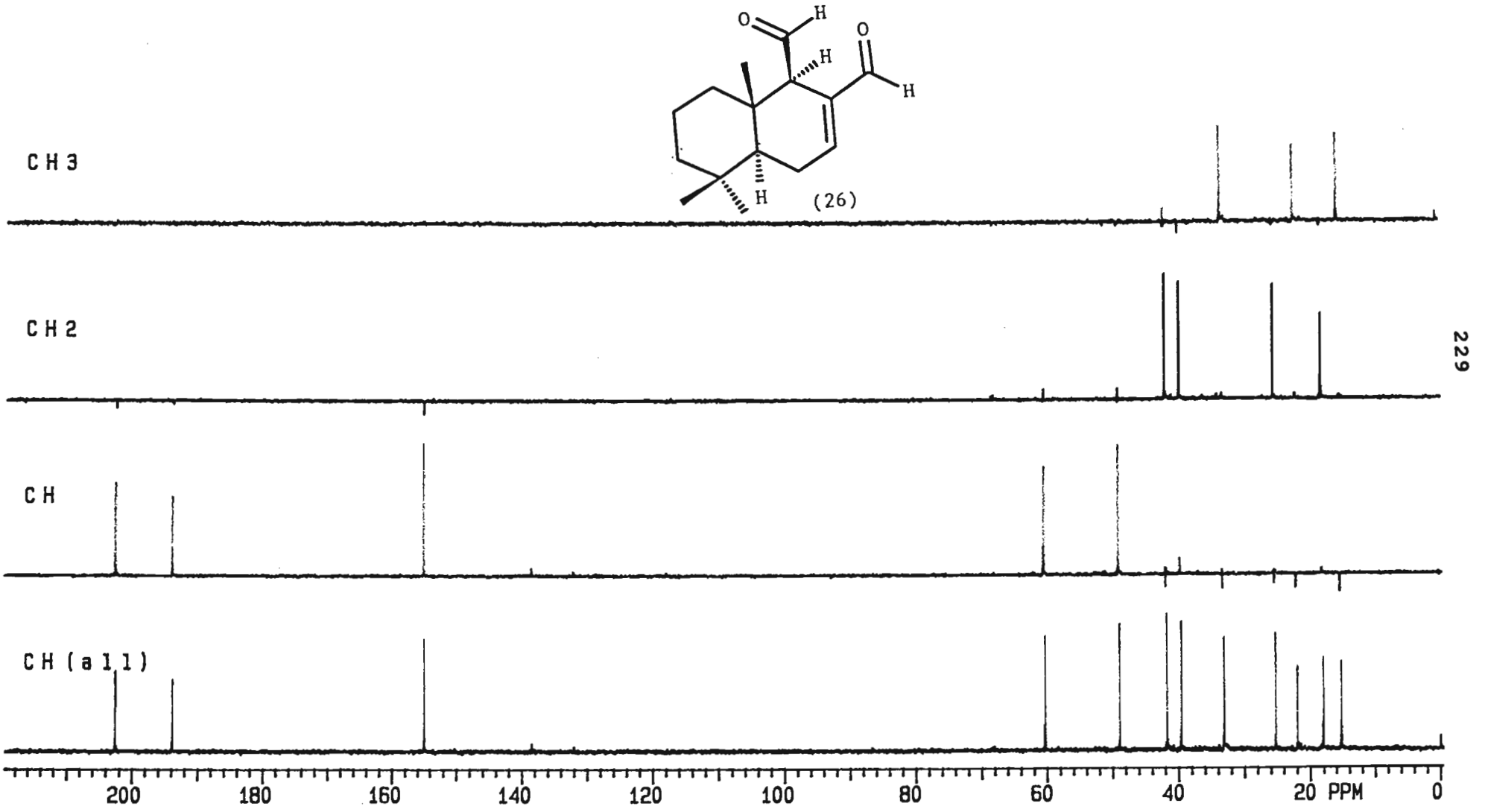


(21)

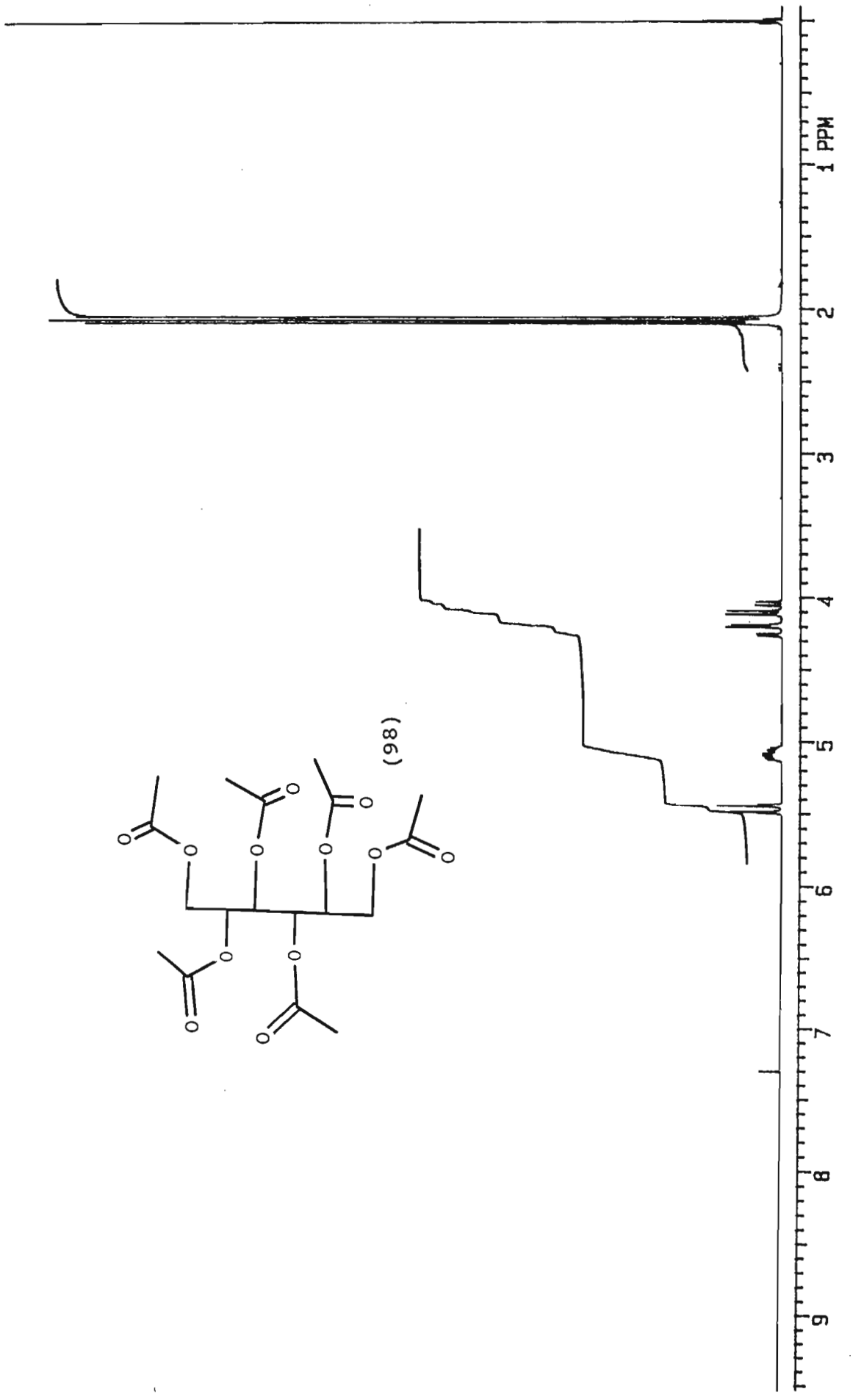

O
H
OH
O
H
H
(21)
CH3
CH2
CH
CH(all)
200
180
160
140
120
100
80
60
40
20
PPM
0


O
H
O
H
H
(26)
7.25 7.20 7.15 7.10 7.05
2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 PPM
9 8 7 6 5 4 3 2 1 PPM

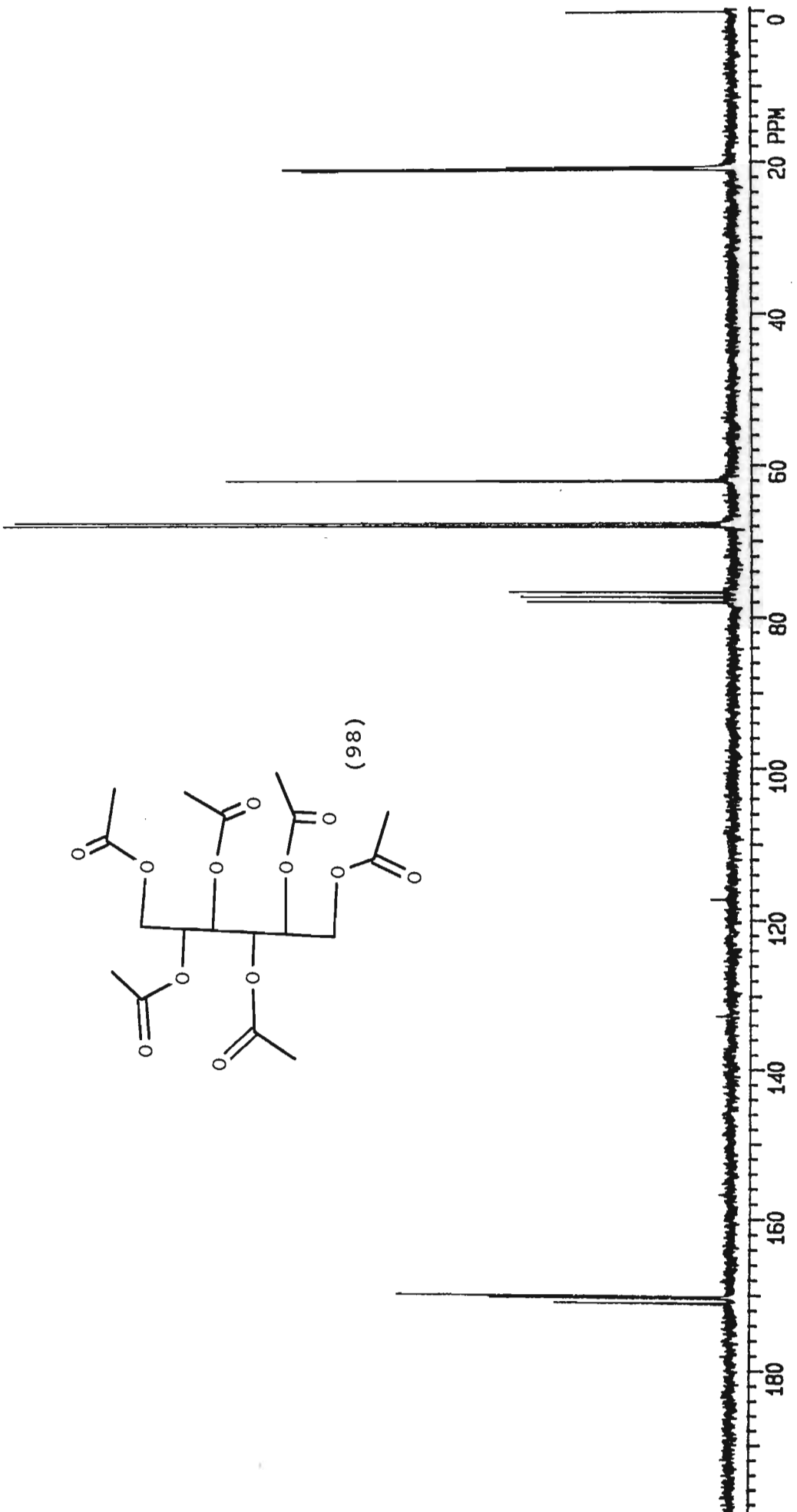

0
20 PPM
40
60
80
100
120
140
160
180
200
O
H
H
O
H
H
(26)



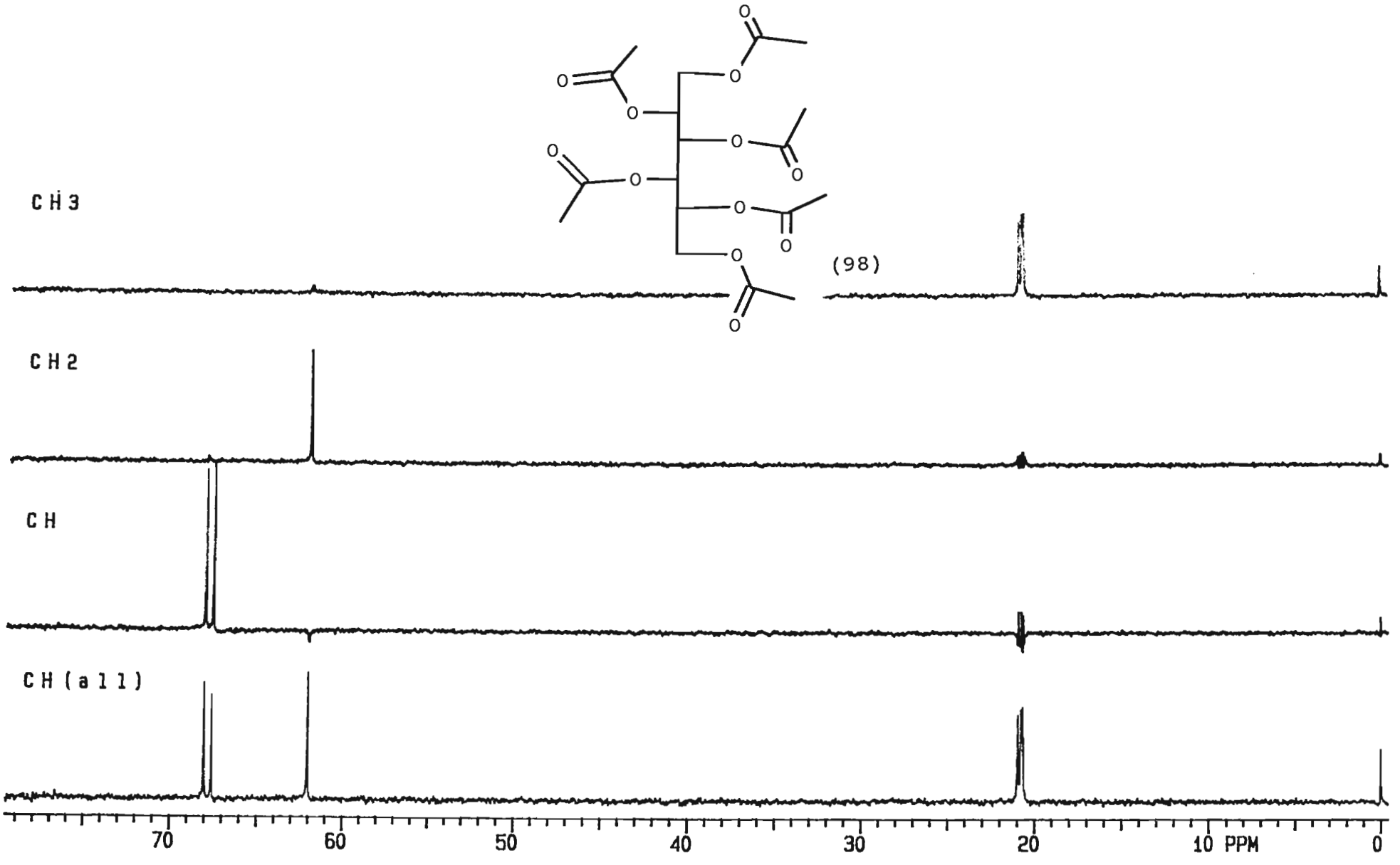
(26)

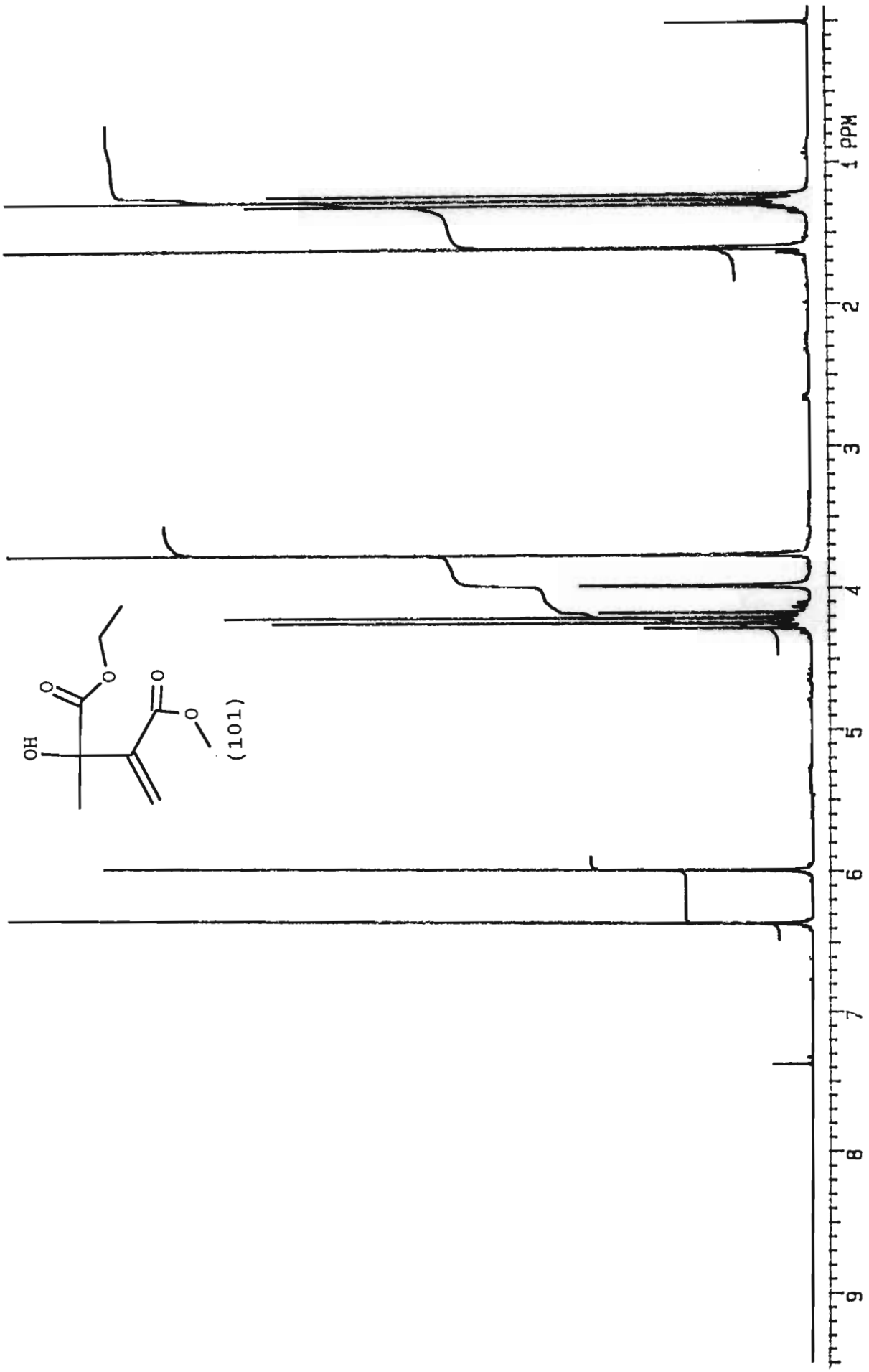


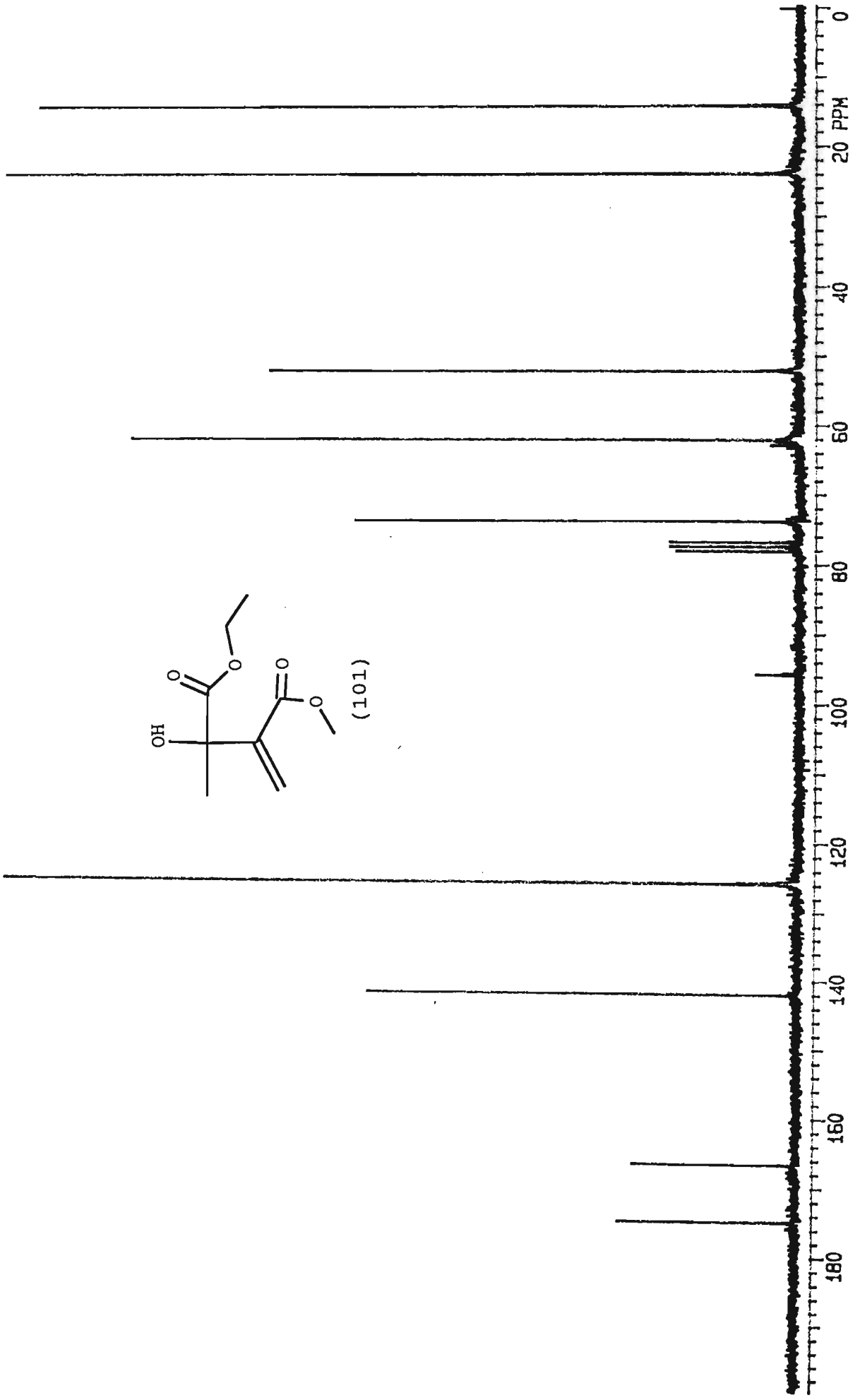
(98)



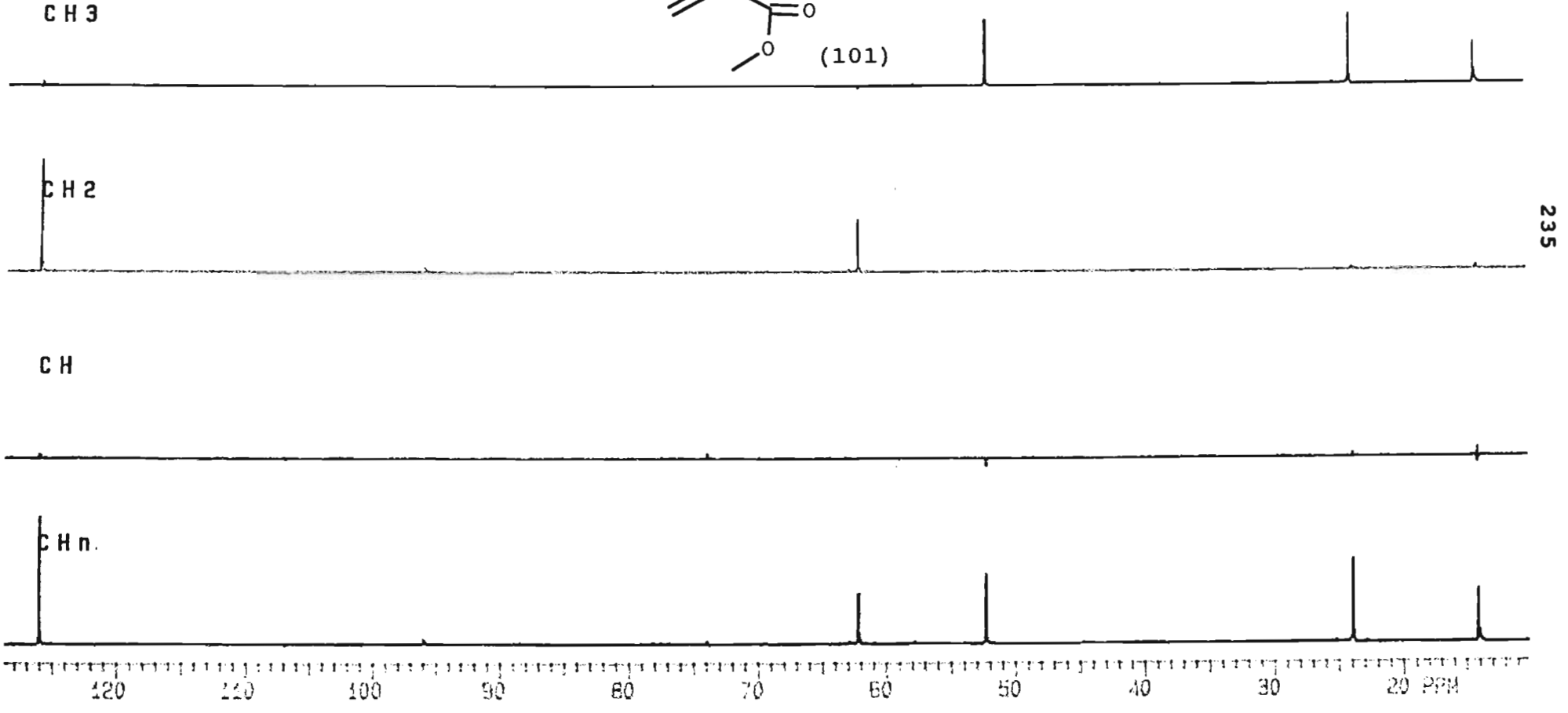
(98)

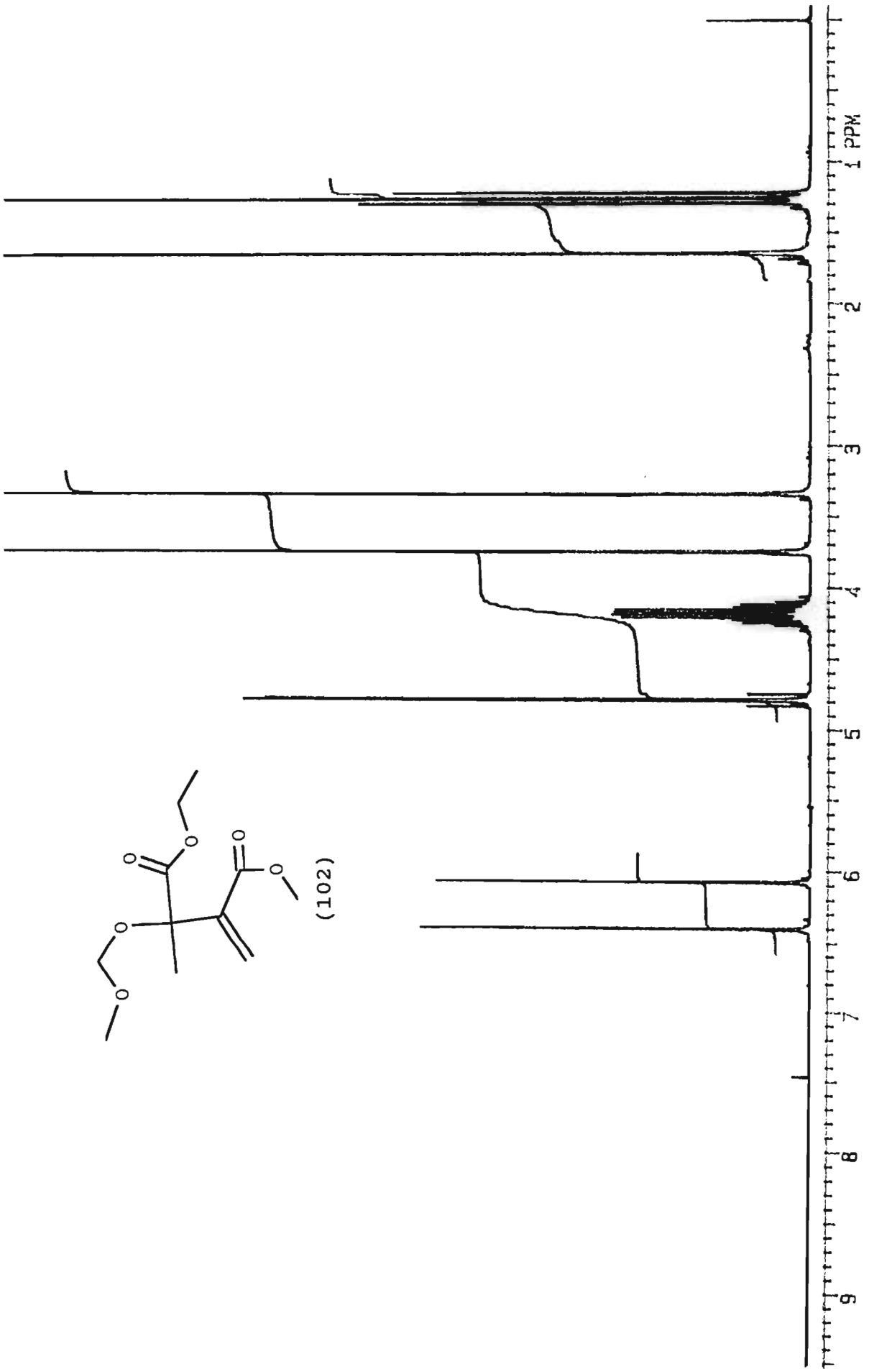

CH3
CH2
CH
CH(all)
(98)
70
60
50
40
30
20
10 PPM
0


OH
O
O
O
O
(101)
1 PPM
2
3
4
5
6
7
8
9

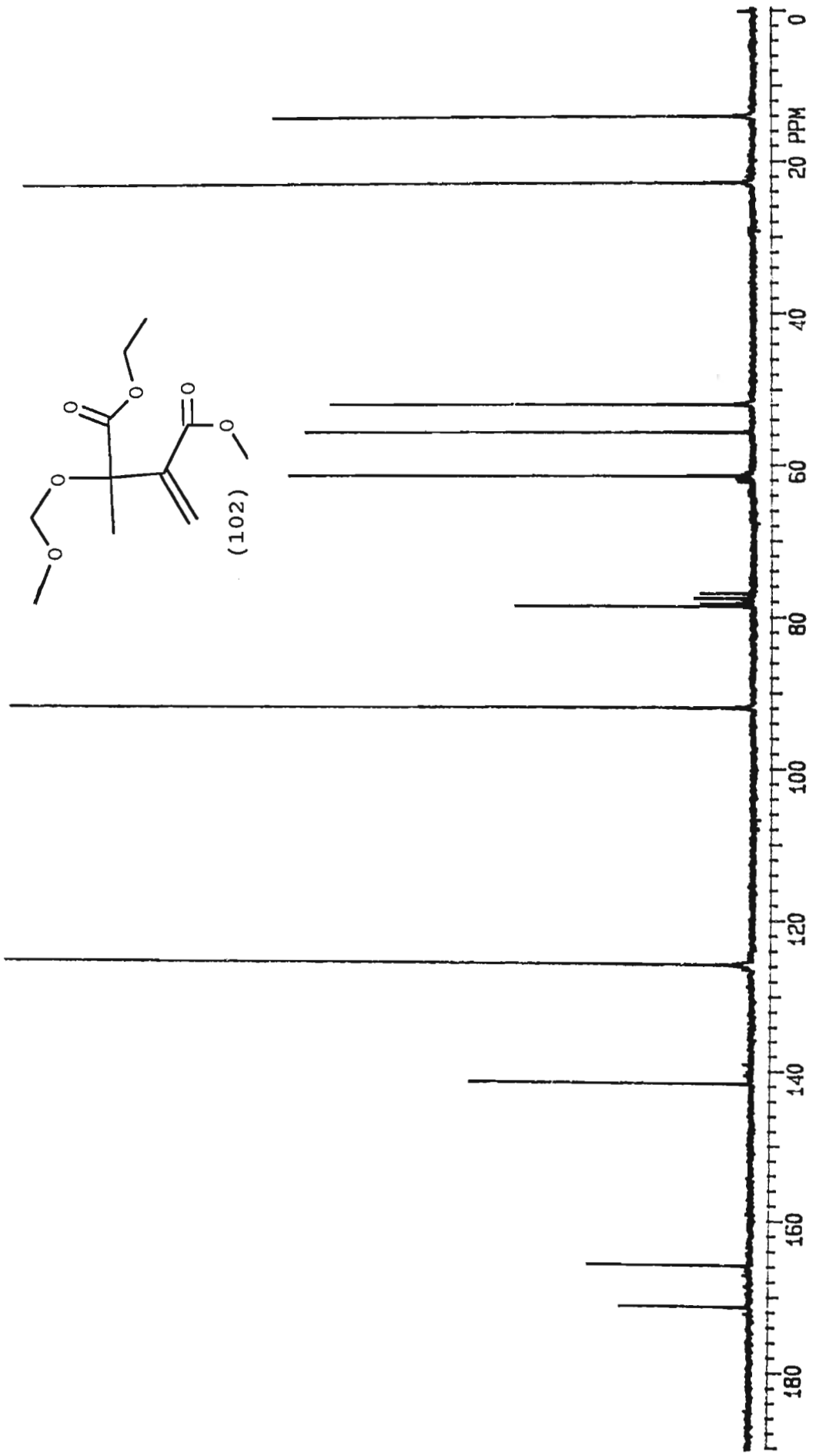

0
20 PPM
40
60
80
100
120
140
160
180
OH
O
O
O
O
(101)

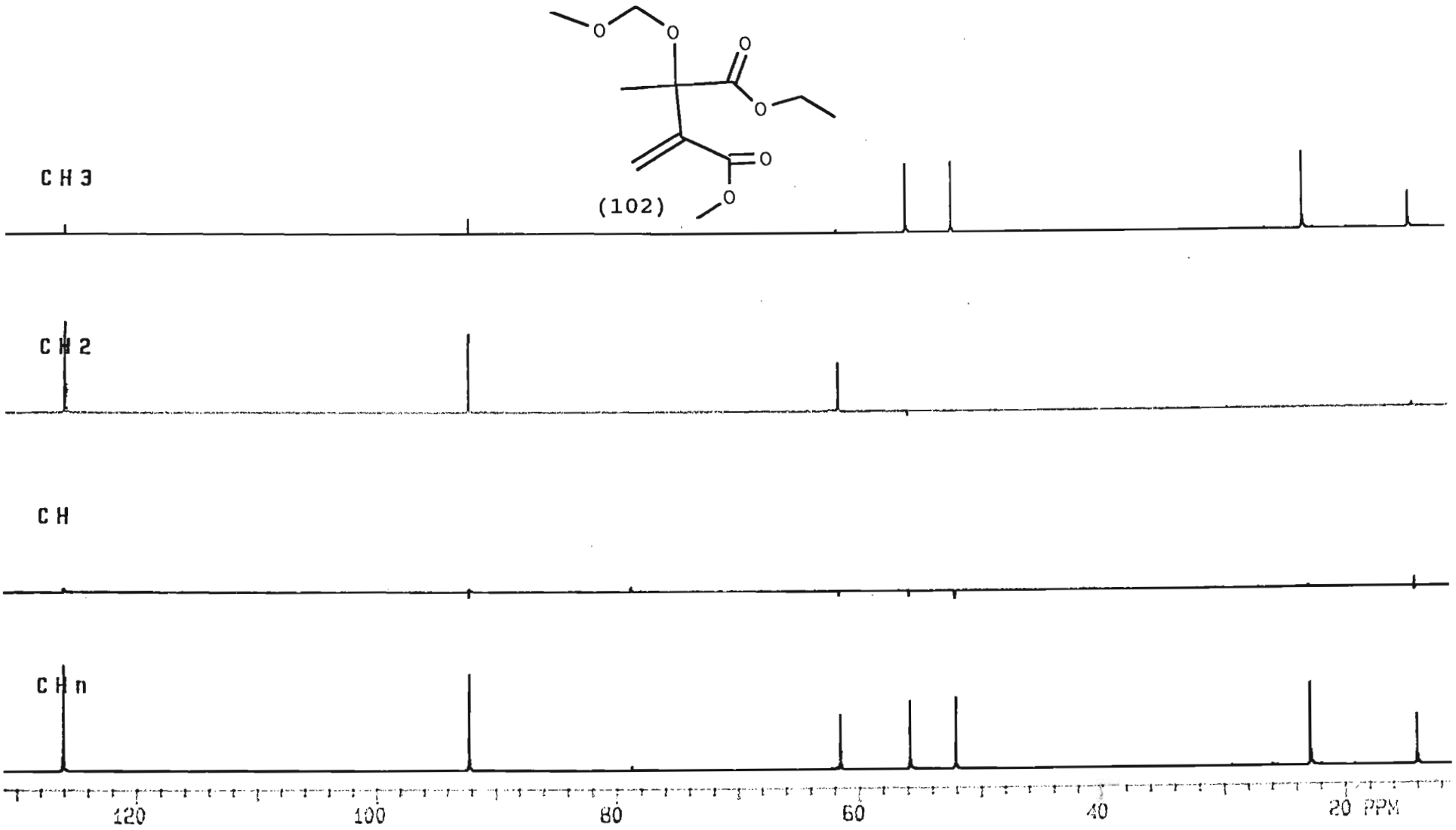
(101)





(102)


(102)
0
20 PPM
40
60
80
100
120
140
160
180



(102)