STEROIDAL AND TRITERPENOIDAL SAPOGENINS FROM RHOICISSUS REVOILII, SESBANIA KENIENSIS AND ALBIZIA GUMMIFERA FROM KENYA

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Work forming part of the requirements for the degree of Doctor of Philosophy in the University of Nairobi

February 1987

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

W. A. Odinga

W. A. Odinga

This thesis has been submitted for examination with our approval as University supervisors.

Helge Kjøsen

Dr.techn. Helge Kjøsen

R. Munavu

Prof. Raphael M. Munavu

To my parents

CONTENTS

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SUMMARY

A system has been developed for the fractionation, purification for the free triterpenes, sugars and steroids of the tuber of Rhoicissus revoilii PLANCH. (Vitaceae).

Hydrolysis of saponins using sodium periodate has been employed and found to be (Chapter 1) gentler than acid hydrolysis.

Structural elucidation of the main triterpenes employing modern chemical and instrumental techniques was successfully accomplished and the triterpenes concluded to be 3β, 23-dihydroxyolean-12-en-28-oic acid (hederagenin) and 2β, 3β-dihydroxyolean-12-en-23,28-dioic acid (medicagenic acid).

The two main steroids in the tuber of Rhoicissus revoilii have been fully analysed and found to be α-spinasterol and chondrillasterol.

The minor steroids detected in R.revoili tuber have been shown by mass spectrometry to be Δ^5-steroids.

The free sugar from R.revoilii tuber has been proved to be sucrose from mass and ^{1}H NMR spectroscopic analysis. Several free fatty acids have been detected from the same plant.

A scheme for the fractionation of Sesbania keniensis (Leguminoseae) seeds has also been developed. Purification of the principal triterpene and steroid in the seeds of Sesbania Keniensis has been accomplished. The triterpene was structurally elucidated and found to be oleanolic acid, while the main sterol was proved to be stigmasterol.

Finally, the stem bark of Albizia gummifera (Leguminoseae) has been investigated both for biological activity of the extracts and chemical constituent of the isolated sapogenin(s). The sapogenin was found to be oleanolic acid from direct spectral comparison with authentic oleanolic acid.

CHAPTER 1

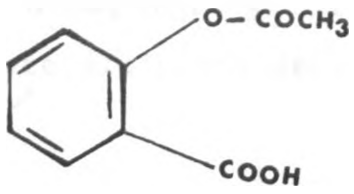
INTRODUCTION

1.0 General

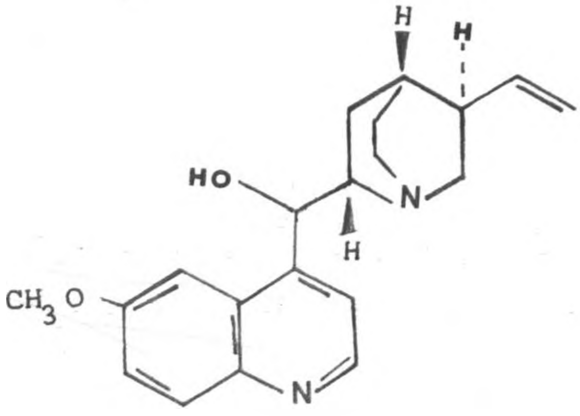
Plants have been used for medicinal purposes throughout man's known history and prehistory. In East Africa, the herbalist is known in Kiswahili as "Bwana Mganga" or medicine man. The use of herbs in Kenya has, however, been vilified with the adoption of Western medicines and the medicine men often arrested when caught administering plant drugs.

Recently, there has been a change in the general attitude towards local herbs, with a lot of focus and interest being directed at plant drugs. Herbalists are now invited by the government officials in Kenya to display their goods to the public [85NAT], with a campaign to let herbs be sold openly to the world at large in progress.

The importance of plant derived medicinals in modern medicine has often been unjustifiably underestimated. Useful compounds such as digitoxin, rutin, papain, morphine, codeine, papaverine, atropine, scopolamine, quinidine, reserpine, caffeine - to mention a few are all plant extractives with many pharmacological activities [66FAR]. The medicines most commonly used by the majority of the population; aspirin (acetyl salicylic acid) 1, extracted from the willow bark as salicylic acid and then converted to its acetate, quinine 2, from cinchona bark and curing or alleviating malaria, morphine 3, the principal

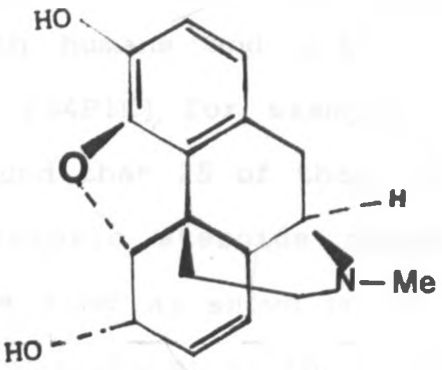


1. -Acetyl salicylic acid (aspirin)

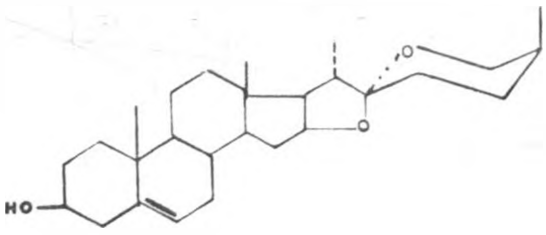


2 Quinine

extract of the opium poppy, an important analgesic, and the oral contraceptives derived from steroidal saponins e.g. diosgenin 4, from Dioscorea sp. (Dioscoreacea)., are all plant derived extractives.



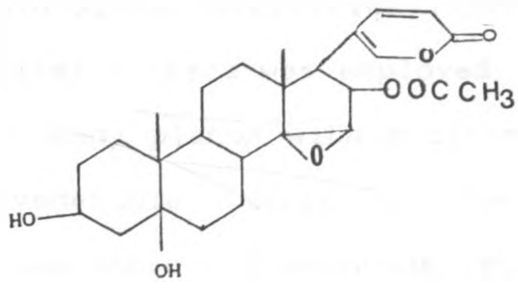
3 Morphine



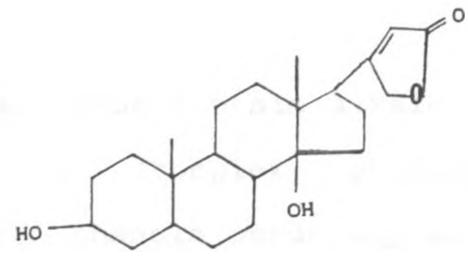
4 Diosgenin

Lack of adequate knowledge of the therapeutic value of plant drugs often leads to overdosage or poisoning. A knowledge of the chemical constituents is therefore desirable. Such knowledge might not only lead to synthesis of other useful derivatives from the isolated compounds, but would also reduce poisonings of both humans and animals by toxic plants. Pike et.al. [64PIK] for example, synthesised 150 steroids and found that 25 of them were cytotoxic. All of the 25 cytotoxic steroids contained an α,β -unsaturated lactone ring as shown in structures 5 and 6 whereas this moiety was absent in the steroids devoid of cytotoxicity.

Cytotoxic unsaturated lactones



5 Cinobufotalin



6 Digitoxigenin

The practice of modern medicine is to isolate the active principles and to discard the undesirables.

It has been established that in 1967 alone, 243 million prescriptions dispensed in the United States contained a secondary plant product as one or more of their active ingredients [73FAR].

Medicinal plants may be classified by three criteria [76KOK].

1) Botanically.
2) Purpose for which it is used.
3) Chemical constituents.

Several approaches are employed to select plants for investigation of their therapeutic value. The most commonly used involves selection of plants used locally as medicines. A second method involves phytochemical screening to establish the presence of certain chemical groups, while a third method is screening randomly selected plants for one or more biological activities [73FAR]. In this project, the first critera was employed.

Many plants with medicinal value contain alkaloids (vegetable bases), tannins (complex phenolic substances), flavonoids (plant phenols occurring both in the free state and as glycosides), saponins (glycosides comprised of one or more sugar units and a polycyclic aglycone or sapogenin), free sterols and triterpenoids.

This project is an effort at studying the chemical constituents of three indigeneous Kenyan medicinal plants, with an emphasis on saponins, free triterpenes and free steroids.

1.1 Plant Saponins

Saponins are widely distributed in the plant kingdom and have been reported to be present in at least 500 plant genera. Their name was derived from the fact that they form a strongly frothing solution with water like soaps. A number of methods have been designed to detect saponins in plant materials [64PER] [59GOT], [54WAL], [61WAL], [51CAI], [64AMA], [61PAS].

Saponins have several characteristic properties that can be used as a basis for simple detection tests:

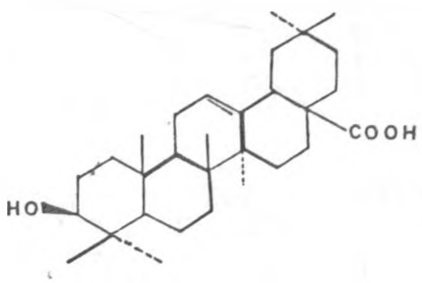
1) They are all capable of haemolyzing red blood cells.
2) In aqueous media they will produce a characteristic honeycomb froth which persists at least 30 minutes after vigorous shaking of the solution.
3) They are toxic to fish, causing paralysis of the gills.
4) They produce characteristic colour reactions in the Liebermann-Burchard test [69STA].

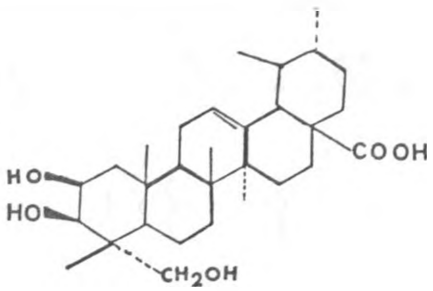
All these properties have been used in one or more screening tests for the detection of saponins in plant materials.

Saponins have been found not only to have emulsifying

to lower surface tension. They have the ability to alter permeability of the cell wall and therefore exert a general toxicity on all organized tissues. Most of the pharmacological activities and their use as fish poisons are based on this general toxicity. Recent studies [66FAR] on saponin induced haemolysis indicate that the corresponding sapogenin is the haemolyzing factor and that hydrolysis of the glycosidic bond precedes haemolysis.

Many saponins have an antibiotic activity, especially against common bacteria. Pharmacological activities include cardiotonic [64NAK], hypotensive [72GUS], [69CHA], anti-sclerotic [72GUS], anti-bacterial [69PET], anti-tumour [70KIM], anti-viral [68SUB], and anti-inflammatory [71KUB] activities. It has been found [63KUB], that a saponin containing the triterpene oleanolic acid 7 is strongly termiticidal while a saponin containing asiatic acid 8 has been found to possess leprosy curative properties [55SKI]. The biogenetic relationships of some triterpenes are shown in Fig. 1.

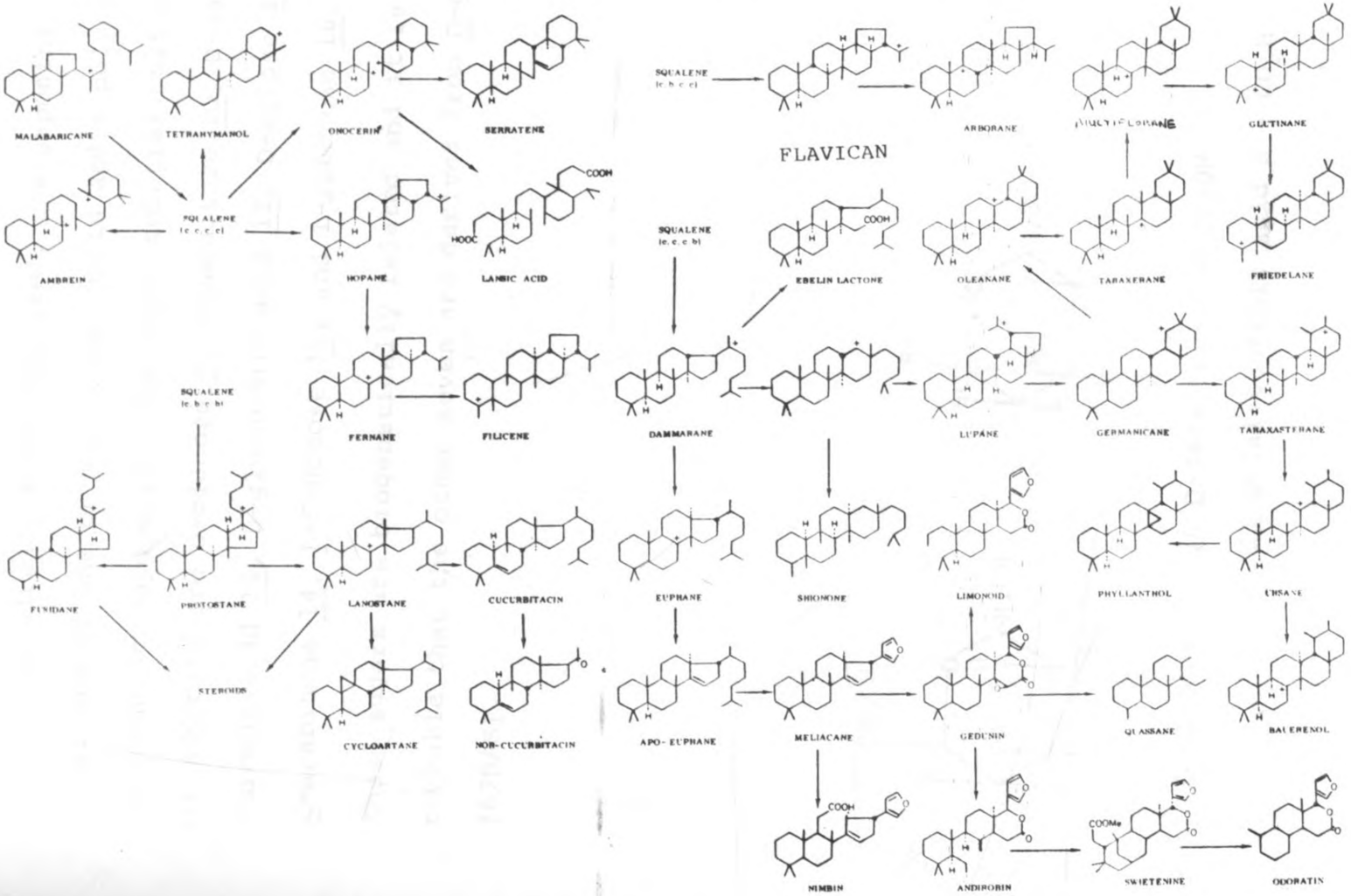




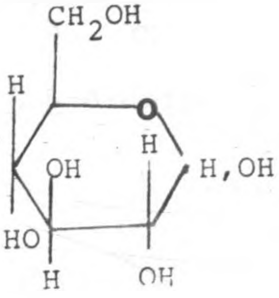
7 Oleanolic acid

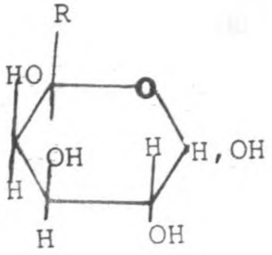
8 Asiatic acid

Figure 1. Some known triterpenoid skeletons [72DEV].



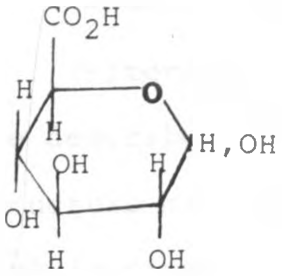
As has already been indicated, the saponins are comprised of one or more sugar units and a polycyclic aglycone or sapogenin. The sugar moieties most common in saponins are D-glucose 9, D-galactose 10, D-galacturonic acid 11, D-glucuronic acid 12, D-xylose 13, L-arabinose 14, L-fructose 15, and L-rhamnose 16. These sugars are biogenetically related and it is probable that the other seven are derived from D-glucose [67BAS].



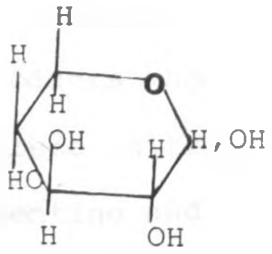


9 D-Glucose 10 D-Galactose R = CH_2OH

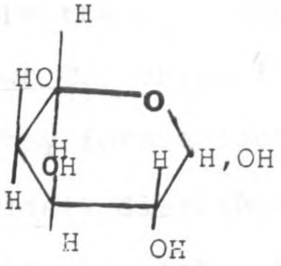
11 D-Galacturonic acid R = CO_2H



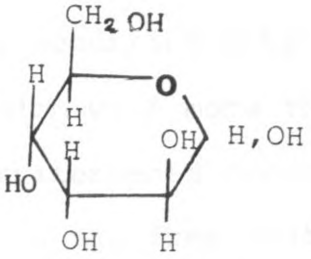
12 D-Glucuronic acid



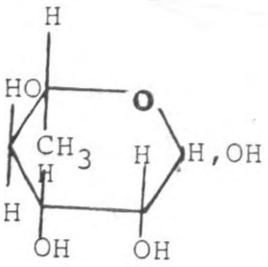
13 D-Xylose



14 L-Arabinose



15 L-Fructose



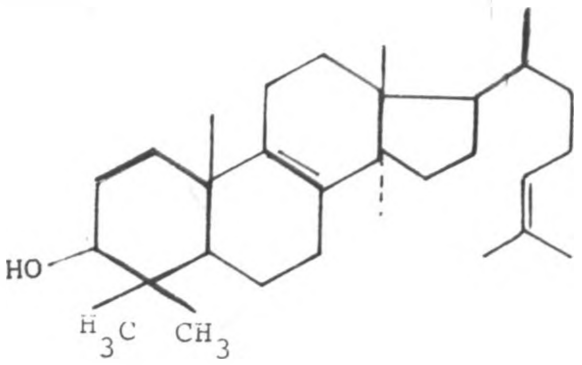
16 L-Rhamnose

The sapogenin has either a triterpenoidal or a steroidal structure.

1.11 Triterpenoidal sapogenins:

Triterpenes are a special group of terpenes, which are described as a group of natural products whose structure may be broken down into isoprene units. Their name is etymologically related to turpentine and alkene and are major components of essential oils of plants. They are classified as monoterpenes (C_{10} compounds), sesquiterpenes (C_{15} compounds), diterpenes (C_{20} compounds) sesterterpenes (C_{25} compounds) and triterpenes which are the C_{30} compounds. The triterpenes are made up of two C_{15} units linked in the middle tail to tail [72HAN]. They form a large group of naturally occurring substances widely distributed in the plant kingdom with more than 750 of established structure. The triterpenes occur in the plants as esters, glycosides or in the free state. A small but important group, including lanosterol 17, is of animal origin.

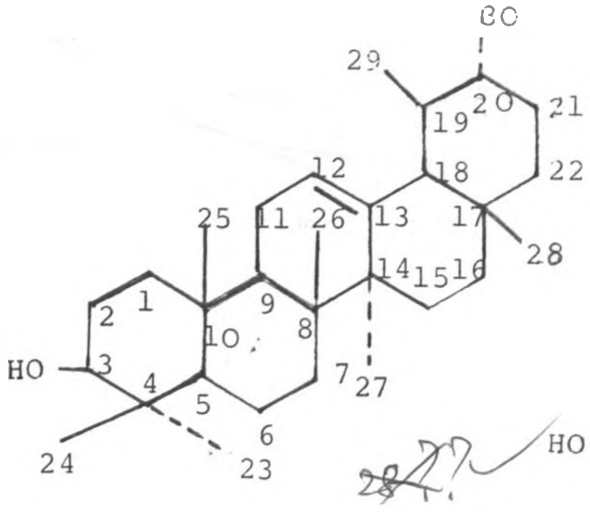
Nearly all triterpenes are polycyclic molecules produced by enzymic cyclization of squalene 18.



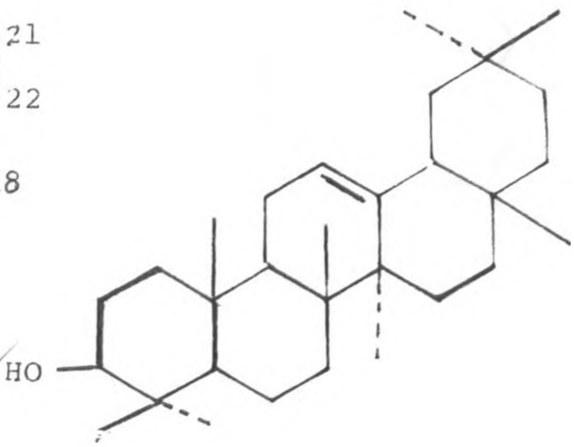
17 Lanosterol

Squalene epoxide from Squalene (18), the immediate biogenetic precursor of all triterpenoids. Triterpenes can either be acyclic, dicyclic, tricyclic, tetracyclic or pentacyclic [83SHI], [72CON].

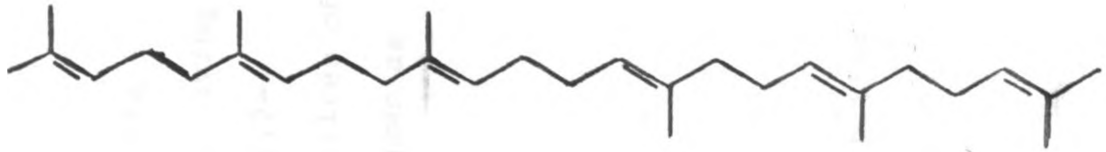
The majority of the triterpenoid sapogenins are pentacyclic, and fall into the lupane/amyrin group as exemplified by α-amyrin 19 (ursane series), β-amyrin 20 (oleanane series) and lupeol 21 (lupane series), whose carbon atoms are numbered as shown in the structures below.



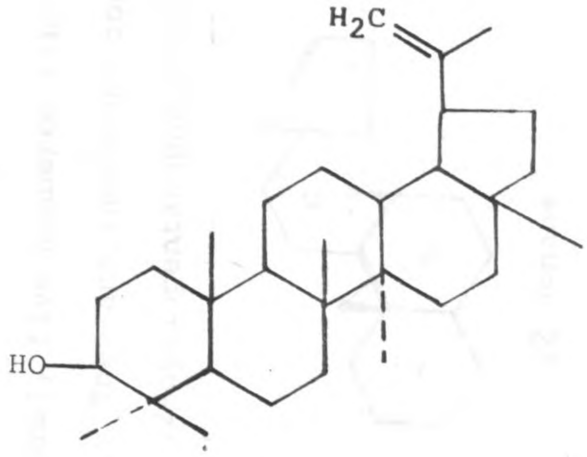
19 α-amyrin



20 β-amyrin



18 Squalene (hexa-ene all trans squalene)

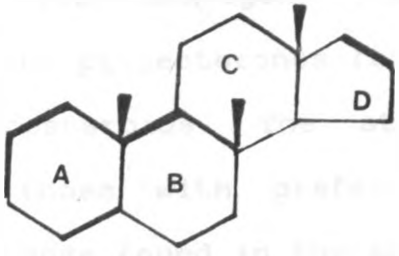


21 Lupeol

Pentacyclic triterpenoid acids of these series are derived through processes in which one or more angular methyl groups are oxidized to a carboxyl group(s), the most common positions being at C4, C17 and C20.

1.12 Steroidal sapogenins

Steroids are characterized structurally by the presence of a saturated phenanthrene ring system having an additional, five membered ring fused at the 1,2-positions. They are therefore considered derivatives of perhydro-1,2-cyclopentanophenanthrene, $C_{17}H_{28}$ known as gonane 22.



22 Gonane

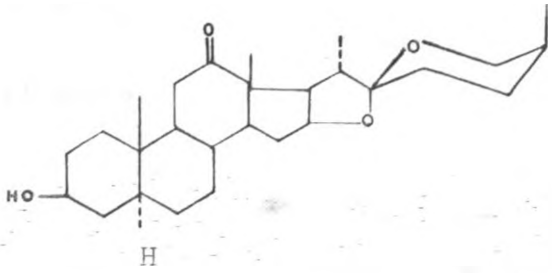
Steroids could be classified as triterpenes, but as sufficient skeletons of steroidal nature are existing, they are generally classified and discussed separately. Biologically they are derived from the tetracyclic triterpenes lanosterol 17 and cycloartenol through a sequential oxidation-decarboxylation of the methyl groups at C-14 and C-4.

Whereas triterpenoidal sapogenins occur both as glycosides and as free triterpenes, steroidal saponins are rarely found as free sapogenins in plant material [66FAR] [52WAL].

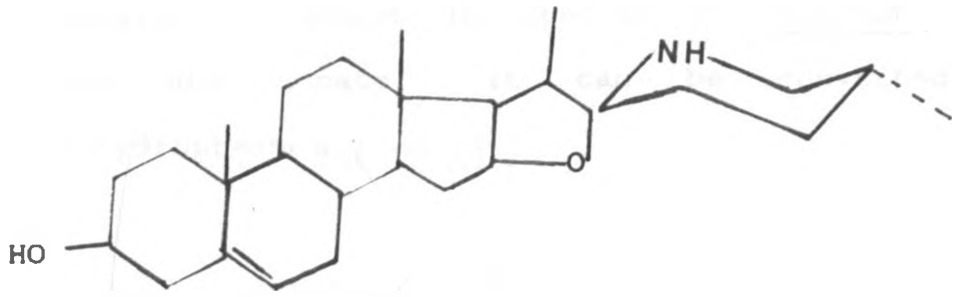
Steroids play a very important role in the manufacture of drugs. In terms of drugs derived from higher plants, steroids are by far the most important group, exceeding the combined usage of drugs such as codeine, atropine, ephedrine, digitoxin and others [77FAR].

The natural steroid hormones found in the body are classified into androgens (the male sex hormones), estrogens and progesterones (the female sex hormones), and corticosteroids. The steroid drugs used are generally those with preferential or more potent action on those found in the body.

The main steroidal sapogenin precursors in the production of other steroids are diosgenin 4, hecogenin 23 and solasodine 24, the latter an example of a steroidal alkaloid which are steroids in which nitrogen is incorporated in the sterol framework.



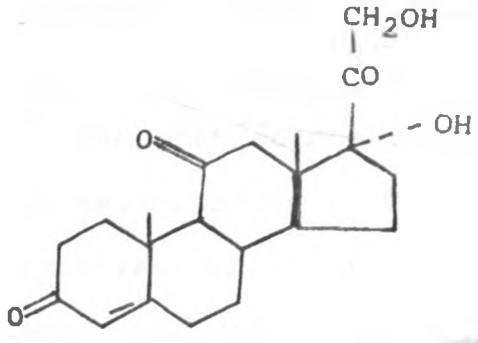
23 Hecogenin $C_{27}H_{42}O_4$.



24 Solasodine $C_{27}H_{43}O_2N$

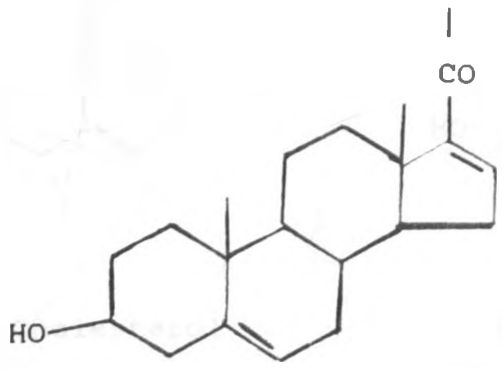
Diosgenin is mostly extracted from the genus Dioscorea (the yam) found in Mexico and Guatemala and is used mainly in the production of oral contraceptives.

Hecogenin in commercial supply is obtained from Agava sisalana Perrine, sisal, of East African origin. It is used mainly in the production of corticosteroids, e.g. cortisone 25



25 Cortisone

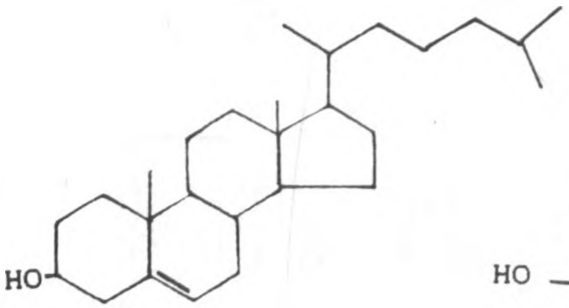
Solasodine is found in species of Solanum, e.g. potato and tomato. It can be converted to 16-dehydropregnenolone 26



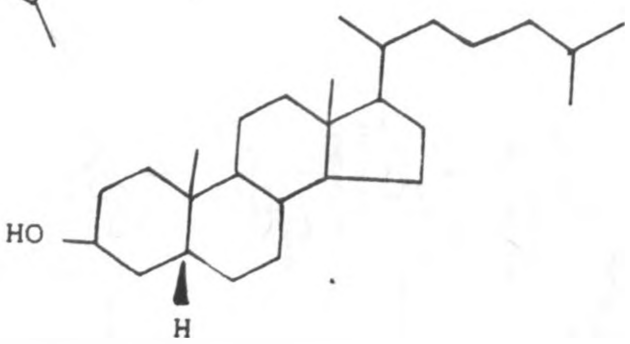
26 16-Dehydropregnenolone

Sterols are crystalline alcohols deriving their name from the greek word Stereos, meaning solid + ol. They are found in the non-saponifiable matter of plants and animals, and have the characteristic steroid skeleton, with a β-hydroxyl group at C3 and an open side chain on C_{17}.

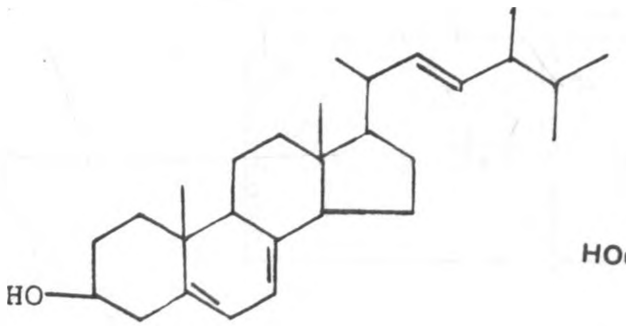
The most common sterol is cholesterol found in all animal tissues. The sterols of animal organism, typified by cholesterol 27 and coprostanol 28, all have the same skeletal structure of twenty-seven carbon atoms. The principal plant sterols, typical examples of which are ergosterol 29 and stigmasterol 30, are either C_{28}- or C_{29} compounds.



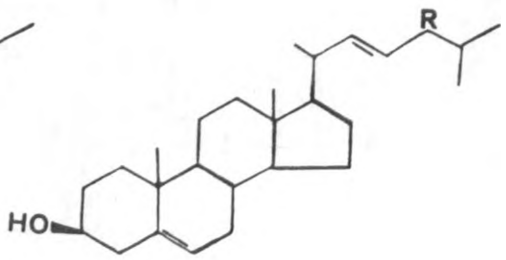
27 Cholesterol



28 Coprostanol

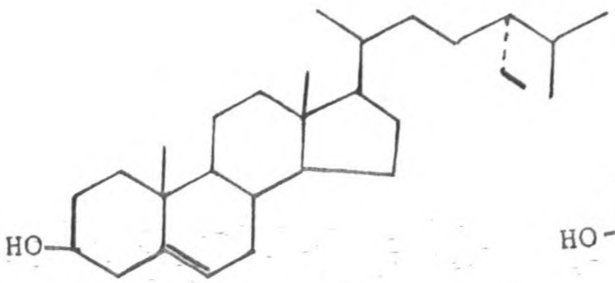


29 Ergosterol

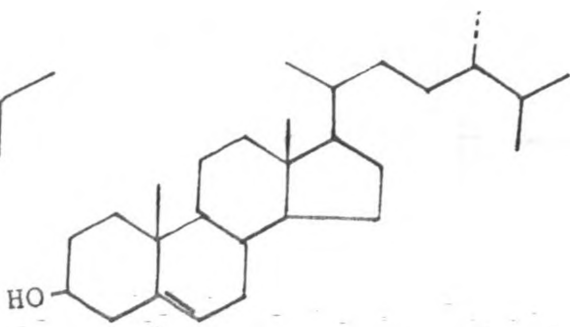


R = 24α-ethyl.

30 Stigmasterol



31 Sitosterol



32 Campesterol

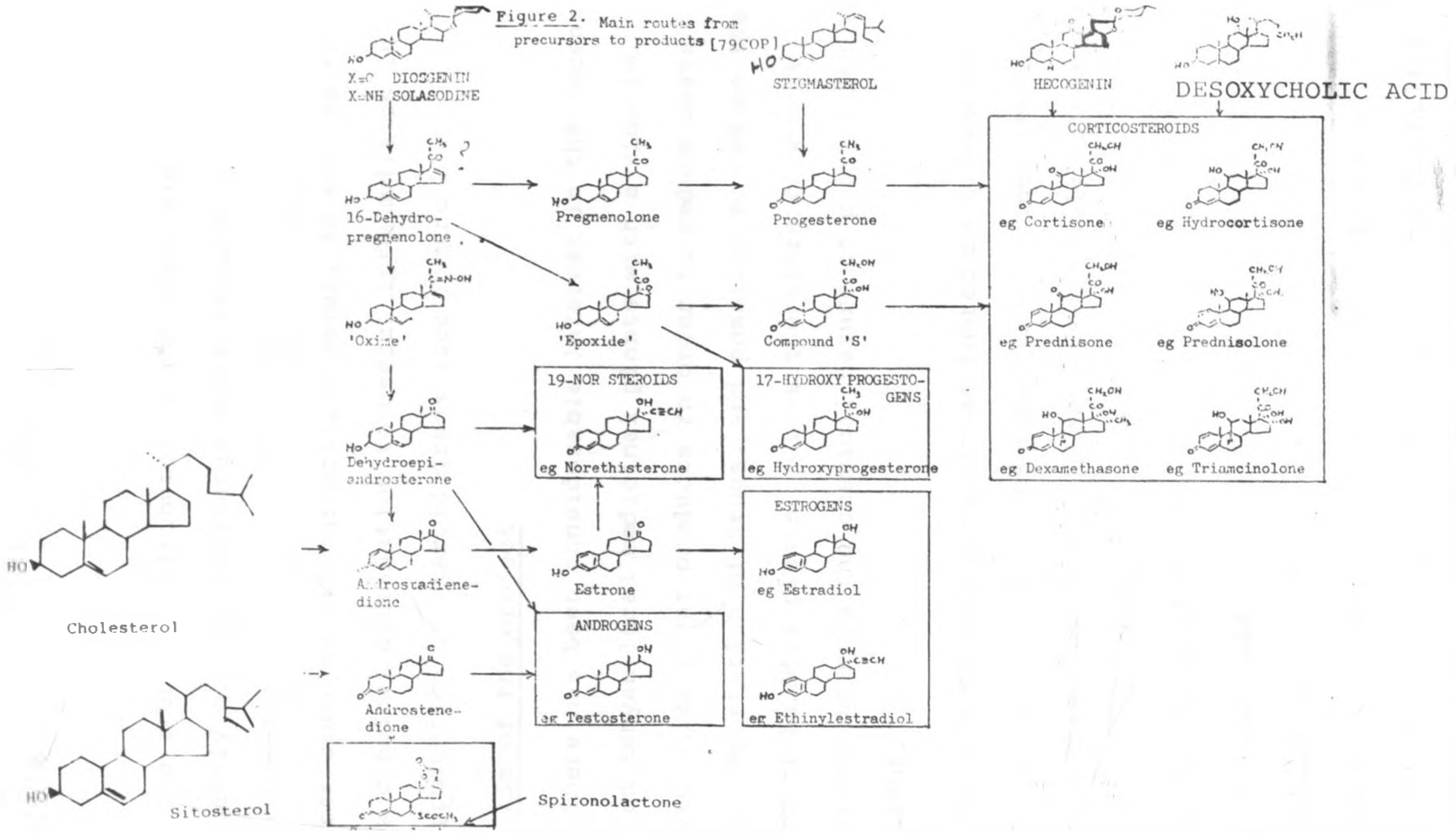


Figure 2. Main routes from precursors to products [79COP]

The sterols distributed in the plant kingdom frequently occur as mixtures where sitosterol 31, campesterol 32 and stigmasterol 30 are common. Only the structure of stigmasterol, however, is such as to allow chemical degradation to useful intermediates in the production of steroid drugs (see Figure 2).

1.2 Aim of the project

There has been considerable interest in the recent past on Kenyan local medicinal plants with a view to commercialize plant products in order to reduce costs due to importation of drugs, and possibly to reduce the intake of harmful plants. It is therefore of utmost importance to know the chemical nature of plants used as medicines.

The aim of this project was therefore to extract, purify and to elucidate the structures of the chemical components of three Kenyan plants used widely as medicines, namely:

Rhoicissus revoilii PLANCH.

Sesbania keniensis

Albizia gummifera (Gmel) C.A. Sm var.

Their local use as milk stimulants and/or for treatment of female related diseases led to the belief that they may contain steroidal sapogenins.

Isolation of the plant products was to be carried out by extracting the different parts of the plants used

as medicines with organic solvents of differing polarities. Separation of the compounds was to follow using chromatographic techniques on the crude products or on their derivatives. Structural elucidation of the isolated compounds was to follow by spectroscopic analysis of the isolated compounds, their derivatives and/or their degradation products prepared in the laboratory. Biological activity tests were to be carried out wherever it was possible.

CHAPTER 2

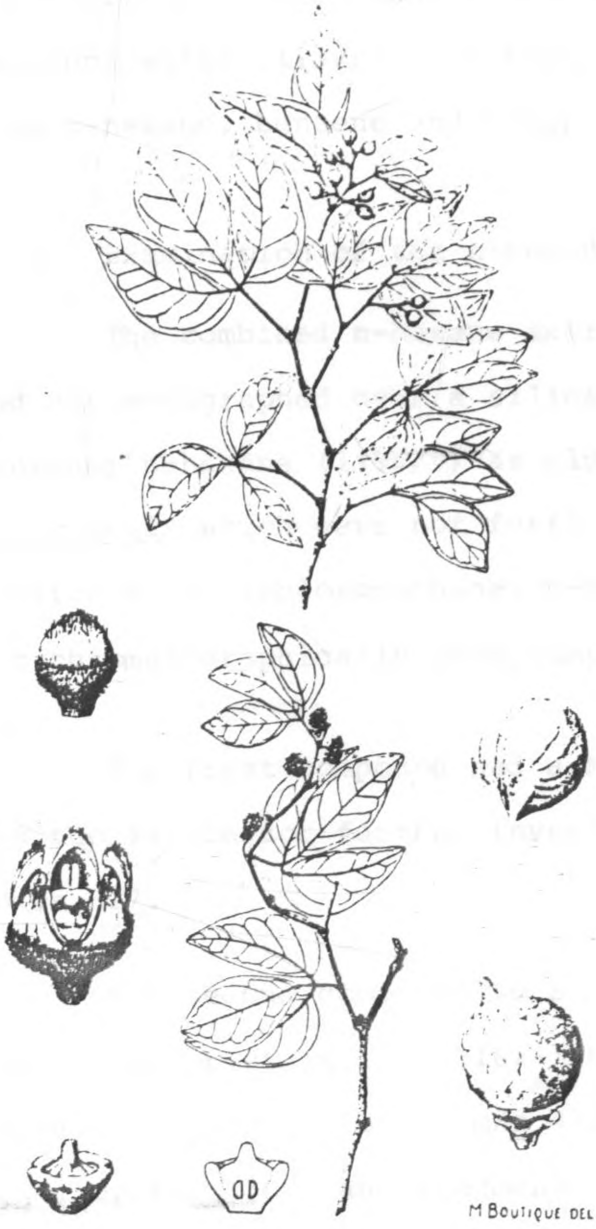
CHEMICAL INVESTIGATION OF RHOICISSUS REVOILII PLANCH

TUBER

2.0 General

Rhoicissus revoilii is a member of the plant family Vitaceae. It is a creeper fairly common in the Ngong Hills and in Karura Forest in Kenya. The leaves are thick and succulent. The tuberous root is generally used for treating wounds, menstrual pains and mixed with milk to feed a calf whose mother is producing little milk and stimulate milk production in cows [76KOK]. The tuberous root is normally pounded, boiled in water and strained before administration. No previous work on the chemical constituents of R. revoilii has been reported. When boiled in water, it formed a strong frothing solution which led to the suspicion that it contained saponins. The local use as a cure for menstrual pains and as a stimulant for producing milk suggested that it may contain either hormonal steroids or steroids which are biogenetically convereted to hormonal steroids in the body.

Fig A.



Rhoicissus Revoilii PLANCH.

2.1 RESULTS AND DISCUSSION

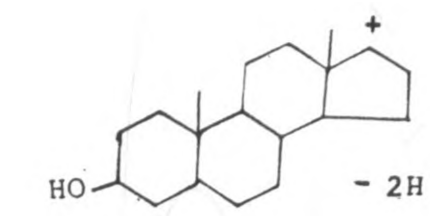
The dried ground tubers of Rhoicissus revoilii PLANCH were extracted with n-hexane and methanol successively. The methanol extract was redissolved in methanol/water (1:3v/v) and partitioned three times with n-hexane, benzene and chloroform respectively.

2.11 Examination of the n-hexane extract

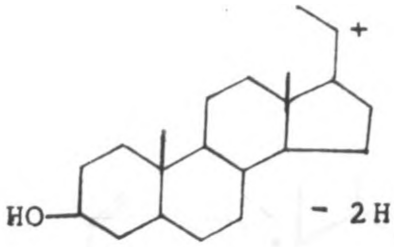
The combined n-hexane extracts were evaporated and chromatographed over a silica gel column using benzene; n-hexane (1:9v/v) as eluant to give a mixture of fatty acids which were not further investigated. Elution with dichloromethane: n-hexane (1:9v/v) gave two chromatographically pure compounds.

The first compound had a Molecular mass 586 but was too little for further investigation and is referred to as H27.

The second compound to be eluted gave white needlelike crystals, m.p. 162-166^{o}. The mass spectrum showed a molecular ion at m/z 412.3701 (calc. for $C_{29}H_{48}O$, 412.3705) and fragment ions at m/z 300 and 271 suggesting that the compound is a steroidal alcohol with two double bonds, one in the side chain while the other is located in one of the rings.



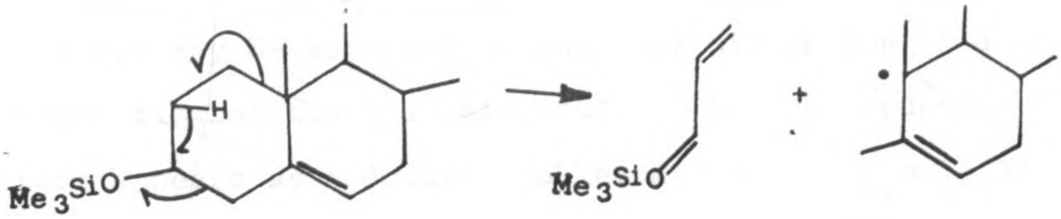
m/z 271



m/z 300

Formation of a monoacetate (M^{+}. at m/z 454) and a monotrimethylsilyl-ether (M^{+}. at m/z 484) under standard acetylation and silylation conditions respectively, confirmed its monohydroxy function, most likely at C3 from biosynthetic considerations. The 24-ethyl substitution of the side chain follows from the mass spectrum which shows losses of 15, 29, 43 and 85 mass units at m/z 397, 383, 369 and 327 respectively. The lack of fragmentation units between the losses of 43 and 85 units indicates a branch point at C24.

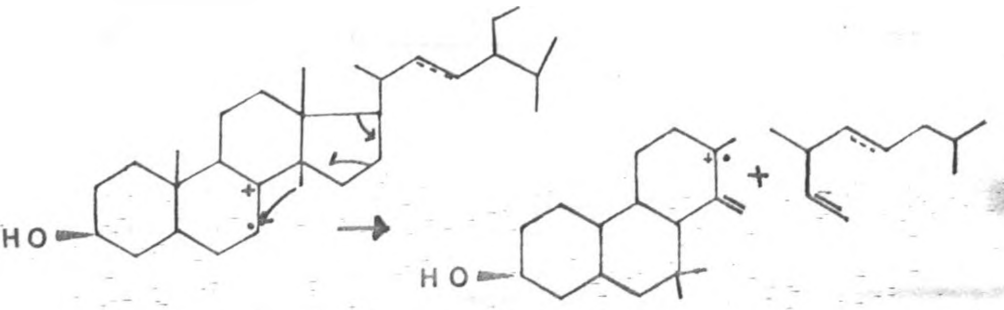
The mass spectra of 3-trimethylsilyloxy-Δ^5 steroids normally show an intense peak at m/z 129 ascribed to the following sequence [67DIE].



m/z 129

No intense ion at m/z 129 was observed in the mass spectrum of the trimethylsilyl ether of the compound. It can therefore not have a 3-hydroxy-Δ^5 structure.

The mass spectrum of the compound shows a moderately intense ion at m/z 246.1988 (calc. for $C_{17}H_{26}O$, 246.1984). Such an ion is normally observed for 3-hydroxy-Δ^7-steroids both with saturated and unsaturated side chains [76ZAR] probably due to the following sequence.



Further evidence for the Δ^7-unsaturated nature comes from the ^{1}H NMR spectrum at 400 MHz of the acetate. A one proton multiplet centered at δ4.69 is in agreement with a 3β-acetoxy function. A one proton double doublet at δ5.021 (J_1= 15.38, J_2= 8.61Hz) may be ascribed to one olefinic proton of a trans disubstituted double bond (Δ^{22}). The counterpart appears as a double doublet at δ5.159 (J_1= 15.38, J_2= 9.23Hz) superimposed on a multiplet at δ5.14 together integrating for two protons. The multiplet at δ5.14 is in agreement with published values for Δ^7-steroids [84GAR], [58SHO], [79SUC], [73ARM] and suggest that the compound is either spinasterol (24S) 33 or chondrillasterol (24R) 34.

Table 1 and 2 give direct comparisons of ^{13}C- and ^{1}H NMR data for spinasterol, chondrillasterol, the isolated sterol and their acetates.

The great similarities in the physical properties of α-spinasterol and chondrillasterol (Table 3) necessitates availability of both reference compounds for GC Co-injection for an unambiguous identification of the sterols from R.revoilii tuber.

Table 1. ^{13}C NMR chemical shifts (δ) of spinasterol, chondrillasterol and the isolated sterol from R.revoilii

Carbon	α-Spinasterol		Chondrillasterol	sterol from R.revoilii.
1		36.8		37.09
2		27.4		28.44
3		73.4		71.03
4		33.8		34.23
5		40.0		40.25
6		29.5		29.61
7		117.3		117.42
8		139.4		138.13
9		49.2		49.44
10		34.2		34.23
11		21.4		21.36
12		39.5		39.44
13		43.2		40.78
14		55.0		55.11
15		23.0		23.00
16	28.5		28.4	28.441
17		55.8		55.87
18		12.0		12.00
19		12.9		12.99
20		40.8		4.78
21	21.1		20.9	21.07
22		138.1		138.13
23		129.4		129.41
24		51.2		51.19
25		31.8		31.83
26	21.4		19.0	21.36
27	19.0		21.4	18.964
28		25.4		
29	12.2		12.4	12.177

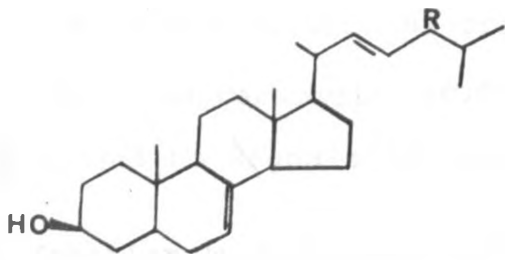
Table 2 ^1H NMR of α-spinaterol, Chondrillasterol, R.revoilii sterol and their acetates.

	C-18 3H,s	C-19 3H,s	C-21 3H,d (J)	C-26/27 3H,d (J)	C-26/27 3H,d (J)	C-29 3H,t (J)	C-3 1H,m	C-7 1H,B_s	C-22/23 1H,dd (J)	C-22/23 1H,dd (J)
α-Spinasterol [84GAR]	0.552	0.800	1.027 (6.5)	0.800 (6.2)	0.851 (6.1)	0.805 (7.3)	3.598	5.172	5.027 (ca.7.5)	5.162 (ca.7.5
Chondrillasterol [84GAR]	0.550	0.800	1.030 (6.5)	0.825 (6.5)	0.845 (6.3)	0.799 (7.2)	3.597	5.172	5.029 (ca 7.5)	5.162 (ca 7.5)
R.revoilii sterol	0.553	0.803	1.027 (6.1)	0.79 (7.0)	0.842 (7.0)	0.809 (7.3)	3.53	5.152	5.019	5.152
α-Spinasteryl-acetate [76SUC]										
[73ARM]	0.55	0.81	1.03 (6.6)	0.80 (7)	0.85 (6.5)	0.81 (6.5)	4.70	5.15	4.97	5.20
Chondrillasteryl-acetate [79CAT]										
[76SUC]	0.54	0.81	1.03 (6.5)	0.79 (7)	0.84 (6.5)	0.81 (7.2)	4.69	5.15	4.97	5.20
R.revoilii sterol acetate	0.548	0.813	1.027 (6.68)	0.800 (6.48)	0.8501 (6.42)	0.806 (7.3)	4.69	5.14	5.02 (15.38,8.61)	5.159 (15.38,9.23)

Table 3: Physical properties [48BER], [50BER], (40ELS) of α-spinasterol, chondrillasterol, their acetates and those of the isolated sterol.

	Melting point	Optical rotation $(\alpha)_D$
α-spinasterol	172-175°	-3°
α-Spinasteryl acetate	185-187°	-5°
Chondrillasterol	168-169°	-2°
Chondrillasteryl acetate	175-176°	-1.0°
R. revoilii sterol	162-166°	
acetate	170-174°	

GLC analysis of the acetate of the sterol, however, showed two peaks with retention times at 43.55 and 44.50 minutes leading to the conclusion that the sterol was a mixture of α-spinasterol 36 and chondrillasterol 37, in the ratio 1:2.



33 R = 24α-ethyl

34 R = 24β-ethyl

Further elution of the column with dichloromethane:ethyl acetate (9:1v/v) and then with ethyl acetate gave a column fraction with one spot on TLC.

This fraction had a series of molecular ions at 14 mass unit intervals from m/z 452.4596 (calc. for $C_{30}H_{60}O_2$, 452.4593) to m/z 368.3660 (calc. for $C_{24}H_{48}O_2$, 368.3668).

A peak at m/z 60 due to McLafferty rearrangement is characteristic for straight chain monocarboxylic acids, unbranched at the α-carbon [81SIL].

The mass spectrum consisted of two series of peaks resulting from cleavage of each C-C bond, with retention of charge either on the oxygen-containing fragment at m/z 45, 59, 73 and so on, or on the alkyl fragment at m/z 29, 43, 57 onwards, constituting clusters at

intervals of 14 units, typical fragmentation pattern for long chain monocarboxylic acids. The ^{1}H NMR spectrum showed no signals for wax esters.

The fraction was therefore deduced to consist of a mixture of seven long chain carboxylic acids of the following structures:

1) $CH_3(CH_2)_{28}COOH$ m/z 452.4596 (calc. for $C_{30}H_{60}O_2$, 452.4593) $(M)^+$.

2) $CH_3(CH_2)_{27}COOH$ m/z 438 $(M)^+$.

3) $CH_3(CH_2)_{26}COOH$ m/z 410.4128 (calc. for $C_{28}H_{56}O_2$, 424.4280 $(M)^+$.

4) $CH_3(CH_2)_{25}COOH$ m/z 410.4128 (calc. for $C_{27}H_{54}O_2$, 410.4124) $(M)^+$.

5) $CH_3(CH_2)_{24}COOH$ m/z 396 $(M)^+$.

6) $CH_3(CH_2)_{23}COOH$ m/z 382 $(M)^+$

7) $CH_3(CH_2)_{22}COOH$ m/z 368.3660 (calc. for $C_{24}H_{48}O_2$, 368.3654 (M).

2.12 Examination of the benzene extract.

The benzene extract was subjected to a silica gel column and eluted successively with the following solvent systems:

1) Fractions 1-25 methanol: chloroform (1:0v/v).

2) Fractions 26-40 methanol: chloroform (1:9v/v).

3) Fractions 41 onwards methanol: chloroform (2:3v/v).

Most of the benzene fractions were obtained in too small amounts to allow more than limited analysis. Some of these are listed in a tabular form (Table 4). They are discussed according to their elution order, as BI - B8.

BI showed an ion at m/z 530.4699 (calc. for $C_{35}H_{62}O_3$, 530.4693). A major peak at m/z 412.3701 (calc. for $C_{29}H_{48}O$, 412.3705) indicates BI to contain spinasterol and chondrillasterol of R. revoilii and other higher molecular weight compounds. This is further confirmed by a fragment at m/z 271.2062) showing BI to contain one double bond in the side chain, and another in the nucleus. A characteristic peak at m/z 246.1986 (calc. for $C_{17}H_{26}O$, 246.1984) is in agreement with a 3-hydroxy-Δ^7 steroid [76ZAR]. The 3-trimethylsilyloxy derivative of BI showed a major peak at m/z 484 leading to the conclusion that BI has a main nucleus

Table 4. TLC and some mass spectral information on the benzene fractions of the methanol extract.

Fraction	Observation on TLC	Designation	Other information obtained
6	1 spot, R_f 0.63	BI	m/z 530.4699 (calc. for $C_{35}H_{62}O_3$, 530.4693)
7	1 spot, R_f 0.56	B2	m/z 412 with higher homologues to 440. Silylether m/z 484.
8-9	2 spot		
10-12	1 spot, R_f 0.40	B3	m/z 414.3861 (calc. for $C_{29}H_{50}O$, 414.3862) Steroid?
13-16	1 spot, R_f 0.28	B4	m/z 486, methylester, m/z 500, diacetate 584.3718 (calc. for $C_{35}H_{52}O_7$, 584.3713) Triterpene.
17-19	2 spots	-	
20-23	1 spot, R_f 0.20	B5	m/z 544.4135 (calc. for $C_{34}H_{56}O_5$, 544.4128) Triterpene.
24-25	2 spots	-	
26-29	1 spot, R_f 0.14	B6	m/z 574.4243 (calc. for $C_{35}H_{58}O_6$, 574.4233)
30-34	2 spots	-	
35-40	1 spot, R_f 0.09	B7	m/z 548
41-46	2 spots	-	
47	1 spot, R_f 0.04	B8	m/z 578

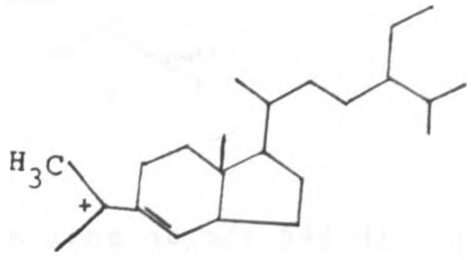
identical to α-spinasterol and chondrillasterol.

B2 had a molecular ion at m/z 412 but with a higher homologue of two methylene groups up to m/z 440 (72THO). A major peak at m/z 354, lacking in that of spinasterol indicates that B2 is different from it. A peak at m/z 246 confirms B2 to be a Δ^7 unsaturated steroid. The TMS ether of B2 had a molecular ion at m/z 484 with two higher methylene groups up to m/z 512 showing B2 to contain one hydroxy group. B2 was deduced to possibly contain the molecule of spinasterol or chondrillasterol with two more methylene groups attached at C24.

The mass spectrum of B3 showed a molecular ion at m/z 414.3861 (calc. for $C_{29}H_{50}O$, 414.3862) and a peak at 412.3705 (calc. for $C_{29}H_{48}O$, 412 3705). GLC analysis of B3 showed two major peaks indicating B3 to be a mixture of two components, possibly a mono and a diunsaturated analogue. An interesting observation occurred when the acetate of B3 was prepared. No molecular ion was observed, an indication that B3 is a Δ^5-unsaturated steroid which have been found to contain no molecular ion in their acetates [67KNI].

The peak at m/z 246, characteristic for Δ^7 unsaturated steroids, is noticeably missing in the mass spectrum of B3. Further confirmation of Δ^5

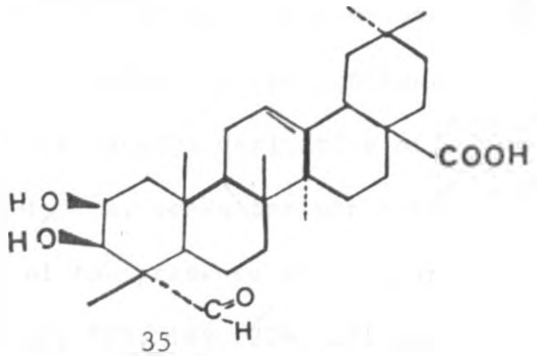
unsaturated nature of B3 is shown by a peak at m/z 303 characteristic of Δ^5 monounsaturated steroids due to the formation of the following fragment [67KNI].



B3 could therefore be a mixture of sitosterol 31 and stigmasterol 30.

The mass spectrum B4 showed a molecular ion at m/z 486 and peaks at 248 and 302 indicating that B4 is a pentacyclic triterpene with a 12-13 double bond with a carboxy group in the DE rings [63DJE]. Preparation of the methyl ester gave a product with a molecular ion at m/z 500 showing B4 to contain one carboxy group. Acetylation of the methyl ester under standard conditions gave a compound with a molecular ion at m/z 584, 3718 (calc. for $C_{35}H_{52}O_7$, 584.3713) confirming B4 to be a dihydroxy compound, with the two hydroxyl groups being located in the AB-rings.

Considering the presence of hederagenin and medicagenic acid in the plant extract, B4 is likely to be 2β, 3β-dihydroxy-23-oxo-olean-12-en-28 oic acid 35.



35

B5 has ions at m/z 544.4135 (calc. for $C_{34}H_{56}O_5$, 544.4128), 454.3082 (calc. for $C_{29}H_{42}O_4$, 454.3083) and at m/z 454.3440 (calc. for $C_{30}H_{46}O_3$, 454.3447). The mass spectral fragmentations show peaks at m/z 248 and 203 indicating B5 to contain a pentacyclic triterpene of the α- or β-amyrin series with a double bond on the 12-13 carbons. The composition of the ions indicated B5 to be a mixture of different compounds and contains one or more pentacyclic triterpene(s). No further purification of B5 was possible. When B5 was methylated, the methyl ester showed no molecular ion, although there were peaks at m/z 486, 468, 262 and 203, further confirmation of presence of a pentacyclic triterpene.

B6 had a molecular ion at m/z 574.4243 (calc. for $C_{35}H_{58}O_6$, 574.4233). A strong peak at m/z 412.3701 (calc. for $C_{39}H_{48}O$, 412.3705) is characteristic for a C29 steroid.

When B6 was acetylated, a product was formed with a molecular ion at m/z 742, leading to the conclusion that B6 had four hydroxyl groups. The tetraacetyl derivative of B6 showed peaks at m/z 43, 103 and 145, typical peaks for peracetylated sugars [64BUD]. Confirmation of the presence of a sugar molecule was seen from the peaks at m/z 109, 169, 229, 271 and a very strong peak at m/z 331 in the mass spectrum of the acetylation product of B6, all characteristic for peracetylated hexosides.

B6 also had a strong ion at m/z 255.2118 (calc. for $C_{19}H_{47}$, 255.2113) characteristic for Δ^7 steroids [67KNI]. A peak at m/z 271 signifies two double bonds, one in the nucleus and the other located in the side chain of the molecule.

From these data, B6 was deduced to be a steroidal hexoside.

B7 had a molecular ion at m/z 548 with fragmented ions at m/z 339, 313, 311 57 and 55. This portion could be a glycoside.

B8 had a series of molecular ions at m/z 578, 564 and at 550 at 14 mass unit intervals with a fragmentation pattern similar to that of carboxylic acids. An ion at m/z 60 is in agreement with a McLafferty rearrangement product for straight chain carboxylic acids. However, when B8 was acetylated, a product was formed which showed no molecular ion, but contained characteristic ions at m/z 331, 211 and 169 for sugars. B8 could be a

mixture of straight chain carboxylic acids and a sugar.

2.13 Examination of the chloroform extract.

The chloroform extract was triturated with methanol to form a precipitate which, when filtered and washed with hot acetone, showed one spot.on TLC. Sodium periodate hydrolysis of this followed by chromatographing over a silica gel column with ethyl acetate: dichloromethane(2:3v/v) as eluant afforded two chromatographically pure compounds.

The first compound to be eluted were white needle like crystals which showed a molecular ion at m/z 502 with a base peak at m/z 248 indicating a pentacyclic triterpene of the β- or α-amyrin series with a carboxyl group situated in the D/E ring with a 12-13 double bond [63DJE]. Methylation of the compound gave a product, m.p. 220-224°, with a molecular ion at m/z 530.3602 (calc. for $C_{32}H_{50}O_6$, 530.3607) confirming the presence of two carboxyl groups in the molecule. Acetylation and trimethylsilylation of the dimethyl ester under normal conditions formed a diacetate, m.p. 221-224°, with a molecular ion at m/z 614 and a disilyl ether with M^+ at m/z 674 respectively, confirming the compound to be a dihydroxy compound.

The ions at m/z 248 in the mass spectrum and that at m/z 262 in the mass spectra of the dimethyl ester and of the dimethyl ester diacetate of the compound indicate that the two hydroxyls and the second carboxyl group are in the A/B rings.

From the Distortionless Enhancement of NMR signals by Polarization Transfer (DEPT) spectrum [82DOD], there are no primary hydroxyl groups present (see Figure 14). The ^{1}H NMR spectrum of the dimethyl ester shows six three proton singlets, attributable to methylgroups at δ 0.725, 0.897, 0.926, 1.127, 1.205 and 1.367.

Two three proton singlets at δ 3.617 and 3.725 (S, 3H) confirm the presence of two methyl ester groups.

A one proton multiplet at δ 5.294 (m, 1H) is characteristic for a vinylic proton and is consistent with presence of a 12, 13 double bond [62SHA]. While the axial 2β-proton is expected to have a large ax-ax coupling with the 1α-proton and two small ax-eq couplings with the 1β- and C3-protons [72CHE], the equatorial 2α-proton is subject to small ax-eq couplings with all three 1α-, 1β- and C3-protons. The small signal width (W 1/2= 11.3 Hz) of the C2-proton at δ 4.18 in the ^{1}H NMR spectrum of the

The hydroxyl group on C2 is therefore in an axial position. Since triterpenes have a fixed conformation, an axial hydroxyl group on C2 can only be in the β-orientation.

The relative stereochemistry of the hydroxyl group on C3 cannot be distinguished from the ^1H NMR spectrum. To ascertain the orientation of the C3 hydroxyl an acetonide was prepared. A product with a molecular ion at m/z 570 was formed indicating successful formation of the acetonide under normal conditions. This confirms that the C3 and the C2 hydroxyls are cis to each other, i.e., both are in the β-orientation.

An AB quartet at δ 2.86 (J = 5.6 Hz, J = 11.2 Hz) is consistent with oleanene type compounds [72CHE]. Further confirmation of the positions of the hydroxyl groups was done by decoupling the C2 proton at δ 5.464 of the diacetate on a 400 MHz ^1H NMR instrument. The C3 proton collapses into a singlet. Decoupling of the vinylic proton signal at δ 5.294 had no effect on either the C3 or the C2 signals. Decoupling of the C2 proton at δ 4.18 of the dimethyl ester results in the doublet at δ 4.017 of the C3 proton collapsing into a singlet. The two hydroxyl groups are concluded to be vicinal in the C2 and C3 positions.

Comparison of the given literature data [69CHE] of the methyl signals (Table 5) of the dimethyl ester diacetate and calculated data for those of the dimethyl esterare in agreement with those of 2β, 3β-diacetoxy olean-12-en-23, 28-dioate and 2β, 3β-dihydroxy olean-12-en-23, 28-dioate (dimethyl medicagenate). Confirmation of the position of the second carboxyl group was done by oxidation and decarboxylation of the monomethyl ester of the compound [57DJE]. When the dimethyl ester was heated with 10% methanolic potassium hydroxide, the product oxidised and decarboxylated, a product was formed with a molecular ion at m/z 470 indicating successful saponification and decarboxylation of one carboxyl group. The second carboxyl can only be in the 23 or 24 positions [57DJE].

Successful formation of a lactone would indicate the second carboxyl to be on the C24 position. An attempt was therefore made to form a lactone. The product obtained did not indicate successful formation of a lactone, indicating the second carboxyl group to be located on C23. Nuclear Overhauser Effect (NOE) experiments irradiating at δ 1446.56 Hz and 1489.46 Hz gave no response also indicating a C23 carboxyl moiety.

Table 5 Calculations of methyl resonance signals [69CHE].

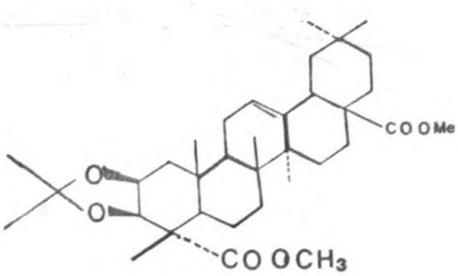
Skeleton	Substituents				Resonance Frequencies of methyl					
	2α	3β	23	23	24	25	26	27	29	30
Methyloleanan-12-en-28-oate	OH	OH	$COOCH_3$	-	1.142	0.958	0.70	1.133	0.866	0.875
	OH	αOH	$COOCH_3$	-	1.208	0.958	0.683	1.117	0.867	0.875
	βOH	βOH	$COOCH_3$	-	1.317	1.22	0.717	1.125	0.85	0.878
	βOH	αOH	$COOCH_3$	-	1.40	1.208	0.708	1.125	0.892	0.90
	Found for R.revoilii dimethyl medicagenate			-	1.367	1.205	0.725	1.127	0.897	0.926
Methyloleanan-12-en-28-oate	βOAc	OAc	$COOCH_3$		1.375	1.183	0.716	1.116	0.883	0.90*
	βOAc	αOAc	$COOCH_3$		1.392	1.158	0.716	1.125	0.883	0.90
	OAc	αOAc	$COOCH_3$		1.242	1.067	0.683	1.117	0.875	0.883
	OAc	αOAc	$COOCH_3$		1.258	1.058	0.683	1.117	0.867	0.875
	Found for R.revoilii dimethyl medicagenate diacetate				1.402	1.205	0.730	1.127	0.906	0.926

*Data was published by Cheung et al [69CHE].

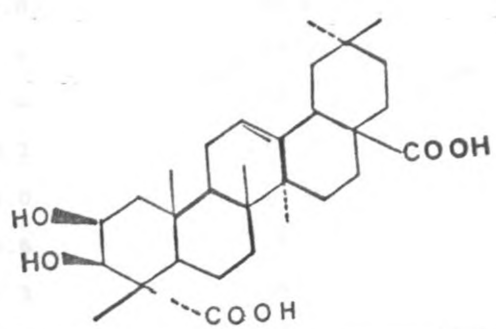
The ^{13}C NMR spectra of both the free acid and that of it's dimethyl ester show great similarities with the reported literature data for both medicagenic acid and its dimethyl ester (see Table 6).

From the given and calculated methyl resonance signals, the dimethyl ester of the isolated triterpene could be either 2β, 3β-dihydroxy olean-12-en-28,23 dioate or 2β, 3α-di-hydroxy olean-12-en-23,28 dioate. Formation of the acetonide indicates the compound to be medicagenic acid, as formation of the acetonide 36 would not be possible had it been 2β, 3α-dihydroxy olean-12-en-23,28-dioate.

The triterpene isolated from R.revoilii is therefore concluded to be 2β, 3β-dihydroxy olean 12-en-23, 28 dioic acid (medicagenic acid) 37.



36. Acetonide



37. Medicagenic acid

Table 6. ^{13}C NMR of isolated medicagenic acid and of its dimethylester as compared to the literature data (83YUN)

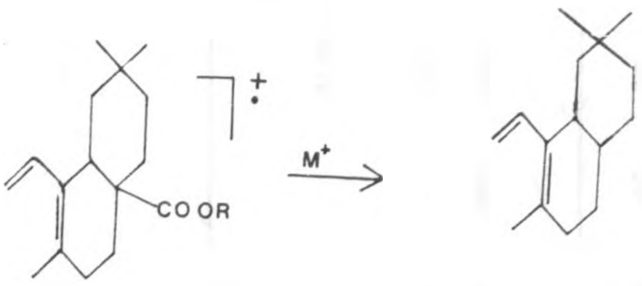
	Medicagenic acid	Medicagenic acid from R.revoilii	Monomethyl medicagenate	dimethyl medicagenate from R.revoilii
1	44.9	45.79	44.2	43.7
2	71.4	70.84	69.9	70.8
3	75.6	75.67	75.6	75.7
4	52.0	52.27	50.2	51.2
5	53.7	53.12	52.1	52.2
6	21.3	20.89	21.0	20.9
7	33.2	33.32	33.2	33.1
8	40.1	39.56	40.1	39.6
9	48.7	48.09	48.6	48.3
10	36.7	37.3	36.6	36.3
11	23.7	23.57	23.7	23.6
12	122.4	122.02	122.5	122.0
13	144.7	143.76	144.7	143.8
14	41.8	41.70	42.0	41.7
15	28.1	27.91	28.0	27.6
16	23.7	23.57	23.4	23.4
17	46.5	46.62	46.5	46.6
18	41.8	41.70	42.3	41.7
19	46.5	46.62	46.5	46.6
20	30.8	30.64	30.0	30.6
21	34.1	33.66	34.4	34.0
22	33.2	33.03	33.4	33.1
23	179.9	178.2	179.1	178.3
24	13.4	14.07	14.0	15.0
25	16.8	16.65	16.6	16.6
26	17.2	16.65	17.3	16.7
27	26.1	26.1	26.0	26.0
28	180.7	178.2	180.9	178.3
29	33.2	33.32	33.2	33.8
30	23.7	23.57	23.7	23.6
			$COOCH_3$	$COOCH_3$
			52.1	52.2

The second compound to be isolated from the chloroform extract gave white plate like crystals, m.p. 331-333° which showed a molecular ion at m/z 472.3542 (calc. for $C_{30}H_{48}O_4$, 472.3553) and an ion at m/z 454.3440 (calc. for $C_{30}H_{46}O_3$, 454.3447) for the loss of water. The base peak at m/z 248 indicates a pentacyclic triterpene with a 12, 13 double bond [63DJE] suggesting an ursane or oleanane skeleton with a carboxy group located somewhere in the D/E rings. Methylation with diazomethane gave a monomethyl ester (M^+ at 486) which in turn formed a monomethyl ester diacetate (M^+ at m/z 570) under normal acetylation conditions showing the compound to be dihydroxy monocarboxylic acid.

An AB quartet (J_1 = 5.6 Hz, J_2 = 11.2 Hz) at δ 2.78 in the ^1H NMR spectrum of the methyl ester is characteristic of the 18β-hydrogen of a 17β-carboxy methyl oleanolate [62SNA], [72CHE] and a one proton multiplet at δ 5.28 confirm the 12, 13 double bond 62SHA . Furthermore a two proton singlet at δ 3.42 and a one proton multiplet at δ 3.87 indicate one primary and one secondary hydroxyl groups.

The ^1H NMR spectrum of the diacetate methyl ester shows a two proton AB quartet centered at δ 3.78 (J = 11.2 Hz) confirming a primary acetoxy function, and a one proton multiplet (W 1/2 = 19.7 Hz) at δ 4.78 suggesting an axial methine proton at C-3 [70YO1], [70YO2].

That both hydroxy functions are located in the AB rings follows from the mass spectral fragmentations of the acid itself and from that of its methyl ester and methyl ester diacetate which show the characteristic retro-Diels-Alder cleavages of the C-ring at m/z 248, 262, and 262 respectively, all fragmenting further to m/z 203.



R = H m/z 248

R = CH_3 m/z 262

m/z 203

The close proximity of the two hydroxy functions follows from the formation of an acetonide (M^+ at m/z 526).

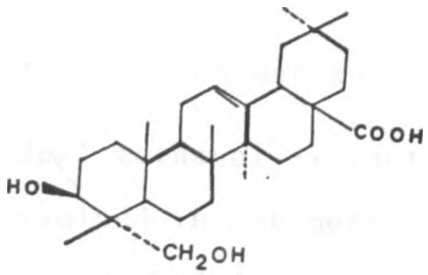
Comparison of the given literature data of both the ^1H NMR methyl resonance frequencies and the ^{13}C NMR spectra (see Tables 7 and 8) confirms the triterpene to be hederagenin (3,23-dihydroxyolean-12-en-28-oic acid) 38.

Table 7. Calculations from Cheung T. et.al [72CHE].

Skeleton	Substituents				Resonance frequencies of methyl groups					
	3β	23	24	23	24	25	26	27	29	30
Methyloleanan-12-en-28 oate	OAc	OAc	H	-	0.842	0.967	0.742	1.12	0.90	0.933
	OAc	H	OAc	0.992	-	0.992	0.717	1.142	0.933	0.941
Found for methyl hederagenate from R.revoilii				-	0.833	0.965	0.73	1.117	0.902	0.926

Table 3. ^{13}C NMR chemical shifts (σ) of methyl hederagenate from R.revoilii as compared to the literature data [74TOR]. [81AOK] of the methylester of hederagenin 38.

	Hederagenin		Observed for methyl hederagenate from R.revoilii
Carbon	[74TOR]	[81AOK]	
1	38.1	38.7	38.094
2	26.4	27.4	26.744
2	76.4	73.7	76.238
4	41.7	42.6	41.78
5	49.7	48.7	49.736
6	18.5	18.5	18.437
7	32.4	32.7	32.361
8	39.3	39.6	39.556
9	47.5	47.9	47.571
10	36.9	37.1	36.924
11	23.1	23.7	23.000
12	122.2	122.9	122.221
13	143.6	144.1	143.691
14	41.7	41.9	41.78
15	27.7	28.0	27.622
16	23.4	23.3	23.351
17	46.7	46.9	46.635
18	41.3	41.7	41.253
19	45.9	46.0	45.816
20	30.7	30.7	30.664
21	33.9	33.9	33.765
22	32.4	32.7	32.361
23	71.3	68.4	72.084
24	11.6	12.8	12.294
25	15.7	15.9	15.629
26	16.9	17.1	16.682
27	26.0	26.1	25.925
28	178.2	179.9	178.207
29	33.1	33.1	33.063
30	23.6	23.7	23.583
28-OCH_3	51.6	28-OCH_3 51.4	28-OCH_3 51.549
15.1 MHz		22.6 MHz	25 MHz
CD_3OD solvent		C_5D_5N solvent	$CDCl_3$ solvent



38 Hederagenin

Acid hydrolysis of the chloroform extract, on TLC comparison with the sodium periodate hydrolysis product showed the same major products, but with some minor products, possibly artefacts produced during hydrolysis. Sodium periodate hydrolysis was therefore a gentler and cleaner method.

The methanol soluble portion of the chloroform extract was chromatographed over a silica gel column and eluted with methanol: chloroform (1:9)v/v) to afford a compound showing one spot on TLC. Recrystallization of this from ether gave white crystals of molecular mass 524.3509 (calc. for $C_{33}H_{48}O_5$, 524.3502) as the major component and an ion at 524.3147 (calc. for $C_{32}H_{44}O_6$, 524.3138) as a minor component. The ^{1}HNMR spectrum was dominated by a signal at δ 1.25 for $-(CH_2)_{\bar{n}}$, making it difficult to make a meaningful deduction of the structure of this compound and is referred to asCF1 in the experimental part.

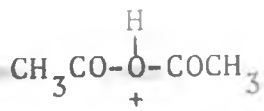
2.14 Examination of the methanol water residue:

The residual methanol extract was a pink powder which was redissolved in hot methanol, the insoluble portion filtered, washed with acetone and chloroform to afford white crystals, mp186-188°.

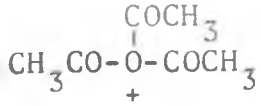
The mass spectrum shows no molecular ion but has fragments at m/z 311 and 293 for the loss of water and a strong fragment at m/z 163.

When acetylated, a product was formed which also showed no molecular ion in the normal spectrum. When magnified and expanded, it showed a molecular ion at m/z 678 and a strong peak at m/z 331 indicating a tetraacetyl hexose residue.

The base peak at m/z 43 for acetylium ion CH_3CO^+ and the two peaks at m/z 103 and 145 for diacetyl (a) and triacetyl (b) oxonium ions



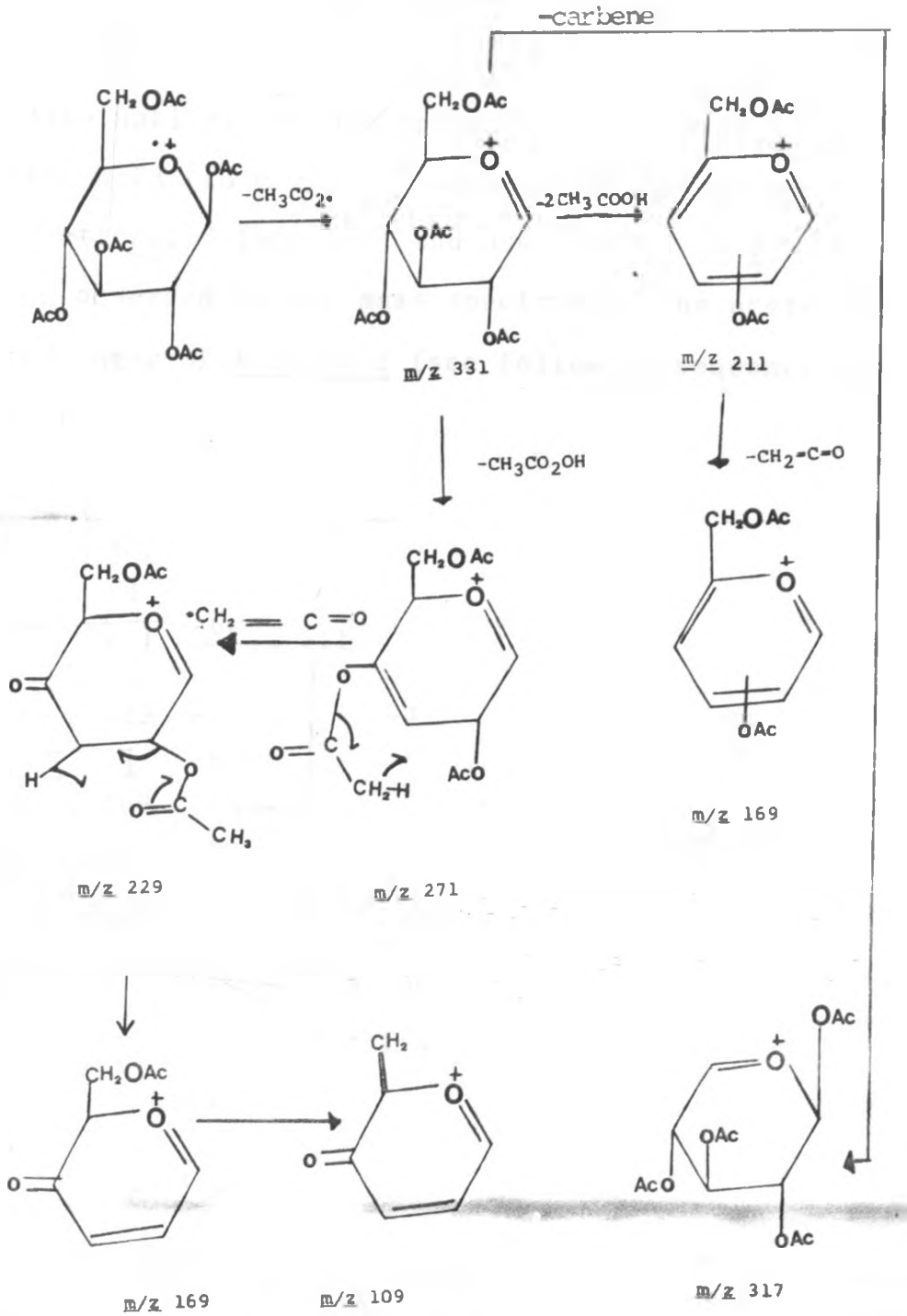
(a) m/z 103



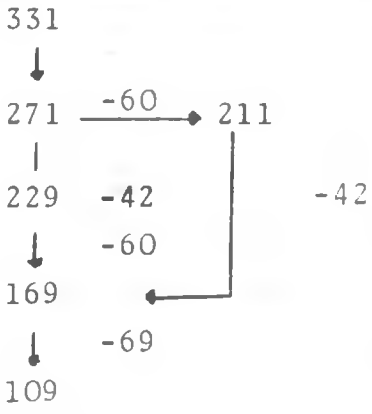
(b) m/z 145

in the mass spectrum of the peracetylated product are in agreement with published spectra for peracetylated sugars [64BUD].

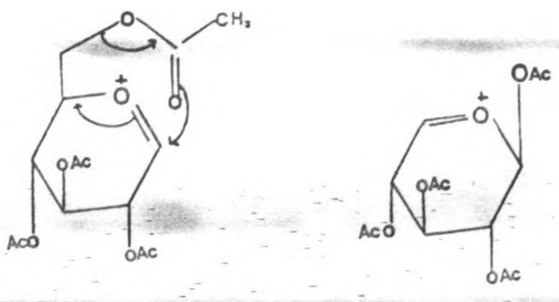
Scheme 2. Postulated fragmentation pathway of per-acetylated sucrose from R. revoilii tuber



The ion at m/z 331 may be explained as derived from α-cleavage of the glycosidic bond resulting in an oxonium ion which decomposes further by the sequential losses of two molecules of acetic acid to give m/z 211. Alternatively it decomposes by sequential losses of acetic acid (to m/z 271), followed by ketene (m/z 229), acetic acid (m/z 169) and acetic acid (m/z 109), all ions observed in the mass spectrum of the acetylated isolated sugar of R. revoilii (see following sequence and Scheme 2).



The ion at m/z 317 may be explained by the loss of carbene from m/z 331 after transfer of the acetoxy function at C-6 to C-1.



m/z 331

From the mass spectral fragmentation pattern, the compound is clearly a glycoside and the molecular ion at m/z 678 of the peracetate is indicative of a disaccharide. For comparison the 1H NMR spectrum of sucrose peracetate was run and compared to that of the disaccharide peracetate. The two proved to be identical (Figures 37 and 38). The mass spectrum of sucrose peracetate was also indistinguishable from that of the peracetate of the sugar isolated from R.revoilii tuber, (Figures 35 and 36), leading to the conclusion that it is identical to sucrose.

The residue obtained when the methanol extract was evaporated was redissolved in methanol and then precipitated with acetone to give a pink powder which, when hydrolysed using sodium periodate and chromatographed, afforded medicagenic acid 37 and hederagenin 38 as from the chloroform extract.

CHAPTER 3

INVESTIGATION OF THE MAJOR CONSTITUENTS OF *SESBANIA KENIENSIS* SEEDS

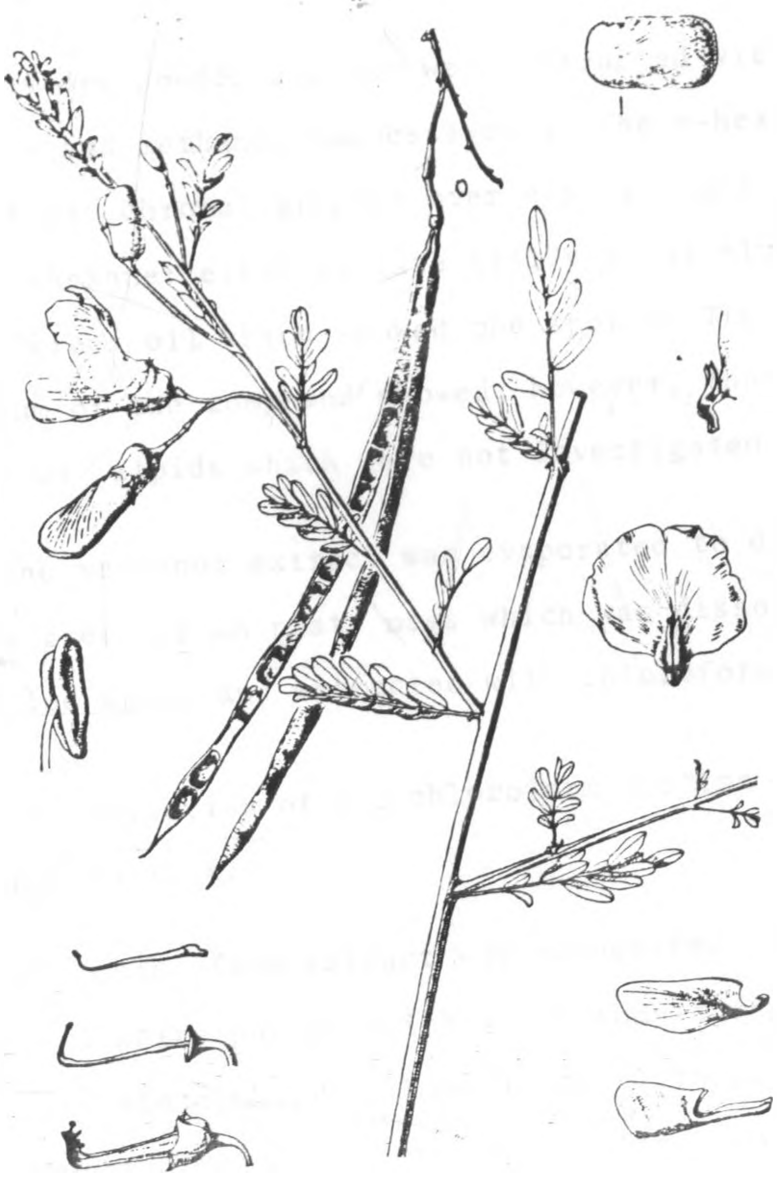
3.0 General

Sesbania keniensis belongs to the plant family Leguminoseae. The pods are narrow and long, containing several small seeds.

Its is commonly used to increase milk in cows and for treatment of swollen parts of the body. It is very common around Nairobi and in Machakos district in Kenya.

A species of Sesbania, *Sesbania drummondii* has been found to be toxic to livestock [64KIN]. *Sesbania drummondii*, *Sesbania vesicaria* and *Sesbania puniceae* have all been found to possess anti-leukemic activity by Powell *et al* [76POW], [79POW], [81POW]. The major triterpenoids of these species of *Sesbania* were found to be oleanolic acid and an isomer of hederagenin, while the major sterols were stigmasterol and sitosterol. No previous published work was found on *Sesbania keniensis* seeds.

Fig B



Sesbania keniensis

0 pod

1 seed

3.1 RESULTS AND DISCUSSION

The dry powdered seeds were extracted with n-hexane and methanol successively. The n-hexane extract was chromatographed over a silica gel column using n-hexane: ethyl acetate (1:19v/v) as eluant to give a green oil which showed one spot on TLC. ^{1}H NMR spectrum of the compound showed, however, that it contained lipids which were not investigated further.

The methanol extract was evaporated to dryness to give a green brown pasty mass which was dissolved in distilled water and extracted with chloroform.

3.11 Examination of the chloroform portion of the methanol extract.

The chloroform extract was evaporated to dryness to form a white powder which was chromatographed using ethyl acetate:dichloromethane (1:4v/v) to give one chromatographically pure compound. Recrystallization from acetone gave white needlelike crystals; m.p. 168-170°

The mass spectrum of the compound showed a prominent molecular ion at m/z 412 with a fragmentation pattern characteristic for sterols. A doublet of peaks at m/z 271 and at m/z 273 initiated by the allylic cleavage of the 17-20 bond is in agreement with the presence of a Δ^{22} unsaturation and a double bond in the nucleus of

the molecule [62FIT], [68WYL]. A fragment ion at m/z 300 caused by the scission at 22-33 bond and ions at m/z 369 (M^+-isopropyl) and m/z 351 (M^+-isopropyl + H_2O), evident in the mass spectrum of the sterol are all confirmation of the presence of Δ^{22} unsaturation in the sterol molecule [76ZAR], [67KNI]. An ion in the mass spectrum of the compound at m/z 255 for the loss of the side chain and water is significant for Δ^5 and Δ^7 unsaturated sterols [67KNI].

Acetylation of the sterol gave a product with no molecular ion, but had a prominent ion at m/z 394 for loss of HOAC, characteristic for Δ^5 steroids [67KNI].

Further confirmation for the presence of a Δ^{22}-bond was obtained from the ^1H NMR spectrum of the sterol which had signals at δ 0.696 (3H, s) for H-18, a vinylic multiplet centered at δ 5.172 and integrating for two protons for H-22 and H-23, whereas a triplet at δ 0.808 (3H, t, J = 7.8 Hz) signifies the presence of a 24-ethyl structure [76NES], [76RUB].

Direct mass and ^1H NMR spectral comparison to an authentic sample of stigmasterol, (Table 9 and Fig 52), showed the compound and stigmasterol to be indistinguishable. Confirmation of the structure of the isolated sterol was done by coinjecting it with the authentic sample of stigmasterol to a Gas Chromatograph.

Table 9. ^{1}H NMR chemical shifts (δ) of stigmasterol [84GAR] and the found values for authentic stigmasterol and *S.reniensis* sterol

Proton	Stigmasterol [84GAR] (J)	Authentic stigmasterol (J)	*S.reniensis* sterol (J)
18 (3H, s)	0.698	0.701	0.696
19 (3H, s)	1.012	1.002	1.009
21 (3H, d)	1.022 (6.5)	1.031 (5.0)	1.031 (5.0)
26/27 (3H, d)	0.795 (6.6)	0.801 (7.7)	0.803 (7.8)
26/27 (3H, d)	0.846 (6.5)	0.843 (5.0)	0.847 (5.0)
29 (3H, t)	0.804 (7.1)	0.813 (6.5)	0.808 (6.4)
3 (1H, m)	3.505	3.48	3.49
6 (1H, Bs)	5.357	5.323	5.323
22/23 (1H, dd)	5.015 (*ca* 7.5)	5.088 (6.4, 7.08)	5.051 (6.4, 7.08)
22/23 (1H, dd)	5.159 (*ca* 7.5)	5.152 (6.4, 7.08)	5.155 (6.4, 7.08)

3.12 Examination of the aqueous phase of the methanol extract.

The aqueous phase of the methanol extract was reduced to a minimum volume. A dark brown precipitate was obtained which was filtered and washed with methanol. Burning of the precipitate on a crucible did not decompose it. The precipitate was therefore deduced to consist of inorganic materials.

The filtrate was evaporated to a small volume, and added slowly to acetone to give an off white precipitate which was then chromatographed over paper cellulose and eluted with n-hexane. Chromatographing of the eluate over a silica gel column with ethyl acetate: dichloromethane (1:4v/v) gave one chromatographically pure compound which was recrystallised from methanol to give white platelike crystals; m.p. 278-280°.

The compound had a molecular ion at m/z 456 and a base peak at m/z 248, indicating that it was a pentacyclic triterpene of the α- or β-amyrin series with a 12-13 double bond and a carboxyl group located in the D/E rings [62DJE],[63DJE], [81OGU].

Methylation of the compound gave a monomethyl ester at m/z 470, which, when acetylated, formed a monomethyl ester monoacetate with a molecular ion at m/z 512, showing the compound to be a monohydroxy monocarboxy compound.

The ^{1}H NMR spectrum of the compound showed seven methyl signals between δ 0.725 and δ 1.132 ppm indicating that none of the methyls, excepting the one bearing the carboxyl group, has a functional group attached to it.

The signal attributed to C-18β-proton is an AB quartet (J_1 = 5.6 Hz, J_2 = 11.2 Hz) centred at δ 2.82 confirming the presence of a 17β-carboxy methyl oleanolate in the ^{1}H NMR spectrum of the methyl ester of the triterpene [72CHE]. The presence of a 12-13 double bond is confirmed by a multiplet at δ 5.28 in the ^{1}H NMR spectrum of the methyl ester of the triterpene.

Retro Diels-Alder fragmentation of the C-ring at m/z 248 and an ion at m/z 203 resulting from loss of COOH from the Diels Alder fragment, confirm the location of the hydroxyl group to be on the AB-rings. The presence of a 3β-hydroxyl can be safely assumed for naturally occurring triterpenoids. The compound is therefore deduced to be oleanolic acid -7.

Spectral comparison (tables 10, 11; figs. 41,42, 44,45) of the isolated triterpene with authentic samples of ursolic acid and oleanolic acid confirms it to be oleanolic acid 7.

CHAPTER 4

BIOLOGICAL AND CHEMICAL INVESTIGATION OF *ALBIZIA GUMMIFERA* G.Mel C. A. Sm. Var STEM BARK

4.0 General

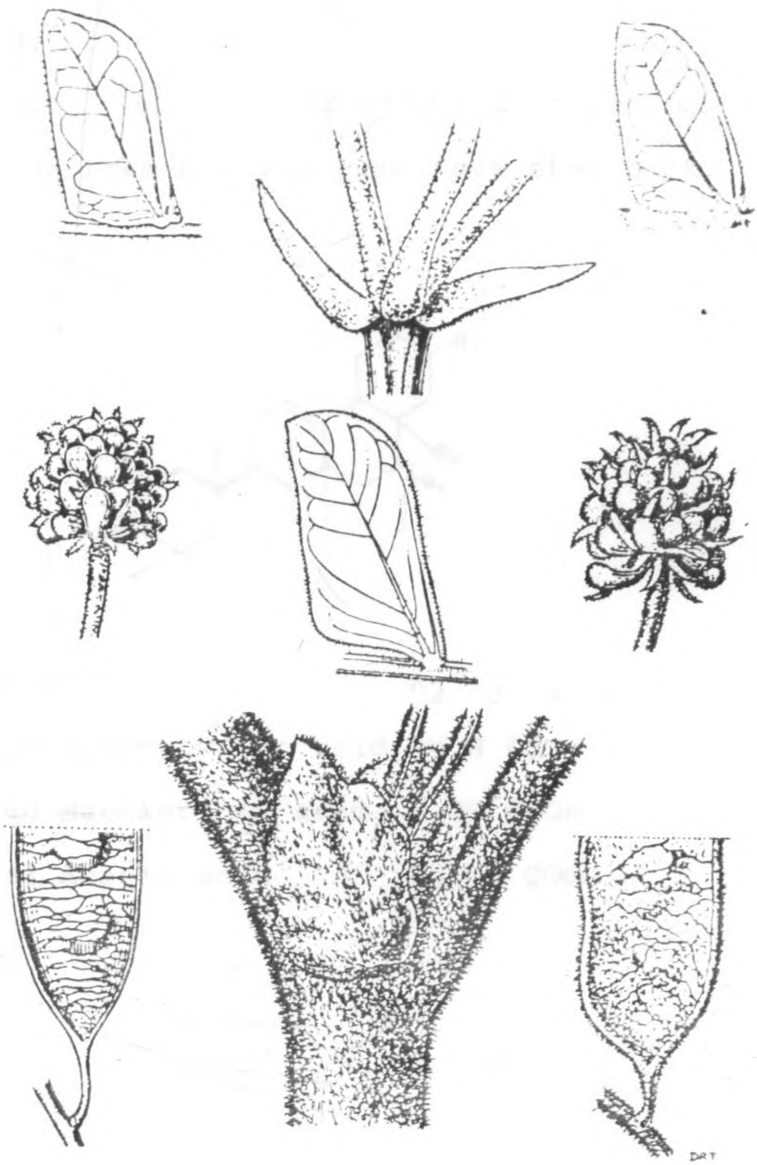
Albizia gummifera is a member of the plant family Leguminoseae and the sub-family Mimoseae. It is a large tree fairly common around Nairobi, the Central province and Kakamega district in Kenya.

An extract from the crushed pods is taken for stomach pains, and that obtained from the pounded roots is mixed with water for bathing to cure skin diseases. A decoction from the bark is used to treat malaria [76KOK]. The pounded bark is also used as fish poison.

Plants belonging to the genus *Albizia* have been reported to be a rich source for saponins and sapogenins [69VAR], [73VAR], [78VAR].

From certain species of Albizia from India, a number of triterpenes are reported to have been isolated, for example, echynocystic acid 39 from *A.lebbek*, *A.odoratissima*, *A.amara*, *A.lucida*, *A.anthelmintica* [57SAN], [59BAR], [62BAR], [61VAR], [62VA1], [62VA2], [62CHA]; oleanolic acid 7 from *A.lebbek*, *A.lucida* [62BAR], [62CHA]; machaerinic acid 40 from *A.procera*, *A.odoratissima* [65VAR], [58PAR];

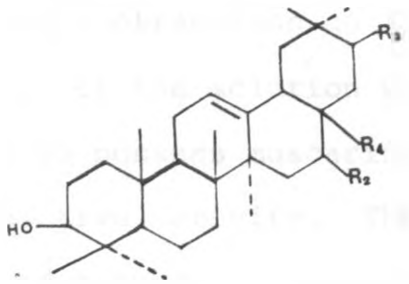
Fig C.



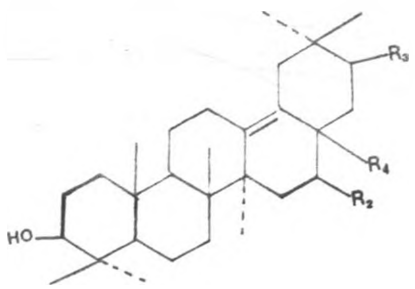
Albizia gummifera

albigenic acid 41, albigenin 42 and acacic acid 43 from A.lebbek and A.odoratissima [59BAR], [62BAR], [65VAR].

Lipton [63LIP] reported isolation of a compound $C_{30}H_{48}O_5$ from Albizia gummifera stem bark.



	R_2	R_3	R_4
39 Echynocystic acid	OH	H	COOH
40 Machaerinic acid	H	OH	COOH
43 Acacic acid	OH	OH	COOH



	R_2	R_3	R_4
41 Albigenic acid	OH	H	COOH
42 Albigenin	O	H	H

4.1 RESULTS AND DISCUSSION

The ground plant material was extracted with hot petroleum ether (40°-60°), methanol and methanol: water (2:1v/v) successively. The petroleum ether extract was a brown viscous oil and was sent for biological activity test. The extract produced spontaneous contractions on the guinea pig ileum when 20 µg of the solution was injected. It was concluded to possess muscarinic activity, but had no cardioactive activity. The methanol extract was found to be a muscarinic antagonist and possibly a nicotinic antagonist. It was found to be cardioactive in high doses.

The methanol extract gave a light pink precipitate in acetone which was filtered and separated from the acetone soluble portion. These two portions were sent for biological activity testing. This acetone soluble portion of the methanol extract had an active ingredient which had a direct effect on the smooth muscle tissue.

The saponin of Albizia gummifera was found to be either a muscarinic, nicotinic antagonist or to contain two elements in it having these effects. It had no cardioactive property. 1g of the isolated saponin was hydrolysed and the hydrolysate recrystallised from ethanol to form white needlelike crystals;

m.p. 278-280°.

Direct TLC, ^{1}H NMR, ^{13}C NMR and mass (Tables 10, 11) spectral comparison of the isolated compound proved it to be identical to authentic oleanolic acid and the oleanolic acid isolated from Sesbania keniensis seeds. The IR spectrum showed characteristic bands for oleanolic acid as given by Snatzke et.al [62SNA]. (Values shown in Experimental part and Figure 57. The compound was therefore concluded to be oleanolic acid 7.

Table 10. Comparison of the ^{13}C NMR chemical shifts (δ) of the literature data of methyloleanolate [75SE0] and the found values for methyl oleanolate from *A.bizia gummifera* bark and methyl oleanolate from *S.keniensis*

C-NO.	Methyloleanolate [75SE0]	from *A.gummifera*	from *S.keniensis*
1	38.5	38.404	38.404
2	27.1	27.194	27.194
3	78.7	78.27	78.611
4	38.7	38.745	38.404
5	55.2	55.218	55.218
6	18.3	18.276	18.324
7	32.6	32.604	32.653
8	39.3	39.232	39.255
9	47.6	47.615	47.615
10	37.0	37.088	37.091
11	23.1	22.954	22.933
12	122.1	122.621	122.377
13	143.4	143.626	143.567
14	41.6	42.572	41.595
15	27.7	27.731	27.682
16	23.4	23.588	23.393
17	46.6	46.543	45.861
18	41.3	40.987	41.279
19	45.8	45.861	45.861
20	30.6	30.655	30.703
21	33.8	33.823	33.871
22	32.3	32.604	32.361
23	28.1	28.072	28.072
24	15.6	15.546	15.546
25	15.3	15.351	15.546
26	16.8	17.106	17.199
27	26.0	25.927	25.927
28	177.9	177.142	177.142
29	33.1	33.092	33.092
30	23.6	23.588	23.637
O-CH_3	51.3	51.694	51.563

Table 11. ^1H NMR chemical shifts (δ) of authentic oleanolic acid, from S.keniensis, from A.gummifera and their methylesters

Types	Oleanolic acid	A	B	Methyloleanolate	from S.keniensis	from A.gummifera
s,3H	0.725	0.725	0.727	0.725	0.725	0.725
s,3H	0.779	0.780	0.771	0.779	0.784	0.784
s,3H	0.902	0.909	0.914	0.902	0.906	0.902
s,3H	0.902	0.909	0.914	9.902	0.906	0.902
s,3H	0.921	0.909	0.914	0.921	0.906	0.902
s,3H	0.985	0.989	0.986	0.985	0.990	0.990
s,3H	1.132	1.136	1.134	1.132	1.132	1.132
dd(J_1 ca 5.6 J_2 ca 11.2)	2.859	2.809	2.82	2.867	2.828	2.860
Tripletlike						
m, 1H	3.211	3.25	3.214	-	-	-
m, 1H	5.284	5.29	5.286	5.28	5.27	5.284
s, 3H				3.622	3.622	3.622

A = Oleanolic acid from S. keniensis

B = Oleanolic acid from A. gummifera

CHAPTER 5

EXPERIMENTAL

5.0 General

All the solvents and chemicals used were standard laboratory reagents. M.ps were determined using an automatic hot stage m.p apparatus and are uncorrected. Hot extraction was carried out in a Soxhlet apparatus. The dried ground plant material was exhaustively extracted with n-hexane and methanol, respectively. Cold extraction was carried out in a glass column of 3 L volume Solvents of rising polarities were used, 2.5 L being used each time. The plant materials were air dried in shadow and ground using a Wiley mill. All the plant materials were identified at the Nairobi University Herbarium.

The saponins were extracted by reducing the crude methanol extract to a minimum volume, transferring to a dropping funnel and adding dropwise to an excess volume of acetone. A precipitate formed which was filtered under reduced pressure, washed with hot acetone and quickly transferred to a desiccator where it was left standing overnight under reduced pressure.

TLC was performed on "Merck Fertigplatten 20 x 20 cm aluminium sheets coated with silica gel 60 without fluorescent indicator and with a layer thickness of 0.2 mm. Approximately 100 ml of solvent was used in the tank.

Preparative TLC was carried out on the same plates as those used for TLC. The plates were sprayed with methanolic rhodamin solution, the bands visualized under 366 nm UV-light, scraped off, extracted with warm ether and filtered under reduced pressure.

Silica gel 60, diameter 0.0400-0.063 mm was used for column chromatography. Two methods were applied for column chromatography, the TLC Mesh Column Chromatography [82TAB] and Flash Chromatography [78STI], [79MEY]. For TLC Mesh Column Chromatography, a modification of the method by Taber [82TAB] was employed. A glass column of 20 mm inner diameter and 150 mm length was used for 200 mg sample, and of 20 mm diameter and 450 mm length for 500 mg sample. A three way stopcock with a T-pattern made of glass was attached to the top of the glass column. The column was first packed dry, then compacted by solvent flow, applying pressure from a bicycle pump. The sample was introduced in solution using a syringe. 10 ml fractions were collected each time. The elution volume Vr, was

calculated using the formula:

$$K' = \frac{Vr'}{Vm} = \frac{Vr-Vm}{Vm}$$

$$\text{where } K' = \frac{1-R_f}{R_f}$$

R_f values were calculated from TLC and V_m = Void volume.

For Flash Chromatography, a glass column of 20 mm diameter and 350 mm length with a blown-up top of 250 ML volume prepared in the institute was used. A slurry of silica gel in the solvent system was first allowed to settle by gravity flow and then further compacted by application of pressure from a bicycle pump. The mixture to be chromatographed was first evaporated onto coarse silica gel to assure even application on the top of the column before introducing the solvent mixture to the blown-up top.

GC was performed on a fused silica capillary column, (length 25 m, inner diameter 0.35 mm), BP1 bonded, on a Varian Model 3700 instrument equipped with a Hewlett-Packard 3390A integrator. Operating conditions were: carrier gas H_2, flow rate 2-4 ML/min; injection temperature 40^{o}C flashed by 120^{o}C/min to 260^{o}C; column temperature 40^{o}C for 5 mins increasing thereafter by 8^{o}C/min to 280^{o}C.

The ^{1}H NMR recordings at 400 MHz, double irradiation, together with Nuclear Overhauer Effect (NOE) experiments were carried out on a Bruker instrument, Model WM450, while the other NMR measurements were recorded on JEOL fX-100, a Pulse-Fourier transform instrument, at 99.6 MHz for ^{1}H NMR and at 25.1 MHz for ^{13}C NMR and DEPT recordings. In all cases deuterochloroform ($CDCl_3$) was used as solvent, with tetramethylsilane (TMS) as internal standard unless stated otherwise. Interpretations were done with extensive use of double irradiation. Mass spectra were recorded on an AEl MS902 instrument, using the direct inlet system. An electron bombardment energy of 70eV was used. Accelerating voltages of 6 and 8kV were employed. The ion source temperature is given in each case. Accurate mass measurements were performed manually or with a computerized on line data processing unit (PDP8) and perfluorokerosene (PFK) used as reference. Infrared spectra were recorded on a Perkin Elmer 521 Grating spectrophotometer. The sample was introduced as nujol mull.

Preparation of diazomethane.

2.14 g of N-methyl-N-nitrosotoluene-p-sulphonamide was dissolved in 30 ml of ether, cooled in ice, and a solution of potassium hydroxide in 10 ml of 96%

ethanol added. After 5 minutes, the ethereal, diazomethane was distilled from a water bath.

Methylation of the acids.

The compound was suspended in a little anhydrous ether or absolute methanol and the above prepared ethereal solution of diazomethane added in small portions until gas evolution ceased and the solution acquired a pale yellow colour. The solvent was evaporated and the product purified by crystallisation.

Acetylation of the hydroxycompounds.

A solution of 1 g sample in 5 ml pyridine and 5 ml acetic anhydride was shaken and left standing overnight. The solvent was removed by warming and blowing with nitrogen gas and the product purified by recrystallisation.

Acid hydrolysis was done by refluxing 5 g sample with 150 ml 8% sulphuric acid for 3 hours, cooling and adding water. In cases where a precipitate formed, the precipitate was filtered and washed with water until washings were neutral. Where there was no precipitate, the hydrolysate was transferred to a separating funnel and partitioned between chloroform and water.

The sapogenins were found in the chloroform phase. Due to the general sensitivity of important triterpenoids towards acids, a more gentle method for the hydrolysis of saponins was attempted.

5.1 RHOICISSUS REVOILII PLANCH. TUBER

5.10 Plant Material

The tuberous roots were dug up at the Ngong Hills near Nairobi, Kenya in July 1982. The tubers were pounded, air dried in shadow and ground. When a sample of the ground plant material was boiled in water, a strong frothing solution was obtained. This suggested that the roots contained saponins.

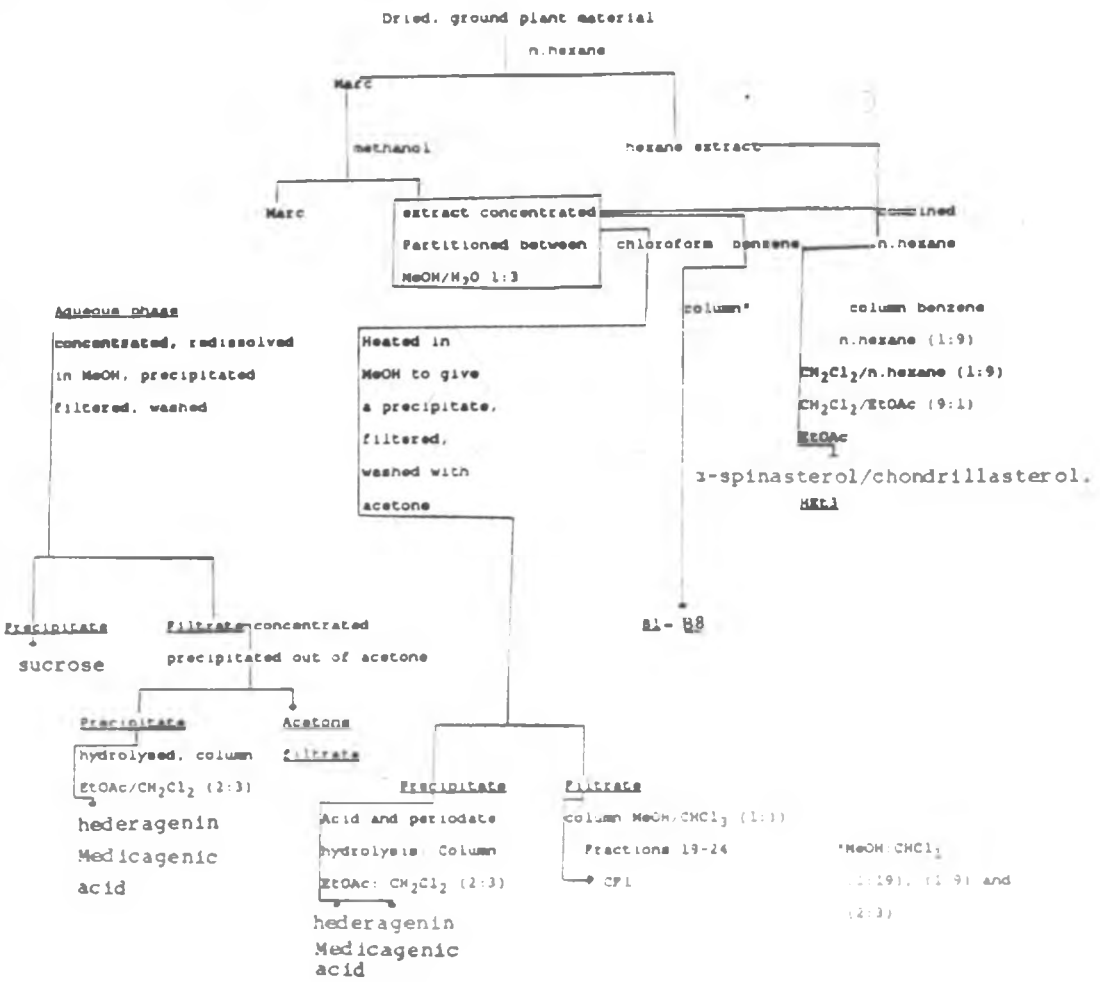
5.11 Isolation of steroids and free sapogenins.

580 g of dry, ground plant material was extracted in a large column with 2.5 L cold n-hexane and 2.5 L methanol successively. The methanol extract was filtered, concentrated and redissolved in methanol/water(1:3v/v). The solution was partitioned three times with n-hexane, benzene and chloroform respectively. The n-hexane portions were combined to give an oily mass in 0.3% of the total plant material when evaporated to dryness.

A) The n.hexane extract:

The combined n.hexane portions were dissolved in benzene: n-hexane (1:9v/v) and subjected to a column of silica gel. Elution was carried out using the following solvent systems:

Scheme 1. Fractionation of Rhoicissus revoilii tuber.



1) Benzene: n-hexane (1:9v/v)

2) Dichloromethane: n-hexane (1:9v/v)

3) Dichloromethane: ethyl acetate (9:1v/v)

4) Ethyl acetate.

The benzene: n-hexane fractions gave a mixture of fatty acids which were not further investigated. Fractions 27 and 28 of the dichloromethane: n hexane system gave one spot on TLC using the same solvent system and was named H27 m/z (rel.int.): 586 (13.5), 558 (45.5), 530 (9), 502 (9.5), 409 (8), 407 (7.7), 391 (9.5) 255 (7.7), 184 (23.6), 177 (25), 69 (81.8), 57 (100), 55 (81.8). Fraction 29 of the same solvent system also gave one spot on TLC. When left standing, it formed long white needlelike crystals in 5% of the crude hexane extract and was identified as consisting of α-spinasterol and chondrillasterol; TLC R_f 0.45; m.p 162-166°C m/z (rel.int.): 412.3701 (calc. for $C_{29}H_{48}O$, 412.3705, 70%), 397 (9.5), 369 (16), 300 (15), 271 (72), 255 (22.5), 246.1988 (calc. $C_{17}H_{26}O$, 246.1984, 16%) 107 (36), δ($CDCl_3$), 400 MHz), 0.553(s, 3H, H18), 0.79 (d, J = 7.0 Hz, 3H, H26), 0.803 (s, 3H, H19), 0.809 (t, J = 7.0 Hz, 3H, H29), 0.842 (d, J = 7.0 Hz, 3H, H27), 1.027 (d, J = 6.1 Hz, 3H, H21), 3.53 (m, 1H), 5.019 (ABq, J_1 = 15.38 Hz, J_2 = 8.61 Hz, H22/23), 5.152 (m, 2H, H7, H23/22), ppm.

The monoacetate of the sterol was prepared by acetylation of the sterol with acetic anhydride in pyridine under normal conditions; m.p 170-174° GC:RT 43.55, 44.50 (according to conditions given in the general experimental part); δ $CDCl_3$, 400 MHz), 0.548 (s, 3H), 0.79 (d, J = 6.5 Hz, 3H), 0.806 (s, 3H), 0.813 (t, J = 7.3 Hz, 3H), 0.850 (d, J = 6.42 Hz, Hz, 3H), 1.027 (d, 3H, J = 6.68 Hz), 4.69 (m, 1H), 5.02 (ABq, J_1 = 15.38 Hz, J_2 = 8.4 Hz, 1H), 5.14 (m, 1H), 5.159 (ABq, J = 15.38 Hz,

The trimethylsilyl ether of the sterol was obtained
' silylation of the sterol mixture with hexamethyl-
.silizane and trimethylchlorosilane in dry pyridine
ed on p.90 above; m/z (rel.int.): 484 (35.3), 469
.1.8), 441 (6.5), 372 (9.4), 343 (47) 255 (58.8)
:1 (100), 83 (94) 73 (100).

Fraction 3 of the ethyl acetate elution fraction
ave one spot on TLC. However, the mass spectrum
owed several molecular ions showing the fraction
) be a mixture identified as comprising seven long
ain carboxylic acids; m/z (rel.int.): 452.4595
calc. for $C_{30}H_{60}O_2$, 452.4593, 0.2%), 424.4286 (calc.
or $C_{28}H_{56}O_2$, 424.4280, 10.6% 410.4128 (calc. for
$_{27}H_{54}O_2$, 410.4124, 5%), 396 (66.7), 382 (16.7),
58.3660 (calc. for $C_{24}H_{48}O_2$, 368.3654, 27.2%), 353 (4.4),
39 (2.7), 325 (3.(), 185 (5.6), 129 (27.2) 97 (28),
3 (44.4), 71 (72.2), 69 (50), 59 (100).

) The benzene extract:

The benzene extract was evaporated to dryness to
ive a brownish gummy mass in 0.2% of the dry plant
aterial. It was subjected to a silica gel column and
luted successively with the following solvent systems:

) Fractions 1-25 methanol: chloroform (1:19v/v)
) Fractions 26-40 methanol: chloroform (1:9v/v)
) Fractions 41 onwards methanol: chloroform (2:3v/v)

BI was collected from the column fraction 6 of the crude benzene extract using solvent system methanol: chloroform (1:19); R_f 0.63, m/z (rel.int.): 530.4699 (calc. for $C_{35}H_{62}O_3$, 530.4693, 3%), 271.2058 (calc. for $C_{19}H_{27}O$, 271.2062, 47%), 255.2115 (calc. for $C_{19}H_{27}$, 255.2113, 9%), 246.1986 (calc. for $C_{17}H_{26}O$, 246.1984, 10%).

Preparation of BI trimethylsilyl ether was carried out by silylation under normal conditions as described on p.90, m/z (rel.int.): 484 (6.7), 467 (10), 255 (1.2), 83 (100).

Column fraction 7 of the above solvent system from the crude benzene extract was named B2 R_f 0.56; m/z (rel.int.), 440 (2.4), 426 (3.2), 412 (12.8), 354 (28), 271 (29), 246 (7.2).

B2 trimethylsilyl ether was prepared by trimethyl silylation of B2 by the method described above; m/z (rel.int.): 484 (38.8), 343 (50), 318 (10.6), 255 (55.6), 73 (100).

B3 were the combined fractions 10-14 of the same column as above R_f 0.28; m/z (rel.int.): 414.3861 (calc. for $C_{29}H_{50}O$, 414.3862, 9.3%), 412.3709 (calc for $C_{29}H_{48}O$, 412.3705; 4.7%), 303 (3.1), 271 (6), 262 (5.3), 256 (6.7), 255 (6) 55 (100).

B4 were fractions 13.16 of the above silica gel column using the same solvent system; R_f 0.28 m/z (rel.int.): 486 (1.3), 454 (12.5), 302 (10.8), 270 (54), 248 (100), 203 (75), 143 (40).

When B4 was methylated with diazomethane, B4 methylester was formed; m/z (rel. int.): 500 (8.3), 482 (4.2), 468 (8.3), 410 (12.5), 262 (66.7), 203 (100).

Acetylation of the methylester under normal conditions gave a methylester diacetate; m/z (rel.int.): 584.3713 (calc. for $C_{35}H_{52}O_7$, 3718, 3.3%), 569 (2.7), 542 (2.7), 524.3509 (calc. for $C_{33}H_{48}O_5$, 524.3502, 5.6%), 510 (5.6), 410.2828 (calc. for $C_{29}H_{38}O_3$, 410.2821, 12%).

B5 was collected from the same column, fractions 20-23; R_f 0.20; m/z (rel.int.): 544.4135 (calc. for $C_{34}H_{56}O_5$, 544.4128, 6.4%), 454.3082 (calc. for $C_{29}H_{42}O_4$, 454.3083 as major peak) and 454.3440 (calc. for $C_{30}H_{46}O_3$, 454.3447 as minor peak; 3.6%), 395 (28), 270.0535 (calc. for $C_{15}H_{10}O_5$, 270.0528, 19.3%), 248 (100), 203 (60).

B6 were fractions 26-29 of the above silica gel methanol: chloroform (1:9; R_f 0.14; m/z (rel.int.): 574.4243 (calc. for $C_{35}H_{56}O_6$, 574.4233, 26.3%), 531.3695 calc. for $C_{32}H_{51}O_6$, 531.3686, 32.7%), 433.2596 (calc. for $C_{25}H_{37}O_6$, 433.2590, 21.8%), 412.3701 (calc. for $C_{29}H_{48}O$, 412.3705; 7.8%), 395.3681 (calc. for $C_{29}H_{47}$, 395.3678, 71%), 271 (6.9) 255.2118 (calc. for $C_{19}H_{47}$, 255.2113, 37%), 229.1950 (calc. for $C_{17}H_{25}$, 229.1956, 18%), 137 (73), 83 (100).

B6 tetraacetate was prepared by acetylation of B6 using acetic anhydride in dry pyridine; m/z (rel.int.): 742 (2.9), 601 (4.3), 385 (42.9), 331 (100), 271 (4), 255 (22.9), 169 (71.4).

B7 was collected from the same column solvent system as B6, fractions 35-40; R_f 0.09; R_f 0.09; m/z (rel.int.): 548 (5), 339 (6.3), 313 (17.5), 311 (9.2), 57 (100), 55 (66.7).

Fraction 47 of the same silica gel column using solvent system methanol: chloroform (2:3) was named B8 R_f 0.04; m/z (rel.int.); 578 (6.3), 515 (1), 354 (3.5), 331 (4.3), 313 (25), 299 (20), 239 (37.5), 149 (50), 57 (100), 55 (77.5).

Acetylation of B8 gave a product with no molecular ion, but with fragments at m/z (rel.int.): 355 (21.8),

331 (37.5), 313 (15.6), 239 (34), 211 (39), 171 (17.2), 169 (51.6), 109 (34), 69 (67) 57 (89), 55 (100).

5.12 Isolation of triterpenes and sugars.

A) From the chloroform extract:

The chloroform extract gave a brown solid on evaporation under reduced pressure in 0.2% of dry plant material. The solid was heated in methanol to form a precipitate which was filtered and washed with hot acetone to give a white powder weighing 0.8 g. TLC using the solvent system ethyl acetate/water/methanol (2:2:1) showed one spot.

0.5 g of this was digested with sodium periodate (75MAS), saponified with ethanolic potassium hydroxide, acidified with dilute hydrochloric acid and extracted with ether. Slow evaporation of the ether extract gave white needlelike crystals in 40% recovery. The mass spectrum, however, showed two peaks of two different molecular ions at 502 and at 472.

Methylation of this compound using diazomethane gave two spots on TLC using solvent system ethyl acetate/dichloromethane (2:3). The faster running spot was identified as the dimethyl ester of medicagenic acid and had an R_f of 0.26. The second component had an R_f of 0.07 and was the methyl ester of hederagenin.

The two compounds were separated on silica gel column using the above solvent.

Fractions 7-12 (Vr = 145.6 ml, medicagenic acid dimethyl ester)

Fraction 16-24 (Vr = 260.6 ml, hederagenin methyl ester).

Vr = Elution volume.

Acid hydrolysis

0.3 g of the white powder was refluxed for 4 hours with 5% methanolic sulphuric acid. The product was precipitated in water, filtered, methylated and compared by means of TLC with the crude periodate hydrolysis product. It was found to contain the same major products, but with some minor products, possibly artefacts produced during hydrolysis.

Medicagenic acid 35 was isolated from R.revoilii tuber as described above in 9.1% of the crude saponin; m/z (rel.int.): 502 (1.1), 456 (3), 248 (100), 203 (67).

Methylation of the medicagenic acid using diazomethane as described in the general experimental part produced dimethyl medicagenate R_f 0.26, (TLC) Vr 145.6 mls in solvent system ethylacetate: dichloromethane (2:3); m.p 220-224°; m/z (rel. int.): 530.3602 (calc. for

$C_{32}H_{50}O_6$, 530.3607, 7%), 515 (1.2), 471 (4.1), 262 (92), 203 (100); δ ($CDCl_3$, 400 MHz) 0.725 (s, 3H), 0.897 (s, 3H), 0.926 (s, 3H), 1.127 (s, 3H), 1.205 (s, 3H), 1.367 (s, 3H), 2.855 (ABq, J_1 = 5.66 Hz, J_2 = 14.5H, H18β), 3.617 (3H, CO_2Me), 3.725 (3H, CO_2Me), 4.017 (d, J = 5.4 Hz, H3), 4.18 (m, W1/2 11.3 Hz, H2), 5.294 (m, 1H, H12) ^{13}C NMR see Table 6.

When dimethyl medicagenate was acetylated using acetic anhydride and pyridine as described, dimethyl medicagenate diacetate was obtained; m/z (rel.int.): 614 (4.3) 554 (5.1), 508 (7.7) 494 (3.1), 435 (3.1) 262 (71.4) 203 (100); δ ($CDCl_3$, 400 MHz) 0.730 (s, 3H), 0.906 (s, 3H), 0.906 (s, 3H), 0.926 (s, 3H) 1.127 (s, 3H), 1.205 (s, 3H), 1.402 (s, 3H), 1.960, 2.058 (2s, OAc), 2.86 (ABq, J = 5.66 Hz, J = 14.5 Hz, H18β), 3.622, 3.676 (2s, 6H, $2CO_2Me$), 5.29 (m, H12), 5.35 (d, J = 5.4 Hz, H3), 5.464 (m, W h/2 = 11.3 Hz, H2).

Dissolving of dimethylmedicagenate in dry acetone and addition of dry copper sulphate as a catalyst resulted in the formation of dimethyl medicagenate acetonide; m/z (rel.int.): 570 (0.5), 262 (42), 203 (100).

Silylation of the dimethyl medicagenate gave dimethyl ester trimethylsilyl ether of medicagenic acid; m/z (rel.int.): 674 (2.5), 584 (6.1), 525 (7), 411 (3.9), 262 (100), 203 (68), 173 (15), 147 (22), 73 (61).

Hederagenin 33 was isolated from the crude Rhoicissus revoilii tuber saponins by the general periodate hydrolysis method described above in 10% of the crude saponin; m/z (rel.int.): 472.3542 (calc. for $C_{30}H_{48}O_4$, 472.3553, 10%), 454.3440 (calc. for 454.3447, 4.5%), 436 (4.6), 248 (100), 203 (60).

Hederagenin monomethyl ester was prepared by methylation of hederagenin with diazomethane; Vr 260.6 mls, R_f 0.07 (TLC) solvent system ethyl acetate: dichloromethane (2:3), m/z (rel.int.): 486 (2.6), 262 (52), 203 (63), δ ($CDCl_3$, 100 MHz) 0.725 (s, 3H), 0.897 (s, 3H), 0.926 (s, 6H), 0.955 (s, 3H), 1.127 (s, 3H), 2.779 (dd, 1H, J_1 = 5.6 Hz, J_2 = 11.2 Hz, H18β), 3.622 (s, 3H, CO_2CH_3), 3.87 (m), 5.284 (m, 1H, H12) ppm. For ^{13}C NMR see Table 3.

Acetylation of hederagenin under standard conditions gave hederagenin diacetate, m/z (rel.int.): 556 (1), 510 (2.1), 496 (2.2), 436 (3.5), 248 (100), 203 (61.2), δ ($CDCl_3$; 100 MHz) 0.833 (s, 3H), 0.926 (s, 6H), 0.970 (s, 3H), 1.127 (s, 3H), 2.024 (s, OAc), 2.068 (s, OAc)

3.902, 3.676 (ABq, 2H J = 11.3 Hz), 5.28 (m, 1H) ppm.

When hederagenin methyl ester was acetylated, hederagenin ester diacetate was obtained; m/z (rel.int.): 570 (4), 526 (1), 510 (8), 262 (84), 203 (100), 189 (26), 133 (11); δ($CDCl_3$, 100 MHz) 0.73 (s, 3H), 0.833 (s, 3H), 0.902 (s, 3H) 0.926 (s, 3H), 0.965 (s, 3H), 1.117 (s, 3H), 2.024 (s, OAc), 2.068 (s, OAc), 2.86 (badly defined dd, H18β), 3.622 (s, 3H, CO_2CH_3), 3.70, 3.85 (d+d, 2H, J = 11.2 Hz, $23H_A$, $23H_B$), 4.78 (tripletlike m, W 1/2 = 19.7 Hz, H3), 5.29 (1H, m, H12) ppm.

Methanol soluble portion of the chloroform extract.

The methanol soluble portion of the chloroform extract was subjected to a column and eluted with methanol/chloroform (1:9). Fractions 19-24 which showed one spot on TLC was named CFI and was recrystallised from ether to give white crystals; m/z (rel.int.): 524.3509 (calc. for $C_{33}H_{48}O_5$, 524.3502) as major peak and 524.3147, (calc. for $C_{32}H_{44}O_6$, 524.3188 as minor peak; 28.5%), 510 (60), 496 (31.5), 383 (90), 369 (100), 263 (26), 249 (31), 189 (30), 133 (60), 129 (35).

B. From the methanol extract

The residual methanol extract, when evaporated, gave a pinkish powder in 6% of the dry plant material. The powder was redissolved in hot methanol, and the insoluble portion filtered and washed with acetone and chloroform. The insoluble portion were white crystals which were found to be sucrose, m.p. 186-188°, m/z (rel.int.): 311 (M-31, 0.1%), (0.1), 275 (0.1), 235 (0.2), 221 (0.3), 205 (0.5), 191 (0.8), 182 (0.7), 165 (0.6), 163 (9), 149 (5.5), 145 (10), 126 (12), 102 (24), 73 (60), 57 (72), 44 (100), δ (MeOD, 100 MHz) 3.338, 3.563, 3.598, 3.662, 3.799, 3.863, 4.039, 4.166 (8s, 14H), 5.399 (d, J = 5.66 Hz, 1H).

Sucrose octaacetate was prepared from sucrose by acetylation under standard conditions; m/z 678, 619, 605, 516, 498, 331, (83), 317 (0.12), 271 (2), 229 (2), 211 (48.3), 169 (73), 43 (100); δ ($CDCl_3$ 100 MHz), 2.03 (d, J = 2.83 Hz), 2.117 (m), 2.176 (s, 23H), 4.176 m, 4.262 (dd, J = 5.65, Hz, J = 14.1 Hz), 4.814 (d, J = 2.82 Hz), 4.917 (d, J = 5.1 Hz), 5.358 (s), 5.541 (m), 5.689 (d, J = 5.67 Hz).

The methanol soluble portion was concentrated to a minimal volume and added dropwise to an excess amount of acetone to give a precipitate, which, when washed with hot acetone, gave a very light pink powder of which 1 g was hydrolysed with 8% methanolic sulphuric

acid and another 1 g hydrolysed with sodium periodate. After separation on silica gel column, the two major products from both hydrolyses were identical to medicagenic acid and hederagenin. The acid hydrolysis products were, however, more difficult to purify due to formation of artefacts. The recovery was also lower, i.e. 3.5% as opposed to 6.0% of the periodate hydrolysis. It was decided to avoid acid hydrolysis in the future.

Sodium periodate hydrolysis [75MAS] of the saponin from R.revoilii: 1 g saponin was treated with 1 g sodium periodate, 1 ml cyclohexylamine and 1 ml acetic acid in 300 mls 50% aqueous methanol. The pH was adjusted by adding cyclohexylamine/acetic acid until the pH of the solution was between 6.2-6.6. The mixture was shaken and left standing for 4 days. Distilled water was added to the product, acidified and extracted with chloroform. The chloroform portion was evaporated to dryness and saponified as described below.

Saponification of the sodium periodate hydrolysed samples.

1 g sample was dissolved in 100 ml hot solution of 96% ethanol and 5 g potassium hydroxide slowly added with stirring. The mixture was left standing one day, water added and extracted with ether to remove non acidic components. To the water solution a few drops of dilute hydrochloric acid was added until a

precipitate formed. This was either filtered or extracted with chloroform.

Preparation of acetonides [74JOH] of medicagenic acid.

50 mg of substance was dissolved in acetone dried over anhydrous copper sulphate. 15 mg of anhydrous copper sulphate was added as catalyst. The reaction mixture was allowed to stand at room temperature for 5 hours. After filtration, the acetone was removed under reduced pressure leaving the product which was recrystallised from acetone.

Preparation of the nor acid [57DJE] of medicagenic acid from R.revoilii

250 mg of the dimethyl ester diacetate was dissolved in 50 ml acetone and was treated at 5-10°C over a period of 25 minutes with a standard chromium trioxide solution (2.7 g chromium trioxide, 7.7 mls water and 23 mls concentrated sulphuric acid) until a slight excess was present. Water was added and the mixture extracted with ether. Removal of the solvent afforded a brown gum. It was heated under reflux for 6 hrs with 10% methanolic potassium hydroxide with a few drops of water to dissolve the potassium hydroxide. The mixture was diluted with water, acidified, extracted with ether and the ether evaporated under reduced pressure. The product was purified by preparative TLC.

Attempt at formation of the lactone of medicagenic acid from R.revoilii

The monomethyl ester acid was treated with 20% methanolic sulphuric acid and left standing for 48 hours. The product was purified using preparative TLC.

Trimethylsilylation of the hydroxy compounds from R.revoilii.

To 10 mg sample in 1 ml dry pyridine was added 0.2 ml hexamethyldisilizane, 0.1 ml trimethylchlorosilane heated at 80^{o} and left standing for 1 hour. The reagents were then removed in a stream of nitrogen in the fume cupboard and the residue dissolved in ethylacetate. The solution was filtered through silica gel to remove any excess reagents.

5.2 SESBANIA KENIENSIS SEEDS

5.20 Plant material

The dry pods were collected near the Bellevue drive in cinema and the Kenya Bureau of Standards, Nairobi, Kenya, in July 1982. The seeds were removed from the pods and ground.

5.21 Defatting of the seeds

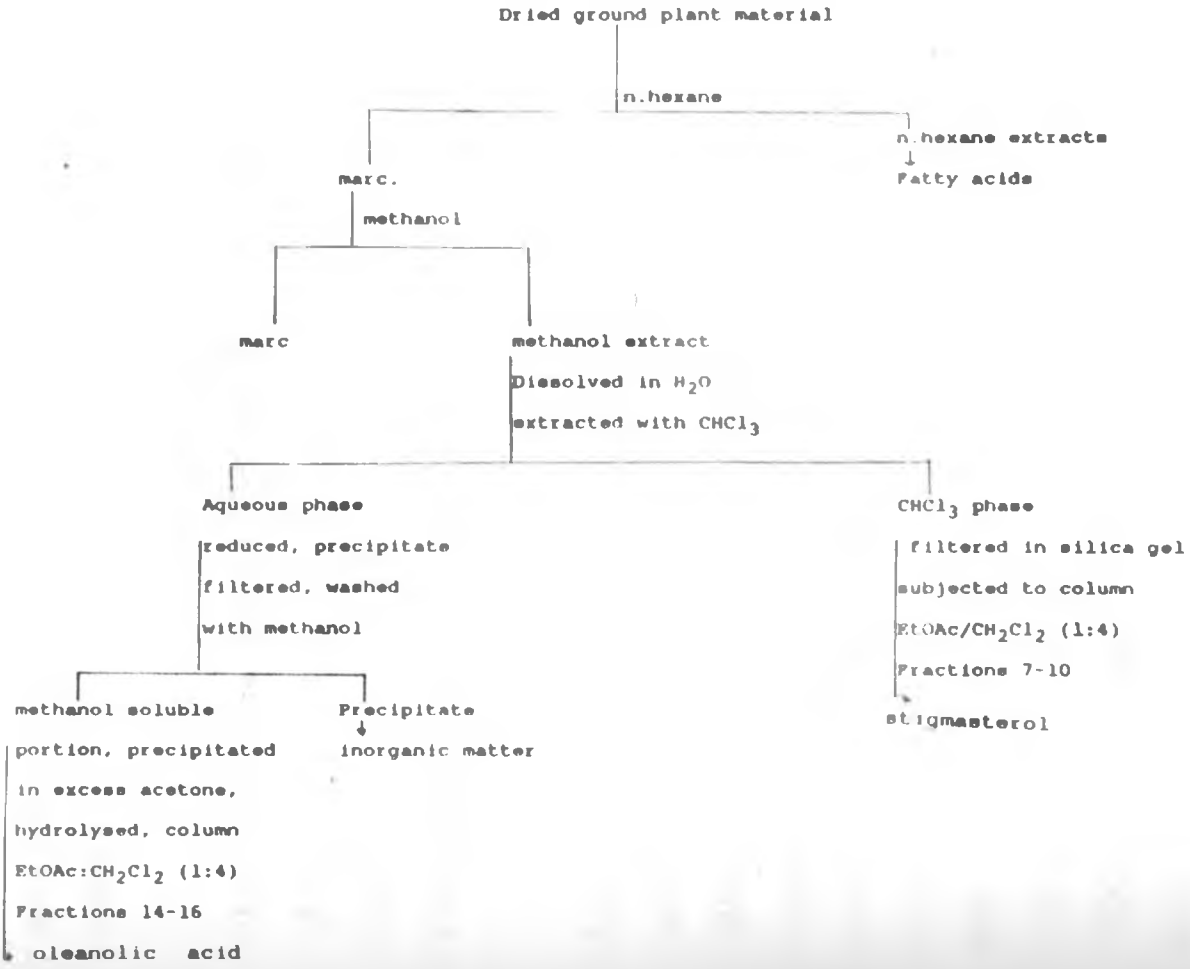
1 kg of the powdered seeds were defatted with L n-hexane on a hot sand bath in a 2 L beaker by stirring with a glassrod and filtering. The operation was repeated four times.

The n-hexane extract was concentrated to dryness to afford a yellowish pasty mass in 10% of the starting dry material. .6 g of this was subjected to a silica gel column and eluted with ethyl acetate: n-hexane (1:19v/v) A green oil was obtained showing one spot on TLC of the same solvent system. ^{1}H NMR spectrum of this showed fatty acids which were not further investigated.

5.22 Extraction with methanol.

The residual plant material was transferred to a soxhlet extractor and extracted with 2.5 L methanol for 12 hours. The extract was filtered and evaporated to dryness to afford a green brown pasty mass in 66.2% of the starting dry plant material.

Scheme 3. Fractionation of Sesbania Keniguais seeds.



The methanol extract was dissolved in distilled water and extracted with chloroform.

A) The chloroform soluble portion of the methanol extract.

The chloroform soluble portion of the methanol extract, when evaporated to dryness, gave a cream white powder in 1% of the total methanol extract.

2.5 g of this was subjected to silica gel in a sintered glass funnel and washed under pressure with chloroform. The eluate was evaporated to dryness, subjected to a silica gel column and eluted with ethyl acetate /dichloromethane (1:4).

Fractions 7-10 gave one major spot on TLC with faster running impurities. They were combined, dried and recrystallised from acetone to give white, needlelike crystals of 38.5 mg in weight and was identified as stigmasterol [30] m.p. 168-170° GC:RT 45.43; m/z (rel. int.): 412 (13.1), 397 (0.7), 369 (2), 351 (1.8), 314 (1.2), 303 (0.8), 300 (5.5), 273 (3.1), 271 (6.6), 255 (7.5), 231 (1.6), 229 (1.7), 215 (3.2), 213, (4.3), 199 (2.4), 187 (2.4), 185 (2.1), 55 (100); 43 (83); δ ($CDCl_3$; 100 MHz) 0.696 (s, 3H), 0.803 (d, 3H, J = 7.8 Hz), 0.808 (t, 3H, J = 6.4 Hz), 0.847 (d, 3H, J = 5.0 Hz), 1.009 (s, 3H), 1.031 (d, 3H, J = 5.0 Hz), 3.49 (m, 1H), 5.051 (dd, 1H, J_1 = 6.4 Hz, J_2 = 7.08 Hz), 5.152 (ABq, 1H, J_1 = 6.4 Hz, J_2 = 7.08 Hz), 5.323 (Bs,

1H) ppm.

When the stigmasterol was acetylated, stigmasteryl acetate was obtained; m/z (rel.int.): 394 (100), 379 (2.9), 351 (8.9), 255 (26.9), 228 (6.7), 213 (74.6).

B) The aqueous phase of the methanol extract.

The aqueous phase of the methanol extract was evaporated to a minimum volume. A dark brown precipitate was obtained which was filtered and washed with small amounts of methanol.

The precipitate was dried. It was found to be insoluble in organic solvents. Burning of a small sample on a crucible did not decompose it, leading to the conclusion that it contained inorganic materials, and was not further investigated.

The filtrate was reduced to a minimum volume of solvent, precipitated by adding dropwise to a large volume of acetone forming a cream white precipitate which was filtered, washed with hot acetone and dried. A white powder was obtained which was hydrolysed with 2N hydrochloric acid, neutralised with sodium hydroxide solution and adsorbed onto cellulose. The cellulose was then eluted with n-hexane. The eluate was evaporated and subjected to a silica gel column and eluted with the solvent system ethyl acetate/dichloromethane (1:4 v/v).

Fractions 14-16 showed one spot on TLC and were pooled together, recrystallised from methanol to form white platelike crystals and was identified to be oleanolic acid 7. m.p. 278-280°C; m/z (rel.int.): 456 (3.1), 448 (0.7), 410 (1.2), 300 (0.8), 248 (100), 203 (43): δ ($CDCl_3$; 100 MHz) 0.725 (s, 3H), 0.78 (s, 3H), 0.909 (s, 9H), 0.989 (s, 3H), 1.136 (s, 3H), 2.809 (dd, 1H, J_1 = 5.65 Hz, J_2 = 11.2 Hz), 3.25 (tripletlike m, 1H), 5.29 (m, 1H) ppm; for ^{13}C NMR, see Fig 47.

Methylation of the oleanolic acid gave methyl oleanolate of m/z (rel.int.): 470 (7.3), 262 (81.8), 203 (100); δ ($CDCl_3$; 100 MHz) 0.725 (S, 3H), 0.784 (s, 3H), 0.906 (s, 9H), 0.990 (s, 3H), 1.132 (s, 3H), 1.544, 2.828 (ABq, 1H, J_1 = 5.6 Hz, J_2 = 11.2 Hz), 3.622 (s, 3H), 5.27 (m, 1H) ppm; for ^{13}C NMR, see Fig 46.

Acetylation of the methyl cleanolate under standard conditions gave oleanolic acid methylester acetate; m/z (rel. int.): 512, 452 (3), 262 (37.1), 203 (60), 43 (100); δ ($CDCl_3$; 100 MHz) 0.715, 0.852, 0.891, 0.916, 1.117, 2.033, 3.612, 4.48, 5.289 ppm.

5.3 ALBIZIA GUMMIFERA (Gmel) C.A. Sm. var. STEM BARK

5.30 Plant material

The stem bark of A.gummifera was collected from Chiromo Campus, University of Nairobi in June 1979. The bark was air dried in shadow and ground.

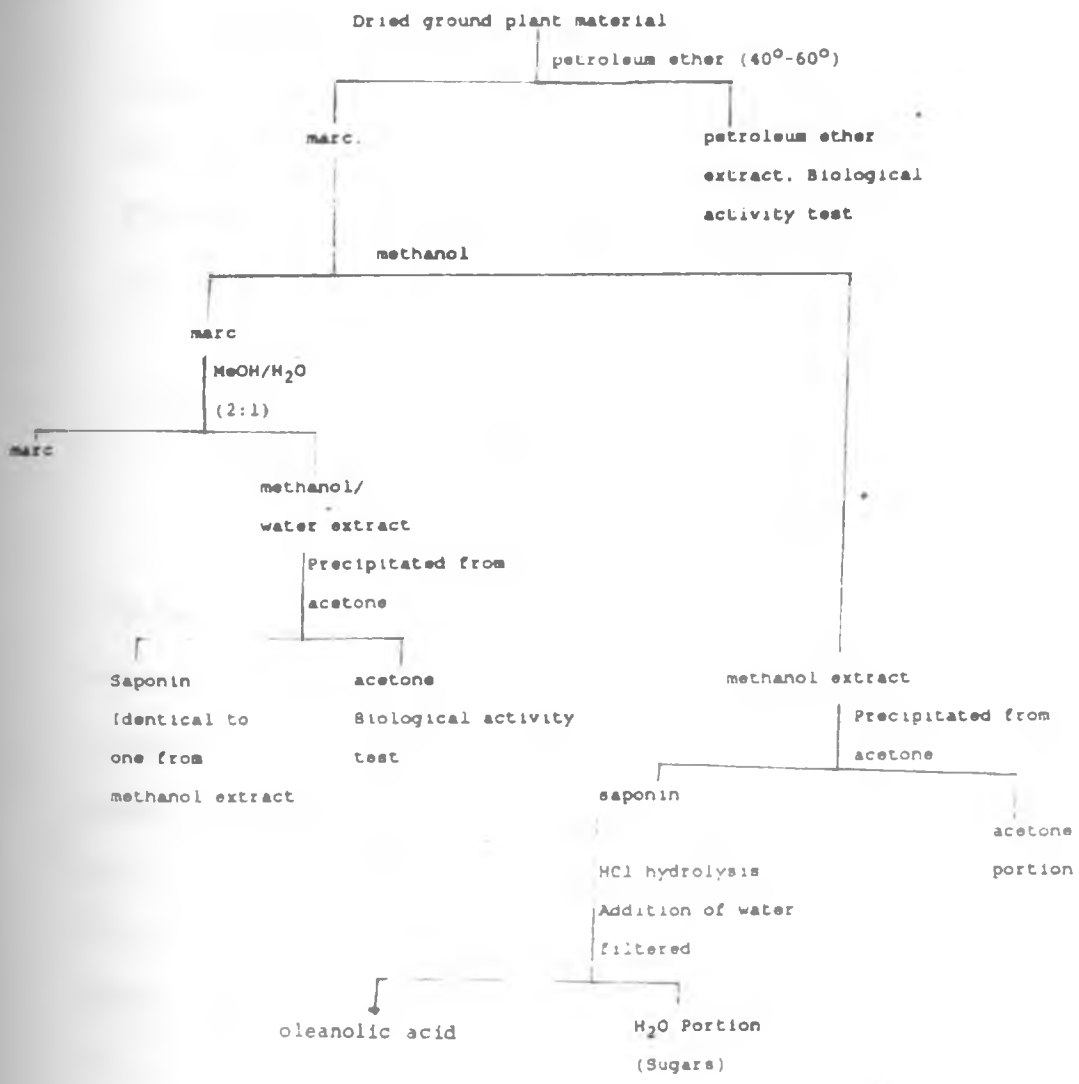
5.31 Extraction and purification.

450 g of the dried ground plant material was extracted successively with hot petroleum ether (40^{o}-60^{o}), hot methanol, and finally with hot methanol:water (2:1v/v) in a soxhlet extractor.

The petroleum ether extract was evaporated to dryness to give a brown viscous oil weighing 35 g. The petroleum ether extract was sent for biological activity test but was not further investigated chemically.

The methanol extract was evaporated to dryness to give a dark brown oily mass of 27.3 g. This was redissolved in a minimum volume of solvent and added dropwise to a large volume of acetone. A light pink precipitate was obtained, which, when washed with hot acetone and dried, gave a light pink powder revealing one major spot on TLC using the solvent systems isopropanol/water/formic acid (18:15:

Scheme 1. Fractionation of the ground bark of *Albizia gummifera*.



5v/v and ethyl acetate/ethanol/water (8:2:1v/v).

The acetone portion of the methanol extract gave an oily mass of 9.5 g when dried and was sent for biological activity testing.

The methanol/water extract was treated as the methanol extract to give a very light pink powder of 5 g in weight which was deduced to be identical to the saponin isolated from the methanol extract from TLC analysis.

5.32 Isolation of the triterpene from the saponin of the stem bark of Albizia gummifera.

1 g of the isolated saponin was hydrolysed with 2N hydrochloric acid. Addition of 50 ml distilled water resulted in a precipitate which was filtered, washed, dried and recrystallised from ethanol. White needlelike crystalls in 5% of the saponin were obtained and was identified as oleanolic acid; m.p. 278-280°C TLC: R_f 0.65; IR ν_{max}^{cm-1}: 1690-1600, 1450-1390, 1364; m/z (rel.int.): 456 (2) 438 (2), 410 (3.22), 392 (2), 248 (100), 233 (7) 219 (2.5); δ ($CDCl_3$; 100 MHz) 0.727 (s, 3H), 0.771 (s, 3H, 0.914 (s, 9H), 0.986 (s, 3H), 1.134 (s, 3H), 2.82 (ABq, J_1 = 5.0 Hz, J_2 = 11.2 Hz, 1H), 3.214 (tripletlike m, 1H), 5.286 (m, 1H).

The methyl oleanolate was prepared from the isolated oleanolic acid by methylation under normal conditions, m/z (rel.int.): 470 (5.8), 455 (1.3), 410 (4.2), 262 (63.6), 203 (100); δ ($CDCl_3$; 100 MHz) 0.725 (s, 3H), 0.784 (s, 3H), 0.902 (s, 9H), 0.990 (s, 3H), 1.132 (s, 3H), 2.860 (ABq, J_1 = 5.65 Hz, J_2 = 11.2 Hz), 3.622 (s, 3H), 5.284 (m, 1H) ppm.

Biological testing for the A. gummifera extracts was done at the Tropical products Institute, London, on guinea pigs. The samples were dissolved in propylene glycol.

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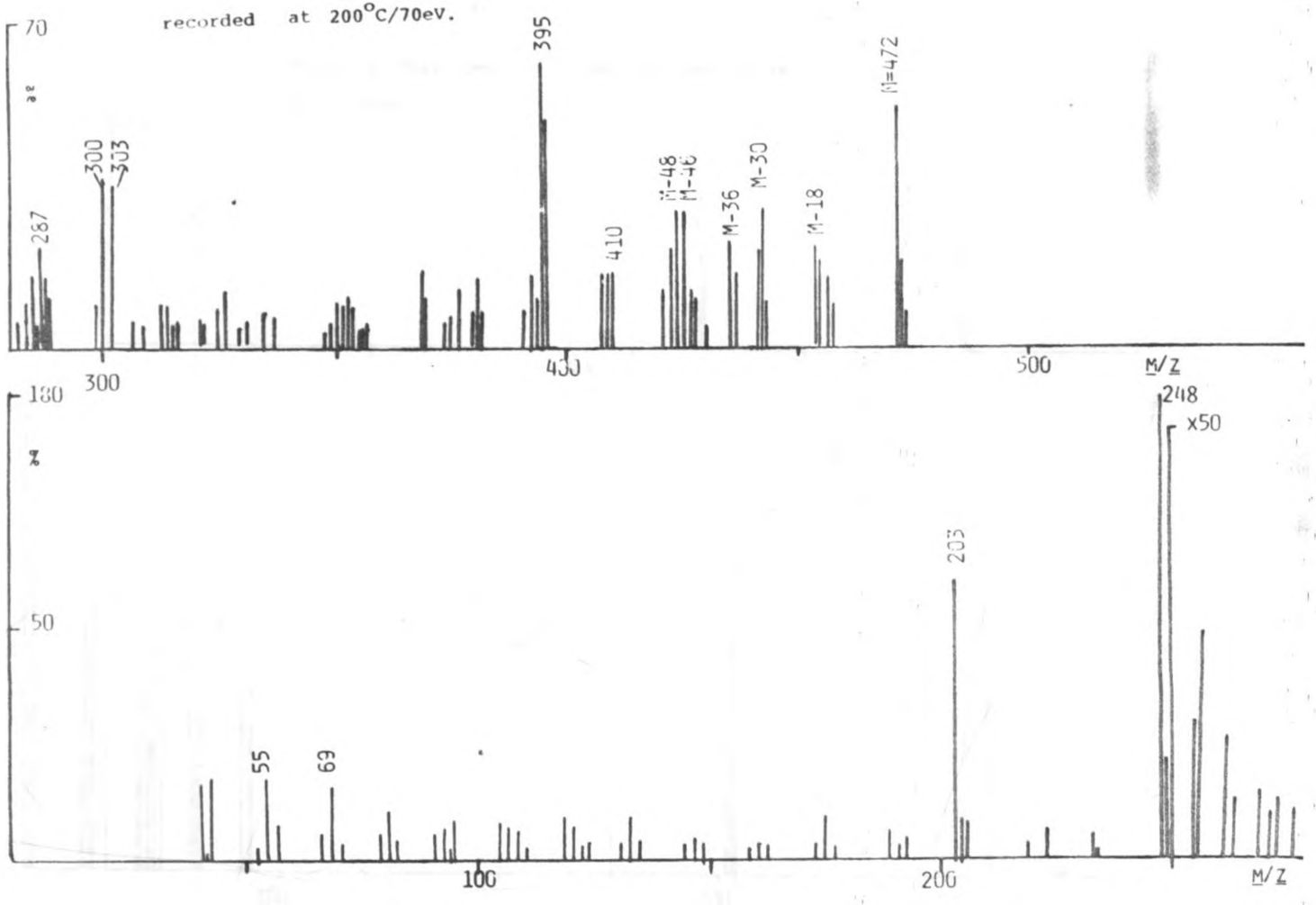
A P P E N D I X

Figure 3. Mass spectrum of hederagenin recorded at 200°C/70eV.



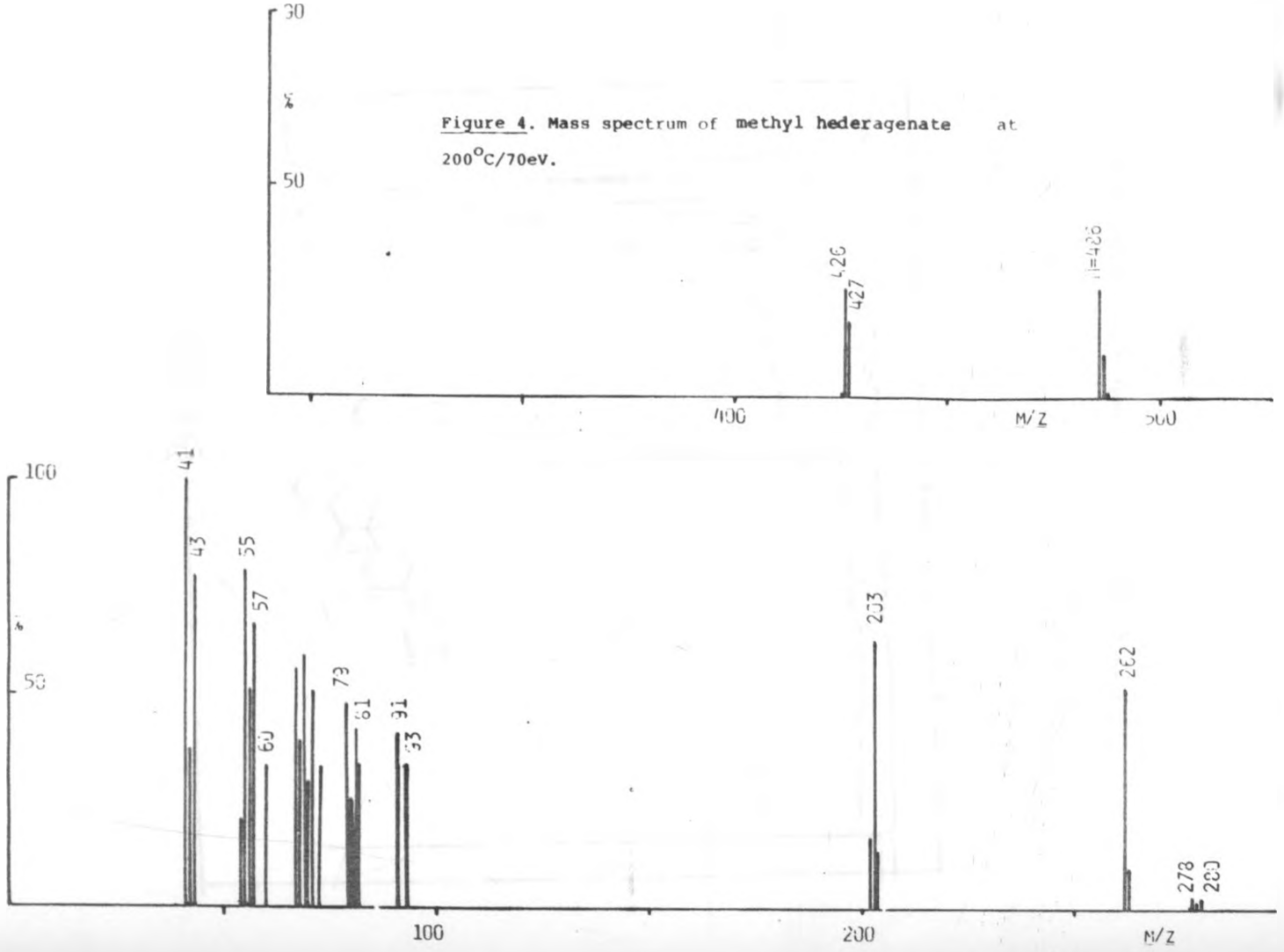


Figure 4. Mass spectrum of methyl hederagenate at 200°C/70eV.

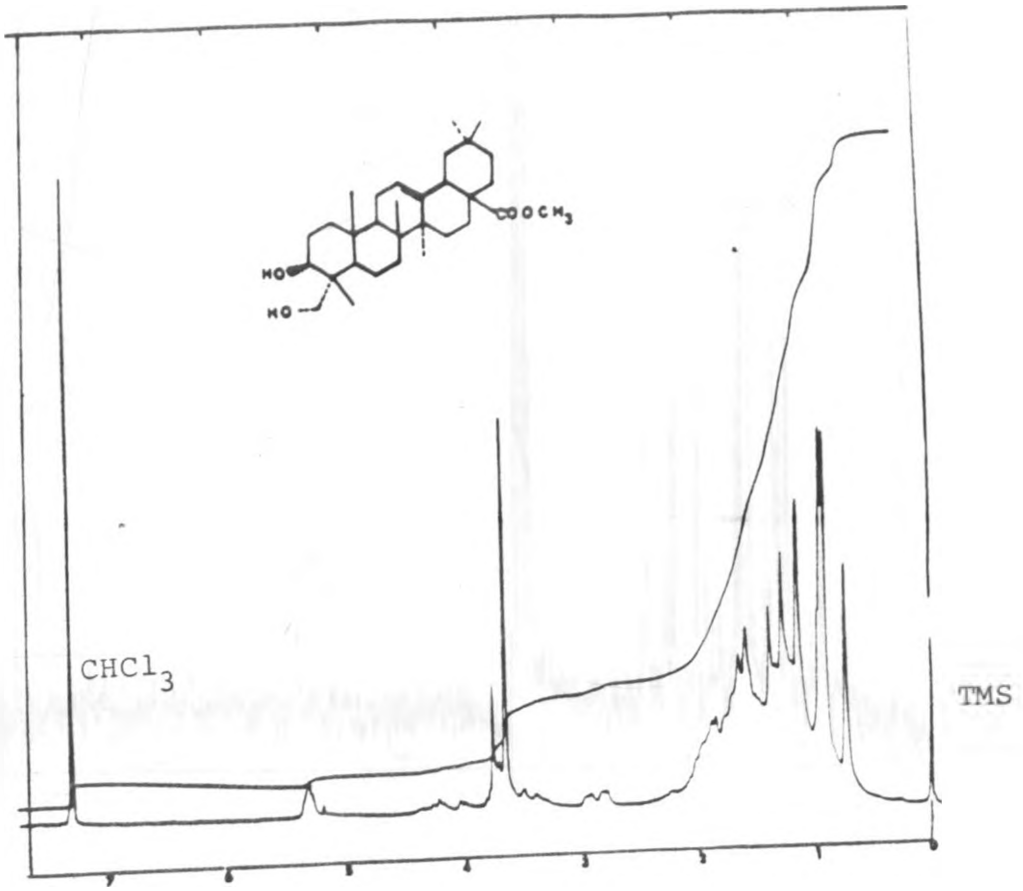


Figure 5. ^{1}H NMR spectrum of hederagenin methyl ester

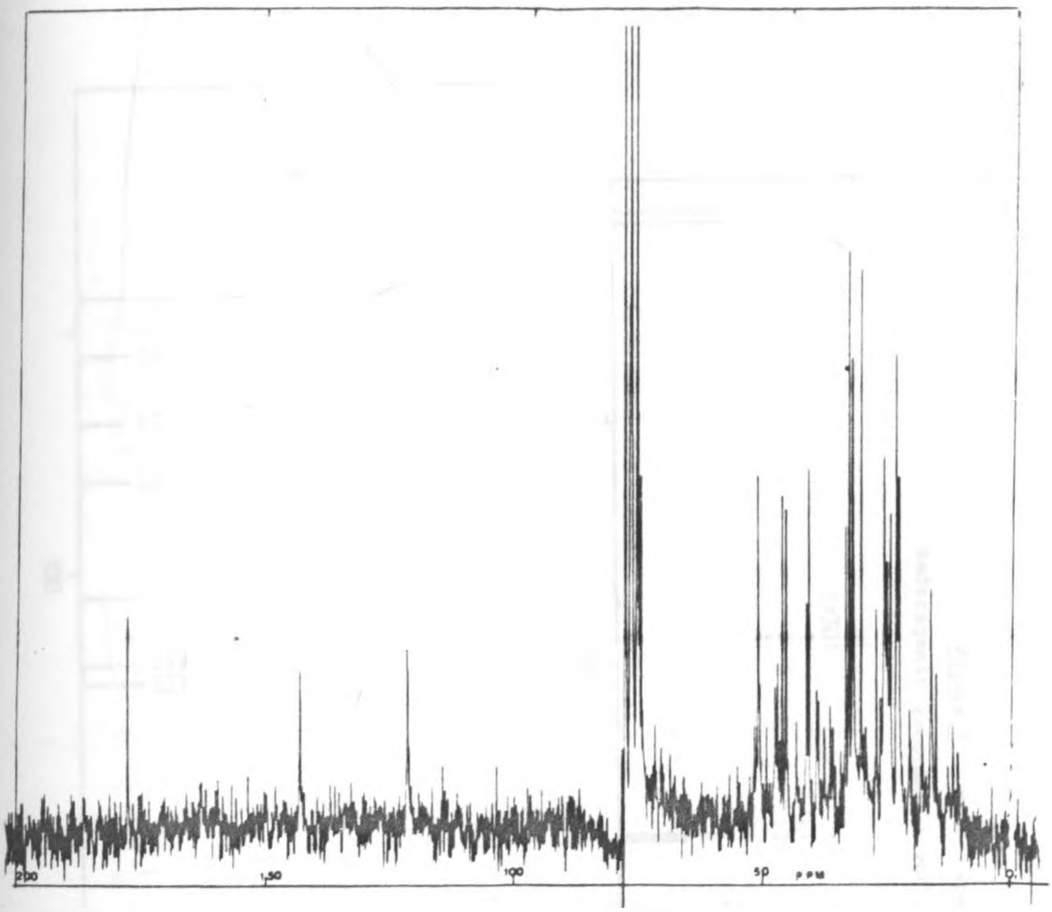


Figure 6. ^{13}C NMR spectrum of hederagenin methyl ester

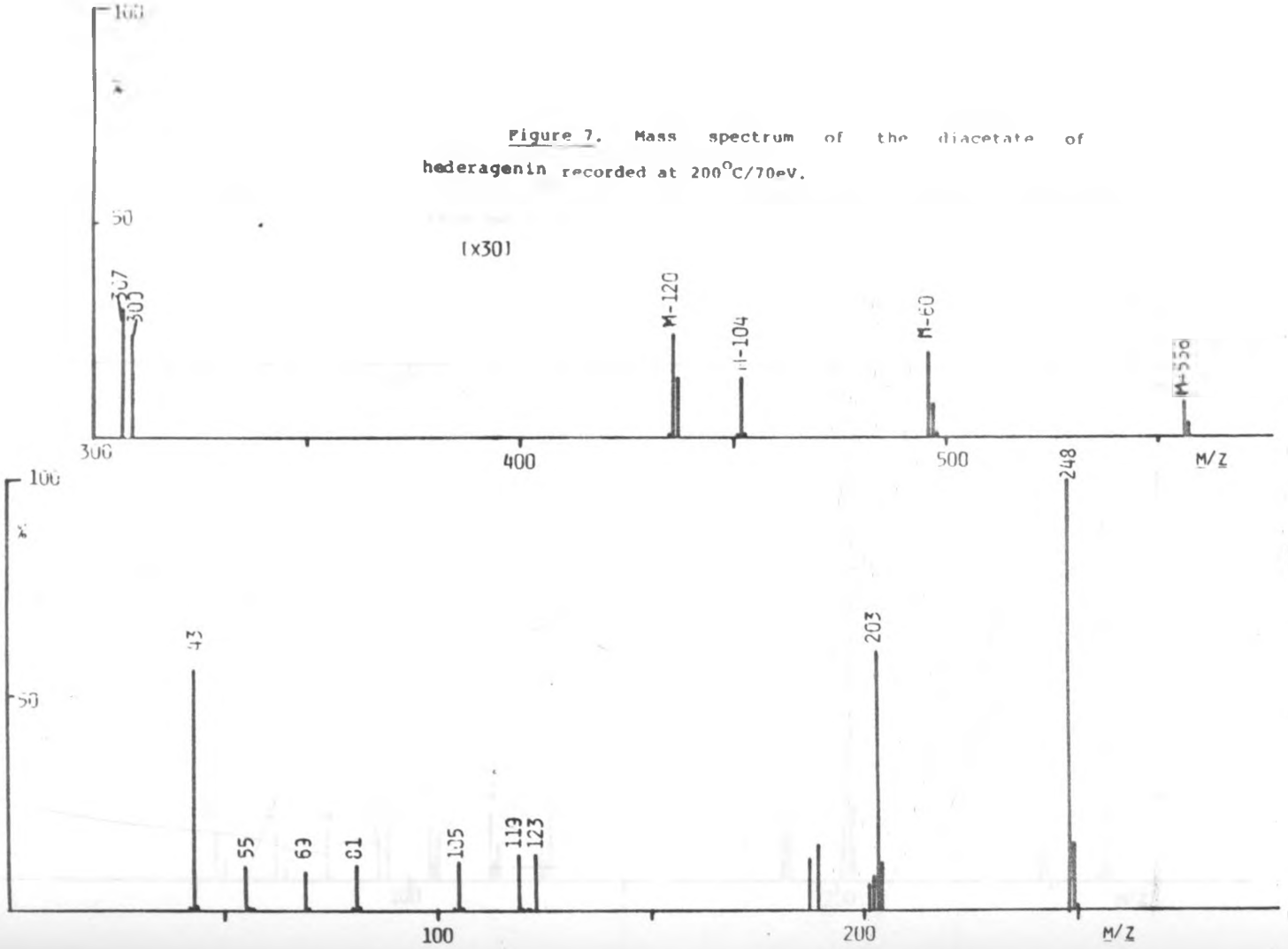


Figure 7. Mass spectrum of the diacetate of hederagenin recorded at 200°C/70eV.

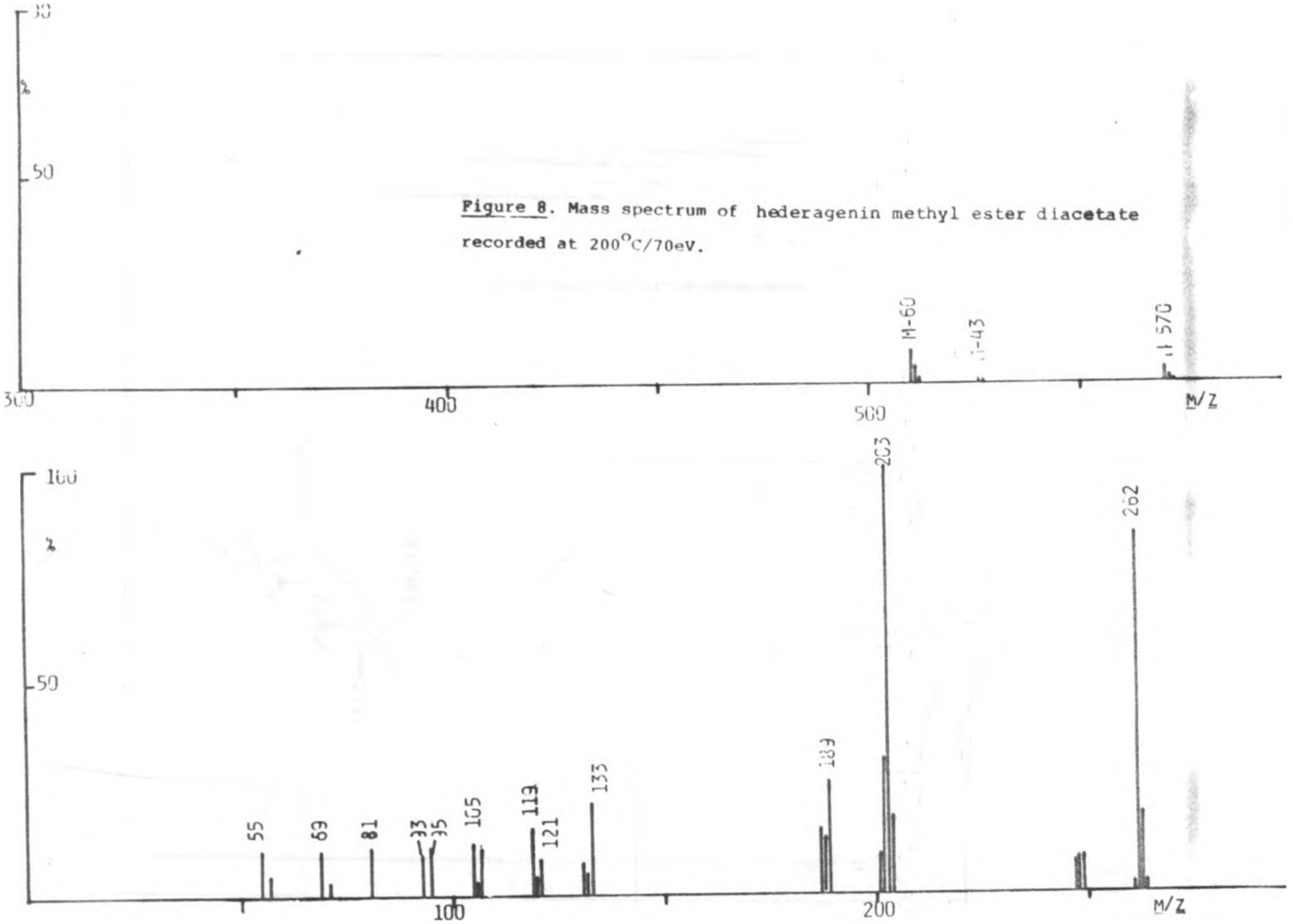


Figure 8. Mass spectrum of hederagenin methyl ester diacetate recorded at 200°C/70eV.

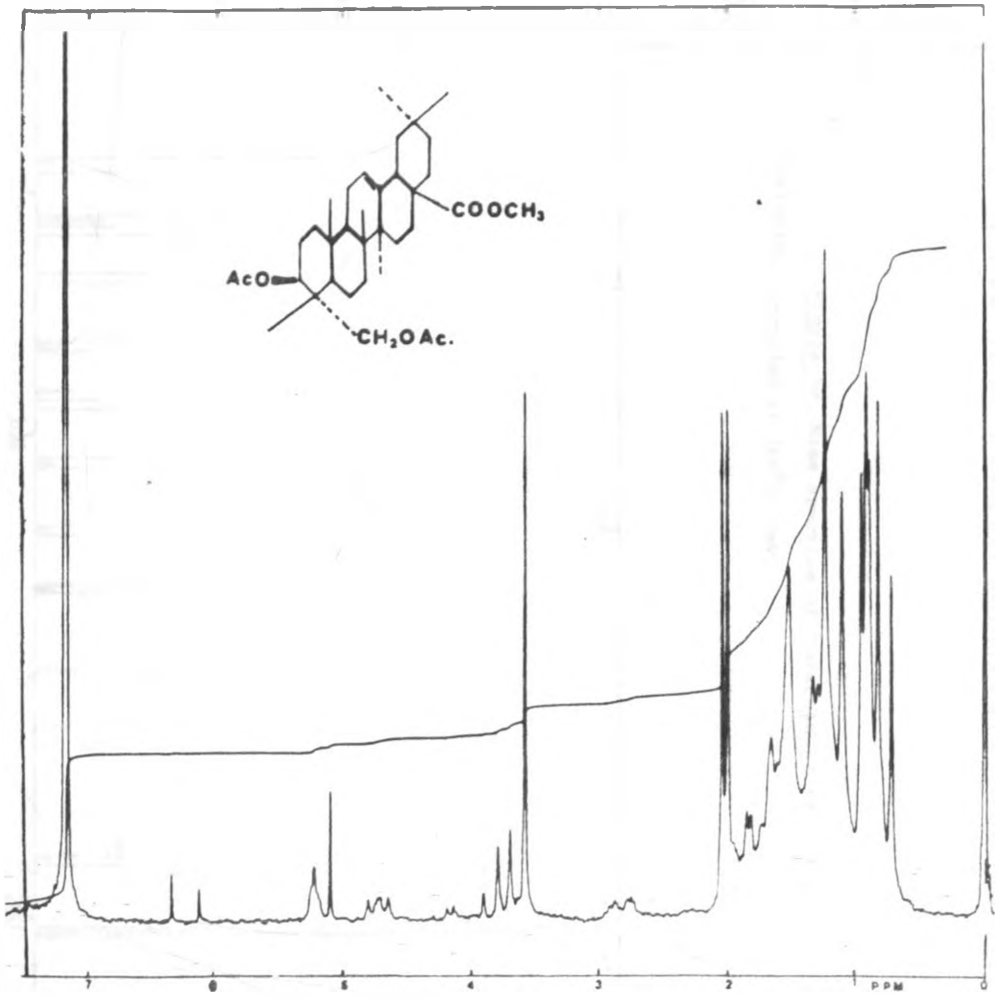


Figure 9. ^{1}H NMR spectrum of hederagenin methyl ester diacetate

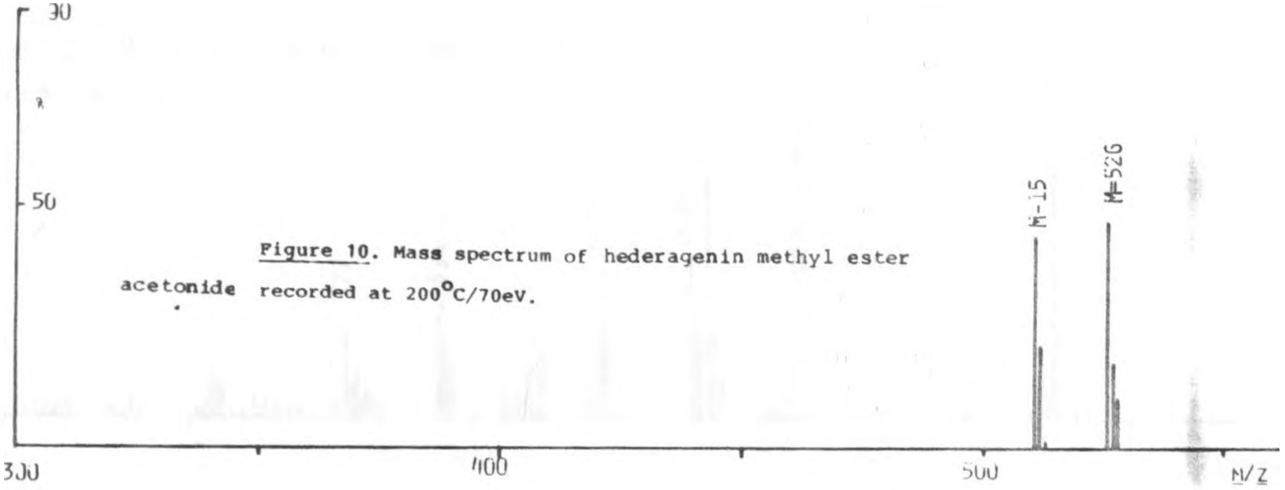
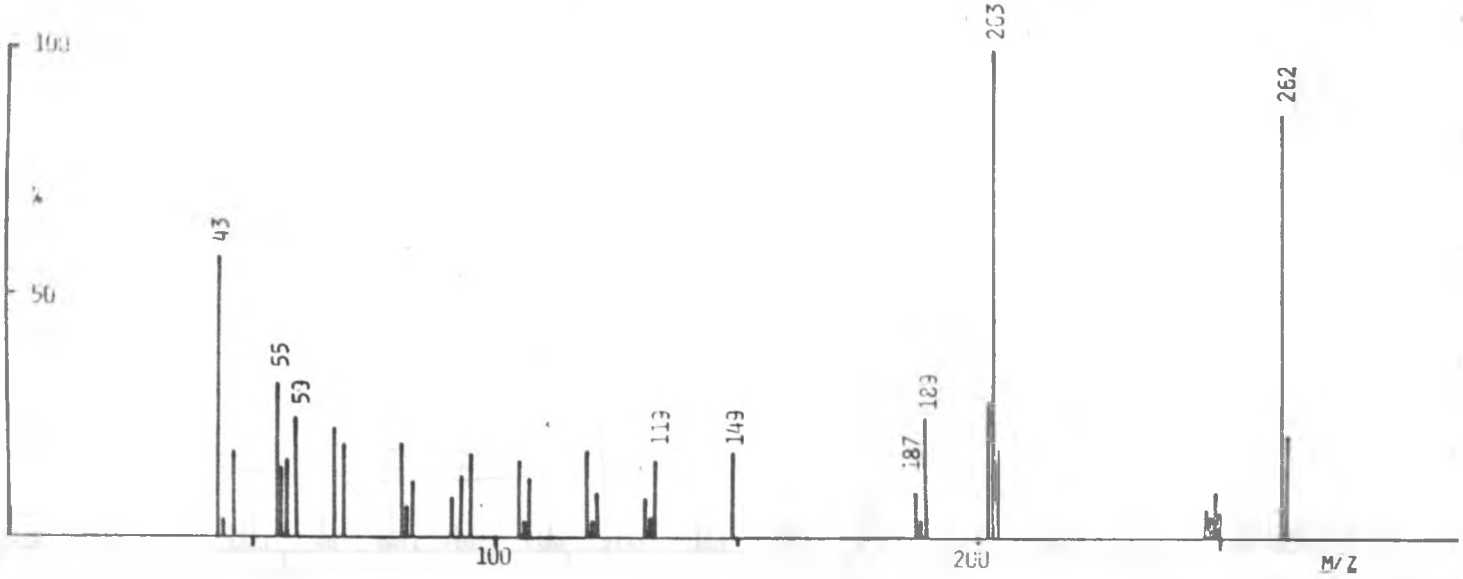


Figure 10. Mass spectrum of hederagenin methyl ester acetonide recorded at 200°C/70eV.



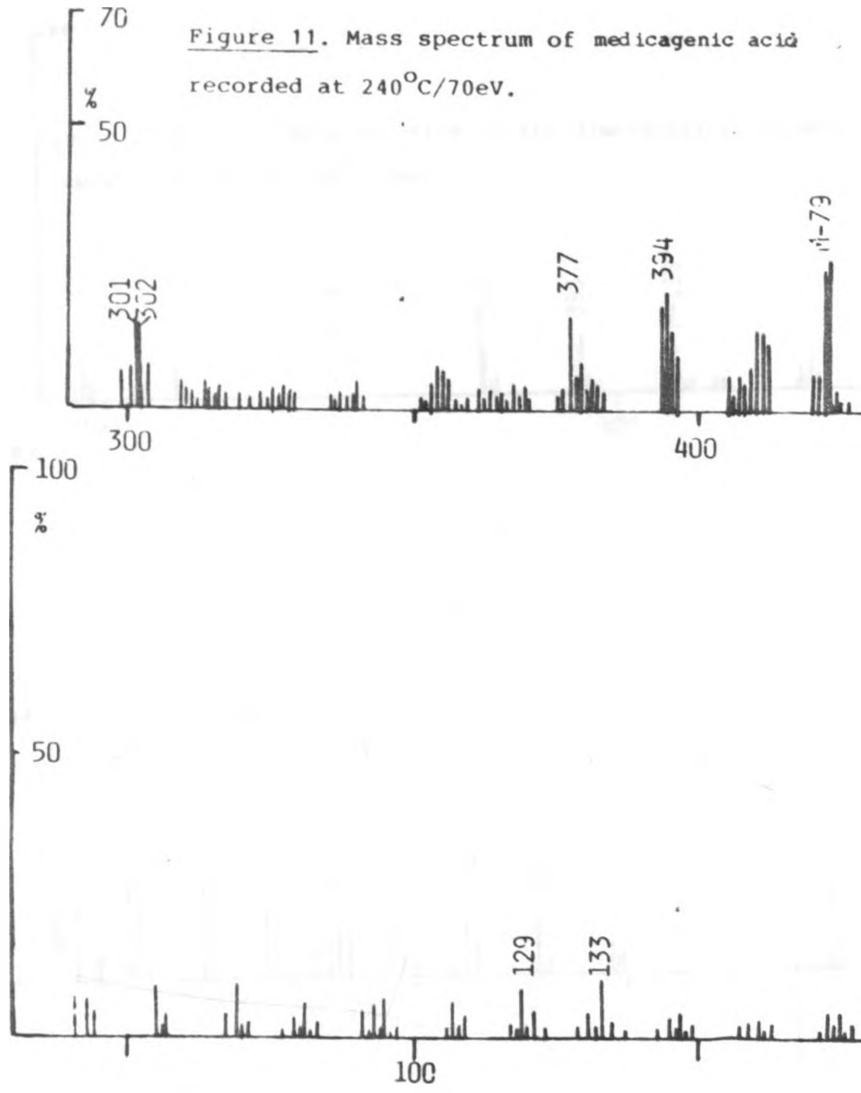


Figure 11. Mass spectrum of medicagenic acid recorded at 240°C/70eV.

M-65
M-61
M-46
(3%)
M-15
M=502
500
M/Z
248
x50
203
273
287
200
300
M/Z

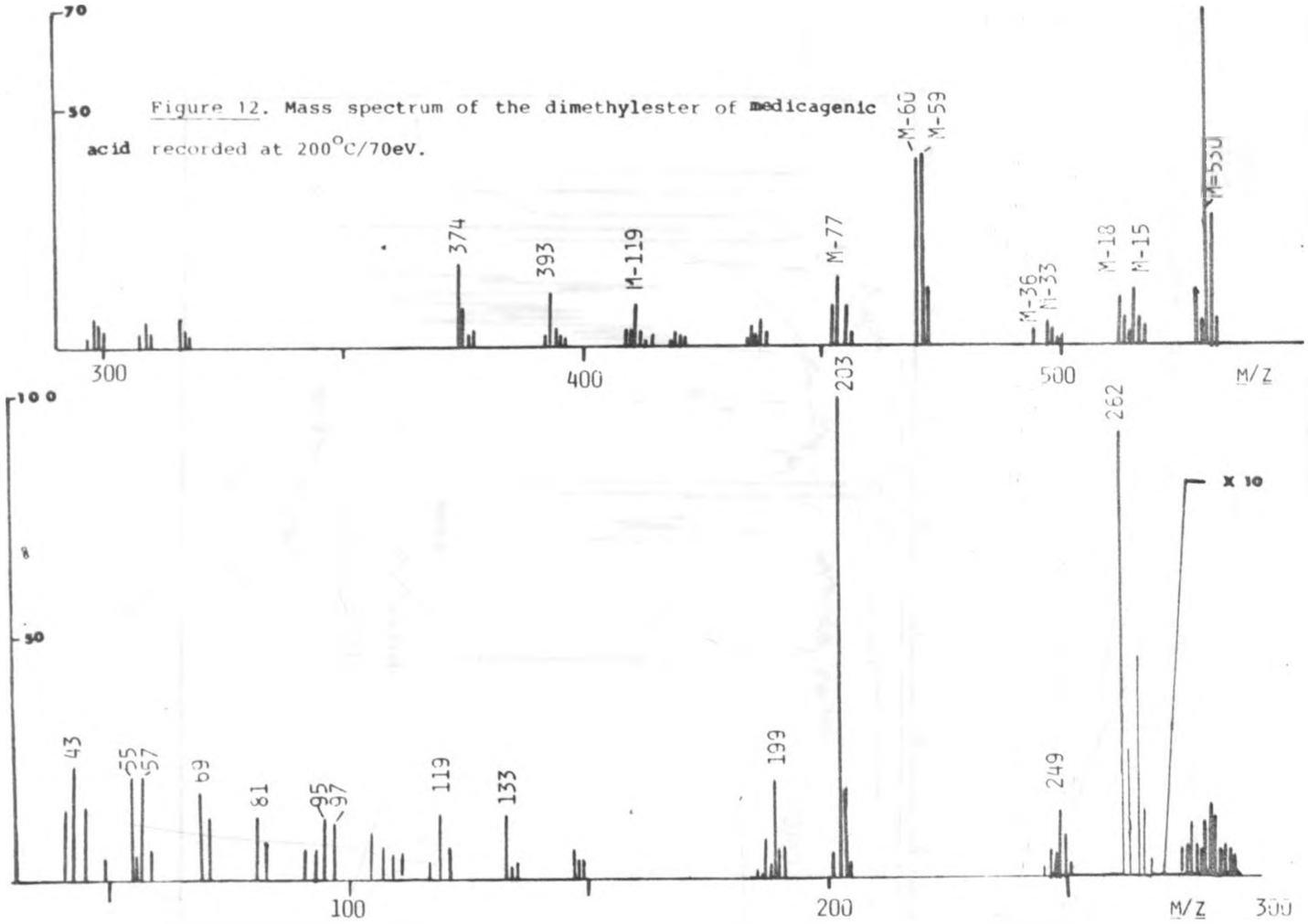


Figure 12. Mass spectrum of the dimethylester of medicagenic acid recorded at 200°C/70eV.

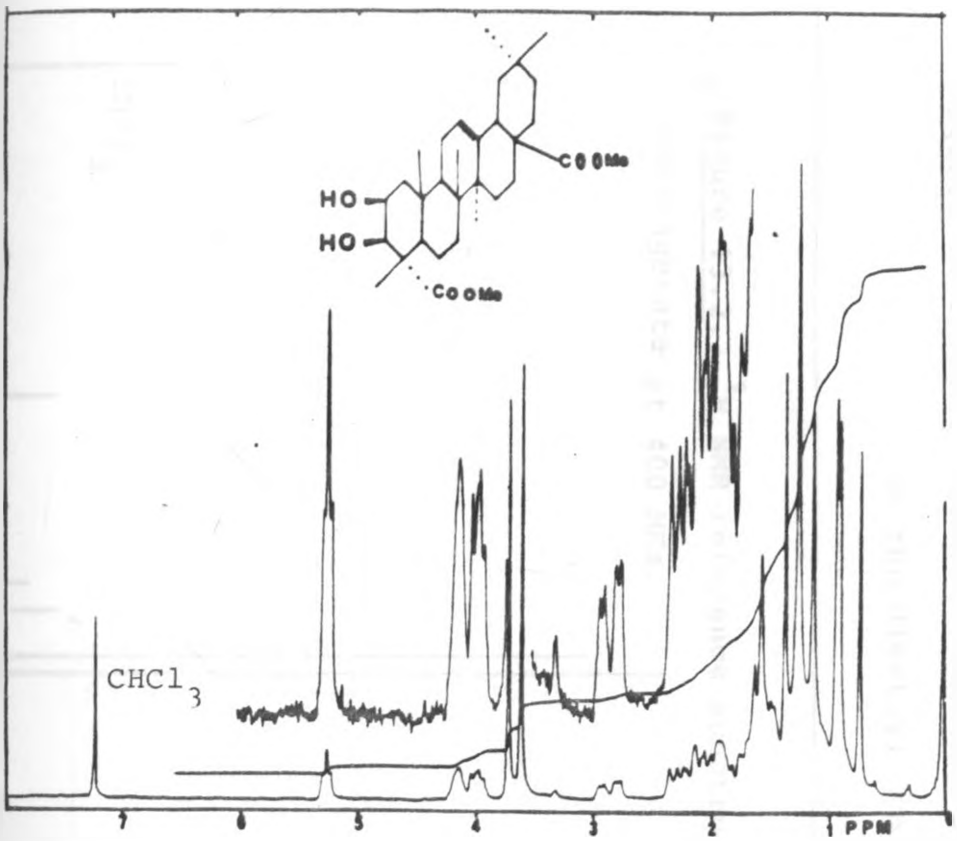


Figure 13.1 ^{1}H NMR spectrum of the dimethyl medicagenate at 100 MHz.

Figures 13.2-13.8. Nuclear Overhauser Effect (NOE) experiments on the dimethyl medicagenate

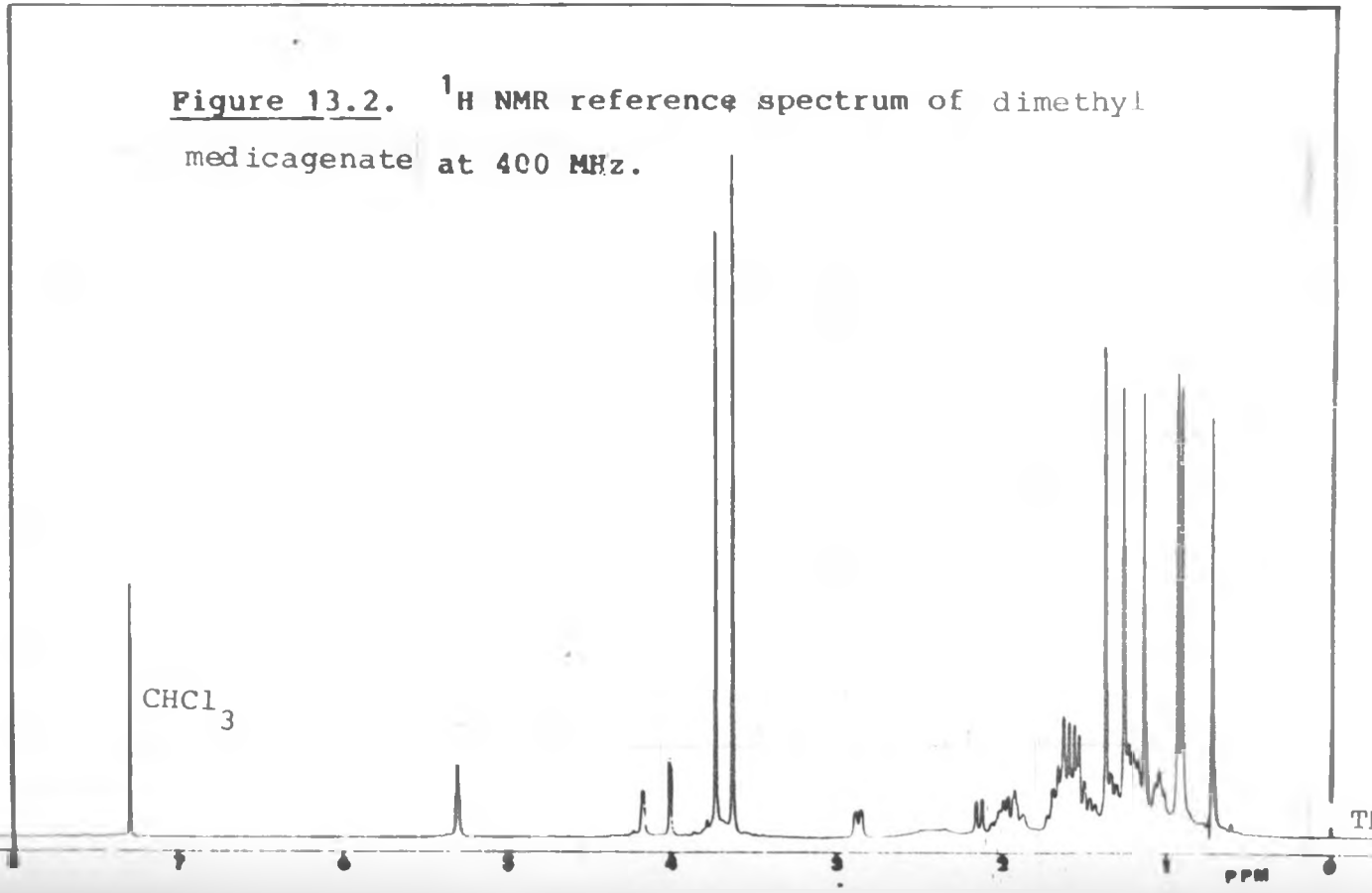
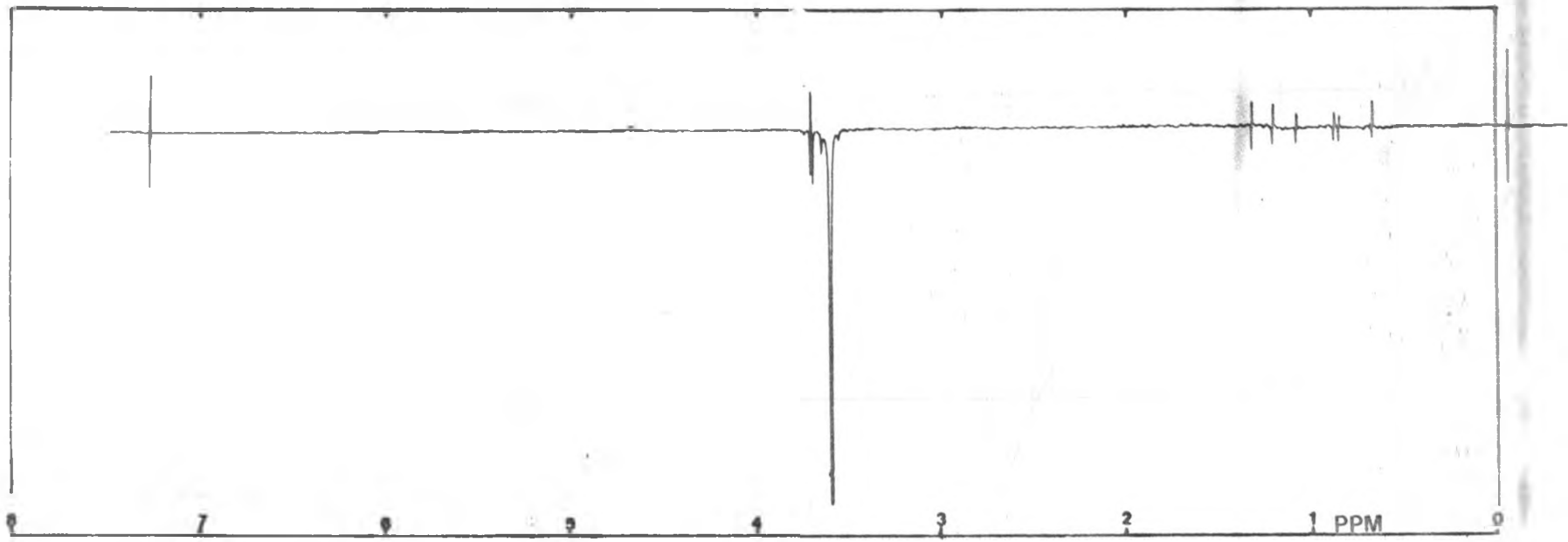


Figure 13.2. ^{1}H NMR reference spectrum of dimethyl medicagenate at 400 MHz.

Figure 13.3. Irradiation at 1446.56 Hz on the signal at δ 3.6152 ppm.



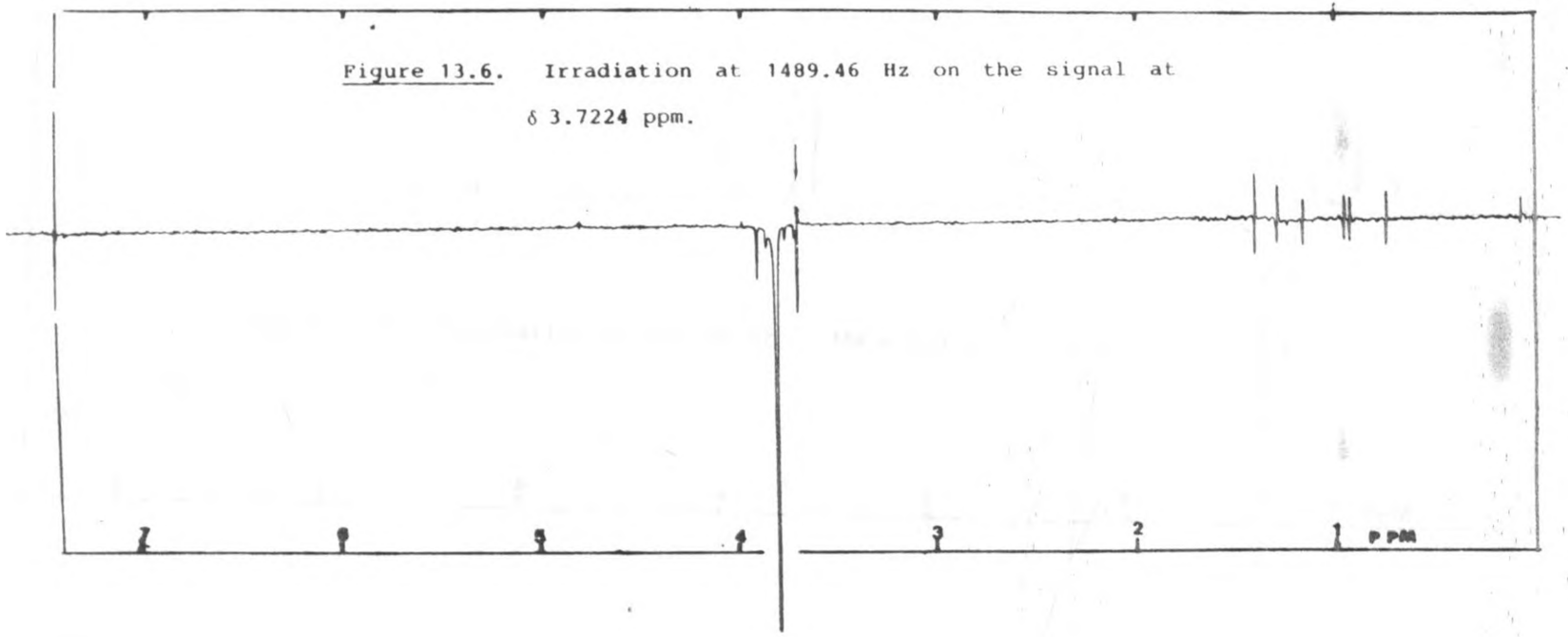


Figure 13.6. Irradiation at 1489.46 Hz on the signal at δ 3.7224 ppm.

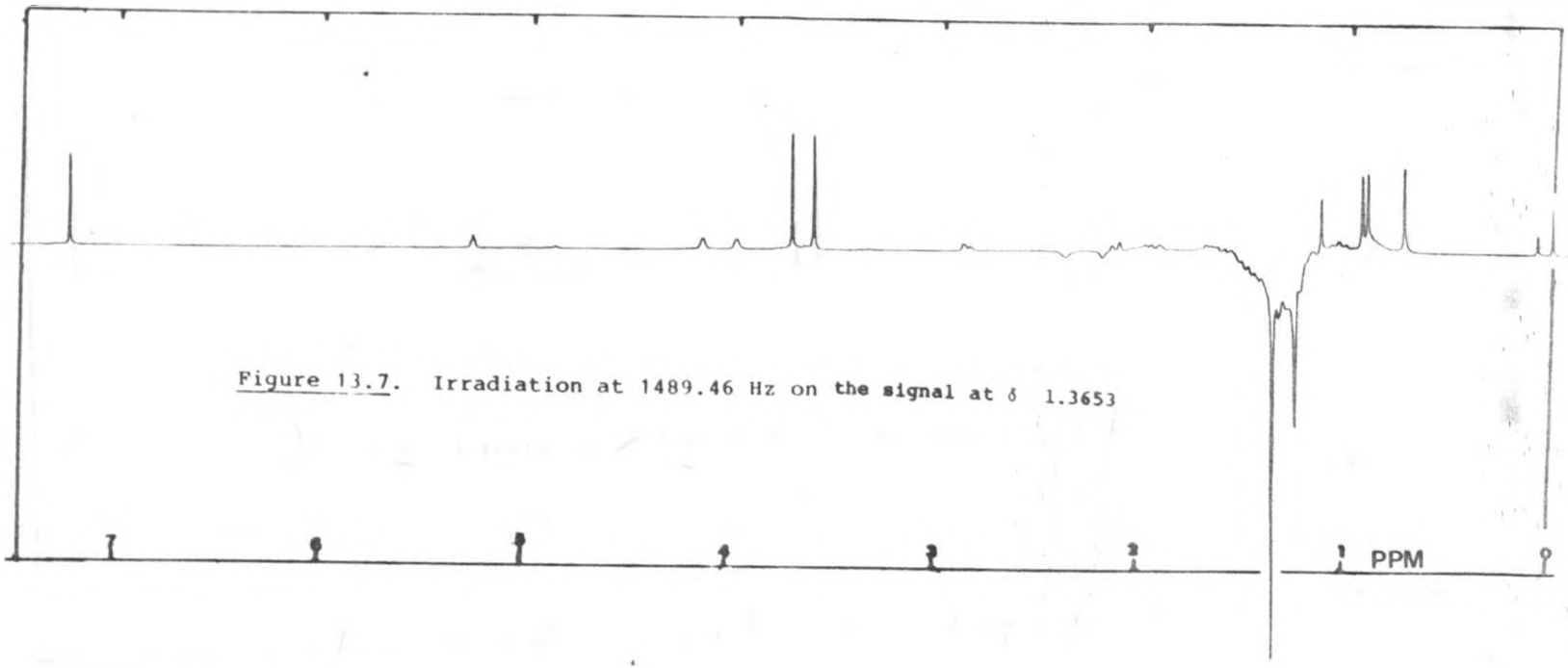


Figure 13.7. Irradiation at 1489.46 Hz on the signal at δ 1.3653

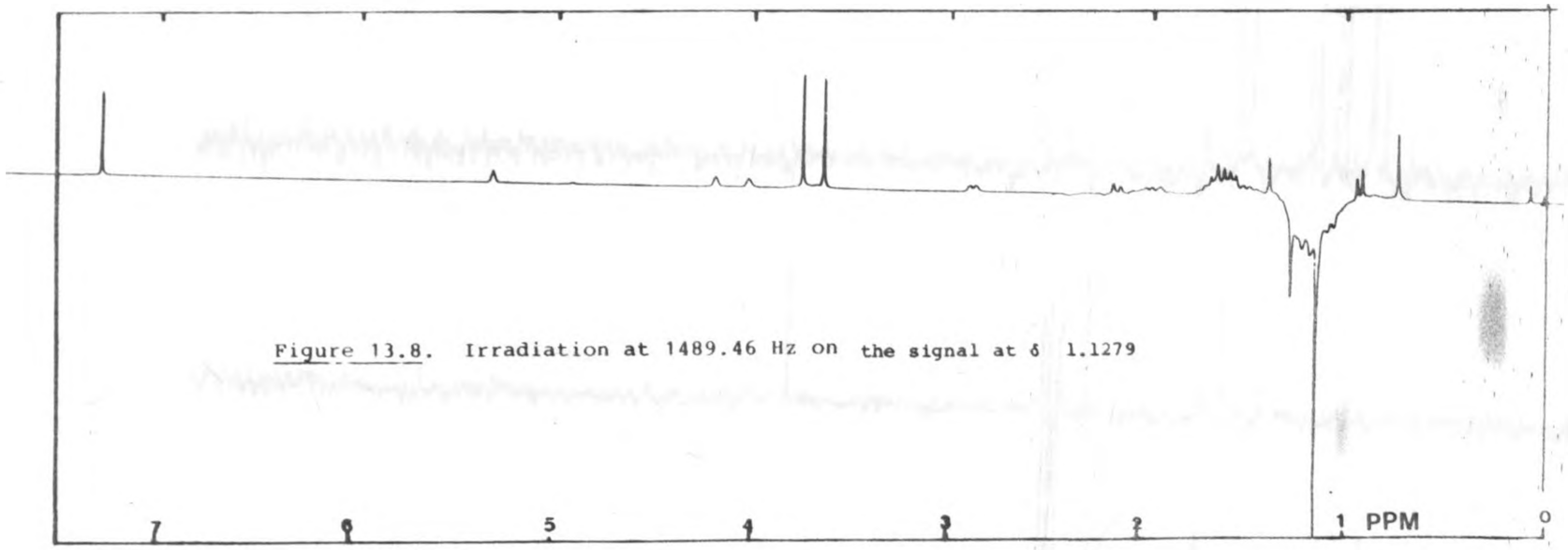
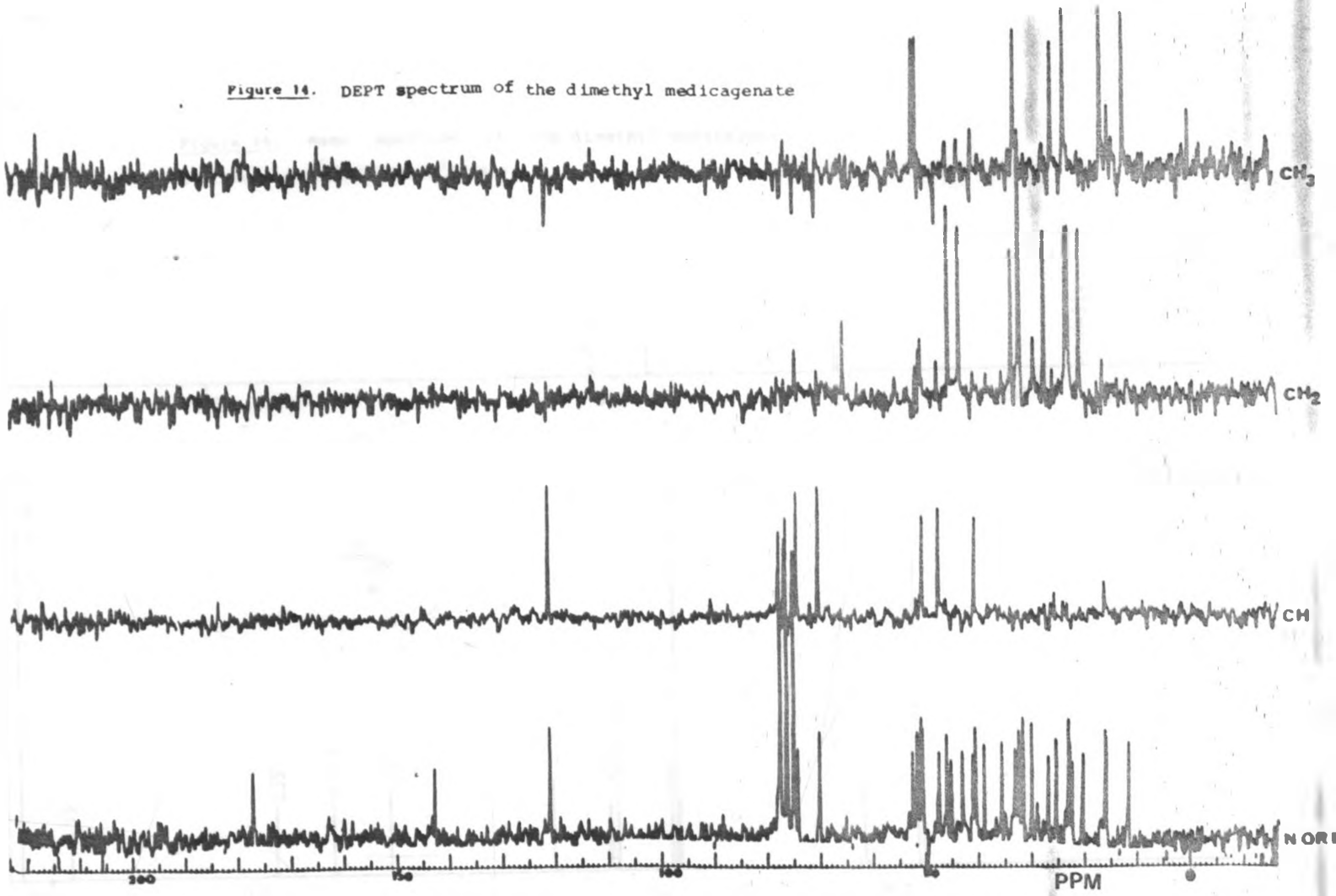


Figure 13.8. Irradiation at 1489.46 Hz on the signal at δ 1.1279

Figure 14. DEPT spectrum of the dimethyl medicagenate



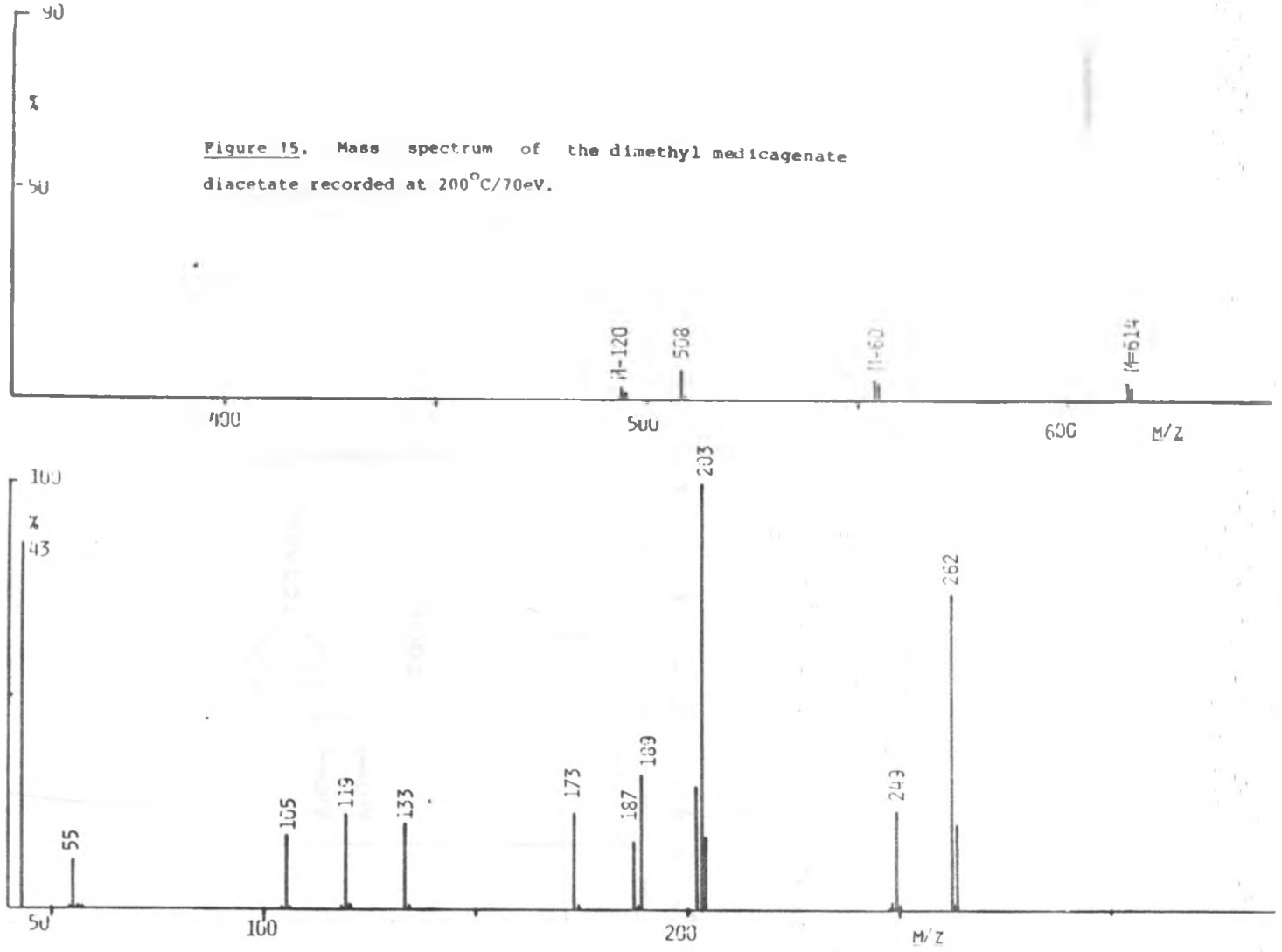


Figure 15. Mass spectrum of the dimethyl medicagenate diacetate recorded at 200°C/70eV.

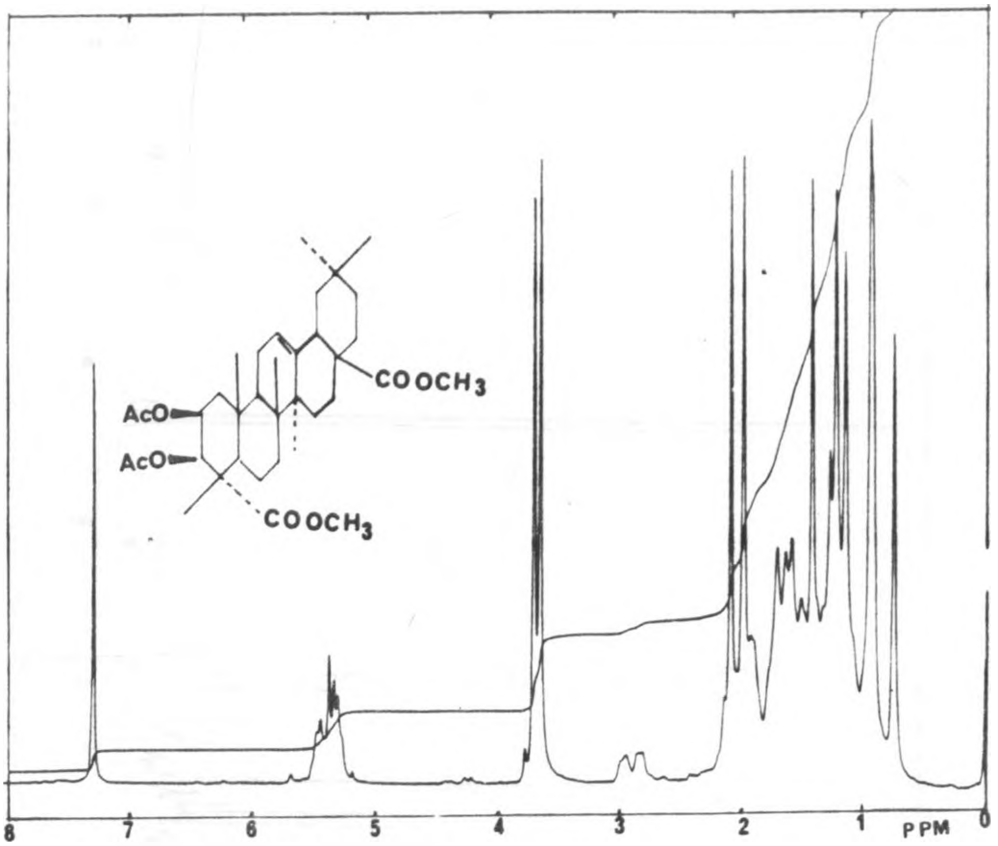
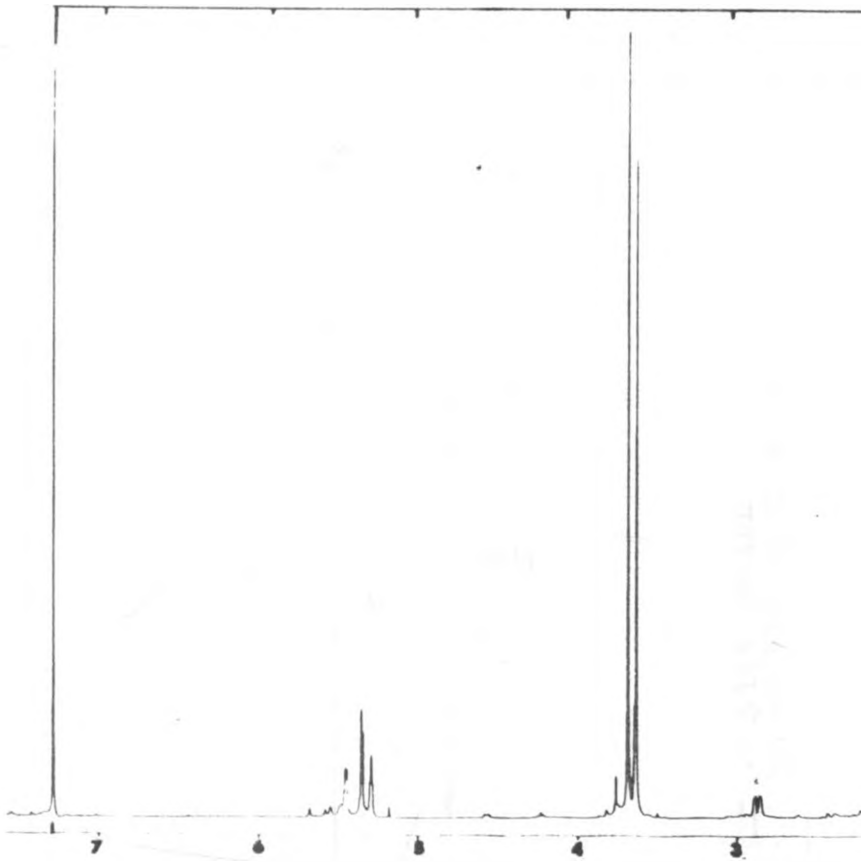


Figure 16. ^{1}H NMR spectrum of dimethyl medicagenate diacetate at 100 MHz.


7
6
5
4
3

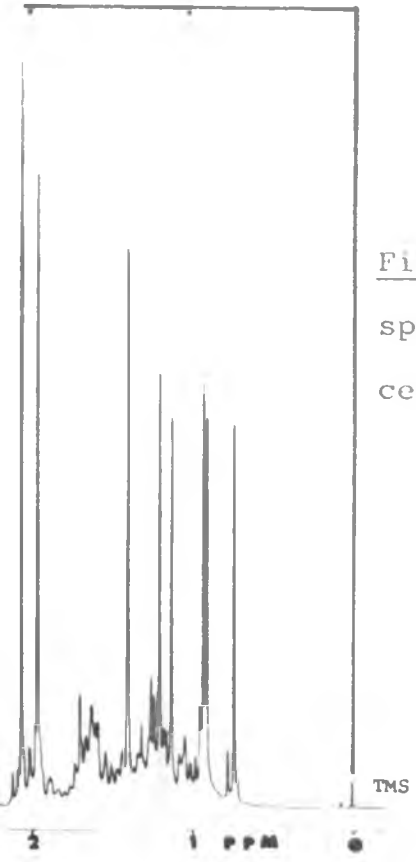


Figure 16.2. ^{1}H NMR reference spectrum of imethyl medicagenate: diacetate at 400 MHz.

Figure 16.3. Decoupling of the signal at δ 5.294 ppm of the dimethylester diacetate of medicagenic acid at 400 MHz.

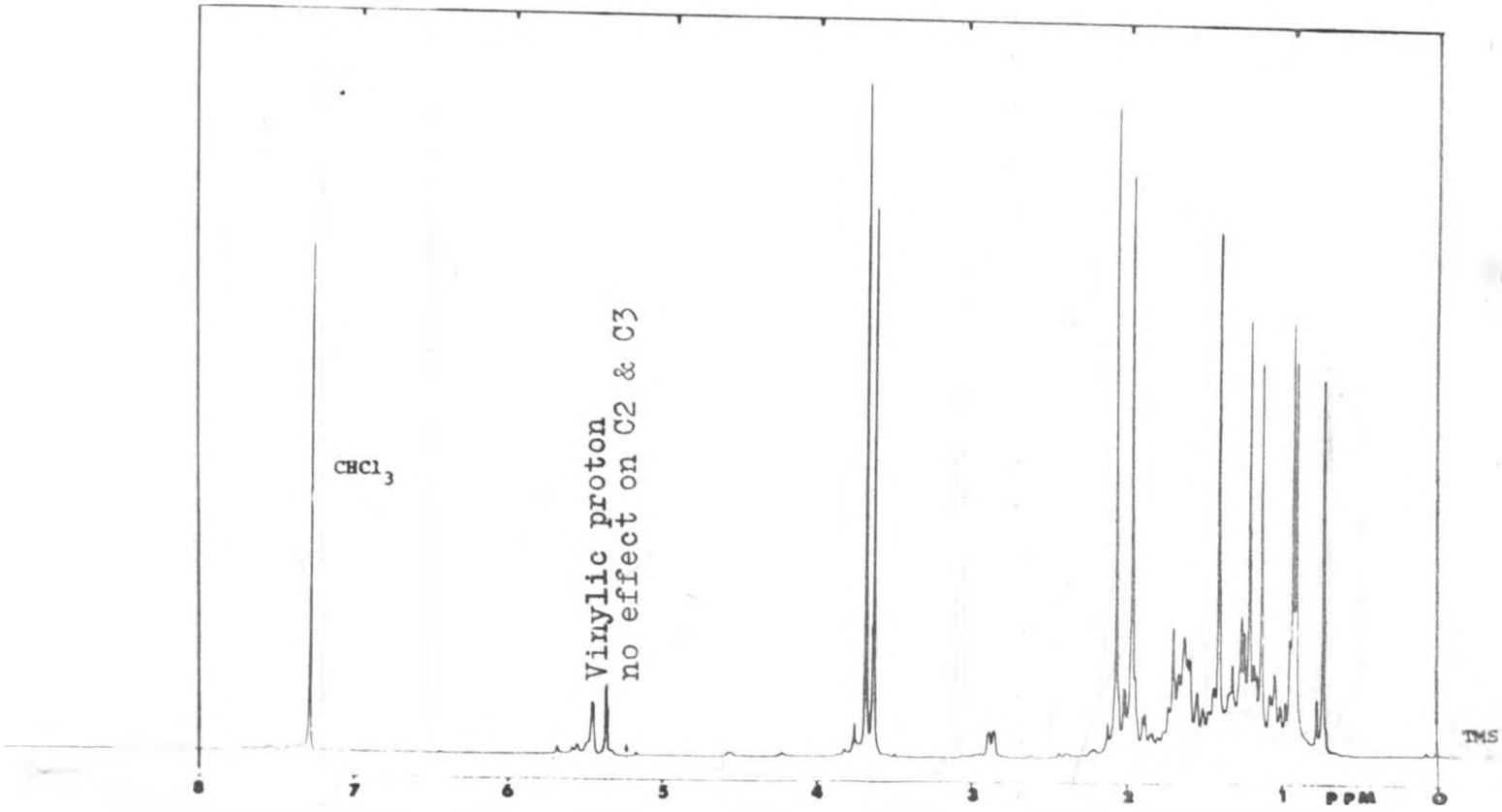
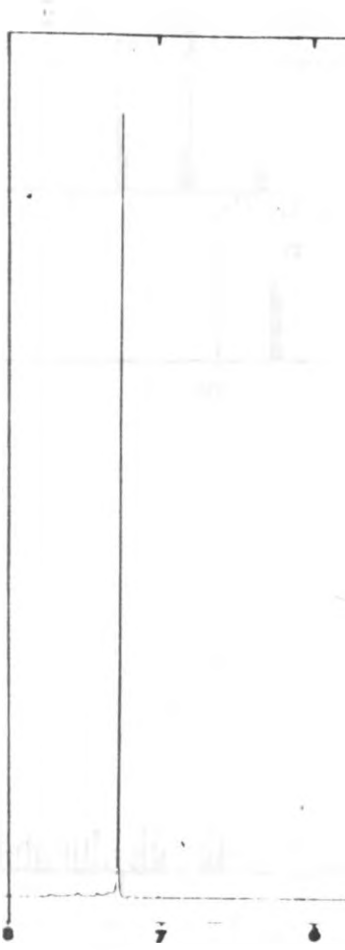
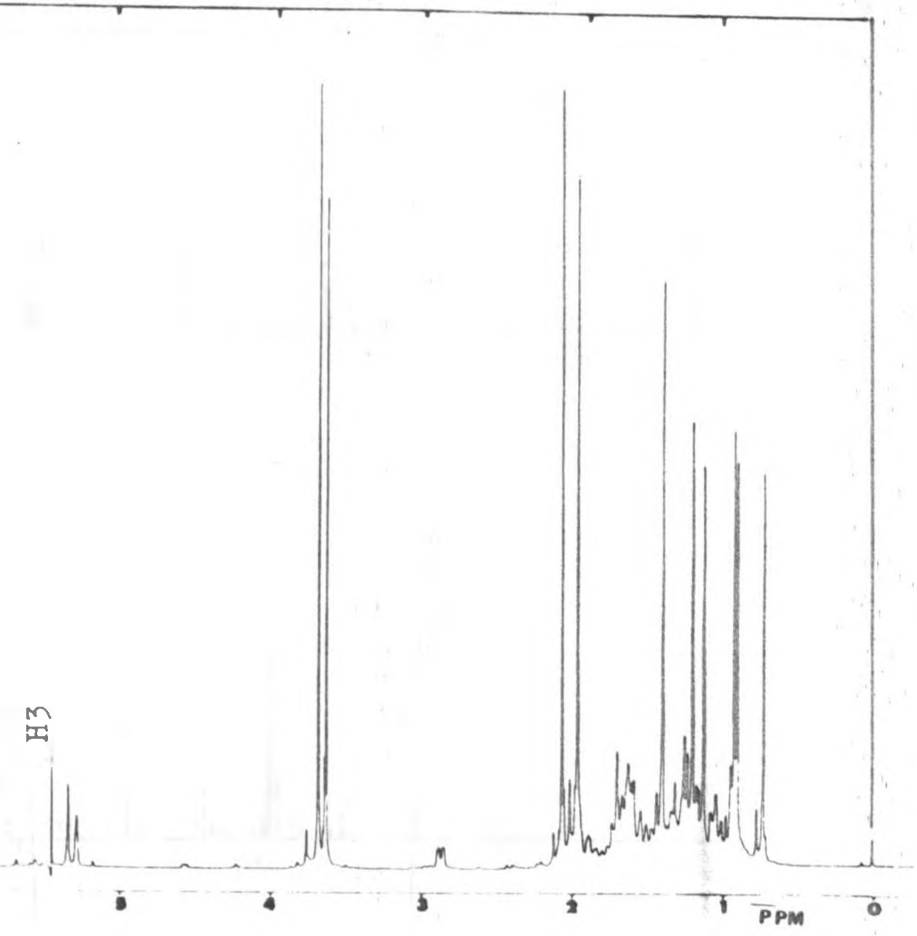


Figure 16.4.



Decoupling of the signal at δ 5.464 ppm of the dimethylester diacetate of medicagenic acid at 400 MHz.



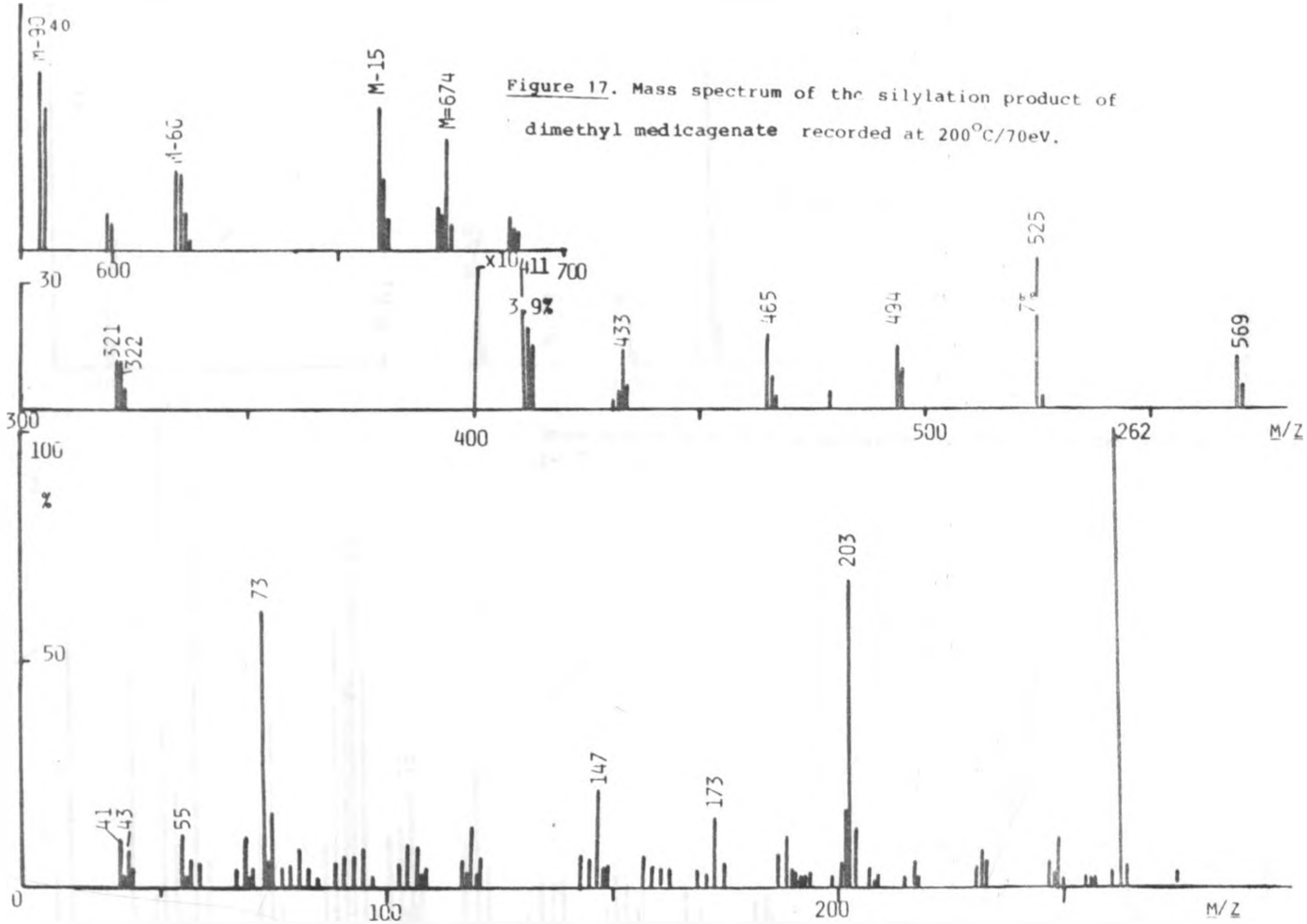
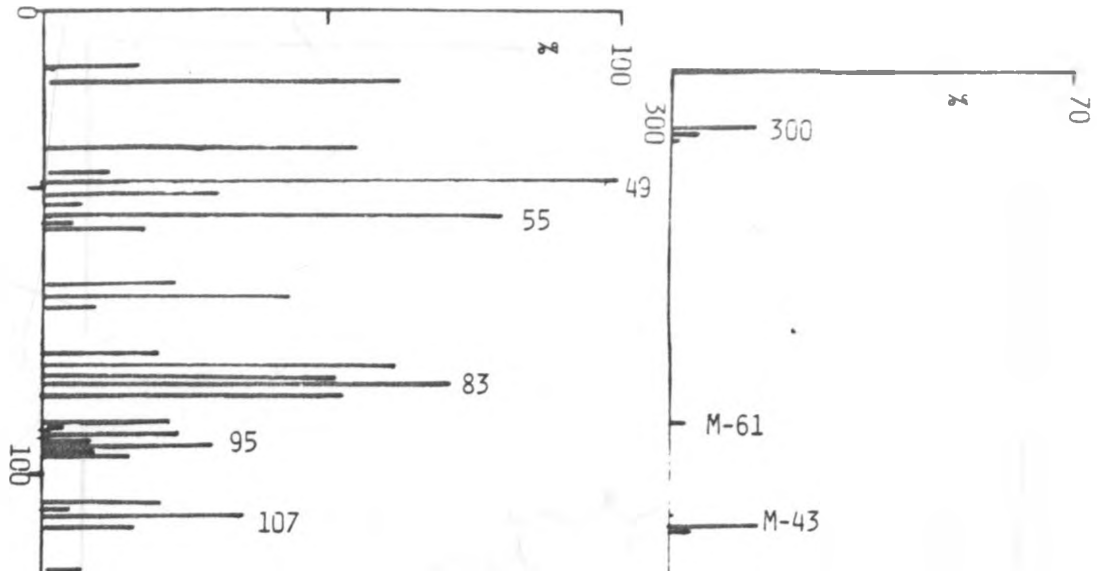


Figure 17. Mass spectrum of the silylation product of dimethyl medicagenate recorded at 200°C/70eV.


0
%
100
49
55
83
95
100
107
300
300
%
70
M-61
M-43

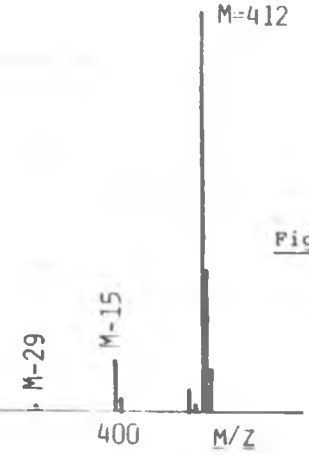
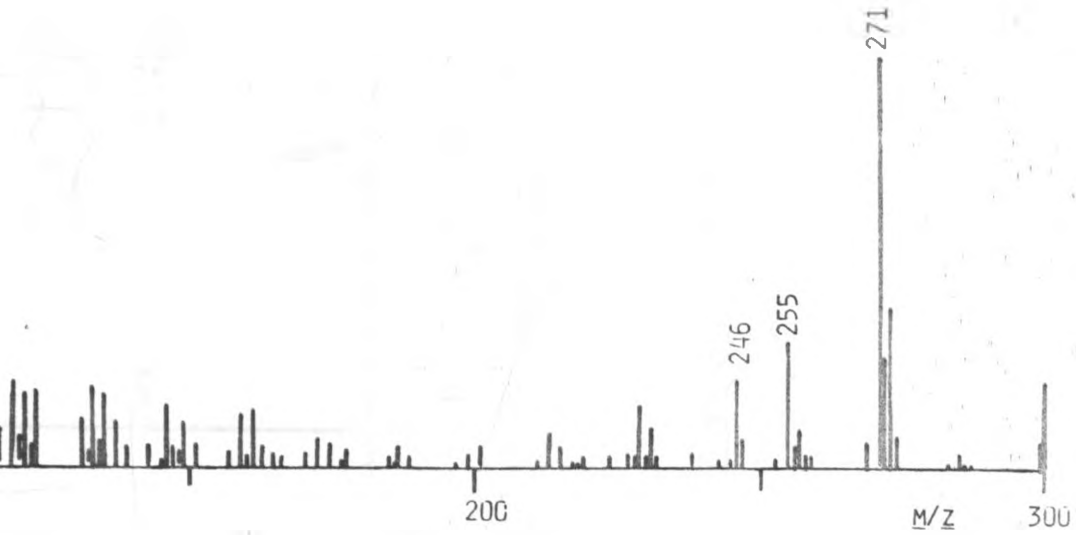


Figure 18.

Mass spectrum of the α-spinasterol/chandrillasterol from R.revoilii recorded at 150°C/70eV.



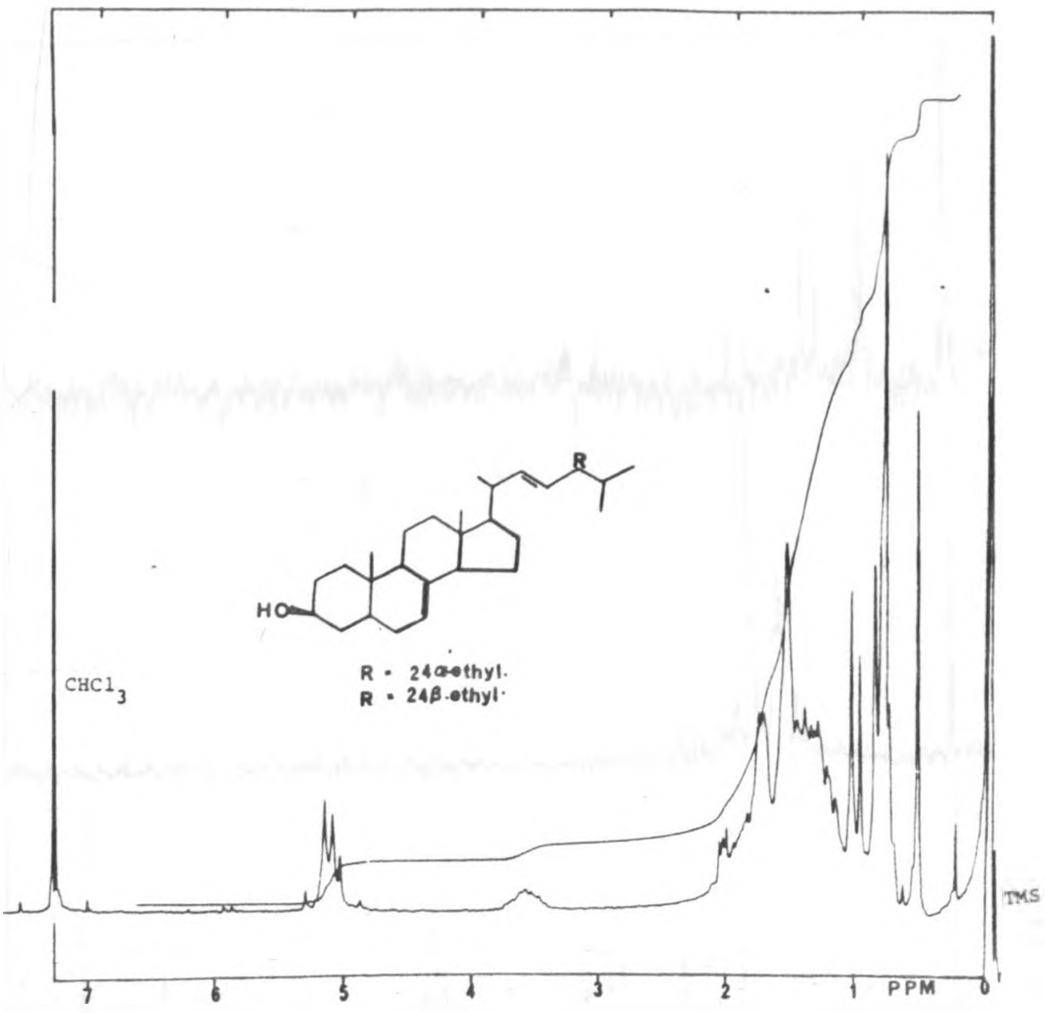


Figure 19. ^{1}H NMR spectrum of the 3-spinasterol/chondrillasterol from Rhoicissus revoilii tuber.

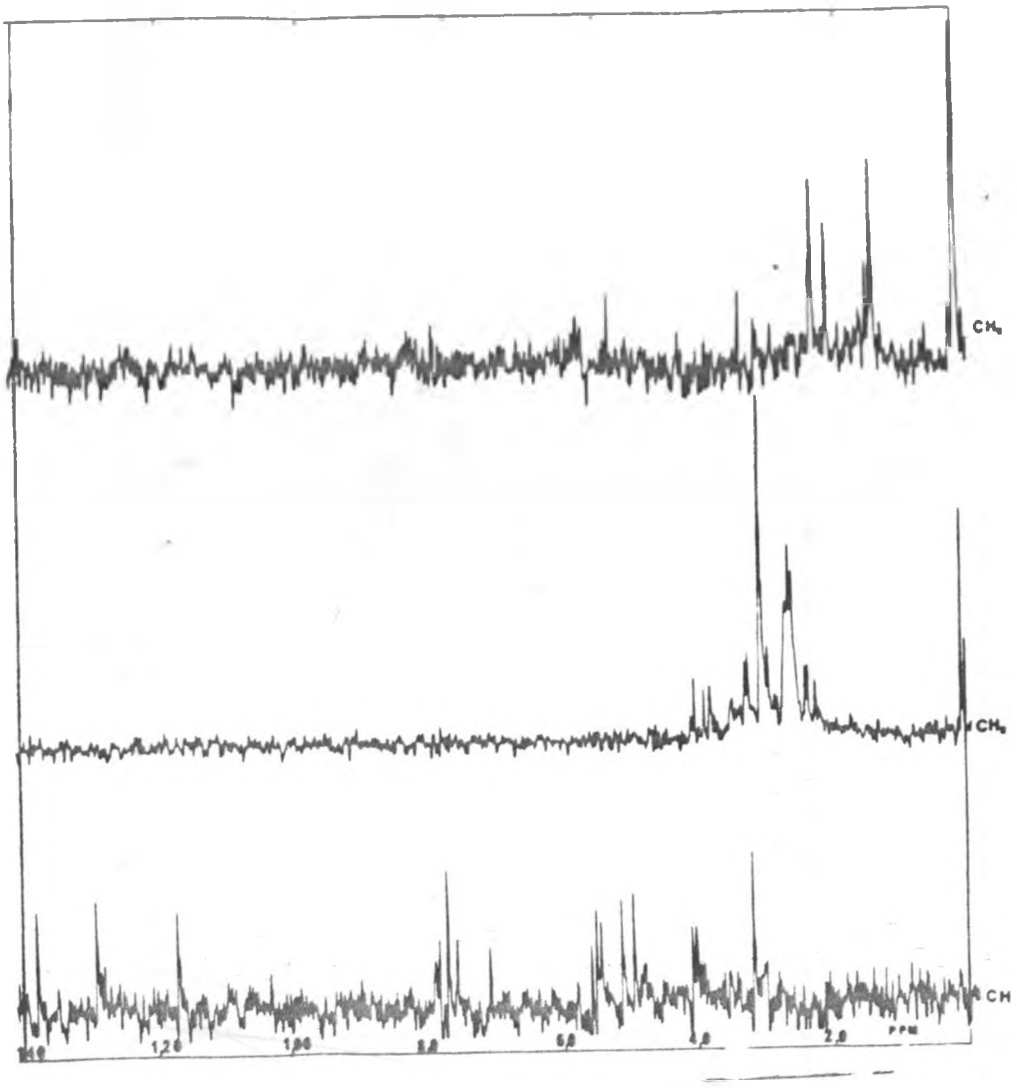
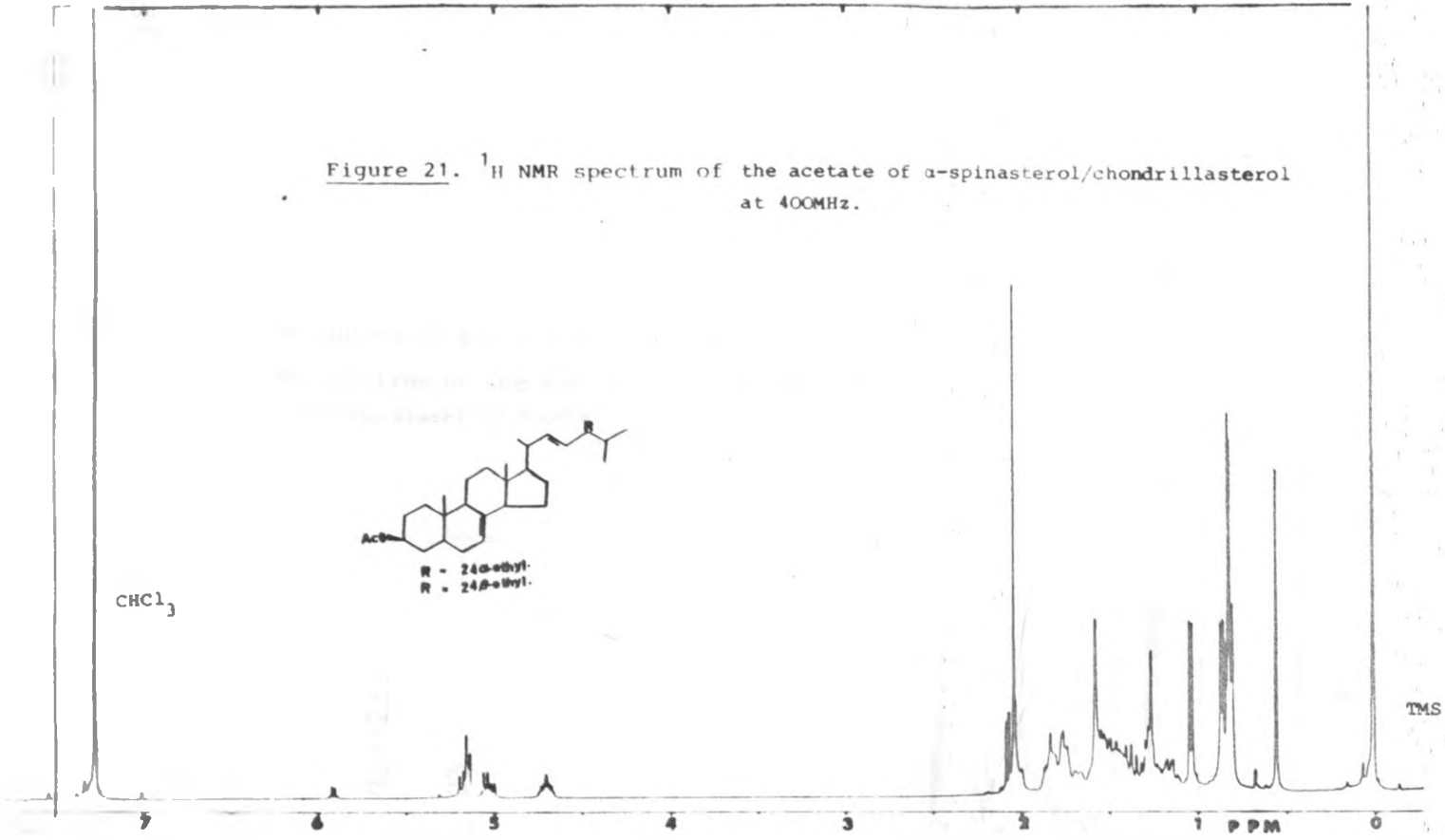


Figure 20. DEPT spectrum of the α-spinasterol/chondrillasterol

Figure 21. ^{1}H NMR spectrum of the acetate of α-spinasterol/chondrillasterol at 400MHz.



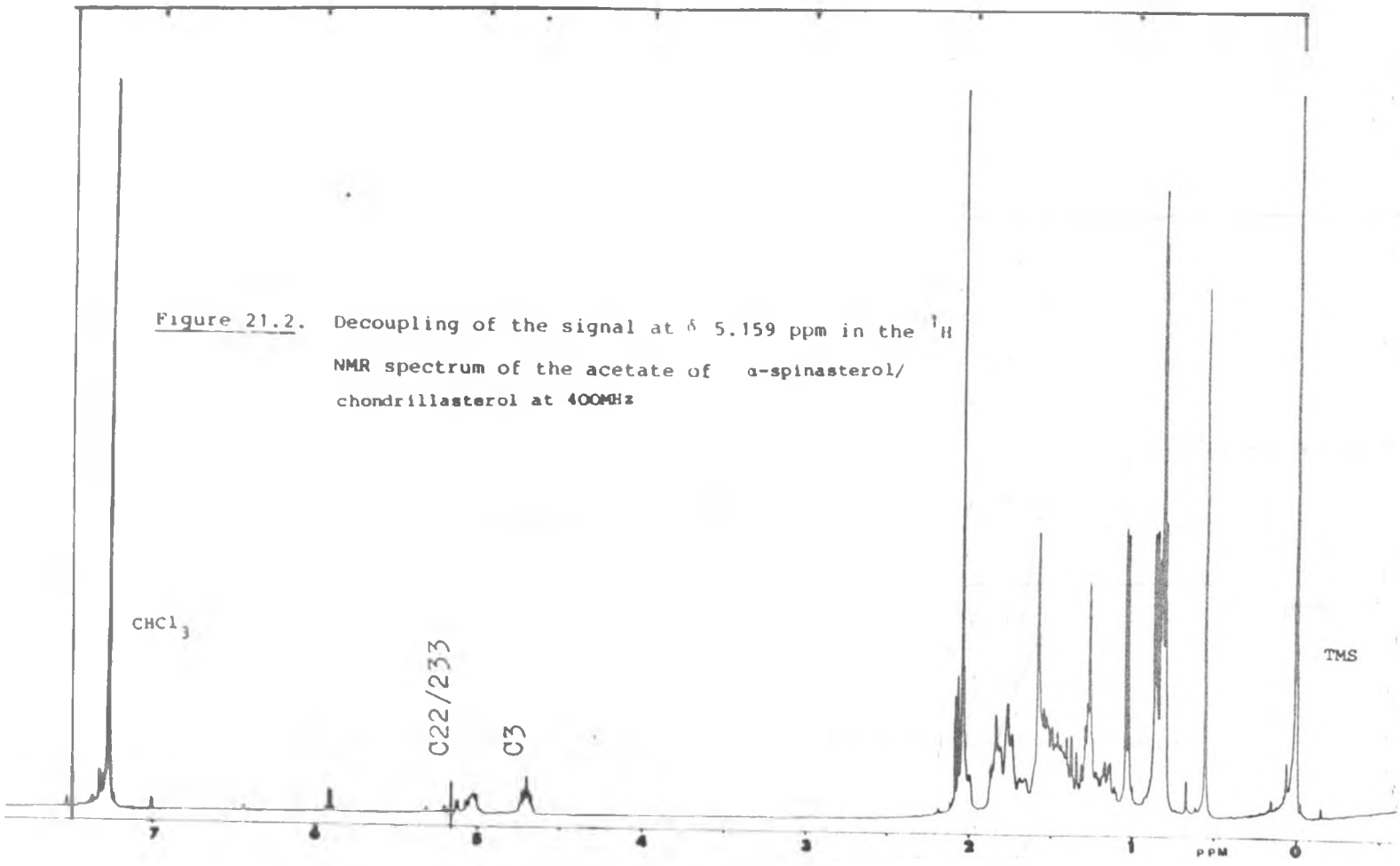


Figure 21.2. Decoupling of the signal at δ 5.159 ppm in the ^1H NMR spectrum of the acetate of α-spinasterol/ chondrillasterol at 400MHz

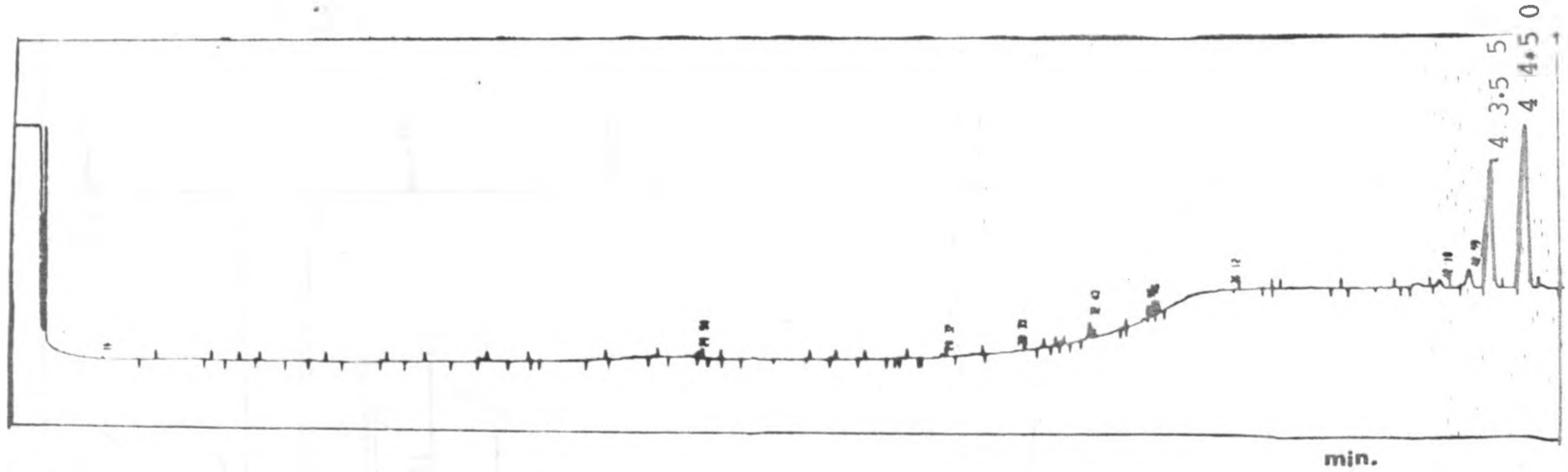


Figure 21.3. GC peaks of the acetate of α-spinasterol/chondrillasterol.

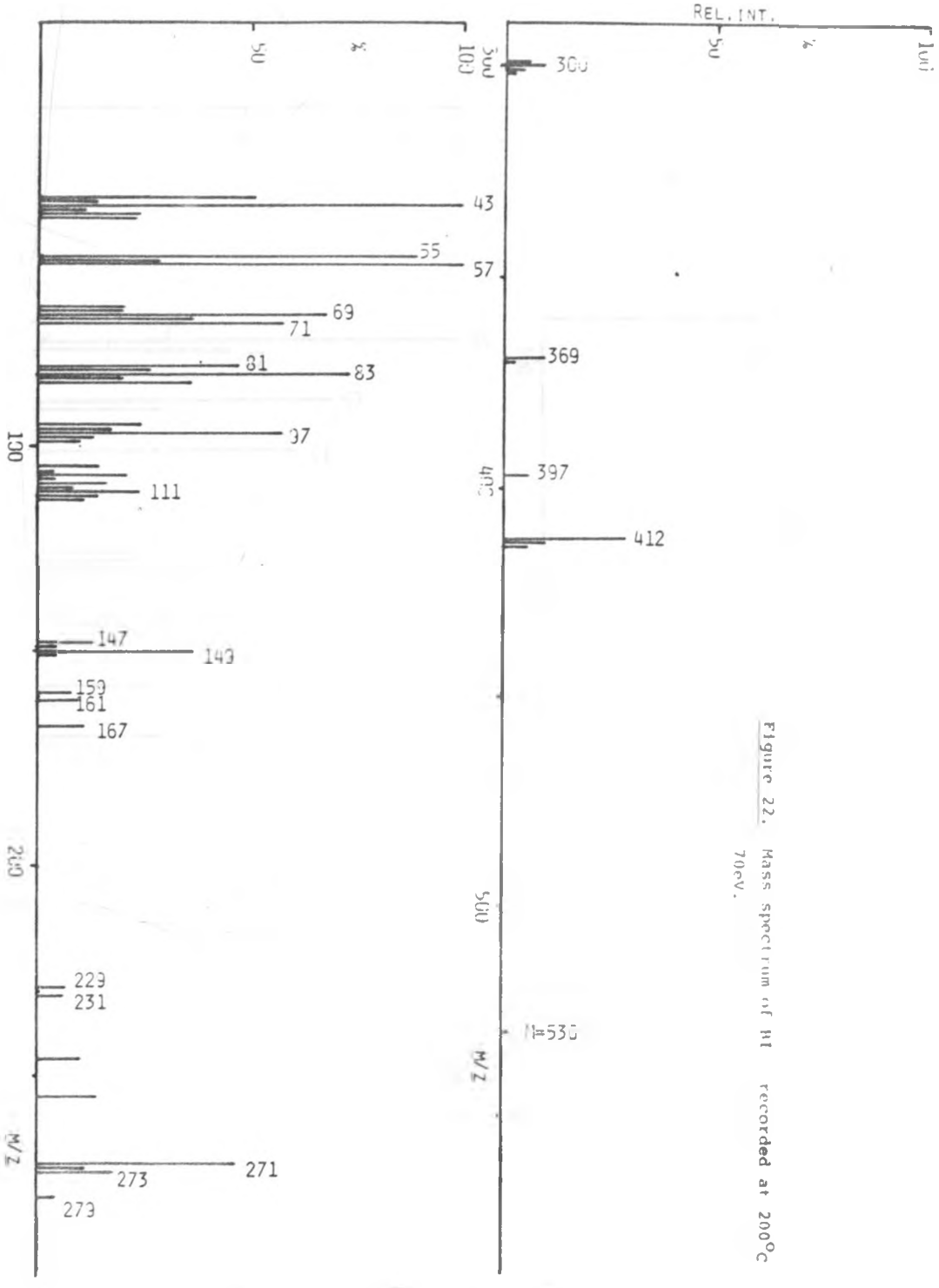


Figure 22. Mass spectrum of III recorded at 200°C 70ev.

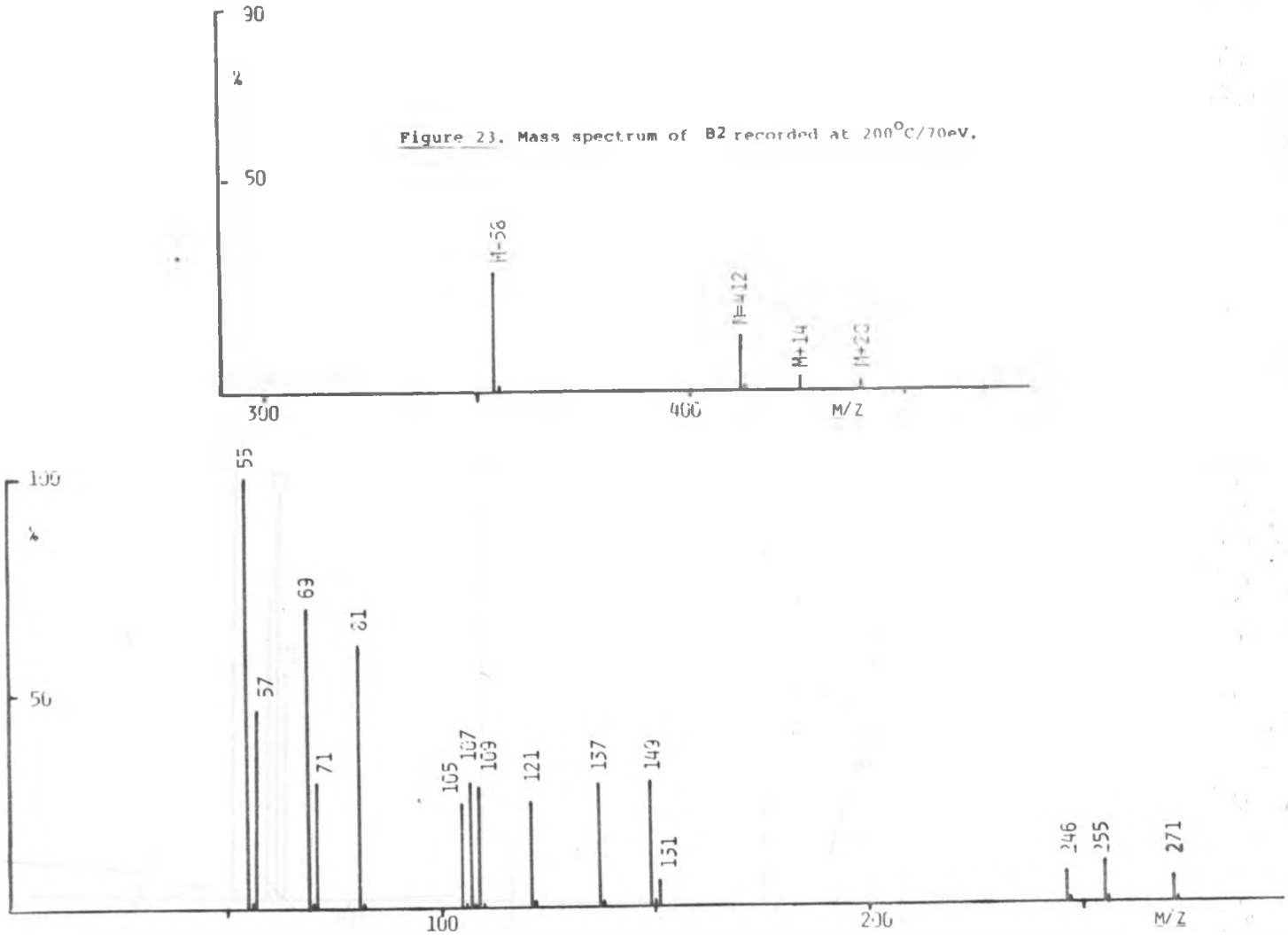


Figure 23. Mass spectrum of B2 recorded at 200°C/70eV.

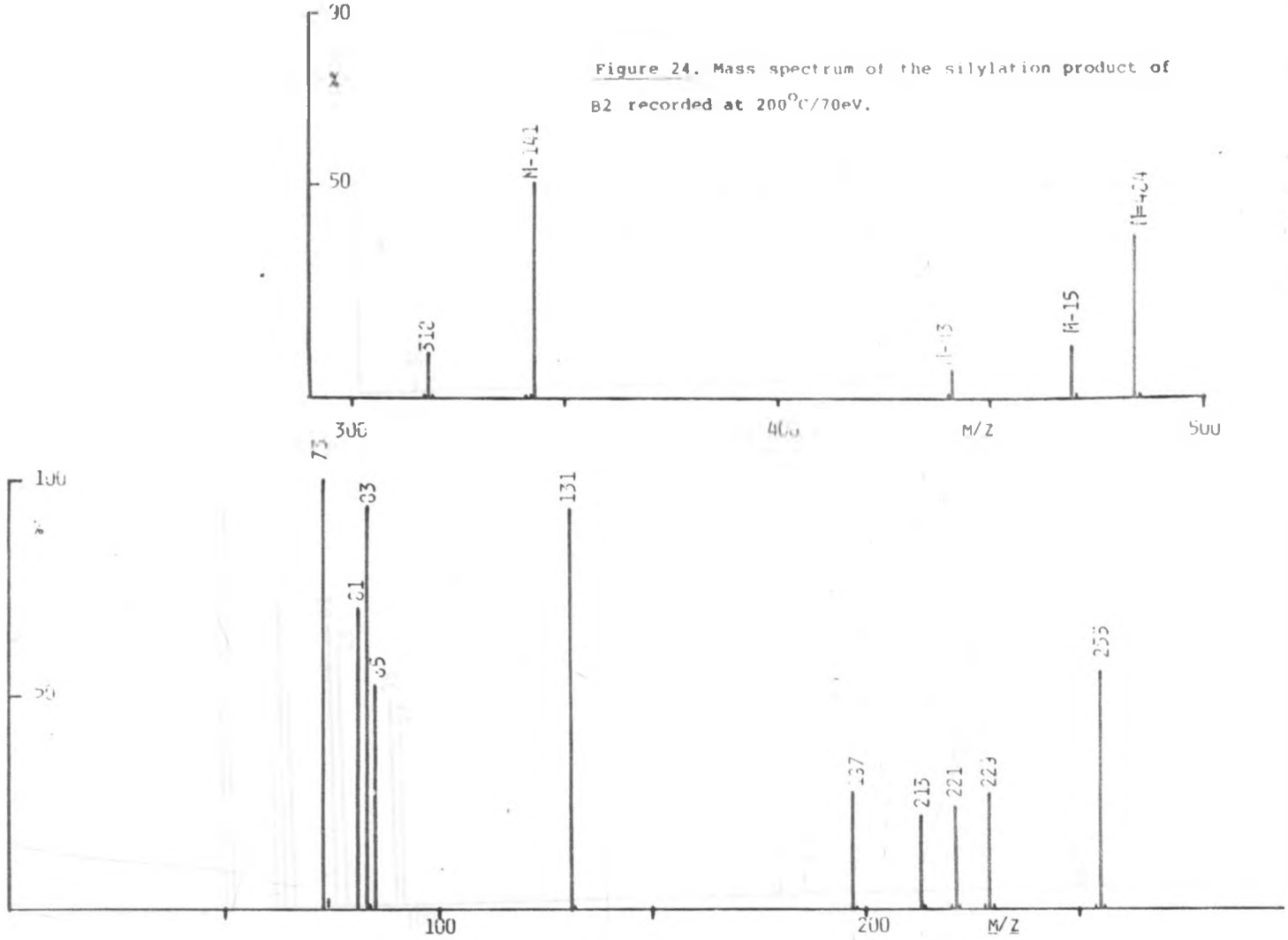
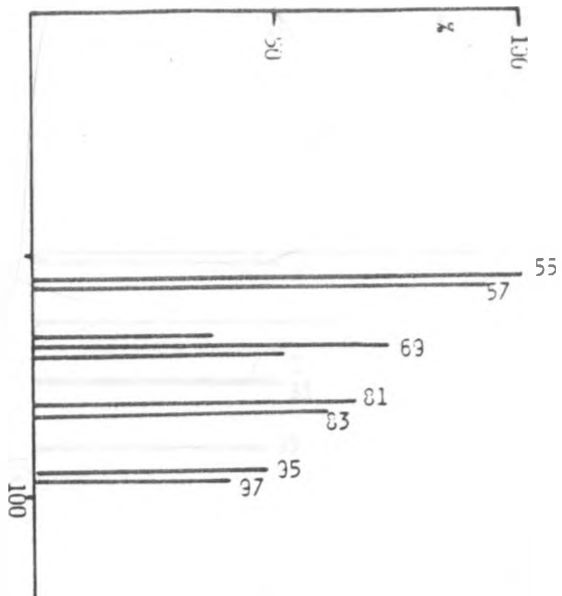


Figure 24. Mass spectrum of the silylation product of B2 recorded at 200°C/70eV.


50
%
100
55
57
63
81
83
35
97

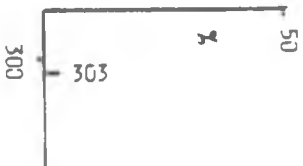
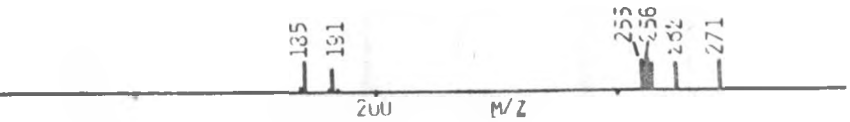

50
%
300
303

Figure 25. Mass spectrum of B3 recorded at 200°C/70eV.



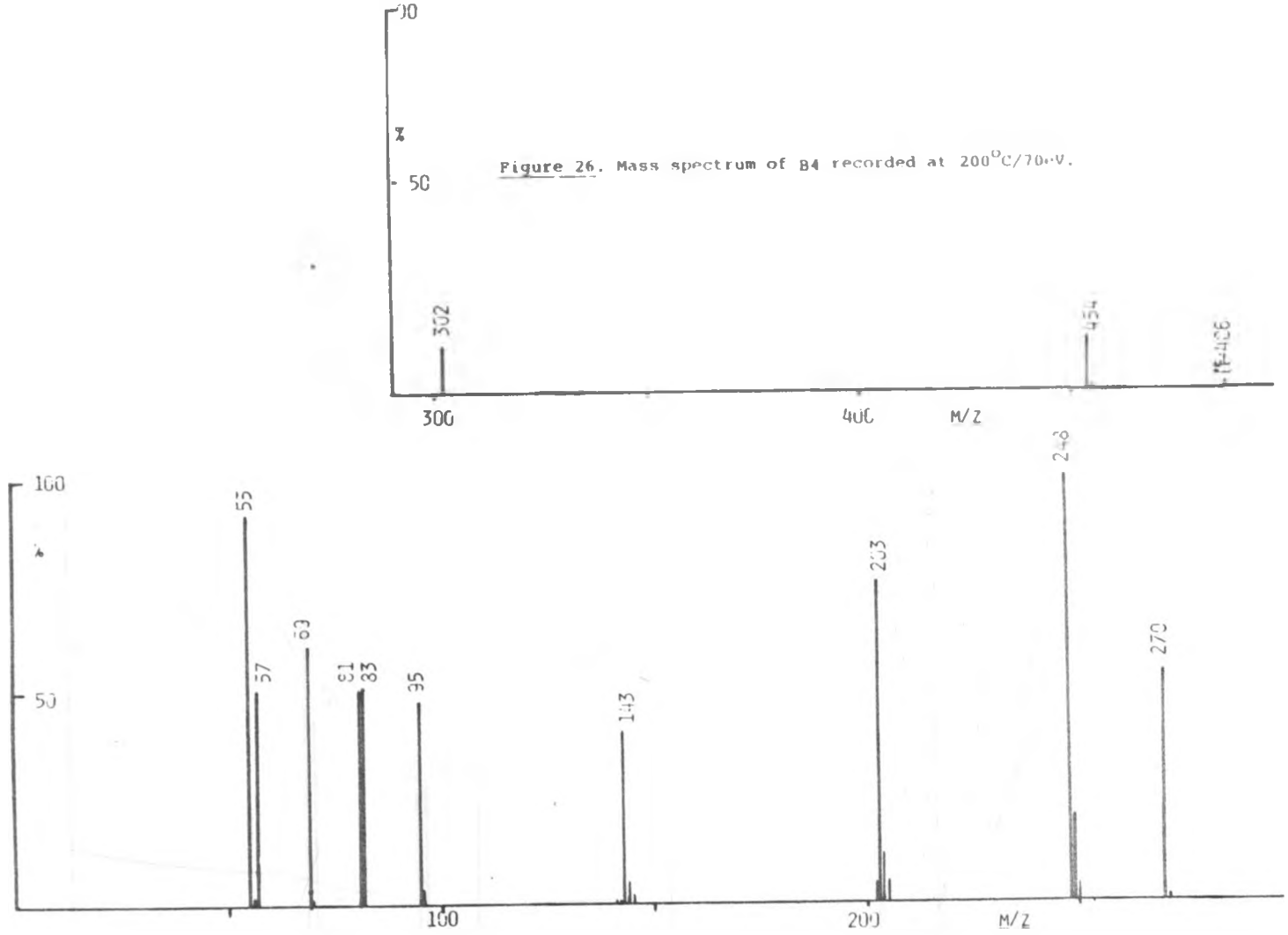


Figure 26. Mass spectrum of B4 recorded at 200°C/70eV.

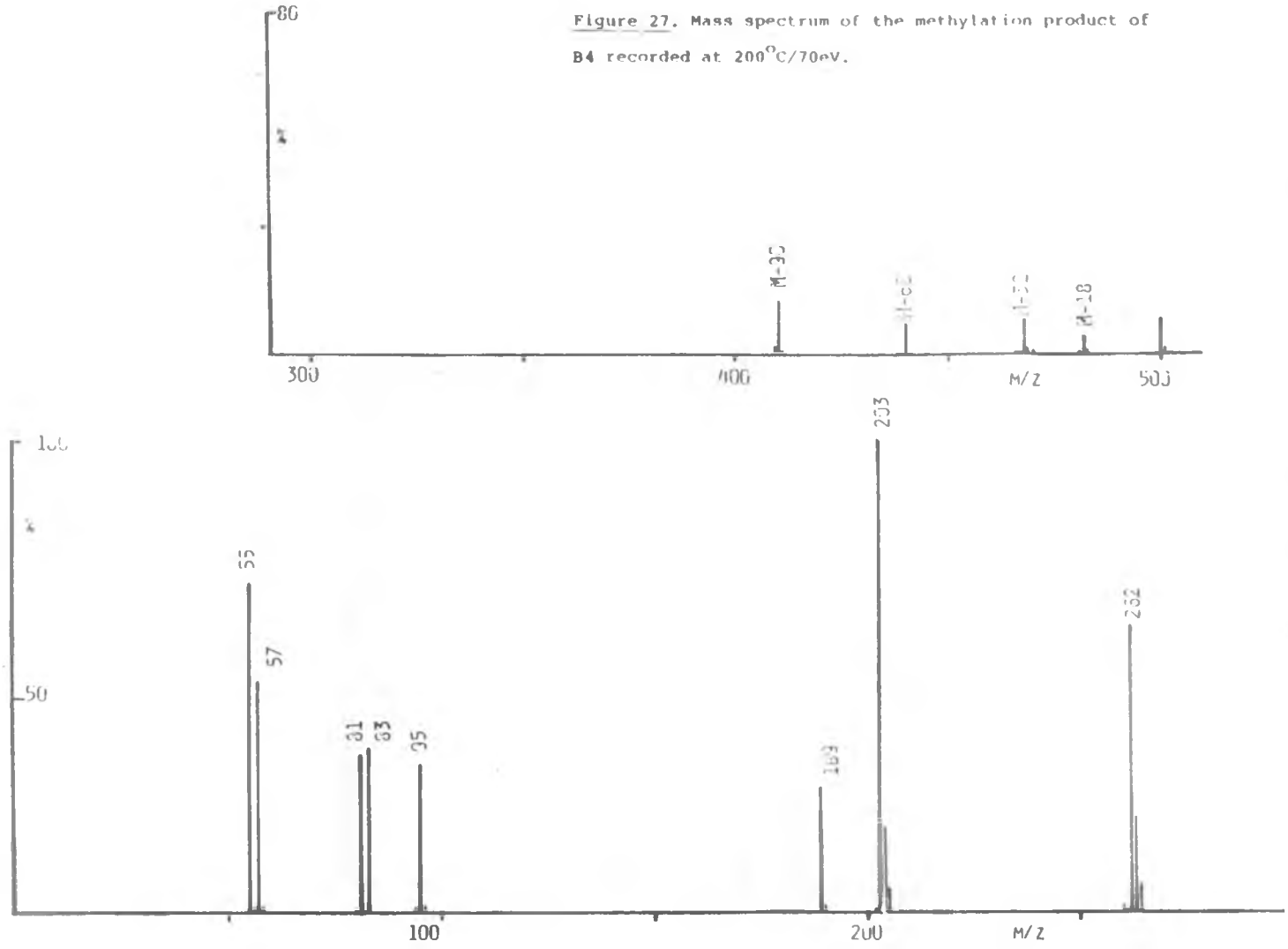


Figure 27. Mass spectrum of the methylation product of B4 recorded at 200°C/70eV.

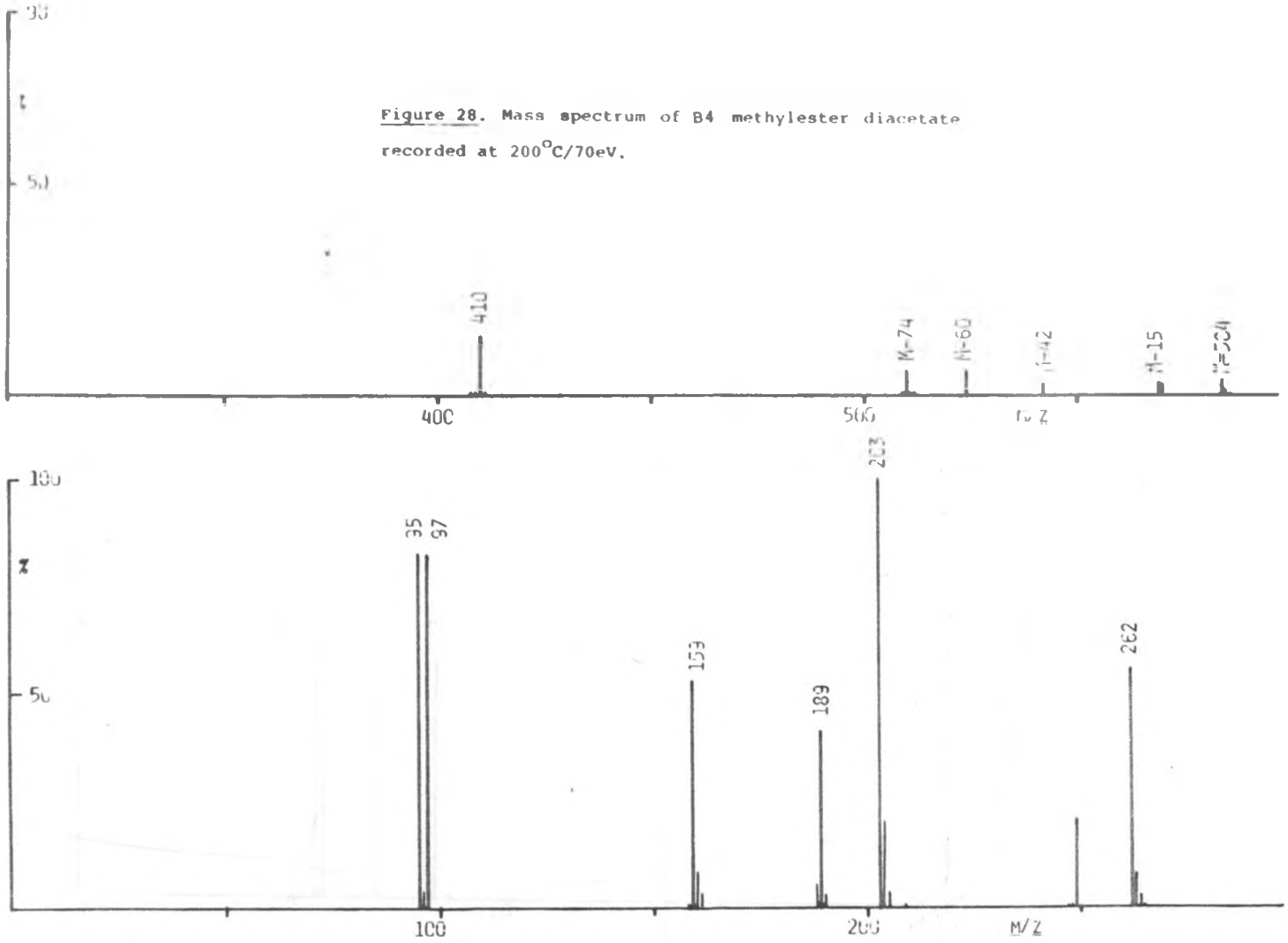


Figure 28. Mass spectrum of B4 methylester diacetate recorded at 200°C/70eV.

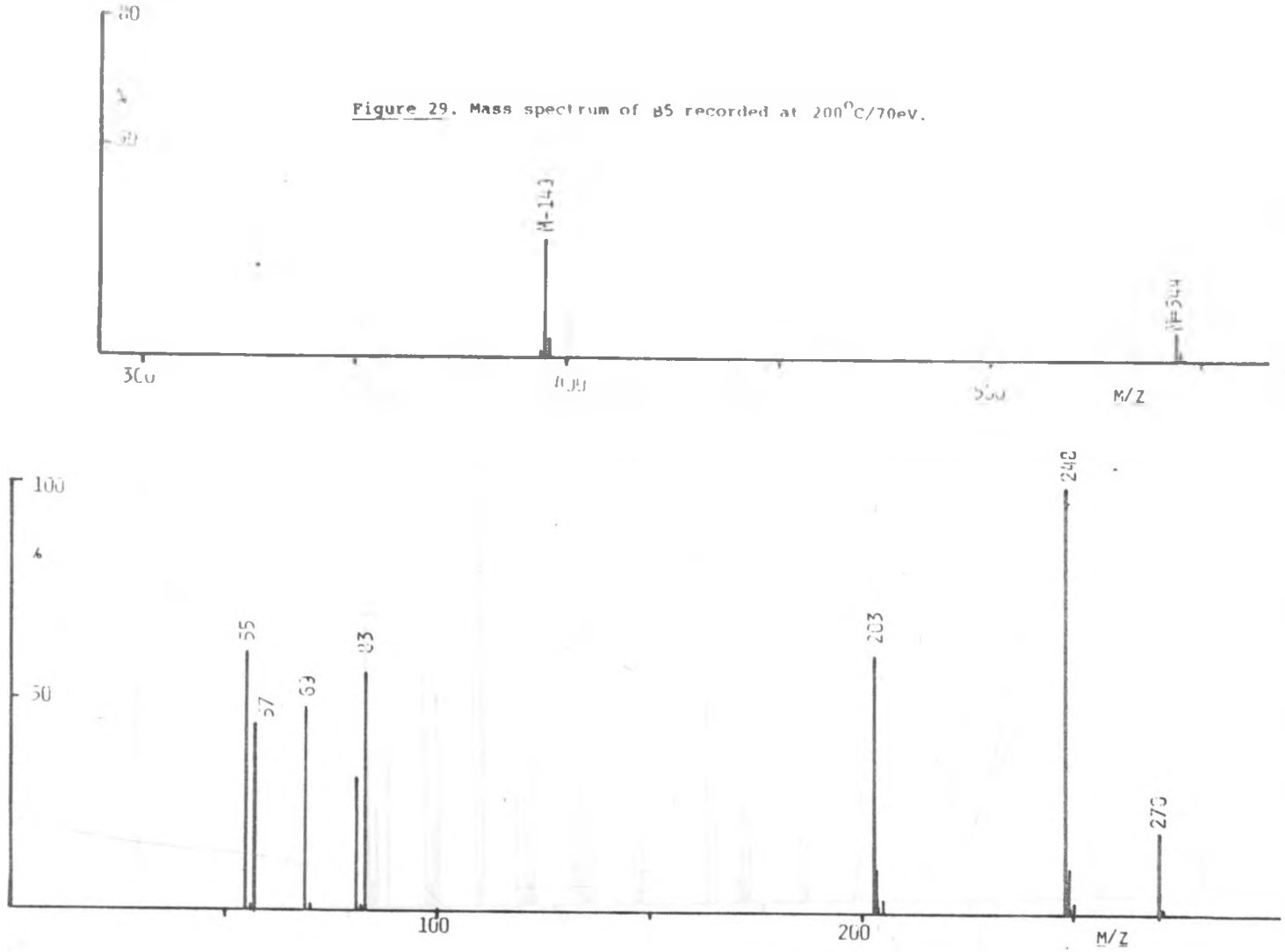


Figure 29. Mass spectrum of B5 recorded at 200°C/70eV.

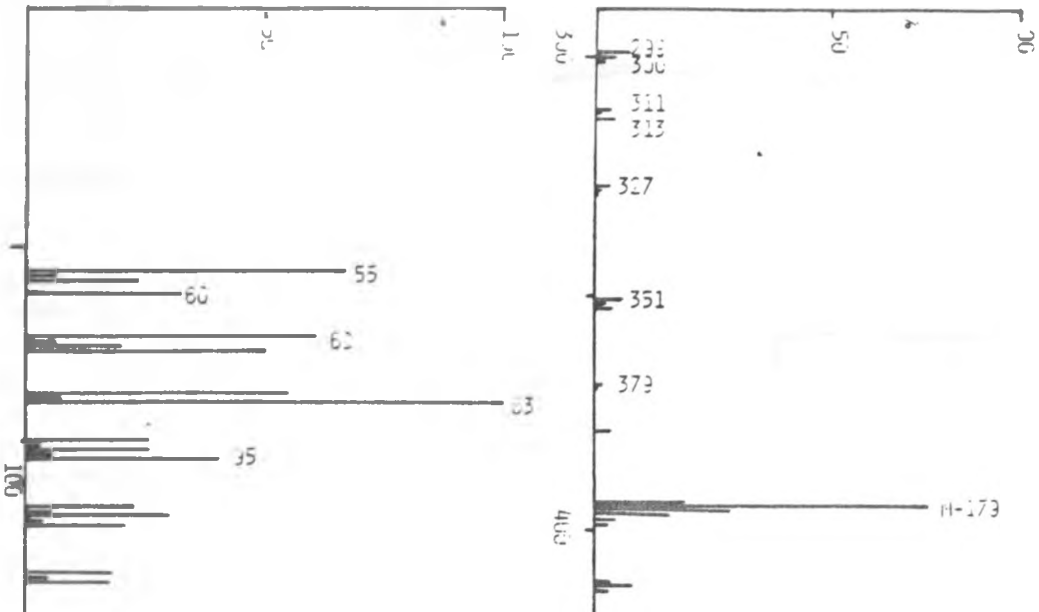
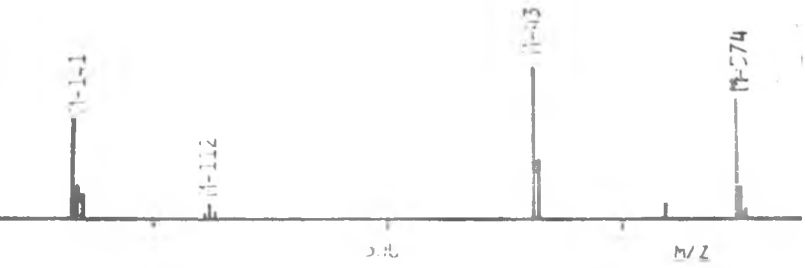
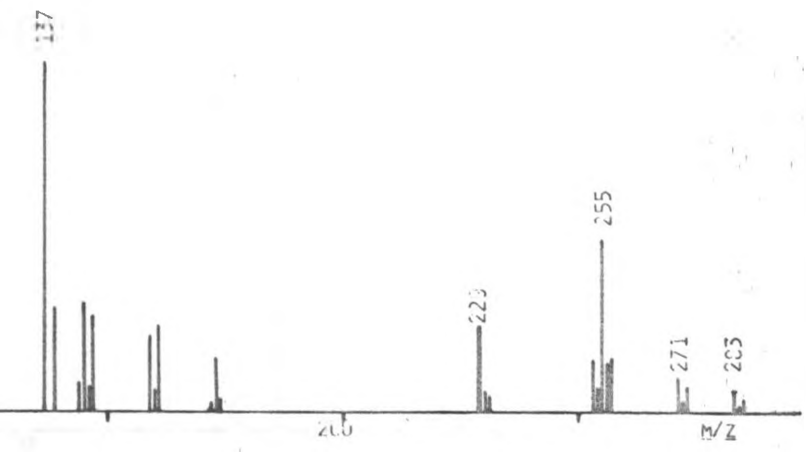

55
60
63
100
M-179

Figure 30. Mass spectrum of B6 recorded at 240°C/70eV.





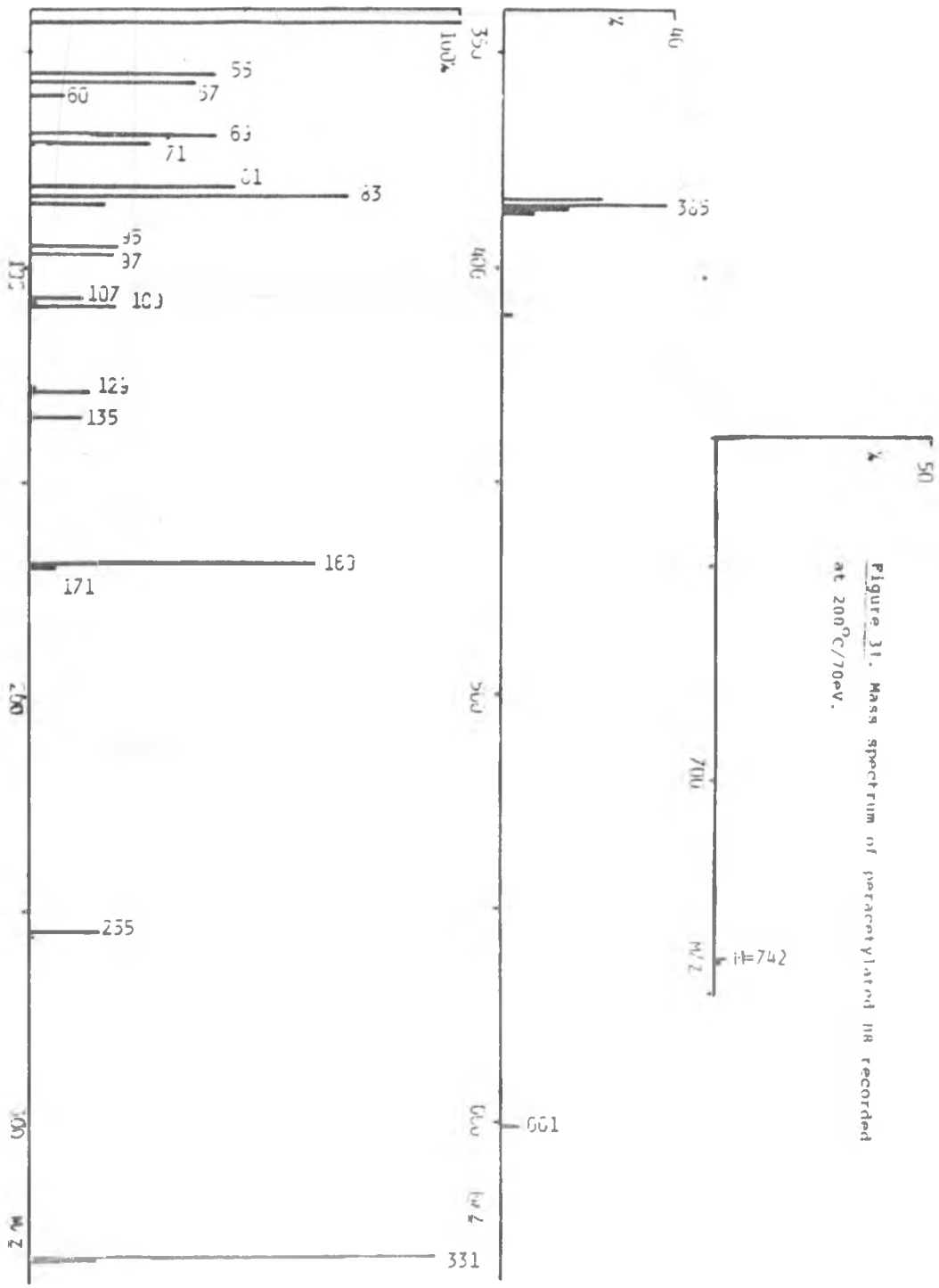
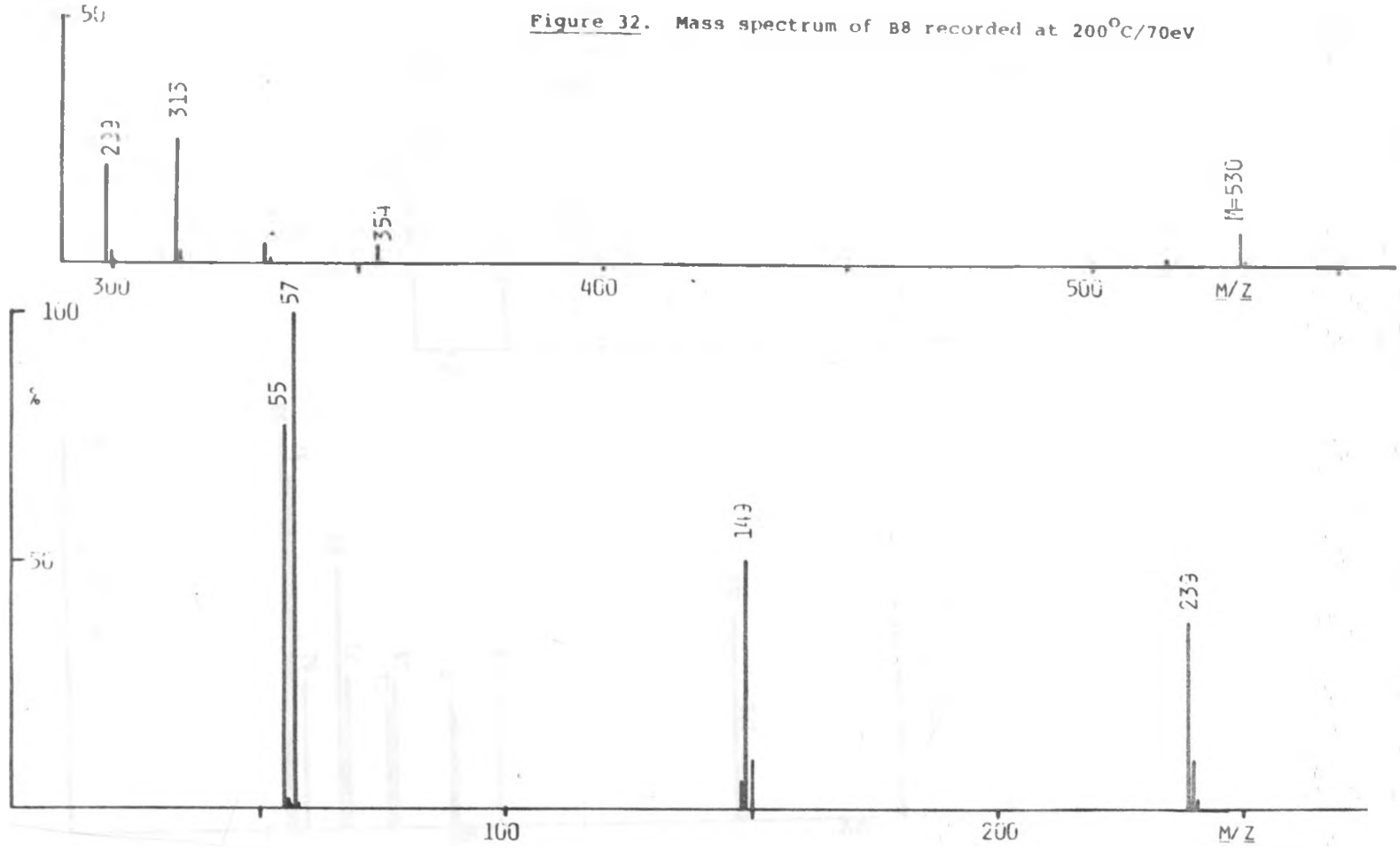
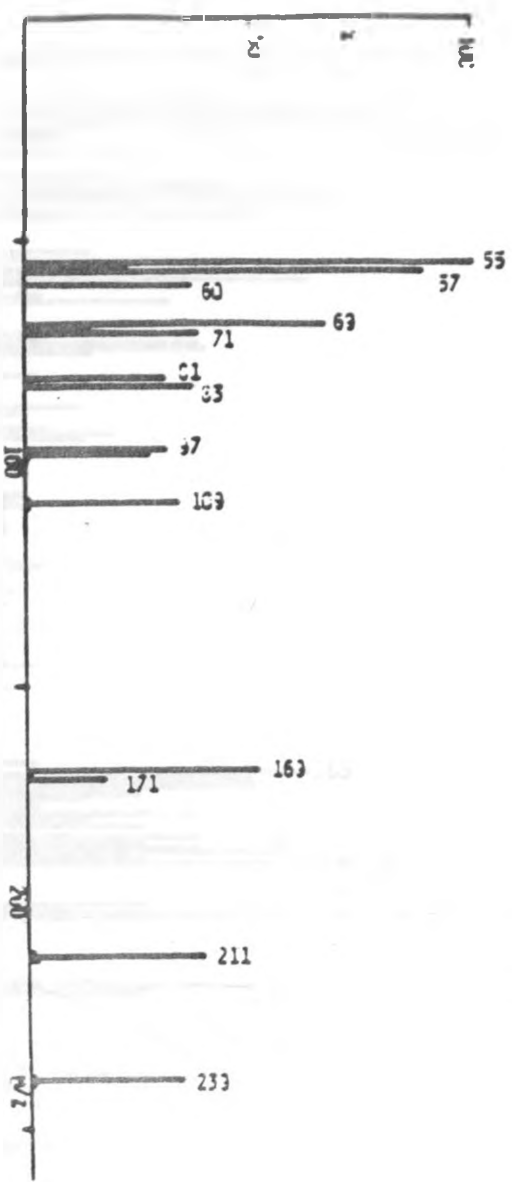


Figure 31. Mass spectrum of peracetylated IHR recorded at 200°C/70eV.

Figure 32. Mass spectrum of B8 recorded at 200°C/70eV





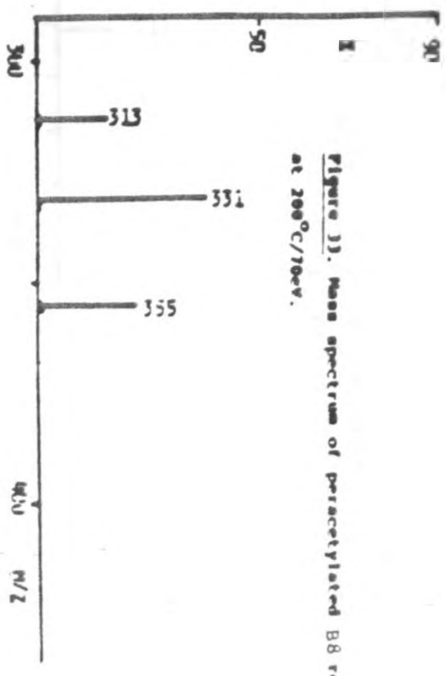


Figure 33. Mass spectrum of peracetylated B8 recorded at 200°C/70eV.

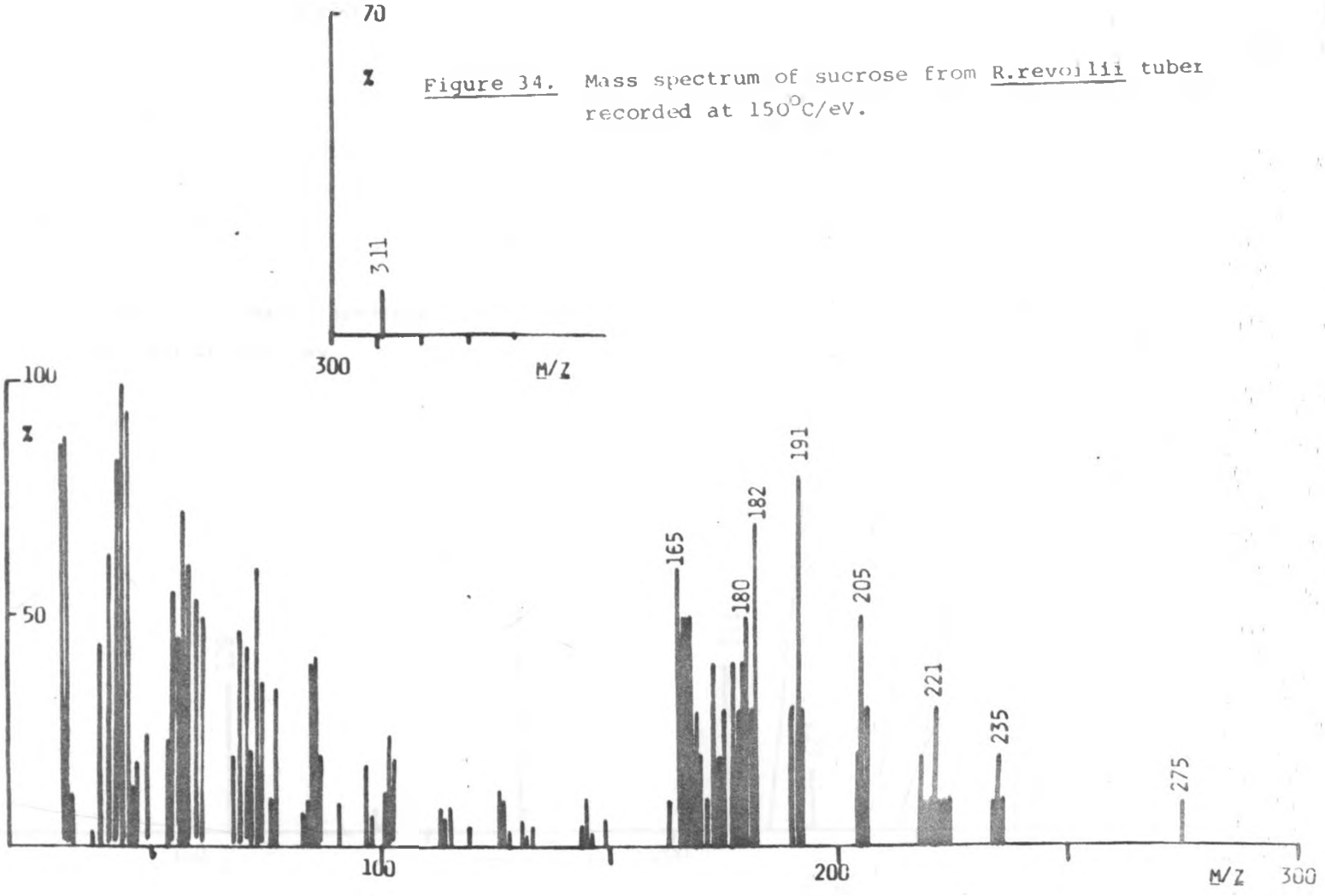
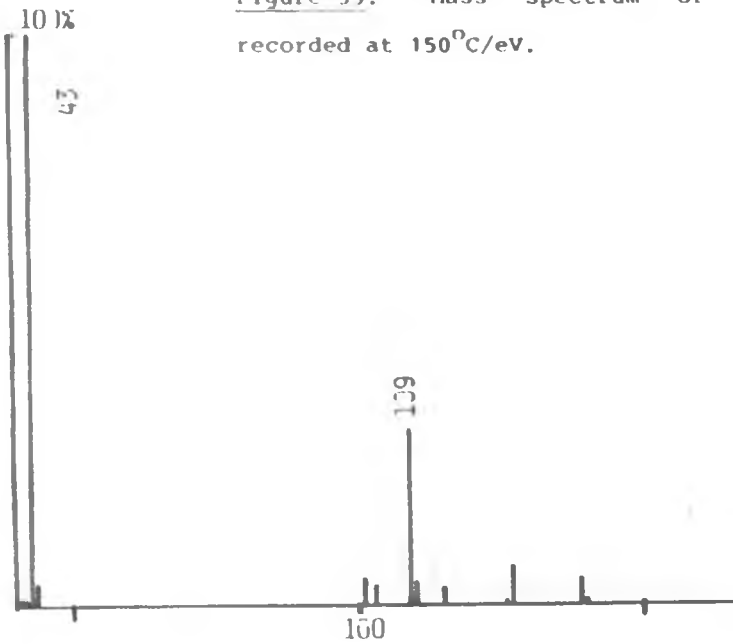


Figure 34. Mass spectrum of sucrose from R.revolii tuber recorded at 150°C/eV.

Figure 35. Mass spectrum of recorded at 150°C/eV.



M=678

peracetylated sucrose from R.revoilii tuber

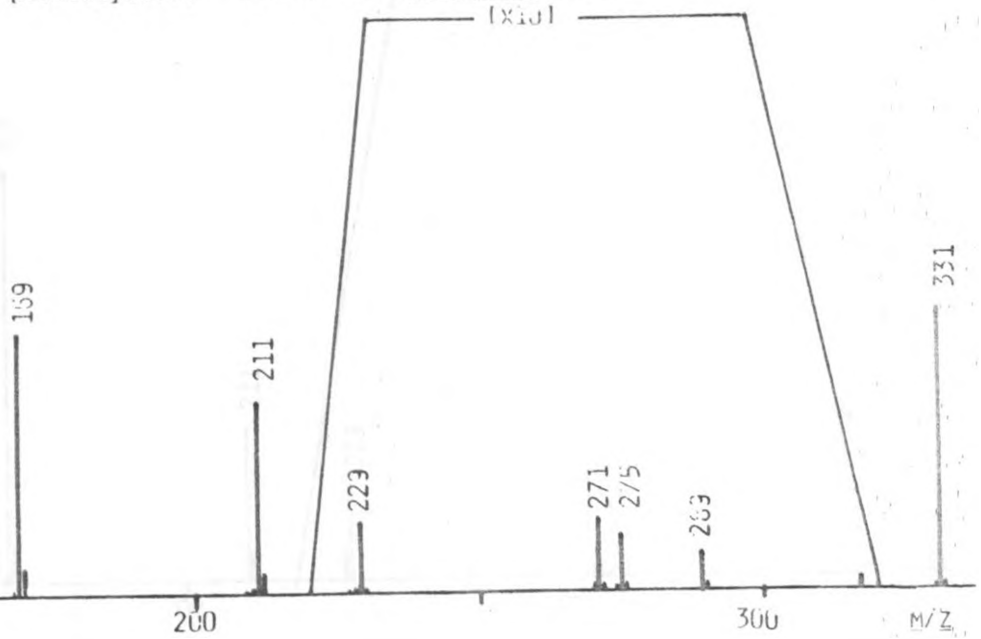
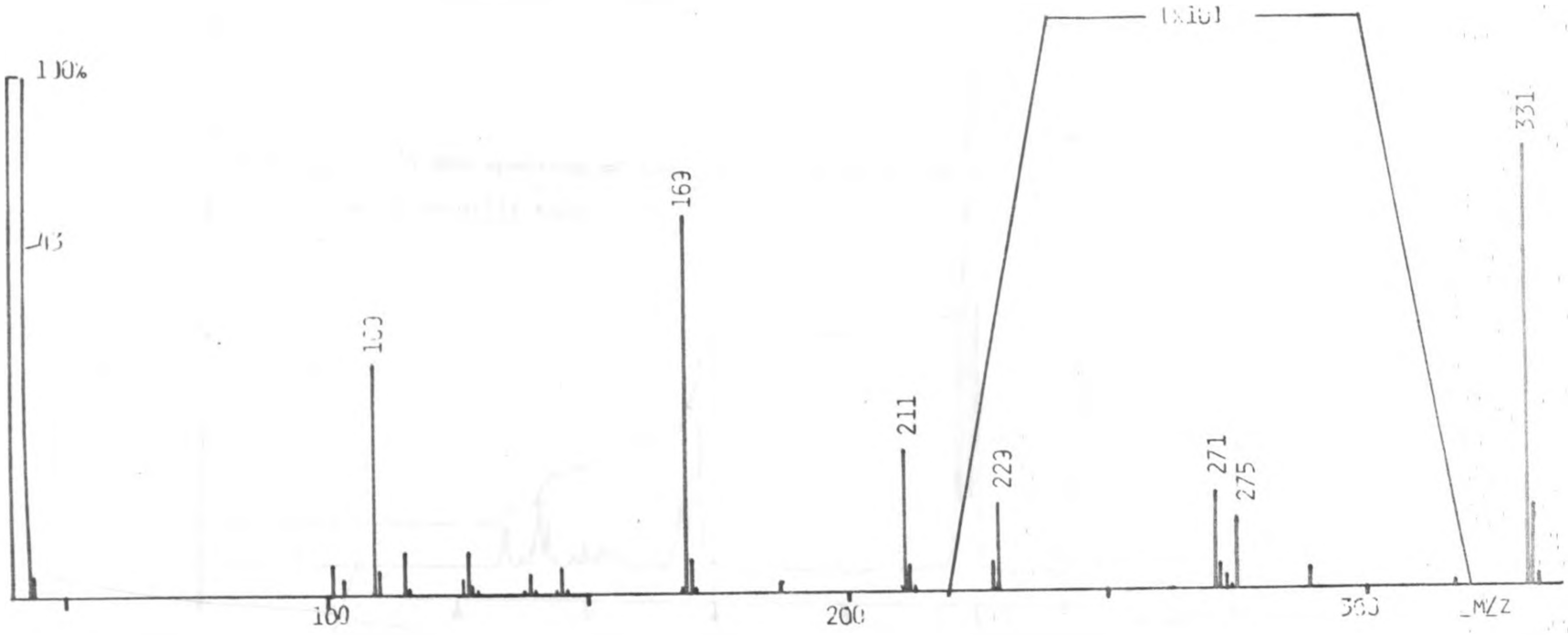


Figure 36. Mass spectrum of authentic sucrose octaacetate recorded at 150°C/70eV.



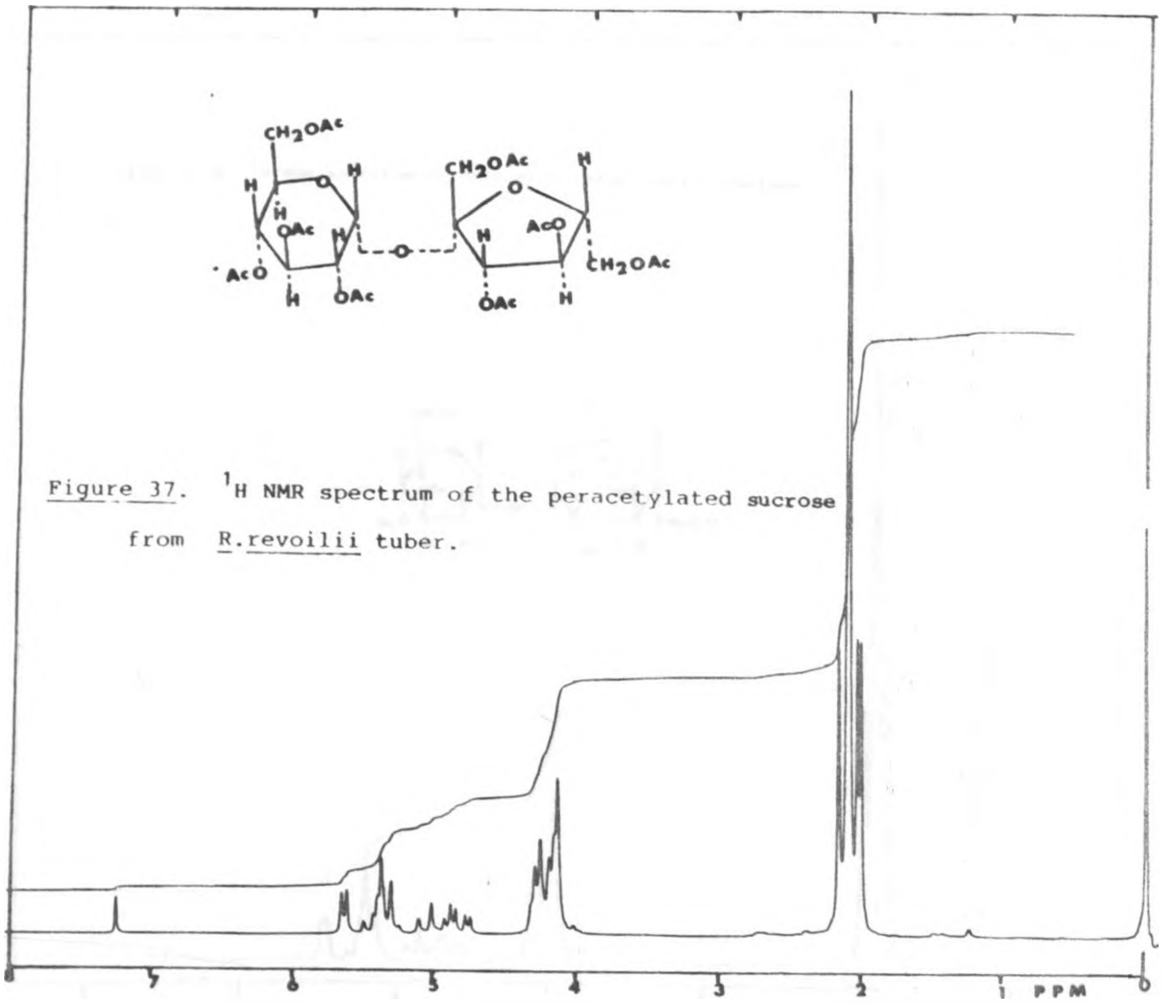


Figure 37. ^{1}H NMR spectrum of the peracetylated sucrose from R.revoilii tuber.

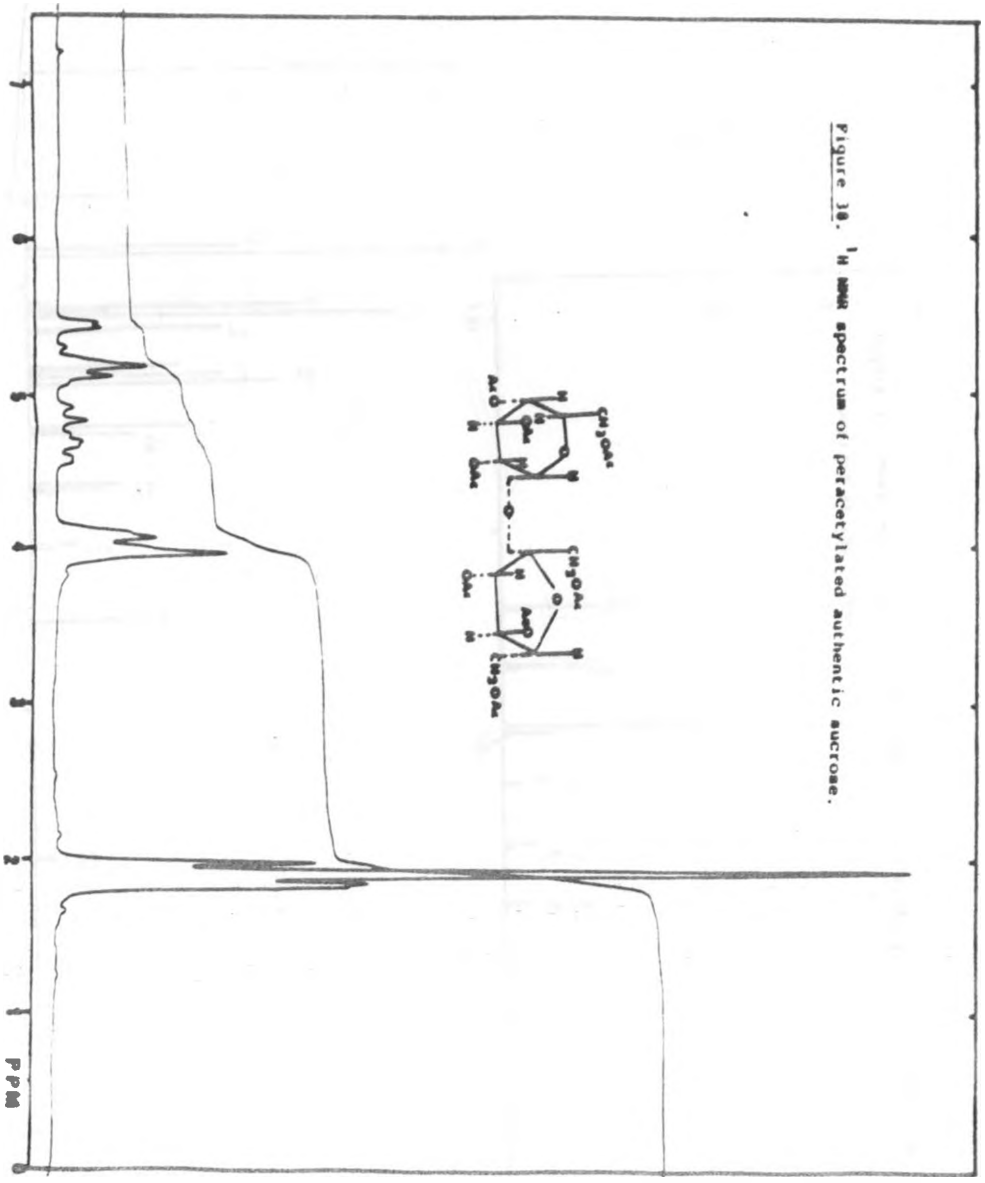


Figure 18. ^1H NMR spectrum of peracetylated authentic sucrose.

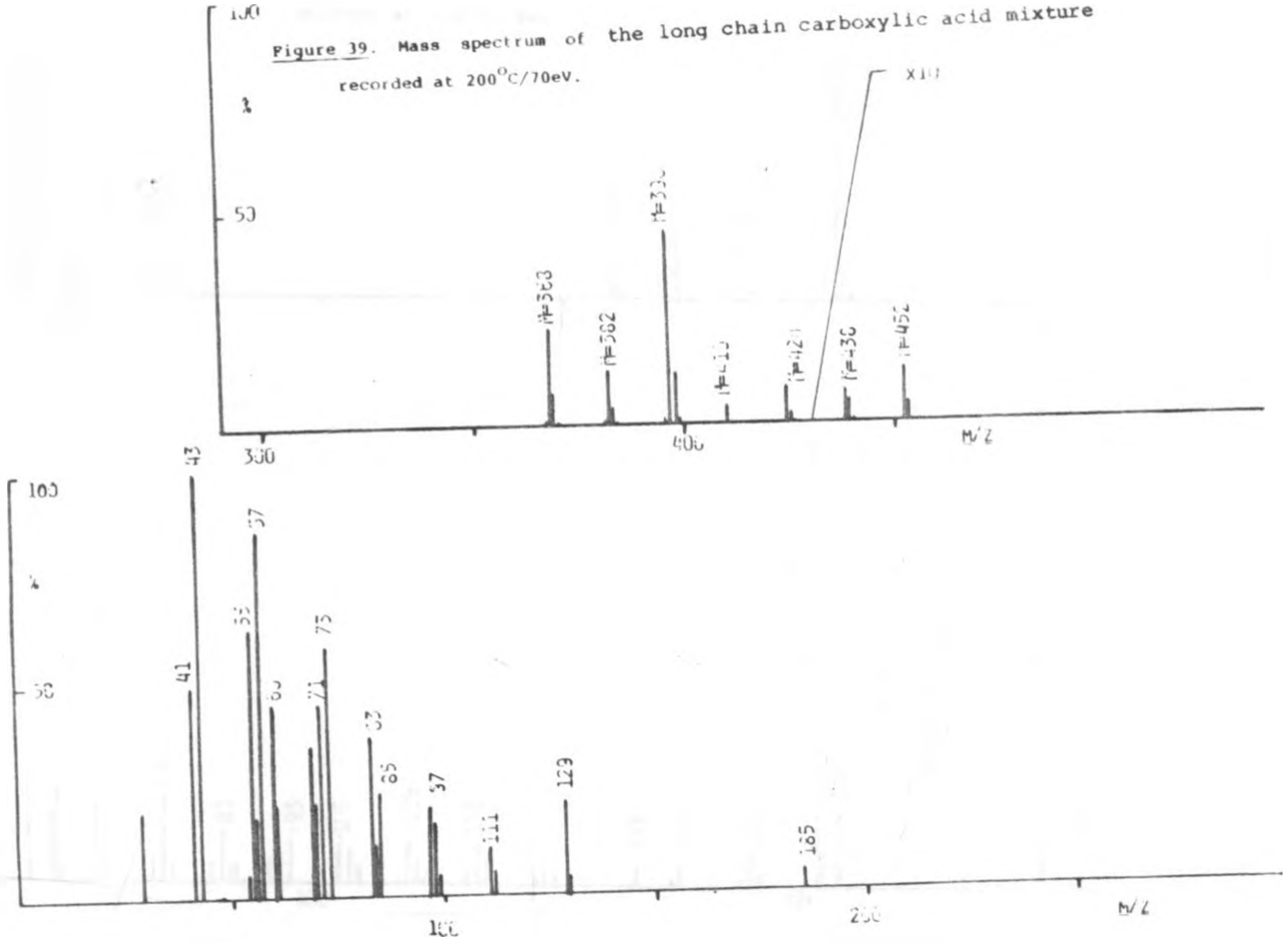
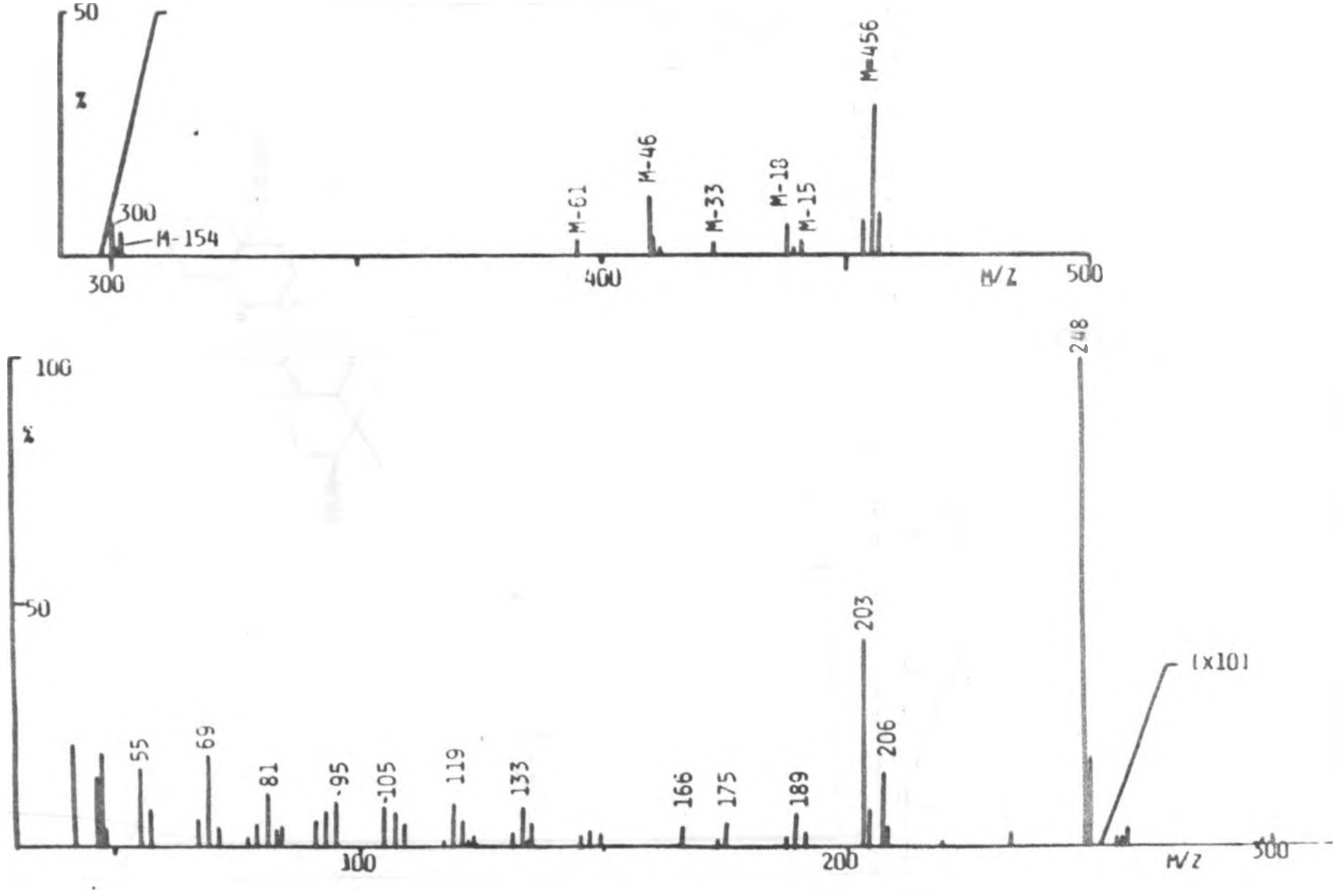


Figure 39. Mass spectrum of the long chain carboxylic acid mixture recorded at 200°C/70eV.

Figure 40. Mass spectrum of oleanolic acid from *S.keniensis* seeds recorded at 200°C/70eV.



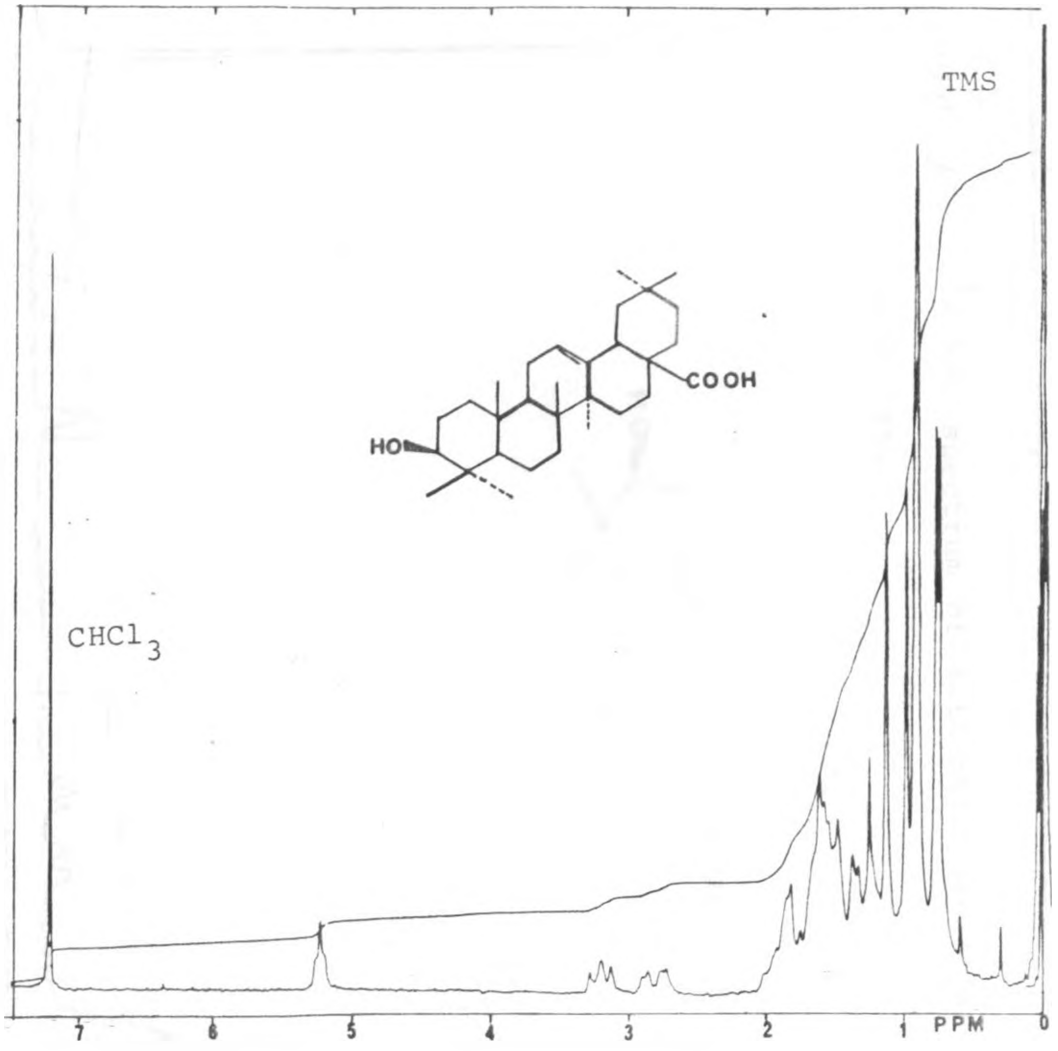
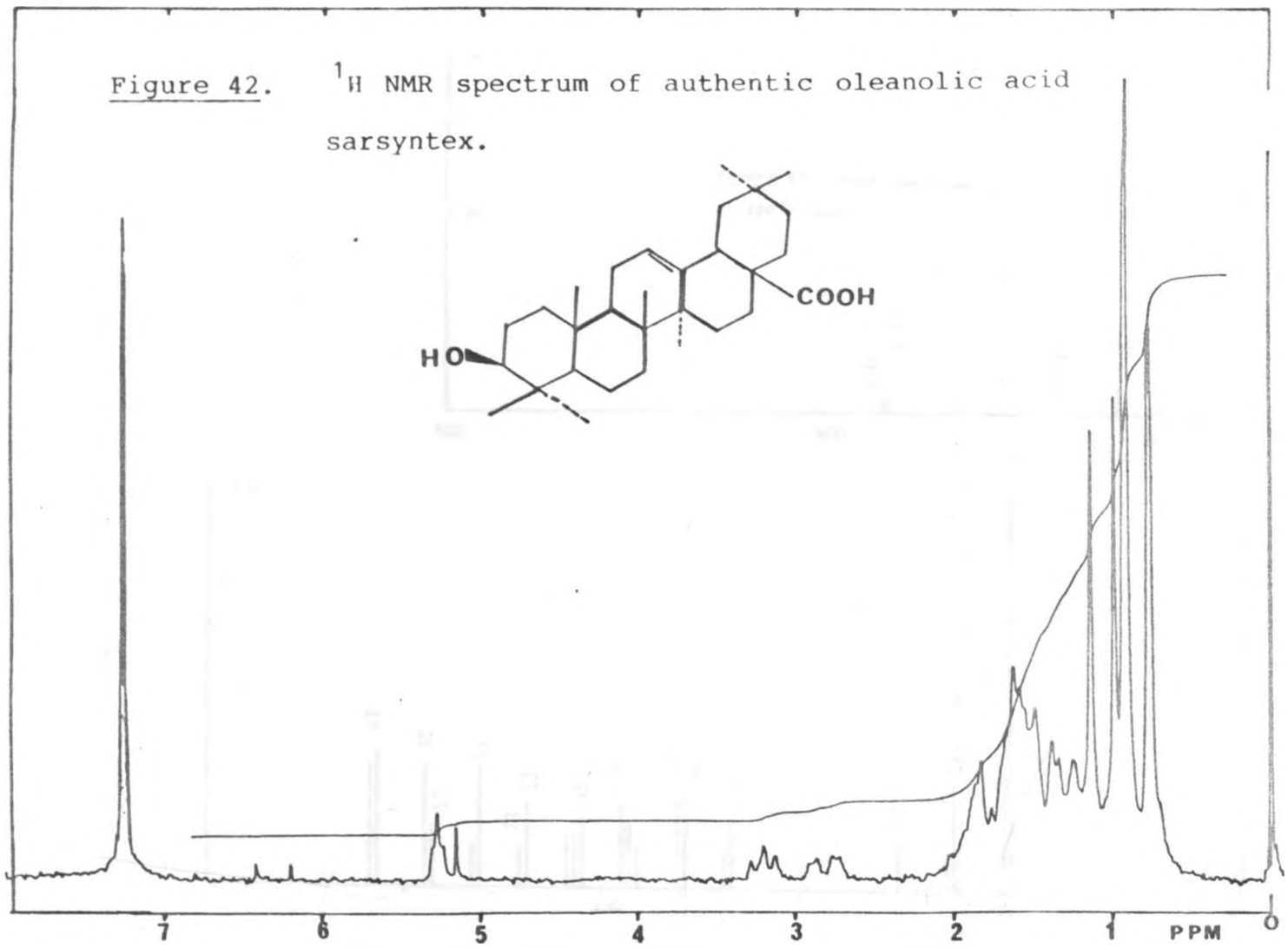


Figure 41. ^{1}H NMR spectrum of the triterpene oleanolic Sesbania keniensis seeds.

Figure 42. ^{1}H NMR spectrum of authentic oleanolic acid sarsyntex.



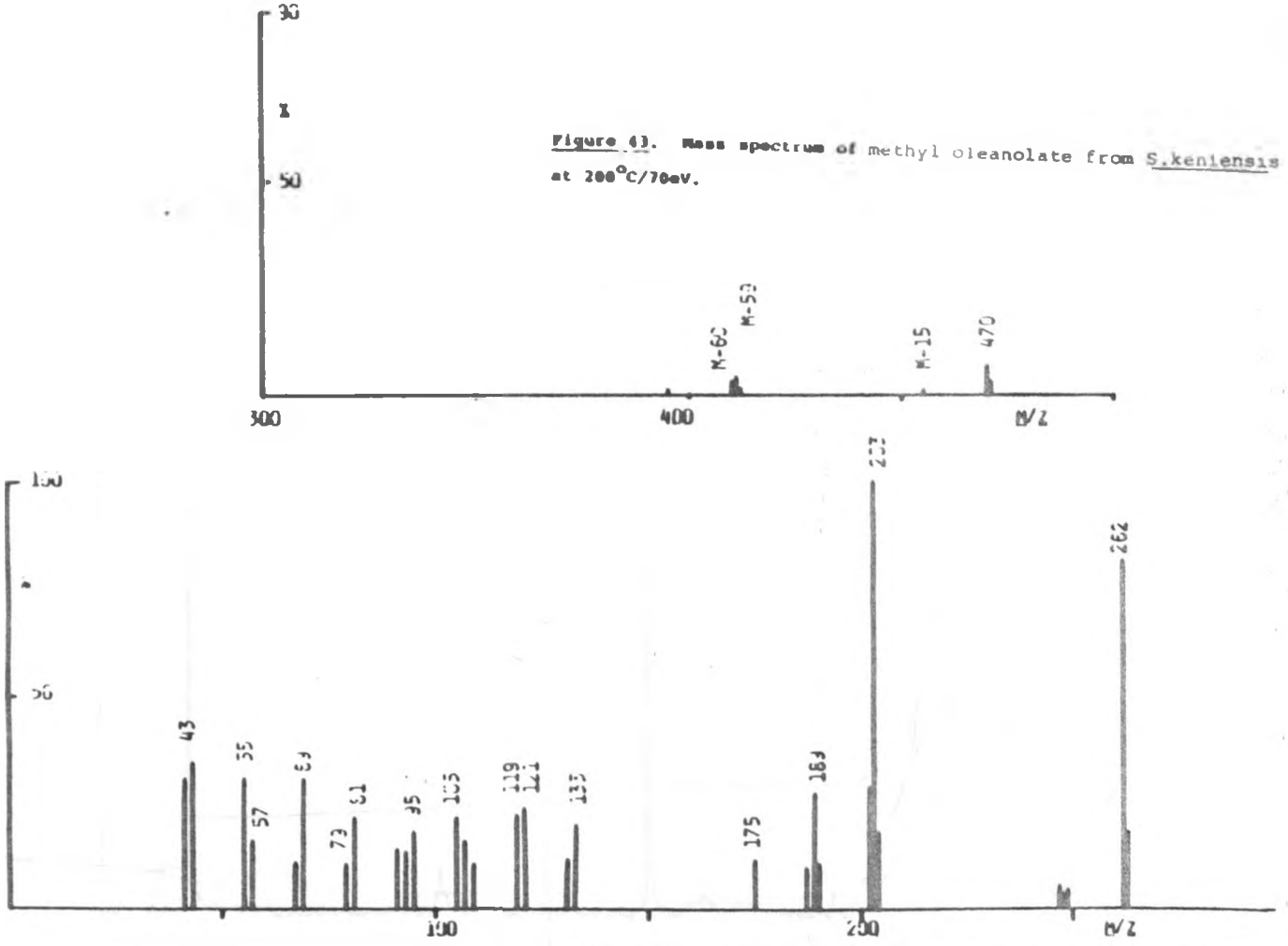
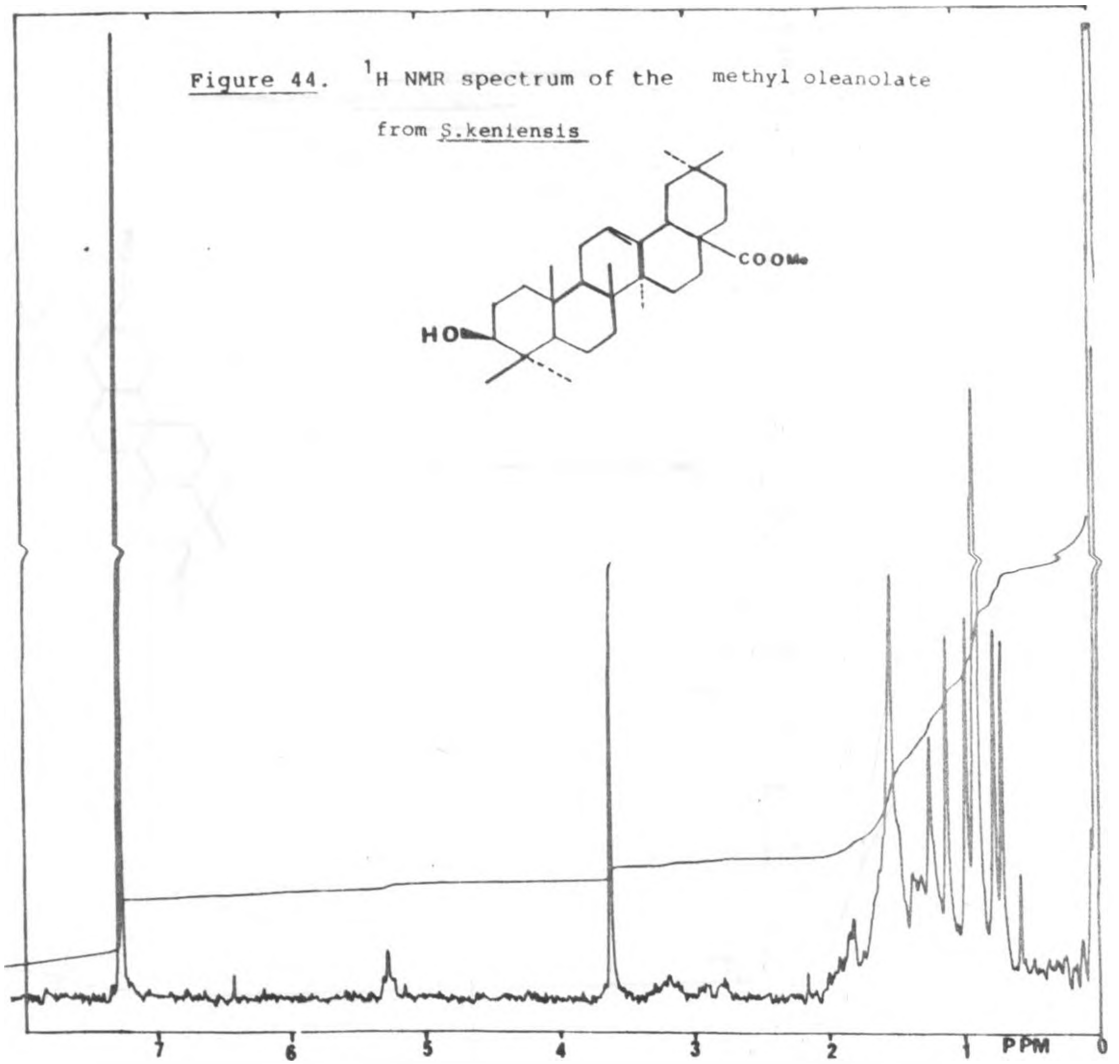


Figure 61. Mass spectrum of methyl oleanolate from S.keniensis recorded at 200°C/70eV.

Figure 44. ^{1}H NMR spectrum of the methyl oleanolate from S.keniensis



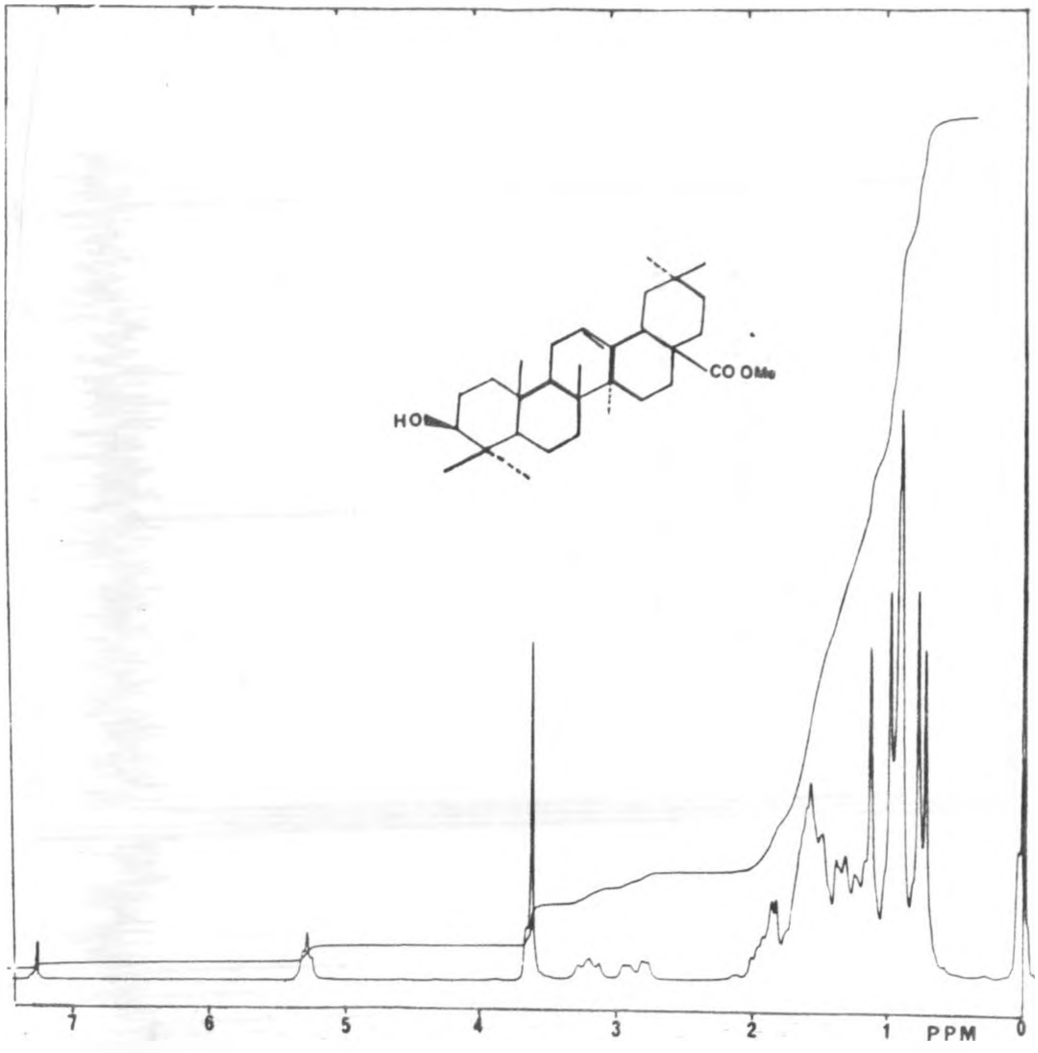


Figure 45. ^{1}H NMR spectrum of the methylester of authentic oleanolic acid sarsyntex.

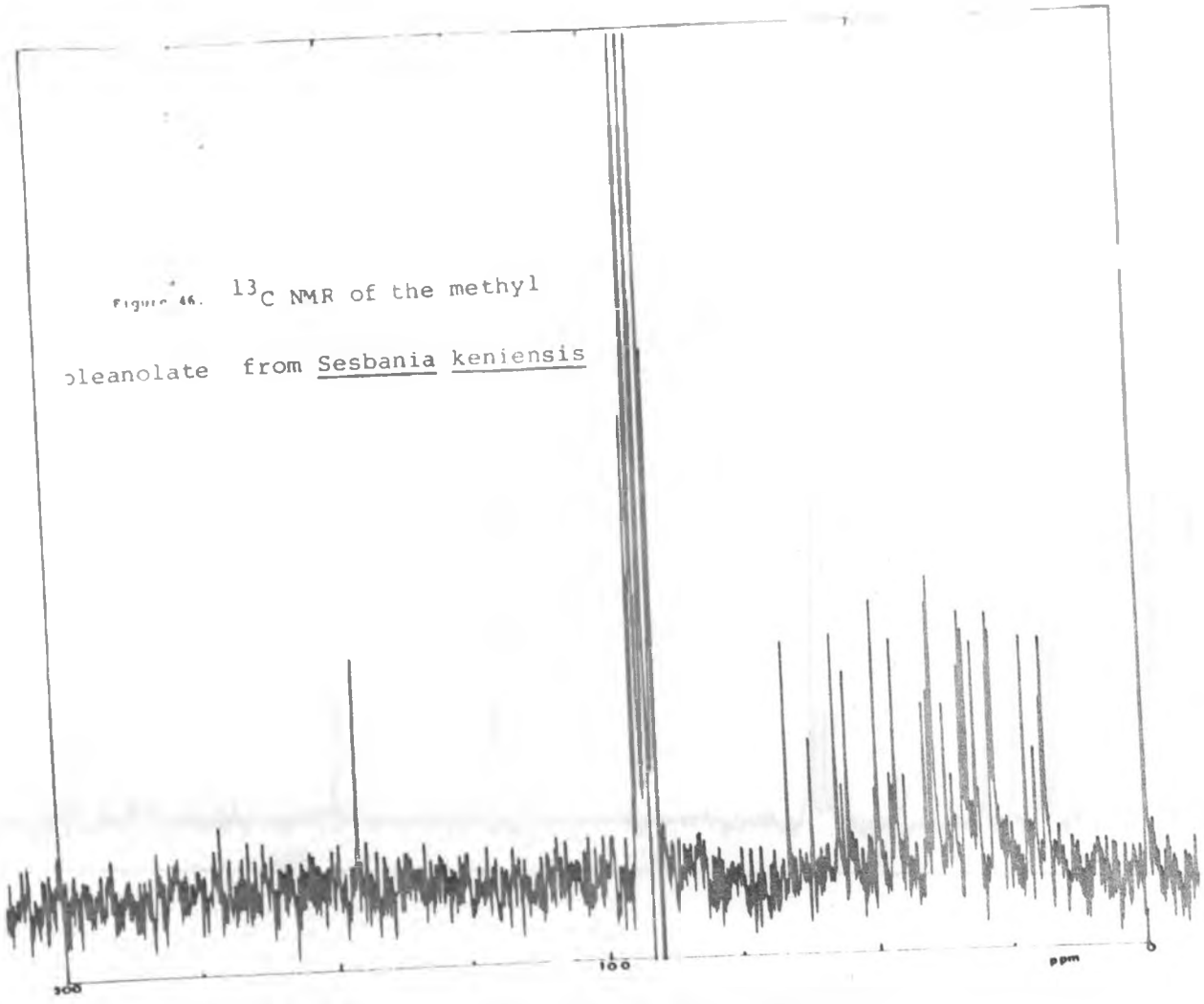


Figure 46. ^{13}C NMR of the methyl oleanolate from Sesbania keniensis

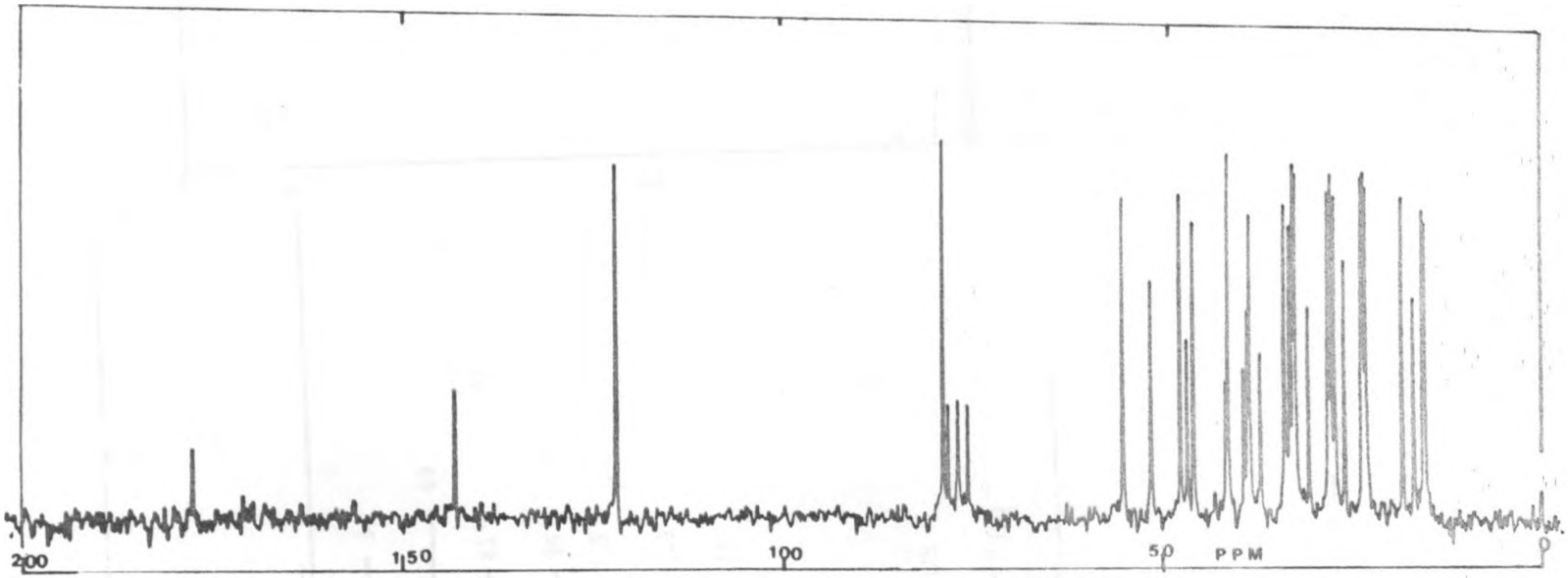
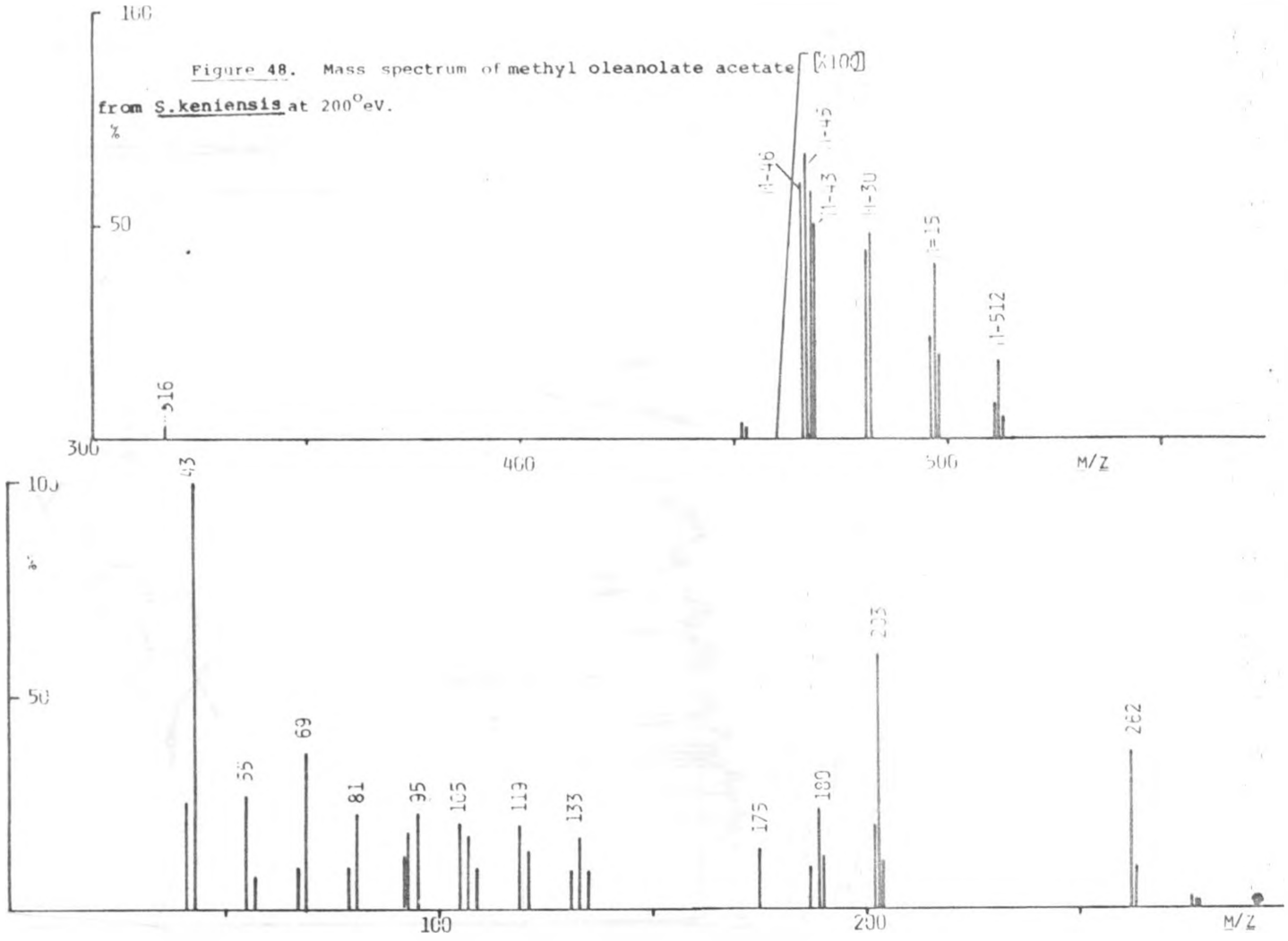


Figure 47. ^{13}C NMR spectrum of authentic oleanolic acid.

Figure 48. Mass spectrum of methyl oleanolate acetate from S.keniensis at 200°eV.



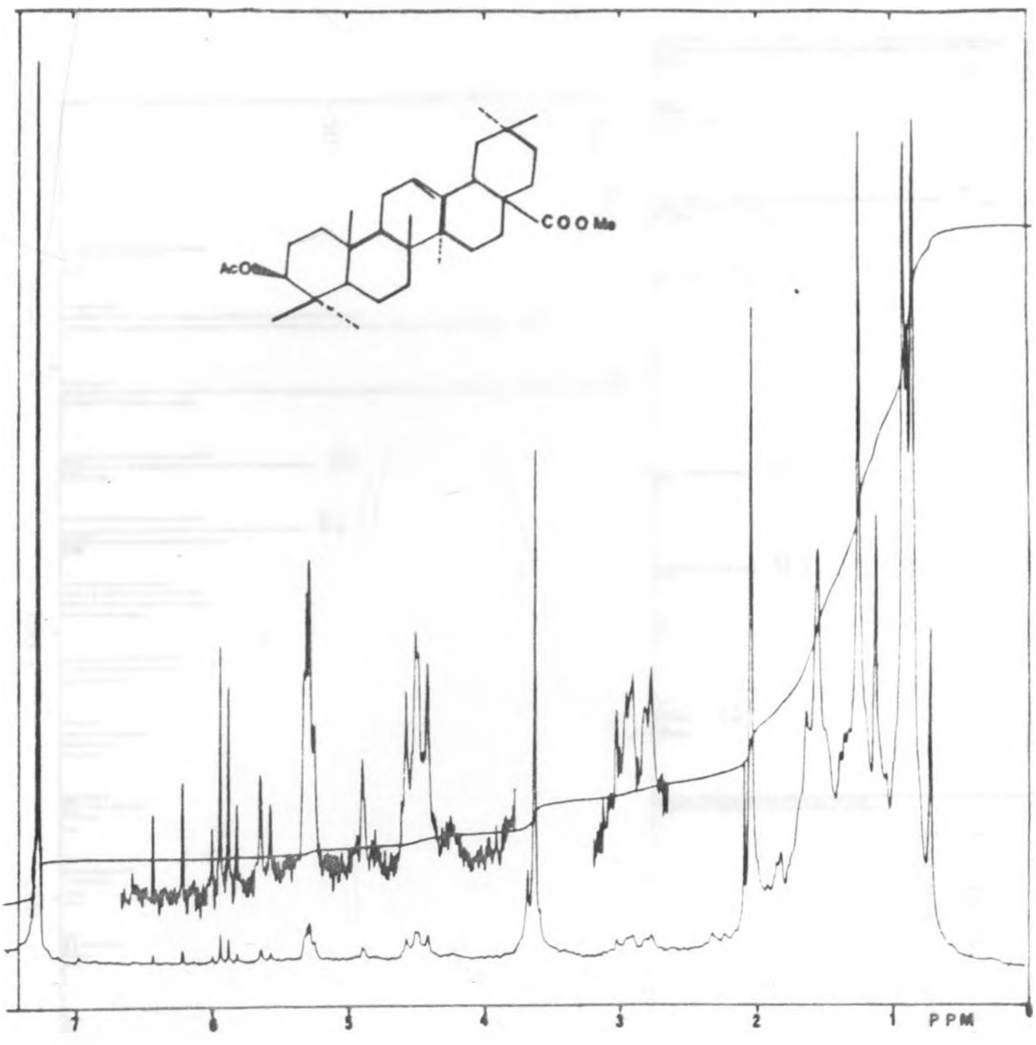
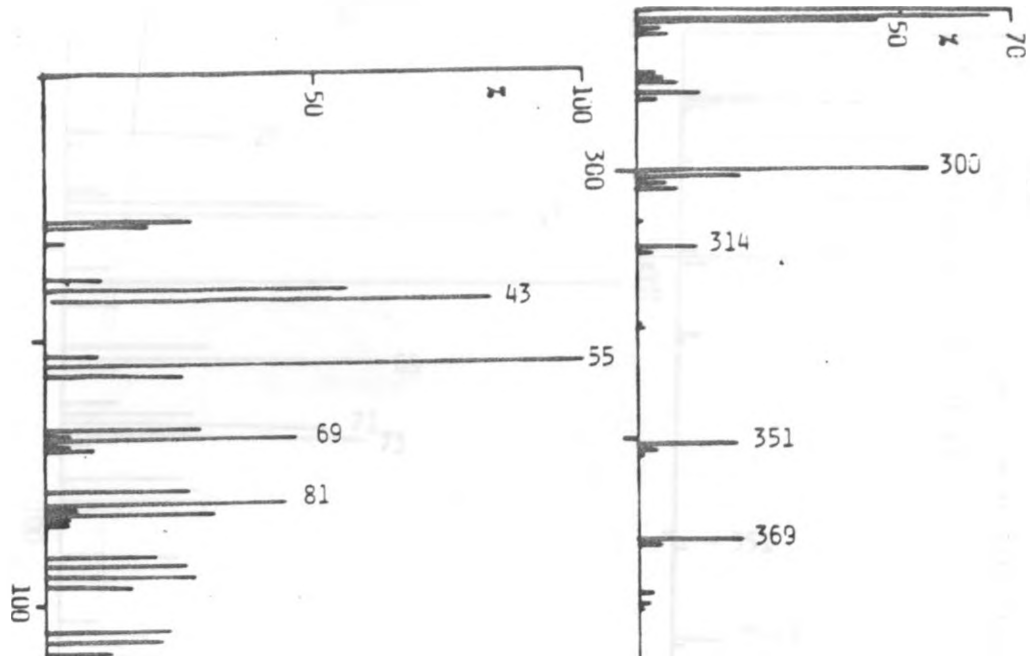


Figure 49. ^{1}H NMR spectrum of methyl oleanolate acetate from S.keniensis


100
50
%
43
55
69
81
100
300
50
%
70
300
314
351
369

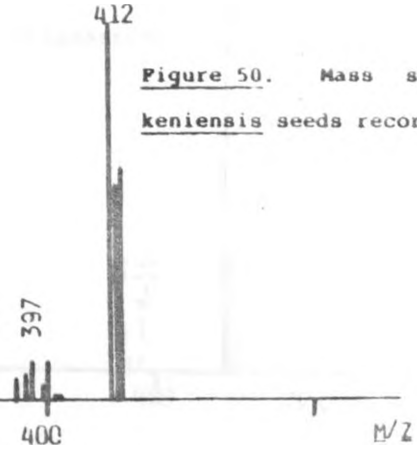
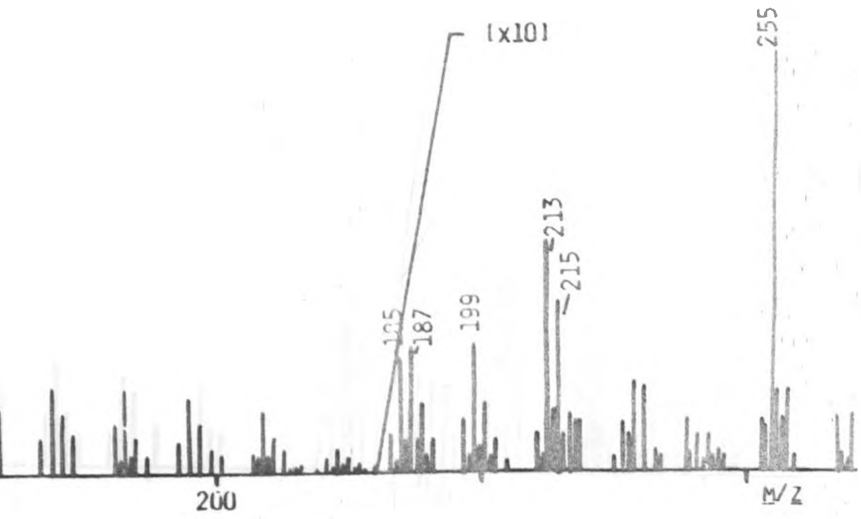


Figure 50. Mass spectrum of stigmasterol from *Sesbania keniensis* seeds recorded at 130°/70eV.



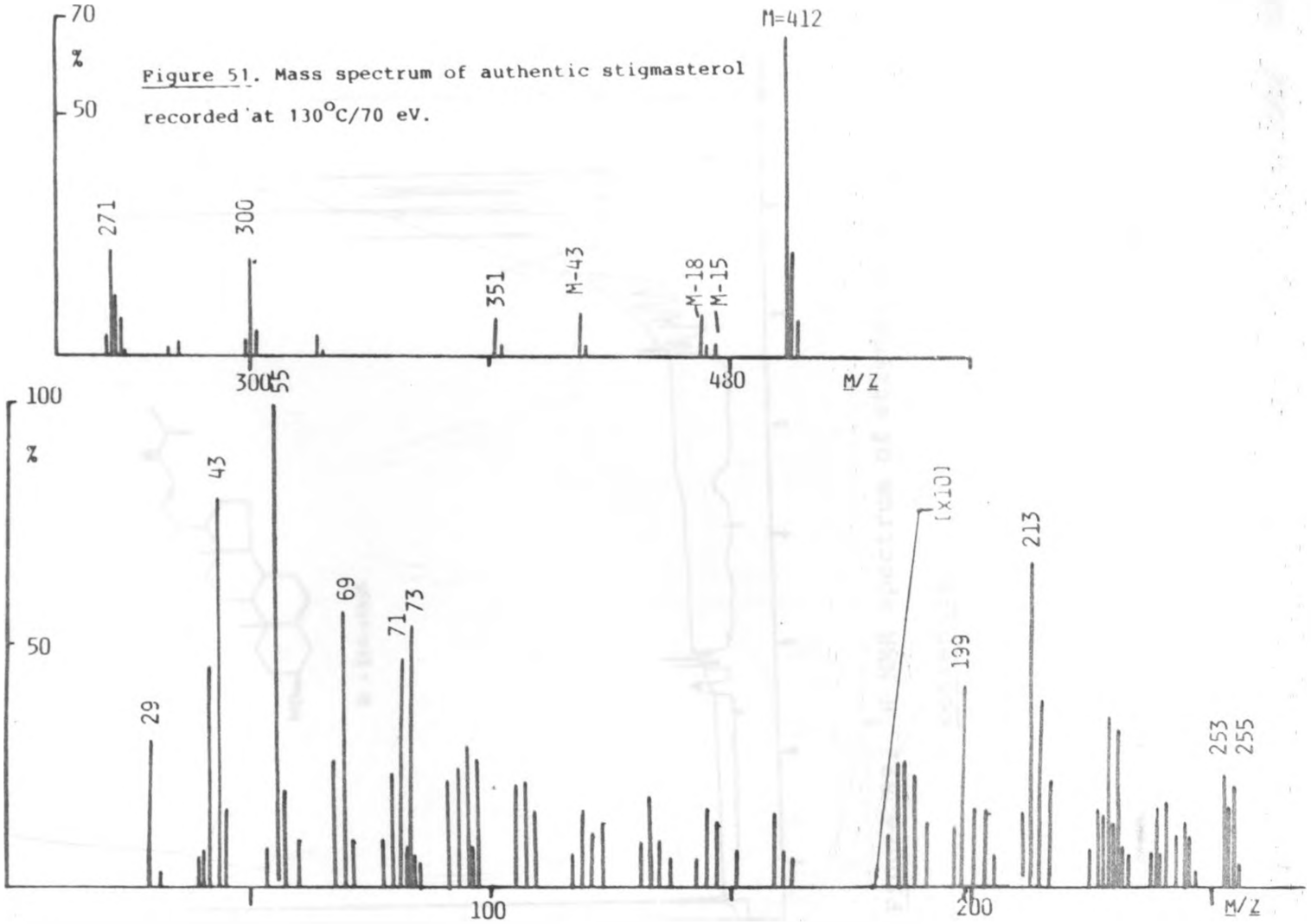


Figure 51. Mass spectrum of authentic stigmasterol recorded at 130°C/70 eV.

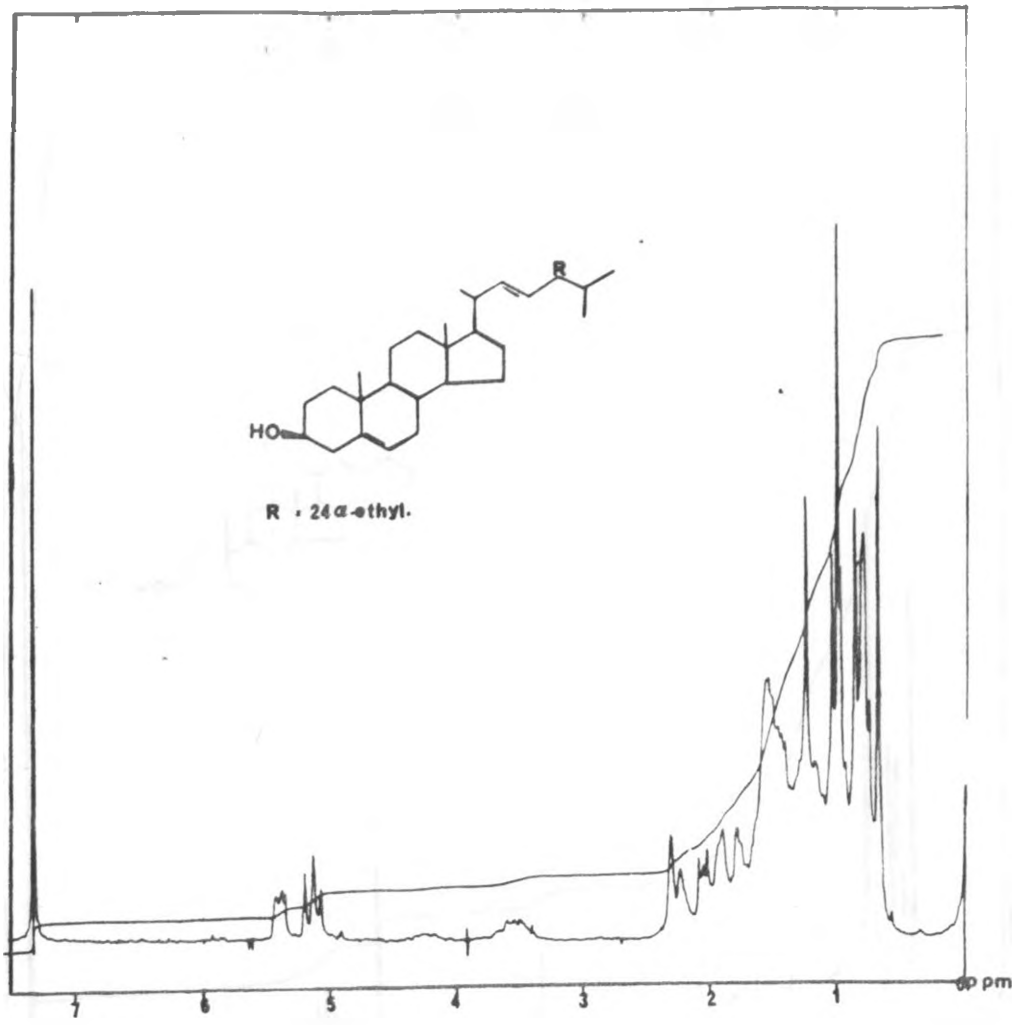


Figure 52. ^{1}H NMR spectrum of stigmasterol from Sesbania keniensis.

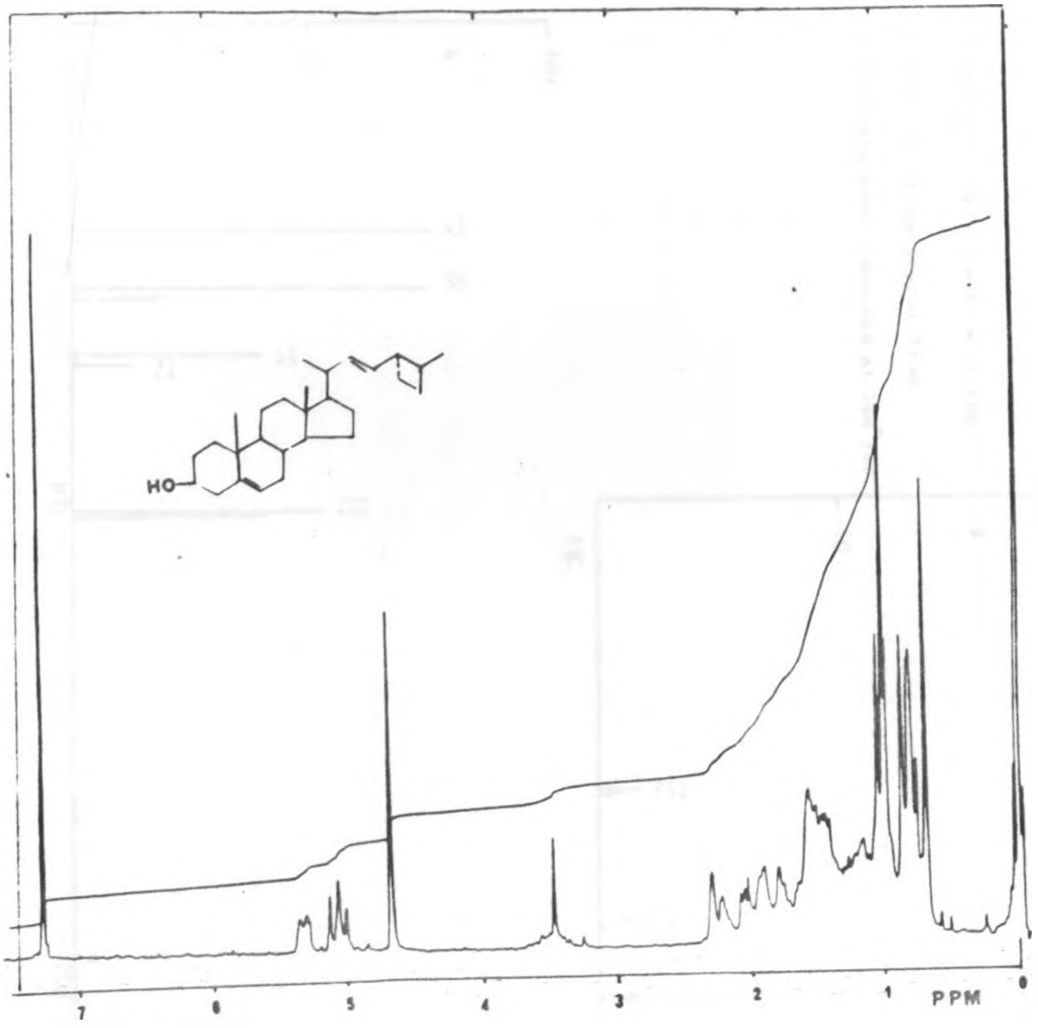


Figure 52.2. ^{1}H NMR spectrum of authentic stigmasterol.

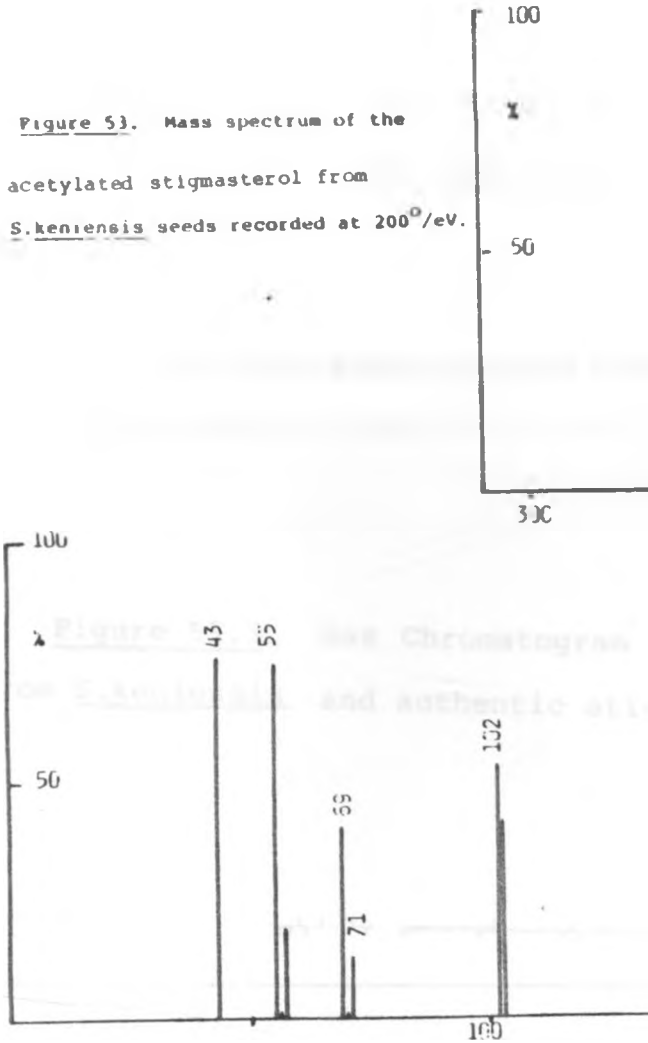


Figure 53. Mass spectrum of the acetylated stigmasterol from S.kenienesis seeds recorded at 200°/eV.

394
351
373
M/Z

213
255
228
200
M/Z

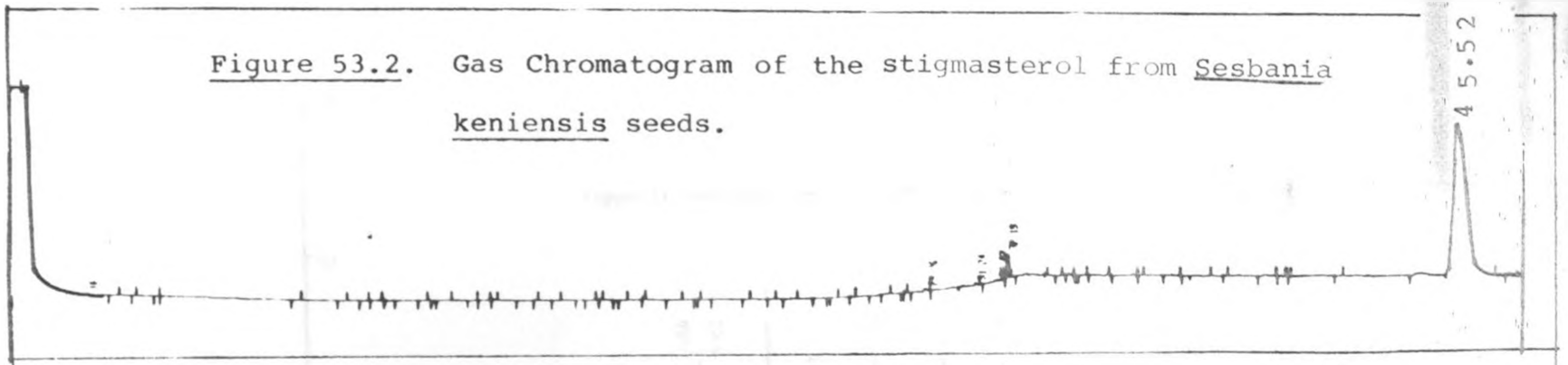


Figure 53.2. Gas Chromatogram of the stigmasterol from Sesbania keniensis seeds.

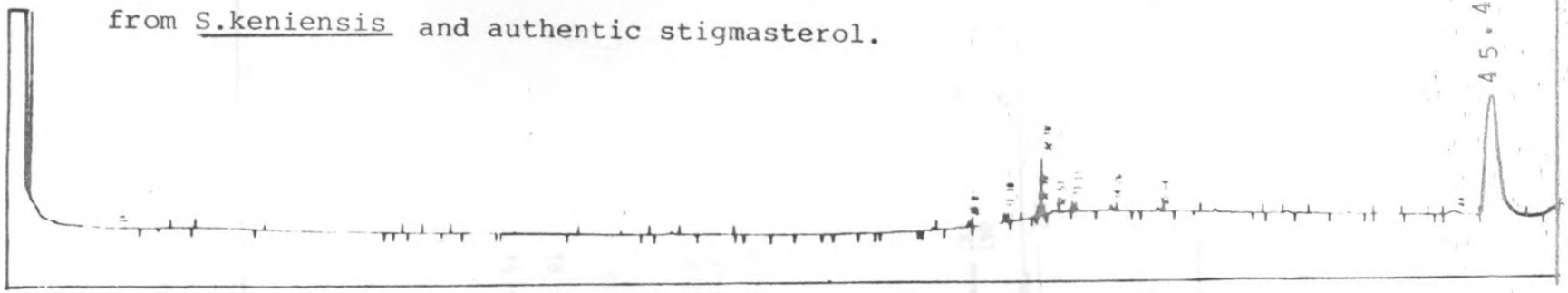
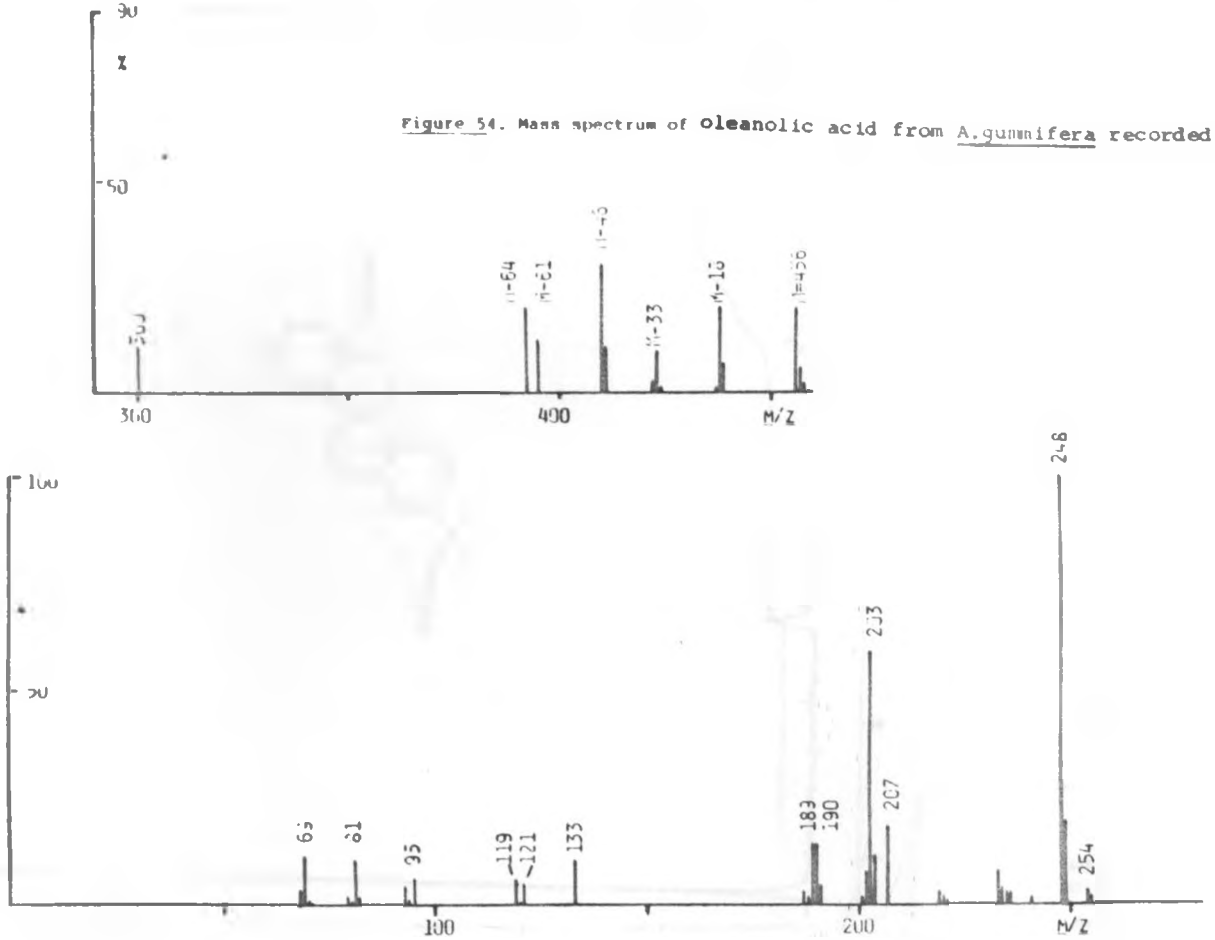


Figure 53.3. Gas Chromatogram peak of the co-injection of stigmasterol from S.keniensis and authentic stigmasterol.

Figure 54. Mass spectrum of oleanolic acid from A.gummifera recorded at 200°/70eV



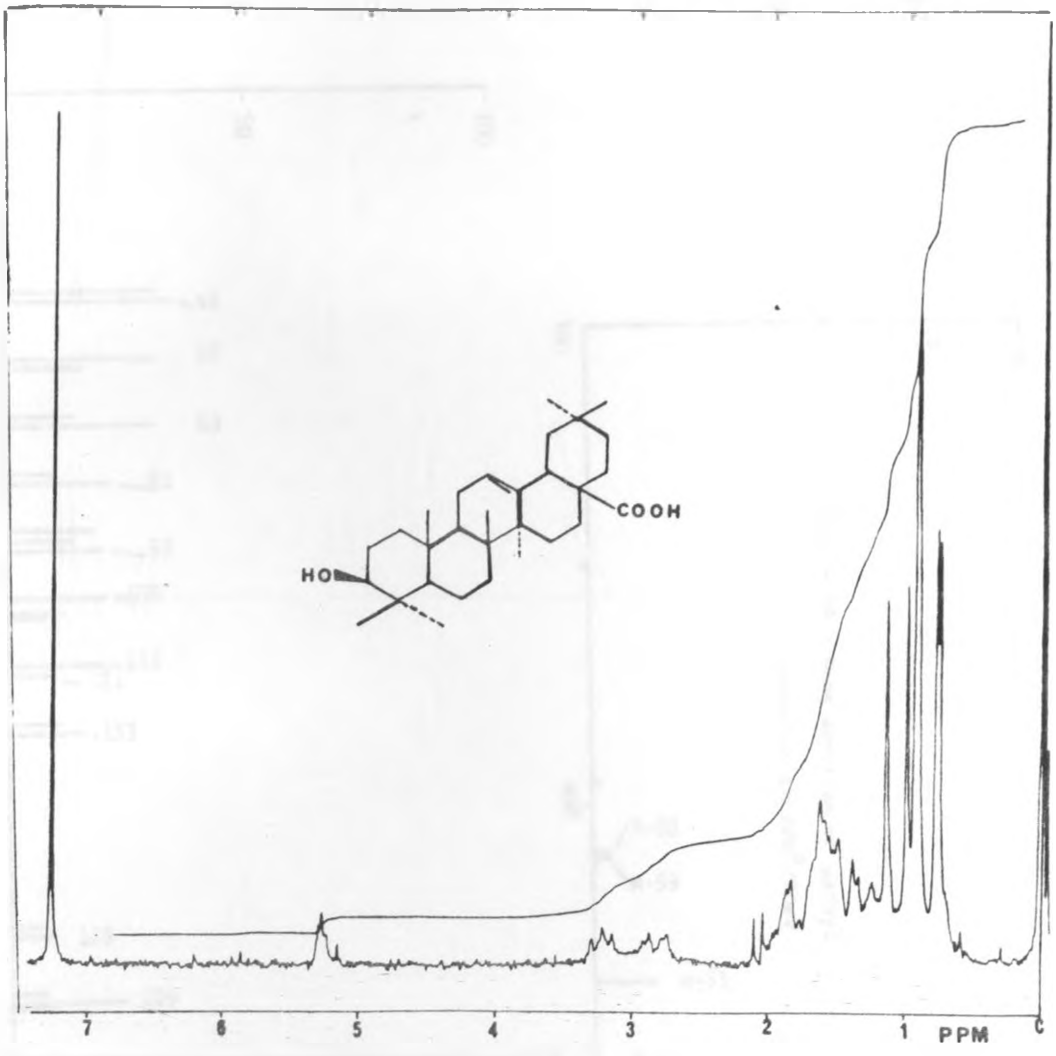


Figure 55. ^{1}H NMR spectrum of the oleanolic acid from Albizia gummifera stem bark.

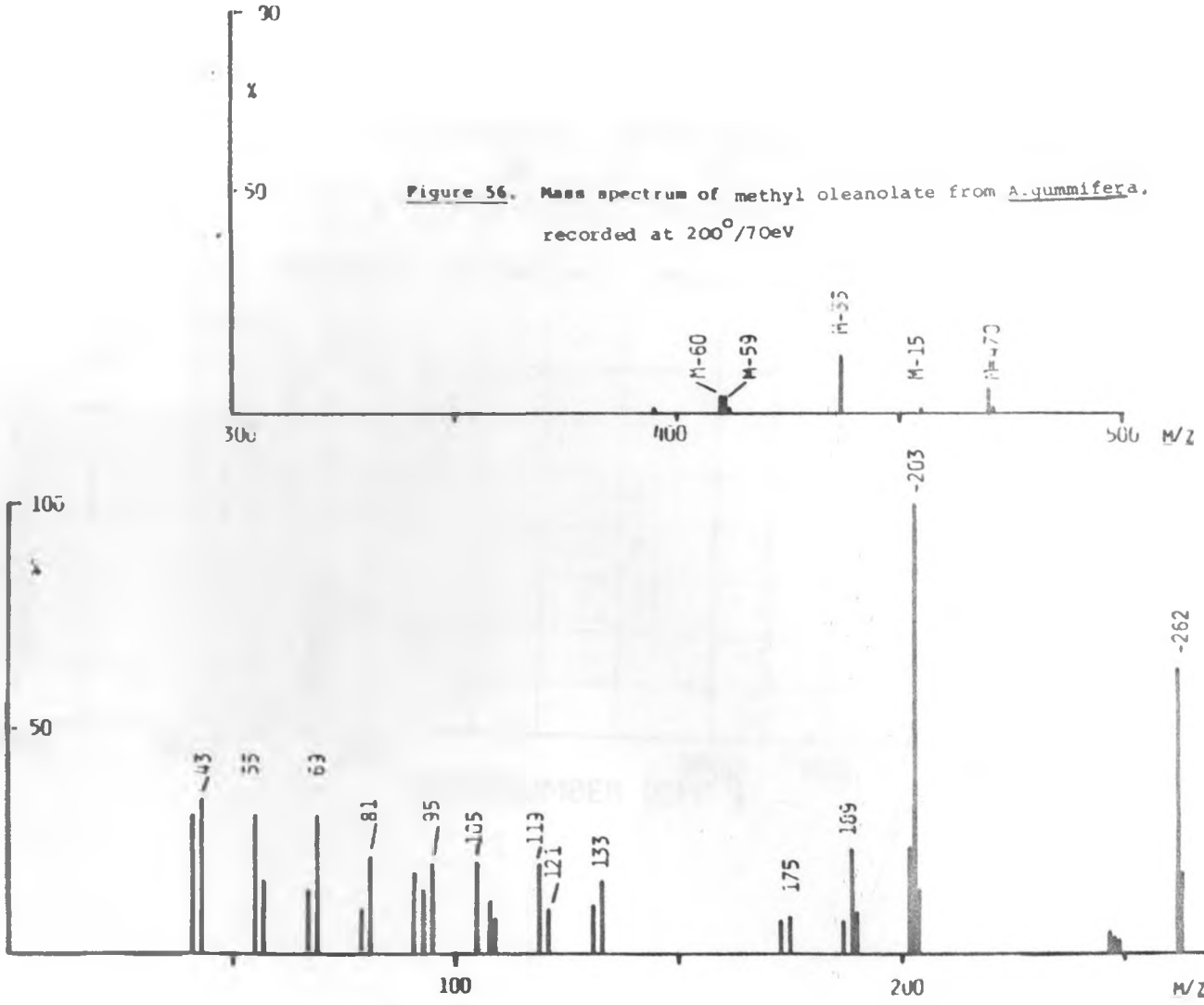


Figure 56. Mass spectrum of methyl oleanolate from A.gummifera, recorded at 200°/70eV

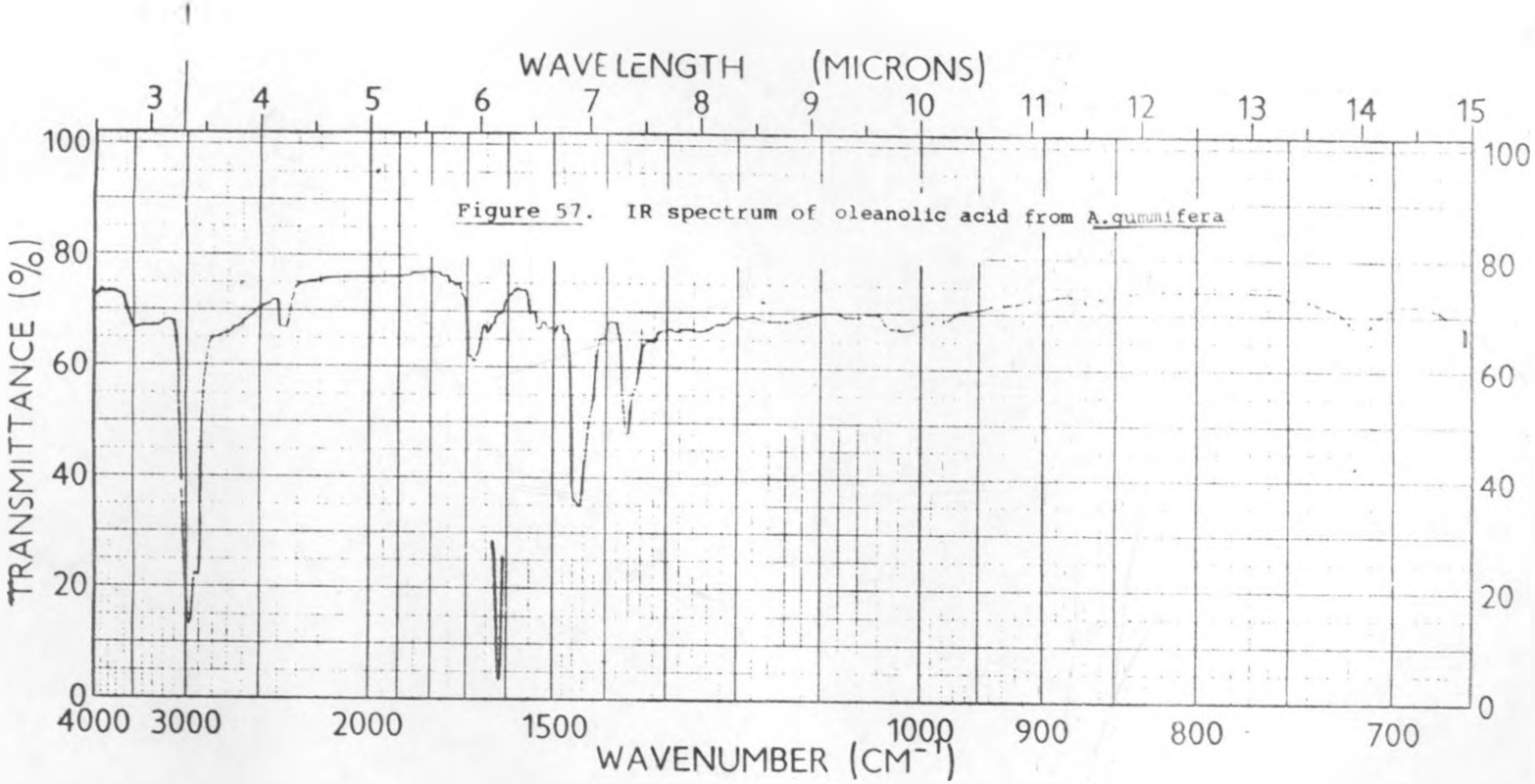


Figure 57. IR spectrum of oleanolic acid from A.gummifera