ISOLATION AND IDENTIFICATION OF POSSIBLE ANALGESICS AND ANTIHYPERTENSIVE AGENTS FROM *ANTIDESMA VENOSUM*

By

Mahlori Jeffrey Mashimbye
B.Sc. (Hons.) (Fort Hare)

MOTIVATION

This investigation originated from a suggestion by Noristan Laboratories, Pretoria, that because Black people were using the roots of A. venosum E. MEY. ex. TUL for treating headache, the plant might contain analgesics.

No previous chemical investigation has been carried out on this plant but from previous work done on other species antihypertensive agents were expected to be present.[45,46,47]

DESCRIPTION OF PLANT

Antidesma venosum E. MEY. ex. TUL family Euphorbiaceae*
[Modulane (N.Sotho); Mphatakwari (Tsonga); Mutshelekwele (Venda); UmGqwagqwa (Zulu); UmHlalanyoni (Swazi); Tassel berry (English)] is a tree, up to 10m high and 35cm in stem-diameter. The bark is grey and smooth.

The leaves are very variable in size and shape, up to 15cm long and 7cm broad. They are usually smaller and more or less elliptic or oblanceolate, somewhat rounded or subacute at the base and apex, sometimes slightly emarginate at the apex, thinly coriaceous or almost membranous, glabrous or pubescent above, varying from thinly pubescent to tomentose beneath, lateral nerves usually 7 on each side, more or less distinctly looped, slightly impressed above and prominent below. The petiole is up to 6mm long and pubescent. The stipules are lanceolate, entire, acute, mostly more of less tomentose.

Male flowers are disposed in catkin-like spikes which are 15cm long, tomentose or pubescent. Bracts are very small. Calyx are more or less pubescent or tomentose, 3 - 5 in number. Stamens are 3 - 5 in number with rudimentary ovary which is either pilose or subgrabrous. Females racemes are 0,4 to 1,0 cm long.

The fruit is a drupe, white when young, turning red, and finally black when ripe, about 8mm long and 5mm in diameter, ellipsoid, slightly flattened, fleshy, pedicelled, with a single hard seed.

The plant is very common in Natal, Zululand, Swaziland, Eastern, North-Eastern and Northern Transvaal, Bechuanaland, Eastern Cape border and South West Africa. It is widely spread throughout tropical Africa as far as the Sudan.[19,42]

*Willis dictionary [84] lists it as belonging to the Stilagnaceae family.

ABBREVIATIONS

^{13}CNMR	-	carbon - 13 magnetic resonance
c	-	concentration
°C	-	degrees Celcius
DMSO	-	dimethyl sulphoxide
^{1}HNMR	-	proton (^{1}H) magnetic resonance
Hz	-	Hertz
I.R.	-	infra-red
m	-	multiplet
m.p.	-	melting point
M.S.	-	mass spectroscopy
NMR	-	nuclear magnetic resonance
ppm	-	parts per million
p	-	page
s	-	singlet
t	-	triplet
TLC	-	thin layer chromatography
TMS	-	tetramethylsilane
U.V.	-	ultra-violet
v/v	-	volume per volume
Ph	-	phenyl group

LIST OF TABLES

I N D E X

CHAPTER ONE

INTRODUCTION

1.1 Definition

Analgesics are pain-relieving natural or synthetic drugs and antihypertensives are cardiovascular agents (natural and synthetic). Because many analgesics and antihypertensive agents are alkaloids, this class of compounds will be briefly reviewed.

1.2 History of alkaloids

Opium is one of the first recorded alkaloids and the earliest descriptions of the psychological and physiological effects of opium appear to have been written in about 300 B.C., although references to opiates have been found dating to about 3000 B.C. opium appears to have been used in early Egyptian, Greek and Arabian cultures primarily for its constipating effect in the treatment of diarrhoea. Later, opium's sleep inducing properties were noted by Greek and Roman writers such as Homer, Virgil and Ovid. Opium was frequently used by the Greeks to treat a variety of problems, including snake bite, asthma, cough, epilepsy, colic, urinary complaints, headache and deafness. The Greeks also appeared to use the drug recreationally, and opium cakes were common, even among high standing military and political figures of Rome.

From the early Greek and Roman days and through the sixteenth and seventeenth centuries, the medicinal and recreational uses of opium were well established through Europe. Since much of the opium came from the Near East and the Orient, a bustling trade in opium existed between East and West. The control of opium was the central issue in the opium wars in China in 1839.[1]

The chemistry and history of alkaloids started in 1805 when F.W. Sertuner[2] announced the isolation of morphine (1). He prepared several salts of morphine and demonstrated that it was the

principle responsible for the physiological effect of opium.[2,3] Later, in 1810, Gomes treated an alcoholic extract of chincona bark and obtained a crystalline precipitate which he named "cinchonino". In 1820, P.J. Pelletier and J.B. Caventon of Faculty of Pharmacy, Paris, showed that "cinchonino" was a mixture which they separated into two new alkaloids called quinine and cinchonine. Subsequently, various investigators isolated more than two dozen additional bases from species of Cinchona and Remijia. Between 1820 and 1850, investigations were intensified and a large number of alkaloids of new and varied types were isolated and characterized.[2] Some of the important representatives discovered during this period are :-

Aconitine - one of the most toxic materials of plant origin known to man.
Atropine - a powerful mydriatic agent.
Colchicine - the alkaloid of the meadow saffron, which is used extensively in the treatment of gout.
Coniine - alkaloid responsible for the death of Socrates when he drank the cup of poison hemlock.
Codeine - a close relative of morphine and a valuable pain killer.
Hyoscyanine - optical active form of atropine.

1.3 Physical properties of alkaloids

They are colourless, crystalline substances containing carbon, hydrogen, oxygen and nitrogen. The few that are liquids are generally free from oxygen. Comparatively few alkaloids are coloured but afford coloured salts. A screening method is used to test the presence of alkaloids in plant extract.[4,5,6] In pure compounds, the fusion method for nitrogen test is preferred.[7]

1.4 Isolation of alkaloids

Alkaloids generally occur as salts which are soluble in alcohol or in water. When they occur as the free base, they may be extracted by alcohol or chloroform.[3,8,9,10,11] When they occur as salts, insoluble in alcohol the free base may be obtained by mixing ground plant with lime or magnesia and then extracting with alcohol, chloroform, ether or petroleum. Extraction in the presence of magnesia or by means of dilute mineral acids should not be resorted to unless no other means is available as many alkaloids suffer decomposition under such treatment.[3] The extract should also be concentrated under reduced pressure in order to avoid decomposition of the alkaloid by excess heat.[3,11] From the concentrated liquors, fat, resin and other impurities are generally

/separated.....

separated by adding water. If necessary dilute mineral acid should also be added to keep the alkaloid in solution. This aqueous or acid solution may then be shaken with an immiscible solvent (e.g. ether) to remove colouring matter, but care should be taken that the alkaloid is not also extracted by solvent, since some of the weakly basic alkaloids can be removed even from acid solution. The concentrated aqueous solution is then made alkaline. A weak base such as dilute ammonia, sodium carbonate or sodium hydrogen carbonate solution is used in preference to solutions of alkali hydroxides.[3] Most of the alkaloids are precipitated by this treatment and may then be separated by shaking out with an immiscible solvent such as chloroform or ether.[3,8,11]

Some alkaloids (e.g. Morphine) contain phenolic hydroxyl groups and dissolve in alkali hydroxide solutions, but as a rule they are precipitated by ammonia or alkali carbonate solutions. Certain alkaloids are miscible with water and are not easily extracted from water by immicible solvents. In such cases precipitation with one of the alkaloidal precipitants mentioned below may be resorted to and the alkaloid recovered from the washed precipitate.[3]

When the "total alkaloid" has been separated from the plant it is necessary to ascertain whether it consists of a single substance or if it is a mixture. Separation into its constituents may be affected by chromatographic techniques such as a column, high pressure and thin layer chromatography. Fractional crystallization can also be resorted to for free alkaloids. The purification of an alkaloid is often facilitated by converting it into a salt.[3]

1.5 Alkaloidal Precipitants

One of the most characteristic properties of alkaloids is that of forming complex double salts with certain metallic halides. These double salts are generally nearly insoluble in water, so that mere traces of alkaloids can be detected by their formation. The following are a few of the most useful precipitants of this kind.

1.5.1 Auric Chloride

A solution of auric chloride gives yellow or orange-coloured precipitates (the aurichlorides) with many alkaloids. The latter should be dissolved in a very slight excess of dilute hydrochloric acid. As a rule, the precipitate can be recrystallized from alcohol, or water, containing a little hydrochloric acid. They have the general composition, $B.HCl.AuCl_3$, but compounds of the

type, $B.AuCl_3$, are also sometimes produced, and under certain conditions aurichloro-derivatives of the alkaloids, containing the group $AuCl_2$ in place of a hydrogen atom of the alkaloid, are formed. Examples of such compounds are referred to under japaconitine and caffeine.[3]

1.5.2 Platinic Chloride

Similar compounds, the platinichlorides, $(B.HCl)_2.PtCl_4$ or $B.H_2PtCl_6$ in the case of diacidic alkaloids, are formed with platinic chloride. They are more soluble than the aurichlorides, especially in dilute hydrochloric acid.

1.5.3 Mercuric Chloride

With solutions of this salt many of the alkaloids give characteristic, crystalline mercurichlorides of the general composition, $B.HCl.HgCl_2$.

Among other precipitants of this class may be mentioned ferric chloride, lead tetrachloride, telluric chloride and thallic chloride.

Solutions of certain double metallic halides form the best known group of alkaloidal precipitants and include the reagents most commonly used for detecting their presence. Among these are the following :-

1.5.4 Potassium Mercuric Iodide (Meyer's Reagent)

It is prepared by adding 6,8g of mercuric chloride, dissolved in water, to 25g of potassium iodide, dissolved in water, and diluting to 1000 ml. This solution gives white, curdy precipitates with minute traces of alkaloids in solutions slightly acidified with hydrochloric or sulphuric acid. The alkaloids may be recovered from these precipitates by suspending them in water and passing a current of sulphuretted hydrogen, when the alkaloidal hydroiodide is formed and may be recovered by concentrating the filtrate.

Similar precipitates are afforded by solutions of potassium bismuth iodide (Dragenodorff's reagent), potassium cadmium iodide (Marme's reagent), iodine in potassium iodide (Wagner's reagent), and other like solutions.

In addition to the foregoing precipitants a number of acids form insoluble alkaloidal salts and therefore act as precipitants.

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1.5.5 Gallotannic Acid

A solution a gallotannic acid gives precipitates of the corresponding tannates with most alkaloids in neutral solution. These precipitates are generally soluble in ammonia and sometimes in dilute acids.

1.5.6 Picric Acid (Hager's Reagent)

The picrates of most of the alkaloids are sparingly soluble in water or dilute acids and are precipitated when a cold, saturated, aqueous solution of picric acid is added to a solution of an alkaloidal salt. They can usually be recrystallized from alcohol and are often characteristic.

1.5.7 Phosphomolybdic Acid (Sommenschein's Reagent)

A solution of this substance gives amorphous yellow precipitates with many alkaloids, and may be used for separating them from associated non-alkaloidal organic matter, since the alkaloids may be regenerated by treating the precipitates with sodium carbonate and extracting rapidly with alcohol.

Phosphotungstic acid and metatungstic acid have been also used as alkaloidal precipitants.

1.6 Nomenclature and classification of alkaloids

The nomenclature of alkaloids has not been systematized. The two commonly used sytems classify alkaloids either according to plant genera in which they occur or on the basis of similarity of molecular structure.[2] Important classes of alkaloids containing generally related members are the aconitum, cinchona, ephedra, lupin, opium, rauwolfia, senecio, solanum and strychnos alkaloids.

Chemically derived alkaloid names are based on the skeletal feature which members of the group possess in common. The classification of alkaloids in this case is based on the heterocyclic systems (fig. 1). Thus, the indole alkaloids (e.g. psilocybin) contain a modified indole nucleus, pyrrolidine alkaloids (e.g. hygrine) contain pyrrolidine ring system and isoquinoline alkaloids (e.g. papaverine) contain an isoquinoline nucleus.[3,6,12]

/1.7 .....

1.7 Biological significance of Alkaloids

Over two thousand alkaloids are known today, and it is estimated that they are present in only 10 - 15% of all vascular plants. The Phanerogans or flowering plants are richer in alkaloids than the Cryptogans or so-called flowerless plants, and of the former class the sub-class dicotyledon is richer in alkaloids than the monocotyledon. In dicotyledons, they occur abundantly in the families : APOCYNACEAE (dogbane, quebracho, Pereiro bark), PAPAVERACEAE (poppies, chelidonium), PAPILIONACEAE (lupins, butterfly-shaped flowers), RANUNCULACEAE (aconitum, delphinium), RUBIACEAE (cinchona bark, ipecacuanlia), RUTACEAE (citrus, fagara), EUPHORBIACEAE and SOLANACEAE (tobacco, deadly nightshade, tomato, potato, thorn apple). The ROSACEAE, GRAMINACEAE and LABIATAE are typically poor in alkaloids whilst the COMPOSITAE occupy an intermediate position. PAPAVERACEAE is an unusual family in that all of its species contain alkaloids.[2]

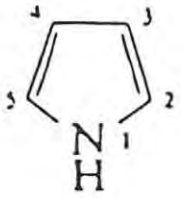
Alkaloids found in a family and expecially in any one genus are usually somewhat closely related; thus the various aconitines form a closely related group, found only in members of genus Aconitum. In some cases a single alkaloid is characteristic of the family, e.g. protopine occurs in many plants of the family Papaveraceae and closely related family Fumariaceae. The purine alkaloids, on the contrary, furnish an instance of closely related alkaloids occuring in plants belonging to different families.

Where investigations have been made of all parts of a plant for alkaloids, as in the cases of hemlock poppy and some solananceous plants, it has been found that alkaloids usually occur in all parts of the plant. It is generally impossible to say with certainty that one particular part of a plant is always richer than another in alkaloids, since the richness of each part varies with the season and with the condition of the plant. Thus in the case of belladoma the amounts of "total alkaloid" recorded vary from 0,15 to 0,60 per cent in the roots and from 0,05 to 0,64 per cent in the leaves. The quantity of alkaloid can be greatly increased by special cultivation and especially by selection. Chevalier has also shown recently that the alkaloidal content of solanaceous plants can be increased by manuring.[3]

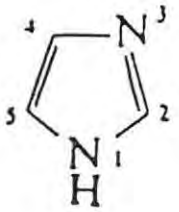
The function of alkaloids in plants remains a subject for speculation and three views have been held :

1) that they are nutritive materials used by plant in metabolism;
2) that they act as protective materials against attack of the plant by animals;

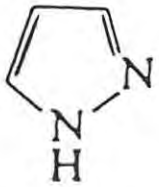
/3).....



Pyrrole



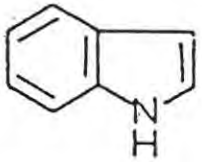
Imidazole



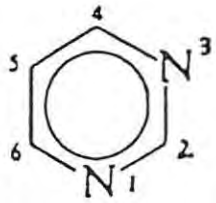
Pyrazole

3-Pyrroline

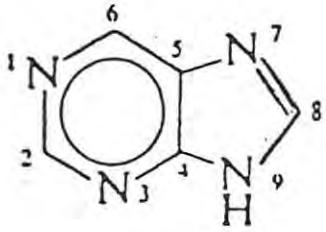
Pyrrolidine



Indole

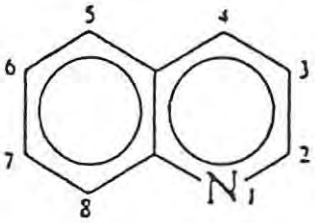


Pyrimidine

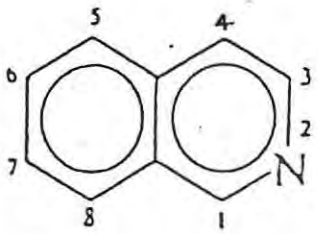


Purine

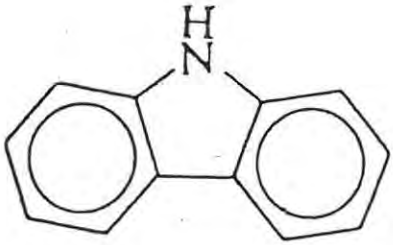
Pyridine



Quinoline

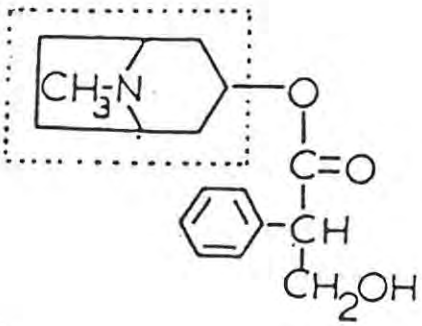


Isoquinoline



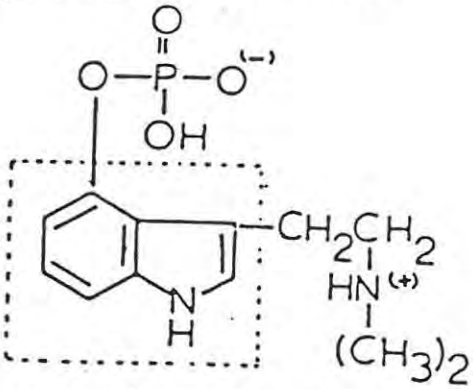
Carbazole

Figure 1 Heterocyclic Systems

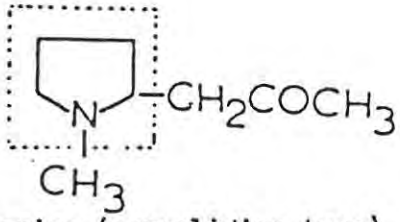


Atropine, Hyoscyamine
(Piperidine-pyrrolidine type)

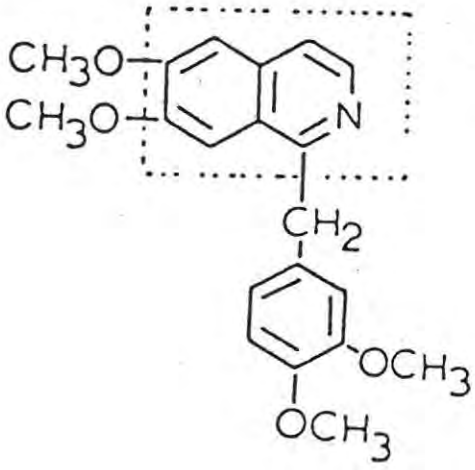
Pseudopelletierine
(pyridine type)



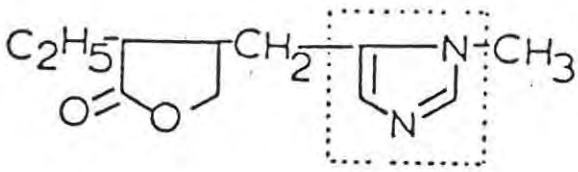
Psilocybin (indole type)



Hygrine (pyrrolidine type)



Papaverine
(isoquinoline type)



Pilocarpine
(Imidazole type)

Figure 2 Note: dashed lines enclose the parent chemical nucleus upon which the type is based.

3) that they are end products of metabolism, rendered harmless to the plant and stored for the most part in special cells where they are not readily reabsorbed into the active plant tissue. [2,3]

1.8 Physiological action of alkaloids

The commercial importance of alkaloids depends wholly on their physiological action, and certain of them, such as quinine and morphine, are, and have long been, among the most commonly used drugs in medicine.

Alkaloid containing plants are distributed all over the world and are used in traditional medicine for the treatment of fevers, colds, colic, etc. Some are used as analgesics and antihypertensive agents. Chemical and pharmacological investigations led to the conclusion of alkaloids as effective constituents. [2, 13]

1.8.1 Analgesics

The accepted, standard analgesic has always been morphine (1). As an analgesic morphine has some disadvantages; it causes respiratory depression, tolerance to its action rapidly develops and it is a drug of primary addiction. [1,2,13] Attempts have been made to eliminate some of the disadvantages by modification of the molecule and by synthesizing compounds that represent only what is believed to be the active section of the molecule. [2] The following generalization, to which of course there are exceptions, may be made :-

1) Activity of morphine is increased by catalytic reduction, methylation, oxidation or elimination of the C(6) hydroxy group, introduction of a C(14) hydroxy or C(5) methyl group, or replacement of the N-methyl by certain groups such as $N\text{-}CH_2CH_2Ph$.

2) Activity is decreased by methylation of the phenolic hydroxyl group, quarternization of the nitrogen, fission of the nitrogen containing ring and opening of the 4,5 - oxygen bridge.

3) Remarkably high activity is found in many derivatives of Diels - Alder adducts of thebaine (2). The ketone (3), for example, is as active as morphine. (4) is 10,000 times as potent as morphine.[2]

/4).....

4) The analgesic activity is found in bases of structures (5), (6), (7), (8) and (9) and many of their derivatives, the structural resemblance of these compounds to morphine (1) is easily understood.

Many alkaloidal analgesics, natural and synthetic, belong to the isoquinoline group (fig 1). The isoquinoline group can further be divided into three subgroups which are morphine, morphinan and 6,7 - benzomorphan.

Among the natural morphines we have nine main members of the group and these may be divided into two enantiomorphic classes namely morphine (1), codeine (12), neopine (13), thebaine (2) and oripavine (14), all isolated from Papaver species and sinomenine (15), hasubanonine (16), metaphanine (17) and protometaphenine (18), all isolated from Japanese plants of the Sinomenium and Stephania species.[2] Synthetic morphine alkaloids include nalorphine (20), naloxone (21) and heroine (19).

The morphinans are members of the class of compounds possessing the main structural skeleton of morphine. The numbering system (1 - 17) and the designation of the rings (A - D) adopted for these compounds are the same as used for morphine.[14] The close relationship existing between these two classes of compounds is best seen by comparing their main structural features as given in page 12.

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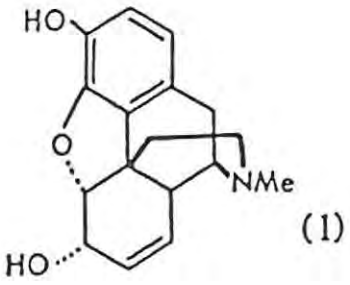
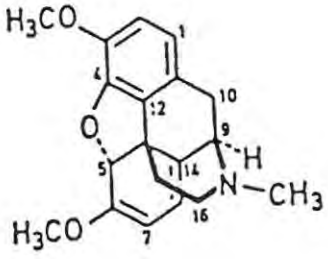
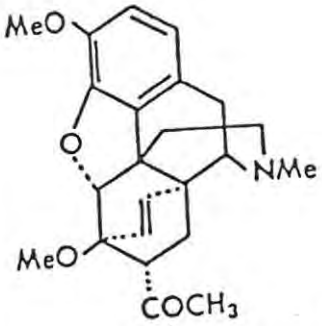


(1)



(2)



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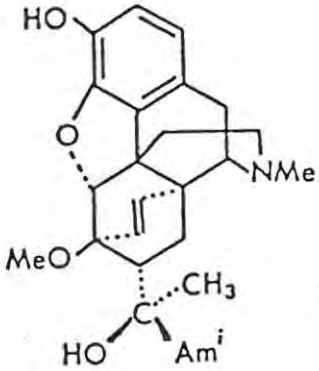

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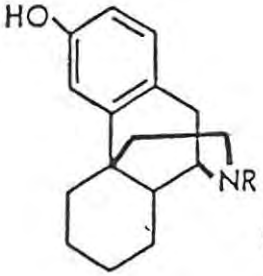

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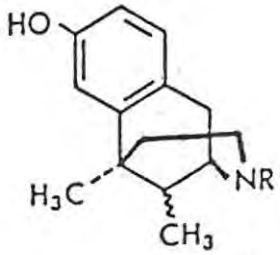

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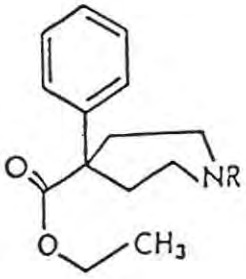

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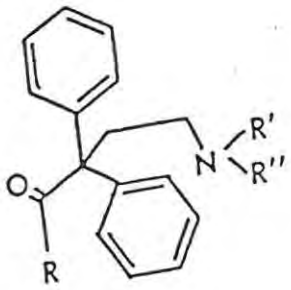

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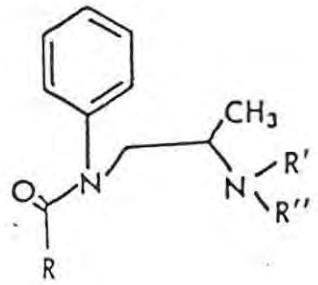

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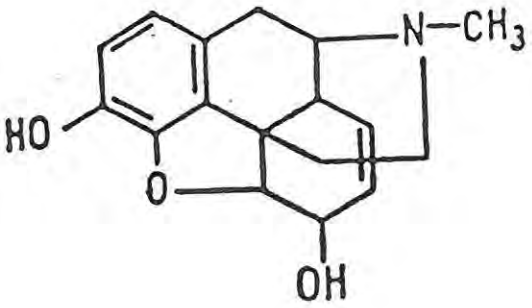
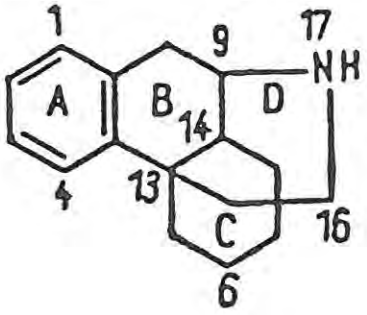
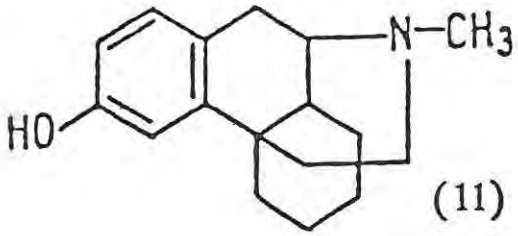


Morphine (1)



Morphinan (10)

The three condensed six-membered rings form the partially hydrogenated phenanthrene fragment, one of which is aromatic (A) while the two other (B and C) are alicyclic. The fusion of rings B and C can either be cis or trans depending upon the configuration at C-13 and C-14 as in decalin. Carbon 13 is quarternary and together with carbon 9 forms the junction with the heterocyclic ring (D). Alkaloids of this subgroup are synthetic. The analgesic, known to be a member of this subgroup is levorphanol (11).

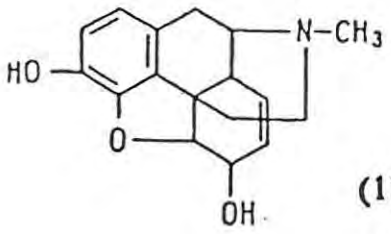


(11)

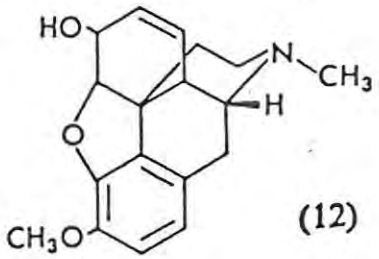
The 6,7-benzomorphans are synthetic alkaloids. The objective in synthesizing these compounds was to maintain the following structural features of morphine and morphinans :

1) The benzene ring.
2) The quarternary carbon (C13 of morphine) attached to the benzene ring and
3) The tertiary nitrogen two methylene groups removed from the quarternary carbon. Finally, optical resolution of any racemate necessarily obtained in ordinary chemical synthesis could be expected to give one analgesic active and one relatively inactive antipode.

/(diagram).....



(1)



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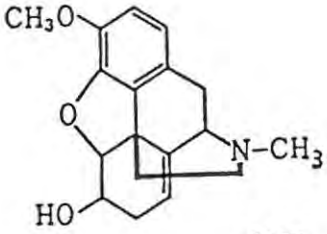

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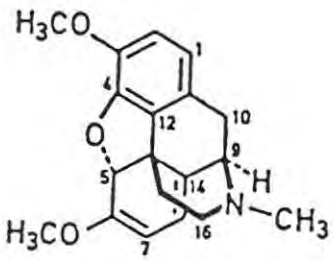

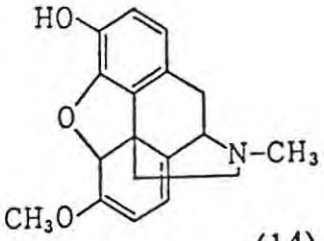

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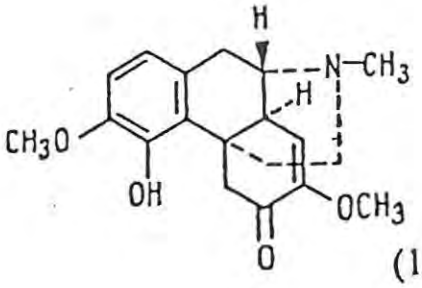

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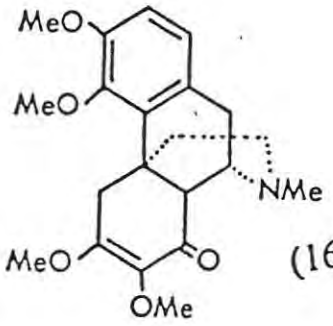

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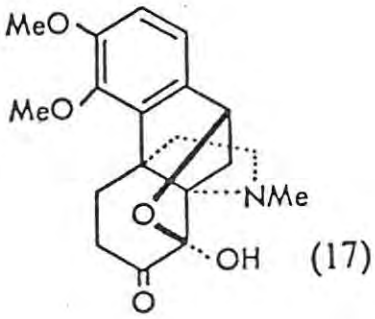

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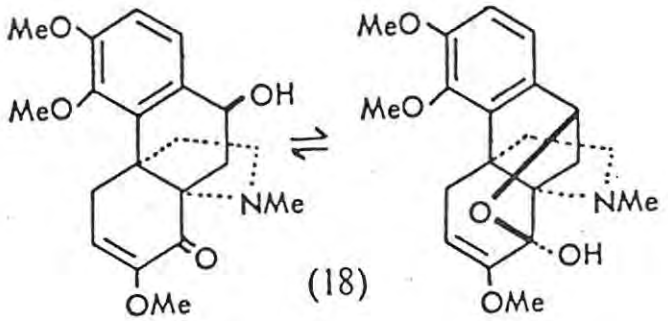

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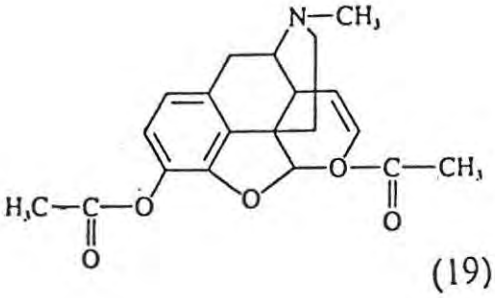

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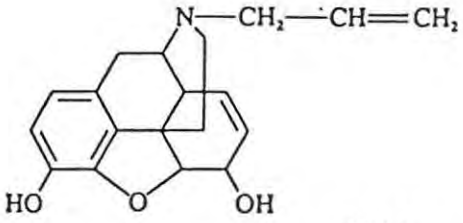

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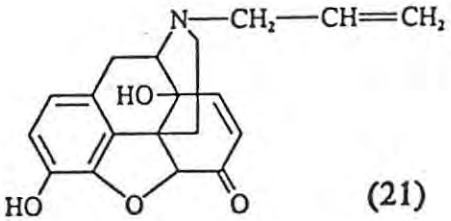

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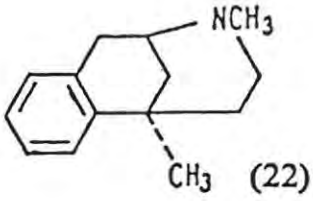

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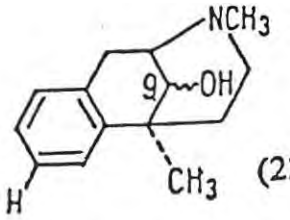


(23)

Analgesics, known to belong to this group are 5-methyl-2(N)-methyl-6,7-benzomorphan (22) and 9-hydroxy-2(N)-methyl-6,7-benzomorphan (23).

1.8.2 Antihypertensive agents

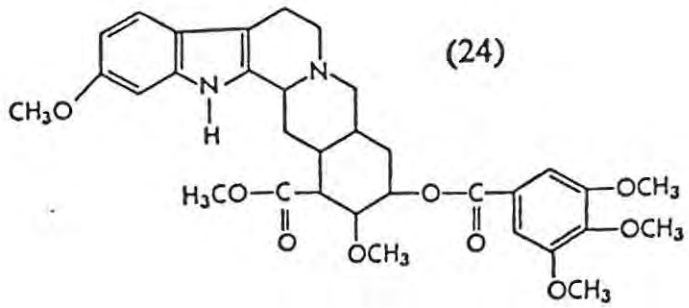
Antihypertensive agents, with the exception of prostaglandins, are alkaloids. They occur naturally and some are synthetically prepared. Antihypertensive agents can be divided into three main groups which are rauwolfia alkaloids, veratrum alkaloids and unclassified antihypertensive agents.

Rauwolfia alkaloids are indole alkaloids extracted from Rauwolfia. [2, 15] Four rauwolfia alkaloids known to be antihypertensive agents are reserpine (24), deserpidine (25), rescinnamine (26) and syrosingopine (27). Reserpine is obtained commercially primarily from R. vomitoria, and to a lesser degree from four other species :- R. canescence, R. micrantha, R. serpentina and R. tetraphylla. [16] Deserpidine differs from reserpine only in the absence of a methoxy group at C-11. It is split by alkaline hydrolysis to give 3,4,5-trimethoxybenzoic acid, methanol and the corresponding indole acid. [17] It has a more rapid onset of action than reserpine. [18] Rescinnamine is the 3,4,5-trimethoxycinnamic acid ester of methyl reserpate. It differs from reserpine only in the acid used to esterify the hydroxyl group at C-18. Alkaline hydrolysis may occur at C-16 and C-18. [17] It is categorized as an antihypertensive agent and tranquilizer. Syrosingopine is also closely related to reserpine, the only difference being the acid used to esterify the hydroxyl group at C-18. It is split by alkaline hydrolysis at C-16 and C-18 to give corresponding acids and methanol. It is effective in the control of some cases of mild hypertension but must be used with other antihypertensive agents in the treatment of severe hypertension. [18]

Veratrum alkaloids are steroidal alkaloids obtained from the species Veratrum album and V. officinalis and other members of the Liliaceae contain ester alkaloids that are potent antihypertensive agents. Of the many steroidal alkaloids from Liliaceae only protoveratrine A(28) and B(29) are useful as medicinal agents. [16]

Numerous unclassified antihypertensive agents are also used. [17,18]

/(diagrams)



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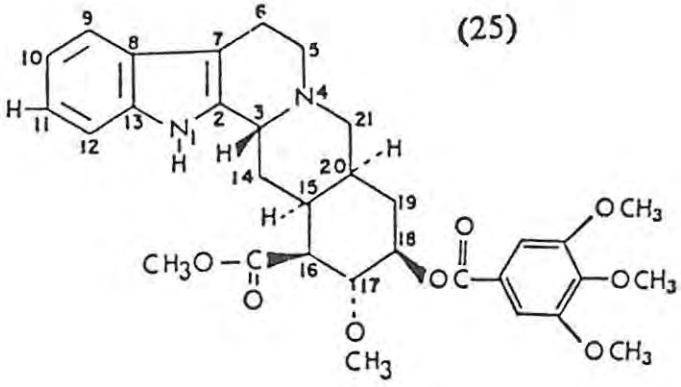

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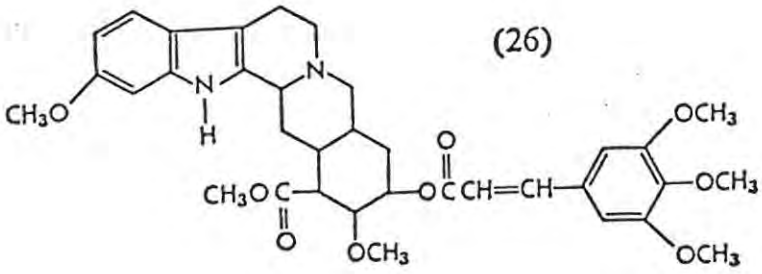

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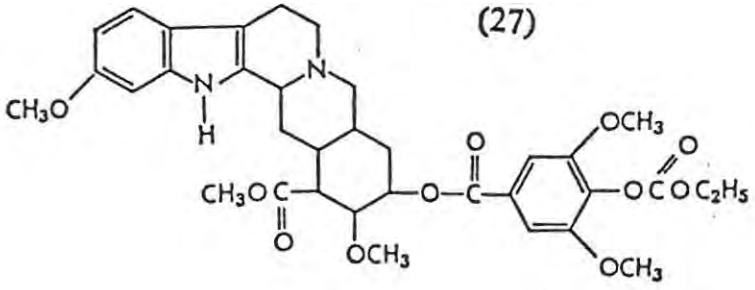
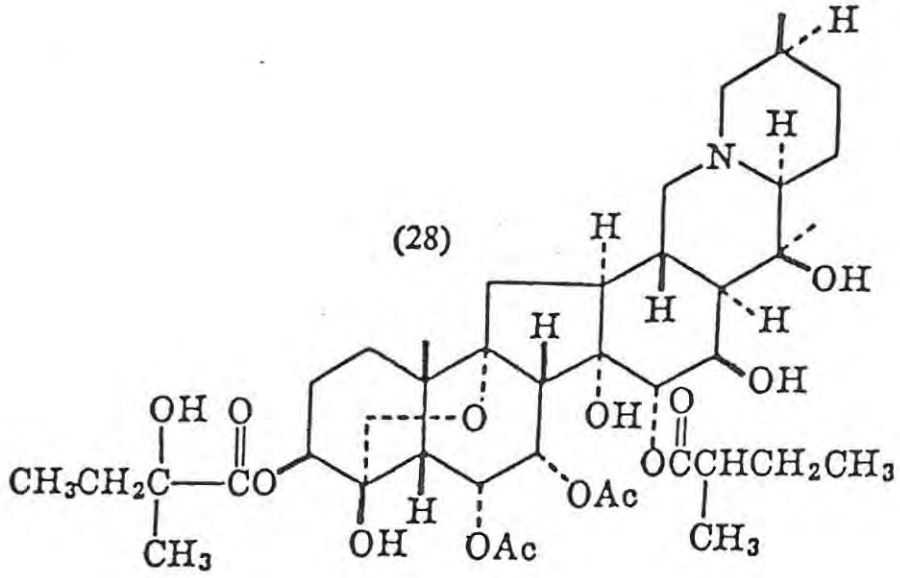


(27)

(28)



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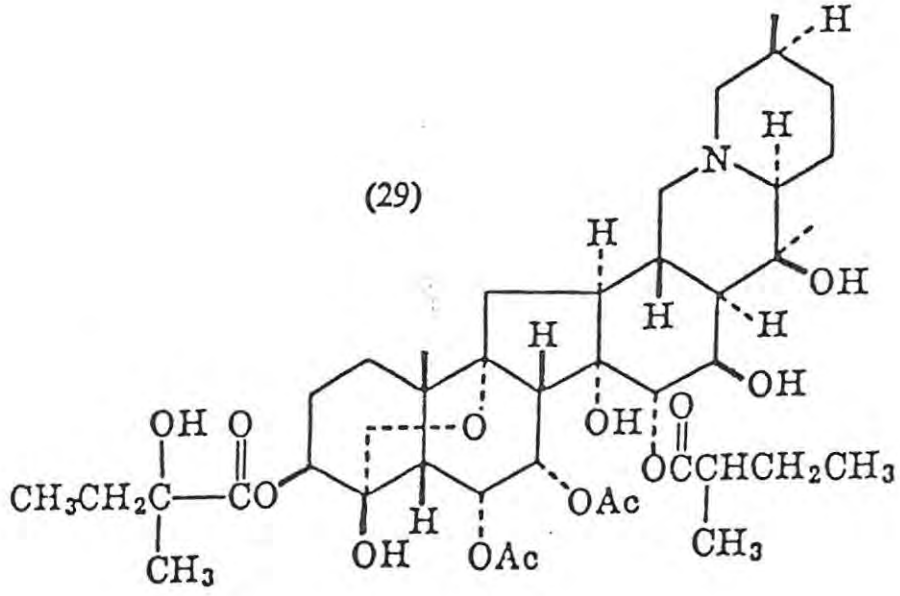


CHAPTER TWO

THE EUPHORBIACEAE

2.1 General Discussion

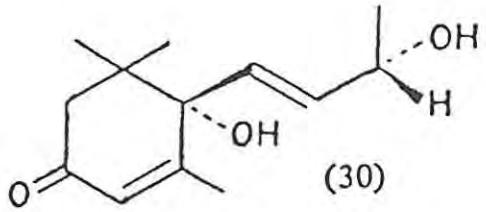
The family Euphorbiaceae is comprised of over 300 genera and species over 5000, mostly natives of the tropics of both hemisphere.[19] The family has been divided into a number of tribes, the largest being the genus Euphorbia which comprises over 2 000 known species. There are close to 200 Euphorbia species in South Africa with a wide distribution.[20]

Plants of the family Euphorbiaceae are used in traditional medicine for different purposes : against fever, as diuretic agents, mouth-wash, treatment of jaundice, gonorrhea, as chewing sticks and as purgatives.[21,22] Some are poisonous and others are used as analgesics and antihypertensive agents.[20,22,23,24] Chemical and pharmacological investigations had been undertaken in some genera. The genera in which investigations were undertaken include; Andrachne, Acalypha, Alchornea, Antidesma, Cleistanthus, Croton, Drypetes, Excocaria, Euphorbia, Fluggea, Jatropha, Macaranga, Maprounea, Phyllanthus, Pterococcus, Securinega, Sapium, Spirostachys, Synadenium and Tragia.

In the genus Croton, investigations were done in Jamaica, India, Italy, Brazil and Venezuela. Species of Croton have yielded alkaloids of the aporphine, proaporphine and morphinandienone type.[15,25] Vomifoliol (30) an antihypertensive agent, was found to be the constituent of Croton lobatus and C. trinitatis.[23]

In Jamaica work has been done on C. lobatus, C. trinitatis, C. humilis, C. linearis and C. plumeri. Analgesics of the isoquinoline group (see fig. 1) were found to be constituents of the genus Croton. In C. flavens, two morphinandione alkaloids, flavinine (31) and flavinantine (32) were isolated while C. Linearis was shown to contain at least eight chloroform soluble alkaloids viz, crotonosine (33), pronuciferine (34) jacularine (38), linearisine (37), L-N-methylcrotonosine (35), 8,14-dihydrosalutaridine (40), 8,14-dihydronorsalutaridine (41) and an unnamed base (39).[26,27] C. flavens growing in the Port Henderson area was shown to have a different alkaloid content for the serrate leaf variety than for the entire leaf type.[10] Norsinoacutine (42) and flavinine (31) were isolated from the serrate leaf type, while norsinoacutine(42), sinoacutine (43)

/and.....



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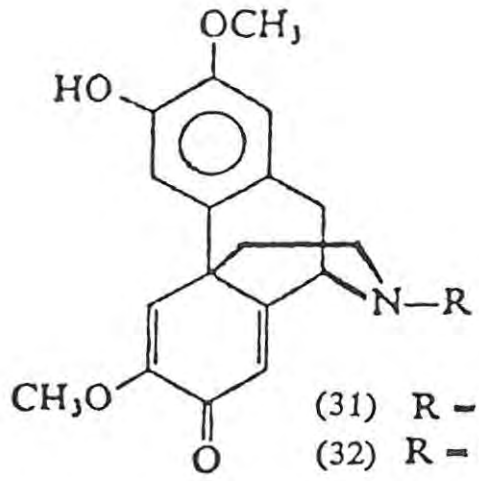
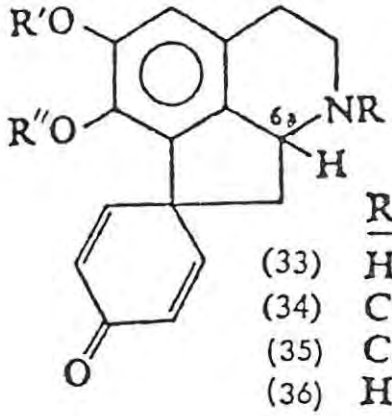
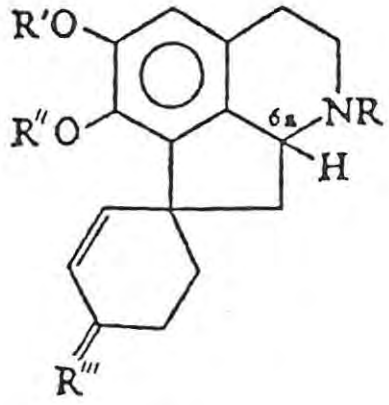


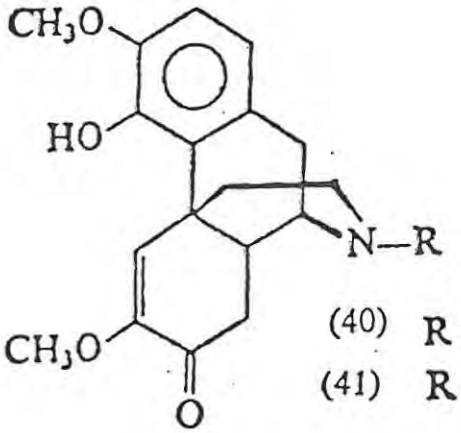
(31) R = H
(32) R = CH_3



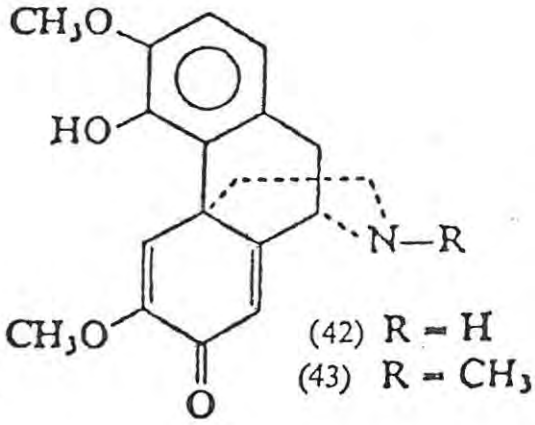
	R	R′	R″	6a Configuration
(33)	H	H	CH_3	R
(34)	CH_3	CH_3	CH_3	R
(35)	CH_3	H	CH_3	S
(36)	H	CH_3	H	S



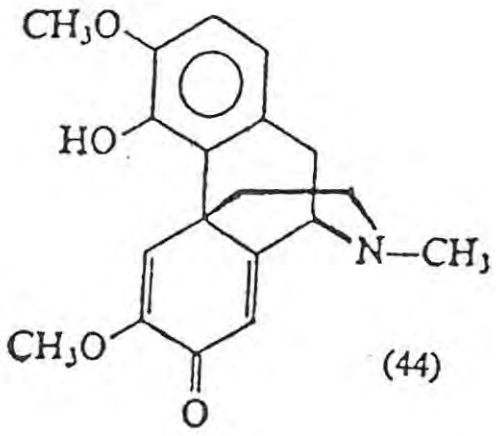
	R	R′	R″	R‴	6a Configuration
(37)	CH_3	H	CH_3	O	S
(38)	CH_3	CH_3	H	H, OH	R
(39)	H	CH_3	H	O	R



(40) R = CH_3
(41) R = H



(42) R = H
(43) R = CH_3



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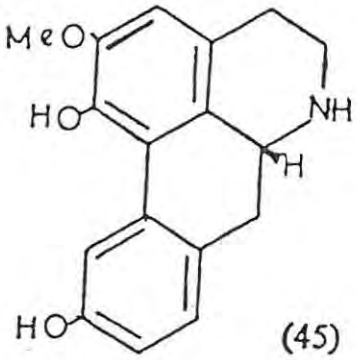

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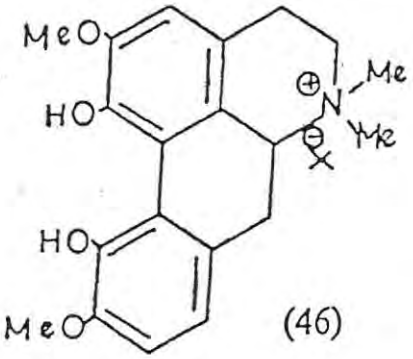

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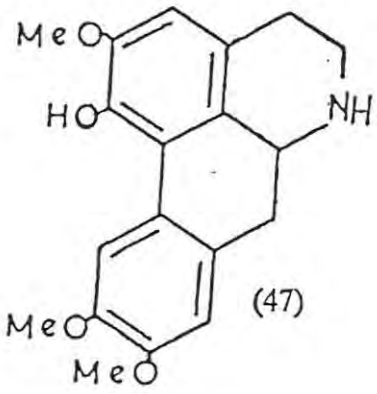

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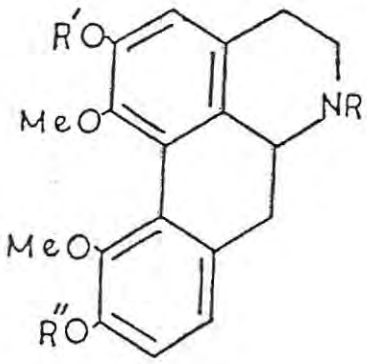
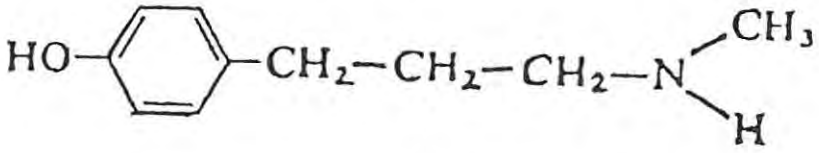


(48) R=R'=R"=H

(49) R=Me, R'=R"=H

(50) R=R'=H, R"=Me

(51) R=R"=Me, R'=H



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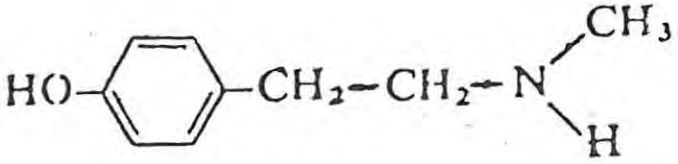

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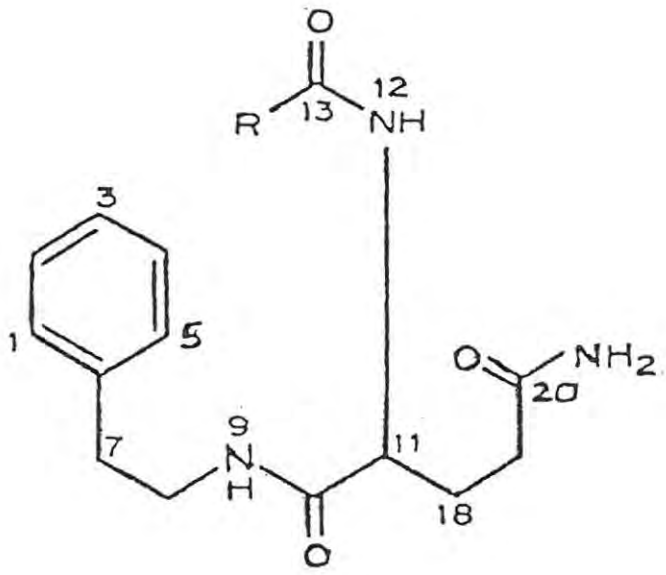
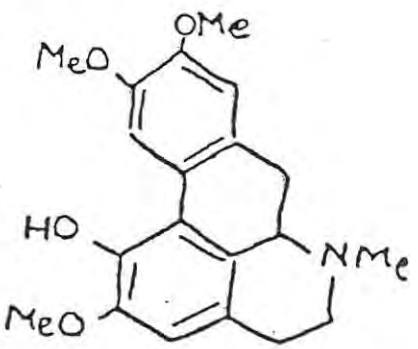


(54) R = —CH(CH3)—CH2CH3

(55) R = —CH(CH3)—CH3



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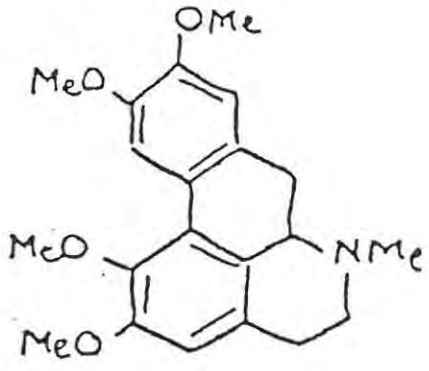

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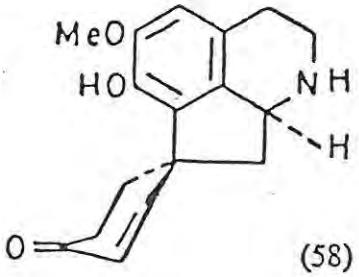

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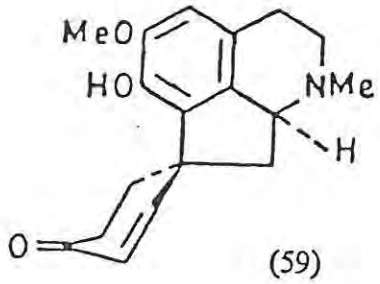

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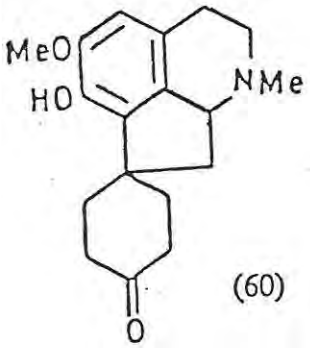

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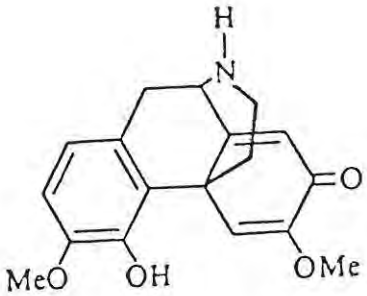

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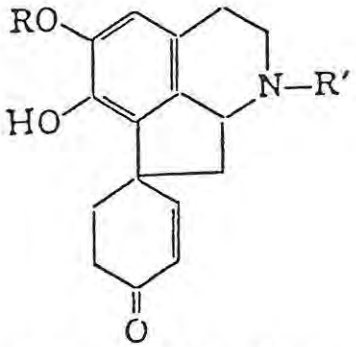
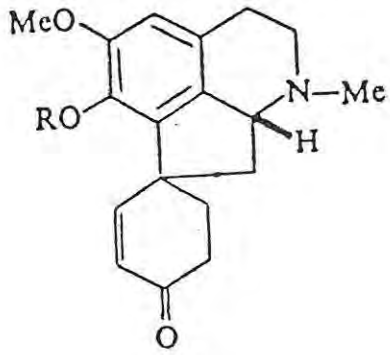


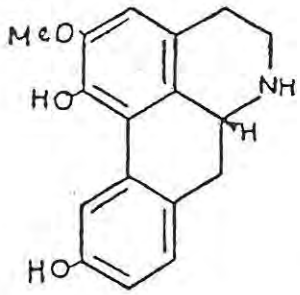
(64), R = R′ = Me

(65), R = Me; R′ = H



(62) R = H

(63) R = Me



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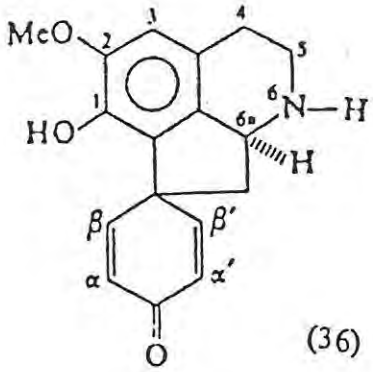

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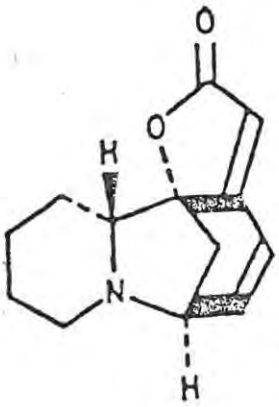

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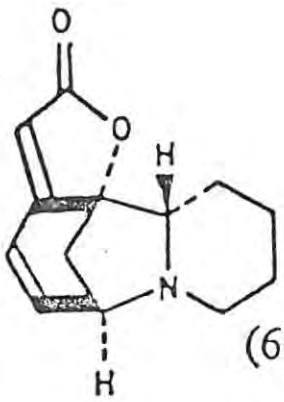

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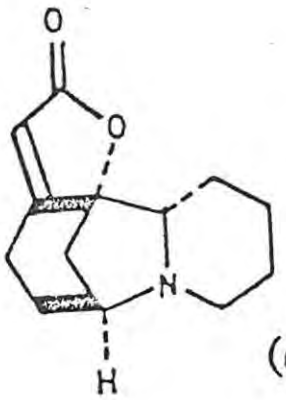

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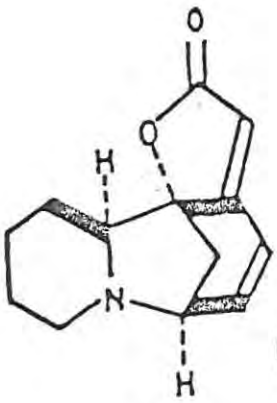

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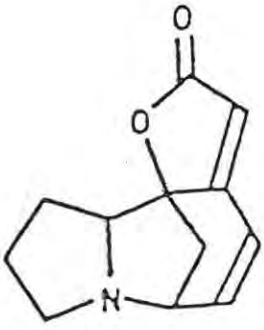

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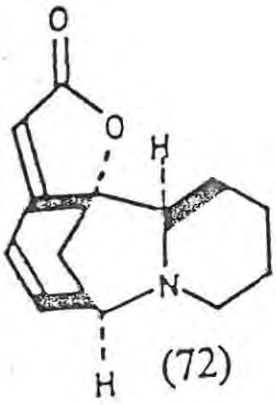

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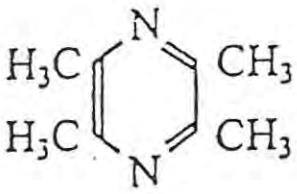


(73)

and flavinantine (32) were found in the entire leaf part. C. plumeri collected from Port Henderson was shown to contain crotonosine (36), L-N-Methylcrotonosine (35), salutaridine (44) and 8,14-dihydrosalutaridine (40).[28] From C. sparsiflorus and C. cumingii two aporphine alkaloids Sparsiflorine (45) and magnoflorine (46) were respectively isolated.[29] Alkaloidal constituents of C. humulis were found to be N-methyltyramine (52), N-methylhomotyramine (53) and two peptidyl alkaloids (54) and (55).[30] Five phenolic aporphines viz. wilsonirine (47), hernovine (48), N-methylhernovine (49), 10-O-methylhernovine (50) and N.methyl-10-O-methylhernovine (51) were isolated from C. wilsonii [29] whilst C. balsamifera contained norsinoacutine and saturaridine.[31]

Thaliporphine (56) and glaucine (57) alkaloids with wound healing activity, have been isolated from Italian C. draconoides.[32] Chemical investigation of C. sparsiflorus showed the presence of three alkaloids : isocrotsparine (58), its N-methyl derivative (59) and tetraidoglaziovine (60).[33] No conclusive structures were obtained for the quarternary alkaloids obtained from Venezuelian C. speciosus, C. xanthochlorus, C. rhamifolius and C. turumiquirensis species.[34]

Chemical investigations of Brazilian C. Salutaris led to the characterization of three alkaloids salutarine (44) salutaridine (44) and N-norsalutaridine (61).[35] An antihypertensive agent, N-methylcrotsparine (62), has been isolated together with crotsparinine (65), N-methylcrotsparinine (64) and sparsiflorine (66) from Indian C. sparsiflorus.[24]

In the genus Securinega work was done in Japan and Russia.[36,37,38] Chemical investigation of Japanese Securinega species reveal pyrrolidine alkaloids to be constituents of the plant species. Securinine, $C_{13}H_{15}NO_2$, (67) was first isolated from Russian Securinega suffruticosa.[37] From Japanese S. virosa pyrrolidine alkaloids : securinine (67), virosecurinine (68), allosecurinine (70), dihydrosecurinine (69), viroallosecurinine (72) and norsecurinine (71) were isolated.[36,37,38]

Plants of the genus Jatropha are known to be very useful in traditional medicine.[21] There are fifteen known species of Jatropha, 8 of which are found in West Africa and 5 in Nigeria.[39] Those found in Nigeria are J. curcas L, J. gossypiifolia L., J. multifida L, J. chevalieri Beille and J. podagrica. J. podagrica Hook is a common shrub found all over West Africa. Its roots, stems, seeds and fruits have been widely used in

/traditional.....

traditional folk medicine in many parts of West Africa. In Nigeria, for example, the Hansas of the former Northern Region usually use the seed oil as an ingredient in the treatment of rheumatic conditions, itch and parasitic skin diseases; whilst the Yoruba-speaking people of the old Western Nigeria usually use the plant in the treatment of fever, as a diuretic agent, as a mouth-wash and chewing stick.[39] Chemical and Pharmacological investigations of stems of J. podagrica revealed the presence of an amide alkaloid, tetramethylpyrazine (73) with neuromuscular, cardiovascular, antibacterial and antihypertensive properties.[21,39]

Examination of the alkaloids of Alchornea javanensis, a small tree of the New Guinea rain forest, has led to a still further extension of the known types of alkaloids of the Euphorbiaceae. Both bark and leaves of A. javanensis contain alkaloids, and two of the alkaloids have been shown to belong to an entirely new class, the hexahydro [1,2-α] pyrimidines (see fig. 1), while two other alkaloids are substituted guanidines.[40,41] The two new hexahydro[1,2-α]pyrimidine alkaloids isolated from A. javanensis are alchornine (74) and alchornidine (75). The other constituents isolated from A. javenensis include two new guanidine alkaloids, N-1, N-1-di-isopentenylguanidine (76) and N-1,N-2,N-3-tri-isopentenylguanidine (70).
From a member of the Euphorbia, E. atoto,[41] the novel alkaloid (+)-9-aza-1-methylbicyclo[3,3,1]nonan-3-one (78) has been isolated.

The genus Phyllanthus was investigated in Nigeria, India and Hong Kong.[21,22,23,25,26,42] Pyrrolidine alkaloids, securinine and allosecurinine, were isolated from Nigerian P. discoides.[43,44]

Not only alkaloids were found to be constituents of the Euphorbiaceae. Lignans, tannins, diterpenes, triterpenes and steroids were also isolated from Euphorbiaceae.[3,13] Some of these natural products were found to be useful for medical purposes.[20,21,45,46,47]

Most species from the genus Euphorbia, which comprises over 2000 species,[13] contain triterpenes and steroids[20,48,49,50] Some triterpenes which have been isolated from the latex of various Euphorbia species are :- obtusifoliol from E. obstusifolia, E. bravoana, E. regis-jubae and E. echinus; germanicol from E. balsamifera; cycloartenol from E. bravoana

/and.....

and E. balsamifera; epigermanicaol, for the first time from natural sources, from E. candelilla var luxurians; friedelin, ß-amyrin and ß-sitosterol from E. hirta var procumbens; taraxerone and taraxerol from E. pilulifera and E. antiquorum; taraxerol from E. royleana; epifriedelanol from E. antiquorum; lanostenol from E. regis-jubae; lanosterol from E. balsamifera and E. regis-jubae; lupeol from E. mauritanica.[20]

Work done on the genus Bridelia revealed triterpenes and steroids to be constituents of B. micrantha, B. monoica and B. moonii species[51,52,53] whilst work done on the genus Cleistanthus disclosed that diterpenes are constituents of C. schlechteri.[54,55]

A survey of Chemical abstracts from 1957 to 1983 showed that no work of a chemical nature had been carried out on any of the following genera : Adenocline, Cavacoa, Caperonia, Clutia, Cephalocroton, Ctenomeria, Jabechampia, Erthrococca, Heywoodia, Hymernocardia, Hydrenanchre, Lachanostylis, Leidesia, Micrococca, Monadenium, Scidelia, Suregada and Sphaerostylis.

2.2 Extractives from the genus Antidesma :

Three species of Antidesma have thus far been investigated. No alkaloids were present in any of these. Two diuretic tripenoids, 16∝-hydroxy-3-ketois-omultiflorene (79) and 3-ß-hydroxy-16-ketoisomultiflorene (80) have been isolated from the hexane extract of Indian A. manasu[47] and friedelin, tritriacotane, canophyllal, canophyllol and 3-keto-16∝-hydroxyfriedelane (81) from the ether extract.[45] A hypolipidemic substance, lupeolactone (83) has been isolated from Japanese A. pantandrum.[46] A. diandrum seeds have yielded a mixture of fatty acids which were found to consist of linolenic, linoleic and oleic acids and a number of unidentified saturated acids.[56,57] The following species of Antidesma are also found in Southern Africa but have not been chemically investigated:- A. natalensi HARV, A. boiviniamum BAILL, A. rufescens TUL, A. membranaceum var. molle MUELL ARG, A. venosum De Wild, A. sassandrae BEILLE and A. fusco-cinerea BEILLE.[42]

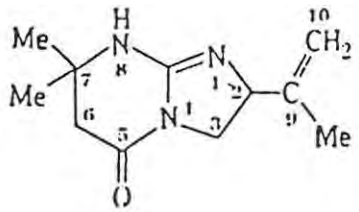
The structures of 16∝-hydroxy-3-ketoisomultiflorene (79) and 3ß-hydroxy-16-ketoisomultiflorene (80) rest chiefly on ^{1}HNMR and M.S. evidence. In addition, the keto group in (79) was removed by Huang-Minlon reduction. The equatorial ∝-orientation of the remaining hydroxyl group in the product

/rests.....

rests on its oxidation with Corey's reagent to the ketone followed by regeneration on reduction with sodium in isoamyl alcohol, a method known to favour production of equatorial∝-alcohols.[58] This method, when applied to a diketone (82), obtained by oxidation of 3-keto-16∝-hydroxyfriedelane, yielded a diol with similar properties to the one obtained from reduction of 3-keto-16∝-hydroxyfriedelane. This indicated the equatorial ∝-orientation of the hydroxyl group.[45]

Only spectroscopic and X-ray crystallographic data were used in determining the structure of lupeolactone (83). Infra-red data showed the presence of a ß-lactone moiety (1810cm^{-1}) and of a vinylidene group (1635 and 880cm^{-1}). The ^{1}HNMR spectrum indicated the presence of five tertiary methyl groups and an isopropenyl group.[46] The ^{13}cnmr spectrum confirmed the presence of a ß-lactone moiety and a vinylidene group. These spectral data suggested that the compound is a lupane triterpenoid having a ß-lactone moiety. This was supported by comparing the ^{13}CNMR spectra of the compound and of lupeol (84).[46] The chemical shifts of the signals are very similar, (see Table 1) except for the signals due to C-4, C-23, C-24 and C-25 which suggest that a ß-lactone moiety is present. The moiety was placed as shown because of replacement of the C-24 signal at δ15,3 in lupeol by a signal at δ175,5. In addition the C-4 suffered a downfield shift from δ38,9 in lupeol to δ5,4 in lupeolactone. From this spectroscopic evidence the structure of lupeolactone was assigned.

The above structure was confirmed by single crystal X-ray crystallographic analysis.[46]



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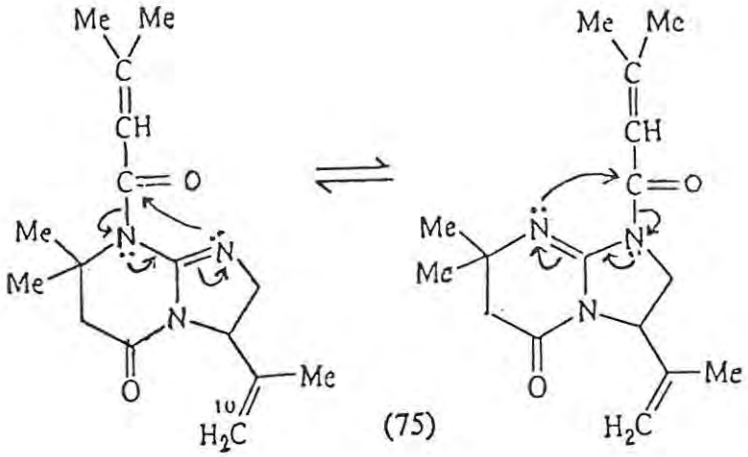

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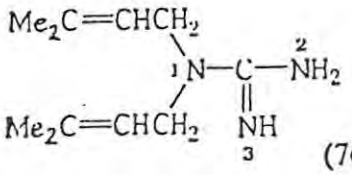

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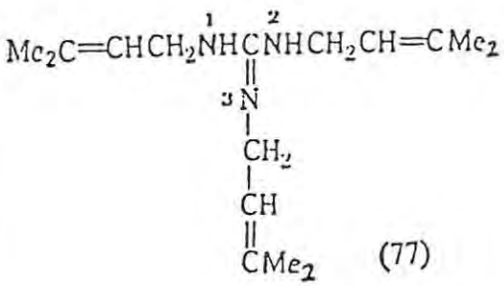

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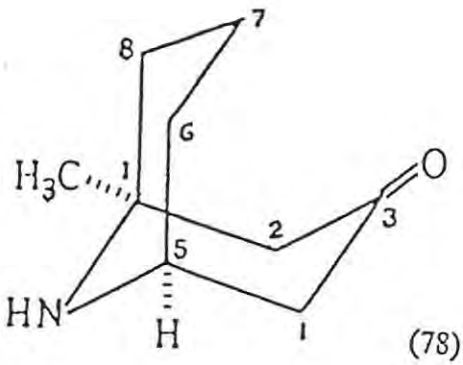

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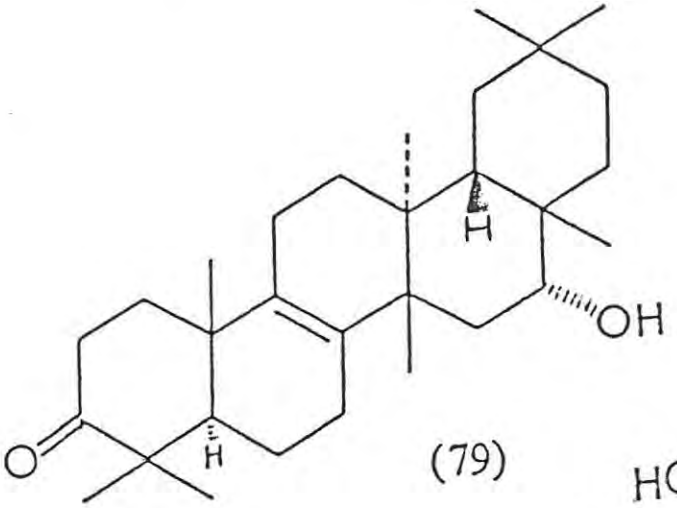

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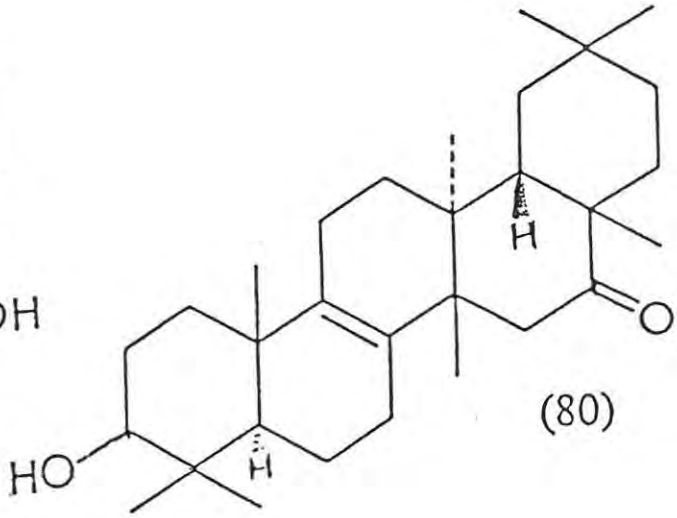

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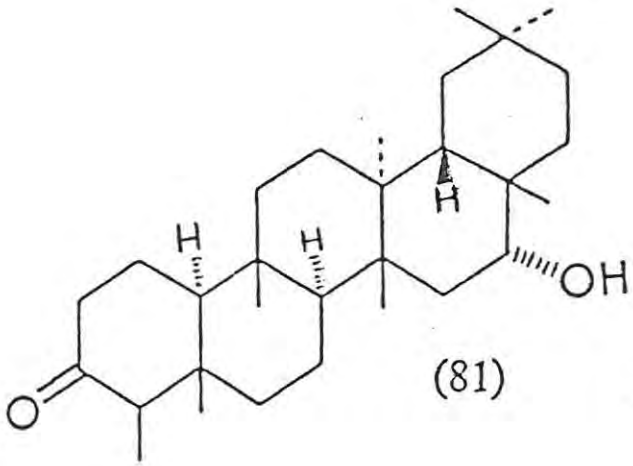

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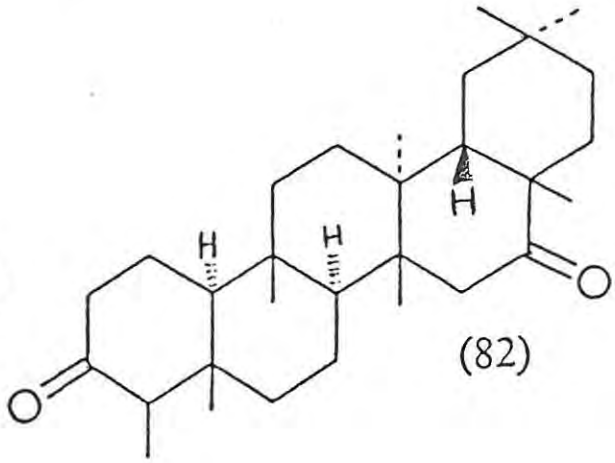

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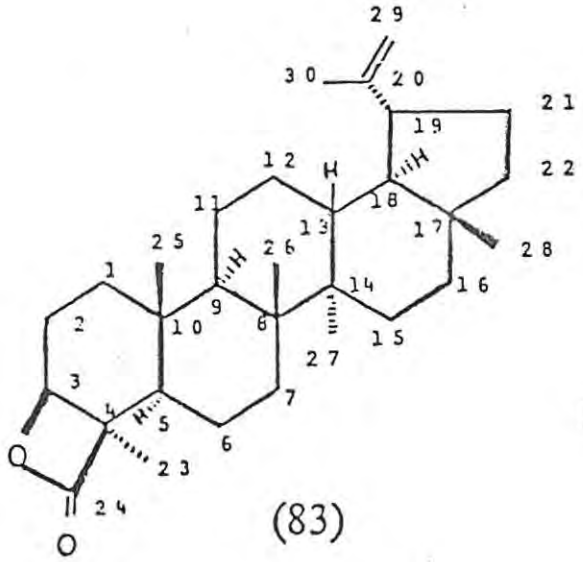

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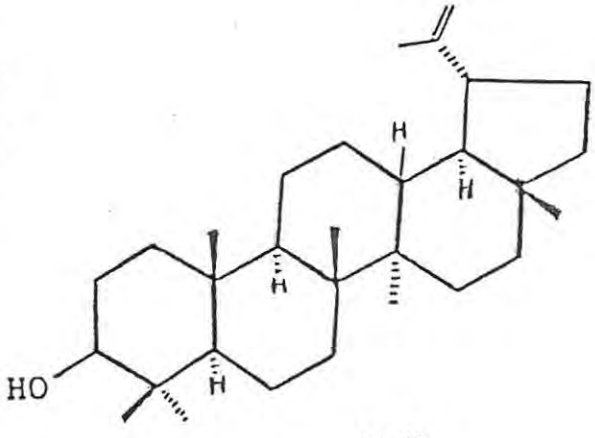


(84)

TABLE 1 Substance (1) = lupeolactone (83)[47]
Substance (2) = lupeol (84)[47]

Number of Carbon	Substance (1)	Substance (2)	Number of Carbon	Substance (1)	Substance (2)
C-1	36.8	38.7	C-16	35.6	35.6
C-2	23.7	27.5	C-17	43.0	43.0
C-3	77.3	79.0	C-18	48.0	48.0
C-4	55.4	38.9	C-19	48.2	48.3
C-5	52.7	55.3	C-20	150.7	150.7
C-6	19.4	18.3	C-21	29.8	29.9
C-7	33.4	34.3	C-22	40.0	40.0
C-8	40.9	40.0	C-23	22.8	28.0
C-9	47.9	50.5	C-24	175.5	15.3
C-10	35.6	37.2	C-25	12.7	16.1
C-11	21.1	21.0	C-26	16.1	16.0
C-12	25.1	25.2	C-27	14.4	14.6
C-13	37.9	38.1	C-28	18.0	18.0
C-14	43.0	42.9	C-29	109.3	109.3
C-15	27.3	27.5	C-30	19.2	19.3

CHAPTER 3

EXPERIMENTAL

3.1 Introduction

All melting points were determined on a Kofler micro-stage apparatus. The optical rotations were recorded on a Perkin-Elmer 141 polarimeter, chloroform being used as solvent for antidesma III and IV and pyridine for antidesma I. The infra-red spectra were recorded either on a Phillips SP3-100 or a Perkin Elmer 180 infra-red spectrophotometer using KBr discs.

^1H nuclear magnetic resonance spectra were recorded using the proton probe of a Brücker spectrometer at 80 MHz, while the carbon probe of the same instrument was used to record the ^{13}CNMR spectra at 20 MHz. Spectra of antidesma III and IV were recorded in deuterochloroform and that of antidesma I in deuterated dimethyl sulphoxide. The δ-values (ppm) are relative to TMS ($\delta = 0$) and were measured from the central peak of the chloroform singlet (δ 7,24) for proton spectra and from the chloroform triplet (δ 77,09) for carbon spectra. Where deuterated dimethyl sulphoxide-d_6 was used the δ - values were relative to the DMSO multiplet (δ 2,49) for proton spectra and (δ 39,5) for carbon spectra.

The high field ^1H spectra on Brücker 360 MHz and mass spectra were kindly provided by the CSIR in Pretoria.

3.2 Chromatographic techniques

3.2.1 Thin Layer Chromatography

TLC analysis was conducted using pre-coated 0,2mm thick silica gel $60F_{254}$ sheets (Merck art. 5714) in chloroform and in hexane-ethyl acetate solutions of variable proportions. The plates were developed by spraying with 5% H_2SO_4(v/v) in methanol and drying at 110°C for three minutes. The spots were also visualized using an ultra.violet lamp before spraying.

3.2.2 Preparative thin layer chromatography

Plates, 20 x 20cm and approximately 1,5mm thick, prepared from silica gel $60F_{254}$ without flourescent indicator, Merck art. 7747, were used.

/3.2.3.....

3.2.3 Column Chromatography

Two different techniques were used for column chromatography. For most separations, open columns containing silica gel 60, grain size 0,063 - 0,2mm (70-230 mesh ASTM, Merck art. 7734) were used, in the approximate ratio of 35g to 1g extract. Alternatively, flash chromatography[59] was employed. These fast columns require silica gel 60, particle size less than 0,063mm (finer than mesh ASTM, merck art. 7729). For the open columns and most of the fast columns mixtures of hexane, benzene, ethyl acetate and chloroform in various proportions were used.

3.3 Extraction of the roots of A. venosum

The dried roots, supplied by Noristan Laboratories, Pretoria, were extracted first with hexane and then with ethanol.

3.3.1 Hexane extract of the roots

Dry, powdered roots (5,0 Kg) were continuously extracted with hexane in a soxhlet apparatus for 24 hours. Crystals formed when the extract was allowed to stand. They were separated by decantation, dissolved in refluxing chloroform containing activated charcoal and the solution filtered. Crystallization from chloroform-methanol afforded colourless needles (0,25g), m.p. 293-295°C, of antidesma I. TLC with chloroform showed that this product consisted of a single substance.

m.p.	293 - 295°C		
Optical Rotation	$[\alpha]_D^{22°C}$	= +6,08°	(c 1,0 in pyridine)
Mass :	Found M^+	= 456,358	
	$C_{30}H_{48}O_3$ requires	456,360	
Analysis	%C = 78,71 , %H = 10,81 , %O = 10,48		
	$C_{30}H_{48}O_3$ requires	%C = 78,90	%H = 10,59
	%O = 10,51		

/Infra-red.....

Infra-red spectrum (Spectrum 1, p64)

max^{KBr} :	3420 cm^{-1}	(OH group)
	1680 cm^{-1}	(C = O group)
	1640 cm^{-1}	(C = C)
	880 cm^{-1}	(out of plane C-H bending of olefins).

Mass Spectrum (Spectrum 8, p71)

m/e : 456 (M^+), 438 (7%), 423 (5,7%), 410 (4,6%), 395 (5,5%), 356 (1,1%), 302 (5,7%), 283 (1,3%), 248 (56,6%), 220 (29,2%), 207 (55,5%), 203 (36,8%), 202 (12,2%), 190 (38,6%), 189 (100%), 175 (36,1%), 174 (5,9%), 1273 (19%), and 109 (39%).

^{1}HNMR

Spectrum 13 (All chemical shifts, p76)

^{13}CNMR

Spectrum 17 (All chemical shifts, p87 for spectrum and table 3,p47).

The hexane mother solution remaining after the removal of the crude (antidesma I) was evaporated and the residue (16g) chromatographed on Merck Silica gel 60 (100g of silica gel was used for every 3g) eluting with different solvent systems.

Fraction No.	Eluting solvent	Vol. of Eluate (ml)	Mass (g)
1	hexane	2 050	4,0
2	hexane-benzene (4:1)	700	0,50
3	hexane-benzene (4:1)	525	0,20
4	hexane-benzene (4:1)	1 525	1,2
5	benzene	800	0,1
6	benzene-chloroform - ethyl acetate (8:1:1)	900	0,08
7	Ethanol	500	0,05

/Fraction 1.....

Fraction 1

TLC (ethyl acetate was used as solvent) indicated that it consisted chiefly of a single substance and was further purified by repeated column chromatography (hexane was used as solvent) followed by preparative TLC which was performed as follows :- 100mg fractions, dissolved in hexane, were applied to the plates which were developed in hexane-benzene (1:1). Side strips were visualized either by spraying with 5% (v/v) sulphuric acid in methanol spray reagent and heating at 110°C or using an ultra-violet lamp. Homogeneous strips of the major product were scraped off the plate and extracted with chloroform. Attempts to crystallize the substance (2,2g) using different solvents were unsuccessful. This substance was named antidesma II.

Fraction 2

This fraction was shown by TLC (chloroform used as solvent) to contain one main constituent and was further column chromatographed on Merck Silica gel 60 (20g), eluting with hexane-benzene (1:1). The solvent was removed and the residue recrystallized from methanol to constant melting point, 82-84°C. Yield 0,20g. The compound was called antidesma III.

Mass :- Found M^+ = 368,31

$C_{24}H_{48}O_2$ requires 368,365

Analysis %C = 78,05 %H = 13,3 %O = 8,82

$C_{24}H_{48}O_2$ requires

%C = 78,20 %H = 13,12 %O = 8,68

Infra-red spectrum (spectrum 3, p66)

ν_{max}^{KBr} : 3430 cm^{-1} (OH group)

1700 cm^{-1} (C = O group)

Mass spectrum (spectrum 10, p73).

/The following.....

The following fragment ions were observed :-

m/e 368,31 (M^+), 311 (1,6%), 297 (2,6%),
241 (3,5%), 213 (3,5%), 185 (7,8%),
183 (1,2%), 169 (1,2%), 155 (1,4%), 141 (2,4%),
129 (20,8%), 127 (3%), 113 (4,3%), 99 (7,4%),
87 (23,9%), 85 (25%), 73 (42,5%),
71 (43,4%), 60 (38%), 59 (8,3%), 57 (82,3%),
45 (4,1%), 43 (100%), and 29 (28,4%).

^{1}HNMR

spectrum 14A, B and C (All chemical shifts, p77-79)

^{13}CNMR

spectrum 18 (All chemical shifts, p88).

Fraction 3

Further chromatography on Merck Silica gel 60 (20g) and elution with hexane afforded a further 0,05g of antidesma III, m.p. 83-85°C. Continued elution with hexane-benzene (4:1) eluted antidesma IV (0,03g) which was crystallized from ethanol, m.p. 137-138°C.

Fraction 4

TLC of the crude collected product indicated the presence of one compound (benzene was used as solvent). Further purification by flash chromatography[72] on Silica gel 60 in chloroform afforded 0,72g of antidesma IV, m.p. 135-138°C (crystallized from ethanol).

Analysis and physical properties of antidesma IV are as follows :-

m.p. 135 - 138°C

Optical Rotation $[\alpha]_D^{21°C}$ = -43,5° (c 1,0 in chloroform).

Mass Found M^+ = 414,386

$C_{29}H_{50}O$ requires 414,386

/Analysis.....

Analysis : %C = 86,26 %H = 12,00 %O = 1,74

$C_{29}H_{50}O$ requires

%C = 84,06 %H = 12,17 %O = 3,77

Infrared spectrum (spectrum 4, p67)

max^{KBr} : 3420 cm^{-1} (broad, OH group).

Mass spectrum (spectrum 11, p74)

The following fragment ions were observed :-

m/e 414 (M^+), 399 (6,3%), 396 (11,7%), 381 (6,6%), 329 (14,2%), 303 (9,7%), 275 (3%), 273 (10,8%), 271 (10%), 255 (19,6%), 231 (10,9%), and 213 (19%).

^{1}HNMR

Spectra 15 A.B.C. and D (All chemical shifts, p80-83) and table 7, p57.

^{13}CHMR

Spectrum 19 (All Chemical shifts, p89) and table 9, p58.

Fraction 5

TLC (chloroform used as solvent) indicated the presence of two compounds. The residue from evaporation of the solvent was chromatographed on silica gel 60 (20g), eluting with benzene-hexane (9:1) and chloroform. The benzene-hexane (9:1) eluate afforded 20mg of antidesma IV, m.p. 136-138°C. The chloroform eluate was pure by TLC (chloroform used as solvent) and crystallized from methanol to give a further 15 mg of antidesma I, m.p. 292-294°C.

Fraction 6

This fraction after chromatography on silica gel 60 (20g) in chloroform yielded more of antidesma I (30mg), m.p. 290-294°C.

/Fraction 7.....

Fraction 7

TLC (ethanol used as solvent) showed the presence of one compound which was purified by chromatography on a preparative TLC plate to afford 20mg of antidesma V, m.p. 272-276°C, after crystallization from ethyl acetate.

Optical Rotation $[\alpha]_D^{21°C}$ = -52° (c 1,0 in DMSO).

Infra-red spectrum (spectrum 6, p69)

max^{KBr} : 3410cm^{-1} (OH group).

3.3.2 Ethanol extract of the roots

After evaporation of the solvent from the hexane extracted root material, an ethanol extraction was carried out for 24 hours at reflux temperatures. Ethanol was removed from the extract under reduced pressure, the residue shaken with 5% H_2SO_4 (v/v) (750ml), and the aqueous layer extraced continuously with chloroform overnight. The chloroform extract was labelled the acidic chloroform extract. The aqueous layer was then basified with NH_4OH and extracted continuously with chloroform until clear. This chloroform extract was labelled the basic chloroform extract.

A portion (0,2g) of the residue from this extract was stirred with 5ml of 1%HCl on a steam bath for two minutes. The hot solution was filtered. When 3 drops of Meyer's reagent was added to 1 ml of this solution no precipitate formed.

The aqueous layer was discarded.

3.3.2.1 Basic chloroform extract

The residue (1,0g) from this extract was chromatographed on silica gel 60 (30g) using chloroform as solvent. A yellow liquid (100mg) was collected. It's R_f value was the same as that of antidesma II.

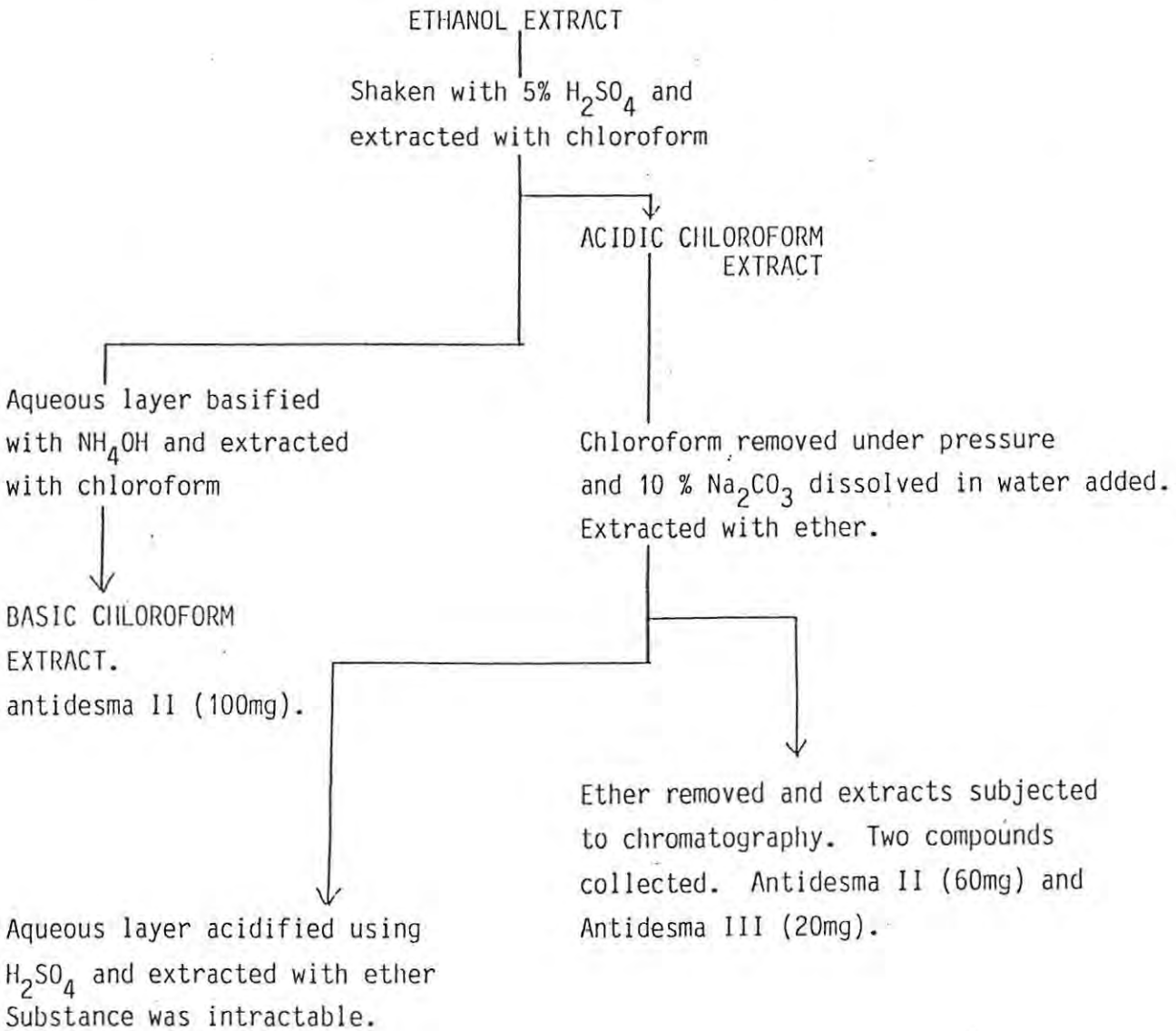
/3.3.2.2.....

3.3.2.2 Acidic Chloroform extract

Chloroform was removed from the acidic chloroform extract and the residue (3,0g) shaken with 10% Na_2CO_3 and extracted with ether until the ether layer became clear. The ether extract was evaporated and the residue (1,0g) subjected to silica gel 60 (35g) chromatography, eluting with hexane and with hexane-benzene (4:1). The hexane eluate was purified by preparative TLC to afford 60mg of antidesma II. The hexane-benzene (4:1) eluate (100mg) was chromatographed on silica gel 60 (20g) using chloroform as solvent, to give 20mg of antidesma III, m.p. 82.84°C, after crystallization from methanol.

The aqueous Na_2CO_3 layer was acidified with H_2SO_4 and extracted several times with ether. The ether extract was evaporated to afford 1,1g of residue which could not be further purified.

The following scheme summarises the separation procedures :



/Details.....

DETAILS OF ROOTS EXTRACT FROM 5,0kg OF A. VENOSUM ARE GIVEN BELOW

Compound	Amount Collected	Yield
antidesma I	0,29g	0,0059%
antidesma II	2,22g	0,044%
antidesma III	0,27g	0,0054%
antidesma IV	0,77g	0,015%
antidesma V	0,02g	0,0004%

3.4 Liebermann-Burchard Test on antidesma IV[60]

A solution of acetic anhydride-sulphuric acid (30:1) was prepared by mixing 30 ml of acetic anhydride and 1 ml sulphuric acid. 2 ml of this mixture was added to 2 ml of a solution of antidesma IV in chloroform (2mg ml^{-1}). When the mixture was shaked a green colour was produced.

3.5 Acetylation of antidesma IV

Method 1[61]

A mixture of acetyl chloride (25ml) and antidesma IV (20mg) was refluxed overnight. The remaining acetyl chloride was evaporated under reduced pressure and the product crystallized from methanol to afford 14mg of product, m.p. 125°C.

/Method 2.....

Method 2[7]

Antidesma IV (10mg), pyridine (1 ml), acetic anhydride (3 ml) and 2 mg of 4-(dimethylamino)pyridine were refluxed for 2 hours. Methanol was added to destroy any residual acetic anhydride and the mixture evaporated under reduced pressure. Toluene was added in 5 ml portions and the solution again evaporated under reduced pressure until all traces of pyridine had been removed. The residue was purified by preparative TLC and crystallized from methanol to provide colourless needles (8 mg), m.p. 126°C.

Optical Rotation $[\alpha]_D^{22°C}$ = -41,5° (c 1,0 in chloroform)

Mass : Found M^+ = 456,44

$C_{31}H_{52}O_2$ requires 456,78

Analysis : %C = 80,53 %H = 11,42 %O = 8,05

$C_{31}H_{52}O_2$ requires

%C = 81,52 %H = 11,48 %O = 7,00

Infra-red Spectrum (spectrum 5, p68)

max^{KBr} : $1722cm^{-1}$ (C = O group)

Mass spectrum (spectrum 12, p75)

The mass spectrum exhibited the following peaks :-

m/e 396 (32,2%), 354 (1,5%), 288 (8%),
255 (14%), 213 (8,3%), 147 (39,4%),
81 (41,5%), and 43 (100%).

^{1}HNMR

Spectra 16 A,B and C (All chemical shifts, p84-86)

^{13}CNMR

Spectra 20 A and B (All chemical shifts, p90-92) and table 10, p59.

/3.6.....

3.6 Acetylation of antidesma V[61]

10mg of antidesma V was refluxed overnight with 10ml of acetyl chloride. The remaining acetyl chloride was removed under reduced pressure and the residue crystallized from methanol to give 7,7mg of colourless needles, m.p. 159-161°C.

Infra-red spectrum (spectrum 7, p70)

max^{KBr} : $1760cm^{-1}$ (C = O group)

3.7 Attempted esterification of antidesma I using diazomethane[62]

Excess ethereal diazomethane was slowly added to an ice-cold solution of 25mg antidesma I in butanol-ether until the yellow colour persisted. The solution was left at 0°C overnight. The solvent was evaporated under reduced pressure and the residue crystallized from methanol to afford 22mg of colourless needles, m.p. 199-200°C.

Infra-red spectrum (spectrum 2, p65)

max^{KBr} :	$3450cm^{-1}$	(OH group)
	$3420cm^{-1}$	(OH group)
	$2210cm^{-1}$	(tripple bond)
	$1690cm^{-1}$	(C = C)
	$880cm^{-1}$	(out of plane C-H bending of olefins)

Analysis :	%C = 69,10		%H = 9,58	
	%N = 2,96		%O = 18,36	

Mass Spectrum (spectrum 9, p72)

The mass spectrum exhibited the following peaks :-

m/e	471 (8,28%),	470 (21,3%),	411,3 (11,66%),	410 (10%),
	395 (2,5%),	393 (2,6%),	330 (2,36%),	316 (3%),
	302 (1%),	301 (2,14%),	283 (1%),	273 (3,5%),
	271 (3,23%),	263 (17%),	262 (70,36%)	250 (13,44%),

249 (18,14%), 248 (17,33%), 233 (12,78%), 220 (33,83%),
215 (10,57%), 207 (54,73%), 206 (10,69%),
205 (10,7%), 204 (13,9%), 203 (52%), 202 (20,3%),
201 (20,3%), 191 (21,65%), 190 (36,36%), 189 (100%),
288 (23,63%), 187 (27,95%), 177 (20,5%), 175 (38,8%),
173 (22,86%), 163 (10,83%), 161 (16,68%), 159 (14,04%),
149 (12,18%), 148 (11,14%), 147 (25,46%), 145 (18%),
135 (32,44%), 133 (34,5%), 131 (19,71%), 121 (34,32%),
119 (45%), 109 (26,44%), 107 (41,77%) and
105 (40,21%),

CHAPTER 4

EXTRACTIVES FROM A. VENOSUM

Chromatography on silica gel of the hexane extracts of dried root material resulted in the isolation of five compounds which were named antidesma I, II, II, IV and V. Antidesma II was obtained as an oil which resisted all attempts at crystallization using different solvents and it was not investigated further. Structures of antidesma I, III, and IV are discussed in sections 4.1, 4.2 and 4.3 respectively. The yield of antidesma V was insufficient even for spectroscopic examination. Antidesma II and III were also isolated from the ethanol extracts.

Chemical reactions and spectroscopic data were used in determining the structures of these compounds.

4.1 Antidesma I

The infra-red spectrum (spectrum 1, p64) showed a peak at 3410cm^{-1} due to a hydroxyl group. A peak at 1680cm^{-1} is considered to arise from a carbonyl group and a peak at 1640cm^{-1} from a vinyl group. The infra-red spectrum also contained a strong band at 880cm^{-1} due to an out of plane C-H bending vibration of an olefin.

The fragmentation pattern observed in the mass spectrum (spectrum 8, p71) strongly suggested that the compound was of the lup - 20(29)-ene type[63,64] and allowed allocation of the carboxyl group at C-17 and the hydroxyl group in ring A (fig.3). The mass data of antidesma I were in fact very similar to those of betulinic acid. The fragment ions at m/e 438 and m/e 411 are due to the loss of H_2O and COOH respectively. Cleavage of ring C between C(12)-C(13) and C(8)-C(14) bonds results in the formation of an ion m/e 220 which on loss of COOH gave rise to fragment ion m/e 175 which on rearrangement loses a proton to give a peak at m/e 174. Alternative cleavage of ring C between C(8)-C(14) and C(9)-C9(11) bonds followed by loss of a proton resulted in ion m/e 207, which on losing H_2O gave rise to the base peak at m/e 189. Loss of hydroxyl group from fragment ion m/e 207 gives rise to a peak at m/e 190. The fragment m/e 248 also results from cleavage of ring C (fig.4) and then loses a carbonyxl group to form a product m/e 203 which rearranges and loses a proton to give rise to a peak at m/e 202. Fragment ions resulting from cleavage of ring D are also observed.

/Cleavage.....

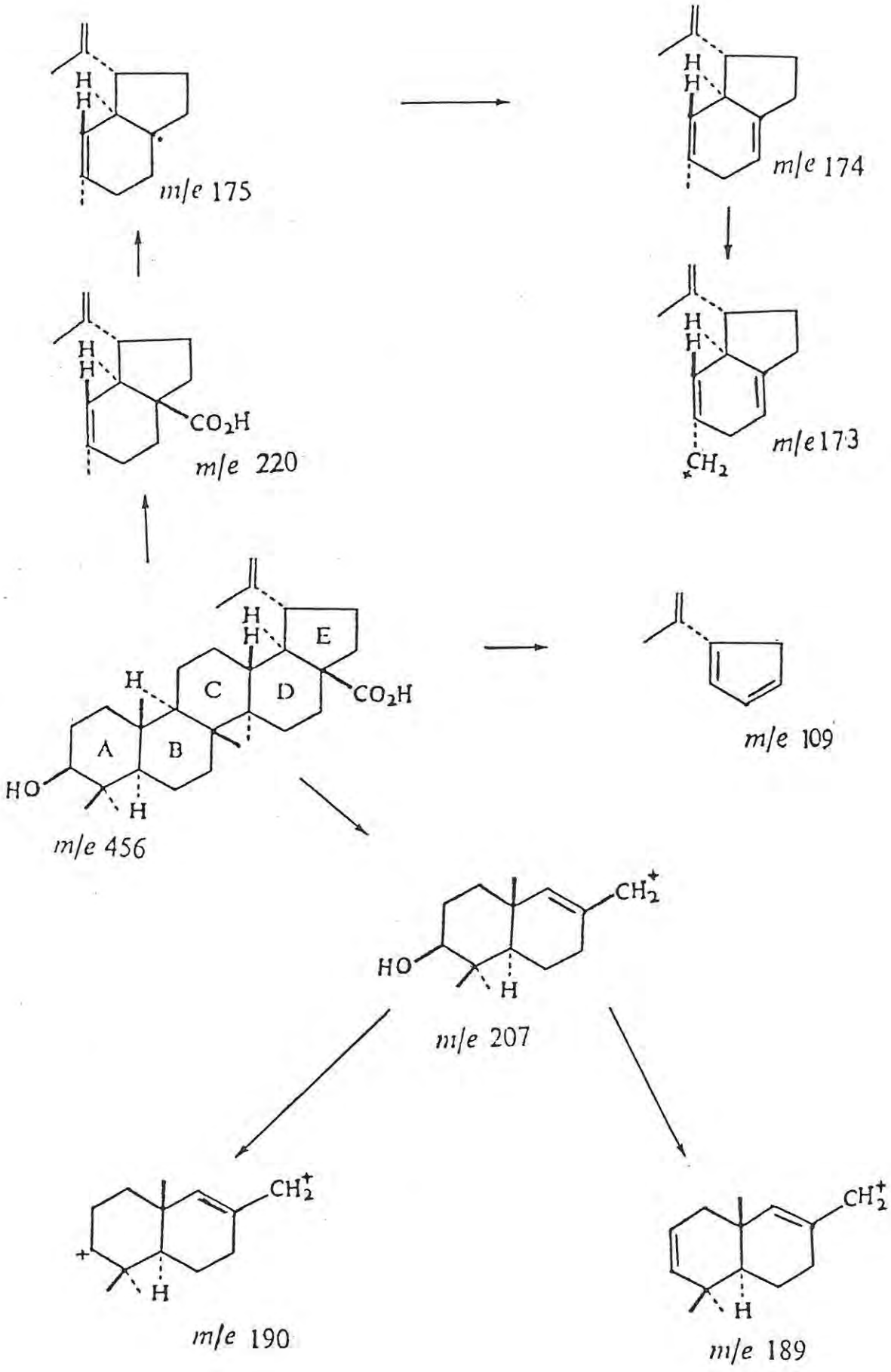


Figure 3

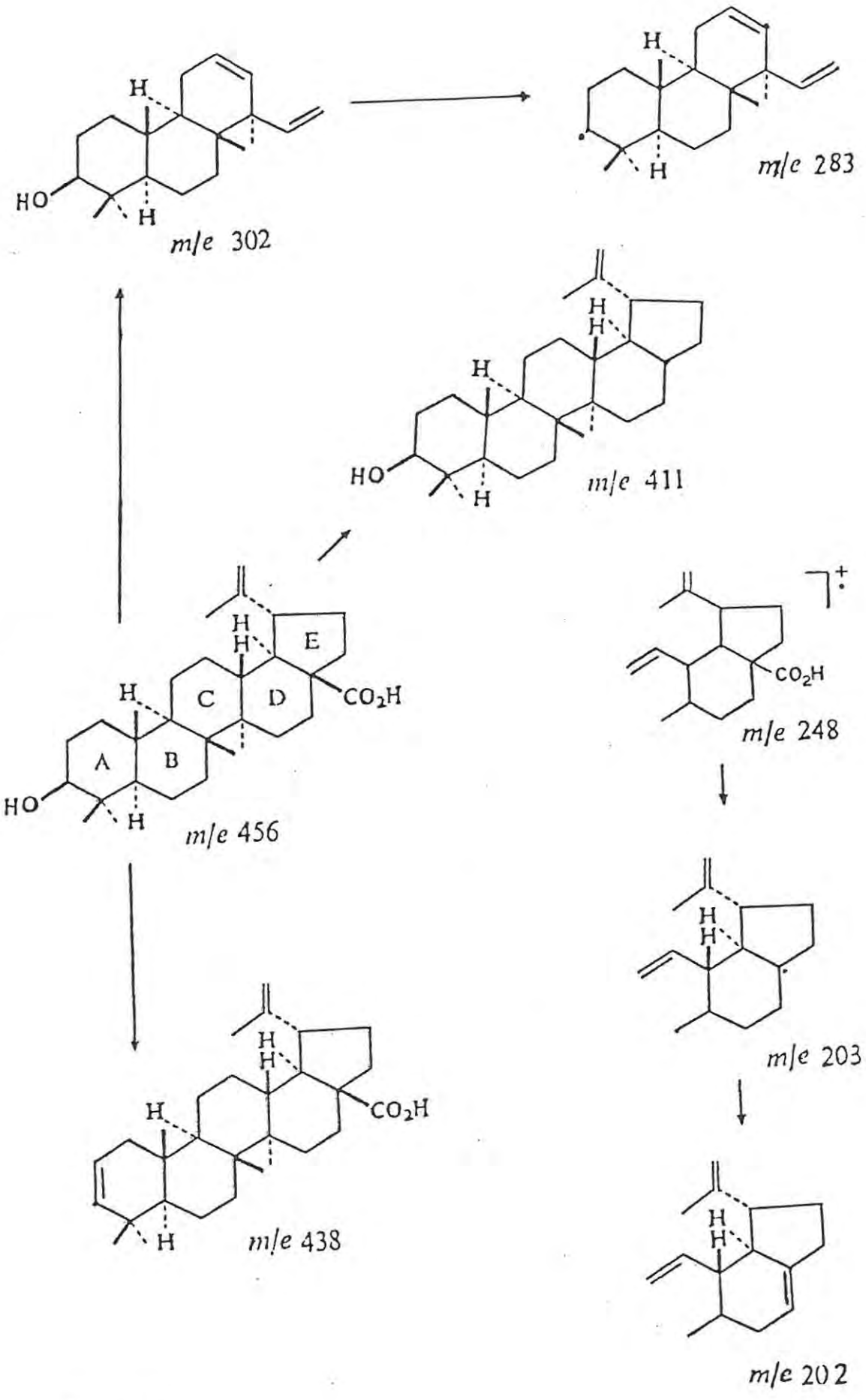

HO
H
m/e 302
m/e 283
m/e 411
A
B
C
D
E
CO2H
m/e 456
m/e 248
m/e 438
m/e 203
m/e 202

Figure 4

Cleavage of the C(13)-C(18) and C(16)-C(17) bonds give rise to a peak at m/e 302 which on losing a hydroxyl group forms an ion m/e 283.

The 80MHz ^{1}HNMR spectrum (spectrum 13, p76), displays six tertiary methyl groups [δ 0,6532 (3H,s); δ 0,7668 (3H,s); δ 0,8720 (6H,s); δ 0,9294 (3H,s) and δ1,6447 (3H,s)], two vinylic protons [δ 4,5630 (1H,m) and δ 4,6742 (1H,m)] long range coupled to a vinylic methyl group [δ1,6447 (3H,s)] and a methine proton [δ 4,1701 (1H, broad)]. The resonances of all the other protons are contained in the methylene envelope.

The proton noise decoupled ^{13}CNMR spectrum (17, p87), of antidesma I revealed the presence of six methyl groups, [δ: 14,4175; 15,8692 ($2CH_3$); 15,6985; 19,0213; and 28,1096], a hydroxy bonded carbon [76,8846], a vinylidene group [δ 109,3806, (C = $\underline{C}H_2$), δ150,3071 ($\underline{C}$ = CH_2)] and a acidic carbonyl group [δ177,023 - $\underline{C}$OOH]. In table 3, p47, the ^{13}CNMR data of antidesma I and lupeol (84)[46] are compared. The chemical shifts of C-17 and C-28 differ significantly. The latter difference is due to a carboxyl group being replaced by a methyl group while the former difference reflects the effect of the adjacent C-28 carboxyl group as opposed to a methyl group.

This spectroscopic evidence coupled with their physical properties (table 2) clearly shows that antidesma I is betulinic acid (85), a fairly common pentacyclic triterpenoid.

An attempt to form the methyl ester using diazomethane was unsuccessful (check analysis and infra-red data in section 3.7). The infra-red absorption frequency at 2210cm^{-1} (check spectrum 2, p65), which is typical of triple bonds, is unexpected in the methyl ester product.

TABLE 2

Comparison of the physical properties of antidesma I and betulinic acid.

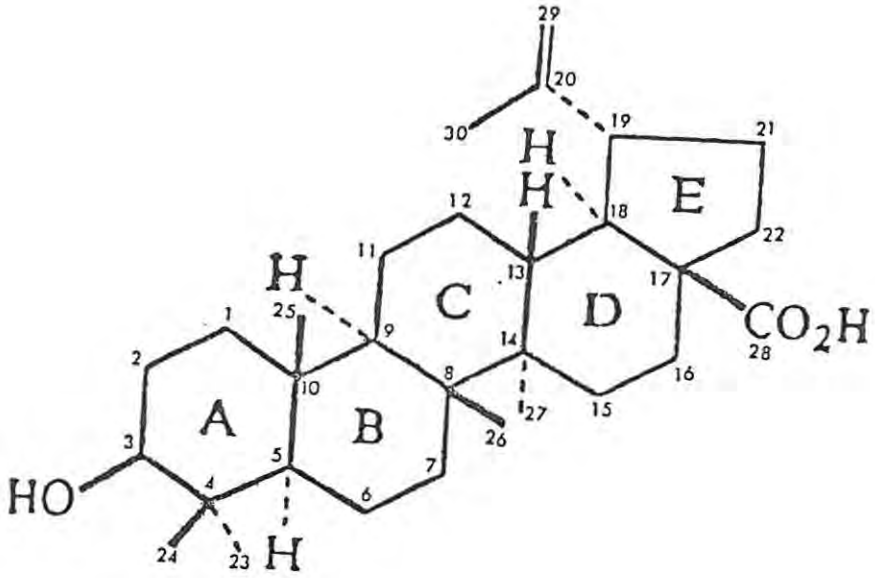
	Antidesma I	Betulinic acid
m.p.	293 - 295°C	295 - 298°C[65] 275 - 278°C[66]
Optical Rotation	$[\alpha]_D^{22°C}$ = +6,08° (c 1,0 in pyridine)	$[\alpha]_D^{22°C}$ = +7,89°[65] (in pyridine) $[\alpha]_D^{22°C}$ = +7,9°[66] (in pyridine)

/Table 3.....

TABLE 3

^{13}CNMR Chemical shifts of Antidesma 1 and lupeol compared.

Number of Carbon	Antidesma 1	Lupeol [46]
C-1	38,7401	38,7
C-2	28,1096	27,5
C-3	76,8846	79,0
C-4	39,7893	38,9
C-5	55,0842	55,3
C-6	17,9933	18,3
C-7	34,0514	34,3
C-8	41,8753	40,9
C-9	50,0842	50,5
C-10	36,8397	37,2
C-11	20,5377	21,0
C-12	25,2038	25,2
C-13	37,7313	38,1
C-14	42,1003	42,9
C-15	27,1897	27,5
C-16	36,3485	35,6
C-17	55,4359	43,0
C-18	48,7706	48,0
C-19	46,6472	48,3
C-20	150,3071	150,9
C-21	29,2145	29,9
C-22	40,8302	40,0
C-23	28,1096	28,0
C-24	14,4175	15,3
C-25	15,8692	16,1
C-26	15,8692	16,0
C-27	15,6985	14,6
C-28	177,0231	18,0
C-29	109,3806	109,3
C-30	19,0213	19,3



(85)

Betulinic acid

4.2 Antidesma III

The infra-red spectrum (spectrum 3, p66) showed peaks at $3430cm^{-1}$ and $1700cm^{-1}$ due to hydroxyl and carboxyl groups respectively.

The mass spectrum (spectrum 10, p73) is strongly reminiscent of that of a typical straight chain hydrocarbon such as heptadecane (fig.5).[67] The fragment ions m/e : 311, 297, 241, 213, 185, 129, 87, 73, 59 and 45 show the characteristic fragmentation pattern of a straightchain monocarboxylic acid (table 5).[68] The fragment ion m/e 60 confirms the presence of a carboxylic acid functionality and is the result of McLafferty rearrangement (fig.6). More details of the fragmentation pattern are provided in table 4. This evidence strongly suggests that antidesma III is tetracosanoic acid. This evidence is supported by the ^{1}H and ^{13}CNMR spectra.

The ^{1}HNMR spectra (spectra 14A, B and C, p77-79) revealed the presence of a methyl group [δ 0,8639 (3H,t)], a hydroxyl proton [δ2,1523 (1H,s)] and two methylene groups [δ 1,6152 (2H,m); δ 2,3258 (2H,t)]. The remaining protons show signals in the region δ1,1976 - δ1,2837.

/Fig. 5.....

FIGURE 5

MASS SPECTRUM OF HEPTADECANE

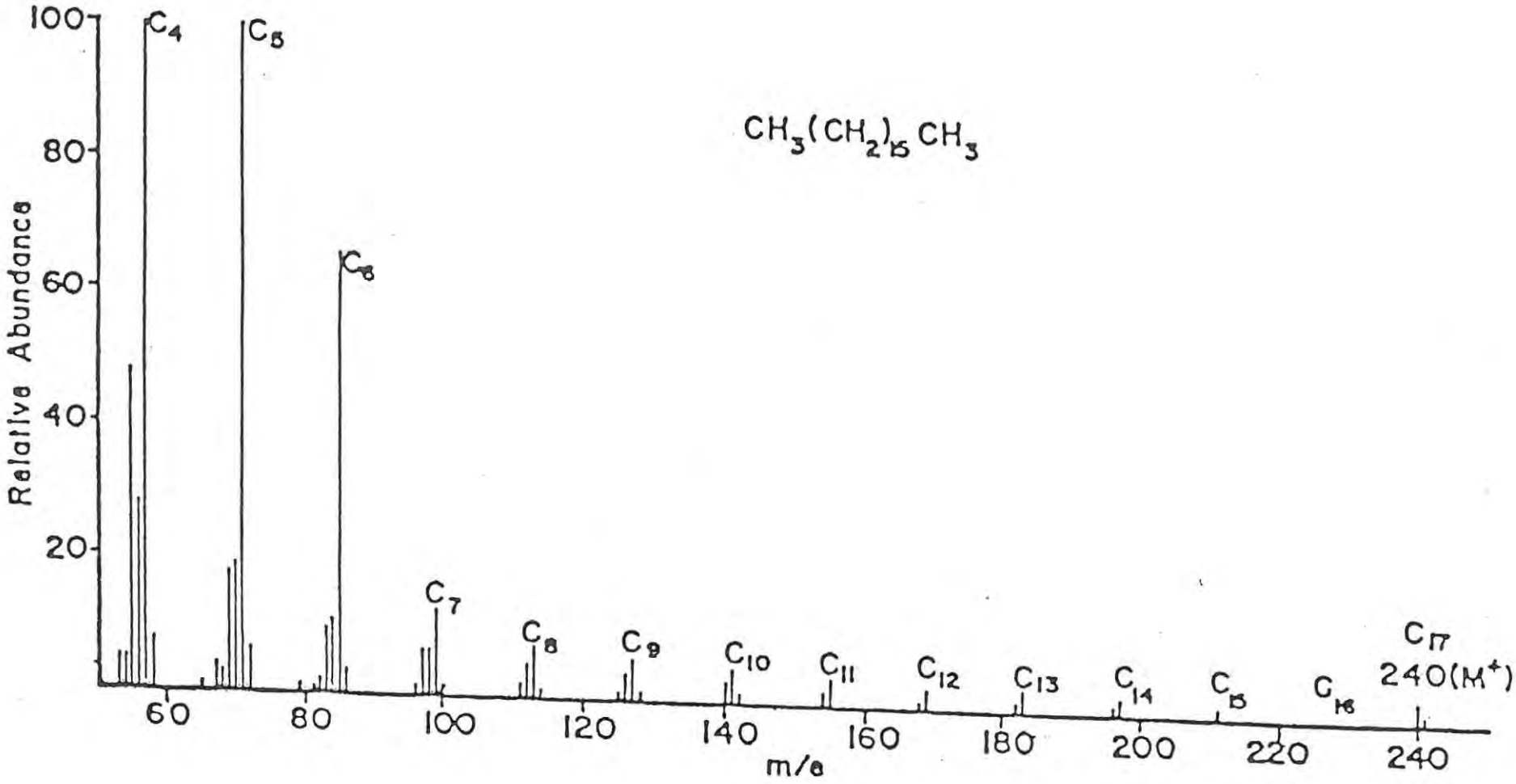


TABLE 4

Fragmentation pattern of antidesma III

m/e	Fragment ions
29	CH_3CH_2
43	$CH_3(CH_2)_2$
57	$CH_3(CH_2)_3$
71	$CH_3(CH_2)_4$
85	$CH_3(CH_2)_5$
99	$CH_3(CH_2)_6$
113	$CH_3(CH_2)_7$
127	$CH_3(CH_2)_8$
141	$CH_3(CH_2)_9$
155	$CH_3(CH_2)_{10}$
169	$CH_3(CH_2)_{11}$
183	$CH_3(CH_2)_{12}$

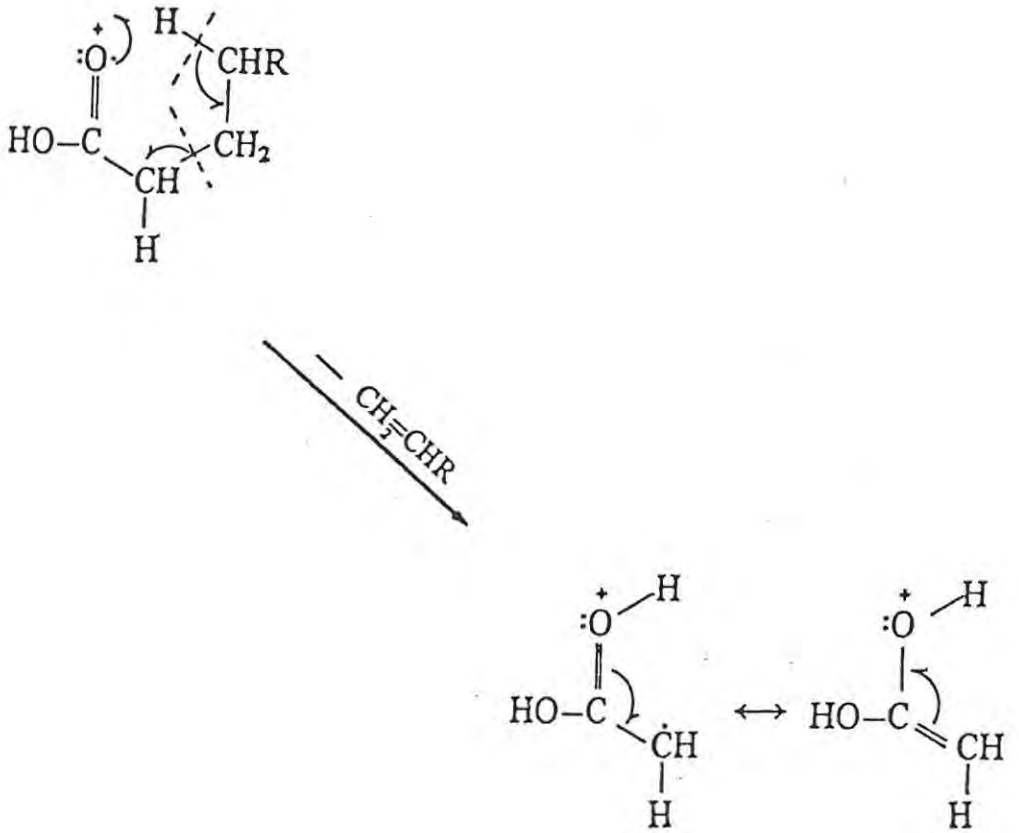
TABLE 5

Fragmentation pattern of antidesma III

m/e	Fragment ions
45	^+COOH
59	$^+CH_2COOH$
73	$^+(CH_2)_2COOH$
87	$^+(CH_2)_3COOH$
129	$^+(CH_2)_6COOH$
185	$^+(CH_2)_{10}COOH$
213	$^+(CH_2)_{12}COOH$
241	$^+(CH_2)_{14}COOH$
297	$^+(CH_2)_{18}COOH$
311	$^+(CH_2)_{19}COOH$

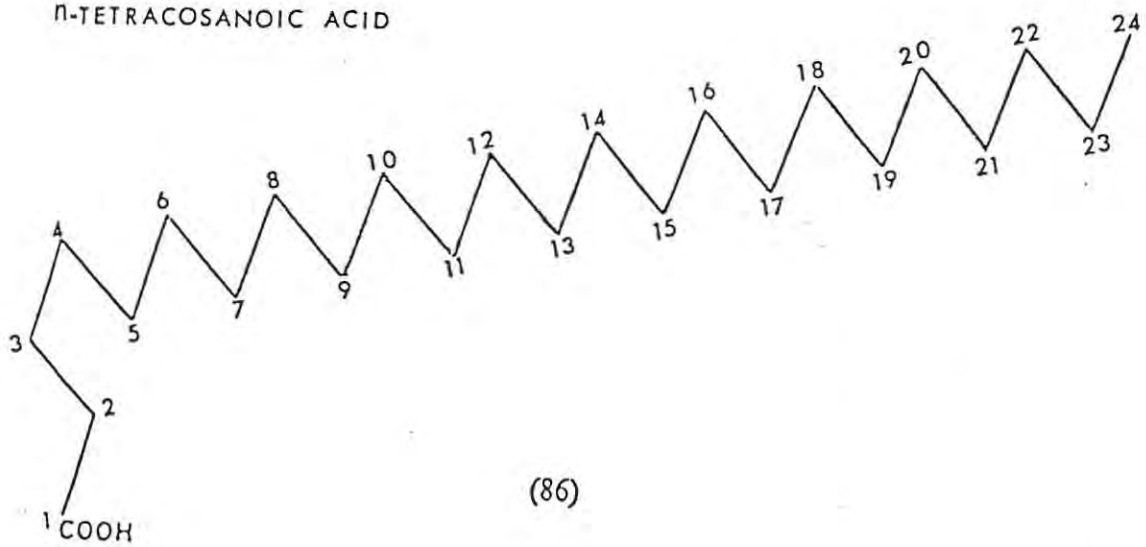
Figure 6

McLafferty rearrangement of monocarboxylic acid.



m/e 60

n-TETRACOSANOIC ACID



(86)

A ^{13}CNMR proton noise decoupled spectrum (spectrum 18) of antidesma III showed seven clear peaks [δ 14,1 ($\underline{C}H_3$-), δ 22,7 (CH_3-$\underline{C}H_2$-), δ 24,7 (CH_3-CH_2-$\underline{C}H_2$-), δ 32,0 ($\underline{C}H_2$-CH_2-COOH), δ 34,0 (-CH_2-CH_2-$\underline{C}H_2$-COOH) and δ 179,7 (-$\underline{C}$OOH)]. The remaining peaks which could not be interpreted appear in the range δ 29,08 - δ 29,7.

Both the spectroscopic evidence and the melting point (Table 6) suggest that antidesma III is n-tetracosanoic acid (86).

TABLE 6

Comparison of the melting points of antidesma III and n-tetracosanoic acid

	Antidesma III	n-tetracosanoic acid
m.p.	82-84°C	81-82°C[69]
		84,5-85,5°C[70]

4.3 Antidesma IV

The infra-red spectrum (spectrum 4, p67) showed a peak at 3420cm^{-1} due to a hydroxyl group whose presence was confirmed by the preparation of a crystalline acetate. Although infra-red data did not indicate the presence of a carbon-carbon double bond, the compound gave a positive Liebermann-Burchard Test.[60]

The fragmentation pattern observed in the mass spectrum (spectrum 11) is characteristic of ß-sitosterol.[71,72,73] The fragment ion m/e 399 is due to cleavage between C-20 and C-21 and the ion m/e 396 results from the loss of a hydroxyl group at C-3 (fig.7). Loss of a C-21 methyl group and hydroxyl group resulted in a peak at m/e 381. The cleavage between C-17 and C-20 gave rise to a peak at m/e 275. Fragmentation of typical tetracyclic steroid to afford the peak at m/e 231 is observed. This kind of fragmentation is generally known as M-(42+R) where R=$C_{10}H_{21}$ for ß-sitosterol. Three paths a, b and c are suggested[71] for this kind of fragmentation (fig.8). The first two would involve the extremely unlikely fragmentation of three bonds without forming energetically favourable fragments, while path c would lead to an allylic carbonium ion (d or e). The mass spectrum (spectrum 12, p75) of the acetyl derivative showed a peak at m/e 396 whick is due to the loss of acetic acid (fig.9).

/The.....

Figure 7

Fragment ions.

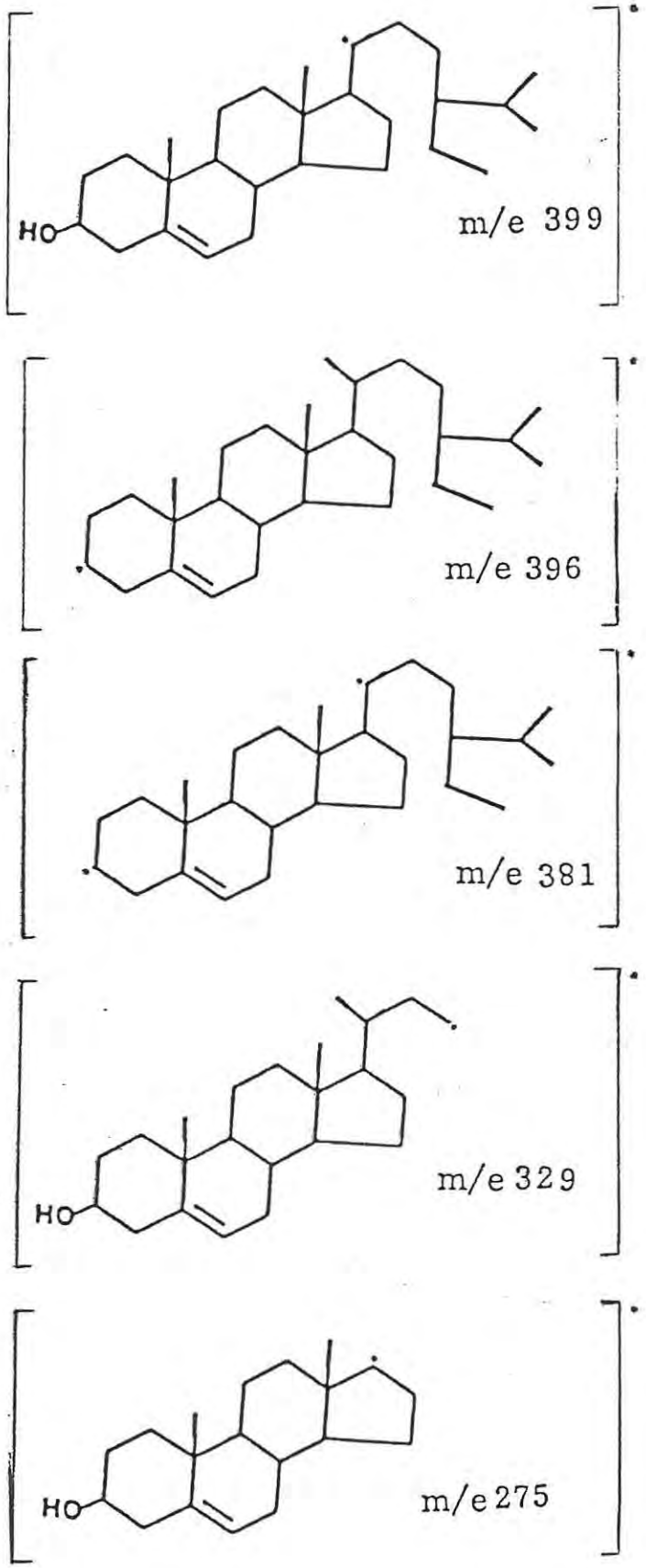


Figure 8

Fragmentation pattern of Tetracyclic steroid. [m - (42 + R) where R = $C_{10}H_{21}$ for sitosterol].

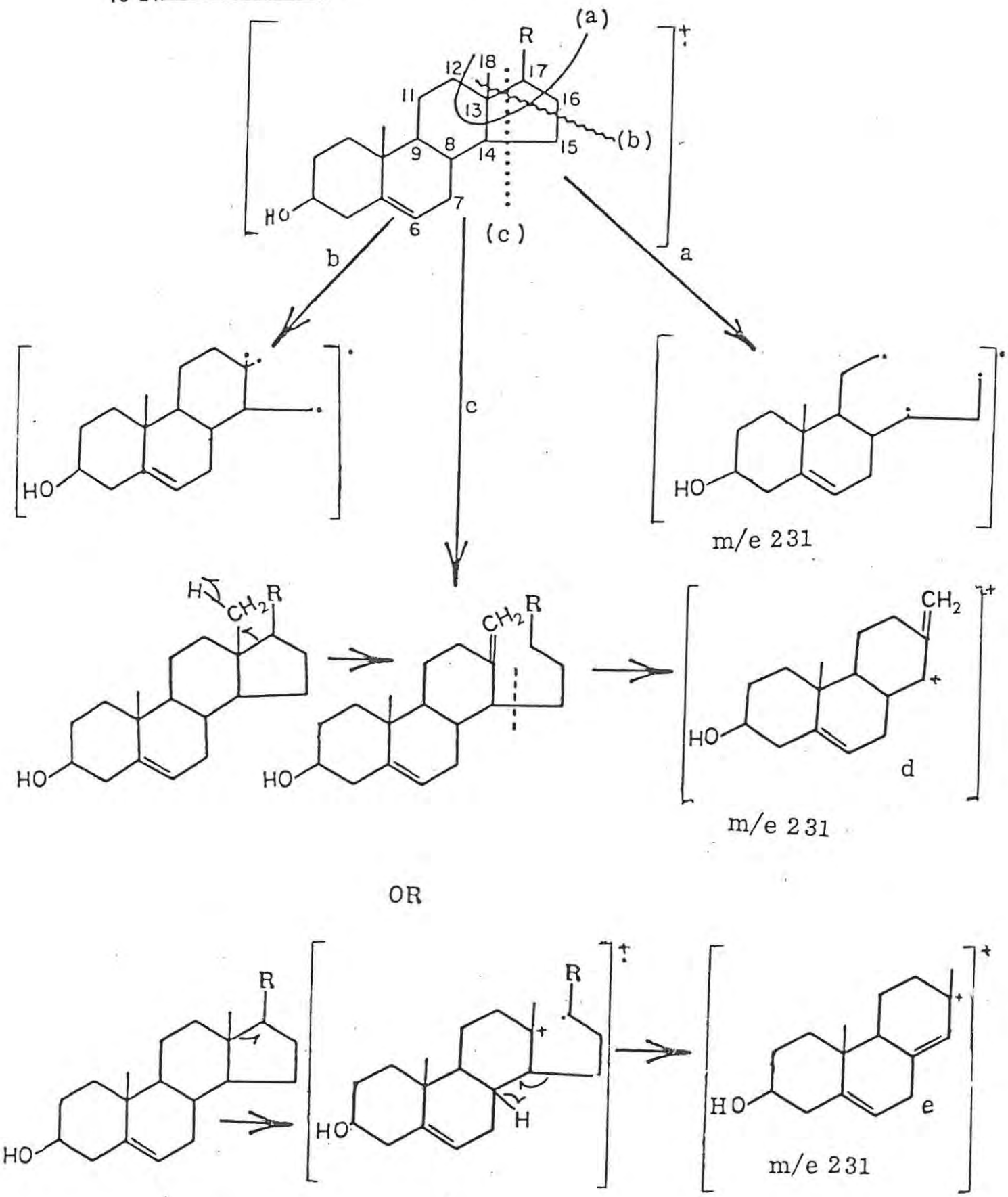
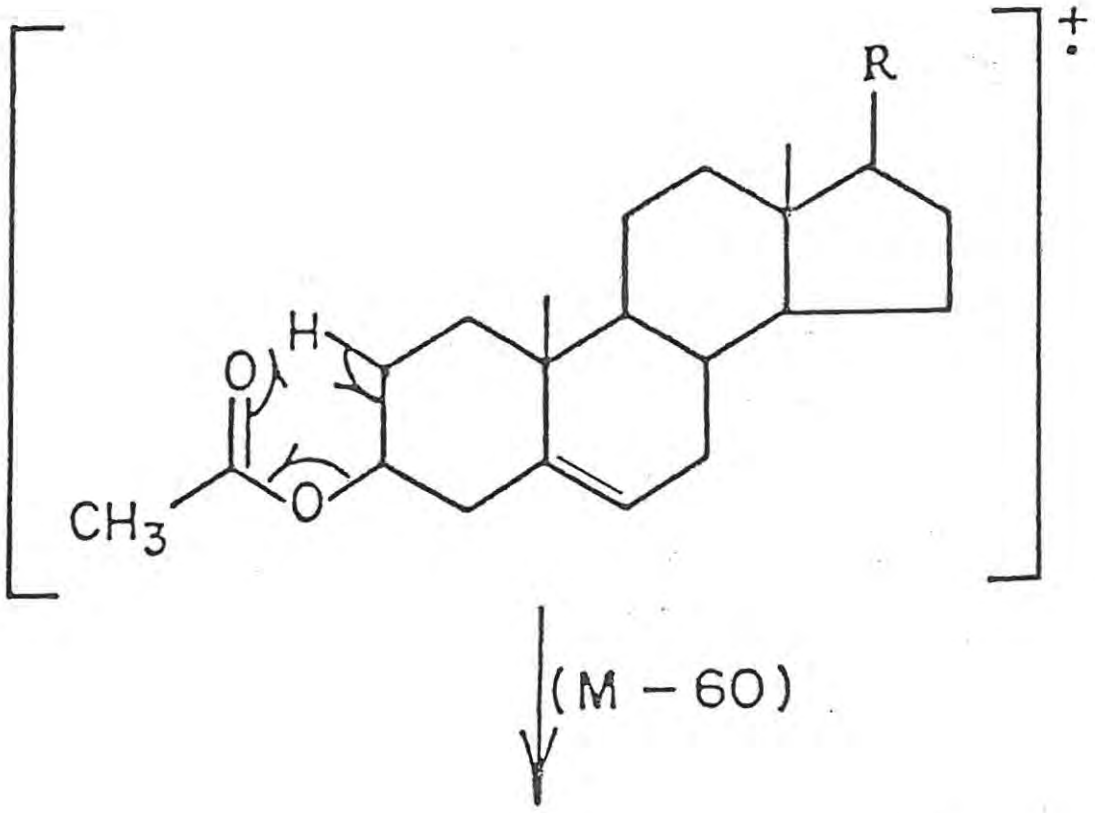
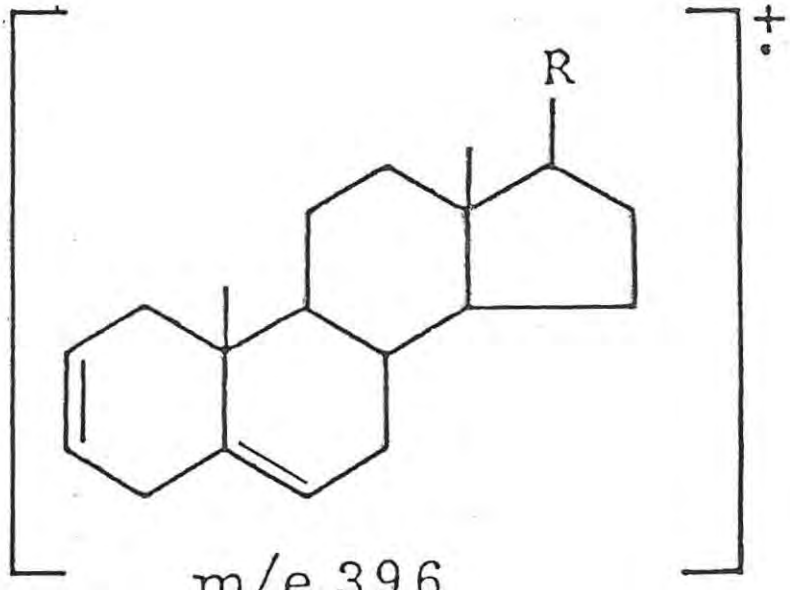


Figure 9

Loss of acetic acid in sitosteryl acetate (R = $C_{10}H_{21}$) .



(M − 60)



m/e 396

The ^{1}HNMR spectra (spectra 15A, B, C and D, p80-83) revealed the presence of six methyl groups [δ 0,6571 (3H,s); δ 0,9048 and δ 0,8918 (3H,d); δ 0,8185, δ 0,8221 and δ 0,7974 (3H,d); δ 0,8048 and δ 0,7839 (3H,d); and δ 0,8185, δ 0,8221 and δ 0,7956 (3H,t)], a methine proton [δ 5,3217(1H,t)] two methylene protons [δ 5,1061 (1H,m) and δ 5,0013 (1H,m)] and a hydroxyl proton [δ 3,4936 (1H,m)]. The remaining protons are so nearly chemical equivalent that they give rise to a broad, almost featureless "hump" (spectrum 15A). Chemical shifts of the methyl groups of antidesma IV and ß-sitosterol[74] are compared in Table 7. The ^{1}HNMR spectra (spectra 16A, B and C, p84-86) of antidesma IV acetate shows the presence of seven methyl groups [δ 0,66 (3H,s); δ 1,0009 (3H,s); δ 0,8975 (3H,d); δ 0,8244 and δ 0,8034 (3H,d); δ 0,8288 and δ 0,8131 (3H,t)] 0-bonded methine [δ 4,5875 (1H,m)], methine proton [δ 5,3575 (1H,t)], two methylene protons [δ 5,1313 (1H,m) and δ 5,0065 (1H,m)] and a methylene group [δ 4,5826 (2H,m)]. In the acetyl derivative, the hydroxymethine multiplet at C-3 is deshielded to δ 4,5875 and the C-2 methylene group is deshielded to δ 4,5826. In table 8 the chemical shifts of the methyl groups of antidesma IV acetate and sitosteryl acetate are compared.

A ^{13}CNMR proton noise decoupled spectrum (spectrum 19, p89) of antidesma IV was recorded. It revealed the presence of six methyl groups [δ : 11, 9598; 18,7900; 18,9875; 19,3900; 21,0622 and 21,2103], a hydroxyl bonded carbon [δ 71,7596] and carbon-carbon double bond [δ 140,7653, (C = C—H) and δ 121,6337, (C = C—H)]. The ^{13}HNMR spectra (spectra 20A and B, p90-91) of antidesma IV acetate showed seven methyl groups [δ : 11,8679; 18,7925; 19,0672; 19,7959; 21,2183; 21,3869 and 27,8034], an oxygenbonded carbon [δ 73,9862], a carbon-carbon double bond [δ 139,6737, (C = C—H) and δ 122,5979, (C = C—H)] and a carbonyl group [δ 206,6515]. The chemical shifts of antidesma IV acetate are recorded in Table 10.

For the sake of comparison, the ^{13}CNMR of antidesma IV and of two tetracyclic steroids,[75,76,77] 3-ketocholestane and cholesterol, are compared in Table 9.

/Table 7.....

TABLE 7 Methyl groups chemical shifts of antidesma IV and ß-sitosterol compared.

Methyl groups	^{1}HNMR Chemical shifts (ppm)	
	Antidesma IV	ß-sitosterol[74]
	360MHz	220MHz
C-18	0,6571	0,680
C-19	0,9845	1,0007
C-21	0,9048	0,919
	0,8918	
C-26 and C-27	0,8181	0,833
	0,7839	
C-29	0,8485	0,842
	0,8221	
	0,7956	

TABLE 8 Methyl groups chemical shifts of Antidesma IV acetate and ß-sitosteryl acetate compared.

Methyl groups	^{1}HNMR Chemical Shifts (ppm)	
	Antidesma IV Acetate	ß-sitosteryl acetate[74]
	360MHz	220MHz
C-18	0,6613	0,676
C-19	1,0009	1,017
C-21	0,9106	0,918
	0,8975	
C-26	0,8244	0,831
	0,8034	
C-27	0,8108	0,815
	0,7902	
C-29	0,8435	0,846
	0,8288	
	0,8134	

From spectroscopic evidence, m.m.p. and physical properties (table 11 and 12) of antidesma IV and its acetyl derivative antidesma IV was found to be ß-sitosterol (87).

TABLE 9 ^{13}CNMR of Antidesma IV, cholesterol and 3-ketocholestane.

Chemical shifts (ppm)

Carbon	Antidesma IV (sitosterol)	3-ketocholestane[75]	Cholesterol[75]
1	37,28	38,5	37,4
2	31,93	38,1	32,1
3	71,76	211,2	71,8
4	42,23	44,6	42,4
5	140,76	46,7	141,0
6	121,63	29,0	121,0
7	31,93	31,7	31,9
8	31,87	35,6	31,1
9	50,18	53,8	50,4
10	36,52	35,4	36,6
11	21,09	21,4	21,0
12	39,80	39,9	39,6
13	40,45	42,5	42,3
14	56,78	56,2	56,8
15	24,36	24,2	24,3
16	28,24	28,2	27,9
17	56,87	56,2	56,3
18	11,96	12,1	11,8
19	19,39	11,4	19,4
20	31,93	35,7	35,8
21	18,99	18,7	18,7
22	36,14	36,1	36,5
23	24,36	23,8	23,8
24	39,70	39,5	39,9
25	29,35	28,0	28,0
26	19,39	22,5	22,6
27	19,39	22,8	22,6
28	24,31		
29	18,79		

/Table 10.....

TABLE 10 ^{13} CNMR Chemical shifts of Antidesma IV Acetate.

Carbon No.	Chemical shifts (ppm)
1	37,0255
2	31,9118
3	73,9862
4	42,3473
5	139,6737
6	122,5979
7	31,9118
8	31,9118
9	50,0993
10	36,1644
11	21,0508
12	39,7640
13	40,4384
14	56,0983
15	24,3050
16	27,8034
17	56,7287
18	11,8679
19	19,7959
20	31,9118
21	19,0672
22	36,6226
23	24,3050
24	39,7640
25	28,8722
26	19,3074
27	19,3074
28	23,1274
29	18,7925
$\underline{CH_3}COO-$	27,8034
$CH_3\underline{C}OO-$	206,6515

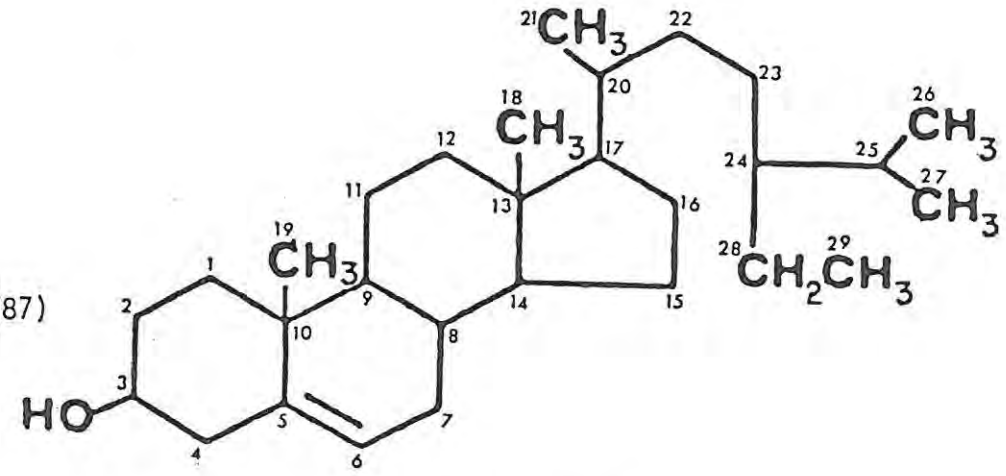
TABLE 11 Physical properties of antidesma IV and ß-sitosterol compared.

	Antidesma IV	ß-sitosterol
m.p.	135 - 138°C	137 - 138°C[78]
Optical Rotation	$[\alpha]_D^{21°C} = -43,5°$ (c 1,0 in chloroform)	$[\alpha]_D^{22°C} = -35°$[79] (in chloroform)
		$[\alpha]_D = -38,2°$[78] (in chloroform)

TABLE 12 Physical properties of antidesma IV acetate and ß-sitosteryl acetate compared.

	Antidesma IV acetate	ß-sitosteryl acetate
m.p.	126°C	125 - 126°C[78]
Optical Rotation	$[\alpha]_D^{22°C} = -41,5°$ (c 1,0 in chloroform)	$[\alpha]_D^{22°C} = -40,3°$[78] (in chloroform)

(87)



ß-sitosterol

From mass spectrum of antidesma IV (spectrum 11, p74) stigmasterol, m/e 412 (10%), and campesterol, m/e 400 (8%), are probable constituents of A. venosum.

4.4 Antidesma V.

Although not enough spectroscopic evidence of antidesma V was collected, its infra-red (spectrum 6, p69), is the same as that of ß-sitosteryl-ß-D-glucoside (88) (spectrum 21, p92), a hypolidemic substance[80] isolated from Hypoxis rooperii[81] and Pygeum africanum.[82]
In table 13, the physical properties of antidesma V, ß-sitosteryl-ß-D-glucoside (88) and their acetyl derivatives are compared.

TABLE 13

Physical properties of antidesma V, ß-sitosteryl-ß-D-glucoside, antidesma V acetate and tetra-acetylsitosteryl-D-glucoside.

1)	Antidesma V	ß-sitosteryl-ß-D-glucoside
m.p.	272 - 276°C	295 - 300°C[83]
Optical Rotation	$[\alpha]_D$ -52° (c 1,0 in DMSO)	
2)	Antidesma V acetate	tetra-acetylsitosteryl-D-glucoside
m.p.	159 - 161°C	166 - 167°C[83]

/4.5 Conclusion.....

4.5 Conclusion

The method used did not isolate alkaloids from A. venosum, however, one cannot say that alkaloids are not constituents of A. venosum. Other methods of isolating alkaloids can be undertaken (see section 1.4). From the work done one can say that A. venosum contains insignificant amounts of ethanol and chloroform soluble alkaloids, hence ethanol and chloroform soluble analgesics and antihypertensive agents (see section 1.1, definition) are not constituents of A. venosum.

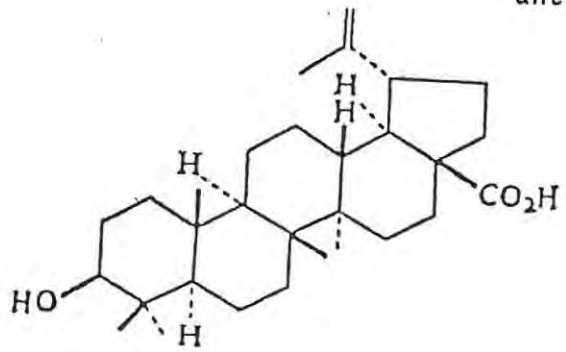
The hexane extract of A. venosum yielded three compounds which were shown to be tetracosanoic acid, ß-sitosterol and betulinic acid. Chemical investigation of A. menasu revealed that two diuretic tripenoids, 16∝-hydroxy-3-ketoisomultiflorene and 3ß-hydroxy-16-ketoisomultiflorene, were isolated from the hexane extract[48] and not the ether extract[46], hence the diuretic tripenoids isolated from A. menasu are not constituents of A. venosum.

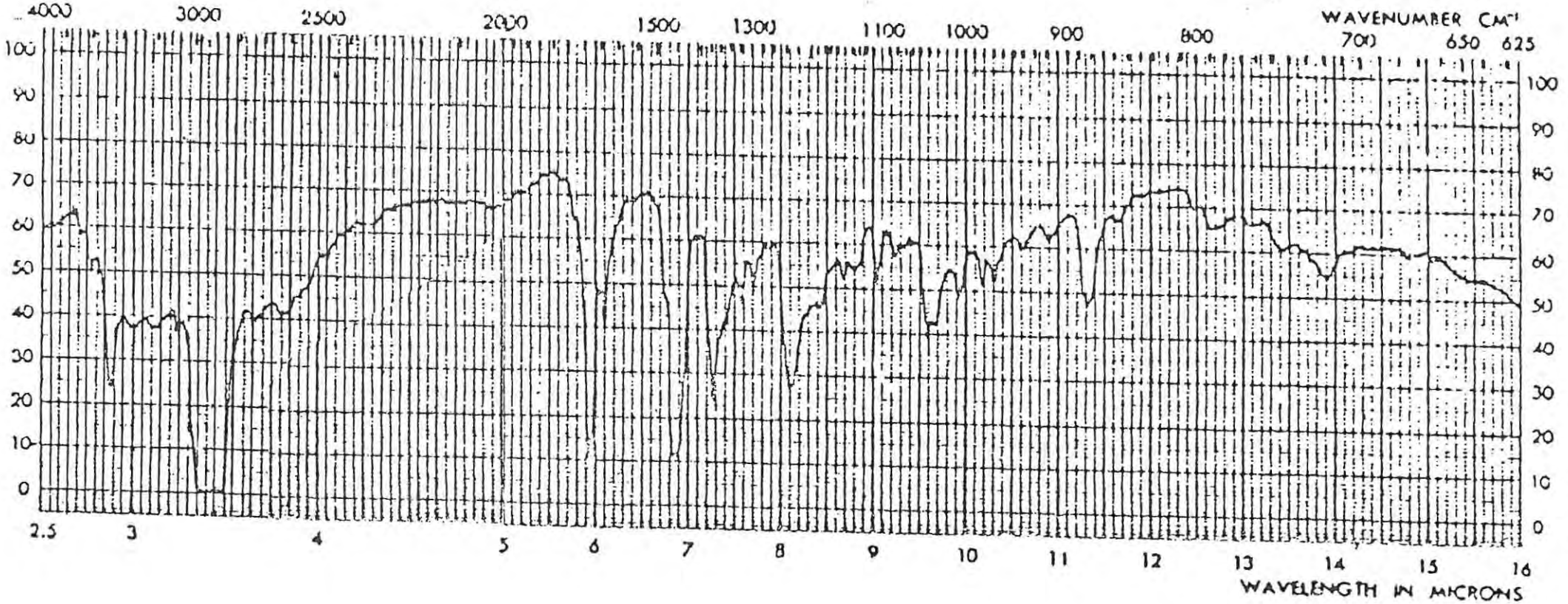
ß-sitosteryl-ß-D-glucoside (88) is the probable active constituent[80] (check section 4.4) of A. venosum.

CHAPTER 5

SPECTRUM 1

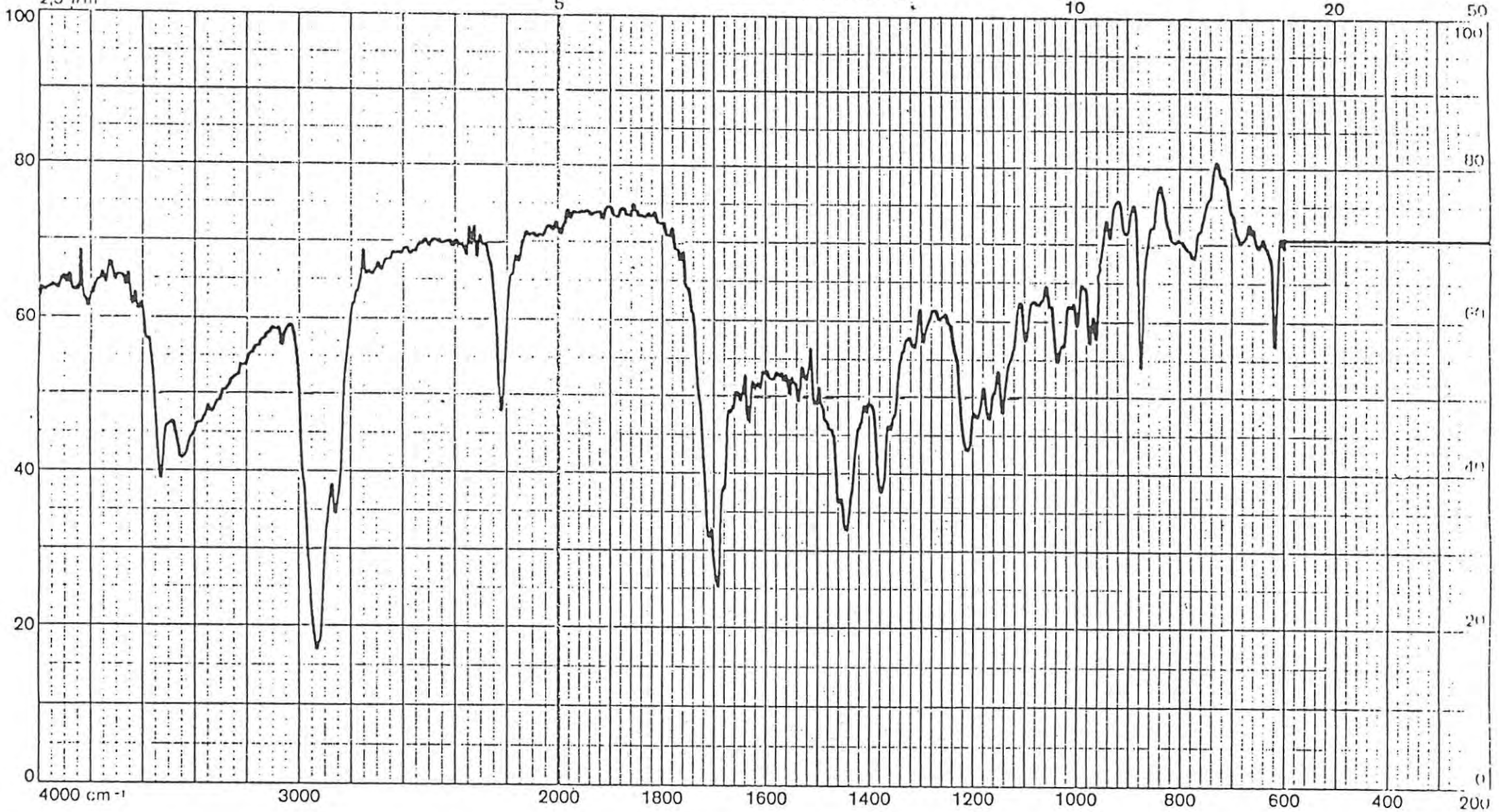
antidesma I.





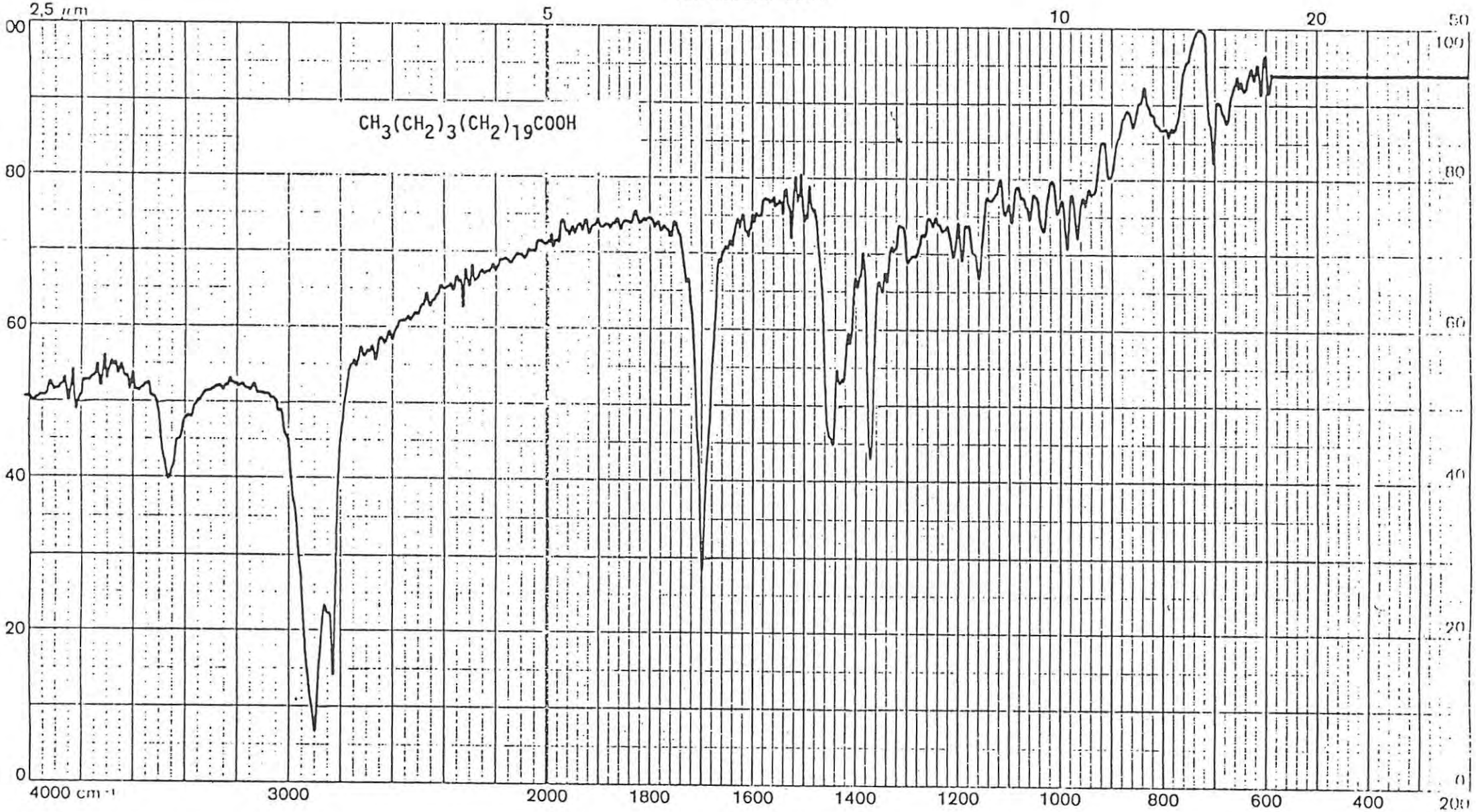
SPECTRUM 2

antidesma I derivative



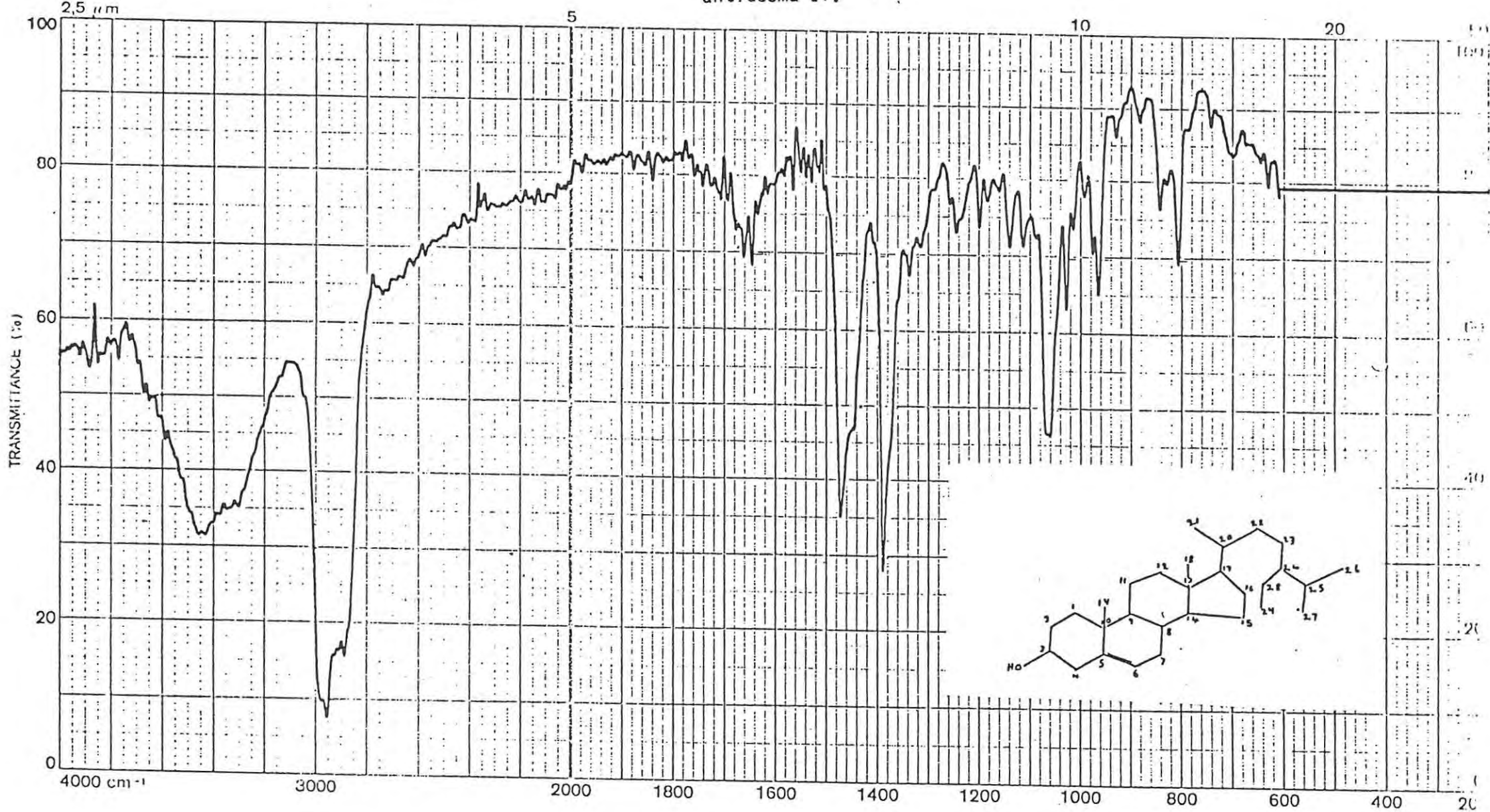
SPECTRUM 3

antidesma III.



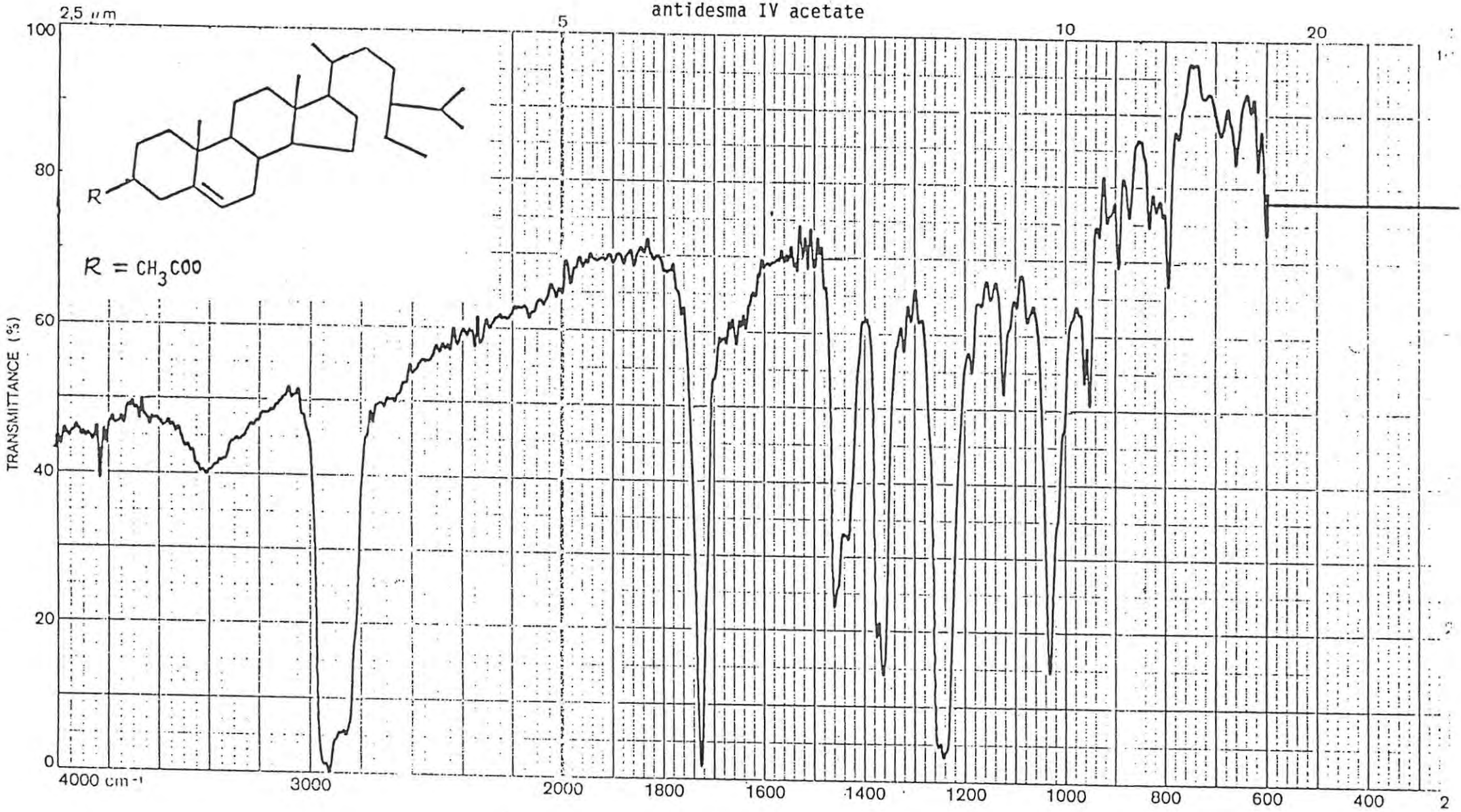
SPECTRUM 4

antidesma IV.



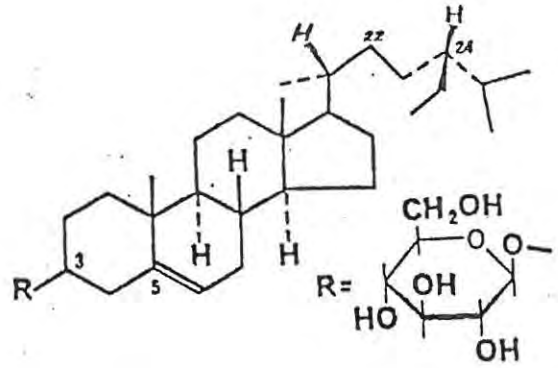
SPECTRUM 5

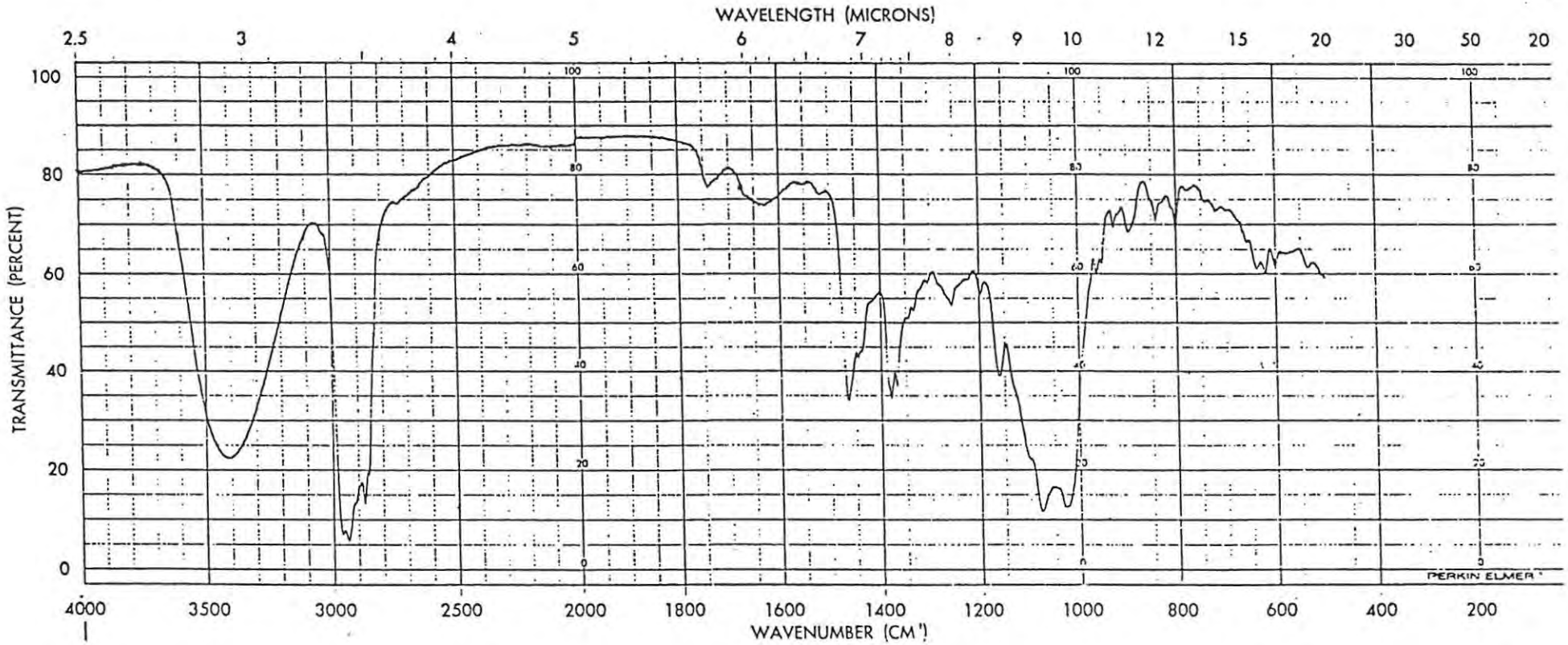
antidesma IV acetate

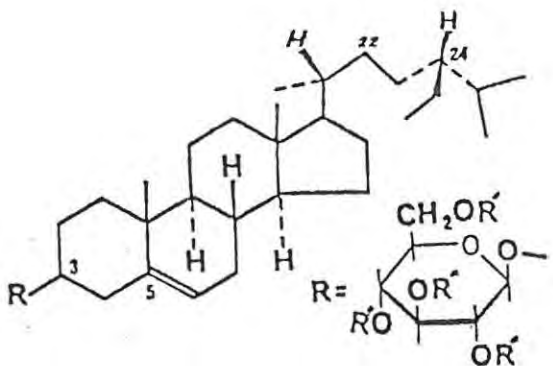


SPECTRUM 6

antidesma V



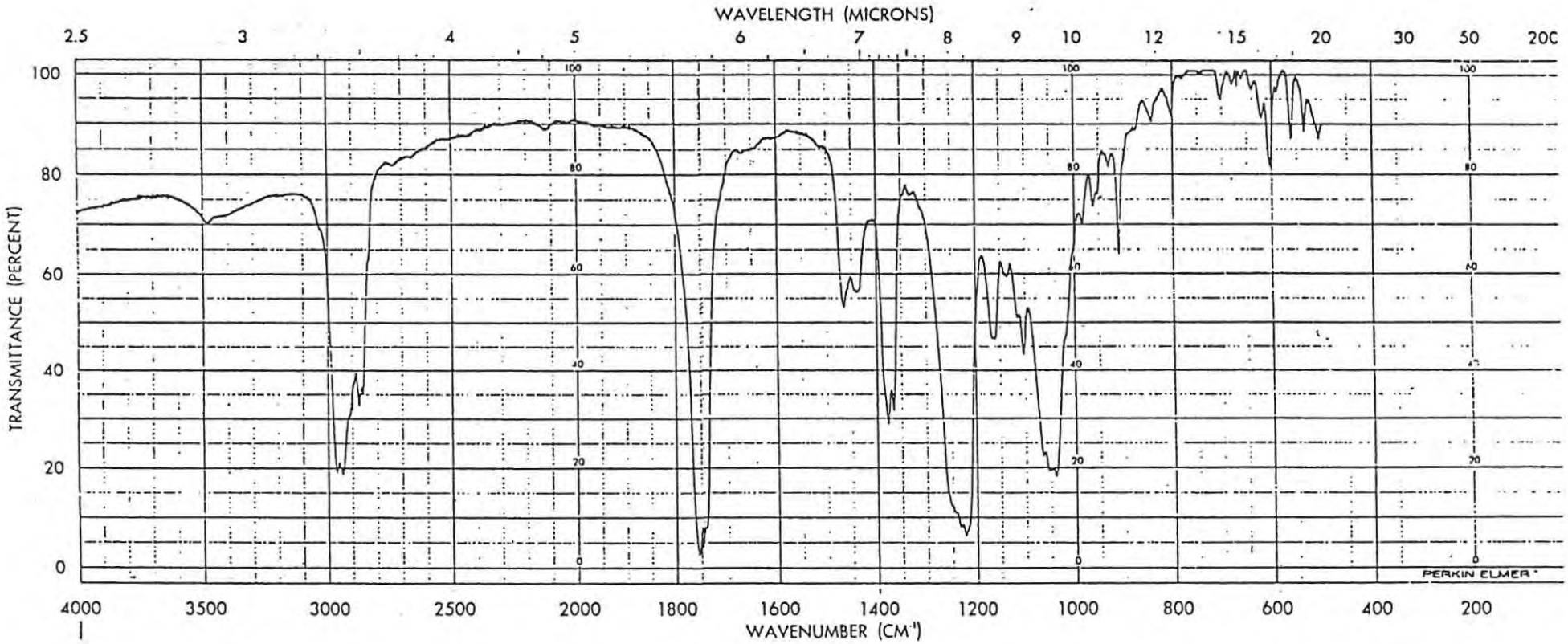




SPECTRUM 7

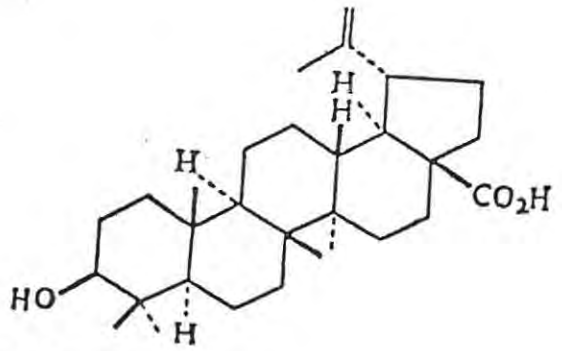
antidesma V acetate.

R'= CH_3CO



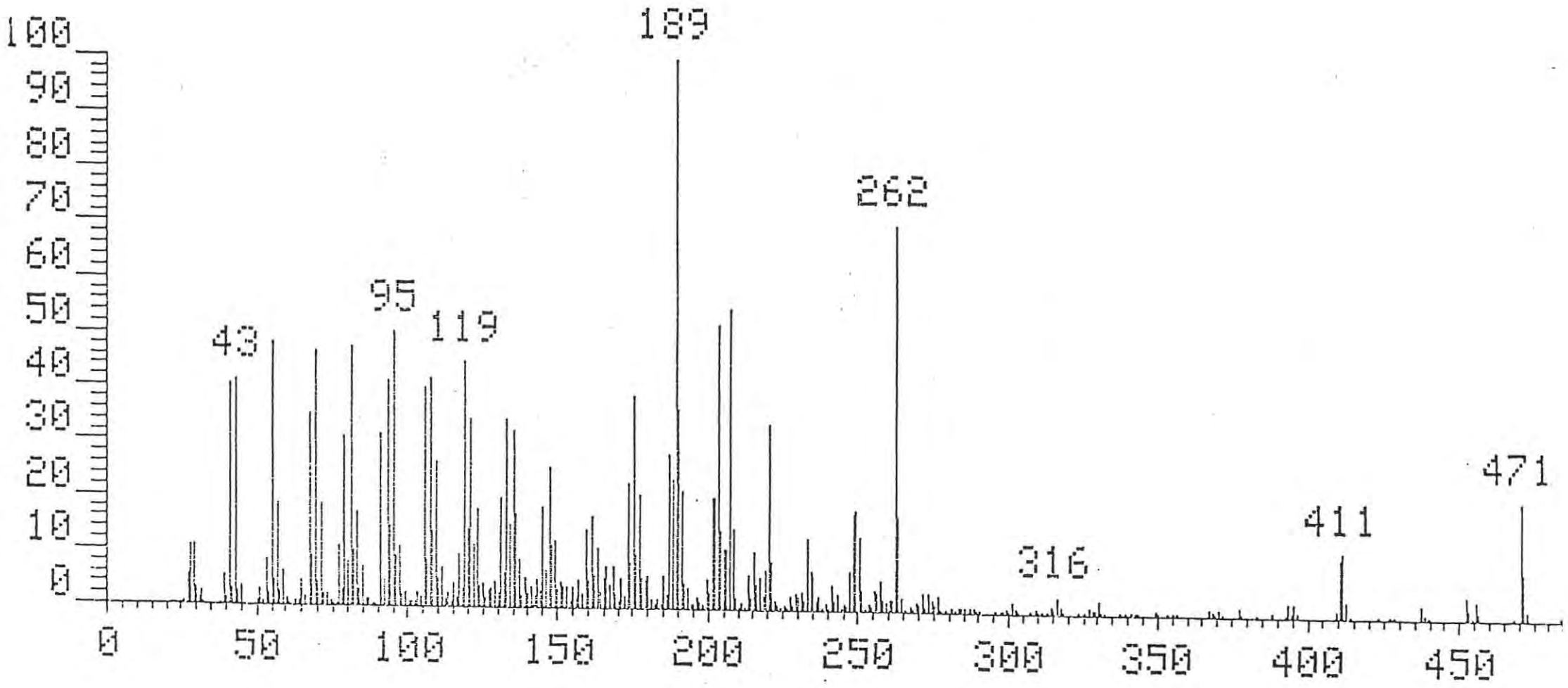
SPECTRUM 8

antidesma I.



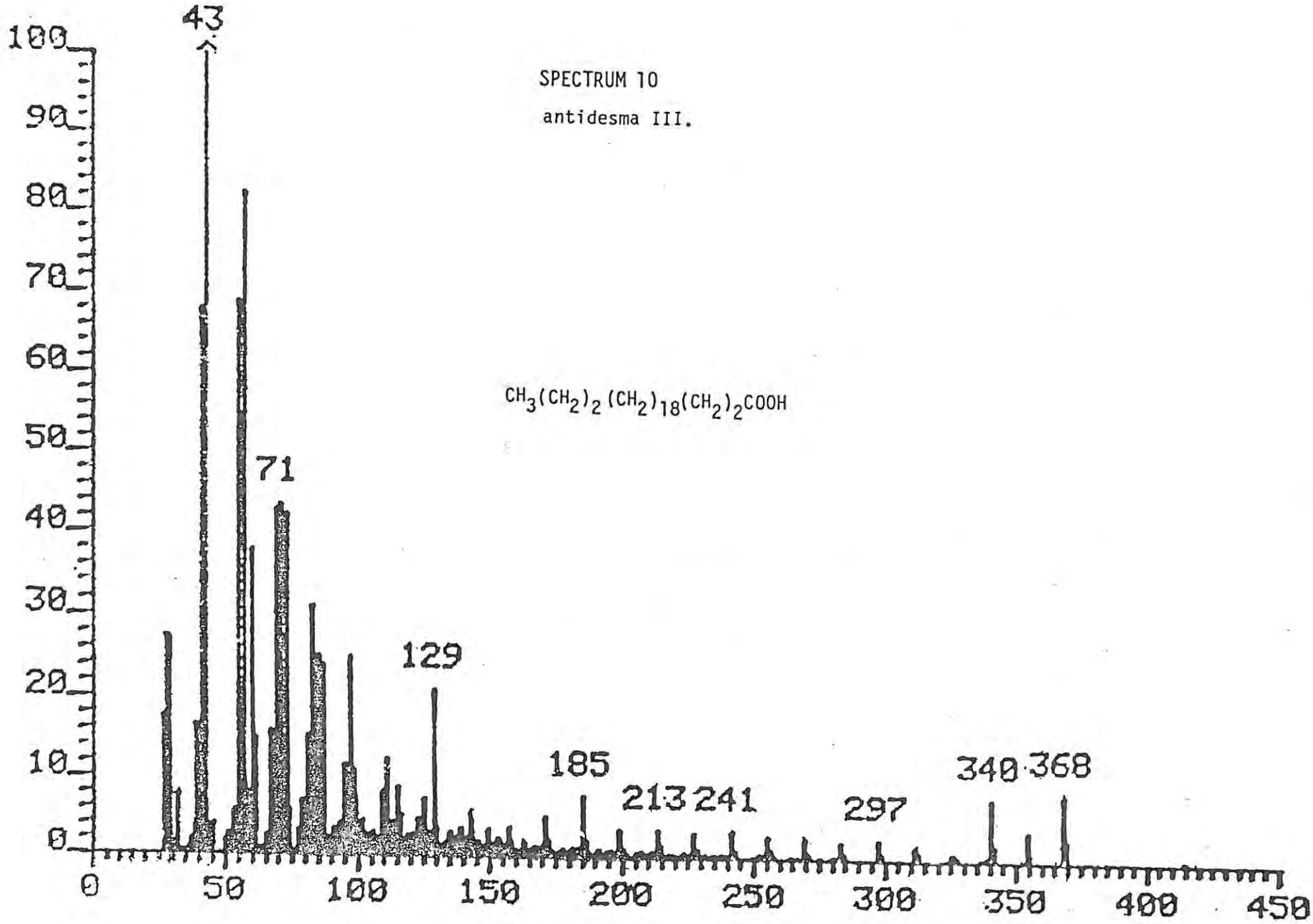
SPECTRUM 9

antidesma I derivative.



SPECTRUM 10

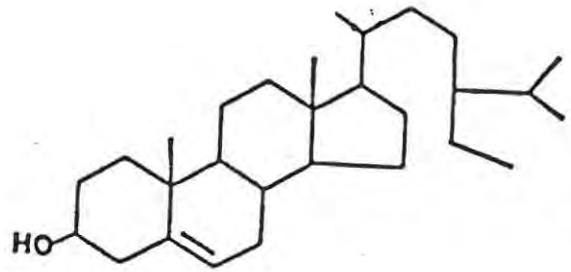
antidesma III.



$CH_3(CH_2)_2(CH_2)_{18}(CH_2)_2COOH$

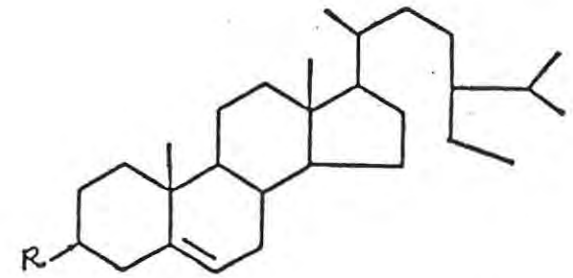
SPECTRUM 11

antidesma IV.



SPECTRUM 12

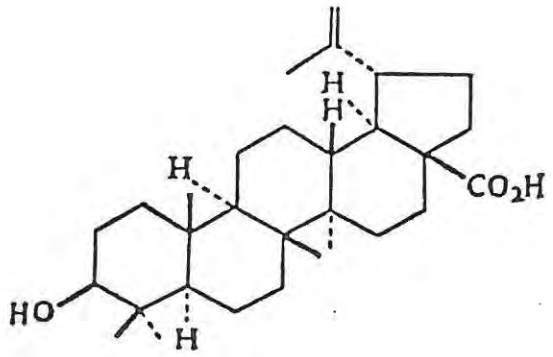
antidesma IV acetate.



$R = CH_3COO$

SPECTRUM 13

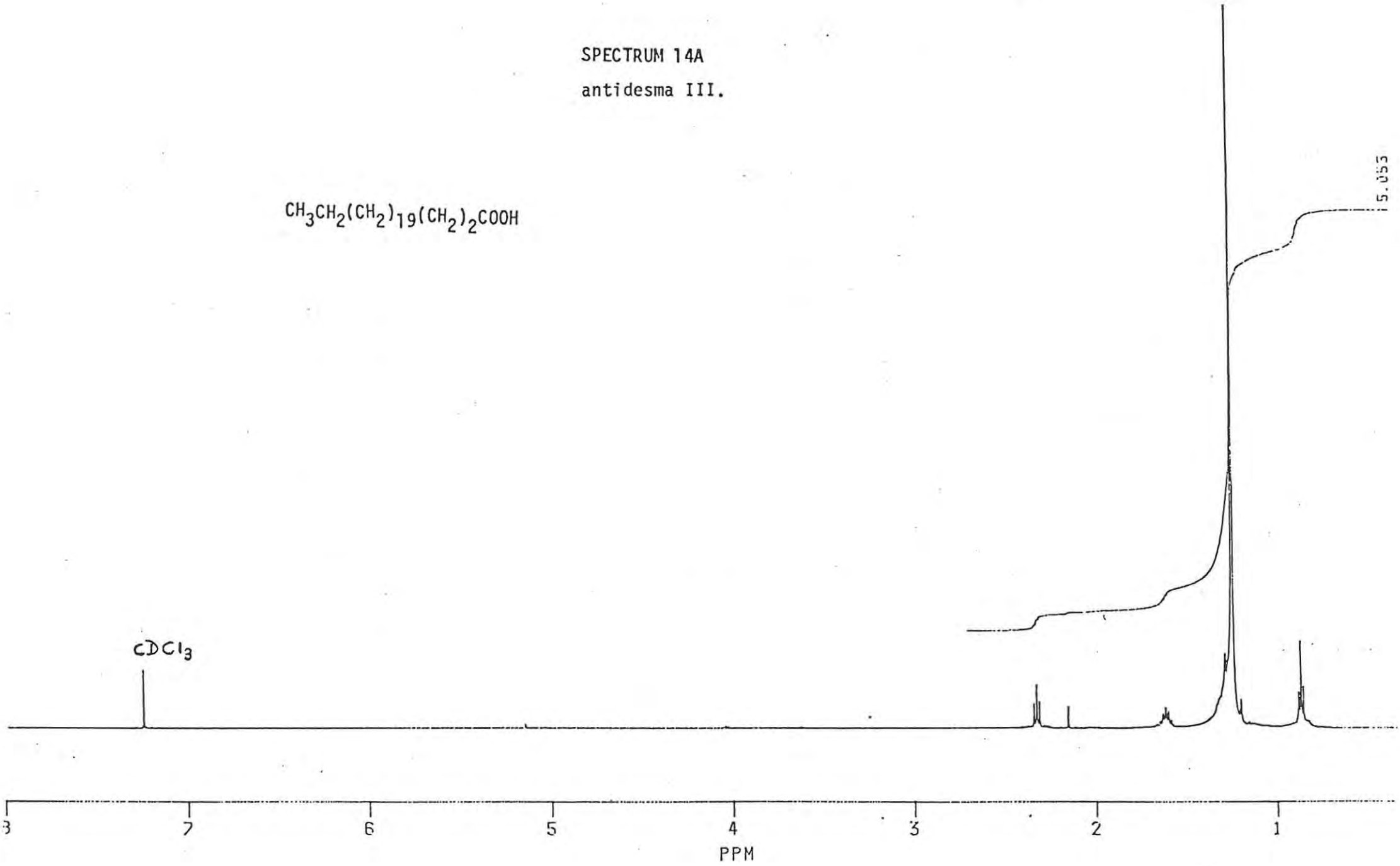
antidesma I



SPECTRUM 14A

antidesma III.

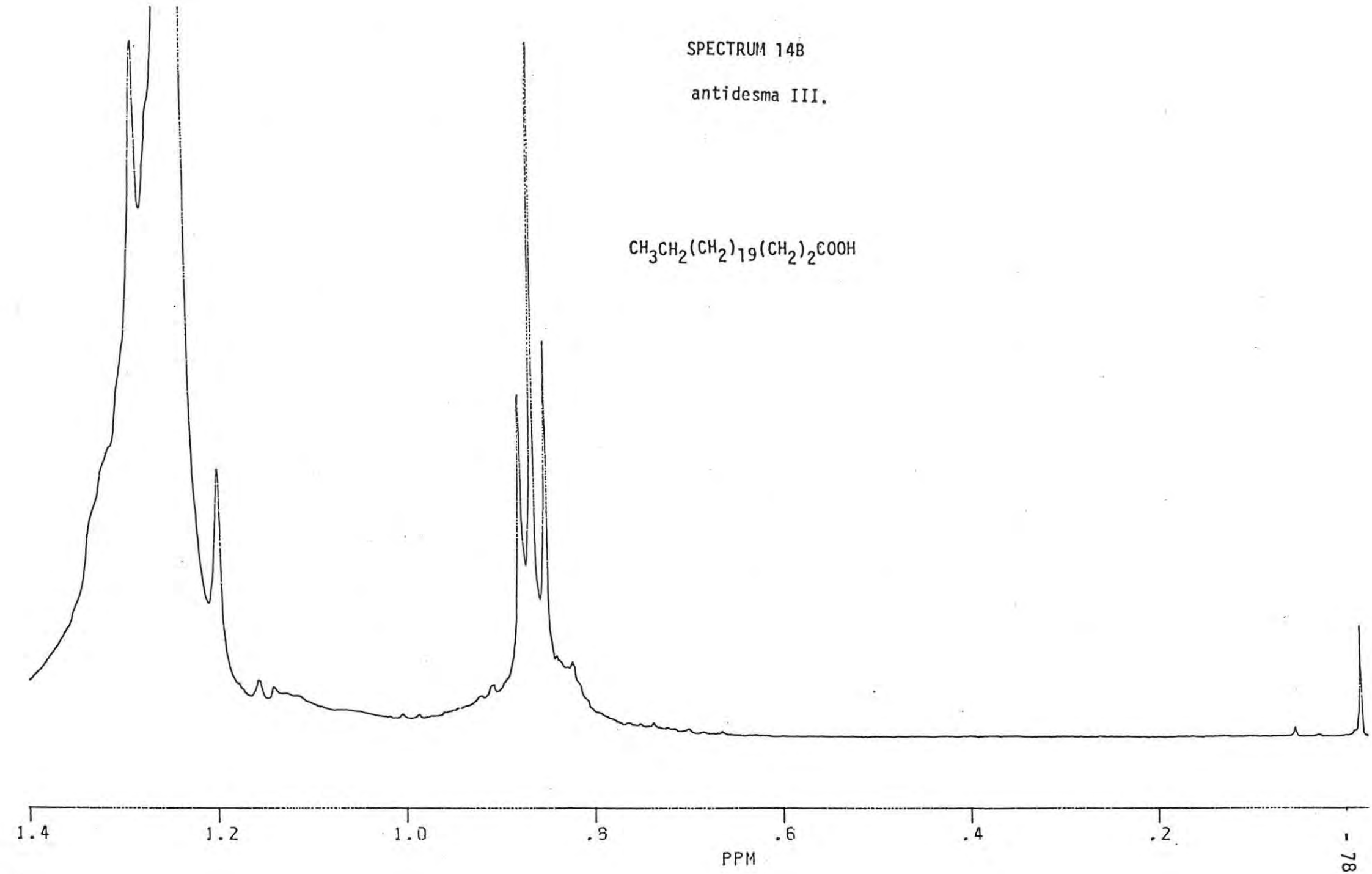
$CH_3CH_2(CH_2)_{19}(CH_2)_2COOH$



SPECTRUM 14B

antidesma III.

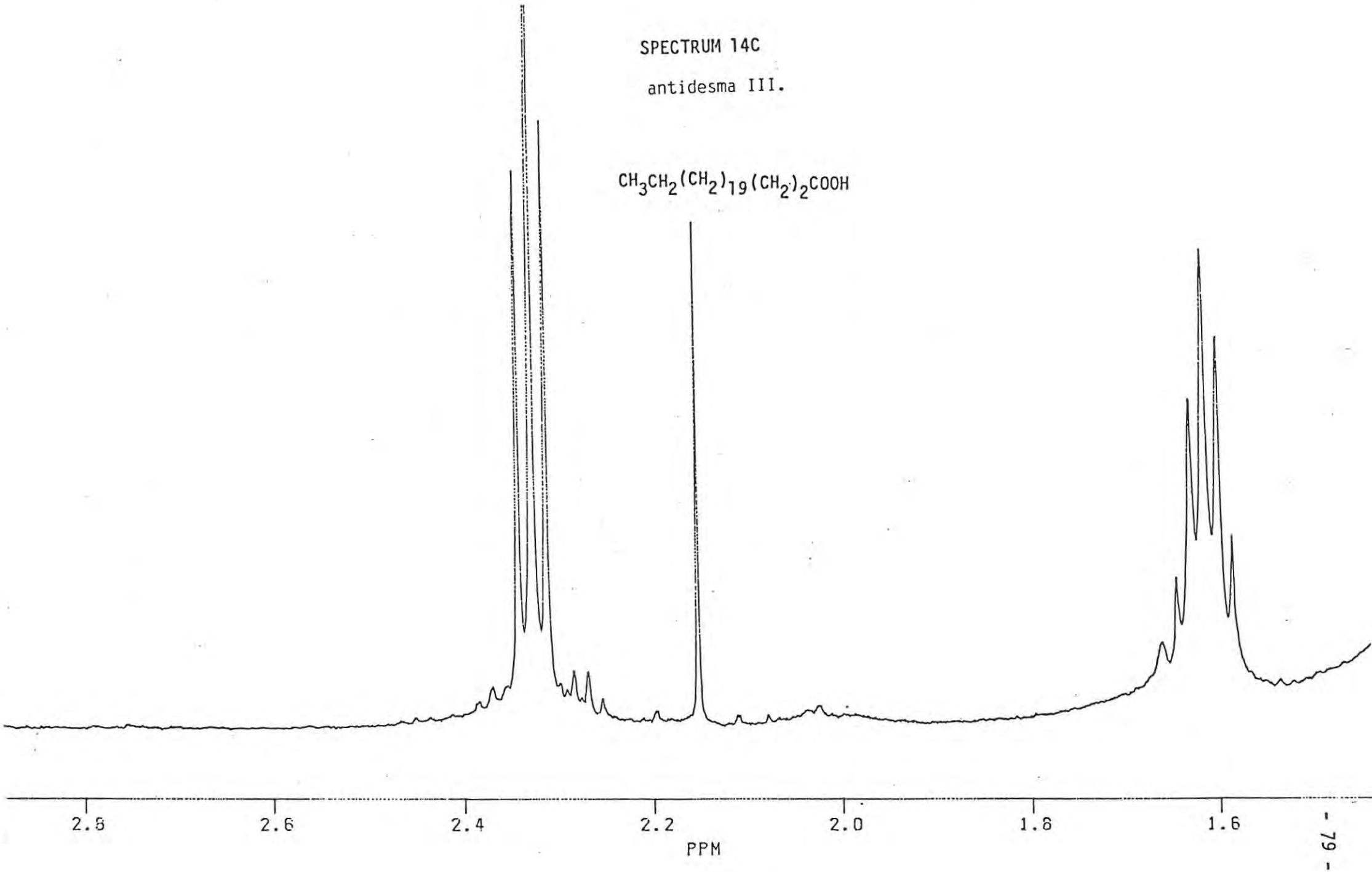
$CH_3CH_2(CH_2)_{19}(CH_2)_2COOH$



SPECTRUM 14C

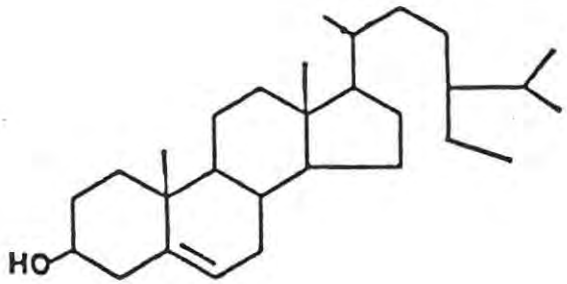
antidesma III.

$CH_3CH_2(CH_2)_{19}(CH_2)_2COOH$



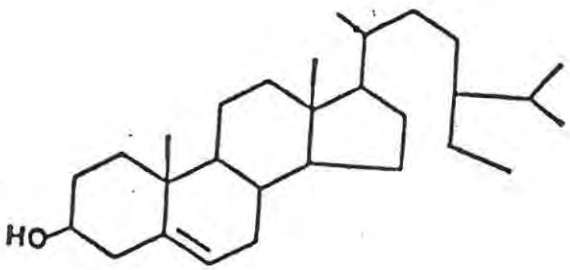
SPECTRUM 15A

antidesma IV.



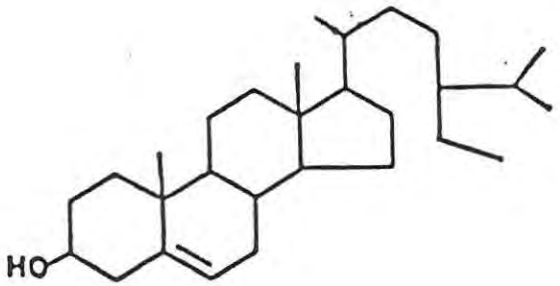
SPECTRUM 15B

antidesma IV.



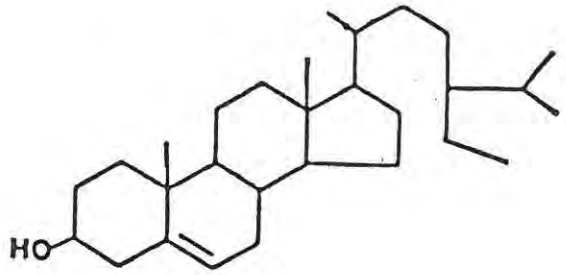
SPECTRUM 15C

antidesma IV.



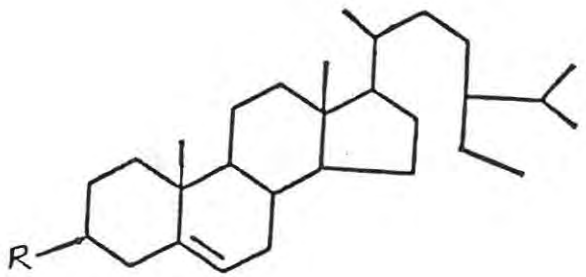
SPECTRUM 15D

antidesma IV



SPECTRUM 16A

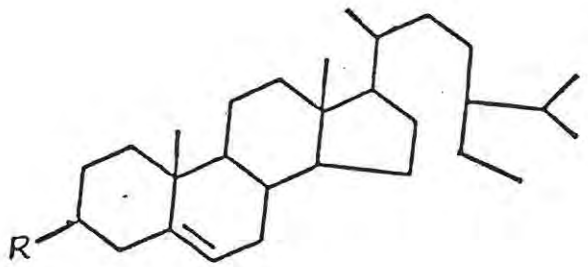
antidesma IV acetate.



R = CH_3COO

SPECTRUM 16B

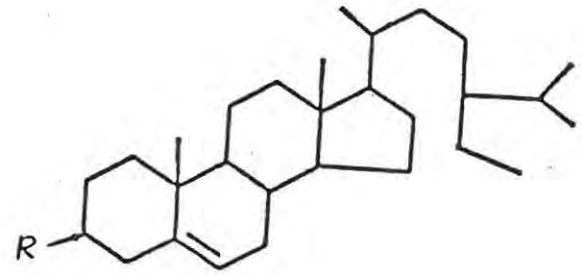
antidesma IV acetate.



$R = CH_3COO$

SPECTRUM 16C

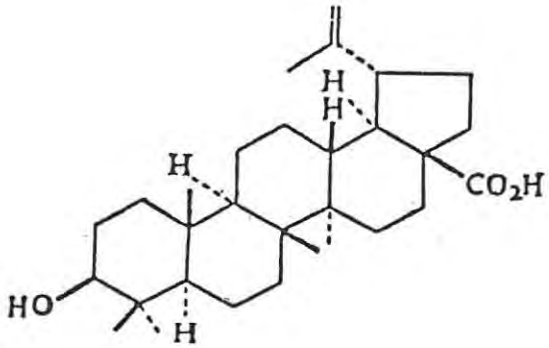
antidesma IV acetate.



$R = CH_3COO$

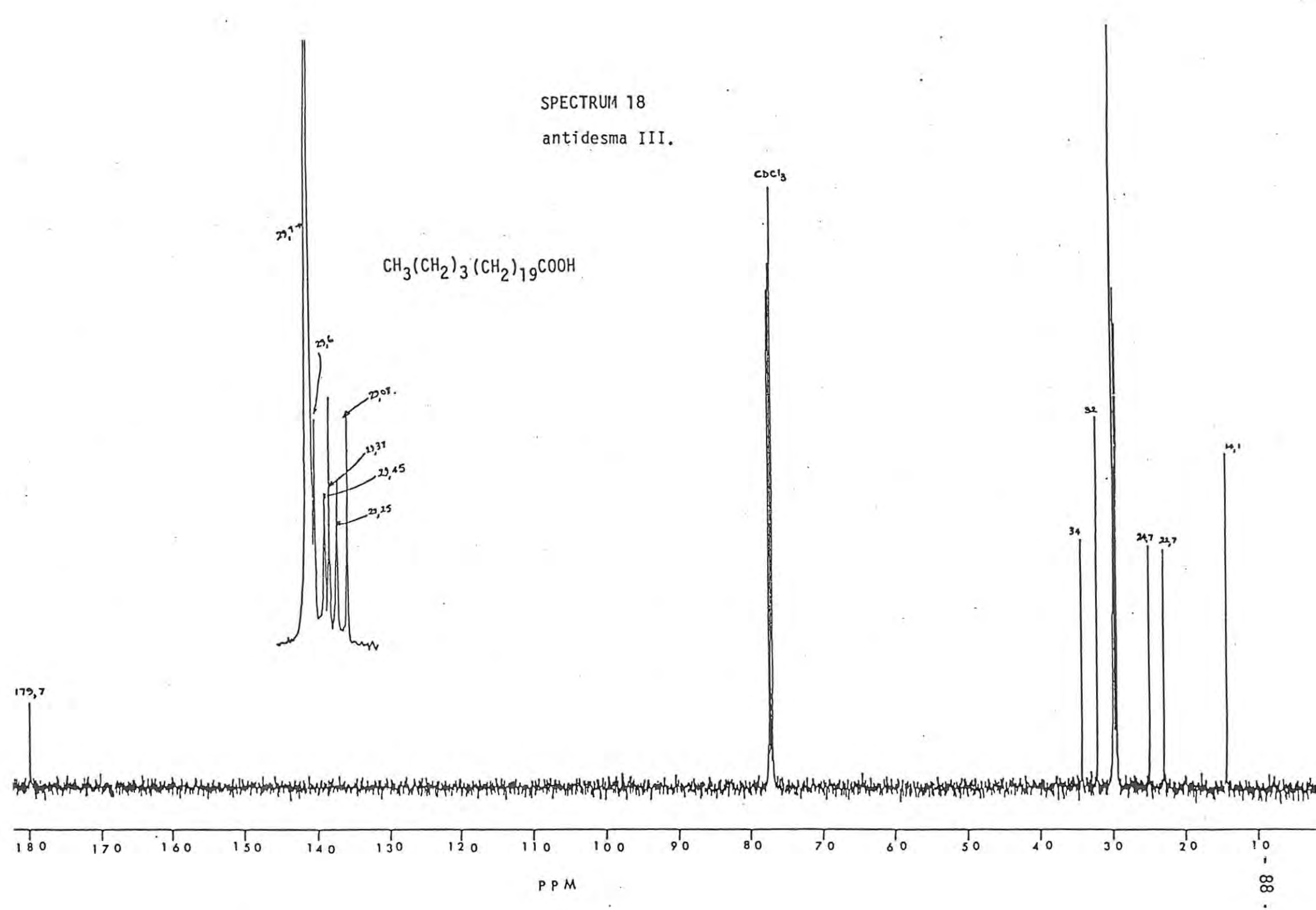
SPECTRUM 17

antidesma I.



SPECTRUM 18

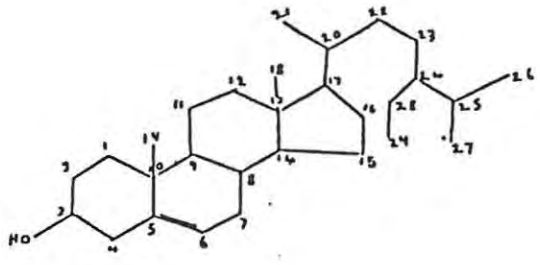
antidesma III.



$CH_3(CH_2)_3(CH_2)_{19}COOH$

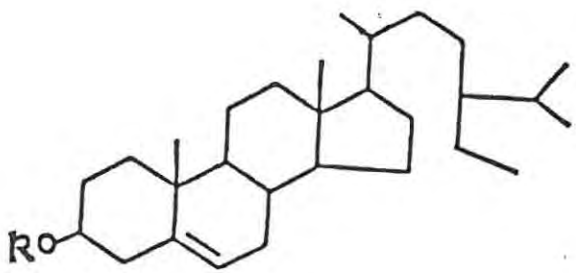
SPECTRUM 19

antidesma IV



SPECTRUM 20A

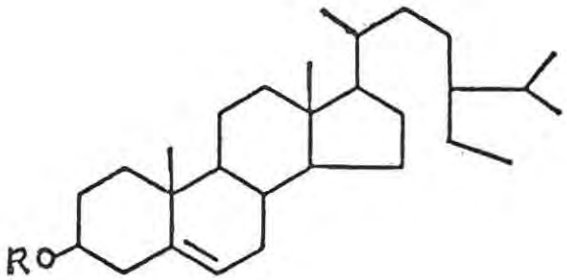
antidesma IV acetate.



$R = CH_3CO$

SPECTRUM 20B

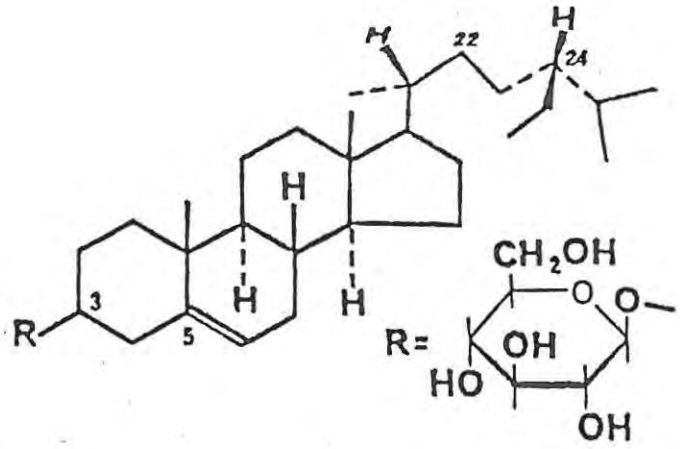
antidesma IV acetate.

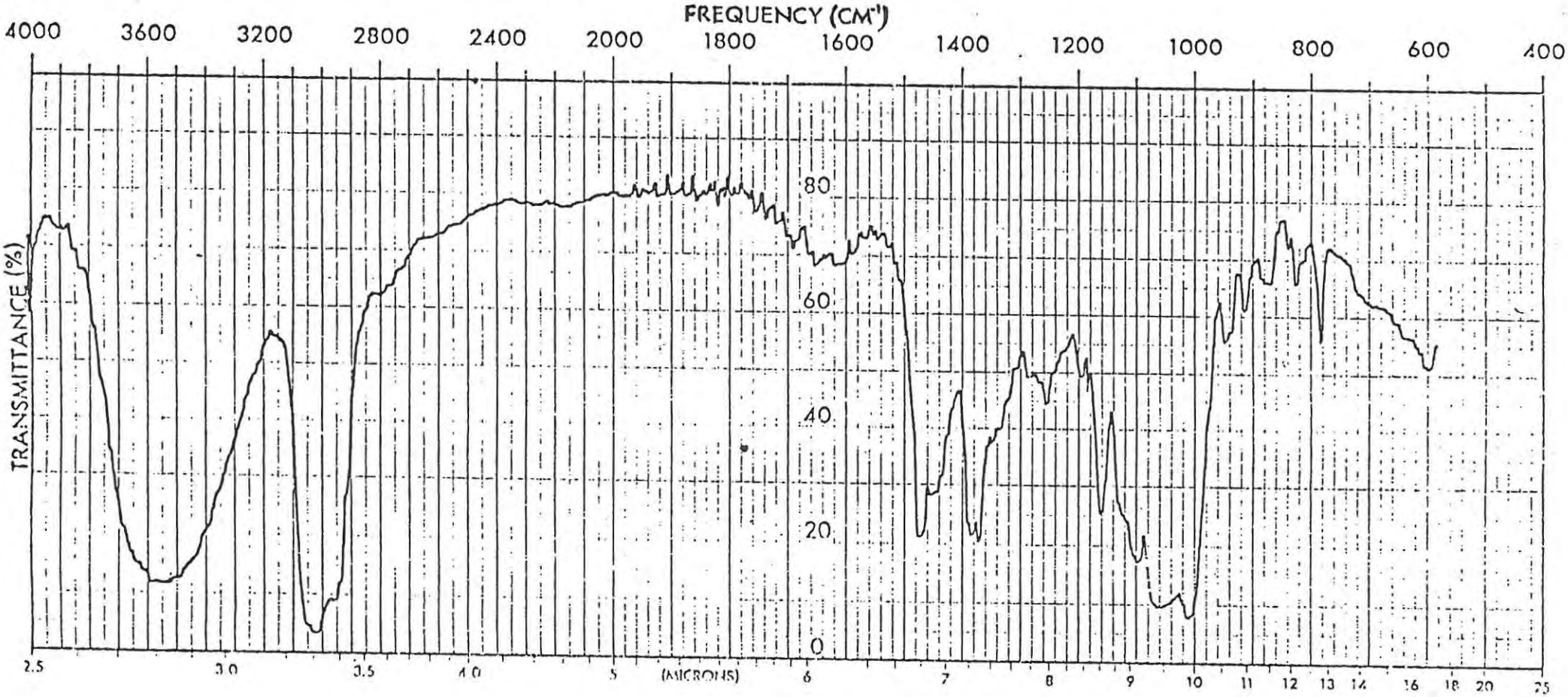


R = CH_3CO

SPECTRUM 21

ß-sitosteryl-ß-D-glucoside





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