PHYTOCHEMICAL INVESTIGATIONS ON *PHASEOLUS SUBTINUS*, *GLEDITSIA TRIACANTHOS* AND *ASTUM GRAVEOLENS*.

BY

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A thesis presented for the degree of

DOCTOR OF PHILOSOPHY

in the

University of Aston in Birmingham

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SUMMARY.

A phytochemical review of the plants under investigation, Phaseolus coccineus, Gleditsia triacanthos and Arium creweolens was made.

The roots, rhizomes and stems of runner bean (Phaseolus coccineus) have been subjected to phytochemical investigation. Fatty acids were isolated, converted to methyl esters and analysed by GLC. The results indicated that the fatty acids of the roots (0.56% w/w) were mainly palmitic and oleic, of the rhizomes (0.50% w/w) mainly palmitic, stearic and oleic, while those of the stems (0.32% w/w) were principally palmitic, lauric and oleic acids.

Further, the unsaponifiable fractions of these parts (0.1% w/w), rhizomes (0.96% w/w) and stems (1.31% w/w) have been examined by spectroscopic techniques resulting in the isolation and identification of three major compounds, namely β-bergamotene, β-sitosterol and 18 n-alkanes [illegible].

A study of the saponin content has revealed that the roots contain four saponins, one of which has been identified as soyasapogenol-C, initially present with D-glucose, D-fructose and L(+) arabinose constituting the sugar moiety. In addition two hydrocarbons have been isolated with the saponin fraction, of which one was identified as n-triacontane. Two sterols in the methanol extract remain unidentified due to insufficient sample obtained. Only soyasapogenol-C has been positively identified in the rhizomes whilst from the stems soyasapogenol-C, soyasapogenol-B and the hydrocarbon n-triacontane have been identified.

Similar studies have also been made on the pods and seeds of Honey-locust (Gleditsia triacanthos). Fatty acids from the pods (0.1% w/w) and seeds (2.0% w/w) have been identified in both cases principally as palmitic and oleic acids.

The unsaponifiable fractions of the pods (0.4% w/w) and the seeds (0.4% w/w) have been found to contain the hydrocarbon, n-triacontane

and the sterols β-sitosterol, stigmasterol and brassicasterol in the former but only the same three sterols in the latter.

A saponin study of the pods has revealed ten genins after hydrolysis of which two have been identified as oleanolic and echinocystic acids along with the sugars D-glucose, L(+) arabinose and L(+) rhamnose. An identical study of the seeds produced seven genins from which the same two acids have been isolated as the major components. The sugars D-glucose, L(+) arabinose, glucuronic and galacturonic acids have also been identified. The aqueous extract of pods revealed ten genins after hydrolysed the saponin fraction of which oleanolic and echinocystic acids and n-triacontane have been isolated as the major components. The sugars D-glucose, L(+) arabinose and galacturonic acid have also been identified.

The alkaloid content of the pods (0.25% w/w) was found to contain tyramine and N-methyl- β -phenyl ethylamine.

Furthermore the pods were found to contain D-galactose and D-mannose as galactomannan (10% w/w).

Finally, a study has been made on celery stems (Apium graveolens). Fatty acids (0.55% w/w) have been isolated and identified as mainly oleic and palmitic acids.

The unsaponifiable fraction (1.7% w/w) has been found to contain nine compounds of which three were isolated in the pure form in sufficient quantity to allow positive identification of two as n-hexacosane and 1,3,5,7-tetra-ene-13-dimethyl-tridecane and the third tentatively as Indosterol which showed a significant anti-inflammatory activity. In addition n-heptacosane, n-undecosane, apigenin-7-glucoside and luteolin have been isolated and identified. A further nine flavonoids have also been separated in trace quantities.

Key words: Phaseolus coccineus, Cladrastis kentukea, Apium graveolens, Chromatography, Spectroscopy

To my family

ACKNOWLEDGEMENTS.

I would like to express my particular thanks to Dr. C. R. A. Veitch for his help and encouragement throughout the experimental work and throughout the preparation of this thesis.

I would like to thank Mr. F. C. G. Edwards for his encouragement.

Dr. L. E. Watts (Plant Breeding Department, Sutton Seeds) is thanked for supplying the runner bean.

Professor P. H. Davis (Royal Botanical Gardens, Edinburgh) is thanked for identifying Honey-locust fruits.

I would like to thank Dr. D. A. Lewis for his help in carrying out the biological studies.

Finally, I would like to express my thanks to the Ministry of Higher Education (Republic of Iraq) for their generous financial support throughout the project.

ABBREVIATIONS.

v.s	very strong
w	weak
s	strong
m	medium
b	broad
eV	electron voltage
KV	accelerator potential
A.A.	Acetic acid
Rt	Retention time
Rf	Rf value
min.	minute
PC	paper chromatography
I.R.	Infra-red
M.S.	Mass spectrum
GLC	Gas-liquid chromatography
NMR	Nuclear magnetic resonance

CONTENTS

I - INTRODUCTION

I-1- General Introduction.

The use of medicinal preparations derived from plants dates back to antiquity[1], but with the introduction of synthetic organic chemicals in the past century the dominant role of such drugs has been increasingly challenged. However the search for new and useful natural products for pharmaceutical purposes continues. This is not surprising since only about 4% of the estimated 350,000 known botanical species have been screened or investigated for biologically active principles[2]. Green plants are essential for all animal life on earth since they convert solar energy into organic carbon compounds which in turn are used to produce essential foods. In addition, all green plants also contain growth regulants and the necessary photosynthetic apparatus.

The formation of these carbon compounds which may be considered the primary activity of green plants, is by no means the most interesting of plant activities. Secondary metabolites have been in use as drugs, as stimulants, as dyes and for many other purposes throughout recorded history[3]. Since these products do not occur in all plants, they were largely ignored until several decades ago by those interested in chemical plant physiology. They have been called secondary metabolites, and have been considered as waste products of the metabolic processes taking place in plants.

Just as each of the billions of individual human beings that exists now or haveexisted in the past, haveor havehad a different set of finger prints, a characteristic mode of walking, and an individual chemistry which makes organ transplantation difficult so has the chemistry of plants been very versatile.

With the aid of chromatography and other modern analytical

methods, the number of new compounds isolated from plants totals about 1000 annually[3].

The fact that a plant is used for food does not necessarily mean that it is harmless. Many plants consumed by man and animals contain harmful ingredients, e.g. potato tubers which turn green on exposure to light build up harmful or even fatal quantities of a poisonous alkaloid (α-solanin). Another example is the toxic properties of the roots of wild runner bean Phaseolus multiflorus[4].

The early scientific workers in the phytochemical field failed to appreciate the extreme complexity of the materials they were trying to investigate and were almost entirely lacking in the techniques necessary for real progress[1]. The need for new drugs such as cortisone which was originally isolated from adrenal cortex and is now commercially synthesized from certain bile acids of cattle, illustrate the principle. Since supply is limited by the number of cattle slaughtered, a cheaper and potentially unlimited plant source of a suitable raw material for the production of this and other therapeutic agents would obviously be of inestimable value[5].

I-2- TAXONOMY AND REVIEW OF THE PLANTS.

I-2-A- Family Leguminoseae

a. Phaseolus coccineus

b. Gleditsia triacanthos

I-2-B- Family Umbellifereae

Apium graveolens

I-2-A Family Leguminoseae.

There is still no general agreement regarding the treatment of the major divisions which are sometimes recognized as separate families. Caesalpiniaceae, Mimosaceae, and Fabaceae "Papilionaceae" or as subfamilies of single family, the Leguminoseae "Fabaceae"-Caesalpinioideae, Mimosoideae and Lotoideae "Papilionoideae, Faboideae, Papilionate". A fourth group, the Swartzioideae, is sometimes recognized as a further subfamily[7]. Bentham[8] treated the Leguminosae as a "vast order, clearly natural, divided into 3 suborders by adequately defined characters", the suborders (equivalent to subfamilies today) were:

I- Papilionaceae

This suborder contains the following tribes, several of which in turn are divided into subtribes .....

1. Podalyrieae (26)
2. Genisteae (56)
3. Trifolieae (6)
4. Loteae (4)
5. Galegeae (54)
6. Hedysareae (46)
7. Vicieae (6)
8. Phaseoleae (47)
9. Dalbergieae (25)
10. Sophoreae (30)
11. Swartzieae (5)

II- Caesalpinieae

This suborder is divided into the following tribes.....

12. Sclerolobieae (10)
13. Eucaesalpinieae (16)
14. Amherstieae (23)
15. Cynometreae (10)
16. Cassieae (11)
17. Bauhinieae (3)
18. Dimorphandreae (3)

III- Mimoseae.

This suborder is divided into the following tribes.....

19. Parkieae (2)
20. Adenanthereae (12)
21. Eumimoseae (5)
22. Acacieae (1)
23. Ingeae (8)

The number of genera are given in brackets after each tribe.

Taubert in "Pflanzenfamilien"[9], recognised three subfamilies like Bentham but in a different sequence, and the tribal arrangements showed some differences in sequence and content:

I- Subfamily Mimosoideae.

This is divided into the following tribes.....

1. Ingeae
2. Acacieae
3. Eumimoseae
4. Adenanthereae
5. Piptadenieae
6. Parkieae

II- Subfamily Caesalpinioideae.

This is divided into the following tribes.....

1. Dimorphandreae
2. Cynometreae
3. Amherstieae
4. Bauhinieae
5. Cassieae
6. Kramerieae
7. Eucaesalpinieae
8. Sclerolobieae
9. Swartzieae

III- Subfamily Papilionatae.

This is divided into the following tribes.....

1. Sophoreae
2. Podalyrieae
3. Genisteae
4. Trifolieae
5. Loteae
6. Galegeae
7. Hedysareae
8. Dalbergieae
9. Vicieae
10. Phaseoleae

The papilionaceae "Lotoideae" is much larger than either of the

other two subfamilies, having about 500 genera and 12,000 species. About 30 genera contain 100 or more species[11]. The subfamily papilionaceae has a world-wide distribution with most of the woody representatives "trees and climbers" in the tropics and the southern hemisphere.

a. Phaseolus coccineus.

Phaseolus coccineus (Synonym Phaseolus multiflorus) belongs to the suborder Papilionaceae. The plants are climbing or scrambling perennials. The leaflets are 5-10 cm. in length and are acuminate and oval in shape (Fig. 1). The racemes are many flowered and are longer than the leaves while the corolla which is usually scarlet in colour sometimes has white wings and a keel. The length is between 15-30 mm. while that of the calyx is 0.5 mm. The beak of the keel forms 1-1$\frac{1}{2}$ of a spiral. The legume with dimensions of 10-20 x 1.5-2 cm. is rough and compressed. The seeds are 13-25 mm. in length and are white or variegated, smooth, flattened and reniform P.coccineus is widely cultivated for its edible legumes and seeds (runner bean).

The genus phaseolus comprises about 160 species. P.coccineus, is the most common variety in the United States, often listed in seed catalogues as an ornamental plant with edible seeds[6] (Fig. 2).

Indigenous runner bean cultivation is most highly developed in the cool humid uplands of Chiapas and Guatemala in the Oakpine regions above 1800 m. The strongly vining runner beans are interplanted with maize in some fields in plots adjacent to houses. In the house gardens, they are treated as perennials with a life span of two to several years. The roots persist for seven years and are regarded as poisonous[6].

Runner beans, unlike P.vulgaris, have the advantage of better resistance to low temperature and strong winds and are only susceptible to a limited number of diseases[10].

The roots of P.coccineus (Fig. 4) are tuberous and therefore this species is perennial in regions where no frost occurs. The aerial

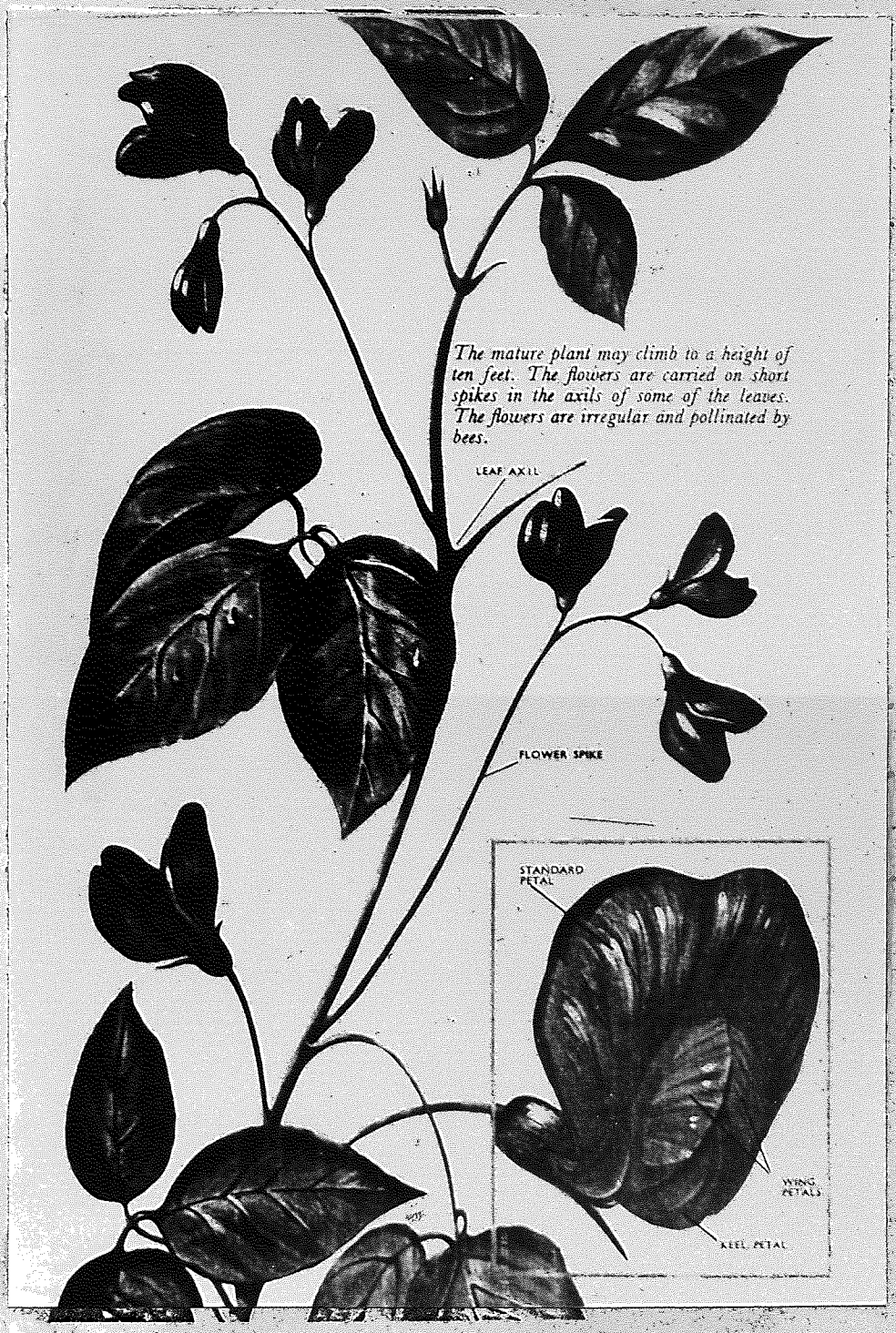


Fig. 1 The mature plant and the flowers of the runner bean.

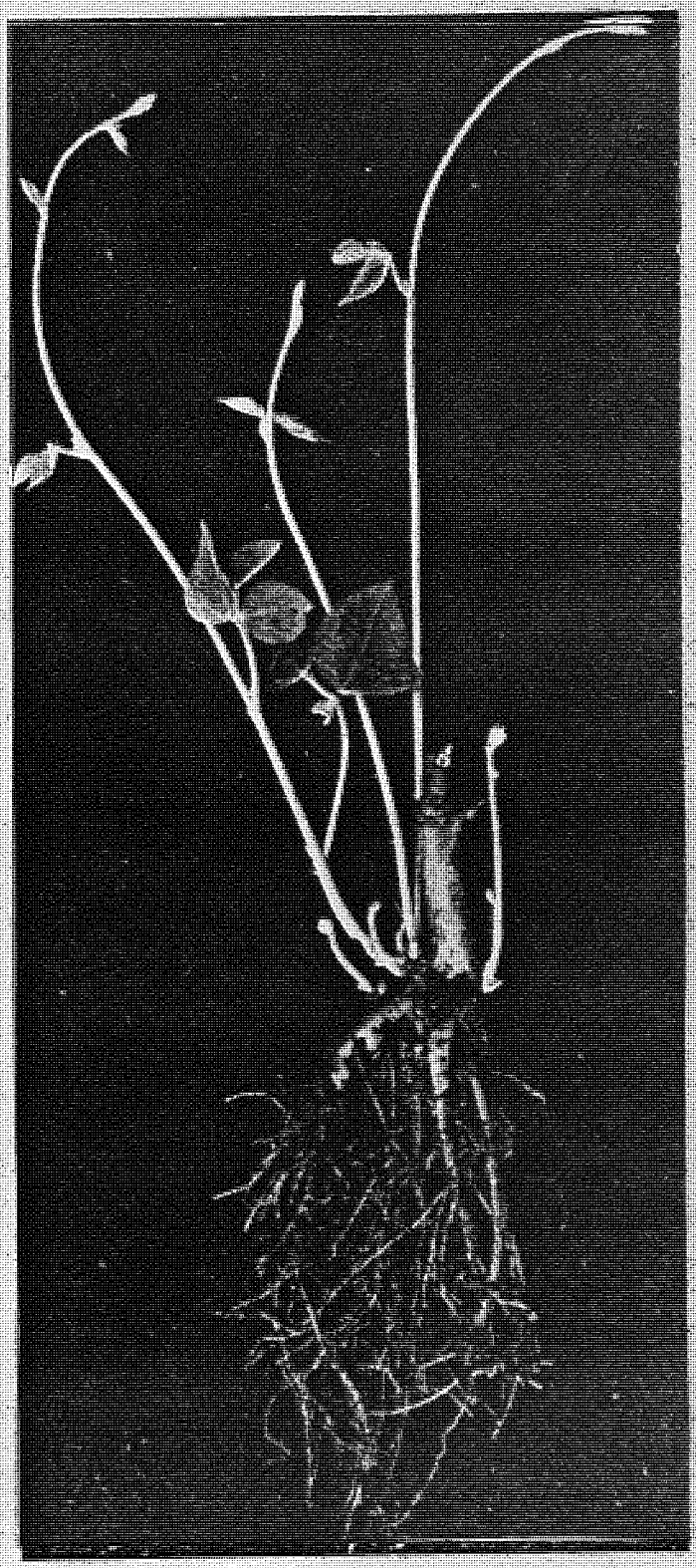


Fig. 2 The mature runner bean Phaseolus coccineus.

Θ 1300 B.C. Rio Zape

O 7500-9000? B.C. Ocampo

X 2200 B.C. Tehuacan

Fig. 3 Archeological distribution of P. coccineus and some early dates in years before present time.

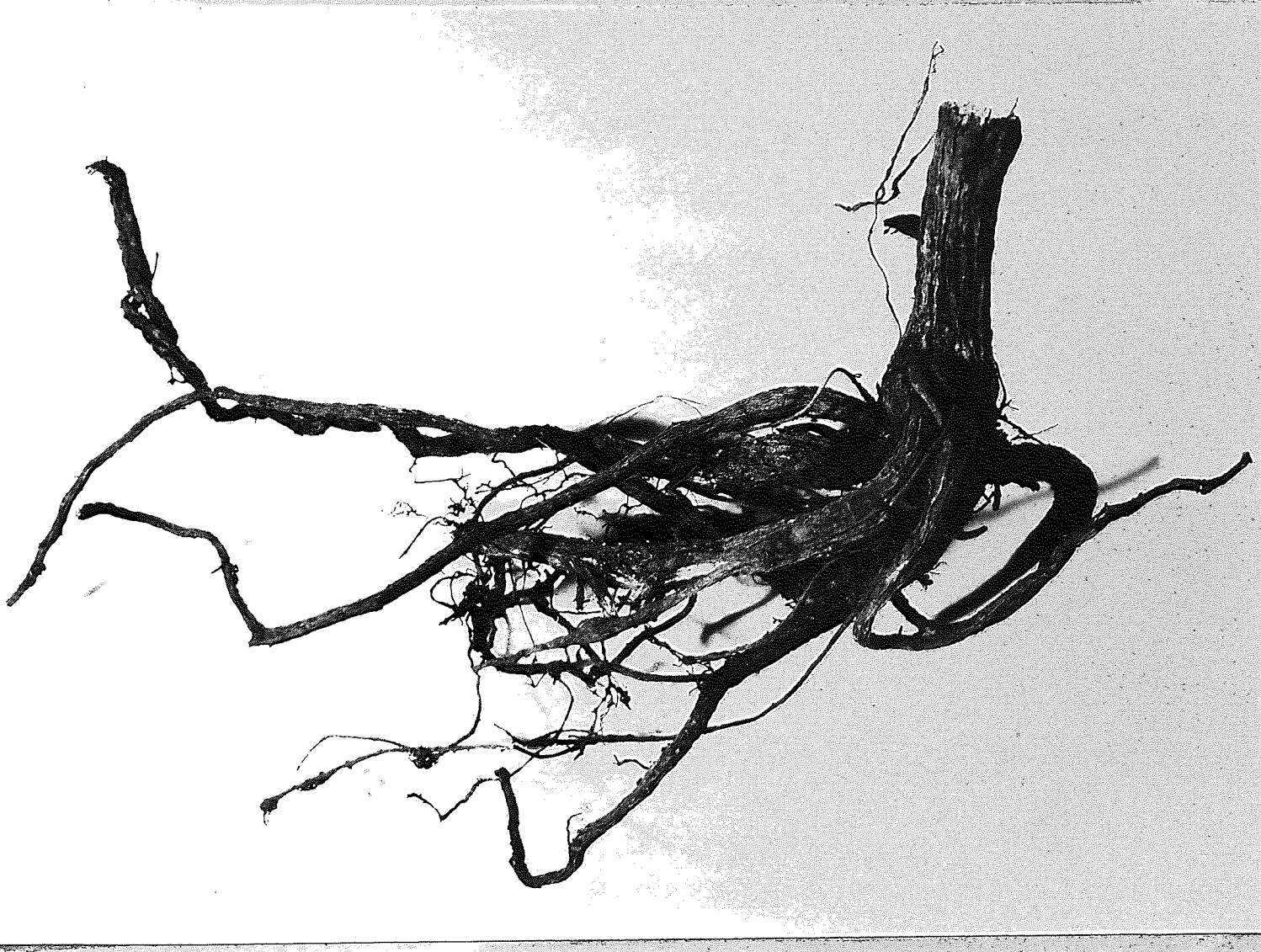


Fig.4 Roots and rhizome of P.coccineus leguminoseae.

parts of the plant may largely die off but the buds in the axils of the seed lobes or those situated higher on the stem break in autumn or else in spring and thus form new stems.

In 1929, Faschingbaver and Kofler[11] reported an account of acute poisoning with cyanosis from the eating of red beans and handling the fire beans (P.coccineus). Marked respiratory and circulatory disturbances were also noted. Several years later Hintz et al[12] conducted a series of experiments to obtain information on the aetiology of "kidney bean toxicosis". Some of the deaths observed among weanling rats fed on high levels of raw kidney beans were attributed to hypoglycaemia. A suggestion was made that hypoglycaemia resulted because raw beans contain factors such as hemagglutinin and trypsin inhibitors that decrease feed intake and feed utilization. Autoclaving or soaking the beans in water for 72 hrs. eliminated the toxic effect[12].

Among 704 legume samples analysed the seed lipid content averaged only about 5.5% while the content of many of the legume seeds used for food is between 1 and 2% only[7]. Few fatty acids and glycerides of leguminoseae have been investigated in any detail, and the literature is scant when compared with published reports on the seed oils from other families such as Cruciferae and Compositae[13].

Relatively complete data is available on the fatty acid composition of seed oils from species in 104 leguminous genera. Oleic and linoleic acids are the principal components. Together they average about 65% of the total acids present. Percentages of these two acids are inversely correlated; some of the legume oils are rich in linoleic, whereas in others oleic acid is in greater amounts[7].

Some saponin-containing members of the leguminoseae family, such as liquorice, have found wide application in industry and medicine. The literature does not cite the presence of saponin in other members of

this family except [redacted] from Phaseolus radiatus[13] a crystalline saponin contained as the aglycone Soyasapogenol-C and, in the carbohydrate moiety glucuronic acid, glucose, arabinose and rhamnose. In 1970, Chirva[14] isolated β-sitosterol and fine saponin glycosides from the seeds of P.vulgaris.

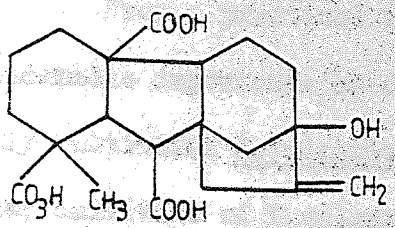
Examination of the secondary constituents from most classes of legumes has been inspired mainly by pharmacological and chemical interests. Much of the work on triterpenoids, particularly on the saponins of soyabean, lucerne and other forage crops, has similarly been initiated because of the potential toxicity of these substances to cattle and other farm animals[15].

A large number of structurally interesting di- and triterpenes have been isolated and characterised. They show considerable promise as taxonomic markers in the family and furthermore intensive, systematic surveys of these substances could be especially rewarding[7]. Although the lower terpenoids "mono- and sesquiterpenes" are clearly present in legume plants, they do not appear to have been studied at all extensively. Only a few species, e.g. Acacia fornesiana[16] have been analysed for these fragrant components, but it appears that essential oils do not occur as abundantly in the leguminoseae.

Bentley in 1952[17] isolated malonic acid together with citric, malic, lactic, succinic, aconitic and fumaric acids from the aqueous extract of runner bean laeves by paper chromatography. He also examined the water extract of the stem tissue, and found 0.7 citric acid, 1.1 malic acid, 2.1 malonic acid, and 0.15 succinic acid mg/gm. fresh weight.

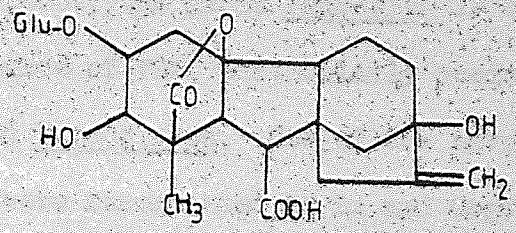
The immature fruits of P.coccineus have yielded no less than seven gibberellins, GA_1, GA_5, GA_6, GA_8, GA_{17}, GA_{19} and GA_{23}[18,19,20,21]. MacMillan et al[21] isolated GA_1 and GA_5 using 87.3 kg. of immature seeds of P.multiflorus and obtained the overall yields of 50 mg. GA_1 and 195 mg.

of GA_5. Of these seven, one GA_{17}(1) is reported for the first time from this plant[19]. In addition, the first sugar-bound gibberellin, namely GA_8-3-β-glucoside (2) has been isolated from this source; overall yield of the glucoside was 4×10^{-5}% and 3760 Kg. of the beans were needed for the isolation[22].



(1)

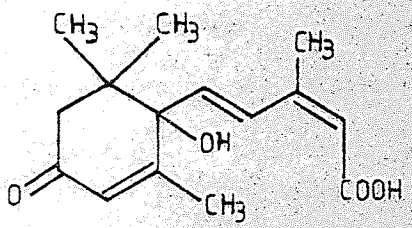
Gibberellin A_{17}



(2)

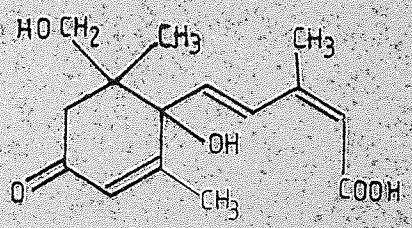
Gibberellin A_8-3-β-glucoside

Phaseic acid (3) has also been found in P.coccineus seeds along with the gibberellins and has most of the structural features associated with abscisins[23] (4) except that one of the gem-dimethyl groups is replaced by $-CH_2OH$[24]. MacMillan et al[25] isolated 6 mg. of phaseic acid from 25 kg. of the immature seeds of P.multiflorus.



(4)

Abscisic acid



(3)

Phaseic acid

It has been known for many years that certain plants contain substances capable of agglutinating erythrocytes. The highest

concentration of these phytohaemagglutinins "PHAS" are present in the seeds, from which they are readily extractrable with normal saline solution. Although the existence of haemagglutinins in various plants had been known for many years[26] the first use of the PHA of P.vulgaris in the separation of leukocytes was made by Li and Osgood[27] in 1949 when they accidentally rediscovered this substance.

From a practical viewpoint, the genus Phaseolus is of considerable importance as a source of PHA, particularly seeds of the widely cultivated P.vulgaris and P.coccineus. Use of the navy and red kidney cultivars of P.vulgaris were recommended by Li and Osgood[27] for the preparation of non-specific PHA suitable for the agglutination and sedimentation of mature erythrocytes from blood samples. The PHA of P.coccineus species agglutinate the erythrocytes of all human blood groups and are therefore described as "non-specific"[28]. PHA is present in the mature seed, but not in the developing seed of P.coccineus [29].

b. Gleditsia triacanthos.

Commonly called the honey locust G.triacanthos, is a leguminous tree which grows up to 20-30 m. in height and is between 0.5-0.7 m. in diameter. Spines which are 5-10 cm. length are usually present. Pinnate "leaflet size 20-30 x 7-12 mm." or bipinnate leaves "leaflet size 8-20 x 3-8 mm. oblong" which are remotely dentate are present. The flowers are greenish-white in colour and are sessile in many flowered racemes. The fruit of G.triacanthos is 17-45 x 2-3 cm. long, brown in colour and contains 10-15 seeds [30] (Fig.5).

G.triacanthos is a native plant of North America but is now found in other countries as well. Honey locust is one of the economically important legumes. It is used as a timber source[7] as well as for ornamental purposes[31].

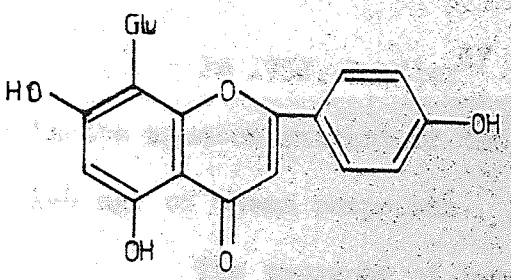
A number of chemical studies have already been done on this plant. As early as 1900, Goret[32] studied the mucilage content of the seeds and he recognized that they consisted mainly of the polysaccharide galactomannan (27%). In 1926, King[33] discovered that the honey locust (G.triacanthos) possessed pharmacodynamic activity. Ruddnck[34] extended these studies. Simons[35] successfully isolated an active principle which possessed marked oxytocic properties. He called this active principle hypoxysin.

Oester[36], in 1934 reported that the aqueous extract of leaves contained two active constituents. One was hypoxysin which had already been isolated by Simons[35] and the second constituent which he was unable to identify had a marked depressor action.

Leschziner[31] in 1970 examined the mucilage of the seeds and reported the homogenous galactomannose (15-20%) was composed of D-mannose and D-galactose in the molar ratios of 3.2-3.5 : 1. In 1974 McCleany and Matheson[37] studied the structural changes in galactomannan

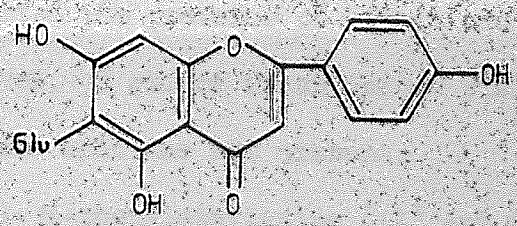
on germination of the honey-locust seeds, by measuring the viscosity, elution volumes on gel filtration and by ultracentrifugation. These changes were slight and consistent with a rapid and complete hydrolysis of a molecule once hydrolysis of the mannan chain started, β- mannanase activity increased and then decreased, paralleling galactomannan depletion. They also discovered that the seeds contained an active α-galactosidase.

Panova and Georgreva[38] studied the flavonoid content of the leaves, they managed to separate six flavonoids by using column chromatography but only three were isolated in the pure state. Flavonoid-A was identified as C-glucoside of saponaretin (isovitexin) type (6). Flavonoid-B was identified as luteolin-glucoside (7) and flavonoid-C was identified as vitexin (5).



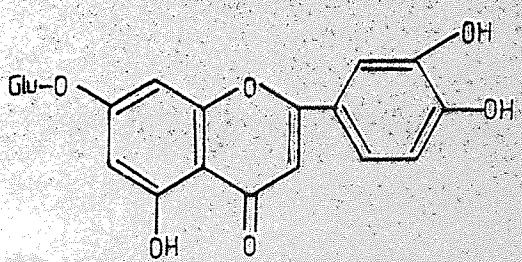
(5)

Vitexin



(6)

Isovitexin

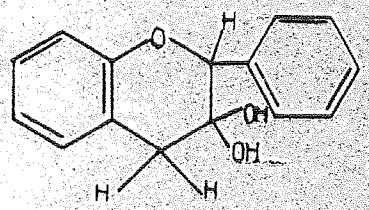


(7)

Luteolin-glucoside

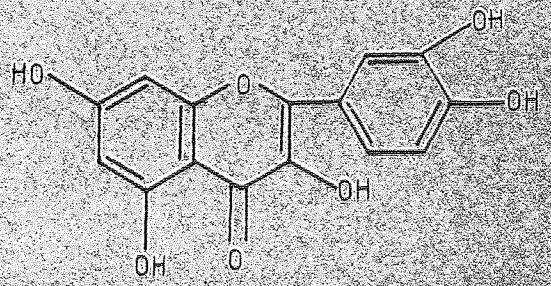
Weinges[39], in 1963 used fresh pods to isolate (+)-catechin (8), a condensed procyanidin, a diglucoside of quercetin (9) (m.p. 204-206°C) and an unknown monoglucoside (m.p. 213-215°C) with a formula of

$C_{21}H_{22-24}O_{12}$. Weinges disagreed with Gakhokidze[40,41] who reported the presence of 3-D-glucosyl-(-)-epicatechin (3,5,7,3,4-pentahydroxy flavone-3-D-glucoside) and flavone aglycone acrammerin (3,4,5,3-7-pentahydroxy-8-methoxy flavone). The reason for this disagreement was that Weinges could not isolate or detect any of these compounds by chromatography.



(8)

Catechin

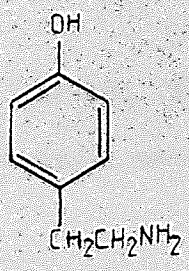


(9)

Quercetin

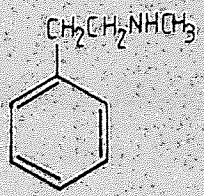
In 1952, Bentley[17] reported the presence of citric and malic acids in the aqueous extract of the leaves. 1 gm. of fresh leaves yielded 1-4 mg. of these compounds. Malonic acid was not present.

The presence of N-methyl- β-phenylethylamine (11) and tyramine (10) in the leaves was reported by Camp[42] in 1966. He reported their presence by comparative studies using thin layer chromatography.



(10)

Tyramine



(11)

N-methyl- β-phenylethylamine

Seriya[43] in 1975 studied the unsaponifiable part of the oil obtained from honey locust seeds and isolated a compound with a m.p. of 139°C (1% yield). This compound was reported to be β-sitosterol. The

m.p. of its acetate was 120-123°C and that of the benzoate was 145-145.5°C. In addition brassicasterol and stigmasterol were also found in small quantities.

In this study, pods and seeds were used which had been collected in Turkey.

Fig. 6 Turkish Vilayets and Grid system. Gleditsia triacanthos fruits sample were collected from the Vilayet Kütahya (28).

Fig. 5 The fruit of Honey Locust Gleditsia triacanthos.

I-2-B Family Umbelliferae.

The family Umbelliferae is often grouped together with two other families in the Umbellales[44], or in the Umbelliflorae[45], all members having small flowers in heads together with solitary ovules. The other two families in this grouping, the Cornuceae and the Araliaceae, are distinguished by being shrubs with succulent fruits and only slightly divided leaves.

The Umbellifereae has been recognised as a natural group since the earliest studies of plants dating back to Theophrastus[46]. The uniformity of the structural features produces considerable difficulty in classifying the members of this family and there has been disagreement between authors from the time of Linnaeus until the present day as to how the family should be subdivided. Koch (1824)[47] divided the family into fifteen tribes and Bentham (1967)[44] recognised three series and nine tribes. Drude's classification[48] was somewhat different from Bentham's as he recognised three subfamilies and twelve tribes. Calestani[49] in 1905 recognised four subfamilies and ten tribes often very different from other authors and Cerceau-Larrival[50] sub-divided the family considerably more than any previous author, producing five sub-families and twenty-seven tribes which were later increased to thirty-one.

The most accepted classification at the sub-family level is that of Drude[48] who recognised the sub-family Hydrocotyloideae, Saniculoideae and Apioideae. His classification is summarised below. It was derived from 231 genera:

Sub-family I Hydrocotyloideae

Tribe 1 Hydrocotyleae

Tribe 2 Mulineae

Sub-family II Saniculoideae

Tribe 3 Saniculeae

Tribe 4 Lagoecieae

Sub-family III Apioideae

Tribe 5 Echinophoreae

Tribe 6 Scandiceae

The tribe Scandiceae is further sub-divided into two sub-tribes:

6a Scandicinae

6b Caucalinae

Tribe 7 Coriandreae

Tribe 8 Smyrnieae

Tribe 9 Ammieae

This tribe is further sub-divided into two sub-tribes:

9a Carinae includes genus Apium.

9b Seselinae

Tribe 10 Peucedaneae

This tribe is divided into three sub-tribes:

10a Angelicinae

10b Ferulinae

10c Tordyliinae

Tribe 11 Laserpitieae

This tribe is divided into three sub-tribes:

11a Silerine

11b Elacosclinae

11c Thapsiineae

Tribe 12 Dauceae

Calestani[49] recognised four sub-families and ten tribes summarised overleaf:

Sub-family	Tribe	Genera
I. Aralineae	1. Hedereae	Hedera
II. Eryngineae	2. Hydrocotyleae	Hydrocotyle
	3. Saniculeae	
	a. Erygieae	Eryngium
	b. Astrantieae	Sanicula, Astrantia
	4. Petagnaeae	
III. Lagoecineae	5. Lagoecieae	Lagoecia
IV. Ferulineae	6. Hohenackerieae	Hohenackeria
	7. Echinophoreae	Echinophora
	8. Ligusticeae	
	This tribe is further sub-divided into 11 sub-tribes, the more important ones being:	
	V. Smyrnieae	Smyrnium, Apium
	VI. Apieae	Conium, Anisum, Bupleurum
	VII. Scandiceae	Chaerophyllum, Scandix, Myrrhis, Cuminum
	VIII. Seseleae	Crithmum, Seseli, Foeniculum, Angelica
	IX. Peucedaneae	Siler, Anethum, Aethus Peucedanum, Ferula, Pastinaca
	X. Caucalideae	Anthriscus, Torilis, Turgenia, Caucalis
	IX. Dauceae	Daucus, Orlaya, Artedia, Laserpitium
	9. Coriandreae	
	a. Anidreae	Anidrum
	b. Eucoriandreae	Coriandrum
	10. Bunieae	Bunium, Conopodium

Calestani's sub-family, Aralineae, is now almost universally accepted as a separate family, the Araliaceae.

The only sub-family that has been extensively investigated is the Apioideae. Volatile oil constituents are found in this sub-family. Aliphatic constituents formed from acetate-malonate, such as hexylacetate, hexylbutyrate, octanol and hexanol have been identified in at least eight species of the genus Heracleum, of the tribe Peucedaneae, sub-tribe Tordyliinae[51]. Other aliphatic compounds have been identified in the dorsal vittae of the unripe fruits and vegetative organs of Coriandrum sativum tribe Coriandreae, in the form of n-decanal and trans-tridec-2-en-1-al[52].

The most commonly encountered volatile oil constituents are the monoterpenes and monoterpenoids formed from acetate-malonate. Cyclic terpene hydrocarbons are widely distributed throughout the Umbelliferae and may be found to some extent in all volatile oils. In the Ammieae, Phellandrene is the major product of Oenanthe aquatica (water fennel)[52], whilst in Apium graveolens (celery) it is limonene.

Oxygenated cyclic monoterpenoids are found in the oils of some species and most of these appear to occur in the Ammieae. Carvone occurs together with limonene in Carum carvi and Anethum graveolens[52,53].

Aromatic substances based on n-propyl benzene derived from shikimic acid appear to be restricted to four tribes, the Scandiceae, the Ammieae, the Peucedaneae and the Dauceae. Harborne et al[54] found n-propyl benzenes in eight genera of the tribe Ammieae. They found myristicin in the fruit oil of Anethum sowa. Myristicin occurs in the leaf of Apium graveolens but not in the fruits of four other Apium species, flowers of Ridolfia segetum also contain myristicin[54].

Sesquiterpenes and sesquiterpenoids are not commonly occurring

in Umbelliferous oils. In the Ammieae they have been identified in only two species. The hydrocarbons azulene and cadinene occurring in the immature fruits of Carum carvi[52], and the hydrocarbons α- and β-selinene and the alcohol eudesmol occurring in Apium graveolens seeds[52].

The number of coumarins and, in particular, furanocoumarin, found and identified in the Umbelliferae increases annually. The highest concentration is found in the roots and, to a lesser extent, in the fruits. The greatest abundance of coumarins and furanocoumarins are found in the tribe Peucedaneae[55]. Coumarins and furanocoumarins occur in other tribes besides the Peucedaneae. Scopoletin has been found in Anethum graveolens[56], bergaptene in Apium graveolens[56] and angelicin in Bupleurum falcatum[57], all occurring in the roots.

Polyacetylene compounds also occur in this family and the first polyacetylenes were identified as toxic principles in Oenanthe crocata, Cicuta virosa and in Aethusa cynapium[58]. Bohlmann et al in 1960[59] were able to conclude that polyacetylenes are widespread, if not universal, in this family.

In a survey of over 300 species for leaf phenolics, Crowden et al[60] in 1969 found that most gave positive results for flavonoids and the few species that were negative (mainly Angelica and Seseli species), generally had large amounts of other phenolics, e.g. coumarins, in their leaves. The flavonoids present in most Umbellifers were found to be kaempferol and quercetin, both flavonols. Luteolin was the main flavone detected in many Umbelliferae[61].

Triterpenoid saponins have also been reported to be present in the family Umbelliferae[62].

Apium graveolens.

Apium graveolens (celery) is one member of this family belonging to the sub-family Apiodeae, tribe Ammieae. It is easily recognized by its characteristic smell. The leaves are bright and pinnate, while the flowers are white, terminal and consist of axilary umbels[63].

In spite of the fact that science was inhibited in the Middle Ages by religious beliefs, herbalism in the care of healing was never discouraged. Herbalism was kept alive by folk medicine, through people who had access to the classical manuscripts of Dioscorides. Traditionally, these herbal remedies were handed down mother to daughter, and it was the women who gathered herbs. Physicians in Germany of the 16th century, were said by the herbalists Fuchs[64], to consider herbals beneath their dignity, and the druggists who were mostly illiterate trusted old women to gather their herbs. Such was the case that the writers of herbals, Simon Corda[64] in the 13th century, attributed much of their knowledge to "highly expert old women".

Fresh celery herb was used as a diuretic in former times and even today as a tea remedy to relieve neuralgia and sciatica.

Most of the work on Apium graveolens has been performed on the seed oil. In 1975 Balbaa[65] studied the fixed oil content of Apium graveolens fruits, and reported a yield of 18.1%. The oil was examined by GLC after it had been saponified. The results revealed eight peaks corresponding to myristic, palmitic, palmitoleic, stearic, petroselinic, oleic and linoleic acids. Petroselinic acid was the major one, reaching up to 60% of the fixed oil while 17.7% oleic acid, 12.4% linoleic acid, 5.9% palmitic acid and 1.55% palmitoleic acid were obtained, the others being found in minor quantities.

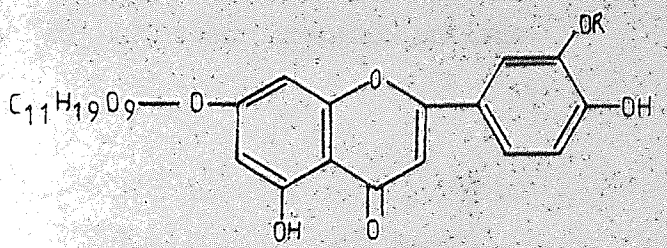
Farooq[66] in his studies using celery fruits obtained 17.0%

of fixed oil, 6.4% of unsaponifiable matter and 82.0% of fatty acids.

In 1973, Singh[67] reported that the leaves of celery contained 0.35% oxalate (0.22% soluble and 0.13% insoluble), 1.81% calcium and 0.94% phosphorous.

Malonic acid has been shown to accumulate in the Umbelliferae compared to the Leguminosae, e.g. the leaves of celery contained more than 1 mg./gm. fresh weight of malonic acid[17].

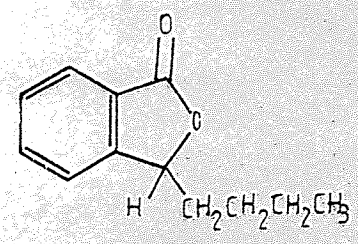
In 1955, Farooq[68] reported the presence of glycoside anthoxanthins as constituents of celery seeds. He isolated flavonoid glycosides and named them as graveobioside-A (12) and graveobioside-B (13). Both these compounds yielded apiose and glucose on hydrolysis. The aglycone of graveobioside-A was found to be luteolin, while that of graveobioside-B was chrysoeriol.



(12) R=H= Graveobioside-A

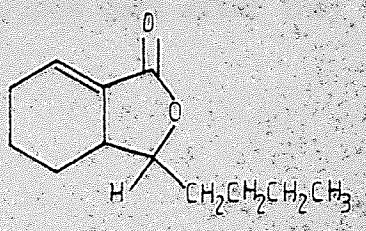
(13) R=CH_3= Graveobioside-B

The characteristic odour of the essential oils of celery is due to a series of phthalides[69], of which 3-n-butyl-phthalide (14) and sedanolide[70] (15)are reported to be the major odour components.



(14)

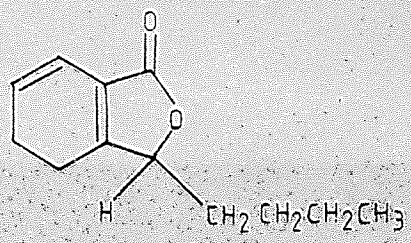
3-n-butyl-phthalide



(15)

Sedanolide

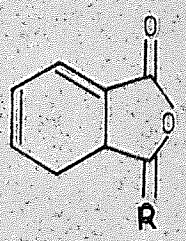
In 1977 Leonard and Bjeldanes[71] discovered the presence of a new compound in the seed oil and called this compound Sedanenolide (16).



(16)

3-n-butyl-4,5-dihydrophthalide

Several other phthalides occur in trace quantities, two phthalide and two dihydrophthalide compounds have been isolated with a strong characteristic odour of celery[72]. These four compounds are: 3-isobutylidene-3a,4-dihydrophthalide (17), 3-isovalidene-3a,4-dihydroph--thalide (18) and their aromatic analogs.



(17) R=CH.CH.$(CH_3)_2$ = 3-isobutylidene-3 ,4-dihydrophthalide

(18) R=CH.CH_2.CH.$(CH_3)_2$ = 3-isovalidene-3 ,4-dihydrophthalide

In this study only the celery stems were used after they had been cleaned from the leaves (Fig.7).

Fig. 7 Celery stems (A.graveolens).

I-3- Chromatographic methods of separation and isolation.

The separation and purification of plant constituents is mainly carried out using chromatographic techniques: paper chromatography (PC), thin layer chromatography (TLC) and gas-liquid chromatography (GLC). The choice of technique depends largely on the solubility and other physical characteristics of the compounds to be separated. Paper chromatography is particularly applicable to water soluble plant constituents, namely the carbohydrates, amino-acids, nucleic acid bases, organic acids, amino sugars and phenolic compounds. Thin layer chromatography is the method of choice for separating all lipid soluble components examples of which are the lipids, steroids, sterols etc. By contrast the third technique, GLC finds its main application with volatile compounds e.g. fatty acids, mono- and sesquiterpenes, hydrocarbons and sulphur compounds or by derivatizing the polar compounds like sugars, amino sugars and sterols, to acetyl trimethylsilyl ethers.

All the above techniques can be used both on micro and macro scales. For preparative work, TLC is carried out on thick layers of adsorbent (0.25-1 mm.) and PC on thick sheets of filter paper. For isolation purposes on an even larger scale than this, it is usual to use column chromatography. This procedure yields purified components in gram amounts. One of the main advantages of PC is the convenience of carrying out separation simply on sheets of filter paper, which serves both as a medium for separation and as a support. Another advantage is the considerable reproducibility of Rf values determined by this technique, so that such measurements are valuable parameters for use in describing new plant compounds. Indeed, for substances such as sugars, the Rf values are the most important means of

describing and distinguishing them. For chromatography on paper or cellulose plates, it may be desirable to alter the composition of the solvent in order to increase or decrease the Rf values, and, in general both pyridine and water cause an increase, while ethyl acetate, butanol and propanol cause a decrease in these values, especially of the sugars[73].

The special advantages of TLC compared to PC include versatility, speed and sensitivity. Versatility is due to the fact that a number of different adsorbents besides cellulose may be spread on to a glass plate. The greater speed of TLC is due to the more compact nature of the adsorbent, while the sensitivity achieved is less than microgram amounts of material[74].

In the last 13 years, the advances made in liquid column chromatography have been comparable with those of flat-bed techniques (TLC and PC) and gas chromatography[75]. In principle there are two reasons for this increasing interest in liquid chromatography. Firstly, the present situation reflects the steadily increasing demands being made upon separation techniques, mainly in biochemistry and drug analysis. Secondly, the theoretical aspects of liquid column chromatography have been developed substantially, mainly as a result of the application of model situations in gas chromatography which resulted in the integration of the knowledge achieved in the techniques and instrumentation of different individual chromatographic variants.

Sterols are the most lipophilic steroids and are easily separated by various types of chromatographic procedures. Solid-liquid adsorption chromatography is the most commonly used technique[76]. The analysis of the hydroxy compounds such as sterols by GLC is a more difficult task than that of the hydrocarbons. The hydroxy compounds have one or more hydroxyl groups which are polar and have the tendency to be adsorbed on silica-type supports. Numerous devices have been

proposed for getting round these difficulties but their effectiveness is often limited[77]. The support material can be treated with acid or alkaline solution, or by silanizing reagent which functions by eliminating OH groups, or by using polar stationary phases (hydroxy types). The best way to overcome these problems is by making derivatives such as the acetates.

The analysis of a complex mixture of fatty acids is one of the greatest success stories in gas chromatography[77]. Nevertheless, all the difficulties mentioned in connection with hydroxy compounds are again evident, together with the added disadvantage that carboxylic acids tend to dimerise. Most of the work on fatty acids (including quantitative analyses) has been carried out following conversion to the methyl esters. Many different methylation procedures have been described and adapted to dealing with extremely small samples[78,79,80]. Among the reagents used in the latter category are diazomethane, 2,2-dimethoxypropane and a mixture of methanol with borontrichloride or borontrifluoride. The latter has the advantage of not introducing the unwanted products due to secondary reactions[81]. Many stationary phases have been used; of these apiezone and polyethyleneglycol succinate are non-polar phases, which allow the separation of unsaturated acids on the basis of their degree of unsaturation[77].

In this study the above techniques were used because of the advantages referred to above and also because the small quantities of pure compounds that were isolated in practice allowed the utilization of these techniques for both separation and isolation purposes. Spectroscopic methods were used for identification purposes only.

I-4- Spectroscopic methods of identification.

Spectroscopic methods are now used at some point in the solution of almost all problems in organic chemistry. Since only small quantities of the compounds were obtained in this study, spectroscopic and chromatographic methods were used for the separation and identification of the compounds isolated.

One of the initial difficulties encountered in the study of the higher terpenoids and of the steroids when first isolated was the accurate determination of molecular weights. Carbon and hydrogen analysis alone, except in a series of derivatives, are not adequate to distinguish between close homologues, for instance between C_{30} and C_{31} terpenoids[82]. While the mass spectrometer has been widely used for substances of low molecular weight, its applicability to the field of natural products, e.g. to steroids and to triterpenoids in particular, was slow to develop because of the complex spectra obtained.

Mass spectrometry has been successfully applied in recent years to a broad range of analytical and structural problems. Applications in the field of petroleum chemistry[83], for example have elucidated hydrocarbon type structures. The mass spectrometer, alone or in combination with other methods of analysis, has proved a valuable tool in the investigation of new compounds.

Physical methods are being increasingly applied to the solution of structural problems in the field of organic chemistry, and the use of infra-red spectroscopy is standard practice in most laboratories today. There is no doubt that of the absorption studies in all parts of the electromagnetic spectrum, those of infra-red measurements are generally the most useful for structural determinations. In the various fields of matural product chemistry, when compounds are often isolated in extremely small amounts and seldom crystalline in

the first instance, infra-red methods are particularly useful.

I-5- Object of the study.

The objects of this research were to isolate and identify as many pure compounds as possible from the three plant species:

1. Phaseolus coccineus (roots, rhizomes and stems).
2. Gleditsia triacanthos (pods and seeds).
3. Apium graveolens (stems).

Thin layer chromatography, using different systems and reagents was used to separate their components and column chromatography to isolate the major ones.

When sufficient amount of the major compounds was isolated, it was screened for any possible biological activity. The minor components present were classified where possible for taxonomic purposes.

In the cases of P.coccineus and G.triacanthos, the amount of materials available were limited. Hence, the methods applied to identify the compounds in this study were those designed for dealing with small quantities of the materials. Chromatographic methods were applied in the cases of separation and isolation, while spectroscopic methods were used for identification purposes.

II - EXTRACTION, SEPARATION AND ISOLATION

II-1- Introduction.

In deciding how extraction may be made that will adequately remove the constituents in plant tissue, the first step must be the selection of a liquid capable of penetrating these tissues and dissolving the constituent or constituents. The next step must be the selection of a suitable process of extraction. A process involving prolonged boiling is not likely to be of value in plants containing volatile constituents nor is a simple method such as steeping in water for a short time likely to produce a useful extract from a dense type of tissue. No single method, however, is suitable for all samples[84].

In this study, the plant material under investigation was examined separately and different extraction procedures were applied to the roots and rhizomes of P.coccineus. Since no previous work on these organs has apparently been reported, the extraction was followed by TLC separation. By experimenting with different extraction procedures, a scheme was devised which was applicable to all three species investigated.

In the scheme, the non-polar compounds were separated first followed by the polar compounds. The crude extracts were subjected to TLC, GLC, PC and then isolation of the components in quantity was achieved by column chromatography.

II-2- MATERIALS AND METHODS

II-2-1- Materials.

II-2-1-1- Plant materials.

1. *Phaseolus coccineus*.

Phaseolus coccineus plants (runner bean) were cultivated in the U.K. by Suttons Seed Ltd. and supplied by L.E.Watts (plant breeding department). They were dug up in September, 1975. Preliminary treatment of the material involved the removal of soil, the division of the plant into three morphological parts: roots, rhizomes and stems. Each plant organ was weighed, chopped and dried under a current of air. It was reweighed after the above procedures. After drying, approximately three quarters of the weight was lost as water. The material was then ground into a coarse powder.

2. *Gleditsia triacanthos* fruits.

These fruits were collected and dried in Turkey. The fruits arrived in the U.K. in August, 1977 and were identified by P.H.Davis (Department of Botany, Edinburgh University, Royal Botanic Gardens, Edinburgh, EH3 5LR). The seeds were separated from the pods and ground into coarse powders.

3. *Apium graveolens* stems.

The celery stems were cultivated in the U.K. and purchased from the local supermarket. After cleaning all the stems were cleared the leaves. The stems were then chopped into slices, dried and ground into a coarse powder; 93.7% of its weight was lost as water during the drying process.

II-2-1-2- Extraction apparatus.

A large soxhlet (40 cm. in length and 10 cm. in width) provided with a cellulose thimble of capacity 400 gm. (19 cm. in length and 8.5 cm. in width) was used.

II-2-1-3- Chromatographic materials.

Silica gel plates were prepared by using 30 gm. of silica gel (silica gel GF_{254} type 60 Merck) mixed with 60 ml. of water. The slurry was spread evenly on five glass plates 20 x 20 cm. to give a layer thickness of 0.25 mm. They were then dried at room temperature for 15 minutes followed by 1 hr. at 110°C in the drying oven.

Aluminium oxide plates were prepared by using 30 gm. of aluminium oxide (Aluminium oxide GF_{254} type 60/E Merck) mixed with 40 ml. of water and the slurry spread evenly over five plates 20 x 20 cm. to give a layer thickness of 0.25 mm. They were dried as described for silica gel plates.

Precoated silica gel sheets (type 60 F_{254} 0.2 mm. thickness Merck).

Precoated cellulose sheets (F_{254} 0.1 mm. thick Merck).

Whatman No.1 paper was used for paper chromatography.

Precoated aluminium oxide sheets (60 F_{254} type E 0.2 mm. thick Merck).

Perkin-Elmer -F11 equipment with flame ionisation detector, utilising Apiezon, SE-30, PEGS and Carbowax columns was used for gas chromatography.

II-2-2- Methods.

II-2-2-1- Phaseolus coccineus.

The schemes that were applied are shown overleaf (scheme 1-3). Each fraction isolated was then subjected to TLC in order to locate the different constituents and to aid identification of the major components.

It was found that only scheme 3 gave satisfactory results. The other schemes yielded only small quantities of the different constituents isolated, even after utilising a large quantity of the original material. Hence, schemes 1 and 2 were studied briefly and only scheme 3 was used to isolate the constituents from all the plant materials used.

Phaseolus coccineus (roots and rhizomes)

II-2-2-1-A- Scheme 1.

Powdered material
Soxhlet extraction, petroleum ether (60 - 80°C), 72 hr.

Marc

Petroleum ether "A"

Dried, moistened and macerated with ammonia-methanol-chloroform, (1:3:6) extracted with chloroform (soxhlet) for 24 hr.

Marc (neglected)

Chloroform extract

Evaporated under reduced pressure and the brown residue was extracted with 1N HCl and filtered

Filtrate

Precipitate "B"

Extracted with chloroform

Aqueous layer

Chloroform layer

Evaporated under reduced pressure after drying over anhydrous Na_2SO_4; brown residue 0.233% w/w non-alkaloid fraction "C"

Adjusted to pH 9.5 (conc. ammonia) and extracted with chloroform

Chloroform extract

Aqueous (neglected)

Evaporated under reduced pressure after drying over anhydrous sodium sulphate (Na_2SO_4); brown residue alkaloid fraction or basic compounds 0.416% w/w "D"

TLC on the fractions obtained from scheme-1.

System 1.

Stationary phase : Silica gel GF_{254}

Mobile phase : Benzene - A.A. 94:1

Location : U.V light and iodine vapour

Fraction : "A"

Results : Table 1 and Fig.8

	U.V light		
Spot No.	254 nm.	366 nm.	Rf
1	Blue	Blue	0.96
2	Blue	Blue	0.79
3	Faint blue	-	0.60
4	Brown	Faint blue	0.26
5	Faint brown	Blue	0.17
6	Faint brown	Blue	0.12
7	Brown	Blue	0.06
8	Purple	Pink	0.00

Table 1 System 1 Fraction A

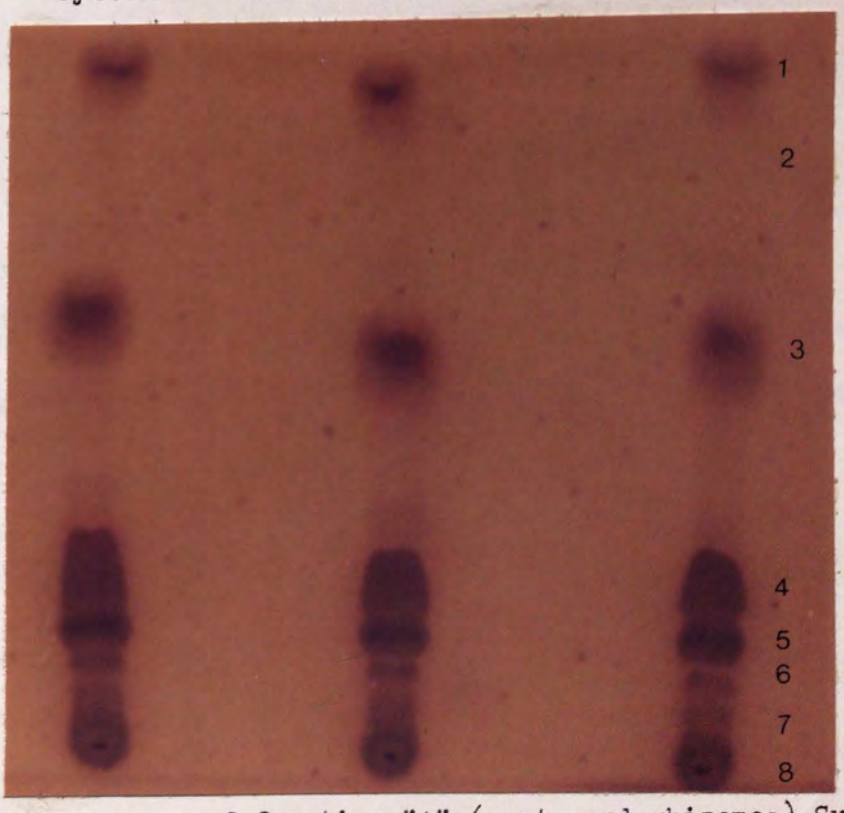


Fig. 8 Chromatogram of fraction "A" (roots and rhizomes) System 1 after exposure to iodine vapour.

Preparative plates were made using the above system and bands corresponding to spots 4-8 were scraped, eluted with chloroform, the chloroform evaporated and the oily concentrate subjected to TLC using system 2.

System 2.

Stationary phase : As under system 1

Mobile phase : Benzene - A.A. 90:12

Location : As under system 1

Results : Table 2

Spot No.	Rf
1	0.66
2	0.53
3	0.50
4	0.28
5	0.25

Table 2 (System 2) Fraction A

In both systems all the spots except spot No.2 gave brown to light brown colour when the chromatograms were exposed to iodine vapour.

System 3.

Stationary phase : As in system 1

Mobile phase : cyclohexane-chloroform-diethylamine 5:4:1

Location : U.V light and dansyl chloride[85] reagent spray

Fractions : "B", "C" and "D".

Results : Tables 3, 4 and 5

Spot No.	Rf
1	0.70
2	0.59
3	0.39
4	0.33

Table 3 (System 3) Fraction B

Spot No.	Rf
1	0.63
2	0.50
3	0.35
4	0.15

Table 4 (System 3) Fraction C

All the spots in both the fractions gave a yellow fluorescence under the U.V light after spraying the plates with dansyl chloride reagent.

Spot No.	U.V 366nm.	Rf
1	Yellow	0.03
2	Faint blue	0.31
3	Faint blue	0.43
4	Faint blue	0.58
5	Blue	0.82
6	Yellow	0.89
7	Yellow	0.93

Table 5 (System 3) Fraction D

Fraction D was then subjected to preparative TLC using system 3. The lower bands (1-4) were scraped off, eluted with chloroform and then subjected to further separation using preparative plates (1 mm. layer thickness) using system 4.

System 4.

Stationary phase : Silica gel GF_{254} (1 mm. thick)

Mobile phase : chloroform-acetone-diethylamine 5:4:1

Location : U.V light

Results : Table 6

Spot No.	U.V 254nm.	Rf
1	Blue fluorescence	0.95
2	Blue fluorescence	0.68
3	Blue fluorescence	0.61
4	Blue fluorescence	0.43

Table 6 (System 4) Fraction "D" lower bands

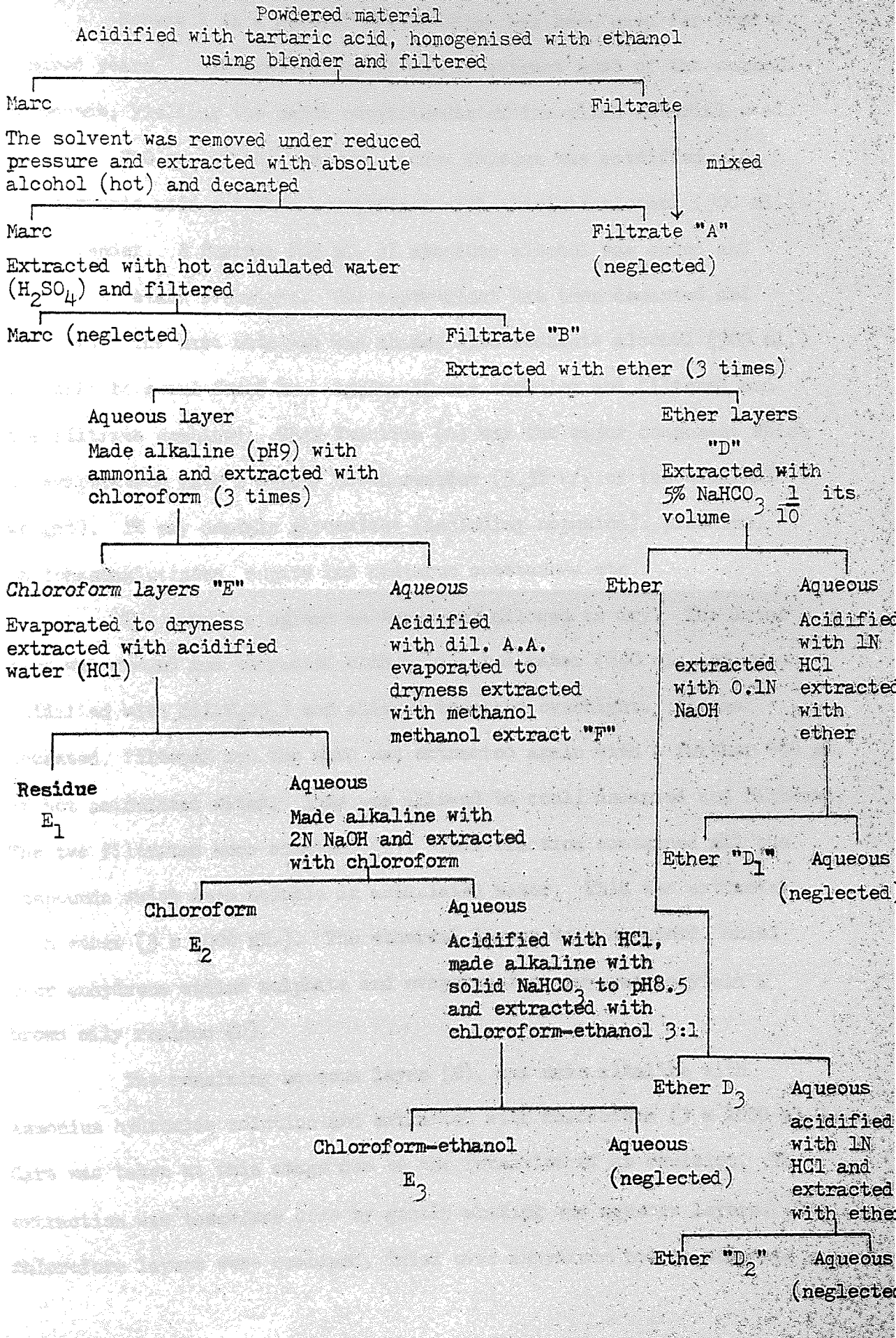
Each of the seven bands (Tables. 5 and 6) were scraped form the preparative plates, eluted with chloroform and evaporated to dryness. All these compounds were present in trace quantities (oily), and the only analytical procedure used was U.V absorptions (Table 7).

Compound No.	λ max 1nm.	λ max 2nm.
1	244	-
2	243	-
3	243	-
4	243	-
5	244	277
6	243	277
7	243	277

Table 7 U.V spectra of fraction D components.

The spectra were measured after dissolving the residue of each compound in 20 ml. methanol.

II-2-2-1-B- Scheme 2.



Scheme 2.

Scheme 2 is a general method which has been used for over a hundred years[84]. Theoretically, it should extract most of the organic compounds, yielding the major constituents of the plant material used.

300 gm. of the powdered, dried rhizome was acidified with 10% tartaric acid and then homogenised with absolute alcohol (300 ml.) in a blender. A further 200 ml. of absolute alcohol was added and allowed to stand overnight. The supernatant was then decanted and filtered. The marc obtained was shaken with absolute alcohol (300 ml.) and left to stand for 2 hr. Again, it was decanted and filtered and the filtrate combined. This fraction (A) was the major component which on evaporation gave a sticky brown residue (5.5% w/w of the original weight). It may contain glycosides (including saponins), proteins, phytohaemaglutinins, sugars and resinous substances etc.

The marc was spread on trays and allowed to dry. The dried marc was ground and extracted with acidulated water (800 ml. of water acidified with dil.H_2SO_4) and allowed to stand overnight. It was decanted, filtered and the marc was extracted again with a further 400 ml. of hot acidulated water. This was allowed to cool, decanted and filtered. The two filtrates were combined (B). This fraction contained all the compounds which were soluble in acidulated water. This was extracted with ether (3 x 1000 ml.). The ethereal layers were combined, dried over anhydrous sodium sulphate and evaporated to dryness to yield a brown oily residue (D).

The remaining aqueous layer (E), was made alkaline with ammonium hydroxide solution and extracted with chloroform (3 x 1000 ml.). Care was taken at this stage due to the formation of an emulsion. The extraction was therefore done by gently shaking the aqueous layers. The chloroform layers were combined, dried over anhydrous sodium sulphate,

evaporated to dryness under reduced pressure to yield residue (E). This fraction might contain the basic chloroform soluble salts, amphoteric chloroform soluble salts and the non-amphoteric chloroform soluble salts. Ultimately this fraction was found to contain three components (E_1, E_2 and E_3).

The aqueous layer remaining after separation of the chloroform layers, contained water soluble, ether insoluble and chloroform insoluble compounds. It was acidified with diluted acetic acid, evaporated to dryness and the residue extracted with methanol. This fraction (F) probably contained quaternary ammonium compounds.

The fraction (D), the acid ether extract contained all the ether soluble acidic and neutral compounds. It was sub-divided into three fractions:

1. D_1 neutral compounds.
2. D_2 weak acids or phenolic compounds.
3. D_3 strong acids.

Fraction (E), the basic chloroform extract, contained all the chloroform-soluble basic compounds:

1. E_1 basic compounds with chloroform-soluble salts.
2. E_2 non-amphoteric basic compounds.
3. E_3 amphoteric compounds.

The marc remaining after the acidulated water extraction, was dried and ground. It was then extracted with chloroform using a soxhlet extraction apparatus for 24 hr. The extract was evaporated to dryness to yield a brown oily residue (C).

Fractionation of the aqueous phase depends on the fact that most organic compounds can be extracted from aqueous solution by immiscible solvents, the neutral and acidic compounds being extracted from acid aqueous solutions, and the basic compounds from alkaline

aqueous solutions.

The neutral compounds can, of course be isolated from either acid or alkaline aqueous solutions but it is usual to co-extract them with acidic compounds rather than basic compounds because the acid group is smaller and usually less of a problem than the basic group.

Thin layer chromatography on fractions from scheme 2.

System 5.

Stationary phase : Aluminium oxide GF_{254}

Mobile phase : n-butanol-5% aqueous solution of citric acid 9:1

Location : U.V light and acidified iodoplatinate solution spray

Results : Tables 8 and 9

Fraction	Spot No.	U.V 254nm.	Iodoplatinate	Rf
C	1	brown	yellowish	0.89
	2	fluorescent blue	-	0.79
	3	fluorescent blue	-	0.52
	4	-	pale yellow	0.07
D	1	fluorescent violet	yellow	0.90
	2	fluorescent blue	-	0.83
	3	blue	-	0.09
	4	yellow	-	0.05
	5	-	pale yellow	0.04
E	1	yellow	yellow	0.90
	2	blue	-	0.69
	3	-	purple	0.64
	4	-	purple	0.37
	5	yellow	-	0.23
	6	-	purple	0.11
F	1	faint blue	-	0.17
	2	-	deep purple	0.11
	3	faint blue	-	0.10

Table 8 Results obtained by using system 5.

Fraction	Spot No.	U.V 254nm.	Rf
E_1	* 1	yellow	0.96
	2	fluorescent violet	0.74
	3	fluorescent violet	0.35
E_2	* 1	yellow	0.92
	* 2	yellow	0.83
	* 3	fluorescent violet	0.70
	4	fluorescent violet	0.44
E_3	* 1	yellow	0.83
	* 2	fluorescent violet	0.70
	3	blue	0.62
	* 4	fluorescent violet	0.48
	5	fluorescent violet	0.23
	6	fluorescent violet	0.19
	7	fluorescent violet	0.15
	8	blue	0.10
	9	fluorescent violet	0.08

Table 9 Results obtained by using system 5.

* These spots gave orange colour with modified Dragendorff's reagent spray and purple colour with acidified iodoplatinate solution spray.

Thin layer chromatography on fractions from scheme 2.

System 6.

Stationary phase : Silica gel GF_{254}

Mobile phase : benzene-ethylacetate-diethylamine 7:2:1

Location : As mentioned under system 5

Results : Table 10

Fraction	Spot No.	U.V light 254nm.	U.V light 366nm.	Iodoplatinate	Rf
C	1	faint blue	-	-	0.95
	2	-	brown	-	0.85
	3	-	brown	-	0.79
	4	-	brown	-	0.58
	5	-	brown	-	0.49
	6	-	brown	-	0.40
	7	-	brown	-	0.21
	8	faint blue	-	-	0.11
	9	fluorescent yellow	-	-	0.07
	10	fluorescent yellow	-	-	0.04
D	1	blue	-	-	0.93
	2	-	-	purple	0.84
	3	fluorescent blue	-	-	0.75
	4	fluorescent blue	-	-	0.25
	5	brown	-	-	0.07
	*6	yellowish white	-	-	0.03
E	1	yellowish blue	-	-	0.95
	2	fluorescent blue	fluorescent blue	-	0.79
	3	fluorescent blue	-	-	0.28
	4	-	brown	-	0.07
F	-	-	-	-	-

Table 10 Results obtained by using system 6.

* This spot was visible yellow.

II-2-2-2- Scheme 3.

The following general scheme of extraction was applied to all the plant material used in this study:

Plant material
Extracted with petroleum ether (60 - 80°C) for 24 hr. in a soxhlet

Marc

Dried and extracted in a soxhlet with methanol for 12 hr. The extract was evaporated to dryness under reduced pressure leaving residue (D). Residue (D) was suspended in water and extracted with ether

Petroleum ether extract evaporated to dryness under reduced pressure leaving residue (A). This was refluxed for 1 hr. with alcoholic KOH (10%)

Unsaponifiable fraction (B)

Saponifiable fraction (C)

Aqueous layer (F)

extracted with butanol saturated with water

Ether extract (E)

refluxed with alcoholic KOH for 1 hr.

Unsaponifiable fraction (E_1)

Saponifiable fraction (E_2)

Aqueous layer (F_2)

Butanol layers (F_1)

Evaporated to dryness under reduced pressure, residue dissolved in minimum amount of methanol and precipitated by the addition of sufficient quantity of ether and filtered

Filtrate (G)

Precipitate (I)

The above scheme of extraction was applied to all the plant materials under investigation. Each fraction was examined separately.

The dried powder was packed in the thimble and extracted for 24 hr. (following mini-scale trials for 6, 12 and24 hr. periods), in a soxhlet with petroleum ether. The petroleum ether was driven off at 70°C using a rotatory vacuum, and the residue (A) was saponified for 1 hr. using freshly prepared alcoholic KOH. The product was concentrated to a small volume and distilled water was added (100 ml.). This was extracted with ether (3 x 100 ml.). The combined ether layers were dried over anhydrous sodium sulphate, filtered and evaporated to dryness under reduced pressure to give the unsaponifiable fraction (B). While the aqueous layer was made acidic with dil. HCl and extracted with ether (3 x 100 ml.). The combined ether layers were dried over anhydrous sodium sulphate and filtered. The ether was evaporated completely under reduced pressure to yield an oily residue (C) corresponding to the saponifiable fraction.

The marc was dried and extracted for a further 12 hr. in the same soxhlet apparatus with methanol. The methanol extract was evaporated and the residue suspended in water (100 ml.), extracted with ether (3 x 200 ml.). The combined ether layers were evaporated to dryness and the residue (E) was saponified as previously described for fraction (A), yielding the saponifiable fraction (E_2) and the unsaponifiable fraction (E_1) which were studied in the same ways as in (C) and (B).

The aqueous layer, was extracted with butanol saturated with water (4 x 200 ml.), the butanol layers were combined and evaporated to dryness and the residue left was dissolved in methanol (10 ml.). Then sufficient quantity of ether was added till no more precipitate was formed. The precipitate was filtered and the filtrate evaporated to dryness under reduced pressure to yield residue (G), while the

precipitate which contained glycosides was washed with more ether and dried (I).

The aqueous layer was evaporated to dryness under reduced pressure to yield residue (F_2).

II-2-2-2-1- Phaseolus coccineus (runner bean).

Roots, rhizomes and stems.

Powdered roots, rhizomes and stems were extracted separately with petroleum ether (60-80°C) using the soxhlet extraction apparatus. Each sample was extracted for 72 hr. Complete extraction was shown to require only 24 hr. The petroleum ether extract was evaporated to dryness under reduced pressure in each case leaving behind a yellow brown, oily residue viz:

a. Roots 1.2% w/w of the original weight.

b. Rhizomes 1.25% w/w of the original weight.

c. Stems 0.75% w/w of the original weight.

Thin layer chromatography on fraction (A) from scheme 3.

The same results were obtained when fraction (A) of the roots, rhizomes and stems was run on system 1 and 7.

System 1.

Stationary phase : Silica gel GF_{254}

Mobile phase : benzene- A.A. 94:1

Location : U.V light and iodine vapour

Results : Table 1

System 7.

Stationary phase : Precoated silica gel F_{254}

Mobile phase : benzene-methanol 9:1

Location : U.V light and phosphomolybdic acid reagent spray

Results : Table 11 and Fig.9.

Spot No.	U.V 254nm.	Phosphomolybdic	Rf
* 1	blue	blue	0.95
2	blue	faint blue	0.85
3	blue	faint blue	0.78
4	-	blue	0.68
5	-	blue	0.53
6	-	blue	0.36
7	pink	-	0.28
8	blue	faint blue	0.25
9	-	blue	0.21
10	blue	blue	0.19
11	-	blue	0.14
12	-	faint blue	0.04

Table 11 Components of roots, rhizomes and stems obtained from fraction A using system 7.

* Spot No. 1 was visible yellow.

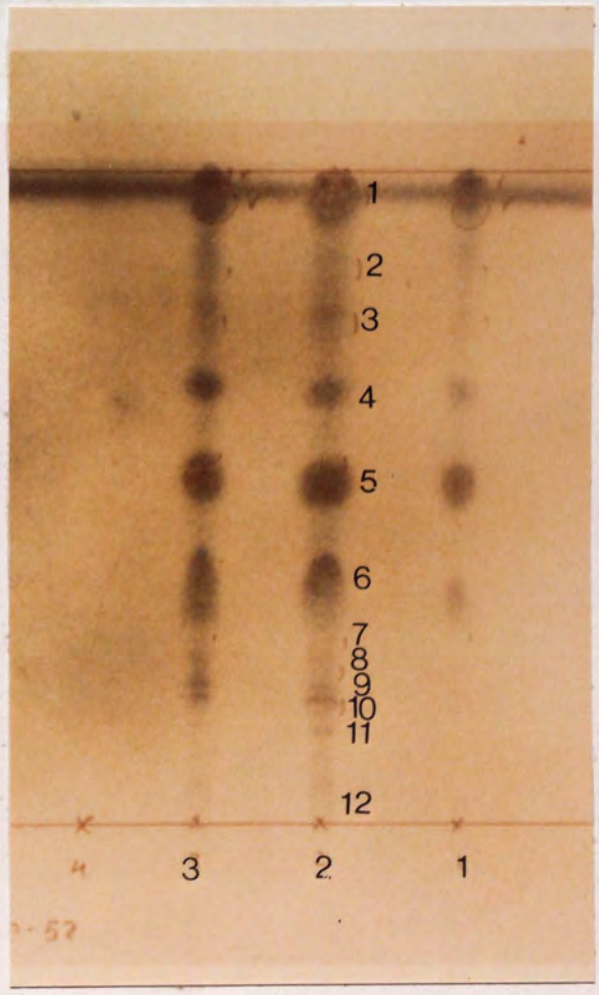


Fig. 9 Chromatogram of system 7.

Fraction A of the : 1. stems; 2. roots; 3. rhizomes.

Thin layer chromatography on fraction(C) from scheme 3.

System 8.

Stationary phase : Precoated silica gel F_{254}

Mobile phase : benzene-A.A. 9:1

Location : Iodine vapour and phosphomolybdic acid reagent spray

Results : Table 12 and Fig. 10

Spot No.	Iodine vapour	Phosphomolybdic	Rf
1	brown	blue	0.74
2	brown	blue	0.66
3	brown	blue	0.59
4	brown	blue	0.53
5	brown	blue	0.49
6	brown	blue	0.47

Table 12 Components of the saponifiable fraction (C) of the roots, rhizomes and stems obtained by using system 8.

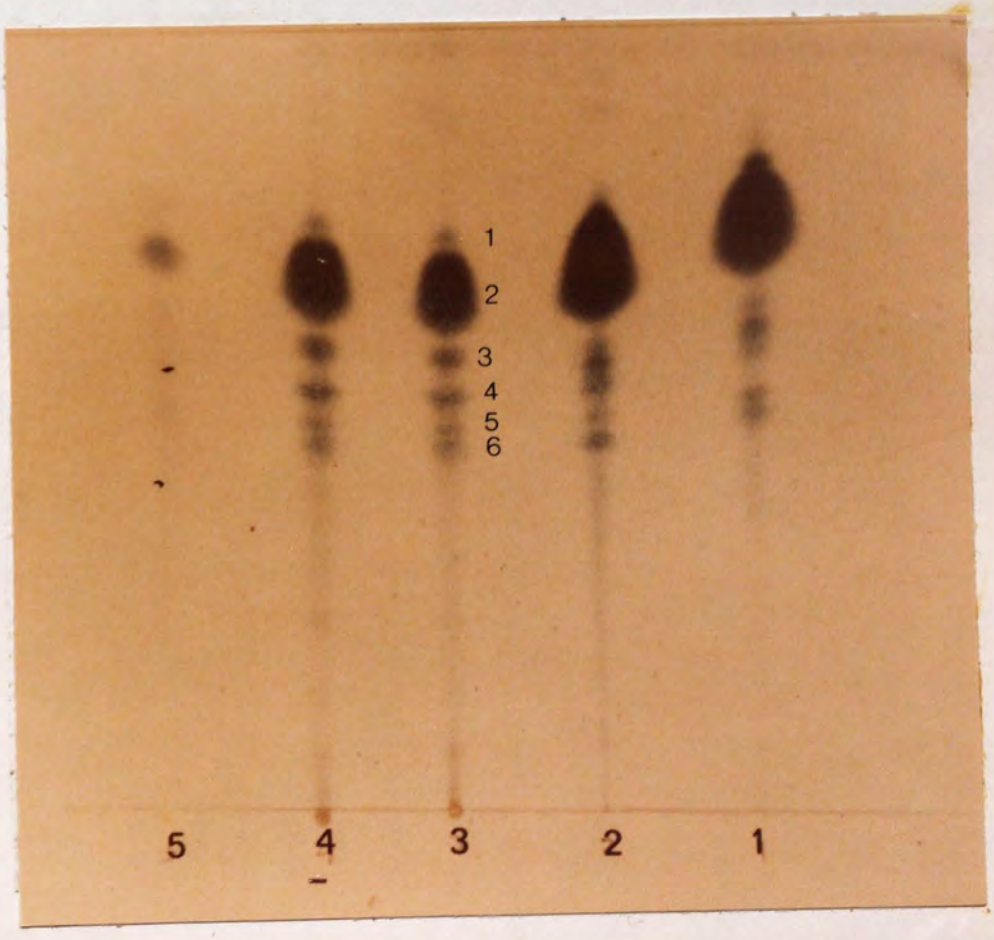


Fig. 10 Chromatogram of system 8 sprayed with phosphomolybdic acid reagent spray.

1). Linoleic acid; 2). Linolenic acid; 3). Fraction C of roots

Thin layer chromatography on fractions from scheme 3 continued

System 9.

Stationary phase : Precoated silica gel F_{254}

Mobile phase : chloroform-methanol 9:1

Location : U.V light and phosphomolybdic acid spray reagent

Results : Table 13 and Fig. 11

Fraction	Spot No.	U.V 254nm.	Phosphomolybdic	Rf
A of roots, rhizomes and stems	1	-	blue	0.96
	2	blue	-	0.92
	3	blue	-	0.82
	4	blue	-	0.71
	5	-	blue	0.69
	6	blue	-	0.56
	7	-	blue	0.41
	8	blue	-	0.34
	9	-	blue	0.27
	10	blue	-	0.17
	11	blue	-	0.13
	12	blue	-	0.07
	13	-	blue	0.04
	14	-	blue	0.02
	15	-	blue	0.00
B of roots, rhizomes and stems	1	-	faint blue	0.96
	2	-	blue	0.69
	3	-	blue	0.41
	4	-	faint blue	0.27
	5	-	blue	0.07
β-sitosterol		-	blue	0.41

Table 13 Results obtained by using system 9.

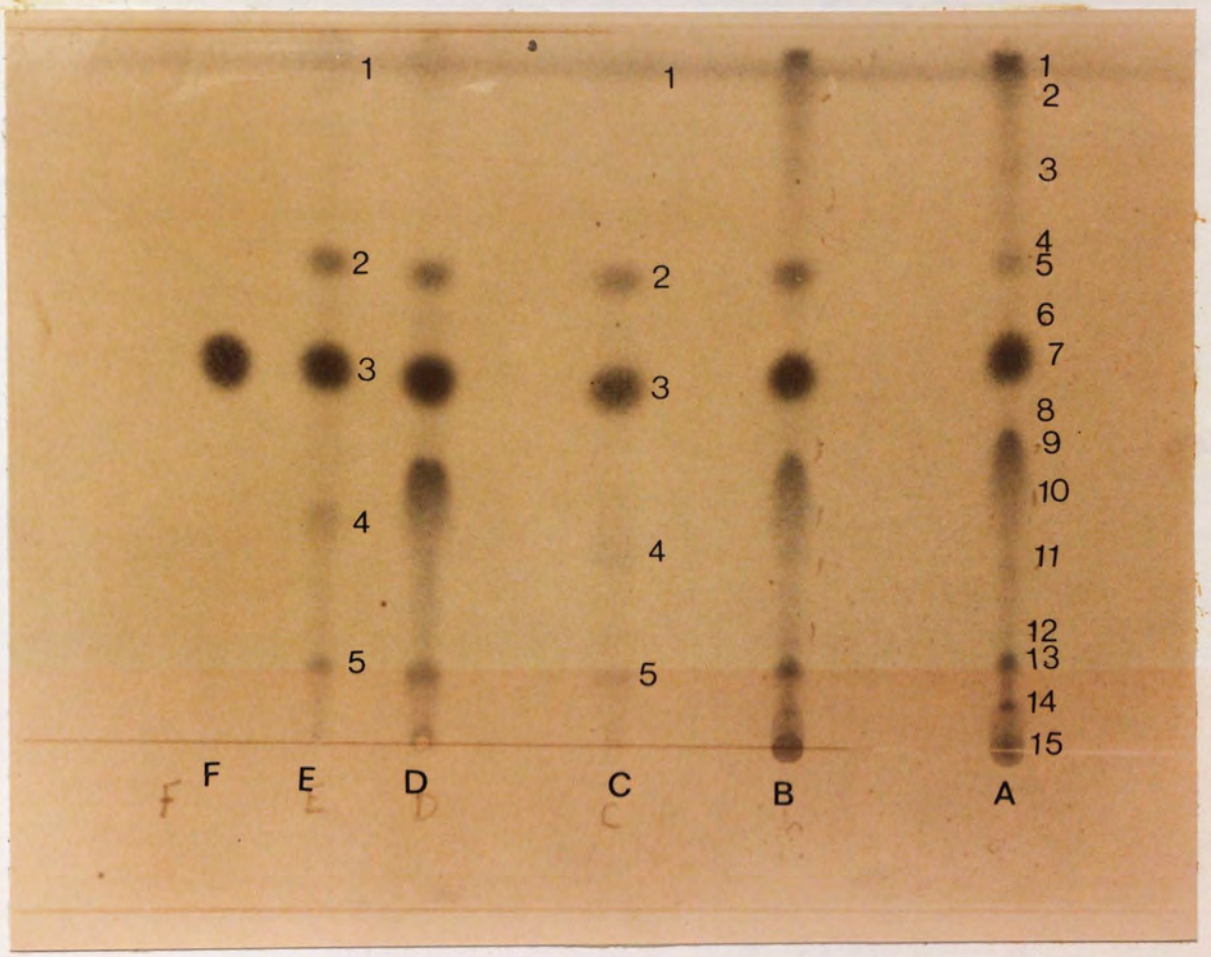


Fig. 11 Chromatogram from system 9.

A. Fraction A of the roots.
B. Fraction A of the rhizomes.
C. Fraction B of the stems.
D. Fraction A of the stems.
E. Fraction B of the roots.
F. β-sitosterol authentic sample.

The chromatogram was sprayed with phosphomolybdic acid reagent spray.

Gas-liquid chromatography of the saponifiable fractions.

Preparation of the sample.

0.5 gm. of the petroleum ether extract was refluxed with 15 ml. of freshly prepared 10% alcoholic KOH for 1 hr. The alcohol was then distilled off and the brown residue was suspended in 25 ml. of distilled water. It was then extracted with ether (3 x 50 ml.) to remove the unsaponifiable fraction (B). The aqueous layer was acidified with 0.2N HCl to release the free fatty acids which were then extracted with petroleum ether (60-80°C) using 3 x 50 ml. the volume. The petroleum ether was distilled off leaving a yellow residue (C).

The percentage of the free fatty acids (C) and the unsaponifiable fractions found in each sample were as follows (Table 14):

Plant material	Fraction C %w/w	Fraction B %w/w
Roots	0.43	0.77
Rhizomes	0.41	0.88
Stems	0.23	0.50

Table 14 The percentages of fractions C and B present in each organ.

About 200 mg. of the oily fraction (C) was placed in a test-tube and 15 ml. of the boron trifluoride methanol reagent was added. The mixture was boiled in a steam bath for 2 min. and the esters were recovered as follows:

The boiled mixture was washed into a separating funnel with 50 ml. of petroleum ether (40-60°C) and 30 ml. of water. The mixture was shaken vigorously and the two layers were allowed to separate. The lower aqueous layer was discarded. The petroleum ether layer was dried over anhydrous sodium sulphate, filtered, evaporated to dryness under reduced pressure and the resulting methyl esters were introduced into the GLC column with ether (1µl).

Standard samples:

Methyl esters of the following acids were used in the 2% ether solution:

1. Capric.
2. Caprylic.
3. Lauric.
4. Myristic.
5. Palmitic.
6. Stearic.
7. Oleic.
8. Linoleic.
9. Linolenic.

The standard methyl esters mixture was used for the identification of the fatty acids present in the sample by direct comparison of the retention times (Rt) of the standard methyl esters with those of the unknown esters in the sample. Also when 0.5 µl. of the 1% of each standard fatty acid methyl ester was injected with 1 µl. of the sample, it was noticed that if the sample contained such a methyl ester, they were eluted out together and the peak corresponding to them became higher.

A number of columns were tried to analyse the presence of fatty acid methyl esters in the sample, but the best results were obtained with the Apiezon 4% column under the following conditions:

System 1.

Column : 4% Apiezon on chromosorb G, mesh (80-100) 1.5 m. in length.

Oven temp.: 250°C

Injection temp. : 300°C

Carrier gas : Nitrogen at 25 p.s.i.

Oxygen & Hydrogen : At 20 p.s.i.

Amplitude : 2×10^2

Chart speed : 10 mm./min.

Volume of sample : 1 µl.

Detector : Flame ionisation detector.

The other columns tried were: 4% SE-30, 3% SE-30, 1.5% SE-30, DGS, OV-17 and Carbowax.

The saponifiable methanol extract.

After petroleum ether extraction, the mare left behind from each of the samplee, was dried and then extracted with methanol for 12 hr. The methanol extract was evaporated to dryness yielding a yellowish brown residue in the case of the roots and rhizomes and a green residue in the case of the stems (Fraction D, scheme 3).

A sample of each residue was studied seperately. Each sample was suspended in water (100 ml.), and extracted with ether (3 x 300 ml.) till the ether layer became almost colourless. The combined ether layers were dried over anhydrous sodium sulphate, filtered and evaporated to dryness under reduced pressure yielding a yellowish brown oily residue (E).

Each sample was saponified as previously described and the saponifiable fraction was studied by GLC (E_2).

It was found that fractions (C) and (E_2) contained the same fatty acids (Table 15).

Plant material	Fraction D%w/w	Fraction E%w/w	Fraction E_2%w/w	Fraction E_1%w/w
Roots	5.27	0.23	0.15	0.08
Rhizomes	5.20	0.17	0.09	0.08
Stems	4.40	0.90	0.09	0.81

Table 15 Percentage of the methanol extract fractions present in the roots, rhizomes and the stems.

Thin layer chromatography of fractions obtained from scheme 3.

System 10.

Stationary phase : Precoated silica gel F_{254}

Mobile phase : benzene-A.A. 9:0.5

Location : Anisaldehyde and phosphomolybdic acid spray reagents.

Results : Table 16 and Fig. 12.

Fraction	Spot No.	Phosphomolybdic	Anisaldehyde	Rf
1 & 2	1	blue	purple	0.76
	2	blue	purple	0.56
	3	blue	purple	0.45
	4	blue	purple	0.43
	5	blue	purple	0.38
	6	blue	purple	0.33
	7	blue	purple	0.30
	8	blue	purple	0.20
	9	blue	purple	0.14
	10	blue	purple	0.08
	11	blue	purple	0.03
	12	blue	purple	0.00
3,4 & 5	1	blue	purple	0.33
6	1	blue	purple	0.38
7 & 8	1	blue	purple	0.00

Table 16 Results obtained from system 10.

The numbers in the column labelled Fraction correspond to the following fractions:

1. Fraction (E_1) of the roots.
2. Fraction (E_1) of the rhizomes.
3. B-sitosterol.
4. Stigmasterol.
5. Cholestane.
6. Cholestene.
7. Fraction (D) of the roots.
8. Fraction (D) of the rhizomes.

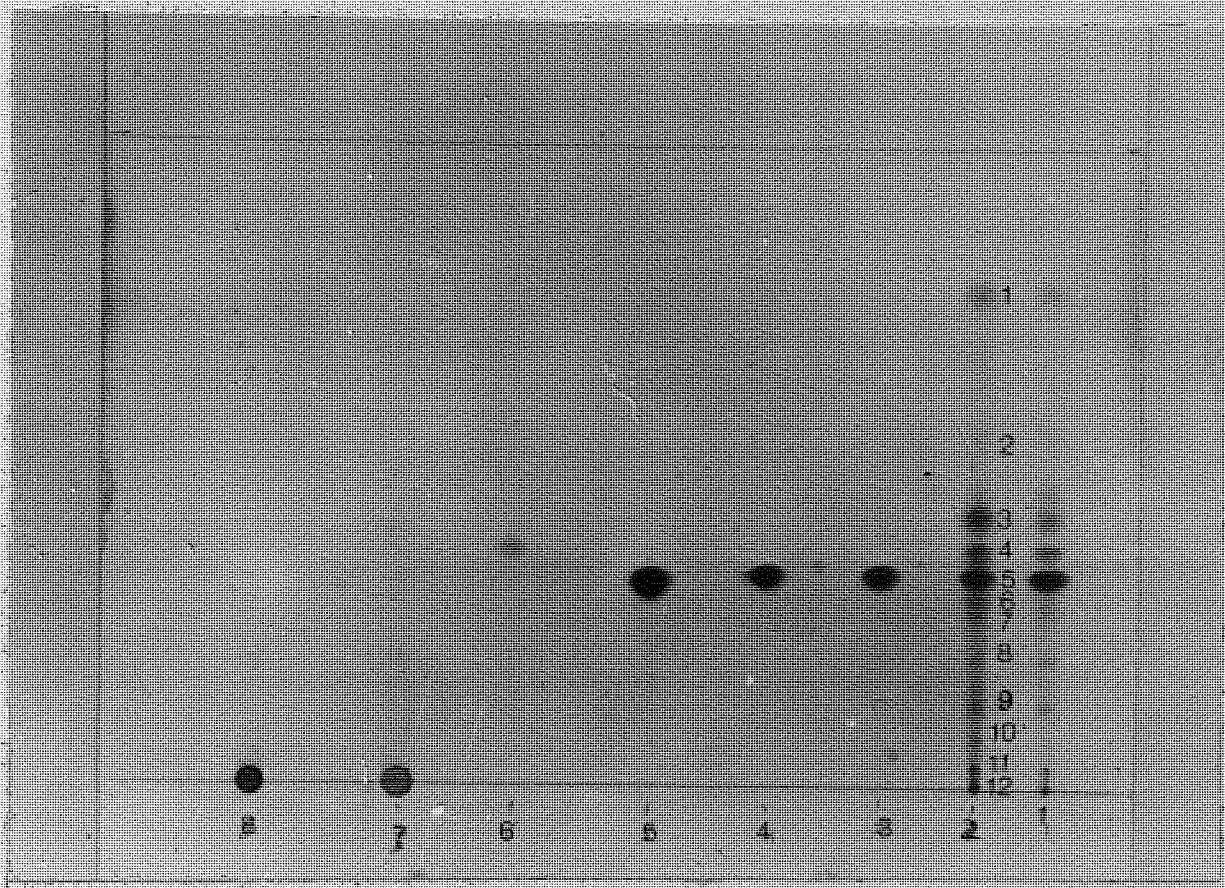


Fig. 12 Chromatogram from system 10 sprayed with anisaldehyde reagent spray.

N.B.

1). Fraction (E_1) of the stems showed same results as fraction (E_1) of the rhizomes.

2). Fraction (D) of the stems showed same results as fraction (D) of the roots and rhizomes.

Isolation of oleic acid (from the roots and rhizomes):

The oily residue (C) obtained from roots and rhizomes was dissolved separately in chloroform (5 ml.), placed on top of silica gel column (40 x 2 cm.) and eluted with chloroform at the rate of 30 drops/min. Fractions of 15 ml. each were collected. Fractions 1-15 contained the minor fatty acids. Fractions 16-22 contained oleic acid. These fractions were examined by TLC using system 1 and the similar fractions were combined, evaporated to dryness under reduced pressure yielding a yellow oily residue.

Isolation of palmitic acid (from the stems):

The oily residue (C) obtained from the stems (2 gm.) was dissolved in toluene (4 ml.) and placed on the top of a silica gel column (40 x 2 cm.). Elution was started at 30 drops/min. using toluene and 10 ml. fractions were collected. Fractions 1-12 eluted a brown band, fractions 13-19 eluted another faint brown band and these fractions were collected using toluene-methanol (95:5). Fractions 20-30 were collected using 10% methanol in toluene.

Each fraction was examined by TLC using syster 11 and the similar fractions were combined and evaporated to dryness. Fractions 13-19 showed two spots, one visible yellow with an Rf value of 0.32 and the other one faint blue with an Rf value of 0.60 with phosphomolybdic acid reagent. The combined fractions were evaporated under reduced pressure and crystallised from methanol giving colourless needle crystals. The crystals were filtered and dried giving a yield of 474 mg. The other fractions showed traces of an oily residue of the other fatty acids.

System 11.

Stationary phase : As in system 1

Mobile phase : chloroform-methanol 9:1

Location : Phosphomolybdic acid reagent spray

Results : Fig. 13

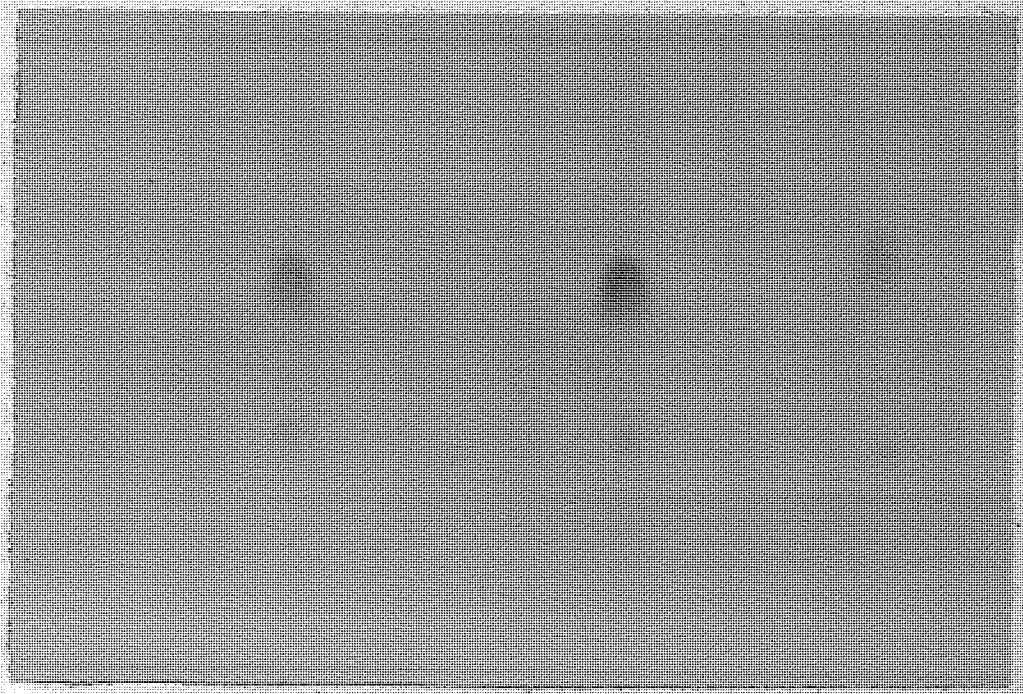


Fig. 13 Results from system 11.

Fractions 13, 16 and 19 were eluted with Toluene-Methanol (95:5).

GLC on the methyl ester derivatives were made on the above fractions (1-12, 13-19 and 20-30) using system 1 and their Rt were compared with those of the standards.

GC-MS of fatty acid methyl esters:

The fatty acid methyl esters obtained from the stems by petroleum ether extraction were analysed by GC-MS using the 3% OV-17 column under the following conditions:

System 2.

GLC operating conditions:

Sample volume : 0.1 µl
Column : 3% OV-17
Detector : Flame ionisation detector
Carrier gas : Helium 0.1 atmos.
Chart speed : 5 mm./min.
Attenuation : 2×10^2

Mass Spectrum conditions:

Multiplier supply : 3.5 KV
Emission control : Scan time : 30 sec.
Span : 100%
Scan : Up

Programmed power supply : 4 KV
V2

Chopper Amplifier CA2 : Amps.f-s. : 10^{-2}
Gain : 10^{-2}
Resp.secs : 0.003

Picoammeter PA2 : Amps.f-s. 10^{-9}
Gain :3

U.V Recorder x1, x3, x10.
Oscillograph : 25 mm./sec.
Track current : 100 µamps.
Ionisation energy : 70 eV

Using the above column, seven peaks were isolated and the

retention times were calculated (Table 17).

Peak No.	Rt in min.
1	0.6
2	1.4
3	2.4
4	3.2
5	4.2
6	6.0
7	8.4

Table 17 The Rt (stems) of fatty acid methyl esters obtained by using system 2.

Thin layer chromatography on the unsaponifiable fractions (B and E_1).

System 12.

Stationary phase : As under system 10

Mobile phase : benzene-methanol 85:15

Location : U.V light, day light and phosphomolybdic acid reagent

Results : Tables 18 and 19.

Fraction	U.V 254nm.	Phosphomolybdic	Day light	Rf
B (stems)				
1	-	blue	yellow	0.97
2	-	blue	-	0.70
3	-	blue	-	0.56
4	-	-	yellow	0.26
5	-	green	-	0.22
β-sitosterol	-	blue		0.56
Stigmasterol	-	blue		0.62
Cholestane	-	blue		0.67

Table 18 Components obtained from fraction B (stems).

Fraction	U.V 254nm.	Phosphomolybdic	Day light	Rf
B (roots & rhizomes)				
1	-	blue	yellow	0.97
2	-	blue	-	0.70
3	-	blue	-	0.56

Table 19 Components obtained from fraction B (roots and rhizomes).

continued..

System 13.

Stationary phase : As under system 10

Mobile phase : benzene-methanol 9:1

Location : Phosphomolybdic acid, anisaldehyde reagent and day light

Results : Table 20

Fraction	Anisaldehyde	Phosphomolybdic	Day light	Rf
B (roots & rhizomes)				
1	brown	faint blue	yellow	0.95
2	pink	blue	-	0.17
3	pink	blue	-	0.10
β-sitosterol	pink	blue	-	0.10
Stigmasterol	pink	blue	-	0.08
Cholestane	pink	blue	-	0.07

Table 20 Components obtained from fraction B (roots and rhizomes).

System 14.

Stationary phase : As under system 10

Mobile phase : benzene-methanol-A.A. 9:0.5:0.5

Location : Phosphomolybdic acid and U.V 254nm.

Results : Table 21 and Fig.14

Fraction	U.V 254nm.	Phosphomolybdic	Rf
B (roots)			
1	-	faint blue	0.93
2	blue	-	0.75
3	-	blue	0.61
4	-	blue	0.45
5	blue	-	0.17
β-sitosterol		blue	0.45
Stigmasterol		blue	0.53

Table 21 Components obtained from fraction B (roots).

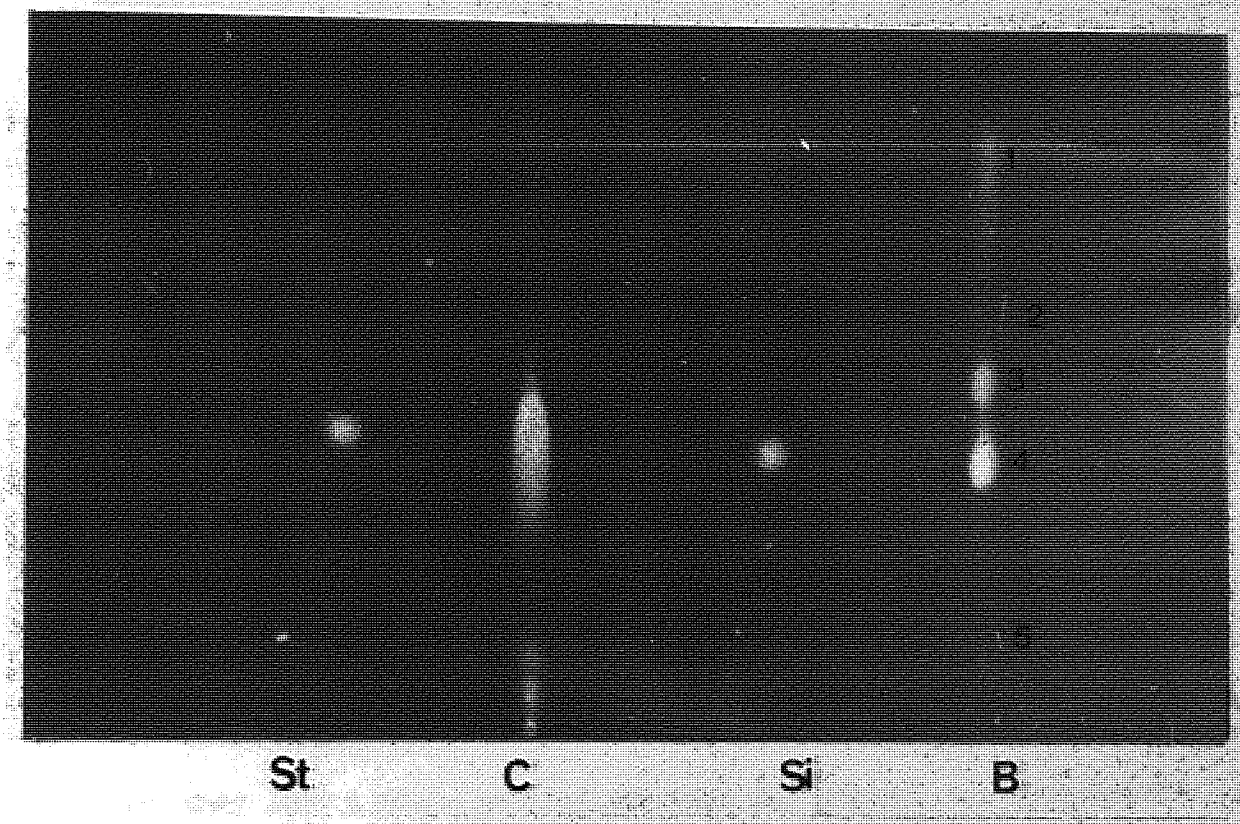


Fig. 14 Chromatogram from system 14 sprayed with phosphomolybdic acid reagent.

B). Fraction (B) of the roots.

Si). β-sitosterol.

C). Fraction (C) of the roots.

St). Stigmasterol.

Isolation of fraction B components by column chromatography.

Only three major components were isolated in pure state i.e. compounds corresponding to spots 1,3 and 4 (Fig.14), from the stems, roots and rhizomes using the following method. This was applied to all three plant fractions.

2.57 gm. of fraction B (roots) was dissolved in benzene (5 ml.) and placed on top of silica gel column (40 cm. length and 2 cm. width) and eluted first with benzene at the rate of 30 drops/min., 10 ml. fractions were collected and then each fraction was examined by TLC using system 14.

Fractions 1-13 showed a yellow colour and on TLC each showed a single spot with an Rf value of 0.93. These fractions were combined, evaporated under reduced pressure and the yellow orange oily residue (25 mg.) was called phaseobone-1.

Fraction 14 gave a colourless oily residue in trace amounts with an Rf value of 0.75.

Fractions 16-22 gave a single spot with an Rf value of 0.61 and they left a white precipitate when the solvent was evaporated to dryness. It was crystallized from methanol leaving white platelet crystals (44 mg.). This compound was called phaseosterol-A.

Fractions 23-26 were a mixture of the above compound and another compound with an Rf value of 0.45.

Fractions 27-35 showed single spots with an Rf value of 0.45. They were combined and the solvent was evaporated completely under reduced pressure leaving a white precipitate which was recrystallized from methanol giving flaky crystals (87 mg.). This compound was called phaseosterol-B.

Fractions 1-15 were eluted by benzene only, while fractions 16-35 were eluted by benzene-methanol in the ratio of 98:2.

No more compounds were located on TLC even when the polarity

of the solvent was increased to benzene-methanol in the ratio of 1:1.

Separation of fraction E_1 components by TLC.

System 14.

Only two spots were isolated from E_1 of the roots, rhizomes and the stems. Their Rf values and the colours were similar to those spots shown in Fig.14 i.e. spots 3 and 4.

System 15.

Stationary phase : As under system 10

Mobile phase : chloroform-methanol 95:5

Location : U.V 254nm. and phosphomolybdic acid reagent

Results : Table 22 and Fig.15

Fraction	U.V 254nm.	Phosphomolybdic	Rf
D (stems)			
1	red	-	0.96
2	blue	-	0.42
3	blue	-	0.32
4	faint blue	-	0.25
5	blue	-	0.16
6	yellowish blue	-	0.12
7	brown	-	0.00
E_1 (stems)			
1	-	blue	0.80
2	-	blue	0.75
3	brown	-	0.57
β-sitosterol	-	blue	0.75
Phaseosterol-B	-	blue	0.75

Table 22 Components obtained from fractions D and E_1 (stems).

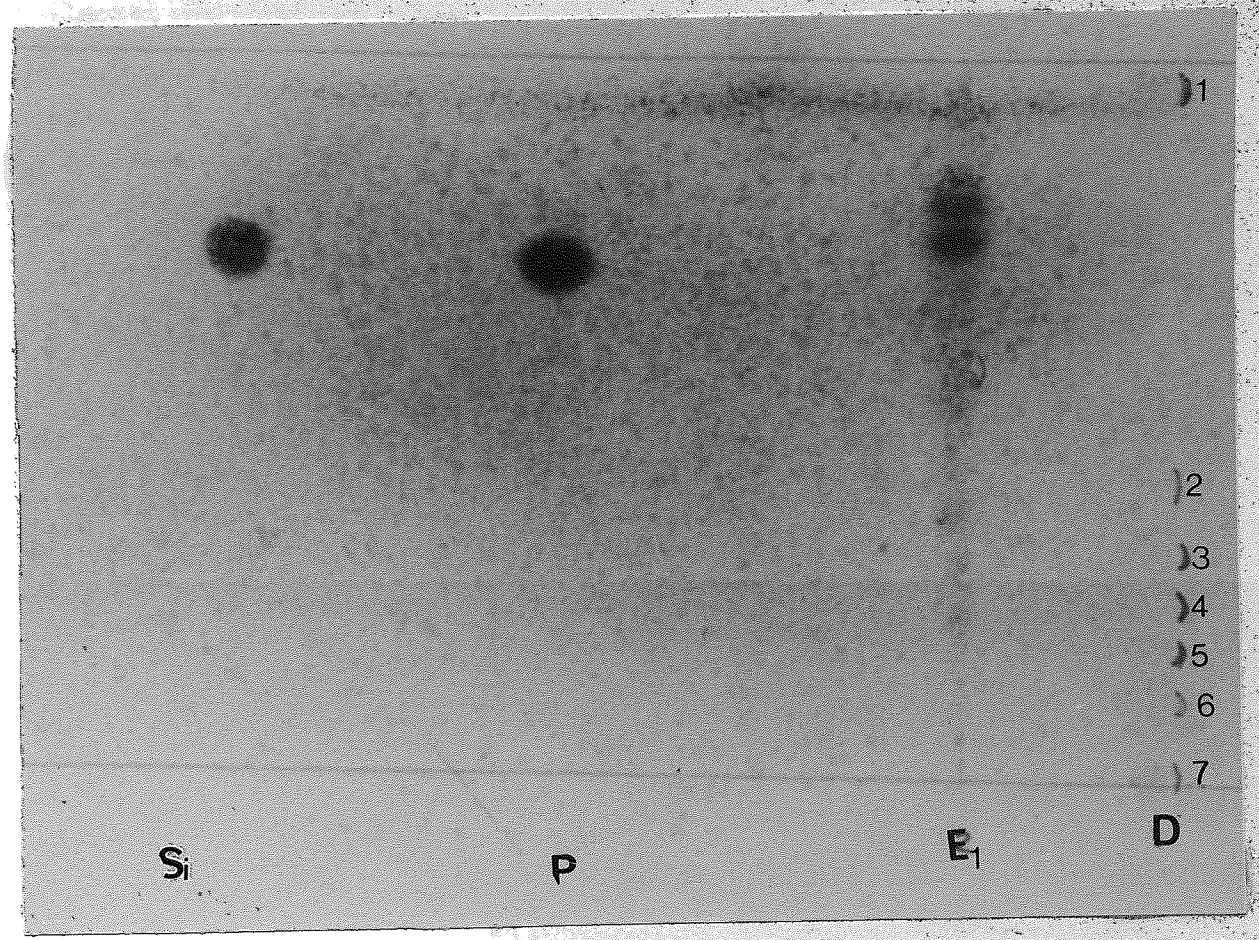


Fig. 15 Chromatogram from system 15 sprayed with phosphomolybdic acid reagent.

P = Phaseosterol-B (stems).

Si = β-sitosterol.

Isolation of fraction E_1 by column chromatography.

0.45 gm. of E_1 (stems) was dissolved in 5 ml. of toluene and transferred onto the silica gel column (50 cm. in length and 2 cm. in diameter). The slurry was made with toluene-methanol (98:2) and the elution was carried out at the rate of 30 drops/min. 10 ml. each fractions were collected and examined by TLC using system 15.

Fractions 1-20 were yellow in colour and each showed a single spot with an Rf value of 0.80 on TLC. Hence, these fractions were combined, evaporated to dryness under reduced pressure and the residue crystallised from methanol to give a white platelet of crystals (phaseosterol-A) weighing 12 mg.

Fractions 21-22 were found to be a mixture of two compounds when examined by TLC using the same system as above with the Rf values of 0.80 and 0.75.

Fractions 23-30 each showed a single spot with the Rf value of 0.75. They were combined, evaporated to dryness and crystallised from methanol leaving flaky crystals (14 mg.). This compound was called phaseosterol-B.

Extraction, separation and isolation of the components of the methanol extract of Phaseolus coccineus.

Each organ (root, rhizome and stem) was studied separately, using the general method of extraction (scheme 3) with slight modifications.

1. The roots.

633 gm. of the dried, powdered roots were extracted for 12 hr. with methanol in a soxhlet extracting apparatus. The brown extract was allowed to cool and the fine white crystals formed were filtered (I). The filtrate was evaporated to dryness under reduced pressure yielding a reddish brown residue (33.4 gm., 5.27% w/w of the original weight). This residue was suspended in water (50 ml.) and extracted with ether (3 x 100 ml.). The ether layers were combined, dried over anhydrous sodium sulphate, filtered and evaporated to dryness under reduced pressure yielding a reddish brown residue (6.8 gm., 1.07% w/w of the original weight). This fraction contained the saponifiable and unsaponifiable fractions which were studied in detail previously.

The aqueous layer was extracted with butanol saturated with water (3 x 100 ml.) and the butanol layers were combined, evaporated to dryness under reduced pressure, leaving a yellowish white precipitate (5.9 gm., 0.93% w/w of the original weight) (II).

The aqueous layer left was evaporated to dryness under reduced pressure leaving a yellowish white precipitate (18.2 gm., 4.97% w/w of the original weight) (III).

Thin-layer chromatography on fractions II and III.

System 16.

Stationary phase : Silica gel GF_{254}

Mobile phase : chloroform-methanol-ethylacetate 5:4:1

Location : U.V and phosphomolybdic acid spray reagent

Results : Table 23

Fraction	Spot No.	U.V 254	Phosphomolybdic	Rf
II	1	blue	-	0.71
	2	-	blue	0.63
	3	-	blue	0.19
	4	-	blue	0.11
III	1	-	blue	0.47
	2	-	blue	0.15

Table 23 Results obtained from system 16.

Hydrolysis of II.

The residue II was presumed to contain saponins due to the fact that frothing occurred when a few ml. of it (in methanol) were shaken with a few ml. of water; a haemolytic effect was produced on red blood cells and it gave a positive Molisch test (general test).

About 5.9 gm. of the residue (II) was dissolved in methanol (10 ml.) and sufficient ether was added until no more precipitate was formed. The precipitate was filtered, washed and dried giving a yellowish white precipitate of crude saponin (5.5 gm., 0.86% w/w of the original weight). The filtrate was neglected as it left a very small amount of a brown oily residue after the ether was evaporated.

Thin layer chromatography on the crude saponin gave the same results as fraction II as shown in Table 23. It was then hydrolysed for 3 hr. with 3N HCl at 100°C, cooled and water (100 ml.) was added. The precipitate of sapogenins was filtered, washed with water and dried yielding a brown precipitate (3 gm., 0.47% w/w of the original weight)(IV).

Thin layer chromatography on sapogenins (IV).

System 17.

Stationary phase : As under system 16

Mobile phase : chloroform-methanol 9:1

Location : As under system 16

Results : Table 24 and Fig.16

Spot No.	U.V 254nm.	Phosphomolybdic	Rf
1	blue	-	0.88
2	blue	-	0.71
3	-	blue	0.38
4	-	blue	0.20
5	-	blue	0.10

Table 24 Results obtained from system 17.

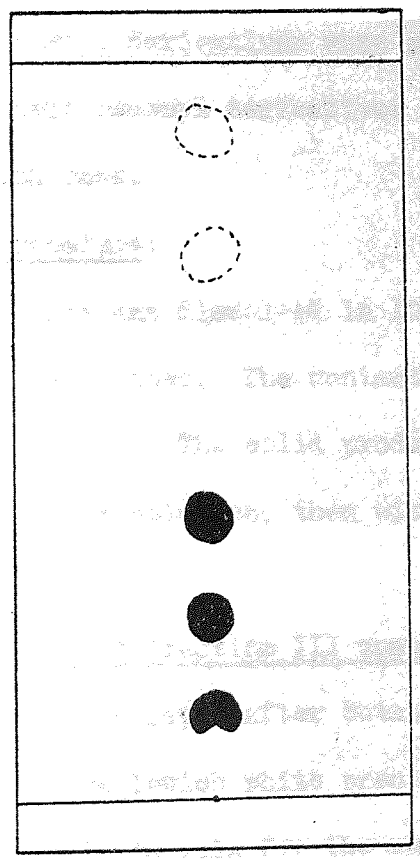


Fig. 16 Chromatogram obtained from system 17.

Isolation of sapogenins (IV) by column-chromatography.

The crude sapogenins (3 gm.) were purified from resinous substances by eluting with methanol from silica gel column (40 x 2 cm.). The methanol eluents were combined and evaporated to dryness leaving a white precipitate (V) (0.5 gm.). TLC on this precipitate gave the same results as in Table 24. The resinous impurities remained on the top of the column.

The precipitate (0.4 gm.) was dissolved in benzene (3 ml.), transferred onto the top of a silica gel column (50 x 2 cm.) and 10 ml. fractions were collected at the rate of 30 drops/min. Elution was carried out with benzene and benzene-methanol mixture. Each fraction was examined by TLC using system 17. The results were summarized in Table 25.

Since the genins (phaseolosides A,B, and C) could not be crystallized, acetyl derivatives were made and when these failed to crystallize, their benzoyl derivatives were made which gave needle crystals in each case.

Benzoylation procedure:

The genin was dissolved in 10% aqueous NaOH (5ml.) and benzoyl chloride (1ml.) was added. The contents were corked and shaken vigorously for 15 min. The solid product obtained was filtered off and washed with NaOH solution, then with water and crystallized from methanol.

Isolation of fraction III components.

The aqueous layer after butanol extraction was evaporated to dryness leaving a yellowish white precipitate. This fraction was also suspected to contain saponin for the reasons previously suggested in fraction II. The crude saponin was precipitated and hydrolysed as under fraction II yielding a white precipitate (0.45 gm., 0.071% w/w of the

Fraction	Solvent	Remarks.
1 -9	benzene	colourless eluent; no spot was observed on TLC.
10 - 14	benzene-methanol 95:5	faint yellow eluent; showed a single spot with an Rf value of 0.88. The fractions were combined, evaporated to dryness and the residue was crystallized from methanol leaving a white platelet of crystals (2 mg.) (phaseobone-2).
15 - 26	benzene-methanol 95:5	colourless eluent; showed a single spot on TLC with an Rf value of 0.71. They were combined, evaporated to dryness and crystallized from methanol leaving a white platelet of crystals (3 mg.) (phaseobone-3).
27 - 60	benzene-methanol 90:10	colourless eluent; showed a single spot on TLC with an Rf value of 0.38. They were combined, evaporated to dryness leaving a white precipitate. It was crystallized from aqueous methanol (90%) leaving a white product (0.031% w/w of the original weight). (phaseoloside-A).
61 - 62	benzene-methanol 90:10	colourless eluent; showed a mixture of two spots with Rf values of 0.38 0.20.
63 - 70	benzene-methanol 85:15	colourless eluent; showed a single spot on TLC with an Rf value of 0.20. They were combined, evaporated to dryness and crystallized from methanol leaving a white product (0.0098% w/w of the original weight). (phaseoloside-B).
71	benzene-methanol 85:15	colourless eluent; showed a mixture of two spots with Rf values of 0.20 and 0.10.
72-100	benzene-methanol 85:15	colourless eluent; showed a single spot with an Rf value of 0.10. They were combined, evaporated to dryness and crystallized from aqueous methanol (90%) leaving a white product (0.0118% w/w of the original product). (phaseoloside-C).

Table 25 Results obtained from the column chromatographic separation of the components of fraction (V).

original weight) (VI).

Thin layer chromatography on fraction (VI).

System 17.

Results : Table 26

Spot No.	U.V 254nm.	Phosphomolybdic	Rf
1	blue	-	0.90
2	blue	-	0.80
3	blue	-	0.69
4	-	blue	0.65

Table 26 Results obtained from system 17 for fraction (VI).

Isolation of fraction (VI) by column chromatography.

The mixture (VI) , 0.45 gm. was dissolved in methanol-chloroform (1:1) (3 ml.) and put onto the top of silica gel column (40 cm. x 2 cm.) which was eluted with methanol-chloroform (1:1). The methanol-chloroform eluents were combined, evaporated to dryness and a yellowish white precipitate was left (0.3 gm., 0.04% w/w of the original weight). The crystallization from aqueous methanol (90% v/v) gave a white amorphous product (phaseoloside-D). TLC showed a single spot with an Rf value of 0.65 (system 17). A benzoyl derivative was made as previously described giving a colourless crystalline product which gave a single spot with an Rf value of 0.91 (system 17).

The other compounds remained on the top of the column together with a resinous substance.

Isolation of fraction II components.

The saponins of this fraction were isolated in pure states as glycosides by two different methods:

a. By preparative TLC.

b. By column chromatography.

a. Preparative TLC on fraction (II).

Fraction II components were separated on TLC using system 16. Four spots (Table 23) were obtained, while on system 18 seven spots (Table 27) were observed. Only two of these spots i.e. spots 5 and 6 were isolated in pure states since the other was present in trace amount (spot 7), while spots 1,2,3 and 4 were not saponins, no colours were produced with either phosphomolybdic acid or with anisaldehyde reagents.

Spots 5 and 6 were isolated as bands from preparative plates (1 mm. thick). These bands were located by spraying the edge of the plate with anisaldehyde reagent. The bands corresponding to spots 5 and 6 were scraped separately, eluted with methanol, the extract was evaporated to dryness and the residue was crystallized from 90% alcohol leaving white products labelled phaseoloside-A_1 and phaseoloside-B_1.

Phaseoloside-A_1 and B_1 were hydrolysed separately and their aglycones were isolated as previously described.

From the m.p. and the Rf values (system 17), it was found that these two aglycones were the same as phaseoloside-A and phaseoloside-B respectively.

System 18.

Stationary phase : Silica gel GF_{254}

Mobile phase : chloroform-methanol 1:1

Location : U.V., anisaldehyde reagent and phosphomolybdic acid reagent

Results : Table 27

Spot No.	U.V 254nm.	Phosphomolybdic	Anisaldehyde	Rf
1	blue	-	-	0.84
2	blue	-	-	0.77
3	blue	-	-	0.64
4	brown	-	-	0.53
5	-	blue	pink	0.30
6	-	blue	pink	0.19
7	-	blue	pink	0.00

Table 27 Results obtained from system 18 for fraction II.

b. Isolation of fraction II components by column chromatography.

0.5 gm. of fraction II was dissolved in chloroform-methanol (9:3) (5 ml.) and transferred onto the top of silica gel column (40cm. x 2cm.). Fractions of 10 ml. each were collected using chloroform and chloroform-methanol mixture at the rate of 30 drops/min. Each fraction was examined by TLC using system 18. Results were summarized in Table 28.

Fraction	Solvent	Remarks
1 - 10	chloroform	colourless eluent; no spot was located by TLC.
11 - 31	chloroform-methanol 95:5	very narrow band was eluted with a faint yellowish green eluent. It left a very small amount of an oily residue which showed a single spot with an Rf value of 0.95 (purple in colour with anisaldehyde).
32 - 36	chloroform-methanol 9:1	yellow band was eluted. It left traces of yellow oily residue when these fractions were evaporated to dryness. It showed four spots on TLC.
37 - 42	chloroform-methanol 9:1	green band was eluted. It showed four spots on TLC and it left traces of green residue when these fractions were evaporated to dryness.
43 - 48	chloroform-methanol 9:1	yellow eluent. Four spots on TLC, traces of yellow residue was left.
49 - 70	chloroform-methanol 8:2	yellow eluent. One spot with an Rf value of 0.03 (purple in colour with anisaldehyde reagent.
71 - 91	chloroform-methanol 8:2	brown band was eluted. One spot on TLC with an Rf value of 0.19.

Table 28 Results obtained from column chromatography for fraction II.

The results obtained by TLC on these fractions are shown in Table 29.

System 18.

Fraction	U.V 254nm.	Anisaldehyde	Rf
1 - 10	-	-	-
11 - 31	-	purple	0.95
32 - 36	1. blue	-	0.76
	2. blue	-	0.62
	3. yellow	-	0.52
	4. blue	-	0.43
37 - 42	1. blue	-	0.38
	2. yellow	-	0.27
	3. blue	-	0.22
	4. -	purple	0.13
43 - 48	as above	as above	as above
49 - 70	-	purple	0.03
71 - 91	faint blue	purple	0.19

Table 29 Results obtained from the fractions eluted by column chromatography of fraction II.

Only fractions (49-70) and (71-91) contained a relatively large amount of material which could be dealt with, while the others were present only in trace amounts.

Fractions (49-70) were combined, evaporated to dryness and crystallized from 90% alcohol leaving a colourless product. The m.p. and the Rf value of this compound and the genin obtained after hydrolysis were similar to that of phaseoloside-A and its glycoside.

Fractions (71-91) were combined, evaporated to dryness and crystallized from 90% alcohol yielding a white product. The m.p. and the Rf value of the glycoside and the genin were similar to that of phaseoloside-B and its glycoside.

Separation and isolation of fraction I components.

Thin layer chromatography on fraction I.

System 19.

Stationary phase : Precoated silica gel F_{254}

Mobile phase : n-butanol-A.A-water 5:4:1

Location : U.V and anisaldehyde reagent

Results : Table 30

Spot No.	U.V 254nm.	Anisaldehyde	Rf
1	blue	orange	0.9
2	fluorescent yellow	-	0.7
3	fluorescent yellow	-	0.6
4	yellow	purple	0.0

Table 30 Results obtained from system 19 for fraction I.

Spot No. 1 and 4 were the major components of fraction 1 and they were isolated in the pure states by preparative TLC using the above system. Spots 2 and 3 were eluted by methanol separately. They left traces of oily residue, while bands corresponding to spots 1 and 4 were eluted by methanol and crystallized from methanol leaving a white platelet of crystals of phaseosterol-C (spot No. 1) (4 mg.,0.0006% w/w of the original weight) and phaseosterol-D (3 mg. 0.0004% w/w of the original weight).

Phaseosterol-D gave a single spot on aluminium oxide F plate with an Rf value of 0.05 using the same solvent system as above.

Spots 2 and 3 were visible yellow and gave purple colour with $FeCl_3$ reagent.

2. The rhizomes.

The crude saponins were isolated and hydrolysed in the same way as previously described for the roots. The isolated genin mixture was chromatographed using system 20.

System 20.

Stationary phase : Silica gel GF_{254}

Mobile phase : chloroform-methanol-ethylacetate 9:5:5

Location : U.V., phosphomolybdic acid and anisaldehyde reagents

Results : Table 31 and Fig.17

Spot No.	U.V 254nm.	Phosphomolybdic	Anisaldehyde	Rf
1	blue	blue	purple	0.89
2	blue	faint blue	faint purple	0.79
3	blue	faint blue	faint purple	0.69

Table 31 Components of rhizome genins isolated by system 20.

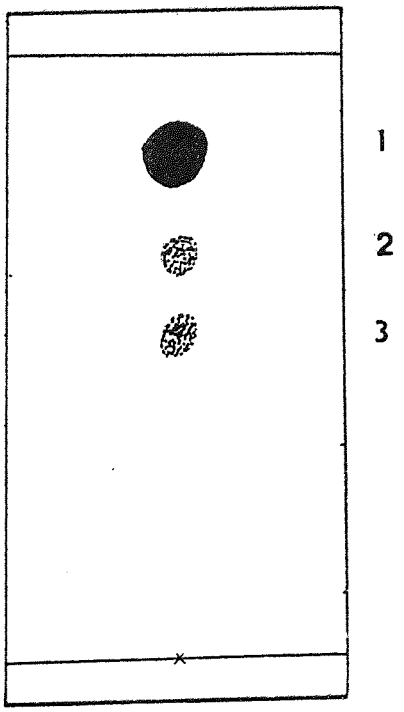


Fig.17 Chromatogram showing the components of rhizome genins isolated by system 20.

The large size of the spot 1 and the dark colour obtained with both reagents indicated that this spot was the major component.

Isolation of the genins by column chromatography.

The mixture (0.3 gm.) was dissolved in benzene (4 ml.) and transferred onto the top of the silica gel column (20 x 2 cm.). Elution was carried out with benzene-methanol (10:0.5) at the rate of 30 drops/min. and 10 ml. fractions were collected and examined by TLC using system 20.

Fractions 1-20 showed a single spot with an Rf value of 0.89, hence they were combined, evaporated to dryness and the white residue was crystallized from methanol leaving a white product (phaseoloside-E) (0.0008% w/w of the original weight).

The other two compounds were eluted with fractions 23-30 as a mixture in very small amounts.

3. The stems.

Extraction.

514.4 gm. of dried, powdered stems were extracted with methanol using a soxhlet apparatus for 12 hr. The green extract was evaporated to dryness under reduced pressure leaving a green residue (3.50% w/w of the original weight). This was suspended in water (100 ml.) and extracted with ether till the ether layers became almost colourless (4 x 200 ml.). The ether layers were combined, dried over anhydrous sodium sulphate and evaporated to dryness under reduced pressure yielding a brown oily residue (saponifiable and unsaponifiable fractions) (1.01% w/w) which was studied in detail under fatty acids and unsaponifiable fraction of the stems.

The aqueous layer was extracted with butanol saturated with water (3 x 100 ml.), the butanol layers combined and evaporated to dryness under reduced pressure leaving a brown residue (VII).

Thin layer chromatography on fraction (VII).

System 21.

Stationary phase : Silica gel GF_{254}

Mobile phase : chloroform-methanol-ethylacetate 9:1:1

Location : U.V. and phosphomolybdic acid reagent spray

Results : Table 32

Spot No.	U.V 254nm.	Phosphomolybdic	Rf
1	blue	blue	0.61
2	blue	blue	0.53
3	blue	blue	0.20

Table 32 Components of fraction (VII) obtained by system 21.

System 22.

Stationary phase : As under system 21

Mobile phase : chloroform-methanol-ethylacetate 5:3.5:1.5

Location : As under system 21

Results : Table 33

Spot No.	U.V 254nm.	Phosphomolybdic	Rf
1	blue	-	0.91
2	brown	-	0.82
3	blue	faint blue	0.75
4	blue	-	0.65
5	blue	-	0.60
6	blue	blue	0.39
7	-	blue	0.31
8	-	blue	0.25

Table 33 Components of fraction (VII) obtained from system 22.

Fraction (VII) was dissolved in hot methanol (10 ml.) and the saponins were precipitated by adding a suffient amount of acetone. The precipitate was filtered off, washed with acetone and dried to give a brown precipitate (VIII). The filtrate was evaporated to dryness leaving a brown oily residue (20 mg.) (IX).

Thin layer chromatography on fraction (IX).

System 22.

Results : Table 34

Spot No.	U.V 254nm.	Phosphomolybdic	Rf
1	blue	-	0.91
2	brown	-	0.82
3	blue	faint blue	0.75
4	blue	-	0.65
5	blue	-	0.60

Table 34 Components of fraction (IX) obtained from system 22.

System 21.

Results : Table 35

Spot No.	U.V 254nm.	Phosphomolybdic	Rf
1	blue	blue	0.09
2	blue	-	0.13
3	blue	-	0.18
4	blue	-	0.33
5	blue	-	0.48

Table 35 Components of fraction (IX) obtained from system 21.

Fraction (VIII) contained saponins (for the same reasons as previously mentioned) and was hydrolysed with 5N HCl at 100°C for 3 hr. The crude genins were filtered, washed with water and dried (X).

Thin layer chromatography on fraction (X).

System 21.

Results : Table 36

Spot No.	U.V 254nm.	Phosphomolybdic	Rf
1	blue	faint blue	0.93
2	blue	-	0.73
3	-	blue	0.69
4	-	blue	0.54
5	-	blue	0.50
6	faint blue	blue	0.42
7	-	blue	0.28
8	-	blue	0.13
9	-	blue	0.00

Table 36 Components of fraction (X) obtained from system 21.

N.B. Spot 6 was the largest one.

Isolation of fraction (X) components by column chromatography.

The mixture (X) was dissolved in toluene (4 ml.) and transferred onto a silica gel column (40 x 2 cm.). Elution was carried out with toluene and toluene-ethanol mixture at the rate of 30 drops/min. 10 ml. fractions were collected and each fraction was examined by TLC using

system 21. The results were summarised in Table 37.

Fraction	Solvent	Remarks
1 - 5	toluene	colourless eluents; showed single spot with an Rf value of 0.93. They were combined, evaporated to dryness and crystallized from methanol leaving a platelet of white crystals (5 mg., 0.0009% w/w of the original weight). Phaseobone-4
6 - 10	toluene	yellow band was eluted. Showed five spots with Rf values of 0.73, 0.69, 0.54, 0.50 and 0.42. They were combined, evaporated to dryness leaving a yellowish white precipitate (3 mg. in weight).
11 - 17	toluene	colourless eluents. Showed a single spot with an Rf value of 0.42. They were combined, evapo--rated to dryness and crystallized from methanol leaving a white product (5 mg., 0.00058% w/w of the original weight). Phaseoloside-F
18 - 19	toluene	colourless eluents and no spot was located.
20 - 26	toluene-methanol 9:1	brown band was eluted. Showed a single spot at the base line. They were combined, evaporated to dryness and crystallized from aqueous methanol (25%) yielding a white product 10 mg., 0.0019% w/w of the original weight) which was crystalline phaseoloside-G

Table 37 Column chtomatography results obtained from fraction (X).

Separation of the sugar components of the saponins.

The filtrate remaining after the aglycones were separated, was neutralised with sodium carbonate, concentrated to a small volume (10 ml.) and separated by TLC and PC using the following systems:

System 23.

Stationary phase : Cellulose F_{254}

Mobile phase : n-butanol-ethanol-water 4:1:2.2

Location : Ammoniacal silver nitrate and aniline hydrogen phthalate reagents

Results : Table 38

Fraction	$AgNO_3$	Aniline phthalate	Rf
II and III	1. purple	brown	0.25
	2. purple	yellowish brown	0.27
	3. purple	orange	0.20
sugars of phaseoloside A and B	1. purple	brown	0.25
	2. purple	yellowish brown	0.27
D-glucose	purple	brown	0.25
D-fructose	purple	yellowish brown	0.27
L(+)Arabinose	purple	red	0.30
Galacturonic acid	purple	orange	0.20

Table 38 Sugar components of the saponins (roots).

System 24.

Stationary phase : Whatman No.1

Mobile phase : As under system 23

Location : aniline hydrogen phthalate reagent

Results : Table 39

Fraction	Aniline phthalate	Rf
II	1. brown	0.30
	2. yellowish white	0.32
	3. red	0.34
	4. orange	0.18
phaseoloside A and B	1. brown	0.30
	2. yellowish white	0.32
	3. red	0.34
rhizomes sugars	1. brown	0.30
	2. red	0.34
	3. brown	0.47
VIII	1. brown	0.30
	2. red	0.34
	3. brown	0.28
D-Glucose	brown	0.30
D-Fructose	yellowish white	0.32
L(+)Rhamnose	brown	0.47
L(+)Arabinose	red	0.34
D-Galacturonic acid	orange	0.18
D-Galactose	brown	0.28
D-Mannose	brown	0.36

Table 39 Sugar components of saponins obtained from the runner bean.

Gas-liquid chromatography of the sugars obtained from the saponin of the roots.

Trimethyl silyl ether derivatives were made of the sugar fractions. The method used was as follows:

The aqueous solution was evaporated to dryness under reduced pressure giving a brown residue. Dry pyridine (0.5 ml.), hexamethyl--disilazane (0.2 ml.) and trimethylchlorosilane (0.1 ml.) were added and the mixture was shaken for 3 min. The excess reagent was evaporated under reduced pressure and the product was extracted with toluene (5 ml.). 2 µl of this was used for GLC analysis.

System 3.

Column	: 3 pc SE-30 on chromosorb G (80-100) mesh, 1.5 m. in length
Oven temp.	: 180°C
Inj. temp.	: 280°C
Carrier gas	: Nitrogen at 30 p.s.i.
Oxygen and Hydrgen	: At 20 p.s.i.
Chart speed	: 20 mm/min.
Amplitude	: 2×10^2
Results	: Table 40 and Figs.18 and 19

N.B. Silyl ether derivatives were made for the standards in the same way as described above.

Fraction	Peak No.	Rt	Area under the peak ($cm.^2$)
II	1	0.85	3.43
	2	1.85	8.54
	3	2.50	7.04
	4	3.50	4.65
III	1	1.85	9.78
	2	2.49	4.32
	3	3.50	0.95
D-Glucose		2.50	-
D-Fructose		1.85	-
L(+)Rhamnose		1.00	-
L(+)Arabinose		0.85	-
D-Gluronic acid		4.50	-
Galacturonic acid		3.45	-
D-Galactose		2.35	-
D-Mannose		1.90	-
Phaseoloside A and B	1	2.50	-
	2	1.85	-
	3	0.85	-

Table 40 GLC of the sugar components of the runner bean roots.

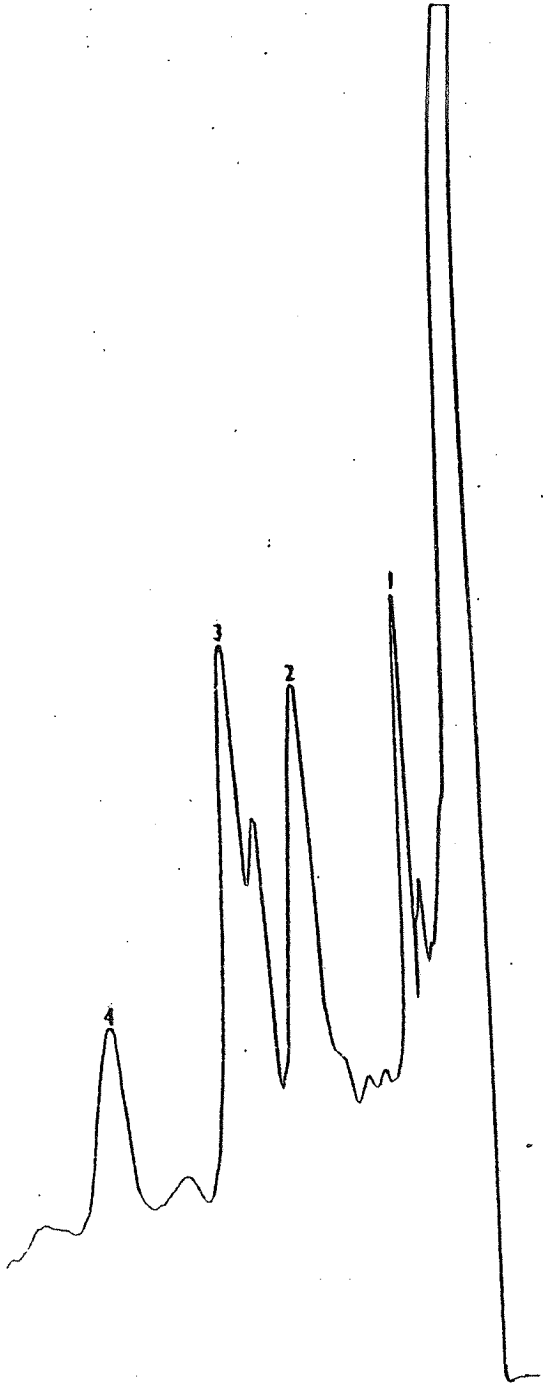


FIG. 18 THE TMS DERIVATIVES OF THE SUGAR COMPONENTS OF FRACTION II (RUNNER BEAN).

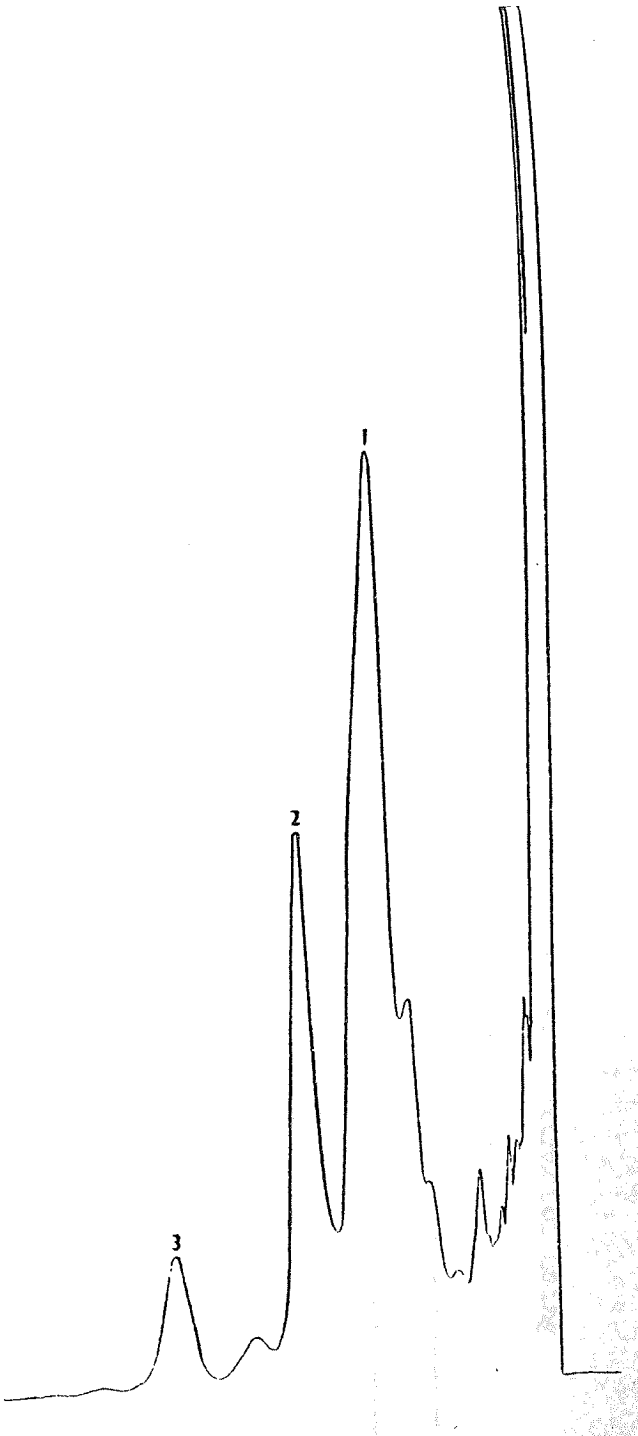


FIG. 19 THE TMS DERIVATIVES OF THE SUGAR COMPONENTS OF FRACTION III (RUNNER BEAN).

II-2-2-2-2- Gleditsia triacanthos (Honey-locust).

A. Honey-locust pods.

Extraction.

14.4 gm. of the dried powdered pods were extracted using scheme 3 and the results obtained are shown in Table 41.

Fraction	Remarks
A	yellow, oily residue (0.95 gm.)
B	yellowish white precipitate (0.61 gm.)
C	yellowish brown oily residue (0.31 gm.)
D	yellowish white precipitate (3.80 gm.)
E	yellow residue (0.40 gm.)
F_1	brown residue (2.00 gm.)
I^1	white precipitate (0.90 gm.)
F_2	The aqueous layer was evaporated to dryness, dissolved in methanol (10 ml.) and the crude saponin was precipitated as for F_1. It gave a white precipitate (0.95 gm.)

Table 41 Scheme 3 fractions obtained from the Honey locust pods.

Thin layer chromatography on fraction B.

System 25.

Stationary phase : Precoated F_{254}

Mobile phase : chloroform-methanol-ethylacetate 7:2:1

Location : U.V and phosphomolybdic acid reagent spray

Results : Table 42

Spot No.	U.V 254nm.	Phosphomolybdic	Rf
1	blue	blue	0.90
2	-	blue	0.79
3	-	blue	0.73
4	-	blue	0.65
β-sitosterol	-	blue	0.73

Table 42 Fraction B components obtained by using system 25.

Isolation of the components of fraction B by column chromatography.

Fraction B (0.3 gm.) was dissolved in toluene (4 ml.) and put onto the top of the silica gel column (40 x 2 cm.). The elution was carried out with toluene at the rate of 30 drops/min. Fractions of 10 ml. each were collected. Each fraction was concentrated to a small volume and examined by TLC using system 25.

Results showed that fractions 1-14 contained a single spot with the Rf value of 0.90. They were combined, evaporated to dryness and crystallized from methanol leaving a white platelet of crystals (25 mg.) "Triacanane-B".

Fractions 16-17 contained a very small quantity of the compound with an Rf value of 0.79. This was neglected due to the small amount present.

Fractions 18-20 gave traces of a compound with an Rf value of 0.73. Although the quantity of this compound was small, a few crystals were isolated in sufficient quantity to do a m.p. It was crystallized from methanol leaving colourless needle crystals "Triacanthosterol-A".

The other fractions (20-22) showed a mixture of spots 3 and 4, while fractions 23-27 showed a single spot with an Rf value of 0.65. Again the quantity was very small and a few crystals were isolated to do the m.p. only. "Triacanthosterol-B".

Separation of fraction C components by GLC.

The fatty acids present in the pods were analysed by GLC using their methyl esters. The derivatives were made as previously described for P.coccineus.

0.31 gm. of the saponifiable fraction was esterified and the methyl esters were dissolved in ether (10 ml.).

The following systems were used:

System 4.

Column : 3% SE-30 on chromosorb G (100-120) mesh, 1.5 m. in length.
Oven temp. : 185°C
Inj. temp. : 300°C
Carrier gas : Nitrogen at 30 p.s.i.
Oxygen and Hydrogen: At 20 p.s.i.
Chart speed : 10 mm./min.
Amplitude : 20 x 10^2
Sample volume : 3 µl
Detector : Flame ionisation.

Standard methyl esters were run separately and by mixing equal volumes of 1% of the standard with the sample under the same conditions. Other fatty acids were identified by comparing their Rt. with those of the published data. Results were shown in Table 43 and Fig. 20.

Peak No.	Rt. min.	Area under peak in $mm.^2$	Percentage present	Probable acid
1	0.6	220.0	8.9	Caprylic
2	0.8	201.0	8.1	Capric
3	0.9	283.5	11.5	Lauric
4	1.3	180.0	7.3	Myristic
5	1.9	744.0	30.3	Palmitic
6	2.5	137.5	5.6	Palmetoleic
7	3.4	321.3	13.0	Oleic
8	3.8	264.0	10.7	Stearic
9	4.6	24.5	0.9	Linoleic
10	5.5	77.0	9.1	Linolenic

Table 43 Fatty acid methyl esters of fraction C (Honey-locust pods) obtained by using system 4.

System 5.

Column : Apiezon 4% on chromosorb G (100-120) mesh, 1.5 m. in length.

Oven temp. : 250°C

Inj. temp. : 300°C

Chart speed : 10 mm./min.

Carrier gas : Nitrogen at 25 p.s.i.

Oxygen and Hydrogen : At 20 p.s.i.

Amplitude : 20×10^2

Sample volume : 3 µl

Detector : Flame ionization.

Results : Table 44 and Fig. 21.

Peak No.	Rt. min.	Area under the peak in $mm.^2$	Percentage present	Probable acid
1	0.9	82.5	3.5	Caprylic
2	1.2	301.5	12.9	Capric
3	1.3	222.0	9.5	Lauric
4	1.4	385.0	16.5	C_{12} alkenoic
5	1.7	130.5	5.6	Myristic
6	2.2	172.5	7.4	Myristoleic
7	2.5	182.0	7.8	C_{14} alkenoic
8	2.8	87.0	3.7	C_{14} alkenoic
9	3.9	480.0	18.5	Palmitic
10	6.4	270.0	11.6	Oleic
11	7.1	58.0	2.4	Linoleic

Table 44 Fatty acid methyl esters of fraction C (Honey-locust pods) obtained by using system 5.

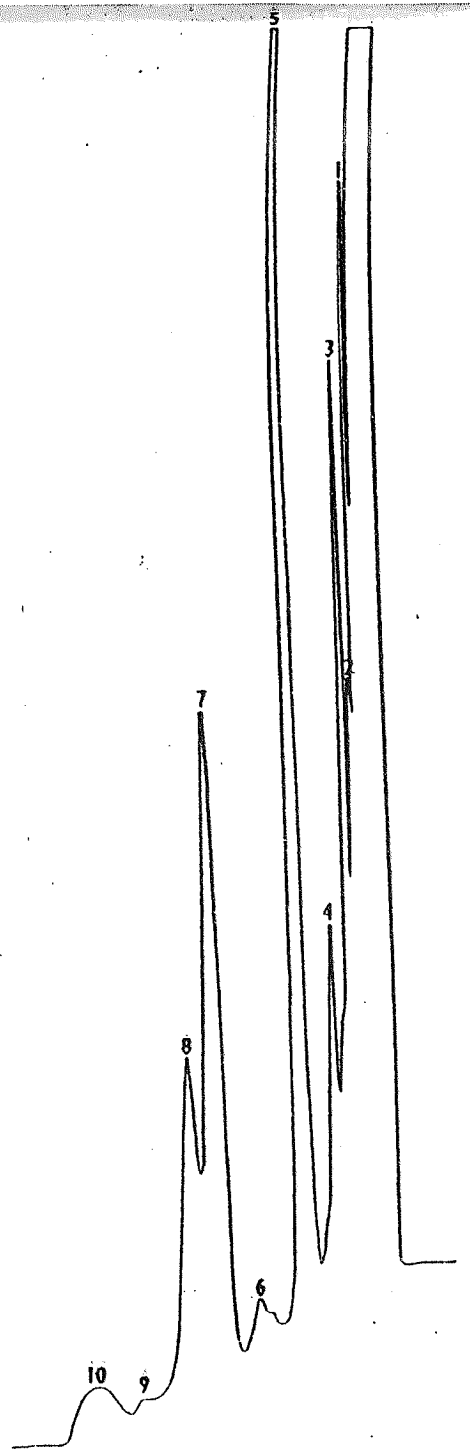


FIG. 20 FATTY ACID METHYL ESTERS OF HONEY LOCUST PODS FROM FRACTION C USING SYSTEM 4.

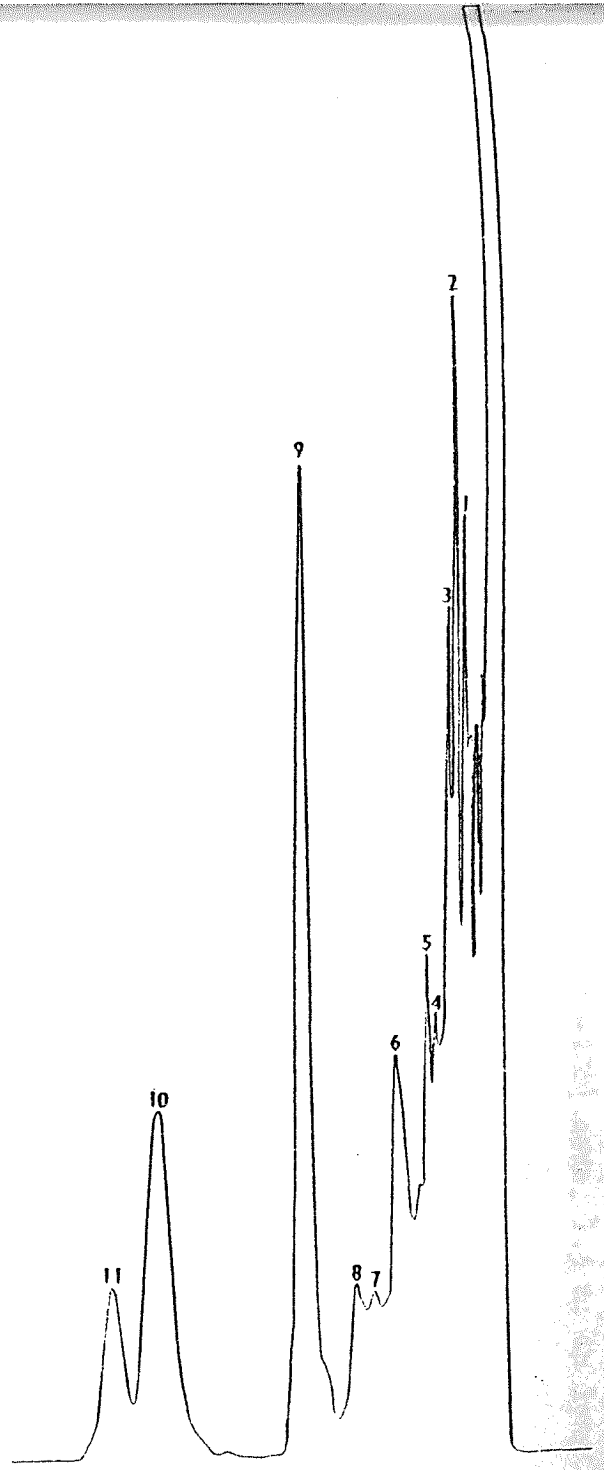


FIG. 21 FATTY ACID METHYL ESTERS OF HONEY LOCUST PODS FROM FRACTION C

System 6.

Column : 5% diethylene glycol polysuccinate on chromosorb G (80-100) mesh, 1.5 m. in length.

Oven temp. : 185°C

Inj. temp. : 280°C

Chart speed : 5 mm./min.

Carrier gas : Nitrogen at 30 p.s.i.

Oxygen and Hydrogen : At 20 p.s.i.

Amplitude : 2×10^2

Sample volume : 3 µl

Results : Table 45.

Peak No.	Rt min.	Probable acid
1	3.4	Palmitoleic
2	4.0	Palmitic
3	7.0	Oleic
4	8.0	Linoleic

Table 45 Fatty acid methyl esters of fraction C (Honey-locust pods) obtained by using system 6.

Separation of fraction E by TLC.

System 26.

Stationary phase : As under system 25

Mobile phase : As under system25

Location : U.V., phosphomolybdic and anisaldehyde reagent sprays

Results : Table 46

Spot No.	U.V 254nm.	Phosphomolybdic	Anisaldehyde	Rf	Remarks
1	blue	blue	purple	0.90	major
2	blue	blue	purple	0.79	
3	blue	blue	purple	0.73	
4	-	blue	purple	0.65	
5	blue	-	-	0.53	
6	-	blue	brown	0.15	visible yellow
7	-	blue	purple	0.00	

Table 46 Fraction E components obtained by using system 26.

N.B. Only spot 6 gave a purple colour when the chromatogram was sprayed with ferric chloride reagent.

Isolation of fraction E components by column chromatography.

The components were isolated using the same procedure as described previosly for fraction B, and only one compound was isolated in sufficient quantity to allow identification. This compound had an Rf value of 0.90 (20 mg.) Triacanane-C.

Separation and isolation of fractions I,G and F.

Fractions F and I contained saponin glycosides (identified by the tests previously described). Fraction G gave trace amounts of a brown resin which was insufficient to allow identification.

Thin layer chromatography on fractions F and I.

System 27.

Stationary phase : Precoated silica gel F_{254}

Mobile phase : n-butanol-A.A-water 4:1:5

Location : U.V., phosphomolybdic and anisaldehyde reagents

Results : Both the fractions gave the same results, namely one spot which gave a blue colour with phosphomol--ybdic acid reagent and a purple colour with anisaldehyde reagent with an Rf value of 0.14.

Hydrolysis of saponins F and I.

900 mg. of fraction I was hydrolysed with 5N HCl at 100°C for 3 hr. The aglycones were filtered, washed with water and extracted by soxhlet with petroleum ether. The extract was evaporated under reduced pressure yielding a white precipitate (500 mg.), which gave a purple colour with Lieberman's reagent.

Fraction F gave the same results as fraction I. The filtrate from F and I fractions was examined for its sugar contents.

Thin layer chromatography on genins F and I.

System 25.

Results: Table 47

Spot No.	U.V 254nm.	Phosphomolybdic	Anisaldehyde	Rf
1	-	faint blue	-	0.90
2	-	blue	purple	0.86
3	-	blue	purple	0.78
4	-	faint blue	pink	0.74
5	-	faint blue	pink	0.64
6	-	faint blue	pink	0.56
7	-	faint blue	pink	0.47
8	-	faint blue	pink	0.41

Table 47 Components of fraction I genins obtained by using system 25.

N.B. Spots No. 2 and 3 were the largest spots and gave intense blue and purple colours with the above reagents.

Isolation of the genins by column chromatography.

The sapogenins mixture (500 mg.) was dissolved in chloroform (4 ml.) and transferred onto the top of a silica gel column (40 x 2 cm.). Fractions of 10 ml. each were collected at the elution rate of 30 drops/min. Each fraction was tested by TLC using system 25. Elution was carried out with chloroform only.

Fractions 1-3 showed a single spot with an Rf value of 0.90. These fractions were combined, evaporated to dryness and the residue was crystallized from methanol leaving a white platelet of crystals (5 mg.) "Triacanane-A".

Fractions 4-10 showed a single spot with an Rf value of 0.86. These fractions were combined, evaporated to dryness and crystallized from methanol yielding a white platelet of crystals (25 mg.) "Triaconthoside-A".

Fraction 11 left traces of white precipitate which when evaporated to dryness showed two spots with Rf values of 0.86 and 0.78.

Fractions 12-24 showed a single spot with an Rf value of 0.78.

These fractions were combined, evaporated to dryness and crystallized from methanol leaving white platelet crystals (25 mg.) "Triacanthoside-B".

Fractions 25-35 left a trace of white precipitate. When they were evaporated to dryness, they showed spots 4, 5, 6, 7 and 8.

The genins obtained from fraction F were subjected to the same procedures i.e. TLC and column chromatography.

On TLC (system 25), only two spots were detected with Rf values of 0.86 and 0.78. They gave a blue colour with phosphomolybdic acid reagent and a purple colour with anisaldehyde reagent.

The genins of fraction F were isolated by column chromatography using the same procedures as described previously. These two compounds were isolated in pure states and they were found to have similar physical properties to triacanthoside-A (20 mg.) and triacanthoside-B (20 mg.).

In the column chromatographic methods for both genin I and genin F, a yellow resinous substance remained on the top of the columns and would not elute even with very polar solvent (methanol).

Aqueous extract of the marc.

The marc (pods) left after petroleum ether and methanol extr--actions was extracted with hot water (150 ml.) with continuous stirring. The reddish brown extract was filtered and evaporated to about 20 ml. using a rotatory vacuum, leaving a brown viscous residue. This residue was dissolved in methanol and the saponin content was precipitated by the addition of sufficient quantity of ether giving a brownish white precipitate which was filtered, washed with ether and dried (XI).

The filtrate was concentrated to a thick syrup. Alcohol was added to the aqueous solution and the white precipitate obtained was filtered and dried (Galactomannan) (XII).

Fraction (XI) was hydrolysed and the crude sapogenins were isolated in the same way as described previously for fractions I and F.

The crude genins (300 mg.) were separated on TLC using system 25 and the results were shown in Table 48 and Fig. 22.

Spot No. 1 was the largest spot in the whole mixture while the others occurred in traces. It was isolated from the others by elution from a silica gel column with toluene, and the fractions containing this spot were combined and evaporated to dryness. The residue was crystallised from methanol leaving platelet crystals (9.5 mg.) "Triacanane-D".

Fraction (XII) was centrifuged and the sticky mass (10% w/w of the original weight) was hydrolysed with 3N sulphuric acid at 100°C for 6 hr. It was neutralised with barium carbonate and filtered. The filtrate was evaporated to a viscous syrup. D-galactose and D-mannose were identified using PC and TLC (systems 28 and 29).

Spot No.	U.V. 254nm.	Phosphomolybdic	Anisaldehyde	Rf.
1	blue	faint blue	-	0.90
2	-	blue	purple	0.84
3	-	blue	pink	0.76
4	blue	blue	pink	0.64
5	-	blue	pink	0.58
6	-	blue	pink	0.52
7	-	blue	pink	0.43
8	-	blue	pink	0.37
9	blue	blue	pink	0.09
10	blue	blue	pink	0.00

Table 48 The genin components of fraction (XI) (Honey-locust pods) obtained by using system 25.

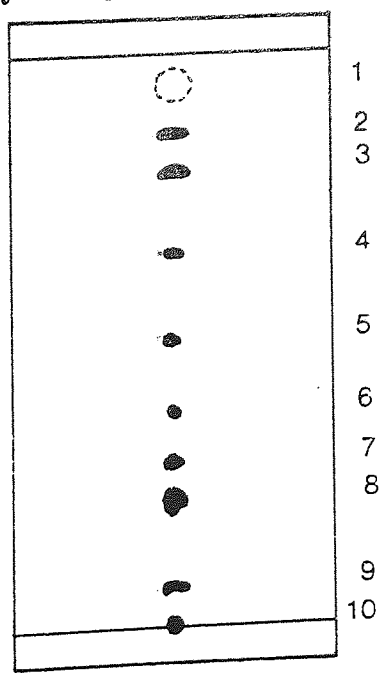


Fig. 22 Chromatogram of genins (XI) obtained by using system 25.

Acetylation of triacanthoside-A and triacanthoside-B.

5 mg. of the genin was dissolved in dry pyridine (3 ml.), acetic anhydride (3 ml.) and glacial acetic acid (3 ml.). The mixture was refluxed for 15 min. The hot solution was then poured onto ice water. The product was filtered, washed with water and crystallised from methanol to yield white platelet crystals.

Extraction and separation of the alkaloid contents.

Using the method of extraction and system of separation after Camp[42], for detecting the alkaloid contents of the leaves of G.triacanthos, the following results were obtained from the pods.

The alkaloid fraction was isolated as brown liquid with a pungent odour (0.28% w/w of the original weight). Thin layer chromatography on the alkaloid fraction using the system after Camp[42] showed the following results (Table 49).

Fraction	Spot No.	U.V 254nm.	Ninhydrin	Rf
Alkaloid	1	blue	red	0.80
	2	blue	red	0.68
Alkaloid HCl	1	-	red	0.46
	2	-	red	0.44

Table 49 Alkaloid content of the Honey-locust pods.

B. Honey-locust seeds.

Using scheme 3 for the extraction, the following results were obtained:

Fraction	Remarks
A	yellow oily residue (2.64% w/w of the original weight)
B	yellowish white precipitate (0.40% w/w of the original weight)
C	yellowish brown oil (2.03% w/w of the original weight)
D	yellowish white precipitate (35% w/w of the original weight)

Table 50 Fractions obtained from the Honey-locust seeds by using scheme 3.

Due to the small amount of the sample available, the other fractions were present in an amount which made the identification impossible.

Thin layer chromatography.

1. Fraction B.

System 25.

Results : Table 51

Spot No.	U.V 254nm.	Phosphomolybdic	Anisaldehyde	Rf
1	blue	faint blue	-	0.90
2	-	blue	purple	0.78
3	-	blue	purple	0.73
4	-	blue	purple	0.65

Table 51 Components of the unsaponifiable fraction B of the Honey-locust seeds obtained by using system 25.

2. Fraction A.

System 25.

Results : Table 52

Spot No.	U.V 254nm.	Phosphomolybdic	Rf
1	blue	blue	0.90
2	-	blue	0.79
3	-	faint blue	0.69
4	-	blue	0.48
5	-	faint blue	0.28
6	-	blue	0.08
7	-	blue	0.00

Table 52 Components of fraction A of the Honey-locust seeds obtained by using system 25.

Separation of the saponifiable fraction C by GLC.

Applying the same procedure as described previously for the fatty acids from pods and using systems 4 and 5, the following results were obtained:

1. System 4.

Results : Table 53 and Fig.23.

Peak No.	Rt min.	Area under peak in sq. mm.^{2}	Percentage present	Probable acid
1	0.6	324.0	19.9	Caprylic
2	0.8	184.5	11.4	Capric
3	1.0	217.5	13.4	Lauric
4	1.1	156.0	9.6	Myristic
5	1.2	88.5	5.5	Myristoleic
6	1.9	204.0	12.6	Palmitic
7	3.4	324.0	19.9	Oleic
8	3.8	78.0	4.8	Stearic
9	4.6	15.0	0.9	Linoleic
10	5.5	33.0	2.0	Linolenic

Table 53 Fatty acid methyl esters of fraction C (Honey-locust seeds) obtained by using system 4.

2. System 5.

Results : Table 54 and Fig.24.

Peak No.	Rt min.	Area under peak in sq. mm.^{2}	Percentage present	Probable acid
1	1.5	404.0	17.4	Caprylic
2	1.7	321.0	13.3	Capric
3	2.4	320.0	13.2	Lauric
4	2.9	244.0	10.1	Myristic
5	3.9	429.0	17.7	Palmitic
6	6.4	565.0	23.3	Oleic
7	7.2	92.0	3.8	Linoleic
8	8.9	40.0	1.6	Linolenic

Table 54 Fatty acid methyl esters of fraction C (Honey-locust seeds) obtained by using system 5.

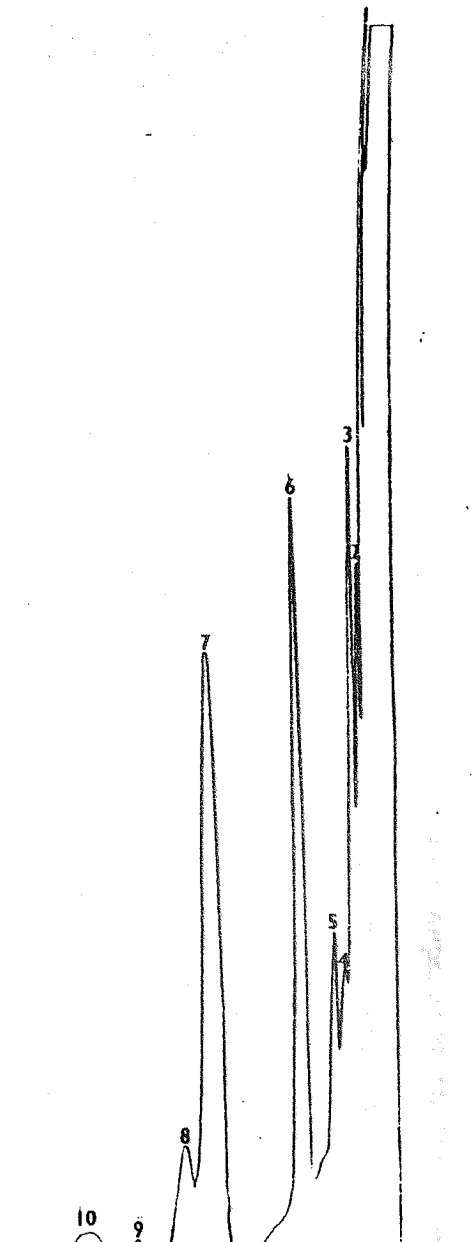


FIG. 23 FATTY ACID METHYL ESTERS OF HONEY LOCUST SEEDS (SYSTEM 4).

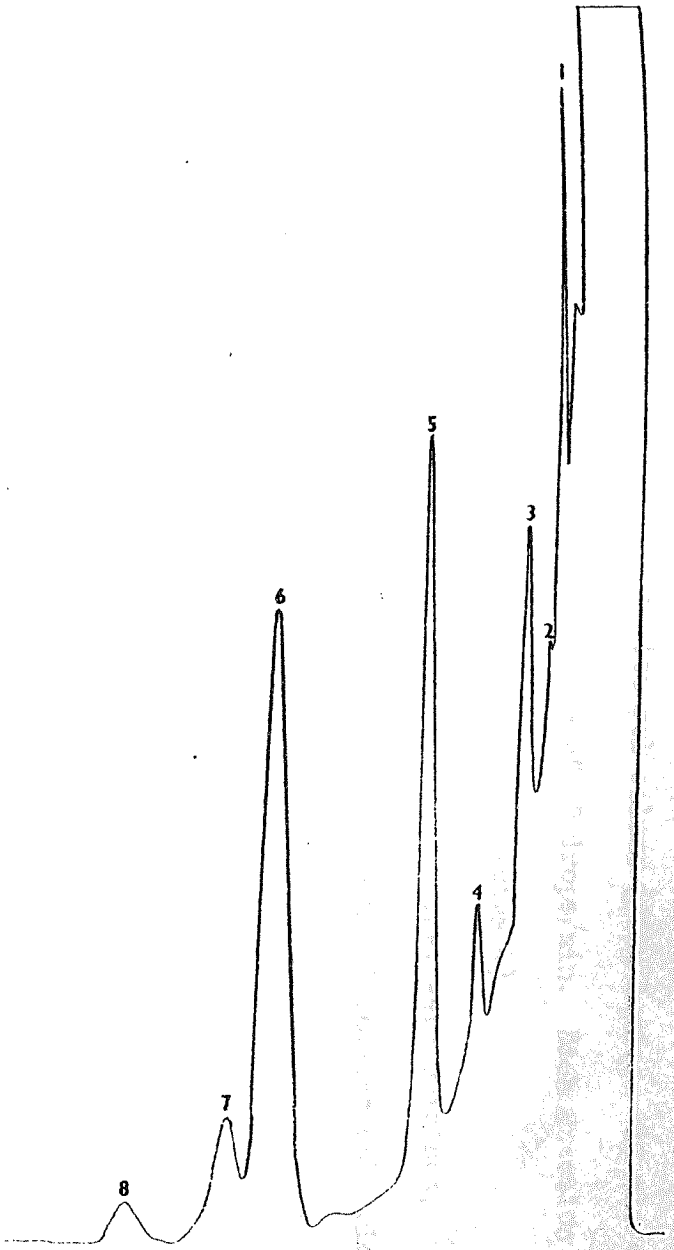


FIG. 24 FATTY ACID METHYL ESTERS FROM THE HONEY LOCUST SEEDS (SYSTEM 5).

Isolation of the saponifiable fraction C by column chromatography.

About 0.2 gm. of fraction C was dissolved in benzene (4 ml.) and put onto the top of the silica gel column (50 x 2 cm.). 10 ml. each fractions were collected at the rate of 30 drops/min. Each fraction was examined by TLC (system 1) and by GLC (system 4).

Fractions 1-5 showed a single spot. They were combined, evaporated to dryness and crystallised from methanol leaving colourless crystals m.p. 63°C. Its methyl ester gave a single peak with Rt of 1.9 min. (system 4).

Fractions 7-10 eluted an oily yellowish brown compound which gave a single spot on TLC and a single peak with Rt of 3.4 min. (system 4) of the methyl ester.

The other fractions showed a mixture of the other fatty acids which occurred in small amounts.

The above two compounds were found to be palmitic and oleic acids by comparing their Rt with those of the standards.

Separation and isolation of fraction D components.

The results obtained from the pods were similar to those obtained from the seeds. The saponins were separated in the same way i.e. hydrolysed and separated by TLC using system 25. The following results were obtained:

Spot No.	U.V 254nm.	Phosphomolybdic	Rf
1	-	faint blue	0.90
2	blue	blue	0.86
3	blue	blue	0.79
4	-	blue	0.73
5	blue	-	0.13
6	blue	-	0.04

Table 55 The genin components of the Honey-locust seeds obtained by using system 25.

Isolation of the sapogenins from the seeds by column chromatography.

The procedure applied was similar to that used for the isolation of sapogenins from the pods.

Only three compounds were isolated in pure states and they were found to be triacanane-A, triacanthoside-A and triacanthoside-B as they had the same m.p. and Rf values as those isolated previously from the pods.

Sugar analysis of seeds and pods sapogenins.

The sugars of the genins isolated from the pods and seeds were separated on TLC and PC using different solvent systems. These were compared with authentic samples.

System 28.

Stationary phase : Whatman No. 1

Mobile phase : n-butanol-A.A.-water 4:1:5

Location : Aniline hydrogen phthalate

Results : Table 56

Fraction	Spot No.	Colour	Rf
A	1	purple	0.29
	2	brown	0.17
B	1	purple	0.29
	2	brown	0.17
D-Glucose		brown	0.17
L(+)Arabinose		purple	0.29
D-Galactose		brown	0.23
D-Mannose		brown	0.29
D-Glucuronic		brown	0.23
D-Galacturonic		brown	0.17
L(+)Rhamnose		purple	0.40

Table 56 Sugar components of fraction I and F_2 obtained by using system 28.

N.B. Fraction A = Sugars from fraction I.

Fraction B = Sugars from fraction F_2.

System 29.

Stationary phase : As under system 23

Mobile phase : As under system 23

Location : Aniline hydrogen phthalate

Results : Table 57 and Fig. 25

Fraction	Spot No.	Colour	Rf
A	1	brown	0.46
	2	reddish brown	0.30
	3	brown	0.28
B	1	orange	0.86
	2	brown	0.46
	3	reddish brown	0.30
	4	brown	0.28
C	1	reddish brown	0.30
	2	brown	0.28
	3	orange	0.20
	4	orange	0.11
D	1	reddish brown	0.30
	2	brown	0.28
	3	orange	0.20
D-Glucose		brown	0.28
L(+)Arabinose		reddish brown	0.30
D-Galactose		brown	0.26
L(+)Rhamnose		brown	0.46
D-Galacturonic		orange	0.20
D-Glucuronic		orange	0.11

Table 57 Sugar components of the Honey-locust seeds and pods.

N.B. C = Sugars from seed saponins.

D = Sugars of the aqueous extract saponins (pods).

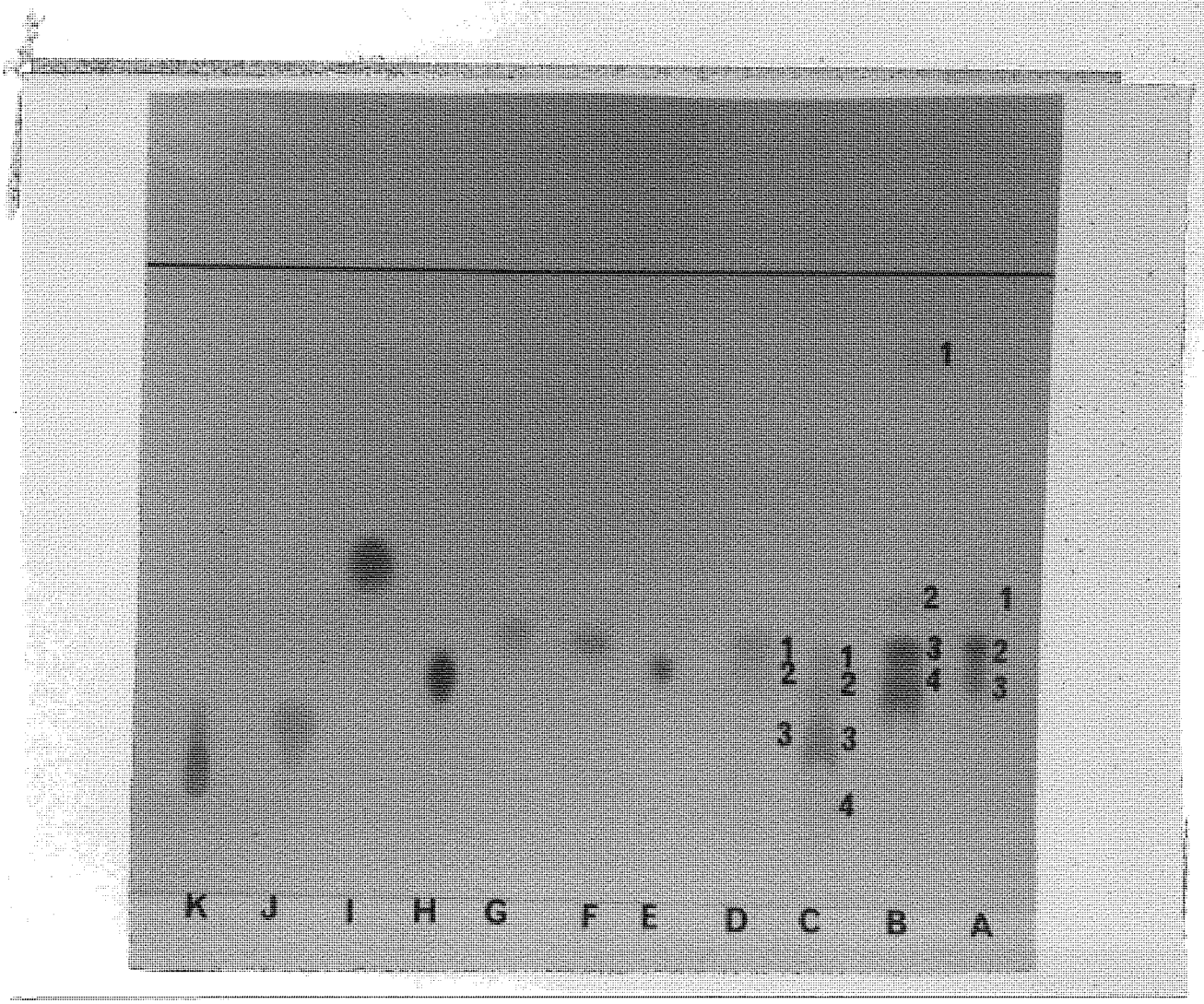


Fig. 25 Sugar components of the Honey-locust saponins (seeds and pods).

A). Sugars from fraction I pods.

B). Sugars from fraction F_2 pods.

C). Sugars from seeds saponins.

D). Sugars of the aqueous extract saponins (pods).

E). D-Glucose.

F). L(+)Arabinose.

G). D-Mannose.

H). D-Galactose.

I). L(+)Rhamnose.

J). D-Galacturonic acid.

K). D-Glucuronic acid.

II-2-2-2-3- Apium graveolens (celery) stems.

Using the same conditions of extraction as previously described (scheme 3), the dried, powdered celery stems were extracted (weight of the sample 373.3 gm.). The results obtained are shown in Table 58.

Fraction	Remarks
A	yellow oily residue (6.3 gm.) (1.6% w/w of the original weight)
B	Orange precipitate (5.0 gm.) (1.3% w/w of the original weight)
C	colourless oil (0.2 gm.) (0.053% w/w of the original weight)
D	brown, viscous residue (26.96% w/w of the original weight)
E	yellowish brown oily residue (1.3% w/w of the original weight)
E_1	yellowish white precipitate (0.4% w/w of the original weight)
E_2	dark brown oily residue (0.5% w/w of the original weight)
F_1	brown, solid residue (1.6% w/w of the original weight)
* F_2	It was evaporated under reduced pressure to a very thick syrup, then it was crystallised twice from aqueous methanol (1:1), leaving colourless needle crystals (graveose-A) (1.0% w/w of the original weight)
I	yellowish brown precipitate (0.33% w/w of the original weight)
G	The ether of precipitation was evaporated to dryness leaving a yellow precipitate (0.16% w/w of the original weight). It was crystallised from methanol leaving needle crystals (0.03% w/w of the original weight).

Table 58 Fractions obtained from scheme 3.

* The mother liquor was evaporated to dryness leaving a white precipitate (2-3% w/w of the original weight) of the inorganic substances.

Thin layer chromatography on fraction A.

System 30.

Stationary phase : Precoated silica gel F_{254}

Mobile phase : toluene-methanol-ethylacetate 9:1:0.5

Location : As under system 9

Results : Table 59 and Fig.26

	U.V			
Spot No.	254 nm.	366 nm.	Phosphomolybdic	Rf
1	-	-	blue	0.00
2	-	-	blue	0.02
3	-	-	blue	0.07
4	-	-	blue	0.14
5	blue	blue	-	0.15
6	-	-	blue	0.23
7	-	-	blue	0.34
8	blue	blue	blue	0.51
9	-	-	blue	0.61
10	fluorescent yellow	blue	-	0.78
11	-	-	blue	0.97

Table 59 Components of fraction A (celery) obtained by using system 30.

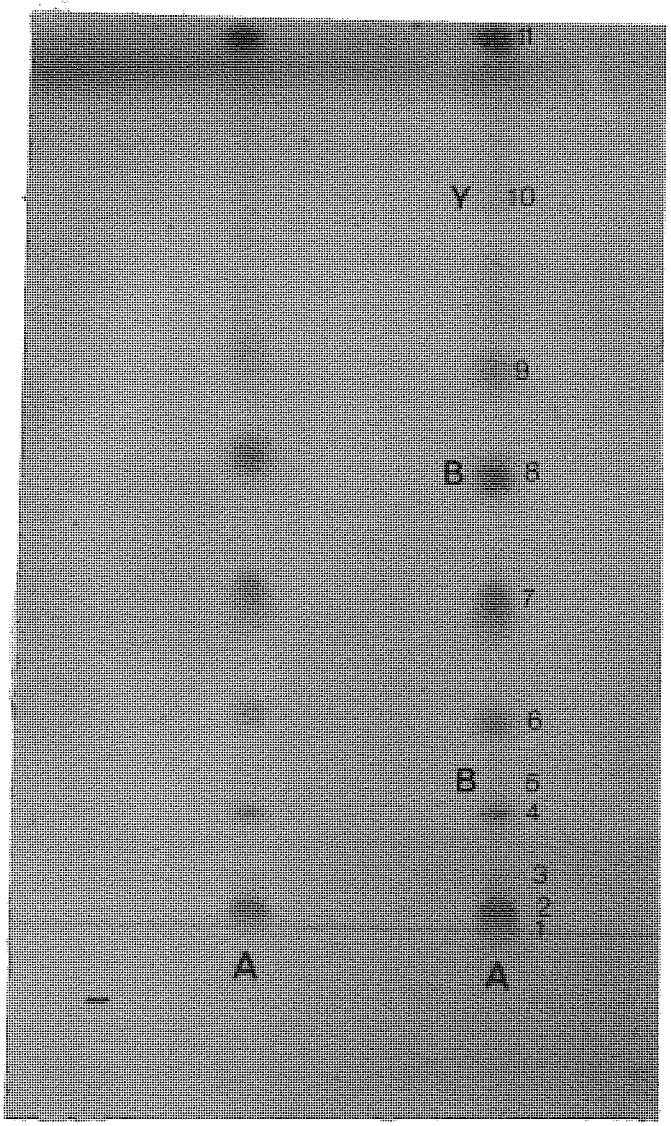


Fig. 26 Chromatogram obtained from system 30 sprayed with phosphomolybdic acid reagent.

Y = Fluorescent yellow under U.V. light 254nm.

B = Blue under U.V. light 254nm.

Thin layer chromatography on fraction B.

System 30.

Results : Table 60 and Fig.27.

Spot No.	U.V 254 nm.	U.V 366 nm.	Phosphomolybdic	Rf
1	fluorescent blue	blue	blue	0.97
2	"	"	blue	0.63
3	"	"	blue	0.49
4	"	"	blue	0.42
5	"	"	blue	0.33
6	"	"	blue	0.16
7	"	"	blue	0.12
8	"	"	blue	0.09
9	"	"	blue	0.05

Table 60 Components of fraction B (celery) obtained by using system 30.

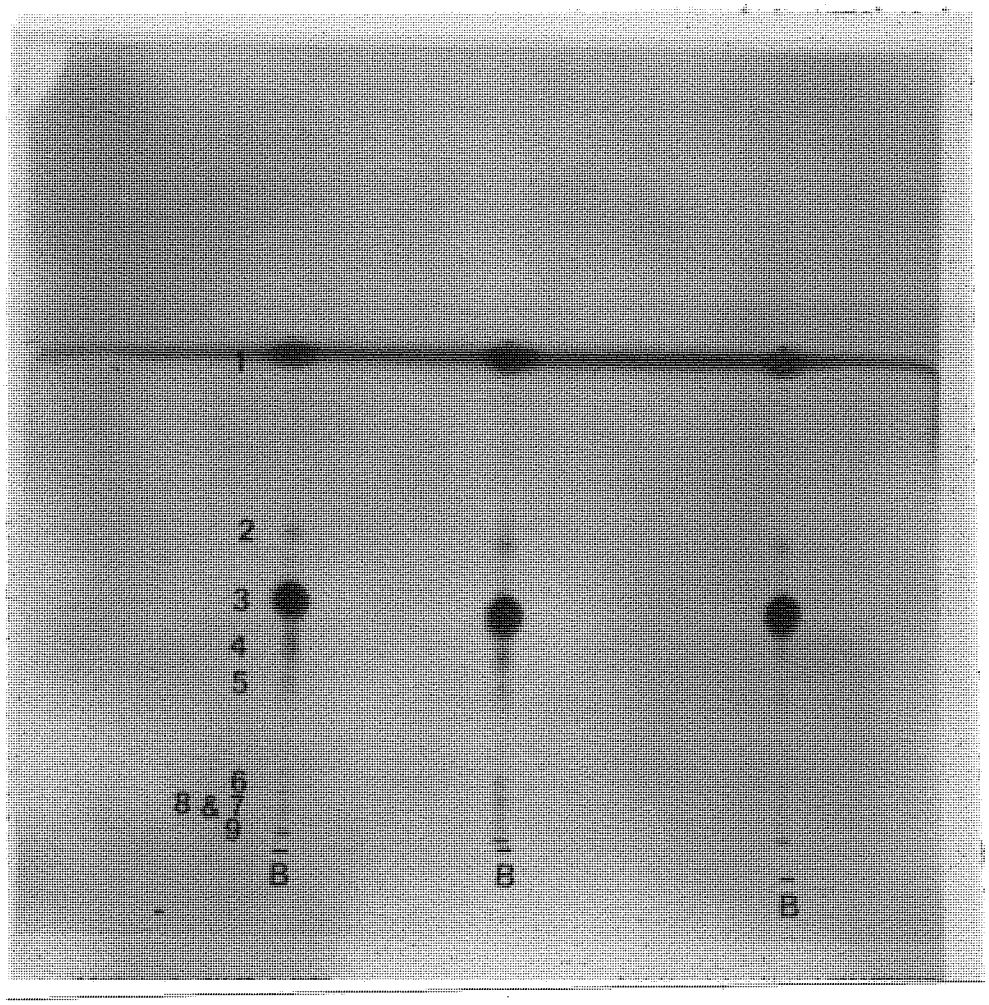


Fig. 27 Chromatogram from system 30 of fraction B sprayed with phosphomolybdic acid reagent.

Isolation of the components of fraction B by column chromatography.

The unsaponifiable fraction B (2.9 gm.) was dissolved in toluene (8 ml.) and loaded onto a silica gel column (30 cm. in length and 3 cm. in diameter). The slurry was made with toluene. Elution rate was at 30 drops/min. and 10 ml. fractions were collected sequentially and each tested by TLC using system 30.

Fractions 1-19 did not show any spot, and the eluent was colourless Fractions 20-40 represented the passage of a yellow band down the column, and each showed a single spot with an Rf value of 0.97. These fractions were combined and the toluene was evaporated under reduced pressure to yield an oily orange residue (20 mg.)(0.68% w/w of the unsaponifiable fraction). The fraction was labelled graveobone-A.

Fractions41-59 showed traces of an oily compound with an Rf value of 0.85, and due to the small quantity obtained it was neglected.

Elution was started at this stage with toluene-methanol (95:5), and fractions60-70 gave traces of a compound with an Rf value of 0.63. This compound was neglected due to the small quantity. Fractions 71-79 did not show any presence of a compound. Fractions 80-110 showed a single spot with an Rf value of 0.49. These fractions were combined and evaporated to dryness yielding a white precipitate which when crystallised from methanol gave colourless needle crystals (m.p. 155°C, 85 mg., 0.039% w/w of the original weight). This compound was labelled graveosterol-A.

Finally the column was eluted completely with methanol and the eluent was evaporated to dryness leaving a very small amount of a brown residue. TLC showed that this brown residue contained a mixture of compounds which corresponded to the spots 4-9 inclusive.

Gas liquid chromatography on graveobone-A.

The oily residue was separated as a single peak on 3 pc SE-30 column with a Rt of 6.5 min. using the following conditions: System 7.

Column	: 3 pc SE-30 on chromosorb G (80-100 mesh), 1.5 m. in length.
Oven temp.	: 150°C.
Inj. temp.	: 250°C.
Concentration	: 1 mg./ml. (toluene).
Volume	: 2 µl.
Carrier gas pressure	: Nitrogen at 20 p.s.i.
Oxygen and Hydrogen	: 20 p.s.i.
Chart speed	: 10 mm./min.
Detector	: Flame ionisation detector.
Results	: Fig. 28.

N.B. Shorter Rt was obtained (2.4 min.) at the oven temperature of 200°C.

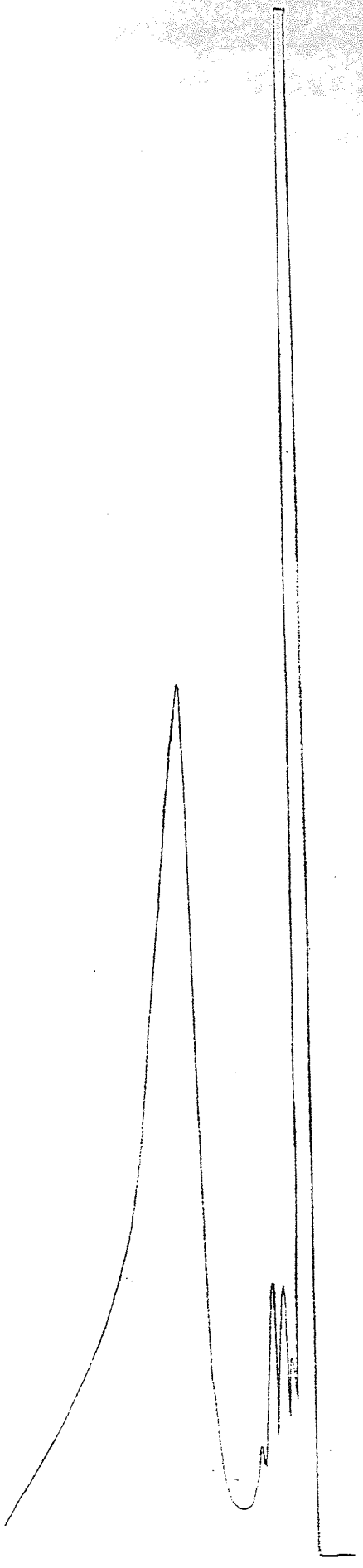


FIG. 28 CHROMATOGRAM OBTAINED BY USING SYSTEM 7 FROM GRAVEOBONE-A (CELERY).

Separation of the components of fraction C and E_2 by GLC.

The saponifiable fractions C and E_2 were analysed by GLC as described previously for the saponifiable fractions of P.coccineus, using different columns:

System 8 (Fraction C).

Column	: 3 pc SE-30 on chromosorb G (80-100 mesh), 1.5 m. in length.
Oven temp.	: 130°C.
Inj. temp.	: 260°C.
Carrier gas pressure	: Nitrogen at 20 p.s.i.
Oxygen and Hydrogen	: 20 p.s.i.
Chart speed	: 10 mm./min.
Volume sample	: 1 µl.
Amplitude	: 2×10^2.
Detector	: Flame ionisation.
Results	: Table 61 and Fig. 29.

Peak No.	Rt (min.)	Area ($cm.^2$)
1	1.0	3.30
2	1.2	4.96
3	1.6	4.10
4	2.3	2.58
5	2.7	3.60
6	3.8	3.26
7	4.0	13.92
8	4.6	13.80
9	6.0	9.60
10	9.9	6.82

Table 61 Fatty acid methyl esters of celery stems.

System 9 (Fraction C).

Column	: 5 pc diethylene glycol polysuccinate on chromosorb G (80-100 mesh), 1.5 m. in length.
Oven temp.	: 185°C.
Inj. temp.	: 280°C.
Carrier gas	: Nitrogen at 20 p.s.i.
Oxygen and Hydrogen	: 20 p.s.i.
Chart speed	: 5 mm. /min.
Volume	: 1 µl.
Amplitude	: 2×10^2.
Detector	: Flame ionisation.
Results	: Table 62 and Fig. 30.

Peak No.	Rt (min.)	Area ($cm.^2$)
1	2.4	0.26
2	3.0	0.36
3	4.2	3.68
4	4.6	0.62
5	5.4	0.26
6	7.4	0.66
7	8.9	0.32
8	10.4	9.80
9	11.4	1.08
10	13.2	1.58
11	18.2	0.90

Table 62 Fatty acid methyl esters of celery stems.

The above system gave better results and standard methyl esters were injected at the same conditions, from which these fatty acid methyl esters were identified.

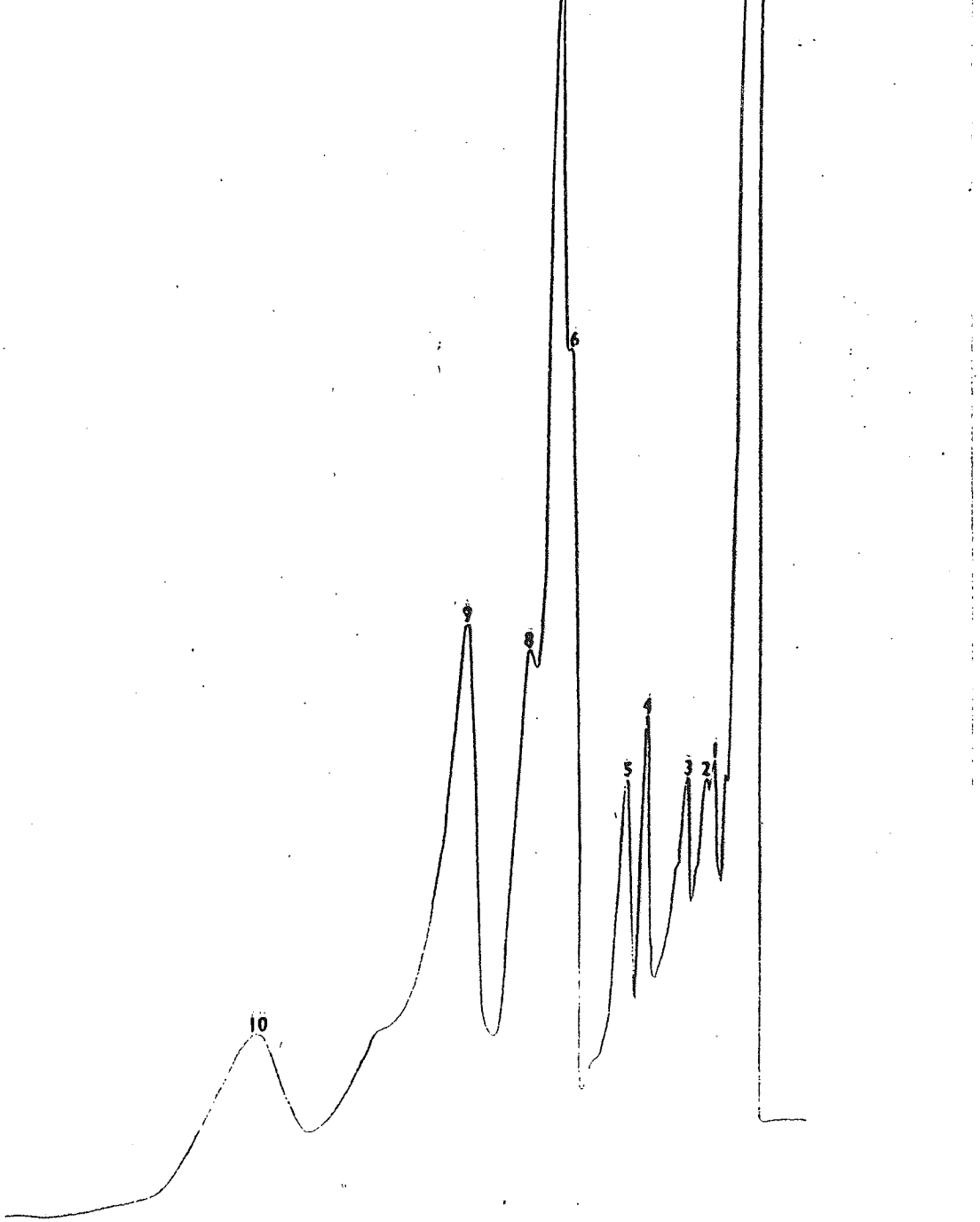


FIG. 29 CHROMATOGRAM OBTAINED FROM FRACTION C USING SYSTEM 8 (CELERY).

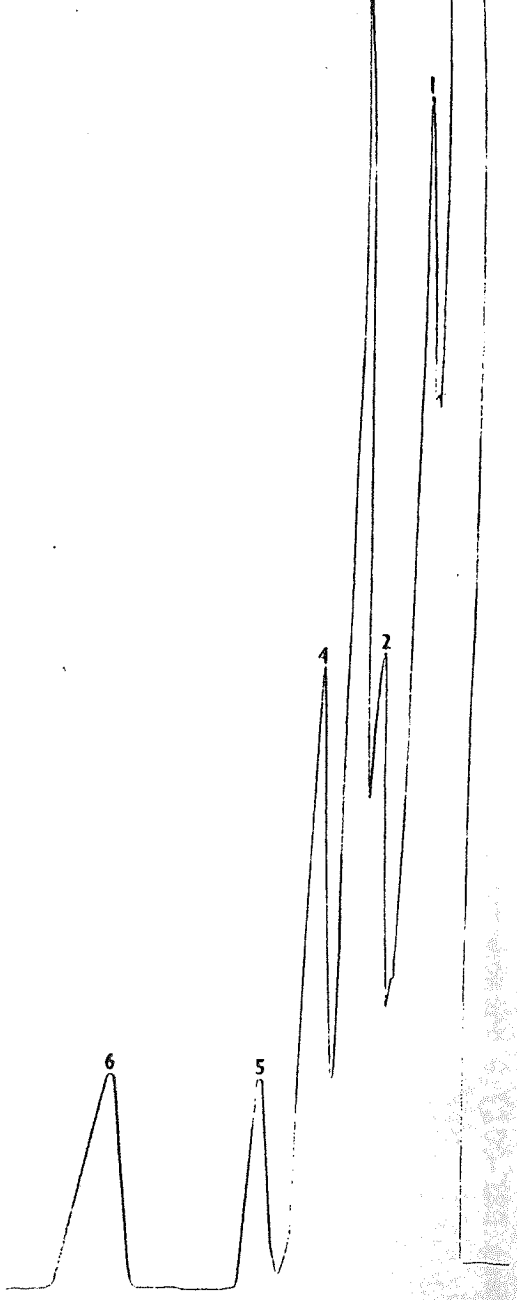


FIG. 31 CHROMATOGRAM OBTAINED FROM FRACTION C USING SYSTEM 10 (CELERY).

System 10 (Fraction C).

Column	: 4 pc Apiezon on chromosorb G (100-120 mesh), 1.5 m. in length.
Oven temp.	: 250°C.
Inj. temp.	: 350 C.
Carrier gas	: Nitrogen at 30 p.s.i.
Oxygen and Hydrogen	: 20 p.s.i.
Volume	: 1 µl.
Amplitude	: 2 x 10^2.
Chart speed	: 10 mm./min.
Results	: Table 63 and Fig. 31.

Peak No.	Rt (min.)	Area ($cm.^2$)
1	1.3	5.18
2	2.1	5.60
3	2.3	8.06
4	2.9	3.82
5	4.0	1.26
6	6.5	2.04

Table 63 Fatty acid methyl esters of celery stems.

System 9 (Fraction E_2).

Results : Table 64 and Fig. 32.

Peak No.	Rt (min.)	Area ($cm.^2$)
1	1.0	1.15
2	1.6	0.42
3	2.4	0.50
4	3.0	0.68
5	4.2	9.72
6	7.4	0.94
7	8.2	1.20
8	10.2	12.09
9	13.2	3.30

Table 64 Fatty acid methyl esters of celery stems (fraction E_2).

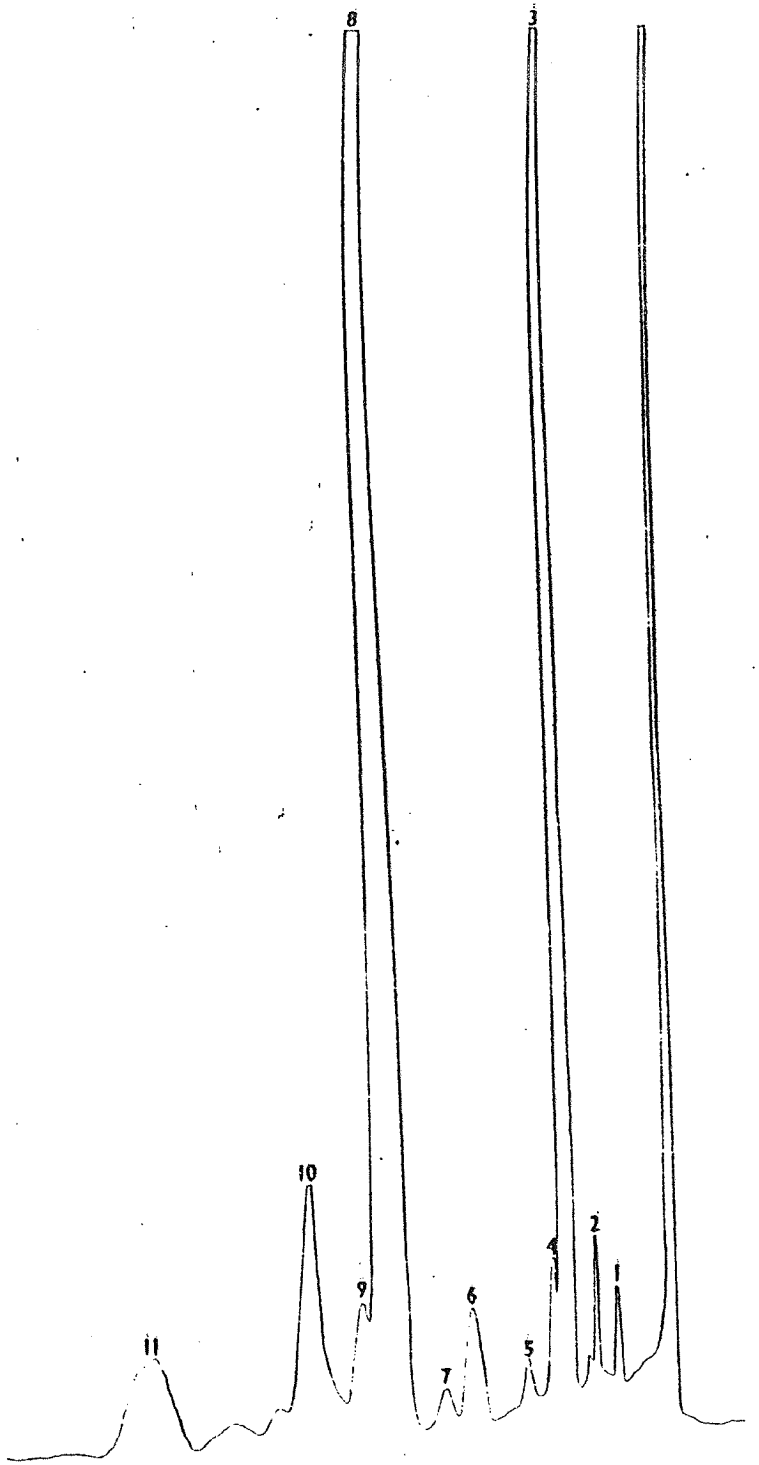


FIG. 30 CHROMATOGRAM OBTAINED FROM FRACTION C USING SYSTEM 9 (CELERY).

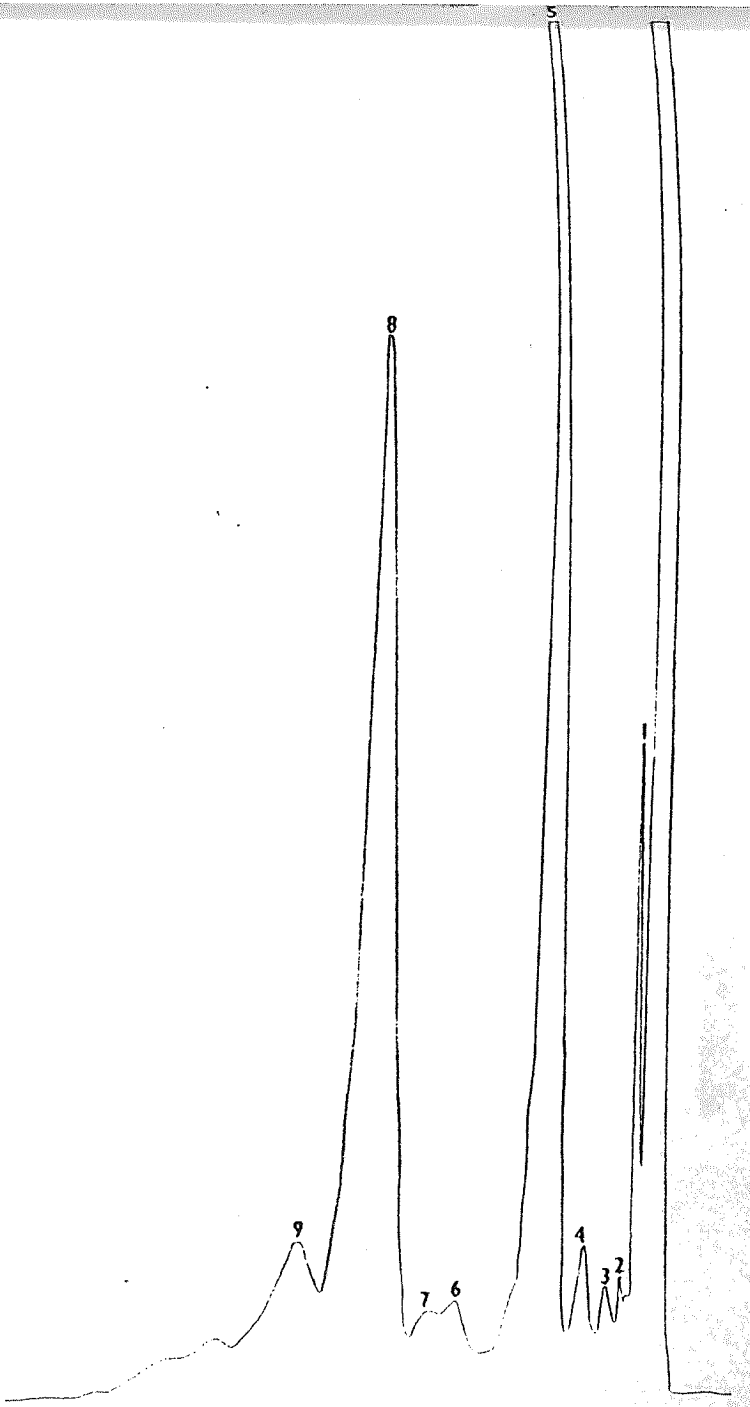


FIG. 32 CHROMATOGRAM OBTAINED FROM FRACTION E_2 USING SYSTEM 9 (CELERY).

Thin layer chromatography on fraction D.

System 31.

Stationary phase : As under system 29

Mobile phase : n-butanol-ethanol-water 5:4:1

Location : As under system 29

Results : Table 65

Spot No.	U.V 254nm.	Phosphomolybdic	Rf
1	-	blue	0.91
2	yellow	-	0.86
3	fluorescent blue	blue	0.78
4	brown	-	0.55
5	brown	-	0.39
6	-	blue	0.34
7	blue	blue	0.21
8	blue	-	0.08
9	blue	-	0.00

Table 65 Components of fraction D (celery) obtained by using system 31.

Thin layer chromatography on fraction E.

System 32.

Stationary phase : As under system 12

Mobile phase : benzene-methanol-ethylacetate 4:1:5

Location : As under system 29

Results : Table 66

Spot No.	U.V 254nm.	Phosphomolybdic	Rf
1	brown	blue	0.91
2	fluorescent blue	-	0.82
3	-	blue	0.73
4	brown	-	0.65
5	brown	faint blue	0.43
6	brown	faint blue	0.04
7	brown	faint blue	0.00
stigmasterol	brown	blue	0.91
β-sitosterol	brown	blue	0.91

Table 66 Components of fraction E (celery) obtained by using system 32.

Thin layer chromatography on fractions E_1 and E_2.

System 33.

Stationary phase : As under system 30

Mobile phase : benzene-methanol 9:1

Location : As under system 30

Results : Tables67 and 68 and Fig. 33.

Spot No.	U.V 254nm.	Phosphomolybdic	Rf
1	blue	blue	0.94
2	-	blue	0.62
3	-	blue	0.40
4	-	blue	0.18
5	-	blue	0.13
6	-	blue	0.11
7	-	blue	0.08
8	-	blue	0.06

Table 67 Components of fraction E_1 obtained by using system 33.

Spot No.	U.V 254nm.	Phosphomolybdic	Rf
1	-	blue	0.45
2	blue	blue	0.15
3	blue	blue	0.10
4	blue	faint blue	0.08
5	blue	faint blue	0.07
6	-	blue	0.06
7	blue	faint blue	0.04
8	blue	brown	0.00

Table 68 Components of fraction E_2 obtained by using system 33.

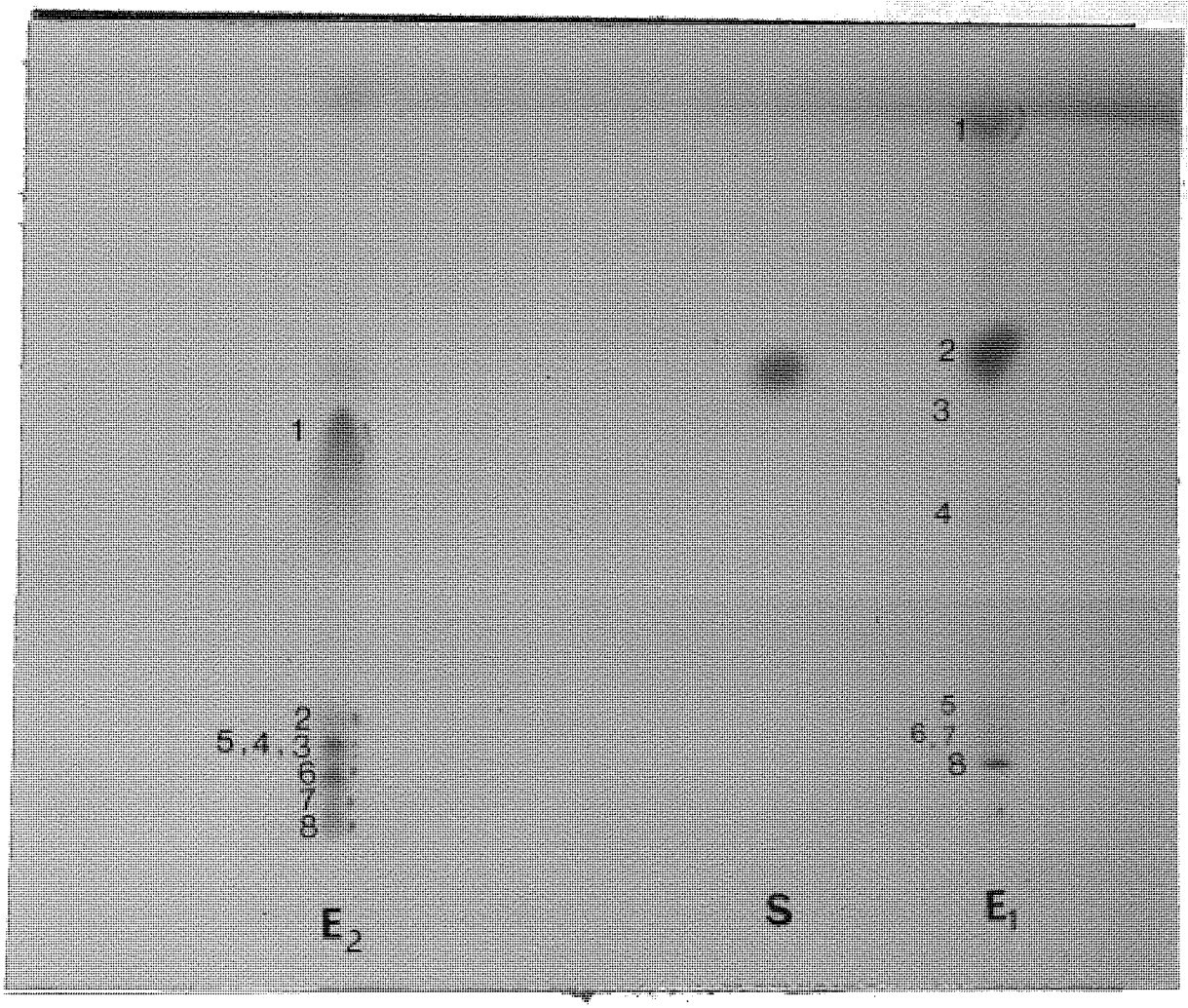


Fig. 33 Chromatogram obtained from system 33.

(The colour changed to brown after standing for a long time).

Fraction S was Stigmasterol.

Thin layer chromatography on fraction E_2.

System 8.

Results: Table 69 and Fig. 34.

Spot No.	U.V 254nm.	Phosphomolybdic	Iodine vapour	Rf
1	blue	blue	brown	0.45
2	-	-	brown	0.41
3	-	blue	brown	0.39
4	-	blue	brown	0.36
5	blue	blue	brown	0.34
6	blue	-	brown	0.30
7	blue	-	brown	0.17
8	-	-	brown	0.13
9	blue	faint blue	brown	0.07
10	blue	-	brown	0.03
11	-	blue	brown	0.02
12	brown	blue	brown	0.00
Palmitic acid	blue	faint blue	faint brown	0.49
Stearic acid	blue	faint blue	faint brown	0.49
Oleic acid	blue	blue	brown	0.45

Table 69 Components of fraction E_2 obtained by using system 8.

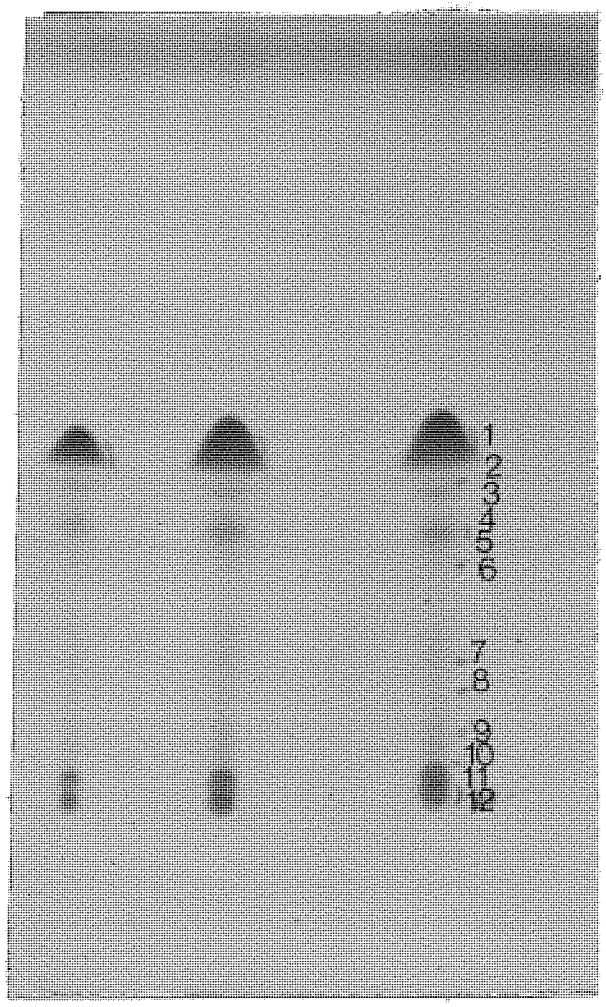


Fig. 34 Chromatogram obtained from system 8.

Isolation of fraction E_1 components by column chromatography.

The unsaponifiable fraction E_1 was dissolved in toluene (4 ml.) and loaded onto a silica gel column (40 cm. in length and 1.5 cm. in diameter). The slurry was made with toluene. Elution rate was 30 drops/min. 10 ml. fractions were collected sequentially and each fraction was tested by TLC using system 33.

Two yellow bands were eluted. The first 15 fractions contained a compound with an Rf value of 0.94 when eluted with toluene. The second band was eluted with 10% w/w methanol in toluene. This band was eluted in fractions 17-33 and had an Rf value of 0.62. The rest of the fractions (33-36), showed a mixture of the other minor components.

Fractions 1-15 were evaporated to dryness and were crystallised from methanol leaving colourless platelet crystals m.p. 59°C (0.015% w/w of the original weight). This compound was labelled graveobone-B.

Fractions 17-33 were combined and evaporated to dryness and crystallised from methanol leaving colourless needle crystals with m.p. 155°C (0.040% w/w of the original weight). This compound was labelled graveosterol-A_1.

Thin layer chromatography on fraction F_1.

1. System 34.

Stationary phase : As under system 30

Mobile phase : n-butanol-ethanol-water 5:4:1

Location : As under system 30

Results : Table 70

Spot No.	U.V 254nm.	Phosphomolybdic	Rf
1	-	blue	0.86
2	blue	-	0.76
3	brown	-	0.65
4	brown	-	0.52
5	yellow	blue	0.45
6	-	blue	0.38
7	blue	-	0.34

Table 70 Components of fraction F_1 obtained by using system 34.

2. System 35.

Stationary phase : As under system 30

Mobile phase : n-butanol-ethanol-A.A.-water 5:4:1:1

Location : As under system 30

Results : Table 71

Spot No.	U.V 254nm.	Phosphomolybdic	Rf
1	-	blue	0.85
2	blue	blue	0.75
3	blue	-	0.72
4	brown	-	0.66
5	-	blue	0.59
6	blue	-	0.40
7	blue	-	0.36

Table 71 Components of fraction F_1 obtained by using system 35.

Thin layer chromatography on fraction G.

As previously described, the ether of precipitation was evaporated completely under reduced pressure leaving a yellow precipitate which was crystallised from methanol giving yellow needle crystals labelled G_1. The mother liquor was labelled G_2. TLC was done on both G_1 and G_2.

System 36.

Ststionary phase : As under system 30

Mobile phase : ethanol-A.A.-water 5:4:1

Location : Day-light and ferric chloride spray reagent

Results : Table 72

Fraction	Spot No.	Day-light	$FeCl_3$ reagent	Rf
G_1	1	yellow	purple	0.36
G_2	1	yellow	purple	0.36
	2	yellow	purple	0.22
	3	yellow	purple	0.18
	4	yellow	purple	0.09
	5	yellow	purple	0.00

Table 72 Components of fraction G obtained by using system 36.

Thin layer chromatography on fraction I.

Precipitate I was hydrolysed with 5N HCl for 6 hr. at 100°C and the aglycones were extracted with chloroform (3 x 30 ml.) till the chloroform layers became almost colourless. The chloroform layers were combined, dried over anhydrous sodium sulphate, filtered and evaporated to dryness under reduced pressure. The yellowish white precipitate left was about 0.03% w/w of the original weight. It was examined by TLC using system 37.

System 37.

Stationary phase : As under system 12

Mobile phase : toluene-methanol-ethylacetate 7:2:1

Location : As under system 30

Results : Table 73

Spot No.	U.V 254nm.	Phosphomolybdic	Rf
1	-	blue	0.50
2	-	blue	0.37
3	-	blue	0.25
4	blue	-	0.18
5	-	blue	0.12
6	-	blue	0.03
7	-	blue	0.00
Stigmasterol	-	blue	0.45

Table 73 Components of fraction I obtained by using system 37.

Isolation of fraction I (aglycones) by column chromatography.

0.4 gm. of the above mixture was dissolved in toluene (4 ml.) and loaded onto the silica gel column (50 cm. in length and 2 cm. in diameter). The slurry was made with toluene and the elution rate was at 30 drops/min. 10 ml. fractions were collected sequentially and each was tested by TLC using system 37.

The results obtained were summarised in Table 74.

Fraction	Solvent used	Labelled	Remarks
1 - 5	toluene	I_1	colourless eluents
7 - 22	toluene	I_2	colourless eluents
23 - 48	toluene	-	no spot located on TLC
49 - 64	toluene-methanol(9:1)	I_3	yellow band
65 - 100	toluene-methanol(9:1)	I_4	yellow eluents

Table 74 Components of fraction I obtained by using column chromatography.

Fractions containing similar compounds were combined and crystallised using methanol. Fractions 1-5 left a colourless platelet product (graveobone-C); 7-22 left colourless needle crystals (graveosterol-A_2) while I_3 which was present in a very small amount left a yellow amorphous product (graveobioside-A).

Product I_4 was a mixture of six spots (system 18) with Rf values of 0.4, 0.32, 0.25, 0.20, 0.19 and 0.0. They were separated by GLC after TMS derivatives were made by dissolving the mixture in pyridine (1 ml.) and trimethylchlorosilane (0.5 ml.) and hexamethyldisilazane (0.5 ml.) were added. The mixture was heated for 5 min. evaporated to dryness under reduced pressure and the TMS derivatives were extracted with toluene (5 ml.) and introduced into the column (system 11).

System 11.

Column : 3 pc SE-30 on chromosorb G (80-100 mesh), 1.5 m. in length.
Oven temp. : 250°C.
Inj. temp. : 400°C.
Carrier gas pressure: Nitrogen at 30 p.s.i.
Hydrogen and Oxygen : 20 p.s.i.
Amplitude : 2×10^2.
Volume : 1 µl.
Chart speed : 20 mm./min.
Detector : Flame ionisation.
Results : Table 75 and Fig. 35.

Peak No.	Rt (min.)	Area (cm.)	Percentage present
1	0.75	4.25	15.71
2	0.90	2.58	9.54
3	1.20	4.04	14.94
4	1.50	3.90	14.42
5	1.85	3.40	12.57
6	2.10	2.66	9.83
7	3.05	2.64	9.76
8	3.90	2.68	9.91
9	5.05	0.90	3.33

Table 75 TMS components of graveobiosides mixture.

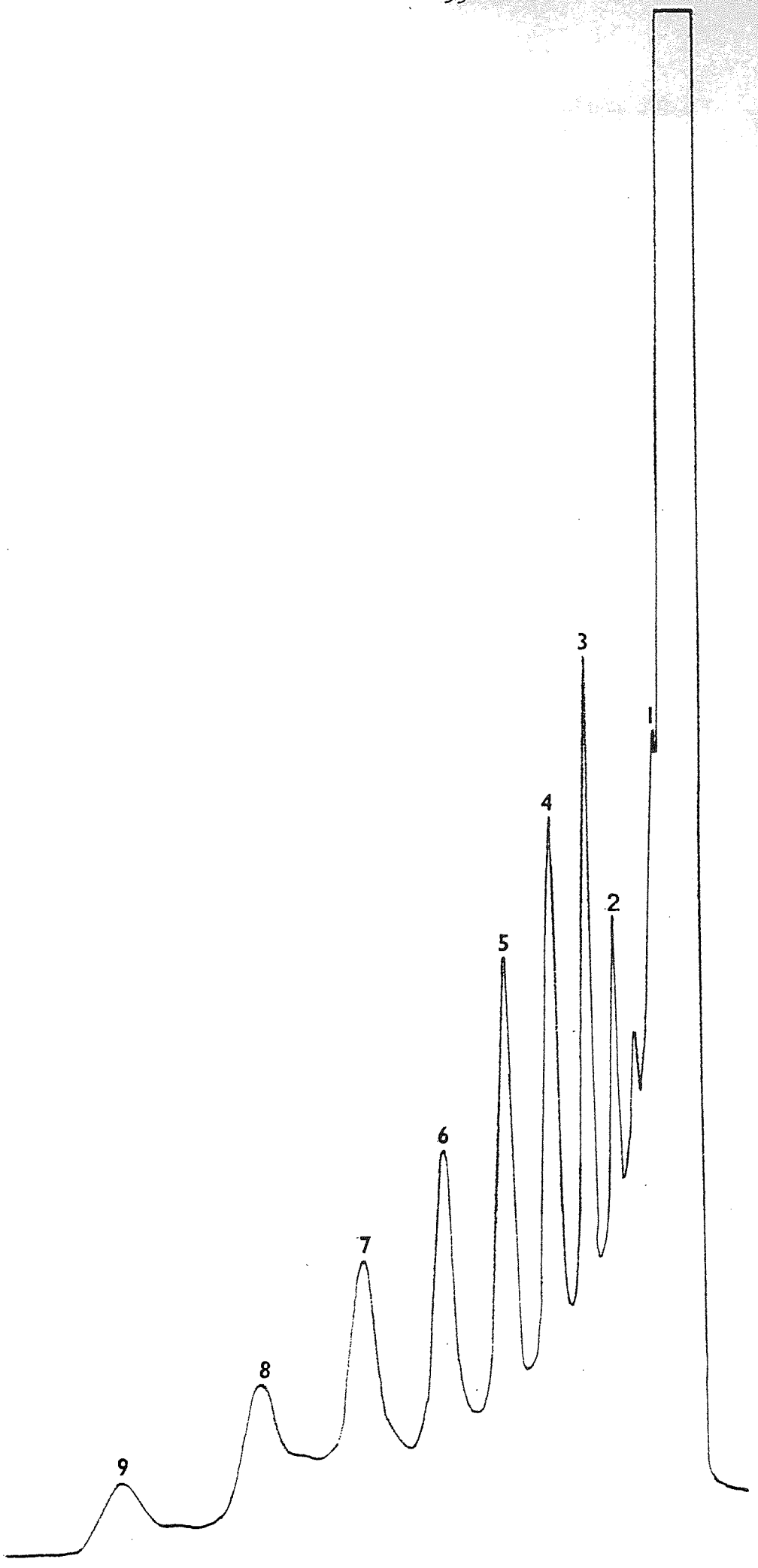


FIG. 35 TMS-DERIVATIVES OF THE FLAVONOID AGLYCONES (SYSTEM 11).

Thin layer chromatography on graveose-A.

a. System 35.

Using the above system, a single spot with an Rf value of 0.07 was located. This spot was faint yellow in colour with phosphomolybdic acid reagent.

b. System 38.

Stationary phase : Cellulose plate

Mobile phase : n-butanol-ethanol-water 4:1:4

Location : Ninhydrin spray

Results : A single spot with an Rf value of 0.08 was located as a pink spot.

c. System 39.

Stationary phase : As under system 38

Mobile phase : n-butanol-A.A.-water 5:4:1

Location : Phosphomolybdic acid and anisaldehyde reagents

Results : A single spot with an Rf value of 0.26 was located as a white spot with anisaldehyde reagent and of a yellow colour with phosphomolybdic acid reagent.

d. System 40.

Stationary phase : Whatman No.1 paper

Mobile phase : As under system 39

Location : Ninhydrin spray

Results : A pink spot with an Rf value of 0.18 was located.

e. System 41.

Stationary phase : As under system 40

Mobile phase : n-butanol-pyridine-water 5:4:5

Location : Ninhydrin spray

Results : A pink spot with an Rf value of 0.18 was located.

Aqueous extract of the celery stems.

The marc (40 gm.) left after petroleum and methanol extractions, was macerated in cold water (150 ml.), filtered and the brown filtrate was extracted with ether (3 x 100 ml.). The ether layers were combined, dried over anhydrous sodium sulphate and evaporated to dryness yielding a yellow residue (XIII). This residue was a mixture of six compounds (TLC system 30). Only one of them was in a relatively large quantity allowing separation in a pure and crystalline form.

System 30.

Results : Table 76.

Spot No.	U.V 254nm.	Phosphomolybdic	Rf
1	-	blue	0.87
2	blue	-	0.62
3	blue	-	0.43
4	-	faint blue	0.18
5	-	faint blue	0.12
6	blue	faint blue	0.00

Table 76 Components of fraction XIII obtained by using system 30.

Only spot No. 1 showed a dark blue colour and a large spot, while the others were present in small amounts.

Compound corresponding to spot No. 1 was isolated by column chromatography, after the mixture was dissolved in toluene (2 ml.) and eluted with toluene. The toluene eluent was evaporated to dryness and the white precipitate left was crystallised from methanol yielding a white platelet crystals labelled graveobone-D.

The aqueous layer was then extracted with butanol saturated with water (3 x 50 ml.). The butanol was evaporated to dryness leaving a yellowish white solid residue. This residue was crystallised from methanol leaving a white amcrphous product labelled graveose-B.

The aqueous layer was then examined by PC using system 42.

This fraction was labelled as XIV . Standard D-glucose and D-fructose were used as references. The spots were located by aniline hydrogen phthalate reagent.

System 42.

Stationary phase : Cellulose F_{254}

Mobile phase : As under system 38

Location : Aniline hydrogen phthalate

Results : Table 77.

Spot No.	Colour	Rf
1	brown	0.21
2	brown	0.16
D-glucose	brown	0.16
D-fructose	brown	0.21

Table 77 Sugar components of fraction XIII.

Thin layer chromatography on graveose-B.

Using the same systems as used for graveose-A, this compound showed the same Rf values and colours as graveose-A.

Acetylation of graveose-A and graveose-B.

About 0.5 gm. of the material was dissolved in dry pyridine (2 ml.) and to this solution glacial acetic acid (3 ml.) and acetic anhydride (3 ml.) were added. The mixture was refluxed for 15 min. and then poured on ice water, filtered, washed with water and crystallised from methanol leaving white platelet crystals in both cases.

III- Results, Identification and Conclusions.

III-1- Instrumental.

1. Melting points (m.p.).

Melting points of the compounds were determined by means of the Electrothermal melting point apparatus (uncorrected).

2. Ultra-Violet absorption spectra (U.V.).

Ultra-Violet spectra were obtained on a PYE UNICAM recording spectrophotometer.

3. Infra-Red absorption spectra (I.R.).

Infra-Red spectra were obtained on a Unicam-SP 200 spectrophotometer. Grubb-Parsons-102 spectrometer was also used for the expansion of some of the spectra. The compounds were examined using KBr discs. Thin-films were used in cases where the compounds were oily. Some unassigned peaks were also recorded.

4. Mass spectra (M.S.).

Mass spectra were determined on an Micromass-12 V.G. Micromass Ltd., at 70 eV at different inlet temperatures at 3 or 4 KV.

5. Nuclear magnetic resonance spectra (NMR).

Nuclear magnetic spectra were obtained on a 60 MHz Perkin-Elmer R 12-A instrument, using deuterochloroform ($CDCl_3$) or deuterium oxide (D_2O). Trimethylsilylether (TMS) was used as internal standard in the case of $CDCl_3$. Other spectra were obtained at 100 MHz and 220 MHz. All the peaks were assigned in δ (ppm) values.

6. Microanalysis.

Microanalysis were carried out by the Butterworth Laboratories Ltd., London.

7. Percentage weight per weight (% w/w).

% w/w infers to the percentage of the original dry weight of the material used unless otherwise specified.

III-2- Introduction.

III-2-1- Saponifiable lipids.

The saponifiable lipids are operationally defined as those materials which are insoluble in water but soluble in organic solvents, and which on heating with alkali form water-soluble soaps. The soaps are salts of long-chain fatty acids, so that these fatty acids are a necessary component of any saponifiable lipid.

Fatty acids occur mainly in plants in a bound form. Lipids occur in considerable amounts in the seeds or fruits of a number of plants, and provide such plants with a storage form of energy to use during germination. Plant fats, unlike animal fats, are rich in unsaturated fatty acids[87].

The saponifiable lipids are classified according to their structures into a few categories:

1. Fatty acids.
2. Simple lipids (fatty acid esters).
3. Phospholipids or phosphatides.
4. Glycolipids.

1. Fatty acids:

Fatty acids found in nature almost always have an even number of carbon atoms, but exceptions do occur. All of the straight chain, odd-carbon acids from C_7-C_{15} have been found free or as esters in higher plants. The vast majority of natural fatty acids have an unbranched carbon chain and differ from one another in chain length and degree of unsaturation. Plants can synthesise all their constituent fatty acids[88].

There are about 50 naturally occurring fatty acids found in the bound state, the most abundant being :

1. Palmitic acid $C_{15}H_{33}COOH$
2. Stearic acid $C_{17}H_{35}COOH$

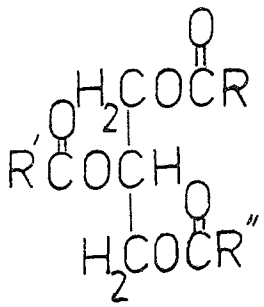
The most widely distributed unsaturated fatty acids are:

1.	Oleic acid	Δ^{9}	$C_{17}H_{33}COOH$
2.	Linoleic acid	$\Delta^{9:12}$	$C_{17}H_{31}COOH$
3.	Linolenic acid	$\Delta^{6:9:12}$	$C_{17}H_{29}COOH$

A number of rarer fatty acids are found as lipid components of plants of a particular taxonomic group rather than being widespread in the plant kingdom. They include acids with acetylenic unsaturation, hydroxyl groups, carbocyclic rings, and branched chains.

2. Triglycerides:

Triglycerides are esters of glycerol with three fatty acid molecules (19).



(19)

R, R′ and R″ represents fatty acid residues.

The normal situation is for the three fatty acids to be different and the molecule therefore is described as a "mixed triglyceride". Those which are solid at room temperature are called fats (saturated fatty acids), whereas liquid triglycerides are called oils (unsaturated fatty acids).

3. Other fatty acid esters:

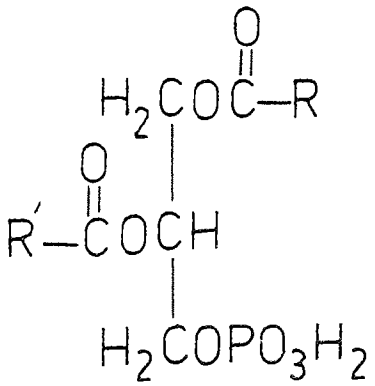
Besides the triglycerides, other simple esters of long-chain fatty acids are commonly found in plants. Whereas the triglycerides usually function as food storage components, the other fatty acid esters seem to be more concerned in protective coatings on leaves, fruits, stems, etc

They are chemical constituents of the substances known botanically as wax, cutin, cork, etc.

Other variants on the long chain ester structure are found in certain plant waxes which have hydroxy acids[89]. When these are present, esters may form between the carboxyl group and the hydroxyl group of the same acid or with the hydroxyl group of another acid.

4. Phospholipids or phosphatides.

Several different types of phosphorus-containing lipids are found in plants. All are esters of phosphoric acid and long chain fatty acids. The simplest phospholipids are phosphatidic acids which have fatty acid groups and phosphoric acid esterified with glycerol (20).



(20)

Phospholipids are polar compounds and are soluble in moderately polar solvents such as methanol. The phospholipids are probably most important in cells because of their involvement in membrane structures and as bridges or binding agents between polar and non-polar cell constit--uents[90]. The major phospholipid of immature soy beans is phosphatidic acid[91].

5. Glycolipids.

Glycolipids are lipids containing sugar units but not phosphorus. Only recently has it become evident that such compounds are widely

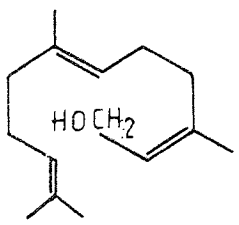
distributed in plants, especially in green leaves[92]. The fatty acids are usually highly unsaturated with linolenic acid predominating[93,94].

III-2-2- Terpenoids and steroids.

The mono- and sesquiterpenoids, substances which are biogenetically derived from two and three isoprene units, respectively, are distributed widely in a variety of plants.

Monoterpenoids are the major components of many essential oils and as such have great impotance as flavours, perfumes and solvents. They are characteristically colourless, water-insoluble, and are optically active. It is difficult to obtain pure compounds because they usually occur in a complex mixture which readily undergoes rearrangement. Typical monoterpene skeletons include acyclic, monocyclic, and bicyclic systems.

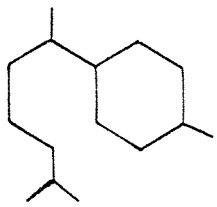
Sesquiterpenoids are C15 compounds, usually regarded as derived from three isoprene residue. Like monoterpenoids they are found as constituents of steam-distillable essential oils. They are found mainly in plants and fungi. Sesquiterpenoids occur as acyclic, monocyclic and bicyclic systems. Farnesol (21) is the most important of the acyclic sesquiterpenoids.



(21)

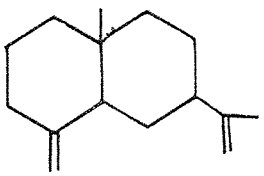
Farnesol

Most of the monocyclic sesquiterpenoids have the skeleton shown below (22) with variations in double bond location and fuctional groups.



(22)

Most of the bicyclic sesquiterpenoids can be divided into naphthalene types and azulene types according to which of these two aromatic structures they give on dehydrogenation. Further subdivision takes into account the locations of substituent groups on the rings. An example of a monocyclic sesquiterpenoid is (s)-abscisic acid (4), and of bicyclic sesquiterpene is β-selenine (23) from A.graveolens seeds[95,96].

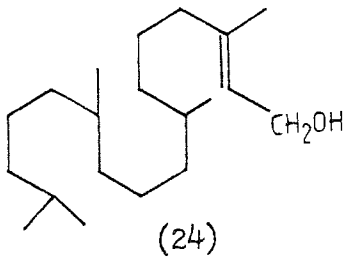


(23)

β-selenine

The diterpenes are C20 compounds which may be formally regarded (with some exceptions) as derived from four isoprenoid residues. They are mainly of plant and fungal origin and usually occur as a mixture of closely related compounds. They include the resin acids and gibberellins. Their great complexity and difficulty of separation has resulted in only a relatively few completely known structures in this group compared to

the vast number which probably occur in nature. As with the lower terpenes, hydrocarbons, alcohols, ethers and acids are all known in this group. The only important acyclic member is the alcohol phytol (24), which is present as the ester attachment in the molecule of chlorophyll. There are three types of diterpenes, resin diterpenes which include the compound abietic acid (25), found in both modern and fossil plant resins[97]. The second type is a group of toxic diterpenes called grayanotoxins, e.g. grayanotoxin-1 (26). They are responsible for the poisonous nature of the foliage of Rhododendron and Kalmia species[74]. The third group of diterpenoids mentioned above are the gibberellins.



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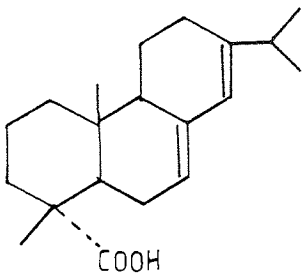

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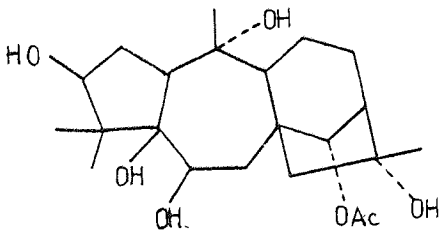
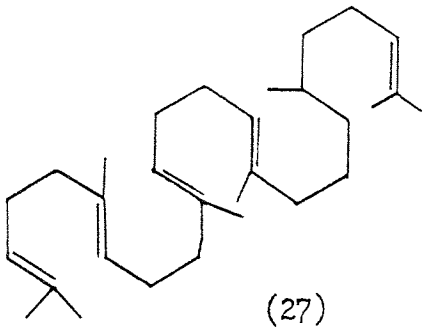


(26)

Triterpenoids are compounds with a carbon skeleton based on six-isoprene units and are derived biosynthetically from the acyclic C30 hydrocarbon, squalene (27). They have relatively complex cyclic structures, most being either alcohols, aldehydes or carboxylic acids. Triterpenoid alcohols occur both free and as glycosides. Many of the glycosides are classed as saponins. Triterpenoid hydrocarbons and

ketones are also known[98,99].

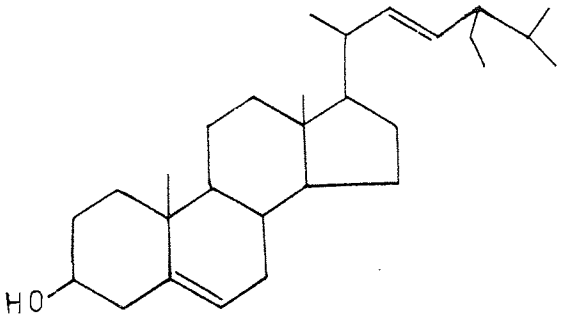


(27)

Squalene

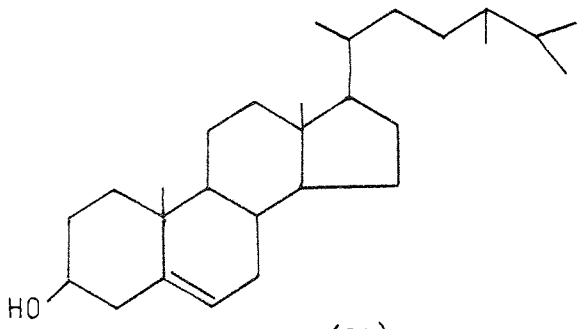
No triterpenoids have so far been found to have monocyclic or dicyclic structures and tricyclic types are rare[88], but several tetracyclic triterpenoids are known[100]. The most important and widely distributed triterpenoids are the pentacyclic compounds. They are most common among the seed plants, both free and as glycosides[101].

Sterols are triterpenes which are based on the cyclopentane perhydrophenanthrene ring system with two methyl groups attached to the ring system, at positions 10 and 13. The eight-carbon side chain found in lanosterol is also present in many steroids, especially from animal sources; but most plant steroids have one or two additional carbon atoms. Sterols are present in animals (e.g. sex hormones, bile acid, etc.) as well as in plants as the so-called 'phytosterols' stigmasterol (28) and campesterol (29). The name 'sterol' applies specifically to steroid alcohols; but since practically all plant steroids are alcohols with a hydroxy group at C3, they are frequently called sterols. The steroid numbering system is shown in formula (30).



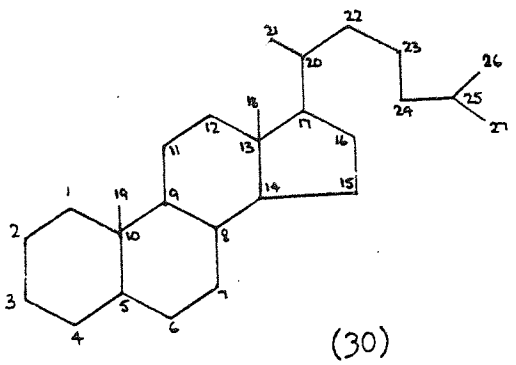
(28)

Stigmasterol



(29)

Campesterol



(30)

Steroids occur throughoutthe plant kingdom as free sterols and their esters in many lipids and as more complex derivatives. In plants they have no known function although they have profound importance in animal metabolism as hormones, coenzymes, bile acids and provitamin D. Certain animal steroids have been shown to influence plant growth strongly, but whether presently unknown plant steroids may act similarly is an open question[102-105]. Cholesterol, the most common animal steroid, has not been found in plants; but there is no sharp distinction between plant and animal steroids, since other members of the group are found in both kingdoms. Pregnane type steroids have recently been found in plants.

Sterols of the zymosterol type are known in the fungi but have not been established yet in any higher plants. The stigmasterol type is most characteristic of higher plants.

Sterolins and Saponins:

As has been mentioned previously, many terpenoid and steroid alcohols exist in nature not as free alcohols but as glycosides.

Sterolins or sterol glycosides are widespread in unrelated plant species. They are found along with free sterols in the unsaponifiable lipid fraction but may be distinguished from free sterols by their much higher melting points and low solubility in such fat solvents as ethyl ether. They are distinguished from saponins by their insolubility in water and lack of toxicity to animals. β-sitosterol glycoside is the most widely distributed plant sterol, so its glycosides are the commonest sterolins.

The saponins were originally named because of their soap-like characteristics. They are powerful surface active agents which cause foaming when shaken with water and in low concentration often produce haemolysis of red blood cells. Certain saponins have become important in recent years because they may be obtained in good yields from some

plants and are used as starting material for the synthesis of steroid hormones to be used in medicine. The saponins have no known function in plants but have been shown to stimulate the growth of pea embryos.

Two types of saponins are recognized: glycosides of triterpenoid alcohols, and glycosides of a particular steroid structure described as having a spiroketal side chain. Both types are soluble in water and ethanol but insoluble in ether. Their aglycones called sapogenins, are prepared by acid or enzymatic hydrolysis and without the sugar residues have the solubility characteristics of other sterols. A few of the steroidal sapogenins are distinguished by having a cis A/B ring structure.

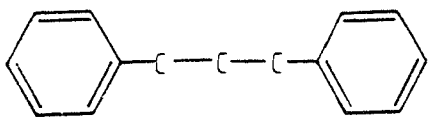
The triterpenoid saponins may have as their aglycones such compounds as oleanolic acid which also occur uncombined with sugars. In some cases though, the aglycones are known only as sapogenins. Oleanane-type sapogenins are much more common than either ursane or lupane types. Glycosylation is generally at C3. Several different monosaccharides are usually present as an oligosaccharide. Uronic acids may also be present as an oligosaccharide. Uronic acids may also be present [106-108].

Examination of legumes for most classes of secondary constituents such as the terpenoids, especially diterpenes (including the gibberellins), triterpenes (saponins and cardiac glycosides) and tetraterpenes (carotenoids), and on other coumarins, cyanogenetic glycosides, polyacetylenes, quinones, phenols and zanthones has been inspired mainly by pharmacological and chemical interests[7]. Much of the work on triterpenoids, particularly on the saponins of soya bean, lucerne and other forage crops, has similarly been initiated because of the potential toxicity of these substances to cattle and other farm animals[15]. Using the most widespread species P.vulgaris (kidney bean) Chirva et al.[14] isolated free β-sitosterol and five triterpene glycosides the 'phaseolosides' from the methanol extract.

III-2-3- The Flavonoids.

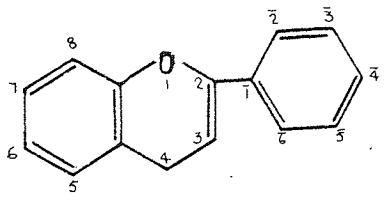
Flavonoids are one of the most numerous and widespread groups of natural constituents. They are important not only because they contribute to plant colour but also because many members (e.g. coumesterol, phloridzin, and rotenone) are physiologically active.

Flavonoids may be described as a series of C6 - C3 - C6 compounds, that is their carbon skeleton consists of two C6 groups (substituted benzene rings) connected by a three-carbon aliphatic chain (30).



(30)

The different classes within the group are distinguished by additional oxygen-hetrocyclic rings and by hydroxyl groups distributed in different patterns. Flavonoids frequently occur as glycosides[88]. The largest group of flavonoids is characterized by containing a pyran ring linking the three-carbon chain with one benzene ring. The numbering system for these flavonoid derivatives is given below (31).



(31)

The flavonoids include many of the most common pigments and occur throughout the entire plant kingdom from fungi to the angiosperms. In higher plants they are found both in vegetative parts and in flowers[71]. Flavonoids are not synthesised by animals but they do accumulate in a few insects, especially butterflies, as a result of the caterpillars feeding habits[61].

Spectrophotometric measurements are now commonly employed in the characterization of flavonoid compounds[109]. The value of spectral data in the identification and structural analysis of these plant pigments has increased considerably by the use of reagents such as aluminium chloride[110] and sodium acetate[111], which produce shifts in the maxima in accordance with the location of various functional groups in the flavonoid molecule. By the use of these spectral methods, it is now possible to determine the structure of some flavonoid compounds on the basis of their spectra alone[109].

The reason for the use of U.V spectroscopy as major technique for the structural analysis of flavonoids is because only small amounts of the pure material are required. Often a single spot on paper or thin layer plate will yield sufficient compound for several U.V. spectra[112], and the amount of structural information gained from the U.V. spectrum is considerably enhanced by the use of specific reagents which react with one or more fuctional groups on the flavonoid nucleus. Reagents which induce such shifts are sodium methoxide, sodium acetate and aluminium chloride[113].

III-2-4- Carbohydrates.

Carbohydrates or sugars occupy a central position in plant metabolism and as early products of photosynthesis, carbohydrates are key compounds in the biochemistry of green plants. Ultimately, all other constituents can be derived from them, so that methods for their detection and estimation are very important to the plant scientist. Not only are sugars the first complex organic compounds formed in the plant as a result of photosynthesis, but also they provide a major source of respiratory energy.

They are easily detected on chromatograms by using one of a range of phenolic or amine reagents (e.g. aniline hydrogen phthalate)[74].

The simple carbohydrates are aldehydes, polyhydroxy or ketones containing a number of hydroxyl groups. They are classified into two broad groups: sugars and polysaccharides. Sugars are either monosaccharides, disaccharides, trisaccharides, tetrasaccharides, and so forth.

Monosaccharides are classified on the basis of the number of carbon atoms in the molecule. Those having three carbon atoms are trioses etc.

The hexitols may be regarded as reduction products of the hexoses.

The story of the hexitols begins with the discovery of D-Mannitol in 1806[113]. Sorbitol, D-Mannitol, L-Iditol are found in plants. D-Mannitol is the most widely distributed of the naturally occurring hexitols, and because of its high tendency to crystallise, it is readily isolated from various mannans in which it occurs.

The hexacetates of the hexitols are admirably suited for identific-ation purposes, being easily crystallisable and possessing sharp melting points.[114]

III-3- Fatty acids.

III-3-1- Fatty acids of P.coccineus.

The percentage of the saponifiable part obtained from the petroleum ether and methanol extracts were as follows:

A. 0.58% w/w from the roots.

B. 0.50% w/w from the rhizomes.

C. 0.32% w/w from the stems.

Thin-layer chromatography of the petroleum ether extract and its saponifiable fraction showed that all the three organs contained the same number of components. The saponifiable fraction of the methanol extract also contained the same components. The free fatty acids (from the petroleum extract) were located on TLC using sytems 1 and 10 were not observed when the methanol extract residue was used. However, after saponification for 1 hr. with alcoholic KOH identical fatty acids were observed in both petroleum ether and methanol extracts. This could be due to the fact that the fatty acids extracted with methanol were actually present as triglycerides and released as free acids on mild hydrolysis with alcoholic KOH, since complete extraction of the free fatty acids took place with petroleum ether, or it could be that the high proportion of saponins and resinous material present in the methanol extract prevent the movement of the fatty acids on the chromatogram.

These fatty acids were separated and identified using the same methods as those applied to the petroleum ether extract. Results indicated that both the saponifiable fractions of petroleum ether (C) and methanol (E_2) extracts contained the same number and proportions of fatty acids.

The fatty acids were separated and identified by GLC using different stationary phases, after they had been converted to their methyl esters with boron trifluoride reagent. This has the advantage of not introducing unwanted products due to secondary reactions[81]. Best results

were obtained with the Apiezon column. The reason for this could possibly be that Apiezon is a non-polar phase, which allows the separation of unsaturated acids on the basis of their degree of unsaturation[77].

Standard methyl esters were injected under the same conditions and their Rt were compared with those obtained from the samples. A dilute solution of the standard methyl ester was also injected together with the sample and the heights of the peaks were compared. The height of the peak of the sample which corresponded to that of the standard increased correspondingly. Finally, the number of carbon atoms was determined by plotting the graph of logarithmic retention time against the number of carbon atoms, using the standards. Hence, the number of carbon atoms present in the unknown fatty acids were determined directly from the graph since the retention times of the unknown acids were measured under identical operating conditions as the standard acids.

Results.

The methyl esters of the standards were run using the same conditions as the samples and the following retention times were obtained as shown in Table 78.

Methylated fatty acid standard giving rise to peak.	Retention time (Rt) mm/min.	Log. Rel. retention time (relative to the Stearate).
C8 - Caprylate	0.60	-1.07
C10- Caprate	0.70	-0.95
C12- Laurate	1.20	-0.77
C14- Myristate	2.00	-0.55
C16- Palmitate	4.10	-0.29
C18- Oleate	6.60	-0.03
C18- Stearate	7.45	0.00
C18- Linoleate	9.30	0.12
C18- Linolenate	11.70	0.22

Table 78 The retention times of the standard methyl esters.

In order to find the number of carbon atoms present in the fatty acids from the oily extract samples the retention times obtained from these unknown acids were compared directly with those obtained form the standards used. A graph was also plotted of the logarithms of the relative retention times of the standards against their carbon atom numbers (Fig. 36). The retention times of the unknown acids were measured under identical operating conditions and hence the carbon numbers were read directly from the graph.

Standard curves were plotted for each sample but only one has been included for reference.

Results obtained from the stems.

Peak number	Rt.	Rel. Rt.	log. Rel. Rt.
1	0.70	0.10	-1.00
2	1.00	0.14	-0.85
3	1.20	0.17	-0.77
4	1.45	0.20	-0.69
5	1.65	0.23	-0.63
6	1.95	0.27	-0.56
7	2.25	0.32	-0.50
8	2.75	0.39	-0.41
9	2.90	0.41	-0.39
10	4.10	0.58	-0.24
11	6.60	0.93	-0.03
12	7.45	1.05	0.02

Table 79 Fatty acid methyl esters obtained from the stems.

The methyl esters of the fatty acids identified by direct comparison were the following:

Peak number	Probable methyl ester	Carbon number of the acid
1	methyl Caprate	10
3	methyl Laurate	12
6	methyl Myristate	14
10	methyl Palmitate	16
11	methyl Oleate	18
12	methyl Linoleate	18

Table 80 Fatty acid methyl esters obtained from the stems.

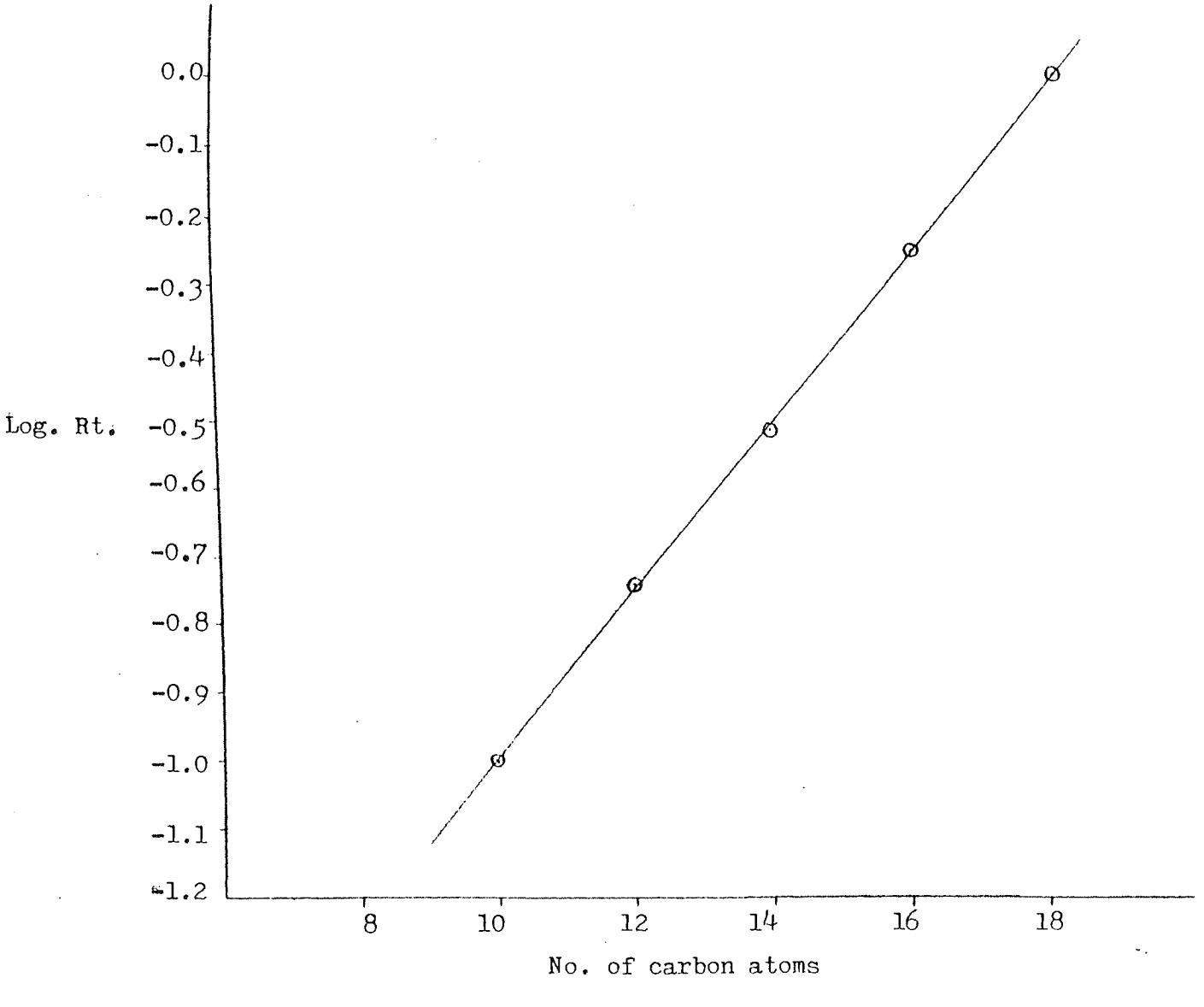


Fig. 36 Calibration graph of the standard methyl esters against the logarithmic time.

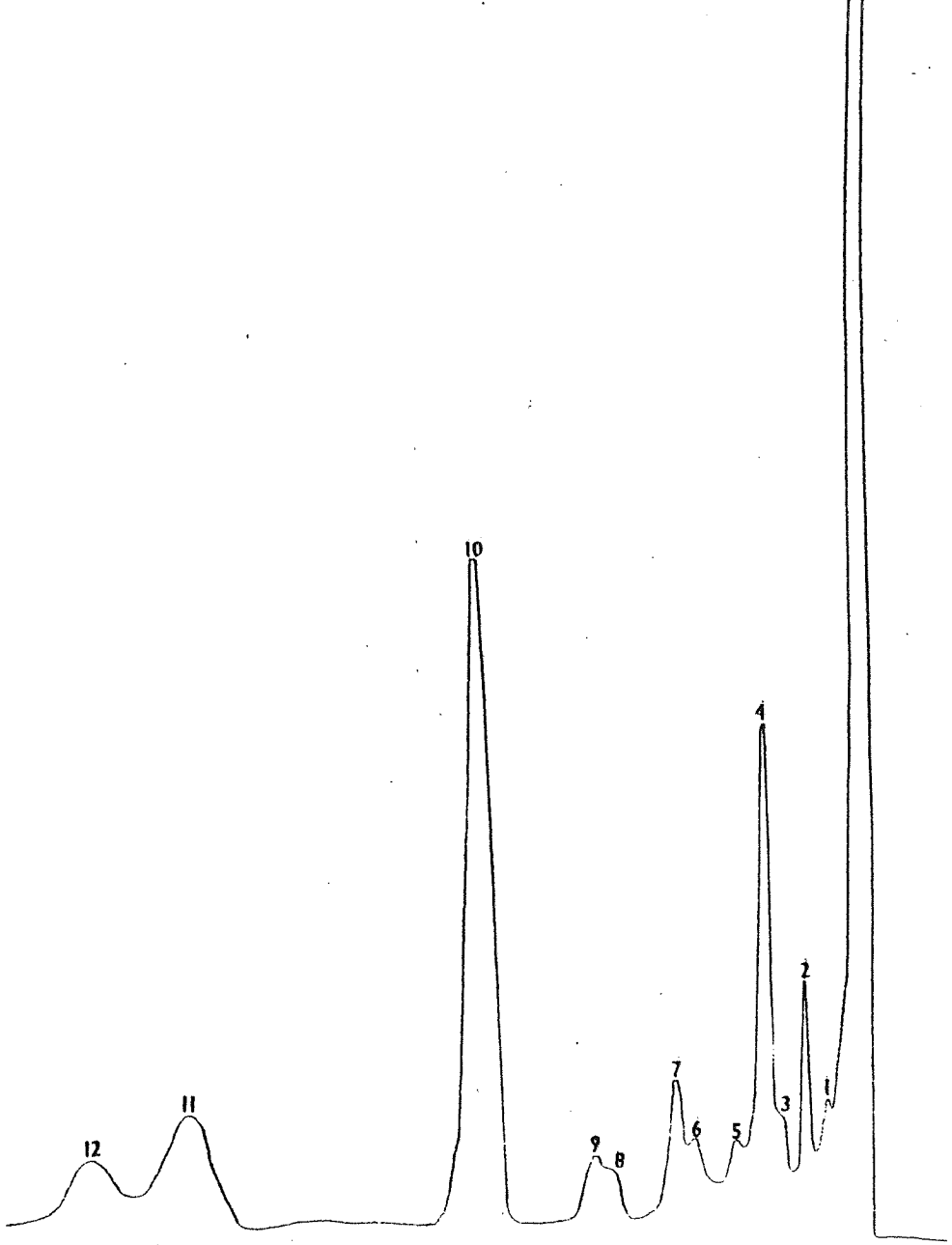


FIG. 37 GLC CHROMATOGRAM OF THE METHYL ESTERS OF THE FATTY ACIDS PRESENT IN THE STEMS(RUNNER BEAN).

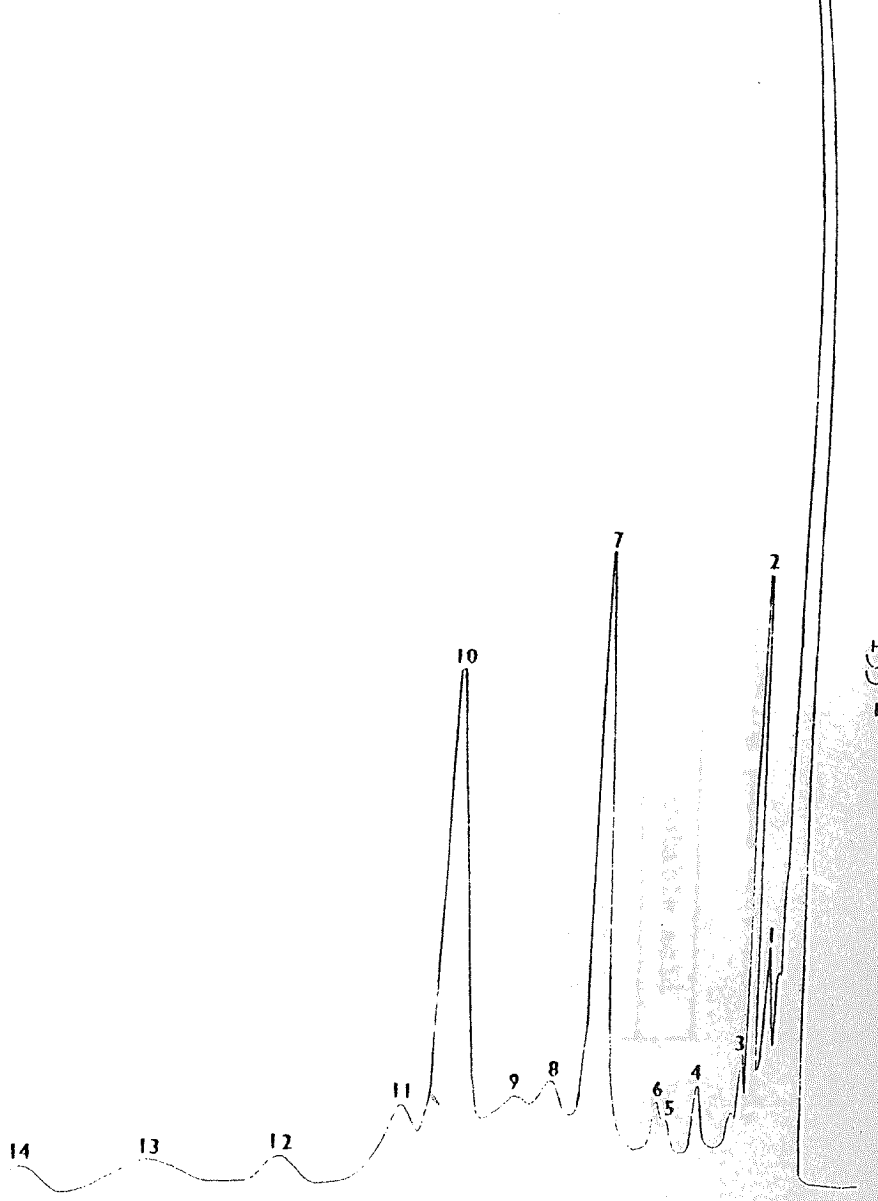


FIG. 39 GLC CHROMATOGRAM OF THE METHYL ESTERS OF THE FATTY ACIDS PRESENT IN THE RHIZOMES(RUNNER BEAN).

From the standard graph the following peaks were identified:

Peak number	Carbon number	Probable acid
1	10	Capric
2	11	C11-alkenoic
3	12	Lauric
4	12	C12-alkenoic
5	13	C13-alkenoic
6	14	Myristic
7	14	C14-alkenoic
8	15	C15-alkenoic
9	15	C15-alkenoic
10	16	Palmitic
11	18	Oleic
12	18	Linoleic

Table 81 Fatty acid methyl esters of the stems.

Results obtained from the roots.

Peak number	Rt.	Rel. Rt.	log. Rel. Rt.
1	0.45	0.06	-1.20
2	0.55	0.07	-1.11
3	0.60	0.08	-1.10
4	0.65	0.09	-1.04
5	0.70	0.10	-1.01
6	0.75	0.11	-0.98
7	0.87	0.12	-0.91
8	1.00	0.14	-0.85
9	1.15	0.16	-0.79
10	1.20	0.17	-0.77
11	1.35	0.19	-0.72
12	1.50	0.21	-0.68
13	1.70	0.24	-0.62
14	1.90	0.27	-0.57
15	2.10	0.30	-0.53
16	2.35	0.33	-0.48
17	2.55	0.34	-0.44
18	3.05	0.43	-0.37
19	3.50	0.49	-0.31
20	4.05	0.57	-0.24
21	4.65	0.65	-0.18
22	5.80	0.82	-0.08
23	6.50	0.92	-0.04
24	6.85	0.96	-0.02

Table 82 The Rt obtained of the methyl esters of fatty acids from the stems.

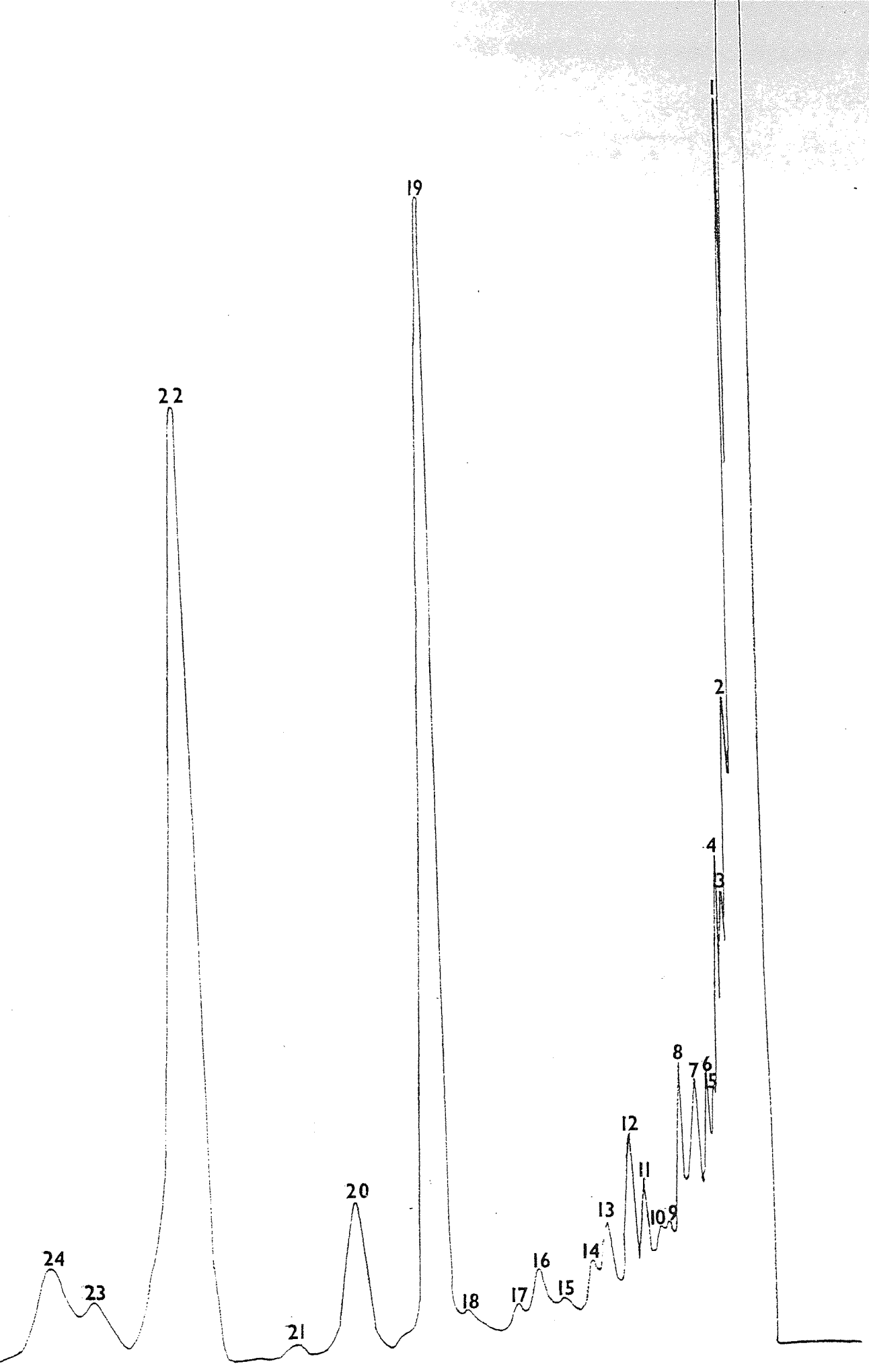


FIG. 38 GLC CHROMATOGRAM OF THE METHYL ESTERS OF THE FATTY ACIDS PRESENT IN THE ROOTS(RUNNER BEAN).

The methyl esters of fatty acids identified by direct comparison were the following:

Peak number	Carbon number	Probable
3	8	Caprylic
6	10	Capric
10	12	Lauric
15	14	Myristic
19	16	Palmitic
22	18	Oleic
23	18	Linoleic
24	18	Linolenic

Table 83 Fatty acid methyl esters obtained from the roots.

From the standard graph the following peaks were identified:

Peak number	Carbon number	Probable acid
1	5	C5 - alkenoic
2	6	C6 - alkenoic
3	8	Caprylic
4	9	C9 - alkenoic
5	10	Capric
6	10	C10 - alkenoic
7	10	C10 - alkenoic
8	11	C11 - alkenoic
9	11	C11 - alkenoic
10	12	Lauric
11	12	C12 - alkenoic
12	12	C12 - alkenoic
13	13	C13 - alkenoic
14	13	C13 - alkenoic
15	14	Myristic
16	14	C14 - alkenoic
17	14	C14 - alkenoic
18	15	C15 - alkenoic
19	16	Palmitic
20	16	C16 - alkenoic
21	17	C17 - alkenoic
22	18	Oleic
23	18	Linoleic
24	18	Linolenic

Table 84 Fatty acid methyl esters obtained from the roots.

Results obtained from the rhizomes:

Peak number	Rt.	Rel. Rt.	log. Rel. Rt.
1	0.7	0.10	-1.01
2	1.0	0.14	-0.85
3	1.2	0.17	-0.77
4	2.0	0.28	-0.55
5	2.5	0.35	-0.45
6	2.6	0.37	-0.44
7	3.6	0.51	-0.29
8	4.5	0.63	-0.20
9	5.2	0.73	-0.14
10	6.3	0.89	-0.05
11	7.1	1.00	-0.00
12	9.3	1.31	-0.12
13	11.4	1.61	-0.21
14	13.2	1.86	-0.27

Table 85 Fatty acid methyl esters obtained from the rhizomes.

The methyl esters of the fatty acids identified by direct comparison were the following:

Peak number	Probable methyl ester	Carbon number of the acid
1	Methyl Caprate	10
3	Methyl Laurate	12
4	Methyl Myristate	14
7	Methyl Palmitate	16
10	Methyl Oleate	18
11	Methyl Stearate	18
12	Methyl Linoleate	18
13	Methyl Linolenate	18

Table 86 Fatty acid methyl esters obtained from the rhizomes.

From the standard graph the following peaks were identified:

Peak number	Carbon number	Probable acid
1	10	Capric
2	11	C11 - alkenoic
3	12	Lauric
4	14	Myristic
5	14	C14 - alkenoic
6	14	C14 - alkenoic
7	16	Palmitic
8	17	C17 - alkenoic
9	17	C17 - alkenoic
10	18	Oleic
11	18	Stearic
12	18	Linoleic
13	18	Linolenic
14	18	C18 - alkenoic

Table 87 Fatty acid methyl esters obtained from the rhizomes.

Number of carbon atoms	Probable acid
C5 alkanoic	Isovaleric
C6 alkanoic	Capric
C9 alkanoic	Pelargonic
C10 alkenoic	Δ^9-Decylenic
C10 dialkenoic	Stillingic
C11 alkanoic	Undecylic
C11 alkenoic	Hendocenoic
C12 alkenoic	Δ^9-Dodecylenic
C13 alkanoic	Tridecyclic
C13 alkenoic	Tridecenoic
C14 alkenoic	Myristoleic
C15 alkenoic	Pentadecylic
C16 alkenoic	Palmitoleic
C17 alkenoic	Margeric acid
C18 trialkenoic	Eleostearic

Table 88 The fatty acids identified from the standard graph.

N.B.

The other alkenoic acids could be mono-, di- or tri-alkenoic acids.

In order to quantify the results obtained by the GLC method, areas under the peaks were calculated by drawing tangents to the sides of the peaks to the base line and then:

$$\text{Area} = \frac{1}{2} \text{ base x height}$$

The areas of each was then expressed as a percentage of the total so that the proportion of each component was obtained in the mixture.

Results:

Stems.

Peak number	Area under the peak (sq.cm.)	Percentage of the fatty acid methyl ester
1	1.55	9.06
2	0.78	3.31
3	0.81	3.45
4	2.94	12.51
5	1.15	4.89
6	1.38	5.87
7	1.36	5.79
8	0.81	4.26
9	0.55	2.34
10	7.04	29.96
11	3.00	12.77
12	2.13	9.06

Table 89 The percentage of the fatty acid methyl esters present in stems.

Rhizomes.

Peak number	Area under the peak (sq.cm.)	Percentage of the fatty acid methyl ester
1	1.30	4.44
2	2.19	7.48
3	0.56	1.91
4	0.55	1.88
5	0.36	1.23
6	0.56	1.91
7	6.44	21.99
8	0.55	1.88
9	0.88	3.00
10	10.08	34.40
11	3.38	11.54
12	0.81	2.77
13	1.00	3.41
14	0.63	2.51

Table 90 The percentage of the fatty acid methyl esters present in the rhizomes.

Roots.

Peak number	Area under the peak (sq.cm.)	Percentage of the fatty acid methyl ester
1	4.62	11.77
2	3.00	7.65
3	0.83	2.12
4	0.91	2.32
5	0.69	1.76
6	1.25	3.19
7	1.47	3.75
8	1.04	2.65
9	1.10	2.80
10	0.95	2.42
11	0.75	1.91
12	0.78	1.99
13	0.77	1.96
14	0.45	1.15
15	0.45	1.15
16	0.49	1.25
17	0.25	0.64
18	0.23	0.49
19	7.46	19.01
20	1.16	2.96
21	0.05	0.13
22	8.75	22.30
23	0.77	1.96
24	1.02	2.60

Table 91 The percentage of the fatty acid methyl esters present in roots.

Results from fraction E_2 and C of the stems (Tables 80 and 81) indicated the presence of twelve fatty acids mainly palmitic acid (29.96%). Other fatty acids present in small amounts were identified either by direct comparison of their Rt with those of the standards or by measuring their logarithmic Rt and the number of carbon atoms obtained from the standard graph are shown in Table 81. Fractions E_2 and C of the roots showed twenty-four fatty acids mainly oleic and palmitic, 22.3% and 19.01% respectively. The others, present in small amount, were identified as previously described under the stems (Table 84). Finally fractions E_2 and C of the rhizomes showed fourteen fatty acids mainly palmitic, stearic and oleic (21.99%, 11.54% and 34.40% respectively), the others were present in small amount (Table 87).

Table 88 shows the probable fatty acids identified from the standard graphs.

Since oleic and palmitic acids were shown to be the major components by GLC techniques, oleic acid was isolated from roots and rhizomes, and palmitic acid from the stems using column chromatography techniques. These two acids were isolated in pure state and identified as follows:

1. Oleic acid.

The pure oleic acid (yellowish brown in colour) showed a single spot on TLC with Rf 0.04 using benzene-methanol 9:1 (system 33) solvent on silica gel plate and Rf 0.66 (system 8). The same Rf value was obtained when the authentic sample was used.

Its iodine value was determined using iodine monochloride method[88] (85.7 Literature value 89.87)[115].

The IR spectrum (thin film) was identical to that of an authentic sample.

A Rt of 6.3 min. was obtained for both the methyl ester of the isolated oleic acid and the standard commercial sample.

2. Palmitic acid.

The m.p. of this compound was found to be 63°C and Rt of its methyl ester using system 1 was found to be 4 min., identical to that of the standard palmitic acid methyl ester.

IR spectrum (KBr disc):

The most remarkable feature noted in the spectrum was the progression of bands between 1320 cm^{-1} (7.4μ) and 1100 cm^{-1} (8.5μ) spaced at approximately equal intervals. Sinclair et al[116] emphasized the fact that these bands are only observed in the spectra of crystalline films, Nujol mulls and potassium bromide discs but not in the spectra of solutions. These bands are associated with methylene wagging and/or twisting vibrations. Johns et al (1952)[117] studied these band progressions and found that the number of bands in the progression is proportional to the chain length of the fatty acid or ester. The chain length of these compounds can be calculated from the formula:

No. of carbon atoms = 2 x No. observed absorption bands + 2

The spectrum of palmitic acid showed 7 of these bands and hence using the above formula, the number of carbon atoms were found to be 16 which confirms the C16 chain length in palmitic acid (Fig.40).

The other important absorption bands noted were 1690 cm^{-1} (s) corresponding to C=O stretching vibration, 1410 cm^{-1}(s), 1430 cm^{-1}(s) and 1460 cm^{-1} (s) corresponding to O-H out-of-plane deformation. Finally the splitting of the two bands at 720 cm^{-1} and 722 cm^{-1} is characteristic of long $-(CH_2)n-$ chain compounds where n is greater than 4.

Mass spectrum of palmitic acid (spectrum 1) (4KeV, 70 eV):

The most characteristic re-arrangement ion occurred at m/e 60 (M.Wt of acetic acid 42%). The parent peak in the mass spectrum was 256. The loss of OH ion from the parent at m/e 238 was not observed in the spectrum indicating that it is an acid and not an ester. Also the peak

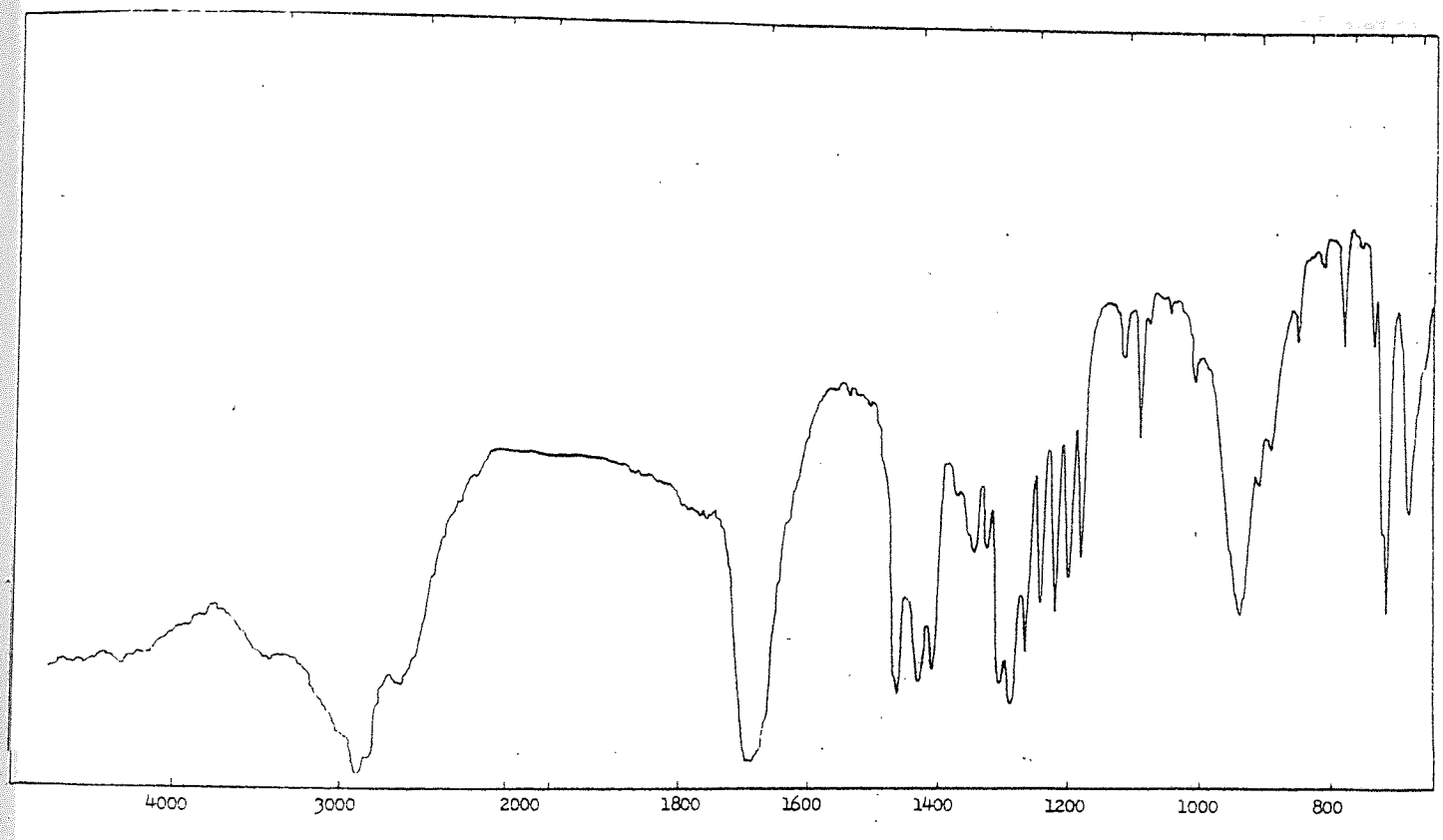


Fig. 40 Infra-red spectrum (KBr disc) of palmitic acid .

corresponding to the loss of COOH was very small (0.4%) relative to the parent peak. The large parent peak indicated the presence of oxygen in the compound showing that the compound being studied was not a hydrocarbon.

The other fractions collected from the column used for isolation of palmitic acid, showed a mixture of fatty acids i.e. 1-12 (GLC of methyl esters, system 1) which were present in a small amount. Fractions 1-12 showed capric, C11 alkenoic, lauric, C12 alkenoic, C13 alkenoic, myristic and C14 alkenoic acids in a mixed form while fractions 20-30 showed oleic and linoleic acids.

Analysis of mass spectra of fatty acid methyl esters.

The fatty acid methyl esters obtained from fraction C of the stems revealed seven peaks under the conditions of GC-MS (system 2), and their mass spectra were calculated. Their Rt are shown in Table 92.

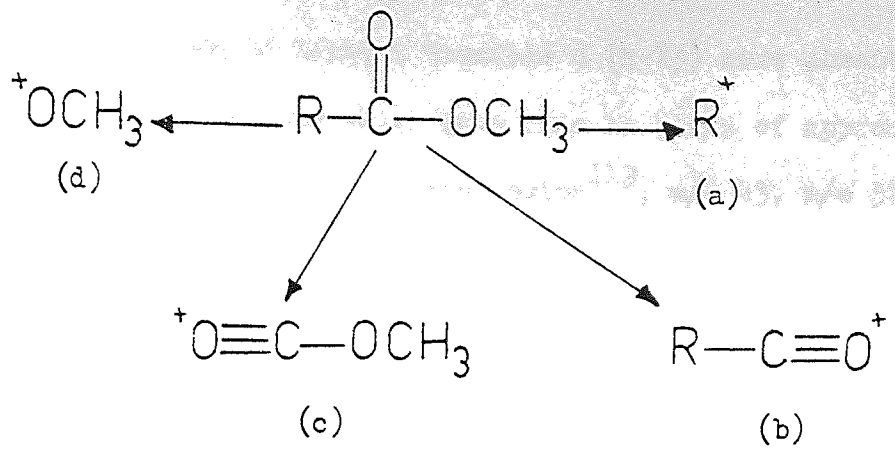
Peak number	Rt min.	Probable acid
1	0.6	Capric
2	1.4	Lauric
3	2.4	C12 dienoic
4	3.2	C13 acid
5	4.2	Palmitic
6	6.0	Oleic
7	8.4	Linoleic

Table 92 Fatty acid methyl esters obtained from the P.coccineus stems.

The mass spectra fragmentation of these methyl ester fatty acids is shown in spectra numbers 2-8 respectively.

Previous detailed studies of the general fragmentation modes of methyl esters[119] have revealed that M^+ is always present in the spectra of unbranched methyl esters, although its intensity relative to the base peak at m/e 74 varies considerably with the chain length.

The cleavage α to the ester carbonyl group can occur in four ways (32).



(32)

Fragment of type (a) and (c) are usually of low abundance or absent. Here the peak at m/e 127 (5%) (M-59) was present in capric acid (peak No. 1) with base peak at m/e 74.

Ion (a) was not observed in the other spectra, while ion (b) M-31 and ion (d) m/e 31, possess a diagnostic value since they are characteristic of the methoxy group in the methyl ester, are as follows:

Peak 1 m/e 31 (20%), M-31, m/e 155 (5%).

Peak 2 m/e 31 (20%), M-31, m/e 183 (33%).

Peak 3 m/e 31 (13%), M-31, m/e 179 (2%).

Peak 4 m/e 31 (11%), M-31, m/e 195 (2%).

Peak 5 m/e 31 (6%), M-31, m/e 239 (7%).

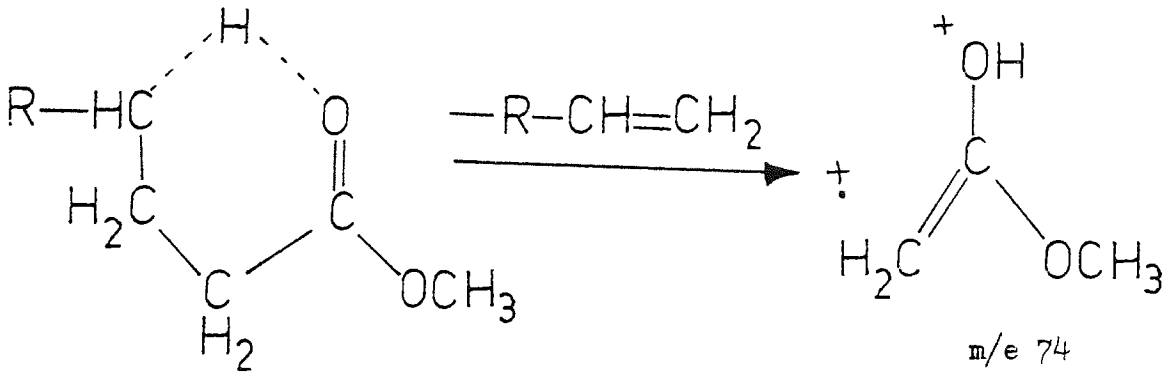
Peak 6 m/e 31 (13%), M-31, m/e 265 (7%).

Peak 7 m/e 31 (27%), M-31, m/e 263 (18%).

The base peak was found at m/e 74 in all the fatty acid methyl esters mentioned above. This mode of formation of the rearranged radical ion has been studied in detail and is known as the McLafferty rearrangement[120] (33).

All the spectra showed fragments corresponding to$((CH_2)_nCOOCH_3)^+$ at m/e 87 (n=2), m/e 101 (n=3), m/e 115 (n=4) etc. These fragments were separated by 14 mass units corresponding to one methylene units. The

peaks representing the ions of highest possible n (M-15) were absent. Finally the hydrocarbon-type ions which gave rise to peaks of appreciable heights were also observed in the low-mass region[119], m/e 43, m/e 57, m/e 71, m/e 85 and m/e 99.



(33)

III-3-2- Fatty acids of Gleditsia triacanthos.

The saponifiable fraction obtained from pods (C) was 2.153% w/w of the original weight, while that of the seeds was 2.030% w/w of the original weight.

Fatty acids of the pods as well as of the seeds were analysed and identified using the methods described previously under P.coccineus and they were as follows:

1. Fatty acids of the pods.

Probable acid	Percentage in (C)
Caprylic	8.9
Capric	8.1
Lauric	11.5
Myristic	7.3
Palmitic	30.3
Palmitoleic	5.6
Oleic	13.0
Stearic	10.7
Linoleic	0.9
Linolenic	9.1

Table 93 Fatty acids obtained from the pods.

Palmitic and oleic acids were the major constituents of the saponifiable lipids (30.3%, 13.0% respectively) which contained eleven fatty acids. Myristoleic acid identified by system 5 was found to be 7.4% of the total precentage.

2. Fatty acids of the seeds.

Probable acid	Percentage in (C)
Caprylic	19.9
Capric	11.4
Lauric	13.4
Myristic	9.6
Myristoleic	5.5
Palmitic	12.6
Oleic	19.9
Stearic	4.8
Linoleic	0.9
Linolenic	2.0

Table 94 Fatty acids obtained from the seeds.

Palmitic and oleic acids were the major constituents in the saponifiable fraction of the seeds, as well as in the pods. Palmitoleic acid which was present in the pods (5.6%) was absent in the seeds and the other fatty acids were present in both at different concentrations as shown in Tables 93 and 94.

Since oleic and palmitic acids were present in high proportions, they were isolated by column chromatography and identified using the method mentioned under palmitic and oleic acids of P.coccineus.

III-3-3- Fatty acids of Apium graveolens.

0.055% w/w of the saponifiable lipids were isolated from the celery stems using petroleum ether (60-80°C) and 0.50% w/w using methanol. The fatty acids fraction (C) and (E_2) were analysed and identified by GLC using their methyl esters (systems 8-10) and by TLC (systems 8 and 33).

TLC showed that oleic/linoleic/linolenic (same Rf values) were present as major constituents (the largest spot), while by GLC eleven peaks in (C) and nine peaks in (E_2) were identified . In both cases oleic and palmitic acids were the major constituents (Tables 95 and 96).

The fatty acids in (E_2) were present as free acids and not as esters or triglycerides as in case of P.coccineus, since the TLC on the methanol extract revealed some of these acids, which were located by system 30. The reason for this could be that methanol is a more suitable solvent than petroleum ether (more polar). A mixture of methanol and petroleum ether is an excellent solvent for the extraction of fatty acids from plant materials, but this solvent mixture also extracts other compounds.

Probable acid	Percentage in (C)
Myristic	1.33
Myristoleic	1.84
Palmitic	18.85
Palmitoleic	3.17
C17 alkanoic	1.33
Stearic	3.38
C18 alkenoic	1.63
Oleic	50.20
Linoleic	5.53
Linolenic	8.09
C18 alkenoic	4.61

Table 95 Fatty acids obtained from the fraction (C).

Probable acid	Percentage in (E_2)
Capric	3.83
Lauric	1.40
Myristic	1.66
Myristoleic	2.26
Palmitic	32.40
Stearic	3.13
C18 alkenoic	4.00
Oleic	40.30
Linolenic	11.00

Table 96 Fatty acids obtained from the fraction (E_2).

III-4- The unsaponifiable fractions.

III-4-1- Phaseolus coccineus.

There were three major components in the unsaponifiable fraction (B) of the roots, rhizomes and stems (systems 26 and 27), two of them i.e. numbers 2 and 3 were also present in fraction (E_1).

These three compounds were isolated in pure state by column chromatography, from the unsaponifiable fraction (B) of the roots, rhizomes and stems, and compounds 2 and 3 isolated by the same technique from fraction (E_1) of the stems only and were compared with the compounds isolated from the petroleum ether extract (B). TLC, IR and m.p. were used for this comparison.

The percentage of (B) and (E_1) as well as the percentages of compounds 1,2, and 3 in these fractions are shown below (Tables 97 and 98).

Fraction	Roots %	Rhizomes %	Stems %
B	0.77	0.88	0.50
E_1	0.08	0.08	0.81

Table 97 The percentage of unsaponifiable fractions of P.coccineus.

Compound No.	Fraction	Roots %	Rhizomes %	Stems %
1	B	0.28	0.30	0.97
2	B	0.95	0.71	1.71
3	B	2.09	3.50	3.39
2	E_1	-	-	2.67
3	E_1	-	-	3.11

Table 98 The percentage of compounds 1,2 and 3 present in the unsaponifiable fractions.

From the Table 97, it could be seen that the unsaponifiable contents of the roots (0.85% w/w), rhizomes (0.96% w/w) were close to each other but the stems (1.31% w/w) contained the largest proportion. From Table 98 phaseosterol-B was present in all three organs in a relatively high

concentration followed by phaseosterol-A and phaseobone-1.

The presence of phaseosterol-A and phaseosterol-B in the methanol extract together with traces of the other unsaponifiable components could be due to two reasons:

Firstly, the insuffient time of extraction with petroleum ether and secondly, it could be due to their greater solubility in methanol than that of petroleum ether.

From the comparative TLC studies between fractions B and E_1 of the roots, rhizomes and stems no appreciable differences were noticed between them.

Phaseosterol-A and phaseosterol-B isolated from fraction E_2 of the stems were found to be identical to those isolated from fraction B of the same organs (TLC, m.p. and I.R spectra).

The identification of the unsaponifiable compounds.

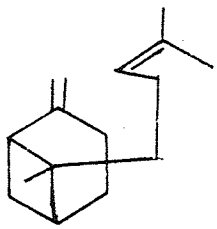
From TLC (system 10) spot 5 which was present in a small amount was found to have the same Rf value and colour as the authentic sample of cholestene. Since it was present in a small amount, its identity was not absolutely established. However, spot 6 had the same Rf value and colour as β-sitosterol and this in addition to phaseobone-1 (spot 1) and phaseosterol-A (spot 4) were isolated in pure states. Other compounds were present only in small amounts.

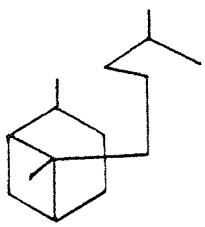
Identification of phaseobone-1.

This compound was isolated as yellow oil, U.V. inactive, and on TLC it gave a faint blue colour with phosphomolybdic acid reagent.

It was identified as β-bergamotane by comparing I.R. spectra with those of the published data[121], which proved to be similar to it except for two weak bands at 1170 cm^{-1} and 1330 cm^{-1} mentioned in the spectra of bergamotane and absent in the spectrum of phaseobone-1. The spectrum (thin film) showed very strong bands at 2850 cm^{-1} and 2925 cm^{-1} corresponding to C-H stretching vibration of asymmetric and symmetric stretch for methylene and methyl groups which overlap each other. The band at 1470 cm^{-1}(s) was due to antisymmetrical deformation of the methyl groups, while the band at 1365 cm^{-1}(s) together with the one at 1383 cm^{-1}(s), suggested an isopropyl group. The spectrum was not identical to that of any saturated bicyclic sesquiterpenoids recorded in literature[227], although it showed close similarity to that of tetrahydro-β-bergamotene (Bergamotane) (34).

Its mass spectrum and NMR confirm the structure.





(34)

β-bergamotene

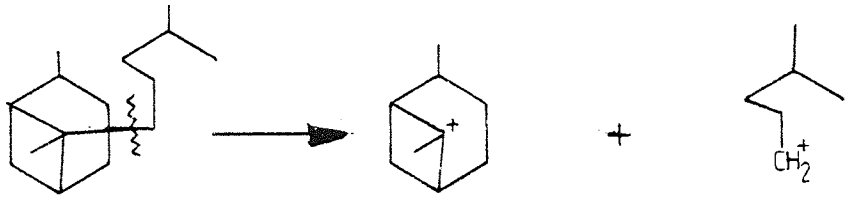
β-bergamotane

The mass spectrum of an alicyclic hydrcarbon is complex and the structure cannot be deduced readily as in cases of aliphatic straight or branched hydrocarbons.

The spectrum (spectrum-9) showed those peaks which are usually found in straight aliphatic hydrocarbons, m/e 43 (78%), 57 (100%), 71 (48%), 85 (78%), 99 (25%) and 113 (16%). The molecular ion (M^+) was relatively small at m/e 208 (2%).

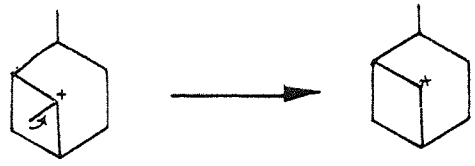
The following fragmentations were suggested:

1. Peak at m/e 123 (16%) could be due to fragment(a)



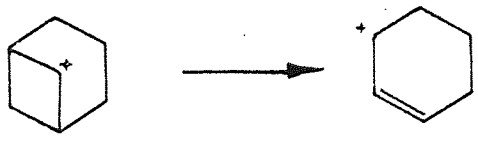
(a) m/e 123 (b) m/e 85

2. Fragment (a) suffers further loss of 14 mass units which could be from the methyl group at C8, involving one hydrogen transfer giving rise to fragment (c) at m/e 109 (23%)



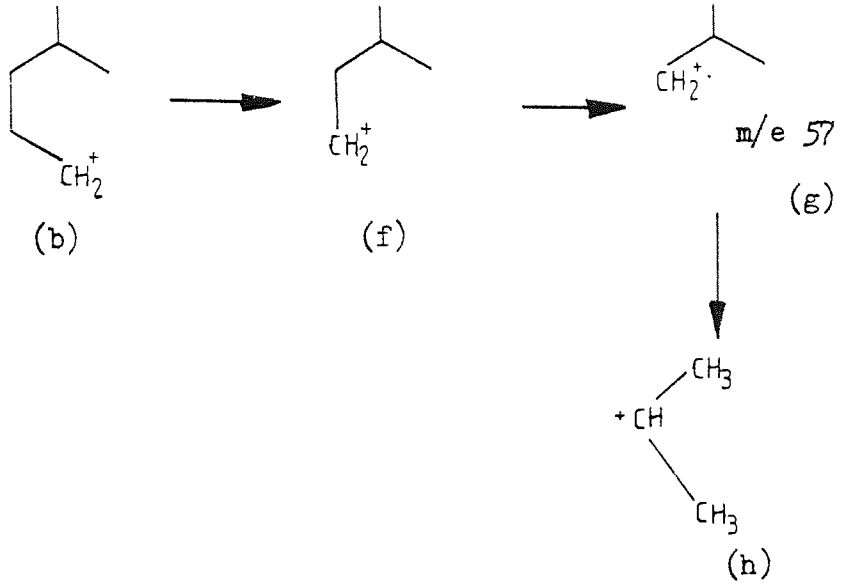
(c)

3. Fragment (c) alternatively loses one further methylene group (could be from C9) giving rise to fragment (d) at m/e 95 (35%)
4. Fragment (d) can lose another methyl group giving rise to a stable ion (e) at m/e 81 (41%)



(d) (e)

5. Fragment (b) loses a methylene group giving rise to ion (f) at m/e 71 (48%) which in turn loses further methylene groups giving rise to the most abundant ion at m/e 57 (100%) (g). This fragment suffers another loss of 14 mass units giving rise to fragment (h)



The NMR spectrum (60 MHz) showed the following absorptions $\delta^{CDCl_3}_{TMS}$ 0.65, 0.825, 0.90, 0.92 corresponding to four methyl groups, while 1.30 corresponding to the methylene protons (16H).

From the above data phaseobone-1 isolated from P.coccineus roots, rhizomes and stems was identified as a sesquiterpene of molecular formula $C_{15}H_{28}$ and structural formula (34) i.e. β-bergamotane.

Identification of phaseosterol-A.

This compound was identified as 18α-oleanan-3-one. It had m.p 243-244°C (Lit. 243°C)[157]. It gave a green colour with Burchard-Liebermann's reagent, but no colour with tetranitromethane. Elemental analysis was found C, 84.5; H, 11.96; O, 3.74; calculated from $C_{30}H_{50}O$ as C, 84.5; H, 11.8; O, 3.75.

Infra-red spectrum (KBr).

The I.R. spectra of the triterpenoids are very similar to those of steroids, with rather stronger absorption in the region of methyl stretching and bending vibrations.

The spectrum of phaseosterol-A showed a very strong bands at 2950 and 2850 cm^{-1} corresponded to the stretching vibrations of-CH_2 and -CH_3 of symmetrical and antisymmetrical types which overlap each other. The band at 1695 cm^{-1}(v.s) was corresponded to the carbonyl absorption and its exact position indicated its location at C_3[122, 157]. It should be noticed that the carbonyl frequencies are not identical with those at equivalent positions on the steroid ring system due most probably to the different mass distribution introduced by extra methyl groups[123].

The absorption regions of the double bonds which are usually observed in three places[124], were not observed in the spectrum of phaseosterol A

The bands at 1390 and 1380 cm^{-1}(s) represented the angular methyl groups between two six-membered rings. Other bands were at 1460, 1440 cm^{-1}(s) due to -CH_2 and -CH_3 bending vibrations.

Mass spectrum (spectrum 10).

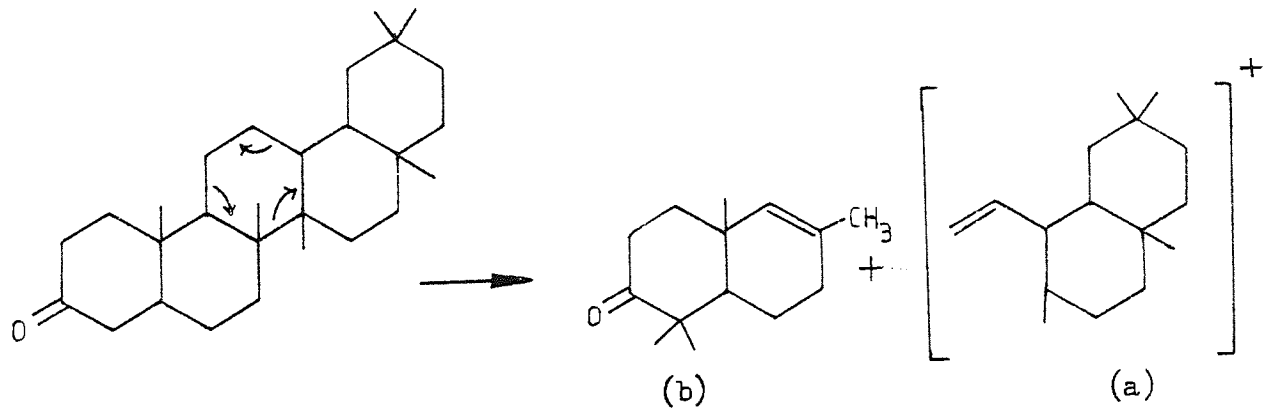
The measured mass was equal to 426 and its calculated mass 426.

The spectrum showed a molecular ion of M^+ 426 (6%), and from the fragmentation pathways of steroids and pentacyclic triterpenoids[158], this compound did not have a side chain substituent, since no loss from the molecular ion was observed. The peaks at m/e 248 (12%) and m/e 203 (12%)

indicated that no substitution was present in rings C, D or E[158].

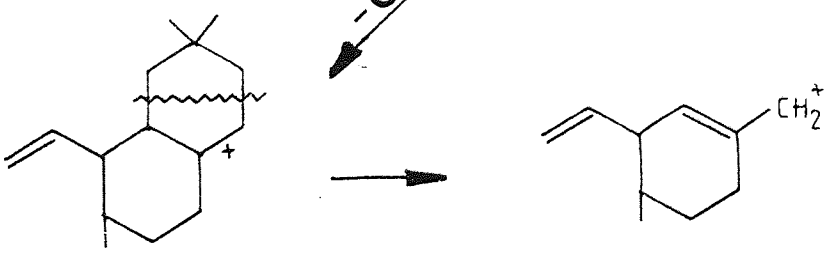
Saturated oleananes, bearing substituents only in rings A and B, were characterized by spectra which were virtually void of any pronounced fragments from the molecular ion[126], with the exception of M^+ and the peaks due to the loss of functional groups such as $M-CH_3$, which was observed at m/e 411 (6%).

The fragmentation of phaseosterol-A can be described by a retro-Diels Alder reaction (RDA)[127] giving rise to fragment (a) at m/e 220 (14%), which in turn loses 15 mass units due to the loss of C-17 methyl group giving



(b) (a)

rise to fragment (c) at m/e 205 (2%)



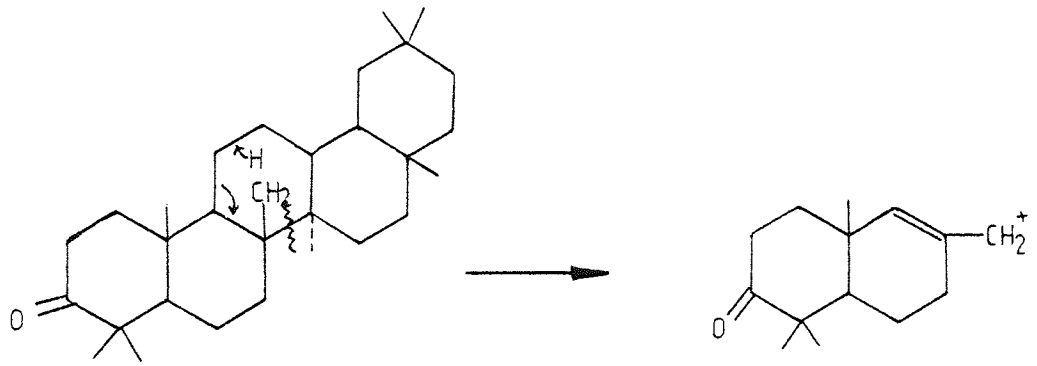
(c) (d)

Fragment (c) loses 70 mass units as indicated by the wavy line giving rise to a stable ion at m/e 135 (20%).

Another important fragment at m/e 205 (2%), which contained

rings A and B and its presence confirmed the C-3 carbonyl group[126]. This fragment is one of the most characteristic fragmentation of saturated pentacyclic triterpenoids (e).

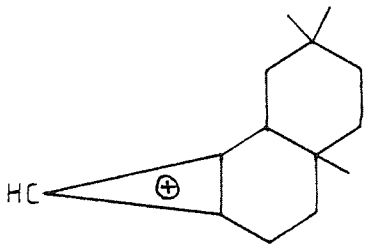
Fragment m/e 206 (16%) was resulting from the loss of 14 mass units from fragment (a).



m/e 205

(e)

Finally, fragment at m/e 191 (20%) corresponded to fragment (f) which involves the right hand portion of the molecule[125].

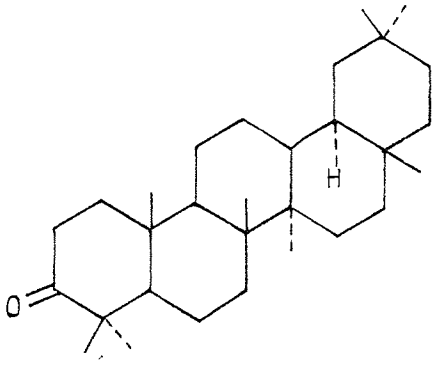


(f)

The NMR spectrum (100 MHz) did not show any overlapping and it showed the following absorptions, $\delta^{CDCl_3}_{TMS}$ 1.18, 1.06. 1.02, 0.92, 0.96, 0.73. 0.93 and 0.87 corresponding to 4α, 4β, 10β, 8β, 14α, 17β, 20β and 20α respectively.

From the above data and by comparing the results with those of published data [157,159] phaseosterol-A was identified as 18α-oleanane-3-one

with the structural formula (35)



(35)

18α-oleanan-3-one

Identification of phaseosterol-B.

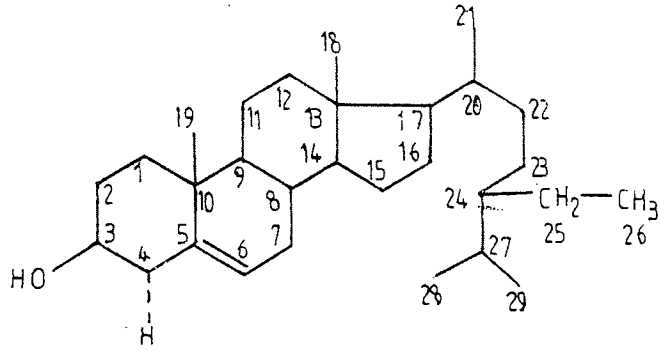
This compound which was isolated from the roots, rhizomes and stems of the runner bean was identified as β-sitosterol (shining white flakes, m.p. 136°C, elemental analysis was found to be C, 84.08; H, 12.01; O, 3.88 and calculated from $C_{29}H_{50}O$ as C, 84.05; H, 12.07; O, 3.86). With Burchard-Liebermann reagent it developed a green colour and with trinitromethane reagent a faint yellow colour. The U.V. was inactive and the I.R. (KBr) spectrum when compared with that of the β-sitosterol was found to be identical.

The strong band at 3425 cm^{-1}(broad) was due to C-H stretching interbonded, while 1060 cm^{-1} was due to C-O stretching in C-OH. One of the main bands at 2950 cm^{-1}(v.s) clearly arose from the C-H bond in a saturated hydrocarbon ($-CH_2$) and since the absorption was very strong, this indicates that the number of methylene groups is high. The absorption band at 2850 cm^{-1}(v.s) which appears on the other side of the previous band and was of the same strength, was due to the C-H symmetrical stretching in the methyl groups. Again, due to the strong absorption, a large number of methyl groups must be present. Therefore, the absorptions at 2950 and 2850 cm^{-1} were due to C-H stretching of CH_2 (2925, 2850) and CH_3 (2962 and 2872 cm^{-1}) which overlapped one another[130]. The strong absorption at 1450 cm^{-1} arose from the methylene scissoring vibration. This band appeared as a shoulder due to overlapping of the high absorption of antisymmetrical deformation of the methyl groups at 1460 cm^{-1}. The isopropyl group at the end of the side chain was diagnosed[130,131] by the presence of strong bands at 1380 cm^{-1}, 1365 $cm^{-1}$and 845 cm^{-1}.

The absorption bands at 1270, 1620, 925 and 885 cm^{-1} arose from CH_2 due to the C-H out-of-plane bending associated with cyclohexane ring.

The double band at C_5 was detected by the weak absorption of the stretching vibration at 1665 cm^{-1} [132,133] and the angular deformation of the

carbon-hydrogen bond in -C=C-H at 803 cm^{-1} 124,134.



(36)

Stigmasta-5-en-3β-ol

Mass spectrum of phaseosterol-B (spectrum-11).

From the structure of β-sitosterol, it is evident that there are several possibilities of loosing a methyl group from the molecule as shown by the first fragment M-15 i.e. m/e 399 (7%). The parent M-15 fragment has also been observed by other investigators in other steroids even in the absence of an alkyl side chain at C_{17}[135]. This parent M-15 fragment may be due to the loss of a methyl group from the side chain or the angular methyl group.

The mass spectra of β-sitosterol and other oxygenated steroids are more complex than that of the non-oxygenated ones due somewhat to thermal instability. The cracking of the basic ring structure is similar in some points in both cases. The following fragmentation took place:

1. m/e 399 (7%) M-15 Loss of CH_3 from the side chain.
2. m/e 273 (25%) M-141 Loss of the side chain[136].
3. m/e 255 (21%) M-159 Loss of the side chain with HOH element from ring A.
4. m/e 213 (25%) In addition to the fragment mentioned in number 3 the breaking of ring D took place at C_{13} - C_{17} and C_{15} - C_{16} carbon-to-carbon bond together with the angular methyl group.
5. m/e 159 (32%) M-255 This corresponded to the rupture of ring C at C_{11} - C_{12} and C_8 - C_{14} carbon-to-carbon bond.
6. m/e 145 (50%) M-269 This corresponded to the breakdown of ring C in another position i.e. C_9 - C_{11} and C_8 - C_{14} carbon-to-carbon bond.
7. m/e 119 (32%) Rupture of ring B at C_{10} - C_9 and C_8 - C_7 carbon-to-carbon bond.
8. m/e 105 (57%) Rupture of ring C at C_{10} - C_9 and C_7 - C_6 carbon-to-carbon bond.
9. m/e 92 (14%) Rupture of ring C at C_{10} - C_9 and C_5 - C_6.
10. m/e 77 (17%) Loss of the angular methyl group at C_{19} together with fragment 9.

In addition to the above fragmentation, m/e 396 (6%) due to M-HOH and m/e 381 due to ($M-CH_3-H-OH$) were observed. The fragment M-111 at m/e 303 (10%) is a characteristic fragment for steroids of C_5 - C_6 unsaturation in ring B[135].

Finally, one of the most general fragmentations of sterols and related C-17 subsituted steroids[156] was the loss of 42 mass units together with C-17 side chain, was found at m/e 331 (17%) which accompanied with m/e 213 (25%) due to the loss of one molecule of water.

Finally the structure of β-sitosterol identified by the previous methods and by comparing these data with the data obtained from an authentic sample, was confirmed by its NMR spectrum. The angular methyl groups were found to give well defined absorptions[137]. However, since β-sitosterol contained a number of such methyl groups, their absorptions were found to be overlapped. The absorptions were observed at $\delta_{TMS}^{CDCl_3}$ 0.70, 0.75, 0.80, 0.85, 0.96, 1.02 and 1.07 which may be assigned respectively to carbon atoms 18; 29; 26, 27; 29; 26, 27; 29; 26, 27 and 29; 21; 19 and 21[138]. Secondary carbinol methine absorption observed at δ3.6, broad, and olefinic proton at δ5.3.

III-4-2- Unsaponifiable fraction of G.triacanthos.

a. G.triacanthos pods.

The percentage of the unsaponifiable fraction was found to be 4.23% w/w of the original weight. It showed four spots on TLC (system-25), only three of which were isolated in pure states by column chromatography. These were:

1. Triacanane-B.

This compound was isolated as white platelet crystals forming 0.17% w/w of the original weight, m.p. 62-63°C, U.V. inactive and it was identified as n-nonacosane (Lit. m.p. 63°C)[139].

Infra-red spectrum (KBr).

The spectrum showed the following absorption bands: 710 (s) (splitting), 885, 1375 (m), 1460 (s), 1470 (s), 2825 (v.s) and 2900 cm^{-1} (v.s).

The spectrum did not indicate any sign of branching in the compound at any region, due to the absence of those bands corresponding to branching at 1385 (s), 1395 (s), 1170, 1155, 1250 and 1250-1200 cm^{-1}. However, it showed a typical spectrum of a straight-chain hydrocarbon, with a structural formula of C_nH_{2n+2}.

The two very strong bands at 2900 and 2825 cm^{-1} corresponded to asymmetrical and symmetrical stretching modes of $-CH_2$ and $-CH_3$ groups[140]. These two groups also gave rise to absorption at 1460 (s) and 1470 cm^{-1} (s) due to hydrogen bending vibrations[141,142]. The intensities of these bands are directly proportional to the number of $-CH_2$ and $-CH_3$ groups[143]. The symmetrical deformation mode of the hydrogen atom of a methyl group in an absorption band at 1375 cm^{-1} (m) which is extremely stable in position provided that the methyl group is attached to another carbon atom[144]. Finally the doublet band at 720 and 710 cm^{-1} was due to a rocking mode of the CH_2[145,146].

Mass spectrum (spectrum-12):

(3 KV, 70 eV, source temperature 300°C, inlet temperature 100°C).

The mass spectrum fragmentation of triacanane-B showed a weak molecular ion, and a typical series of $C_nH^+_{2n+1}$, and to a lesser extent $C_nH^+_{2n-1}$ plus an ion with abundance maxima at C_3 (m/e 43). This agreed with the fragmentation of straight-chain hydrocarbons[147].

Viallard and Magat[148] studied homologous series of unbranched hydrocarbons to gain information on the relative probabilities of cleavage at the various types of bonds. They found that in each homologous series the percentage of the fragment formed by breaking C-H bonds, without rupture of C-C bonds, decreased as one proceeded to higher molecular weight (from 84% in ethane to 0.008% for n-octane). These fragments observed in the above spectrum were, m/e 43 (100%), 57 (92%), 71 (83%), 85 (75%), 99 (67%), 113 (58%) etc. Consideration of fragment ions formed by breaking C-C bonds showed that those fragments containing an odd number of hydrogen atoms were more abundant than those containing even numbers, m/e 44 (21%), 56 (21%), 70 (25%), 84 (21%), because the ions of the radicals are more favoured than the molecular ion. Simultaneous rupture of two C-C bonds never occurs[149].

It is always possible to measure the molecular weight from the mass spectrum of a straight-chain alkane[149]. Although hydrocarbons are generally considered to be thermally stable at normal ionization chamber temperature, the heavier molecular weight paraffins from about C_{12} upwards, required a heated sample system for their examination. This was the reason for an inlet temperature of 100°C and a source temperature of 300°C.

From the above data it was concluded that triacanane-B was a straight chain hydrocarbon with a molecular weight of 408, the base peak at m/e 43 corresponding to loss of C_3H_7, followed by fragments corresponding to C_nH_{2n+1} which was accompanied by fragments of lower intensities, which

disappeared near the end of the spectrum, corresponding to C_nH_{2n-1} and C_nH_{2n}, m/e 393 (0.5%) corresponding to the loss of $-CH_3$ from the parent ion.

The results were confirmed by elemental analysis which was found to be C, 85.70%; H, 14.63% calculated from $C_{29}H_{60}$ C, 85.29%; H, 14.70%.

Triacanane-B was therefore shown to be n-nonacosane with the structural formula (37),

$$CH_3\ (CH_2)_{27}\ CH_3$$

(37)

2. Triacanthosterol-A.

This compound was present in a small amount (about 0.01% w/w) and was isolated as colourless needle crystals, m.p. 138-139°C. The TLC properties of this compound were identical to those of an authentic sample of β-sitosterol. Since the amount isolated was very small, no more information was obtained, and since Seriya[43] isolated this compound from the seed and identified it as β-sitosterol, Triacanthosterol-A could be β-sitosterol. A comparative TLC study was performed with the same fraction obtained from the seed and this was found to be identical to β-sitosterol.

The same results were obtained with Triacanthosterol-B (0.009% w/w of the original weight, m.p. 175-176°C (D)) and identified on the same basis as stigmasterol (28). Finally spot number four (Triacanthosterol-C) could be brassicasterol (38).

Fraction (E) (the unsaponifiable fraction of methanol extract) revealed in addition to the above four compounds, a further two compounds with Rf values of 0.53 and 0.15 respectively (system 26). The former compound was not steroidal in nature (it gave no colour with phosphomolybdic acid and anisaldehyde reagents), while the latter compound with an Rf value

of 0.15 (not a steroid as well), gave a purple colour with ferric chloride reagent and it was visible yellow. This compound could be a free flavonoid. These two compounds were unidentifiable due to the small amounts present. Finally, at the base line, a steroidal compound was located.

From fraction (E) only one compound was isolated in pure state (triacanane-C, 0.13% w/w) and it was identified as n-noncosane (37) by comparing its m.p., I.R. and M.S. with those of triacanane-B. The total amount present in the pods is therefore 0.30% w/w.

b. G.triacanthos seeds.

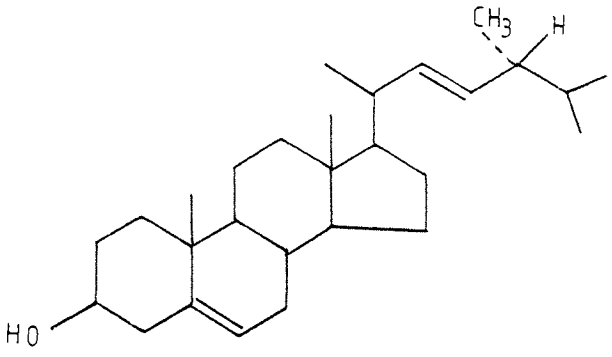
The percentage of the unsaponifiable fraction obtained from the seeds forms about 0.4% w/w of the original weight. The components were separated on TLC (system 25) and compared with those obtained from the pods. Since similar results were obtained, the same type and number of compounds were indicated in both seeds and pods.

Three of these compounds were identified as β-sitosterol, stigmasterol and brassicasterol. on the basis of Seriya's[43] conclusions. He isolated β-sitosterol and identified it by a comparative study (I.R., M.S. and m.p.) with authentic sample, while the other two were examined by a TLC study only.

Table 99 explains the results obtained from both seeds and pods.

Spot No.	Name of the compound	Identified as
1	Triacanane-B	n-nonacosane
	Triacanane-C	n-nonacosane
2	Triacanthosterol-A	β-sitosterol
3	Triacanthosterol-B	Stigmasterol
4	Triacanthosterol-C	Brassicasterol

Table 99 Compounds obtained from the Honey-locust pods and seeds.



(38)

Brassicasterol

III-4-3- Unsaponifiable fraction of Apium graveolens.

The unsaponifiable fraction (1.7 % w/w) contained two major compounds, together with seven minor compounds (system 30). Spots 1 and 2 were the major ones, which were isolated in pure states by column chromatography (graveobone-A and graveosterol-A respectively). Graveosterol-A was again isolated from the unsaponifiable fraction of methanol extract (E_1) together with another compound labelled graveobone-B.

All the compounds except graveobone-A and B located on the chromatograms (system 30) could be steroidal in nature due to the fact that they gave the colour reaction with steroidal reagents. Only three of them were isolated and identified completely i.e. graveobone-A, graveosterol-A and graveobone-B.

1. Graveobone-A

It was isolated as a yellow oil (0.0092% w/w) by column chromatography from fraction (B). It gave a single peak with Rt (6.5 min.) when run on GLC using (system 7). On TLC it gave a single spot with fluorescent blue colour under the U.V. light (254 nm.) and a blue colour with phosphomolybdic acid reagent and an Rf 0.97 (system 30).

This compound was not β-selinene, β-selinane or bi- or monocyclic sesquiterpenes, since its spectra (I.R., M.S. and NMR) were not identical to any of these compounds recorded in the literature[96,150,151].

Its I.R. spectrum (thin film) indicated that this compound was an aliphatic hydrocarbon with conjugated double bonds.

The spectrum was quite complex in the region of C=C stretching vibrations. It showed four absorptions at 1600 (s), 1640 (m), 1680 (w) and 1718 (w) cm^{-1}. These could be due to two conjugated systems.

One of the most valuable means of detection of double bonds through infra-red is the examination of the region near 3000 cm^{-1}. The spectrum showed an absorption band at 3090 cm^{-1} (m.shoulder) corresponding

to C-H stretching frequencies of the $=CH_2$[140]. Another band was observed as a shoulder at 3025 cm^{-1} which corresponded to =CH- vibration. By the use of these two bands it is possible to differentiate the structure as $-CH=CH_2$ (In the cases of $-C=CH_2$ and -C=CH- only one band would be observed at 3079 and 3019 cm^{-1} respectively[143] and hence these structures can be eliminated).

The normal CH stretching vibrations of the saturated part were observed at 2925 (v.s) and 2825 (v.s), and the bending vibrations observed at 1465 (s) and 1450 (s) cm^{-1}.

The third region for HC=CH was observed at 1000 (s), 910 (s) and 900 (s) cm^{-1} due to out-of-plane =CH deformation vibrations. The influence of conjugation on the position of these bands is weak, and there is usually a shift towards higher frequencies, particularly in long conjugated chains, the frequency reaching a limiting value of about 1000 cm^{-1}[152]

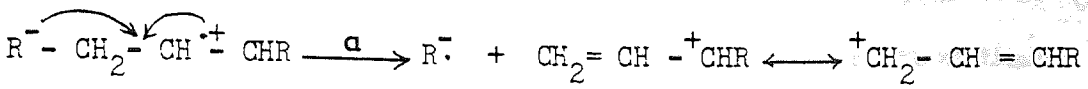
Other bands were observed at 730 (s) cm^{-1} which corresponded to a rocking mode of the CH_2[145,146] and two strong bands at 1385 and 1365 cm^{-1} which could be due to an isopropyl group at the end of the chain[130].

The U.V. spectrum (in chloroform) confirmed the conjugation, it showed λmax 268, 297, 317 and 339 nm.

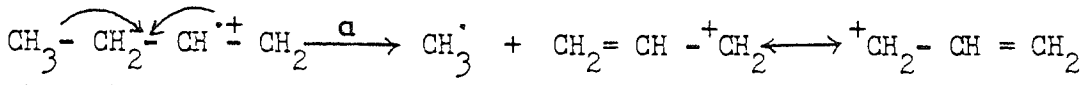
Mass spectrum (spectrum-13).

The addition of a double bond to an alkane increases the abundance of the $C_nH_{2n-1}^+$ and $C_nH_{2n}^+$ ion series. The spectrum of graveobone-A showed these ions:
m/e 41 (100%), 42 (17%), 55 (48%), 56 (26%), 69 (52%), 70 (22%), 83 (26%), 84 (13%), 97 (22%) and 98 (9%) and in addition it showed a series of fragments corresponding to $C_nH_{2n+1}^+$ i.e. m/e 43 (74%), 57 (83%), 71 (48%) and 85 (35%). These fragments disappeared at the end of the spectrum.

Alkene ions exhibit allylic cleavage (a), but also show a strong tendency to isomerize through migration of the double bond.



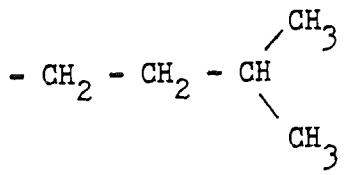
(a)



m/e 41 (100%)

(b)

Finally the relatively intense peak at m/e 147 (26%) (M-57) could be due to the loss of the isopropyl group with one methylene group, while m/e 161 (26%) corresponded to the loss of the isopropyl group at the end of the chain, and m/e 133 (35%) was due to the loss of fragment (c)

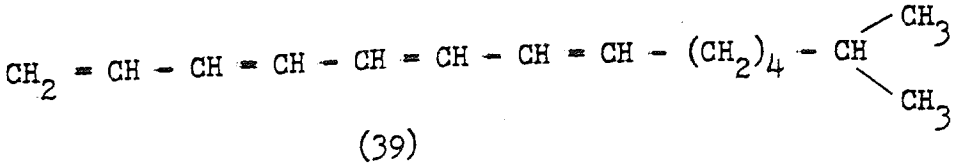


(c)

Fragment m/e 119 (30%) was due to the loss of another $-CH_2$ group and also with m/e 105 (65%) and m/e 91 (39%). This indicated that the conjugations are at the end of the chain opposite to the isopropyl end.

The NMR spectrum in $CDCl_3$ showed the following absorption bands: $\delta^{CDCl_3}_{TMS}$ 0.85 and 0.875 corresponding to two methyl groups. The ethylenic double bonds were observed at δ3.65 as a triplet and at δ4.7 and at δ5.0.

From the above data graveobone-A was identified as 1,3,5,7-tetra-ene-13-dimethyl-tridecane with the structural formula (39)



(39)

2. Graveosterol-A.

This compound was isolated as colourless needle crystals (0.039% w/w), m.p. 155°C, acetate 135°C. It gave a green colour with Liebermann-Burchard reagent and a yellow colour with tetranitromethane, but its U.V. was inactive.

The microanalysis was found to be C, 83.74; H, 12.26; O, 4.08 calculated from $C_{29}H_{48}O$ as C, 84.44; H, 11.65; O, 3.88.

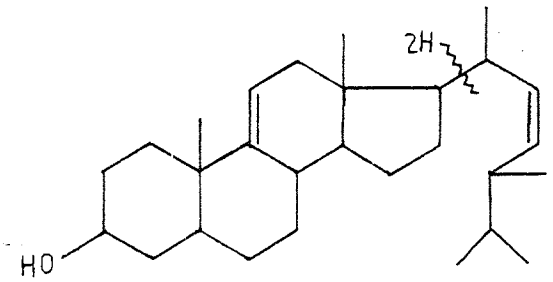
In addition to the characteristic bands of a steroidal molecule (which were discussed under β-sitosterol), the I.R. spectrum of graveosterol-A showed the following bands: 3400 (s.broad) corresponding to O-H stretching vibration, 2925 (v.s), 2850 (v.s) corresponding to $-CH_2$ and $-CH_3$ (C-H) stretching vibrations, while bands at 1470 (s), 1450 (s) corresponded to C-H bending vibrations of $-CH_2$ and $-CH_3$. The bands at 1385 and 1370 cm^{-1} corresponded to the isopropyl group at the end of the side chain[130], together with the band at 842 cm^{-1} [131]. The weak absorption at 1648 cm^{-1} corresponded to the stretching vibration of H-C=C at C_9 and the angular deformation of carbon-hydrogen bond in -C=C-H at $\Delta^{9(11)}$ was found at 810 cm^{-1} [138]. The band at 960 cm^{-1} corresponded to the double bond in the side-chain (Δ^{22-23}) [153]. While the =CH stretching vibration which should appear near 3040-3030 cm^{-1} was not observed due to the weak absorption of this band, the use of KBr disc and due to overlapping caused by $-CH_2$, $-CH_3$ stretching vibration bands which were very strong.

The acetate spectrum (KBr) showed strong absorption at 1720 (v.s) and at 1240 (v.s) cm^{-1} corresponding to the carbonyl of the acetyl group and the disappearance of the band at 3400 cm^{-1}.

The mass spectrum of graveosterol-A (spectrum-14) showed characteristic peaks at m/e 369 (12%), 351 (21%), 314 (9%), 300 (18%), 273 (18%) (M^+ - 139, the side chain), 271 (21%) (M^+ - (139+2)), 255 (45%), 231 (21%), 213 (36%), 159 (45%), 83 (100%), 81 (57%) and 43 (57%).

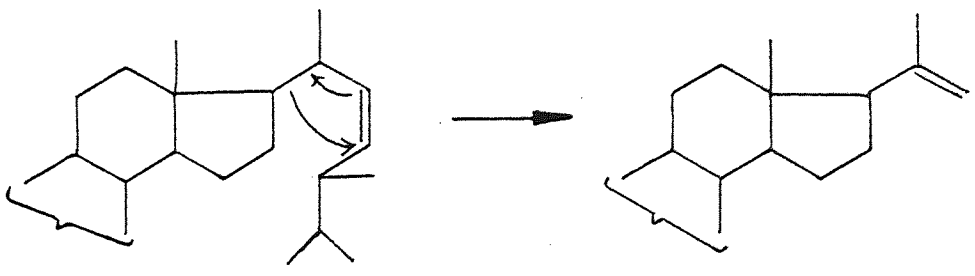
The peak at m/e 271 (28%) was more intense than the peak at m/e 314 (9%) and m/e 273 (18%). This indicated that the double bond was at Δ^9 and not at Δ^5 or Δ^7 [138]. Generally the m/e 271 peak is more intense than m/e 314 if the sterol has $\Delta^{7(8)}$ double bond[154], while the reverse situation exists in the case of $\Delta^{5(6)}$ isomers. Furthermore, the m/e 271 is found to be the base peak in the case of $\Delta^{7(8)}$ steroid[155].

The presence of a Δ^{22-23} double bond was diagnosed by the peak at m/e 273 and m/e 300[154]. The peak at m/e 271 corresponded to the loss of the side chain together with two hydrogen atoms from the steroid nucleus.



m/e 271

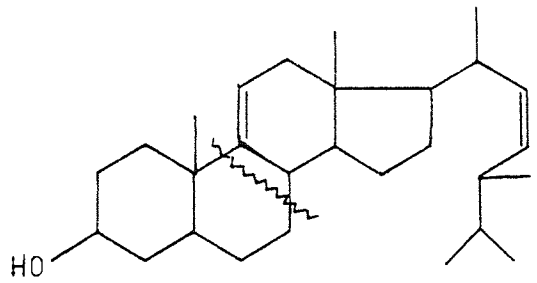
The presence of the peak at m/e 271 in the spectrum indicated that this compound has the usual sterol nucleus together with a double bond in the side chain[154]. The m/e 314 was due to the "McLafferty" type of rearrangement (a) to (b).



(a) (b)

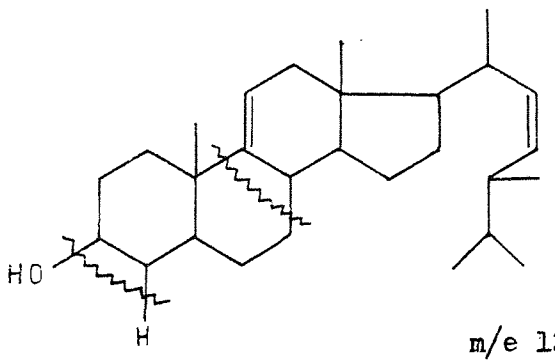
The loss of H_2O from the parent ion was observed at m/e 394 (9%) and the loss of CH_3COOH from the acetate (spectrum-15) was also observed at m/e 394 (59%). The loss of the C-17 side chain from graveosterol-A was observed at m/e 273 (18%) and the loss of the side chain together with a molecule of water was observed at m/e 255 (45%) (M-157) and (M-199) m/e 255 (55%) in the case of the acetate derivative.

The cleavage of the nuclear structure at the 9-10 and 7-8 carbon-to-carbon bonds resulted in two fragments (a) and (b) at m/e 171 (15%) andm/e 121 (27%) respectively.



m/e 171 (15%) Graveosterol-A
m/e 171 (10%) Graveosterol-A acetate

(a)

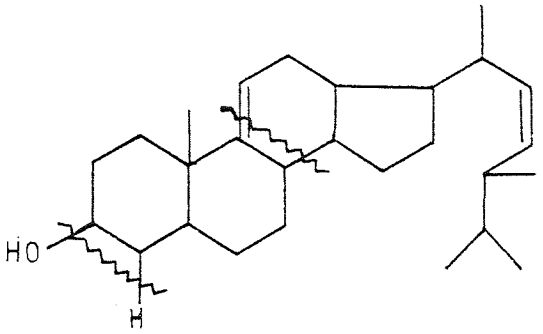


m/e 121 (27%) Graveosterol-A
m/e 121 (29%) Graveosterol-A acetate

(b)

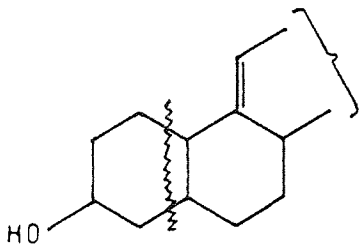
The absence of the fragment which corresponds to loss 111 mass units from the parent peak i.e. m/e 301 indicated the complete saturation of ring B[135]. The peak at m/e 147 (36%) corresponding to the fragment resulting from the breaking down of ring C at the 8-14 and 9-11 carbon-to-carbon bonds and the skeletal structure of rings A and B remains essentially intact.

As usual, compounds having a C_3 hydroxyl group show the loss of HOH peak.



m/e 147 (36%) Graveosterol-A
m/e 147 (50%) Graveosterol-A acetate

The loss of 72 mass units from the parent ion was observed at m/e 340 (12%). This fragmentation was not observed when ring B contained a double bond between C_5 and C_6. The formation of this ion apparently involves rupture of ring A [135].

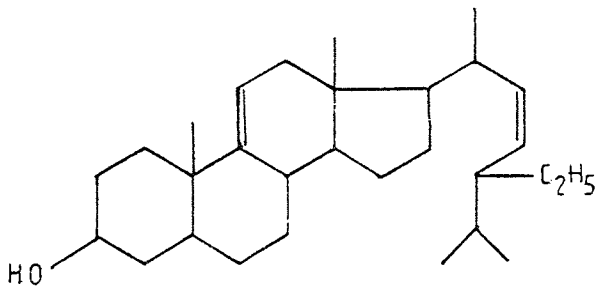


m/e 340 (12%) Graveosterol-A
m/e 382 (17%) Graveosterol-A acetate

Finally the peak at m/e 231 (21%) due to the loss of the side chain together with 42 mass units, which accompanied by m/e 213 (31%) due to the loss of HOH was described by Tokes et al[156]. The formation of the above peaks revealed the absence of any double bonds in the ring D.

The NMR spectrum (in $CDCl_3$ at 60 MHz) of graveosterol-A showed the presence of methyl signals at $\delta^{CDCl_3}_{TMS}$ 0.70, 0.75, 0.80, 0.85, 0.95, 0.97, 1.00 and 1.07 which may be assigned respectively at carbon atoms 18; 29; 26, 27; 29; 26, 27 and 29; 21; 19 and 21[111]. The secondary carbinol methin was observed at 3.6 (broad) and the olefinic proton at δ5.12 (2 H) ($-CH^{22}= CH^{23}-$) and at δ5.35 (1 H) of Δ^9. The protons of $-CH_2$ were observed at δ2.45.

From the above data, graeosterol-A would appear to be indosterol with structural formula (40)



(40)

3-β-hydroxy-5α-stigmasta-9(11),22(23)-diene

3. Graveobone-B.

This compound (0.01% w/w) was identified as n-hexacosane, m.p. 59°C, U.V. inactive. It was isolated as white platelet crystals and gave negative tests for triterpenoid and unsaturation. Its I.R. spectrum (KBr) was similar to that of triacanane-B i.e. straight chain hydrocarbon, giving the following strong absorption bands: 722, 735, 1470, 1480, 2850 and 2925 cm^{-1}. The mass spectrum (spectrum-16) showed the same fragmentation pathways as that of triacanane-B. The previous discussion on n-nonacosane of I.R. and M.S. is also applicable here.

The NMR spectrum (60 MHz) showed two absorption bands (singlets);

$\delta^{CDCl_3}_{TMS}$ 0.9 corresponding to six protons belonging to the two methyl groups at the two ends of the molecule, and 1.3 corresponding to fourty-eight protons of the methylene groups (1:8).

From the above data graveobone-B was identified as n-hexacosane with structural formula (41)

$$CH_3(CH_2)_{24}CH_3$$

(41)

III-5- Methanol extract components.

III-5-1- Phaseolus coccineus roots.

The compounds isolated from the methanol extract were as follows:

1. Phaseobone-2.

This compound was isolated as a platelet crystals (m.p. 53°C, 0.00061% w/w). It was not a sapogenin since it gave no colour with Lieberman's reagent. But it may be hydrocarbon due to its low Rf and m.p. Identification was not made due to the small amount present.

2. Phaseobone-3.

This compound was isolated as white platelet crystals (m.p. 69-70°C, 0.0009% w/w). It was identified as n-tritriacontane (42) by its m.p., I.R. and M.S. It gave no colour with Lieberman's and tetranitromethane reagents.

The I.R. (KBr) spectrum showed typical straight-chain hydrocarbon absorptions in the following positions, 710 (s), 720 (s), 1380 (m), 1460 (s), 1470 (s), 2825 (v.s) and 2900 (v.s) cm^{-1}. The spectrum has been previously described under Triacanane-B.

The mass spectrum (spectrum-17), has been previously described under n-nonacosane.

$$CH_3(CH_2)_{31}CH_3$$

(42)

3. Phaseoloside-A.

This compound was isolated as a glycoside (saponin) and as an aglycone (sapogenin), by two different methods.

The glycoside was isolated as white amorphous product (0.13% w/w, m.p. 125-128°C (D)). The aglycone isolated as a white product which failed to crystallise from many solvents e.g. methanol, ethanol and their aqueous solutions, etc.

The sugar moiety was identified as D-glucose, L(+) arabinose and D-fructose, by paper chromatography (system-24) and by GLC using their silyl ether derivatives (system-3).

The aglycone (0.03% w/w, m.p. 205-207°C (D)) gave a benzoyl derivative as colourless, needle crystals, m.p. 110-112°C but only in a minute quantity. On treatment with Lieberman's and tetranitromethane reagents, green and yellow colours respectively were obtained. The I.R. (KBr) spectrum showed bands at 2925 (s), and 2850 (s) corresponding to C-H stretching vibrations of $-CH_2$ and $-CH_3$ groups and strong bands at 1720 and 1260 cm^{-1} corresponding to the carbonyl group or groups of the benzoate. Bands at 1600, 1550 and 710 cm^{-1} confirmed the aromatic ring or rings.

The I.R. (KBr) spectrum of the aglycone was not clear due to the massive bands present at the absorption frequencies of the hydroxyl groups at 3400-3500 and 1040 cm^{-1}. The other bands were observed as weak absorptions.

The NMR spectrum (60 MHz) showed the absorptions at $\delta^{CDCl_3}_{TMS}$ 0.8, 0.85, 0.90, 0.975, 1.1 and 1.125 corresponding to the angular seven methyl groups (0.90 corresponded to two methyl groups). The absorption at δ5.2 could be due to the ethylenic double bond.

Due to lack of sufficient compound, the structure of phaseoloside-A remains unconfirmed.

4. Phaseoloside-B.

This compound was isolated as a glycoside and as an aglycone by two different methods.

The glycoside was isolated as white morphous product (0.328% w/w, m.p. 272-275°C (D)).

The sugar moiety was identified by paper chromatography (system-24) and by GLC (system-3) as D-glucose (2 molecules) L(+) arabinose and

D-fructose.

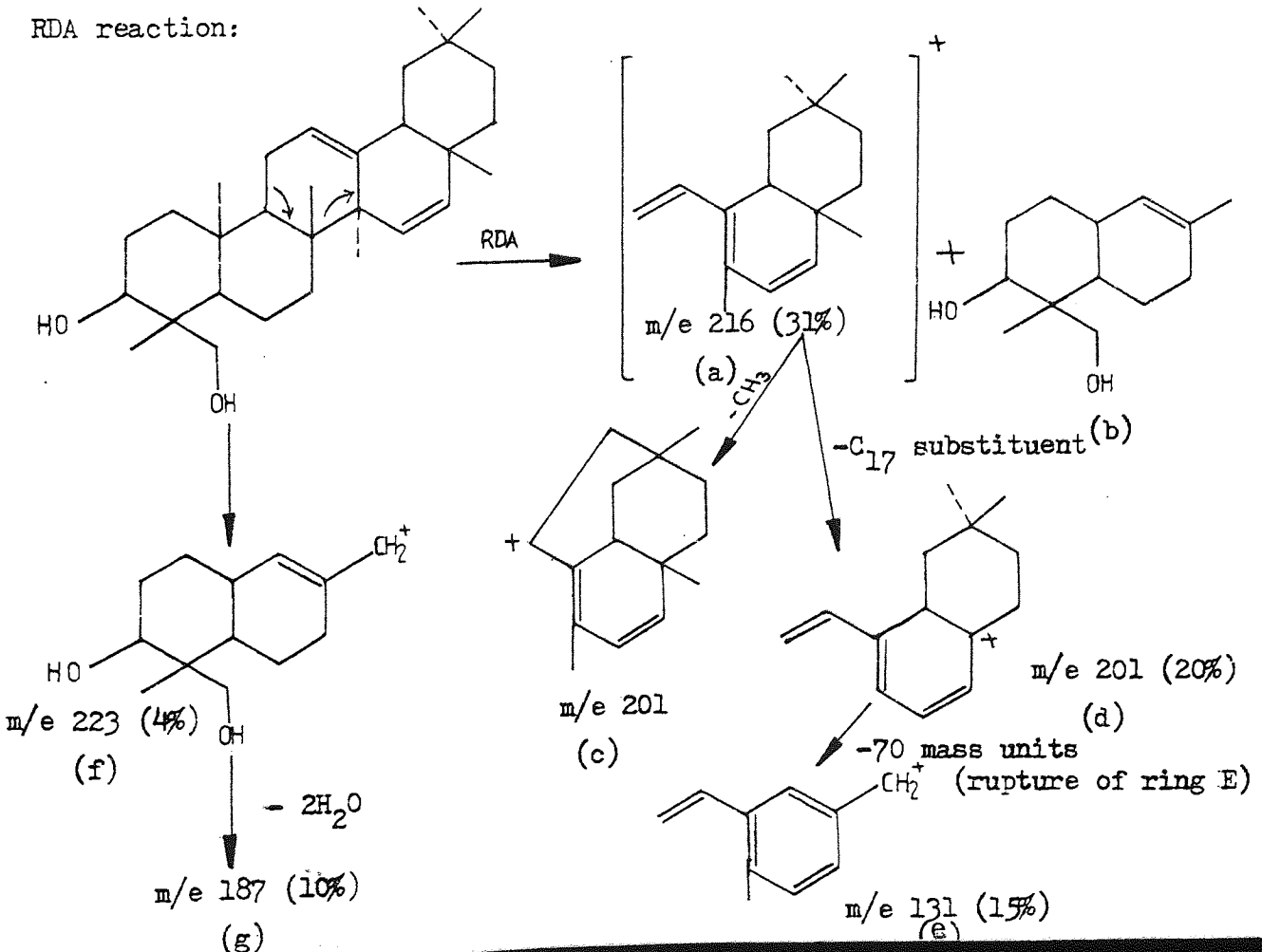
After hydrolysis the aglycone was isolated as amorphous white product (m.p. 238-239°C). It gave a yellow colour with tetranitromethane reagent and a green colour with Lieberman's reagent. It gave colourless needle crystals of a benzoyl derivative (m.p. 116-118°C).

The I.R. (KBr) spectrum of the genin showed a broad and strong absorption band at 3350 cm^{-1} which corresponded to the O-H stretching vibration. The band at 1690 (m) cm^{-1} corresponded to the C=C-H stretching vibration, while the angular deformation of C-H bonds representing the two double bonds at Δ^{12} and Δ^{15} were observed at 810 (m) and 760 (m) cm^{-1}.

The I.R. spectrum of benzoate was similar to that of phaseoloside-A benzoate.

The mass spectrum showed the characteristic fragmentation pathway of pentacyclic triterpenoids (spectrum-18).

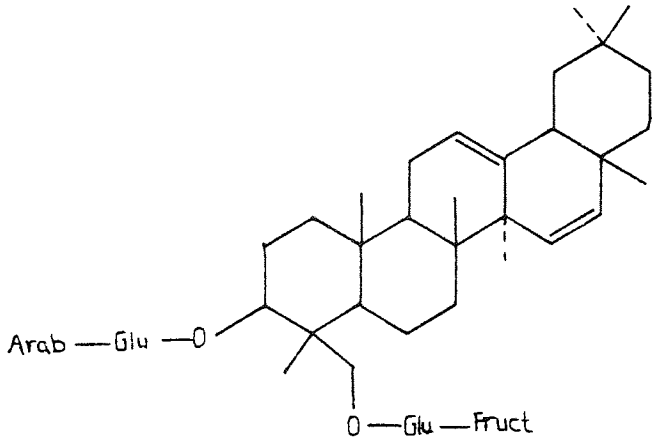
This compound was of oleanene type since the peak at m/e 201 (fragment d) was more intense than the peak at m/e 187 (fragment g)[125]. The fragmentation pathway of this genin is summarized below applying the RDA reaction:



The mass spectrum of the benzoate showed the same fragmentation pathway and the most important peaks were: m/e 234 (30%), 219 (35%), 216 (28%), 203 (8%), 201 (19%), 191 (3%), 187 (10%), 189 (10%), 175 (10%), 131 (5%), 122 (90%), 105 (100%), 77 (70%), 69 (8%), 51 (33%) and m/e 44 (35%). The other fragments including the molecular ion were of very low abundances. The details of the spectrum is described later under phaseoloside-C.

The NMR spectrum (60 MHz) showed the following absorptions: $\delta^{CDCl_3}_{TMS}$ 0.75, 0.80, 0.85, 0.95, 1.05 and 1.1 corresponding to seven methyl groups (δ 0.85 corresponding to two methyl groups overlapping each other).

The data obtained were compared with the published data (m.p. 238-239, 239-240, and 240-241°C)[160, 161, 162] and the compound was tentatively identified as soyasapogenol-C. From the sugar analysis the structure of phaseoloside-B could be inferred to have a formula (43). Lack of material prevented a conclusive elemental analysis being preformed, and to study a stepwise hydrolysis of the molecule. The structure proposed is therefore tentative.



(43)

Phaseoloside-B

5. Phaseoloside-C.

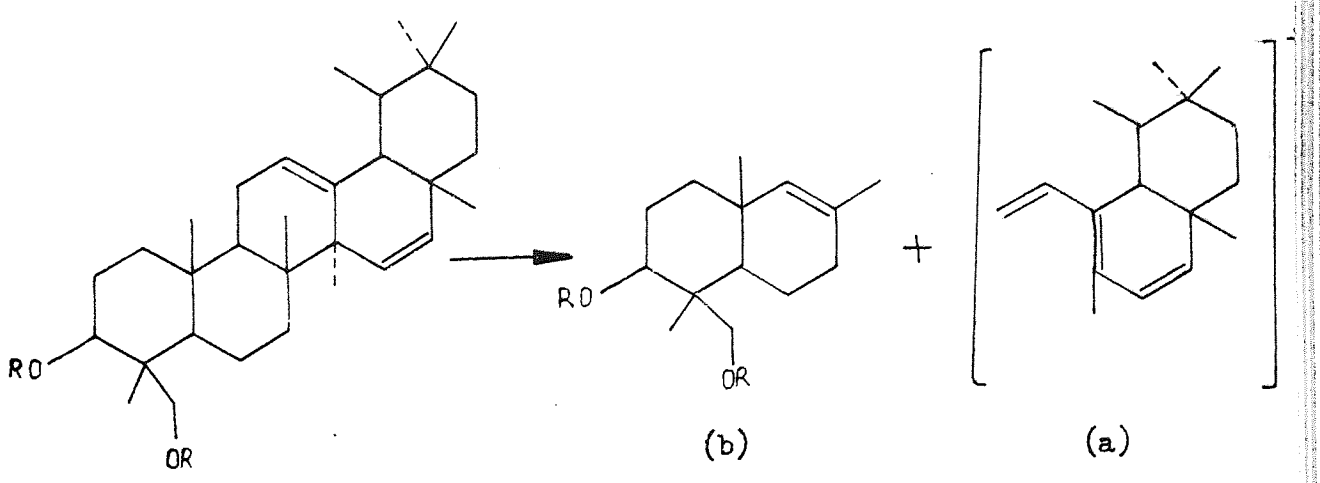
This compound was present in a small amount and it was isolated as a sapogenin (0.01% w/w, m.p. 262-265°C). It gave a green colour with

Lieberman's reagent and a yellow colour with tetranitromethane. Since both the genin and its acetyl derivative were uncrystallizable, its benzoyl derivative was made which yielded colourless crystalline product (m.p. 128-129°C).

The I.R. (KBr) spectrum of the genin was similar to that of phaseoloside-B. The I.R. of the benzoate showed the following absorption bands: 710, 980, 1000, 1030, 1070, 1090, 1110, 1170, 1265, 1315, 1450, 1600, 1720, 2850 and 2900 cm^{-1} (all strong), 680, 805, 860, 920, 940, 1380, 1555 and 1610 cm^{-1}(all medium).

The mass spectrum of the benzoate (spectrum-19) showed only one major difference between phaseoloside-B and C and that is the peak at m/e 201 was less intense than the peak at m/e 187 in case of phaseoloside-C. This indicated that phaseoloside-C was of Δ^{12}-ursene type[125].

Applying the RDA rule[125], the following fragmentation pathway was observed in the benzoyl derivative of phaseoloside-B and C.



R=COC$_6$H$_5$ (b) (a)

m/e 203

Phaseoloside-B (8%)

Phaseoloside-C (6%)

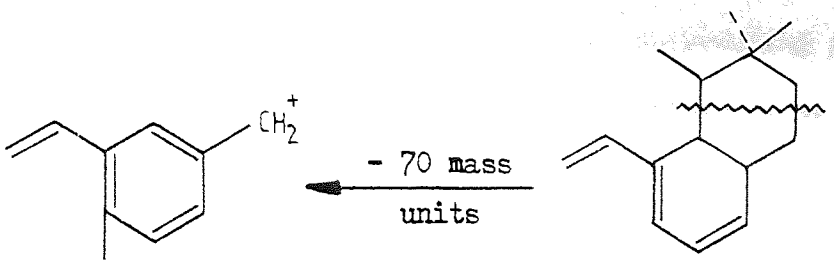
- 13 mass units

Phaseoloside-B m/e 216 (28%)

Phaseoloside-C m/e 216 (10%)

- C_{17} substituent

continued overleaf.....



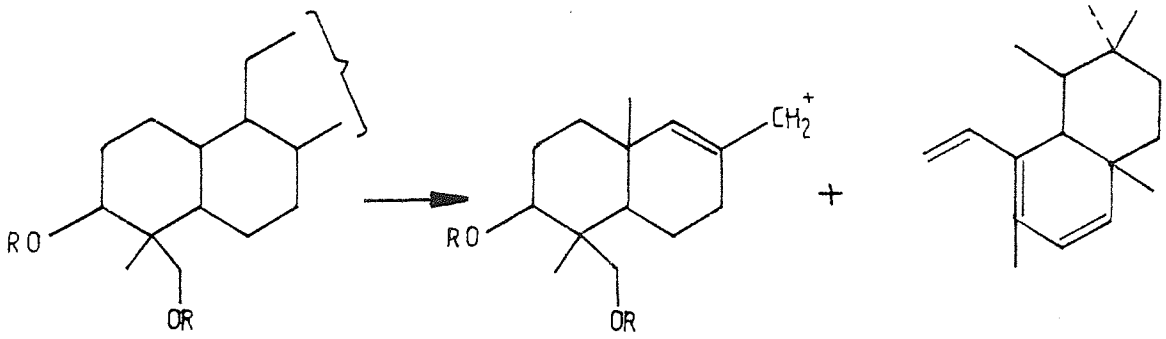
m/e 131

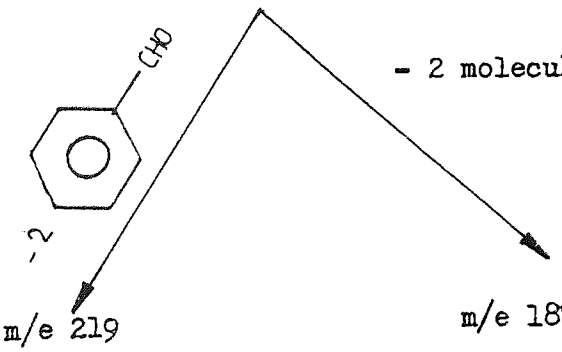
m/e 201

(c)

Phaseoloside-B (5%)
Phaseoloside-C (13%)

Phaseoloside-B (19%)
Phaseoloside-C (6%)





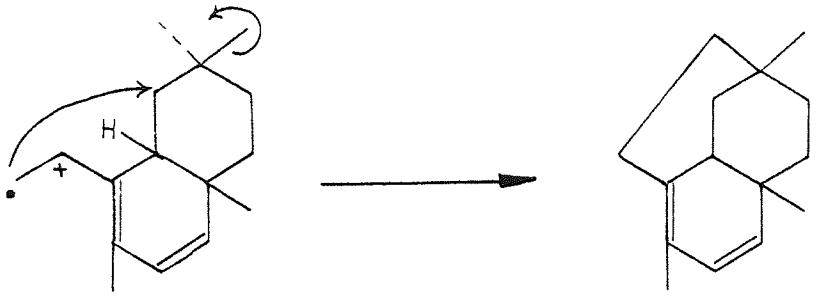
m/e 219

Phaseoloside-B (35%)
Phaseoloside-C (31%)

m/e 187

Phaseoloside-B and C (10%)

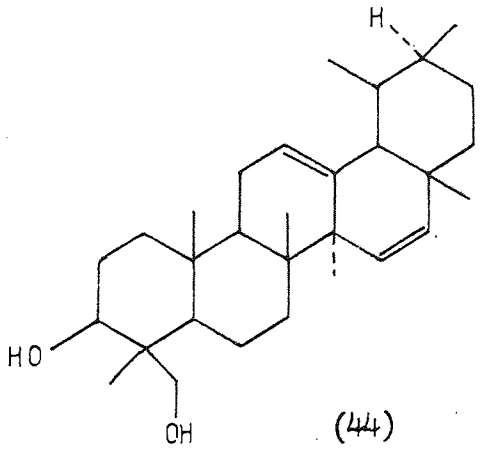
Other fragments observed were the peak at m/e 122 (49%) which corresponded to benzoic acid and the base peak at m/e 105 corresponding to $C_6H_5CO^+$. The peak at m/e 201 was the most interesting peak from a mechanistic standpoint and it resulted from a further loss of 15 mass units from RDA fragment (a).



m/e 201

The centre of unsaturation at Δ^{15} exhibits only a secondary effect on the general fragmentation pathway of Δ^{12}-ursene and Δ^{12}-oleanene and has no effect at all on the expulsion of the C-17 substituent[126].

Lack of material also prevented a conclusive elemental analysis being performed; the structure proposed is therefore tentative as Δ^{15}-3, 24-dihydroxy-Δ^{12}-ursene with the structural formula (44)



(44)

Phaseoloside-C

6. Phaseoloside-D.

This compound was isolated from fraction (III) (see the experimental

part) of the methanol extract as a white product. It was not crystallisable from aqueous methanol or aqueous ethanol (0.04% w/w, m.p. 303-305°C). The benzoate had a m.p. of 203-205°C and it gave a green colour with Lieberman's reagent and a yellow colour with tetranitromethane.

This compound was unidentified due to the small amount available.

7. Phaseosterol-C.

This compound was isolated by preparative layer chromatography as a white platelet of crystals (0.0006% w/w, m.p. 50°C). It gave an orange colour with anisaldehyde reagent on TLC and a green colour with Lieberman's reagent but no colour with tetranitromethane.

The U.V. spectrum (methanol) showed λmax. of 252 nm. The I.R. (KBr) showed very strong absorptions at 2925 and 2850 cm^{-1} corresponding to C-H stretching vibrations of CH_2 and CH_3 groups, while the band at 1722 cm^{-1}(s) corresponded to the C=O stretching vibrations. The band at 1470 cm^{-1}(s) was due to CH_2 and CH_3 bending vibrations. Other bands were weak and were as follows: 1630, 990, 980, 900, 880, 820, 795, 745, 730 and 705 cm^{-1}.

The mass spectrum (spectrum-20) (3KV, 70eV, source temperature at 200°C) showed a base peak at 83 and the molecular ion at 324 (42%). The spectrum was complicated and the identification of this compound from the above data was impossible.

8. Phaseosterol-D.

This compound was isolated by preparative layer chromatography as white platelet of crystals (0.0004% w/w, m.p. 60°C). It gave a purple colour on TLC with anisaldehyde reagent, and a green colour with Lieberman's reagent while a yellow colour was produced with tetranitromethane.

The U.V. spectrum showed λmax. at 250 nm. and at 295 nm. The I.R. spectrum (KBr) showed (in addition to the bands previously mentioned under phaseosterol-C) bands at 1710 (s) and 3350 (s,broad) cm^{-1}.

The mass spectrum (spectrum-21) (3 KV, 70 eV and source

temperature 200°C) showed the base peak at 41 with the molecular ion at m/e 304 (4%).

This compound was also unidentified due to the same reasons as mentioned for phaseosterol-C.

III-5-2- Phaseolus coccineus rhizomes.

One sapogenin (phaseoloside-E) was isolated from the rhizomes by column chromatography. The product was a white compound (0.0008% w/w, m.p. 239-241°C). It was U.V. inactive and gave a yellow colour with tetranitromethane, indicating unsaturation, and a green colour with Lieberman's reagent.

The elemental analysis was found to be C, 81.62%; H, 10.98%; O, 7.30% calculated as C, 81.8%; H, 10.90%; O, 7.27% from $C_{30}H_{48}O_2$.

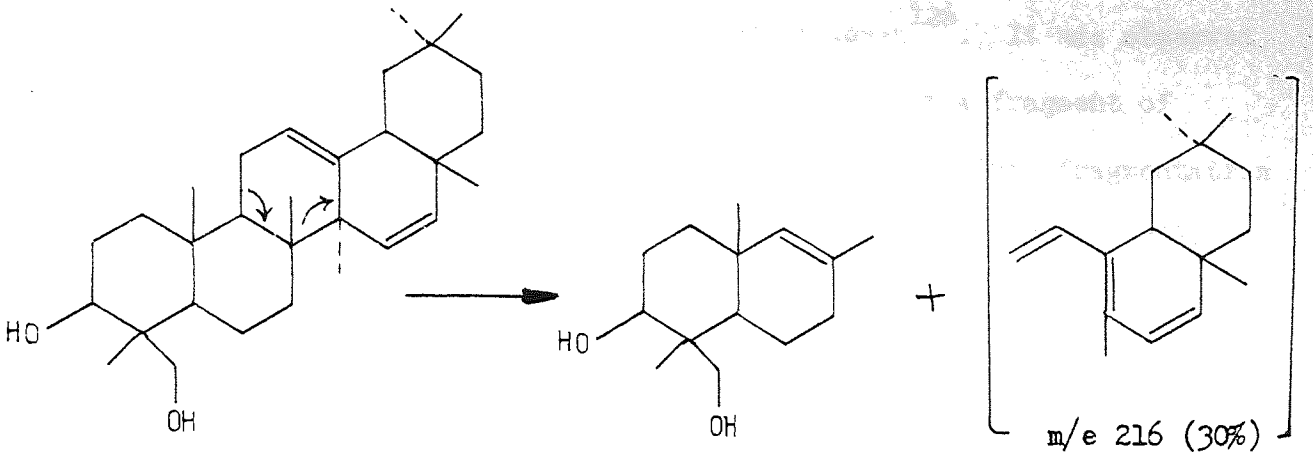
The I.R. spectrum (KBr) showed a strong band at 3350 cm^{-1} corresponding to -OH stretching vibration. Two overlapping bands at 2900 (v.s) cm^{-1} and 2825 (v.s) cm^{-1} corresponded to symmetrical and asymmetrical stretching vibrations of $-CH_2$ and $-CH_3$ groups (2926, 2853 and 2962, 2872 cm^{-1} respectively)[140]. The bands at 1460 (s) and 1450 (s.sh) were due to C-H bending vibration of $-CH_2$ and $-CH_3$ groups of an asymmetrical mode[163]. The symmetrical mode was observed at 1375 (s) cm^{-1}. This band also corresponded to the bending vibrations of the methyl groups between two six-membered rings. The intensity of this absorption band was related to the number of $-CH_3$ groups.

The band at 1040 (s) cm^{-1} was due to C-O stretching and O-H deformation vibrations.

Finally the band at 1685 cm^{-1} (m) corresponded to C=C-H stretching deformation at C_{12} and C_{15}, while bands at 815 (w) and 760 (m) cm^{-1} were due to angular deformation of C-H bonds at C_{12} and C_{15}. This was confirmed by the M.S. fragmentation pathway.

The mass spectrum of phaseoloside-E (4 KV, 70 eV, source temperature 300°C) (spectrum 22) showed that m/e 201 was more intense than that of m/e 187. This indicated that this compound was of oleanene type[125].

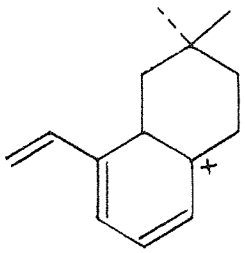
The fragmentation pattern of this compound can be described by RDA reaction (fragment a).



(b) (a)

The presence of an unsaturated centre at C_{15} exhibits only slight secondary effect on RDA reaction[126]. Its presence had no effect on the expulsion of the C-17 substituent.

Fragment (a) was subjected to further fragmentation with a loss of 15 mass units from C-17 giving rise to a fragment (c) at m/e 201 (24%).

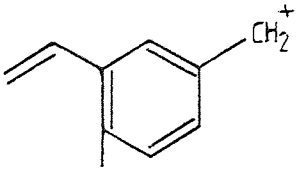


m/e 201 (24%)

(c)

Hence the relative intensities of fragment (a) and (c) offered an important indication about the attachment of a methyl group at C-17.

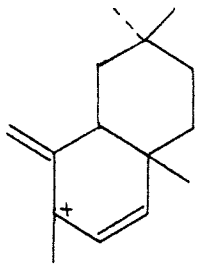
Ion (c) suffered a further loss of 70 mass units yielding fragment (d) at m/e 131.



m/e 131 (16%)

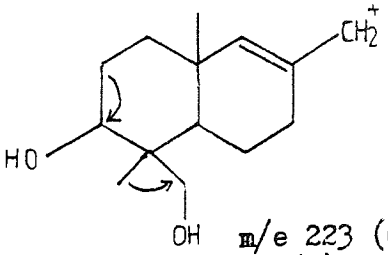
(d)

The above fragment was a highly stabilized ion and was produced probably from the partial loss of ring (e). Species (c) is usually accompanied by a less intense ion 14 mass units lower[126]. It was observed at m/e 187 (12%), while fragment (a) was accompanied by a fragment of relatively low abundance containing 13 mass units less. This fragmentation involved one hydrogen transfer yielding fragment (e) at m/e 203% (11%).

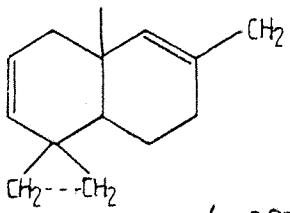


m/e 203 (11%)
(e)

Finally the fragment at m/e 223 (6%) which was composed of rings A and B, could be due to fragment (f). This fragment undergoes further loss of two molecules of water give rise to more abundant fragment (g) at m/e 187 (12%)



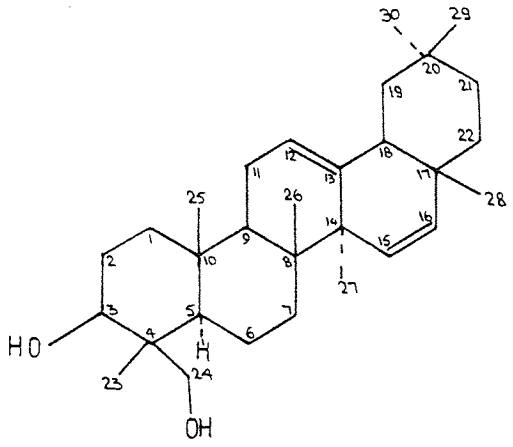
m/e 223 (6%)
(f)



m/e 187 (12%)
(g)

The end of the spectrum showed the molecular ion which was of low abundance (M^+ 440 (0.5%)) together with fragments at m/e 422 (0.5%) corresponding to $M^+ - H_2O$ and m/e 405 (0.8%) corresponding to $M^+ - (H_2O\text{-}HO)$.

From the above results and by comparing the physical properties with the published data[160,161,162], this compound was identified as soyasapogenol-C with the structural formula (45) as shown overleaf.



(45)

Soyasapogenol-C (Phaseoloside-E)

Δ^{15}-ene-3,24-dihydroxy oleanene

III-5-3- Phaseolus coccineus stems.

Three compounds were isolated in pure states from the saponin fraction and they were as follows:

1. Phaseobone-4.

This compound was isolated by column chromatography as white platelet crystals (0.00097% w/w). It was not a sapogenin because it had a low m.p. 65°C (Lit. 66°C) and also no colour was produced with both Lieberman's and tetranitromethane reagents. The U.V. was inactive, and its I.R. spectrum (KBr) showed a typical straight-chain hydrocarbon with the following bands: 720 (s), 1380 (m), 1470 (s), 2825 (v.s) and 2900 (v.s)cm^{-1} The I.R. has already been discussed under n-nonacosane.

The mass spectrum (3 KV, 70 eV, source temperature 300°C) (spectrum-23) indicated that this compound was a straight-chain hydrocarbon. The maximum abundance was observed at m/e 57 and the molecular ion at m/e 422 was of low abundance. The details of the spectrum has been previously described under n-nonacosane.

The elemental analysis was found to be C, 84.57; H, 15.43; and was calculated as C, 85.3; H, 14.69 from $C_{30}H_{62}$.

From the above data this compound was identified as n-triacontane with a structural formula (46) as shown below.

$$CH_3(CH_2)_{28}CH_3$$

(46)

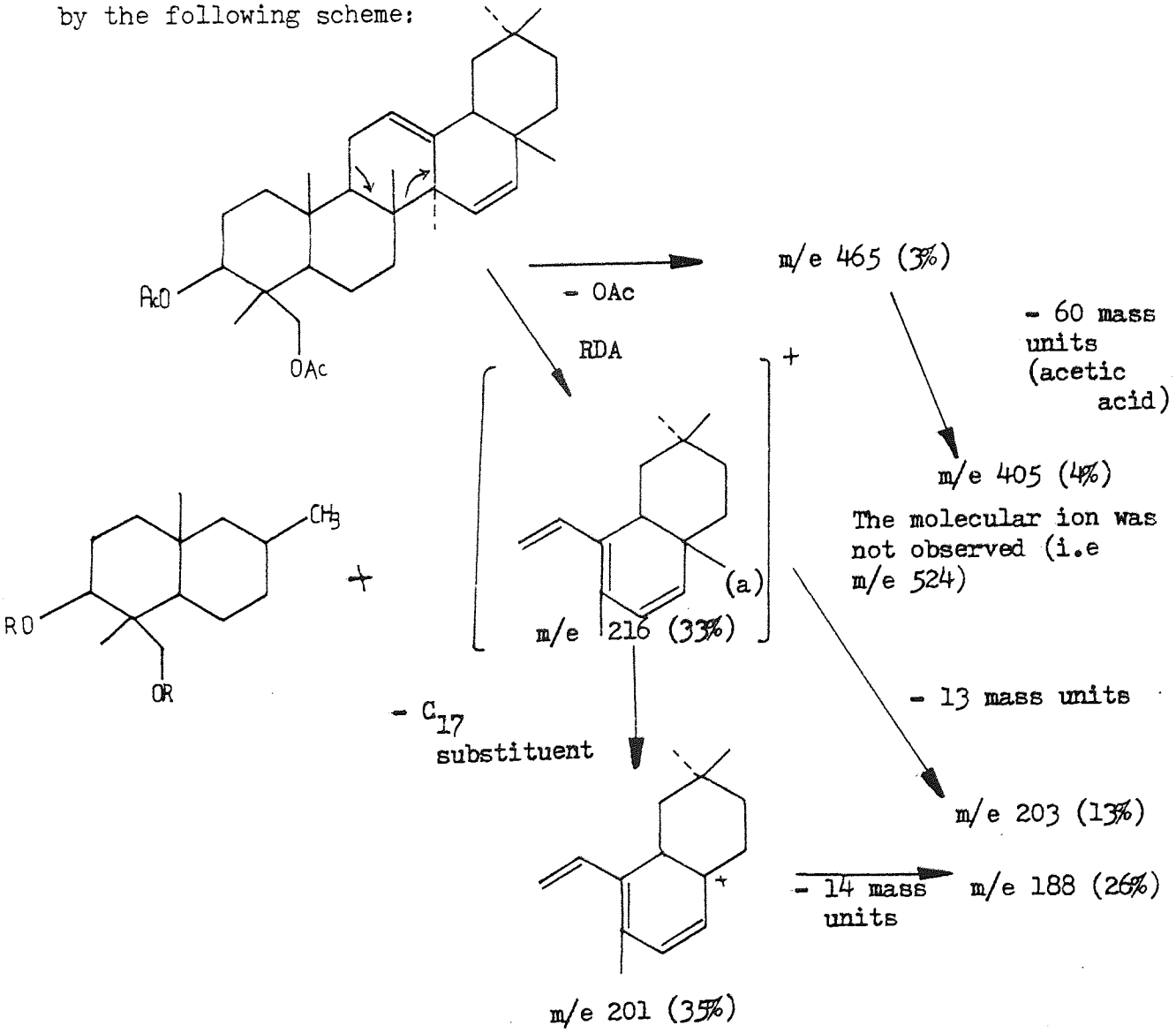
2. Phaseoloside-F.

This compound was isolated as a white product (0.0019% w/w, m.p. 239-241°C, acetate 200-203°C). It gave a yellow colour with tetranitromethane and a green colour with Lieberman's reagent. The U.V. was inactive but the I.R. and mass spectrum of this genin were similar

to those of phaseoloside-E obtained from the rhizomes.

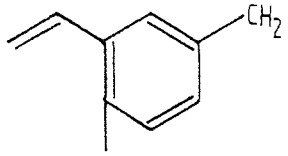
The acetate derivative of this compound was studied by I.R. and M.S. The I.R. (KBr) showed strong bands at 1760 and 1730 cm^{-1} corresponding to the carbonyl groups of the acetate at C_{24} and C_3 respectively. The strong band at 1240 cm^{-1} corresponded to the acetate groups while the band corresponding to -OH stretching vibration had disappeared indicating that complete acetylation had taken place.

The mass spectrum of the diacetate (spectrum-24) showed the same fragmentation pathway as discussed earlier under phaseoloside-E of the rhizomes indicating that the acetate groups at C_3 and C_{24} did not effect the general fragmentation pathway. This pathway maybe explained by the following scheme:

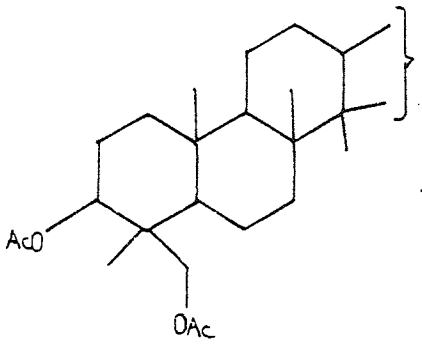


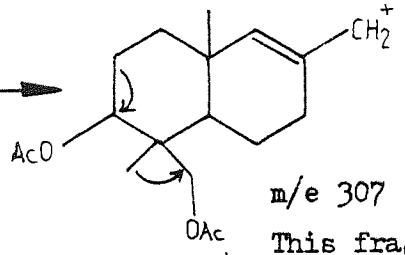
continued overleaf.....

- 70 mass units partial cleavage of ring E



m/e 131 (14%)

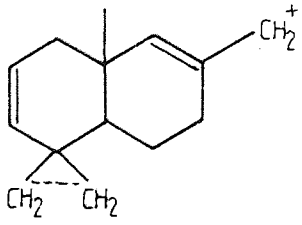




m/e 307

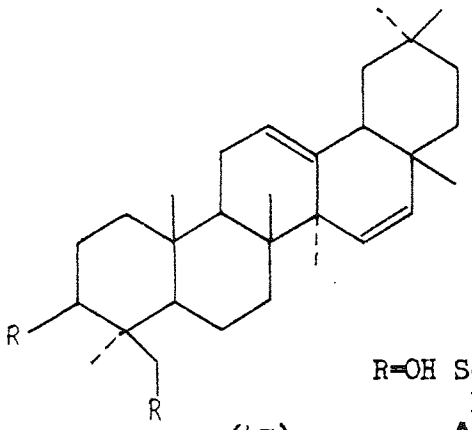
This fragment was not observed in the spectrum

- 2 molecules of acetic acid



m/e 187 (26%)

From the above and by comparing the results with those of published data[160,161,162], phaseoloside-F of the stems was identified as soyasapogenol-C (Lit. m.p. 238-239°C; diacetate 202-203°C), with a structural formula (47)



(47)

R=OH Soyasapogenol-C
Δ^{15}-ene-3,24-dihydroxy oleanene

R=OAc Soyasapogenol-C diacetate
Δ^{15}-ene-3,24-diacetyl oleanene

3. Phaseoloside-G.

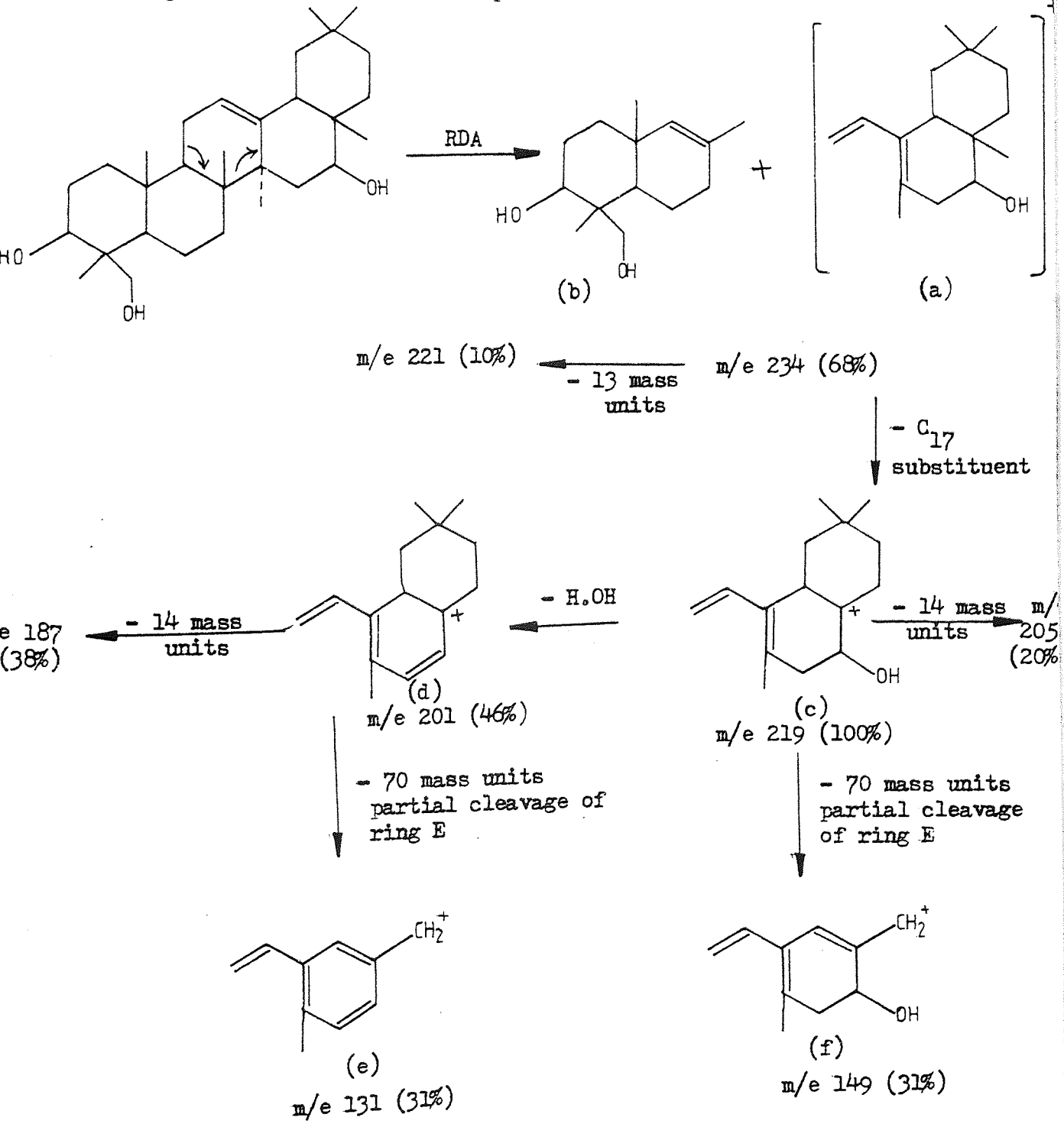
This compound was isolated as a white product (0.00058% w/w, m.p. 259-260°C (Lit. 260-261°C)). The U.V. was inactive but the substance gave a green colour with Lieberman's reagent and a yellow colour with tetranitromethane reagent.

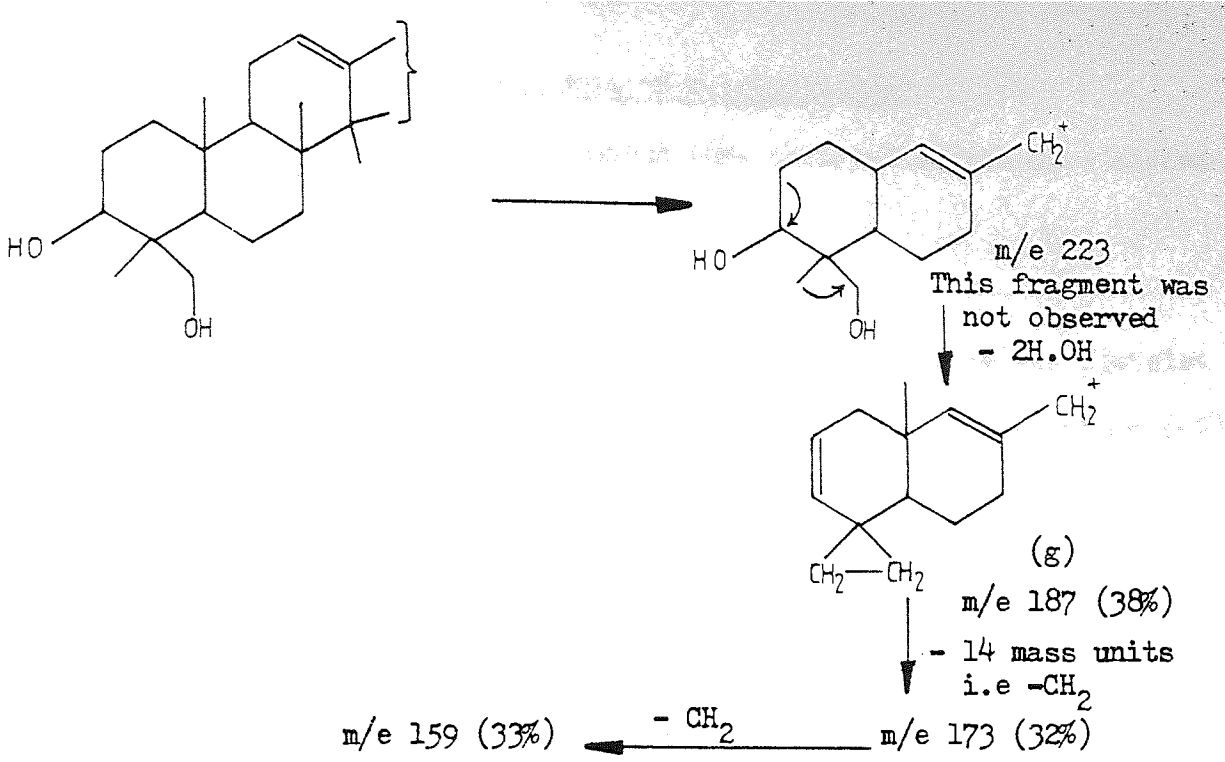
The I.R. spectrum (KBr) showed a similar spectrum to that of phaseoloside-F. It showed bands at 3400 (br.s), 2925 (v.s), 2850 (v.s), 1465 (s), 1450 (s), 1385 (s), 1140 (m), 1098 (m), 1080 (m), 1040 (v.s), 1030 (v.s), 1000 (s), 980 (m), 920 (m), 865 (m) and 1630 (m) cm^{-1}. The discussion of the spectrum was similar to that of phaseoloside-F in which the most important bands were at 3400 cm^{-1} which corresponded to -OH stretching vibration and the bands at 1040, 1030 and 1000 cm^{-1} which corresponded to C-O stretching and O-H deformation vibrations.

The bands at 2925 and 2850 cm^{-1} were due to symmetrical and asymmetrical stretching vibrations of $-CH_2$ and $-CH_3$, while the bands at 1465 cm^{-1} and 1450 cm^{-1} were due to $-CH_2$ and $-CH_3$ bending vibrations.

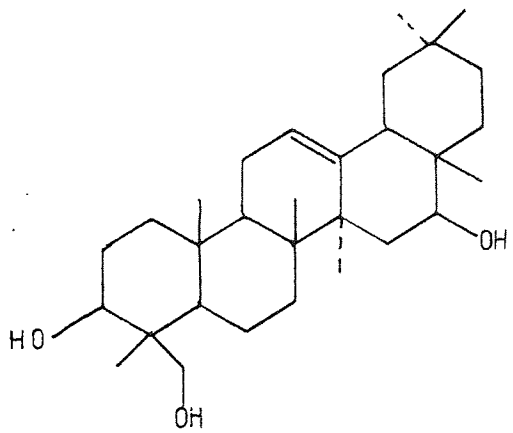
Finally, the bands at 1630 cm^{-1} corresponded to C=C-H stretching deformation at C_{12} while the band at 865 cm^{-1} could be due to the angular deformation of C-H bond at Δ^{12}. This was confirmed by the mass spectrum fragmentation pathways.

The mass spectrum of phaseoloside-G (spectrum-25) (4 KV, 70 eV, source temperature 300°C , probe temperature 150°C), showed that this compound was of oleanene type due to the fact that m/e 219 (c) was more intense than m/e 187 (g)[125]. The fragmentation pathway agreed with the general fragmentation pathway of pentacyclic triterpenoids and the following scheme was devised to explain it.





Finally the molecular ion was observed at M^+ 458 which was of low abundance (1%) and M-H_2O at m/e 440 (3%). From the above data and by comparing the results with the published data[160,161,162], phaseoloside-G was identified as soyasapogenol-B with the structural formula (48) as shown below.



(48)

Soyasapogenol-B

3,16,24 trihydroxy-Δ^{12}-oleanene

III-5-4- Methanol extract components of G.triacanthos .

Two genins and one hydrocarbon were isolated in the pure states from the seeds and pods:

1. Triacanane-A.

This compound was isolated from the pods as colourless platelet crystals (0.034% w/w, m.p. 65°C). It was identified as n-triacontane (49), by its m.p., I.R.(KBr) and by M.S. data. The discussion related to the spectra has been described previously under triacane-B.

$$CH_3\ (CH_2)_{28}\ CH_3$$

(49)

2. Triacanthoside-A.

This compound was isolated from the saponin fractions of both seeds and pods (0.10% w/w and 0.17% w/w respectively, of the original weight). It was identified as oleanolic acid (50), by spectroscopic and physical data obtained by these methods of analysis. The results were compared with the published data[165] on this compound.

The m.p. was found to be 308-309°C (Lit. 308-310°C) while the m.p. of it's acetate was 230°C. The U.V. was inactive and a purple colour was produced with Lieberman's reagent and a yellow colour with tetranitrometh--ane reagent.

Elemental analysis was found to give C; 74.94%, H; 10.78%, O; 13.50% $C_{30}H_{48}O_3\ 1\frac{1}{2}\ H_2O$ requires C, 74.53%; H, 10.55%; O, 13.25%.

The Infra-red spectrum (KBr).

Cyclic terpenoid substances are an ideal class of compound for I.R. study, since they exist in very large numbers based on relatively few types of molecular skeleton. They differ mainly in the nature and position of a few fairly non-polar substituents[166]. Most of the I.R. work on steroids and terpenoids has been concerned with the identification and

location of hydroxyl groups, carbonyl groups and ethylenic double bonds. In triacanthoside-A, the band corresponding to the hydroxyl group (stretching vibration) was observed as a strong broad band at 3450 cm^{-1}. The broadening of this absorption band was due to intramolecular hydrogen bonding, because of the high concentration and due to the solid state[166]. The methyl and methylene bending vibrations were observed at 1440 (s) and 1450 (s) cm^{-1}. The stretching vibrations of these groups were observed at 2900 (v.s) and 2825 (v.s) cm^{-1}. A band at 1380 cm^{-1} was due to the bending vibration of the angular methyl groups between two six-membered rings. The absorption associated with the stretching motion of the C=C bond which should have been observed at 1650 cm^{-1} was overlapped by the strong absorption of the carbonyl group of COOH at 1687 cm^{-1}, while the angular deformation of the carbon-hydrogen bond in -C=C-H was observed at 850 (w) and 940 (w) cm^{-1}.

The very intense peak at 1687 cm^{-1} corresponded to acid carbonyl group at C_{17}[123]. The spectrum of the acetyl derivative showed strong absorption bands at 1720 and 1240 cm^{-1} corresponding to the acetate group with consequent loss of the band at 3450 cm^{-1}. The other bands observed in the spectrum of triacanthoside-A were 990 (s), 1000 (s), 1020 (s), 1040 (s), 1360 (s), 1380 (s) and 2600 (m) cm^{-1}.

The mass spectra of triacanthoside-A and its acetate (spectra 26 and 27).

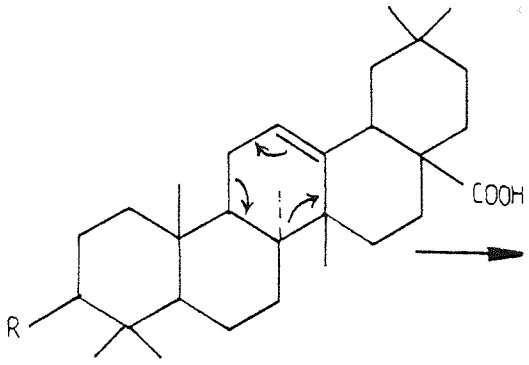
The spectrum of triacanthoside-A showed a molecular ion at m/e 456 (3%). From the fragmentation pathways of steroids and pentacyclic terpenoids[158], this compound did not have any side chain substituents, since no loss from the molecular ion was observed. The peaks at m/e 248 (100%) and m/e 203 (41%) indicated that no substitution was present in rings C, D and E[158].

Fragmentation patterns in the mass spectra of Δ^{12}- unsaturated oleanenes and their derivatives can be described by a RDA reaction in ring C, the charge remaining in the diene portion. The correctness of this

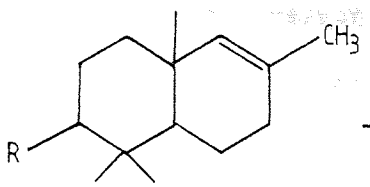
assignment is shown by the fact that substitution in ring A and B does not effect the mass of fragment (a) that is the diene portion, while alteration in rings D and E results in appropriate mass shifts.

In triacanthoside-A, the above mechanism yielded fragment (a) at m/e 248 (100%), while its acetate showed the same fragment but it was of low abundance (23%). This indicated that addition of an acetyl group to ring A did not shift the fragment (a)[125]. Ion (a) was subjected to a further loss of 45 mass unit (-COOH) yielding fragment (c) at m/e 203. This fragment was also observed in the acetyl derivative. The fragment at m/e 248 (100% in triacanthoside-A and 23% in its acetyl derivative) was more intense than fragment (e) and hence the compound is oleanene and not ursene type[125]. Species (c) suffer further decomposition by the loss of 70 mass units giving rise to a fragment (d) at m/e 133 (35%). This cleavage was probably due to the partial loss of ring E yielding the highly stabilized ion (d). Another important fragment was the fragment which contained rings A and B and its formation involved one hydrogen transfer yielding fragment (e). The loss of a molecule of water (from oleanolic acid) or acetic acid (from the acetate) yielded species (f) at m/e 189. The loss of C-17 substituent was observed at fragment m/e 203 corresponding to the loss of -COOH at C_{17}. This ion was not abundant in the acetate m/e 452 (2%).

The following scheme summarises the fragmentation pathway of triacanthoside-A and its acetate:



R a=OH
b=OAc



(b)

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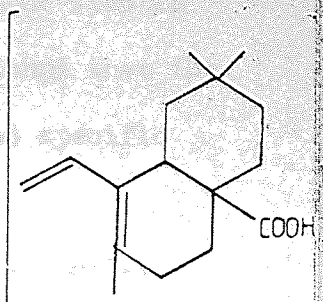
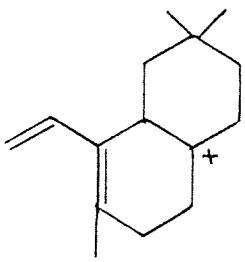
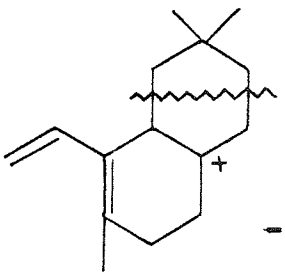


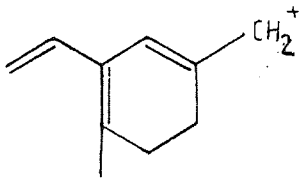
(a)
m/e a=248 (100%)
b=248 (23%)



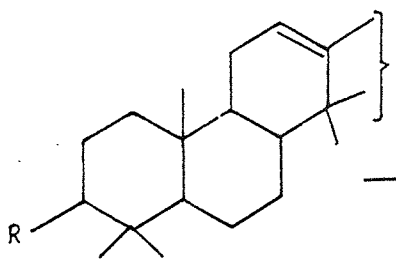
(c) m/e a=203 (41%)
b=203 (51%)

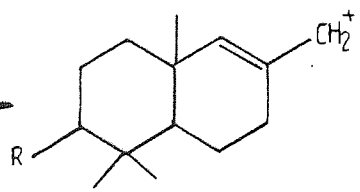


- 70 mass units



(d)
m/e a=133 (35%)
b=133 (45%)





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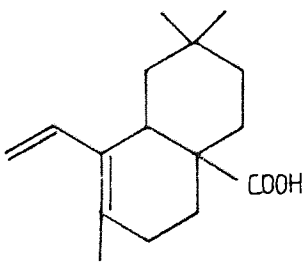
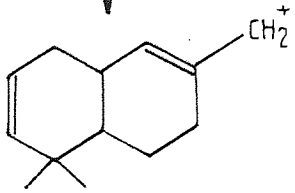


(e)
m/e a=207 (38%)
b=249 (8%)



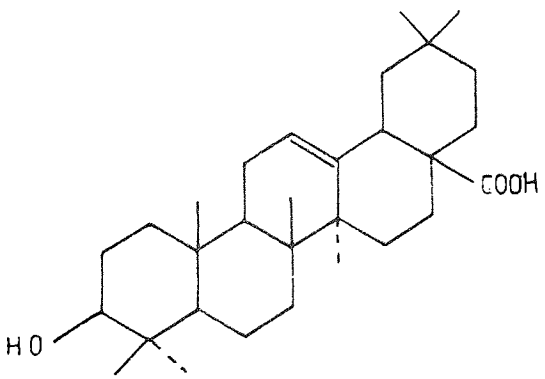
(f)
m/e a=189 (93%)
b=189 (48%)

The NMR spectrum.

Shamma[129] investigated the nuclear magnetic resonance spectra of Δ^{12}-oleanene and Δ^{12}-ursene derivatives at 40 MHz and found that the spectra revealed overlapping signals in the methyl region and specific assignments to most of the methyl groups could not be made.

The spectra of Δ^{12}-oleanenes and ursenes are quite complex even at 100 MHz[125] owing to secondary pertubation effects exhibited by the two secondary methyl groups.

The spectrum of triacanthoside-A (220 MHz) showed the following well defined absorption peaks: $\delta^{CDCl_3}_{TMS}$ 0.775, 0.80, 0.90, 0.925, 0.95, 1.0, 1.12 and 1.22 corresponding to methyl groups of C_{27}, C_{30}, C_{29}, C_{26}, C_{25}, C_{23} and C_{24} respectively[157]. The proton of the trisubstituted double bond was observed at δ4.75 being the least shielded in the molecule. From the above data, triacanthoside-A was identified as oleanolic acid with a structural formula (50)



(50)

3β-hydroxy, Δ^{12}-oleanene-28-oic acid

3. Triacanthoside-B.

This compound was isolated from the saponin fractions of both the seeds and pods as colourless needle crystals (0.10% w/w and 0.15% w/w respectively). It was identified as echinocystic acid (51) by its physical properties, the spectroscopic data obtained, and by the colour reaction on the compound itself and on TLC. Its m.p. was found to be 299-301°C

(Lit. 296-299°C)[167] and its diacetate was 265-266°C (Lit. 265-267°C)[167]. A yellow colour was produced with tetranitromethane and a purple colour with Lieberman's reagent. Elemental analysis gave C, 72.24%; H, 9.33%; O, 17.82% and was calculated $C_{30}H_{48}O_4$ $1\frac{1}{2}$ H_2O as C, 72.14%; H, 9.61%; and O, 17.63%.

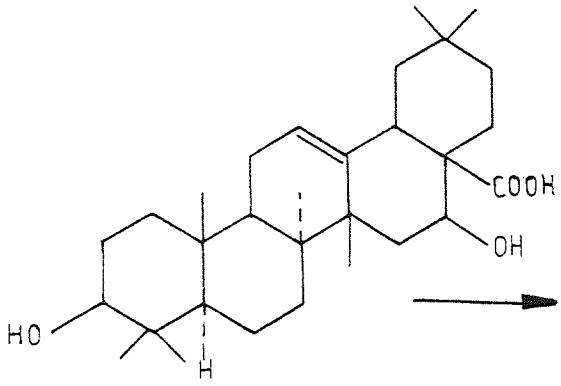
The infra-red spectrum (KBr) when compared with that of triacanthoside-A did not show much difference except that the band corresponding to the carbonyl group was observed at 1670 (s) cm^{-1}. The spectrum obtained from the acetyl derivative showed distinct differences for the carbonyl absorption. In addition to the carbonyl group shift to 1680 cm^{-1}, it showed two other strong absorptions at 1710 and 1720 cm^{-1} corresponding to two acetyl groups (1240 (s) and 1260 (s) cm^{-1}). This indicated that two hydroxyl groups were acetylated.

Table (100) shows the difference in m.p. of echinocystic acid obtained from different plant sources:

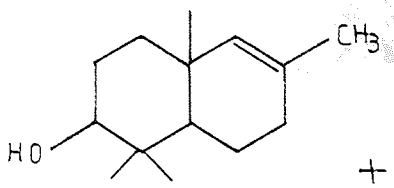
Compound	G.triacanthos	E.fabacea[168]	A.lebbek[169]	A.odoratissima[167]
Echinocystic	299-301°C	305-312°C	291-293°C	296-299°C
Diacetate	265-266°C	272-275°C	248-249°C	265-276°C

Table 100 The melting points of echinocystic acid and its diacetate obtained from different plant sources.

The mass spectra of echinocystic acid and its diacetate (spectra 28 and 29), showed the same fragmentation pathway of pentacyclic triterpenoid. The pathways are summarised below:



(51)



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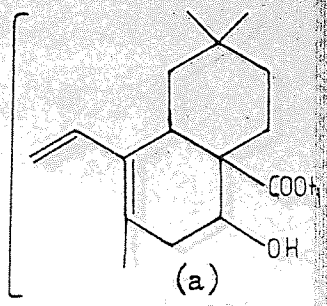
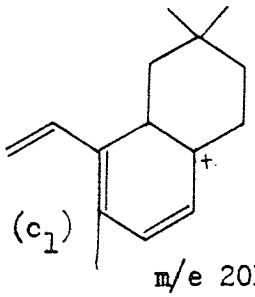


(a)

m/e 264 (8%)

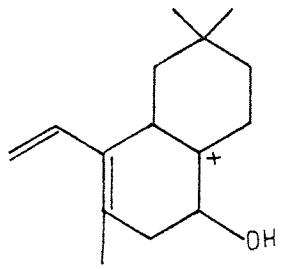
3β-16α-dihydroxy, Δ^{12}-oleanene-28-oic acid



(c_1)

m/e 201 (18%)

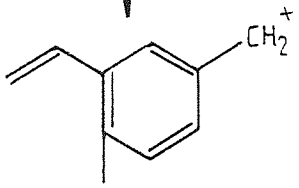
- H_2O



(c)

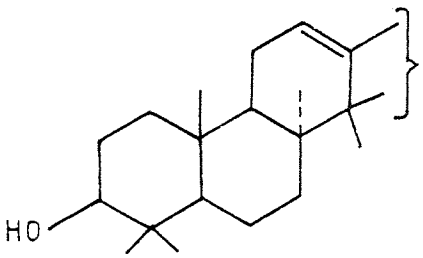
m/e 219 (5%)

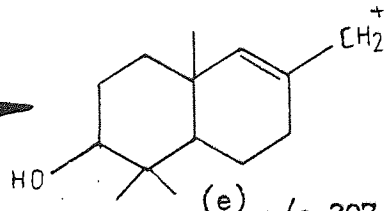
- 70 mass units



(d)

m/e 131 (20%)





(e) m/e 207 (7%)

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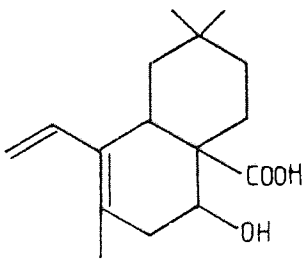
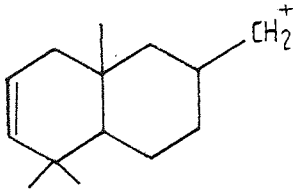
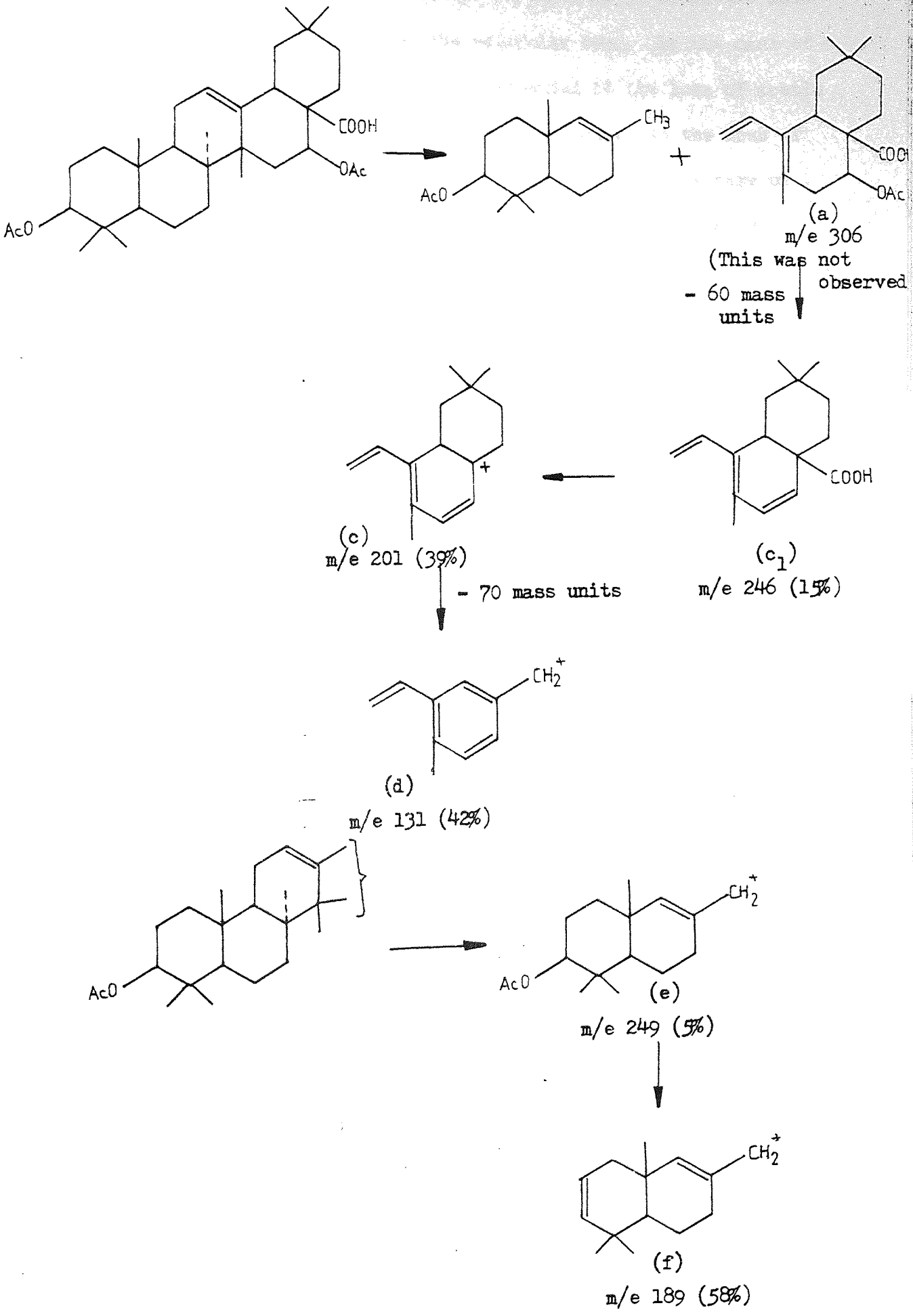


- H_2O



(f)

m/e 189 (16%)


COOH
OAc
AcO
CH3
+
COOH
OAc
(a)
m/e 306
(This was not observed
- 60 mass units
COOH
(c)
m/e 201 (39%)
(c1)
m/e 246 (15%)
- 70 mass units
CH2+
(d)
m/e 131 (42%)
AcO
CH2+
(e)
m/e 249 (5%)
CH2+
(f)
m/e 189 (58%)

The mass spectra of the acetyl derivatives of oleanolic acid and echinocystic acid did not show the molecular ions. In the case of oleanolic acid acetate, m/e 438 (1%) corresponded to the loss of acetic acid from the parent ion and m/e 452 (2%) corresponded to the loss of a molecule of formic acid from the parent ion, while, in the case of echinosystic acid diacetate it showed m/e 452 (5%) which probably corresponded to the loss of acetyl and formyl groups from the parent ion.

The NMR spectrum of echinocystic acid showed similar results to that of oleanolic acid.

III-5-5- Methanol extract components of A.graveolens.

The following compounds were isolated from the methanol extract (the glycoside fraction):

1. Graveobone-C.

This compound was isolated as platelet crystals (0.0025% w/w, m.p. 60°C). Its U.V. was inactive and no colour was obtained with tetranitromethane and Lieberman's reagents. The elemental analysis was found to be C, 85.3%; H, 14.60% calculated from $C_{27}H_{56}$ as C, 85.20%; H, 14.73%.

The I.R. spectrum (KBr) showed a typical straight chain hydrocarbon (720(s), 730(s), 1380(m), 1465(s), 2850(v.s) and 2925(v.s) cm^{-1}).

The mass spectrum (spectrum 30), also showed a typical fragmentation pattern of saturated straight chain hydrocarbon. The mass and I.R. spectra were discussed previously under compound triacanane-B.

From the above data graveobone-C was identified as n-heptacosane with the structural formula (52).

$$CH_3(CH_2)_{25}CH_3$$

(52)

2. Graveosterol-A_2.

It was isolated as acicular crystals (0.0040% w/w, m.p. 155°C) and was identified possibly as the aglycone (indosterol) by comparing its m.p., I.R., M.S. and TLC properties with that isolated from the petroleum extract.

The sugar moiety of indosterol glycoside was not identified since it was hydrolysed together with the other glycosides.

3. Graveobioside-A.

It was isolated as a pale yellow powder (0.003% w/w, m.p. 349-351°C) (dec.). Comparison of U.V. and I.R. spectra with those of graveobioside-B

showed the compounds to be similar. This compound was identified as the aglycone of graveobioside-B in accordance with its m.p., I.R. and U.V. data (i.e. Apigenine m.p. 348-350°C)[170]. The details of identification have been described under Graveobioside-B.

4. Graveobioside mixture.

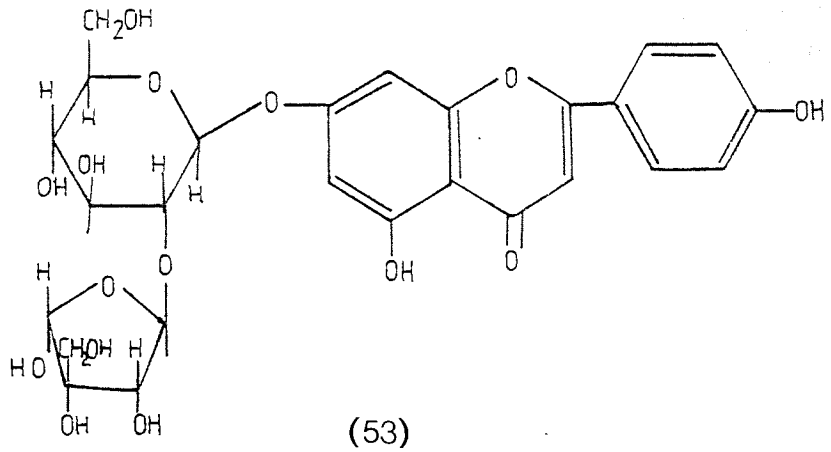
This mixture of compounds was isolated in trace amounts and TLC examination revealed six compounds. They were visible yellow and gave a purple colour with ferric chloride (system 18). Nine peaks were detected by GLC when their TMS derivatives were used (system 7). These compounds could probably be flavonoids present in small amounts which made their isolation in the pure states impossible.

These nine compounds could be the aglycones of those isolated together with graveobioside-B from the ether of precipitation (fraction G_2).

5. Graveobioside-B.

This compound was isolated as yellow needle crystals (0.03% w/w, m.p. 195°C). It was identified as apigenin-7-apioglucoside,(53), found C, 52.74%; H, 5.63% ; O, 40.85%, calculated from $C_{26}H_{29}O_{15}\ \frac{1}{2}\ H_2O$, C, 52.88%; H, 4.91%; and O, 40.67%.

The I.R. spectrum (KBr) showed a strong absorption band at 1660 cm^{-1} corresponding to the carbonyl group at C_4 and its low frequency was due to the chelation effect[171]. The band at 3400 cm^{-1} (v.s and broad) corresponding to the OH stretching vibration. Bands at 1605(s), 1570(s), 1560(s) and 1500(s) cm^{-1} were due to C-H stretching vibration of the phenyl skeleton. Bands at 1245(s), 1180(s), 1120(m), 1070(s) and 1030(m) cm^{-1} corresponded to -CH or =C-O-C in plane aromatic. Bands at 840(s) cm^{-1} and 825(m) cm^{-1} were due to (C-O)[172]. Other bands observed were 1300(m), 1370(s), 1385(m), 1420(w), 1450(s), 2850(m) and 2925 cm^{-1}.



(53)

Ultra-Violet spectra.

A solution of 1 mg./100 ml. methanol showed two characteristic absorption bands:

Band I 335 nm.

Band II 268 nm.

These absorption bands were examined under the effect of the following reagents:

1. Sodium methoxide.

   Band I shifted to 392 nm. with an increase in intensity.

   Band II unchanged.

2. Sodium hydroxide.

   Band I shifted to 380 nm. with decreased intensity.

   Band II shifted to 270 nm. and decreased in intensity.

3. Sodium acetate.

   No bathcchromic shift was observed but a decrease in intensity observed.

4. Sodium acetate/Boric acid.

   As under 3.

5. Aluminium chloride.

   Band I a. 386 nm.

   b. 351 nm.

Band II a. 300 nm.

b. 276 nm.

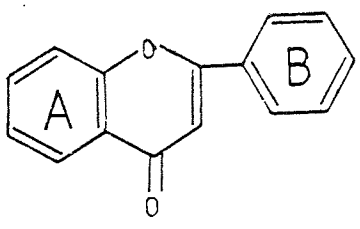
6. Aluminium chloride.

As in 5.

The spectra obtained are shown in Fig. 43.

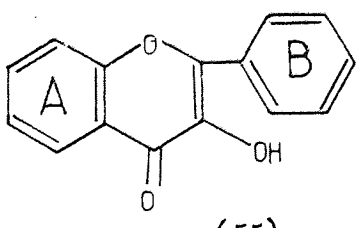
Spectrophotometric measurements are now employed in the characterization of flavonoid compounds. The value of such spectra is increased by the use of reagents such as aluminium chloride, sodium methoxide, sodium acetate (fused) and boric acid. Sodium acetate produces a shift in the maxima in accordance with the location of the various functional groups in the flavonoid molecules[109]. By the use of these spectra alone it is now possible to determine the structure of some flavonoid compounds.

Flavones (54) and flavonols (55) generally exhibit high intensity absorption in the 320-338 nm. region (Band I) and the 240-270 nm. region (Band II). The position and the intensity of the λmax. of each of these bands varies with the relative resonance contributions of the benzoyl (56), cinnamoyl (57) and pyrone (58) ring groupings to the total resonance of the flavone molecule.



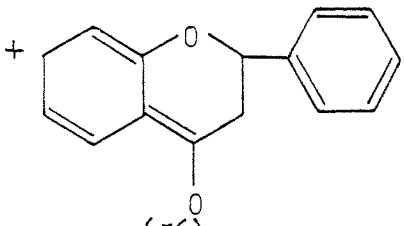
(54)

Flavone



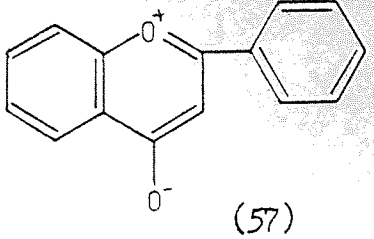
(55)

Flavonol



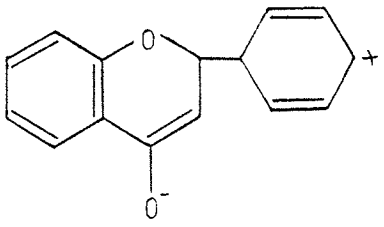
(56)

Benzoyl group



(57)

Pyrone ring group



(58)

Cinnamoyl group

Although these groupings undoubtedly interact, the spectra of substituted flavones and flavonols in neutral and alkaline solutions suggests that Band I is associated chiefly with absorption in the cinnamoyl grouping and Band II with absorption in the benzoyl grouping[109]. Thus, the introduction of electron-donating groups such as hydroxyl into the ring B increase its relative resonance contribution and consequently produce a considerable bathochromic shift of Band I. Introduction of hydroxyl or methoxyl groups into the ring A, on the other hand, primarily increases the resonance contribution of this ring and tend to increase the wavelength and intensity of maximum absorption of Band II. For example, the spectra of apigenin results in bathochromic shift of Band I to 336 nm. and a considerable increase in its intensity relative to that of Band II.

The introduction of an hydroxyl or methoxyl group into the unconjugated 3-position increases the resonance contribution of an adjacent 4-hydroxyl substituent. For poly-hydroxyflavones a consistent bathochromic shift of 10-15 nm. of Band I is thereby obtained; apigenin λmax. 336 (59), luteolin λmax. 350 (60).

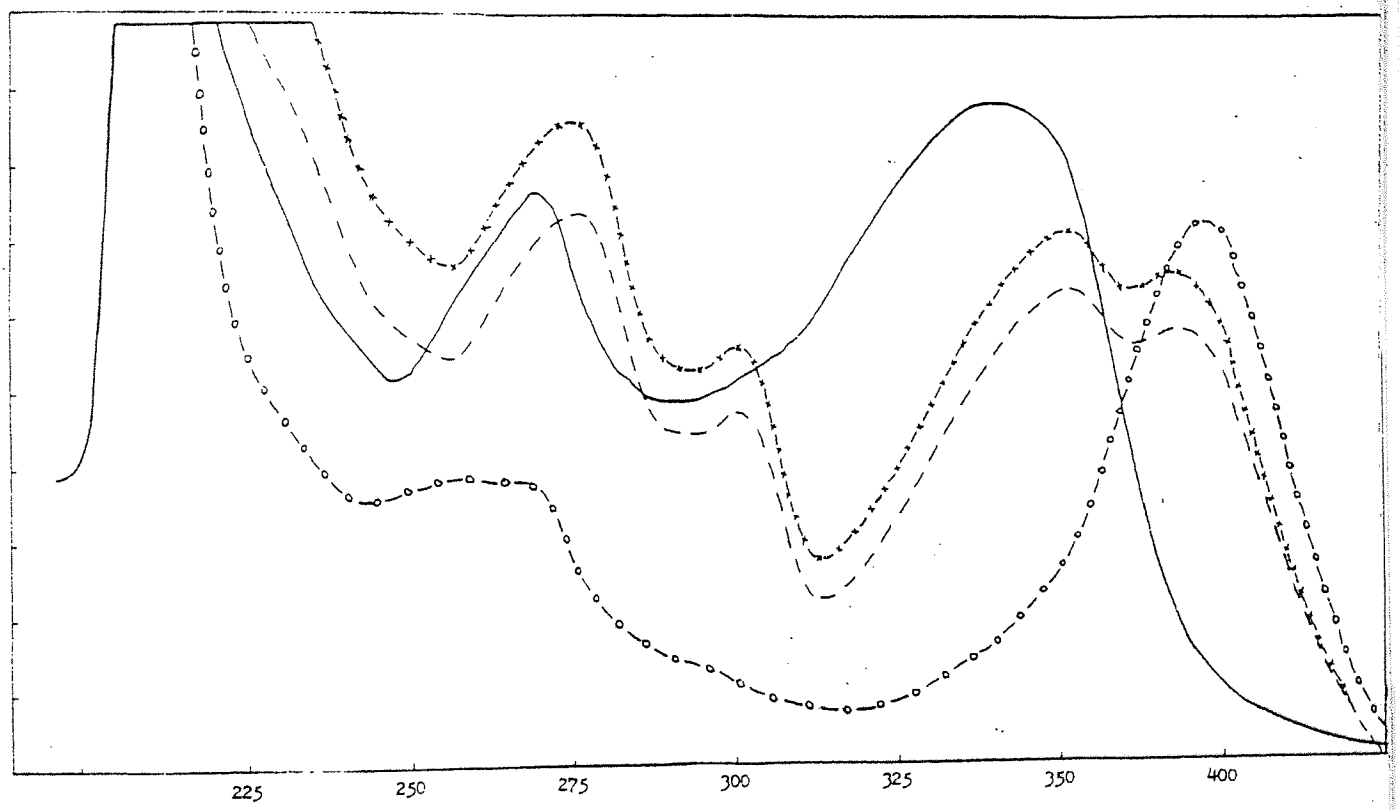


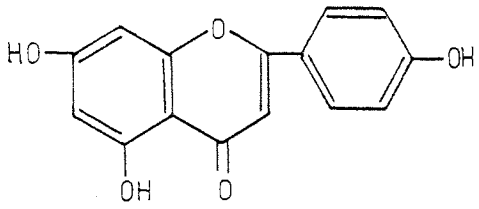
Fig. 43 The ultra-violet spectra of graveobioside-B.

x - x - x Aluminium chloride.

--------- Aluminium chloride/Hydrochloric acid.

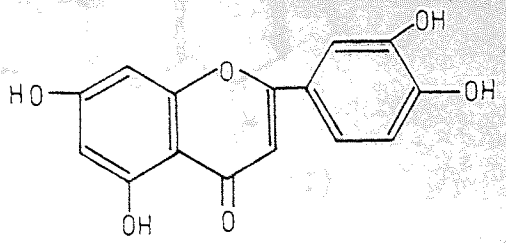
- o - o - Sodium methoxide.

——————— Graveobioside-A in methanol without any reagent.



(59)

Apigenin

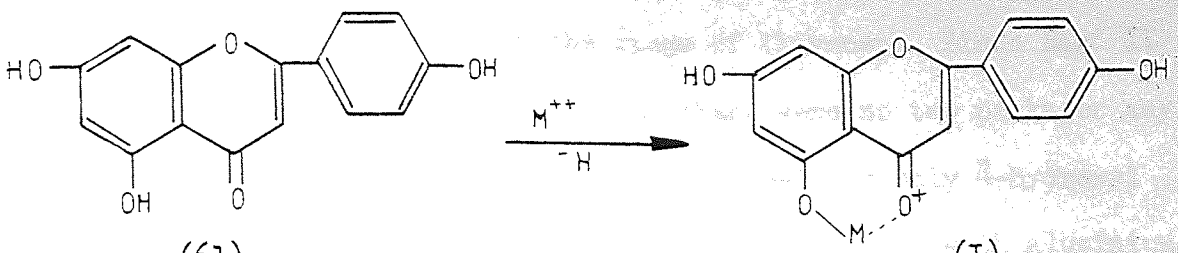


(60)

Luteolin

Band II of flavones and flavonols which contain only a 4-substituent in the ring B, e.g. apigenin and kaempferol, has a single well-defined peak. For flavones and flavonols which have hydroxyl or methoxyl substituents in both the 3- and 4-position e.g. luteolin and quercetin, Band II shows two definite peaks or one peak and a pronounced inflection. This observation is particularly useful for identification purposes. When three substituents are present in the ring B e.g. tricin and myricetin, Band II has only a single peak.

Flavones and flavonols which do not contain a free 5- or 3-hydrxyl group do not form complexes on addition of few drops of aluminium chloride solution. 5-hydroxyflavones and 5-hydroxyflavonols in which the 3-hydroxyl group is protected form stable yellow complexes (I). This complex formation results in considerable bathochromic shifts of Bands I and II. Each of these bands in the spectrum of aluminium chloride complex characteristically exhibit two distinct peaks or inflections. The bathochromic shift of the flavone Band I to the complex Band Ia is 20-45 nm.[109,173].



(61) (I)

Ia	386
Ib	351
IIa	300
IIb	276

Location of a 7-hydroxyl group by sodium acetate.

Sodium acetate is sufficiently basic to ionize hydroxyls located at positions 7,3, and 4 of the flavone nucleus. Hydroxyls located elsewhere are unaffected. Ionization of 3- and 4-hydroxyls produces bathochromic shifts of Band I but does not affect the position of Band II. Since Band II is associated mainly with absorption in the ring A, however, ionization of a 7-hydroxyl group results in a pronounced bathochromic shift of this band.

In the presence of sodium acetate, boric acid chelates with phenolic compounds containing o-dihydroxyl groups. The λmax. of Band I of flavones and flavonols which contain an o-dihydroxyl group e.g. luteolin therefore undergoes a bathochromic shift of 15-30 nm. on the addition of a mixture of boric acid and sodium acetate. The spectrum of compounds which do not contain o-dihydroxyl group are not appreciably affected.

All hydroxyl groups on the flavonoid nucleus are ionized to some extent by the strong base, sodium methoxide. Hence, for most hydroxylated flavonoids, shifts to longer wavelength are observed in both bands. The presence of a 4-hydroxyl group is evidenced by a Band I bathochromic of 57 nm. without a decrease in intensity.

From the above explanations we can conclude that compound graveobioside-B is a flavone and not flavonol due to the absorption at

λ max. 268 nm. and 335 nm. in the range of flavones. Since Band II showed a single well-defined peak and there were no two peaks or any inflection, this suggests that graveobioside-B has only 4-hydroxyl substituent in ring B[109]. The formation of a complex with aluminium chloride indicated the presence of a 5-hydroxyl group. This complex results in considerable bathochromic shifts of both bands. Each of these bands characteristically exhibits two distinct peaks[173](62)

Ia = 386

Ib = 351

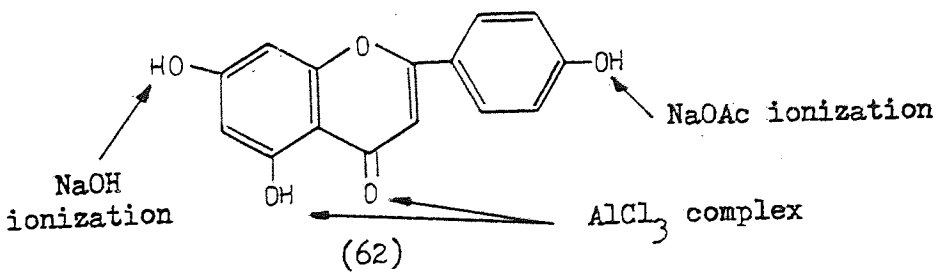
IIa = 300

IIb = 276

The presence of 4-hydroxyl group was evidenced by the bathochromic shift of Band I by 340 (5 nm.) due to ionization of 4-hydroxyl group, while no effect on 7-hydroxyl group was observed due to the presence of 5-hydroxyl group[109].

There are no o-dihydroxyl groups due to the fact that sodium acetate/boric acid had no effect on Band I which should have shown bathochromic shift if there were any o-dihydroxyl groups in rings A or B by 12-30 nm. The bathochromic shift of Band I by 58 nm. produced by the addition of the strong base sodium methoxide was an evidence of 4-hydroxyl group (sodium hydroxide causes a bathochromic shift of 44 nm.).

From the above results, graveobioside-B was proposed to have the following structure (53). The U.V., I.R., TLC data, elemental analysis together with the comparative study with the published data[68,72] also pointed out for graveobioside-B to have the structure proposed before (53).



(62)

6. Graveose-A.

This compound was isolated from the methanol extract together with inorganic nitrate, oxalate and phosphate compounds (3.3% w/w) from which it was purified by double crystallization from aqueous methanol leaving colourless needle crystals (1.0% w/w, m.p. 166°C and the m.p. of its acetate 125-126°C (published data 126°C))[177]. The elemental analysis was found to be C, 39.6; H, 7.68; calculated from $C_6H_{14}O_6$ as C, 39.5; H, 7.68 and O, 52.74. The elemantal analysis of its acetate was found to be C, 48.29; H, 5.86; O, 42.30; calculated from $C_{18}H_{26}O_{12}$ H_2O as C, 47.78; H, 5.75; and O, 42.47.

This compound was identified as D-mannitol (64) from its physical properties by comparing them with those of published data[178], as well as from its I.R., M.S. and NMR spectra.

The I.R.(KBr) showed a broad absorption band at 3400 cm^{-1} which corresponded to the OH stretching vibration while the absorption bands at 1080 (s), 1030 (s) were due to C-O stretching vibration. A band at 2900 (m) cm^{-1} corresponded to C-H stretching vibration. The other bands observed of medium intensities were 1382, 1320, 1260, 1195, 925 and 895 cm^{-1}.

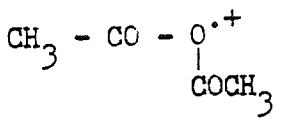
The I.R. spectrum of the acetate derivative showed the presence of a strong absorption band at 1730 cm^{-1}, corresponding to the carbonyl of the acetate groups. In addition to the strong bands at 1242 and 1220 cm^{-1} and the disappearance of the band corresponding to the OH groups was also noticed.

The mass spectrum.

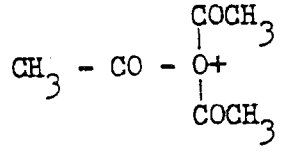
Although mass spectra have been obtained for simple sugars[179], the spectrum of graveose-A did not show the molecular ion and the spectrum did not give any information on the structure of the compound. It showed a maximum abundance at m/e 40, in addition the following m/e were observed: m/e 41 (8%), 42 (7%), 43 (31%), 44 (45%), 45 (15%), 56 (8%), 57 (11%),

60 (10%), 61 (19%), 73 (23%), 74 (7%), 91 (3%), 103 (9%), 105 (7%) and 122 (5%). This could be due to the volatility and thermal stability of free sugar which renders this procedure impractical[119], therefore derivatives are normally employed. Acetates, methyl esters and trimethylsilyl ethers are among those which are commonly used[180,181].

The spectrum of the hexacetate (Spectrum 31) showed three important ions which were derived from the acetate groups. That at m/e 43, the most abundant fragment in the whole spectrum, was the base peak in all published spectra of polyacetate carbohydrates[128], and corresponded to the acetylium ion, CH_3CO^+. The other two ions were found at m/e 103 (48%) and at m/e 145 (43%) corresponding to the di- and tri-acetyl groups respectively.



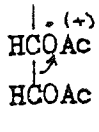
m/e 103



m/e 145

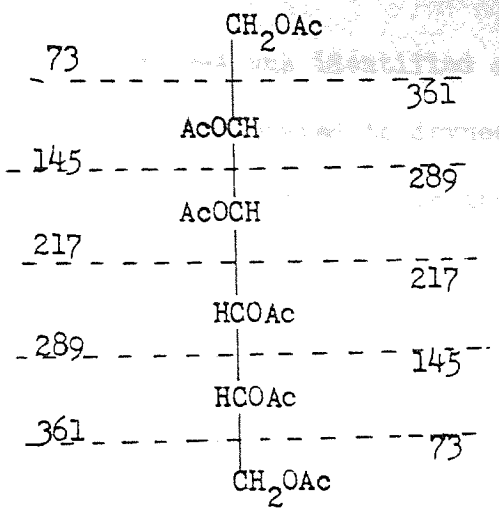
Carbohydrate derivatives in general give weak or no molecular ions on electron impact mass spectrometry and systematic studies on the M.S. of alditol acetates have revealed a simple mode of fragmentation for this class of compounds[182]. Mannitol hexacetate like most alditol acetates did not give a molecular ion, but $(M-CH_3COO^{\cdot})^+$ was found in a low abundance at m/e 375 (8%).

Primary ions were formed from mannitol hexacetate by elimination of an acetoxyl group or by cleavage of the chain as shown in (a).



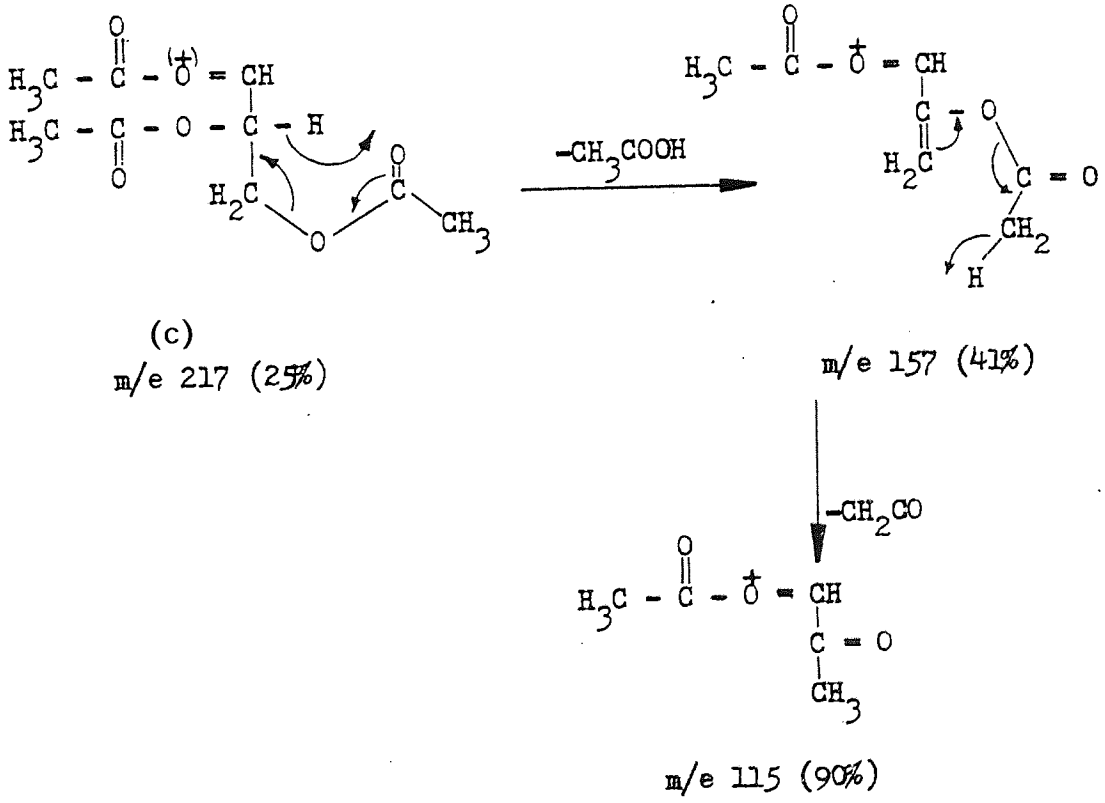
(a)

In this way, m/e 375 $(M-CH_3COO^{\cdot})^+$ and five other primary fragments are formed as shown:



(b)

The primary fragments are degraded by serial elimination of acetic acid (60 mass units) or ketone (42 mass units) as shown in (c);



(c)
m/e 217 (25%)

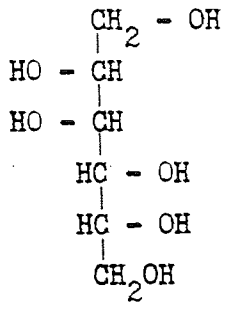
m/e 157 (41%)

m/e 115 (90%)

The NMR spectrum of mannitol in D_2O showed the following absorptions as doublets at $\delta \overset{D_2O}{TMS}$ 3.7, 3.75, 3.9, 3.95 corresponding to one proton of C_2, C_3, C_4 and C_5, while the singlet at $\delta \overset{D_2O}{TMS}$ 3.8 corresponded to four protons of C_1 and C_6.

From above graveose-A was identified as D-mannitol (64).

N.B. The mother liquor was evaporated to dryness leaving 1.2 gm. of a white precipitate which was examined for its inorganic content and found to contain oxalate, phosphate and nitrate.



(64)

III-6- The aqueous extract components.

III-6-1- Honey-locust pods.

The aqueous extract of the pods showed the presence of saponins, hydrocarbons and galactomannan.

The genin fraction was separated on TLC using System 25. Nine genins giving rise to spots 2-10 were located. Spot No.1 was not a genin as it did not give any colour with anisaldehyde reagent (Table 48).

The compound corresponding to spot No.1 was the only compound present in a considerable amount, while the genins were present in trace quantities. Hence this compound (Triacanane-D) was isolated from the mixture by column chromatography and it yielded white platelet crystals (0.065% w/w; m.p. 64-66°C). It did not give any colour with Lieberman's and tetranitromethane reagents and was U.V. inactive.

Triacanane-D was identified as n-triacontane (46). Its elemental analysis was found to be C, 84.47%; H, 15.27 calculated as C, 85.3%; H, 14.69% from $C_{30}H_{60}$. The I.R. spectrum (KBr) was identical to that of compound triaconane-B and the mass spectrum showed a weak molecular ion at m/e 422 (3%) and the typical fragmentations of a straight-chain hydrocarbon which were similar to those of triacanane-A with the exception that it exceeded by only 14 mass units i.e. $-CH_2$ group.

The I.R. and M.S. have been previously discussed in detail under triacanane-B.

From the above data this compound was identified as triacontane with structural formula (46).

The white precipitate (XII) was found to contain D-galactose and D-mannose by comparing their colours and Rf values with those of authentic samples using systems 28 and 29.

This residue has already been isolated by many workers[174,175,176] using the seeds of honey-locust and has been named galactomannose (yield

in seeds 15-20% w/w, and in pods 10% w/w). The molar ratios of D-galactose and D-mannose were analysed to be 1:3.2-3.5[176].

III-6-2- Celery stems.

Fraction XIII showed six spots on TLC (System 30). Only one of them was isolated in pure form (Rf 0.87), while the others (spots 2,3,4,5, and 6) were present in small amounts.

The above compound graveobone-D was isolated by column-chromatography as white platelet crystals (0.002% w/w, m.p. 67-68°C). It was U.V. inactive and produced no colour with Lieberman's and tetranitromethane reagents. The I.R.(KBr) showed strong bands at 720, 1380, 1465, 2825 and 2900 cm^{-1}. Its M.S. (3 KV, 70 eV, source temperature 300°C) showed the same fragmentation pathway as mentioned previously under compound triacanane-B. This compound was identified as n-Undecosane having the structural formula (63).

$$CH_3(CH_2)_{29}CH_3$$

(63)

Graveobone-D

The other fractions obtained from the aqueous extract were graveose-B and fraction XIV.

Fraction XIV was analysed on TLC using System 42. D-glucose and D-fructose were located, their Rf values and colours were compared with those of authentic samples.

Graveose-B.

2.2% of this compound was isolated from the aqueous extract. From its m.p., optical rotation, TLC, I.R., NMR, and M.S. data, this compound was found to identical to graveose-A i.e. D-mannitol.

Becker (1968)[178] isolated 0.1% of D-mannitol from PASCAL celery petioles.

N.B. Slow crystallization yielded needle crystals while rapid crystallization yielded amorphous product.

III-7- Identification of the sugar components of saponins isolated from the methanol and aqueous extracts.

III-7-1- Sugars from runner beans.

The sugar components of the saponins isolated from fraction II of the roots were identified by PC (systems 23 and 24) and by GLC using their silyl ether derivatives (system 3) as D-glucose, D-fructose, L(+) arabinose and D-galacturonic acid, by comparing their Rt and Rf values with those of authentic samples.

Under the same conditions sugars from fraction III were identified as: D-fructose, D-glucose and D-galacturonic acid. Sugars of phaseoloside-A and phaseoloside-B were identified as D-glucose (2 molecules the area under the peak by GLC), D-fructose and L(+)arabinose. The sugars from the genins isolated from the rhizomes were identified as D-glucose, L(+)arabinose and L(+)rhamnose.

III-7-2- Sugars from Honey-locust.

The sugars were identified by PC and TLC (systems 28 and 29), by comparing their Rf values with those of authentic samples.

The sugars of the genins isolated from methanol extract (fraction I) of the pods were identified as D-glucose, L(+)rhamnose and L(+)arabinose, while the sugars of the same genins isolated from fraction F_2 were identified as D-glucose, L(+)rhamnose, L(+)arabinose and an unidentified sugar.

The sugars of the saponin components of the seeds were identified as follows: D-glucose, L(+)arabinose, D-galacturonic acid and D-glucuronic acid.

Finally the sugar contents of the aqueous extract of pods were as follows: D-glucose, L(+)arabinose and D-galacturonic acid.

From the above results, it can be concluded that even though the aglycones isolated from the same and from different organs and fractions were identical, the sugar moiety was different in their glycosides. This

could explain the difference in their physical properties such as the solubilities.

IV- Reagents.

1. Iodoplatinate spray reagent[84].

Platinic chloride (0.25 gm.) and potassium iodide (5 gm.) were dissolved in sufficient water to produce 100 ml.

This reagent was used as a general location reagent for nitrogenous bases on TLC and PC.

2. Acidified Iodoplatinate spray reagent.

2 ml. of hydrochloric acid was added to iodoplatinate reagent (100 ml inorder to increase the sensitivity of the reagent and to locate even small amounts of compounds.

3. Modified Dragendorff's reagent[184].

a. Bismuth subnitrate (850 mg.) was dissolved in water (10 ml.) and glacial acetic acid (10 ml.).

b. Potassium iodide (8 gm.) was dissolved in water (20 ml.).

The solutions a and b were mixed and glacial acetic acid (20 ml.), water (100 ml.) was added to the mother liquor (10 ml.).

The reagent must be used fresh. This reagent is sensitive to small amounts of alkaloidal compounds on TLC as orange spots.

4. Dansyl chloride spray reagent[86].

0.05% of dansyl chloride (1-dimethylaminonaphthalene-5-sulphonyl chloride).

This reagent proved to be more sensitive than any of the alkaloid reagent and it was used to visualise the non-phenolic alkaloids. It gave fluorescent yellow spots under U.V. light (254nm).

5. Phosphomolybdic acid in reagent[183].

10% phosphomolybdic acid in methanol. This reagent gives a blue colour with strongly reducing compounds poly-oxygenated steroids, and most terpenoids. The blue background can be overcome by exposing the chromatogram to ammonia vapour.

6. Aniline hydrogen phthalate[74].

Aniline (9.2 ml.) and phthalic acid (16 gm.) were dissolved in butanol (490 ml.), ether (490 ml.) and water (20 ml.).

7. Ninhydrin reagent[73].

0.1% w/v in acetone. This reagent was used to locate free amino sugars on TLC and PC as reddish-purple colour.

8. Anisaldehyde reagent[183].

Mix 1 ml. of conc. H_2SO_4 and 100 ml. acetic acid and add 0.1 ml. anisaldehyde immediately before use.

In all cases the plates were sprayed with 10 ml. of the reagent and dried at 105°C in an oven for 5 minutes.

In paper chromatography, the paper was dipped in the reagent for a few seconds, dried between two filter papers and then dried in the oven for 5 minutes at 105°C.

V. BIOLOGICAL ACTIVITIES.

Since relatively large amounts of graveose-A and graveosterol-A were isolated from the celery stems, they were tested for the presence of any biological activity using the anti-inflammatory test.

The antiquity of rheumatic diseases has been the subject of some debate. The ability of extracts of the Meadow saffron Colchicum autumnale to reduce joint pain was described in 550 A.D. by Alexander of Tralles, a Greek physician. This herb contains the alkaloid colchicine and until quite recently was a drug of choice in the relief of gout. Colchicine, which is a plant hormone, is thought to impair microtubule integrity and so reduce enzyme release. It inhibits mitosis and thus prevents phagocytosis of urate crystals by leucocytes, thus preventing perpetuation of the inflammatory process[185].

The bark of the cinchona species of tree, known as Jesuit's or Peruvian powder and indigenous to South America, gained a considerable reputation for its apparent ability to cure malaria. Its principle active constituents are the alkaloids quinine and cinchonine. By 1639 its fame reached Spain and in 1677 it first appeared in the London pharmacopoeia under the name Cortex peruanus[1]. This drug was also used in the treatment of rheumatism but its scarcity in England during the Napoleonic Wars led to substitution by other drugs.

The use of the bark of the white willow and other species of Salix had long been known in folk lore for the relief of fever and pain. Consequently, the scarcity of cinchona led to the use in 1798 of the bark of Salix Alba by William White, apothecary to the Mineral Water Hospital in Bath. This drug contains Salicin, a glucoside, which is metabolised to salicylic acid. Salycilic acid was used by Hoffman in 1898 for the historic synthesis of acetylsalicylic acid, whose efficacy and safety are

still recognised in the treatment of rheumatic diseases.

In 1949, Hench et al.[186] described the dramatic anti-inflammatory properties of cortisone, one of the naturally occurring corticosteroids, and this was followed soon afterwards by reports of the ability of phenylbutazone to reduce the pain and swelling associated with rheumatoid arthritis[187]. These drugs and their derivatives are still widely used in clinical medicine.

Today a wide range of totally dissimilar chemical compounds are used in the treatment of rheumatic diseases. These include Indomethacin[188], organic gold salts[189], the fenematis[190], Ibuprofen[191] and Penicillamine[192].

Assessment of the Carrageenin-Induced inflammation.

An acute inflammation of short duration was produced by the method of Winter, Risley and Nuss (1962)[193]. Rat hind paws were injected subcutaneously with 0.05 ml. of a suspension of carrageenin (extract of the Irish Moss, Chondrus crispus). Initial experiments were carried out[194] to establish the optimum concentration of carrageenin and for all further experiments a 2% suspension was used. The optimum time for measurement of the inflammation was found to be between three and six hours.

Inflammation was assessed by immersing the paws up to the hair-line in a mercury plethysmograph connected to a pressure transducer (Devices). The mercury filled manometer consisted of a U-tube with one wide arm and one narrow arm. The wide arm was approximately 2.5 cm. in diameter, open to the atmosphere and was used to immerse the paws. In the narrow arm, the mercury was in contact with water, which filled the pressure transducer and connecting tube. Air bubbles were removed and pressure adjustments made using a water-filled syringe connected by a three way tap.

The recorder was calibrated before and checked during each experiment using a ground-glass syringe plunger immersed in the mercury bath.

Results:

1. Experiment 1.

Group of four rats each were used.

G_1 = Control

G_2 = Phenylbutazone 50 mg./ml.

G_3 = Graveose-A 200 mg./ml.

G_4 = Graveosterol-A 50 mg./Kg.

	Mean Volume 3 hrs.	6 hrs.
G_1	0.2083 ∓ 0.0436	0.3000 ∓ 0.0408
G_2	0.2083 ∓ 0.0239	0.2417 ∓ 0.0271
		t= 1.1903
		not significant
G_3	0.1917 ∓ 0.0455	0.2083 ∓ 0.0473
		t= 1.4680
		P<0.1
		not significant
G_4	0.1917 ∓ 0.0154	0.2000 ∓ 0.0258
		t= 2.0716
		P<0.05

Carrageenin concentration was 2% suspension of crude carrageenin (this contained both the active and the inactive carrageenin).

2. Experiment 2.

Group of four rats each were used.

G_1 = Control

G_2 = Indomethacin 50 mg./ml.

G_3 = Graveosterol-A 50 mg./ml.

G_4 = Graveosterol-A 100 mg./ml.

	Mean Volume 3 hrs.		6 hrs.	
G_1	1.0143		0.8500	
	∓ 0.0472		∓ 0.0535	
G_2	0.3786	t=7.0854	0.4571	t=4.5204
	∓ 0.0763	P<0.0005	∓ 0.0685	P<0.0005
G_3	0.8286	t=3.1060	0.7571	t=1.1752
	∓ 0.0596	P<0.005	∓ 0.0582	not significant
G_4	0.8786	t=16691	0.7500	t=1.2330
		P<0.10	∓ 0.0607	not significant

In this experiment, pure carrageenin which was more potent was used (marine colloid).

Conclusions:

From Experiment 1, it was found that indosterol (graveosterol-A) was significantly anti-inflammatory while graveose-A (D-mannitol) was inactive. In the second experiment, as a pure and more irritant form of carrageenin was used, 100 mg./ml. of indosterol was also showed anti-inflammatory activity (P<0.10).

VI. CONCLUSIONS.

VI-1- Chemotaxonomy.

Although there is no agreement regarding the treatment of the major divisions of the Leguminoseae, several authors [7,195] have attempted to discuss the taxonomy of this family in terms of either the yield of galactomannan derived from the endosperm or of the D-mannose:D-galactose ratio of the galactomannan. Since galactomannans have been identified in 70 species of this family[196], the data available is insufficient to permit the drawing of meaningful conclusions.

Chemotaxonomy should be employed since it gives the impression that there is an approach to taxonomy in which chemical data are more important than are other classes. We must look forward to the time when chemical data, not only for Leguminoseae and Umbelliferea, but for all plant kingdom families, can be more regularly used. Obviously, much research is required to work out the distribution of various kinds of compounds throughout the plant kingdom in respect of comparative plant anatomy[197] and such work is being performed[7].

The fatty acids, free sterols, hydrocarbons, sugars, flavonoids and saponins isolated and identified from runner bean, honey-locust and celery provide additional data to aid chemotaxonomy in the Leguminoseae and Umbelliferae.

VI-2- The general schemes of extraction and separation.

Under Scheme-1, roots and rhizomes (runner bean) were studied separately and the results obtained showed little difference between these two organs concerning the quantities, numbers and types of compounds separated. In this scheme alkaloids and basic compounds were separated by TLC.

Fraction B revealed four spots on TLC (System 3). All the spots gave a yellow fluorescence with dansyl chloride under U.V. light (254 nm.).

These four compounds could be non-phenolic alkaloids. Since dansyl chloride (1-dimethylamino-naphthalene-5-sulphonyl chloride) proved to be more sensitive than any of the other alkaloid reagents tested, it was used routinely to visualize the non-phenolic alkaloids[86].

Fraction C revealed four different non-phenolic alkaloids on the same system.

Fraction D showed seven compounds but none of them gave the fluorescent yellow colour with dansyl chloride.

All the above fractions gave very small amounts of yellowish brown residues, and isolation of the above components in pure states plus comparative studies on TLC with authentic samples were impossible.

Under scheme 2 a general method of extraction for non-volatile organic compounds was used. From the scheme it was noticed that the major constituents occurred in fraction A (5.5% w/w). The components of (A), were isolated and studied in detail under scheme 3, while the minor constituents were studied briefly by TLC after they were separated into neutral and acidic fraction (D) and basic Fraction (E).

Fractions C,D, and E were present as yellow, oily residues (0.009-0.01% w/w).

Fraction C which contained the water insoluble and chloroform soluble compounds revealed four spots on TLC (System 5). Two of them showed a yellowish white colour with iodoplatinate reagent which could be primary or secondary amine[73,84]. Two other primary or secondary amines were located with the same system in fraction D, which revealed five spots. After fractionation of (D) to acidic and neutral fractions (D_1, D_2 and D_3), no difference was observed between these fractions which indicated that these fractions were identical.

The basic fraction (E) revealed six spots (System 5) of which only four showed the alkaloidal colour reaction. The basic fraction E_1

revealed three spots and the one with an Rf value of 0.96 (System 5) gave an orange colour with the modified Dragendorff's reagent and a purple colour with the iodoplatinate reagent. Hence, it could be tertiary amine type. The amphoteric fraction E_2 showed four spots. Three of them being of tertiary amine type had Rf values of 0.92, 0.83 and 0.72 (System 5) respectively. The non-amphoteric fraction (E_3) showed nine compounds of which three had tertiary amine character (at Rf = 0.83, 0.70 and 0.48). It was concluded that fraction E contained five tertiary amine compounds with Rf values of 0.96, 0.92, 0.83, 0.70 and 0.48.

Scheme 3 was found to be the best scheme for isolation of the components from the three plants under investigation. It was more specific than the other two schemes. The fatty acids were separated from the other compounds by the simple saponification process.

VI-3- Runner bean.

Under Scheme 3 it was found that the roots, rhizomes and stems of runner bean contained identical constituents in fractions A, B and C (by TLC). System 7 revealed twelve compounds from the saponifiable and unsaponifiable fractions, while System 9 revealed fifteen. The saponifiable fraction (C) showed six spots, the major one of which proved to be oleic acid (Fig. 10). The unsaponifiable fraction (B) revealed five spots (System 9), of which three were present in relatively large amounts. These three compounds were isolated in pure states by column chromatography and were identified as β-bergamotane, 18α-oleanane-3-one and β-sitosterol.

Using the column chromatographic technique for isolation, it was found that saturated hydrocarbons migrated most rapidly as these were adsorbed little or not at all. Adsorption of unsaturated hydrocarbons increases with the number of those which are conjugated. The presence of a functional group or groups affected the adsorption affinity which increases in the following sequence:

$$R\text{-}CH_3 \longrightarrow R\text{-}O\text{-}alkyl \longrightarrow >R\text{-}C{=}O \longrightarrow R\text{-}COOH$$

This was true in the cases when β-bergamotane, 18α-oleanane-3-one and β-sitosterol were separated by System 14 and isolated by column chromatography in the above sequence. The above sequence was also observed in all the TLC systems and column chromatographic techniques used in this study.

The unsaponifiable fraction (E_1) obtained from the methanol extract showed twelve compounds (System 10). In addition to 18α-oleanane-3--one and β-sitosterol, the other ten compounds were steroidal in nature due to the fact that they gave a purple colour with anisaldehyde and a blue colour with phosphomolybdic acid reagents. Their isolation and identification were impossible since they occurred in too small amounts.

The amounts of fatty acids obtained from fractions(C) and (E_2) with petroleum ether and methanol were 0.58, 0.50 and 0.32% w/w in the roots, rhizomes and stems respectively. By GLC it was found that oleic and palmitic acids were the major constituents of the fatty acids present in the three organs (22.30, 19.01; 34.40, 21.99; 29.96, 12.51% w/w of the total acids respectively). Oleic acid was isolated by column chromatography from the roots and rhizomes, while palmitic acid was isolated from the stems. The other fatty acids present in moderate or small amounts were also identified (Tables 81, 84 and 87).

The more polar components were extracted with methanol from which the fatty acids and sterols were separated first by filtration after cooling (phaseosterol-C and phaseosterol-D), and secondly by extracting the methanol residue with ether from an aqueous suspension. It was found that the percentages of the fatty acids and free sterols extracted by Scheme 3 were larger than those extracted by methanol only (1.43-1.07 and 1.65-1.01% w/w in the case of roots and stems respectively).

The crude saponins were separated from the other impurities by precipitate using aqueous methanol and ether (saponin contents were found to be 0.93, 0.90 and 0.75% w/w in the roots, rhizomes and stems respectively).

The relatively high proportion of saponins present in these organs could be the cause of the reported deaths of cattle and other farm animals after eating these organs.

From the saponin fraction of the roots, two hydrocarbons and four sapogenins were isolated. One of the hydrocarbons was identified as n-nonacosane. From the saponins, phaseoloside-A could not be identified due to the lack of material while phaseoloside-B was tentatively identified as soyasapogenol-C (with a sugar moiety of D-glucose (2 molecules), L(+) arabinose and D-fructose). Phaseoloside-C was also tentatively identified as Δ^{15}-3-24-dihydroxy-Δ^{12}-ursene. Finally, phaseoloside-D could not be identified due to the lack of material.

Only one sapogenin was isolated (phaseoloside-E) from the rhizomes and was identified as soyasapogenol-C.

From the stems one hydrocarbon (phaseobone-4) was isolated and identified as n-triacontane. In addition two sapogenins were isolated (phaseoloside-F and phaseoloside-G) and identified as soyasapogenol-C and soyasapogenol-B respectively.

VI-4- Honey-locust fruits.

The total unsaponifiable fraction obtained from the pods (petroleum ether and methanol extracts (B and E) formed about 7.0% w/w. Fraction (B) revealed four compounds on TLC (System 25), which were identified as n-nonacosane, β-sitosterol, stigmasterol and brassicasterol. Fraction (E) on the other hand revealed seven compounds (System 26). In addition to the above four, these additional three compounds had Rf values of 0.53, 0.15 and 0. The compound with an Rf value of 0.53 was not steroidal in nature and was present in a small amount. The other with an Rf value of 0.15 could be flavanoid since it was a visible yellow spot and also it produced a purple colour with ferric chloride reagent. Again it occurred in trace amounts only. Finally, the steroidal compound at the

base line was also in too small amount to allow isolation and identification.

The unsaponifiable fraction of the seeds was also studied and was found to be identical to fraction (B) of the pods i.e. it showed one hydrocarbon and three sterols.

The fatty acids (2.15% w/w) of the pods were identified. Ten of these were separated by GLC (System 4) and identified as Caprylic (8.9%), Capric (8.1%), Lauric (11.5%), Myristic (7.3%), Palmitic (30.3%), Palmitoleic (5.6%), Oleic (13.0%), Stearic (10.7%), Linoleic (0.9%) and Linolenic (9.1% w/w of the total fatty acids present).

The fatty acids of the seeds formed about 2.03%, and showed the following acids on System 4: Caprylic (19.9%), Capric (11.4%), Lauric (13.4%), Myristic (9.6%), Myristoleic (5.5%), Palmitic (12.6%), Oleic (19.9%), Stearic (4.8%), Linoleic (0.9%) and Linolenic (2.0% w/w of the total acids). Oleic and palmitic acids were isolated in pure states by column chromatography from fraction (C) of the seeds. Since they were present in high concentrations, the methods applied for identification were similar to those applied for runner bean.

The saponin content of the pods showed a single spot on TLC (System 27), but after hydrolysis eight compounds (System 25, Table 47) were detected of which only one was not a genin in nature (Rf 0.90). From the genin mixture only the above compound and two other genins were isolated in pure states by column chromatography and were identified as n-triacontane, oleanolic acid and echinocystic acid.

The sugar components were identified by paper and thin layer chromatography as D-glucose, D-mannose, L(+) arabinose and L(+) rhamnose. One sugar remained unidentified and had a Rf value of 0.86 (System 29 and Table 57).

The sapogenins content of the aqueous extract showed ten compounds, of which only one was not a genin (System 25, Table 48, Fig. 22). This compound was present in a relatively large amount and it was isolated

from the above mixture, then identified as n-triacontane. The sugar content of the genins separated from the aqueous extract was identified as L(+) arabinose, D-glucose and D-galacturonic acid.

The sapogenin content of the seed was found to contain six compounds. Two of them were genin in nature (Rf 0.86, 0.79) which were isolated and identified as oleanolic and echinocystic acids. In addition one hydrocarbon (Rf 0.90) was isolated and identified as n-triacontane. The other compounds (Rf 0.73, 0.13 and 0.04, System 25, Table 55) were not genin in nature and occurred in trace amounts which made their isolation impossible.

The sugar contents of the sapogenins obtained from the seeds were identified as D-glucose, L(+) arabinose. D-galacturonic acid and D-glucuronic acid.

The alkaloidal contents (0.28% w/w) of the pods were identified by TLC and were found to be tyramine and N-methyl-β-phenyl ethyl amine.

The galactomannan content of the pods (10% w/w) was determined by PC and TLC using Systems 28 and 29 and it was found to contain D-galactose and D-mannose.

VI-5- Celery stems.

The total unsaponifiable fraction obtained from the petroleum ether and methanol extracts formed 1.7% w/w. Fraction (B) showed nine compounds on TLC (System 30, Table 60). Only two of them (Rf 0.97 and 0.63) occurred in relatively large amounts and were isolated in pure states and identified as 1,3,5,7-tetra-ene-13-dimethyl tridecane (0.009% w/w) and tentatively as 3 β-hydroxy-5 α-stigmasta-9(11),22(23) diene (Indosterol (0.039% w/w)). This latter compound was found to be an active anti-inflammatory compound. The remaining compounds were steroidal in nature and were present in small amounts. On the other hand fraction (E_1) revealed eight compounds (System 33, Table 67). Only two of them were

isolated and identified as n-hexacosane (0.015% w/w) and tentatively as Indosterol (0.04% w/w). The other six compounds present in fraction (E_1) were steroidal in nature and occurred in trace amounts.

The fatty acids of celery stems (0.55% w/w) were separated and identified by TLC and GLC. These fatty acids were identified to be as follows: Capric (3.83), Lauric (1.40), Myristic (2.99), Myristoleic (4.10), Palmitic (51.25), Palmitoleic (3.17), C_{17} alkanoic (1.33), Stearic (6.51), C_{18} alkenoic (5.63), Oleic (90.5), Linoleic (5.53), Linolenic (19.09) and C_{18} alkenoic (4.61% w/w of the total acids present in E_2 and C fractions).

From the ether of precipitation used to precipitate the glycoside content, a pure crystalline flavanoid was isolated and was identified as apigenin-7-apioglucoside (0.03% w/w), while from the glycoside fraction Indosterol (0.004% w/w), n-heptacosane (0.0025% w/w), apigenin (0.0026% w/w) and a mixture of nine flavanoids were isolated.

The flavanoid mixture was separated by GLC and TLC (Systems 11 and 36 respectively). Due to the trace amounts present, identification was impossible. Finally, D-mannitol (1% w/w), inorganic phosphate and oxalate (2.3%) were isolated from the methanol extract.

It is worthwhile to mention that Singh[67] reported the presence of 0.35 gm.% w/w oxalate, 1.81 gm.% w/w calcium and 0.94 gm.% w/w phosphorus in celery leaves.

The aqueous extract of celery was also studied and was found to contain six compounds. One of these was isolated in a pure state and identified as n-undecosane (0.002% w/w) (System 30, Table 76). D-mannitol (2.2% w/w), D-fructose and D-glucose were also isolated from the aqueous extract.

Celery stems have a long history of culinary use and have frequently been used therapeutically in local folk medicine for the treatment of neuralgia and sciatica. The pharmacological investigation of Indosterol, present in celery stems, suggests that it is this compound which may be primarily responsible for the therapeutic properties attributed to celery stems. It is interesting to note that although celery seeds, which have also been used as an anti-inflammatory agent, have been the subject of several phytochemical investigations, there is no reported evidence of Indosterol in the seeds.

Indosterol is an unusual sterol in as much as it contains a double band in Ring C of the nucleus (Δ^{9-11}) and the only other report of its occurrence in nature is by Gupta[138] who isolated and characterised it, from another Umbelliferus plant - Seseli indicum. Gupta isolated the compound as the free sterol from its unsaponifiable matter in the light petroleum extract but it was found to occur in celery stems, not only in a free sterol in the unsaponifiable matter of the light petroleum extract but also as a glycoside in the methanol extracts. This would suggest its possible involvement in metabolic reactions in the stem. It is probable that it is synthesised in the leaves and moves down the stem to the root system or the glycoside with excess Indosterol being also stored in the stem. The celery stems examined were obtained from cultivated plants and it would therefore be of interest to examine the wild Apium graveolens.

The genus Apium presents a number of taxonomic problems and it may well be that the presence of this unusual sterol may indicate a taxonomic link with the genus Seseli.

In view of the fact that the major fatty acid in Umbelliferus seeds is petroselenic acid (C-16, $\Delta^{9(10)}$), up to 60% of the total

fatty acid - acid is characteristic of the family, it was surprising not to find this acid in the extract of the stem where the two major fatty acids are palmitic and stearic.

Honey locust fruits

Of the components found in the unsaponifiable fraction from both fruit and seeds, nonacosane had not previously been reported, the earlier work of Seriya[43] having indicated the presence of β-sitosterol, stigmasterol and brassicasterol only.

Catechin, a diglucoside of quercetin, reported in the fresh pods by Weinges[39] could not be detected in either the dried pods or seeds. This may be due to the conditions of drying and storage of the fruit after collection with the possibility of the formation of polymerised insoluble compounds.

Runner bean

The present study reveals differences in the saponin content of various Phaseolus species. Chirva[14] reported three saponins in P. vulgaris each being based on the aglycone soyasapogenol-C with different sugar moieties while P. radiatus showed only one saponin also based on soyasapogenol-C. In P. coccineus there were several saponins - although in the rhizomes the one saponin was based on soyasapogenol-C, the stems contained saponins based on soyasapogenol-B as well as soyasapogenol-C. The saponin in the roots however, were based on soyasapogenol-C and on Δ^{15}-3, 24 - dihydroxy - Δ^{12} - ursene. Thus while soyasapogenol-C is a triterpenoid common to all three species of Phaseolus, and to the roots, rhizomes and stems of P. coccineus, there are different triterpenoids specific to the different plant organs. This raises several interesting points regarding their biogenesis and their movement in the plant and will indicate the specificity of certain enzymes in the separate plant organs.

VII. MASS SPECTRAL DATA.

Spectrum 1.

m/e (I%) 27 (25), 29 (42), 39 (13), 40 (11), 41 (71), 42 (15), 43 (75), 44 (11), 45 (0.5), 55 (51), 56 (13), 57 (28), 60 (42), 69 (23), 71 (19), 73 (46), 83 (11), 85 (9), 87 (7), 97 (7), 117 (7), 131 (13), 185 (3), 213 (7), 256 (100).

Spectrum 2 (peak 1).

m/e (I%) 30 (20), 31 (20), 43 (27), 45 (13), 56 (16), 57 (20), 59 (7), 71 (14), 74 (100), 85 (12), 87 (18), 99 (12), 101 (13), 115 (8), 123 (5), 127 (5), 137 (3), 155 (5), 158 (5), 168 (5).

Spectrum 3 (peak 2).

m/e (I%) 31 (20), 43 (41), 55 (30), 56 (15), 57 (52), 71 (33), 74 (100), 85 (22), 99 (11), 101 (15), 109 (11), 113 (11), 127 (11), 141 (33), 143 (35), 171 (33), 183 (33), 185 (19), 214 (15).

Spectrum 4 (peak 3).

m/e (I%) 31 (13), 43 (27), 55 (24), 57 (22), 71 (11), 74 (100), 85 (8), 87 (49), 99 (3), 143 (8), 179 (2), 199 (5), 210 (3).

Spectrum 5 (peak 4).

m/e (I%) 31 (11), 43 (15), 57 (25), 71 (12), 74 (100), 85 (7), 87 (37), 99 (5), 101 (7), 127 (5), 143 (7), 180 (4), 195 (2), 214 (5), 226 (4).

Spectrum 6 (peak 5).

m/e (I%) 31 (6), 43 (10), 57 (21), 71 (47), 74 (100), 85 (10), 87 (65), 99 (4), 101 (19), 115 (3), 143 (46), 199 (7), 227 (15), 239 (7), 270 (6).

Spectrum 7 (peak 6).

m/e (I%) 31 (13), 43 (53), 57 (40), 71 (17), 74 (100), 85 (13), 87 (93), 99 (20), 101 (7), 113 (17), 127 (10), 143 (20), 199 (7), 240 (7), 265 (7), 296 (7).

Spectrum 8 (peak 7).

m/e (I%) 31 (27), 41 (64), 43 (82), 57 (91), 71 (45), 74 (100), 85 (36), 87 (73), 99 (27), 127 (36), 141 (36), 153 (27), 199 (27), 249 (36), 258 (36), 263 (18), 294 (27).

Spectrum 9.

m/e (I%) 41 (80), 42 (25), 44 (25), 55 (78), 56 (50), 57 (100), 67 (33), 69 (78), 70 (48), 81 (41), 85 (78), 91 (26), 95 (35), 95 (35), 97 (50), 99 (25), 105 (43), 109 (23), 111 (21), 113 (16), 119 (33), 123 (16), 133 (46), 147 (16), 161 (8), 169 (8), 207 (4), 208 (2).

Spectrum 10.

m/e (I%) 40 (40), 41 (46), 43 (54), 55 (74), 57 (50), 59 (42), 67 (36), 68 (22), 69 (100), 71 (24), 78 (32), 81 (58), 82 (40), 83 (40), 95 (72), 107 (32), 109 (62), 111 (20), 123 (54), 125 (62), 133 (15), 135 (20), 138 (26), 164 (22), 180 (28), 189 (14), 191 (20), 203 (12), 204 (15), 205 (25), 206 (15), 218 (28), 220 (14), 232 (20), 234 (10), 246 (18), 248 (12), 257 (5), 273 (20), 411 (6), 426 (6).

Spectrum 11.

m/e (I%) 40 (7), 41 (25), 43 (100), 44 (71), 55 (82), 57 (78),
67 (42), 69 (53), 71 (42), 77 (17), 79 (39), 81 (82),
83 (35), 85 (25), 91 (50), 92 (14), 93 (46), 94 (17),
95 (60), 97 (28), 105 (57), 107 (53), 108 (17), 109 (35),
111 (14), 117 (17), 119 (32), 120 (25), 121 (39), 123 (21),
131 (28), 133 (35), 135 (32), 145 (50), 147 (50), 159 (32),
161 (25), 163 (17), 173 (14), 213 (25), 231 (17), 255 (21),
271 (2), 273 (25), 303 (10), 314 (8), 381 (7), 382 (10),
398 (14), 399 (7), 414 (10).

Spectrum 12.

m/e (I%) 42 (17), 43 (100), 44 (21), 56 (21), 57 (92), 69 (25),
70 (25), 71 (83), 84 (21), 85 (75), 86 (21), 99 (67),
113 (58), 127 (54), 141 (42), 155 (38), 169 (28), 183 (22),
197 (17), 211 (13), 225 (10), 239 (9), 253 (7), 267 (6),
281 (4), 295 (4), 309 (4), 323 (3), 337 (3), 351 (3),
365 (2), 379 (2), 393 (0.5), 406 (5), 407 (0.5), 408 (0.2).

Spectrum 13.

m/e (I%) 30 (13), 39 (22), 40 (74), 41 (100), 42 (17), 43 (74),
44 (48), 55 (48), 56 (26), 57 (83), 60 (13), 67 (30),
68 (17), 69 (52), 70 (22), 71 (48), 73 (35), 74 (35),
75 (13), 77 (35), 78 (9), 79 (35), 80 (13), 81 (39),
82 (22), 83 (26), 84 (13), 85 (35), 91 (39), 92 (13),
93 (48), 94 (17), 95 (30), 96 (9), 97 (22), 98 (9),
99 (9), 105 (65), 106 (22), 107 (30), 108 (17), 109 (22),
111 (9), 119 (30), 120 (30), 121 (26), 122 (26), 123 (9),
124 (9), 133 (35), 134 (13), 135 (13), 147 (26), 148 (13),
149 (9), 161 (26), 175 (13), 189 (17), 204 (17).

Spectrum 14.

m/e (I%) 41 (30), 43 (57), 55 (73), 57 (73), 67 (30), 68 (24), 69 (76), 70 (30), 71 (54), 81 (57), 82 (36), 83 (100), 84 (27), 85 (36), 93 (45), 97 (79), 98 (21), 99 (15), 105 (36), 107 (36), 109 (30), 111 (42), 119 (27), 120 (18), 121 (27), 123 (27), 125 (27), 131 (24), 133 (39), 135 (27), 145 (45), 147 (36), 151 (15), 159 (45), 161 (27), 163 (24), 171 (15), 173 (24), 199 (21), 213 (36), 231 (21), 255 (45), 271 (21), 272 (15), 273 (18), 300 (18), 314 (9), 340 (12), 351 (21), 369 (12), 379 (12), 380 (12), 381 (9), 390 (9), 397 (9), 394 (9), 410 (2), 412 (30).

Spectrum 15.

m/e (I%) 41 (45), 42 (10), 43 (83), 55 (100), 56 (31), 57 (83), 61 (31), 67 (43), 68 (19), 69 (86), 70 (31), 71 (55), 79 (29), 81 (86), 83 (93), 84 (24), 85 (36), 91 (33), 93 (42), 95 (45), 96 (19), 97 (74), 98 (19), 105 (40), 107 (40), 109 (33), 111 (38), 119 (29), 120 (19), 121 (29), 123 (24), 125 (19), 131 (29), 133 (43), 135 (29), 145 (50), 146 (26), 147 (50), 148 (14), 149 (17), 157 (24), 159 (40), 161 (29), 171 (10), 173 (19), 175 (14), 189 (12), 199 (17), 213 (31), 255 (55), 271 (8), 273 (10), 274 (10), 275 (10), 281 (12), 282 (14), 283 (12), 351 (21), 379 (14), 381 (12), 382 (17), 394 (59), 395 (24), 396 (24), 412 (8), 454 (1).

Spectrum 16.

m/e (I%) 42 (10), 43 (100), 56 (20), 57 (95), 71 (82), 84 (20), 85 (72), 98 (10), 99 (62), 113 (59), 127 (55), 141 (40), 155 (40), 169 (38), 183 (25), 197 (20), 211 (15), 225 (12), 239 (10), 253 (8), 267 (8), 281 (7), 295 (7), 309 (5), 323 (5), 337 (4), 351 (3), 366 (3).

Spectrum 17.

m/e (I%) 42 (65), 43 (88), 56 (65), 57 (100), 69 (47), 70 (50), 71 (59), 84 (44), 85 (56), 98 (44), 99 (56), 112 (41), 113 (56), 126 (41), 127 (56), 140 (35), 141 (55), 154 (35), 155 (56), 168 (32), 169 (55), 182 (32), 183 (47), 197 (44), 211 (35), 225 (32), 239 (32), 253 (21), 267 (18), 281 (15), 295 (14), 309 (11), 323 (8), 337 (10), 351 (11), 365 (10), 379 (6), 393 (4), 407 (3), 421 (2), 435 (0.5), 449 (0.25), 464(0.25).

Spectrum 18.

m/e (I%) 43 (67), 55 (100), 56 (60), 57 (94), 67 (57), 71 (65), 81 (60), 83 (60), 85 (70), 91 (60), 95 (60), 97 (60), 105 (50), 107 (30), 109 (50), 111 (58), 119 (39), 131 (15), 132 (15), 133 (35), 149 (35), 187 (10), 189 (10), 191 (8), 201 (20), 203 (19), 216 (31), 223 (4), 255 (4), 422 (0.5), 440 (1), 441 (1).

Spectrum 19.

m/e (I%) 41 (22), 43 (28), 44 (79), 50 (30), 51 (42), 57 (30), 69 (28), 71 (13), 77 (70), 81 (25), 95 (30), 105 (100), 106 (28), 107 (25), 109 (30), 111 (9), 122 (49), 131 (13), 133 (13), 147 (13), 163 (10), 175 (30), 176 (30), 187 (10), 189 (10), 191 (22), 201 (6), 203 (6), 216 (10), 219 (31), 234 (61), 235 (12), 273 (2), 285 (1), 287 (1), 341 (1), 407 (1), 409 (1), 425 (0.8), 440 (1), 456 (2), 475 (0.5), 579 (0.5), 580 (0.14).

Spectrum 20.

m/e (I%) 40 (93), 41 (86), 42 (81), 53 (91), 54 (84), 55 (90), 67 (90), 68 (54), 71 (50), 81 (65), 83 (100), 89 (25),

93 (25), 95 (54), 97 (40), 103 (54), 109 (79), 111 (42), 124 (25), 126 (33), 129 (33), 139 (33), 186 (42), 218 (21), 269 (37), 280 (20), 296 (17), 311 (12), 320 (12), 324 (42).

Spectrum 21.

m/e (I%) 41 (100), 42 (62), 56 (72), 57 (72), 60 (49), 70 (43), 72 (52), 74 (60), 74 (33), 79 (33), 80 (31), 86 (37), 95 (25), 98 (28), 106 (31), 110 (25), 148 (60), 286 (29), 304 (4).

Spectrum 22.

m/e (I%) 43 (67), 55 (100), 56 (60), 57 (94), 67 (57), 70 (50), 71 (67), 79 (25), 81 (67), 82 (33), 83 (64), 84 (31), 85 (72), 91 (64), 93 (28), 95 (61), 96 (30), 97 (64), 98 (22), 99 (33), 105 (55), 107 (32), 109 (59), 111 (61), 113 (22), 119 (41), 120 (15), 121 (27), 123 (35), 131 (16), 132 (18), 133 (37), 135 (24), 149 (40), 187 (12), 189 (12), 191 (8), 201 (24), 203 (11), 216 (30), 223 (6), 255 (3), 391 (1), 405 (0.8), 422 (0.5), 440 (0.5).

Spectrum 23.

m/e (I%) 43 (85), 57 (100), 71 (90), 85 (80), 99 (80), 113 (70), 127 (65), 141 (61), 142 (21), 155 (50), 156 (16), 168 (23), 169 (50), 182 (18), 183 (55), 196 (16), 197 (43), 210 (12), 211 (31), 225 (25), 239 (20), 253 (17), 267 (13), 281 (10), 295 (7), 309 (5), 323 (10), 337 (10), 351 (9), 365 (6), 379 (3), 393 (2), 407 (2), 422 (1).

Spectrum 24.

m/e (I%) 41 (50), 42 (44), 43 (85), 44 (69), 45 (56), 55 (67), 57 (95), 60 (48), 69 (60), 71 (80), 81 (100), 83 (47), 85 (68), 95 (72), 97 (39), 107 (32), 109 (30), 119 (34),

131 (14), 133 (29), 137 (36), 145 (25), 147 (20), 159 (22),
161 (19), 169 (18), 173 (19), 175 (19), 187 (26), 188 (13),
191 (3), 201 (35), 203 (13), 216 (33), 255 (5), 268 (16),
270 (16), 286 (10), 328 (9), 372 (5), 405 (4), 465 (3).

Spectrum 25.

m/e (I%) 41 (81), 43 (88), 57 (74), 69 (77), 71 (38), 79 (46),
81 (81), 83 (50), 85 (35), 91 (48), 93 (67), 95 (88),
97 (31), 99 (27), 105 (74), 107 (79), 108 (24), 109 (59),
119 (78), 121 (72), 122 (46), 123 (42), 131 (31), 133 (62),
135 (62), 136 (35), 145 (39), 147 (51), 149 (31), 159 (33),
161 (55), 163 (27), 173 (32), 175 (67), 176 (80), 177 (35),
187 (38), 189 (29), 191 (10), 201 (46), 203 (29), 205 (20),
216 (41), 217 (24), 219 (100), 234 (68), 235 (35), 250 (20),
273 (6), 407 (5), 425 (4), 440 (3), 456 (2), 458 (1).

Spectrum 26.

m/e (I%) 40 (58), 41 (58), 43 (58), 44 (58), 56 (58), 57 (58),
67 (65), 69 (65), 70 (62), 71 (41), 79 (58), 81 (58),
83 (67), 85 (58), 91 (74), 93 (72), 95 (91), 97 (43),
99 (65), 105 (37), 107 (44), 109 (58), 117 (43), 119 (37),
120 (93), 121 (65), 129 (37), 131 (35), 132 (41), 133 (35),
135 (46), 143 (46), 145 (67), 147 (52), 149 (37), 155 (39),
157 (43), 159 (50), 161 (38), 169 (38), 171 (41), 173 (67),
175 (65), 176 (24), 187 (42), 188 (43), 189 (93), 190 (72),
190 (10), 201 (41), 202 (55), 203 (41), 207 (38), 213 (36),
235 (10), 248 (100), 375 (10), 377 (10), 390 (19), 392 (19),
408 (21), 410 (16), 423 (2), 438 (1), 456 (3).

Spectrum 27.

m/e (I%) 43 (62), 44 (60), 56 (60), 57 (67), 58 (60), 71 (100),

81 (58), 83 (60), 85 (62), 91 (40), 93 (38), 95 (60), 96 (60), 97 (60), 98 (22), 99 (62), 105 (53), 107 (42), 109 (39), 111 (37), 113 (39), 119 (47), 121 (36), 123 (25), 125 (17), 127 (23), 129 (16), 131 (35), 133 (45), 134 (19), 135 (27), 141 (28), 143 (20), 145 (36), 147 (36), 149 (38), 155 (21), 157 (19), 159 (28), 161 (21), 169 (35), 171 (18), 173 (37), 175 (39), 187 (37), 189 (48), 190 (35), 191 (10), 201 (20), 202 (26), 203 (51), 213 (18), 215 (18), 225 (9), 227 (10), 239 (14), 248 (23), 249 (8), 377 (6), 390 (8), 392 (10), 408 (4), 410 (4), 423 (0.5), 438 (1), 452 (2).

Spectrum 28.

m/e (I%) 41 (43), 43 (25), 44 (25), 55 (25), 56 (8), 57 (20), 76 (45), 82 (38), 83 (100), 84 (91), 104 (47), 119 (47), 131 (20), 132 (20), 149 (27), 173 (22), 187 (19), 189 (18), 201 (16), 202 (16), 203 (16), 207 (7), 218 (10), 219 (6), 246 (11), 264 (8), 377 (3), 393 (3), 395 (3), 410 (8), 424 (11), 439 (1), 454 (4), 472 (7).

Spectrum 29.

m/e (I%) 43 (40), 55 (40), 56 (40), 57 (45), 69 (48), 71 (55), 79 (51), 91 (52), 109 (56), 111 (73), 113 (86), 119 (57), 121 (52), 127 (58), 131 (42), 145 (37), 147 (35), 149 (100), 150 (55), 155 (36), 167 (36), 173 (32), 175 (37), 187 (67), 188 (41), 189 (58), 201 (39), 246 (15), 249 (5), 264 (10), 410 (7), 452 (5).

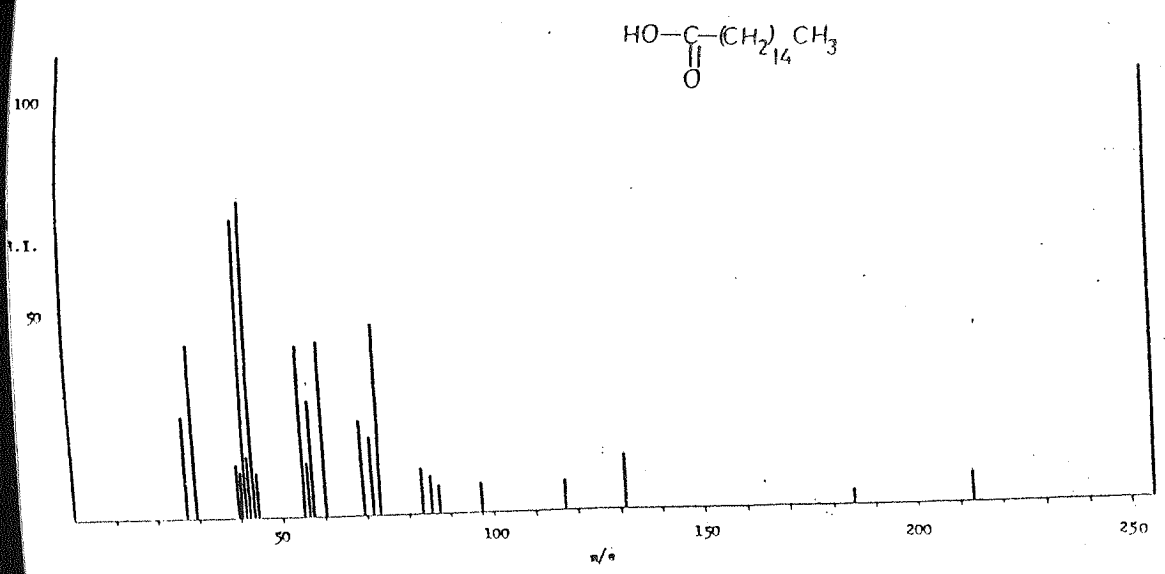
Spectrum 30.

m/e (I%) 41 (22), 42 (6), 43 (77), 55 (23), 56 (16), 57 (100), 70 (14), 71 (46), 85 (70), 99 (30), 113 (17), 127 (14), 141 (11), 155 (8), 169 (6), 183 (5), 197 (5), 211 (4),

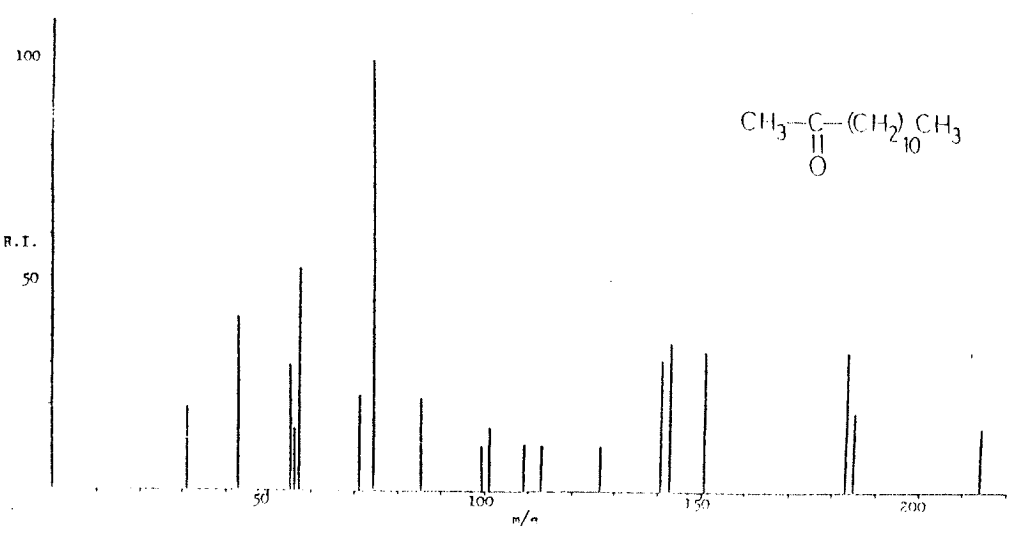
225 (3), 239 (2), 253 (2), 267 (1), 281 (1), 295 (0.7), 309 (0.7), 323 (0.4), 337 (0.4), 351 (0.4), 365 (0.4), 380 (0.4).

Spectrum 31.

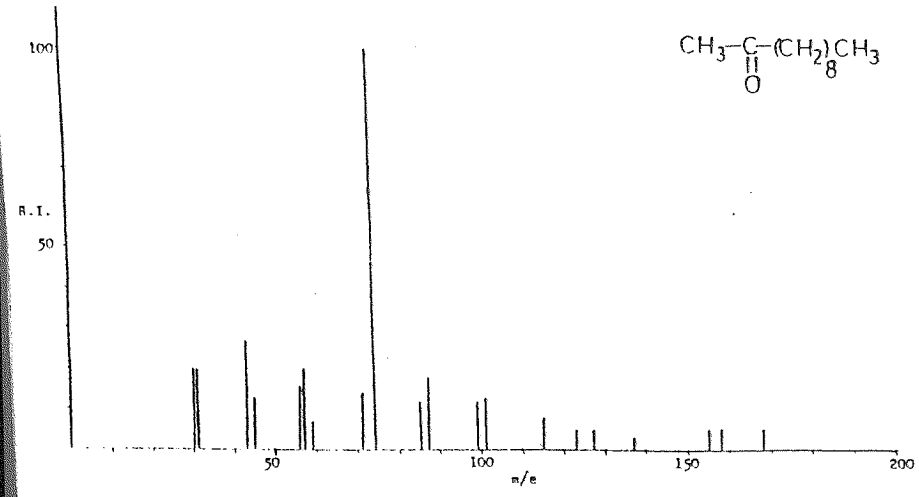
m/e (I%) 43 (100), 73 (20), 85 (31), 97 (26), 103 (48), 115 (90), 127 (39), 128 (42), 139 (55), 145 (43), 157 (41), 170 (30), 174 (14), 187 (55), 217 (25), 259 (23), 289 (15), 361 (8), 375 (8).



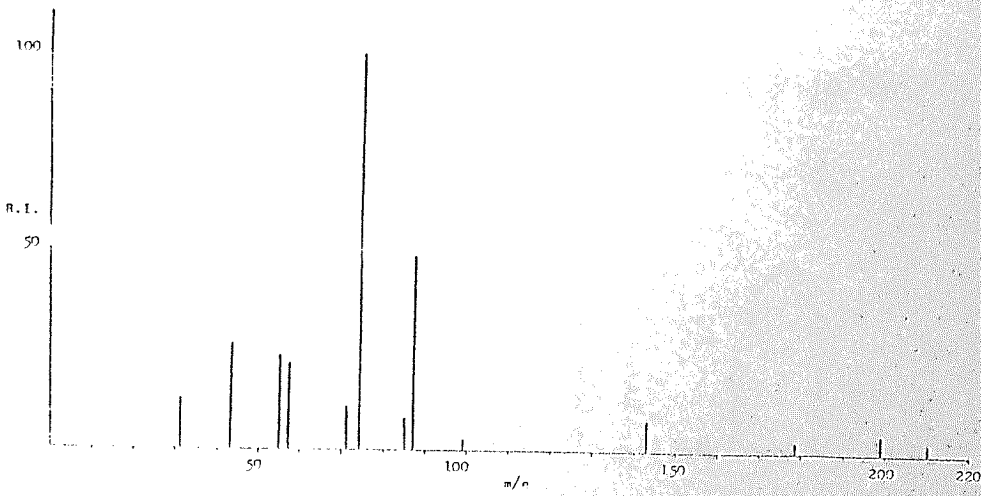
SPECTRUM 1 PALMITIC ACID (RUNNER BEAN)



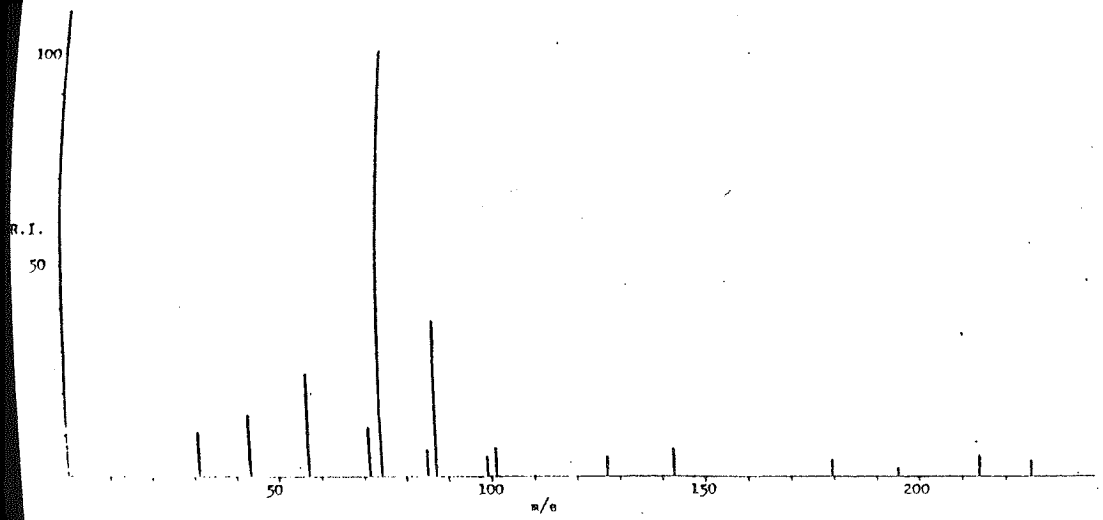
SPECTRUM 3 METHYL LAURATE (RUNNER BEAN)



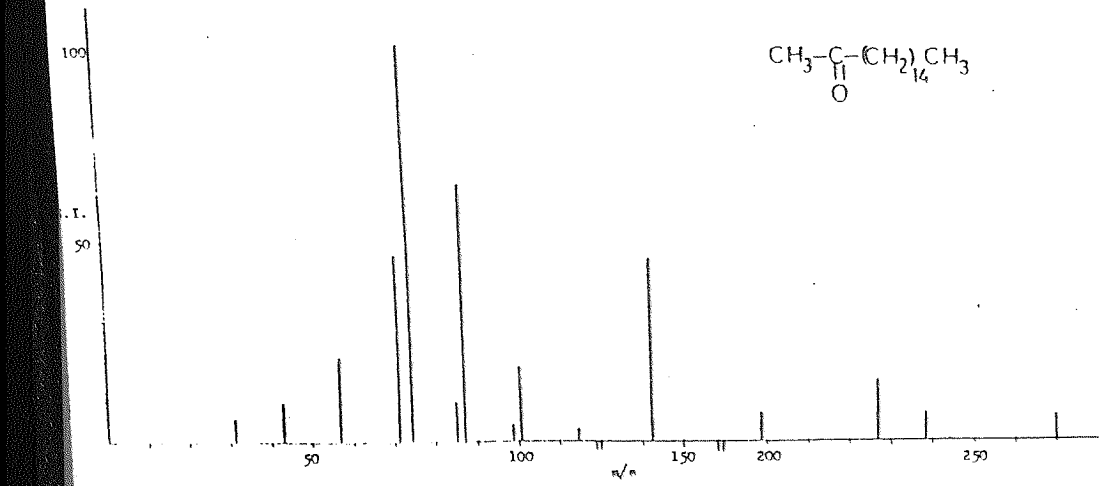
SPECTRUM 2 METHYL CAPRATE (RUNNER BEAN)



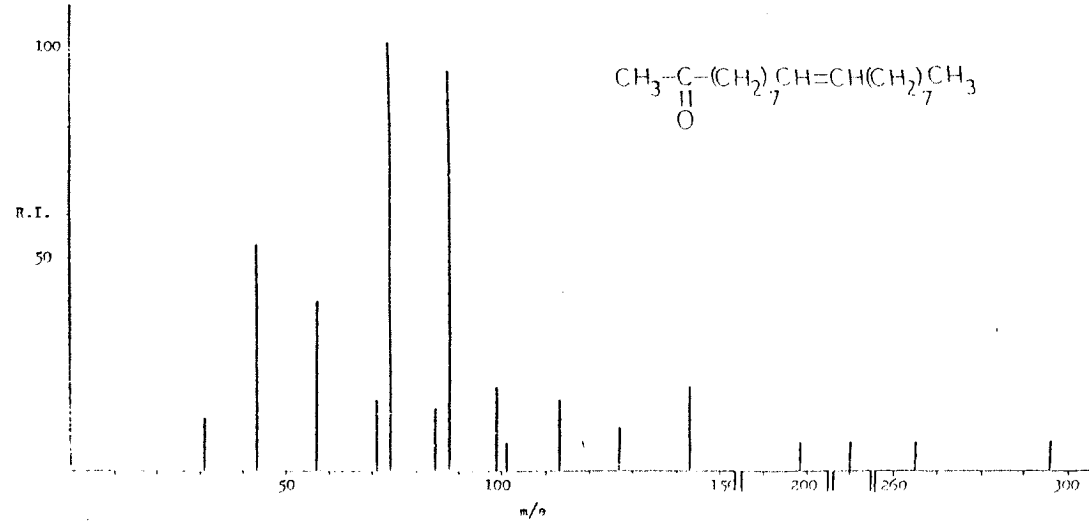
SPECTRUM 4 C_{12}- DIENOIC METHYL ESTER (RUNNER BEAN)



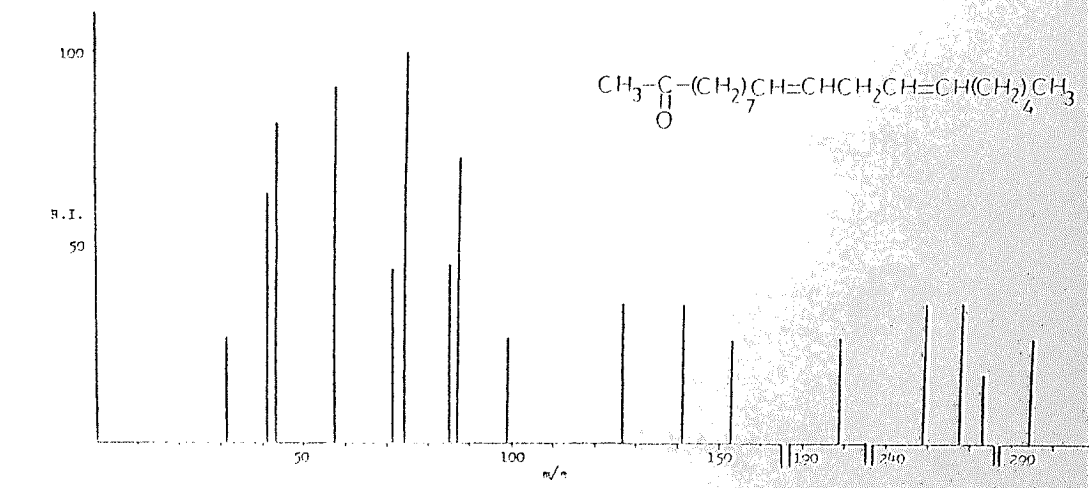
SPECTRUM 5 C_{13}- METHYL ESTER (RUNNER BEAN)



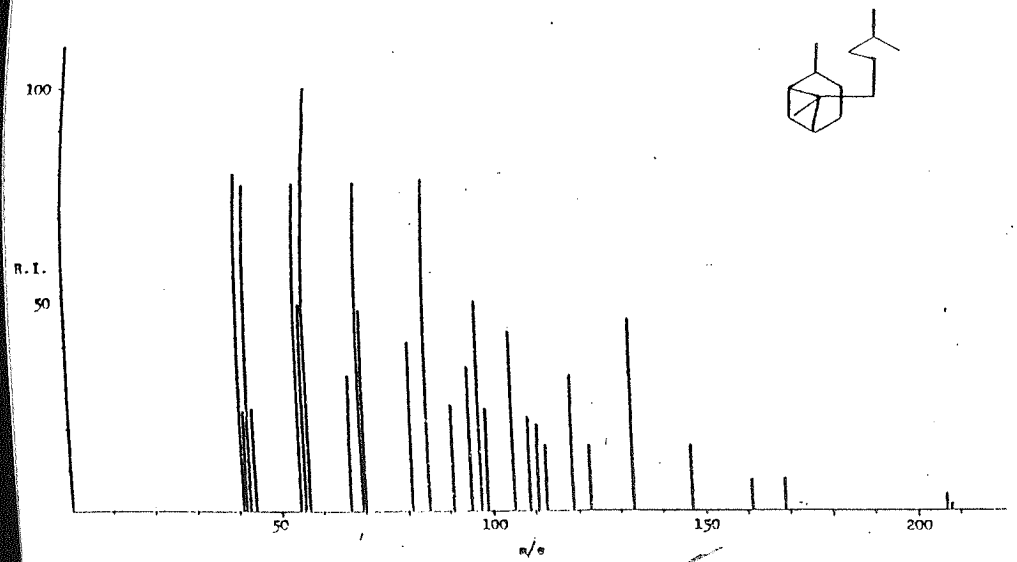
SPECTRUM 6 METHYL PALMITATE (RUNNER BEAN)



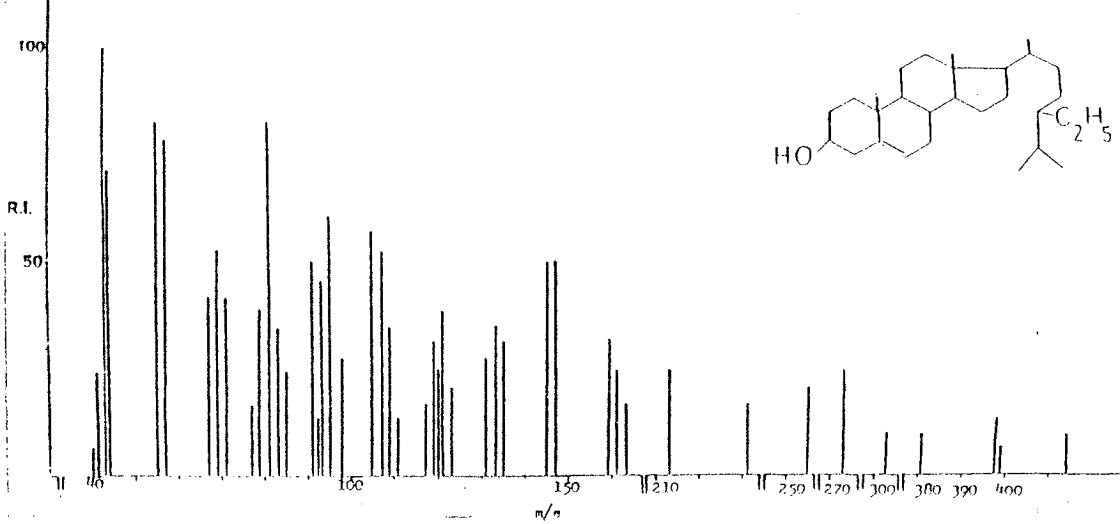
SPECTRUM 7 METHYL OLEATE (RUNNER BEAN)



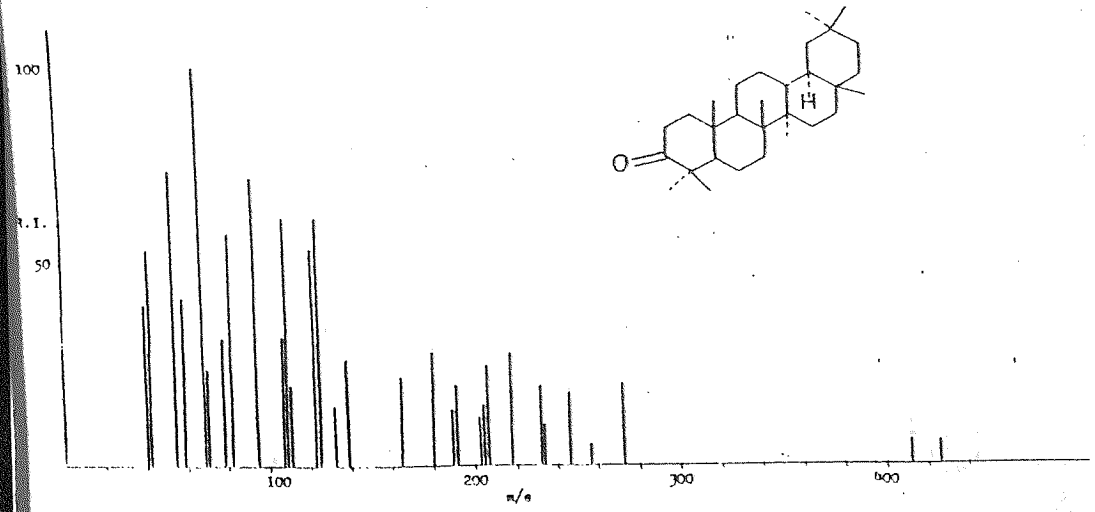
SPECTRUM 8 METHYL LINOLEATE (RUNNER BEAN)



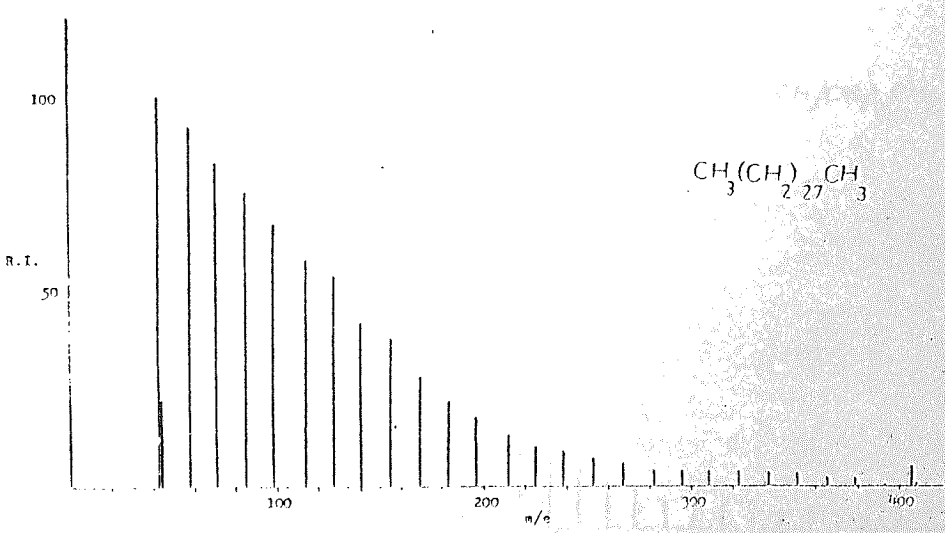
SPECTRUM 9 β-BERGAMOTANE (RUNNER BEAN)



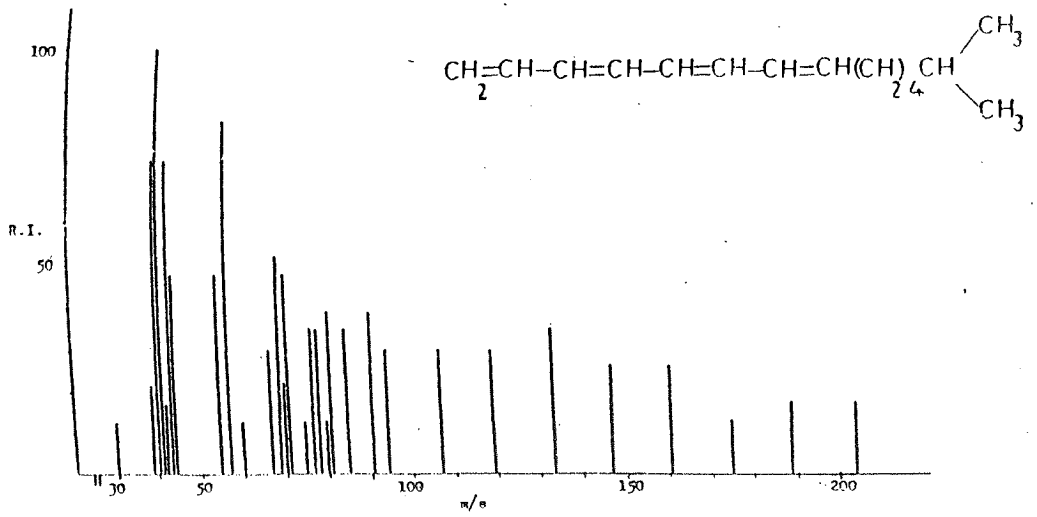
SPECTRUM 11 PHASEOSTEROL-B (RUNNER BEAN)



SPECTRUM 10 PHASEOSTEROL-A (RUNNER BEAN)
18 α-OLEANAN-ONE

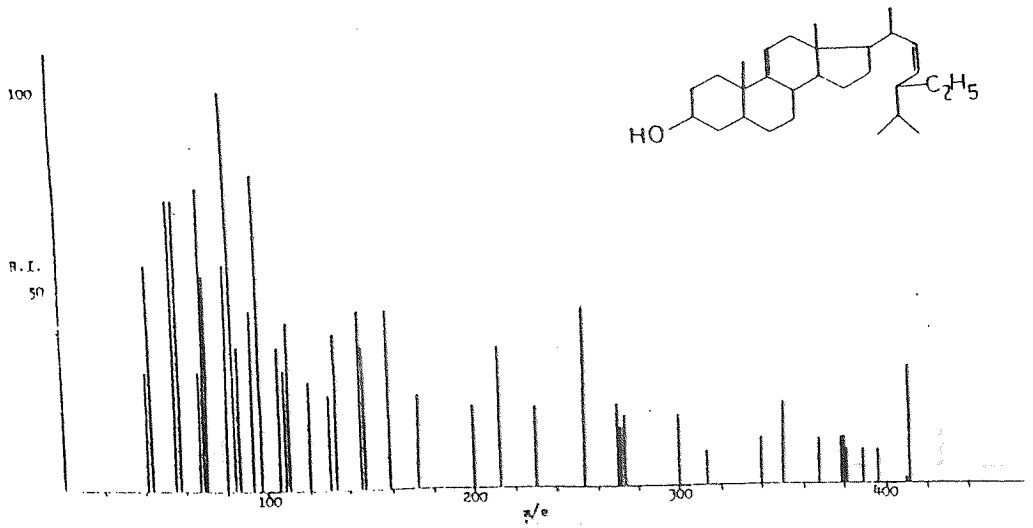


SPECTRUM 12 TRIACANANE-B (HONEY LOCUST)
n-nonacosane



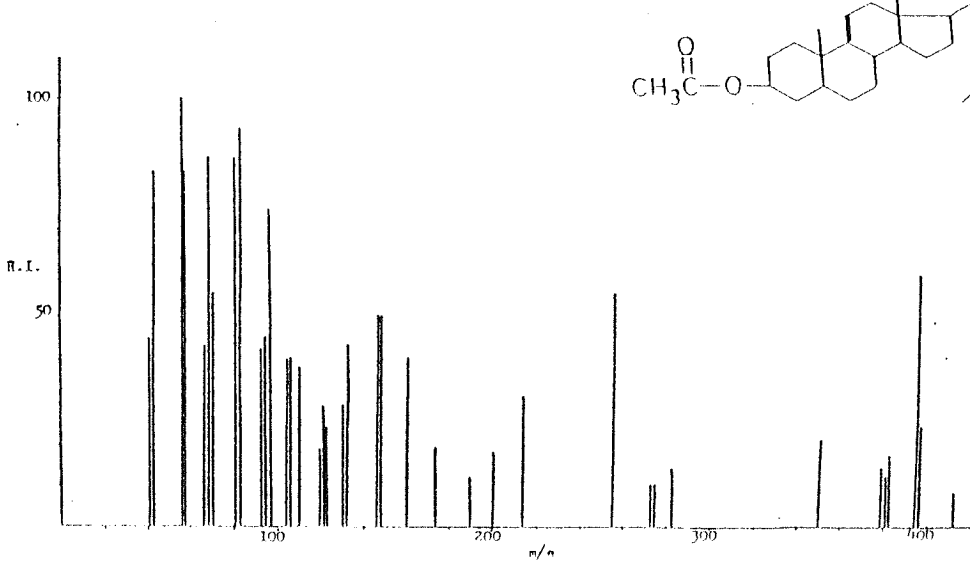
SPECTRUM 13 GRAVEOBONE-A (CELERY)

1,3,5,7-tetra-ene-13-dimethyl-tridecane



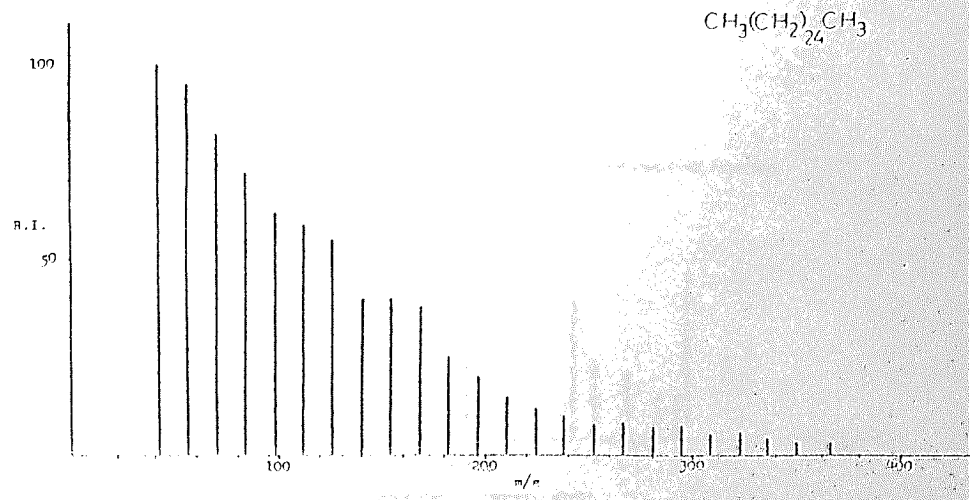
SPECTRUM 14 GRAVEOSTEROL-A (CELERY)

INDOSTEROL



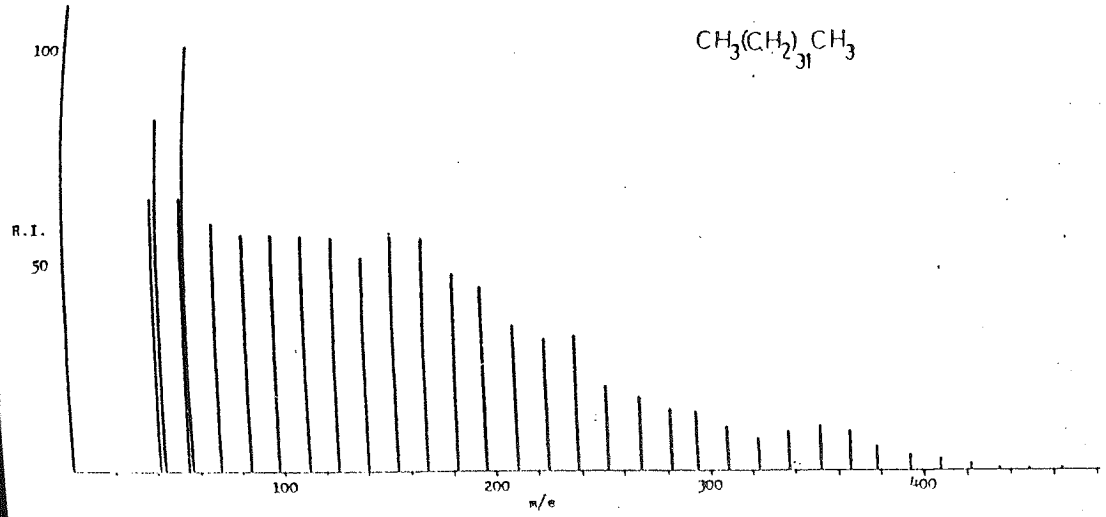
SPECTRUM 15 GRAVEOSTEROL-A ACETATE (CELERY)

(INDOSTEROL ACETATE)



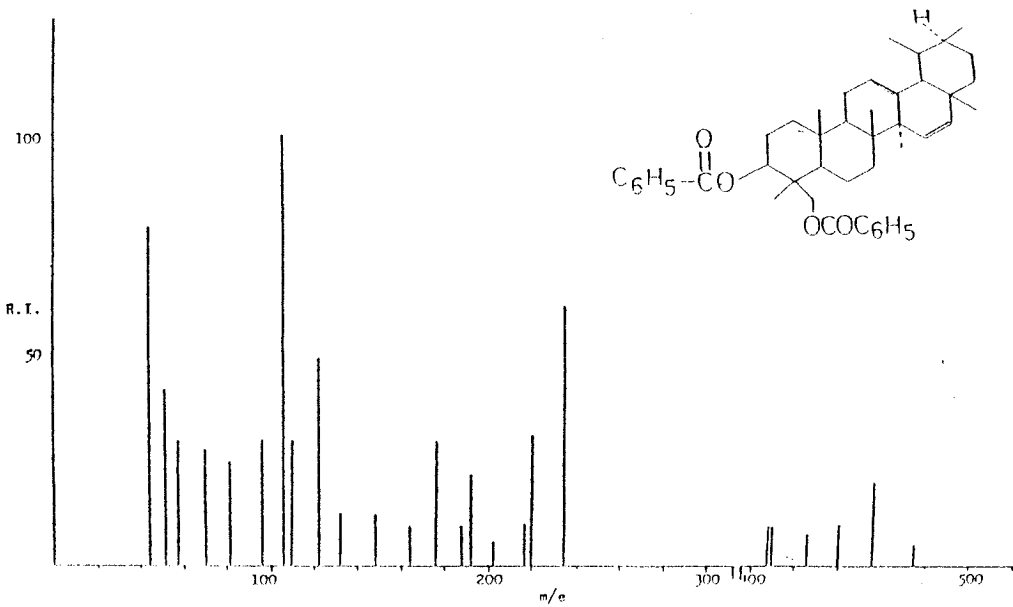
SPECTRUM 16 GRAVEOBONE-B (CELERY)

(n-HEXACOSANE)

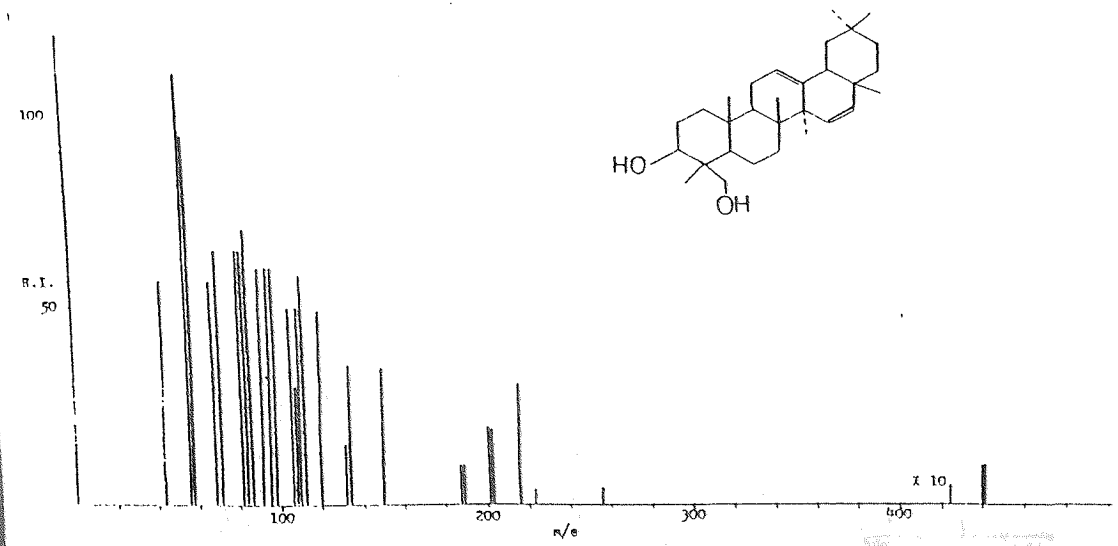


$CH_3(CH_2)_{31}CH_3$

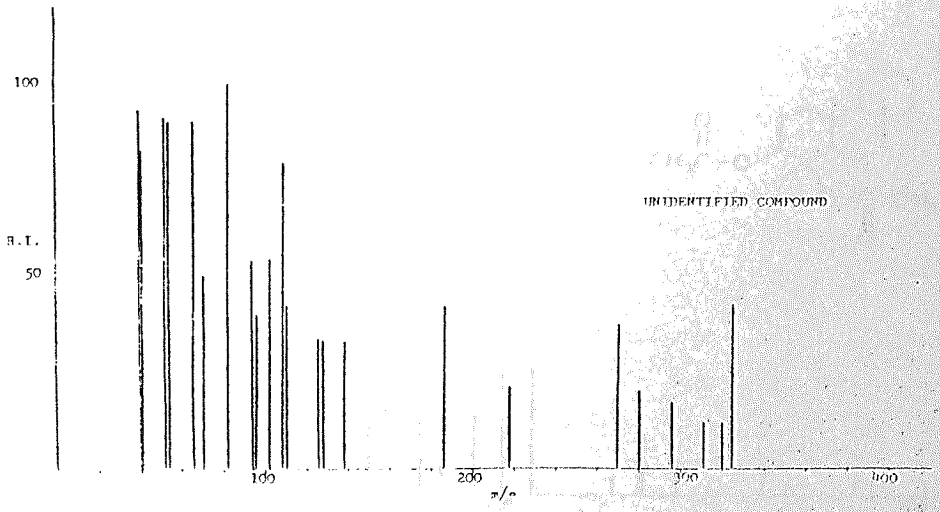
SPECTRUM 17 PHASEOBONE-2 (RUNNER BEAN ROOTS)
n-TRITRIACONTANE



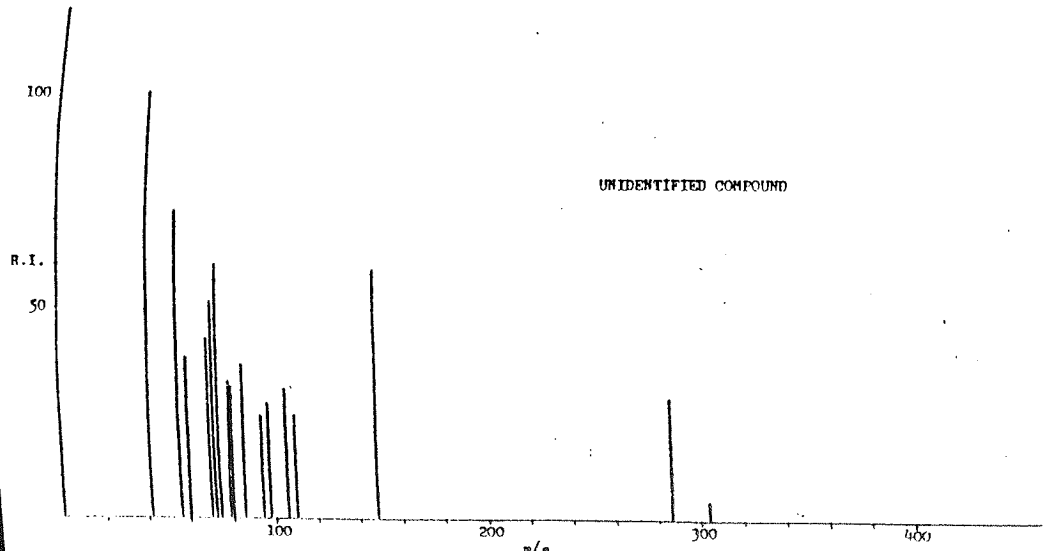
SPECTRUM 19 PHASEOLOSIDE-C BENZOATE (RUNNER BEAN)
Δ^{15}-ENE- 3,24 DIBENZOYL- Δ^{12} - URSENE



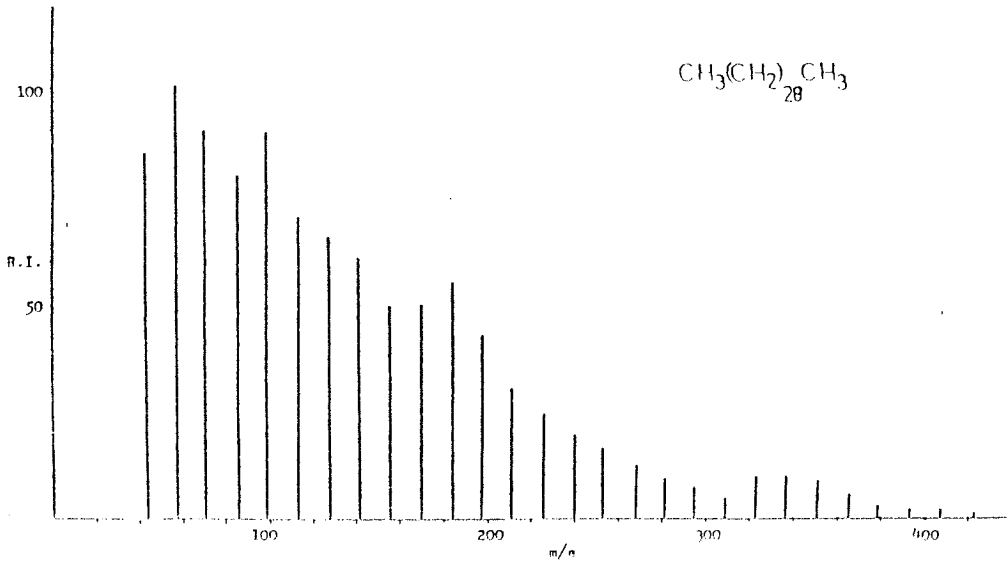
SPECTRUM 18 PHASEOLOSIDE-8 (RUNNER BEAN)
SOYASAPOGENOL-C



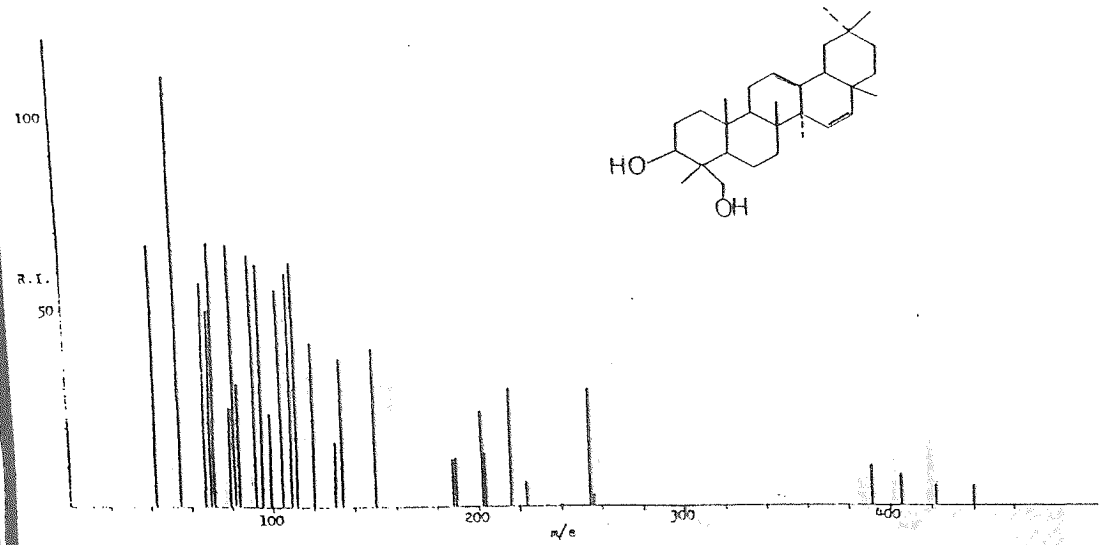
SPECTRUM 20 PHASEOSTEROL-C (RUNNER BEAN)



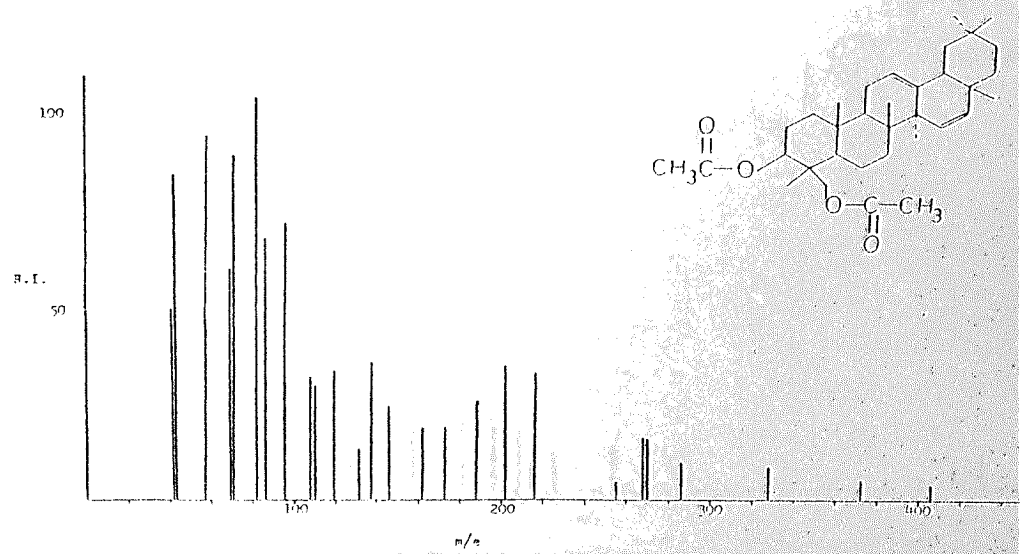
SPECTRUM 21 PHASEOSTEROL-D (RUNNER BEAN)



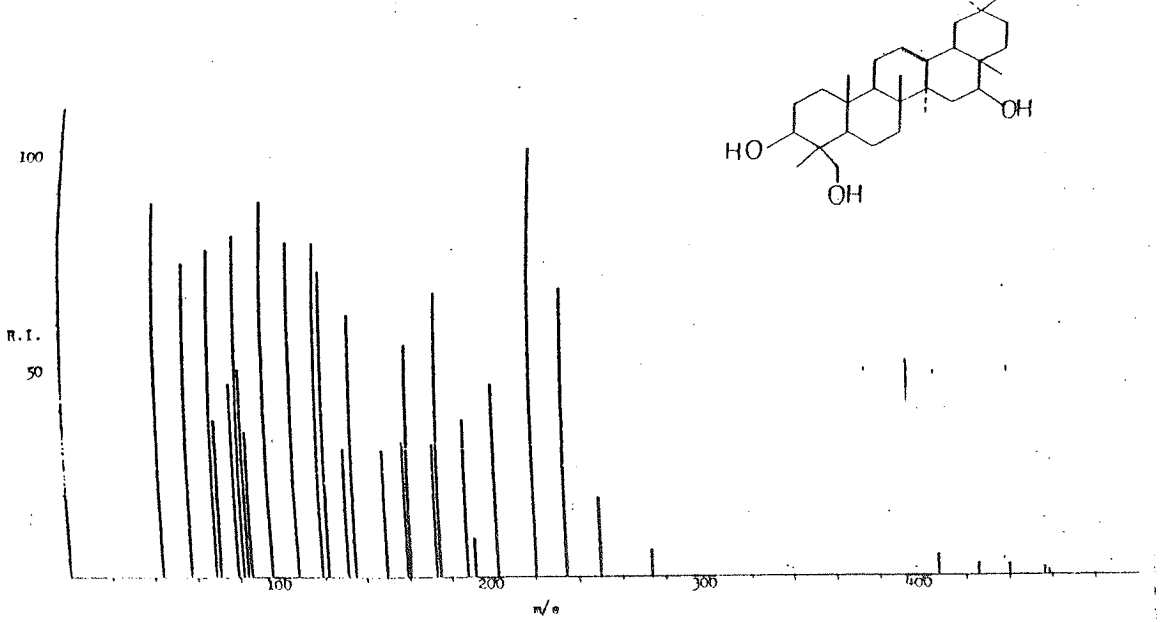
SPECTRUM 23 PHASEOBONE-A (RUNNER BEAN)
n-TRIACONTANE



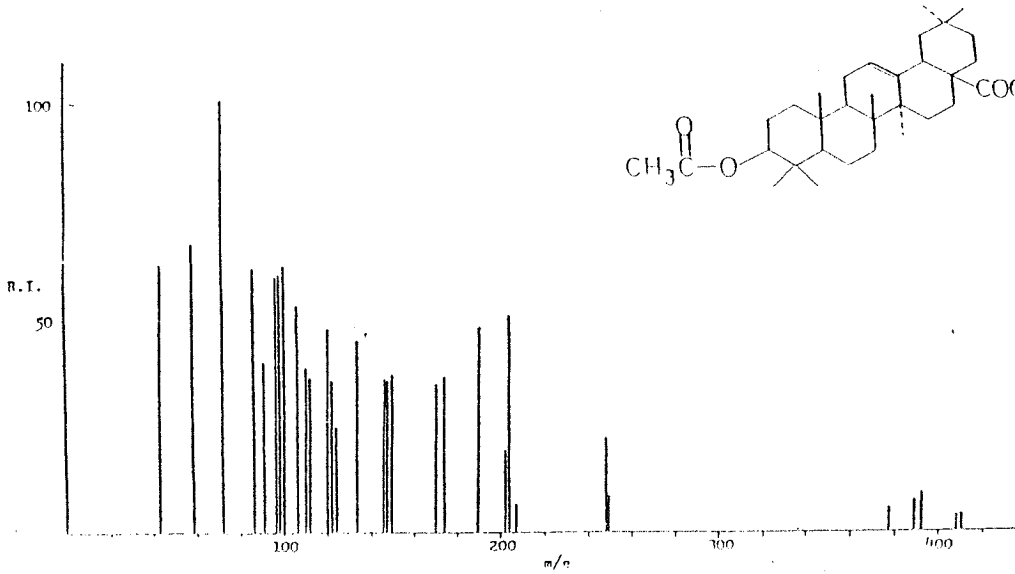
SPECTRUM 22 PHASEOLOSIDE-E (RUNNER BEAN)
SOYASAPOGENOL-C



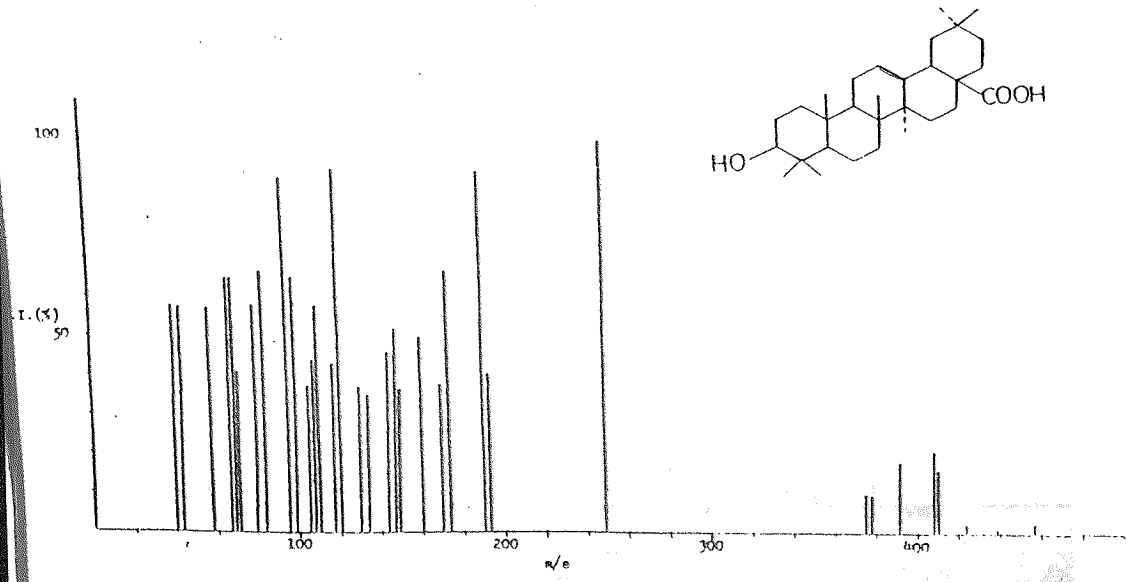
SPECTRUM 24 PHASEOLOSIDE-E DIACETATE (RUNNER BEAN)
SOYASAPOGENOL-C DIACETATE



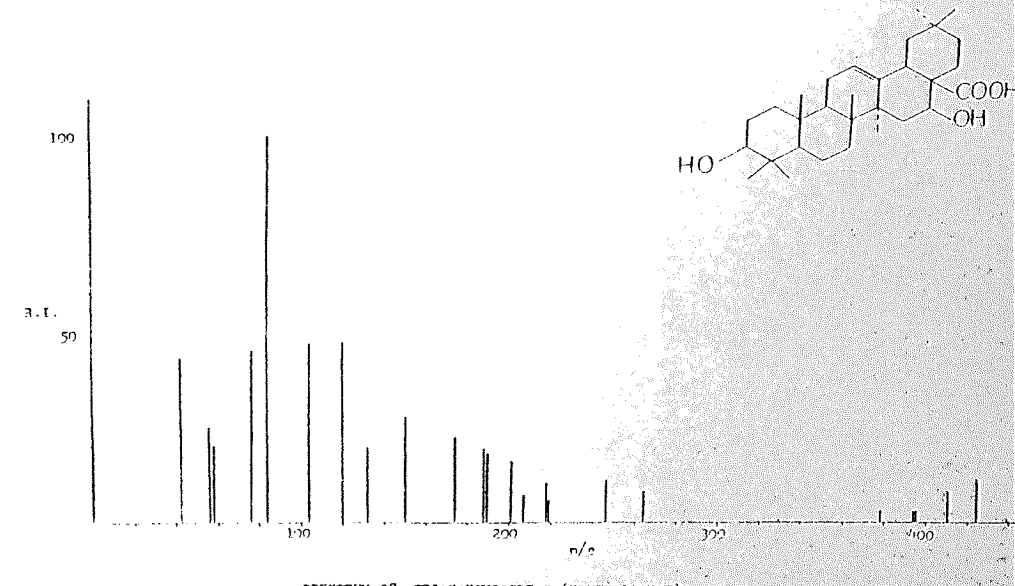
SPECTRUM 25 PHASEOLOSIDE-G (RUNNER BEAN)
(SOYASAPOGENOL-B)



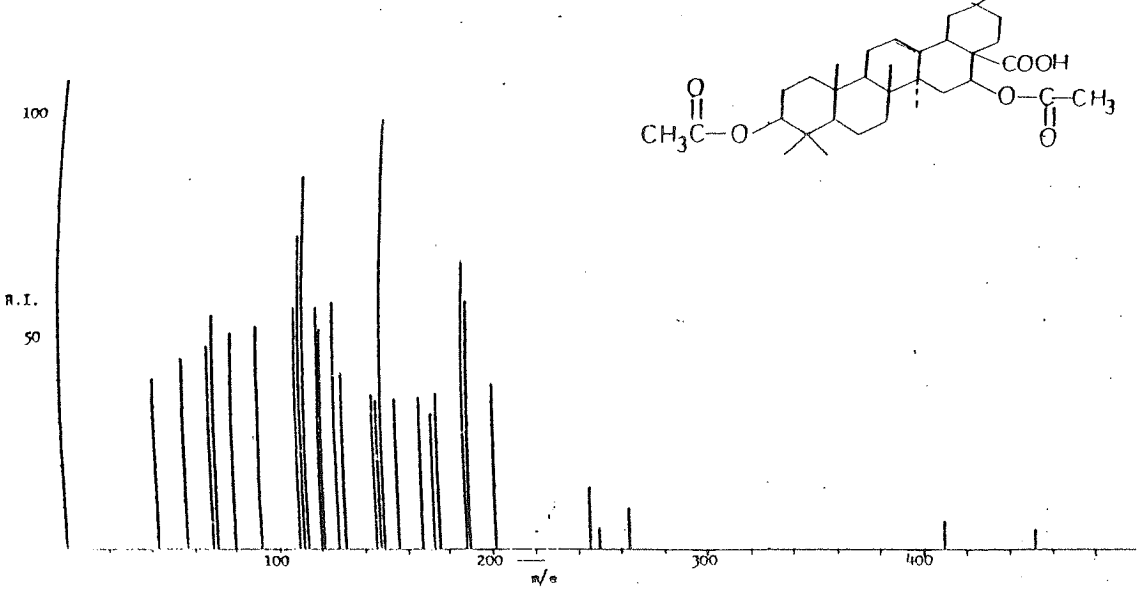
SPECTRUM 27 TRIACANTHOSIDE-A ACETATE (HONEY LOCUST)
(OLEANIC ACID ACETATE)



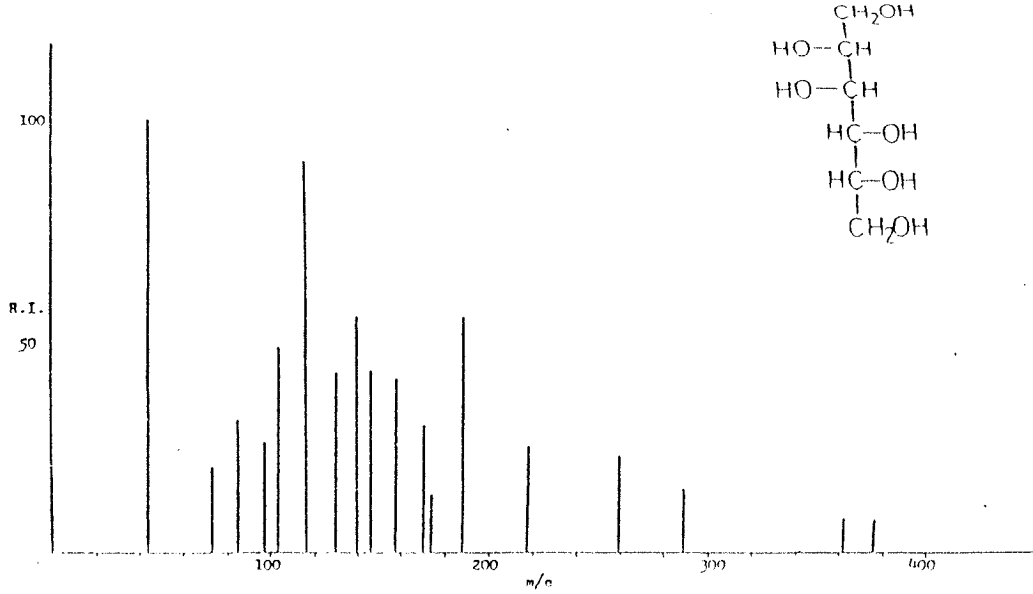
SPECTRUM 26 TRIACANTHOSIDE-A (HONEY LOCUST)
(OLEANOLIC ACID)



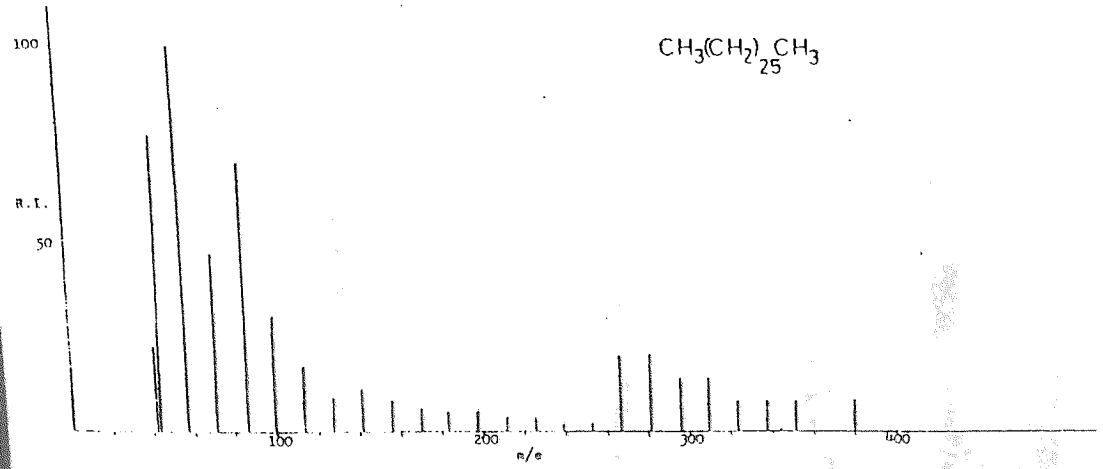
SPECTRUM 28 TRIACANTHOSIDE-B (HONEY LOCUST)
(ECHINOCYSTIC ACID)



SPECTRUM 29 TRIACANTHOSIDE-B DIACETATE (HONEY LOCUST)
(ECHINOCYSTIC ACID DIACETATE)



SPECTRUM 31 GRAVEOSE-A HEXAACETATE (CELERY)
(D-MANNITOL)



SPECTRUM 30 GRAVEOBONE-C (CELERY)
(n-HEPTOCOSANE)

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