CHEMICAL INVESTIGATIONS OF NATURAL PRODUCTS OF POTENTIAL BIOLOGICAL INTEREST.

A thesis submitted to the University of Glasgow

for the degree of

Doctor of Philosophy

in the

Faculty of Science

by

Ganapathy Subramanian M.Sc.,

September 1966

Medicinal Chemistry Section,
Department of Pharmacy,
University of Strathclyde.

(formerly the Royal College of Science and Technology)

TABLE OF CONTENTS

Section II. Triterpenoids From *Gaultheria subcorymbosa* And *Gaultheria antipoda*

Section III. Chemical Observations On Some *Hebe* Species.

Section V. Chemistry Of Arundoin.

ACKNOWLEDGEMENTS

The author expresses his thanks to Professor J.B. Stenlake, for providing the opportunity to carry out this research, and to Professor P.L. Pauson, Department of Pure and Applied Chemistry, University of Strathclyde for making freely available the facilities of his department.

He also takes this opportunity to express his sincere gratitude to Dr. M. Martin-Smith, Senior Lecturer, Medicinal Chemistry Section, Department of Pharmacy, University of Strathclyde, for suggesting the problems, for his continued guidance and constant encouragement and for kindly providing the extractives of the species of Cortaderia.

The appreciation of the author is extended to Mr. T.A. Bryce, Department of Chemistry, University of Glasgow, for kindly carrying out the mass spectral determinations, Dr. G. Eglinton, Department of Chemistry, University of Glasgow, for making available the facilities of his section, and Dr. P. Bladon, Department of Pure and Applied Chemistry, University of Strathclyde, for measuring the nuclear magnetic resonance spectra.

The author is indebted to Miss Lucy B. Moore of Botany Division, D.S.I.R. (New Zealand) for providing the two species of Hebe, to Dr. H.E. Connor of Botany Division, D.S.I.R. (New Zealand) for providing the authentic species of Cortaderia

and for his interest and collaboration, to Dr. R. Hodges for kindly collecting and extracting the *Cortaderia* from Raglan, to Dr. K. Schreiber, Gatersleben, East Germany, for providing the triterpene methyl ether fraction from Cuban sugar cane wax, to Dr. S. Natori, National Institute of Hygenic Sciences, Japan, for a gift of cylindrin, to Dr. A.S. Rao, National Chemical Laboratory, Poona, India, for a gift of the synthetic methyl ether from *O*-desmethylarundoin, to Dr. S. Abe, Yamazaki Works, Japan, for gifts of miliacin, isomiliacin, sawamilletin and isosawamilletin, to Dr. C.J.W. Brooks, Department of Chemistry, University of Glasgow, for a gift of taraxerol, to Dr. W. Lawrie, Department of Pure and Applied Chemistry, University of Strathclyde, for gifts of methyl ursolate, uvaol and *O*-acetyl methylursolate, to Dr. A.F.G. Dixon and Mr. G. Ross, Department of Zoology, University of Glasgow, for culturing the aphid *Megoura viciae*, to (Mrs.) June Grady, and Dr. T.C. Muir of Experimental Pharmacology, University of Glasgow and Mr. J.L. Paterson, University of Strathclyde for carrying out the pharmacological assays, and to Mr. J.M.L. Cameron, B.Sc., for kindly carrying out the microanalyses.

Thanks are due to all my colleagues in the Medicinal Chemistry Section, Department of Pharmacy, University of Strathclyde, especially Dr. W.D. Williams for his invaluable assistance throughout the research. It is a great pleasure

to acknowledge the technical help given by Mr. R. Nugent and his able staff.

The typing of this thesis was carried out by Mrs. R. Nugent.

Finally the author wishes to express his deep appreciation to his parents for their generous financial assistance during the period of this work.

ABSTRACT

This thesis is divided into five distinct and self-contained sections whose unifying theme is contained in the general title 'CHEMICAL INVESTIGATIONS OF NATURAL PRODUCTS OF POTENTIAL BIOLOGICAL INTEREST .

Section I gives an account of a chemical investigation of the aphid Megoura viciae Buckton. This study was made, in the light of reports of the toxicity of this insect to the predatory insect Adalia decempunctata. However the presence of a toxic principle could not be demonstrated. A brief survey of the different types of toxic principles found in insects is given to place the work in perspective.

Section II deals with the isolation of the triterpene acid fractions from the native New Zealand plants Gaultheria antipoda and Gaultheria subcorymbosa. The investigation was undertaken in the light of what were considered to be reliable reports that the plant Gaultheria antipoda, contained a galactogenic principle. Arguments in support of the belief that this principle could possibly be a triterpene acid are advanced. It was found that ursolic acid was the sole triterpene acid present in both plants.

Section III describes attempts at the isolation and characterisation of the constipatory principle present in several species of Hebe, one of which, Hebe stricta is well

established as being effective in arresting loose bowel movements. Chemical examination revealed D-mannitol and condensed tannins to be major constituents of these plants and pharmacological studies indicated that the constipatory properties could be attributed to the condensed tannins in agreement with earlier assumptions. At the same time the opportunity was taken to perform gas-liquid chromatographic analyses of the alkanes and the components of the long chain esters present; as an extension of earlier work directed towards a possible chemotaxonomic differentiation within the genus Hebe.

Section IV gives an account of an investigation of five species of Cortaderia for the presence of triterpene methyl ethers. This work was stimulated by the absence of arundoin in a sample of Cortaderia from Raglan, by a recent taxonomic revision of the grass family Arundinoideae and because of certain detailed botanical studies. Triterpene methyl ethers were found to be absent from Cortaderia atacamensis and Cortaderia selloana. Cortaderia fulvida and Cortaderia richardii were found to contain arundoin. Cortaderia toetoe was found to contain a mixture of three triterpene methyl ethers, which, after determination of the relative gas liquid chromatographic retention times of nine authentic triterpene methyl ethers on four different stationary phases and application of mass spectrometry, could be positively identified as arundoin,

β-amyrin methyl ether and α-amyrin methyl ether. In the case of α-amyrin methyl ether this would appear to be the first report of its natural occurrence.

In addition, gas liquid chromatographic analyses were performed on the alkane fractions and on the components of the long chain esters present in the leaf surface wax of each species of Cortaderia. The results of these analyses in conjunction with the triterpene methyl ether analyses resulted in a partial chemotaxonomic differentiation of the five species of Cortaderia.

The two major triterpene methyl ethers present in the wax of Cuban sugar cane were identified as sawamilletin (taraxerol methyl ether) and arundoin. A third minor component had gas liquid chromatographic retention times corresponding to bauerenol methyl ether. A triterpene alcohol present in Artemisia vulgaris was shown to be O-desmethylarundoin (fernenol) through direct comparison of the derived methyl ether with arundoin. The mass spectra and nuclear magnetic resonance spectra of triterpene methyl ethers are discussed. An interesting facet of the mass spectra is the loss of neutral methanol as a major fragmentation process while the nuclear magnetic resonance spectra reveal the absorption of the 3α proton (ie the proton on the carbon atom bearing the methoxyl group) to occur at surprisingly high field. The preparation of synthetic

triterpene methyl ethers is described.

The co-occurrence of the three triterpene methyl ethers in Cortaderia toetoe and Cuban sugar cane has interesting biogenetic implications, so in order to set these in perspective, an account is given of the current postulates on the derivation of different triterpenes and steroids in terms of different conformational foldings of all trans squalene during cyclisation.

Section V describes the chemistry of the pentacyclic triterpene arundoin and the preparation of the 9(11)-dienes from the methyl ethers of bauerenol and multiflorenol for the purposes of a direct comparison with the 9 (11)-diene derived from arundoin.

SECTION I

CONSTITUENTS OF *MEGOURA VICIAE* BUCKTON

INTRODUCTION

In the course of a study of the escape responses of certain aphids to the presence of the coccinellid Adalia decempunctata (L), Dixon[1] observed that within two minutes of ingesting the aphid Megoura viciae Buckton, the larvae of Adalia decempunctata frequently regurgitated their gut contents; and, moreover, that a certain percentage of fourth instar larvae provided with Megoura viciae as food, died within a few days. These observations served to suggest the presence within the aphid, of a physiologically active agent and accordingly it was decided to initiate chemical studies of Megoura viciae in an endeavour to establish the nature of any toxin or toxins present.

The possibility of virus transmission[2,3] or toxic polypeptides[4], being the factors involved appeared to be ruled out by the extremely short time interval, which in some instances was as little as two minutes, between the ingestion of the aphid and the onset of the symptoms of distress in the Adalia decempunctata larvae. Hence attention was focussed on a search for a toxic principle of relatively low molecular weight.

SURVEY OF INSECT TOXINS

The existence of relatively simple toxic principles in insects and their secretions is well established. Such

compounds usually play a role in defence mechanisms or else afford a means of paralysing prey. In addition, insect secretions having other primary roles may occasionally exhibit incidental toxicity to other organisms. Among these other primary roles of insect secretions, may be listed surfactant activity for propulsion on water[5]; formation of odour trails from food to the nest; function as pheromones for communicative purposes[7]; and Mullerian mimicry[8] [where scent patterns are used to mislead colour-blind predators]. It is also of considerable interest that certain aphids secrete an oily liquid which they smear over predatory coccinellids, immobilising them as the secretion solidifies. This phenomenon has been termed waxing.[1]

The effects of insect secretory toxins on predators are generally of short-term duration, except in the case of venoms which are actually injected into the bodies of the victims, although the secretions of nasute termites and ants of the genus Iridomyrmex are known to kill certain other insects.[9,10]

The toxic principles elaborated by insects vary greatly in their chemical nature. On the one hand they may be simple inorganic compounds such as hydrochloric acid,[11] hydrocyanic acid,[12] nitrous acid,[13] ammonia,[14] or potassium hydroxide,[15,16] whilst on the other hand they may be organic compounds of widely varying complexity.

Table I, lists certain of the simpler organic toxins elaborated by insects together with references to original literature. These toxins embrace fatty acids, aldehydes, alcohols, esters, ketones, simpler aromatic compounds, quinones and terpenoids.

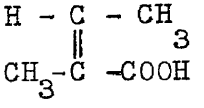
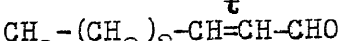
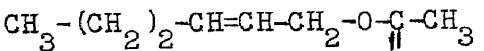
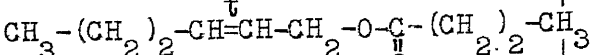
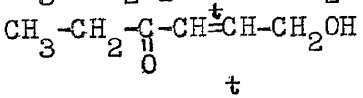
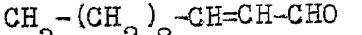
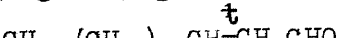
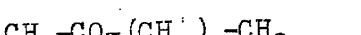
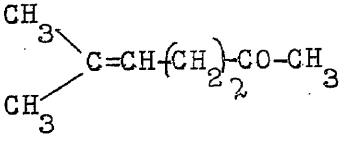
The biological effects produced by the lower fatty acids have been attributed solely to a lowering of pH[75,76]. Of the aldehydes listed in Table I *trans*-2-hexenal occurs in several orders of insects. Thus it has been isolated from the West African black cocktail ant (*Atpogyne africana*)[25], the cockroach (*Eurycotis floridana*)[25] and the bug *Acantho-cephala femorata*[26]. The related *trans*-2-heptenal is found in the rice stink bug, *Oebalus pugnax*[31], whilst the related ester 2-hexen-1-ol acetate has been obtained from the male water bug, *Belostoma indica*.[27] Of considerable interest is the discovery that higher unsaturated alcohols act as sex attractants in insects. Thus the sex-attractant of the silk moth has recently been shown to be *trans*-10-*cis*-12-hexadecadien-*1*-ol[77] whilst that of the female gypsy moth has been identified as (+)10-acetoxy-*cis*-7-hexadecen-*1*-ol[77]. Also of interest in this connection is the suggestion that the attraction of the mulberry for silkworms[78] may be due to 2-hexenal and 3-hexenal which are known to be constituents of the leaves.

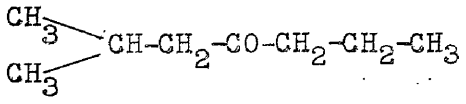
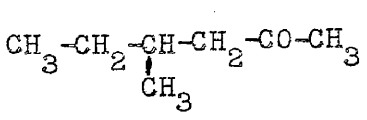
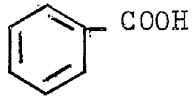
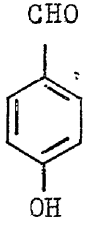
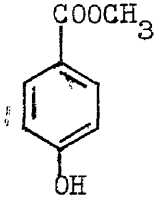
The presence of salicyl aldehyde in the secretions of certain insects has been known since the last century and it has been suggested that biogenetically this aldehyde may

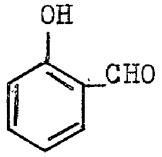
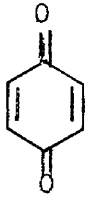
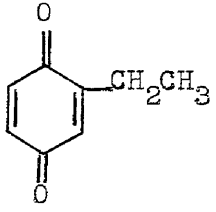
TABLE I.

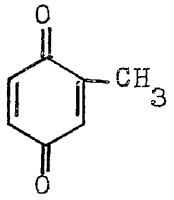
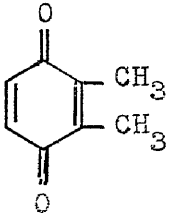
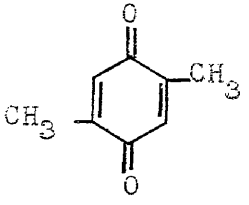
SIMPLER ORGANIC CONSTITUENTS OF INSECT SECRETIONS AND VENOMS

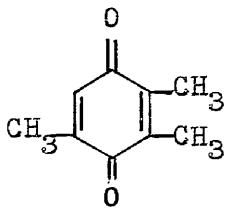
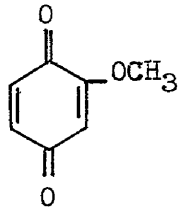
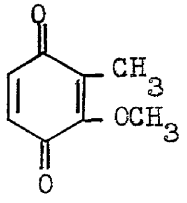
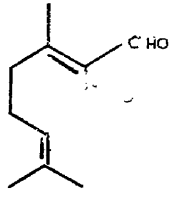
No.	COMPOUND	FORMULA	OCCURRENCE	REF.
	Lower Aliphatic Compounds:			
	(A) Fatty Acids.			
1	Formic acid	H-COOH	Various ants of families Formicinae, Myrmicinae	17-20
2	Acetic acid	CH_3-COOH	Mastigoproctus giganteus (Lucas).	21
			Myrmicaria natalensis (Fred).	5
3	Propionic acid	CH_3-CH_2-COOH	Myrmicaria natalensis (Fred).	5
4	Isobutryic acid	$(CH_3)_2$CH-COOH	Myrmicaria natalensis (Fred).	5
5	Isovaleric acid	$(CH_3)_2$CH-CH_2-COOH	Myrmicaria natalensis (Fred).	5
6	Caprylic acid	CH_3-$(CH_2)_6$-COOH	Mastigoproctus giganteus (Lucas).	21
7	9-Hydroxydec-trans-2-enoic acid	CH_3-CH(OH)-$(CH_2)_5$-CH=CH-COOH	Apis mellifera (L).	23
8	Methacrylic acid	CH_2=C(CH_3)-COOH	Abax ater (de Vill).	24

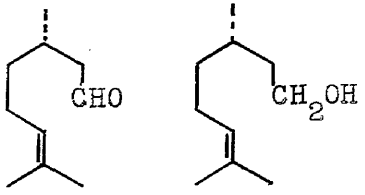
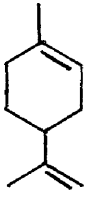
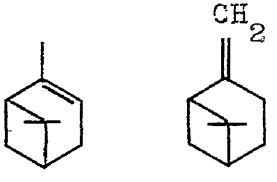
9	Tiglic acid		Abax ater (de Vill).	24
(B)	Unsaturated Aliphatic Alcohols, Aldehydes And Esters:			
10	Trans2-Hexenal		Eurycotis floridana. Atpogyne africana. Acanthocephala femorata. Brochymena quadripustulata.	25 25 26 22
11	1-Acetoxyhex-2-ene		Belostoma indica.	27
12	Trans 2-Hexen-1-ol butyrate		Lethocerus indicus.	28
13	Trans 4-Oxohex-2-en-1-ol		Nazara viridula (Fabricus).	29
14	Trans 2-Dodecenal		Oebalus pugnax (Fabricus).	31
15	Trans 2-Heptenal		Euchistus servus Nazara viridula (Fabricus). Oebalus pugnax (Fabricus).	31 31 31
(C)	Ketones:-			
16	Methyl amyl ketone		Iridomyrmex parcuisnosus Iridomyrmex detectus (F)	32 33
17	6-Methylhept-5-en-2-one		Iridomyrmex conifer (F) Iridomyrmex nitidiceps. Tapinoma nigerrimum (Nyl). Dolichoderus scabridus.	34 35 36 37

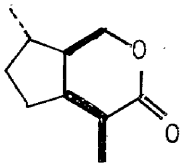
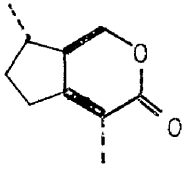
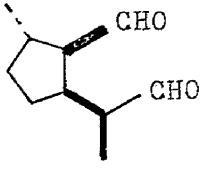
18	Propyl isobutyl ketone		Tapinoma nigerrimum (Nyl).	36
19	4-Methylhexan-2-one		Dolichoderus clarki.	38
(D)	Aromatic Compounds.			
20	Benzoic acid		Dytiscus marginalis (L)	39
21	Para-Hydroxybenzaldehyde		Dytiscus marginalis (L)	39,40
22	Methyl para-hydroxybenzoate		Dytiscus marginalis (L)	39,40

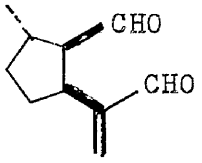
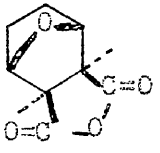
23	Salicylaldehyde		*Phyllodecta vitellinae* (L) *Aromia moschata* (L)	41,42 30
(E)	Quinones:-			
24	*Para*-Benzoquinone		*Brachinus crepitans* (L) *Tenebrio obscurcus* (Fab). *Blaps gigas* (L) *Diplopter punctata*. *Julus terrestris*. *Spirostreptus castaneus* (Atems).	43 44 45 46 47,48 49
25	2-Ethyl-1,4-benzoquinone		*Tribolium confusum* *Tribolium castaneum* (Herb). *Diaperis maculata* (Oliver) *Gnaptor spinimanus* (Pallas). *Blaps lethifera* (Marsh). *Blaps mortisaga* (L). *Blaps mucronata* (Lat).	50,51 50,52,53 46 44 44 44 44

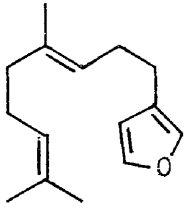
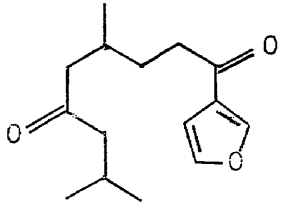
26	2-Methyl-1,4-benzoquinone		*Brachinus crepitans* (L) 43 *Tribolium confusum*. 50-53 *Tribolium castaneum* (Herb). 50-53 *Diaperis maculata* (Oliver) 46,53 *Diploptera punctata* 46 *Spirostreptus virgator* (Silvestri) 49 *Archiulus sabulosus* (L) 54 *Pachybolus laminatus* (Cook) 49 *Forficula auricularia* (L) 56
27	2,3-Dimethyl-1,4-benzoquinone		*Forficula auricularia* (L) 56 *Heteropachyloidellus robustus* (Roewer). 57,58
28	2,5-Dimethyl-1,4-benzoquinone		*Heteropachyloidellus robustus* (Roewer). 57,58

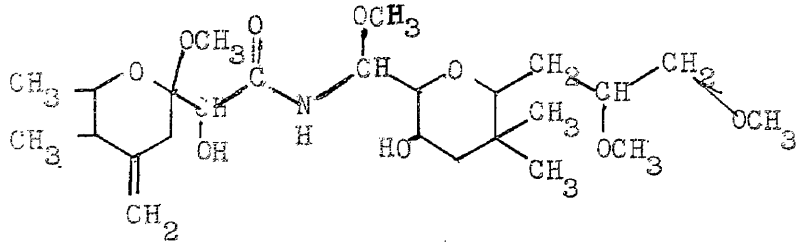
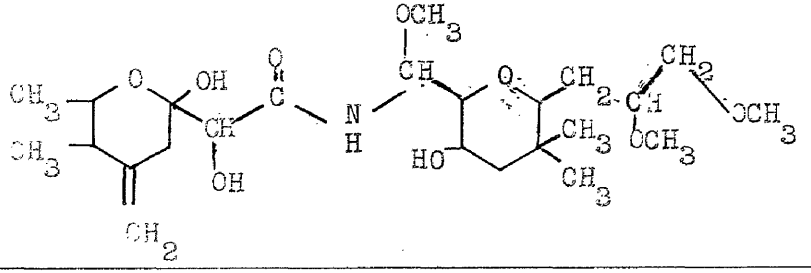
29	2,3,5-Trimethyl-1,4-benzoquinone		Heteropachyloidellus robustus (Roewer).	57,58
30	2-Methoxy-1,4-benzoquinone		Tribolium castaneum (Herb).	50,52,53
31	2-Methyl-3-methoxy-1,4-benzoquinone		Archiulus sabulosus (L)	54
(F)	Terpenoids:-			
	(1) Monoterpenoids			
32	Citral		Atta sexdens (Ford). Acanthomyops claviger (Roger)	59 60

33	Citronellal and Citronellol		Acanthomyops claviger. (Roger)	60
34	dl - Limonene		Myrmicaria natalensurfed.	61
35	∝-and β-pinene		Nasutitermes (Sp).	62

36	Iridomyrmecin		*Iridomyrmex humilis* (Myr).	34,63,64
37	Isoiridomyrmecin (*syn* Iridolactone, Matatabilactone).		*Iridomyrmex nitidus* *Dolichoderus scabridus* (Syn *Diceratoclinea scabridus*).	34 38
38	Iridodial		*Iridomyrmex detectus* (Fred Smith) *Iridomyrmex conifer* (Forel). *Iridomyrmex nitidiceps* *Tapinoma nigerrimum* (Nyl) *Dolichoderus scabridus*.	34 65 65 36 38

39	Dolichodial (syn Anisomorphol)		Iridomyrmex rufoniger Iridomyrmex myrmecodial. Dolichoderus clarki (Syn. Acanthoclinea clarki). Dolichoderus dentata (Syn. Acanthoclinea dentata). Anisomorpha buprestoides (Stoll).	38 38 38 38 66
40	Cantharidin		Numerous beetles of family Meloidae.	67

(2)	Sesquiterpenoids			
41	Dendrolasin		Lasius fulginosus (Lat). (Syn: Dendrolasius fulginosus). Lasius umbratus (Nyl). (Syn: Chronolasius umbratus).	68 69
42	Myroporone		Myroporum bentiodes (Gray)	70

(G)	Miscellaneous			
43	Pederin		Paederus fuscipes (Curt.).	71-74
44	Pseudopederin		Paederus fuscipes (Curt.).	71,74

After the manuscript of this section of the thesis had been completed an important review on ant secretions and venoms appeared, written by Cavill, Science, 1965, 149, 1337.

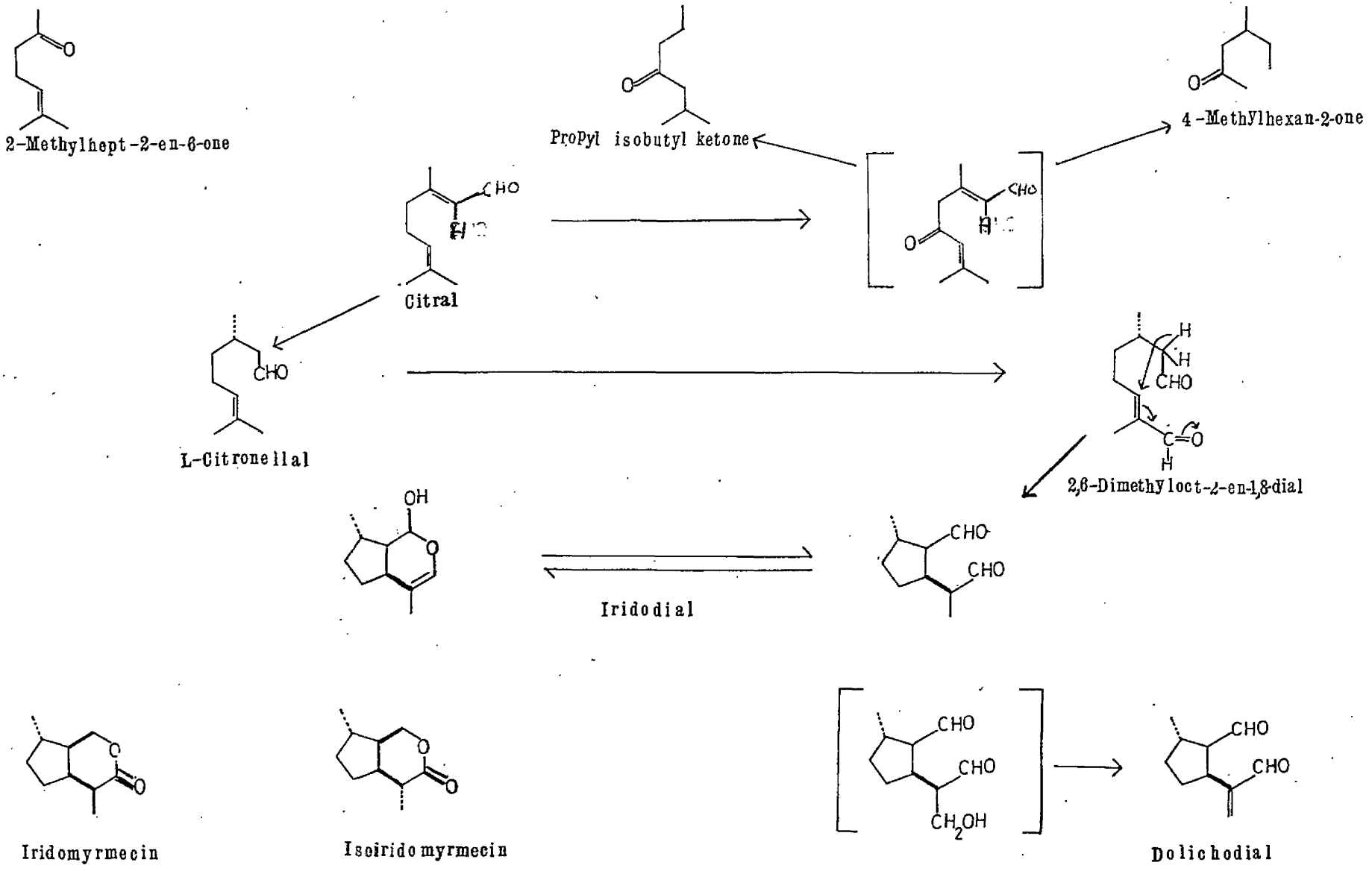
arise from populin and salicin ingested during feeding.[41]

The occurrence of quinonoid compounds in arthropod poisons, including spider[58] as well as insect poisons, is well established. A notable feature of these quinone poisons is that, unlike the quinones formed from vegetable tannins which are ortho-benzoquinones, all the examples of insect quinones known so far are para-benzoquinones. An interesting phenomenon occurs with the beetle Brachinus crepitans where a mixture of approximately equal parts of para-benzoquinone and 2-methyl-1,4- benzoquinone is explosively ejected by means of gas under pressure.[43]

Although they have been included as monoterpenoids in Table I, the isoprenoid origin of the cyclopentanoid insect toxins, iridomyrmecin, iso-iridomyrmecin (syn iridolactone), iridodial, anisomorphal and dolichodial has not been unequivocally proven.[79] However the recent successful laboratory synthesis of iridodial from L-(-) citronellal[80] might suggest that citral serves as the in vivo precursor[81] especially as citral has been obtained from the mandibular glands of the leaf cutting ant Atta sexdens,[59] although it has not so far been identified in members of the Dolichoderinae. Certainly the co-existence of methylheptenone, propyl isobutyl ketone and methyl hexanone with iridodial in the ants would not be inconsistent with citral acting as a biogenetic precursor of iridodial since these compounds could conceivably

Fig. A.

Scheme for the Biogenetic Origin of the Dolichoderine Ant Extractives. [adapted from Cavill and Hinterberger in International Congress for Entomology, Vienna, 1960, 53.]



also arise from citral as indicated in Fig. A. The evidence that the ant Acanthomyops claviger (Roger) incorporates labelled acetate and mevalonate into citral [81a] further suggests that the normal 'mevalonic acid' pathway of terpene biosynthesis is being utilised. [81b]

The pharmacology of iridomyrmecin has been investigated in detail by Pavan, [82] but it would appear to be without significant activity in higher organisms.

Another crystalline compound occurring in certain insects of the family Meloidae [67] is the potent vesicant cantharidin which has also been suggested to be terpenoid in origin [83]. Cantharidin has a marked toxicity for epithelial tissue, especially that of the kidneys [84], and in sufficient dosage it produces severe nephritis. Its use as an aphrodisiac in cattle breeding stemmed from its ability to induce a reflex erection by irritation of the urethral mucous membrane. [85] Cantharidin has pronounced irritant properties as indicated by its ability to produce vesication even in the skin of corpses [86] and recently a renewed interest has been shown in the biochemical mechanism of cantharidin acantholysis. [87] The inhibition of tumour induction by carcinogenic tar [88,89] may also be attributable to its irritant properties which results in a sloughing of the embryonic tumour.

Myroporone and dendrolasin differ from the other insect terpene toxins in being not monoterpenoids but sesquiterpenoids.

TABLE II

PEPTIDES AND PROTEINOGENIC AMINES IN INSECT VENOMS

No.	Substance	Occurrence	Ref.
1.	Acetylcholine	Vespa crabro (L)	92
2.	Cholinesterase	Vespula vulgaris (L)	93
3.	Histidine	Apis mellifera (L)	92
4.	Histamine	Apis mellifera (L)	94
		Vespula vulgaris (L)	95
		Vespa crabro (L)	96
5.	5-Hydroxytryptamine	Vespa crabro (L)	96,97
		Vespula vulgaris (L)	95,97
		Polistes gallicus	98
		Dolichovespula media	99
		Leiurus quinquestriatus	100
6.	Hyaluronidase	Apis mellifera (L)	92
		Vespula vulgaris (L)	93
		Bombus pratorum (L)	93
7.	(a) Kinin (b) New Kinin	Vespula vulgaris (L) Vespa crabro (L)	95,101 102
8.	Lecithin and Lecithinase	Apis mellifera (L)	103,104
9.	Phospholipase 'A'	Apis mellifera (L)	92
10.	Phospholipase 'B'	Vespula vulgaris (L)	105

Dendrolasin is further unique in being a selective contact insecticide, acting specifically on ants and having little or no action on certain beetles, bugs, orthoptera and other insects.[90]

In the ant Chthonolasius umbratus two non-isoprenoid substances undecane ($C_{11}H_{24}$) and methyl-n-undecyl ketone (CH_3-CO-$C_{11}H_{23}$) have been identified[69] and these doubtlessly represent two of the components of the insect wax. Other work on insect waxes has been well reviewed[91]. The chemical constitutions of pederin and pseudopederin have recently been reported.[74] Pederin exerts an anaesthetic effect on the skin[90] and acts as a powerful phytoinhibitor.

Table II lists various peptides, proteinogenic amines and allied compounds which have been found in insect venoms.

The toxic principles shown in Table II occur in a specific class of insect secretions referred to as venoms. These are usually injected into the victim by means of organs specifically adapted for the purpose e.g. the sting of the bee, but poisons of a protein nature[106] may also occur on appendages such as the urticating hairs.[107] These protein poisons of the urticating hairs produce erythema, vesication, urticaria, swelling and burning at the site of contact[108,109], as well as systemic responses which include paralytic symptoms, nausea and other reactions of an allergic nature. Owing to the complex chemical nature of the active principles of the venoms from the hairs or spines of various insects very little is as yet known of their detailed constitutions.

Wasp and bee venoms are especially complex and despite many years of work, little is known, other than that they contain peptides and proteinogenic amines.[110] So far bee venom has been shown to contain three components from electrophoretic studies. These are designated as:

(1) Fraction F_0 possessing no pharmacological activity.[111]

(2) Fraction F_1 which contains 13 amino acids, none of which contain sulphur.

and (3) Fraction F_2 containing the same 13 amino acids as fraction F_1 plus cystine-cysteine, histidine, methionine, phenylalanine and tyrosine.[112] In addition fraction F_2 is known to contain at least two enzymes-hyaluronidase and phospholipase A.[111]

Fraction F_1, which has also been designated melitin, largely accounts for the local and general toxicity. Melitin haemolyzes serum-free erythrocytes,[113] enhances the permeability of skin capillaries,[114] exerts a peripheral vasodepressor action[114,115] and produces respiratory paralysis,[116]

Fraction F_2 does not exert the above effects, but it appears to supplement them, chiefly through the two enzyme components, phospholipase A and hyaluronidase.[104]

The spreading factor hyaluronidase[117] is also present in wasp venom, and has been identified in Vespula vulgaris (L),[114] Vespa crabro (L)[118] and Polisters omissa (Weyrauch).[95] Wasp venom also contains 5-hydroxytryptamine and free amino

acids,[119] while the venom of the hornet V. crabro contains acetylcholine.[114,76] Jaques and Schachter[95] found in the venom of Vespula vulgaris a slowly dialyzable peptide, kinin, which is a potent hypotensive agent. A non-proteinaceous, strongly alkaline venom[120,121] is secreted by the fire ant Solenopsis solvissima Richteri.[122]

DISCUSSION

The Megoura viciae used in the present work was cultured on young Vicia faba plants in a glasshouse and harvested at intervals with the specimens being stored in chloroform until 160 gms had accumulated.[123,124] Previous studies had indicated the inability of Megoura viciae to synthesise and excrete melezitose, a trisaccharide believed to be toxic to certain insects,[125] and in the present work, paper chromatography confirmed the absence of this compound from the honeydew of our cultures.[123,124] In contrast to the earlier work, however, sucrose was the only sugar detectable in our honeydew indicating the absence of intestinal invertase and transglucosidase activities (known to be influenced by factors such as temperature[122,126]) in the aphid and simple excretion of sucrose in excess of nutritional requirements under the conditions of culture - it being well established[123] that sucrose is the sole sugar of plant phloem.

Unlike certain other aphids[127] Megoura viciae proved to be non-toxic to mammals as evidenced by the absence of any

discernible effect on mice or guinea pigs after oral administration of the whole dried carcasses, or of the unfractionated extractives separately obtained from the aphid with the solvents chloroform, acetone, ethanol and water.

The chloroform extractives of Megoura viciae consisted mainly of alkanes and fatty acid esters. The alkane fraction was separated from the other components for gas-liquid-chromatographic analysis through saponification of the esters, followed by treatment of the neutral fraction with 2,4-dinitrophenylhydrazine to remove ketonic material, and chromatography over alumina. Application of gas-liquid-chromatography to the alkane fraction so obtained as described by Eglinton et al[128] showed it to consist predominantly of C_{27}, C_{28} and C_{29}, normal hydrocarbons with an appreciable quantity of C_{27} and C_{28} isoparaffins. The full paraffin distribution pattern is summarised in Table III.

The alkanes of the leaf surface wax of the Vicia faba plants upon which the Megoura viciae had been feeding were analysed for comparison and the results are also shown in Table III. From the table it is clear that the alkane distribution patterns are quite different, thus parallelling the observations of Schreiber[129] with respect to the alkanes present in the larvae of the potato beetle, Leptinotarsa decemlineata Say, and in leaves of the potato plants on which they were feeding; although in his case the paraffins

TABLE III

Distribution In Mole Percentage Of The Alkanes* Of *Megoura viciae*

And Of The Leaf Surface Wax Of *Vicia Faba*

	Total Alkane Fraction‡	C_{23}		C_{24}		C_{25}		C_{26}		C_{27}		C_{28}		C_{29}		C_{30}		C_{31}		C_{32}		C_{33}	
		n	iso	n	iso	n	iso	n	iso	n	iso	n	iso	n	iso	n	iso	n	iso	n	iso	n	iso
Megoura Viciae	4%	3	-	2	1	3	1	7	2	16	16	16	6	21	-	6	-	-	-	-	-	-	-
Vicia Faba	3%		-	-	-	-	-	4	-	10	-	5	-	16	-	4	-	35	-	8	-	18	-

‡ Total percentage of alkane hydrocarbons calculated from total weight of petrol extractives

* The content of an individual alkane is expressed as a mole percentage of the total hydrocarbon content from C_{23} - C_{33}. The mole percentage is taken as being equivalent to the area percentage i.e. $100An/\sum_{23}^{33}An$, where An is the area of the peak corresponding to the hydrocarbon $C_nH_{(2n+2)}$ as measured by planimeter. The values are approximated to the nearest 1%. The branched alkanes are designated as 'iso'.

of the beetle larvae proved to be of surprisingly high molecular weight. It is noteworthy that branched chain alkanes are present in Megoura viciae but absent from the leaves of Vicia faba. This latter fact is also of interest since isoparaffins are absent from the leaf wax of the related plant Phaseolus aureus Roxb.[130] (the stringbean).

Saponification of the mixed fatty acid esters from Megoura viciae gave a mixture of carboxylic acids, which after conversion into the methyl esters by means of an excess of diazomethane in ether, was subjected to gas-liquid-chromatography on a butane-1,4-diol succinate polyester column. This showed the presence of seven peaks. Five of the seven esters so resolved were identified as methyl myristate, methyl palmitate, methyl stearate, methyl oleate and methyl linoleate (major constituent) by intensification of the appropriate peaks on addition of authentic material, while the linear plot of carbon atom number against log retention time for the saturated esters indicated the other two to be methyl pentadecylate and methyl margarate.

Acetylation of the total mixed aliphatic alcohols liberated in the saponification and application of gas liquid chromatography to the resulting mixture of acetates with appropriate intensification experiments, employing authentic n-alkyl acetates, showed the three alcohols present to be octan-1-ol, decan-1-ol and myristyl alcohol.

No organic bases or sterols were detected in the extractives from the aphid. The absence of sterols is of interest since insects are believed to be unable to effect the biosynthesis of steroids [131,132] and to rely on their food as sole source of sterols which are then converted into essential hormones, such as the juvenile hormone ecdysone [133-140] (2ξ,3β,14α,22,25-pentahydroxy-5β-cholest-7-en-6-one [141]) much in the same way as indogenous cholesterol is converted into various steroid hormones by mammals. Sucking insects such as Megoura viciae which feed on plant phloem might therefore be expected not to contain high percentages of sterols. Detailed studies by Schreiber et al [142] on the sterols of adult, larval and pupal forms of the Colorado potato beetle, Leptinotarsa decemlineata Say, have shown that the same sterols and triterpenoids are present in the insect as are present in the leaves of the food plant, but in different proportions - presumably reflecting a differential metabolism of these compounds by the insect.

Steam distillation of freshly killed specimens of Megoura viciae revealed the absence of volatile organic components as checked by extraction of the steam distillates with carbon tetrachloride followed by infra-red analysis.

The water-soluble and ethanol-soluble extractives from the aphid consisted mainly of amino acids, peptides and the two sugars D-glucose and D-ribose (identified by paper chromatography). The presence of D-glucose was further

confirmed by conversion into the penta-acetate which proved identical with an authentic sample.

Application of the standard tests[143] to living specimens of Megoura viciae showed the presence of aphin pigments, known to be without pronounced toxic properties.[144] In view of the difficulties in making positive identification of individual aphins[145], these compounds were not further investigated.

The present studies, therefore, have failed to establish the basis for the observed toxicity of Megoura viciae to the predatory insect Adalia decempunctata L. A possibility, which might not have been detected by the techniques presently employed, would be the secretion by Megoura viciae of a potent highly volatile toxic principle which was lost in the course of the chemical work-up, so that any further work on the problem should be so designed as to take this possibility into account.

A short account of this work has been published as a note in the Journal of the Chemical Society[146] and a reprint is included in the Appendix to this thesis.

EXPERIMENTAL

Megoura Viciae (260 g) in chloroform (200 ml) was homogenised in a Waring Blendor for 2 minutes. Kieselguhr (3g) was stirred into the mixture which was then filtered through a thin layer of kieselguhr. The filter cake was again extracted with chloroform (200 ml) in the Waring Blendor and

filtered as before. The combined chloroform extracts were centrifuged in order to permit separation from an aqueous phase originating from the body fluid of the Megoura viciae, and then taken to dryness to yield a residue of 10 g. The filter cake of kieselguhr and carcass material was successively extracted with ethanol, acetone and water to yield separate fractions of ethanol-soluble, acetone-soluble and water-soluble extractives, this last being combined with the aqueous body fluid solubles.

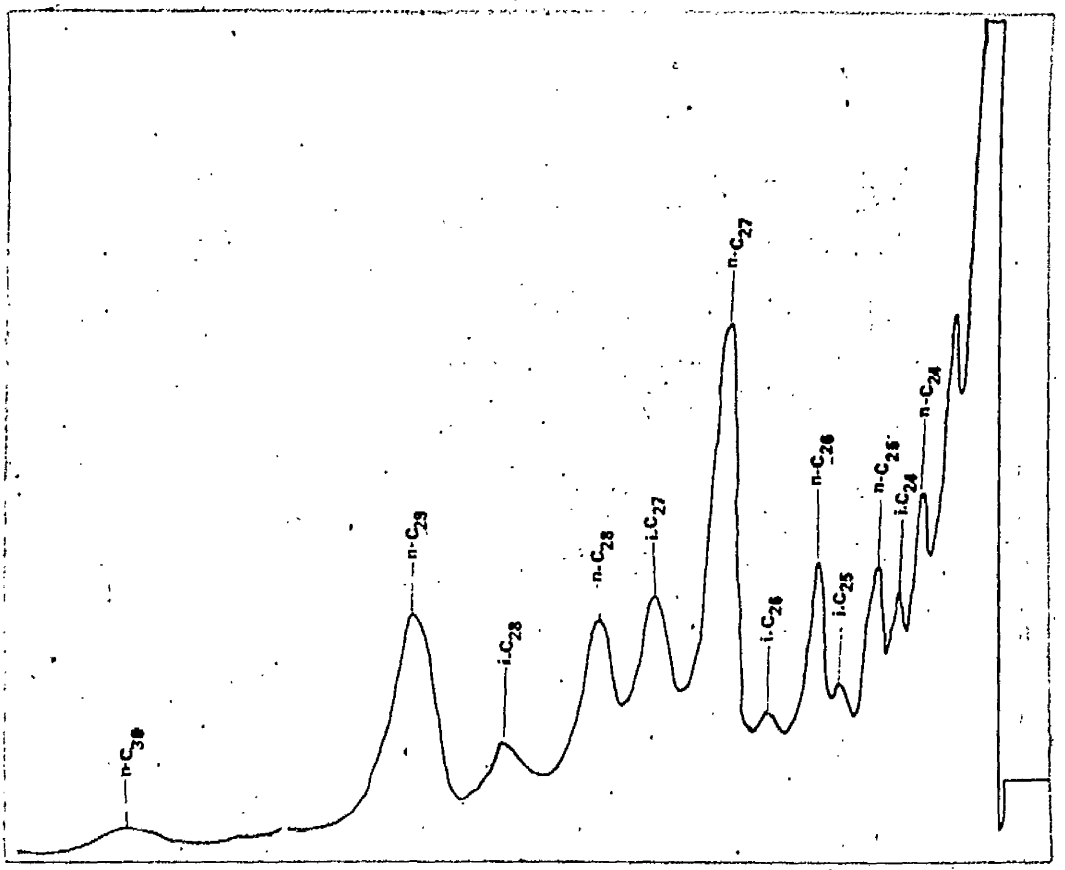
The chloroform-soluble material was completely soluble in light petroleum and consisted mainly of hydrocarbons and long-chain fatty esters.

Isolation of Alkanes.

The light petroleum-soluble fraction (200 mg) obtained from the chloroform extractives of Megoura viciae was refluxed with 2,4-dinitrophenylhydrazine (200 mg) and conc. HCl (0.2 ml) in ethanol (10 ml) for 2 hours, in order to convert any carbonyl compounds present, into the corresponding 2,4-dinitrophenylhydrazones. The solvent was then removed under reduced pressure, and the residue exhaustively extracted with redistilled light petroleum of b.p. 40-60°. The light petroleum-soluble material so obtained, after removal of solvent, was refluxed for 2 hours in aqueous ethanol (1:2, 10 ml) containing sodium hydroxide (0.2 g) in order to saponify the ester fraction present. The solution was then taken to dryness

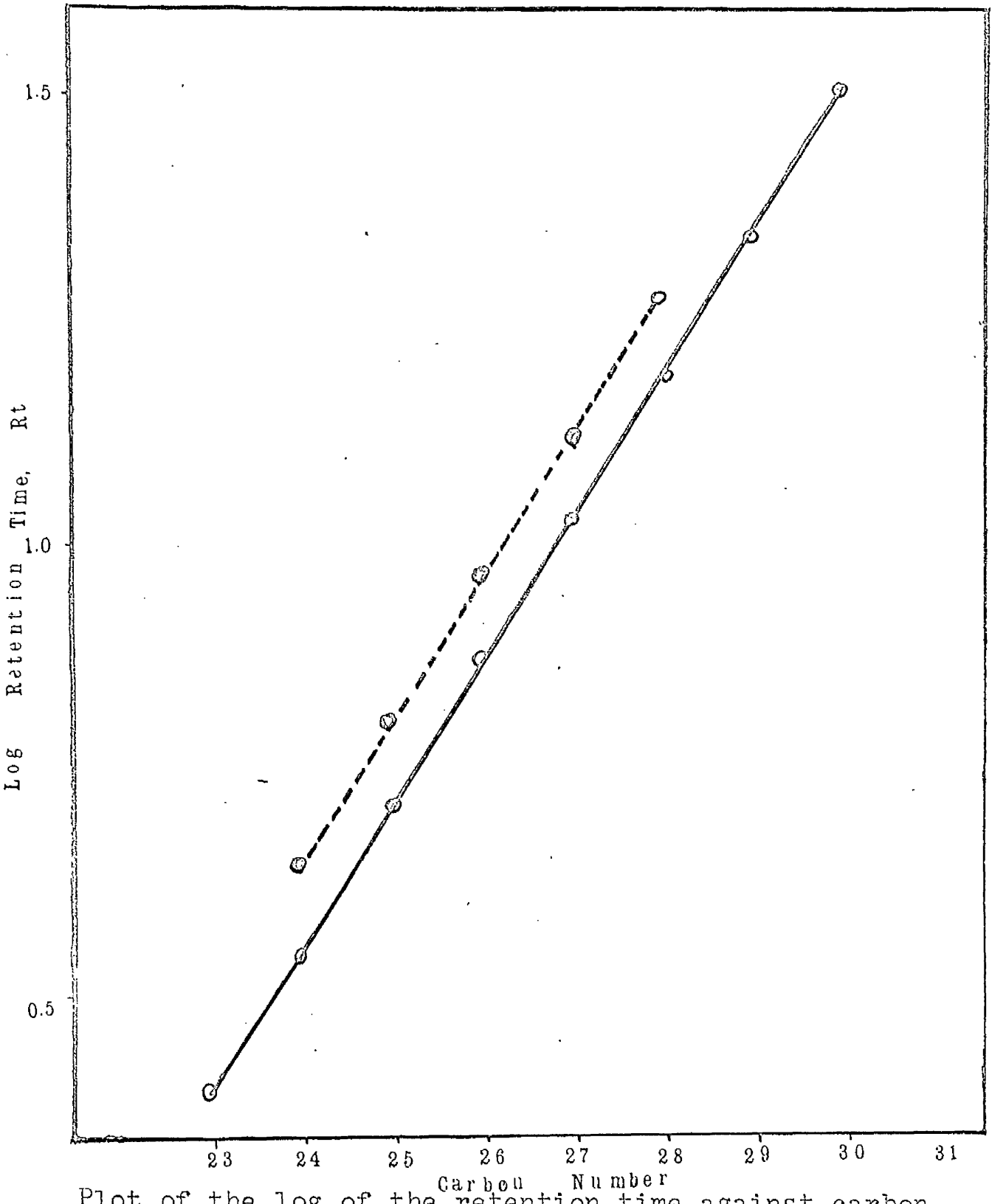
FIG. B.

Gas-Liquid Chromatogram* Of Hydrocarbon Fraction Derived From The Petroleum Extractives of Megoura viciae.



* Load 0.1 µl of a solution (ca 5%) of the total hydrocarbon fraction in AnalaR chloroform: column 0.5% Apiezon L on celite, 80 - 100 mesh; gas flow, 45 ml of argon per min; detector voltage 1250V, altenuation x 10.

FIG C



Plot of the log of the retention time against carbon number for the peaks assigned to the n-and iso-alkane series. Data from the gas-liquid chromatogram (Fig. 'B') of the hydrocarbon fraction of Megoura viciae. O-O=n-alkanes, O--O=iso-alkanes.

under reduced pressure and the residue thoroughly extracted with redistilled light petroleum of b.p. 40-60°. The resulting extract was filtered through alumina (Woelm, neutral 2 g) when the hydrocarbon fraction was found to be completely eluted with further light petroleum. Removal of solvent afforded mixed alkanes (9 mg) uncontaminated with compounds of any other chemical class, as shown by infra-red analysis.

Gas-Liquid-Chromatography of the Hydrocarbon fraction.

The instrument used was a 'Pye Argon' gas chromatograph fitted with a 90Sr detector and a 120 x 0.5 cm. column of celite (80 - 100 mesh) coated with 0.5 per cent Apiezon-L grease deposited from light petroleum of b.p. 60-80° C. The hydrocarbon mixture (ca.2 mg) obtained from Megoura viciae as outlined above, was dissolved in hexane (ca. 50 mℓ) and applied to the heated column (ca. 225° C) in a 0.1 μl load. In order to check the reproducibility, several separate runs were made and the results of one such run are summarised in Fig. 'B'. This shows 13 peaks of which 8 were concluded to be n-alkanes of carbon atom numbers 23 to 30 since the intensification of the appropriate peaks on addition of authentic C_{27} and C_{29} alkanes to the mixture, permitted assignment of carbon atom number to the linear plot, of log retention time (Rt) against carbon atom content (Fig. C). The remaining peaks fell on a parallel straight line in the

plot of log retention time against carbon atom content (Fig. C) and were consequently attributed to the isoalkanes C_{24} to C_{28}. This conclusion was confirmed by addition of authentic C_{27} and C_{29} isoalkanes to the alkane mixture from Megoura viciae, and a plot of log retention time against carbon atom number.

Esters:- Light petroleum-solubles (200 mg) from the chloroform extractives of Megoura viciae were refluxed for 18 hours, with sodium hydroxide (4 g) in ethanol (30 ml) and water (14 ml). After removal of the solvents under reduced pressure, the residue was treated with water and extracted repeatedly with ether (8 x 100 ml). The combined ether extracts were then dried over anhydrous Na_2SO_4, filtered and the solvent evaporated. The ether soluble material (62 mg) thus obtained was acetylated by heating on steam bath with acetic anhydride (2 ml) for one hour. The reaction mixture was cooled, poured into ice cold water and the resulting solution extracted with ether (100 ml). The ethereal layer was filtered through alumina (Woelm neutral 5 g) to give a mixture of acetates (40 mg) which were then subjected to gas-liquid-chromatography on a 'Pye Argon' gas chromatograph using a 120 x 0.5 cm column of celite (80 - 100 mesh) coated with 10% butane-1,4-diol polysuccinate at 175°C. This showed the acetate mixture to consist of 3 components. That they were the acetates of octan-1-ol, decan-1-ol and myristyl

alcohol, was shown by the intensification of the appropriate peak on addition of the authentic esters, one at a time, to the original mixture and further gas-liquid chromatography after each addition.

The basic aqueous solution from the saponification of the light petroleum extractives was acidified with 6.N.HCl and extracted with ether. The ethereal solution was then dried over anhydrous Na_2SO_4 and the solvent removed under reduced pressure to yield a mixture of long chain fatty acids (115 mg) showing the characteristic split $(CH_2)_4$ peak at 725 cm^{-1} in the infra red. The acids were taken up in anhydrous ether and treated with an excess of an ethereal solution of diazomethane to yield their methyl esters (95 mg).

The methyl ester mixture when subjected to gas-liquid chromatography on a Pye Panchromatograph instrument using a butane -1,4-diol succinate polyester column at 175°C was resolved into seven major components. Of these, five were shown to be methyl myristate, methyl palmitate, methyl stearate methyl oleate and methyl linoleate by intensification experiments with added authentic specimens. The linear plot of carbon atom content against log retention time for the saturated esters indicated the two remaining (minor) components to be methyl pentadecanoate and methyl margarate.

Sugars:- The ethanol-soluble (0.5 g) and water-soluble fractions (1.2g) of Megoura viciae were separately concentrated

and shown to consist mostly of sugars as evidenced by infra-red analysis and paper chromatography. Chromatography of the concentrated ethanolic and aqueous extracts together with selected sugars on Whatman No. 1 filter paper using butanol-acetone-water (2:7:1) as developing solvent[147] and aniline-diphenylamine phosphate as detecting reagent[148] indicated the presence of D-glucose and D-ribose. Admixture of authentic D-glucose and D-ribose to the aqueous and ethanolic extracts followed by further chromatography under the same conditions confirmed the identity of the aphid sugars.

Aqueous extractives (1g) from Megoura viciae were acetylated by refluxing in acetic anhydride (25 ml) in the presence of zinc chloride (1g) for 3 hours. The reaction mixture was poured into 250 ml of water and the crude crystalline material which separated was collected by filtration and washed with water. The residue (0.8 g) was then chromatographed on alumina (15 g) (Woelm, acid). Elution with benzene afforded a crystalline solid (0.6 g) which was recrystallised from methanol as white needles of D-glucose pentaacetate m.p. 109°C (lit.[149] for D-glucose-pentaacetate, 110°C). There was no melting point depression on admixture with authentic material. The infra-red spectra were identical.

Honeydew of Megoura viciae:-

Honeydew excreted by Megoura viciae was collected

by means of micropipettes and chromatographed on Whatman No. 1 filter paper using butanol-acetone-water (2:7:1) as a developing solvent[147] and aniline-diphenylamine phosphate as a detecting reagent.[148] A single spot only was observed and that this was sucrose was shown by comparative paper chromatography.

Examination of Megoura viciae for the presence of aphin pigments:-

Tests for the presence of aphin pigments were performed by the method of Todd and his co-workers.[143]

Thus living Megoura viciae (20 individuals) were crushed and stirred in 80% aqueous acetone (V/V-0.5 ml) in a small test tube. Light petroleum (0.5 ml) was added and the mixture shaken, centrifuged and the two layers separated- the aqueous layer being designated 'A' and the light petroleum layer being designated 'B'. A second similar sample of insects was crushed in phosphate buffer pH 6.5 (0.3 ml) and the mixture kept for 3 minutes. Acetone (0.5 ml) and light petroleum (0.5 ml) were added and the aqueous and light petroleum layers separated as before - the aqueous layer being designated 'C' and the light petroleum layer 'D'.

Layer 'A' was non-fluorescent and its red colour was reversibly changed to yellow on acidification. Layers 'B' and 'C' contained no pigments. Layer 'D' was yellow,

showing a greenish yellow fluorescence, and exhibited absorption bands at 4330, and 4620 A°. On the addition of cold formic acid the solution gave a yellow fluorescence (absorption bands at 4570 and 4880 A°). Addition of concentrated HCl to layer 'D' gave an orange fluorescence with absorption bands at 5890, 5640, 5210 and 4520 A°. These tests therefore show that aphin pigments are present in Megoura viciae. In view of the difficulties in making positive identification of individual aphins[145] and of the known non-toxic nature[144] of these compounds they were not investigated further.

Isolation and identification of alkanes from Vicia faba.

Leaves of Vicia faba (10 g) were dipped for 30 seconds in each of three successive volumes (20 ml, 10 ml, and 15 ml) of chloroform. The combined chloroform extracts so obtained were filtered to remove suspended matter and the filtrate evaporated to dryness under reduced pressure. The residue (250 mg) was found to consist mainly of hydrocarbons and fatty acid esters.

Isolation of the alkane fraction (6 mg), uncontaminated with compounds of other chemical groups, was achieved by an identical procedure to that employed in the isolation of the alkanes from Megoura viciae, using 200 mg of total chloroform extractives. Gas-liquid chromatography (under the same conditions as described for the alkanes from Megoura viciae)

showed the alkane fraction of *Vicia faba* to consist solely of the normal alkanes from C_{26} to C_{33}.

BIBLIOGRAPHY

1. Dixon, Trans. Roy. Entomol. Soc., London, 1958, 110, 319.

2. cf. Sylvester, in Biological Transmission of Disease Agents Ed. K. Maramorsch, Academic Press, New York, 1962, p. 11.

3. Maramorsch, Ann. Rev. Entomol., 1963, 8, 369.

4. cf. Kazda, Experientia, 1962, 18, 270.

5. Quilico, Grunanger, and Pavan, XI Inter. Cong. Entomol., Vienna, 1960, p. 66.

6. Shackelford, Michalowicz, and Schwartzman, J. Org. Chem., 1962, 27, 1631.

7. Boch and Shearer, Nature, 1964, 202. 320.

8. Rothschild, XI Inter. Cong. Entomol., Vienna, 1960, p. 257.

9. Ernst, Rev. Suisse de Zool., 1959, 66, 289.

10. Pavan, Mem. Soc. Ent., (Ital.), 1951, 30, 107.

11. Denhan, Insect Life, 1888, 1, 143.

12. Chopard, Nature (Paris), 1962, No. 3325, 206.

13. Vialli, Rev. Biol. Colon., 1939, 2, 273.

14. Osman and Brander, Z. Naturforschg., 1961, 16b, 749.

15. Poulton, Trans. Entomol. Soc., London, 1866, p. 137.

16. Latter, Trans. Entomol. Soc., London, 1892, 287.

17. Stumper, C.R. Acad. Sci., 1952, 234, 149.

18. Pavan, Ric. Sci., 1956, 26, 144.

19. Quilico, Fiozzi and Pavan, Ric. Sci., 1956, 26, 177.

20. Schildknecht and Weis, Z. Naturforschg., 1961, 16b, 361.

21. Eisner, Meinwald, Monroe and Ghent, XI Inter. Cong. Entomol., Vienna, 1960, p. 110.

22. Blum, Ann. Entomol. Soc., Amer., 1961, 54, 410.

23. Butler, Callow and Chapman, Nature, 1964, 201, 733.

24. Schildknecht and Weis, Z. Naturforschg., 1962, 17 b, 439.

25. Roth, Niegisch and Stahl, Science, 1956, 123, 670.

26. Blum, Crain and Chidester, Nature, 1961, 189, 245.

27. Butenandt and Tant, Z. Physiol. Chem., 1957, 308, 277.

28. Villai, Devakul and Maarse, Anal. Biochem, 1964, 7, 269.

29. Gilby and Waterhouse, Austral. J. Chem., 1964, 17, 1311.

30. Hollande, Ann. Unive. Grenoble., Sect. Sci., Med., 1909, 21, 459.

31. Blum and Traynham, XI Inter. Cong. Entomol., Vienna, 1960, p. 48.

32. Blum, Warter, Monrose and Chidester, J. Insect Physiol., 1963, 9, 881.

33. Cavill, Ford and Locksley, Chem. and Ind., 1956, 465.

34. Cavill, Ford and Locksley, Austral. J. Chem. 1956, 9, 288.

35. Cavill, Ford and Locksley, Austral. J. Chem., 1957, 10, 352.

36. Trave and Pavan, Chim. e. Indstr., 1956, 38,1015.

37. Pavan and Trave, Insects Sociauz., 1958, 5, 299.

38. Cavill and Hinterberger, Austral. J. Chem., 1960, 13, 514.

39. Schildknecht and Weis, Z. Naturforschg., 1962, 17b, 448.

40. Schildknecht, Holoubek and Wolkenstoerfer, Z. Naturforschg., 1962, 17b, 81.

41. Pavan, IV Inter. Cong. Biochem., 1958, p. 15.

42. Eisner, Swithenbank and Meinwald, Ann. Entomol. Soc., Amer., 1963, 56, 37.

43. Schildknecht, Angew. Chem., 1957, 69, 62.

44. Schildknect and Weis, Z. Naturforschg., 1960, 15b, 757.

45. Moreau, Bull. Soc. Linn. Provence., (Marseille)., 1932, 5, 34.

46. Roth and Stay, J. Insect Physiol., 1958, 1, 305.

47. Phisalix and Behal, Bull. Soc. Chim., 1900, 25, 88.

48. Phisalix, Animaux Venimeux et Venins. Masson., Paris, 1922.

49. Barbier and Leaderer, Biochimia, 1957, 22, 236.

50. Hackman, Pryor and Todd, Biochem. J., 1948, 43, 474.

51. Roth and Howland, Ann. Entomol. Soc., Amer., 1941, 34, 151.

52. Alexander and Barton, Biochem. J., 1943, 37, 463.

53. Laconti and Roth, Ann. Entomol. Soc. Amer., 1953, 46, 281.

54. Trave, Garate and Pavan, Chim. Industr., 1959, 41, 19.

55. Schildknechtand Weis, Z. Naturforschg., 1961, 16b, 810.

56. Schildknecht and Weis, Z. Naturforschg., 1960, 15b, 755.

57. Eastable, Ardao, Brasil and Fieser, J. Amer. Chem. Soc., 1955, 77, 4942.

58. Fieser and Ardao, J. Amer. Chem. Soc., 1956, 78, 774.

59. Butenandt, Linzen and Lindaur, Naturwiss., 1959, 46, 461.

60. Chadha, Eisner, Monro and Meinwald, J. Insect Physiol., 1962, 8, 175.

61. Grunanger, Quilico and Pavan, Lincei, Rend. Sc. Fis. Mar e Nat., 1960, 28, 293.

62. Moore, J. Insect Physiol., 1964, 10, 371.

63. Pavan and Nascimbene, Boll. Soc. Med. Chiv. Pavia., 1948, 72, 193.

64. Fusco, Trave and Vercellone, Chim. e Industr., 1955, 37, 251.

65. Cavill and Ford, Austral. J. Chem., 1960, 13, 296.

66. Meinwald, Chadha, Hurst and Eisner, Tetrahedron Letters, 1961, nol, 29.

67. Dixon, Martin-Smith and Smith, Canad. Pharmaceutical. J., 1963, 96, 501.

68. Quilico, Piozzi and Pavan, Ric. Scientifica., 1956, 26, 177.

69. Quilico, Piozzi and Pavan, Tetrahedron, 1957, 1, 177.

70. Kubota and Matsura, Chem. And. Ind., 1957, 491.

71. Pavan and Bo, Physiol. Comp. et. Oecol., 1953, 3, 307.

72. Quilico, Cardani, Ghiringhelli and Pavan, Chim. ed Ind., (Milan), 1961, 43, 1434.

73. Pavan and Bo, Mem. Soc. Entom., (Italy) 1952, 31, 67.

74. Cardani, Ghiringhelli, Mondelli and Quilico, Tetrahedron Letters, 1965, No. 29, 2537.

75. Stumper, Naturwiss, 1960, 47, 457.

76. Weckering, XI Inter. Cong. Entomol., Vienna, 1960, 3, 102.

77. Butenandt and Hecker, Angew. Chem., 1961, 73, 349.

78. Watnabe, Nature, 1958, 182, 325.

79. Valenta, Wiesner, Babier, Bogri, Forrest, Fried and Reinshagen, Experientia, 1962, 18, 111.

80. Clark, Frav, Jaeger and Robinson, Tetrahedron, 1959, 6, 217.

81. cf. Ruzicka. Experientia, 1953, 9, 357.

81a. Happ and Meinwald, J. Amer. Chem. Soc., 1965, 87, 2507.

81b. Richards and Hendrickson 'The Biosynthesis of Steroids, Terpenes and Acetogenins' Ed. W.A. Benjamin Inc. New York.

82. Pavan, Arch. Int. Pharmacodyn, 1952, 89, 223.

83. Martin-Smith and Khatoon, Progress In Drug Research, Ed. E. Jucker. 1963, 6, 279.

84. Sakamato, Proc. Jap. Pharmacol. Sci., 1933, 7, 118.

85. Viehoever and Cohen, Amer. J. Pharm., 1938, 110, 226.

86. Hahn and Jaeger, Klin. Wschr., 1931, 10, 489.

87. Weakley and Einbinder, J. Invest. Dermat., 1962, 39, 39.

88. Berenblum, J..Path. Bact., 1935, 40, 359.

89. Dubois and Ball, Bull. Acad. Med., Paris, 1933, 110, 791.

90. Pavan, Significato, Chemico, e Biologico. Dialginni, Veleni Di Insette., (Pavia), 1958, 1, 1.

91. Eglinton and Hamilton, in Chemical Plant Taxonomy Ed. T. Swain, Academic Press, New York, 1963, p, 197.

92. Newmann and Habermann, Arch. Exp. Path. Pharmak., 1954, 222, 367.

93. Jaques, Helv. Physiol. Acta, 1955, 13, 113.

94. Slotta and Borchert, Mem. Inst. Butantan, 1954, 26, 279.

95. Jaques and Schachter, Brit. J. Pharmacol., 1954, 9, 53.

96. Bhola, Calli and Schachter, J. Physiol., London, 1960, 151, 35.

97. Michl, ML. Chem. 1957, 88, 70.

98. Erspamer, Medicina, (Pharma)., 1955, 5, 1.

99. Pavan, Chim. Industr., 1955, 37, 714.

100. Adam and Weiss, Nature, 1956, 178, 421.

101. Schachter and Thain, Brit. J. Pharmacol., 1954, 9, 352.

102. Bhola, Calli and Schachter, J..Physiol., London, 1961, 159, 167.

103. Flury, Arch. Exp. Path. Pharmak., 1920, 85, 319.

104. Neumann, Habermann and Hansen, Arch. Exp. Path. Pharmak., 1953, 217, 130.

105. Contardi and Latzer, Biochem. Z., 1928, 197, 222.

106. Jones and Miller, Arch. Dermatol., 1959, 79, 81.

107. Kephart, J. Parasitol., 1914, 1, 95.

108. Bishopp, U.S. Dept. of Agri. Cir. No., 228, 1923.

109. Randel and Doan, in Venoms., Buckley and Progress Ed. Amer. Assoc. Advance. Sci. Pub., No. 44 Washington, D.C., 1956, p.111.

110. Lauter and Vrla, J. Econ. Entomol., 1939, 32, 806.

111. Neumann and Habermann, Arch. Exptl. Pathol. Pharmakol., 1954, 222, 367.

112. Fischer and Dorfel, Biochem. Z., 1953, 324, 465.

113. Habermann and Neumann, Z. Physiol. Chem., 1954, 297, 179.

114. Neumann and Habermann, in Venoms Buckley and Progress, Ed. Amer. Assoc. Advance Sci.Pub. No. 44 Washington D.C., 1956, p. 171.

115. Habermann, Arch. Exptl. Pathol. Pharmakol., 1954, 222, 173.

116. Hodgson, Bee World, 1955, 36, 217.

117. Jaques, in Venoms, Buckley and Progress Ed. Amer. Assoc. Advance Sci Pub. No. 44. Washington D.C., 1956, 291.

118. Said, Bull. Soc., Entomol., (Egypt), 1960, 44, 167.

119. Kaiser and Michel, Die Biochemie Der Tiertschen. Gifte., Franz. Deuticke, Vienna, 1958, p. 256.

120. Blum and Callahan, XI Inter. Cong. Entomol., Vienna, 1960, p. 290.

121. Blum, Walker, Callahan and Novak, Science, 1958, 128, 306.

122. Caro, Derbes and Jung, Arch. Dermatol., 1957, 75, 475.

123. Von Dehn, Z. Vergliech Physiol., 1961, 45, 88.

124. Ehrhardt, Z. Vergliech Physiol., 1962, 46, 169.

125. Zoebelein, Z. Angew. Entomol., 1956, 39, 129.

126. Auclair, Ann. Rev. Entomol., 1963, 8, 439.

127. Volker, Frohner's Lehrbuch Der Toxikologie Tierärzte, 6th Ed. Stuttgart, Ferdinand Enke. Verlag., 1950, p. 386, and References cited.

128. Eglinton, Gonzalez, Hamilton and Raphael, Phytochemistry, 1962, 1, 89.

129. K..Schreiber, Personal Communication.

130. Wanless, King and Ritter, Biochem. J., 1955, 59, 684.

131. Gilmour, Biochemistry of Insects, New York, Academic Press, 1961, p. 225.

132. Clayton, J. Lipid Res., 1964, 5, 3.

133. Karlson, Hoffmeister, Hoppe and Huber, Ann. Chem., 1963, 622, 1.

134. Karlson and Hoffmeister, Z. Physiol. Chem., 1963, 331, 298.

135. Kopec, Biol. Bull., 1922, 42, 323.

136. Wigglesworth, Quart. J. Microscop. Sci., 1934, 77, 191.

137. Fraenkel, Proc. Roy. Soc., 1935, B118, 1.

138. Fukuda, Proc. Imp. Acad., Japan, 1940, 16, 414.

139. Williams, Biol. Bull., 1947, 93, 89.

140. Butenandt and Karlson, Z. Naturforschg., 1952, 7b, 80.

141. Hoppe and Huber, Chem. Ber., 1965, 98, 2353.

142. Von Ardenne, Osske, Schreiber, Steinfelder and Tummler, J. Insect Physiol., 1965, 11, 1365.

143. Duewell, Humann, Johnson, Macdonald and Todd, J..Chem. Soc., 1950, 3304.

144. Lord Todd, Personal Communication.

145. Humann, Johnson, Macdonald and Todd, J. Chem. Sco., 1950, 477.

146. Dixon, Martin-Smith and Subramanian, J. Chem. Soc., 1965, 1562.

147. Giri and Nigam, Naturwiss., 1953, 40, 343.

148. Buchan and Savage, Analyst, 1952, 77, 401.

149. Hudson and Dale, J. Amer. Chem. Soc., 1915, 37,1264.

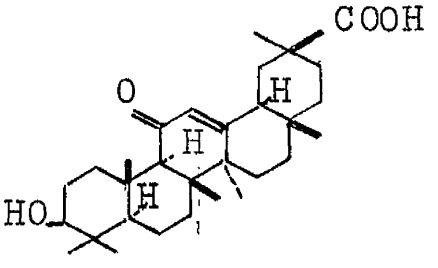
SECTION II

TRITERPENOID FROM *GAULTHERIA SUBCORYMBOSA* Col. AND *GAULTHERIA ANTIPODA*.

INTRODUCTION

On the basis of what were considered to be reliable reports[1] that the native New Zealand shrub Gaultheria antipoda (common name, snowberry; family Ericaceae) contained in active galactogenic principle, quantities of twigs and leaves from this plant were collected in New Zealand in 1959 by Dr. M. Martin-Smith and brought to these laboratories for detailed chemical investigation of the organic constituents present. At the same time the opportunity was taken to collect quantities of the more readily available related species, Gaultheria subcorymbosa, although there appeared to be no reports that this species had ever been employed by the Maori as a galactogen as was the case with Gaultheria antipoda.[1]

Preliminary investigations[2] indicated that both plants, in common with other members of the Ericaceae[3-9], contained appreciable quantities of a triterpene acid fraction. This suggested, by analogy with the established ability of the triterpene glycyrrhetinic acid (I) to intensify the action of glucocorticoid hormones[10-13] (by preventing their enzymatic



I

conversion into inactive compounds[14]) that the reputed galactogenic activity of Gaultheria antipoda might possibly reside in a triterpenoid capable of interfering with the action of steroid hormones involved in the control of lactogenesis. Although still not fully understood, milk secretion is a complex process[15-18] which would appear to be influenced both by protein hormones secreted by the anterior pituitary gland and by steroid hormones secreted by other endocrine glands. There is thus at least a superficial parallel to other physiological control mechanisms involving a complex interaction of pituitary protein hormones and steroid hormones, such as the control of carbohydrate metabolism (ACTH from the pituitary; hydrocortisone from the adrenal cortex) or the control of the female menstrual cycle (follicle stimulating hormone and luteinizing hormone from the pituitary; steroidal oestrogens and progesterone from the ovary). In the case of lactogenesis there would appear to be primary control by a lactogenic hormone complex secreted by the anterior pituitary gland,[15] of which the most important component has been variously termed prolactin,[19] galactin,[20] mammotrophin,[21] lactogen,[22] and leutotrophin,[23] and a secondary influence by ovarian and adrenocortical steroids.[15]

As in the control of the female cycle it would appear that relative concentrations of different steroids as well as the absolute concentration of any one steroid play an

important role in the 'feedback' process leading to the release of prolactin. Thus secretion of prolactin appears to be promoted by low blood levels of oestrogen and inhibited by high blood levels of oestrogen or by low blood levels of oestrogen in the presence of high concentrations of progesterone.[24,25] Hence in addition to the possibility that it could procure the direct release of prolactin in its own right, any galactogenic triterpenoid present in Gaultheria antipoda could conceivably produce its effect by securing a critical change in the normal absolute and relative concentrations of oestrogens and progesterone. As with glycyrrhetinic acid, this could be effected through the inhibition of the destruction of one or other of these steroid hormones, or it could be effected by a direct antimetabolite action in which either oestrogen or progesterone is prevented from acting on the anterior pituitary gland.

The main function of cortical steroids in lactation is still uncertain,[15] but it is considered that they may be responsible for securing the necessary levels of milk precursors in the blood. It is conceivable therefore that any galactogenic principle from Gaultheria antipoda could also exert its influence by affecting the action of the adrenocortical steroids in some way.

With these considerations in mind, attention was

concentrated on characterising the components present in the triterpene acid fractions from both Gaultheria antipoda and Gaultheria subcorymbosa.

DISCUSSION

The more abundantly available Gaultheria subcorymbosa was worked up for triterpene acids by two separate procedures as a check against failure to detect individual components in any single procedure.

In the first method, the finely ground dried leaves and twigs were exhaustively extracted with hot ethanol and the resulting solid extractives extracted in turn with light petroleum and chloroform. After unsuccessful attempts to fully purify the triterpene acid or acids in the light petroleum and chloroform extractives by means of crystallisation, the crude acid fractions were treated with an ethereal solution of diazomethane in order to obtain the corresponding methyl esters which were then subjected to purification by alumina column chromatography.

In the second procedure, the total ethanolic extractives were treated directly with diazomethane and the resulting

mixture then subjected to alumina column chromatography when the triterpene methyl ester fractions eluted in crystalline form with light petroleum/ether (1:1) after preliminary elution of alkanes and long chain fatty esters with light petroleum.

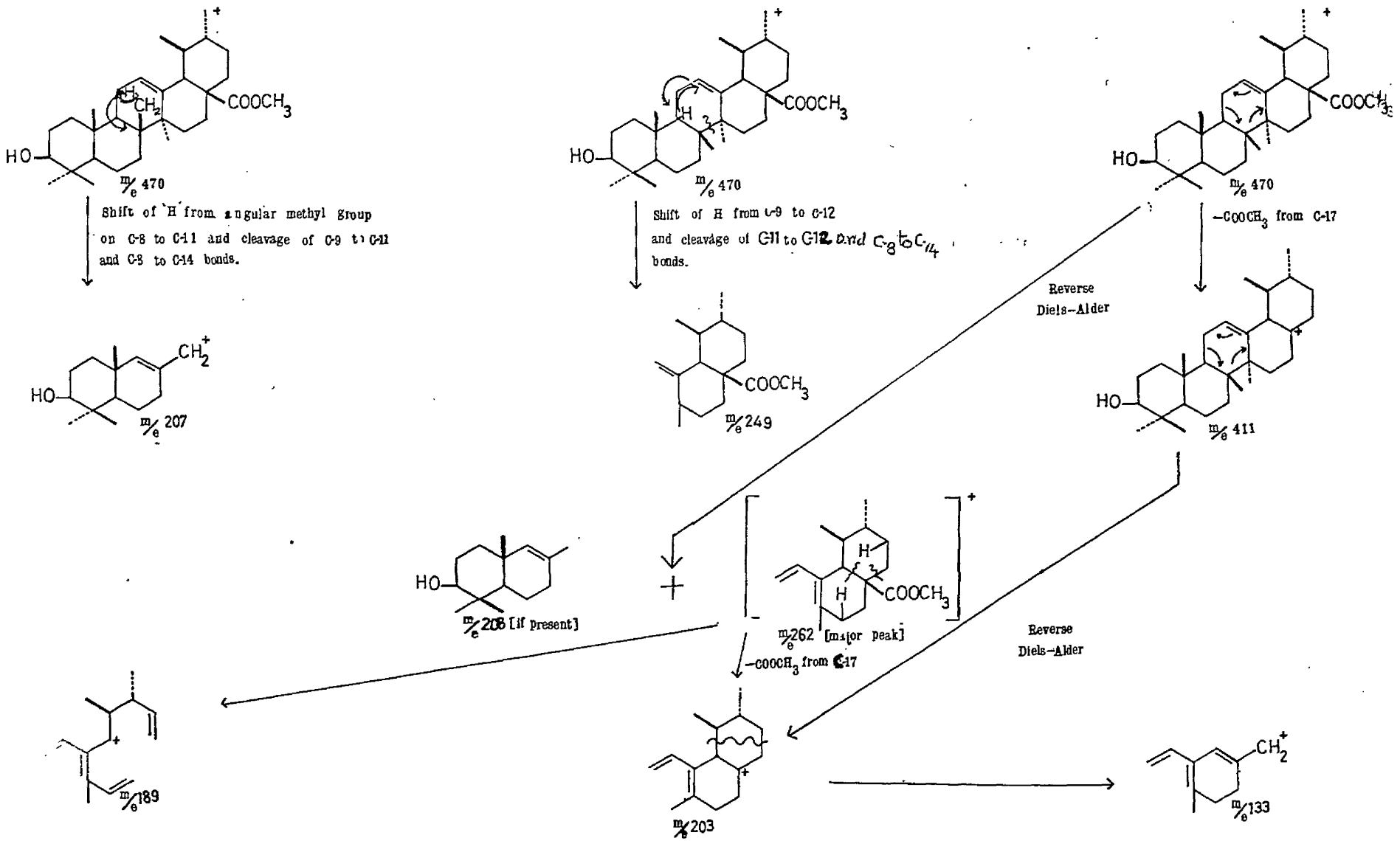
In the case of the less abundant Gaultheria antipoda the finely ground dried leaves and twigs were exhaustively extracted with hot chloroform and the total extractives treated with diazomethane before application of alumina column chromatography which afforded the triterpene methyl ester fraction in crystalline form.

All triterpene methyl ester fractions obtained from G. subcorymbosa in both procedures showed marked variation in melting points on crystallisation from different solvents, although they appeared homogenous on thin layer chromatography. Thus it was decided to examine them by mass spectrometry in an endeavour to utilize the established diagnostic mass spectral fragmentation patterns of different triterpenoids as a criterion of both purity and identity. 26-30

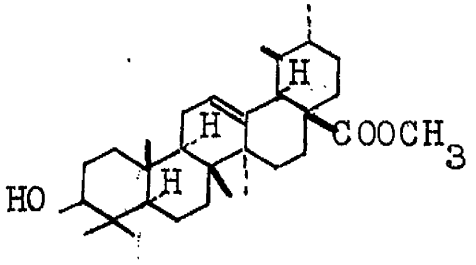
In the event, mass spectrometry, kindly carried out by Mr. T.A. Bryce, to whom the present author wishes to express

Fig.1

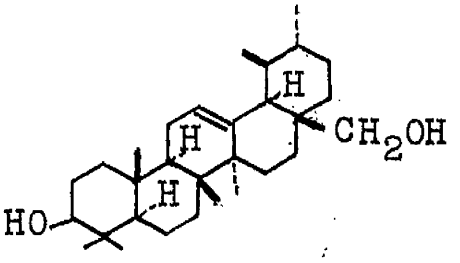
MASS SPECTRAL FRAGMENTATION OF METHYL URSOLATE. [after Budzikiewicz, Wilson and Djerassi. J.Amer.Chem.Soc., 1963, 85, 3688.]



his sincere appreciation, showed that all the triterpene methyl ester fractions obtained from both the chloroform-soluble and light petroleum-soluble extractives of Gaultheria subcorymbosa gave identical spectra (within the normal variation to be expected from factors such as temperature differences within the mass spectrometer), thus indicating that different solvations and/or different crystalline forms of the same compound had been giving rise to the observed differing melting points. Moreover, these spectra were identical with that reported for methyl ursolate (II)[28,31], with a parent molecular peak of 470 mass units (corresponding to $C_{31}H_{50}O_3$) and prominent peaks at m/e 411, 262, 249, 207, 203, 189 and 133. The origin of these peaks has been fully discussed by Djerassi and his colleagues[29] and is shown schematically in Fig. 1.



II



III

Indication that this scheme is indeed correct is provided by the appearance of metastable ions corresponding to the appropriate transitions, e.g. m/e 262 ⟶ m/e 203, metastable ion at $\frac{203^2}{262}$ = 157.3.

Further confirmation of the identity of the methyl ester was obtained by comparison of its infra red spectrum with that of authentic material. Acetylation of the methyl ester obtained from the Gaultheria subcorymbosa by means of acetic anhydride in pyridine gave material, identical with authentic O-acetyl methyl ursolate (mixed melting point, infra red spectrum, $[\alpha]_D$, mass spectrum). Reduction of the methyl ester obtained from the Gaultheria subcorymbosa with lithium aluminium hydride gave material identical with authentic uvaol (III) (mixed melting point, infra red spectrum, $[\alpha]_D$, mass spectrum).

There was no indication in either work-up procedure of the presence of any appreciable quantities of triterpene acids other than ursolic acid in Gaultheria subcorymbosa whilst examination of Gaultheria antipoda showed ursolic acid to be the only detectable triterpene acid in this plant also. A short account of this work has been published as a note in the Journal of the Chemical Society[32] and a reprint is included in the Appendix of this thesis.

It is of some interest that during the drying of the Gaultheria antipoda a pronounced smell of methyl salicylate was noted[33] since oil of wintergreen is obtained from the closely related plants Gaultheria procumbens[34] and Gaultheria fragrantissima Wall.[35]

As to whether or not ursolic acid, which the present work

has shown to be the sole triterpene acid of G. antipoda and G. subcorymbosa does possess galactogenic properties awaits to be determined experimentally.

Very little attention appears to have been paid to the pharmacological properties of triterpene acids, or indeed, of triterpenes in general. What investigations have been made have been summarised by Martin-Smith and Khatoon.[36]

EXPERIMENTAL

Melting points were taken on a Kofler block. Optical rotations were taken in chloroform on a Bellingham and Stanley polarimeter.

Infra-red spectra were measured on a Perkin-Elmer 237 instrument, in carbon tetrachloride solution unless otherwise stated. Light petroleum refers to the fraction of b.p. 60-80. The mass spectra were determined, through the kind co-operation of Mr. T.A. Bryce, with an A.E.I. M.S. 9 double-focussing mass spectrometer using a direct inlet system. The energy of ionizing electrons was 70 V, the ionizing current was 100μ a. and the source temperature was 90 - 110°. The authentic specimens of methyl ursolate, O-acetyl methyl ursolate and uvaol were kindly supplied by Dr. W. Lawrie.

Isolation of Ursolic Acid from Gaultheria subcorymbosa.

A. First Method.

The finely ground dried leaves and twigs of Gaultheria

subcorymbosa (800 g) were exhaustively extracted with boiling ethanol (1500 ml) in a Soxhlet apparatus (24 hours) and the solvent removed from the extract under reduced pressure. The residue (40 g) was extracted in turn with light petroleum, chloroform and water. Removal of solvents yielded respectively 5g, 20g, and 12g of material, leaving a residue weighing 3 g.

Attempts to redissolve the light petroleum soluble fraction in light petroleum resulted in the uptake of 3 g of material (consisting mostly of alkanes[37] and fatty material) leaving a residue of approximately 2 g which was treated with ether to yield an ether-soluble fraction and a residue which on recrystallisation from ethanol yielded a white solid (400 mg) m.p. 260° - 280°. The ether-soluble portion on crystallisation from ether yielded a white crystalline solid (300 mg) m.p. 224-244°.

The original chloroform extract after evaporation of the solvent and crystallisation of the residue from ethanol yielded a white crystalline solid (3 g) m.p. 280-284°.

All the above crystalline fractions had the properties of triterpene acids and infra red analysis indicated that they were very similar in constitution. Aliquots of each triterpene acid fraction (250 mg) were separately esterified with an ethereal solution of diazomethane and the products chromatographed on alumina(Woelm, neutral 20g). Elution

with light petroleum/ether (1 : 1) yielded, (1) from the ether-soluble fraction, 210 mg. m.p. 110-114°; (2) from the ether insoluble fraction, 205 mg. m.p. 108 - 110°; and (3) from the chloroform soluble fraction 220 mg. m.p. 112 - 114°, of the methyl esters. The esters in each case showed identical behavour on thin layer chromatography (on silica gel plates, using light petroleum/ether (1:1) as solvent system and concentrated sulphuric acid as detecting agent), running as a single compound but still showing different melting points on crystallisation from different solvents.

B. Second Method.

Dried finely ground twigs and leaves of Gaultheria subcorymbosa (800 g) were exhaustively extracted with boiling ethanol (1500 ml) in a Soxhlet apparatus (24 hours). The ethanolic solution was treated directly with an excess of an ethereal solution of diazomethane and the solvents removed under reduced pressure to give a residue (38 g). Chromatography of an aliquot of this material (3 g) over alumina (Woelm neutral 30 g) yielded a white crystalline ester fraction 1.72 g) eluted by means of light petroleum/ether (1 : 1). Recrystallisation from ether gave white needles, m.p. 112-114°; from ethyl acetate m.p. 162 - 164°; from ethanol m.p. 169-171°, $[\alpha]_D$ = + 58 (c = 2.0) (lit.[38] for methyl ursolate m.p. 169 - 170° $[\alpha]_D$ = + 62). The melting point of the specimen

crystallised from ethanol was undepressed on admixture with authentic methyl ursolate crystallised from ethanol. The infra red spectra of the two specimens were identical.

Application of mass spectrometry to the esters obtained from both methods A and B, above gave in all cases a parent molecular peak at 470 mass units (corresponding to $C_{31}H_{50}O_3$) and a cracking pattern strictly comparable to that of authentic methyl ursolate[28,31]; m/e 411, 410, 262 (intense), 249, 207, 203 (intense), 189, 133 with metastable peaks at 157.3, 87.2 and 172.8 corresponding to the transitions $262^+ \longrightarrow 203^+$, $203^+ \longrightarrow 133^+$ and $207^+ \longrightarrow 189^+$ respectively.

O-Acetyl Methyl Ursolate:-

Methyl ester (1 g) derived from Gaultheria subcorymbosa was acetylated by adaptation of the method of Sengupta and Khastgir[39] by heating on the steam bath with acetic anhydride (10 ml) in pyridine (10 ml) for 4 hours. The reaction mixture was poured into ice cold water with constant stirring. The crystalline product was filtered, washed with water and re-crystallised from ethanol to yield the crystalline acetate (1 g) m.p. 244 - 245°, $[\alpha]_D$ = + 57 (c = 2.5) (lit.[40] for O-acetyl methyl ursolate, m.p. 244 - 247° $[\alpha]_D$ = +58). There was no melting point depression on admixture with authentic material and the infra red spectra were identical. The same compound was obtained by acetylation of the methyl ester of the triterpene acid from Gaultheria antipoda.

Mass spectrum: parent molecular peak 512 mass units, (corresponding to the formula $C_{33}H_{52}O_4$) m/e. 262, 249, 203 (intense), 190, 189, 130 with metastable peaks at 157.3, 87.2 and 172.8 corresponding to the transitions $262^+ \longrightarrow 203^+$ $203^+ \longrightarrow 133^+$ and $207^+ \longrightarrow 189^+$ respectively.

Uvaol

Methyl ester (200 mg) prepared from the acid isolated from Gaultheria subcorymbosa was treated in refluxing dry ether with an excess of lithium aluminium hydride (1 g) for 8 hours. Excess of reagent was destroyed by careful addition of water and the mixture was then treated with 6N. HCl. Extraction with ether afforded the diol (0.8 g) m.p. 223 - 225° from ether. $[\alpha]_D$ = + 76 (c = 2.0) (lit.[41] for uvaol, m.p. 222 - 224°, $[\alpha]_D$ = +72). There was no mixed melting point depression with authentic material and the infra red spectrum in chloroform was identical with that of authentic uvaol. Mass spectrum: parent molecular peak 442 mass units (corresponding to $C_{30}H_{50}O_2$), m/e. 411, 234 (intense), 221, 207, 203 (intense), 189, 133 with metastable peaks at 176.2, 87.0 and 72.6 corresponding to the transitions $234^+ \longrightarrow 203^+$, $203^+ \longrightarrow 133^+$ and $207^+ \longrightarrow 189^+$ respectively.

Isolation of Methyl Ursolate from Gaultheria antipoda.

Dried finely ground twigs and leaves of Gaultheria antipoda (750 g) were exhaustively extracted with chloroform (1 litre) in a Soxhlet apparatus for 24 hours. Removal of

solvent from the extract under reduced pressure yielded a green residue (52 g).

An aliquot (5 g) of the chloroform extractives was taken into ethanol and treated with an ethereal solution of diazomethane. The crude product (4.8 g) was chromatographed on alumina (Woelm, neutral 50 g). The initial fraction obtained by elution with light petroleum consisted mainly of alkanes[37] and fatty material. The fraction which eluted with light petroleum/ether (1 :1) was crystalline (2.8 g) and on recrystallisation from ethanol had m.p. 168 - 169°, $[\alpha]_D$ = +60 (c = 2.5) (lit[38] for methyl ursolate m.p. 169-170°, $[\alpha]_D$ = +62). There was no depression in melting point on admixture with authentic methyl ursolate. The infra red spectra were identical. Acetylation under the same conditions as employed with the esters from Gaultheria subcorymbosa yielded O-acetyl methyl ursolate identical with authentic material.

BIBLIOGRAPHY

1. Dr. A.K. North. Personal communication.

2. Dr. M. Alauddin, Unpublished work.

3. Tatsuo, Kariyone and Yohei Hashimoto, J. Pharm. Soc. Japan. 1949, 69, 313.

4. Sosa, Bull. Soc. Chim. biol., 1950, 32, 344.

5. Riichi Kawaglichi, Ki Gu-Kim and Ho-Kei Kim, J. Pharm. Soc. Japan, 1942, 62, 4.

6. Arthur and Hui, J. Chem. Soc., 1954, 4633.

7. Arthur and Hui, J. Chem. Soc., 1954, 2782.

8. Mario Passerini, Mario Ridi and Pieropapini, Ann. Chim. (Rome) 1954, 44, 783.

9. Arthur, Lee and Ma, J. Chem. Soc., 1956, 1461.

10. Hart, Brit. Med. J., 1957, i, 417.

11. Hudson, Mittelman and Podberezec, New Engl. J. Med., 1954, 251, 641.

12. Elmadjian, Hope and Pincus, J. Clin. Endocrin., 1956, 16, 338.

13. Louis and Conn, J. Lab. Clin. Med., 1956, 47, 20.

14. Atherden, Biochem. J., 1958, 69, 75.

15. Cowie and Folley in Sex and Internal Secretions, ed. W.C. Young, 3rd edn., Williams and Wilkins, Baltimore, 1961, pp 590-642.

16. Folley, Dairy Science Abstracts, 1961, 23, 511.

17. Kon and Cowie, Milk: The Mammary Gland and Its Secretion, Academic Press, New York, 1961.

18. Benson, Cowie, Folley and Tindal, in Recent Progress in the Endocrinology of Reproduction, ed. C.W. Lloyd, Academic Press, New York, 1959, p.457.

19. Riddle, Bates and Dykshorn, Anat. Record, 1932, 54, 25.

20. Gardner and Turner, Research Bull., Missouri Agr. Expt. Sta., 1933, p. 196.

21. Lyons and Catchpole, Proc. Soc. Exptl. Biol. Med., 1933, 31, 305.

22. Reece and Turner, Research Bull. Missouri Agr. Expt. Sta., 1937, 266.

23. Astwood, J. Endocrinol., 1941, 28, 309.

24. Folley, Recherches Recentes sur la Physiologie et la Biochimie de la Secretion Lactee, Masson, Paris, 1954.

25. Folley, The Physiology and Biochemistry of Lactation, Oliver and Boyd, London, 1956.

26. Courtney and Shannon, Tetrahedron Letters, 1963, no13, 173.

27. Shannon, Austral. J. Chem., 1963, 16, 683.

28. Djerassi, Budzikiewicz and Wilson, Tetrahedron Letters, 1962, No. 7, 263.

29. Budzikiewicz, Wilson and Djerassi, J. Amer. Chem. Soc., 1963, 85, 3688.

30. Djerassi, 'Structure Elucidation of Natural Products' (Vol. 2: Steroids, Terpenoids, Sugars and Miscellaneous Classes). Holden Day Inc., San Francisco. London, Amsterdam. 1964. p. 122.

31. Huneck and Snatzke, Chem. Ber., 1965, 98, 120.

32. Alauddin, Bryce, Clayton, Martin-Smith and Subramanian, J. Chem. Soc., 1965, 4611.

33. Dr. M. Martin-Smith Personal Communication.

34. Cahours, Annalen, 1843, 48, 60.

35. Chopra, Indigenous Drugs of India, Dhur and Sons, Calcutta, 1958, p.179.

36. Martin-Smith and Khatoon, in Progress in Drug Research, ed. E. Jucker, vol. 6, 1963, pp 279-346.

37. Eglinton, Hamilton and Martin-Smith, Phytochemistry, 1962, 1, 137.

38. Fuji and Osumi, J. Pharm. Soc., Japan, 1939, 59, 176.

39. Sengupta and Khastgir, Tetrahedron, 1963, 19, 123.

40. Mills and Werner, J. Chem. Soc., 1955, 3132.

41. Zürcher, Jeger and Ruzicka, Helv. Chim. Acta, 1954, 37, 2145.

SECTION III

CHEMICAL OBSERVATIONS ON SOME HERE SPECIES.

INTRODUCTION

Some of the larger-leaved shrubs of the genus Hebe (Family - Scrophulariaceae; Botanical names as in H.N. Allan, Flora of New Zealand, Vol. I, 1961, Wellington Government printer) have long enjoyed a medicinal reputation in New Zealand, the leaves and tender shoots being employed by the Maori in the treatment of certain skin deseases[1] and for arresting the loose bowel movements of such conditions as dysentery and diarrhoea[2 - 10]. Also there are reports that decoctions of these plants were used in the treatment of ulcers[11] and venereal diseases[11].

Of these varied uses, however, there would seem little doubt that extracts from certain Hebe species are truly effective in arresting loose bowel movements, their efficacy being confirmed by Pakeha and Maori alike. Accordingly it appeared a worthwhile project to attempt the isolation and characterisation of the chemical substance or substances responsible for this activity and submit such material for pharmacological investigation.

Earlier a cursory chemical investigation had been carried out on one member of the genus[9] which at that time was designated Veronica salicifolia and the tentative conclusion was reached that the active constipatory agents were tannins[9], although there was some dispute over this, as well as a number of conflicting reports concerning the efficacy of various extracts of the plant prepared by

different methods[4,5,9]. Aqueous extracts of the leaves of this plant (now known as Hebe stricta (Benth.) L.B. Moore) have however been shown to be without action against micro-organisms producing amoebic and bacillary dysentery[12].

The present work described in this thesis represents an extension of studies already carried out at the University of Glasgow[13] with the two species H. odora and H. stricta, to the further species H. corriganii and H. bollonsii of which the dried leaves were kindly provided by Miss Lucy B. Moore. At the same time as the main work directed towards the isolation and characterisation of any constipatory principles present was being carried out, the opportunity was taken to perform a gas liquid chromatographic analysis of the alkanes and components of the long chain esters, in a continuation of earlier studies[14] designed to secure a possible chemo-taxonomic differentiation within the genus Hebe, which is characterised by an extreme ease of hybridisation[15].

DISCUSSION

The methods employed for the isolation of the chemical constituents of both Hebe corriganii and Hebe bollonsii were the same in each case: thus the dried leaves were finely ground and exhaustively extracted with ethanol using a Soxhlet apparatus and the solid extractives so obtained divided into light petroleum-soluble, chloroform-soluble and residual

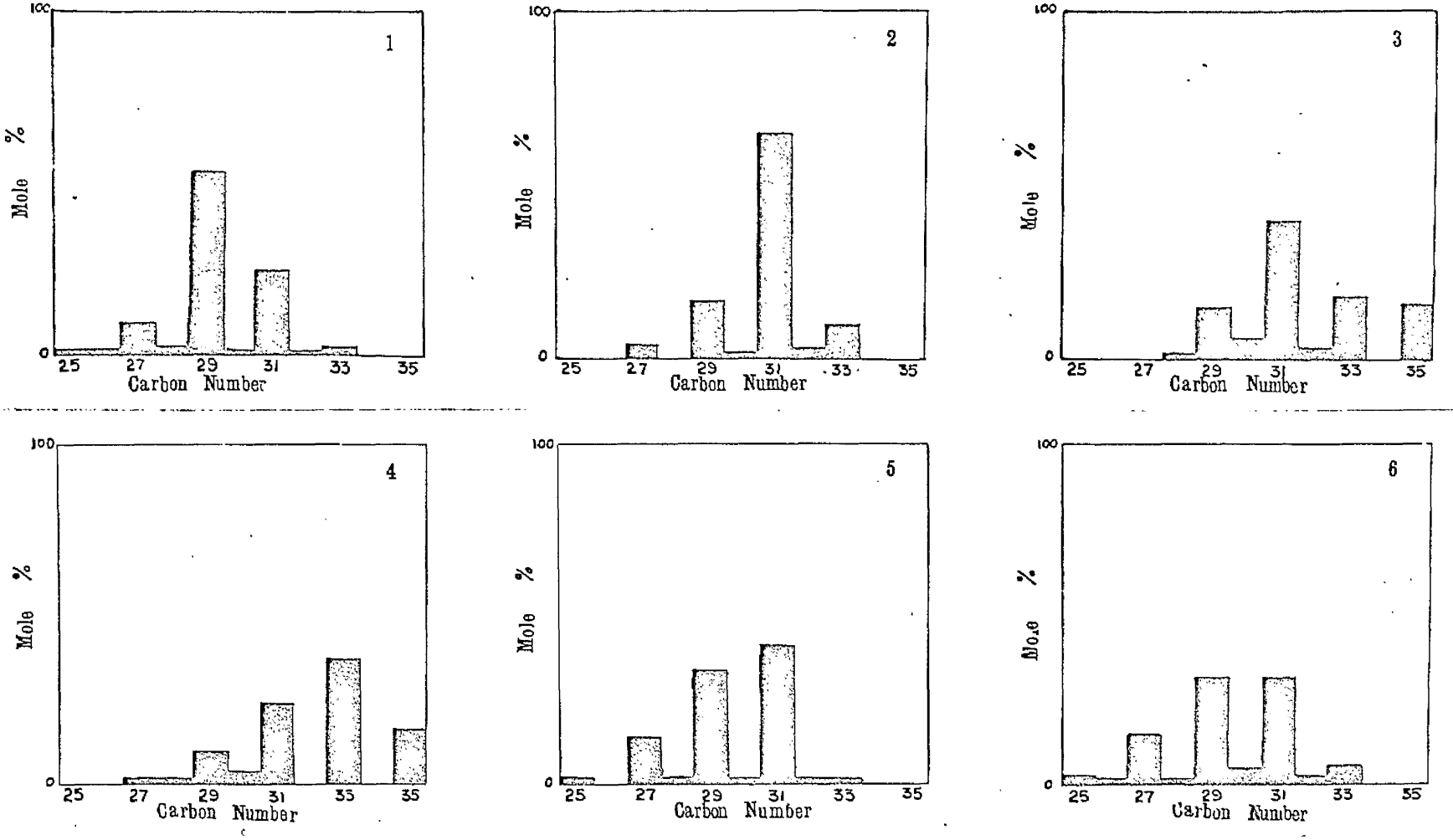
ethanol-soluble fractions.

The alkane fraction, uncontaminated with compounds of other chemical groups as shown by infrared analysis, was obtained from the light petroleum-soluble fraction by the method of Eglinton *et al.*[14] and subjected to gas liquid chromatographic analysis. The alkane distribution patterns are shown in histogram form in Fig. I, while the percentage of each alkane in the total alkane fraction is shown in Table I. Also included in Figure I and Table I for comparative purposes are the corresponding data obtained from earlier work[14] with four other *Hebe* species. It is to be noted that none of the species contains more than traces of branched alkanes (none being detected in *H. odora*, *H. corriganii* or *H. bollonsii*). It may be noted also that *H. corriganii* and *H. bollonsii* possess extremely similar alkane distribution patterns, suggesting that the utility of plant alkane analysis as a method of 'fingerprinting' individual species to aid taxonomic and pharmacognostic differentation may prove more limited than originally hoped.[16,17]

It is nevertheless of interest that there seems to be considerable variation among the species of *Hebe* so far examined as to the major *n*-alkane present. Thus in *H. odora* it is the C_{29} compound; in *H. parviflora*, *H. diosmifolia* and *H. corriganii* it is the C_{31} compound; in *H. stricta* it is the C_{33} compound; while in *H. bollonsii* an

Figure 1.

Distribution In Mole Percentage Of n-Alkanes C_{25}-C_{35} In Surface Wax Of Hebe Species As Shown In Histogram Form.



The numbers in the top right hand corners of the individual histograms serve to identify the individual Hebe species and correspond to the numbers in Table 1.

TABLE I

Distribution In Mole Percentage Of The Alkanes From Different Hebe Species*

No.	Plant	Portions Extracted	Total Petrol Extracts‡	Total Alkane Fraction§	C_{23}		C_{24}		C_{25}		C_{26}		C_{27}		C_{28}		C_{29}		C_{30}		C_{31}		C_{32}		C_{33}		C_{34}		C_{35}		Reference
					iso	n	iso	n	iso	n	iso	n	iso	n	iso	n	iso	n	iso	n	iso	n	iso	n	iso	n	iso	n	iso	n	
1	Hebe odora	Stems and leaves	3.9	4		+		1		2		2		10		3		52		2		25		2		3						14
2	H. parvifolia	''	2.5	10						1		+	+	4		1	+	17		2	+	65		3		10						14
3	H. diosmifolia	''	3.2	10										1		2	+	15		6	+	40		3	+	18		+		16		14
4	H. stricta	''	1.7	4.5						+		1		2		2	+	10		4	+	24		1	1	37		1	1	16		14
5	H. corriganii	leaves	1.8	3						2		1		14		2		34		2		41		2		2						
6	H. bollonsii	''	2.0	3.7						3		2		15		2		32		5		32		3		6						

* The content of an individual alkane is expressed as a mole percentage of the total hydrocarbon content from C_{23}-C_{35} inclusive. The mole percentage is taken as being equivalent to the area percentage i.e. $100\, A_n / \sum_{23}^{35} A_n$, where A_n is the area of the peak corresponding to the hydrocarbon $C_nH_{(2n+2)}$, as measured by planimeter. The values are approximated to the nearest 1 per cent; peaks of relative area less than 1 per cent are indicated by +. The branched chain alkanes are designated iso-

‡ As per cent dry weight-values to the nearest 0.05 per cent.

§ Per cent total alkane hydrocarbons calculated on total weight of petrol extractives to the nearest 0.5 per cent.

equal mixture of the C_{31} and C_{33} compounds represents the preponderant alkanes. In accord with modern concepts of biogenesis[18] in which n-alkanes are considered to arise via decarboxylation of aliphatic carboxylic acids built up from linear condensation of acetate units by a process involving acetyl coenzyme A or malonyl coenzyme A and so possessing an even number of carbon atoms, n-alkanes with an odd number of carbon atoms form the major proportion of the total alkane content of each species of Hebe.

The long chain ester fractions of H. corriganii and H. bollonsii occurring in the light petroleum-soluble fractions were saponified and the acids so liberated were identified by means of gas liquid chromatography of their methyl esters which were prepared by means of diazomethane. The alcohols liberated during the saponification were identified by means of gas liquid chromatography of their acetates.

The results of the fatty acid and long chain alcohol analyses are shown in Tables II and III which also include the corresponding analysis for H. odora which was not undertaken during the earlier studies on this plant.[14] The results of both the acid and alcohol analyses would, however, indicate little potential value of such analyses in chemotaxonomy since the total numbers of representatives in each series in no case exceeds five with dodecan-1-ol the predominant alcohol in all cases and lauric acid the predominant acid, except with H. odora, where capric acid is the major acid. The

TABLE II

Fatty Acids From Different Hebe Species*

No.	Plant	Total acid fraction present (based on dry weight of leaves)	Capric (Decanoic)	Lauric (Dodecanoic)	Myristic (Tetradecanoic)	Palmitic (Hexadecanoic)	Stearic (Octadecanoic)
1	Hebe odora		32%	28%	22%	12%	8%
2	Hebe corriganii	0.93%	26%	42%	31%	11%	2%
3	Hebe bollonsii	1.12%	1%	49%	29%	21%	

* The quantities of the individual acid are expressed as a percentage of the total acids isolated.

TABLE III

Alcohols From Different Hebe Species*

No.	Plant	Total alcohol fraction present (based on dry weight of leaves)	Octan-1-ol	Decan-1-ol	Dodecan-1-ol	Hexadecan-1-ol
1	Hebe odora		24%	46%	19%	14%
2	Hebe corriganii	0.68%	29%	43%	17%	12%
3	Hebe bollonsii	0.64%	30%	48%	22%	

*The quantities of the individual alcohols are expressed as a percentage of the total alcohol isolated.

complete absence of unsaturated acids is of interest as are the short chain lengths of the acids (C_{10} - C_{18}) and alcohols (C_8 - C_{16}) as compared to the chain lengths of the alkanes (C_{25} - C_{33}). The latter situation may be contrasted to that pertaining with respect to the stem wax of the sugar cane *Saccharum officinarum*[19], the cuticle wax of the carnuba palm, *Copernicia corifera*,[20] and the cuticle wax of the apple fruit, *Pyrus malus*[21] where the constituent alkanes, acids and alcohols all have carbon atom numbers of the same order. At the same time it lends further support to the conclusion of Eglinton and Hamilton[18] that there appears to be no consistent relationship between the distribution patterns of the alkanes, alcohols and acids.

Again in keeping with current biogenetic theory[18,22,23] the acids and alcohols in all the species of *Hebe* appear restricted to those with an even number of carbon atoms.

The residual ethanol extractives which were insoluble in both light petroleum and chloroform, were then investigated in order to attempt the isolation of the active constipatory agent. Fractional crystallisation from ethanol, gave in the case of both *H. corriganii* and *H. bollonsii*, crystalline D-mannitol (identical in all respects with authentic material) as the least soluble fraction. This is of some interest as earlier work with *H.* odora and *H. stricta*[13] had also shown D-mannitol to be present in these plants.

The non-crystalline solid cream-coloured residue remaining after removal of the D-mannitol in the case of both H. corriganii and H. bollonsii was bitter in taste, but application of standard tests[24] showed the absence of alkaloids, which appear to be of but rare occurrence in the Scrophulariaceae, having been reported in only some ten species[25]. The residues, however, showed reactions characteristic of catechin-type condensed tannins[26].

Application of paper chromatography showed the tannin fractions to consist of several components with some apparently common to both species. However all attempts to isolate the individual compounds present in crystalline form were without success. Application of standard colour tests[27] to the total tannin fraction indicated the absence of compounds of the chromone type[28] whilst application of the Gibbs test indicated the absence of phenols possessing a free para position.

In view of the lack of success in securing the individual components of the tannin mixtures in pure crystalline form and since condensed tannins are known to be complex products of which the constitutions are still largely unknown[29], work was discontinued at this stage. However, the total crude tannin fraction obtained from H. odora in the earlier studies at the University of Glasgow[13] was subjected to pharmacological screening and the results (kindly made available by Mrs. June Grady and Dr. T.C. Muir) are in entire agreement

with the active constipatory principles of the Hebe species being condensed tannins.

Indeed tannins as a group, of which the condensed tannins (phlobatannins or non-hydrolysable tannins) are but one of two sub-groups[29] - the other being the hydrolysable tannins which are split by acids, alkalis or enzymes into the constituent polyhydric alcohols and phenolic acids - have at one time seen considerable use in medicine primarily on account of their astringent properties. Since tannins react with tissue proteins[30] they have in the past seen application in the treatment of diarrhoea[31], mucosal inflammation[30], burns[32] and certain microbial infections[30], but their high toxicity[32-35] has caused their virtual disappearance from modern medicine.

An account of this work has been submitted to the Journal of Pharmacy and Pharmacology[37].

EXPERIMENTAL

The dried finely ground leaves (80 g) of the particular species under investigation were exhaustively extracted with ethanol (300 ml) in a Soxhlet apparatus and the solvent removed under reduced pressure. The residues (3 to 4g) were successively extracted with light petroleum (b.p. 40-60) and with chloroform. The chloroform extractions yielded little material and were not further investigated.

Isolation of alkanes.

The total alkane fraction was isolated by the method of

Eglinton *et al.*[14] Thus the light petroleum extractives (1g) were refluxed with 2,4-dinitrophenylhydrazine (1g) and conc. HCl (0.5 ml) in ethanol (20 ml) for 2 hours in order to convert any carbonyl compounds which might be present into their 2,4-dinitrophenylhydrazones. The solvent was removed under reduced pressure and the residue exhaustively extracted with light petroleum. After removal of the solvent the petrol-soluble material was refluxed for 2 hours in aqueous ethanol (2:1, 20 ml) containing sodium hydroxide (1g) in order to hydrolyse the esters present. The solution was taken to dryness under reduced pressure, and the residue thoroughly extracted with light petroleum. The petroleum extract was filtered through alumina (Woelm neutral 5 g) and the hydrocarbon fraction completely eluted with further light petroleum. Infrared analysis showed the alkane fraction so obtained to be uncontaminated with compounds of other chemical classes.

Gas-liquid chromatographic analysis of the alkane fraction in chloroform on a 'Panchromatograph' instrument with 0.5% Apiezon 'L' on Embacel (80-100 mesh) at 225°C showed the presence of nine components which were characterised as being the C_{25} to C_{33} *n*-alkanes by employing authentic C_{27}, C_{29} and C_{31} *n*-alkanes as markers on a second run.

Components of the esters of *H. corriganii*, *H. bollonsii* and *H. odora*:-

The crude light petroleum-soluble material (200 mg) from

the ethanolic extractives of each plant was refluxed for 4 hours with 15% ethanolic potassium hydroxide solution (25 ml). After removal of the solvent under reduced pressure, 100 ml of water was added and the mixture extracted exhaustively with ether (3 x 80 ml). The combined ether extracts were dried over anhydrous sodium sulphate, filtered, and on evaporation of the solvent yielded the neutral components.

The total crude ether soluble material was acetylated by refluxing with acetic anhydride (2 ml) in pyridine (2 ml) for 1 hour and the reaction mixture cooled and poured into water. The crystalline material resulting was collected by filtration, washed with water, taken up in ethanol and passed through alumina (Woelm neutral 5 g) to give the acetates of the alcoholic components.

Gas-liquid chromatographic analysis of the acetates on a 10% polyethylene glycol adipate polyester column at 175°C showed the presence of several components. In the cases of H. odora and H. corriganii, these were identified as the acetates of octan-1-ol, decan-1-ol, dodecan-1-ol and hexadecan-1-ol and in the case of H. bollonsii as the acetates of octan-1-ol, decan-1-ol and dodecan-1-ol by adding authentic acetates to the mixture and further gas-liquid chromatography.

The basic aqueous solution from the saponification, on acidification with dil. HCl and extraction with ether yielded a mixture of the free carboxylic acids which were converted

into the methyl esters by treatment with an excess of ethereal diazomethane. The esters were subjected to chromatography on alumina (Woelm neutral 5 g) being eluted with light petroleum.

Gas-liquid chromatographic analysis employing a 10% polyethylene glycol adipate polyester column at 175°C permitted separation of the individual esters. These were then identified by addition of authentic specimens, as the esters of capric, lauric, myristic, palmitic and stearic acids in the case of H. odora and H. corriganii, and as the esters of capric, lauric, myristic and palmitic acids in the case of H. bollonsii.

Isolation of D-mannitol from H. corriganii and H. bollonsii:-

The ethanol-soluble residue (1.5 g) remaining after removal of the light petroleum-soluble and chloroform-soluble fractions from the total ethanolic extractives of each plant was taken up in hot 95% ethanol, and successively concentrated to afford several crops of crystalline compound which on further recrystallisation from ethanol had m.p. 163-165°C (lit.[36] for D-mannitol. m.p. 160°C). The absence of a mixed melting point depression on admixture with authentic D-mannitol and infrared analysis confirmed the identity of the material. The yields based on dry weight of leaves were H. corriganii 3.7% and H. bollonsii 2.9%.

Condensed Tannins.

After removal of D-mannitol, the remaining ethanolic

extractives from each plant (2 to 3 g) were taken to dryness under reduced pressure to yield glassy non-crystalline solids possessing a creamish colour and a tendency to gain a pink tinge on prolonged exposure to air, and proving to be very hygroscopic. These residues gave a greenish colouration with ferric chloride, were bitter in taste and gave precipitates with gelatin solution, phenazone, lead acetate and bromine water. On boiling with dil. HCl phlobaphenes were formed confirming the material to contain condensed tannins. Paper chromatography on Whatman No. 1 sheet and thin layer chromatography on silica employing butanol/acetone/water as solvent showed the presence of several components. Column chromatography employing paper rolls, charcoal-kieselguhr, or alumina pre-treated with acetic acid, failed to afford individual components in crystalline form.

BIBLIOGRAPHY

1. Goldie, Trans. and Proc. N. Z. Inst., 1904, 37,1.

2. Newman, Trans. and Proc. N.Z. Inst., 1879, 12,433.

3. Kesteven, Lancet, 1880, ii, 457.

4. Baber, Trans and Proc. N.Z. Inst., 1886, 19, 319.

5. Bell, N.Z. med. J., 1890, 3, 129.

6. Martindale and Westcott, Extra Pharmacopeia, 1898, 9th edn., p. 463.

7. Best, J. Polynesian Soc., 1905, 14, 1.

8. Beattie, Trans. and Proc. N.Z. Inst., 1920, 52,53.

9. Gardner, N.Z. J. Sci. Tech., 1923, 6, 147.

10. Wall and Cranwell, The Botany of Auckland, 1943, 2nd edn., p. 35. Wilson and Horton, Auckland.

11. Neil, The New Zealand Family Herb Doctor, 1891, 2nd edn., p. 524. Mills, Dick and Co., Dunedin.

12. Briggs, Personal communication, 1959.

13. Mrs. June Grady and Dr. Martin-Smith, unpublished.

14. Eglinton, Hamilton and Martin-Smith, Phytochemistry, 1962, 1, 137.

15. Cockayne and Allan, Ann. Bot. 1934, 48, 1.

16. Eglinton, Gonzalez, Hamilton and Raphael, Phytochemistry, 1962, 1, 89.

17. Eglinton, Hamilton, Raphael and Gonzalez, Nature, 1962, 193, 739.

18. Eglinton and Hamilton, in Chemical Plant Taxonomy, 1963, p. 197. editor T. Swain, New York, Academic Press.

19. Kranz, Lamberton, Murray and Redcliffe, Austal.J. Chem., 1960, 13, 498.

20. Mazliak, C.R. Acad. Sci., Paris, 1960, 251, 2393.

21. Mazliak, J. Agric. Tropical et de Botanique Applique, 1961, 8, 180.

22. Chibnall, Piper, Pollard, Williams and Sahai, Biochem.J. 1934, 28, 2189.

23. Waldron, Gowers, Chibnall and Piper, Biochem. J., 1961, 78, 435.

24. Paech and Tracey, Modern Methods of Plant Analysis, 1955, 4, 373. Berlin, Springer-Verlag.

25. Brown, Ph.D., Thesis, University of Glasgow, 1962.

26. Haslam, Haworth, Jones and Rogers, J. Chem. Soc., 1961, 1829.

27. Campbell, in Chemistry of Carbon Compounds, 1959, Vol. IVB, p. 904, editor E.H. Rodd, Elsevier, London.

28. Gibbs, J. Biol. Chem., 1927, 72, 649.

29. Haworth, Proc. Chem. Soc., 1961, 401.

30. Ramstad, Modern Pharmacognosy, 1959, p. 214, McGraw-Hill Book Company Inc., New York.

31. Burger, Medicinal Chemistry, 1960, 2nd edn., p. 538, Interscience Publishers, New York.

32. Armstrong, Clarke and Cotchin, J..Pharm. Pharmacol., 1957, 9, 98.

33. Wells, Humphrey and Coll, New Engl. J. Med., 1942, 226, 629.

34. Paton, Lancet, 1964, 3, 934.

35. McAllister, Anderson, Bloomberg and Margulis, Radiology, 1963, 80, 765.

36. Fischer, Ber. dtsch. Chem. Ges., 1890, 23, 383.

37. Grady, Lewis, Martin-Smith, Muir and Subramanian, manuscript submitted to J. Pharm. Pharmacol., 1966.

SECTION IV

CHEMICAL COMPARISON OF *CORTADERIA* SPECIES AND GAS LIQUID CHROMATOGRAPHIC STUDIES WITH TRITERPENE METHYL ETHERS.

INTRODUCTION

During an investigation of the leaf alkane distribution pattern of the New Zealand toe-toe grass [collected for chemical and pharmacological studies in view of reports[1] of its use in the treatment of kidney complaints by the Maoris] strong absorption by the alkane-containing fraction was observed at 1104 cm^{-1} in the infrared[2]. Such absorption is characteristic of the ether function[3], and further studies[4] by Dr. R.J. Hamilton at the University of Glasgow led to the successful isolation of two ethers, Ether 'A' and Ether 'B' and strong indications[5] from gas liquid chromatographic experiments that a third ether, Ether 'C' was also present. Ether 'B' was concluded[4] to be β-amyrin methyl ether, whilst Ether 'A' was designated 'arundoin' and shown to be a second triterpene methyl ether of unknown structure.

In order to complete the elucidation of the structure of arundoin, as described in Section V of the present thesis, further quantities of the compound were required, but plant material kindly collected in March 1965 at Raglan, New Zealand, and extracted by Dr. R. Hodges failed to provide any arundoin. This seemed of considerable interest in the light of botanical work[6] [published after the original collection of plant material in September 1959] in which the New Zealand toe-toe grass, originally designated Arundo conspicua Forst. f., has been

differentiated into three separate species designated Cortaderia toetoe, Zotov, Cortaderia fulvida [Buch.] Zotov and Cortaderia richardii [Endl.] Zotov.

In the circumstances it thus seemed desirable to undertake a comparison of the chemical constituents of the leaf surface waxes of these three individual Cortaderia species concurrently with the further work directed towards the determination of the constitution of arundoin, new supplies of which had become available as a result of a return visit to the site of the first collection at Plimmerton, New Zealand, by Dr. M. Martin-Smith, under the auspices of the Wellcome Trust. In addition, the opportunity was taken to examine two South American Cortaderia species naturalised in New Zealand viz., Cortaderia selloana [Schult.] Aschers. et Graebn. and Cortaderia atacamensis [Philippi] Pilger as well as Poa anceps, another native New Zealand grass. Leaves of the five authenticated Cortaderia species were kindly supplied by Dr. H.E. Connor, Botany Division, D.S.I.R., Christchurch, New Zealand. Accordingly gas liquid chromatographic analyses were performed on the alkane fractions and on the components of the long chain esters present in the leaf surface wax of each grass in an attempt to secure chemotaxonomic differentiation within the genus Cortaderia. Also an investigation was made into the presence or absence of triterpene methyl ethers in the wax of each plant, and where such compounds were present,

identification of them was made with heavy reliance on gas liquid chromatography and mass spectrometry. At the same time similar analyses were carried out on the light petroleum extractives supplied by Dr. Hodges from the unidentified Raglan species of Cortaderia and on the unidentified Cortaderia species growing at Plimmerton, New Zealand, from which the large scale isolations of arundoin had been made, in order to see whether positive identification of the species concerned could be made on the basis of such analyses. In addition a comparison between the constituents of the wax from the leaves and of the wax from the rhizomes of the Plimmerton species was made. This last work was deemed desirable in the light of the recent isolation of arundoin from the rhizomes of the grass Imperata cylindrica P. Beauv var. media Hubbard [=var. koenigii Durand et Schinz] by Natori and his coworkers[7,8].

The gas liquid chromatographic studies undertaken in connection with the identification of the triterpene methyl ethers, where present, in the various Cortaderia species pointed to the need for determining the retention times of known triterpene methyl ethers under standard conditions. Since the retention times of steroids[9-14] and triterpenoids[15,16] are conventionally determined relative to the retention time of 5α-cholestane, the retention times of 9 triterpene methyl ethers [readily available from natural sources or from methylation of the parent alcohol], relative to that of 5α-cholestane, were determined on 0.5% Apiezon L, 1.5% SE-30, 1.5% QF-1 and

1.0% CDMS columns. These experiments showed that no resolution of the five ethers derived from the oleanane or rearranged oleanane skeleton [viz. the methyl ethers of germanicol, δ-amyrin, β-amyrin, taraxerol and multiflorenol] was possible on any of the columns used, although separation of any one of these five ethers from cylindrin, arundoin, bauerenol methyl ether and α-amyrin methyl ether was feasible with all four columns. The data obtained from this work was then applied to the identification of ethers 'B' and 'C' from Cortaderia toetoe and to the identification of the triterpene methyl ethers present in the wax of Cuban sugar cane. Application of mass spectrometry permitted identification of individual methyl ethers of the oleanane type. Finally, that a triterpene alcohol occurring in Artemisia vulgaris L. was O-desmethyl arundoin was shown by direct comparison of the synthetic methyl ether from this alcohol [kindly supplied by Dr. A.S. Rao of the National Chemical Laboratory, Poona, India] with authentic arundoin.

DISCUSSION

A. Chemotaxonomic Studies With Cortaderia Species

1. Alkane Distribution Patterns

The total leaf surface alkane fraction, uncontaminated with compounds of other chemical groups, as indicated by infrared analysis, was obtained from the light petrol extractives of

TABLE I

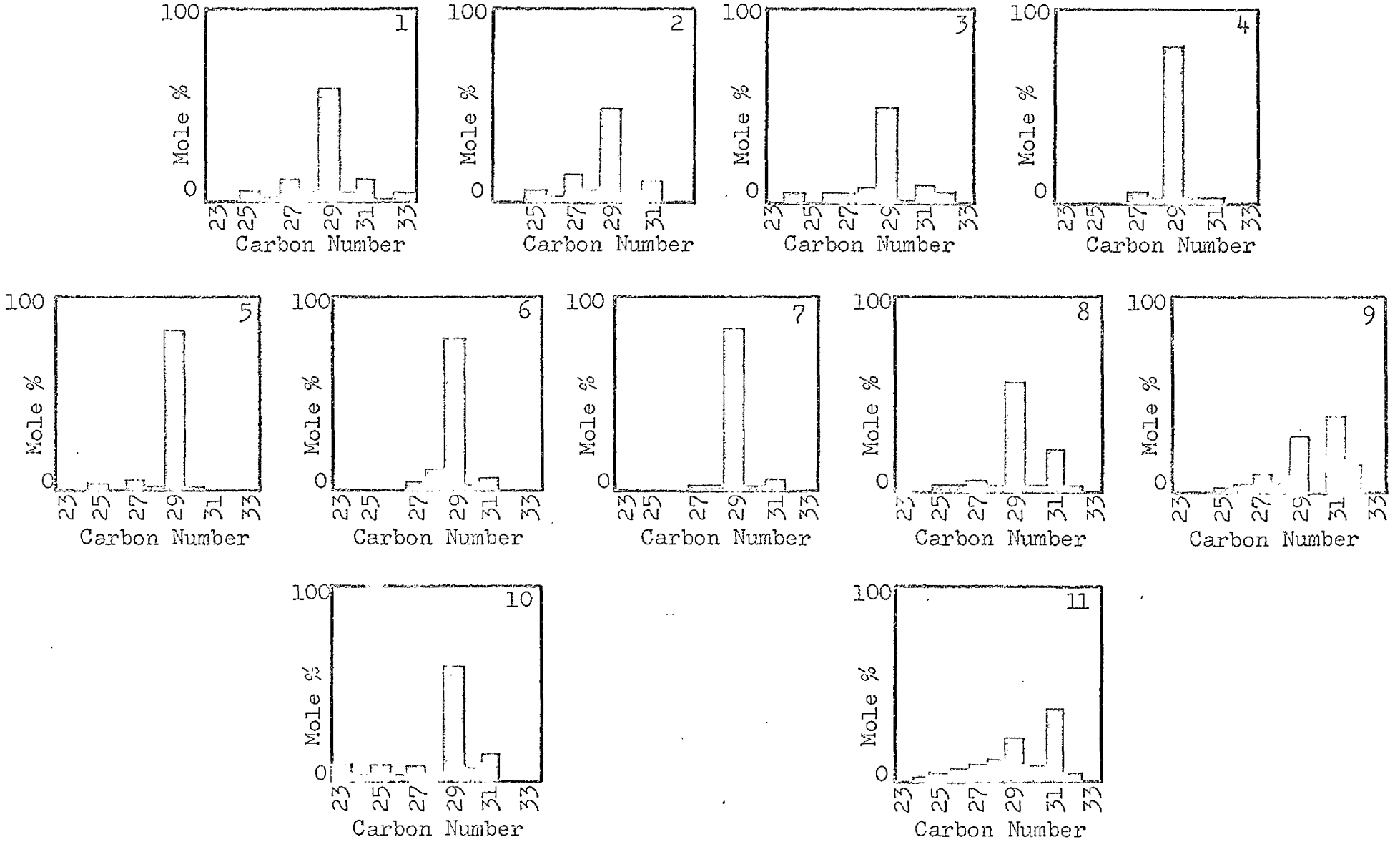
Percentage Composition of Surface Alkanes As Determined By Gas Liquid Chromatography On 0.5% Apiezon 'L' Columns at 240°C.*

No.	Plant	Portion Extracted	n-Alkane Defined By Carbon Atom Content												
	(A) NEW ZEALAND CORTADERIA SPECIES		C_{22}	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	Ref.
1	Cortaderia species collected Sept. 1959 at Plimmerton.	Total Leaves		+	+	4	2	9	5	60	5	12	2	3	2
2	Cortaderia species collected Dec. 1961 at Plimmerton.	Leaves		+	+	7	6	14	9	50	4	10			2
3	Cortaderia species collected June 1965 at Plimmerton.	Rhizomes			6	+	6	6	8	51	3	9	6		
4	Cortaderia species collected June 1965 at Plimmerton.	Leaves	+	+	+	+	+	5	4	82	3	3			
5	Cortaderia toetoe	Leaves	+	+	+	3	+	5	2	83	2	+			
6	Cortaderia fulvida	Leaves				+	+	3	9	80	2	4			
7	Cortaderia richardii	Leaves	+	+	+	+	+	2	2	86	2	6			
	(B) SOUTH AMERICAN CORTADERIA SPECIES														
8	Cortaderia selloana	Leaves				2	2	4	3	58	3	23	2		
9	Cortaderia atacamensis	Leaves			+	2	3	9	4	29	+	39	13		
10	(C) Cortaderia species collected March 1965 at Raglan	Leaves	+	5	1	6	2	5	3	58	7	13			
11	(D) Poa anceps	Leaves			2	3	5	8	10	21	8	35	3		

* The content of an individual alkane is expressed as a mole percentage of the total hydrocarbon content from C_{22}-C_{33} inclusive. The mole percentage is taken as being equivalent to the area percentage, i.e. $100\ A_n / \sum_{22}^{33} A_n$, where A_n is the area of the peak corresponding to the hydrocarbon $C_nH_{(2n+2)}$, as measured by planimeter. The values are approximated to the nearest 1 per cent and peaks of relative area less than

FIGURE 1

Distribution In Mole Percentage Of n-Alkanes C23-33 In Surface Wax As Shown In Histogram Form



The numbers in the top right hand corners of the individual histograms serve to identify the individual grass and correspond to the numbers in Table I.

the unmacerated fresh leaves of each individual grass, after removal of carbonyl compounds by treatment with 2,4-dinitrophenylhydrazine, of esters through saponification, and of ethers through treatment with concentrated sulphuric acid, followed by alumina column chromatography, as described by Eglinton *et al.*[2] Each total alkane fraction thus obtained was then subjected to gas liquid chromatographic analysis employing 0.5% Apiezon L as the stationary phase. After the gas liquid chromatographic trace of the natural mixture had been obtained, selected authentic *n*-alkanes were added to the mixture and further traces obtained. Intensification of the peaks corresponding to the added known alkanes then permitted their identification in the mixture whilst a plot of log retention time against carbon atom number for the peaks on the original gas liquid chromatographic trace permitted a complete analysis of the alkanes present, since such a plot for an homologous series gives a straight line.[17] The various leaf surface alkane distribution patterns determined as a result of this work are shown in histogram form in Fig. 1, whilst the percentage of each alkane in the total alkane fraction is shown in Table I. Also included in Fig. 1 and Table I for comparative purposes are the corresponding data obtained from the original work[2] with '*Arundo conspicua*'.

Two important facts are readily apparent from Fig. 1 and Table I. Firstly, there is considerable divergence between

the percentage compositions of the surface alkane fractions obtained from the rhizomes and from the leaves of the Cortaderia species growing at Plimmerton from which the second large scale extraction of arundoin was made in June 1965. Secondly, there is considerable divergence between the percentage compositions of the surface alkane fractions obtained from the leaves collected at the different periods September 1959, December 1961 and June 1965 of the Cortaderia species growing at Plimmerton, although the alkane pattern of the leaves collected in 1965 would appear to be in sufficient agreement with that of authentic Cortaderia toetoe from Dr. Connor to give further support to the evidence presented below that the unidentified Plimmerton species is indeed Cortaderia toetoe. However, all three New Zealand Cortaderia species, viz. C. toetoe, C. fulvida and C. richardii show similar leaf surface alkane distributions. This fact, coupled with the occurrence of anatomical and seasonal variations in alkane distribution pattern as made apparent by the studies with the Plimmerton material already mentioned, raises considerable doubt as to the fulfilment of the original hopes[18,19] of employing plant alkane analysis as a taxonomic tool. In this connection it may also be noted [as kindly pointed out by Dr. Connor[20]] that the surface wax of Cortaderia species appears most abundant on the young developing tillers and that there may be little wax left on the older leaves, which again raises the possibility of variation in the composition of the wax as well. Other studies

on the presence of cyanogenetic glycosides in Cortaderia species[20,21] have shown that these compounds, although present in young plants, may be absent from older plants. Again, experiments on the total quantity of leaf surface wax present in the grass Poa colensoi Hook, f. [blue tussock][22] have shown that the amount of wax present increases in inverse ratio to the quantity of rainfall and increases slightly with increase in temperature[22]. It was also suggested that wind might influence the total quantity of surface wax present.[22] No determinations of the compositions of the wax were, however, attempted in this work.

From the foregoing it is abundantly clear that considerable caution must be applied to any attempted application of plant alkane analysis to chemotaxonomy. Certainly it is apparent that a systematic investigation into the possible influence of seasonal, climatic, geographical and age factors on the composition of plant surface waxes is essential before the method can be unqualifiedly accepted. At the same time it might be profitable to undertake a detailed comparison of different techniques[2,23] of isolating the total paraffin fraction in order to prove complete reproducibility in the isolation of the alkane fractions. In the present work all g.l.c. determinations were done in duplicate or triplicate with concordant results, but no indication was obtained as to any possible variations between different workers or between different laboratories.

In the present work, there would, perhaps, appear to be a distinction between the native New Zealand Cortaderia species and the South American Cortaderia species introduced into New Zealand, in that the former seem to have a lower percentage of the C_{31} component [as compared to the C_{29} component] in the leaf wax than the latter. Indeed in the case of the South American species Cortaderia atacamensis the C_{31} component is found in the present work to be the major constituent rather than the C_{29} component which is the major constituent in the other four species.

The alkane analysis of the Cortaderia species collected by Dr. Hodges at Raglan in March 1965, does not permit of any assignment of identity to this species, which on botanical identification[20] would seem to be Cortaderia toetoe, although there would appear to be some differences from typical Cortaderia toetoe plants from Wellington province[20]. It is perhaps pertinent to note, though, that personal observations by Dr. Connor[20] suggest that the most abundant Cortaderia species in the Raglan district is the South American Cortaderia selloana with some of the New Zealand Cortaderia fulvida also present. Any possibility of hybridisation between C. selloana and C. fulvida such as has been suggested by Dawson[24] and repeated by Dansereau[25] [see too, Connor[26]] would seem quite impossible[20,26] on account of the widely different flowering times of the two species [late November-December for C. fulvida and mid March - late

April for C. selloana [26] and on account of genetical difficulties arising from differences in chromosome numbers [20] which are C. fulvida, 2n=90 and C. selloana, 2n=72. Similarly, differences in flowering times of the New Zealand C. toetoe [late January-February] and the South American C. selloana [mid March - late April] again coupled with differences in chromosome numbers make it extremely unlikely that hybridisation can occur between these two species. Thus, as pointed out by Connor [26], there seems little likelihood of hybridisation between indigenous and introduced species of Cortaderia in New Zealand, although hybridisation is known between the two indigenous New Zealand species C. richardii and C. fulvida [20].

It is noteworthy that none of the grasses examined appeared to contain any branched alkanes since none were detectable by gas liquid chromatographic analysis under the conditions employed. All the peaks obtained on the gas liquid chromatographic traces fell on the one straight line when log retention time was plotted against carbon atom number. Where isoalkanes are present, they give rise to a second straight line which is not coincident with the n-alkane line, when log retention time is plotted against carbon atom number [19,27,28].

In connection with the alkane distribution pattern of Poa anceps, it might be of some interest if the alkane distribution pattern were to be determined for the related Poa colensoi - especially in the light of the studies on the

variation in the quantities of total surface wax with climatic conditions which have been reported for the latter grass.[22]

2. Analyses Of The Total Fatty Acids And Alkanols Present In The Surface Waxes

These analyses were performed without distinction between the free and combined [as esters] acids and alcohols. Thus, the total light petroleum extractives from each plant were separately saponified. The resulting total mixed acid fraction in each case was converted into a mixture of the derived methyl esters by the action of diazomethane and the esters so obtained subjected to gas liquid chromatography on 10% PEGA columns at 175°. After the g.l.c. trace of the esters present in each mixture of natural origin had been determined, further g.l.c. experiments involving the addition of authentic methyl esters to the mixtures of natural origin were performed. In this way identification of the components of the unknown mixtures was achieved through the intensification of the appropriate peaks. A plot of log retention time against carbon atom number then permitted further identifications.

Similarly, each total alcohol fraction obtained from the saponifications was converted into a mixture of acetates by means of acetic anhydride and the derived acetates identified in each case through gas liquid chromatography, with the aid of separate intensification experiments involving the addition

TABLE II

Percentage Distribution Of Fatty Acids In Surface Wax Components As Determined By Gas Liquid Chromatography Of The Derived Methyl Esters On 10% PEGA Columns At 175°C *

Plant	Portion Extracted	Lauric Acid	Myristic Acid	Palmitic Acid	Stearic Acid	Oleic Acid
(A) NEW ZEALAND CORTADERIA SPECIES						
Cortaderia species collected June 1965 at Plimmerton	Rhizomes	26	20	32	15	8
Cortaderia species collected June 1965 at Plimmerton	Leaves	22	19	39	14	6
Cortaderia toetoe	Leaves	24	29	39	8	
Cortaderia fulvida	Leaves	28	26	32	13	
Cortaderia richardii	Leaves	26	30	39	14	
(B) SOUTH AMERICAN CORTADERIA SPECIES						
Cortaderia selloana	Leaves	23	28	40	9	
Cortaderia atacamensis	Leaves	24	26	37	13	
(C) Poa anceps	Leaves	25	32	39	13	

* The content of an individual acid is expressed as a percentage of the total acid content. Percentages were obtained by gravimetric integration of the areas of the recording paper enclosed under the peaks on the trace of the derived methyl esters.

TABLE III

Percentage Distribution Of n-Alkanols In Surface Wax Components
As Determined By Gas Liquid Chromatography Of The Derived Acetates
On 10% PEGA Columns At 175°C. *

Plant	Portion Extracted	Hexan-1-ol	Octan-1-ol	Decan-1-ol	Dodecan-1-ol	Tetradecan-1-ol
(A) NEW ZEALAND CORTADERIA SPECIES						
Cortaderia species collected June 1965 at Plimmerton	Rhizomes	20	28	39	12	9
Cortaderia species collected June 1965 at Plimmerton	Leaves	22	29	41	6	2
Cortaderia toetoe	Leaves	26	20	38	14	
Cortaderia fulvida	Leaves	24	19	40	17	
Cortaderia richardii	Leaves	23	26	38	13	
(B) SOUTH AMERICAN CORTADERIA SPECIES						
Cortaderia selloana	Leaves	18	29	42	19	
Cortaderia atacamensis	Leaves	26	24	38	20	
(C)						
Poa anceps	Leaves	22	30	41	7	

* The content of an individual alcohol is expressed as a percentage of the total alcohol content. Percentages were obtained by gravimetric integration of the areas of the recording paper enclosed under the peaks on the trace of the derived acetates.

of selected authentic acetates.

The results of the fatty acid and long chain alcohol analyses are shown in Table II and Table III respectively. A certain amount of difficulty was encountered in performing direct integration of the areas under the peaks on the traces, so, in order to determine the percentage compositions of the different mixtures, resort was made to a gravimetric integration procedure in which the area enclosed by each peak on a trace was cut out and weighed.

The results of the fatty acid and long chain alcohol analyses would seem to indicate that such analyses have little potential application in chemotaxonomic distinction of the five Cortaderia species. In no case did the total number of acids, nor the total number of alcohols exceed five, whilst the same four acids [lauric acid, myristic acid, palmitic acid and stearic acid, i.e. the usual acids of the glyceride pool] and the same four alcohols [n-hexanol, n-octanol, n-decanol and n-dodecanol] were present in all five Cortaderia species in comparable relative amounts. Moreover, palmitic acid was the preponderant acid and n-decanol was the preponderant alcohol in all cases.

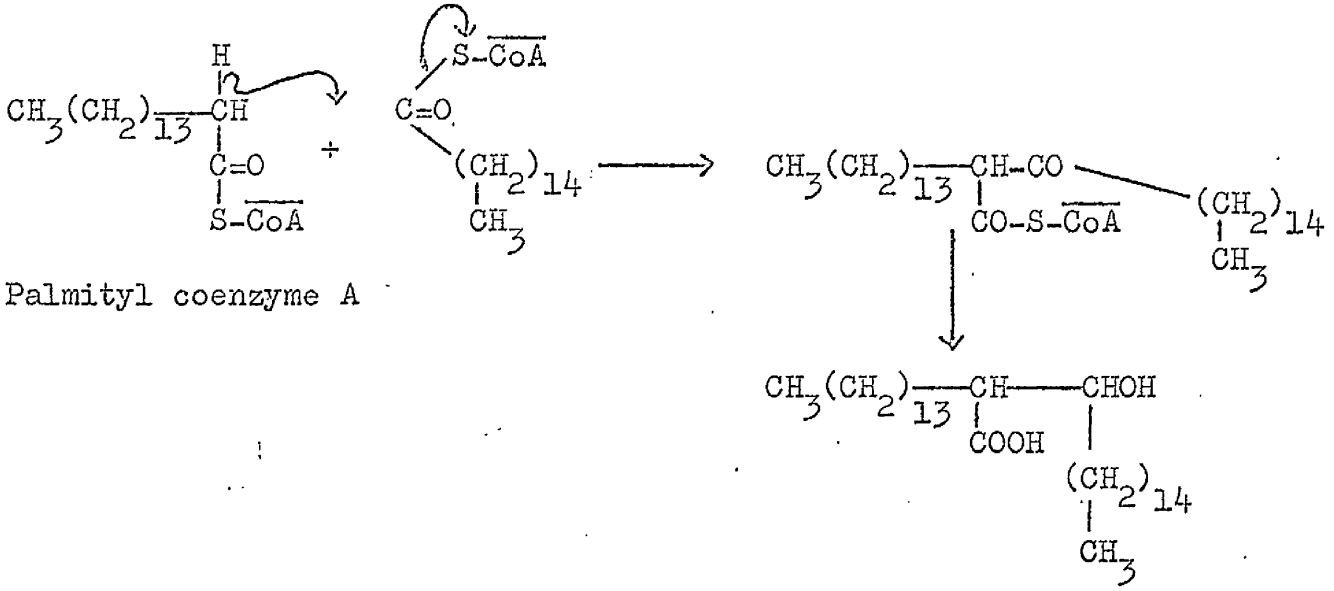
The detection of the unsaturated acid, oleic acid, together with tetradecanol in the Cortaderia species from Plimmerton [both leaves and rhizomes] and its apparent absence from the other Cortaderia species [especially C. toetoe with which the Plimmerton species is concluded to be identical -

FIGURE 2

Parallel Between Proposed Biogenesis Of n-Alkanes

And Biogenesis Of Corynomycolic Acid

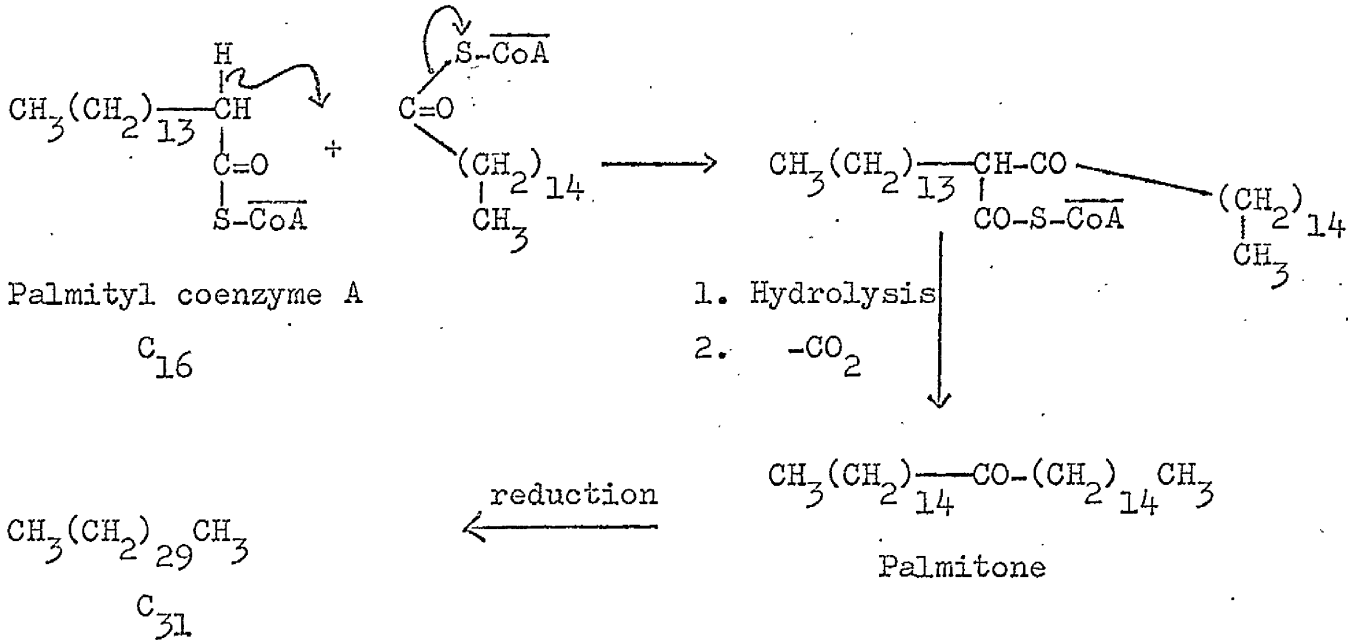
A. Biogenesis Of Corynomycolic Acid[30]



Palmityl coenzyme A

Corynomycolic acid

B. Proposed Biogenesis Of n-Alkanes[29]



Palmityl coenzyme A

C_{16}

Palmitone

C_{31}

[vide infra] may reflect geographical or climatic variation or may result from sampling error since far greater quantities of total extract were available from the Plimmerton material than were available from the other Cortaderias. Seasonal variation would not be a possibility here since the 1965 Plimmerton material and the five authenticated Cortaderia species were all collected at the same time of year.

A noteworthy feature of Table III is the short chain lengths of the alcohols [C_6-C_{14}]. The chain lengths of the acids in Table II [C_{12}-C_{18}] in relation to the chain lengths of the n-alkanes in Table I [C_{23}-C_{33}] are as to be expected in terms of current biogenetic theory in which it is considered[29] that one route at least leading to the formation of n-alkanes involves the coupling of two molecules of fatty acid before decarboxylation and reduction to the paraffin occurs, much as in the established biogenesis of corynomycolic acid.[30] The apparent parallel between this potential route for the biogenesis of the n-alkanes and the biogenesis of corynomycolic acid is portrayed in Fig. 2.

The results of the acid and alcohol analyses as given in Tables II and III are in agreement with the contention of Eglinton and Hamilton[29] that there appears to be no consistent relationship between the distribution patterns of alkanes, alcohols and acids in plants. In agreement with present concepts of biogenesis[29] n-alkanes with an odd number of carbon

atoms form the major alkane components of all the Cortaderia species, whilst the alcohols and acids appear restricted to those with an even number of carbon atoms.

3. Determination of the Presence or Absence of Triterpene Methyl Ethers.

Infrared analyses of the total light petrol extractives from the surface wax of each grass revealed the presence of absorption characteristic of the ether function at 1104 $cm.^{-1}$ in the light petroleum extractives of Cortaderia toetoe, Cortaderia fulvida, Cortaderia richardii and of the Plimmerton Cortaderia species [both leaves and rhizomes] from which the large scale extractions of arundoin had been made. On the other hand no absorption characteristic of the ether function was observed with the total light petrol extractives of either of the two South American Cortaderia species, of the unidentified Raglan Cortaderia species, or of Poa anceps.

In order to check the possibility that trace amounts of triterpene ethers, insufficient to reveal themselves in the infrared spectrum of the total light petrol extracts, could still be present in the South American Cortaderia species or the Raglan material, the light petrol extracts from these plants were worked up [as described below] as if they did contain ethers. However, no ethers could be detected at the appropriate stage in the chromatography. Hence, it can be concluded that the South American and the Raglan Cortaderia

species contain no appreciable quantities of triterpene methyl ethers.

The general procedure employed for the isolation of the triterpene ethers from Cortaderia toetoe, Cortaderia fulvida and Cortaderia richardii was as previously described by Hamilton[4] for his original isolation of ether 'B' and arundoin. Thus the total light petrol extractives were chromatographed over alumina, employing light petrol as eluant, and, after rejection of the initially eluted fatty material, the crystalline triterpene methyl ether fractions were collected.

In this way pure arundoin having identical infrared spectrum and m.p. with authentic material was isolated from C. richardii, C. fulvida and C. toetoe. However, the later fractions from C. toetoe showed m.p.s. lower than that of pure arundoin and were therefore assumed to be mixtures [in accord with the work of Hamilton[4] on 'Arundo conspicua'].

Application of gas liquid chromatography using 0.5% Apiezon L and 1.5% SE-30 showed that only one peak, corresponding in retention time to arundoin, was present in the total triterpene methyl ether fractions from both C. richardii and C. fulvida, but that the later fractions from the alumina column chromatography of the triterpene methyl ether fraction from C. toetoe gave rise to 3 peaks on the trace. These same 3 peaks were present in the traces obtained with the triterpene ether fractions from both the rhizomes and the leaves of the

Plimmerton Cortaderia species - indicating that this species must in all probability be Cortaderia toetoe. Certainly, the habitat of this material in swampy ground at Plimmerton would be in agreement with this conclusion[20].

Hence the five authenticated Cortaderia species investigated can be divided into 3 classes on the basis of the triterpene methyl ether analysis: i. the two South American Cortaderia species which contain no triterpene methyl ethers; ii. Cortaderia richardii and Cortaderia fulvida which each contain arundoin as the sole triterpene methyl ether; and iii. Cortaderia toetoe which contains arundoin and at least two other triterpene methyl ethers.

Addition of 5α-cholestane to the mixture of triterpene methyl ethers obtained from Cortaderia toetoe showed that on the different g.l.c. columns the retention times of the peaks observed, relative to 5α-cholestane [=1.00], were as follows:-

	0.5% Apiezon L	1.5% SE-30
Peak I [Ether 'B']	2.79	2.44
Peak II [Ether 'C']	3.18	2.72
Peak III [arundoin]	4.28	3.21

Preparative gas liquid chromatography was successful in separating the materials responsible for Peaks I and II which were then identified by their mass spectra and subsequent direct comparison with authentic specimens as β-amyrin and α-amyrin

methyl ethers respectively. This work is described in detail in sub-section C - see on. Before this work was successfully undertaken, however, in an attempt to facilitate the identification of individual triterpene methyl ethers present in mixtures of natural origin, a series of qualitative gas liquid chromatographic studies with pure authentic triterpene methyl ethers was undertaken as described in the following sub-section B.

Chemotaxonomic Conclusions with Respect to Cortaderia Species

As is readily seen from the foregoing discussion, studies on the chemical constituents of the leaf surface waxes of the various Cortaderia species, obtained by cold light petrol extraction of the unmacerated fresh leaves, has given rise to a certain degree of differentiation within the genus. Although the fatty ester analyses gave no distinction and the alkane analyses really only distinguished Cortaderia atacamensis from the other four species, the triterpene methyl ether analyses gave clear distinction between Cortaderia toetoe where arundoin and the methyl ethers of α-amyrin and β-amyrin were present; the two other New Zealand species, viz. Cortaderia fulvida and Cortaderia richardii where arundoin only was present; and the two South American Species, viz. Cortaderia selloana and Cortaderia atacamensis where no triterpene methyl ethers were present. Since the alkane distribution patterns would appear to differentiate Cortaderia selloana and Cortaderia atacamensis,

the work has failed only in not providing a distinction between Cortaderia fulvida and Cortaderia richardii as far as the authenticated plants are concerned.

The work has also served to strongly suggest that the unidentified Plimmerton species is Cortaderia toetoe, but the position with respect to the Raglan species is most unsatisfactory. The botanical identification of this material as Cortaderia toetoe, but the absence of triterpene methyl ethers is difficult to explain unless a genetic mutation is responsible. Certainly the production of triterpene methyl ethers in Cortaderia toetoe must be under genetic and not environmental control since the same three ethers are present in the Plimmerton plants [swampy habitat] and the authentic plants [habitat, open field].

The marked chemical difference between the three New Zealand species, which elaborate triterpene methyl ethers, and the two South American species, which do not, is also parallelled by fundamental botanical differences[20]. For instance, the three New Zealand species have sterile stamens in female plants whilst the two South American species have staminodes[20]. Also there is a major difference in lemma differentiation between the two groups[20].

B. Gas-Liquid Chromatographic Studies With Triterpene Methyl Ethers

As pointed out in an earlier sub-section, it was considered desirable to have available data on the relative g.l.c. retention times of different triterpene methyl ethers, in order to facilitate their ready identification in mixtures of natural origin. Accordingly nine authentic triterpene methyl ethers, obtained either from natural sources or by methylation of the corresponding triterpene alcohol by adaptation of the method of Morice and Simpson[31], were subjected to a series of gas liquid chromatographic experiments using different stationary phases and employing 5α-cholestane [relative retention time =1] as internal standard. The structures of these compounds are shown in Fig. 3 and their retention times relative to 5α-cholestane on 0.5% Apiezon L, 1.5% SE-30, 1.5% QF-1 and 1.0% CDMS columns are shown in Table IV. Preliminary experiments showed that lower temperatures led to increased 'tailing'. 'Tailing' was also pronounced on the QF-1 columns at 240°. The most satisfactory separations were those with the Apiezon L columns which gave high absolute retention times.

Although the feasibility of separating certain triterpene methyl ethers by means of gas liquid chromatography had been demonstrated by Hamilton[4] in his successful separation of Ether 'B' from arundoin, the present work would appear to

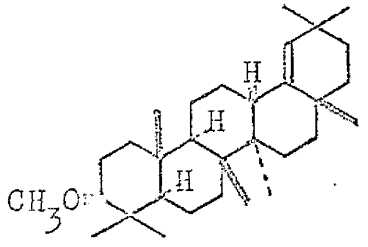
TABLE IV

Relative Gas Liquid Chromatographic Retention Times Of Triterpene Methyl Ethers *

Compound		0.5% Apiezon L Column Temperature 240°	1.5% SE-30 Column Temperature 240°	1.5% QF-1 Column Temperature 225°	1.0% CDMS Column Temperature 240°
5α-Cholestane (internal reference standard)		1.00(11-14 mins)	1.00 (3-4 mins)	1.00($2\frac{1}{4}$-$2\frac{3}{4}$ mins)	1.00 ($2\frac{1}{2}$-3 mins)
Germanicol Methyl Ether (Miliacin)	I	2.83	2.54	2.66	3.79
δ-Amyrin Methyl Ether (Isomiliacin)	II	2.80	2.44	2.78	3.79
β-Amyrin Methyl Ether (Isosawamilletin)	III	2.79	2.45	2.89	3.77
Taraxerol Methyl Ether (Sawamilletin)	IV	2.74	2.45	2.75	3.67
Multiflorenol Methyl Ether	V	2.74	2.46	2.93	3.77
α-Amyrin Methyl Ether	VI	3.20	2.73	3.17	4.25
Bauerenol Methyl Ether	VII	4.11	3.24	3.42	5.59
3β-Methoxy-E:C-friedoisohop-9(11)-ene (Arundoin)	VIII	4.31	3.20	3.52	5.50
Isoarborinol Methyl Ether (Cylindrin)	IX	4.95	3.43	3.47	6.25

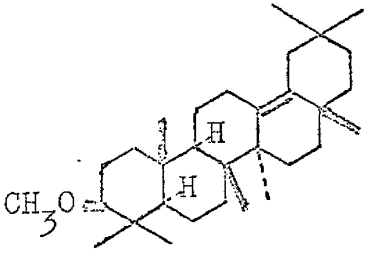
* The stationary phase was supported on Gas Chrom Z, 100-120 mesh; column temperatures as shown; detector temperature 248°; carrier gas argon, 60 mℓ/min; for further details see Experimental Section. The relative retention times shown represent the mean of at least three determinations. These values are approximate to ± 0.10 see calculation of experimental error in Experimental Section.

Triterpene Methyl Ethers Subjected To Gas Liquid Chromatography



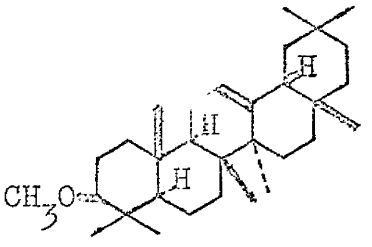
I

Germanicol Methyl Ether



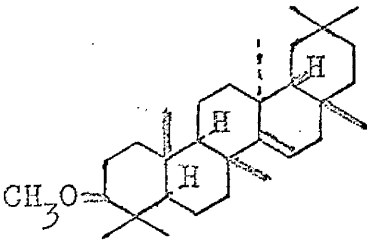
II

δ-Amyrin Methyl Ether



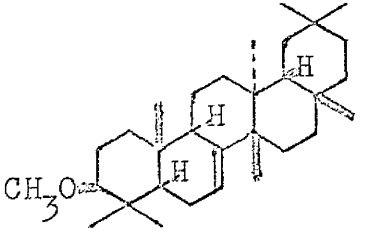
III

β-Amyrin Methyl Ether



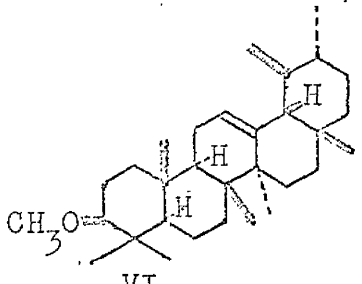
IV

Taraxerol Methyl Ether



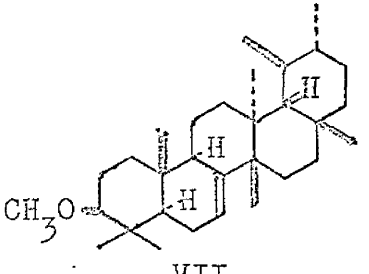
V

Multiflorenol Methyl Ether



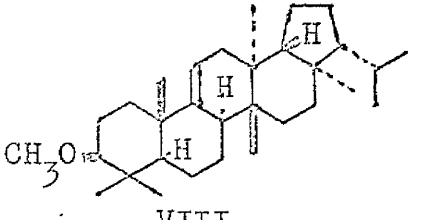
VI

α-Amyrin Methyl Ether



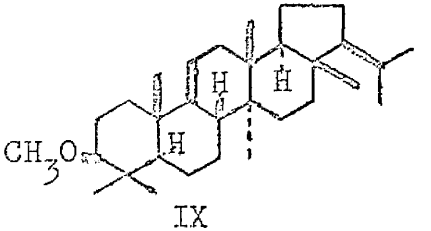
VII

Bauerenol Methyl Ether



VIII

Arundoin



IX

Cylindrin

represent the first systematic study of the gas liquid chromatography of triterpene methyl ethers. There have, however, been published several papers on the gas liquid chromatography of other triterpenoids[15,16,32] including several triterpene trimethylsilyl ethers[15,16].

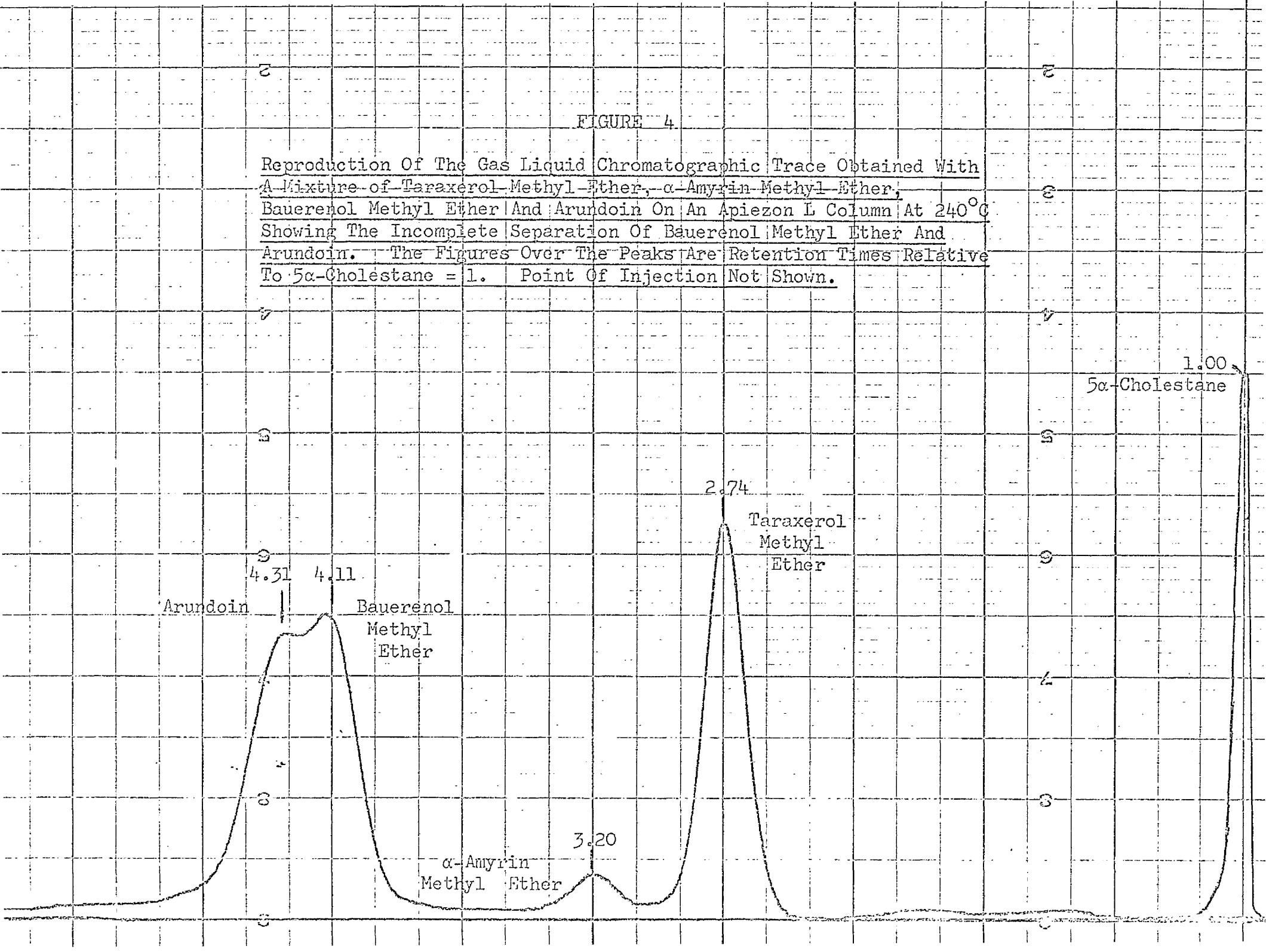
Examination of Table IV reveals that none of the four stationary phases employed gave rise to any clear-cut distinction between the five ethers [compounds I-V in Fig. 3] derived from the oleanane or rearranged oleanane skeleton - a result in agreement with the earlier observation by Hamilton[4] that β-amyrin methyl ether and taraxerol methyl ether showed identical retention times on Apiezon L columns - so despite the ease of preparation of triterpene methyl ethers from the parent alcohol by the method of Morice and Simpson[31], it would appear from the present work that gas liquid chromatography of methyl ethers may not be of as useful application in the triterpene field as it is in the steroid field[9-11]. Gas liquid chromatography employing silver nitrate in the stationary phase, as has been successfully used in the separation of other mixtures of olefin isomers[33] would not seem applicable to the present situation, in view of the high temperatures required in the g.l.c. of the triterpene methyl ethers. Separations of mixtures of the five triterpene methyl ethers I-V, might prove feasible with thin layer chromatography incorporating silver nitrate or mercuric salts in the adsorbant, but this was not attempted in the present work. Preliminary attempts at

temperature-programmed g.l.c. with an SE-30 column failed to achieve a separation of taraxerol methyl ether, and β-amyrin methyl ether, and this approach was not further investigated.

The identical [within the limits of experimental error - see experimental section] retention times of ethers I-V were further substantiated when various mixtures of ethers from this group, taken two at a time were subjected to gas liquid chromatography. Thus a single symmetrical peak, giving no hint of the presence of two components was shown by mixtures of β-amyrin methyl ether with taraxerol methyl ether, of β-amyrin methyl ether with germanicol methyl ether, of taraxerol methyl ether with germanicol methyl ether, and of δ-amyrin methyl ether with germanicol methyl ether, on the Apiezon L. column. Similar results were obtained on the SE-30 column and on the QF-1 column. In fact the only case where the presence of two components was even suggested was with the pair germanicol methyl ether and taraxerol methyl ether on the SE-30 column and here the asymmetry of the peak was barely discernible. Similar asymmetry in the peak obtained with a mixture of germanicol methyl ether and β-amyrin methyl ether on the SE-30 column was not detectable. Hence it is apparent that differences in relative retention time of the order of 0.1 [germanicol methyl ether = 2.54 and taraxerol methyl ether = 2.45 on SE-30] give no separation. On the other hand the SE-30 column gave a clear separation of any one of the oleanane group

FIGURE 4

Reproduction Of The Gas Liquid Chromatographic Trace Obtained With A Mixture of Taraxerol Methyl Ether, α-Amyrin Methyl Ether, Bauerenol Methyl Ether And Arundoin On An Apiezon L Column At 240°C Showing The Incomplete Separation Of Bauerenol Methyl Ether And Arundoin. The Figures Over The Peaks Are Retention Times Relative To 5α-Cholestane = 1. Point Of Injection Not Shown.



ethers from α-amyrin methyl ether [relative retention time 2.73].

A mixture of bauerenol methyl ether and arundoin, although not fully resolved, clearly showed as a mixture of two components on the Apiezon L column [Fig. 4] but gave only a single symmetrical peak on the SE-30 and QF-1 columns. This difference in the relative order of retention times on the Apiezon L and SE-30 and QF-1 columns is in accord with other studies of the gas liquid chromatography of triterpenes on different stationary phases[15,16].

The inability to achieve separation between the methyl ethers derived from the oleanane skeleton on any of the stationary phases employed might suggest that, at the high temperature involved [240°C], backbone rearrangement was occurring on the columns to give the same thermodynamically stable compound [which would be expected to be δ-amyrin methyl ether] as the sole species eluting from the columns. That this was not the case, however, was shown by employing preparative g.l.c. columns in place of the analytical columns and collecting the eluted material. In this way, utilizing the diagnostic mass spectral cracking patterns of pentacyclic triterpenes[34,35], it was shown that taraxerol methyl ether and β-amyrin methyl ether emerged unchanged from 1.5% SE-30 and 1.0% Apiezon L columns at 240°, whilst employing infrared spectral characteristics it was shown that multiflorenol methyl ether emerged unchanged

from a 1.0% Apiezon L column at 240°. That no rearrangement was occurring with the ursane skeleton was apparent since bauerenol methyl ether and α-amyrin methyl ether were readily resolved on the Apiezon L and SE-30 columns at their different characteristic retention times at 240°, although they were not resolved on the QF-1 column.

It would thus appear with respect to the methyl ethers derived from the oleanane or rearranged oleanane skeleton that differences in polarity or conformational restriction are insufficient to permit resolution of mixtures of these compounds on gas liquid chromatography under the conditions employed in the present work. These two factors of polarity and conformational restriction together with molecular weight [in the present case, constant] are recognised[9] to be the main influences on relative retention times .

In connection with the virtually identical retention times of the five methyl ethers of the oleanane group it is of interest that very close retention time values for β-amyrin [3.23] and taraxerol [3.14] on 1.3% SE-30 columns have been reported[15] by the Japanese workers. Similarly the trimethylsilyl ethers of β-amyrin and taraxerol appear to have very similar retention times, being 3.34 and 3.15; 3.12 and 3.12; and 3.42 and 3.33 on 2% CNSi, 1.5% QF-1 and 1% NGS columns respectively[15].

The spread of relative retention time values on the SE-30 columns shown in Table IV, viz. 2.44 - 3.43, is somewhat lower

than the range of 3 - 6 previously reported[15] for monosubstituted pentacyclic triterpenes on SE-30 columns where the substituents are hydroxyl, keto, acetoxyl, methoxycarbonyl etc., but again this would be in accord with the relatively non-polar nature of the methoxyl group.

The extensive gas liquid chromatographic studies carried out in the steroid field have permitted detailed analysis of the influence of given substituents in given nuclear positions on the retention time and tables of 'group retention factors' which are the relative changes in retention accompanying the introduction of particular substituents [especially double bonds] are available[9-12]. The 'group retention factor' is defined[9] as the relative retention time of the steroid bearing substituent x divided by the relative retention time of the analogue lacking substituent x - with each relative retention time normally measured with respect to 5α-cholestane = 1. In the present work, however, no such detailed correlations are feasible owing to the constitution of the compounds studied and the absence of saturated analogues, and parent hydrocarbons as reference standards. It may be noted that within the nine triterpene methyl ethers studied [for formulae see Fig. 3] there are seven different nuclear systems represented. Thus three of the compounds [the methyl ethers of germanicol (I), δ-amyrin (II) and β-amyrin (III) possess the unrearranged oleanane skeleton, one [the methyl ether of taraxerol (IV)]

is a derivative of D-friedo oleanane[36], one [the methyl ether of multiflorenol (V)] is a derivative of D:C-friedo oleanane, one [α-amyrin methyl ether (VI)] is a derivative of ursane, one [bauerenol methyl ether (VII)] is a derivative of D:C-friedo ursane, one [arundoin (VIII) is a derivative of E:C-friedo isohopane and one [cylindrin (IX)] is an E:C-friedo derivative of the as yet unnamed parent compound giving rise to the arborinol series.[37,38]

It is nevertheless of interest that the ratio of the relative retention times of β-amyrin methyl ether and multiflorenol methyl ether of the oleanane group is not the same as the ratio of the relative retention times of the corresponding ursane analogues, viz. α-amyrin methyl ether and bauerenol methyl ether on any of the stationary phases.

When the data given in Table IV are taken in conjunction with the limited data available from other studies[15,16,32] of the g.l.c. behaviour of triterpenes it would appear that a generalisation may be emerging in that compounds derived from the fundamental oleanane skeleton would seem to have lower retention times than analogous compounds based on the ursane skeleton, which in turn would seem to have lower retention times than analogous compounds based on the isohopane skeleton. However, further detailed studies are required in order to ascertain whether or not this trend will be confirmed. In this connection, further g.l.c. studies with the methyl ethers of lupeol, glutinol, friedelan -3 β-ol, phyllanthol, taraxasterol,

Ψ-taraxasterol and hydroxyhopane might profitably be undertaken.

C. Identification Of The Triterpene Methyl Ethers Of Cortaderia toetoe And Their Biogenetic Implications.

In terms of the analytical gas liquid chromatographic results discussed in the previous section B and summarised in Table IV, it is apparent from the retention time data given on Page 72 for the g.l.c. analyses of the mixture of triterpene methyl ethers from Cortaderia toetoe, that, in addition to arundoin, the mixture probably contains α-amyrin methyl ether [hitherto unreported in nature] and at least one methyl ether from the oleanane group. In the absence of any gas liquid chromatographic system proven capable of resolving mixtures of the five methyl ethers of the oleanane group (compounds I-V) and in the knowledge that no separation occurred with thin layer chromatography using silica gel or alumina as adsorbants with a variety of different moving phases, it was decided to obtain the material responsible for Peak I and the material responsible for Peak II in the g.l.c. traces by means of preparative g.l.c. and then subject each sample to mass spectral analysis in an attempt at further identification. Preparative gas liquid chromatography, employing a 1% Apiezon L column at 240°C was successful in separately providing the two materials and the mass spectral analysis showed that the material corresponding

to Peak I had a mass spectrum identical with that of authentic β-amyrin methyl ether [thus confirming the conclusions of Hamilton[4] as to the identity of Ether 'B'] whilst the material corresponding to Peak II [ie. Hamilton's Ether 'C'] had a mass spectrum identical with that of authentic α-amyrin methyl ether.[34,35] The observed and predicted major mass spectral cracking peaks for various triterpene methyl ethers are shown in Table VI, subsection F - see later.

Although the presence of other triterpene methyl ethers of the oleanane group in Cortaderia toetoe can not be ruled out, the fact that their presence could not be detected by mass spectrometry, nor in the infrared spectrum of Ether 'B' which was identical with that of synthetic β-amyrin methyl ether, would tend to indicate that, if such compounds are present, it is only in very small amounts. Similarly the mass spectrum and infrared spectrum of Ether 'C' showed that this material was predominantly, if not entirely, the methyl ether of α-amyrin, so that any other methyl ethers present, having the same relative retention times as the methyl ether of α-amyrin, could also only be present in trace quantities.

Biogenetic Considerations

The co-occurrence in Cortaderia toetoe of methyl ethers derivable from the oleanane, ursane and isohopane skeletons is of considerable interest in terms of current biogenetic theory, since it would imply the existence of two separate

cyclisation mechanisms of squalene in the same plant. In order that this may be seen more clearly a brief resumé of present-day concepts relating to the biogenesis of triterpenes is desirable. Moreover such a survey would seem particularly timely in view of the heightened current interest in plant triterpene biogenesis resulting from plant tissue culture experiments[37,40] which promise to afford a sophisticated method of experimental verification of the finer details of the theory.

Current Theory On The Biogenesis Of Triterpenes

Elegant deductions by Eschenmoser, Ruzicka and their colleagues[41-43] in Switzerland, extending earlier proposals on the biogenesis of cholesterol from squalene via lanosterol by Woodward and Bloch[44], have indicated that all triterpenes and steroids of established structure can, theoretically at least, if not in reality, be derived from all trans squalene according to one of several separate cyclisation mechanisms, each of which gives rise to the observed absolute stereochemistry of the resultant products. There would now appear to be at least seven of these separate cyclisations operative in Nature, although of course others are theoretically possible and compounds formed via other modes may still remain to be discovered. These seven cyclisations of all trans squalene [itself formed via mevalonic acid and the isoprenoid route by a mechanism involving the tail-to-tail condensation of two

molecules of farnesyl pyrophosphate [45] may be briefly outlined as follows:-

1. Cyclisation in chair, boat, chair, boat conformational sequence.

Synchronous cyclisation of all trans squalene in chair, boat, chair, boat conformational sequence gives a fundamental tetracyclic carbonium ion from which several groups of triterpenes and the steroids can be derived. It has been customary to regard the initiation of this cyclisation as taking place through the intercession of the biological equivalent of OH^+ [46] but recently emphasis has been given [47] to the view that cyclisation is in reality induced by H^+ to give a hydrocarbon, which, while still bound to the enzyme in some way, then undergoes selective hydroxylation at what is C-3 in steroid and triterpene numbering. This interpretation as well as doing away with the need to postulate such an unlikely species as OH^+ would perhaps explain the occurrence in Nature of various triterpene hydrocarbons such as taraxerene and the fern triterpene hydrocarbons, as well as the incorporation of labelled lanostadiene into lanosterol in cell-free systems from yeast [47]. It would also be in accord with established hydroxylations at other saturated secondary carbon atoms of the steroid nucleus by mammalian and microorganismic enzyme systems in which atmospheric oxygen [not oxygen from water] is involved to give a species believed to be akin to $\cdot OH$. [48] The chair, boat, chair, boat mode of cyclisation of all trans

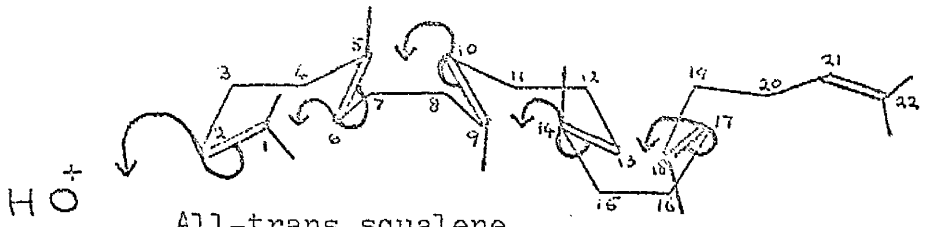
squalene is shown schematically in Figure 5 in which the usually portrayed OH^+ is employed. Moreover, for the sake of simplicity, the fundamental carbonium ion, designated α, has been portrayed as a classical carbonium ion, although representation of α in this way does not depict the origin of the observed control of the absolute configuration developed at the carbon atom marked as number 18 in the subsequent history of α. In order to show the development of specific configuration at C-18 [which is renumbered as C-20 in the conventional steroid and tetracyclic triterpene numbering systems [36,49]] it is customary to depict α as being equivalent to certain bridged non-classical carbonium ions. The numbering systems employed in Fig. 5, and in the subsequent Figs. 7,10,15, 17,18 is as in squalene, and not as in the conventional triterpene and steroid numbering systems, in order that the origin of the nuclear carbon atoms in the various cyclisation products may be more readily recognised.

It may be noted that formation of the 9,14 bond to enclose the third 6-membered ring, ring C, [Fig. 5] involves an anti-Markownikoff addition with respect to the 13,14 double bond. Formation of the 13,17 bond to create the 5-membered ring D avoids a second anti-Markownikoff addition with respect to the 17,18 double bond, as would be involved in the formation of a 13,18 bond with a 6-membered ring D.

The fundamental carbonium ion α then acts as a common precursor [41-43] for several distinct skeletal types, namely,

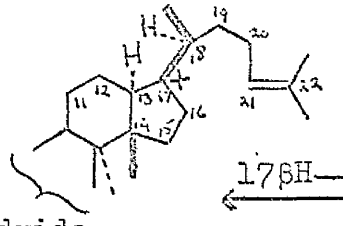
FIGURE 5

Derivation Of Compounds Resulting From Cyclisation Of All Trans Squalene In Chair, Boat, Chair, Boat Conformational Sequence

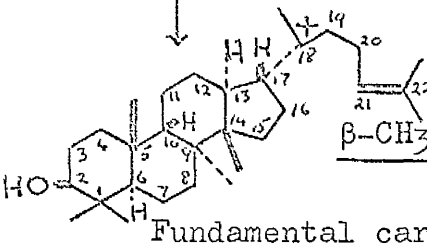


All-trans squalene

Loss of 17βH. Various oxidations. Loss of β-CH_3 from C-1 → FUSIDIC ACID series



17βH → 18S

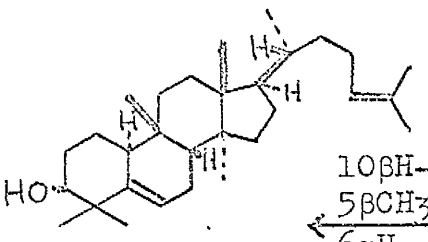


Fundamental carbonium ion α

Further hydride and methyl shifts. Loss of proton. → SARGASTEROL type.

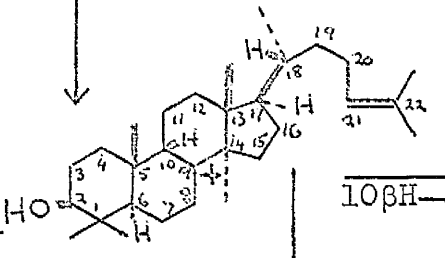
Friedel backbone rearrangements
17βH → 18R (i.e. 20R in lanosterol numbering)
13αH → 17α, 14βCH_3 → 13β,
9αCH_3 → 14α.

PARKEOL type.



10βH → 9β
5βCH_3 → 10β
6αH → 5α
loss of 7βH as proton.

BRYOGENIN -CUCURBITACIN series



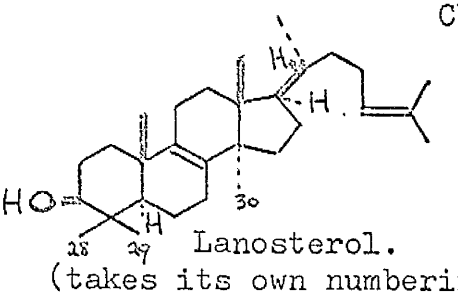
10βH → 9β

Loss of 11αH as proton.

Cyclopropane ring formation

Loss of 10βH as proton

CYCLOARTENOL series

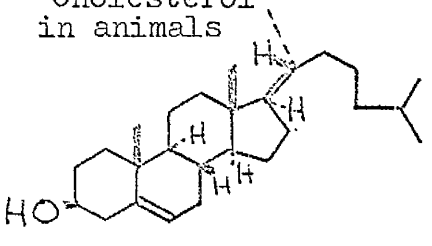


Lanosterol.
(takes its own numbering system).

Certain ALKALOIDS and ? PLANT STEROIDS

Loss of C-28, C-29 and C-30 with other oxidations and reductions.

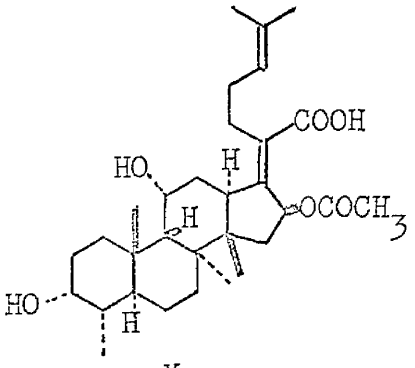
STEROIDS e.g. Cholesterol in animals



In mammalian body →
- Vitamin D_3
- Bile Acids
- Oestrogens
- Progesterone
- Androgens
- Adrenocorticoids

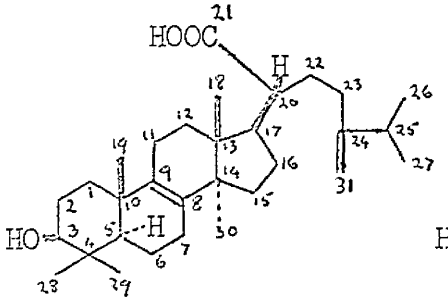
FIGURE 6

Some Representative Individual Triterpenes And Steroids Arising From The Synchronous Cyclisation Of All Trans Squalene In Chair,Boat,Chair,Boat Conformational Sequence By The Routes Outlined In Figure 5. Conventional Numbering.



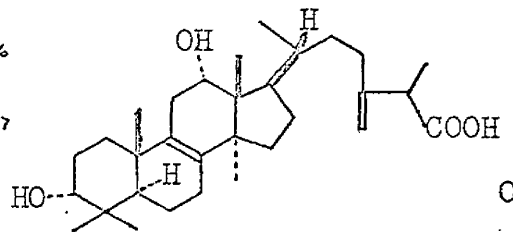
X

Fusidic Acid



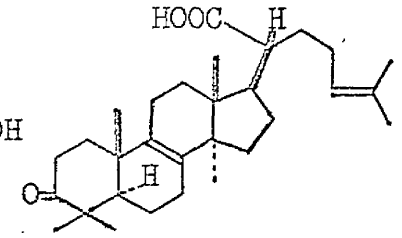
XI

Eburicoic Acid



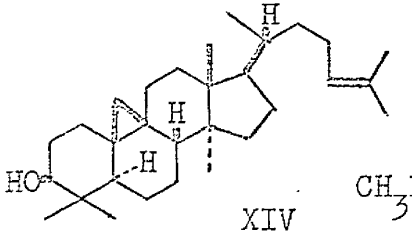
XII

Polyporenic Acid A



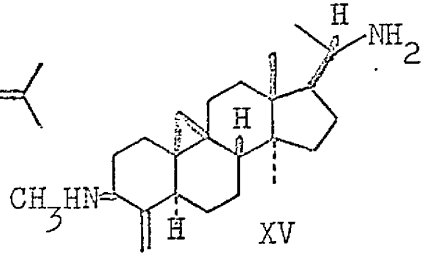
XIII

Pinicolic Acid



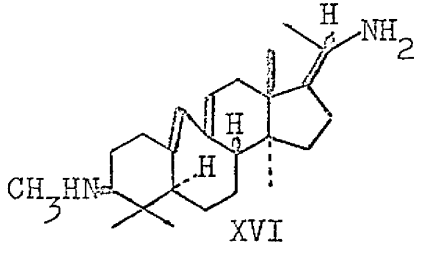
XIV

Cycloartenol



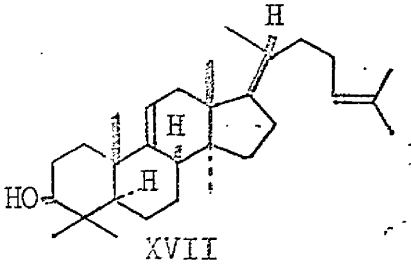
XV

Cyclobuxine



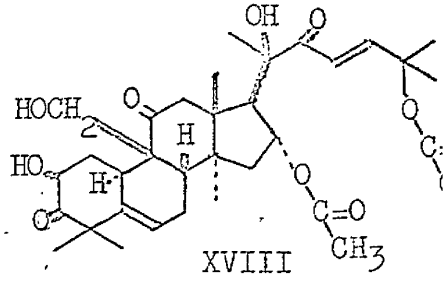
XVI

Buxenine-G



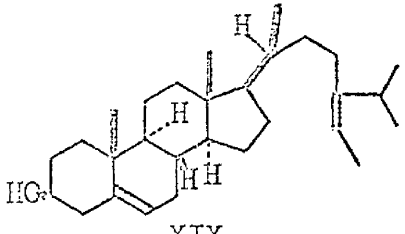
XVII

Parkeol



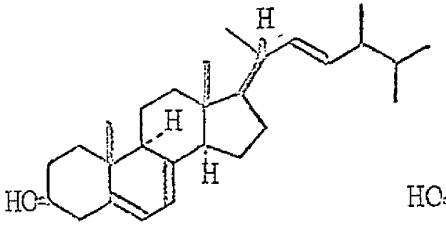
XVIII

Cucurbitacin A



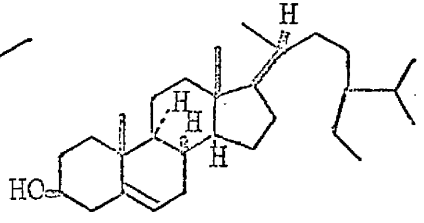
XIX

Sargasterol



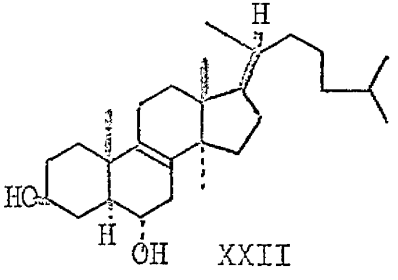
XX

Ergosterol



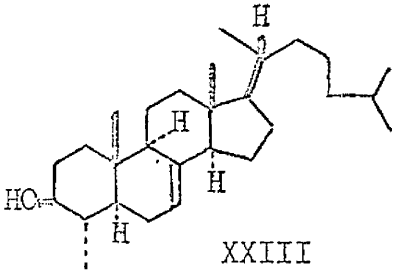
XXI

β-Sitosterol



XXII

Macdougallin



XXIII

Lophenol

tetracyclic triterpenes of the fusidic acid-helvolic acid-cephalosporin P_1 group[50], tetracyclic triterpenes of the lanosterol group, tetracyclic triterpenes of the bryogenin-cucurbitacin group, pentacyclic triterpenes of the cycloartenol group, and steroids with both normal [i.e. R] and abnormal [i.e. S] configuration at C-20 [steroid numbering]*. The routes to these different groups from α are outlined in Fig. 5. These different routes are defined by the intercession or otherwise of the type of backbone rearrangements involving stereospecific 1,2 hydride shifts and Wagner-Meerwein-like 1,2 methyl group migrations[51] upon which Allard and Ourisson[36] based their friedo nomenclature for rearranged triterpene skeletons. The various representatives in each group then differ from their skeletal prototype with respect to their degree of oxidation.

Thus the fusidic acid group of tetracyclic triterpenes [for which the skeletal prototype has not yet been isolated] arises without rearrangement of α, through loss of the 17β proton. Subsequent oxidations on the prototype so formed, including oxidative removal of the β-methyl group from C-1 of α[50] then give rise to fusidic acid (X)[50], the structure of which is shown in Fig. 6.

The lanosterol series of tetracyclic triterpenes which includes lanosterol itself[52], dihydrolanosterol[53], certain

* R and S convention for specifying the absolute configuration of asymmetric carbon atoms as described by Cahn, Ingold and Prelog, Experientia, 1956, 12, 81.

derived compounds in which an extra carbon atom derived from formate[54] [probably via S-adenosylmethionine[55]] has been inserted at C-24 [lanosterol numbering], e.g. eburicoic acid (XI)[56], and various other more highly oxidised derivatives such as the polyporenic acids, e.g. polyporenic acid A (XII)[57], pinicolic acid (XIII)[58] tumulosinic acid[59] and cimigenol[60] are derived from carbonium ion α by a backbone rearrangement[61] involving the following shifts:

17βH ⟶ 18R [20R in lanosterol numbering]

13αH ⟶ 17α

14βCH_3 ⟶ 13β

9αCH_3 ⟶ 14α

followed by loss of the proton from the 10β-position.

The cycloartenol group [which includes compounds such as cycloläudenol[62], which has an additional carbon atom in the side chain, and cycloeucalenol[63], which has lost the β-methyl group from the C-1 position of α, as well as having an additional carbon atom in the side chain] can be regarded as being derived from carbonium ion α via the same rearrangement as is involved in the formation of lanosterol except that, instead of loss of the 10β proton, the 10β proton migrates to the 9β position with cyclopropane ring formation occurring through loss of a proton from the methyl group attached to C-5 and bond formation to the 10β position. The structure of cycloartenol (XIV)[64] is given in Fig. 6. Further transformations including oxidative shortening of the side chain

in the cycloartenol prototype are then believed to give rise to alkaloids of the cyclobuxine[65], cyclomicrophylline[66] and buxenine[67] types. The structure of cyclobuxine (XV)[65] is shown in Fig. 6 as is the structure of buxenine G (XVI)[67]. This last compound is of further interest, since in addition to other changes in the cycloartenol molecule, the cyclopropane ring has been cleaved giving rise to a seven-membered ring B.

The formation of parkeol (XVIII)[68] can be considered to follow the pathway common to the biogenesis of lanosterol and cycloartenol but after the migration of $9\alpha CH_3 \Longrightarrow 14\alpha$ and of $10\beta H \Longrightarrow 9\beta$ a proton is then lost from C-11 of α.

Formation of the bryogenin-cucurbitacin series which includes gratiogenin[70] involves the same series of 1,2 shifts as were described for the formation of lanosterol plus the following subsequent additional non fully concerted shifts:

$$10\beta H \longrightarrow 9\beta$$

$$5\beta CH_3 \longrightarrow 10\beta$$

$$6\alpha H \longrightarrow 5\alpha$$

followed by loss of the 7β hydrogen atom as a proton. Further oxidations of the resulting skeletal prototype then lead to the formation of bryogenin, gratiogenin and the cucurbitacins. The structure of cucurbitacin A (XVIII)[71] is shown in Fig. 6.

The great majority of naturally occurring steroids [i.e. those with 20R configuration] of both the plant and animal kingdoms have generally been assumed to arise through subsequent modifications to the lanosterol molecule[41-43], although recently

it has been suggested that, in some plants at least, the plant sterols may in fact arise from cycloartenol rather than from lanosterol[40]. Certainly this would be in accord with the apparent relatively common occurrence of cycloartenol in plants and with the rare instances of the isolation of lanosterol [and also cholesterol[72]] from the plant kingdom. Steroids such as sargasterol (XIX)[73] occurring in algae, which have 20S configuration would appear to arise from the as yet unisolated 20-isolanosterol or 20-isocycloartenol [lanosterol numbering], the formation of which must be analogous to the formation of lanosterol or cycloartenol, except that migration of the 17β hydrogen atom in α is controlled in such a way that opposite stereochemistry is developed in the first carbon atom of the side chain.

Formation of plant sterols of the ergosterol (XX)[74] and β-sitosterol (XXI)[75] types from lanosterol [or cycloartenol] involves the addition of a one-carbon unit and of two one-carbon units[76,77] respectively at C-24 [lanosterol numbering] in addition to the oxidative loss of methyl groups from C-4 and C-14 [lanosterol numbering] which is also involved in the biogenesis of the key animal sterol, cholesterol. The exact sequence followed in the loss of these methyl groups is as yet inconclusively established,[78] but the natural occurrence in cacti of the plant sterols macdougallin (XXII)[79] which has lost the two methyl groups from C-4 [lanosterol numbering],

and lophenol (XXIII)[80] which has lost the methyl group from C-14 and the β-methyl group from C-4 [lanosterol numbering], would perhaps indicate that removal of the methyl groups does not necessarily always occur in the same order in all organisms. In animals it would seem that the methyl group attached to C-14 [lanosterol numbering] is the first to be lost in the biogenetic route to cholesterol since the intermediary sterols 3β-hydroxy-4,4-dimethyl cholest-8,24-diene[81], 3β-hydroxy-4α-methyl cholest-8-ene[82] and 3β-hydroxy-4α-methyl cholest-7-ene[83] (lophenol, XXIII) have been isolated from animal sources. There is also evidence that saturation of the side chain double bond of lanosterol occurs at a late stage in the formation of the true sterols. Indeed in animals conversion of desmosterol [24-dehydrocholesterol] into cholesterol had been assumed to be the last stage in the biosynthesis of cholesterol since certain drugs which are employed as anti-hypercholesterolaemic agents in attempts to prevent conditions such as atherosclerosis produce an accumulation of desmosterol[84], but recent evidence has cast doubts on the validity of this assumption.[78] In the plant kingdom too, it is probable that saturation of the C_8H_{15} side chain derived from lanosterol [or cycloartenol], where it occurs, is also a late stage and that other plant steroids formed without loss of carbon atoms from this side chain, e.g. the steroidal sapogenins and the steroidal alkaloids

based on the spirosolane, solanocapsine[85], solanidane, veralkamine[86], jervine, veratramine and cevane skeletons, may well arise while the side chain is still unsaturated. Oxidative cleavage of the lanosterol or cycloartenol side chain in plants, on the other hand, can be considered to give rise to the plant pregnane group[87] [including alkaloids of the pregnane, conanine and paravallarine groups[88]], the pregnane derived cardenolides[89], the scilladienolides, the plant androstane group[90], and the plant oestrane group[91]. With respect to the suggestion[40] mentioned earlier, that cycloartenol rather than lanosterol may be the precursor of a number of plant steroids, it is of interest that feeding experiments with cholesterol labelled in the 4-position with ^{14}C have shown that the labelling is incorporated in the Holarrhena alkaloids, holaphyllamine and holaphylline[92].

The animal sterol, cholesterol, in which the lanosterol side chain has been saturated, likewise gives rise to a variety of different steroids via oxidative cleavage of the side chain. Hydroxylation at C-20 and C-22 [cholesterol numbering] followed by cleavage of the 20,22 bond to yield pregnenolone and isocaproic acid appears to be the main route by which the steroid hormones are formed - with pregnenolone then acting as key precursor of progesterone, the adrenocorticoids, the androgens and the oestrogens[77,93]. The bile acids are formed from cholesterol via nuclear hydroxylation, oxidation to the 4-en-3-one, reduction to the 3α-hydroxy-5β-compound and

oxidative degradation of the side chain involving hydroxylation at C-26. A summary of the groups of mammalian steroids formed from lanosterol via cholesterol is included in Fig. 5.

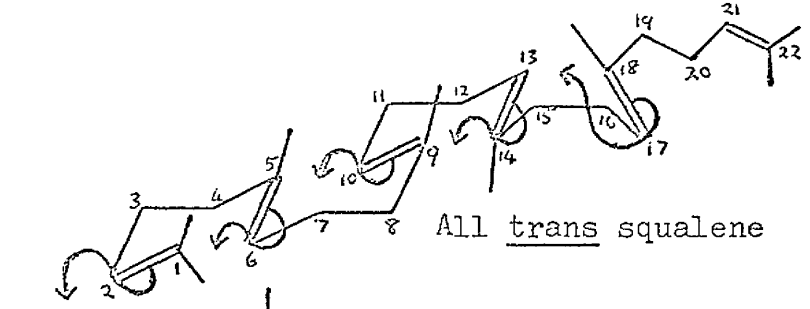
Compared with the carbonium ions δ, ε and ζ resulting from other modes of cyclisation of all trans squalene [Figs. 7 and 15], ion α in Fig. 5 would seem to give rise to relatively few derived skeletal types in nature. This is apparently due to the unfavourable trans-syn-trans relationship of the A/B and B/C ring fusions and the boat form of ring B in ion α which serve to provide a driving force for the introduction of an 8,9 double bond [lanosterol numbering], as in the lanosterol and 20-isolanosterol series; of a cyclopropane ring involving C-9, C-10 and C-19 [lanosterol numbering] as in the cycloartenol series; of a 9,11 double bond [lanosterol numbering] as in parkeol; or of a cis B/C ring junction, as in the bryogenin-cucurbitacin series. The existence in nature of the fusidic acid series[50], however, shows that this driving force to relieve the trans-syn-trans A/B, B/C relationship is not so strong as to deny the existence of this system. It may be noted, nevertheless, that subsequent reduction of the 8,9 double bond of lanosterol proceeds in such a manner as to give the more favoured trans-anti-trans relationship of rings A, B and C with a chair ring B in the derived steroids.

Unlike carbonium ion β [Fig. 7] carbonium ion ∝ [Fig. 5]

FIGURE 7

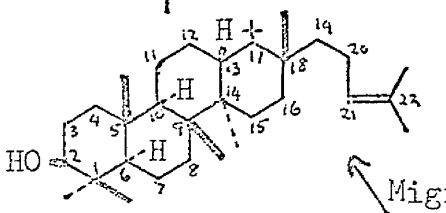
SHIONONE
(renumbered)

Derivation Of Compounds Resulting From Cyclisation Of All Trans Squalene In Chair,Chair,Chair,Boat Conformational Sequence.



All trans squalene

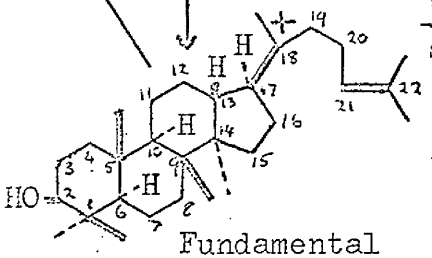
Complete friedo backbone rearrangement starting with 13βH ⟶ 17



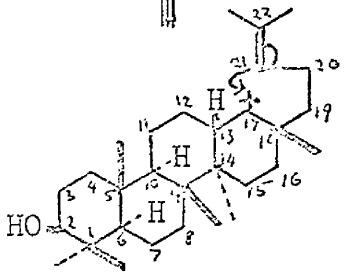
Migration of 16,17 bond to 16,18 position

Without skeletal rearrangement ⟶ TETRACYCLICS of Protopanaxadiol and Dammar resin groups (renumbered)

With friedo skeletal rearrangement ⟶ TETRACYCLICS of Euphol, Tirucallol, Butyrospermol and Masticadienonic Acid types (renumbered)



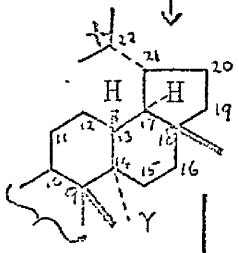
Fundamental Carbonium ion β



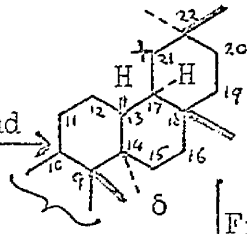
Formation of 17,21 bond

TARAXASTEROL (renumbered)

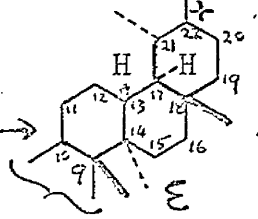
Loss of proton from methyl group on C-22



Migration of 20,21 bond to 20,22 position



Migration of 22αCH3 to 21α



Loss of 20H ⟶ Ψ-TARAXASTEROL (renumbered)

OH⁻ ⟶ MONOGYNOL A (renumbered)

Loss of proton from methyl group on C-22 ⟶ LUPEOL (renumbered)

Loss of 17αH ⟶ GERMANICOL (renumbered)

Friedo rearrangements starting with 17αH ⟶ 21α ⟶ FRIEDO OLEANANES (renumbered)

Friedo rearrangements starting with 21βH ⟶ 22β ⟶ FRIEDO URSANES (renumbered)

does not appear to give rise to pentacyclic triterpenes [excluding those with cyclopropane rings].

2. Cyclisation in chair, chair, chair, boat conformational sequence.

Synchronous cyclisation of all trans squalene in chair, chair, chair, boat conformational sequence, as illustrated in Fig. 7, affords the fundamental tetracyclic carbonium ion β, [squalene numbering], which acts as the common precursor of a large number of tetracyclic and pentacyclic triterpenes belonging to a number of different skeletal types. Once again, as in the formation of ion α [Fig. 5], the formation of the 9,14 bond in β involves an anti-Markownikoff addition with respect to the 13,14 double bond.

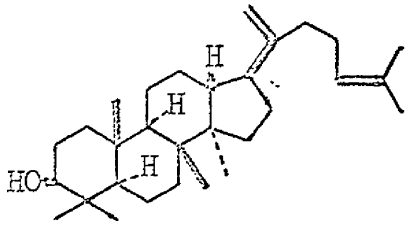
The unrearranged skeleton possessed by carbonium ion β appears in tetracyclic triterpenes of the dammar resin group[94] which are either formed by loss of a proton from the methyl group attached to C-13 in β [e.g. dammadienol (XXIV)] or by direct nucleophilic attack by OH^- at C-18 in β as in the dammarenediols I and II (XXV) which differ, only in the stereochemistry developed at this centre. Dipterocarpol[95], aglaiol[96], carnaubadiol[97] and octotillol[98] are other representatives of this group. Dammarenolic acid (XXVI)[99] or 20-hydroxy-C:4-secodammar-4[30],24-dien-3-oic acid is an interesting member of the series in which there has been cleavage of ring A. Two of the products obtained by acid

hydrolysis of the saponins present in the root of Panax ginseng C.A. Meyer namely panaxadiol[100] and panaxatriol[101] also possess the unrearranged skeleton present in carbonium ion β. These compounds are, however, artefacts derived by acid catalysed addition of the 20-OH group of a dammarenediol-type compound to the 24,25 double bond to give an α,α-dimethyl-tetrahydropyran ring in the side chain[100]. In the case of panaxadiol, the open chain precursor, or true genin, proto-panaxadiol has been successfully isolated.[101]

What can also be regarded as an unrearranged skeleton based on carbonium ion β also occurs where there is a hydride shift from the 17α position to the 18 position in β followed by loss of proton from the 13β position since asymmetry at C-17 is lost with the introduction of the 13,17 double bond. Isotirucallenol (XXVII)[102], for example, in which there is S configuration at C-20 [lanosterol numbering] if it is found in Nature would be formed in this way. However the 1,2 shifts 17αH ⟶ 18, 13β ⟶ 17β in carbonium ion β result in opposite stereochemistry, with the side chain in the α-orientation, being developed at C-17. Friedo backbone rearrangements initiated by these two 1,2 shifts give rise to the euphol, tirucallol, butyrospermol and masticodienonic acid types and their various oxidised derivatives. Thus backbone rearrangement of ion β with

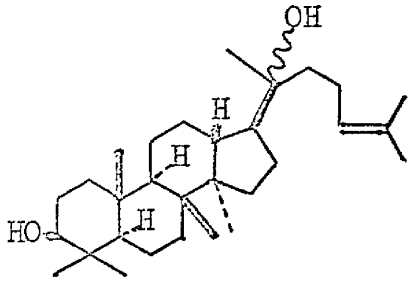
FIGURE 8

Some Representative Individual Tetracyclic Triterpenes Arising From The Synchronous Cyclisation Of All Trans Squalene In Chair,Chair,Chair,Boat Conformational Sequence By The Routes Outlined In Figure 7.



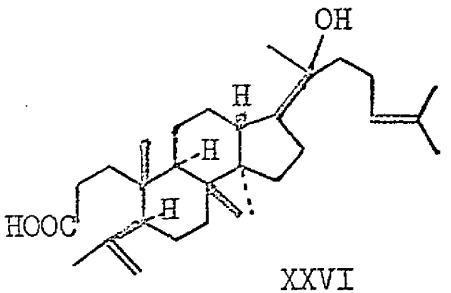
XXIV

Dammadienol



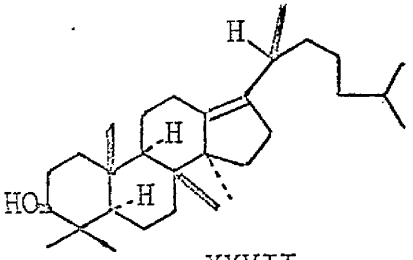
XXV

Dammarenediols I & II



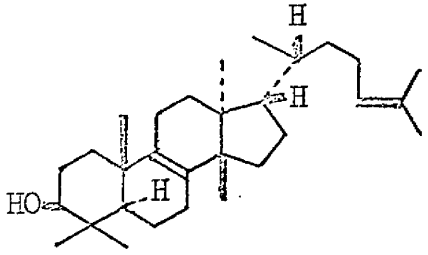
XXVI

Dammarenolic acid



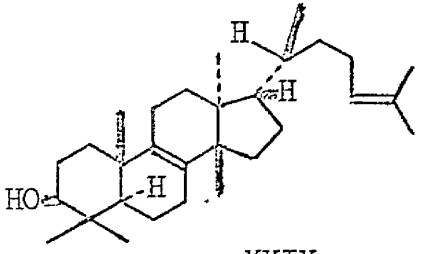
XXVII

Isotirucallenol



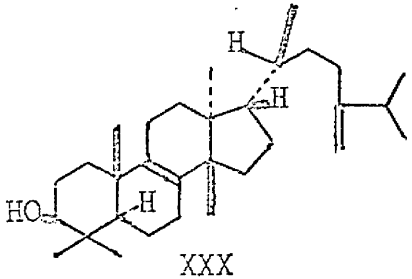
XXVIII

Euphol (20R)



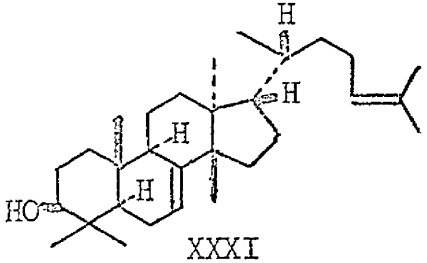
XXIX

Tirucallol (20S)



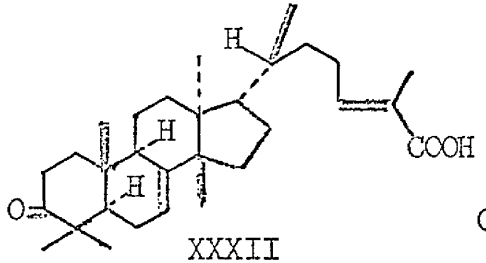
XXX

Euphorbol



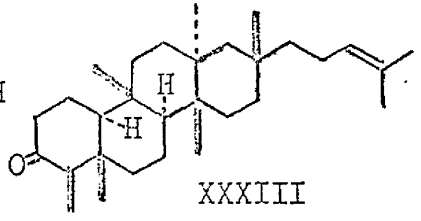
XXXI

Butyrospermol



XXXII

Masticadienonic acid



XXXIII

Shionone

$17\alpha H \longrightarrow 18R$

$13\beta H \longrightarrow 17\beta$

$14\alpha CH_3 \longrightarrow 13\alpha$

$9\beta CH_3 \longrightarrow 14\beta$

and loss of $10\alpha H$ gives euphol (XXVIII)[103] whilst the analogous rearrangement having $17\alpha H \longrightarrow 18S$ gives tirucallol (XXIX)[102] and the derived euphorbol (XXX) which has an additional carbon atom in the side chain[102]. A similar process involving

$17\alpha H \longrightarrow 18$

$13\beta H \longrightarrow 17\beta$

$14\alpha CH_3 \longrightarrow 13\alpha$

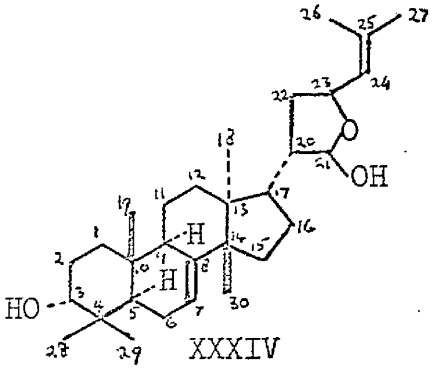
$9\beta CH_3 \longrightarrow 14\beta$

with loss of proton from C-8 in carbonium ion β leads to the formation of compounds such as butyrospermol (XXXI)[104] where configuration is 18R and masticodienonic acid (XXXII)[105] where configuration is 18S.

Euphol (XXVIII) or butyrospermol (XXXI) appear to be of considerable further biogenetical significance since either of these compounds [or conceivably a closely related substance in which the friedo backbone rearrangement of carbonium ion β [Fig. 7] has stopped after the 1,2 shifts $17\alpha H \longrightarrow 18R$, $13\beta H \longrightarrow 17\beta$, $14\alpha CH_3 \longrightarrow 13\alpha$] would seem to be acting[57,106] via oxidative ring cleavages, as a biogenetic precursor of the highly oxygenated bitter principles of the limonin group of C_{26} 'triterpenoids', of the C_{25} compound simarolide, and of

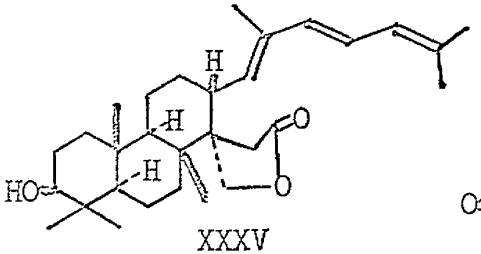
FIGURE 9

Selected Highly Oxidised Compounds Of Natural Occurrence Believed To Arise Biogenetically Through Oxidative Ring Cleavages Of Tetracyclic Ring Systems Derived From Carbonium Ion β in Fig. 7.



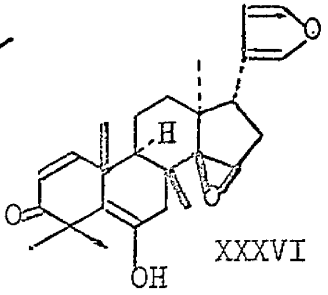
XXXIV

Flindissol
(retains full butyrospermol ring system)



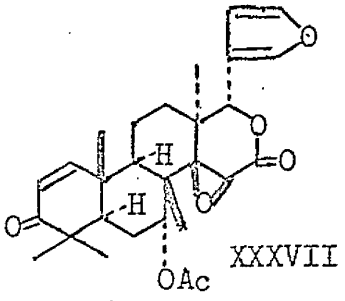
XXXV

Ebelin lactone
(ring D of dammadienol type cleaved)



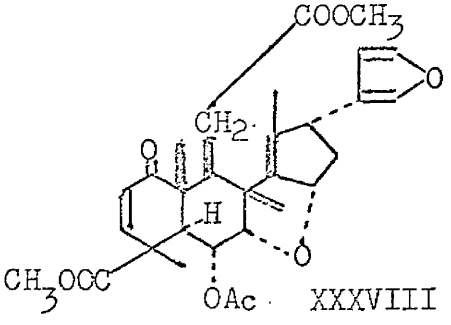
XXXVI

Cedrelone
(side chain shortened)



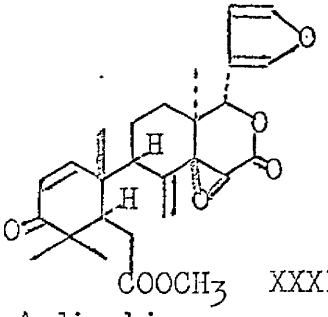
XXXVII

Gedunin
(ring D cleaved)



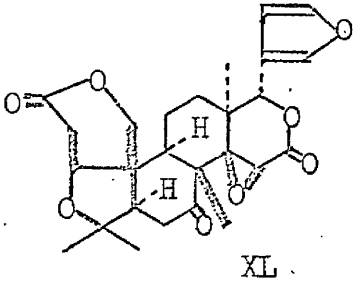
XXXVIII

Nimbin
(ring C cleaved)



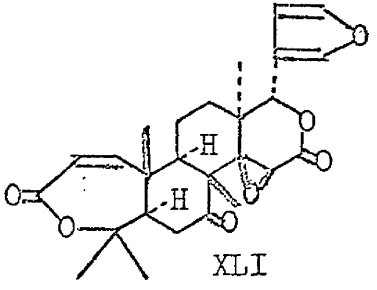
XXXIX

Andirobin
(rings B and D cleaved)



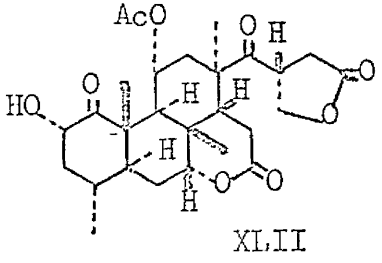
XL

Limonin
(rings A and D cleaved)



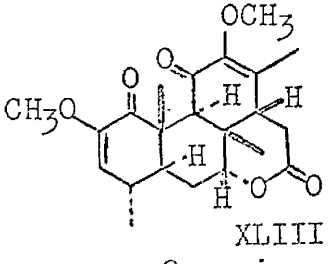
XLI

Obacunone



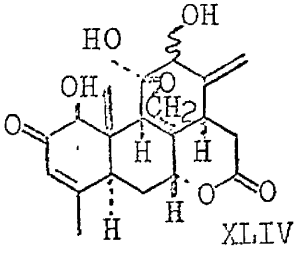
XLII

Simarolide



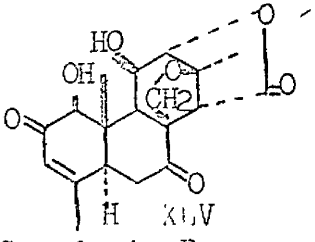
XLIII

Quassin



XLIV

Ailanthone



XLV

Samaderin B.

the quassin group of C_{20} 'triterpenoids'. Further circumstantial evidence in support of this contention would seem to be provided by the existence in Nature of compounds such as flindissol (XXXIV)[107] the related 24, 25 epoxide turraeanthin[108] and ebelin lactone (XXXV)[109] which can be regarded as being formed by the operation of the early stages only of the oxidative processes giving rise to the limonin and quassin groups. Thus in flindissol, the structure of which is closely related to the elemi acids[107], and turraeanthin the full butyrospermol ring system has been retained intact, with formation of a tetrahydrofuran ring system in the side chain, without loss of carbon atoms. This tetrahydrofuran ring system can be regarded as a formal precursor of the furan ring present in cedrelone (XXXVI)[110], gedunin (XXXVII)[111], nimbin (XXXVIII)[112], andirobin (XXXIX)[113], limonin (XL)[114] and obacunone (XLI)[115], the structures of which are shown in Fig. 9, as well as of the furan ring system present in other related compounds, such as veprisone[116], hiritin[117], swietenolide[118] and carapin[119]. In all these compounds the formation of the furan ring has been accompanied by the loss of a 4-carbon fragment from the side chain [i.e. C_{21} and C_{22} with its two appended methyl groups from the side chain inherited from carbonium ion β in Fig. 7]. Ebelin lactone (XXXV)[109] represents the operation of another early oxidative stage - namely oxidative cleavage of ring D - and this compound can be regarded as being formed

from a dammadienol-type precursor through such oxidative cleavage of ring D, lactonisation on to the C-14 methyl group and introduction of a third double bond into the side chain.

Simarolide (XLII)[120] and the various members of the quassin group, of which the representative members quassin (XLIII)[121], ailanthone (XLIV)[122] and samaderin B (XLV)[123] are portrayed in Fig. 9, then result from further oxidations and new ring closures involving oxygen. Gascardic acid[124] would appear to be another compound resulting from cleavage of a tetracyclic triterpene derived from carbonium ion β [Fig. 7] - in this case cleavage of ring A.

In addition to backbone rearrangements of the type already discussed, ion β [Fig. 7] also gives rise to another type of rearrangement involving what, in terms of classical carbonium ion chemistry, can be regarded as a migration of the 16,17 bond to the 16,18 position. This rearrangement may, or may not be accompanied by synchronous attack by the π electrons of the 21,22 double bond on C-17. Thus it has been proposed[125] that shionone which has been assigned the formula XXXIII [Fig. 8] arises from such a migration of the 16,17 bond to the 16,18 position followed by the complete friedo backbone rearrangement

$13\beta H \longrightarrow 17\beta$

$14\alpha CH_3 \longrightarrow 13\alpha$

$9\beta CH_3 \longrightarrow 14\beta$

$10\alpha H \longrightarrow 9\alpha$

$5\beta CH_3 \longrightarrow 10\beta$

$6\alpha H \longrightarrow 5\alpha$

$1\beta CH_3 \longrightarrow 6\beta$

and loss of the α hydrogen atom from C-2 as a proton. Tautomerism of the resultant enol into the keto form then gives shionone. This complete backbone rearrangement is strictly analogous to the formation of friedelin by complete backbone rearrangement of carbonium ion δ as is shown in Fig. 12.

Where migration of the 16,17 bond to the 16,18 position is accompanied by synchronous attack by the π electrons of the 21,22 double bond on C-17, a new pentacyclic carbonium ion having a 6-membered ring D in chair conformation and a 5-membered ring E is formed. This new carbonium ion is shown as γ in Fig. 7. Ion γ can then generate ion δ via what can again be pictured in terms of classical carbonium ion chemistry as a migration of the 20,21 bond to the 20,22 position. Ion δ in turn can generate ion ε by migration of the α-methyl group from C-22 to C-21.

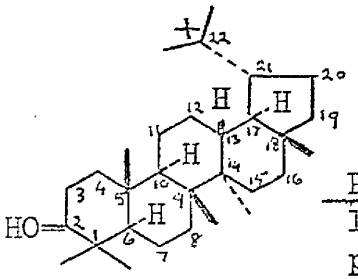
The above rearrangements have been shown in terms of classical carbonium ions for the sake of simplicity.

Portrayal as bridged non-classical carbonium ions, however, serves to emphasise the stereochemical factors which are playing an important part in procuring the ordinarily thermodynamically unfavoured generation of what is essentially a secondary carbonium ion (δ) from what is essentially a tertiary carbonium ion (γ) and so providing the driving force, which together with the influence of the enzymes concerned, gives rise to the various products in their observed configurations.

At first sight other possible rearrangements might appear feasible for ions β and γ. For instance it might seem that in place of the migration of the 16,17 bond to the 16,18 position, which leads to the production of ion γ from ion β, migration of the 13,17 bond of β to the 13,18 position [equivalent to the direct anti-Markownikoff formation of a 6-membered ring D from all <u>trans</u> squalene in a chair, chair, chair, boat cyclisation sequence] could occur instead. However, if this were to occur, any synchronous attack by the π electrons of the 21,22 double bond would leave ring D as a boat in the resulting pentacyclic compound. This situation might be expected to be thermodynamically unfavoured, thus explaining the apparent absence of compounds of this type in Nature. On the other hand, if migration of the 13,17 bond in β to the 13,18 position were to occur with pause for conformational adjustment of ring D to a chair, before attack by the π

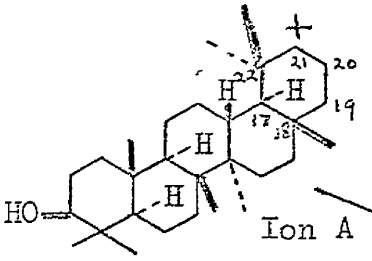
FIGURE 10

Postulated Alternative Carbonium Ion Formation In Cyclisation Of All Trans Squalene In Chair,Chair,Chair,Boat Conformational Sequence. Numbering As In Squalene.



Carbonium ion γ formed as portrayed in Figure 7.

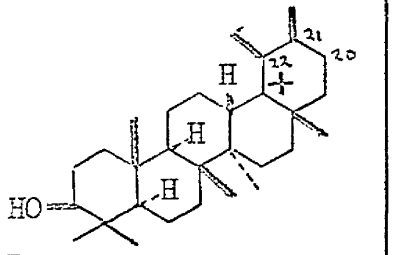
Proposed migration of 17,21 bond to 17,22 position



Ion A

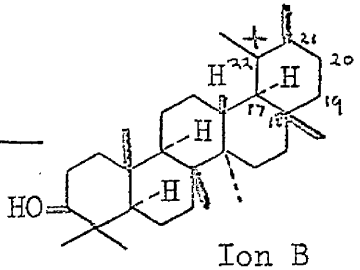
Migration of $22\beta CH_3 \longrightarrow 21\beta$

Migration of $22\alpha CH_3 \longrightarrow 21\alpha$

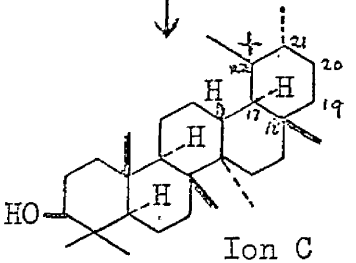


Potential precursor of systems unknown as yet in Nature.

Migration of $17\alpha H \longrightarrow 22\alpha$

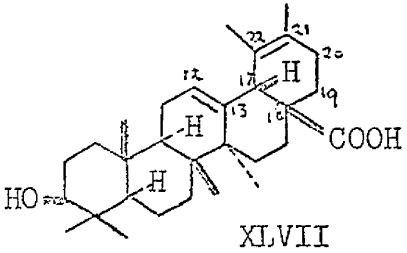


Ion B



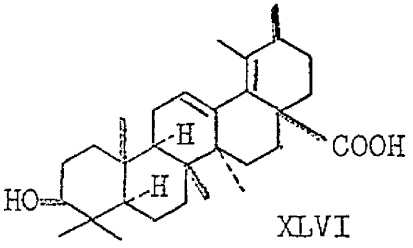
Ion C

Migration of $17\alpha H \longrightarrow 22\alpha$



XLVII

Tomentosolic acid



XLVI

Vanguerolic acid

URSANE and FRIEDOURSANE series

electrons of the 21,22 bond to generate a new 17,21 bond, then there is complete equivalence with either the chair, chair, chair, chair, boat or chair, chair, chair, chair, chair cyclisation sequences of squalene which are considered separately as the fourth and fifth basic modes of cyclisation.

In the case of ion ε, migration of the 17,21 bond to the 17,22 position might seem an alternative to the observed migration of the 20,21 bond to the 20,22 position which gives rise to ion δ. Such a migration by the 17,21 bond would be expected to give ion A as shown in Fig. 10. Migration of either the 22β-methyl group or the 22α-methyl group to C-21 would then be theoretically possible to give ion B or ion C respectively. The further migration of the α hydrogen atom from the carbon atoms designated as 17 to the carbon atoms designated as 22 in ions B and C [Fig. 10] will then result in β configuration being developed for the methyl group at C-22. Thus ion C becomes completely equivalent to ion ξ [Fig. 7] as far as a friedo backbone rearrangement is concerned, and so represents an alternative route of formation for α-amyrin and the friedo ursanes, but ion B would give rise to a pentacyclic system having both the methyl groups on C-21 and C-22 in the β configuration. However no pentacyclic systems based on ion B would seem to have been found in Nature. Although vanguerolic acid (XLVI)[126] might conceivably arise from ion B with subsequent introduction of the 12,13 double bond [e.g. by elimination of a leaving group from the 12-position] the occurrence of tomentosolic

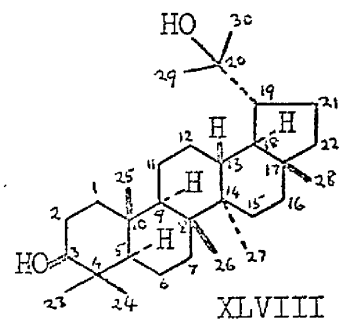
acid (XLVII)[126] with its βH on C-17 [squalene numbering] would make it clear that both compounds are derived from α-amyrin [see page 108] and that introduction of the ring E double bonds in these compounds is a subsequent independent process.

Two courses of reaction might be anticipated for ions γ δ and ε [in Fig. 7] - viz. i. stabilisation by loss of a neighbouring proton or through reaction with a nucleophilic species, and ii. backbone rearrangements of the friedo type already discussed with respect to carbonium ion α [Fig. 5] and carbonium ion β [Fig. 7]. Which of these two theoretically available reaction pathways is actually observed will of course depend upon the particular energetics favourable to each individual ion.

Thus it would seem that ion γ, which can be regarded as the fundamental ion of the lupane series, is not particularly disposed to undergo a friedo backbone rearrangement since no friedolupane derivatives would appear to have been found as yet in Nature. Instead ion γ either picks up OH^- as in the formation of monogynol A (XLVIII)[127], or loses a proton from one of the methyl groups attached to C-22 to give lupeol (XLIX)[128,129]. Lupeol or monogynol A then act as parent compounds for more highly oxygenated derivatives such as betulin (L)[128], betulinic acid (LI)[128], melaleucic acid (LII)[130], alphitolic acid (LIII)[131], thurberogenin (LIV)[132], stellatogenin (LV)[129], the interesting 29-norlupane derivative, platanic acid (LVI)[133], and the A-norlupane

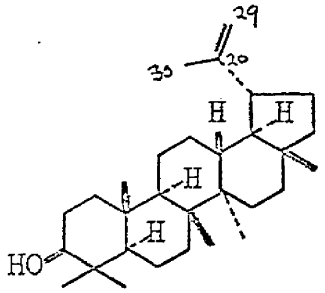
FIGURE 11

Some Naturally-Occurring Triterpenes Derived From The Fundamental Lupane Carbonium Ion γ Of Figure 7.



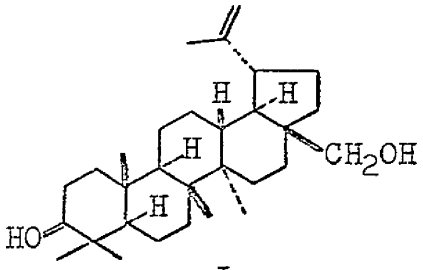
XLVIII

Monogynol A



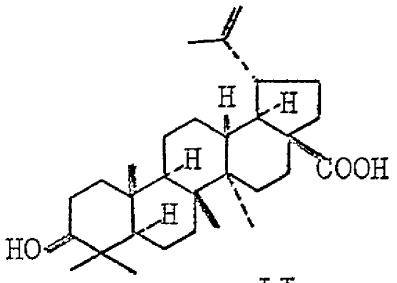
XLIX

Lupeol



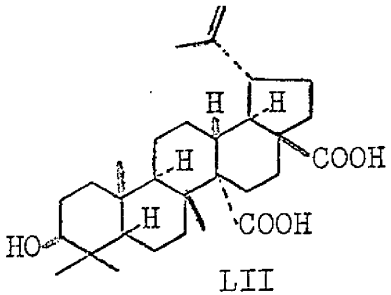
L

Betulin



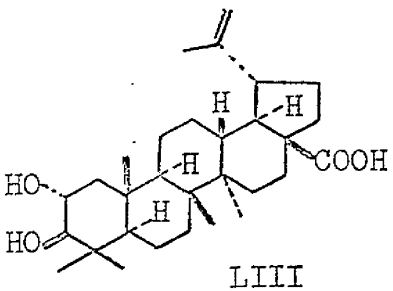
LI

Betulinic Acid



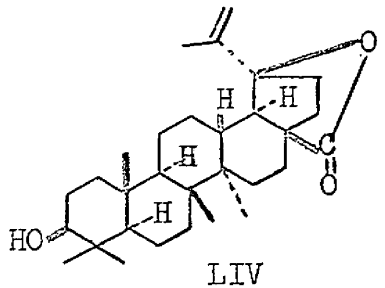
LII

Melaleucic acid



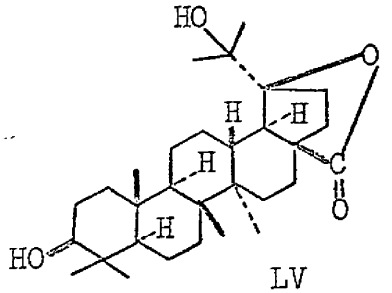
LIII

Alphitolic acid



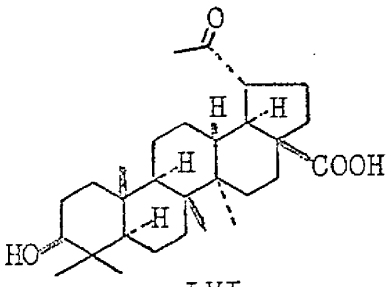
LIV

Thurberogenin



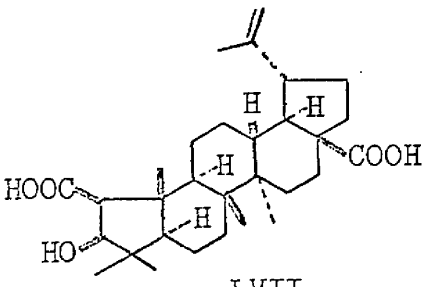
LV

Stellatogenin



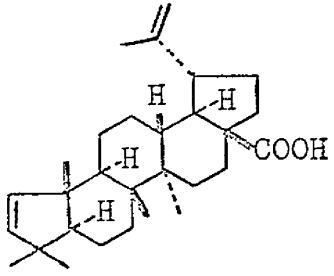
LVI

Platanic acid



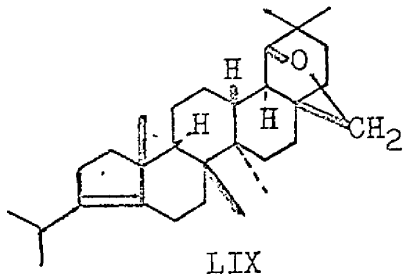
LVII

Ceanothic acid



LVIII

Ceanothenic acid



LIX

α-Apoallobetulin

derivatives, ceanothic acid (LVII)[134] and ceanothenic acid (LVIII)[135]. The structures of these compounds are given in Fig. 11 as is the structure of α-apoallobetulin (LIX)[136] which has been isolated from montan wax. This last compound is of interest with respect to the ring E system since like all compounds of the allobetulin series it can be regarded as being formed from a fundamental lupeol-type molecule through Markownikoff addition of a proton to the isopropylene double bond with synchronous bond migration to give a six-membered ring E and attack by oxygen situated on the carbon in the D/E junction on the transient secondary carbonium ion so formed. This process is of course entirely analogous to the formation of ion δ from ion ϰ [Fig. 7] i.e. to the process by which the fundamental oleanane carbonium ion ε is generated from the fundamental lupane carbonium ion ϱ and so the allobetulin series, whilst strictly oleanane derivatives, are generally regarded as rearranged lupane derivatives. Any absolute distinction is, however, meaningless.

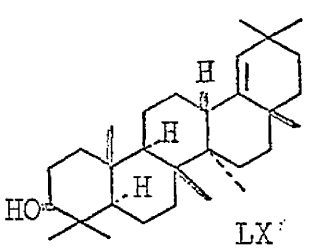
A further point of interest concerning α-apoallobetulin (LIX) is that it has suffered a neo rearrangement[36] in ring A with extrusion of a leaving group from the 3β position followed by migration of the 6,1 bond to the 6,2 position [numbering as in ions β and ϰ, Fig. 7] and loss of proton.

Two other compounds of interest belonging to the lupane series are canaric acid[137], the 2,3-seco acid of the lupane series corresponding to nyctanthic acid (LXXXI, Fig. 13)

FIGURE 12

Representative Individual Pentacyclic Triterpenes Arising From Friedo Backbone Rearrangement In The Parent Oleanane Carbonium Ion δ (Fig. 7). Numbering As In Oleanane.

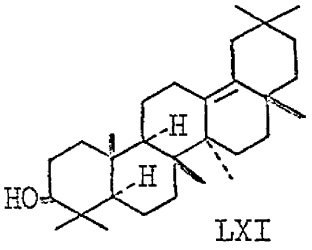
(Superscript arabic numbers are reference numbers - see bibliography)



LX

Germanicol[128]
(an olean-18-ene)

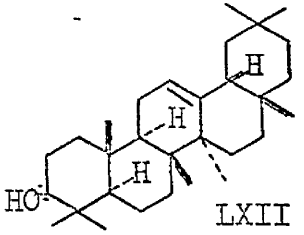
Loss of 18αH as proton



LXI

δ-Amyrin[139]
(an olean-13(18)-ene)

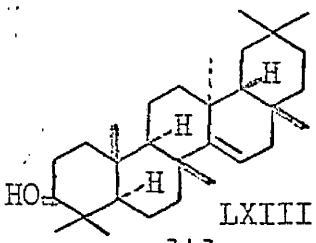
Loss of 13βH as proton



LXII

β-Amyrin[140]
(an olean-12-ene)

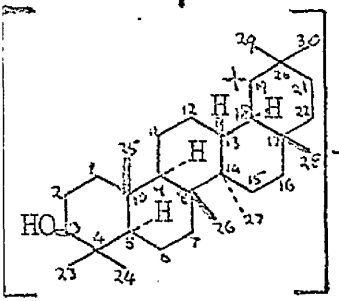
Loss of 12αH as proton



LXIII

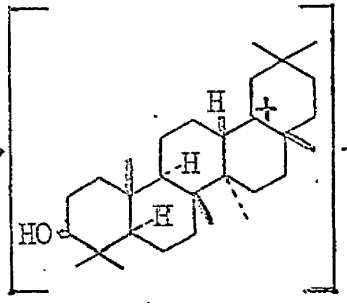
Taraxerol[141]
(a D friedoolean-14-ene)

Loss of 15βH as proton

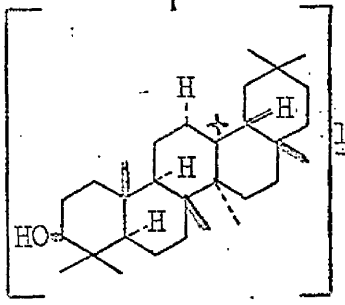


Fundamental oleanane carbonium ion δ formed as portrayed in Fig.7.

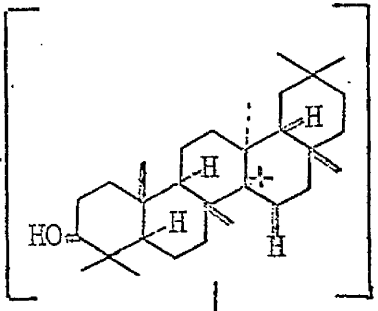
18αH → 19α



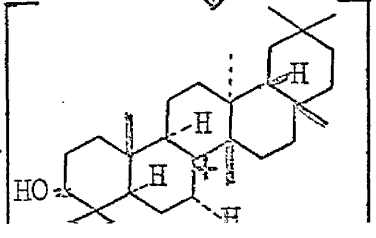
13βH → 18β



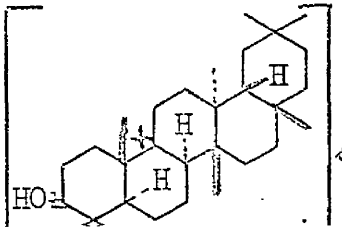
14αCH$_3$ → 13α



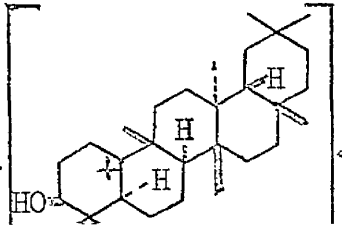
8βCH$_3$ → 14β



9αH → 8α



10βCH$_3$ → 9β



Loss of 1αH as proton

D:B friedooleanan-10(1)-enes. Not yet found in nature.

as proton

D:B friedoolean-5(10)-enes.
Not yet found in nature.

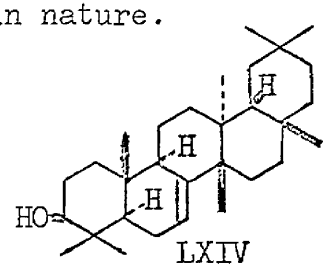
5αH → 10α

as proton

D:C friedoolean-9(11)-enes.
Not yet found in nature.

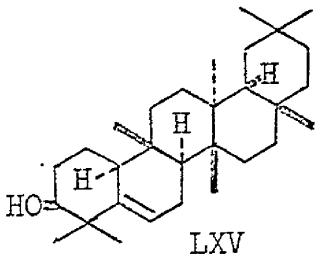
as proton

D:C friedoolean-8-enes.
Not yet found in nature.



LXIV

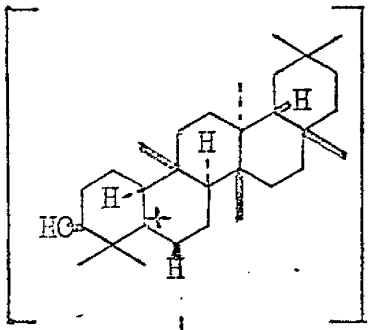
Multiflorenol[142]
(a D:C-friedoolean-7-ene)



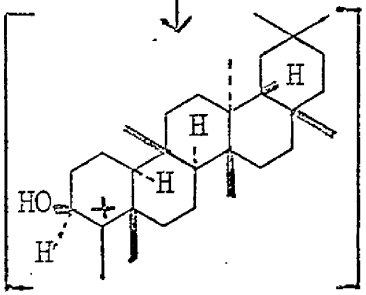
LXV

Glutinol[143]
(a D:B friedoolean-5-ene)

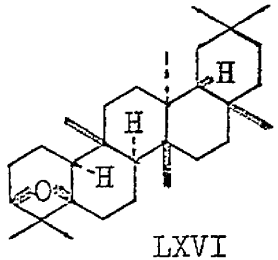
Loss of 6βH as proton



4βCH$_3$ → 5β



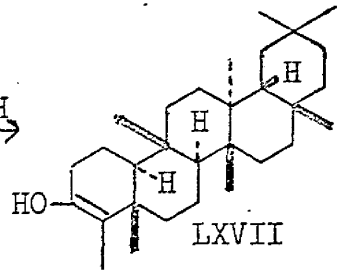
Attack by 3βOH



LXVI

Dendropanoxide[144]
(a D:B friedooleanane)

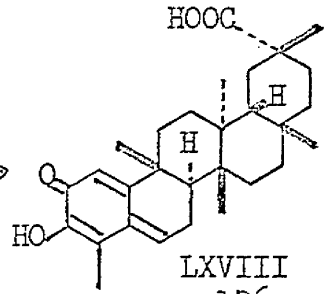
Loss of 3αH as proton



LXVII

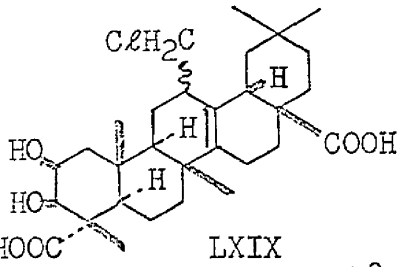
Enol form of Friedelin[145]
(a D:A friedoolean-3-ene)

oxidation



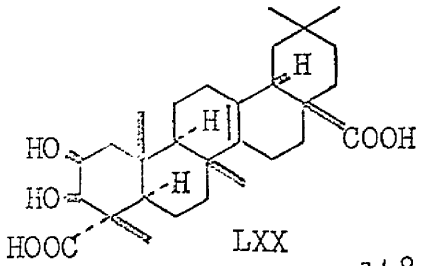
LXVIII

Celastrol[146]



LXIX

Senegenin[148]



LXX

Senegenic acid[148]

of the β-amyrin series, and emmolactone[138] a bis nor triterpene related in skeletal type to ceanothenic acid, but lacking C-28.

In contrast to ion ɣ which does not appear to undergo friedo backbone rearrangement, ion δ (Fig. 7) which can be regarded as the fundamental ion of the oleanane series reacts both via stabilisation by loss of proton and via friedo backbone rearrangement. Thus simple loss of the proton from C-17 in ion δ gives rise to germanicol (LX)[128].

Friedo backbone rearrangement of ion δ seems very highly favoured and indeed a virtually complete series of friedo oleanane derivatives is now known. It is to be noted, however, that since the first two 1,2 shifts involved with respect to friedo backbone rearrangement of ion δ are hydride shifts viz. 18αH ⟶ 19α and 13βH ⟶ 18β [oleanane numbering], the compounds resulting from subsequent proton loss from the 13β and 12α positions (δ-amyrin (LXI)[139] and β-amyrin (LXII)[140] respectively) still retain an unrearranged carbon skeleton apart from the stereochemistry at C-18. Representative examples of known naturally-occurring friedo-oleanane types are shown in Fig. 12. The only four additional types which are theoretically possible and which do not appear to have as yet been found in Nature are the D:C-friedooolean-8-ene, the D:C-friedooolean-9(11)-ene, the D:B-friedooolean-10(1)-ene and the D:B-friedooolean-5(10)-ene types.

The product resulting from the complete friedo backbone rearrangement of carbonium ion 6 is friedelin (LXVII)[145] which lends its name to describe such backbone rearrangements. Friedelin is of further interest since it is believed to be the precursor of celastrol (LXVIII)[146] and its derived methyl ester, pristimerin, which are the most highly oxidised pentacyclic triterpenes so far to be discovered in Nature.

Another compound of interest resulting from friedo backbone rearrangement of carbonium ion 6 is dendropanoxide (LXVI)[144] which is also shown in Fig. 12. This compound can be regarded as resulting from intramolecular attack by the oxygen atom of the 3β hydroxyl group on the same classical carbonium ion as gives rise to glutinol (LXV)[143] with resultant formation of a 3β,5? ether bridge and loss of proton off oxygen.

Also included in Fig. 12 are the structures for senegenin (LXIX)[147,148] and the 27- nor triterpenoid compound senegenic acid (LXX)[148], although both compounds are known to be artefacts produced during the acid hydrolysis of the true saponin of Polygala senega - namely presenegenin (2β,3β, 27-trihydroxyolean-12-en-23,28-dioic acid)[200]. The formation of nor triterpenoids on the acid hydrolysis of triterpenoid saponins is of not uncommon occurrence as evidenced by the isolation of the 28 - noroleanane derivatives norechinocystadienol (LXXVIII - Fig. 13)[149], albigenin[150] and 'Triterpene B'[151] under conditions of acid hydrolysis.

Ion ε, which can be regarded as the fundamental ion of the taraxerstane-ursane series, like ion δ, also reacts by both immediate stabilisation and by friedo backbone rearrangement. Loss of a proton from the methyl group on C-22 gives taraxasterol[152] whilst loss of a proton from C-20 gives ψ-taraxasterol[152,153] from which are derived the corresponding 12-hydroxylated compounds arnidiol and faradiol respectively[154] as well as the lactone (28-COOH to 20βOH) phillyrigigenin[155]. On the other hand, friedo backbone rearrangements starting with

$$21\beta H \longrightarrow 22\beta$$

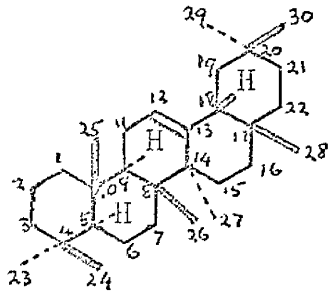
$$17\alpha H \longrightarrow 21\alpha \text{ etc.}$$

give rise to several friedo ursanes. Fewer friedo derivatives appear at present to be known in the ursane series than in the oleanane series, but undoubtedly more representatives will be discovered. However, one friedo ursane derivative of interest, for which an oleanane analogue does not yet appear to have been discovered in Nature, is phyllanthol (XCIV)[156] the structure of which is shown in Fig. 14. This compound can be assumed to arise in an analogous manner to that described for cycloartenol on page 88. In the case of phyllanthol the carbonium ion corresponding to that giving rise to α-amyrin [i.e. the carbonium ion in which friedo backbone rearrangement of ion ε has progressed as far as localisation of the positive charge on C-13] suffers attack by the 14α-methyl group with loss of proton, in place of losing the 12α proton for formatic

TABLE V

Naturally-Occurring Triterpenes Containing The β-Amyrin Nucleus

(Aglycones Only Are Listed)



Compound	Functional Groups					Refs.
	C=C (other than 12,13)	OH	C=O	COOH	OAc	
Acacic Acid		3β, 16β, 21β		28		157
Aegiceradiol (LXXI)	15	3β, 28				158
Aescinidin (Barringtogenol C)		3β, 16α, 21α, 22β, 28				159
β-Amyrenone			3			160
β-Amyrin acetate					3	161
Ajunolic acid		2α, 3β, 23		28		162
A_1-Barrigenol		3β, 15β, 16β, 27, 28				163
Barringtogenic acid		2α, 3β		23, 28		164
Barringtogenol		2α, 3β, 23, 28				164
Barringtogenol D*		3β, 22β, 28 (16α*, 21α*)				165
Bassic acid	5	2β, 3β, 23		28		166
Bayogenin		2β, 3β, 23		28		167

Compound	Functional Groups					Refs
	C=C (other than 12,13)	OH	C=O	COOH	OAc	
α-Boswellic acid (LXXII)		3		24		168,169
Bredemolic acid		2β,3α		28		170
Caccigenin		3β,15,16β,23		28		171
Chichipegenin		3β,16β,22α,28				172
Cincholic acid		3β		27,28		173
Cochalic acid		3β,16β		28		174
Commic acid C		2β,3β		24		175
Crataegolic acid (Maslinic Acid) (LXXV)		2α,3β		28		176,193
Dihydroicterogenin*		3β,24,22β*		28		177
Dihydrorehmannic acid**		3β,22β**		28		177
Dumortierigenin***		3β,15β***		28***		178
Echinocystic acid		3β,16α		28		168,179
Entagenic acid		3,15,16 (or 3, 21,22)		28		180
Erythrodiol		3β,28				181
Glabric acid		3β,18α	11	30		182
Glycyrrhetic acid (LXXIII)		3β	11	30		168,183
Gummosogenin		3β,16β	28			184
Gypsogenin		3β	23	28		168,185
Hederagenin (LXXIV)		3β,23		28		168

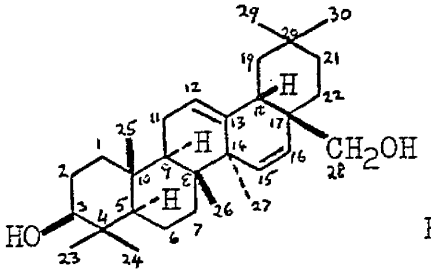
Compound	Functional Groups					Refs.
	C=C (other than 12,13)	OH	C=O	COOH	OAc	
7β-Hydroxy A_1-barrigenol		3β,7β,15β,16β 27,28				186
Icterogenin**		22β**,24	3	28		187
Isosawamilletin		3β,OCH_3				188
Katonic acid		3α		29		189
Lantadene B†		22β†	3	28		190
Liquoric Acid		3β,(16α*,21α*)	11	30		191
Longispinogenin		3β,16β,28				181
Machaeric acid		3β	21	28		192
Machaerinic acid		3β,21β		28		192
Maniladiol		3β,16β				140,168
Medicagenic acid		2α,3β		23,28		194
Myrtillogenic acid		3β,16β,28		29		195
Oleanaldehyde (LXXVI)		3β	28			196
Oleanolic acid		3β		28		140,168
Oleanolic acid acetate				28		197
Oleanonic acid			3	28		198
3-epi Oleanolic acid		3α		28		198
Phytolaccogenin		2β,3β,23		28,30		199
Presenegenin		2β,3β,27		23,28		200
Primulagenin A		3β,16α,28				140,201

Compound	Functional Groups					Refs.
	C=C (other than 12,13)	OH	C=O	COOH	OAc	
Polygalacic acid		2β,3β,16α,23		28		202
Protoaescigenin (LXXVII)		3β,16α,21α 22β,24,28				203
Queretaroic acid		3β,30		28		204
Quillaic acid		3β,16α	23	28		168,205
Rehmannic acid**		22β**	3	28		187
Saikogenin B	9(11)	3β,16β,28				206
Siaresinolic acid		3β,19α		28		168,207
Sophoradiol		3β,7α (or 3β,22β)				208
Soyasapogenol A		3β,21α,22α,24 (or 3β,21β,22β, 24)				209
Soyasapogenol B		3β,22β,24				209
Soyasapogenol C	21	3β,24				209
Spergulagenic Acid		3β		28,29		210
Stryphnodendron sapogenin B††		3β,21β††		28††		211
Stryphnodendron sapogenin F††		2α,3β,21β††		28††		211
Sumaresinolic acid		3β,6β		28		168,212
Terminolic acid		2α,3β,6β,23		28		213
Treleasegic acid		3β,21β,30		28		214
Tomentosic acid		2α,3β,19β,23		28		215

*16α,21α-oxide **22β-angeloyloxy ***15β,28-lactone †22β-β,β-dimethylacryloxy ††21β,28-lactone

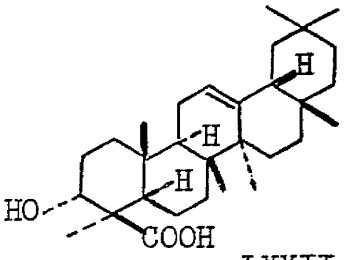
FIGURE 13

Selected Representative Naturally-Occurring Triterpenes Derived From β-Amyrin



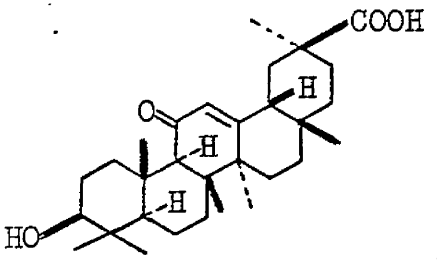
LXXI

Aegiceradiol



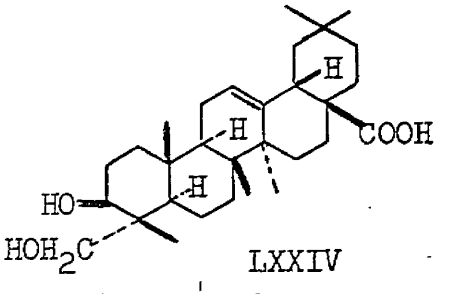
LXXII

α-Boswellic acid



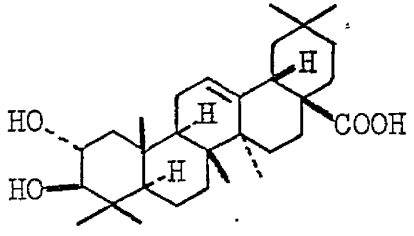
LXXIII

Glycyrrhetic acid



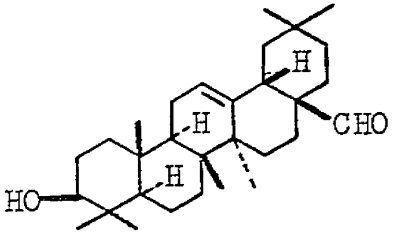
LXXIV

Hederagenin



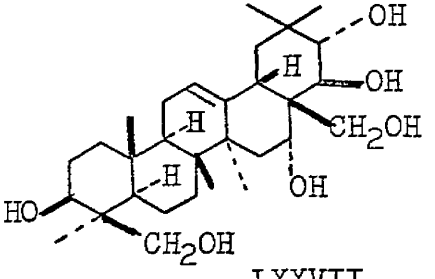
LXXV

Crataegolic acid



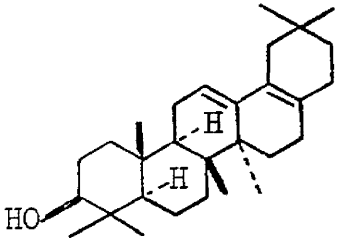
LXXVI

Oleanaldehyde



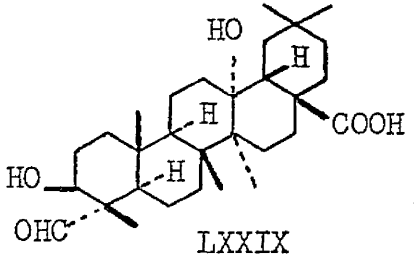
LXXVII

Protoaescigenin



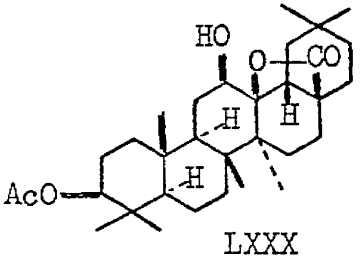
LXXVIII

Norechinocystadienol



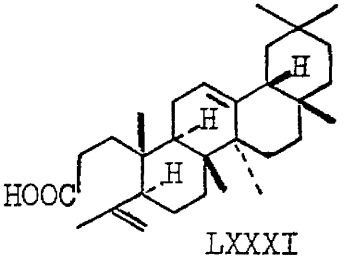
LXXIX

12-H,13α-Hydroxygypsogenin



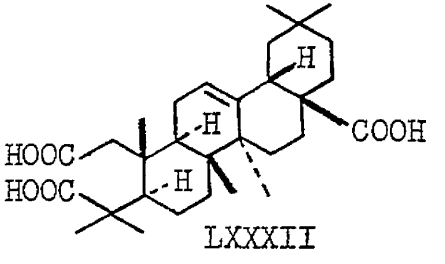
LXXX

12-Hydroxyoleanolic lactone acetate



LXXXI

Nyctanthic acid



LXXXII

2,3-secoolean-12-en-2,3,28-trioic acid

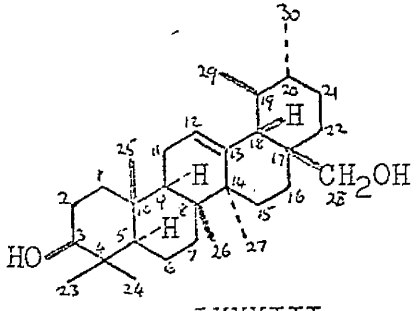
of α-amyrin.

It may be noted that ursane, which has been assigned the role of a fundamental triterpene hydrocarbon for the purpose of nomenclature[36,49] is in essence itself a friedo-taraxerstane and it is perhaps unfortunate that this was not taken into account in its assignment as a fundamental system. It is also to be noted that once the migrations 21βH ⟶ 22β and 17αH ⟶ 21α have occurred in carbonium ion ε to generate the ursane stereochemistry at C-22 and C-21 no further change in the carbon skeleton results from the migration 13βH ⟶ 17β.

Of the various friedo rearranged compounds derivable from ions δ and ε those with a 12,13 double bond seem particularly common in Nature. Thus β-amyrin (LXII)[140] and its ursane counterpart, α-amyrin[216], can be regarded as the parent systems of a large number of variously oxygenated naturally occurring pentacyclic triterpenes. In the case of β-amyrin some seventy derivatives still retaining the unchanged basic β-amyrin nucleus are now known[217] and these are listed in Table V. The structures of selected examples are shown in Fig. 13 which also shows the structures of other compounds such as norechinocystadienol (LXXVIII)[149], 12-H, 13α-hydroxy-gypsogenin (LXXIX)[218], 12-hydroxy-oleanolic lactone acetate (LXXX)[219], nyctanthic acid (LXXXI)[220] and 2,3-secoolean-12-en-2,3,28-trioic acid (LXXXII)[221] which can be regarded fundamentally as β-amyrin derivatives in which there have been further changes other than simple insertion of oxygen functions

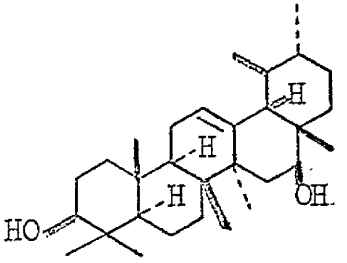
FIGURE 14

Selected Representative Naturally-Occurring Triterpenes Derived From α-Amyrin.



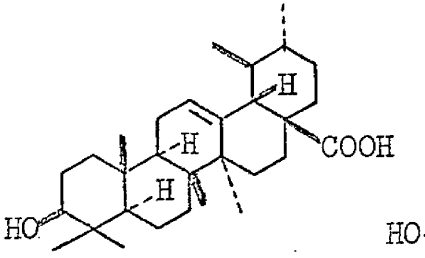
LXXXIII

Uvaol



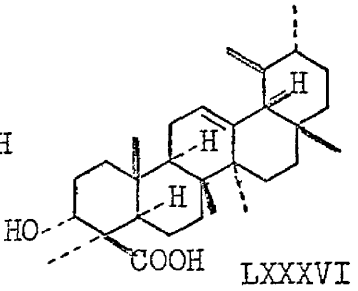
LXXXIV

Brein



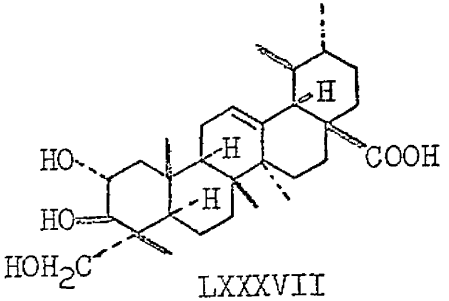
LXXXV

Ursolic acid



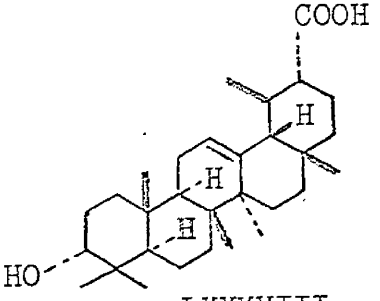
LXXXVI

β-Boswellic acid



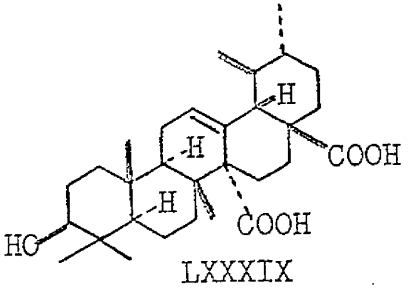
LXXXVII

Asiatic acid



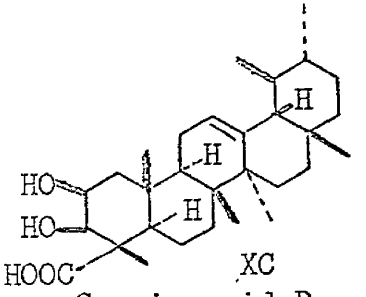
LXXXVIII

Ifflaionic acid



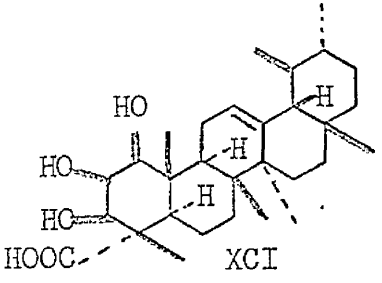
LXXXIX

Quinovic acid



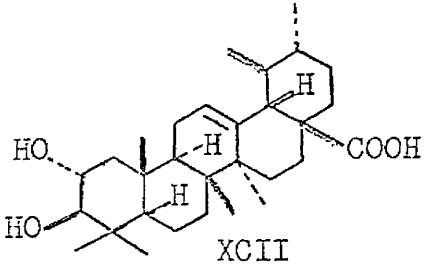
XC

Commic acid D



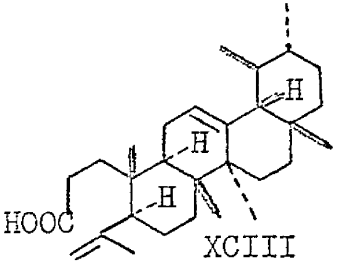
XCI

Commic acid E



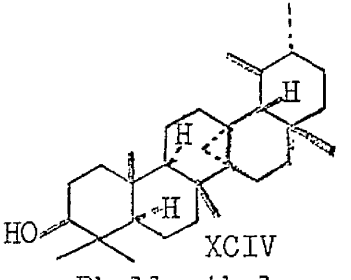
XCII

2α,3β-Dihydroxyurs-12-en-28-oic acid



XCIII

Roburic acid

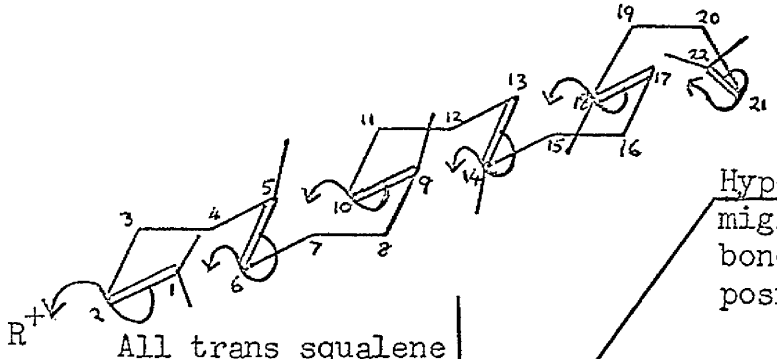


XCIV

Phyllanthol

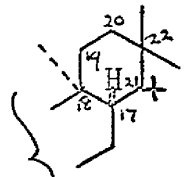
FIGURE 15

Derivation Of Compounds Resulting From Cyclisation Of All Trans Squalene In Chair,Chair,Chair,Chair,Boat Conformational Sequence



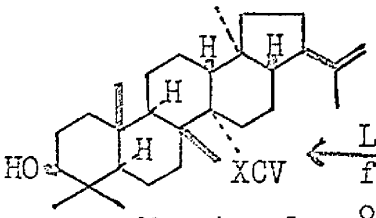
All trans squalene

Hypothetical migration of 20,21 bond to 20,22 position.



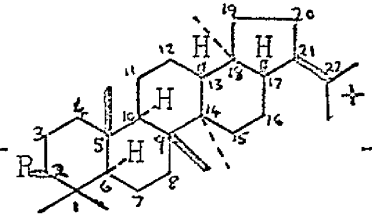
Ion Z

Compounds formed from this ion still unknown in Nature.



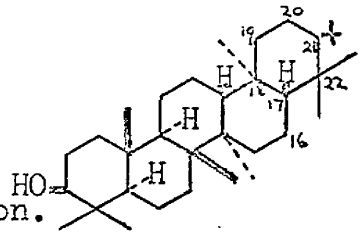
Moretenol

Loss of proton from methyl group on C-22.



Fundamental Isohopane Carbonium ion ζ

Migration of 17,21 bond to the 17,22 position.

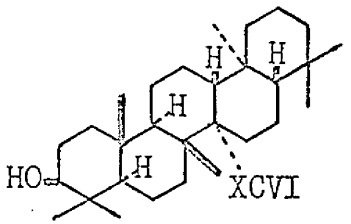


Ion W

Friedo backbone rearrangements

Various Derivatives as shown in Figure 16

?



Tetrahymanol

(OH, C=O, COOH). Thus norechinocystadienol has lost C-28 [oleanane numbering], nyctanthic acid and 2,3-secoolean-12-en-2,3,28-trioic acid have suffered an oxidative cleavage of ring A of β-amyrin, whilst 12-H, 13α-hydroxygypsogenin and 12-hydroxyoleanolic lactone acetate have suffered changes involving the 12,13 double bond of β-amyrin.

Fewer derivatives based on α-amyrin have so far been discovered in Nature - there being only some twenty so far known[217]. Representative examples are shown in Fig. 14. These are uvaol (LXXXIII)[168,222], brein (LXXXIV)[223], ursolic acid (LXXXV)[216,222], β-boswellic acid (LXXXVI)[224], asiatic acid (LXXXVII)[225], ifflaionic acid (LXXXVIII)[226], quinovic acid (LXXXIX)[168,222], commic acid D (XC)[175], commic acid E (XCI)[227], 2α,3β-dihydroxyurs-12-en-28-oic acid (XCII)[228], the ring A cleaved roburic acid (XCIII)[229] and the cyclopropane compound phyllanthol (XCIV)[156] which has already been discussed [page 106].

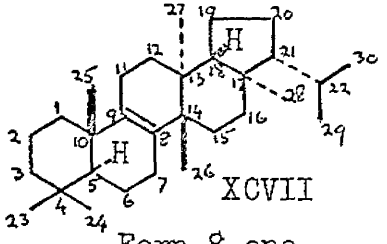
3. Cyclisation in chair, chair, chair, chair, boat conformational sequence

Synchronous cyclisation of all trans squalene in chair, chair, chair, chair, boat conformational sequence gives rise to the fundamental pentacyclic ion **S** [Fig. 15] of the moretane or isohopane skeleton in a one stage process which involves anti-Markownikoff additions with respect to both the 13,14 and the 17,18 double bonds.

The pentacyclic triterpenes derived from carbonium ion

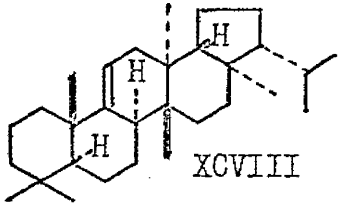
FIGURE 16

Some Representative Individual Pentacyclic Triterpenes Arising From The Synchronous Cyclisation Of All Trans Squalene In Chair,Chair,Chair, Chair,Boat Conformational Sequence Via Friedo Backbone Rearrangements Of Carbonium Ion 5 In Figure 15



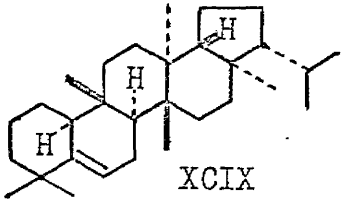
XCVII

Fern-8-ene



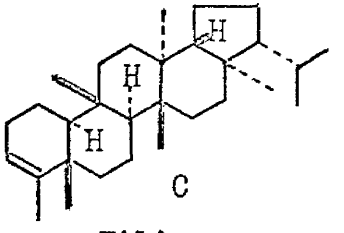
XCVIII

Fernene



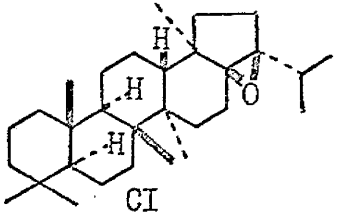
XCIX

Adianene

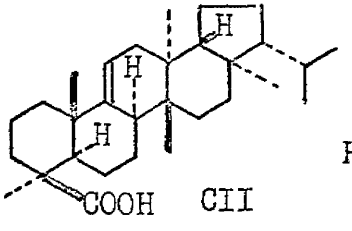


C

Filicene

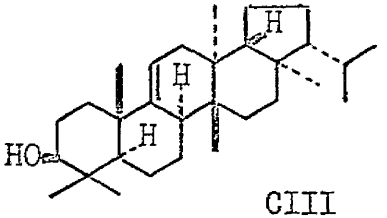


CI



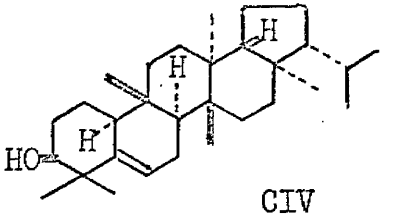
COOH CII

Davallic Acid



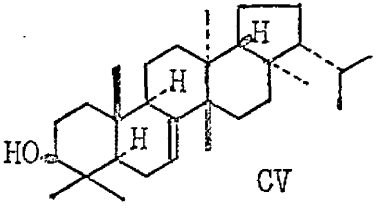
HO CIII

Fernenol



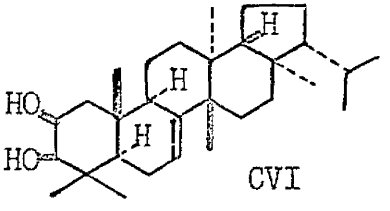
HO CIV

Simiarenol



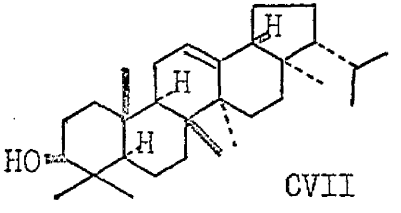
HO CV

Motiol



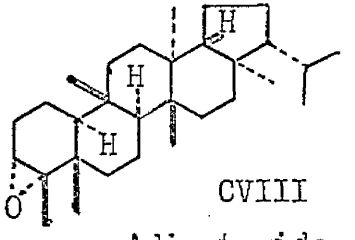
HO HO CVI

Motidiol



HO CVII

Neomotiol



O CVIII

Adiantoxide

may have a special evolutionary significance in terms of the hypothesis, advanced by Barton,[47] that cyclisation of squalene is initiated by H^+ [rather than by the customarily proposed OH^+] with subsequent introduction of an hydroxyl group while the newly cyclised squalene molecule is still bound to the enzyme surface. Thus the triterpenoids derived from carbonium ion S (e.g. fern-8-ene (XCVII)[230], fernene (XCVIII)[231], adianene (XCIV)[230], filicene (C)[230] the epoxide, CI[232], and davallic acid (CII)[233] which are found in ferns lack a 3-oxygen function, whereas the triterpenoids derived from carbonium ion S which occur in higher plants (e.g. moretenol (XCV)[234] which occurs in Ficus macrophylla Dest., arundoin (VIII, Fig. 3) which occurs in various grasses[7,8,38,190], fernenol (CIII) which occurs in various grasses[38] and Artemisa vulgaris L. [family Compositae][235] and simiarenol (CIV)[236], its 2β-hydroxylated derivative, adianendiol[237], motiol (CV)[237], motidiol (CVI)[237] and neomotiol (CVII)[237] which occur in Rhododendron species [family Ericaceae] all possess a 3-oxygen function. It is therefore tempting to consider that in the more primitive ferns development of the hydroxylation step subsequent to the cyclisation step has not occurred, whereas in the higher, more recently evolved plants, this hydroxylation step has been developed. The only possible contradiction to this conclusion in the light of present day knowledge would seem to be the occurrence of the 3,4-epoxide, adiantoxide (CVIII) in the fern Adiantum capillus Veneris[238]

should the epoxide function be formed via an original hydroxyl function at C-2 [numbering as in carbonium ion ξ]. However, formation of the epoxide in this way would seem unlikely on two counts. Firstly the α-configuration of the epoxide in adiantoxide is difficult to explain in terms of the usual β-configuration of the hydroxyl group at C-3 in steroids and triterpenes and secondly the existence of the epoxide CI, clearly derived from carbonium ion ξ after the 1,2 shift 21αH⟶22 suggests that epoxide formation does not occur through attack of an hydroxyl group on a carbonium ion [sited on an adjacent carbon atom] which is developed during the fundamental friedo backbone rearrangement process. Rather such epoxide formation would seem to take place via a separate process occurring subsequently to the friedo backbone rearrangements, e.g. glycol formation at a double bond or insertion of an hydroxyl group at a saturated carbon atom α to a carbon atom already bearing an hydroxyl group, with one of the vic hydroxyl groups then serving as a leaving group [e.g. through conversion into some type of phosphate ester].

Carbonium ion ξ [Fig. 15] can apparently stabilise without rearrangement by loss of a proton from one of the methyl groups on C-22 to give moretenol (XCV)[234]. It can also apparently give rise to an extensive series of friedo backbone rearrangement products as is apparent from the

representative compounds shown in Fig. 16, all of which can be regarded as being formed by successive 1,2-shifts and proton eliminations completely analogous to those portrayed in detail in Fig. 12 for the fundamental oleanane carbonium ion δ. At present relatively fewer compounds based on the fundamental isohopane ion ζ have been discovered in Nature than are known based on carbonium ions γ, δ and ϵ of Fig. 7, but undoubtedly many more will be found. Nevertheless, at the present time, of the 15 theoretically possible positions for double bond generation resulting from 1,2 shifts starting with 21αH $\longrightarrow$ 22 in carbonium ion ζ, six have been established as occurring in Nature. Thus the E-friedoisohop-12-ene system is present in neomotiol (CVII)[237], the E:C-friedoisohop-7-ene system is present in motiol (CV)[237] and motidiol (CVI)[237], the E:C-friedoisohop-8-ene system is present in fern-8-ene (XCVII)[230], the E:C-friedoisohop-9(11)-ene system is present in fernene (XCVIII)[231], fernenol (CIII)[38,235], arundoin (VIII)[8] and davallic acid (CII)[233], the E:B-friedoisohop-5-ene system is present in adianene (XCIX)[230], simiarenol (CIV)[236] and adianenediol[237] and the E:A-friedoisohop-3-ene system is present in filicene (C)[230].

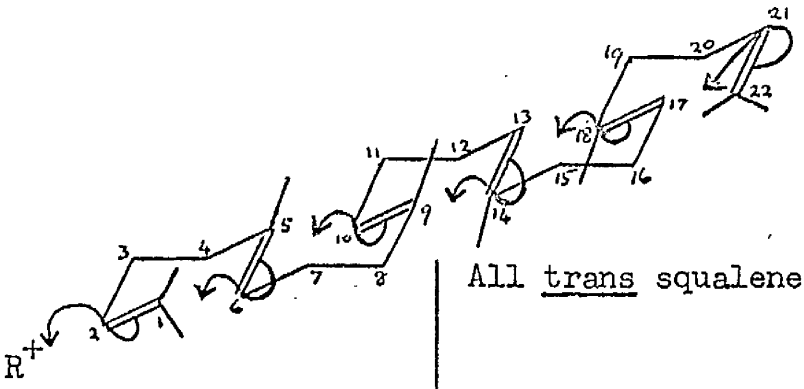
Interestingly, the E:C-friedo-, E:B-friedo- and E.A-friedo isohopane derivatives, fern-8-ene (XCVII), adianene (XCIX) and filicene (C) respectively all occur in the same fern, Adiantum monochlamys Eaton[230], together with diploptene

(syn hopene-b)(CIX)[231] which can be regarded as being formed via a chair, chair, chair, chair, chair cyclisation sequence of all trans squalene [vide infra]. In this connection it may be noted that fern-8-ene, adianene and filicene could also arise via friedo backbone rearrangement of the fundamental carbonium ion resulting from such a chair, chair, chair, chair, chair cyclisation sequence, but if they do, then it must be via a non-concerted process. A fully concerted process with respect to the 1,2 shifts 21αH $\longrightarrow$ 22, 17βH $\longrightarrow$ 21β is only possible with the fundamental isohopane carbonium ion ζ. The analgous shifts in the fundamental hopane carbonium ion η, [Fig. 17] would have to be 21βH $\longrightarrow$ 22 and 17βH $\longrightarrow$ 21β in order to account for the correct stereochemistry in fern-8-ene, adianene and filicene were they to be formed from this ion.

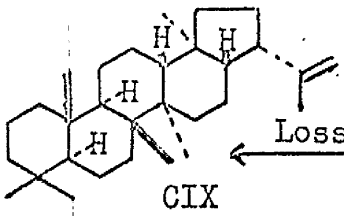
That ion ε [Fig. 15] may undergo rearrangements analogous to those undergone by ion γ to give ions δ and ε [Fig. 7] is perhaps suggested by the occurrence of tetrahymanol (XCVI)[239], a possible biogenesis of which from ion ε via ion W, is shown in Fig. 15. However, it must be pointed out that the gamma-cerane skeleton present in tetrahymanol which is the first pentacyclic triterpene to be isolated from an organism of the animal kingdom, is also formally derivable from the fundamental onocerane biscarbonium ion [Fig. 20] as well as from rearrangement of ion η [Fig. 17], so no firm conclusions are possible. Certainly it would appear that no compounds

FIGURE 17

Derivation Of Compounds Resulting From Cyclisation Of All Trans Squalene In Chair,Chair,Chair,Chair,Chair Conformational Sequence



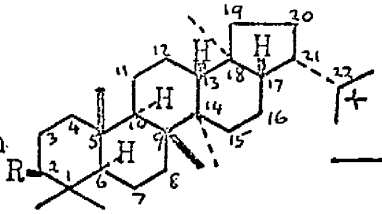
All trans squalene



CIX

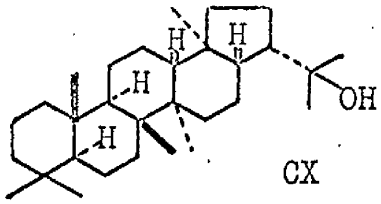
Diploptene

Loss of proton



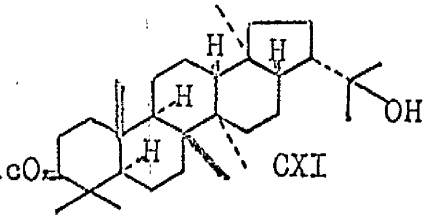
Fundamental Hopane Carbonium Ion

OH^-



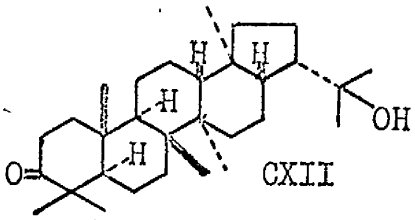
CX

Hydroxyhop



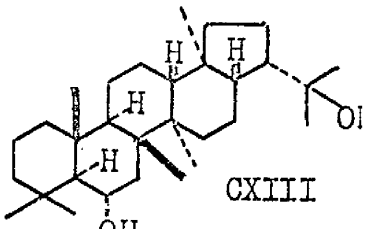
CXI

β-Acetoxy-hydroxyhopane



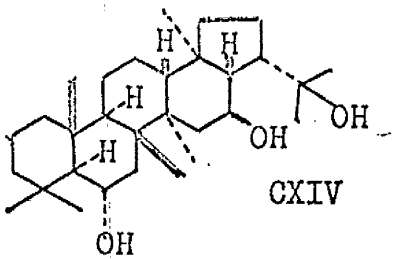
CXII

Hydroxyhopanone



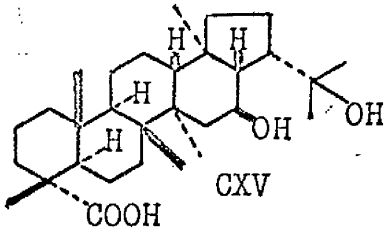
CXIII

Zeorin



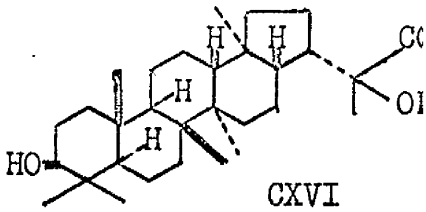
CXIV

Leucotylin



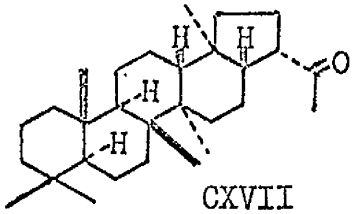
CXV

Leucotylic Acid



CXVI

Pyxinic Acid



CXVII

Adiantone

based on ion ζ [Fig. 15], which would result from the alternative migration of the 20, 21 bond to the 20, 22 position in ε in place of a migration of the 17, 21 bond to the 17,22 position, have as yet been discovered in Nature.

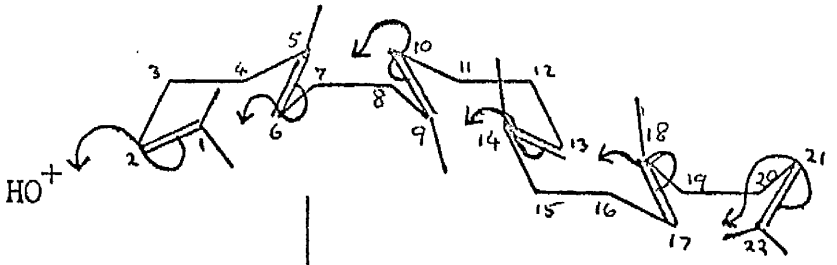
4. Cyclisation in chair, chair, chair, chair, chair conformational sequence

Synchronous cyclisation of all trans squalene in chair, chair, chair, chair, chair conformational sequence gives rise to the fundamental pentacyclic ion η [Fig. 17] of the hopane skelton in a one stage process, which, like the cyclisation in chair, chair, chair, chair, boat conformational sequence to give the fundamental isohopane carbonium ion ε [Fig. 15], involves anti-Markownikoff additions with respect to both the 13,14 and 17,18 double bonds. A third anti-Markownikoff addition [with respect to the 21,22 double bond] is avoided by formation of a 5-membered, and not a 6-membered ring E.

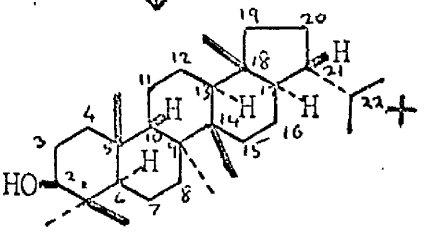
Carbonium ion η [Fig. 17] can stabilise either by loss of a proton from one of the methyl groups on C-22 as in the formation of diploptene (CIX)[231] or by picking up OH^- as in the formation of hydroxyhopane [syn diplopterol] (CX)[240], 3β-acetoxy-hydroxyhopane (CXI)[241], hydroxyhopanone (CXII)[242], zeorin (CXIII)[243,244], leucotylin (CXIV)[244], leucotylic acid (CXV)[245] and pyxinic acid (CXVI)[246]. Adiantone (CXVII)[247] is of interest, since like platanic acid (LVI)[133] of the lupeol series [Fig. 11] it has lost the methylene group of

FIGURE 18

Derivation Of Compounds Resulting From Cyclisation Of All Trans Squalene In Chair,Boat,Chair,Chair,Boat Conformational Sequence



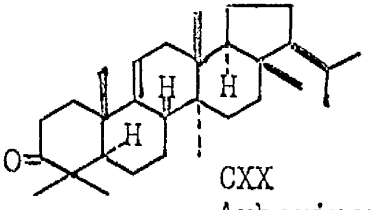
All trans squalene



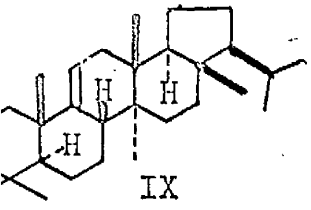
Fundamental
Carbonium Ion 0

Friedo backbone rearrangement

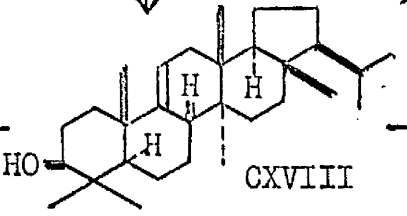
21βH → 22
17αH → 21α
18βCH$_3$ → 17β
13αH → 18α
14βCH$_3$ → 13β
9αCH$_3$ → 14α
10βH → 9β
Loss of 11αH as proton



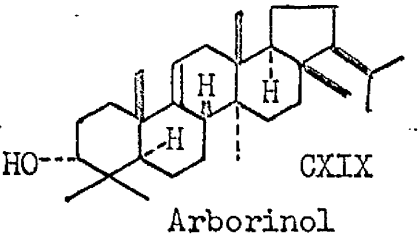
CXX
Arborinone



IX
Cylindrin



CXVIII
Isoarborinol



CXIX
Arborinol

the side chain by oxidation.

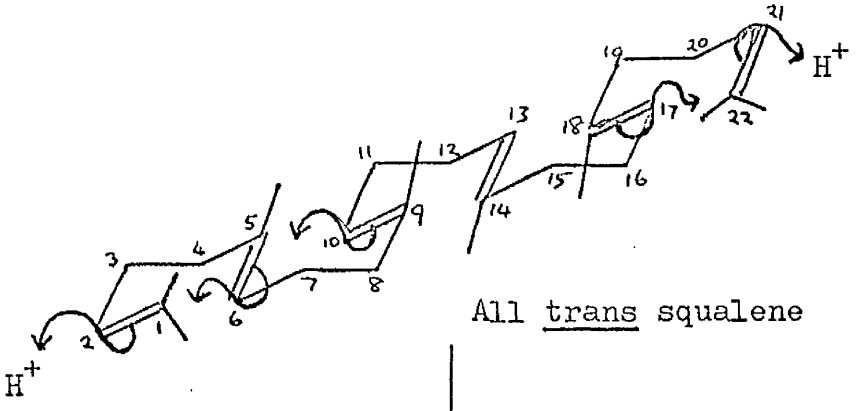
The position regarding possible friedo backbone rearrangement of ion ϒ has already been briefly discussed [page 112]. Should a non-concerted rearrangement occur, then the fundamental hopane ion can be regarded as the parent of the various compounds shown in Fig. 16, but if friedo backbone rearrangements in the isohopane - hopane series are fully concerted, ion ϒ will not give rise to friedo-derivatives.

5. Cyclisation in chair, boat, chair, chair, boat conformational sequence

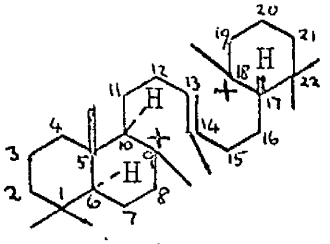
Synchronous cyclisation of all trans squalene in chair, boat, chair, chair, boat conformational sequence can be considered to give rise to the fundamental pentacyclic carbonium ion Ω [Fig. 18][37,38] in a one stage process which involves anti-Markownikoff additions with respect to both the 13,14 and 17,18 double bonds. At the present time, there would appear to be known, no naturally occurring pentacyclic triterpenes based on the unrearranged carbonium ion Ω, only the E:C-friedo derivatives isoarborinol (CXVIII)[248], its methyl ether cylindrin (IX)[7], arborinol (CXIX)[37,248] and arborinone (CXX)[249] having a 9(11) double bond. Although the trans-syn-trans relationship of the A/B and B/C ring fusions [cf. the identical situation in carbonium ion α of Fig. 5] of carbonium ion Ω would be expected to provide a good driving force for friedo backbone rearrangements at

FIGURE 19

Proposed Derivation Of Ambrein

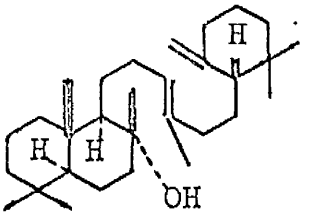


All trans squalene



Fundamental ambrene
biscarbonium ion

Attack by OH^- at C-9
Loss of proton from methyl group on C-18



CXXI

Ambrein

least as far as the E:C-friedo 9(11)-enes it will nevertheless be interesting to see whether compounds based on the unrearranged ion ϑ, or on skeletons resulting from a lesser degree of friedo backbone rearrangement in ϑ, will be discovered in Nature.

Comparison of Figs. 15, 17 and 18 with Fig. 7 shows that a major difference between ions ε, η and ϑ on the one hand and ions γ, δ and ε on the other is the inversion of position of carbon atoms 17 and 18 of squalene in the two groups, which serves to underline the necessity of showing all fundamental carbonium ions of the triterpene series in the squalene numbering system, rather than in their own assigned systems.

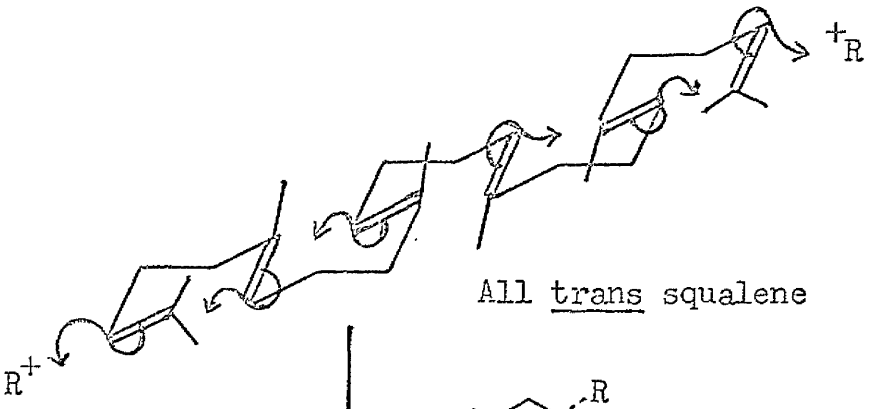
6. Cyclisation simultaneously from both ends to give two 6-membered rings at one end and one 6-membered ring at the other end of the resulting compounds.

Cyclisation, initiated by H^+, of all trans squalene simultaneously from both ends with formation of two 6-membered rings at one end and of one 6-membered ring at the other end, can be considered to give rise to the fundamental ambrene biscarbonium ion from which is derived ambrein (CXXI)[250] as shown in Fig. 19.

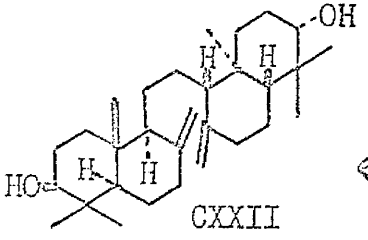
7. Cyclisation simultaneously from both ends to give two 6-membered rings at each end of the resulting compounds.

Cyclisation of all trans squalene simultaneously from both ends with formation of two 6-membered rings at each end,

Cyclisation Of All Trans Squalene, Simultaneously From Both Ends To Give Two Six-Membered Rings At Each End



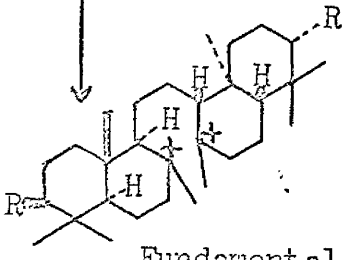
All trans squalene



CXXII

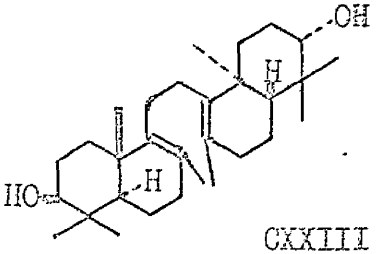
α-Onocerin

Proton Loss



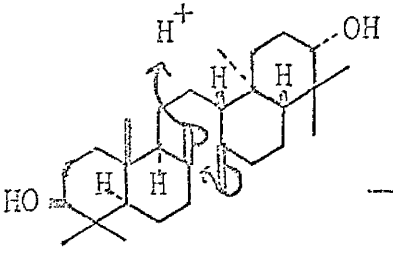
Fundamental Onocerane biscarbonium ion

Proton Loss



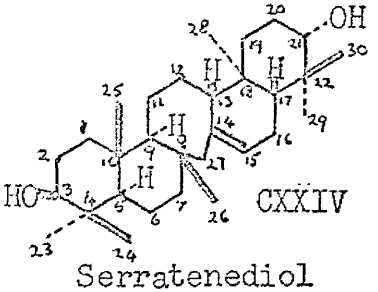
CXXIII

β-Onocerin



α-Onocerin

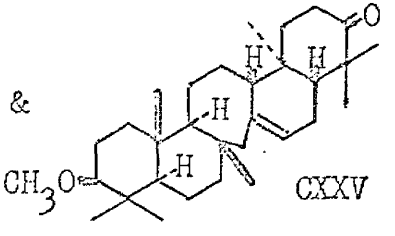
Acid catalysis



CXXIV

Serratenediol

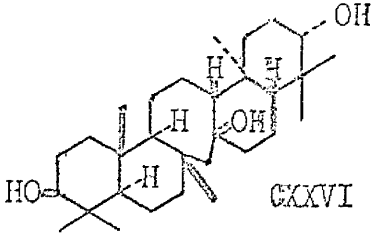
&



CXXV

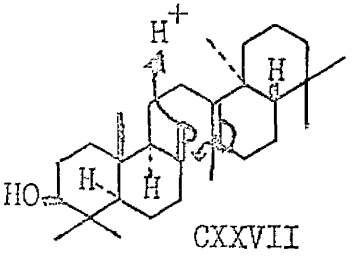
3β-Methoxy-21-ketoserrat-14-ene

&



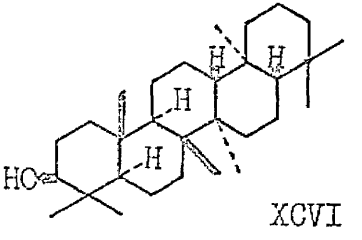
CXXVI

Tohogenol



CXXVII

Acid catalysis



XCVI

Tetrahymanol

can be considered to give rise to the fundamental onocerane biscarbonium ion from which are directly derived α-onocerin (CXXII)[251] and β-onocerin (CXXIII)[251] as shown in Fig. 20. Acid catalysed ring closure of the α-onocerin skeleton can then be considered to give rise to serratenediol (CXXIV)[252] and its various keto and methoxyl derivatives occurring in pine barks (e.g. 3β-methoxy-21-ketoserrat-14-ene (CXXV))[253] as well as to tohogenol (CXXVI)[254] and its 24-hydroxylated derivative tohogeninol[254]. Similarly acid catalysed ring closure of a compound such as CXXVII in which one double bond is as in α-onocerin and one double bond is as in β-onocerin, can be considered to represent a possible alternative route to that portrayed in Fig. 15 for the biogenesis of the gammacerane derivative, tetrahymanol (XCVI). Support for such a biogenetic route via CXXVII is provided by the laboratory synthesis of γ-onocerin diacetate via acid catalysed ring closure of α-onocerin diacetate[251], a route which was extended to provide a laboratory synthesis of tetrahymanol itself[239].

It is to be noted that both the cyclisation sequence of all <u>trans</u> squalene shown in Fig. 20 and the previous cyclisation sequence giving rise to ambrein [Fig. 19] appear to involve all chair conformations from the resultant stereochemistry, and that the independent cyclisations from both ends of the squalene molecule avoid any anti-Markownikoff additions.

It is thus seen, on the basis of the above discussion that numerous variants on a basic theme satisfactorily account for all of the wide variety of triterpene types so far found in Nature. Moreover a considerable body of direct experimental evidence has now been advanced which would support the essential correctness of the proposals outlined above.

Thus experiments with ^{14}C acetic acid [labelled either in the carboxyl group, or in the methyl group] have shown the broad essentials of the conversions of acetate into mevalonate, of mevalonate into isopentenyl pyrophosphate, of isopentenyl pyrophosphate into farnesyl pyrophosphate and of farnesyl pyrophosphate into squalene. These experiments have been admirably summarised in a recent review by Clayton[78] and will not be discussed in detail here.

Total degradation of squalene[255] and of cholesterol[256] biosynthesised from labelled acetate, with identification of the origin of each carbon atom of both compounds, gave results in entire agreement with the scheme outlined in Fig. 5 for the biogenesis of cholesterol via lanosterol, originally proposed by Woodward and Bloch[44], and later incorporated by Eschenmoser and Ruzicka[41-43] in their more comprehensive theory interrelating the biogenesis of all triterpenes and steroids. Further evidence was produced when it was shown that lanosterol was synthesised in rat tissue both <u>in vitro</u>[257] and <u>in vivo</u>[258]

and in turn was efficiently metabolised to cholesterol.

That squalene plays a central role as a biological precursor of polycyclic triterpenes and steroids in plants as well as in animals is supported by experiments showing that labelled mevalonate is rapidly converted into squalene in the tissues of higher plants[259] and that the labelling pattern of the squalene so formed is identical with that produced in animal tissues.[260]

That the 13β and 14α methyl groups of lanosterol take up their positions by 1,2-shifts from the corresponding 14β and 8α positions [lanosterol numbering] respectively in the fundamental tetracyclic carbonium ion α [Fig.5] has been demonstrated by appropriately designed labelling experiments performed by Bloch *et al*[261] and by Cornforth *et al*[262].

Radio-tracer studies have shown that the nuclear transformations involved in the conversion of lanosterol into cholesterol in animal liver tissue can apparently occur with equal facility in both lanosterol and 24,25-dihydrolanosterol,[78] so it has not proved feasible to elucidate a definite sequence of stages for this conversion.

It is of interest that recent work[263,264] might suggest that 3β-hydroxy lanost-7,24-diene[78] or lanosta-8,24-diene[47] rather than lanosterol is the key product of cyclisation of squalene from which the steroids arise.

In the case of the plant sterols, experiments with

radiotracers on the biogenesis of ergosterol (XX)[265] and eburicoic acid (XI)[266] have shown that the same intermediates as far as the fundamental carbonium ion α of Fig. 5 are involved, as are involved in the biogenesis of cholesterol in animals.

Relatively less experimental work has been done with respect to proving that the biogenesis of the pentacyclic triterpenes follows the routes proposed in Figs. 7, 12, 15, 17 and 18 but it has been shown that lupeol (XLIX) biosynthesised from [2-^{14}C] mevalonate possessed[43,267] a labelling pattern in complete accord with the theory as did soyasapogenol D[268], betulin (L)[267] and betulinic acid (LI)[267]. The stereospecificity of the process is indicated by the fact that in neither lupeol nor soyasapogenol D do the terminal methyl groups of squalene, only one of which is labelled, at each end of the molecule, become equivalent.

Other experiments have shown that radio-labelled mevalonic acid is incorporated into β-amyrin[269] and oleanolic acid[270].

It would seem probable that the triterpenes and steroids so far discovered in Nature, have by no means exhausted all possible variations of triterpenoid biogenesis, and that many new variants remain to be discovered. Some of these possibilities are obvious from the foregoing discussion, but other less obvious types may also be discovered. As examples of the more obvious gaps in the series, as at present known,

may be cited the absence of the ursane analogue of friedelin and filicene, the absence of the friedo oleanane and friedo isohopane analogues of phyllanthol, and the absence of the ursane and oleanane analogues of ceanothic and ceanothenic acids. The less obvious gaps may well be filled through the discovery of new compounds derived from modes of cyclisation of all trans squalene other than the seven given above, of new compounds arising from squalenes in which one or more of the double bonds have the cis configuration [compare the role of the isomeric all trans and trans-trans-cis farnesyl-pyrophosphates in sesquiterpene biogenesis [271]] or of new compounds derived from further rearrangements of the carbonium ions discussed above. It is highly probable that new types may be represented among the forty odd pentacyclic triterpenes of unknown structure listed by Halsall and Aplin [217], and with the very recent elucidation of the structures of the friedo isohopane series [230,231] and of arborinol [37], it may well prove possible to relate some of these unknown compounds to compounds of established structure.

The Biogenetic Significance Of The Triterpene Methyl Ethers Of Cortaderia toetoe

From what has been discussed it is apparent that the co-occurrence of arundoin [derivable from the isohopane skeleton] with the methyl ether of β-amyrin [derivable from the oleanane skeleton] and the methyl ether of α-amyrin

[derivable from the ursane skeleton] in Cortaderia toetoe implies that two separate cyclisations of all trans squalene are occurring side by side in the same plant - viz. chair, chair, chair, chair, boat cyclisation in the case of arundoin and chair, chair, chair, boat cyclisation in the case of the methyl ethers of α- and β-amyrin.

A similar situation but with the co-occurrence of three separate modes of cyclisation appears to exist with respect to Cuban sugar cane, since work described in subsection D of this thesis has shown that arundoin, a friedo ursane derivative [bauerenol methyl ether] and a friedo oleanane derivative [taraxerol methyl ether] appear to co-exist in the wax of this plant together with the previously reported[272] sterols β-sitosterol, stigmasterol, campesterol, 24-methyl-enelophenol and 24-ethylidenelophenol.

Such co-existence of more than one cyclisation mechanism of squalene in the one plant is not without precedent, since it must be present wherever plant sterols and pentacyclic triterpenes co-exist. Examples of this situation which may be cited are the co-occurrence of cardiac glycosides with ursolic and oleanolic acids in Nerium odorum[273], the co-occurrence of stigmasterol and the soyasapogenols in the soya bean[274], the co-occurrence of β-sitosterol and α-amyrin in the pea plant[275] and the co-occurrence of β-sitosterol and various pentacyclic triterpenes in Salvia officinalis[276]. Again, parkeol (XCII) [chair, boat, chair, boat sequence]

and butyrospermol (XXXI), lupeol (XLIX) and β-amyrin (LXII) [chair, chair, chair, boat sequence][277] occur in shea-oil whilst citrostadienol [chair, boat, chair, boat sequence] and the butyrospermol-derived citrus bitter principle limonin, both occur in the grapefruit plant.[278] A particularly appropriate analogy to the present instances with Cortaderia toetoe and Cuban sugar cane wax with their co-occurrence of chair, chair, chair, chair, boat and chair, chair, chair, boat cyclisation sequences is afforded by Ficus macrophylla Desf. which elaborates[234] moretenol [chair, chair, chair, chair, boat sequence], lupeol and butyrospermol [chair, chair, chair, boat sequence] and cycloartenol [chair, boat, chair, boat sequence].[7] This situation can also be compared with the coexistence in Imperata cylindrica of arundoin and cylindrin which implies the co-occurrence of chair, chair, chair, chair, boat and chair boat, chair, chair, boat cyclisation sequences respectively.

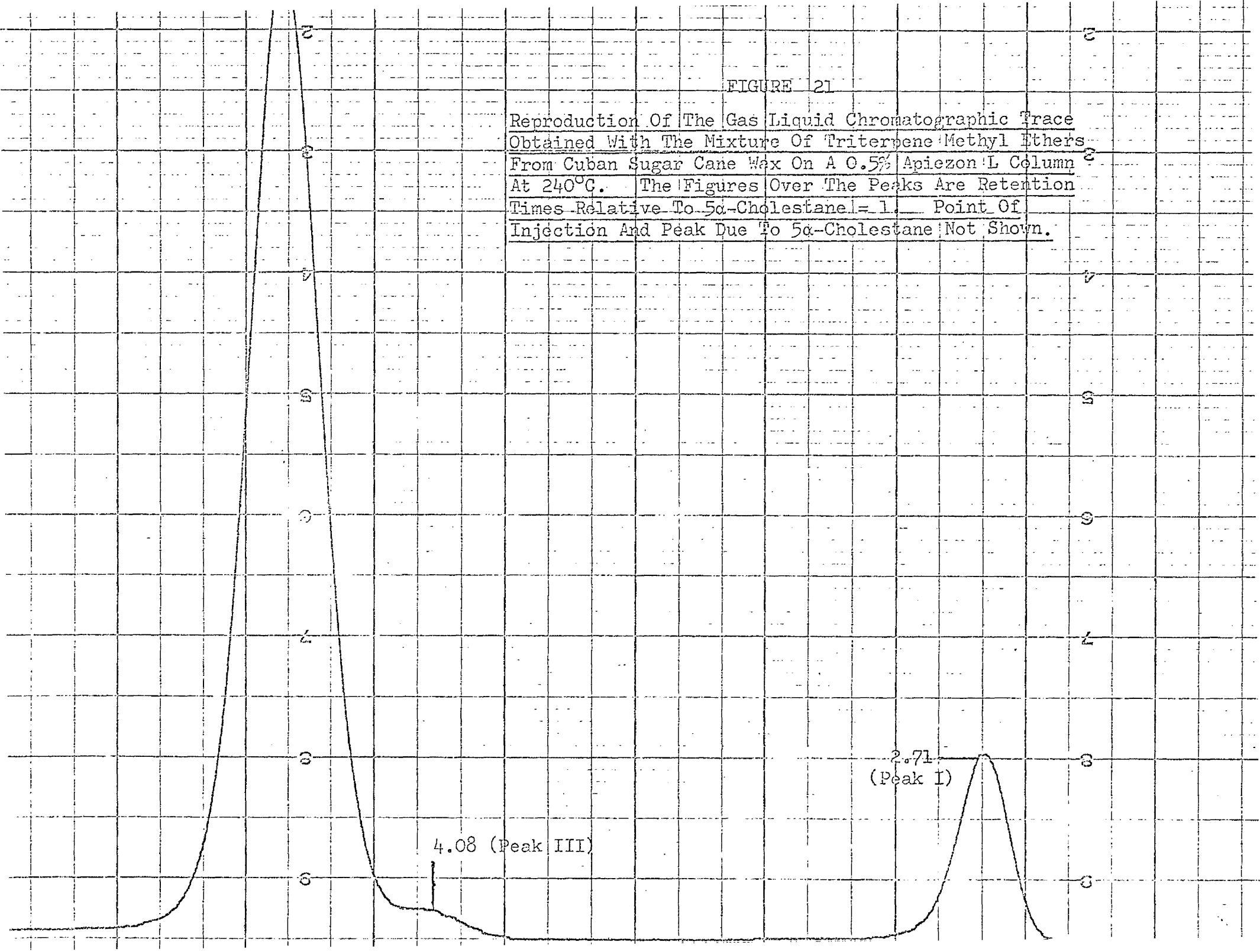
In view of the absolute stereospecificity of the enzyme systems concerned in steroid and triterpene biogenesis as apparent from the unique configurations invariably observed in these compounds, the presence of more than one cyclisation pathway must be taken as implying the coexistence of separate enzyme systems - itself a fact of no small interest in terms of the exact role played by triterpenes and steroids in plants, which is still unknown.

D. Triterpene Methyl Ethers From Cuban Sugar Cane Wax.

While the gas liquid chromatographic studies with triterpene methyl ethers described in the preceding sub-section B were being conducted, Dr. K. Schreiber at Gatersleben, East Germany, became aware of the work and kindly supplied for identification a triterpene ether fraction which he had isolated from the wax of Cuban sugar cane [Saccharum officinarum L.][272]. The occurrence of triterpene methyl ethers in this plant is of some interest since it is closely related to Imperata cylindrica from which the two triterpene methyl ethers, arundoin and cylindrin have been isolated[7,8] - both plants belonging to the tribe Andropogoneae. The fact that triterpene methyl ethers were detected in sugar cane wax only as late as 1964 is also of considerable interest in view of the extensive chemical investigations[279], including several on the wax[280,281], which have been performed on Saccharum officinarum on account of its value as a food source and the consequent availability of large quantities of material, and reflects the power of modern methods of plant analysis. Prior to the introduction of infrared spectrometers and chromatographic methods, including g.l.c. the presence of triterpene methyl ethers in a plant would not have been suspected, as such compounds have physical properties very akin to those of the paraffins and would, in all probability, have been rejected with the 'fatty material' during preliminary defatting operations in the

FIGURE 21

Reproduction Of The Gas Liquid Chromatographic Trace Obtained With The Mixture Of Triterpene Methyl Ethers From Cuban Sugar Cane Wax On A 0.5% Apiezon L Column At 240°C. The Figures Over The Peaks Are Retention Times Relative To 5α-Cholestane = 1. Point Of Injection And Peak Due To 5α-Cholestane Not Shown.



chemical work-up of the plant material.

The apparent failure[280,281] to detect triterpene methyl ethers in the wax of Australian sugar cane and their successful isolation[272] from the wax of Cuban sugar cane may be explicable in terms of geographical factors or in terms of different strains being cultivated in the two countries.

Until the recent isolation of triterpene methyl ethers from pine bark[253], these compounds appeared confined to grasses[7,8,38,190,232,286] so it now becomes interesting to speculate on their possible isolation from representatives of other plant families now that a deliberate search for them can be undertaken.

In the present studies preliminary analytical gas liquid chromatographic experiments with the material supplied by Dr. Schreiber employing a 0.5% Apiezon L column with argon as carrier gas showed the presence of 2 prominent well-resolved peaks with a third incompletely resolved peak of low intensity on the low retention side of the second main peak [Fig. 21]. However, gas liquid chromatography employing 1.5% SE-30, 1.5% QF-1 and 1% CDMS as the stationary phases with argon as carrier gas, or employing 2.5% SE-30 and 2% XE-60 columns with nitrogen as carrier gas revealed the presence of only two peaks.

Further analytical gas liquid chromatographic experiments,

with added 5α-cholestane as internal standard, showed the relative retention times of the peaks to be as follows:-

Component	0.5% Apiezon L	1.5% SE-30
I	2.71	2.43
II (major component)	4.30	3.21
III (minor component)	4.08	-

Comparison of these relative retention time values with the data given in Table IV strongly suggests that the mixture provided by Dr. Schreiber could contain arundoin, the methyl ether of bauerenol and one or more of the methyl ethers of germanicol, α-amyrin, β-amyrin, taraxerol or multiflorenol.

Indeed addition of authentic arundoin to the mixture intensified the peak on the Apiezon L column corresponding to component II, addition of bauerenol methyl ether intensified the peak corresponding to component III and addition of taraxerol methyl ether, β-amyrin methyl ether, or multiflorenol methyl ether intensified the peak corresponding to component I. Moreover the disappearance of the peak due to component III on changing from an Apiezon L to a SE-30 column is completely in accord with the gas liquid chromatographic behaviour of bauerenol methyl ether (see Table IV).

In order to obtain further information as to the identity of the components in Dr. Schreiber's mixture it was decided to invoke preparative gas liquid chromatography and mass

FIGURE 22

Line Diagram Of The Mass Spectrum, Determined Using A Direct Inlet System, Of The Material Responsible For Peak II In The Preparative Gas Liquid Chromatography Of The Triterpene Methyl Ethers Occurring In Cuban Sugar Cane Wax Employing SE-30 As Stationary Phase.

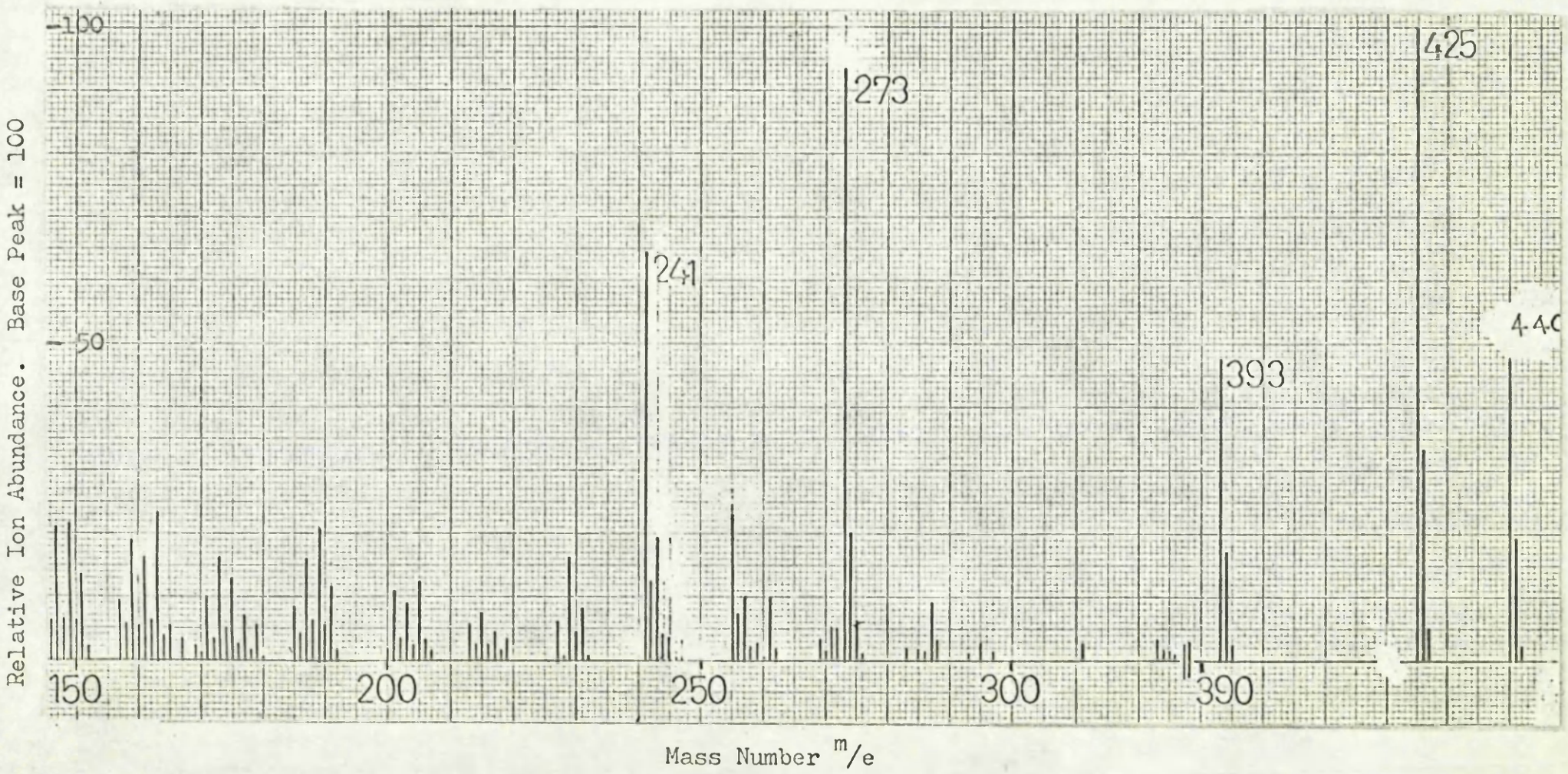
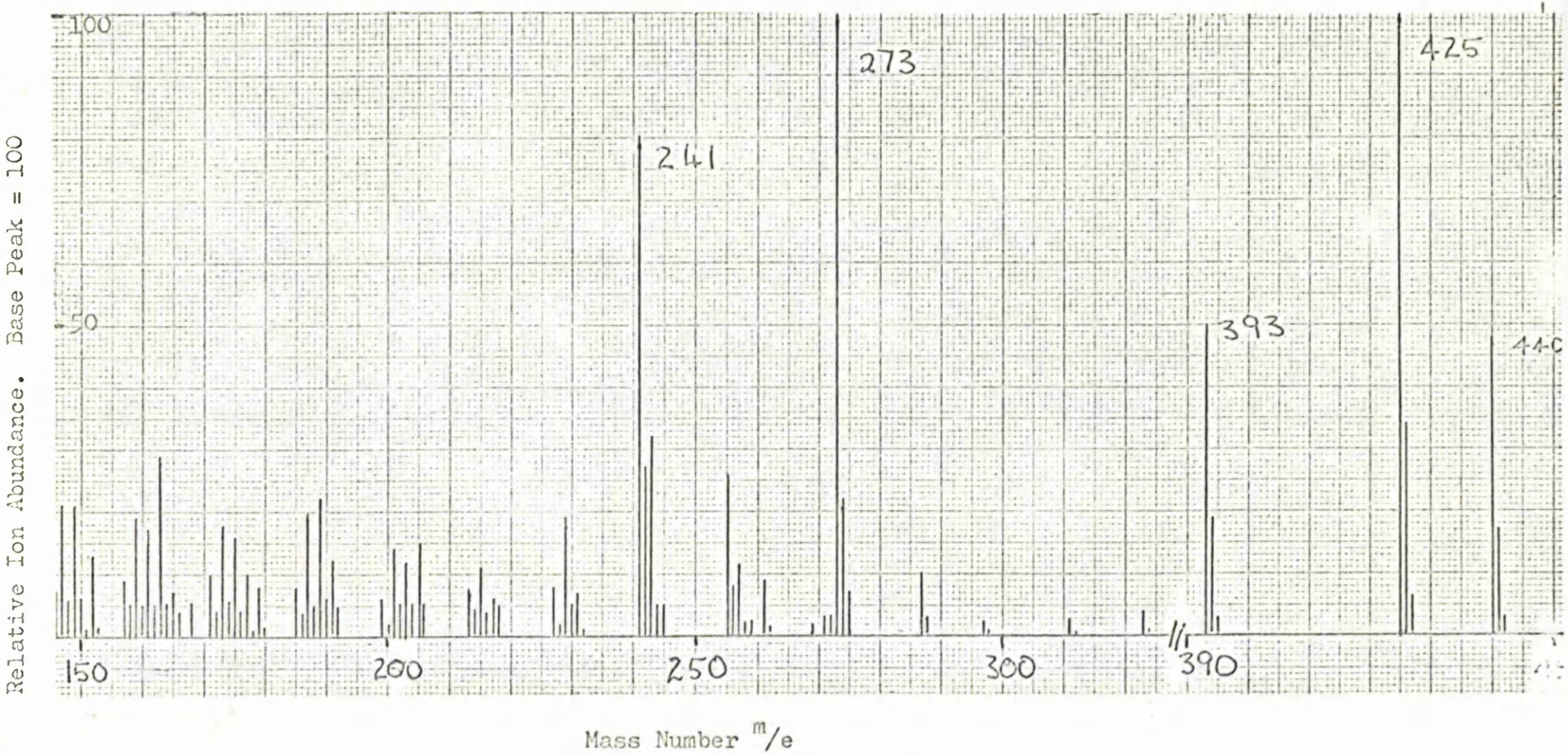


FIGURE 23

Line Diagram Of The Mass Spectrum Of Authentic Arundoin, Determined Using A Direct Inlet System



spectrometry. No success was achieved in attempts to secure the material responsible for Peak III on Apiezon L columns, but a preparative SE-30 column permitted clean separation of two fractions, one corresponding to Peak I and one corresponding to Peak II plus Peak III [of the Apiezon L columns].

Application of mass spectrometry [kindly undertaken by Mr. T.A. Bryce] showed that the material of Peak II from the SE-30 preparative column gave a mass spectrum [Fig. 22] virtually identical with that of authentic arundoin [Fig. 23], as determined using a direct inlet system, with peaks at $\frac{m}{e}$ 440 [parent], 425 [strong], 287 [weak], 273 [base peak] and 261 [weak] characteristic of a $\Delta^{9(11)}$ triterpene with methyl groups at C-13 and C-14[34,35]. In addition prominent peaks were present in both spectra at $\frac{m}{e}$ 393 and 241. The origin of these peaks, which are considered to arise from the ions $\frac{m}{e}$ 425 and $\frac{m}{e}$ 273 by loss of methanol, is discussed in detail in subsection F.

No evidence for the presence of bauerenol methyl ether in admixture with the arundoin from Peak II of the preparative gas liquid chromatography can be advanced from a comparison of Figs. 22 and 23. This is perhaps to be expected since both arundoin and bauerenol methyl ether give rise to extremely similar mass spectral cracking patterns[34,35]. Thus both compounds would be expected[34,35] to give rise to

FIGURE 24

Line Diagram Of The Mass Spectrum, Determined Using A Direct Inlet System, Of The Material Responsible For Peak I In The Preparative Gas Liquid Chromatography Of The Triterpene Methyl Ethers Occurring In Cuban Sugar Cane Wax Employing SE-30 As Stationary Phase.

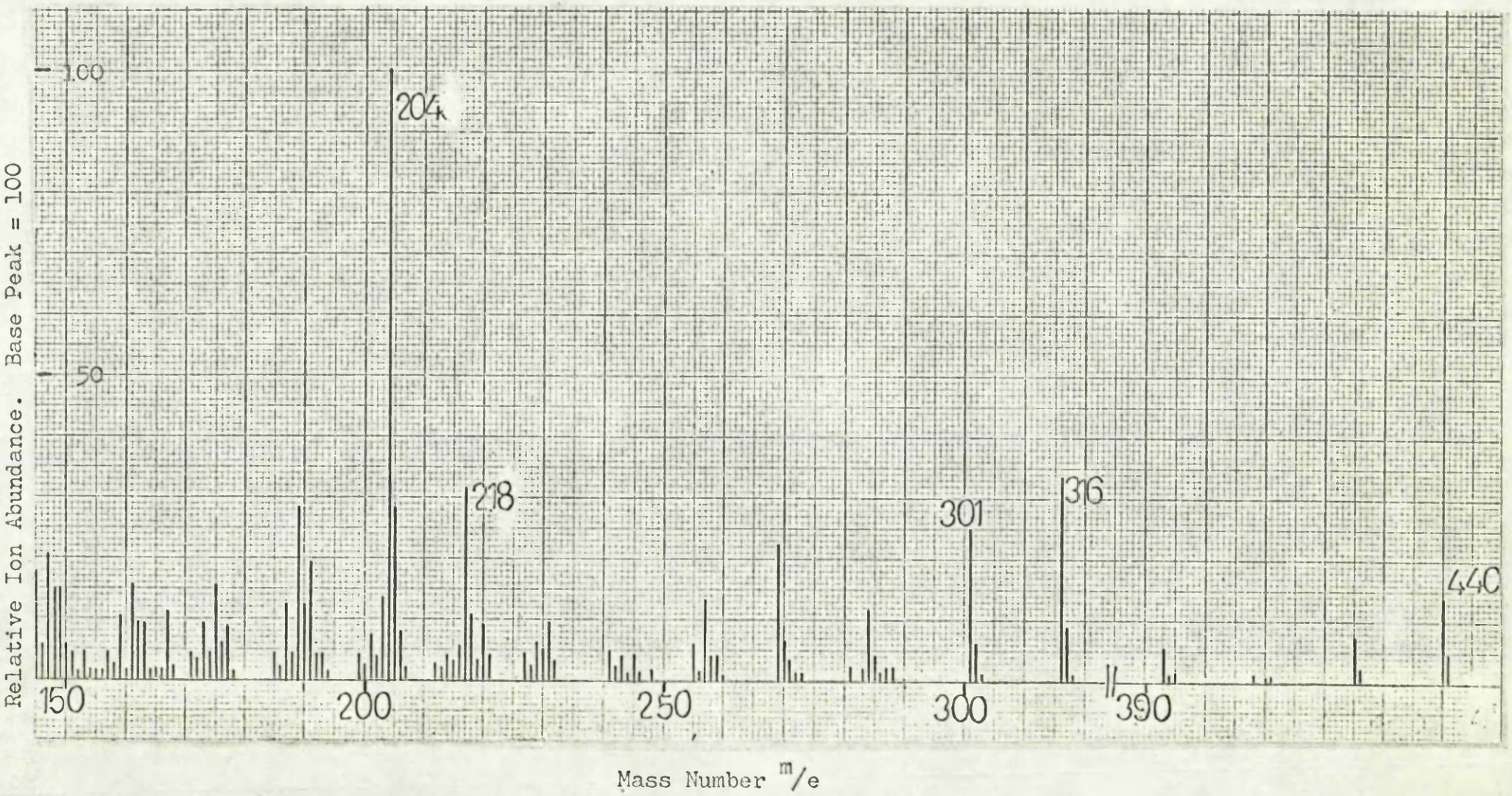
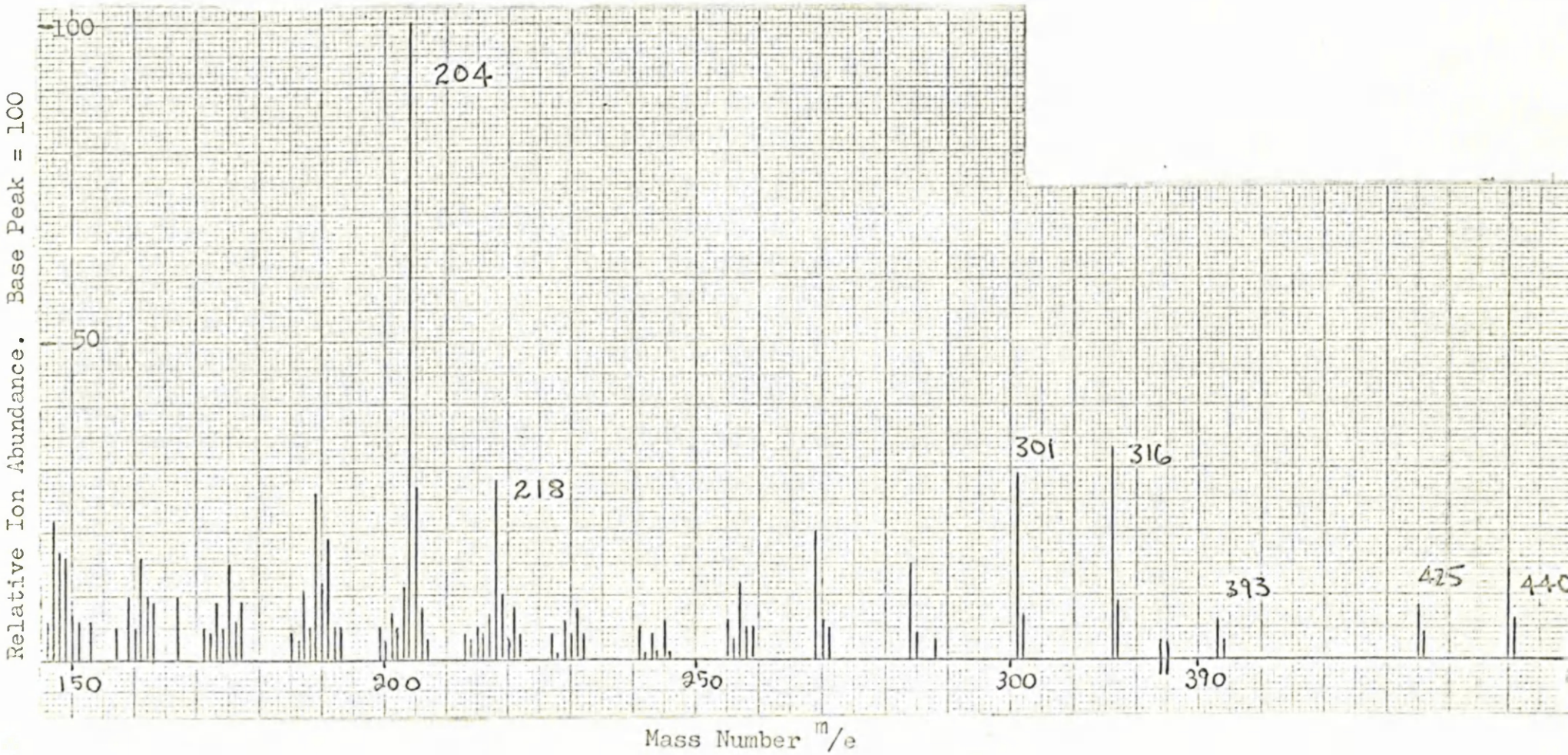


FIGURE 25

Line Diagram Of The Mass Spectrum Of Authentic Taraxerol Methyl Ether (Sawamilletin), Determined Using A Direct Inlet System.



ions $\frac{m}{e}$ 440, $\frac{m}{e}$ 425, $\frac{m}{e}$ 287, $\frac{m}{e}$ 273 and $\frac{m}{e}$ 261. Any differences which might be expected at $\frac{m}{e}$ 234 and $\frac{m}{e}$ 205, which are ions characteristic of the mass spectrum of bauerenol methyl ether[35], should be relatively minor since these ions have low abundance and so differences in their intensity as between Fig. 22 and Fig. 23 would only be discernible if relatively large quantities of bauerenol methyl ether were present with the arundoin from the sugar cane wax. The g.l.c. trace [Fig. 21] shows that this is not so.

Direct comparison of the material of Peak II of the preparative gas liquid chromatography with authentic arundoin showed identity of infrared spectra in KCl disc and absence of any mixed melting point depression.

The mass spectrum of the material of Peak I of the preparative gas liquid chromatography was strictly comparable with that of authentic taraxerol methyl ether. The two spectra are shown in line diagram form in Fig. 24 and Fig. 25 respectively. Comparison of Figs. 24 and 25 serves to indicate that if any other triterpene methyl ethers of the oleanane group are co-occurring with taraxerol methyl ether in Cuban sugar cane wax, then they must be present in very small amounts. Thus the absence of any ion of $\frac{m}{e}$ 234 [the expected base peak in the mass spectrum of multiflorenol methyl ether[34,35]] in Fig. 24 indicates the absence of multiflorenol methyl ether as a contaminant of

taraxerol methyl ether in the sugar cane wax. Similarly the identical relative abundance of the ions $\frac{m}{e}$ 203, 204, 205 and 218 in Figs. 24 and 25 would seem to rule out the presence of any appreciable quantities of germanicol methyl ether (medium peaks at $\frac{m}{e}$ 203, 204, 205[34,35]), δ-amyrin methyl ether (base peak $\frac{m}{e}$ 205[34,35]) or β-amyrin methyl ether (base peak $\frac{m}{e}$ 218[34,35]), although the possibility that one or more of these compounds is present in trace amounts can not be ruled out.

Direct comparison of the material corresponding to Peak I on the preparative SE-30 column with authentic taraxerol methyl ether showed identity of infrared spectra in KCl disc and absence of any mixed melting point depression - again demonstrating the insignificance of the quantities of any other components which could be present.

In summary then, the present investigations have shown Dr. Schreiber's mixture to consist of at least 3 different triterpene methyl ethers. Arundoin and sawamilletin [taraxerol methyl ether] have been identified as the major constituents with a strong possibility that the minor third component is bauerenol methyl ether.

E. Confirmation of The Occurrence of O-Desmethylarundoin in Artemisia vulgaris L.

During the time the present studies were in progress, Dr. A.S. Rao of the National Chemical Laboratory, Poona,

India, provided a specimen of a synthetic triterpene methyl ether, prepared from a hitherto unreported pentacyclic triterpene alcohol of natural occurrence in Artemisia vulgaris L. which he believed to be O-desmethylarundoin. Gas liquid chromatographic studies employing 0.5% Apiezon L columns and 5α-cholestane as internal standard showed the synthetic methyl ether to exhibit a single symmetrical peak which was identical in retention time with that of pure arundoin. A mixture of Rao's compound with arundoin showed a single symmetrical peak on the trace which again had identical retention with that of pure arundoin. Conclusive proof that Rao's naturally occurring triterpene alcohol was indeed O-desmethylarundoin was achieved by comparison of the infrared spectrum of the synthetic methyl ether in KCl disc with the infrared spectrum of arundoin. The two spectra were indisputedly identical. Further there was no mixed m.p. depression on admixture of Rao's methyl ether and arundoin.

F. Remarks On The Mass Spectra Of Triterpene Methyl Ethers

As apparent from subsections C and D, mass spectrometry played an indispensable role in the identification of the triterpene methyl ethers present in Cortaderia toetoe and Cuban sugar cane wax. In the course of this work an interesting facet of the mass spectra of the triterpene methyl

TABLE VI.

Major Peaks In The Mass Spectra Of Triterpene Methyl Ethers

Compound	Predicted* Mass Spectral Peaks (Observed Mass Spectral Peaks Relative Ion Abundances Given As %age Of Base Peak = 100)			Metastable Peaks For Loss Of Methanol From Ion Of Series A To Corresponding Ion Of Series B.	
		Series A Corresponding To Predicted Ions	Series B Arising By Loss Of Methanol	Observed	Calculated
Arundoin (VIII) (Heated Inlet System) Cf. Fig. 35	m/e 440 (Parent) 425 (h) 355 (l) 287 (m) 273 (b) 261 (l)	m/e 440 (15%) 425 (60%) 365 (14%) 355 (1%) 287 (8%) 273 (58%) 261 (5%)	m/e 408 (16%) 393 (100%) 333 (4%) 323 (3%) 255 (31%) 241 (76%) 229 (14%)	378.3 363.4 ? 294.0 226.5 212.8 201.0	378.3 363.4 303.8 293.9 226.6 212.8 200.9
Arundoin (VIII) (Direct Inlet System) Cf. Fig. 23	m/e 440 (Parent) 425 (h) 355 (l) 287 (m) 273 (b) 261 (l)	m/e 440 (48%) 425 (100%) 355 (1%) 287 (10%) 273 (100%) 261 (9%)	m/e 393 (50%) 323 (4%) 255 (26%) 241 (80%) 229 (19%)	363.4 294.0 226.5 212.8 201.0	363.4 293.9 226.6 212.8 200.9
Taraxerol Methyl Ether (IV) (Direct Inlet System) Cf. Fig. 25	m/e 440 (Parent) 425 (l) 316 (m) 301 (m) 218 (m) 204 (b) 189 (m)	m/e 440 (14%) 425 (8%) 316 (33%) 301 (29%) 218 (28%) 204 (100%) 189 (26%)	m/e 393 (6%) 284 (15%) 269 (20%)	363.4 255.2 240.4	363.4 255.2 240.4
Bauerenol Methyl Ether (VII) (Direct Inlet System) Cf. Fig. 26	m/e 440 (Parent) 425 (m) 287 (l) 273 (l) 261 (b) 234 (l) 205 (m)	m/e 440 (46%) 425 (32%) 287 (6%) 273 (20%) 261 (100%) 234 (8%) 205 (24%)	m/e 393 (21%) 255 (10%) 241 (18%) 229 (77%) 202 (8%)	363.4 226.6 212.8 201.0 ?	363.4 226.6 212.8 200.9 174.4
β-Amyrin Methyl Ether (III) (Direct Inlet System) Cf. Fig. 27b	m/e 440 (Parent) 425 (l) 222 (l) 221 (l) 218 (b) 205 (l) 203 (m) 189 (m) 133 (l)	m/e 440 (7%) 425 (2%) 222 (3%) 221 (7%) 218 (100%) 205 (3%) 203 (36%) 189 (14%) 133 (10%)	m/e 408 (1%) 393 (1%) 190 (15%) 189	378.5 363.4 162.6 161.6	378.3 363.4 162.6 161.6
α-Amyrin Methyl Ether (VI) (Direct Inlet System) Cf. Fig. 27a	m/e 440 (Parent) 425 (l) 222 (l) 221 (l) 218 (b) 205 (l) 203 (m) 189 (m) 133 (l)	m/e 440 (4%) 425 (1%) 222 (5%) 221 (11%) 218 (100%) 205 (2%) 203 (14%) 189 (14%) 133 (8%)	m/e 408 (1%) 393 190 (10%) 189	378.5 363.4 162.6 161.6	378.3 363.4 162.6 161.6
Cylindrin (IX) (Direct Inlet System Cf. Fig. 29	m/e 440 (Parent) 425 (h) 355 (l) 287 (m) 273 (b) 261 (l)	m/e 440 (70%) 425 (100%) 355 (9%) 287 (16%) 273 (87%) 261 (10%)	m/e 393 (61%) 323 (13%) 255 (30%) 241 (52%) 229 (22%)	363.4 294.0 226.6 212.8 201.0	363.4 293.9 226.6 212.8 200.9
Germanicol Methyl Ether (I) (Direct Inlet System) Cf. Fig. 28	m/e 440 (Parent) 425 (m) 221 (l) 218 (l) 205 (m) 204 (b) 203 (m) 190 (m) 189 (h) 177 (h)	m/e 440 (50%) 425 (35%) 221 (18%) 218 (24%) 205 (35%) 204 (96%) 203 (26%) 190 (36%) 189 (100%) 177 (70%)	m/e 408 (5%) 393 (7%) 189	378.3 363.4 161.6	378.3 363.4 161.6

* Calculated from fragmentation patterns established by H. Budzikiewicz, J. M. Wilson and C. Djerassi[35]

l = low intensity
m = medium intensity
h = high intensity
b = base peak

The abundances quoted for the ions arising by loss of methanol are not corrected for contributions from fragments of the same mass arising from other modes of breakdown.

ethers was observed which warrants further discussion and this will now be considered in terms of Table VI which summarises the major ions observed in the mass spectra of the various triterpene methyl ethers studied, as well as listing the major ions to be predicted from the work of Djerassi and his colleagues[34,35].

It is readily seen from Table VI that the presence of the methoxyl group on the triterpene nucleus gives rise to an extra series of ions differing from those predicted on the basis of Djerassi's work by loss of 32 mass units, but that apart from this there is excellent agreement between the observed and predicted spectra. That the observed subsidiary series of ions are related to the predicted series by loss of methanol [mass 32] in a one stage process is confirmed by the appearance of the appropriate metastable peaks in the spectra. Thus in the mass spectrum of bauerenol methyl ether [Fig. 26] metastable peaks are observed at 363.4 (calcd. for $\frac{m}{e}$ 425 $\longrightarrow$ $\frac{m}{e}$ 393, $\frac{393^2}{425}$ = 363.4), at 212,8 (calcd. for $\frac{m}{e}$ 273 $\longrightarrow$ $\frac{m}{e}$ 241, $\frac{241^2}{273}$ =212.8) and at 201.0 (calcd. for $\frac{m}{e}$ 261 $\longrightarrow$ $\frac{m}{e}$ 229, $\frac{229^2}{261}$ = 200.9). Similarly in the mass spectrum of arundoin [Fig. 23] metastable peaks are observed at 363.4 (calcd. for $\frac{m}{e}$ 425 $\longrightarrow$ $\frac{m}{e}$ 393, $\frac{393^2}{425}$ = 363.4), at 226.5 (calcd. for $\frac{m}{e}$ 287 $\longrightarrow$ $\frac{m}{e}$ 255, $\frac{255^2}{287}$ = 226.6) and at 212.8 (calcd. for $\frac{m}{e}$ 273 $\longrightarrow$ $\frac{m}{e}$ 241, $\frac{241^2}{273}$ = 212.8), whilst in the mass spectrum of taraxerol methyl ether

FIGURE 26

Line Diagram Of The Mass Spectrum Of Authentic Bauerenol Methyl Ether, Determined Using A Direct Inlet System.

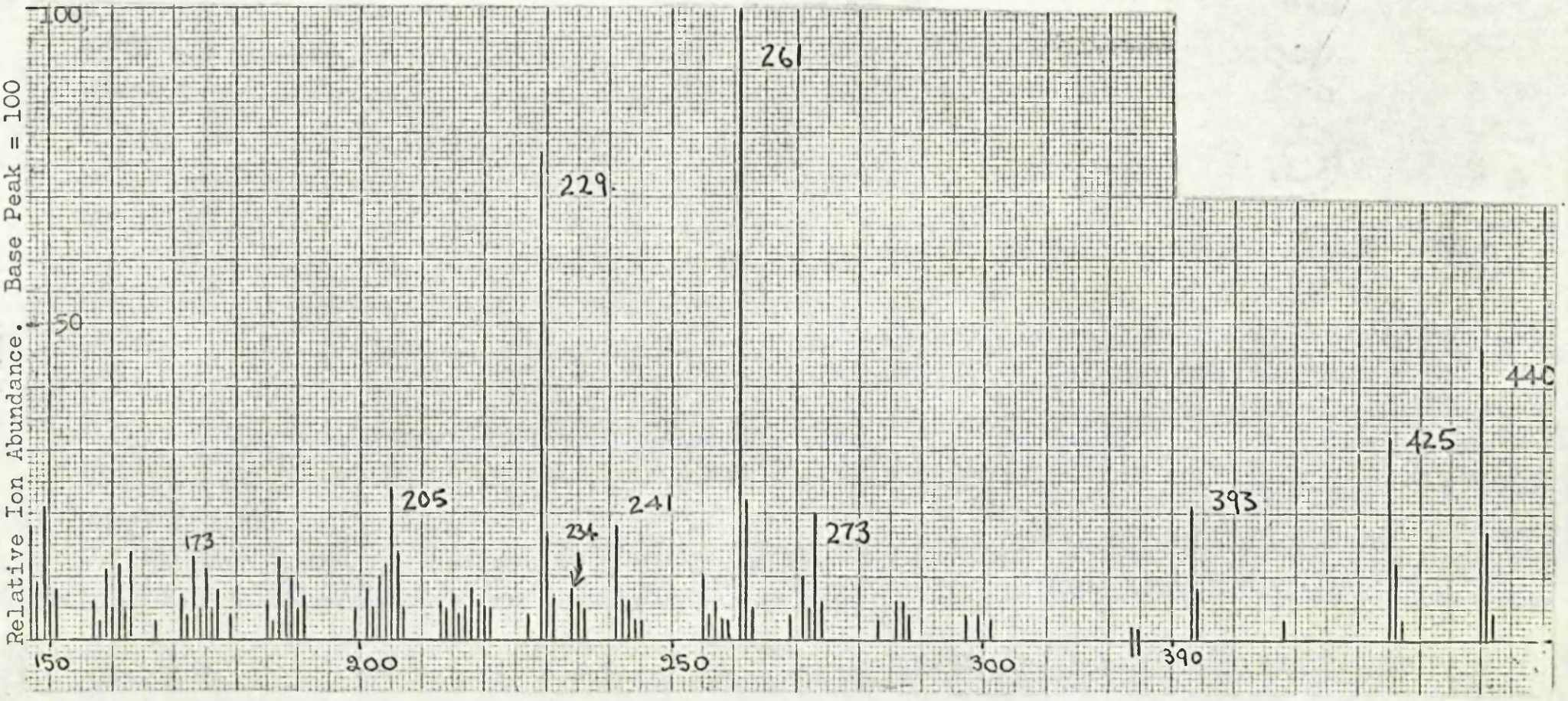


FIGURE 27 a

Line Diagram Of The Mass Spectrum Of Authentic α-Amyrin Methyl Ether Determined Using A Direct Inlet System

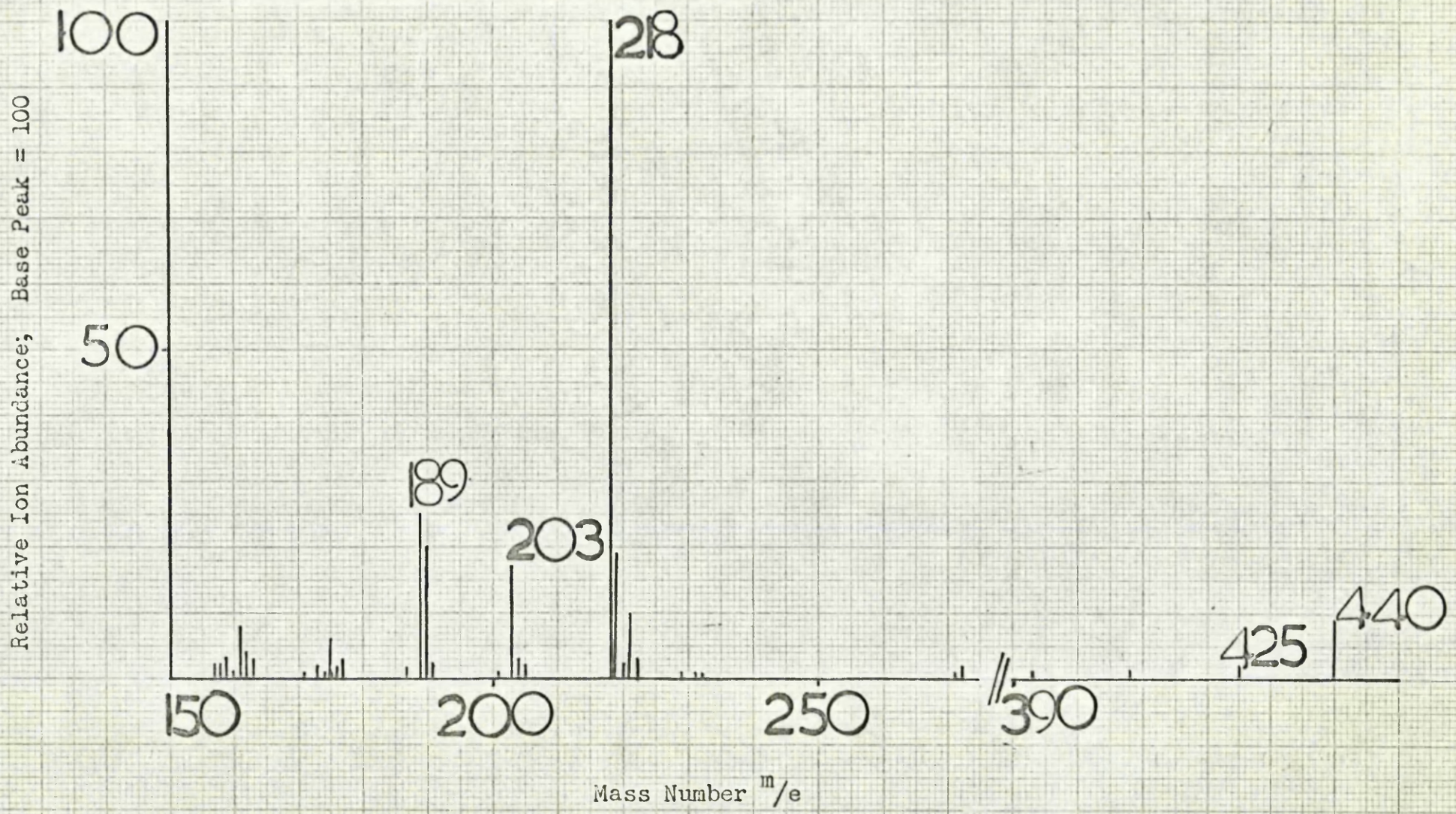


FIGURE 27 b

Line Diagram Of The Mass Spectrum Of Authentic β-Amyrin Methyl Ether Determined Using A Direct Inlet System.

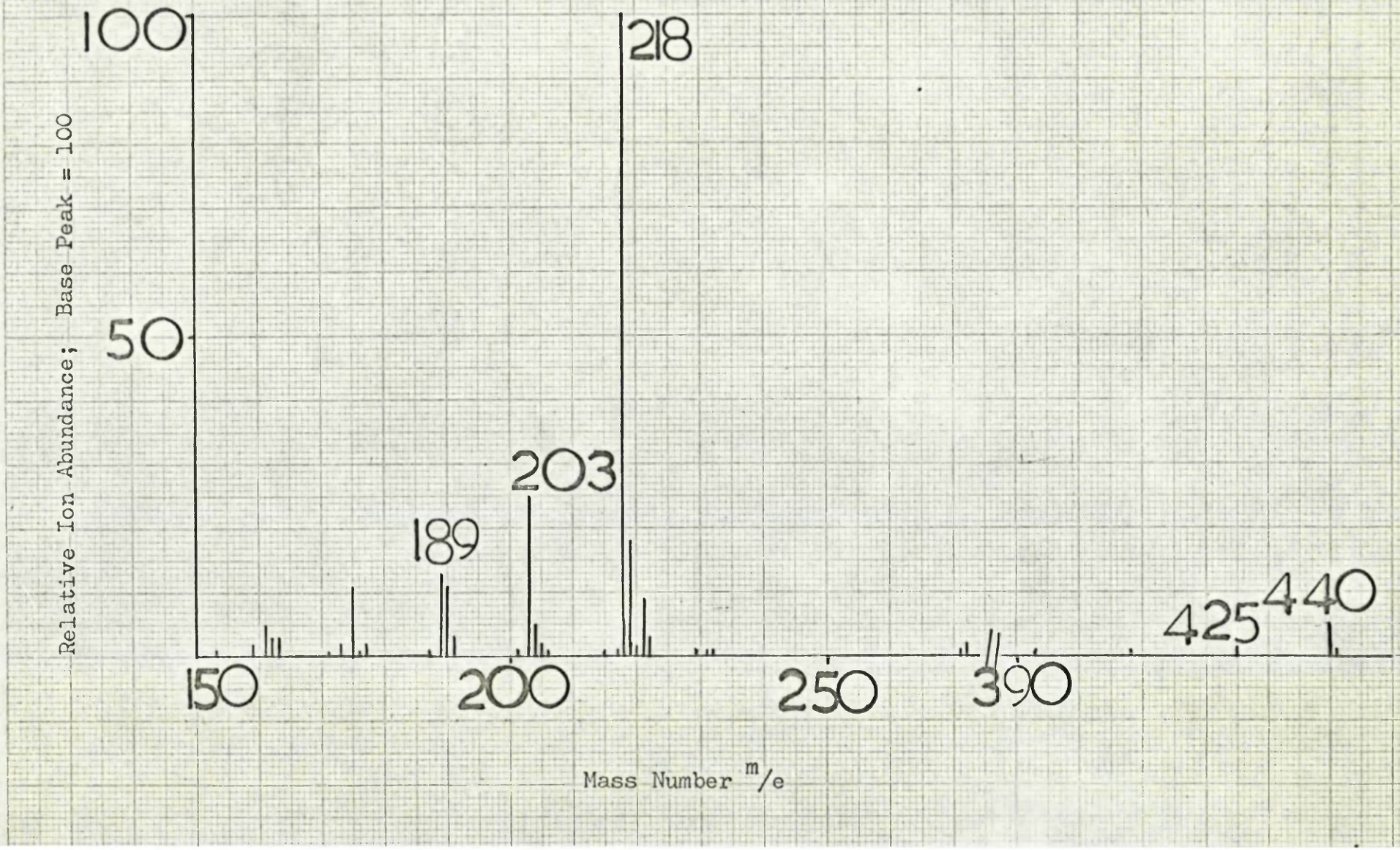


FIGURE 28

Line Diagram Of The Mass Spectrum Of Authentic Germanicol Methyl Ether Determined Using A Direct Inlet System.

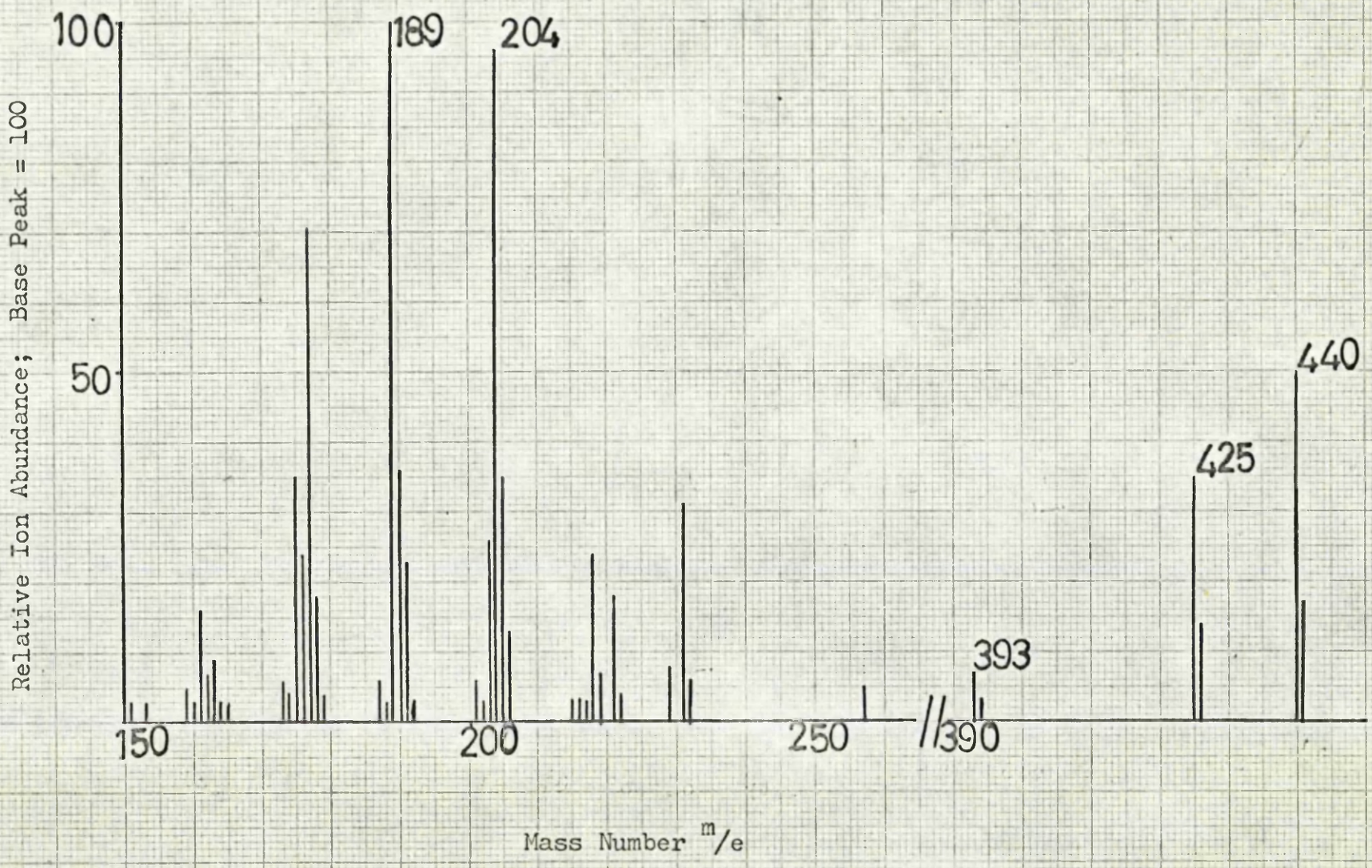


FIGURE 29

Line Diagram Of The Mass Spectrum Of Authentic Cylindrin Determined Using A Direct Inlet System.

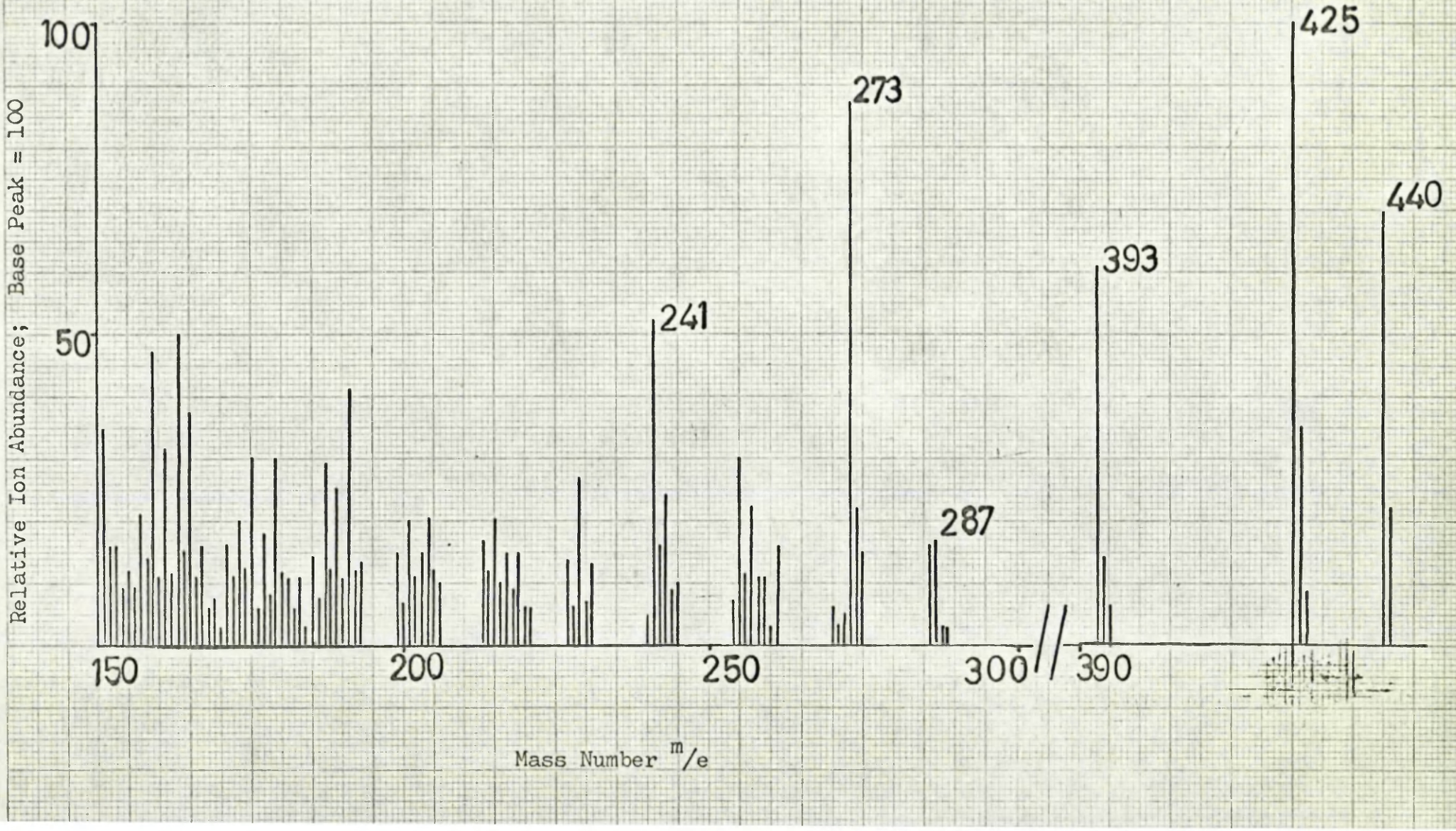
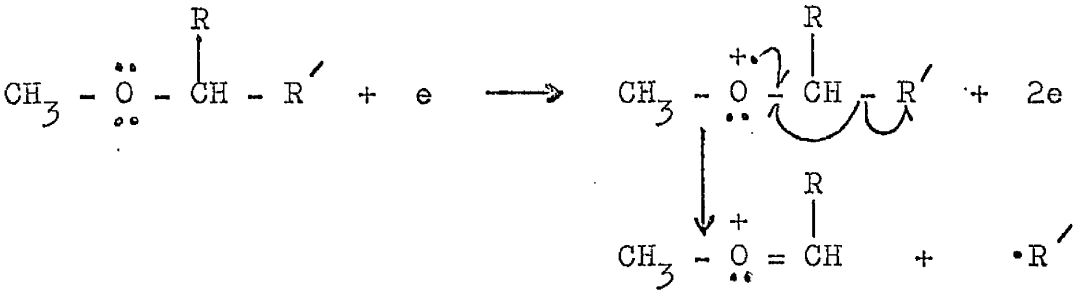


FIGURE 30

Characteristic Mass Spectral Fragmentation Of Simpler Ethers In Which One Of The Radicals Is Methyl.



where R' is a higher alkyl radical than R.

FIGURE 31

Characteristic Mass Spectral Fragmentation Of Symmetrical And Non-Branched Ethers.

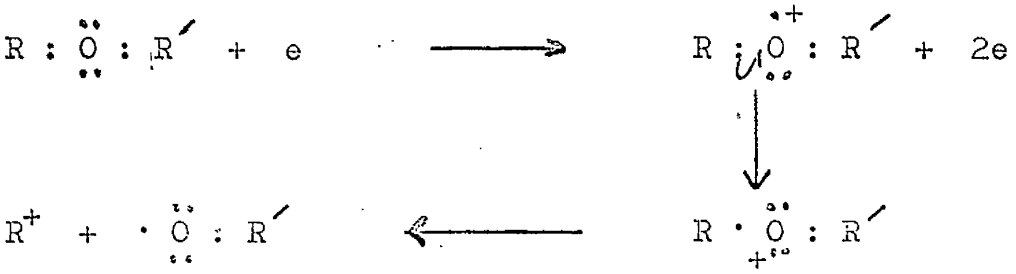


FIGURE 32

Characteristic Mass Spectral Fragmentation Of Branched Ethers Where R Is A Higher Alkyl Radical Than Methyl.

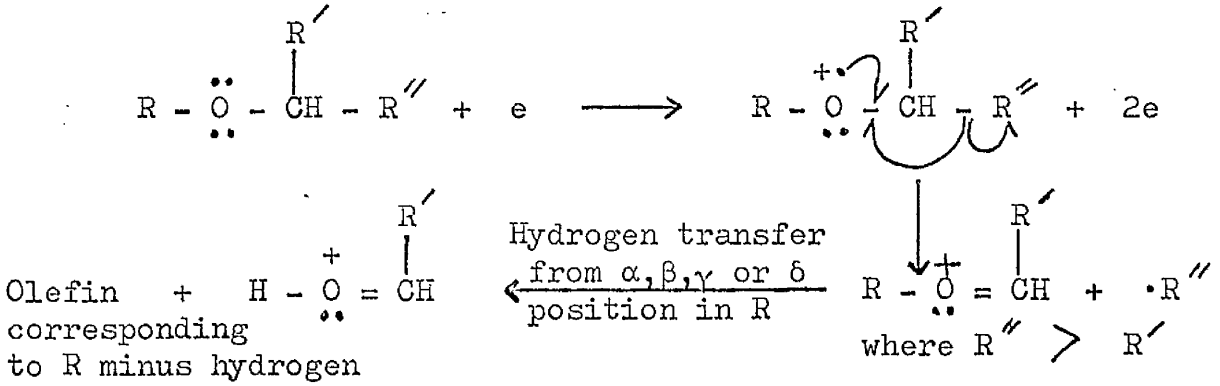


TABLE VII

Relative Abundance Of Parent -32 Ions In The Mass Spectra Of Simple Methyl Ethers. Adapted From McLafferty, Analytical Chemistry, 1957, 29, 1782.

Ether	Base Ion (Relative Abundance = 100)	Parent Ion	Parent Ion minus 32	
			m/e	Relative Abundance*
$CH_3COCH_2CH_3$	$CH_3\overset{+}{O}=CH_2$ m/e 45	60	28	8%
$CH_3COCH_2CH_2CH_3$	$CH_3\overset{+}{O}=CH_2$ m/e 45	74	42	2%
$CH_3COCH(CH_3)_2$	$CH_3\overset{+}{O}=CHCH_3$ m/e 59	74	42	9%
$CH_3COCH_2CH_2CH_2CH_3$	$CH_3\overset{+}{O}=CH_2$ m/e 45	88	56	20%
$CH_3COCH_2CH(CH_3)_2$	$CH_3\overset{+}{O}=CH_2$ m/e 45	88	56	4%
$CH_3COCH(CH_3)CH_2CH_3$	$CH_3\overset{+}{O}=CHCH_3$ m/e 59	88	56	4%
$CH_3COC(CH_3)_3$	$CH_3\overset{+}{O}=C(CH_3)_2$ m/e 73	88	56	4%

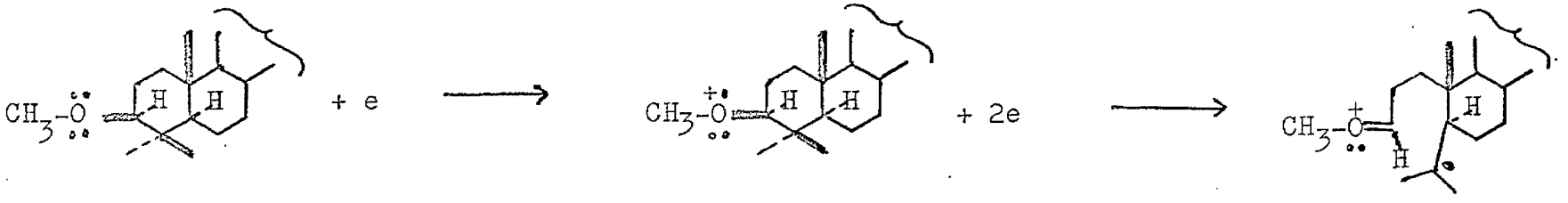
* The abundances quoted are not corrected for contributions from fragments of the same mass arising from other modes of breakdown.

[Fig. 25] metastable peaks are observed at 363.4 (calcd. for $\frac{m}{e}$ 425$\rightarrow\frac{m}{e}$ 393, $\frac{393^2}{425}$ = 363.4), at 255.2 (calcd. for $\frac{m}{e}$ 316$\rightarrow\frac{m}{e}$ 284, $\frac{284^2}{316}$ = 255.2) and at 240.4 (calcd. for $\frac{m}{e}$ 301$\rightarrow\frac{m}{e}$ 269, $\frac{269^2}{301}$ = 240.4). These metastable peaks, corresponding to loss of methanol, are included in the last two columns of Table VI.

This loss of methanol has analogy in the loss of a molecule of alcohol from certain higher molecular weight ethers in the mass spectrometer, as commented on by McLafferty[287]. Loss of methanol also appears to be a minor route of fragmentation of simple methyl ethers as is apparent from Table VII which has been constructed from the original data of McLafferty[287], although this author makes no comment on this process in his discussion. However, none of the triterpene methyl ethers studied followed the characteristic fragmentation pattern of simple methyl ethers [Fig. 30][287], which is itself different from the fragmentations undergone by other simple ethers[287,288] as seen from Fig. 31 and Fig. 32. Fragmentation of the triterpene methyl ethers according to the mechanism depicted in Fig. 30 would give rise to the species CXV shown in Fig. 33. This species, already bearing a positive charge, would then have to give rise to ions of the series $\frac{m}{2e}$ should it be formed prior to and in preference to the characteristic mass spectral fragmentation of the triterpene nucleus. Inspection of

FIGURE 33

Predicted Mass Spectral Behaviour Of The Methyl Ether System Of Triterpene Methyl Ethers Should This Be A Prime Site Of Electron Attack.



Interpretation Of The Observed Mass Spectral Cracking Pattern Of Arundoin (Based On Budzikiewicz, Wilson and Djerassi, J. Amer. Chem. Soc.,1963, 85, 3688).

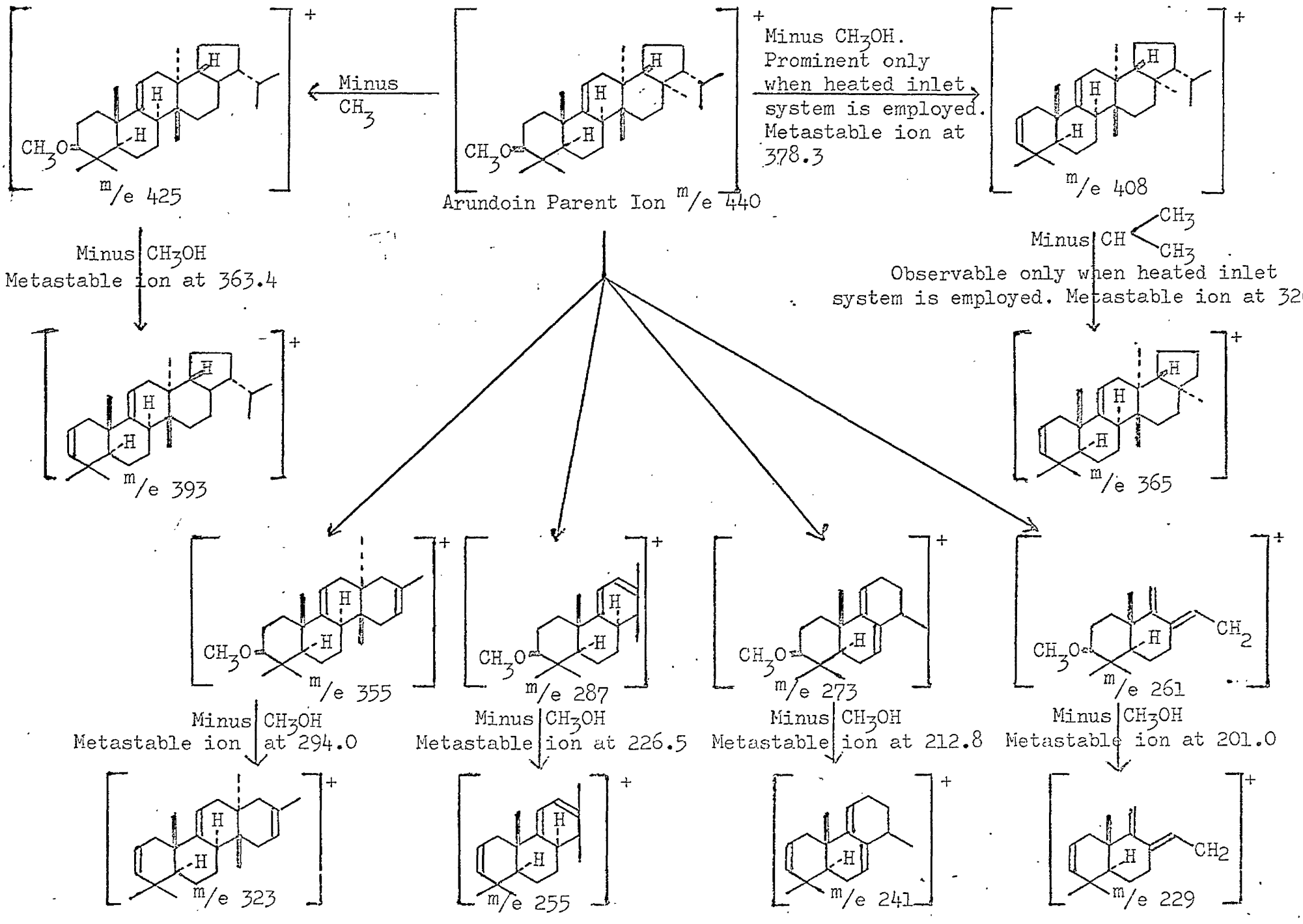
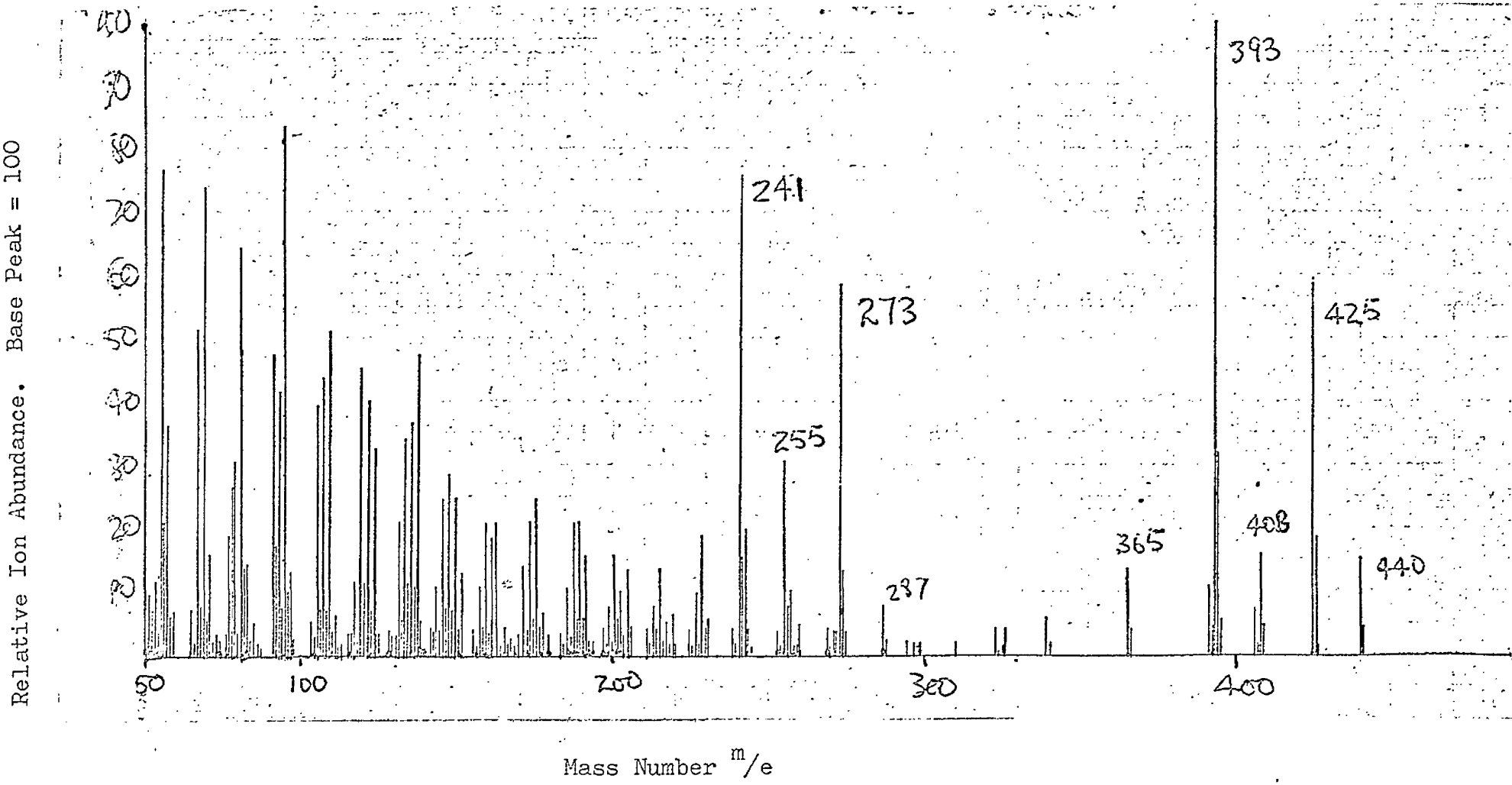


FIGURE 35

Line Diagram Of The Mass Spectrum Of Authentic Arundoin, Determined Using A Heated Inlet System.



the observed spectra shows that such ions are of extremely low abundance, if present at all, and so it can be concluded that the process shown in Fig. 33 is not at all favoured. Rather the mass spectral cracking must follow the established triterpene pattern[34,35] [as is also true for triterpene alcohols, which do not exhibit[34,35] the strong tendency for cleavage of the C —— COH bond which is observed with simple aliphatic alcohols[289]] with loss of neutral methanol from the various fundamental ions. The pattern for arundoin is shown in Fig. 34.

It is also of interest that the mass spectra of the triterpene methyl ethers revealed no additional peaks at parent ion plus one mass unit as has been reported as being characteristic of simple ethers[287]. The observed abundance of the ion of mass parent plus one at $\frac{m}{e}$ 441 can be fully accounted for in terms of the natural abundance of ^{13}C which for $C_{31}H_{52}O$ = 440 should give a peak at parent plus one of intensity ca. 34% that of the parent ion[290].

It is of considerable interest that when the mass spectrum of arundoin was determined using a heated inlet system [Fig. 35] in place of a direct inlet system [Fig. 23], loss of methanol became more pronounced. Thus a peak at $\frac{m}{e}$ 408 (parent minus 32 with an observed metastable ion at 378.3 - calculated 378.3) becomes pronounced whilst the ions at $\frac{m}{e}$ 393 ($\frac{m}{e}$ 425-32) $\frac{m}{e}$ 241 ($\frac{m}{e}$ 273-32) and $\frac{m}{e}$ 255 ($\frac{m}{e}$ 287-32) are more abundant than the ions from which they

FIGURE 36

N.M.R. Spectrum Of Arundoin As Determined In Saturated $CDCl_3$ Solution On A 40 Megacycle Instrument With Added Tetramethylsilane As Reference Standard.

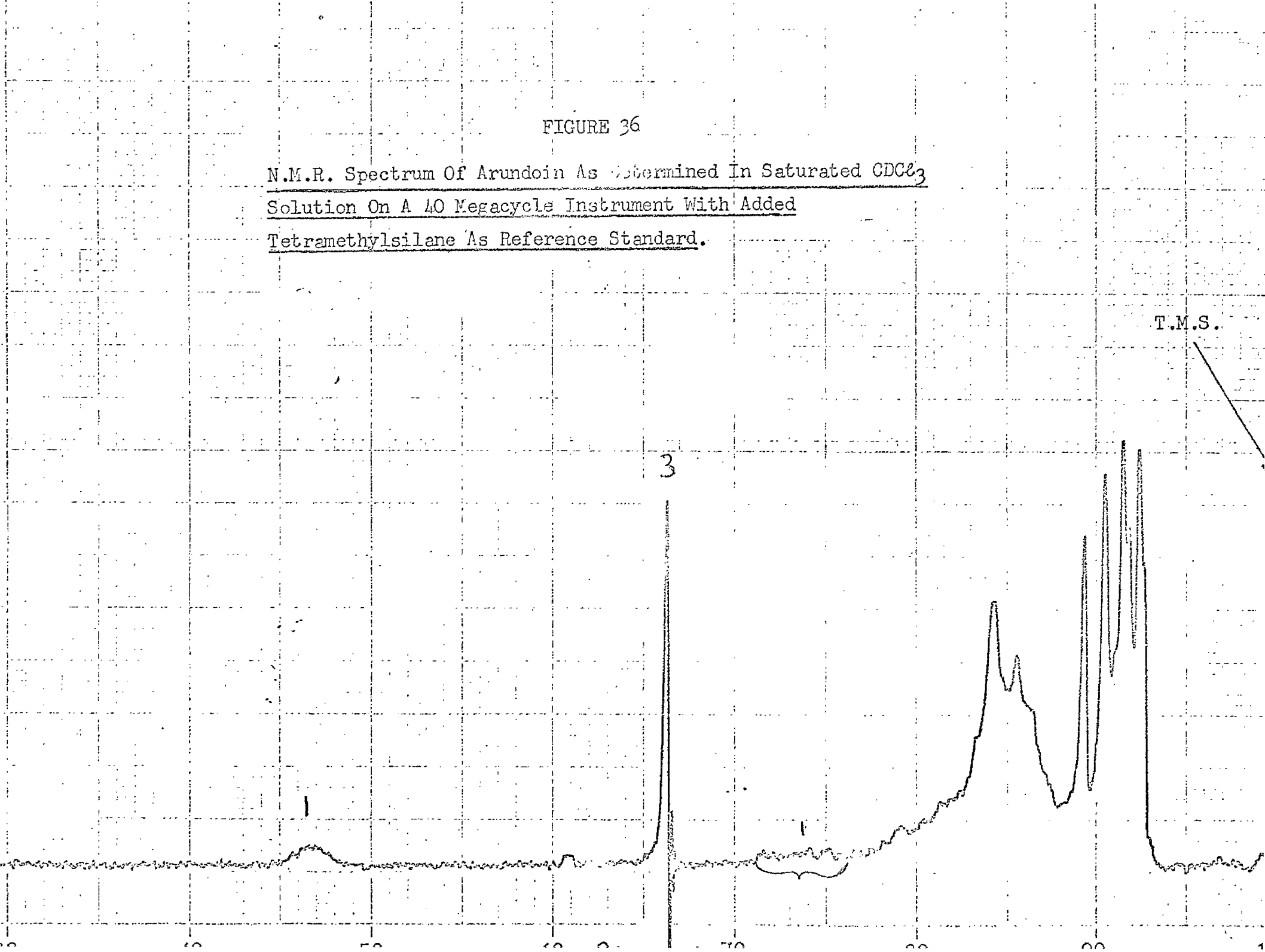


FIGURE 37

N.M.R. Spectrum Of α-Amyrin Methyl Ether As Determined In $CDCl_3$ Solution On A 40 Megacycle Instrument With Added Tetramethylsilane As Reference Standard.

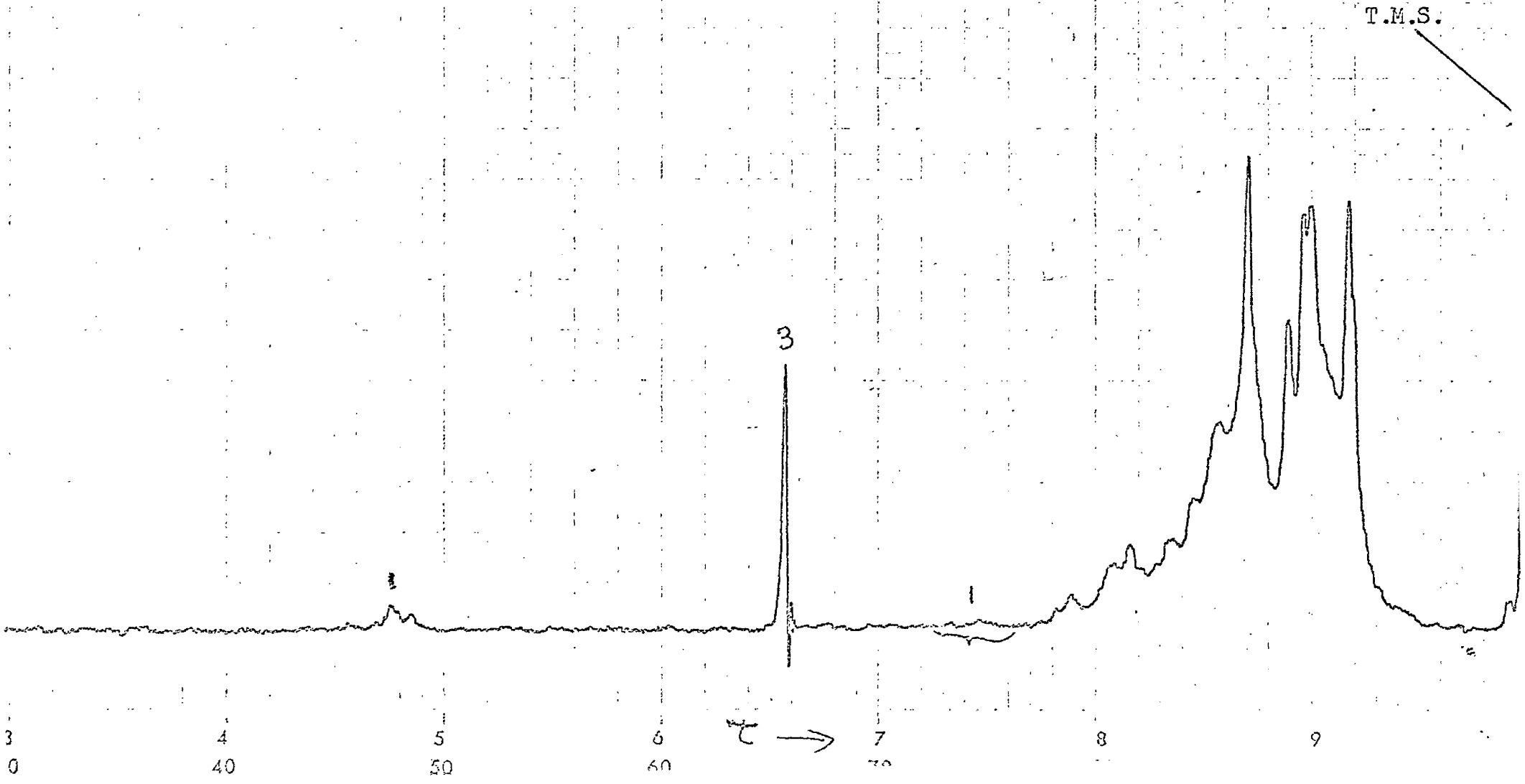


FIGURE 38

N.M.R. Spectrum of β-Amyrin Methyl Ether As Determined In $CDCl_3$ Solution On A 40 Megacycle Instrument With Added Tetramethylsilane As Reference Standard.

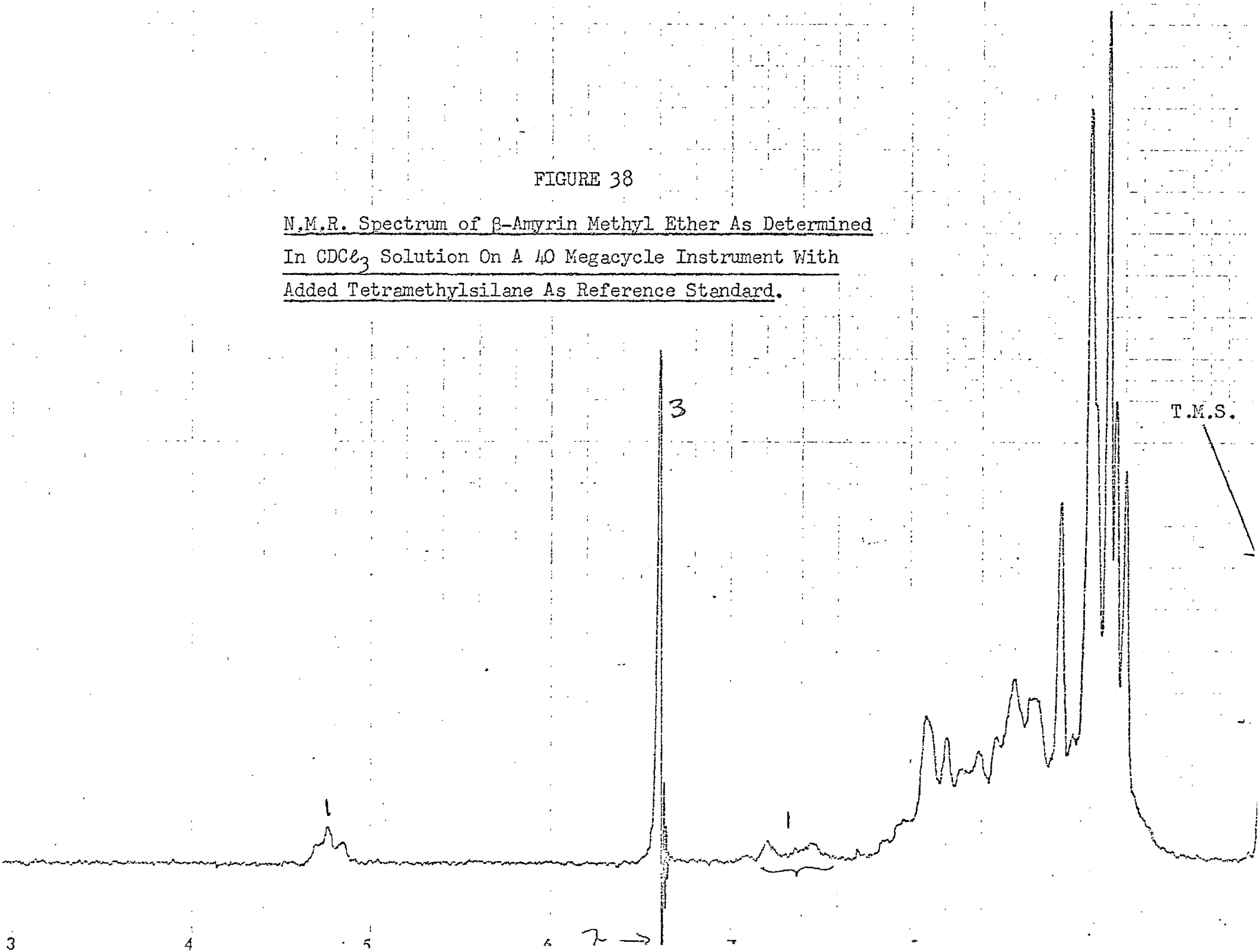
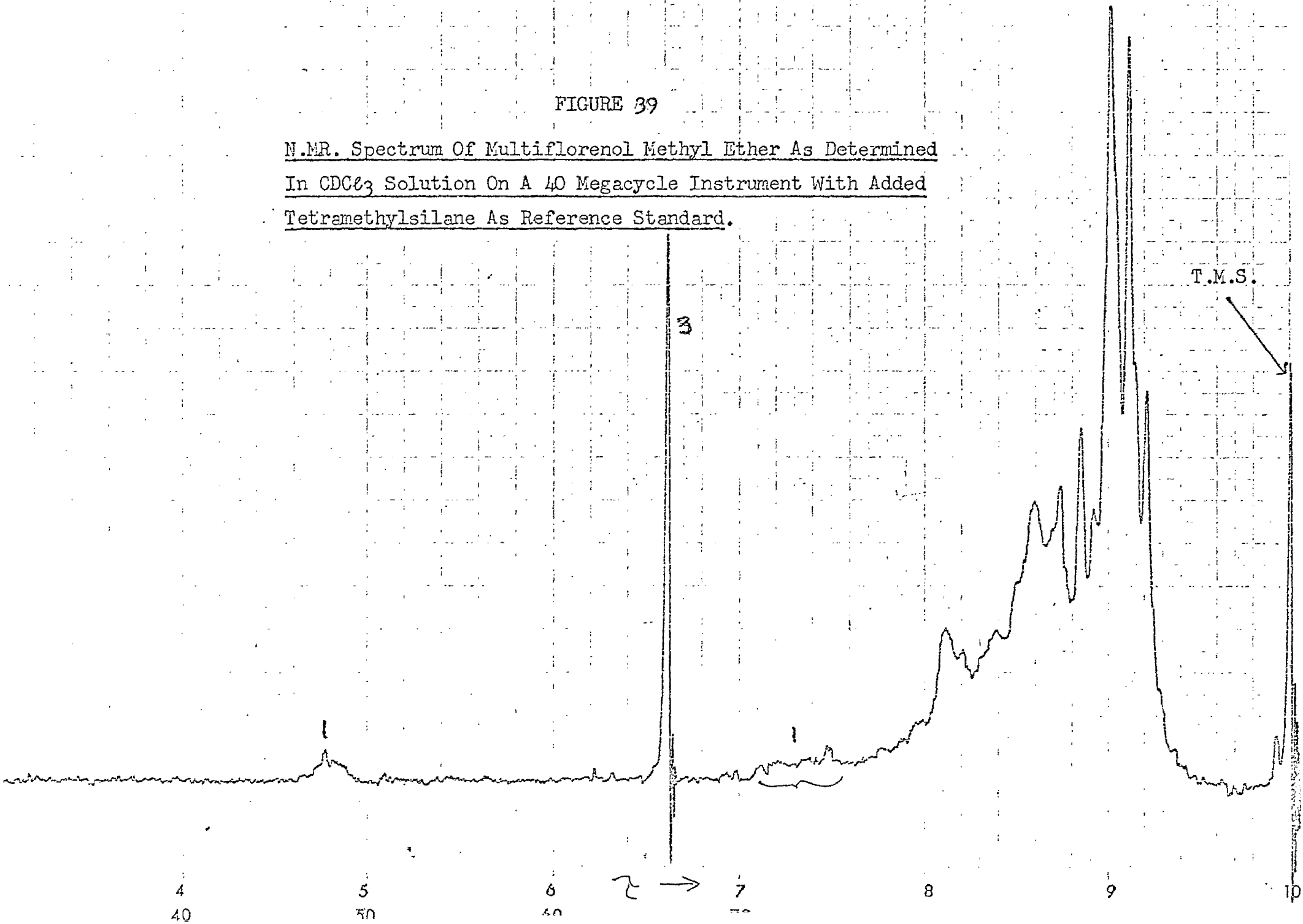


FIGURE 39

N.MR. Spectrum Of Multiflorenol Methyl Ether As Determined In $CDC\ell_3$ Solution On A 40 Megacycle Instrument With Added Tetramethylsilane As Reference Standard.



are derived through loss of methanol.

A further important fact is the appearance of an ion at $\frac{m}{e}$ 365 in the mass spectrum of arundoin when the heated inlet system is employed. That this peak, which is absent in the corresponding spectrum obtained using a direct inlet system [Fig. 23], is derived from loss of 43 mass units from the ion $\frac{m}{e}$ 408 is apparent from the existence of a metastable ion at 326.5 (calculated for $\frac{m}{e}$ 408 $\longrightarrow$ $\frac{m}{e}$ 365, $\frac{365^2}{408}$ = 326.5). This corresponds to the loss of the isopropyl side chain from ring E of arundoin, and its non-appearance in the mass spectrum of arundoin using a direct inlet system was one of several unfortunate factors which contributed to the wrong assignment of structure to arundoin[188], since it has been well established that loss of side chain is one of the characteristic fragmentations undergone by steroids and triterpenes in the mass spectrometer[291].

G. Remarks On The Nuclear Magnetic Resonance Spectra Of Triterpene Methyl Ethers

In the course of the present work the n.m.r. spectra of several of the triterpene methyl ethers were determined in $CDCl_3$ using a Perkin Elmer 40 megacycle instrument. These n.m.r. spectra are shown as Figs. 36-39.

The resolution with the 40 megacycle instrument unfortunately does not permit detailed assignment of the methyl proton absorptions and this shortcoming, like the failure of the mass spectrum using a direct inlet system to reveal

FIGURE 40

N.M.R. Spectrum Of α-Amyrin As Determined In $CDCl_3$ Solution On A 40 Megacycle Instrument With Added Tetramethylsilane As Reference Standard.

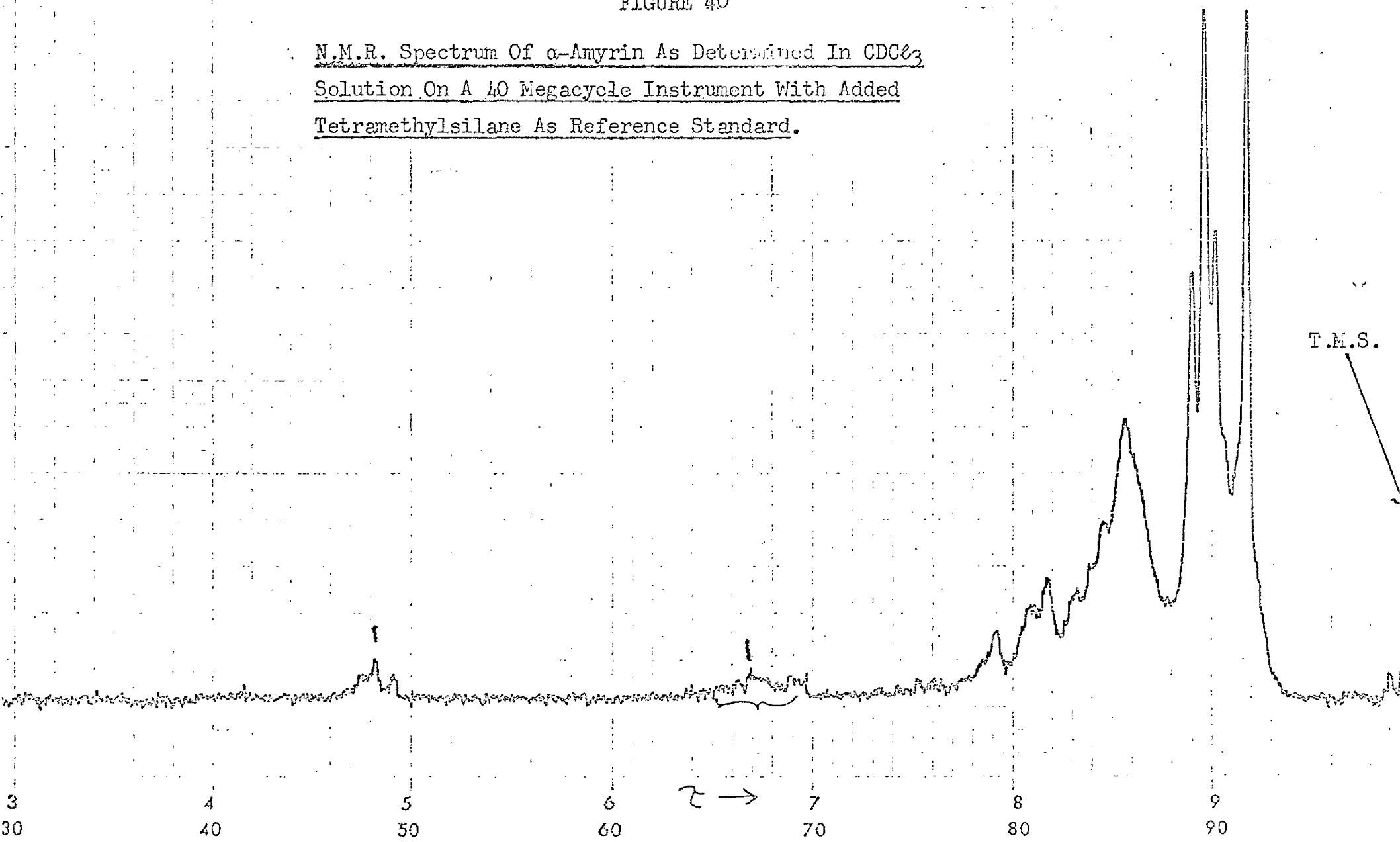
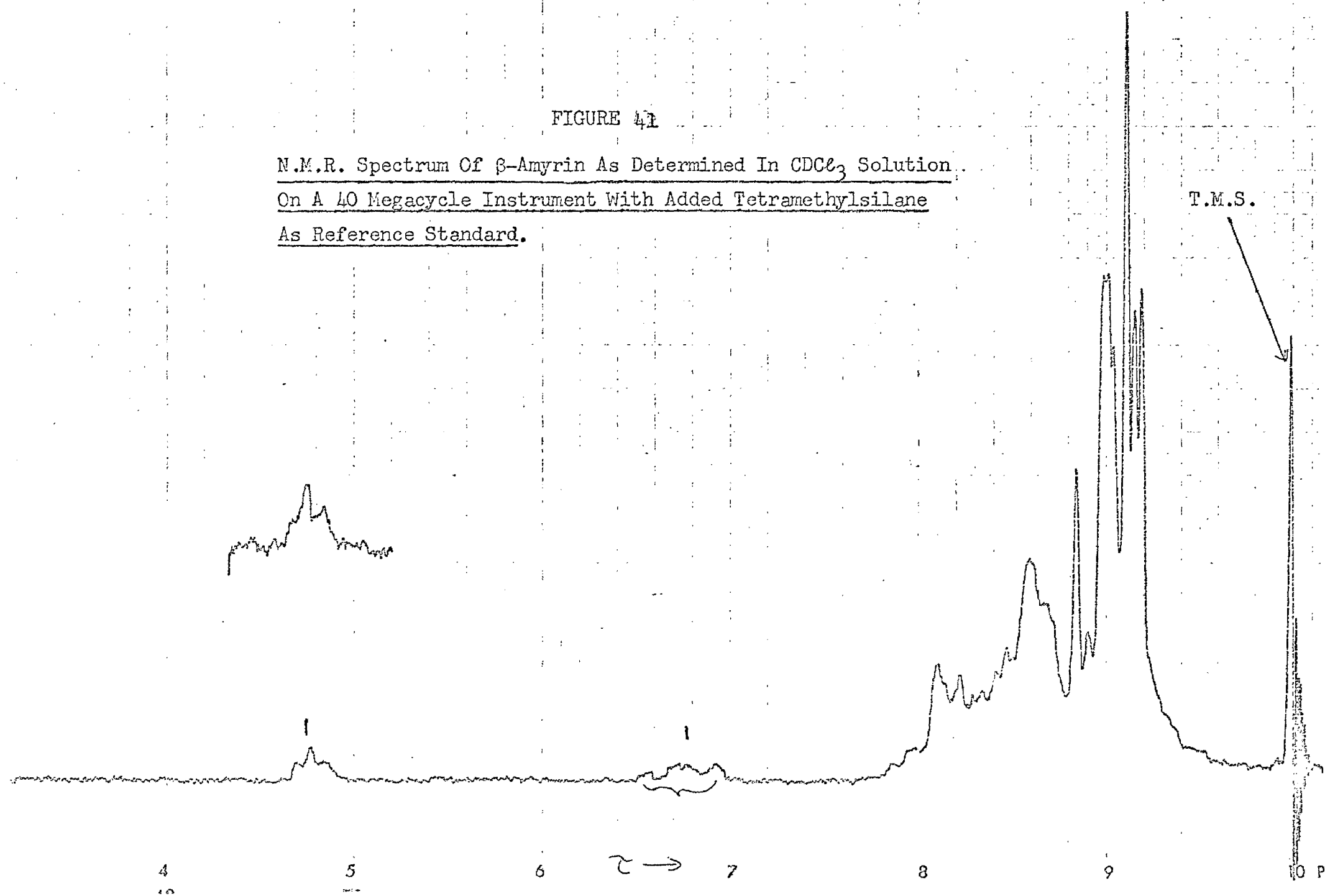


FIGURE 41

N.M.R. Spectrum Of β-Amyrin As Determined In $CDCl_3$ Solution On A 40 Megacycle Instrument With Added Tetramethylsilane As Reference Standard.



the presence of the isopropyl group in arundoin, was partially responsible for the incorrect deductions[188] as to the structure of arundoin.

Nevertheless an interesting aspect of the n.m.r. spectra shown in Figs. 36-39 is the high field at which the signal from the 3α proton occurs. In the original interpretation of the n.m.r. spectrum of arundoin[188], the absorption at 6.62 τ was concluded to have an intensity of 4 protons, but careful examination of the n.m.r. spectra of arundoin and the methyl ethers of α-amyrin, β-amyrin, bauerenol and multiflorenol shows that the absorption at ca. 6.62 τ is of intensity 3 protons and so attributable to the $O-CH_3$ group, whilst the low diffuse absorption, intensity 1 proton, centred at ca. 7.4 τ and ca. 30 c.p.s. broad must be arising from the O-CH proton. This same high field absorption by the 3α proton has also been observed in arundoin and cylindrin by Dr. Natori[292] who employed a 100 megacycle instrument with which he was also able to conclusively demonstrate the doublets in the spectrum of arundoin due to the isopropyl methyl groups. The absorption of the 3α proton in triterpene methyl ethers is certainly at higher field than might have been anticipated from the diffuse low intensity axial C-3 proton absorption centred at 6.8 τ and 25 c.p.s. broad found in α-amyrin [Fig. 40] and β-amyrin [Fig. 41] and the similar low intensity C-3 proton absorption centred at ca. 5.6 τ and 30 c.p.s. broad found in 3β-acetoxytriterpenes[293], and it must be

attributed to a high degree of shielding by the methoxyl group.

The signal from the single vinylic proton present in arundoin and the methyl ethers of α-amyrin, β-amyrin, bauerenol and multiflorenol is clearly distinguishable as a multiplet at ca. 4.7-4.8 τ, whilst the corresponding vinylic proton absorption of α-amyrin and β-amyrin occurs at ca. 4.8 τ.

EXPERIMENTAL

Materials and Methods

Mass Spectrometry. All the mass spectra were determined with an A.E.I. M.S.9 double-focusing mass spectrometer using a direct inlet system, except in the case of arundoin where the mass spectrum was determined both with the direct inlet system and with a heated inlet system. The energy of ionising electrons was 70 V, the ionising current was 100μA and the source temperature was 150 °C.

Gas Liquid Chromatography. The instrument employed in the analytical gas liquid chromatographic studies was a standard Pye Panchromatograph, giving preheating of the argon carrier gas and fitted with standard glass tubes, containing the column packing, of 5 feet in length and internal diameter ca $\frac{3}{16}$ inch. The detector was the standard Lovelock argon ionisation type, fitted with a ^{90}Sr source and the current from the detector was fed into a Honeywell Brown [Newhouse, Lanarkshire, Scotland] pen recorder with sensitivity 0-10mV.

Direct injections (0.2-0.3μl. of a chloroform solution of the compounds under investigation) were made on to the column through a silicone-rubber 'blind hole' stopper with a 1μl. syringe [Hamilton Co. Inc. Whittier, Calif., U.S.A.]. Standard conditions were as follows: column temperature, 240 ± 1 °; detector temperature, 248 ± 1 °; argon flow rate,

60 ml/min. at outlet (inlet pressure 10-12 lb./in.^{2}); nominal detector voltage, 1000 V; sensitivity setting, $1X10^{-8}$ amp. However different column temperatures were employed, where appropriate, e.g. 175° for the polyethylene glycol adipate columns [see Tables II and III] and 225° for the QF-1 column [see Table IV].

The instruments employed in the preparative gas liquid chromatographic work were an Aerograph - A.90P$_3$ [Wilkens Instrument and Research Inc., Walnut Creek, California] using helium as the carrier gas and fitted with standard copper tubes, containing the column packing, of 10 feet in length and internal diameter ca $\frac{1}{4}$ inch. and an Aerograph A.700 [Wilkens Instrument and Research Inc., Walnut Creek, California] using helium as the carrier gas and fitted with standard copper tubes, containing the column packing of 6 feet in length and internal diameter ca $\frac{1}{4}$ inch. The detector used with both instruments was of the thermal conductivity type and the current from the detector was fed into either a Kent Mark 3 recorder with sensitivity 0-10mV or into a Honeywell Brown [Newhouse, Lanarkshire, Scotland] pen recorder with sensitivity 0-10mV.

Direct injections [15 to 20 µl.] of a chloroform solution of the compounds under investigation were made on to the column through a silicone-rubber 'blind hole' stopper with a 50 µl. syringe [Hamilton Co., Inc., Whittier, California, U.S.A.]. Standard conditions were as follows:

column temperature, 280 $\pm$ 1° [Aerograph A.90. P_3] or 240 $\pm$ 1° [Aerograph A. 700]; detector temperature 315 $\pm$ 1° [Aerograph A.90. P_3] or 280 $\pm$ 1° [Aerograph A. 700]; helium gas flow rate, 100 ml/min. [Aerograph A. 90 P_3] or 60 ml/min. [Aerograph A. 700] at outlet; filament current 195 milliamps [Aerograph A. 90. P_3] or 150 [Aerograph A. 700], attenuation 32.

Preparation of columns. Column packings for the Pye Panchromatograph were prepared on the silane-treated support, Gas-Chrom Z [Applied Science Laboratories Inc., State College, Pennsylvania, U.S.A.] of 100-120 mesh. The coating with stationary phase was achieved by weighing out the required quantity of the desired stationary phase, viz. Apiezon L grease [Edwards High Vacuum Ltd., Manor Royal, Crawley, Sussex, U.K.]; silicone polymer, SE-30, [General Electric Co., Schenectady, N.Y., U.S.A.]; fluorosilicone polymer, QF-1 (FS-1265), [Wilkens Instrument and Research Inc., Walnut Creek, California, U.S.A.]; cyclohexane dimethanol succinate, CDMS [Applied Science Laboratories Inc., State College, Pennsylvania, U.S.A.]; or polyethyleneglycol adipate, PEGA, [Pye Instruments Ltd., Cambridge, U.K.], dissolving in AnalaR chloroform and adding the correctly weighed quantity of support to the solution so obtained. The chloroform was then removed by distillation *in vacuo* at 100°C. with the minimum of agitation and the coated supporting phase further

dried for 1 hour *in vacuo* at 100°C. Column packings so prepared contained 0.5% ($\frac{W}{V}$) Apiezon L, 1.5% ($\frac{W}{V}$) SE-30, 1.5% ($\frac{W}{V}$) QF-1, 1.0% ($\frac{W}{V}$) CDMS and 10% ($\frac{W}{V}$) PEGA.

The glass tubes were then filled with the required column packing with repeated gentle tapping. Before any freshly packed column was used for chromatography it was stabilised by heating at 250° for 24 hours in a slow stream of argon.

Column packings for the Aerograph-A.90.P_3 and the Aerograph A.700 were prepared on the silane-treated support Gas-Chrom Z [Applied Science Laboratories Inc., State College, Pennsylvania, U.S.A.] of 100-120 mesh. The coating of the stationary phase was achieved by suspending the correctly weighed quantity of support in 100 ml of 3% SE-30 or 1% Apiezon L in toluene and applying a gentle vacuum to remove occluded air. After 15-20 minutes the suspension was poured into a Buchner funnel with gentle suction - the vacuum being released as soon as filtrate ceased to flow. The moist support was transferred to a filter paper, and after air-drying it was dried in an oven at 80° for 6 hours.

The copper column used for the packing was treated with dichlorodimethylsilane in toluene, and then washed well with toluene and methanol and dried before use. The column was packed by gradual addition of the coated support and repeated tapping. Columns thus prepared were coiled and stabilised before use, by heating at 300° for 24 hours in a

slow stream of argon.

Determination of retention data. Measurements of retention times on the Pye Panchromatograph were made between the first displacement of the recorder pen after the injection and the point corresponding to the peak of the response to the compound concerned. The recorded response to the injection was observed 20-25 sec. after the moment of injection and coincided with the return of the outlet flow rate from an elevated level [due to the pressure wave from the evaporation of chloroform] to 60 ml./min. 5α-Cholestane was included in most solutions used to measure retention times and these were expressed as ratios relative to 5α-cholestane, but in a number of experiments arundoin was employed as a secondary reference standard. Good agreement between the relative retention times determined with each standard was obtained.

Evaluation of Probable Experimental Error in the Determination of Relative Retention Times.

In order to assess the reproducibility of the retention times it is necessary to consider the probable experimental error involved in making the measurements on the g.l.c. trace.

It is estimated that the absolute error in measuring the distance between the first displacement of the recorder pen [due to the pressure wave from the evaporation of chloroform on injection of the solution] and the peak of the response to the compound concerned is ± 0.05 cm - a value largely

determined by the width of the trace line. Selected examples from actual data can then be chosen to afford an illustration of the errors to be expected in the retention times as determined relative to that of 5α-cholestane. The absolute error [at the constant chart speed of 12'' per hour which was routinely employed] will be the same with all stationary phases, but the %age error will be highest for low absolute retention times [i.e. with the QF-1 and CDMS columns] and lowest for high absolute retention times [i.e. with the Apiezon L columns]. The following calculations serve to afford representative indications of the expected %age errors.

(a) For cylindrin on an Apiezon L column:

In one experiment the distances between the first displacement of the recorder pen and the peak of the responses were:

5α-cholestane	5.60 cm.
cylindrin	28.00 cm.

so that these distances could be in fact 5.60 ± 0.05 cm. and 28.00 ± 0.05 cm. Hence extreme limits for the actual retention time of cylindrin relative to 5α-cholestane are $\frac{28.00 + 0.05}{5.60 - 0.05}$ and $\frac{28.00 - 0.05}{5.60 + 0.05}$ viz. 5.06 and 4.94. This gives a percentage error of $\frac{0.06}{5.00} \times 100 = 1.2\%$.

(b) For taraxerol methyl ether on an Apiezon L column:

In one experiment the distances between the first

displacement of the recorder pen and the peak of the responses were:

5α-cholestane	5.85 cm.
taraxerol methyl ether	15.90 cm.

so that the distances could be in fact 5.85 $\pm$ 0.05 cm. and 15.90 $\pm$ 0.05 cm. Hence extreme limits for the actual retention time of taraxerol methyl ether relative to 5α-cholestane are $\frac{15.90 + 0.05}{5.85 - 0.05}$ and $\frac{15.90 - 0.05}{5.85 + 0.05}$ viz. 2.76 and 2.68.

This gives a percentage error of $\frac{0.04}{2.72} \times 100 = 1.45\%$.

(c) For α-amyrin methyl ether on an SE-30 column:

In one experiment the distances between the first displacement of the recorder pen and the peak of the responses were:

5α-cholestane	1.70 cm.
α-amyrin methyl ether	4.65 cm.

so that these distances could be in fact 1.70 $\pm$ 0.05 cm. and 4.65 $\pm$ 0.05 cm. Hence extreme limits for the actual retention time of α-amyrin methyl ether relative to 5α-cholestane are $\frac{4.65 + 0.05}{1.70 - 0.05}$ and $\frac{4.65 - 0.05}{1.70 + 0.05}$ viz. 2.84 and 2.62. This gives a percentage error of $\frac{0.11}{2.73} \times \frac{100}{1} = 4.0\%$

(d) For β-amyrin methyl ether on a QF-1 column:

In one experiment the distances between the first displacement of the recorder pen and the peak of the responses were:

5α-cholestane	1.15 cm.
β-amyrin methyl ether	3.35 cm.

so that these distances could be in fact 1.15 ± 0.05 cm. and 3.35 ± 0.05 cm. Hence extreme limits for the actual retention time of β-amyrin methyl ether relative to 5α-cholestane are $\frac{3.35 + 0.05}{1.15 - 0.05}$ and $\frac{3.35 - 0.05}{1.15 + 0.05}$ viz. 3.09 and 2.75. This gives a percentage error of $\frac{0.17}{2.92} \times 100 = 5.8\%$.

It is thus seen that the estimated experimental error in determining the relative retention time increases from 1.2% for the longest retention time [cylindrin] to 1.45% for the shortest retention time [taraxerol methyl ether] on the Apiezon L column and that the experimental error increases still further to a maximum value of 5.8% for the shorter retention times [e.g. β-amyrin methyl ether] on the QF-1 columns.

The largest absolute error of ± 0.17 is observed with the QF-1 column.

However, the fact that all the retention times shown in Table IV are the mean of at least three determinations, with some values [particularly those on the Apiezon L and SE-30 columns] being the means of as many as ten determinations, means the actual absolute error should be considerably less than the maximum of ± 0.17. Indeed the self consistency of the actual results allows the limits of ± 0.10 to be set [as

indicated in the footnote to Table IV].

Reproducibility of data.

The reproducibility of the relative retention times of the nine triterpene methyl ethers on Apiezon L and SE-30 columns was tested by performing separate determinations on different freshly prepared columns after an interval of three months. The results were within the estimated experimental error, as calculated above.

Efficiency of columns.

The efficiency of all columns was somewhat less than would be desired, with the Apiezon L column being by far the best. Employing the standard formula[294] of $\frac{16y^2}{x^2}$ for calculation of theoretical plates [where x is the base width of the peak and y is the retention value measured in the same units as x], the efficiency of the various columns in terms of theoretical plates was as follows:

Compound	0.5% Apiezon L	1.5% SE-30	1.5% QF-1	1% CDMS
5α-cholestane	2160	408	115	115
β-amyrin methyl ether	2544	484	184	108
α-amyrin methyl ether	2304	449	116	195
arundoin	2272	449	154	193

Triterpene Methyl Ethers.

The cylindrin employed in the gas liquid chromatographic

studies was kindly provided by Dr. S. Natori, National Institute of Hygenic Sciences, Tokyo, Japan, to whom the present author wishes to express his appreciation. He also wishes to cordially thank Dr. S. Abe, Yamazaki Works, Japan for gifts of miliacin, isomiliacin, sawamilletin and isosawamilletin, and Dr. C.J.W. Brooks of the University of Glasgow for a gift of taraxerol.

The methyl ethers of multiflorenol and bauerenol were prepared from multiflorenol and bauerenol [isolated from the bark of Gelonium multiflorum A. Juss by the procedure of Sengupta and Khastgir[142]] by the same general procedure as was used in the preparation of the methyl ethers of taraxerol, β-amyrin and α-amyrin, through adaptation of the method of Morice and Simpson[31].

Triterpene alcohol (400 mg) and potassium sand (400 mg) were stirred in dry benzene (5 ml) at room temperature under nitrogen for 3 hours. Methyl iodide (1 ml) in dry benzene (2 ml) was added every 2 hours with refluxing for 12 hours. Methyl alcohol was added to decompose unreacted potassium, water added and the benzene layer separated and washed with water. The solid residue obtained from the organic layer, on removal of the solvent, was subjected to infrared spectral analysis in carbon tetrachloride solution, to ensure the absence of hydroxyl absorption, and then crystallised from ethyl acetate to constant melting point.

In this way were prepared in ca 90% yield:-

Multiflorenol methyl ether, m.p. 190-193°, $[\alpha]_D$ = -32 (c = 1.9 in $CHCl_3$), ε 4,300 at 205 mμ. Found: C, 84.4; H, 11.4. $C_{31}H_{52}O$ requires C, 84.5; H, 11.9%.

Bauerenol methyl ether, m.p. 212-215°, $[\alpha]_D$ = -32 (c = 1.2 in $CHCl_3$), ε 4,100 at 205 mμ. Found: C, 84.6 H, 11.9. $C_{31}H_{52}O$ requires C, 84.5; H, 11.9%

Methyl ether of α-amyrin, m.p. 221-223°, $[\alpha]_D$ = +92 (c =2.0 in $CHCl_3$). Literature [31], m.p. 221-222°, $[\alpha]_D$ = +93 (in $CHCl_3$).

Methyl ether of β-amyrin, m.p. 247-248°, $[\alpha]_D$ = +98 (c = 2.0 in $CHCl_3$). Literature [31,282], m.p. 247-248°, $[\alpha]_D$ = +98 (in $CHCl_3$). Taraxerol methyl ether, m.p. 276-278°. Literature [282,284] m.p. 278°.

Isolation of Surface Waxes

Fresh leaves [or rhizomes] of the particular grass under investigation, in varying quantities as available, were cut into 10 inch lengths and immersed in redistilled light petroleum of b.p. 40-60° for 16 hours at room temperature. The light petroleum extractives were then obtained by decantation and removal of the solvent under reduced pressure on a rotary film evaporator. In this way total light petroleum extractives were obtained as pale yellow waxes from the following:-

Cortaderia toetoe	G3793,	Green Leaves,	(4 lbs)	10.5g
Cortaderia fulvida	G3794,	Green Leaves,	(4 lbs)	8.5g
Cortaderia richardii	G3817,	Green Leaves,	(4 lbs)	7.5g
Cortaderia selloana	TAITAPU,	Green Leaves,	(4 lbs)	7.0g
Cortaderia atacamensis	G4786,	Green Leaves,	(4 lbs)	6.0g
Cortaderia, Plimmerton species,		Green Leaves,	(325 lbs)	350g
Cortaderia, Plimmerton species,		Fresh Rhizomes,	(2 lbs)	3.2g
Poa anceps		Green Leaves,	($2\frac{1}{2}$ lbs)	3.5g

Isolation of The Alkane Fractions

In all cases the following procedure was followed:

Total light petroleum extractives (1.0g) from the particular grass under investigation were refluxed with an excess of 2,4-dinitrophenylhydrazine (1.0 g) and conc. HCl (0.5 ml) in ethanol (20ml) for 2 hr. to convert carbonyl compounds into 2,4-dinitrophenylhydrazones. The solvent was then removed under reduced pressure and the residue exhaustively extracted with redistilled light petroleum of b.p. 40-60°. After removal of the solvent, the light petroleum-soluble material was refluxed for 2 hr. in aqueous ethanol (1:2, 20 ml) containing NaOH (1.0 g). The solution was taken to dryness under reduced pressure and the residue thoroughly extracted with redistilled light petroleum of b.p. 40-60°. The resulting solution was then chromatographed over basic alumina (Woelm grade I, 5 g) and the hydrocarbon fraction completely eluted with further redistilled light petroleum of b.p. 40-60°. The residue obtained on removal of the solvent was subjected to infrared analysis in KCl disc and if no absorption other than that due to alkanes was observed the sample was submitted directly to gas liquid

chromatographic analysis on a 0.5% Apiezon L column on the Pye Panchromatograph under the standard conditions described on page 137.

Where ether absorption at 1104 cm^{-1} was present in the hydrocarbon fraction [viz. with Cortaderia toetoe, Cortaderia fulvida, Cortaderia richardii and the Plimmerton Cortaderia species], the alkane containing fraction was treated with conc H_2SO_4 (5 ml) at 140° for 4 hr before being taken up in redistilled light petroleum of b.p 40-60° and rechromatographed prior to gas liquid chromatographic analysis.

After a satisfactory g.l.c. trace had been obtained for the alkane mixture from the particular grass under investigation, authentic n-nonacosane was added to a sample of the natural mixture and a second g.l.c. trace obtained with the new mixture, with identification of the peak which was intensified with respect to the first trace. Repetition with addition of authentic n-untriacontane in place of the n-nonacosane permitted identification of the peak due to the C_{31} n-alkane.

A plot was then made of log retention time against carbon atom number [using the two identified peaks] and the remaining n-alkanes of the natural mixture identified from their positions on this plot, which was a straight line.

Integration of the areas under each peak then permitted determination of the mole percentages of the individual alkanes, which are given in Table I.

No extraneous peaks were observed on any of the n-alkane plots, thus showing the absence of iso- or anteiso alkanes.

Analysis of Fatty Acids and n-Alkanols In Surface Wax Components

Total light petroleum extractives (1g) from the particular grass under investigation were refluxed for 2 hr in aqueous ethanol (1:2, 20 ml) containing NaOH (3g). The solution was taken to dryness and thoroughly extracted with dry ether. The combined ethereal solutions were then taken to dryness and the residue refluxed in acetic anhydride (5 ml) for 4 hr. to convert the constituent alcohols into the corresponding acetates. The reaction mixture was allowed to cool to room temperature, water (20 ml) added and the solution left to stand for 24 hr. to hydrolyse the excess of acetic anhydride. The mixture was then carefully neutralised with sodium bicarbonate solution and extracted with ether. Removal of solvent from the resulting ethereal solution afforded the mixed acetates ready for g.l.c. analysis.

The ether-insoluble residue resulting from the aqueous ethanolic saponification of the light petroleum extractives of each grass was taken up in water (30 ml), the solution acidified to liberate the free carboxylic acids from their sodium salts, and extracted with ether to permit isolation of the acids. After removal of solvent from the ethereal solution the residual material was dissolved in methanol and

the resulting solution treated with an excess of an ethereal solution of diazomethane. Removal of solvents under reduced pressure then afforded the corresponding methyl esters ready for g.l.c. analysis.

The g.l.c. analysis of both the alcohol acetates and the methyl esters of the acids were conducted on 10% PEGA columns on the Pye Panchromatograph at 175° under the standard conditions described on page 166. Authentic n-hexyl acetate, n-octyl acetate and n-decyl acetate were employed to aid identification of the unknown acetates, through intensification experiments analogous to those described for the g.l.c. alkane analyses described above. Similarly authentic methyl laurate, methyl palmitate, methyl stearate and methyl oleate were employed in analogous fashion to aid identification of the unknown methyl esters.

For the alcohol acetates, all the peaks fell on the one straight line when log retention time was plotted against carbon atom number, whilst the only peak not falling on the one straight line for the analogous plot for the methyl esters was that identified as methyl oleate by addition of authentic material.

Integration of the areas under the peaks [using a gravimetric procedure] afforded the %age of each component present. The results are given in Tables II and III.

Isolation of Triterpene Methyl Ethers

Total light petroleum extractives (2.0g) from the

particular grass under investigation, were dissolved in light petroleum of b.p 40-60° and chromatographed over neutral alumina (Woelm, grade I, 50 g), using further light petroleum of b.p 40-60° to develop the column. The initial eluants contained mainly paraffins, but subsequent fractions yielded crystalline material which proved to be the triterpene methyl ethers.

In the case of Cortaderia fulvida and Cortaderia richardii the total triterpene methyl ether fraction, on g.l.c. employing a 0.5% Apiezon L column, showed a single peak corresponding in retention time to that of arundoin. On recrystallisation from ethyl acetate both samples showed a m.p. of 235-237° and gave no mixed melting point depression with authentic arundoin. The infrared spectra were identical.

In the case of Cortaderia toetoe and the Plimmerton Cortaderia species the m.p.s of successive fractions of the triterpene methyl ethers progressively decreased from 235° to ca 210° and g.l.c. analysis revealed that the later fractions were becoming progressively enriched with Ether 'B' and Ether 'C'.

Isolation of Arundoin, β-Amyrin Methyl Ether and α-Amyrin Methyl Ether

Total light petroleum extractives (80 g) from the Plimmerton Cortaderia species were dissolved in light petroleum of b.p. 40-60° and chromatographed over neutral

alumina (B.D.H. 1200 g) using light petroleum of b.p. 40-60° to develop the column. The initial eluants were rejected and the main triterpene fraction, which eluted subsequently, collected.

Gradual increase of the polarity of the solvent through addition of progressively greater quantities of benzene, to pure benzene and then through addition of progressively greater quantities of chloroform to pure chloroform gave an ester fraction followed by an alcohol fraction. These fractions were not further investigated in the present work.

The crude triterpene methyl ether fraction (5.5 g - 6.9% of the total light petroleum extractives) was dissolved in light petroleum, b.p. 40-60° and rechromatographed over neutral alumina (Woelm, grade I, 100 g) using further light petroleum to develop the column. After rejection of an initial small paraffin fraction, subsequent eluants afforded pure arundoin (2.7g) and then a mixture of arundoin with Ether 'B' and Ether 'C'.

The latter fractions (80 mg), containing the highest proportions of Ether 'B' and Ether 'C' were dissolved in AnalaR chloroform (0.25 ml) and the solution automatically injected, 15 to 20µl at a time onto a 1%-Apiezon 'L' column in the Aerograph -A700 instrument. The fractions corresponding to the three well resolved peaks on the trace were collected as they eluted from the column in capillary glass tubes. After 9 cycles were obtained Ether 'B' (12mg),

Ether 'C' (5 mg) and Arundoin (20 mg).

Ether 'B' had m.p. 246-248°, which was undepressed on admixture with authentic β-amyrin methyl ether. Further identification of Ether 'B' as β-amyrin methyl ether was achieved through comparison of infrared and mass spectra.

Ether 'C' had m.p. 220-222°, which was undepressed on admixture with authentic α-amyrin methyl ether. Further evidence that Ether 'C' was indeed α-amyrin methyl ether was achieved through comparison of infrared and mass spectra.

Isolation of Arundoin and Sawamilletin from Cuban Sugar Cane Wax

Substance 'W' (40 mg) isolated as previously described[272] was dissolved in AnalaR chloroform (0.2 ml) and the solution automatically injected, 15 to 20μl at a time onto a 3%-SE-30 column in the Aerograph - A.90. P_3 instrument. The fractions corresponding to the two well resolved peaks on the trace were collected as they eluted from the column in capillary glass tubes. After eight cycles were obtained Fraction 'A' corresponding to peak I (10mg) and Fraction 'B' corresponding to peak II and III (12 mg).

Fraction 'A' had m.p. 276-278° which was undepressed on admixture with authentic taraxerol methyl ether. Further identification of Fraction 'A' as taraxerol methyl ether was achieved through comparison of infrared and mass spectra

Fraction 'B' had m.p. 235-236° which was undepressed on admixture with authentic arundoin. The small amounts of the material corresponding to peak II which were present had no perceptible influence on either the mass spectrum or the infrared spectrum of Fraction B, which were identical with those of authentic arundoin.

BIBLIOGRAPHY

1. L. Smith, cited by Brooker and Cooper in 'New Zealand Medicinal Plants', Handbook of the Auckland War Memorial Museum, Unity Press, Auckland, 1962, p.23.

2. Eglinton, Hamilton and Martin-Smith, Phytochemistry, 1962, 1, 137.

3. Jones and Sandorfy in 'Technique of Organic Chemistry' ed, A. Weissberger, vol IX, 'Chemical Applications of Spectroscopy', Interscience, New York, 1956, pp. 247-580.

4. Hamilton, Ph.D. Thesis, University of Glasgow, 1962.

5. R. J. Hamilton, personal communication.

6. Zotov, N.Z. J. Bot., 1963, 1, 78.

7. Ohmoto, Nishimoto, Ito and Natori, Chem Pharm. Bull., Tokyo, 1965, 13, 224.

8. Nishimoto, Ito, Natori and Ohmoto, Tetrahedron Letters, 1965, no. 27, 2245.

9. Clayton, Nature, 1961, 190, 1071.

10. Clayton, Nature, 1961, 192, 524.

11. Clayton, Biochemistry, 1962, 1, 357.

12. Brooks and Hanaineh, Biochem. J., 1963, 87, 151.

13. Horning, VandenHeuvel and Creech, in 'Methods of Biochemical Analysis' ed. D. Glick, Interscience, New York, vol. IX, 1963, p. 69.

14. Knights, J. Gas Chromatog., 1964, 2, 160.

15. Ikekawa, Natori, Itokawa, Tobinaga and Matsui, Chem. Pharm. Bull., Tokyo, 1965, 13, 316.

16. Ikekawa, Natori, Ageta, Iwata and Matsui, Chem. Pharm. Bull., Tokyo, 1965, 13, 320.

17. James in 'Methods of Biochemical Analysis' ed. D. Glick, Interscience, New York, vol, VIII, 1960, 1.

18. Eglinton, Gonzalez, Hamilton and Raphael, Nature, 1962, 193, 739.

19. Eglinton, Gonzalez, Hamilton and Raphael, Phytochemistry, 1962, 1, 89.

20. Dr. H. E. Connor, personal communication.

21. Dr. E. P. White, personal communication.

22. Daly, J. Exptl. Bot., 1964, 15, 160.

23. Inter alia Kreger, Rec. Trav. Bot. Neerl., 1948, 41, 603; Chibnall, Piper Pollard, Williams and Sahai, Biochem. J., 1934, 28, 2189; Chibnall and Piper, Biochem. J., 1934, 28, 2209; Martin and Batt, Ann. Appl. Biol., 1958, 46, 375; Martin, J. Sci. Fd. Agric., 1960, 11, 635; Dewey, Hartley and MacLauchlan, Proc. Roy. Soc., 1962, B155, 532; Savidan, Thesis, University of Paris, 1956; Mazliak, C.R. Acad., Sci., Paris, 1960, 251, 2393; Mazliak, J. Agric. Tropicale et de Botanique Applique, 1961, 8, 180; Mazliak, Supplement to Bull. Int. Inst. Refrig., 1961, p.77.

24. Dawson, Tuatara, 1958, 7, 1.

25. Dansereau, Bull. Torrey bot. Cl., 1964, 91, 114.

26. Connor, N.Z. J. Bot., 1965, 3, 17.

27. Downing, Kranz and Murray, Australian J. Chem., 1960, 13, 80.

28. Levy, Doyle, Brown and Melpolder, Analyt. Chem., 1961, 33, 698.

29. Eglinton and Hamilton in 'Chemical Plant Taxonomy' ed. T. Swain, Academic Press, New York, 1963, pp. 187-217.

30. Gastambide-Odier and Lederer, Nature, 1959, 184, 1563.

31. Morice and Simpson, J. Chem. Soc., 1942, 198.

32. Inter alia Eglinton, Hamilton, Hodges and Raphael, Chem. and Ind., 1959, 955; Capella, Fedeli and Cirimele, Chem. and Ind., 1963, 1590; Shimizu, Uchimaru and Ohta, Chem. Pharm. Bull., Tokyo, 1964, 12, 74; Ageta, Iwata and Yonezawa, Chem. Pharm. Bull., Tokyo, 1963, 11, 408; Ageta, Iwata and Natori, Tetrahedron Letters, 1963, no.22, 1447.

33. E.g. Bradford, Harvey and Chalkley, J. Inst. Petroleum, 1955, 41, 80; van de Craats, Anal. Chim. Acta, 1956, 14, 136.

34. Djerassi, Budzikiewicz and Wilson, Tetrahedron Letters, 1962, no. 7 263.

35. Budzikiewicz, Wilson and Djerassi, J. Amer. Chem. Soc., 1963, 85, 3688.

36. Allard and Ourisson, Tetrahedron, 1957, 1, 277.

37. Kennard, Riva di Sanseverino, Vorbrüggen and Djerassi, Tetrahedron Letters, 1965, no. 39, 3433.

38. Nishimoto, Ito, Natori and Ohmoto, Chem. Pharm. Bull., Tokyo, 1966, 14, 97.

39. Benveniste, Hirth and Ourisson, Phytochemistry, 1966, 5, 31.

40. Benveniste, Hirth and Ourisson, Phytochemistry, 1966, 5, 45.

41. Eschenmoser, Ruzicka, Jeger and Arigoni, Helv. Chim. Acta, 1955, 38, 1890.

42. Ruzicka, Proc. Chem. Soc., 1959, 341.

43. Ruzicka, Pure and Applied Chem., 1963, 6, 493.

44. Woodward and Bloch, J. Amer. Chem. Soc., 1953, 75, 2023.

45. Inter alia Lynen, Agranoff, Eggerer, Henning and Moslein, Angew. Chem., 1959, 71, 657; Popjak and Cornforth, Adv. Enzymol., 1960, 22, 281; Popjak, Goodman, Cornforth, Cornforth and Ryhage, J. Biol. Chem., 1961, 236, 1934; Cornforth, J. Lipid Res., 1959, 1, 3.

46. Cf. Tchen and Bloch. J. Amer. Chem. Soc., 1956, 78, 1516; Tchen and Bloch, J. Biol. Chem., 1957, 226, 931.

47. Barton, Paper delivered at Anniversary Meetings of the Chemical Society, Oxford, March 1966; Barton and Moss, Chem. Comm., 1966, no. 9, 261.

48. Inter alia Staudinger, Kirsch and Leonhauser, Ann. N.Y. Acad. Sci., 1961, 92, 195; Grant, Ann. Reports Progr. Chem., 1955, 52, 316; Hayano et al, Rec. Progr. Hormone Res., 1956, 12, 79.

49. Mathieu and Ourisson 'Pouvoir Rotatoire Naturel', 1958, vol. 2, Triterpenes, Pergamon Press, London.

50. Godtfredsen, von Daehne, Vangedal, Marquet, Arigoni and Melera, Tetrahedron, 1965, 21, 3505.

51. Cf. Cornforth, Cornforth, Pelter, Horning and Popjak, Tetrahedron, 1959, 5, 311; Maugdal, Tchen and Bloch, J. Amer. Chem. Soc., 1958, 80, 2589.

52. Voser, Mijovic, Heusser, Jeger and Ruzicka, Helv. Chem. Acta, 1952, 35, 2414.

53. Ruzicka, Rey and Muhr. Helv. Chim. Acta, 1944, 27, 472.

54. Danielsson and Bloch, J. Amer. Chem. Soc., 1957, 79, 500; Dauben, Fonken and Boswell, J. Amer. Chem. Soc., 1957, 79,1000; Alexander and Schwenk, J. Amer. Chem. Soc., 1957, 79, 4554.

55. Cf. Parks, J. Amer. Chem. Soc.,1958, 80, 2023; Lederer, Experientia, 1964, 20, 473.

56. Holker, Powell, Robertson, Simes, Wright and Gascoigne, J. Chem. Soc., 1953, 2422.

57. E.g. Ourisson and Crabbé, 'Les Triterpenes Tetracycliques' 1961, Hermann, Paris.

58. Halsall and Hodges, J. Chem. Soc., 1954, 2385.

59. Guider, Halsall and Jones, J. Chem. Soc., 1954, 4471.

60. Guider, Halsall, Hodges and Jones, J. Chem. Soc., 1954, 3234.

61. Corsano, Mellor and Ourisson, Chem. Comm., 1965, no.10, 185.

62. Henry, Irvine and Spring, J. Chem. Soc., 1955, 1607.

63. Cox, King and King, J. Chem. Soc., 1959, 514.

64. Barton, Page and Warnhoff, J. Chem. Soc., 1954, 2715; Irvine, Henry and Spring, J. Chem. Soc., 1955, 1316.

65. Brown and Kupchan, J. Amer. Chem. Soc., 1962, 84, 4592.

66. Nikano and Terao, Tetrahedron Letters, 1964, no. 18, 1035, 1045.

67. Kupchan and Asbun, Tetrahedron Letters, 1964, no. 42, 3145.

68. Lawrie, Spring and Watson, Chem. and Ind., 1956, 1458.

69. Biglino, Lehn and Ourisson, Tetrahedron Letters, 1963, no. 24, 1651.

70. Tschesche, Biernoth and Snatzke, Annalen, 1964, 674, 196.

71. de Kock, Enslin, Norton, Barton, Sklarz and Bothner-By, J. Chem. Soc., 1963, 3828; Lavie, Shvo, Gottlieb and Glotter, J. Org. Chem., 1963, 28, 1790.

72. Eg. Tsuda, Agaki, Kishida, Hayatsu and Saka, Chem. Pharm. Bull., Tokyo, 1958, 6, 724; Johnston Bennet and Heftmann, Science, 1963, 140, 198; Schreiber and Osske, Tetrahedron, 1964, 20, 2575.

73. Tsuda, Hayatsu, Kishida and Akagi, J. Amer. Chem. Soc., 1958, 80, 921.

74. Windaus, Inhoffen and von Reichel, Annalen, 1934, 510, 248.

75. Dirscherl and Nahm, Annalen, 1943, 555, 57.

76. Castle, Blondin and Nes, J. Amer. Chem. Soc., 1963, 85, 3306.

77. Clayton, Quart. Rev., 1965, 19, 201.

78. Clayton, Quart. Rev., 1965, 19, 168.

79. Djerassi, Knight and Wilkinson, J. Amer. Chem. Soc., 1963, 85, 835.

80. Djerassi, Krakower, Lemin, Liu, Mills and Villotti, J. Amer. Chem. Soc., 1958, 80, 6284.

81. Gautschi and Bloch, J. Amer. Chem. Soc., 1957, 79, 684; Gautschi and Bloch, J. Biol. Chem., 1958, 233, 1343.

82. Kandutsch and Russell, J. Biol. Chem., 1959, 234, 2037; Kandutsch and Russell, J. Amer. Chem. Soc., 1959, 81, 4114.

83. Neiderhiser and Wells, Arch. Biochem. Biophys., 1959, 81, 300.

84. Inter alia Dvornik and Kraml, Proc. Soc. Exp. Biol., N.Y., 1963, 112, 1012; Gordon, Cantrall, Cekleniak, Albers, Littell and Bernstein, Biochem. Biophys. Res. Comm., 1961, 6, 359; Phillips and Avigan, Proc. Soc. Exp. Biol. N.Y., 1963, 112, 233; Ranney, Cook, Hambourger and Counsell, J. Pharmacol., 1963, 142, 132; Sachs and Wolfman, Metabolism, 1963, 12, 608; Blohm, Kariya and Laughlin, Arch. Biochem. Biophys., 1959, 85, 250.

85. Schreiber and Ripperger, Annalen, 1962, 655, 114.

86. Tomko and Bendik, Coll. Czech. Chem. Comm., 1962, 27, 1404.

87. Inter alia Tschesche, Angew. Chem. 1961, 73, 727; Tschesche, Brugmann and Snatzke, Tetrahedron Letters, 1964, no. 9, 473; Satoh, Ishii and Oyama, Chem. Pharm. Bull., Tokyo, 1960, 8, 657; Mitsuhashi and Shimizu, Steroids, 1963, 2, 373; Mitsuhashi and Nomura, Steroids, 1964, 3, 271; Shoppee, Lack and Robertson, J. Chem. Soc., 1962, 3610; Shoppee, Lack and Sternhell, J. Chem. Soc., 1963, 3281; Jaeggi, Weiss and Reichstein, Helv. Chim. Acta, 1963, 46, 694.

88. Goutarel 'Les Alcaloides Stéroïdiques des Apocynacées', 1964, Hermann, Paris. See also Tomita, Uyeo and Kikuchi, Tetrahedron Letters, 1964, no. 18. 1053; no. 25, 1641.

89. von Euw and Reichstein, Helv. Chim. Acta, 1964, 47, 711.

90. Zalkow, Burke and Keen, Tetrahedron Letters, 1964, no. 4, 217.

91. Inter alia Butenandt and Jacobi, Z. Physiol. Chem., 1933, 218,104; Bennett, Ko and Heftmann, Phytochemistry, 1966, 5, 231.

92. Bennett and Heftmann, Arch. Biochem. Biophys. 1965, 112, 616.

93. James, Ann. Repts. Progr. Chem., 1962, 59, 426.

94. Mills and Werner, J. Chem. Soc., 1955, 3132; Mills, Chem. and Ind., 1956, 189; Mills, J. Chem. Soc., 1956, 2196.

95. Godson, King and King, Chem. and Ind., 1956, 190; Cosserat, Ourisson and Takahashi, Chem. and Ind., 1956, 190.

96. Shiengthong, Verasaran, Nanonggai- Swanrath and Warnhoff, Tetrahedron, 1965, 21, 917.

97. Barnes, Galbraith, Ritchie and Taylor, Austral. J. Chem. 1965, 18, 141.

98. Halls and Warnhoff, Chem. and Ind., 1963, 1986.

99. Arigoni, Barton, Bernasconi, Djerassi, Mills and Wolff, Proc. Chem. Soc., 1959, 306.

100. Tanaka, Nagai and Shibata, Tetrahedron Letters, 1964, no. 33, 2291.

101. Shibata, Tanaka, Soma, Iida, Ando and Nakamura, Tetrahedron Letters, 1965, no. 3, 207; Elyakov, Dzizenko and Elkin, Tetrahedron Letters, 1966, no. 2, 141.

102. Arigoni, Jeger and Ruzicka, Helv. Chim. Acta, 1955, 38, 222.

103. Arigoni, Viterbo, Dunnenberger, Jeger and Ruzicka, Helv. Chim. Acta, 1954, 37, 2306; Barton, McGhie, Pradhan and Knight, J. Chem. Soc., 1955, 876.

104. Dawson, Halsall, Jones, Meakins and Rhillips, J. Chem. Soc.,1956, 3172; Irvine, Lawrie, McNab and Spring, J. Chem. Soc.,1956, 2029; Lawrie, Hamilton, Spring and Watson, J. Org. Chem., 1956, 21, 491; Lawrie, Hamilton, Spring and Watson, J. Chem. Soc., 1956, 3272.

105. Barton and Seoane, J. Chem. Soc., 1956, 4150.

106. Polonsky, Fouquey and Gaudemer, Bull. Soc., Chim., France, 1964, 1827; Son Bredenberg, Chem. and Ind, 1964, 73; Dreyer, Experientia, 1964, 20, 297.

107. Birch, Collins, Muhammad and Turnbull, J. Chem. Soc., 1963, 2762.

108. Bevan, Ekong, Halsall and Toft, Chem. Comm., 1965, no. 24, 636.

109. Barclay, Eade, Simes, Simes and Taylor, Chem. and Ind., 1963, 1206; Eade, Rosslen, Simes and Simes, Austral. J. Chem., 1965, 18, 1451.

110. Grant, Hamilton, Hamor, Hodges, McGeachin, Raphael, Robertson and Sim, Proc. Chem. Soc., 1961, 445.

111. Sutherland, Sim and Robertson, Proc. Chem. Soc., 1962, 222.

112. Narayanan, Pachapurkar, Pradhan, Shah and Narashimhan, Indian J. Chem., 1964, 2, 108.

113. Ollis, Ward and Zelnik, Tetrahedron Letters, 1964, no. 37, 2607.

114. Arigoni, Barton, Corey, Jeger, Caglioti, Sukh Dev, Ferrini, Glazier, Melera, Pradhan, Schaffner, Sternhell, Templeton and Tobinaga, Experientia, 1960, 26, 41; Arnott, Davie, Robertson, Sim and Watson, Experientia, 1960, 26, 49.

115. Kubota, Matsuura, Tokoroyama, Kamikawa and Matsumoto, Tetrahedron Letters, 1961, no. 10. 325.

116. Govindachari, Joshi and Sundararajan, Tetrahedron, 1964, 20, 2985.

117. Chan and Taylor, Chem. Comm., 1966, no. 7, 206.

118. Connolly, McCrindle, Overton and Warnock, Tetrahedron Letters, 1965, no. 33, 2937.

119. Arene, Bevan, Powell and Taylor, Chem. Comm., 1965, no. 14, 302,

120. Polonsky, Proc. Chem. Soc., 1964, 292; Brown and Sim, Proc. Chem. Soc., 1964, 293.

121. Carman and Ward, Tetrahedron Letters, 1961, no. 10, 317; Valenta, Papadopoulos and Podesva, Tetrahedron, 1961, 15, 100.

122. Polonsky and Fourrey, Tetrahedron Letters, 1964, no. 52, 3983; Carinovi, Ceccherelli, Grandolini and Bellavita, Tetrahedron Letters, 1964, no. 52, 3991.

123. Zybler and Polonsky, Bull. Soc. Chim., France, 1964, 2016.

124. Brochere and Polonsky, Bull. Soc. Chim., France, 1960, 963.

125. Patil, Ourisson, Tanahashi and Takahashi, Bull. Soc., Chim. France, 1964, 1422; Kamisako and Takahashi, Yakagaku Zasshi, 1965, 85, 888.

126. Barton, Cheung, Daniels, Lewis and McGhie, J. Chem. Soc., 1962, 5163.

127. Chatterjee, Anand and Dhar, J. Sci. Ind. Res., India, 1959, 18B, 262.

128. Dietrich and Jeger, Helv. Chim. Acta, 1950, 33, 711.

129. Djerassi, Farkas, Liu and Thomas, J. Amer. Chem. Soc., 1955, 77, 5330.

130. Chopra, Fuller, Thieberg, Shaw, White, Hall and Maslen, Tetrahedron Letters, 1963, no. 27, 1847.

131. Guise, Ritchie and Taylor, Austral. J. Chem., 1962, 15, 314.

132. Djerassi and Hodges, J. Amer. Chem. Soc., 1956, 78, 3534.

133. Aplin, Halsall and Norin, J. Chem. Soc., 1963, 3269.

134. Mechoulam, Chem. and Ind., 1961, 1835; de Mayo and Starratt, Canad. J. Chem., 1962, 40, 788.

135. de Mayo and Starratt, Canad. J. Chem. 1962, 40, 1632.

136. Jarolim, Hejno and Sorm, Coll. Czech. Chem. Comm., 1963, 28, 2443.

137. Carman and Cowley, Austral. J. Chem., 1965, 18,213.

138. Eade, Ellis and Simes, Chem. Comm., 1966, no. 8, 246.

139. Musgraves, Stark and Spring, J. Chem. Soc., 1952, 4393.

140. Bischof, Jeger and Ruzicka, Helv. Chim. Acta, 1949, 32, 1911; Abd El Rahim and Carlisle, Chem. and Ind., 1954, 279.

141. Brooks, J. Chem. Soc., 1955, 1675; Beaton, Spring, Stevenson and Stewart, Chem. and Ind., 1954, 1454.

142. Sengupta and Khastgir, Tetrahedron, 1963, 19, 123.

143. Fischer and Seiler, Annalen, 1961, 644, 162.

144. Kimura, Hashimoto and Agata, Chem. Pharm. Bull., Tokyo, 1960, 8, 1145.

145. Corey and Ursprung, J. Amer. Chem. Soc., 1956, 78, 5041.

146. Harada, Kakisawa, Kobayashi, Musya, Nakanishi and Takahashi, Tetrahedron Letters, 1962, no. 14, 603; Johnson, Juby, King and Tam, J. Chem. Soc., 1963, 2884.

147. Duggan, de Mayo and Starratt, Proc. Chem. Soc., 1964, 264.

148. Duggan, de Mayo and Starratt, Tetrahedron Letters, 1964, no. 37, 2567; Pelletier, Adityachaudhury, Tomasz, Reynolds and Mechoulam, Tetrahedron Letters, 1964, no. 41, 3065.

149. Rao and Bose, J. Org. Chem., 1962, 27, 1470.

150. Barua and Raman, Tetrahedron, 1962, 18, 155.

151. Breton and Gonzalez, J. Chem. Soc., 1963, 1401.

152. Ames, Beton, Bowers, Halsall and Jones, J. Chem. Soc., 1954, 1905.

153. Beton, Bowers, Halsall and Jones, Chem. and Ind., 1953, 847.

154. Santer and Stevenson, J. Org. Chem., 1962, 27, 3204.

155. Chopra, White and Melrose, Tetrahedron, 1965, 21, 2585.

156. Barton, Page and Warnhoff, J. Chem. Soc., 1954, 2715.

157. Varshney, Shamsuddin and Beyler, Tetrahedron Letters, 1965, no. 17. 1187.

158. Rao and Bose, Tetrahedron, 1962, 18, 461.

159. Tschesche and Wulff, Tetrahedron Letters, 1965, no. 12, 1569.

160. Dr. J. McLean and Dr. W. Lawrie, personal communication in ref. 217.

161. Ultee, Pharm. Weekblad., 1949, 84, 65; Mitra and Misra, Phytochemistry, 1965, 4, 345.

162. King, King and White, J. Chem. Soc., 1958, 2830.

163. Cole, Downing, Watkins and White, Chem. and Ind., 1955, 254.

164. Anantaraman and Pillai, J. Chem. Soc., 1956, 4369.

165. Chakraborti and Barua, Experientia, 1962, 18, 66.

166. King and Yardley, Proc. Chem. Soc., 1959, 393; J. Chem. Soc., 1961, 4308.

167. Eade, Simes and Stevenson, Austral. J. Chem., 1963, 16, 900.

168. Elsevier's Encyclopedia of Organic Chemistry. vol. 14, p. 526; vol. 14 supplement, p. 939S. Elsevier, New York, 1940 and 1952.

169. Vogel, Jeger and Ruzicka, Helv. Chim. Acta, 1951, 34, 2321.

170. Tschesche, Henckel and Snatzke, Tetrahedron Letters, 1963, no. 10, 613.

171. Ayengar and Rangaswami, Tetrahedron Letters, 1966, no. 18, 1947.

172. Sandoval, Manjarrez, Leeming, Thomas and Djerassi, J. Amer. Chem. Soc., 1957, 79, 4468.

173. Tschesche, Duphorn and Snatzke, Annalen, 1963, 667, 151.

174. Djerassi, Thomas and Monsimer, J. Amer. Chem. Soc., 1955, 77, 3579.

175. Thomas, Tetrahedron, 1961, 15, 212.

176. Tschesche, Henckel and Snatzke, Annalen, 1964, 676, 175.

177. Anderson, de Kock and Enslin, J. South African Chem. Inst., 1961, 14, 58.

178. Djerassi, Robinson and Thomas, J. Amer. Chem. Soc., 1956, 78, 5685.

179. Frazier and Noller, J. Amer. Chem. Soc., 1944, 66, 1267.

180. Barua, Naturwissenschaften, 1956, 43, 250.

181. Djerassi, McDonald and Lemin, J. Amer. Chem. Soc., 1953, 75, 5940; Djerassi and Lippman, J. Amer. Chem. Soc., 1954, 76, 5780.

182. Beaton and Spring, J. Chem. Soc., 1956, 2417.

183. Ruzicka and Jeger, Helv. Chim. Acta, 1942, 25, 775; Beaton and Spring, J. Chem. Soc., 1955, 3126.

184. Djerassi, Geller and Lemin, J. Amer. Chem. Soc., 1954, 76, 4089.

185. Vogel, Jeger and Ruzicka, Helv. Chim. Acta, 1951, 34, 2321.

186. Knight and White, Tetrahedron Letters, 1961, no. 3, 100.

187. Barton and de Mayo, J. Chem. Soc., 1954, 887, 900; Barton, de Mayo and Orr, J. Chem. Soc., 1956, 4160.

188. Eglinton, Hamilton, Martin-Smith, Smith and Subramanian, Tetrahedron Letters, 1964, no. 34, 2323.

189. King and Morgan, J. Chem. Soc., 1960, 4738.

190. Barton, de Mayo, Warnhoff, Jeger and Perold, J. Chem. Soc., 1954, 3689.

191. Elgamal, Fayez and Snatzke, Tetrahedron, 1965, 21, 2109.

192. Djerassi and Lippman, J. Amer. Chem. Soc., 1955, 77, 1825.

193. Caglioti, Cainelli and Minutilli, Gazzetta, 1961, 91, 1387; Caglioti and Cainelli, Tetrahedron, 1962, 18, 1061.

194. Djerassi, Thomas, Livingston and Thompson, J. Amer. Chem. Soc., 1957, 79, 5292.

195. Djerassi and Monsimer, J. Amer. Chem. Soc., 1957, 79, 2901.

196. Shamma and Rosenstock, J. Org. Chem., 1959, 24, 726.

197. Ruzicka, Frame, Leicester, Liguori and Brungger, Helv. Chim. Acta, 1934, 17, 426; White and Zampetti, J. Chem. Soc., 1952, 5040.

198. Huneck, Tetrahedron, 1963, 19, 479.

199. Stout, Malofsky and Stout, J. Amer. Chem. Soc., 1964, 86, 957.

200. Shimizu and Pelletier, Chem. and Ind., 1965, 2098; Dugan and de Mayo, Canad. J. Chem., 1965, 43, 2033.

201. De Maheas, C.R. hebd. Seances Acad. Sci., 1959, 249, 1799; 1961, 252, 805.

202. Rondest and Polonsky, Bull. Soc. Chim., France, 1963, 1253.

203. Kuhn and Low, Annalen, 1963, 669, 183.

204. Djerassi, Henry, Lemin, Rios and Thomas, J. Amer. Chem. Soc., 1956, 78, 3783.

205. Bilham and Kon, J. Chem. Soc., 1940, 1469; 1941, 552.

206. Kubota, Tonami and Hinoh, Tetrahedron Letters, 1966, no. 7, 701.

207. Ruzicka, Grob, Egli and Jeger, Helv. Chim. Acta, 1943, 26, 1218.

208. Ishimasa, J. Pharm. Soc., Japan, 1960, 80, 304.

209. Cainelli, Britt, Arigoni and Jeger, Helv. Chim. Acta, 1958, 41, 2053; Smith, Smith and Spring, Chem. and Ind., 1958, 889; Smith, Smith and Spring, Tetrahedron, 1958, 4, 111.

210. Chakrabarti, Mukherjee and Barua, Tetrahedron, 1966, 22, 1431.

211. Tursch, Tursch, Harrison, da Silva, Monteiro, Gilbert, Mors and Djerassi, J. Org. Chem., 1963, 28, 2390.

212. Djerassi, Thomas and Jeger, Helv. Chim. Acta, 1955, 38, 1304.

213. King and King, J. Chem. Soc., 1956, 4469.

214. Djerassi and Mills, J. Amer. Chem. Soc., 1958, 80, 1236.

215. Row and Subba Rao, Tetrahedron, 1962, 18, 827.

216. Corey and Cantrall, J. Amer. Chem. Soc., 1959, 81,1745.

217. Halsall and Aplin, Fortschritte der Chemie Organischer Naturstoffe, ed. L. Zechmeister, vol. 22, 1964, pp. 153-202.

218. Khorlin, Ovodov and Kochetkov, Zhurn. Obschei Khimii, 1962, 32, 782 (Engl. transl. 1962, 32, 778).

219. Chan. Halsall and Jones cited in ref. 217.

220. Arigoni, Barton, Bernasconi, Djerassi, Mills and Wolff, J. Chem. Soc., 1960, 1900; Whitham, J. Chem. Soc., 1960, 2016.

221. Crowley, Proc. Chem. Soc., 1962, 27.

222. Zurcher, Jeger and Ruzicka, Helv. Chim. Acta, 1954, 37, 2145.

223. Laird, Spring and Stevenson, J. Amer. Chem. Soc., 1960, 82, 4108.

224. Beton, Halsall and Jones, J. Chem. Soc., 1956, 2904.

225. Polonsky and Zybler, Bull. Soc., Chim., France, 1961, 1586.

226. Bosson, Galbraith, Ritchie and Taylor, Austral. J. Chem. 1963, 16, 491.

227. Thomas, Heusler and Muller, Tetrahedron, 1961, 16, 264.

228. Glen, Lawrie, McLean and El-Garby Younes, Chem. and Ind., 1965, 1908.

229. Mangoni and Belardini, Tetrahedron Letters, 1963, no. 14, 921.

230. Ageta, Iwata and Natori, Tetrahedron Letters, 1964, no. 46, 3413.

231. Ageta, Iwata and Natori, Tetrahedron Letters, 1963, no. 22, 1447; Ageta, Iwata and Yonezawa, Chem. Pharm. Bull., Tokyo, 1963, 11, 408.

232. Berti, Bottari, Marsili and Morelli, Tetrahedron Letters, 1966, no. 9, 979.

233. Nakanishi, Lin, Kakisawa, Hsu and Hsiu, Tetrahedron Letters, 1963, no. 22, 1451.

234. Galbraith, Miller, Rawson, Ritchie, Shannon and Taylor, Austral J. Chem., 1965, 18, 226.

235. Kundu, Chatterjee and Rao, Tetrahedron Letters, 1966, no. 10. 1043.

236. Arthur, Hui and Aplin, Tetrahedron Letters, 1965, no. 14, 937.

237. Nakamura, Yamada, Wada, Inoue, Gioto and Hirata, Tetrahedron Letters, 1965, no. 24, 2017.

238. Berti, Bottari and Marsili, Tetrahedron Letters, 1964, no. 1, 1.

239. Tsuda, Morimoto, Sano, Inubushi, Mallory and Gordon, Tetrahedron Letters, 1965, no. 19, 1427.

240. Ageta, Iwata and Yonezawa, Chem. Pharm. Bull., Tokyo, 1963, 11, 407.

241. Cerny, Vystrcil and Huneck, Chem. Ber., 1963, 96, 3021.

242. Baddeley, Halsall and Jones, J. Chem. Soc., 1961, 3891.

243. Huneck and Lehn, Bull. Soc. Chim., France, 1963, 1702.

244. Huneck, Chem. Ber., 1961, 94, 614.

245. Yosioka, Nakanishi and Tsuda, Tetrahedron Letters, 1966, no. 6, 607.

246. Yosioka, Matsuda and Kitagawa, Tetrahedron Letters, 1966, no. 6, 613.

247. Berti, Bottari, Marsili, Lehn, Witz and Ourisson, Tetrahedron Letters, 1963, no. 20, 1283.

248. Vorbruggen, Pakrashi and Djerassi, Annalen, 1963, 668, 57.

249. Hui and Lam, Phytochemistry, 1965, 4, 333.

250. Lederer, Industrie parfum., 1953, 8, 189.

251. Barton and Overton, J. Chem. Soc., 1955, 2639.

252. Inubushi, Sano and Tsuda, Tetrahedron Letters, 1964, no. 21, 1303.

253. Rowe and Bower, Tetrahedron Letters, 1965, no. 32, 2745.

254. Inubushi, Tsuda and Sano, Chem. Pharm. Bull. Tokyo, 1965, 13, 750.

255. Cornforth and Popjak, Biochem. J., 1954, 58, 403.

256. Cornforth, Hunter and Popjak, Biochem. J., 1953, 54, 590, 597; Cornforth, Gore and Popjak, Biochem. J., 1957, 65, 94.

257. Clayton and Bloch, J. Biol. Chem., 1956, 218, 305, 319.

258. Schneider, Clayton and Bloch, J. Biol. Chem., 1957, 224, 175.

259. Nicholas, J. Biol. Chem., 1962, 237, 1485.

260. Nes and Rosin, 6th International Congress of Biochemistry, New York, 1964, p. 588.

261. Maudgal, Tchen and Bloch, J. Amer. Chem. Soc., 1958, 80, 2589.

262. Cornforth, Cornforth, Pelter, Horning and Popjak, Tetrahedron, 1959, 5, 311.

263. Clayton, Nelson and Frantz, L. Lipid Res., 1963, 4, 166.

264. Gaylor, J. Biol. Chem., 1963, 238, 1643, 1649.

265. Hanahan and Wakil, J. Amer. Chem. Soc., 1963, 75, 273; Dauben and Hutton, J. Amer. Chem. Soc., 1956, 78, 2647; Dauben, Hutton and Boswell, J. Amer. Chem. Soc., 1959, 81, 403.

266. Dauben, Ban and Richards, J. Amer. Chem. Soc., 1957, 79, 968; Dauben and Richards, J. Amer. Chem. Soc., 1956, 78, 5329; Lawrie, McLean, Pauson and Watson, Chem. Comm., 1965, no. 24, 623.

267. Guglielmetti, Dissertation, E.T.H., Zurich, 1963, Cited in ref. 217.

268. Arigoni, Experientia, 1958, 14, 153.

269. Baisted and Nes, J. Biol. Chem., 1963, 238, 1947.

270. Nicholas, J. Biol. Chem., 1962, 237, 1481.

271. Hendrickson, Tetrahedron, 1959, 7, 82.

272. Osske and Schreiber, Tetrahedron, 1965, 21, 1559.

273. Ishidate, Tamura and Okada, J. Pharm. Soc., Japan, 1947, 67, 206, in Chem. Abstr., 1951, 45, 9068; Takemoto and Kometani, J. Pharm. Soc., Japan, 1954, 74, 1263, in Chem. Abstr., 1955, 49, 4233; Rittel, Hunger and Reichstein, Helv. Chim. Acta, 1953, 36,

274. Matthes and Dahle, Arch. Pharm., 1911, 249, 436; Meyer, Jeger and Ruzicka, Helv. Chim. Acta, 1950, 33, 672, 1835.

275. Baisted, Capstack and Nes, Biochemistry, 1962, 1, 537; Capstack, Baisted, Newschwander, Blondin, Rosin and Nes, Biochemistry, 1962, 1, 1178.

276. Nicholas, J. Biol. Chem., 1962, 237, 1476.

277. Bauer and Moll, Fette u Seifen, 1939, 560; Dawson, Halsall, Jones and Robins, J. Chem. Soc., 1953, 586.

278. Mazur, Weizmann and Sondheimer, J. Amer. Chem. Soc., 1958, 80, 1007, 6293; Bernay, Annalen, 1841, 40, 317.

279. Eg. Balch, 'Wax and Fatty By-Products From Sugar Cane' Technol. Rep. Ser. no. 3, [Sugar Research Foundation Inc., New York,] 1947. Whyte and Hengeveld, J. Amer. Oil Chem. Soc., 1950, 27, 57; Wiedenhof, J. Amer. Oil Chem. Soc., 1959, 36, 297; Horn and Matic, J. Sci. Fd. Agric., 1957, 8, 571; Bose and Gupta, Proc. Ann. Conv. Sugar Technologists' Assoc., India, 1961, 29, 70, in Chem. Abstr., 1964, 60, 13423.

280. Lamberton and Redcliffe, Austral. J. Chem., 1960, 13, 261.

281. Kranz, Lamberton, Murray and Redcliffe, Austral. J. Chem., 1960, 13, 498.

282. Ito, J. Chem. Soc., Japan, 1938, 59, 274; Abe and Obara, Nippon Kagaku Zasshi, 1959, 80, 1487.

283. Ito, J. Faculty Agr. Hokkaido Imp. Univ., 1934, 37, 1; Abe, Bull. Chem. Soc., Japan, 1960, 33, 271; Sugiyama and Abe, Nippon Kagaku Zasshi, 1961, 82, 1051; Abe, Ibid., 1961, 82, 1054, 1057.

284. Abe, Nippon Kagaku Zasshi, 1959, 80, 677, 1491.

285. Nishimoto, Ito, Natori and Ohmoto, Tetrahedron Letters, 1965, no. 27, 2245.

286. Ohmoto, Nishimoto, Ito and Natori, Chem. Pharm. Bull, Tokyo, 1965, 13, 224.

287. McLafferty, Anal. Chem., 1957, 29, 1782.

288. Djerassi and Fenselau, J. Amer. Chem. Soc., 1965, 87, 5747.

289. Friedel and Sharkey, Anal. Chem., 1956, 28, 940; McLafferty, Dow Chemical Co. Tech. Rept., November, 1955.

290. Beynon and Williams, 'Mass and Abundance Tables for Use in Mass Spectrometry', Elsevier, Amsterdam, 1963.

291. Reed and de Mayo, Chem. and Ind., 1956, 1481; Budzikiewicz and Djerassi, J. Amer. Chem. Soc., 1962, 84, 1430.

292. Dr. S. Natori, personal communication.

293. Sharma, Glick and Mumma, J. Org. Chem., 1962, 27, 4512.

294. Pye Panchromatograph Handbook, page 52.

SECTION V

CHEMISTRY OF ARUNDOIN

INTRODUCTION.

As indicated in the previous Section of this thesis, observation of strong absorption in the infrared at 1104 cm^{-1} in the alkane-containing fraction from a sample of New Zealand 'toetoe' grass[1], at that time designated 'Arundo conspicua' Forst. f. but now identified [Section IV of present thesis] as Cortaderia toetoe Zotov, led to the isolation of a crystalline compound, named arundoin, which was characterised as a triterpene methyl ether of unknown constitution.[2] The studies presently described are concerned with the elucidation of the chemical structure of arundoin.

Earlier work by Hamilton[2] had established that arundoin, which exhibited dimorphism with the two forms melting at 235-237° and 271-273° and which showed $[\alpha]_D$ -9° in $CHCl_3$, had a molecular weight of 440 [mass spectrum]. This value was in agreement with the analytical figures obtained by Hamilton which fitted a molecular formula $C_{31}H_{52}O$ - ie a formula having 6 double bond equivalents ($C_nH_{2n+2} = C_{31}H_{64}$; $\frac{64-52}{2} = 6$) which is the number of double bond equivalents imposed upon triterpenes by their mode of biogenesis[3], provided that there is no subsequent introduction of additional unsaturation. A strong singlet in the n.m.r. spectrum of arundoin at 6·62 τ - earlier considered to have an intensity of 4 protons but now known to have an intensity of 3 protons [page134 this thesis] - coupled with the presence in the mass spectrum of arundoin of

ions at $\frac{m}{e}$ P-15-32 and $\frac{m}{e}$ 273-32 were deemed evidence for the presence on a secondary carbon atom of a methoxyl group[2]. The methoxyl function could not, however, be demonstrated directly in a Zeisel determination owing to the insolubility of arundoin in the hydriodic acid reagent.

The presence of a double bond in arundoin was deduced from the production of a yellow colour with tetranitromethane, infrared absorption (CCl_4 solution) at 3028 cm^{-1}, 1639 cm^{-1} and 810 cm^{-1}, and ultraviolet end absorption at 203 mμ (ε=7,450). The original n.m.r. spectrum of arundoin available to Hamilton did not permit the identification of any olefinic proton absorption, but that the double bond was trisubstituted was deduced from the infrared absorption, from the ratio of $\frac{\varepsilon 210 m\mu}{\varepsilon 220 m\mu}$ which at 6·0 was in the correct range for a trisubstituted double bond[4], and from the failure to achieve reduction of the double bond employing a prereduced platinum catalyst in an ethyl acetate-acetic acid medium in the light of the known resistance of triterpene trisubstituted double bonds to hydrogenation[5]. A later n.m.r. spectrum obtained in the course of work by Dr. S.J. Smith[6] clearly showed a multiplet at 4·65 τ of intensity one proton, thus conclusively demonstrating, when taken in conjunction with the U.V. data quoted above, the presence of but one double bond in arundoin, which was indeed trisubstituted. Both Hamilton[2] and Smith[6] reported failure to isomerise this double bond with hydrochloric acid in chloroform. More vigorous attack by means of concentrated sulphuric acid was

reported by both workers[2,6] to yield, after the production of a red colour, a mixture showing carbonyl absorption but no ether absorption at 1104 cm^{-1} in the infrared, indicating attack on the methyl ether function, but neither worker isolated any pure crystalline compound from the mixture. Smith[6] also attempted selenium dioxide oxidation about the double bond of arundoin, but reported no success.

The presence of but one double bond in a compound having a formula requiring 6 double bond equivalents demands that the compound be pentacyclic. Indeed evidence that arundoin was a pentacyclic and not a tetracyclic triterpene was adduced by Hamilton from the fact that no ions corresponding to loss of side chain (a C_8 unit for lanostane and cholestane derivatives a C_9 unit for 24-methyllanostane and ergostane derivatives, or a C_{10} unit for 24-ethyllanostane and stigmastane derivatives) as is characteristic of tetracyclic triterpenes and steroids[7], were present in the mass spectrum of arundoin.

From the above data, it is apparent [as concluded by both Hamilton[2] and Smith[6]] that arundoin must be a pentacyclic triterpene having a trisubstituted double bond and bearing a methoxyl group on a secondary carbon atom.

At the time of Hamilton's work in 1962, the mass spectral fragmentation of arundoin could not be correlated with any of the then known[8] triterpene cracking patterns, and so the position of the double bond could not be placed, other than

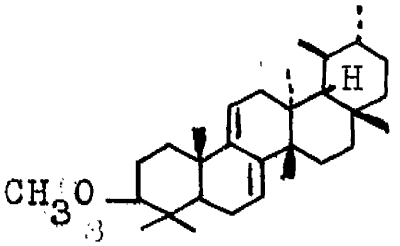
that it could not be in the 12, 13(18), 14 or 18 positions.

The absence of any peak derived by the loss of 43 mass units [isopropyl group] in the mass spectrum of arundoin determined using a direct inlet system led Hamilton[2] to the conclusion that arundoin should belong to either the ursane group or the oleanane group. He sought to distinguish between these two possibilities by quantitative infrared studies in CCl_4 solution in which the ratio of the ε value of the angular methyl C-CH_3 stretching absorption at 1376 cm^{-1} to the ε value of the gemdimethyl C-CH_3 stretching absorption at 1364 cm^{-1} was compared to the analogous ratios for taraxerol methyl ether and α-amyrene[9]. From the result it was concluded by Hamilton[2] that 2 pairs of gemdimethyl groups were probably present in arundoin.

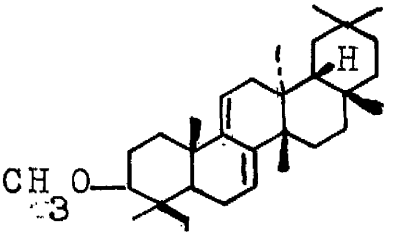
Hamilton[2] also considered molecular rotational differences between known triterpene alcohols and their derived methyl ethers, but in view of the extreme similarity of molecular rotational differences in the various pentacyclic triterpene skeletal types[10], no conclusions could be drawn. Nor could he draw any conclusions from considerations of the melting points of various appropriately chosen triterpenes.

DISCUSSION

Work on the elucidation of the structure of arundoin was considerably aided by the publication in 1963 of an important paper on the mass spectral fragmentation of a



I



II

wide variety of triterpene types by Budzikiewicz, Wilson and Djerassi[11] which permitted immediate identification of the mass spectrum of arundoin as being characteristic of a 9(11)-ene. Accordingly, on the assumption that arundoin was indeed a 3-methoxy pentacyclic triterpene possessing a 9(11) double bond, as suggested by the n.m.r. and mass spectral evidence as summarised above, in the present work it was decided to convert arundoin into the corresponding, 7,9(11)-diene in order that direct comparison could be made with the 7,9(11)-dienes of established structure derivable from the methyl ethers of triterpenes such as multiflorenol[12] and bauerenol.[12,13]

On the basis of the report by Smith[6] that arundoin did not undergo selenium dioxide dehydrogenation into the 7,9(11)-diene under the normal conditions, arundoin was converted into the corresponding 7,9(11)-diene by a sequence analogous to that employed in the conversion of arborinol into its 7,9(11)-diene[14]. This sequence involved epoxidation of the 9(11) double bond with trifluoroperacetic acid, acid catalysed elimination from the resulting epoxide and chromatographic purification of the product.

The 'diene' so obtained from arundoin was clearly not identical with the diene(I) prepared from the methyl ether of bauerenol (infrared in CCl_4 and mixed m.p.]. However there was a marked similarity[4] between the infrared spectra

measured in CCl_4 solution of the diene prepared from arundoin and that of the 7,9(11)-diene (II) prepared from the methyl ether of multiflorenol and this coupled with a lack of melting point depression between the two specimens gave rise to the erroneous conclusion that arundoin was 3β-methoxy-D:C friedo oleana-9(11) ene - a conclusion which was considered to have biogenetic support in the identification of a second oleanane derivative, β-amyrin methyl ether (see section IV of this thesis), as a minor component of 'Arundo conspicua' by Hamilton[2].

The lack of any loss of 43 mass units in the mass spectrum of arundoin [corresponding to loss of an isopropyl group] when a direct inlet system was employed [see page 133] and the lack of resolution of the methyl group proton absorptions in the 9τ region of the n.m.r. spectrum of arundoin obtained with a 40 megacycle instrument caused an E:C-friedolup-9(11)-ene or an E:C-friedoisohop-9(11)-ene structure to be omitted from consideration.

Accordingly a preliminary announcement[15] of the structure of arundoin as 3β-methoxy-D:C-friedooleana-9(11)-ene was made. However, on its appearance, Dr. S. Natori kindly informed us of his own work on a triterpene methyl ether, which he had isolated, together with cylindrin [isoarborinol methyl ether] from the grass Imperata cylindrica.[16] He believed this compound to be identical with arundoin from the published physical constants[15], but he could not agree to its structure

being 3β-methoxy-D:C-friedooleana-9(11)-ene on the basis of his own experimental work. Direct comparisons of specimens of arundoin and Dr. Natori's triterpene methyl ether in both the Glasgow and Tokyo laboratories (i.r. in KCl disc, g.l.c., mixed m.p.) indisputedly proved their identity, whilst proof that arundoin was in fact 3β-methoxy-E:C friedoisohop-9(11)-ene (III) was advanced by Dr. Natori[17].

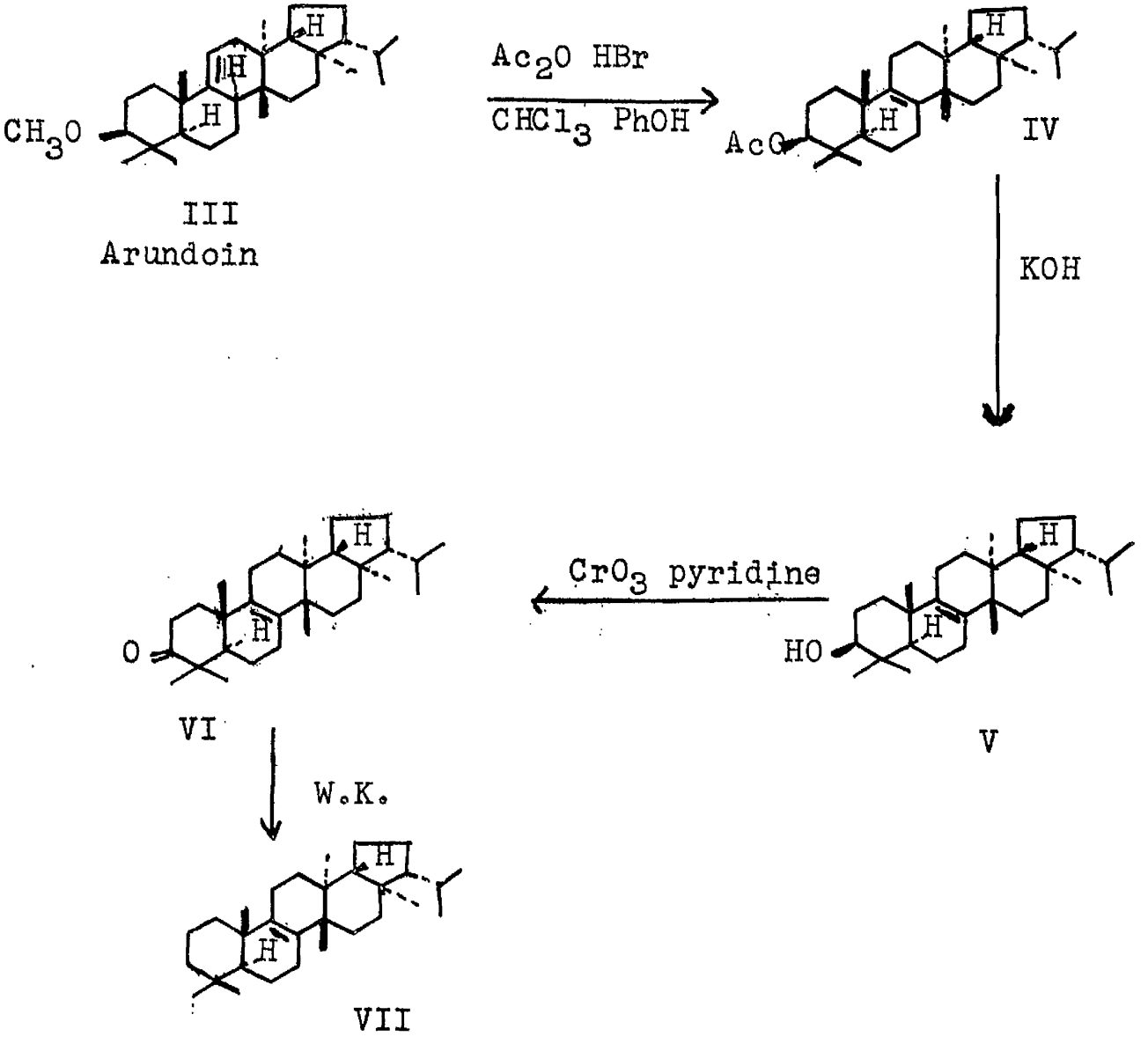
The first important clue to the true identity of arundoin was furnished by the n.m.r. spectrum obtained by Dr. Natori with a 100 megacycle instrument, which, unlike the n.m.r. spectrum available to Smith[6] from a 40 megacycle instrument, showed clear resolution of a doublet in the 9 τ region with each peak having an intensity of 1·5 protons, thus indicating the presence of a methyl group on a carbon atom bearing a hydrogen atom. Such a situation is of course incompatible with an oleanane skeleton, but in keeping with the presence of an isopropyl group.

A further clue to the true constitution of arundoin obtained by Dr. Natori[17] came from the product of chromic acid oxidation of arundoin which was a conjugated enone having an O.R.D. curve coinciding with those of fern-9(11)-en-12-one and methyl 12-ketodavallate.

Final proof of the constitution of arundoin as 3β-methoxy-E:C friedoisohop-9(11)-ene was then obtained by Dr. Natori[17] through its conversion as shown in Fig. 1 into fern-8-ene (VII), identical in all respects with an authentic specimen. Thus

Figure 1

Conversion of Arundoin into Fern-8-ene.

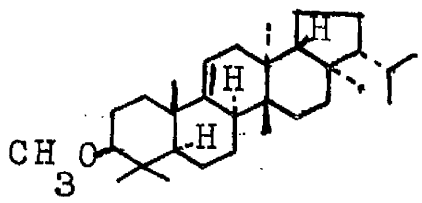


treatment of arundoin with a mixture of acetic anhydride, hydrobromic acid, chloroform and phenol replaced the 3β-methoxyl group by a 3β-acetoxy group [configuration deduced from n.m.r. data] and at the same time isomerised the double bond from the 9(11)-position into the 8-position as shown by the mass spectral fragmentation pattern to give compound IV. A separate experiment clearly demonstrated that, in direct negation of the claims of Hamilton[2] and Smith[6] the double bond in arundoin was smoothly isomerised from the 9(11)-position to the 8-position on treatment with acid.[17]

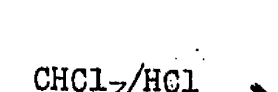
Hydrolysis of the 3β-acetoxy-8-ene (IV) resulting from the treatment of arundoin with the acetic anhydride/HBr reagent in chloroform phenol gave the corresponding 3β-hydroxy compound (V) which was in turn oxidised by means of chromic oxide in pyridine to the 3-ketone (VI). Wolff-Kishner reduction of this ketone then afforded fern-8-ene (VII).

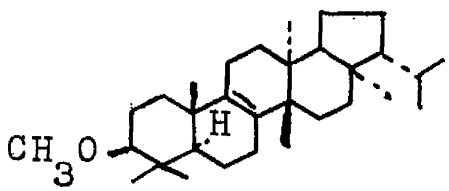
As a result of this work by the Japanese group it became necessary to put into train further work to rectify the erroneous conclusions made earlier in the Glasgow laboratories.

Accordingly the action of HCl in $CHCl_3$ on arundoin was first reinvestigated, and it was found that in agreement with the Japanese work[17] arundoin was smoothly converted into an isomer of m.p. 223-224° and $[\alpha]_D$ + 28° ($CHCl_3$) which on the grounds of molecular rotation differences and the absence of double bond absorption in the i.r. can be assigned the structure of 3β-methoxyfern-8-ene (VIII). Redetermination

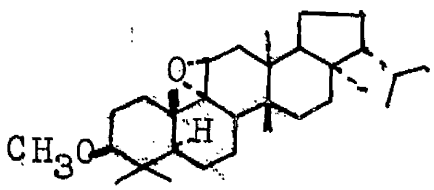


III

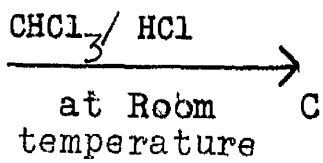


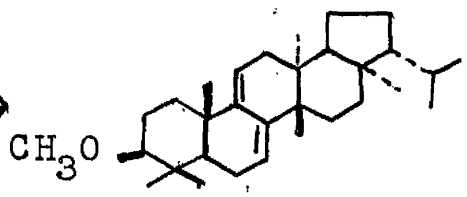


VIII



IX





X

of the $[\alpha]_D$ of arundoin gave a value of $-5°7^{\circ}$ rather than the -9° reported by Hamilton[2] in good agreement with the value of $-5°3^{\circ}$ quoted by the Japanese[17], but our specimen still melted at 237° rather than the $242-243^{\circ}$ quoted by the Japanese[17].

Application of gas liquid chromatography to the original specimen of our 7,9(11)-diene prepared from multiflorenol, showed it to be a mixture, so its preparation was again carried out and a pure specimen obtained. In view of the discrepancy of physical constants between our arundoin epoxide (m.p. $225-226^{\circ}$, $[\alpha]_D$ + 36 in $CHCl_3$) prepared by the action of trifluoroacetic acid and hydrogen peroxide on arundoin, and the epoxide from arundoin (m.p. $271-272^{\circ}$)[17] prepared by the Japanese by the action of perbenzoic acid on arundoin, attempts were made to compare the products obtained by both procedures. All attempts to repeat the trifluoroacetic acid/H_2O_2 procedure failed, but employing perbenzoic acid, a product (IX) resulted having m.p. $269-271^{\circ}$ in good agreement with the Japanese work[17].

Treatment of this compound with sulphuric acid followed by alumina chromatography of the product afforded the 7,9(11)-diene (X) in pure form [as shown by g.l.c. analysis] having m.p. $228-229^{\circ}$ and λ max 232, 239, 247·5mμ (ε 15,500; 16,900; 9,900) in hexane. The ultraviolet spectrum was thus in good agreement with that obtained by the Japanese[17] and with

the spectra characteristically given by 7,9(11)-dienes of both tetracyclic and pentacyclic triterpenes where the methyl group on C-13 has the α-configuration and the methyl group on C-14 has the β-configuration[12,13,18] - such ultraviolet spectra being quite distinct from those of 7,9(11)-dienes having an α-methyl group at C-14 and a β-methyl on C-13[14,18]. Direct comparison of the 7,9(11)-diene thus obtained from arundoin with the 7,9(11)-diene derived from multiflorenol methyl ether showed that they were non-identical.

Re-determination of the mass spectrum of arundoin employing a heated inlet system in place of a direct inlet system revealed the presence of an ion at $\frac{m}{e}$ 365 derived by a one-stage loss of 43 mass units (isopropyl group) from the ion at $\frac{m}{e}$ 408 as proved by the appearance of a metastable ion at 326·5 (calcd. for $\frac{m}{e}$ 408 $\longrightarrow$ $\frac{m}{e}$ 365, $\frac{365^2}{408}$ = 326·5), as has already been discussed [page 133, Section IV of this thesis], so all discrepancies between our original report[15] and the Japanese work[17] were thus resolved and the structure of arundoin, as determined by the Japanese, accepted by us as 3β-methoxy-E:C friedoisohop-9(11)-ene.

EXPERIMENTAL

Materials and Method:

Melting points were taken on a Kofler block. Optical rotations were measured in chloroform solution, in 1 decimeter cells using a Unicam, 'PEPOL-66', No. 558801 [Bellingham and Stanley Ltd., London.] instrument. Infrared spectra were taken on a Perkin-Elmer-237 instrument in KCl disc. Ultra violet absorptions were measured in hexane.

Isomerisation of Arundoin:

Into a solution of arundoin (50 mg) [obtained from Cortaderia toetoe, section IV of this thesis] in chloroform (10 ml) dry hydrogen chloride was passed for half an hour. The solution was then washed well with water and sodium bicarbonate solution, and the organic layer separated, dried over anhydrous sodium sulphate, and filtered. Removal of solvent and crystallisation of the solid residue from hexane gave the isomerised product, 3β-methoxy fern-8-ene (VIII), having m.p 223-225°; $[\alpha]_D$ = +30° (c=2). Found: C,84.55; H,11.8; calculated for $C_{31}H_{52}O$: C,84.5; H,11.9% Literature[17] m.p. 223-224°; $[\alpha]_D$=+28.9(chloroform).

Epoxide from Arundoin:

To a solution of arundoin (75 mg) in chloroform (5 ml) was added 0.5ml of a 0.817N solution of perbenzoic acid in

chloroform. After standing for 24 hr. at room temperature the solution was shaken with several portion of aqueous sodium carbonate and the chloroform layer dried over anhydrous sodium sulphate. Removal of solvent and crystallisation of the residue from hexane gave the epoxide of arundoin (IX) (29mg) m.p. 269-271°; $[\alpha]_D$ = +79.6 (C=1.8); Found: C81.46; H,11.47 Calculated for $C_{31}H_{52}O_2$: C,81.5; H,11.5% Literature[17], m.p. 271-272.

3β-Methoxy-D:C-friedooleana-7,9(11)-diene: (II)

To a suspension of multiflorenol methyl ether (100 mg) [obtained from multiflorenol as described in section IV] in glacial acetic acid (25ml) was added a solution of selenium dioxide (100mg) in acetic acid (2.6ml) and the mixture heated on a steam bath for 1 hr. After cooling and separation of deposited selenium by filtration, the mixture was diluted with water and the precipitated solid collected and dried. It was then taken up in light petroleum (50ml) and filtered through a column of alumina [Woelm, basic, 3g] employing a further 150 ml of light petroleum to complete the elution of crystalline 3β-methoxy-D:C-friedooleana-7,9(11)-diene (II) having m.p. 226-228°. Found: C,84.6; H,11.47; $C_{31}H_{50}O$ requires C, 84.84; H, 11.49%. λmax 232, 239 and 248mμ (ε-16,100,16,800 and 9,500).

3β-Methoxy-D:C-friedoursa-7,9(11)-diene (I)

To a solution of selenium dioxide (150mg) in acetic

acid (2.5ml) was added a suspension of bauerenol methyl ether (150mg) [prepared from bauerenol as described in section IV] in glacial acetic acid (25ml) and the mixture heated on the steam bath for 1 hr. After cooling, filtration from the deposited selenium, and dilution with water, a crystalline solid separated. This was washed thoroughly with water and then dissolved in light petroleum, b.p. 40-60° (50ml) and filtered through alumina [Woelm, basic, 3g]. Elution of the column with further light petroleum (100ml) and combination of the total eluants afforded, after removal of solvent, 3β-methoxy-D:C-friedoursa-7,9(11)-diene(I) (100mg) which on recrystallisation from ethyl acetate had m.p. 182-183° λmax 232, 239.5, 248mμ (ε16,600, 17,400 and 10,200). Found: C,84.2; H,11.5; $C_{31}H_{50}O$ requires C,84.84; H,11.49%.

3-β-methoxy-fern-7, 9(11)-diene (X).

To a solution of the epoxide of arundoin (25mg) in chloroform (5ml) was added concentrated sulphuric acid (1ml) and water (3ml) and the mixture agitated on a mechanical shaker at room temperature for 48 hr. The organic layer was separated, washed with sodium bicarbonate solution, and then with water, dried over anhydrous sodium sulphate and finally taken to dryness. The residue was taken up in light petroleum b.p. 40-60° and chromatographed over alumina [Woelm, neutral 2g]. The initial eluants obtained with light petroleum

afforded colourless needles of 3β-methoxy-fern-7, 9(11)-diene (X) m.p. 228-229° $[\alpha]_D$=-154.5° (C=1.5), λ max. 232,239 247.5mμ (ε15,500, 16,900, 9,900) Found: C,84.76; H,11.47; calculated for $C_{31}H_{50}O$: C,84.84; H,11.49%. Literature[17] m.p. 228-231° $[\alpha]_D$=-157° ($CHCl_3$).

BIBLIOGRAPHY.

1. Eglinton, Hamilton and Martin-Smith, Phytochemistry, 1962, 1, 137.

2. R.J. Hamilton, Ph.D. Thesis, University of Glasgow, 1962.

3. Ruzicka, Proc. Chem. Soc., 1959, 341.

4. Bladon, Henbest and Wood, J. Chem. Soc., 1952, 2737.

5. Eg. Smith, Smith and Spring, Tetrahedron, 1958, 4, 111.

6. Dr. S..J. Smith personal communication.

7. Reed and De Mayo, Chem. and Ind., 1956, 1481; Budzikiewicz and Djerassi, J. Amer. Chem. Soc., 1962, 84, 1430.

8. Djerassi, Budzikiewicz and Wilson, Tetrahedron Letters, 1962, 263.

9. Barton, Warnhoff and Page, Chem. and Ind., 1954, 220.

10. Ageta, Iwata and Natori, Tetrahedron Letters, 1964, 3413.

11. Budzikiewicz, Wilson and Djerassi, J. Amer. Chem. Soc., 1963, 85, 3688.

12. Lakey and Leeding, Proc. Chem. Soc., 1958, 342.

13. Sengupta and Khastgir, Tetrahedron, 1963, 19, 123.

14. Vorbrüggen, Pakrashi and Djerassi, Liebigs Annalen, 1963, 668, 57.

15. Eglinton, Hamilton, Martin-Smith, Smith and Subramanian, Tetrahedron Letters, 1964, 2323.

16. Ohmoto, Nishimoto, Ito and Natori, Chem. Pharm. Bull., Tokyo, 1965, 13, 224.

17. Nishimoto, Ito, Natori and Ohmoto, Tetrahedron Letters, 1965, 2245.

18. Dawson, Halsall and Swayne, J. Chem. Soc., 1953, 590.

APPENDIX