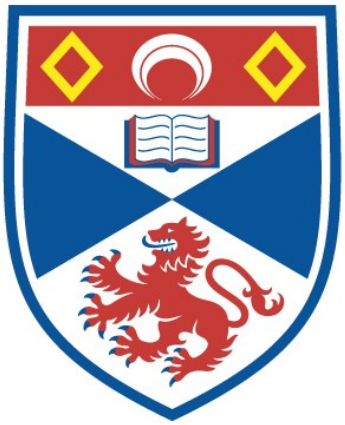
STUDIES OF SOME FATTY OILS

Anthony John Sealy

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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STUDIES OF SOME FATTY OILS

being a Thesis

presented by

ANTHONY JOHN SEALY, B.Sc.

to the

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in application for

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DECLARATION

I hereby declare that the following Thesis is based on results of experiments carried out by me, that the Thesis is my own composition, and that it has not been presented previously for a Higher Degree.

The research was carried out in the Chemical Research Laboratories of the United College in the University of St. Andrews, under the direction of Dr. F. D. Gunstone.

CERTIFICATE

I hereby certify that Mr. Anthony John Sealy has spent eleven terms at research work under my supervision, has fulfilled the conditions of Ordinance 16 (St. Andrews), and that he is qualified to submit the accompanying thesis in application for the degree of Doctor of Philosophy.

Research Supervisor.

UNIVERSITY CAREER

I entered the United College of St. Salvator and St. Leonard, University of St. Andrews, in October 1956 and was awarded a Harkness Residential Scholarship in October 1957. I pursued a recognised course for graduation in Science and graduated B. Sc. with Second Class Honours in Chemistry in 1960.

I was admitted as a Research student in September 1960 and was awarded a D.S.I.R. studentship which I held until September 1963.

ACKNOWLEDGMENTS

I wish to record my sincere thanks to Dr. F.D. Gunstone for his able guidance, constant interest and encouragement throughout this work. I am also grateful for his help and advice in many matters.

I also wish to thank Mr. M. Zochowski for carrying out the infra-red determinations, and Mr. T. Norris for his help from time to time.

Thanks must also be expressed to Dr. S.H. Bell, D.S.I.R. Paint Research Station, for the gift of isano oil; Mr. Peter Davis, Warden of the Bird Observatory, Fair Isle, Shetland, for the fulmar oil; Mr. J.L. Mowat, Curator, University Botanic Garden, St. Andrews, for the gifts of Conium maculatum and Heracleum mantegazzianum; Messrs. T.B. Lock and Sons Ltd., Yeovil, Somerset, for supplying the other seeds of the Umbelliferae which were examined and Dr. L.J. Morris, Brunel College, for obtaining a sample of petroselinic acid.

Finally I would like to express my thanks to the D.S.I.R. for financial assistance throughout the work.

Paper based on part of this work accepted for publication

Fatty Acids (XII) The Acetylenic Acids of Isano (Boleko) Oil

(F.D.Gunstone and A.J.Sealy, J. Chem. Soc., 1963)

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Part III.

Seed Oils of the Umbelliferae

SUMMARY

I.

The seed of the tree Onguekoa Gore (Olacaceae) contains nine acetylenic C_{18} acids comprising three quarters of the total glyceride acids. Four of these acids also contain a hydroxyl group attached to $C_{(8)}$. 8-hydroxyoctadec-trans-11-en-9-ynoic acid is isolated for the first time from Santalum album seed oil.

II.

The stomach oil of the fulmar petrel (Fulmarus glacialis) is shown to be similar to a typical fish oil; acids containing 14 - 22 carbon atoms are present, including docosahexaenoic and eicosapentaenoic acids.

III.

The classical method of analysis of the seed oils of the Umbelliferae was unsatisfactory and a more accurate method is now devised.

ISANO OIL

Isano (Boleko) Oil

Isano oil, also called boleko oil or ongokea oil, is derived from the nut Onguekoa Gore Engler specie (syn. Ongokea kleineana Pierre) (Olacaeae). The trees, which grow abundantly in Equatorial Africa, produce nuts which are one third shell and two thirds kernels. The kernels contain 60 % of an oil which turns red on exposure to daylight and explodes violently on heating.

Although the oil is highly unsaturated it does not dry when exposed to the atmosphere as a thin film and hence commercial development has been slow.

Isano oil is soluble to a limited extent in light petroleum, ethanol and hexane but is completely miscible with benzene, acetone, diethyl ether, carbon tetrachloride and chloroform.[1,2]

The degree of unsaturation of isano oil is not accurately measurable by Wijs or Hanus iodine value methods because of conjugated triple bonds.[2] The theoretical iodine value as determined by hydrogenation is 316.

This oil has been the subject of several investigations, but when the present work was undertaken there was no general agreement on its chemical nature. The early work will be reviewed, and since this shows that the oil contains acetylenic and hydroxy acetylenic acids, and our present work confirms this view, the natural occurrence of such acids in vegetable fats will also be summarised.

Fatty Acids of Isano Oil

Saturated

The presence of small amounts (2 - 3 %) of saturated acids has been reported by several workers.[3,4,5,6] Sehor[7,8,9] isolated stearic acid from the oil, while Castille[10] considered caproic, caprylic, lauric, palmitic, stearic and arachidic acids in the oil.

Olefinic C_{18} Acids

The presence of oleic, elaidic and linoleic acids has been reported.[4,6,7]

Isanic (Erythrogenic) Acid

Octadec-17-en-9,11-diynoic acid (m.pt. 40°C) is unusual in that it is one of the few acids occurring naturally as glycerides which contains an acetylenic group and also a vinyl group. Early workers[11,12,13] assigned the names isanic or erythrogenic acid to this highly unsaturated acid and Castille[10] suggested two possible structures (I and II) for this acid, based on his observations that catalytic reduction gave stearic acid after absorption of hydrogen (5 mols.) and that ozonolysis yielded formaldehyde and formic, oxalic and adipic acids.

I $CH_2=CH.(CH_2)_4.C\equiv C.C\equiv C.(CH_2)_7.COOH$

II $CH_2=CH.C\equiv C.(CH_2)_4.C\equiv C.(CH_2)_7.COOH$

These deductions were confirmed by Steger and van Loon[14] who proved that isanic acid was I and Castille[15] showed dihydroxyisanic acid to be 17,18-dihydroxyoctadec-9,11-diynoic acid. Further evidence for this structure came from Armitage, Cook, Entwistle, Jones and Whiting[16] who considered the ultra-violet spectrum reported by Castille was inconsistent with structure II.

Black and Weedon[17] have synthesised isanic acid by the oxidative coupling of oct-1-en-7-yne and ω-decynoic acid.

The content of this acid in isano oil as reported by various workers is summarised in Table I.

Bolekic Acid

Meade[18] noticed that natural isanic acid exhibited a different ultra-violet spectrum from the synthetic acid as prepared by Black and Weedon. The absorption bands characteristic of a diyne are present together with absorption bands at longer wavelengths characteristic of an enediyne chromophore. He isolated this impurity (2 - 3 %) by its inability to form a urea complex at -6°C. Bolekic acid is hydrogenated to stearic acid adsorbing 4·9 mols. of hydrogen and shows cis unsaturation by infra-red absorption.

Dupont, Dulou and Pouliquen[3] isolated bolekic acid by chromatographing isano oil on a silicic acid column. These workers stated the acid to be octadec-7-en-9,11-diynoic acid.

Table I

Composition of Isano (Boleko) Oil

References	2	3	4	5	6	7	10	12	18
Acid %									
Saturated	-	3	5	3	s	a	a*	2	-
Unsaturated	-	-	-	-	-	-	-	98	-
Isanic	46	48	41	-	A	a	a	-	35
Isanolic	44	36	40	-	44	a	-	-	a
Bolekic	9	3	-	-	-	-	-	-	a
Bolekoic	-	4	-	-	-	-	-	-	5
Elaidic	-	-	-	-	-	a	-	-	-
Linolenic	-	6	2	-	s	a	-	-	-
Ethylenic	-	-	-	9	-	-	-	-	-
Hydroxy-acetylenic	-	-	-	40-50	-	-	-	-	-
Nonhydroxy-acetylenic	-	-	-	30-40	-	-	-	-	-
Others	-	-	4	-	-	-	-	-	-

a = acid present, but percentage not given.

s = small amount present.

A = appreciable amount present.

a* = includes caproic, caprylic, lauric, palmitic, stearic and arachidic acids.

Isanolic Acid

From the hydrogenated mixed esters of isano oil Steger and van Loon[14] obtained a hydroxy acid (m.pt. 75 - 78°C) which they considered to be a monohydroxy stearic acid not identical with 12-hydroxy stearic acid. Riley[19] obtained a concentrate of the hydroxy acid by partition of the mixed acids at 0°C. Hydrogenation of this acid gave 8-hydroxy stearic acid whose structure was proved by oximation, Beckmann rearrangement and acid hydrolysis of the keto acid. Kaufmann, Baltes and Herminghaus[6] assigned the structure 8-hydroxyoctadec-17-en-9,11-diynoic acid based on a comparison of the ultra-violet spectra of isanic and isanolic acids but failed to realise that both spectra were contaminated with a small percentage of an enediyne chromophore.

Meade[18] showed that two derivatives of 8-hydroxy stearic acid, containing a diyne and an enediyne chromophore respectively, were present.

Bolekoic Acid

Seher[8] isolated from isano oil a hydroxy acid to which he assigned the structure 8-hydroxyoctadec-14-en-10,12-diynoic acid, based on degradation products obtained from ozonolysis of the methyl ester of the keto acid.

Natural Acetylenic Acids of Glyceride Origin

Although the number of natural acetylenic compounds is very large,[1,20] the number occurring as triglycerides is fairly small. The majority

of these have been discovered in the past two decades, only two acetylenic acids being known prior to 1940; many of them were reported only during the course of this investigation.

Tariric Acid

Octadec-6-ynoic acid (tariric) was first noted by Arnaud[21] in 1892 as a constituent of the seed oil tariric (bitterbush), Picramnia Sow. (family Simarubaceae). Steger and van Loon[22] reported this acid to comprise 95 % of the total fatty acids.

Ximenynic Acid

Octadec-trans-11-en-9-ynoic acid occurs in the seed oils of the Ximenia genus (20 - 25 %) of the Olacaceae family[24,25] and in several members of the Santalum genus (40 - 50 %) of the Santalaceae.[26,27,28] When hydroxylated with performic acid it gives 11,12-dihydroxyoctadec-9-ynoic acid.

Octadec-trans-11-trans-13-dien-9-ynoic Acid was isolated from the crude acids from the bark of Ximenia americana roots by reversed phase chromatography.[28] The acid is also present in the cotyledons of the mature plants, Sweet Quandong, Santalum Acuminatum D.C.[31]

Octadec-trans-13-en-9,11-diynoic Acid

Although the seed fat of Exocarpus cupressiformis Labill

(Santalaceae) contains 60 % of ximenynic acid as glycerides, the roots of the tree contain a fat with octadec-trans-13-en-9,11-diynoic acid forming 59 % of the mixed acids.[29] The structure of this acid was assigned on the following evidence: hydrogenation effects the addition of hydrogen (5 moles) and gives pure stearic acid. Oxidation with neutral permanganate gave valeric, oxalic and azelaic acids. The infra-red absorbs strongly at 955 cm.$^{-1}$ showing the ethylene bond is trans.

Octadec-trans-12-trans-14-dien-8,10-diynoic Acid

This acid was isolated from the somatic lipids from the seed fat of the Leptomeria aphylla R.Br.[28] (Santalaceae). The structure was proved by mild oxidation of the maleic anhydride adduct when suberic acid was the main dicarboxylic acid formed. The isomeric octadec-13,15-dien-9,11-diynoic acid is present in the root lipids of Sweet Quandong, Santalum Acuminatum D.C.[31]

Octadec-15-en-9,11,13-triynoic Acid

This acid is present in the root lipids of the mature plants of Sweet Quandong, Santalum Acuminatum D.C. and is accompanied by smaller amounts of octadec-15,17-dien-9,11,13-triynoic acid and possibly oxygenated derivatives of octadec-15-en-9,11,13-triynoic acid.[31]

Hydroxy Acids of Glyceride Origin

Long chain hydroxy acids are not common component acids of seed fats. However they occur frequently, and sometimes in large amounts, in wool wax, insect waxes, bacterial waxes, waxes of coniferous plants, brain lipids, etc..

Ricinoleic Acid or (+)-12-hydroxyoctadec-cis-9-enoic acid is the best known hydroxy alkenoic acid. It was first isolated by Saalmüller[32] in 1848 from castor oil in which it is the principal constituent (91 -95 % of the fatty acids). The present structure was first assigned by Goldsobel[33] in 1894 and was confirmed by Walden[34] in the same year, and several workers since then.[35]

Lesquerolic Acid (+)-14-hydroxyeicos-11-enoic acid is present in Lesquerella lasiocarpa (40 - 45 %) and L. lindheimerii (51- 72 %). The pure methyl ester was obtained by fractionation of the mixed esters between acetonitrile - hexane.[36] The position of the double bond was determined by von Rudloff oxidation of o-acetyl lesquerolic acid. Infra-red evidence indicated the hydroxyl group was β to the double bond.

9-hydroxyoctadec-cis-12-enoic Acid was first shown by Gunstone[37] to be present in the seed oil of Strophanthus sarmentosus (family Apocynaceae) in which it forms 6·6 % of the fatty acids. This acid seems to occur generally throughout the Strophanthus genus.[38]

Densipolic Acid, 12-hydroxyoctadec-cis-9-cis-15-dienoic acid, is a major constituent (50 %) of Lesquerella densipila (Cruciferae).[39] The structure was deduced chemically by oxidative degradation and corroborated by n.m.r. spectra.

Dimorphecolic Acid, Artemisic Acid

Dimorphecolic acid, 9-hydroxyoctadec-trans-10-trans-12-dienoic acid, is the chief constituent fatty acid (48 %) of the Dimorphotheca aurantiaca seed oil (Compositae).[40] The structure of this acid was confirmed by Fontell[41] who showed also that other species - Artemisia Cosmos and Helianthus (Compositae), Calliandra (Leguminosae), and Balanites (Zygophyllaceae) - contain a mixture of 9-hydroxyoctadec-trans-10-cis-12-dienoic acid and 13-hydroxyoctadec-cis-9-trans-11-dienoic acid (Artemisic acid).

The seed oil of the Tragopon porrifolus (Compositae) also contains about 4 % of the conjugated diene hydroxy acids[42] identified as 9-hydroxyoctadec-10,12-dienoic acid and 13-hydroxyoctadec-9,11-dienoic acid. The double bond configuration was either cis-trans or trans-cis.

Ximenynolic Acid, 8-hydroxyoctadec-trans-11-en-9-ynoic acid, occurs as a minor component in Ximenia caffra oil.[43] Isolated by solvent partition, the acid was characterised by its ultra-violet absorption, oxidation and hydrogenation to 8-hydroxy stearic acid.

Kamlolenic Acid, 18-hydroxyoctadec-cis-9-trans-11-trans-13-trienoic acid, is the major constituent of kamala oil Mallotus Philippinensis

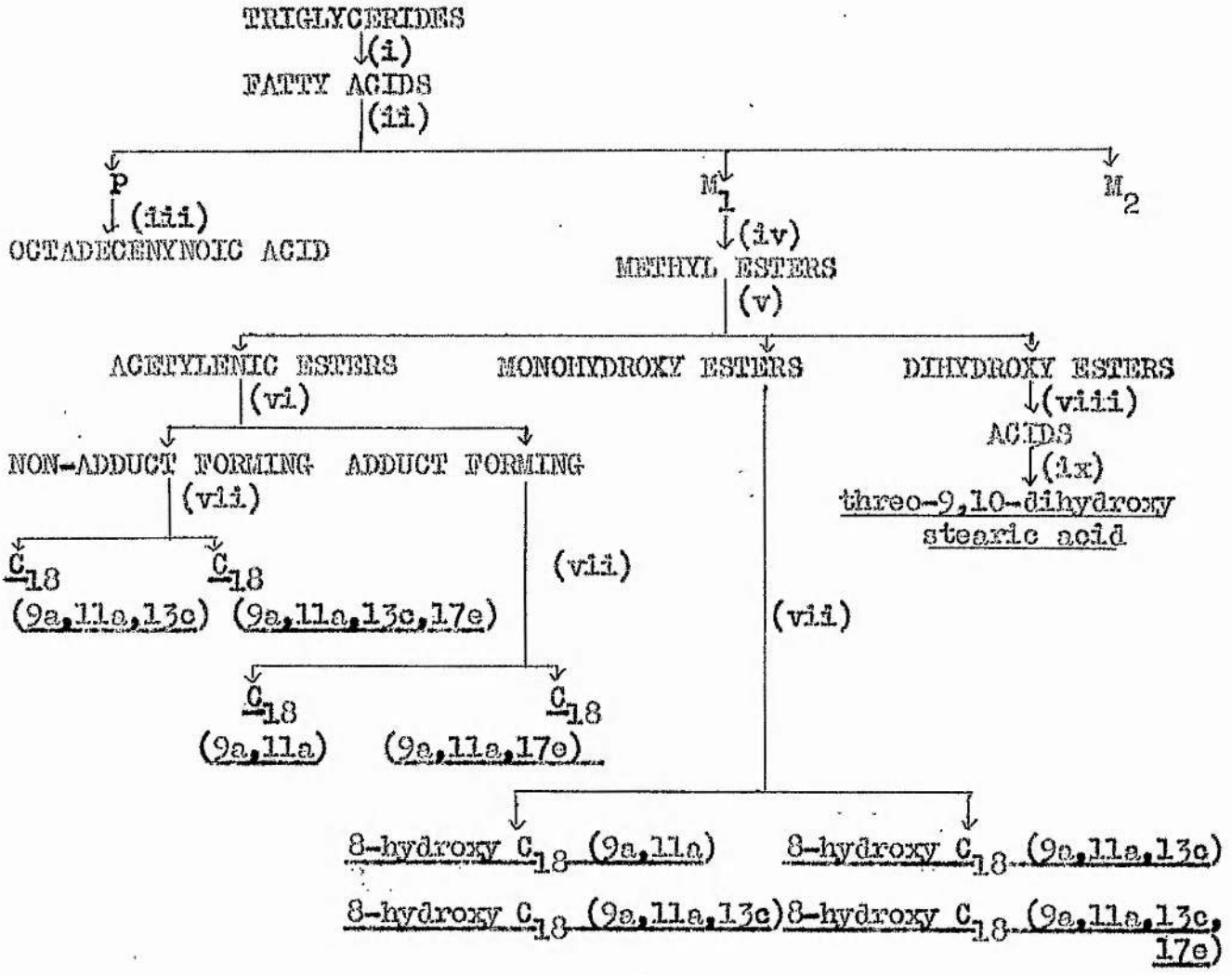
(Euphorbiaceae). It comprises up to 60 % of the total fatty acids of this oil. The structure was proved by Calderwood and Gunstone[44] and Gupta et alia.[45]

More recently, Hatt and Redcliffe[46] showed the acid to be present in the seed fats of the Australian species of Mallotus, namely M. discolor and M. claoxyloids.

The first known dihydroxy acid occurring in seed fats was 9,10-dihydroxy stearic, isolated from castor oil (Ricinis communis L.) in 1925 by Eibner and Munzing[47] and since then by a number of others in amounts varying from 0·6 to 2·4 %. The natural acid is optically active and melts at 141°C.

Three-12,13-dihydroxyoctadec-9-enoic acid has been reported to occur in the seed oil of Cephalocroton cordofanus (Euphorbiaceae)[48] and in some of the Vernonia seed oils (Compositae)[49]. Tulloch[50] has recently presented evidence to show that an epoxide-hydrolysing enzyme system is present in the uredospores of Puccinia graminis. Incubation of the spores results in a partial conversion of 9,10-epoxyoctadecanoic acid to (+)-three-9,10-dihydroxyoctadecanoic acid. A similar enzyme may be present in the oils of the Cephalocroton and Vernonia genera.

Scheme of Analysis of Isano Oil



The symbols indicate acetylenic bond (a), cis-ethylenic bond(c), and ethylenic bond of unspecified configuration (e).

(i) Saponification, extraction of unsaponifiable material and acidification.
(ii) Partition between light petroleum and aqueous methanol.
(iii) Reversed phase chromatography.
(iv) Methylation (MeOH - HCl).
(v) Neutral alumina chromatography.
(vi) Urea fractionation.
(vii) $AgNO_3$ - silicic acid chromatography.
(viii) Hydrolysis and acidification.
(ix) Recrystallisation from ethyl acetate.

DISCUSSION

The separation of isano oil into the component acids has been achieved by a combination of partition between light petroleum and aqueous methanol, chromatography on a column of alumina and of silica impregnated with silver nitrate, and urea fractionation. (See page 11.)

Isano oil was hydrolysed and the unsaponifiable material (6 %) was removed. The mixed acids were divided into three fractions by partition between light petroleum and 80 % aqueous methanol.[51] The acids from each fraction were subsequently methylated and the methyl esters were sampled on a Pye Argon Chromatograph. The petrol fraction (P, 50 %) contained saturated, olefinic and some acetylenic acids, a methanol fraction, (M_2, 12 %) contained acetylenic (80 %) and monohydroxy acids (20 %), another methanol fraction (M_1, 38 %) was almost entirely mono and dihydroxy acids.

The possibilities of reversed - phase chromatography as a means of separating acids was first demonstrated by Howard and Martin.[52] The mobile phase is aqueous acetone and the stationary phase is liquid paraffin contained on a column of kieselguhr which has been made non-wetting by treatment with dichlorodimethylsilane. The acids of the petrol fraction, (P), were loaded on to a column and eluted with three portions of 48 % aqueous acetone. The first fraction contained C_{18} enediyne and C_{18} diyne acids, whilst the second fraction contained approximately 50 % of an enyne acid recognised by its ultra-violet absorption spectrum and its behaviour on the gas-liquid chromatograph.

This method of separation was eventually abandoned in view of the time taken to run a column and the relatively small loads (100 mg.) that could be separated.

The M_1 esters were adsorbed on a column of neutral alumina[54] and separated into non-hydroxy esters (M_1A_1, 10 %, eluted with benzene), monohydroxy esters (M_1A_2, 80 %, eluted with ether), and dihydroxy esters (M_1A_3, 8 %, eluted with ether containing 5 % of methanol). The purity of each fraction was determined by thin-layer and gas-liquid chromatography.

Examination of ultra-violet and infra-red absorption spectra of the non-hydroxy fraction M_1A_1 showed it to contain a mixture of diyne and cis-enediyne esters. The cis-olefinic group makes this molecule non-linear thereby reducing the likelihood of forming a urea adduct. The non-hydroxy esters (M_1A_1), submitted to repeated crystallisation with urea at $0^{\circ}C$, gave a non-adduct-forming fraction ($M_1A_1U_1$, 10 %), rich in enediyne esters, and an adduct-forming fraction ($M_1A_1U_2$, 90 %), of almost pure diynoic esters.

The ability of olefins to form co-ordination complexes with certain metal ions has long been recognised, and the compounds formed between the silver ion in particular and a variety of unsaturated hydrocarbons have been studied extensively by Lucas and his co-workers.[55] Dutton[56] et alia separated methyl oleate and methyl elaidate by counter-current distribution in the system isooctane/0·2M silver nitrate in 90 % methanol, and de Vries[57] was able to separate cis and trans isomers by chromatography on a column of silicic acid impregnated with silver nitrate. This adsorbent has also been used

in thin-layer chromatography.[53] The fractions $M_1A_1U_1$ and $M_1A_1U_2$ were further separated, on a column of silica impregnated with silver nitrate into esters containing, and esters not containing, a vinyl group. The separations were monitored by infra-red spectroscopy, which was used to detect the vinyl group; in some fractions absorption at 1645 $cm.^{-1}$ was practically nil, in others it was such that the ratio of the intensity of absorption at the vinyl peak to that of the ester peak (1739 $cm.^{-1}$) was close to that measured with methyl undec-10-enoate. Gas-liquid chromatography was used only in a limited way because these closely-related esters in the M_1A_1 fraction were separated into two peaks only, one due to esters containing the diyne chromophore and the other due to esters containing the enediyne chromophore. These separation procedures yielded four non-hydroxy acids.

Octadec-cis-13-en-9,11-diynoic Acid

The u.v. spectrum showed $\lambda_{max.}$ 214·5, 227, 239, 252·5, 267, 282·5 mμ ($E^{1\%}_{1cm.}$ 1180, 112, 175, 317, 478, 378.) The i.r. spectrum showed bands at 2248 $cm.^{-1}$ (C≡C, St) and 1695 $cm.^{-1}$ (C=O, St). The absorption at 985 $cm.^{-1}$ was zero, indicating the double bond to be cis. Hydrogenation afforded stearic acid, ozonolysis gave valeraldehyde, and oxidation, with potassium permanganate and potassium periodate, gave valeric and nonandioic acids.

Octadec-cis-13,17-dien-9,11-diynoic Acid

The u.v. spectrum showed $\lambda_{max.}$ 214·5, 227, 239, 252·5, 267, 282·5 mμ

($E^{1\%}_{1cm.}$ 1250, 118, 184, 334, 504, 398.) The i.r. spectrum showed bands at 2248 cm.$^{-1}$ (C≡C,St), 1690 cm.$^{-1}$ (C=O,St), and 1645 cm.$^{-1}$ (CH_2=CH,St). Hydrogenation gave stearic acid, ozonolysis gave succindialdehyde, and oxidation gave succinic and nonandioic acids.

Octadec-9,11-diynoic Acid

The u.v. spectrum showed $\lambda_{max.}$ 227, 239, 252·5 mμ. ($E^{1\%}_{1cm.}$ 13, 12, 4.) The i.r. spectrum showed bands at 2248 cm.$^{-1}$ (C≡C,St) and 1695 cm.$^{-1}$ (C=O,St). Hydrogenation gave stearic acid and oxidation, with potassium permanganate and potassium periodate, gave a mixture of heptanoic and nonandioic acids.

Octadec-17-en-9,11-diynoic Acid

The u.v. spectrum showed $\lambda_{max.}$ 227, 239, 252·5 mμ. ($E^{1\%}_{1cm.}$ 13, 12, 4.) The i.r. spectrum showed bands at 2248 cm.$^{-1}$ (C≡C,St), 1695 cm.$^{-1}$ (C=O,St) and 1645 cm.$^{-1}$ (CH_2=CH,St). Hydrogenation afforded stearic acid and oxidation, with potassium permanganate and potassium periodate gave hexandioic and nonandioic acids.

Monohydroxy Esters (M_1A_2)

The monohydroxy esters, after hydrogenation, when examined on the gas-liquid chromatograph, showed three peaks with carbon numbers[59] 18·0, 19·3, 19·6, which are identified as stearate, ketostearate and hydroxystearate. These assignments were based on the known chromatographic behaviour of these esters and on the changes in the hydrogenated esters when oxidised, reduced, and acetylated. The three components

of the hydrogenated M_1A_2 esters were separated on a column of neutral alumina. Light petroleum and benzene (9:1) eluted methyl stearate, benzene eluted methyl ketostearate, and ether and benzene (1:1) eluted methyl hydroxystearate. The ketostearate and an authentic sample of methyl 12-ketostearate showed infra-red absorption peaks at 1727 cm.$^{-1}$ and 1698 cm.$^{-1}$ due to ester and carbonyl groups respectively, while only the former peak is present in the infra-red absorption curve of the hydroxy ester.

The hydroxystearate was oxidised to ketostearate, and this and the methyl ketostearate isolated from isano oil, were separately treated with hydroxylamine and sodium acetate, and then with sulphuric acid.[19] Under these conditions an oxime is formed which undergoes Beckmann rearrangement and is then hydrolysed. In each case octandioic and undecanoic acids were recognised by gas-liquid chromatography, showing that the acids present in the hydrogenated M_1A_2 fraction are 8-hydroxystearic and 8-ketostearicacids. Similar conclusions were obtained by oxidation of these acids with potassium permanganate[60] in acetic acid and identification of the products by gas-liquid chromatography.

The ketostearate present after hydrogenation is considered to be produced during the hydrogenation of the unsaturated hydroxy esters and not to arise from unsaturated keto ester. It has been reported previously that reduction of unsaturated hydroxy acid gives some keto acid,[61] presumably through double bond migration. Methyl ketostearate results during the catalytic hydrogenation of methyl ricinoleate,

methyl ricinstearolate and methyl 8-hydroxyxlmenynate (isolated for the first time from Santalum Album seed oil), and from the M_1A_2 fraction after reduction with sodium borohydride. In addition, attempts to separate keto compounds from the unhydrogenated esters by Girard's reagent were completely unsuccessful.

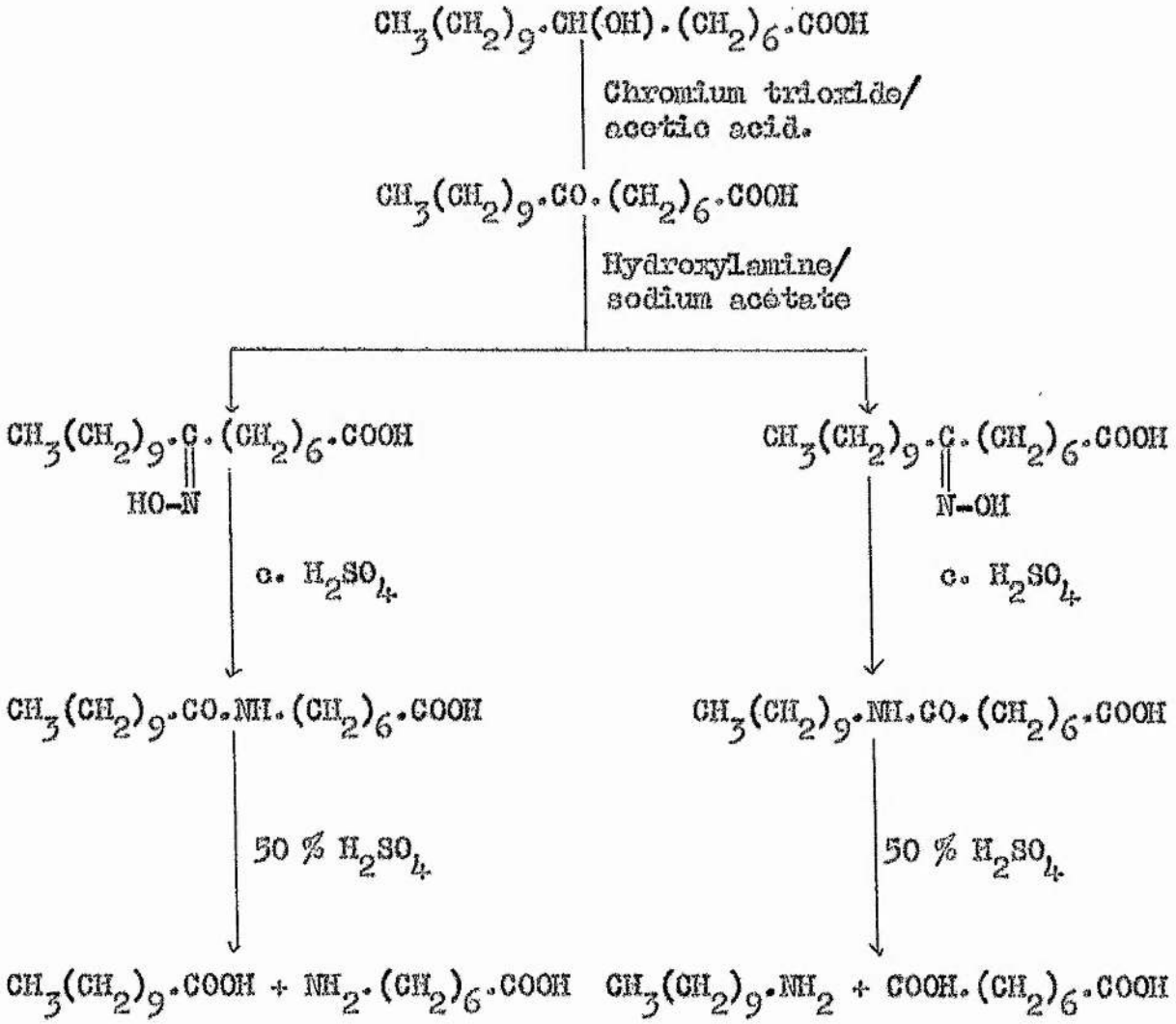
The M_1A_2 fraction showed the characteristic enediyne chromophore to the extent of 9 %. Separation of the hydroxy esters, into a fraction containing the enediyne chromophore, and a fraction not containing this chromophore, by urea fractionation, was unsuccessful. The silica-silver nitrate column separates the hydroxy esters into vinyl and non-vinyl fractions, but each fraction still contains some hydroxy enediynoic ester.

The non-vinyl fraction was found to contain 8-hydroxyoctadecendiynoic acid and 8-hydroxyoctadec-9,11-diynoic acid, based on the following evidence. The u.v. spectrum showed $\lambda_{max.}$ 214·5, 227, 239, 252·5, 267, 282·5 mμ. ($E^{1\%}_{1cm.}$ 212, 37, 45, 52, 67, 52.) The i.r. produced bands at 2248 $cm.^{-1}$ (C≡C,St) and 1695 $cm.^{-1}$ (C=O,St). Hydrogenation afforded mainly 8-hydroxystearic acid and oxidation with potassium permanganate and potassium periodate gave a mixture of heptanoic and octandioic acids.

The vinyl fraction was found to contain an 8-hydroxyoctadecdiendiynoic acid and 8-hydroxyoctadec-17-en-9,11-diynoic acid, based on the following evidence. The u.v. spectrum showed $\lambda_{max.}$ 214·5, 227, 239, 252·5, 267, 282·5 mμ. ($E^{1\%}_{1cm.}$ 197, 35, 46, 57, 70, 57.) The i.r. produced bands at 2248 $cm.^{-1}$ (C≡C,St), 1695 $cm.^{-1}$ (C=O,St) and 1645 $cm.^{-1}$

Table III

Scheme of Hydroxy Group Position Determination



(CH_2=CH,St). Hydrogenation afforded 8-hydroxystearic acid, and oxidation with potassium permanganate and potassium periodate gave hexandioic and octandioic acids.

The shorter chain degradation fragments obtained from the oxidation of the small amount of conjugated enediynoic acid in both fractions could not be adequately recognised but the hydroxydiendiynoic acid in the vinyl fraction can only be the 9a,11a,13c,17e or the 10a,12a,14c,17e - C_{18} acid, and the former structure is preferred. By analogy, the hydroxyendiynoic acid in the non-vinyl fraction is probably the 9c,11a,13c - C_{18} acid.

In this connection, ricinoleic and ricinstearolic acids were degraded with potassium permanganate and potassium periodate to investigate the behaviour of β unsaturated hydroxy acids to these reagents. Ricinstearolic acid gave only two products, identified by gas-liquid chromatography and infra-red spectroscopy as nonandioic and 3-hydroxynonanoic acids. Ricinoleic acid gave a mixture of products, the main components being heptanoic, nonandioic and 3-hydroxynonanoic acids. A β-hydroxy dibasic acid was not recognised among the products of degradation of the isano hydroxy acids.

Further evidence for the hydroxyl being α to the unsaturated systems in the conjugated enediyne acids is presented by Morris.[62] The near infra-red spectrum showed a sharp symmetrical band due to hydroxyl stretching at 3611 $cm.^{-1}$ with no trace of a band at 3580 $cm.^{-1}$ Compounds with unsaturated centres $\beta\gamma$ to a hydroxyl group show a free hydroxyl band near 3625 $cm.^{-1}$ and an associated hydroxyl band near 3580 $cm.^{-1}$ due to intramolecular hydrogen bonding of the hydroxyl

group to the π-electron system of the unsaturated bond, whereas vicinally unsaturated hydroxy compounds show only a single associated band.

Seher[8] considered isano oil to contain 8-hydroxyoctadec-12-en-8,10-diynoic acid. After Oppenauer oxidation of the petrol-insoluble mixed esters, he isolated the unsaturated keto ester by Girard's reagent, purified it on an alumina column, ozonised it, and recognised butyraldehyde, glyoxal and half ester of 3-ketononandioic acid as degradation products.

$$CH_3.(CH_2)_2.CH{=}CH.C{\equiv}C.C{\equiv}C.CH_2.\underset{\underset{O}{\|}}{C}.(CH_2)_6.COOCH_3$$

$$\downarrow$$

$$CH_3.(CH_2)_2CHO + OHC.COOH + HOOC.COOH + HOOC.CH_2.\underset{\underset{O}{\|}}{C}.(CH_2)_6.COOCH_3$$

The material submitted to ozonolysis represented 14 % of the total esters; no evidence for an acid of this structure in such a large amount was found during this investigation. The two hydroxy enediynoic acids of uncertain structure in the oil examined did not exceed 3 % of the total.

Other reactions carried out to settle the structures of the two hydroxy enediynoic acids gave inconclusive results, mainly because of the difficulty of recovering and recognising small amounts of reaction products from reactants which were always more than 90 % diyne acids and less than 10 % enediynoic acids.

Since long-chain acids containing a hydroxyl group α to the double bond are readily dehydrated by hot glacial acetic acid,[40] the reaction was carried out on the M_1A_2 fraction as a possible means of

distinguishing between $\alpha\beta$ and $\beta\gamma$ unsaturated alcohols. After reaction, no change in the ultra-violet spectrum could be observed even though most of the fraction is known to consist of the $\alpha\beta$ acetylenic hydroxyl compounds.

$$-C\equiv C.C\equiv C.CH(OH)CH_2 \xrightarrow{\text{glacial acetic acid}} -C\equiv C.C\equiv C.CH=CH-$$

$$\downarrow H_2/\text{Lindlar catalyst}$$

$$-CH=CH.CH=CH.CH(OH)CH_2 \xrightarrow{\text{glacial acetic acid}} -CH=CH.CH=CH.CH=CH-$$

It appears that acetylenic hydroxyl compounds differ from their ethylenic analogues in this respect, for after semi-hydrogenation in the presence of Lindlar's catalyst,[63] the ester was dehydrated and the product showed strong triene but no tetraene absorption. It is uncertain whether this indicates that the enediyne acids have their hydroxyl groups $\beta\gamma$ to the unsaturation, or whether, in the partial hydrogenation, the small amounts of hydroxyenediynoic esters have been reduced beyond the hydroxytriene stage.

Lithium aluminium hydride reduces $\alpha\beta$ acetylenic alcohols to trans-olefinic alcohols but does not react with $\beta\gamma$ acetylenic alcohols.

$$-C\equiv C-C\equiv C-CH(OH)- \xrightarrow{LiAlH_4} -C\equiv C-CH=CH-CH(OH)-$$

$$-CH=CH-C\equiv C-C\equiv C-CH(OH)- \xrightarrow{LiAlH_4} -CH=CH-C\equiv C-CH=CH-CH(OH)-$$

$$-CH=CH-C\equiv C-C\equiv C-(CH_2)_n-CH(OH)- \xrightarrow{LiAlH_4} -CH=CH-C\equiv C-C\equiv C-(CH_2)_n-CH(OH)-$$

When the hydroxy esters (M_1A_2) were reduced with lithium aluminium hydride there was spectroscopic evidence of a new enyne chromophore resulting from the diynoic hydroxy esters, but it was impossible to decide whether there had been any change from the enediyne to a dienyne chromophore as the positions of the absorption maxima of these two chromophores are very similar.

The oxidation of the hydroxy esters with chromium trioxide-pyridine[40] and manganese dioxide[64] has also been examined. Manganese dioxide is a selective reagent, oxidising only α/β unsaturated alcohols to α/β unsaturated ketones.

$$-CH{=}CH-C{\equiv}C-C{\equiv}C-CH(OH)- \xrightarrow[CrO_3\text{-pyr}]{MnO_2} -CH{=}CH-C{\equiv}C-C{\equiv}C.\overset{O}{\overset{\|}{C}}-$$

$$-CH{=}CH-C{\equiv}C-C{\equiv}C-(CH_2)_n-CH(OH)- \xrightarrow{CrO_3\text{-pyr}} -CH{=}CH-C{\equiv}C-C{\equiv}C-(CH_2)_n.\overset{O}{\overset{\|}{C}}-$$

Although both of these reagents oxidised all the hydroxy esters to keto esters, (the absence of any residual hydroxy esters being confirmed by infra-red absorption and thin-layer chromatography), the ultra-violet absorption indicated the presence of a $-C{\equiv}C-C{\equiv}C-C{=}O$ but not of any $-CH{=}CH-C{\equiv}C-C{\equiv}C-C{=}O$. The absorption spectrum of the $-CH{=}CH-C{\equiv}C-C{\equiv}C-C{=}O$ chromophore is not recorded in the literature, but Bohlmann and Herbst[65] have reported the spectrum of $CH_3.(C{\equiv}C)_2.CH{=}CH.CHO$.

The silica-silver nitrate column separated the keto esters into a vinyl and a non-vinyl fraction and each fraction, after hydrolysis, and oxidation with potassium permanganate and potassium

periodate, gave the same degradation products as for unsaturated hydroxy esters. After hydrogenation the keto esters were examined by gas-liquid chromatography showing four peaks due to stearate, ketostearate, hydroxystearate and an unknown compound of carbon number '18·6' (Apiezon L) and '19·1' (QF-I). This ester was unaffected by sodium borohydride reduction and was isolated by adsorption chromatography on alumina as a liquid of molecular formula $C_{19}H_{36}O_3$. Iodination, followed by reduction with activated zinc in the presence of anhydrous methanolic hydrogen chloride, gave methyl stearate[67]; oxidation by chromium trioxide in acetic acid gave mainly octandioic and nonandioic acids. This evidence suggests that the unknown ester is probably a 5- or 6-membered oxygen heterocycle.

The Dihydroxy Ester

The fraction M_1A_3, rich in dihydroxy esters, was hydrolysed. Crystallisation of the product from ethyl acetate[66] at 0°C gave three-9,10-dihydroxystearic acid. This did not depress the melting point of an authentic sample, and was oxidised by potassium permanganate to nonanoic and azelaic acids. This dihydroxy acid, comprising 2 % of the mixed acids, has not previously been reported from the seed oil but may be related to the epoxy acid discovered by Morris.[62]

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Isano oil is seen to be an unusual seed oil in that it contains major proportions of five C_{18} acetylenic acids (51 %), four 8-hydroxy C_{18} acetylenic acids (22 %), and three-9,10-dihydroxystearic acid (2 %). The results of a quantitative study[53] of this oil are summarized in Table IV.

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Table IV

Component Acids (%-wt) of Isano Oil

	%-wt.
Saturated acids	
(C_{14} 1%, C_{16} 4%, C_{18} 1%)	6
Olefinic acids	
(oleic 14%, linoleic 5%)	19
Acetylenic acids	
(9a,11e 1%; 9a,11a 10%; 9a,11a,17e 32%; 9a,11a,13e 2%; 9a,11a,13e,17e 6%)	51
8-Monohydroxyacetylenic acids	
(9a11a 4%; 9a,11a,13e 1%; 9a,11a,17e 15%; 9a,11a,13e,17e 2%)	22
9,10-Dihydroxystearic acid	2

EXPERIMENTAL

Thin-layer Chromatography

Thin layers of kieselgel G (according to Stahl) were applied to plates (20x5 cm.) in a thickness of 250 μ by a commercial applicator. The plates, dried and activated by heating at 110°C for half an hour, were stored in a desiccator.

0·01 ml. of a chloroform solution (10 %) of the ester mixtures was applied to the plates 1-2 cm. apart on a line 1-2 cm. from the bottom of the plate. The plates were developed by ascending elution in sealed gas jars containing eluting solvent to a depth of 0·5 cm. The jars were lined with solvent-soaked filter paper to give a vapour-saturated atmosphere in the jar. The spots were made visible by exposure to iodine vapour.

Silver nitrate impregnated plates.

These plates were impregnated when required by spraying with a saturated methanolic solution of silver nitrate. After spraying, the plates were dried for an hour in an oven at 110°C. Samples were applied, and the plates were developed as described previously. The spots were made visible by spraying with a 0·2 % solution of 2',7'-dichlorofluorescein in 98 % ethanol, and viewing under ultra-violet light.

Gas-liquid Chromatography

Gas chromatography of the methyl esters was carried out on a Pye Argon Chromatograph with a Sr^{90} β ray ionisation detector. The glass columns, which were 4 feet long, were packed with either 5 % or 10 % Apiezon L grease on alkali-washed celite or 10 % Dow-Corning fluoro-alkyl silicone polymer (QF-I) on untreated celite. The columns were operated at 100 -200°C with an argon flow rate of 35 ml. per minute. Samples either as liquids or as ether solutions were injected by stopping the gas flow, removing the gas lead, discharging the sample from a 0·025 or 0·1 μl. pipette, replacing the gas lead and restoring the argon flow. Retention times were measured from the negative air peaks and the results expressed as carbon numbers[59] which were found from a straight line plot of log. V_R for a range of suitable standards against chain length of the saturated acids.

Acidic products were converted to the methyl esters by refluxing for 2 hours with a twenty-fold excess of dry methanolic hydrogen chloride (0·5 N). At the end of this time water was added and the methanolic solution was extracted with ether. The ether was washed with 0·5 N sodium bicarbonate to remove mineral acid, then with water; it was dried over anhydrous sodium sulphate.

Ultra-violet absorption was measured on a Unicam SP700 using methanolic solutions, and infra-red absorption was measured on thin films with a Grubb-Parsons GS2A double beam grating instrument.

Hydrolysis of Isano Oil

The oil (30 g.) was refluxed with 0·5 N alcoholic potassium hydroxide (400 ml.) for one hour in an atmosphere of nitrogen. Water (2 volumes) was added and the unsaponifiable matter (6 %) was extracted with ether. The fatty acids (27 g.) were liberated by acidification with 5N hydrochloric acid and extracted with ether.

Partition of the Mixed Acids between Aqueous Methanol (1:4) and Light Petroleum[51]

Light petroleum (1100 ml.) and aqueous methanol (800 ml.) were equilibrated by shaking together in a separating funnel.

To 500 ml. of light petroleum in funnel 1 was added 33·20 g. of mixed acids, and 200 ml. of the same solvent was placed in funnels 2,3 and 4. Aqueous methanol (200 ml.) was added to the first funnel and after equilibration was passed to each of the other three funnels in turn. This was followed by three similar portions of aqueous methanol. The distribution of the acids was as follows:-

	Light petroleum solutions	Methanol extracts	
No. of fraction	1, 2, 3, 4	4, 3, 2,	1
Weight	16·52 g. (50 %)	4·15 g. (12 %)	12·53 g. (38 %)

Table V

Carbon Numbers		Fractions			Identity
Apiezon L	QF-I	M_1	M_2	P	
12·0	12·0	-	-	+	Lauric
14·0	14·0	-	-	+	Myristic
16·0	16·0	-	-	+	Palmitic
17·6	18·0	-	-	+	Linoleic
17·8	18·0	-	-	+	Oleic
18·0	18·0	-	-	+	Stearic
18·5	-	-	-	+	Ximenynic
19·3	21·0	+	+	+	C_{18} 9a,11a,13c
					C_{18} 9a,11a,13c,17c
19·7	22·0	+	+	+	C_{18} 9a,11a
					C_{18} 9a,11a,17e
20·9	23·65	+	+	-	Hydroxy acids

+ present - absent

The symbols indicate acetylenic bond (a), cis-ethylenic bond (c) and ethylenic bond of unspecified configuration (e).

Ultra-Violet Spectra of Three Fractions

	$\lambda_{max.}$ mμ.	214·5	227	239	252·5	267	282·5
$E^{1\%}_{1cm.}$	Methanol(1); M_1	260	66·7	72	78	89	80
	Methanol(2,3,4); M_2	295	63	69	87	109	89
	Petrol(1,2,3,4); P	152	32	35	37	50	41

The acids in each fraction were subsequently methylated and sampled on the gas-liquid chromatograph at 200°C. (See Table V).

Separation of the Acids of the Petrol Fraction by Reversed Phase Chromatography[52]

Materials

Non-wetting kieselguhr was prepared by exposing to the vapours of dichlorodimethylsilane in a partially evacuated desiccator. The siliconised material was washed free of acid with methanol and dried at 110°C. Liquid paraffin was neutralised by filtration through a column of alumina.

The stationary phase was liquid paraffin on siliconised kieselguhr (7:5).

The mobile phase was a range of aqueous acetones.

Preparation of a Column

75 g. of the liquid paraffin/kieselguhr was poured into the column (35x1·3 cm.) as a slurry with 70 % aqueous acetone. The mixture of fatty acids (100 mg.) was mixed into a slurry with paraffin impregnated kieselguhr and 35 % aqueous acetone. Before loading the acids 10 ml. of 35 % aqueous acetone was run on to the column.

Eluting Solvent	Weight (mg.)	Carbon Numbers (G.L.C.)
(1) 48 % Acetone (250 ml.)	41	19·3,19·7.
(2) 48 % Acetone (250 ml.)	20	18·5,19·3,19·7.
(3) 70 % Acetone (250 ml.)	37	12·0,14·0,16·0, 17·8,17·6,18·0.

The ultra-violet absorption maxima in mμ fractions (1) and (2) are recorded below. Values of $E^{1\%}_{1cm.}$ are in parentheses.

(1) $\lambda_{max.}$ 214·5 (290), 227 (62), 239 (67), 252·5 (86), 267 (101), 282·5 (87).

(2) $\lambda_{max.}$ 229 (290).

Separation of the M_1A_1 Esters into Two Fractions by Urea Fractionation

Urea (13 g.) was added to a solution of the M_1A_1 esters (2·6g.) in hot methanol (78 ml.). The solution was left to crystallise at 0°C, and the adduct filtered off and washed with methanol previously saturated with urea. Water (150 ml.) was added to the filtrate, the esters were extracted with ether (3x50 ml.), and the ethereal extracts washed with water, dried (sodium sulphate) and evaporated. After repeating the above process five times, a fraction ($M_1A_1U_1$, 250 mg.) of esters enriched in enediyne chromophore was obtained.

The adduct was decomposed with water, extracted with ether (2x50 ml.), and the ethereal extracts were washed with water, dried (sodium sulphate) and evaporated, yielding liquid esters ($M_1A_1U_2$, 2·24g.).

$\lambda_{max.}$ mμ.	214·5	227	239	252·5	267	282·5
$E^{1\%}_{1cm.}$ $M_1A_1U_1$	1250	118	184	334	504	398
$E^{1\%}_{1cm.}$ $M_1A_1U_2$	-	13	12	4	-	-

Separation of the Urea Fractions by Silver Nitrate - Silicic Acid Column Chromatography

Preparation of Silver Nitrate - Silicic Acid Adsorbent[57]

Silicic acid (100.g.) was suspended in aqueous solution of silver nitrate (200 ml.) - analytical grade (500 g. silver nitrate per litre). This mixture was heated for 30 minutes at 100°C, and after cooling to room temperature, the mass was filtered off on a Buchner funnel, and dried for sixteen hours at 120°C. After grinding, the adsorbent was ready for use. It contains 0·27 g. silver nitrate/g.

Preparation of the Column

A mixture of adsorbent (10 g.), Hyflo Super Cel (5 g.) and light petroleum (50 ml.) was heated to boiling for 5 minutes while stirring. After cooling to room temperature, the slurry was brought into the chromatography tube. The column was cooled with tap water (17°C) and shielded from light.

A solution of methyl esters which did not form an adduct with urea (250 mg.) in light petroleum / ether (4:1, 5 ml.) was added to the column. Elution was carried out with a further 100 ml. of this solvent.

Fraction	Solvent(ml.)	Weight(mg.)	% Vinyl as shown by I.R.
1	10	-	-
2	10	15	-
3	10	30	-
4	10	14	-
5	10	1	-
6	10	10	100
7	10	41	100
8	10	80	100
9	10	46	100
10	10	5	100

The purity of each fraction was checked by thin-layer chromatography on silver nitrate impregnated plates.

Fractions 1 - 5, and also fractions 6 - 10, were combined.

$\lambda_{max.}$	mμ.	214·5	227	239	252·5	267	282·5
$E^{1\%}_{1cm.}$	(1-5)	1180	112	175	317	478	378
$E^{1\%}_{1cm.}$	(6-10)	1250	118	184	334	504	398

A similar separation was carried on the fraction of the esters (M_1A_1) forming an adduct with urea.

Fraction	Eluant(ml.)	Weight(mg.)	% Vinyl as shown by I.R.
1	10	-	-
2	10	17	-
3	10	29	-
4	10	11	-
5	10	2	-
6	10	14	100
7	10	36	100
8	10	82	100
9	10	39	100
10	10	5	100

Ultra-violet Absorption

$\lambda_{max.}$	mμ.	227	239	252·5
$E^{1\%}_{1cm.}$	(1-5)	13	12	4
$E^{1\%}_{1cm.}$	(6-10)	12·5	11·5	4

von Rudloff Oxidation of Unsaturated Acids

The esters were hydrolysed by refluxing with a 100 % excess of 0·5N sodium hydroxide for ½ hour in an atmosphere of nitrogen.

The acids (60 mg.) were dissolved in a solution of potassium carbonate (380 mg.) in water (32 ml.) and a solution of potassium periodate (1300 mg.) and potassium permanganate (15 mg.) in water (64 ml.) was added. The mixture was shaken for 24 hours at room temperature and the excess oxidant destroyed with sulphur dioxide.

(When products of low molecular weight were expected, this solution was neutralised with potassium carbonate and reduced in volume to 10 ml. on a rotary film evaporator.) The acidified solution was saturated with sodium chloride and was extracted with ether (5x30 ml.) Evaporation of the ether afforded the acids (69 mg.) which were esterified with methanolic hydrogen chloride and examined by gas-liquid chromatography on both Apiezon L and QF-I columns at appropriate temperatures.

Ester	Vinyl group (%)	U.V. absorption $\lambda_{max.}$	$E^{1\%}_{1cm.}$	Degradation products mono.	di.	Structure (C_{18} acids)
		214·5,227,239,252·5,267,282·5				
$M_1A_1U_1$	Nil	1180,	112,175,317, 478,378	C_5	C_9	9a,11a,13c
$M_1A_1U_1$	100	1250	118,184,334,504, 398	-	C_4C_9	9a,11a,13c,17e
$M_1A_1U_2$	Nil		13, 12, 4	C_7	C_9	9a,11a,
$M_1A_1U_2$	100		13, 12, 4	-	C_6C_9	9a,11a,17e

a - acetylenic bond

c - cis-ethylenic bond

e - ethylenic bond of unspecified configuration

The infra-red absorption in the 960 $cm.^{-1}$ region was zero showing that the Δ^{13} ethylenic bond in acids $M_1A_1U_1$ is cis.

The Hydroxy Esters in Fraction M_1A_2

The esters (250 mg.) of fraction M_1A_2 in methanol (30 ml.) were hydrogenated by shaking in an atmosphere of hydrogen for 24 hours, using a 10 % palladium / charcoal (50 mg.) as catalyst. The catalyst was filtered off and the filtrate evaporatedto dryness, affording the saturated esters (244 mg.).

A solution of the saturated esters (250 mg.) in light petroleum (10 ml.) was adsorbed on neutral alumina (15 g.) on a column (24x1·2)cm.).

Eluant (50 ml.)	Weight (mg.)	Melting point (lit.)[68] ester	acid
Light petroleum	25	38·5-39° (37·8)	71·2-71·7° (70·1)
Benzene	38	45·5-46° (46·5-46·9)	83-83·5° (83·6-83·8)
Ether/Benzene (1:1)	158	55-55·5° (55·3-55·6)	80·5-81° (81·5-81·7)

All these fractions showed an ester peak (1727 $cm.^{-1}$) in their infra-red spectra but only the second fraction a carbonyl peak at 1698 $cm.^{-1}$.

Oxidation of the Hydrogenated M_1A_2 Esters with Chromium Trioxide

A 10 % solution of chromium trioxide in acetic acid (0·24 ml.) was added to the esters (60 mg.) in acetic acid (0·6 ml.). The initial reaction was exothermic and was moderated by cooling in running water. After 30 minutes at room temperature, the mixture was poured into water and the excess oxidant destroyed with sulphur dioxide. The product was extracted with ether (3x30 ml.). The ethereal extracts, after washing with sodium carbonate and water, were dried (sodium sulphate) and the solvent evaporated affording the esters (57 mg.).

Reduction of the Hydrogenated M_1A_2 Esters

Sodium borohydride (4mg.) was added to a solution of the esters (50 mg.) in 95 % methanol (0.5 ml.). After ten minutes, water (5 ml.) was added and the solution heated to boiling. The reduced esters (47 mg.) were recovered by ether extraction.

Acetylation of the Hydrogenated M_1A_2 Esters

The methyl esters (50 mg.) were refluxed for two hours with a 1:3 mixture of acetic anhydride and pyridine (5 ml.). The solution was poured into water (30 ml.) and extracted with ether (2x50 ml.). The ethereal extracts, after washing several times with dilute hydrochloric acid and water, afforded a clear oil (46 mg.).

	Carbon Numbers (Apiezon L)			
Hydrogenated M_1A_2	18·0	19·3	19·6	
After CrO_3 / acetic acid	18·0	19·3		
After sodium borohydride	18·0		19·6	
Acetylation	18·0	19·3		19·75
Identity	stearate	keto stearate	hydroxy stearate	acetoxy stearate

Oxidation of 8-hydroxystearic Acid with Potassium Permanganate[60]

To the hydroxy esters (40 mg.) dissolved in Analar acetic acid (1 ml.), powdered potassium permanganate (200 mg.) was added portionwise so that the temperature did not exceed 50°C. After three hours at 50°C the acetic acid was removed under reduced pressure. The residue was diluted with water (10 ml.) containing some sulphuric acid and decolourised with sulphur dioxide.

The resulting monobasic acids were extracted with light petroleum (2x50 ml.). Evaporation of the solvent gave acids (15 mg.) which on esterification (methanolic hydrogen chloride) and sampling on the gas-liquid chromatograph gave peaks corresponding to undecanoic and decanoic acids with minor amounts of nonanoic and octanoic acids.

The dibasic acids were extracted with ether (3x50 ml.) after saturating the solution with salt. Esterification of the residue (5 mg.) after evaporation of the solvent, and sampling on the gas-liquid chromatograph gave peaks corresponding to octandioic and heptandioic acids, accompanied by minor amounts of shorter chain dibasic acids.

Oximation, Beckmann Rearrangement and Hydrolysis of the Keto Esters[19]

A solution of methyl esters of 8-ketooctadecanoic acid (95 mg.), hydroxylamine hydrochloride (60 mg.), sodium acetate (90 mg.) and water (0·2 ml.) in ethanol (2 ml.) was boiled for two hours. Water (4 ml.) was then added and the solution extracted with ether. Evaporation of the ether afforded mixed oximes (90 mg.), which were heated in concentrated sulphuric acid (1 ml.) for one hour at 100°C, the solution diluted with water (1 vol.), and refluxed for four hours. The mixture was further diluted with water (20 ml.) and extracted with ether (5x20 ml.). Evaporation of the ether gave a mixture (79 mg.) of undecanoic and octandioic acids, identified by esterification (methanolic hydrogen chloride) and sampling on the gas-liquid chromatograph.

The Separation of the Methyl Esters M_1A_2 on a Silver Nitrate - Silicic Acid Column

The column was prepared as for the non-hydroxy esters M_1A_1. (see page 32)

The methyl hydroxy esters (M_1A_2, 250 mg.) in a solution of light petroleum / ether (3:2, 5 ml.) were adsorbed on the column and eluted with this solvent mixture (100 ml.).

Fraction	Eluant(ml.)	Weight(mg.)	% Vinyl as shown by I.R.
1	10	1	-
2	10	20	-
3	10	19	-
4	10	7	-
5	10	17	100
6	10	30	100
7	10	57	100
8	10	44	100
9	10	28	100
10	10	20	100

The purity of each fraction was checked by thin-layer chromatography on silver nitrate - impregnated silicic acid plates.

Fractions 1-4, and also fractions 5-10, were combined. Both fractions were hydrolysed and the acids oxidised by potassium permanganate and potassium periodate. The products were esterified (methanolic hydrogen chloride) and the results obtained are shown below.

Fraction	U.V. absorption $\lambda_{max.}$ mμ. $E^{1\%}_{1cm.}$						Degradation products (acids)		Structure (8-hydroxy C_{18} acids)
	214·5,	227,	239,	252·5,	267,	282·5	Mono.	Di.	
1 - 4	212	37	45	52	67	52	C_7	C_8	9a,11a.
5 - 10	197	35	46	57	70	57		C_6 C_8	9a,11a,17e

a - acetylenic bond, e - ethylenic bond of unspecified configuration.

Attempted Dehydration of the Hydroxy Esters $(M_1A_2)^{40}$

The hydroxy esters (100 mg.) were heated with glacial acetic acid (12 ml.) for four hours, water (50 ml.) added, the product extracted with ether (50 ml.) and the ether extracts washed several times with water to remove the acetic acid.

The ultra-violet absorption spectrum of the product showed no change from that of the starting material.

Reduction of M_1A_2 with Hydrogen / Lindlar's Catalyst

The hydroxy esters (330 mg.) in ethyl acetate (10 ml.) containing quinoline(50 mg.) were semi-hydrogenated to olefinic esters by shaking in an atmosphere of hydrogen with Lindlar's catalyst (300 mg.). Filtration of the catalyst and removal of the solvent gave olefinic esters (289 mg.), which were successfully dehydrated with acetic acid as shown by the following spectroscopic results.

After reduction	$\lambda_{max.}$ at 235 mμ.	($E^{1\%}_{1cm.}$ 725)
After reduction and dehydration	$\lambda_{max.}$ at 265, 275 and 287 mμ.	($E^{1\%}_{1cm.}$ 1245)

Reduction of M_1A_2 esters with Lithium Aluminium Hydride.

A solution of the hydroxy esters (300 mg.) in dry ether (25 ml.) was added dropwise to lithium aluminium hydride (300 mg.) suspended in dry ether (25 ml.) and the mixture was refluxed for two hours and allowed to stand overnight. Sufficient wet ether was added to decompose the excess lithium aluminium hydride, and the

mixture extracted with 10 % sodium hydroxide to remove inorganic matter. The ethereal solution, after drying (sodium sulphate) and evaporation, yielded alcohols (286 mg.) $\lambda_{max.}$ 229 mμ. ($E^{1\%}_{1cm.}$ 637), 240 (inflexion).

Oxidation of the M_1A_2 Esters with Chromium Trioxide / Pyridine

A solution of the hydroxy esters (600 mg.) in dry pyridine (3·5 ml.) was added to a solution of chromium trioxide (700 mg.) in dry pyridine (7 ml.). The mixture was shaken for 24 hours at room temperature, and was then diluted with water and extracted with ether (3x30 ml.). The combined ether extracts were washed with 2N hydrochloric acid and then water, dried over sodium sulphate, and evaporated. The product, a dark reddish oil (558 mg.), was purified by filtration through a column of neutral alumina.

The infra-red absorbed at 1727 $cm.^{-1}$ and 1667 $cm.^{-1}$ due to an ester and α/β unsaturated keto carbonyls respectively.

Ultra-violet absorption	214·5	227	240	252·5	267	282·5 mμ
$E^{1\%}_{1cm.}$ after oxidation	303	136	157	186	228	193
$E^{1\%}_{1cm.}$ before oxidation	201	55	56	61	68	56

Preparation of Manganese Dioxide

A solution of manganese sulphate (111 g.) in water (150 ml.) and a solution of sodium hydroxide (40 %, 117 ml.) were added simultaneously during one hour to a hot stirred solution of potassium permanganate (960 g.) in water (600ml.). Manganese dioxide was precipitated soon after the start as a fine brown solid. Stirring was continued for a further hour and the solid was then filtered and washed with water until the washings were colourless. The solid was dried in an oven at 120°C and ground to a fine powder (90 g.) before use.

Oxidation of the M_1A_2 fraction with Manganese Dioxide[64]

The hydroxy esters (620 mg.) were stirred with manganese dioxide (6·2 g.) in benzene (100 ml.) for three hours. After filtration of the manganese dioxide, evaporation of the benzene yielded keto esters (612 mg.).

The infra-red absorbed at 1727 $cm.^{-1}$ (ester carbonyl) and 1667 $cm.^{-1}$ ($\alpha\beta$ unsaturated ketone).

Ultra-violet absorption	214·5	227	239	252·5	267	282·5	mμ.
$E^{1\%}_{1cm.}$ after oxidation	300	134	155	184	223	190	
$E^{1\%}_{1cm.}$ before oxidation	201	55	56	61	68	56	

Thin-layer chromatography of the keto esters showed only one spot on an untreated plate but two spots on a plate impregnated with silver nitrate.

Hydrogenation of the Keto Esters

The keto esters were hydrogenated and the product was (a) reduced with sodium borohydride and (b) oxidised with chromium trioxide / acetic acid. Each product, sampled on the gas-liquid chromatograph, gave the results summarised below.

		Carbon Numbers			
Hydrogenated	(Apiezon L)	18·0	18·6	19·3	19·6
keto esters	(QF - I)	18·0	19·1	23·2	22·0
After sodium borohydride	(Apiezon L)	18·0	18·6	-	19·6
reduction	(QF - I)	18·0	19·1	-	22·0
After chromium trioxide	(Apiezon L)	18·0	-	19·3	-
acetic acid oxidation	(QF - I)	18·0	-	23·2	-

The hydrogenated keto esters (250 mg.) after sodium borohydride reduction were loaded on to a column of neutral alumina (15 g.) and separated into three fractions.

Eluant	Volume(ml.)	Weight(mg.)	M.Pt.	
Light petroleum	100	10	38·5-39°	stearate
Benzene	100	52	liquid	unknown
Benzene / Ether (1:1)	100	179	54·5-55°	hydroxy-stearate

The infra-red spectrum of the unknown showed no unusual features. (Found: C,72·4; H,11·2. Calc. for $C_{19}H_{36}O_3$: C,73·0; H,11·5%) When treated with chromium trioxide / acetic acid it was degraded, mainly to octandioic and heptandioic acids.

Chain - Length Determination of the Unidentified Oxidation Product[67]

The esters (50 mg.) were heated with iodine (250 mg.) and red phosphorus (600 mg.) for one hour at 100°C. After dilution with water (50 ml.) and extraction with ether (2x50 ml.), the ether solution was washed with a 5 % solution of sodium hydrogen sulphite, dried and evaporated. The product was dissolved in 10 ml. of a 5 % solution of methanolic hydrogen chloride and boiled with activated zinc for four hours. Filtration, dilution with water (50 ml.) and extracted with ether (2x50 ml.) afforded methyl stearate (42 mg.).

threo-9,10-dihydroxystearic Acid

The esters (M_1A_3) were hydrolysed with an excess of 0·5N methanolic sodium hydroxide. The recovered acid (29 mg.) was dissolved in ethyl acetate (1·5 ml.), cooled to 0°C and allowed to stand overnight.[66] The dihydroxy acid (14 mg.) was filtered off and recrystallised from more ethyl acetate. It melted at 94-94·5°C alone or when mixed with an authentic sample of threo-9,10-dihydroxy-stearic acid. Oxidation with potassium permanganate and potassium periodate gave nonanoic and nonandioic acids, which were recognised, after esterification, by gas-liquid chromatography.

The Hydroxy Acid in Santalum Album

The evergreen tree Santalum Album (Linn.) of the Santalaceae genus is well known for its highly scented wood (sandalwood) which is used extensively throughout India. The tree varies in height from twelve to thirty-five feet, and bears fruit twice a year. Gunstone and Russell[69] showed the oil to contain 88 % of ximenynic glyceride and the mixed acids (excluding unsaponifiable material) to contain 95 % of ximenynic acid.

This present work demonstrates the presence of 1 % of a 8-hydroxyoctadec-trans-11-en-9-ynoic acid.

The dried seeds, $\frac{1}{4}$" in diameter, had an average weight of 0·15 g. and gave a pale greenish-yellow oil (53·5 %) when extracted with light petroleum. The oil was hydrolysed by boiling with 0·5N alcoholic potassium hydroxide for one hour, during which time a gum separated; this was rejected before saponification and ether extraction of the mixed acids, which were subsequently divided into two fractions by partition between light petroleum and 80 % aqueous methanol.[51] The petrol fraction (91 %) contained mainly octadec-trans-11-en-9-ynoic acid together with small amounts of palmitic and oleic acids. The aqueous methanol fraction (9 %) contained octadec-trans-11-en-9-ynoic acid and a hydroxy acid. The methyl esters of the methanol fraction were adsorbed on a column of neutral alumina[54] and separated into methyl octadec-trans-11-en-9-

ynoate (85 %, eluted with benzene) and a hydroxy ester (15 %, eluted with ether). These fractionations were monitored by gas-liquid chromatography.

Hydrogenation of this monohydroxy ester gave a mixture of methyl stearate, 8-hydroxystearate, and 8-ketostearate separated by adsorption on a column of neutral alumina. The position of the oxygenated function was determined by converting through ketones, oximes, and amides to a mixture of degradation fragments.[19] Ozonolysis of the unsaturated hydroxy ester in methanol at -40°C, followed by reduction of the ozonides by shaking in an atmosphere of hydrogen in the presence of Lindlar's catalyst gave heptanal, which was identified as its dimethyl acetal on the gas-liquid chromatograph.[70] The ultra-violet absorption spectrum was characteristic of an enyne chromophore and infra-red absorption at 955 $cm.^{-1}$ showed the ethylenic bond to be trans. This evidence is consistent with the structure 8-hydroxyoctadec-trans-11-en-9-ynoic acid. This acid has been previously reported to accompany ximenynic acid in Ximenia caffra oil.[43]

EXPERIMENTAL

Isolation of 8-hydroxyoctadec-trans-11-en-9-ynoic Acid

Santalum Album seed oil (12 g.) was hydrolysed by a 100 % excess of 0·5N boiling alcoholic potassium hydroxide, and the mixed acids were partitioned between petroleum ether and 80 % aqueous methanol. The acids in each extract were subsequently methylated (anhydrous methanolic hydrogen chloride) and both fractions were examined on a gas-liquid chromatograph using a 10 % Apiezon L / Celite column at 200°C, with the results shown below.

Carbon Number	Petroleum Ether Fraction (91 %)	Aqueous Methanol Fraction (9 %)	Identity
16·0	+	-	Palmitic
17·7	+	-	Oleic
18·5	+	+	Ximenynic
19·9	-	+	Hydroxy-ximenynic
20·0	+	-	(Arachidic)

(+ present, - absent)

Separation of the Methyl Esters of the Methanol Fraction on Neutral Alumina

A solution of the methyl esters (250 mg.) in benzene (5 ml.) was loaded on a column of neutral alumina (15 g.) and the esters were separated into two fractions.

Eluant	Volume(ml.)	Weight(mg.)	$\lambda_{max.}$ mμ.	$E^{1\%}_{1cm.}$	
Benzene	50	196	229	512	Ximenynic
Ether	50	35	229	448	Hydroxy-ximenynic

The infra-red spectrum of the ether extract showed bands at 960 cm.$^{-1}$ (trans double bond), 1735 cm.$^{-1}$ (ester carbonyl) and 3620 cm.$^{-1}$ (hydroxyl).

Ozonolysis of the Hydroxy Ester

A solution of the esters (50 mg.) in methanol (30 ml.) was ozonised at -40°C with a stream of ozonised oxygen (2·5 - 3·0 %) obtained by silent high tension electric discharges. The effluent gas was bubbled through a solution of potassium iodide and starch in dilute sulphuric acid. The reaction was stopped when the starch indicator turned blue and pure nitrogen was bubbled through the reaction mixture to remove dissolved oxygen and ozone. Lindlar's catalyst (50 mg.) was added to the solution which was shaken in an atmosphere of hydrogen for thirty minutes. Filtration of the catalyst and evaporation of the solvent afforded a mixture of aldehydes and acids (45 mg.).

The mixture of aldehydes and acids was methylated[70] by refluxing for two hours with 2 % anhydrous methanolic hydrogen chloride. After cooling, the methanolic solution was neutralised by the addition of a slight excess of anhydrous sodium carbonate. The

product was extracted with light petroleum (3x50 ml.) from the methanol. The light petroleum solution, after shaking with saturated sodium bisulphite and water and drying over sodium sulphate, was evaporated, affording a mixture of acetals and esters (46 mg.). The mixture was refluxed for two hours with 0·5N methanolic sodium hydroxide to convert the methyl esters to sodium salts. The solution was cooled, diluted with water and the acetals extracted with light petroleum (3x50 ml.). The light petroleum, after washing with water and drying (sodium sulphate), was evaporated affording the dimethylacetal of heptanal (14 mg.), which was identified by gas-liquid chromatography.

Hydrogenation of the Hydroxy Ester

The hydroxy ester (25 mg.) in methanol (10 ml.) was hydrogenated by shaking in an atmosphere of hydrogen for twenty-four hours, using 10 % palladium / charcoal (5 mg.) as a catalyst. The catalyst was filtered off and the filtrate evaporated, affording the saturated esters (22 mg.).

A solution of the saturated esters (22 mg.) in light petroleum (5 ml.) was adsorbed on a column of neutral alumina (15 g.).

Eluant	Volume(ml.)	Weight(mg.)	M.Pt.	Infra-red absorption	Methyl Ester
Light petroleum	50	2	38-39°	1727 cm.$^{-1}$	Stearate
Benzene	50	2	45·5-46°	1727, 1698	Keto-stearate
Ether / Benzene(1:1)	50	16	55°	1727 cm.$^{-1}$	Hydroxy-stearate

Oximation, Beckmann Rearrangement and Hydrolysis of the Keto Esters[19]

The hydroxy esters were oxidised as previously (see Page 37) to the keto esters.

A solution of the keto esters (15 mg.), hydroxylamine hydrochloride (10 mg.), sodium acetate (15 mg.), and water (0·1 ml.) in ethanol (1 ml.) was boiled for two hours. Water (4 ml.) was added and the solution extracted with ether. Evaporation of the ether gave mixed oximes (12 mg.) which were heated in concentrated sulphuric acid (1 ml.) for one hour at 100°C, the solution diluted with water (1 vol.), and refluxed for four hours. The mixture was further diluted with water (10 ml.) and extracted with ether (5x20 ml.). Evaporation of the ether gave a mixture (11 mg.) of undecanoic and octandioic acids, identified by esterification (methanolic hydrogen chloride) and sampling on the gas-liquid chromatograph.

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FULMAR OIL

INTRODUCTION

The fulmar petrel[5] (Fulmarus glacialis) breed in enormous numbers in St. Kilda and the Orkney and Shetland islands, but have also been reported as far south as Cornwall. In 1878 it was estimated that twelve fulmars were breeding in Britain; by 1949 that number had risen to 1,800,000. They are purely oceanic wanderers, and seldom, if ever, come to land, except for the purpose of breeding. When disturbed the bird ejects, as a defensive measure, with considerable force, some of the oil stored in the stomach. The oil is a clear yellow colour and begins to solidify at 10°C and finally sets to a translucent solid at 0°C. It is reported that this bird also uses its stomach oil for preening and courtship and to calm rough seas when landing on the ocean. Fulmar oil has recently been used medicinally[1] and it seemed of interest to discover whether there was anything unusual about the composition of the oil.

The oil has previously been examined by Rosenheim and Webster[2] and by Lovern[3] but these results do not agree. Rosenheim and Webster considered that the oil was not a glyceride but a liquid wax containing, after hydrolysis, nearly forty per cent of unsaponifiable material, which consisted mainly of unsaturated higher alcohols of the same type as those found in sperm oil. The fatty acids had an iodine value of 156, and 20·9 % of the acids gave an insoluble bromide which was shown to be the decabromide of a docosapentaenoic acid.

Lovern has also investigated the body oil of the fulmar and

the results he obtained are summarised below.

Number of specimens = 6 Fat content = 15·2 %

Iodine value of the oil = 130·2 Non-saponifiable material = 7·8 %

Component Acids

Saturated (per cent)

C_{10}	C_{14}	C_{16}	C_{18}	C_{20}
3	2	13·9	3·2	nil

Unsaturated (per cent)

C_{14}	C_{16}	C_{18}	C_{20}	C_{22}	C_{24}
0·9	3·9	26·9	26·8	22·1	trace
	(-2·0H)	(-2·8H)	(-4·0H)	(-6·6H)	

DISCUSSION

The present study of fulmar oil was carried out in three stages:

(a) Qualitative examination of the oil and the component acids - isolation and recognition of the major unsaturated components.

(b) Quantitative analysis of the component acids by gas-liquid chromatography.

(c) Study of the glyceride structure of the oil.

These are discussed below; the separation procedures are shown schematically in Table I.

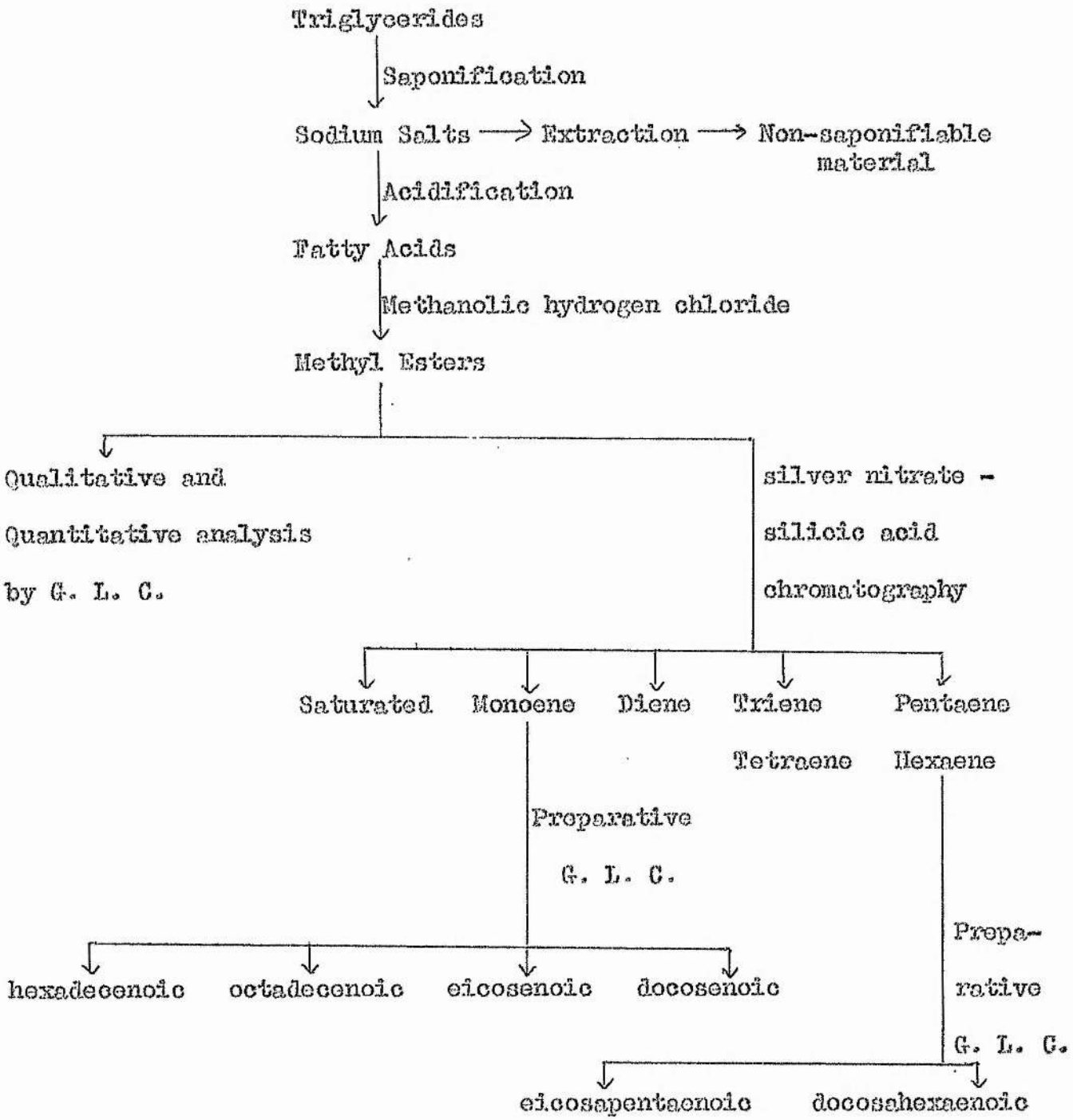
(a) Qualitative Analysis

Rosenheim and Webster[2] in their analysis of the stomach oil of the fulmar stated it was a liquid wax containing forty per cent of non-saponifiable material, mainly long-chain alcohols. When the oil used in the present investigation was examined by thin-layer chromatography on silicic acid it behaved as a triglyceride containing only a minor proportion of unsaponifiable material.[4]

Hydrolysis of the oil was achieved by refluxing with alcoholic potassium hydroxide. The mixed acids, after extraction of the non-saponifiable material (4 %), were converted to their methyl esters which were subsequently examined by gas-liquid chromatography. The esters were well resolved on a column of Apiezon L (10 %) but on a column of QF - I (10 %) the esters were separated according to

Table I

Separation Procedures of Fulmar Oil



chain length but not with respect to unsaturation, i.e. there was no separation of stearic, oleic, linoleic etc.. The methyl esters were also examined on a column of polyethylene glycol adipate (5 %), the temperature not exceeding 170°C. If the percentage of stationary phase exceeds five or the temperature exceeds 170°C, the bleed of stationary phase from the column becomes too high and the sensitivity of the detector is greatly reduced. The gas-liquid chromatographic analysis of the mixed esters and hydrogenated methyl esters on two stationary phases are given in Table II.

Chromatograms of the methyl esters showed twelve major and ten minor peaks; after hydrogenation there were five major and five minor peaks. Comparison with the chromatograms of the original ester shows that nearly all the myristate, about half the palmitate and a small amount of the stearate were present in the original ester, but the remainder of the saturated esters present in the fully hydrogenated sample had been derived from unsaturated compounds.

Isolation of the Six Major Unsaturated Esters by Silver Nitrate - Silicic Acid Chromatography and Preparative Gas- Liquid Chromatography

The mixed esters were adsorbed on a column of silver nitrate - silicic acid[8] and separated into a saturated fraction (eluted with light petroleum containing 1 % of ether), a monoene fraction (eluted with light petroleum containing 3 % of ether), a diene fraction (eluted with light petroleum containing 5 % of ether), a triene and tetra-

Table II

Analysis of Fulmar Oil Methyl Esters

Carbon Numbers of Eluted Esters (a) before and (b) after hydrogenation				Identification
(a) Mixed Esters		(b) Hydrogenated Esters		
Ap. L.	P.E.G.A.	Ap. L.	P.E.G.A.	
14·0	14·0	14·0	14·0	14:0
13·8	14·4			14:1
14·4	14·4	14·4	14·4	15br
15·0	15·0	15·0	15·0	15:0
16·0	16·0	16·0	16·0	16:0
15·8	16·4			16:1
15·5	16·9			16:2
16·45	16·4	16·4	16·4	17br
17·0	17·0	17·0	17·0	17:0
18·0	18·0	18·0	18·0	18:0
17·7	18·4			18:1
17·5	18·9			18:2
17·5	19·5			18:3
17·2	19·9			18:4
19·0	19·0	19·0	19·0	19:0
20·0	20·0	20·0	20·0	20:0
19·75	20·4			20:1
19·2	20·9			20:3
19·0	22·1			20:5
		22·0	22·0	22:0
21·65	22·4			22:1
20·75	23·8			22:5
20·55	24·0			22:6

In the column headed Identification, the first figures (14-22) indicate the number of carbon atoms in the acid and the figures after the colon the number of double bonds. br - branched chain ester

These assignations are based on retention values quoted by James[6] and DeWitt.[7]

ene fraction (eluted with light petroleum containing 10 % of ether) and a combined pentaene and hexaene fraction (eluted with ether). This fractionation was monitored by gas-liquid chromatography.

During the course of this investigation a Perkin Elmer Fractometer (Model 451) with preparative column became available, and the monoene fraction and the pentaene and hexaene fraction were separated into their component esters. The esters were loaded on a column(90x2·1 cm.) packed with Apiezon L (20 %) on Celite and the fractions were collected by allowing the effluent gas to pass through a cotton-wool packed U-tube immersed in an acetone-cardice trap kept at -40°C. The purity of each fraction was checked by resampling on the Pye Argon chromatograph.

The monoenoic esters were divided into four fractions containing esters of C_{16}, C_{18}, C_{20}, and C_{22} acids. Each was hydrolysed and the resulting acid oxidised by potassium permanganate and potassium periodate[9] with the following results:-

Monobasic Acid	Dibasic Acid	Monoenoic Acid
heptanoic	nonandioic	hexadec-9-enoic
nonanoic	nonandioic	octadec-9-enoic
nonanoic	undecandioic	eicos-11-enoic
undecanoic	undecandioic	docos-11-enoic

The pentaene-hexaene fraction was similarly divided into two components but because of difficulty with the preparative column only a small quantity of each pure component was obtained; these were

hydrolysed and oxidised as described above. The eicosapentaenoic and docosahexaenoic acids gave glutaric and succinic acids respectively as the only recognised degradation products. This suggests that these acids are:- eicosa-5,8,11,14,17-pentaenoic acid

docosa-4,7,10,13,16,19-hexaenoic acid.

A mixture of these two was submitted to partial oxidation[11] and the results obtained supported the structures suggested above for the eicosapentaenoic and docosahexaenoic acids. The mixture of the two acids was submitted to the following treatment:- hydroxylation with $\gtrsim$ 0·6 mol. of performic acid, giving a mixture of the possible dihydroxy acids, a trace of more extensively oxidised acid and unchanged starting material; catalytic hydrogenation of the remaining unsaturated centres; oxidative cleavage by von Rudloff's reagent of the saturated dihydroxy acids to give a mixture of mono- and di-basic acids; after esterification these were examined by gas-liquid chromatography on Apiezon L (10 %) andQF-I (10 %) columns at 100°C, 150°C and 200°C with the following results:-

Chain Length of Acids

Monobasic 5, 6, 7, 8, 9, (10), 11, 12, (13), 14, 15, (16), (17), 18, 20, 22.

Dibasic 4, 5, (6), 7, 8, (9), 10, 11, (12), 13, 14, (15), 16, 17, (18), 19.

The figures given in parentheses indicate minor amounts.

These results are consistent with the presence of double bonds at positions 4, 5, 7, 8, 10, 11, 13, 14, 16, 17 and 19 in one or both of these acids.

The monoenoic and polyenoic acids isolated here are common constituents of fish oils. Hexadec-9-enoic is as widely distributed in fish oils and marine bird oils as is oleic, but generally in much smaller amounts (8 - 15 %). A recent examination of menhaden body oil showed hexadec-9-enoic acid to be accompanied by the Δ 8 isomer.[12]

Two positionally isomeric acids, namely eicosa-9-enoic and eicosa-11-enoic, have been isolated from natural fats, and both have been reported present in fish oils and marine animal oils.[13]

Toyama[14] was the first to establish the identity of docosa-11-enoic acid which had previously been believed to be identical with the isomeric erucic (docosa-13-enoic) acid found in vegetable oils.

The gas-liquid chromatographic analyses suggested an eicosatrienoic acid to be present in fulmar oil. Klenk et alia[15] reported the presence of an eicosa-5,8,11- and -8,11,14-trienoic acids in herring oil and Baudart[16] found a small proportion of the 8,11,14 isomer in shark liver oil.

The presence of eicosapentaenoic acid in fish oils has been established for a long time. The acid was first characterised as a 4,8,12,15,18 isomer.[17] Recently a 5,8,11,14,17 isomer was reported in pilchard oil,[18] menhaden body oil[12] and herring oil.[15]

In 1938 Farmer and van den Heuvel[20] reported the isolation of a docosahexaenoic acid by molecular distillation of the methyl esters of cod liver oil and showed it to be structurally pure and non-conjugated. The structure of the docosahexaenoic acid occurring in pilchard oil was first established by Whitcutt[19] in 1957 and the same acid was found in menhaden body oil.[12]

(b) Quantitative Analysis by Gas - Liquid Chromatography

Before analysing the fulmar esters, two standard mixtures of esters were examined by gas-liquid chromatography, and it was shown (i) that the area of the peak delineated by the detector is directly proportional to the molar concentration of that component and(ii) that the detector response increases linearly with increasing quantity of each component.

The area of the peaks was determined directly by a Pye Integram fitted to the machine or by measuring the peak areas on a normal chromatogram after triangulation.

Quantitative Analysis of Fulmar Oil Acids

This analysis was carried out on an Apieson L (10 %) column at 200°C. The argon flow rate was 30 ml./minute. The esters were clearly resolved except in the C_{18} region and further analysis on a polyethylene glycol adipate column (161°C) was made to separate these.

Component Acids (per cent weight)

Chain	Double Bonds										Hydrogenated		
Length	0	1	2	3	4	5	6	Br.	U	Total	Sat.	Br.	U.
14	3·3	0·3	-	-	-	-	-	-	-	3·6	3·1	-	-
15	0·4	-	-	-	-	-	-	0·2	0·2	0·8	-	0·4	-
16	14·5	6·1	1·0	-	-	-	-	-	-	21·6	22·6	-	-
17	0·7	-	-	-	-	-	-	0·5	-	1·2	0·7	0·6	-
18	2·3	12·8	0·2	-	2·3	-	-	-	-	17·6	18·4	-	-
20	0·2	16·5	-	0·2	-	11·0	-	-	-	27·9	28·0	-	-
22	-	18·1	-	-	-	1·0	8·0	-	-	27·1	26·2	-	-
Total	21·4	53·8	1·2	0·2	2·3	12·0	8·0	0·7	0·2	1000		100·0	

U - unknown, Br - branched

Comparison of the Analyses of Cod Liver Oil, Menhaden Body Oil and Herring Oil with Fulmar Oil

	Fulmar				Cod Liver Oil[7]			
Chain length	s	m	p		s	m	p	
14	3	-	-	(3)	3	-	-	(3)
16	14	6	1	(21)	12	9	1	(22)
18	2	13	2	(17)	3	25	5	(33)
20	-	17	11	(28)	-	13	10	(23)
22	-	18	9	(27)	-	6	10	(16)
	19	54	23		18	53	26	
		96				97		

	Menhaden Body Oil[12]				Herring Oil[15]			
Chain length	s	m	p		s	m	p	
14	7	-	-	(7)	6	-	-	(6)
16	17	10	5	(32)	12	6	1	(18)
18	3	15	7	(25)	1	18	6	(25)
20	-	2	16	(18)	-	15	7	(22)
22	-	-	11	(11)	-	26	5	(31)
	27	27	39		19	64	19	
		93				102		

s - saturated, m - monoethenoid, p - polyethenoid.

Figures given are percentages by weight.

Comments

Fulmar oil is seen to be similar to a typical fish oil in the following respects -

(a) The wide range of acids present; acids containing 14 - 22 carbon atoms are present.

(b) The low content of saturated acids; this is mainly palmitic with small quantities of myristic, stearic and arachidic.

(c) The high unsaturation of the C_{20} and C_{22} acids.

(d) The eicosapentaenoic and docosahexaenoic acids are of the "linolenic" type ie. the first double bond, numbered from the methyl end, is at carbon atom '3'. This is in contrast to the polyethenoid acids in the fats of land animals which are mainly of the"linoleic" type.

Fulmar oil is seen to be similar to cod liver oil in the proportions of saturated, monoethenoid and polyethenoid acids present but differs from menhaden oil which has only small percentages of eicosenoic and docosenoic acids but higher percentages of saturated and polyethenoid acids.

(c) The Glyceride Structure of Fulmar Oil

The various experimental procedures available in 1956 for examining the glyceride structure of a fat were reviewed by Hilditch.[19] Since then the most important contributions have been the development by Dutton and Scholfield[20] of countercurrent distribution as an effective means of separating glycerides, and hydrolysis by pancreatic lipase which preferentially removes the acyl groups attached to the two primary alcohol groups of glycerol leaving a 2-monoglyceride.

Use has already been made of the data obtained from pancreatic hydrolysis for the investigation of fats. Both Mattson et alia[23] and Desnuelle et alia[24] have concluded that fatty acid distribution in seed oils is not random, confirming investigations made by other methods. The data so far obtained has been discussed by several authors.[25]

The fulmar oil was hydrolysed (50 -60 %) by pancreatic lipase at constant temperature and constant pH.[26] The mixture of oil and acids* was extracted with ether. The oil remaining after removal of the free acids by percolation through a column of Amberlite resin, was adsorbed on a column of Davidson Silica[27] and separated into a triglyceride

* A small sample of the oil and acids was methylated (boron trifluoride/ methanol)[31] and sampled on the gas-liquid chromatograph. It was assumed that during the methylation only the acids were converted to esters and none of the glycerides was hydrolysed.

fraction (eluted with benzene), a diglyceride fraction (eluted with benzene containing 10 % of ether) and a monoglyceride fraction (eluted with ether). This fractionation was monitored by thin-layer chromatography.[28]

The monoglyceride fraction was hydrolysed by boiling with alcoholic potassium hydroxide, and the acids were converted to their methyl esters which were subsequently examined quantitatively by gas-liquid chromatography.

The following table gives the component acids (% molar) for the mixed acids and for those attached to the C_2 (β) position of glycerol determined from the monoglycerides, and to the C_1 and C_3 (α) positions determined from the acids liberated by pancreatic lipase. The column headed α^* is calculated from the mixed acids and the values obtained for acids attached to the C_2 (β) position.

Acid	Mixed Acids	α	β	α^*
14:0	4·2	1·0	12·9	-
14:1	0·3	0·3	0·1	0·4
15br	0·2	0·1	0·4	0·1
15unk	0·2	0·2	-	0·3
15:0	0·5	0·6	0·5	0·5
16:0	16·4	10·4	27·0	11·1
16:1	7·1	5·6	10·3	5·5
16:2	1·0	0·5	3·2	-
17br	0·5	0·7	0·4	0·6
unk	tr			
17:0	0·7	1·0	0·4	0·8
18:0	2·3	3·4	0·5	3·2
18:1	13·3	16·0	7·2	16·4
18:4	2·4	1·8	3·8	1·7
19:0	tr	-	-	-
20:0	0·2	-	-	-
20:1	15·7	21·5	4·4	21·4
20:3	0·2	0·2	0·5	0·1
20:5	10·7	10·8	11·8	10·2
22:1	16·0	21·0	4·8	21·4
22:5	1·0	0·2	2·0	0·5
22:6	7·0	4·7	11·5	5·0

$$\alpha^* = \frac{(\text{mixed acids} \times 3) - \beta}{2}$$

Distribution of Acyl Groups between the Primary and Secondary Alcohol Groups of Glycerol

Comparison of Fulmar Oil with Other Fish Oils[29]

	14:0	16:0	16:1	16:2	18:1	20:1	22:1	20:5	22:6
Cod Liver	5	13	23	1	35	15	6	2	1
	10	27	18	1	10	9	4	10	11
Cod Muscle	5	25	15	13	22	8	4	3	6
	9	21	12	4	19	8	6	3	19
Scallop Muscle	3	44	5	13	16	4	7	3	1
	6	16	9	5	23	3	4	25	5
Lobster Liver	4	10	9	9	18	11	6	10	4
	2	9	7	6	15	10	1	20	9
Fulmar	1	11	5	4	16	21	21	10	5
	13	27	10	3	7	4	5	12	12

Figures give molar percentages.

Comments

(i) The results obtained experimentally for the component acids in the C_1 and C_3 (α) positions, as determined from the acids liberated by pancreatic lipase, agree well with their calculated values. (columns headed α and α^*, page 71.)

(ii) There is a marked absence of C_{18}, C_{20}, C_{22} monoethenoid in the 2 positionsof fulmar oil. Saturated and polyethenoid acids are preferentially acylated at the 2 position, but these tendencies are not so clearly marked as in vegetable triglycerides.

EXPERIMENTAL

Preparation of 5 % Polyethylene Glycol Adipate / Celite for Gas - Liquid Chromatography[30]

Celite 545 (100 - 120 mesh) was covered with concentrated hydrochloric acid for three hours at room temperature, and the mixture stirred frequently. The acid was diluted with distilled water, decanted and the solids transferred to a Buchner funnel, and washed free of residual acid. The support was then treated in a similar manner with a mixture of one part of ammonium hydroxide and three parts of water, washed free of base and dried at 145°C overnight.

Polyethylene glycol adipate (1 g.) was dissolved in chloroform (100 ml.) and celite (20 g.) was added. The chloroform was removed on a rotary film evaporator and the material was dried at 100°C under vacuum.

A column was packed with the polyethylene glycol adipate / celite and purged for thirty hours at 170°C before use.

Gas Chromatographic Analysis of Standard Mixtures

Analysis of Methyl Esters of Saturated Acids

(a) Acids	Per cent Molar	(b) Per cent Found	Standard Deviation
14:0	8·0	7·5	± 1·4
16:0	31·6	31·2	± 1·5
18:0	24·7	25·0	± 0·5
20:0	24·0	24·2	± 1·4
22:0	11·7	12·1	± 1·7

Notes to above Table

(a) Chromatography on an Apiezon L (10 %) column at 200°C.

Argon flow rate - 33·3 ml. / minute. Detector voltage - 1000.

(b) Mean results of five determinations.

Analysis of Methyl Esters of Saturated and Unsaturated Acids

(a) Acid	Per cent Molar	(b) Per cent Found	Standard Deviation
Palmitic	24	25	± 1·4
Oleic	36	37	± 1·6
Linoleic	10	10	± 1·8
Arachidonic	30	28	± 1·7

Notes to above Table

(a) Chromatography on a 10 % Apiezon L column at 200°C corrected in the oleic/linoleic region with a further analysis with polyethylene glycol

adipate at 161°C (5 %). Argon flow rate - 33·3 ml./minute.
Detector voltage - 1000.

(b) Mean results of five determinations.

Analysis of Fulmar Oil by Thin - Layer Chromatography[4]

10 μl. of a chloroform solution (10 %) of the following (a) fulmar oil (b) a triglyceride (c) a wax ester were spotted on to a thin layer of silicic acid on glass. The silicic acid was developed with a mixture of light petroleum, ether and acetic acid (90:10:1), and the spots detected bybexposure to iodine vapour. It was found that the fulmar oil behaved as a triglyceride under these conditions.

Hydrolysis of the Fulmar Oil

The oil (2g.), collected at Fair Isle Bird Observatory, Shetland, was refluxed with 0·5N alcoholic potassium hydroxide (30 ml.) for three hours in an atmosphere of nitrogen. Water (2 volumes) was added and the unsaponifiable material (4 %) was extracted with ether. The fatty acids (1·8 g.) were liberated by acidification with 5N hydrochloric acid and extracted with ether.

The acids (1·8 g.) were converted to their methyl esters (1·8 g.) by refluxing with methanolic hydrogen chloride (0·5N, 20 ml.). The esters were sampled on a Pye Argon chromatograph using Apiezon L/celite (10 %) and polyethylene glycol adipate (5 %) columns.

Iodine value of methyl esters (observed) = 165 (calculated) = 163

Separation of the Methyl Esters into Four Fractions by Silver Nitrate - Silicic Acid Chromatography[8]

A mixture of the silver nitrate - silica adsorbent (10 g., see page 32), Hyflo Super Cel (5 g.) and light petroleum (50 ml.) was heated to boiling for five minutes while stirring. After cooling to room temperature, the slurry was brought into the chromatography tube. The column was cooled with tap water (17°C) and shielded from light.

A solution of methyl esters (250 mg.) in light petroleum (5 ml.) was added to the column.

Eluant	Volume(ml.)	Weight(mg.)	%	Identity
Ether:Light petroleum				
1:99	25	24	23(21)	Saturated
1:99	25	29		
3:97	25	31		
3:97	25	81	57(54)	Monoene
3:97	25	15		
5:95	25	3	1 (1)	Diene
10:90	25	6	2 (3)	Triene and Tetraene
Ether	25	21	18(21)	Pentaene and Hexaene
Ether	25	19		
		229		

This fractionation was monitored by gas-liquid chromatography. Figures in parentheses were determined by gas-liquid chromatography.(see page66.)

Preparative Gas - Liquid Chromatography on a Perkin - Elmer Fractometer

The monoene fraction (127 mg.) was subjected to preparative gas-liquid chromatography on an Apiezon L (20 %) column at 220°C, utilizing a thermal conductivity detector. Four fractions were collected by passing the effluent gas through cotton-wool packed U-tubes immersed in a cardice-acetone trap at -40°C.

Each fraction was weighed and its purity checked by resampling on the Pye Argon chromatograph.

Fraction	Weight(mg.)	Identification
1	9	Hexadecenoic
2	15	Octadecenoic
3	21	Eicosenoic
4	20	Docosenoic

The hexaene and pentaene fraction (40 mg.) was similarly separated into two fractions by preparative gas-liquid chromatography.

Fraction	Weight(mg.)	Identification
1	3	Pentaene
2	4	Hexaene

von Rudloff Oxidation [9] of the Unsaturated Acids

The esters were hydrolysed by refluxing with a 100% excess of 0·5N sodium hydroxide for one hour in an atmosphere of nitrogen.

The acids (10 mg.) were dissolved in a solution of potassium carbonate (180 mg.) in water (5 ml.), and a solution of potassium periodate (70 mg.) and potassium permanganate (1 mg.) in water (20 ml.) was added. The mixture was shaken for 24 hours at room temperature, and the excess oxidant destroyed with sulphur dioxide. This solution was neutralised and the volume reduced by half on a rotary film evaporator. The acidified solution was saturated sodium chloride and extracted with ether (5x30 ml.). Evaporation of the ether afforded acids (9 mg.), which were esterified and examined by gas-liquid chromatography on Apiezon L (10 %- and QF - I (10 %) columns at 150°C.

(The quantities of oxidant given above are for monounsaturated acids; for the degradation of the pentaene and hexaene acids, five and six times as much oxidant respectively was used.)

Partial Oxidation [11] of the Eicosapentaenoic and Docosahexaenoic Acids

The unsaturated acids (60 mg.), 30 % hydrogen peroxide(0·01 ml.) and 98 % formic acid (0·03 ml.) were shaken together for fifteen minutes at 30°C. Any unchanged peracid was then destroyed with sulphur dioxide and the resulting solution hydrolysed with an excess of 2N sodium hydroxide for one hour at 100°C. The unsaturated dihydroxy acids were liberated with dilute sulphuric acid, extracted with ether, and

hydrogenated in methanol by shaking in an atmosphere of hydrogen in the presence of 10 % palladium-charcoal for 24 hours. After filtration of the catalyst and removal of the solvent the product was degraded by von Rudloff's reagent to a mixture of mono- and dibasic acids, identified by gas-liquid chromatography after esterification.

Partial Hydrolysis of Fulmar Oil[26]

The triglycerides of fulmar oil (1 g.) were dispersed in 1·2M ammonium chloride/ammonium hydroxide buffer (10 ml.), pH = 8·5; a calcium chloride solution (22 %, 2·0 ml.) and sodium taurocholate (10 mg.) were added together with pork pancreatic lipase (100 mg.) which had been purified by homogenising with acetone, centrifuging, and drying in a vacuum desiccator. Hydrolysis was carried out at 40°C and the pH was maintained at 8·5 throughout by the addition of 0·880 S.G. ammonium hydroxide from a burette. When two-thirds of the acids had been liberated, the pH was brought to 1·0 by the addition of 10N hydrochloric acid, and the mixture extracted with ether (3x30 ml.)

The ether solution, after passing through an IR400 "Amberlite" resin column (30 g.) to remove the free fatty acids, was dried (sodium sulphate) and evaporated affording neutral glycerides (506 mg.)

Davidson Grade 923 silica gel (100 - 200 mesh) was heated overnight in an oven at 120°C, and 5 % of water was added. A portion (30 g.) of this was made into a slurry with light petroleum (50 ml.) and was packed into a column (400x19 m.m.). The sample of glycerides

(506 mg.) was added in a chloroform solution and separated into three fractions by extraction with benzene, benzene containing 10 % of ether, and ether.[27] The stopcock at the bottom of the column was adjusted so that 1·5 - 2 ml. of effluent was collected per minute. The fractionation was monitored by thin-layer chromatography.[28]

Eluant	Volume(ml.)	Weight(mg.)	Identity
Benzene	200	4	Triglyceride
Benzene containing 10 % ether	200	121	Diglyceride
Ether	200	269	Monoglyceride

Hydrolysis of the Monoglyceride Fraction

The monoglycerides (269 mg.) were hydrolysed by boiling alcoholic potassium hydroxide (0·5N, 4 ml.) in an atmosphere of nitrogen. The acids were converted to their methyl esters (methanolic hydrogen chloride) and the esters were examined qualitatively and quantitatively by gas-liquid chromatography.

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SEED OILS OF THE UMBELLIFERAE

INTRODUCTION

In 1909 Vongerichten and Köhler[1] established the presence of an isomer of oleic acid, petroselinic (octadec-cis-6-enoic) acid, in the fatty oil of parsley seeds (Petroselinum sativum). Scherer (1909)[2] observed a solid oleic acid in the seed fats of two other Umbellates, Pimpinella anisum and Foeniculum capillaceum. This acid is the predominant constituent (ca. 70 %) of the seed oils of the Umbelliferae family and is usually accompanied by oleic, linoleic and palmitic acids. (See Table I). Petroselinic acid is also present in the seed oils of the closely related Araliaceae family[3] and in the seed fat of Picrasma quassioides (Simarubaceae)[4] and in human hair fat.[5] Kurono et alia[6] reported that petroselaidic (octadec-trans-6-enoic) acid was a normal constituent of the oil obtained from the fruits of Anthriscus sylvestris but only in small amounts. These authors also reported that irradation with ultra-violet of plants containing petroselinic acid resulted in the production of petroselaidic acid.

The classical procedure for the analysis of the seed oils of the Umbelliferae was devised by Hilditch.[7] The mixed fatty acids from the saponified fats were treated with lead acetate in alcoholic solution and a preliminary separation was thus effected into (1) saturated acids, petroselinic and probably a little oleic acid and (2) oleic and linoleic acids possibly accompanied by traces of palmitic and petroselinic acids. Each group of acids was then converted to the methyl esters and the latter submitted to fractional distillation at low pressure. All C_{18} monoethenoid

Component Acids of Seed Fats of the Umbelliferae and Araliaceae

Umbelliferae	Palmitic	Petroselinic	Oleic	Linoleic	Ref.
Ammi visnaga (Sudan)	5	50	42	3	10
Ammi visnaga (Argentine)	5	44	30	19	11
Angelica glabra		P			12
Angelica polyclada	P	P*		P	13
Angelica sylvestris	4	19	44	33	14
Angelica ursina	P	P*		P	15
Anthriscus cerefolium	5	41	0·5	53·5	7
Anthriscus cerefolium	6	59		30	16
Anthriscus sylvestris		70			17
Apium graveolens	3	51	26	20	7
Apium graveolens	P	P*	P	P	18
Apium graveolens (Argentine)	12	41	30	10	19
Bupleurum falcatum	P	P*		P	20
Carum ajowan	P	P*	P	P	18
Carum carvi	3	26	40	31	7
Carum carvi	3	17	61	29	21
Carum copticum	5	48	24	20	22
Chamaele decumbens	P	P*	P	P	18
Conium maculatum		67			14
Conioselinum univittatum		P			23
Coriandrum sativum	8	53	32	7	7

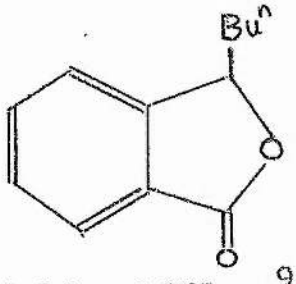
Umbelliferae	Palmitic	Petroselinic	Oleic	Linoleic	Ref.
Coriandrum sativum	9·7	38·5	37·8	14	24
Daucus carota	4	5·8	14	24	7
Daucus carota	7	55	23	15	25
Daucus carota	1	—— 81 ——		13	16
Foeniculum officinale	4	60	32	14	7
Foeniculum vulgare		86			26
Heracleum candicans	1	—— 66 ——		18	27
Heracleum nipponicum	P	P*	P	P	28
Heracleum sphondylium	4	19	52	25	14
Lingusticum acutilobum	P	P*	P	P	29
Mallotus japonicus	P	P		P	30
Oenanthe stolonifera		75			17
Osmothiza aristata	P	P*	P	P	31
Panax schinseng		P*	P		32
Pastinaca sativa	1	46	32	21	7
Petroselinum sativum	3	76	15	6	33
Petroselinum sativum	3	70	9	18	34
Petroselinum sativum	5	—— 82 ——		13	35
Phellopterus littoralis	P	P*		P	36
Pimpinella anisum	3	24	56	17	21
Pimpinella anisum	14	17·5	43·5	25	37
Pleurospermum kamtschaticum	P	P*	P	P	31
Pimpinella antisetum	3	25	61	10	38
Seseli indicum	6	46	31	13	39

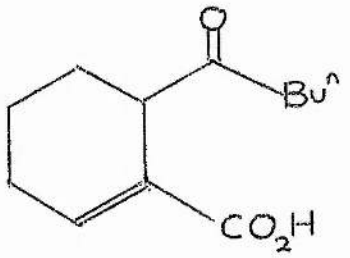
Umbelliferae	Palmitic	Petro-selinic	Oleic	Linoleic	Ref.
Seseli libanotis		21·3			31
Seseli vidicum		P		P	18
Ternstroemia japonica	P	P		P	40
Araliaceae					
Aralia elata	P	P*		P	41
Hedera helix	5	60	20	13	3

(P - present, * - also reported to contain small amounts of petroselaidic acid, figures give percentages by weight)

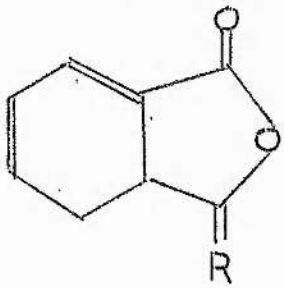
acid in the crystalline fraction is calculated as petroselinic acid, and all C_{18} monoethenoid acid in the mother liquors as oleic acid. A complication from the point of view of quantitative work was the presence of resinous matter (lactonic) in many of the ester fractions (notably celery and parsnip). Recently Barton and de Vries[8] showed celery oil to furnish

3 n-butylphthalide and sedanoic acid.





while Gold and Wilson[9] reported the presence of alkylidene dihydrophthalides in the oil.



(i) R = $CH{-}CH(CH_3)_2$

(ii) R = $CH.CH_2.CH(CH_3)_2$

New studies of the glyceride composition of vegetable fats have recently been started in this department and it is planned to include a number of fats containing petroselinic acid in this study. The work now to be described is an attempt to devise a more satisfactory method of determining the component acids of such fats. This has been achieved, but the glyceride study of these fats awaits the development of new methods of glyceride examination now being studied by Gunstone, Padley and Qureshi (unpublished work).

DISCUSSION

Oleic and petroselinic esters were not adequately resolved by gas-liquid chromatography nor adequately separated by thin-layer chromatography plates layered with silica gel and silver nitrate. The method of examination finally devised involved the following stages:-

(1) Analysis by quantitative gas-liquid chromatography in terms of palmitic, palmitoleic, stearic, octadecenoic acids, and linoleic acid.

(2) Isolation of the monoenoic acids by column chromatography on silica/silver nitrate.[42]

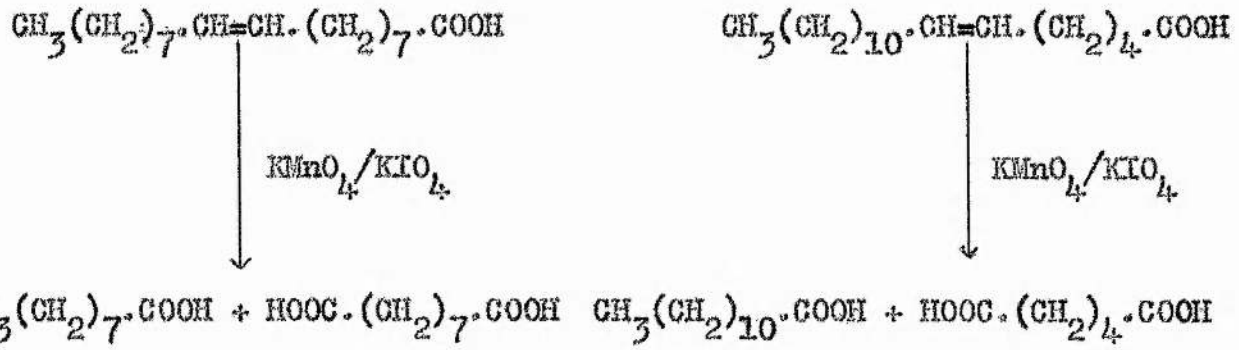
(3) The analysis of the monoenoic acid fraction by determination of the ratio of methyl dodecanoate (from petroselinic acid) to dimethyl nonandioate (from oleic acid) after oxidation by the von Rudloff procedure.[43]

The mixed acids, free from unsaponifiable material, were converted to the methyl esters which were examined by gas-liquid chromatography using a polyethylene glycol adipate (5 %) column at 161°C and an Apiezon L (10 %) column at 200°C. Under these conditions the oleic and petroselinic esters were not separated and appeared as only one peak on the chromatogram.

A solution of the methyl esters in light petroleum was adsorbed on a column of silica impregnated with silver nitrate and separated into a saturated fraction (eluted with light petroleum containing 1 % of ether), a monoene fraction (eluted with light petroleum containing 3 % of ether) and a diene fraction (eluted with

light petroleum containing 5 % of ether). These separations were monitored by gas-liquid and thin-layer chromatography.

The monoene fraction was hydrolysed and the acids were oxidised by potassium permanganate and potassium periodate to hexandioic, nonandioic, nonanoic and dodecanoic acids, which were subsequently methylated and identified by gas-liquid chromatography on an Apiezon L / Celite (20 %) column at 150°C.



Several synthetic mixtures of oleic and petroselinic acids were oxidised by potassium permanganate and potassium periodate, and it was found that a comparison of the areas of methyl dodecanoate and dimethyl noandioate peaks gave the most consistent analyses of the constituents of the starting mixture.

The diene fraction, after hydrolysis and oxidation, afforded only hexanoic and nonandioic acids. There was no nonanoic and hexandioic acids in the degradation products as would be expected from an octadec-6,9-dienoic acid.

$$CH_3(CH_2)_4 \cdot CH{=}CH \cdot CH_2 \cdot CH{=}CH \cdot (CH_2)_7 \cdot COOH$$

$$\downarrow KMnO_4/KIO_4$$

$$CH_3(CH_2)_4 \cdot COOH + HOOC \cdot CH_2 \cdot COOH + HOOC \cdot (CH_2)_7 \cdot COOH$$

$$CH_3(CH_2)_7 \cdot CH{=}CH \cdot CH_2 \cdot CH{=}CH \cdot (CH_2)_4 \cdot COOH$$

$$\downarrow KMnO_4/KIO_4$$

$$CH_3(CH_2)_7 \cdot COOH + HOOC \cdot CH_2 \cdot COOH + HOOC \cdot (CH_2)_4 \cdot COOH$$

Quantitative Examination by Gas-Liquid Chromatography

In some instances, the peak areas were measured by a Pye Integram fitted to the machine, and in others the areas were measured mechanically by triangulation. Several synthetic mixtures of palmitic oleic and linoleic esters were examined. The detector response to these non-oxygenated fatty esters was proportional to the molar percentages in the synthetic mixture. No correction factor was required.

Synthetic mixtures of methyl dodecanoate and dimethyl nonandioate were also examined, to determine detector response. The peak area corresponding to dimethyl nonandioate relative to methyl dodecanoate was underestimated and to obtain accurate quantitative data it was necessary to multiply the peak area of the nonandioate by a correction factor of 1·3.

Results Table II

Component Acids (per cent weight)

	Palmitic	Palmitoleic	Stearic	Oleic	Petroselinic	Linoleic	Iodine value obs.	Iodine value calc
Petroselinum sativum	3	Tr	3	5	77	12	92	90
Daucus carota	4	0	Tr	18	65	13	95	93
Coriandrum sativum	2	Tr	Tr	9	72	17	100	98
Conium Maculatum	4	Tr	Tr	17	61	18	96	97
Heracleum montegayzianum	6	Tr	Tr	15	54	25	107	103
Anthriscus cerefolium	7	1	1	6	49	36	109	110
Carum carvi	6	Tr	1	18	38	37	110	111

Tr - Trace

Comments

(1) The minimum amount of any seed oil required for a total analysis is 500 mg.

(2) The saturated acid content (almost all palmitic) is low but shows a tendency to be slightly higher in the oils of higher unsaturation.

(3) Changes in iodine value depend almost entirely on diene / monoene ratio.

(4) The oleic acid content varies (5 - 18 %) but is always lower than petroselinic acid content (38 - 77 %).

(5) The highest percentage of petroselinic acid is accompanied by

the lowest percentage of linoleic acid.

(6) Petroselinum sativum (parsley) is the best source of petroselinic acid. The seeds contain 14 % of oil, which contains a high percentage of petroselinic acid accompanied by a low percentage of oleic acid.

(7) There was no evidence of any octadec-6,9-dienoic acid in the diene fraction. An acid similar to this in the monoethenoid ($\Delta 6$) and triethenoid ($\Delta 6,9,12$)[44] series occurs in vegetable fats. Octadec-6,9-dienoic acid is reported only in human hair fat[5] and in menhaden oil.[45]

(8) A comparison of results obtained in the present investigation with previous work is shown in Table III.

Table III

Comparison of the Present Work with Previous Results

	Ref.	Palmitic	Stearic	Petroselinic	Oleic	Linoleic
Petroselinum sativum	P	3	3	77	5	12
	35	5		—— 82 ——		13
	34	3		70	9	18
	33	3		76	15	6
Daucus carota	P	4		65	18	13
	7	4		58	14	24
	25	7		55	23	15
	16	1		—— 81 ——		13
Coriandrum sativum	P	2		72	9	17
	7	8		53	32	7
	24	9·7		38·5	37·8	14·0
Anthriscus cerefolium	P	7		49	6	36
	7	5		41	5	53·5
	16	6		—— 59 ——		30
Carum carvi	P	6		38	18	37
	7	3		26	40	31
	21	3		17	61	20

P - present work

EXPERIMENTAL

Gas-Liquid Chromatography

The analyses of the mixed esters of the seed oils was carried out on two stationary phases (a) 5 % polyethylene glycol adipate on acid and alkali washed celite at 161°C, and (b) 10 % Apiezon L grease on alkali washed celite at 200°C. Quantitative analyses are an average from four chromatograms.

Extraction, Hydrolysis and Methylation of the Seed Oils

The seeds were coarsely ground and extracted with light petroleum for 24 hours. The oil was hydrolysed by boiling with a 100 % excess of alcoholic potassium hydroxide (0·5N). The mixed acids, after removal of the non-saponifiable material, were converted to the methyl esters by refluxing for two hours with a twenty-fold excess of dry methanolic hydrogen chloride (0·5N).

Silver Nitrate - Silicic Acid Chromatography

The silver nitrate - silicic acid adsorbent was prepared as previously (p. 32.). The columns were packed with 10 g. of the adsorbent and 5 g. of Hyflo Super Cel.

von Rudloff Oxidation of (a) Monoene Acids (b) Diene Acids

The esters were hydrolysed by refluxing with 100 % excess of 0·5N sodium hydroxide for one hour.

The acids (50 mg.) were dissolved in a solution of potassium carbonate (86 mg.) in water (20 ml.), and a solution of potassium periodate (368 mg.) and potassium permanganate (4 mg.) in water (60 ml.) was added. The mixture was shaken for 24 hours at room temperature, and the excess oxidant destroyed with sulphur dioxide. This solution was neutralised with potassium carbonate and reduced in volume to 10 ml. on a rotary film evaporator. The acidified solution was saturated with sodium chloride and extracted with ether (7x30 ml.) Evaporation of the ether afforded acids which were esterified and examined by gas-liquid chromatography on a 20 % Apiezon L column at 150°C.

Gas Chromatographic Analysis of Standard Mixtures

Column 20 % Apiezon L at 150°C

The analyses given are mean results of six determinations, the load varying between 0·025 and 0·1 µl.

Mixture I	Mol. (%)	Found	Standard Deviation
C_{12} monobasic	48·5	55	± 1·5
C_9 dibasic	51·5	45	± 1·4

Correction factor for C_9 dibasic = 1·30

Mixture II			
C_{12} monobasic	41·4	50·1	± 1·3
C_9 dibasic	58·6	49·4	± 1·2

Correction factor for C_9 dibasic = 1·30

Mixture III			
C_{12} monobasic	73	77	± 0·7
C_9 dibasic	27	23	± 1·8

Correction factor for C_9 dibasic = 1·29

Three synthetic mixtures of petroselinic and oleic acids were oxidised by the von Rudloff procedure and the quantitative analyses of the dodecanoate and nonandioate were determined by gas-liquid chromatography on a 20 % Apiezon L column at 150°C.

Mixture I	Mol. (%)	Found	Standard Deviation
Petroselinic	51·4	57·3	± 1·6
Oleic	48·6	42·6	± 1·5

Correction factor for C_9 dibasic = 1·3

Mixture II			
Petroselinic	62·5	69·5	± 1·3
Oleic	37·5	30·5	± 1·4

Correction factor for C_9 dibasic = 1·28

Mixture III			
Petrodelinic	73	77	± 0·5
Oleic	27	23	± 1·7

Correction factor for C_9 dibasic = 1·29

The results are a mean from four determinations.

Petroselinum sativum (Parsley)

Per cent fat content in seeds = 17

Per cent non-saponifiable material = 12

Iodine value of methyl esters (observed) = 92 (calculated) = 90

Silver nitrate - Silicic Acid Chromatography

The esters (250 mg.) in light petroleum (5 ml.) were adsorbed on to the column.

Eluant	Volume(ml.)	Weight(mg.)	%	Identity
Ether:light petroleum				
1:99	25	6	2·4	Saturated
3:97	25	49		
3:97	25	135	86	Monoene
3:97	25	26		
5:95	25	27	11	Diene
		243		

Per cent recovery = 97

Component Acids	Per cent Molar	Per cent Weight
Palmitic	3·7	3·3
Palmitoleic	0·3	0·3
Stearic	2·8	2·8
Petroselinic	77·1	77·3
Oleic	4·6	4·6
Linoleic	11·7	11·6

Daucus carota (Carrot)

Per cent fat content in seeds = 14

Per cent non-saponifiable material = 12

Iodine value of methyl esters (observed) = 95 (calculated) = 93

Silver Nitrate - Silicic Acid Chromatography

The esters (250 mg.) in light petroleum (5 ml.) were adsorbed on to the column.

Eluant	Volume(ml.)	Weight(mg.)	%	Identity
Ether:light petroleum				
1:99	25	6	2·6	Saturated
3:97	25	55		
3:97	25	101	84	Monoene
3:97	25	34		
5:95	25	31	13·6	Diene
		227		

Per cent recovery = 90

Component Acids	Per cent Molar	Per cent Weight
Palmitic	4·2	3·8
Palmitoleic	-	-
Stearic	0·3	0·3
Petroselinic	65·2	65·4
Oleic	18·0	18·1
Linoleic	12·5	12·4

Coriandrum sativum (Coriander)

Per cent fat content in seeds = 9

Per cent non-saponifiable material = 10

Iodine value of methyl esters (observed) = 100 (calculated) = 98

Silver Nitrate - Silicic Acid Chromatography

The esters (250 mg.) in light petroleum (5 ml.) were adsorbed on to the column.

Eluant	Volume(ml.)	Weight(mg.)	%	Identity
Ether:light petroleum				
1:99	25	6	2·5	Saturated
3:97	25	56		
3:97	25	101	80	Monoene
3:97	25	34		
5:95	25	42	17·5	Diene
		239		

Per cent recovery = 96

Component Acids	Per cent Molar	Per cent Weight
Palmitic	2·3	2·1
Palmitoleic	0·3	0·3
Stearic	0·3	0·3
Petroselinic	71·2	71·4
Oleic	9·2	9·2
Linoleic	16·7	16·7

Anthriscus cerefolium (Chervil)

Per cent fat content in seeds = 11

Per cent non-saponifiable material = 3

Iodine value of methyl esters (observed) = 107 (calculated) = 110

Silver Nitrate - Silicic Acid Chromatography

The esters (250 mg.) in light petroleum (5 ml.) were adsorbed on to the column.

Eluant	Volume(ml.)	Weight(mg.)	%	Identity
Ether:light petroleum				
1:99	25	17	7·0	Saturated
3:97	25	31		
3:97	25	63	55·7	Monoene
3:97	25	41		
5:95	25	61	37	Diene
5:95	25	29		
		242		

Per cent recovery = 97

Component Acids	Per cent Molar	Per cent Weight
Palmitic	7·7	7·0
Palmitoleic	0·5	0·5
Stearic	1·1	1·1
Petroselinic	48·3	48·8
Oleic	6·3	6·3
Linoleic	36·0	36·1

Carum carvi (Caraway)

Per cent fat content in seeds = 9

Per cent non-saponifiable material =13

Iodine value of methyl esters (observed) = 110 (calculated) = 111

Silver Nitrate - Silicic Acid Chromatography

The esters (250 mg.) in light petroleum (5 ml.) were adsorbed on to the column.

Eluent	Volume(ml.)	Weight(mg.)	%	Identity
Ether:light petroleum				
1:99	25	15	6·2	Saturated
3:97	25	37		
3:97	25	85	56·4	Monoene
3:97	25	13		
5:95	25	52	36·4	Diene
5:95	25	37		
		239		

Per cent recovery = 96

Component Acids	Per cent Molar	Per cent Weight
Palmitic	6·0	5·5
Palmitoleic	0·4	0·4
Stearic	1·3	1·3
Petroselinic	37·6	37·9
Oleic	18·1	18·3
Linoleic	36·5	36·6

Conium maculatum

Per cent fat content in seeds = 10

Per cent non-saponifiable material = 6

Iodine value of methyl esters (observed) = 94. (calculated) = 97

Silver Nitrate - Silicic Acid Chromatography

The esters (250 mg.) in light petroleum (5 ml.) were adsorbed on to the column.

Eluent	Volume(ml.)	Weight(mg.)	%	Identity
Ether:light petroleum				
1:99	25	11	4.4	Saturated
3:97	25	53		
3:97	25	107	77	Monoene
3:97	25	35		
5:95	25	45	18	Diene
		245		

Per cent recovery = 98

Component Acids	Per cent Molar	Per cent Weight
Palmitic	4.0	3.6
Palmitoleic	0.3	0.3
Stearic	0.3	0.3
Petroselinic	60.3	60.6
Oleic	16.8	16.9
Linoleic	18.3	18.3

Heracleum mantegazzianum

Per cent fat content in seeds = 9

Per cent non-saponifiable material = 7

Iodine value of methyl esters (observed) = 107 (calculated) = 103

Silver Nitrate - Silicic Acid Chromatography

The esters (250 mg.) in light petroleum (5 ml.) were adsorbed on to the column.

Eluant	Volume(ml.)	Weight(mg.)	%	Identity
Ether:light petroleum				
1:99	25	15	6·2	Saturated
3:97	25	52		
3:97	25	80	68	Monoene
3:97	25	31		
5:95	25	62	26	Diene
		240		

Per cent recovery = 96

Component Acids	Per cent Molar	Per cent Weight
Palmitic	6·0	5·5
Palmitoleic	0·2	0·2
Stearic	0·3	0·3
Petroselinic	53·5	53·6
Oleic	15	15·2
Linoleic	25	25·2

Apium graveolens (Celery)

Per cent fat content in seeds =14

The mixed acids, after extraction of non-saponifiable material, were converted to the methyl esters and examined on the gas-liquid chromatograph at 161°C using 5 % polyethylene glycol adipate / celite as the stationary phase.

	Carbon Numbers	
Mixed Esters	Hydrogenated Mixed Esters	Identity
13·3	13·3	-
16·0	16·0	Palmitic
16·5	16·4	-
17·7	17·6	-
18·0	18·0	Stearic
18·3	-	Petroselinic/ Oleic
18·9	-	Linoleic
19·3	19·3	-
19·6	-	-

The unidentified esters are presumably resinous material which makes the quantitative analysis of the celery seed esters given below only approximate.

Component Acids	Per cent Molar	Per cent Weight
Palmitic	8·0	7·5
Palmitoleic	0·3	0·5
Stearic	0·5	0·5
Petroselinic / Oleic	65·0	65·5
Linoleic	26·0	26·0

Table IV

Comparison of (a) Analyses obtained by Silver Nitrate/Silicic Acid Chromatography and (b) Analyses obtained by Gas-Liquid Chromatography

		(a)	(b)
	Saturated	2·5	6·5
Petroselinum sativum	Monoene	86	82
	Diene	11	12
	Saturated	2·6	4·5
Daucus carota	Monoene	84	83·2
	Diene	13·4	12·4
	Saturated	2·5	2·6
Coriandrum sativum	Monoene	80	80·7
	Diene	17·5	16·7
	Saturated	7·0	9·0
Anthriscus cerefolium	Monoene	55·7	56·0
	Diene	37	36
	Saturated	6·2	6·8
Carum carvi	Monoene	56·4	56·2
	Diene	36·4	36·6

Table IV (cont.)

		(a)	(b)
	Saturated	4.4	3·9
Conium maculatum	Monoene	77	78
	Diene	18	18·1
	Saturated	6·2	5·8
Heracleum mantegazzianum	Monoene	68	69
	Diene	26	25·2

The figures given are percentages by weight.

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