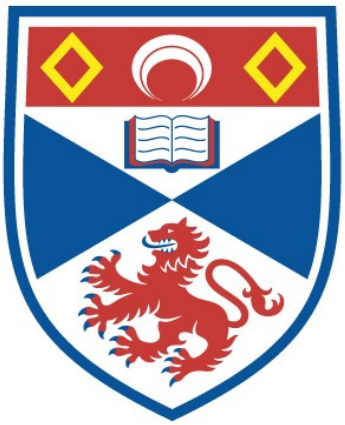
OCCURRENCE AND REACTIONS OF LONG-CHAIN EPOXY ACIDS

Henry Brown Stuart Conacher

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



1968

OCCURRENCE AND REACTIONS OF LONG-CHAIN EPOXY ACIDS

being a thesis

presented by

HENRY BROWN STEWART CONACHER, B.Sc.

to the

UNIVERSITY OF ST. ANDREWS

in application for

THE DEGREE OF DOCTOR OF PHILOSOPHY.

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DECLARATION.

I hereby declare that this Thesis is a record of the results of my own experiments, that it is my own composition, and that it has not previously been presented in application for a higher degree.

The research was carried out in the Chemistry Research Department of the University of St. Andrews under the direction of F.D. Gunstone, D.Sc., F.R.I.C.

CERTIFICATE.

I hereby certify that Mr. Henry Brown Stewart Conacher has spent twelve 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 1957. I pursued a recognised course and graduated B.Sc. with Second Class Honours in Chemistry in 1961.

From 1961 to 1965 I carried out research in the Chromatography Research Laboratory, J. Lyons and Co. Ltd., London.

I was admitted as a Research Student in the United College, University of St. Andrews, in October 1965 and was awarded an S.R.C. Studentship which I held until October 1968.

ACKNOWLEDGEMENTS.

I wish to record my sincere thanks to Dr. F.D. Gunstone for suggesting such an interesting research topic and for his able guidance, constant interest and encouragement throughout this work.

I wish also to express my gratitude to Professor J.I.G. Cadogan in whose department the work was done; to Mr. R. Morris and the technicians of the department for their assistance; and to the Northern Regional Research Laboratory, Peoria, for the gift of the following seeds: Crepis aurea, Crepis vesicaria, Cephalaria joppica, Cephalaria leucantha and Helichrysum bracteatum.

Thanks must also be expressed to Mr. C.D. Sinclair of the Statistics Department for introducing me to the mysteries of computer programming and for his invaluable assistance in composing both programmes.

I must also express my deepest thanks to my wife for sacrificing many long hours in typing this thesis.

Finally, I am indebted to the S.R.C. for financial support.

PUBLICATIONS.

(i) Rearrangement of an Unsaturated Epoxy Ester to a Cyclopropane Compound.

H.B.S. Conacher and F.D. Gunstone, Chem. Comm., 1967, 984.

(ii) Base-catalysed Isomerisation of Epoxy Esters: The Partial Synthesis of Methyl Coriolate from Methyl Vernolate and of Racemic Methyl Helenynolate from Methyl Crepenynate.

H.B.S. Conacher and F.D. Gunstone, Chem. Comm., 1968, 281.

LECTURE.

'The Rearrangement of Methyl Epoxyoleate and related Epoxy Esters.'

H.B.S. Conacher, presented to the Oils and Fats Group, Chemistry and Industry, London, February 16th, 1968.

CONTENTS.

SUMMARY.

The possibility that long-chain epoxy acids may be key intermediates in the biosynthesis of acids with conjugated unsaturation has been investigated. By isomerisation of several epoxy acids with acidic and basic catalysts it was hoped to provide a chemical model to support this postulate.

Acid-catalysed rearrangement of methyl vernolate, although giving no support for the above hypothesis, yielded a novel oxocyclopropane ester, identified by degradative and synthetic techniques as methyl 12-oxo-9,10-methyleneheptadecanoate. This reaction was studied in two solvents under different conditions, and a mechanism has been proposed for the formation of this unusual ester. Under optimum conditions the ester was obtained in 34% yield.

Base-catalysed rearrangement of suitably activated epoxy esters provided useful support for the biosynthetic postulate. By this means partial syntheses were effected of methyl coriolate from vernolate, racemic methyl α-dimorphecolate from 9,10-epoxyoctadec-12-enoate, and racemic methyl helenynolate from methyl 9,10-epoxyoctadec-12-ynoate. A synthesis of methyl parinarate

from methyl linolenate via an epoxy intermediate was also attempted.

Re-examination of three seed oils for unknown epoxy acids which would be biosynthetic intermediates, led to the discovery of a new epoxy acid, cis-9,10-epoxyoctadec-12-ynoic acid, in Helichrysum bracteatum seed oil.

Finally, the inter- and intraglyceride distribution of vernolic acid in six seed oils was examined. It was shown that vernolic acid, like oleic and linoleic acids, competes effectively for the 2-position in the triglycerides.

ABBREVIATIONS.

GLC - Gas-liquid chromatography.

DEGS - Diethyleneglycolsuccinate polyester.

ApL - Apiezon L grease.

C.No - Carbon number.

TMS - Trimethylsilyl ether.

(prep.) TLC - (preparative) Thin layer chromatography on Silica Gel G.

(prep.) Ag^{+}/TLC - (preparative) Thin layer chromatography on Silica Gel G impregnated with silver nitrate.

NMR - Nuclear magnetic resonance.

IR - Infra red.

UV - Ultra violet.

For acids.

18:1 - First number gives the chain length, the second number the number of double bonds.

Chapter I.

GENERAL INTRODUCTION.

OCCURRENCE AND POSSIBLE BIOSYNTHETIC SIGNIFICANCE OF LONG-CHAIN EPOXY ACIDS.

1. Occurrence and structure of epoxy acids.

The first epoxy acid shown to occur naturally, cis-12,13-epoxyoctadec-cis-9-enoic acid, was discovered and characterised by Gunstone[1] in 1954. This acid, vernolic acid, comprised 72% of the mixed acids of Vernonia anthelmintica seed oil. It was later isolated from this oil by Smith et al.[2] who confirmed Gunstone's work.

The same workers[3,4] also described the occurrence of its isomer, cis-9,10-epoxyoctadec-cis-12-enoic acid, as a component of Chrysanthemum coronarium seed oil.

These two acids are obviously related to linoleic acid and epoxy acids structurally similar to oleic and linolenic acids have also been characterised (Table 1, p. 3).

cis-9,10-Epoxystearic acid was first identified by Hopkins and Chisholm[5] as comprising 3% of the acids of Tragopogon porrifolius seed oil. Shortly afterwards the same acid was observed by Tulloch and co-workers[6-9] in many fungal spores.

An acid related to linolenic, cis-15,16-epoxyoctadeca-cis-9,cis-12-dienoic acid, has been found in small amount in the seed oil of Camelina sativa[10] and Morris[11] considers, on the

basis of chromatographic evidence only, that it may be present in trace amounts in several other seed oils. It seems likely that the other monoepoxides of linolenic acid (9,10-epoxyoctadeca-cis-12,cis-15- and 12,13-epoxyoctadeca-cis-9,cis-15- dienoic acids) also occur and will be discovered in the future.

The present work has led to the discovery of a new epoxy acid, cis-9,10-epoxyoctadec-12-ynoic acid, in Helichrysum bracteatum seed oil[12].

Some of these acids exist in several stereoisomeric forms. 12,13-Epoxyoctadec-9-enoic acid has been shown to occur in both enantiomorphic forms[13] and the 9,10-epoxystearic in both geometric forms[5,14]. The absolute configuration of the naturally occurring epoxy acids has also been the subject of several recent studies. The elegant work of Morris and Wharry[15] established that the (+)-epoxyoleic acid (vernolic) from Vernonia oil has the 12D,13D configuration. They also concluded that the (-)-epoxyoleic acid from the Malvaceae seed oils[13] must have the 12L,13L configuration. By similar studies Powell et al.[16] confirmed the absolute configuration of vernolic acid and showed that the two 9,10-epoxy acids present in Xeranthemum annuum seed oil, 9,10-epoxystearic acid and 9,10-epoxyoctadec-12-enoic acid, both have the 9L,10L configuration.

These epoxy acids have been the subject of two recent reviews by Wolff[17] and Krewson[18] and their wide-spread

distribution is now accepted; Gunstone[19] has indicated their occurrence in more than 40 species from 12 different plant families. Sometimes two or more of these acids occur in the same seed oil[11,14,16].

Table 1.

Common acid	Epoxy acid
$CH_3 \cdot (CH_2)_7 \cdot CH{:}CH \cdot (CH_2)_7 \cdot CO_2H$	
oleic	$CH_3 \cdot (CH_2)_7 \cdot \underset{\diagdown O \diagup}{CH \cdot CH} \cdot (CH_2)_7 \cdot CO_2H$
$CH_3 \cdot (CH_2)_4 \cdot CH{:}CH \cdot CH_2 \cdot CH{:}CH \cdot (CH_2)_7 \cdot CO_2H$	
linoleic	$CH_3 \cdot (CH_2)_4 \cdot \underset{\diagdown O \diagup}{CH \cdot CH} \cdot CH_2 \cdot CH{:}CH \cdot (CH_2)_7 \cdot CO_2H$
	vernolic
	$CH_3 \cdot (CH_2)_4 \cdot CH{:}CH \cdot CH_2 \cdot \underset{\diagdown O \diagup}{CH \cdot CH} \cdot (CH_2)_7 \cdot CO_2H$
	coronaric
$CH_3 \cdot CH_2 \cdot CH{:}CH \cdot CH_2 \cdot CH{:}CH \cdot CH_2 \cdot CH{:}CH \cdot (CH_2)_7 \cdot CO_2H$	
linolenic	$CH_3 \cdot CH_2 \cdot \underset{\diagdown O \diagup}{CH \cdot CH} \cdot CH_2 \cdot CH{:}CH \cdot CH_2 \cdot CH{:}CH \cdot (CH_2)_7 \cdot CO_2H$

2. The role of epoxy acids in the biosynthesis of conjugated polyenoic acids.

Natural long-chain polyene acids are predominantly methylene interrupted compounds having cis configuration. Linoleic acid (18:2; 9c,12c) and linolenic acid (18:3; 9c,12c,15c) are the best known members of this class which also includes many C_{16}, C_{20} and C_{22} acids[20].

In addition there are a few polyenoic acids with conjugated unsaturation which is partly cis and partly trans. Those of lipid origin are almost entirely C_{18} acids and the more important members are listed in Table 2.

Table 2.

Conjugated acid	Trivial name	Reference
13-OH,9c,11t	coriolic	21
9-OH,10t,12c	α-dimorphecolic	22
9-OH,10t,12t	β-dimorphecolic	23
8t,10t,12c	calendic	24
8c,10t,12c	jacaric	25
9c,11t,13t	α-eleostearic	26
9c,11t,13c	punicic	27
9t,11t,13c	catalpic	28
9c,11t,13t,15c	parinaric	29

These include three hydroxydienes, five trienes and one tetraene. It is increasingly held that these arise from linoleic and linolenic acid and early suggestions about how this might occur were made by Gunstone and by Morris.

Gunstone[30] suggested that most of these acids might be formed in the seed from linoleic acid via 11-hydroxyoctadeca-9,12-dienoic acid. Morris[31], however, considered this intermediate to be "neither necessary nor likely" and proposed that these acids are formed from linoleic acid by initial enzymic peroxidation, followed by rearrangement and reduction, to produce hydroxy conjugated diene acids. Dolev et al.[32,33] and Samuelsson and Hamberg[34,35] have recently demonstrated the high positional specificity of some lipoxidase systems on unsaturated acids and this may be viewed as support of Morris' hypothesis.

The possibility that the conjugated trienes arise from the hydroxydienes is an obvious one[23,24] as is the relation between oleic, linoleic and linolenic acid and the natural epoxy acids discussed in pp. 1-3. Gunstone[19] recently suggested that the two epoxyoctadecenoates (vernolic and coronaric) could rearrange to hydroxydienes to provide a complete biosynthetic sequence which can be simply represented:

linoleic $\longrightarrow$ epoxymonoene $\longrightarrow$ hydroxydiene $\longrightarrow$ conjugated triene

This can be elaborated to provide the following sequences:

(i) linoleic → vernolic → coriolic → punicic and α-eleostearic acids (Table 3, p. 7).

(ii) linoleic → coronaric → α-dimorphecolic → jacaric and calendic acids (Table 3, p. 7).

(iii) crepenynic → monoepoxidised crepenynic → helenynolic acid (Table 4, p. 8).

(iv) linolenic → 12,13-epoxidised linolenic → 12-hydroxy and 13-hydroxy triene → parinaric acid (Table 4, p. 8).

The ultimate proof of these biosynthetic sequences must be provided by the use of labelled compounds with the whole plant, parts of the plant, or with isolated enzyme systems. Short of this however, it is possible to provide valuable supporting evidence along the following lines:

(a) The intermediates are likely to occur naturally with the correct structure both in gross terms and stereochemically.

(b) There might be evidence of co-occurrence of some or all of the compounds involved in the total sequence.

(c) The reactions must be chemically feasible.

Items (a) and (b) will be discussed here though some additional experimental evidence is cited in Chapter IV. The main part of this thesis is concerned with further examination of item (c).

Table 3.

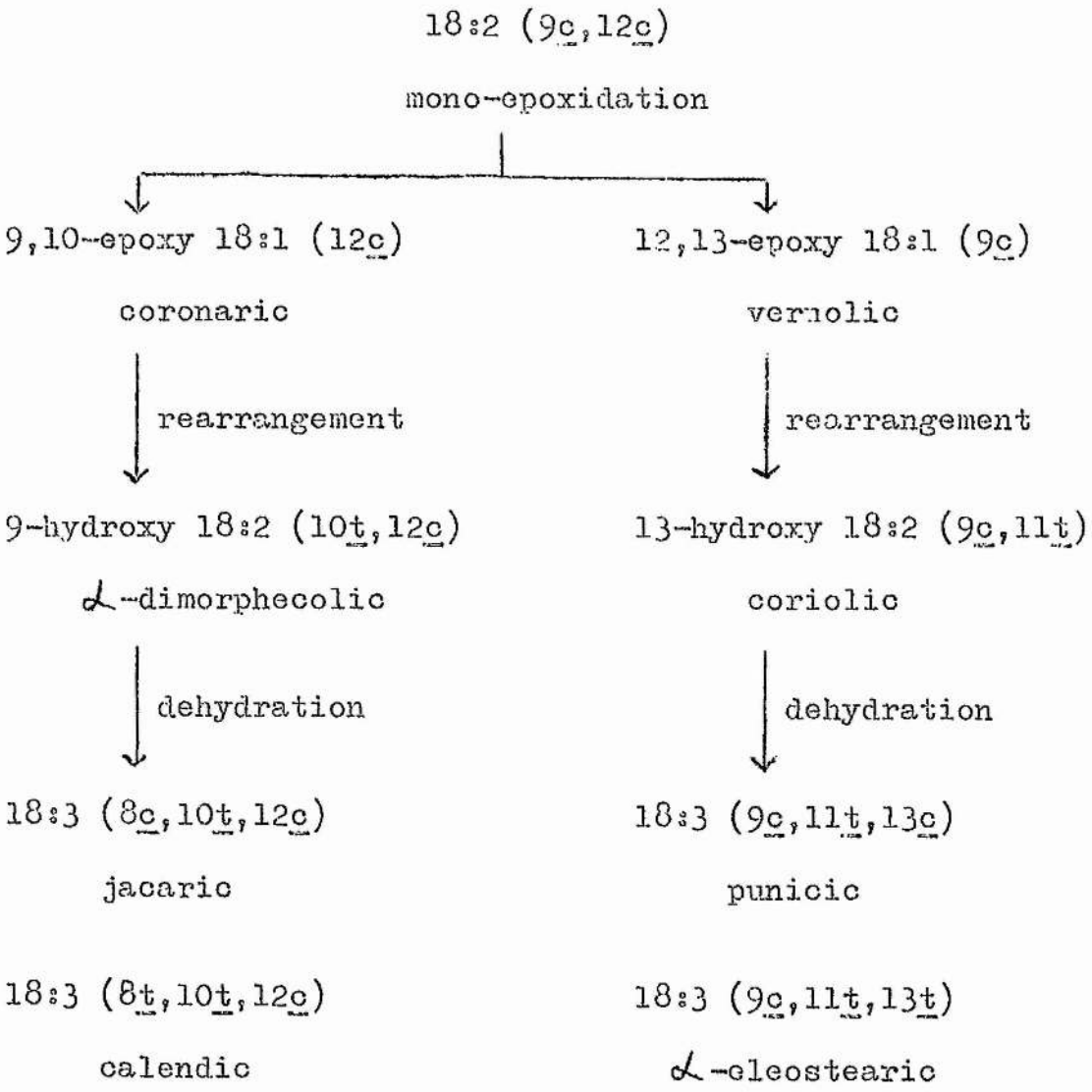

18:2 (9c,12c)
mono-epoxidation
9,10-epoxy 18:1 (12c)
coronaric
rearrangement
9-hydroxy 18:2 (10t,12c)
α-dimorphecolic
dehydration
18:3 (8c,10t,12c)
jacaric
18:3 (8t,10t,12c)
calendic
12,13-epoxy 18:1 (9c)
vernolic
rearrangement
13-hydroxy 18:2 (9c,11t)
coriolic
dehydration
18:3 (9c,11t,13c)
punicic
18:3 (9c,11t,13t)
α-eleostearic

Table 4.

18:2 (9c,12a)	18:3 (9c,12c,15c)
crepenynic	linolenic
↓ epoxidation	↓ monoepoxidation
9,10-epoxy 18:1 (12a)	12,13-epoxy 18:2 (9c,15c)
↓ rearrangement	↓ rearrangement
9-hydroxy 18:2 (10t,12a)	12-hydroxy 18:3 (9c,13t,15c) and 13-hydroxy 18:3 (9c,11t,15c)
helenynolic	↓ dehydration
	18:4 (9c,11t,13t,15c)
	parinaric

(a) Structure of intermediates and products.

If these epoxy acids, hydroxy acids and conjugated trione and tetraene acids are related to the more common linoleic and linolenic acids then in all cases except two (β -dimorphecolic and catalpic) double bonds in the original position (9 or 12 or 15) retain the cis configuration. Thus, vernolic, coriolic,

α-eleostearic and punicic acids still have the 9c unit from linoleic, and coronaric, α-dimorphecolic, calendic and jacaric acids still have the 12c unit from linoleic. Similarly, parinaric retains the 9c and 15c units from linolenic.

The proposal also has important consequences in respect of the absolute configuration of the intermediate oxygenated acids as it would be expected that the absolute configuration of the epoxy acid and the derived hydroxydiene acid would be the same. Of the hydroxydiene acids, coriolic acid[21] has the 13D configuration, and α-dimorphecolic[22,36] and β-dimorphecolic[37] acids both have the 9D configuration. Thus in sequence (i), vernolic acid (12D,13D) could be the precursor of coriolic acid (13D) but in sequence (ii), coronaric (9L,10L) is unlikely to be the precursor of α-dimorphecolic acid (9D). However, as 12,13-epoxyoleate has been shown to occur in both 12D,13D and 12L,13L configurations, it is possible that the 9,10-epoxyoctadec-12-enoic acid might do so likewise; the 9D,10D isomer would then be the precursor of α-dimorphecolic acid.

In the dehydration of the hydroxydienes to the conjugated trienes both 1,2-*cis* and 1,2-*trans* dehydration appears to be possible[38].

With sequence (iii) the intermediate monoepoxidised crepenynate was not known but on the basis of this hypothesis it

was sought and found. This is described in Chapter IV.

In sequence (iv), linolenic ⟶ parinaric acid, no useful comments can be made regarding the stereochemistry of the epoxydiene or the hydroxytriene(s) intermediates as none of these is known. The epoxydiene (cis-15,16-epoxyoctadeca-cis-9,cis-12-dienoic acid) that has been reported by Gunstone and Morris[10] could by similar rearrangements give rise to a 16-hydroxy 9c,12c,14t acid and thence to the 9c,12c,14t,16c and 9c,12c,14t,16t tetraene acids, but acids with these structures have not yet been discovered.

The two acids which do not fit conveniently into these schemes, β-dimorphecolic and catalpic acids, are known to co-exist with the 9c,12t[31] and 9t,12t[39] isomers of linoleic from which they may be derived by similar epoxidation-rearrangement-dehydration sequences.

(b) Co-occurrence of members of the biosynthetic sequences.

Linoleic with the other acids. Linoleic acid is present in almost every seed oil so its co-occurrence with any other acid is not significant. The co-occurrence of the 9c,12t isomer with β-dimorphecolic acid in Dimorphotheca sinuata seed oil[31], and of the 9t,12t isomer with catalpic acid in Catalpa bignonioides[39], is, however, of considerable significance and again suggests a clear relation between the non-conjugated polyene acids,

hydroxydiene acids, and conjugated triene acids.

Hydroxydienes and conjugated trienes. The general co-occurrence of these two classes of acids in seed oils from many different plant families (although especially the Compositae) has been adequately demonstrated by the research group at Peoria[40-42]. Their efforts, however, did not distinguish between the various hydroxydiene isomers or those of the conjugated triene acids and this remains a problem which can only be solved by further investigation.

Attempts have also been made to show a more specific relationship. Hopkins and Chisholm[43] found a hydroxydiene and a conjugated triene in different species of the Osteospermum genus, and suggested this as evidence for a biogenetic relationship between the two. They considered that although the hydroxydiene acid in question (9-OH 10t,12t) was not likely to be the precursor of the triene (8t,10t,12c), the latter might arise from the 9-OH 10t,12c isomer also known to occur in the Compositae family. The co-existence of the 9-OH 10t,12c acid with the 8t,10t,12c triene acid was later demonstrated in Calendula officinalis seed oil by Badami and Morris[36].

While the frequent co-occurrence of hydroxydiene acids with conjugated triene acids is accepted, there remain unusual features in this relationship. For instance, there are many cases[12,22,24,43,44] where the 9-OH 10t,12c acid and its

13-OH 9c,11t isomer occur together in the same species, yet sources of conjugated triene acids have never been found to contain more than one isomer in one species. Hopkins and Chisholm[38], however, have reported two triene isomers (9c,11t, 13t and 9c,11t,13c) co-occurring in one genus (Momordica).

Epoxy and hydroxydiene acids. Morris and co-workers[11,44] first demonstrated, on the basis of TLC and GLC evidence, the co-occurrence of epoxy acids with hydroxydiene acids and the same group[45] also described a method, using near IR spectroscopy, for the estimation of small amounts of epoxy acids in the presence of large amounts of hydroxydiene components. Almost simultaneously, Hopkins and Chisholm[24] found a small amount of hydroxydiene acids in Tragopogon porrifolius seed oil in which they had previously found an epoxy acid. Recently the studies of Powell et al.[12,46] have shown the generality of this co-existence and, in particular, in Xeranthemum annuum seed oil[22,46] they have reported the co-occurrence of the two possible monoepoxy acids from linoleic acid along with the two related hydroxydiene acids.

3. General biological significance of epoxides.

Support for Gunstone's proposal is to be found in a growing awareness of the potential importance of epoxides as biosynthetic intermediates in other classes of natural products.

Epoxides might be involved in the biosynthesis of acetylenic compounds. James[47], in his studies on the biosynthesis of crepenynic acid, has considered vernolic acid as a possible precursor. This is in agreement with the observation of Earle and co-workers[48] who, in their studies on Crepis oils, found a definite inverse relationship between linoleic and the sum of vernolic and crepenynic acid. Recently, however, Bohlman[49] found no conversion of 'labelled' vernolic acid into crepenynic acid.

The occurrence of polyacetylenic epoxides has been described by Bohlman et al.[50] and by Jones and Stevenson[51]. Particularly significant is the discovery by the last mentioned authors[51] of the co-occurrence in the Coprinus quadrifidus metabolites of a polyacetylenic trans-epoxy alcohol (II) with the corresponding olefin (I) and the 1,2-diol (III). This suggests a close biogenetic relationship.

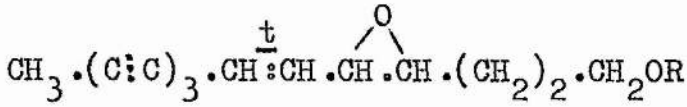
$$HC{:}C.C{:}C.C{:}C.\overset{t}{CH}{:}CH.CH_2OH \qquad (I)$$

$$HC{:}C.C{:}C.C{:}C.\underset{O}{CH}.CH.CH_2OH \qquad (II)$$

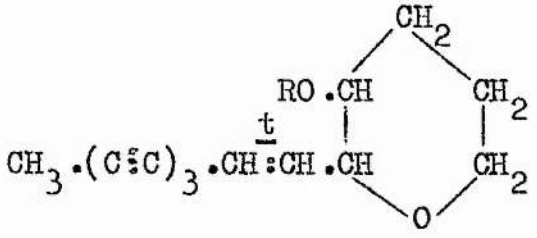
$$HC{:}C.C{:}C.C{:}C.\underset{\text{erythro}}{CH(OH).CH(OH)}.CH_2OH \qquad (III)$$

In a recent review[52] on the biogenesis of the poly-ynes of Tribus Anthemideae, Bohlman considers the polyacetylenic

tetrahydropyran derivatives (V) to be derived from naturally occurring epoxides (IV).



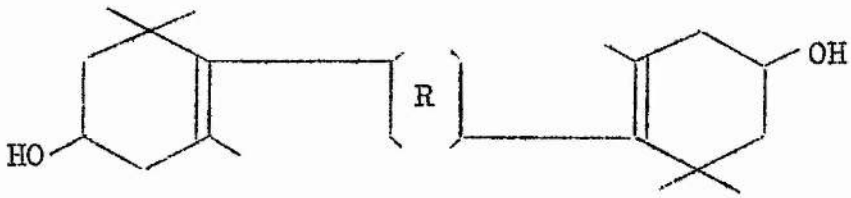
(IV)



(V)

R = H,Ac

A neat picture of biogenetic relations within a class of natural products is presented by the carotenoids. Structural determinations[53,54] on the pigments of red pepper have revealed a class of carotenoids containing five-membered rings. Examples of these include capsorubin (IX) and capsanthin (X). It is considered[53,54] that these compounds are formed biosynthetically from zeaxanthin (VI) by pinacolic rearrangement (XI) of the natural epoxides violaxanthin (VII) and antheraxanthin (VIII) respectively.



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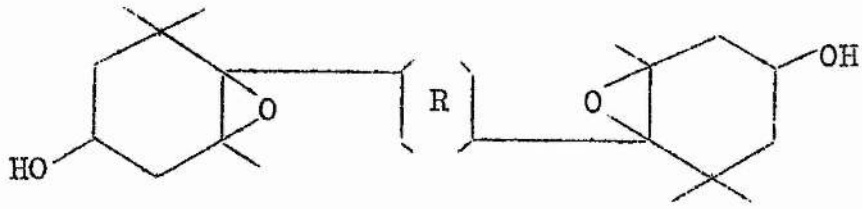

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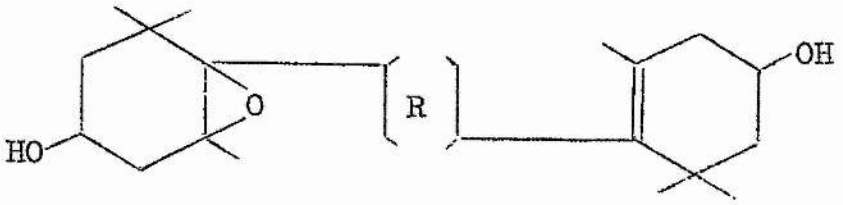
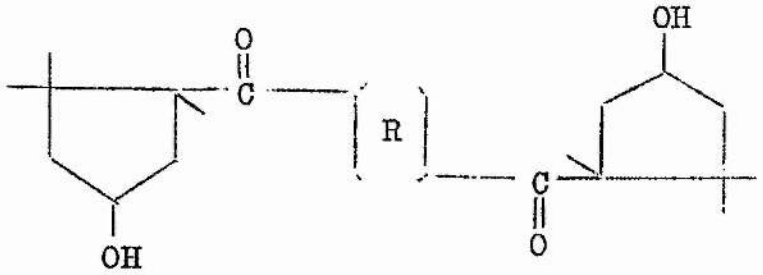


(VIII)



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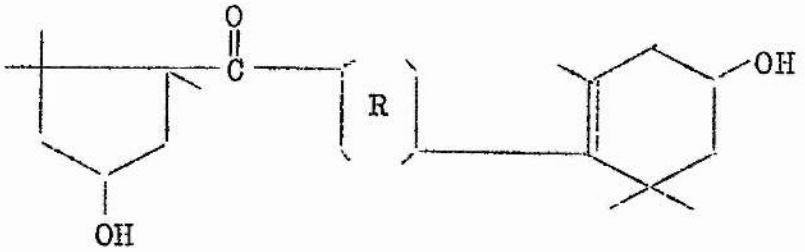
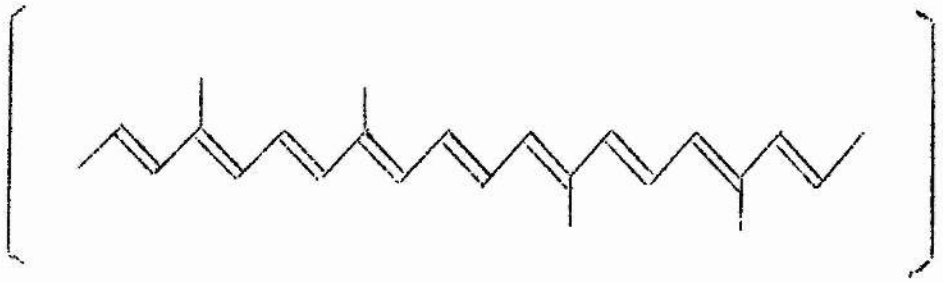
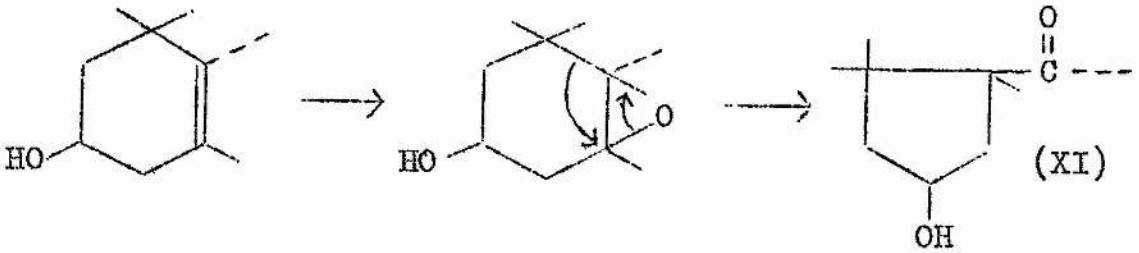


(X)

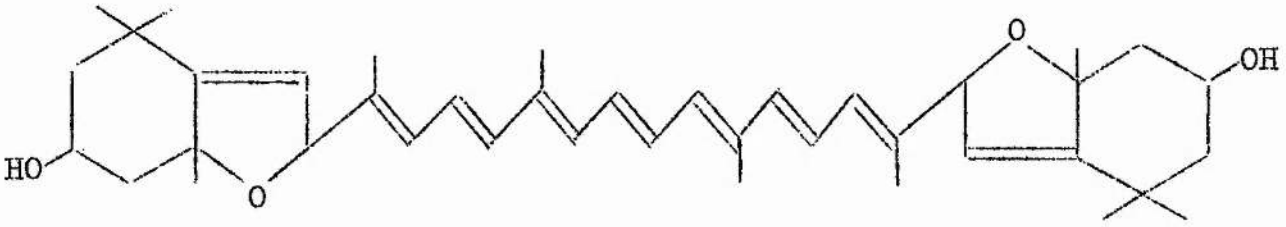
R =





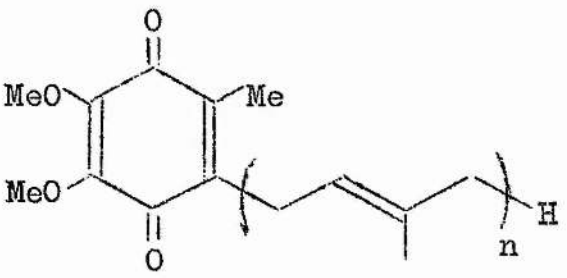
(XI)

The naturally occurring furanoid oxide, auroxanthin (XII), is also believed to be derived via an epoxide[55] as this type of rearrangement is effected under mild chemical conditions[56].

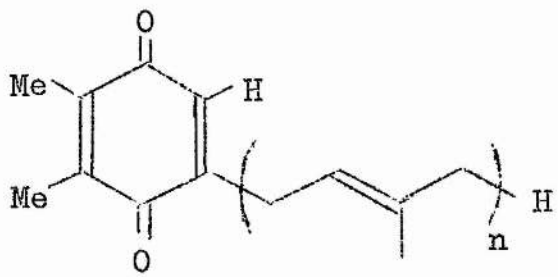


(XII)

Recently the occurrence of five epoxy derivatives of ubiquinone-10 (XIII) have been described[57]. These have the epoxide in the isoprenoid side chain. It had been previously demonstrated[58,59] that in the related plastoquinones a series of minor components with hydroxyl groups in the side chain co-occurred with plastoquinone-9 (XIV). It was therefore tentatively suggested that epoxides might be the precursors of the hydroxylated derivatives[57].



(XIII) n = 10



(XIV) n = 9

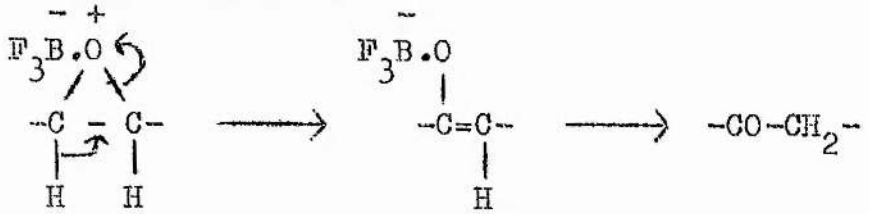
Finally, in the steroid field, Breuer and Knuppen[60] demonstrated the presence of a steroid epoxidase in animal tissue in their work on oestratetraenol. More recently the studies of Corey et al.[61,62] and van Tamelen et al.[63] have shown that 2,3-epoxysqualene is a key intermediate in the biosynthesis of sterols from squalene.

Chapter II.

ACID-CATALYSED ISOMERISATION.

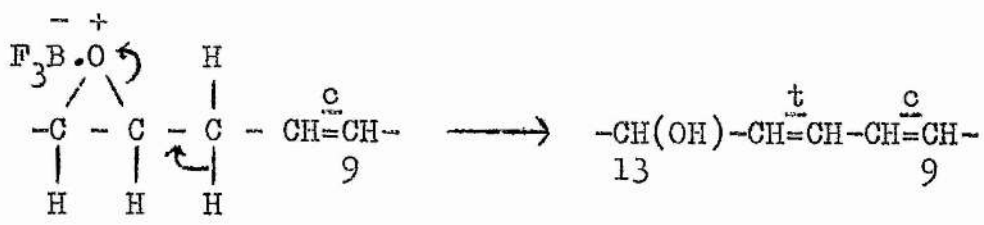
INTRODUCTION.

The rearrangement of epoxides to carbonyl derivatives under the action of acidic catalysts is well known and has been the subject of two comprehensive reviews[64,65]. Recently, this rearrangement has been applied to fatty acid epoxides by Walens et al.[66] who demonstrated that high yields of 9- and 10-oxo-stearates could be obtained by the reaction of boron trifluoride etherate on 9,10-epoxystearate in boiling dioxan. This reaction probably occurs via the intermediate (I) which then affords the enolic form of these ketones.



(I)

It was hoped that a similar reaction on methyl vernolate (12,13-epoxyoleate) might involve the hydrogen atom of the activated methylene group at C(11) and furnish the allylic alcohol derivative (methyl coriolate) via intermediate (II).

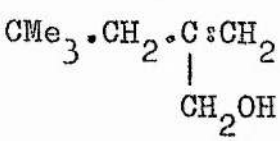


(II)

Several acid-catalysed reactions of epoxides have been reported in the literature in which allylic alcohols have been obtained.

Byers and Hickinbottom[67] found that the action of acid on 1:2-epoxy-2:4:4-trimethylpentane (III) gave, in addition to the expected diol, two unsaturated alcohols: 4:4-dimethyl-2-(hydroxymethyl)pent-1-ene (IV) and 2:4:4-trimethylpent-2-en-1-ol (V).

(III)



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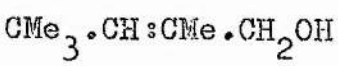
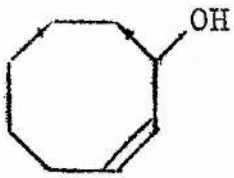
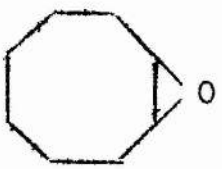


(V)

Similarly, Cope and co-workers[68] obtained products including an allylic alcohol (VI) from the reaction of formic acid with cis cyclo-octene oxide (VII).



(VI)



(VII)

In addition, in their studies on the rearrangement of epoxysteroids with boron trifluoride, Henbest and Wrigley[69] obtained steroids containing a conjugated diene grouping which

they considered to arise by dehydration of an intermediate allylic alcohol.

Studies were commenced on the reaction between methyl vernolate and boron trifluoride under the conditions used by Walens et al.[66] in an attempt to convert it to methyl coriolate; the possibility of further dehydration to the conjugated triene(s) was also realised.

DISCUSSION.

Methyl vernolate was isolated from Vernonia anthelmintica seed oil and adjudged pure by TLC and GLC.

1. Isomerisation of methyl vernolate with boron trifluoride etherate in refluxing dioxan.

Methyl vernolate was refluxed for three hours in anhydrous dioxan with three equivalents of boron trifluoride. GLC of the isolated reaction products indicated the complete disappearance of the epoxy ester with formation of several new components (Table 5).

Table 5.

C.No (DEGS)	% Area	C.No (ApL)	% Area
24.8	13	18.8	15
25.3	69	19.1	65
26.8	18	19.9	20

Notes.

a) Under these conditions methyl vernolate had carbon numbers of 24.6 and 19.1.

b) In addition to the three peaks given above a series of peaks ranging from carbon number 20-24 appeared as a rather wavy baseline on the DEGS column. These were discounted in the above table.

TLC analysis showed four reasonably distinct fractions (A, B, C and D, in order of decreasing Rf value) which corresponded approximately in polarity to 'non-oxygenated' esters (A), 'oxo' esters (B), 'mono-hydroxy' esters (C) and 'di-hydroxy' esters (D). The infra red spectrum, in addition to the usual ester features, indicated hydroxyl and oxo groups and a trace of trans unsaturation. The ultra violet spectrum showed conjugated triene absorption and absorption at λ max 225mμ. The reaction product was separated by silica gel column chromatography into four fractions, A (10%), B (70%), C (10%) and D (10%), in the proportions indicated. Examination of these fractions by TLC indicated C and D to be fairly complex, possibly containing polymeric material.

Each fraction was analysed in greater detail.

1.1 Fraction D.

This fraction, as mentioned above, contained several components, one of which was characterised as methyl 12,13-dihydroxyoleate by its IR spectrum and by von Rudloff oxidation before and after hydrogenation. Walons et al.[66] had previously demonstrated the presence of some 9,10-dihydroxystearate in their isomerisation of methyl 9,10-epoxystearate. No further work was carried out on this fraction.

1.2 Fraction A.

The infra red spectrum indicated complex cis,trans and trans,trans conjugation[70] and the ultra violet spectrum showed conjugated diene and conjugated triene chromophores. von Rudloff oxidation of the fraction indicated unsaturation beginning both at C(8) and at C(9), and hydrogenation gave methyl stearate.

GLC analysis of the fraction showed two distinct groups of peaks of approximately equal area (Table 6).

Table 6.

C.No (DEGS)	C.No (ApL)	% Area
20.8, 21.2, 21.7	18.0, 18.3, 18.5	50
23.2, 23.7	19.2, 19.5	50

The last group corresponded in carbon number to methyl α-eicostearate (23.3, 23.7, DEGS; 19.2, 19.5, ApL) and the first group may be conjugated diene esters with an isolated double bond.

The fraction was partially separated by prep. Ag^+/TLC. An attempt to identify a conjugated diene ester subfraction by partial reduction with hydrazine[71] failed both due to the complexity of products and to lack of material.

1.3 Fraction C.

Investigations on this fraction are discussed in detail on page 28.

1.4 Fraction B.

It was expected that this fraction would show essentially one peak on GLC corresponding to the 12(13)-oxo-oleates (cf. Walens et al.[66]). Instead, GLC showed three components (Table 7).

Table 7.

	C.No (DEGS)	% Area	C.No (ApL)	% Area
B1	24.8	13	18.8	15
B2	25.3	69	19.1	65
B3	26.8	18	19.9	20
	After hydrogenation			
	24.9	100	18.8	18
			19.4	82

The infra red spectrum indicated oxo (1710cm^{-1}), conjugated oxo (1670, 1685cm^{-1}) and trans-unsaturation (970cm^{-1}), and the ultra violet spectrum showed a conjugated oxo absorption at λ max 225mμ. Catalytic hydrogenation yielded a component

corresponding to a methyl oxostearate (24.9, DEGS; 19.4, ApL)* together with, apparently, the unchanged component B1 (Table 7, p. 24). Reduction with sodium borohydride to a mixture of hydroxy esters confirmed the presence of an oxo group.

Fraction B was initially separated into concentrates of the three components by a combination of prep. Ag^+/TLC and direct TLC. (See Separation Scheme 1, p. 57.)

Component B3.

This component was characterised as methyl 12-oxo-octadec-trans-10-enoate on the following evidence:

(i) The ultra violet spectrum showed a strong absorption at λ max 225mμ ($E^{1\%}_{1cm}$ 480) indicative of an $\alpha\beta$-unsaturated ketone[72], and the infra red spectrum indicated trans-unsaturation (970cm^{-1}) and a conjugated oxo group (1670, 1685cm^{-1}).

(ii) Catalytic hydrogenation yielded an oxostearate (24.9, DEGS; 19.4, ApL) which was oxidised by chromic acid to the C_{11}- and C_{12}- dibasic acids. This shows the presence of an oxo group on C(12).

(iii) von Rudloff oxidation of the original ester gave the C_{10}- dibasic acid.

As $\alpha\beta$-unsaturated ketones have been shown to isomerise

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* In parentheses, numbers used with DEGS or ApL should be assumed to be carbon numbers unless otherwise stated.

readily to the conjugated form under hot acidic conditions[72,73] it seemed possible that this component arose from the isomerisation of the methyl 12-oxo-oleate formed during the reaction. It was shown that an authentic sample of this last ester was partially isomerised (30%) to the conjugated trans isomer when heated with boron trifluoride in dioxan.

When the original reaction on methyl vernolate was carried out at room temperature with only one equivalent of boron trifluoride, the conjugated component, B3, was not formed. Except for the absence of B3 this milder technique yielded major products qualitatively similar to those obtained from the initial isomerisation and subsequent identifications were carried out on compounds isolated from this milder isomerisation. It was hoped that without the trans isomer, B3, the other 'B' components would be more readily separated.

2. Isomerisation of methyl vernolate with boron trifluoride etherate in dioxan at room temperature.

Methyl vernolate in anhydrous dioxan was allowed to react overnight at room temperature with one equivalent of boron trifluoride. GLC analysis of the isolated reaction product indicated the complete disappearance of the epoxy ester with the formation of new components (Table 8, p. 27).

Table 8.

C.No (DEGS)	% Area
22.3	0.5
22.7	0.5
23.2	4.0
23.9	3.0
24.8	8.0
25.3	80.0
26.0	4.0

(Methyl vernolate had a C.No of 24.6)

The four fractions, A (2%), B (84%), C (9%) and D (5%), were isolated in the proportions shown by column chromatography. TLC of these fractions again indicated Fractions C and D to be fairly complex. Fraction D was presumed to be similar to that obtained in the earlier experiment (p. 21) and was not re-examined further.

2.1 Fraction A.

GLC analysis indicated two groups of peaks (Table 9, p. 28). This fraction was qualitatively similar to that described previously (p. 23) and was not investigated further.

Table 9.

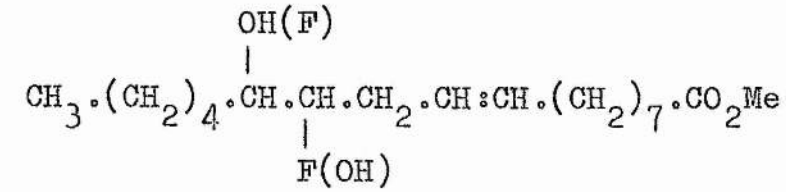
C.No (DEGS)	C.No (ApL)	% Area
20.8	18.0	
21.3	18.4	30
21.7	18.8	
23.2	19.2	70
23.6	19.5	

2.2 Fraction C.

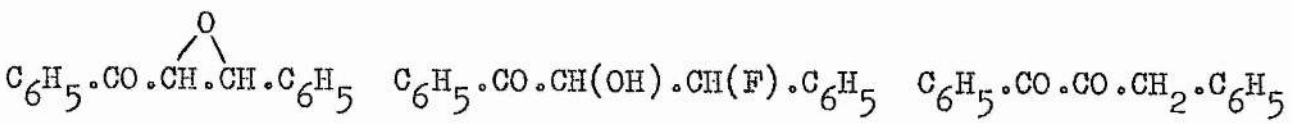
TLC indicated this fraction to be fairly complex but it did show two major components running with Rf values slightly less than chlorohydrin esters prepared from epoxyoleate. GLC analysis on a polar column gave two peaks of high carbon number (27.6, 27.9); on a non-polar phase, only broad 'humps' were observed indicative of polar hydroxy compounds. The ultra violet spectrum showed no significant absorption but the infra red spectrum, in addition to the usual ester features, indicated hydroxyl ($3590cm^{-1}$), and an absorption at $1070cm^{-1}$, possibly due to C-F stretching[74].

When Fraction C was submitted to the original vigorous reaction conditions (3 moles of boron trifluoride in refluxing dioxan) it furnished products qualitatively similar to those obtained from epoxyoleate (p. 21). It was tentatively concluded from this evidence that part of Fraction C comprised

fluorohydrin esters.

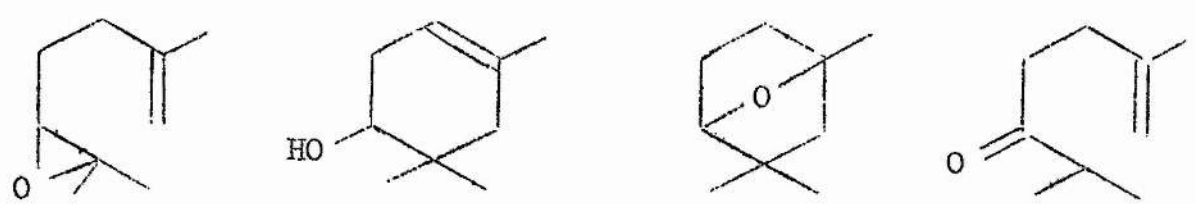


A precedent for this type of behaviour can be drawn from the work of House[75] who observed that treatment of the oxide (I) with a limited amount of boron trifluoride ($\leq$ molar equiv) in ether led to fluorohydrin (II). Further treatment of the latter with an excess of boron trifluoride in ether then produced the dicarbonyl compound (III) which was also obtained directly from reaction of the original epoxide with an excess of boron trifluoride.



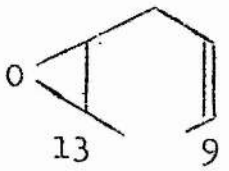
(I) (II) (III)

The presence of cyclised components in this fraction was also considered. Goldsmith[76] has shown that geraniolene monoepoxide (IV) is isomerised in benzene solution to the products (V), (VI) and (VII).

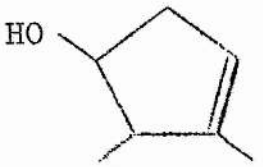


(IV) (V) (VI) (VII)

Epoxyoleate (VIII) might similarly rearrange to the cyclopent-enol ester (IX).



(VIII)



(IX)

When Fraction C was hydrogenated and then oxidised it yielded an oxo ester with different GLC behaviour (24.5, DEGS) from authentic 12-oxostearate (24.9, DEGS). The infra red spectrum of this oxo ester showed carbonyl absorption (1720cm^{-1}) at a slightly higher frequency than that of an oxostearate (1710-1715cm^{-1}) but lower than that expected of a five-membered ring ketone (1740cm^{-1})[77]. Removal of the oxo function by reduction of its tosylhydrazone[78] yielded a component identical in GLC behaviour to methyl stearate. Chromic acid oxidation of this desoxo ester gave no useful information.

Fraction C was assumed to contain fluorohydrin esters and was not characterised further.

2.3 Fraction B.

GLC analysis (Table 10, p. 31) gave a similar pattern to that obtained from the total reaction product. No absorption was observed in the ultra violet, and the infra red spectrum was similar to that of an authentic sample of methyl 12-oxo-octadec-

9-enoate. The major component B2 (81%) had similar carbon numbers on polar and non-polar phases (25.3, 19.1) to 12-oxo-oleate.

Table 10.

C.No (DEGS)	% Area
22.3	0.5
22.7	0.5
23.2	4.0
24.8	8.0
25.3	81.0
26.0*	6.0

* This component varied in amount in different GLC analyses of the same sample.

Attempted separation of Fraction B by Ag^+/TLC.

Considerable difficulty was experienced for some time with this separation until eventually it was realised that autoxidation of one component (methyl 12-oxo-octadec-9-enoate) was occurring on the plate.

These problems are discussed in the following pages (pp. 31-35) and the main discussion on the identity of the components of Fraction B is resumed on page 35.

A typical prep. Ag^+/TLC separation gave the results shown in Table 11 (p. 32) (see Separation Scheme 2, p. 58).

Table 11.

Fraction	% Weight	C.No (DEGS)	C.No (ApL)	% Area
B1	8	22.7	18.6	4
		24.8	18.8	96
		25.6	19.2	
B1a	7	22.3	18.5	9
		23.2	19.5	51
		24.8	18.8	12
		25.3	19.2	28
B2	72	25.3	19.0	100
B2a	10	-	-	-
B4	3	-	-	-

Notes.

a) The components are in order of decreasing Rf value and are designated B1, B1a,...etc. to retain the original nomenclature for the components.

b) The major fraction, B2, occasionally seemed to comprise two components, however this was extracted initially as one fraction.

c) Fractions B2a and B4 were not eluted under the GLC conditions used.

Attempts to identify B2.

Re-chromatography of this major fraction by prep. Ag^+/TLC gave two components of approximately equal weight. Both had the same carbon number on GLC (25.3, DEGS; 19.0, ApL) although it

was noted that considerably more of the lower band had to be injected to give a peak of similar area to that obtained from the upper band. This suggested that a minor component only was being observed.

Two possible reasons for this separation were considered:

(i) Separation of cis and trans isomers. If this were the case the trans isomer would probably be the upper band and the cis isomer the lower band. Infra red evidence, however, was not consistent with this view. The lower band only, showed a small trans absorption ($970cm^{-1}$) and in both its infra red ($1685cm^{-1}$) and ultra violet spectra (λmax 225mμ) a conjugated oxo group was indicated.

(ii) Separation of 12- and 13- oxo-oleates. As Fraction B2 was presumably a mixture of the 12- and 13- oxo-oleates (GLC and IR) it was possible that a separation of positional isomers was occurring. An attempt to check this idea by determination of the position of the oxo group in each band was unsuccessful because in each case hydrogenation gave a considerable amount ($>$ 40%) of unexpected by-products in addition to the expected oxostearates.

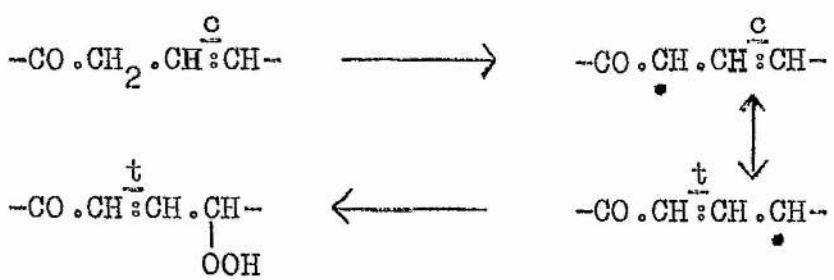
These results suggested that one or more components were undergoing change during separation.

Appropriate experiments then showed that Fraction B was unchanged by chromatography on thin layers of silica and continued to run as a single spot, but that in the presence of silver ions

five fractions were obtained (Table 11, p. 32), some of which no longer gave a single spot on silica layers.

This may be due to a silver ion-catalysed autoxidation of methyl 12-oxo-octadec-9-enoate which is known to be readily autoxidised[79,80].

The five fractions from the Ag^+/TLC separation were run on a silica plate. When this was sprayed with a solution of potassium iodide, Fractions B2, B2a and B4 each showed dark brown spots indicative of hydroperoxides[81]. The hydroperoxide fractions were not studied further but if autoxidation follows the sequence set out below, the final product, (X), being an $\alpha\beta$-trans-enone, would account for the infra red and ultra violet spectra (p. 33).



(X)

During hydrogenation (X) might cyclise to esters having a furan ring and thereby account for the unusual hydrogenation products.

To check this, samples of methyl 12-oxo-octadec-9-enoate and 9-oxo-octadec-12-enoate were prepared and chromatographed by Ag^+/TLC. When re-examined on silica layers, the 12-oxo compound,

but not the 9-oxo ester*, showed polar impurities revealed as brown spots when sprayed with potassium iodide solution.

Main discussion continued.

Component B2.

It was thus clear that the Ag^+/TLC technique was not appropriate for the separation of Component B2 from Fraction B and the former was therefore identified using the whole Fraction B.

It proved to be a mixture of methyl 12-oxo (70%) and methyl 13-oxo-oleates (30%). No significant absorption was observed in the ultra violet spectrum and the infra red spectrum was identical to that of authentic 12-oxo-oleate. von Rudloff oxidation gave only the C_9- dibasic acid, apart from unchanged B1 and the component of carbon number 22.7. Hydrogenation gave methyl oxostearates, again accompanied by the same unchanged components. Chromic acid oxidation of the hydrogenated esters yielded the C_{11}-, C_{12}- and C_{13}- dibasic acids, (along with smaller amounts of C_7-, C_8- and C_9- dibasic acids now known to arise from B1), placing oxo groups at C(12) and C(13). To determine the relative proportions of the 12- and 13- oxo isomers,

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* This ester was chosen as being analogous to methyl 13-oxo-octadec- 9-enoate since both have two methylene groups between the double bond and the oxo function.

the hydrogenated oxo esters were converted to oximes, rearranged to amides, and hydrolysed to various products including the C_{12}- and C_{13}- dibasic acids[82]. The relative areas of the C_{12}- and C_{13}- dibasic esters, as determined by GLC, gave the ratio of the oxo isomers. (Fraction B1 was later shown to give no significant peaks when submitted to this rearrangement.)

Component B1.

Having discovered that B1 was unchanged after von Rudloff oxidation and hydrogenation, it was separated from B2 by reaction with methanolic mercuric acetate as described by Cocker et al.[83]. The unreacted material was isolated by prep. TLC and whenever GLC indicated adduct formation to be incomplete, the saturated components were purified by Ag^+/TLC which has no deleterious effect on this component.

GLC showed B1 to be a mixture of two compounds which we could not separate by any other technique. These proved to be the cis and trans isomers of the oxocyclopropane ester (XI) on the following evidence:

$$CH_3.(CH_2)_4.CO.CH_2.\underset{\diagdown\,\diagup}{\overset{CH_2}{CH.CH}}.(CH_2)_7.CO_2Me \qquad (XI)$$

Chromatographic behaviour. On TLC and Ag^+/TLC, component B1 behaved like saturated oxo or epoxy esters. GLC indicated two components in relative amounts 80:20 on both polar (24.8, 25.6,

DEGS) and non-polar (18.8, 19.2, ApL) phases respectively.

Spectroscopic evidence. No absorption was observed in the ultra violet and the infra red spectrum showed absorption at 1730cm^{-1} (ester carbonyl), 1710cm^{-1} (oxo), and bands at 1020cm^{-1} and 3050cm^{-1} indicative of a cyclopropane unit[84]. The NMR spectrum also indicated the presence of a cyclopropane group with complex absorption in the region 9.5-10.3τ. The greater intensity of absorption below 10.0τ indicated predominantly a trans cyclopropane configuration[85,86]. This suggested that the two constituents observed by GLC could be the cis and trans cyclopropane isomers with the major constituent (24.8, DEGS; 18.8, ApL) having the trans configuration. The absence of olefinic protons was confirmed by the NMR spectrum.

The mass spectra of the two compounds, after separation by GLC, were identical, further confirming their isomeric nature. Both showed a molecular ion peak at m/e 310. Peaks corresponding to α-cleavage (m/e 99, 239 and 207 (239 - 32)) and β-cleavage (m/e 114, 254 and 222 (254 - 32)) to the oxo group placed the latter at C(13)[87]. The mass spectra at this stage* gave no indication as to the cyclopropane ring position[88-90].

Chemical methods. B1 appeared to be saturated since it was

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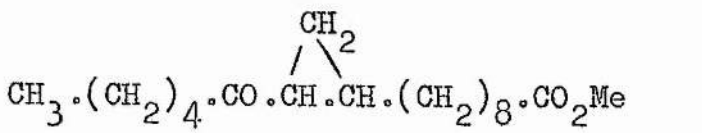
* The significance of two relatively intense peaks at m/e 127 and 139 will be discussed later (p.44).

unchanged by von Rudloff oxidation and by catalytic hydrogenation. Sodium borohydride reduction yielded a pair of compounds behaving like hydroxy esters on TLC and GLC (25.7, 26.4, DEGS). Chromic acid oxidation gave the C_7-, C_8- and C_9- dibasic acids and the C_5- and C_6- monobasic acids as the only major fragmentation products.

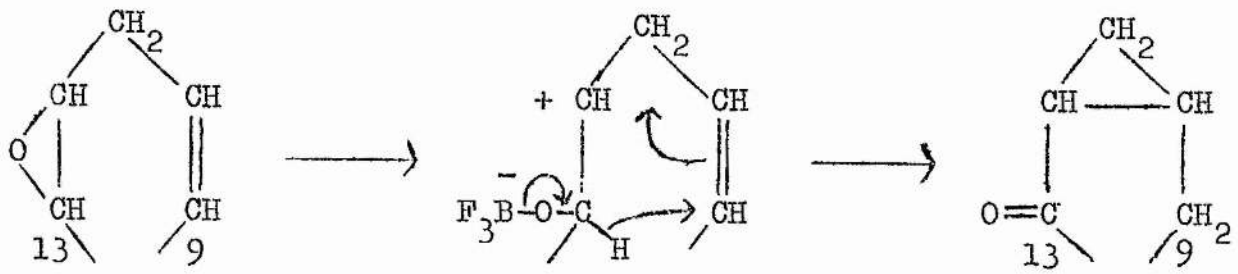
Removal of the carbonyl group by reduction of its tosyl-hydrazone[78] gave an ester of infra red spectrum identical with that of a methyl 9,10-methylene-octadecanoate (kindly supplied by Dr. W.W. Christie). The desoxo ester behaved like a mixture of trans and cis methyl methyleneheptadecanoates on GLC[89] with carbon numbers of 18.0 and 18.6 (DEGS) and 17.4 and 17.8 (ApL).

Possible structure of cyclopropane esters.

In view of the evidence at this stage and of the possible rearrangement of homoallylic cations to cyclopropane derivatives[91-93], it was considered that B1 was the oxocyclopropane ester (XII) arising by the mechanism:



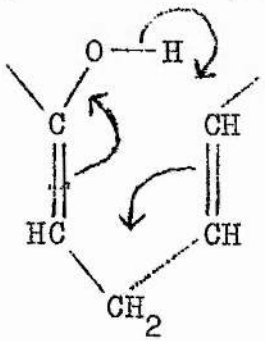
(XII)



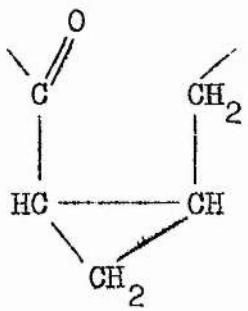
(XIII)

The homoallyl cation (XIII) rearranges to a cyclopropane and the new carbonium ion centre at C(9) is 'satisfied' by a 1-5 hydride shift from C(13). The driving force for this reaction is presumably provided by oxo formation at C(13) leading to the stable conjugated oxocyclopropane group.

Roberts et al.[94] have recently shown that pyrolysis of such an oxocyclopropane derivative gives a $\gamma\delta$-unsaturated ketone and it therefore seemed possible that, by a reversal of this process, the 13-oxo-octadec-9-enoate could give, via its enol (XIV), the oxocyclopropane ester (XII),



(XIV)



(XII)

This idea was not pursued when it was shown that methyl 9-oxo-octadec-12-enoate was unchanged after treatment with boron trifluoride in refluxing dioxan.

To confirm the postulated oxocyclopropane structure (XII) this substance was synthesised by reaction of the diazoketone (XV) and methyl undecylenate (XVI) following the procedure described by Lefort et al[95].

$CH_3 \cdot (CH_2)_4 \cdot CO \cdot CHN_2$ (XV)

$CH_2 : CH \cdot (CH_2)_8 \cdot CO_2Me$ (XVI)

$CH_3 \cdot (CH_2)_4 \cdot CO \cdot \underset{\diagdown\,\diagup}{CH_2} \cdot CH \cdot (CH_2)_8 \cdot CO_2Me$ (XII)

The product, isolated by prep. TLC, was found to differ from B1 both spectroscopically and chromatographically (Table 12).

Table 12.

	B1	Synthetic oxocyclopropane
IR	$1710cm^{-1}$ (oxo)	$1685cm^{-1}$ (oxo)
	$1730cm^{-1}$ (ester)	$1730cm^{-1}$ (ester)
	$1020cm^{-1}$, $3050cm^{-1}$ (cyclopropane)	$1030cm^{-1}$, $3070cm^{-1}$ (cyclopropane)
NMR	9.8-10.3τ	9.5τ (small)
GLC	24.8, 25.6 (DEGS)	25.2 (DEGS)
	18.8, 19.2 (ApL)	19.1 (ApL)

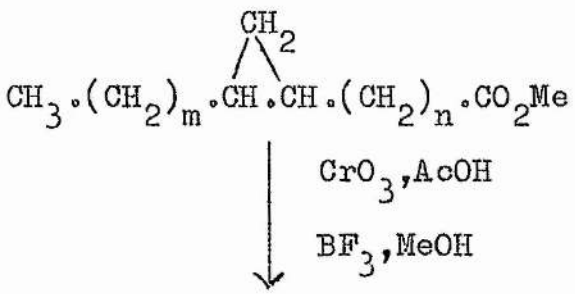
The difference between the properties of these two esters suggested that whilst the synthetic compound had a conjugated cyclopropane ring and oxo group, in B1 these two functions were not conjugated. When this conjugation was removed from the synthetic ester (XII), by reduction with sodium borohydride, the resulting hydroxy derivative exhibited spectral properties

more like those of B1.

An important clue to the position of the cyclopropane ring in B1 was obtained by observing that chromic acid oxidation of the synthetic ester (XII) gave C_8-, C_9- and C_{10}- dibasic acids whilst B1 gave C_7-, C_8- and C_9- dibasic acids.

It was shown that cyclopropane esters (kindly supplied by Dr. W.W. Christie) consistently furnished three dibasic acids and three monobasic acids when oxidised (Table 13). The dibasic acids are in the approximate ratio 60:25:15, longest to shortest.

Table 13.

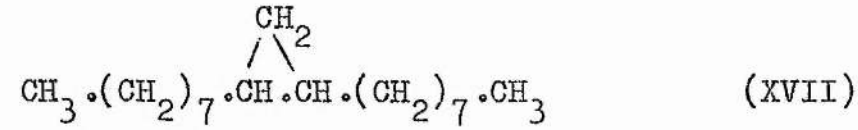


$CH_3.(CH_2)_p.CO_2Me$ $\qquad$ $MeO_2C.(CH_2)_r.CO_2Me$

p = m, m-1 and m-2 $\qquad$ r = n, n-1 and n-2

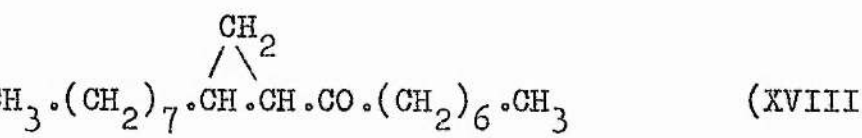
Several methods[96-99] for determination of the position of the cyclopropane ring in aliphatic esters have been described, but this oxidation procedure, followed by GLC examination of the acidic fragments, has the advantage of simplicity and speed of determination and does not require a mass spectrometer.

Shortly after this work was completed, Prome and Asselineau[100] described the chromic acid oxidation of aliphatic hydrocarbons such as (XVII) containing cyclopropane rings.



(XVII)

They obtained as their major oxidation product the 9,10-methylene octadecan-8-one (XVIII). (Prome[90] later used the mass spectrum of this major oxidation product (XVIII) for determination of the ring position.)



(XVIII)

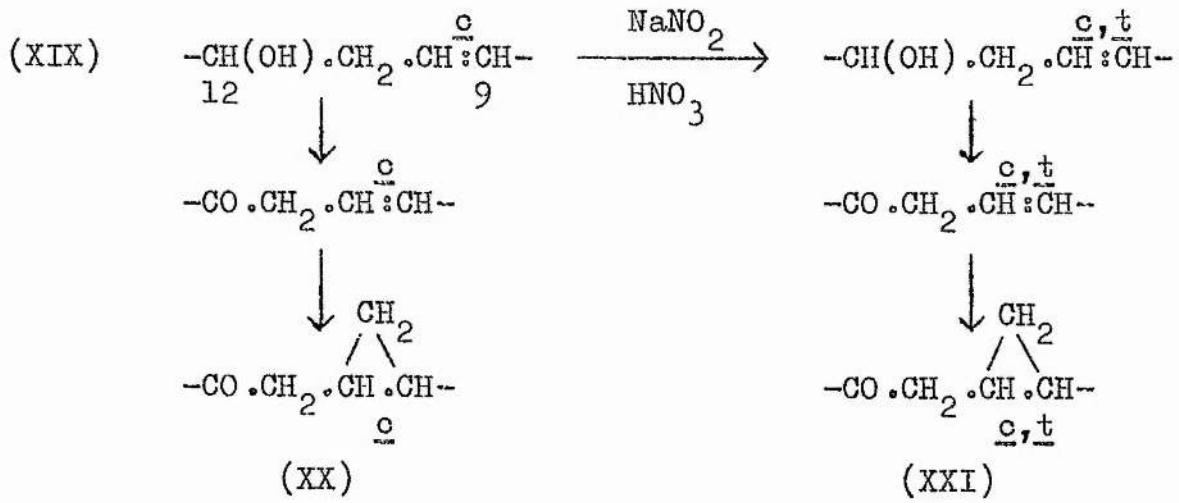
They also reported, however, as principal products of secondary oxidation the C_7-, C_8- and C_9- monobasic acids (3%, 5% and 12% respectively).

Thus, while it appears that the acidic fragments observed by us are only secondary oxidation fragments, the procedure is still considered to be valuable.

Fraction B1 after 'de-ketonation' and chromic acid oxidation gave C_7-, C_8- and C_9- dibasic acids (18%, 25% and 57% respectively) and C_6-, C_7- and C_8- monobasic acids. These facts indicated the revised structure (XI) for B1.

$$CH_3 \cdot (CH_2)_4 \cdot CO \cdot CH_2 \cdot \underset{\diagdown\,/}{\overset{CH_2}{CH}} \cdot CH \cdot (CH_2)_7 \cdot CO_2Me \qquad (XI)$$

Synthesis of a compound of this structure was not convenient but its homologue was readily prepared from methyl ricinoleate (XIX) in both cis (XX) and mixed cis,trans (XXI) forms.



The cis homologue was prepared by oxidation of ricinoleate to the 12-oxo ester[101] followed by conversion to the oxocyclopropane ester (XX) by reaction with methylene iodide and zinc-copper couple[89]. The cis,trans homologues (XXI) were prepared similarly after stereomutation of methyl ricinoleate to a mixture of cis and trans isomers[102].

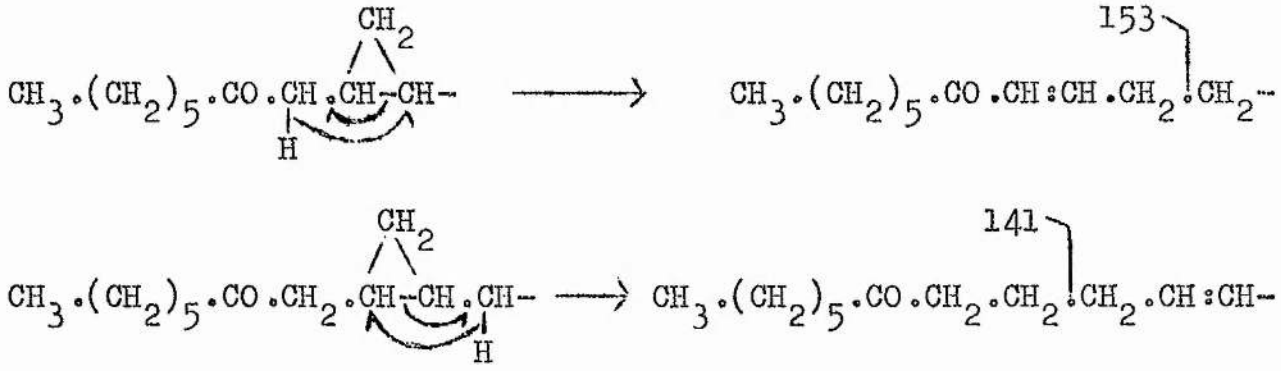
The synthetic cis,trans homologues had NMR and IR spectroscopic properties identical with B1 and carbon numbers expected of its homologues (Table 14, p. 44). The cis homologue also

exhibited the expected spectroscopic and chromatographic properties. Its GLC behaviour confirmed the earlier predictions (p. 37) regarding the stereochemical nature of the two compounds observed in B1.

Table 14.

B1		cis,trans homologues		cis homologue	
DEGS	ApL	DEGS	ApL	DEGS	ApL
24.8	18.8	25.8	19.8	-	-
25.6	19.2	26.6	20.2	26.6	20.2

The mass spectra of the synthetic compounds were identical, both showing a molecular ion peak at m/e 324. The expected peaks corresponding to α-cleavage (m/e 113, 239 and 207 (239 - 32)) and β-cleavage (m/e 128, 254 and 222 (254 - 32)) to the oxo group were also apparent. In addition, two relatively intense peaks were observed at m/e 141 and 153 which may be compared with those of m/e 127 and 139 observed with B1 (p. 37). These can be rationalised as shown in the scheme below



Collapse of the cyclopropane ring with transfer of a hydrogen atom is followed by allylic cleavage to the newly formed double bond.

Finally, these homologues gave the expected dibasic acids (C_7, C_8 and C_9) on chromic acid oxidation and their 'de-ketonated' derivatives gave the expected dibasic (C_7, C_8 and C_9) and monobasic acids (C_7, C_8 and C_9).

Component B1a (from prep. Ag^+/TLC separation of Fraction B).

GLC analysis on polar and non-polar columns gave the results tabulated in Table 15.

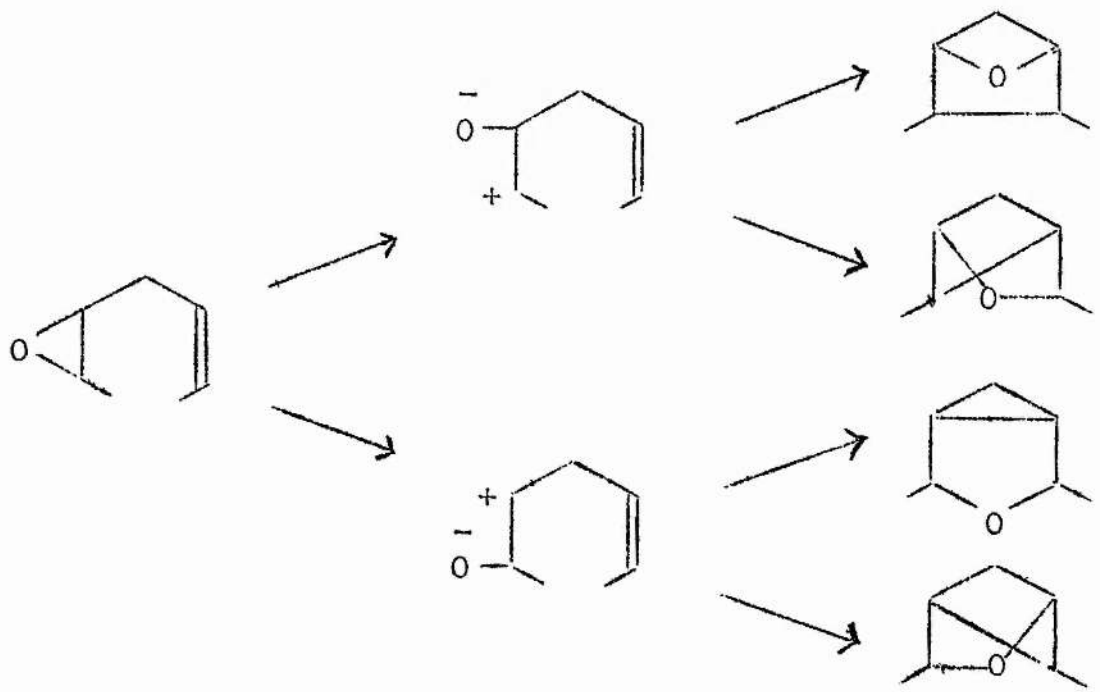
Table 15.

C.No (DEGS)	C.No (ApL)	% Area
22.3	18.5	9
23.2	19.5	51
24.8	18.8	12
25.3 25.6	19.1	28

By a rather laborious technique involving separation of small amounts on silica plates using double-development, this fraction was further separated into three sub-fractions: S1 47%, S2 40% and S3 13% in order of decreasing Rf value.

Band S1. GLC analysis indicated this band to comprise two components of carbon numbers 22.3, 23.2 (DEGS) and 18.5, 19.5

(ApL). The infra red spectrum indicated no carbonyl (other than the ester carbonyl at 1730cm^{-1}) and no trans-unsaturation but showed absorption at 1055cm^{-1} and 1215cm^{-1}, indicative of an ether linkage[103]. Goldsmith[76] has reported cyclic ether formation by the reaction of geraniolene monoepoxide with boron trifluoride (see p. 29), and it is tempting to suggest that these ether-containing esters are of the form shown below. These compounds were not identified further because of lack of material.



Band S2. GLC analysis indicated this to be predominantly the component of carbon number 25.3 (DEGS) and 19.1 (ApL). These carbon numbers correspond closely with authentic oxo-oleate. Hydrogenation gave the expected oxostearate (24.9, DEGS; 19.4, ApL). The infra red spectrum was similar to that of authentic oxo-oleate but indicated trans-unsaturation (970cm^{-1}). von

Rudloff oxidation indicated a C_9- dibasic acid and a fragment of carbon numbers 15.2 and 10.3 (DEGS and ApL), expected of a 4-oxo-nonanoate.

These facts are consistent with a structure of methyl 13-oxo-octadec-trans-9-enoate.

Band S3. Although separable from B1 on TLC, both GLC and infra red evidence indicated this fraction as being identical to B1. The nature of this fraction is discussed later (p. 54).

3. Isomerisation of methyl vernolate with boron trifluoride in benzene solution.

In accordance with several reports[75,76,104] that isomerisation gave different products in polar and non-polar solvents the reaction between methyl vernolate and boron trifluoride was examined in benzene solution. As with the isomerisations in dioxan it was proposed to study the reaction at room temperature and at the boiling point of the solvent. Under vigorous conditions (3 equivalents boron trifluoride in refluxing benzene) a large amount of polymeric material (TLC) was obtained. This procedure was therefore abandoned and studies were concentrated on the milder conditions.

Methyl vernolate was allowed to react at room temperature for thirty minutes with one equivalent of boron trifluoride. GLC of

the isolated products indicated three major components of similar carbon numbers to those obtained in dioxan, but quantitatively different (Table 16).

Table 16.

C.No (DEGS)	% Area	
	benzene	dioxan
22.3	-	0.5
22.7	-	0.5
23.2	3.0	4.0
23.7	5.0	-
23.9	-	3.0
24.8	24.0	8.0
25.3	42.0	80.0
25.6	26.0	-
26.0	-	4.0

TLC analysis showed the usual four fractions which were separated by column chromatography to give A (6%), B (65%), C (19%) and D (10%).

Fractions C and D were shown by IR and TLC to be similar to those obtained with dioxan and were not examined further.

3.1 Fraction A.

GLC analysis gave the results shown in Table 17 (p. 49). These results were unusual in that the major peaks on DEGS (23.2,

23.7) suggested conjugated triene esters as the main constituents, yet on the non-polar column the main constituents were observed at carbon numbers considerably lower (18.4, 18.8) than those expected for conjugated triene esters (19.2, 19.5).

Table 17.

C.No (DEGS)	% Area	C.No (ApL)	% Area
20.8 }		18.0	9
21.2 }	9	18.4	31
21.7	6	18.8	32
23.2	37	19.2	13
23.7	48	19.5	15

Combination of the results from both columns gave the following approximate composition for Fraction A (Table 18).

Table 18.

Possible component	C.No (DEGS)	C.No (ApL)	% Area
Conjugated diene with isolated double bond	21.2	18.0 }	15
	21.7	18.4 }	
Conjugated triene	23.2	19.2 }	28
	23.7	19.5 }	
Unknown	23.2	18.4 }	57
	23.7	18.8 }	

The ultra violet spectrum showed absorption at λmax 230mμ ($E^{1\%}_{1cm}$ 615), possibly indicative of conjugated diene (ca. 50%), and at λmax 267mμ ($E^{1\%}_{1cm}$ 385) indicative of conjugated triene (ca. 20%). The infra red spectrum, in addition to the usual ester features, indicated complex cis,trans configurations with absorption at 942, 958, 980 and 990cm^{-1}.

Prep. Ag^+/TLC separation gave two fractions of equal weight, A1 (the upper band) and A2. GLC analysis of these fractions gave the results in Table 19.

Table 19.

C.No (DEGS)				C.No (ApL)			
A1	% Area	A2	% Area	A1	% Area	A2	% Area
21.0	4	20.8	5	18.0	4	18.0	-
21.3	5	21.7	8	18.4	3	18.4	64
23.2	7	23.2	67	18.8	80	18.8	16
23.7	84	23.7	20	19.2	4	19.2	11
				19.6	9	19.6	9

Again, combination of the results from the two phases indicated the approximate compositions:

Possible component	% Area A1	% Area A2	Calculated A*
Conjugated diene	9	13	11
Conjugated triene	13	20	17
Unknown	78	67	72

* Calculated A = (A1+A2)/2

The infra red spectra suggested that both fractions still possessed complex cis,trans configuration; A1 gave absorption at 958, 981 and 989cm^{-1} and A2 at 942, 958 and 980cm^{-1}. In both cases conjugated diene and triene chromophores were observed in the ultra violet spectrum with the former being predominant.

von Rudloff oxidation of A1 and A2 gave in both cases C_8- and C_9- dibasic acids and an unidentified component of carbon number 21.0, on a polar column. No monobasic fractions were observed.

Attempts to separate A1 and A2 further failed. After treatment with maleic anhydride Fraction A yielded an unreacted component with UV, IR and GLC behaviour similar to A2. This result is in accordance with a cis,trans separation on Ag^+/TLC. These results also suggest that the major unknown components possessed a cis,trans (23.3, DEGS; 18.4, ApL) and trans,trans (23.7, DEGS; 18.8, ApL) configuration. Mikolajczak et al.[105] have recently demonstrated that an ester possessing 'en-allene' conjugation underwent cyclisation under GLC conditions. While there is no evidence that the unknown components are 'en-allenes', it is conceivable that they might be polyene esters of such configuration as to undergo a cyclisation readily under GLC conditions. Such an alteration on GLC might also explain why A (calculated) is different from Fraction A.

No preparative GLC facilities were available at the time for

further study of these fractions and no further work was carried out.

3.2 Fraction B.

GLC analysis gave the same qualitative picture as obtained from the dioxan reaction but quantitatively the results were different: more of the oxocyclopropane ester was formed. In addition, only traces of the peaks (22.3, 23.2, DEGS) formerly designated as ether-containing esters (p. 46) were apparent (Table 20).

Table 20.

C.No (DEGS)	% Area	C.No (ApL)	% Area *
24.8	26	18.8	-
25.3	45	19.1	-
25.6	29	19.2	-

* % Areas were not readily calculated on this phase because of the poor separation of the peaks of carbon number 19.1 and 19.2.

The ultra violet spectrum contained no absorption band but the infra red spectrum now showed the presence of a cyclopropane group (1020, 3050cm^{-1}) and some trans-unsaturation (965cm^{-1}). The NMR spectrum also indicated both cis and trans cyclopropane rings.

Catalytic hydrogenation followed by GLC analysis gave the expected products, unchanged oxocyclopropane esters and an oxo-stearate. Chromic acid oxidation of the hydrogenated products gave the expected C_7-, C_8- and C_9- dibasic acids (from the oxo-cyclopropane ester) and, unexpectedly, only C_{12}- and C_{13}- dibasic acids. These last two dibasic acids indicate the presence of a 13-oxo ester only. This was confirmed by the oximation - rearrangement procedure described earlier (p. 36) when only the C_{13}- and the C_9- dibasic acid (90% and 10%) were observed.

It was noted in the C_7-, C_8- and C_9- dibasic acids arising from chromic acid oxidation that the C_8- dibasic acid was the major component. From a 13-oxocyclopropane ester only, it was expected that the dibasic acids would be formed in the order $C_9 > C_8 > C_7$ (ca. 60:25:15). This, taken in conjunction with the C_9- dibasic acid obtained in the oximation-rearrangement procedure, could indicate an oxo group at C(9). The implications of these observations are discussed later (p. 54).

Further separation of B. Having ascertained that none of the 12-oxo-oleate was present in this fraction, it (Fraction B) was separated by prep. Ag^+/TLC into three closely separated bands: B1 (40%), B1a (24%) and B2 (36%). (See Separation Scheme 3, p. 58)

Component B1.

This was identified spectroscopically and chromatographically as the cis (50%) and trans (50%) isomers of the 13-oxocyclopropane

ester. Chromic acid oxidation yielded the three expected dibasic acids in the proportions $C_9 > C_8 > C_7$.

Component B2.

The band was identified as the methyl 13-oxo-octadec-cis-9-enoate by the usual spectroscopic, chromatographic and degradative studies.

Component B1a.

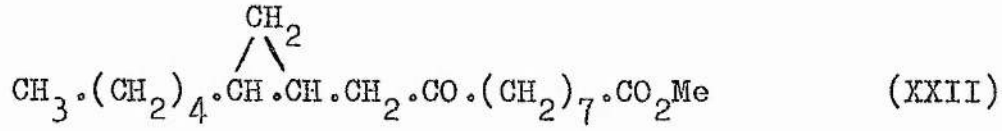
This band compared in composition with the band B1a isolated from the dioxan isomerisation (p. 45), containing peaks corresponding to oxocyclopropane esters (24.8, 25.6, DEGS) and oxo-oleate (25.3, DEGS). No 'ether' esters were apparent in this case. The relative amounts, determined by GLC were 24.8 (34%), 25.3 (48%) and 25.6 (18%).

The infra red spectrum, in addition to the expected oxocyclopropane absorptions, showed trans-unsaturation (965cm^{-1}). Hydrogenation gave unchanged oxocyclopropane and an oxostearate. von Rudloff oxidation yielded C_9- dibasic acid, 4-oxo-nonanoic acid and unchanged oxocyclopropane compounds.

From this evidence the compound of carbon number 25.3 was identified as the methyl 13-oxo-octadec-trans-9-enoate.

Compounds of carbon numbers 24.8 and 25.6. These compounds had GLC and spectroscopic properties identical with the 13-oxo-cyclopropane esters in B1 and it was tempting to suggest they arose from contamination of B1a with B1. However, the TLC

separation, although small, was real and therefore some explanation was necessary to account for this behaviour. It is tentatively suggested that these compounds are the 9-oxocyclopropane isomers (XXII) of the 13-oxocyclopropane esters in B1 and that the basis of separation is the position of the oxygenated group[106].



(XXII)

A compound of this structure would account for the unusual chromic acid oxidation of hydrogenated Fraction B which yielded a higher proportion than expected of the C_8- dibasic acid* and also account for the C_9- dibasic acid (10%) observed after the oximation-rearrangement procedure of Fraction B.

The mass spectrum of the component of carbon number 24.8 (DEGS) strengthened the possibility of such a structure. Peaks were observed corresponding to those expected from α-cleavage (m/e 153, 185 and 153 (185 - 32)) and β-cleavage (m/e 168, 200 and 168 (200 - 32)) to an oxo group at C(9). The previously

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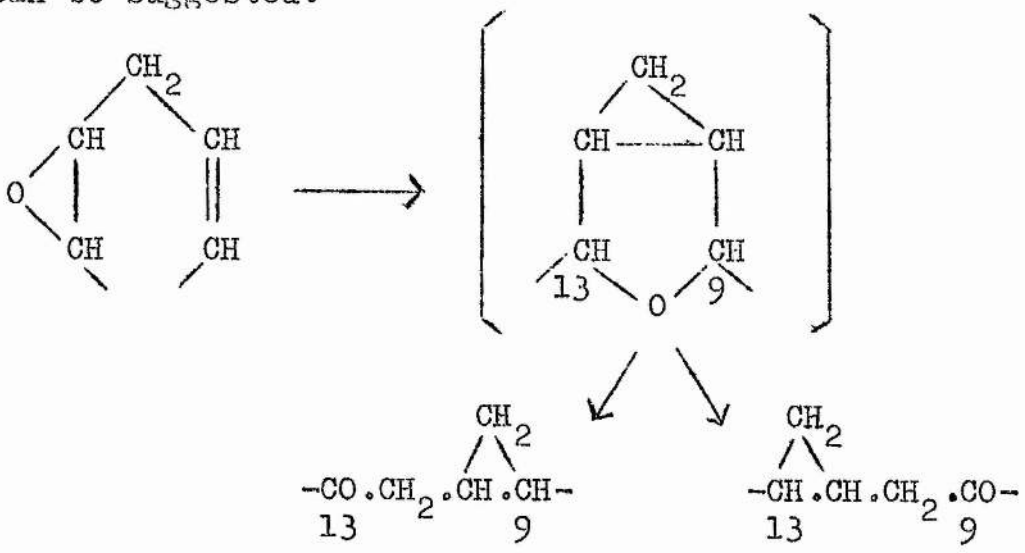
* In our hands chromic acid oxidation of a 9-oxo ester yields two dibasic acids: $C_8 > C_9$. Similar oxidation of a 9,10-methylene ester yields three dibasic acids: $C_9 > C_8 > C_7$. Oxidation of a mixture of these two might therefore yield dibasic acids: $C_8 > C_9 > C_7$.

invoked allylic cleavage (after collapse of the cyclopropane ring and hydrogen transfer) (pp. 44-45) also accounted for peaks at m/e 225 and 193 (225 - 32), and m/e 213 and 181 (213 - 32). An authentic 9-oxocyclopropane ester for mass spectral comparison with this component could be obtained by isomerisation of methyl coronarate* but the latter ester was not available so attempts to identify these components were not pressed further.

The formation of small amounts of the 9-oxocyclopropane esters from a 12,13-epoxyoleate could be explained by some form of 'ether-linked' intermediate[76]. As these have been observed in the dioxan isomerisation (p. 46) the following possible reaction scheme can be suggested:



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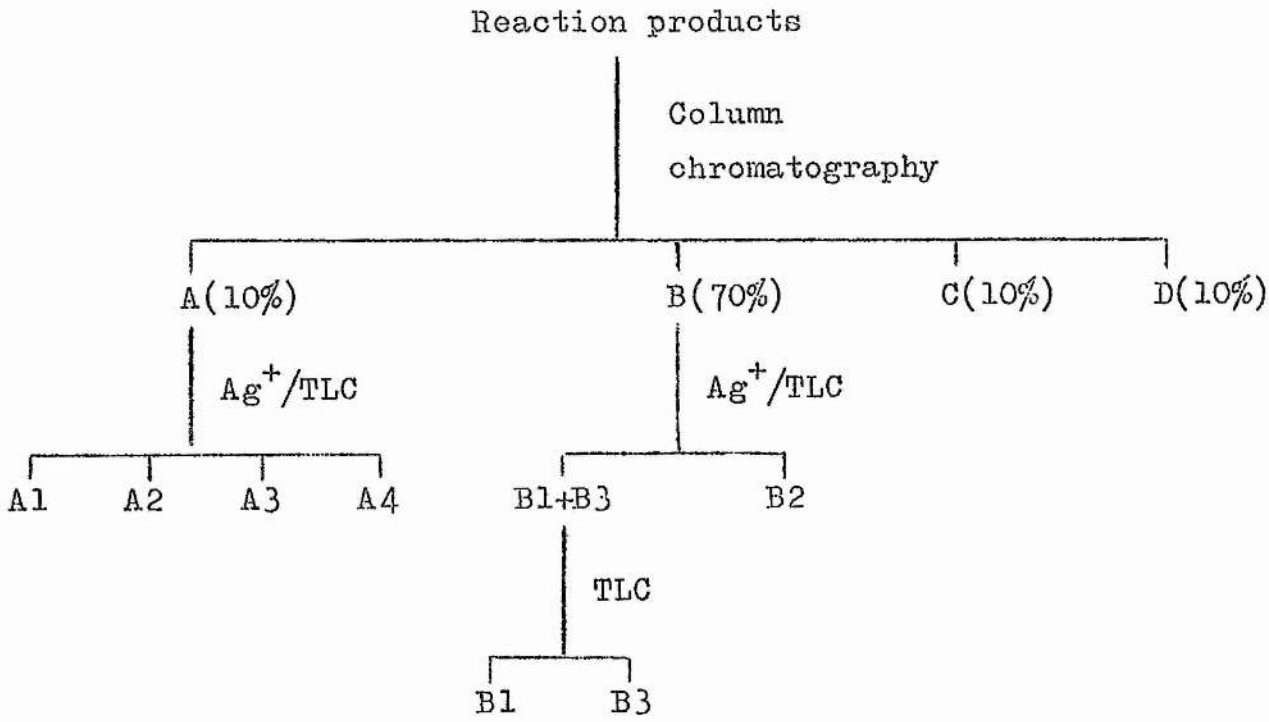
* The absence of methyl coronarate in the methyl vernolate was established.

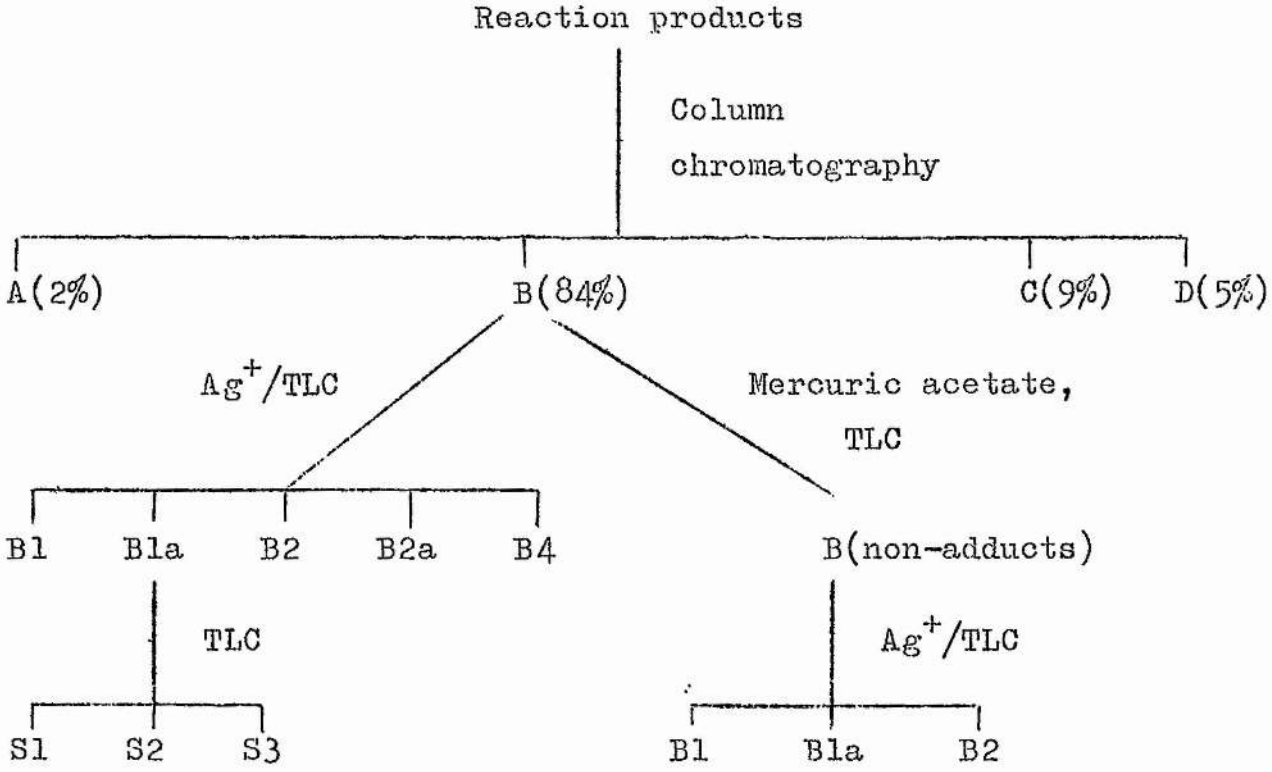
A more detailed study of the ether intermediates might give more information.

The relatively small amount of the 9-oxocyclopropane ester compared with the 13-oxo isomer suggests that this route via ether intermediates is only a minor pathway.

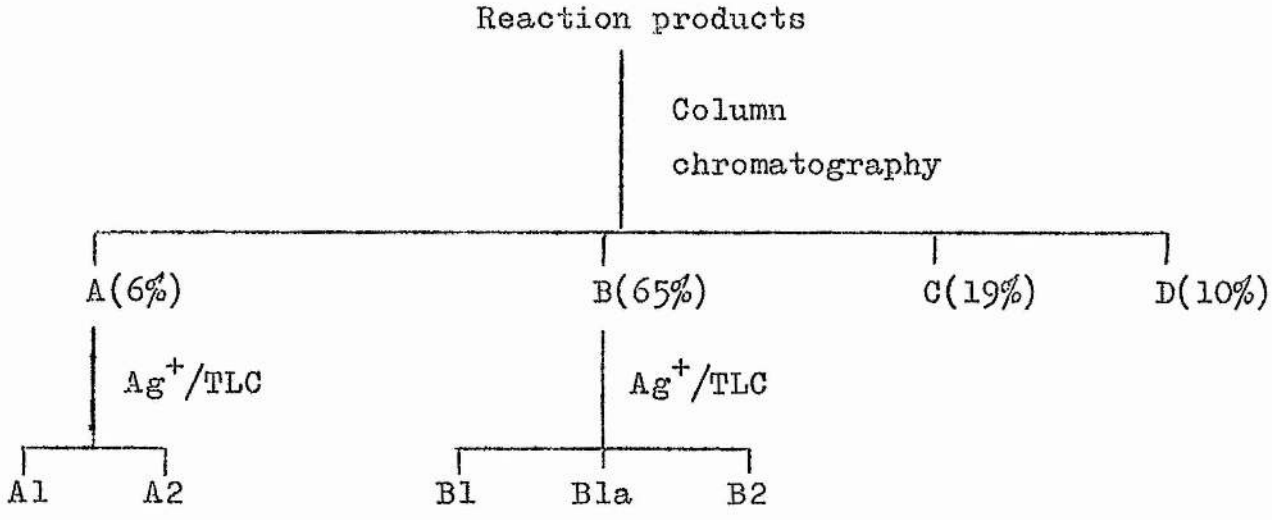
Separation Scheme 1



Separation Scheme 2



Separation Scheme 3



The results from the three isomerisation experiments are summarised in Table 21.

Table 21.

		1.*	2.*	3.*
(A)		10	2	6
(B)		70	84	65
	13-oxocyclopropane (trans)	10	6	13
	13-oxocyclopropane (cis)		1	13
	9-oxocyclopropane (cis & trans)	-	1	8
	13-oxo-9c	18	20	23
	13-oxo-9t	-	2	8
	12-oxo-9c	28	51	-
	'ether' ester	-	3	-
	12-oxo-10t	14	-	-
(C)		10	9	19
(D)		10	5	10

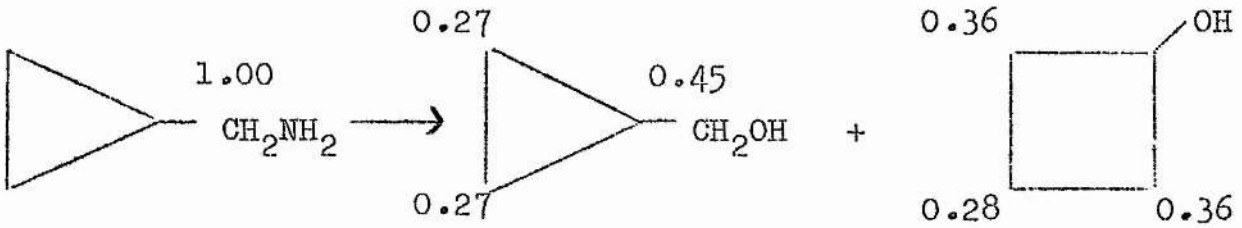
* 1. Isomerisation in refluxing dioxan (p. 21).
2. Isomerisation in dioxan at room temperature (p. 26).
3. Isomerisation in benzene at room temperature (p. 47).

4. Mechanistic features.

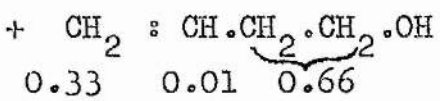
4.1 Possible mechanism of rearrangement.

Labelling experiments with cyclopropylmethylamine-1-^{14}C (XXIII) have been shown to give products (XXIV) in which the label

is nearly, but not quite, statistically distributed among the three methylene groups[107,108].

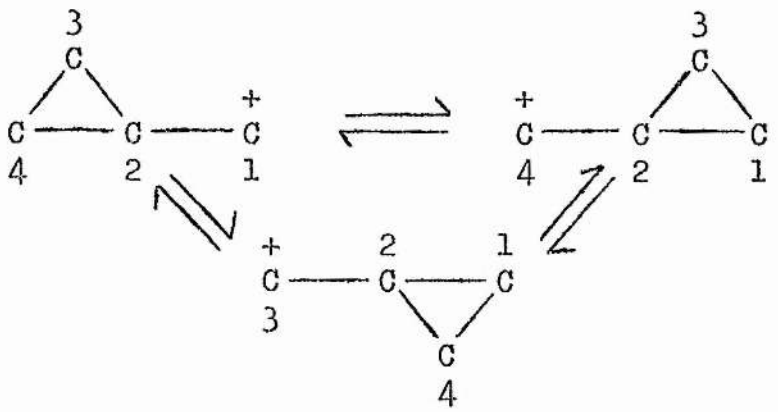


(XXIII)



(XXIV)

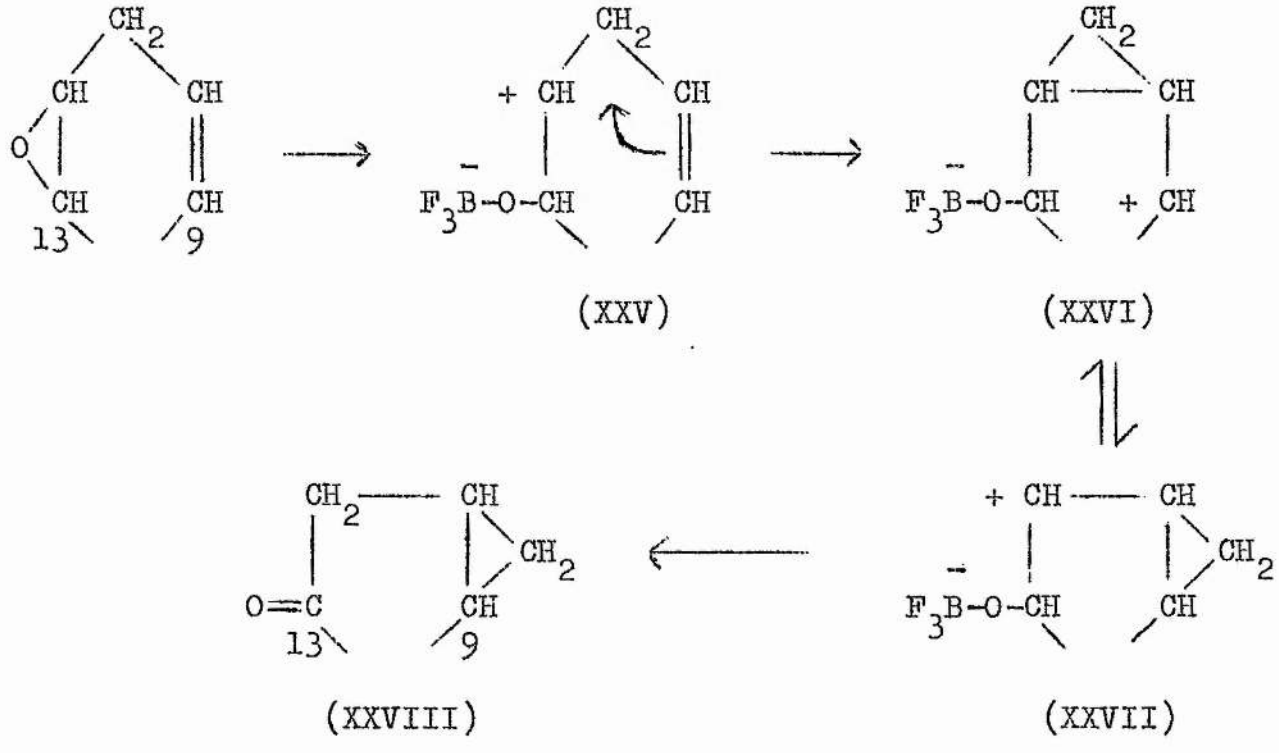
The above information indicates that the following interconversions of cyclopropylmethyl cations are possible:



It is therefore possible that our rearrangement might proceed as shown in Scheme 4 (p. 61). This involves rearrangement of the homo-allylic cation (XXV) to the cyclopropane derivative (XXVI), (as postulated before (p. 38)). This isomer (XXVI), with its carbonium ion centre at C(9), is in equilibrium with

cyclopropane carbonium ion (XXVII) in accordance with the interconversions shown on the previous page. Oxo formation, which may occur by 1,2-hydride shift or by proton loss to yield an enol, disturbs the equilibrium between the cyclopropyl carbonium ions in the direction of the final product (XXVIII). This reaction involves a skeletal rearrangement of the carbon chain.

Scheme 4.



4.2 Solvent effects.

The following facts require rational explanation:

(i) The relatively small yield of oxocyclopropane ester in the dioxan isomerisation (8-10%) compared with that in the benzene isomerisation (34%).

(ii) In dioxan, the oxocyclopropane ester is predominantly trans (80%) yet in benzene a 1:1 (cis:trans) ratio is found.

(iii) In dioxan, the 12-oxo and 13-oxo compounds are in the ratio 6:4 yet in benzene the product is entirely 13-oxo (100%).

To gain more information on the influence of $\beta\gamma$-unsaturated centres on epoxide ring opening, the isomerisations of methyl 12,13-epoxystearate and 9,10-epoxyoctadec-12-ynoate were studied in both dioxan and benzene solvents. The major Fraction B was isolated from both (where possible) and the ratio of oxo isomers determined as before. The results are summarised in Table 22.

Table 22.

	DIOXAN			BENZENE		
	B(%)	12-oxo	13-oxo	B(%)	12-oxo	13-oxo
12,13-epoxystearate	81	50	50	-	-	-
12,13-epoxyoleate	84	60	40	65	-	100
9,10-epoxyoctadec-12-ynoate	89	95*	5*	-	-	-

* This ester actually gave 10-oxo and 9-oxo (95:5) compounds but these are represented in the equivalent 12-oxo:13-oxo ratio. 9,10-Epoxystearate when isomerised gave similar results to those obtained from the 12,13-epoxystearate.

In the more polar solvent, dioxan, carbonium ions are formed on C(12) and C(13): these lead to the 13-oxo and 12-oxo compounds respectively. With 12,13-epoxystearate (and 9,10-epoxystearate) the two possible oxo isomers are formed in equal amounts. In the case of the $\beta\gamma$-olefinic epoxide the double bond influences ring opening to give slight preferential cleavage of the ether bond attached to C(13). This preferential cleavage is even more pronounced with the $\beta\delta$-acetylenic bond which appears to influence ring opening to such an extent as to give almost complete cleavage of the C(13) ether bond. Morris[15] has recently demonstrated a similar preferential cleavage in polar solvents of the ether bond attached to C(13) in epoxyoleate. The reason why this bond is preferentially cleaved is not yet fully understood.

In the non-polar solvent, benzene, a more rational explanation can be put forward. With the $\beta\delta$-olefinic epoxide, the epoxide ring opens completely with cleavage of the ether bond attached to C(12) to give the more stable homo-allylic cation. The latter then rearranges to give various products. In the case of the saturated epoxide and the acetylenic epoxide no such stabilised cation can be formed and the non-stabilised cations which are formed polymerise extremely rapidly so that no ketones are formed.

It is further considered that in dioxan, because of stabilisation of the intermediate carbonium ions by solvation, the

reaction comes mainly under thermodynamic control, yielding the more stable trans cyclopropane isomer. In benzene, with little or no stabilisation by the solvent, the reaction is mainly under kinetic control and yields equal amounts of the two isomers.

In kinetically controlled reactions the ratio of products (i.e. cyclic:open chain) depends on the stability and structure of the intermediates. Although in the ground state a cyclopropyl system is about 10K.cal/mole more strained than an open chain system, an ionic cyclopropyl intermediate is more stable than the corresponding open chain intermediate[109]. This might then account for the higher percentage of cyclic products in benzene than in dioxan. Under thermodynamic control (dioxan) the open chain intermediate might be favoured giving predominantly acyclic oxo compounds.

Conclusions.

Boron trifluoride-catalysed isomerisation of methyl vernolate has led to very little, if any, of the desired methyl coriolate, although a small amount of unidentified conjugated triene esters were observed which may or may not have arisen via this hydroxy-diene ester.

The reaction has nevertheless proved to be very interesting for under different conditions in two solvents, it has yielded an oxocyclopropane ester in moderate yield and a possible mechanism

for its formation has been discussed. Although long-chain cyclopropane esters occur naturally, this is the first report of their formation by interaction of a double bond and some ionic centre produced during a reaction. Further studies in our laboratory, initiated by this work, have resulted in the formation of cyclopropane isomers from methyl ricinoleate.

No cyclic compounds were observed from similar reactions on a $\beta\gamma$-acetylenic epoxy ester.

Chapter III.

BASE-CATALYSED ISOMERISATION.

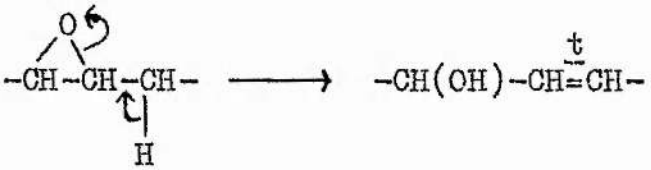
INTRODUCTION.

Base-catalysed isomerisation of epoxides can proceed by two routes:

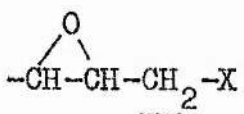
(i) α-elimination, in which the initial event is direct proton abstraction from the oxide ring. This may be followed by redistribution of the bonding electrons to give ultimately one or more carbonyl compounds[110-112].

Alternatively, saturated alcohols have been obtained via a carbenoid insertion process[111-114].

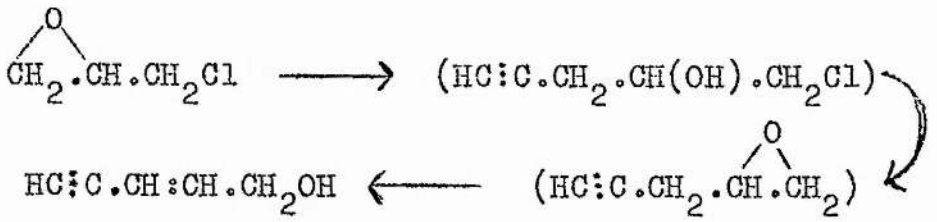
(ii) β-elimination, in which reaction is initiated by proton abstraction from a carbon atom adjacent to the ring, usually yielding the allylic alcohol.



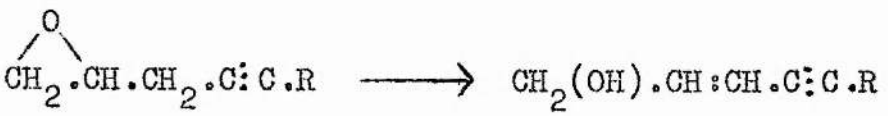
Although it has been shown[111,115-117] that this mode of isomerisation occurs with several saturated epoxides, β-elimination occurs more readily when the methylene group, adjacent to the ring, is activated by a group (X) such as CN[118], $PhSO_2$[118], CO[119-122] or CO_2Me[123].



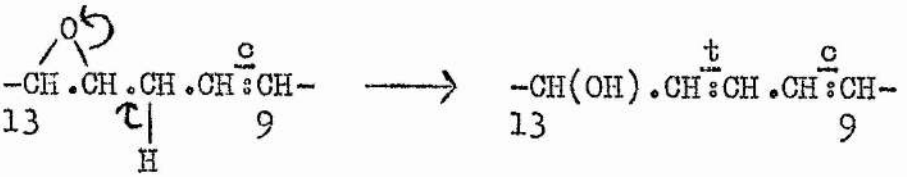
A related process, involving activation by an acetylenic group, was invoked by Haynes et al.[124] to explain the formation of 1-hydroxy-2-penten-4-yne from the condensation of epichlorohydrin with sodium acetylide.



More recently, Russian workers[125] have demonstrated the facile isomerisation of $\beta\gamma$-acetylenic epoxides to hydroxyenyne isomers using powdered alkali in ether.



In methyl vernolate, the C(11) methylene group is activated by the 9,10 double bond and it was considered that β-elimination might yield the desired 13-hydroxyoctadeca-cis-9,trans-11-dienoate.



DISCUSSION.

Initial experiments were carried out with methyl epoxyoleate and some common bases (potassium methoxide, ethoxide and tertiary butoxide) in various solvents. The UV spectrum was used as a sufficient indication of whether the desired reaction occurred.

Potassium methoxide and ethoxide.

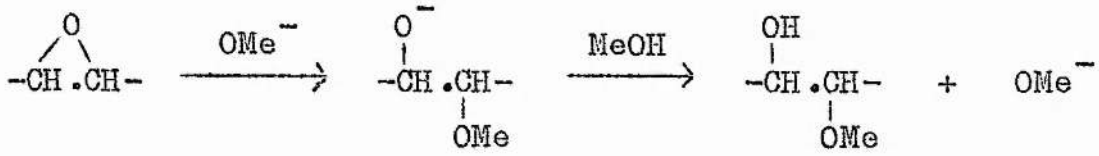
Although potassium methoxide is used in the transesterification of epoxy oils[126] without oxirane destruction it was hoped that stronger solutions might effect the required isomerisation. With 10:1 ratios of base:epoxide however, no diene conjugation was observed under various reaction conditions in methanol or dioxan solution. Similar negative results were obtained with ethoxide.

Potassium tertiary butoxide.

Ugelstad et al.[127] have shown that the rate of isomerisation of linseed oil in a solution of potassium butoxide in butanol was increased enormously by the presence of an excess of dimethylsulphoxide (DMSO) or dimethylformamide (DMF), even at room temperature. When epoxyoleate was treated with excess tertiary butoxide in DMF, very little conjugated diene could be observed. Under similar conditions a sample of methyl linoleate gave the conjugated isomer in 20% yield.

Reaction at room temperature using tertiary butoxide in DMSO[115] was found to give 10% conversion to the conjugated OH diene after a reaction time of three days. This reagent had the disadvantage of saponifying the ester group.

The major reaction occurring with these bases is probably nucleophilic substitution, giving the alkoxy-alcohol[113].



Accordingly, attention was focussed on lithium diethylamide which has been used extensively by Cope and co-workers[113] and more recently by Crandall and Chang[111,128]. This is a poor nucleophile but a strong base.

Lithium diethylamide isomerisation.

Following Cope[113], the reagent, prepared by the interaction of phenyllithium[129] and diethylamine, was refluxed for eight hours with methyl vernolate in ether solution. Chromatography (GLC and TLC) showed that the product contained hydroxy dienes, and the high ultra violet absorption at λ max 233mμ ($E^{1\%}_{1cm}$ 718 and 701, in two experiments) indicated a high yield of conjugated dienes. Prep. TLC furnished two major products (30% and 34%) which were shown to be the 13-hydroxyoctadeca-cis-9,trans-11-dienoate and its N,N-diethylamide, as described later.

In an endeavour to decrease the amount of amide (and increase the yield of OH diene ester) the reaction was studied at lower temperatures and for shorter reaction times. It was shown that the rearrangement was almost complete in one hour at $0^{\circ}C$. Under these conditions the diene ester (recovered in 60% yield) was accompanied by less of its diethylamide (20%).

Characterisation of hydroxy ester and its diethylamide.

a) The hydroxy ester was isolated by TLC on silica followed by Ag^{+}/TLC to separate some artefacts formed during the preparation of lithium diethylamide. The purified product showed absorption at λmax 233mμ ($E^{1\%}_{1cm}$ 850) in the ultra violet, and at 3595, 1730, 980 and 945cm^{-1} in the infra red, indicative of a hydroxy ester with a conjugated cis,trans diene system. Its GLC showed three peaks having carbon numbers of 23.3, 23.7 and 28.0 (DEGS). The first two (23.3, 23.7) are conjugated trienes formed by dehydration of the hydroxy-diene ester during chromatography[130] and the latter is presumably the hydroxy ester itself. In this respect this ester differed from methyl dimorphecolate (9-hydroxyoctadeca-trans-10,trans-12-dienoate) which showed only the two conjugated trienoic ester peaks. A naturally occurring sample of methyl coriolate (13-hydroxyoctadeca-cis-9,trans-11-dienoate) isolated from Coriaria myrtifolia seed oil[131] showed similar GLC behaviour to the synthetic ester.

The NMR spectrum showed a peak at 5.9τ (CHOH) and a complex

series of peaks at 3.3-4.9τ arising from the four conjugated olefinic protons. The spectrum was identical with that published for coriolic ester[21].

Hydrogenation gave a mixture of hydroxystearate and oxo-stearate, and chromic acid oxidation of this gave C_{12}- and C_{13}- dibasic acids as the major products.

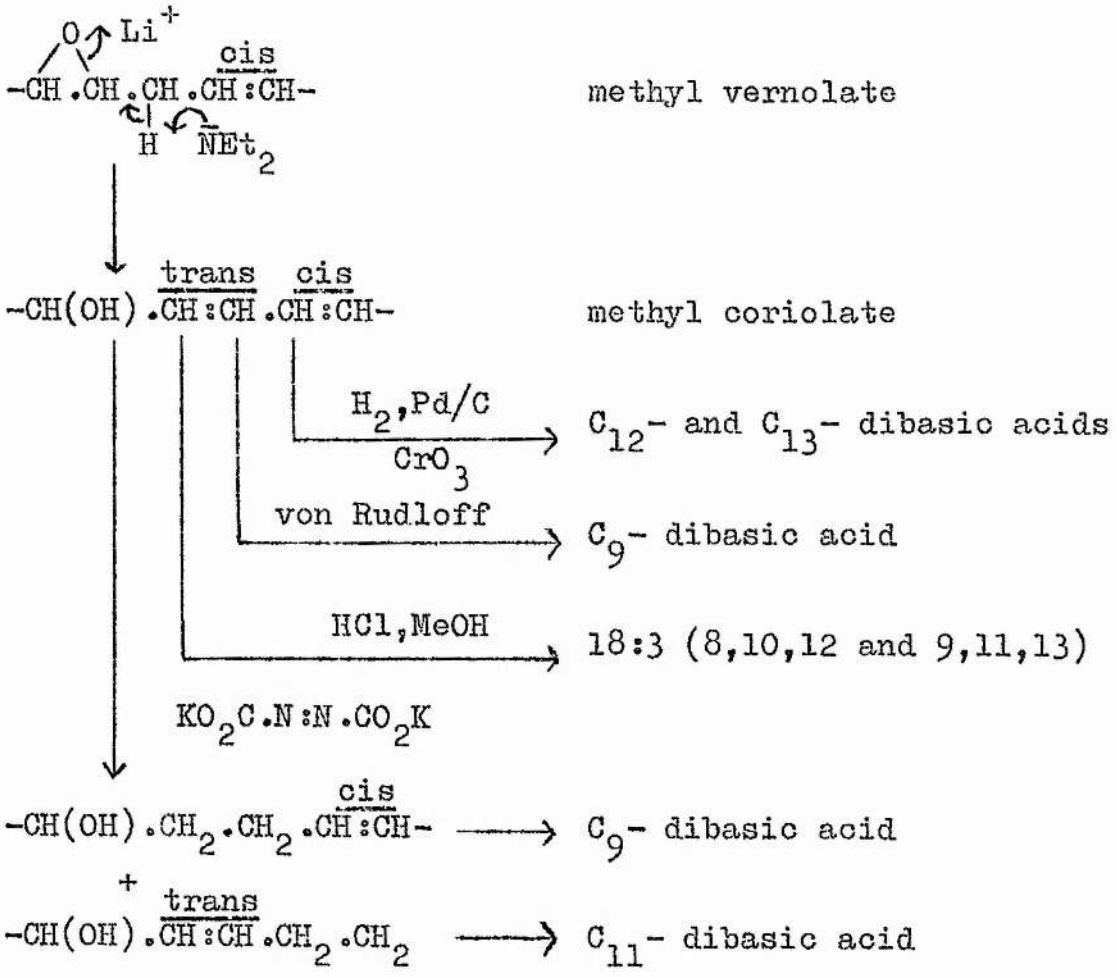
von Rudloff oxidation gave the C_9- dibasic acid, indicating that unsaturation started at C(9).

The hydroxy ester was readily dehydrated by acidic methanol to conjugated triene esters. von Rudloff oxidation of the latter indicated a mixture of 8,10,12- and 9,11,13- trienoates, arising from 1,2 and 1,6 dehydration of the dienol system[43].

Partial reduction with potassium azodicarboxylate[22] gave two hydroxy monoene esters which were separated by Ag^+/TLC. The cis-isomer gave the C_9- dibasic acid when oxidised; the trans-isomer gave the C_{11}- dibasic acid along with about 5% of the C_9- dibasic acid. The latter (C_9- dibasic) probably resulted from some unreacted diene ester. These changes are summarised in Scheme 5 (p. 72).

Although it has not been possible to make any optical measurements, this synthetic ester, prepared from optically active methyl vernolate, is considered to be optically active and to have the 13D configuration of the naturally occurring ester[21].

Scheme 5.



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b) The second major component (34%) contained a cis,trans conjugated diene group (UV and IR) and a hydroxyl group (IR). It was not, however, an ester (IR) and its NMR spectrum showed a quartet at 6.5-6.9τ indicative of a diethylamide. When methyl stearate was treated with lithium diethylamide a component was isolated (15%) having the spectroscopic and chromatographic properties shown by authentic N,N-diethylstearamide. It was therefore concluded that this isomerisation product (34%) was the

diethylamide of the 13-OH diene ester resulting from nucleophilic attack of the reagent on the ester group.

Some transformations of synthetic methyl coriolate.

9-Hydroxyoctadeca-10,12-dienoic acid (dimorphecolic) occurs naturally as the 10t,12c[22] and the 10t,12t[23] isomers and also in an oxidised form as the 9-oxo 10t,12t[132] acid. It seems possible that the isomeric 13-hydroxyoctadeca-9,11-dienoic acid (coriolic) will occur in similar forms and these have been prepared from the synthetic coriolate.

Treated with iodine in carbon disulphide solution[133] the 13-hydroxy 9c,11t dienoic ester gave a mixture of cis,trans and trans,trans isomers which were separated with difficulty by prep. Ag^+/TLC. The isolated 13-hydroxy 9t,11t ester was about 95% pure as shown by GLC of its trimethylsilyl ether[134]. Its NMR, UV and IR spectra (except for a small absorption at 945cm^{-1}) were similar to that of an authentic sample of the isomeric ester, methyl dimorphecolate (9-OH 10t,12t), isolated from Dimorphotheca pluvialis ringens seed oil.

Oxidation of the cis,trans hydroxy ester with chromic acid in pyridine[135] gave an oxo compound in which the major product (9c,11t) was accompanied by some trans,trans isomer. The amount of the latter was increased by iodine isomerisation and was reduced, but not eliminated, by avoiding acidic conditions during

the reaction work-up. Attempts to separate pure trans,trans oxo ester from the mixture of isomers after iodine isomerisation by Ag^+/TLC were unsuccessful, and finally this isomer was prepared by chromic acid oxidation of the 13-hydroxy 9t,11t ester. The resulting oxo ester had similar spectral properties to an authentic sample of 9-oxo-octadeca-trans-10,trans-12-dienoate from Dimorphotheca pluvialis ringens seed oil.

The naturally occurring 9-hydroxy and 9-oxo isomers could be prepared by similar reactions from methyl 9D,10D-epoxyoctadec-12-enoate but as yet no source of this ester is available.

Rearrangement of other epoxy esters with lithium diethylamide.

Although the base-catalysed rearrangement of methyl vernolate gave coriolate in good yield, the reaction failed with epoxy-stearate and monoepoxidised ximenynate; presumably because there is no activation of the methylene group adjacent to the epoxy function. The reaction was successfully applied to a number of selected synthetic epoxy esters which contained such an activated methylene group and resulted in the partial syntheses of racemic methyl α-dimorphecolate, racemic methyl helenynolate, and methyl parinarate.

Partial synthesis of racemic α-dimorphecolate.

Monoepoxidation of methyl linoleate gave a mixture of two

epoxides which was rearranged in about 60% yield to 9- and 13-hydroxyoctadecadienoates. These positional isomers were separated by prep. TLC in approximately equal amounts thus confirming the observation by Maerker et al.[136] that epoxidation of linoleate occurred equally at the 9,10 and 12,13 positions.

The lower band, on TLC, was identified as the 9-hydroxy-octadeca-*trans*-10,*cis*-12-dienoate by spectroscopic and degradative studies similar to those described for methyl coriolate. Although this ester will be in the racemic form, an optically active acid could presumably be obtained by isomerisation of natural coronaric acid[4].

Partial synthesis of racemic methyl helenynolate.

Methyl helenynolate (9-hydroxyoctadec-*trans*-10,12-ynoate) has recently been found in *Helichrysum bracteatum* seed oil by Powell et al.[137]. Co-occurring in the same oil was crepenynic acid (18:2 (9c,12a)) and it may be that helenynolic acid is produced biosynthetically from crepenynic acid via an epoxide. This pathway is illustrated in Table 4 (p. 8).

Methyl crepenynate, isolated by prep. Ag^+/TLC from *Afzelia cuanzensis* methyl esters[138], was monoepoxidised and submitted to base-catalysed rearrangement. Prep. TLC furnished a hydroxy enynoic ester in 50% yield which was shown to be identical to methyl helenynolate on the following evidence.

(i) The ester showed absorption at λ max 228mμ ($E^{1\%}_{1cm}$ 600) and 237mμ ($E^{1\%}_{1cm}$ 510) in the ultra violet spectrum, and at 3595, 1730 and 950cm^{-1} in the infra red, indicative of a hydroxy ester possessing a conjugated trans enyne system. The NMR spectrum contained a complex series of peaks in the region 3.7-4.6τ (olefinic protons) and a peak at 5.9τ due to the proton on the carbon atom also attached to the hydroxyl group.

(ii) The hydroxy ester decomposed on both polar and non-polar columns[137] but as its TMS derivative, it had a carbon number of 23.4 (DEGS). On thin layers of silica the hydroxy ester was slightly more polar than a standard 9-OH 10t,12c ester but in the presence of silver ions the position was reversed, the enyne ester running with a slightly higher Rf value.

(iii) Chromic acid oxidation of the hydrogenated ester gave essentially C_9- and C_8- dibasic acids, placing the hydroxyl group on C(9).

(iv) von Rudloff oxidation of the ester yielded a C_9- dibasic acid and a C_6- monobasic acid, which placed the enyne system between C(10) and C(13).

(v) Lithium aluminium hydride reduction[137] yielded a diol showing characteristic 'allene' absorption in the infra red spectrum at 1950cm^{-1}.

(vi) Refluxing with methanolic hydrogen chloride yielded an ester still possessing conjugated enyne absorption (UV) but

which now contained a methyl ether linkage as evidenced by its infra red spectrum (1080, 1100cm^{-1}).

(vii) As additional proof of structure the ester was partially reduced with potassium azodicarboxylate to give a product which was separable into three bands by Ag^{+}/TLC. These bands, in order of decreasing Rf, were characterised by a combination of IR and GLC (TMS derivatives) as (1) unchanged ester, (2) hydroxy ester with conjugated cis,trans unsaturation and (3) hydroxy ester with an acetylenic group. von Rudloff oxidation of band (3) gave a C_6- monobasic ester along with a γ-lactone. The latter was identified as the lactone of a 4-hydroxydodecanedioic acid by its infra red spectrum (1770cm^{-1}) and by comparison of its GLC characteristics with the lactone formed from oxidation of natural methyl 9-hydroxyoctadec-12-enoate.

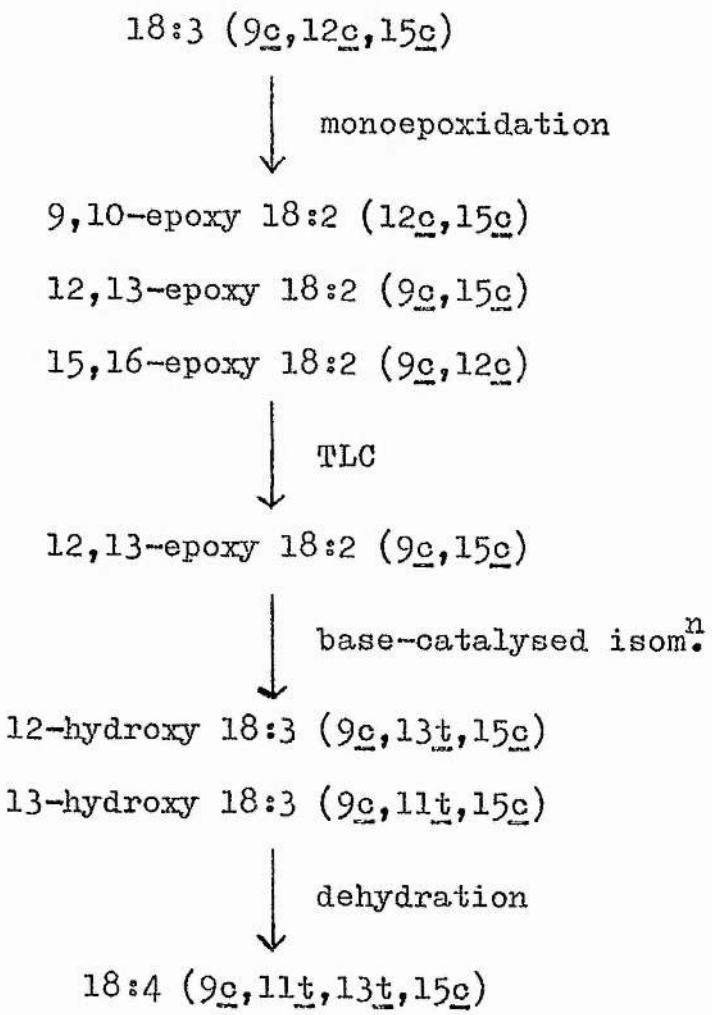
These facts prove that the product of rearrangement of the monoepoxidised methyl crepenynate was methyl 9-hydroxyoctadec-trans-10-en,12-ynoate.

As a consequence of this successful conversion of crepenynic ester to methyl helenynolate the epoxy esters in Helichrysum bracteatum have been re-examined and it has been shown that 9,10-epoxyoctadec-12-ynoic acid accompanies the coronaric acid previously identified[12]. The discovery and structural proof of this new epoxy ester is discussed in Chapter IV.

Attempted synthesis of methyl parinarate.

Parinaric acid, the only non-oxygenated conjugated tetraenoic long-chain acid presently known in nature, was discovered in 1933[139] but its configuration was only verified recently when Bagby et al.[29] showed it to be the 18:4 (9c,11t,13t,15c) isomer.

An attempt has been made to prepare this ester from methyl linolenate by the following scheme based on the biosynthetic pathway postulated by Gunstone.



Methyl linolenate was monoepoxidised in 50% yield with monoperphthalic acid. During purification by prep. TLC it was observed that the monoepoxides were partially separated into two spots which appeared to be in an approximate ratio of 1:2 (upper::lower). Since the upper spot had an Rf value similar to that of authentic methyl 12,13-epoxyoleate it was concluded that this was the 12,13-epoxy isomer while the lower composite spot comprised the 9,10-epoxy and the 15,16-epoxy isomers. This separation is in general agreement with the observation of Morris[106] that 12-hydroxystearate has a higher Rf value than either the 9-hydroxy or the 15-hydroxy isomer.

Accordingly, by rather laborious chromatography on thin layers of silica, using multiple development, a small amount of the upper component was isolated from the monoepoxidised fraction. After base-catalysed isomerisation and prep. TLC, this yielded a monohydroxy ester (40%) with the spectroscopic properties expected of a hydroxy ester possessing conjugated cis,trans unsaturation.

The lower component was also isomerised to monohydroxy esters with similar spectral properties, and preliminary acid-catalysed dehydrations were carried out on this monohydroxy ester. Reaction at room temperature with anhydrous dioxan/sulphuric acid converted the hydroxy ester to conjugated tetraene esters only, as evidenced by complete replacement of the conjugated diene chromophore by conjugated tetraene (UV spectrum). This tetraene is probably a

mixture of 9t,11t,13t,15c and 9c,11t,13t,15t isomers arising from 1,6 dehydration of the 9-OH 10t,12c,15c and the 16-OH 9c,12c,14t isomers respectively. Acid-catalysed dehydration of methyl coriolate 13-OH 9c,11t by both 1,2 and 1,6 dehydration gives, predominantly, the all trans trienes. The 1,6 dehydration described above is presumably due to additional activation of C(14) and C(11) by the extra double bond.

It was hoped that acid-catalysed dehydration of the upper band would lead to 1,2 trans dehydration only, to yield the 9c,11t,13t,15c tetraene ester. When this hydroxy trienoate was reacted with the dioxan/sulphuric acid reagent, an ester was obtained with strong tetraene absorption in its UV spectrum (λ max 302mμ, $E^{1\%}_{1cm}$ 2300). The infra red spectrum showed a strong absorption at 996cm^{-1} and weak bands at 975, 951 and 925cm^{-1}.

von Rudloff oxidation of this ester gave only a C_9- dibasic acid indicating the conjugated system began at C(9).

The IR evidence is not completely consistent with that reported for methyl parinarate. Bagby et al.[29] observed four bands in the region 900-1000cm^{-1}, corresponding to those given above, but found slightly different relative intensities. Later, Gunstone and Subbarao[140] claimed only two bands in this region, 993 and 951cm^{-1}.

While it is considered that the main product is the 9c,11t,13t,15c isomer, some cis,trans isomerisation may have

occurred in the dehydration step leading to small amounts of the c,t,t,t isomers. In view of the rather laborious TLC separation of the monoepoxy isomers it may also be that slight contamination of the upper fraction by the lower fraction occurred, again leading to a stereochemically impure product.

It is possible that a base-catalysed dehydration of the p-toluenesulphonate derivative[141] of the hydroxydiene ester might lead to a more stereospecific product.

Conclusions.

By base-catalysed isomerisation of the appropriate epoxy ester, partial syntheses have been effected of methyl coriolate, racemic α-dimorphecolate, and racemic helenynolate. A synthesis of methyl parinarate from methyl linolenate via epoxy intermediates has also been described.

While this does not prove any biosynthetic link between the epoxy esters and the hydroxydiene (or enyne) esters, the ease of conversion (> 80%, including the diethylamide derivatives) provides useful supporting evidence for Gunstone's proposals outlined in Chapter I.

Chapter IV.

RE-EXAMINATION OF SELECTED SEED OILS: A SEARCH FOR UNKNOWN EPOXY ACIDS OF POSSIBLE BIOSYNTHETIC IMPORTANCE.

INTRODUCTION.

In the previous chapter, the ready conversion of unsaturated epoxy esters to hydroxy esters with conjugated unsaturation has provided a good chemical analogue to Gunstone's postulated biosynthetic pathway. The next logical step would be to attempt to demonstrate similar conversions in the plant itself using labelled epoxides. As no facilities were available for this, it was decided to extend the co-occurrence aspect (discussed in Chapter I) to an examination of some seed oils with a view to identifying epoxy acid intermediates predicted by Gunstone's theory.

Three seed oils have been examined:

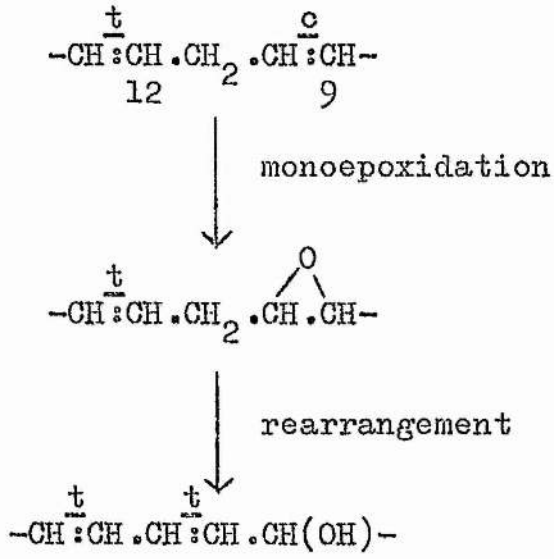
(i) Helichrysum bracteatum seed oil.

This seed oil is known to contain coronaric acid[12], an appropriate isomer of which might be the precursor of the α-dimorphecolic acid also present. Since this oil also contains crepenynic and helenynolic acid it seemed useful to see if the predicted intermediate (Table 4, p. 8), 9,10-epoxyoctadec-12-ynoic acid, might also be present.

(ii) Dimorphotheca pluvialis ringens seed oil and Dimorphotheca aurantiaca seed oil.

Morris and Marshall[31] have recently described the occurrence of β-dimorphecolic acid (9-OH 10t,12t) with an isomer of linoleic

(9c,12t) in D. aurantiaca seed oil which is also considered[11] to contain a small amount of epoxy acid. This oil and the closely related D. pluvialis ringens have therefore been re-examined to see if they contain any 9,10-epoxyoctadec-trans-12-enoic acid. This latter is the expected intermediate between the linoleic isomer and the β-dimorphecolic acid.



DISCUSSION.

1. Helichrysum bracteatum seed oil.

The seed oil was converted to methyl esters by reaction at room temperature with sodium methoxide in anhydrous methanol[126]. The monoepoxy fraction (14%) was isolated by prep. TLC of the methyl esters, and GLC (DEGS) indicated only three components: X (C.No 24.0, 6%), Y (C.No 24.6, 69%) and Z (C.No 26.0, 25%). These correspond in carbon number to authentic samples of methyl epoxystearate, epoxyoctadecenoate and epoxyoctadecynoate. Attempted separation of this fraction by Ag^+/TLC was unsuccessful and finally a scheme was adopted which, although changing component Y, allowed subsequent separation of components X and Z. This was considered acceptable as the epoxyoctadecenoate (Y) had previously been identified by Powell et al.[12] as methyl coronarate, and the other two components were of more interest.

The monoepoxy fraction was treated with an excess of peracid which reacted only with component Y. The monoepoxides (X and Z), unchanged on GLC and TLC, were then readily separated by prep. Ag^+/TLC. Using this technique the components X (7%), Z (24%) and Y (69%, now diepoxide) were recovered in the proportions (by weight) shown. These recoveries (% wt) compare very favourably with the original GLC results (% area) and suggest that no unusual

changes occurred during the isolation procedure.

Characterisation of component Z.

Chromatographically and spectroscopically, component Z and synthetic 9,10-epoxyoctadecynoate ester (prepared by epoxidation of methyl crepenynate) were indistinguishable. They showed identical behaviour on thin layers of silica and silica/silver nitrate, and had the same carbon number on polar (26.0, DEGS) and non-polar (19.1, ApL) columns. Their IR and NMR spectra were also identical.

A portion of component Z was subjected to acetolysis and then saponified according to Gunstone's procedure[1]. The resulting dihydroxy unsaturated acid was esterified and submitted to von Rudloff oxidation before and after hydrogenation. Before hydrogenation it gave a C_9- dibasic acid and a C_6- monobasic acid; after hydrogenation it gave a C_9- dibasic and a C_9- monobasic acid.

These facts place the epoxy group at the 9,10 position, and an unsaturated centre between carbon atoms 12 and 13.

On thin layers of silica impregnated with boric acid[142] the hydrogenated dihydroxy ester ran with an authentic threo-9,10-dihydroxystearate. This proves that the epoxide ring in Z has the cis configuration.

Finally, base-catalysed isomerisation of component Z, with lithium diethylamide, yielded a hydroxy ester possessing trans-enyne unsaturation (UV and IR spectra). This confirms the

presence of an acetylenic linkage in the original component Z and since such isomerisations require an activated methylene group adjacent to the oxirane ring the acetylenic linkage must be between C(12) and C(13).

It is considered that this evidence identified component Z as methyl cis-9,10-epoxyoctadec-12-ynoate.

As methyl helenynolate has recently been shown to possess the 9D absolute configuration[143] it is predicted that this epoxy ester will have the 9D,10D configuration.

Characterisation of component X.

Component X was shown to be cis-9,10-epoxystearate by similar procedures which are detailed in the experimental section.

2. Dimorphotheca pluvialis ringens seed oil.

The seed oil was converted to methyl esters with sodium methoxide in anhydrous methanol. Prep. TLC of these esters yielded a small monoepoxy fraction (1%) shown by GLC (DEGS) to contain two components of carbon number 24.0 (10%) and 24.6 (90%), indicative of an epoxystearate and an epoxyoctadecenoate respectively. The IR spectrum indicated no trans-unsaturation. With care this fraction was separated by TLC into two bands: an upper (24.6, DEGS), which ran with an Rf value similar to 12,13-epoxyoleate, and a lower of carbon numbers 24.0 (20%) and 24.6 (80%),

which ran with 9,10-epoxystearate. Neither showed trans-unsaturation in the infra red.

It is considered that this evidence suggests the presence of methyl 9,10-epoxystearate, methyl 12,13-epoxyoctadecenoate and methyl 9,10-epoxyoctadecenoate. There was no evidence of a trans enoic epoxy acid and no further attempt was made to identify the other acids.

3. Dimorphotheca aurantiaca seed oil.

This oil gave similar results to the D. pluvialis ringens oil. No trans epoxy ester was detected.

Conclusions.

The identification of a predicted epoxy intermediate, methyl 9,10-epoxyoctadec-12-ynoate, in Helichrysum bracteatum seed oil has provided further support for the biosynthetic importance of epoxy acids.

Chapter V.

DISTRIBUTION OF VERNOLIC ACID IN SEED OIL TRIGLYCERIDES.

INTRODUCTION.

It is now well established that in most vegetable seed oils, containing the common saturated and unsaturated acids, the triglycerides are preferentially acylated at the 2-position by the unsaturated C_{18} acids[144,145]. In view of the proposed biosynthetic relationship between linoleic acid and vernolic acid it was considered useful to study the distribution of vernoloyl groups in seed oil triglycerides.

Initially, very little information on this distribution was available. Krewson et al.[146] had shown that the vernoloyl groups in Vernonia anthelmintica seed oil were present almost entirely as trivernolin, yet in Euphorbia lagascae seed oil[147] which also contained a high proportion of vernolic acid, the vernoloyl groups were more randomly distributed between the mono-, di- and triepoxy triglycerides.

After our work commenced however, Tallent et al.[148] reported the identification and distribution of vernoloyl groups in several seed oils. Their studies revealed a general preference of the vernoloyl groups for the 2-position of the triglyceride molecules.

In the present work, six seed oils from three plant families have been examined for both intra- and interglyceride distribution of vernoloyl groups. With four oils, the triglyceride composition of the non-epoxy and monoepoxy fractions has been calculated from

lipolysis and from Ag^+/TLC results.

These oils together with their vernolic acid content are summarised in Table 23.

Table 23.

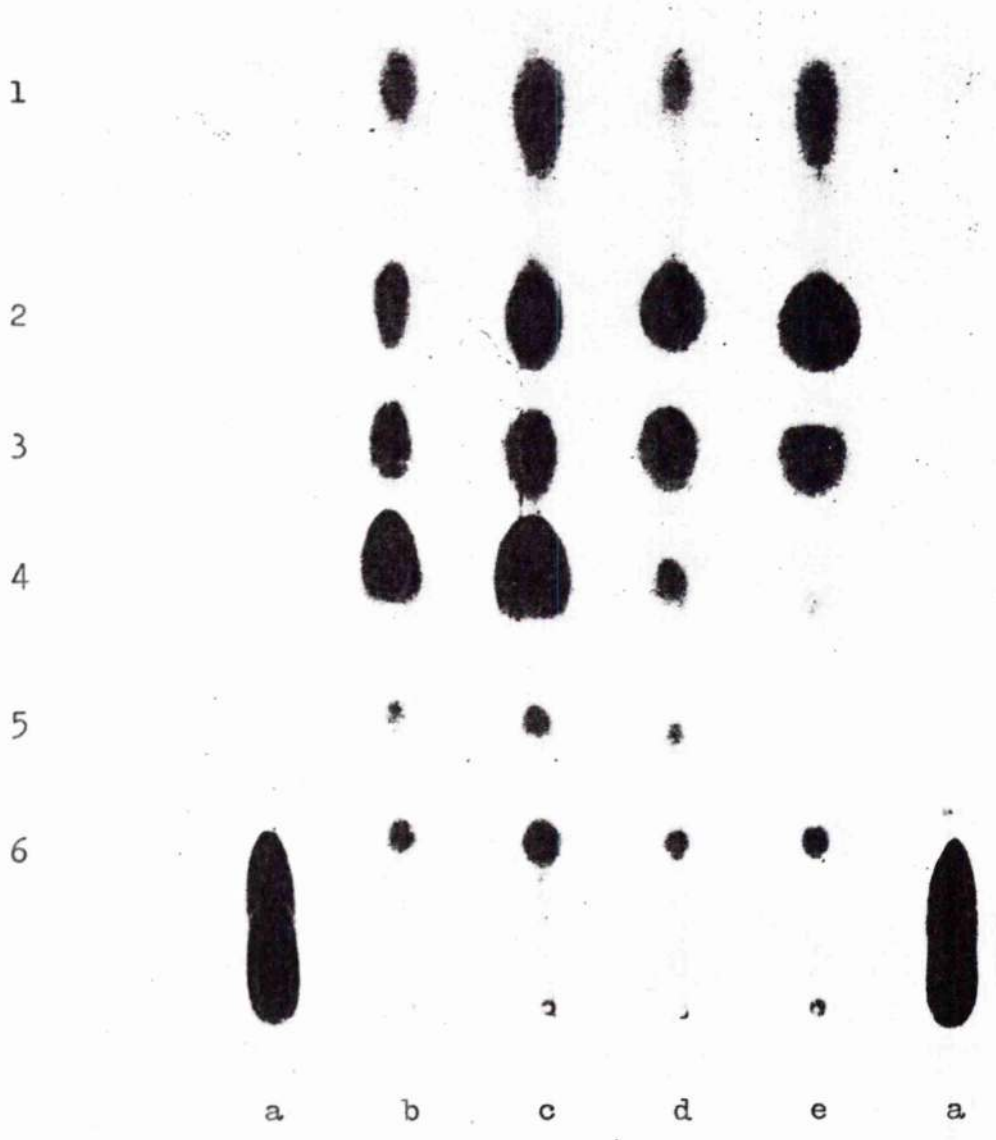
	Species name	Abbrev. name	Family	Content of vernolic*
1)	Cephalocroton peuschelli	Cp	Euphorbiaceae	72
2)	Cephalocroton cordofanus	Cc	Euphorbiaceae	67
3)	Crepis aurea	Ca	Compositae	60
4)	Crepis vesicaria	Cv	Compositae	52
5)	Cephalaria joppica	Cj	Dipsacaceae	36
6)	Cephalaria leucantha	Cl	Dipsacaceae	19

Notes.

1) and 2) have a high content of vernolic acid, 5) and 6) have a low content of this acid, 3) and 4) show intermediate values.

* In all tables in this chapter all figures are quoted as % mole.

Plate I.



TLC separation (PE25) of four epoxy seed oils.

a. Castor oil. b. C. aurea. c. C. vesicaria.
d. C. joppica. e. C. leucantha.

Components visualised by iodine vapour.

DISCUSSION.

1. Methods.

a) Extraction. Both Vernonia anthelmintica[146] and Euphorbia lagascae[149] seeds have been shown to contain a hydrolytic enzyme which became active in the crushed seeds. To avoid any hydrolysis arising from a similar mechanism, all seeds were ground under petrol and subsequently extracted with the same solvent.

b) GLC analyses. Methyl esters were prepared from glycerides by mild transesterification with sodium methoxide in methanol[126]. GLC analyses were carried out on a DEGS column held at 190°C as described in the general methods (p. 99). Under these conditions methyl vernolate was eluted as a reasonably symmetrical peak (sometimes followed by a minor peak) of carbon number 24.6. As reported by Herb et al.[150], methyl vernolate gave a low response, relative to the normal fatty acids, on this phase and a correction factor of 1.26 was obtained using standard mixtures of pure vernolate and methyl heptadecanoate. This factor has been included in all calculations.

c) Separation of triglycerides. As illustrated in Plate I, prep. TLC proved an excellent method for separating these oils into fractions based on the number of vernoloyl groups present in the triglyceride molecules. Each oil was separated into six

fractions by this technique. The top fraction (1) was non-saponifiable matter and was discarded. The others were non-epoxy (2), monoepoxy (3), diepoxy (4) and triepoxy (5) triglycerides, in order of decreasing Rf value. The lowest fraction (6) probably contained sterol(s) and partial glycerides.

Prep. Ag^+/TLC separations and recovery of fractions were carried out essentially as described by Gunstone and Padley[144].

d) Lipolysis. Lipase hydrolyses, which have been previously shown to proceed satisfactorily with vernoloyl groups[151], were effected on the non-epoxy, monoepoxy and diepoxy triglyceride fractions of each oil by the semi-micro procedure of Luddy et al.[152].

e) Calculations. Relative amounts of triglyceride fractions from both prep. TLC and prep. Ag^+/TLC separations were calculated using methyl heptadecanoate as internal standard[144].

As an aid to the rather laborious calculations involved, two short computer programmes were composed. The first (C1) converted peak areas for each fraction to relative mole percent. The second (C2) converted peak areas for each fraction to increment mole percent. taking into account the relative amounts of each fraction.

Glyceride compositions from Ag^+/TLC separations were calculated for each sub-fraction in terms of a tertiary (or simpler) mixture, from the molar composition of the esters and

from its Ag^+/TLC behaviour.

Triglyceride composition of the fractions were calculated from lipolysis data as described by Coleman[153] and Vander Wal[154]. With the monoepoxy triglyceride the calculation was modified to allow one epoxyacyl group in each triglyceride.

2. Results.

Interglyceride distribution of vernoloyl groups.

In the following discussion epoxyacyl groups are referred to as E and all other acyl groups as X.

The results obtained by prep. TLC of the six oils are summarised in Table 24 (p. 93) in terms of the four triglyceride classes: non-epoxy triglycerides (X_3), monoepoxy triglycerides (X_2E), diepoxy triglycerides (XE_2) and trivernolin (E_3).

The sum of the esters obtained from each fraction compared favourably with the original oil, providing a useful check on the recovery and quantitation of the separated fractions. There was also, in general, good agreement between the theoretical values of vernolic for the monoepoxy (33.3%) and diepoxy (66.7%) triglyceride fractions, and those actually obtained. (Full details are given in Tables E17 - E22 in the Experimental section.)

Table 24.

	X_3			X_2E			XE_2			E_3		
	A	B	C	A	B	C	A	B	C	A	B	C
Cp	3	2	2	14	17	17	43	44	44	40	37	37
Cc	4	3	4	18	19	23	44	44	45	34	34	28
Ca	9	6	7	18	29	29	59	43	43	14	22	21
Cv	14	11	11	15	36	36	60	39	39	11	14	14
Cj	31	26	25	38	44	46	25	25	25	6*	5	4
Cl	55	53	51	32	37	39	10	9	9	3**	1	1

A = % mole found.

B = % mole calculated assuming 1,2,3-random distribution[155], and treating all acids other than vernolic as a single group.

C = % mole calculated by Gunstone's theory[156], treating vernolic as a typical unsaturated C_{18} acid.

* E_3, by GLC, contained 75% vernolic. Figure given assumes 100% vernolic, hence true E_3 will be less.

** E_3, by GLC, contained 58% vernolic. Figure given assumes 100% vernolic.

For oils of high vernolic content (Cp and Cc) and a low vernolic content (Cj and Cl) the amounts of the triglyceride fractions agree fairly well with predictions based on a 1,2,3-random distribution[155] and with Gunstone's distribution theory[156]. This does not appear to hold with oils of intermediate epoxy content (Ca and Cv) where the results do not agree with either

distributional theory. A similar non-random distribution was found by Tallent et al[148] for C. aurea and C. joppica.

Intraglyceride distribution of vernoloyl groups.

The results from lipolysis of the non-epoxy (fraction 2), monoepoxy (fraction 3) and diepoxy (fraction 4) triglycerides from each oil are summarised in Table 25 in terms of an enrichment factor[157]. This latter is the ratio of the concentration (molar) of an acid group in the 2-position to its concentration in the total triglyceride. Full lipolysis details are given in Tables E17 - E22 of the Experimental section.

Table 25.

	fraction 2		fraction 3			fraction 4		
	18:1*	18:2	18:1	18:2	E	18:1	18:2	E
Cp	1.2	1.4	1.3	1.1	1.1	0.9	0.8	1.2
Cc	1.2	1.4	1.2	1.0	1.2	0.9	0.9	1.1
Ca	1.1	1.6	0.5	1.1	1.7	0.1	0.2	1.5
Cv	1.0	1.4	0.6	0.9	1.6	0.2	0.1	1.4
Cj	1.2	1.6	1.2	1.4	1.5	1.0	1.3	1.3
Cl	1.5	1.6	1.1	1.4	1.6	1.1	1.4	1.3

Note.
The saturated acids have been omitted.

* These figures indicate the number of carbon atoms and double bonds per acid molecule; thus 18:1 represents octadecenoic acid.

In all the oils examined there is a general tendency for the vernoloyl group to be attached at the 2-position in the monoepoxy and diepoxy triglyceride fractions, as noted by Tallent et al.[148]. The last authors also reported however an exception to this general trend in Euphorbia lagascae, where the vernoloyl groups in the monoepoxy fraction appeared to be preferentially attached to the 1(3)-position(s). They hinted that this might be a general trend in the Euphorbiaceae family. This is not confirmed from our results on the Cephalocroton oils.

It is also interesting to note that crepenynic acid (present in C. aurea and C. vesicaria) appears to be attached at the 1(3)-position(s) in contrast to the common unsaturated acids. This acid may however behave unusually under lipolysis conditions.

Triglyceride structure of the non-epoxy and the monoepoxy fractions from four oils calculated from lipolysis and from Ag^+/TLC results.

The results from the non-epoxy fractions are summarised in Table 26 (p. 96) and those from the monoepoxy fractions in Table 27 (p. 96).

Table 26.

	001*	011	002	111	012	112	022	122	222
Cp2									
Ag^+/TLC	6	7	11	6	20	13	19	13	5
Lipolysis	5	8	8	3	22	13	16	17	8
Cc2									
Ag^+/TLC	6	9	9	7	21	10	18	12	8
Lipolysis	5	8	8	3	22	12	16	18	8
Cj2									
Ag^+/TLC	5	9	9	2	28	10	24	8	5
Lipolysis	8	8	14	2	25	9	17	12	5
C12									
Ag^+/TLC	9	8	19	3	19	6	21	10	5
Lipolysis	10	6	19	1	23	5	20	10	6

* These figures indicate the number of double bonds in the three acyl chains. Each symbol includes all positional isomers.

Table 27.

	00E	01E	11E	02E	12E	22E
Cp3						
Ag^+/TLC	1	13	12	26	26	22
Lipolysis	3	11	8	21	29	28
Cc3						
Ag^+/TLC	1	10	9	28	26	26
Lipolysis	3	9	7	21	28	32
Cj3						
Ag^+/TLC	19	21	7	30	14	9
Lipolysis	18	22	6	30	15	9
C13						
Ag^+/TLC	17	23	8	31	12	9
Lipolysis	18	22	6	31	14	9

To assess the reproducibility and accuracy of the Ag^+/TLC method, two fractions from one oil (C. peuschelli) were analysed a second time. These results are compared below:

	001	011	002	111	012	112	022	122	222
Cp2									
Ag^+/TLC(1)	6	7	11	6	20	13	19	13	5
Ag^+/TLC(2)	8	9	9	4	19	12	20	12	7

	00E	01E	11E	02E	12E	22E
Cp3						
Ag^+/TLC(1)	1	13	12	26	26	22
Ag^+/TLC(2)*	-	12	10	26	28	23

In general the triglycerides determined by prep. Ag^+/TLC and by lipolysis are of the same order, though individual values occasionally show some discrepancy.

Conclusions.

Vernolic acid, like linoleic and the other common C_{18} unsaturated acids, appears to be preferentially attached at the 2-position in its triglycerides.

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* A small amount of non-epoxy triglyceride (1%) was observed in this case.

EXPERIMENTAL.

(1) Purification of solvents.

All solvents were distilled before use. Dioxan, benzene and ether were dried by distillation from sodium wire. Dry methanol was prepared by Vogel's procedure[158]. Carbon disulphide for infra red analyses was dried over calcium chloride, carefully distilled and stored over calcium chloride in the dark. Petroleum ether (bp. 40-60°C) was used throughout and is designated simply as petrol.

(2) Thin layer chromatography (TLC).

TLC analyses were carried out on layers of Silica Gel G (0.3mm, wet thickness) and on layers of Silica Gel G impregnated with silver nitrate (15%). The former is referred to as TLC or direct TLC and the latter as Ag^+/TLC. Preparative separations were done on thicker layers (1.0mm, wet thickness) and are designated prep. TLC and prep. Ag^+/TLC respectively. Glass plates, 20cm x 20cm, were used throughout.

Mixtures of ether/petrol or ether/benzene were generally used as solvents and are designated in abbreviated forms such as PE20 or BE15. The letters P, E and B represent petrol, ether and benzene respectively and the number indicates the percentage by volume of the second component in the mixture.

Qualitative TLC plates were visualised by exposure to iodine vapour but on Ag^+/TLC and on all preparative plates, the

components were made visible by spraying with an ethanolic solution (0.2%) of 2,7-dichlorofluorescein and viewing under ultra violet light.

Components were recovered from preparative plates by scraping off the marked bands and extracting them with ether by either slurrying or by soxhlet extraction. Fractions are normally presented in order of decreasing Rf value.

(3) Gas-liquid chromatography (GLC).

For normal analysis of methyl esters two machines were used, one fitted with a polar column (a) and the other with a non-polar column (b).

(a) A Pye 104, Model 24, with twin flame ionisation detectors. Columns were of stainless steel (5' x $\frac{1}{4}$") packed with Gas Chrom Z (70-80 mesh) coated with 20% diethylene glycol succinate polyester (DEGS). Normal operating conditions were 190°C with a flow rate of 50ml/min (nitrogen).

(b) A Pye Argon chromatograph with a β-ray ionisation detector. Columns were of glass (4' x $\frac{1}{4}$") packed with Gas Chrom Z (70-80 mesh) coated with 5% Apiezon L grease (ApL). Normal operating conditions were 210°C with a flow rate of 40ml/min (argon).

Free acids ($<C_{12}$ monobasic) were analysed, using the Pye 104, on a 20% Carbowax 20M phase impregnated with phosphoric acid.

The support was acid-washed celite (80-100 mesh) and the column (5' x $\frac{1}{4}$") was operated at 135°C with a carrier gas flow rate of 50ml/min.

Esters, as ether solutions (1%), were injected directly on to the columns using a 10µl Hamilton syringe fitted with a long needle (7.5cm). Free acids, as ether solutions (1%), were injected into a flash heater, held at 190°C, using a 10µl Hamilton syringe fitted with a shorter needle (4.0cm).

Peak areas were estimated by multiplying peak height by peak width at half height[159] and retention times are reported as carbon numbers[160].

(4) Spectroscopic analyses.

(i) Infra red spectra (IR).

Rapid qualitative spectra were run on Perkin Elmer Infracords 137 and 237. More accurate spectra were recorded on a Perkin Elmer 621 grating spectrophotometer. Samples were run as thin films on sodium chloride discs or as solutions (1%) in carbon disulphide using 1mm pathlength liquid cells with sodium chloride windows.

When quoting IR spectra results, figures are usually given only for absorptions additional to those normally observed in a long chain methyl ester with no other functional groups.

(ii) Ultra violet spectra (UV).

Ultra violet spectra were recorded in methanol solution on a Unicam SP 800 spectrophotometer.

(iii) Nuclear magnetic resonance spectra (NMR).

Spectra were recorded on 15% solutions in carbon tetrachloride, with tetramethylsilane as internal standard, using a Perkin Elmer R10 spectrometer operating at 60Mc/sec.

(iv) Mass spectra (MS).

GLC/MS analyses were kindly carried out by Dr. Kelly of Unilever Ltd.

(5) General chemical methods.

(i) Hydrogenation.

Hydrogenations were carried out in a hydrogen atmosphere for one hour at room temperature in methanol or glacial acetic acid solution using 10% palladium/charcoal (Pd/C) as catalyst. The latter was removed by filtration and the material recovered by evaporation of the solvent under vacuum. Yields were usually high ($>90\%$). The following gives an indication of the relative amounts used: sample (20mg), Pd/C (20mg) and solvent (5ml); sample (100mg), Pd/C (40mg) and solvent (10ml).

(ii) Esterification.

Esterifications were carried out by refluxing for three minutes with an excess of the boron trifluoride/methanol complex

(12½%) in methanol[161]. The reaction mixture was poured into water saturated with sodium chloride and extracted with petrol. Normally the boron trifluoride/methanol complex would be diluted with ca. 5 volumes of methanol before use, e.g. sample (5mg), boron trifluoride complex (1ml) and methanol (5ml); sample (50mg), boron trifluoride complex (2ml) and methanol (10ml).

(iii) von Rudloff oxidation[162].

The position of unsaturated centres in esters was determined by von Rudloff oxidation as described by Craig and Tulloch[163].

Stock oxidising solution was prepared by dissolving potassium periodate (22.4g, 0.0975mole) and potassium permanganate (0.4g, 0.0025mole) in one litre of water. The ester (5mg) was shaken overnight with tert. butanol/water (5ml, 7:1), potassium carbonate solution (1ml, 0.5%) and the oxidising solution (2ml). Excess of oxidising agent was destroyed with sulphur dioxide, the solution was basified with solid potassium hydroxide, and the solvent removed under vacuum. The residue was acidified with sulphuric acid (10%), saturated with sodium chloride and extracted with ether (2 x 10ml). Free monobasic acids were analysed by GLC after careful evaporation of the dried ether extracts under vacuum at $< 30^{\circ}C$. After esterification with methanol (5ml) and boron trifluoride/methanol (0.5ml), the esters were extracted from the diluted aqueous solution with petrol (2 x 10ml) and analysed by GLC at the appropriate temperature.

(iv) Chromic acid oxidation[164].

This technique was used to determine the position of oxo, hydroxyl and cyclopropane groups in long-chain esters.

The hydrogenated ester (15mg) was dissolved in glacial acetic acid (2ml) and stirred at room temperature for two hours with a solution of chromium trioxide (120mg) in glacial acetic acid (2ml). The reaction mixture was diluted with water (25ml), excess oxidant destroyed with sulphur dioxide, and the products extracted with petrol (2 x 10ml). After esterification with methanol (5ml) and boron trifluoride/methanol (1ml) the esters were extracted with petrol (2 x 10ml) and analysed by GLC.

(v) Beckmann rearrangement[82,165].

This procedure was used to determine positional oxo isomers of aliphatic esters present in a hydrogenated mixture. It involves reduction of olefinic centres, oximation, rearrangement of the oximes to amides, followed by hydrolysis of the amides, and is outlined below using hydrogenated Fraction B (2.3, p. 118).

Hydrogenated Fraction B (60mg) was refluxed in aqueous ethanol (water/ethanol (1:4), 3ml) with hydroxylamine hydrochloride (50mg) and fused sodium acetate (60mg). After four hours the reaction mixture was diluted with water (25ml) and extracted with ether (2 x 25ml). The recovered oximes (60mg) were heated at 110°C for two hours with conc. sulphuric acid (2ml). Water (2ml) was carefully added, through the condenser, to the cooled acidic

solution of the amides and the mixture refluxed for two hours. After extraction and esterification the recovered dibasic esters were analysed by GLC.

This total procedure will normally be referred to as the oximation rearrangement throughout the experimental sections.

ACID-CATALYSED ISOMERISATION.

Isolation of methyl vernolate.

Vernonia anthelmintica seed oil (20g) was neutralised by passage through a short alumina column (4" x 1" column, 100-120 mesh alumina, Type H, P. Spence and Sons Ltd.) using chloroform (500ml) as solvent. Evaporation of the chloroform yielded neutralised oil (16.9g).

Transesterification[126].

Vernonia oil (16.9g) was shaken gently overnight with dry methanolic sodium methoxide (220ml, 0.02N). The reaction mixture was poured into water (750ml), carefully acidified (pH4) with sulphuric acid (0.1N), and immediately extracted with ether (3 x 250ml) to yield a yellow oil (13.4g, 80%).

TLC (PE40) indicated complete esterification.

Column chromatography

Mixed Vernonia esters (7.2g) were chromatographed on silica gel (Whatman SG 31, 250g) eluting with gradually increasing proportions of ether in petrol (250ml) and collecting 125ml fractions.

Eluted fractions were monitored by TLC and appropriate ones combined. Methyl vernolate (2.9g) was eluted predominantly by the PE20 solvent*. It was over 98% pure (GLC and TLC) and

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* For solvent abbreviation see p. 98.

contained only traces of methyl oleate and linoleate. The ester was stored as a solution in petrol at 0°C.

1. Isomerisation of methyl vernolate with boron trifluoride etherate in refluxing dioxan[66].

Methyl vernolate (350mg, 1.3mmole) was refluxed for three hours in anhydrous dioxan (20ml) with boron trifluoride etherate (0.25ml, 4.1mmole). The reaction mixture was diluted with water (50ml) and extracted with ether (3 x 50ml). Ether extracts were washed with water (3 x 10ml), dried over sodium sulphate and evaporated under vacuum to yield a light brown oil (345mg, 90%)*.

The reaction product was analysed by GLC (Table 5, p. 21), TLC (PE30) (p. 22), UV and IR.

The UV spectrum showed absorption at λ max 225mμ ($E^{1\%}_{1cm}$ 94) and λ max 267mμ ($E^{1\%}_{1cm}$ 60), and the IR spectrum indicated absorptions at 3595cm^{-1} (hydroxyl), 1710cm^{-1} (oxo), 1685cm^{-1} (conjugated oxo) and 970cm^{-1} (trans).

Separation by column chromatography. The reaction product (320mg) was chromatographed on silica gel (Whatman SG 31, 100g) eluting with petrol/ether mixtures and collecting 100ml fractions.

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* Hereafter, this recovery procedure is described as "worked up in the usual way", etc.

Eluted fractions were monitored by TLC(PE30) and combined as shown in Table E1. Elution was practically complete (315mg, 98%).

Table E1.

Fraction	Solvent	Weight (mg)	
1	PE5	-	
2	PE5	-	
3	PE10	25	A, 31mg, 10%
4	PE10	12*	
5	PE20	34	
6	PE20	134	B, 232mg, 74%
7	PE40	58	
8	PE40	23	C, 29mg, 9%
9	PE60	6	
10	PE60	7	D, 23mg, 7%
11	E	10	

* This fraction was shown (TLC) to contain approximately equal amounts of A and B and for the purpose of calculation it has been equally divided between each.

Subsequent experiments gave the following results:

Methyl vernolate	Reaction product	Column recovery	A	B	C	D
330mg	320mg	311mg	10%	69%	10%	11%
330mg	325mg	321mg	9%	73%	9%	9%
300mg	294mg	288mg	7%	64%	16%	13%

1.1 Fraction D.

These esters showed O-H stretching in the IR spectrum ($3595cm^{-1}$). Fraction D (10mg) was hydrogenated in methanol (5ml) solution in the presence of Pd/C (10mg). von Rudloff oxidation was carried out on Fraction D (5mg) and on the hydrogenated derivative (5mg). These gave the C_9- and C_{12}- dibasic acids respectively which were recognised by GLC on DEGS and ApL columns.

1.2 Fraction A.

GLC analysis on both DEGS and ApL columns gave the results summarised in Table 6 (p. 23).

The IR spectrum showed absorption at 945, 970 and $980cm^{-1}$, indicative of complex cis,trans conjugation, and the UV spectrum gave absorption expected from conjugated diene (λ max 233mμ) and conjugated triene chromophores (λ max 257, 267 and 277mμ).

von Rudloff oxidation of A (3mg) gave C_8- and C_9- dibasic acids (GLC) and hydrogenation of A (5mg) gave methyl stearate (GLC).

By prep. Ag^+/TLC (BE10), A (90mg) gave four poorly separated sub-fractions A1 (18mg), A2 (42mg), A3 (9mg) and A4 (6mg). Each fraction had the carbon numbers (DEGS) shown in Table E2 (p. 109).

Table E2.

Fraction (and % wt)	Carbon number					
A	20.8	21.3	21.7	22.1(tr)	23.2	23.7
A1 (24%)	-	-	-	-	-	23.7
A2 (56%)	20.8	21.3	21.7	22.1	23.2	23.7
A3 (12%)	-	21.3	21.7	22.1	-	-
A4 (8%)	-	21.3	-	-	-	-

Hydrazine reduction[71]. Aqueous hydrazine (0.5ml, 4%) and glacial acetic acid (10mg) were added to A3 (8mg) dissolved in methanol (3ml) and the solution heated at 50°C for six hours. Air was bubbled through the solution continuously. The solution was acidified and extracted with ether. The products (7mg) were analysed by GLC (Table E3).

Ag^+/TLC (PE15) of the reduced product gave five fractions A3(I) - A3(V) (Table E3), von Rudloff oxidation of each band gave no recognisable acidic fragments.

Table E3.

Fraction	Carbon number (DEGS)							
A3	-	-	-	-	-	21.4	21.8	22.3
A3(reduced)	18.0	18.6	19.3	20.7	21.1	-	21.8	22.3
A3(I)	18.0	-	-	-	-	-	-	-
A3(II)	18.0	18.6	-	-	21.1	-	-	-
A3(III)	-	18.8	-	20.7	-	-	-	-
A3(IV)	-	18.6	-	-	-	-	21.6	-
A3(V)	-	-	-	-	-	-	21.6	22.3

A similar reduction of methyl α-eleostearate gave *inter alia* *c,t* conjugated diene (20.5) and *t,t* conjugated diene (21.2) with the carbon numbers (DEGS) indicated.

1.4 Fraction B.

GLC results, before and after hydrogenation, are shown in Table 7 (p. 24).

The IR spectrum had absorption bands at 1710, 1670, 1685 and 970cm^{-1}, and the UV spectrum showed absorption at λ max 225mμ ($E^{1\%}_{1cm}$ 100).

Fraction B was separated into a number of sub-fractions by a combination of prep. Ag^+/TLC and prep. TLC respectively as follows:

Prep. Ag^+/TLC (PE25) of B (120mg) gave an upper, BU (46mg), and lower fraction, BL (52mg). Further prep. TLC (PE10, two developments) of BU (46mg) yielded BU1 (23mg) and BU2 (20mg). GLC (DEGS) showed these four fractions to contain the compounds B1, B2 and B3 as shown in Table E4.

Table E4.

	BU		BU1		BU2		BL	
	C.No	% Area	C.No	% Area	C.No	% Area	C.No	% Area
B1	24.8	28	24.8	43	24.8	5	-	-
B2	25.3	28	25.3	53	25.3	2	25.3	100
B3	26.8	44	26.8	4	26.8	93	-	-

Reduction with sodium borohydride. B (30mg) was stirred with sodium borohydride (50mg) in methanol (10ml) for 30 minutes. The solution was diluted with water and the product (25mg) extracted with ether (2 x 20ml).

The product showed absorption at 3595cm^{-1} (OH) in the IR spectrum and gave carbon numbers of 25.7 and 26.3 on a DEGS column. Component B3 (i.e. fraction BU2).

The UV spectrum showed strong absorption at λ max 225mμ ($E^{1\%}_{1cm}$ 480) and the IR spectrum gave bands at 1670, 1685 and 970cm^{-1}.

von Rudloff oxidation of B3 (4mg) gave a C_{10}- dibasic acid (95%) and a C_9- dibasic acid (5%). After hydrogenation this component had carbon numbers of 24.9 on DEGS, and 19.4 (96%) and 18.8 (4%) on ApL. Chromic acid oxidation of the hydrogenated ester (10mg) gave essentially C_{11}- and C_{12}- dibasic acids (GLC).

Preparation of methyl 12-oxo-octadec-cis-9-enoate[101]. To castor oil methyl esters (340mg) in glacial acetic acid (3.5ml) was added, all at once, a solution of sodium dichromate (230mg), conc. sulphuric acid (0.12ml), water (0.30ml) and glacial acetic acid (2ml). After thirty seconds, the reaction mixture was diluted with water (50ml), and extracted with ether (3 x 25ml). The ether extracts, after washing with sodium carbonate solution (2 x 10ml, 10%) and water (2 x 10ml), yielded a product (270mg) from which methyl 12-oxo-oleate (120mg) was recovered by prep. TLC (PE30).

Isomerisation of methyl 12-oxo-octadec-cis-9-enoate with boron trifluoride in refluxing dioxan. The oxo-oleate (30mg, 0.1mmole) was refluxed for three hours in anhydrous dioxan (5ml) with boron trifluoride etherate (0.4ml, 0.3mmole). The reaction product (28mg) was isolated in the usual way. The oxo-oleate had carbon numbers 19.1 (ApL) before isomerisation and 19.1 (70%) and 19.9 (30%) after isomerisation. This last (19.9) is the carbon number of component B3.

2. Isomerisation of methyl vernolate with boron trifluoride etherate in dioxan at room temperature.

Methyl vernolate (2.0g, 6.5mmole) was stirred overnight at room temperature in anhydrous dioxan (100ml) with boron trifluoride etherate (0.4ml, 6.7mmole). The reaction product (1.98g), extracted in the usual way, gave the GLC results shown in Table 8 (p. 27).

This product (1.80g) was chromatographed on silica gel (200g) as described previously, to give the results shown in Table E5 (p. 113). Recovered material amounted to 1.74g (96%).

2.1 Fraction A.

GLC results are summarised in Table 9 (p. 28). The IR spectrum showed complex cis,trans absorption as before (p. 108),

and the UV spectrum indicated conjugated triene at λ max 267mμ ($E^{1\%}_{1cm}$ 980).

Table E5.

Fraction	Solvent	Weight (mg)	
1	PE5		
2	PE5		
3	PE10	40	A
4	PE10		
5	PE20	1463	B
6	PE20		
7	PE40		
8	PE40		
9	PE60	152	C
10	PE60		
11	PE80	82	D
12	PE80		
13	E		
14	E		

2.2 Fraction C.

This had carbon numbers of 27.6 and 27.9 on DEGS but showed only very broad peaks on ApL. Absorption at 3590 and 1070cm^{-1} was observed in the IR spectrum.

Isomerisation of C with boron trifluoride etherate in refluxing dioxan. Fraction C (32mg, 0.1mmole) was refluxed for

three hours in anhydrous dioxan (5ml) with boron trifluoride etherate (0.4ml, 0.3mmole). The reaction product (24mg) was examined spectroscopically and chromatographically.

UV spectrum: λ max 230mμ ($E^{1\%}_{1cm}$ 250), λmax 267mμ ($E^{1\%}_{1cm}$ 250) (Fr. C showed no UV absorption). The carbon numbers of Fraction C, before and after re-treatment with boron trifluoride, were as follows:

DEGS	before	27.6, 27.9
	after	20.9, 21.3, 21.8, 22.1, 22.6, 23.3, 23.7, 24.8, 25.3, 26.1, 26.5, 27.5
ApL	before	-
	after	18.6, 18.8, 19.1, 19.6, 20.7

The reaction product (21mg) was separated by prep. TLC (PE30) into four fractions: A' (2mg), B' (8mg), C' (7mg) and D' (1mg). A' had carbon numbers (DEGS) of 20.9, 21.3, 21.8, 22.1, 23.3 and 23.7, and B' had carbon numbers (DEGS) of 22.6, 24.8, 25.3, 26.1 and 26.5.

von Rudloff oxidation of A' (2mg) gave C_8- and C_9- dibasic acids (GLC).

Removal of the hydroxy group from C. This was achieved by hydrogenation, oxidation to an oxo ester, followed by sodium borohydride reduction of its tosylhydrazone.

C (120mg) was smoothly hydrogenated and the product (110mg),

in acetic acid (2ml), was stirred for one hour at room temperature with chromium trioxide (100mg) dissolved in glacial acetic acid (2ml). The oxo ester (55mg, oxo absorption at 1720cm^{-1}, carbon numbers 24.5 (DEGS) and 19.1 (ApL)) was isolated by prep. TLC (PE30). Dissolved in methanol (10ml), this was refluxed for one hour with tosylhydrazine (100mg) and methanol/sulphuric acid (3ml, 3%) to give the tosylhydrazone (50mg). Reduction with sodium borohydride (200mg) in methanol (10ml) converted the tosyl-hydrazone to a product (30mg) which after purification by prep. TLC (PE20) amounted to 12mg and proved to be methyl stearate (C.No 18.0 on DEGS and ApL).

2.3 Fraction B.

GLC results are shown in Table 10 (p. 31). The IR spectrum showed oxo stretching (1710cm^{-1}).

Fraction B (400mg) was separated by prep. Ag^+/TLC (PE25) into five fractions, B1 (30mg), B1a (25mg), B2 (267mg) B2a (37mg) and B4 (11mg), with carbon numbers summarised in Table 11 (p. 32).

Re-chromatography (prep. Ag^+/TLC (PE25)) of the major sub-fraction B2 (110mg) gave upper and lower fractions, B2U (44mg) and B2L (47mg), which were shown to have identical carbon numbers (25.3, DEGS and 19.1 ApL). The IR spectra of B2U and B2L showed the following absorptions:

	B2U	B2L
UV (λmax, $E^{1\%}_{1cm}$)	223mμ (30)	223mμ (150)
IR (cm^{-1})	1710 (oxo)	1710 (oxo), 1685 (conjugated oxo), 970 (trans)

B2 (25mg), B2U (25mg) and B2L (25mg) were each hydrogenated in glacial acetic acid solution (5ml), Pd/C (20mg), and the resulting products had the carbon numbers (DEGS) shown in Table E6.

Table E6.

B2 hydrogenated		B2U hydrogenated		B2L hydrogenated	
C.No	% Area	C.No	% Area	C.No	% Area
21.3	22	21.3	36	21.3	46
21.6	6	21.6	2	21.6	25
22.2	4	24.9	62	24.9	29
22.7	2				
24.9	66				

Alteration in B during Ag^+/TLC. Fraction B (20mg) was chromatographed by direct TLC (PE30) to give unchanged B (19mg) (TLC and GLC).

Fraction B (10mg) was separated into five fractions (see p.115) by prep. Ag^+/TLC (PE25). Examination of each fraction by direct TLC (PE30) showed Fractions B2, B2a and B4 to contain polar impurities, of lower Rf than Fraction B, revealed as brown spots when sprayed with aqueous potassium iodide solution (10%).

Preparation of methyl 9-oxo-octadec-cis-12-enoate. A concentrate of methyl 9-acetoxyoctadec-cis-12-enoate was available in the laboratory.

The acetoxy derivative (2g), refluxed for one hour with sulphuric acid/methanol (50ml, 1%), yielded the 9-hydroxy ester (1.00g). The latter (90mg) was oxidised to the 9-oxo derivative (65mg) with chromium trioxide (100mg) in glacial acetic acid solution.

Alteration of 12-oxo-octadec-cis-9-enoate during Ag^+/TLC. The 9-oxo ester (10mg) and the 12-oxo ester (10mg) were chromatographed separately by Ag^+/TLC (PE25) and the recovered materials re-examined by direct TLC (PE30). The recovered 12-oxo ester, but not the 9-oxo isomer, contained polar impurities revealed as brown spots with potassium iodide spray.

Component B2 (using Fraction B).

GLC results are described in Table 10 (p. 31) and the IR spectrum showed absorption at 1710cm^{-1}.

von Rudloff oxidation of Fraction B (5mg) followed by GLC (DEGS) gave a C_9- dibasic acid together with unchanged components of carbon number 22.7, 24.8 and 25.6. Hydrogenation of B (100mg) gave products (95mg) having the carbon numbers shown in Table E7 (p. 118).

Table E7.

C.No (DEGS)	% Area	C.No (ApL)	% Area
22.7	tr	18.6	tr
24.9	100	18.8	8
		19.2	
		19.4	92

Chromic acid oxidation of hydrogenated B (15mg) gave predominantly C_{11}-, C_{12}- and C_{13}- dibasic acids with smaller amounts of C_7-, C_8- and C_9- dibasic acids. Oximation and Beckmann rearrangement of the hydrogenated products (60mg) yielded C_{12}- (70%) and C_{13}- (30%) dibasic acids (GLC, DEGS and ApL).

Reaction of B with mercuric acetate. Stock reactant solution was prepared by allowing mercuric oxide (1.4g) to dissolve in a solution of methanol (25ml), glacial acetic acid (0.3ml) and water (1.0ml)[83].

Fraction B (100mg) was allowed to stand overnight at room temperature with stock solution (2ml). The solvent was evaporated at $<30^{\circ}C$ under reduced pressure and the residue extracted with ether. Unreacted material (15mg), recovered by prep. TLC (PE30), had carbon numbers (DEGS) 22.2 (2%), 22.7 (5%), 23.2 (tr), 24.8 (74%), 25.3 (9%) and 25.6 (10%).

Prep. Ag^+/TLC (PE25) of the unreacted material (15mg) gave three fractions, B1 (10mg), B1a (1mg) and B2 (2mg), analysed by GLC (DEGS) to give the results in Table E8 (p. 119).

Repetition of these experiments yielded B1 (72mg) from B (900mg).

Table E8.

B1		B1a		B2	
C.No	% Area	C.No	% Area	C.No	% Area
22.7	7	22.2	5	25.3	100
24.8	80	23.2	3		
25.6	13	24.8	51		
		25.3	37		
		25.6	4		

Component B1.

B1 showed essentially two major peaks on DEGS (C.No 22.7 (4%), 24.8 (80%) and 25.6 (16%)) and ApL columns (C.No 18.6 (2%), 18.8 (81%) and 19.2 (17%)). Its IR spectrum showed absorption at 1710, 1020 and 3050cm^{-1}. GLC/MS showed the two major constituents to be similar and the mass spectrum of one (25.6, DEGS) is summarised in Table E9 (p. 120).

The NMR spectrum, in addition to absorptions expected of methyl oxostearate, showed absorption at 9.7τ (trans-cyclopropane) and at 10.2τ (cis-cyclopropane, small).

von Rudloff oxidation and hydrogenation gave unchanged B1 (GLC, TLC) and reduction of B1 (10mg) with sodium borohydride (20mg) in methanol (5ml) gave a reduced product (8mg) (25.7 and 26.4, DEGS; IR spectrum: 3595cm^{-1} (OH), 1020 and 3050cm^{-1}

(cyclopropane)) which ran on TLC (PE40) with methyl ricinoleate. Chromic acid oxidation of B1 (15mg) gave (GLC) C_7-, C_8- and C_9- dibasic acids (15%, 30% and 55% respectively) and C_5- and C_6- monobasic acids. The oxo group in B1 was converted to a methylene group as described previously (p. 114). B1 (17mg) was refluxed with tosylhydrazine (27mg) in methanol containing concentrated sulphuric acid (6ml, 1%). The recovered tosylhydrazones (32mg) were refluxed overnight with sodium borohydride (100mg) in methanol (10ml) to give a product which after purification by prep. TLC (PE20) amounted to 5mg. Its IR spectrum showed cyclopropane absorptions (1020, 3050cm^{-1}) and GLC analysis gave the carbon numbers 18.0 (80%), 18.6 (20%), and 17.4 (80%), 17.8 (20%) on DEGS and ApL columns respectively.

Table E9.

m/e	I	m/e	I	m/e	I	m/e	I	m/e	I
310	3	196	5	152	3	127	17	109	7
295	3	195	1	151	3	125	5	108	3
279	5	194	1	149	2	123	7	107	3
267	7	181	2	147	5	122	5	105	2
254	3	180	3	141	3	121	5	100	9
239	1	179	4	140	3	120	3	99	100
237	1	167	5	139	21	119	3	98	8
236	3	165	7	137	5	115	3	97	10
235	3	164	10	136	4	114	3	96	7
222	2	161	3	135	3	113	3	95	17
211	1	154	2	133	1	112	5	94	5
207	3	153	6	128	3	111	5	93	5
						110	3	91	2

Attempted conversion of methyl 9-oxo-octadec-12-enoate to a cyclopropane compound. The ester (30mg, 0.1mmole) was refluxed for three hours in anhydrous dioxan (5ml) with boron trifluoride etherate (0.4ml, 0.3mmole). The product (28mg), extracted in the usual way, was shown to be unchanged by GLC (25.4, DEGS), IR (1710cm^{-1}) and Ag^{+}/TLC (PE25).

Synthesis of methyl 12-oxo-10,11-methyleneheptadecanoate. A dried ethereal solution (250ml) of diazomethane (~2.8g) was prepared from p-tolylsulphonylmethylnitrosamide (21.5g) by the standard method[166]. Caproyl chloride was obtained from caproic acid (15g) by reaction with thionyl chloride (30ml). Excess thionyl chloride was removed at 30°C under vacuum and caproyl chloride (12g) purified by vacuum distillation (55°C/10mm). Methyl undecenoate was prepared by esterification of undecenoic acid (20g) with boron trifluoride/methanol reagent (12½%, 10ml) in methanol (40ml). Passage through a Florisil column gave the purified ester (18g). Cyclohexane was dried by distillation over sodium wire, and powdered copper sulphate was heated in a muffle oven at 200°C for four hours immediately prior to use.

Preparation of diazoketone. Caproyl chloride (2.8g, 0.021 mole) in anhydrous ether (50ml) was added gradually to a stirred dry ethereal solution of diazomethane (250ml, 0.067mole). Ether and excess diazomethane were removed under nitrogen and the residue taken up in anhydrous petrol (100ml) and dried over sodium

sulphate to yield diazoketone (2.8g).

Reaction of diazoketone with methyl undecenoate. Methyl undecenoate (7.0g, 0.035mole) in cyclohexane (15ml) containing anhydrous copper sulphate (1.5g) was heated to 87-90°C with stirring. To this was added, dropwise, over a period of four hours, a solution of the diazoketone (2.8g, 0.020mole) and methyl undecenoate (3.0g, 0.015mole) in cyclohexane (15ml). Thereafter the solution was cooled, filtered and the cyclohexane removed under vacuum to yield a yellow-green viscous residue (13.0g). The oxocyclopropane ester was recovered from the reaction product by prep. TLC (PE20).

The IR, NMR and GLC properties of this ester are summarised in Table 12 (p. 40).

Chromic acid oxidation of the synthetic ester gave C_8-, C_9- and C_{10}- dibasic acids (GLC).

Sodium borohydride reduction of the ester (60mg) gave a reduced product (55mg) which had a carbon number of 25.7 (DEGS) and an IR spectrum showing hydroxyl (3595cm^{-1}) and cyclopropane (1020, 3050cm^{-1}) absorptions. The NMR spectrum also showed distinctive cyclopropane absorptions at 9.55τ and 9.75τ.

Chromic acid oxidation of B1 after 'de-ketonation'. B1 (60mg), by reduction of its tosylhydrazone (p. 120), gave 'de-ketonated' product (17mg) which gave C_7-, C_8- and C_9- dibasic acids (18%, 25% and 57%) and C_6-, C_7- and C_8- monobasic acids

(GLC) when oxidised with chromic acid.

Chromic acid oxidation of methyl methylene-octadecanoates. Three isomeric esters (15mg) were oxidised as previously described. Each yielded three dibasic acids and three monobasic acids on GLC as shown in Table E10.

Table E10.

Position of methylene group	Dibasic acid	% Area*	Monobasic acid
10,11	C_{10}	49	C_8
	C_9	28	C_7
	C_8	23	C_6
9,10	C_9	56	C_9
	C_8	27	C_8
	C_7	17	C_7
8,9	C_8	61	C_{10}
	C_7	24	C_9
	C_6	15	C_8

* These areas were determined on the dibasic acids only because of recovery difficulties with the monobasic acids.

Synthesis of 12-oxo-cis-9,10-methylene-octadecanoate[89]. Methyl 12-oxo-cis-9-enoate (850mg) was obtained by oxidation of castor oil methyl esters (3.0g) as previously described (p. 111).

Preparation of zinc/copper couple. Zinc dust (2.0g) was added to vigorously stirred, nearly boiling glacial acetic acid.

After one minute cupric acetate monohydrate (0.4g) was added and the mixture stirred for two minutes until the blue colour disappeared. The hot supernatant liquid was decanted and the couple thoroughly washed with glacial acetic acid (5 x 20ml) and then with anhydrous ether (5 x 20ml).

Preparation of the cyclopropane derivative. To the zinc/ /copper couple in anhydrous ether (10ml) was added di-iodomethane (4ml) and the 12-oxo ester (215mg) in ether (5ml), and the solution refluxed overnight. The ether was then decanted and washed with cold dilute hydrochloric acid (10ml N, x 3) and water (3 x 10ml). Excess di-iodomethane was removed (after evaporation of ether) at 100°C under high vacuum (0.5mm). Prep. Ag^+/TLC (PE25) yielded pure oxocyclopropane ester (150mg).

Synthesis of 12-oxo-cis,trans-9,10-methylene-octadecanoate. A mixture of castor oil methyl esters (2.16g) and nitric acid (2ml, 50%) was stirred vigorously for fifteen minutes at 60°C with a solution of sodium nitrite (1ml, 15%). The partially elaidinised product (1.86g) was oxidised to an oxo ester concentrate (1.25g) by the procedure described previously (p. 111) and pure 12-oxo ester (565mg) isolated by prep. TLC (PE30). This oxo ester (260mg) yielded pure cis,trans oxocyclopropane derivative (85mg) as described above.

Properties of synthetic cis and cis,trans oxocyclopropanes. Both showed identical absorption at 1710cm^{-1} (oxo), 1020 and

3050cm^{-1} (cyclopropane) in their IR spectra. In their NMR spectra the cis isomer showed cyclopropane absorption at 10.2τ, and the cis,trans isomer indicated cyclopropane protons at 9.7 and 10.2τ. Their mass spectra were also identical; that of the cis isomer is given in Table E11. GLC analyses are shown in Table 14 (p. 44).

Table E11.

m/e	I	m/e	I	m/e	I	m/e	I	m/e	I
324	5	207	4	163	2	138	2	114	9
309	2	197	2	162	2	137	5	113	100
293	5	196	7	161	4	136	5	112	5
282	1	195	2	155	2	135	4	111	6
281	2	194	2	154	4	133	2	110	4
267	8	189	1	153	24	129	3	109	7
255	1	183	1	152	3	128	3	107	4
254	6	182	2	151	3	127	2	105	3
251	1	181	5	150	1	126	2	101	2
249	1	180	3	149	2	125	3	99	4
239	2	179	4	148	1	124	2	98	9
237	1	168	2	147	5	123	9	97	13
236	3	167	5	142	3	122	6	96	7
235	4	166	2	141	20	121	5	95	25
223	1	165	8	140	2	120	2	94	4
222	2	164	10	139	3	119	2	93	5
						115	2	91	3

I represents the peak intensity relative to the base peak (113).

Chromic acid oxidation of these esters (15mg) yielded C_7-, C_8- and C_9- dibasic acids (9%, 34% and 57% respectively), and C_6- and C_7- monobasic acids. The cis,trans oxocyclopropane isomer (60mg), by reduction of its tosylhydrazone (p. 120), yielded a desoxo derivative (20mg) which gave C_7-, C_8- and C_9- dibasic acids (16%, 24% and 60%), and C_7-, C_8- and C_9- monobasic acids, on chromic acid oxidation.

Component B1a.

Fraction B1a, (25mg), obtained from prep. Ag^+/TLC of Fraction B, gave the results shown in Table 15 (p. 45) on GLC analysis. It, (25mg), was separated by prep. TLC (PE20, two developments) into three sub-fractions: S1 (10mg), S2 (9mg) and S3 (3mg), which were analysed by GLC (DEGS and ApL) to give the results in Table E12.

Table E12.

Fraction	C.No (ApL)				C.No (DEGS)			
B1a	18.5	18.8	19.1	19.5	22.3	23.2	24.8	25.3
S1	18.5	-	-	19.5	22.3	23.2	-	-
S2	-	-	19.1	-	-	-	-	25.3
S3	-	18.8	19.2	-	-	-	24.8*	-

* Component of carbon number 25.6 (ca. 5%) was also present.

The IR spectrum of each fraction indicated S1 to contain ether linkages (1055, 1215cm^{-1}), S2 to contain oxo (1710cm^{-1}) and trans-unsaturation (970cm^{-1}), and S3 to have both oxo (1710cm^{-1}) and cyclopropane (1020, 3050cm^{-1}) groups.

von Rudloff oxidation of S2 (5mg) gave a C_9- dibasic acid and a component with carbon numbers of 15.2 (DEGS) and 10.3 (ApL). Fraction S2, after hydrogenation, had carbon numbers 24.9 (DEGS) and 19.4 (ApL).

3. Isomerisation of methyl vernolate with boron trifluoride in benzene.

Isomerisation in refluxing benzene.

Methyl vernolate (100mg, 0.3mmole) was refluxed for three hours in anhydrous benzene (10ml) with boron trifluoride etherate (0.6ml, 1.0mmole). The dark brown reaction product (90mg) gave a continuous 'streak' from the origin on TLC (PE30), and showed small ill-defined peaks on GLC (DEGS).

Isomerisation in benzene at room temperature.

Methyl vernolate (1.14g, 3.7mmole) was allowed to react in anhydrous benzene (50ml) for thirty minutes at room temperature with boron trifluoride etherate (0.25ml, 4.2mmole). The reaction product (1.09g, 96%) was extracted in the usual way and analysed by GLC (Table 16, p. 48).

Column chromatographic separation of the reaction product (1.00g) on silica gel (200g) gave the results shown in Table E13. Recovered material amounted to 0.95g (95%).

Table E13.

Fraction	Solvent	Weight (mg)	
1	PE5	-	
2	PE5	55	A, 61mg, 6%
3	PE10		
4	PE10	13*	
5	PE20	614	B, 620mg, 65%
6	PE20		
7	PE40		
8	PE40		
9	PE60	161	C, 181mg, 19%
10	PE60		
11	PE80	48*	
12	PE80	62	D, 90mg, 10%
13	E		
14	E		

* For the purpose of calculation, these fractions, shown by TLC to be mixtures, have been distributed between the relevant major fractions.

Fraction C showed absorption at 3595cm^{-1} (OH) and 1070cm^{-1} (C-F) in its IR spectrum, and peaks on GLC of carbon number 27.6 and 27.9 (DEGS). Fraction D showed hydroxyl (3595cm^{-1}) absorption.

3.1 Fraction A.

GLC results are shown in Table 17 (p. 49). Fraction A showed absorption at 230 ($E^{1\%}_{1cm}$ 615), 257, 267 ($E^{1\%}_{1cm}$ 385) and 277mμ in the UV, and complex absorption in the IR at 990, 980, 958 and 942cm^{-1}.

Prep. Ag^+/TLC (PE15) of A (45mg) gave A1 (19mg) and A2 (20mg), having the carbon numbers shown in Table 19 (p. 50). Both fractions showed complex absorption in their IR spectrum (A1: 989, 981, 958cm^{-1}, and A2: 980, 958, 942cm^{-1}) and in their UV spectrum absorption was observed at 230mμ, and 257, 267 and 277mμ.

Reaction with maleic anhydride. Fraction A (10mg) in benzene (3ml), was refluxed for one hour with maleic anhydride (20mg). The unreacted material (3mg), isolated by prep. TLC (PE20), showed similar chromatographic (GLC, TLC, Ag^+/TLC) and spectroscopic (IR, UV) properties to Fraction A2.

3.2 Fraction B.

GLC results are given in Table 20 (p. 52). The IR spectrum showed absorption at 1710, 1020 and 3050cm^{-1}, and its NMR spectrum indicated cis (10.2τ) and trans (9.7τ) cyclopropane isomers.

Fraction B (100mg) was hydrogenated in methanol (10ml) with Pd/C (40mg) to give hydrogenated product (95mg) (24.9 and 25.6, DEGS; 18.8, 19.2 and 19.4, ApL) which on oxidation with chromic acid gave C_7-, C_8- and C_9- dibasic acids (19%, 44% and 37%), and

C_{12}- and C_{13}- dibasic acids. Oximation and Beckmann rearrangement of hydrogenated B (50mg) gave C_9- and C_{13}- dibasic acids (10% and 90%).

Prep. Ag^+/TLC (PE25) of Fraction B (90mg) gave B1 (36mg), B1a (19mg) and B2 (29mg) of GLC composition shown in Table E14.

Table E14.

B1		B1a		B2	
C.No (DEGS)	% Area	C.No (DEGS)	% Area	C.No (DEGS)	% Area
24.8	50	24.8	34	25.3	100
25.6	50	25.3	48		
		25.6	18		

Component B1.

Except for the different ratio (GLC, NMR) of the two cyclopropane isomers (cis and trans) this fraction was similar, spectroscopically and chromatographically, to Fraction B1 from the dioxan isomerisation (p. 119).

Component B2.

This component showed oxo absorption (1710cm^{-1}) in the IR spectrum and had carbon numbers of 25.3 (DEGS) and 19.0 (ApL). It was hydrogenated to a component (24.9, DEGS; 19.4, ApL) which gave C_{12}- and C_{13}- dibasic acids as major fragments when oxidised with chromic acid. von Rudloff oxidation gave a C_9- dibasic acid and a component (15.2, DEGS; 10.3, ApL) corresponding to methyl 4-oxononanoate.

Component B1a.

This fraction showed absorption in its IR spectrum at 1710cm^{-1} (oxo), 1020 and 3050cm^{-1} (cyclopropane) and 965cm^{-1} (trans), and gave peaks of carbon number 24.8, 25.3, 25.6 (DEGS) and 18.8, 19.1, 19.2 (ApL). Hydrogenation of B1a gave unchanged cyclopropane esters (24.8 and 25.6, DEGS; 18.8 and 19.2, ApL) and an oxostearate (24.9, DEGS; 19.4, ApL). von Rudloff oxidation gave a C_9- dibasic acid, a component corresponding to a 4-oxo-nonanoic acid, and unchanged oxocyclopropane esters.

The mass spectrum of the trans-cyclopropane isomer (18.8. ApL; 24.8, DEGS) is tabulated below (Table E15).

Table E15.

m/e	I	m/e	I	m/e	I	m/e	I	m/e	I
310	11	207	5	158	5	136	5	111	34
295	8	200	5	157	8	135	16	110	47
279	13	195	5	155	5	130	8	109	18
268	5	193	5	154	5	127	11	108	13
263	3	186	8	153	37	126	16	107	21
254	3	185	68	152	5	125	97	99	37
253	5	182	11	151	5	124	5	98	26
239	3	181	58	150	5	123	11	97	100
237	3	179	5	143	16	122	5	96	13
225	18	169	11	142	5	121	5	95	26
223	3	168	42	140	13	115	11	94	8
221	3	167	5	139	13	113	5	93	13
213	5	164	5	137	5	112	11		

5. Isomerisation of methyl 12,13-epoxystearate with boron trifluoride in dioxan and in benzene.

Preparation of methyl 12,13-epoxystearate. Methyl octadec-12-enoate (350mg, 1.2mmole) (kindly supplied by Dr. I.A. Ismail) was converted to its epoxide by reaction overnight at room temperature with an ethereal solution of monoperphthalic acid (5ml, 2.2mmole). The epoxy ester (320mg), isolated by prep. TLC (PE30) of the reaction mixture, had carbon numbers of 24.0 (DEGS) and 19.3 (ApL).

Isomerisation in dioxan.

Methyl 12,13-epoxystearate (125mg, 0.40mmole) was stirred overnight at room temperature in anhydrous dioxan (10ml) with boron trifluoride etherate (0.25ml, 0.42mmole). The product (120mg) showed one peak on GLC (24.9, DEGS; 19.4, ApL), and three spots on TLC (PE30). Prep. TLC (PE30) of this product (105mg) gave B (81mg, 81%), C (10mg, 10%) and D (8mg, 8%).

Fraction B. GLC analysis indicated one component (24.9, DEGS; 19.4, ApL) and the IR spectrum indicated oxo absorption ($1710cm^{-1}$). Oximation and Beckmann rearrangement of Fraction B (40mg) gave C_{12}- and C_{13}- dibasic acids (51% and 49%).

Fraction C. Its IR spectrum indicated hydroxyl ($3595cm^{-1}$) and a C-F absorption ($1070cm^{-1}$), GLC analysis showed one component (27.5, DEGS).

Fraction D. The IR spectrum indicated the presence of a hydroxyl group (3595cm^{-1}).

Isomerisation in benzene.

Methyl 12,13-epoxystearate (125mg, 0.40mmole) in anhydrous benzene (10ml) was allowed to react at room temperature for thirty minutes with boron trifluoride etherate (0.25ml, 0.42mmole). The extracted product (115mg) showed only one peak on GLC (27.5, DEGS), and on TLC (PE30) it gave a streak from the origin up to an Rf value corresponding to a monohydroxy ester. Its IR spectrum showed absorption at 3595cm^{-1} (hydroxy) and 1070cm^{-1} (C-F).

Isomerisation in benzene with one fifth equiv boron trifluoride.

The epoxystearate (31mg, 0.10mmole) was stirred at room temperature for fifteen minutes in anhydrous benzene (5ml) with boron trifluoride etherate (0.25ml, 0.02mmole). The reaction product (28mg) gave two peaks (GLC) of carbon numbers 24.0 and 27.5 (DEGS), and on TLC (PE30) showed one spot with Rf value of the original epoxy ester along with a streak from the origin to an Rf value corresponding to a monohydroxy ester.

Attempted isomerisation of 9(10)-oxostearates with boron trifluoride in benzene.

Oxo esters (31mg, 0.1mmole) were stirred at room temperature for thirty minutes in anhydrous benzene (5ml) with boron trifluoride etherate (0.5ml, 0.1mmole). The reaction product (30mg) was unchanged oxo ester (GLC (24.9, DEGS; 19.4, ApL) and TLC (PE30)).

6. Isomerisation of 9,10-epoxystearate in dioxan and benzene.

This oxo ester gave identical results to those obtained with the 12,13-epoxystearate.

7. Isomerisation of 9,10-epoxyoctadec-12-ynoate in dioxan and benzene.

Methyl 9,10-epoxyoctadec-12-ynoate was prepared by epoxidation of Afzelia cuanzensis methyl esters and isolated by prep. Ag^+/TLC (p. 149).

Isomerisation in dioxan.

The epoxy ester (100mg, 0.3mmole) was treated overnight at room temperature in anhydrous dioxan (10ml) with boron trifluoride (0.7ml, 0.3mmole). The reaction product (100mg) isolated in the usual way gave no peaks on GLC (DEGS and ApL). The IR spectrum indicated oxo absorption ($1720cm^{-1}$) and no absorption was observed in the UV spectrum. Prep. TLC (PE30) of this product (100mg) gave B (81mg, 8%), C (7mg, 8%) and D (3mg, 3%).

Fraction B. The IR spectrum had an absorption band at $1720cm^{-1}$ (oxo). No peaks were observed on GLC but after hydrogenation, GLC showed one component (24.9, DEGS; 19.4, ApL). Oximation and Beckmann rearrangement of hydrogenated B (40mg) gave C_9- and C_{10}- dibasic acids (5% and 95%) respectively.

Sodium borohydride reduction of Fraction B (10mg) yielded product BR (8mg) which, as its TMS derivative, showed one component of carbon number 21.4 (DEGS). Hydrogenation of BR (4mg) followed by GLC of its TMS derivative again showed one component of carbon number 19.8 (DEGS).

Fraction C. This showed no significant absorption in the UV spectrum.

Isomerisation in benzene.

The epoxyacetylenic ester (20mg) treated with one equivalent of boron trifluoride etherate in the usual way, gave polymeric material (TLC) and no identifiable components (GLC).

BASE-CATALYSED ISOMERISATION.

Attempted isomerisation of methyl vernolate with (a) potassium methoxide and (b) potassium ethoxide.

(a) Methyl vernolate (100mg, 0.3mmole) was stirred overnight with potassium methoxide (210mg, 3.0mmole) in anhydrous methanol (10ml). An aliquot (1ml) was taken and the remaining solution was refluxed, further aliquots (1ml) being taken after fifteen minutes, thirty minutes and sixty minutes. Each aliquot was diluted with water and extracted with ether. The ether extracts were washed with dilute hydrochloric acid, water, and dried over sodium sulphate. The recovered residues were each quantitatively diluted (50ml) with methanol and examined in the ultra violet but no absorption was observed at 225-235mμ.

(b) Methyl vernolate (100mg, 0.3mmole) was treated as above with potassium ethoxide (250mg, 3.0mmole) in anhydrous ethanol (10ml). Again no UV absorption was apparent.

(c) Similar experiments in anhydrous dioxan also gave products with no UV absorption.

Attempted isomerisation of methyl vernolate with potassium tert. butoxide in tert. butanol/dimethylformamide mixture.

A stock solution of potassium tert. butoxide was prepared by dissolving the tert. butoxide (540mg, 5mmole) in tert. butanol (10ml).

To methyl vernolate (22mg, 0.07mmole), dissolved in dimethylformamide (5ml), was added stock tert. butoxide solution (1ml, 0.5mmole) and the solution stirred at room temperature for one hour. An aliquot (1ml) was withdrawn and the remaining solution was heated at 70°C for one hour when a further aliquot (1ml) was removed. After acidification and ether extraction, each product was examined in the ultra violet.

A control experiment was carried out simultaneously on methyl linoleate (22mg).

	λ max 233mμ ($E^{1\%}_{1cm}$) room temperature	λ max 233mμ ($E^{1\%}_{1cm}$) 70°C
Linoleate	100	250
Vernolate	-	30

Isomerisation of methyl vernolate with potassium tert. butoxide in dimethylsulphoxide.

Dimethylsulphoxide (DMSO) was dried over and distilled (70°C/10mm) from calcium hydride pellets.

Methyl vernolate (175mg, 0.56mmole) was stirred with potassium tert. butoxide (112mg, 1.00mmole) in anhydrous DMSO (10ml) for three days at room temperature. The reaction mixture was then poured into water and extracted with ether to give product (35mg). Acidification of the aqueous layers and extraction with ether yielded a further product (123mg). Both showed low absorption at 233mμ($E^{1\%}_{1cm}$ 100).

Attempted isomerisation of methyl vernolate with potassium tert. butoxide in (a) dimethylformamide, (b) dioxan and (c) benzene.

Methyl vernolate (31mg, 0.1mmole) was stirred overnight in anhydrous dimethylformamide (dioxan, benzene) (5ml) with potassium tert. butoxide (12mg, 0.1mmole). After acidification and extraction with ether the product was examined in the ultra violet but in no case was there significant absorption around 233mμ.

Rearrangement of methyl vernolate with lithium diethylamide.

Preparation of lithium diethylamide.

Bromobenzene was dried over calcium chloride and purified by distillation (45°C/10mm). Diethylamine was distilled from potassium hydroxide pellets. All reactions were carried out in dry apparatus in a nitrogen atmosphere and were stirred magnetically.

Bromobenzene (2.86g, 0.0175mole) in anhydrous ether (20ml) was added to a stirred mixture of lithium (0.28g, 0.04g atom, in small pieces) in anhydrous ether (20ml) at such a rate as to maintain reflux. The resulting solution of phenyllithium was filtered through glass wool and the filtrate diluted to 100ml with anhydrous ether. To this solution (90ml, 0.015mole), cooled to 0°C, diethylamine (1.12g, 0.015mole) in anhydrous ether (10ml), was added, dropwise, over fifteen minutes.

Rearrangement.

Methyl vernolate (1.2g, 0.004mole) in anhydrous ether (20ml) was added to a solution of lithium diethylamide (100ml, 0.015mole) at 0°C. The ice-bath was then removed and the solution refluxed for eight hours. The resulting mixture, diluted with water and extracted with ether, yielded a yellow oil (1.3g) which was examined in the ultra violet and by GLC.

Its UV spectrum showed strong absorption at λmax 233mμ ($E^{1\%}_{1cm}$ 718; a duplicate experiment gave $E^{1\%}_{1cm}$ 701). GLC analysis (DEGS) showed several components of carbon number 22.9, 23.3, 23.7, 24.4 and 28.0, in addition to several peaks of carbon number $<$19.0.

A blank experiment, without vernolate, showed the components of carbon number $<$19.0, 22.9 and 24.4 to be artefacts from the lithium diethylamide preparation.

Prep. TLC (PE45) of the reaction product (375mg) yielded eight fractions in the following proportions: A (9mg, 3%), B (106mg, 30%), C (12mg, 3%), D (20mg, 6%), E (16mg, 4%), F (45mg, 13%), G (30mg, 8%) and H (120mg, 34%). The major fractions B and H were examined by GLC, TLC and UV.

Fraction B.

GLC (DEGS) analysis showed five components of carbon number 22.9, 23.3, 23.7, 24.4 and 28.0. Its IR spectrum showed absorption at 3595, 1730, 980 and 945cm^{-1}, and in the UV spectrum it

gave a strong maximum at 233mμ ($E^{1\%}_{1cm}$ 836).

Prep. Ag^+/TLC (PE25) of B (90mg) yielded two bands, B1 (5mg, 6%) and B2 (80mg, 94%), analysed by GLC (DEGS) to give components of carbon number 22.9 and 24.4, and 23.3, 23.7 and 28.0 respectively.

Fraction H.

No peaks were observed on GLC (DEGS). The IR spectrum showed absorption at 3595, 3400 (broad absorption), 1630, 980 and 945cm^{-1} but none at 1730cm^{-1}. Its UV spectrum gave a strong maximum at 233mμ ($E^{1\%}_{1cm}$ 809). The NMR spectrum showed a quartet at 6.5-6.9τ.

N,N-diethylstearamide. Stearoyl chloride (500mg) was prepared by reaction of thionyl chloride with stearic acid, and diethylamine (5ml) was carefully added. After sixteen hours at room temperature, excess diethylamine was removed under vacuum and the product (400mg) purified by prep. TLC (PE50). Its IR spectrum showed absorption at 1630cm^{-1}, and in its NMR spectrum a characteristic quartet was observed at 6.5-6.9τ.

Reaction of methyl stearate with lithium diethylamide. Methyl stearate (400mg) in anhydrous ether (10ml) was added to lithium diethylamide (50ml, 0.008mole) at 0°C and the resulting mixture refluxed for eight hours. The product (440mg) was separated by prep. TLC (PE40) into several fractions, and the one with the lowest Rf value (60mg, 15% (based on original methyl

stearate)) was shown by its IR and NMR spectra to be identical to authentic N,N diethylstearamide.

Optimum reaction conditions for rearrangement.

Methyl vernolate (1.2g, 0.004mole) was allowed to react with lithium diethylamide reagent (0.015mole) at 0°C in anhydrous ether (70ml). Aliquots (10ml) were withdrawn after fifteen, thirty and sixty minutes, and after refluxing for a further two hours. The aliquots, extracted in the usual way, were analysed by GLC (DEGS) and TLC.

After sixty minutes at 0°C the methyl vernolate (24.6, DEGS) had almost completely reacted and there were three new components of carbon number (DEGS) 23.3, 23.7 and 28.0 together with those of carbon number <19.0. The two artefacts (22.9, 24.4, DEGS) were now present in only trace amounts. TLC (PE45) of this reaction product showed two main fractions corresponding to the previous fractions B and H.

Preparative isomerisation at 0°C.

Lithium diethylamide (0.015mole) in anhydrous ether (40ml), prepared from stock phenyllithium solution* (20ml, 0.015mole) and

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* It was found more convenient to prepare a stock ether solution (100ml) of phenyllithium (0.075mole) and use aliquots to prepare the diethylamide reagent immediately prior to use. This stock solution was stored at 0°C under nitrogen and was still satisfactory at the end of a month.

diethylamine (1.12g, 0.015mole) in anhydrous ether (20ml), was allowed to react with methyl vernolate (1.26g, 0.004mole) in anhydrous ether (20ml) for one hour at 0°C. Ether extraction yielded a yellow oil (1.36g).

Prep. TLC (BE25) of the reaction product (250mg) gave six fractions: A' (10mg, 4%), B' (10mg, 4%), C' (144mg, 63%), D' (10mg, 4%), E' (15mg, 6%) and F' (44mg, 19%). Fractions A' (<19.0, DEGS) and D' (22.9, 24.4, DEGS) were of non-lipid origin (p. 139) and Fraction B' was epoxyoleate (24.6, DEGS). Fraction E' is probably the diethylamide of methyl vernolate. Fractions C' and F' were equivalent to previous Fractions B and H respectively.

Structure of the hydroxydiene ester (Fraction C').

GLC analysis indicated three components (23.3, 23.7 and 28.0, DEGS) in this fraction. It showed absorption at 3595, 1730, 980 and 945cm^{-1} in its IR spectrum, and a strong absorption at 233mμ ($E^{1\%}_{1cm}$ 850) in its UV spectrum. The NMR spectrum gave the information shown in Table E16 (p. 143).

Hydrogenated, in acetic acid solution using Pd/C (15mg), the hydroxy ester (16mg) gave a mixture (15mg) of oxo- (24.9, DEGS) and hydroxystearate (25.9, DEGS). Chromic acid oxidation of the hydrogenated product (15mg) gave C_{12}- and C_{13}- dibasic acids (GLC).

von Rudloff oxidation of the unsaturated hydroxy ester (5mg) yielded the C_9- dibasic acid (GLC).

Table E16.

Assignment	Appearance	τ value	No. of protons
$C\underline{H}_3$, terminal	irregular triplet	9.1	3
$C\underline{H}_2$, in chain	broad peak	8.65	18
$OC\underline{H}_3$	singlet	6.4	3
$C\underline{H}_2.COOCH_3$ $C\underline{H}_2.CH:$ $O\underline{H}$	multiplet	7.7-8.1	5
$C\underline{H}OH$	apparent doublet	6.0	1
$(C\underline{H}:C\underline{H})_2$	multiplet	3.3-4.9	4

Dehydration of the ester (12mg) was effected by boiling with methanolic hydrogen chloride (10ml, 0.1N) for one hour. The product showed only conjugated triene absorption (257, 267 and 277mμ), and furnished C_8- and C_9- dibasic acids when oxidised under von Rudloff conditions.

Partial reduction by di-imide. Potassium azodicarboxylate was prepared from azoformamide (10g) by stirring in an ice-cooled vessel with potassium hydroxide solution (25ml, 50%). The crystals were filtered off under nitrogen atmosphere, dissolved in water at 0°C, and refiltered into ethanol (5 volumes) at 0°C. This gave a yellow precipitate which was washed with methanol and dried in a vacuum desiccator over concentrated sulphuric acid. Diene ester (105mg) was stirred with potassium azodicarboxylate (1.8g) in dry methanol (9ml) during dropwise addition over one hour

of a mixture of methanol, acetic acid and water (2ml, 1:1:1). The mixture was diluted with water and the product (100mg) thoroughly extracted with ether. It (80mg) was separated by prep. Ag^+/TLC (BE25) into saturated hydroxy ester (10mg), trans monoene (9mg), original diene (41mg) and cis monoene (16mg). von Rudloff oxidation of the cis monoene gave a C_9- dibasic acid; the trans isomer gave a C_{11}- dibasic acid (95%) along with a C_9- dibasic acid (5%).

Isolation of authentic methyl coriolate.

Coriaria myrtifolia seeds (1.14g) were thoroughly ground in a mortar and the oil (204mg, 20%) extracted by petrol (six hours). The oil (10mg) was converted to methyl esters by reaction at room temperature overnight with sodium methoxide in anhydrous methanol (5ml, 0.1%). Methyl coriolate, isolated by TLC (PE40), gave peaks of carbon number 23.3, 23.7 and 28.0 (DEGS).

Preparation of methyl 13-hydroxyoctadeca-trans-9,trans-11-dienoate.

The cis,trans diene ester (160mg) dissolved in iodine/carbon disulphide solution (5ml, 30mg%), was placed under a 100 watt light bulb for two hours with occasional shaking. The recovered product (150mg) was separated by prep. Ag^+/TLC (BE25, two developments) into an upper (60mg, 57%) and a lower band (45mg, 43%): the latter ran with the same Rf value as the original c,t hydroxy ester. Additional bands of much higher Rf value were also

observed but these were neither isolated nor characterised.

The upper band. GLC analysis (DEGS) of this fraction, as its TMS derivative, indicated two components of carbon number 20.7 (5%) and 21.6 (95%). The TMS derivative of the original cis,trans hydroxy ester had a carbon number of 20.7 (DEGS). Its IR spectrum showed absorption at 3595, 1710, 982 and a very weak absorption at 945cm^{-1}, and in the UV spectrum it gave a strong absorption at 231mμ ($E^{1\%}_{1cm}$ 980). The NMR spectrum was similar to that obtained with the hydroxy cis,trans isomer except that the complex multiplet due to the conjugated olefinic protons extended only from 3.7-4.8τ and in this multiplet one major absorption was apparent at 4.1τ. An authentic sample of methyl dimorphecolate obtained from Dimorphotheca pluvialis ringens (p. 159) had an identical spectrum.

The lower band. This fraction ran on Ag^+/TLC (PE25) with a similar Rf value to the original cis,trans ester. GLC analysis (DEGS) of its TMS derivative indicated two components of carbon number 20.7 (90%) and 21.6 (10%). Its IR spectrum showed absorption at 3595, 1730, 980 and 945cm^{-1}; the intensity of absorption at 980cm^{-1} being slightly greater than that at 945cm^{-1}.

Preparation of methyl 13-oxo-octadeca-9,11-dienoates.

Chromium trioxide (400mg) was slowly stirred into pyridine (4ml) until the yellow complex precipitated. After addition of the hydroxydiene ester (320mg) in pyridine (2ml), the slurry was

stirred overnight. Ice-water was then added and the product (256mg) recovered by ether extraction. TLC (PE40) indicated complete oxidation of the hydroxy ester and GLC (ApL) showed a major peak of carbon number 20.1 (98%) accompanied by a minor peak of carbon number 21.0 (2%). The IR spectrum showed the presence of a conjugated dienone (1730, 1680, 1660, 1630 and 1580cm^{-1}) with cis,trans configuration (990 and 952cm^{-1}), and the UV spectrum contained an absorption maximum at 275mμ. When acid was used in the recovery of the product the minor peak (21.0, ApL) was slightly larger (ca. 5-10%). A change in the relative proportions of the two components was also observed (in one experiment) after prep. TLC (PE40); the minor component (21.0, ApL) then amounted to nearly 50% of the mixture.

When the cis,trans dienone (120mg) was isomerised with iodine in carbon disulphide, the product (105mg) showed the same two peaks of carbon number 20.1 (now only 10%) and 21.0 (now 90%). From its IR spectrum the product was mainly the trans,trans isomer (993cm^{-1}) of a conjugated dienone (1730, 1680, 1660, 1630 and 1580cm^{-1}). Attempted prep. Ag^{+}/TLC (BE20 and PE25, 2 developments in each solvent) gave no separation of the two components.

The all trans dienone was better prepared by chromium trioxide/pyridine (100mg in 2ml) oxidation of the 13-hydroxy trans,trans diene ester (60mg) in pyridine (2ml). The recovered product (41mg) contained no unreacted hydroxy ester (TLC, PE40)

and was mainly the component of carbon number 21.0 (96%) together with that of carbon number 20.6 (4%). Its IR spectrum was as described above and its NMR spectrum is described below.

Assignment	Appearance	τ value	No. of protons
$C\underline{H}_3$, terminal	irregular triplet	9.1	3
$C\underline{H}_2$, in chain	broad peak	8.65	16
$OC\underline{H}_3$	singlet	6.4	3
$C\underline{H}_2.COOCH_3$ $C\underline{H}_2.CO$ $C\underline{H}_2.CH:$	multiplet	7.4-8.0	6
$(C\underline{H}:C\underline{H})_2$	multiplet	3.7-4.2	3
	multiplet	2.7-3.2	1

In these respects (GLC, IR and NMR) the ester was identical with authentic methyl 9-oxo-octadeca-trans-10,trans-12-dienoate isolated from Dimorphotheca pluvialis ringens seed oil (p. 159).

Attempted rearrangement of methyl 9,10-epoxystearate and mono-epoxidised methyl ximenynate.

A stock solution of monoperphthalic acid in ether (500ml containing 0.22mole peracid), prepared by the standard prodedure[167], was stored at 0°C over anhydrous sodium sulphate.

Olive oil methyl esters (942mg, 3.1mmole) were epoxidised by reaction overnight at room temperature with stock peracid solution (20ml, 8.8mmole). The reaction mixture was poured into aqueous

alkali (15%) and extracted with ether. Methyl 9,10-epoxystearate (500mg) (24.0, DEGS) was isolated from the reaction product (760mg) by prep. TLC (PE30).

Methyl ximenynate (570mg, 2.0mmole) was reacted overnight at room temperature with stock peracid solution (10ml, 4.4mmole). The reaction mixture, separated directly by prep. TLC (PE30), yielded monoepoxidised methyl ximenynate (240mg) and unchanged methyl ximenynate (240mg).

Base-catalysed rearrangement.*

In a nitrogen atmosphere, diethylamine (0.4ml) in anhydrous ether (10ml) was slowly added, dropwise, to an ice-cold solution of stock phenyllithium (5ml) (p. 141) in anhydrous ether (10ml). After ten minutes the epoxy ester ($\leqslant$250mg), dissolved in anhydrous ether (10ml), was added and the whole stirred at 0°C for one hour. The reaction product was recovered by ether extraction.

Rearrangement of methyl 9,10-epoxystearate (180mg) gave a product (202mg) which was shown by GLC (24.0, DEGS) and TLC (PE30) to be mostly unchanged epoxystearate.

Similarly, monoepoxidised ximenynate (200mg) yielded a product (215mg) which was predominantly unreacted epoxy ester (GLC: broad peak centred at 26.2, DEGS).

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* All base-catalysed reactions on epoxy esters ($\leqslant$250mg) described hereafter will be carried out using these conditions unless otherwise stated.

Partial synthesis of racemic methyl α-dimorphecolate.

Methyl linoleate (422mg, 1.4mmole) was treated overnight with peracid solution (4ml, 1.8mmole). Prep. TLC (PE30) of the reaction mixture yielded a monoepoxy fraction* (220mg).

Base-catalysed isomerisation (p. 148) of the monoepoxide (220mg) yielded a product (233mg) from which hydroxydiene esters* (120mg) were separated by prep. TLC (BE25). The hydroxydiene fraction (100mg) was further separated by prep. TLC (PE25, two developments) into an upper (44mg) and a lower band (49mg). Both fractions showed the characteristic IR, UV and NMR spectra of a hydroxydiene ester with cis,trans conjugated unsaturation (p. 142) and both (5mg, each) were dehydrated when boiled for one hour with methanolic hydrogen chloride (5ml, 0.1N).

The upper fraction (20mg) was hydrogenated in acetic acid (5ml), using Pd/C (20mg) as catalyst, to a mixture of hydroxy- and oxostearates (GLC). These were oxidised by chromic acid to give C_{12}- and C_{13}- dibasic acids; the lower fraction after hydrogenation gave predominantly C_8- and C_9- dibasic acids. von Rudloff oxidation of each fraction yielded the C_9- dibasic acid.

Partial synthesis of racemic methyl helenynolate.

Afzelia cuanzensis seed oil (2.36g) was refluxed for fifteen

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* Prep. TLC showed this fraction to consist of two components in approximately equal amounts.

minutes with sodium methoxide in methanol (25ml, 0.1N). The acidified reaction mixture was extracted with petrol to yield methyl esters (2.28g) which contained methyl crepenynate (40%, 21.6, DEGS). Ag^+/TLC (PE25) of the methyl esters gave five fractions, shown by GLC to be saturates, monoenoates, dehydro-crepenynate (22.4, DEGS), crepenynate and linoleate respectively. Methyl crepenynate (285mg) containing a trace of methyl linoleate (3%, GLC) was isolated by prep. Ag^+/TLC (PE25).

Methyl crepenynate (285mg, 1mmole) was monoepoxidised by reaction overnight at room temperature with stock peracid solution (5ml, 2.2mmole). The reaction product was separated by prep. TLC (PE30) into monoepoxidised crepenynate (202mg) (26.0, DEGS), unchanged crepenynate (24mg) and a diepoxy fraction (10mg).

It was later found more convenient to treat the Afzelia esters directly with a ten-fold excess of peracid. Prep. TLC (PE30) of the reaction mixture then yielded a fraction containing only monoepoxidised methyl crepenynate (26.0, DEGS) and epoxy-stearate (24.0, DEGS). Further separation of this mixture by prep. Ag^+/TLC gave pure monoepoxidised crepenynate as the lower band. By this means, Afzelia esters (1.0g) yielded monoepoxidised crepenynate (240mg).

Isomerisation (p. 148) of monoepoxidised crepenynate (250mg) yielded a reaction product (270mg) separated by prep. TLC (BE25) into four main fractions: C1 (20mg, 9%), C2 (120mg, 53%),

C3 (28mg, 12%) and C4 (60mg, 26%); a band (absorbing under UV light) of Rf value higher than that of C1 was discounted in these calculations.

Fraction C1 contained a major component of carbon number 26.0 (DEGS) and Fractions C3 and C4 were presumed to be diethylamides (their IR spectra showed strong absorption at 1630cm^{-1}).

The major Fraction C2.

Compared with methyl 9-hydroxyoctadeca-trans-10,cis-12-dienoate, Fraction C2 had a slightly lower Rf value on TLC (PE40) but a higher value on Ag^+/TLC (BE25). It had absorption bands at 3595, 1730 and 950cm^{-1} in its IR spectrum, and there was a strong absorption at 228mμ ($E^{1\%}_{1cm}$ 600) and an inflexion at 238mμ ($E^{1\%}_{1cm}$ 510) in its UV spectrum. The NMR spectrum, with complex absorption in the region 3.7-4.7τ is summarised below:

Assignment	Appearance	τ value	No. of protons
CH_3, terminal	irregular triplet	9.1	3
CH_2, in chain	broad peak	8.65	18
OCH_3	singlet	6.4	3
$CH_2.COOCH_3$, $CH_2.C:$, OH	multiplet	7.5-8.10	5
CHOH	apparent doublet	6.0	1
CH:CH.C:	two triplets	4.25-4.7	1
CH:CH.C:	two doublets	3.7-4.25	1

TMS derivative. Hexamethyldisilazane (0.2ml) and trimethylchlorosilane (0.1ml) were added to the ester (5mg) dissolved in dry pyridine (1ml). After five minutes the pyridine was removed under vacuum and the residue taken up in ether. GLC analysis (DEGS) showed one component of carbon number 23.4.

Fraction C (20mg) was hydrogenated in methanol (5ml) using Pd/C (20mg) as catalyst, to give an oxostearate (24.9, DEGS) and a hydroxystearate (25.9, DEGS). Chromic acid oxidation of these products gave C_8- and C_9- dibasic acids.

von Rudloff oxidation of the hydroxy ester furnished a C_9- dibasic acid and a C_6- monobasic acid.

Lithium aluminium hydride reduction. The hydroxy ester (43mg) dissolved in anhydrous ether (3ml) was added, dropwise, to a suspension of lithium aluminium hydride (200mg) in anhydrous ether (2ml), and the mixture was refluxed for two hours. Excess reducing agent was destroyed by the addition of wet ether, followed by sulphuric acid. Extracted product (37mg) was separated by prep. TLC (PE60) and the major fraction (33mg) recovered. Its IR spectrum showed absorption due to allene ($1950cm^{-1}$) and hydroxyl ($3595cm^{-1}$) groups.

Fraction C (24mg) was refluxed for one hour with methanolic hydrogen chloride (7ml, 0.1N). The major component (18mg), recovered by prep. TLC (PE30), contained an ether linkage (1080 and $1100cm^{-1}$). Its GLC showed one major component (25.2, DEGS)

and three minor ones (22.6, 23.4 and 25.6, DEGS).

Partial reduction with di-imide. The hydroxy ester (40mg) was stirred with potassium azodicarboxylate (1.2g) in anhydrous methanol (3ml). A mixture of methanol/acetic acid/water (1:1:1) was slowly added, dropwise, until the yellow colour disappeared. The extracted product (34mg), analysed by GLC (DEGS, TMS derivative), gave three main peaks of carbon number 20.8 (10%), 21.6 (26%) and 23.4 (64%), together with small peaks of carbon number 20.1 and 21.0. Prep. Ag^+/TLC (BE15) of this reduced product furnished three fractions, H1 (17mg, 63%), H2 (3mg, 11%) and H3 (7mg, 26%), which were examined by GLC (DEGS, TMS derivative) and IR, with the results given below:

Fraction	C.No	IR absorption (cm^{-1})	Assignment
H1	23.4	3595 and 950	unchanged hydroxy-enyne
H2	20.8	3595, 980 and 945	hydroxy c,t diene
H3	21.6	3595	hydroxy-yne

Fractions H1 and H2 each yielded the C_9- dibasic acid and the C_6- monobasic acid, after von Rudloff oxidation.

von Rudloff oxidation of Fraction H3 gave a C_6- monobasic acid, and a component of carbon number 29.0 (DEGS) and 17.2 (ApL). The oxidised products showed strong IR absorption at 1770 and 1730cm^{-1}. Similar products were obtained by oxidative cleavage

of methyl 9-hydroxyoctadec-cis-12-enoate.

Attempted synthesis of methyl parinarate.

Linseed oil (4g) was treated overnight at room temperature with sodium methoxide in anhydrous methanol (30ml, 0.5%). Acidification and petrol extraction yielded methyl esters (3.7g) which were separated by prep. Ag^+/TLC (PE30) to give methyl linolenate (98% pure by GLC). This ester (680mg, 2.3mmole) was epoxidised overnight at room temperature with stock peracid solution (6ml, 2.6mmole) to yield, by prep. TLC (PE30), a monoepoxy fraction (372mg). When examined by TLC (PE25), this showed two components, in approximate ratio 1:2 (upper:lower); the upper component ran with an Rf value similar to that of methyl vernolate.

Prep. TLC (PE10, followed by PE20) of the monoepoxy fraction (245mg) gave an upper band (59mg) and a lower band (167mg)*.

Isomerisation (p. 148) of the upper and lower fractions, followed by prep. TLC (BE25), gave two monohydroxy esters (33mg and 85mg respectively). Their UV spectra (λ max 234mμ and λ max 237mμ) and their IR spectra (980 and 945cm^{-1}) indicated conjugated cis,trans diene systems.

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* As care was taken to avoid contamination of the upper fraction with the lower one, these two weights do not give a true indication of the relative amounts of these two fractions.

Dehydration experiments.

A stock acidic solution was prepared by diluting concentrated sulphuric acid (3ml) to 50ml with anhydrous dioxan. The mono-hydroxy ester (10mg), from the lower fraction, dissolved in anhydrous dioxan (10ml), was stirred at room temperature with stock sulphuric acid/dioxan (0.5ml). Aliquots (1ml) were taken at intervals, poured into water and ether extracted. The recovered material was diluted to 100ml with methanol and examined in the ultra violet. After a reaction time of one hour, no conjugated diene absorption remained and only tetraene absorption (λ max 301mμ) was observed.

Dehydration of hydroxy ester from upper component.

The hydroxy ester (20mg) dissolved in anhydrous dioxan (20ml) was stirred at room temperature for one hour with stock sulphuric acid/dioxan solution (1ml). An aliquot (1ml) had strong tetraene absorption (λ max 302mμ) but no absorption at 234mμ. The remaining reaction mixture was poured into water, saturated with sodium chloride, and extracted with petrol. The latter, dried over sodium sulphate, was carefully evaporated under vacuum at $<30^{\circ}C$ to yield a product (14mg). Strong absorption peaks were observed in the UV spectrum at 277, 288, 302 ($E^{1\%}_{1cm}$ 2300) and 316mμ, and the IR spectrum showed strong absorption at 996cm^{-1} with weaker bands at 975, 951 and 925cm^{-1}. von Rudloff oxidation of the product (3mg) gave only a C_9- dibasic acid.

SELECTED SEED OILS.

1. Helichrysum bracteatum seed oil.

Extraction and transesterification.

Helichrysum bracteatum seeds (9.8g) were thoroughly ground in a mortar and extracted for four hours with petrol. The seeds were then re-ground and extracted for a further four hours to yield a light yellow oil (2.02g, 20%). This oil (2.02g) was shaken overnight at room temperature with sodium in anhydrous methanol (25ml, 0.1%) to give methyl esters (1.67, 82%). TLC (PE30) indicated complete transesterification.

Isolation of monoepoxy fraction.

Prep. TLC (PE30) of the methyl esters (1.60g) gave four distinct fractions: A (1.01g, 68%), B (0.21g, 14%), C (0.19g, 13%) and D (0.08g, 5%). Fraction B had the same Rf value as authentic 12,13-epoxyoleate and showed three components on GLC (DEGS): X (C.No 24.0, 6%), Y (C.No 24.6, 69%) and Z (C.No 26.0, 25%). Attempted prep. Ag^+/TLC (PE25, BE15) of Fraction B was unsuccessful.

Separation of Fraction B.

Fraction B (200mg) was allowed to stand overnight at room temperature with stock peracid solution (5ml, 2.2mmole). The reaction product, separated by prep. TLC (PE30), gave a monoepoxy

fraction (56mg) and a diepoxy fraction (121mg). GLC (DEGS) analysis of the recovered monoepoxy fraction showed two components: X (C.No 24.0, 20%) and Z (C.No 26.0, 80%). Prep. Ag^+/TLC (PE30) of this fraction (56mg) separated X (11mg), as the upper band, from Z (42mg).

Characterisation of component Z.

On TLC (PE30), component Z ran with authentic methyl 9,10-epoxyoctadec-12-ynoate and both had identical carbon numbers (26.0, DEGS; 19.1, ApL) on GLC. The IR and NMR spectra of both were also identical; the IR spectrum showed no significant features, and in the NMR spectrum the epoxy ring protons produced a broad multiplet centred on 7.27τ. No olefinic protons were observed.

Position of epoxide group. The ester Z (20mg) was refluxed for two hours with glacial acetic acid (2ml). After removal of the latter under vacuum the residue was refluxed (2hr) with sodium hydroxide (8%) in water/methanol (1:4, 5ml). Acidification, extraction and esterification yielded product P (18mg), part of which (10mg) was hydrogenated in methanol (5ml) with Pd/C (10mg) catalyst, to yield PH (10mg). von Rudloff oxidation of P (5mg) gave C_9- dibasic and C_6- monobasic acids; similar oxidation of PH (5mg) gave C_9- dibasic and C_9- monobasic acids.

Fraction PH, when examined (PE50) on silica gel G impregnated with boric acid (0.3mm, wet thickness, 5% boric acid), ran with an

authentic threo-9,10-dihydroxystearate.

The authentic 9,10-epoxyoctadec-12-ynoate gave identical results.

Rearrangement. Base-catalysed isomerisation of component Z (17mg) gave a product (27mg) which, after prep. TLC (PE45), yielded a hydroxy ester (7mg). This hydroxy ester showed absorption in its UV spectrum at λ max 228mμ ($E^{1\%}_{1cm}$ 500) and λ max 238mμ ($E^{1\%}_{1cm}$ 430), and its IR spectrum indicated hydroxyl (3595cm^{-1}) and trans-enyne (950cm^{-1}) absorptions.

Base-catalysed isomerisation of the authentic 9,10-epoxy-octadec-12-ynoate ester (20mg) yielded a hydroxy ester (9mg) with the same spectral properties.

Characterisation of component X.

Component X had GLC (24.0, DEGS; 19.3, ApL) and TLC (PE30) retention characteristics identical with authentic methyl cis-9,10-epoxyoctadecanoate.

Position of epoxide group. The ester (10mg) was converted to its dihydroxy derivative (10mg) with acetic acid (2ml) etc. as described above. von Rudloff oxidation of the dihydroxy ester gave a C_9- dibasic and a C_9- monobasic acid.

On boric acid impregnated plates, the dihydroxy ester ran with authentic methyl threo-9,10-dihydroxystearate.

2. Dimorphotheca pluvialis ringens seed oil.

Extraction and transesterification.

Seeds (2.5g), after grinding and soxhlet extraction (petrol), yielded an oil (800mg, 32%) which was converted to methyl esters (682mg) by reaction overnight at room temperature with sodium methoxide in anhydrous methanol (20ml, 0.1%).

Isolation of monoepoxy fraction.

Prep. TLC (PE30) of the methyl esters (330mg) gave four bands: A (90mg, 31%), B (3mg, 1%), C (16mg, 5%) and D (185mg, 63%). Fraction B had the same Rf value as authentic methyl vernolate and GLC (DEGS) indicated two components of carbon number 24.0 (10%) and 24.6 (90%). Its IR spectrum was similar to that of methyl vernolate.

Separation of monoepoxy fraction.

Fraction B (3mg) was separated by TLC (PE20, two developments) into two approximately equal fractions. GLC (DEGS) showed the upper fraction to contain one component of carbon number 24.6, and the lower to contain components of carbon numbers 24.0 (20%) and 24.6 (80%). Their IR spectra showed no absorption between 900 and 1000cm^{-1}.

3. Dimorphotheca aurantiaca seed oil.

The monoepoxy fraction (1%) was isolated as described for D. pluvialis ringens.

Seeds (5.41g) yielded oil (1.56g, 29%) which was converted to methyl esters (1.26g). Prep. TLC (PE30) gave a monoepoxy fraction (10mg) which showed two components (GLC, DEGS) of carbon number 24.0 (15%) and 24.6 (85%). The IR spectrum of the monoepoxy fraction showed no trans absorption.

TRIGLYCERIDE STUDIES.

Extraction of oil.

All oils were ground in a mortar under petrol and extracted with petrol in a soxhlet. Operations were carried out as quickly as possible. The oil content of the seeds is given in Tables E17 - E22.

Transesterification.

A dilute solution of sodium methoxide in anhydrous methanol (5ml, 0.05%) was used with all fractions ($\leqslant$ 5mg) at room temperature overnight. No acidification was used in the extraction with ether.

Prep. TLC separation.

Oils (ca. 200mg) were separated by prep. TLC (PE25) into six fractions using ten 20 x 20cm plates, and glycerides recovered from the silica by soxhlet extraction with ether. Fractions were diluted to 100ml with petrol and stored at 0°C. Aliquots were taken for quantitation, prep. Ag^+/TLC and lipolysis as required. The results are given in Tables E17 - E22.

Lipolysis procedure.

Treatment of lipase. Pancreatic lipase (available in the laboratory) was homogenised for two minutes with acetone in an

Ato-mix. After centrifuging, the lipase was dried overnight in a vacuum desiccator at room temperature and stored in a tightly corked bottle in the refrigerator.

Preparation of M 'TRIS' buffer. Trihydroxymethylaminomethane (TRIS) (12.11g) was dissolved in distilled water (20ml) and titrated with M HCl to pH 8.0. Finally the whole was diluted to 100ml with water. The final pH was 8.2.

Procedure. Preliminary reactions were carried out on cottonseed oil triglycerides (5mg) and trivernolin (5mg, isolated from C. cordofanus) to establish conditions for 20-25% recovery of monoglycerides (based on the weight of original triglyceride), the latter being quantitated with methyl heptadecanoate as internal standard.

Triglyceride (5mg), dissolved in ether, was added to a centrifuge tube (fitted with a B19 socket), and the ether evaporated off under nitrogen. Panoreatic lipase (15mg) was dispersed in 'TRIS' buffer (10ml) and an aliquot (1ml) added to the centrifuge tube containing the triglyceride. Calcium chloride solution (2.2%, 0.1ml) and bile salt solution (0.05%, 0.3ml) were quickly added and the mixture held at 40°C for one minute. Thereafter the mixture was stirred (at 40°C) for 8 minutes with a mini-stirrer. Finally the whole was poured into water, ether extracted, and the monoglyceride fraction recovered by prep. TLC using a solvent of chloroform:acetone:ammonia (80:20:1)[144].

Results are given in Tables E17 - E22.

Prep. Ag^+/TLC separation.

Non-epoxy triglycerides (6mg) were separated by prep. Ag^+/TLC (BE10) and the monoepoxy triglycerides (6mg) by prep. Ag^+/TLC (BE25).

Fractions were recovered from the silica by slurrying with methanol:ether:water (5:5:1)[144] at which stage methyl heptadecanoate (0.2mg) was added to each as internal standard. The triglycerides were then re-extracted into ether prior to transesterification. Results are summarised in Tables E17, E18, E21 and E22.

Table E17.

Cephalocroton peuschelli (29% oil).

a) Component esters*.

16:0	18:0	18:1	18:2	18:3	Epoxy
3.7	2.9	7.4	13.1	0.9	72.0

b) Prep. TLC (PE25).

	16:0	18:0	18:1	18:2	18:3	Epoxy	Amount (% mole)
Cp2	16.0	9.7	30.4	40.7	3.2	-	2.8
Cp3	7.3	5.2	18.7	32.1	2.1	34.6	13.6
Cp4	4.4	3.3	8.7	15.2	1.0	67.4	41.2
Cp5	-	-	-	-	-	100.0	38.8
Cp6	10.2	6.0	16.6	34.6	1.9	30.7	3.6
Total	3.6	2.6	7.6	13.0	0.8	72.4	

c) Lipolysis studies**.

	16:0	18:0	18:1	18:2	18:3	Epoxy
Cp2						
TG	14.5	9.7	31.4	41.1	3.3	-
MG	1.9	-	36.5	57.4	4.2	-
Cp3						
TG	7.4	5.4	19.3	34.3	2.0	31.6
MG	1.3	-	24.1	37.8	1.6	35.2
Cp4						
TG	4.3	3.6	8.7	15.7	1.2	66.5
MG	0.5	-	7.8	13.0	-	78.7

* All values given in Tables E17 - E22, are quoted as % mole.

** Figures for Cp2, Cp3 etc. are slightly different from those given in b). Samples had been kept (at 0°C) for some time before analysis.

d) Ag^+/TLC (BE10) of Cp2.

Fr.	14:0	16:0	18:0	18:1	18:2	18:3	20:0	Amount (% mole)
1	4.9	35.4	22.9	32.9	1.6	-	2.3	7.0
2	0.8	21.7	17.0	44.0	14.1	1.4	1.0	22.3
3	0.7	17.6	11.4	35.4	32.4	1.3	1.2	19.1
4	0.4	4.0	1.9	59.2	32.0	2.5	-	11.8
5	0.7	18.3	11.3	5.4	63.0	-	1.3	13.2
6	0.7	5.2	2.3	28.7	60.4	2.7	-	15.0
7	1.0	5.3	2.1	9.5	77.7	4.4	-	11.6
Total	1.0	15.0	9.9	31.9	39.6	1.8	0.8	
Cp2	-	16.0	9.7	30.4	40.7	3.2	-	

d') Ag^+/TLC (BE10) of Cp2 (duplicate).

Fr.	14:0	16:0	18:0	18:1	18:2	18:3	20:0	Amount (% mole)
1	4.2	36.1	26.9	32.8	-	-	-	7.1
2	1.7	20.2	14.9	60.5	0.9	1.2	0.6	9.9
3	1.2	24.5	17.0	33.2	22.4	1.0	0.7	13.9
4	0.7	18.2	11.3	34.5	32.5	1.8	1.0	17.9
5	1.2	4.6	1.1	57.6	33.6	1.9	-	11.0
6	0.9	13.5	7.2	14.4	62.6	1.4	-	23.5
7	0.6	5.2	2.0	11.5	68.6	12.1	-	16.7
Total	1.2	15.8	9.9	30.8	38.9	3.1	0.3	
Cp2	-	16.0	9.7	30.4	40.7	3.2	-	

e) Ag^+/TLC (BE25) of Cp3.

Fr.	14:0	16:0	18:0	18:1	18:2	18:3	Epoxy	Amount (% mole)
1	1.1	20.3	14.8	31.9	0.3	0.8	30.8	13.8
2	0.2	1.9	0.7	65.7	1.8	1.3	28.4	12.8
3	0.6	17.6	14.4	1.3	33.9	-	32.2	23.9
4	0.2	1.2	-	32.3	34.5	1.4	30.4	25.3
5	0.2	1.8	0.5	1.9	59.8	4.4	31.4	24.2
Total	0.4	8.0	5.7	21.8	31.5	1.7	30.9	
Cp3	-	7.3	5.2	18.7	32.1	2.1	34.6	

e') Ag^+/TLC (BE25) of Cp3 (duplicate).

Fr.	14:0	16:0	18:0	18:1	18:2	18:3	Epoxy	Amount (% mole)
1a	-	50.4	29.9	19.7	-	-	-	1.4
1b	1.5	20.0	13.3	31.6	0.6	1.4	31.6	11.2
2	1.0	3.1	1.0	59.8	2.7	1.4	31.0	11.5
3	0.4	17.4	14.3	2.2	34.5	-	31.2	23.2
4	0.5	1.2	-	32.4	33.3	2.1	30.5	27.1
5	0.4	1.6	0.7	2.0	59.3	4.9	31.1	25.6
Total	0.6	8.1	5.5	20.5	32.6	2.1	30.6	
Cp3	-	7.3	5.2	18.7	32.1	2.1	34.6	

Table E18.

Cephalocroton cordofanus (30% oil).

a) Component esters.

16:0	18:0	18:1	18:2	18:3	Epoxy
4.5	3.2	8.3	16.4	0.9	66.7

b) Prep. TLC (PE25).

	16:0	18:0	18:1	18:2	18:3	Epoxy	Amount (% mole)
Cc2	13.5	10.5	31.1	41.8	3.1	-	3.8
Cc3	6.6	5.4	17.0	35.6	1.8	33.6	16.7
Cc4	3.5	3.1	7.4	17.7	0.9	67.4	40.6
Cc5	0.3	0.1	0.4	0.7	-	98.5	31.8
Cc6	8.6	5.9	16.1	30.6	1.1	37.7	7.1
Total	3.7	3.0	8.3	17.2	0.9	66.9	

c) Lipolysis studies.

	16:0	18:0	18:1	18:2	18:3	Epoxy
Cc2						
TG	13.5	10.5	31.1	41.8	3.1	-
MG	2.7	-	37.5	57.0	2.8	-
Cc3						
TG	6.6	5.4	17.0	35.6	1.8	33.6
MG	1.4	1.2	20.3	36.1	1.8	39.2
Cc4						
TG	3.5	3.1	7.4	17.7	0.9	67.4
MG	0.8	0.4	6.3	15.5	0.5	76.5

d) Ag^+/TLC (BE10) of Cc2.

Fr.	16:0	18:0	18:1	18:2	18:3	Amount (% mole)
1	44.8	25.1	30.1	-	-	6.4
2	21.7	12.8	65.5	-	-	8.8
3	34.5	25.3	10.6	29.6	-	7.8
4	14.4	3.4	72.3	7.0	2.9	8.0
5	18.9	13.1	35.6	32.4	-	19.0
6	6.0	2.1	56.5	33.4	2.0	9.9
7	18.5	11.9	6.9	62.7	-	14.1
8	6.1	1.8	27.9	61.3	2.9	11.9
9	4.2	1.4	9.2	74.1	11.1	14.1
Total	16.7	9.7	32.2	39.0	2.4	
Cc2	13.5	10.5	31.1	41.8	3.1	

e) Ag^+/TLC (BE25) of Cc3.

Fr.	16:0	18:0	18:1	18:2	18:3	Epoxy	Amount (% mole)
1	21.8	14.1	32.5	-	-	31.6	10.0
2	2.6	0.9	64.5	2.0	-	30.0	9.9
3	18.7	12.6	1.6	34.0	-	33.1	26.6
4	1.3	-	31.9	33.7	1.6	31.5	23.7
5	1.4	0.7	1.6	64.5	0.7	31.1	27.7
6	12.0	1.9	16.8	23.2	24.5	21.6	2.1
Total	8.3	5.1	18.4	35.7	1.1	31.4	
Cc3	6.6	5.4	17.0	35.6	1.8	33.6	

Table E19.

Crepis aurea (30% oil).

a) Component esters.

16:0	18:0	18:1	18:2	Crep*	Epoxy
3.8	2.3	10.8	20.5	3.0	59.6

b) Prep. TLC (PE25).

	16:0	18:0	18:1	18:2	18:3	20.0	Crep	Epoxy	Amount (% mole)
Ca2	11.4	6.8	22.0	47.1	1.7	1.0	10.0	-	8.7
Ca3	5.1	2.4	16.6	33.4	-	-	10.0	32.5	17.2
Ca4	3.9	2.0	8.8	18.6	-	-	0.5	66.2	55.2
Ca5	1.1	0.5	1.6	3.0	-	-	0.5	93.3	12.8
Ca6	4.4	1.8	8.5	25.5	-	-	-	59.8	6.1
Total	4.4	2.3	10.4	22.0	0.2	0.1	2.9	57.7	

c) Lipolysis studies.

	16:0	18:0	18:1	18:2	18:3	20:0	Crep	Epoxy
Ca2								
TG	11.4	6.7	22.0	47.1	1.7	1.1	10.0	-
MG	-	-	24.8	73.1	2.1	-	-	-
Ca3								
TG	5.1	2.4	16.6	33.4	-	-	10.0	32.5
MG	-	-	9.0	35.0	-	-	-	56.0
Ca4								
TG	3.9	2.0	8.8	18.6	-	-	0.5	66.2
MG	-	-	1.2	2.8	-	-	-	96.0

* Methyl crepenynate.

Table E20.

Crepis vesicaria (12% oil).

a) Component esters.

16:0	18:0	18:1	18:2	Crep	Epoxy
5.5	2.1	7.4	31.6	1.1	52.3

b) Prep. TLC (PE25).

	16:0	18:0	18:1	18:2	18:3	Crep	Epoxy	Amount (% mole)
Cv2	13.1	5.5	16.6	59.1	1.2	4.5	-	13.5
Cv3	6.1	2.5	12.3	43.1	-.	2.2	33.8	14.6
Cv4	3.8	1.6	4.5	22.4	-.	-	67.7	57.5
Cv5	1.3	0.6	1.1	5.8	-	-	91.2	9.9
Cv6	5.3	1.8	4.6	36.0	-.	-	52.3	4.5
Total	5.2	2.2	6.9	29.4	0.2	0.9	55.2	

c) Lipolysis studies.

	16:0	18:0	18:1	18:2	18:3	Crep	Epoxy
Cv2							
TG	13.1	5.5	16.6	59.1	1.2	4.5	-
MG	-	-	15.8	82.5	1.7	-	-
Cv3							
TG	6.1	2.5	12.3	43.1	-	2.2	33.8
MG	-	-	6.7	39.5	-	-	53.8
Cv4							
TG	3.8	1.6	4.5	22.4	-	-	67.7
MG	-	-	1.0	2.9	-	-	96.1

Table E21.

Cephalaria joppica (18% oil).

a) Component esters.

14:0	16:0	18:0	18:1	18:2	Epoxy
9.2	14.4	2.8	15.3	22.7	35.6

b) Prep. TLC (PE25).

	14:0	16:0	18:0	18:1	18:2	Epoxy	Amount (% mole)
Cj2	10.1	18.2	3.5	28.6	39.6	-	28.8
Cj3	9.8	16.5	3.1	16.0	21.4	33.2	35.4
Cj4	7.3	12.2	2.2	6.9	7.5	63.9	23.7
Cj5	3.1	6.2	1.2	6.6	7.6	75.3	6.7
Cj6	6.8	13.4	2.6	14.8	14.8	47.6	5.4
Total	8.7	15.1	2.8	16.8	22.1	34.5	

c) Lipolysis studies.

	14:0	16:0	18:0	18:1	18:2	Epoxy
Cj2						
TG	10.1	18.2	3.5	28.6	39.6	-
MG	0.6	0.9	0.4	35.0	63.1	-
Cj3						
TG	9.8	16.5	3.1	16.0	21.4	33.2
MG	0.7	0.8	0.5	18.5	29.3	50.2
Cj4						
TG	7.3	12.2	2.2	6.9	7.5	63.9
MG	0.6	1.0	0.4	7.1	9.9	81.0

d) Ag^+/TLC (BE10) of Cj2*.

Fr.	14:0	16:0	18:0	18:1	18:2	Amount (% mole)
1	10.1	37.1	9.4	41.6	1.8	7.6
2	7.2	21.0	4.4	64.2	3.2	8.5
3	11.9	27.6	6.5	21.9	32.1	16.4
4	9.9	19.8	3.9	32.9	33.5	19.6
5	1.7	2.8	-	63.4	32.1	8.5
6	7.5	14.8	2.8	11.2	63.7	30.6
7	1.2	3.6	-	9.8	85.4	8.8
Total	7.8	18.1	3.8	28.3	42.0	
Cj2	10.1	18.2	3.5	28.6	39.6	

e) Ag^+/TLC (BE25) of Cj3*.

Fr.	12:0	14:0	16:0	18:0	18:1	18:2	Epoxy	Amount (% mole)
1	1.9	25.4	29.7	6.2	2.9	-	33.9	18.6
2	1.1	14.3	17.7	3.2	29.3	-	34.4	20.2
3	-	2.7	4.1	-	55.6	3.1	34.5	8.9
4	0.4	9.5	16.7	3.6	1.0	33.6	35.2	29.4
5	-	1.0	1.6	-	28.6	32.2	36.6	13.5
6	-	0.7	2.7	-	1.8	63.8	31.0	9.4
Total	0.7	10.9	14.8	2.9	15.7	20.5	34.5	
Cj3	-	9.8	16.5	3.1	16.0	21.4	33.2	

* Peak areas measured with integrator attached to GLC.

Table E22.

Cephalaria leucantha (15% oil).

a) Component esters.

12:0	14:0	16:0	18:0	18:1	18:2	Epoxy
10.0	9.8	8.1	1.5	19.5	31.7	19.4

b) Prep. TLC (PE25).

	12:0	14:0	16:0	18:0	18:1	18:2	Epoxy	Amount (% mole)
C12	13.5	11.4	9.2	1.6	23.0	41.3	-	53.5
C13	10.7	9.5	7.7	1.5	15.9	21.6	33.1	30.7
C14	6.8	7.1	5.3	1.0	9.1	10.9	59.8	9.9
C15	5.5	6.2	5.5	1.0	10.3	13.4	58.1	2.9
C16	7.4	8.0	9.3	2.0	19.1	24.1	30.1	3.0
Total	11.6	10.1	8.3	1.5	18.9	31.0	18.6	

c) Lipolysis studies.

	12:0	14:0	16:0	18:0	18:1	18:2	Epoxy
C12							
TG	13.5	11.4	9.2	1.6	23.0	41.3	-
MG	-	-	-	-	34.1	65.9	-
C13							
TG	10.7	9.5	7.7	1.5	15.9	21.6	33.1
MG	-	-	-	-	17.7	31.0	51.3
C14							
TG	6.8	7.1	5.3	1.0	9.1	10.9	59.8
MG	-	-	-	-	10.1	15.4	74.5

d) Ag^+/TLC (BE10) of C12*.

Fr.	12:0	14:0	16:0	18:0	18:1	18:2	Amount (% mole)
1	18.8	25.8	18.5	4.4	32.5	-	8.9
2	7.4	11.2	9.2	2.4	69.8	-	8.4
3	19.4	21.6	14.6	2.8	12.6	29.0	21.2
4	9.4	12.5	9.7	2.0	32.7	33.7	19.0
5	1.2	1.2	1.6	-	61.8	34.2	6.9
6	5.9	9.0	7.6	1.3	8.9	67.3	27.3
7	0.8	1.3	2.1	-	10.7	85.1	8.3
Total	9.9	12.8	9.8	1.9	25.2	40.4	
C12	13.5	11.4	9.2	1.6	23.0	41.3	

e) Ag^+/TLC (BE25) of C13*.

Fr.	12:0	14:0	16:0	18:0	18:1	18:2	Epoxy	Amount (% mole)
1	24.5	25.7	19.7	4.0	2.9	-	23.2	14.5
2	14.4	12.4	9.2	1.4	28.5	-	34.1	23.4
3	2.0	1.8	2.8	-	60.4	1.7	31.3	9.0
4	8.5	10.9	10.5	1.6	0.9	32.6	35.0	28.6
5	1.4	1.1	1.5	-	31.6	30.7	33.7	13.2
6	0.5	1.2	2.2	-	1.7	62.6	31.8	11.3
Total	9.8	10.2	8.7	1.4	17.1	20.6	32.2	
C13	10.7	9.5	7.7	1.5	15.9	21.6	33.1	

* Peak areas measured with integrator attached to GLC.

Computer programme C1.

```
      DIMENSION A(20),PA(20),GMOL(20),WMOL(20),PMOL(20)
      READ 5,K
      DO 80L=1,K
      READ 5,N
    5 FORMAT(I10)
      READ 10,(A(I),I=1,N)
   10 FORMAT(9F8.3)
      READ 10,(GMOL(I),I=1,N)
      SUM=0.0
      DO 20I=1,N
   20 SUM=SUM+A(I)
      DO 30I=1,N
   30 PA(I)=100.0*A(I)/SUM
      DO 40I=1,N
   40 WMOL(I)=PA(I)/GMOL(I)
      WSUM=0.0
      DO 50I=1,N
   50 WSUM=WSUM+WMOL(I)
      DO 60I=1,N
   60 PMOL(I)=100.0*WMOL(I)/WSUM
      PRINT 61
   61 FORMAT(20H ORIGINAL PEAK AREAS)
      PRINT 70,(A(I),I=1,N)
      PRINT 62
   62 FORMAT(//)
      PRINT 63
   63 FORMAT(13H AREA PERCENT)
      PRINT 70,(PA(I),I=1,N)
      PRINT 64
   64 FORMAT(//)
      PRINT 65
   65 FORMAT(14H MOLES PERCENT)
      PRINT 70,(PMOL(I),I=1,N)
      PRINT 66
   66 FORMAT(//)
   80 CONTINUE
   70 FORMAT(14F8.3)
      CALL EXIT
      END
```

Computer programme C2.

```
      DIMENSION A(10,15),ADJ(10,15),WMOL(10,15),PMOL(10,15),GMOL(15),CHE
     1CK(15),SUM(10),RAT(10),PRAT(10),SMOL(10)
      READ 2,K
    2 FORMAT(I2)
      DO 1000L=1,K
      DO 14I=1,10
      DO 14J=1,15
   11 A(I,J)=0.0
   12 ADJ(I,J)=0.0
   13 WMOL(I,J)=0.0
   14 PMOL(I,J)=0.0
      DO 15J=1,15
   15 GMOL(J)=0.0
      DO 17I=1,10
   16 RAT(I)=0.0
   17 PRAT(I)=0.0
      READ 10,M,N
   10 FORMAT(I2,I2)
      DO 40J=1,N
   20 READ 30,(A(I,J),I=1,M)
   30 FORMAT(9F8.3)
   40 CONTINUE
      N1=N-1
      READ 50,(GMOL(J),J=1,N1)
   50 FORMAT(9F8.3)
      DO 60I=1,M
      SUM(I)=0.0
      DO 60J=1,N1
   60 SUM(I)=SUM(I)+A(I,J)
      DO 70I=1,M
   70 RAT(I)=SUM(I)/A(I,N)
      SRAT=0.0
      DO 80I=1,M
   80 SRAT=SRAT+RAT(I)
      DO 90I=1,M
   90 PRAT(I)=RAT(I)*100.0/SRAT
      DO 100I=1,M
      DO 100J=1,N1
  100 ADJ(I,J)=A(I,J)*PRAT(I)/SUM(I)
      DO 110I=1,M
      DO 110J=1,N1
  110 WMOL(I,J)=ADJ(I,J)*1000.0/GMOL(J)
      DO 120I=1,M
      SMOL(I)=0.0
      DO 120J=1,N1
  120 SMOL(I)=SMOL(I)+WMOL(I,J)
      SSMOL=0.0
      DO 125I=1,M
  125 SSMOL=SSMOL+SMOL(I)
      DO 130I=1,M
      DO 130J=1,N1
  130 PMOL(I,J)=WMOL(I,J)*100.0/SSMOL
      DO 140J=1,N1
      CHECK(J)=0.0
      DO 140I=1,M
  140 CHECK(J)=CHECK(J)+PMOL(I,J)
      PRINT 150
  150 FORMAT(20H ORIGINAL PEAK AREAS///)
      DO 180I=1,M
  160 PRINT 170,(A(I,J),J=1,N)
  170 FORMAT(15F8.3)
  180 CONTINUE
      PRINT 190
  190 FORMAT(////)
      PRINT 200
  200 FORMAT(20H ADJUSTED PEAK AREAS///)
      DO 230I=1,M
  210 PRINT 220,(ADJ(I,J),J=1,N1)
  220 FORMAT(14F8.3)
  230 CONTINUE
      PRINT 240
  240 FORMAT(////)
      PRINT 250
  250 FORMAT(5H WMOL///)
      DO 280I=1,M
  260 PRINT 270,(WMOL(I,J),J=1,N1)
  270 FORMAT (14F8.3)
  280 CONTINUE
      PRINT 290
  290 FORMAT(////)
      PRINT 300
  300 FORMAT(14H MOLES PERCENT///)
      DO 330I=1,M
  310 PRINT 320,(PMOL(I,J),J=1,N1)
  320 FORMAT(14F8.3)
  330 CONTINUE
      PRINT 340
  340 FORMAT(1H1)
      PRINT 350
  350 FORMAT(18H MOLECULAR WEIGHTS)
      PRINT 360,(GMOL(J),J=1,N1)
  360 FORMAT(14F8.3)
      PRINT 370
  370 FORMAT (23H CHECK ON MOLES PERCENT)
      PRINT 360,(CHECK(J),J=1,N1)
      PRINT 380
  380 FORMAT (30H TOTAL PEAK AREA FOR EACH BAND)
      PRINT 390,(SUM(I),I=1,M)
  390 FORMAT(10F8.3)
      PRINT 400
  400 FORMAT(11H BAND RATIO)
      PRINT 390,(RAT(I),I=1,M)
      PRINT 410
  410 FORMAT(22H PERCENTAGE BAND RATIO)
      PRINT 390,(PRAT(I),I=1,M)
      PRINT 420
  420 FORMAT(18H BAND SUM OF MOLES)
      PRINT 390,(SMOL(I),I=1,M)
      PRINT 430
  430 FORMAT(1H1)
 1000 CONTINUE
      CALL EXIT
      END
```

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