EPOXIDATION OF UNSATURATED ESTERS

Hermann Robert Schuler

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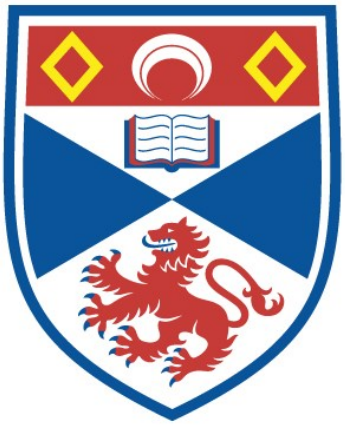

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Epoxidation of Unsaturated Esters

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presented by

Hermann Robert Schuler

to the

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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 Department of Chemistry, United College of St. Salvator and St. Leonard, University of St. Andrews, under the supervision of Professor F.D. Gunstone,D.Sc., F.R.I.C.

Certificate

I hereby certify that Hermann Robert Schuler has completed twelve terms of research work under my supervision, has fulfilled the conditions of the Resolution of the University Court 1967, No. 1 (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

After obtaining the Abitur at the Hebel Gymnasium in Lörrach, West Germany, I entered Basel University in April 1966 and graduated as Diplom Chemiker in June 1971.

I was admitted as a research student in the United College, University of St. Andrews, in October 1971 and was awarded the Elizabeth Soutar Scholarship which I held until October 1974.

Acknowledgements

My greatest debt is to Professor F.D. Gunstone who always showed interest in my concerns and the progress of my work. I wish to express to him my deep gratitude for the many helpful discussions and his continuous encouragement.

Thanks are due to all my friends and colleagues in our research group and in the Chemistry Department whose company and stimulating discussions I enjoyed so much.

I would also like to thank Mrs. M. Smith and Mr. C. Millar for running the NMR and the mass spectra. Particular thanks go to Mrs. W. Pogorzelec for her experience and patience in typing this thesis.

Last, but by no means least, I want to thank Professors Lord Tedder and P.A.H. Wyatt who kindly accepted me as a research student in their Department and who made available to me the first award of the Elizabeth Soutar Scholarship which enabled me to spend three happy years in this marvellous town of St. Andrews.

To my parents

and

to Marie-Louise

Contents

Abbreviations

Ac	acetate
ApL	Apiezon L
DEGS	diethylene glycol succinate
DMF	dimethylformamide
E	diethyl ether
ECL	equivalent chain length
GLC	gas-liquid chromatography
IR	infrared
M	molecular ion
Me	methyl
MS	mass spectrometry
NMR	nuclear magnetic resonance
P	petroleum ether
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl
UV	ultraviolet

A short writing is frequently used instead of systematic names of fatty acids and their methyl esters. For example, octadec-*cis*-9-enoic acid, ie. oleic acid is abbreviated 18:1 (9c). The numbers 18 and 1 give the number of carbon atoms and the number of unsaturated centres, respectively. The parenthetical expression (9c) indicates that the unsaturated centre is located between C(9) and C(10) and is a *cis* double bond; "t" denotes a *trans* double bond and "a" stands for an acetylenic bond.

Summary

Eight isomeric 9,12-diunsaturated C_{18} methyl esters and seven isomers from two series of positionally isomeric methyl octadeca-cis,cis-dienoates, ie. 18:2 (5c12c - 8c12c) and (6c9c - 6c11c) were epoxidised to furnish monounsaturated monoepoxy esters and diepoxy esters. The monoepoxides and diepoxides were separated in pure form by column and thin layer chromatography. A comparative study was made of the chromatographic (TLC and GLC) behaviour and the NMR spectra of the unsaturated epoxy esters.

TLC gave some indication of the position and geometry of the epoxy group and the nature of the unsaturated centre. The geometry of the epoxy group and the olefinic or acetylenic nature of the unsaturated centre were also revealed by GLC.

220 MHz NMR spectra, recorded for all unsaturated epoxy esters and diepoxy esters, exhibited up to nine more or less resolved signals which showed the long range deshielding influences of the ester group, the unsaturated centre, and the epoxy group. These influences were noticeable even when five methylene groups separated the deshielding group and the proton under consideration. Even though the NMR spectra did not give a complete structural analysis of the epoxy esters, they nevertheless revealed the geometry of the epoxy group as well as the nature of the unsaturated centre.

The epoxides were converted to vicinal diether derivatives the mass spectrometric examination of which allowed the unambiguous location of the epoxy group and therefore also of the original double bond.

Melting points of the vicinal dihydroxy acids, derived from all the twenty-six unsaturated epoxy esters by acetolysis and hydrolysis, were determined. The melting point differences between the various isomers reflected such structural features as the geometry and position of the α-diol group and of the accompanying

unsaturated centre.

Two diepoxy esters, methyl cis-6,7,cis-9,10-diepoxystearate and methyl cis-8,9,cis-12,13-diepoxystearate, were reacted with boron trifluoride etherate. Instead of the expected dioxo derivatives, various cyclic ethers were obtained.

A minor study was concerned with the hydroboration of various unsaturated long chain esters. The intermediate organoboranes were subjected to oxidation, protonolysis and coupling reactions to give in good yields hydroxy, oxo and hydroperoxy esters as well as hydrogenated and coupled products.

PART I

Epoxidation of Unsaturated Esters

INTRODUCTION

Natural occurrence of epoxy fatty acids

Several of the numerous natural epoxides can be classified as derivatives of fatty acids. The first of the epoxy acids was discovered in 1954 when Gunstone[1] found this compound as the major fatty acid component in the seed oil of Vernonia anthelmintica and identified it as cis-12,13-epoxyoctadec-cis-9-enoic acid. Since its discovery in 1954 this epoxy acid, also called vernolic acid, has been isolated from the seed oils of many species of several plant families[2-5]. Its isomer, coronaric acid, ie. cis-9,10-epoxyoctadec-cis-12-enoic acid, also occurs in various seed oils, though at a considerably lower level[6,7].

Both these epoxy acids have a structural similarity to linoleic acid (18:2 9c12c) from which they can be considered to be formally derived by replacement of one double bond by an epoxide ring. By analogy, 9,10-epoxystearic acid is formally related to oleic acid, the most common unsaturated fatty acid. It is therefore not surprising that cis-9,10-epoxystearic acid is widely met in seed oils[8] and in spore oils of various plant rusts[9]. Similarly, three dienoic epoxy acids might conceivably derive from linolenic acid (18:3 9c12c15c). So far only one of these, cis-15,16-epoxyoctadeca-cis-9,cis-12-dienoic acid, has been found, again as glyceride ester in a seed oil[10,11].

Helichrysum bracteatum seed oil contains an acetylenic epoxide, cis-9,10-epoxyoctadec-12-ynoic acid, and its olefinic analogue, coronaric acid. It is of interest that this seed oil also contains octadec-cis-9-en-12-ynoic acid and linoleic acid which might be the precursors of the epoxy compounds[12].

Small quantities of cis-9,10-epoxyoctadeca-trans-3,cis-12-dienoic acid have been detected in the seeds of Stenachaenum macrocephalum after prolonged storage[13]. This epoxy acid is thought to be enzymatically produced in the seeds from endogenous

octadeca-trans-3,cis-9,cis-12-trienoic acid. There are several reports suggesting that in various seeds a lipoxygenase is present which after harvest catalyses the conversion of unsaturated fatty acids to oxygenated products like epoxy acids[11,14,15].

Graveland[16] showed that cis-12,13-epoxy-9-hydroxyoctadec-trans-10-enoic acid and the isomeric cis-9,10-epoxy-12-hydroxy-octadec-trans-11-enoic acid are primary products of an enzymatic oxidation of linoleic acid in doughs.

In all the epoxy acids mentioned so far the epoxide group has the cis configuration. The only naturally occurring trans epoxy acid is trans-9,10-epoxystearic acid which Vioque et al.[17] discovered in the lipid extract from pressed olive pulp.

Another family of epoxy acids has been isolated from the cuticle layers of leaves and fruits of various plants. The cutin epoxy acids, some of which constituted up to 60% of the total fatty acid content of the cutin polymer, are ω-oxygenated C_{18}-acids with one cis-epoxy group in the 9,10-position. The wide occurrence of cis-9,10-epoxy-18-hydroxystearic acid[18-23] and of its monounsaturated[21-23] and diunsaturated[24] analogues, cis-9,10-epoxy-18-hydroxy-18:1 (12c) and cis-9,10-epoxy-18-hydroxy- 18:2 (12c15c), is well documented. The ω-hydroxy group can also be further oxidised as shown by the more recent discoveries of 9,10-epoxy-18-oxostearic acid[25] and of 9,10-epoxyoctadecane-1,18-dioic acid[23].

In contrast to all these epoxy fatty acids isolated from plant materials only one epoxy acid has been discovered in animal tissue. This is cis-10,11-epoxy-7-ethyl-3,11-dimethyltrideca-trans-2,trans-6-dienoic acid. It differs from the previously described plant epoxy acids in its branched structure which is partly isoprenoid and indicates a different biogenesis. In the form of its methyl ester this epoxy acid acts as a hormone in the juvenile giant silk worm moth[26,27].

Biological aspects of epoxy fatty acids

The two carbon atoms of the epoxide ring are asymmetrically substituted in all the epoxides described above. Those natural epoxy acids where optical rotation has been studied have all been found optically active. For some of them the absolute configuration of the epoxide group has been determined and their structure has thus been completely revealed[28-31]. For example, (+)vernolic acid which is found in several plant families has been shown to have the D configuration (S-12, R-13). Its (-)enantiomer, occurring only in one plant family, has the L configuration (R-12,S-13)[28]. The optical activity of the natural epoxy fatty acids is taken as evidence for their being biosynthetic products rather than autoxidation products, since racemates are generally not produced in nature.

Little is known about the biosynthesis of epoxy fatty acids and about their metabolic role. Scott et al.[32] indicated an enzymatic activity possibly present in the seeds of Vernonia anthelmintica. This enzyme function which becomes active only when the seeds are crushed catalyses the conversion of vernolic acid to threo-12,13-dihydroxyoleic acid. Morris and Crouchman[33] demonstrated that this hydration proceeds in a stereospecific way. The epoxide ring is opened with inversion at C-12 and the epoxide oxygen is retained in its original configuration at C-13.

Similar enzymes which catalyse the stereospecific hydration of cis-9,10-epoxystearic acid have been discovered in Claviceps species[34] and in the spores of various plant rusts[35,36]. Tulloch[35] made the tentative suggestion that the threo-9,10-dihydroxystearic acid formed may be a precursor for nonanal which stimulates spore germination. Another hydratase has been found in a strain of Pseudomonas. Both cis- and trans-9,10-epoxystearic acid are hydrated, but from racemic mixtures of either cis-

or trans-epoxides only one enantiomeric epoxide is converted to one enantiomeric dihydroxy acid[37-39]. So epoxy acids have been established as precursors of dihydroxy acids.

Miwa et al.[40] concluded that dihydroxy acids can also be precursors of epoxy acids. In their investigation of the fatty acid composition in maturing Vernonia anthelmintica they found that during maturation formation of dihydroxyoleic acid preceded the formation of vernolic acid and that the content in dihydroxy acid decreased as the proportion of epoxy acid increased. The authors suggested the presence of three enzymatic activities which may catalyse the oxygenation of oleic and linoleic acid, the dehydration of the dihydroxy to the epoxy acid and finally the esterification to the triglyceride oil.

In a comprehensive study of the biosynthesis of cis-9,10-epoxystearic acid in red stem rust infected wheat Knoche[41] showed that acetate is an active substrate for the biosynthesis of the epoxy acid as also are stearic acid and oleic acid. These C_{18} substrates are transformed to the epoxy acid without undergoing β-oxidation followed by resynthesis of the carbon chain. The experimental results provide evidence that oleic acid is the immediate precursor of the cis-9,10-epoxystearic acid. Knoche also showed that the epoxide oxygen originates from molecular oxygen.

Similar enzymatic epoxidations are known to play key roles in the metabolism of various natural and unnatural compounds. An example is the detoxication of naphthalene. When fed to animals it is converted to naphthylmercapturic acid. 1,2-Dihydro-1,2-dihydroxynaphthalene is the first isolated oxygenated product in this sequence and there is good evidence that it is produced from an epoxide intermediate[42,43]. Similarly the degradation of alkaloids such as kynurenic acid is believed to proceed via an epoxide intermediate[44]. Another important example of an enzymatic epoxidation is that of the acyclic triterpenoid squalene. Two research groups[45-47] found independently that rat and swine liver preparations contain

an enzyme responsible for the formation of 2,3-epoxy-2,3-dihydro squalene. Another enzyme controls the cyclisation of 2,3-epoxy-2,3-dihydrosqualene to lanosterol. Hence 2,3-epoxy-2,3-dihydro-squalene is a very important intermediate in the conversion of squalene to sterols which is initiated by this epoxidation step. A recent report[48] discussed the enzymatic epoxidation of aliphatic alkenes. The catalysing enzyme system consisted of three separate protein components and required NADH and molecular oxygen.

In the lipid field, the allylic hydroxy group in the aliphatic part of prostaglandin molecules is thought to be introduced via an intermediate epoxide function resulting from enzymatic oxygenation of the appropriate olefinic bond. The position of this hydroxy group in the prostaglandin supports this hypothesis[49]. The biosynthesis of the various C_{18}-cutin fatty acids has been extensively studied by Kolattukudy and coworkers[24,50]. They demonstrated that for fatty acids to be precursors of in-chain oxygenated cutin acids it is essential to possess a $\Delta 9$ double bond. Fatty acids which meet this requirement, ie. oleic, linoleic and linolenic acid, are converted to the 18-hydroxy acids, the cis-9,10-epoxy-18-hydroxy and the 9,10-18-trihydroxy acids. 1-^{14}C-stearic acid only undergoes ω-hydroxylation but no further internal oxygenation.

So far epoxy fatty acids have been shown to be precursors for hydroxy and dihydroxy derivatives, and it is considered that they themselves are derived from unsaturated fatty acids. Further suggestions have been made that they also could be intermediates in the formation of new olefinic and acetylenic unsaturation. Wolff[2] pointed to a possible biosynthetic relationship between linoleic (18:2 9c12c), vernolic (cis-12,13-epoxy 18:1 9c) and crepenynic acid (18:2 9c12a). Gunstone devised a scheme which relates a number of conjugated polyunsaturated fatty acids to the most common unsaturated fatty acids, oleic and linoleic. The key

intermediates in this scheme are epoxides (12,51). However, no biochemical evidence exists so far which would substantiate this hypothesis. Labelled vernolic acid was not converted into crepenynic acid[52]. Similarly, Gurr and Bloch[53] using cis-9,10-epoxystearic acid bound to the acyl carrier protein ACP could not find any formation of oleic acid.

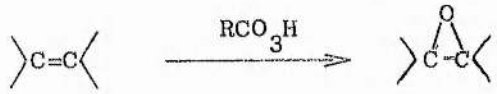
Utilisation of epoxy fatty acids

The great importance of epoxides depends on the wide range of reactions which these compounds can undergo. Large quantities of epoxides are produced industrially for a variety of purposes the most prominent of which is the manufacture of epoxy resins. The potential of epoxidised fatty materials was quickly recognised. Vernonia anthelmintica oil with its high epoxy acid content (70%) has been evaluated as a stabiliser and plasticiser for polyvinyl chloride[54,56]. The stabilising qualities are mainly due to the ability of the epoxy groups to react with residual hydrogen chloride in the polymer with chlorohydrin formation. Soybean, cottonseed and linseed oils, to name just a few oils rich in unsaturated fatty acids, are commercially epoxidised for use as stabilisers and plasticisers. The epoxy derivatives serve also as monomers for curing with agents like phthalic anhydride to give epoxy resins[57,58] and are valuable intermediates for a great number of chemicals.

Peracid epoxidation and its mechanism

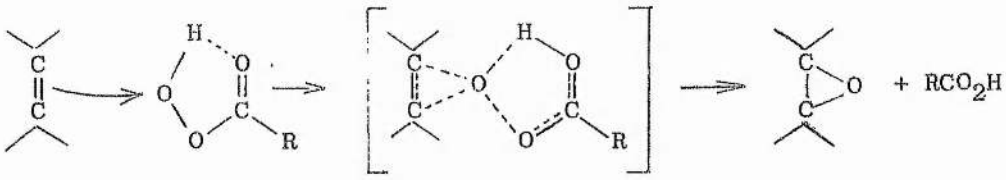
Epoxides show great versatility in organic synthesis as a source of many other functional groups. Hence reactions leading to epoxy compounds are of major importance and the various preparative procedures have been reviewed by Swern[59] and Rosowsky[60]. The earliest epoxidation method, which is still used to some extent, employs the hypohalogenation of an olefin followed by

dehydrohalogenation[61]. This method has been largely replaced by an epoxidation reaction based on the discovery by Priloschajew[62] that alkenes are smoothly epoxidised by peroxy acids:



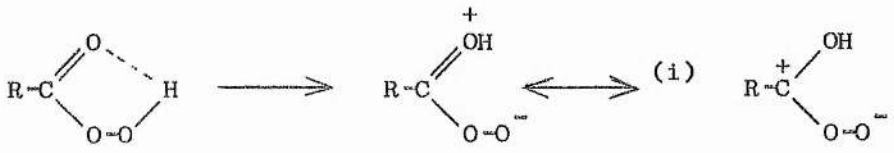
The reaction proceeds quantitatively and results in an electrophilic cis-addition of oxygen across the double bond so that the epoxide geometry is the same as that of the unsaturated centre[63-65]. The stereospecificity and the high yields make this method extremely useful. The reaction times of this second order reaction are normally short. Cis olefins react faster than trans olefins[66], whilst acetylenic bonds react very slowly so that olefinic centres can be selectively epoxidised in compounds containing both olefinic and acetylenic unsaturation[67].

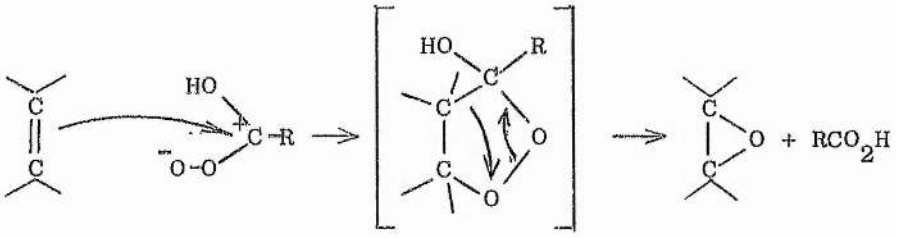
Of the various mechanisms proposed for this peroxy acid oxidation two are still disputed. Bartlett[68] suggested the following 1,1-addition mechanism:



Kinetic studies indicate a non-ionic mechanism[69] and a rather high negative entropy of activation[70] consistent with the postulated bicyclic intermediate. The strong intramolecular H-bonding in aliphatic and aromatic peroxy acids can be taken as further support for this mechanism.

However, reactivity parameters of this epoxidation reaction led Kwart and Hoffmann to the postulation of a 1,3-dipolar addition mechanism[71]:





They showed that the hydroxy carbonyloxide species (i), produced in the ozonisation of suitable olefinic compounds like ascorbic acid, can effect the epoxidation of olefins in the same way as peroxy acids.

A MO-study on the hydrogen bonded and the dipolar forms of peroxy acids revealed only a small difference between the total π-electron energies of the two forms. It was concluded therefore that both mechanisms should be theoretically possible. The 1,1-mechanism is expected to be favoured in nonpolar solvents and the 1,3-mechanism in polar media[72].

DISCUSSION

Synthesis of epoxides related to diunsaturated fatty acids: monounsaturated epoxides and diepoxides

The conversion of monounsaturated fatty acids and their derivatives to epoxides has been extensively studied. The interest in the epoxides of polyunsaturated fatty acid materials grew rapidly when their natural occurrence and the scope of their commercial utilisation became apparent. Swern[73] reported that methyl linoleate can be converted in good yields to mono- and di-epoxides according to the amount of peracid used in the epoxidation. Two Russian authors claimed the synthesis of methyl 12,13-epoxyoctadec-9-enoate via the monoepoxidation of methyl linoleate with peracetic acid[74]. The first total synthesis of this naturally occurring epoxyacid, ie. vernolic acid, was achieved by Osbond[75]. By selective epoxidation of synthetic crepenynic acid (18:2 9c12a) he obtained (±)cis-9,10-epoxyoctadec-12-ynoic acid which he then managed to hydrogenate partially to (±) vernolic acid without concurrent opening of the epoxide ring. The acetylenic epoxide was later prepared again by Conacher and Gunstone[12] in order to prove the structural identity of the natural cis-9,10-epoxyoctadec-12-ynoic acid they had discovered in the seed oil of Helichrysum bracteatum. By a sequence of stereospecific reactions[65] trans-12,13-epoxyoctadec-cis-9-enoic acid was obtained from vernolic acid[30] Maerker et al.[76] investigating the possibility of positional selectivity in the monoepoxidation of methyl linoleate found that of the two unsaturated monoepoxides methyl coronarate was formed to a slightly larger extent (54%) than methyl vernolate (46%). Ferrari et al.[77] showed, however, that the monoepoxidation of methyl linoleate yielded 39% of coronarate and 40% of vernolate. These authors were the first to demonstrate that the two isomeric monoepoxides can be separated by column chromatography on a preparative scale. IR, NMR and mass spectra were recorded for the

two pure epoxides, but the IR and NMR spectra showed no characteristic differences.

Apart from this last mentioned report and a few sporadic references no systematic study of the chromatographic and spectroscopic characteristics of epoxides derived from polyunsaturated fatty materials has been undertaken so far. Jacobsberg and Gunstone[78] demonstrated that position and configuration of the epoxide group in all the isomeric methyl epoxystearates can be established on the basis of the mass and NMR spectra without resort to chemical degradation. Position and geometry of the double bond in the parent olefinic esters can be inferred because of the clear stereospecificity of the epoxidation reaction.

Since Jacobsberg's study was confined to saturated epoxides derived from all the methyl cis- and trans-octadecenoates we decided to investigate the various epoxidation products related to a selection of fifteen synthetic diunsaturated C_{18} esters. Most of them had been prepared by Lie Ken Jie[79] and Jacobsberg[80] but a few were newly synthesised by routes already described in literature. The diunsaturated esters used in this epoxidation can be divided into four series which comprise the following acids:

n		
5	18:2 (5c12c)	
4	18:2 (6c12c)	18:2 (6c12c)
3	18:2 (7c12c)	18:2 (6c11c)
2	18:2 (8c12c)	18:2 (6c10c)
1	18:2 (9c12c)	18:2 (6c9c)
1	18:2 (9c12c)	18:2 (9a12c)
1	18:2 (9c12t)	18:2 (9a12t)
1	18:2 (9t12c)	18:2 (9c12a)
1	18:2 (9t12t)	18:2 (9t12a)

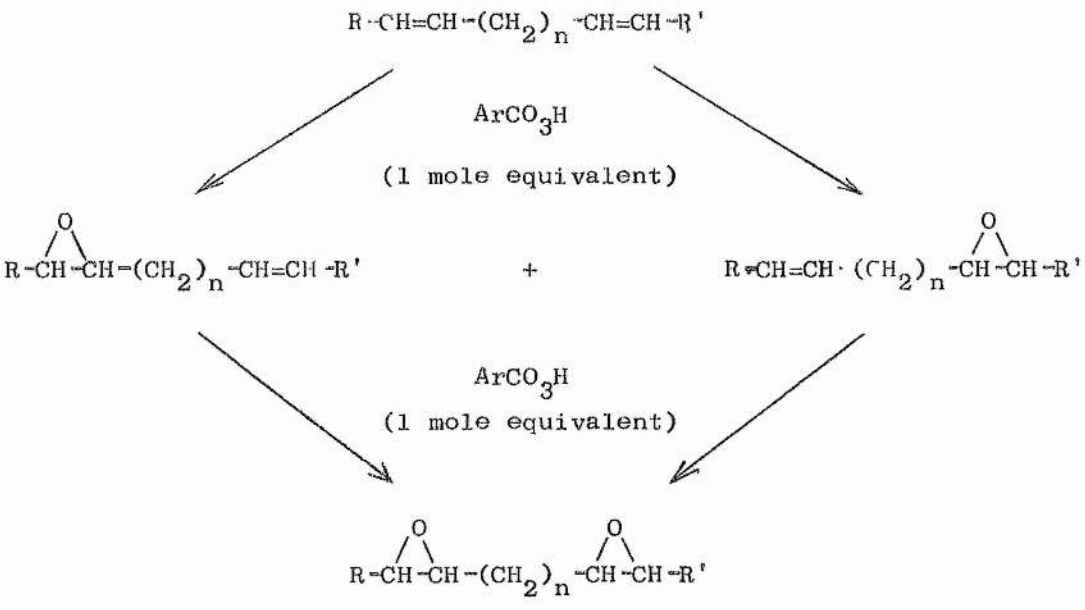
The symbol 18:2 (5c12c) indicates a C_{18} acid with two unsaturated centres, Δ5 and Δ12, both of which have cis geometry. The symbols c and t stand for cis and trans olefinic unsaturation, a indicates an acetylenic bond, and n is the number of methylene

groups which separate the two unsaturated centres. In the first two series one of the two cis double bonds is kept in a fixed position, ie. Δ12 and Δ6, whilst the position of the other double bond is varied so that n = 1 to 5. In the other two series the position of the two unsaturated centres is fixed (9,12 n = 1) and their nature varied.

The epoxidation reactions were carried out on a millimole scale using meta-chloroperbenzoic acid as epoxidising agent. This peroxy acid is a stable, crystalline solid with a relatively high molecular weight which can be accurately weighed in the small amounts required for stoichiometric control of the reaction.

Epoxidation of dienoic esters furnishes in the first instance a mixture of the two racemic and positionally isomeric monounsaturated epoxides which are then further epoxidised to a racemic mixture of four stereoisomeric diepoxides (as shown in Scheme 1).

Scheme 1 Epoxidation of Dienoic Esters



$R = -(CH_2)_pCOOCH_3$

$R' = -(CH_2)_mCH_3$

$n = 1-5$

Our preliminary experiments indicated that the isomeric monoepoxides could be isolated and separated from each other without great difficulty and that they offer more diagnostic information than the related diepoxides. To obtain the maximum yield of monoepoxides the diene esters were each treated with one mole equivalent of peracid in chloroform solution at room temperature for five hours. Iodometric titration showed that all the peracid was consumed in about four hours.

If the two double bonds in the dienoic esters each had the same reactivity as the double bond remaining in the unsaturated monoepoxide, the monoepoxy and diepoxy products would be expected in a 2:1 ratio. However, in our reactions this ratio was consistently higher (Table 1).

Table 1

Related diene ester	n	Proportions of monoepoxy to diepoxy products	
5c12c	5	72	28
6c12c	4	69	31
7c12c	3	69	31
8c12c	2	72	28
9c12c	1	80	20
9c12t	1	83	17
9t12c	1	80	20
9t12t	1	88	12
6c11c	3	70	30
6c10c	2	81	19
6c9c	1	82	18

These results, which are based on the amounts of mono- and diepoxides separated by column chromatography, show that the double bonds in the dienoic esters are more susceptible to electrophilic attack by peracid than is the double bond in the unsaturated monoepoxide. In the monoepoxide the double bond is deactivated especially when only one methylene group (n=1) removed from the epoxide group which apparently causes the deactivation. Maerker et al[81] also drew attention to the observation that in the epoxidation of methyl

linoleate the monoepoxidation step proceeds faster than the second stage leading to the diepoxide. The products of our epoxidations were separated by silica column chromatography into unreacted dienoates (10-25%), the pair of monoepoxy products (50-70%) and the diepoxy ester (10-25%). The two monoepoxides which were only partly resolved by column chromatography could be separated by preparative TLC. For convenience we classified the two isomeric unsaturated monoepoxy esters from each diene as A and B. In the A isomer the epoxy group is further removed from the carbomethoxy group than is the unsaturated centre, whereas in the B isomer the epoxy group is located in between the ester function and the unsaturated centre. So methyl cis-12,13-epoxyoctadec-cis-9-enoate is by our definition the A epoxide and methyl cis-9,10-epoxyoctadec-cis-12-enoate the B epoxide derived from the common precursor methyl linoleate (18:2 9c12c). This is abbreviated to 18:2 9c12c and 18:2 9c12c, respectively. The underlined part gives the position and configuration of the epoxide function. As all our epoxides were derived from diunsaturated C_{18} esters the code describing the chain length and number of unsaturations, 18:2 is usually ommitted:

18:2 (9c12c) ⟶ 9c12c + 9c12c ⟶ 9c12c

linoleate	A isomer monoepoxide	B isomer monoepoxide	diepoxide

It is noteworthy that monoepoxidation of our dienoic esters did not produce the A and B monoepoxides in equal amounts suggesting that the two alkene centres have a different reactivity towards the peracid as indicated by Table 2. The values are based on the amounts of pure monoepoxides obtained by preparative TLC separation of the total monoepoxide fractions. The monoepoxide mixture from methyl linoleate could not be analysed fully, because the separation proved particularly difficult. Numbers marked with an asterisk refer to GLC determinations.

With one exception the quantity of the A monoepoxide always

Table 2

monoepoxides derived from		A %	B %
18:2	5c12c	71	29
	6c12c	64	36
	6c11c	62	38
	6c10c	69	31
	6c9c	60	40
	7c12c	60	40
	8c12c	58	42
	9c12c	-	-
	9c12t	47*	53*
	9t12c	54*	46*
	9t12t	55	45

exceeds that of the corresponding B isomer. As the distance between the carbomethoxy group and the first double bond is reduced the inequality of the two double bonds becomes more pronounced. The carbomethoxy group apparently deactivates the nearer double bond so that the peroxy acid attacks preferably the more distant centre to furnish the A epoxide.

The monoepoxidation of 18:2 (9c12t) constitutes the only exception where the B monoepoxide (9c12t) is formed in greater yield than the A isomer (9c12t). To produce either of these the cis-Δ9 or the trans-Δ12 double bond has to be epoxidised. As already mentioned cis olefins are known to react with peracids faster than trans olefins, so it is not surprising that the proportion of cis-9,10-epoxide (B) exceeds that of trans-12,13-epoxide (A) and that the general pattern, A > B is reversed in this instance.

The structural features of all our unsaturated epoxy esters were determined on the basis of the chromatographic and spectroscopic characteristics which are detailed in the following sections. There it is shown how far each of these physical properties can be correlated with the structure of the epoxy

ester and to what extent these properties can be used for the structure analysis of unsaturated epoxy esters.

Thin layer chromatography (TLC)

Few inventions have caused such basic changes in chemical and biological research as that of the various chromatographic techniques. Of these, TLC has proved enormously successful, if not indispensable in the field of lipid chemistry. It seems rather astonishing today that about two decades had to pass between its first published application[82] and its wide and general recognition[83].

As normally employed, TLC like column chromatography is a form of adsorption chromatography. Mixtures can be separated into individual components depending on the rate at which they travel through a column or across a plate under the influence of an eluting or developing solvent. It is obvious that by altering the nature of the stationary phase and the solvent the migration characteristics of a particular compound can be influenced in a controlled way. By this procedure it is possible to separate not only compounds differing widely in chemical composition, but also such similar compounds as positional and geometrical isomers. This can sometimes be achieved on TLC even when it is not possible by GLC and such separation can be effected on an analytical or preparative scale for a very small expenditure of time and money.

The success of this research project depended on the separation

in pure form of the two isomeric unsaturated epoxides formed in the epoxidation of diunsaturated esters. Our hopes of accomplishing such separations were raised by a number of publications which concern the TLC characteristics of some epoxy esters. Morris et al.[84-86] reported appreciable differences between the isomeric unsaturated epoxy esters methyl vernolate and methyl coronarate. In a comprehensive study of various oxygenated methyl stearates they found that the position of the functional groups markedly influences the rate of migration on TLC resulting in a sinusoidal pattern of the R_f values[86]. The R_f minimum was observed when the substituent was attached to C(5) or C(6) and the R_f maximum was observed with compounds having the substituent at C(12) or C(13). In their study of the TLC behaviour of all the isomeric cis- and trans-epoxystearates Jacobsberg and Gunstone[78] verified not only the sinusoidal pattern resulting from the position of the epoxy function, but also a distinct separation of the isomeric cis and trans epoxides which had been earlier noted by Vioque et al.[87].

The extent by which our isomeric pairs of unsaturated epoxides are resolved on thin layers of untreated silica gel is shown in Figures 1, 2 and 3. R_f values were not evaluated since the chromatograms were obtained by prolonged or double development. Figures 1 and 2 demonstrate that the separation becomes more distinct as the number (n) of methylene groups separating the unsaturated and epoxidised centres increases. With the structural assignment based on mass spectroscopic determinations, some more observations can be rationalised:

(I) The effect caused by the position of the epoxy group is marked, but variation of the double bond position has relatively little influence on the TLC characteristics.

(II) In our series of isomeric pairs of unsaturated epoxides the mobility is always greater for the isomer in which the epoxy group is further from the carbomethoxy group.

The members of the series of epoxides derived from all the 9,12-diunsaturated esters (Figures 3 and 4) differ in three structural features: (i) the position of the epoxy group which may be 9,10- or 12,13- (ii) the geometry of the epoxy group and (iii) the type and geometry of the unsaturated centre.

All these structural features make their characteristic contribution to the observed overall TLC behaviour of the respective compound. The individual contributions are relatively small but still noticeable. Isomers, where these influences counteract each other, are either separated poorly or not at all. However, modification of the silica by impregnation with $AgNO_3$ allows satisfactory separation in such instances.

(i) Observation (II) concerning the position of the epoxy group is confirmed: 12,13-epoxides are more mobile on silica than their 9,10-isomers with similar unsaturation.

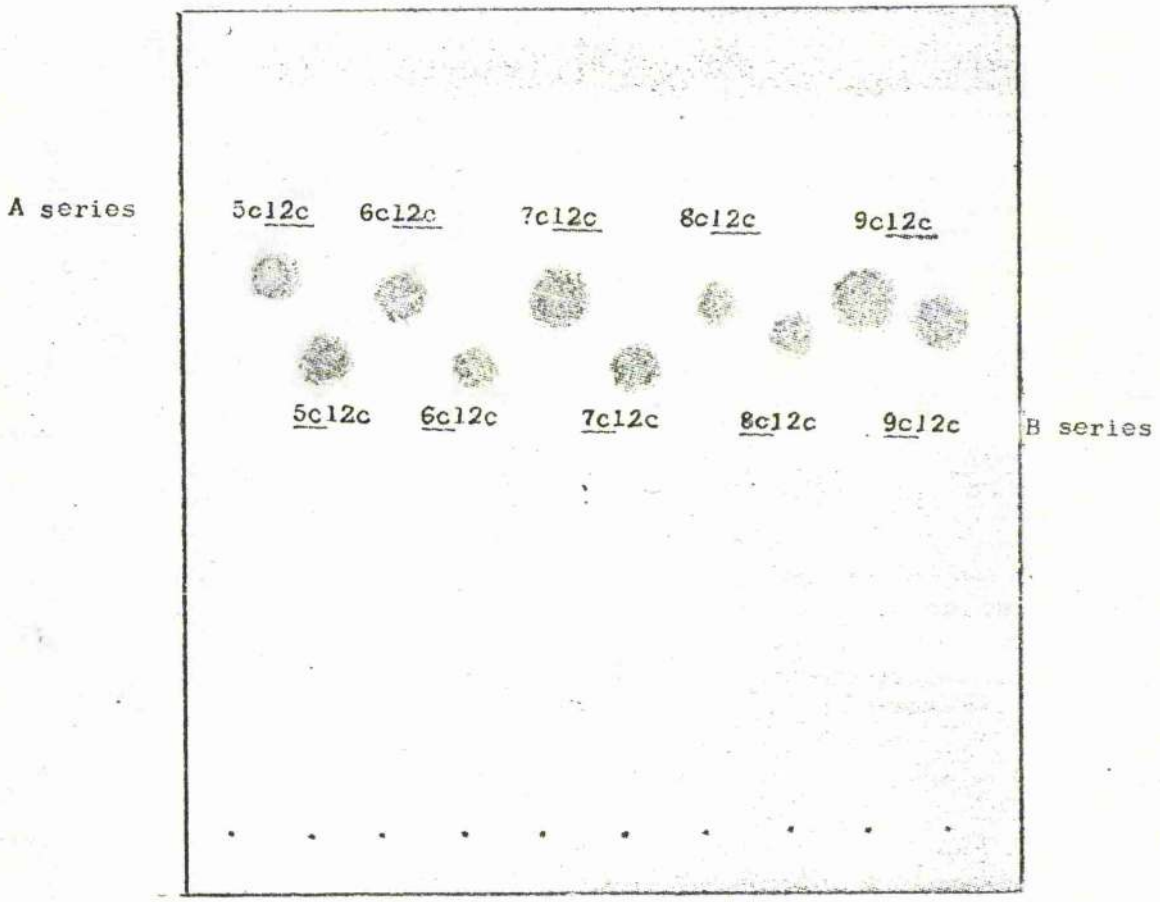
(ii) Cis geometry of the epoxy group leads to greater polarity than trans geometry. This is noticeable on normal silica (Figure 3) but is even more evident (Figure 4) when the separation is carried out on silica impregnated with $AgNO_3$ as discussed below.

(iii) For an unsaturated epoxide with defined position and geometry of the epoxy group mobility is in the order cis > trans $\cong$ acetylenic, and this effect is more significant when the unsaturation is $\Delta 9$ rather than $\Delta 12$.

On silica plates impregnated with $AgNO_3$ epoxy cis and trans alkenoates can clearly be distinguished. Silver ions are known to form π-complexes more strongly with cis than with trans olefins[88,89] and so it is possible to separate the two monoepoxides of methyl 18:2 (9t12c) which are not separable on normal silica plates. This is shown in Figure 4. Also seen is the effect of the geometry of the epoxy group which is somewhat obscured on Figure 3 by the other influencing factors [(i) and (iii)]. The trans epoxides are clearly less polar than the comparable cis epoxides.

Figure 1

TLC of monoepoxy derivatives of 18:2 methyl esters

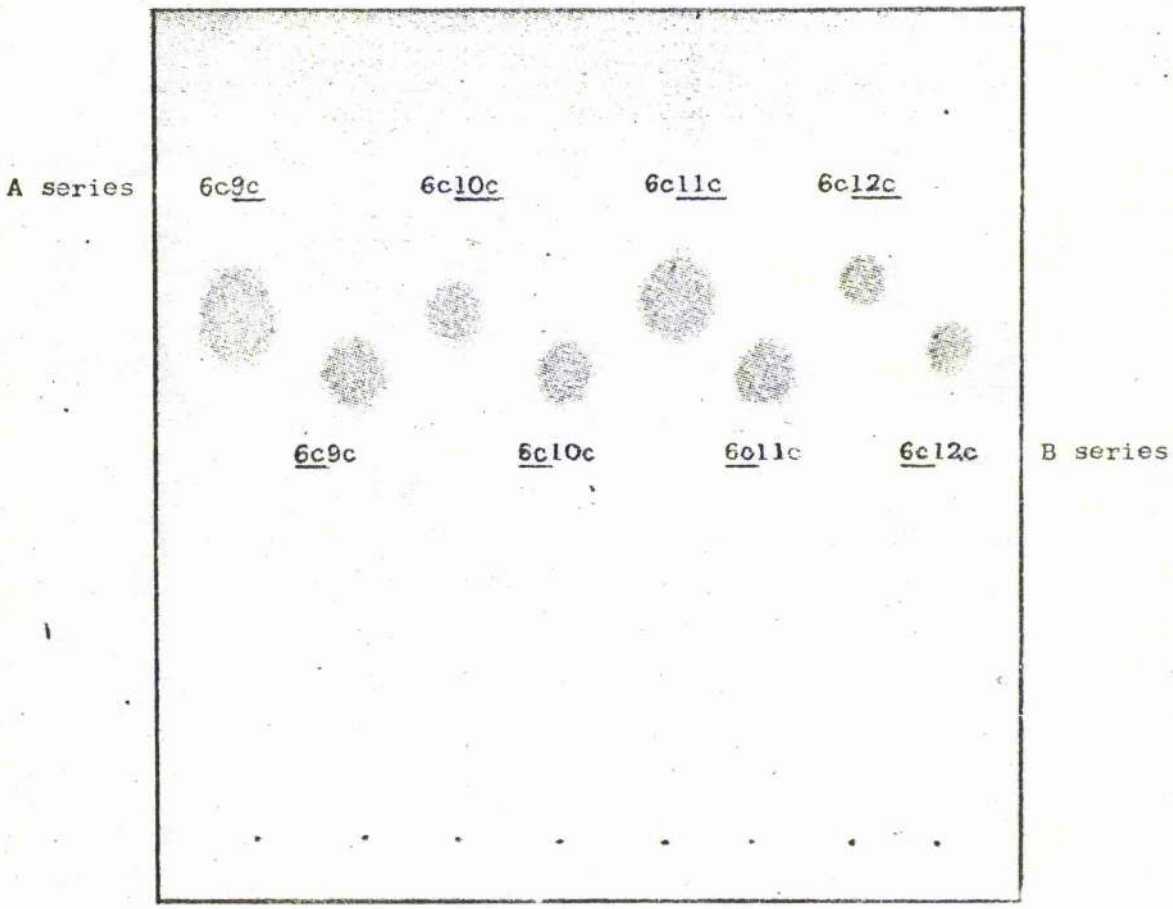


Adsorbent: Silica gel G

Solvent: PE30

Figure 2

TLC of monoepoxy derivatives of 18:2 methyl esters

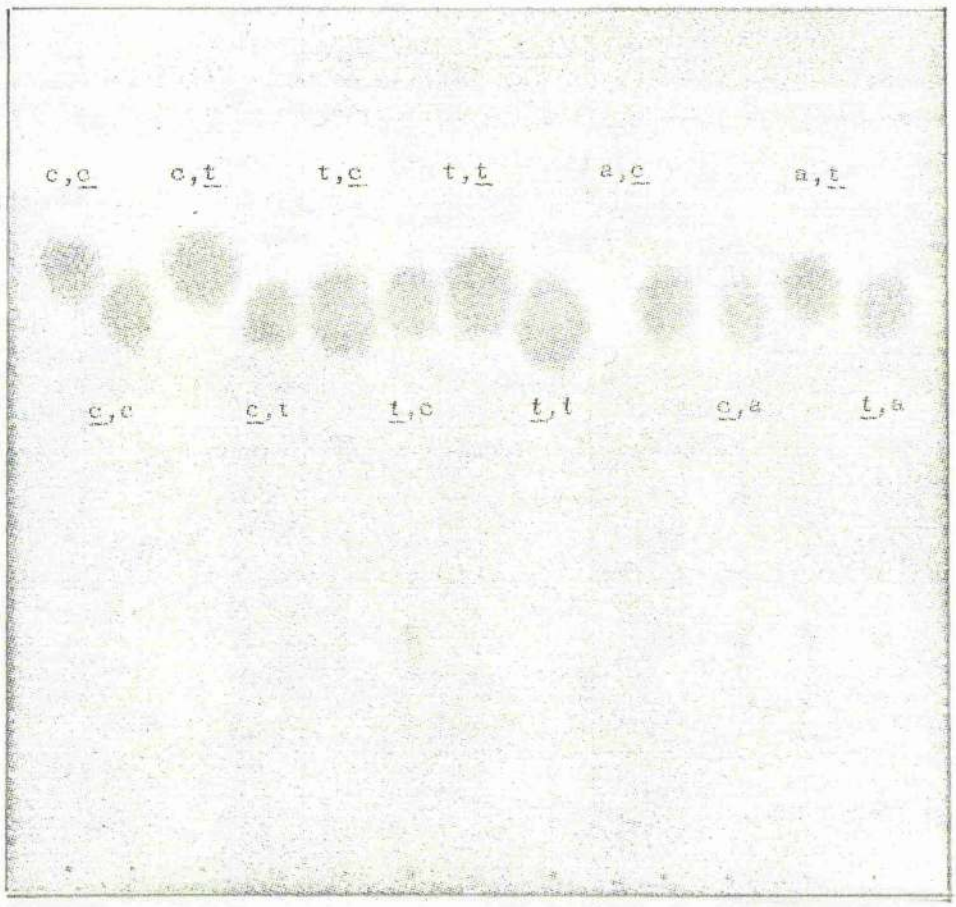


Adsorbent: Silica gel G

Solvent: PE30

Figure 3

TLC of monoepoxy derivatives of 9,12-diunsaturated C_{18} methyl esters



(1st letter = 9,10 position, 2nd letter = 12,13 position, epoxide underlined)

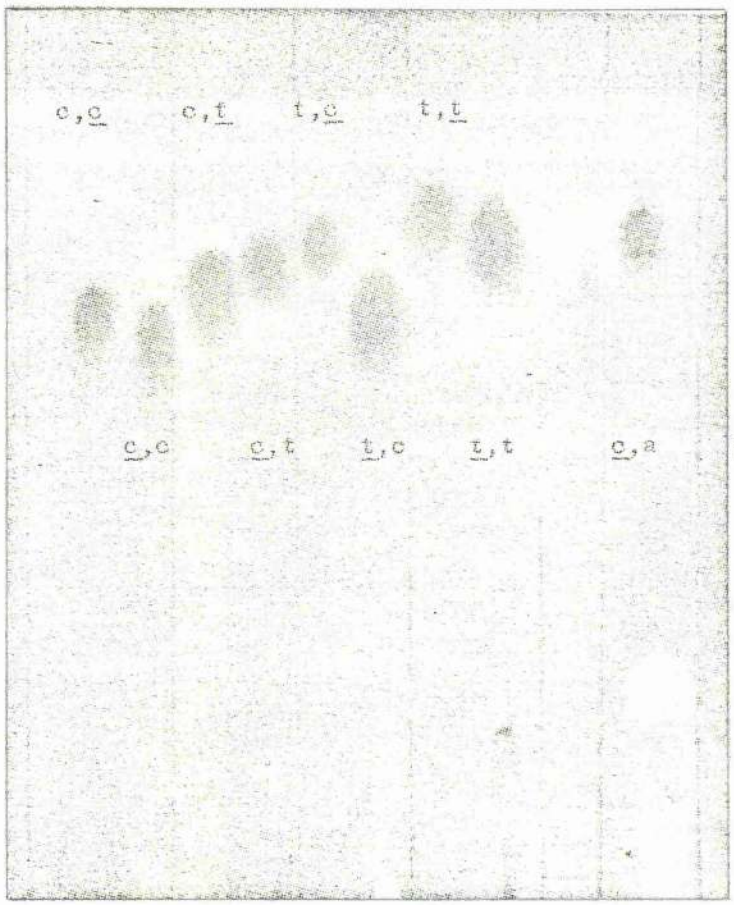
Adsorbent: Silica gel G

Solvent: PE30

Figure 4

TLC of monoepoxy derivatives of 9,12-diunsaturated C_{18} methyl esters

A series



B series

(1st letter = 9,10 position, 2nd letter = 12,13 position, epoxide underlined)

Adsorbent: 10% $AgNO_3$ in Silica gel G

Solvents for double development: (i) Toluene; (ii) PE30

Gas-liquid chromatography (GLC)

One of the first applications of GLC was the analysis of some short chain acids by James and Martin in 1952[90]. Since then GLC has established itself as the most widely used technique in lipid chemistry allowing the qualitative identification as well as quantitative analysis of a variety of compounds. Suitable stationary phases and supporting materials have been developed for a wide range of analytical problems. Improvements in instrumentation also have led to a widening of the scope of GLC. Combined with mass spectrometry, GLC has gained still greater importance in the more recent past.

As its analytical power has gradually become evident, attempts have been made to correlate GLC data with structural features of the compound chromatographed. Two groups[91,92] found independently that a logarithmic relationship exists between the retention time and the chain length of methyl esters of saturated straight-chain monocarboxylic acids: the logarithm of the retention time of such an ester is a linear function of the number of carbon atoms in the corresponding acid. On this basis an equivalent chain length (ECL) can be calculated from the logarithmic retention time of any substance chromatographed under operational conditions identical with those used for the saturated reference esters.

For a given stationary phase and within a limited temperature range each compound has a characteristic ECL value which indicates its GLC behaviour more directly than the retention time itself. Thus the ECL concept has greatly facilitated comparison of GLC data obtained by different research groups. The increasing availability of ECL data has already proved to be particularly useful in the gas chromatographic screening of lipid extracts from numerous natural sources. In view of the importance of unsaturated epoxy esters and the scarcity of reports of their GLC characteristics (compare Table 3) we examined our series of twenty-six unsaturated

epoxy esters on packed polar (DEGS) and nonpolar (ApL) columns. The results are given in Table 4. Tables 5 and 6 show the GLC characteristics of the bis-TMS ether and of the mixed vicinal methyl TMS diether derivatives which were produced and used for mass spectrometric studies. These derivatives were obtained from the various epoxy esters by hydrolysis or methanolysis of the epoxy group and subsequent silylation of free hydroxy groups. The course and stereochemistry of these reactions are described in some detail in the section on mass spectrometry (pages 46 and 47).

Attempts to rationalise ECL values of various fatty acid derivatives by correlating them with the structural features of these derivatives were made by Ackman[94] who pointed out that functional groups in fatty acid derivatives give rise to characteristic ECL changes when compared with the saturated straight chain homologue. The magnitude of such a change depends mainly on the nature of the functional group and to a much lesser extent on

Table 3 Published ECL values of unsaturated C_{18} epoxy esters

	ApL	DEGS
methyl vernolate (9c12c)	19.1[93]	24.6[93]
	19.2[40,92]	
	19.1[0]	24.2[0]
methyl coronarate (9c12c)	19.0[29]	
	19.0[13]	
	19.1[0]	
methyl cis-9,10-epoxyoctadec-12-ynoate (9c12a)	19.1[93]	26.0[93]
	19.0[29]	
	19.2[0]	25.7[0]

0 = our results

its position along the carbon chain. Ackman defined the contribution which a functional group makes to the ECL as the fractional chain length (FCL). ECL predictions on the basis of

FCL values have been successfully made for various unsaturated fatty acids[95,96]. The treatment rests on the assumption that all the functional groups present in a compound make their characteristic contributions (FCL) towards the overall retention behaviour of the compound (ECL) in a simple additive manner. We have derived ECL values for our unsaturated epoxides on the basis of previously collected ECL data of the appropriate monounsaturated and monoepoxy C_{18} esters[79,96,97]. These calculated ECL values are given in Tables 7 and 8.

In most cases the ECL values given in Tables 4-6 are based on a single determination. For several isomers up to five repeated determinations have been carried out and these showed that the maximum variation from an averaged ECL is about 0.1 when ApL and about 0.2 when DEGS was used as stationary phase. Each of the unsaturated epoxy esters except one appeared as a single peak on the chromatogram. The exception was methyl cis-5,6-epoxyoctadec-cis-12-enoate which gave several peaks on both columns. It is concluded that this epoxide is at least partially decomposed. This observation, as well as the ECL values of the decomposition products, parallels the findings of Jacobsberg and Gunstone[78] who also reported decomposition for the methyl 5,6-epoxyoctadecanoates: the cis isomer showed partial decomposition on the DEGS column only, the trans isomer on both columns.

The number of theoretical plates[98] was about 1400 for our ApL and about 1700 for our DEGS columns. At these column efficiencies compounds had to differ by at least 0.2 ECL units before any separation of peaks could be observed. For complete separation an ECL difference of 0.4 to 0.6 units was required. As Table 4, for the unsaturated ester, shows, only the cis and trans epoxide pairs derived from methyl 18:2 (9c12t) and methyl 18:2 (9t12c) meet this requirement and show some degree of separation. On DEGS the separation of this pair is almost complete whereas on ApL the isomeric

pairs are only partly resolved. On both stationary phases the trans epoxides have smaller ECL than the cis epoxides irrespective of the nature of the accompanying unsaturation. This is in agreement with the findings for saturated cis and trans epoxystearates[78]. Emken[99] using EGSS-X columns also achieved partial separation of the two unsaturated epoxy esters of 18:2 (9c12t) whereas the two monoepoxides derived from 18:2 (9t12t) appeared as one peak as they did in our studies.

On polar phases like DEGS acetylenic epoxides are characterised by considerably greater ECL values than the olefinic isomers. Analogous ECL differences between acetylenic and olefinic diethers are exhibited in Tables 5 and 6. The stereochemistry of the two vicinal ether groups of the mixed acetylenic methyl TMS diethers is reflected in the different ECL values of the threo and erythro isomers: partial separation on ApL and complete separation on DEGS is obtained for the acetylenic threo and erythro methyl TMS diethers. Small differences are also observed between olefinic threo and erythro diethers: in the case of bis-TMS ethers the erythro isomer has the greater ECL, whereas amongst the mixed methyl TMS diethers it is the threo isomer which has the larger retention time.

On DEGS, the series of vicinal bis-TMS ethers where the position of the ether groups or of the double bond is varied (n = 1 to 5) show a noteworthy alternation of ECL which allows the separation of certain positional isomers. The alternation pattern is the same for A and B isomers with the A isomer having the slightly greater ECL. In the 6,x (x = 9 to 12) series the alternation is interrupted between the bis-TMS derivatives of (6c10c) and (6c11c). A similar break in alternation has been observed for the melting points of octadecadiynoic acids[79].

Tables 7 and 8 show the comparison between observed and calculated ECL values of our unsaturated epoxy esters. For ApL the observed and calculated ECL are in good agreement whereas for DEGS their difference is significant and consistently

Table 4 ECL values of monounsaturated monoepoxy derivatives of methyl octadecanoate

A refers to the isomer in which the unsaturated centre is located between the carbomethoxy and the ether group

B refers to the isomer in which the ether group is located between the carbomethoxy group and the unsaturated centre.

unsaturations in the parent diunsaturated ester	ApL		DEGS	
	A	B	A	B
5c12c	19.0	18.7 * 19.0	24.0	21.4 * 25.3
6c12c	19.0	19.1	24.2	24.2
7c12c	18.8	18.9	24.0	24.1
8c12c	19.0	19.1	24.1	24.2
9c12c	19.1	19.1	24.2	24.2
9c12c	19.1	19.1	24.2	24.2
9c12t	18.9^{t}	19.3	23.9^{t}	24.3
9t12c	19.2	19.0^{t}	24.3	23.9^{t}
9t12t	18.9^{t}	19.0^{t}	23.8^{t}	23.8^{t}
9a12c	19.2		25.8	
9a12t	18.9^{t}		25.4^{t}	
9c12a		19.2		25.7
9t12a		19.1^{t}		25.4^{t}
6c9c	18.9	18.9	24.1	23.9
6c10c	19.0	18.9	24.2	23.9
6c11c	18.8	18.8	24.1	24.1
6c12c	19.0	19.1	24.2	24.2

* partial decomposition

t trans epoxide (all other values relate to cis epoxides)

Table 5 ECL values of monounsaturated vicinal bis-TMS ether derivatives of methyl octadecanoate

For explanation of A and B see Table 4

	ApL		DEGS	
	A	B	A	B
5c12c	19.9	19.7	19.7	19.6
6c12c	19.8	19.5	20.6	20.3
7c12c	19.8	19.6	19.9	19.8
8c12c	19.6	19.7	20.8	20.7
9c12c	19.7	19.6	19.7	19.7
9c12c	19.7	19.6	19.7	19.7
9c12t	19.8[e]	19.9	19.9[e]	19.9
9t12c	19.9	19.7[e]	20.0	19.8[e]
9t12t	20.2[e]	19.9[e]	20.2[e]	19.9[e]
9a12c	20.1		21.3	
9a12t	20.0[e]		21.3[e]	
9c12a		20.0		21.2
9t12a		20.0[e]		21.2[e]
6c9c	19.4	19.5	20.3	20.2
6c10c	19.6	19.7	19.7	19.6
6c11c	19.4	19.4	19.8	19.7
6c12c	19.8	19.5	20.6	20.3

e erythro bis-TMS ether (all other values relate to threo bis-TMS ethers)

Table 6 ECL values of monounsaturated vicinal methyl TMS diether derivatives of methyl octadecanoate

For explanation of A and B see Table 4

	ApL		DEGS	
	A	B	A	B
5c12c	19.7	19.9	21.6	21.4
6c12c	19.5	19.5	21.6	21.5
7c12c	18.8	18.9	21.7	21.6
8c12c	19.6	19.5	21.6	21.4
9c12c	19.6	19.6	21.6	21.6
9c12c	19.6	19.6	21.6	21.6
9c12t	19.6[e]	19.8	21.5[e]	21.6
9t12c	19.8	19.6[e]	21.8	21.6[e]
9t12t	19.7[e]	19.6[e]	21.7[e]	21.6[e]
9a12c	19.9		23.3	
9a12t	19.7[e]		22.7[e]	
9c12a		20.0		23.2
9t12a		19.8[e]		22.7[e]
6c9c	19.4	19.4	21.3	21.2
6c10c	19.6	19.5	21.4	21.3
6c11c	19.6	19.6	21.5	21.3
6c12c	19.5	19.5	21.6	21.5

e erythro methyl TMS ether (all other values relate to threo methyl TMS diethers)

Table 7 Observed and calculated ECL values of monounsaturated monoepoxy derivatives of methyl octadecanoate on ApL

	A			B		
	obs	calc	obs-calc	obs	calc	obs-calc
5c12c	19.0	19.0	0		19.0	
6c12c	19.0	19.0	0	19.1	19.0	+0.1
7c12c	18.8	19.0	-0.2	18.9	19.0	-0.1
8c12c	19.0	19.0	0	19.1	19.0	+0.1
9c12c	19.1	19.0	+0.1	19.1	19.1	0
9c12c	19.1	19.0	+0.1	19.1	19.1	0
9c12t	18.9	18.9	0	19.3	19.1	+0.2
9t12c	19.2	19.1	+0.1	19.0	18.9	+0.1
9t12t	18.9	19.0	-0.1	19.0	19.0	0
9a12c	19.2	19.2	0			
9a12t	18.9	19.0	-0.1			
9c12a				19.2	19.3	-0.1
9t12a				19.1	19.2	-0.1
6c9c	18.9	19.0	-0.1	18.9	19.0	-0.1
6c10c	19.0	19.0	0	18.9	19.0	-0.1
6c11c	18.8	18.9	-0.1	18.8	19.0	-0.2
6c12c	19.0	19.0	0	19.1	19.0	+0.1

Calculated ECL values are obtained as in the following example:

FCL (cis Δ5) = ECL (18:1 5c) - ECL (18:0) = -0.34

FCL (cis-12,13-epoxy) = ECL (cis-12,13-epoxy 18:0) - ECL (18:0) = 1.35

∴ calculated ECL (5c12c) = ECL (18:0) + FCL (cis Δ5)
+ FCL (cis-12,13-epoxy)
= 18.0 + (-0.34) + 1.35 = 19.01

Table 8 Observed and calculated ECL values of monounsaturated monoepoxy derivatives of methyl octadecanoate on DEGS

For explanation of A and B see Table 4

	obs	calc	obs-calc	obs	calc	obs-calc
5c12c	24.0	24.5	-0.5		24.4	
6c12c	24.2	24.6	-0.4	24.2	24.6	-0.4
7c12c	24.0	24.6	-0.6	24.1	24.7	-0.6
8c12c	24.1	24.6	-0.5	24.2	24.6	-0.4
9c12c	24.2	24.5	-0.3	24.2	24.5	-0.3
9c12c	24.2	24.5	-0.3	24.2	24.5	-0.3
9c12t	23.9	24.2	-0.3	24.3	24.4	-0.1
9t12c	24.3	24.6	-0.3	23.9	24.4	-0.5
9t12t	23.8	24.1	-0.3	23.8	24.3	-0.5
9a12c	25.8	26.0	-0.2			
9a12t	25.4	25.7	-0.3			
9c12a				25.7	26.0	-0.3
9t12a				25.4	25.9	-0.5
6c9c	24.1	24.4	-0.3	23.9	24.4	-0.5
6c10c	24.2	24.6	-0.4	23.9	24.5	-0.6
6c11c	24.1	24.6	-0.5	24.1	24.5	-0.4
6c12c	24.2	24.6	-0.4	24.2	24.6	-0.4

negative. Bearing in mind the limited reproducibility of ECL determinations on DEGS columns[100] and the fact that this comparison is based on results obtained by three different research workers using different DEGS columns and operating conditions, the consistent discrepancy between observed and calculated ECL values (Δ = -0.4 to -0.5 ECL) does not actually contradict Ackman's FCL hypothesis.

Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR spectroscopy is based on the fact that certain nuclei including, in particular, the proton have a magnetic moment which can be affected by a magnetic field. When fields oscillating in the radio frequency range are applied, transitions can be induced between different energy states of the nuclear magnetic moment. The absorption of small amounts of radio frequency energy connected with such transitions can be detected instrumentally. The various protons of an organic compound give rise to a number of signals depending on their chemical environment producing a characteristic spectrum. This is due to the shielding of the protons by the surrounding electron clouds. Coupling of nuclear spins via valence electrons is a second differentiating factor. Thus by virtue of chemical shift and spin coupling parameters both of which are characteristic for specific groups of compounds, NMR spectroscopy has become a widely used technique for the structural identification of organic molecules.

Unfortunately structural assignment is often complicated when both parameters are of comparable magnitude. However, advantage

can be taken of their intrinsic difference: whereas the spin coupling constant is independent of the magnetic field, chemical shifts are proportional to the applied field and working at the highest possible field strength gives optimum resolution of the signals. The introduction of high frequency spectrometers has therefore improved NMR spectroscopy greatly.

Empirical correlations of chemical shifts, coupling constants and band shapes with certain structures play a dominant role in theinterpretion of NMR spectra since theoretical calculation of these spectroscopic characteristics is still a very complex problem. No systematic study of unsaturated epoxy fatty acids has been carried out so far, although one of the first applications of NMR spectroscopy in the field of lipid chemistry was concerned with the detection of epoxy groups. Hopkins et al.[101,102] examined the 40 MHz spectra of cis-9,10-epoxystearic and cis-12,13-epoxyoleic acid. From the NMR spectra they inferred the presence of these acids in certain glyceride oils and confirmed this conclusion by chemical analysis. Ferrari et al.[77] reported the 60 MHz spectra of both monoepoxides derived from methyl linoleate and found no difference between the two. A few other unsaturated epoxy fatty acids examined by NMR are not representative of this class of compounds, because their epoxide protons or olefinic protons are strongly influenced by additional functional groups[16,27]. So we thought it worthwhile to carry out a systematic NMR study of unsaturated epoxy esters extending the work by Jacobsberg and Gunstone[78] on the methyl epoxyoctadecanoates. The spectra of several diepoxides are included in our studies.

All our data are derived from 220 MHz spectra which were obtained at room temperature, using approximately 10% solutions of epoxide in carbon tetrachloride. Chemical shifts are given in ppm downfield from internal tetramethylsilane (δ=0), whilst spin coupling constants are given in Hz.

The spectra of our monoenoic epoxy esters exhibit eight distinguishable major signals which can be attributed to protons of the following groups in order of increasing chemical shifts:

1) C-methyl group
2) methylene groups
3) methylene group β to $-CO_2Me$
4) methylene groups α to the double bond
5) methylene group α to $-CO_2Me$
6) epoxy group
7) ester methyl group
8) double bond

This pattern is changed somewhat when the double bond is exchanged for an acetylenic bond or a second epoxy group. Differences in the NMR spectra of esters belonging to the same class are caused by the deshielding of the various protons by the unsaturated centre, the carbomethoxy and the epoxy groups. Even when these groups are up to four methylene groups apart from a proton, slight deshielding influences can be recognised. Tables 9a to e and Table 10 show the shift values of all the major signals and these are discussed below.

1) C-methyl Group

The three protons of the terminal CH_3 group give a distorted triplet (J=7) at 0.88 to 0.92δ. A deshielding influence is noticeable for various groups occupying the 12,13-position. With a cis-epoxy group also present in 9,10-position the order of this deshielding effect is:

12,13 functional group	a	epoxy	cis	trans	saturated
δ	0.92	0.91	0.90	0.89	0.88[103]

The effect falls off rapidly when more than four CH_2 groups separate the methyl group from either the unsaturated centre or the epoxide group. It is expected that this effect would be more significant with groups closer to the terminal methyl group, but there were no examples of this type among our compounds.

2) Chain Methylene Groups

The protons of methylene groups only weakly deshielded produce signals of complex shapes in the region of 1.28 to 1.48δ and constitute the most intense signals of the spectrum. Because some methylene groups are slightly more deshielded than others, two peaks (sometimes three) are observed which give some indication of the relative positions of the strongly deshielding double bond, ester and epoxy groups. As the position of these groups differ in the various isomers, the intensities of the low and high field parts of the band are found to vary too. The integrals of the signals suggest, on a qualitative basis, that the signal at lower field ($\sim$ 1.48δ) is caused by CH_2 groups which are α to an epoxy group or β to an unsaturated centre or γ to the ester function.

3) Methylene Group β to $-CO_2Me$

The signals for the protons of this group always separate from the polymethylene band and appear as a distinct signal in the range from 1.57 to 1.74δ. Superimposed on the strong deshielding effect exerted by the ester function, weaker deshielding influences are noticeable for the epoxy group and the double bond. This influence is relatively prominent when either of the two groups is located between C(5) and C(6), but decreases rapidly as the distance between C(3) and this deshielding group increases. The epoxy group appears to have the stronger effect of the two. Generally the signal for this CH_2 group appears as the expected quintet (J=7). In the spectra of some diepoxides this signal is somewhat obscured by a multiplet due to the CH_2 group deshielded by two neighbouring epoxy groups.

4) Methylene Groups Adjacent to Unsaturated Centres

All the unsaturated epoxides examined (n≥1) contain two CH_2 groups next to the unsaturated centre. Since these two CH_2 groups are not equivalent, they give rise to rather complex signals. For

olefinic epoxides these signals appear in the region from 1.98 up to 2.26δ where they are overshadowed by the strong triplet due to the C(2) protons. Unpublished results by Frost[103] confirm our observation that in certain olefinic epoxy esters the signals of allylic protons overlap with that of the protons attached to C(2).

When n = 4 or 5 both CH_2 groups α to the double bond are quasi equivalent, and only one signal is observed at 1.98-2.02δ. When n becomes smaller the internal CH_2 group comes under the additional deshielding influence of the epoxy group resulting in the broadening and eventually the splitting of the signal into two parts. The spectrum of methyl cis-6,7-epoxy 18:1(10c) for example (n=2) shows two fully resolved quartets centred at 2.01δ (J=6.5) and 2.15δ (J=7) each representing two protons. For the other olefinic epoxides where n = 2 or 1 the signal for the internal allylic CH_2 group appears to be shifted even further downfield, so that it overlaps with the triplet caused by the C(2) protons and is sometimes completely obscured by this always very prominent triplet. Nevertheless, integration confirms that four protons are contributing to the overlapping signals of this region, two of them being allylic. The less deshielded external CH_2 group is always seen as a multiplet of four or more lines at 1.98 to 2.07δ.

In acetylenic epoxides with n=1 the external propargylic protons produce a strongly distorted triplet (J=7) around 2.11δ. The protons of the internal methylene groups being strongly deshielded by both the triple bond and the epoxy group are considered to give rise to a doublet (J=17) which is centred at 2.51δ and exhibits a complex fine structure.

5) Methylene Groups Adjacent to $-CO_2Me$

The protons of the methylene groups α to the ester group produce a triplet (J=7.5) with the centre around 2.20 to 2.31δ. The shift values vary slightly depending on the position of the epoxy group. The variations parallel those of the shifts associated

with the C(3) protons. The deshielding effects caused by the double bond in variable positions are comparably weak. In the spectrum of the mono- or diepoxide with an epoxy group in the 5,6-position, a multiplet of six lines is observed which can be analysed into two triplets (J=7.5) separated by 4Hz. A similar nonequivalence of the two C(2) protons is found in the 220 MHz spectra of the methyl 5,6-epoxyoctadecanoates for which Frost[103] reports values of 13.5 and 1 Hz for the cis and trans epoxides respectively.

6) Epoxide Protons

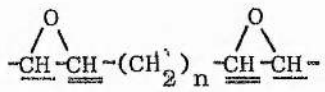
The signal for the two epoxide protons of the unsaturated epoxy esters examined is a poorly resolved multiplet. Its shift depends in the first instance on the geometry of the epoxy groups and to a lesser extent on its position in the molecule.

For cis epoxides (n=1 to 5) signals are found between 2.69 and 2.75δ, whereas for the trans epoxides (n=1) the signal appears around 2.50δ). These values are in close agreement with the results obtained for the corresponding saturated cis respectively trans epoxides[78]. The slight variations of these shifts indicate a weak deshielding effect caused by the double bond. In the 5,6- and 6,7-epoxides another influence seems to come from the carbomethoxy groups. Influenced by both the double bond and the ester group the epoxide protons of methyl cis-6,7-epoxy 18:1 (9c) exhibits the greatest shift (2.75δ) amongst our isomeric monoenoic epoxy esters. The geometry of the double bond does not make any difference to the shift of epoxy protons.

When the double bond is replaced by an acetylenic bond and n=1, not only the chemical shift but also the band shape is clearly changed. In the spectra of the two acetylenic cis epoxides two clearly separated signals emerge at 2.77δ and 2.92 or 2.93δ. The signal at lower field is a quintet (J=4) and represents the epoxide proton nearer to the acetylenic bond. The other signal, though

poorly resolved, also represents one proton. For the two acetylenic trans epoxides only one signal centred at 2.62 or 2.63δ is observed. It is a complex multiplet which overlaps partly with the signal resulting from the inner propargylic protons.

In the diepoxides the two epoxy groups influence each other, and so the shift varies slightly from 2.72δ for n=5 to 2.77δ for n=2. With n=1, the two protons of each epoxy group differ so much



that they give rise to two separate signals. Two partly resolved complex multiplets with centres around either 2.79 and 2.91δ or 2.52 and 2.65δ are seen in the spectra of cis,cis and trans,trans diepoxides respectively. The low field part of the signal represents the pair of inner protons [C(10) and C(12)], whereas the other part stands for the less deshielded pair of outer protons [C(9) and C(13)]. In the mixed cis,trans diepoxides four signals are observed each representing one proton. The signals appear at 2.55 and 2.66δ (trans-epoxy group) and at around 2.78 and 2.92δ (cis-epoxy group).

7) Ester Methyl Group

All the spectra show a sharp singlet at 3.58 to 3.61δ which is due to the three protons of the carbomethoxy group.

8) Olefinic Protons

The olefinic protons are the most highly deshielded in the spectra of the olefinic epoxy esters. They give rise to a rather complex multiplet which is most clearly resolved when n=1. A more or less symmetrical signal of nine lines is then obtained. The separation of these lines varies between 6 and 7.5 Hz. No consistent differences concerning pattern and shift of the signal are observed between cis- and trans-olefinic protons.

Small variations in the chemical shifts of the olefinic protons can be related to the relative position of the epoxide

and the carbomethoxy groups. So the compound with the double bond furthest removed from both these deshielding groups, ie. methyl cis-5,6-epoxy 18:1 (12c), produces the signal with the smallest shift (5.27δ), whilst the signal of methyl cis-9,10-epoxy 18:1 (6c), where both groups are nearest, show the largest shift (5.42δ).

The results discussed on the preceding pages show that NMR spectroscopy can provide much information about the structural features of various monounsaturated epoxy esters and diepoxy esters. Not only the types of functional groups are revealed (carbomethoxy groups, epoxide, alkene or alkyne), but to some extent also their relative positions.

Diagnostic differences are caused by the deshielding influence of the three functional groups. Though strongest for protons on adjacent carbon atoms, deshielding influences are noticeable on protons which are separated by up to four methylene groups from the deshielding centre.

The chemical shifts for protons of cis and trans epoxy groups differ clearly by 0.23 ppm. So NMR spectroscopy, even if it does not provide a complete structural analysis of unsaturated epoxy esters, shows unambiguously the geometry of the epoxy groups which was not revealed by our mass spectrometric examinations.

Table 9a Principal NMR features of methyl epoxyoctadec-noates

$\underline{CH_3}$-$(CH_2)_n$- (C-methyl group)

	monoepoxide A	monoepoxide B	diepoxide
5c12c	0.91	0.89	0.90
6c12c	0.91	0.89	0.91
7c12c	0.91	0.89	0.91
8c12c	0.91	0.89	0.91
9c12c	0.91	0.90	0.91
6c9c	0.88	0.88	0.88
6c10c	0.89	0.88	0.89
6c11c	0.90	0.89	0.89
6c12c	0.91	0.89	0.91
9c12c	0.91	0.90	0.91
9c12t	0.89	0.89	0.91
9t12c	0.91	0.89	0.91
9t12t	0.90	0.89	0.91

-$\underline{CH_2}$-CH_2-CO_2Me (methylene group β to -CO_2Me)

5c12c	1.64	1.73	1.74
6c12c	1.60	1.67	1.66
7c12c	1.58	1.62	1.60*
8c12c	1.57	1.60	1.60**
9c12c	1.58	1.59	1.60**
6c9c	1.61	1.67	1.64**
6c10c	1.59	1.67	1.66*
6c11c	1.61	1.67	1.60*
6c12c	1.60	1.67	1.66
9c12c	1.58	1.59	1.60**
9c12t	1.57	1.58	1.58**
9t12c	1.57	1.58	1.58**
9t12t	1.57	1.57	1.57**

* partial overlap with proton signals of CH_2-groups deshielded by epoxy groups

** overlap with proton signals of CH_2-groups deshielded by epoxy groups

For J values see text

Table 9b Principal NMR features of methyl epoxyoctadecenoates

$-\underline{CH_2}-CO_2Me$ (methylene group α to $-CO_2Me$)

	monoepoxide A	monoepoxide B	diepoxide
5c12c	2.22	2.29	2.31
6c12c	2.22	2.26	2.26
7c12c	2.21	2.23	2.23
8c12c	2.21	2.22	2.22
9c12c	2.21	2.22	2.21
6c9c	2.23	2.26	2.27
6c10c	2.23	2.26	2.26
6c11c	2.22	2.26	2.26
6c12c	2.22	2.26	2.26
9c12c	2.21	2.22	2.21
9c12t	2.20	2.21	2.21
9t12c	2.20	2.21	2.21
9t12t	2.21	2.21	2.21

$-COO\underline{CH_3}$ (ester methyl group)

5c12c	3.59	3.61	3.61
6c12c	3.58	3.60	3.60
7c12c	3.58	3.59	3.59
8c12c	3.59	3.59	3.59
9c12c	3.60	3.60	3.59
6c9c	3.59	3.60	3.60
6c10c	3.59	3.60	3.60
6c11c	3.59	3.61	3.60
6c12c	3.58	3.60	3.60
9c12c	3.60	3.60	3.59
9c12t	3.58	3.58	3.58
9t12c	3.58	3.58	3.58
9t12t	3.58	3.58	3.58

For J values see text

Table 9c Principal NMR features of methyl epoxyoctadecenoates

(epoxide protons)

	monoepoxide A	monoepoxide B	diepoxide
5c12c	2.69	2.72	2.72
6c12c	2.69	2.72	2.73
7c12c	2.71	2.71	2.73
8c12c	2.73	2.73	2.76
9c12c	2.73	2.73	2.79,2.91
6c9c	2.73	2.75	2.81,2.93
6c10c	2.73	2.73	2.77
6c11c	2.71	2.73	2.75
6c12c	2.69	2.72	2.73
9c12c	2.73	2.73	2.79,2.91
9c12t	2.50	2.73	2.55,2.66, 2.78,2.91
9t12c	2.73	2.49	2.55,2.66, 2.78,2.93
9t12t	2.50	2.49	2.52,2.65

For J values see text

Table 9d Principal NMR features of methyl epoxyoctadecenoates

$-\underline{CH_2}-CH=CH-\underline{CH_2}-$ (allylic protons)

	monoepoxide A	monoepoxide B
5c12c	2.02	1.98
6c12c	2.02	2.01
7c12c	2.03	2.01 2.06
8c12c	2.02 2.14*	2.02 2.13*
9c12c	2.03** —	2.03** 2.12
6c9c	2.06** —	2.02 2.12
6c10c	2.07 2.15 2.20**	2.01 2.15
6c11c	2.06	2.01 2.05
6c12c	2.02	2.01

Table 9d continued on next page

Table 9d (cont)

	monoepoxide A	monoepoxide B
9c12c	2.03** —	2.03** 2.12
9c12t	2.00** —	2.00*
9t12c	2.00* 2.07	2.00** —
9t12t	1.98 2.11* 2.15	1.98 2.08* 2.16

* partial overlap with the signal for C(2)-protons

** extensive overlap with the signal for C(2)-protons

For J values see text

Table 9e Principal NMR features of methyl epoxyoctadecenoates

-CH=CH- (olefinic protons)

	monoepoxide A	monoepoxide B
5c12c	5.31	5.27
6c12c	5.31	5.29
7c12c	5.31	5.31
8c12c	5.33	5.34
9c12c	5.41	5.41
6c9c	5.42	5.41
6c10c	5.36	5.34
6c11c	5.33	5.32
6c12c	5.31	5.29
9c12c	5.41	5.41
9c12t	5.36	5.41
9t12c	5.41	5.36
9t12t	5.38	5.38

For J values see text

Table 10 Principal NMR features of methyl cis- and trans-epoxyoctadecynoates

	9al2c	9cl2a	9al2t	9tl2a
$\underline{CH_3}\text{-}(CH_2)_n\text{-}$	0.91	0.92	0.91	0.91
$\text{-}\underline{CH_2}\text{-}CH_2CO_2Me$	1.58	1.59	1.59	1.58
$\text{-}C\equiv C\text{-}\underline{CH_2}\text{-}(CH_2)\text{-}$	1.99 2.11	2.00 2.11	2.11	2.10
$\text{-}\underline{CH_2}\text{-}CO_2Me$	2.21	2.21	2.21	2.21
$\text{-}C\equiv C\text{-}\underline{CH_2}\text{-}\underset{\diagdown\,\diagup}{\overset{O}{CH}}\text{-}CH\text{-}$	2.47 2.55	2.48 2.56 2.63	2.47 2.55	2.48 2.56
$\underset{\diagdown\,\diagup}{\overset{O}{\underline{CH}}}\text{-}\underline{CH}\text{-}$	2.77 2.92	2.77 2.93	2.62	2.63
$\text{-}CO_2\underline{CH_3}$	3.58	3.59	3.59	3.59

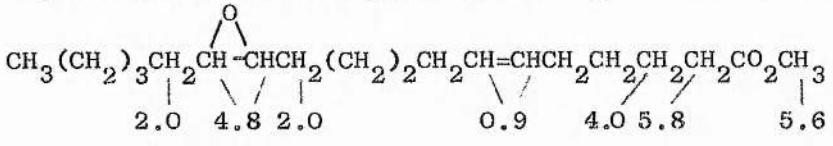
For J values see text

Swern and Wineburg[104,105] showed that much more information can be derived from the NMR spectra of long chain esters with the help of lanthanide shift reagents. These form complexes with molecules, which contain sufficiently basic groups, and deshield very strongly protons at or near the site of coordination. In unsubstituted esters, the ester group constitutes the only site of coordination, and discrete signals are observed for protons as far away as C(5). With a second complexing group in a suitable position it should be possible to make assignments for an even larger number of protons. Swern and Wineburg[105] demonstrated this with methyl 12-hydroxystearate and methyl ricinoleate.

We carried out an analogous study of the two unsaturated epoxy esters methyl cis-12,13-epoxy 18:1 (6c) and methyl cis-9,10-epoxy 18:1 (6c). Epoxy esters promised to be suitable for such examinations, since the epoxy group is known to be at least as good a ligand as the ester function. Like Swern and Wineburg we used tris-(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-

octane-4,6-dionato)eupopium (III) ($Eu(fod)_3$) as shift reagent. We recorded the 100 MHz spectrum of each epoxy ester at seven different [$Eu(fod)_3$] to [epoxy ester] ratios (r) in order to examine the relative effects of the shift reagent on the various protons. Because we had only small quantities of both the epoxy ester and the shift reagent at our disposal, we changed the ratio r by adding small quantities of epoxy ester to a constant quantity of $Eu(fod)_3$. So at large ratios ($r > 1.3$), ie. very low concentrations of epoxy ester, the signals observed were rather weak. Hence it was not possible to determine such features as multiplicity of the signal, coupling constants and integrated area under the signal, all of which would be essential pieces of information for a complete structural analysis of the epoxy ester.

The seven spectra ($r = 0.3$ to 2.6) of methyl cis-12,13-epoxy 18:1 (6c) indicate that the ester group acts as a better ligand than the epoxy groups. This is reflected in the relative increases of chemical shifts associated with protons of these groups and of protons in their close neighbourhood. Complex formation with the ester group apparently affects protons up to C(5). The signal of the epoxy protons is affected less than the carbomethoxy protons and the protons on C(2). The relative differences of shifts induced by complex formation with the ester and the epoxy group are even more pronounced when the shifts for the protons adjacent to these groups are compared. The following values (ppm) show to what extent the respective proton signals are shifted downfield from the characteristic shift values observed in the normal NMR spectrum. The given values are derived from the spectrum where the [$Eu(fod)_3$] to [epoxy ester] ratio was 1.6 At least eleven signals could be distinguished although some of them



were only partly resolved. By bringing the epoxy group and the double bond closer together we hoped to eliminate some ambiguities and to show the nonequivalence of even more protons.

The complexity of the spectra of the complexed methyl cis-9,10-epoxy 18:1 (6c) confirmed our proposition though not in a desired way. As the number of signals increased, more of them overlapped. So association of the signals with certain protons was only possible in few cases. One conclusion, which is in contrast with the results discussed above, can be drawn however: for all the seven ratios from 0.3 up to 1.9 the epoxide protons experience stronger deshielding effects than the C(2) protons or those of the carbomethoxy group.

Although the outcome of these studies was not very successful it does not contradict the potential of NMR shift reagents. If the required quantities of epoxy esters and shift reagent had been used, the spectra would have been much clearer and could have given all the information needed for a complete structural analysis.

Mass Spectrometry

Mass spectrometry holds a place of prime importance amongst the techniques used for identification of fatty acid derivatives. Its usefulness has been increasingly revealed and exploited during the last fifteen years and much useful information on a variety of fatty acid derivatives is now available.

However, the scope of mass spectrometry is somewhat reduced when unsaturated fatty acids or their ester derivatives are subjected to electron impact. As the double bond migrates under the energetic conditions in the spectrometer, its position along the

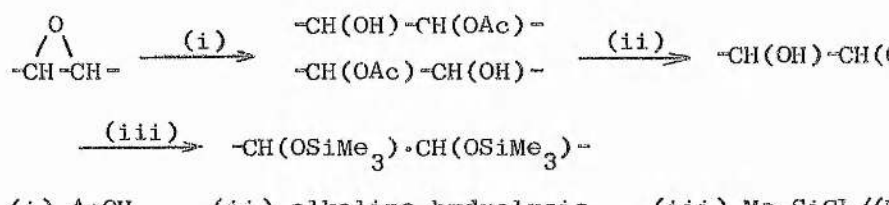
fatty acid chain cannot be directly determined. To overcome this problem the alkene derivative may be converted to a compound which has functional groups in place of the original double bond. The mass spectrum of such a derivative should then indicate the position and if possible the geometry of the double bond. Deuterisation, epoxidation, hydroxylation, methoxymercuration-demercuration and other derivatisations have been applied to this end[106-111].

Jacobsberg and Gunstone[78] examined the mass spectra of the complete series of methyl cis- and trans-epoxyoctadecanoates and showed like others before them that the double bond position in a monounsaturated ester can be determined by epoxidation followed by mass spectrometric examination. We wanted to extend this examination to a range of unsaturated monoepoxides derived from diunsaturated esters, but the spectra of these epoxides proved to be rather complex and difficult to interpret. Vacheron et al.[112] hoped to solve this problem by hydrogenating unsaturated epoxy esters and examining the spectra of the saturated epoxides, but this approach raises new problems and was therefore not attempted.

Instead of hydrogenating the double bond, modification of the epoxy group to functional groups more suitable for mass spectrometric studies could produce a more satisfactory solution. Trimethylsilyl (TMS) ethers are known to give simple mass spectra which show only a few abundant and diagnostic ions[113-115].

Epoxides are easily and quantitatively converted to the corresponding bis-TMS ethers according to the following scheme (2):

Scheme 2 Conversion of epoxides into bis-TMS ethers

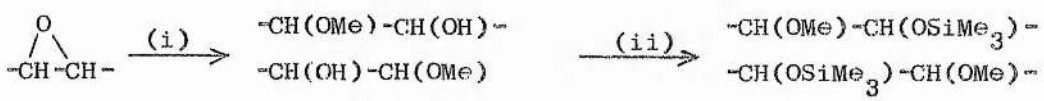


(i) AcOH (ii) alkaline hydrolysis (iii) $Me_3SiCl/(Me_3Si)_2NH$, pyridine

The opening (i) of the epoxide ring is a nucleophilic substitution which proceeds with inversion of configuration at the carbon atom where substitution occurs. Since reactions (ii) and (iii) occur without configurational change, cis epoxides are converted to threo-bis-TMS ethers whereas trans epoxides furnish erythro-bis-TMS ethers.

The same relationship, cis → threo and trans → erythro, applies also to the reaction sequence by which mixed methyl TMS diethers are produced (scheme 3):

Scheme 3 Conversion of epoxides into methyl TMS diethers



(i) $MeOH/BF_3$ (ii) $Me_3SiCl/(Me_3Si)_2NH$, pyridine

In two recent reports these derivatives were shown to be very suitable for mass spectrometry. For example, Holloway et al.[23] elucidated in this way the structure of a new epoxy acid present in plant cutins and suberins. In a detailed study of the mass spectra of unsaturated oxygenated fatty acid derivatives, methyl vernolate and methyl coronarate were examined by Kleiman and Spencer[116] after they had converted these epoxides to the corresponding methyl TMS diether derivatives.

For our mass spectrometric studies we converted each of the unsaturated epoxy esters both to the corresponding bis-TMS ethers and to the mixed methyl TMS diethers. The mass spectra were recorded using an ionisation energy of 17 eV. With a potential of 70 eV the intensity of the diagnostic ions was decreased and a variety of smaller fragment ions became more prominent thus complicating the interpretation of the spectra.

In order to compare general fragmentation patterns we determined the percentage of total ion current carried by each fragment ion and examined the results for ions with high abundance.

The percentage of total ion current is based on the sum of all the intensities from mass 51 up to the highest observable peak which is generally the molecular ion M or the fragment ion M-15. The results are given in Tables 11-14.

Tables 11a and b refer to bis-TMS ethers and Tables 12a and b refer to mixed methyl TMS diethers. Our results are divided into two groups according to the relative position of the ether groups and the unsaturated centre with respect to the carbomethoxy group. In the A group (Tables 11a and 12a) the unsaturated centre is nearer to, whilst in the B group (Tables 11b and 12b) it is further from the ester group than are the ether functions.

The headings of the various columns indicate the structure of the compounds to which the data belong. These abbreviated representations show the position and nature of the unsaturated centre and the position and configuration of the epoxide (underlined) from which the ether derivatives have been produced. Thus the column headed 5c12c refers to derivatives of cis-12,13-epoxyoctadec-cis-5-enoic acid.

The various fragments to which the data of the respective rows correspond are described in some detail later. The contributions of all the cited fragments to the total ion current are summed in the row entitled TOTAL. These values show that generally a third to a half of the total ion current is accounted for by the given selection of fragment ions. The remainder of the total ion current is carried by a multiplicity of ions generally of small abundance. Some spectra, however, exhibit a few moderately strong peaks (2-5% $\sum_{51}^{M}$). Some other frequently recurring ions include those of m/e 159, 149, 146, 142, 135, 129, 111, 109 and 103. Explanations have been offered in the literature[107,115,117] for those which are underlined, but not for the remainder.

In all the spectra only five prominent peaks ($> 5\% \sum_{51}^{M}$) could not be explained. Their occurrence is indicated by an asterisk. An accompanying footnote specifies the respective fragment.

The row designated $\%\sum_{51}^{99}$ gives the fraction of the total ion current which is carried by ions of mass 51 up to 99. It is noteworthy that the values of $\%\sum_{51}^{99}$ are generally complementary to the values given as TOTAL which comprise all the specified and rather large fragments. This may be due to variations of the ionising potential between different recordings: for low voltages primary fragmentation prevails, whilst bombardment with electrons of higher voltages induces further fragmentation which is apparent in the increased abundance of the smaller ions, ie. $\%\sum_{51}^{99}$ is relatively large. The ionisation potential was in all cases nominally 17 eV.

The values in the rows designated by $\sum$ (a) and $\sum$ (b) represent the sums of all the contributions to the ion current which can be related to the fragmentation a/a' and b/b' respectively.

Two fragmentation modes were found in the spectra of both ether derivatives:

(i) The most abundant ions are formed by cleavage of the carbon-carbon bond adjacent to an ether function (α-cleavage). Since there are two ether groups present in vicinal positions, three α-cleavages are possible giving rise to a total of six fragments:

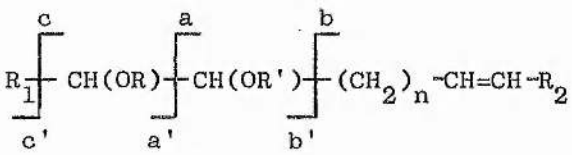
a,a' by cleavage of the bond between the two oxygenated carbon atoms (When the fragmentation of mixed methyl TMS diethers is described, the subscripts OMe and OTMS denote which of these groups is retained in the respective fragment);

b,b' by cleavage of the bond between the ether carbon and the unsaturated section of the molecule;

c,c' by cleavage of the bond between the ether carbon and the saturated section of the molecule.

The unprimed fragments contain the unsaturated section, whereas the primed fragments contain the saturated section. The double

bond has been selected as the reference point rather than the ester function, since this gives a more consistent set of results.



(ii) A second set of ions (I to VI) are possibly derived from radical intermediates produced by migration of a TMS group to the carbomethoxy groups[115]. These ions are generally less abundant than those described under (i). Scheme 4 illustrates a likely course for the formation of these fragments (I to VI).

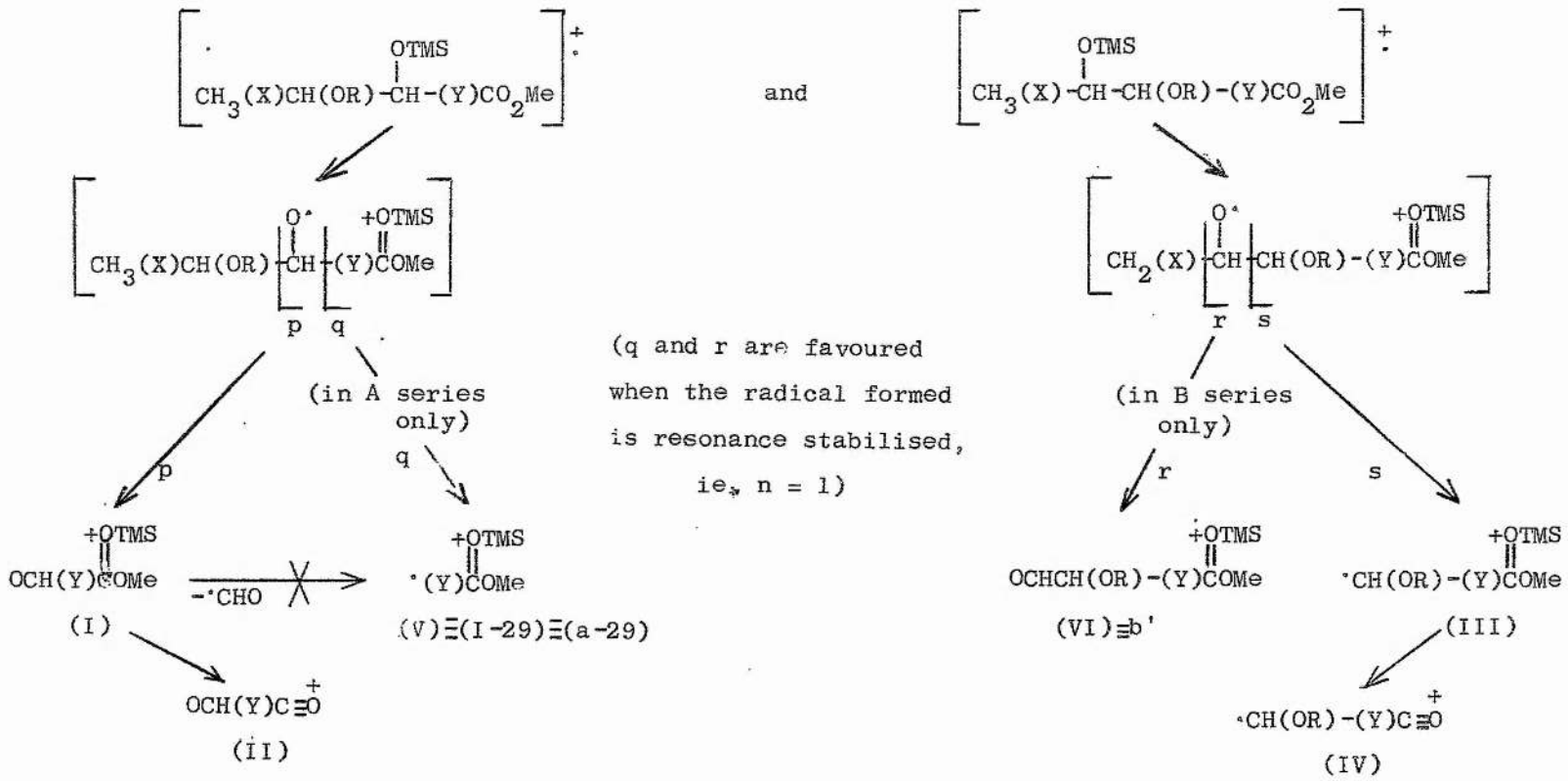
Some of the ions described under (i) and (ii) suffer further fragmentation. Recurring features are the loss of 90 ($-Me_3SiOH$), 32 (-MeOH), 29 (-CHO) and 18 ($-H_2O$) mass units. Many spectra contain metastable peaks related to each of these decomposition reactions. Only for the hypothetical decomposition I (or a) → (I-29) with concurrent loss of CHO was no metastable peak observed. This might justify the designation of this fragment ion as V rather than as either (I-29) or (a-29) and be evidence for its formation according to the cleavage q shown in Scheme 4.

Perusal of the results shown in Tables 11 and 12 leads to a possible classification of our series of ether derivatives into three categories based on their mass spectrometric characteristics viz:

(i) olefinic ethers with $n > 1$
(ii) olefinic ethers with $n = 1$
(iii) acetylenic ethers with $n = 1$

The common features of the bis-TMS ether derivatives are summarised in Table 13. In the compounds where $n > 1$ the only fragments of significant abundance appear to be a' and a. When $n = 1$, two more fragmentation modes are noticeably favoured, since one of the pair of fragments produced is stabilised by resonance with a double or triple bond. Thus the ions b' and b'-90 as well as fragment V become prominent.

Scheme 4


and
(q and r are favoured when the radical formed is resonance stabilised, ie, n = 1)
(in A series only)
(in B series only)
p
q
r
s
-·CHO
(I)
(II)
(III)
(IV)
(V)≡(I-29)≡(a-29)
(VI)≡b'

The results of the mixed methyl TMS diethers are summarised in an analogous way in Table 14. It is evident that fragments containing a TMS ether group overshadow those with a methyl ether group. The conclusion that the charge is more strongly retained by the TMS group accords with the findings of both Holloway et al.[23] and Kleiman and Spencer[116].

In view of the close structural relationship between the bis-TMS ethers and the mixed methyl TMS diethers it is not surprising to find a great number of similarities between their mass spectra. However one contrasting feature concerns the relative abundance of the two fragments a and a'. In the spectra of the bis-TMS ethers fragment a' always predominates over a. This is not generally found for the mixed methyl TMS diethers where a'_{OTMS} exceed a_{OTMS} for all the olefinic members where $n > 1$, but is reversed for three out of four of the acetylenic compounds with $n = 1$. It may be significant that in the 9,12-diene derivatives ($n = 1$) there is the pattern shown below between a'_{OTMS} and a_{OTMS}. The comparison between the abundances of a'_{OTMS} and a_{OTMS} is possible only in the A series and is ruled out in the B series by the fact that there are two fragment ions, a'_{OTMS} and b'-90, with the same m/e value so that the ion current at this m/e ratio is the sum of the contributions from both of them.

(threo diether)	9c12c	$a'_{OTMS} < a_{OTMS}$
(threo diether)	9t12c	$a'_{OTMS} < a_{OTMS}$
(erythro diether)	9c12t	$a'_{OTMS} > a_{OTMS}$
(erythro diether)	9t12t	$a'_{OTMS} > a_{OTMS}$

Apart from this feature and other minor differences in the abundances of various fragment ions, threo and erythro isomers give very similar spectra. Suitable as both the bis-TMS ether and the mixed methyl TMS diether derivatives of epoxides are for the unambiguous determination of the position of the epoxy group, they

cannot provide conclusive information as to the geometry of the epoxy group and hence of the double bond which had been reacted to furnish the epoxy group. This structural feature, however, is easily determined by the GLC behaviour or the NMR spectra of the epoxy ester.

Recently two basically different approaches have been investigated for the determination of the double bond position in unsaturated fatty acid derivatives. As alternative to the derivatisation of the double bond, Vetter et al.[118] pointed out that modification of the carboxyl group can furnish derivatives suitable for the mass spectrometric location of olefinic centres. They found that pyrrolidides were appropriate for this objective. The pyrrolidide group apparently stabilises the positive charge in the molecular ion and the fragment ions so that the double bond does not migrate as in the acid or ester. Andersson and Holman[119] confirmed the results of Vetter et al. by studying a larger selection of unsaturated pyrrolidides. However, they found no significant differences between the spectra of cis- and trans-enoic isomers.

Weinkam[120] showed that chemical ionisation instead of the usual ionisation by electron bombardment induces a fragmentation which makes it possible to determine the position, but not the geometry of the double bond. Unfortunately, the diagnostic fragment ions are not very abundant so that the structural analysis is rather complicated even in the case of monoenoic compounds.

Tables 11-14

Fraction of the ion current carried by the most abundant fragments in the mass spectra (17 eV) of compounds derived from a series of methyl epoxyoctadecenoates and methyl epoxyoctadecynoates.

Table 11a bis-TMS ethers

fragment	5c12c*	6c12c	7c12c	8c12c	9c12c	6c9c	6c10c	6c11c	6c12c	9c12c	9c12t	9t12c	9t12t	9a12c	9a12t
a≡I	12	4	2	10	9	13	11	2	4	9	3	5	3		
a-90	1	12	8					13	12						
a'	30	19	25	23	26	19	24	20	19	26	4	16	12	41	33
b'					10	8				10	4	9	6	5	7
b'-90					2	4				2	1	4	1	1	2
c'	1			1			1	1			4	1	4		
IV-90	1	9	3	1		1			9		1	1			
V					3	4				3	1	4	2	1	2
TOTAL	45	44	38	35	50	49	36	36	44	50	18	40	28	48	44
$\sum_{51}^{99}$	19	12	10	24	16	15	21	20	12	16	50	23	42	16	18
$\sum$(a)	43	35	35	33	35	32	35	35	35	35	7	21	15	41	33
$\sum$(b)					12	12				12	5	13	7	6	9

* 5c12c : m/e 159, 6% $\sum_{51}^{M}$

Table 11b bis-TMS ethers

fragment	5c12c	6c12c	7c12c	8c12c	9c12c	6c9c	6c10c*	6c11c	6c12c	9c12c	9c12t	9t12c	9t12t	9c12a	9t12a
a	3	5	4	7	12	5		2	5	12	10	9	5	3	3
a-90	1	5	9	4	1		5	7	5	1	1	1	1	1	1
a'≡I	22	15	19	11	17	17	30	14	15	17	17	13	13	36	38
a'-90	1			1										1	2
b												1	1	1	1
b'≡VI	1				4	1				4	7	2	3	2	4
b'-90			2		8	18	1			8	11	6	8	5	4
c'			4	1											
II	1	2	2	3	5	2	1	1	2	5	4	6	4	7	7
III	2	1	1	2	1	1	2	1	1	1	1				
IV		3	1			3	3	1	3						
TOTAL	31	31	42	29	48	47	42	26	31	48	51	38	35	56	60
$\sum_{51}^{99}$	25	32	31	28	13	13	2	34	32	13	13	22	25	15	10
$\sum$(a)	27	25	32	23	30	22	35	23	25	30	28	23	19	41	44
$\sum$(b)	1		2		12	19	1			12	18	9	12	8	9

* 6c10c : m/e 142, 7% $\sum_{51}^{M}$

Table 12a mixed methyl TMS diethers

fragment	5c12c	6c12c	7c12c	8c12c*	9c12c	6c9c	6c10c	6c11c	6c12c	9c12c	9c12t	9t12c	9t12t	9a12c	9a12t
a'_{OMe}	1	1	1	1	3	1	1	2	1	3	1	1	4	1	1
$a_{OTMS} \pm 1$	8	3	3	11	8	15	6	7	3	8	9	14	4	1	1
$a_{OTMS}{}^{-90}_{-32}$ / d_{OMe}	2	6	8	1			1	12	6					1	1
a_{OMe}			1		1	1	1			1					
a'_{OTMS}	22	17	23	14	5	10	7	21	17	5	13	13	11	1	2
b'	1	1			8	14			1	8	14	10	12	6	6
c'		1	1	1	1		1	1	1	1	1	1	2	2	2
II	1		1	1	2		1	1		2	1	1	1		
II-18	5	7	4	1		2	4	10	7		1			2	1
III	1					1									
IV		1	1		1	1		2	1	1	1		1		
V					2	3	1			2	3	3	2	3	2
TOTAL	41	37	43	30	31	48	23	56	37	31	44	43	37	17	16
$\sum_{51}^{99}$	17	20	14	15	21	12	26	8	20	21	18	22	34	26	20
$\sum$ (a)	33	27	36	27	17	27	16	42	27	17	23	28	19	4	5
$\sum$ (b)	1	1			8	14			1	8	14	10	12	6	6

* 8c12c : m/e 319, 11% $\sum_{51}^{M}$

Table 12b mixed methyl TMS diethers

fragment	5c12c	5c12c	7c12c	8c12c	9c12c	8c9c	6c10c	6c11c	5c12c	9c12c	9c12t	9t12c*	9t12t	9c12a	9t12a**
a'_{OMe}	4	2	2	2	1		2	2	2	1	1	1	2	5	3
a_{OTMS}	3	4	4	6	(18)	4	3	6	4	(18)	(16)	(10)	(14)	5	6
$a_{OTMS}-90$ / $a_{OMe}-32$	1	4	9	6	1		3	6	4	1	1	1	1	1	1
$a_{OMe} \equiv I$					(4)					(4)	(3)	(2)	(3)	1	1
a'_{OTMS}	24	17	20	15	12	4	14	25	17	12	12	6	11	3	4
$a'_{OTMS}-32$	3	5	1	1		3	5	5	5						
$a'_{OTMS}-90$ / $a'_{OMe}-32$	3	4	1	1	1	3	4	2	4	1			1	2	1
b		5				1	2	2	5					1	1
b' ≡VI					5	2				5	6	1	4	2	3
b'-32			1		5	4				5	4	2	4	3	3
b'-90		1			(18)	16	1	1	1	(18)	(16)	(10)	(14)	7	8
II	3	3	2	4	(4)	2	2	2	3	(4)	(3)	(2)	(3)	2	2
III	2	3	3	2	2	2	2	4	3	2	2		1	1	1
IV		1							1					1	
TOTAL	43	49	43	37	49	41	39	55	49	49	45	23	40	34	34
$\sum_{51}^{99}$	24	22	21	26	10	17	24	15	22	10	17	38	14	24	22
$\sum\sum$ (a)	38	36	37	31		14	31	46	36					17	16
$\sum\sum$ (b)		6	1			23	3	3	6					13	15

* m/e 103, 7% $\sum_{51}^{M}$

** m/e 126, 6% $\sum_{51}^{M}$

Table 13 Summarised fragmentation features of bis TMS ethers

A refers to the A-series (Table 11a)

B refers to the B-series (Table 11b)

th = threo bis TMS ethers

e = erythro bis TMS ethers

fragment	n>1		n=1 olefinic		n=1 acetylenic	
	A	B	A	B	A	B
a'	>>10*		>10		>>>10	
a	9-15**	4-12**	th 5,9,13 e 3,3	th 5,10,12 e 5,9	< 1	3
a-90			≤ 1		< 1	
b'	< 1		th 8,9,10 e 4,6	th 1,4,7 e 2,3	5,7	2,4
b'-90	≤ 2		th 2,4,4 e 1,1	th 8,11,18 e 6,8	1,2	4,5
c'	≤ 1 ‡		th< 1 e 4,4	<1	< 1	
II	< 1	1-3	< 1	2-6	< 1	7
V(A)	< 1		th 3,4,4 e 1,2	- -	1,2	-

* generally more prominent in A than in B

** sum of (a) and (a-90) which are complementary

‡ 7c12c : 4

Table 14 Summarised fragmentation features of the mixed methyl TMS ethers

A refers to the A-series (Table 12b)

B refers to the B-series (Table 12b)

th = threo methyl TMS ethers

e = erythro methyl TMS ethers

fragment	n>1		n=1			
			olefinic		acetylenic	
	A	B	A	B	A	B
a'_{OMe}	2	2-4	1-4	1-2	1,1	3,5
a_{OMe}	$\leqslant 1$		$\leqslant 1$ [(i)]		$\leqslant 1$	
a'_{OTMS}	$\gg 10$ [(ii)]		5-13	4-12	1,2	3,4
a'_{OTMS}-32(B)		1-5		< 1 [(iii)]		< 1
a'_{OTMS}-90(B) / a'_{OMe}-32(B)		1-4		$\leqslant 1$ [(iii)]		1,2
a_{OTMS}	3-11	3-6	4-15	6c9c:4 [(iv)]	1,1	5,6
a_{OTMS}-90	6-12 (n=3 or 4)	3-9 (n=2-4)	< 1	$\leqslant 1$	1	
a_{OMe}-32	$\leqslant 2$ (n=2or5)	1 (n=5)				
b'	< 1		8-14	4-6 [(v)]	6,6	2,3
b'-32(B)		$\leqslant 1$		4-5 [(vi)]		3,3
b'-90(B)		$\leqslant 1$		6c9c:16 [(iv)]		7,8
II-18 (A)	4-10 [(vii)]		< 1 [(viii)]		1,2	
V (A)	$\leqslant 1$		2-3		2,3	

(i) unless representative for two fragments; (ii) except for 6c10c:7 ; (iii) except for 6c9c:3; (iv) 9,12 B series: a_{OTMS} and b'-90 overlapping; (v) except for 9t12c: 1, and 6c9c:2; (vi) except for 9t12c:2; (vii) except for 8c12c:1 ; (viii) except for 6c9c:2

Melting points of some vicinal dihydroxyoctadec-enoic and -ynoic acids

As epoxyoctadecenoic acids appear to be liquid at ambient temperature, we decided to determine the melting points of the dihydroxy acids derived from the epoxy esters by acetolysis followed by alkaline hydrolysis. By this reaction sequence, which has been discussed on pages 46 and 47, cis epoxides are converted to threo diols and trans epoxides to erythro diols.

The melting points are listed in Table 15. In the x,12 (x = 5 to 9) and the 6,y (y = 9 to 12) series, a distinct pattern of alternation is observed. This alternation is not consistent for the B members of the latter series, ie. threo-6,7-dihydroxy 18:1 (9c-12c).

Of the two isomeric dihydroxy acids (A and B) related to the same dienoic acid it generally is the B isomer which has the higher melting point. This pattern is broken only in the case of the dihydroxy derivatives of 18:2 (9c12t) where the A isomer has the erythro configuration (erythro-12,13-dihydroxy 18:1 9c) and the B isomer has the threo configuration (threo-9,10-dihydroxy 18:1 12t).

Within the series of the diols related to the 9,12-dienoic C_{18} acids, both the configuration of the diol group and the geometry of the double bond are reflected in the widely differing melting points. Erythro diols and trans alkenes have higher melting points than their threo and cis isomers, respectively. The melting point differences are far greater between isomers differing in the configuration of the diol group than between those isomers which differ in the geometry of the double bond.

Similarly, of the four acetylenic dihydroxy acids examined the erythro diols have considerably (ca. 35^{o}) higher melting points than the threo isomers. Small melting point differences are also observed between positional isomers: the two 9,10-dihydroxyoctadec-12-ynoic acids melt at higher (ca. 10^{o}) temperatures than the corresponding 12,13-dihydroxyoctadec-9-ynoic acids.

Table 15 Melting points of unsaturated dihydroxy C_{18} acids

5,6th:12c denotes threo-5,6-dihydroxyoctadec-cis-12-enoic acid

12,13e:9t denotes erythro-12,13-dihydroxyoctadec-trans-9-enoic acid

a stands for acetylenic unsaturation

B-series	°C	A series	°C
5,6th:12c	51-54	12,13th:5c	45-50
6,7th:12c	72-73	12,13th:6c	53-54
7,8th:12c	52-54	12,13th:7c	40.5-42
8,9th:12c	62-63	12,13th:8c	51-52
9,10th:12c	62-63[a]	12,13th:9c	50-52
6,7th:9c	71.5-72.5	9,10th:6c	64.5-65
6,7th:10c	58.5-60	10,11th:6c	53-55.5
6,7th:11c	65-66	11,12th:6c	59.5-61
6,7th:12c	72-73	12,13th:6c	53-54
9,10th:12c	62-63[a]	12,13th:9c	50-52[b]
9,10th:12t	71-74	12,13e:9c	73-76[c]
9,10e:12c	96-99	12,13th:9t	65-68[d]
9,10e:12t	119-120.5	12,13e:9t	103-105
9,10th:12a	68-70[e]		
9,10e:12a	105.5-107		
		12,13th:9a	59-62
		12,13e:9a	93.5-95

a 60.5-61 °C[14]; 53-57 °C[7]

b 50-52 °C[7]; 52-53 °C[75]; 53-54 °C[1]
(+)-enantiomer 61-61.5 °C[121]; 63-63.5 °C[31]
(-)-enantiomer 61-61.5 °C[121]; 62.5-63 °C[31]
1:1 mixture 52.5-53 °C[121,31]

c 87-88 °C[122]

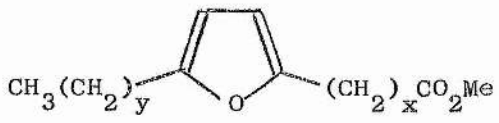
d 67.5-69.5 °C[1]

e 71-72 °C[123]; 72-72.5 °C[75,124]

Reaction of methyl cis-6,7,cis-9,10-diepoxystearate and of methyl cis-8,9,cis-12,13-diepoxystearate with boron trifluoride etherate

Introduction

Saturated epoxy esters can be converted in high yields to isomeric oxo esters by reaction with boron trifluoride in boiling dioxan, but when methyl cis-9,10,cis-12,13-diepoxystearate is treated in the same way, the yield of dicarbonyl compounds is rather poor[125]. Abbot and Gunstone[126] carried out this isomerisation reaction at room temperature and found that only a fraction (20%) of the reaction product consisted of a mixture of 9,12- and 10,13-dioxostearates. From chromatographic and spectroscopic data and from chemical evidence they concluded that the other possible dioxo isomers (10,12- and 9,13-) were absent. Beside a mixture of 9,12- and 10,13-furanoid esters [(1), 21%] they isolated



9,12-(x=7,y=5)

10,13-(x=8,y=4)

(1)

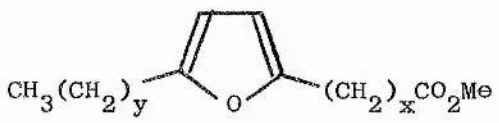
a fraction (17%) for which they proposed a bicyclic ether structure.

Results

Methyl cis-6,7,cis-9,10-diepoxystearate

For a reexamination of these findings we reacted methyl cis-6,7,cis-9,10-diepoxystearate (n=1) with boron trifluoride in dioxan at room temperature for fifteen hours. The reaction product was separated by preparative TLC into five fractions (A-E). The least polar fraction A constituting a third of the reaction product resembled in its TLC and GLC characteristics the furanoid esters which Abbot had found. This product was identified on the basis of spectroscopic data (IR, UV, NMR and MS) as a mixture of 6,9- and

7,10-furanoid esters (2). Since furans can be obtained by acid



(2)

6,9- (x=4,y=8)

7,10-(x=5,y=7)

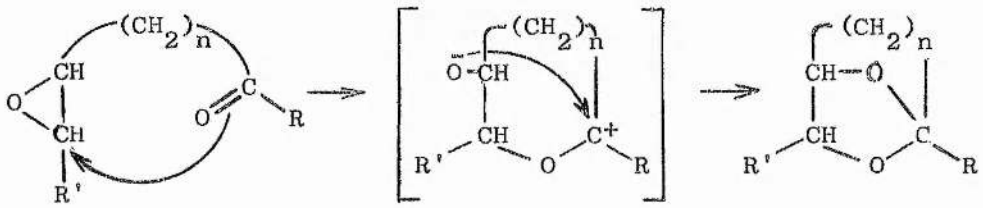
catalysed reaction of 1,4-dicarbonyl compounds it is possible that these furanoid esters derive from the 6,9- and 7,10-dioxostearates which are, along with the 6,10- and 7,9- isomers, the expected dioxo products of the isomerisation of 6,9-diepoxystearate. Should any 7,9-dioxoester be formed, it is unlikely to react further giving an unfavoured four membered cyclic ether. Fraction D (15%) showed strong oxo- and ester-carbonyl absorption (1710 and 1740 cm^{-1}) in the infrared. The NMR spectrum had a sharp two proton singlet at 2.55δ and a broad six proton multiplet at 2.1-2.5δ which we take as evidence for the presence of methyl 7,9-dioxo-stearate. Mass spectrometric examination supports this assignment.

The other fractions consisted of compounds containing oxo and hydroxy or epoxy groups, but methyl 6,10-dioxostearate could not be detected.

Methyl cis-8,9,cis-12,13-diepoxystearate

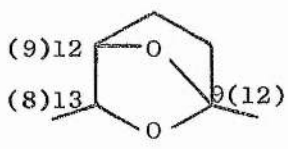
After confirming and extending what Abbot had found, we were interested to discover if the acid catalysed isomerisation of methyl cis-8,9,cis-12,13-diepoxystearate where two methylene groups (n=2) separate the epoxy groups yielded diketones rather than cyclic ethers as in the previous experiment (n=1). Methyl cis-8,9,cis-12,13-diepoxystearate was treated with boron trifluoride and the reaction product was separated by preparative TLC into five fractions (A-E) none of which contained any furanoid ester. Band A (25%), which was the most mobile fraction and separated clearly from the other fractions, was more polar than the furanoid esters derived from cis-6,7,cis-9,10-diepoxystearate but less polar than

the diepoxy starting material. The IR spectrum did not show any hydroxy or oxo absorption and the picric acid test[127] for α,β-epoxides proved negative too. The NMR spectrum contained a singlet at 3.68δ representing the carbomethoxy protons and a complex two proton signal between 3.8 and 3.958δ which is the region typical for protons of a secondary alcohol or ether group. The mass spectrum showed a molecular ion peak at m/e 326 which corresponds with a molecular formula of $C_{19}H_{34}O_4$. The carbomethoxy group contains two of the four oxygen atoms. The other two are most probably part of a bicyclic ketal structure which accords with the above observations. Intramolecular reaction of epoxyketones may lead to such bicyclic ketal structures



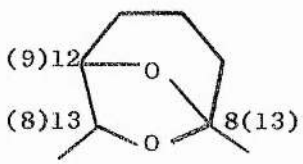
n = 2 or 3

As the isomerisation of the cis-8,9,cis-12,13-diepoxystearate could yield four isomeric epoxy ketones, ie. the cis-8,9-epoxy-12- and 13-oxo- and the cis-12,13-epoxy-8- and 9-oxostearates, the following four isomeric ketals might be formed:



8,9-12
12,13-9

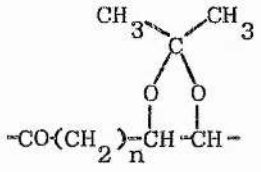
and



8,9-13
12,13-8

The mass spectrum of compound A provided convincing evidence for a ketal link between C(8) and C(13), but did not indicate whether the five and six membered (8,9-12 and 12,13-9) or the six and seven (8,9-13 and 12,13-8) bicyclic ethers were formed exclusively.

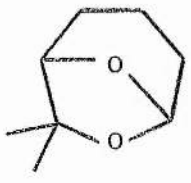
In order to locate the ketal-carbonyl carbon we thought it necessary to open the ketal ring. Attempts to achieve this by refluxing with methanolic sulphuric acid failed. Refluxing with aqueous sulphuric acid resulted in the hydrolysis of the ester group, but the ketal ring survived unchanged as shown by the NMR and IR spectra. However, the reaction with sulphuric acid in acetone proved successful. The methylated product showed strong oxo but no hydroxy absorption in the infrared which is in agreement with the expected oxo-isopropylidene structure (3). Sodium



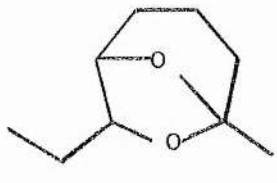
(3)

borohydride reduction then gave the hydroxy isopropylidene ester which was then silylated. The mass spectrum of this derivative showed strong fragmentation α to the TMS ether group and indicated that each of the four carbon atoms [C(8), C(9), C(12) and C(13)] may bear the trimethylsilyloxy group and therefore be the ketal forming carbonyl group. Sc five and six membered as well as six and seven membered bicyclic ketals appear to be formed in this acid catalysed isomerisation reaction of the diepoxide separated by two methylene groups.

Two similar bicyclic ketals have been discovered in natural sources, one of them (4) being a component of hop oil[128], the other (5) acting as sex attractant for beetles[129]. Both compounds



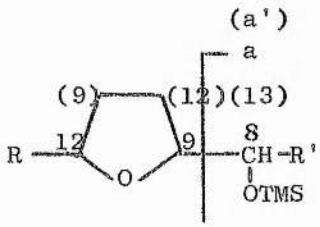
(4)



(5)

contain a six and a seven membered ketal ring and also exhibit unexpected chemical stability.

Fraction C(21%) of the isomerisation product showed hydroxy and oxo absorption in the infrared and the test with picric acid indicated the presence of an epoxide ring. When the silylation product was analysed on TLC, two bands were obtained. One of them with unchanged mobility on TLC appeared to be an epoxy oxo-ester. The other less polar band did not give any oxo absorption in the infrared nor did it react with picric acid. The mass spectrum showed a distinct molecular ion peak and two prominent fragments (a and a') which suggested an exocyclic hydroxy-tetrahydrofuran (6) or -tetrahydropyran (7) structure.



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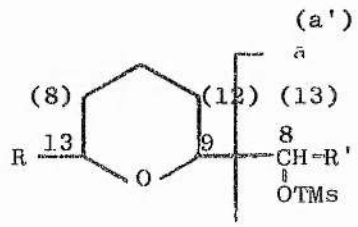


(7)

The ions a and a' could arise from both cyclic ethers (6 and 7), and we have no evidence to distinguish between (6) and (7).

The more polar bands D and E containing a rather complex mixture of hydroxy and oxo compounds were not investigated further.

Conclusion

The acid catalysed isomerisation of the two diepoxides methyl cis-6,7,cis-9,10-diepoxystearate (n=1) and methyl cis-8,9,cis-12,13-diepoxystearate (n=2) gives significant amounts of cyclic ethers with greatly differing ring structures. The expected dicarbonyl compounds could not be isolated apart from one isomer, ie. methyl 7,9-dioxostearate, whose cyclisation to a four membered cyclic ether is apparently not favoured.

PART II

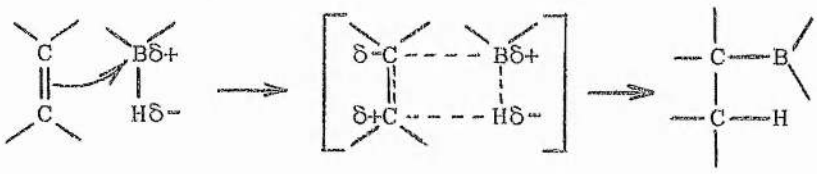
Hydroboration Studies

Hydroboration Studies

Introduction

Within the past fifteen years, organoboranes have gained great importance in organic chemistry. Most of the credit for this goes to H. C. Brown and his coworkers who not only discovered the hydroboration reaction furnishing organoboranes but who also revealed the many ways by which organoboranes can be transformed to compounds with widely differing substituents[130-132].

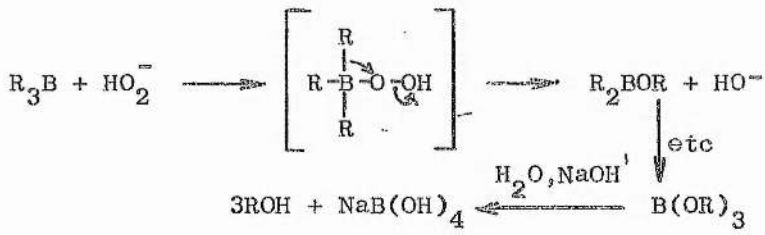
Hydroboration occurs readily when olefins and acetylenes are reacted with diborane. The reaction involves the cis addition of a boron-hydrogen bond of diborane across the unsaturated centre. The polarisation of the boron-hydrogen bond explains the anti-Markovnikov course of this addition:



The four-centre transition state is also consistent with the observation that the addition takes place in a cis manner. Alkenes with little steric hindrance such as the mono- and disubstituted alkenes used in this study are readily hydroborated by diborane or the borane-ether complex (R_2O-BH_3) to give trialkylboranes (R'_3B) which can then be converted to a great variety of products. The few reactions employed in this study are (I) oxidation with alkaline hydrogen peroxide, (II) oxidation with chromic acid, (III) reaction with oxygen followed by hydrogen peroxide, (IV) protonolysis and (V) the coupling reaction with silver oxide.

(I) Oxidation with alkaline hydrogen peroxide[133,134].

Organoboranes can be converted in excellent yields to alcohols by treatment with alkaline hydrogen peroxide. The reaction takes place with retention of configuration as illustrated by the given mechanism.



Vinylboranes obtained by the monohydroboration of alkynes react in an analogous way to give enols which tautomerise to carbonyl compounds[135].

(II) Oxidation with chromic acid[136]

The oxidation of organoboranes with aqueous chromic acid yields carbonyl compounds as would the successive oxidation reactions with alkaline hydrogen peroxide and chromic acid.

(III) Oxidation with oxygen followed by hydrogen peroxide[137,138]

This reaction sequence constitutes a convenient synthesis of alkyl hydroperoxides. The rapid oxidation of organoboranes with oxygen has been shown to proceed via a free radical chain:

$$R_3B + {}^{\cdot}O\text{-}O^{\cdot} \longrightarrow R_2BO_2^{\cdot} + R^{\cdot}$$

$$R^{\cdot} + {}^{\cdot}O\text{-}O^{\cdot} \longrightarrow RO_2^{\cdot}$$

$$RO_2^{\cdot} + R_3B \longrightarrow R_2BO_2R \ (1) + R^{\cdot}$$

$$R_2BO_2R + RO_2^{\cdot} \longrightarrow RB(O_2R)_2 \ (2) + R^{\cdot}$$

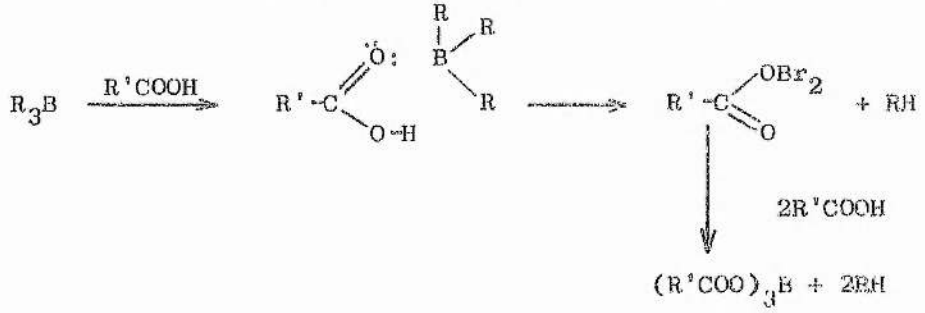
$$R_2BO_2R + R_3B \longrightarrow 2R_2BOR \ (3)$$

The initial product, the alkylperoxyborane R_2BO_2R (1), can either react with a second alkylperoxy radical to give the bis-alkylperoxy product (2) or oxidise a trialkylborane to give two moles of mono-alkoxyborane (3). At low temperature (-78°C) or in highly dilute solution (0.01-0.05 M), this intermolecular redox reaction is insignificant, whereas the formation of the bis-alkylperoxyborane (2) proceeds quantitatively. Subsequent oxidation with hydrogen peroxide furnishes two moles of the hydroperoxide and one mole of alcohol:

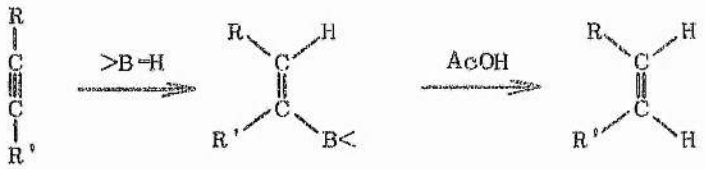
$$R_3B \xrightarrow{2O_2} RB(O_2R)_2 \xrightarrow[H_2O]{H_2O_2} 2ROOH + ROH$$

(IV) Protonolysis[139,140]

Whilst fairly stable towards water and aqueous mineral acids, organoboranes react readily with carboxylic acids to give alkanes:



Protonolysis of vinyl type organoboranes obtained by the monohydroboration of alkynes proceeds smoothly yielding cis alkenes of high purity:



(V) Coupling reaction with silver oxide[141-143]

Treatment of organoboranes with aqueous solutions of silver nitrate and sodium hydroxide provides a means of linking two alkyl groups of the organoboranes by a carbon-carbon bond. The coupling reaction probably proceeds via a silver alkyl intermediate which, being unstable under the reaction condition, breaks down into silver and an alkyl radical. Combination of such radicals then gives the coupled product.

Discussion and results

The selective reaction of diborane with an unsaturated centre in compounds also containing other functional groups is one of the most promising features of the hydroboration reaction. So, for example, it has been established that an ester group is far less reactive towards diborane than a double bond. Fore and Bickford[144] found that hydroboration of methyl oleate followed by oxidation

with alkaline hydrogen peroxide yielded an equimolar mixture of methyl 9- and 10-hydroxystearate together with some unreacted methyl oleate. No significant amounts of products arising from the reduction of the ester function were obtained. Methyl hendec-10-enoate could be similarly converted in about 50% yield to methyl 11-hydroxyhendecanoate. The reaction product did not contain any component resulting from the possible reduction of the ester group[145].

No further investigation has been made into the hydroboration of unsaturated long chain esters despite their apparent suitability for this reaction and subsequent conversions. We therefore decided to examine the hydroboration of a wider range of unsaturated fatty esters and to study the versatility of the organoboranes formed.

The reaction between diborane and the unsaturated substrate was carried out in THF at temperatures between 0°C and 20°C. The application of diborane in stoichiometric amounts, which were essential in all our reactions performed on a millimole scale, was possible by using standardised solutions of diborane in THF. No attempts were made to isolate and examine the organoboranes but their conversion to the various derivatives normally allowed the inference of the extent and also of the course of the hydroboration step.

On the following pages an account is given of the various conversions (I-V) of organoboranes which were derived from a number of unsaturated compounds (i-ix).

(I) Oxidation with alkaline hydrogen peroxide

(i) Methyl oleate [18:1 (9c)]

Hydroboration followed by oxidation with alkaline hydrogen peroxide is a well studied procedure for the preparation of hydroxy compounds in excellent yields (>90%). As the hydroxy products derived from methyl oleate can be easily isolated and

identified, we used methyl oleate as standard substrate to work out optimum reaction conditions. The products obtained after hydroboration and oxidation were examined by TLC and GLC and the components were separated by preparative TLC for their spectroscopic characterisation.

Varying the ratio (r) of the reactants of the hydroboration step, ie. borane-hydride (>B-H) to methyl oleate (r=[>B-H]:[18:1(9c)]), gave different product mixtures. Reaction of equimolar amounts of >B-H and methyl oleate (r=1) which should have furnished the trialkylborane (R_3B) gave only a 60% conversion of oleate to hydroxystearate with the remainder of the product being unreacted starting material. That an incomplete hydroboration, but not the oxidation reaction, was to blame for the rather low yield, was proved when a 50% excess of >B-H (r=1.5) was used. The yield of hydroxystearate was now improved to 95%. The TMS ether derivative, appearing on GLC as a single peak, gave a mass spectrum which showed it to be an equimolar mixture of the TMS derivatives of methyl 9- and 10-hydroxystearates. This was indicated by the equal intensities of the ion pairs a , b' and a-104 which originate from α-cleavage at the TMS ether bearing carbon atoms C(9) and C(10).

$$CH_3(CH_2)_y\overset{a}{\underset{b'}{|}}CH(OTMS)|(CH_2)_xCOOCH_3$$

	x	y	a	b'	a-104
9-TMSO 18:0	7	8	259(100%)	229(100%)	155(35%)
10-TMSO 18:0	8	7	273(100%)	215(100%)	169(35%)

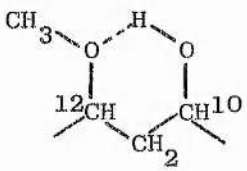
When a three-fold excess of >B-H was employed in the hydroboration of methyl oleate the yield of hydroxystearate dropped to 80% whilst another component (16%) appeared which was identified as a mixture of 1,9- and 1,10-dihydroxyoctadecane. Obviously the ester group is to some extent susceptible to reduction by diborane, but this reduction does not seriously interfere with the much faster hydroboration of the Δ9 double bond. Even with a six-fold

excess of >B-H (r=6) only 60% of the product consisted of the diol derivative.

Having achieved the quantitative hydroboration of methyl oleate as seen by its complete conversion to methyl 9(10)-hydroxystearate, we were interested in how the hydroboration reaction would be effected by the presence of other functional groups. We confined our study to 12-hydroxyoleate, ie. ricinoleate,and its methyl ether and acetoxy derivative.

(ii) Methyl 12-methoxyoleate [12-OH 18:1 (9c)]

When methyl 12-methoxyoleate was reacted with a 60% excess of >B-H (r=1.6) and then subjected to oxidation with alkaline hydrogen peroxide, only one third of the unsaturated ester appeared to have been transformed to a mixture of two hydroxy methoxy esters. Their mobilities on TLC differed sufficiently to allow a preparative separation. The more polar fraction (PE50, Rf 0.14) was identified by mass spectrometric examination of its TMS ether derivative as methyl 9-hydroxy-12-methoxystearate. The less polar fraction (Rf 0.25) was lost by accident, but we assume it to be the other expected hydroxy isomer, ie. methyl 10-hydroxy-12-methoxystearate. Its greater mobility on TLC supports this assignment as a hydrogen bonded six-membered cyclic structure is conceivable. Analogous



hydrogen bonding in the 9-hydroxy isomer would result in a less favourable seven membered ring.

(iii) Methyl 12-acetoxyoleate [12-OAc 18:1 (9c)]

Rather unexpected results were obtained for the hydroboration and subsequent oxidation of methyl 12-acetoxyoleate. Instead of the expected acetoxy-hydroxy products, methyl 12-hydroxyoleate and an equimolar mixture of 9,12- and 10,12-dihydroxystearates were

found in the products. By varying the amounts of diborane these products were formed in different ratios. The results based on gas chromatographic analysis are summarised below:

r=[>B-H]:[12-OAc 18:1 (9c)]	1.1	1.65	2.2
12-OAc 18:1 (9c) (%)	8	2	-
12-OH 18:1 (9c) (%)	64	60	34
9,12-diOH 18:0 (%)	14	19	31
10,12-diOH 18:0 (%)	14	19	35

Hydration of the Δ9 double bond occurred in little more than a third of the reaction of methyl 12-hydroxyoleate where r = 1.65. Similarly, only a 34% yield of methyl 9(10)-hydroxy-12-methoxy-stearate was obtained in the analogous reaction of methyl 12-methoxyoleate (r=1.6), whilst a complete conversion of methyl oleate to methyl 9(10)-hydroxystearate was achieved by using a 50% excess of diborane (r=1.5) . So the acetoxy and the methoxy substituents at C(12) seem to deactivate the Δ9 double bond.

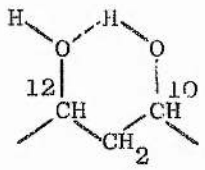
The conversion of the acetoxy group at C(12) into the hydroxy substituent was probably due to hydrolysis occurring during the treatment with alkaline hydrogen peroxide and not due to reductive reaction of diborane.

(iv) Methyl 12-hydroxyoleate [12-OH 18:1 (9c)]

When methyl 12-hydroxyoleate was first treated with diborane and then oxidised with alkaline hydrogen peroxide, three different types of compounds were encountered in the reaction product. They comprised unreacted methyl hydroxyoleate, a pair of methyl dihydroxy stearates and another pair of polyhydroxy compounds which did not show any carbonyl absorption in the infrared.

The two dihydroxy esters could be separated by preparative TLC and were identified by mass spectrometric examination of their bis-TMS ether derivatives as methyl 9,12-dihydroxystearate and methyl 10,12-dihydroxystearate. The 10,12-dihydroxy ester was the isomer

with greater mobility on TLC, probably due to intramolecular hydrogen bonding resulting in a six-membered ring structure:



The gas chromatogram of the silylated polyhydroxy fraction showed two partly resolved peaks of similar shape and height. One of them corresponded with the ECL characteristic for 1,9,12 tris(trimethylsilyloxy)-octadecane obtained by $LiAlH_4$ reduction and silylation of methyl 9,12-dihydroxystearate. If reduction of the ester group of the two 9,12- and 10,12-dihydroxy esters, which were found in equal quantities, had led to the trihydroxy products, these also should be found in equal amounts and this was clearly indicated by GLC. GLC also showed that methyl 9,12-bis(trimethylsilyloxy)-stearate has the greater ECL than methyl 10,12-bis(trimethylsilyloxy)stearate and that 1,9,12-tris(trimethylsilyloxy)octadecane similarly corresponds with the greater ECL than its 1,10,12 tris-TMS ether isomer.

The relative yields of these di- and trihydroxy products depended on the amounts of diborane used in the hydroboration reaction as shown below (the given percentages are based on the gas chromatographic analysis of the silylated reaction mixtures):

r=[>B-H]:[12-OH 18:1 (9c)]	1.2	2.4	3.6
12-OH 18:1 (9c) (%)	84	16	-
9,12-diOH 18:0 (%)	8	40	30
10,12-diOH 18:0 (%)	8	40	30
1,9,12-triOH-octadecane (%)	-	2	20
1,10,12-triOH-octadecane (%)	-	2	20

Most of the first moleequivalent of >B-H was used without apparent change of the starting material. From this we infer that before attacking the double bond, diborane might be engaged in a reaction with the hydroxy group. It is conceivable that a borate,

$B(OR)_3$, is formed which is hydrolysed in the subsequent reaction with alkaline hydrogen peroxide to give unchanged 12-hydroxyoleate.

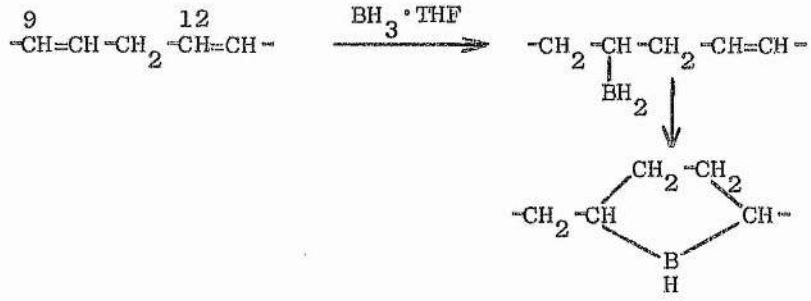
The dihydroxystearates were, however, obtained in good yields when the hydroboration was carried out with double the amount of diborane. When the excess of diborane was increased even further, trihydroxy products were formed to a significant extent by reduction of the ester function. For the hydroboration of methyl 12-hydroxy-oleate the order of reactivity towards diborane was:

$-OH > -CH=CH- > -COOCH_3$.

(v) Methyl linoleate [18:2 (9c12c)]

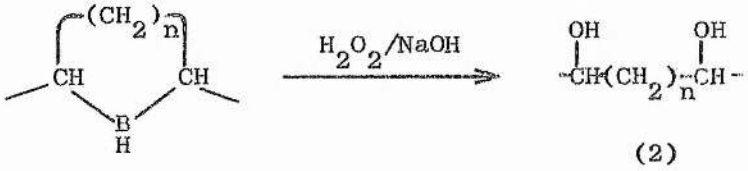
The results discussed so far indicate that the hydroboration of an internal double bond (RCH=CHR') leads to a random addition of boron at either carbon atom of the unsaturated centre giving rise to a 1:1 mixture of positionally isomeric organoboranes. Oxidation then yields a 1:1 mixture of isomeric hydroxy derivatives.

Assuming that the dihydroboration of methyl linoleate proceeds similarly in two uncoordinated steps, oxidation of the intermediate organoborane is expected to yield the four dihydroxy-stearates, 9,12-, 10,12-, 9,13- and 10,13-, in equal amounts. Unequal proportions of these four isomeric dihydroxystearates are expected, however, if the hydroboration of the two double bonds of methyl linoleate involves formation of a cyclic organoborane as for example:



In the initial hydroboration step, boron can be placed at four different positions in the linoleate molecule, ie. at C(9), (10), (12) or (13), and the second addition of a boron-hydrogen bond can

occur in two ways leading to a total of four isomeric cyclic organoboranes (1). These yield on oxidation with alkaline hydrogen peroxide four isomeric dihydroxy compounds (2):



(1) (2)

n	number of ring members (n+3)	-diOH 18:0
1	4	10,12
2	5	9,12;10,13
3	6	9,13

As a four-membered ring structure (n=1) is least favoured, the yield of methyl 10,12-dihydroxystearate should be smaller than the yields of each of the other three isomers.

Analysis of the reaction mixtures by preparative TLC gave methyl linoleate, unsaturated hydroxy esters and two dihydroxy-stearate fractions. The less polar dihydroxy ester fraction was identified as methyl 10,12-dihydroxystearate and amounted to more than a third of the more polar fraction which we consider to be a mixture of methyl 9,12-, 10,13- and 9,13-dihydroxystearates.

r=[>B-H]:[18:2 (9c12c)]		3	4
18:2 (9c12c)	(%)	20	8
OH 18:1	(%)	41	22
10,12-diOH 18:0	(%)	11	21
9,12- 10,13- 9,13- } diOH 18:0	(%)	28	49

The high yield of methyl 10,12-dihydroxystearate indicates that the hydroboration of the two double bonds in methyl linoleate occurs in two uncoordinated steps.

(vi) Methyl stearolate [18:1 (9a)]

As no compound containing both an acetylenic and an ester group had been hydroborated so far, we were interested in finding out how these two groups would compete for diborane. With no interference from the ester group, hydroboration of the acetylenic bond with one moleequivalent of >B-H is expected to lead to a vinyl type organoborane (>CH=C-B<) the oxidation of which would furnish the oxo compound (-CH_2CO-).

With a 50% excess of >B-H (r=1.5) the monohydroboration of the acetylenic bond proceeded smoothly as the expected mixture of methyl 9- and 10-oxostearate was obtained in 87% yield.

(II) Oxidation with chromic acid

When the organoborane derived from methyl oleate was refluxed with aqueous chromic acid, a product was obtained which consisted mainly of methyl 9(10)-oxostearate accompanied by small amounts of some hydroxy ester (7%) and methyl oleate (4%).

(III) Oxidation with oxygen followed by oxidation with hydrogen peroxide

(viii) Cyclohexene

The conversion of cyclohexene to cyclohexyl hydroperoxide by this route had already been described in the literature[137,138]. Our experiment (r=1.2) furnished the expected hydroperoxy and hydroxy products in yields of 64% and 29%, respectively.

(ix) Tridec-1-ene

When tridec-1-ene was reacted in the same way only 14% of tridecyl hydroperoxide was formed whilst tridecanol was obtained in 81% yield. Oxidation of the intermediate tris(tridecyl)borane

(R_3B) with alkaline hydrogen peroxide gave tridecanol in 88% yield which confirmed the completeness of the hydroboration step. So the results suggest that the reaction of the intermediate organoborane with oxygen did not go to completion probably because the organoborane was only partially soluble under the conditions of this oxidation (0.5 M solution in THF, -40°C).

The NMR spectrum of the hydroxy product showed a two proton triplet (J=6 Hz) at 3.50δ which is consistent with the alcohol being tridecan-1-ol ($CH_3(CH_2)_{11}\underline{CH}_2OH$). Similarly, the NMR spectrum of the hydroperoxide contains a triplet (J=6Hz) at 3.88δ which shows the attachment of the hydroperoxy group at C(1), ie. $CH_3(CH_2)_{11}\underline{CH}_2OOH$.

(i) Methyl oleate [18:1 (9c)]

Methyl oleate was hydroborated in the usual way. The completeness of the conversion to the organoborane was checked by oxidation of a sample of the hydroboration product with alkaline hydrogen peroxide. A 90% yield of hydroxystearate indicated that the hydroboration had occurred almost quantitatively. The organoborane was oxidised with oxygen in a 0.05 M THF solution cooled to -40°C. Subsequent treatment with hydrogen peroxide gave a product which could be separated by preparative TLC into two polar components. The more polar one was identical with methyl hydroxystearate. The other component (56%) gave a distinct reaction with ferrous thiocyanate reagent indicating its being the expected hydroperoxy ester.

The NMR spectrum provided further evidence for its hydroperoxide structure as it contained a broad one-proton signal at 3.75δ which we consider to represent the proton of the methine group carrying the hydroperoxy group, ie. $>\underline{CH}\text{-}OOH$. The corresponding signal for the hydroxy derivative appears at 3.45δ ($>\underline{CH}\text{-}OH$). For the hydroxy and hydroperoxy derivatives of cyclohexene and tridec-1-ene similar shift differences were observed:

	$-CH_2OOH$	$-CH_2OH$	Δ(ppm)
n-tridecyl hydroperoxide	3.88	3.50	0.38
	>CHOOH	>CHOH	
cyclohexyl hydroperoxide	3.86	3.49	0.37
methyl 9(10)-hydroperoxy-stearate	3.75	3.45	0.30

The hydroperoxide is expected to be an equimolar mixture of methyl 9- and 10-hydroperoxystearate. The yield of its formation was 85% of the theory or 56% based on methyl oleate.

(IV) Protonolysis

The vinyl type organoborane obtained by the monohydroboration of methyl stearolate (compare p. 77) was treated with glacial acetic acid at room temperature. Analysis of the product by TLC and GLC showed two components with retention characteristics comparable with those of methyl oleate and methyl 9(10)-oxostearate. The infrared spectrum of the more mobile component (82%) indicated that the geometry of the double bond was purely cis. So the major product appears to be methyl oleate.

(V) Coupling reaction

(ix) Tridec-1-ene

Tridec-1-ene was hydroborated (r=1.2) and then reacted with aqueous solutions of sodium hydroxide and silver nitrate. The product gave a single spot on TLC with a Rf value similar to the starting material. GLC, however, did not show any appreciable amount of tridec-1-ene but two peaks (5% and 86%) with retention times to be expected for a coupled product. The mass spectrum

had a distinct molecular ion peak at m/e 366 corresponding with a hydrocarbon of the composition $C_{26}H_{54}$, which shows that the coupling reaction had been successfully achieved.

(vii) Methyl hendec-10-enoate [11:1 (10)]

Methyl hendec-10-enoate was quantitatively reacted to the organoborane as shown by the 95% yield of methyl 11-hydroxyhendecanoate which was obtained by oxidation of a small sample of the hydroboration product with alkaline hydrogen peroxide. Treatment of the organoborane with aqueous sodium hydroxide and silver nitrate gave a product which TLC showed to consist of one major component with an Rf value comparable to that of methyl hendec-10-enoate. GLC gave two peaks (5% and 88%) with ECL values compatible with an α,ω-C_{22} diester.

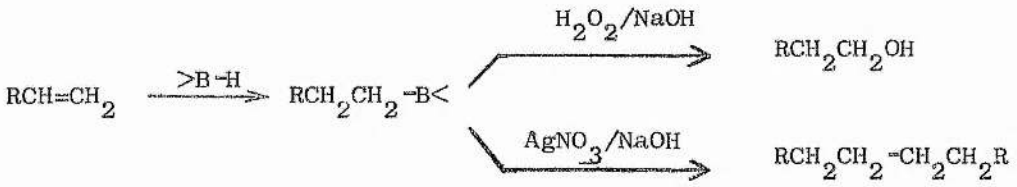
The mass spectrum provided conclusive evidence for such a structure as it contained two intense peaks at m/e 366 (M-32) and 324 (M-74) which both derive from the missing molecular ion ($C_{24}H_{46}O_4$). Other prominent peaks at m/e 112, 98 and 84 are characteristic features in the mass spectra of the dimethyl esters of long chain α,ω-dicarboxylic acids[108].

The NMR spectrum contained all the signals typical for a long chain ester with the exception of the triplet around 0.9δ (CH_3-C). The absence of any signal in this region (0-1.3δ) showed that the coupling product consisted predominantly of 1,20-dicarbomethoxy eicosane (1) and only little, if any, 1,19-dicarbomethoxy-10-methylnonadecane (2).

$CH_3OOC(CH_2)_{20}COOCH_3$ (1)

$CH_3OOC(CH_2)_8-\underset{\displaystyle CH_3}{\underset{|}{CH}}-(CH_2)_{10}COOCH_3$ (2)

This confirms the anti-Markovnikov addition of >B-H across the terminal double bond of methyl hendec-10-enoate:



$R = CH_3OOC(CH_2)_8-$

When recrystallised from ether-petrol, the ester melted sharply at 72°C which is the melting point reported for 1,20-dicarbomethoxy-eicosane[146].

Conclusions and Summary

Various fatty acid derivatives could be obtained in good yields via the hydroboration of unsaturated esters followed by treatment with various reagents. The ester group was generally far less reactive towards diborane than the unsaturated centre, thus allowing the selective hydroboration of the latter, which may be either a double or triple bond.

The hydroboration of a terminal double bond led almost exclusively to the addition of boron at the terminal carbon and for this reason to a single substitution product as the functional group is always introduced into the molecule at the site of the boron-carbon bond in the intermediate organoborane.

The products obtained from alkenes with an internal double bond always consisted of mixtures of positional isomers which reflect the random addition of boron at either carbon atom of the unsaturated centre.

Our hopes that various functional groups in the near neighbourhood of the unsaturated centre would possibly have a directing influence on the course of the hydroboration did not materialise.

Substrate (methyl ester)	organoborane reacted with	derivative	yield %
(i) 18:1 (9c)	H_2O_2/NaOH	9(10)-OH 18:0	95
	$HCrO_4$	9(10)-oxo 18:0	82
	O_2/H_2O_2	9(10)-OOH 18:0	56
(ii) 12-OCH_3 18:1 (9c)	H_2O_2/NaOH	9(10)-OH,12-OCH_3 18:0	34
(iii) 12-OAc 18:1 (9c)	H_2O_2/NaOH	9(10),12-diOH 18:0	66
(iv) 12-OH 18:1 (9c)	H_2O_2/NaOH	9(10),12-diOH 18:0	80
(v) 18:2(9c12c)	H_2O_2/NaOH	9(10),12(13)-diOH 18:0	70
(vi) 18:1 (9a)	H_2O_2/NaOH	9(10)-oxo 18:0	87
	CH_3COOH	18:1 (9c)	82
(vii) 11:1 (10)	H_2O_2/NaOH	11-OH 11:0	9-
	$AgNO_3$/NaOH	$CH_3OOC(CH_2)_{20}COOCH_3$	93
(viii) cyclohexene	O_2/H_2O_2	hydroperoxy cyclohexane	65
(ix) tridec-1-ene	H_2O_2/NaOH	1-OH-tridecane	88
	O_2/H_2O_2	1-OOH-tridecane	14
	$AgNO_3$/NaOH	hexacosane	91

EXPERIMENTAL

Solvents

All solvents were reagent grade unless otherwise stated. Petroleum was distilled and the fraction boiling between 40°C and 60°C was used. Ether was first dried by standing over anhydrous calcium chloride. After decanting it was distilled and stored over sodium wire. Benzene and toluene were dried and stored in the same way. Tetrahydrofuran (THF) was first dried over sodium and then refluxed with lithium aluminium hydride for two hours and distilled. Diglyme (dimethyl ether of diethylene glycol) was stored over calcium hydride for three days. After decantation it was distilled from lithium aluminium hydride. Acetone was dried by storage over anhydrous sodium sulphate. Methanol was dried by Vogel's method[147]. Pyridine was refluxed for two hours with potassium hydroxide pellets before distillation. The dry pyridine was stored over potassium hydroxide.

Chromatographic analysis

Thin layer chromatography (TLC)

Analytical TLC was carried out on glass plates coated with 0.25 mm layers (wet thickness) of silica gel G. For silver ion TLC the silica gel contained silver nitrate (10% w.w). The plates were activated for 1 h at 120°C. For separation on a preparative scale, plates (20 cm x 20 cm) with a silica layer of 1 mm (wet thickness) were activated for 2 h at 120°C.

Ether-petroleum mixtures were used as developing solvents. The abbreviation PE30 stands for a mixture of petroleum and ether in a ratio of 70 to 30 (v:v).

Spots on analytical TLC were generally detected by spraying with an ethanolic solution of phosphomolybdic acid (10%) followed by heating for ca. 15 min at 120°C [148].

For the specific detection of epoxy compounds, the plates were sprayed with ethanolic picric acid (0.05 M) and placed for 30 min in a tank saturated with the vapours of an ether-ethanol-acetic acid mixture (80:2:1, v:v:v). Exposure to ammonia vapour showed the epoxides as orange spots on a yellow background[149].

Peroxides were apparent as red-brown spots after spraying with a reagent prepared by dissolving ferrous sulphate (4 g) and ammonium thiocyanate (4 g) in hydrochloric acid (1M, 70 ml). The spray reagent was decolourised prior to use by washing with pentan-1-ol. The quickly fading peroxide spots had to be marked before the phosphomolybdic acid spray was applied to show any other components.

Preparative plates were sprayed with an ethanolic solution of 2,7-dichlorofluorescein (0.2%) and viewed under ultraviolet light. Bands were scraped off and extracted with ether or ether-methanol mixtures. Where necessary, residual 2,7-dichlorofluorescein was removed by percolation through a column of Florisil using ether as eluting solvent.

The order of the separated bands according to their decreasing Rf values is A, B, C etc.

Gas liquid chromatography (GLC)

GLC was carried out with a Pye 104 chromatograph fitted with a flame ionisation detector. The columns used were of stainless steel (1.52 m x 4.75 mm i.d.) and contained two different stationary phases: diethylene glycol succinate (DEGS, 20% coated on HMDS chromosorb W, 80-100 mesh) or Apiezon L (ApL, 3% coated on AW-OCMS chromosorb G, 80-100 mesh). The number of theoretical plates[98] was about 1400 for the ApL and about 1700 for the DEGS column, the latter of which deteriorated markedly with use. Nitrogen was used as carrier gas. The flow rate was usually 40 ml/min. The normal oven temperatures were 210°C and 190°C for ApL and DEGS

columns, respectively. These operating conditions were varied according to the nature of the substance under consideration.

Peak height times peak width at half height gave peak areas. Saturated straight chain methyl esters were used as internal (or external) standards for the determination of ECL values[94].

Spectroscopic analysis

Infrared spectroscopy (IR)

IR spectra were recorded on Perkin-Elmer 237 and 257 spectrometers. Samples were either run as films between sodium chloride discs or as ca. 1% solutions in carbon tetrachloride or carbon disulphide using sodium chloride cells, 1 mm wide.

Nuclear magnetic resonance spectroscopy (NMR)

Spectra were recorded at 60 MHz on a Perkin-Elmer R10 spectrometer and at 100 MHz on a Varian HA100 instrument using ca. 15% solutions in carbon tetrachloride which contained some tetramethylsilane as internal standard. 220 MHz spectra were recorded under an SRC scheme on a Varian HR220 instrument at the PCMU, Harwell, Didcot, Berks.

All shift values are given in ppm downfield from tetramethylsilane (δ=0). Coupling constants are given in Hz. The following abbreviations are used in the text for describing the appearance of NMR signals: s (singlet), d (doublet), t (triplet), qua (quartet), qui (quintet), and m (multiplet).

Mass spectrometry (MS)

Mass spectra were recorded with direct-probe insertion of samples into the source of an AEI MS902 mass spectrometer. The source pressure was 2×10^{-7} torr and the temperature ca. 200°C. The ionisation potential was 17 or 70 eV with respective ion currents of 100 and 500 μA.

Melting points

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected.

General chemical procedures

Esterification

For small scale methylations the fatty acid (up to 500 mg) was refluxed for 30 min with a 2% solution of boron trifluoride-methanol complex[150] in dry methanol (20 ml). Larger scale methylations were carried out by refluxing the acid for 1 h with methanolic sulphuric acid (0.5 M). The cooled reaction mixture was poured into brine and extracted with ether (2x). The ether extracts were washed with aqueous sodium hydrogen carbonate (5%, 2x) and brine (2x), combined and dried over anhydrous sodium sulphate.

Transesterification

Glycerides were converted to methyl esters by refluxing for 30 min with methanolic sodium methoxide (0.1 M). The reaction mixture was carefully acidified, diluted with water and extracted with ether.

Trimethylsilylation[151]

The hydroxy compound (5 mg) dissolved in dry pyridine (1 ml) was shaken for 1 min with hexamethyldisilazane (0.2 ml) and trimethylchlorosilane (0.1 ml). After standing for 5 min the pyridine was removed under vacuum and the residue extracted with ether.

Purdie methylation[152]

Hydroxy esters (100 mg) were converted to their methyl ether derivatives by refluxing for 6 h with freshly prepared silver oxide (85 mg) and methyl iodide (2.5 ml). The reaction mixture was cooled, diluted with ether and filtered. The product was purified by preparative TLC.

Preparation of methanesulphonates (mesylation) of vicinal dihydroxy esters and their conversion to alkenoates[153]

Methanesulphonyl chloride (1.4 g) was slowly added to a stirred solution of dihydroxy ester (1 g) in dry pyridine (15 ml). The reaction mixture was cooled so that the temperature did not exceed 20°C. After stirring for 4 h, ice (20 g) was added followed by hydrochloric acid (4 M, 60 ml) and the dimesyl derivative was extracted with ether. The completeness of the reaction was affirmed by the absence of any OH stretching in the IR spectrum and by the 1:2 intensities of the NMR signals at 3.68 ($-COOCH_3$) and 4.08 ($-SO_2CH_3$).

A mixture of the dimesylate (1.4 g), sodium iodide (4.0 g) and acid-washed and dried zinc dust (2.8 g) in DMF (30 ml) was refluxed for 6 h. The reaction mixture was cooled and filtered. The filtrate was poured into brine, acidified with dilute hydrochloric acid (4 M) and extracted with ether. The ether extracted product containing some free acid was reesterified to give the methyl alkenoate (0.7 g).

Bromination-dehydrobromination

This procedure was used to convert enoic esters into ynoic compounds: bromine was added dropwise to a stirred solution of alkenoate (0.5 g) in carbon tetrachloride (30 ml) until a yellow colour persisted. After stirring for 30 min the mixture was washed with aqueous sodium thiosulphate (5%, 2x) and water (2x) and dried over anhydrous sodium sulphate. The crude dibromide (0.76 g) remaining after removal of the solvent was refluxed for 2 h with ethanolic potassium hydroxide (1 M, 10 ml). After acidification with dilute sulphuric acid (2 M) and dilution with water, the alkynoic acid was extracted with ether.

Partial hydrogenation[154]

The acetylenic compound to be hydrogenated was purified by percolation through a Florisil column prior to partial hydrogenation which was effected with a Lindlar catalyst (ROCHE) at atmospheric pressure using methanol as solvent. When the conversion of alkyne to cis-alkene was complete - usually within 5 min - the hydrogen uptake ceased sharply. The catalyst was filtered off and the solvent removed under vacuum.

Reduction with lithium aluminium hydride

For conversion to alcohols, esters or acids (100 mg) dissolved in dry ether (5 ml) were added to a well stirred suspension of lithium aluminium hydride (20 mg) in dry ether (2 ml). After stirring at room temperature for 15 min, wet ether and then water was added to destroy excess hydride. The mixture was acidified with hydrochloric acid (4 M) and extracted with ether.

Reduction with sodium borohydride

Selective reduction of oxo esters to hydroxy esters: sodium borohydride (30 mg) was added to the stirred solution of methyl oxo ester (30 mg) in methanol (3 ml). After 30 min the mixture was diluted with water, acidified and extracted with ether.

Synthesis of some Δ9,12-diunsaturated C_{18} esters and epoxidation and hydroxylation procedures

Methyl octadec-trans-12-en-9-ynoate (18:2 9a12t)

methyl threo-12,13-dihydroxy 18:1 (9a)

↓ (i) MsCl, pyridine
↓ (ii) NaI, Zn, DMF

methyl 18:2 (9a12t)

Methyl threo-12,13-dihydroxyoctadec-9-ynoate (4.1 g), which was available in pure form (its bis-TMS ether derivative having an ECL of 20.1 on ApL and 21.8 on DEGS), was converted to the dimesyl ester (5.6 g) which did not show any hydroxy absorption in the infrared. Its NMR spectrum contained two strong signals at δ3.04 and 3.08, each representing three protons. Demesylation with sodium iodide and zinc in DMF yielded a product (3.3 g) which was eluted from a Sorbsil column (50 x 2 cm) with 200 ml portions of P, PE5, PE10 and PE20. Methyl octadec-trans-12-en-9-ynoate (2.45 g, 67% yield based on the dihydroxy ester) was eluted with PE5 and PE10 and had ECL values of 18.0 (ApL) and 21.4 (DEGS). Its NMR spectrum contained signals at δ 0.89 (t, CH_3CH_2-), 1.33 (m, in chain -(CH_2)-), 1.60 (m, -$CH_2CH_2CO_2Me$), 1.97 (m, -$CH_2CH{=}CHCH_2C{\equiv}C$-), 2.12 (m, -CH=CH-$CH_2C{\equiv}CCH_2$), 2.23 (t, -$CH_2COOMe$), 2.80 (m, -CH=CH$CH_2C{\equiv}C$-), 3.59 (s, -$COOCH_3$), 5.39 and 5.52 (m, -CH=CH-).

Methyl octadeca-cis-9,trans-12-dienoate (18:2 9c12t)

Partial hydrogenation of methyl octadeca-trans-12-en-9-ynoate with Lindlar's catalyst gave one major product contaminated with several minor components as indicated by GLC. (ECL 17.7 (95%), 18.1 (2%), 20.2 (2%) on ApL and 18.5 (4%), 19.3 (92%), 21.4 (1%), 23.8 (3%) on DEGS). Pure methyl octadeca-cis-9,trans-12-dienoate [ECL 17.7 (ApL), 19.3 (DEGS)] was isolated by preparative silver ion TLC (PE30). Its NMR showed absorption at δ 0.89 (t, CH_3CH_2-),

1.30 (m, in chain $-(CH_2)-$), 1.58 (qui, 7Hz, $-CH_2CH_2CH_2CO_2Me$), 1.96 (m, $-CH_2CH=CHCH_2-CH=CH_2CH_2-$), 2.21 (t, $-CH_2CO_2Me$), 2.62 (m $-CH=CH-CH_2CH=CH-$), 3.58 (s, $COOCH_3$), and 5.33 (m, $-CH=CH-$).

Methyl octadec-cis,12-en-9-ynoate (18:2 9a12c)

methyl erythro-12,13-dihydroxy 18:1 (9c)

(i) Ac_2O
(ii) Br_2,CCl_4
(iii) KOH,EtOH
(iv) MeOH,H_2SO_4
(v) MsCl,pyridine
(vi) NaI,Zn,DMF

methyl 18:2 (9a12c)

Methyl erythro-12,13-dihydroxyoctadec-cis-9-enoate (5.2 g), which was available, was acetylated by refluxing with acetic anhydride. The diacetoxy ester was then brominated, dehydrobrominated with ethanolic potassium hydroxide and remethylated. The acetylenic erythro-12,13-dihydroxy ester (1.78 g, 35%) was eluted from a Sorbsil column with PE50 and PE60. After mesylation, demesylation and remethylation, methyl octadec-cis-12-en-9-ynoate (1.05 g, 56%) was eluted from a Sorbsil column with PE5 and PE7.5. Silver ion TLC (PE30) and the NMR spectrum showed the ester to be free of any trans olefinic by-product. Its ECL was 17.9 on ApL and 21.4 on DEGS.

Methyl octadeca-trans-9,trans-12-dienoate (18:2 9t12t)

Linoleic acid (97% , 2.64 g), dissolved in ether (100 ml) was shaken for 20 min with an ice cold mixture of aqueous sodium nitrite (2 M, 35 ml), and nitric acid (6 M, 23 ml)[155]. The ether layer was washed with an aqueous solution of sodium thiosulphate (5%, 2x) and brine (2x). The solvent was removed and the product methylated. Preparative silver ion chromatography (PE20) gave three bands. The least mobile band (C, 4%) had the same Rf value as methyl linoleate, whilst band B (38%) was comparable with methyl 18:2 (9c12t). Band A (0.56 g, 57%) had ECL values of 17.65 (ApL) and

19.2 (DEGS) and showed strong absorption at 975 cm^{-1}. Its NMR spectrum had signals at δ 0.89 (t, CH_3-C), 1.30 (m, in chain -CH_2-), 1.58 (qui, 7 Hz, -$CH_2CH_2CO_2Me$), 1.96 (m, -CH_2-CH=CH-CH_2-CH=CH-CH_2-), 2.21 (t, -CH_2CO_2Me), 2.62 (m, -CH=CHCH_2CH=CH-), 3.58 (s, -$COOCH_3$)- and 5.33(-CH=CH-).

Methyl octadec-cis-9-en-12-ynoate (18:2 9c12a) (methyl crepenynate) (A) Attempted synthesis from ricinoleate

$$CH_3(CH_2)_5CH(OH)CH_2\underset{cis}{CH=CH}(CH_2)_7COOGL$$

(Castor oil, ca. 85%, ricinoleate)

↓ (i) Br_2, CCl_4 (ii) KOH, EtOH (iii) MeOH, H_2SO_4

$$CH_3(CH_2)_5CH(OH)CH_2C\equiv C(CH_2)_7COOCH_3 \qquad (1)$$

↓ $Pb(OAc)_4$

$$AcOCH_2C\equiv C(CH_2)_7COOCH_3 \qquad (2)$$

↓ (i) H_2, Lindlar's catalyst (ii) NaOMe, MeOH (iii) PBr_3

$$BrCH_2\underset{cis}{CH=CH}(CH_2)_7COOCH_3 \qquad (3)$$

↓ $CH_3(CH_2)_4C\equiv CMgBr, Cu_2(CN)_2$

methyl 18:2 (9c12a) (4)

Methyl 12-hydroxyoctadec-9-ynoate (1)

Castor oil (40 g, ricinoleic acid constituting 85% of its fatty acid content) was brominated, dehydrobrominated and finally methylated to give a crude product (38.5 g) which had a broad IR absorption band at 3300-3600 cm^{-1} but did not show any other absorption above 3000 cm^{-1}. Its TMS ether derivative gave several peaks on GLC corresponding with ECL of 18.3 (7%) and 19.3 (91%) on ApL and 20.4 (4%), 21.2 (87%) and 21.8 (8%) on DEGS. A sample of the major component, ie. the expected ricinstearolate, which was

obtained by preparative TLC (PE40) gave an NMR spectrum with signals at δ 0.86 (t, CH_3-C), 1.3-1.4 (m, in chain -CH_2-), 1.58 (m, -$CH_2CH_2CO_2Me$), 2.0-2.35 (m, -CH_2CO_2Me and -$CH_2C{\equiv}CCH_2$-), 3.55 (s, -$COOCH_3$ obscuring a broad m -CH(OH)-). No absorption for olefinic protons was observed.

Methyl 11-acetoxyhendec-9-ynoate (2) [compare ref. 156]

A mixture of crude methyl ricinstearolate (85%, 36.1 g), lead tetraacetate (53.6 g, 25% excess) and calcium carbonate (12.5 g) in dry benzene (1200 ml) was refluxed for 20 h with continuous drying of the solvent by passage through a Soxhlet containing anhydrous calcium chloride. The cooled reaction mixture was filtered using a Büchner funnel. The filtrate was washed with brine, aqueous sodium hydrogen carbonate (5%, 2x) and finally with brine (2x). After drying over anhydrous sodium sulphate, the solvent was removed and an oily product (36.9 g) was obtained. The IR spectrum had no O-H stretching and TLC (PE20) showed some more mobile components in addition to the major spot with Rf 0.45, but no ricinstearolate (Rf 0.23). The reaction product was subjected to a vacuum distillation under nitrogen. The first fraction (2.2 g, 81-84°C/2 mm Hg) was a mixture of compounds with ECL (ApL) smaller than 10. The second fraction (23.2 g, 152-157°C/1 mm Hg) had the following ECL : 13.8 (7%), 14.6 (92%) on ApL and 20.4 (2%), 21.8 (3%) and 23.0 (91%) on DEGS. The IR spectrum indicated an acetylenic bond (2250 cm^{-1}) and two ester functions (1740, 1230 and 1175 cm^{-1}). It also showed an unexplained absorption band at 970 cm^{-1} which did not arise from the bending vibration of a trans olefinic C-H bond as the NMR spectrum contained no signal for any olefinic proton. NMR signals were observed at δ 1.4 (m, in chain -CH_2-), 2.03 (s, CH_3COO-), 2.25 (m, -$C{\equiv}CCH_2CH_2$- and -CH_2CO_2Me), 3.58 (s, -$COOCH_3$), and 4.54 (m, 3Hz, $AcOCH_2C{\equiv}C$-).

Methyl 11-bromohendec-cis-9-enoate (3)

Two batches (ca. 5 g) of methyl 11-acetoxyhendec-9-ynoate were percolated with PE20 through a Florosil column and then partially hydrogenated in methanol (120 ml) using Lindlar's catalyst (250 mg). The product showed IR absorption bands at 3020, 1730 and 1240-1160 cm^{-1}, whilst the band at 2250 cm^{-1}, observed in the spectrum of the acetylenic starting material had disappeared. GLC also indicated that all acetylenic ester had been hydrogenated mainly to the olefinic product which was accompanied by some minor products [ECL 11.1 (6%), 14.3 (93%) on ApL and 12.4 (12%), 20.2 (2%), 20.8 (86%) on DEGS].

The olefinic acetoxy ester (9.1 g) was dissolved in methanolic sodium methylate (0.25 M, 125 ml) and stirred at room temperature for 18 h. After acidification (HCl, 2 M), the product was extracted with ether (3x) and washed neutral with brine (3x). The oil obtained (6.7 g) was eluted from a Sorbsil column (70 x 2 cm) with 150 ml oortions of P, PE5, PE10, PE20 and 200 ml portions of PE40, PE50 and PE60. Methyl 11-hydroxyhendec-cis-9-enoate (4.75 g, 71%) was eluted with PE40 and PE50 and appeared to be pure on GLC [ECL 13.3 (ApL) and for the TMS ether derivative 16.1 (DEGS)]. The IR spectrum showed O-H stretching and the NMR spectrum contained signals at δ 1.34 (m, $-(CH_2)_4-$), 1.57 (m, $-CH_2CH_2CO_2Me$), 2.02 (m, $-CH=CHCH_2CH_2-$), 2.22 (t, $-CH_2CO_2Me$), 3.60 (s, $-COOCH_3$), 4.04 (d, 5Hz, $HOCH_2CH=CH-$) and 5.32 (m, $-CH=CH-$). A less polar compound (1.2 g) eluted with PE10 had an ECL of 11.1 (ApL) and showed no O-H stretching in the infrared. Its NMR spectrum had signals at δ 1.30 (8H), 1.57 (5H), 1.96 (2H), 2.21 (2H), 3.58 (3H) and 5.30 (2H). The signal at δ 1.57, representing five protons, was an intense doublet (5 Hz) superimposed upon the weak multiplet for the methylene group β to the methoxy group. As the signal at δ 4.04 ($HOCH_2CH=CH-$) was missing, this compound was thought to be methyl hendec-cis-9-enoate formed by hydrogenolysis during the

partial hydrogenation of the acetylenic acetoxy ester.

Phosphorus tribromide (3.42 g) was slowly added to a well stirred and cooled solution of methyl 11-hydroxyhendec-cis 9-enoate (4.42 g) in dry ether (15 ml) and dry pyridine (1 ml) and the reaction mixture was refluxed for 4 h. After cooling, water was added and the product was extracted with ether (2x). The ether extracts were washed with brine, aqueous sodium hydrogen carbonate (5%, 2x) and finally with brine (2x). The product (5.85 g) gave only one spot on TLC (PE30) and showed no O-H stretching in the infrared. Its NMR spectrum had signals at 1.32 (m, in chain $-CH_2-$), 1.58 (m, $-CH_2CH_2CO_2Me$), 2.06 (m, $-CH=CHCH_2CH_2-$), 2.22 (t, $-CH_2CO_2Me$), 3.60 (s, $-COOCH_3$), 3.88 (d, 6 Hz, $-BrCH_2CH=CH-$), and 5.67δ (qui, $-CH=CH-$).

Methyl octadec-cis-9-en-12-ynoate (4)

A three-necked flask equipped with a reflux condenser, dropping funnel and magnetic stirrer was flushed with nitrogen and charged with acid washed magnesium turnings (106 mg), dry THF (10 ml) and a few iodine crystals. Bromoethane (476 mg) was added dropwise and the reaction was started by gentle heating. After stirring the Grignard reaction mixture at room temperature for 1.5 h, a solution of hept-1-yne (383 mg, prepared according to Gunstone and Lie Ken Jie[79]) in dry THF (5 ml) was added slowly and stirring was continued at room temperature for 2.5 h. Then cupric cyanide (50 mg) was added under vigorous stirring followed after 10 min by methyl 11-bromo-hendec-cis-9-enoate (1.220 g) in one portion. The reaction mixture was refluxed for 18 h and then poured on ice. The ether extracted product was separated by preparative TLC (PE15) into three fractions A (Rf 0.83), B (Rf 0.77) and C (Rf 0.70). TLC and the NMR spectrum indicated that fraction C (30%) contained unreacted bromo ester, whereas fraction B (51%) gave a similar NMR spectrum which showed a triplet (7 Hz) at 1.23δ and a quartet (7Hz) at 4.05δ instead of

the usual methoxy signal at 3.60δ. Band B was possibly ethyl 11-bromo-hendec-cis-9-enoate. The NMR spectrum of band A (17%) showed it to be the expected condensation product with signals at δ 0.91 (t, 6Hz, CH_3-C), 1.32 (m, in chain -CH_2-), 1.59 (m, 7Hz, -$CH_2CH_2CO_2Me$), 2.05 (m, C(8) and C(13) methylene groups), 2.22 (t, 7 Hz, -CH_2CO_2Me), 2.79 (m, -CH=CHCH_2C≡C-), 3.60 (s, -$COOCH_3$) and 5.37 (m, 6Hz, -CH=CH-).

Attempts to condense the bromo ester with the lithium derivative of hept-1-yne proved even less successful in my hands than the condensation via the Grignard compound of hept-1-yne. No further efforts were made to improve this condensation step and methyl crepenynate was synthesised by a somewhat modified route B, devised by Bradshaw et al.[157].

(B) Synthesis of methyl crepenynate via a Wittig reaction

$CH_3(CH_2)_4C{\equiv}CH$

↓ (i) $LiNH_2$, liquid NH_3; (ii) ethylene oxide

$CH_3(CH_2)_4C{\equiv}CCH_2CH_2OH$ (1)

↓ PBr_3

$CH_3(CH_2)_4C{\equiv}CCH_2CH_2Br$ (2)

↓ PPh_3, toluene

$CH_3(CH_2)_4C{\equiv}CCH_2CH_2\overset{+}{P}Ph_3Br^-$ (3)

↓ (i) n-butyllithium, Et_2O; (ii) $OCH(CH_2)_7CO_2Me$ (4)

$CH_3(CH_2)_4C{\equiv}CCH_2\underset{cis}{CH=CH}(CH_2)_7CO_2Me$ (5)

Non-3-yn-1-ol (1)

Hept-1-yne (22.5 g), which had been prepared from 1-bromo-pentane and sodium acetylide[79], was added in dry ether (50 ml) over a period of 50 min to a solution of lithium amide prepared from lithium (2 g), ferric nitrate (0.2 g), and liquid ammonia (500 ml)

cooled in an acetone-CO_2 bath. After stirring for 3.5 h, ethylene oxide (15.5 g, 50% excess) in dry ether (50ml) was added in one portion. The reaction mixture was stirred overnight and then treated successively with ammonium sulphate, water and dilute sulphuric acid (2 M). The ether extracted product was shown by GLC to contain hept-1-yne (32%). Non-3-yn-1-ol (17.7 g, 54%), obtained by vacuum distillation (58-59°C/2 mm Hg), appeared as a single peak on both ApL and DEGS columns. Its NMR spectrum contained signals at δ 0.91 (t, CH_3-), 1.37 (m, -$(CH_2)_3$-), 2.10 (m, 2Hz and approx. 7Hz, -$(CH_2)_3CH_2C{\equiv}C$-), 2.32 (m, 2Hz and 7Hz, -$C{\equiv}CCH_2CH_2OH$), 2.84 (s, broad, -O-H), and 3.57 (t, 7Hz, -CH_2OH).

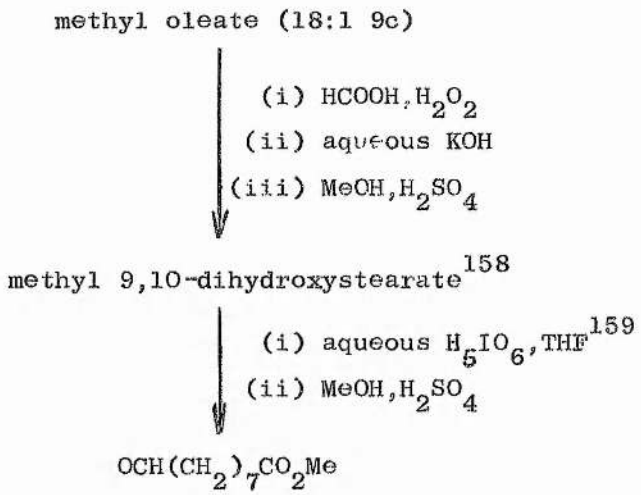
1-Bromonon-3-yne (2)

Freshly distilled phosphorus tribromide (14.6 g) was slowly added to a stirred solution of non-3-yn-1-ol (15.4 g) in dry ether (50 ml) at 0°C. After refluxing for 3 h, the reaction mixture was poured into ice water and extracted with ether (2x). The product was then distilled to yield 1-bromonon-3-yne (7.4 g, 31%, b.p. 72-74°C/3 mm Hg). The NMR showed signals at δ 0.91 (t, CH_3-), 1.37 (m, -$(CH_2)_3$-), 2.10 (m, 2Hz and approx. 7Hz, -$(CH_2)_3CH_2C{\equiv}C$-), 2.65 (m, 2Hz and 7.5Hz, -$C{\equiv}CCH_2CH_2Br$) and 3.35 (t, 7.5Hz, -CH_2Br).

Non-3-yn-1-yltriphenylphosphonium bromide (3)

A mixture of 1-bromonon-3-yne (7.2 g) and triphenylphosphine (12.25 g, 30% excess) in dry toluene (100 ml) was refluxed under nitrogen for 72 h. After two thirds of the solvent had been distilled off, the oily product was triturated with dry hexane (100 ml) to give crude non-3-yn-1-yltriphenylphosphonium bromide (14.2 g, 85%) which after recrystallisation from ethyl acetate melted at 128-132°C (lit. 133-134°C [157]).

Methyl 8-formyloctanoate (4)



Hydrogen peroxide (30%, 4.2 g) was added during 15 min to a well stirred solution of methyl oleate (83%, the other components being methyl palmitate and methyl stearate, 12.2 g) in formic acid (90%, 40 ml). The reaction temperature was not allowed to exceed 40°C. After 1.5 h sodium hydrogen sulphite (0.2 g) was added and the formic acid was distilled off under reduced pressure. The oily reaction product was washed with water (15 ml, 3x) and boiled with aqueous sodium hydroxide (3M, 100 ml) for 3 h. The reaction mixture was poured on to an excess of hot dilute hydrochloric acid (4 M). The oily product separated and solidified on cooling. The aqueous layer was discarded and the solid remelted and washed with hot water. The crude dihydroxy acid (m.p. 78-82°C) was refluxed for 1 h with methanolic sulphuric acid (2%, 50 ml) to give the solid dihydroxy ester (11.5 g). GLC of its bis-TMS ether derivative showed that the conversion of oleate to dihydroxystearate had been quantitative (ECL 19.9 (85%) on ApL and 20.4 (87%) on DEGS).

Potassium periodate (5.8 g, 20% excess) in aqueous sulphuric acid (1 M, 30 ml), at 20°C was rapidly added to a solution of methyl 9,10-dihydroxystearate (87%, 8.0 g) in THF (40 ml) at 40°C. The reaction mixture was shaken vigorously for 30 min and after dilution with water it was extracted with ether (2x). The ether

extracts were washed with saturated aqueous sodium hydrogen carbonate (2x) and with brine (2x). Distillation afforded methyl 8-formyloctanoate (3.54 g, 90%, b.p. 105-109°C/2 mm Hg, ECL (ApL) 16.8). Its NMR spectrum showed signals at δ 1.32 (m. $-(CH_2)_4-$), 1.59 (m, $-CH_2CH_2CO_2Me$), 2.22 (t, $-CH_2CO_2Me$) partially overlapping with 2.36 (m, 1.5 Hz and 7Hz, $-CH_2CHO$), 3.59 (s, $-COOCH_3$) and 9.66 (t, 1.5Hz, $-CHO$).

Methyl crepenynate (5)

A solution of n-butyllithium[160] in hexane (1.13 M, 20 ml) was slowly added to a rapidly stirred suspension of non-3-yn-1-yltriphenylphosphonium bromide (12.25 g) in dry ether (60 ml) contained in a flask fitted with a condenser and a mercury-sealed gas outlet tube. The orange-red suspension was stirred for 15 min under nitrogen atmosphere and protected from light. The methyl 8-formyloctanoate (3.48 g) in dry ether (15 ml) was added. The orange colour faded and a white precipitate formed. The reaction mixture was stirred at room temperature for 1.5 h and refluxed for another hour. The cooled solution was filtered, diluted with water and acidified with dilute hydrochloric acid (2 M). The ether extracted product (5.5 g) was eluted from a Sorbsil column (50 x 2 cm) with 150 ml portions of P, PE1, PE2, PE3, PE4, PE5, PE7.5 and PE10. A colourless oily liquid (2.47 g, 45%) was recovered from the eluates PE3, PE4 and PE5. TLC (PE20) showed three poorly separated components (Rf 0.63, 0.60 and 0.56) the most polar of which resembled methyl crepenynate isolated from Afzelia cuanzensis oil[161]. Silver ion TLC (PE25) also indicated three components with marginally different Rf values. GLC (DEGS) gave three major peaks corresponding with ECL values of 21.4 (59%), 23.2 (7%) and 23.6 (14%). Repeated separation by preparative TLC (PE12.5) yielded the most polar fraction [ECL 17.9 (ApL) and 21.4 (DEGS)] in pure form. An IR absorption band at 3020 cm^{-1} and the absence

of any distinct absorption around 975 cm^{-1} indicated a cis double bond. The NMR spectrum contained signals at δ 0.91 (t, CH_3-C), 1.31 (m, in chain -CH_2-), 1.59(qui, 6.5Hz, -$CH_2CH_2CO_2Me$), 2.05 (m (four protons), -CH_2CH=$CHCH_2C{\equiv}CCH_2$-), 2.23 (t, 7Hz, -CH_2CO_2Me), 2.80 (m, -CH=$CHCH_2C{\equiv}C$-), 3.60 (s, -$COOCH_3$) and 5.34 (m, -CH=CH-).

The purification of methyl crepenynate by preparative TLC also yielded fractions enriched in the more mobile components A and B. The NMR spectrum of component A [DEGS, ECL 23.2 (85%)] differed from that of methyl crepenynate only in signals for protons of the alcohol part of the ester: instead of the singlet at δ 3.60 (s, -$COOCH_3$) a two proton triplet (7Hz) was observed at δ 3.99. The integration of the triplet at δ 0.94 (CH_3C) indicated contributions from six protons, ie. two CH_3- groups. The signal at δ 1.32 (in chain -CH_2-) represented at least nine methylene groups, ie. at least two more than expected for methyl crepenynate. Compound A was probably n-butyl crepenynate. Transesterification with methanolic sodium methylate afforded methyl crepenynate. Component B (ECL (DEGS) 23.6) was considered to be ethyl crepenynate as its NMR spectrum contained a quartet (7Hz) at δ 4.05 (-$COOCH_2CH_3$) and a triplet (7Hz) at δ 1.21 (-$COOCH_2CH_3$).

Methyl octadec-trans-9-en-12-ynoate (18:2 9t12a)

methyl 18:2 (9c12a)

(i) $ArCO_3H$, $CHCl_3$
(ii) AcOH
(iii) KOH, MeOH
(iv) MeOH, H_2SO_4
(v) MsCl, pyridine
(vi) NaI, Zn, DMF

methyl 18:2 (9t12a)

Methyl cis-9,10-epoxyoctadec-12-ynoate (1.42 g), obtained by

epoxidation of methyl crepenynate, was refluxed in glacial acetic acid (40 ml) for 6 h. The acetic acid was distilled off and the residual oil poured into cold water and extracted with ether (2x). The ether extract was refluxed with methanolic potassium hydroxide (3 M, 50 ml) for 1 h. After cooling and acidification with sulphuric acid (2 M), the product was extracted with ether and boiled with methanolic sulphuric acid (0.45 M, 70 ml) to give methyl threo-9,10-dihydroxyoctadec-12-ynoate (1.40 g, 93%). The solid dihydroxy ester was mesylated and demesylated with sodium iodide and zinc in DMF solution. The product containing an appreciable amount of free acid was remethylated. The product which appeared on GLC as asingle peak (ECL 18.0 on ApL and 21.3 on DEGS) was separated by preparative silver ion TLC (PE20) into two bands A and B. The less polar band A (87%) had a strong absorption band at 975 cm^{-1} which was missing in the otherwise identical IR spectrum of band B (13%). The NMR spectrum of band A contained signals at δ 0.91 (t, CH_3-C), 1.30 and 1.38 (m, in chain -CH_2-), 1.55 (m, -$CH_2CH_2CO_2Me$), 2.03 (m (four protons), -$CH_2CH{=}CHCH_2C{\equiv}CCH_2$-), 2.21 (t, -$CH_2CO_2Me$), 2.78 (m, -CH=CHCH$_2$C≡C-), 3.58 (s, -$COOCH_3$), 5.37 and 5.50 (m, -CH=CH-).

Methyl octadeca-trans-9,cis-12-dienoate (18:2 9t12c)

Partial hydrogenation of methyl octadec-trans-9-en-12-ynoate using Lindlar's catalyst gave methyl octadeca-trans-9,cis-12-dienoate in quantitative yield. The dienoic ester was shown to be pure by silver ion TLC (PE20) and GLC (ECL 17.7 on ApL and 19.4 on DEGS). Its NMR spectrum contained signals at δ 0.89 (t, CH_3-C), 1.30 (m, in chain -CH_2-), 1.57 (m, -$CH_2CH_2CO_2Me$), 1.97 (m, -$CH_2CH{=}CHCH_2CH{=}CHCH_2$-), 2.21 (t, -$CH_2CO_2Me$), 2.66 (m, -CH=CHCH$_2$CH=CH-), 3.58 (s, -$COOCH_3$), and 5.30 (six line m, -CH=CH-).

Monoepoxidation of diunsaturated C_{18} esters

(a) m-Chloroperbenzoic acid

For determination of the peroxy content, a methanolic solution of m-chloroperbenzoic acid was allowed to react with an excess of aqueous potassium iodide. The liberated iodine was titrated with a standardised solution of sodium thiosulphate. The purity of m-chloroperbenzoic acid determined in this way was 91.5%.

(b) Diunsaturated C_{18} esters

All the diunsaturated methyl esters were purified by column chromatography (Sorbsil) before being epoxidised.

(c) Typical monoepoxidation procedure

A solution of m-chloroperbenzoic acid (91.5%, 320 mg, 1.7 mmole) in chloroform (10 ml) was added to a stirred solution of methyl octadecadienoate (500 mg, 1.7 mmole) in chloroform (10 ml,. The reaction mixture was stirred at room temperature for 5 h and then washed successively with aqueous sodium hydrogen sulphite (5%, 1x), sodium hydrogen carbonate (5%, 2x), and finally with brine (2x). All the aqueous solutions were extracted with ether (50 ml). The organic layers were combined and dried over anhydrous sodium sulphate. The product obtained after removal of the solvent was eluted from a Sorbsil column (M60, 50 g), with 75 ml fractions of petroleum mixed with increasing amounts of ether (2.5, 5, 7.5, 10, 12.5, 15, 20, 30, 40, 50, 60%). The order of elution was: unreacted ester (PE5), the two isomeric monoepoxy esters (PE10, 12.5 and 15) and the diepoxy ester (PE30 and 40) Recovery was usually above 90%. The two isomeric monoepoxides were then separated by preparative TLC (PE30) which had to be repeated once or twice where the resolution of the two isomers (A and B) was poor. Generally more than 80% of the material chromatographed could be recovered after each TLC separation.

Vic- dihydroxyoctadecenoic acids

The monoepoxy ester (30 mg) was refluxed with glacial acetic acid (2 ml) for 6 h, water was added and the product was extracted with ether (75 ml, 2x). The product was then boiled with methanolic potassium hydroxide (1 M, 2 ml) for 1 h. The reaction mixture was diluted with water, acidified with dilute hydrochloric acid (4 M) and extracted with ether (7.5 ml, 2x). Removal of the solvent yielded the crude dihydroxy acid which was recrystallised repeatedly from ether-petroleum mixtures. The melting points determined are listed on page 61.

Methyl bis(trimethylsilyloxy)-octadecenoates

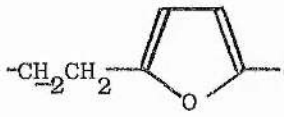
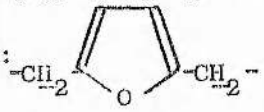
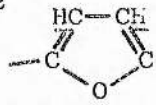
Each unsaturated dihydroxy acid (10 mg) was converted to the methyl ester by reaction with methanolic boron trifluoride. Subsequent silylation gave the crude bis-TMS ether derivative which was purified by preparative TLC (PE10) for gas chromatographic and mass spectroscopic examination.

Mixed methyl TMS diether derivatives

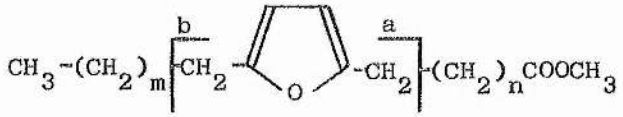
Each unsaturated monoepoxy ester (10 mg) was refluxed for 30 min with methanolic boron trifluoride (2%, 2 ml). Water was added to the cooled reaction mixture which was then extracted with ether (7.5 ml, 2x). The ether extracts were washed with aqueous sodium hydrogen carbonate (2x) and brine (2x) and dried over anhydrous sodium sulphate. After removal of the solvent, the hydroxy methoxy ester was silylated. The methyl TMS diether was purified by preparative TLC (PE20) for characterisation by GLC and MS.

Rearrangement of methyl cis 6,7,cis-9,10-diepoxystearate

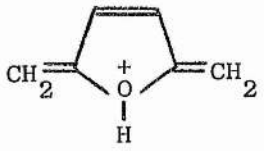
A mixture of methyl cis-6,7,cis-9,10-diepoxystearate (387 mg, 1.19 mmole) and dioxan (20 ml) containing boron trifluoride etherate (0.15 ml, 1.20 mmole) was stirred at room temperature for 18 h. After dilution with water, the product (376 mg) was isolated by extraction with ether. Its GLC showed major peaks with ECL 18.0 and 20.3 on ApL and 21.2 and 29.6 on DEGS. Separation by preparative TLC (PE35) gave five fractions: A (117 mg, 33%), B (18 mg, 5%), C (17 mg, 5%), D (54 mg, 15%) and E (152 mg, 42%).

Fraction A [33%, ECL 18.0 (ApL) and 21.2 (DEGS)] showed absorption at 3100, 1740, 1570 and 1020 cm^{-1} in the infrared and at 224 nm in the ultraviolet. Its NMR spectrum contained signals characteristic for long chain esters [δ 0.88, 1.28, 2.24 and 3.58] along with a multiplet at 1.62 δ (six protons: ), a distorted triplet at 2.52δ (J=7Hz, four protons: ) and a poorly resolved multiplet at 5.72δ (two protons ). The most prominent feature of its mass spectrum are listed below. (Intensities (I) are expressed as percentages related to the underlined base peak)

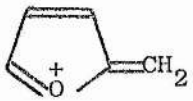
	m/e	I		m/e	I
(M)	308	16		149	14
(M-31)	277	6		135	22
(b,n=4)	209	32		121	90
(a,m=7)	207	15		107	56
(b,n=3)	195	22	(c)	95	100
(a,m=6)	193	32	(d)	81	42
(209-32)	177	16	(e)	69	21
(195-32)	163	18			



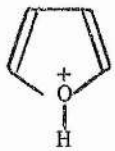
n = 3, m = 7
n = 4, m = 6



(c)



(d)



(e)

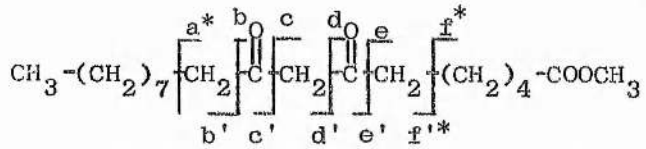
The fragment ions (c), (d) and (e) are stabilised by delocalisation of the positive charge over the furan ring system.

Fraction B (5%) had broad IR absorption bands at 3500 and at 1740-1710 cm^{-1} and gave a faint reaction with picric acid. No further examination was carried out.

Fraction C (5%). Only the IR spectrum was recorded. This showed absorption at 3000, 1740, and 1070 cm^{-1}.

Fraction D [15%, ECL 20.3 (ApL) and 29.6 (DEGS)] had infrared peaks at 1740 and 1710 cm^{-1}, but none above 3000 cm^{-1}. The test with picric acid did not indicate any 1,2-epoxide structure. The NMR spectrum contained signals at δ 0.88 (t, CH_3-CH_2-), 1.27 (m, in chain -CH_2-), 1.57 (m, six protons: -$CH_2CH_2COCH_2COCH_2CH_2CH_2CH_2CH_2CO_2Me$), 2.15-2.45 (m, six protons: -$CH_2COCH_2COCH_2(CH_2)_3CH_2CO_2Me$), 2.55 (s, -$COCH_2CO$-); and 3.59 (s, -$COOCH_3$). Some details of the mass spectrum are given below and tentative assignments are made for some of the fragments:

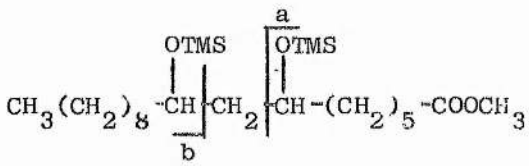
	m/e	I		m/e	I		m/e	I
(M)	326	2		196	35		153	30
(M-31	295	3		185	12		143	50
	249	5		183	10		141	30
	228	6	(a^*-32)	182	15	(c-32)	139	60
(a^*)	214	11		181	15	(b')	127	35
(f'*)	212	6	(c)	171	35	(d-32)	125	40
	211	9	(d')	169	10	(i^*)	114	60
	210	10	(b-32)	167	35		113	30
(b)	199	4	(d)	157	15		111	100
(e')	197	12	(c')	155	20	(e-32)	97	60

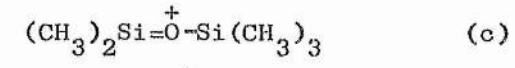


Fragments marked with an asterisk arise from McLafferty rearrangement.

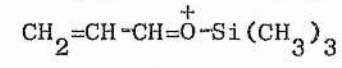
Part of fraction D was reduced with sodium borohydride in dry methanol to a compound which showed absorption peaks at 1740 cm^{-1} and 3350 to 3650 cm^{-1}. The mass spectrum of its TMS derivatives contained the following peaks:

	m/e	I		m/e	I		m/e	I
(M-15)	459	< 1	(a)	231	17		170	9
	271	16	(b)	229	21		149	11
	269	10		217	23	(c)	147	20
	257	29		215	17	(d)	129	49
	255	10		185	8	(e)	73	100

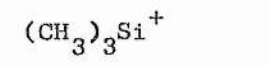




(c)



(d)



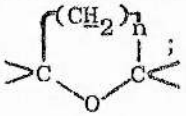
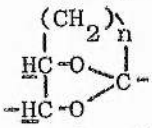
(e)

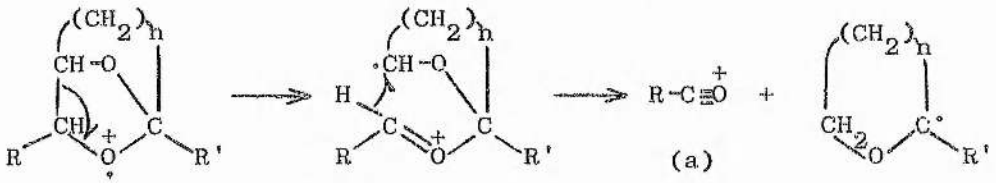
Fraction E (42%) consisted of several components which were not further investigated.

Rearrangement of methyl cis-8,9,cis-12,13-diepoxystearate

Methyl cis-8,9,cis-12,13-diepoxystearate (424 mg, 1.30 mmole) was reacted in dioxan (25 ml) containing boron trifluoride etherate (0.175 ml, 1.39 mmole) as in the previous experiment. Separation by preparative TLC (PE30) afforded five fractions (in the order of increasing polarity): A (94 mg, 25%), B (16 mg, 4%), C (77 mg, 21%), D (46 mg, 12%) and E (141 mg, 38%).

Fraction A [25%, ECL 18.8 (ApL) and 23.4 (DEGS)] had a similar mobility (Rf 0.47) on TLC (PE35) as monoepoxy esters but clearly differed from the diepoxy substrate (Rf 0.19) and from the furanoid

esters (Rf 0.65) of the previous experiment. It did not give any hydroxy or oxo absorption in the infrared nor did it react with picric acid, thus showing the absence of any 1,2-epoxide structure. Its NMR spectrum contained signals at δ0.89 (t, CH_3-CH_2-), 1.21 (m, in chain -CH_2-), 1.31 (m,  ; n = 2 or 3), 2.22 (t, -CH_2CO_2Me), 3.58 (s, -$COOCH_3$), and 3.79-3.95 (a distorted triplet, ). Details of its mass spectrum are given below with explanations for some of the fragment ions. High resolution of the peak with m/e = 171 gave the exact mass of 171.1012 which corresponds with a molecular formula of $C_9H_{15}O_3$ (calculated mass = 171.1021). This indicated a fragment ion (a) $[\overset{+}{O}\equiv C(CH_2)_6COOCH_3]$ which we consider to originate from the following fragmentation of the bicyclic ketal structure (8,9-12 and 8,9-13):



(M) (a)

R = $-(CH_2)_6COOCH_3$ n = 1,2 (a)

R = $-(CH_2)_4CH_3$ n = 1,2 (a')

Analogous fragmention of the isomeric ketals 12,13-8 and 12,13-9 leads to the ion (a') with m/e = 99 which was the base peak of the spectrum.

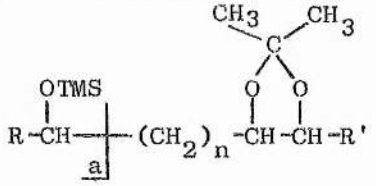
	m/e	I		m/e	I
(M)	326	< 1	(a-32)	139	30
(M-32)	294	2		127	11
	228	8		121	6
	199	5	(a-60)	111	49
	185	6	(a')	99	100
(a)	171	87		83	53
	156	18		71	70

Attempts to open the bicyclic ketal structure:

(i) After refluxing with methanolic sulphuric acid (3%) for 1 h compound A gave a product identical with compound A as judged by TLC, GLC and its IR spectrum.

(ii) When compound A (73 mg, 0.22 mmole) was refluxed with a mixture of dioxan (5 ml) and aqueous sulphuric acid (1 M, 8 ml) a product A‡ was obtained which had broad IR absorption bands at 1710 and 2500-3500 cm^{-1}. Its NMR spectrum closely resembled that of compound A except that there was no methoxy signal at 3.6 δ. Methylation of A‡ regenerated compound A.

(iii) Isopropylidene formation Compound A (65 mg, 0.2 mmole) was dissolved in dry acetone (1 ml) containing conc. sulphuric acid (1.0 mmole) and stirred at room temperature for 3h. The reaction mixture was then neutralised with methanolic potassium hydroxide (1 M) and extracted with ether. TLC showed that the product contained two components A' (80%) and B' (20%). A' was identical with A, whilst B' was more polar and had strong oxo and ester carbonyl absorption in the infrared, but no hydroxy absorption. Its ECL on ApL was 21.0. Reduction with sodium borohydride in methanol produced a hydroxy ester whose TMS derivative gave a single peak on GLC having an ECL of 20.8 (ApL) and 23.4 (DFGS). Mass spectroscopic examination showed strong α-cleavage at the carbon atoms bearing the TMS ether group:



oxo-diol from which the TMS ether - isopropylidene is derived		n	R	m/e	I
8	12,13	3	$-(CH_2)_6CO_2Me$	245	45
9	12,13	2	$-(CH_2)_7CO_2Me$	259	33
12	8,9	2	$-(CH_2)_5CH_3$	187	9
13	8,9	3	$-(CH_2)_4CH_3$	173	100

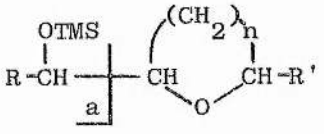
Other peaks were observed at 443 (M-15, 8), 329 (13), 149 (40) and

129 (CH_2=CH-CH=$\overset{+}{O}$-Si$(CH_3)_3$, 100).

Fraction B (4%) was not examined.

Fraction C (21%) showed IR absorption at 3500, 1740 and 1710 cm^{-1}. An epoxide structure was indicated by a positive reaction with picric acid. When the TMS derivative was examined on TLC two bands, C_1' (70%) and C_2' (30%), were obtained. Band C_2' having the same polarity on TLC as fraction C absorbed at 1740 and 1710 cm^{-1} and gave a positive reaction with picric acid. We consider it to be an epoxy oxo ester, but were unable to locate the epoxy and oxo groups by MS.

The less polar fraction C_1' had no hydroxy or oxo absorption in the infrared, nor did it react with picric acid. The main GLC peaks corresponded with ECL values of 19.6 on ApL and 22.4 and 23.6 on DEGS. The mass spectrum contained a molecular ion (M^+) at m/e 400 which indicated a cyclic system. We suggest a tetrahydrofuran and/or tetrahydropyran structure



n = (2,3) m/e

R = -$(CH_2)_6CO_2Me$ 245 a

R = -$(CH_2)_4CH_3$ 173 a'

	m/e	I		m/e	I
(M)	400	4	(a')	173	100
400-15	385	1		149	21
(a)	245	41		143	21
245-32	213	7	245-104*	141	27
	211	7		129	25
	199	11		103	41

* 104 : $(CH_3)_3SiOCH_3$

Fraction D (12%) gave an IR spectrum with peaks at 3500, 1740 and 1710 cm^{-1}, but was not investigated further.

Fraction E (38%) showed broad hydroxy and ester- and oxo-carbonyl absorption in the infrared. Its silylation product was examined on TLC (PE30) and found to consist of several not clearly separated components. No attempt was made to characterise them.

Materials and General Procedures

Methyl oleate [18:1 (9c)]

Olive oil was transesterified with methanolic sodium methylate. Methyl oleate was purified by urea fractionation[162] of the mixed methyl esters. Fractions of 93% and 97% methyl oleate were obtained with palmitate and traces of stearate as the only contaminants. These saturated esters did not interfere during the hydroboration and the subsequent reactions, but served as internal GLC standards for checking overall recoveries.

Methyl stearolate [18:1 (9a)]

Methyl stearolate was obtained from methyl oleate by bromination and dehydrobromination followed by methylation.

Methyl linoleate [18:2 (9c12c)]

Methyl linoleate (97%) was prepared from corn oil (ca. 65% in linoleate) by transesterification and repeated urea fractionation.

Methyl ricinoleate [12OH 18:1 (9c)]

Castor oil (7 g) was neutralised by percolation through a short alumina column using chloroform as eluting solvent. The recovered neutral oil (5.7 g) was transesterified to the mixed methyl esters (5.5 g) which were then chromatographed on a Sorbsil column (70 x 2 cm) using 200 ml portions of PE5, PE10, PE20, PE40, PE50 and PE60 as eluting solvents. The pure methyl ricinoleate (4.5 g) was eluted by PE40 and PE50.

Methyl 12-methoxyoleate [12OMe 18:1 (9c)]

This was prepared from methyl 12-hydroxyoleate by Purdie methylation with silver oxide and methyl iodide and purification by preparative TLC (PE25).

Methyl 12-acetoxyoleate [12OAc 18:1 (9c)]

Methyl 12-acetoxyoleate was obtained by refluxing methyl ricinoleate with acetyl chloride for 1 h.

Methyl hendec-10-enoate as well as tridec-1-ene were commercially available.

Preparation of a solution of diborane in tetrahydrofuran[134]

Sodium borohydride (3.78 g, 0.1 mole) in dry diglyme (100 ml) was slowly added from a pressure-equalising dropping funnel to a stirred solution of boron trifluoride etherate (28.4 g, 0.2 mole, 50% excess) in dry diglyme (20 ml). The liberated diborane gas was passed through a reflux condenser and through a diglyme solution of sodium borohydride (0.5 M, 10 ml) to remove traces of boron trifluoride into tetrahydrofuran (55 ml) which was stirred and cooled in an ice-bath. Throughout the reaction a slow stream of dry nitrogen was applied as carrier gas for the diborane. A gas outlet via a mercury safety valve ensured the exclusion of air. When the addition of sodium borohydride was complete (1 h), the reaction mixture was heated for 1 h at 70-80°C whilst the flow of dry nitrogen was maintained. The flask containing the diborane solution (ca. 0.8 M) was disconnected and sealed for storage at 0°C.

As the diborane concentration slowly decreased with time, the molarity of the solution had to be determined before use. This was done by injecting samples of diborane solution into water and measuring the volume of hydrogen liberated. The aqueous solutions containing the hydrolysis product, ie. boric acid, after addition of mannitol were titrated with aqueous sodium hydroxide. Both

volumetric and acidometric determinations gave identical diborane concentrations for the freshly prepared diborane solution. However, over a period of four weeks the >B-H concentration determined volumetrically decreased by up to 30% whereas the diborane concentration determined acidometrically stayed more or less unchanged.

For all hydroborations diborane solutions were employed on the basis of their volumetrically determined >B-H concentration.

Hydroboration procedure

The flask containing the monounsaturated compound (1.25 mmole) in dry THF (2 ml) was flushed with dry nitrogen and closed with a rubber septum. The solution was cooled to 0°C and stirred whilst the solution of diborane in THF (0.64 M, 0.39 ml; 1.5 mmole >B-H, r = 1.2) was slowly added (2 min) by means of a hypodermic syringe. (Exclusion of air and moisture was very important for the success of the hydroboration.) After 30 min at 0°C, the reaction mixture was stirred for 1 h at room temperature and then subjected to further treatment with various reagents.

Hydroborations using greater excesses of diborane were carried out in a similar way.

(I) Oxidation of the organoborane with alkaline hydrogen peroxide

Aqueous solutions of sodium hydroxide (3 M, 0.6 ml) and hydrogen peroxide (9.1 M [by iodometric titration], 0.35 ml) were carefully added to the THF solution of the organoborane derived from the monoene (1.25 mmole). The mixture was refluxed for 3 h. After cooling and acidification the product was extracted with ether.

(III) Oxidation of the organoborane with oxygen followed by oxidation with hydrogen peroxide

The monoene (3 mmole) was treated with diborane in THF (3.9 mmole >B-H, r =1.3). After completion of the hydroboration (checked by oxidation of a sample of the organoborane with alkaline hydrogen peroxide) dry THF was added to make the organoborane solution about 0.06 M. The solution was cooled to -40°C and stirred whilst oxygen was passed through a sintered gas dispersion tube into the solution. After 4 h the flow of oxygen was stopped and the temperature was allowed to rise to 0°C. Then hydrogen peroxide (9.1 M, 0.5 ml) was added dropwise to the stirred solution. Stirring was continued for 30 min at room temperature. Brine was added and the product was extracted with ether (3x). The ether extracts were washed with water (2x) and then dried over anhydrous sodium sulphate. The solvent was distilled off at reduced pressure (T < 30°C).

(V) Coupling reaction with potassium hydroxide and silver nitrate

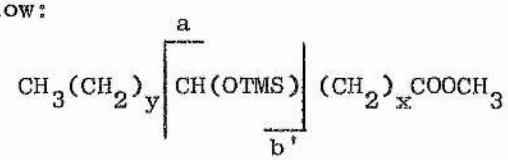
Methanolic potassium hydroxide (2 M, 4 ml), and aqueous silver nitrate (5 M, 0.8 ml) were added to the organoborane solution prepared by hydroboration (r = 1.2) of a monoene (3 mmole). After stirring for 2 h the reaction mixture was diluted with water and acidified with dilute sulphuric acid (2 M). Extraction with ether yielded the product.

(i) Hydroboration reactions with methyl oleate

(I) Hydroboration followed by oxidation with alkaline hydrogen peroxide (See general procedures)

Hydroboration (r=1.5) of methyl oleate (330 mg, 93% oleate, 7% palmitate, 1.03 mmole) followed by oxidation with alkaline hydrogen peroxide yielded a product (325 mg) which was reesterified with methanol-boron trifluoride. TLC (PE30) gave two spots with Rf

0.85 and 0.28. GLC of silylated product showed three peaks with ECL (DEGS) of 16.0 (8%), 18.5 (2%) and 19.5 (89%). The major component, purified by preparative TLC (PE30), showed O-H stretching in the infrared and had an NMR spectrum with signals at δ 0.89 (t, CH_3-CH_2-), 1.30 (m, -CH_2-), 1.58 (m, -$CH_2CH_2CO_2Me$), 1.78 (s, -O-H), 2.22 (t, -CH_2-CO_2Me), 3.45 (m, >CH-OH) and 3.60 (s, -$COOCH_3$). The main features of the mass spectrum of the silylated hydroxy ester are listed below:

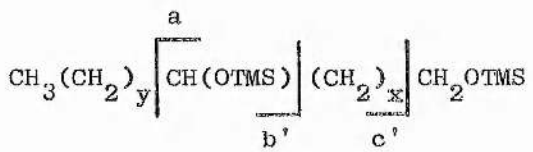


9-OTMS (x=7,y=8)
10-OTMS (x=8,y=7)

	m/e	I(%)	m/e	I(%)
a(10-OTMS)	273	100	149	87
a(9-OTMS)	259	100	129	34
b'(9-OTMS)	229	100	103	51
b'(10-OTMS)	215	100	83	77
273-104*	169	35	75	100
259-104*	155	35	73	100

*104: $(CH_3)_3SiOCH_3$

When a larger excess of diborane (r=3.0) was employed in the hydroboration step, the oxidation product contained a further component (16%) which did not show any carbonyl absorption in the infrared. Its TMS ether derivative had an ECL (DEGS) of 16.7, identical with that of 1,9(10)-bis(trimethylsilyloxy)octadecane derived from methyl 9(10)-hydroxystearate by reduction with lithium aluminium hydride followed by silylation. The mass spectrum confirmed this identity:



9-OTMS (x=7,y=8)
10-TOMS (x=8,y=7)

	m/e	I(%)
M	430	1
c'	327	14
a(10-OTMS)	317	52
a(9-OTMS)	303	68
b'(9-OTMS)	229	100
b'(10-OTMS)	215	100

(II) Hydroboration followed by oxidation with chromic acid

Methyl oleate (382 mg, 93% oleate, 7% palmitate, 1.20 mmole) was hydroborated (r=1.5). Aqueous sulphuric acid (4 M, 1 ml) and sodium dichromate (2 M, 0.6 ml, 50% excess) were added and the mixture was refluxed for 3 h. The ether extracted material was reesterified to yield the product (385 mg). GLC of the silylated product showed a major component with an ECL (DEGS) of 24.5 (77%) accompanied by some minor peaks corresponding to ECL values of 16.0 (9%), 18.4 (4%) and 19.5 (7%). The major component was separated by preparative TLC (PE40) in 70% yield. The IR spectrum had ester- and oxo-carbonyl absorption bands at 1740 and 1710 cm^{-1}. The NMR spectrum contained signals at δ 0.88 (t, CH_3-CH_2), 1.2[illegible] (m, -CH_2-), 1.49 (m, J=7Hz, -$CH_2CH_2CO_2Me$), a four line multiplet arising from two partly overlapping triplets (J=J'=7Hz) at δ 2.20 (-CH_2CO_2Me) and δ 2.27 (-CH_2COCH_2-), and 3.57 (s, -$COOCH_3$). The main features of the mass spectrum are listed below:

α-cleavage

$$CH_3(CH_2)_{y-1}\overset{a^*}{\overbrace{CH_2}}\overset{b}{\overbrace{CO}}\overset{c}{\overbrace{CH_2}}(CH_2)_{x-1}COOCH_3$$

c' d'^*

9-oxo (x=7,y=8)

10-oxo (x=8,y=7)

	m/e	I(%)
M	312	1
312-31	281	7
a^*(10-oxo)	214	10
a^*(9-oxo)	200	12
b(10-oxo)	199	10
b(9-oxo)	185	15
c(10-oxo)	171	7
d'^*(9-oxo)	170	17
c(9-oxo)	157	26
d'^*(10-oxo)	156	26
c'(9-oxo)	155	19
	150	100
c'(10-oxo)	141	25

*fragments a^* and d'^* result from the McLafferty rearrangement

(III) Hydroboration and oxidation with oxygen and hydrogen peroxide

Methyl oleate (674 mg, 90% oleate, other constituents being saturated esters, 2.05 mmole) was treated with diborane (>B-H 4.1 mmole, r = 2.0) in THF. (The completeness of the hydroboration was checked by oxidising a small sample of the organoborane solution with alkaline hydrogen peroxide. Only traces of methyl oleate were found in the product containing hydroxystearate and saturated esters.) The organoborane solution was diluted with THF and subjected to oxidation with oxygen and hydrogen peroxide (see general procedure p. 113).

The ether extracted oxidation product was separated by preparative TLC (PE30) into four bands: A (Rf 0.74, 9%), B (Rf 0.59, 6%), C (Rf 0.52, 51%) and D (Rf 0.22, 34%). Band A contained saturated esters and band B (ECL (DEGS) 24.5) appeared to consist of oxostearate. Band D (ECL (DEGS) 25.3 and 19.5 after silylation) was identical with methyl 9(10)-hydroxystearate (IR,NMR).

Band C gave a positive reaction with ferrous thiocyanate indicating a peroxy group. The NMR spectrum contained signals at δ 0.87 (t, $C\underline{H}_3$-CH_2-), 1.26 (m, -$C\underline{H}_2$-), 1.50 (m, -$CH_2C\underline{H}_2CO_2Me$), 2.22 (t, -$C\underline{H}_2CO_2Me$), 3.59 (s, -$COOC\underline{H}_3$) and 3.75 (m, >C$\underline{H}$OOH). GLC showed two peaks with ECL (DEGS) of 24.5 (82%) and 25.3 (18%) which are ECL values characteristic for methyl 9(10)-oxostearate and methyl 9(10)-hydroxystearate. A second analogous hydroboration-oxidation reaction of methyl oleate yielded a hydroperoxy ester (47%) which was identical, as indicated by TLC and NMR, with the hydroperoxy product described above. However, this time, the gas chromatogram showed two equally-sized peaks corresponding to ECL 24.5 and 25.3 fraction C being the expected methyl 9(10)-hydroperoxystearate is likely to give two GLC peaks as decomposition to oxo- and hydroxy-esters probably takes place at the temperature (190°C) in the gas chromatograph.

(ii) Hydroboration reaction with methyl 12-methoxyoleate

(I) Hydroboration followed by oxidation with alkaline hydrogen peroxide

Methyl 12-methoxyoleate (ECL (DEGS) 21.6; 198 mg, 0.61 mmole) was hydroborated (r=1.6), then oxidised with alkaline hydrogen peroxide and finally reesterified to give the product (195 mg). TLC (PE50) showed three spots with Rf values of 0.60, 0.25 and 0.14. The main component which was the most mobile one was chromatographically identical with methyl 12-methoxyoleate. GLC analysis of a silylated sample of the total product showed a major peak with ECL (DEGS) 21.6 and two minor components with ECL 22.2 (9%) and 23.8 (1%). Separation by preparative TLC (PE50) afforded three bands, A (66%), B (18%) and C(16%). Band A was identical with the starting material. Band B was lost by accident. Band C had an O-H stretching band in the IR spectrum. The TMS ether derivative gave a single GLC peak (ECL 22.2). Some details of its mass spectrum are given below:

$$CH_3(CH_2)_5\underset{c'}{\underline{CH(OCH_3)}}(CH_2)_2\overset{b}{\underset{a'}{\underline{CH(OTMS)}}}(CH_2)_7COOCH_3$$

	m/e	I(%)
a' and/or b	259	100
259-18	241	30
259-32	227	100
259-90*	169	17
259-104**	155	45
	149	100
c' and/or $CH_2=CH-CH=\overset{+}{O}Si(CH_3)_3$	129	80

* 90 : $(CH_3)_3SiOH$,

** 104 : $(CH_3)_3SiOCH_3$

(iii) Hydroboration reactions of methyl 12-acetoxyoleate

(I) Hydroboration followed by oxidation with alkaline hydrogen peroxide

Methyl 12-acetoxyoleate (ECL (DEGS) 24.6, ca. 1 mmole) was hydroborated with varying amounts of diborane (r=1.1, 1.65 and 2.25). Oxidation with alkaline hydrogen peroxide was followed by reesterification and the products were examined by TLC and GLC. Four components occurring in varying proportions were detected and characterised on the basis of their retention characteristics in comparison with those of compounds with known structures. [Compare the hydroboration and oxidation of methyl 12-hydroxyoleate (see section iv)]. They were

	Rf(PE50)
12-OAc 18:1 (9c)	0.85
12-OH 18:1 (9c)	0.55
10,12-diOH 18:0	0.09
9,12-diOH 18:0	0.03

	ECL (DEGS)	r=1.1 (%)	r=1.65(%)	r=2.25(%)
12-OAc 18:1 (9c)	24.6	8	2	-
12-OTMS 18:1 (9c)	19.8	64	60	34
10,12-diOTMS 18:0	20.5	14	19	31
9,12-diOTMS 18:0	20.8	14	19	35

(iv) Hydroboration reactions with methyl 12-hydroxyoleate

(I) Hydroboration followed by oxidation with alkaline hydrogen peroxide

Methyl 12-hydroxyoleate was hydroborated with varying amounts of diborane (r=1.2, 2.4 and 3.6). The organoboranes obtained were subjected to treatment with alkaline hydrogen peroxide followed by reaction with methanolic boron trifluoride. Analysis by TLC (PE50) showed that the products contained various components which had the following Rf values:

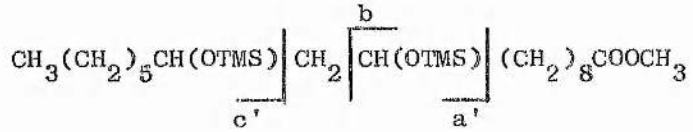
Rf(PE50)	0.52	0.10	0.03	0.00
r=1.2	+	+	+	
r=2.4	+	+	+	
r=3.6		+	+	+

The starting material, methyl 12-hydroxyoleate, appeared as a spot with Rf 0.52. The product where the largest excess of diborane had been used (r=3.6) was free of any unreacted hydroxyoleate but contained another very polar component (Rf 0.00).

Samples of the three reaction products were silylated and analysed by GLC. The results are summarised below:

ECL (DEGS)	18.9(%)	19.3(%)	19.8(%)	20.5(%)	20.8(%)
r=1.2	-	-	84	8	8
r=2.4	2	2	16	40	40
r=3.6	20	20	-	30	30

Methyl 12-trimethylsilyloxyoleate had an ECL of 19.8. The product where r=3.6 was separated by preparative TLC (PE60) into three bands, A (34%), B (31%) and C (35%). The TMS ether derivative of the most mobile band A gave a single peak of ECL 20.5. The mass spectrum contained the following peaks:



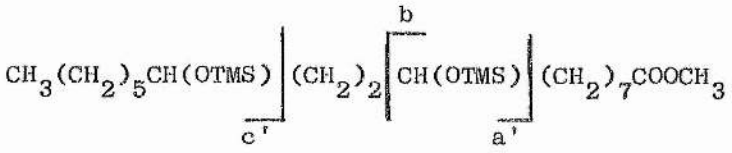
	m/e	I(%)
	299	3
b	273	56
a'-90*	213	5
c'	187	100
273-104**	169	22
	149	10
	147	26
	129	14

* 90 : $(CH_3)_3SiOH$

** 104 : $(CH_3)_3SiOCH_3$

A mass spectrum with the same main features was obtained for the bis-TMS ether derivative (ECL (DEGS) 20.5) of methyl 10,12-dihydroxy-stearate which had been prepared by hydroxymercuration and demercuration of methyl 12-hydroxyoleate[163].

Band B was also converted to the TMS ether derivative (ECL (DEGS) 20.8) which gave a mass spectrum with the following main features:



	m/e	I(%)
a'	317	21
	299	22
b	259	25
317-90*	227	39
c'	187	100
	178	15
	159	21
259-104**	155	27

* 90 : $(CH_3)_3SiOH$

** 104 : $(CH_3)_3SiOCH_3$

The bis-TMS ether of methy 9,12-dihydroxystearate prepared by hydroxymercuration and demercuration of methyl 12-hydroxyoleate[163] gave a very similar mass spectrum and had also an ECL value of 20.8.

Band C did not have any carbonyl absorption band in the IR spectrum. GLC examination of the TMS ether derivative showed two components with ECL values of 18.9 (52%) and 19.3 (48%) 1,9,12-Tris-(trimethylsilyloxy)octadecane obtained from methyl 9,12-dihydroxystearate by reduction with lithium aluminium hydride and silylation had an ECL of 19.3

(v) Hydroboration reactions with methyl linoleate

(I) Hydroboration followed by oxidation with alkaline hydrogen peroxide

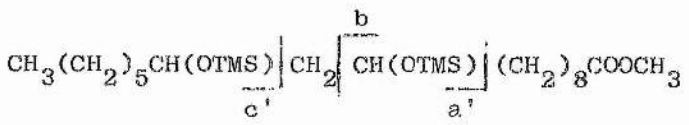
Methyl linoleate was hydroborated using a 50% (r=3.0) and a 100% excess of diborane (r=4.0). Oxidation and reesterification yielded the reaction products which were separated by preparative TLC (PE50) into four bands (A-D):

	Rf(PE50)	(r=3)%	(r=4)%
band A	0.95	20	8
band B	0.70	41	22
band C	0.16	11	21
band D	0.05	28	49

Band A was identical with methyl linoleate (TLC,GLC,IR). All the other bands (B-D) showed strong O-H stretching as well as ester carbonyl absorption in the infrared. The IR spectrum of band B also had a distinct absorption band at 3020 cm^{-1}, indicating the presence of olefinic protons.

The NMR spectrum of band B contained signals at δ 0.88 (t, CH_3-CH_2-), 1.31 (m, -CH_2-), 1.50 (m, -$CH_2CH_2CO_2Me$), 2.00-2.18 (m, J=7Hz, -CH_2-CH=CH-CH_2-CH(OH)-), 2.21 (t, J=7Hz, -CH_2CO_2Me), 3.50 (m, >CHOH), 3.59 (s, -$COOCH_3$) and 5.39 (m, -CH=CH-). It closely resembled the NMR spectrum of methyl 12-hydroxyoleate. The TMS ether derivative had an ECL (DEGS) of 19.9 (methyl 12-OTMS 18:0, ECL 19.8). So band B consisted of methyl hydroxyoctadecenoate. No attempt was made to locate the position(s) of the hydroxy groups.

Band C was converted to the TMS ether derivative which closely resembled methyl 10,12-bis-(trimethylsilyloxy)stearate derived from methyl 12-hydroxyoleate by hydroboration, oxidation, methylation and silylation (compare p. 118). The silylated band C had an ECL of 20.5 and gave a mass spectrum with the following main features:

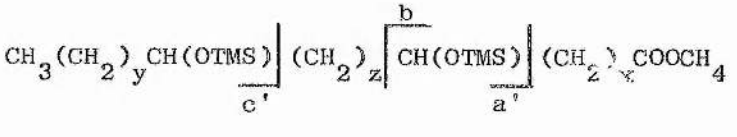


	m/e	I(%)
	299	4
b	273	20
a'-90*	213	12
	199	8
c'	187	93
273-104**	169	14
	149	43
	147	100
	129	45

* 90 : $(CH_3)_3SiOH$

** 104 : $(CH_3)_3SiOCH_3$

The TMS ether derivative of band D had an ECL value of 20.9. Its mass spectrum is summarised below:



	x	y	z
9,12-diOTMS 18:0	7	5	2
9,13-diOTMS 18:0	7	4	3
10,13-diOTMS 18:0	8	4	3

	m/e	I(%)
	299	26
b(10)	273	32
b(9)	259	46
a'(9)-90*	227	53
a'(10)	213	32
c'(12)	187	85
c'(13)	173	100
273-104**	169	54
	159	59
259-104**	155	85

(a', b and c' denote cleavages α to a TMS ether bearing >CH- group; numbers in brackets indicate the position of this >CH(OTMS) group in the ester chain.

* 90 : $(CH_3)_3SiOH$, ** 104: $(CH_3)_3SiOCH_3$

(vi) Hydroboration reactions with methyl stearolate

(I) Hydroboration followed by oxidation with alkaline hydrogen peroxide

Methyl stearolate (ECL (DEGS) 20.3, (ApL) 17.9) was hydroborated (r=1.5), oxidised with alkaline hydrogen peroxide and refluxed with methanolic trifluoride. The product (349 mg), examined by TLC (PE40), showed one major component (Rf 0.69) and three faint spots with Rf values of 0.80, 0.38 and 0.05. Silylation did not affect the mobility of the major component. The gas chromatogram (DEGS) of the silylated product showed one major component (87%) with an ECL of 24.5 accompanied by several minor peaks corresponding to ECL values of 18.4 (4%), 19.5 (4%), 20.3 (3%) and 20.7 (2%). The major component, isolated by preparative TLC (PE40), was identical with methyl 9(10)-oxostearate derived from methyl oleate by hydroboration and oxidation with chromic acid (compare p. 115), as seen by TLC, GLC, IR, NMR and MS.

(IV) Hydroboration followed by protonolysis

Glacial acetic acid (0.6 ml) was added to the organoborane obtained by hydroboration (r=1.5) of methyl stearolate (352 mg). The mixture was stirred at room temperature for 20 min, diluted with water and extracted with ether. The reesterified product (336 mg) was analysed by TLC (PE20) which gave two distinct spots with Rf values of 0.67 and 0.39, corresponding to methyl oleate and methyl 9(10)-oxostearate respectively. The gas chromatogram (DEGS) contained two peaks with ECL 18.5 (82%) and 24.5 (12%) which again represent methyl oleate and methyl 9(10)-oxostearate respectively. The IR spectrum contained a distinct absorption band at 3020 cm^{-1}, but none between 950 and 1000 cm^{-1}, and therefore indicated cis configuration of the double bond.

(vii) Hydroboration reactions of methyl hendec-10-enoate

(I) Hydroboration followed by oxidation with alkaline hydrogen peroxide

Methyl hendec-10-enoate (251 mg, 1.27 mmole) in dry THF (2 ml) was hydroborated (r=1.3) and subsequently oxidised with alkaline hydrogen peroxide. The product (244 mg) obtained after methylation using methanolic boron trifluoride appeared as a single TLC spot with Rf (PE20) 0.10. The gas chromatogram (DEGS) of the silylated product showed a major peak (95%) with ECL 15.1 and only a trace (1%) of methyl hendec-10-enoate (ECL 12.1). The mass spectrum of the TMS ether derivative contained the following peaks:

$TMSOCH_2(CH_2)_9COOCH_3$

(or less likely $CH_3CH(OTMS)(CH_2)_8COOCH_3$)

	m/e	I(%)
M-15	273	100
M-30	258	10
M-(15+32)	241	87
	159	16
	129	14
	117	43
	107	55
	103	82

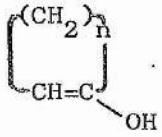
(V) Hydroboration followed by reaction with alkaline silver oxide

Methyl hendec-10-enoate (0.607 mg, 3.3 mmole) was hydroborated (r=1.3). The solution containing the organoborane was treated with methanolic potassium hydroxide (2M, 4 ml) and aqueous silver nitrate (5 M, 0.8 ml). The product appeared as a single TLC spot with a Rf value of 0.65 (PE20) being identical with methyl hendec-10-enoate. Analysis by GLC (ApL) showed, however, no trace of the starting material, but two peaks with ECL 23.9 (6%) and 24.6 (92%). Analysis on a DEGS column similarly gave two peaks corresponding to ECL 28.9 (5%) and 29.8 (88%). The NMR spectrum showed signals at

δ 1.26 (m, $-CH_2-$), 1.56 (m, $-CH_2CH_2CO_2Me$), 2.20 (t, $-CH_2CO_2Me$) and 3.58 (s, $-COOCH_3$). The main features of the mass spectrum are listed below:

$CH_3OOC(CH_2)_{20}COOCH_3$ (and $CH_3OOC(CH_2)_{10}CH(CH_3)(CH_2)_8COOCH_3$)

	m/e	I(%)
M-32	366	5
M-74	324	4
a(n=5)	112	18
a(n=4)	98	62
	87	59
a(n=3)	84	33
	83	33
b	74	100

a  b $CH_2=C(\overset{+}{O}H)OCH_3$

The product, recrystallised from ether-petrol, melting sharply at 72°C.

(viii) Hydroboration reaction with cyclohexene

(III) Hydroboration followed by oxidation with oxygen and hydrogen peroxide

Cyclohexene (795 mg) was hydroborated (r=1.2). The solution containing the organoborane was made 0.5 M by dilution with THF and then cooled to -40°C and oxidised with oxygen. After warming up to room temperature the oxidation was completed with hydrogen peroxide. The ether extracted product (653 mg) did not contain any cyclohexene as seen by GLC. Separation by preparative TLC (PE30) afforded three bands: A (Rf 0.58, 65%), B (Rf 0.26, 29%) and C (Rf 0.05, 6%). Band B was chromatographically (TLC and GLC) identical with cyclohexanol. Its NMR spectrum had signals at δ 1.23, 1.53, 1.76 (all $>CH_2$ groups) and 3.49 (m, >CHOH) and 3.8 (OH). Band A gave a reaction with ferrous thiocyanate reagent. The gas chromatogram (3% ApL, 70°C) showed a single peak with a retention

time of 36 min compared with a retention time of 10 min for cyclohexanol. The NMR spectrum contained a rather complex set of signals at δ 1.32, 1.52, 1.76, 1.96 as well as signals at δ 3.86 (m, >CHOOH) and 8.46 (s, -OOH). Band C was no further examined.

(ix) Hydroboration reactions with tridec-1-ene

(I) Hydroboration followed by oxidation with alkaline hydrogen peroxide

Tridec-1-ene (728 mg, 4 mmole) in dry THF (4 ml) was reacted with diborane solution (0.74 M, 1.35 ml, r =1.5). Treatment with hydrogen peroxide (9.1 M, 1 ml) and aqueous sodium hydroxide (6 M, 1.5 ml) afforded the product (773 mg). Analysis by GLC indicated three components (88%, 8% and 4%). One of the minor components (8%) had the same retention time as tridec-1-ene. The main component separated by preparative TLC (PE40) gave an NMR spectrum with signals at δ 0.88 (t, CH_3-CH_2-), 1.25 (m, -CH_2-), 1.48 (m, -CH_2CH_2OH), 2.40 (s, -OH) and 3.50 (t, J=6Hz, -CH_2OH).

(III) Hydroboration followed by oxidation with oxygen and hydrogen peroxide

Tridec-1-ene was hydroborated as above. The resulting organoborane solution was diluted with THF to 0.35 M concentration. Oxygen passed through the cooled (-40°C) solution ignoring a white precipitate. Subsequent warming up to room temperature and treatment with hydrogen peroxide gave the product which was separated by preparative TLC (PE40) into three bands: A (Rf 0.91, 5%), B (Rf 0.72, 14%) and C (Rf 0.43, 81%). Band A was chromatographically (TLC and GLC) identical with tridec-1-ene and band C was identical with 1-hydroxytridecane. Band B gave a reaction with the ferrous thiocyanate reagent. The (100 MHz) NMR spectrum showed signals at δ 0.87 (t, CH_3-CH_2-), 1.24 (m, -CH_2-), 1.46 (m, -CH_2CH_2OOH) and 3.88 (t, J=6Hz, -CH_2OOH).

(V) Hydroboration followed by reaction with alkaline silver oxide

Methanolic potassium hydroxide (2 M, 4 ml) and aqueous silver nitrate (5 M, 0.8 ml) was added to the organoborane solution obtained by hydroboration (r=1.2) of tridec-1-ene (607 mg, 3.3 mmole). After stirring for 2h the mixture was acidified and extracted with ether. TLC examination of the product (524 mg) gave only one spot with an Rf value similar to that of tridec-1-ene. GLC (ApL) showed little (3%) tridec-1-ene, but two peaks with ECL 22.4 (5%) and 23.1 (86%). The mass spectrum of the product exhibited a distinct molecular ion peak at m/e 366, to which a series of peaks differing by 14 mass units led up.

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