OXYMERCURATION REACTIONS OF LONG-CHAIN COMPOUNDS

being a thesis
presented by

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to the

UNIVERSITY OF ST. ANDREWS

in application for

THE DEGREE OF DOCTOR OF PHILOSOPHY

August 1971

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 Ronald Peter Inglis has completed twelve terms of research work under my supervision, has fulfilled the conditions of Ordinance 16 (St. Andrews) and that he is qualified to submit the accompanying thesis in application for the degree of Doctor of Philosophy.

Research Supervisor.

UNIVERSITY CAREER

I entered the United College of St. Salvator and St. Leonard, University of St. Andrews in October, 1964 and subsequently graduated B.Sc. with First Class Honours in Chemistry in June,1968.

I was admitted as a research student in the United College, University of St. Andrews in October, 1968 and was awarded an S.R.C. Studentship which I held until October, 1971.

PUBLICATIONS

(i) "Substituent Effects on the Oxymercuration - Demercuration of Long-Chain Unsaturated Esters."
F.D. Gunstone and R.P. Inglis, Chem. Comm., 1970, 877.

(ii) "NMR Spectra of Fatty Acids and Related Compounds."
F.D. Gunstone and R.P. Inglis, Topics in Lipid Chemistry, ed. F.D. Gunstone, Logos Press, 1971, vol. 2, p.287.

LECTURE

"New method for the location of $\Delta 3$, $\Delta 4$ and $\Delta 5$ double bonds in long-chain compounds."
R.P. Inglis, presented to the Scottish Lipid Discussion Group, Aberdeen, 6th. May, 1971.

ACKNOWLEDGEMENTS

I wish to express my sincere thanks to Professor F.D. Gunstone for his help and guidance and for his interest and encouragement throughout this work.

I am deeply grateful to my wife for her constant encouragement and for painstakingly typing this thesis.

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

CONTENTS

Summary

The reaction of methyl oleate with a mercuric salt was investigated in the presence of various nucleophiles to determine the scope and facility of the oxymercuration addition reaction. Methoxy, ethoxy, acetoxy, hydroxy and amido long-chain esters were readily prepared by this reaction. Attempts to produce phenoxy, higher alkoxy, peroxy and amino esters were less successful.

The position of the double bond in relation to other substituent groups (methoxy, acetoxy, carbomethoxy, hydroxy, other double bonds, end of the chain) in a long-chain molecule was shown to influence the position of nucleophilic attack and hence the distribution of positional isomers in the product.

Attack of the intermediate mercurinium ion by a suitably situated internal nucleophilic group afforded heterocyclic products. Several hydroxy monoene systems including octadecenols gave cyclic ethers by reaction with mercuric acetate in a non-participating solvent. This cyclisation by oxymercuration provided a better yield of 1,4-epoxides than previous synthetic methods. Hydroxymercuration of diene esters gave cyclic ether products.

A simple quantitative method of analysis for $\Delta 3$, $\Delta 4$ or $\Delta 5$ unsaturated fatty acids in an oil was developed. This method, which involved the cyclo-oxymercuration reaction, was also used to isolate polyunsaturated alcohols with double bonds in these positions.

A report claiming that $\Delta 5$ and $\Delta 7$ fatty acids were present in Umbelliferae seed oils was shown to be incorrect by a combination of the new analytical technique and ~~by~~ oxidative degradation studies.

Finally, methoxymercuration - demercuration of three natural oils gave methoxylated triglycerides. The viscosity, refractive index and melting behaviour of these products and of the original oils were compared.

ABBREVIATIONS

Ac *O*	–	Acetate
Ag^+ TLC	–	Thin-layer chromatography on silica gel G impregnated with silver nitrate
CE	–	Cyclic ether
DMF	–	Dimethylformamide
E	–	Diethyl ether
ECL	–	Equivalent chain length [127]
GLC	–	Gas - liquid chromatography
HCE	–	Hydrogenated cyclic ether
HFO	–	Hydrogenated fish oil
IR	–	Infra-red
LAH	–	Lithium aluminium hydride
M	–	Molecular ion (MS)
MS	–	Mass spectrum
NMR	–	Nuclear magnetic resonance
P	–	Petroleum ether
prep	–	preparative
THF	–	Tetrahydrofuran
THP	–	Tetrahydropyran
TLC	–	Thin-layer chromatography
UV	–	Ultra-violet

INTRODUCTION

Although the first addition compound of a metallic salt with an olefin was isolated and analysed by Zeise before 1830 [1,2,3] as $KCl.PtCl_2.C_2H_4$, it was not until 1900 that the first addition of a mercury salt across a double bond was observed. Hofmann and Sand [4,5] isolated mercury-containing products by reacting ethylene, propene or but-1-ene with a mercury salt in the presence of water. Typically, ethylene and mercuric chloride gave products (1) and (2) in addition to a number of substitution and polymerised products.

$HOCH_2CH_2HgCl$ (1)

$O(CH_2CH_2HgCl)_2$ (2)

Schoeller and co-workers [6] showed that the solvent played an important role. For example, mercuric acetate, which was found to be more reactive than mercuric halides by Hofmann and Sand, and ethanol shaken in an ethylene atmosphere gave the product (3) in 95% yield.

$CH_3COOHgCH_2CH_2OCH_2CH_3$ (3)

Hugel and Hibou [7] demonstrated that ethylene was rapidly absorbed by mercuric acetate in acetic acid thus:

$$Hg(OCOCH_3)_2 + CH_2{=}CH_2 \xrightarrow{CH_3COOH} CH_3COOCH_2CH_2HgOCOCH_3$$

Although other types of oxymetalation reaction (oxythallation, oxyplumbation, oxypalladation and oxyplatination) are now known [8], they have not been investigated or exploited to the same extent as oxymercuration. Early oxymercuration investigations (pre 1950) are lucidly reviewed in the article by Chatt [9]. A more recent review (1968) by Kitching [8] brings the subject almost up to date.

Structure of the oxymercurated products

A controversy concerning the structure of the mercuric salt - olefin products arose in 1920 when Manchot and Klüg [10,11] suggested (mainly because of the ease of regeneration of the parent olefin) that these compounds were co-ordination complexes of the type (4) rather than addition compounds of the type (1) above.

$$CH_2{=}CH_2.Hg(OH)Cl$$

(4)

Sand [12] had earlier concluded that there was a tautomeric equilibrium between compounds of type (1) and type (4). Several

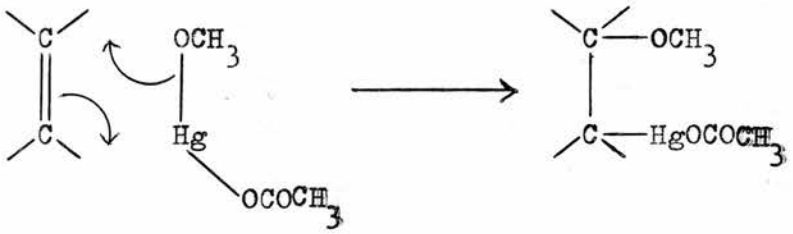
American workers [13,14,15] rejected Manchot's hypothesis and Marvel [16,17] clearly demonstrated the fallacy of the proposed metal ion - olefin π-complex structure by isolating diastereoisomers with different rotations after methoxymercuration of optically active menthyl cinnamate. Marvel's findings were later corroborated by nuclear magnetic resonance studies at 40MHz [18] and at 60MHz [19] which unambiguously showed that the π-olefin linkage disappeared on oxymercuration. It has been repeatedly shown that adduct formation takes place in the Markownikov sense.

Stereochemistry of oxymercuration

There are many examples in the literature which show that the oxymercuration of unstrained olefins is stereospecific [15,20,21,22]. This is clearly demonstrated in the case of an optically active olefin where oxymercuration of one pure enantiomer yields only one pair of diastereoisomers.

Wright [20,23] suggested erroneously that addition to a double bond in an oxymercuration reaction was cis, as he believed that mercuric acetate in methanol gave an equilibrium to produce methoxymercuric acetate (5) which he claimed added as a discrete unit to the olefin:

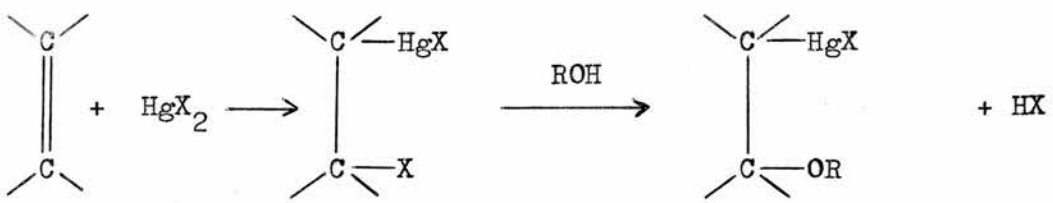
$$Hg(OCOCH_3)_2 \rightleftharpoons^{CH_3OH} CH_3OHgOCOCH_3 + CH_3COOH \qquad (5)$$



Lucas et al [24] argued mechanistically that by analogy with the bromination of an olefin (vide infra) oxymercuration should give rise to a trans adduct. This prediction was vindicated by X-ray [25] and NMR [26,27] studies of cyclohexene methoxy- and hydroxy-(deuteroxy-)mercurated products respectively.

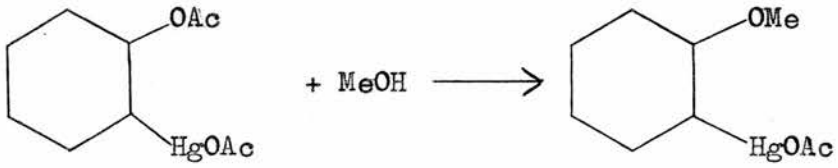
Mechanism of oxymercuration

Early workers [4,20] in this field believed that a molecular combination of mercuric salt and olefin preceded a solvent displacement reaction:



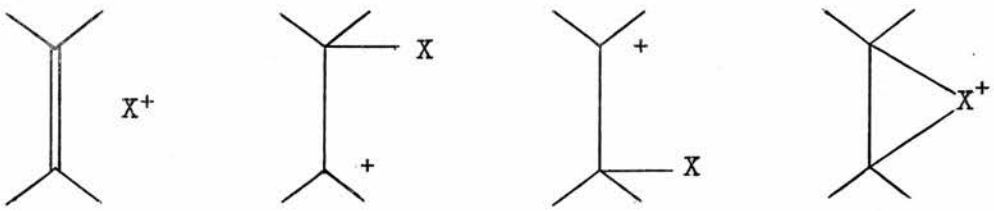
Brook and Wright [28] disproved this hypothesis by preparing the acetoxy mercuric acetate from cyclohexene and showing

that the rate of exchange of acetoxy by methoxy in methanol was so slow as to render this mechanism implausible:



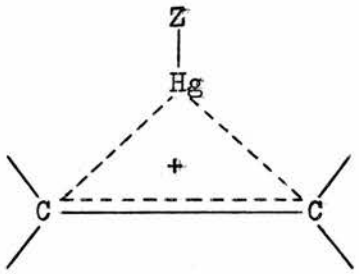
The stereospecificity observed in oxymercuration reactions rules out the possibility of a radical mechanism.

Although the metal ion - olefin π-complex had been rejected as a structure for the final products of oxymercuration, there was a distinct possibility that it might exist as a short-lived intermediate in the oxymercuration reaction. Evidence for an intermediate of this type was presented by Lucas et al [24] who compared it with the known silver ion - olefin co-ordination [29,30] and bromonium ion [31] complexes. Canonical forms (6 - 9) shown below were considered for bromination (X = Br^+), oxymercuration (X = $HgOAc^+$) or silver - olefin co-ordination (X = Ag^+) complexes.



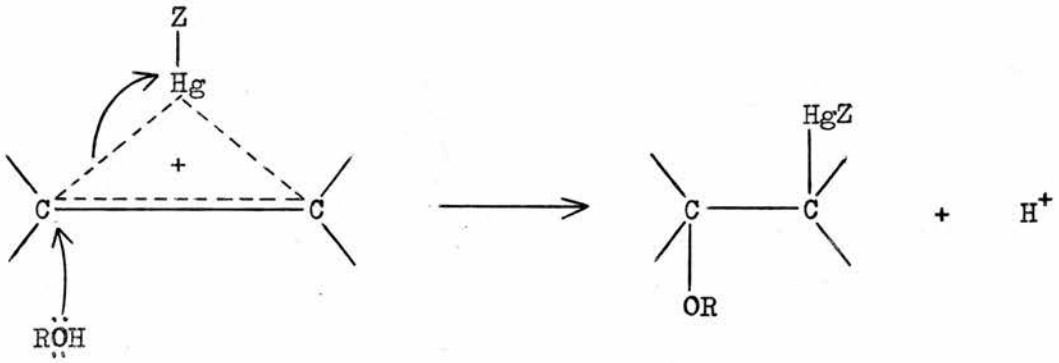
(6) (7) (8) (9)

The mercurinium ion can therefore be conveniently represented thus:



The formation of a three-membered cyclic mercurinium ion intermediate of this type readily explains why the overall addition in an oxymercuration reaction is <u>trans</u>. Attack by a nucleophilic group on the same side of the mercurinium ion as the bulky mercury atom is obviously sterically unfavourable.

Therefore:



i.e. <u>trans</u> addition

Consideration of resonance hybrids (7) and (8) indicate why oxymercuration of unsymmetrical olefins normally occurs in the Markownikov sense as shown previously by NMR studies [32,33].

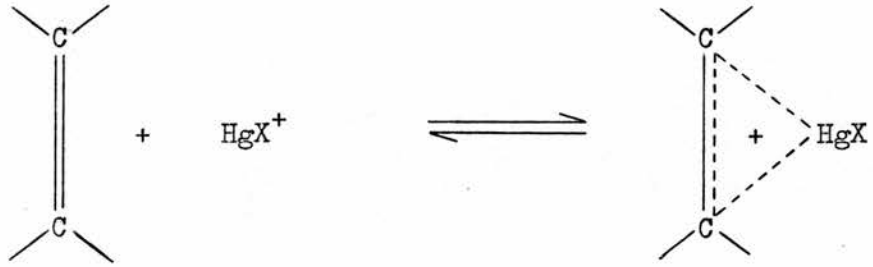
Although early attempts to detect the presence of mercurinium ions by NMR spectroscopy failed and Whitham [34] was unsuccessful in an attempt to trap a mercurinium ion in an olefin oxymercuration reaction mixture containing catalytic quantities of mineral acid (reaction becomes thermodynamically controlled), a recent molecular orbital approach to electrophilic addition of olefins [35] implied that under appropriate experimental conditions mercurinium ions could exist as independent entities. Olah and Clifford [36] have recently observed the existence of the mercurinium ion (10) by NMR studies at -30° (singlet at 2.32 τ; J = 190 Hz; ^{199}Hg-^{1}H).

$$CH_3OCH_2CH_2HgCl \xrightarrow[SO_2]{FSO_3H\text{-}SbF_5} CH_3OH_2^+ + \text{(10)}$$

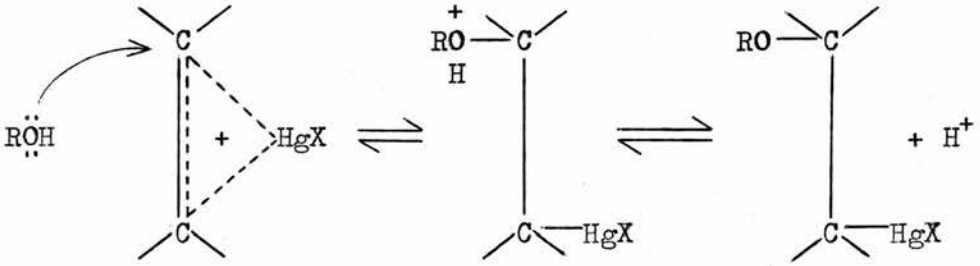
(10)

Although a group of French workers [37,38] have postulated an ionic cyclic bis-mercury bis-olefin intermediate in the oxymercuration of methyl oleate, the overall mechanism of the reaction is generally accepted to involve initial dissociation (11) of the mercuric salt followed by attack of the cationic species on the olefin in a rate-determining step (12) to yield the intermediate mercurinium ion. Antiperiplanar addition of a solvent molecule (13) or of an internal nucleophilic group (vide infra) produces the trans-substituted Markownikov product.

$$HgX_2 \rightleftharpoons HgX^+ + X^- \qquad (11)$$



(12)

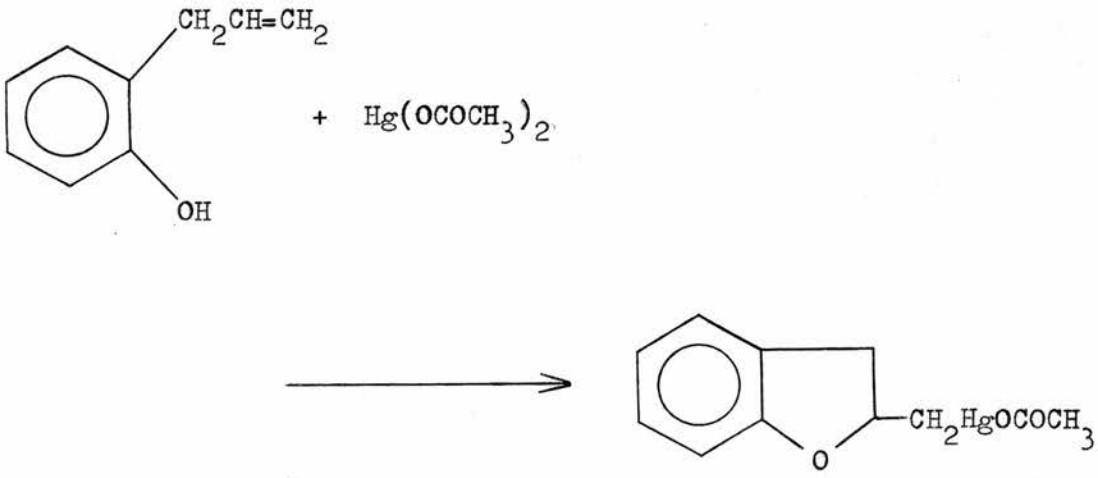


(13)

This mechanism readily explains the observed second order kinetics (first order in mercury salt and first order in olefin).[15,20,21,22,23] Furthermore, steric considerations show why 1,2-dialkyl <u>cis</u>-substituted olefins react much more rapidly than their <u>trans</u> isomers.[15,20,21]

If the olefin is suitably substituted, attack on the intermediate mercurinium ion by an internal group with nucleophilic character can yield a heterocyclic product. This is particularly favourable if the ring so formed is

five-membered.[13,39,40] For example a quantitative yield of a dihydro substituted benzofuran was obtained by the oxymercuration of ortho-allyl phenol.[13]



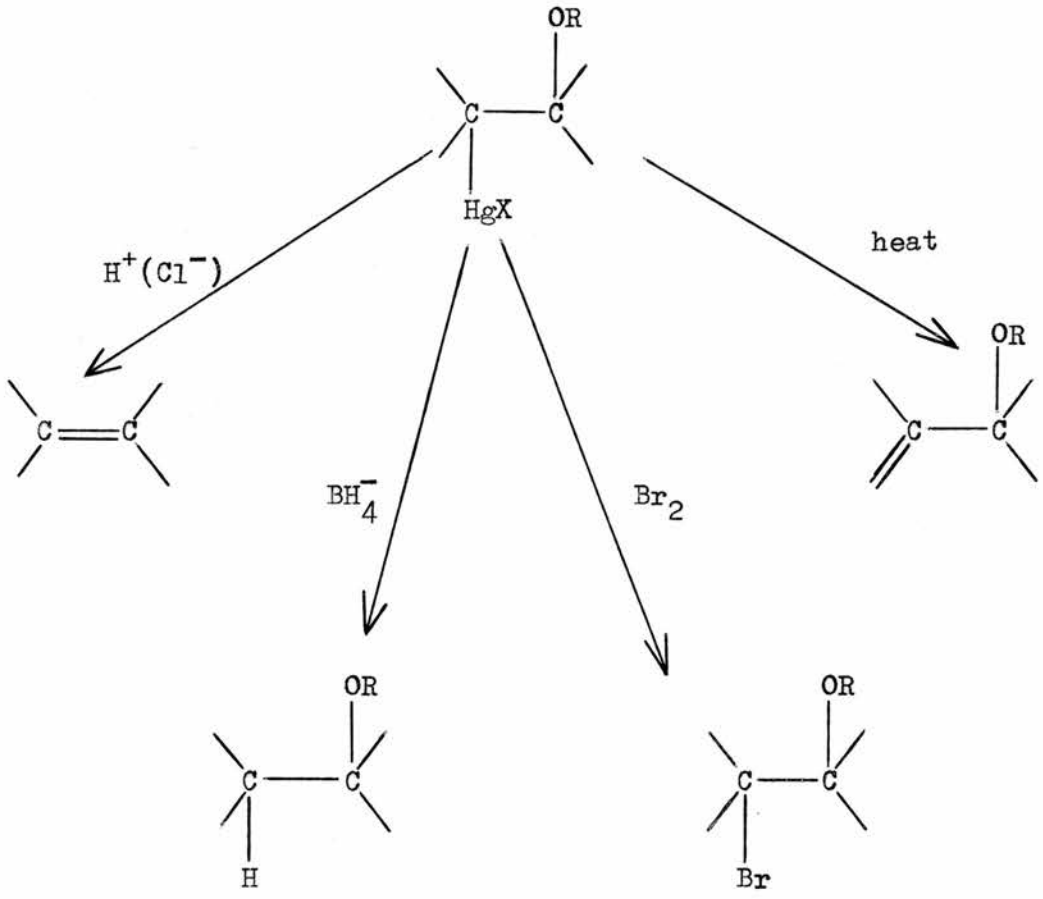
Further examples from the literature on this type of internal ether formation are discussed later (Chapter 3).

Reactions of oxymercurials

Of the four major reactions of oxymercurials outlined in the diagram below, only acid regeneration of the olefin and sodium borohydride reduction of the oxymercurial are used extensively in this thesis. While our investigations were in progress Japanese workers reported the preparation and thermal decomposition of acetoxymercurials from methyl linoleate [41], methyl ricinoleate[42] and methyl acetoxyricinoleate.[42] A group of French workers [37,38]

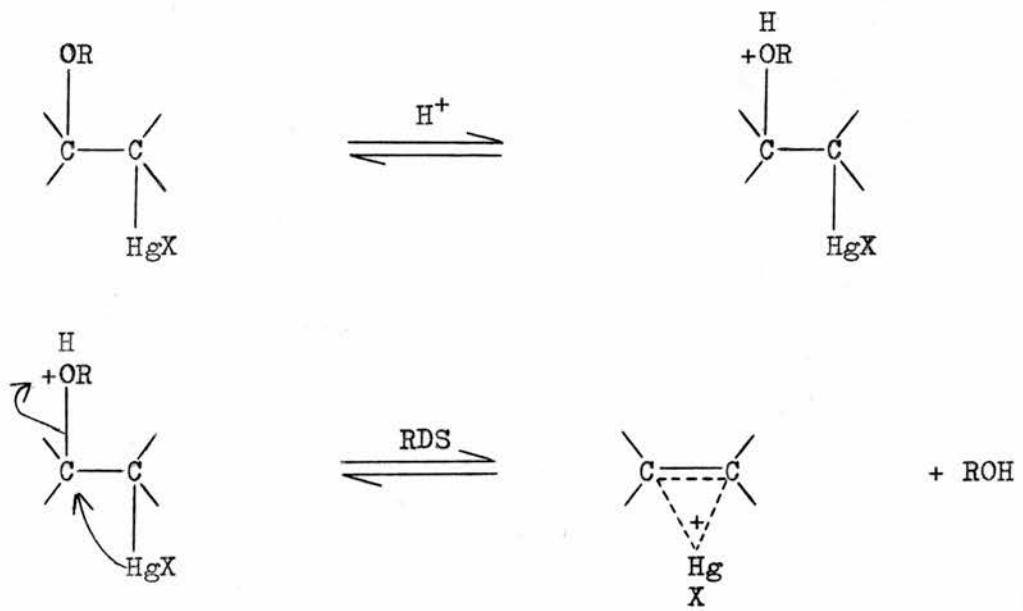
have reported the preparation and decomposition in various solvents of the acetoxy-mercurial from methyl oleate. Allylic acetates were the major products in all cases.

Bromination and thermal decomposition of oxymercurated products are not discussed further.



Acid regeneration (deoxymercuration)

Kreevoy and co-workers [43] showed by extensive kinetic experimental work that deoxymercuration of α-methoxy-β-iodomercuri compounds with aqueous perchloric acid containing iodide ion proceeded via a mercurinium ion formed from the protonated substrate in a rate-determining step:



Although the presence of halide ion is very necessary for the regeneration of the olefin its exact role in the deoxymercuration reaction has not been conclusively evaluated despite involved studies by Kreevoy [43] and Ichikawa et al [44]. This dependence on the presence of halide ion is strikingly demonstrated by the fact that $HOCH_2CH_2HgNO_3$ in 5% sulphuric acid is converted only in 20% yield to the ether compound $O(CH_2CH_2HgNO_3)_2$ in one

week.[12] In contrast, 5% hydrochloric acid regenerates ethylene quantitatively from the same hydroxy-mercurial in a few seconds.

As the overall result of oxymercuration is normally a trans-addition and treatment of the mercury adduct with halide acid gives trans-elimination, it is clear that oxymercuration - deoxymercuration is a stereospecific process. This property has been widely utilised in lipid chemistry for the separation and isolation of polyenoic long-chain esters.[45,46,47,48] Although methyl stearate, oleate, linoleate etc. can be separated chromatographically (thin layer [49] and column [50]) using the silver ion technique, separation of components is often minimal and can only be carried out on a relatively small scale. Improved separation on a larger scale of a mixture of saturated and unsaturated long-chain esters can be effected by reacting the mixture with mercuric acetate in methanol. Chromatography [46] or liquid - liquid partition [45,47] of the resultant methoxy-mercurials is considerably simplified due to the magnified differences in polarity between non-adducted material (from saturated esters), mono-adducts (from monoenes), di-adducts (from dienes) etc. After separation, the unsaturated esters can be regenerated stereospecifically and quantitatively by treatment of the fractions with 5% hydrochloric acid. Jantzen and Andreas [51] have also used this methoxymercuration - deoxymercuration technique to separate methyl oleate (which reacts quickly) from methyl elaidate (reacts more slowly).

Reduction of oxymercurials (demercuration)

Sodium amalgam,[52,53] sodium borohydride,[40,54] lithium aluminium hydride or electrolytic reduction [55] of a mercury adduct readily replaces the -HgX substituent by -H without affecting the alkoxy or hydroxy group. Sodium amalgam in D_2O,[56] sodium borodeuteride [57] and lithium aluminium deuteride reductions have been successfully used to replace -HgX by -D enabling the site of mercuration to be readily determined by NMR spectroscopy. Sodium stannite [39,58] and hydrazine reductions [40] of mercury adducts tend to give rather large amounts of regenerated olefin as well as saturated product, particularly if there is an alkoxy group β to the mercury function as there is in the products of alkoxymercuration.

Sodium borohydride reduction of alkyl mercurials appears to have been first used by Henbest and Nicholls [40] in ether-methanol solution. Traylor and Baker [56] and Robson and Wright [54] were the first to effect reductive demercuration in aqueous solution. Traylor [59] carried out the demercuration by in situ borohydride reduction but obtained a low yield (25%) of product. Brown and Geoghegan [60] improved on Traylor's technique and obtained an essentially quantitative yield of hexan-2-ol by the reaction of hex-1-ene and mercuric acetate in a water - tetrahydrofuran mixture followed by in situ reduction with aqueous alkaline sodium borohydride. Brown and his co-workers have extended the procedure to effect the Markownikov synthesis

of ethers [61] and amines [62] from terminal olefins. The advantage of the *in situ* reduction is that apart from the obvious convenience of removing a step in the procedure, handling of the highly toxic organo-mercury compounds is reduced to a minimum.

Although borohydride reductions of oxymercurated products are normally carried out in aqueous alkaline conditions, the reduction takes place rapidly and smoothly in neutral conditions.[57] It has been suggested [8] that the presence of alkali is responsible for the absence of deoxymercuration during reductive demercuration with sodium borohydride but deoxymercuration was not found to be a serious side-reaction during the present investigations where the addition of aqueous sodium hydroxide was avoided to prevent total or partial hydrolysis of the esters under examination.

Analytical applications of oxymercuration

Martin [63] devised a method for the accurate ($\pm$0.5%) determination of the amount of olefinic material present in a sample by titrating the acetic acid released by methoxymercuration of the sample with standard sodium hydroxide. Das [64] improved the procedure by showing that unreacted mercuric acetate could be measured titrimetrically in glycolic solution.

Minnikin [65] *et al* have recently developed a method of locating double bonds in long-chain mono- and poly-unsaturated esters by examining the mass spectrum of the methoxymercurated - reduced esters. This method had been used for the location of

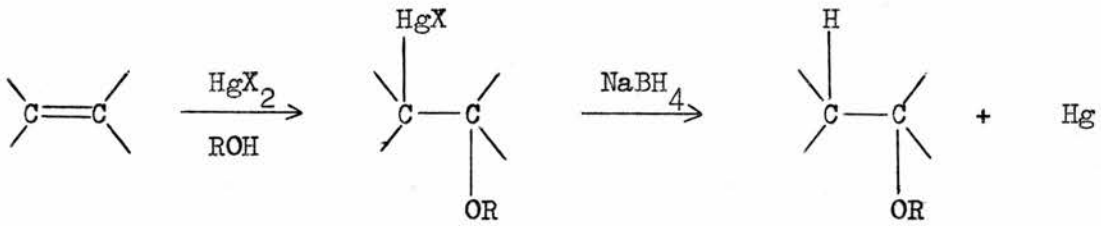
the sites of alkoxy- and hydroxymercuration (and therefore location of the sites of unsaturation) in the present investigations before Minnikin published his results.

The use of sodium borodeuteride demercuration has already been discussed.

CHAPTER 1

VARIATION OF ATTACKING NUCLEOPHILE

The reaction of olefins with a mercuric salt (usually mercuric acetate) in the presence of a nucleophile to give an organomercury compound which can readily be reduced by sodium borohydride [40] to a mercury-free product is well established.



The synthetic usefulness of this reaction has not been extensively exploited, particularly with unsaturated long-chain esters, and the experiments in this chapter were carried out to see how far the attacking nucleophile could be varied. Increased interest in this field is shown by the appearance of several significant papers[61,62,66] whilst our work was in progress.

Methyl oleate was normally used as substrate though tridec-1-ene was used (1.9 and 1.10) for comparison purposes when difficulty in identifying the oleate products was encountered. The methyl oleate used in this work was almost pure (98%) and contained traces of saturated esters (14:0 and 16:0) as the only impurities. These have been neglected in describing the products of each reaction.

Unless otherwise stated, oxymercuration reactions were carried out using mercuric acetate and reductions were effected with aqueous sodium borohydride.

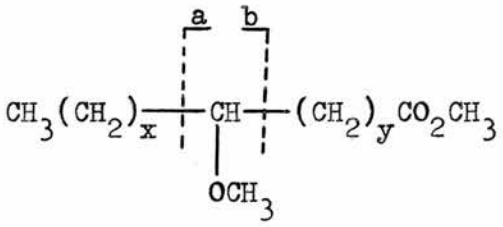
1.1 Methanol

Methoxymercuration is the most widely studied of the oxymercuration reactions [6,15,21,61] and we have used this as the standard reaction with which other oxymercuration reactions are compared.

The methoxymercuration - demercuration product from methyl oleate was found by GLC to contain only two materials (ECLs 18.5 and 21.2) which were shown to be methyl oleate (2%) and methyl 9(10)-methoxystearates (98%). The major product, isolated by prep TLC and examined by mass spectroscopy, proved to be a mixture (1:1) of the 9- and 10-methoxystearates. Methyl 9-methoxystearate was made by methylation of the corresponding hydroxy ester for comparison purposes.

The major fragments, which show that the mixture of 9- and 10-methoxystearates is a 1:1 mixture, are tabulated overleaf.

MS 1.1[1]



	a	b	a-32[4,5]	M+	M-31[5]
x = 8)	201[2]	171	169	328	297
y = 7)	(82)[3]	(53)	(11)	(0.4)	(6)

				Base peak
x = 7)	215	157	183	69
y = 8)	(78)	(63)	(12)	(100)

Notes (relevant to all tabulations of mass spectral data)

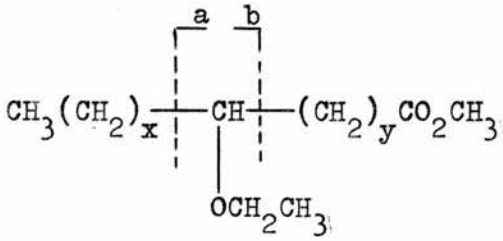
1. Stick diagrams are reproduced in appendix 1.
2. This figure is the $^{m}/e$ value for the fragment.
3. Intensities are expressed as percentages relative to the largest peak in the spectrum (base peak = 100%).
4. Proposed transitions in the mass spectrometer were usually confirmed by the presence of a metastable peak M* in the spectrum at an $^{m}/e$ value equal to ($^{m}/e$ daughter fragment)^2 ÷ ($^{m}/e$ parent fragment).[67]
5. Loss of 31 and 32 amu were due to the loss of CH_3O and CH_3OH respectively.

1.2 Ethanol [6,61]

Oxymercuration - reduction of methyl oleate in absolute ethanol yielded methyl oleate (ECL 18.5, 5%) and methyl 9(10)-ethoxystearate (ECL 20.7, 95%). The major product, which was less polar than methyl 9-methoxystearate, showed the same GLC and TLC behaviour as an authentic sample of methyl 9-ethoxystearate.

The abundance of the major fragments in the mass spectra of the authentic ester (MS 1.2a) and of the reaction product (MS 1.2b) show the latter to be a 1:1 mixture of the 9- and 10-substituted esters.

MS 1.2a and MS 1.2b



		a	b	a-28[1]	a-32	a-46[2]	a-60[3]	b-28[1]
1.2a	x = 8) y = 7)	215 (100)	185 (100)	187 (3)	183 (9)	169 (3)	155 (60)	157 (2)
1.2b	x = 8) y = 7)	215 (100)	185 (81)	187 (9)	183 (8)[4]	169 (21)[4]	155 (23)	157 (20)
	x = 7) y = 8)	229 (90)	171 (96)	201 (8)	197 (5)	183 (8)[4]	169 (21)[4]	143 (33)

Notes: overleaf.

Notes:

1. Loss of 28 amu is presumably due to the loss of ethylene (or the atoms thereof) from the ethoxy side chain.
2. Loss of 46 amu is due to the loss of ethanol.
3. Loss of 60 amu is due to the loss of 28 amu + 32 amu (ethylene + methanol).
4. Peak is produced by both isomers.

1.3 t-Butanol; 1.4 n-Butanol; 1.5 n-Hexanol

Brown and Rei [61] have shown that the oxymercuration of several simple terminal and cyclic olefins proceeds with ease in the presence of methanol, ethanol and iso-propanol. In the case of t-butanol they discovered that the rate of oxymercuration was considerably slower and the yield of alkyl ether (if any) much lower than with the other alcohols.

Attempted alkoxy- and aryloxymercurations of methyl oleate with mercuric acetate in the presence of t-butanol, n-butanol (THF as co-solvent) and n-hexanol (THF as co-solvent) and with mercuric trifluoroacetate* in the presence of t-butanol were unsuccessful. Unchanged methyl oleate was obtained in every case.

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* Brown [61] suggested that for optimum results the mercuric salt should be varied so as to avoid competition of the mercuric salt anion with the nucleophile for the mercurinium intermediate. The order of nucleophilicity of anions used in these studies is acetate > trifluoroacetate > nitrate.

1.6 Phenol

It has been reported that phenols can be added to alkenes photolytically [68] or by acid catalysis [69]. A convenient method of attaching polyhydric phenols with anti-oxidant properties to unsaturated fatty acids would solve the physical problem of solubility encountered in straightforward mixing of fatty acid and anti-oxidant.

Unfortunately, attempts to react phenol in THF with mercuric acetate, and in THF and in DMF with mercuric trifluoroacetate, were unsuccessful.

1.7 Water (+ co-solvent)

Although the first oxymercuration studies were conducted in aqueous solution [4,5,9] it was some time before the technique was used synthetically to effect the Markownikov hydration of a double bond. Brown and Geoghegan [66] studied this reaction with a series of terminal olefins in aqueous THF and found that the rate of reaction and the yield of product were reduced by changing the THF:H_2O solvent composition from 1:1 to 3:1. They also showed that variation of the temperature of reduction (30° - 60°) was unimportant. Brown expressed his surprise at the length of reaction time used by earlier workers compared with a reaction time of approx. one hour for the terminal olefins studied by him. Longer reaction times were necessary in the present experiments presumably due to the fact

that attack of HgX^+ on the olefin (rate-determining step) is much slower in the case of a 1,2-dialkyl substituted olefin due to steric hindrance than for a terminal olefin.

Methyl oleate was hydrated by oxymercuration - demercuration with water as the nucleophilic reagent in the presence of THF or DMF as co-solvents. When the ratio of water to co-solvent was 1:9 ($^v/_v$) the yield of hydroxystearate was low (5%) but this rose to 70 - 80% with a 1:1 ratio. It was shown that the rate of addition of aqueous sodium borohydride during the reduction step did not affect the yield of hydroxy ester.

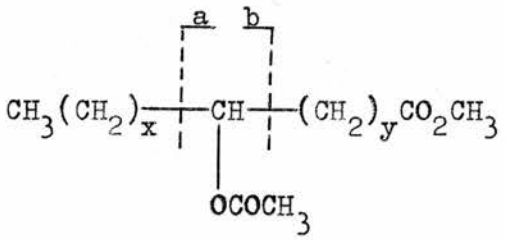
The major product (ECL 25.6) was isolated by prep TLC and the MS of its trimethylsilyl ether (ECL 19.9) indicated that it was a mixture (approx. 1:1) of methyl 9- and 10-hydroxystearates by consideration of the major fragments as before.
(See MS 1.7 appendix 1)

1.8 Acetic acid

Hugel and Hibou [7] were the first to report the acetoxymercuration of ethylene and higher olefins. Recently, French [37,38] and Japanese [41,42] workers have obtained acetoxymercurated adducts from methyl oleate, linoleate, ricinoleate and acetylated ricinoleate.

Acetoxymercuration - reduction of methyl oleate in glacial acetic acid gave a major product (82%, ECL 24.2) which was isolated by prep TLC and identified by its IR (1,235cm^{-1} and 1,725cm^{-1}) and mass spectrum as a 1:1 mixture of 9- and 10-acetoxystearates. Chromatographic comparison with authentic methyl esters showed the only other products to be methyl oleate (ECL 18.5, 13%) and methyl hydroxystearate (ECL 26.1, 5%).

MS 1.8



	a	a-32	a-42	a-60	a-74	b	b-42	b-60
x = 8)	229	197	187	169*	155	199	157	139
y = 7)	(17)	(7)	(75)	(60)	(63)	(5)	(28)	(12)
x = 7)	243	211	201	183	169*	185	143	125
y = 8)	(16)	(6)	(75)	(16)	(60)	(7)	(39)	(28)

continued overleaf:

M^+	M-31	M-42	M-43	M-60	M-74	M-75	M-91	M-92	base peak
356 (0.1)	325 (0.3)	314 (3)	313 (12)	296 (9)	282 (5)	281 (21)	265 (36)	264 (51)	74 (100)

* occurs twice

Losses are due to the following fragments:

-31 (CH_3O); -32(CH_3OH); -42 (CH_2CO); -43(CH_3CO); -60 (CH_3CO_2H); -74 (-32-42); -75 (-32-43); -91 (-31-60); -92 (-32-60).

The presence of metastable peaks confirmed all proposed transitions.

1.9 Ethylene glycol (ethanediol)

Oxymercuration with this dihydric alcohol as nucleophile was studied to see if it was possible to obtain the 2:1 (olefin: nucleophile) adduct, described as the bis-adduct.

There was no reaction between methyl oleate and ethylene glycol in a 2:1 molar ratio using mercuric acetate or mercuric trifluoroactetate.

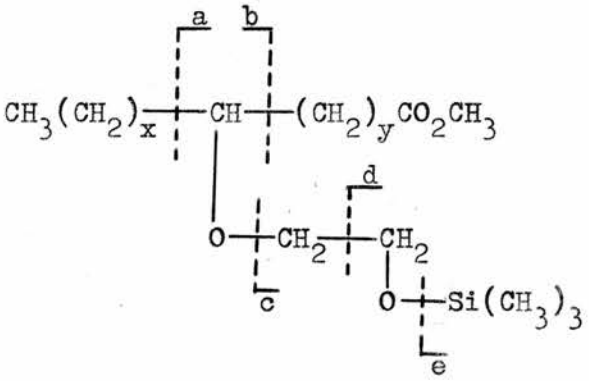
Reaction occurred with a large excess of ethylene glycol in DMF solution and, after reduction, the product was separated into three components (C_1, C_2, C_3) by prep TLC.

The least polar component (C_3, 36%) was shown by GLC (ECL 18.5) and TLC to be unchanged methyl oleate.

Component C_2 (11%, ECL 24.0) was shown by spectroscopy (IR 1,235cm^{-1}, 1,725cm^{-1}; NMR 8.06τ, 3H, singlet, $\underline{CH_3}COO-$; MS 1.9 C_2 appendix 1) and by its TLC and GLC behaviour to be identical to the 1:1 mixture of methyl 9- and 10-acetoxystearates obtained in the previous section (1.8).

The most polar component (C_1, 53%, ECL 28.5) was shown to be the oleate - ethylene glycol mono-adduct by elemental analysis. Mass spectral measurement of the TMS ether (ECL 25.5) indicated a 1:1 mixture of the 9- and 10-isomers. No bis-adduct was formed possibly because addition of the mono-adduct to a second oleate mercurinium ion is sterically inhibited.

MS 1.9 C_1



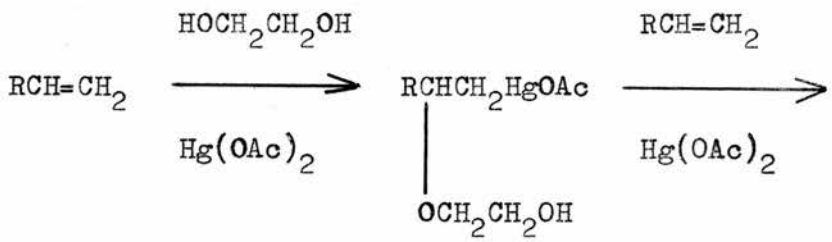
Fragments are tabulated overleaf.

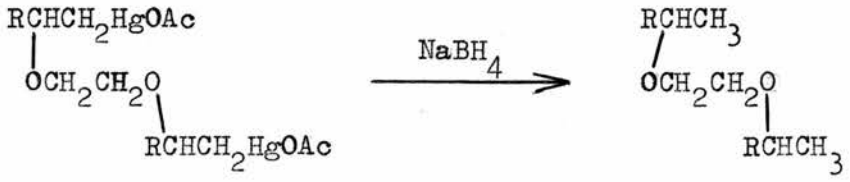
	a	b
x = 8)	303	273
y = 7)	(12)	(26)
x = 7)	317	259
y = 8)	(11)	(31)

c	d	e	M^+	M-31	M-d	M-c	M-c-16
117 (100)	103 (11)	73 (87)	430 (-)	399 (1)	327 (0.5)	313 (4)	297 (6)

An attempt to produce the bis-adduct by reaction of tridecene and ethylene glycol (2:1 molar ratio) with mercuric acetate in DMF was also unsuccessful. The mono-adduct (26%) was formed along with 2-acetoxy (53%) and 2-hydroxytridecane (8%).

Expected:

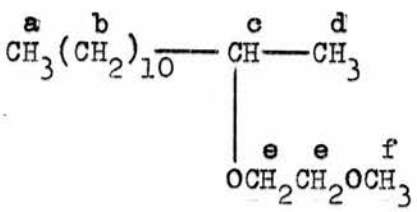




Sodium borohydride reduction of the oxymercuration reaction mixture gave a product which was methylated and separated into components D_1 - D_4 by prep TLC.

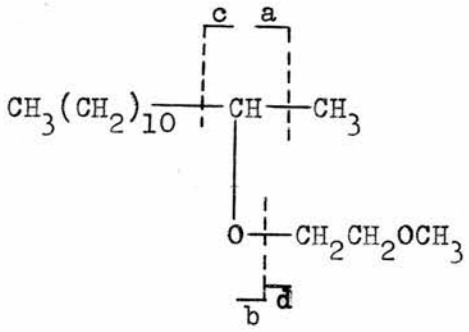
The most polar component D_1 (26%, ECL 16.3 before methylation, ECL 13.4 after methylation) was shown to be 2-(2'-methoxyethoxy)tridecane by NMR (1.9 D_1, p.144) and MS (1.9 D_1).

NMR 1.9 D_1



	τ value	Appearance	No. of protons
a	9.12	irreg. triplet	3
b	8.74	broad singlet	20
c	ca6.7	multiplet	1
d	8.93	doublet	3
e	6.59	singlet	4
f	6.73	singlet	3

MS 1.9 D_1

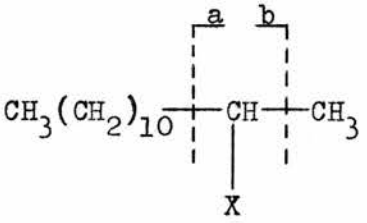


M^+	M-1	a	b	c	d
258 (-)	257 (0.6)	243 (5)	199 (6)	103 (100)	59 (96)

Component D_2 (53%, ECL 12.4 unchanged by methylation) was shown to be 2-acetoxytridecane by IR (1,235cm^{-1}, 1,725cm^{-1}), NMR (8.06 τ, 3H, singlet, $C\underline{H}_3CO$ O-) and MS (1.9 D_2; X = OAc in table below).

Component D_3 (8%, ECL 13.1 before methylation, ECL 9.3 after methylation) was identified as 2-methoxytridecane by IR (1,100cm^{-1} - ether linkage) and MS (1.9 D_3, X = OMe in table). The 2-hydroxytridecane which gave the 2-methoxy product by methylation must have been formed by the presence of water as an impurity (probably in the ethylene glycol) during the oxymercuration reaction.

MS 1.9 D_2 and MS 1.9 D_3



	a	b	M^+	
1.9 D_2, X = OAc	87 (100)	227 (0.5)	242 (0.3)	$^m/e$ 43 v. large (CH_3CO)
1.9 D_3, X = OMe	59 (*)	199 (22)	214 (1.5)	

* Peak with $^m/e$ 59 was much too large to allow measurement. The peak with $^m/e$ 69 was used as base peak instead.

Component D_4 (13%) was shown to be tridecene by GLC, TLC and MS (M^+ 182).

1.10 t-Butylhydroperoxide

While these investigations were in progress, several papers were published describing t-butylperoxymercuration. Bloodworth and co-workers [70,71] described the reaction of t-butylhydroperoxide with terminal olefins and mercuric acetate in dichloromethane. The peroxides were isolated by silica gel chromatography and demercurated by sodium borohydride reduction. Contamination of the peroxymercurials with 15 - 20mole% of acetoxymercurials was reported. These have been reported as contaminants in the hydroxymercuration of norbornene [56,72] and are not unexpected.

Schmitz et al [73] have also reported the t-butylperoxymercuration of several terminal olefins (50 - 90% yields) and of cyclohexene (53% yield). Bloodworth and Bunce [74] have recently described the t-butylperoxymercuration of α,β-unsaturated ketones and esters.

Oxymercuration - demercuration of methyl oleate in t-butylhydroperoxide gave 5 fractions (A_1 - A_5) by prep TLC.

The most polar fraction (A_1, 8%) was shown to be a mixture of several components by TLC and was not examined further.

Fraction A_2 (19%, ECL 25.5 and ECL as TMS ether 19.9) was shown to be methyl hydroxystearates by comparison with authentic methyl 9-hydroxystearate.

Fraction A_3 (6%) gave 7 peaks on GLC and was not examined further due to its complexity.

Fraction A_4 (44%) with ECLs 23.3 (86%) and 23.5 (11%) had an NMR spectrum similar to that of authentic cis-9,10-epoxystearic acid [75] except for the presence of the ester methyl protons. The signal at ca 7.52 τ (due to the epoxide methine protons) was irregular and unresolved, implying that A_4 was a mixture of methyl trans- and cis-epoxystearates. The mass spectrum (MS 1.10 A_4) was very similar to that of synthetic methyl 9,10-epoxystearate [76]. Co-injection of fraction A_4 with authentic epoxystearates showed the major component (ECL 23.3) to be the trans isomer and the other component (ECL 23.5) to be the cis isomer.

Fraction A_5 (23%, ECL 18.5) was found to be unchanged methyl oleate (GLC and TLC).

The hydroxy esters (A_2) are probably formed from water, present as an impurity in the t-butylhydroperoxide (practical grade) which was used. The complete absence of any t-butylperoxyoleate is presumably again due to steric interaction between the 1,2-disubstituted olefin and the bulky nucleophilic group.

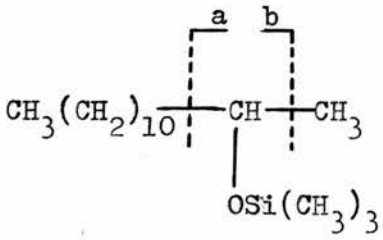
Epoxidation of the double bond to yield A_4 (44%) was not expected. Mercuric ion catalysis is the most probable explanation for this side reaction.

Tridecene in t-butylhydroperoxide gave four bands (B_1 - B_4) on prep TLC after oxymercuration - reduction.

Fraction B_1 (8%, most polar) appeared very complex on TLC(E) and was not examined further.

Fraction B_2 (9%, ECL 13.0) contained a hydroxyl group (IR and GLC of TMS ether) and was shown by MS of the TMS ether (MS 1.10 B_2) to be 2-hydroxytridecane.

MS 1.10 B_2



M^+	M-1	a	b
272 (0.6)	271 (1.2)	173 (*)	257 (27)

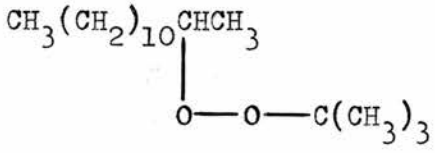
* Peak with $^m/e$ 173 was far too large to allow measurement. The peak with $^m/e$ 73 was taken as base peak.

Fraction B_3 (46%, ECL 12.1) was shown by comparison of its NMR spectrum with that of synthetic methyl 17,18-epoxystearate [77] (triplet, 8.03 τ, 1H; doublet, 7.5 τ, 2H) to be 1,2-epoxytridecane. The mass spectrum (MS 1.10 B_3) with a molecular ion peak at $^m/e$ 198 was in agreement with this structure.

Fraction B_4 (37%) showed a singlet at 8.82τin the NMR spectrum (9H, $(C\underline{H}_3)_3COO-$; cf NMR of t-butylhydroperoxide) and a signal at 9.1τ (6H, 2 x terminal $C\underline{H}_3-$) which implied that it was 2-<u>t</u>-butylperoxytridecane. The mass spectrum (MS 1.10 B_4)

supported this assignment. Fraction B_4 decomposed on GLC to give a major peak with an ECL similar to that of fraction B_3 (1,2-epoxytridecane). This suggests that B_4 may lose t-butanol on the GLC column to give the 1,2- (or possibly 2,3-) epoxytridecane.

MS 1.10 B_4



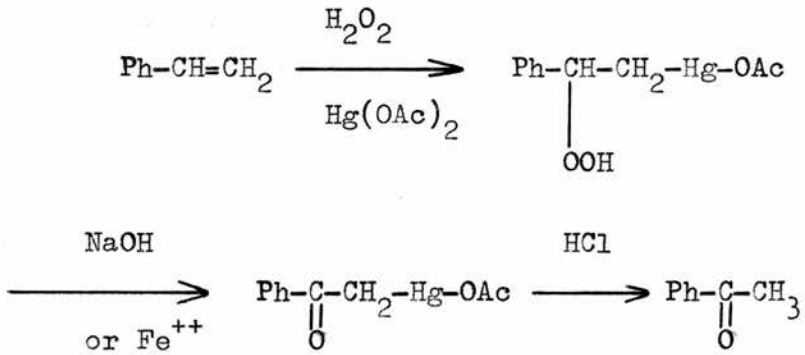
M^+	$M-Bu^tO$	$M-Bu^tOH$	$M-Bu^tOO$	$M-Bu^tOOH$
272 (23)	199 (2)	198 (3)	183 (20)	182 (100)

The peak with $^m/_e$ 57 (Bu^t-) was much too large to measure and the peak with $^m/_e$ 182 was used as base peak.

1.11 Hydrogen peroxide

Schmitz et al [73] have studied the oxymercuration of styrene and cyclohexene with hydrogen peroxide and mercuric acetate. They obtained the hydroperoxy mercury adducts in yields of 75% and 36% respectively. Treatment of these adducts with sodium hydroxide or with ferrous sulphate gave the corresponding ketones. Demercuration of the latter could be effected with hydrochloric acid.

e.g.



Attempted hydroperoxymercuration - reduction of methyl oleate in THF with hydrogen peroxide yielded a product which gave six fractions (A - F) on prep TLC.

Fraction A (12%, most polar) was shown to contain mercury and was not examined further.

Fractions B (30%) and C (11%) appeared to contain peroxide linkages (ammonium thiocyanate / ferrous sulphate spray reagent [78]) and a high oxygen to carbon ratio of approx. 1 to 5

(by elemental analysis). Despite NMR, IR and MS measurements these fractions were not conclusively identified. Attempts to reduce these fractions with (a) more sodium borohydride and (b) stannous chloride in methanolic hydrochloric acid [79] were unsuccessful.

Fraction D (8%) was shown to be methyl hydroxystearate by comparison with authentic hydroxystearates on TLC and GLC (also as TMS ether). The hydroxyl group may have become attached to the carbon chain during the oxymercuration step (50% aqueous hydrogen peroxide was used) and / or be formed by reduction of a hydroperoxy adduct during reaction with sodium borohydride.

Fraction E (29%) was identical to authentic methyl 9,10-trans-epoxystearate by TLC, GLC and MS (MS 1.11). The epoxide methine protons gave a signal at 7.54τ in the NMR spectrum (carbon tetrachloride as solvent) compared to a reported value (solvent not quoted) of 7.34τ [80]. Gunstone and Jacobsberg [77] have shown that the methine protons of the epoxide ring in synthetic methyl 9,10-trans-epoxystearate give a signal at 7.54τ. The difference in values may be due to solvent, concentration or instrument shifts.

A blank reaction with methyl oleate and hydrogen peroxide in THF without mercuric acetate gave unchanged methyl oleate as the only product. This supports the suggestion made in the last section that mercuric ion catalysed epoxidation may occur in the presence of peroxidic reagents.

Fraction F (10%) consisted of unchanged (or regenerated) oleate and saturated impurities present in the original methyl oleate.

Although a simple synthesis of hydroperoxy substituted fatty acids (particularly of allylic hydroperoxides) would be extremely valuable in the study of the autoxidation of fats [81]*, hydroperoxymercuration is unsuitable for this synthesis as demercuration of the adduct (eg. by sodium borohydride reduction) causes the decomposition of the hydroperoxide function.

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* Review on hydroperoxides.

1.12 Acetonitrile

Brown and Kurek [62] have recently reported the successful amidomercuration in high yield of four terminal olefins (hex-1-ene; dec-1-ene; 3,3-dimethylbut-1-ene and styrene) and two cyclic olefins (cyclopentene and cyclohexene) with mercuric nitrate (see footnote on p.20) in acetonitrile. The reaction is thought to proceed thus:

$$RCH{=}CH_2 + CH_3CN + Hg(NO_3)_2 \longrightarrow RCHCH_2HgNO_3 \quad (N{=}CCH_3,\ ONO_2)$$

$$RCHCH_2HgNO_3 \ (N{=}CCH_3,\ ONO_2) \xrightarrow{NaBH_4} RCHCH_3 \ (NHCOCH_3) + Hg$$

Brown added aqueous sodium hydroxide (3M) to the reaction mixture before addition of sodium borohydride (0.5M) in aqueous sodium hydroxide (3M). The use of sodium hydroxide was found to be unnecessary in the present studies.

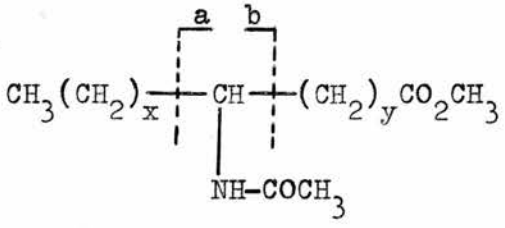
Methyl oleate and mercuric nitrate dihydrate in acetonitrile gave a product after borohydride reduction which was shown to contain ester and amide functions:-

IR spectrum:	3,420cm^{-1}	N-H stretching
	1,733cm^{-1}	ester C=O stretching
	1,672cm^{-1}	amide C=O stretching

NMR spectrum:	8.1 τ	3H	singlet	$\underline{CH}_3CO.NH-$
	6.4 τ	3H	singlet	$-CO.O\underline{CH}_3$

The mass spectrum showed the product to be a 1:1 mixture of methyl 9- and 10-acetylaminostearates.

MS 1.12



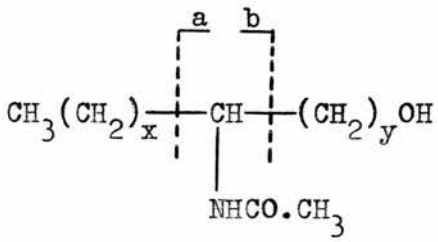
	a	b	a-32	a-42	a-74	b-42
x = 8) y = 7)	228 (41)	198 (47)	196 (12)	186 (44)	154 (18)	156 (82)
x = 7) y = 8)	242 (35)	184 (53)	210 (14)	200 (41)	168 (14)	142 (91)

M+1	M	M-1	M-31	M-43	M-75	Loss of fragments explained overleaf:
356 (3)	355 (3)	354 (2)	324 (8)	312 (4)	282 (6)	

Major fragments: $-31(CH_3O)$; $-32(CH_3OH)$; $-42(CH_2CO)$; $-43(CH_3CO)$; $-74(-31-43)$ or $(-32-42)$; $-75(-32-43)$.

An attempt to obtain the corresponding methyl aminostearates by refluxing the amides in methanolic sulphuric acid (aqueous sulphuric acid would have given the amphoteric amino acid which might have been difficult to isolate) was unsuccessful.

Sodium metal was added to the amide in methanol. The product was isolated after refluxing (10hr) and shown to be more polar on TLC than the starting material. The IR spectrum of this product showed that the amide carbonyl ($1,650cm^{-1}$) had not been markedly affected but that the ester carbonyl ($1,733cm^{-1}$) had disappeared and new peaks due to O-H absorption were now present. The NMR spectra of this product and of its methylated derivative (6.78τ, 5H, $-C\underline{H}_2OC\underline{H}_3$) confirmed that the methyl ester function had been reduced to an alcohol, presumably by a Bouveault - Blanc type of reaction. The major fragments in the mass spectrum of this product, all of which had even $^m/_e$ values due to the presence of a nitrogen atom, were consistent with α-cleavage to the amide group in a mixture of 9- and 10-acetylamino-octadecanols as shown overleaf.



	a	b	a-42	b-42
x = 8) y = 8)	198	200	156	158
x = 7) y = 9)	184	214	142	172

Lithium aluminium hydride reduction of a sample of methyl 9(10)-acetylaminostearates in ether gave the same alcohol (TLC, IR, NMR) as obtained above by reaction with sodium in methanol.

Attempts to obtain primary amino-octadecanols from the acetylamino-octadecanols by treatment with refluxing methanolic hydrochloric acid or by heating with 90% orthophosphoric acid at 100° and 180° gave products which were not conclusively identified but which were shown by IR, TLC and NMR not to contain an amine function.

An attempt to reduce the acetylamino-octadecanols to the corresponding ethylamino-octadecanols with diborane [82] in THF was unsuccessful.

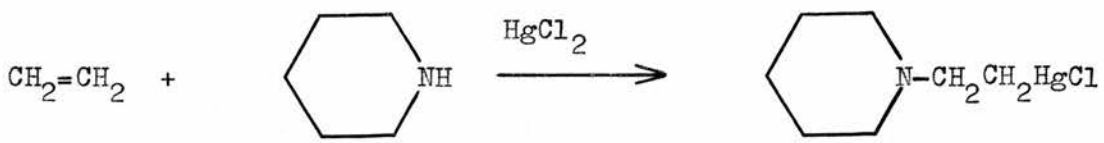
The reason for the stability of the long-chain amide is explained by Smith [83] who states -

> "Hydrolysis (of amides) is appreciably slowed down by branching in the neighbourhood of the amido group, as a result of interference with the approach of H_2O (or OH^-) to the carbonyl carbon, and highly hindered amides may become virtually unhydrolyzable."

The reason for the resistance of the acetylaminostearates (and amido-octadecanols) to reduction may also be that the long alkyl chains twist around the amido group and effectively protect it from attack.

1.13 Diethylamine and 1.14 Piperidine

Russian workers [84] first reacted ethylene and mercuric chloride in piperidine solution to obtain the adduct as shown:



Périé and Lattes [85,86,87] have recently described the aminomercuration of several simple terminal olefins using piperidine, pyrrolidine and aniline.

Hydrogen sulphide, sodium hydrogen sulphide, ammonia or diethylamine produced dense precipitates when added to a solution of mercuric acetate in DMF. These precipitates were presumably due to the formation of insoluble mercury-containing complexes. No precipitate appeared if the amount of added diethylamine was limited.

Attempted aminomercuration of methyl oleate with mercuric acetate and a limited amount of diethylamine in DMF gave unchanged methyl oleate after borohydride reduction.

An attempt to produce methyl 9(10)-piperidinylstearate by reacting oleate and mercuric chloride in piperidine gave unreacted oleate after borohydride reduction. Addition of a catalytic amount of di-t-butylperoxide to the mercuration reaction mixture did not alter the result.

Summary of results

Methyl oleate as substrate:

Nucleophile	Co-solvent (if any)	Products[1]	%[2]
methanol	-	9(10)-methoxy	98
ethanol	-	9(10)-ethoxy	95
t-butanol[3]	-	no reaction	-
n-butanol	THF	no reaction	-
n-hexanol	THF	no reaction	-
phenol[3]	THF	no reaction	-
phenol	DMF	no reaction	-
water	THF (1:1)	9(10)-hydroxy	78
water	DMF (1:1)	9(10)-hydroxy	72
acetic acid	-	9(10)-acetoxy	82
ethylene glycol	DMF	(9(10)-2'-hydroxyethoxy (9(10)-acetoxy	53 11
t-butylhydroperoxide	-	(9(10)-hydroxy (9,10-trans-epoxy (9,10-cis-epoxy (unidentified	19 38 6 29
hydrogen peroxide	THF	(9(10)-hydroxy (9,10-trans-epoxy (unidentified (peroxidic material (unidentified)	8 29 12 41
acetonitrile[4]	-	9(10)-acetylamino	100
diethylamine	-	no reaction	-
piperidine[5]	-	no reaction	-

Notes overleaf:

Notes:

1. All products are substituted methyl stearates.
2. Where the percentages or sum of percentages is less than 100, the difference is due to unchanged methyl oleate in the product.
3. These reactions were also attempted using mercuric trifluoroacetate with the same result.
4. Mercuric nitrate was used instead of mercuric acetate.
5. Mercuric chloride was used.

Tridecene as substrate:

Nucleophile	Co-solvent	Products[1]	%
ethylene glycol	DMF	2-2'-hydroxyethoxy	26
		2-acetoxy	53
		2-hydroxy	8
		tridecene	13
t-butylhydroperoxide	-	2-hydroxy	9
		1,2-epoxy	46
		2-t-butylperoxy	37
		unidentified	8

Note:

1. Products are 2-substituted tridecanes.

CHAPTER 2

SUBSTITUENT EFFECTS

This section is concerned with the influence of neighbouring functional groups on the oxymercuration of alkene esters. It has already been well established [60,61,71] that the Markownikov-orientated product results from the alkoxy-, hydroxy- or peroxymercuration of terminal olefins. This was confirmed by our methoxymercuration - demercuration of methyl hendec-10-enoate (section 2.4) which yielded the 10-methoxy ester only.

A series of esters of general structure $RCH(X)(CH_2)_nCH{=}CHR'$ were examined. In these, X was -OMe or -OAc (n = 1,2) and the double bond was either cis or trans. In addition, several cis and trans octadecenoates ($RCH{=}CH(CH_2)_nCOOCH_3$, n = 0 - 4) were examined. When n was very large, as in methyl oleate (n = 7, see section 1.1), the two possible methoxy isomers were obtained in equal amounts. At smaller values of n, the two isomeric products were formed in differing proportions. Our experiments showed that for the following sequence:

$$\underset{\displaystyle X}{\overset{\displaystyle |}{RCH(CH_2)_nCH{=}CHR'}} \xrightarrow[\text{ii. } NaBH_4]{\text{i. } Hg(OAc)_2,\ MeOH} \underset{\displaystyle X}{\overset{\displaystyle |}{RCH(CH_2)_n}}\underset{\displaystyle OMe}{\overset{\displaystyle |}{CH}}{-}CH_2R' \quad + \quad \underset{\displaystyle X}{\overset{\displaystyle |}{RCH(CH_2)_nCH_2}}{-}\underset{\displaystyle OMe}{\overset{\displaystyle |}{CHR'}}$$

(1) (2)

(i) The predominant product was that in which the group introduced was as far from the substituent (X) as possible. i.e. product (2).

(ii) The disparity between the proportion of the two products decreased as n increased.

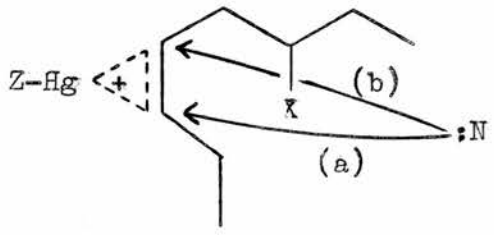
(iii) The results were virtually unaffected by the configuration of the double bond.

(iv) In reactions of appropriate dienes, the oxymercuration of one double bond gave a substituted monoene in which the substituent influenced the reaction of the remaining double bond.

(v) Different results were obtained when the substituent X was a hydroxy group (see section 3.1).

This partial selectivity of the position of nucleophilic attack, which led to a predominance of the product in which there was maximum separation of the two substituent groups, was probably due to steric hindrance of the attacking nucleophile by the substituent (X) already attached to the chain. Molecular models supported this hypothesis.

Diagrammatically: Reaction (a) is more favourable than reaction (b).



The presence of a substituent had no effect on the hydrochloric acid induced reversal of the oxymercuration reaction, except in two special systems (see sections 2.3 b and 2.7) where both cis and trans methoxymercurated alkenes furnished the trans isomers on deoxymercuration.

2.1 Methyl acetoxyoctadecenoates

The methoxymercuration - demercuration reactions of the cis and trans isomers of 12-acetoxy $\Delta 9$ (3) and 9-acetoxy $\Delta 12$ (4) C_{18} esters were examined.

$CH_3(CH_2)_5CH(OAc)CH_2CH=CH(CH_2)_7COOCH_3$ (3)

$CH_3(CH_2)_4CH=CH(CH_2)_2CH(OAc)(CH_2)_7COOCH_3$ (4)

The cis and trans isomers of (3) gave 12-acetoxy-10-methoxy and 12-acetoxy-9-methoxy esters and the isomers of (4) gave 9-acetoxy-12-methoxy and 9-acetoxy-13-methoxy esters. These were identified by comparison with authentic samples after conversion to the hydroxy-methoxy and the dimethoxy esters. The isomeric products were sufficiently separated by GLC to allow the proportion of each to be determined.

Relative amounts of acetoxy methoxy positional isomers produced.

β & ɣ acetoxy alkenes (18:1)	acetoxy methoxy esters 10,12-	9,12-	9,13-
9c 12-OAc (β)	20	80	-
9t 12-OAc (β)	14	86	-
12c 9-OAc (ɣ)	-	21	79
12t 9-OAc (ɣ)	-	34	66

The values reported above may be inaccurate due to the errors inherent in the area measurement of closely overlapping peaks (see note 4 to table on p.155). Nevertheless, the ratio of positional isomers in the product from the β-acetoxy alkene system was not very different from that observed for the ɣ-acetoxy alkene products. A larger change in the product isomer ratio occurred with other substituents (-OMe and -COOMe, see sections 2.2 and 2.3) as they changed position in relation to the double bond.

Unreacted starting material was obtained in each reaction (usually 2 - 4%) but this was inexplicably high (30%) in the reaction of the 12-acetoxy 9t methyl ester.

2.2 Methyl methoxyoctadecenoates

Methoxymercuration - demercuration of the four isomeric methyl methoxyoctadecenoates (5 and 6) gave the expected pairs of dimethoxystearates which were identified by chromatographic comparison with authentic samples of 9,12- and 10,12-dimethoxystearates.

$$CH_3(CH_2)_5CH(OMe)CH_2\overset{c}{\underline{CH}}=\overset{t}{\underline{CH}}(CH_2)_7COOCH_3 \qquad (5)$$

$$\downarrow$$

$$CH_3(CH_2)_5CH(OMe)CH_2CH_2CH(OMe)(CH_2)_7COOCH_3 \qquad (9,12-)$$

$$+$$

$$CH_3(CH_2)_5CH(OMe)CH_2CH(OMe)(CH_2)_8COOCH_3 \qquad (10,12-)$$

$$CH_3(CH_2)_4\overset{c}{\underline{CH}}=\overset{t}{\underline{CH}}(CH_2)_2CH(OMe)(CH_2)_7COOCH_3 \qquad (6)$$

$$\downarrow$$

$$CH_3(CH_2)_4CH(OMe)(CH_2)_3CH(OMe)(CH_2)_7COOCH_3 \qquad (9,13-)$$

$$+$$

$$CH_3(CH_2)_5CH(OMe)CH_2CH_2CH(OMe)(CH_2)_7COOCH_3 \qquad (9,12-)$$

Small amounts of starting materials found in the reaction products were presumably formed by regeneration during the sodium borohydride reduction step. As in section 2.1, the 9-trans ester gave an unusually large amount (13%) of regenerated starting material.

Relative amounts of dimethoxy positional isomers produced

β & ɣ methoxy monoenes (18:1)	dimethoxy esters 10,12-	9,12-	9,13-
9c 12-OMe (β)	19	81	-
9t 12-OMe (β)	20	80	-
12c 9-OMe (ɣ)	-	42	58
12t 9-OMe (ɣ)	-	45	55

2.3 Methyl octadecenoates

(a) Methoxymercuration - demercuration

Several sythetic C_{18} monoene esters [88] in which the double bond was near the carbomethoxy function gave the expected isomeric methoxy products by methoxymercuration - demercuration.

$$RC\underline{H}{=}{=}\underline{C}H(CH_2)_nCOOCH_3 \longrightarrow RCH(OMe)(CH_2)_{n+1}COOCH_3 + RCH_2CH(OMe)(CH_2)_nCOOCH_3$$

The ratio of the isomeric methoxystearates produced from each monoenoic ester was readily determined if these isomers could be separated on a capillary GLC column. Assignments of ECL values to the isomers were made by comparison of the different products on GLC as in the following example:

18:1 3-cis gave products with ECLs 20.3 and 20.6.

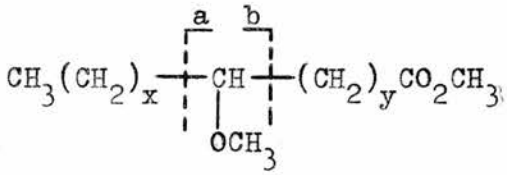
Expected products: 3-methoxy- + 4-methoxystearate.

18:1 4-cis gave products with ECLs 20.6 (20%) and 20.8 (80%)

Expected products: 4-methoxy- + 5-methoxystearate.

Thus methyl 4-methoxystearate must have an ECL of 20.6 and the appropriate ECLs can be assigned to the 3- and 5-methoxy esters. This conclusion was confirmed by the mass spectral analysis of the reaction products. For example, the mass spectrum for the 4-cis ester product showed a predominance of the α-cleavage fragments from 5-methoxystearate consistent with the assignment already made.

MS 2.3



		a	b	a-32
4-methoxy	(x = 13) (y = 2)	131 (22)	241 (3)	99 (4)
5-methoxy	(x = 12) (y = 3)	145 (40)	227 (9)	113 (15)

When the isomeric reaction products were eluted as a single peak on capillary GLC, the isomer ratio was approximately determined* by considering the α-cleavage fragments in the mass spectrum.

Relative amounts of each methoxy isomer

ester	position of methoxy group in product							
	2-	3-	4-	5-	6-	7-	9-	10-
2-*cis*	0	78						
2-*trans***	0	94						
3-*cis*		4	96					
3-*trans*		7	90					
4-*cis*			20	80				
4-*trans*			21	79				
5-*cis*				50	50			
6-*cis*					50	50		
9-*cis*							50	50

** When comparing ECL values, an adjustment of -0.3 ECL units had to be made for the 2-*trans* ester products as they were *ethyl* ester. All others were methyl esters.

* Caution must be exercised when analysing the mass spectrum of a mixture of positional isomers as one particular peak in the spectrum may result from more than one fragment. Furthermore, quantitative comparison of similar breakdown fragments from two isomers in a mixture can only give an approximate answer, particularly if the position of substitution is near other functional groups or near the end of a long chain.

The reaction product from the 2-cis ester contained methyl octadec-trans-2-enoate (20%) which was isolated by prep TLC and identified by its TLC and MS behaviour and by its IR spectrum ($975cm^{-1}$, trans). Starting material (2%) was also present.

The 2-trans and the 3-trans products contained 6% and 3% respectively of unreacted (or regenerated) starting material.

The steric (and possibly electronic) effect was seen to be particularly strong in the case of reaction with the 2- and 3-unsaturated alkenoates but died away rapidly thereafter. The geometry of the double bond appeared to have little effect on the regiospecificity of the reaction.

(b) Methoxymercuration - deoxymercuration

Aliquots were removed from several of the methoxymercuration reaction mixtures in part (a) and deoxymercurated with methanolic hydrochloric acid. Regeneration of the esters was stereospecific and the products were identified by comparison with authentic monoene esters on GLC and Ag^{+} TLC. The only exception was methyl octadec-cis-2-enoate (ECL 18.3) which gave the trans isomer (ECL 19.6) in 100% yield. The latter was identified by comparison with ethyl octadec-trans-2-enoate on GLC and TLC and from the IR spectrum which showed strong absorption at $975cm^{-1}$. The UV spectrum (λ_{max} 218nm) confirmed that it was an α,β-unsaturated ester. Grimmer and Hildebrandt [89] had previously

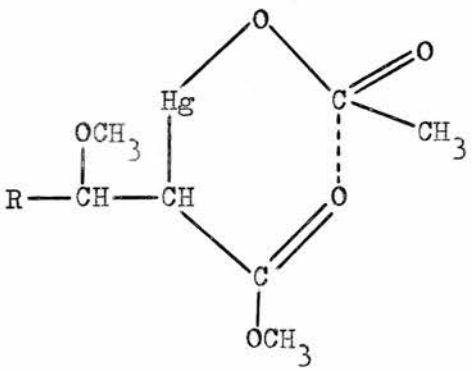
obtained a mixture of isomers by treatment of some methoxymercurated 2-cis methyl alkenoates with mineral acid. No experimental details were included, and it was not clear how they identified their reaction products.

As the normal methoxymercuration of a double bond proceeds via an intermediate mercurinium ion to give overall trans-addition, and halide acid induced deoxymercuration proceeds via a similar intermediate to give overall trans-elimination [44] (by the principle of microscopic reversibility), this abnormal stereomutation must proceed by one (or both) of the following routes:

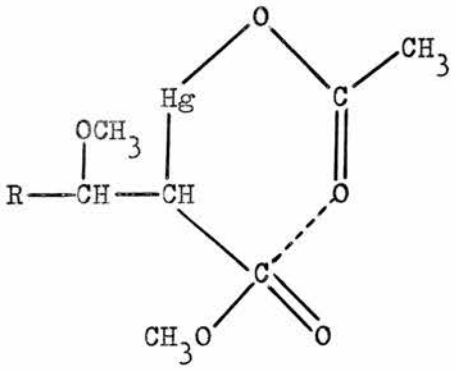
trans-addition then cis-elimination (7)

cis-addition then trans-elimination (8)

From the borohydride reduction products, it is clear that the mercury substituent in the adduct is only on the second carbon atom. This being so, at least two 6-membered configurational-holding type structures can be drawn:



(9)



(10)

Although it is not immediately obvious, it is conceivable that electronic interactions of this type could lead to a cis-elimination. Alternatively, interaction between the intermediate mercurinium ion and the carbomethoxy group might favour cis-addition (8) or cis-elimination (7) but not both.

To determine whether the anion (acetate) was significant in this reaction, methoxymercuration with mercuric chloride was attempted. Since borohydride reduction gave only starting material, it was assumed that there was no oxymercuration with this salt.

Ethyl octadec-trans-2-enoate, which contained 7% of the cis-isomer as an impurity, gave the pure trans-isomer after methoxymercuration and acid regeneration.

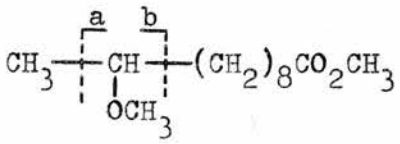
This mild stereomutation procedure for an α,β-cis-unsaturated ester might be adaptable to other conjugated enone systems and could be useful in synthetic preparations where this type of isomerism was required.

2.4 Methyl hendec-10-enoate

Methoxymercuration - demercuration of methyl hendec-10-enoate gave starting material (ECL 12.0, 9%) and methyl 10-methoxyhendecanoate (ECL 15.9, 91%). The latter was isolated by prep TLC and identified by its NMR spectrum which showed a doublet at 8.95 τ(3H) indicating that the terminal methyl group

was adjacent to a methine group. i.e. $C\underline{H}_3-CH(OCH_3)-$ The absence of a triplet at ca 6.8τ ($CH_3OC\underline{H}_2-$) showed that the 11-methoxy isomer was not formed. This was confirmed by the mass spectrum and by the fact that, if present, we would have expected it to separate from the 10-methoxy product on GLC. The mass spectrum confirmed the structure of the product.

MS 2.4



$\underline{M^+}$	<u>M-1</u>	<u>M-31</u>	<u>a</u>	<u>b</u>	<u>a-32</u>
230	229	199	215	59	183
(0.2)	(1)	(7)	(22)	(100)	(7)

This result was in agreement with the report by Brown <u>et al</u> [61] who obtained Markownikov orientated products from several simple mono-olefins.

2.5 Methyl linoleate

Methyl linoleate containing methyl oleate (2%) and methyl linolenate (2%) as the only impurities was reacted with excess mercuric acetate in methanol. Aliquots were removed at various times (1hr - 100hr) and reduced with borohydride.

The products were examined by GLC (see table p.161) and it was found that the time of reaction had little effect on the composition of the products.

A partially reacted (or partially regenerated) product (ECL 21.7, ca 20%) was shown by its GLC and TLC behaviour to be methoxymonoene. The amount of the latter was reduced to 7% in another experiment using fresh sodium borohydride for the reduction step. Partial regeneration from the mercury di-adduct seems to be the more feasible source of this product which was isolated by prep TLC and hydrogenated to yield methyl methoxystearates (ECL 21.2).

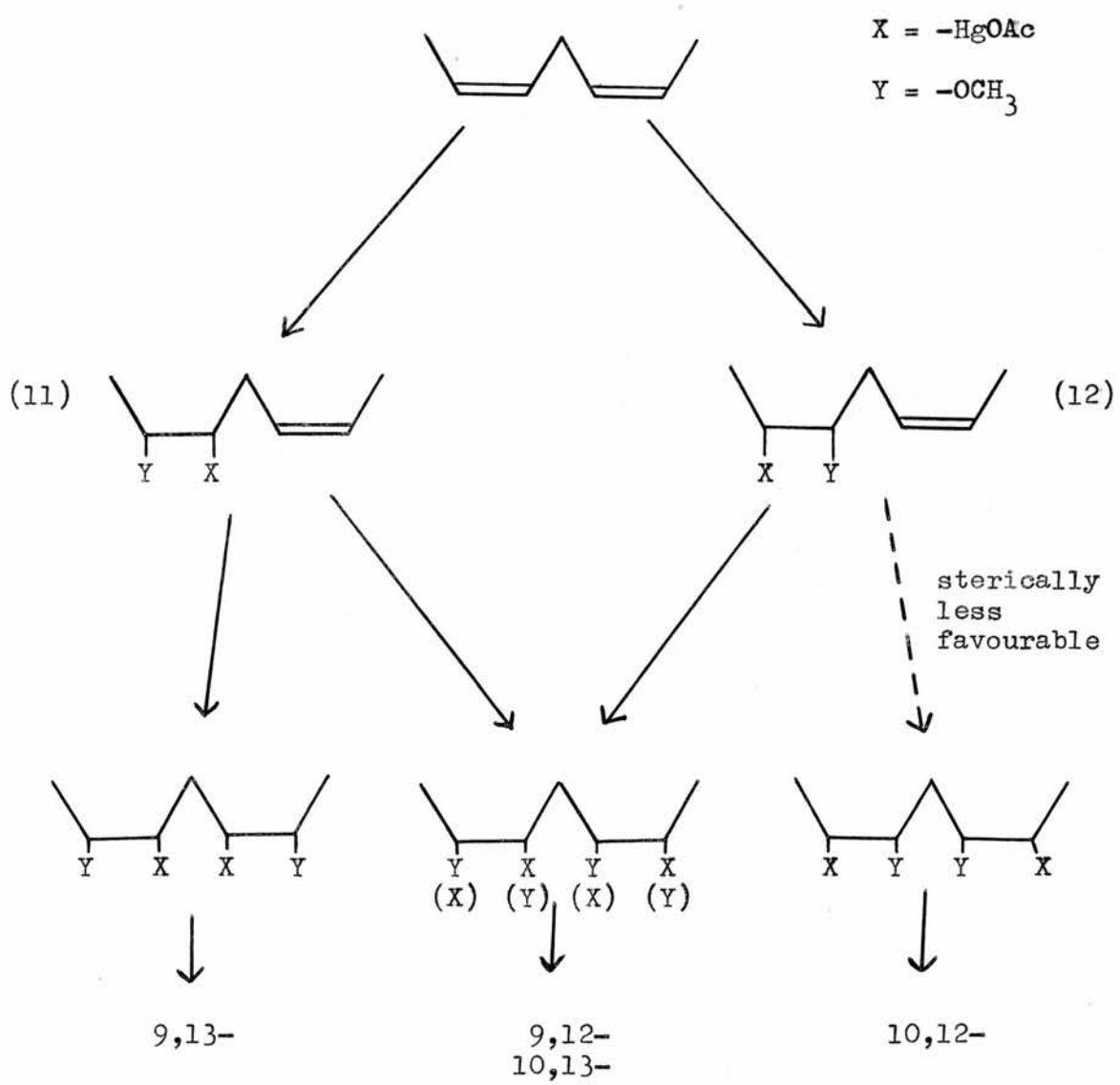
The major products of this reaction with ECLs 23.6 (11%) and 24.2 (79%) were chromatographically identical to authentic methyl 10,12- and 9,12-dimethoxystearates * respectively. The major component (79%) probably contained methyl 9,12-; 10,13- and 9,13-dimethoxystearates, all of which would be expected to have similar TLC and GLC behaviour. The small amount of methyl methoxystearate (ECL 21.2, 3%) also obtained in this reaction was probably formed by the methoxymercuration - demercuration of the oleate impurity.

Assuming that the double bonds of linoleate were methoxymercurated one after the other, two types of system (11 and 12) could result after one double bond had reacted. Each of these

---

* These dimethoxy esters were isolated and characterised as described in section 3.1.

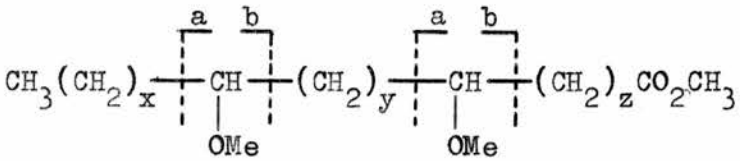
systems could give rise to two dimethoxy isomers after the second bond was attacked. Assuming that nucleophilic attack took place preferentially at the intermediate mercurinium ion carbon atom furthest away from the neighbouring substituents, we can see why the 10,12- isomer was formed in low yield (11%) compared with the sum of the three other possible isomers (79%).



2.6 Methyl octadec-cis-8-cis-12-dienoate

The methoxymercuration - demercuration and deoxymercuration reactions of synthetic 18:2 8c,12c ester were examined. Acid-induced regeneration gave starting material as shown by GLC, TLC and IR (no absorption at 970cm^{-1}). Sodium borohydride demercuration gave a mixture of the possible isomeric dimethoxystearates (8,12-; 9,12-; 8,13- and 9,13-). This mixture gave a broad peak on GLC (ECL 24.0) and had the same TLC behaviour as authentic methyl 9,12-dimethoxystearate. The major fragments in the mass spectrum showed that there was a greater amount of substitution on carbon atoms 8 and 13 than on carbon atoms 9 and 12. This was in accordance with the steric hindrance hypothesis.

MS 2.6



Position of substituent	a	b	a-32	b-32	a-64
z = 6 C(8)	187 (42)	215 (29)	155 (8)	183 (8)	
z = 7 C(9)	201* (41)	201* (41)	169* (14)	169* (14)	
x = 5 C(12)	273 (10)	129 (26)	241 (8)	97 (16)	209 (2)
x = 4 C(13)	287 (18)	115 (53)	255 (4)	83 (24)	223 (8)

M^+	M-15	M-31	M-32	M-63	M-64	base peak
358 (-)	343 (0.4)	327 (0.6)	326 (1)	295 (2)	294 (3)	71 (100)

* These fragments were produced from two sources.

2.7 Conjugated octadecadienoates

Mills [90] has shown that the cis, trans conjugated diene in the diterpenoid alcohol cis-abienol can be converted in 80% yield to the trans, trans isomer by treatment with mercuric acetate in acetic acid followed by deoxymercuration in ether solution with hydrochloric acid, or with zinc and acetic acid. He then found that the isomeration could be effected in methanol or in acetic acid but not in ether with catalytic amounts (0.1mole) of mercuric acetate. McNeely and Wright [91] showed that methoxymercuration of buta-1,3-diene with one equivalent of mercuric acetate gave only 4-chloromercuri-3-methoxybut-1-ene after exchange of acetate by chloride anion. No evidence of a 1,4-addition product was found.

The purity of conjugated diene esters (ca 43%) obtained from dehydrated castor oil by methanolysis and prep TLC was considered too low for the present investigation. An improved starting material (93% conjugated diene) was obtained by the thermal decomposition of the methanesulphonate ester from methyl ricinoleate in a heterocyclic base by the method of Gunstone and Said [92]. Isomeric conjugated dienes were identified by their ECL values which were known. The mixture of conjugated dienes was treated with mercuric acetate in methanol. Aliquots were removed and were deoxymercurated with methanolic hydrochloric acid. The GLC results show that there was some stereomutation to give trans, trans from cis,trans from cis,cis isomers.

viz:

ECL	Assignment (18:2)	starting material	deoxymercurated product
19.3	9c,12c	7	7
20.3	9c,11t	73	60
20.6	9c,11c	17	13
20.9	9t,11t	3	20

As in section 2.3 b, either addition or elimination (but not both) must be able to proceed by a cis mechanism for this isomerisation to occur.

Sodium borohydride reduction of the methoxymercuration reaction mixture gave methyl methoxyoctadecenoates (ECL 21.3, 30%), methyl 10,12- and 9,11-dimethoxystearates (ECL 23.3, 62%) and methyl 9,12-dimethoxystearate (ECL 23.9, 8%). These were identified by comparison with authentic esters on TLC and GLC. The partially reacted diene (methoxy monoene) showed trans absorption in the IR spectrum (970cm^{-1}). Methyl 10,11-dimethoxystearate, if present, would have had a different ECL on GLC from the other dimethoxy esters.

Unlike other experiments in this section, the major product is not the one in which the substituent groups are furthest apart. This may be due to an increase in the carbonium ion character of the mercurinium ion carbon atom adjacent to the second double bond due to stabilisation of the carbonium ion by the

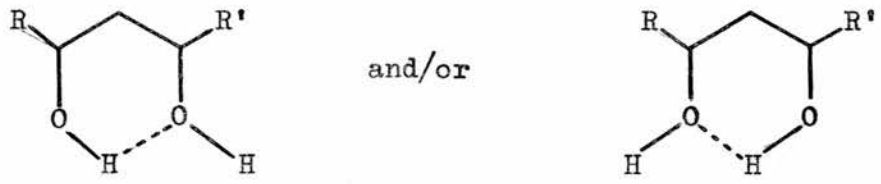
double bond. This would explain the higher incidence of nucleophilic attack at carbons 10 and 11. Subsequent attack of the second double bond would then be governed by the normal steric considerations, yielding the 10,12- and 9,11-dimethoxy isomers as the major products.

2.8 Methyl ricinoleate

Hydroxymercuration - demercuration of methyl ricinoleate in aqueous tetrahydrofuran (1:1 v/v) gave products which were examined on GLC as TMS ethers. Prep TLC(PE30) of this product gave methyl 9,12- and 10,12-dihydroxystearates (79%), starting material (4%) and methyl 9,12-epoxystearates (17%).

The epoxystearates (ECLs 21.1, 94% and 21.4, 6%) were identified by chromatographic comparison with authentic material which was obtained in higher yield and characterised as described in section 3.1. The formation of this cyclic ether is also discussed in that section.

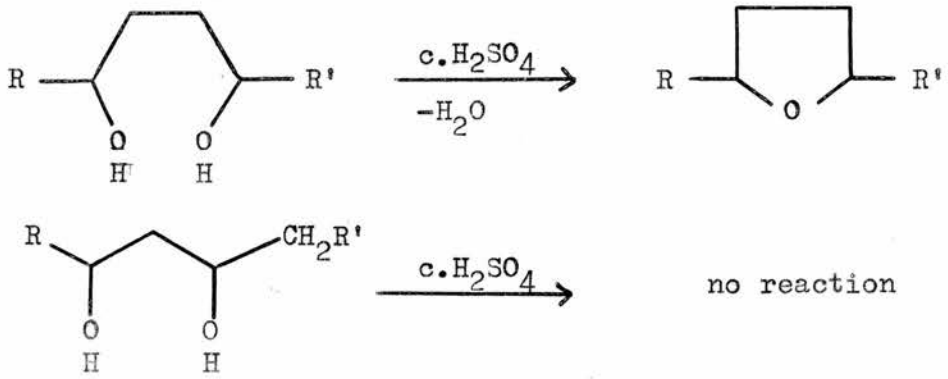
The methyl dihydroxystearates were examined on GLC as bis-TMS ethers (ECLs 20.4, 12% and 20.7, 88%). TLC(PE70) of the dihydroxy esters showed two spots, the smaller of which was the less polar. This suggested that the minor component was the 10,12-dihydroxy ester which would undergo intramolecular hydrogen bonding as shown:



Similar hydrogen bonding with the 9,12-dihydroxy ester would involve a 7-membered ring.

This tentative identification of the 10,12-dihydroxy ester as the minor component was confirmed by dehydration of the mixture with methanolic sulphuric acid. TLC of the dehydrated product showed the minor spot to be unchanged, whereas the larger spot was replaced by a much less polar component, formed by the cyclo-dehydration of the 9,12-dihydroxy ester:

i.e.



Prep TLC of the total dehydration product gave methyl dihydroxy ester (16%, ECL 20.4 as TMS ether) and the cyclised product (84%, ECLs 21.1, 78% and 21.4, 22%). The latter was identified as a mixture of methyl <u>cis</u>- and <u>trans</u>-9,12-epoxystearates

by comparison with the products obtained and characterised in section 3.1.

The unchanged dihydroxy material was methylated and was shown by comparison with authentic methyl dimethoxystearates to be predominantly methyl 10,12-dimethoxystearate (ECL 23.3, 78%) with some uncyclised methyl 9,12-dimethoxystearate(ECL 23.9, 22%) present.

The products from the hydroxymercuration - demercuration of ricinoleate were therefore:

starting material	4%
methyl 9,12-epoxystearates	17%
methyl 10,12-dihydroxystearate	10%
methyl 9,12-dihydroxystearate	69%

The relative amounts of the two dihydroxy isomers were consistent with the steric hindrance proposition.

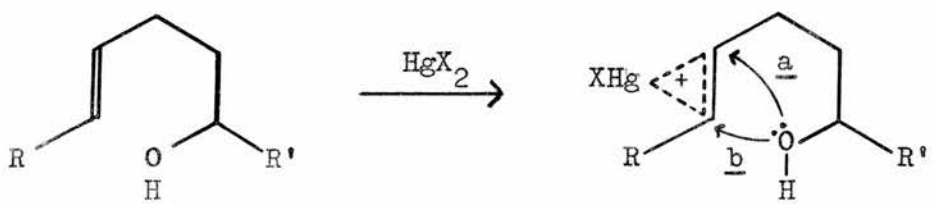
CHAPTER 3

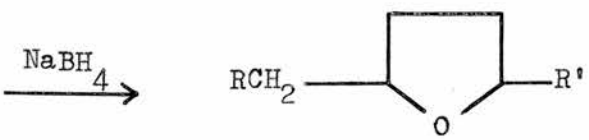
NEIGHBOURING GROUP PARTICIPATION

This chapter concerns the oxymercuration of several unsaturated long-chain compounds in which an oxygenated substituent (-OH, -COOH, -CH(O)CH-) near the double bond can attack the intermediate mercurinium ion to yield O-heterocyclic products. Hydroxymercuration of two non-conjugated diene esters to give cyclic ethers is also described.

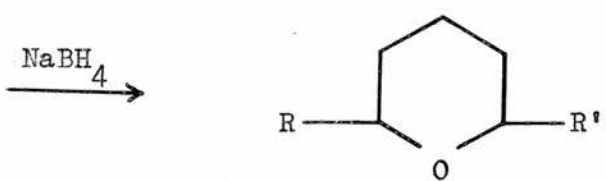
There are many examples in the literature describing the formation of internal cyclic ethers by the oxymercuration of hydroxy alkenes.[13,39,53] This cyclisation reaction is particularly favourable when the product is a 5- rather than a 6-membered ring. Although the conformational ring strain is greater in the former, this is outweighed by the higher probability that the two positions in a chain of atoms will come close enough together to form a 5- rather than a 6-membered ring. In the following investigations, a di-substituted tetrahydrofuran (1) was always formed in preference to a di-substituted tetrahydropyran (2) when either could have been formed.

i.e.



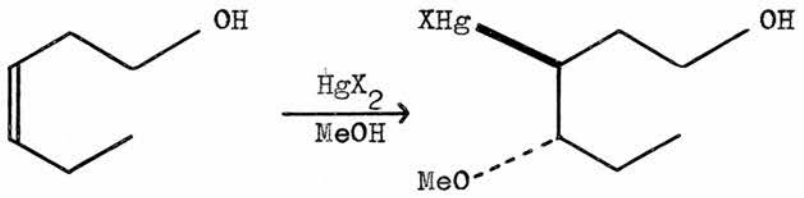


(1)

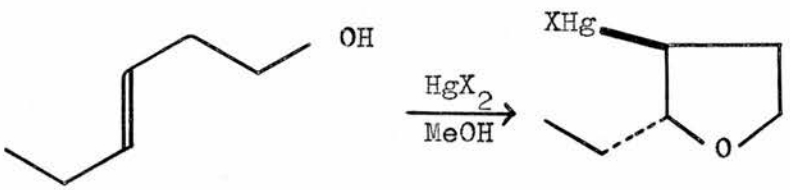


(2)

Henbest and Nicholls [40] demonstrated the importance of the double bond geometry in the methoxymercuration of a β-hydroxy monoene by showing that cis-hex-3-enol (3) and trans-hex-3-enol (4) reacted in methanol with a mercuric salt to give different products:



(3)



(4)

3.1 Hydroxy monoene esters

Oxymercuration

Four isomeric methyl hydroxyoctadecenoates (see table, A - D) were subjected to methoxymercuration. Although TLC showed the absence of starting material after one hour, sodium borohydride reduction after the same time yielded a large amount of unchanged hydroxy esters which were identified by TLC and GLC. Sodium

borohydride reduction of aliquots after 2 days gave products which were found to contain little, if any, starting material. These products were examined on a capillary GLC column (see table below).

Acid induced deoxymercuration of aliquots from the methoxymercuration reaction mixtures of the two hydroxy cis-alkenoates (A and C) gave stereospecifically regenerated hydroxy monoenoates as shown by GLC, TLC and IR (no trans absorption at 970cm^{-1}).

Capillary GLC results (borohydride products)

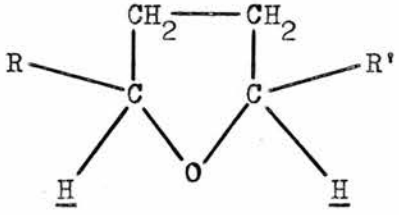
expt	A	B	C	D
methyl esters	9c 12-OH	9t 12-OH	12c 9-OH	12t 9-OH
products (ECL)				
9,13-epoxide (20.0)	-	-	-	8%
9,12-epoxides (21.3)	8%	20%	33%	35%
(21.6)	2%	79%	67%	57%
starting material (26.2)	6%	1%	-	-
10-OMe,12-OH (28.0)	18%	-	-	-
9-OMe,12-OH (29.0)	66%	-	-	-

Identification of cyclic ethers

The cyclic ether with ECL 20.0 (D_1) was separated from the total demercurated product D by prep TLC. The mass spectrum (MS 3.1 D_1, see overleaf) showed conclusively that this component was methyl 9,13-epoxystearate.

The 9,12- cyclic ethers with ECLs 21.3 and 21.6 (D_2) were

also isolated from product D by prep TLC and were examined by MS (see MS 3.1 D_2 below). The NMR spectrum of product B, which was essentially the same as D_2 (see previous table), showed an irregular multiplet (ca 2H) under the sharp ester methyl singlet at 6.4τ. This was probably due to resonance of the protons on the carbon atoms adjacent to the ring oxygen:



The NMR spectra of <u>cis</u> and <u>trans</u> 2,5-dimethyltetrahydrofuran has been described by Gagnaire and Monzeglio [93]. They reported τ-values of 6.14 and 5.98 for these ring protons in the <u>cis</u> and <u>trans</u> isomers respectively.

The two peaks observed on GLC for this product are considered to be due to the <u>cis</u> and <u>trans</u> isomers, both of which gave exactly the same mass spectral fragmentation pattern when examined separately by the GLC - MS technique. We were unable to relate the GLC peaks to the <u>cis</u> and <u>trans</u> isomers.

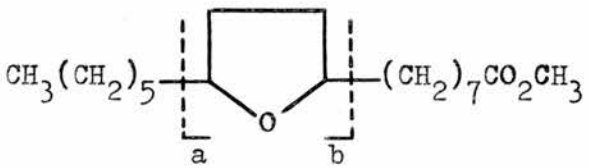
The 1,5-epoxide (D_1) can presumably also exist in <u>cis</u> and <u>trans</u> forms and although it gave a single peak on GLC, this did not preclude the possibility that both isomers were formed in the reaction.

These long-chain epoxides have been obtained by other

methods and characterised by Abbott and Gunstone [94]. Extensive isotopic studies by Brandt and Djerassi [95] on long-chain 1,4-epoxy esters established the major mechanistic fragmentation pathways in the mass spectrometer. Loss of 18 units is explained by the loss of H_2O.

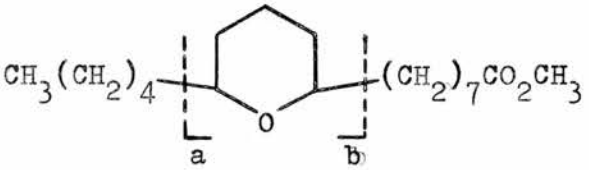
The major fragments are tabulated and assigned below:

MS 3.1 D_2



M+1	M	M-1	M-18	M-31	M-49*	a	a-18	a-32	a-50*	b	b-18
313 (1)	312 (2)	311 (1)	294 (1)	281 (2)	263 (2)	227 (48)	209 (12)	195 (50)	177 (15)	155 (100)	137 (38)

MS 3.1 D_1



M+1	M	M-1	M-18	M-31	M-49*	a	a-18	a-32	a-50*	b	b-18
313 (1)	312 (3)	311 (1)	294 (2)	281 (2)	263 (2)	241 (15)	223 (25)	209 (27)	191 (13)	155 (98)	137 (58)

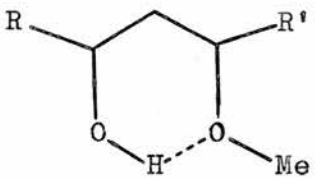
* -49 = -31-18; -50 = -32-18.

Aliquots from the methoxymercuration reaction mixture B were reduced with sodium borohydride after two days and after one year at 0°. Both reductions gave isomeric methyl 9,12-epoxystearates, but GLC of the one-year product showed an increase of 5% in the area of

the first isomer peak (ECL 21.3) with a corresponding decrease in the area of the second (ECL 21.6). This suggested that the first eluted isomer is the more thermodynamically stable of the two.

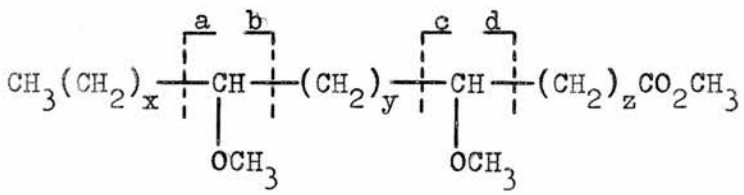
Identification of hydroxy methoxy esters.

The hydroxy methoxy esters (9,12- and 10,12-), obtained as major products from methyl ricinoleate in reaction A, were separated by prep TLC. The 10,12- isomer proved to be the less polar on GLC and TLC, due to the fact that it alone can form a hydrogen bonded 6-membered ring:



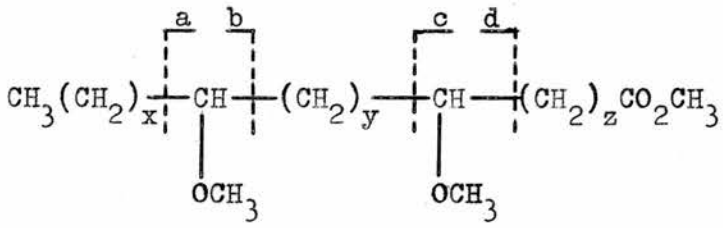
Methylation of the hydroxy methoxy esters gave dimethoxy products which were conclusively identified by the ∝-cleavage fragments in their mass spectra.

MS 3.1 A'_1 and MS 3.1 A'_2



M^+	M-31	M-32	M-31-32	M-32-32
358 (-)	327 (2)	326 (5)	295 (4)	294 (4)

MS 3.1 A_1' and MS 3.1 A_2'



	a	a-32	a-64	b	c	c-32	d	d-32
MS 3.1 A_1' (9,12-)	273 (12)	241 (60)	209 (13)	129 (64)	201* (100)	169* (86)	201* (100)	169* (86)
MS 3.1 A_2' (10,12-)	273 (-)	241 (25)	209 (3)	129 (100)	215 (100)	183 (40)	187 (8)	155 (58)

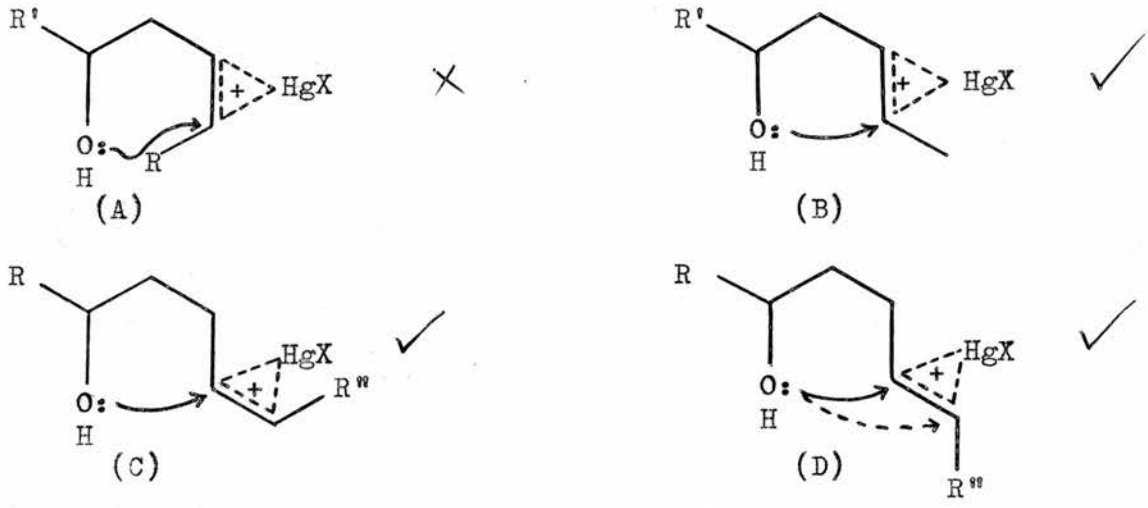
* These peaks occur twice.

Ease of ring formation.

The hydroxymercuration - demercuration of methyl ricinoleate in aqueous THF described in section 2.8, gave a low yield (22%) of isomeric 9,12-epoxystearates. An attempt to increase the amount of cyclic ether from this β-hydroxy alkenoate by reacting it with mercuric acetate in a non-participating solvent (THF) gave only 9% of cyclic ethers (ECLs 21.3, 7% and 21.6, 2%) after demercuration. In contrast, oxymercuration - demercuration of the 9-hydroxy 12-*cis* C_{18} ester in a non-participating solvent gave a high yield (89%) of cyclic ethers with ECLs 21.3 (28%) and 21.6 (61%) as well as some starting material (11%).

This consistent reluctance on the part of the 12-hydroxy 9-cis C_{18} ester to form a cyclic ether is probably caused by steric hindrance of hydroxyl attack on the mercurinium ion (see A below). Molecular models show that this attack is much more sterically favourable for the trans isomer (B) and for the cis and trans γ-hydroxy alkenoates (C and D) than for methyl ricinoleate (β-hydroxy alkenoate).

Diagrammatically:

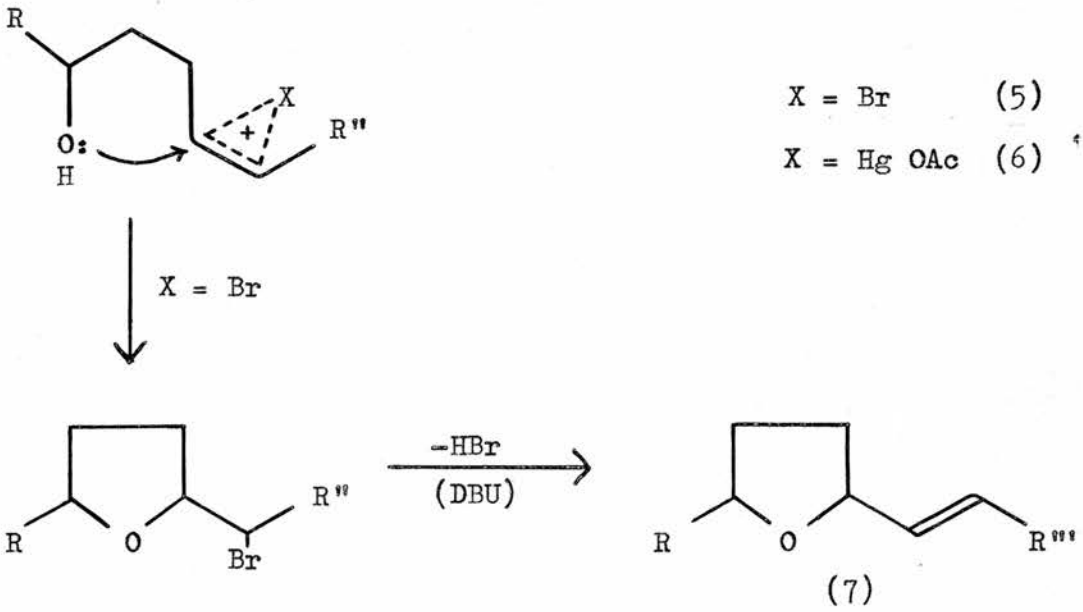


X = -OAc; R = $-(CH_2)_7CO_2CH_3$; R' = $-(CH_2)_5CH_3$; R'' = $-(CH_2)_4CH_3$

In an attempt to prepare methyl 9-hydroxyoctadec-trans-12-enoate for this experiment (see appendix 2), bromination - dehydrobromination of the cis isomer (to give the acetylenic analogue which was saponified and treated with lithium in liquid ammonia to yield the required trans acid) unexpectedly gave 40% of methyl 9,12-epoxyoctadec-13-enoate (7). This might be explained

by the internal nucleophilic attack of the hydroxyl group on the intermediate bromonium ion (5), in the same way as the intermediate mercurinium ion (6) was attacked in the previous cyclo-oxymercuration reactions.

i.e.

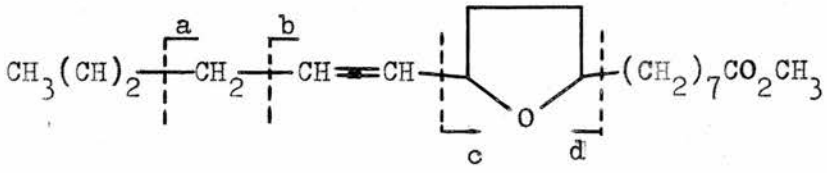


X = Br (5)

X = Hg OAc (6)

(7)

The dehydrobrominated product (7) was separated by column chromatography from the other products (acetylene and ene-bromide) and identified by the major MS fragments as shown:



M^+	M-18	M-31	a	b	b-32	c	c-18	c-32	d	d-18
310	292	279	267	253	221	227	209	195	153	135

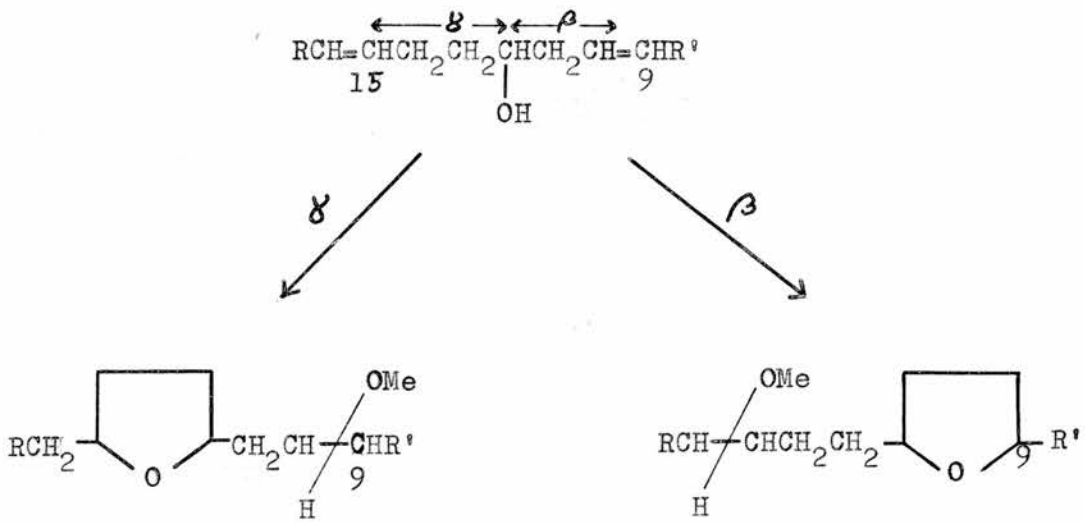
No evidence for the presence of 9,13-epoxide (tetrahydropyran) was found in the mass spectrum (i.e. very small peaks at $^{m}/e$ 241, 223).

Hydrogenation of this ester gave methyl 9,12-epoxystearate which was identified by comparison with authentic material on TLC, GLC and MS.

3.2 Methyl densipolate [96] (18:2 9c,15c 12-hydroxy)

(a) Methanol as solvent

Methoxymercuration - demercuration of methyl densipolate could give two possible 1,4-epoxide products (8 and 9) as it contains both β- and γ-hydroxy alkene systems. The reaction products are further complicated by normal methoxymercuration of the double bond not involved in cyclic ether formation as shown:



(8) (9)

A 1,5-epoxide (tetrahydropyran) might also be produced by the γ-hydroxy system.

From experiments A and C in section 3.1, we would expect product (8) to be formed in preference to (9). This was shown to be true by examination of the mass spectrum of the reaction products (MS 3.2 a, appendix 1). Fragments produced by α-cleavage to the methoxyl group and to the heterocyclic ring showed that the major product of this reaction contained a 12,15- but not a 12,16-epoxide. In addition, it was shown that there was more methoxyl substitution on C_9 than on C_{10}. The GLC trace of the total reaction product could be rationalised in terms of these products.

i.e.

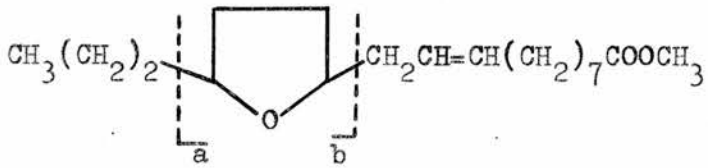
ECL (area%)		Product (methyl octadecanoates)
23.7 (2) 23.9 (14)	——	cis and trans-12,15-epoxy-10-methoxy
24.3 (20) 24.6 (62)	——	cis and trans-12,15-epoxy-9-methoxy

(b) DMF as solvent

Oxymercuration of methyl densipolate in DMF gave simpler products as one double bond remained intact after demercuration. Unreacted starting material (14%, ECL 26.1; ECL

as TMS ether 20.8) was separated from the major reaction product (10) by prep TLC and was identified by chromatographic comparison with authentic material. The major reaction product showed two peaks on GLC with ECLs 21.9 (33%) and 22.1 (67%). GLC - MS of the components showed them to be almost identical. Consideration of α-cleavage fragments indicated a 12,15-epoxide only (see MS 3.2 "21.9" and MS 3.2 "22.1"" in appendix 1). von Rudloff oxidation gave a C_9 dibasic ester (56%) indicating that the only double bond remaining was between carbon atoms 9 and 10, as well as other oxidation fragments with ECLs 12.3 (12%) and 12.6 (22%) probably due to methyl cis and trans-3,6-epoxynonanoates.

MS "21.9"



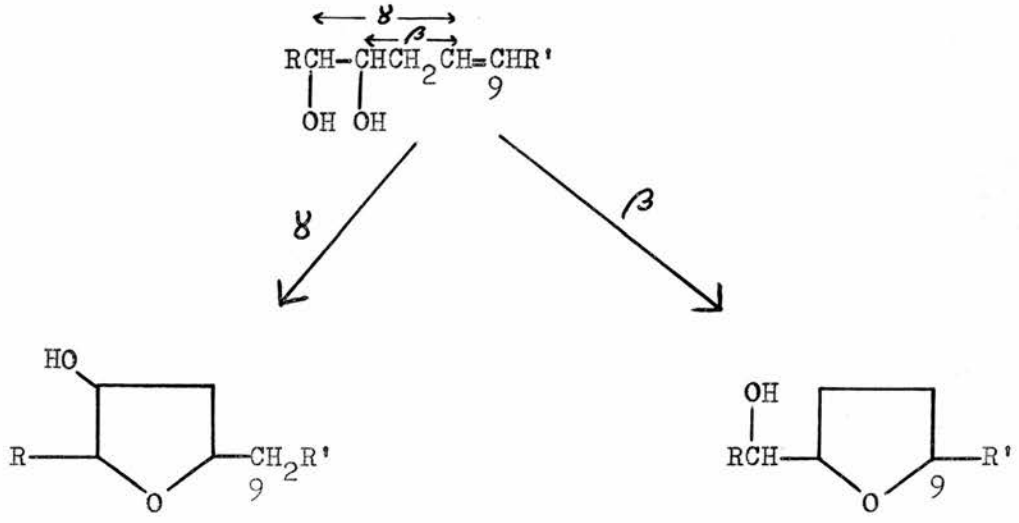
(10)

M^+	M-31	a	a-18	a-32	b	b-18
310 (0.5)	279 (1.0)	267 (-)	249 (0.4)	235 (0.4)	113 (100)	95 (86)

The 9,12- and 12,16-epoxides were shown to be absent by the absence of the relevant fragments in the mass spectrum.

3.3 Methyl 12,13-dihydroxyoleate

Methoxymercuration of methyl 12,13-dihydroxyoleate could give rise to a 10,13-epoxide with an endocyclic hydroxyl group (11) or to a 9,12-epoxide with an exocyclic hydroxyl group (12). By our previous investigations, the formation of (11) rather than (12) was predicted as the former is produced from a γ-hydroxy-alkene system whereas (12) would be derived from a β-hydroxy-cis-alkene. This prediction was found to be true.



(11) (12)

Examination by GLC of the total product before and after methylation and trimethylsilylation showed that 5 - 6% of starting material was present. The major products had ECLs as shown overleaf:

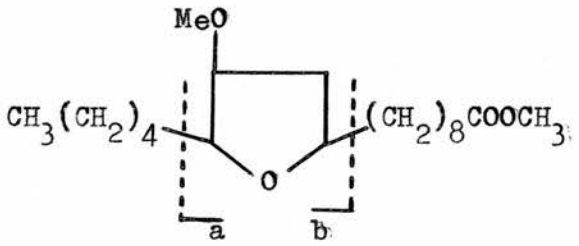
Product		as methyl ethers		as TMS ethers	
ECL	Area%[1]	ECL	Area%	ECL	Area%
24.2	1	23.4[2]	6	20.1[2]	5
29.3	10	24.3[3]	11	22.5	13
29.9	89	24.8[3]	81	22.9	82
		26.5	2		

Notes:

1. These values are slightly high as the very polar dihydroxy starting material was not eluted.
2. Methylated starting material had an ECL of 23.4. The TMS ether had an ECL of 20.1.
3. These values are comparable with those obtained in section 3.2 a.

The major methylated products were isolated by prep TLC and, as predicted, were shown to be the cis and trans methyl 10,13-epoxy-12-methoxyoctadecanoates by consideration of the α-cleavage fragments in the mass spectrum.

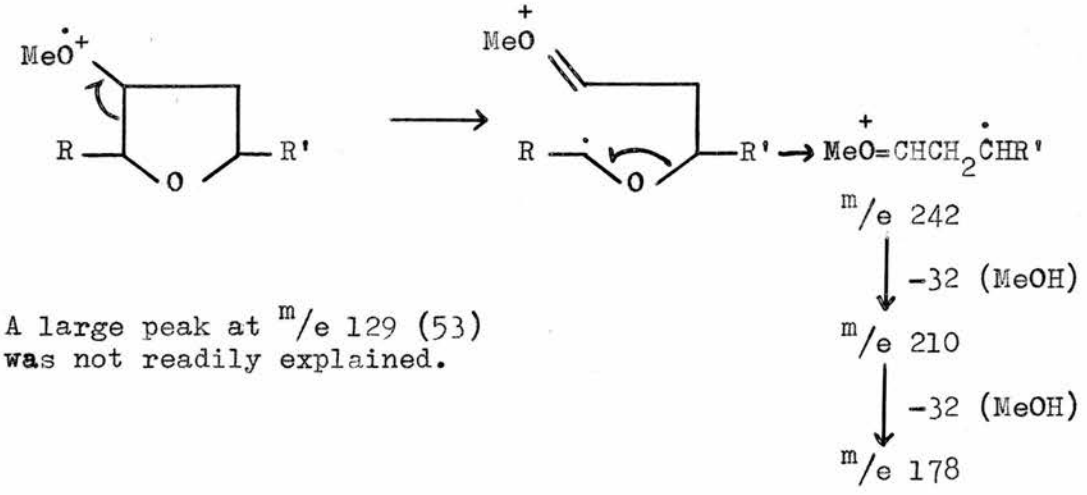
i.e. **MS 3.3**



M+1	M	M-1	M-18	M-31	M-32	M-18-31	M-31-32
343 (0.2)	342 (-)	341 (0.2)	324 (0.4)	311 (6)	310 (2)	293 (0.4)	279 (4)

a	a-18	a-32	b	b-18	b-32	base peak
271 (1.2)	253 (2.5)	239 (1.9)	171 (20)	153 (-)	139 (10)	71 (100)

The presence of peaks with $^{m}/e$ values 242(3), 210(12) and 178(25) can be explained by the mechanism outlined below:

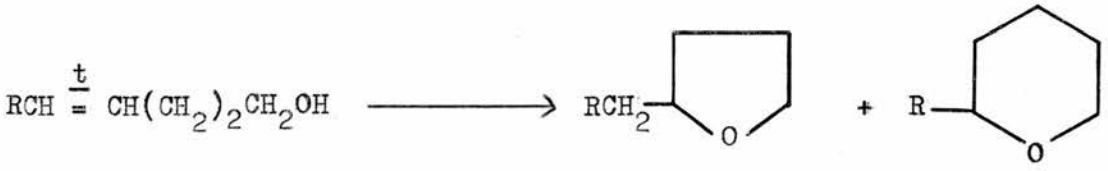


A large peak at $^{m}/e$ 129 (53) was not readily explained.

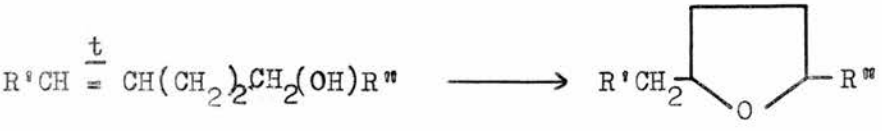
3.4 Octadecenols

Introduction

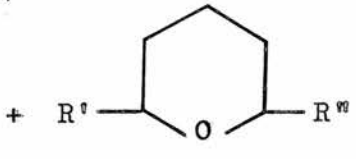
In this section, methoxymercuration - demercuration reactions are described for octadecenols of general structure $RCH{=}CH(CH_2)_nOH$ (n = 1 - 5, cis and n = 1 - 4, trans). The β- and γ-hydroxy alkene systems (n = 2 and 3 respectively) behaved in a very similar manner to the corresponding systems examined in section 3.1. For, example, octadec-trans-4-enol(γ-; n = 3) gave a tetrahydropyran (13) and a tetrahydrofuran (14) in the yields shown below. Methyl 9-hydroxyoctadec-trans-12-enoate (also γ-; section 3.1) gave products (15,16) as indicated.



(13) 88% (14) 12%

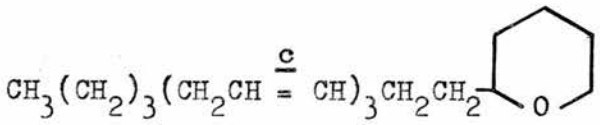


(15) 92%



(16) 8%

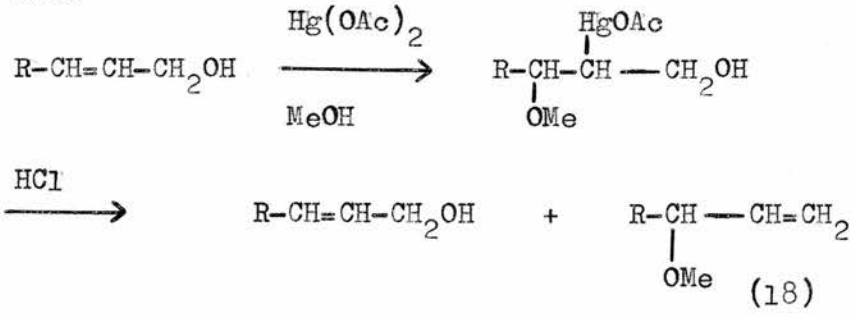
As before, a 5-membered ring was formed in preference to a 6-membered ring when either could have been formed. The $\Delta 5$ alcohols (ω-; n = 4) could have formed 6- or 7-membered rings, but only the former were observed. Cyclo-oxymercuration was shown to be equally facile in a non-participating solvent. The $\Delta 2$ and the $\Delta 6$ alcohols gave non-cyclic products. An attempt to force the $\Delta 6$ <u>cis</u> alcohol to form a 7-membered ring by oxymercuration in the absence of a nucleophilic solvent was unsuccessful. Arachidonyl alcohol gave a cyclic ether (17) which was required for comparison with a product obtained in section 4.2.



(17)

Treatment of the oxymercurated octadec-2-enols with methanolic hydrochloric acid gave considerable quantities of 3-methoxyoctadec-1-ene (18). Protonation of the more electronegative -OH group in preference to the -OMe group provides a simple explanation for this, although elimination can, and does, take place in both directions.

i.e.



(18)

Isomeric octadecenols ($\Delta 3$ to $\Delta 6$) were either available in the laboratory or were produced by lithium aluminium hydride (LAH) reduction of the corresponding monoene acids or esters. The reduction technique had to be modified to prepare the 2-cis and 2-trans octadecenols as LAH reduction of the α,β-unsaturated esters gave large amounts of the saturated alcohol. The IR spectrum of the 2-cis alcohol showed strong O-H absorption (3,590cm^{-1} and 1,365cm^{-1}) and also showed the absence of trans isomer (no absorption at 970cm^{-1}). Arachidonyl alcohol (20:4 5c,8c,11c,14c) was produced by LAH reduction of ethyl arachidonate. All alcohols were examined by GLC before use.

(a) Oxymercuration - demercuration

The unsaturated alcohols were reacted with mercuric acetate in methanol and/or DMF. Aliquots from the reaction mixtures containing the $\Delta 2$ and $\Delta 3$ octadecenols were deoxymercurated with methanolic hydrochloric acid (see part b). Demercuration with sodium borohydride gave products as outlined overleaf.

Demercuration products

Reaction in methanol

alcohol	product	
(18:1)	ECL (area %)	assignment
2-cis	19.5 (18)	octadecanol[1]
	20.3 (2)	starting material
	21.8 (9)	2-methoxyoctadecanol
	22.0 (66)	3-methoxyoctadecanol
2-trans	14.1 (36)	3-methoxyoctadec-1-ene[2]
	19.6 (5)	octadecanol[1]
	20.4 (4)	starting material
	21.9 (7)	2-methoxyoctadecanol
	22.2 (31)	3-methoxyoctadecanol
3-cis (β)	15.8 (7)	2-tetradecyl-THF
	22.5 (18)	3-methoxyoctadecanol
	22.9 (72)	4-methoxyoctadecanol
3-trans (β)	15.7 (100)	2-tetradecyl-THF
4-cis (γ)	15.8 (92)	2-tetradecyl-THF
	20.4 (8)	starting material
4-trans (γ)	15.2 (12)	2-tridecyl-THP
	15.8 (88)	2-tetradecyl-THF
5-cis (δ)	15.1 (100)	2-tridecyl-THP
5-trans (δ)	15.1 (100)	2-tridecyl-THP
6-cis	21.2 (5)	unidentified
	22.7 (95)	6- + 7-methoxyoctadecanol
Reaction in DMF (18:1)		
4-trans	15.2 (8)	2-tridecyl-THP
	15.8 (90)	2-tetradecyl-THF
6-cis	20.3 (100)	starting material
(20:4)		
5,8,11,14 - all cis	18.6 (97)	2-pentadecatrienyl-THP

Notes:

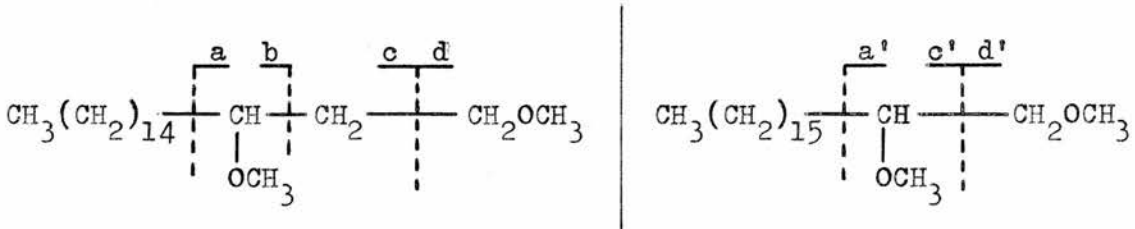
1. This was present in the starting material.
2. Production of this is explained in the acid regeneration section (b).

Identification of products

Product from 2-cis alcohol

Methylation of the total product gave dimethoxyoctadecanes (ECL 17.7, 82%) which were separated from the methylated octadecanol (ECL 14.9; octadecanol was present as an impurity in the starting material) by prep TLC. The mass spectrum (below) showed this product to be mainly 1,3- with some 1,2-dimethoxyoctadecane.

MS 3.4 - $1H^-$/MeI



M^+	M-31	a	a-32	b	c(=c')	d(=d')	a'	a'-32
314 (1)	283 (3)	103 (100)	71 (43)	255 (33)	269 (10)	45 (77)	89 (-)	57 (29)

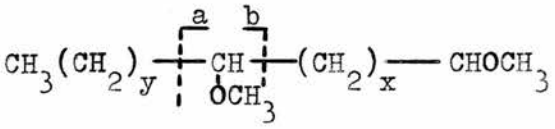
Product from 2-trans alcohol

The methoxyoctadecanols from the 2-trans isomer reaction were identified by comparison with the products obtained from the cis isomer above. A major component (ECL 14.1, 36%) was identified as 3-methoxyoctadec-1-ene by comparison on TLC, GLC and MS (see MS 3.4 $-2H^{-}$ in appendix 1) with the same material obtained by acid regeneration (see part b).

Product from 3-cis alcohol

As with other β-hydroxy cis-alkene systems studied (see sections 3.1, 3.2, 3.3), octadec-cis-3-enol gave a low yield (7%) of substituted tetrahydrofuran which was identified by comparison on GLC (ECL 15.8) and TLC with the total 3-trans isomer product (vide infra). The major products with ECLs 22.5 (18%) and 22.9 (72%) were methylated to give a mixture of 1,3- and 1,4-dimethoxyoctadecanes (ECL 17.7, single peak). The mass spectrum of this mixture showed, as expected, that the 1,4- isomer predominated:

MS 3.4 - 3A



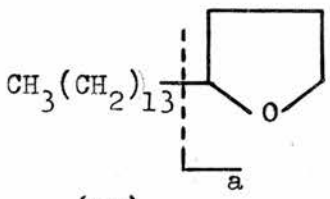
		a	a-32	b	M^+	M-1	M-32	M-64
x = 1 y = 14	(1,3-)	103 (40)	71* (100)	255 (10)	314 (-)	313 (1)	282 (2)	250 (6)
x = 2 y = 13	(1,4-)	117 (71)	85 (96)	241 (27)				

* This peak was probably only partly due to fragment a-32.

Products from 3-trans, 4-cis, 4-trans, 5-cis and 5-trans alcohols

The methoxymercuration - reduction products from the 3-trans and from the 5-cis isomers with ECLs 15.7 and 15.1 respectively, were shown by their mass spectra to be the 2-alkyl substituted cyclic ethers (19) and (20) respectively.

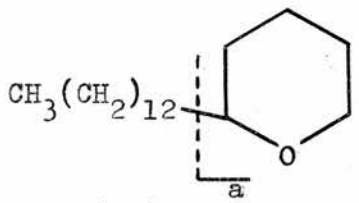
MS 3.4 - 3B



(19)

M^+	M-18	a	base peak
268 (10)	250 (45)	71 (*)	72 (100)

MS 3.4 - 3E



(20)

M^+	M-18	a	base peak(a-18)
268 (2)	250 (5)	85 (*)	67 (100)

* These peaks were too large to be measured. The second largest peaks in the spectra were taken as base peaks.

Methoxymercuration—demercuration of the 4-cis, 4-trans and 5-trans octadecenols gave products which were identified by comparison on TLC, GLC and MS with the cyclic ethers obtained above.

The cyclo-oxymercuration of octadec-trans-4-enol (and presumably of the 3-trans, 4-cis, 5-cis and 5-trans isomers) was shown to be equally facile in a non-participating solvent (DMF). This has important consequences for chapter 4.

Product from 6-cis alcohol

Methoxymercuration - reduction of octadec-cis-6-enol gave the expected mixture of 6- and 7-methoxyoctadecanols (ca 1:1) as shown by the mass spectrum of the methylated product (ECL 17.8). The mass spectrum (MS 3.4 - 3G) is reproduced in appendix 1. An attempt to form a 7-membered cyclic ether by reaction of the 6-cis alcohol with mercuric acetate in DMF failed. Unchanged starting material was obtained after the demercuration step.

Product from arachidonyl alcohol

Oxymercuration of arachidonyl alcohol (ECL 24.1) in DMF and reduction with sodium borohydride gave the expected cyclic ether (ECL 18.6) which was hydrogenated to give 2-pentadecyltetrahydropyran with ECL 17.0 (cf. 2-tridecyltetrahydropyran above with ECL 15.1).

(b) Oxymercuration - deoxymercuration

Aliquots from the octadec-3-enol (cis and trans) methoxymercuration reaction mixtures were treated with methanolic hydrochloric acid. The products were shown by GLC, TLC and IR to be stereospecifically regenerated starting materials.

The major acid deoxymercurated products from the 2-cis and 2-trans monoene alcohols were as shown overleaf:

Deoxymercurated products

alcohol (18:1)	ECL (area %)	assignment
2-cis	14.1 (71)	3-methoxyoctadec-1-ene
	19.5 (13)	octadecanol*
	20.3 (13)	starting material
2-trans	14.0 (73)	3-methoxyoctadec-1-ene
	20.4 (20)	starting material

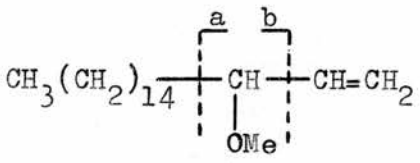
* This was present in the starting material.

Prep TLC was used to separate the regenerated octadecenols from the new component. The former were identified by GLC, TLC and IR (no trans absorption at 970cm^{-1} for cis isomer product). The new component, which was previously obtained in 36% yield by the borohydride reduction of the methoxymercurated octadec-trans-2-enol in section 3.4a, was identified by its mass spectrum before (MS 3.4 $-2H^-$) and after (MS 3.4 -1BH) hydrogenation.

---

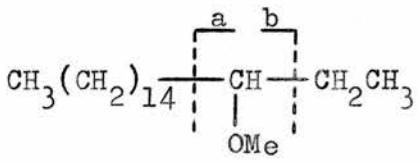
i.e.

MS 3.4 $-2H^-$



M^+	a	b
282 (1)	71 (100)	255 (2)

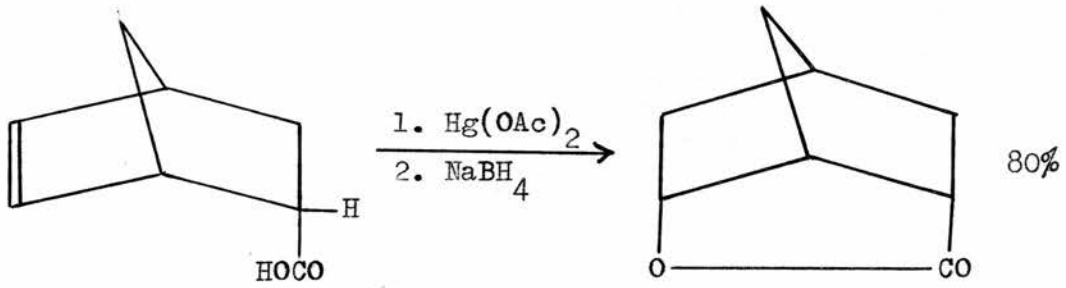
MS 3.4 -1BH



M^+	a	b
284 (1)	73 (100)	255 (20)

3.5 Octadecenoic acids

Henbest and Nicholls [40] obtained a lactone (22) by reacting the unsaturated acid (21) with mercuric acetate in methanol and reducing the product with sodium borohydride in ether - methanol.



(21) (22)

In the present section, the oxymercuration - aqueous sodium borohydride reduction of three monoenoic acids (9-cis, 4-trans and 5-trans) was investigated. Only the 5-trans acid gave any positive evidence for lactone formation.

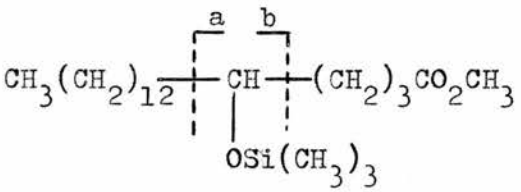
Methoxymercuration - demercuration of oleic acid (9-cis) gave products which were esterified and then identified on TLC and GLC as methyl oleate (ECL 18.4, 13%) and methyl methoxystearates (ECL 21.0, 87%).

The methoxymercuration - aqueous borohydride reduction of octadec-trans-4-enoic acid was carried out twice. In each case the product was esterified and was shown to be identical to authentic methyl octadec-trans-4-enoate by GLC, TLC and NMR (triplet at 4.6 τ, 2H, $C\underset{t}{H}=C\underset{t}{H}$). Surprisingly, no methyl 4(5)-methoxystearates were obtained, implying that the double bond had been involved in some sort of reversible reaction which prevented it from forming

the normal mercury adduct. The most plausible explanation is that a lactone was formed during oxymercuration, but the double bond was regenerated by the aqueous sodium borohydride.

Methoxymercuration - demercuration - esterification of the 5-trans acid gave starting material (14% as methyl ester), methyl 5 (6)-methoxystearates (ECL 20.9, 64%) and methyl 5-hydroxystearate (ECL 26.1 and ECL of TMS ether 20.0, 23%). GLC - MS analysis of the trimethylsilylated product showed that the component with ECL 20.9 was a 1:1 mixture of the 5- and 6-methoxy isomers (see MS 3.5b in appendix 1). The mass spectrum of the component with ECL 20.0 (MS 3.5a) showed that there was a hydroxyl group on C_5 only. This implied that a δ-lactone formed in the methoxymercuration step had been subsequently hydrolysed by the aqueous borohydride step.

MS 3.5a

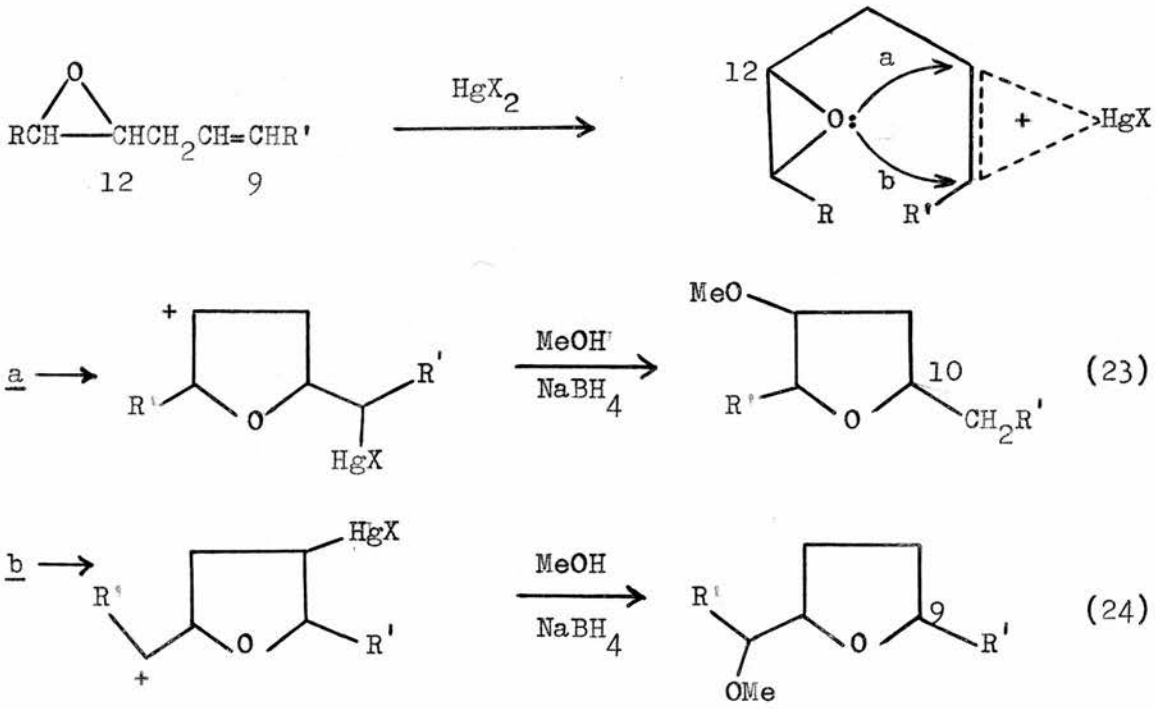


M^+	M-15	M-31	M-15-32	a	a-32	b
386 (-)	371 (5)	355 (2)	339 (5)	203 (84)	171 (12)	285 (29)
			6-isomer ⟶	217 (3)	185 (-)	271 (2)

An attempt to oxymercurate the 5-trans acid in a non-participating solvent (DMF) gave only starting material after borohydride reduction.

3.6 Methyl vernolate (18:1 9c 12,13-cis-epoxy)

Methoxymercuration - reduction of methyl vernolate might be expected to give products (23) and (24) as shown.



The methoxymercuration - reduction product from methyl vernolate gave two major bands on prep TLC. The upper band (22%) with ECLs 24.3 (11%) and 24.7 (89%) was shown to be cis and trans methyl 10,13-epoxy-12-methoxyoctadecanoate (23) by comparison of

its TLC, GLC and MS behaviour with the same product obtained from methyl 12,13-dihydroxyoleate as in section 3.3. The mass spectrum (see MS 3.6 B in appendix 1) did not entirely rule out the presence of ester (24).

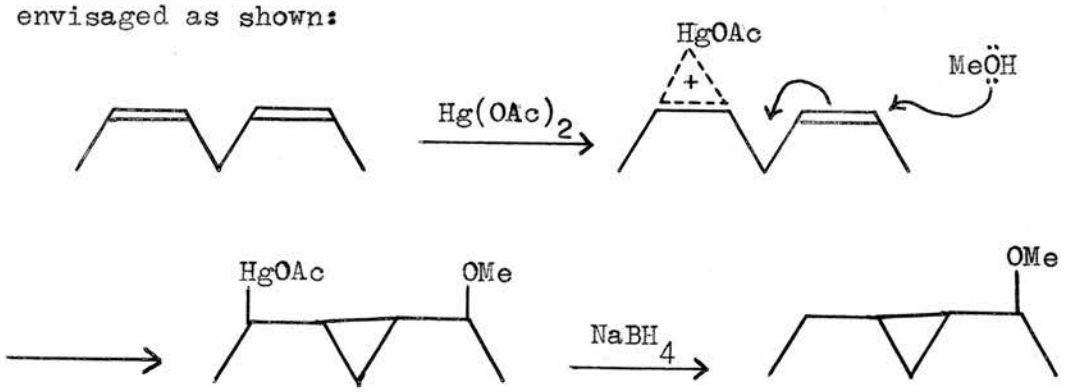
The lower band on TLC (78%) showed a major peak on GLC (ECL 27.6, 78%). The mass spectrum (MS 3.6 A in appendix 1) showed this major product to be a 1:1 mixture of the normal methoxy adducts.

i.e. methyl 9(10)-methoxy-12,13-epoxystearates.

3.7 Methyl octadecadienoates

Introduction

Methoxymercuration - reduction experiments with conjugated and non-conjugated dienes described earlier (see sections 2.5, 2.6, 2.7), were carried out with the possibility of carbocyclisation in mind. For example, a 1,5-addition to methyl linoleate was envisaged as shown:

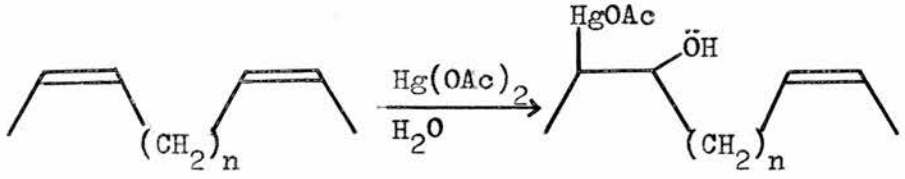


Products with TLC and GLC behaviour consistent with a methoxy cyclopropane ester were formed in these earlier reactions, but were shown to be partially reacted dienes (i.e. methoxy monoenes) by IR and by hydrogenation.

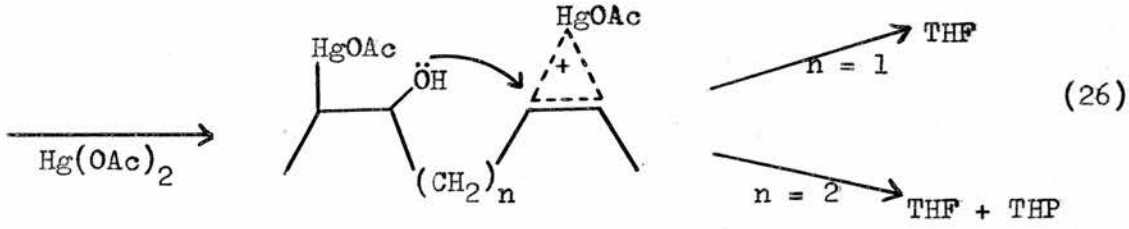
In the present section, reaction of methyl linoleate with mercuric acetate in DMF gave a product after sodium borohydride reduction which was shown to contain starting material (81%) and a mixture of acetoxy monoenoates (19%). The latter (ECL 24.4) were isolated by prep TLC and were shown to contain a double bond by hydrogenation and GLC (ECL 24.1). The mass spectrum of the hydrogenated product (MS 3.7 - 1 in appendix 1) showed it to be a mixture of 9-, 10-, 12- and 13-acetoxystearates. Therefore in the absence of other suitable nucleophiles, the mercury salt anion has attacked the intermediate mercurinium ion to give the expected products.

Although no carbocyclic products were produced in these experiments, treatment of 18:2 9c, 12c and 18:2 8c, 12c methyl esters in aqueous DMF with mercuric acetate gave appreciable amounts of O-heterocyclic products. These were presumably formed by hydroxymercuration of one double bond (25) followed by cyclo-oxymercuration involving the second double bond (26).

i.e.



(25)



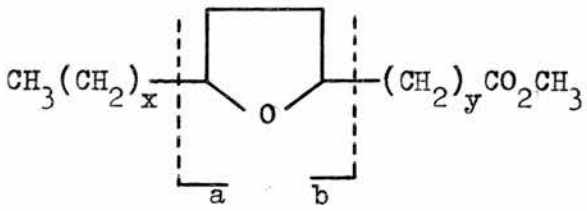
(26)

Methyl linoleate (n = 1)

Hydroxymercuration - demercuration of linoleate gave two bands on prep TLC. The less polar band (48%) contained a trace of starting material and a 1:1 mixture of methyl 9,12- and 10,13-epoxystearates (cis and trans). These were identified by their chromatographic behaviour and by mass spectrometry (see MS 3.7 - 2A below). The polar TLC band contained methyl di-hydroxystearates as shown by GLC and MS of the bis-TMS ethers (see MS 3.7 - 2B in appendix 1). Consideration of the major fragments showed there was more substitution on C_{10} than on C_9 and more on C_{12} than on C_{13}.

The overall yield of cyclic ethers was increased to 70% in a second hydroxymercuration - reduction experiment with methyl linoleate by cyclodehydrating some of the di-hydroxy ester product with refluxing methanolic sulphuric acid.

MS 3.7 - 2A



	a	a-18	a-32	a-18-32	b	b-18
x = 5, y = 7	227 (35)	209* (34)	195 (31)	177 (8)	155 (72)	137 (22)
x = 4, y = 8	241 (33)	223 (4)	209* (34)	191 (4)	141 (80)	123 (42)

M+1	M^+	M-1	M-18	M-31	M-18-31
313 (2.4)	312 (1.7)	311 (1.1)	294 (1.3)	281 (1.5)	263 (2)

* This peak occurs twice.

A large scale hydroxymercuration - demercuration was carried out on methyl linoleate (21g) under nitrogen. The 9,12- and 10,13-epoxystearates (8g) were isolated by column chromatography and were shown to be pure by TLC and GLC.

We are grateful to G. Silverstone (V. Wolf Ltd.) who tested the stabilising properties of this mixture of 9,12- and 10,13-epoxides in poly(vinyl chloride). Unlike long-chain vic-epoxides, he showed that our 1,4-epoxystearates did not act as plastic stabilising agents. At the time of writing, these epoxides (and other cyclic products obtained in this chapter) are being

investigated for pharmacological activity by Allen & Hanbury Ltd..

Methyl octadeca-cis-8,cis-12-dienoate

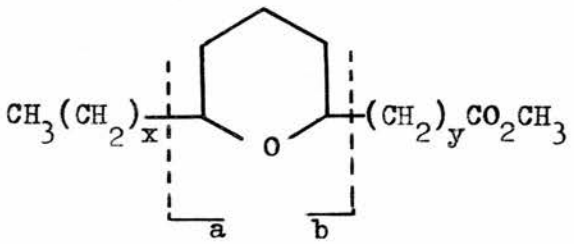
Hydroxymercuration (in aqueous DMF) - sodium borohydride reduction of the 18:2 8c, 12c methyl ester gave a product which was separated by prep TLC into the fractions shown below:

Methyl esters	amount[1]	ECL
starting material[2]	18%	19.2
acetoxy monoenoates[2]	9%	24.5
dihydroxy material[2]	14%	(20.2, 21.8, 22.0)[3]
9,12-epoxystearates[4]	23%	21.0 (5%), 21.3 (18%)
8,12- + 9,13-epoxystearates[5]	36%	19.7

Notes:

1. The amounts were calculated from GLC and prep TLC results.
2. These were identified by comparison of their TLC and GLC behaviour with authentic esters.
3. These ECLs are of the TMS ethers.
4. The only 1,4-epoxides present were cis and trans methyl 9,12-epoxystearates as shown by mass spectroscopy (see MS 3.7 - 3B in appendix 1).
5. These were shown by mass spectroscopy (MS 3.7 - 3C) to be a 1:1 mixture of the two positional isomers.

MS 3.7 - 3C



	a	a-18	a-32	a-50	b	b-18
x = 5, y = 6	227 (16)	209* (41)	195 (25)	177 (12)	169 (61)	151 (26)
x = 4, y = 7	241 (17)	223 (16)	209* (41)	191 (8)	155 (100)	137 (39)

M+1	M^+	M-1	M-18	M-31	M-18-31
313 (3.9)	312 (6.4)	311 (1.4)	294 (2.3)	281 (3.2)	263 (3.4)

* This peak occurs twice.

3.8 Long-chain keto-esters

Extensive studies involving the oxymercuration - demercuration of β- and γ-unsaturated keto-esters were abandoned due to the complexity of the products obtained.

3.9 Addendum

After the discussion to section 3.7 was written, a paper* in a new journal concerning the hydroxymercuration - demercuration of a series of dienes was brought to our notice. The reactions described in Brown's paper concern short-chain and cyclic dienes and the results obtained were markedly similar to our results with long-chain diene esters. Three empirical generalisations concerning the directive influence of the hydroxyl group upon the products obtained from oxymercuration -demercuration were made by Brown and co-workers who state:

"

(1) Irrespective of the original hydroxyl group, Markownikov addition occurs.

(2) Tetrahydrofuran or tetrahydropyran derivatives will be formed if the original hydroxyl group is so located that a five- or six-membered ring can be formed by participation of the hydroxyl group. Exceptions are found when the hydroxyl group and the double bond are contained within small rings. If not so located, then the corresponding diol will be obtained.

(3) If both carbons of the double bond are equally Markownikov, then addition occurs in such a position as to place the incoming oxygen nucleophile most remote positionally and configurationally from the original hydroxyl group. The positional preference diminishes with increasing distance between the original hydroxyl and the double bond. "

* H.C. Brown, P.J. Geoghegan, J.T. Kurek and G.J. Lynch, Organometallics in Chemical Synthesis, 1970/71,1,7.

CHAPTER 4

SOME APPLICATIONS OF OXYMERCURATION REACTIONS ACCOMPANIED BY CYCLISATION

Privett [97] has recently reviewed the natural sources and methods of isolation of most of the known polyunsaturated fatty acids. These acids are generally isolated from natural sources by the combination of suitable techniques which include distillation [98]; fractionation of derivatives (e.g. bromides [99], acetoxymercuri-methoxy adducts [51], salts [100]); fractionation as urea complexes [101]; low-temperature fractional crystallisation [102]; liquid-liquid partition chromatography [103]; countercurrent distribution [104]; column or thin-layer adsorption chromatography [105] (especially silver ion chromatography [50,106]) and preparative gas-liquid chromatography [107]. The selection of these techniques and the order in which they are used depends largely on the nature and amount of the required fatty acid in the original oil.

Once isolated, the position of the double bonds can be found by mass spectrometry of the deuterated [108], epoxidised [109] or oxymercurated [65] polyene esters. Alternatively, oxidative cleavage with permanganate or by ozonolysis gives fragments which can be identified by GLC. Privett [110] has also reviewed the determination of the structure of unsaturated fatty acids via these degradative methods.

These procedures are frequently tedious and the quantitative results are not always reliable. Furthermore, separation of two esters with the same number of double bonds e.g. 18:2 5c,9c and 18:2 9c,12c is extremely difficult by any

of the methods already mentioned. The relative amount of each isomer can only be determined by oxidative cleavage and measurement of the oxidised fragments by GLC. Over-oxidation, loss of volatile fragments and preferential solubility losses in the work up of the products tend to make this an inaccurate quantitative procedure.

Analytical procedure

A general analytical method has been devised using the results of section 3.4 to simplify the detection and quantitation of fatty acids containing $\Delta 3t$, $\Delta 4$ or $\Delta 5$ unsaturation in natural or in adulterated oils. The procedure depends on the fact that only long-chain alcohols with unsaturation in these positions will give cyclic ethers by oxymercuration - sodium borohydride reduction. Once produced, these cyclic ethers can readily be separated from the unreacted alcohols by virtue of their greatly decreased polarity. Quantitative prep TLC shows how much cyclic ether there is and therefore how much $\Delta 3t$, $\Delta 4$ and $\Delta 5$ fatty acid was present in the original oil. Comparison of these cyclic ethers (CEs) and of their hydrogenated derivatives (HCEs) on GLC with the 2-alkyl cyclic ethers obtained in section 3.4 permits identification of the individual components to be made. The experimental procedure is outlined overleaf.

$$\text{oil} \xrightarrow[\text{ether}]{\text{LAH}} \text{alcohols} \xrightarrow[(ii)\,NaBH_4]{(i)\,Hg(OAc)_2,\,DMF} \text{alcohols} + \text{cyclic ethers}$$

$$\xrightarrow{\text{prep TLC}} \text{cyclic ethers} \xrightarrow[Pd/C]{H_2} \text{hydrogenated cyclic ethers}$$

To recapitulate, the results of section 3.4 were as tabulated:

alcohol (18:1)	area %	
	2-alkyl-THF	2-alkyl-THP
3c	8	-
3t	100	-
4c	92	-
4t	88	12
5c	-	100
5t	-	100
ECL	15.7	15.1

Thus an HCE obtained from a natural oil by this procedure with an ECL of, say, 17.1 must originate from a $C_{20}\Delta5$ acid. As each double bond introduced into a long-chain ester is known to increase the ECL value by approximately 0.5 - 0.6 units, comparison of the ECL values of the CE and of the

hydrogenated cyclic ether indicate how many double bonds were present in the original acid, apart from the one involved in the cyclo-oxymercuration reaction. If the HCE has an ECL of 17.1, as in the hypothetical case above and the CE has an ECL of 18.8, we can see that the cyclic ether must contain 3 double bonds. It is then clear that the original acid was a C_{20} tetraene with the first double bond in the 5-position. Care must be exercised to avoid confusion between the increment in ECL value due to an additional double bond and the difference in the ECLs values (ca 0.6 units) between a 2-alkyl-THP and the corresponding THF.

A major weakness in the quantitation of the total cyclic ether content was the necessity of weighing the CEs extracted from the TLC plate. This was particularly undesirable when the CE content amounted to only a few percent of the total reduction product. This difficulty was overcome by the addition of a carefully measured amount of an appropriate* methyl ester as an internal standard to a known quantity of the original oil before LAH reduction and oxymercuration. The mixture of cyclic ethers produced from the internal standard and from the original oil were isolated by prep TLC but were not weighed. Comparison

---

* Authentic alcohols (e.g. 18:1 5c or 18:1 4c) which would give cyclic ethers by oxymercuration - demercuration were used as internal standards providing these alcohols were not present in the original oil.

of the CE peak areas on GLC with the peak area of the internal standard CE provided relatively accurate* quantitative results.

Several animal, vegetable and partially hydrogenated fish oils reputed to contain $\Delta 3$, 4 or 5 unsaturation were successfully examined by the methods outlined above.

Isolation procedure

An extension of the procedure described above has been used to effect the simple isolation of pure polyunsaturated fatty alcohols with $\Delta 3t$, $\Delta 4$ or $\Delta 5$ unsaturation. In general, an increase in the degree of unsaturation of a fatty acid results in an increase in the difficulty of its purification, but the method of isolation described below gave polyunsaturated alcohols rapidly and quantitatively.

Initial attempts to find a simple method of oxidising these alcohols to the corresponding acids without affecting the double bonds were unsuccessful and there has not been time to re-examine this part of the problem.

Oxymercuration of the LAH reduced oils in DMF gave a mixture of unreacted alcohols and mercury-containing CEs. Because of their greatly increased polarity, the latter were

---

* It was assumed that the GLC flame-ionisation detector response was the same for all 2-alkyl cyclic ethers obtained.

easily separated by TLC or by column chromatography. Treatment of these isolated mercury adducts with methanolic hydrochloric acid gave the stereospecifically regenerated parent alcohols. In one instance, the unreacted alcohols were removed from the mercurated material on a silica gel column using ether as developing solvent, followed by elution of the regenerated alcohols by passing methanolic hydrochloric acid down the column. The regenerated fatty alcohols could then have been investigated by partial reduction - oxidative degradation to determine the positions of the other double bonds.

4.1 Seed oil esters

Four seed oils, reported to contain unusual fatty acids, were examined by GLC as esters and also as alcohols. A rough correlation could be made between the GLC results as ($ECL_{alcohol} - ECL_{ester}$) was normally about 1.7 ECL units (see experimental section).

The following experiments were carried out at an early stage in the development of this analytical technique. Apart from section (a) all oxymercurations were carried out in a non-participating solvent (DMF).

(a) Carlina corymbosa

It has been reported [111] that this seed oil contains 18:1 5c (21%) and a trace of 16:1 5c. The oxymercuration of the alcohols from this seed oil was carried out in methanol in an

early experiment before the cyclo-oxymercuration reaction in a non-participating solvent was investigated. Normal methoxymercuration of double bonds not involved in the cyclisation reaction complicated the product, and quantitation of the cyclic ethers by measurement of the total product on GLC gave very inaccurate results due to the large range of ECLs involved. The cyclic ethers were not isolated by prep TLC.

Results:

alcohols			total product		
	ECL	Area%	ECL	Area%	Assignment
14:0	16.0	3	13.0	2	16:0 THP
16:0	17.7	12	14.9	46	18:0 THP
18:0	19.7	11	16.0	3	14:0 (alcohol)
18:1	20.4	34	17.7	12	16:0
18:2	21.2	40	19.6	11	18:0
			23.0	8	methoxy 18:0
			23.5	8	methoxy 18:1
			25.9	10	dimethoxy 18:0

Although evidence for the presence of the 16:1 and 18:1 $\Delta 5$ acids in the original oil was obtained, quantitation was impossible.

(b) Teucrium depressum (A), Thalictrum flavum (B) and Calea uniflora (C) seed oils were examined on GLC after LAH reduction - oxymercuration - demercuration. The presence of cyclic ethers in the total products was obvious from the GLC traces (see tables in experimental section). The cyclic ethers were isolated by quantitative prep TLC and identified by GLC. It was not necessary to hydrogenate these CEs as the level of unsaturation in the products was low.

Results:

Oil	% of CEs by TLC	ECL(area%)	Assignment	Amount in original oil: acid	found	reported
A	9.5%	15.5 (6)	18:1 THP	18:2 $\Delta 5$[1]	0.6%	0.7%
		16.2 (94)	18:2 THP	18:3 $\Delta 5$[1]	8.9%	8.7%
B	35%	13.1 (4)	16:0 THP	16:1 $\Delta 5$[2]	1.4%	-
		15.1 (24)	18:0 THP	18:1 $\Delta 5$[2]	8.4%	-
		15.6 (15)	18:1 THP	18:2 $\Delta 5$	5.1%	-
		16.2 (57)	18:2 THP	18:3 $\Delta 5$	19.4%	-
C	11%[3]	16.1 (5)	18:1 THF	18:2[4]	0.5%	tr
		16.8 (95)	18:2 THF	18:3[4]	10.5%	17%

Notes:

1. It has been reported [112] that oil A contains 18:3 5c,9c,12c (6.7%); 18:3 5t,9c,12c (2.0%) and 18:2 5c,9c (0.7%).
2. These two acids have been found* in oil B but have not been quantitatively measured.
3. This value was low due to spillage during recovery of the CEs (4mg) from the TLC plate.
4. These acids could have been $\Delta 4c$ or $\Delta 3t$. Oil C has been found* to contain 18:3 3t,9c,12c (17%) and 18:2 3t, 9c (tr).

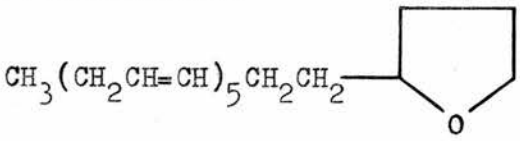
* Dr. I.A. Wolff, personal communication.

Although identification of the component acids was relatively simple, quantitation by prep TLC was not always accurate because of the errors and losses involved in extracting and weighing a very small amount of material.

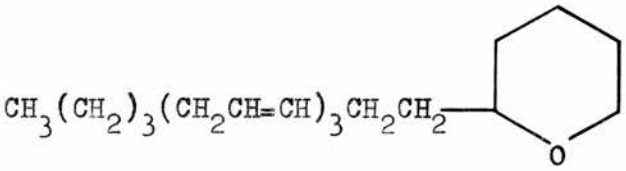
4.2 Rat liver oil

Analysis

Fatty acids free from non-saponifiable impurities were extracted from four rat livers and then reduced with LAH to the corresponding alcohols. Oxymercuration (in DMF) and demercuration furnished two new components as indicated by GLC. These were separated from the unreacted alcohols and from each other by prep TLC and were shown to be cyclic ethers (1) and (2) by virtue of their TLC behaviour (2-alkyl-THP is less polar than 2-alkyl-THF) and by consideration of their ECL values before and after hydrogenation.



(1)



(2)

The GLC trace of the total mercurated - reduced product showed new peaks with ECLs 18.6 (25%) and 22.7 (8%) corresponding to cyclic ethers (2) and (1) respectively. These area percentages were not

regarded as being very accurate due to the large range of ECLs involved and because the GLC flame-ionaisation detector response may be different for cyclic ethers and alcohols.

A better measure of the amount of each cyclic ether present in the total reaction mixture was obtained by quantitative prep TLC*. Cyclic ether (1), which amounted to 4% of the total product, had an ECL of 22.7 which was changed to 19.8 after hydrogenation. This indicated that it was a C_{22} THF with a pentaene side chain which must have been derived from a C_{22} hexaene fatty acid with a $\Delta 3t$ or a $\Delta 4$ double bond. As trans unsaturation is uncommon in animal fatty acids, it is almost certain that the fatty acid in question was 22:6 4,7,10,13,16,19 - all cis.

Cyclic ether (2), 13% of the total product, had the same ECL (18.5) as the cyclic ether obtained from arachidonyl alcohol in section 3.4. Hydrogenation gave the same saturated cyclic ether (ECL 17.0) in both cases, confirming that the parent acid was a C_{20} tetraene with $\Delta 5$ unsaturation. The TLC band containing cyclic ether (2) also contained traces of material with ECL values of 17.7, 19.5 and 19.9 (16.1, 18.1 and 19.0 after hydrogenation).

The rat livers examined were thus shown to contain the

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* An unidentified TLC fraction (8%) which was not eluted on GLC may have been non-lipid material.

following fatty acids:

4% of 22:6 $\Delta 4$ (probably 4,7,10,13,16,19 - all cis) and

13% of 20:4 $\Delta 5$ (probably 5,8,11,14 all cis) including

traces of 19:4 $\Delta 5$, 21:4 $\Delta 5$ and 22:3 $\Delta 5$.

The 22:6 alcohol (expected ECL 27.0) was not observed on the GLC trace of the original rat liver alcohols. We believe that loss of this component takes place on the GLC column due to the presence of the hydroxyl group and/or to the high degree of unsaturation. This belief was supported (vide infra) by GLC of a mixture containing the 22:6 alcohol. When examined as alcohols, no peak was observed for the latter, but when examined as TMS ethers, effectively cutting down the time of residence on the GLC column, the 22:6 TMS ether was eluted normally.

Isolation

The components listed above were readily isolated as the corresponding alcohols by reacting another sample of the rat liver alcohols with mercuric acetate in DMF and extracting the total product with ether.* The mercury-containing cyclic ethers

* An attempt to extract the unreacted alcohols only from the total reaction mixture with petrol was unsuccessful. Alternative liquid - liquid partition systems, which would provide a viable process for large-scale isolation of the mercury-containing material, were not investigated.

were separated from the unreacted alcohols by TLC and the 20:4 and 22:6 alcohols (13%) were regenerated with methanolic hydrochloric acid. The IR and UV spectra of these alcohols showed very little evidence of any trans unsaturation (970cm^{-1}) and less than 0.5% of conjugated diene (233nm). Another mercury-containing band which ran slightly ahead of the unreacted alcohols on TLC was also isolated and treated with acid.

The product (12%), contaminated with some of the unreacted alcohols due to the poor separation on TLC, had an unusual appearance on TLC and gave no peaks on GLC suggesting that it was non-lipid in origin. This component and the unidentified component (8%) found in the analytical experiment were probably the same and may have been present in the original oil.

The regenerated alcohols gave a major peak on GLC (ECL 23.7) due to the arachidonyl alcohol in addition to small peaks due to trace components. There was no evidence of the 22:6 alcohol (expected ECL ca 27.0). GLC of the TMS ethers however gave two major peaks with ECLs 18.9 (76%) and 21.8 (24%). The fact that there was no evidence of these peaks on the GLC trace of the unreacted alcohols run as TMS ethers indicated that the separation had been highly efficient.

4.3 Tall oil

Crude tall oil is a by-product of the sulphate paper process and is fractionated by distillation into fatty acids and rosin. Tall oil fatty acids are used mainly in surface coatings, detergents, emulsifiers and in the manufacture of epoxidised esters used in the stabilisation of poly(vinyl chloride). An important property is their resistance to yellowing on exposure to air and light. This has been attributed to the absence of linolenic acid in tall oil acids. Ennor and Oxley [113] have described the composition and fractionation of tall oil.

Finnish workers discovered and identified octadeca-5c,9c,12c-trienoic acid [114], eicosa-5c,11c,14c-trienoic acid [115] and octadeca-5c,9c-dienoic acid [116] in several tall oil and pine wood fatty acids. To isolate the 20:3 acid they used urea complex formation followed by liquid - liquid extraction of the acetoxymercuri-methoxy adduct. The regenerated fatty acids were then fractionated on a spinning band column and further purified by repetition of the acetoxymercuri-methoxy adduct and liquid - liquid extraction steps. The product, then 96 - 97% pure, was subjected to oxidative fission and the double bond positions were determined by identification of the oxidised fragments on GLC.

GLC analysis of the total tall oil fatty acids was used by these investigators to determine how much of the $\Delta 5$ components were present in a given sample.

Analysis without an internal standard

The Δ5 components were readily identified and measured by the cyclo-oxymercuration technique.

The tall oil acids were examined on GLC as methyl esters and as alcohols. Correlation between the ECL values and the peak areas was good (see table in experimental section). Oxymercuration - reduction of these alcohols gave cyclic ethers (10%) which were separated from the unreacted alcohols on TLC and identified by GLC before and after hydrogenation.

i.e.

HCEs		CEs		parent acid[2](%)[3]
ECL (%)	assignment	ECL (%)	assignment	
15.0 (85)	18:0 THP	13.1 (1)	16:0 THP	16:1 (0.1)
15.6[1](5)	18:1 THP	15.5 (11)	18:1 THP	18:2 (1.1)
17.0 (10)	20:0 THP	16.3 (69)	18:2 THP	18:3 (6.9)
		17.4 (10)	20:1 THP	20:2 (1.0)
		18.0 (9)	20:2 THP	20:3 (0.9)

Notes:

1. This might have been 18:0 THF but as only one cyclic ether band was isolated from the TLC plate it seemed more probable that it was incompletely hydrogenated 18:2 THP.
2. All acids were Δ5.
3. The amounts of these components present in the original oil were calculated from the fact that prep TLC gave 10% of cyclic ethers.

Analysis with an internal standard

The quantitative measurement of these Δ5 components was improved by the addition of 4.55mole % of octadec-*cis*-5-enoate to the total tall oil fatty acids. The normal procedure was carried out and the total cyclic ethers, including 18:0 THP from the internal standard, were isolated without weighing by prep TLC. Comparison of the cyclic ether peaks on GLC with the peak from the standard allowed the mole % of each component in the original oil to be calculated. A correction was made for the addition of the internal standard. This gave results as shown below.

	CEs		
ECL (%)	assignment	parent acid	mole %
11.0 (1)	14:0 THP	14:1(all Δ5)	0.1
15.0 (34)	18:0 THP	18:1*	-
15.6 (6)	18:1 THP	18:2	0.8
16.3 (51)	18:2 THP	18:3	7.2
17.5 (4)	20:1 THP	20:2	0.6
18.1 (4)	20:2 THP	20:3	0.6

* internal standard

Although von Rudloff oxidation of the cyclic ethers to determine the other double bond positions was not carried out, it is clear that the tall oil fatty acids studied contained the components already reported as shown:

	found	reported
18:2 5c,9c	0.8%	1 - 2%[20] (1%)*
18:3 5c,9c,12c	7.2%	4 - 10%[21] (11%)
20:3 5c,11c,14c	0.6%	traces[22] (?)

* The figures in brackets were quoted by the suppliers of our tall oil sample. Although C_{20} acids (12%) were reported, no information was given on the double bond positions.

In addition to these reported components, our results showed the presence of a 20:2 $\Delta 5$ (possibly 5c,11c) acid (0.56%) in the tall oil acids examined.

Linoleic acid

GLC of the tall oil acids as esters and as alcohols showed 11% and 9% respectively of the 18:3 component. As tabulated above, the suppliers quote a value of 11% for the triene. The disparity between these values and the value of 7.2% obtained by us for the 18:3 5c,9c,12c component was shown to be due to linolenic acid not previously recognised as a component of tall oil.

The triene methyl esters (ca 9%) were obtained by prep Ag^+ TLC of the esterified tall oil fatty acids. GLC of these

esters gave ECLs as shown:

ECL	area%	assignment
19.7	93	18:3
20.2	4	18:4*
21.6	3	20:3

* The small amount of this component would not have been noticeable on the TLC plate and must have been inadvertently scraped off with the trienes.

LAH reduction of these trienes and oxymercuration (in DMF) – demercuration gave a mixture of cyclic ethers and unreacted alcohols which were separated by prep TLC. The cyclic ethers (65%) had ECLs 16.2 (96%) and 18.2 (4%), corresponding, as above, to 18:2 and 20:2 THPs. The unreacted alcohols (35%) were shown to contain a $\Delta 9$ double bond as the first position of unsaturation by comparison of the von Rudloff oxidation products with those obtained by oxidation of authentic oleyl alcohol. This confirmed the presence of 2 - 4% of linolenic acid in the tall oil fatty acid sample examined.

4.4 Fish oils and hydrogenated fish oils

The degree of unsaturation found in fish oils is generally greater than that found in land animals or in plants. A review by Swain [117] describes the composition and properties of fish oils. Reviews on the production of marine oils and on the utilisation of fish oils can be found in the same volume (p.139 and p.149 respectively). Lovern [118] has stated that the most common unsaturated marine oils are as shown:

C atom	No. of double bonds
16	1, or 3
18	1, or 4
20	1, or 4, or 5
22	1, or 5, or 6

Hydrogenated and partially hydrogenated fish oils are widely used in the food and allied industries. As catalytic hydrogenation causes considerable migration of double bonds, partially hydrogenated fish oils would be expected to contain a complex mixture of positional isomers. This isomeration is discussed in detail in a review by Dutton [119] on the hydrogenation of fats.

(a) Analysis

Two fish oils (cod and pilchard) and eight hydrogenated fish oils (HFOs) were quantitatively reduced to the corresponding alcohols which were examined on GLC (see tables in experimental section). Assignments were made to the peaks on GLC from the

ECL values. As the HFO alcohols were very alike on GLC, only four of the eight were examined further. Cyclic ethers were obtained by quantitative prep TLC of the oxymercuration - demercuration products from the two fish oils and from the four HFOs. These products were examined on GLC before and after hydrogenation (see experimental section).

HFOs

Positive assignments could be made for the HCEs by comparison of their ECLs with authentic compounds. As mentioned earlier, a double bond adds about the same amount to an ECL value as the difference in the ECL between a THP and a THF with the same number of carbon atoms. This fact complicated the identification of the unsaturated CEs, but it was evident that only saturated, monoene and diene cyclic ethers were present, indicating that the HFO alcohols contained a maximum of 3 double bonds. The GLC results and the assignments made are tabulated in the experimental section. Inspection of these tables show that the major acids present in the HFOs were as outlined overleaf:

acid	%
16:1 5 *	1 - 3
16:1 4	1 - 2
18:1 5	1 - 4
18:1 4) 18:2 5)	2 - 5
18:2 4	1
20:1 5	2 - 9
20:1 4) 20:2 5)	14 - 19
20:2 4) 20:3 5)	7 - 14
20:3 4	0 - 2

acid	%
22:1 5	1 - 7
22:1 4) 22:2 5)	7 - 10
22:2 4) 22:3 5)	15 - 17
22:3 4	10 - 20
24:1 5	3 - 6
24:1 4) 24:2 5)	1 - 3
24:2 4) 24:3 5)	1 - 5
24:3 4	trace

* First position of unsaturation

Fish oils

As expected, the fish oil cyclic ethers and HCEs were much simpler than those from the HFOs. The same two major products were evident in both pilchard and cod liver oils as shown by GLC.

ECL (HCE)	Cod (area%)	Pilchard (area%)	assignment
17.0	48	67	20:0 THP
19.7	44	21	22:0 THF
(CE)			
19.4	45	66	20:4 THP
22.6	47	22	22:5 THF

As the cod and pilchard oxymercuration - reduction products gave 10.5% and 19% of cyclic ethers respectively by prep TLC, the oils must have contained fatty acids in the amounts shown below:

acid		cod	pilchard
20:5	5,8,11,14,17 *	5.0%	12.7%
22:6	4,7,10,13,16,19 *	5.0%	4.1%

* The positions of the double bonds are probably as shown as these products contained very little conjugated material (vide infra). They are both $\omega 3$ acids.

Minor amounts of other acids (less than 1%) were identified as 20:4 $\Delta 4$; 20:5 $\Delta 5$ and 26:3 $\Delta 5$ by inspection of the GLC results tabulated in the experimental section.

As in the rat liver experiment (section 4.2), the highly unsaturated long-chain alcohols were not observed by GLC of the total fish oil alcohols.

In another experiment, cod liver oil alcohols gave a product by oxymercuration - demercuration as before. To ensure that the more highly unsaturated CEs did not decompose on the TLC plate, the total demercurated product was hydrogenated. The HCEs (10.9%) were then isolated by prep TLC and were shown by GLC to be identical to the HCEs obtained earlier by separation of the CEs on TLC before hydrogenation.

(b) Isolation

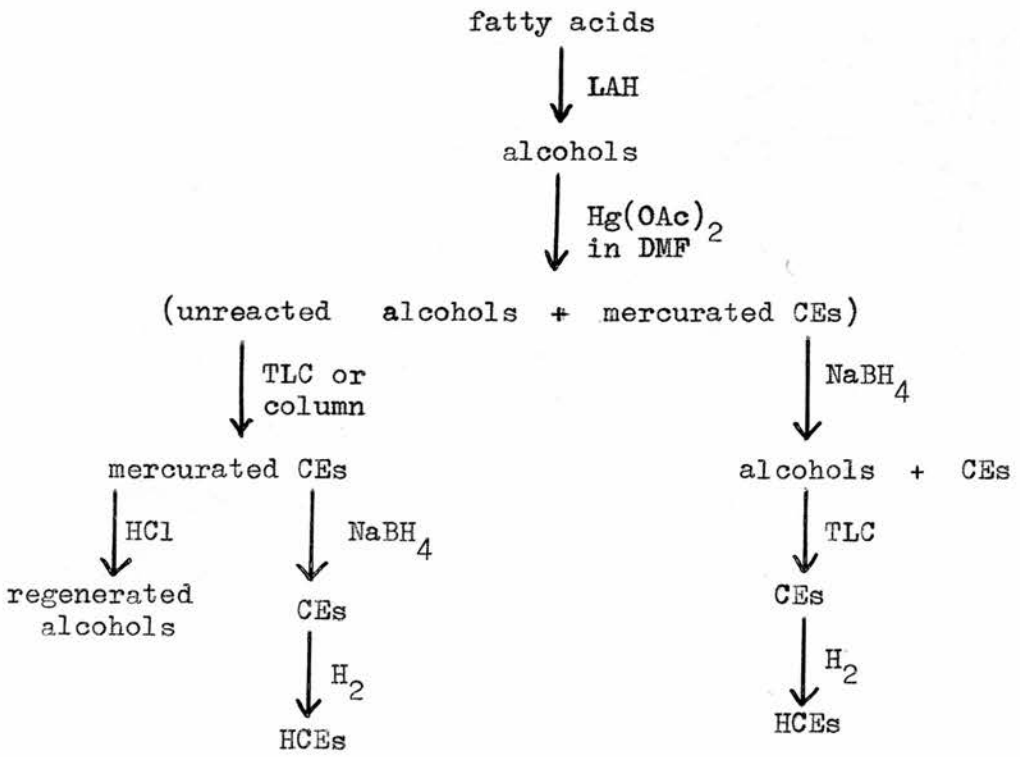
Cod liver oil alcohols were allowed to react with mercuric acetate in DMF. The total organic product was extracted with ether and was introduced on to a silica gel column. Unreacted alcohols were removed using ether as the developing solvent. Methanolic hydrochloric acid was then passed through the column and the regenerated 20:5 and 22:6 alcohols were extracted from the methanol fractions with ether. The alcohols were examined by TLC and by GLC. Although the 20:5 alcohol was eluted from the GLC column (ECL 24.7, 74%) with minor amounts of other components (see experimental), there was no indication of the presence of the 22:6 alcohol until the product was run on GLC as TMS ethers. these had ECLs of 13.5 (0.5%), 18.8 (1%), 19.4 (45%) and 21.7 (53%). The unreacted alcohols, examined on GLC as TMS ethers, did not contain any components with an ECL greater than 19.1, indicating that the separation had been efficient and that there were no 20:5 and 22:6 acids in cod liver oil apart from 20:5 $\Delta 5$ and 22:6 $\Delta 4$.

The IR spectrum of the regenerated alcohols indicated the

presence of a trace of <u>trans</u> unsaturation ($970cm^{-1}$). The quantitative UV spectrum showed absorption at 233nm (ε = 700) and at 303nm (ε = 260) indicating the presence of 2% conjugated diene and 0.3% of conjugated tetraene. These chromophores may have been present in the original acids or may have been produced during the isolation procedure.

It is clear from the ease and efficiency of separation that this process could be used concurrently to isolate and to identify components with $\Delta 3\underline{t}$, $\Delta 4$ or $\Delta 5$ unsaturation. This would involve a comparison of the TMS ethers of the regenerated alcohols against standard TMS ethers or alternatively production of cyclic ethers by sodium borohydride reduction of an aliquot from the isolated oxymercuration product.

Summary of methods



CHAPTER 5

INVESTIGATION OF FOUR UMBELLIFERAE SEED OILS

It is well known that seed oils from Umbelliferae plants contain octadec-cis-9-enoic acid and octadec-cis-6-enoic acid (petroselenic acid) as major components. Recently, Kartha has claimed that these seed oils also contain the 5c [120] and 7c [121] isomers in quantities of 2 - 5% and greater than 10% respectively. He has also claimed [122] that the first double bonds in animal and vegetable fats are not located in discrete positions, but are distributed in major amounts over 3 or more positions.

We examined four Umbelliferae seed oils by the normal oxymercuration in DMF – reduction technique, and although a component similar to authentic 2-alkyl-THP on GLC and TLC was obtained, implying that the 18:1 Δ5 fatty acid was present in the oils, the mass spectrum showed that this component was not 18:0 THP. Oxidative degradation experiments confirmed that there was very little if any Δ5 or Δ7 monoenoic acids in the seed oils examined. While this work was in progress, Kuemmel [123] questioned the validity of Kartha's findings [122] and pointed to the great number of examples in the literature which conflict with Kartha's rather suspect results.

Methyl octadec-cis-4-enoate was added in accurately weighed amounts as an internal standard to known weights of the seed oils examined. LAH reduction of the mixtures gave alcohols which were reacted with mercuric acetate in DMF and later reduced with borohydride to give products from which the cyclic ethers were isolated by prep TLC. GLC of these CEs gave ECLs as shown overleaf. The whole procedure was repeated on

coriander seed oil without the internal standard to ensure that there was no 18:1 Δ4 acid in the oil. From these GLC results and from the known amounts of added internal standard, the amounts of the components with ECLs 13.7 and 15.1 were calculated. A correction was made for the presence of the internal standard in the original oil.

	Area %				
ECL	carrot	coriander	parsley	caraway	assignment
13.7	2(0.1)[1]	1(0.1)	0.5(0,1)	1(0.1)	16:0 THF
15.1	75(4.0)	52(6.7)	26(5.8)	32(3.7)	unidentified
15.7[2]	23	47	25	64	18:0 THF[2]
18.8	-	-	49	3	non-lipid[3]

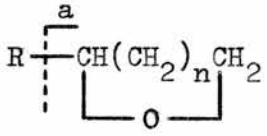
Notes:

1. The figures in brackets are the calculated amounts (mole%) of the components in the original oils.
2. This was produced by the 18:1 4c internal standard.
3. This product had an unusual appearance on TLC and partly masked the THF cyclic ether band.

The mass spectra of the Umbelliferae cyclic ethers showed a molecular ion peak at $^{m}/e$ 268. A peak at M-18 ($^{m}/e$ 250) was also present. A very intense peak at $^{m}/e$ 99 and a less intense peak at $^{m}/e$ 81 (-18) were observed as the principal features in all spectra. Peaks with $^{m}/e$ 71 were observed in the

mass spectra of cyclic ethers which contained the 18:0 THF as an internal standard. The 2-alkyl-THF and 2-alkyl-THP produced by authentic 18:1 4c and 18:1 5c alcohols showed very intense peaks at $^{m}/e$ values 71 and 85 respectively in their mass spectra. These peaks are almost certainly produced by α-cleavage to the heterocyclic ring.

i.e.



n = 2, a = 71

n = 3, a = 85

The intense peaks at $^{m}/e$ 99 and at $^{m}/e$ 81($-H_2O$) in the mass spectra of the Umbelliferae CE implied that this cyclic ether was either:

(i) a 7-membered heterocycle

(ii) a methyl-substituted THP

(iii) a dimethyl or ethyl-substituted THF

A larger amount of this cyclic ether was obtained by the normal oxymercuration procedure from carrot seed oil alcohols. The IR spectrum of this CE showed normal ether absorptions (1,118cm^{-1} and 1,360cm^{-1}) and a weak unidentified absorption at 1,725cm^{-1}. The 100MHz NMR spectra of 18:0 THF, 18:0 THP and this cyclic ether were compared (see table in experimental section) but no positive identification of the product could be made.

The non-saponifiable material was extracted from coriander seed oil and the non-lipid material was isolated from caraway seed oil by prep TLC. LAH reduction and then oxymercuration - demercuration of these materials did not yield any of the unidentified

component with ECL 15.1. This must therefore have been produced by a fatty acid rather than by some non-lipid component in the oils.

von Rudloff oxidation studies

The methyl monoene esters from caraway seed oil esters were isolated by prep Ag^+ TLC. von Rudloff oxidation of these monoenes gave monobasic and dibasic esters which were separated by prep TLC and shown by GLC to be C_6 and C_9 dibasics and C_9 and C_{12} monobasics. These are the fragments expected from the permanganate oxidation of 18:1 $\Delta 6$ and $\Delta 9$ esters. Apart from a very small trace of C_{11} monobasic ester, there was no evidence of $\Delta 5$ or $\Delta 7$ unsaturation.

Authentic methyl octadec-cis-5-enoate (1%) and methyl octadec-cis-7-enoate (1%) were added to coriander methyl esters as internal standards. Isolation of the monoene esters and von Rudloff oxidation as described above gave monobasic and dibasic esters which were examined by GLC. The monobasic esters clearly contained C_{11} and C_{13} fragments in addition to the C_9 and C_{12} fragments obtained earlier and the dibasic esters clearly showed the presence of C_5 and C_7 fragments in addition to the C_6 and C_9 fragments obtained earlier. This showed that 5-cis and 7-cis 18:1 esters could readily be detected at the 1% level by this method and suggested that there was probably less than 0.1% (if any) of the $\Delta 5$ or $\Delta 7$ monoenoates in the oils examined.

These results confirmed work done by Miss A. Mowat in an undergraduate final-year research project.

CHAPTER 6

METHOXYMERCURATION OF OLIVE, CORN AND LINSEED OILS

Methoxymercuration - demercuration

Three common triglycerides (olive, corn and linseed oils) containing oleic acid (82%), linoleic (54%) and linolenic acid (47%) respectively as the major components were successfully methoxylated on a large scale by methoxymercuration - reduction. Examination of these oils by GLC as methyl esters before and after the reaction showed that all the double bonds had not reacted. By inspection of the table of GLC results in the experimental section, we can see that for 100 molecules of product (as methyl esters) the number of methoxyl groups to the number of residual double bonds was as shown:

oil	no. of methoxyls	no. of double bonds
olive	74	11
corn	104	7
linseed	130	11

Physical properties

The relative viscosities of the original oils and of the methoxylated products were measured in chloroform solution. The results showed that viscosity increased when double bonds were replaced by methoxyl groups but decreased as the degree of unsaturation increased.

The refractive indices of the methoxylated oils were slightly lower than those of the original oils.

The most striking physical change observed was the difference between the melting behaviour of these methoxylated oils and the melting behaviour of the starting materials. A crude comparison of the relative melting points was carried out by measuring the time taken for equal weights of the oils to slip down the sides of glass sample tubes after these had been inverted and removed from the refrigerator at -15°. If the oils were left at -15° for less than 2 days before this procedure was carried out, anomalous results were obtained. This was probably due to incomplete crystallisation of the oils.

oil	relative viscosity (sec) [1]	refractive index [2]	average slip time (sec)
olive	68.4	1.4692	430
methoxylated olive	70.0	1.4640	47
corn	68.1	1.4729	140
methoxylated corn	70.3	1.4688	82
linseed	67.8	1.4811	84
methoxylated linseed	69.3	1.4691	70

Notes:

1. Measured as 10.0% $^{w}/_{v}$ solutions in chloroform at 30.0°. Chloroform had a flow time of 45.5sec.
2. Measured at 21.7°.

Hydroxymercuration

Attempts to hydroxymercurate olive and corn oil with mercuric acetate in DMF and water (4:1 v/v) gave virtually unchanged starting material (TLC and GLC of methyl esters) after sodium borohydride reduction. This may have been due to insolubility of the triglycerides in the solvent system used.

EXPERIMENTAL

GENERAL PROCEDURES

Purification of solvents

All solvents were reagent grade unless otherwise stated. Carbon disulphide was distilled and stored over molecular sieve (type 4A). Dimethylformamide (DMF) was dried by the addition of benzene and the removal of water as an azeotrope (b.p. 78°). The DMF was subsequently distilled (b.p. 153°) and stored over molecular sieve (type 4A). Diethyl ether was dried by standing over calcium chloride. After decantation and distillation, it was stored over sodium wire. Methanol was dried by reaction with magnesium and iodine according to Vogel's procedure.[124] Petroleum ether was distilled. The fraction with b.p. 40 - 60° was used. Pyridine was distilled from potassium hydroxide pellets. Tetrahydrofuran was dried over anhydrous sodium sulphate, distilled at 63 -65°, and stored over sodium wire.

Spectroscopic Analysis

Infra-red (IR)

Spectra were recorded on a Perkin-Elmer 257 grating spectrometer. Samples were normally run as 1% solutions in carbon disulphide or carbon tetrachloride using sodium chloride cells of 1mm path length.

Mass spectra (MS)[67]

Spectra were recorded on an AEI MS 902 mass spectrometer. Samples were introduced by direct insertion or by a Pye 104 chromatograph. Spectra were normally run at 70 ev with a source pressure of 10^{-6} torr and a temperature of 200°.

Nuclear Magnetic Resonance (NMR)

Spectra were recorded on 15% solutions in carbon tetrachloride using a Perkin-Elmer R-10 spectrometer operating at 60 MHz or a Varian HA-100 operating at 100 MHz. Chemical shifts

were measured in ppm downfield from internal tetramethylsilane (τ=10).

Ultra-violet(UV)

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

Chromatographic Techniques

Thin Layer Chromatography (TLC)

Analytical TLC was carried out on glass plates (20cm x 5cm) with a layer of silica gel G (0.25mm wet thickness) or with silica gel G containing 10% silver nitrate (Ag^+ TLC).[106]

Preparative TLC (prep TLC) was carried out on glass plates (20cm x 20cm) with a silica layer of 1mm wet thickness. Preparative silver nitrate TLC is abbreviated to prep Ag^+ TLC. Prep TLC plates were pre-cleaned with distilled ether.

Ether - petroleum mixtures were normally used as developing solvents. The abbreviation PE20 indicates a mixture of 20% ether and 80% petrol by volume.

Separated components on analytical plates were made visible by iodine vapour and/or by spraying with a 10% solution of dodeca-phosphomolybdic acid in ethanol followed by heating at approx. 120° for 10 min.[125] Mercury - containing compounds were apparent as intense blue spots after spraying with a 0.2% solution of 1,5-diphenylcarbazone in ethanol.[126] Dodeca-phosphomolybdic acid could be used successfully after the diphenylcarbazone spray.

Components on preparative plates fluoresced under UV light after the plates had been sprayed with a 0.2% solution of 2,7-dichlorofluorescein in methanol. Bands were scraped off, slurried with distilled ether or methanol and filtered. Residual dichlorofluorescein was removed by percolation through a short column (5cm x 1cm) of florisil with ether.

Gas - Liquid Chromatography (GLC)

A Pye 104 model 4 chromatograph with a flame ionisation detector was used throughout. Glass columns (5ft by $\frac{1}{4}$ in i.d.) were packed with Gas Chrom Z (70 - 80 mesh) coated with 20% diethylene glycol succinate polyester (DEGS). Samples were injected directly on to the column using a 10μl SGE syringe with an 11cm needle. Operating conditions were varied according to the nature of the substance under investigation. Carrier gas (oxygen-free nitrogen) flow rate was varied from 15 to 60ml/min. Oven temperature was varied from 120 to 190°. Peak areas (uncorrected for detector response) were measured by one or more of the following methods:

(i) Peak height x peak width at half height

(ii) Peak height x retention time

(iii) Dupont curve analyser

Saturated straight-chain methyl esters were used as external standards or as internal standards by co-injection. Retention times are reported as equivalent chain lengths (ECLs).[127] Apparent inconsistencies in ECL values reported in the text were due to deterioration of the liquid phase with use. Whenever possible, product ECLs were compared with the ECLs of authentic materials run consecutively.

General Chemical Procedures

Esterification [128]

Acids were refluxed for 20minutes with a 2% solution of boron trifluoride-methanol complex in dry methanol. The reaction mixture was cooled, poured into brine and extracted with ether (x2). Ether extracts were combined, washed with 5% aqueous sodium bicarbonate solution (x2) and water (x2) and dried over anhydrous sodium sulphate.

Methanolysis

Glycerides were converted to methyl esters and acetoxy esters to hydroxy esters by shaking overnight at room temperature or refluxing for 30 minutes with dry methanolic sodium methoxide (0.1M). The reaction mixture was carefully acidified, poured into brine and extracted with ether. (Free acids were removed from glycerides before methanolysis by percolation through an alumina column using chloroform as solvent.)

Trimethylsilylation [129]

Long-chain hydroxy compounds were converted into their trimethylsilyl ethers (TMS ethers) to facilitate examination by GLC. To ca 5mg of material in pyridine (1ml) was added hexamethyldisilazane (0.2ml) and trimethylchlorosilane (0.1ml). After 5 minutes the pyridine was removed under vacuum and the TMS ethers dissolved in ether.

Purdie methylation [130]

Long-chain hydroxy compounds were converted into their corresponding methyl ethers to facilitate GLC, TLC and MS examination.

Methyl iodide (1ml) and freshly prepared silver oxide [131] (5mg) were added to the material under investigation (10mg). The reaction mixture was heated under reflux for 3 hours, cooled and then filtered. Excess methyl iodide was removed under vacuum.

Lithium aluminium hydride reduction

Acids, esters and glycerides were converted to long - chain alcohols by reaction with excess lithium aluminium hydride (LAH) in dry ether. To a stirred suspension of LAH (20mg) in dry ether (2ml) was added dropwise a solution of lipid material (100mg) in dry ether (2 to 5ml). After stirring for 10 minutes at room temperature excess hydride was destroyed by the cautious addition of wet ether and then water. Dilute sulphuric acid (20ml, 2M) was added and the product extracted with ether (2x20ml) and dried over anhydrous sodium sulphate.

Hydrogenation

Samples (10mg) in methanol (5ml) with 10% palladium/ charcoal (10mg) as catalyst were shaken in a hydrogen atmosphere for one hour at room temperature. The catalyst was removed by filtration and the material recovered in high yield (>90%) by evaporation of the solvent under vacuum.

von Rudloff oxidation [132,133]

Oxidative cleavage was used to determine the position of unsaturated centres in long-chain compounds.

An oxidising solution of potassium periodate (22.4g, 0.0975mole) and potassium permanganate (0.4g, 0.0025mole) in one litre of water was used.

The unsaturated material (5mg) in distilled t-butanol (7ml) and water (1ml) was shaken overnight with 5% aqueous potassium carbonate (1ml) and oxidising solution (2ml). Excess oxidising agent was destroyed with sulphur dioxide and the solution made alkaline (potassium hydroxide pellets). After most of the solvent had been carefully removed under vacuum the residue was acidified (2M HCl), saturated with sodium chloride and extracted with ether (2x20ml). The resulting acids were esterified (boron trifluoride - methanol) and the products analysed by GLC.

N.B. In order to minimise loss of volatile short-chain products all ether extracts were carefully evaporated at atmospheric pressure.

Oxymercuration

A typical procedure is outlined below. Specific details are included in the text.

Excess mercuric acetate (100mg, 0.31mmole) and methyl oleate (50mg, 0.17mmole) in methanol (10ml) were left in a stoppered flask at room temperature for 2 to 4 days. (Mechanical shaking was only necessary when the reaction mixture was not homogeneous. For example, when water was used as solvent a dense yellow precipitate was formed.)

Borohydride reduction of oxymercuration product

Excess sodium borohydride (20mg) dissolved in water (10ml) was added dropwise with stirring to the oxymercuration reaction mixture (above) at 0° (ice bath). The reaction mixture was stirred at room temperature for 30 min, saturated with sodium chloride and extracted with ether (2x20ml).

Acid regeneration of oxymercuration product

An aliquot (1 - 2ml) from the oxymercuration reaction was stirred with methanolic hydrochloric acid (0.5M, 10ml) for 10 min at room temperature. The product was extracted with ether.

PREPARATION OR SOURCE OF STARTING MATERIALS

Oleic acid (98%) was obtained from olive oil acids by urea fractionation as described by Schlenk and Holman.[101] The acid was esterified with methanolic hydrogen chloride (10% $^{w}/_{v}$).

Linoleic acid (96%) was prepared from corn oil acids (ca 60% linoleic) by removal of oleic acid and other impurities as crystalline urea complexes from methanol. The ester was obtained by refluxing the acid for 20min with methanolic hydrogen chloride (10% $^{w}/_{v}$).

Methyl 12-hydroxyoctadec-cis-9-enoate (ricinoleate) was prepared pure from castor oil. This was neutralised by percolation through a short alumina column (100 - 120 mesh) using chloroform as solvent. The recovered neutralised oil was converted to methyl esters (methanolic sodium methoxide) which were chromatographed on silica gel (Sorbsil M60) using petroleum mixed with increasing amounts of ether as developing solvent.

Methyl 12,13-epoxyoctadec-cis-9-enoate (vernolate) was obtained (99% pure) from Cephalacroton cordofanus seed oil by the same chromatographic procedure as outlined above. Because of the acid-labile nature of the oxirane function[134], care must be exercised when extracting the methyl esters from the trans-esterification reaction mixture to prevent the solution becoming acidic.

Methyl 9-hydroxyoctadec-cis-12-enoate was isolated from Strophanthus courmontii seed oil by partition of the seed oil acids between 20% aqueous methanol and petrol[135] to give a 90% concentrate of the required acid in the methanol layer. This concentrate was esterified and further purified by column or thin-layer chromatography as required.

Methyl 12-hydroxyoctadec-trans-9-enoate (ricinelaidate) was obtained by stereomutation of the corresponding cis isomer (0.1M) with 3-mercaptopropionic acid (0.2M) in absolute ethanol for 2 days at 30° by the method of Kircher et al[136]. Careful separation of the mixture of cis and trans isomers by 15% Ag^+ prep TLC(PE40) gave a 60% yield of the required trans isomer (IR 970cm^{-1}).

Early attempts to stereomutate the hydroxy monoene acids by shaking in ethereal solution with nitric acid (6M) and aqueous sodium nitrite (2M)[137] produced large amounts of nitrogen-containing adducts (TLC) and were abandoned.

Methyl 9-hydroxyoctadec-trans-12-enoate could not be prepared by Kircher stereomutation of the cis isomer, as large amounts of adduct material (very polar on TLC) and very little trans ester (IR and Ag^+ TLC) were produced. The difference between this attempt and the successful stereomutation of methyl ricinoleate must be due to some interaction between the hydroxyl group and the double bond in the 9-hydroxy 12c ester. This was overcome by protecting the hydroxyl group by acetylation. The ester was refluxed in acetic anhydride for 6hr and the acetoxy ester was then successfully stereomutated. The trans isomer was isolated by 15% Ag^+ TLC(PE30). The required hydroxy ester was obtained by leaving the acetoxy ester overnight in methanolic sodium methoxide solution (0.5M, 10ml).

An alternative method of preparing the 12t hydroxy ester involving bromination - dehydrobromination of the cis isomer to give the 12a ester (and other interesting products) followed by lithium - ammonia reduction to give the required 12t product is described in appendix 2.

Methyl 12,13-threo-dihydroxyoctadec-cis-9-enoate[138] was prepared from Vernonia anthelmentica seed oil by refluxing the oil in glacial acetic acid (10hr) and hydrolysing the product (aqueous methanolic potassium hydroxide) to give free

di-hydroxy acid which was recrystallised from ethyl acetate at 0° (x 7) and esterified (boron trifluoride - methanol) to give the required ester. The product was shown to be pure by GLC, TLC and GLC of the bis-TMS ether.

Methyl 9,10-cis- and 9,10-trans-epoxystearates were prepared by leaving methyl oleate and methyl elaidate respectively in an ethereal solution of mono-perphthalic acid overnight. Excess epoxidising agent was removed by washing the ether solution with aqueous sodium hydroxide (2M) and water. Prep TLC(PE30) was used to remove unreacted and saturated methyl esters. Monoperphthalic acid was prepared and standardised according to the method described by Payne.[139]

Mercuric trifluoroacetate [61] was prepared from mercuric oxide (7.2g, 0.033mole) dissolved in trifluoroacetic acid (10ml) by the dropwise addition of trifluoroacetic anhydride (7.0g, 0.033mole). The reaction mixture was refluxed (10min) and the solvent distilled off. The salt (11.1g, 0.026mole, m.p. 164 -166°, lit. m.p. 165 - 167° [140]) was obtained by recrystallisation (x 2) from trifluoroacetic acid.

Several synthetic acids, esters and alcohols were available in the laboratory. References to the syntheses are given in the main text.

I wish to express my thanks to the various suppliers for the gifts of samples listed below.

sample	source
rat livers	Biochemistry Department, University of St. Andrews.
fish oils and hydrogenated fish oils	Dr. F.B. Padley (Unilever) and Dr. B. Bulleymore (J. Lyons)
tall oil acids	Mr. J.A. Ashley (B.O.C.)
Carlina corymbosa, *Calea uniflora*, *Teucrium depressum* and *Thalictrum flavum* oils	Dr. I.A. Wolff (then of Northern Regional Research Laboratories, Peoria.)

Umbelliferae seeds were purchased from T.B. Lock & Sons Ltd. (Yeovil, Somerset).

1. VARIATION OF ATTACKING NUCLEOPHILE

Methyl oleate (98% pure by GLC and TLC) with methyl palmitate and methyl myristate as the major impurities was allowed to react with mercuric acetate in the presence of various nucleophiles. The oxymercurated products were reduced *in situ* with sodium borohydride. (see General Procedures)

1.1 Methanol

Methyl oleate (510mg, 1.72mmole) in anhydrous methanol (40ml) yielded a product (560mg) after oxymercuration and reduction. GLC analysis showed two peaks with ECLs of 18.5 (2%) and 21.2 (98%). The minor component had the same ECL and the same Rf value on TLC (0.75, PE30) as methyl oleate. The major component, purified by prep TLC (PE30), was submitted for mass spectral analysis. (see MS 1.1 in appendix 1) Authentic methyl 9-methoxystearate had an ECL of 21.2.

1.2 Ethanol

Methyl oleate (75mg, 0.25mmole) in 5ml absolute ethanol yielded a product (87mg) after oxymercuration and reduction. GLC analysis gave two peaks with ECLs of 18.5 (5%) and 20.7 (95%). The major component (Rf 0.69, PE30) appeared to be slightly less polar than the methyl methoxystearates (Rf 0.66) obtained in part 1.1.

Methyl 9-ethoxystearate was prepared from pure methyl 9-hydroxyoctadec-*cis*-12-enoate. Hydrogenation gave methyl 9-hydroxystearate (ECL 26.1, 90%) containing methyl stearate (ECL 18.0, 5%) and methyl oxostearate (ECL 24.9, 5%) as impurities. The total hydrogenated product was submitted to Purdie alkylation with ethyl iodide (2ml, b.p. 78°) and freshly-prepared silver oxide (40mg) for 3 hours. GLC analysis indicated that etherification of the hydroxyester was only about 50% complete but pure methyl 9-ethoxystearate (ECL 20.64) was obtained by prep TLC (PE30) of the total product.

The major product from the oxymercuration in ethanol and the synthetic methyl 9-ethoxystearate had the same Rf value on TLC (PE30), the same ECL on GLC but different mass spectra (MS 1.2a and MS 1.2b respectively) as the mercuration product was a mixture of the 9- and 10-ethoxystearates.

1.3 t-Butanol

(a) Reaction with mercuric acetate

Methyl oleate (150mg, 0.51mmole) in t-butanol (10ml) was shaken* with mercuric acetate (175mg, 0.55mmole) at room temperature. After 3 days, 5ml of the reaction mixture was removed, reduced with sodium borohydride, and residual butanol was removed by blowing with a stream of nitrogen. After a further 3 days the remaining reaction mixture was reduced in the same manner. GLC and TLC analysis indicated that both products consisted solely of methyl oleate (ECL 18.5, Rf 0.75, PE30). The IR spectrum of the 6-day product showed very little absorption at 970cm^{-1}.

(b) Reaction with mercuric trifluoroacetate

Methyl oleate (100mg, 0.34mmole) in t-butanol (6ml) with mercuric trifluoroacetate (160mg, 0.375mmole) gave a homogeneous solution which was left at room temperature for 3 days. GLC and TLC analysis after sodium borohydride reduction indicated that very little reaction had taken place. (TLC showed a minor component with the same Rf as methyl hydroxystearate.)

1.4 n-Butanol

Methyl oleate (100mg, 0.34mmole) in n-butanol (10ml) and dry THF (20ml) was shaken with mercuric acetate for 3 days. After borohydride reduction and the removal of residual solvent under a stream of nitrogen, the product (103mg) was examined by GLC and TLC and shown to be identical to methyl oleate.

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* Mercuric acetate appeared to be completely insoluble in t-butanol.

1.5 n-Hexanol

The procedure of 1.4 was followed using n-hexanol instead of n-butanol. The product was again identical to methyl oleate.

1.6 Phenol

Excess phenol (1g, 0.106mole), methyl oleate (150mg, 0.51mmole) and mercuric acetate (200mg, 0.55mmole) were dissolved in anhydrous THF (10ml). After 30min, the clear solution had become white and opaque and was subsequently shaken for 3 days. In addition to the normal work up after sodium borhydride reduction, the ether extracts were washed with aqueous sodium hydroxide (1M, 2 x 20ml) to remove excess phenol.

The experiment was repeated using mercuric trifluoroacetate (236mg, 0.55mmole) and phenol (200mg, 2.13mmole) in THF (6ml) and again with mercuric acetate (200mg, 0.55mmole), methyl oleate (100mg, 0.34mmole) and phenol (50mg, 0.53mmole) in DMF (5ml). Unchanged methyl oleate was obtained in all cases.

1.7 Water + co-solvent (THF or DMF)

Methyl oleate (100mg, 0.34mmole) and mercuric acetate (120mg, 0.38mmole) were added to each of the following systems:

Expt.	Water (ml)	THF (ml)	DMF (ml)
A	1	9	-
B	1	-	9
C	5	5	-
D	5	-	5

A dense yellow precipitate was produced on the addition of the mercury salt. The reaction mixtures were shaken for 2 - 3 days at room temperature and then A, B and D were reduced with sodium borohydride. In an attempt to discover if the rate of addition of sodium borohydride affected the relative amounts of components in a product, reaction C was separated into two 5ml portions (C_1 and C_2). C_1 was reduced by the rapid addition of aqueous

borohydride (40mg in 5ml water), but the borohydride solution was added dropwise over 5 min to C_2.

Reults are summarised below:

expt	product (mg)	oleate[1]	hydroxystearate[1]	TLC (PE30)	
				Rf 0.75	Rf 0.32
A	94	95%	5%	major	minor
B	97	96%	4%	major	minor
C_1	53	23%	77%	minor	major
C_2	54	23%	77%	minor	major
D	98	29%	71%	minor	major

Notes

1. Products were identified by chromatographic comparison with authentic methyl oleate (ECL 18.5, Rf 0.75) and with authentic methyl 12-hydroxystearate (ECL 25.6, Rf 0.32) and its TMS ether (ECL 19.9).
2. Traces of methyl acetoxystearates (ECL 24.0) were also obtained in all cases.

The mass spectrum of the TMS ethers of the methyl hydroxystearates isolated from product D by prep TLC (PE30) are included in appendix 1 (MS 1.7).

1.8 Acetic acid

Methyl oleate (100mg, 0.34mmole) in glacial acetic acid (5ml) was allowed to react with mercuric acetate (120mg, 0.38mmole) for 2 days at room temperature. Aqueous sodium hydroxide (3M) was carefully added until the solution was alkaline (pH 8 - 9) immediately before the addition of sodium borohydride. The product gave 3 peaks on GLC with ECLs of 18.5 (13%), 24.2 (82%) and 26.1 (5%). The major product was isolated by prep TLC (Rf 0.57, PE30) and checked by GLC (24.2, 100%). The minor components correspond to

methyl oleate and methyl hydroxystearate on GLC and TLC. They were not examined further.

The IR spectrum of the major component shows strong absorption bands at 1,725cm^{-1} (carbonyl stretching) and at 1,235cm^{-1} (C-O stretching). The latter is considerably stronger than the same band in the IR spectrum of methyl stearate. The mass spectrum (MS 1.8) is reproduced in appendix I.

1.9 Ethylene glycol

The following reaction mixtures were allowed to react at room temperature for 3 days and then reduced with sodium borohydride:

A Methyl oleate (150mg, 0.51mmole) and ethylene glycol (19mg, 0.31mmole) in THF (10ml) with mercuric acetate (200mg, 0.63mmole). Mechanical shaking was necessary.

B Methyl oleate (100mg, 0.34mmole) and ethylene glycol (11mg, 0.18mmole) in THF (5ml) with mercuric trifluoroacetate (200mg, 0.47mmole).

C Methyl oleate (100mg, 0.34mmole) and ethylene glycol (1g) in DMF (5ml) with mercuric acetate (200mg, 0.63mmole).

D Tridecene (1g, 0.55mole) and ethylene glycol (170mg, 0.027mole) in DMF (25ml) with mercuric acetate (2g, 0.63mole).

A and B gave products (144mg and 98mg respectively) which were identical on GLC and TLC to methyl oleate. They were not examined further.

C gave a product (105mg) which showed 3 peaks on GLC with ECLs 18.5, 24.0 and 28.5. The TMS ethers were prepared (5mg) and shown to have ECLs of 18.5, 19.5 (minor), 23.5 and 24.0. Prep TLC (PE20) on 57mg of product provided 3 bands - C_1(Rf 0.07), C_2(Rf 0.34) and C_3(Rf 0.59). Components were extracted from band C_1 by ether and methanol (50% v/v) and from bands C_2, C_3 by ether alone.

C_1 (28.6mg, 53%) gave ECLs of 28.5 and 25.5 (trace). Elemental analysis gave C - 70.9%, H - 12.2%. Calculation for $C_{21}H_{42}O_4$ gave C - 70.4%, H - 11.8%.

The TMS ether (ECL 23.5) was prepared and submitted for mass spectral measurement (MS 1.9 C_1, appendix 1).

C_2 (6.0mg, 11%) gave ECLs of 24.0, 21.1 (trace) and 26.7 (trace). Strong IR absorption bands at 1,725cm^{-1} and 1.235cm^{-1} indicated the presence of an acetoxy function. The 60MHz NMR spectrum showed a singlet at 8.06τ(3H) in addition to the normal polymethylene, terminal methyl and ester methyl signals. The mass spectrum (MS 1.9 C_2) is shown in appendix 1.

C_3 (19.2mg, 36%) with ECL 18.5 was identical to methyl oleate on GLC and TLC and was not examined further.

D gave a product (1.2g), a portion of which (132mg) was methylated (methyl iodide / silver oxide) to yield a product (148mg). GLC of the original product and of the etherified derivative gave a peak corresponding to authentic tridecene which was eluted immediately after the solvent ether peak. Due to the range of ECLs of the products, measurement of peak areas was approximate. GLC results are tabulated below:

Total product		Methylated product	
ECL	Area %	ECL	Area %
tridecene	22	tridecene	20
12.4	41	9.3	10
13.1	8	12.4	41
16.3	20	13.4	20
22.4	10	22.4	10

Prep TLC (PE5) of the methylated product (145mg) gave 4 bands - D_1(Rf 0.22), D_2(Rf 0.41), D_3(Rf 0.47) and D_4(Rf 0.78). Components were extracted with ether and examined by GLC, MS, IR and NMR.

D_1 (37mg, 26%) had an ECL of 13.4. The purity was checked by TLC (E) which gave one spot (Rf 0.6) and showed no evidence of dimeric material. The mass spectrum (MS 1.9 D_1) is reproduced

in appendix 1. The 60MHz NMR spectrum is outlined below:

NMR 1.9 D_1

Assignment	Appearance	τ value	No. of protons
CH_3-, terminal	irregular triplet	9.12	3
CH_3-CH($OCH_2CH_2OCH_3$)-	doublet	8.93	3
-CH_2-, in chain	broad singlet	8.74	20
-$O(CH_2)_2O$-	singlet	6.59	4
CH_3O-	singlet	6.73	4
>$CHO(CH_2)_2OCH_3$	multiplet	ca6.70	

D_2 (75mg, 53%) had ECLs of 9.3 (minor), 12.4 (>90%) and 22.4 (trace) on GLC. The IR spectrum showed strong absorption at 1,725cm^{-1} and 1,235cm^{-1} indicating the presence of an acetoxy function. The mass spectrum was recorded (MS 1.9 D_2, appendix 1). The NMR spectrum is summarised below:

NMR 1.9 D_2

Assignment	Appearance	τ value	No. of protons
CH_3, terminal	irregular triplet	9.12	3
CH_3-CH(OAc)-	doublet	8.84	3
-CH_2-, in chain	broad singlet	8.8-8.4	20-21
$CH_3CO.O$-	singlet	8.06	3
>CH-OAc	multiplet	5.22	0-1

An extremely small pair of singlets (6.6 and 6.8τ) were presumably due to the minor amount of impurities (ECLs 9.3 and 22.4) present in this fraction.

Component D_3 (11.2mg, 7.9%) had an ECL of 9.3. The IR spectrum showed the normal C-H absorptions and a band at 1,100cm^{-1} indicating the presence of an ether linkage. The mass spectrum is tabulated below:

MS 1.9 D_3

m/e	I	m/e	I	m/e	I
214	1.5	123	5	85	35
213	2.3	113	8	83	62
199	22	111	31	71	93
182	35	109	10	69	100
149	15	98	15	59	*
126	20	97	54		
125	12	95	15		

* Peak m/e 59 was far too large to allow intensity measurement.

Component D_4 (19.2mg, 13.5%) was identical to authentic tridecene on GLC, TLC and MS (M^+ 182).

1.10 t-Butylhydroperoxide A. Methyl oleate

Mercuric acetate (200mg, 0.63mmole) was dissolved in t-butylhydroperoxide (10ml) by gentle warming on a water bath (<40°). Methyl oleate (150mg, 0.51mmole) was added and the mixture was shaken gently as a slight pale-yellow ppt. was formed. After borohydride reduction and extraction of the product with ether, some t-butylhydroperoxide remained. Most of this was removed by blowing with a stream of nitrogen, but a strong smell of t-butylhydroperoxide persisted in the residue (176mg). GLC of this product gave ECLs 18.5(25%), 23.3(45%), 23.5(6%), 24.0(3%), 24.4(6%) and 25.4(15%). The product (110mg) was separated into five fractions by prep TLC (PE30) as shown overleaf:

fraction	Rf	weight (mg)	%	ECLs of fraction
A_1	0.05	8.1	8	---
A_2	0.16	18.5	19	23.3(2%), 24.3(1%), 25.4*(97%)
A_3	0.22	6.3	6	22.6(5%), 23.3(6%), 23.5(11%), 24.4(37%), 25.1(7%), 25.3(13%), 26.1(22%).
A_4	0.38	44	44	23.3(86%), 23.5(11%), 24.0(3%)
A_5	0.53	23	23	18.5

* ECL of TMS ether 19.9.

Additional information:

Fraction A_1 gave no significant peaks on GLC. TLC(E) showed that it was a mixture of several components, some of which contained mercury, and it was not examined further.

Fraction A_2 was not separated from authentic methyl 9-hydroxystearate (ECL 25.5 and 19.9 as TMS ether) by TLC(PE40).

The complex mixture A_3 was not examined further.

Fraction A_4 showed no unusual features in its IR spectrum. The mass spectrum (MS 1.10 A_4) is reproduced in appendix 1. The NMR spectrum is outlined below:

NMR 1.10 A_4

Assignment	Appearance	τ Value	No. of protons
$C\underline{H}_3$-, terminal	unresolved	9.1	40
$-C\underline{H}_2$-, in chain	broad doublet	8.4 - 8.8	
$-C\underline{H}_2.CO_2CH_3$	masked triplet	7.78	2
$-CO_2C\underline{H}_3$	sharp singlet	6.4	3
	broad multiplet	7.52	2

Methyl trans- and cis—9,10-epoxystearates have ECLs of 23.3 and 23.5 respectively and were eluted with the components of ECL 23.3 and 23.5 when co-injected with fraction A_4.

A_5 was identical to methyl oleate on TLC and GLC.

1.10 t-Butylhydroperoxide B. Tridecene

Tridecene (500mg, 2.74mmole) and mercuric acetate (1.5g, 4.7mmole) in t-butylhydroperoxide were shaken at room temperature for 2 days. After sodium borohydride reduction and work up, the product (700mg) still had a strong smell of t-butylhydroperoxide. Prep TLC(PE30) of 300mg of product gave 4 bands - B_1(Rf 0.07), B_2 (Rf 0.23), B_3 (Rf 0.71) and B_4 (Rf 0.89). On extraction with ether B_1 gave a product (18mg, 8%) which showed several spots on TLC(E). It was not examined further.

B_2 gave 20mg(9%) of material with an ECL of 13.0. The IR spectrum of B_2 showed strong O-H absorption at 3,600cm^{-1}. The TMS ether of B_2 had a very low ECL on GLC and its mass spectrum (MS 1.10 B_2, appendix 1) indicated that B_2 was 2-hydroxytridecane.

B_3 yielded 102mg (46%) of material with ECLs of 10.6 (trace) and 12.1. The mass spectrum which was consistent with a 1,2-epoxide structure is recorded in appendix 1 (MS 1.10 B_3). The NMR spectrum is outlined below:

NMR 1.10 B_3

Assignment	Appearance	τ Value	No. of protons
$C\underline{H}_3$-, terminal	unresolved triplet	9.10	3
$-C\underline{H}_2$-, in chain	broad singlet	8.73	22
	a triplet	8.03	1
	b doublet	7.52	2

This spectrum was compared with the 100MHz NMR spectrum of synthetic methyl 17,18-epoxystearate.[77]

B_4 gave 81.6mg (37%) of material which appeared to decompose on GLC to give a very broad peak with an ECL of approx. 12.4. The mass spectrum (MS 1.10 B_4, appendix 1) suggests that B_4 is t-butylperoxytridecane. The NMR spectrum is outlined below:

NMR 1.10 B_4

Assignment	Appearance	τValue	No. of protons
$C\underline{H}_3$, terminal	unresolved triplet	9.11	3
$C\underline{H}_3CH(OOBu^t)$	part of doublet	8.93	1.5
$(C\underline{H}_3)_3COO-$	singlet	8.82	9
$-C\underline{H}_2-$, in chain	broad singlet	8.73	18
$-C\underline{H}_2CH(OOBu^t)$	multiplet	8.10	2

The NMR spectrum of t-butylhydroperoxide gave a singlet at 8.82τ.

1.11 Hydrogen peroxide

Methyl oleate (500mg, 1.57mmole) and mercuric acetate (600mg, 1.88 mmole) in THF (5ml) and aqueous hydrogen peroxide (14.7M, 5ml) gave an orange ppt. which was shaken at room temperature for 30hr. Sodium borohydride reduction afforded a product (460mg) which gave two major peaks on GLC with ECLs 23.4(84%) and 25.8(16%) with a minor peak ECL 16.9. The TMS derivative of the total product had ECLs 16.9(minor), 19.6(18%), 20.4(8%), 20.6(15%), 23.4(51%) and 23.7(9%). Prep TLC(PE35) on part of the product (205mg) gave six major bands: A(Rf 0.12, 21mg,12%) which gave several minor peaks on GLC with low ECLs (mainly 19.0); B(Rf 0.24, 51mg, 30%) with an ECL of 16.9 and many minor low ECL peaks on GLC; C(Rf 0.29, 19mg, 11%) with the same GLC behaviour as B; D(Rf 0.35, 14mg, 8%) with the same GLC behaviour (ECL 25.8 and ECL of TMS ether 19.6) as methyl hydroxystearate; E(Rf 0.65, 49mg, 29%, ECL 23.4) and F(Rf 0.8, 17mg, 10%) with ECLs 14.0, 16.0, 18.0 (present as impurities in the starting material) and 18.5.

Band A was shown to contain mercury (1,5-diphenylcarbazone spray) and was not examined further.

Bands B and C gave products which were examined on TLC(PE70). The TLC plate was sprayed with ammonium thiocyanate in acetone (1.3%) and after drying with aqueous ferrous sulphate solution (4%).[78] Components B and C appeared as brown spots with Rf values 0.49 and 0.60 respectively. The IR spectra of B and C showed absorption in the 3,200cm^{-1} - 3,600cm^{-1} region. Shoulders at 1,710cm^{-1} on the ester carbonyl peak were also observed. The NMR spectrum of component B showed no unusual characteristics. Component C showed a broad multiplet (ca 1 proton) centred at 6.0τ. Elemental analysis of component B gave C-67.49% and H-11.94%. Analysis of component C gave C-66.59% and H-11.43%. The high resolution mass spectrum of component B gave a molecular ion peak at $^{m}/e$ 299.294648. Component C gave a molecular ion peak at 299.294353. Attempts to reduce the components from bands B and C with sodium borohydride in methanol and with stannous chloride in methanolic hydrochloric acid (0.5M) gave very little methyl hydroxystearate (ECL 25.8).

Band E gave a component which had the same TLC, GLC and MS(MS 1.11, appendix 1) behaviour as methyl 9,10-<u>trans</u> - epoxystearate.[77] The NMR spectrum of this product (in carbon tetrachloride) showed a triplet centred at 7.53τ(60MHz), 7.55τ (100MHz) equivalent to two protons, in addition to the normal long-chain methyl ester signals.

A blank reaction mixture with methyl oleate (100mg) in THF (5ml) and aqueous hydrogen peroxide (14.7M, 5ml) was shaken at room temperature. After 3 days, an aliquot (1ml) was removed and extracted with ether and the remainder (4ml) was reduced with sodium borohydride. Both products were shown to be unreacted methyl oleate (TLC and GLC). No evidence of methyl epoxystearate (ECL 23.4) was found.

1.12 Acetonitrile

Methyl oleate (100mg, 0.34mmole) and mercuric nitrate dihydrate (200mg, 0.55mmole) were dissolved in distilled acetonitrile (10ml, b.p. 82°). After two days, the reaction mixture was reduced with sodium borohydride to yield a pale-yellow solid (130mg) which was not eluted on GLC. GLC and TLC indicated that no oleate remained and only 1 spot (Rf 0.4) was obtained on TLC(E). The mass spectrum of this product is reproduced in appendix 1 (MS 1.12). The IR spectrum in carbon disulphide showed major absorption bands at 3,420cm^{-1} (N-H stretching), 1,733cm^{-1} (ester carbonyl stretching), and 1,672cm^{-1} (amide carbonyl stretching). The NMR spectrum in carbon tetrachloride is summarised below:

NMR 1.12 a

Assignment	Appearance	τ value	No. of protons
$C\underline{H}_3$-, terminal	unresolved triplet	9.1	3
-$C\underline{H}_2$-, in chain	broad singlet	8.71	27
$C\underline{H}_3CO.NH$-	singlet	8.1	3
-$C\underline{H}_2CO_2CH_3$	triplet	7.76	3
-$COOC\underline{H}_3$	singlet	6.4	3
>$N\underline{H}$	broad singlet	3.5	~0

Treatment with acid

The product (73mg) was refluxed for 12hr with methanolic sulphuric acid (4.5M, 25ml). The reaction mixture was cooled, diluted carefully with water and neutralised with aqueous sodium hydroxide (3M), then extracted with ether and dried over anhydrous sodium sulphate. The product (66mg) was identical to the starting material (methyl acetylaminostearate) on TLC(E) and IR (no reduction in the intensity of the amide carbonyl absorption).

Treatment with base

The original product (76mg) was refluxed for 10hr with dry methanolic sodium methoxide (4M, 10ml). After cooling and

acidifying with dilute hydrochloric acid, the reaction mixture was neutralised with sodium bicarbonate. A solid product (69mg), obtained by ether extraction (2 x 20ml) and drying over sodium sulphate, showed only one component (Rf 0.21) more polar than the starting material (Rf 0.38) on TLC(E). NMR of this product in deutereochloroform showed the total absence of an ester methyl group (normally at 6.4τ). The spectrum is detailed below:

NMR 1.12 b

Assignment	Appearance	τ value	No. of protons
$C\underline{H}_3$-, terminal	unresolved triplet	9.11	4
$-C\underline{H}_2-$, in chain	broad singlet	8.73	29
$C\underline{H}_3CO.NH-$	singlet	7.95	3
$-C\underline{H}_2OH$	triplet	6.35	2
$>N\underline{H}$	broad singlet	4.5	~ 0
$-O\underline{H}$	broad singlet	5.65	1

The IR spectrum in chloroform showed amide carbonyl ($1,650cm^{-1}$) but no ester carbonyl. There was a broad O-H peak with a sharper N-H peak superimposed on it at $3,400cm^{-1}$. The mass spectrum was consistent with that expected from a mixture of 9- and 10-acetylamino-octadecanols. Major peaks were observed at $^m/_e$ values of 214, 200, 198, 184, 172, 158, 156 and 142. The peak at $^m/_e$ 74 (due to McLafferty rearrangement of an ester [67]) which was the base peak in the mass spectrum of the starting material was very small (1%). The TMS ether was not eluted under normal GLC operating conditions.

A portion of the product (40mg) was methylated (methyl iodide / silver oxide) and examined by NMR in carbon tetrachloride solution. Comparison with NMR 1.12 b showed that the triplet at 6.35τ(2H, $-C\underline{H}_2OH$) and the broad singlet at 5.65τ(1H, $-O\underline{H}$) had disappeared. New peaks were observed at 6.78τ(5H, singlet superimposed on a multiplet, $C\underline{H}_3O\underline{C}H_2-$). Shifts in the $>N\underline{H}$ signal

(4.5τ to 4.2τ) and in the $\underline{CH}_3CONH$- signal (7.95τ to 8.15τ) were attributed to the change in solvent. Minor singlets at 6.12τ(1H) and at 6.41τ(1H) were also observed.

A larger quantity of methyl acetylaminostearates was prepared as before by the reaction of methyl oleate (500mg) and mercury salt (1g) in acetonitrile (20ml) followed by the normal sodium borohydride reduction. The purity of the product (618mg) was checked by IR, TLC and GLC (no oleate eluted).

LAH reduction

The ester (319mg) in dry ether (10ml) was added dropwise to a stirred suspension of LAH (200mg) in dry ether (10ml). The reaction mixture was stirred for 10min at room temperature and then heated under reflux for 10min. Excess reducing agent was destroyed by the careful addition of wet ether. Dilute sulphuric acid (1M, 20ml) was added and the product (290mg) isolated by ether extraction. The TLC behaviour, the IR spectrum and the NMR spectrum (apart from slight shifts in the -O-$\underline{H}$ and $>$N-$\underline{H}$, signals presumably due to different concentrations) were identical to those obtained for the product of the reaction with sodium methoxide.

Samples of the acetylamino-octadecanols obtained from the LAH reduction were:

(a) Refluxed with hydrochloric acid (6M, 10ml) in methanol (10ml) for 4 hours.

(b) Heated at 100° with orthophosphoric acid (90%) for 2 hours.

(c) Heated at 180° with orthophosphoric acid (90%) for 2 hours.

The product in each case was examined by TLC after extraction from aqueous alkaline solution with ether. The product from (a) gave two spots on TLC(E). The lower spot corresponded to the starting material. As the other spot was much less polar (Rf 0.55) it could not have been the required amine. The IR spectrum of the total product appeared to be identical to that of

the starting material. Products from (b) and (c) gave similar but complex patterns on TLC. The major product from (c) (29%) was isolated by prep TLC (PE20, Rf 0.65). The NMR spectrum of this product showed a triplet at 7.7τ, a broad singlet at 8.72τ and an unresolved triplet at 9.1τ with integrals in the ratio of 9:49:21. The IR spectrum showed normal C-H absorption and a strong peak at 1,700cm^{-1}. There was no evidence for N-H or O-H.

As the objective was to obtain a high yield of amine, these products were not examined further. An attempt to reduce the acetylaminostearates to the corresponding secondary amines by refluxing the former in dry THF with excess diborane (prepared by dropping sodium borohydride in diglyme on to boron trifluoride - diethyl etherate in diglyme [141]) for 2 hr was unsuccessful.

1.13 Diethylamine

When hydrogen sulphide, sodium hydrogen sulphide, ammonia or diethylamine were added to a solution of mercuric acetate in DMF, dense precipitates were immediately formed. No precipitate appeared if the amount of diethylamine was limited.

Methyl oleate (100mg, 0.31mmole) in DMF (10ml) with redistilled diethylamine (b.p.55°, 0.5ml) and mercuric acetate (200mg, 0.63mmole) was left at room temperature for 4 days. The product obtained after sodium borohydride reduction was shown to be unchanged methyl oleate (TLC and GLC).

1.14 Piperidine

Methyl oleate (200mg, 0.62mmole) and mercuric chloride (400mg, 1.47mmole) were shaken in piperidine until the reaction mixture was homogeneous (about 2 hr) and then left at room temperature. After 2 days, 5ml of the reaction mixture were withdrawn and reduced in the normal manner. Di-t-butylperoxide (0.2ml) was added to the remaining reaction mixture, which was reduced with borohydride 4 days later.

Both products were shown to be identical to methyl oleate by TLC and GLC.

2. SUBSTITUENT EFFECTS

2.1 Methyl acetoxyoctadecenoates

Methyl acetoxyoctadecenoates (see table) were prepared by refluxing the corresponding hydroxy esters for 10hr with acetic anhydride (5ml). The purity of each product was checked by TLC and GLC (ECL 24.8).

The acetoxy esters were reacted with excess mercuric acetate in dry methanol in reactions A - D (see table overleaf). After 2 days aliquots (2ml) were removed from reaction mixtures **A** and C, and reduced with aqueous sodium borohydride. The bulk of each reaction mixture was reduced after a further 5 days. The 2 - day and 7 - day products were identical on TLC and GLC. Reaction mixtures B and D were reduced after 2 days.

TLC(PE30) of each product showed a major component (Rf 0.35) and a minor component (Rf 0.52) corresponding to the starting material. The major spot from products **A** and B appeared to consist of two parts, the smaller of which was the less polar.

Products A and C(acetoxymethoxy esters)were converted into the corresponding hydroxymethoxy esters (increased polarity on TLC) by methanolysis. Results are tabulated overleaf.

Expt	Acetoxy methyl ester (18:1)	Ester (mg)	Product[1] (mg)	ECLs of product[2,3,4]
A	12-OAc 9c	145	150	24.8(2%), 26.6(20%), 27.2(78%)
B	12-OAc 9t	39	37	24.6(30%), 26.4(10%), 26.9(60%)
C	9-OAc 12c	165	176	24.9(3%), 27.2(20%), 27.4(77%)
D	9-OAc 12t	30	38	24.7(4%), 26.9(32%), 27.1(64%)

Notes:

1. Corrected in A and C for removal of aliquot.
2. Products A and C also contained minor components (ECLs 21.4 and 21.7) which were not identified.
3. The components with ECLs 24.6 - 24.9 correspond to unreacted starting material.
4. Area percentages are very approximate when adjacent peaks have a difference in ECL of less than 0.3.

After methanolysis, A and C gave products A' with ECLs of 27.9(19%) and 28.9(81%) and C' with ECLs of 26.6(3%), 29.0(30%) and 29.2(67%). Authentic methyl 12-hydroxy-10-methoxyoctadecanoate and authentic methyl 12-hydroxy-9-methoxyoctadecanoate had ECLs of 27.9 and 28.9 respectively.

2.2 Methyl methoxyoctadecenoates

Unsaturated methoxy esters were prepared from the corresponding hydroxy esters by Purdie alkylation and purified by prep TLC(PE30) when necessary. The esters were adjudged pure by GLC and Ag^+ TLC.

The methoxy esters were reacted with excess mercuric acetate in dry methanol and reduced after 2 - 3 days with aqueous sodium borohydride. Results are tabulated overleaf.

Expt	Methoxy methyl ester (18:1)	Ester (mg)	Product (mg)	ECLs of product[1,2]
A	12-OMe 9c	60	68	21.6(3%), 23.4(18%), 24.0(79%)
B	12-OMe 9t	38	43	21.6(13%), 23.5(17%), 24.0(70%)
C	9-OMe 12c	60	61	21.6(3%), 23.9(41%), 24.1(56%)
D	9-OMe 12t	56	62	21.6(4%), 23.9(38%), 24.1(58%)

Notes:

1. All the methoxyoctadecenoates had an ECL of 21.6.
2. Co-injection of the products from A and C on GLC showed that the material with ECL 24.0 in A is eluted with the component of ECL 23.9 in C. This indicated that the order of elution of dimethoxystearates was 10,12- (ECL 23.4) before 9,12- (ECL 23.9) before 9,13- (ECL 24.1).

TLC(PE30) of the products showed a major component (Rf 0.56) and a minor component (Rf 0.77) with the same chromatographic behaviour as authentic 9,12-dimethoxystearate and starting material respectively. In addition, products C and D contained a component (Rf 0.62) with the same TLC behaviour as authentic 10,12-dimethoxystearate.

2.3 Methyl octadecenoates

Synthetic cis-2-, cis-4-, cis-5- and cis-6- methyl and trans-2- ethyl octadecenoates and cis-3-, trans-3- and trans-4-octadecenoic acids were available in the laboratory.[88] The acids were esterified with methanolic boron trifluoride (125mg 18:1 3c gave 126mg of ester; 48mg 18:1 3t gave 52mg of ester; 40mg 18:1 4t gave 42mg of ester). Esters were greater than 99% pure (GLC) except for 18:1 2t ethyl ester (impurity: 7% 18:1 2c ethyl ester with ECL 18.5); 18:1 3t methyl ester (impurity: 2% 18:1 3a methyl ester with ECL 20.6); and 18:1 4t methyl ester (impurity: 5% 18:1 4a methyl ester with ECL 20.3). The acetylenic impurities were

discounted from area percentage calculations on the reaction products.

The esters were reacted in dry methanol (5ml) with excess mercuric acetate for 2 - 3 days. Aliquots (1ml) were removed from reaction mixtures A - E (see table) and stirred for 10min with methanolic hydrochloric acid (1M, 5ml). Products were extracted with ether. The bulk reaction mixtures were reduced with sodium borohydride.

Results are tabulated below:

Expt	Ester[1] (18:1)	Ester (mg)	Product[2] (mg)	Ester (ECL)	H^- product[3] (ECL)	H^+ product (ECL)
A	2c	34	36	18.26	19.6(20%), 20.3(78%)	19.57
B	2t	100	111	19.93[5]	19.9(6%), 20.6(94%)	19.93
C	3c	60	63	18.77	20.3(4%), 20.6(96%)	18.79
D	3t	52	59	18.75	20.3(7%), 20.6(90%)	18.76
E	4c	32	38	18.34	20.6(20%), 20.8(80%)	18.34
F	4t	42	47	18.29	20.6(21%), 20.8(79%)	---
G[4]	5c	40	42	18.41	20.8(100%)	---
H	6c	30	29	18.43	20.8(100%)	---

Notes:

1. Starting materials were all methyl esters except B which was an ethyl ester.
2. Corrected in expts. A - E for removal of aliquot.
3. ECLs and area percentages were measured on a capillary GLC column. The borohydride products from A and D contained 2 - 3% of starting material.
4. The borohydride product from expt. G was co-injected on capillary GLC with the methyl 9(10)-methoxystearates obtained in part 1.1. A single peak was observed.
5. The starting material in B (ECL 19.93) contained an impurity (7% 2c ethyl ester with ECL 18.5). The H^+ product (ECL 19.93) was 100% pure.

The hydrochloric acid product from A showed strong absorption in the IR spectrum at 975cm^{-1} and in the UV spectrum at 218nm indicating the presence of a conjugated trans enone. The IR spectra of the acid regenerated products from C and E showed no evidence of trans unsaturation. These cis products separated from the corresponding trans isomers on Ag^+ TLC(PE20).

The borohydride product from A (36mg) was separated by prep TLC(PE30) into two components: A_1 (Rf 0.76, 22.5mg, 76%, ECL 20.3) and A_2 (Rf 0.86, 7.3mg, 24%. ECL 19.6). The IR spectrum of A_2 showed strong absorption at 975cm^{-1}.

The most significant peaks in the mass spectra of A_1 and of the mixtures of isomeric methyl methoxystearates obtained by borohydride reduction in expts. C, E, G and H are outlined below. The corresponding peaks from the mass spectrum of the methyl 9(10)-methoxystearates obtained from methyl oleate (MS 1.1) are included. All spectra had a molecular ion peak at $^m/_e$ 328.
(Nos. in brackets are intensities expressed as percentages of the base peak(s) which are underlined.)

A_1	255(11),	117(92),	85(10)	and	75(100)
C	255(3),	117(4),	85(14)		
	241(16),	131(72),	99(5)	and	74(100)
E	241(3),	131(22),	99(4)		
	227(9),	145(40),	113(15)	and	74(100)
G	227(24),	145(100),	113(42)		
	213(48),	159(100),	127(80)		
H	213(62),	159(100),	127(87)		
	199(83),	173(100),	141(78)		
1.1	171(54),	201(81),	169(11)		
	157(64),	215(81),	183(12)	and	69(100)

The mass spectrum of A_2 had a molecular ion peak (M^+) at $^m/_e$ 296(7) and peaks M-31 at $^m/_e$ 265(15); M-32 at $^m/_e$ 264(24) and a base peak at $^m/_e$ 87(100) as its principle features. The lack of significant peaks at $^m/_e$ values of 269, 103 and 71 indicated the absence of methyl 2-methoxystearate in A_2.

An attempted oxymercuration of methyl octadec-cis-2-enoate (26mg) in methanol(5ml) with mercuric chloride (50mg) for 3 days at room temperature gave no reaction (GLC, TLC and IR of sodium borohydride product).

2.4 Methyl hendec-10-enoate

Hendec-10-enoic acid (2g) was esterified with boron trifluoride - methanol. The ester (2g) was shown to be >99% pure by GLC (120°).

A sample of the methyl ester (125mg,0.63mmole) and mercuric acetate (210mg, 0.66mmole) in methanol (10ml) were left at room temperature for 1 day. Sodium borohydride reduction of 5ml of the reaction mixture afforded a product (77mg) which gave 2 peaks on GLC with ECLs 12.0 (14%) and 15.94 (86%). (The remainder of the reaction mixture reduced after a further 2 days yielded an identical product.) A portion of the product (55mg) was separated into two bands with Rf values of 0.72 (minor) and 0.56. The minor band gave 4.5mg(9%) of material (ECL 12.0) which was identical to the starting material on GLC and TLC. The major product (43mg, 91%, ECL 15.9) was examined by MS (2.4, appendix 1) and NMR (overleaf).

NMR 2.4

Assignment	Appearance	τ value	No. of protons
$C\underline{H}_3CH(OCH_3)$-	doublet	8.95	3
$-C\underline{H}_2-$, in chain	broad singlet	8.7	14
$-C\underline{H}_2CO_2CH_3$	triplet	7.77	2
$C\underline{H}_3OC\underline{H}<$	singlet + multiplet	6.78	4
$-CO_2C\underline{H}_3$	singlet	6.40	3

2.5 Methyl linoleate

Preliminary oxymercuration experiments on methyl linoleate in methanol, in which the mercurated mono- and di- adducts were isolated and separated by prep TLC or by column chromatography before sodium borohydride reduction, were partially successful. These were superseded by later experiments utilising the *in situ* borohydride reduction technique.[60] Addition of aqueous sodium hydroxide (3M) immediately before borohydride reduction was found to be unnecessary.

Methyl linoleate (100mg, 0.34mmole) containing methyl oleate (2%) and methyl linolenate(2%) as the only impurities was dissolved in dry methanol (10ml) with mercuric acetate (300mg). Aliquots (0.1ml) were removed 30min, 1hr and 2hr after the addition of the mercuric salt. TLC(PE30) indicated that all starting material had disappeared after 30min. Larger aliquots (1ml) were removed after various times (see table) and reduced with sodium borohydride. TLC (PE30) of all the aliquot reduction products showed a major component which was chromatographically identical to methyl 9,12-dimethoxystearate Minor components which had the same TLC behaviour as methyl linoleate and methyl 12-methoxyoleate were also observed. GLC results are tabulated overleaf.

ECLs[1]	1hr[3]	2hr	4hr	6hr	50hr	100hr	X (see later)
19.4	5	2	3	3	2	5	-
21.2[2]	2	3	3	3	4	3	3
21.7	23	23	20	19	16	25	7
23.6	6	7	6	6	9	6	11
24.2	64	64	68	69	69	61	79

Notes:

1. Under identical GLC conditions, methyl linoleate had an ECL of 19.4, methyl 12-methoxyoctadec-cis-9-enoate an ECL of 21.6, methyl 10,12-dimethoxystearate an ECL of 23.8, and methyl 9,12-dimethoxystearate an ECL of 24.2.
2. The product with ECL 21.2 was probably mono-methoxy stearate produced from the 2% methyl oleate present as an impurity in in the linoleate.
3. Reduction by aqueous sodium borohydride containing sodium hydroxide (20mg, 0.5mmole) of another aliquot (1ml) removed after 1hr gave exactly the same result.

At a later date, the methoxymercuration of methyl linoleate (62mg, 0.21mmole) in dry methanol (5mg) with a fresh sample of mercuric acetate (240mg, 0.75mmole) was allowed to proceed for 3 days. The reaction mixture was reduced with fresh sodium borohydride and the product examined by GLC (see X in previous table). Prep TLC(PE30) of the product (53mg) allowed the isolation of the component (4.4mg, 8.3%) with the same TLC behaviour as methyl 12-methoxystearate. This mixed product (ECLs 21.2, 26% and 21.6, 74%) gave only one peak (ECL 21.2) after hydrogenation (Pd/C) in methanol. The significance of this result is discussed in section 3.7.

2.6 Methyl octadec-cis-8, cis-12-dienoate

The synthetic 18:2 8c, 12c methyl ester (ECL 19.2, 100%) was available in the laboratory. The ester (120mg, 0.41mmole) and mercuric acetate (360mg, 1.13mmole) were left at room temperature for 5 days in dry methanol (10ml). An aliquot (2ml) was stirred with methanolic hydrochloric acid (1M, 10ml) for 30min. As the product (ECL 19.2, 100%) showed no absorption in the 950 - 1,000cm^{-1} region of the IR spectrum and had the same TLC behaviour as the starting material, it was not examined further.

The bulk of the oxymercuration reaction mixture was reduced with sodium borohydride to yield a product (120mg) with ECLs of 21.0 (2%), 21.5 (3%) and 24.0 (95%) on GLC. The broad shape of the peak with ECL 24.0 suggested that more than one component was present. This product, chromatographically similar to methyl 9,12-dimethoxystearate, was isolated by prep TLC(PE20, Rf 0.5). The mass spectrum (MS 2.6) is reproduced in appendix 1.

2.7 Conjugated octadecadienoates

Dehydrated castor oil (2g) was transesterified with methanolic sodium methoxide to give methyl esters (1.98g). Prep TLC(PE20) of these esters (390mg) gave a major component (276mg) with the same Rf value as methyl stearate. GLC of this material (A) showed ca 43% of conjugated octadecadienoates (see table overleaf).

A better starting material (B) was obtained by heating the methanesulphonate ester from methyl ricinoleate with 1,5-diazobicyclo(5.4.0)hendec-5-ene according to the method of Gunstone and Said.[92]

The methyl esters were examined by GLC (see table overleaf). These esters (37mg, 0.13mmole) were left with mercuric acetate (150mg, 0.47mmole) in dry methanol (5ml) for 3 days. A portion of the reaction mixture (1ml) was stirred with methanolic hydrochloric acid (0.5M, 5ml) for 10min. The product (C, 7.3mg) gave a single spot on TLC(PE30, Rf 0.83) with the same polarity as the starting material. GLC results are tabulated overleaf.

ECL	Assignment[1] (18:2)	Area % A[2]	B	C
19.3	9c, 12c	49	7	7
20.3	9c, 11t	27	73	60
20.6	9c, 11c	9	17	13
20.9	9t, 11t	7	3	20

Notes:

1. Assignments were made by comparison with authentic diene esters available in the laboratory.
2. Minor peaks with ECLs 14.0 (1%), 16.0 (1%), 18.0 (1%) and 18.5 (4%) were also observed.

The remaining oxymercuration reaction mixture was reduced with sodium borohydride. The product (33.8mg) had ECLs of 21.2 (30%), 23.3 (62%) and 23.9 (8%) on GLC. Prep TLC(PE15, double development) on part of the product (23mg) gave D (Rf 0.74, 6mg, 32%) and two overlapping bands (Rf 0.52) which were scraped off the plate together and extracted to give E (13mg, 68%). D (ECL 21.3, 100%) showed strong absorption in the IR spectrum at 950cm^{-1}, 970cm^{-1} and 1,050cm^{-1} but no significant absorption at 3,070cm^{-1}, implying the absence of a cyclopropane ring. E had ECLs of 23.3 (85%) and 23.9 (15%) corresponding to authentic methyl 10,12- and 9,12-dimethoxystearate respectively.

2.8 Methyl ricinoleate (water + THF as solvent)

Pure methyl ricinoleate (105mg, 0.34mmole) was shaken with mercuric acetate (200mg, 0.63mmole) in water (5ml) and THF (5ml) for 2 days and then reduced with sodium borohydride to give a product (119mg) which was examined on GLC as TMS ethers:

ECL	Area %	Assignment*
19.7	4	starting material
20.4	11	dihydroxy stearates
20.7	63	
21.1	18	9,12-epoxystearates
21.4	4	

* By comparison with ECLs of authentic compounds.

Prep TLC(PE30) on the product (100mg) gave 3 bands: A (Rf 0.04, 72mg, 79%) which had ECLs (run as TMS ethers) of 20.4 (12%) and 20.7 (88%); B (Rf 0.21, 3.5mg, 4%) which had the same TLC and GLC behaviour as methyl ricinoleate and C (Rf 0.53, 16.1mg, 17%) which had ECLs of 21.1 (94%) and 21.4 (6%) and was shown to be methyl trans- and cis-9,12-epoxystearates by chromatography and mass spectrometry.

Cyclodehydration of fraction A

TLC(PE70) of fraction A showed a large spot (Rf 0.23) and a smaller double spot (Rf 0.39). These methyl dihydroxystearates (50mg) were refluxed with methanol (9ml) and conc. sulphuric acid (1ml) for 6hr. (Ether extracts were washed with 5% aqueous sodium bicarbonate to remove traces of acid.) The product (45.3mg) gave a large spot at the solvent front on TLC(PE70), the double spot (Rf 0.39) remained and there was no trace of the spot with Rf 0.23. GLC results are shown overleaf.

ECL	Product	Product (as TMS ethers)
21.1	78%	66%
21.4	22%	18%
20.4	—	16%

Part of the dehydrated product (42mg) was separated by prep TLC(PE30) into bands A_1 (Rf 0.05, 6mg, 16%) and A_2 (Rf 0.57, 32mg, 84%). Component A_2 was chromatographically and spectroscopically identical to methyl 9,12-epoxystearates. Component A_1 was methylated to give methyl dimethoxystearates which were examined by GLC (ECLs * 23.3, 78% and 23.9, 22%). The mass spectrum showed that there were methoxyl groups on carbon atoms 9, 10 and 12.

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* Under the same GLC conditions, methyl 10,12- and 9,12-dimethoxystearates had ECLs of 23.3 and 23.9 respectively.

3. NEIGHBOURING GROUP PARTICIPATION

Sections 2.5 - 2.8 are partly relevant to this chapter.

3.1 Hydroxy monoene esters

(a) Methanol as solvent

Early investigations on these esters involving the isolation of the mercury adducts and the addition of base before borohydride reduction were superseded by the following investigations (A -D, see table).

The hydroxy esters (65 - 125mg) in methanol (10ml) were left at room temperature with a 50% molar excess of mercuric acetate. After 2hr, TLC showed that no starting material remained. Reduction of an aliquot (1ml) at this stage with sodium borohydride gave a product which was shown by GLC and TLC to contain a large amount of starting material. After 2 days aliquots (2ml) were drawn from each reaction mixture and reduced with sodium borohydride. The products (quantitative) were examined by TLC and GLC (see table):

expt	A	B	C	D	
methyl	9c	9t	12c	12t	
ester (18:1)	12-OH	12-OH	9-OH	9-OH	
ECL	Area % [1]				TLC Rf value [2]
20.0	-	-	-	8%	0.59
21.3	8%	20%	33%	35%	0.53
21.6	2%	79%	67%	57%	
26.2	6%	1%	-	-	0.29
28.0	18%	-	-	-	0.23
29.0	66%	-	-	-	0.15

Notes:

1. Area percentages were measured on a capillary DEGS GLC column.
2. The relative sizes of spots on analytical TLC(PE30) of the total products were in accordance with the relative area percentages of components by GLC.

The component with ECL 20.0, Rf 0.59 (D_1, 1,5-epoxide) was separated from the components with ECLs 21.3 and 21.6, Rf 0.53 (D_2, 1,4-epoxide) in expt D by prep TLC(PE30). The mass spectra (MS 3.1 D_1 and MS 3.1 D_2 respectively) are reproduced in appendix 1.

Reaction product A was separated by prep TLC(PE30) into components A_1 (Rf 0.15), A_2 (Rf 0.23), A_3 (Rf 0.29) and A_4 (Rf 0.53). A_1 and A_2 were methylated (methyl iodide / silver oxide) to give A_1'(ECL 23.9, MS 3.1 A_1'appendix 1) and A_2'(ECL 23.3, MS A_2' appendix 1) which had the same chromatographic behaviour as methyl 9,12- and 10,12-dimethoxystearate respectively. Component A_3 was identical to methyl ricinoleate (TLC, GLC and GLC of TMS ether). Component A_4 was identical to product D_2 above apart from the relative

amounts of the two isomeric constituents (GLC).

The NMR spectrum of reaction product B showed no unusual features apart from an irregular broad multiplet (ca 2H) under the ester methyl signal at 6.4τ.

The bulk reaction mixtures D and B were kept at 0° and reduced with sodium borohydride after one week and one year respectively. D gave exactly the same product as was obtained after 2 days. The only difference in the one-year product from B was a slight increase (ca 5%) in the area of the first peak (ECL 21.3) with a corresponding decrease in the area of the second peak (ECL 21.6) as compared with the 2-day reduction product on GLC.

Acid regeneration

Aliquots (1ml) from reactions A and C were removed after 2 days and stirred with methanolic hydrochloric acid (0.5M, 10ml) for 10min. The products were identical to the starting materials (GLC, TLC and IR).

(b) Water + THF as solvents

The reaction between methyl ricinoleate and mercuric acetate in water and THF is described in section 2.8.

(c) THF as solvent

Pure methyl ricinoleate (70mg, 0.22mmole) and mercuric acetate (140mg, 0.44mmole) in dry THF (5ml) were shaken gently. TLC(PE50) on aliquots taken after 1, 2, 4 and 7 days showed very little evidence for the formation of a mercury adduct. An aliquot (1ml) was reduced with sodium borohydride after 8 days to give a product (14mg) with ECLs 21.3 (7%), 21.6 (2%) and 26.1 (91%). The major component corresponded to starting material and the

minor components to the isomeric 1,4-epoxides obtained previously (GLC and TLC).

(d) DMF as solvent

Pure methyl 9-hydroxyoctadec-cis-12-enoate (22mg, 0.07mmole) and mercuric acetate (50mg, 0.16mmole) in dry DMF (1ml) were left to react at room temperature (4 days). Sodium borohydride reduction gave a product (21mg) with ECLs 21.3 (28%), 21.6 (61%) and 26.1 (11%) on GLC.

3.2 Methyl densipolate (18:2 9c,15c 12-hydroxy)

(a) Methanol as solvent

Methyl densipolate (20mg, 0.06mmole) and mercuric acetate (50mg, 0.16mmole) in methanol (2ml) were left at room temperature for 2 days. Sodium borohydride reduction yielded a product (23.2mg) which gave a double spot (Rf 0.48), less polar than the starting material (Rf 0.36) on TLC(PE30). GLC of the product gave 4 peaks with ECLs 23.7 (2%), 23.9 (14%), 24.3 (20%) and 24.6 (62%). No change in the GLC trace was observed after trimethylsilylation. The mass spectrum (MS 3.2a) is recorded in appendix 1.

(b) DMF as solvent

Methyl densipolate (18mg, 0.05mmole) and mercuric acetate (30mg, 0.09mmole) in dry DMF (10ml) were left at room temperature. Sodium borohydride reduction after 2 days gave a product (20mg) with ECLs 21.9 (28%), 22.1 (58%) and 26.1 (14%). Prep TLC(PE 30) on 15mg of this product gave band A (Rf 0.32, 2mg, 14%) with ECL 26.1 and ECL of TMS ether 20.8 and band B (Rf 0.57, 12mg, 86%) with ECLs 21.9 (33%) and 22.1 (67%).

Component A had the same TLC and GLC behaviour as methyl densipolate (ECL 26.1, ECL of TMS ether 20.8). The mass spectra of the two components in B were measured separately by GLC - MS

and shown to be almost identical (see MS 3.2 "21.9" and MS 3.2 "22.1" in appendix 1). A portion of B (2mg) was subjected to von Rudloff oxidation. GLC of the products (170°) gave a double peak with ECLs (monobasic) 12.3 (12%) and 12.6 (22%) and a peak with ECL 9.0 (56%) on a dibasic ester scale. Traces of C_6 and C_8 dibasic esters were also observed.

3.3 Methyl threo-12,13-dihydroxyoleate (methanol as solvent)

Methyl threo-12,13-dihydroxyoleate (100mg, 0.30mmole), adjudged pure by TLC(PE50) and GLC of the TMS ether, was allowed to react with mercuric acetate (150mg, 0.47mmole) for 2 days in dry methanol (10ml). Sodium borohydride reduction yielded a product (98mg) which gave a complex spot (Rf 0.29) on TLC(PE65), intermediate in polarity between starting material and monohydroxy ester. Part of the product was methylated and gave a major spot (Rf 0.93) with traces of slightly more polar material on TLC(PE65). The mass spectrum of the methylated product (MS 3.3) is included in appendix 1. GLC results on the product, the methylated product and the trimethylsilylated product are tabulated below:

Product		Methyl ethers		TMS ethers	
ECL	Area %	ECL	Area %	ECL	Area %
24.2	1	23.4	6	20.1	5
29.3	10	24.3	11	22.5	13
29.9	89	24.8	81	22.9	82
		26.5	2		

Samples of starting material were methylated (ECL 23.4) and converted to the bis-TMS ether (ECL 20.1).

3.4 Octadecenols

1. Octadec-cis-2-enol (methanol as solvent)

Preparation:

Early attempts to reduce methyl octadec-cis-2-enoate to the allylic alcohol with excess and with controlled amounts of LAH gave products which contained a high percentage of saturated alcohol (ECL 19.7).

A more successful reduction was carried out by stirring the ester (116mg, 0.39mmole) in dry ether (5ml) for 2hr with 2ml of a stock solution consisting of LAH (75mg, 1.97mmole) and dry methanol (62.5mg, 1.95mmole) in dry ether (5ml). The reduced product (108mg) showed two spots on Ag^+ TLC(PE40) with Rf values of 0.64 (minor) and 0.36 (major). GLC gave peaks with ECLs 14.6 (trace), 17.7 (trace), 19.5 (20%) and 20.3 (80%). Octadecanol had an Rf value of 0.64 and an ECL of 19.5 under the same chromatographic conditions. The IR spectrum of the reduction product showed strong absorption at 3,590cm^{-1}, 1,365cm^{-1} and 1,115cm^{-1}. No absorption was observed between 950 and 1,000cm^{-1}.

Oxymercuration:

Octadec-cis-2-enol (41mg, 0.15mmole) with 20% octadecanol (ECL 19.5) as impurity was left for 3 days with mercuric acetate (50mg, 0.16mmole) in methanol (5ml). An aliquot (1ml) was treated with methanolic hydrochloric acid (1M, 5ml) and the remainder (4ml) reduced with sodium borohydride.

The hydrochloric acid product (8mg) was examined by GLC (see table) and separated by prep TLC(PE30) into bands A (Rf 0.30, 2.9mg, 41%) with ECLs 19.5 (49%) and 20.3 (51%) and B (Rf 0.83, 4.2mg, 59%) with ECL 14.1. Part of B (2mg) was hydrogenated to give BH (ECL 13.8, MS 3.4-1BH in appendix 1). The IR spectrum of A showed a very slight irregularity at 970cm^{-1}.

The borohydride product (44mg) was examined by GLC (see

table) and TLC(PE30, Rf 0.18 major, Rf 0.29 minor). A portion of this product (ca 5mg) was methylated and re-examined by GLC. The major component from the methylated product (ECL 17.7) was isolated by prep TLC(PE20) and submitted for MS measurement (MS 3.4-1H⁻/ MeI in appendix 1).

GLC results:

		borohydride product	
starting material ECL(area%)[1]	hydrochloric acid product ECL(area%)[1]	before methylation ECL(area%)[1]	after methylation ECL(area%)
14.6(tr)	14.1(71)	14.1(2)	14.1(2)
17.7(tr)	17.7(2)	15.9(3)	14.9(13)[2]
19.5(20)[2]	18.4(1)	19.5(18)[2]	15.8(3)
20.3(80)	19.5(13)[2]	20.3(2)	17.7(82)
	20.3(13)	21.8(9)	
		22.0(66)	

Notes:

1. Due to the range of ECLs, area percentages are approximate.
2. Octadecanol and its methyl ether had ECLs 19.5 and 14.9 respectively.

2. Octadec-trans-2-enol (methanol as solvent)

Preparation:

Ethyl octadec-trans-2-enoate (369mg, 1.19mmole, ECL 19.93) in dry ether (2ml) was stirred for 2hr with the ethereal LAH - methanol stock solution (3ml, 3mmole). The product (360mg) gave 3 peaks on GLC with ECLs 19.6 (25%), 19.9 (50%) and 20.4 (25%). Prep TLC(PE20) on this product (180mg) gave three poorly separated bands, the most polar of which yielded material (40mg) with ECLs

17.6 (1%), 19.6 (6%), 19.9 (5%) and 20.4 (88%) and an IR spectrum which showed strong absorption at 3,595cm^{-1}, 1,118cm^{-1} and 969cm^{-1}.

Oxymercuration:

Octadec-trans-2-enol (25mg, 0.09mmole, 88% pure) and mercuric acetate (50mg, 0.16mmole) were dissolved in dry methanol (6ml). After 4 days, 2ml were withdrawn and stirred with methanolic hydrochloric acid (1M, 10ml). The remaining 4ml were reduced with borohydride.

The acid - treated product (9.5mg) was examined by GLC (see table below) and TLC(PE30) which gave two spots with Rf values of 0.30 and 0.83. The less polar component (Rf 0.83) was isolated by prep TLC(PE30) and its mass spectrum was shown to be identical to MS 3.4 - 2H$^-$ (see below).

The borohydride - reduced product was examined by GLC (see table) and TLC(PE30) which showed two major spots with Rf values of 0.18 and 0.83. The less polar component (Rf 0.83, ECL 14.1) was isolated by prep TLC(PE20) and submitted for MS measurement (MS 3.4-2H$^-$, appendix 1).

GLC results:

starting material ECL(area%)	hydrochloric acid product ECL(area%)*	borohydride product ECL(area%)*
17.6(1)	14.0(73)	14.1(36)
19.6(6)	18.4(4)	16.5(6)
19.9(5)	18.7(3)	17.6(1)
20.4(88)	20.4(20)	18.4(4)
		19.6(5)
		20.4(4)
		20.7(6)
		21.9(7)
		22.2(31)

* Due to the range of ECLs, area percentages are approximate.

3. Octadec-*cis*-3 to 6-enols, octadec-*trans*-3 to 5-enols (methanol as solvent)

Synthetic octadec-*cis*-3 to 6-enols were available in the laboratory. The *trans*-3- ester (99mg) was reduced with LAH in dry ether to octadec-*trans*-3-enol (98mg). Synthetic *trans*-4- and *trans*-5- acids (100mg and 206mg) were reduced to the corresponding alcohols (95mg and 200mg). Alcohols were checked for purity by GLC and TLC before use.

The alcohols were allowed to react with mercuric acetate in dry methanol (5 - 10ml) for 2 days. Aliquots (1ml) were drawn from A and B (see table) and stirred with methanolic hydrochloric acid (0.5M, 10ml) to give products which were shown to be identical (GLC, TLC and IR) to the corresponding starting materials. The remainder of reaction mixture A and reaction mixture B and all other reaction mixtures were reduced with sodium borohydride. Results are tabulated overleaf.

expt	alcohols			products		TLC
	18:1	ECL	wt(mg)	wt(mg)[1]	ECL(area%)	Rf(PE20)[2]
A	3c	20.3	69	68	15.8(7),16.9(2) 22.5(18),22.9(72)	0.13[3](0.75)
B	3t	19.9	50	52	15.7(100)	0.75
C	4c	20.4	14	16	15.8(92),20.4(8)	0.75(0.35)
D	4t	20.2	70	65	15.2(12),15.8(88)	0.75(0.86)
E	5c	20.4	21	22	15.1(100)	0.86
F	5t	20.3	55	52	15.1(100)	0.86
G	6c	20.3	24	26	21.2(5),22.7(95)	0.11

Notes:

1. Corrected in A and B for removal of aliquot.
2. Rf of minor components shown in brackets.
3. Appears as double spot.

Products A and G were methylated and examined by GLC and MS (see MS 3.4-3A and MS 3.4-3G in appendix 1). GLC of the methylated products showed a major peak with ECL 17.8 in each case.

Mass spectra of products B and E (MS 3.4-3B and MS 3.4-3E) are reproduced in appendix 1. The mass spectrum of product C was very similar to that of product B (peak with $^{m}/e$ 71 far greater than any other peaks in the spectrum). In addition to the very intense peak at $^{m}/e$ 71 the spectrum of product D had a smaller but appreciable peak at $^{m}/e$ 85. Product F had a very similar mass spectrum to that of product E (very intense peak at $^{m}/e$ 85). In addition, the mass spectra of products B - F showed a molecular ion peak at $^{m}/e$ 268 and a large peak at $^{m}/e$ 250.

4. Octadec-cis-6-enol (DMF as solvent)

Octadec-cis-6-enol (20mg, 0.074mmole) and mercuric acetate (50mg, 0.157mmole) in dry DMF (5ml) were left at room temperature for 2 days before reduction with sodium borohydride. The product (20mg) had exactly the same GLC and TLC behaviour as the starting material.

5. Octadec-trans-4-enol (DMF as solvent)

Octadec-trans-4-enol (10mg, 0.037mmole) and mercuric acetate (30mg, 0.094mmole) in DMF (5ml) were left at room temperature for 2 days and then reduced with sodium borohydride. The product gave two major peaks on GLC with ECLs 15.2 (8%) and 15.8 (90%). Minor peaks (ECLs 20.0, 21.3) were also observed. The product had the same TLC behaviour as the product obtained by the oxymercuration - reduction of octadec-trans-4-enol in methanol (section 3.4-3).

6. Arachidonyl alcohol (20:4 5c,8c,11c,14c)

Ethyl arachidonate supplied by Hofman le Roche was shown to be 96% pure by GLC (ECL 22.4 and 21.8, 4%). The ester (60mg, 0.19mmole) was reduced to the alcohol (56mg, ECL 24.1 and 3% impurity ECL 23.5) with LAH in dry ether.

The alcohol (49mg, 0.15mmole) and mercuric acetate (200mg, 0.63mmole) in DMF (5ml) were left at room temperature for 18hr. An aliquot (1ml) was removed and reduced with sodium borohydride to give a product (8.5mg, ECLs 18.6, 97% and 17.1, 3%). Hydrogenation of this product gave a material which showed a single peak (ECL 17.0) on GLC.

3.5 Octadecenoic acids (methanol as solvent)

Synthetic trans-4- and trans-5-octadecenoic acids were available in the laboratory. Their purity was checked on GLC after esterification (boron trifluoride - methanol) of a small sample of each acid. Oleic acid was obtained by alkaline hydrolysis of methyl oleate. Small amounts of saturated acids present in the oleic acid (checked as esters on GLC) are ignored in area percentage calculations.

1. Octadec-cis-9-enoic acid (oleic)

Oleic acid (100mg, 0.35mmole) and mercuric acetate (150mg, 0.47mmole) in methanol (10ml) gave a milky solution which cleared with gentle warming ($<40^{\circ}$) and shaking. After 36hr, sodium borohydride reduction gave a product (104mg) which was converted to methyl esters (103mg, boron trifluoride - methanol). These esters had ECLs of 18.4 (13%) and 21.0 (87%) corresponding to methyl oleate and methyl methoxystearate respectively. TLC (PE30) supported this assignment.

2. Octadec-trans-4-enoic acid

The acid (50mg, 0.18mmole) in methanol (15ml) with mercuric acetate (100mg, 0.31mmole) gave a dense white precipitate which disappeared on shaking for 2 - 3 minutes. After 4 days, sodium borohydride reduction afforded a product (42mg) which was esterified (boron trifluoride - methanol) and shown to have the same behaviour on GLC and TLC as the starting material. The NMR spectrum of the non-esterified product was identical to the NMR spectrum of octadec-trans-4-enoic acid (triplet at 4.6τ, 2H, $\underset{t}{\underline{CH}} = \underline{CH}$).

3. Octadec-trans-5-enoic acid

Samples of the acid (50mg, 0.18mmole) and mercuric acetate (100mg, 0.31mmole) were added to methanol (5ml, experiment A) and to DMF (5ml, experiment B). Reaction mixture A formed an

immediate white precipitate which disappeared after shaking for a number of hours. Reaction mixture B also produced a dense white precipitate which persisted even after shaking for 36hr. Sodium borohydride reductions were carried out on reaction mixtures A and B after 36hr. The reduced products (34mg and 39mg respectively) were esterified and examined by TLC(PE30) and GLC.

The esters from A (33mg) showed 3 spots on TLC and had ECLs 18.5 (14%), 20.9 (64%) and 26.1 (22%). The TLC Rf values and the ECLs corresponded to those of methyl oleate, methyl methoxystearate and methyl hydroxystearate respectively. The TMS ethers of product A with ECLs 18.5 (13%), 20.0 (24%), 20.9 (61%) and 21.7 (3%), were submitted for GLC - MS measurement. The mass spectra of components with ECLs 20.0 (MS 3.5a) and 20.9 (MS 3.5b) are recorded in appendix 1.

The product from reaction B (40mg) had the same chromatographic behaviour as esterified starting material.

3.6 Methyl vernolate (18:1 9c 12,13-cis-epoxy)

Methyl vernolate (85mg, 0.27mmole) and mercuric acetate (150mg, 0.47mmole) in methanol (10ml) were left at room temperature for 4 days. Sodium borohydride reduction furnished a product (90mg) which was examined by GLC (see table) and separated by prep TLC(PE20, double development) into bands A (Rf 0.46, 56mg, 78%) and B (Rf 0.57, 16mg, 22%). GLC results are tabulated overleaf.

ECLs*	total product Area %	band B Area %	band A Area %
24.3	5	11	-
24.7	21	89	-
26.2	5	-	6
26.5	8	-	12
26.8	4	-	-
27.1	3	-	4
27.6	54	-	78

* Methyl vernolate had an ECL of 24.2 and contained an unidentified impurity with ECL 25.1 (8%).

The mass spectra of bands A and B (MS 3.6A and MS 3.6B) are reproduced in appendix 1.

3.7 Methyl octadecadienoates

1. Methyl linoleate (DMF as solvent)

Methyl linoleate (60mg, 0.2mmole) and mercuric acetate (240mg, 0.75mmole) in dry DMF (5ml) were left at room temperature for 3 days before reduction with sodium borohydride. The product (61mg) had ECLs 19.2 (81%) and 24.4 (19%). Prep TLC(PE 20) on part of this product (42mg) gave a major band with the same Rf value as methyl linoleate (ECL 19.2) and a more polar minor band (8.9mg, 21%, ECL 24.4). This latter component was chromatographically identical to methyl acetoxystearate (TLC and GLC, ECL 24.1) after hydrogenation. The mass spectrum (MS 3.7 - 1, appendix 1) showed that this hydrogenated product was a mixture of methyl 9-, 10-, 12- and 13-acetoxystearates.

2. Methyl linoleate (water + DMF as solvent)

Methyl linoleate (96mg, 0.32mmole) and mercuric acetate (210mg, 0.66mmole) in water (5ml) and DMF (10ml) produced a yellow precipitate. The reaction mixture was shaken at room temperature (36hr) and reduced with sodium borohydride to give a product (103mg), 100mg of which was separated by prep TLC (PE 30) into bands A (Rf 0.56, 45.5mg, 48%) and B (Rf 0.09, 48.5mg, 52%).

Component A had ECLs 19.2 (trace), 21.0 (46%) and 21.3 (54%) and was identical to authentic methyl 9,12-epoxystearate (cis and trans) on GLC and TLC. (Changes in ECLs of 0.3 from previous values for 1,4-epoxides were due to deterioration of the GLC column with age.) The mass spectrum (MS 3.7-2A, appendix 1) was recorded.

TMS ethers of component B had ECLs 19.9 (5%), 20.1 (13%), 21.5 (17%) and 21.8 (64%). The mass spectrum of these TMS ethers (MS 3.7-2B) confirmed that they were mainly bis-trimethylsilyloxystearates.

The oxymercuration procedure was repeated with methyl linoleate (94mg, 0.32mmole) and mercuric acetate (220mg, 0.69mmole) in the same solvent system. The total borohydride reduction product (92mg) was refluxed for 6hr in methanolic sulphuric acid (0.5M, 20ml). Prep TLC(PE30) was used to isolate the 1,4-epoxides (52mg, 70%) from part of the product (74mg).

A large scale preparation of methyl 9,12- + 10,13-epoxystearates (cis and trans) was effected by vigorously stirring methyl linoleate (21.1g, 0.07mole, containing about 8% of methyl oleate) with mercuric acetate (50g, 0.16mole) in water (100ml) and DMF (750ml) under a nitrogen atmosphere for 3 days. Sodium borohydride (10g, 0.26mole) in water (500ml) was added dropwise over 10min to the stirred reaction mixture at 0° (ice-bath). After stirring at room temperature (1hr), the product (23.0g) was extracted with petrol (2 x 300ml). Recrystallisation of the

methyl dihydroxystearates from petrol (150ml, -10°) left a mother liquor which contained the required 1,4-epoxides, which were only 70 - 80% pure by GLC of the trimethylsilylated mother liquor. As a purification step was still necessary, the fractions were recombined and separated on a Sorbsil column (75cm x 3cm) eluting with P (6 x 200ml), PE5 (4 x 200ml), PE7.5 (3 x 200ml then 3 x 100ml) and PE10 (9 x 200ml then 2 x 400ml). The eluant was monitored by TLC(PE40). Non-polar material (saturates and unreacted linoleate) was obtained in fractions 11 - 15. Fractions 17 - 24, containing pure 1,4-epoxides (TLC and GLC), were combined to give 8.0g of material. Fractions 24 - 27 (0.8g) contained impure 1,4-epoxides. Methyl dihydroxystearates (12.2g) were washed off the column with methanol (21).

3. Methyl octadeca-cis-8,cis-12-dienoate (water + DMF as solvent)

Synthetic 8c,12c-dienoate [88] (ECL 19.2, 120mg, 0.41mmole) and mercuric acetate (260mg, 0.82mmole) in water (1ml) and DMF (9ml) were shaken for 3 days and reduced with sodium borohydride to yield 126mg of product. Prep TLC(PE20) on part of this product (95mg) gave bands A (Rf 0.34, 12.5mg, 14%), B (two overlapping bands Rf 0.69, 28mg, 32%), C (Rf 0.83, 31mg, 36%) and D (Rf 0.88, 16mg, 18%).

Component A (as TMS ethers) gave several peaks on GLC the largest of which had ECLs 20.2, 21.8 and 22.0. GLC of the other components are tabulated overleaf.

ECL	Assignment[1]	Area %			
		Total product	B	C[2]	D
19.2	18:2	24	-	5	100
19.7	1,5-epoxide	42	-	95	-
21.0	1,4-epoxide	5	18	-	-
21.3		18	55	-	-
24.5	18:1 acetoxy	11	27	-	-

Note:

1. Assignments were made by comparison with authentic materials on TLC and GLC.
2. The mass spectrum of component C (MS 3.7-3C) is recorded in appendix 1.

3.8 Long chain keto-esters

Methyl 12-oxostearate (20mg, 0.06mmole) and mercuric acetate (50mg, 0.16mmole) in methanol (3ml) were left at room temperature. After 2 days an aliquot was removed and examined by TLC. There was no evidence of mercury-containing material. Sodium borohydride reduction of the reaction mixture afforded a product which had the same GLC and TLC behaviour as methyl hydroxystearate.

Several oxymercuration-reduction procedures were effected in methanol with varying amounts of mercuric acetate on 18:1 9c 12-oxo and 18:1 12c 9-oxo esters prepared by chromium trioxide oxidation of the corresponding hydroxy esters. Despite extensive attempts to identify the many reaction products using prep TLC, prep GLC and MS techniques, the investigation was abandoned due to the complexity of the products.

A later attempt to oxymercurate 18:1 12c 9-oxo methyl ester in DMF was also abandoned for the same reason.

4. SOME APPLICATIONS OF OXYMERCURATION REACTIONS ACCOMPANIED BY CYCLISATION

4.1a Seed oil esters (methanol as solvent)

Carlina corymbosa esters [111] (15.8mg) were reduced with LAH in dry ether to alcohols which were examined on GLC (see table below) and then reacted with mercuric acetate in methanol (normal procedure). After 2 days at room temperature the oxymercuration reaction was reduced with sodium borohydride to yield a product (23mg) which was examined by TLC and GLC. TLC(PE20) showed spots corresponding to dimethoxyoctadecanol (Rf 0.07), methoxyoctadecanol (Rf 0.11), octadecanol (Rf 0.19) and 2-alkyl-THP (Rf 0.70). A further spot (Rf 0.93) was shown to be due to an impurity (peroxide ?) in the ether used for extracting the borohydride - reduction product.

GLC results are tabulated below:

esters		alcohols		products	
ECL	Area%	ECL	Area%	ECL	Area% [1]
16.0	10	16.0	3	13.0	2
16.5	1	17.7	12	14.9[3]	46
18.0[2]	9	19.7[2]	11	16.0	3
18.4	34	20.4	34	17.7	12
19.2	46	21.2	40	19.6	11
				23.0	8
				23.5	8
				25.9	10

Notes:

1. Area percentages are very approximate due to the range of ECLs.
2. The increase in ECL on reduction of an ester to the corresponding alcohol is ca 1.7 ECL units.
3. The ECL of 2-tridecyl-THP under the same GLC conditions was 15.0.

4.1b Seed oil esters (DMF as solvent)

Three seed oil esters (A, B, C) were reduced (LAH in ether) to the corresponding alcohols which were examined on GLC (see table below). Samples of these alcohols were dissolved in dry DMF (5ml) with excess mercuric acetate and left to react at room temperature for 2 - 3 days. Sodium borohydride reduction furnished products which were examined by GLC and TLC. Prep TLC(PE30) was used to isolate the 2-alkyl cyclic ethers which were examined on GLC.

A. Teucrium depressum esters (20mg) gave a product (19mg) after LAH reduction and oxymercuration - reduction procedures which was separated on prep TLC(PE30) to give a component (Rf 0.61, 9.5%) with the same TLC behaviour as 2-alkyl-THP. This component gave two spots on Ag^+ TLC(PE20) with Rf values 0.35 and 0.58. GLC results are tabulated below.

esters	alcohols	products	cyclic ethers[2,3]	
ECL(area%)	ECL(area%)	ECL(area%)	ECL(area%)	assignment
16.0(13)	17.6(15)	17.6(13)	15.5(6)	18:1THP
16.7(1)	18.1(1)	18.1(1)	16.2(94)	18:2THP
18.0(6)	19.6(7)	19.6(5)		
18.5(25)	20.0(25)	20.0(25)		
19.2(45)	20.8(43)	20.8(42)		
19.6(10)	21.4(7)	21.3(4)		
20.0(tr)	21.8(1)	21.8(1)		
20.1(tr)				
		13.8(0.5)[1]		
		15.5(0.5)[1]		
		15.8(0.5)[1]		
		16.2(8)[1]		

Notes:

1. New peaks.
2. Minor peaks with ECLs 12.5, 16.5 and 18.4 were shown to be due to ether impurities.
3. 2-Tridecyltetrahydropyran (referred to hereafter as 1β:0 THP) had an ECL of 15.0 under the same GLC conditions.

B. Thalictrum flavum esters (20mg) gave a product (20mg) after LAH reduction and oxymercuration - reduction. Prep TLC(PE30) afforded a component (Rf 0.61, 35% *) with the same TLC behaviour as 2-alkyl-THP. Ag^+ TLC(PE20) showed a major spot (Rf 0.35) and two smaller spots (Rf 0.58 and Rf 0.65). GLC results are tabulated below:

esters ECL(area%)	alcohols[1] ECL(area%)	products ECL(area%)	cyclic ethers ECL(area%)	assignment
16.1(7)	17.6(8)	17.6(7)	13.1(4)	16:0THP
16.5(3)	18.1(4)	18.1(2)	15.1(24)	18:0THP
18.0(5)	19.6(4)	19.6(3)	15.6(15)	18:1THP
18.4(22)	20.0(26)	20.0(10)	16.2(57)	18:2THP
18.9(7)	20.6(8)	20.5(3)		
19.2(20)	20.8(19)	20.8(11)		
19.6(36)	21.3(31)	21.3(7)		
20.3(tr)				
		13.1(3)[2]		
		15.1(14)[2]		
		15.6(9)[2]		
		16.2(34)[2]		

Notes:

1. Trace components with ECLs 14.8, 15.7, 16.0, 16.8 and 19.2 were also observed.
2. New peaks.

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* Inaccurate due to solvent ether impurities.

C. *Calea uniflora* esters (53mg) were reduced (LAH) to give the corresponding alcohols (52mg) which gave a product (50mg) after oxymercuration and sodium borohydride reduction. Prep TLC(PE30) on 36 mg gave a component (4mg, 11%) with the same Rf value as 2-alkyl-THF (slightly more polar than the THP bands obtained in B and C). GLC results are tabulated below:

esters ECL(area%)	alcohols[1] ECL(area%)	products ECL(area%)	cyclic ethers[3,4] ECL(area%)	assignment[4]
16.0(12)	17.6(13)	17.6(10)	16.1(5)	18:1THF
16.6(tr)	18.2(tr)	18.2(tr)	16.8(95)	18:2THF
18.0(4)	19.6(5)	19.6(3)		
18.4(14)	20.0(16)	20.0(14)		
19.2(53)	20.7(48)	20.7(51)		
19.9(17)	20.9(17)			
		16.1(1)[2]		
		16.9(21)[2]		

Notes:

1. Trace components with ECLs 14.0 and 16.1 were also observed.
2. New peaks.
3. Hydrogenation (Pd/C) of these cyclic ethers gave a product which showed only 1 peak (ECL 15.7) on GLC.
4. 2-Tetradecyl-THF had an ECL of 15.7.

4.2 Rat liver oil

(a) Isolation of rat liver oil

Four livers (21.9g) were obtained from 4-month old female Wister rats (body weights ca 200g) and homogenised in 3 batches in methanolic potassium hydroxide (1M, 25ml per 7g) on an MSE overhead homogeniser at 2,500rpm. The homogenates (bilious green suspensions) were refluxed on a steam bath (1hr) to ensure complete hydrolysis. Extraneous solid material was removed by centrifugation and the non-saponifiable material extracted from the supernatant liquid with ether (3 x 200ml). The aqueous solution of potassium salts was acidified with hydrochloric acid (1M) and extracted with ether (4 x 150ml). Ether extracts were combined, washed with water (3 x 200ml), reduced in volume to ca 100ml and dried over anhydrous sodium sulphate. The recovered acids (301mg) gave a typical free acid streak on TLC(PE30) and had a particularly nauseous smell. The acids were quantitatively reduced to the corresponding alcohols (LAH in dry ether) and examined by GLC (see table overleaf).

(b) Partial identification of constituent alcohols

The rat liver alcohols (50mg) and mercuric acetate (150mg, 0.47mmole) in DMF (10ml) were left at room temperature for 2 days. Sodium borohydride reduction afforded products which were examined by GLC (see table overleaf).

rat liver alcohols

ECL	13.6	14.4	15.6	16.0	16.6	17.6	18.3
Area%	0.3	0.1	0.8	0.1	0.6	32	2
	18.7	19.2	19.6	20.2	21.0	22.0	24.1
	1.5	0.5	26	12	16	2	7

products of cyclo - oxymercuration

ECL	13.6	14.6	15.1	15.7	16.7	17.6	18.2
Area%	0.3	0.2	0.1	0.5	0.6	23	0.7
	18.6	19.2	19.6	20.1	20.9	22.7	
	25	0.7	19	10	12	8	

Part of the product (35mg) was separated by prep TLC(PE15, double development) into four bands:

A (Rf 0.22, 21.5mg, 75%); B (Rf 0.52, 2.3mg, 8%);
C (Rf 0.59, 1.2mg, 4%) and D (Rf 0.73, 3.5mg, 13%).

Component A was shown by TLC and GLC to consist of unreacted alcohols. Component B gave no appreciable peaks on GLC and was not examined further. The material from band C had an ECL of 22.7 and an ECL of 19.8 after hydrogenation (reduction of 5 double bonds). The Rf value (TLC) of component C was consistent with that of a 2-substituted THF. The product from band D gave a major peak on GLC with ECL 18.5 (17.0 after hydrogenation, reduction of 3 double bonds) and traces of material with ECLs 17.7, 19.5 and 19.9 (16.1, 18.1 and 19.0 after hydrogenation).

(c) Isolation of 20:4 and 22:6 alcohols

Rat liver alcohols (164mg) and mercuric acetate (250mg, 0.78mmole) were allowed to react in DMF (10ml) for 2 days. An attempt to extract non-mercurated material only with petrol (2 x 20ml) was unsuccessful as the petrol extracts contained mercury (tested with 1,5-diphenylcarbazone). Petrol and DMF fractions were combined and ether (50ml) was added. The total solution was washed with water (3 x 50ml) and dried over sodium sulphate. Part of the product (140mg) was run on prep TLC(E). A narrow strip at one side of the plate was sprayed with ethanolic 1,5-diphenylcarbazone (0.2%). This showed a major mercury-containing spot (A) at the origin and a minor mercury-containing spot (B) at the solvent front. The remainder of the prep TLC plate showed a major band immediately below band B, with the same TLC behaviour as the starting material. The bands corresponding to spots A and B were scraped off and stirred separately with methanolic hydrochloric acid (0.5M, 15ml) for 10min. After filtration, addition of ether (50ml) and washing with water (2 x 50ml), products A' (17.5mg, 13%) and B' (16.5mg,12%) were obtained.

TLC(PE20) of product B' gave a minor spot with an Rf value of 0.27 and a larger, diffuse red spot (Rf 0.84) which did not fluoresce under UV light. No appreciable peaks were observed on GLC and B' was not examined further.

Product A' gave a large spot (Rf 0.27) and several less polar traces of material on TLC(PE 20). GLC gave a major peak (ECL 23.7) and minor peaks with ECLs 19.9, 20.6 and 21.8. GLC of the TMS ethers of A' gave major peaks with ECLs 18.9 (76%) and 21.8 (24%) and minor peaks with ECLs 13.5, 14.5, 15.7 and 16.1. Arachidonyl alcohol had an Rf of 0.27 and an ECL of 23.7 (ECL 18.9 as TMS ether) under the same chromatographic conditions. The IR spectrum of A' showed only a small irregularity at 970cm^{-1}, and the quantitative UV spectrum in methanol indicated the presence of $< 0.5\%$ of conjugated diene.

Attempts to oxidise tetradecanol to tetradecanoic acid as a model for the oxidation of isolated arachidonyl alcohol with chromium trioxide in acetone - water and ether - water solvent systems were abandoned as very low yields of acid were obtained.

4.3 Tall oil

(a) No internal standard

Tall oil acids (110mg) supplied by British Oxygen Company were esterified (boron trifluoride - methanol). The esters (120mg) were examined by GLC (see table). Another sample of acids (200mg) was reduced with LAH in dry ether to yield alcohols (181mg). These alcohols (99mg) and mercuric acetate (300mg, 0.94mmole) were left to react in DMF (5ml) at room temperature. Sodium borohydride reduction after 2 days yielded a product (99mg), most of which (90mg) was separated by TLC(PE30) into cyclic ethers (7mg, 10%) and unreacted alcohols (65mg, 90%). A sample of the former (ca 2mg) was hydrogenated. GLC results are tabulated overleaf.

esters[1] ECL(area%)	alcohols[2] ECL(area%)	cyclic ethers[3,5] ECL(area%)	hydrogenated[4,6] cyclic ethers ECL(area%)
18.4(36)	20.1(40)	13.1(1)	15.0(85)
19.2(41)	20.9(41)	15.5(11)	15.6(5)
19.6(11)	21.6(9)	16.3(69)	17.0(10)
20.3(6)	22.0(4)	17.4(10)	
20.8(4)	22.5(3)	18.0(9)	
21.1(1)			
21.5(1)			

Notes:

1. Traces of material with ECLs 16.0, 16.6 and 18.0 were also present.
2. Traces of material with ECLs 16.0, 17.7, 18.0, 18.4, 18.8, 19.2, 19.6 and 23.0 were also present.
3. Traces of material with ECLs 18.8 and 19.2 were also present.
4. Traces of material with ECLs 17.0, 17.3, 17.9, 19.6, 22.7 and 24.1 were also present.
5. GLC of the total oxymercuration - reduction product clearly showed the presence of these cyclic ethers when compared with the GLC trace of the original alcohols.
6. 2-Tridecyltetrahydropyran and 2-tetradecyltetrahydrofuran had ECLs of 15.0 and 15.6 respectively under the same GLC conditions.

(b) With internal standard

Tall oil acids (396.2mg, 1.405mmole) and pure synthetic methyl octadec-cis-5-enoate (20.9mg, 0.0706mmole) were dissolved in dry ether (5ml) and reduced with LAH in ether to yield alcohols (389mg) which were checked by GLC. Apart from a slight increase in the area % of the peak with ECL 20.1, the GLC of these alcohols was identical to that of the alcohols obtained in part (a) - see table above.

Alcohols (80mg, 0.30mmole) and mercuric acetate (200mg, 0.63mmole) in DMF (10ml) were allowed to react at room temperature for 2 days. Sodium borohydride reduction afforded a product (77mg) from which the alkyl THPs were isolated by prep TLC(PE 20). The material extracted from the TLC band was not weighed. GLC of this product is outlined below:

ECL	area% [1]	assignment [2]	mole % in mixture [3]	mole % in original oil [4]
8.5	0.3	?		
11.0	1	14:0 THP	0.13	0.14
15.0	34	18:0 THP	4.55 [3]	-
15.6	6	18:1 THP	0.80	0.84
16.3	51	18:2 THP	6.82	7.16
17.5	4	20:1 THP	0.53	0.56
18.1	4	20:2 THP	0.53	0.56

Notes:

1. These are average values of several measurements using different measuring techniques.
2. Assignments were made by comparison with ECLs of authentic saturated cyclic ethers and with allowance for double bonds.
3. These values were calculated from known amount of added octadec-cis-5-enoate.
4. Values were corrected for the presence of the added standard.

As the results from this experiment gave a lower value (7%) for all cis-octadec-5,9,12-trienoic acid than the supplier's published value of 11% (see discussion), the triene methyl esters (23.8mg, ca 9%) were isolated from tall oil methyl esters (280mg) by Ag^{+} TLC(PE20). GLC gave three peaks with ECLs 19.7 (93%),

20.2 (4%) and 21.6 (3%). LAH reduction of these triene esters gave the corresponding alcohols (21mg, 0.08mmole) which were allowed to react with mercuric acetate (60mg, 0.19mmole) in DMF (5ml) for 2 days. Sodium borohydride reduction furnished a product (19.0mg) from which a non-polar band (alkyl-THPs, 9.0mg, 65%) with ECLs 16.2 (96%) and 18.2 (4%), and a polar band (long-chain alcohols, 4.9mg, 35%) were isolated by prep TLC(PE30). von Rudloff oxidation of the latter component yielded a product which gave a large peak (ECL 18.9) and several minor peaks on GLC. von Rudloff oxidation of oleyl alcohol (prepared by LAH reduction of methyl oleate) also gave a product with ECL 18.9 (minor products ECLs 17.3 and 17.9).

4.4 Fish oils and hydrogenated fish oils (HFOs)

(a) Oxymercuration - reduction

Two fish oils (cod and pilchard) and eight hydrogenated fish oils (HFO 1 - 8) were reduced with LAH in ether. The resultant alcohols were examined by GLC. Due to the similarity of the composition of the HFO alcohols (see GLC results) only HFOs 1 - 4 and the two unadulterated fish oils were examined further. The alcohols (ca 200mg) were allowed to react with mercuric acetate (ca 300mg) in DMF (10ml) for 2 days. Sodium borohydride reduction gave products(table below) from which the cyclic ethers (THFs and THPs) were isolated by prep TLC(PE30) and examined by GLC. These cyclic ethers were hydrogenated and re-examined by GLC (see following pages).

oil	oil (mg)	alcohols (mg)	total product (mg)[1]	cyclic ether (%)[2]
cod	200	175	176	10.5
pilchard	199	174	168	19
HFO 1	200	184	174	4.7
HFO 2	201	178	170	5.0
HFO 3	200	182	189	6.2
HFO 4	206	178	177	6.9
HFO 5	201	180	-	-
HFO 6	201	179	-	-
HFO 7	206	178	-	-
HFO 8	200	198	-	-

Notes:

1. These values were corrected for the removal of GLC and TLC aliquots.
2. These were values obtained from the weight of components extracted from prep TLC bands.

GLC of fish oil and HFO alcohols

ECL[1]	(X:Y)[2]	Cod	Pilch.	1	2	3	4	5	6	7	8
15.7	(14:0)	9	14	14	16	15	16	16	14	18	13
16.7	(15:0)	1	1	1	1	1	1	2	1	1	1
17.7	(16:0)	22	30	29	29	26	30	27	27	27	24
18.2	(16:1)	16	17	8	9	9	12	9	9	9	8
18.8	(16:2)	tr	-	2	2	2	2	2	2	2	2
19.2	(16:3)	2	4	1	2	1	2	2	2	1	2
19.7	(18:0)	4	4	12	7	5	6	6	7	4	9
20.1	(18:1)	30	12	14	19	21	19	18	19	16	22
20.8	(18:2)	3	6	3	4	4	3	4	5	4	5
21.7	(20:0)	-	-	5	2	2	1	3	7	2	3
22.1	(20:1)	7	2	7	7	9	6	8	2	10	7
22.7	(20:2)	3	4	2	1	1	1	2	1	2	2
23.1	(20:3)	tr	1	-	-	-	-	-	-	-	-
24.0	(22:0)	2	1	1	1	3	tr	2	3	4	2
25.2	(22:2)	1	4	-	-	-	-	-	-	-	-

Notes:

1. In addition, all samples showed peaks with ECLs 9.6 (8:0), 10.6 (9:0), 11.6 (10:0), 13.6 (12:0) and 14.7 (13:0) in trace amounts.
2. Assignment: X = no. of C atoms in chain;
   Y = no. of double bonds.

GLC of HFO cyclic ethers

ECL	1	2	3	4	assignment THF &/or THP	
9.6	tr	tr	tr	tr	12:0	-
10.6	-	-	2	tr	13:0	-
11.1	tr	tr	-	2	14:0	-
11.8	tr	tr	1	1	14:0	14:1
13.0	3	2	1	2	-	16:0
13.8	2	2	1	1	16:0	16:1
15.1	4	3	1	2	-	18:0
15.7	4	5	4	2	18:0	18:1
16.3	tr	tr	1	1	18:1	-
17.1	9	6	4	2	-	20:0
17.6	14	19	18	14	20:0	20:1
18.2	7	11	14	13	20:1	20:2
18.8	-	-	1	2	20:2	-
19.1	7	2	1	1	-	22:0
19.5	~ 7	-	-	-	-	22:1
19.7	~ 10	10	7	9	22:0	22:1
20.2	17	17	15	17	22:1	22:2
20.7	10	16	15	20	22:2	-
21.2	3	4	6	4	-	24:0
21.5	1	2	3	2	24:0	24:1
22.2	2	tr	5	3	24:1	24:2
22.8	tr	tr	1	tr	24:2	-

GLC of hydrogenated HFO cyclic ethers

ECL	1	2	3[1]	4[2]	assignment
12.1	tr	-	1	tr	?
13.0	3	3	2	3	16:0 THP
13.7	2	2	2	1	16:0 THF
15.0	7	8	8	6	18:0 THP
15.7	3	2	2	1	18:0 THF
17.0	25	30	32	29	20:0 THP
17.7	9	6	4	7	20:0 THF
19.0	21	18	15	21	22:0 THP
19.7	30	30	29	30	22:0 THF

Notes:

1. Unidentified components (total 5%) with ECLs 20.3, 21.5 and 22.5 were also observed.
2. Unidentified components (total 2%) with ECLs 9.0, 16.6, 20.4, 21.1, 21.6 and 22.5 were also observed.

GLC of cod and pilchard cyclic ethers
and hydrogenated cyclic ethers

(a) Cyclic ethers			(b) Hydrogenated cyclic ethers			
	Area%			Area%		
ECL[1]	Cod	Pilchard	ECL[2]	Cod	Pilchard	Assignment[3]
17.7	1	2	15.1	2	3	18:0 THP
18.5	2	1	17.0	48	67	20:0 THP
19.4	45	66	17.7	tr	2	20:0 THF
22.6	47	22	19.1	1	tr	22:0 THP
23.0	3	tr	19.7	44	21	22:0 THF
24.4	tr	5	23.1	tr	2	26:0 THP

Notes:

1. Minor peaks with ECLs 11.0, 11.7, 12.4, 13.1, 13.7, 14.0, 15.4, 15.8, 17.3, 18.0 and 21.7 (2 - 4% in total) were also observed.
2. Minor peaks with ECLs 11.0, 11.7, 12.7, 13.8, 14.8, 15.3, 16.0, 18.0, 18.4, 21.6 and 22.6 (5% in total) were observed.
3. Assignments were made by comparison with authentic cyclic ethers.

Part of the oxymercurated - reduced cod alcohol product (81mg) was hydrogenated immediately after recovery from the sodium borohydride reaction mixture. The hydrogenated cyclic ethers (THFs and THPs, 10.9%) were isolated by prep TLC(PE 25) and were shown to be identical (GLC) to those obtained previously when the cyclic ethers were isolated by prep TLC before hydrogenation.

(b) Isolation of the 20:5 and 22:6 alcohols from cod liver oil

Cod liver oil (500mg) was reduced with LAH in ether to yield alcohols (457mg) which were dissolved in dry DMF (20ml) with mercuric acetate (150mg). After 2 days water (50ml) was added to the reaction mixture which was extracted with ether (3 x 40ml) and dried over sodium sulphate. The product (495mg) showed a large spot with the same polarity as octadecanol and a polar mercury-containing spot at the origin on TLC (E). The total product (495mg) was introduced on to a sorbsil column (16cm x 1.5cm) in ether. Ether fractions (3 x 100ml) were collected. The first fraction was shown to contain the unreacted alcohols (343mg) by GLC. Methanolic hydrochloric acid (0.5M, 50ml) was then passed through the column. Brine (100ml) was added to the eluant, and the product (78mg) was extracted with ether (2 x 50ml). TLC (E) gave a single spot (no mercury) with the same polarity as octadecanol. The IR spectrum showed strong absorption at 3,605cm^{-1} (O-H) and at the usual C-H absorption frequencies. There were very small irregularities at 925cm^{-1} and 970cm^{-1}. The UV spectrum in methanol showed absorption at 233nm (ε = 700) and at 303nm (triplet, ε = 260). GLC of the product and of its trimethylsilylated derivative are shown overleaf.

ECL	(area%)
16.3	(2)
16.7	(2)
18.2	(6)
20.0	(2)
20.2	(2)
23.8	(4)
24.7	(74)
26.9	(8)

TMS ethers	
ECL	(area%)
13.5	(0.5)
18.8	(1)
19.4	(45)
21.7	(53)

GLC of the TMS ethers of the alcohols which were unaffected by oxymercuration (first ether fraction from column separation) gave ECLs 11.3 (6%), 13.5 (22%), 15.7 (39%), 17.0 (5%), 17.8 (18%) and 19.1 (10%) only.

5. INVESTIGATION OF FOUR UMBELLIFERAE SEED OILS

(a) Isolation and oxymercuration

Finely ground seeds (10.0g) from 4 Umbelliferae plants gave seed oils (see table below) by soxhlet extraction for 6hr using petrol (200ml). Samples of each oil, with an accurately weighed amount of methyl octadec-cis-4-enoate added as an internal standard, were reduced with LAH in dry ether. The recovered alcohols were submitted to the oxymercuration procedure in DMF (10ml) with mercuric acetate (ca 2 x weight of alcohol used) for 2 days followed by sodium borohydride reduction. Results are tabulated below:

seed	total oil (g)	oil + 18:1 4c (mg)	alcohols (mg)	product (mg)
carrot	0.867	215	2.42	172	168
coriander	0.840	174	10.50	164	143
parsley	1.094	228	12.80	202	200
caraway	1.057	228	17.10	208	204

GLC of the total alcohols gave very similar chromatograms. Typically, the alcohols from carrot seed oil had components with ECLs 12.0 (1%), 13.2 (2%), 15.4 (6%), 17.6 (5%), 18.0 (5%), 20.1 (70%) and 20.9 (11%) and trace components with ECLs 16.0, 16.8, 19.1 and 19.6.

TLC(PE10) of the borohydride reduction products showed a large spot near the origin (Rf 0.27) corresponding to octadecanol in polarity and two small spots (Rf values 0.67 and 0.89)

corresponding to 2-alkyl-THF and 2-alkyl-THP respectively. In addition minor non-lipid components appeared as red, non-fluorescent spots when the TLC plate was sprayed with ethanolic dichlorofluorescein but did not interfere with the cyclic ethers except in the case of the parsley product where the 2-alkyl-THF band was badly masked by this non-lipid material. The cyclic ethers were isolated (but not weighed) from each product by prep TLC(PE10) and examined by GLC (see table overleaf).

A blank reaction (without an internal standard) was carried out on coriander alcohols (115mg) which were obtained by LAH reduction of the seed oil (148mg). These alcohols gave a product (103mg) after oxymercuration in DMF and reduction with sodium borohydride, from which the cyclic ethers (4.5%) were isolated by prep TLC(PE10). (Although there was no evidence on the TLC plate of 2-alkyl-THF the appropriate region of the plate was scraped off with the 2-alkyl-THP band.) The recovered cyclic ethers were examined by GLC.

GLC results are tabulated overleaf.

	Area %				
ECL	carrot	coriander	parsley	caraway	coriander[1]
13.7	2	1	0.5	1	3
15.1	75	52	26	32	97
15.7[2]	23	47	25	64	-
18.8	-	-	49	3	-

Notes:

1. No internal standard was used.
2. 2-Tridecyltetrahydrofuran from methyl octadec-cis-4-enoate (internal standard).

The mass spectrum of the cyclic ethers from coriander seed oil (without an internal standard) showed a very intense peak with $^{m}/e$ 99, a large peak at $^{m}/e$ 81, a molecular ion peak at $^{m}/e$ 268 and a peak at $^{m}/e$ 250 as its principle features. The mass spectra of the cyclic ethers from the other oils were very similar except for an additional large peak at $^{m}/e$ 71. None of these spectra showed any evidence of a peak at $^{m}/e$ 85.

A larger amount of cyclic ether (10mg) was obtained from carrot oil alcohols (430mg) by oxymercuration - reduction and prep TLC. The IR spectrum showed strong absorptions at 1,118cm^{-1} and 1,360cm^{-1} in addition to the normal C-H absorptions. A weak absorption at 1,725cm^{-1} was also observed.

The 100MHz NMR spectrum of this cyclic ether (1mg in carbon tetrachloride in a microcell) was compared with the 100MHz NMR spectra of authentic 2-tridecyltetrahydropyran and 2-tetradecyltetrahydrofuran. Results are tabulated below:

NMR 5a

τ value	appearance	No. of protons		
		carrot cyclic ether	18:0 THP	18:0 THF
9.1	unresolved triplet	6	3	4
8.7	broad singlet	16	28	26
8.2	broad multiplet	8	2	4
6.9	broad multiplet	-	3	-
6.2	multiplet	4	2	3

(b) Examination of non-saponifiable material

Coriander oil (454mg) in methanol (7ml) was refluxed with aqueous potassium hydroxide (0.5M, 3ml) for one hour. Water (50ml) was added and the non-saponifiable material (20mg) extracted with ether (3 x 30ml). The non-saponifiable material was examined by GLC before being reduced with LAH in dry ether to give products which were also examined by GLC. These products were subjected to oxymercuration in DMF (2ml) with mercuric acetate (10mg) for 2 days before borohydride reduction. The final product had the same GLC behaviour as the LAH reduction product with ECLs 9.7 and 20.1 (major), 13.3 (medium) and 11.8, 16.0, 17.8 and 18.8 (all minor). The only one of these peaks not observed on GLC of the initial non-saponifiable material was the largest (ECL 20.1).

Non-lipid material, obtained from caraway seed oil (160mg) by prep TLC(PE30), was contained in bands A (3.3mg, 2.6%) and B (15.2mg, 12%) immediately above and below the major lipid band (88mg, 70%) respectively. A much more polar band near the origin of the TLC plate gave material (18.6mg, 15%) which was shown to be diglyceride by its TLC behaviour and by GLC of the LAH reduction products. The non-lipid bands A (ECL 22.6) and B (ECLs 13.4 major and 17.5 minor) remained unchanged by LAH reduction and by subsequent oxymercuration - reduction of the LAH product.

(c) von Rudloff oxidations

Coriander oil (121mg) and caraway oil (157mg) were refluxed separately in methanolic sodium methoxide (0.1M, 10ml) for 30min. The monoene methyl esters (Rf 0.71) were isolated from the total products by Ag^+ prep TLC(PE15) using methyl oleate at the side of the TLC plate as a standard. In both cases the monoene esters had ECLs 16.5 (0.5%) and 18.5 (99.5%) on GLC. von Rudloff oxidation on ca 5mg of each monoene ester gave products which were examined by GLC after separation by prep TLC(PE10) into dibasic esters (C_6 and C_9 with a trace of C_8) and monobasic esters (C_9 and C_{12} with traces of C_{10} and C_{11}).

Coriander acids (394mg) from which the non-saponifiable matter had been removed (see earlier) were esterified (boron trifluoride - methanol). Synthetic methyl octadec-cis-5-enoate (10.3mg) and methyl octadec-cis-7-enoate (10.4mg) were dissolved in dry ether to give 100.0ml of solution. After thorough mixing, 10.0ml of the solution were removed and added to part of the coriander methyl esters (97.8mg). Monoene esters isolated by prep Ag^+ TLC(PE15) of the mixture were subjected to von Rudloff oxidation. GLC of the oxidation products clearly showed the presence of C_5 and C_7 dibasic and C_{11} and C_{13} monobasic esters in addition to the oxidation fragments obtained earlier (without the addition of standards).

6. METHOXYMERCURATION OF OLIVE, CORN AND LINSEED OILS

(a) Methoxymercuration

Samples of olive, corn and linseed oils (10.0g, 2.0g and 2.0g respectively) and mercuric acetate (13g, 5.4g and 8.1g respectively) * in methanol - THF (2:1 v/v; 150ml, 75ml and 90ml respectively) were shaken at room temperature for 5 days. Reduction of the organomercurial reaction mixtures by the cautious dropwise addition of aqueous sodium borohydride solution (addition of hydrochloric acid (1M) during the extraction process helped to dispel the grey sludge formed during the reduction) afforded products (10.99g, 2.36g and 2.50g respectively) which were considerably more polar on TLC(PE15) than the starting materials (Rf 0.57).

Samples (ca 50mg) of the reaction products and of the starting materials were converted into their methyl esters by refluxing in methanolic sodium methoxide (0.5M, 10ml) for one hour. GLC of these esters gave results as tabulated overleaf.

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* A 25% molar excess of mercuric acetate was used on the assumption that olive oil was 100% triolein, corn oil was 100% trilinolein and linseed oil was 100% trilinolenin.

ECL[1]	olive oil		corn oil		linseed oil	
	before	after	before	after	before	after[2]
16.0	10	12	16	19	9	16
18.0	2	2	1	4	5	5
18.6	82	10	28	4	17	3
19.4	6	-	54	-	22	-
20.3	-	-	1	-	47	-
21.0	-	65	-	31	-	18
21.5	-	2	-	7	-	4
23.3	-	tr	-	5	-	4
23.9	-	4	-	30	-	12
24.6	-	-	-	-	-	6
24.7	-	-	-	-	-	4
26.1	-	5	-	-	-	5
26.6	-	-	-	-	-	6
27.1	-	-	-	-	-	12

Notes:

1. Methyl esters of the following authentic materials had ECLs under identical GLC conditions as shown:
   oleate 18.6; linoleate 19.4; linolenate 20.3;
   12-methoxystearate 21.0; 12-methoxyoleate 21.5;
   10,12-dimethoxystearate 23.3; 9,12-dimethoxystearate 23.9.
2. In addition, an unidentified peak with ECL 22.4 (5%) was observed.

(b) Physical measurements

Viscosity

The oils and their methoxylated products were too viscous to be used neat in the viscometer available. Solutions of each sample (1.00g) in chloroform (10.0ml) were run in an Ubbelohde suspended level viscometer immersed in a thermostatically controlled water bath at 30.0°. After immersion in the water bath, 30min were allowed for equilibration. Runs were repeated (normally about 10 times) until 3 consecutive values within a time of 0.1sec were obtained. Results are tabulated overleaf.

Refractive Index

Refractive indices were measured at 21.7° on a polarising refractometer (model: Abber 60). Results are tabulated overleaf.

Slip time

Samples (1.0g) of each oil and of each methoxylated oil were frozen at -15° for at least two days in screw-top sample tubes (1.5cm x 0.5cm) which were as nearly equal in weight, and consequently in wall thickness, as could be found. The tubes were inverted, removed from the refrigerator and the time until the oils were sufficiently molten to slip down the walls of the sample tubes was measured. This procedure was repeated (x 10) and the average "slip time" was calculated for each oil. Room temperature varied between 21.5° and 23°. It was found that anomalous results were obtained if the oils were left at -15° for less than two days. The methoxylated olive oil tended to flow slowly even at -15°.

Results

oil	relative viscosity[1] (sec)	refractive index[2]	average slip time[3] (sec)
olive	68.4	1.4692	430
methoxylated olive	70.0	1.4640	47
corn	68.1	1.4729	140
methoxylated corn	70.3	1.4688	82
linseed	67.8	1.4811	84
methoxylated linseed	69.3	1.4691	70

error ± 10%

Notes:

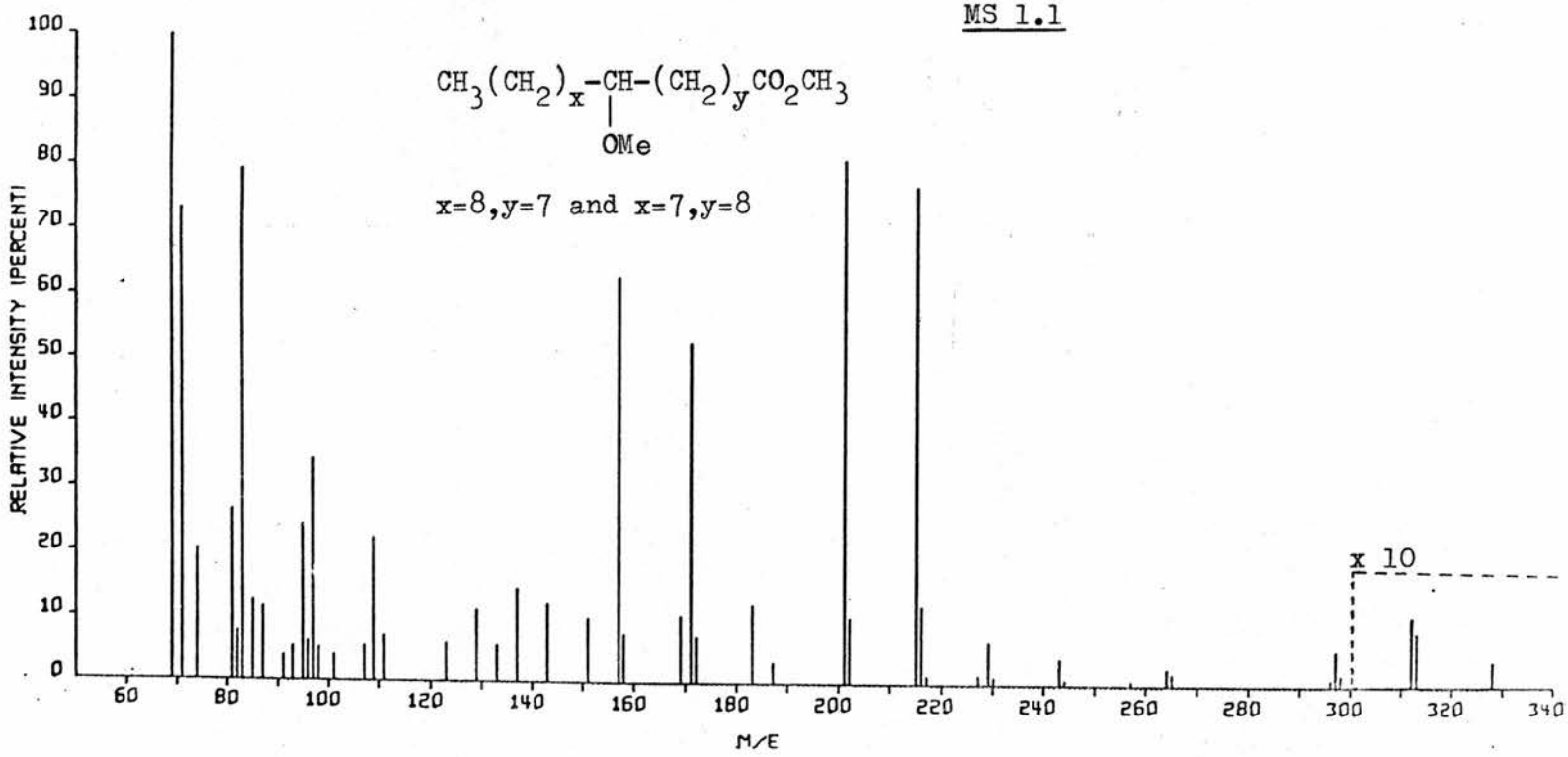
1. Chloroform (solvent) had a flow time of 45.5sec.
2. Lit. values [142] at 25°:
   olive oil 1.4657 - 1.4667;
   corn oil 1.4733;
   linseed oil 1.4797 - 1.4802.
3. Measured at ~22°.

(c) Hydroxymercuration

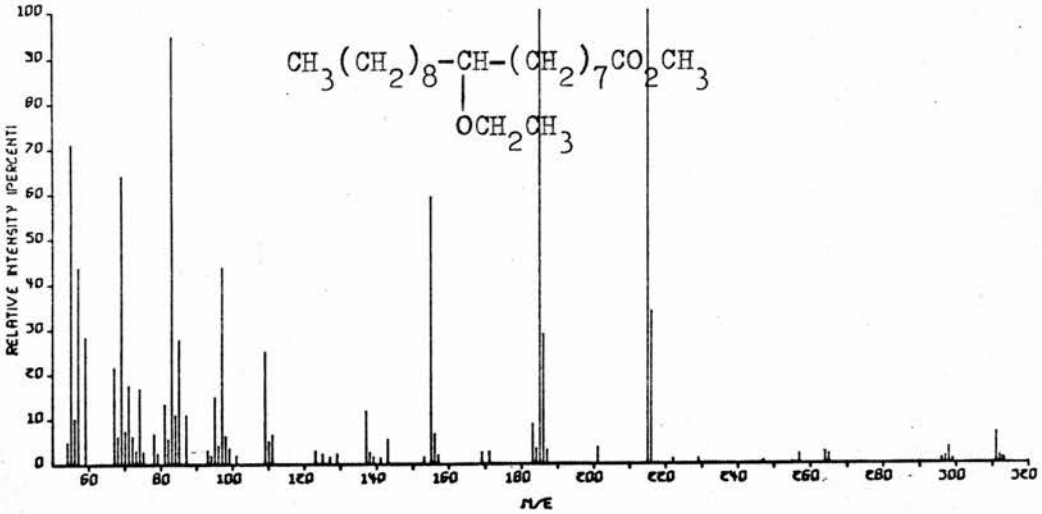
Attempts to react olive oil (5g) and corn oil (5g) separately with mercuric acetate (10g) in DMF (20ml) and water (5ml) gave virtually unchanged olive and corn oils (TLC and GLC of methyl esters) after sodium borohydride reduction.

APPENDIX 1

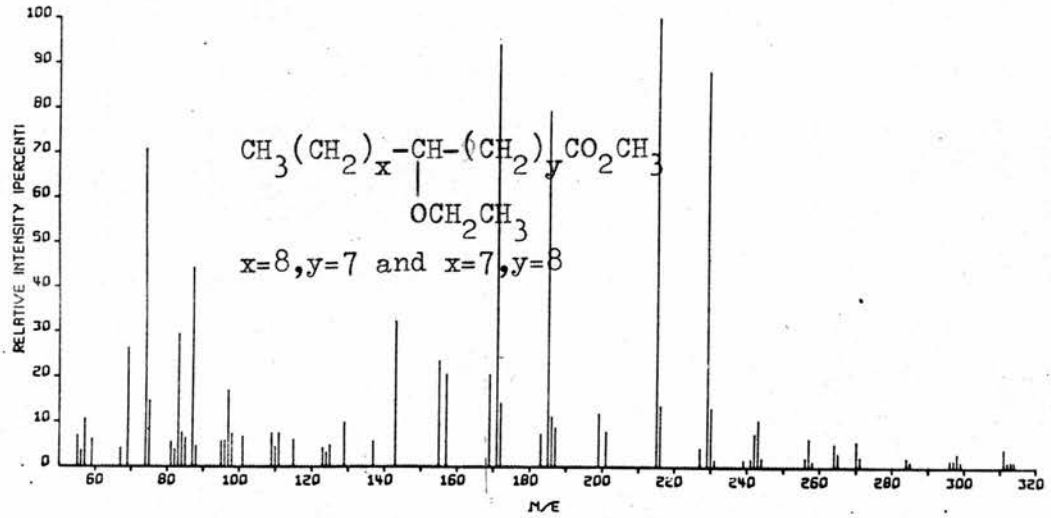
MS 1.1


CH3(CH2)x-CH-(CH2)yCO2CH3
OMe
x=8,y=7 and x=7,y=8
RELATIVE INTENSITY (PERCENT)
M/E
x 10

MS 1.2a


CH3(CH2)8-CH-(CH2)7CO2CH3
OCH2CH3
RELATIVE INTENSITY (PERCENT)
M/E

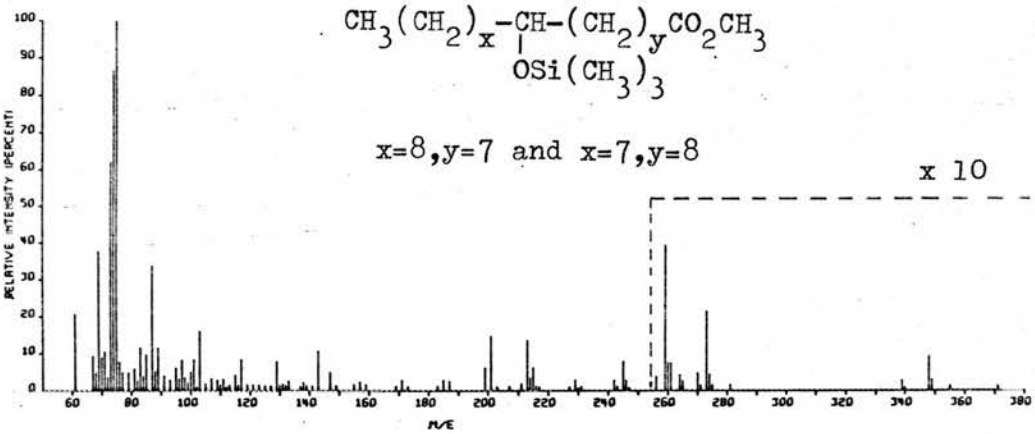
MS 1.2b


CH3(CH2)x-CH-(CH2)y CO2CH3
OCH2CH3
x=8,y=7 and x=7,y=8
RELATIVE INTENSITY (PERCENT)
M/E

MS 1.7

$CH_3(CH_2)_x-CH(OSi(CH_3)_3)-(CH_2)_yCO_2CH_3$

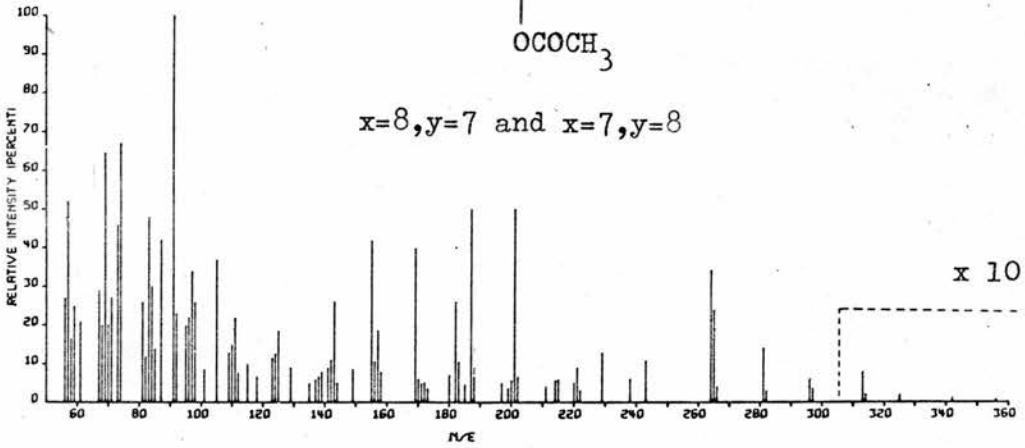
x=8,y=7 and x=7,y=8



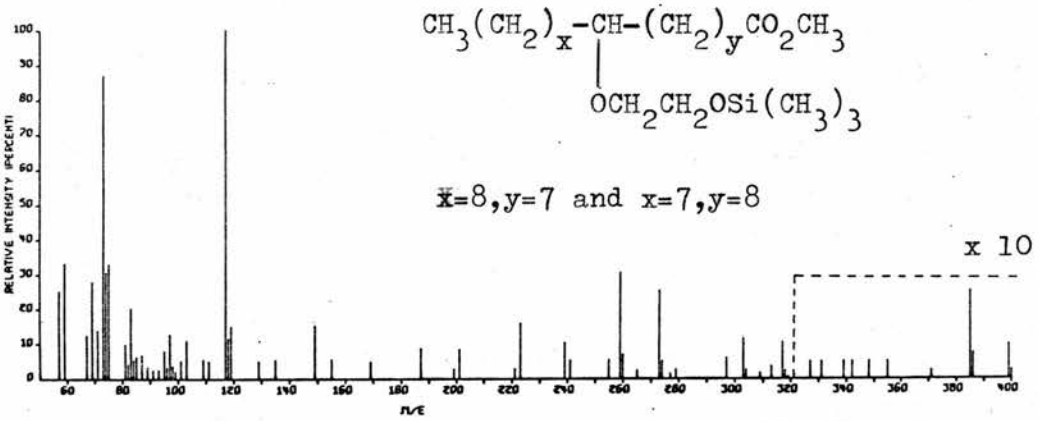
MS 1.8

$CH_3(CH_2)_x-CH(OCOCH_3)-(CH_2)_yCO_2CH_3$

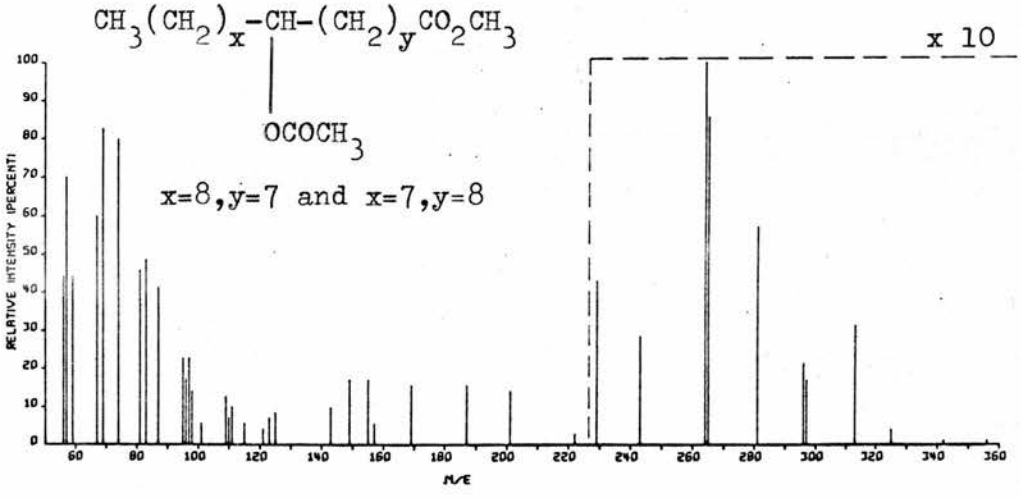
x=8,y=7 and x=7,y=8

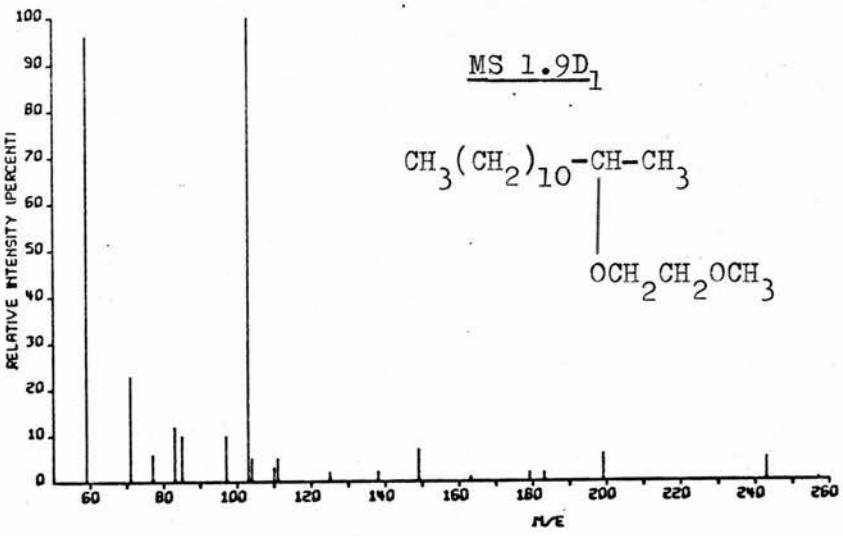


MS 1.9C$_1$



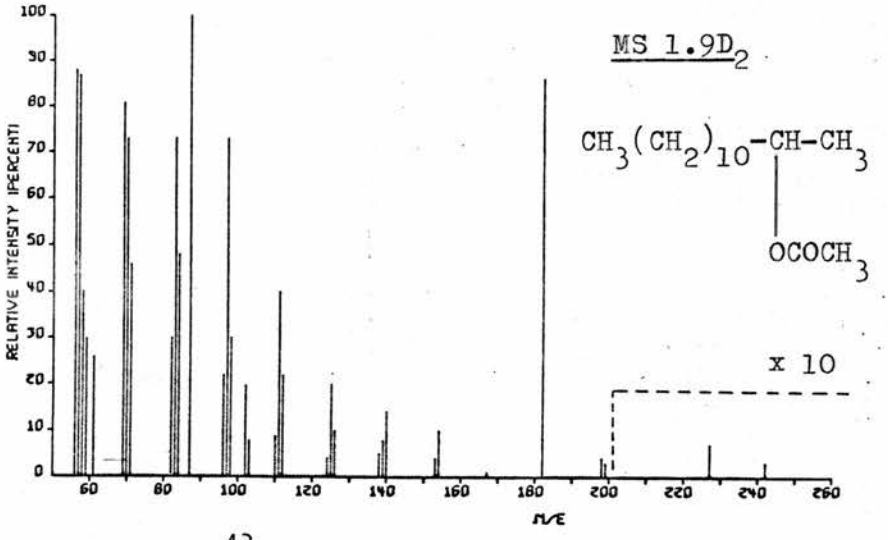
MS 1.9C$_2$





MS 1.9D$_1$

$CH_3(CH_2)_{10}-CH(OCH_2CH_2OCH_3)-CH_3$



MS 1.9D$_2$

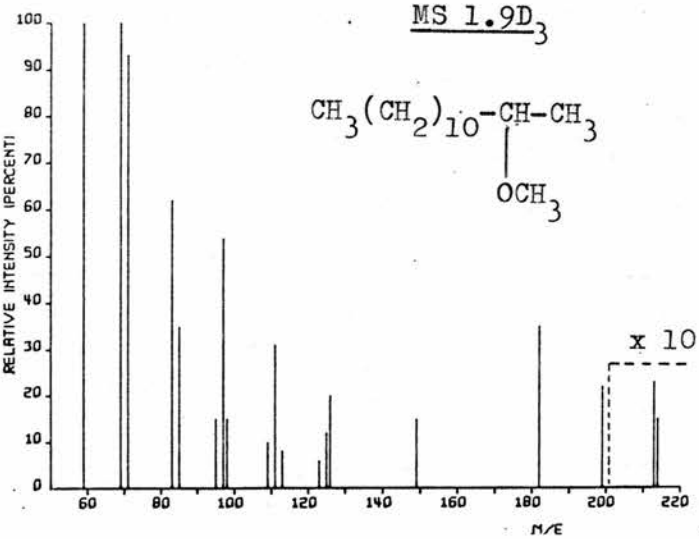
$CH_3(CH_2)_{10}-CH(OCOCH_3)-CH_3$

x 10

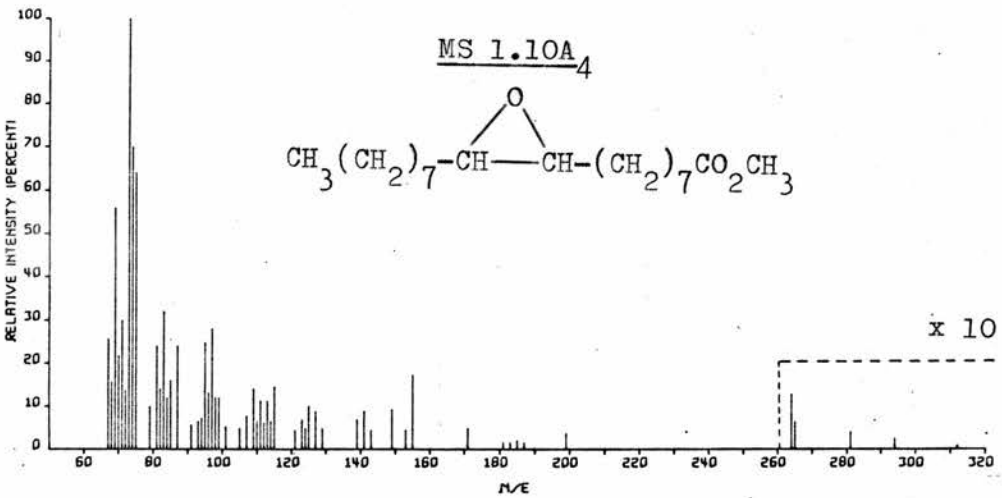
43

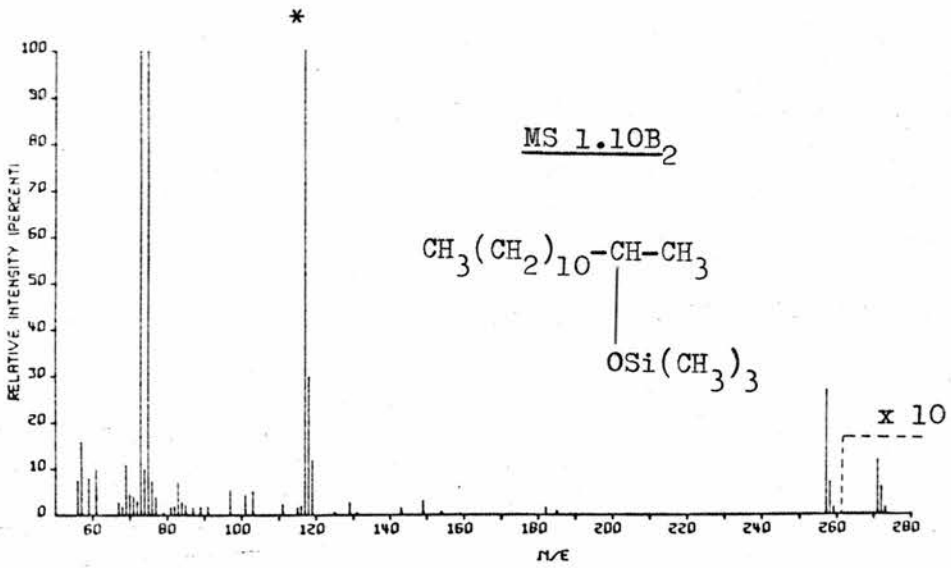
Peak at $^{m}/_{e}$ was very intense.

MS 1.9D$_3$

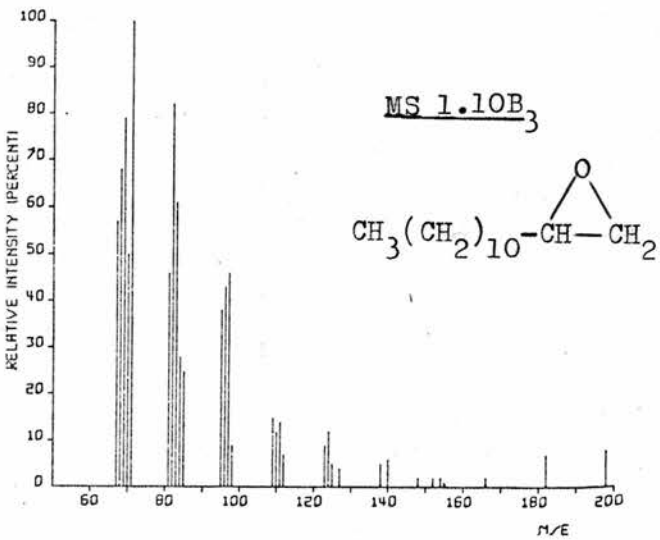


MS 1.10A$_4$

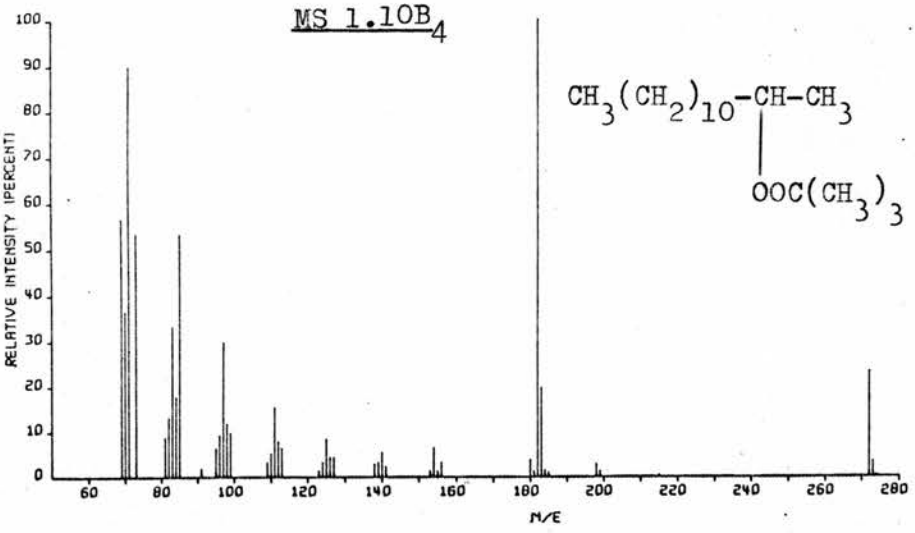




* Peak at $^{m}/e$ 117 was too intense to allow measurement.

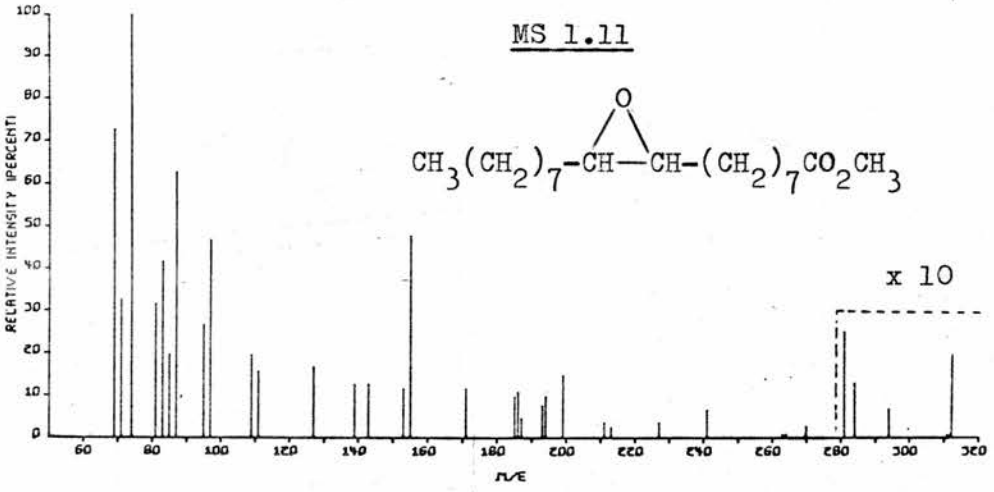


MS 1.10B$_4$

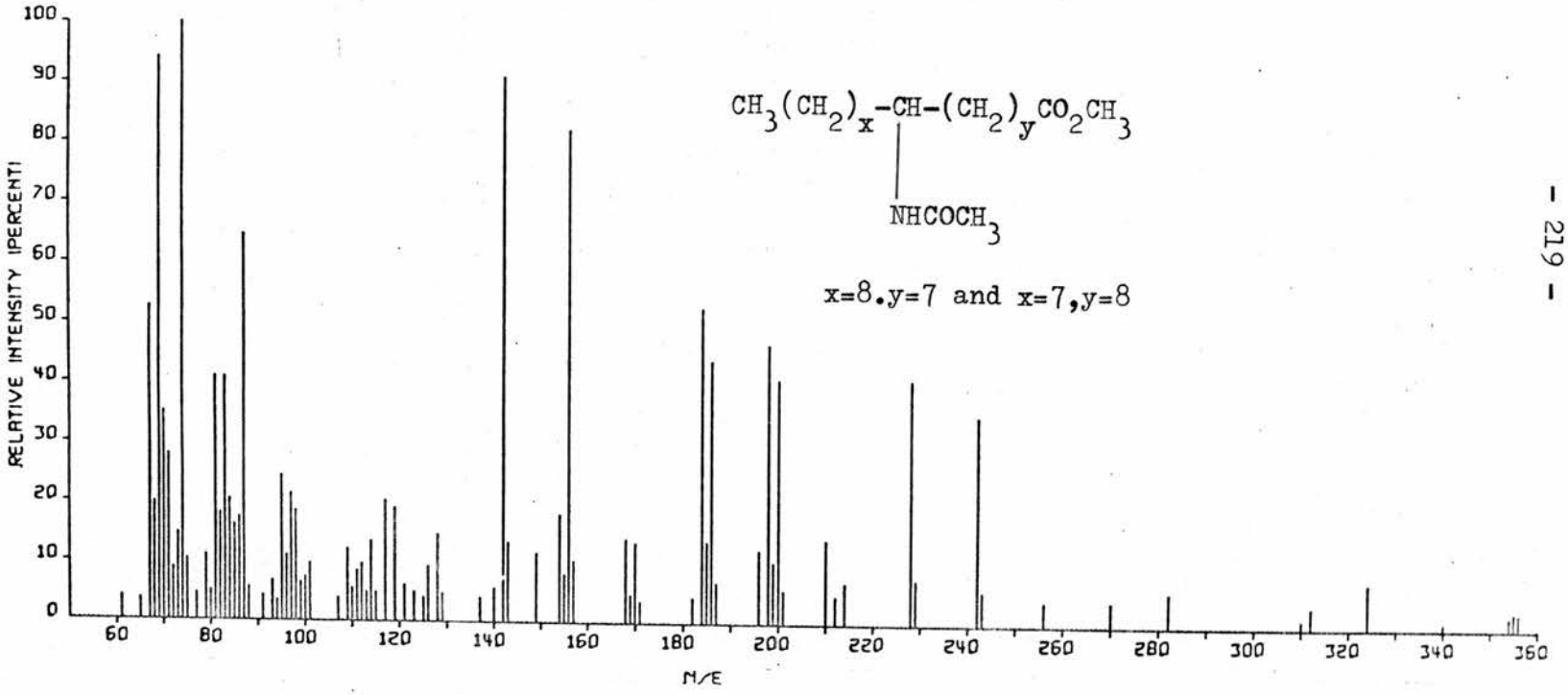


Peak at $^{m}/_{e}$ 57 was very intense.

MS 1.11



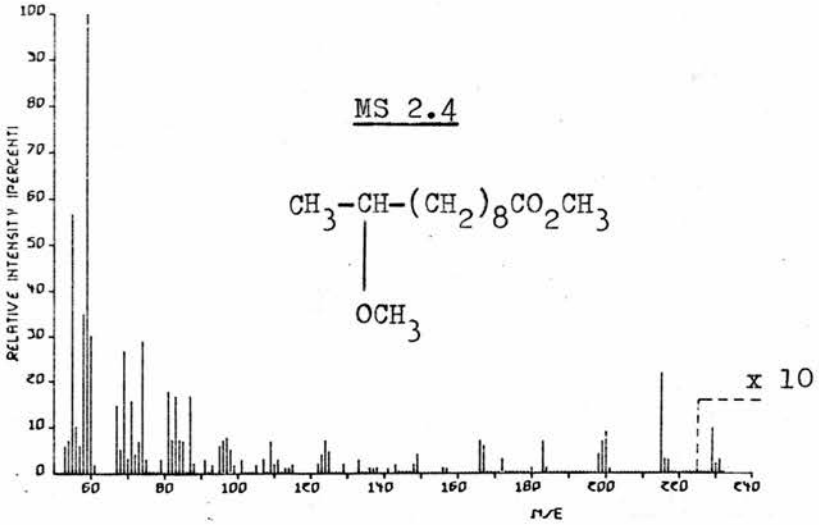
MS 1.12



$CH_3(CH_2)_x-CH-(CH_2)_yCO_2CH_3$

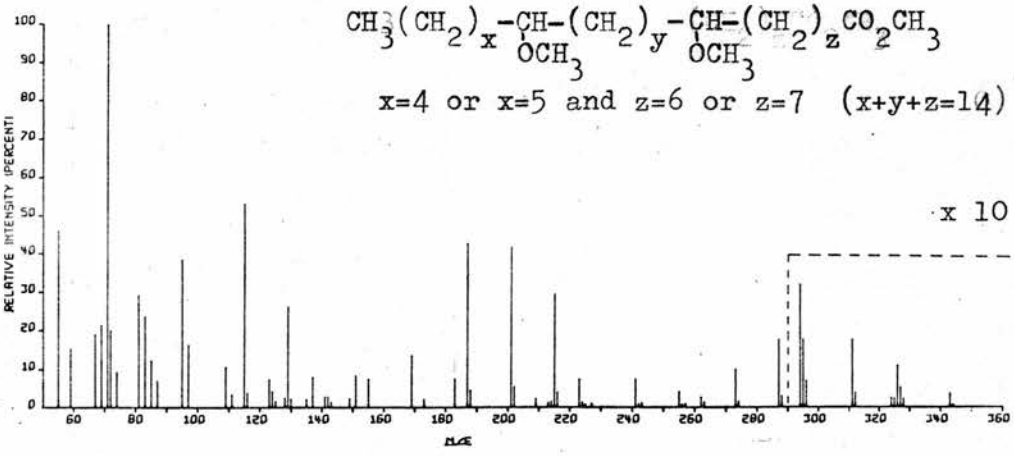
$NHCOCH_3$

x=8.y=7 and x=7,y=8



MS 2.4

$CH_3-CH(OCH_3)-(CH_2)_8CO_2CH_3$

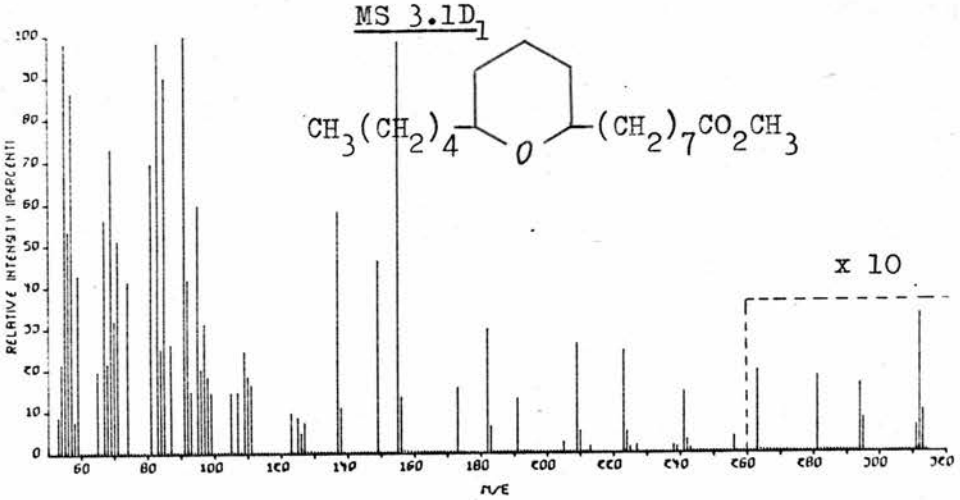


MS 2.6

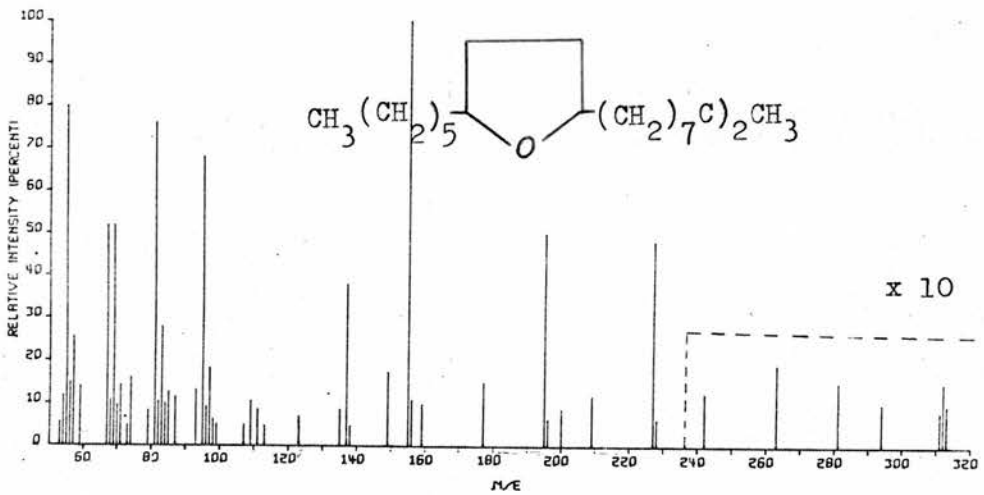
$CH_3(CH_2)_x-CH(OCH_3)-(CH_2)_y-CH(OCH_3)-(CH_2)_zCO_2CH_3$

x=4 or x=5 and z=6 or z=7 (x+y+z=14)

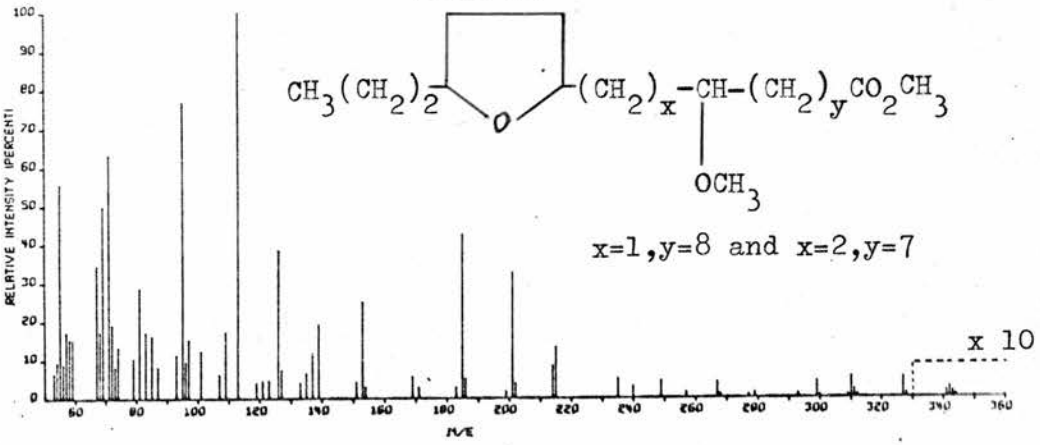
MS 3.1D$_1$



MS 3.1D$_2$

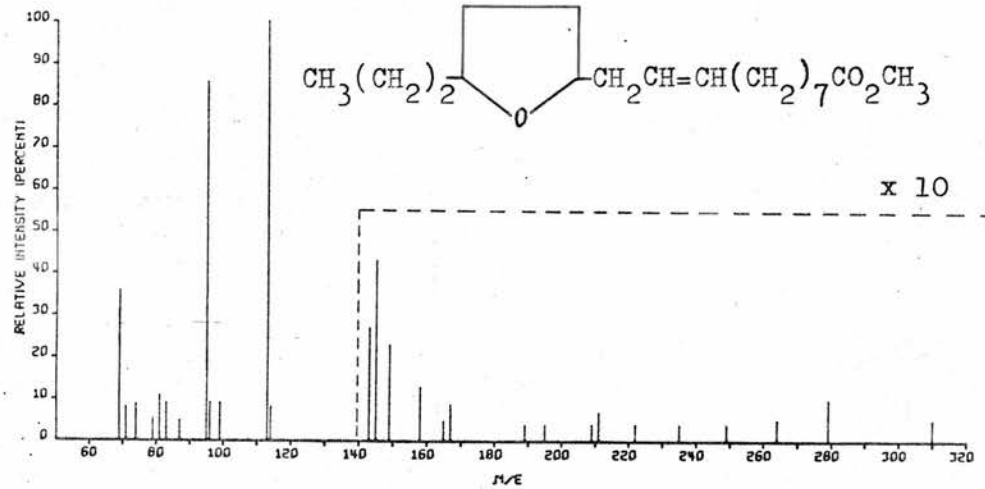


MS 3.2a

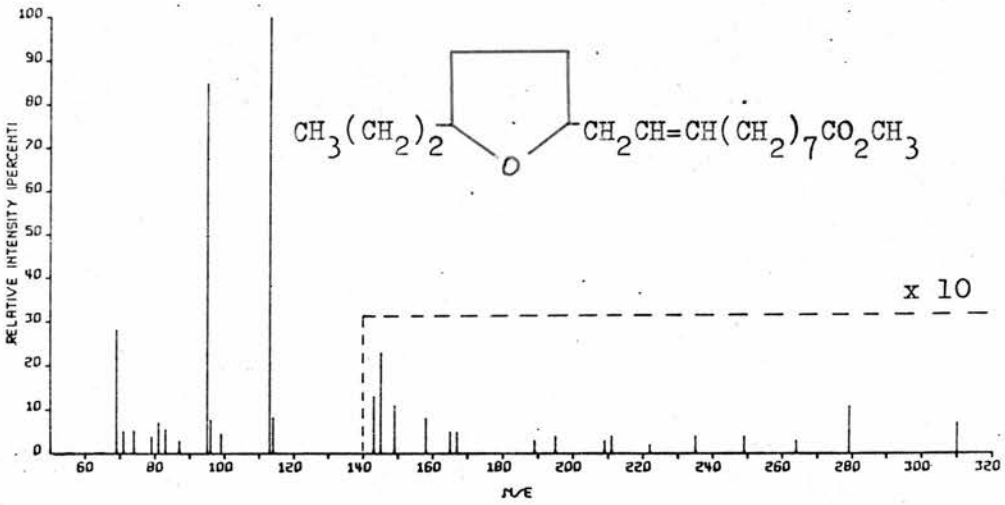


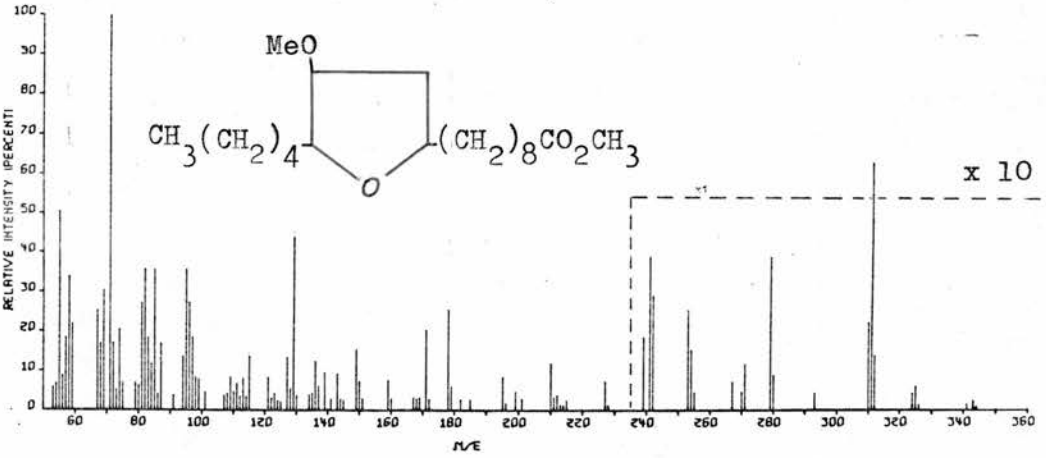
x=1,y=8 and x=2,y=7

MS 3.2 "21.9"

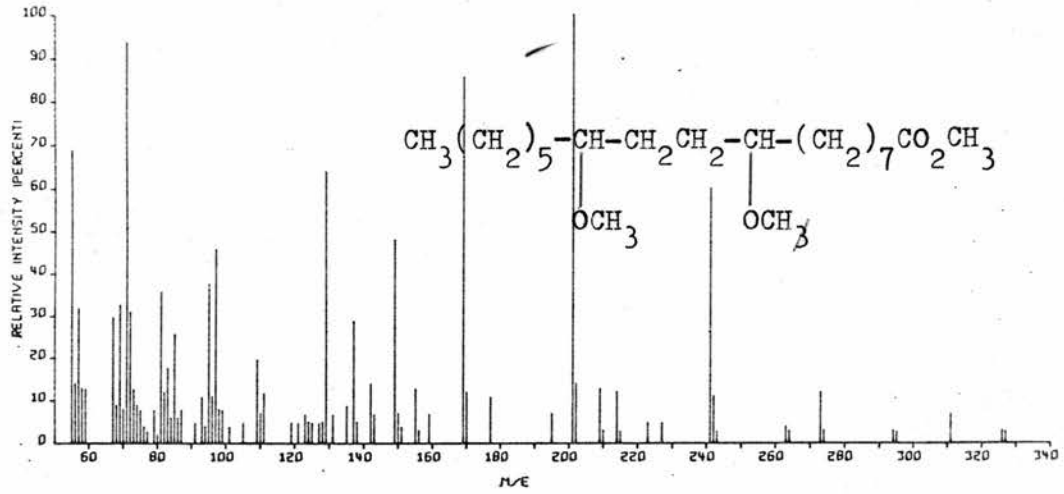


MS 3.2 "22.1"

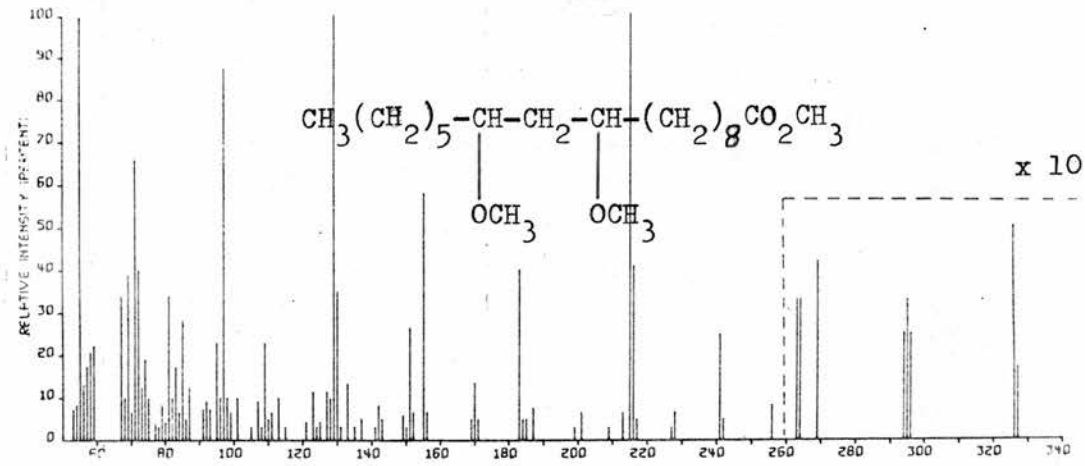

RELATIVE INTENSITY (PERCENT)
M/E
x 10


MeO
RELATIVE INTENSITY (PERCENT)
M/E
x 10

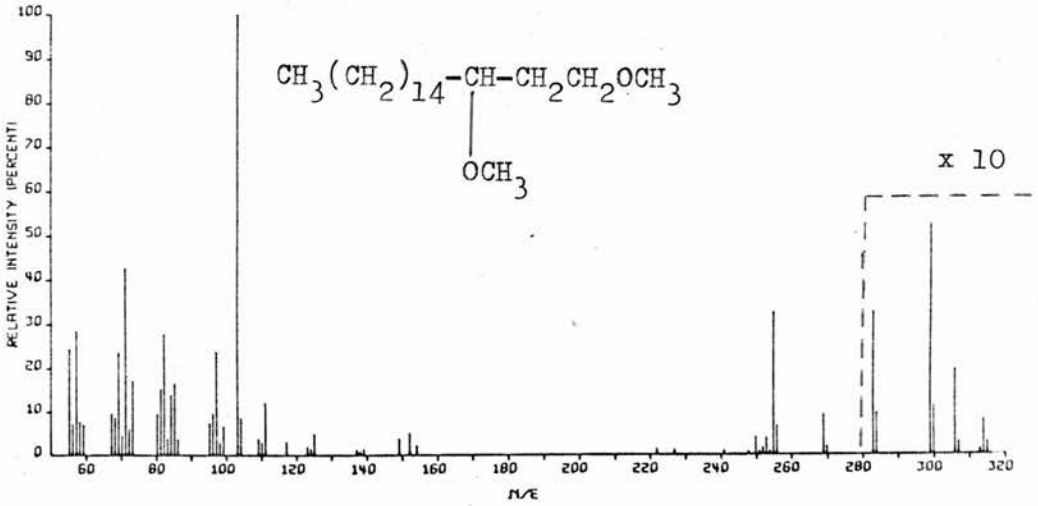
MS 3.1 A'_1



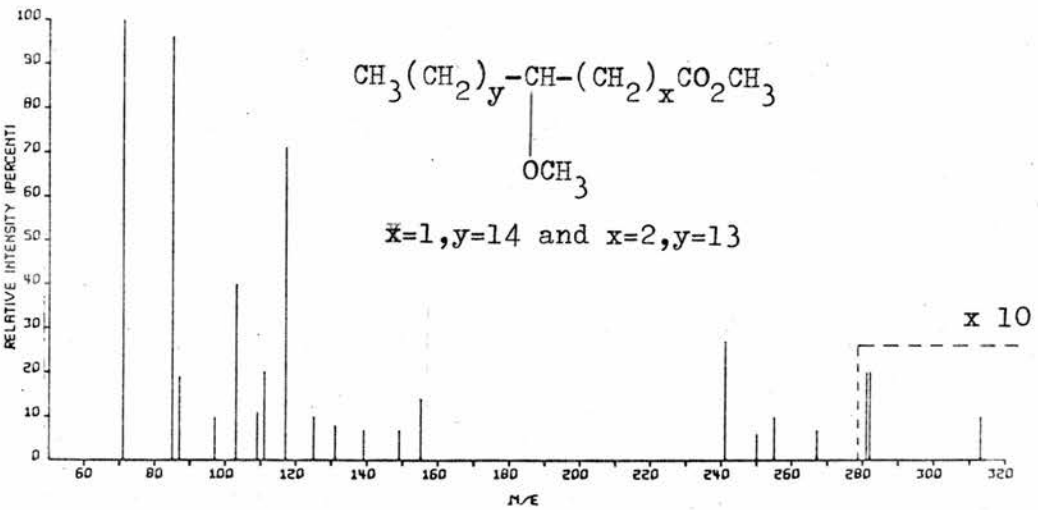
MS 3.1 A'_2



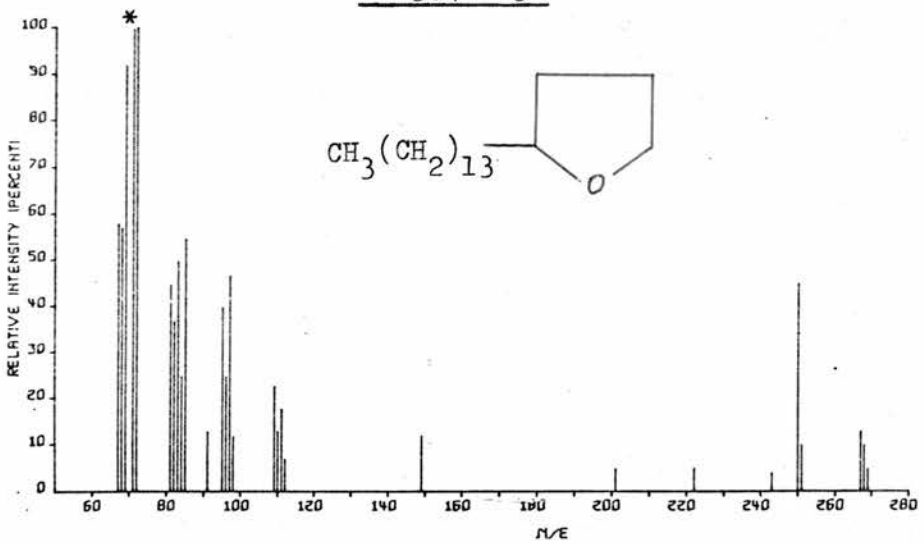
MS 3.4 - $1H^-$/MeI



MS 3.4 - 3A

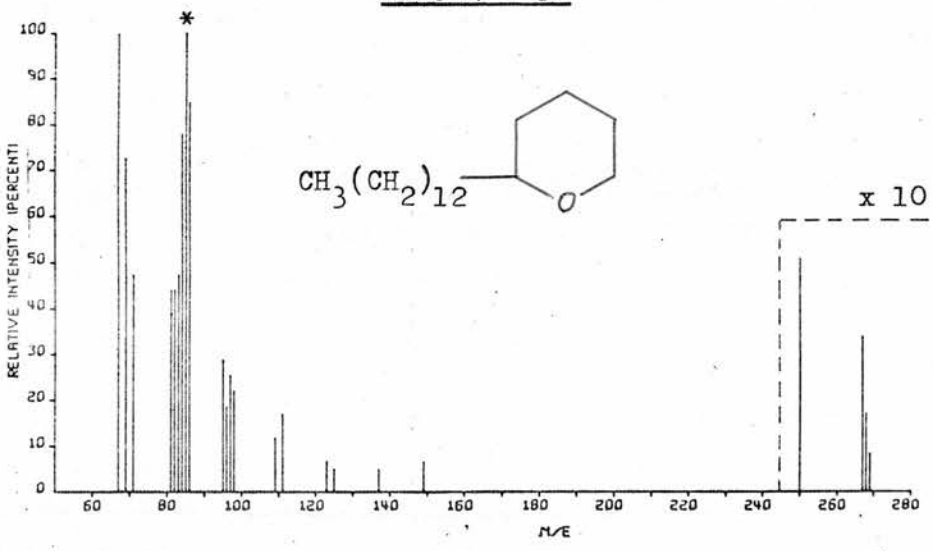


MS 3.4 - 3B



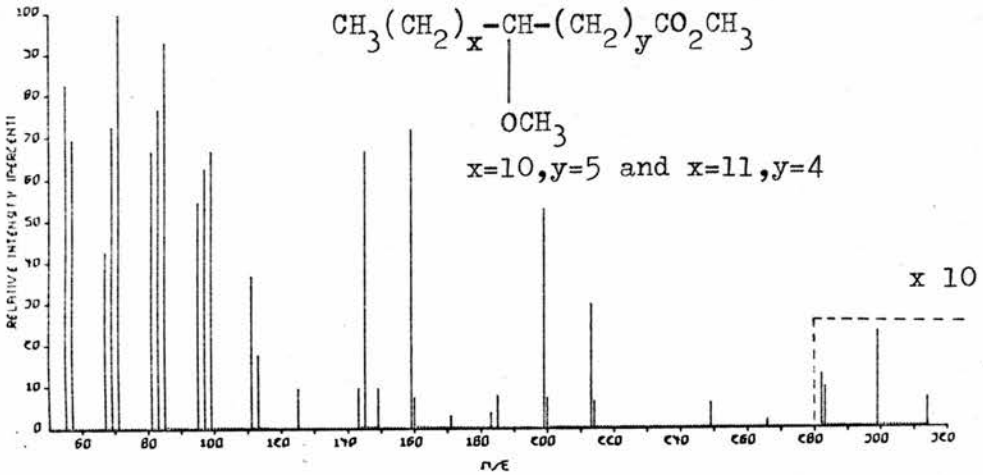
* Peak at $^{m}/e$ 71 was too intense to allow measurement.

MS 3.4 - 3E

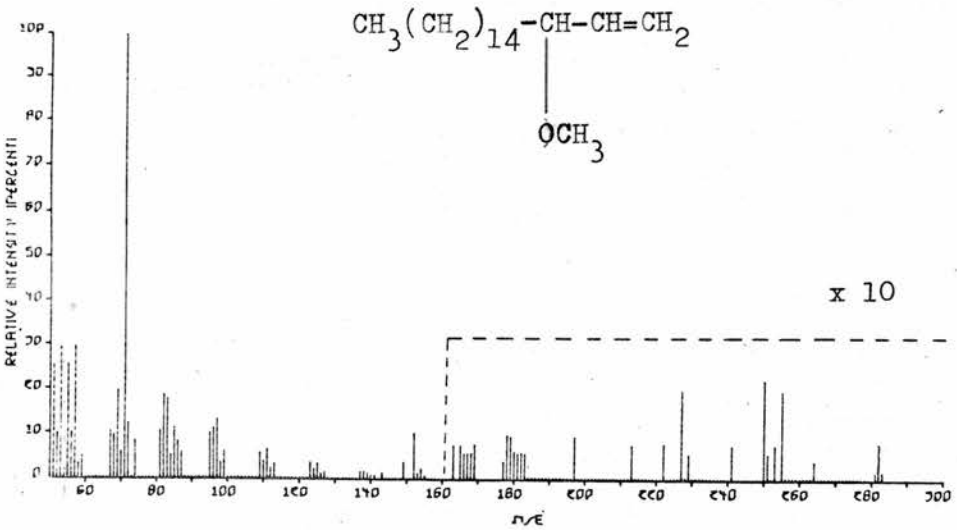


* Peak at $^{m}/e$ 85 was too intense to allow measurement.

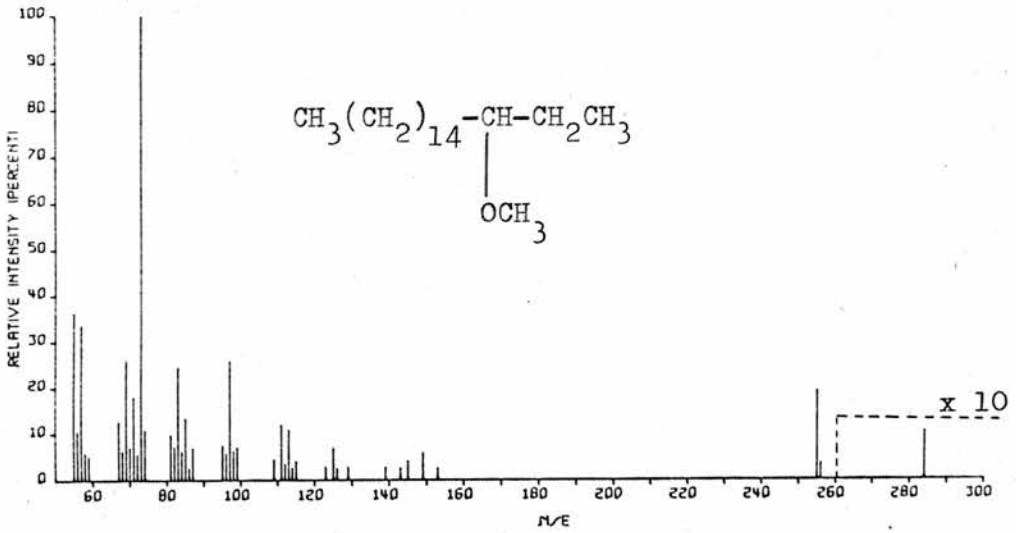
MS 3.4 - 3G



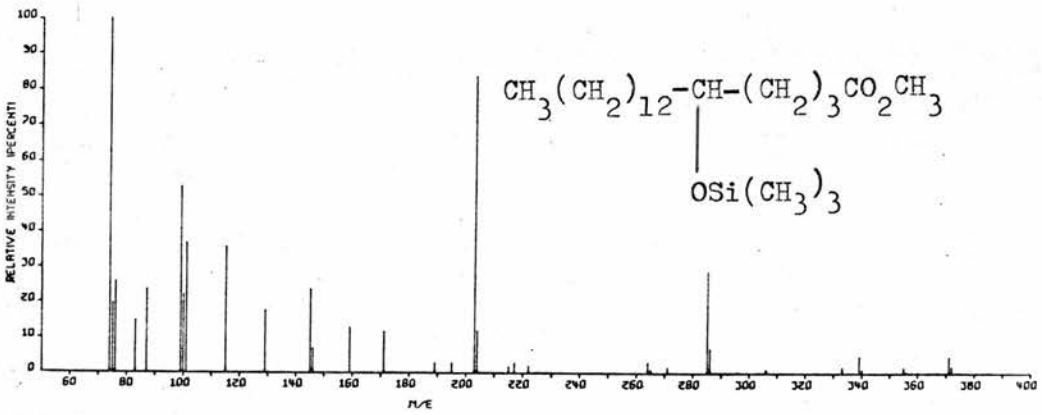
MS 3.4 - $2H^-$



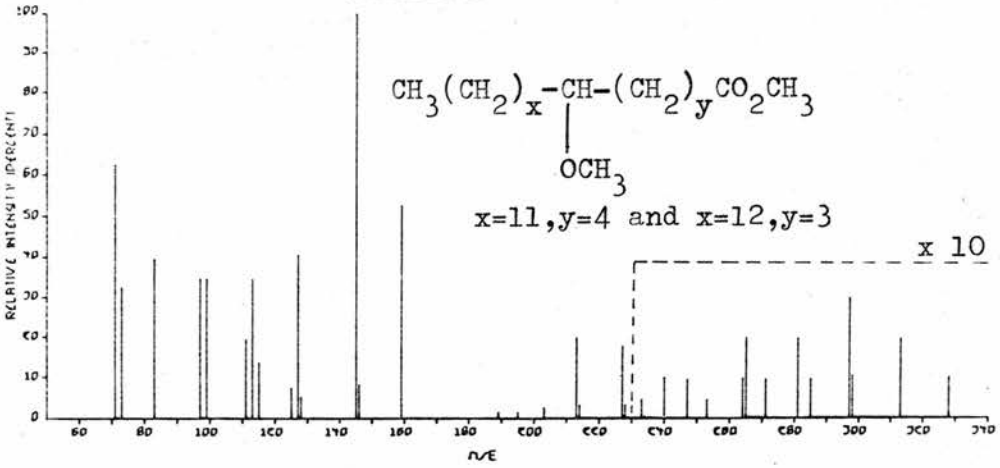
MS 3.4 - 1BH



MS 3.5a



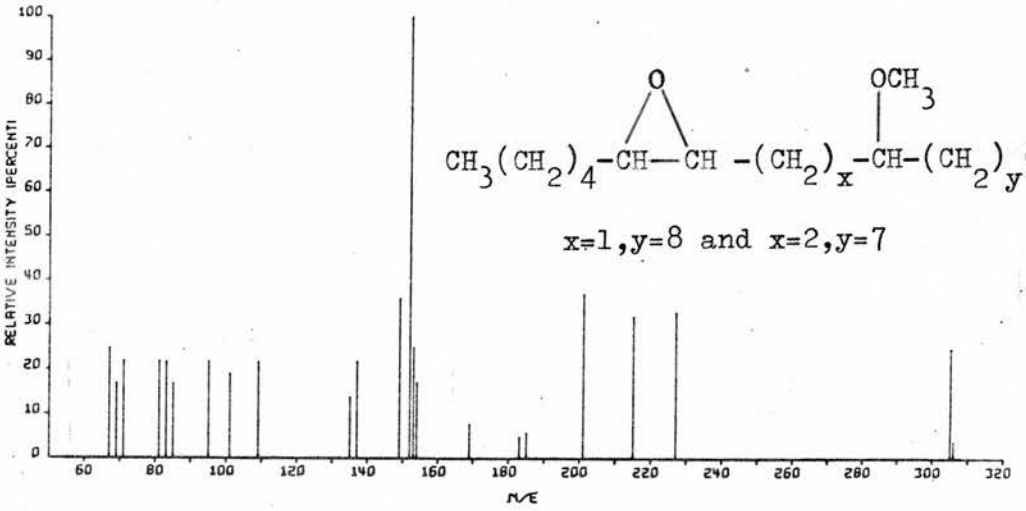
MS 3.5b



$CH_3(CH_2)_x-CH(OCH_3)-(CH_2)_yCO_2CH_3$

x=11,y=4 and x=12,y=3

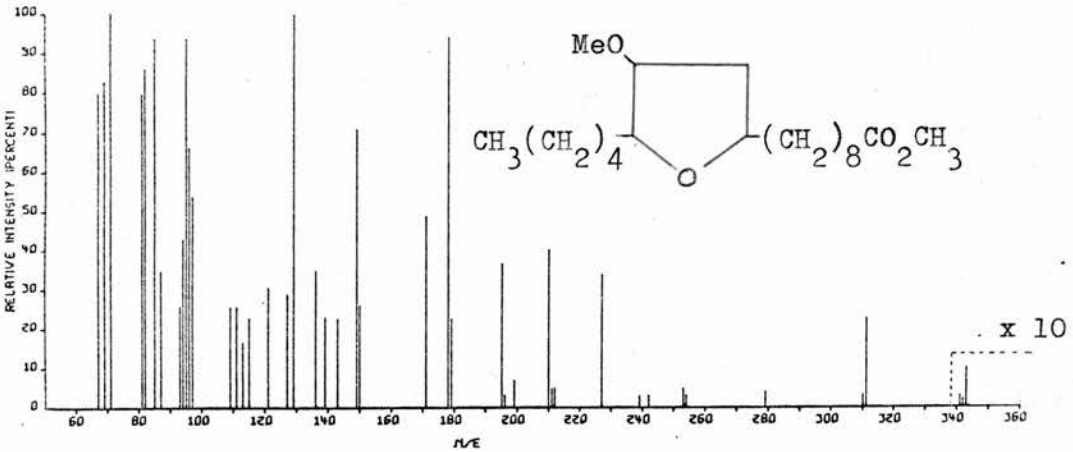
MS 3.6 A



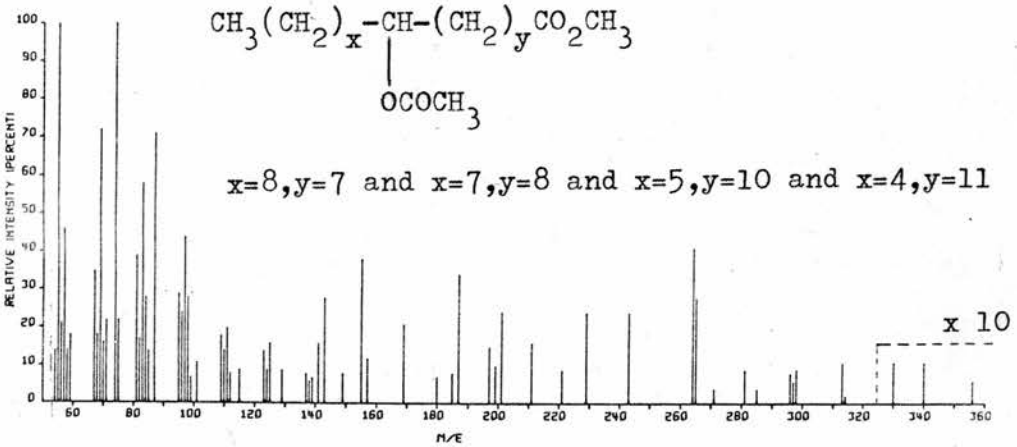
$CH_3(CH_2)_4-CH(O)-CH-(CH_2)_x-CH(OCH_3)-(CH_2)_yCO_2CH_3$

x=1,y=8 and x=2,y=7

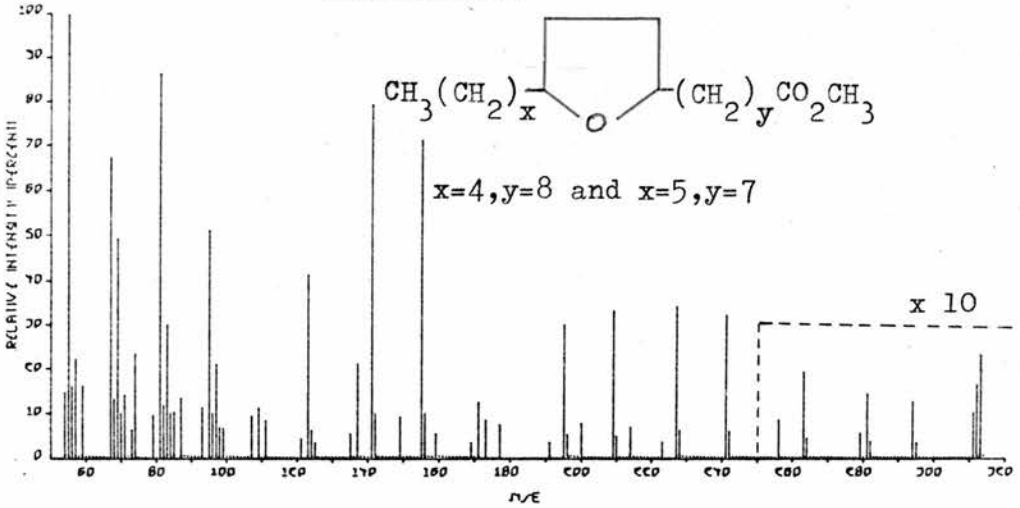
MS 3.6B


MeO
CH3(CH2)4
(CH2)8CO2CH3
O
x 10
RELATIVE INTENSITY (PERCENT)
M/E

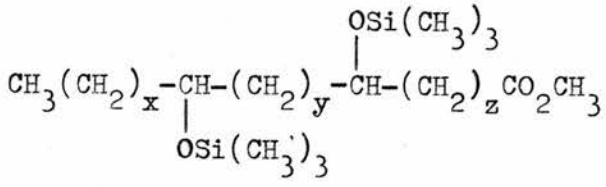
MS 3.7 - 1


CH3(CH2)x-CH-(CH2)yCO2CH3
OCOCH3
x=8,y=7 and x=7,y=8 and x=5,y=10 and x=4,y=11
x 10
RELATIVE INTENSITY (PERCENT)
M/E

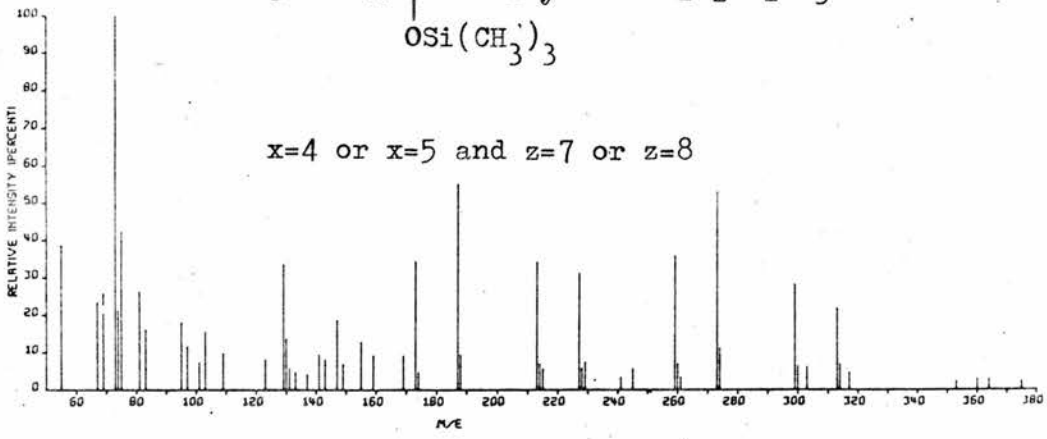
MS 3.7 - 2A



MS 3.7 - 2B

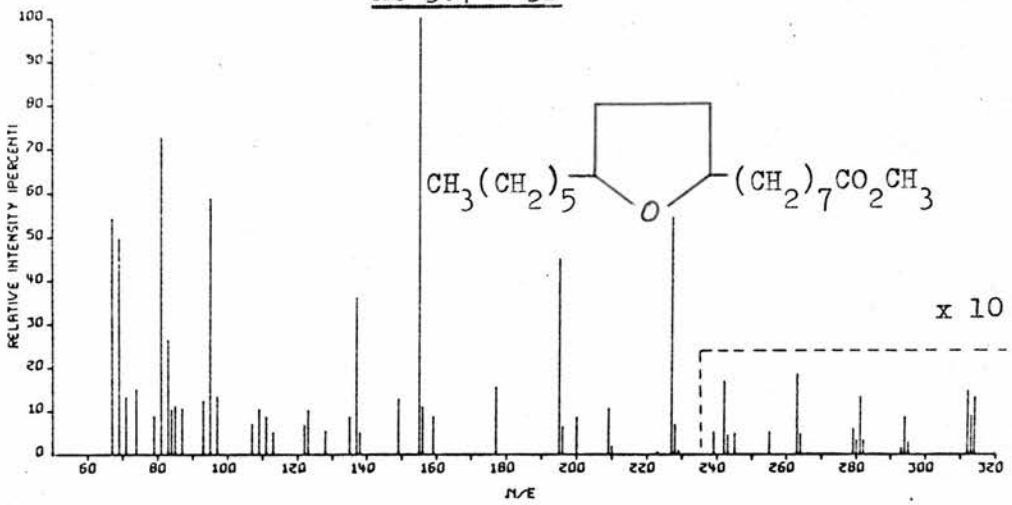


x=4 or x=5 and z=7 or z=8

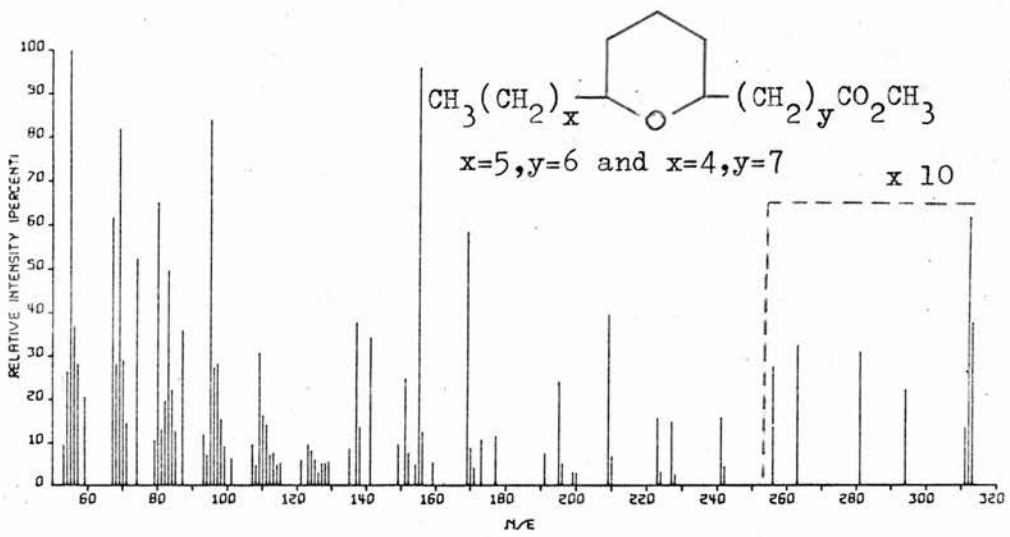


+ higher peaks

MS 3.7 - 3B



MS 3.7 - 3C



APPENDIX 2

Alternative preparation of methyl 9-hydroxyoctadec-trans-12-enoate

Methyl 9-hydroxyoctadec-cis-12enoate (1.75g, 0.565mmole) was dissolved in carbon tetrachloride and cooled to 0°. Bromine in carbon tetrachloride (10%) was added dropwise with stirring until the bromine colour persisted and the reaction mixture was stirred at room temperature for a further 20min. Removal of the solvent under vacuum gave a product (2.44g) which showed two spots on TLC. The bromo esters were heated on a steam bath for 5hr with 1,5-diazobicyclo(5.4.0)hendec-5-ene (3.42g, 22mmole). Water (20ml) was added to the reaction mixture which was acidified with hydrochloric acid (1M). The organic product (1.75g) was extracted with ether (2 x 20ml) and separated by column chromatography (silica gel, Sorbsil M60, 25cm x 2.5cm) monitored by TLC(PE30), into two major components. The minor component (40%) was shown to be methyl 9,12-epoxyoctadec-13-enoate by GLC and MS and by comparison of the component after hydrogenation with authentic methyl 9,12-epoxystearates (MS, TLC and GLC).

The major component (60%) had ECLs of 28.3 (83%) and 31.3 (17%). A small sample of authentic methyl ricinstearolate had an ECL of 28.4.

A sample of this major product (610mg) was refluxed for 45min in a 50% aqueous methanolic solution of potassium hydroxide (2M, 10ml). The recovered acids (570mg) in anhydrous THF (2ml) were added to liquid ammonia (200ml). The solution was stirred and clean lithium metal added at a rate to maintain a blue colouration in the solution. After 2hr, excess lithium was carefully destroyed by the addition of water, the ammonia was allowed to evaporate, and the product (470mg) was recovered by ether extraction.

Esterification of the product gave material (479mg) which showed 2 peaks on GLC with ECLs 26.5 (82%) and 28.3 (18%) corresponding to monoene and monoyne hydroxy esters respectively. The required 9-hydroxyoctadec-trans-12-enoate (ECL 26.5, IR 970cm^{-1}) was purified by Ag^{+} prep TLC(PE40).

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