THE ANALYSIS AND SYNTHESIS OF TRIGLYCERIDES

by

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SYNOPSIS

The first part of this thesis deals with the analysis of triglyceride mixtures and two aspects of triglyceride analysis have been investigated. Firstly, the high temperature gas-liquid chromatographic separation of triglyceride mixtures has been carried out using selected high temperature (325°) stationary phases (OV-17, OV-22, Dexsil 300GC, PmPE (high polymer) and Poly-S-179). They have been evaluated as to their resolution and quantitative recovery of model monoacid triglyceride mixtures consisting of saturated and unsaturated triglycerides. Low separation factors, combined with almost quantitative recovery of the sample makes OV-17 one of the stationary phases of choice when separating triglyceride mixtures on the basis of molecular weight. Poly-S-179 has been shown to have sufficient polarity to permit improved separation of triglyceride mixtures based on the degree of unsaturation. Secondly, a method has been developed for the quantitative determination of the fully saturated triglyceride composition of fats. The method involves a quantitative oxidation of the unsaturated triglycerides, in the fat, to a mixture of α-hydroxy oxo and dihydroxy compounds, using aqueous alkaline potassium permanganate and a phase transfer catalyst in a two phase reaction. The fully saturated triglycerides are fractionated from the oxidation products using column chromatography and quantified by gas-liquid chromatography. The oxidation procedure is particularly useful for the analysis of fats

of high mono-unsaturated triglyceride composition and for triglyceride samples of varying chain length and polarity.

The second part of the thesis deals with finding the best experimental details for the synthesis of structurally pure triglycerides, using 1,3-dibromo propan-2-ol (I) as substrate. Previous work in this field, particularly the related nucleophilic substitution reactions, has been repeated, and the products examined by modern analytical and spectroscopic methods. As a result, it has been found that the substrate (I) must initially be converted into the 2-acyl derivative. For the subsequent nucleophilic substitution by the carboxylate anion at carbons 1- and 3 a judicious choice of not only solvent, relative concentrations of reactants and temperature but also a suitably exposed nucleophile is important. For the maximum yield of triglyceride a 50mole% excess of tricaprylmethylammonium carboxylate (which provides a highly lipophilic counter-ion, and consequently a more 'naked' carboxylate ion), a non-polar solvent (hexane or toluene) at reflux temperature and a reaction time of 3 hours have been found to be necessary. Using the above reaction conditions, the following symmetrically substituted diacid triglycerides have been synthesised; glycerol 1,3-distearate-2-palmitate, glycerol 1,3-distearate-2-oleate, glycerol 1,3-distearate-2-acetate, glycerol 1,3-diacetate-2-palmitate, glycerol 1,3-dipalmitate-2-oleate and glycerol 1,3-dioleate-2-palmitate. In addition, a triacid triglyceride-glycerol 1-stearate-2-palmitate-3-oleate and an unsymmetrically substituted diacid triglyceride-glycerol 1-stearate-2,3-

dipalmitate have been synthesised to demonstrate the overall utility of the method. The structural purity of all of the synthesised triglycerides has been demonstrated using an enzymatic lipolysis procedure.

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General Introduction to Fats and Oils.

Fats and oils have been recognised as a separate category of foodstuffs since prehistoric times and are generally considered to refer to substances which have a similar chemical structure and which have the same metabolism in the animal body. They play an important role in the human diet, besides providing calories they act as vehicles for such vitamins as A, D, E and K. They are also the source of several of the essential fatty acids such as linoleic, linolenic and arachidonic acids. They have the unique quality of improving palatability of foods, and because of this they are used in a wide variety of foods. According to Markley,[1] the use of fats as foods is probably instinctive; the application of fats as illuminants, in cosmetics, in medicinals and for use as lubricants, dates back before our earliest records of civilised man.

Chevreul[2], as early as 1823 was able to establish the fact that the main component of fats and oils is the triester formed from one mole of glycerol and three moles of fatty acid. These triesters of glycerol are known as triglycerides. In fact, Schule had obtained glycerol in 1779 by the saponification of olive oil with litharge, but he did not recognise how this fact was related to the structure of fats and oils. Fats are considered as those substances which are solids at ordinary temperatures, while oils are liquids under similar circumstances, however, what may be classed as a

fat in one locality may be considered as an oil in a warmer climate.

The three most important conditions which regulate the nature of a fat are:-

1) The chain length of the fatty acids.

2) The degree of unsaturation of the fatty acids.

3) Their positional distribution among the three hydroxyl groups of the glycerol backbone.

Generally, those fats that have a high proportion of saturated fatty acids are solids at ambient temperatures and those in which the unsaturated fatty acids are preponderant are liquid. Nature endows every oil and fat with a certain distribution pattern of fatty acids among the glycerides, which also determines their consistency as either solid or liquid. Because of this distribution, the use of any given oil or fat is limited. The fatty acids present in animal and vegetable fats are predominantly composed of fatty acids having an even number of carbon atoms. Furthermore, they consist almost exclusively of straight chain acids rather than branched chain components, in a few isolated cases ring compounds are found to occur as part of the fatty acid molecule.

The fatty acids most frequently occurring as components of natural fats and oils can be classified into several series. The first of these is the saturated fatty acid series, they have no unsaturated linkages and cannot be altered by hydrogenation or halogenation. The second series is characterized by the presence of one double

bond and is called the monoethenoid series. A third group is the diethenoid series which is characterized by two unsaturated linkages. The tri- and tetraethenoid acids may be classified under the general group of polyethenoid acids. The fatty acids having more than one double bond make up some of the most important of the fatty acids and since they cannot be synthesised by the higher animals, and some of them are required by the animal, they are of considerable importance from a nutritional standpoint.

The saturated fatty acids have the empirical formula $C_nH_{2n}O_2$. In the case of natural fats, n is, in almost every case, an even number. A number of odd-carbon acids occur in the isoacids in wool fat.[3]

The monoethenoid acids have the general empirical formula of $C_nH_{2n-2}O_2$. The most common acid is oleic acid (octadec-9-enoic acid) which is found in most oils and fats. The simplest monoethenoid acid found naturally is crotonic acid (but-2-enoic acid) found in the inedible croton oil. Oleic acid is so widely distributed in natural oils to be considered the predominant acid. It is present to the extent of nearly 85% in olive oil and 74% in cashew kernel oil. It also seems to be the chief component of the fats of warm blooded animals in contrast to palmitoleic acid (hexadec-9-enoic acid) which is largely present in the fat of cold blooded animals. The monoethenoid acids can exhibit cis/trans isomerism, but it is unusual for the trans isomers to be found in natural products.

The acids which have more than one double bond are of

great importance in animal nutrition and for industrial uses. They include the so called essential fatty acids which are required by animals since they cannot be synthesised from other fatty acids or carbohydrates. They used to be of considerable importance in the paint industry as well as wherever drying oils are employed. It is by virtue of the relative ease with which such acids can be hydrogenated that the vegetable shortening and margarine industries have been able to develop. Linoleic acid is the diethenoid fatty acid that is ordinarily found in fats and oils. Many of the common food oils may contain 50% or more of the acid (hempseed, poppyseed, sunflower seed, cotton seed and soybean oils). The absence of large amounts of this acid in animal fats is apparently related to the inability of the animal cells to synthesise acids with more than one unsaturated linkage, or at least, to form it at a rate demanded by the growing organism.[4]

In order to extend the use of fats and oils, such processing steps as fractionation, hydrogenation and interesterification, or a combination of these, are usually applied. Fractionation separates an oil or fat into a solid and liquid fraction, each of which can be used separately. Hydrogenation converts a liquid oil into a semi-solid or solid fat, thus extending its use. Interesterification alters the original order of distribution of fatty acids on the glycerol backbone, producing fats with different melting and crystallisation characteristics than the parent.

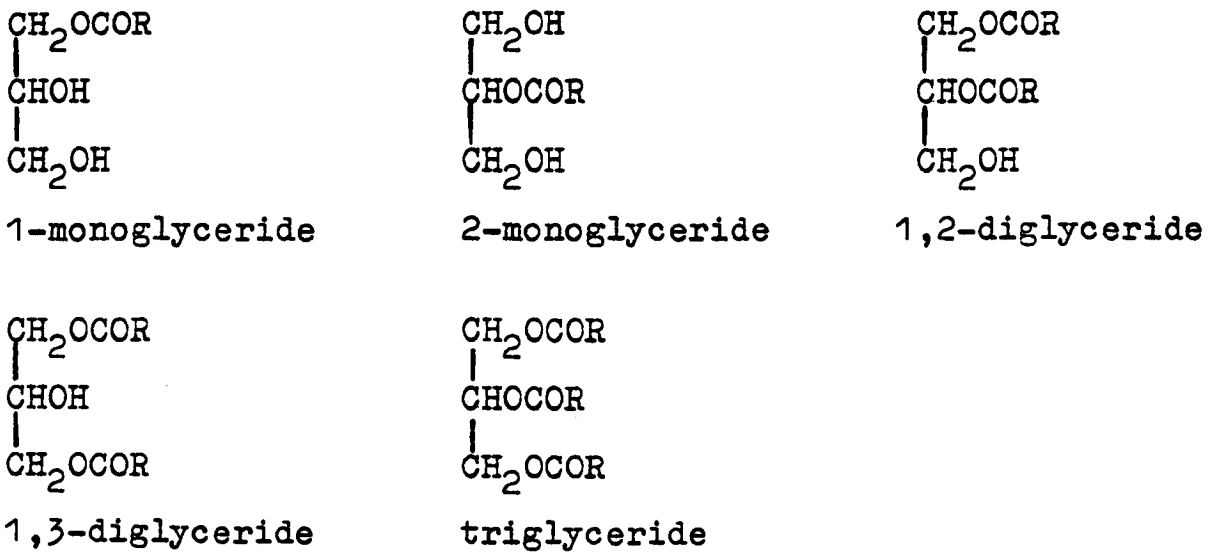
PART 1: THE ANALYSIS OF TRIGLYCERIDES

Chapter 1

1.1 Introduction to the analysis of fats and oils.

Five derivatives are possible on the esterification of glycerol with a fatty acid (RCO_2H) and these are shown in Figure 1.

Fig.1 Esterification products of a fatty acid with glycerol.



In mammalian tissues triglycerides are present to the largest extent, but mono- and diglycerides may also be present in certain tissues.

The number of distinct triglycerides, N, that can be prepared from n different fatty acids can be shown[5] to be given by the expression:-

$$N = \frac{(n^2 + n^3)}{2}$$

in which only positional isomers are distinguished,

enantiomorphic forms are not. If only the major component acids are considered, a large number of natural fats contain at least four, including palmitic, stearic, oleic and linoleic, which means that 40 different triglycerides may be present in the sample. If the two primary hydroxyls are esterified with different fatty acids, the central glycerol carbon atom becomes asymmetric and such triglycerides can exist in two enantiomorphic forms which adds to the total number of possible individual triglycerides. The complete analysis of a complex triglyceride mixture, as found in oils and fats, using only one technique is not possible, since it is necessary to determine:-

(a) The overall fatty acid composition.

(b) The distribution of these acids between the various triglycerides present.

(c) The distribution of the acids between the primary and secondary hydroxyls, within each mixed triglyceride molecule.

(d) The distribution between the two primary hydroxyls of each asymmetric, mixed triglyceride molecule.

Instead, a number of techniques must be employed and the combined information enables the composition of the triglycerides in the sample to be determined. Some of the methods of analysis are outlined below.

(a) A number of methods are available for fatty acid analysis[6], but for most purposes the speed and high resolution of gas-liquid chromatography (GLC) renders it superior to other methods. It is very versatile and

probably the easiest method to operate for quantitative analyses, and in biological work the small size of the sample required is a further advantage. James[7] has comprehensively reviewed the application of GLC to fatty acid analysis.

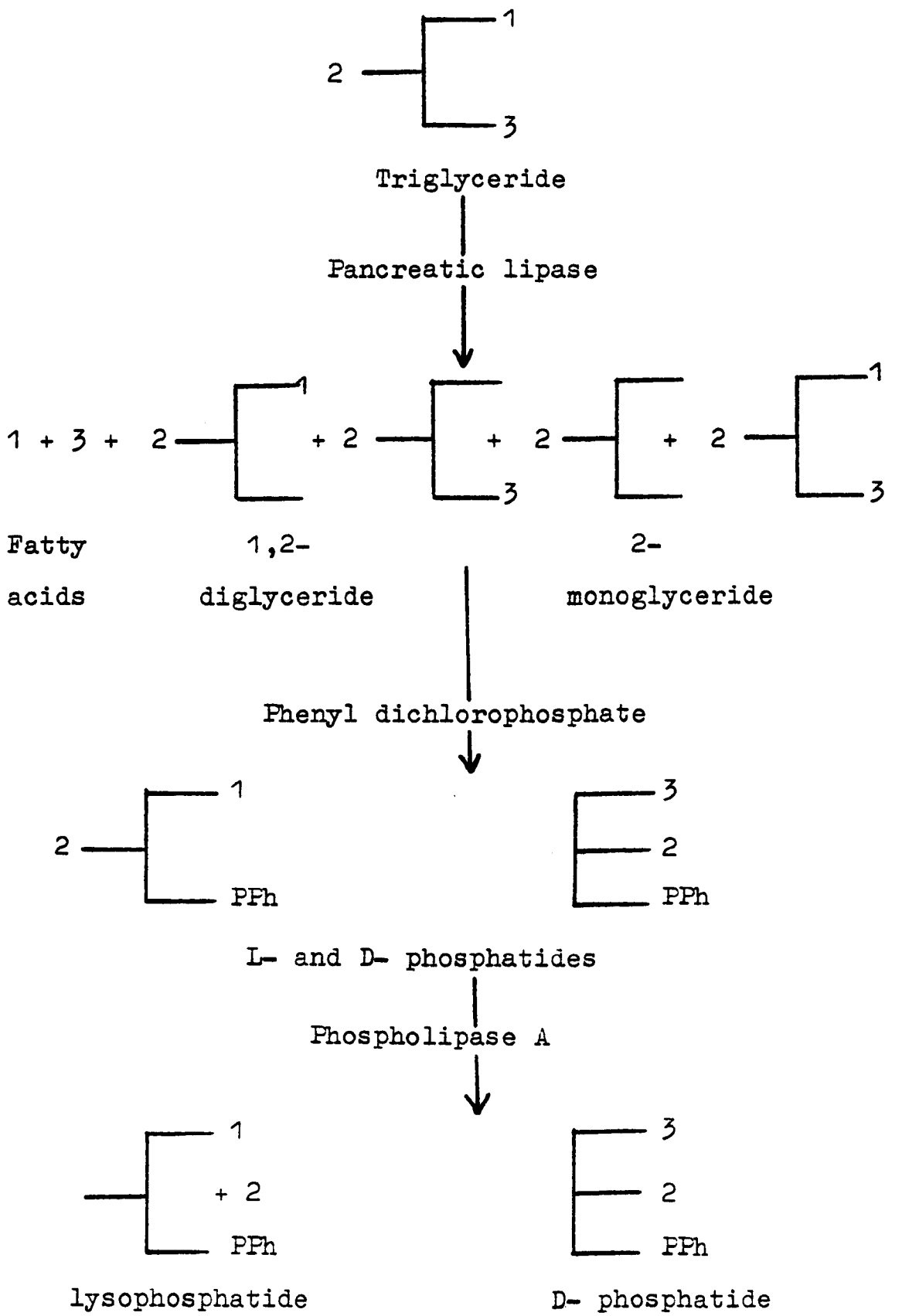
(b) The two methods that have gained most importance for the distribution of fatty acids between the various triglycerides are the separation of triglycerides by degree of unsaturation on silica gel impregnated with silver nitrate using column[8] or thin layer chromatography (TLC)[9]; and by molecular weight using GLC.[10] Neither of these methods, alone or in combination can effect the complete resolution of a triglyceride mixture. Only a limited number of positional isomers can be separated by silver nitrate chromatography and a number of triglycerides are known with different fatty acid compositions but with the same molecular weight and containing the same number of double bonds.

(c) Undoubtedly the simplest technique for determining the distribution of fatty acids between the primary and secondary positions of a triglyceride is to subject it to hydrolysis by pancreatic lipase.[11,12] This enzyme catalyses the hydrolysis of fatty acid residues from the primary positions of the triglyceride; so the 2-monoglyceride produced contains the acids origi nally present at the 2-position of the triglyceride. The other acids, known to be present from the overall fatty acid analysis must therefore have been present at the 1- and 3-positions.

The positional specificity of pancreatic lipase has been demonstrated by the use of synthetic mixed triglycerides.[11,12] The partial glycerides produced are liable to undergo acyl migration, so that the acids of the 2-positions, by migration to the 1- or 3-positions, may be hydrolysed and appear in the free fatty acids. There is the additional complication that different acids may be hydrolysed at different rates from the primary positions.[13,14] The anomalous behaviour of triglycerides containing short chain acids is now well established.[13,14]

(d) The work of Brockerhoff[15] introduced a method in which the fatty acid composition of the 1-position may be determined independently of those at the 3-position. The method is outlined in figure 2 and involves removing one fatty acid residue from the triglyceride with pancreatic lipase followed by converting the diglyceride to a phosphatidyl phenol with phenyl dichlorophosphate and subjecting this to hydrolysis with phospholipase A of snake venom. Since the hydrolysis is stereospecific, only the L-isomer is attacked. The fatty acids found in this lysophosphatidyl phenol are derived from the original 1-position of the triglycerides. The composition of the acids of the 2-positions is known from that of the 2-monoglycerides produced in the original hydrolysis; and the composition of the 1-, 2- and 3-positions is known from the overall fatty acid analysis of the original fat. The composition of the 3-position may then be calculated by difference.

Fig. 2 Differentiation of the 1- and 3-positions by the Brockerhoff method.



Several modern techniques have been applied to the analysis of fats and oils to provide reliable data on the triglyceride compositions. Two such techniques are carbon-13 nuclear magnetic resonance spectrometry (^{13}C NMR) and high performance liquid chromatography (HPLC).

(^{13}C NMR)

The quantitative analysis of mixtures of organic compounds is carried out routinely using a variety of analytical techniques. NMR so far has been competitive only in a few cases with more sensitive and less expensive techniques such as GLC, ultra-violet (UV) and infra-red (IR) spectroscopy, etc. and is usually limited to those cases where the orders of magnitude of the constituents to be analysed are comparable. The strength of NMR, on the one hand, is its selectivity combined with its power to detect as well as identify an unknown sample. The following example illustrates how ^{13}C NMR could be used as an alternative to GLC in a typical quantitative problem encountered in food technology. Linseed oil which contains mainly stearic, oleic, linoleic, linolenic and a small amount of palmitic acid was investigated by ^{13}C NMR.[16] The sample was run as a 0.04M solution in $CDCl_3$ containing $Cr(Acac)_3$ and the fatty acid composition of the oil was subsequently determined and showed good agreement with the results from a GLC analysis. The

information also enabled the degree of unsaturation, usually expressed as the iodine number, to be determined.

HPLC

The recent rapid development of HPLC has been of great importance in many areas, but its potential for lipid analyses is still under active investigation. One of the main problems has been the detection of non-UV absorbing lipids in effluents of liquid chromatography columns,[17] particularly when gradient elution is used. The most widely used approach was evaporation of the solvent, followed by flame-ionization detection of the solute on a moving wire. Only very few of these prototypes have been developed into commercially available models, and even these are often difficult to operate and have relatively low sensitivity. As a consequence they are not suitable for routine analysis even though they have been the subject of much research.[18-23] UV detection at 210 nm is not generally applicable because of the large number of solvents and solutes that have strong absorptions at that wavelength. The refractive index (RI) detector is not sensitive enough and a thermally stable system is needed for maximum use of the detector. Also, the response of the RI detector is greater for saturated glycerides than for unsaturated ones which makes accurate quantitation a severe problem. An IR detector is finding greater use as a good alternative to the RI detector.

An important advantage of HPLC is that fractions can be collected and re-analysed using other solvent systems or alternative techniques.

In recent years, a few papers on the HPLC separation of triglycerides have appeared in the literature, all of which deal with reverse phase chromatography.[24-28] Heslof,[29] using two different solvent systems, completely separated model compounds differing by two methylene groups, he also extended the separations to coconut oil and palm kernel oil.

The separation of triglyceride mixtures on the basis of their total degree of unsaturation by the addition of halogen to the double bonds has been reported.[30] Separations are improved and the technique allows the use of a UV absorption detector which shows a very high sensitivity towards halogen derivatives of poly-unsaturated glycerides.

An interesting method has been described[31] for the separation of triglyceride mixtures including the positional isomers 1,3-disaturated-2-unsaturated (SUS) and 1-unsaturated-2,3-disaturated (USS) by argentation HPLC. The quantitation of trisaturated (SSS), SUS and USS in fat mixtures, using an internal standard is described. The separations were carried out on a column packed with 10% silver nitrate on Partisil 5 using benzene at 6.8° as the mobile phase.

The preceeding introduction has shown that no one technique can provide the necessary information on the triglycerides present in a fat, instead a combination of

methods must be used.

Two particular methods;

(1) High temperature GLC separations,

(2) Phase transfer (PT) catalysed permanganate oxidation of unsaturated triglycerides,

have been studied to improve and extend their usefulness for triglyceride analyses.

Chapter 2

High temperature GLC separations of triglycerides.

2.1 Introduction

Analytical techniques are becoming increasingly sophisticated and the separation of triglycerides by GLC is amongst one of the modern analytical methods available to the lipid chemist. It has quickly become established as an indispensible technique since its development by Martin and James[32] in 1952. The GLC separations of triglyceride mixtures are by molecular weight[33-36] or the more commonly termed carbon number separations (according to the total number of carbon atoms in the fatty acid chains.). This is a severe limitation of GLC analysis in that unsaturated triglycerides are not distinguished from the saturated triglycerides having the same carbon number. However, the triglyceride analysis does give information about the distribution of the fatty acids within a given fat, which cannot be obtained by fatty acid analysis or even lipolysis. For example, a simple fat containing only palmitic and stearic acid can have the same fatty acid composition but widely different triglyceride composition depending on how the acids are distributed. For this reason GLC can be an invaluable method for distinguishing between interesterified and non-interesterified fats. Because of the low vapour pressure of triglycerides, present methods are based upon the use of short columns

of low stationary phase loadings and using high temperatures. Excellent reviews by Litchfield[37] and earlier by Pierce[38] optimise conditions relating to the quantitative analysis of triglyceride mixtures.

Many compounds have been reported as suitable for use as stationary phases in GLC, but little information is available as to their applicability to the separation and quantitative recovery of triglyceride mixtures. Polyesters of succinic acid, a cyanopropyl phenyl siloxane[39] (Silar 5CP) and more recently a polar siloxane[40] (Silar 10C) have been used for the resolution of saturated and unsaturated diglycerides. Unfortunately they have only moderate thermal stability and similar separations using triglycerides cannot be satisfactorily effected. Silar 10C showed some resolution of triglycerides by degree of unsaturation but the columns could only be used for a few months at 250-270°.

The major criterion in selecting a stationary phase suitable for triglyceride analysis is its thermal stability with only those which can operate at 325° or greater being considered suitable. Hence OV-1, OV-101, OV-17, OV-22, SE-30, SE-52, JXR, Dexsil 300 and PmPE have been the stationary phases of choice, but all tend to be non-polar compared to the polyester phases which are used in fatty acid analysis.

Liquid crystals are becoming increasingly important as stationary phases in GLC, but as yet there are insufficient stationary phases with both good separation properties and

the ability to operate at temperatures above 200°. High temperature liquid crystalline phases usually exhibit high molecular weights and low vapour pressures, therefore they do not easily bleed off the column and can be used in temperature programmed work.[41] Separations of polycyclic aromatic hydrocarbons, naphthalene homologues and their geometric isomers[42-45] have been reported. They have also been used in the separation of isomers of benoxaprofen,[46] epimers of steroids[47] and polynuclear azaheterocyclic compounds. Most of the separations were according to boiling point which is similar to that observed on conventional non-polar stationary phases. A liquid crystal stationary phase, N,N'-Bis-(p-phenylbenzylidene)α,α'-bi-p-toluidine (BPhBT), has been reported[43] with a nematic range of 257-403°, it has been used at 275° without excessive deterioration of column performance being observed.

In the present study, several thermally stable stationary phases have been evaluated as to their resolution and quantitative recovery of model monoacid triglyceride mixtures, consisting of saturated and unsaturated triglycerides. The stationary phases evaluated were OV-17, OV-22, Dexsil 300GC, PmPE (High polymer), a polyphenyl ether sulphone phase (Poly-S-179) and BPhBT. The comparison should enable a single stationary phase to be nominated as most suitable for triglyceride analysis. The upper thermal limits for the stationary phases are given in table 1.

Table 1. Recommended upper thermal limits for the stationary phases.*

Stationary phase	Type of phase	Recommended thermal limit
OV-17	50% methyl phenyl silicone	375°
OV-22	35% methyl phenyl silicone	350°
Dexsil 300GC	Carborane methyl silicone	500°
PmPE (High polymer)	Polyphenyl ether	450°
Poly-S-179	Polyphenyl ether sulphone	400°
BPhBT	Liquid crystal	275°

* Ex Phase Separations Ltd.

2.2 Results and Discussion.

The response of the flame-ionization detector is known to vary with load levels.[48] No attempt was made to ascertain this variation or to correct for it, instead, a constant weight of the reference triglyceride mixture was used to evaluate the stationary phases. The response factors given in table 2 relate the actual composition of the test mixture to the area response recorded on the chromatogram. These response factors give an accurate indication of sample recovery.

The Van Deemter equation[49] provides a useful guide for optimising parameters related to column design and operation. Practical considerations show that the best resolutions are obtained with low stationary phase loadings on solid supports of small uniform mesh. There is, however, a limit to the minimum mesh size of the solid support which can be used, since the increased pressure drop which accompanies small mesh size is particularly critical in triglyceride chromatography. Standard diatomaceous earth supports are commonly used, but special chemical treatment is necessary to reduce adsorption effect which cause tailing. A commercial solid support, Supelcoport 100/120 mesh is chemically treated and is suitable for the preparation of column packings.

The BPhBT stationary phase was found to be unsuitable for triglyceride analysis. The upper temperature limit of 275° was too low to elute the higher molecular weight

Table 2. Experimentally determined weight and molar response factors on 2% loadings of the stationary phases in 0.45m x 2.5mm I.D. glass columns.

Stationary phase	Weight calibration factors (F_w)						Molar calibration factors (F_m)					
	36**	42	48	54	$54^{3=}$***	$54^{6=}$	36	42	48	54	$54^{3=}$	$54^{6=}$
Theoretical*	1.00	0.97	0.95	0.93								
OV-17	1.00	0.99	1.08	1.24			1.00	0.88	0.85	0.93		
OV-22	1.00	0.99	1.11	1.26			1.00	0.87	0.87	0.92		
Dexsil 300GC	1.00	1.01	1.24	1.55			1.00	0.89	0.97	1.10		
PmPE	1.00	1.25	1.97	4.17			1.00	1.12	1.56	3.00		
Poly-S-179	1.00	0.78	0.85	1.24	1.70	2.63	1.00	0.89	0.88	1.14	1.22	1.90

* Theoretical F_w response factors for specific triglycerides, calculated by assuming that all of the injected sample reaches the detector and that the flame-ionisation detector response is proportional to the hydrocarbon content of each triglyceride.

** Total number of carbon atoms in the fatty acid acyl groups of each triglyceride, referred to as the carbon number.

*** Refers to the total number of alkenic linkages in each triglyceride. Therefore, for monoacid triglycerides, $54^{3=}$ is triolein.

triglycerides. Excessive use beyond 275° resulted in deterioration of the column, the stationary phase was driven off almost completely when temperatures in the range 325-350° were used. Even the triglycerides that were eluted at 275° exhibited excessive solute retention and broad peak elution.

On short columns (0.45m), OV-17, OV-22, Dexsil 300GC and PmPE separate triglycerides only on the basis of carbon number. The separation factors (ΔC values) are given in table 3. Good resolution, combined with good recovery, is obtained for most of these stationary phases in an acceptable analysis time of less than 45min. However, PmPE gave unacceptably high response factors, indicating large losses of the higher molecular weight triglycerides, and is therefore unsuitable for use in the quantitative analysis of triglyceride mixtures. These losses could be due to pyrolysis during vapourisation of the sample, on-column degradation, adsorption or condensation. Since the experimental conditions were the same for all of the stationary phases, then adsorption of triglycerides onto the column is the most likely cause of sample loss. No low molecular weight fragments could be detected which would indicate pyrolysis or degradation.

The ΔC values are a more practical guide than the number of theoretical plates in determining if a specific column would yield a desired separation. Litchfield et al[50] defined an empirical ΔC value equal to the minimum carbon number difference between two triglycerides that could be

Table 3. Experimentally determined separation factors in the C_{42-48} and C_{48-54} regions of the chromatogram.

Stationary phase*	ΔC_{42-48}	ΔC_{48-54}
OV-17	1.9	2.1
OV-22	2.2	2.6
Dexsil 300GC	1.9	2.1
PmPE	1.9	2.1
Poly-S-179	2.1	2.2

* All the stationary phases were used as 2% (wt/wt) coatings on Supelcoport (100-120 mesh).

separated with baseline resolution in the C_{42-48} region of the chromatogram. In the present study, ΔC values in both the C_{42-48} and C_{48-54} regions of the chromatogram were calculated since most natural fats contain C_{48}, C_{50}, C_{52} and C_{54} triglycerides. A linear relationship exists between retention time and the number of carbon atoms in a homologous series of compounds, for temperature programmed operation. However, because of a continuously decreasing difference in volatility between higher molecular weight pairs of triglycerides, they are eluted at progressively closer intervals and so the linear relationship exists only over a limited range of the triglyceride series. This is shown by an increase in the ΔC_{48-54} value compared to the ΔC_{42-48} value for all of the stationary phases evaluated. The low separation factors obtained on the OV-17 column, combined with almost quantitative recovery of the sample, make it one of the stationary phases of choice when resolving and quantitatively analysing triglyceride mixtures on the basis of molecular weight. The loading of 2% (wt/wt) stationary phase gave good recovery and resolution without having to utilise temperatures beyond 350° to elute the higher molecular weight samples. The resolution of the triglyceride mixture on a loading of 1% (wt/wt) stationary phase is given for comparison in table 4, as can be seen, there is a deterioration in sample resolution.

Poly-S-179 was the only stationary phase to resolve triglycerides of the same carbon number, but differing in

Table 4. Experimentally determined separation factors in the C_{42-48} and C_{48-54} regions of the chromatogram.

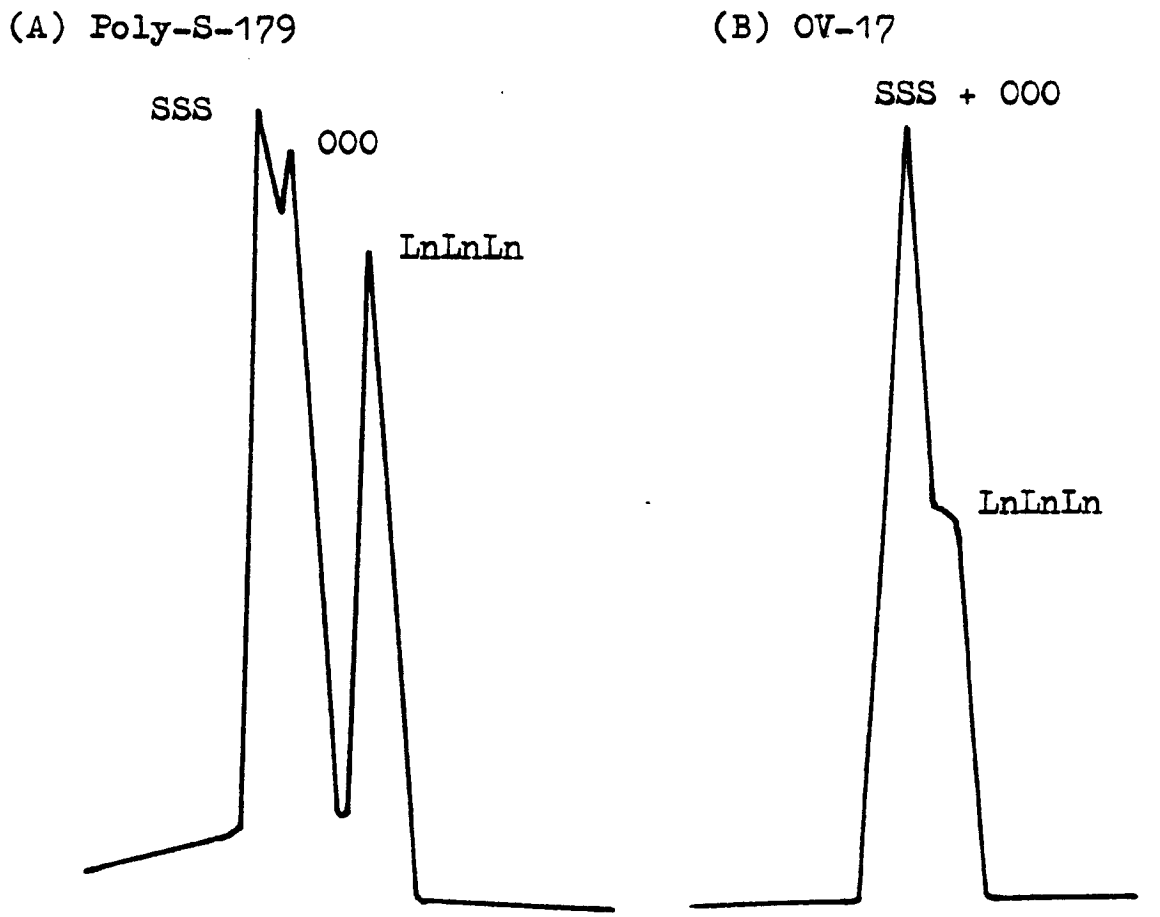
Stationary phase*	ΔC_{42-48}	ΔC_{48-54}
OV-17	2.1	2.4
OV-22	2.3	2.8
Dexsil 300GC	2.0	2.4
PmPE	2.1	2.4
Poly-S-179	2.2	2.5

* All the stationary phases were used as 1% (wt/wt) coatings on Supelcoport (100-120 mesh).

degree of unsaturation, on a 0.45m column. Figure 3 shows typical chromatograms for the resolution of a mixture of saturated and unsaturated triglycerides on Poly-S-179 and OV-17 phases. Using Poly-S-179 the triglycerides are eluted in order of increasing molecular weight and increasing degree of unsaturation. Attempts to increase the resolution of the C_{54} triglycerides by moving to longer column lengths met with only partial success, as the increase in resolution was accompanied by a marked increase in the amount of on-column degradation. On a 1.06m column containing 2% Poly-S-179, tristearin and triolein were completely resolved with baseline resolution, but the chromatogram showed marked amounts of broad ill-defined material eluting immediately before the tristearin peak making a quantitative analysis troublesome. This on-column degradation places a practical limit on column length. Weight and molar response factors were calculated for triolein and trilinolein on the Poly-S-179 phase and these are given in table 2. The attempted resolution of tristearin, triolein and trilinolein on a 0.45m column containing 2% OV-17 was unsuccessful. The saturated and unsaturated triglycerides were not resolved, with trilinolein distorting the descending edge of the main C_{54} peak.

The reference mixture of simple monoacid triglycerides was eluted from the 2% Poly-S-179 column at a temperature approximately 25° below that on the other phases. This is probably due to the low affinity of the triglycerides, which have a non-polar character, for the polar stationary

Fig. 3. Chromatogram of a synthetic mixture of tristearin (SSS), triolein (OOO) and trilinolein (LnLnLn) on Poly-S-179 and OV-17 stationary phases.



Columns:- 0.45m x 2.5mm I.D. glass columns packed with 2% loadings of stationary phase on Supelcoport (100-120 mesh).

Operating conditions are given on Page 27.

phase. The response factors for the unsaturated triglycerides increased with the degree of unsaturation, indicating an increase in irreversible adsorption onto the column and an increase in on-column degradation.

In conclusion, a polyphenyl ether sulphone stationary phase (Poly-S-179) has sufficient polarity to permit improved separations of triglycerides based on the degree of unsaturation. It also has sufficient thermal stability to allow its regular application to triglyceride analysis. For separations based on carbon number, the OV-17 phase must be regarded as a phase of choice for regular work.

2.3 Experimental

2.3.1 Materials

Dexsil 300GC, OV-17 and OV-22 were purchased from Phase Separations Ltd. (Queensferry, Great Britain). PmPE (High polymer) was purchased from Varian Aerograph, Walnut Creek, Calif., U.S.A.. Poly-S-179 was purchased from Supelco. Inc. (Bellefonte, Pa., U.S.A.). Supelcoport (100-120 mesh) was purchased from Supelco. Inc. (chemically treated to reduce adsorption effects). Trilaurin, trimyristin, tripalmitin, tristearin, triolein and trilinolein (all 99% purity) were purchased from Sigma Chem. Co. Ltd. (St. Louis, Mo., U.S.A.).

2.3.2 Gas-liquid chromatography

A Pye Unicam model 104 gas chromatograph equipped with flame-ionisation detectors was used for the analyses.

Column packings were prepared by coating the stationary phases onto the solid support, from chloroform solution, by the solvent evaporation technique.[51] Glass columns 0.45m x 2.5mm I.D. were filled with the column packings with the aid of a vacuum pump and a vibrator to ensure closely packed columns. The columns were satisfactorily conditioned by heating at 350° for 8hr with a nitrogen flow rate of 80ml/min. The injection port and detector temperatures were maintained at 350°. A reference saturated triglyceride mixture was prepared (Table 5) and analysed on each column. Analyses were continuously repeated until steady calibration factors were obtained. A temperature program in the range 220-350° at 3-4°/min with a nitrogen flow rate of 80ml/min was used to elute the triglycerides.

Table 5. Composition of the saturated monoacid triglyceride mixture.

Triglyceride	Weight taken to 100 cm^3(g)	Weight%	Mole%
Trilaurin	0.1763	25.39	29.92
Trimyristin	0.1728	24.91	25.94
Tripalmitin	0.1708	24.62	22.96
Tristearin	0.1740	25.08	21.18

2.3.3 Evaluation methods

2.3.3a Recovery of triglycerides

The recovery of the model saturated triglyceride mixture (Table 5) was evaluated by determining the weight and molar response factors for the eluted triglycerides. Peak areas were measured by triangulation, quantitative weight response factors (F_w) and molar response factors (F_m) for individual triglycerides were calculated by the internal normalisation technique. Here F_w= actual wt%/ area% and F_m= actual mole%/area%. A value of 1.00 was assigned to F_w and F_m for trilaurin (primary standard) which was assumed to be completely recovered from the column. The primary standard was then included in all calibration mixtures so that the calibration factors from all GLC runs were comparable.

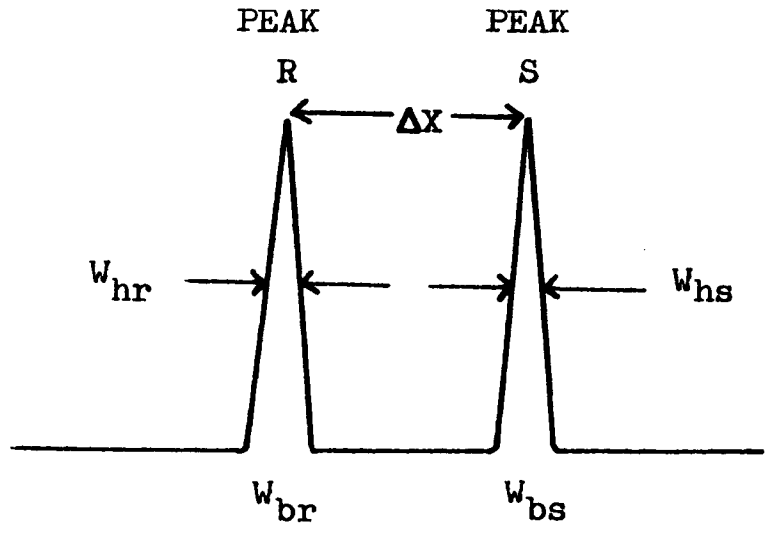
2.3.3b Resolution of saturated triglycerides

The resolution of the triglyceride peaks was measured by determining the separation factor $\Delta C,^{50}$ defined as the minimum carbon number difference between two adjacent triglycerides that can be separated with baseline resolution. (Figure 4)

2.3.3c Resolution and recovery of a saturated and unsaturated triglyceride mixture

The resolution of saturated and unsaturated trigly-

Fig. 4. Calculation of the Separation Factor ΔC



(a) Any two triglyceride peaks R and S with a difference in carbon number may be chosen.

(b) The average baseline width (W_{brs}) of triglyceride peaks in this region was found using the relationship $W_{brs} = 1.6(W_{hr} + W_{hs})$

(c) The distance (ΔX) between the apices of the two peaks was measured.

(d) The maximum number of peaks (M) which can be separated with baseline resolution is:-

$M = \Delta X/W_{brs} = \Delta t/1.6(W_{hr} + W_{hs})$.

(e) The minimum carbon number difference which can be resolved with baseline resolution (ΔC) is 6/M, therefore $\Delta C = 9.6(W_{hr} + W_{hs})/\Delta X$.

cerides of the same carbon number was determined by analysing a reference mixture of tristearin, triolein and trilinolein. Response factors for triolein and trilinolein were only calculated for stationary phases that resolved the reference mixture.

Chapter 3

Phase transfer catalyzed oxidation of unsaturated triglycerides.

3.1 Introduction

A small number of fatty acids gives rise to a comparatively large number of combinations of distinct triglycerides. The quantitative analysis of triglycerides in a fat has posed a difficult problem because of the similarity in physical properties of adjacent triglycerides in a series. The problem can be simplified, at the expense of obtaining less information, by several methods. For example, fractionation of the triglycerides can be carried out directly on the fat,[52-57] or fractionation carried out after oxidation of unsaturated fatty acid components.[58-60] A micro method has been described[61,62] which involves ozonolysis of double bonds, followed by catalytic reduction of the ozonides. The ozonides, as well as the aldehyde cores obtained on reduction were separated and quantitatively estimated by TLC.

Hilditch and Lea[63] determined the amount of fully saturated triglycerides as the neutral fraction recovered after oxidation with potassium permanganate ($KMnO_4$) in acetone. In a later study, Kartha[64,65] showed that the oxidation procedure used by Hilditch and Lea resulted in partial hydrolysis of the triglycerides, he avoided this hydrolysis by the addition of acetic acid to the acetone

and extended the method by fractionating the oxidised triglycerides as their magnesium salts. Since neither the oxidation nor the fractionation were entirely quantitative, the accuracy of the results is difficult to assess, and further work is needed to obtain quantitative results on the fully saturated triglyceride composition of complex triglyceride mixtures.

An alternative method for the determination of fully saturated triglycerides is to remove the unsaturated triglycerides by oxidation with $KMnO_4$ in a two phase reaction. However, reaction between two substances located in different phases of a mixture is often inhibited because of the inability of reagents to come together. The stirred heterogeneous mixture of triglycerides in dichloromethane and an aqueous $KMnO_4$ solution is an example where little or no oxidation of the unsaturated triglycerides is reported even when vigorous stirring is used.[66] Traditionally this problem has been solved by the use of an appropriate mutual solvent or a water miscible cosolvent such as an alcohol to obtain a homogeneous medium. However, if a hydroxylic solvent is selected the reaction may still proceed slowly owing to extensive solvation of the anions. The use of highly polar aprotic solvents such as dimethyl sulphoxide (DMSO), dimethyl formamide (DMF) and hexamethyl phosphoramide (HMPA) has permitted reactions to take place between inorganic and organic species, but those solvents often require absolutely anhydrous conditions and their recovery or complete removal is sometimes troublesome and

time consuming. A large increase in the surface area of reactants, exemplified by micellar systems[67] represent another useful way of speeding up reactions, but the preparation and particularly the breaking of emulsions[68] in order to isolate the reaction products can be a formidable task.

An alternative solution to the heterogeneity problem is to employ a phase transfer catalyst (PTC). The catalyst transfers the water soluble reactant across the aqueous-organic interface into the organic phase where a homogeneous reaction can take place. The requirements for a substance to function as a catalyst are that it must be lipophilic and have either a charge-diffuse or buried charge cation capable of pairing with an anion. Quaternary ammonium cations (Q^+) serve effectively as catalysts[69] and Herriott and Picker[70] have found that two factors favour catalyst efficiency. Firstly, a large number of carbon atoms to give a high lipophilicity and secondly a symmetrical disposition of these carbon atoms about the heteroatom. Apparently the more shielded is the positive charge, the more effective will be the catalyst. Other related systems are also effective catalysts, both quaternary phosphonium[66,71] and arsonium[72] compounds have been used. An alternative to the use of a quaternary heteroatom catalyst is the use of an agent which can complex an alkali metal cation, solvate it, and provide a lipophilic exterior which can be solvated by the organic medium, examples of these are crown ethers[73] and cryptates.[74-76]

The phase transfer function is a known equilibrium[77-79] between anions in an aqueous phase and anions associated with Q^+ in an organic phase. Q^+ cations in a non polar environment preferentially associate with large and minimally hydrated ions and also with anions having considerable organic structure. This selectivity is important to the reaction step since in anion transfer reactions it dictates which anion will predominate in the organic phase. Under normal conditions the PTC is not consumed but performs the transport function repeatedly. Several explanations have been proposed for the effectiveness of quaternary ammonium ions as catalysts[66,71,80,81] but the phase transfer mechanism proposed by Starks[66] accounts for the major features of the reactions. The subject of PTC has been reviewed[82-84] and the variables controlling efficient ion transfer have begun to be understood.

For the phase transfer catalysed permanganate oxidation of a complex triglyceride mixture to be useful for the determination of the fully saturated triglyceride composition of fats and oils then three factors need to be satisfied. Firstly, all of the unsaturated acyl groups in the triglyceride mixture should be oxidised. Secondly, there should be no loss of fully saturated triglycerides due to hydrolysis or interesterification. Finally, the fully saturated triglycerides should be easily isolated from the oxidation products. With these aims, the oxidation procedure was studied and applied to several fats and oils to determine if it could be an analytically useful technique.

3.2 Results and Discussion.

Several quaternary ammonium salts and a crown ether have been evaluated as to their ability to transfer permanganate ions from an aqueous phase into a dichloromethane phase. The anion partitioning equilibrium represented by equation (1) was measured to determine the relative amounts of the two anions associated with the quaternary ammonium salt.

Equation 1

$$(QX)_{org} + (MnO_4^-)_{aq} \rightleftharpoons (QMnO_4)_{org} + (X^-)_{aq}$$

(X^-)= halogen

The results are given in Table 6, the phase transfer agents are placed in only approximate rank order as some of the differences are small and probably not significant. The data shows that in all cases the quaternary ammonium salts are more efficient than crown ether complexation for the transfer of permanganate into dichloromethane. The quaternary ammonium salts also offer flexibility and overcome the disadvantages accompanying crown ether complexation. For example, their catalytic ability is applicable to all cationic species whereas a specific crown should be selected for each cation for optimum performance. They are cheaper and perhaps more important, non toxic, therefore "aliquat 336" was selected as the phase transfer agent for the oxidation of unsaturated triglycerides.

Table 6. *Efficiency of various phase transfer agents (PTA) for the transfer of permanganate from an aqueous to an organic phase.*

PTA	Mole% PTA combined with permanganate in the organic phase
Aliquat 336	97
Hyamine 1622	96
TBAB	95
CTAB	93
18-Crown-6	53

Aliquat 336:- Tricapryl methyl ammonium chloride

Hyamine 1622:- Diisobutylphenoxyethoxyethyl-dimethyl-benzyl-ammonium chloride monohydrate

TBAB:- Tetrabutyl ammonium bromide

CTAB:- Cetyl trimethyl ammonium bromide

18-Crown-6:- 1,4,7,10,13,16 hexaoxa cyclooctadecane

The oxidation of unsaturated triglycerides using neutral aqueous $KMnO_4$ and "aliquat 336" was unsatisfactory as a quantitative procedure for the elimination of unsaturated triglycerides from a complex triglyceride mixture. Fatty acid analysis of the oxidised triglycerides as their methyl esters (Table 7), showed the presence of a complex mixture of products which typically analysed as:- methyl 9,10- and 10,9-hydroxy oxo stearate (43%), methyl 9,10-dioxo stearate (5%) and methyl 9,10-dihydroxy stearate (trace). Cleavage products included monobasic and dibasic acids and secondary decomposition products, they represented about 50% of the total reaction products. The column fractionation of the fully saturated triglycerides from the oxidation products was time consuming, as it required two or more column separations to completely remove the dioxo and acidic products.

In contrast, the oxidation procedure using aqueous alkaline potassium permanganate and "aliquat 336" was satisfactory for the quantitative oxidation of unsaturated triglycerides into more polar derivatives. For example, Figure 5 shows the fatty acid analysis of cocoa butter triglycerides before oxidation and after fractionation from the oxidation products, there is a complete absence of unsaturated acyl groups in the oxidised sample.

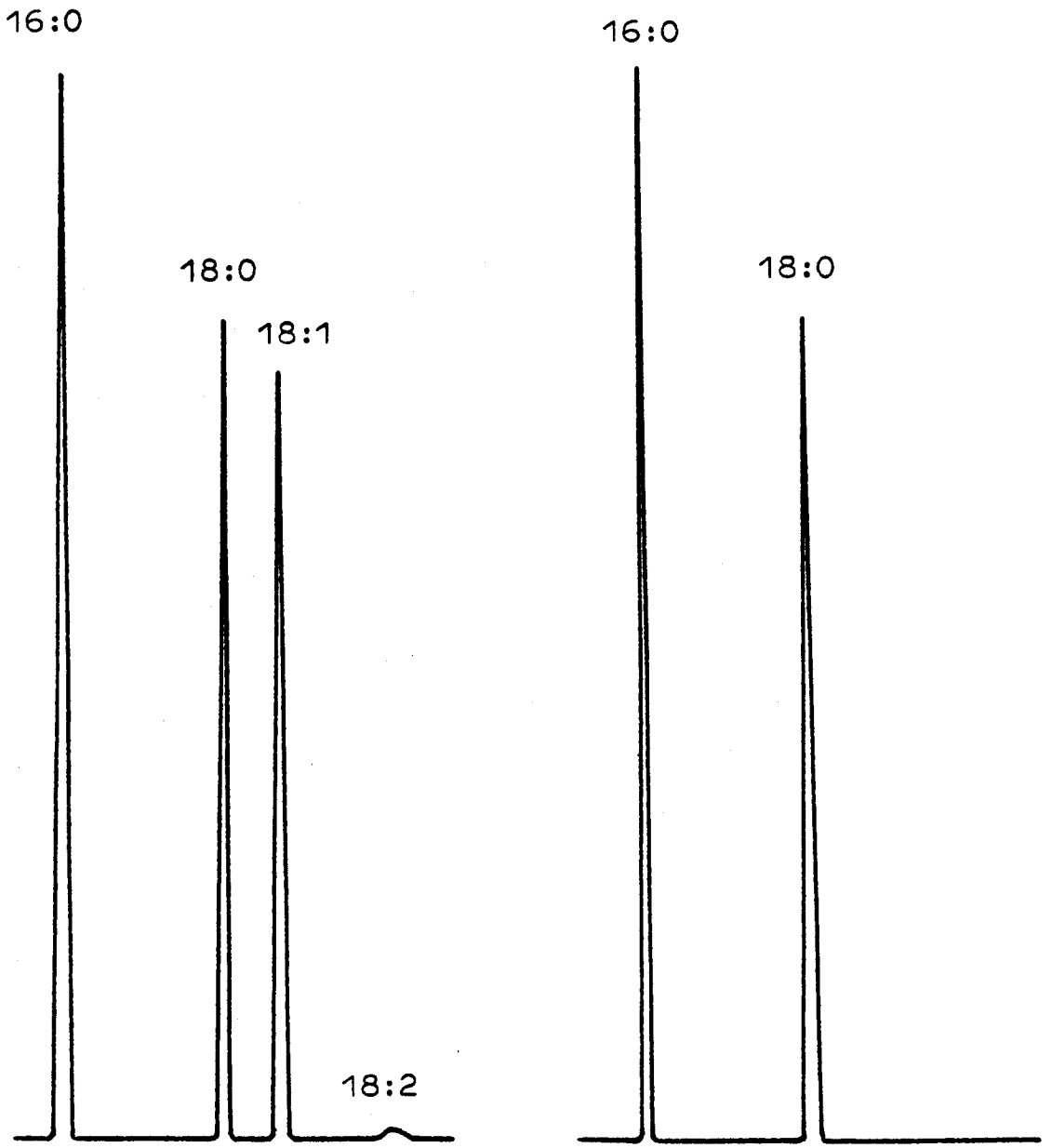
The optimum reaction conditions were determined using methyl oleate as a model unsaturated acyl group in a triglyceride and varying the mole ratios of the oxidant, sodium hydroxide and "aliquat 336." A mole ratio of 1 part

Table 7. Identification of the oxidation products using neutral aqueous $KMnO_4$ and "aliquat 336" in a two phase reaction.

TLC spot	Rf value	GLC retention time (min)	Identified as:-
1	0.0	Complex thermal fragmentation pattern	PTC
2	0.16	30.0	Methyl 9,10-dihydroxy stearate
3	0.41	14.0	Monomethyl azelaic acid
4	0.52	28.0	Mixture of methyl 9,10- and 10,9-hydroxy oxo stearate
5	0.68	8.0	Monobasic acid components, mainly nonoic acid
6	0.88	26.8	Methyl 9,10-dioxo stearate
7	0.90	22.4	Residual methyl oleate

N.B. Analytical details are given in the experimental section.

Figure 5. Fatty acid analysis of Cocoa Butter triglycerides.



(a) Methyl esters of fatty acids derived from cocoa butter before oxidation.

(b) Methyl esters of fatty acids derived from the neutral triglycerides fractionated from the crude oxidation mixture.

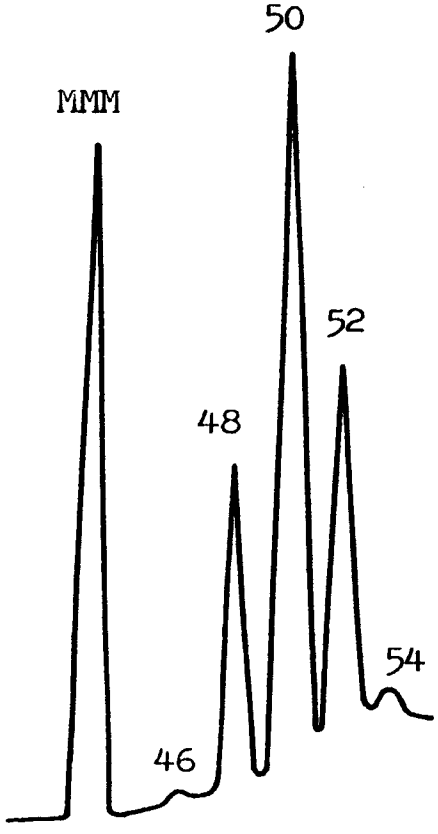
methyl oleate, 5 parts oxidant, 7 parts sodium hydroxide and 1 part "aliquat 336" gave complete oxidation of the ethylenic linkages in a two hour reaction at room temperature. The reaction products were 9,10- and 10,9-hydroxy oxo methyl stearate and 9,10-dihydroxy methyl stearate formed in approximately equi-molar amounts. Lowering the temperature of the reaction to 0-5° did not alter the ratio of reaction products but resulted in a decrease in the reaction rate. Increasing the mole ratio of sodium hydroxide from 7 parts to 55 parts only slightly increased the yield of 9,10-dihydroxy methyl stearate. Reducing the concentration of PTC reduced the rate of reaction. An excess of $KMnO_4$ was needed for complete reaction, this is particularly important when highly unsaturated fats are to be oxidised.

The oxidation with alkaline potassium permanganate and a PTC occurs because of the ability of the PTC to transfer both permanganate and hydroxide ion into the organic phase. Table 6 shows that 97 mole% of the catalyst is in the form of $Q^+MnO_4^-$ in the organic phase. With hydroxide ion, only 10 mole% of the catalyst is in the form Q^+OH^- the remainder is present as Q^+Cl^-. Therefore the partition coefficients of the permanganate and hydroxide anions between the two phases greatly favours the formation of $Q^+MnO_4^-$ ion pairs. As a consequence, complete formation of the dihydroxy compound cannot be achieved using the present catalyst. Experimentally the reactions are very easy to perform, and the saturated triglycerides

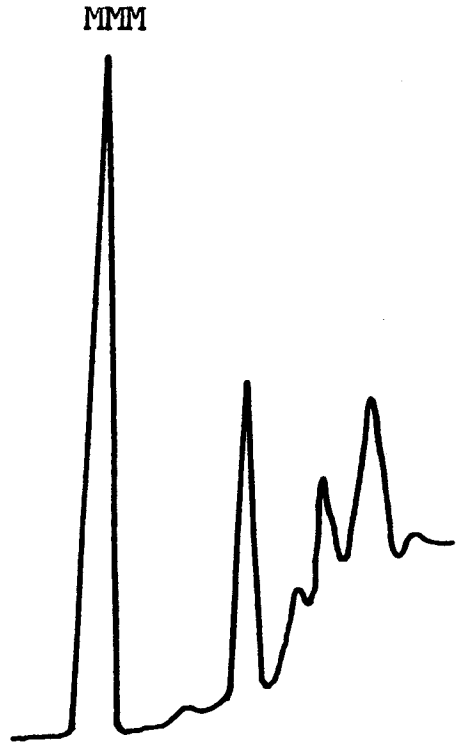
are readily isolated from the two phase reaction mixture. The possible loss of saturated triglycerides by hydrolysis or interesterification during the oxidation procedure and subsequent column fractionation was checked by GLC. A known weight of trimyristin (0.09g) was added to a fat sample prior to oxidation. Trimyristin was selected as the reference compound because none of the fats examined contained any C_{42} triglycerides. Recovery of the trimyristin after oxidation and fractionation was quantitative, showing that fully saturated triglycerides were stable to the oxidation conditions.

Typical gas-liquid chromatograms of samples before and after oxidation are shown in Figures 6 and 7. The crude oxidation mixture from the stearin fraction of palm oil (Figure 6B) chromatographs as a number of ill-defined peaks from which no quantitative information can be extracted. This chromatogram demonstrates the necessity of a fractionation step. The fully saturated triglyceride compositions for a number of fats, determined by the PTC oxidation procedure, are given in Table 8. Quantitative molar calibration factors for the GLC analyses were determined using a reference triglyceride mixture. The fully saturated triglyceride compositions given in Table 8 compare favourably with literature results determined by silver nitrate TLC.[85] The oxidation method is particularly useful for the analysis of fats of high mono-unsaturated triglyceride composition and for triglyceride samples of varying chain length and polarity when overlap

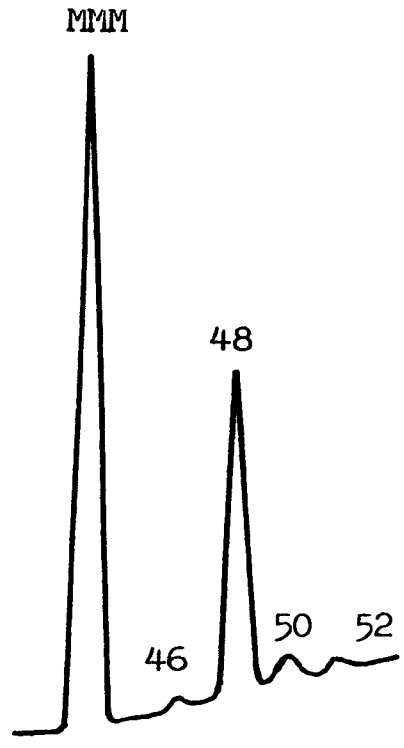
Figure 6. Chromatograms used in the determination of the fully saturated triglyceride composition of the stearin fraction of Palm Oil.



(a) Total triglycerides in the stearin fraction of Palm Oil

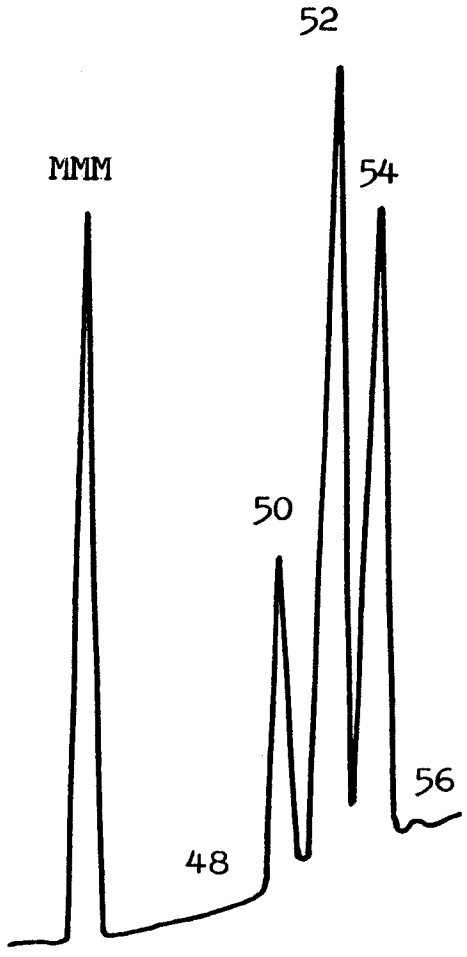


(b) Crude oxidation products before fractionation

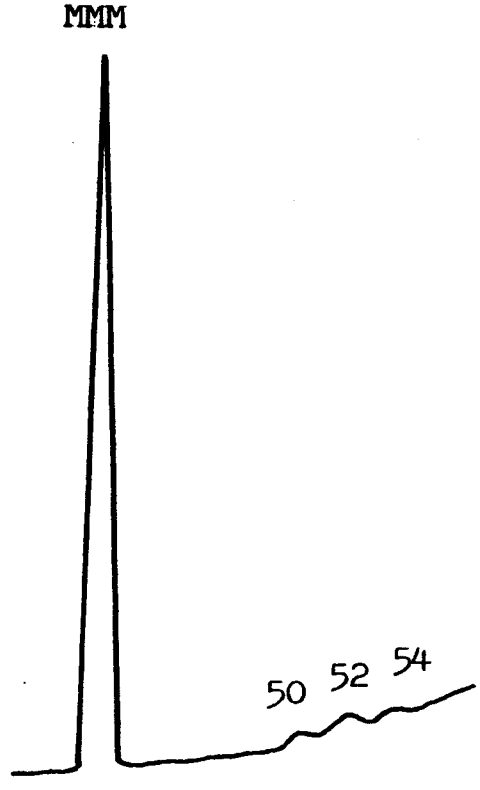


(c) Fully saturated triglycerides

Figure 7. Chromatograms used in the determination of the fully saturated triglyceride composition of Cocoa Butter.



(a) Total triglycerides in Cocoa Butter



(b) Fully saturated triglycerides

Table 8. Fully saturated triglyceride compositions (S_3) of various natural oils and fats determined by the PTC-oxidation procedure.

No. of acyl carbon atoms	Triglyceride Composition (Mole%)											
	Cocoa Butter		Lard		Crude Palm Oil		Stearin fraction of Palm Oil		Olive Oil		Soya bean Oil	
	Total	S_3	Total	S_3	Total	S_3	Total	S_3	Total	S_3	Total	S_3
46	0.0	0.0	0.3	0.1	0.2	0.2	0.6	0.6	0.0	0.0	0.0	0.0
48	0.05	0.05	1.7	0.6	3.9	3.5	19.3	19.3	0.0	0.0	0.0	0.0
50	17.95	0.55	16.3	1.9	42.3	0.5	54.6	2.4	4.0	0.0	1.3	0.0
52	45.1	0.9	63.8	3.0	43.6	0.0	24.8	1.0	32.2	0.0	24.4	0.0
54	36.4	0.6	15.2	0.2	10.0	0.0	0.7	0.0	63.4	0.0	74.3	0.0
56	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0
Total	100.0	2.1	100.0	5.8	100.0	4.2	100.0	23.3	100.0	0.0	100.0	0.0

on silver nitrate TLC is a problem.

Although 100mg of sample was used for the analyses, lower sample weights can be used and the method may lend itself to the analysis of lipids from body fluids.

3.3 Experimental

3.3.1 Materials

Trilaurin, trimyristin, tripalmitin, tristearin, triolein and methyl oleate (all of 99% purity) were purchased from Sigma Chemicals Ltd.

Dichloromethane and potassium permanganate were of Analar purity.

OV-17 stationary phase was purchased from Phase Separations Ltd.

DEGS-PS stationary phase was purchased from Supelco Inc.

Kieselgel 100 (35-70mesh) was used for silicic acid column chromatography.

"Aliquat 336" was purchased from Fluka Chemicals Ltd. The alkyl groups are a mixture of C_8-C_{12} straight chains with an average chain length of ten carbon atoms and a molecular weight of approximately 507.

TBAB was purchased from Fluka Chemicals Ltd.

CTAB was purchased from BDH Chemicals Ltd.

"Hyamine 1622" was purchased from Fluka Chemicals Ltd.

18-Crown-6 was purchased from Aldrich Chem. Co. Ltd.

Olive oil, soybean oil and lard were commercial samples. Cocoa butter was supplied by J. Bibby Ltd.

Crude palm oil (ex Malaysia) and palm oil stearin fraction were supplied by Dr. B.K.Tan (Malaysian Agricultural Research and Development Institute).

3.3.2 Distribution coefficients between the organic and aqueous phases.

3.3.2a Hydroxide ion

"Aliquat 336" (1.3mmole) dissolved in dichloromethane ($25cm^3$) was thoroughly shaken with 0.1M sodium hydroxide solution ($25cm^3$). An aliquot of the aqueous phase ($5cm^3$) was removed and titrated with standard hydrochloric acid to a phenolphthalein end-point. An aliquot of the origi nal sodium hydroxide solution ($5cm^3$) was titrated to determine the number of moles originally present.

3.3.2b Permanganate ion

The permanganate in the solvent phase was determined iodometrically. The PTC (0.25mmole) dissolved in dichloromethane ($25cm^3$) was thoroughly shaken with 0.02M aqueous $KMnO_4$ solution ($25cm^3$). An aliquot of the aqueous phase ($5cm^3$) was dissolved in 2M acetic acid ($20cm^3$) and aqueous potassium iodide was added ($5cm^3$ of a 30% solution). The liberated iodine was titrated against standard sodium thiosulphate solution using starch indicator near the end-point. The dichloromethane solution was analysed similarly except that acetone ($30cm^3$) was added before the potassium iodide to obtain a homogeneous solution.

3.3.3 TLC

Silicic acid preparative TLC was used for the isolation of the oxidation products for identification by GLC and spectrophotometric methods. For this purpose, plates of silica gel G (20cm x 20cm, 0.5mm thick) were prepared by standard methods,[86] and activated at 110° for one hour before use. A mixture of petroleum ether (Bpt 40-60°), diethyl ether, formic acid (59:40:0.1v/v/v) was used as the eluting solvent system. Bands were located by spraying the plate with a 0.2% alcoholic solution of 2,7 dichlorofluorescein and viewing under UV light. For identification and quantitation the spots were located and the silica gel of each area immediately scraped off the plate, collected in separate glass tubes and covered with a solution of 5% methanol in diethyl ether. After filtration of the suspension and several washings with further portions of the solvent mixture, the eluted compounds were identified by GLC and spectrophotometric methods.

3.3.4 GLC

3.3.4a GLC of neutral triglycerides

The gas chromatograph used was a Pye Unicam model 104 equipped with flame-ionisation detectors, automatic temperature programming and a 0.45m x 2.5mm I.D. glass column containing 2% OV-17 on Supelcoport (100-120mesh). The column was programmed from 250-360° at 4°/min with a nitrogen flow rate of 80ml/min. The peaks were identified

by cochromatography with known triglyceride reference compounds. Peak areas were measured by triangulation and triglyceride compositions were reported in mole percentage.

3.3.4b GLC of unoxidised fatty acids.

Fatty acid compositions of the fat prior to oxidation and the triglycerides fractionated after oxidation were determined by the GLC analysis of their methyl esters prepared by the Brockerhoff method.[87] The methyl esters were analysed on a 2.1m x 2.5mm I.D. glass column containing 10% DEGS-PS on Supelcoport (100-120mesh). The column temperature was held constant at 175° and samples were analysed isothermally with a nitrogen flow rate of 80ml/min. Typical chromatograms for cocoa butter are given in Figure 5.

3.3.4c GLC of oxidised fatty acids.

The crude oxidation mixture before fractionation on silicic acid contained a range of compounds of varying polarity. The methyl esters of the oxidised triglycerides were analysed on a 1.83m x 2.5mm I.D. glass column containing 3% OV-17 on Supelcoport (100-120mesh), programmed from 85-300° at 6°/min with a nitrogen flow rate of 80ml/min. The fatty acids were identified on the basis of retention time and cochromatography with known standards as well as IR spectroscopy and mass spectrometry.

3.3.5 Oxidations with potassium permanganate.

3.3.5a Aqueous alkaline potassium permanganate.

The fat (0.1g) and "aliquat 336" (0.25g) dissolved in dichloromethane ($20cm^3$) were placed in a $100cm^3$ round bottomed flask. Sodium hydroxide (0.1g) dissolved in distilled water ($8cm^3$) was added to the flask and the two phase mixture was vigorously stirred at room temperature for three minutes. Potassium permanganate (0.4g), sodium hydroxide (0.1g) dissolved in distilled water ($10cm^3$) was added to the flask and the mixture stirred vigorously for two hours. After reaction, the excess potassium permanganate and manganese dioxide produced during the oxidation were reduced by bubbling sulphur dioxide gas into the mixture until the pH of the aqueous phase was 2-3. The organic layer was separated and the residual aqueous layer washed with dichloromethane ($3 \times 5cm^3$). The combined organic extracts were washed with distilled water ($2 \times 8cm^3$), dried over anhydrous magnesium sulphate and the volume reduced almost to dryness. This extract (crude oxidation mixture) was fractionated on a 10cm x 1cm I.D. glass column fitted with a sintered glass base and packed with silicic acid (2.5g). The eluting solvent was a mixture of petroleum ether (Bpt 40-60°), diethyl ether (90:10v/v). The fully saturated triglycerides were eluted in the first $25cm^3$, which was reduced in volume and the triglycerides quantified by GLC using OV-17 as the stationary phase. Examination of the methyl esters of this eluted fraction showed a complete absence of unsaturated acyl groups.

3.3.5b Aqueous neutral potassium permanganate.

The oxidation procedure was as outlined above except that the sodium hydroxide was omitted. A reaction time of two and one half hours was required for complete reaction of the unsaturated triglycerides. The fractionation step outlined above was only partially successful in separating the fully saturated triglycerides from the oxidation products, the monobasic acid components were also partially eluted in the triglyceride fraction.

REFERENCES

1. Markley, K.S., Fatty acids, Interscience, New York, 1947.

2. Chevreul, M.E., Recherches chimique sur les corps gras d'origine animale, Levrault, Paris, 1823. Cited by Lawrie, J.W., Glycerol and the Glycols, Reinhold, New York, 1928, pp17-18.

3. Weitkamp, A.W., J. Amer. Chem. Soc., 1945, 67, 447.

4. Williams, H.H. and Anderson, W.E., Oil and Soap, 1935, 12, 42.

5. Kaufman, H.P. and Wessels, H., Fette u. Seifen., 1964, 66, 13.

6. Coleman, M.H., in "Advances in Lipid Research," Ed. Paoletti, R. and Kritchevsky, D., 1963, Vol.1, p.1.

7. James, A.T., in "Methods of Biochemical Analysis," Ed. Glick, D., 1960, Vol.8, p.1.

8. De Vries, B., Chem. and Ind. (London), 1962, 1049.

9. Barrett, C.B., Dallas, M.S.J. and Padley, F.B., ibid., 1962, 1050.

10. Kuksis, A. and McCarthy, H.J., Canad. J. Biochem. Physiol., 1962, 40, 679.

11. Mattson, F.H. and Beck, L.W., J. Biol. Chem., 1955, 214, 115.

12. Savary, P. and Desnuelle, P., Compt. rend., 1955, 240, 2571.

13. Entressangles, B., Pasero, L., Savary, P., Desnuelle, P. and Sarda, L., Bull. Soc. Chim. biol., 1961, 43, 583.

14. Clement, G., Clement, J. and Bezard, J., Biochem. Biophys. Res. Comm., 1962, 8, 238.

15. Brockerhoff, H., J. Lipid Res., 1965, 6, 10.

16. Shoolery, J.N., Applications Note Number 3, Varian Assoc., Palo Alto, California, 1975.

17. Aitzetmuller, K., J. Chromatog., 1975, 113, 231.

18. James, A.T., Ravenhill, J.R. and Scott, R.P.W., Chem. and Ind., 1964, 746.

19. Karmen, A., Separation Sci., 1967, 2, 387.

20. Scott, R.P.W. and Kucera, P., Analyt. Chem., 1973, 45, 749.

21. Scott, R.P.W. and Lawrence, J.G., J. Chromatog. Sci., 1970, 8, 619.

22. Aitzetmuller, K., J. Chromatog. Sci., 1975, 13, 454.

23. Kluchi, K., Ohta, T. and Ebine, H., ibid., 1975, 13, 461.

24. Pei, P., Henley, R. and Ramanchandran, S., Lipids, 1975, 10, 152.

25. Wada, S., Koizumi, C. and Nonaka, I., Yukakagu, 1977, 26, 92.

26. Wada, S., Koizumi, C., Takiguchi, A. and Nonaka, I., ibid., 1978, 27, 579.

27. Plattner, R., Spencer, G. and Kleiman, R., J. Amer. Oil Chemists' Soc., 1977, 54, 511.

28. Plattner, R., Wade, K. and Kleiman, R., ibid., 1978, 55, 381.

29. Herslof, B., Podlaha, O. and Toregard, B., ibid., 1979, 56, 864.

30. Karleskind, A., Valmalle, G., Midler, O. and Blank, M., Analusis, 1977, 5(2), 79.

31. Smith, E.C., Jones, A.D. and Hammond, E.W., J. Chromatog., 1980, 188, 205.

32. Martin, A.J.P. and James, A.T., J. Biochem., 1952, 50, 679.

33. Fryer, F.H., Ormand, W.L. and Crump, G.B., J. Amer. Oil Chemists' Soc., 1960, 37, 589.

34. Kuksis, A. and McCarthy, M.J., Canad. J. Biochem. Physiol., 1962, 40, 679.

35. Huebner, V.R., J. Amer. Oil Chemists' Soc., 1959, 36, 262.

36. Huebner, V.R., Pap. Los Angeles Meet Amer. Oil Chemists' Soc., Sept. 1959, Paper no. 4.

37. Litchfield, C., Analysis of Triglycerides, Academic press, New York, 1972.

38. Pierce, A.E., Silylation of Organic Compounds, Pierce Chemical Co., Rockford, Ill., 1968, p.75.

39. Myher, J.J. and Kuksis, A., J. Chromatog. Sci., 1975, 13, 138.

40. Takagi, T. and Itabashi, Y., Lipids, 1977, 12, 1062.

41. Kelker, H., Scheurle, B., Sabel, J., Jainz, J. and Winterscheidt, H., Mol. Cryst. Liq. Cryst., 1971, 12, 113.

42. Janini, G.M., Johnston, K. and Zielinski, W.L., Anal. Chem., 1975, 47, 670.

43. Janini, G.M., Muschik, G.M., Schroer, J.A. and Zielinski, W.L., ibid., 1976, 48, 1879.

44. Janini, G.M., Muschik, G.M. and Zielinski, W.L., ibid., 1976, 48, 809.

45. Tesarik, K., Frycka, J. and Ghyezy, S., J. Chromatog., 1978, 148, 223.

46. Hall, M. and Mallen, D.N.B., J. Chromatog. 1976, 118, 268.

47. Zielinski, W.L., Johnston, K. and Muschik, G.M., Anal. Chem., 1976, 48, 907.

48. Aerograph Research Notes, Wilkins Instruement and Research Inc., Palo Alto, Calif., Winter 1960.

49. Littlewood, A.B., in "Third International Gas Chromatography Symposium, Ed. Brenner, N., Callen, J.E. and Weiss, M.D., Academic press, New York, 1962, p.141.

50. Litchfield, C., Harlow, R.D. and Reiser, R., J. Amer. Oil Chemists' Soc., 1965, 42, 849.

51. Burchfield, H.P. and Storrs, E.E., Biochemical Applications of Gas Chromatography, Academic press, New York, 1962, p.45.

52. Dutton, H.J. and Cannon, J.A., J. Amer. Oil Chemists' Soc., 1956, 33, 46.

53. Quimby, O.Y., Willie, R.L. and Lutton, E.S., ibid., 1953, 30, 186.

54. Riemenschneider, R.W., ibid., 1954, 31, 266.

55. Scholfield, C.R. and Dutton, H.J., ibid., 1958, 35, 493.

56. Scholfield, C.R. and Dutton, H.J., ibid., 1959, 36, 325.

57. Scholfield, C.R. and Hicks, M.A., ibid., 1957, 34, 77.

58. Hilditch, T.P. and Lea, C.H., J. Chem. Soc., 1927, 3106.

59. Kartha, A.R.S., J. Amer. Oil Chemists' Soc., 1953, 30, 280.

60. Youngs, C.G., ibid., 1961, 38, 62.

61. Privett, O.S. and Blank, M.L., J. Lipid Res., 1961, 2, 37.

62. Privett, O.S. and Blank, M.L., J. Amer. Oil Chemists' Soc., 1963, 40, 70.

63. Hilditch, T.P. and Lea, C.H., J. Chem. Soc., 1907, 3106.

64. Kartha, A.R.S., "Studies on the Natural Fats," Vol.1. Published by the author, Ernakulam, India, 1949.

65. Kartha, A.R.S., J. Amer. Oil Chemists' Soc., 1953, 30, 280.

66. Starks, C.M., J. Amer. Chem. Soc., 1971, 93, 195.

67. Fendler, J.H., "Catalysis in Micellar and Macromolecular Systems," Academic press, New York, 1976.

68. Coleman, J.E. and Swern, D., J. Amer. Oil Chemists' Soc., 1958, 35, 674.

69. Herriott, A.W. and Picker, D., Tetrahedron Letters, 1972, 4517.

70. Herriott, A.W. and Picker, D., J. Amer. Chem. Soc., 1975, 97, 2345.

71. Starks, C.M. and Owens, R.M., ibid., 1973, 95, 3613.

72. Gibson, N.A. and Hosking, J.W., Austral. J. Chem., 1965, 18, 123.

73. Gokel, G.W. and Durst, H.D., Synthesis, 1976, 168.

74. Dietrich, B. and Lehn, J.M., Tetrahedron Letters, 1973, 1225.

75. Lehn, J.M., Structure and Bonding, 1973, 16, 1.

76. Clement, D., Damm, F. and Lehn, J.M., Heterocycles, 1976, 5, 477.

77. Seeley, F.G. and Crouse, D.J., J. Chem. Eng. Data, 1966, 11, 424.

78. Cerai, E., Chromatog. Rev., 1964, 6, 154.

79. Scibona, G., Byrum, J.F., Kimura, K. and Irvine, J.W., Solvent Extr. Chem. Proc. Int. Conf., 1965, 398.

80. Makosza, M. and Wawrzyniewicz, M., Tetrahedron Letters, 1969, 4659.

81. Makosza, M. and Bialecka, B., Tetrahedron Letters, 1971, 4517.

82. Dockx, J., Synthesis, 1973, 8, 441.

83. Dehmlow, E.V., Chem. Technol., 1975, 5, 210.

84. Dehmlow, E.V., Angew. Chem. Int. Ed. Eng. 1977, 16, 493.

85. Jurriens, G. and Kroesen, A.C.J., J. Amer. Oil Chemists' Soc., 1965, 42, 9.

86. Mangold, H.K., in "Thin Layer Chromatography," Ed. Stahl, E., Academic press, New York, 1965, pp 137-186.

87. Brockerhoff, H., Arch. Biochem. Biophys. 1965, 110, 586.

PART 11: THE SYNTHESIS OF TRIGLYCERIDES

Chapter 1

1.1 Introduction

Except for monoacid triglycerides, pure authentic mixed acid triglycerides are not commercially available at reasonable price. Authentic triglycerides are required for metabolic studies and in the manufacture of a wide variety of foodstuffs and cosmetics. They are also required as reference compounds for the development of structure elucidation techniques and modern analytical methods such as GLC, TLC, HPLC, NMR and mass spectrometry (MS). At present, only mono- and diglycerides prepared by the glycerolysis of vegetable and animal fats are permitted for use in foodstuffs and cosmetics. However, pure synthetic glycerides may be allowed in future providing the method of synthesis does not introduce toxic substances into the final products.

There have been many attempts to synthesise triglycerides, and synthetic glycerides of short chain fatty acids have been shown[1] to be easily assimilated. The methods of synthesis can best be discussed by initially considering the starting materials that have been used and secondly considering nucleophilic substitution reactions in glycerol derivatives as it has a direct bearing on the synthetic work undertaken in Part II of the thesis.

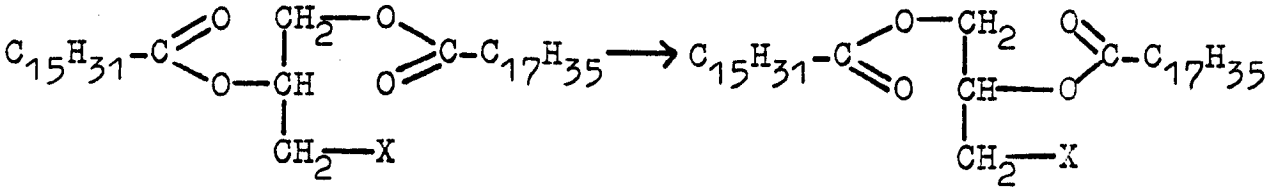
One of the main problems encountered in the synthesis of triglycerides is that of acyl migration within partial glycerides. This is discussed to draw attention to the problems

that are encountered in the design of a suitable reaction sequence.

1.2 Acyl Migration

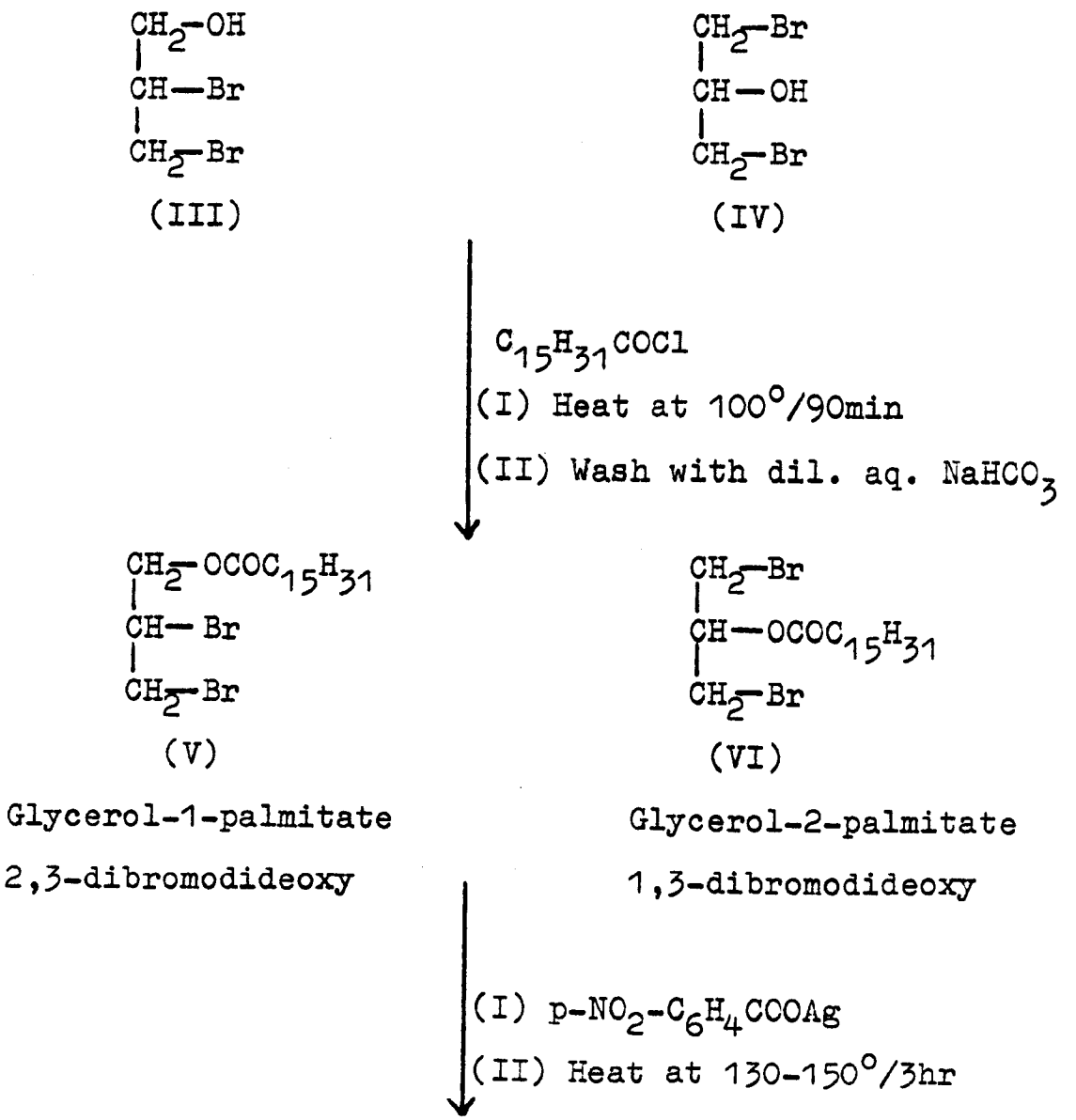
Fischer[2] in an attempt to synthesise 1,2-diglycerides from 1-iodoglycerol observed that the products obtained were not those expected, but the corresponding 1,3-diglycerides, the formation of which involved migration of the acyl group from the 2- to the 3-position. By this observation Fischer was able to explain both his own and the anomalous results of other workers,[3-6] but perhaps more importantly he drew attention to one of the most important factors upon which the structure of a synthetic glyceride may depend. Little attention was paid to the discovery until ten years later when Fairbourne[7] revived and extended Fischer's explanation into what is generally known as acyl migration. He observed that the acyl migration is not confined to the interchange between a hydroxyl group and an acyl group, but it can occur between two acyl groups or between an acyl group and another group such as phthalimido. An acyl migration between two acyl groups is shown in Figure 1.

Fig. 1 Acyl migration in a glycerol derivative

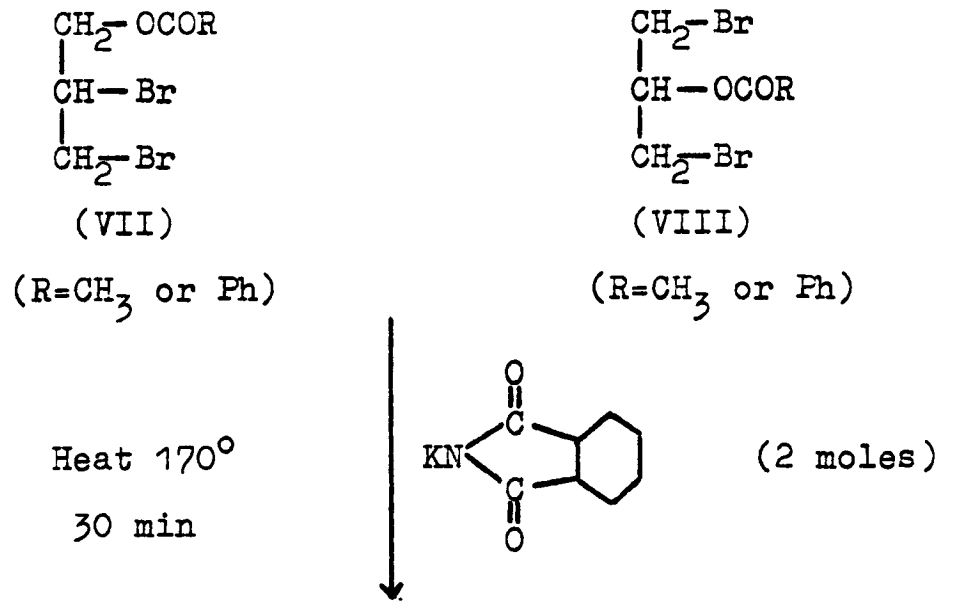


(I) (II)

The isomeric halohydrins, 2,3-dibromo propan-1-ol (III) and 1,3-dibromo propan-2-ol (IV) are typical of substrates in which acyl migration ultimately results in the same product being formed rather than isomeric products.



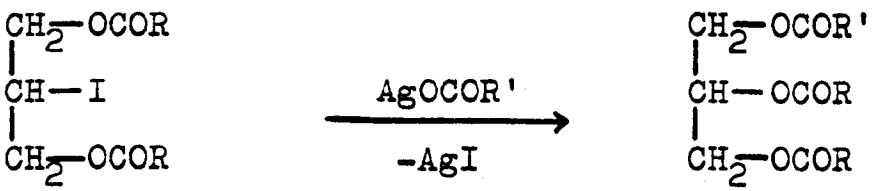
The same glycerol-2-palmitate 1,3-di-p-nitrobenzoate derivative was obtained[8] from both (V) and (VI). When (VI) is subject to hydrolysis, by silver nitrite in aqueous ethanol, the glycerol-1-palmitate and not the expected glycerol-2-palmitate is obtained.



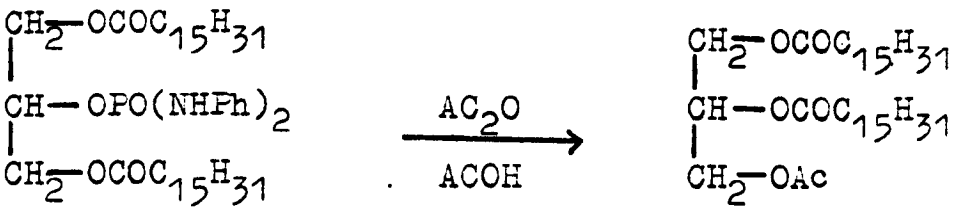
The same glycerol-diphthalimido-acylate was obtained from both (VII) and (VIII).

The following examples have been observed by Malkin et al.[9,10]

(a) Acyl migration can occur from the 1- to the 2- position.

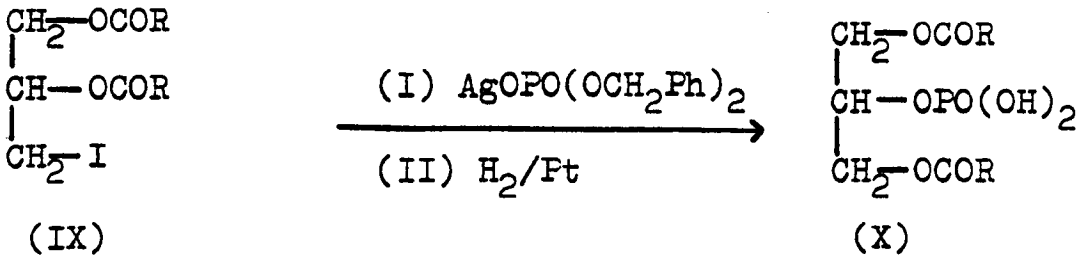


(b) Acyl migration can occur from the 2- to the 1- position.



Besides the above mentioned examples of acyl migration

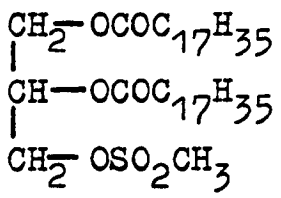
there are several others e.g. the formation of glycerol 1,3-diacyl-2-phosphate (X) by the reaction[11] of glycerol 1,2-diacyl-3-iododeoxy (IX) with silver phosphate.



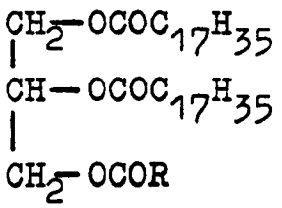
Aneja et al.[12] have examined this reaction using the more bulky silver dibenzyl phosphate. It was found that the acyl migration takes place but only to the extent of 2% with their substrate.

The acyl migrations, observed during attempted glyceride synthesis, take place in acidic,[13] basic[10,14] and even neutral media.[15] They are also observed on mere heating.[16] The extent of the migration, whether from the 2- to the 1-position or vice versa, depends upon the structure and stereochemistry of the glyceride concerned, the experimental conditions and reagents used. Martin[17] observed that 2-monoglycerides, in chloroform solution containing some ethanol, on treatment with 56% aqueous perchloric acid, lead within 10-30min, to an equilibrium mixture containing 90-92% of 1-mono and 8-10% of 2-monoglyceride. Aneja and Davies[15] found that in solvents such as acetonitrile or benzene, glycerol 1,2-distearate-3-methanesulphonate (XI) gave on heating for 2hr. with tetrabutylammonium oleate a triglyceride mixture comprising 92% of glycerol 1,2-

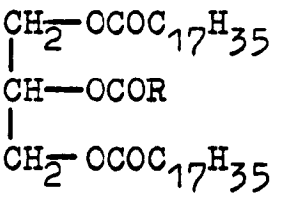
distearate-3-oleate (XII, R=$C_{17}H_{33}$) and 8% of glycerol 1,3-distearate-2-oleate (XIII, R=$C_{17}H_{33}$). Using sodium



(XI)

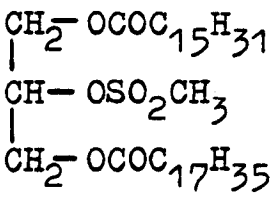


(XII)

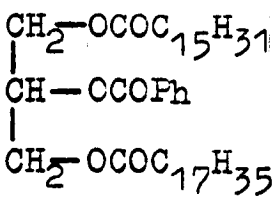


(XIII)

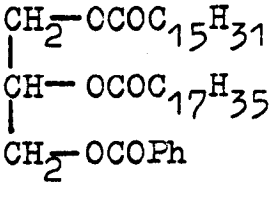
benzoate in place of tetrabutylammonium oleate, they obtained a triglyceride mixture which comprised 95% of (XII, R=Ph) and 5% of (XIII, R=Ph). Under the same conditions glycerol 1-palmitate-3-stearate-2-methanesulphonate (XIV) gave them a triglyceride mixture which comprised 5% of glycerol 1-palmitate-3-stearate-2-benzoate (XV) and 95% of an equimolar mixture of glycerol 1-palmitate-2-stearate-3-benzoate (XVI) and glycerol 1-benzoate-2-palmitate-3-stearate (XVII).



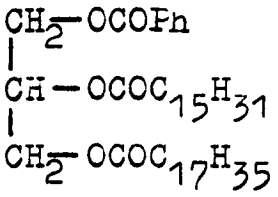
(XIV)



(XV)

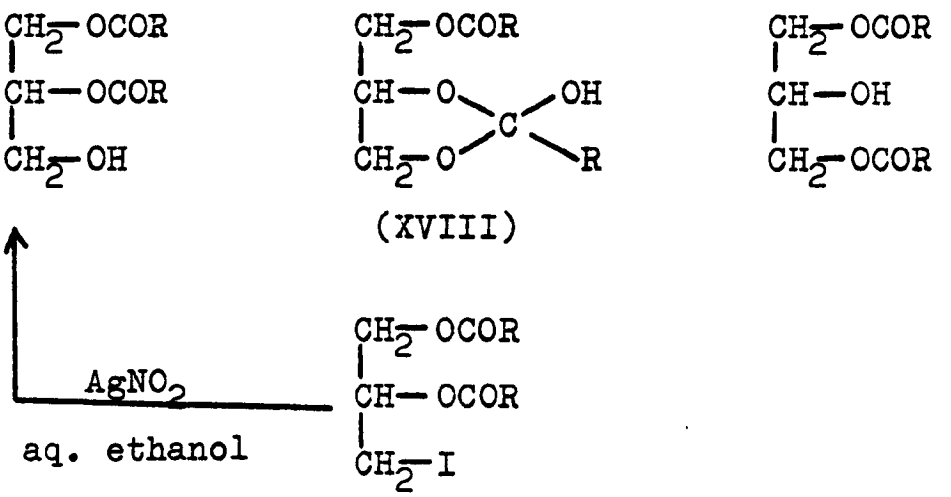


(XVI)

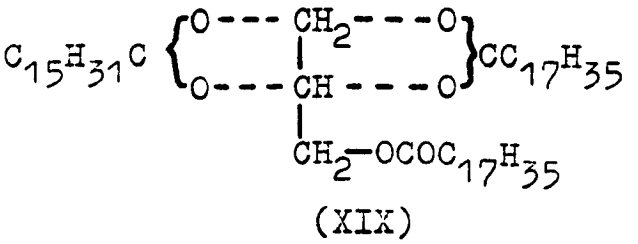


(XVII)

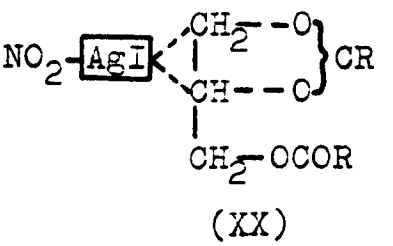
In the above examples, both the acyl and acyloxy groups are involved in the migration. Fischer[2] suggested that the formation of 1,3-diglycerides from either 1,2-diglycerides in acidic medium or from glycerol 1,2-diacyl-3-iododeoxy upon treatment with silver nitrite in hot aqueous ethanol, a dioxolane intermediate[18] (XVIII) was involved, and that the free hydroxyl group originally present or generated in situ played an important role in the acyl migration.



Fairbourne[7] attributed the thermal isomerization of triglycerides to valency rearrangements which pass through a common intermediate (XIX). A similar intermediate (XX) being involved in the formation of the 1,3-diglyceride from glycerol 1,3-diacyl-2-iododeoxy.

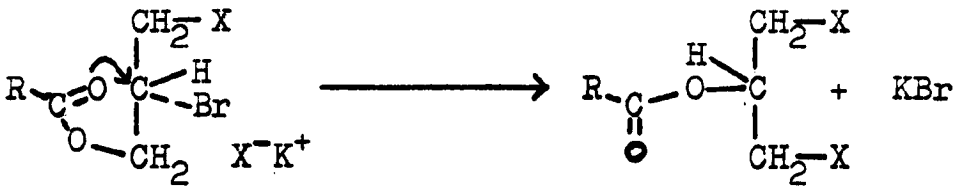


(XIX)



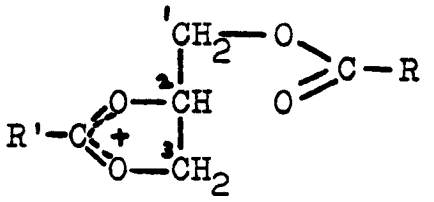
(XX)

Malkin et al[10] (cf examples (a) and (b) p.62) explained their results by implying that the acyloxy migration from 1- to the 2-position is accompanied by Walden inversion.



They also stated that similar changes are involved in the dephosphorylation of derivatives of 1,3-dipalmitate-2-phosphatidic acid. A close scrutiny of their experimental results does not completely support the involvement of Walden inversion and warrants consideration of other possible explanations. An alternative explanation is the Winstein-Buckles dioxolenium ion[19] which Malkin et al did consider but rejected on the grounds that with such an intermediate acyl migration could occur from C-1 to C-2 or C-2 to C-1, whereas they only detected the former in their experiments.

Aneja and Davies[12,15] using modern separative techniques and spectroscopic methods, for examining reaction products, have inferred that the displacement of the sulphonate group in (XI) and (XIV) occurred with the participation of the neighbouring carboxylate-ester group and proceed via the Winstein-Buckles type of dioxolenium ion (XXI) as the reaction intermediate.This is attacked by the incoming nucleophile regioselectively towards the terminal C-3 due to the inductive effect[11] of the carboxylate group at C-1



(XXI)

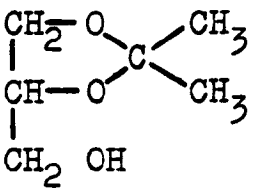
but perhaps more important due to the steric effect (ease of approach) of the nucleophile, which favours the less hindered and more accessible C-3 to the detriment of C-2. On the basis of the unsymmetrical intermediate it is possible to explain the results of Malkin et al., except the involvement of Walden inversion. Although many of the anomalous results due to acyl or acyloxy migrations appear to be explicable satisfactorily by the intermediacy of the unsymmetrical dioxolenium ion, the possibility of direct nucleophilic substitution involving Walden inversion cannot be ruled out without checking, on the one hand, results of Malkin et al and on the other hand, collecting compelling evidence for retention of configuration.

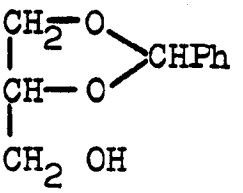
Prior to the recognition of acyl migration, specially during the first quater of this century, there was great controversy among chemists concerning the synthesis and and analytical data of 2-monoglycerides and 1,2-diglycerides, some of which later were proven to be 1-monoglycerides and 1,3-diglycerides respectively, formed by acyl migration. However, it is now possible to choose experimental conditions and sequence of steps such as to minimise acyl migration and also turn it to one's advantage. For example, Martin[17]

by a combination of periodic acid titrations and perchloric acid induced acyl migrations, has developed a method of estimating 1-monoglycerides and total monoglycerides in a mixture.

1.3 Starting materials for Triglyceride synthesis.

1. Glycerol
2. Compounds which are either simple derivatives of glycerol, or its distant relatives.

(I) 

(II) 

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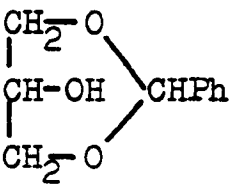
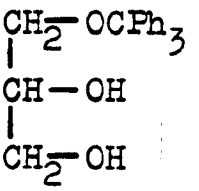


(III) 

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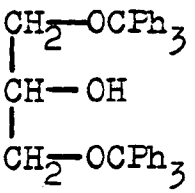
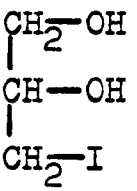
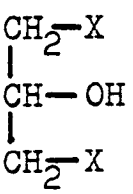
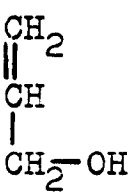
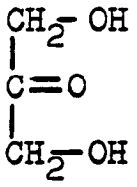


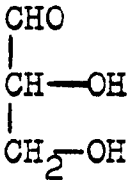
(IV) 

(V) 

(X=Cl,Br)

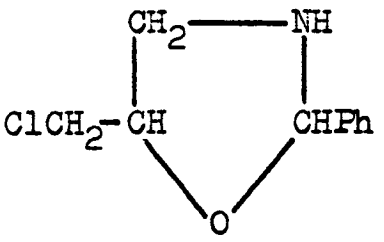
(VI) 

(VIII) 

(IX) 

3. D- or L- mannitol, involving intermediates like 2(1) above but yielding active and 'cryptoactive' glycerides. 'Cryptoactive' triglycerides are those which from their mode of synthesis and the nature of the starting material are expected to possess optical activity, but the latter cannot be detected by polarimetry at the sodium D-line or even in the UV region. The majority of the long chain fatty acid triglycerides synthesised by Schlenk[20] belong to this category.

4. Miscellaneous compounds.

(1) 

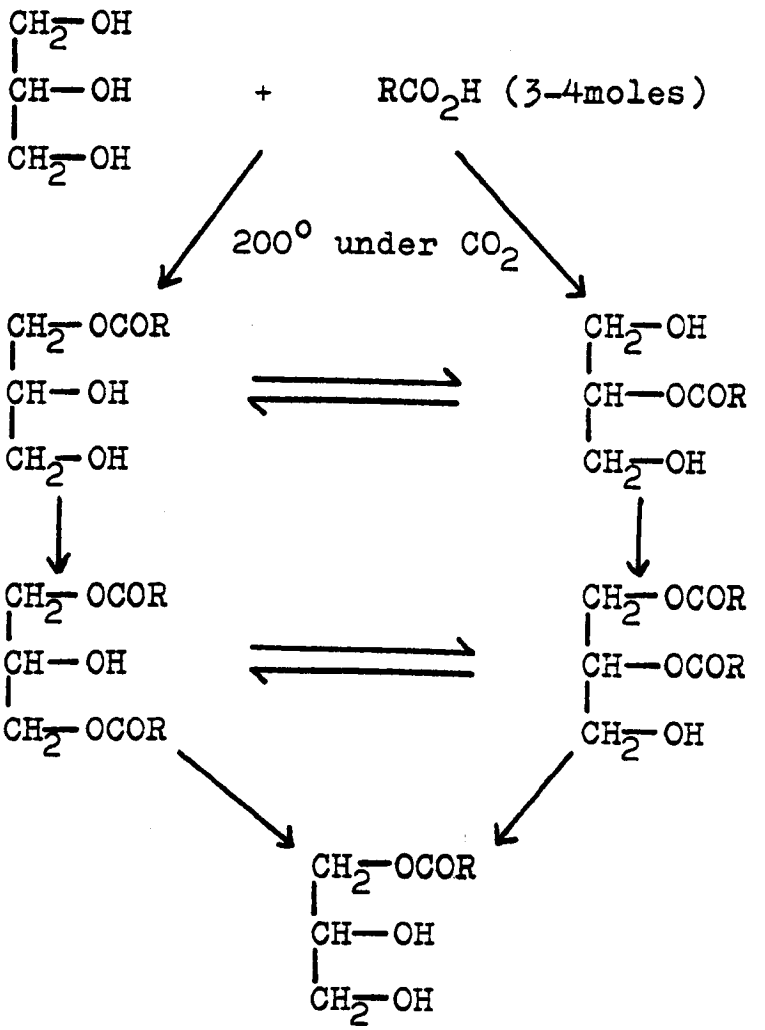
2-phenyl-5-chloromethyloxazolidine

(11) $HOCH_2-CO_2H$ The diazoketone route.

5. Interesterification

1. Glycerol

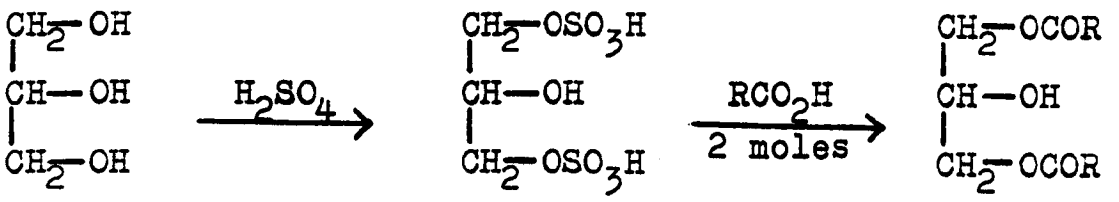
The esterification of glycerol with a fatty acid is a step-wise process.



This method of making simple triglycerides is due to Garner.[21] It is not, however, suitable for the synthesis of mixed acid triglycerides. It is a modification of Berthelot's[22] original method in which glycerol was heated with a fatty acid in a sealed tube at 200°. Industrially the reaction is performed at 180-230° for 3-6hr under a vacuum of 20mm. Provided that the acid is present in an excess of 5 to 20% the reaction can be brought to a stage

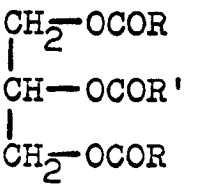
where no monoglyceride and only a little diglyceride remains. Esterification is accelerated by the use of a catalyst such as naphthalene- -sulphonic acid. A wide variety of inorganic catalysts[23] have been used including $AlCl_3.6H_2O$, $CdCl_2.2H_2O$, FeO, MgO, MnO_2, NaOH, PbO, $SnCl_2.2H_2O$, $ZnCl_2$ and ZnO. Acetoglycerides can be prepared by heating a monoglyceride mixture with acetic anhydride at 110° for 1 to 4hr in a dry inert atmosphere.[24,25]

(II) Esterification via sulphuric acid esters of glycerol



(XXII)

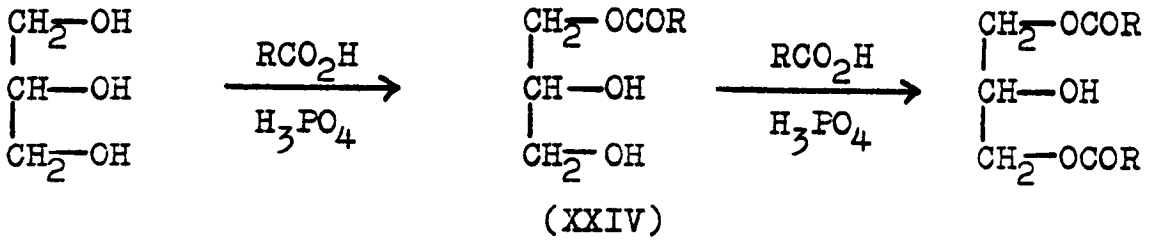
R'COCl



(XXIII)

This method is due to Grun and co-workers.[26-28] The monoacid diglyceride (XXII) can be converted into a diacid triglyceride (XXIII) by reaction with an acid chloride in the presence of a base such as pyridine or quinoline.

(III) Esterification in the presence of syrupy phosphoric acid.[29,30]

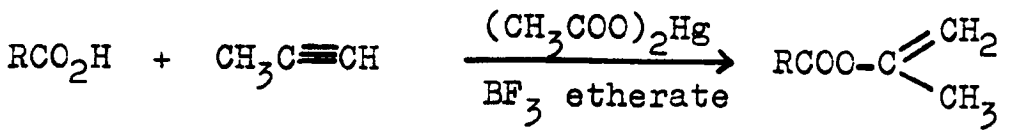


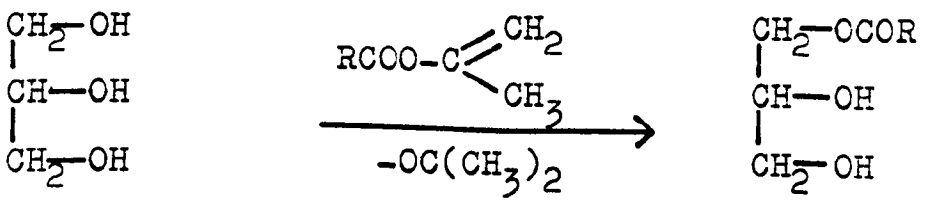
(XXIV)

R=CH_3 or $CH_3CH_2CH_2$

A further modification[31] is to employ trisodium phosphate in place of syrupy phosphoric acid, and heat the mixture under anhydrous conditions. In both cases the reaction mixture is initially non-homogeneous but becomes progressively homogeneous as the monoglyceride first formed (XXIV) dissolves the unreacted fatty acid. This observation inevitably means that the formation of the diglyceride will occur to a large extent even when the fatty acid is present in low concentrations. The method is unsuitable for the preparation of triglycerides. The solvolysis of glycerol phosphates with the soaps of fatty acids is another alternative which does not seem to have been reported.

(IV) Glycerol esters via isopropenyl esters of fatty acids[32]

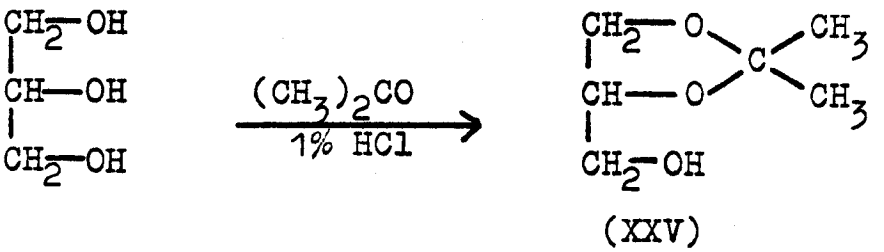




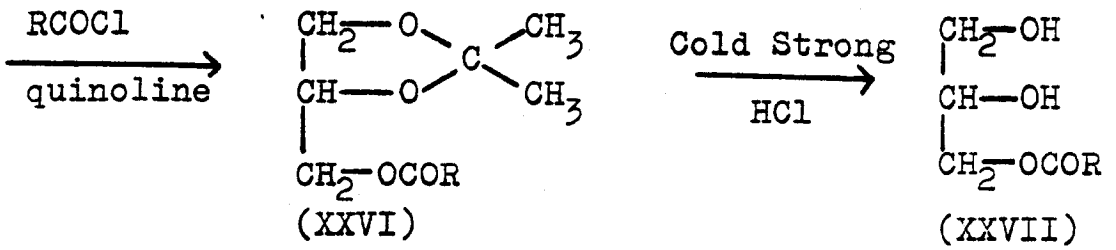
Fatty acids react with methyl acetylene, in the presence of mercuric acetate and boron trifluoride etherate, to give isopropenyl esters which react with glycerol to give the corresponding monoglyceride and acetone.
N.B. Thermolysis (170-220°) of the isopropenyl ester, in the presence of a trace of toluene-p-sulphonic acid, yields the reactive intermediate (R-CH=C=O) which acylates glycerol.

2. Compounds related to or derived from glycerol

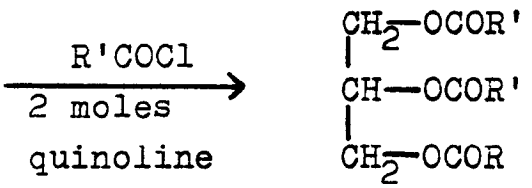
(I) Use of the isopropylidene protecting group



(XXV)



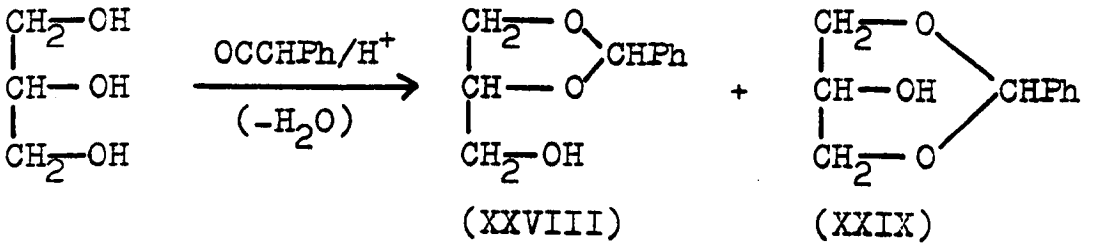
(XXVI) (XXVII)



This is the most widely used route[33] for the synthesis of monoglycerides and diacid triglycerides, the yields are usually very good. The conversion of 1,2-isopropylidene-

rac-glycerol (XXV) into the 3-acyl derivative (XXVI) can also be effected by using the free fatty acid, in the presence of toluene-p-sulphonic acid. The preparation of (XXVI) from glycerol has been shown to be a one pot reaction.[34] It has also been prepared by trans-esterification of (XXV) with methyl ricinoleate in the presence of sodium methoxide. Boric acid has been found[35] to be the most effective agent for the deketalisation of (XXVI) as it does not result in acyl migration within the partial glyceride. The synthesis of glycerides with unsaturated fatty acids, eg oleic, linoleic acids, has also been reported.[36] The acid is first converted into the dibromo derivative which is then transformed into the acid chloride which is used in the normal way. The double bonds are regenerated by treatment with zinc and ethanol.

(II) Use of the benzylidene protecting group.

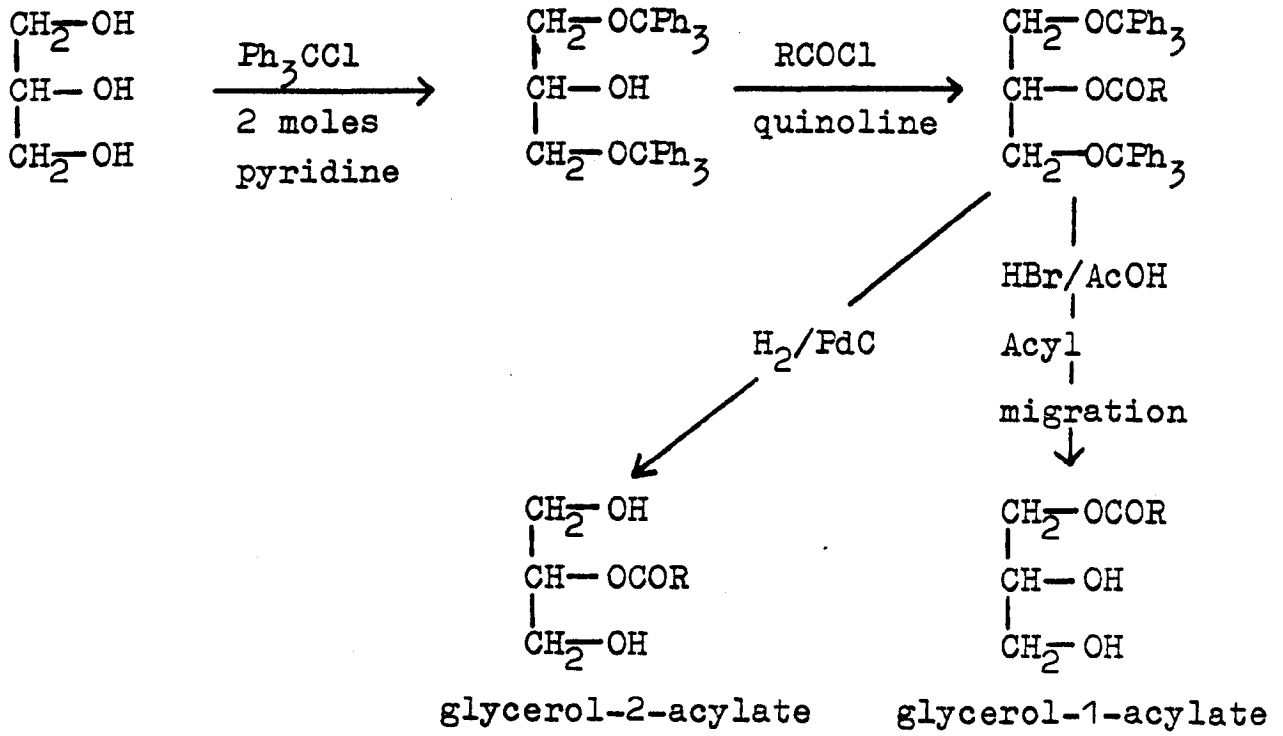


(XXVIII) Major product + (XXIX) Minor product

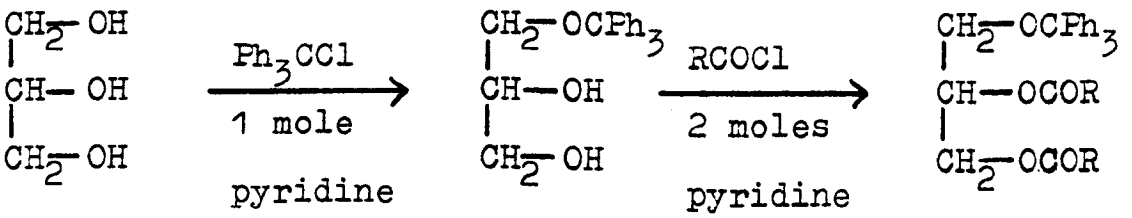
The isomeric benzylidene glycerols (XXVIII) and (XXIX) were separated by fractional crystallisation and converted into the corresponding 3- and 2-acyl derivatives on treatment with the corresponding acid chloride in the presence of a base (quinoline or pyridine). Removal of the benzyl-

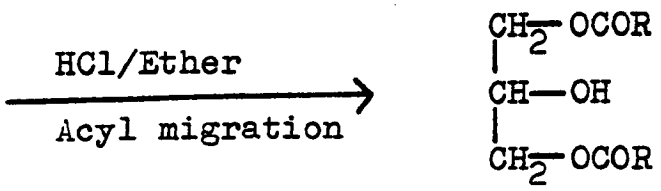
idene group using cold acid results in migration of the acyl group from the 2-position to the 1- or 3-positions. The removal using catalytic hydrogenation or more recently using boric acid in triethylborate has enabled pure 2-monoglycerides to be prepared, as the reagents do not cause acyl migration.

(III) Use of the trityl protecting group.[37-39]

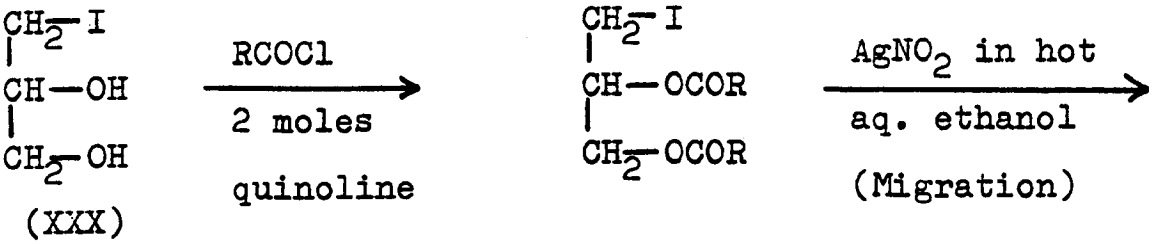


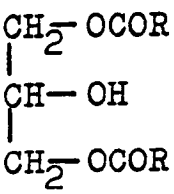
The trityl protecting group has also been used to prepare 1,3-diglycerides of both saturated and unsaturated fatty acids,[40,41] as outlined below.





(IV) 1-iodoglycerol ('Alival')



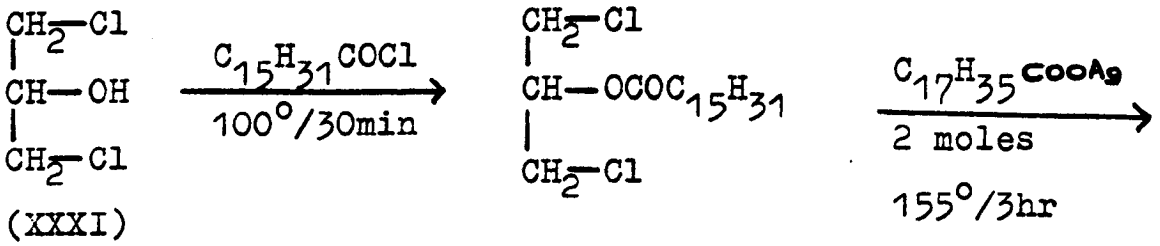


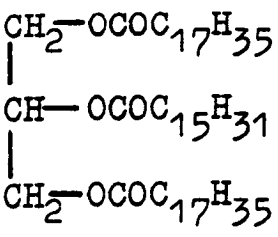
The method[42-44] gives excellent yields of pure 1,3-diacylglycerides. The silver nitrite treatment hydrolyses with concomitant acyl migration so the method cannot be used to prepare 1,2-diglycerides. The difficulty of preparing the starting material (XXX) in high yield has been solved.[45,46]

(V) Glycerol 1,3-dihalogendideoxy

The use of the 1,3-dihalo derivative has met with little success for the synthesis of triglycerides. Reaction with an alkali metal salt of a carboxylic acid results in some substitution but predominantly dehydrohalogenation.

The method was originally attempted by Grun.[47] The following variation[48,49] has led to triglycerides but the structural purity of the starting material (XXXI) and product (XXXII) are doubtful.

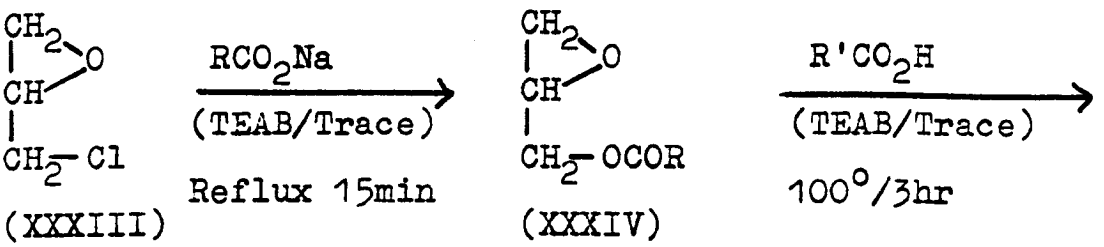


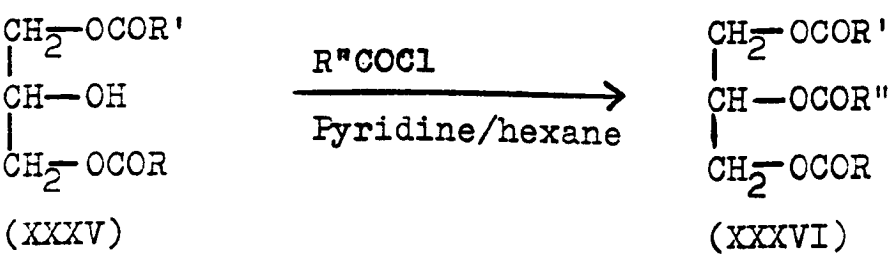


(XXXII)

Although it was claimed[48,49] that the HCl evolved did not cause any acyl migration in the initially formed monoacyl derivative, Fairbourne[50] showed that it did.

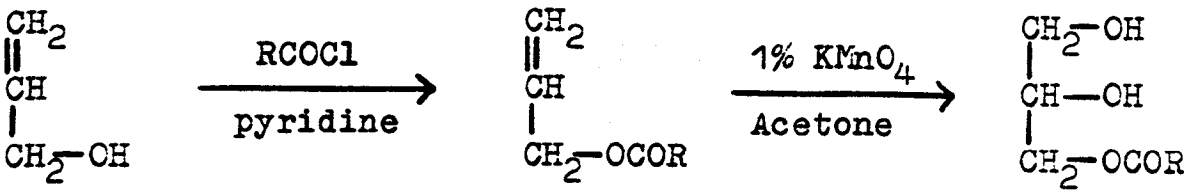
(VI) Epichlorohydrin route[51]



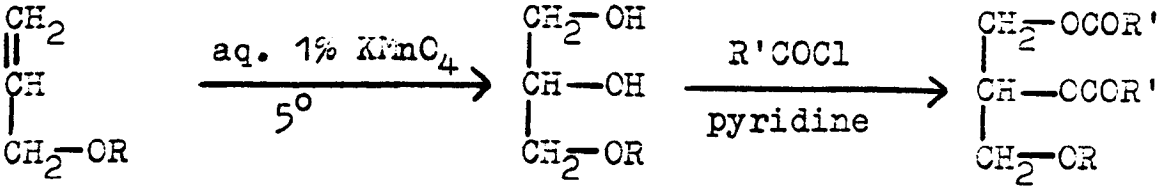


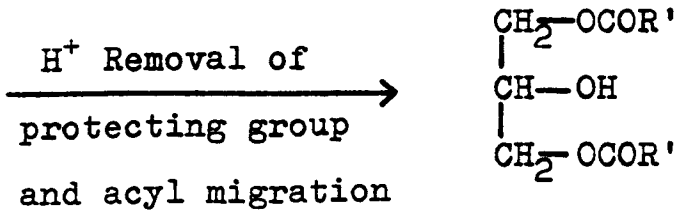
Mank et al[51] describe the best conditions for the synthesis of the glycidyl esters (XXXIV) in yields of 90-93%. The conversion of this glycidyl ester into the diglyceride by heating with free fatty acid at 130° was known[52] but yields were only 25-40%. By the use of tetraethyl ammonium bromide (TEAB) and heating the degassed glycidyl ester to 100° Mank et al were able to isolate in high yields a mixture of 1,3- and 1,2-diglycerides which they isomerised to the 1,3-isomer by heating the solid mixture at 5-10° below their melting point. On recrystallisation they obtained 1,3-diglycerides of 99% purity. The triglycerides (XXXVI) were obtained in high yields (84-95%). It is a flexible route, however, the inability to satisfactorily isomerise a mixture of 1,3- and 1,2-diglycerides of unsaturated acids is a severe problem.

(VII) Allyl alcohol[53,54]

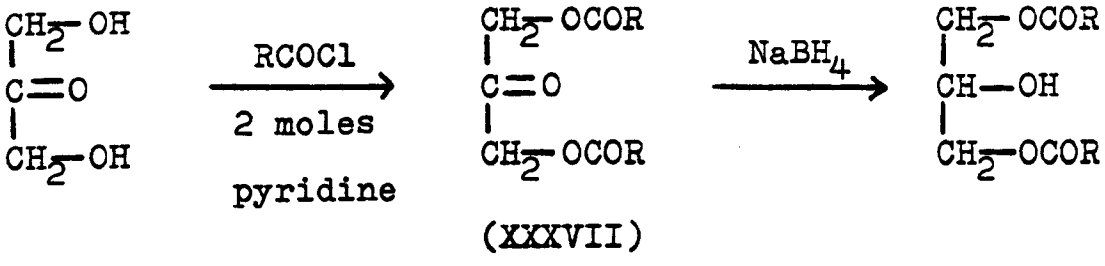


One of the main problems is a suitable solvent for the oxidation. An alternative[55] route is to convert allyl alcohol into the tetrahydropyranyl ether and follow the scheme:-





(VIII) Dihydroxy acetone (1,3-dihydroxy propan-2-one)



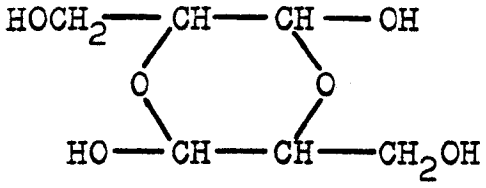
(XXXVII)

This route was explored by Grun and Wittka[56] who reduced the oxo group of the intermediate (XXXVII) catalytically. This reduction method limited its use to only saturated fatty acids. Barry and Craig[57] also examined the route and had an elaborate synthetic sequence requiring the protection of the oxo group as a mercaptal. However, Bentley and McCrae[58] overcame previous limitations by employing sodium borohydride for the reduction step. The yields in both steps are high (76-99%). The purity of products from long chain fatty acids, such as palmitic and oleic acids is high (there were no detectable amounts of 1,2-diglyceride in the product). However synthesis of short chain diglycerides is less satisfactory, (eg glycerol 1,3-diacetate) as NMR has shown the presence of 10% of the 1,2-isomer.

(IX) Glyceraldehyde

Glyceraldehyde would appear to be an interesting

compound for glyceride synthesis, but it does not appear to have been used, presumably for the two following reasons. Firstly, ordinary glyceraldehyde exists as a dimer (XXXVIII)



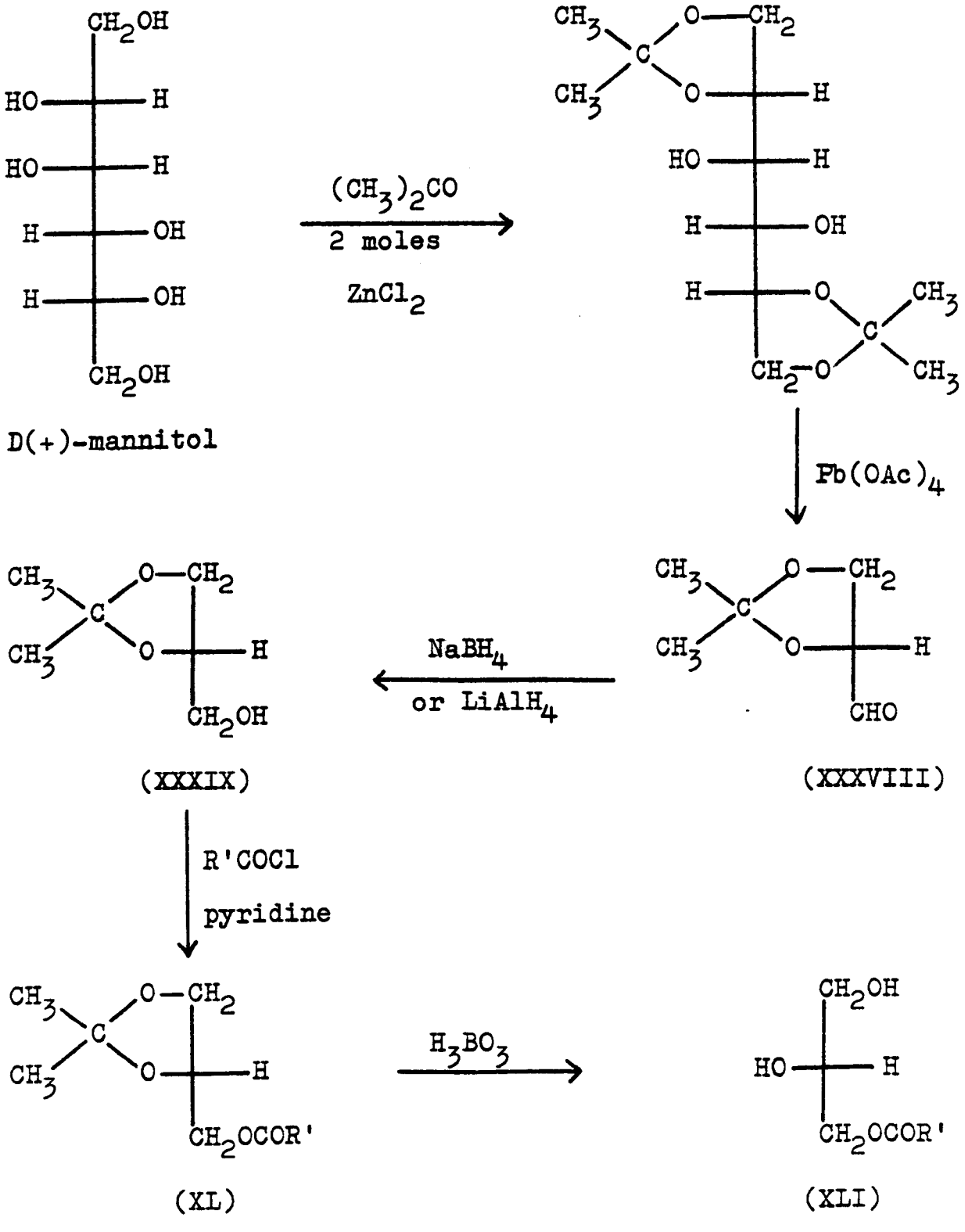
(XXXVIII)

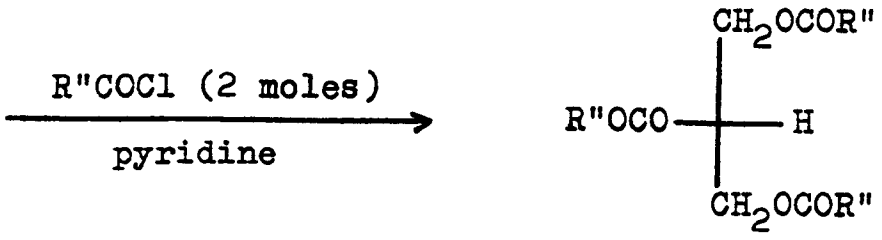
and attempts to convert it into the monomeric form may lead to its isomerisation[59] to dihydroxyacetone. Secondly, isopropylidene derivatives of glyceraldehyde have been prepared indirectly from D- or L- mannitol. The latter have the added advantage of yielding optically active glycerides.

3. <u>D- or L- mannitol for the synthesis of optically active and 'cryptoactive' glycerides.</u>

This widely used route was first worked out by Baer and Fischer[60-63] and later improved by others.[20,64-67] The method has led to optically active 1-monoglycerides, 1,2- and 1,3-diglycerides and triglycerides of various acids. A reaction sequence is outlined below, for the synthesis of optically active monoglycerides (XLI) or diacid triglycerides (XLII). The stepwise reaction of (XLI) with two different fatty acid chlorides will result in an optically active 1,3-diglyceride followed by a triacid triglyceride. The use of L-mannitol leads to the corresponding enantiomeric compounds. It has been shown[66,67] that even enantiomeric monoacid diglycerides (sn-glycerol 1,2-diacylate

and sn-glycerol 2,3-diacylate) can be prepared from the same intermediate (XXXIX).

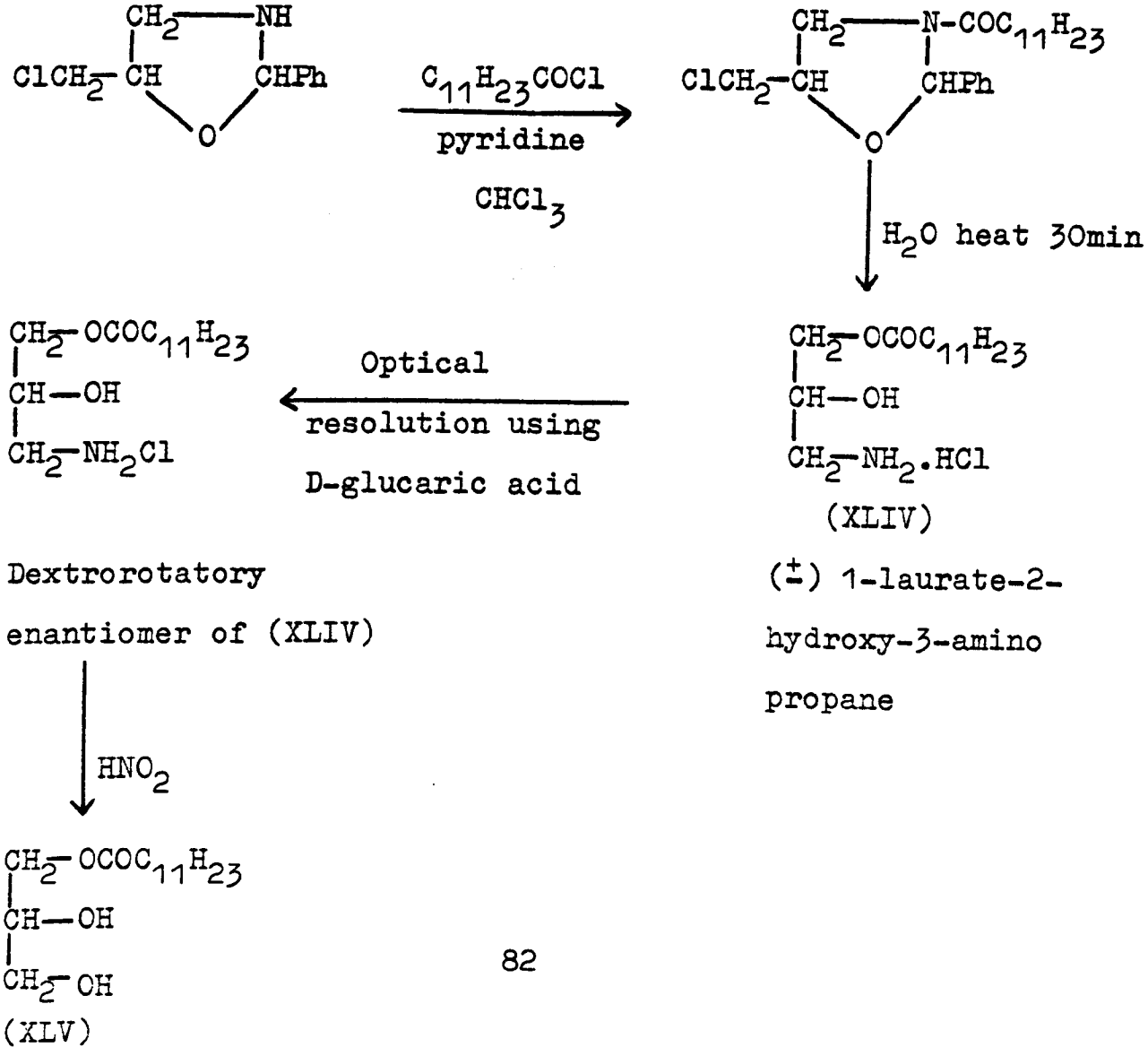




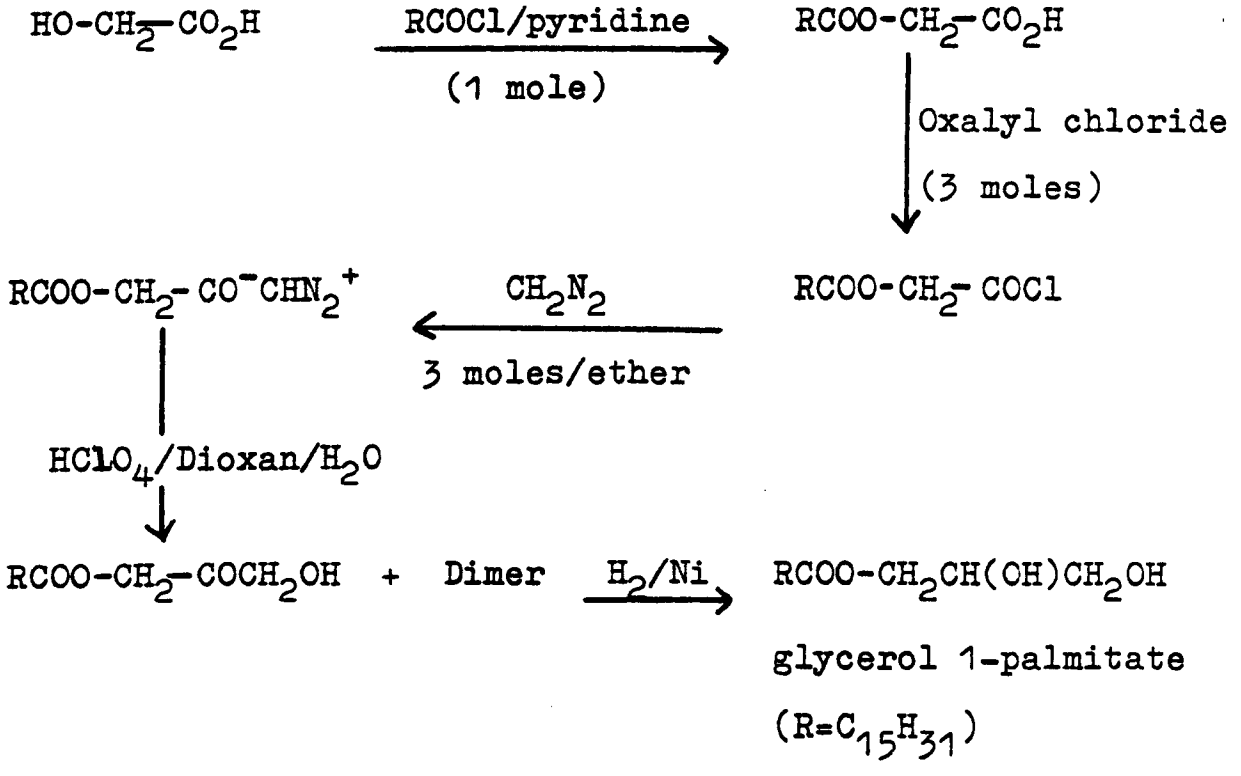
(XLII)

4. Miscellaneous compounds as starting materials.

(I) Few attempts[68,69] were made to prepare optically active glycerides prior to the discovery of the mannitol route. For example, 2-phenyl-5-chloromethyloxazolidine (XLIII) was converted into optically inactive glycerol 1-laurate (XLV). No further work has been done to develop this route.



(II) Glycolic acid-the diazoketone route.

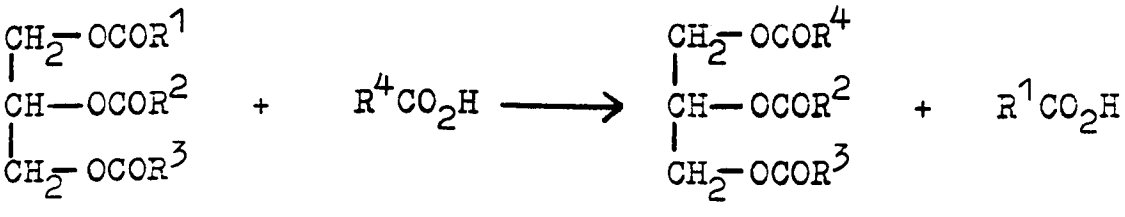


This route has provided monoacid and diacid diglycerides of saturated fatty acids only, and it has no advantage over the dihydroxyacetone route. However, it consists of the stepwise building up of a desired molecule and thereby offers scope to incorporate isotopic labelling to yield radioactive glycerides which may be useful in metabolic and mechanistic studies.

5. Interesterification.

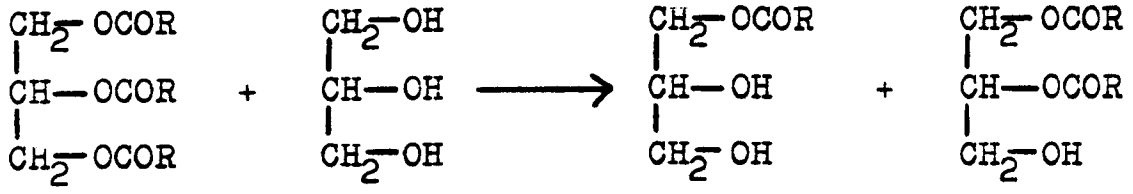
In the early literature[70] interesterification is divided into three classes:-

(I) Acidolysis[71] where a fatty ester is reacted with an acid.



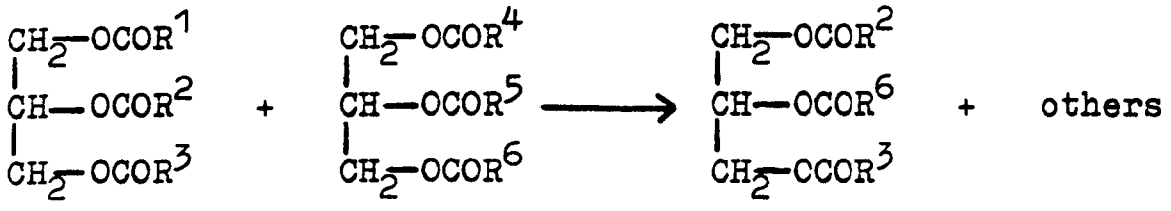
Acidolysis is covered by a number of patents but none are used in industry on a large scale.

(II) Alcoholysis[72] where a fatty ester is reacted with an alcohol.



Sorbitol and sucrose esters have also been made by this technique.

(III) Ester interchange where the acyl groups are exchanged from one triglyceride to another.



This is the most important of the interesterification methods in commercial use and is usually subdivided into random where the acyl groups move freely from one position to another until the fatty acids are arranged on the glycerol backbone in a statistical distribution, and directed interesterification where the equilibrium mixture is disturbed by crystallisation to produce a desired product.

It has been found necessary to use a catalyst, which can be metals (Li, Pb, Fe, Sn) their oxides and salts, alkali metals (Na, K and their alloy) their alcoholates,

hydroxides, hydrides and amides. Mineral acids and sulphonic acids have also been used. The most popular catalysts can be divided into high temperature ones (250-500°F)[73,74] eg zinc chloride, sodium hydroxide with and without glycerol, sodium stearate and low temperature ones (45-375°F)[75], which include sodium methylate, sodium or sodium-potassium alloy, sodium hydride and sodium amide. They are used at low levels, 0.02-2%. The catalyst can be inactivated by moisture, free fatty acids and peroxides, although it has been shown[76] on a laboratory scale that alcoholysis with sodium methoxide in methanol proceeds smoothly in the presence of a large amount of water. Various examples can be cited[77-81] in which interesterification has been used on a commercial scale to produce margarines and confectionary fats.

1.4 Nucleophilic substitution in glycerol derivatives.

Most of the nucleophilic substitution reactions used in glyceride synthesis involve reactions such as:-
(a) Substitution of a halogen anion by the carboxylate anion, in which the anionic species is generated in situ from the corresponding alkali-metal (Na,K) or silver salt.
(b) Substitution of a sulphonate (mesylate or tosylate) or a phosphate anion by the carboxylate anion.

The well known method for the preparation of carboxylate esters by the reaction of alkyl halides with the sodium or potassium salts of carboxylic acids is not a generally useful synthetic method[82] owing to poor yields and conver-

sions, along with the competing side reaction of dehydrohalogenation. Reactions involving silver ion assisted nucleophilic substitutions provide a more efficient system[83,84] resulting in higher yields of substitution products and low amounts of dehydrohalogenation products. However, the high cost of silver restricts their use to laboratory scale preparations.There are several ways in which the utility of the nucleophilic substitution reaction can be extended and some of these methods are briefly outlined below.

Cesium carboxylates

A facile procedure has been described[85] for the preparation of a wide variety of carboxylate esters derived from protected amino acids and peptides. The cesium carboxylate is allowed to react with different alkyl halides in DMF solution, to form the corresponding esters. The results indicated that it is a superior method to the sodium or potassium salts, but it is still not a generally applicable method.

Mercuric carboxylates

Mercuric carboxylates in the presence of catalytic amounts of triacy loxyboranes have been reported[86] as an efficient reagent for the esterification of alkyl halides. The results were compared with reactions using silver and sodium carboxylates. Esterification of primary alkyl halides by silver acetate closely paralleled the mercuric acetate reaction, except that yields in the latter tended to be higher. Both silver and mercuric salts were superior to

sodium salts. However, the high cost of mercuric salts greatly limits their practical usefulness.

Copper(I) oxide-base system

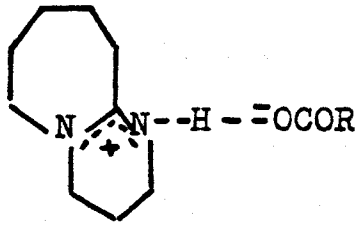
A method has been reported[87] for the esterification of a carboxylic acid with an alkyl halide using a copper(1) oxide-base system. Isonitrile or pyridine are suitable as the base component. It is assumed that the reaction proceeds via the key intermediate of cuprous carboxylate which reacts with the alkyl halide to form the desired ester. Therefore, the reaction can be compared with the similar reaction using alkali metal carboxylates. The yields in the copper system are superior to those for the alkali metal salts.

DBU-carboxylic acid system

One of the most interesting procedures for the esterification of carboxylic acids is based on the reaction of carboxylic acids with alkyl halides in the presence of 1,8 diazabicyclo(5.4.0)-undec-7-ene (DBU).[88] The reaction proceeds in a non polar solvent, such as benzene, to give esters in good yields. It is stated that the main advantages of the system include (a) The reaction of carboxylate ions with alkyl halides can be carried out in a non polar solvent. (b) No prior preparation of carboxylate ions is necessary. (c) Very small amounts of dehydrohalogenation side reactions.

The results show that the carboxylate ions formed by the reaction of carboxylic acids with DBU, in benzene, are

sufficiently reactive to alkyl halides to give esters in good yields, but their basicity is very low. Evidently the basicity of DBU and carboxylate ions in the system is greatly reduced by protonation (XLVI).



(XLVI)

The comparable reaction in the presence of triethylamine instead of DBU proceeds too slowly to be useful. The principle of esterification using DBU in benzene resembles that of ion-pair extractive alkylation or crown ether complexation. The biggest difference is that (XLVI) is not a free ion-pair but is hydrogen bonded between DBU and the carboxyl. It is this hydrogen bond that plays an important role in controlling the reactivity of the carboxylate anion, noticeably the low basicity.

Crown ether complexation.

The cyclic polyethers described by Pedersen[89-92] are of special interest because of their remarkable complexing properties. They form complexes with many salts, especially those of alkali and alkaline earth cations, both in solution and in the crystalline state. Anions of these salts, eg carboxylate anions, have been shown to be unusually reactive.

In the presence of a crown ether, (18-Crown-6), high concentrations of potassium carboxylates can be achieved in non polar solvents. This carboxylate anion shows enhanced reactivity and can be used to prepare esters, from alkyl halides, in high yields.

Quaternary ammonium carboxylates

Replacement of the cation in an alkali metal carboxylate by a highly lipophilic quaternary ammonium cation results in a highly reactive carboxylate anion[93] which can be used in solvents ranging from polar, non polar to dipolar aprotic. The enhanced reactivity can be attributed to increased solubility in the organic phase and a reduction in the cation-anion interaction energy. Results are analagous to those obtained by crown ether complexation of an alkali metal cation, and in some cases the results are superior. They offer an excellent alternative to the alkali metal cations.

Dipolar aprotic solvents

The above examples can be summarized as nucleophile activation by variation of the cation. One synthetic improvement is to perform carboxylate displacements using dipolar aprotic solvents.[94] They have two main properties that recommend them for synthetic work:-

(I) A wide variety of organic compounds and inorganic compounds are soluble, especially in solvents such as DMF, DMSO and HMPA.

(2) Anions react much more readily in displacement reactions

because they have no general hydrogen bonding interaction with the solvent, as they have in protic solvents, and are thus much less solvated and more reactive in the dipolar aprotic solvents.

As would appear from the discussion on acyl migration, the nucleophilic substitution reactions observed are of two possible types:-

(I) Those in which a chiral substrate leads to a product of opposite configuration, suggesting involvement of the Walden inversion.

(2) Those in which a chiral substrate leads to a product of the same configuration, indicating retention of configuration.

Because of the unique structural features of the substituted glycerol molecule used as substrate, reactions of type (2) predominate and further, these are dominated by participation of an appropriate group on the neighbouring carbon atom.

Aneja and co-workers[12,15,95-101] have closely studied both the above types of nucleophilic substitution reactions. They found that acylglycerols carrying an OH group at C-2 when reacted with the reagent system triphenylphosphine-carbon tetrachloride or triphenylphosphine-diethylazodicarboxylate-carboxylic acid, proceed without concomitant acyloxy migration and lead to a product in which the configuration is opposite to that of the substrate. By a study of the halogendeoxygenation of the toluene-p-sulphonate of 2-phenyl-1,3-dioxan-5-ol with lithium bromide in boiling

acetonitrile they have shown[98] that rearrangements under essentially neutral conditions can occur, and that great caution should be exercised in the choice of starting material and reaction route for the synthesis of pure glycerides.

In nucleophilic substitution reactions which are accompanied by acyl or acyloxy migrations, not only the unsymmetrical dioxolenium ion (XXI)[15] but other species, depending on the structure of the substrate, can be a reaction intermediate. For example, in the synthesis of 1,3-diglycerides from 1,3-dihalogendeoxyglycerol (route 2(V)) a glycidyl ester has been detected.[102] An epoxy intermediate (XLVII) can also explain why Bergmann and Sabetay[69] obtained racemic 1-monolaurin (XLV) from pure dextrorotatory amine hydrochloride (XLIV)

Aneja and co-workers[101] have also discussed the strategy for the regiospecific synthesis of glycerolipids via nucleophilic substitution reactions. They found that the parameters which insured a high degree of specificity are:-

(I) Choice of leaving group eg triphenylphosphonium.

(2) Use of an aprotic polarising solvent eg HMPA.

(3) Application of a tetraalkylammonium cation as counter-ion.

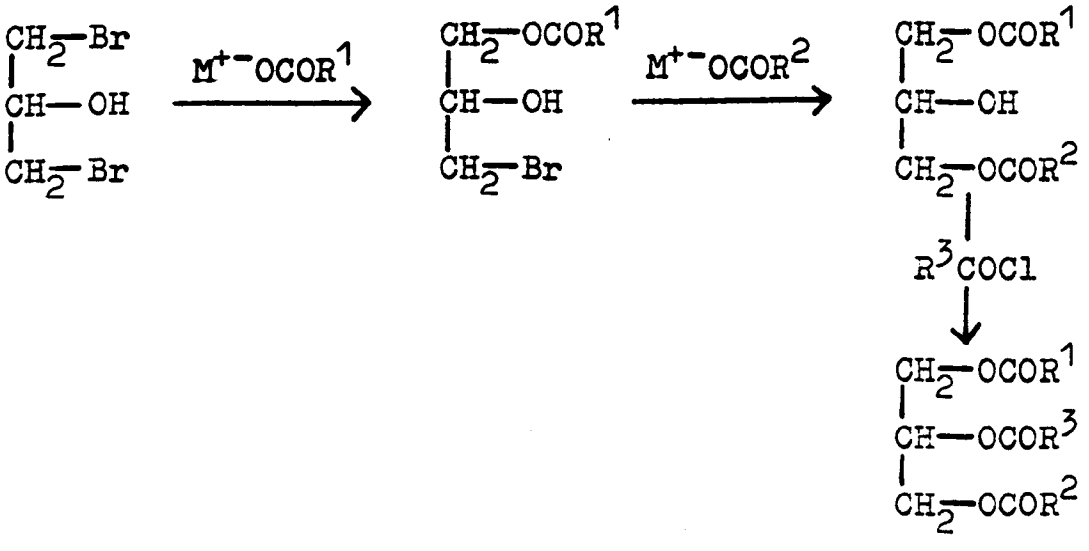
Nucleophilic substitution reactions in glycerol derivatives offers economy in the number of synthetic steps that are needed to produce a desired mixed-acid triglyceride. A substrate, 1,3-dibromopropan-2-ol, which has been shown to be unsuitable for glyceride synthesis[47] was critically

re-examined in the light of advances in nucleophilic substitution techniques. The aim of the investigation was to determine if the substrate could be used as a reliable precurs or for structurally pure mixed acid triglycerides.

2.1 Proposed route for the synthesis of triacid triglycerides of high structural purity.

The reaction sequence outlined in scheme 1 may appear attractive as a general route for the synthesis of a triacid triglyceride. The implied simple three stage reaction does not involve the use of protecting groups, whose removal can sometimes catalyse acyl migration[37-39,55] within the resulting partial glyceride. The scheme offers great diversity in the preparation of triglycerides, theoretically any triacid triglyceride could be synthesised merely by altering the carboxylate ester groups to be introduced.

Scheme 1



Where:- M^+ = Na^+, K^+ or Ag^+

R^1COO^- = Preferably the stearate anion

R^2COO^- = Another desired fatty acid anion

R^3COCl = A suitable fatty acid chloride

2.2 Results and Discussion

2.2.1 Reaction of 1,3-dibromo propan-2-ol with an equimolar amount of dry sodium-, potassium- or silver-stearate.

The stearate anion ($^{-}OCOC_{17}H_{35}$) was chosen to be introduced in the first stage of the reaction as the resulting product would be an easily isolatable product. The results given in Table 1 are a typical analysis, and were found to be independent of the counter ion used.

Table 1 Products from the reaction of 1,3-dibromo propan-2-ol with sodium-, potassium- or silver stearate.

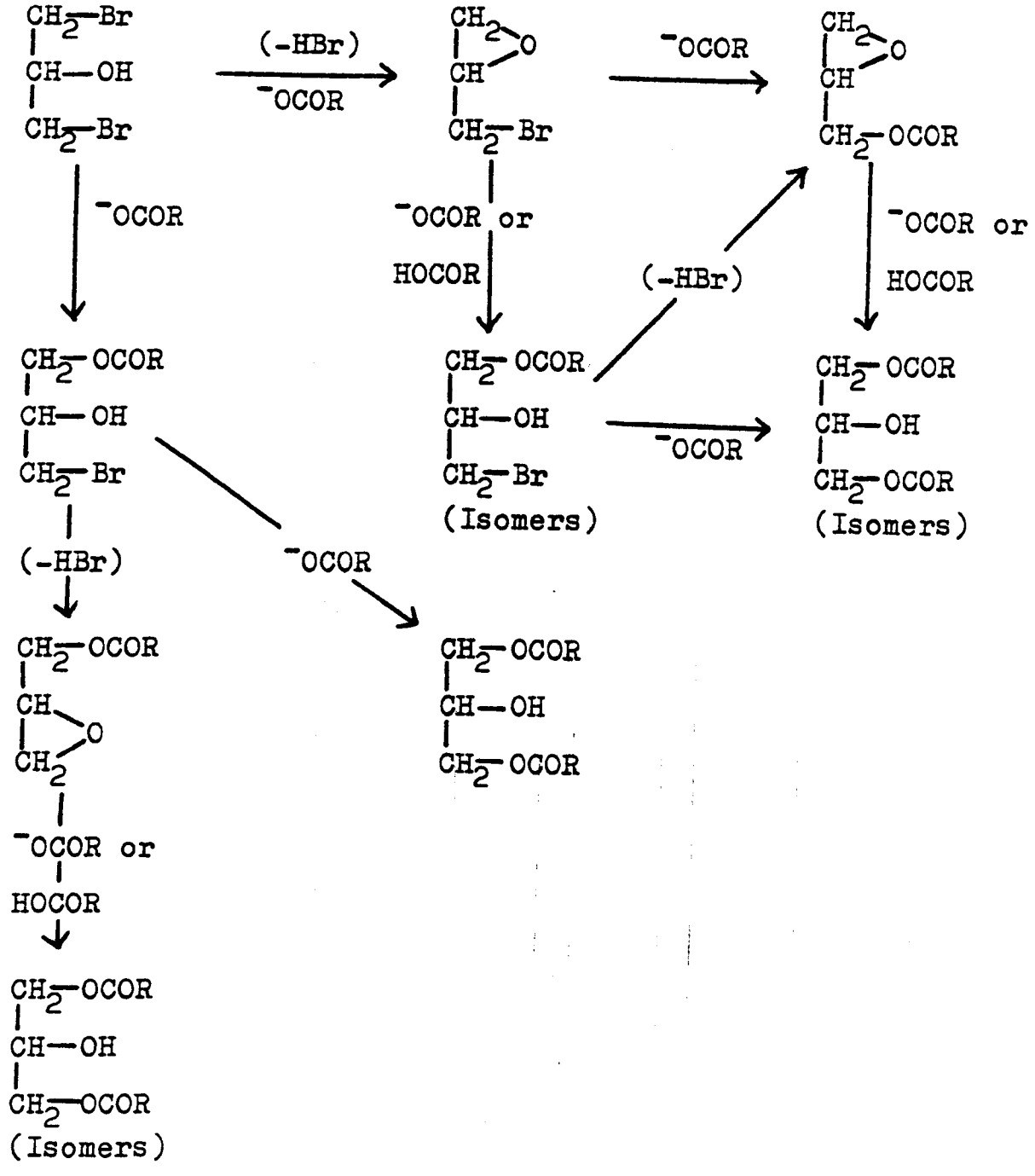
Compound	Moles x 10^{3}
Stearate anion introduced into the reaction flask.	6.43
Stearic acid (XLVIII)	1.24
Glycidyl stearate (XLVIX)	0.46
Glycerol 1-stearate-3-bromodeoxy and glycerol 2-stearate-3-bromodeoxy (L)	0.53
Glycerol 1,3- and 1,2-distearin (LI)	1.04

The reaction did not proceed as expected, dehydrobromination in the parent substrate being the major reaction, yielding epibromohydrin and the free carboxylic acid which obviously arises by the reaction of HBr and the carboxylate

salt. The high yield of glycerol distearin suggests that there is an effective excess of species that can react with the epoxide. This is understandable as the low boiling epibromohydrin partly escapes from the reaction vessel and is trapped at the base of the condenser. The residual substrate that has survived dehydrobromination can undergo the desired nucleophilic substitution and yields the 1- or 3-stearate derivative. If excess nucleophile is present, the final product would be the glycerol distearin.

The carboxylate anion can react with the epibromohydrin at all three carbon atoms. Direct displacement of bromide for carboxylate produces glycidyl stearate. Attack at either of the carbon atoms of the epoxide ring will produce the carboxylate ester but also an alkoxide ion. This alkoxide ion can abstract a proton from a suitable substrate to regenerate the hydroxyl group or undergo intramolecular nucleophilic substitution to reform an epoxide. Alternatively the free stearic acid could attack the epoxide ring, forming the stearate ester. There are evidently numerous alternative reaction routes which lead to both the wanted and the unwanted products. The major products encountered and their possible modes of formation are shown in Figure 2. The isomeric compounds will be glycerol 1-stearate -3-bromodeoxy and glycerol -2-stearate-3-bromodeoxy for the monoacyl compound a mixture of 1,3- and 1,2-distearin for the diacyl compound. The results indicate that the reaction of 1,3-dibromo propan-2-ol with the metal carboxylates is not satisfactory for the synthesis of the monostearate

Figure 2. Formation of reaction products from metal carboxylates and 1,3-dibromo propan-2-ol.



Where R= $C_{17}H_{35}$

derivative.

The reaction mixture in the above study was not homogeneous, as the metal carboxylates have low solubility in the substrate. In an attempt to provide a homogeneous reaction mixture, two dipolar aprotic solvents were investigated to determine if they could be used to exert a beneficial effect upon the course of the reaction, and in particular arrest the loss of HBr from the substrate.

2.2.2 Reaction of 1,3-dibromo propan-2-ol with an equimolar amount of dry potassium stearate in DMF and HMPA.

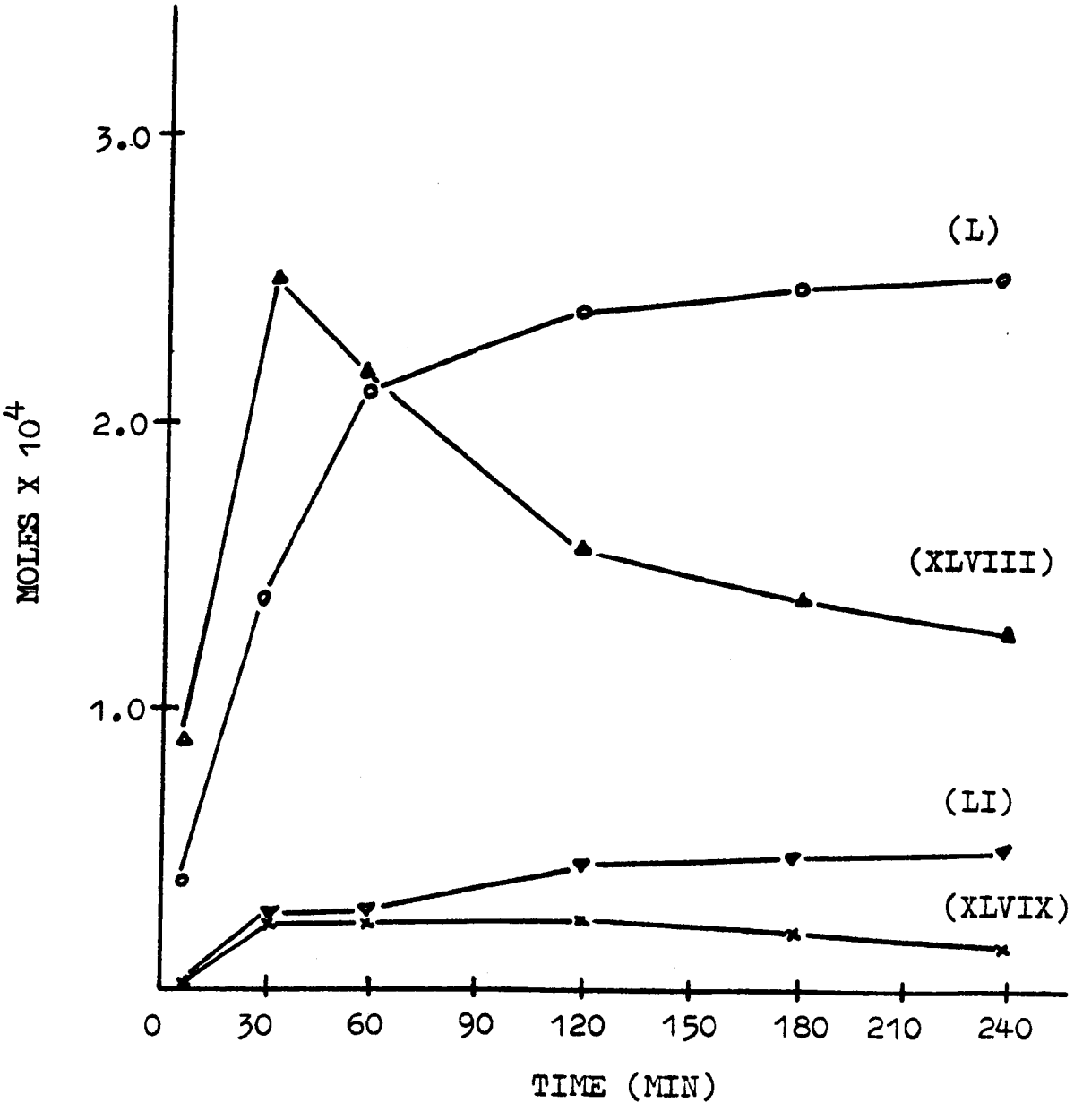
Dipolar aprotic solvents have found much use in preparative organic chemistry because of their ability to solubilize inorganic salts.[94] Anions in these solvents have no general hydrogen-bonding interaction with the solvent, as they have in protic solvents, and are therefore, much less solvated and more reactive. The dipolar aprotic solvents also solvate cations strongly and have fairly high dielectric constants, so that electrolytes are strong in these solvents, leaving the anions free to actively participate in reactions without the stabilizing influence of ion-pair formation. Another factor in their favour is that they are strong hydrogen bond acceptors and, therefore, interact specifically and strongly with dipolar hydrogen bond donors such as alcohols. This interaction may find use as a "partial protection" of the hydroxyl in the substrate and hinder loss of HBr. The common dipolar aprotic solvents are acetone, acetonitrile, DMSO, DMF and HMPA.

The products from reactions conducted in DMF and HMPA are shown in Figures 3 and 4. Both sets of results show that loss of HBr from the substrate is still a dominant reaction. Free stearic acid (XLVIII) is produced at the beginning of the reaction and is partially consumed as the reaction proceeds. The graphs provide evidence for the participation of stearic acid in the formation of the monostearate derivative (L).

In HMPA, the rise in concentration of (XLVIII) is almost paralleled by a rise in the concentration of the monoacylated product (L). The glycidyl ester (XLVIX) and diglyceride (LI) also increase in concentration at the start of the reaction. The products will be formed by the competition between substitution and elimination reactions in the substrate. After 30min, the concentration of (XLVIII) falls and the concentration of (L) rises, indicating that the acid is participating in the formation of (L), since the concentration of carboxylate ion will be small. Reaction of epibromohydrin with (XLVIII) produces a mixture of the two isomers of (L). The formation of (LI), in the latter part of the reaction, probably arises from the reaction of (XLVIII) with (XLVIX).

A similar situation exists when DMF is used as solvent. The proportion of (XLVIII) is higher with a decrease in concentration of all other compounds. More dehydrobromination results, which could be due to the decrease in solubility of the potassium stearate, compared to HMPA as solvent, leading to slower reaction with the liberated epibromohydrin.

Fig. 3 Reaction of the substrate with an equimolar amount of potassium stearate in HMPA at 70°.



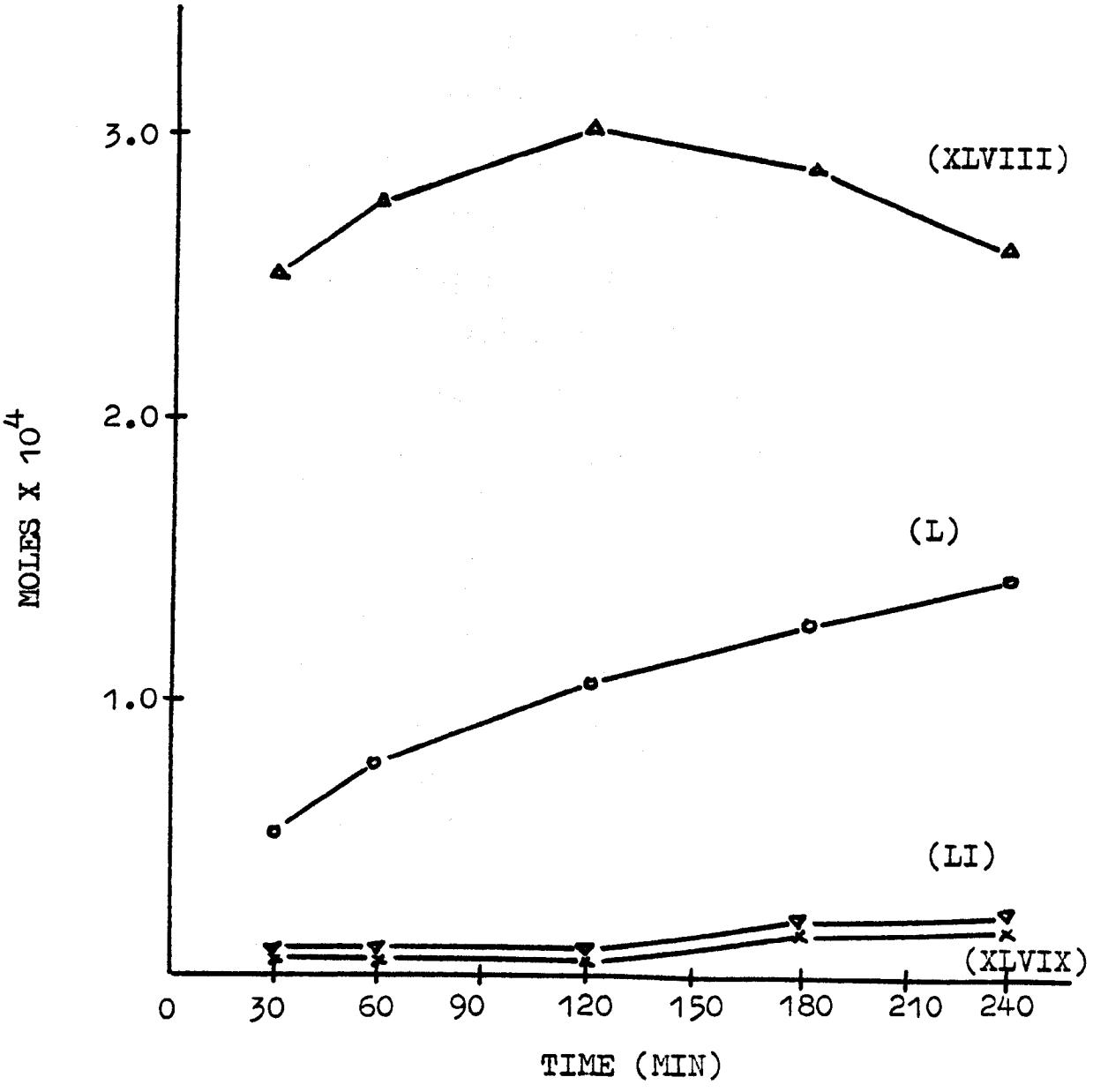
(XLVIII)= Stearic acid

(XLVIX) = Glycidyl stearate

(L)= Glycerol 1-stearate-3-bromodeoxy and glycerol 2-stearate-3-bromodeoxy

(LI)= 1,3- and 1,2-distearin

Fig. 4 Reaction of the substrate with an equimolar amount of potassium stearate in DMF at 70°.



Noticeably, the overall yield of (LI) in HMPA and DMF is far less than when the solvent is omitted (cf p.94). The increased reactivity of the stearate anion combined with efficient stirring of the homogeneous mixture apparently allows the epibromohydrin to react further, rather than escape from the reaction vessel.

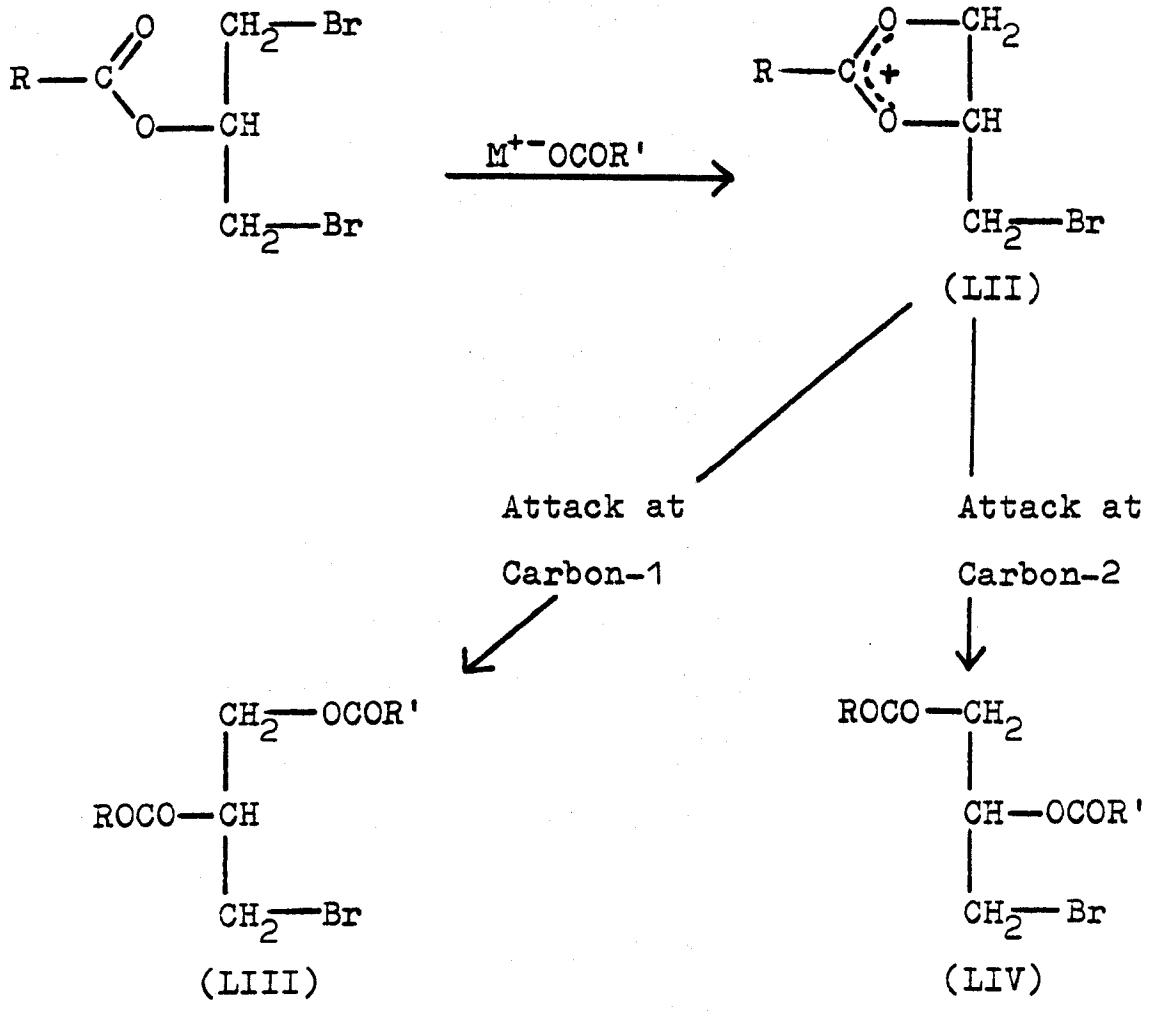
In conclusion, the reaction in HMPA and DMF has certain advantages over the reaction without solvents, but the yield and probable structural purity of compound (L) are disappointing. The isolation of (L) from the other products is an arduous task and results in a low overall yield. The proposed stepwise route has thus no merit for the synthesis of high purity triglycerides as the substrate is too prone to the loss of HBr under the conditions employed.

3.1 Glycerol 1,3-dibromodideoxy-2-palmitate as a substrate for the synthesis of triglycerides.

From the fore-going account it would seem that the proton on the hydroxyl group at carbon-2 in 1,3-dibromopropan-2-ol is readily lost on treatment with the carboxylate anion. Treatment of the halohydrin with a fatty acid chloride removes this proton and generates the first carboxylic ester group at carbon-2. This has the dual advantage of removing the ability of the substrate to form an epoxide and at the same time preparing the monoester. Subsequent nucleophilic substitution reactions at carbons-1 and -3 with a different carboxylate anion will produce a mixed acid triglyceride. However, the introduction of the ester group at carbon-2 introduces the unwelcome possibility of side reactions, notably the concomitant acyloxy migration[12,15] from either carbon-2 to carbon-1 or carbon-2 to carbon-3 and involves a dioxolenium ion such as (LII) as a reaction intermediate. Reaction of this intermediate (Figure 5) can give rise to product (LIII) or the rearranged product (LIV). Therefore any triglycerides synthesised from the 2-acyl precurs or must be analysed by a lipolysis procedure to determine if acyloxy migration has occured. With this aim, 1,3-dibromo propan-2-ol was acylated with palmityl chloride to yield the 2-palmitate derivative as the new reaction substrate.

The synthesis of a diacid triglyceride (glycerol 1,3-

Figure 5 Formation of isomeric compounds from the same precurssor.



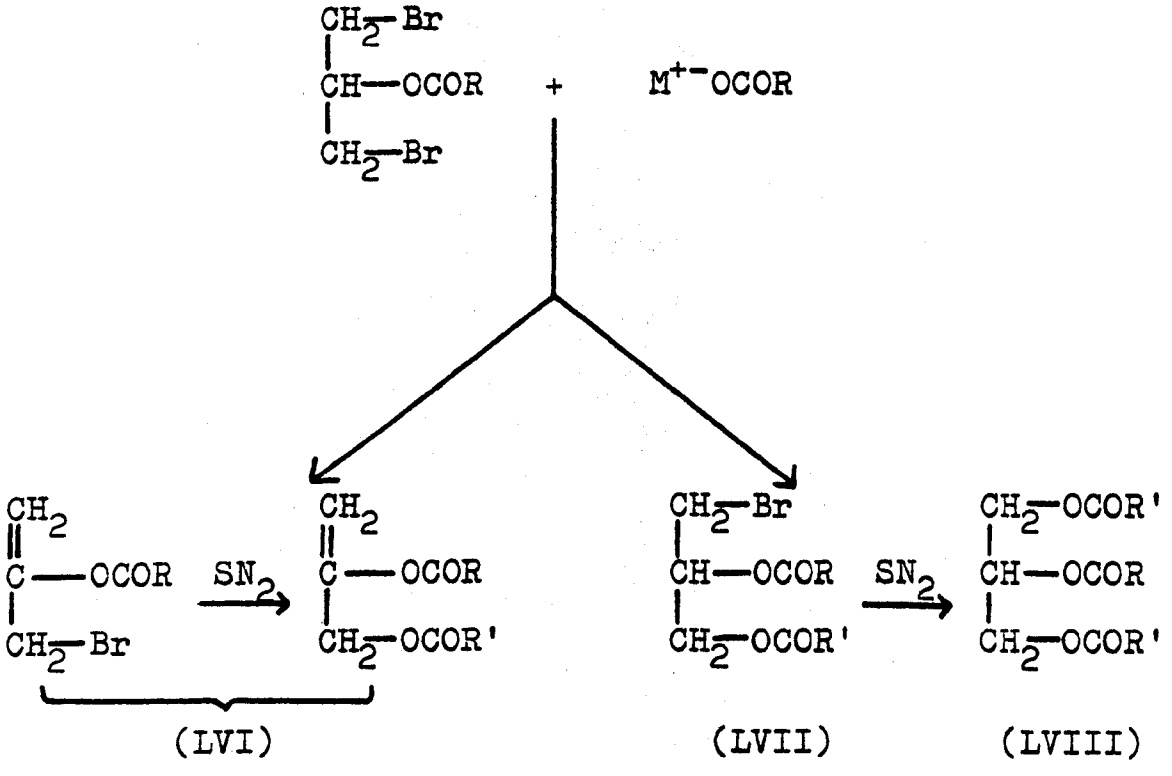
distearate-2-palmitate) was studied in detail to evaluate the effectiveness of using glycerol 1,3-dibromodideoxy-2-palmitate as a substrate for the synthesis of high purity mixed acid triglycerides. The substrate is a primary alkyl halide possessing one hydrogen atom at carbon-2 and can, therefore, simultaneously undergo bimolecular substitution and elimination on treatment with a nucleophilic reagent. Also, nucleophilic substitution in the substrate, by, for example, the stearate anion, can yield solely the non-rearranged glycerol 1,3-distearate-2-palmitate or it could be contaminated with glycerol 1,2-distearate-3-palmitate, if acyl migration has occured. All these possibilities are depicted schematically in Figure 6.

The solvent, and the counterion (M^+) in the stearate salt ($M^+{}^-OCOC_{17}H_{35}$) were varied to examine their effect upon the yield of substitution and elimination products. The solvents examined (n-hexane, toluene, tetrahydrofuran (THF), acetone, DMF and HMPA) ranged from non-polar to dipolar aprotic, with dielectric constants in the range 1.9 to 36.7.

Traditionally, for nucleophilic substitution reactions involving carboxylate anions, the alkali metal cations Na^+ and K^+ have been used,[82] but because of their insolubility in most solvents their synthetic utility is restricted to dipolar aprotic solvents such as DMF and HMPA. The more effective silver-ion assisted nucleophilic substitution[83,84] provides a useful alternative to the alkali metal ions. But, perhaps the most interesting counter ions are the

(continued on p. 106)

Figure 6 Substitution and elimination products arising from the reaction of glycerol 1,3-dibromo-dideoxy-2-palmitate with $M^{+-}OCOR'$



M^+ = Na^+, K^+, Ag^+, $(C_2H_5)_4N^+$, $(C_{10}H_{21})_3N^+CH_3$ and 18-Crown-6/K^+

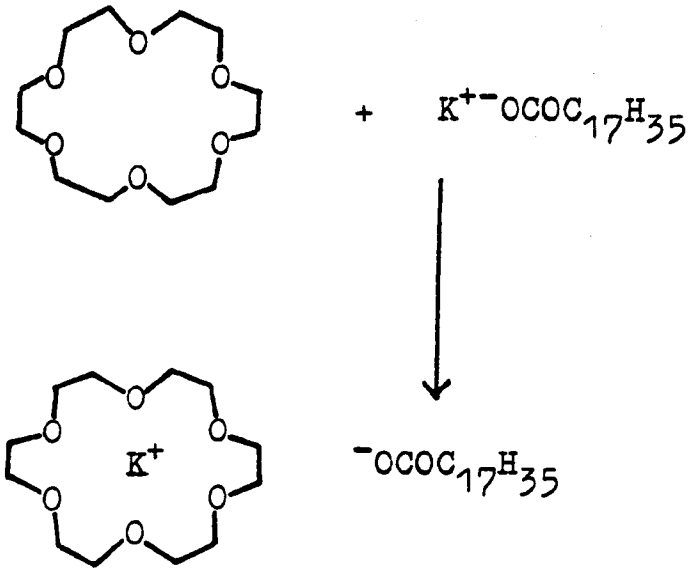
R = $C_{15}H_{31}$ (n-pentadecyl)

R' = $C_{17}H_{35}$ (n-heptadecyl)

Product (LVI) is the total concentration of elimination products in the reaction mixture, comprising the initial elimination product and its monosubstitution derivative.

quaternary ammonium[93] and crown ether complexed alkali metal cations.[89-92] They derive their synthetic utility from their ability to solubilize inorganic reagents and salts in aprotic non-polar solvents. The anions of these solubilized salts possess tremendous reactivity as a result of a reduced cation-anion interaction energy. This arises, in the case of quaternary ammonium salts, from the steric effect of the alkyl groups of the cation preventing close approach of the anion. In the case of the crown ether complex, the bulky polyether ring shields the charge of the cation very effectively and thereby decreases the interionic attraction. The complex of potassium stearate and 18-Crown-6 (1,4,7,-10,13,16 hexaoxacyclooctadecane) shown in Figure 7 was selected for crown ether evaluation.

Figure 7 The complex of 18-Crown-6 with potassium stearate.



The cation is located in the centre of the main ring in the plane, or almost in the plane, of the oxygen atoms, being held there principally by electrostatic, (ion-dipole) forces.

The quaternary ammonium cations used were tetraethylammonium and the highly lipophilic tricaprylmethylammonium ions.

3.2 Results and Discussion.

3.2.1 Variation of solvent and counter-ion on the reaction of $M^{+-}OCOC_{17}H_{35}$ with glycerol 1,3-dibromodideoxy-2-palmitate.

The results of the experiments are given in Tables 2A-2F, on pages 108-110.

Rationalisation of the results requires consideration of the actual anion species involved in the reaction. In the low polarity solvents (n-hexane and toluene) ion-pairs ($M^{+-}OCOC_{17}H_{35}$) and aggregates of ion-pairs $(M^{+-}OCOC_{17}H_{35})_n$ represent the prevailing species in the solution,[103,104] they can be collectively termed "Associated species." The crown ether complexes and quaternary ammonium cations (cf Tables A and B) have maximum values for the proportion of substitution products, and minimum values for the amount of products formed by elimination reactions, in these solvents.

In the dipolar aprotic solvents (cf Tables E and F) there is little difference between the crown ether complexes, the quaternary ammonium cations or the Na^+ and K^+ ions. This suggests that the solvent separated ion-pairs or free

(continued on p.111)

Table 2 Variation of solvent and counter-ion on the reaction of $M^+{}^-OCOC_{17}H_{35}$ with glycerol 1,3-dibromodideoxy-2-palmitate.

Dielectric constant at 25° = E

(A) Solvent: n-hexane (E=1.9) 3hr reaction at 69°

M^+	Mole% substrate consumed	Mole% products LVI*	LVII*	LVIII*
Na	<1	<1	<1	<1
K	<1	<1	<1	<1
Ag	<1	<1	<1	<1
$(C_2H_5)_4N$	33	2	29	2
$(C_{10}H_{21})_3NCH_3$	100	9	25	66
18-Crown-6/K	60	5	51	4

(B) Solvent: Toluene (E=2.4) 3hr reaction at 69°

M^+	Mole% substrate consumed	Mole% products LVI*	LVII*	LVIII*
Na	<1	<1	<1	<1
K	<1	<1	<1	<1
Ag	<1	<1	<1	<1
$(C_2H_5)_4N$	27	1	24	2
$(C_{10}H_{21})_3NCH_3$	100	11	29	60
18-Crown-6/K	52	3	45	4

* See structures on p.105

(C) Solvent: THF (E=7.6) 3hr reaction at 69°

M^+	Mole% substrate consumed	Mole% products LVI	LVII	LVIII
Na	<1	<1	<1	<1
K	<1	<1	<1	<1
Ag	<1	<1	<1	<1
$(C_2H_5)_4N$	37	4	31	2
$(C_{10}H_{21})_3NCH_3$	100	15	15	70
18-Crown-6/K	100	23	44	33

(D) Solvent: Acetone (E=20.7) 3hr reaction at reflux.

M^+	Mole% substrate consumed	Mole% products LVI	LVII	LVIII
Na	<1	<1	<1	<1
K	<1	<1	<1	<1
Ag	<1	<1	<1	<1
$(C_2H_5)_4N$	28	5	22	1
$(C_{10}H_{21})_3NCH_3$	100	18	58	24
18-Crown-6/K	83	17	45	21

(E) Solvent: HMPA (E=30.0) 3hr reaction at 69°

M^+	Mole% substrate consumed	Mole% products LVI	LVII	LVIII
Na	100	39	3	58
K	100	44	1	55
Ag	19	3	16	0
$(C_2H_5)_4N$	100	40	0	60
$(C_{10}H_{21})_3NCH_3$	100	42	0	58
18-Crown-6/K	100	40	2	58

(F) Solvent: DMF (E=36.7) 3hr reaction at 69°

M^+	Mole% substrate consumed	Mole% products LVI	LVII	LVIII
Na	24	5	16	3
K	40	13	19	8
Ag	3	1	2	0
$(C_2H_5)_4N$	100	40	42	18
$(C_{10}H_{21})_3NCH_3$	100	50	0	50
18-Crown-6/K	100	46	4	50

ions ($M^+ + {}^-OCOC_{17}H_{35}$) prevail in these solvents, these species can be collectively termed "Dissociated species." The proportion of elimination product, for all of the cations, is maximised in DMF or HMPA. The observed enhanced basicity of the carboxylate anion, in the dipolar aprotic solvents, can be attributed, at least in part, to solvent assistance in carbanion formation at carbon-2 in the substrate.

In THF, where solvent assistance in carbanion formation is limited, the reactivity enhancement of the crown ether complexes and the tricaprylmethylammonium counter-ions is more apparent in the nucleophilic sense than the basic sense, even though the proportion of elimination product is still marked.

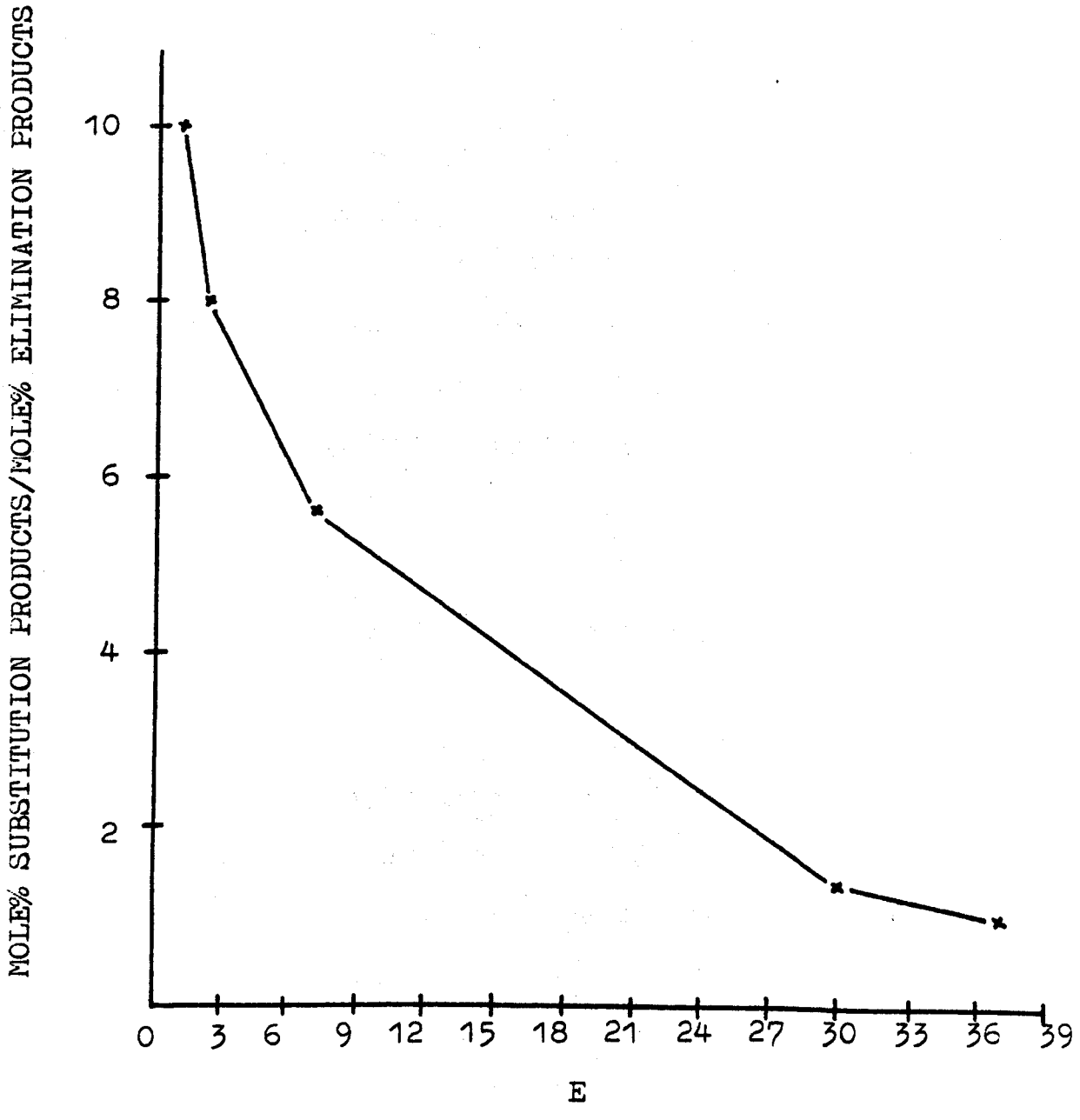
Evidently, the associated nucleophile found in non-polar solvents has a smaller propensity to take part in the elimination reaction (E_2) as compared to the dissociated species, this implies that the basicities of the associated and dissociated form of the nucleophile are very different. The lowering of base strength induced by ionic association explains why the most favourable proportion of substitution products is found in the non-polar solvents. The attractive ion-dipole interactions between the counter-ion of the associated nucleophile and the leaving group may also play a part in determining the proportion of substitution to elimination products. It has been proposed on previous occasions that the attractive interactions can operate in E_2[105-107] and SN_2[108] reactions, and stabilize the transition states, in dependence on the proximity of the interacting

groups. The results from Tables 2A-2F show that free-ions, ion-pairs and ionic aggregates are distinct species endowed with their own reactivities. Since the aim of the synthesis is to maximise the yield of substitution products and minimise any elimination products formed, the combination of tricaprylmethylammonium stearate (TMAS) as the source of carboxylate ion and n-hexane as solvent was studied further to maximise the yield of the substitution products.The graph of mole% substitution products/ mole% elimination products versus the dielectric constant of the solvent shown in Figure 8 demonstrates the necessity of a non-polar solvent.

3.2.2 Effect of dilution on the reaction of TMAS with glycerol 1,3-dibromodideoxy-2-palmitate in n-hexane solution.

When both the cation and anion of a quaternary ammonium salt have large organic structures with high solubility in the organic solvent, there will be insufficient insolubility to promote aggregation.[109] However, amines with longer (or more) alkyl chains eg TMAS have larger molecular volumes and are therefore less soluble in any given solvent (as predicted by the theory of regular solutions). As a consequence, the ions of the quaternary ammonium salt in n-hexane are aggregated to some extent. The question of whether this aggregation could be called an "inverted micelle" requires identification of some discontinuity in property as a function of concentration. With this aim, the reaction of excess TMAS with the substrate, in n-hexane

Fig. 8 Graph of substitution/elimination ratio versus dielectric constant of the solvent.



solution, was monitored over a large concentration range to determine if any discontinuity in the extent of reaction or product distribution could be detected. The results of the experiments are shown graphically in Figures 9 and 10.

The critical micelle concentration (CMC) for reversed micelles[110] is not so general as in the case of aqueous systems, and not so sharp if it exists. This characteristic results sometimes from stepwise aggregation: monomer $\longleftrightarrow$ dimer $\longleftrightarrow$ trimer $\longleftrightarrow$ n-mer. Figures 9 and 10 show that at a concentration of 1 x $10^{-2}Ml^{-1}$ TMAS there is an abrupt increase in the extent of reaction and a marked decrease in the ratio of substitution to elimination products which could represent an effective CMC for TMAS in n-hexane. It is noticeable that the nucleophilicity and basicity in the postulated aggregated species (1 x $10^{-2}Ml^{-1}$) is different from that of the monomeric species (0.5 x $10^{-2}Ml^{-1}$). As the concentration increases from 1 x $10^{-2}Ml^{-1}$ to 8 x 10^{-2} Ml^{-1} TMAS there is an increase in the extent of reaction, with the ratio of substitution to elimination products remaining constant. From 8 x $10^{-2}Ml^{-1}$ to 40 x $10^{-2}Ml^{-1}$ TMAS there is a gradual decrease in the extent of reaction and only a minor decrease in the ratio of substitution to elimination products. This could be attributed to a difficulty in efficiently stirring the increasingly viscous reaction solution.

The reasons for aggregation of TMAS in n-hexane are different from those applying to aqueous solutions.[111-112] In a non-polar solvent, there is the possibility of a reduction of the interfacial energy between the head groups

Fig. 9 Graph of extent of reaction versus the concentration of TMAS in n-hexane

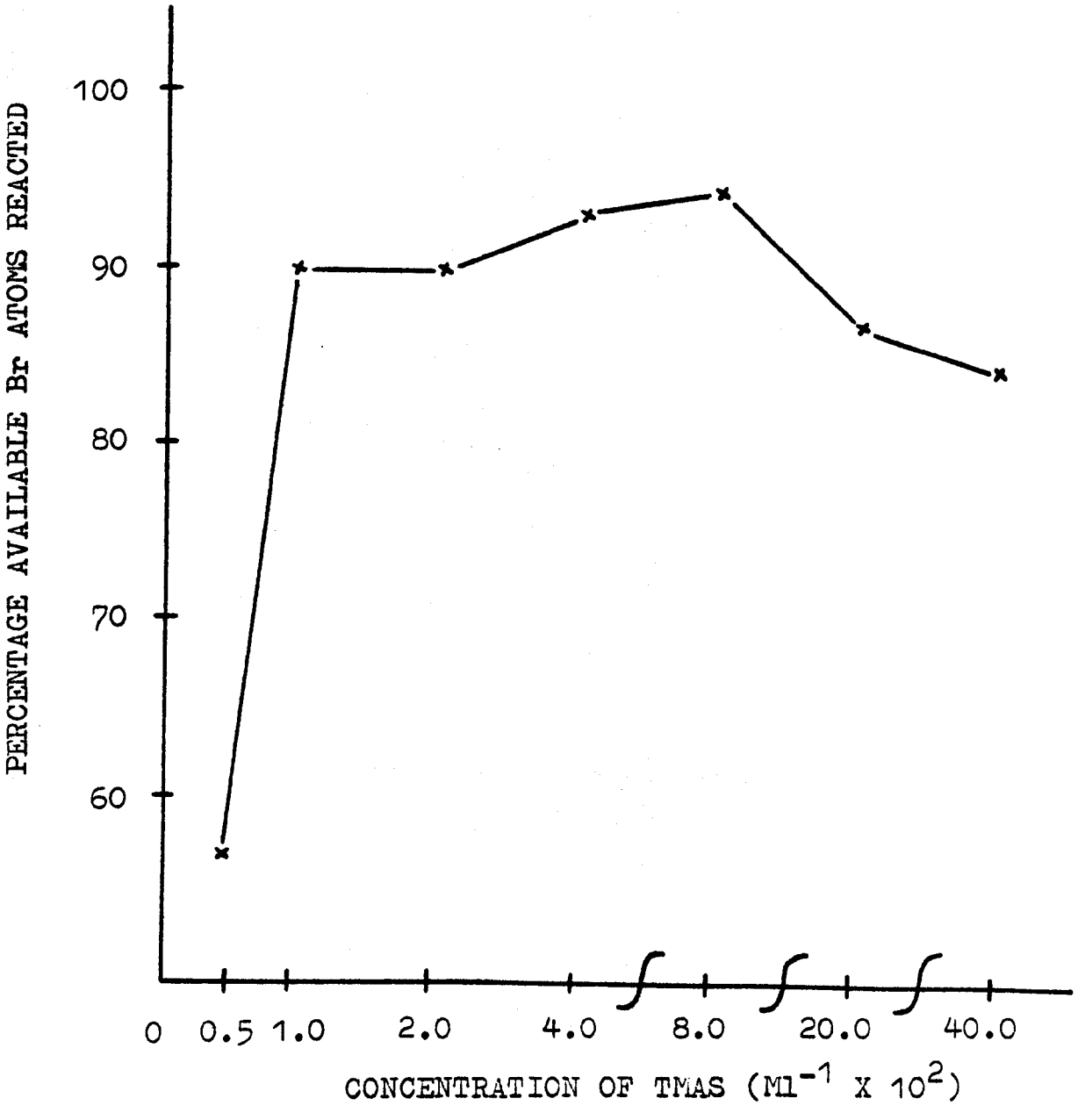
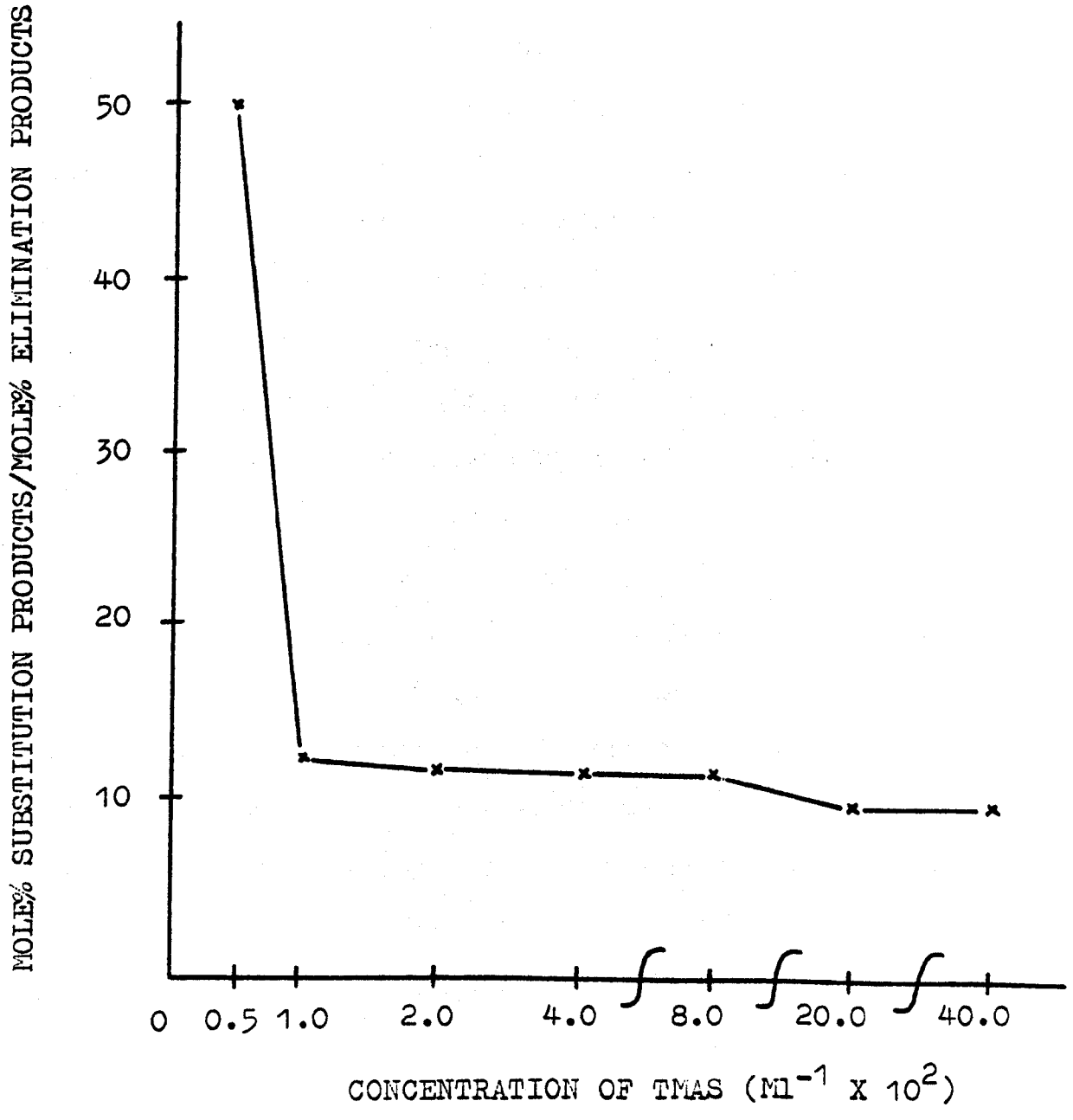


Fig. 10 Graph of substitution/elimination ratio versus the concentration of TMAS in n-hexane.



and solvent on aggregation, as the head groups are situated in the aggregate core remote from the solvent. The largest energy changes arise from dipole-dipole interaction between head groups in the aggregate core, and it is these head group effects that provide the forces for aggregation. Opposing aggregation will be the possible loss of translational, vibrational and rotational freedom of the monomers when placed in an aggregate. It is to be expected that the three long alkyl chains in the cation will prevent close approach of other monomers and severely restrict the aggregation number. The aggregation properties of alkylammonium carboxylate surfactants in benzene and carbon tetrachloride have been reported,[113] the data indicated small aggregates each containing 3-7 monomers and these have been termed inverted micelles.

The high propensity of carboxylate anions associated with quaternary ammonium ions in non-polar media to undergo displacement reactions is particularly suited to the synthesis of triglycerides. In non-polar media, the ion-pair/or aggregates of ion-pairs are the reaction entity and the abnormal reactivity can be explained by a reduced cation-anion interaction energy. However, an alternative possibility is the formation of a weak complex in which the alkyl halide penetrates deeply into the web of alkyl groups around the cation. Such complex formation is favoured not only by coulombic ion-dipole interactions but also by Van der Waals attraction between the alkyl groups of the quaternary ammonium cation and that of the alkyl halide. From such a complex, a "push-pull" transition stat

as described by Swain,[114] can be readily formed, although in this case it is not necessary that the displacing ion be separated from the cation (Ionised).

The origin of the "aggregation effect" can be described in terms of substrate partitioning between the aggregate and bulk solvent phases. It has been reported[115] that surfactant aggregates in benzene are capable of solubilizing a large molecule such as vitamin B_{12a}. Solutions containing aggregates of TMAS in n-hexane are homogeneous by the usual criteria of physical chemistry, yet, the system consists of regions of high TMAS concentrations separated by essentially pure solvent. The substrate, when added to the system will distribute itself between the region occupied by the aggregates and the remainder of the available volume. If conditions in these two environments are different, then the aggregates will exert either a beneficial or a detrimental effect upon the desired course of the reaction. The solubilized substrate in the aggregate core will be in close proximity to the ammonium and carboxylate ions and probably held in a relatively rigid configuration via hydrogen bonding and other interactions. The experimental results indicate that the elimination reaction occurs to a greater extent in the aggregated system compared to the non-aggregated system present at a concentration of $0.5 \times 10^{-2} Ml^{-1}$ TMAS. However, for most purposes, this concentration is too low for the synthesis of triglycerides, even though it represents the conditions in which the elimination reaction is least favoured, since the volume of solvent needed for the preparation of more

than very small quantities, is prohibitive. As a compromise, a concentration of 20 x $10^{-2}Ml^{-1}$ TMAS was adopted, as it allowed the preparation of significant amounts of product from one reaction. At this concentration, the formation of the elimination product as an impurity is unavoidable.

3.2.3 Effect of variation in the number of mole equivalents of glycerol 1,3-dibromodideoxy-2-palmitate and TMAS on the distribution of reaction products.

The results of the experiments are shown graphically in Figures 11 and 12. As the number of moles of TMAS increases relative to the substrate, the proportion of elimination product increases as shown by a marked decrease in the substitution/elimination ratio. This trend can be explained by considering the extent of aggregation as the concentration of TMAS increases. It is reasonable to assume that only a certain proportion of the substrate is associated with the TMAS aggregate, the remainder is distributed throughout the bulk solvent phase. Therefore, as the extent of aggregation increases, so will the proportion of the substrate associated with the aggregates, and this inevitably results in a higher yield of the elimination product. The observed higher concentration of elimination product at high nucleophile concentration places a practical limit on the mole ratio of nucleophile that should be used. The optimum mole ratio of TMAS to substrate is 3:1, increasing the mole ratio beyond this figure results in an increase in the proportion of elimination product.

Fig. 11 Effect of variation in the number of mole equivalents of TMAS and substrate on the distribution of reaction products.

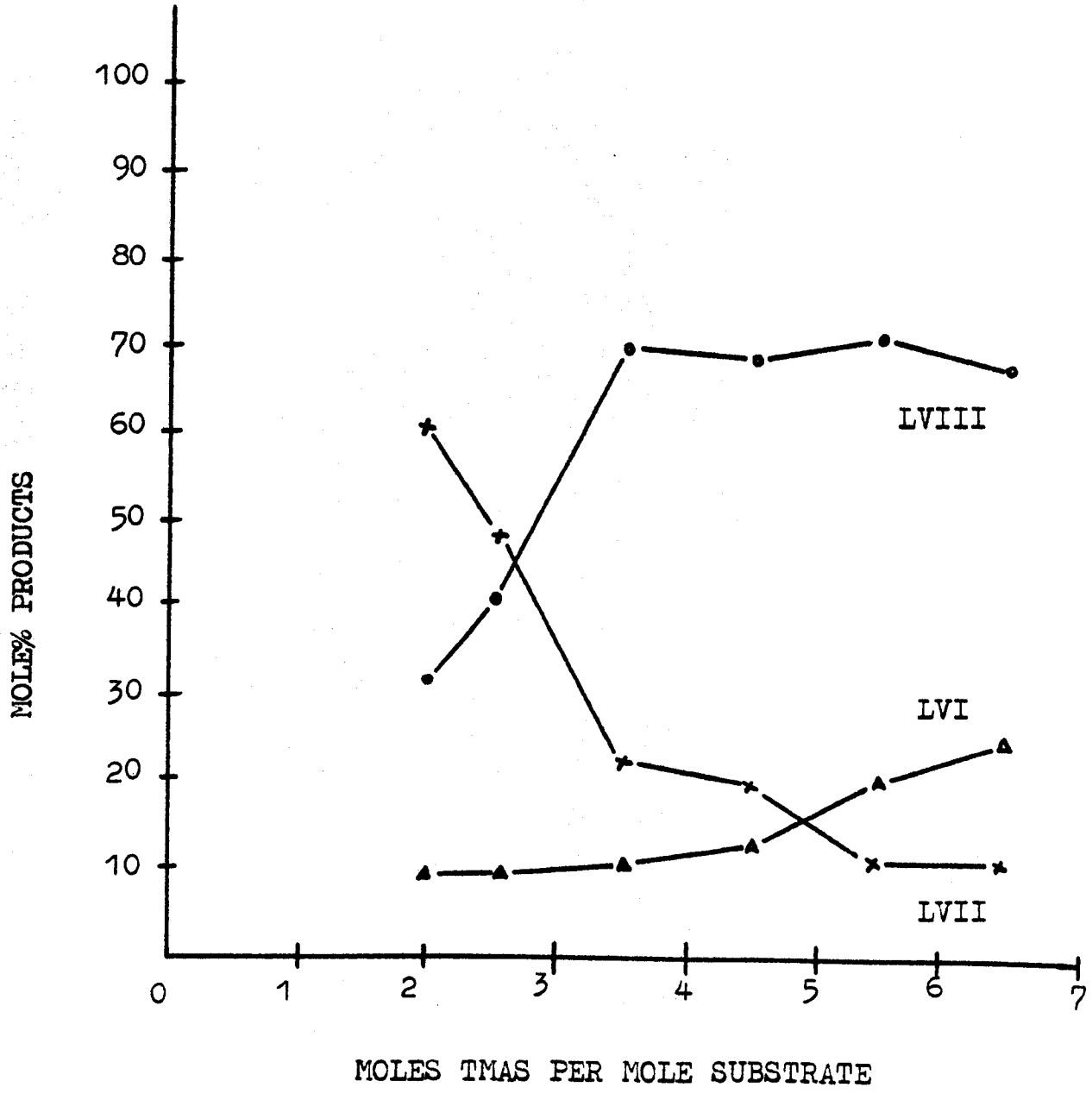
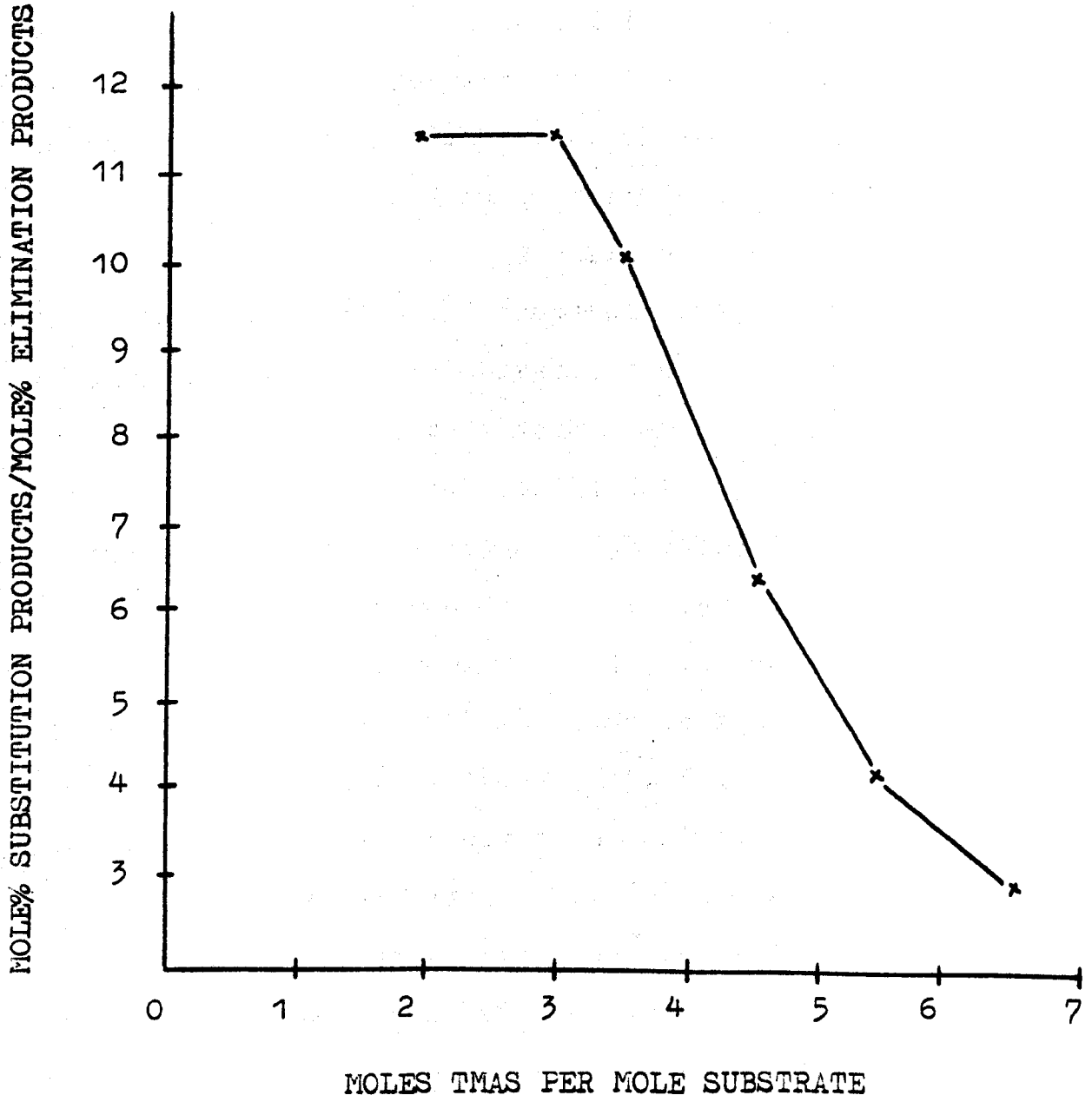


Fig. 12 **Effect of variation in the number of mole equivalents of TMAS and substrate on the substitution/elimination ratio.**

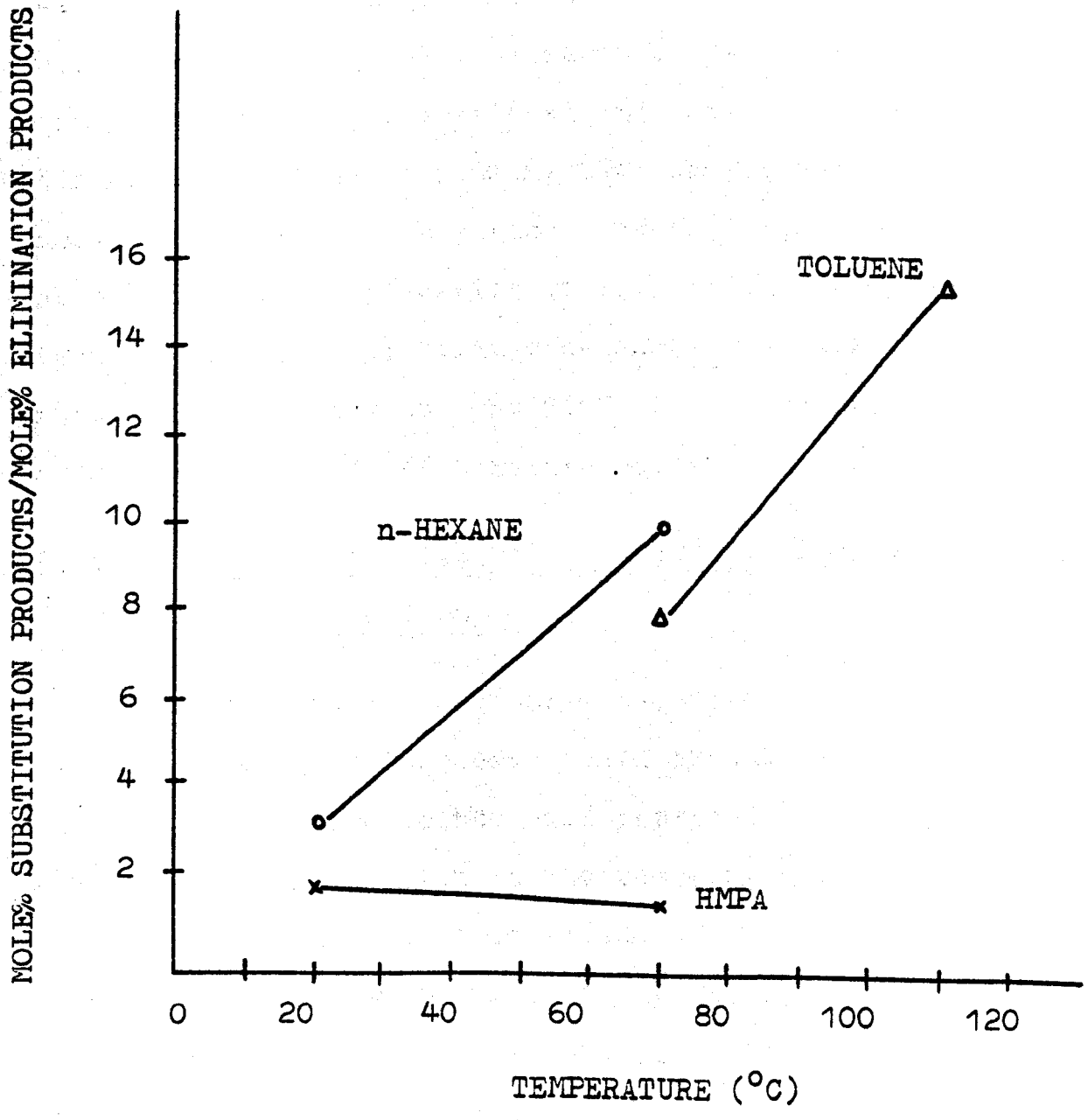


3.2.4 Effect of temperature on the distribution of substitution and elimination products.

Most of the synthetic work was carried out using n-hexane as solvent, but the boiling point of 69° is rather low, toluene (boiling point 111°) has the advantage that the reaction can be studied in a non-polar solvent over a larger temperature range. The effect of temperature on the distribution of substitution and elimination products was monitored to gain further information about the effect of aggregation on the yield of reaction products. HMPA was also studied and compared with the non-polar solvents since, in this solvent, the reacting species are undoubtedly either solvent separated ion-pairs or "free-ions"[116] in which aggregation is not a problem. The results of the experiments are shown graphically in Figure 13.

Elimination is favoured with respect to substitution by an increase in temperature, this is probably due to elimination leading to an increase in the number of particles, whereas substitution does not. Elimination thus has the more favourable entropy term, and because this ($\Delta S^{\ddagger}$) is multiplied by the temperature (T) in the relation for the free energy of activation, $\Delta G^{\ddagger}$ ($\Delta G^{\ddagger}=\Delta H^{\ddagger}-T\Delta S^{\ddagger}$) it will increasingly outweigh a less favourable enthalpy of activation ($\Delta H^{\ddagger}$) term as the temperature rises. The expected trend is therefore for a decrease in the substitution/elimination ratio, for a given solvent, on raising the temperature. This expected trend is shown for HMPA, but the reverse holds for both of the non-polar solvents. These

Fig. 13 Effect of temperature on the substitution/ elimination ratio, for three solvents.



results can be rationalised, once again, by considering the state of aggregation of TMAS at the different temperatures. In n-hexane and toluene, the substitution/elimination ratio increases with increasing temperature, these results suggest that the extent of aggregation decreases with increasing temperature. This can be seen as an overall breaking of the aggregate structure due to an increase in the translational, vibrational and rotational energy of the TMAS molecules on raising the temperature. Figure 13 shows that the maximum yield of substitution products is obtained by the application of refluxing toluene as solvent instead of the lower boiling n-hexane. The results also show that TMAS is stable at 111° and can be used as an effective source of carboxylate anion.

3.2.5 Reaction of glycerol 1,3-dibromodideoxy-2-palmitate with molten TMAS.

TMAS is a viscous semi-solid at ambient temperature, as the temperature increases, TMAS becomes a mobile readily stirred liquid. The molten salt represents an interesting reaction medium in which it has the dual roles of solvent and reactant. Since both ion-association and anion solvation are absent in such melts[117,118] the reactivity of the carboxylate anion should be enhanced. The molten salt can be regarded as the ultimate step in the progression from protic solvent, dipolar aprotic solvent to molten salt.

Previous reactions (cf p.107 and 114) have shown that TMAS in a dipolar aprotic solvent or aggregated TMAS in a non-polar solvent results in an enhancement in basicity as

well as nucleophilicity. The results of the experiments using molten TMAS are shown in Figures 14 and 15, they contrast markedly with the results obtained from concentrated solutions of TMAS in n-hexane. The molten salt shows a high substitution/elimination ratio indicating a highly nucleophilic and weakly basic anion as the reacting species. Another interesting observation is that the elimination product is formed at the start of the reaction and does not increase in concentration as the reaction proceeds. This is indicative of the elimination product arising solely from glycerol 1,3-dibromodideoxy-2-palmitate and not the intermediate glycerol 1-stearate-2-palmitate-3-bromodeoxy compound. Indeed, later experiments using n-hexane and HMPA as solvents and the intermediate compound as substrate confirm this observation. It would seem reasonable to infer that the elimination product arises while the glycerol 1,3-dibromodideoxy-2-palmitate is being distributed uniformly throughout the molten salt where the geometrical arrangement of the nucleophile and substrate may temporarily aid the elimination reaction.

The utilisation of a molten quaternary ammonium salt as solvent and reactant is a useful alternative to the classical technique of employing a cosolvent. However, its practical application is limited to those reactions in which an excess of the molten salt is desired. Low ratios of molten salt to substrate result in a non-homogeneous reaction mixture that is difficult to homogenise. It does however, provide the conditions for the maximum yield of substitution products.

Fig. 14 Formation of products with time from the reaction of molten TMAS with the substrate.

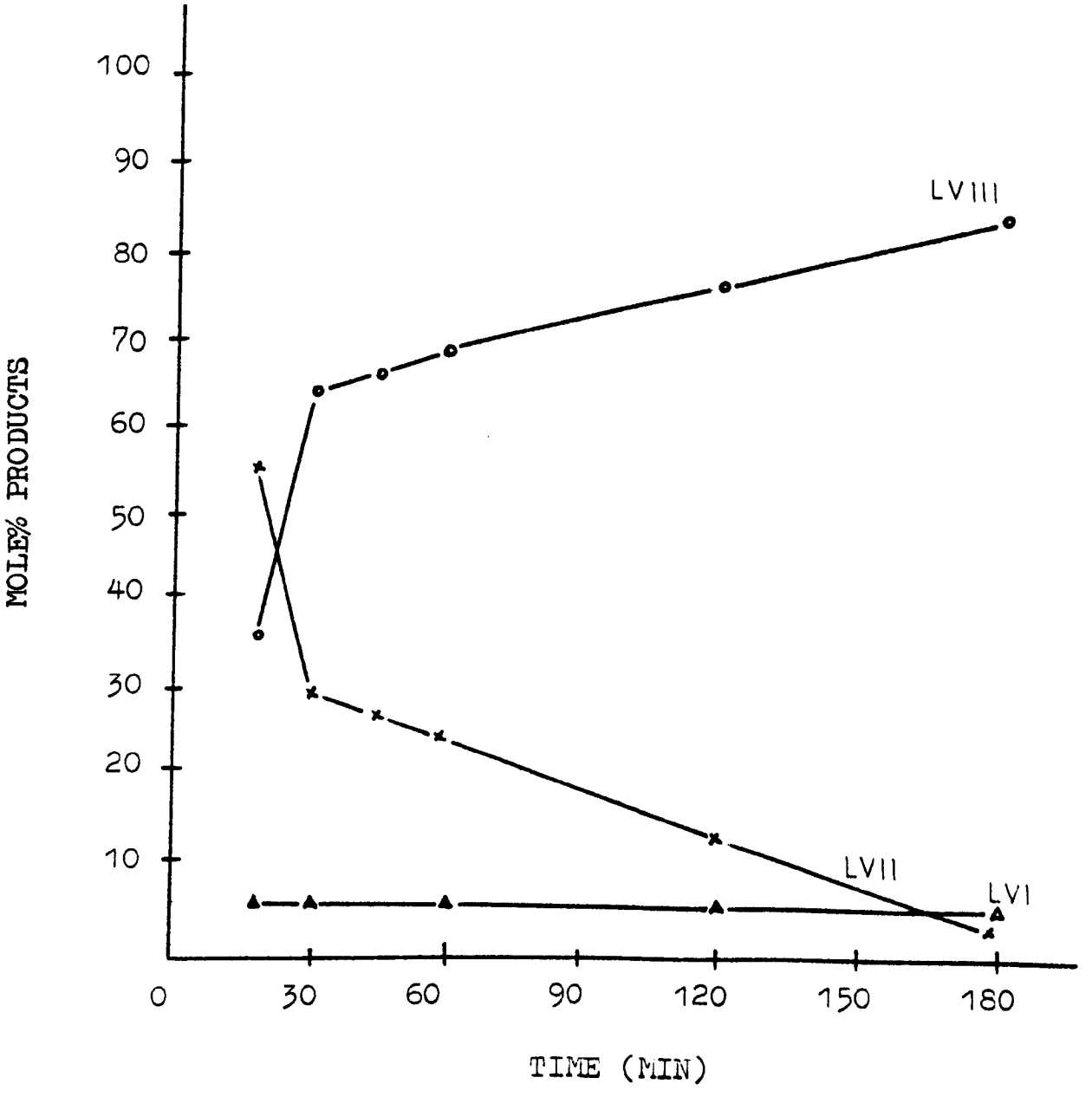
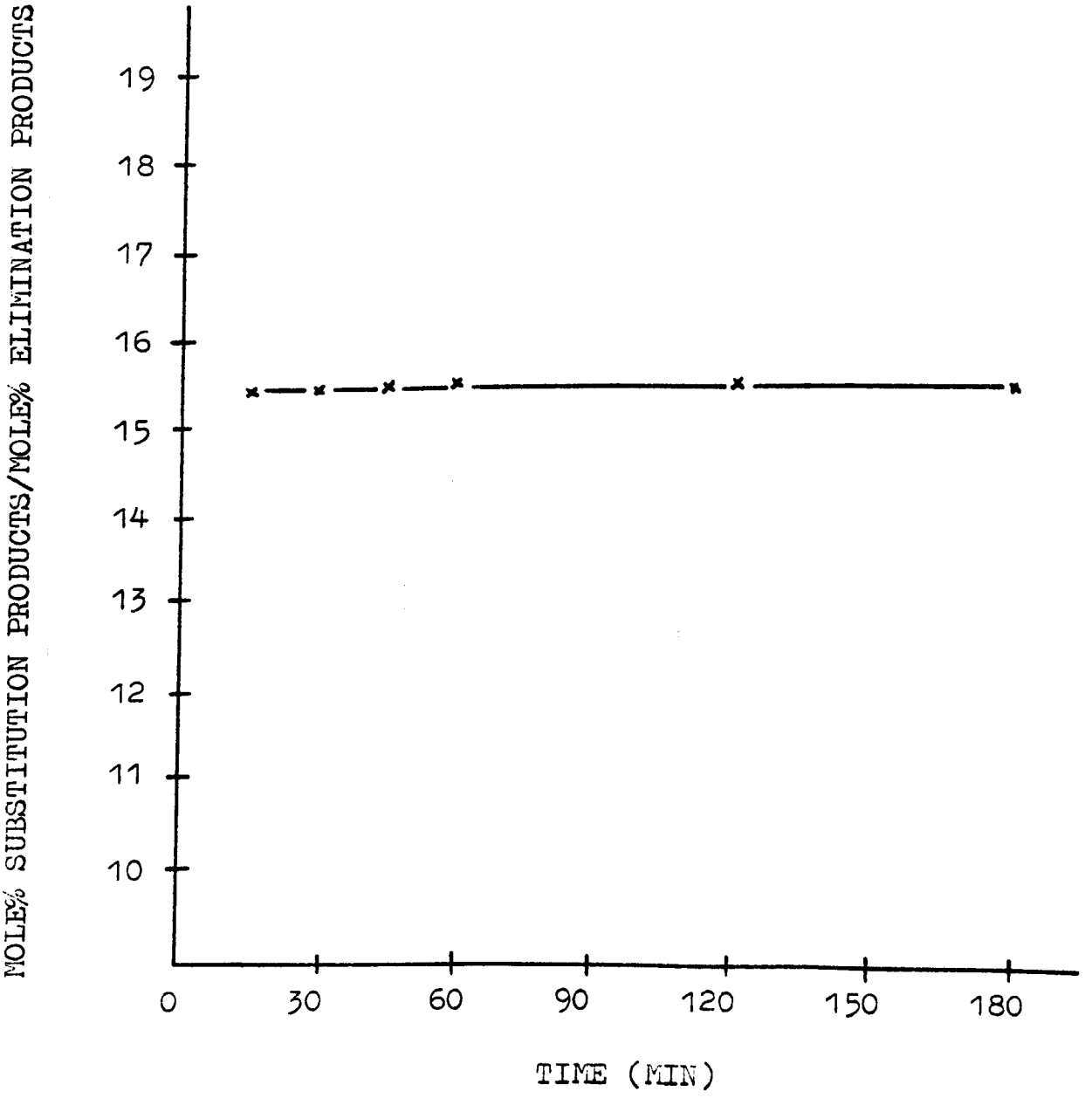


Fig. 15 Graph of substitution/elimination ratio versus time for the reaction of molten TMAS with the substrate.



3.2.6 Reaction of glycerol 1,3-dibromodideoxy-2-palmitate with silver stearate.

The silver-ion assisted nucleophilic substitution of bromide by the stearate anion is an effective method for the synthesis of a diacid triglyceride. The yield of triglyceride is high (95 mole%) with the intermediate compound (LVII) present at 3 mole% and a small amount of elimination product (LVI,2 mole%) also being detected. Although the reaction proceeds almost to completion, experimental evidence is presented in a later section (3.2.8) which shows that small amounts of acyl migration have occurred resulting in the formation of glycerol 1,2-distearate-3-palmitate as an impurity. In general the separation of isomeric triglycerides from mixtures is difficult, therefore the silver carboxylate can only be used when reliable and easy means of purifying the resulting triglycerides are available. Even though the reaction with silver stearate is efficient, it is of no practical use for larger than laboratory scale work because of the high cost, compared to alternative counter-ions.

3.2.7 Reaction of glycerol 1-stearate-2-palmitate-3-bromodeoxy with TMAS in n-hexane and HMPA.

Hexane

The reaction yielded 76 mole% glycerol 1,3-distearate-2-palmitate and 24 mole% unreacted substrate. No elimination product was detected in the products.

HMPA

The reaction yielded 96 mole% glycerol 1,3-distearate-2-palmitate and 4 mole% unreacted substrate. No elimination product was detected in the products.

Previous results (cf p.107) have shown that TMAS in HMPA behaves as a potent nucleophile and base, and will readily abstract a proton from glycerol 1,3-dibromodideoxy-2-palmitate. The same elimination reaction is not observed in glycerol 1-stearate-2-palmitate-3-bromodeoxy, indicating that the proton on carbon-2 may be shielded from the attacking anion. This steric effect probably arises from the stearate group attached to carbon-1. The close proximity of the ester carbonyl to carbon-2, and the long alkyl chain of the stearate group prevents close approach of the incoming anion and thereby hinders proton abstraction.

3.2.8 Structural purity of the synthesised triglycerides.

The structural purity of the synthesised triglycerides was determined from data obtained by pancreatic lipase hydrolysis. The pancreatic lipase hydrolyses[119,120] only the primary ester linkages in a triglyceride leaving the secondary ester linkages intact. The specificity of the enzyme for the primary positions of the triglycerides is believed to be absolute. Any release of the fatty acids at the 2-position is probably due to acyl migration within the resulting partial glyceride or due to contamination with a non-specific lipase. The development and application of pancreatic lipase hydrolysis to triglyceride analysis

has been comprehensively reviewed.[121] Of the hydrolytic products, the monoglycerides are representative of the acyl groups at the 2-position and are commonly used in determining the distribution of fatty acids in the triglycerides. Isomerisation of the 2-monoglycerides to the 1- or 3-monoglyceride may occur, but does not cause the total monoglyceride composition to be unrepresentative since the 1- or 3-monoglycerides are almost entirely derived from the 2-monoglycerides.

Samples of glycerol 1,3-distearate-2-palmitate synthesised from glycerol 1,3-dibromodideoxy-2-palmitate and TMAS in n-hexane solution, were analysed by the lipolysis procedure. It contained 99 mole% of the desired isomer and 1 mole% of glycerol 1,2-distearate-3-palmitate as an impurity. This probably arises from the small amount of 2,3-dibromo propan-1-ol that was detected in the original 1,3-dibromo propan-2-ol before acylation with palmityl chloride. This point shows the importance of using pure starting materials for the synthesis of triglycerides, many of the aberrant analytical values reported for so-called pure triglycerides may well be the result of failing to ensure adequate purity of the starting material. The encouraging high yield and almost absolute structural purity of glycerol 1,3-distearate-2-palmitate have enabled a number of other symmetrical diacid triglycerides to be synthesised. The overall method is seen to be applicable to triglycerides containing both long and short acyl chains and differing in degree of unsaturation. The following symmetrical diacid triglycerides have been synthesised:-

1. Glycerol 1,3-distearate-2-palmitate (SPS)
2. Glycerol 1,3-distearate-2-oleate (SOS)
3. Glycerol 1,3-distearate-2-acetate (SAcS)
4. Glycerol 1,3-diacetate-2-palmitate (AcPAc)
5. Glycerol 1,3-dipalmitate-2-oleate (POP)
6. Glycerol 1,3-dioleate-2-palmitate (OPO)

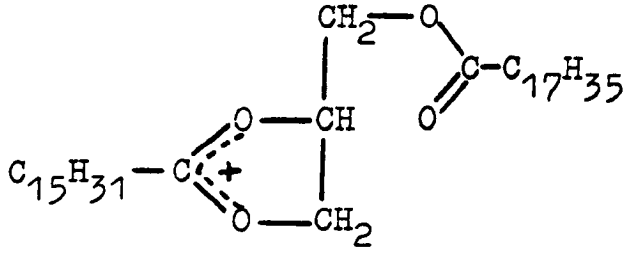
The synthesis of a specific triacid triglyceride (eg glycerol 1-stearate-2-palmitate-3-oleate) requires glycerol 1-stearate-2-palmitate-3-bromodeoxy as precursor free from the isomeric glycerol 1-palmitate-2-stearate-3-bromodeoxy. The maximum yield of the intermediate compound is obtained using silver stearate, but lipolysis of the product revealed that it contained approximately 5 mole% of the unwanted isomer. TMAS in refluxing toluene results in solely the desired isomer, but the yield is rather low (45%). Therefore, the main problem encountered in the synthesis of any triacid triglyceride or unsymmetrically substituted diacid triglyceride is the isolation of the precursor in good yield and high structural purity. This is an even greater problem when the precursor is a liquid and recrystallisation cannot be used as a method of purification. To show that triacid and unsymmetrical diacid triglycerides could be synthesised, glycerol 1-stearate-2-palmitate-3-bromodeoxy was isolated and purified for the final substitution reaction. Two triglycerides, namely, glycerol 1-stearate-2-palmitate-3-oleate (SPO) and glycerol 1-stearate-2,3-dipalmitate (SPP) were synthesised as examples of the utility of the reaction. All of the

synthesised triglycerides had a high degree of structural purity (99%).

3.2.9 Factors influencing regioselectivity in nucleophilic substitution reactions in glycerol 1,3-dibromodideoxy-2-palmitate.

In the silver ion catalysed substitution reaction, the unwanted glycerol 1-palmitate-2,3-distearate was produced to the extent of 5%. When the bulky TMAS, in a non-polar solvent, was used the reaction produced exclusively the desired 2-palmitate isomer.

Nucleophilic substitution reactions at a tetrahedral carbon atom in a glycerol ester derivative proceed [15] with participation of a neighbouring acyl group and involves a dioxolenium ion, such as LIX, as a reaction intermediate.



(LIX)

Verkade suggested[11] that the mode of reaction of LIX is dictated by the inductive (electron withdrawing) effect of the acyloxy group at carbon-1. However, it has been suggested[15] that the steric requirements, ie the relative

ease of approach of the incoming nucleophile near carbons 2 or 3 in LIX must be regarded as an additional, possibly more important factor. This suggestion is supported by the evidence from the reactions using silver stearate and TMAS as sources of the carboxylate ion. TMAS, in a non-polar solvent, will be present in the form of aggregates of ion-pairs. The exceptional steric requirement of the bulky aggregates results in exclusive attack at the much more accessible terminal carbon atom resulting in only the non-rearranged product being formed. By comparison, the much less bulky silver salt undergoes a small amount of reaction at the central carbon atom even though the majority of the reaction is at the terminal carbon atom. These results cannot be rationalised by the inductive effect as suggested by Verkade, but can be satisfactorily explained on steric grounds.

3.2.10 Conclusion.

The preparation of structurally pure glycerol 1,3-distearate-2-palmitate, using 1,3-dibromopropan-2-ol as substrate, has been studied and the desired triglyceride successfully prepared. The two step reaction involves the initial preparation of glycerol 1,3-dibromodideoxy-2-palmitate, from the substrate, with subsequent nucleophilic substitution, by the stearate anion, at carbons 1 and 3 without any concomitant acyl migration. Optimum reaction conditions for the nucleophilic substitution step involve a 50% mole excess of a quaternary ammonium stearate (TMAS), in refluxing n-hexane or toluene. The method has been applied to the synthesis of a number of triglycerides in which the acyl groups have varying chain lengths and degree of

unsaturation. All of the triglycerides have been prepared in high yield and demonstrated to be structurally pure.

3.3 Experimental

3.3.1 Preparation of starting materials and reactants.

3.3.1a Preparation of 1,3-dibromo propan-2-ol.

The viscous liquid was prepared from glycerol, red phosphorus and bromine by a standard procedure.[122] As recommended, the product was isolated by collecting the fraction that distilled at 110-112° at a pressure of 20mm Hg. The yield was 46% of theory.

GLC/MS analysis of the reaction product.

The gas chromatograph used was a Perkin Elmer Sigma 3 fitted with a 1.83m x 2.5mm ID glass column containing 3% OV-17 on Supelcoport 100/120 mesh. The column was programmed from 85-200° at a heating rate of 6°/min with a nitrogen flow rate of 80ml/min. The injection port was maintained at 250° and the detector at 275°. The effluent from the column was split in the ratio 1:1 between a flame ionisation detector and a VG MM 12F mass spectrometer via a jet separator. Electron impact (EI) spectra were obtained at a resolution of ~1:800, electron energy of 70eV, emission current of 200uA and accelerating voltage of 2.5KV. Chemical ionisation spectra were obtained using isobutane as reagent gas at a source ionising pressure of ~8 x 10^{-5} torr. The source was operated with 100eV electron beam an emission current of 500uA and a

2KV accelerating voltage.

Three compounds were detected and identified.

Compound 1 (GLC retention time: 4.1 min. Concentration 80%)

m/e 44, 79/81, 80/82, 93/95, 122/124, 123/125, 136/138 216/218/220

Consistent with 1,3-dibromo propan-2-ol

Compound 2 (GLC retention time: 4.9 min. Concentration 8%)

m/e 93/95, 106/108, 136/138, 185/187/189, 216/218/220

Consistent with 2,3-dibromo propan-1-ol

Compound 3 (GLC retention time: 6.2 min. Concentration 12%)

m/e 39, 79/81, 80/82, 93/95, 185/187/189, 199/201/203

Consistent with 1,2,3-tribromo propane

The product was re-distilled, and the fraction collected that distilled at 58° under a pressure of 1.5mm Hg. Analysis of this fraction showed 1,3-dibromo propan-2-ol (98%), 2,3-dibromo propan-1-ol (0.8%) and 1,2,3-tribromo propane (1.2%). Attempts to purify the compound to a higher purity by further re-distillation were unsuccessful. The structure of the purified material was confirmed by ^{1}H and ^{13}C NMR.

^{1}H NMR (100MHz)

$BrC\underline{H}_2-C\underline{H}(O\underline{H})-C\underline{H}_2Br$

(a) (b)(c) (a)

Protons	δ	Integration
(a)	3.58	4
(b)	3.97	1
(c)	2.66	1

^{13}C NMR

CH_2 Br	35.6	2
CH CH	70.1	1

All chemical shifts are relative to TMS.

3.3.1b Preparation of glycerol 1,3-dibromodideoxy-2-palmitate.

Dry n-hexane (150cm^3) was added to a mixture of 1,3-dibromo propan-2-ol (45.9g, 0.211mole) and pyridine (18.4g, 0.233mole) in a 500cm^3 round bottomed flask fitted with an addition funnel and a calcium chloride drying tube. The two phase mixture was stirred vigorously and palmityl chloride (51.2g, 0.186mole) dissolved in dry hexane (100cm^3) was slowly added over 30 min., so that the temperature did not rise above 30^o. The reaction was monitored by IR spectroscopy, and stopped when all of the acid chloride (ν 1809cm^{-1}) had been consumed (4.5hr). The reaction mixture was transferred to a 500cm^3 separating funnel, washed with water (2 x 30cm^3), and dilute HCl (2 x 30cm^3). The hexane fraction was washed with water until neutral, dried over anhydrous sodium sulphate, filtered and the solvent removed under reduced pressure on a rotary evaporator. The crude white product was dissolved in hot methanol and recrystall-

ised at 0° to yield, on drying at 25° in a vacuum oven at 0.1mm Hg, 78g of a white product. (Yield 92% based on the weight of palmityl chloride used.)

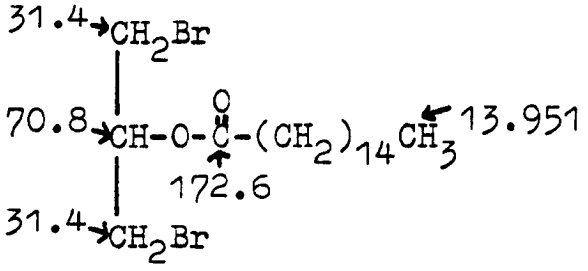
Analytical data

Melting point 35.5°

IR (Thin film) Ester carbonyl ν 1743cm^{-1}

MS (Major/significant ions) m/e 119/121, 199/201/203, 239, 255,256,257, 375/377, 411/413/415, 454/456/458

^{13}C NMR (Chemical shifts δppm relative to TMS)



All of the above information is in accord with the structure shown above.

3.3.1c Preparation of glycerol 1,3-dibromodideoxy-2-oleate.

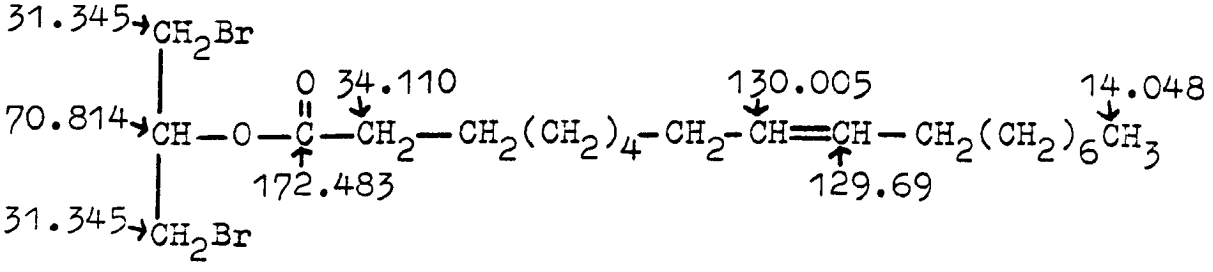
The product was prepared in excellent yield (94%) from 1,3-dibromo propan-2-ol (5.0g, 0.023mole), pyridine (1.97g, 0.025mole) and oleoyl chloride (6.1g, 0.0203mole) using the method described in 3.3.1b. The product was recrystallised from methanol at -15°to yield, on drying in a vacuum oven, 9.2g of a water white liquid.

Analytical data

IR (Thin film) Ester carbonyl ν 1743cm^{-1}

MS (Major/significant ions) m/e 79/81, 80/82, 119/121, 137/139, 199/201/203, 263, 264, 265, 401/403

^{13}C NMR (Chemical shifts δ ppm relative to TMS)



All of the above information is in accord with the structure shown above.

3.3.1d Preparation of glycerol 1,3-dibromodideoxy-2-acetate.

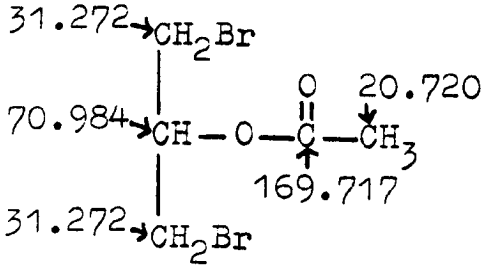
The product was prepared from 1,3-dibromo propan-2-ol (5.0g, 0.023mole), pyridine (1.97g, 0.025mole) and acetyl chloride (1.6g, 0.0204mole) using the method described in 3.3.1b. The product was isolated in 90% yield.

Analytical data

IR (Thin film) Ester carbonyl ν 1740cm^{-1}

MS (Major/significant ions) m/e 79/81, 80/82, 93/95, 119/121, 165/167, 179/181, 199/201/203

^{13}C NMR (Chemical shifts δ ppm relative to TMS)



All of the information is in accord with the structure shown.

3.3.1e Preparation of palmityl chloride.

Analytically pure palmitic acid (99%) was treated with a 1.4 molar excess of re-distilled thionyl chloride. The reaction was completed by refluxing, with the exclusion of moisture, for 2hr. The excess thionyl chloride was removed under reduced pressure. Hexane was added to the residue and it was washed three times with ice water, followed by immediately drying over anhydrous magnesium sulphate. The hexane solution was filtered and the solvent removed by evaporation under reduced pressure on a rotary evaporator. The acid chloride was obtained as a water white liquid, it was stored at 0^{o} in a tightly stoppered round bottomed flask until used.

3.3.1f Preparation of oleoyl chloride.

Pure oleic acid (99%) was treated with a 0.8molar excess of oxalyl chloride and stirred for 3days at room temperature, with the exclusion of moisture. The acid chloride was isolated and stored as described in 3.3.1e.

3.3.1g Preparation of sodium stearate.

Analytically pure stearic acid (15.8g, 0.0555mole) was dissolved in warm acetone ($300cm^3$). 5M sodium hydroxide solution ($10.55cm^3$) was added dropwise over a 10min period, the contents were stirred vigorously with warming for a further 2hr. The sodium stearate was filtered under suction

washed with ice-cold water (2 x 70cm^3) and air-dried. The course granular powder was ground to a fine powder and extracted with diethyl ether, using a Soxhlet apparatus, for 24hr. The product was dried to constant weight, over phosphorus pentoxide, in a vacuum oven at 50° and a pressure of 0.1mm Hg. Analysis of the product by IR showed it to be free of moisture and carboxylic acid.

3.3.1h Preparation of potassium stearate.

5M potassium hydroxide (11cm^3) was slowly added to a solution of pure stearic acid (16.5g, 0.058mole) in hot methanol (200cm^3). The mixture was stirred vigorously for one hour at 50°. The solvent was removed using a rotary evaporator to yield the product, which was triturated with ice-cold water (75cm^3), filtered under suction and allowed to air-dry. The product was extracted with diethyl ether and dried as described in 3.3.1g.

3.3.1i Preparation of silver stearate.

The silver stearate was prepared by a standard method[123] which involved adding an ammoniacal solution of silver nitrate to an ethanolic solution of pure stearic acid. The silver stearate was extracted with diethyl ether and dried as described in 3.3.1g with all operations being conducted in the dark. The pure, dried salt was stored in a dark bottle.

3.3.1j Preparation of tetraethylammonium stearate.

Since only moderate amounts of this salt were needed

it was convenient to prepare it from the excess silver stearate.

Tetraethylammonium bromide (5.0g, 0.0238mole) was dissolved in methanol (75cm^3). Silver stearate (9.3g, 0.0238mole) was added and the mixture was refluxed with stirring for 30min. The solution was allowed to cool to room temperature and the insoluble silver bromide was filtered off. The tetraethylammonium stearate was isolated by removal of the methanol, under reduced pressure, on a rotary evaporator. The salt was dried to constant weight, over phosphorus pentoxide, in a vacuum oven at 50° and a pressure of 0.1mm Hg.

3.3.1k Preparation of tricaprylmethylammonium stearate.

5M methanolic potassium hydroxide (12cm^3) was added to a solution of tricaprylmethylammonium chloride (30.4g, 0.060mole) in methanol (75cm^3). The mixture was stirred for 15min., cooled to 0° and stirred for a further 15min. The chilled solution was filtered into a warm methanolic solution of pure stearic acid (0.060mole). The warm mixture was stirred vigorously for 30min., the solvent was removed using a rotary evaporator to yield a viscous liquid. The product was dissolved in hexane (200cm^3), dried over anhydrous magnesium sulphate, filtered, and the hexane removed using a rotary evaporator. The product was dried to constant weight, over phosphorus pentoxide, in a vacuum oven at 35° and a pressure of 0.1mm Hg.

3.3.1l Preparation of 18-Crown-6/potassium stearate complex.

18-Crown-6 (4.0g, 0.0151mole) was dissolved in methanol ($150cm^3$) in a $250cm^3$ round bottomed flask. Potassium stearate (4.88g, 0.0151mole) was added and the mixture was stirred for 30min at 50°. The methanol was slowly removed under reduced pressure using a rotary evaporator to yield the product as a slightly tacky, off-white solid.

3.3.2 Reaction of 1,3-dibromo propan-2-ol with an equimolar amount of sodium-, potassium- or silver stearate.

1,3-dibromo propan-2-ol (1.4g, 0.00643mole) and the desired stearate salt (0.00643mole) were added to a $50cm^3$ round bottomed flask, fitted with a water-cooled condenser and a calcium chloride guard tube. The flask was immersed in an oil bath maintained at 70° and the contents were stirred vigorously for 12hr. The reaction using silver stearate was conducted in the dark. The reaction products were dissolved in chloroform ($15cm^3$), the inorganic salts were filtered off and washed with chloroform ($2 \times 10cm^3$). The combined chloroform extracts were transferred to a $50cm^3$ volumetric flask containing tripalmitin as an internal standard (0.569g, 0.00071mole). The flask was made up to $50cm^3$ with chloroform and thoroughly shaken. An aliquot of this solution was silylated with TCMS/HMDS/pyridine reagent ($1.5cm^3$), by allowing to stand at 45° for 10min. Typically, an aliquot of $0.5cm^3$ of the chloroform solution was taken for silylation. The reaction products were analysed and quantified by GLC.

3.3.2a GLC/MS analysis of the reaction products.

The gas chromatograph used was a Perkin Elmer Sigma 3, equipped with flame ionisation detectors, automatic temperature programming and a 0.45m x 2.5mm ID glass column containing 2% OV-17 on Supelcoport 100/120 mesh. The column was programmed from 160-350° at a heating rate of 6°/min, and a nitrogen flow rate of 80ml/min. The injection port was maintained at 290° and the detectors maintained at 375°. Effluent from the column was split in the ratio 1:1 between a flame ionisation detector and a VG MM 12F mass spectrometer via a jet separator. Operating conditions for the MS are given on p.134.

3.3.2b Identification of reaction products.

Four compounds were detected by GLC and identified by MS and co-chromatography with reference compounds, when available.

Compound XLVIII (GLC retention time: 3.20min.)

CI m/e 357
EI m/e 73, 75, 117, 129, 132, 145, 341, 342, 356
Identified as the trimethylsilylether of stearic acid. An authentic sample of silylated stearic acid exhibited the same retention time.

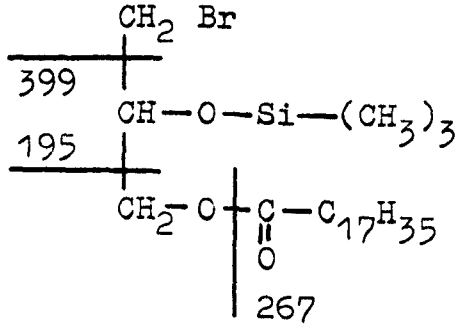
Compound XLVIX (GLC retention time: 10.1min.)

There was no evidence from the MS for the presence of any silyl groups, indicating the absence of a hydroxyl group

in the un-derivatised sample. The only high mass ion at m/e 267 (stearyl) with significant fragments at m/e 116, 112 and 98.
CI gives the base ion at m/e 341 with the major fragment ion at m/e 267. The data is consistent with glycidyl stearate.

Compound L (GLC retention time: 11.1min.)

CI m/e 493/495 The isotopic ratio is that expected for one bromine atom.
EI m/e 73, 75, 116, 195/197, 208/210, 267, 340, 370, 399, 477/479
The data can be rationalised by the trimethylsilylether of glycerol 1-stearate-3-bromodeoxy.



Compound LI (GLC retention time: 25.4min.)

The MS is that expected of the trimethylsilylether of distearin. An authentic sample of silylated distearin exhibited the same retention time.

3.3.3 Reaction of 1,3-dibromo propan-2-ol with an equimolar amount of potassium stearate in HMPA or DMF.

1,3-dibromo propan-2-ol (0.155g, 0.71mmole), potassium stearate (0.229g, 0.71mmole) and tripalmitin (0.219g, 0.27mmole) were added to a 50cm^3 round bottomed flask. HMPA or DMF (25cm^3) was added and a water cooled condenser fitted. The flask was immersed in an oil bath held at 70^o and the contents stirred vigorously. Aliquots of the hot reaction mixture (1.5-2.0cm^3) were removed at certain time intervals (5, 30, 60, 120, 180 and 240min.). The aliquot was added to water (2cm^3) and the mixture saturated with sodium chloride. The insoluble products were filtered off under suction, washed with ice-cold water (2 x 1cm^3) and air-dried. The organic products were dissolved in chloroform (2cm^3) and the residual potassium stearate filtered under suction. The volume of chloroform was reduced to 0.5cm^3 under nitrogen and the solution silylated with TCMS/HMDS/ pyridine reagent (0.5cm^3). The reaction products were analysed and quantified by GLC using tripalmitin as the internal standard. The analytical conditions are those used in 3.3.2a.

3.3.4 Variation of the counter-ion and solvent on the reaction of glycerol 1,3-dibromodideoxy-2-palmitate with the stearate anion.

The stearate salt can be represented by $M^+ \ ^-OCOC_{17}H_{35}$, where M^+ is the counter-ion (Na^+, K^+, Ag^+, $(C_2H_5)_4N^+$, $(C_{10}H_{21})_3CH_3N^+$, and 18-Crown-6/K^+)

The solvents varied were n-hexane, toluene, THF, acetone, DMF and HMPA.

Glycerol 1,3-dibromodideoxy-2-palmitate (1.03g, 2.26

mmole) and the stearate salt (7.23mmole) were added to a 100cm^3 round bottomed flask. The solvent (40cm^3) was added and a water cooled condenser added. The flask was immersed in an oil bath held at 70^o and the contents stirred vigorously for 3hr., or in the case of acetone, refluxed at 56^o for 3hr.

Isolation of reaction products

a) M^+ = Na^+, K^+, Ag^+ and $(C_2H_5)_4N^+$

1) Solvents: N-hexane, toluene, THF and acetone.

The reaction mixture was cooled to room temperature and the solvent removed, under reduced pressure, on a rotary evaporator. Chloroform (30cm^3) was added to the residue, the insoluble salts were filtered off and washed with chloroform (2 x 10cm^3). The organic extracts were combined and analysed for residual starting material and products, by GLC, using the conditions described in 3.3.2a.

2) Solvents: DMF and HMPA

The reaction mixture was cooled to room temperature and poured into water (60cm^3) to precipitate the products. Sodium chloride was added, with stirring, until the solvent was clear. The products were partitioned into warm hexane (3 x 40cm^3) and the combined extracts washed with water (3 x 20cm^3), dried over anhydrous sodium sulphate and the solution filtered. Chloroform (20cm^3) was added to the hexane solution to ensure that the products remained in solution.

b) $M^+ = (C_{10}H_{21})_3CH_3N^+$

1) Solvents: N-hexane, toluene, THF, and acetone.

The reaction mixture was cooled to room temperature and the solvent removed on a rotary evaporator. The products were precipitated from the residue by adding 30cm^3 of a methanol-water mixture (87/13 v/v). The mixture was cooled to 0° and allowed to stand for 15min. The products were filtered under suction and washed with further portions of the ice-cold methanol-water mixture (2 x 10cm^3). The products were dissolved in chloroform (30cm^3), dried over anhydrous sodium sulphate, filtered and analysed by GLC.

2) Solvents: DMF and HMPA

The products and quaternary ammonium salts were partitioned into hexane, as described in a.2. The hexane was removed using a rotary evaporator and the products isolated using methanol-water as described in b.1.

c) M^+ = 18-Crown-6/K^+

1) Solvents: N-hexane, toluene, THF and acetone

The reaction mixture was cooled to room temperature and the solvent removed on a rotary evaporator. The products were dissolved in ice-cold chloroform (20cm^3) and the insoluble salts were filtered off and washed with ice-cold chloroform (2 x 10cm^3). The chloroform was removed on a rotary evaporator and the products isolated from a methanol-water mixture as described in b.1.

2) Solvents: DMF and HMPA

The products were partitioned into hexane as described in a.2. The hexane was removed using a rotary evaporator and the products isolated as described in c.1.

3.3.4a Identification of the reaction products.

The reaction products, isolated as previously described, were analysed by preparative TLC and GLC. The same products were formed in all of the reactions, but the proportions varied markedly.

Preparative TLC

Plates of silica gel G (20cm x 20cm, 0.5mm thick) were prepared by standard methods[124] and activated at 110° for 1hr before use. A mixture of petroleum ether (bp 40-60°), diethyl ether (95:5 v/v) was used as the eluting solvent system. Bands were located by spraying the plate with a 0.2% alcoholic solution of 2,7' dichlorofluorescein and viewing under UV light. For identification, the spots were located and the silica gel of each area immediately scraped off the plate, collected in separate glass tubes and extracted with a solution of 5% methanol in chloroform. After filtration of the suspension and several washings with further portions of the solvent mixture, the eluted compounds were identified by GLC/MS, and when necessary, other instru mental techniques.

Product 1. TLC Rf 0.15, GLC retention time: 2.4min.

No suitable EI spectrum was obtained.

CI m/e 285 suggests stearic acid. An authentic sample of stearic acid exhibited the same retention characteristics on the TLC and GLC analyses.

Product 2. TLC Rf 0.24, GLC retention time: 33.8min.

EI spectrum contains significant ions at m/e 607 (M^+ - palmitate), 579 (M^+ - stearate), 395, 367, 341, 315, 313, 267 (stearate) and 239 (palmitate) indicating the compound to be glycerol 1,3-distearate-2-palmitate. An authentic sample exhibited the same retention characteristics on the TLC and GLC analyses.
Melting point 61-61.5°

Product 3. TLC Rf 0.46, GLC retention time: 23.4min.

EI spectrum gives ions at m/e 578, 395, 367, 267 and 239. By the GLC retention time, and assuming m/e 578 to be the $M^+ - H_2O$ ion, would suggest this to be glycerol 1-stearate-2-palmitate. The CI spectrum however, is dominated by bromine containing ion-pairs at m/e 659/661, 403/405 and 375/377 together with ions at m/e 579 and 359 which indicate that one atom of bromine is present and the compound is glycerol 1-stearate-2-palmitate-3-bromodeoxy.
Melting point 57-58°

Product 4. TLC Rf 0.51, GLC retention time: 4.6min.

EI gives the base ion at m/e 295 with significant ion at m/e 239. The CI spectrum gives pseudomolecular ions at m/e 375/377 indicating one atom of bromine. The above data and the GLC retention time, indicate the compound to be

propylene-2-palmitate-3-bromo.

Product 5. TLC Rf 0.51, GLC retention time: 22.0min.

EI spectrum gives the base ion at m/e 239 indicating palmitate present, m/e 267 indicates stearate present and 295 the $(CH{=}C(CH_2)C{-}O{-}C(O)(CH_2)_{14}CH_3)^+$ fragment. The CI spectrum contains ions at m/e 239, 267, 295, 341 and 579. This could be consistent with propylene-2-palmitate-3-stearate. The concentration of this compound varied markedly throughout the experiments and its structure was investigated further to confirm its identification.

^{13}C NMR

The ^{13}C NMR spectrum at 50.32MHz shows a typical long chain saturated hydrocarbon spectrum, but with additional peaks at 105.07ppm and 62.64ppm. The non-protonated carbons are too weak to be observed under the conditions employed. The peak at 105.07ppm is in the $C{=}\underline{C}H_2$ region and that at 62.64ppm is in the $-\underline{C}H_2{-}OR$ region. Also a peak at about 34ppm usually associated with a $\underline{C}H_2{-}\underline{C}{=}O$ group is split, indicating two different environments. Thus, all of the evidence from ^{13}C NMR indicates that the basic structural elements of the proposed structure are possible.

^{1}H NMR

The proton NMR spectrum at 200MHz provides further evidence for the proposed structure. The spectrum shows six resonance groups associated with the compound. Assuming

that the triplet at 0.90δ is equivalent to 2(CH_3-) then the number of protons involved with the other resonances are as follows:- Starting at the low field end of the spectrum, the first two peaks (5.08δ and 5.01δ -2 proton integral) are in the olefinic region and show non-equivalence of a C=CH_2 group, where the two geminal protons are coupled to each other (1.8Hz). In addition, the peak at 5.08δ shows long range coupling to the peak at 4.64δ. The next peak (4.64δ -2 proton integral) is a singlet which shows long range coupling and has a δ value typical of a -CH_2—OR group. The next multiplet (2.38δ -4 proton integral) is in the region expected for R—CH_2—CO—OR. Decoupling experiments show that this resonance is from two triplets, ie the -CH_2 groups from the long chain fatty acid resonate at different values. The broad resonance centred at 1.64δ (4 proton integral) is the next -CH_2 down the chain ie R—CH_2—CH_2—COOR, and finally the other -CH_2 groups give rise to the large peak at 1.27δ (52 proton integral). The integral value indicates that the compound contains both palmitate and stearate. Additional evidence for the double bond is the fact that the R—CH_2—OR resonance is a singlet, ie no protons on the adjacent carbon and the long range coupling can only occur in "fixed" systems, ie a double bond.

IR

The IR spectrum shows the presence of a long chain compound, from the size of the aliphatic -CH stretching band. The ester carbonyl stretching is at 1745cm^{-1}. One or

both of the weak peaks at 3050-3070cm^{-1} could be attributed to a $>C=CH_2$ group. A double bond is also indicated by a weak C=C stretching band near 1675cm^{-1}. The available evidence therefore, points to the proposed structure being correct.

Melting point 57-58.5°

Product 6 TLC Rf 0.67, GLC retention time: 8.2min.

The major significant ions in the spectrum are m/e 119/121, 199/201/203, 239, 255, 256, 257, 375/377, 411/413/415 and 454/456/458. The isotope ratio indicates that two atoms of bromine are present. The data is consistent with glycerol 1,3-dibromodideoxy-2-palmitate. An authentic sample exhibited the same retention characteristics on the TLC and GLC analyses.

Melting point 35.5°

3.3.5 Effect of dilution on the reaction of glycerol 1,3-dibromodideoxy-2-palmitate with excess TMAS in refluxing n-hexane.

Glycerol 1,3-dibromodideoxy-2-palmitate (0.50g, 1.1 mmole) and TMAS (2.66g, 3.52mmole) were dissolved in various volumes of n-hexane. Seven experiments were conducted in which the reactants were separately dissolved in 704cm^3, 352cm^3, 176cm^3, 88cm^3, 44cm^3, 17.6cm^3 and 8.8cm^3 of n-hexane. The solutions were refluxed with stirring for three hours in appropriately sized round-bottomed flasks. The solutions were allowed to cool and the solvent was removed, under reduced pressure, using a rotary evaporator.

The products were isolated as described in 3.3.4(b1.), from 20cm^3 of the methanol-water mixture.

3.3.6 Effect of variation in the number of mole equivalents of glycerol 1,3-dibromodideoxy-2-palmitate and TMAS on the distribution of reaction products.

Glycerol 1,3-dibromodideoxy-2-palmitate (1.03g, 2.26 mmole) was added to a 100cm^3 round bottomed flask and dissolved in n-hexane (40cm^3). TMAS (4.52, 5.65, 7.91, 10.17, 12.43, and 14.69mmole) was added to the flask in individual experiments. The contents were stirred vigorously, at reflux temperature, for three hours. The products were isolated as described in 3.3.4(b1.), and analysed and quantified by GLC.

3.3.7 Effect of temperature on the distribution of reaction products.

Glycerol 1,3-dibromodideoxy-2-palmitate (1.03g, 2.26 mmole) and TMAS (5.46g, 7.23mmole) were added to a 100cm^3 round bottomed flask. Hexane, toluene and HMPA (40cm^3) were added, in individual experiments, and a water cooled condenser, fitted with a calcium chloride guard tube, was attached. The contents were stirred vigorously at the following temperatures and for the specified times.

1. Hexane for 28hr at 20°.
2. Hexane for 8hr at reflux.
3. Toluene for 3hr at 70°.
4. Toluene for 3hr at 110°.
5. HMPA for 15hr at 20°.
6. HMPA for 3hr at 70°.

The reaction products from reactions 1-4 were isolated as described in 3.3.4(b1.). The products from reactions 5 and 6 were isolated as described in 3.3.4(b2.). The products were analysed and quantified by GLC.

3.3.8 Reaction of glycerol 1,3-dibromodideoxy-2-palmitate with molten TMAS.

Glycerol 1,3-dibromodideoxy-2-palmitate (1.03g, 2.26 mmole) and TMAS (5.46g, 7.23mmole) were added to a 25cm^3 round bottomed flask. The flask was stoppered and immersed in an oil bath held at 70°. The contents were stirred vigorously for a pre-determined time, (15, 30, 45, 60, 120, and 180min.) in separate experiments, and the products were isolated as described in 3.3.4(b1.).

3.3.9 Reaction of glycerol 1,3-dibromodideoxy-2-palmitate with an equi-molar amount of silver stearate.

Glycerol 1,3-dibromodideoxy-2-palmitate (3.0g, 6.58 mmole) and silver stearate (2.57g, 6.58mmole) were added to a 50cm^3 round bottomed flask and it was tightly stoppered. The flask was wrapped in aluminium foil and immersed in an oil bath held at 70° and the contents stirred vigorously for 15hr. Chloroform (20cm^3) was added and the insoluble silver salts were filtered off and washed with a further portion of chloroform (50cm^3). The combined extracts were analysed by GLC and shown to contain glycerol 1-stearate-2-palmitate-3-bromodeoxy (74mole%), glycerol 1,3-distearate-2-palmitate (13mole%) and residual starting material (13 mole%).

Isolation of glycerol 1-stearate-2-palmitate-3-bromodeoxy.

The chloroform was removed using a rotary evaporator and the residual products dissolved in warm acetone ($70cm^3$). The solution was allowed to cool to room temperature and water was slowly added, dropwise, with continuous stirring until the solution became turbid. The solution was allowed to stand until the precipitate had completely settled and the solvent phase was clear. The precipitate (triglyceride) was filtered off and the solvent phase isolated. More water was added dropwise, with continuous stirring, until the diacyl compound just started to precipitate out. Stirring was continued and the product was allowed to settle for 15 min, filtered off and air dried. The yield was 2.82g (65% theory). Analysis by GLC showed the purity to be 98%.

Analysis of the product by a lipolysis procedure (3.3.13) showed the presence of 3mole% glycerol 1-palmitate-2-stearate -3-bromodeoxy.

Analytical data

GLC retention time 23.4min.
TLC (0.5mm silica gel; eluting solvent system: petroleum ether (boiling point 40-60°), diethyl ether (95:5 v/v)
Rf 0.46.
Melting point (ex TLC purification) 57-58°.

3.3.10 Reaction of glycerol 1,3-dibromodideoxy-2-palmitate with 2moles of silver stearate.

Glycerol 1,3-dibromodideoxy-2-palmitate (2.0g, 4.38 mmole) and silver stearate (3.43g, 8.77mmole) were reacted

and the products isolated in chloroform solution as described in 3.3.9. Analysis of the triglyceride fraction by the lipolysis procedure (3.3.13) showed the presence of 5mole% of glycerol 1,2-distearate-3-palmitate.

3.3.11 Reaction of glycerol 1-stearate-2-palmitate-3-bromodeoxy with TMAS in n-hexane and HMPA.

Glycerol 1-stearate-2-palmitate-3-bromodeoxy (1.2g, 1.82mmole) and TMAS (2.75g, 3.64mmole) were added to a 50cm^3 round bottomed flask. Hexane or HMPA (20cm^3) were added and a water cooled condenser fitted. The hexane solution was refluxed for 3hr, whilst the HMPA solution was stirred vigorously for 3hr at 70°. The products from the hexane reaction were isolated as described in 3.3.4(b1.) and the products from the HMPA reaction were isolated as described in 3.3.4(b2.). The products were analysed and quantified by GLC.

3.3.12 Synthesis of symmetrical diacid triglycerides.

(1) Glycerol 1,3-distearate-2-palmitate (SPS)

Glycerol 1,3-dibromodideoxy-2-palmitate (1.37g, 3mmole) and TMAS (7.25g, 9.6mmole) were weighed into a 100cm^3 round bottomed flask fitted with a water cooled condenser. The reactants were dissolved in toluene (50cm^3) and the mixture was refluxed for 4hr. The solvent was removed, under reduced pressure, using a rotary evaporator and the products isolated from the quaternary ammonium salts as described in 3.3.4(b1). The yield of triglyceride, as determined by GLC, was 94% of theory. A small amount (50mg) of the total products was

purified by preparative TLC using the system described in 3.3.4a, to obtain an analytically pure sample of the synthesised triglyceride.

Analytical data

MS (Major/significant ions) m/e 239, 267, 313, 341, 367, 565, 579, 607.
GLC retention time 34.2min.
Melting point 61-61.5°
Structural purity by lipolysis 99%

(2) Glycerol 1,3-distearate-2-oleate (SOS)

The triglyceride was prepared from glycerol 1,3-dibromodideoxy-2-oleate (1.45g, 3mmole) and TMAS (7.23g, 9.6mmole) as described in 3.3.12(1). The same isolation and purification procedure was also followed, the yield of triglyceride was 93%.

Analytical data

MS (Major/significant ions) m/e 264, 265, 266, 267, 339, 341, 591, 604, 605, 606, 607.
GLC retention time 36.6min.
Melting point 35.5-36.5°
Structural purity by lipolysis 99%

(3) Glycerol 1,3-dipalmitate-2-oleate (POP)

The triglyceride was prepared from glycerol 1,3-dibromodideoxy-2-oleate (1.45g, 3mmole) and tricapryl methyl ammonium palmitate (7.0g, 9.6mmole) as described in 3.3.12(1).

The same isolation and purification procedure was also followed, the yield of triglyceride was 94%.

Analytical data

MS (Major/significant ions) m/e 239, 264, 265, 313, 338, 339, 367, 393, 551, 563, 576, 577.
GLC retention time 32.0min.
Melting point 34°
Structural purity by lipolysis 99%.

(4) Glycerol 1,3-dioleate-2-palmitate (OPO)

The triglyceride was prepared from glycerol 1,3-dibromo-dideoxy-2-palmitate (1.37g, 3mmole) and tricaprylmethyl-ammonium oleate (7.23g, 9.6mmole) as described in 3.3.12(1). The same isolation procedure was followed but at -20°, the product was also purified as previously described. The yield of triglyceride was 90%.

Analytical data

MS (Major/significant ions) m/e 239, 264, 265, 313, 338, 339, 367, 393, 563, 576, 577, 603.
GLC retention time 34.2min.
Structural purity by lipolysis 99%.

(5) Glycerol 1,3-distearate-2-acetate (SAcS)

The triglyceride was prepared from glycerol 1,3-dibromo-dideoxy-2-acetate (0.78g, 3mmole) and TMAS (7.25g, 9.6mmole) as described in 3.3.12(1). The same isolation and purification procedure was also followed, the yield of triglyceride

was 93%.

MS (Major/significant ions) m/e 267, 341, 369, 383, 393, 606, 607, 666.
GLC retention time 25.2min.
Melting point 56.5°
Structural purity by lipolysis 99%.

(6) Glycerol 1,3-diacetate-2-palmitate (AcPAc)

The triglyceride was prepared from glycerol 1,3-dibromo-dideoxy-2-palmitate (1.37g, 3mmole) and tricaprylmethylammonium acetate (5.1g, 9.6mmole) as described in 3.3.12(1). The same isolation procedure was followed but at -20°, the product was also purified as previously described. The yield of triglyceride was 90%.

Analytical data

MS (Major/significant ions) m/e 158, 159, 239, 341, 354, 355.
Structural purity by lipolysis 99%.

3.3.12a. Synthesis of an unsymmetrical triglyceride.

Glycerol 1-stearate-2,3-dipalmitate (SPP)

The triglyceride was prepared from glycerol 1-stearate-2-palmitate-3-bromodeoxy (1.98g, 3mmole) and tricaprylmethylammonium palmitate (3.49g, 4.8mmole) using the conditions described in 3.3.12(1). The same isolation and purification procedure was also followed, the yield of triglyceride was 96%.

Analytical data

MS (Major/significant ions) m/e 239, 267, 313, 341, 367, 395, 537, 551, 565, 579.

GLC retention time 32.8min.

Structural purity by lipolysis 99%.

3.3.12b. Synthesis of a triacid triglyceride

Glycerol 1-stearate-2-palmitate-3-oleate (SPO)

The triglyceride was prepared from glycerol 1-stearate-2-palmitate-3-bromodeoxy (1.98g, 3mmole) and tricaprylmethylammonium oleate (3.61g, 4.8mmole) as described in 3.3.12(1). The same isolation and purification procedure was also followed, the yield of triglyceride was 96%.

Analytical data

MS (Major/significant ions) m/e 239, 264, 265, 267, 313, 338, 339, 341, 367, 393, 395, 577, 579, 605.

GLC retention time 34.2min.

Melting point 50-51°

Structural purity by lipolysis 99%.

3.3.13 Lipolysis procedure

(1) Reagents

Pancreatin (Ex Porcine Pancreas, purchased from the Sigma Chemical Company.)

Sodium tauroglycocholate solution (0.04%wt/vol) made up in buffer solution.

Buffer solution of NH_4Cl-NH_4OH, pH 8.4 (1.2M NH_4Cl and

10% NH_4OH solutions)

Calcium chloride.$6H_2O$ solution (22% wt/vol)

Solvents: N-hexane and diethyl ether.

(2) Defatting Pancreatin

Acetone ($50cm^3$) was added to pancreatin (10g) in a $100cm^3$ beaker. The mixture was stirred and filtered under vacuum, using a Buchner funnel and filter flask. The residue was washed with acetone ($50cm^3$) followed by diethyl ether (2 x $50cm^3$) and finally allowed to air-dry.

(3) Semi-micro method of lipolysis

To the sample (40mg) in a screw-capped phial (diameter 2cm, height 7.5cm) was added n-hexane ($0.5cm^3$) and bile salt solution ($2cm^3$). The mixture was gently shaken in a water bath held at 39°, for 5min, by means of a shaking machine. The triglyceride is dissolved in the hexane phase. Calcium chloride solution ($0.25cm^3$) and pancreatin (60mg) were added, the top was tightly secured and the mixture vigorously agitated for 10min. The reaction mixture was extracted with diethyl ether (5 x $5cm^3$). The combined ether extracts were washed with water (3 x $2cm^3$) and dried over anhydrous sodium sulphate.

The lipolytic products were separated into mono-, di-, triglycerides and free fatty acids by thin layer chromatography on 20 x 20cm plates using an eluting solvent system of iso-octane: diethyl ether: formic acid (60:40:1 v/v/v). The separated bands were visualised by spraying the plate with a 0.2% ethanolic solution of 2,7 dichlorofluorescein and

viewing under UV light. To determine the fatty acid composition at the 2-position, the monoglyceride band was scraped off the plate and covered with warm 5% methanol in chloroform ($3cm^3$). The solution was filtered from the silica, and the silica washed with further portions of the warm solvent mixture (2 x $1cm^3$). The extracts were combined and the solvent removed under a stream of nitrogen, to yield the monoglyceride.

(4) Preparation of methyl esters.

0.5M sodium methylate solution ($5cm^3$) was added to the monoglyceride fraction, and the mixture refluxed for 5min. The transesterified mixture was cooled and 0.5M sulphuric acid added ($5cm^3$). The esters were extracted with Analar grade chloroform (2 x $10cm^3$), the chloroform extracts were washed with water until neutral, dried over anhydrous sodium sulphate and the volume reduced to $1cm^3$ under a stream of nitrogen.

(5) Analysis of the methyl esters.

The gas chromatograph used was a Pye Unicam model 104 equipped with flame ionisation detectors and a 2.1m x 2.5mm ID glass column containing 10% DEGS-PS on Supelcoport 100/120 mesh. The methyl esters were analysed isothermally at 175° with a nitrogen flow rate of 80ml/min. The methyl esters were identified by co-chromatography with reference compounds.

REFERENCES

1. Personal Communication from Dr. F.B. Padley.

2. Fischer, E., Ber., 1920, 53, 1621.

3. Guth, F., Z. Biol., 1903, 44, 78.

4. Renshaw, R.R., J. Amer. Chem. Soc., 1914, 36, 537.

5. Kreis, H. and Hafner, F., Ber., 1903, 36, 1123.

6. Grun, A. and Theimer, E., Ber., 1907, 40, 1792.

7. Fairbourne, A., J. Chem. Soc., 1930, 369.

8. Fairbourne, A. and Cowdry, G.W., J. Chem. Soc., 1929, 129.

9. Malkin, T. and Bevan, T.H., "Prog. in Chem. Fats and other Lipids," Ed. Holman, R.T., Lundberg, W.G. and Malkin, T., Pergamon Press (Oxford) 1957, Vol.14 p.64 and references cited therein.

10. Bevan, T.H., Brown, D.A., Gregory, G.I. and Malkin, T., J. Chem. Soc., 1953, 127.

11. Verkade, P.E., Bull. Soc. Chim. France. 1963, 10, 1993.

12. Aneja, R. and Davies, A.P., Chem. Phys. Lipids, 1974, 12, 39.

13. Verkade, P.E., Chimie et Industrie, 1953, 69, 239.

14. Daubert, B.F. and King, C.G., J. Amer. Chem. Soc., 1938, 60, 3008.

15. Aneja, R. and Davies, A.P., Tetrahedron Letters, 1972, 4497.

16. Mank, A.P.J., Ward, J.P. and Van Dorp, D.A., Chem. Phys. Lipids, 1976, 16, 107.

17. Martin, J.B., J. Amer. Chem. Soc., 1953, 75, 5483.

18. Hibbert, H. and Grieg, M.E., Can. J. Res, 1931, 4, 254. They actually isolated a dioxolone derivative in their attempt to isolate the monotrichloroacetate of ethylene glycol.

19. Winstein, S. and Buckles, R.E., J. Amer. Chem. Soc., 1942, 64, 2780.

20. Schlenk, W., J. Amer. Oil Chemists' Soc., 1965, 42, 945.

21. Garner, T.L., J. Soc. Chem. Ind., 1928, 47, 278,801.

22. Berthelot, M., Ann. Chim. (France), 1854, 41, 216.

23. Feuge, R.O., Kraemer, E.A. and Bailey, A.E., Oil and

24. Feuge, R.O., Vicknair, E.J. and Lovegren, N.B., J. Amer. Oil Chemists' Soc., 1953, 30, 283.

25. Alfin-Slater, R.B., Coleman, R.D., Feuge, R.O. and Altschul, A.M., J. Amer. Oil Chemists' Soc., 1958, 35, 122.

26. Grun, A., Ber., 1905, 38, 2284.

27. Grun, A. and Schacht, P., ibid., 1907, 40, 1778.

28. Grun, A. and Theimer, E., ibid., 1907, 40, 1792.

29. Schuette, H.A. and Hale, J.T., J. Amer. Chem. Soc., 1924, 53, 2829.

30. Hilditch, T.P. and Rigg, J.G., J. Chem. Soc., 1935, 1774.

31. Young, H.H. and Black, H.C., J. Amer. Chem. Soc., 1938, 60, 2803.

32. Rothman, E.S., J. Amer. Oil Chemists' Soc., 1968, 45, 189.

33. Fischer, E., Bergmann, M. and Barwind, E., Ber., 1920, 53, 1589.

34. Hartman, L., Chem. and Ind., 1960, 711.

35. Martin, J.B., J. Amer. Chem. Soc., 1953, 75, 5482.

36. Black, H.C. and Overlay, C.A., J. Amer. Chem. Soc., 1939, 61, 3051.

37. Verkade, P.E., Chimie et Industrie, 1953, 69, 239.

38. Verkade, P.E., Van der Lee, J. and Meerburg, W., (a) Rec. Trav. Chim. 1935, 54, 716. (b) 1937, 56, 635.

39. Helferich, B. and Sieber, H., Z. physoil. Chem., 1927, 170, 31; 1928, 175, 311.

40. Rose, W.G., J. Amer. Chem. Soc., 1947, 69, 1384.

41. Daubert, B. F. and Lutton, E.S., ibid., 1947, 69, 1449.

42. Fischer, E., Ber., 1920, 53, 1621.

43. Grun, A. and Wohl, H., Inaug. Diss. Technische Hochschule, Munchen 1927.

44. Grun, A. and Kirch, A., Inaug. Diss. Technische Hochschule, Dresden 1928.

45. Devan, T.H., Malkin, T. and Smith, D.B., J. Chem. Soc., 1955, 1383.

46. Verkade, P.E., Hessel, L.W. and Vanhinsen, O.E., Rec. Trav. Chim., 1954, 73, 842.

47. (a) Grun, A. and Schreyer, B., Ber., 1912, 45, 3420.
(b) Grun, A. and Limpacher, R., Ber., 1926, 59, 690.

48. Whitley, G.S., Trans. Roy. Soc. Canada, 1919, Section III, 255.

49. Thomson, W.F., ibid., 1912, 85, 284.

50. Fairbourne, A., J. Chem. Soc., 1930, 369.

51. Mank, A.P.J., Ward, J.P. and Van Dorp, D.A., Chem. Phys. Lipids, 1976, 16, 107.

52. Hunter, J.R., Roberts, P.L. and Kestner, E.B., J. Amer. Chem. Soc., 1948, 70, 3214.

53. Fairbourne, A. and Cowdry, G.W., J. Chem. Soc., 1929, 129.

54. Fairbourne, A. and Foster, G.E., ibid., 1926, 3146.

55. Barry, P.J. and Craig, B.M., Canad. J. Chem., 1955, 33, 716.

56. Grun, A. and Wittka, F., Chem-Ztg., 1926, 50, 753.

57. Barry, P.J. and Craig, B.M., Canad. J. Chem. 1955, 33, 716.

58. Bentley, P.H. and McCrae, W., J. Org. Chem. 1970, 35, 2082.

59. Fischer, H.O.L., Taube, C. and Baer, E., Ber., 1927, 60, 479.

60. Baer, E. and Fischer, H.O.L., J. Biol. Chem., 1939, 128, 475.

61. Baer, E. and Fischer, H.O.L., J. Amer. Chem. Soc., 1939, 61, 761.

62. Fischer, H.O.L. and Baer, E., ibid., 1945, 67, 2031.

63. Sowden, J.C. and Fischer, H.O.L., ibid., 1941, 63, 3244.

64. Buchea, D. and Baer, E., J. Lipid Res., 1960, 1, 405.

65. Gigg, J. and Gigg, R., J. Chem. Soc. (C), 1967, 431.

66. Pfeiffer, F.R., Cohen, S.R., Williams, K.R. and Weisbach, J.A., Tetrahedron Letters 1968, 3549.

67. Pfeiffer, F.R., Miao, C.K. and Weisbach, J.A., J. Org. Chem., 1970, 35, 221.

68. Abderhalden, E. and Eichwald, E., Ber., 1915, 48, 1847.

69. Bergmann, M. and Sabetay, S., Z. physoil. Chem., 1924, 137, 47.

70. Eckey, E.W., "Vegetable Fats and Oils," Reinhold Publishing Co., N.Y., 1954, p.142.

71. Eckey, E.W., Procter and Gamble Co. U.S. Pat. 2,378,006 (1945).

72. Wright, H.J., Segur, J.B., Clark, H.V., Coburn, S,K., Langdon, E.E. and DuPuis, R.N., Oil and Soap, 1944, 21, 145.

73. Firma-oel Werke Germania GmbH and Norman, W., German Pat. 417,215 (1920).

74. Dominick, W.E. and Nelson, D., (Swift and Co.) U.S. Pat. 2,625,485 (1953).

75. Hawley, H.K. and Dobson, R.D., Procter and Gamble Co. U.S. Pat. 2,733,251 (1956).

76. Personal Communication from Dr. P.A.T. Swoboda.

77. Sreenivasan, B., J. Amer. Oil Chemists' Soc., 1978, 55, 796.

78. List, G.R., Emken, E.A., Kowlek, W.F., Simpson, T.D. and Dutton, H.J., J. Amer. Oil Chemists' Soc., 1977, 54, 408.

79. Bell, R.J., Campbell, R.L., Gibson, R. and Sims, J.F., U.S. Pat. 3,396,037 (1968).

80. Babin, A., Oleagine, 1974, 29, 375.

81. Chobanov, D. and Chobanov, R., J. Amer. Oil Chemists' Soc., 1977, 54, 47.

82. Mills, R.H., Farrar, M.W. and Weinkauff, C.J., Chem. Ind. (London), 1962, 2144.

83. Purdie, T. and Neave, G.B., J. Chem. Soc., 1910, 97, 1517.

84. Finch, N. and Schlittler, E., Tetrahedron, 1968, 24, 5421.

85. Wang, S.S., Gisin, B.F., Winter, D.P., Makofske, R., Kulesha, I.D., Tzougraki, C. and Meienhofer, J., J. Org. Chem., 1977, 42(8), 1286.

86. Larock, R.C., J. Org. Chem., 1974, 39(25), 3721.

87. Saegusa, T. and Murase, I., Synthetic Communications, 1972, 2(1), 1.

88. Ono, N., Yamada, T., Saito, T., Tanaka, K., Kaji, A., Bull. Chem. Soc. Japan, 1978, 51(8), 2401.

89. Pedersen, C.J., J. Amer. Chem. Soc., 1967, 89, 7017.

90. Pedersen, C.J., ibid., 1970, 92, 386.

91. Pedersen, C.J., ibid., 1970, 92, 391.

92. Pedersen, C.J., Fed. Proc. Fed. Amer. Soc. Exp. Biol., 1968, 27, 1305.

93. Wagenknecht, J.H., Baizer, M.W. and Chruma, J.L., Synthetic Communications, 1972, 2(4), 215.

94. Parker, A.J., Chemical Reviews, 1969, 69(1), 1.

95. Aneja, R. and Davies, A.P., J.C.S. Chem. Comm. 1972, 727.

96. Aneja, R., Davies, A.P. and Knaggs, J.A., J.C.S. Chem. Comm., 1973, 110.

97. Aneja, R., Davies, A.P., Harkes, A. and Knaggs, J.A., ibid., 1974, 963.

98. Aneja, R. and Davies, A.P., J. Chem. Soc.(1). 1974, 141.

99. Aneja, R., Chadha, J.S. and Knaggs, J.A., Chem. Phys. Lipids 1973, 11, 89.

100. Aneja, R. and Chadha, J.S., Biochim. Biophys. Acta. 1971, 239, 84.

101. Aneja, R., Davies, A.P. and Wilson, M., Abstract 0607 of the 14th World Congress I.S.F., 17-22 September 1978, Brighton.

102. Aneja, R., Bhati, A., Hamilton, R.J., Padley, F.B. and Steven, D.A., unpublished results.

103. Halaska, V., Lochman, L. and Lim, D., Coll. Czech. Chem. Commun., 1968, 33, 3245.

104. Schlosser, M., Jan, G., Byrne, E. and Sicher, J., Helv. Chim. Acta., 1973, 56, 1630.

105. Zavada, J., Pankova, M. and Svoboda, M., Coll. Czech. Chem. Commun., 1976, 41, 3778.

106. Zavada, J. and Pankova, M., ibid., 1977, 42, 3421.

107. Zavada, J., Pankova, M., Svoboda, M. and Schlosser, M., J. Chem. Soc. Chem. Commun., 1973, 168.

108. Kornblum, N., Seltzer, R. and Haberfield, P., J. Amer. Chem. Soc., 1963, 85, 1148.

109. Fowkes, F.M., "Solvent properties of Surfactant solutions." Published 1967 Marcel Dekker, New York. Editor Shinoda, K.

110. Kitahara, A., "Advances in Colloid and Interface Sci.," 1980, 12, 109-140.

111. Pilpel, Chem.Rev., 1963, 63, 221.

112. Singleterry, C.R., J. Amer. Oil Chemists' Soc., 1955, 32, 446.

113. El Seoud, O.A., Fendler, E.J., Fendler, J.H. and Medary, R.T., J.Phys. Chem., 1973, 77(15), 1876.

114. Swain, C.G. and Eddy, R.W., J. Amer. Chem. Soc., 1948, 70, 2989. Swain, C.G., ibid., 1950, 72, 4583.

115. Fendler, J. H., Nome, F. and Van Woert, H.C., J. Amer. Chem. Soc., 1974, 96(21), 6745.

116. Parker, A.J., Adv. Org. Chem., 1965, 5, 1-46.

117. Seward, R.P., J. Amer. Chem. Soc., 1951, 73, 515.

118. Kenausis, L.C., Evers, E.C. and Kraus, C.A., Proc. Natl. Acad. Sci. US., 1962, 48, 121, 1963, 49, 141.

119. Mattson, F.H. and Beck, L.W., J. Biol. Chem., 1955, 214, 115.

120. Savary, P. and Desnuelle, P., Compt. Rend., 1955, 240, 2571.

121. Litchfield, C., "Analysis of Triglycerides," Academic Press, New York, 1972, pp 105-137.
Jurriens, G., Analysis and Characterization of Oils, Fats and Fat Products, Vol.2, Ed. Boekenoogen, H.A., Interscience, London, 1968, pp 219-298.
Coleman, M.H., Advances in Lipid Research, Vol. 1, Ed. Paoletti, R. and Kritchevsky, D., Academic Press, New York, 1963, pp 2-64.

122. Organic Syntheses. Collective Vol. 2. P. 305.
Ed. Blatt, A.H., Publishers Wiley and son, New York.

123. Whitby, G.W., Trans. Roy. Soc. Canada, 1919, 13, 257.
Whitmore, W.F. and Lauro, M., Ind. Eng. Chem., 1930, 22, 646-649.

124. Mangold, H.K., in "Thin Layer Chromatography," Ed. Stahl, E., Academic Press, New York, 1965, pp 137-186.