SYNTHETIC AND ANALYTICAL INVESTIGATIONS RELATED TO VEGETABLE OILS AND ANIMAL FATS

by

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Synopsis

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The work presented here has developed along two lines: analysis and synthesis of triacylglycerols. A new application is reported in the use of the infrared detector in HPLC for the detection and preparative and quantitative separation of triacylglycerols of seed fats and oils, acylglycerols from lipolysis mixtures and synthetic acylglycerols. The composition of the total triacylglycerol of some of these fats and oils, and the distribution of fatty acids in the sn-2 position in them are determined for the first time. The n-hydrocarbon composition of most of these fats and oils is also determined for the first time.

Three new routes, namely, the tosylate, the silyl ether and the phosphate route, have been explored. In the tosylate route, best conditions are found for the preparation of glycerol-1,3-ditosylate and its subsequent conversion into 1,3-diacylglycerols by nucleophilic substitution. From the 1,3-diacylglycerols, five symmetrical diacid triacylglycerols have been prepared by reaction with an appropriate acid chloride. By the silyl ether route symmetrical diacylglycerols have been prepared from the 1,3-disilyl derivative of 1,3-dihydroxy-acetone. In this regard,it is noted that selective silylation of glycerol does not take place, and the 1,3-disilyl derivative of glycerol (prepared indirectly) does not undergo nucleophilic substitution. However,this route does not offer any advantage over existing routes. In connection with the phosphate route, preliminary experiments have shown that the diphenyl phosphate group can be substituted easily and under mild conditions by a carboxylate anion in a primary alkyl phosphate derivative in both non-polar and polar protic and aprotic solvents. The product from the reaction of diphenyl phosphoryl chloride and glycerol contained only a small amount of the desired 1,3-diphosphate derivative which has been converted into the 1,3-diacylglycerols by nucleophilic substitution.

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GENERAL INTRODUCTION

Lipids, proteins and polysaccharides are the main constituents of the bulk of the solid material in living organisms. Lipids can be divided broadly into two classes: simple lipids, which comprise triacylglycerols and wax esters and compound lipids which include phospholipids and glycolipids. The lipids may appear on review to be a heterogeneous collection of molecules but they are all derivatives of fatty acids, formed synthetically or biosynthetically. Their distinctive property is a high solubility in most organic solvents and a low solubility in water. In food storage in living organisms lipids act as a part of the efficient and specific transport process which is capable of overcoming the difficult problems of transporting water-insoluble compounds in an aqueous medium. Lipids also have a great potential for variation in structure, depending on geographical and physiological location, growth conditions and other factors. Neutral lipids, i.e. triacylglycerols, represent a mean by which energy rich molecules can be stored in the cell. They produce more energy in the form of ATP on a mole basis than do polysaccharides. Triacylglycerols are the major constituents of fats and oils and play a unique role in the cooking and preserving of food. In the body they are the only form of transport of essential water-insoluble compounds, such as the vitamins A, D, E and K. In this transport process, emulsifiers keep the water-insoluble components in a highly-dispersed state, by incorporating them into micelles and the stabilizers (water-trapping proteins or polysaccharides) prevent these food micelles from gathering and coalescing. The lipid, glycerol-monostearate, is the only synthetic emulsifier legal in Britain. It is as good as lecithin in promoting

micelle formation. By adding glycerol-monostearate to edible fats, which are largely triacylglycerols, their dispersive power is much improved. In vertebrates, both digestion and absorption of fat rely heavily on bile, which is secreted in the liver. Lecithin and bile salts are its main constituents. The monoacylglycerols and fatty acids produced, by hydrolysis, catalysed by pancreatic lipase from bile juice, are further dispersed as mixed micelles of bile salts, fatty acids and monoacylglycerols. The hydrolysed triacylglycerols are regenerated in the liver and adipose tissues.

All fatty acids can be described by the formula RCOOH where R is usually a straight chain of CH_2 units, sometimes separated by -CH=CH- groups. More complex triacylglycerols contain fatty acids with branched chains, hydroxyl groups or cyclic substituents. In nature, fatty acids usually have an even number of carbon atoms. The most common fatty acids in both plants and animal fats are the unsaturated oleic and linoleic acids and the saturated palmitic and stearic acids. Whereas mono- and di-unsaturated fatty acids occur widely in vegetable and in animal fats, the polyunsaturated fatty acids are not of common occurrence in higher animals or at least they are not produced by animal cells at the rate required by the organism.[1] Consequently the poly-unsaturated fatty acids are of great importance in an animal diet. It has been reported recently that Australian[132] fish oils are rich sources of polyunsaturated fatty acids. Prostaglandins,[2] which appear to be involved in every system in the body and any form of disease, are biosynthesised from arachidonic acid and other fatty acids, such as dihomo-γ-linolenic and cis-eicosapentenoic acids. Polyunsaturated fatty acids are widely used also in industry, e.g. in the manufacture of margarines and paints. Naturally-occurring unsaturated fatty acids

contain cis rather than trans carbon-to-carbon double bonds. This is important because with this cis geometry there is a restriction on close-packing between molecules and as a result the formation of a more open fluid arrangement of any lipid aggregate. Our use of fats and oils as illuminants, in cosmetics in medicines and as lubricants dates from before the earliest records of civilised man.

As early as 1854,[3] the triesters formed from one mole of glycerol and three moles of fatty acids were established as the main components of fats and oils. They are known as triacylglycerols. They are synthesised completely from their simplest components, fatty acids and glycerol or from partial acylglycerol which then are further acylated. In nature there is a tendency towards synthesis of mixed triacylglycerols. In addition to the four different fatty acids mentioned above, natural triacylglycerols also contain shorter-chain fatty acids e.g. myristic, lauric, caproic and other acids. Milk fats are unusual amongst animal fats; they have a relatively high proportion of short chain fatty acids. When studying a particular natural fat qualitative as well as quantitative analyses must be conducted. Consequently, it is very important to consider not only the different fatty acids but also the proportion of these acids present. A clear picture of the chemical constitution of natural fats and oils is dependent basically upon our knowledge of the molecular structure of the individual triacylglycerols. Thus, in addition to the fatty acid composition, it is necessary to know the position in the glycerol molecule which each fatty acid occupies.

The upsurge of interests in lipids during the 1950's and 1960's was

due mainly to the development of powerful new techniques[4-10] for the separation, analysis and identification of these components. The advent of chromatographic methods has increased the ease with which some of these components can be determined and has permitted even the identification of some compounds which was not possible previously. Defining the patterns by which nature assembles glycerol and fatty acids into a complex mixture of triacylglycerols is the ultimate aim of the analyses. In most cases, synthesis of possible positional isomers and comparison of each with the natural acylglycerols was the only method available for structure determination. It has not been easy always to elucidate acylglycerol structures by classical degradative studies or by spectroscopic methods. So far enzymatic methods are the only methods available for the determination of triacylglycerol positional isomers. For the development of the above techniques and other modern analytical methods, such as gas-liquid chromatography, thin layer chromatography and high-performance liquid chromatography, authentic acylglycerols are not easy to obtain and they can be expensive. It has therefore become a tradition among workers interested in the study of acylglycerols to synthesise their own compounds. Metabolic studies, the manufacture of a variety of foodstuffs and cosmetics also require authentic acylglyerols.

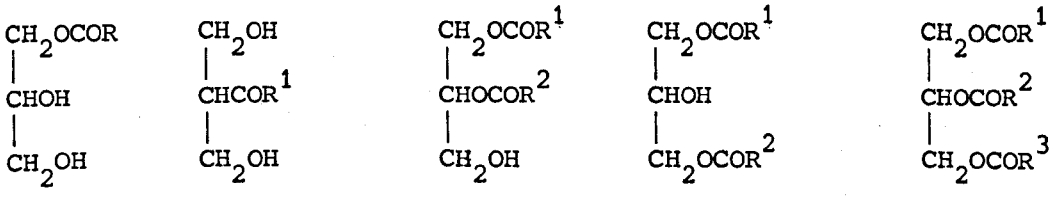
The work presented here has developed along two lines, firstly, studies of the analytical methods for the detection and determination of the triacylglycerols in natural fats and oils using chromatographic and enzymatic methods and secondly, exploration of satisfactory methods for the synthesis of specific diacid triacylglycerols by nucleophilic substitution reactions.

PART A: DETECTION AND DETERMINATION OF TRIACYLGLYCEROLS IN NATURAL FATS AND OILS

1. INTRODUCTION TO THE ANALYSIS OF TRIACYLGLYCEROLS.

The understanding of the structural composition of triacylglycerols is increasing with the continual improvement in the techniques and methods of their separation. No single technique can separate adequately the very complex mixture of individual triacylglycerols in natural fats and oils. However a combination of modern techniques can give a clearer picture of the triacylglycerol compositions.

Neutral lipids contain mostly triacylglycerols and small amounts of di- and monoacylglycerols (Fig. 1), hydrocarbons, esters and free fatty acids.

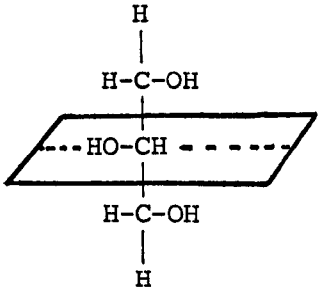


(a) (b) (c)

Fig. 1: glycerol derivatives (a) monoacylglycerols (b) diacylglycerols and (c) triacylglycerols

R^1, R^2, R^3 = $-(CH_2)_nCH_3$; n = whole number.

The glycerol molecule is completely symmetrical, although the two ends are not stereochemically identical in many enzymic reactions. However if one of the primary hydroxyls is esterified or if the two



plane of symmetry

primary hydroxyls are esterified each with a different acid, then the plane of symmetry is lost and the molecule acquires a chiral centre at the middle carbon atom. The Hirshmann[11] convention is now the unambiguous system adopted in 1967 by the IUPAC and IUB and used universally to number the three hydroxyl groups of glycerol. If the central carbon atom of the glycerol molecule is viewed with the C-H bond pointing away from the viewer, then each of the three remaining bonds leads to an hydroxyl group. The three hydroxyl groups viewed in this manner are numbered in a clockwise order with the 2-position already defined as the position carrying the hydroxyl attached directly to the central carbon atom (Fig. 2). This is equivalent to a Fisher projection in which the middle hydroxyl group is located on the left side of the glycerol carbon-chain, and the carbon atoms are numbered in the conventional top-to-bottom sequence (Fig. 3).

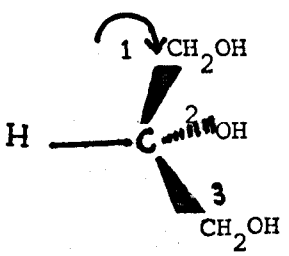


Fig. 2: diagram illustrating the Hirshmann stereospecific numbering convention

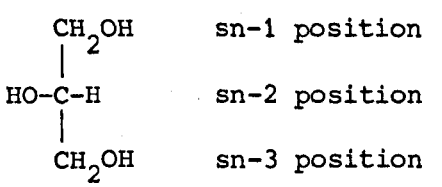
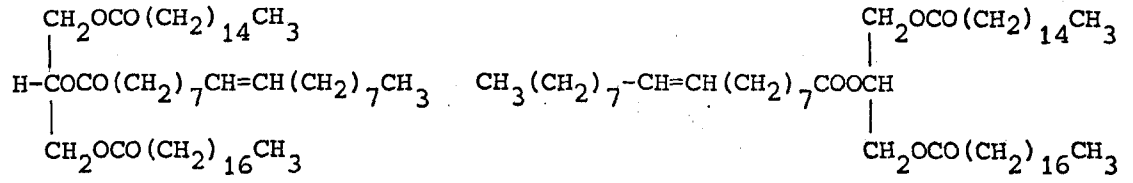


Fig. 3: stereoscopic convention applied to the Fisher planar projection

The use of the system is illustrated in Fig. 4.



sn-glycerol-1-stearate-2-oleate-3-palmitate

sn-glycerol-1-palmitate-2-oleate-3-stearate

Fig. 4: application of the Hirshmann convention to the naming of triacylglycerols

The Hirshmann convention is very useful in the description of ways in which any three fatty acids can be esterified to glycerol.

Natural sources of lipids usually contain mixed triacylglycerols. When a mixture of n different fatty acids is esterified with glycerol, the number of possible triacylglycerols which can be formed[12] is n^3, if all isomers are distinguishable. The triacylglycerol mixtures found in many plant seeds contain 5-10 different fatty acids, which can give rise to 125 to 1000 possible triacylglycerol molecular species of which 50 to 80% are known to exist.[14] Fat from animal sources is more complex, and may contain 10 to 40 different fatty acids; the most complex fat is butter fat[13] which contains at least 140 different fatty acids and this can give rise to 2,744,000 triacylglycerol molecular species of which 50 to 80% are known to exist.[14] This complexity makes the analysis of natural triacylglycerols extremely difficult.

When analysing a particular fat or oil, it may be necessary to separate the individual triacylglycerols, to determine the total fatty acid composition and the distribution of the fatty acids in the individual triacylglycerols.

Separation and analysis of lipids have been reviewed frequently.[14-16] Some of the modern analytical techniques are described below.

1.1 Enzymatic analysis of triacylglycerols.

The first technique used to determine the distribution of fatty acids between the primary and secondary position of a triacylglycerol was that described by Mattson in 1955.[17] The enzyme used was pancreatic lipase which catalyses the hydrolysis of fatty acids from the primary positions of triacylglycerols. The monoacylglycerols so produced contain the acids originally present at the position-2 of the triacylglycerols. The method has been developed since and refined to avoid acyl migration or indiscriminate hydrolysis[18-20] and applied to smaller amounts of sample.[20] It is preferable to analyse the fatty acids left in the sn-2 position rather than the free fatty acids produced by the hydrolysis. It was found that under certain conditions pancreatic lipase can hydrolyse all three positions of a triacylglycerol molecule.[19] There is also the possibility that different fatty acids may be hydrolysed from the primary positions at different rates.[21,22] The anomalous action of pancreatic lipase on triacylglycerols containing short-chain fatty acids has been established.[21,22]

The full stereospecific analysis of triacylglycerols was published by Brockerhoff in 1965.[10] He demonstrated that the fatty acid compositions of the sn-1 and sn-3 sites could be analysed separately based on the hydrolytic stereospecificity of phospholipase A, obtained from lyophilised snake venom (Fig. 4). The first step is deacylation of the triacylglycerol sample to produce representative diacylglycerols, using pancreatic lipase or a Grignard reagent. The next step is isolation of

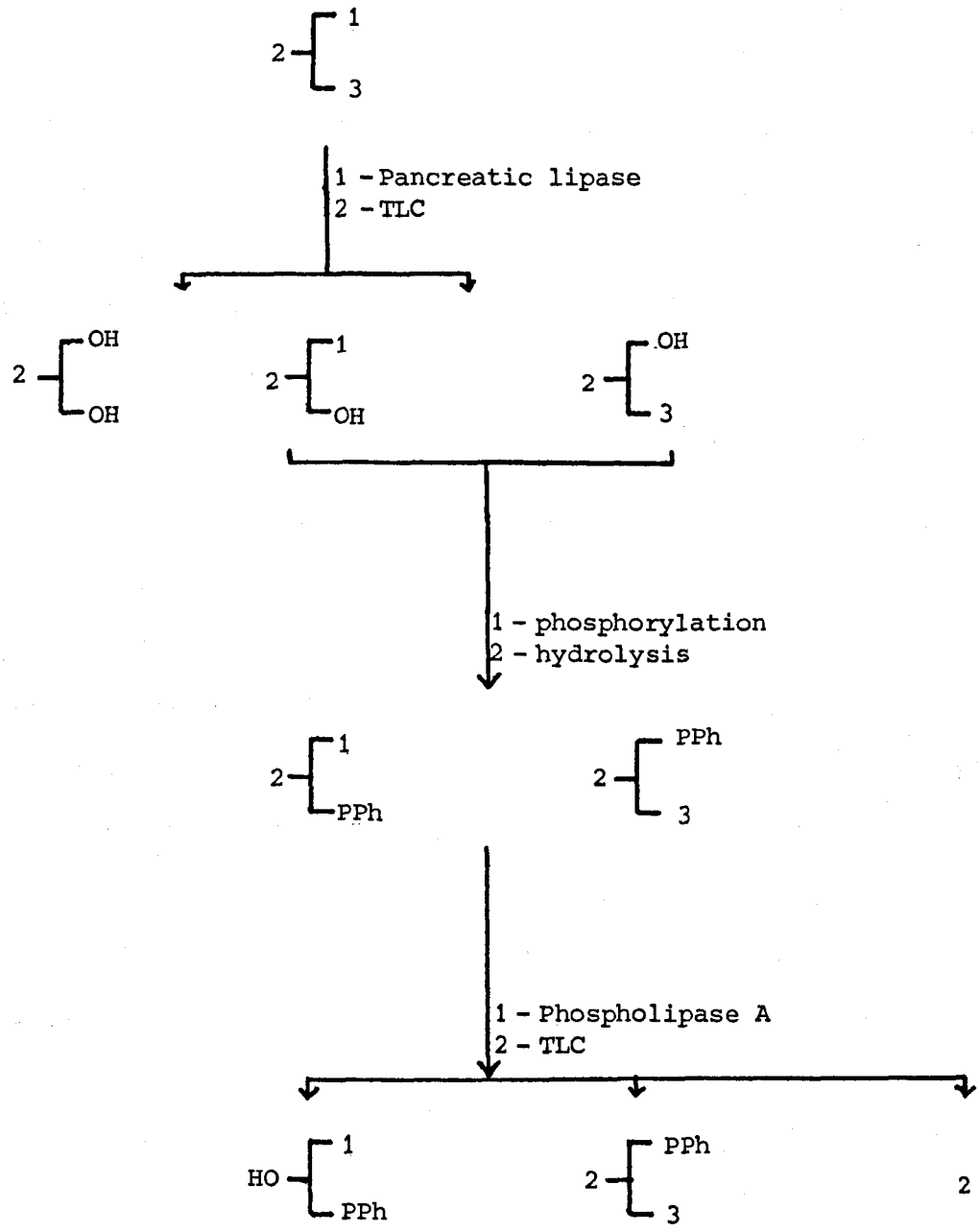


Fig. 4: Stereospecific analysis of triacylglycerols as described by Brockerhoff.[10]

PPh = phenyl phosphate group; 1,2,3 refer to fatty acids at sn-1, sn-2 and sn-3 positions respectively.

the diacylglycerols by TLC and converting them into phospholipids using phenyl dichlorophosphate. The final step in this stereospecific analysis is the hydrolysis of the glycerol diacylphospholipids using phospholipase A, separation of the reaction products and determination of their fatty acid compositions. Phospholipase A hydrolyses only the 2-position fatty acids of sn-glycerol-1,2-diacyl-3-phosphatide and not that of the enantiomer sn-glycerol-2,3-diacyl-1-phosphatide.[23,24] In presence of sn-glycerol-1,3-diacyl-2-phosphatide, phospholipase A hydrolyses the fatty acids in the sn-1-position only.[23,25]

Separation and analyses of the fatty acids of the reaction products permit the determination of the composition of the sn-1, sn-2 and sn-3 -positions in the original triacylglycerols as follows.

sn-1 = sn-glycerol-1-acyl-3-phosphatide from phospholipase A hydrolysis.

sn-2 = monoacylglycerol from pancreatic lipase hydrolysis or from free fatty acids from phospholipase A hydrolysis.

sn-3 = original triacylglycerol - monoacylglycerols from pancreatic lipase - monoacylglycerols from hydrolysed lysophosphatide

or

sn-3 = unhydrolysed phosphatide - monoacylglycerols from pancreatic lipase.

There are other alternative ways for the stereospecific determination of triacylglycerols.[133]

1.2 Gas Liquid Chromatography (GLC).

The gas chromatograph is by far the most-used instrument in the analytical field. Packed columns of length 1.8 m and 3.5 m are more frequently used than capillary columns. These latter are becoming more and more popular with both surface coated open tubular (SCOT) and wall coated open tubular (WCOT) columns.[26-29] Rapid identification of fats and oils can be achieved with more information than when using packed columns. Capillary columns have a high resolving power, shorter retention times and are highly efficient. However, packed columns are cheaper, easy to make, have longer life-times and can take a high load of solvent and sample. For most purposes GLC with its universal flame ionization detector (FID), with its speed of analysis and with its high resolution is superior to other methods. There are a number of stationary phases which are used in the separation of lipid components.[30] In previous work from this laboratory, several stationary phases were studied for their suitability for the analysis of triacylglycerols.[134] For the analysis of fatty acids from fats and oils, highly developed procedures are used.[31-33,40] Triacylglycerol analysis on packed columns has been performed for nearly 25 years.[6,12,30,31,34-37] It is possible to separate the triacylglycerols according to their total carbon number on non-polar stationary phases. Attempts have been made to separate triacylglycerols based on their degree of unsaturation.[31,38] Some problems may arise in the analysis of triacylglycerols by GLC, such as thermal degradation, if precautions are not taken.[39]

1.3 Thin Layer Chromatography (TLC).

Silicic acid, containing calcium sulphate as a binder (Kieselgel G) is used normally for the separation of almost all types of lipids, the separation being based on functional groups. The triacylglycerols are separated according to their degree of unsaturation using silver nitrate-impregnated TLC plates.[12,41,42] Argentation TLC and GLC separations by molecular weight have improved the information about triacylglycerols and their fatty acid distribution. The separation of positional isomers by argentation TLC on the basis of double bond position was achieved by increasing the amount of silver nitrate[43,44] (25% w/w). Traditionally TLC has not been used as a quantitative method. Early developments in the field of quantification involved slit scanning densitometry, the technique, however, showed difficulties due to imperfections in the thin layer, background colours and distortions of spot density and shape. Errors can be as high as 20%. Tubular TLC[45] and Iatroscan[46,47] are two new forms of quantitative TLC. In tubular TLC the chromatogram is moved through a ring furnace and the pyrolysis products are swept into an FID by a flow of inert carrier gas. In the Iatroscan TH10 (Chromarods) the thin layer chromatogram is a quartz rod coated with silica and passed directly through an FID. The quartz rods can be reused. These new techniques are attracting increasing applications in the lipid field but they still suffer from high levels of errors[48,49,131].

1.4 High Performance Liquid Chromatography (HPLC).

During the last ten years HPLC has never ceased to increase in popularity. It is not in direct competition with GLC, since the two techniques are considered to be complementary. The major advantage of HPLC is its ability to handle compounds of limited thermal stability and volatility. However in some cases HPLC has proved more effective, e.g. in reverse-phase HPLC or high performance reverse-phase chromatography (HPRC) of triacylglycerols. Increasingly efficient separation of triacylglycerols by HPRC has been achieved by μ-Bondapac C_{18},[50-54] Spherisorb 5-ODS,[55] Vydac,[55] Zorbax,[56,57] Supelcosil LC-8, Supelcosil LC-18 and Partisil ODS-1 and ODS-2[57] column packings using various mobile phases. Triacylglycerols were also separated on columns packed with 10% (w/w) silver nitrate on Partisil-5.[58] Mobile phases containing silver nitrate[59] have aided the separation of saturated and unsaturated triacylglycerols. However argentation liquid chromatography showed a lack of reproducibility of K' value.[60] Furthermore, possible silver mirror formation on the detector cell window remains a strong deterrent to its general use.[60] There have been several reports to evaluate various stationary and mobile phases.[54,57,59,60] The development of highly efficient octadecyl-bonded column packing has enabled the separation of critical pairs and triacylglycerol isomers without resorting to argentation chromatography. The critical pairs (CP) are defined as:

$$CP = CN - 2n$$

where CN is the actual carbon number and n is the number of double bonds per triacylglycerol molecule. Separation of triacylglycerols according to chain length and degree of unsaturation was achieved on a

Nucleosil 5μ C_{18}[54] (Macherey-Nagel) using UV and RI detectors. Separation has also been effected using Supelcosil LC-18 columns packed with a 5 μ octadecyl-bonded spherical silica[60] with a RI detector. Fats and oil mixtures have been separated in this way.

The limitations of HPLC lie mainly in the choice of the detector. The detectors are either of a low sensitivity or are non specific or both. According to Aitzemüller[61] normal phase HPLC involves adsorption rather than partition, so the separations of components of varying polarities may involve more than one solvent. The detectors in use have several restrictions, particularly when gradient elution is used. For the UV detector, components which do not absorb at long wavelengths are determined between 215 nm and 195 nm. However most solvents absorb in this region and impurities in solvents also have a high absorbance in this region of the spectrum. The use of the UV detector is illustrated by the separation of triacylglycerols on the basis of their degree of unsaturation by the addition of a halogen (I_2) to the double bond.[62] The separation is enhanced and halogen derivatives of polyunsaturated acylglycerols have a high absorbance in the UV region (265 nm). The refractive index (RI) detector is less sensitive than the UV detector but it is more universal. However it can be used only under isocratic conditions and a thermally stable system is needed for maximum benefit. UV[63] as well as RI[54] detectors were found to have characteristic responses towards unsaturated and unsaturated triacylglycerols. Phase transformation detectors, i.e. moving wire detectors, are the third type of detectors[64-69] used in HPLC. The solvents are evaporated on a moving wire (disc or tape) while the solute is pyrolysed or converted to methane and analysed by

the FID. Detectors of this type although useful do not enjoy a lot of popularity: they are often difficult to operate and have a low sensitivity.[61] The infrared (ir) detector is finding greater use; although reported by few workers,[70,71] its potential has not been investigated fully.

From the analytical methods described above, it becomes obvious why triacylglycerols require even more powerful procedures if we are to get a complete picture of their composition. Neutral lipids extracted from one natural source vary depending on the solvent mixture. The solvent usually used for the extraction of neutral lipids is petroleum spirit (40-60° fraction). Triacylglycerols are the major constituents in seed fats and oils. The other constituents are hydrocarbons, esters, free fatty acids, di- and monoacylglycerols. Once isolated from their natural environment, these components are prone to changes. Deterioration of the sample may be due mainly to acid- or base-catalysed hydrolysis, or to oxidation initiated by the presence of oxygen or irradiation. In this latter case, once the reaction is initiated, it will be autocatalytic. Samples containing high levels of unsaturation are relatively unstable. Some fats and oils (e.g. cocoa butter), containing natural antioxidant (tocopherol), show good stability on storage. The degree of unsaturation determines the stability of the sample. The level of linolenic acid and fatty acids with greater unsaturation is a critical factor in predicting oxidative stability. Oxygen can attack highly unsaturated lipids even at low temperatures. Sufficient care must be taken to ensure that the sample is free from acid, base or active enzyme and that it is not exposed to heat or any of those factors for any length of time. The samples are best kept at sub-zero temperatures in brown glass bottles with screw tops, since plastic

bottles are porous to gases and metallic ones have catalytic properties.

Preliminary separation into chemical classes is a necessity prior to any qualitative or quantitative analysis of the neutral lipid fraction. Group or class separation predominates in work on lipids.[72-84] Traditionally these separations are carried out by adsorption on silica gel. TLC[85-88] is the accepted method for the preliminary separation of the constituents of fats and oils for quantities below 50 mg. Column chromatography[88-90] was used for quantities much higher than that. Lipids also were analysed without any class separation[26,91,92] and that may result in some unwanted unknowns.[91]

Lipid classes have been separated by HPLC and the method is well established.[71,80,93,96] Most separations were achieved using moving wire detectors which are now withdrawn from the market. Since Parris's[70] first report on the use of the infrared (ir) detector in lipid separation by HPLC, few publications have followed. In his paper, Parris described the possibilities of using the ir detector in the analysis of carbonyl-containing compounds under gradient elution. Since then there have been reports on the separation of other lipid classes,[71] i.e. hydrocarbons, methyl esters, ketones and acylglycerols also under gradient elution. Triacylglycerols also were separated from estolides[94] under isocratic conditions using the ir detector. There was also a report on the separation of triacylglycerols from mono- and diacylglycerols under gradient elution.[95] In this last paper, the authors reported the separation of a lipolytic mixture containing more 1,3-diacylglycerols than 1,2-diacylglycerols. The ir detector also was used in the separation of component acylglycerols. It was compared with a RI detector under isocratic conditions.[54] The two detectors were found to be

equally sensitive. The use of an ir detector often was associated with lack of suitable spectral "windows", i.e. wavelengths where the optical transmission is sufficiently high to enable sensitive detection of components eluting from the chromatographic column. Acetonitrile, chloroform and n-heptane have high transmittance in the region where the carbonyl group absorbs (5.75 μm) and since they represent a wide range of polarities they may be well-suited for the separation of lipid classes by adsorption chromatography.

In the present study HPLC equipped with an infrared detector (HPLC-ir) was used for the preparative separation of individual chemical classes in seed fats and oils and lipolysis mixtures. The ir detector was studied under isocractic, stepwise and gradient elution. The component triacylglycerols and their fatty acids were analysed. Preparative TLC was used in the same way for comparison. It is TLC and not traditional column chromatography which is really competing with HPLC. The resolving power of the HPLC is very adequate in the handling of many components in natural fats and oils, but in several cases, when carried out on good plates, TLC can produce similar results.

2. RESULTS AND DISCUSSION.

2.1 Oil extraction.

The seven samples of seed kernels studied were peanut, almond, sesame, perilla, babassu, Borneo tallow and hazelnut. The mature seeds came from commercial sources and the neutral lipids were extracted using petroleum spirit (40-60°). Only very minor quantities of the polar lipids will be extracted under these conditions. The seed kernels were ground and extracted for 20 hours using a Soxhlet

apparatus. The amounts of lipid extracted ranged from 59 to 41% (w/w) (Table 1).

Seeds	% (w/w) Seeds fats and oils extracted	Literature data % (w/w)
Peanut	41.7	41.2-48.1[97]
Almond	59.0	54.35-61.6[98]
Sesame	48.5	53.1[99]
Perilla	40.8	44.7[100]
*Babassu	-	65-68[101]
Borneo tallow	50.2	48-50[102]
Hazelnut	51.2	54[103]

*Babassu was received as a fat

Table 1; Amount of fat and oil extracted from seed kernels.

The amounts of fats and oils extracted from the various seed kernels are in good agreement with the literature data (Table 1). The amount of fat extracted depends very much on the method of extraction used and on the origin of the seeds. Perilla and peanut kernels contain less oil than any of the other seeds while almond and babassu kernels have the highest contents. The neutral lipids isolated contain hydrolysable esters and unsaturated centres vulnerable to oxidation. The precautions taken to prevent deterioration of the samples were mentioned earlier. None of the samples studied was protected with antioxidants. The

samples, solid fat in particular, were homogenised before analysis, to prevent settling during storage, since otherwise the fraction being analysed will not be truly representative of the whole fat.

2.2 Preparative separation of lipid classes by TLC and HPLC.

The fats and oils isolated from the seed kernels were separated into lipid classes using TLC and HPLC equipped with an infrared detector (HPLC-ir) i.e. the two techniques were used for the separation of the triacylglycerols from crude fat and oil. Both techniques were compared for their capacity to separate monoacylglycerols from lipolysis mixtures. TLC was first used to isolate the lipid classes. In preparative TLC, the amount of sample loaded onto the plate is limited. If the lipid mixture is solid, the quantity taken must be $\leqslant$ 25 mg, if it is liquid, the quantity is reasonably higher ($\leqslant$ 30 mg). The glass plates (20 x 20 cm) were coated with 0.5 mm Kieselgel 60 G. If the amounts taken are higher, band overlapping may occur. In cases where the samples contain components with high percentage of poly-unsaturated fatty acids (perilla) or short chain fatty acids (babassu), the overlapping is increased. These effects are shown in Table 2 where the R_f values for the triacylglycerols, fatty acids, esters and hydrocarbons are given. The difference in R_f values (ΔR_f) between the fatty acids and the triacylglycerols was highest for Borneo tallow (ΔR_f = 0.33) and the standards (ΔR_f = 0.3) which were glycerol trimyristate and stearic acid. The ΔR_f values for babassu (0.26) and perilla (0.16) were much lower. Therefore, these results re-emphasize the need for caution when separating triacylglycerols with different chain length fatty acids.

Fats and oils	R_f values				
	Fatty acids	Triacyl-glycerols	Esters	Diacyl-glycerols	Hydro-carbons
Standards	0.19	0.49	0.79	0.03	0.98
Borneo tallow	0.20	0.53	0.92	0.03	0.98
Babassu	0.12	0.38	0.65 0.80	0.03	0.98
Perilla	0.19	0.36	0.64 0.77	0.03	0.98

Table 2: Effect of acid composition on the separation of triacylglycerols from neutral lipids by TLC. Elution system: 80:19.5:0.5 (v/v) Petroleum spirit:Et_2O:Acetic acid.

Most of the samples had a triacylglycerol content of 99.1% ± 0.8% (w/w). In babassu, which was received as a fat, the triacylglycerol content was low mainly due to hydrolysis which was shown by its high free fatty acid content. These results are in accord with the generally accepted levels of triacylglycerols in neutral lipids, i.e. hazelnut (98%),[104] almond (99.6%),[105] peanut (98%),[106] sesame (98.3%),[107] perilla (98.9%)*,[110] Borneo tallow (99.5%)*[108] and babassu (99.7%)*.[109]

The hydrocarbons were also separated by TLC. They constitute only a small percentage of the neutral lipid mixture (Table 3).

*The triacylglycerol content was taken as a difference between the total oil and the unsaponifiable matter contents.

Seed fats and oils	Hydrocarbons content in neutral lipid fraction: % (w/w)	Hydrocarbons content: literature values: % (w/w)
Peanut	0.02	
Almond	0.03-0.02	0.03-0.02[105]
Sesame	0.03-0.01	
Perilla	0.02-0.01	
Babassu	0.04-0.02	
Borneo tallow	0.02-0.01	
Hazelnut	0.02-0.01	

Table 3: hydrocarbons content of some seed oils from TLC preparative separations.

The amounts of triacylglycerols from any one fat or oil which can be separated on one TLC plate were sufficient for the three subsequent analyses to be performed, namely (a) GLC of intact triacylglycerols, (b) interesterification of the total triacylglycerols and (c) lipase hydrolysis. Each TLC run lasted 40 min plus the time required for visualisation and extraction. In the case of the monoacylglycerols there was less sample available and the whole plate was visualised. This resulted in the sample being contaminated with 2,7'-dichlorofluorescein, the visualising agent. The samples then were separated from the 2,7'-dichlorofluorescein by running them again without spraying the TLC plates. The band to be extracted was estimated from the R_f

value obtained from the previous run.

The second technique to isolate the lipid classes from seed fats and oils was based on HPLC-ir. The chromatographic conditions are given in the experimental section (3). The solvents used were n-heptane and chloroform for the separation of triacylglycerols and n-heptane, chloroform and acetonitrile for the separation of monoacylglycerols.

In the separation of triacylglycerols up to 3.7 mg of a sample were injected at a time on the HPLC column. The relative standard deviation on the weight of the sample recovered was σ_{n-1} = 0.05 mg for six successive runs. The solvent in each sample firstly was evaporated off under a stream of nitrogen and the residues were weighed on a micro-balance. The following weights were recorded: 4 x (3.2 mg) and 2 x (3.3 mg). The triacylglycerol content in the neutral lipid fraction separated by HPLC-ir varied between 97 and 98% (w/w) for most of the samples. For babassu fat it was only 81% (w.w). All the values were 1 to 2% lower than those in the TLC preparative separations: this may be due to the fact that TLC is an open column and therefore the samples are extracted instead of being recovered as is the case in column chromatography.

2.3 The chromatographic system needed for the separation of neutral lipids by HPLC using the infrared detector (HPLC-ir).

The neutral lipid fraction contains mainly triacylglycerols with three carbonyl groups in each molecule. The minor components such as esters, free fatty acids and mono- and diacylglycerols also contain carbonyl functional groups. The detector was set at a fixed wavelength of 5.75 μm (1740 cm^{-1}), the absorption wavelength for the carbonyl group.

As indicated in solvent profile 1 (section 3), the two solvents used for the separation of the triacylglycerols were n-heptane and chloroform. Each solvent has a high transmittance at 5.75 μm, but n-heptane has a higher absorbance than chloroform. The system was run initially with 100% n-heptane for 5 min. Hydrocarbons elute under these conditions.[71] A linear gradient elution was achieved by pumping chloroform regularly into the system to reach a selected maximum polarity of 70:30 (v/v) n-heptane:chloroform in 15 min. As more and more chloroform is pumped into the system, the baseline acquired a gentle negative slope (Figs. 5 & 6) on which any carbonyl-containing compounds could be detected. The triacylglycerols eluted after 21.5 min. The percentage relative standard deviation (% RSD) on the retention time for the triacylglycerols was 7.4 (4 runs). After the maximum polarity was reached, the baseline became horizontal again and it returned to its initial level, when 100% equilibrium was attained. Free fatty acids and diacylglycerols eluted successively after the triacylglycerols and during the return to the initial conditions. The level of detection for the triacylglycerols was 4.6 μg (glycerol trimyristate) at 0.1 attenuation (Section 3).

In the separation of monoacylglycerols from the lipolysis mixture, a three solvent system was used. Although n-heptane, chloroform and acetonitrile have high transmittances at 5.75 μm, they have different absorbances in that region. n-Heptane and acetonitrile have higher absorbances than chloroform. As pointed out by Parris,[70] a horizontal baseline could be obtained if the initial and final stages of the gradient elution are of similar baseline level. This procedure could be achieved but it has its disadvantages, viz. loss of resolution, longer retention times and (because the pumping system varies from one

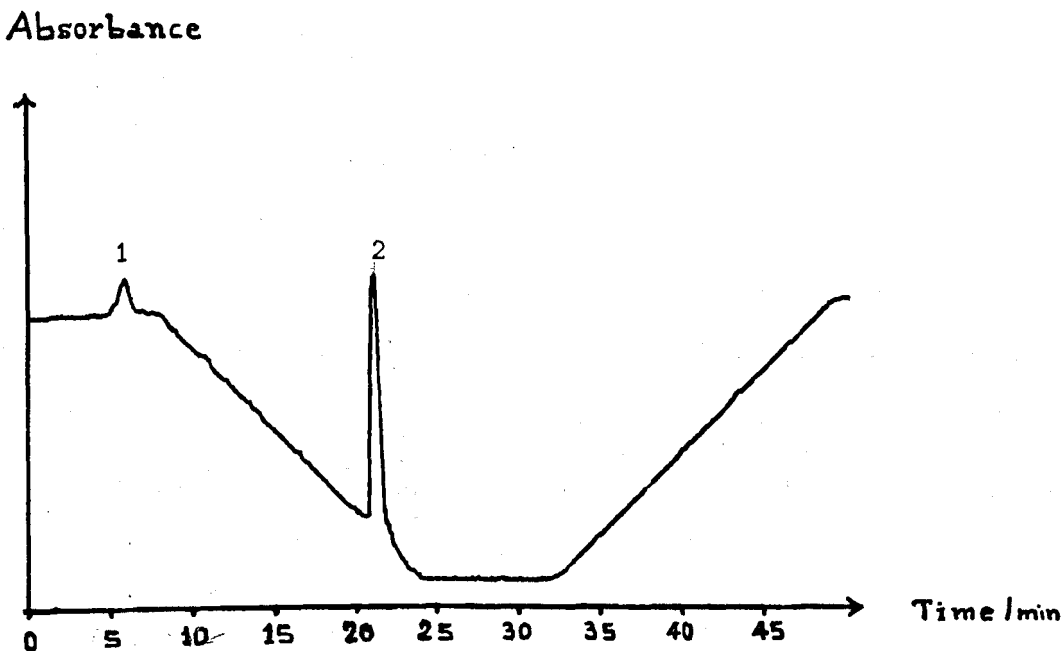


Fig. 5: separation of lipid classes by HPLC-ir: separation of a standard mixture using solvent profile 1 (Sec.3.2.2); 1: methyl stearate, 2: trimyristate

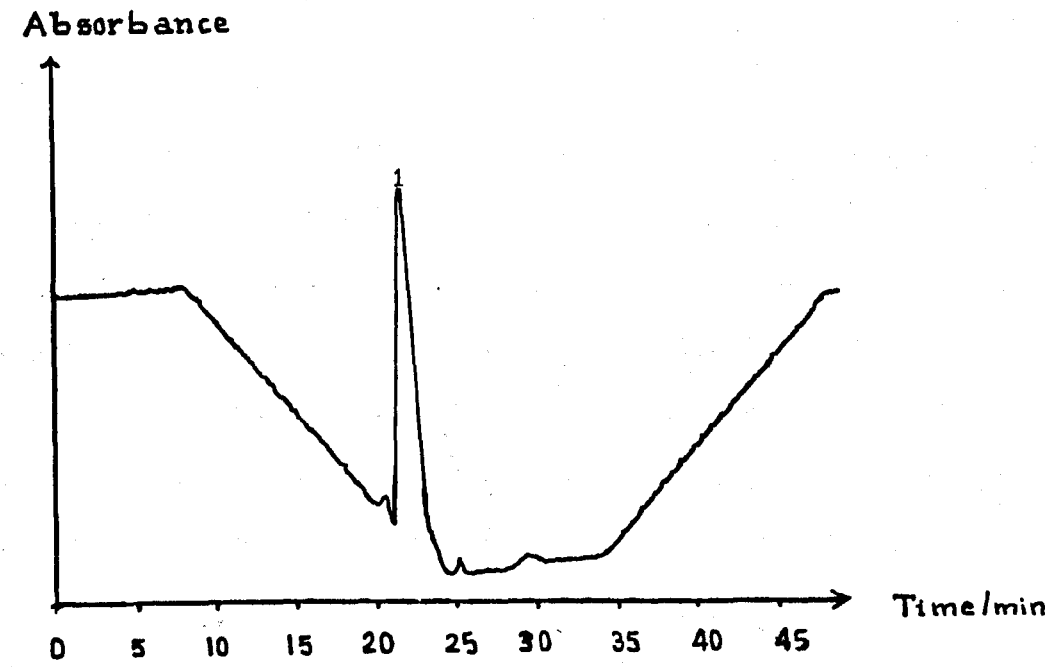


Fig. 6: separation of lipid classes by HPLC-ir: separation of triacylglycerols of Borneo tallow using solvent profile 1 (Sec.3.2.2); 1: triacylglycerol mixture

instrument to another) non-reproducible conditions. A stepwise gradient elution was adopted instead as shown in solvent profile 2 (section 3) and good resolution and short retention times were obtained (Figs. 7 and 8). 70:30 (v/v) n-heptane:chloroform was pumped into the system, at first triacylglycerols eluted after 3 min and then 1,3-diacylglycerols after 13 min. After 14 min the 95:5 (v/v) chloroform:acetonitrile was introduced. There was a sharp drop in the baseline which lasted for a few seconds, after which the baseline was horizontal and regular. The 1,2-diacylglycerols eluted after 17 min and monoacylglycerols after 20.1 min. A small artefact peak appeared in some runs even when injecting pure eluent with a retention time of 17.3 min (Fig. 8). It may be due to the deviation of the system from the "ideal" conditions.[130] The % RSD for the monoacylglycerols retention times was 5.2 (5 runs). The minimum level of detection for the monoacylglycerols was 9.1 μg (glycerol monostearate) at 0.1 attenuation. Neither the UV nor the RI detectors could have achieved such a level of detection and performance under similar conditions. The conditions used were very simple and have wide applications in preparative and quantitative separation of triacylglycerols from neutral lipids (Fig. 6) from different sources, separation of monoacylglycerols from lipolysis mixtures and 1,2-diacylglycerols for full stereoscopic analysis of the triacylglycerols structure (Fig. 8). It can be applied also to the preparative and quantitative separation of the acylglycerols in synthetic mixtures, foodstuff emulsifiers and food chemistry in general. It is the first time such an application is described and applied to fats and oils and synthetic acylglycerols.

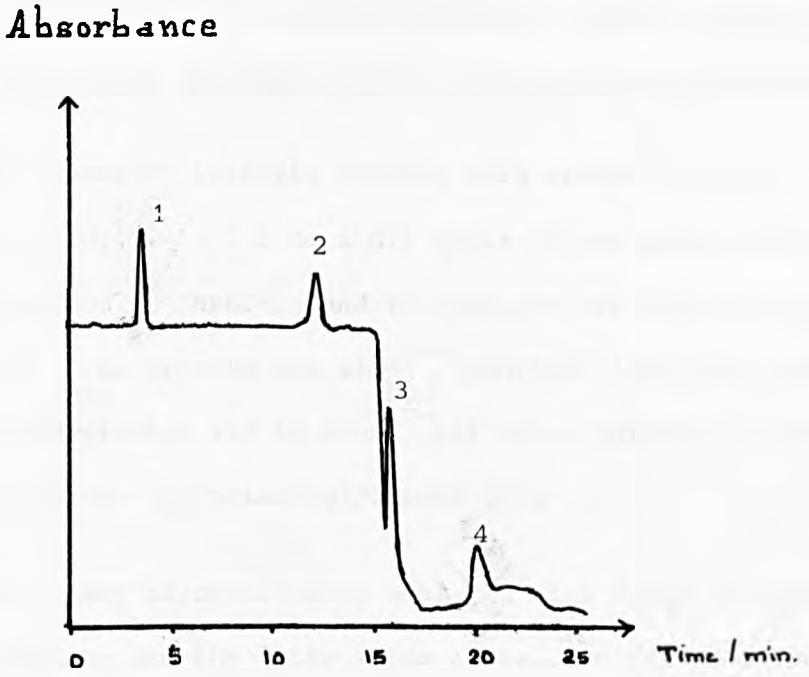


Fig. 7: separation of mono-, di- and triacylglycerols by HPLC-ir using solvent profile 2 (Sec.3.2.2); 1: Trimyristate; 2: sn-glycerol-1,3-distearate; 3: glycerol-1,2-distearate; 4: glycerol monostearate.

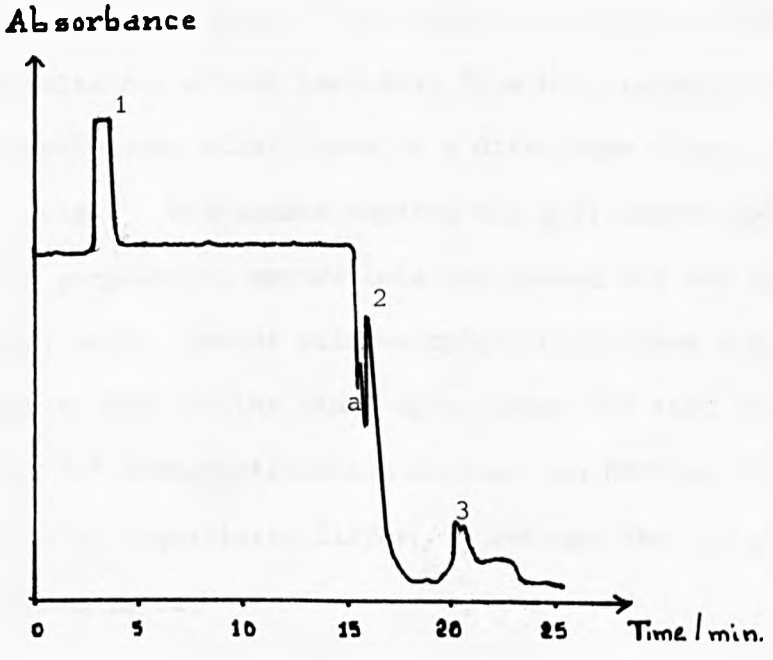


Fig. 8: separation of a lipolytic mixture of hazelnut acylglycerols by HPLC-ir using solvent profile 2 (Sec.3.2.2); 1: triacylglycerols, 2: 1,2-diacylglycerols, 3: monoacylglycerols, a: artefact peak

2.4 GLC analysis of triacylglycerols and methyl esters of their fatty acids from the HPLC-ir and TLC preparative separations

The separated triacylglycerols were analysed by high temperature GLC on a (0.45 m x 2.5 mm i.d.) glass column packed with 3% OV17. The results from the HPLC-ir and TLC preparative separations are given in Table 4. The results are almost identical from both techniques. The highest deviation was in peanut oil triacylglycerols with a value of ±2.9% for the C_{52} triacylglycerols (Fig. 9).

The triacylglycerols were analysed also for their total fatty acids compositions and the fatty acids present in the sn-2 position. The fatty acids were analysed as methyl esters on two different columns, the 3% OV17 (1.85 m x 2.5 mm i.d.) glass column which separates them according to their chain length and the 10% DEGS (3.00 m x 2.5 mm i.d.) glass column which separates them according to their chain length and degree of unsaturation. The results are given in Tables 5, 6, 7 and 8. The results are almost identical from both techniques, though for almond and sesame oils, there is a difference of up to 7.8% in linoleic acid content. Histograms showing the differences between the HPLC-ir and TLC preparative separations for peanut oil and babassu fat are given in Figs. 9-14. Peanut oil triacylglycerols have a composition pattern similar to most of the other oils except for that from babassu fat. The Student "t" test distribution between the HPLC-ir and TLC showed that there is no significant difference between the two methods at 95% confidence level.

$$t = \frac{\bar{D}}{Sd} \times \sqrt{N}$$

(cont. p.31)

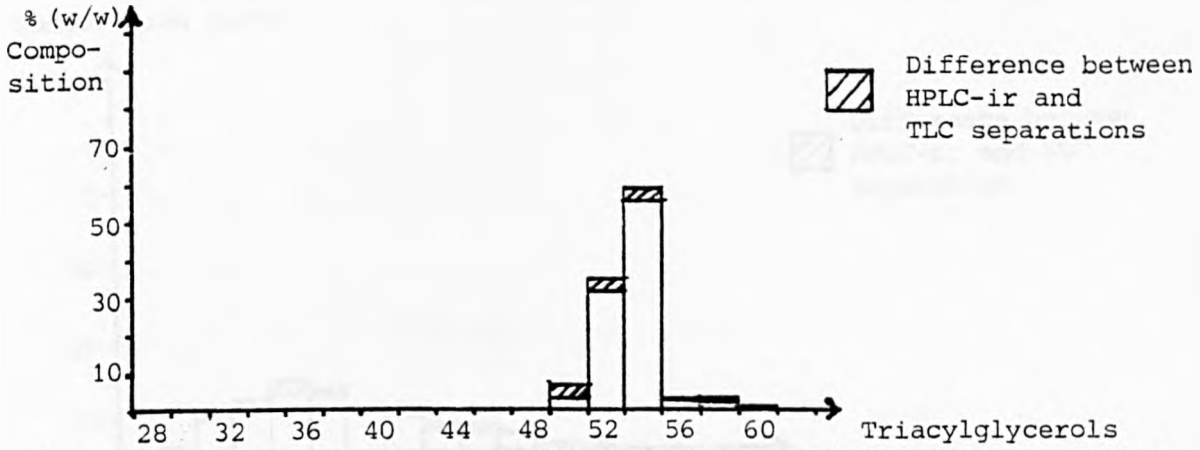


Fig. 9: histogram showing the difference in triacylglycerol composition between HPLC-ir and TLC separations in peanut oil

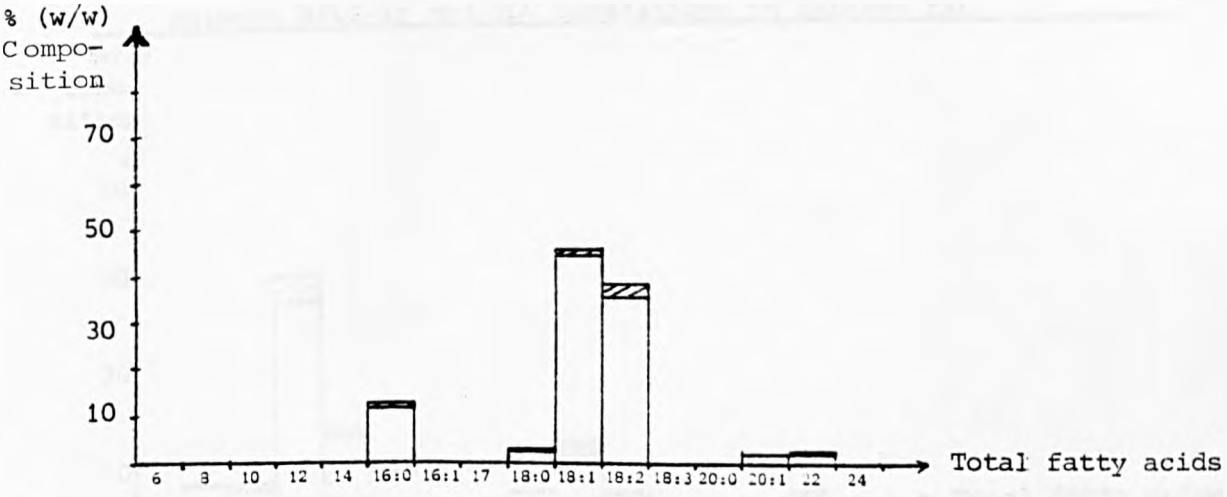


Fig. 10: histograms showing the difference in total fatty acid composition between HPLC-ir and TLC separations in peanut oil

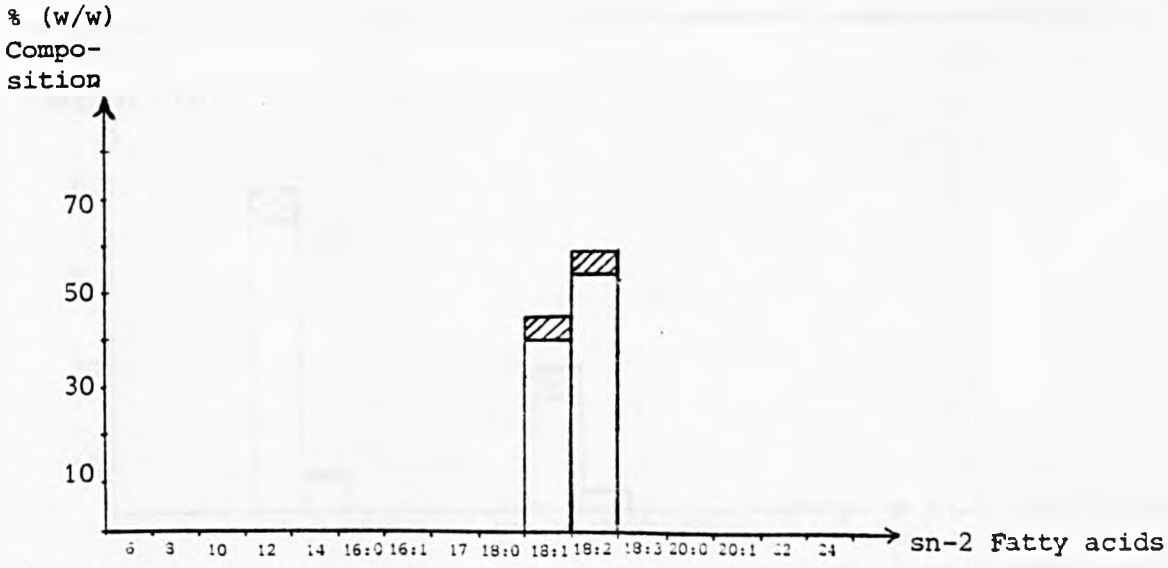


Fig. 11: histogram showing the difference in sn-2 fatty acid composition of the triacylglycerols between HPLC-ir and TLC separations in peanut oil

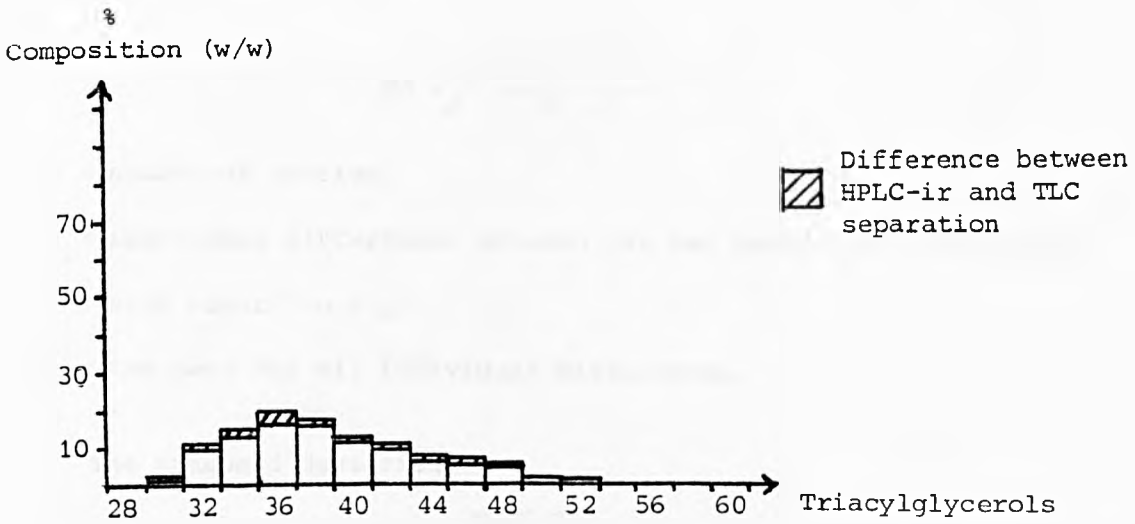


Fig. 12: histogram showing the difference in triacylglycerol composition between HPLC-ir and TLC separations in babassu fat.

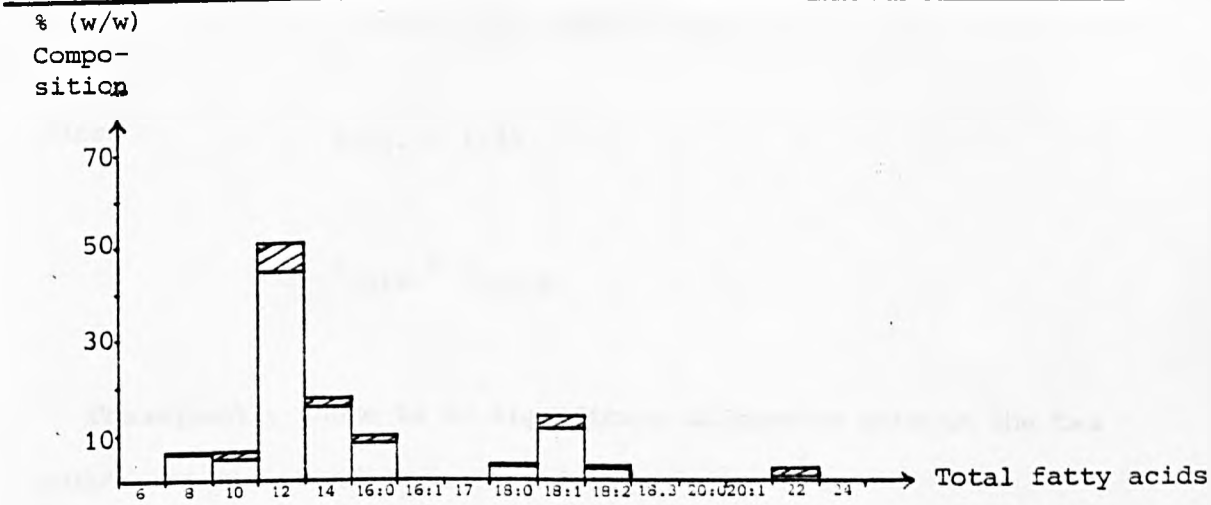


Fig. 13: histogram showing the difference in total fatty acid composition between HPLC-ir and TLC separations in babassu fat

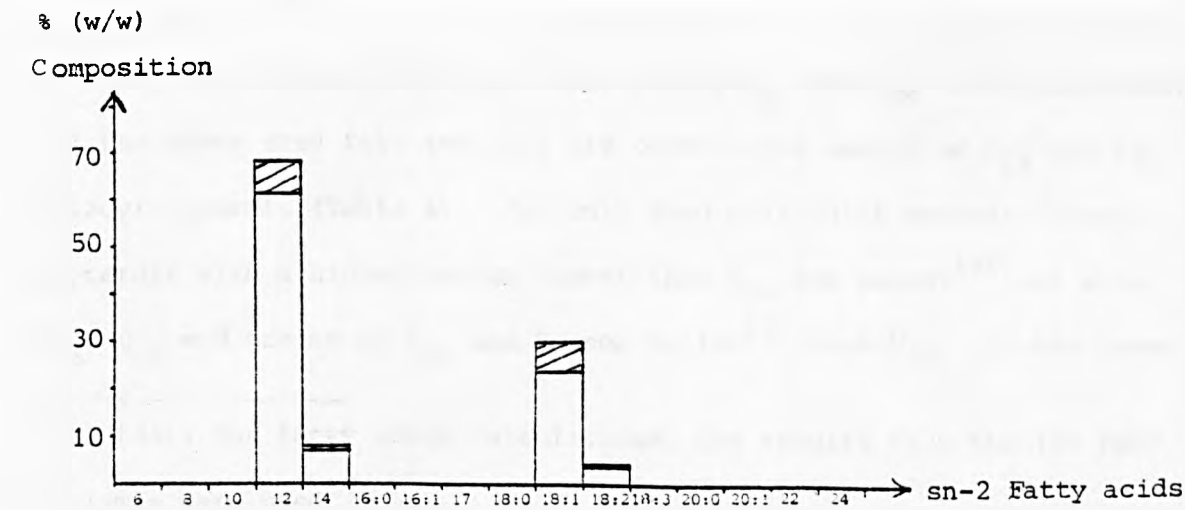


Fig. 14: histogram showing the difference in sn-2-fatty acid composition of the triacylglycerols between HPLC-ir and TLC separation in babassu fat

$$Sd = \sqrt{\frac{\Sigma (D_i - \bar{D})^2}{N - 1}}$$

N = number of entries.

D_i = individual differences between the two methods for each result with regard to sign.

$\bar{D}$ = the mean for all individual differences.

The standard deviation is

$$Sd = \sqrt{\frac{764.50}{9.5}} = 2.84$$

$\therefore$ $$t^* = \frac{0.11}{2.84} \times \sqrt{96} = 0.38$$

Since $t_{\infty 95\%} = 1.95$

$\therefore$ $t_{calc} < t_{table}$.

Consequently there is no significant difference between the two methods at this confidence test (95%).

2.5 Triacylglycerols and fatty acid distribution in the seed fats and oils studied.

Except for babassu which contains mainly C_{36} and C_{38} triacylglycerols, all the other seed fats and oils are constituted mainly of C_{54} and C_{52} triacylglycerols (Table 4). The only seed oils which contain triacylglycerols with a higher carbon number than C_{54} are peanut[120] oil with C_{56}, C_{58} and traces of C_{60} and Borneo tallow[112] with C_{56}. In the lower

*"t" test: for fatty acids calculations, the results from the 10% DEGS columns were used.

Table 4: GLC of triacylglycerols separated by TLC and HPLC-ir (% w/w)

Seed fats and oils \ Triacylglycerols		C_{28}	C_{30}	C_{32}	C_{34}	C_{36}	C_{38}	C_{40}	C_{42}	C_{44}	C_{46}	C_{48}	C_{50}	C_{52}	C_{54}	C_{56}	C_{58}	C_{60}
Peanut	1								tr.	tr.		tr.	2.7	34.7	55.8	2.9	3.3	0.6
	2											tr.	4.4	31.8	58.5	3.0	2.3	tr.
Almond	1								tr.		tr.	tr.	0.8	23.8	75.4			
	2											tr.	1.4	21.3	77.3			
Sesame	1											tr.	3.4	30.9	65.7			
	2										tr.	tr.	3.2	28.3	68.5			
Perilla	1											1.6	3.4	26.4	68.6			
	2											tr.	2.4	27.9	69.7			
Babussu	1	tr.	1.2	9.1	12.2	17.4	16.7	12.3	10.5	7.2	6.2	4.9	1.5	0.8	tr.			
	2	tr.	2.1	10.8	14.6	19.8	15.3	11.1	8.8	5.8	5.0	4.2	1.6	0.9	tr.			
Borneo tallow	1											tr.	10.3	41.8	45.6	2.3		
	2											tr.	9.6	42.6	45.7	2.1		
Hazelnuts	1										tr.	tr.	0.6	16.3	83.1			
	2										tr.	tr.	0.8	15.8	83.4	tr.		

*Perilla hydrogenated. tr. ⩽ 0.5; 1. Triacylglycerols from TLC separation; 2. Triacylglycerols from HPLC-ir separation

carbon number triacylglycerols, sesame oil[114] and perilla oil (hydrogenated) contain similar amounts of C_{52} while hazelnut[91,112] and almond[91,112] have much more marked similarities in all their triacylglycerol contents (i.e. C_{50}, C_{52} and C_{54}).

Perilla triacylglycerols contain a high percentage of polyunsaturated fatty acids and this made their analysis by GLC very difficult, due to peak broadening and overlapping between peaks, unless they were hydrogenated. Hydrogenation does not affect the chain length distribution of the triacylglycerol composition, since the triacylglycerols are separated according to their carbon number. The results for the triacylglycerols for all the seed fats and oils studied (Table 4) agree with the literature data.[91,112,114,120] No data are available for perilla oil or babassu.

In the total fatty acid content (Tables 5 and 6), all seed fats and oils contained at least five different fatty acids. Peanut, babassu and Borneo tallow contain more saturated fatty acids than the rest of the seed fats and oils. Peanut oil[85,88,111] has the longest fatty acid chains [eicosenoic ($C_{20:1}$), behenic ($C_{22:0}$) and lignoceric ($C_{24:0}$) acids]. Borneo tallow[112] contains only eicosenoic ($C_{20:1}$) and behenic ($C_{22:0}$) acids, while babassu fat[116,117] is very rich in short-chain fatty acids and contains 11 different fatty acids with hexanoic (C_6) and behenic ($C_{22:0}$) being the shortest and the longest respectively. Babassu and Borneo tallow are the only fats and oils which have saturated fatty acids as the main components; in babassu it is lauric acid ($C_{12:0}$ $\cong$ 45%) and in Borneo tallow it is stearic acid ($C_{18:0}$ $\cong$ 45%). In the rest of the seed oils saturated fatty acids are the minor constituents. The most abundant saturated fatty acid in these seeds is palmitic acid

(cont. p.36)

Table 5: GLC of fatty acids in triacylglycerols separated by TLC (% w/w)

Seed fats and oils \ Fatty acids		C_6	C_8	C_{10}	C_{12}	C_{14}	$C_{16:0}$	$C_{16:1}$	C_{17}	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$	$C_{20:1}$	C_{22}	C_{24}
Peanut	1						10.8			81.1				1.0		5.0	2.1
	2						11.6			2.5	44.5	38.0		tr.	1.7	1.7	tr.
Almond	1					tr.	5.7			92.8				0.6		0.9	
	2					tr.	6.0	0.9		0.7	65.2	27.2		tr.		tr.	
Sesame	1						8.6			91.4				tr.		tr.	
	2					tr.	9.7			5.7	41.3	43.3					
Perilla	1						7.8			92.2							
	2						6.1			1.8	10.4	22.2	59.5				
Babassu	1	tr.	5.2	4.2	47.8	16.2	8.0			15.6						3.0	
	2	tr.	5.2	4.4	50.2	15.2	7.4			2.9	10.5	1.9				2.3	
Borneo tallow	1						18.8			81.2							
	2						20.1			43.9	33.6	0.8	1.6				
Hazelnuts	1						4.3		tr.	95.7						tr.	
	2						3.4		0.6	1.4	88.3	6.3					

tr. $\leq$ 0.5; 1: 3% OV17 analyses; 2: 10% DEGS analyses.

Table 6: GLC of fatty acids in triacylglycerols separated by HPLC (% w/w)

Seed fats and oils \ Fatty acids		C_6	C_8	C_{10}	C_{12}	C_{14}	$C_{16:0}$	$C_{16:1}$	C_{17}	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$	$C_{20:1}$	C_{22}	C_{24}
Peanut	1						11.8		tr.	85.1				1.0		2.1	tr.
	2						12.2		tr.	2.8	45.6	35.2		tr.	1.8	2.4	tr.
Almond	1						5.9			94.1							
	2						6.2	0.6		1.1	72.0	20.1					
Sesame	1						10.2			89.8							
	2						10.8	tr.		5.5	47.3	35.5	0.9			tr.	
Perilla	1						40.0			60.0							
	2						8.9	tr.		1.6	11.5	17.5	60.5				
Babassu	1	tr.	4.7	4.4	45.6	17.3	9.4			18.6							
	2	tr.	5.7	5.8	44.0	16.9	8.9			2.4	13.2	2.4		tr.		0.7	
Borneo tallow	1						17.6			81.2				1.2			
	2						17.8			46.2	32.9	tr.	1.4	tr.	1.1	0.6	
Hazelnuts	1						6.4			93.6							
	2						7.3			2.0	86.3	4.4					

tr. ⩽ 0.5; 1: 3% OV17 analyses; 2: 10% DEGS analyses

($C_{16:0}$), then come stearic ($C_{18:0}$) and behenic ($C_{22:0}$) acids. Almond[112] and hazelnut[112] oils contain very similar amounts of unsaturated fatty acids [oleic ($C_{18:1}$) and linoleic ($C_{18:2}$)] but they differ in their individual compositions. Almond oil contains more linoleic acid acid ($C_{18:2}$) than hazelnut. However peanut and sesame[114] oils which also contain those two fatty acids, i.e. $C_{18:1}$ and $C_{18:2}$, have them in nearly equal proportions. Perilla[115] is the only oil which contains a polyunsaturated fatty acid which is also the main component in the fatty acids present, viz, linolenic acid ($C_{18:3}$). The fatty acids which are common to all the seed fats and oils analysed are palmitic ($C_{16:0}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$) and linoleic ($C_{18:2}$). All the results given (Tables 5 and 6) agree favourably with the literature data.[85,88,111,112,114-117]

The fatty acids present in the sn-2 position of the triacylglycerols of the seed fats and oils are given in Tables 7 and 8. C_{18} unsaturated fatty acids are present in the sn-2 position as the major components (> 98% w/w) in all seed oils, except for babassu which contains mainly lauric acid ($C_{12:0}$) as the main constituent. In most of the oils, as shown by the enrichment factors[90] (Table 9), there is a preference of linoleic ($C_{18:2}$) over oleic ($C_{18:1}$) in the sn-2 position. In babassu fat more than 60% (w/w) of lauric acid ($C_{12:0}$) is present in the sn-2 position, while its content of oleic ($C_{18:1}$) in the same position has increased more than its content of linoleic acid ($C_{18:2}$). Borneo tallow,[119] which is mainly made of saturated fatty acids ($\geq$ 60% w/w), has the sn-2 position composed of the unsaturated oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) acids, with oleic acid as the major component. In cases where linolenic ($C_{18:3}$) is present together with

(cont. p.40)

Table 7: GLC of fatty acids in 2-monoacylglycerols separated by TLC (% w/w)

Seed fats and oils \ Fatty acids		C_6	C_8	C_{10}	C_{12}	C_{14}	$C_{16:0}$	$C_{16:1}$	C_{17}	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$	$C_{20:1}$	C_{22}	C_{24}
Peanut	1						tr.			100							
	2						tr.				40.5	59.5			tr.	tr.	tr.
Almond	1									100							
	2										59.8	40.2					
Sesame	1									100							
	2										39.1	60.9					
Perilla	1									100							
	2										13.0	21.8	65.2				
Babassu	1				67.4	6.5				26.1							
	2			tr.	67.7	6.5	tr.			tr.	22.8	3.0					
Borneo tallow	1									100							
	2										97.7	2.3					
Hazelnuts	1						2.0			98.0							
	2						1.0			1.3	92.4	5.3					

tr. ≤ 0.5; 1: 3% OV17 analyses; 2: 10% DEGS analyses

Table 8: GLC of fatty acids in 2-monoacylglycerols separated by HPLC (% w/w)

Seed fats and oils \ Fatty acids		C_6	C_8	C_{10}	C_{12}	C_{14}	$C_{16:0}$	$C_{16:1}$	C_{17}	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$	$C_{20:1}$	C_{22}	C_{24}
Peanut	1						tr.			100							
	2										45.3	54.7					
Almond	1						tr.			100							
	2						tr.			tr.	62.8	37.2					
Sesame	1									100							
	2									tr.	43.4	56.6					
Perilla	1									100							
	2									tr.	19.1	20.2	60.7				
Babassu	1				58.7	7.3				34.0							
	2			tr.	60.5	7.1	tr.			tr.	29.0	3.4					
Borneo tallow	1									100							
	2									tr.	100	tr.					
Hazelnuts	1						tr.			100							
	2						tr.			tr.	100	tr.					

tr. ⩽ 0.5. 1: 3% OV17 analyses; 2: 10% DEGS analyses.

Seed fats and oils \ Fatty acids		C_{12}	C_{14}	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Peanut	1				0.9	1.6	
	2				1.0	1.8	
Almond	1				0.9	1.5	
	2				0.9	1.9	
Sesame	1				1.0	1.4	
	2				0.9	1.6	
Perilla	1				1.2	1.0	1.1
	2				1.7	1.2	1.0
Babassu	1	1.4	0.4		2.2	1.6	
	2	1.4	0.4		2.2	1.4	
Borneo tallow	1				2.9	2.9	
	2				3.0		
Hazelnut	1			0.9	1.1	0.8	
	2				1.2		

Table 9: enrichment factors[90] 1: TLC preparative separation;
2: HPLC preparative separation.

oleic ($C_{18:1}$) and linoleic ($C_{18:2}$), as in Borneo tallow and perilla, oleic and linoleic acids are favoured in the sn-2 position. The observations made above are similar to those made by Gunstone[90,121] and Mattson and Volpenheim[113] in their analysis of seed triacylglycerols. Up to now there is no report available in the literature for the fatty acids present at the sn-2 position in perilla oil and babassu fat to enable a comparison to be made with the results in Tables 7 and 8. As far as the babassu triacylglycerols are concerned, there are some seeds[122] where lauric acid ($C_{12:0}$) is present mainly in the sn-2 position, as in babassu seeds themselves and in other seeds where it is present mainly in the sn-1 and sn-3 positions. The results given in Tables 7 and 8 for the fatty acid contents of the rest of the seed oils compare favourably with the literature data.[85,88,111,113,114,118,119]

2.6 n-Hydrocarbons present in seed fats and oils studied.

The hydrocarbons fractions were analysed using a 1.85 m x 2.5 m i.d. glass column packed with 3% OV17 stationary phase on Supelcoport (cf. sec. 3). The number of n-alkanes, unknowns and major components are given in Table 10 and the relative percentage compositions of the n-alkanes are given in Table 11. In each of the seven fats and oils studied there is a different major hydrocarbon. n-C_{21} is one of the major components in all seed oils except Borneo tallow and babassu fat while the n-C_{22} is one of the three major components in almond, sesame and hazelnut oils. Almond and sesame oils have the same three major components, n-C_{22}, n-C_{28} and n-C_{21}. Sesame oil has another component, n-C_{23}, in common with hazelnut and Borneo tallow. There is no regular pattern which would show any relation between the n-hydrocarbons and the fatty acids in the triacylglycerols or their degree of unsaturation. There are very few reports on the

Seed fats and oils	n-Hydrocarbons present	Number of unknown hydrocarbons	Major n-hydrocarbons
Peanut	C_{15}-C_{34}	33	C_{18},C_{21},C_{17}
Almond	C_{18}-C_{34}	12	C_{22},C_{28},C_{21}
Sesame	C_{18}-C_{34}	17	C_{23},C_{28},C_{22},C_{21}
Perilla	C_{15},C_{19}-C_{34}	22	C_{31},C_{21},C_{29}
Babassu	C_{15},C_{17},C_{18},C_{20}-C_{22} C_{24}-C_{32},C_{34}	30	C_{25},C_{30},C_{24}
Borneo tallow	C_{14},C_{15},C_{20},C_{22}-C_{28}, C_{30},C_{32}-C_{34}	21	C_{23},C_{33},C_{34}
Hazelnut	C_{15},C_{17},C_{19}-C_{34}	15	C_{21},C_{22},C_{23}

Table 10: analysis of the hydrocarbons of some seed fats and oils.

hydrocarbons of seed oils.[123,124,104,105] In one of the reports[123] where the hydrocarbon composition (C_{14}-C_{23}) is given for Spanish almond and hazelnut oils, one of the samples of almond oil has the same pattern of n-hydrocarbons as the almond oil sample studied here, but the percentage composition is different (Figs. 15,16). In the hazelnut oil case, comparison can be made only in terms of major components. The hazelnut oil studied here and one of the samples of the Spanish hazelnut oil have n-C_{19}, n-C_{21} and n-C_{22} as major components (Figs. 19,20). In the same report it is shown that the hydrocarbon composition varies considerably not only between the samples of the same oil in different locations but also between the samples from the same location (Figs. 16-18 and 20-22). The hydrocarbon composition

(cont. p.45)

Table 11: n-hydrocarbons content of some seed fats and oils (% w/w)

% Hydro-carbons / Seed fats and oils	C_{14}	C_{15}	C_{16}	C_{17}	C_{18}	C_{19}	C_{20}	C_{21}	C_{22}	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	C_{34}
Peanuts		1.9	8.9	10.5	13.5	5.8	3.1	11.1	7.9	5.9	6.0	4.3	3.8	2.9	5.5	2.6	1.2	2.0	1.2	0.5	1.4
Almond					0.4	1.0	1.3	13.8	28.5	7.7	5.3	7.0	4.9	6.3	15.5	2.4	1.6	1.0	0.9	1.0	1.4
Sesame					0.4	1.3	2.0	9.9	10.0	13.2	8.0	6.4	5.7	9.3	13.3	5.5	2.2	2.2	3.3	1.5	5.8
Perilla		0.8				0.3	0.3	15.4	9.9	3.8	6.8	4.6	2.9	6.5	4.9	11.9	2.9	22.1	4.0	0.8	2.1
Babassu		0.7		0.5	1.9		14.3	3.4	1.2		16.0	28.6	3.4	0.3	0.3	8.0	16.5	1.0	1.2		2.7
Borneo tallow	1.0	1.9					3.2		6.8	21.5	3.7	3.5	6.1	4.9	6.6		3.9		5.2	14.7	17.0
Hazelnut		1.4		2.1		7.2	1.8	29.4	12.4	7.6	5.1	3.1	5.7	3.5	2.8	3.2	2.7	2.6	2.7	0.8	5.9

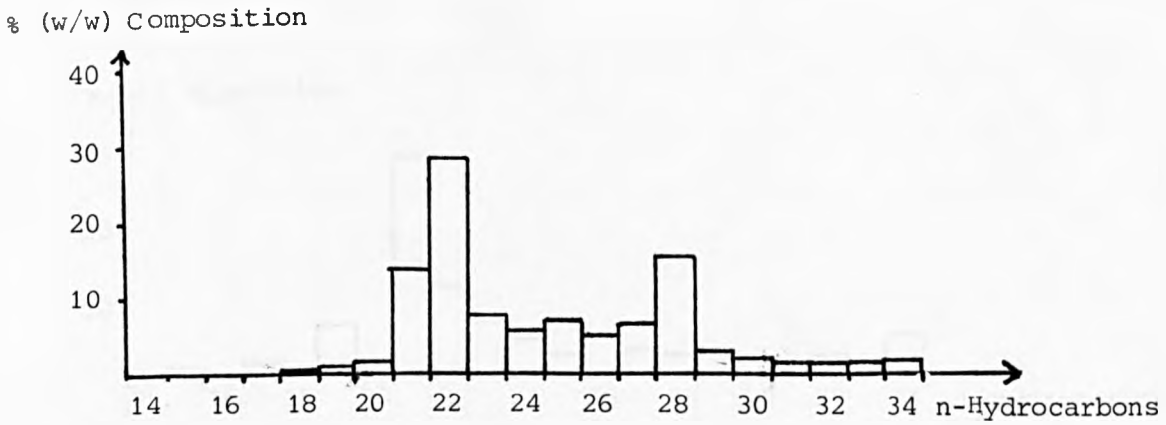


Fig. 15: % composition of n-hydrocarbons in the almond oil studied

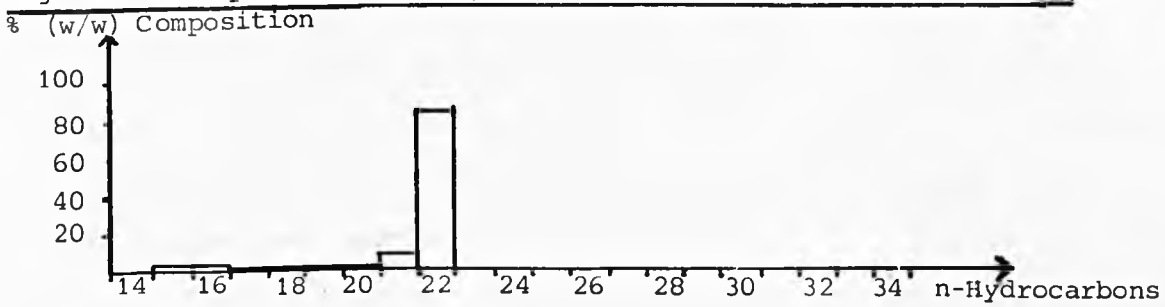


Fig. 16: % composition of n-hydrocarbons in Spanish almond oil (Gr.1)[123]

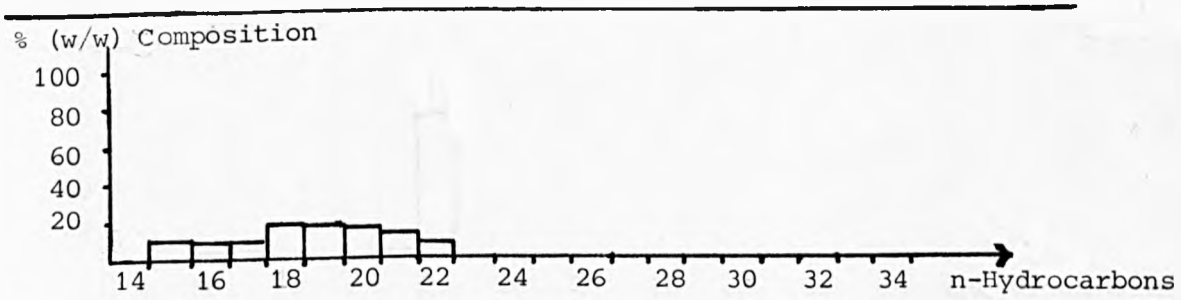


Fig. 17: % composition of n-hydrocarbons in Spanish almond oil (Gr.3)[123]

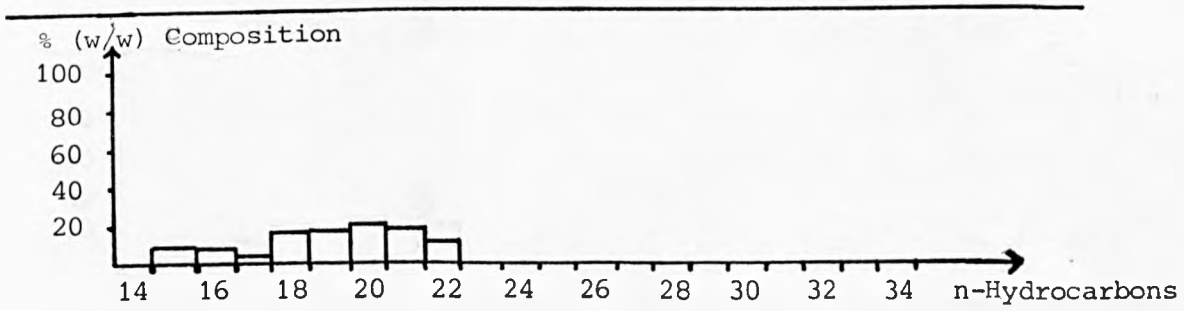


Fig. 18: % composition of n-hydrocarbons in Spanish almond oil (Gr.4)[123]

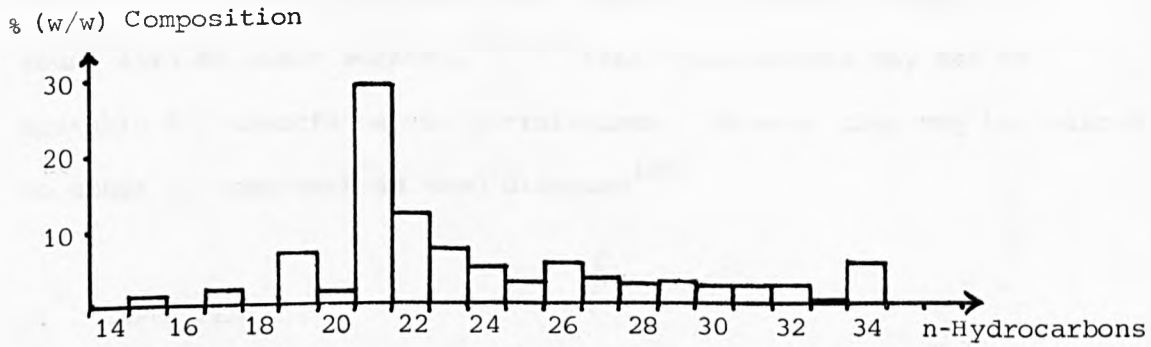


Fig. 19: % composition of n-hydrocarbons in the hazelnut oil studied

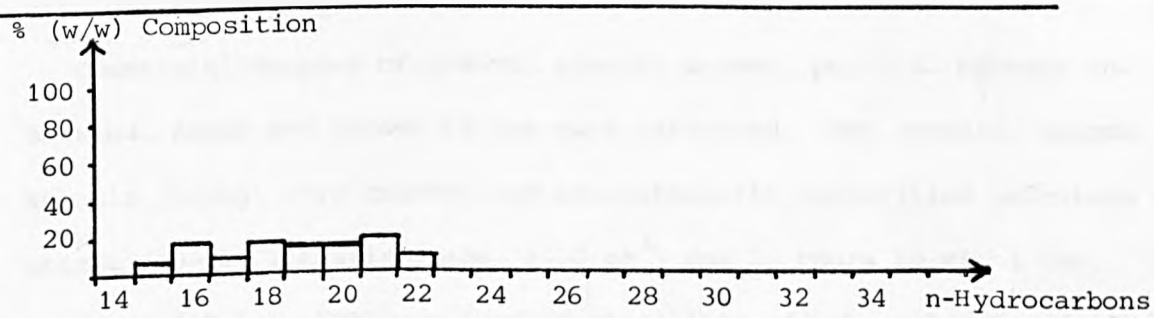


Fig. 20: % composition of n-hydrocarbons in Spanish hazelnut oil (Ta.6)[123]

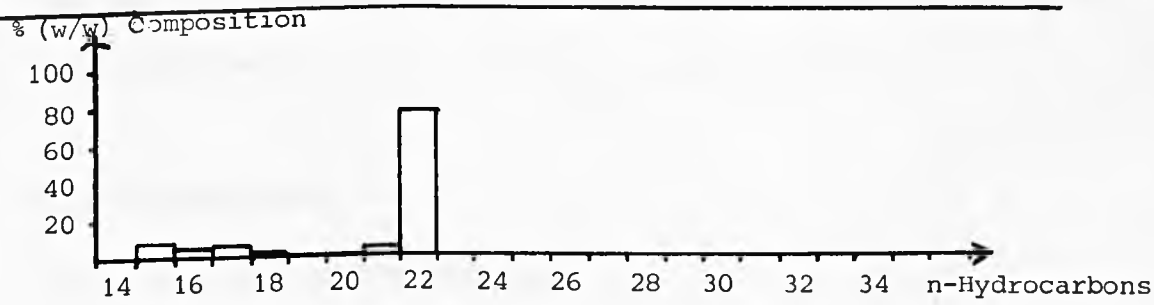


Fig. 21: % composition of n-hydrocarbons in Spanish hazelnut oil (Ta.4)[123]

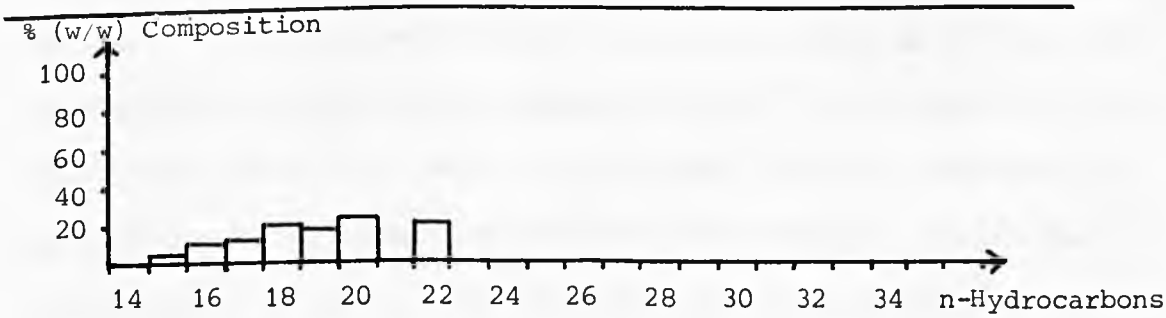


Fig. 22: % composition of n-hydrocarbons in Spanish hazelnut oil (Ta.3)[123]

of any particular oil also may vary considerably from one report to another.[105&108,106&124] Despite the variation in hydrocarbon composition and contents in the seed fats and oils studied, it appears, as found also by other workers,[105,126] that hydrocarbons may not be suitable for chemotaxonomic correlations. However they may be related to other factors such as seed diseases.[106]

3. EXPERIMENTAL.

3.1 Oil extraction.

Commercial samples of peanut, almond, sesame, perilla, babassu and hazelnut seeds and Borneo tallow were extracted. For example, almond kernels (84.0 g) were crushed and extracted with redistilled petroleum spirit (40-60°), Analar grade, 2000 cm^3) for 20 hours to yield the seed oil (49.4 g, 59% w/w) after distilling off the solvent. Seed fats and oils were kept under nitrogen in brown screw-cap containers in the refrigerator.

3.2 Chromatography.

3.2.1 Thin layer chromatography.

All solvents used were of Analar grade and all were redistilled. Plates of chloroform-washed Merck's silica gel (G60: 20 x 20 cm, 0.5 mm thick) were prepared by a standard method[128] and activated at 110°C for 1 hour before use. Bands or spots were located by spraying the plates with 0.2% ethanolic solution of 2,7'-dichlorofluroscein and viewing under UV light. For identification and quantification the bands were located using reference standards and the silica gel corresponding to each spot immediately scraped off the plates and

washed with hot chloroform (3 x 10 cm^3). A solvent mixture of methanol and chloroform (1:9, v/v) was used to wash the monoacylglycerols off the TLC plates. The solvent then was distilled off using a rotary thin film evaporator.

Peanut oil (0.025 g) was applied to the TLC plates which were eluted with a mixture of petroleum spirit, diethyl ether and acetic acid (80, 19.5 and 0.5 % by volume respectively). Two bands were extracted corresponding to R_f: 0.98 (hydrocarbons) and R_f: 0.51 (triacylglycerols). The triacylglycerols and hydrocarbons were kept under nitrogen in chloroform and in screw-top brown containers for further analysis. The lipolysis mixture was applied to the TLC plates which were then eluted with a chloroform:methanol:acetic acid solvent (94.5:5.0:0.5, v/v) and the bands corresponding to R_f: 0.30 were extracted to yield the 2-monoacylglycerols. The 2-monoacyl-glycerols were interesterified immediately with sodium methoxide in dry methanol to give the corresponding methyl esters.

3.2.2 High Performance Liquid Chromatography

A SP 8700 solvent delivery system, a SP 8750 organiser and a Foxboro-Wilks Miran infrared detector were used. The ir detector was operated at 5.75 μm (response 1, attenuation 0.25, slit width 1.0 mm and cell length of 1.5 mm). The stainless steel column (25 cm x 4.6 mm i.d.) was packed with 5 μm Spherisorb S5W. The solvents used were acetonitrile (HPLC grade), chloroform (Analar grade) and n-heptane (Analar grade and GPR redistilled).

The triacylglycerols were separated from the seed fats and oils

using the solvent profile 1. Each fat or oil (0.035 g) was injected as a chloroform-n-heptane solution and the triacylglycerol fraction was collected and the solvent removed on the rotary thin-film evaporator. The collected samples were redissolved in chloroform and kept under nitrogen in brown containers with screw tops.

Time/min	n-Heptane (%)	Chloroform (%)
0.0	100	00
5.0	100	00
20.0	70	30
30.0	70	30
45.0	100	00

Solvent profile 1

Time/min	n-Heptane (%)	Chloroform (%)	Acetonitrile (%)
0.0	70	30	00
14.0	70	30	00
14.1	00	90	10
32.0	00	90	10
32.1	70	30	00

Solvent profile 2

For the separation of the monoacylglycerols, the lipolysis mixture was dissolved in 10 µl chloroform and injected. The vial then was washed with another 10 µl of chloroform and this also injected. The mono-

acylglycerols were separated from the other constituents using the solvent profile 2, collected and the solvent mixture removed using the rotary thin-film evaporator. The 2-monoacylglycerols were inter-esterified immediately using sodium methoxide in dry methanol to give the corresponding methyl esters.

3.2.3 Gas-liquid chromatography.

The gas chromatograph used was a Pye 104 with dual flame ionization detectors. The triacylglycerols were analysed using a glass column (0.45 m x 2.5 mm i.d.) packed with 3% OV17 on Supelcoport (100-120) mesh) under the following conditions: detector temperature, 370°C; initial column temperature, 240°C (increased by 6°C min^{-1} to 340°C); the carrier gas was nitrogen with a flow rate of 60 ml min^{-1}. The triacylglycerols were identified using reference standards. The amount of each triacylglycerol was corrected using calibration factors.[12] The calibration factors varied frequently and they had to be determined for every new analysis. Perilla oil which is very rich in polyunsaturated fatty acids was hydrogenated using a standard procedure[12] before the analysis of its triacylglycerols by GLC. For the analysis of the methyl esters two columns were used: the first glass column (1.85 m x 2.5 mm) was packed with 3% OV17 on Supelcoport (100-120 mesh). The initial column temperature was 100°C and it was increased at a rate of 6°C min^{-1} to 340°C. The second glass column (3.00 m x 2.5 mm i.d.) was packed with 10% DEGS on Supelcoport (100-120 mesh). The initial column temperature was 100°C and it was increased to 190°C at a rate of 3°C min^{-1}. For both columns used the detector operating temperature was 370°C and the carrier gas (nitrogen)

flow rate was 40 ml min^{-1}. The methyl esters were identified using reference standards. All peak areas were measured by triangulation.

3.3 Preparation of methyl esters.

Sodium (0.25 g) was added to magnesium-dried methanol[129] (5 cm^3) and the solution was added to the acylglycerols sample. The mixture was refluxed for 5 min. The transesterified sample was left to cool and 1.0 mol dm^{-3} sulphuric acid were added (10 cm^3). The esters were extracted with chloroform (3 x 10 cm^3). The combined chloroform extracts were washed with water until neutral, dried over anhydrous magnesium sulphate and the solvent distilled off using the rotary thin-film evaporator. The samples were transferred to containers with screw-tops and each made up to a volume depending on the quantities obtained, by dissolving each in chloroform, followed by analysis by GLC.

3.4 Lipolysis.

Lipase hydrolysis was carried out on samples (Ca. 0.005 g) according to the procedure of Luddy,[20] but before using the drying agent each sample was washed with saturated sodium carbonate (2 x 10 cm^3) then dried over anhydrous magnesium sulphate. The hydrolysis products thus obtained were isolated individually either by TLC (3.2.1) or HPLC (3.2.2), immediately converted to methyl esters (3.3) and analysed by GLC (3.2.3).

4. FINDINGS AND CONCLUSIONS.

A new application is reported of the use of the infrared detector in HPLC for the detection and preparative and quantitative separation of triacylglycerols of seed fats and oils, acylglycerols from lipolysis mixtures and synthetic acylglycerols. Fats and oils and lipolysis mixtures with short and polyunsaturated fatty acids were chromatographed. The system is very simple, very stable and highly reproducible. The results from HPLC-ir were found to be comparable to those from TLC, although the latter is time-consuming and involves the possibility of air oxidation if precautions are not taken. The fatty acids from the triacylglycerols of seed fats and oils were analysed using gas-liquid chromatography and it was found that unsaturated fatty acids occur mainly in the sn-2 position.and that there is generally a preference for linoleic acid over both oleic and linolenic acids, and further there is a preference for oleic acid over linolenic acid. The triacylglycerols of perilla oil and babassu fat are given for the first time with the fatty acid composition at the sn-2 position. Also given for the first time are the n-hydrocarbons for all seed fats and oils except for almond oil and the percentage composition of the n-hydrocarbons of peanut, sesame, perilla, Borneo tallow and babassu oils and fats.

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PART B

SYNTHESIS OF SYMMETRICAL DIACID TRIACYLGLYCEROLS

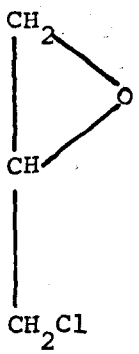
1. INTRODUCTION TO THE SYNTHESIS OF TRIACYLGLYCEROLS.

Synthesis of triacylglycerols is important in metabolic studies, analysis of natural products and in industrial processes. Firstly in metabolic studies, synthetic triacylglycerols are needed in structure determination for the understanding of the distribution of fatty acids in animal and vegetable fat,[1] the synthesis of triacylglycerols by the liver and adipose tissue[2,3] and the deposition and modification of fatty acids in the adipose tissue.[4,5] Secondly in the analysis of natural products, authentic triacylglycerols are needed for the development of modern analytical techniques such as enzymatic analysis, high temperature and capillary gas liquid chromatography, reverse phase high performance liquid chromatography, mass spectrometry and nuclear magnetic resonance spectroscopy. Authentic acylglycerols and mixed triacylglycerols in particular are either expensive or not available. Thirdly and finally, triacylglycerols are used in industrial processes. Synthetic triacylglycerols may be used in foodstuffs and cosmetics if the final products are free from toxic substances. The only synthetic acylglycerol permitted in Britain is glycerol monostearate which is used as an emulsifier. There was also a report[6] of the synthesis of cocoa butter - like fat from glycerol-1-palmitate-3-stearate and oleoylanhydride. Triacylglycerols with short chain fatty acids[7] are known to be assimilated easily by infants and old people. None of the methods available today is satisfactory for the preparation of all types of triacylglycerols. The methods of preparation have been reviewed by several workers.[8-11] A sensible choice of a synthetic route is especially important in the preparation of partial acylglycerols to be used as intermediates in the synthesis of di- and triacylglycerols.

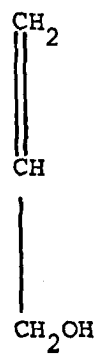
The introduction of saturated fatty acids, if there are any, as early as possible, yields products of high purity through recrystallization. The importance of using pure starting materials in the synthesis of acylglycerols is necessary in achieving high yields of high purity products. The synthesis of mixed acid triacylglycerols of specific structure usually requires the synthesis of monoacylglycerols or diacylglycerols. The methods for preparing triacylglycerols may be divided as follows. (1.1) Acyglycerols from miscellaneous compounds. (1.2) Acylglycerols from direct acylation of glycerols. (1.3) Acyglycerols from protected glycerol derivatives. (1.4) Acylglycerols from suitable glycerol derivatives by nucleophilic substitution reactions.

1.1 Acylglycerols from miscellaneous compounds.

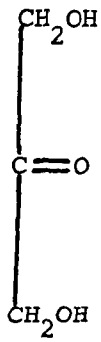
The following substrates have been used:



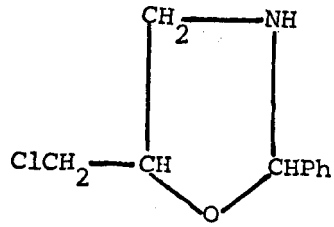
1.1.1: 1-Chloro-2,3-epoxypropane



1.1.2: Allyl alcohol



1.1.3:1,3-Dihydroxyacetone



1.1.4:2-Phenyl-5-chloromethyl-oxazolidene

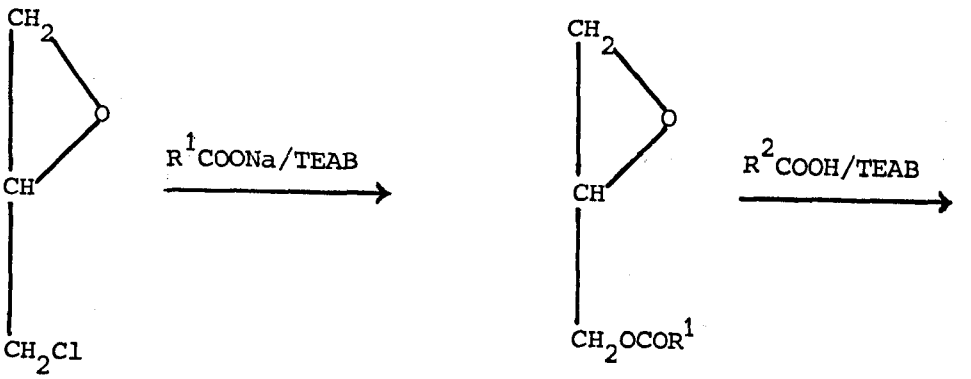
$HOCH_2CO_2H$

1.1.5:Glycolic acid

Some of the substrates were used because of their unique suitability in forming specific acylglycerols.

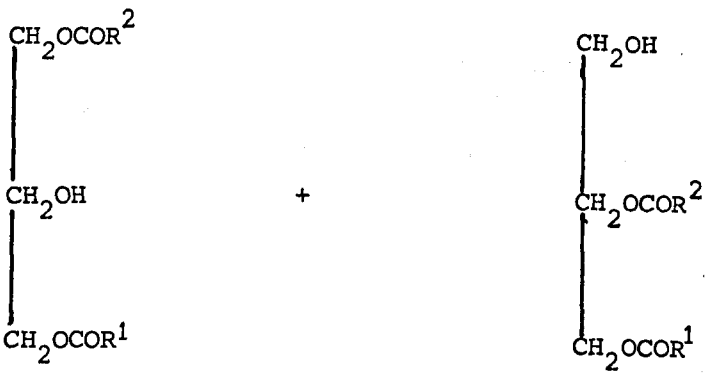
1.1.1 1-Chloro-2,3-epoxypropane (epichlorohydrin).

The method was successfully developed[12] for the preparation of 1,3- and 1,2-diacylglycerols of saturated fatty acids from the reaction of the 2,3-propyloxy ester derivative with fatty acids in the presence of tetraethyl ammonium bromide (TEAB) acting as a catalyst (Scheme: 1). The diacylglycerols were isomerised to the 1,3-isomers by heating the mixture at 5 to 10° below the melting point of the diacylglycerol mixture. The method is unsuitable for unsaturated and short chain fatty acids. Triacylglycerols can be prepared by acylation of diacylglycerols using an acid chloride.



1-Chloro-2-3-epoxy-propane

1-Acyl-2,3-epoxy-propane



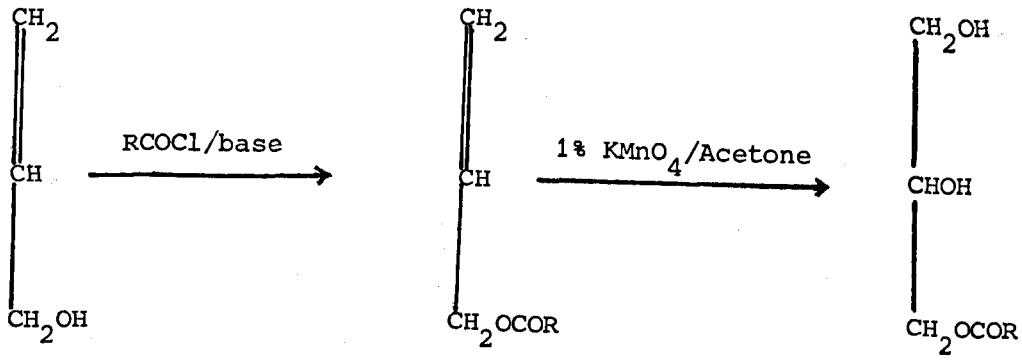
1,3-Diacid diacylglycerol

1,2-Diacid diacylglycerol

Scheme: 1

1.1.2 Allyl alcohol.

The allyl alcohol method was used mainly for the preparation of monoacylglycerols[13] (Scheme: 2).

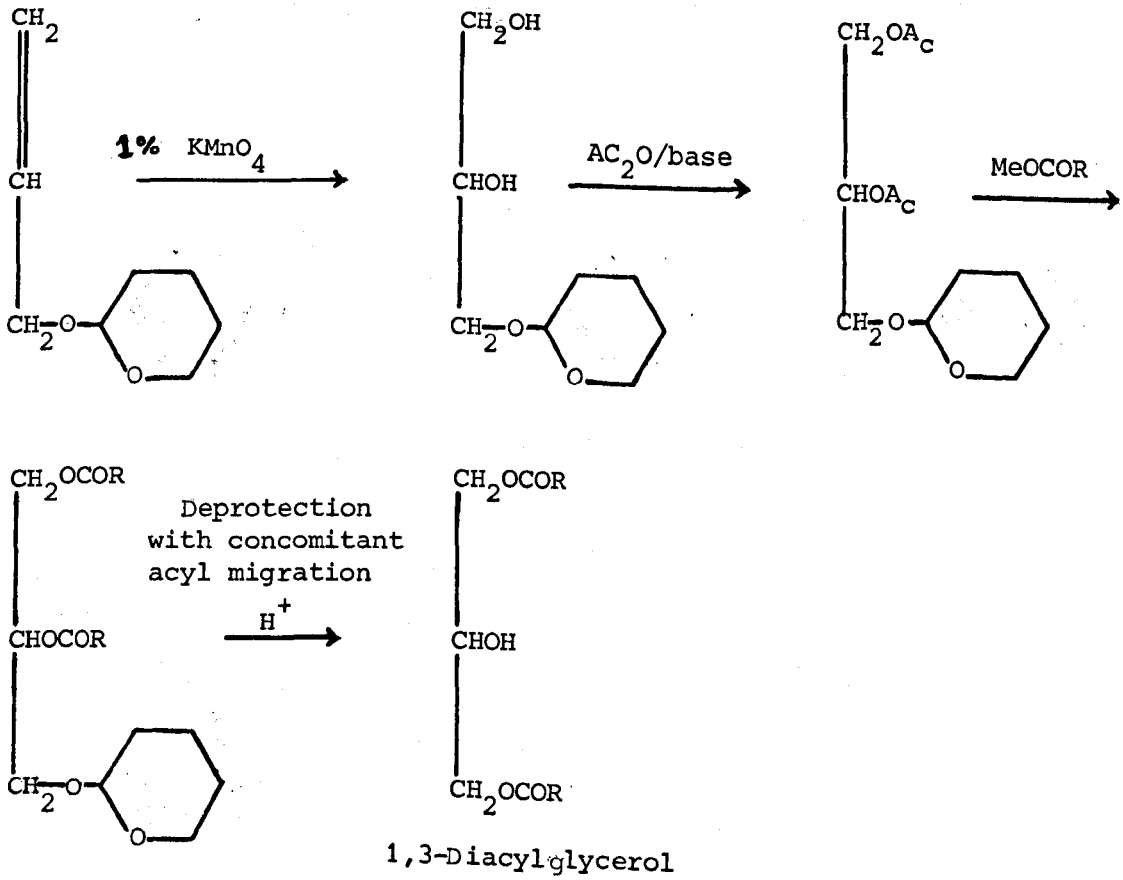


Allyl alcohol

Monoacylglycerol

Scheme: 2

The method was applied later to the preparation of 1,3-diacylglycerols[14] from the hydroxylated tetrahydropyranyl ether derivative (Scheme: 3).



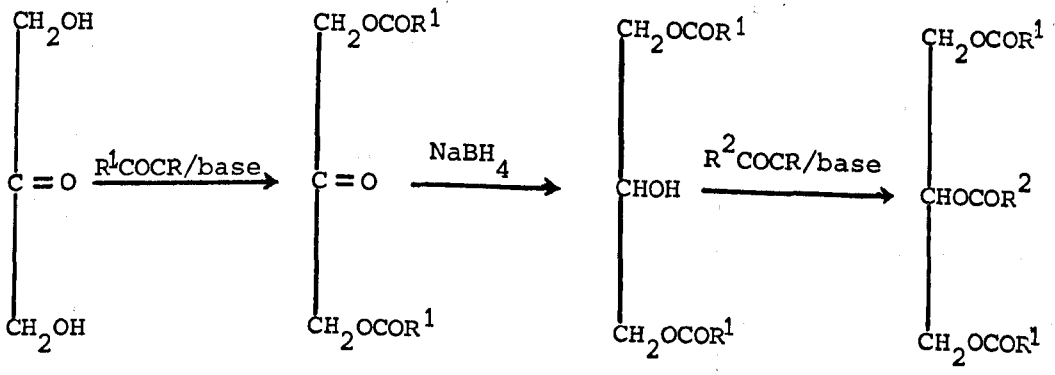
1,3-Diacylglycerol

derivative

Scheme: 3

1.1.3 1,3-Dihydroxyacetone.

1,3-Dihydroxyacetone is a good substrate for the preparation of pure symmetrical diacylglycerols and diacid triacylglycerols.[14,15] Since sodium borohydride was introduced in the reduction step,[16] the method has been extended to include unsaturated fatty acids (Scheme: 4). The method is not suitable for the preparation of 1,3-diacylglycerol with short chain fatty acids due to the isomerization of the 1,3-diacylglycerol to the 1,2-diacylglycerol.[16]

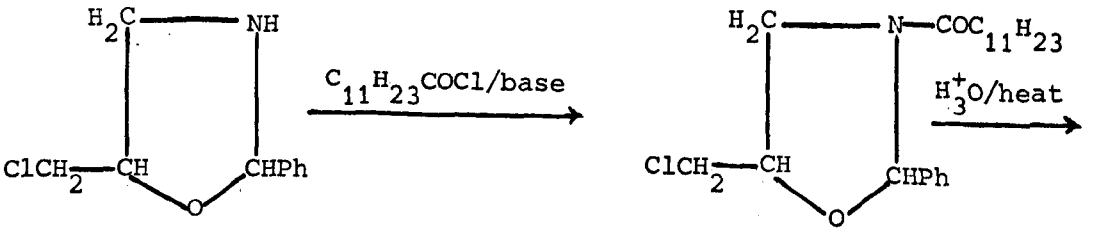


1,3-Dihydroxyacetone — 1,3-Diacylglyerol — Symmetrical diacid triacylglycerol

Scheme: 4

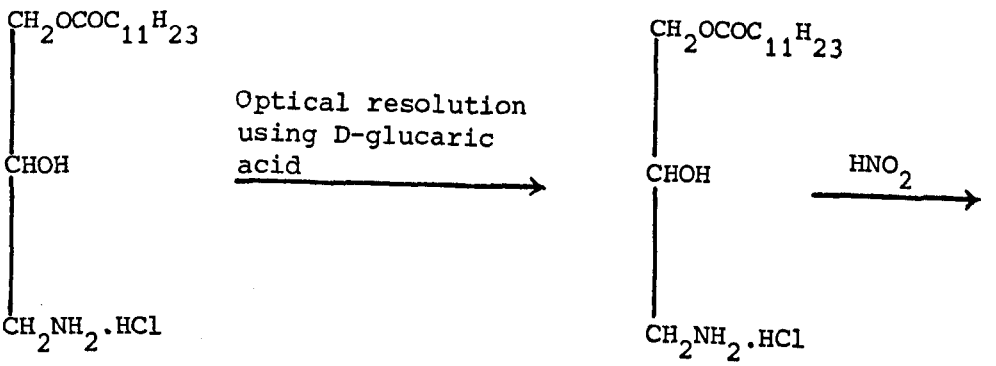
1.1.4 2-Phenyl-5-chloromethyloxazolidene.

Before the mannitol route (cf. 1.3) was discovered few attempts were made to prepare optically active triacylglycerols,[17,18] e.g. an attempt was made using 2-phenyl-5-chloromethyloxazolidene, but this resulted in the isolation of glycerol-1-monolaurate (Scheme: 5).



2-Phenyl-5-chloro-

methyloxazolidene

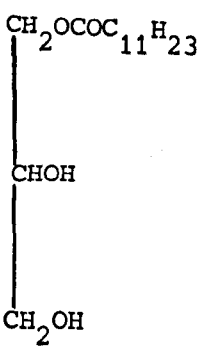


(±)-1-Lauroyloxy-2-hydroxy-

3-aminopropane

Dextrorotatory

enantiomer



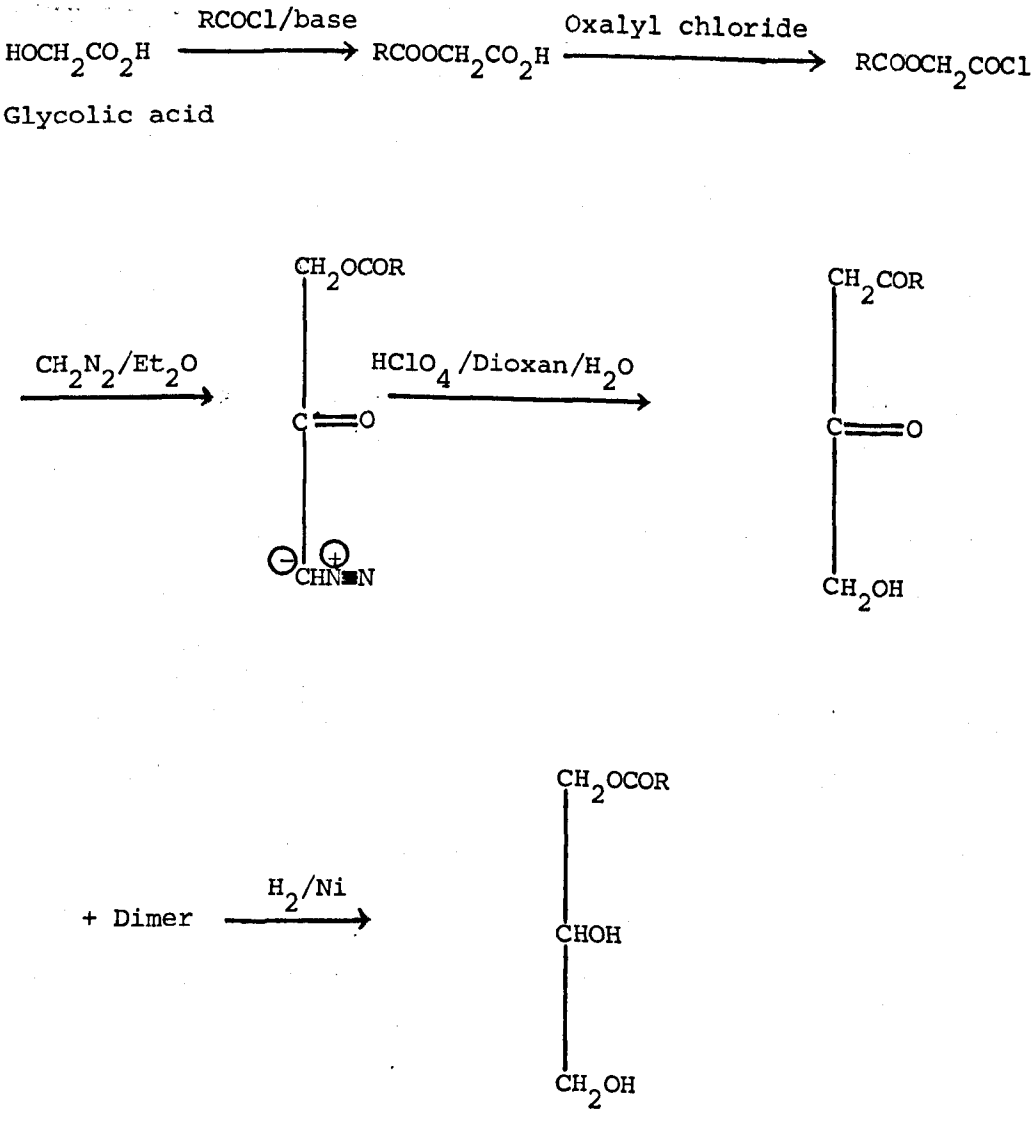
Glycerol mono-

laurate

Scheme: 5

1.1.5 Glycolic acid.

The glycolic acid route[19] can be used for the preparation of mono- and diacid diacylglycerols of saturated fatty acids (Scheme: 6). It is limited compared to the 1,3-dihydroxyacetone route, but it has one advantage, it can be used to incorporate an isotopic label which may be useful in metabolic and mechanistic studies.



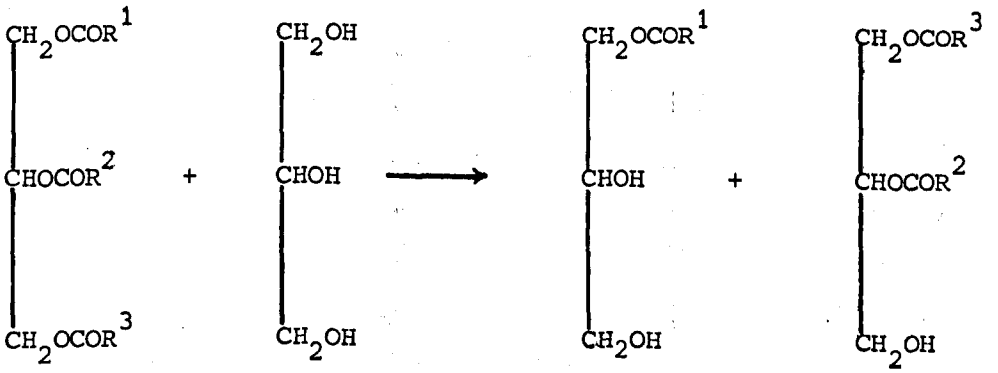
Scheme: 6

1.2 Acylglycerols from direct acylation of glycerol.

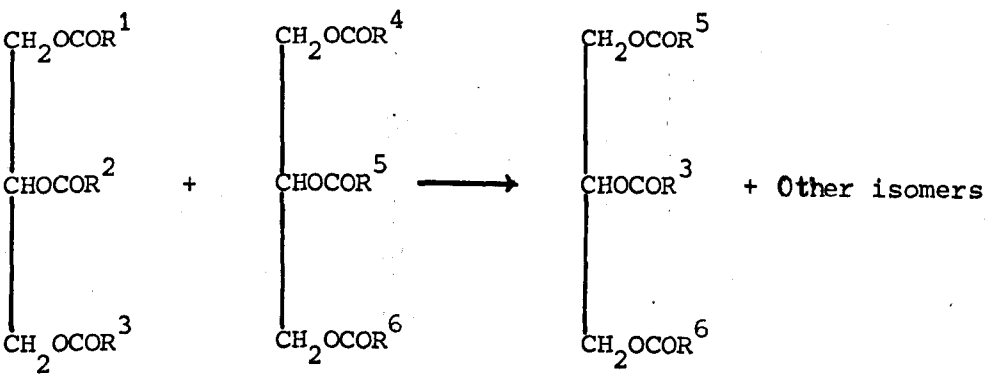
This goes back to Berthelot[20] who prepared monoacid acylglycerols by heating glycerol with fatty acids in a sealed tube at 200°C. Other workers[21] developed the method by mixing equivalent amounts of glycerol and fatty acids and heated the mixture at 200°C in an atmosphere of carbon dioxide to obtain almost theoretical yield of triacylglycerols. With the use of the appropriate catalyst, free fatty acids, methyl esters and acid chlorides can be used to acylate glycerol or partial acylglycerols. Phosphoric acid and trisodium phosphate have been used as catalysts[22,23] in the preparation of mono- and diacylglycerols by the reaction of glycerol with a fatty acid. The esterification process is also accelerated by using catalysts such as naphthalene-sulphuric acid. Inorganic catalysts include[24] $AlCl_3 6H_2O$, $CdCl_2.2H_2O$, FeO, MgO, NaOH, PbO, $SnCl_2.2H_2O$, $ZnCl_2$ and ZnO.

In all these reactions, acylation is random and, therefore, the acylglycerols obtained are not specific but rather a mixture of different isomers. This process of randomization which involves acyl migration or ester interchange is used to a great extent in industry, either for the preparation of partially acylated acylglycerols, from the reaction of glycerol with a triacylglycerol (alcolholysis,[28] Scheme: 7) or by the reaction of two different triacylglycerols (ester interchange, Scheme: 8). The process of alcoholysis is used for the production of emulsifiers while the process of ester interchange is used for the production of margarines,[25] salad oils[26] and confectionery fat.[27] The processes of interesterification are enhanced by the use of catalysts which are usually metals (Li, Pb, Fe, Sn), their oxides and salts, alkali-metals (Na, K and their alloys), their alcoholates, hydroxides and hydrides, amides and mineral acids. Although covered by many

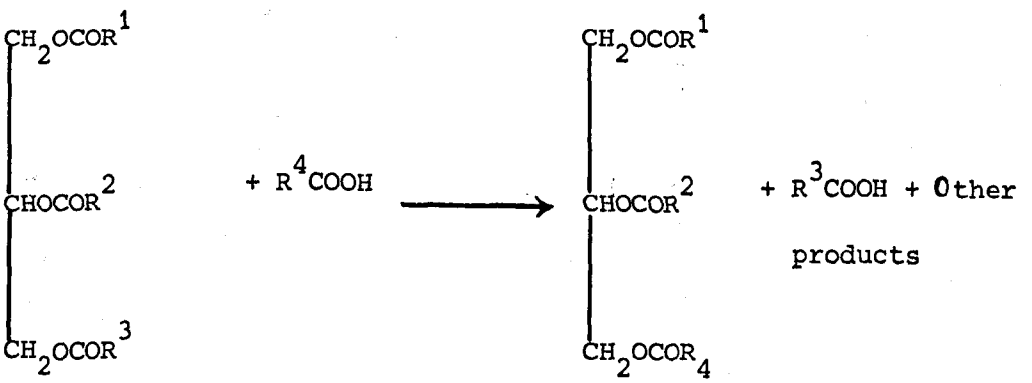
patents, the reaction of acylglycerols with fatty acids (Acidolysis,[29] Scheme: 9) is the least used process in the interesterification procedures.



Scheme: 7. Alcoholysis[28]



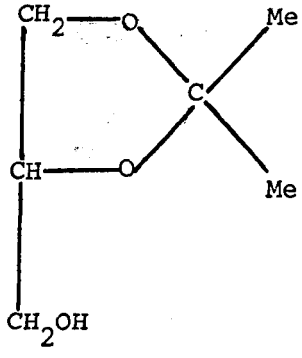
Scheme: 8. Ester-interchange[25-27]



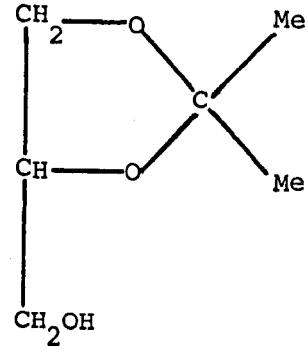
Scheme: 9. Acidolysis[29]

1.3 Acylglycerols from protected glycerol derivatives.

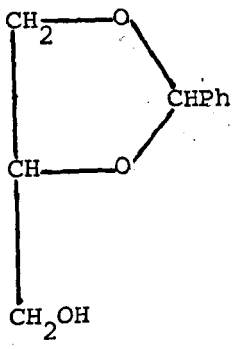
The esterification of a specific hydroxyl group or groups of the glycerol moiety, requires that the remaining hydroxyl group(s) be blocked prior to esterification. Several blocking groups have been used. Three protecting groups are being used extensively. If a particularly acyl group is to be introduced into the primary position, 1,2-isopropylidene and also 1,2-benzylidene derivatives are used. If the secondary hydroxyl group of the glycerol molecule is to be acylated, then 1,3-benzylidene glycerol is used. In both cases, the trityl (or triphenylmethyl) group has been used extensively for protecting one or the two primary hydroxyl groups. Isopropylidene and benzyl and trityl ethers have been used also in the preparation of optically active acylglycerols.[40] The procedures consist of esterifying the protected glycerol moiety with an acid chloride, removing the blocking group and finally acylating the remaining hydroxyl group(s) in order to get the required triacylglycerol. For this purpose the following starting materials have been used.



1.3.1 1,2-Isopropylidene-rac-glycerol



1.3.2 sn-Glycerol-1,2 or 2,3-isopropylidene



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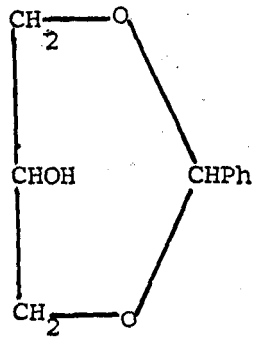
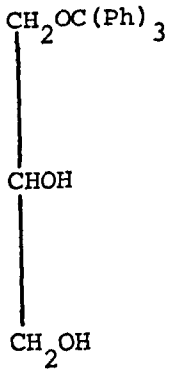
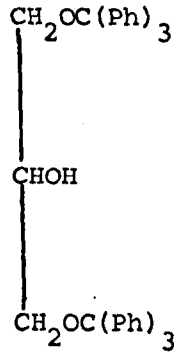


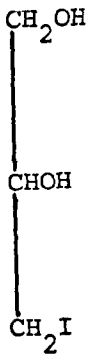
1.3.3 1,2-Benzylidene glycerol and 1,3-Benzylidene glycerol



and



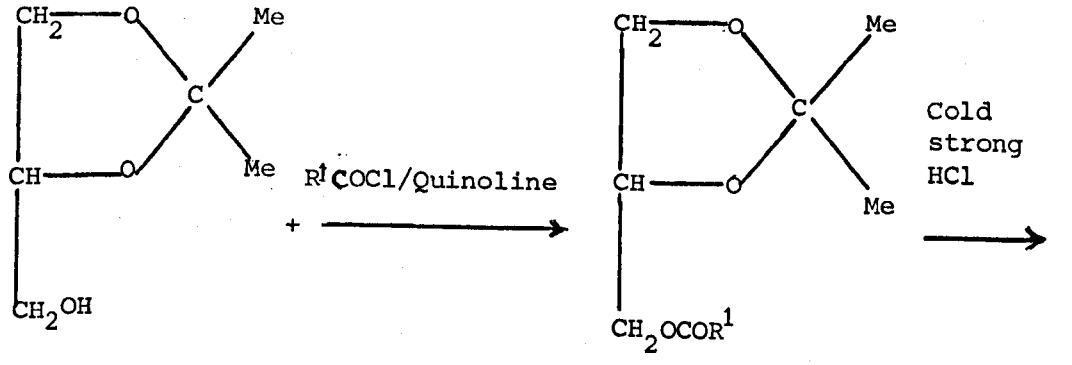
1.3.4 1-Tritylglycerol and 1,3-Bis-tritylglycerol

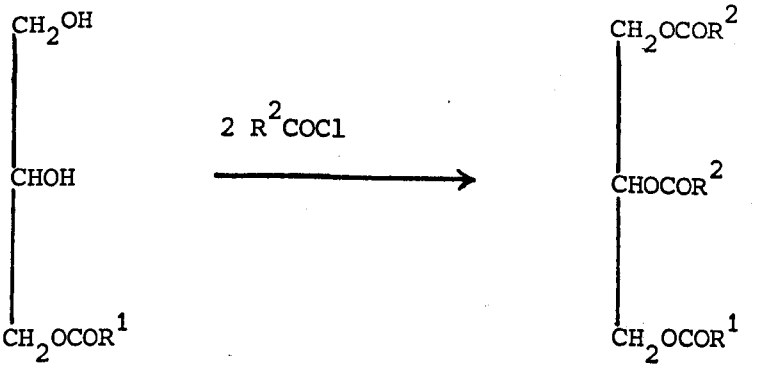


1.3.5 1-Iodoglycerol (Alival)

1.3.1 1,2-Isoprolylidene-rac-glycerol.

1,2-Isopropylidene-rac-glycerol is available commercially. It may be prepared by reacting glycerol and acetone in the presence of 1% HCl as a catalyst.[30] This is a good route[31] for the preparation of specific monoacylglycerols and unsymmetrical diacid triacylglycerols (Scheme: 10).



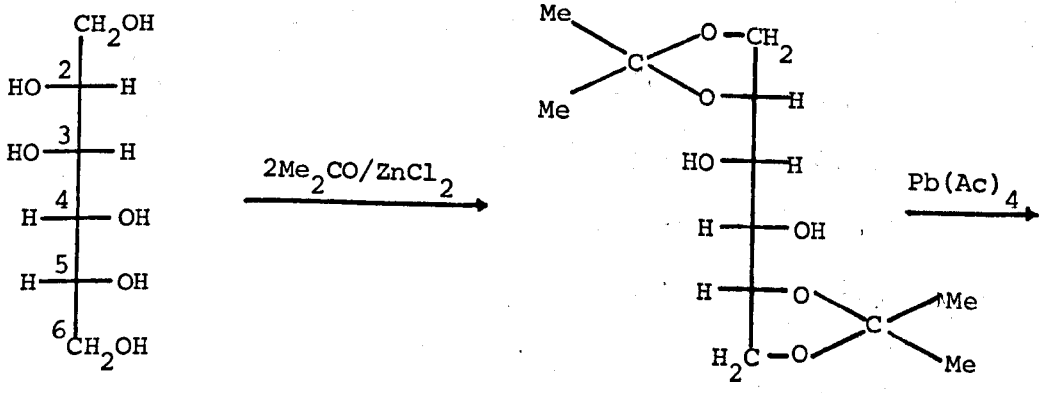


Scheme: 10

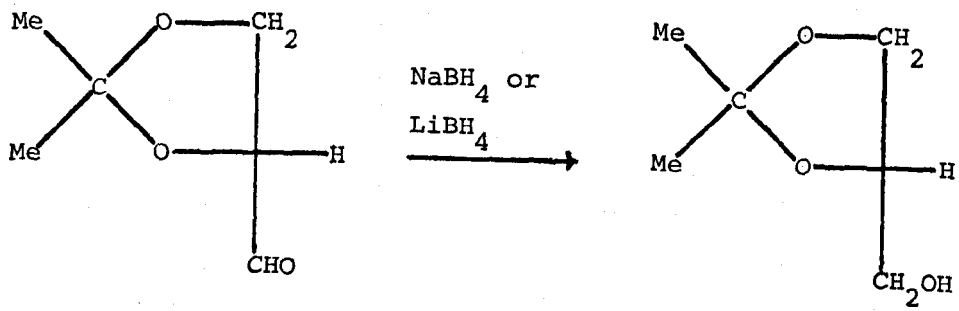
Since an acid catalyst was used in the preparation of the 1,2-isopropylidene-rac-glycerol, free fatty acids were used in the esterification to make it a one pot reaction.[30] Saturated and unsaturated fatty acids were used also in the preparation of symmetrical diacid triacylglycerols.[32] The use of methyl esters of fatty acids and basic catalysts such as sodium methoxide lead to the monoacylglycerols.[33] The best results for cleaving the protecting group were obtained using boric acid in trimethyl borate, isomerization through acyl migration was kept to a minimum.[34]

1.3.2 sn-Glycerol 1,2 and 2,3-isopropylidene derivatives.

The preparation of optically active acylglycerols was made possible from the synthesis of sn-1,2- and 2,3-isopropylidene glycerol. The two substrates were synthesised from D- and L-mannitols[35,36] by preserving the optical activity at carbons 2 and 5 of the substrates (Scheme: 11). The route was pioneered by Baer and Fischer.



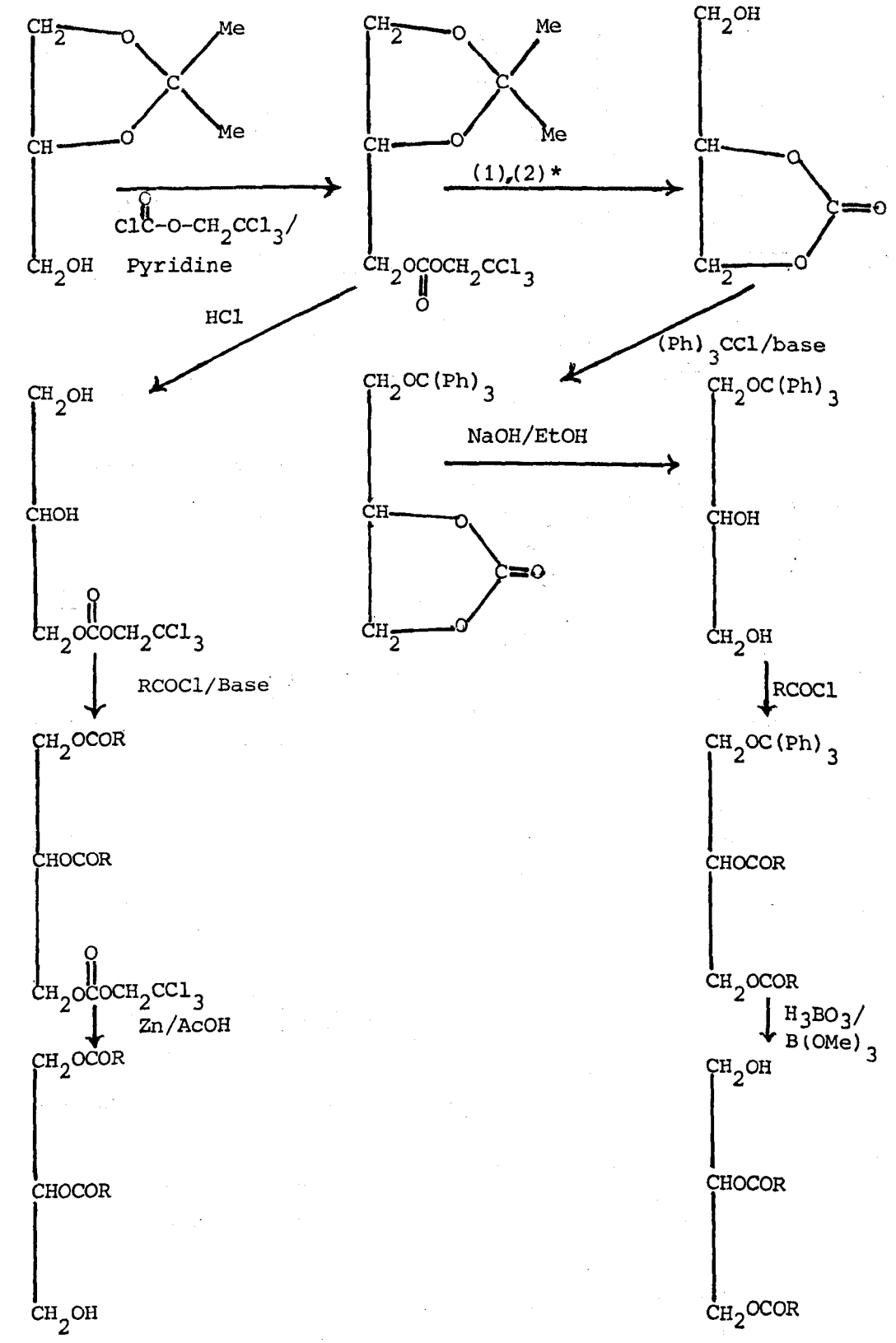
D-mannitol



sn(+)-1,2-Isopropylidene glycerol

Scheme: 11

However, L-mannitol can only be prepared by a tedious procedure starting from L-arabinose.[36] Other workers improved Baer and Fischer's method in order to prepare optically active mono- and diacylglycerol enantiomers from sn(+)1,2-isopropylidene glycerol,[37,38] obtained from D-mannitol (Scheme: 12). Acyl migration occurs only to a little extent or not at all and the products are obtained in pure state by crystallization. The enantiomeric diacid triacylglycerols can be obtained from the respective optically active diacylglycerols. Natural triacylglycerols are cryptoactive[30] because their optical activity is not apparent. It is too small to allow measurements and this is mainly due to the little difference in terms of carbon numbers between the fatty acids constituting the individual triacylglycerols. Enantiomeric diacid diacylglycerols have also been prepared from D-mannitol using benzyl and trityl protecting groups.[40] Chiral acyl-glycerols were also prepared from D- and L-serine.[41]

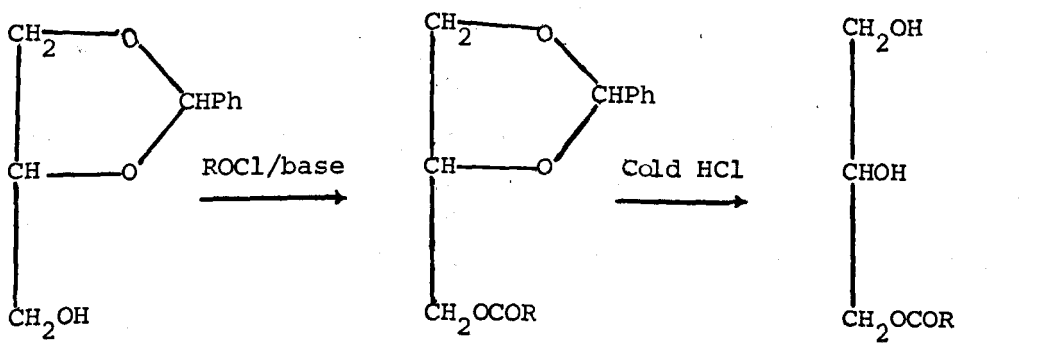


Scheme: 12

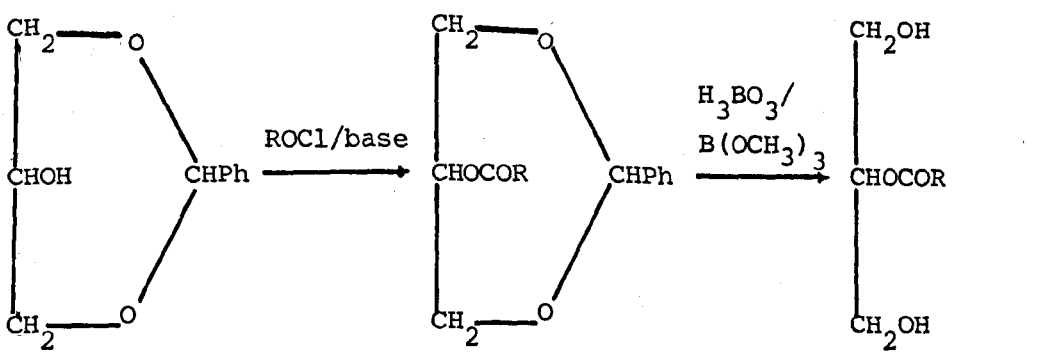
*(1) $H_3BO_3/B(OCH_3)_3$; (2) Separation on florisil (10% H_3BO_3)

1.3.3 Benzylidene derivatives of glycerol.

The two benzylidene isomers can be obtained from the reaction of anhydrous glycerol with benzaldehyde and a catalytic amount of toluene-p-sulphonic acid.[42] The two isomers are separated by fractional crystallization. After acylation, the blocking groups are removed[43] by treating the substrates with aqueous acetic or hydrochloric acids (Scheme: 13), but acyl migration is known to take place under these conditions. Hydrogenolysis can be used to cleave the



1-Monoacylglycerol



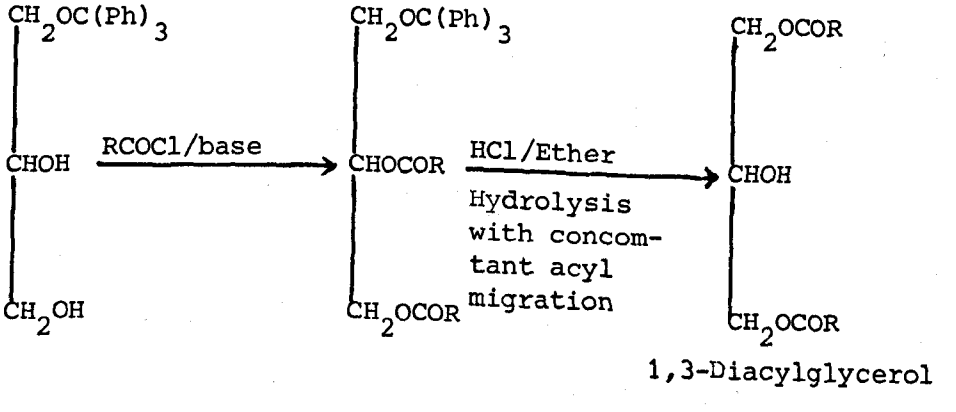
2-Monoacylglycerol

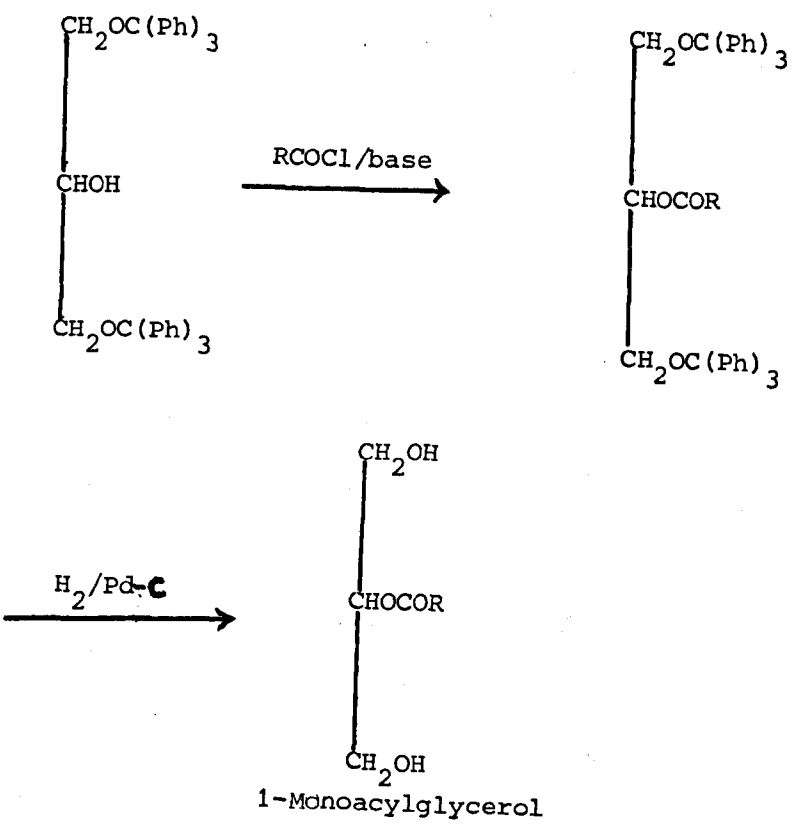
Scheme: 13

benzylidene without isomerization taking place. However it cannot be used if unprotected unsaturated fatty acids are present in the monoacylglycerols. The process of isomerization is reduced to a minimum if the benzylidene groups are cleaved with boric acid in trimethyl- or triethylborate.[26] The benzylidene route can be used for the preparation of symmetrical and unsymmetrical diacid triacylglycerols.

1.3.4 The trityl derivatives of glycerol.

The trityl derivatives are prepared from the reaction of glycerol and triphenyl methyl chloride with pyridine acting as a catalyst.[41,45] The triphenyl methyl chloride reacts preferentially with the primary hydroxyl groups of the glycerol molecule first. After acylation the trityl derivatives are cleaved by acid hydrolysis which results in concomitant acyl migration[46] or by hydrogenolysis where acyl migration is avoided but is restricted to acylglycerols of saturated fatty acids as mentioned above. The trityl route is suitable for the preparation of 1- and 2-monoacylglycerols, 1,2- and 1,3-diacylglycerols[47,48] and diacid triacylglycerols (Scheme: 14). The triacylglycerols are obtained by acylating the remaining hydroxyl groups with an acid chloride.

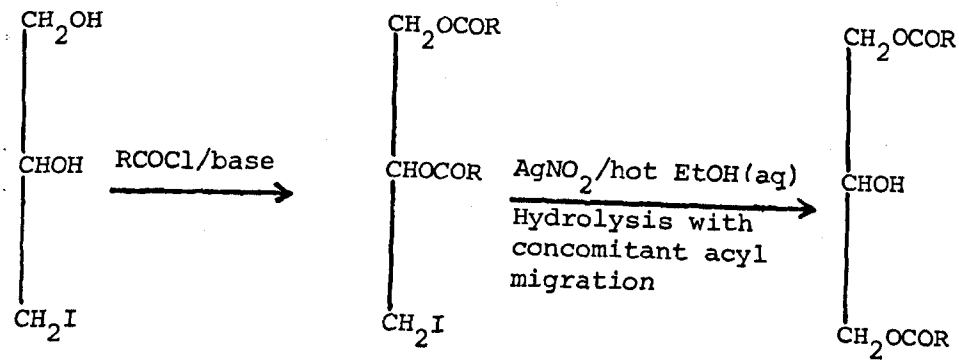




Scheme: 14

1.3.5 1-Iodoglycerols (Alival).

Although 1-Iodoglycerol is not strictly a protected glycerol molecule, the iodide is serving the same purpose as the groups described above and the hydroxyl group can be easily regenerated. There are several ways of preparing 1-Iodoglycerol,[49] one of them is by reacting 1,2-benzylidene glycerol with triphenylphosphite methiodide. The benzylidene is cleaved by acid hydrolysis. It is suitable for the preparation of symmetrical diacylglycerols and diacid triacylglycerols[50,51] (Scheme: 15).

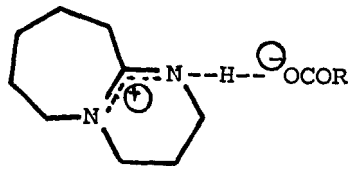


Scheme: 15

1.4 Acylglycerols from suitable glycerol derivatives by nucleophilic substitution reactions.

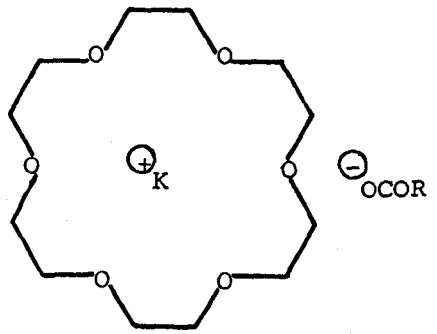
Nucleophilic substitution reactions cannot be carried out on glycerol because of the difficulty in displacing a hydroxyl group by a carboxylate anion. Instead the hydroxyl groups are replaced by easy leaving groups such as halides or sulphonates. The glycerol molecule is then activated for nucleophilic substitution reactions. These halides and sulphonates act as protecting groups similar to the ones described in sec. 1.3 above and allow acylation of any free hydroxyl present. However they are much more resistant to the conditions for cleaving them. Nucleophilic substitution reactions usually take place under mild conditions using fatty acid salts or free fatty acids as the nucleophilic source. As it is important to choose the right leaving group it is also important to choose the right fatty acid salt and the right solvent. There are a number of fatty acid salts which are being used. Despite their popularity the Na and K salts give very poor yield and high percentage of by-products.[52] Silver[53,54] and mercuric salts are

much more efficient in their reactivity with the alkyl halides. Reactions involving these salts result in the formation of high substitution products and low amounts of dehydrohalogenation products. Esterification of alkyl halides using mercuric carboxylates in the presence of catalytic amounts of triacycloxyboranes[55] gave yields higher than those obtained with silver salts. However due to their high costs, the mercuric and silver salts are restricted to laboratory preparations. The use of caesium salts has been reported also. Caesium carboxylates[56] derived from protected amino acids and peptides were reacted with different alkyl halides in dimethyl formamide (DMF). The yields of esters were higher than those obtained from the corresponding sodium and potassium salts. Alkyl halides have been substituted also by free fatty acids in presence of a copper(I) oxide-base system.[57] Presumably the substitution reaction occurs via a cuprous carboxylate intermediate which reacts with the alkyl halide.. The yields are superior to those of the alkali metal salts. The reactions of the free fatty acids can be improved if the bond between the proton of the hydroxyl group and the carboxylate anion is weakened. 1,8-diazabicyclo (5.4.0)-undec-7-ene (DBU)[58] was used to complex with free fatty acids in their reactions with alkyl halides in benzene resulting in good yields of the corresponding esters. The reaction takes place in non-polar solvents, such as benzene, with no prior preparation of the carboxylate anions and gives small amounts of dehydrohalogenation products. DBU complexes with a carboxylic acid by means of the hydrogen bonding as indicated below. The hydrogen bonding and the basicity character of the complexation control the reactivity of the carboxylate anion. The more basic is the complexing agent, the more reactive is the carboxylate anion. DBU was



DBU-carboxylic acid complex

found much more basic than triethylamine. In their turn alkali metal salts can be made more reactive if the association between the metal cation and the carboxylate anion is loosened. The cyclic polyethers, known as crown ethers, have very important complexing properties.[59,60] Their utility comes from their complexation with alkali metals in particular, in solution and in the crystalline state. The cation is

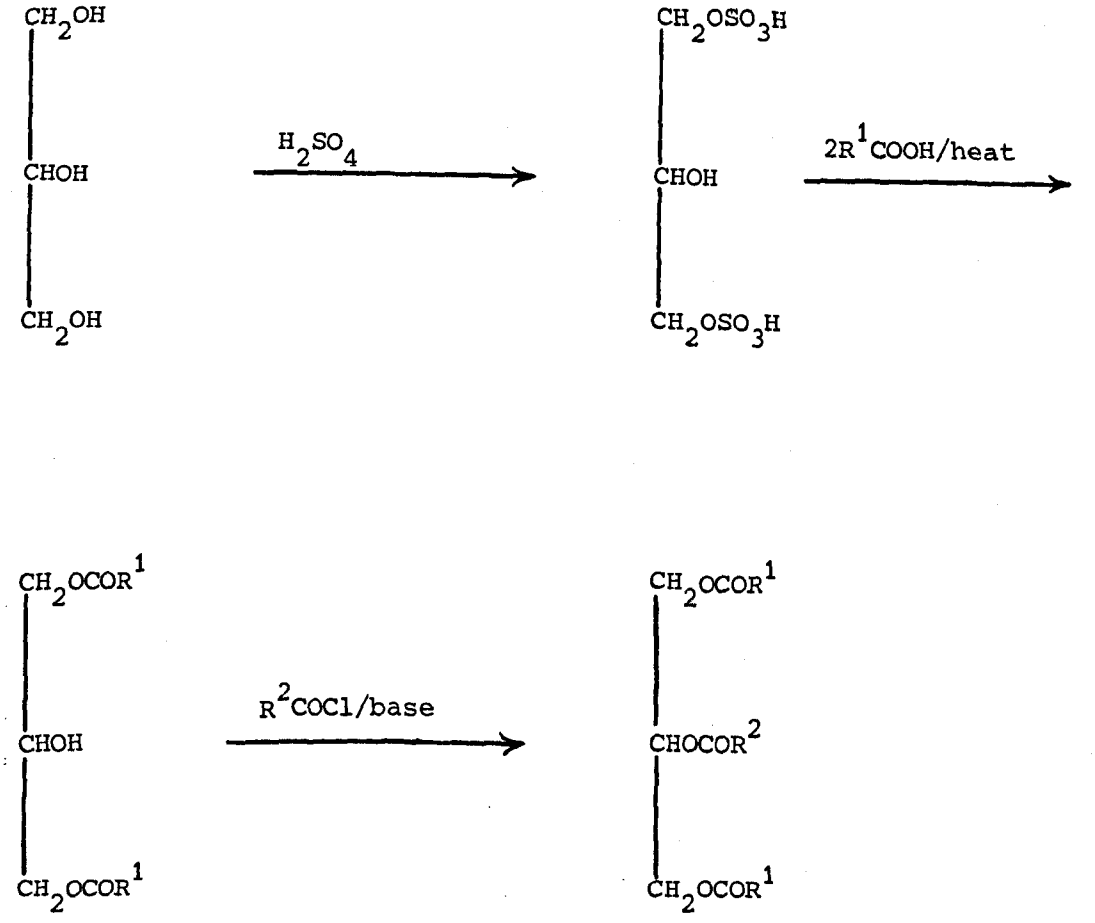


18-Crown-6-ether potassium carboxylate complex

located in the centre of the main ring held principally by electrostatic forces. The resulting anions formed are unusually reactive and high yield of esters were obtained from alkali metals complexed to crown ethers and alkyl halides. The reduction in the cation-anion interaction enhances the solubility of the carboxylate anions and hence the

reactivity with the substrate. The replacement of the alkali-metal salts, by quaternary ammonium salts, such as tricaprylmethyl ammonium salts, gives results similar to those obtained using crown ethers and in some cases the yields are much better. Quaternary ammonium salts are soluble in a wide range of solvents. The symmetrical disposition of the hydrocarbon chains around the nitrogen, provides a good shield of the cation from the carboxylate anion and, therefore, reduces their interaction. The anion is more exposed, "naked", and therefore more susceptible to undergo nucleophilic substitution reactions.[61] A wide range of solvents are available for the preparation of acylglycerols depending on the reagents. Solvents such as hexamethylphospharamide (HMPA), dimethylformamide (DMF), dimethyl sulphoxide (DMSO), tetrahydrofuran (THF) and acetonitrile are used more and more in modern organic synthesis[62] because of their high solvation power of a wide range of organic and inorganic compounds and also because anions are less solvated due to the lack of general hydrogen bonding with the solvent.

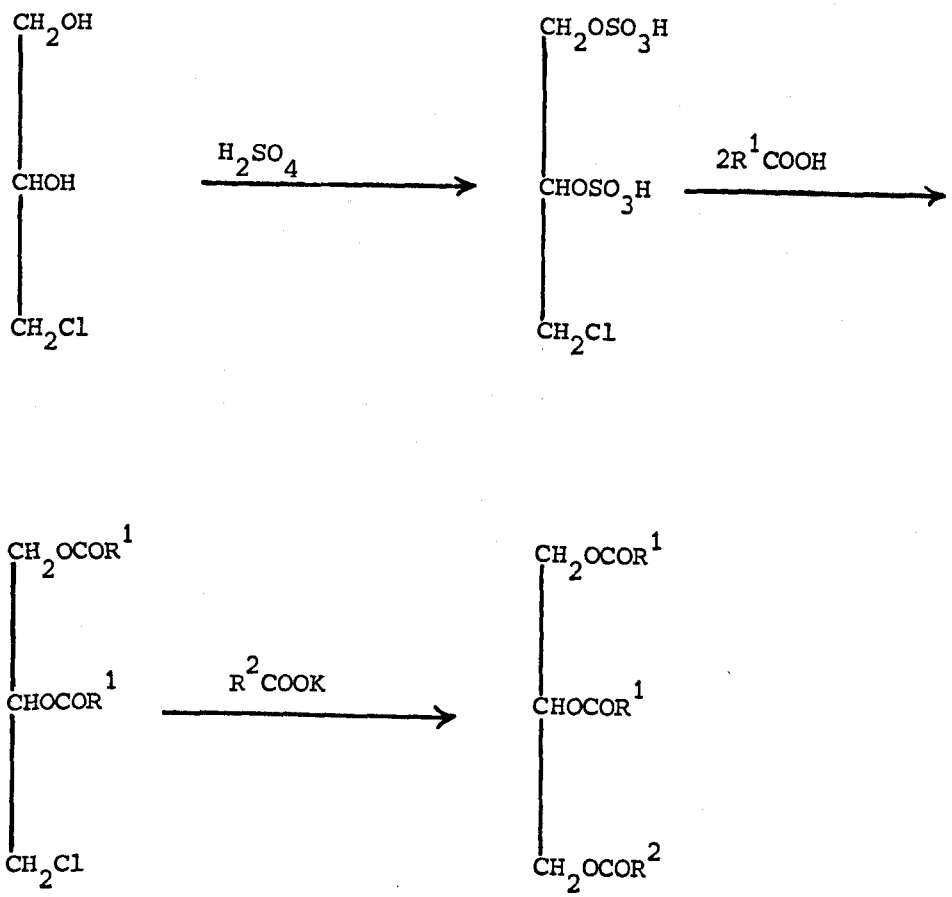
Two glycerol derivatives have been studied for their nucleophilic substitution reactions, the sulphuric acid esters of glycerol and the halogeno-glycerol derivatives. The methods for the preparation of mixed triacylglycerols started only at the beginning of 1900. They were mainly those of Grun and co-workers.[63-65] The mixed triacylglycerols were prepared by reacting glycerol with sulphuric acid and the glycerol-disulphuric acid ester produced was reacted with an equimolar amount of a fatty acid. The product was acylated with an acid chloride to give the corresponding symmetrical diacid triacylglycerols (Scheme: 16a).



Symmetrical diacid triacylglycerol

Scheme: 16a

Unsymmetrical diacid triacylglycerols were prepared from the sulphuric acid esters of 1-chloroglycerol (Scheme: 16b).



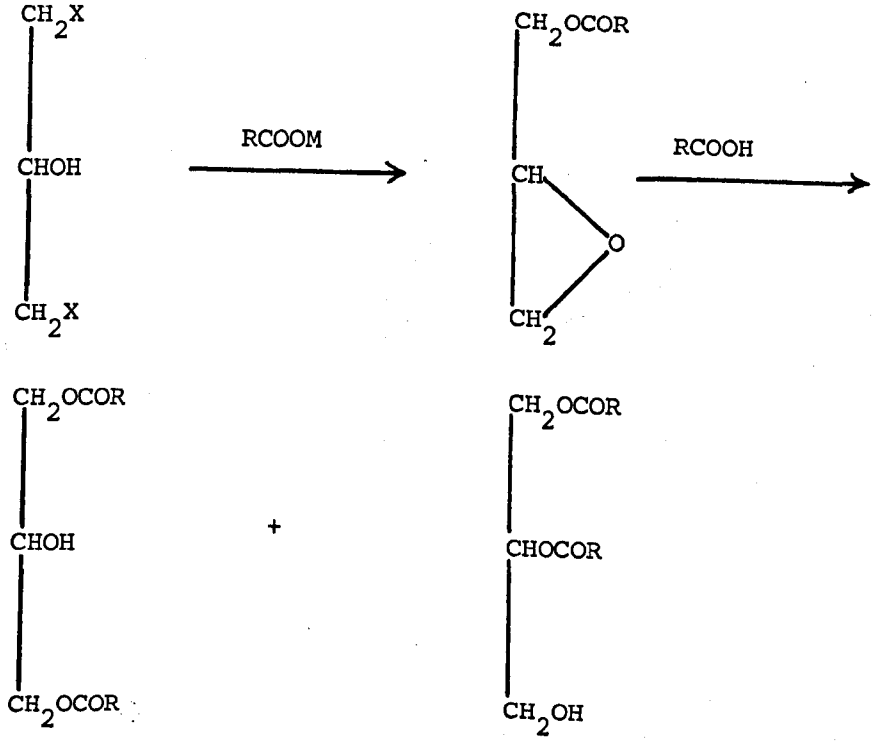
Unsymmetrical diacid triacylglycerols

Scheme: 16b

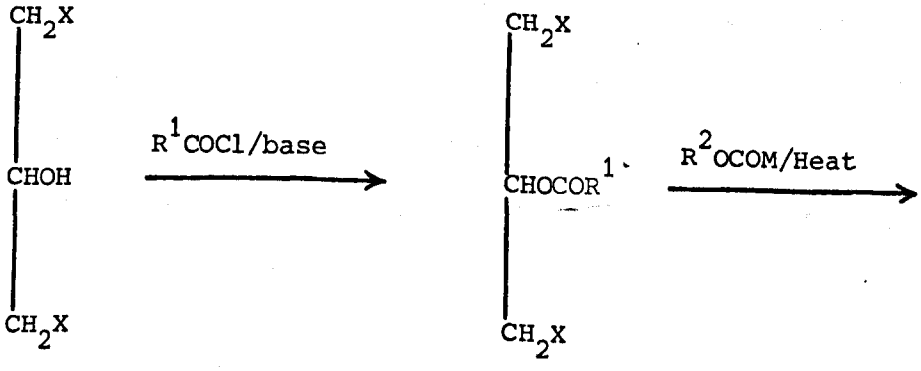
In the preparation of triacylglycerols, the authors[66] noticed the formation of other acylglycerols and free fatty acids. The above methods were investigated by other workers[67,68] and were found to be unsatisfactory due to: the reaction conditions, the uncertainties about the structure of the intermediate products and the possibility of rearrangements.

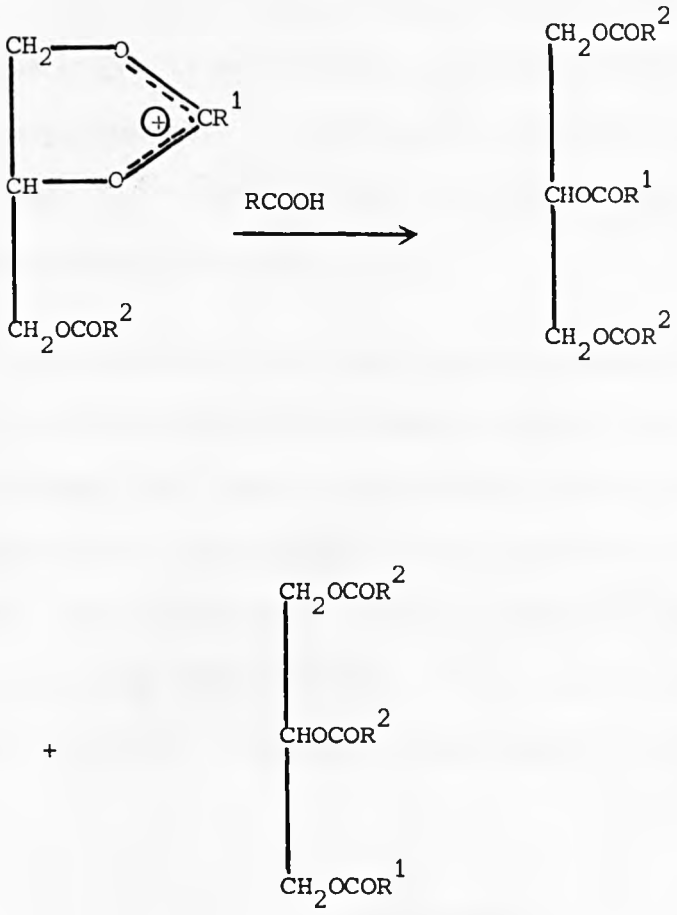
The halogen derivatives of glycerol have been studied extensively for the preparation of mixed triacylglycerols. The dibromo derivatives

can be prepared from glycerol, red phosphorus and bromine.[69] The 1,3-dibromoglycerol is separated from the other isomers by distillation. When the method was adopted for the preparation of acylglycerols,[70,71] there was doubt about the purity of the products[68] due to dehydrohalogenation resulting from neighbouring group participation (Schemes 17 and 18).



Scheme: 17



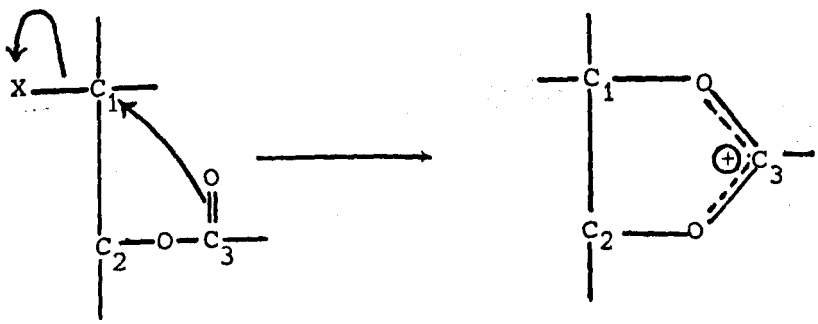


Scheme: 18

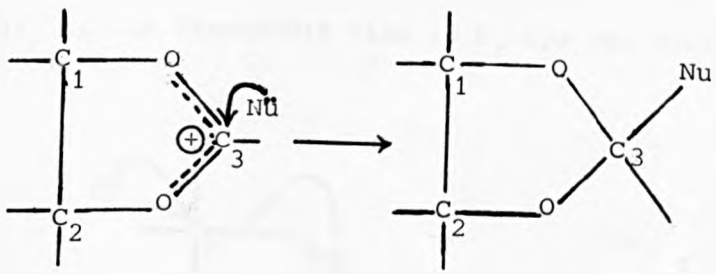
Specific diacid triacylglycerols were prepared[72] by a simple two-step procedure from mono- and disubstituted halogenoglycerols. The nucleophilic substitution reactions were conducted in polar aprotic solvents with reaction times varying from 1.5 to 117 hours and reaction temperatures varying from 75° to 153° . The yields obtained were above average (48 to 58%). The author assumes that because there is no hydroxyl group adjacent to an acyl group at any time during the synthesis, acyl migration cannot occur. But as depicted in

Scheme 18 acyl migration could occur. Recently ideal conditions have been found for the preparation of diacid triacylglycerols, without any concomitant acyl migration,[73] starting from dihalogeno-monoacyl-glycerols. The best yields were obtained using quaternary ammonium salts in non-polar aprotic solvents.

Glycerol and its acyl derivatives have three functional groups in close proximity to each others, and are ideally suited for intra-molecular interactions, e.g. acyl or acyloxy migration which was mentioned in the previous description of the preparation of acylglycerols (1.1-1.4). Such interactions can be reduced or enhanced sometimes in order to get the right products. There are two basic ways for rearrangement to take place. The acyl oxygen attacks the carbon C_1

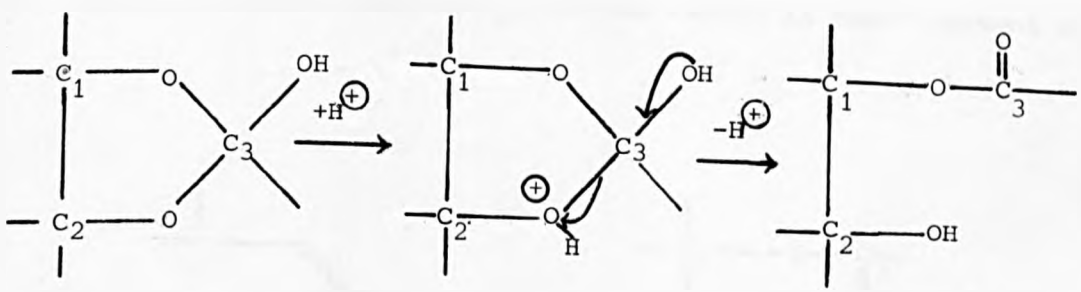


and replaces the leaving group in an S_N2 process to form a five membered ring. The leaving group may be a halide, ^-OH, ^-OTs or any other good leaving group. The nucleophile can attack either the carbonyl carbon (C_3) or one of the other two carbons (C_1 or C_2). The most favourable site attracts the nucleophile. Firstly if the nucleophile attacks the carbonyl carbon, C_3, there will be no further rearrangements if the nucleophile is RO^- or $RCOO^-$. However if the



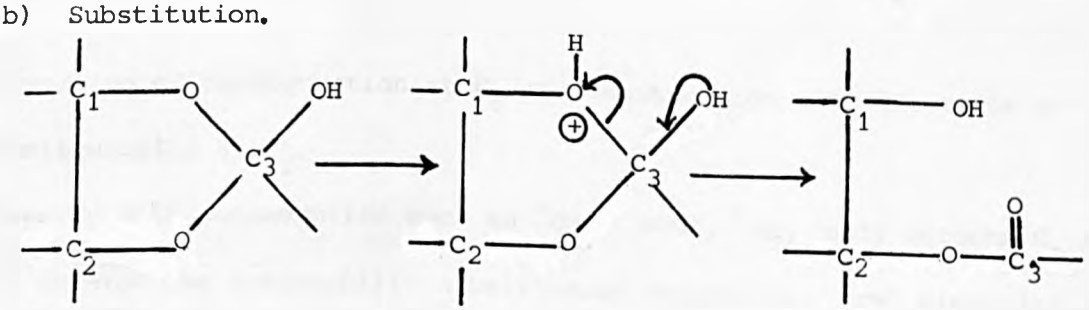
nucleophile is H_2O then there are two possibilities for the ring to open with,

(a) substitution and rearrangement.



The net result is rearrangement with inversion at carbon C_1 and substitution with retention of configuration at carbon C_2.

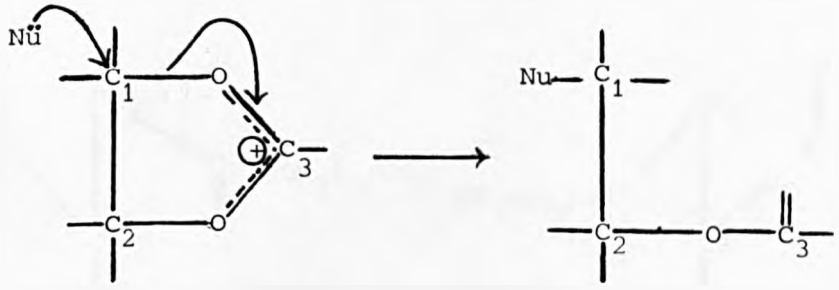
(b) Substitution.



Here the net result is substitution with inversion at carbon C_1 and retention of configuration at carbon C_2.

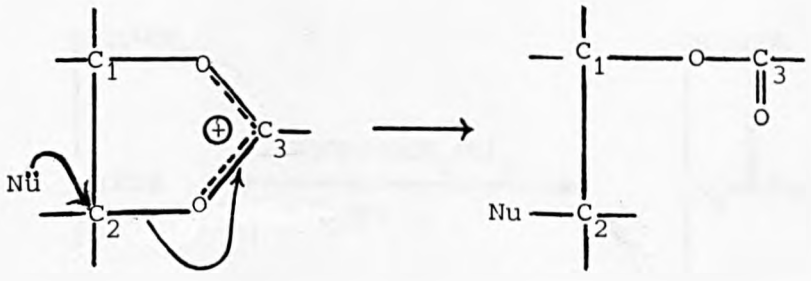
Secondly the nucleophile can attack the sites at carbons C_1 and C_2.

(a) If the favourable site is C_1, the net result is substitution



with retention of configuration at both C_1 and C_2.

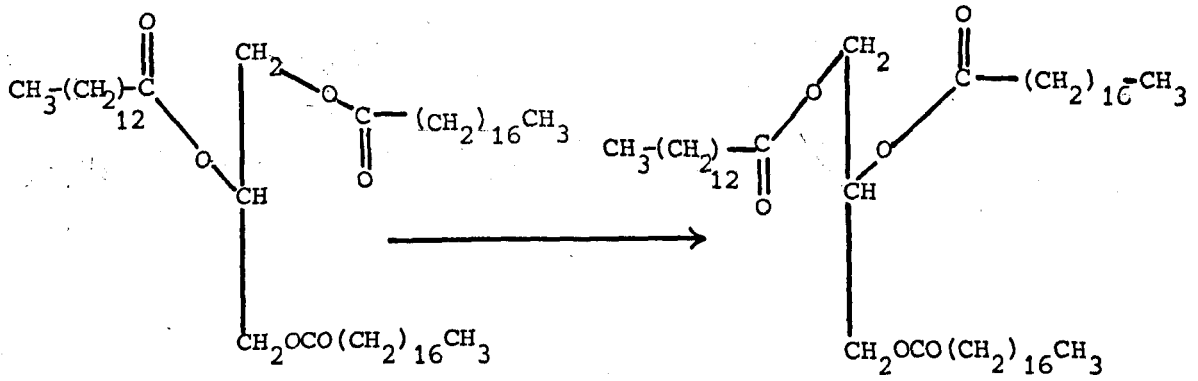
(b) If the favourable site is C_2, the net result is rearrangement with



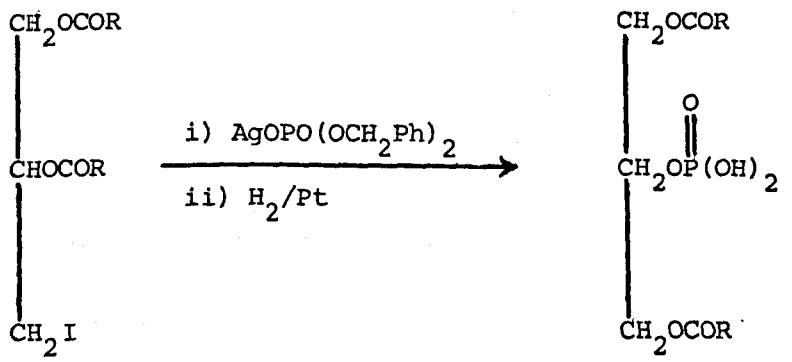
inversion of configuration at C_1 and substitution with inversion of configuration at C_2.

However with nucleophiles such as ^{-}OR, $^{-}OCOR$, ^{-}OH, only carbons C_1 and C_2 undergo the nucleophilic substitution reactions. Acyl migration was first observed by Fisher[50] in his preparation of diacylglycerols.

Instead of the 1,2-isomer, he obtained the 1,3-isomer (Scheme: 15). Fairbourne[68] observed that acyl migration can occur between a hydroxyl group and an acyl group (Scheme: 17) or between two acyl groups (Scheme: 19) or between an acyl group and another group such as the diphthalimido group or the phosphate group (Scheme: 20).

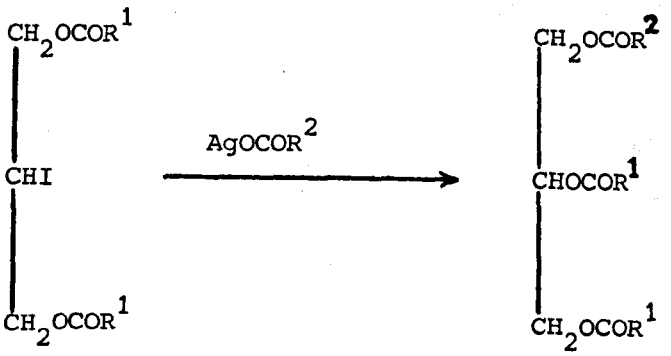


Scheme: 19



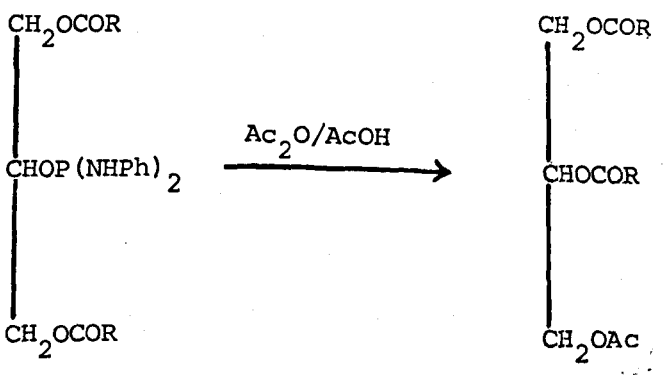
Scheme: 20

The extent of the acyl migration, whether it is from the 2-position of the glycerol molecule to the 1/3-position[75] (Scheme: 21), or from the



Scheme: 21

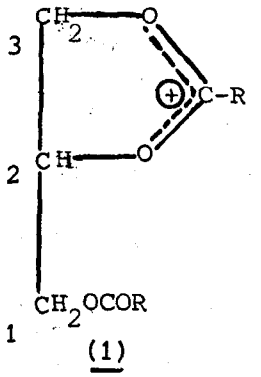
1/3-position to the 2-position[75] (Scheme: 22) depends on the structure



Scheme: 22

of the acylglycerols, reaction conditions and reagents. For example by heating a mixture of 1,3- and 1,2-diacylglycerols it was possible to

isolate 99% pure 1,3-diacylglycerols on recrystallization.[10,12] Further more Martin[26] succeeded in determining monoacylglycerols in mixtures which contained both the 2- and 1-monoacylglycerols. By heating the mixture with perchloric acid, the 2-monoacylglycerol is converted into the 1-monoacylglycerol. The total amount of 1-monoacylglycerol is estimated then by periodic acid oxidation. Acyl migration can be reduced in presence of bulky groups e.g. Aneja and Davies[76] using the bulky silver dibenzyl phosphate found that acyl migration shown in Scheme: 20 takes place only to the extent of 2%. In many preparations of acylglycerols the anomalous results can be explained as either due to acyl or acyloxy migration involving the dioxolenium ion as intermediate (the Winstein-Buckles[77] dioxolenium ion (1)). Aneja

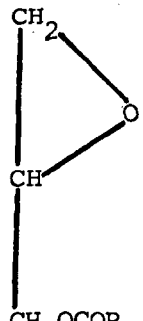


(1)

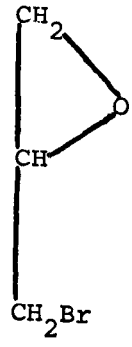
Winstein-Buckles dioxolenium ion

and Davies[76,78] found that the dioxolenium ion intermediate is attacked by the incoming nucleophile at the terminal C_3, mainly due to the steric effects (ease of approach of the nucleophile) which favours the less hindered and more accessible C_3 to C_2. Direct nucleophilic substitution reactions involving the Walden inversion are also possible[80] (S_N2 type). In nucleophilic substitution reactions where the acyl group is not participating, other intermediates are involved and these lead to the

formation of isomeric acylglycerols e.g. in the preparation of diacylglycerols from 1,3-dihalogenoglycerols, 1,3- as well as 1,2-diacylglycerols were obtained. The 1,2-isomer is thought to have resulted from the 1-acyl-2,3-epoxypropane (2) or 1-bromo-2,3-epoxypropane. Both intermediates[73] were isolated.



(2)



1-bromo2,3-epoxypropane

Aneja[79] and co-workers have studied the regiospecific synthesis of glycerophospholipids via S_N reactions, and found that the parameters which insured high specificity were:

(a) choice of leaving group e.g. triphenylphosphonium

(b) use of an aprotic polarising solvent e.g. HMPA

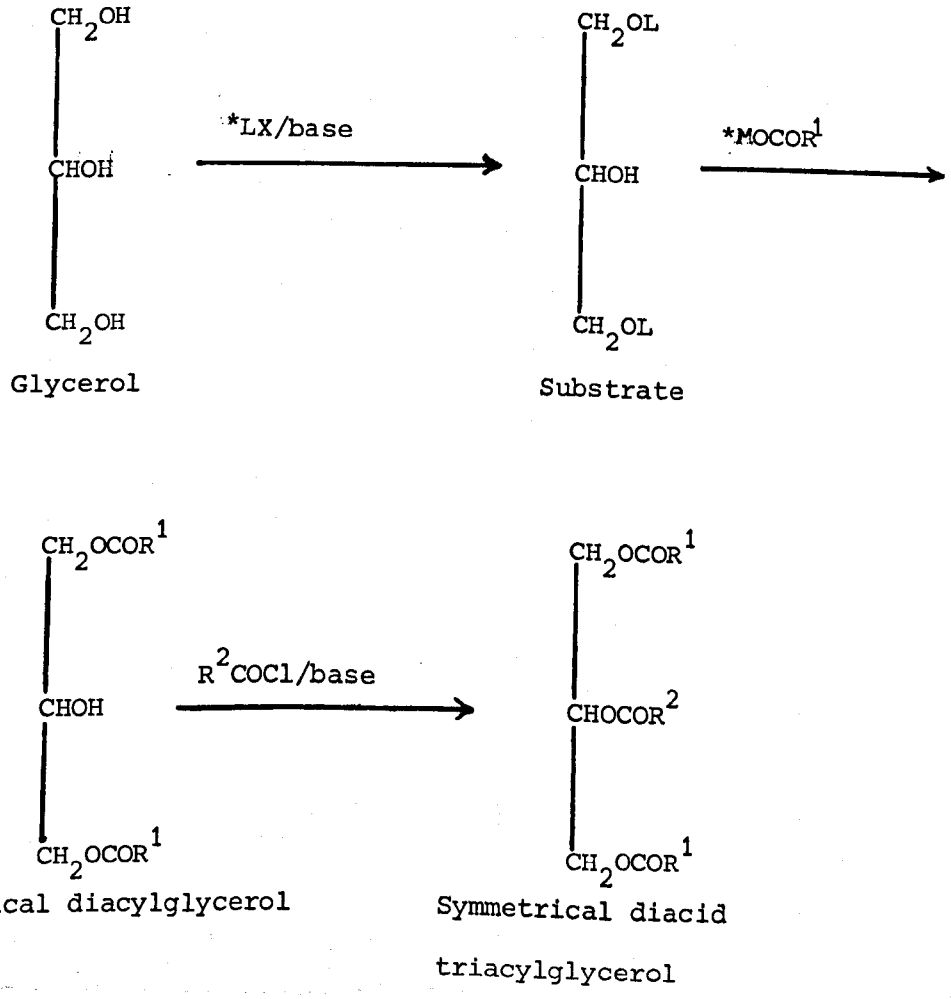
(c) Application of a tetraalkyl ammonium cation as counter-ion.

Symmetrical diacid triacylglycerols have been obtained by different procedures as mentioned earlier, most of which involve protecting groups and their subsequent removal by chemical and catalytic cleavage. However their preparation by nucleophilic substitution reactions on glycerol derivatives offer economy in the number of synthetic steps which are needed to produce the desired diacid triacylglycerols.
In the present work three new routes, for the preparation of symmetri-

cal diacid triacylglycerols by nucleophilic substitution reactions are explored and their possible use for the preparation of pure diacid triacylglycerols are considered. The three routes are as follows.

1. The tosylate route
2. The silyl ether route
3. The phosphate route.

In all these routes the first step involves the preparation of the appropriate derivative of glycerol which is then subjected to nucleophilic substitution, followed by acylation of the resultant symmetrical diacylglycerol with an acid chloride (Scheme: 23).



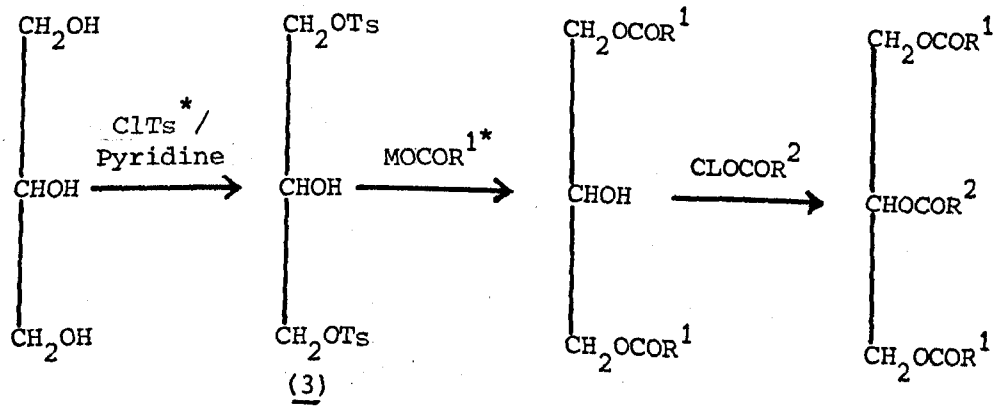
Scheme: 23

*LX: halide derivative of the leaving group; MOCOR: fatty acid salt

2.1 THE TOSYLATE ROUTE

2.1.1 INTRODUCTION.

Apart from Grün's work[63-65] on sulphuric acid esters of glycerol and related halide derivatives,[12,70-73,81-82] very little work has been done using other substrates utilizing substitution reactions. Although halides are the commonest leaving groups for synthetic purposes in nucleophilic substitution reactions, the sulphonic esters are much better leaving groups. Sulphonic esters are most frequently prepared by treatment of the corresponding acid halides with alcohols in the presence of a base. The base is often pyridine which functions as a nucleophilic catalyst.[84] Sulphonyl halides react more quickly with primary than with secondary or tertiary alcohols.[85] The toluene-p-sulphonyl (tosylate) groups have been used in partially acylated glycerols or related derivatives[78] but not for the synthesis of specific triacylglycerols, starting from glycerol. The proposed procedure for the synthesis of symmetrical diacid triacylglycerols using toluene-p-sulphonyl derivative of glycerol as a substrate is similar to that outlined in scheme: 23 (L=Ts).



Scheme: 23

* Ts = $SO_2\text{-}C_6H_5.CH_3$; R^1 or R^2 = $-(CH_2)_nCH_3$, n is a whole number.

2.1.2 RESULTS AND DISCUSSION.

2.1.2.1 Preparation of the 1,3-ditosylate derivatives of glycerol.

Using the selectivity of its reactions with primary and secondary alcohols, toluene-p-sulphonyl chloride was reacted with anhydrous glycerol (1 mol of glycerol to 2 mol of toluene-p-sulphonyl chloride) in pyridine at 0°. After 44 hours the reaction mixture was analysed by TLC (5% (v/v) methanol in dichloromethane) and the three components detected were isolated and quantified (table 1). The TLC

R_f	0.70	0.55	0.24
Composition/ % (w/w)	7.4	90.0	2.6

Table 1: reaction of glycerol with tosyl chloride in pyridine (44 hours); TLC: 5% v/v CH_3OH in CH_2Cl_2.

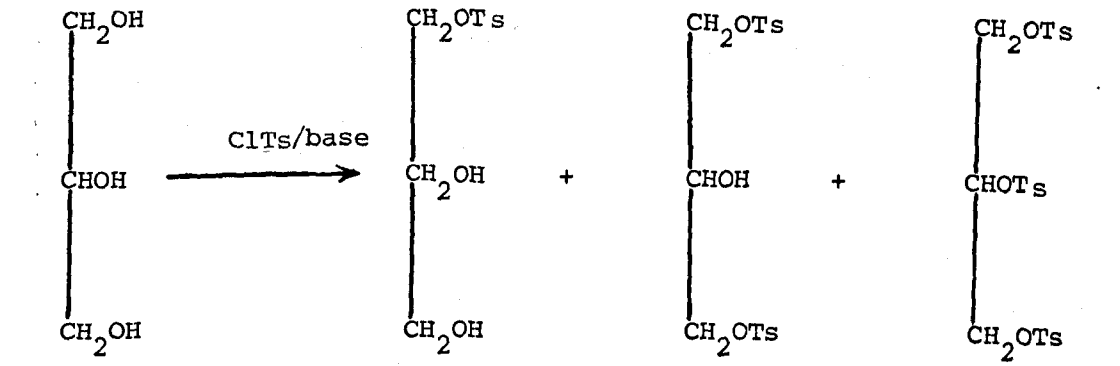
analysis showed also that all the tosyl chloride (R_f 0.66) had reacted. The isolated components were analysed further by high performance liquid chromatography (HPLC) under isochratic conditions using a UV detector with a fixed wavelength (254 nm) and a silica column. The elution system was 1% (v/v) methanol in chloroform. The absorbances were calculated from the peak areas by the method of triangulation. The three components eluted at different retention times and had the following relative absorbances: the component of R_f 0.70 was 2.63, the component of R_f 0.55 was 1.83 and the component of R_f 0.24 was 1.00 with ratios of 3:2:1 respectively (table 10, 2.1.3.1). The tosyl chloride had an

absorbance of 1.00 under the same conditions. Therefore the products of R_f 0.24, 0.55 and 0.70 were the mono- di- and tritosylate derivatives of glycerol (Scheme: 24). The 1,3-and 1,2-isomers of glycerol (acylglycerols and silyl ethers (2.2)) can be separated by TLC and further analysis (2.1.3) showed that the disubstituted glycerol was glycerol-1,3-ditosylate,(3). Ideal conditions were found to produce a maximum amount of (3). The use of pyridine as a solvent gave a higher yield of (3) than the use of chloroform (table 2). This was due mainly to the insolubility of glycerol in chloroform. The yield of (3) was increased when the reaction time was increased from 20 to 44 hours (table 2). The amount of (3) did not increase significantly when the time was increased further. It is worth adding that the reactions

Reaction time/hours	20	44	44
Solvent	Pyridine	Chloroform	Pyridine
% (w/w) Glycerol-1,3-ditosylate (3) in product mixture	85	51	90

Table 2: effects of solvent and time on the yield of glycerol-1,3-ditosylate (3).

of (3) or the component at R_f 0.24 (glycerol monotosylate) with excess toluene-p-sulphonyl chloride both yielded the component of R_f 0.70 (glycerol tritosylate).



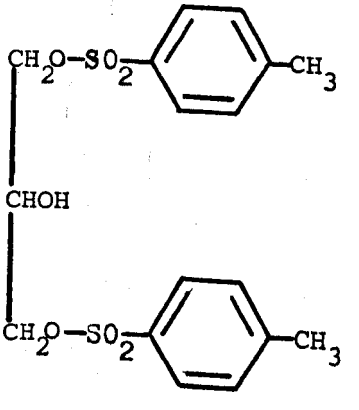
Glycerol | Glycerol mono-tosylate | (3) | Glycerol trito-sylate

Scheme: 24

From the experimental data it appears that with glycerol, toluene-p-sulphonyl chloride reacts firstly with the primary OH group. It reacts with the secondary OH group in the glycerol molecule only when the primary alcohol sites have reacted. Even when the two primary alcohol sites of a particular glycerol molecule have reacted, other unreacted primary alcohol sites on other glycerol molecules are much more likely to react than the secondary alcohol of the already disubstituted glycerol molecule. This explains the high yield of (3) compared with that of glycerol-tritosylate (table 1). This regioselectivity is mainly due to steric hindrance and the greater the substitution at the primary alcohol sites the greater is the steric hindrance at the secondary alcohol site and consequently the less reactive it becomes.

2.1.2.2 Separation of glycerol-1,3-ditosylate,(3).

(3) was separated by flash chromatography using a procedure (2.1.3.1) slightly different from that described by Clark.[86] The product mixture was eluted through a column packed with silica gel 60 (230-400 mesh) with 2% (v/v) methanol in chloroform as the elution system. Fractions of 20 cm^3 were collected and analysed by TLC. Fractions from 1 to 4 contained glycerol tritosylate with fraction 3 containing most. Most of (3) was collected in fractions 7, 8 and 9 but some was present also in fraction 6. Fraction 10 did contain (3) but was contaminated with glycerol monotosylate. The full results are given in Table 3 and also in the form of a histogram (fig. 1). Glycerol-1,3-ditosylate,(3),was a colourless liquid which showed neither decomposition when stored at 3° nor did it change when refluxed in dry hexane for 4 hours. It solidified after long-term storage but still did not decompose (TLC). The % (w/w) of (3) recovered was 80.4% of the product mixture against 90.4% yield produced from the reaction.



(3)

Fraction number	1	2	3	4	5	6	7	8	9
Composition % (w/w)	0.17	0.19	7.33	1.32	0.12	3.09	15.94	38.95	22.41
Components present	Glycerol tritosylate					(3)			

Fraction number	10	11	12	13	14	15	16	17	
Composition % (w/w)	5.26	4.62	0.25	0.07	0.04	0.12	0.06	0.06	
Components present	(3) + glycerol monoto-sylate		Traces of glycerol monotosylate						

Table 3: product mixture from the reaction of glycerol with toluene-p-sulphonyl chloride, separated by flash chromatography (2.1.2.2 and 2.1.3).

2.1.2.3 Reactions of glycerol-1,3-ditosylate,(3).

To assess the suitability of (3) for the preparation of pure triacylglycerols, its nucleophilic substitution was investigated using two counter-ions, sodium ($Na^{\oplus}$) and tricaprylmethyl ammonium ($[C_{10}H_{21})_3CH_3]N^{\oplus}$) ions, and two aprotic solvents of differing polarities namely n-hexane (E^* = 1.9) and dimethylsulphoxide (DMSO; E =46.7) The carboxylate

*E = dielectric constant at 25°

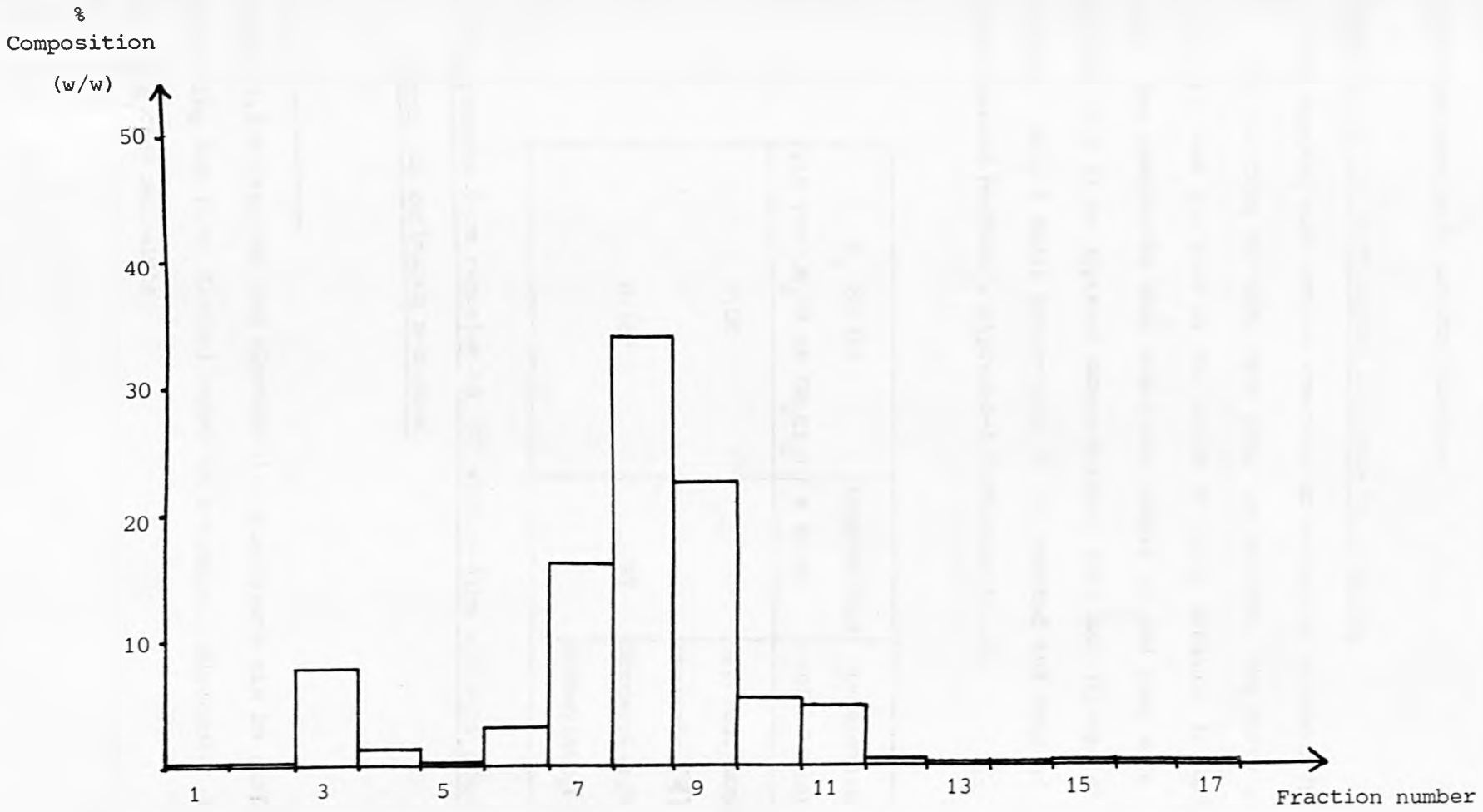


Fig. 1: separation of the product mixture from the reaction of glycerol with toluene-p-sulphonyl chloride by flash chromatography.

anion used was stearate since the diacylglycerols, if produced, would be separated readily by crystallization.

a. Reaction of (3) with sodium stearate in n-hexane.

(3) was reacted with sodium stearate in refluxing n-hexane for 4 hours. The reaction mixture, free from the solvent, the fatty acid and its salt, was analysed by TLC using 1% (v/v) methanol in dichloromethane. Two components were separated (table 4) and they were identified (2.1.3) as glycerol monostearate, (4), and glycerol-1,3-ditosylate. Only a small percentage of (3) reacted and none of it gave the desired product , glycerol-1,3-distearate,(5).

R_f values (1% v/v CH_3OH in CH_2Cl_2)	Composition % (w/w)	Inferences (cf. 2.1.3)
0.00	3	Glycerol monostearate,(4).
0.15*	97	Glycerol-1,3-ditosylate,(3).

Table 4: products from reaction of (3) with sodium stearate after 4 hours in refluxing n-hexane.

*Glycerol-1,3-ditosylate and glycerol-1,2-distearate can be differentiated using 40% (v/v) diethyl ether in n-hexane. Glycerol-1,3-ditosylate does not elute.

b. Reaction of (3) with sodium stearate in DMSO.

(3) was reacted with sodium stearate in DMSO for 4 hours at 79°. The reaction mixture was treated and separated by TLC as in (a). Six components were separated (table 5) and they were identified (2.1.3). More than half the amount of (3) reacted to give, amongst other products, the desired product (5) (6% w/w) and 1-stearate-2,3-epoxypropane (7) (26% w/w). The amount of (7) in the product mixture was surprisingly high.

R_f values (1% v/v CH_3OH in CH_2Cl_2)	Composition % (w/w)	Inference (cf. 2.1.3)
0.00	13	Glycerol monostearate, (4).
0.15*	43	Glycerol-1,3-ditosylate, (3).
0.30	6	Glycerol-1,3-distearate, (5).
0.47	5	Not identified
0.59	26	1-stearate-2,3-epoxypropane, (7).
0.80	6	Not identified

Table 5: products from reaction of (3) with sodium stearate in DMSO after 4 hours at 79°.

*Glycerol-1,3-ditosylate and glycerol-1,2-distearate can be differentiated using 40% (v/v) diethyl ether in n-hexane. Glycerol-1,3-ditosylate does not elute

c. Reaction of (3) with Tricaprylmethyl ammonium stearate (TCMAS) in DMSO.

(3) was reacted with TCMAS in DMSO for 4 hours at 79°. The reaction mixture was treated and analysed as in (a). Six components were

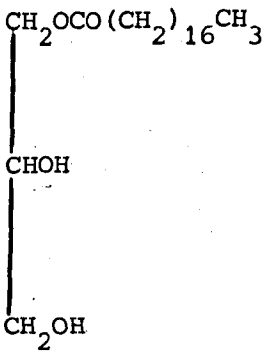
R_f values (1% v/v CH_3OH in CH_2Cl_2	Composition % (w/w)	Inference (cf. 2.1.3)
0.00	4	Glycerol monostearate, (4).
0.16*	24	Glycerol-1,2-distearate, (6).
0.29	58	Glycerol-1,3-distearate, (5).
0.72	1	Not identified
0.84	8	Not identified
0.95	5	Not identified

Table 6: products from reaction (3) with TCMAS in DMSO for 4 hours at 79°.

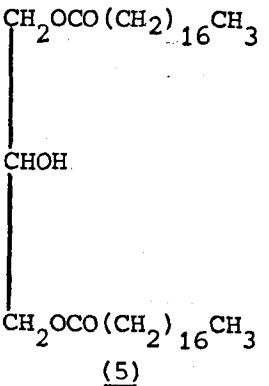
separated (Table 6). Three of these components were identified (2.1.3) as glycerol monostearate (4), glycerol-1,2-distearate (6) and glycerol-1,3-distearate (5). It may be noticed that all the substrate (3) had reacted, yet the amount of the desired product (5) was still low and considering the structure of (3), the amount of (6) produced was high.

---

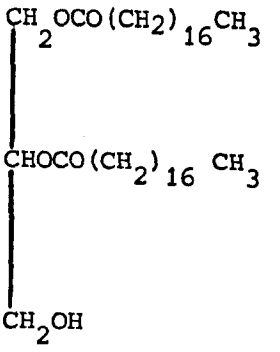
*Glycerol-1,3-ditosylate and glycerol-1,2-distearate can be differentiated using 40% (v/v) diethyl ether in n-hexane as the elution system. Glycerol-1,3-ditosylate does not elute.



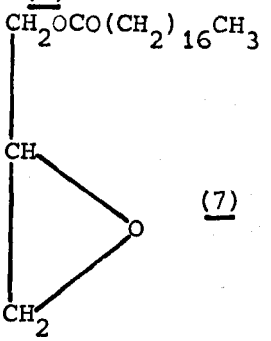
(4)



(5)



(6)



(7)

d. Reaction of (3) with TCMAS in n-hexane.

(3) was reacted with TCMAS in refluxing n-hexane for 4 hours. The reaction mixture was treated and analysed as in (a). Two components were separated (table 7). They were identified (2.1.3) as (5) (90%

R_f values (1% v/v CH_3OH in CH_2Cl_2)	Composition % (w/w)	Inference (cf. 2.1.3)
0.16*	10	Glycerol-1,2-distearate, (6).
0.29	90	Glycerol-1,3-distearate, (5).

Table 7: products from reaction of (3) with TCMAS in refluxing n-hexane.

*Glycerol-1,3-ditosylate and glycerol-1,2-distearate can be differentiated using 40% (v/v) diethyl ether in n-hexane. Glycerol-1,3-distosylate does not elute.

(w/w)) and (6) (10% (w/w)). It may be noticed that the number of by-products decreased dramatically and that the conditions used are the best so far for a maximum yield of (5) and a minimum yield of (6).

The results above can be summarised as follows:

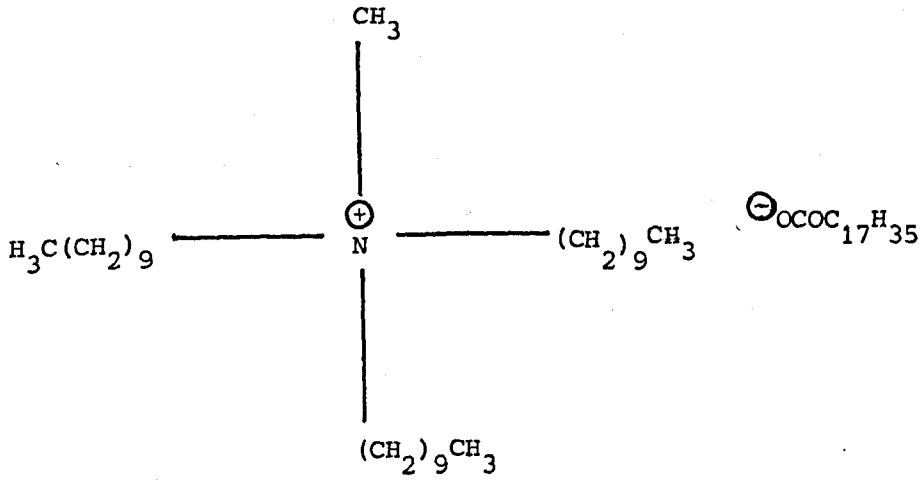
a. (3) + $NaOCO(CH_2)_{16}CH_3$ $\xrightarrow{\text{n-hexane}}$ 97% unreacted substrate.

b. (3) + $NaOCO(CH_2)_{16}CH_3$ $\xrightarrow{\text{DMSO}}$ 43% unreacted substrate, 6% (5) and 26% (7).

c. (3) + TCMAS $\xrightarrow{\text{DMSO}}$ 58% (5) and 24% (6).

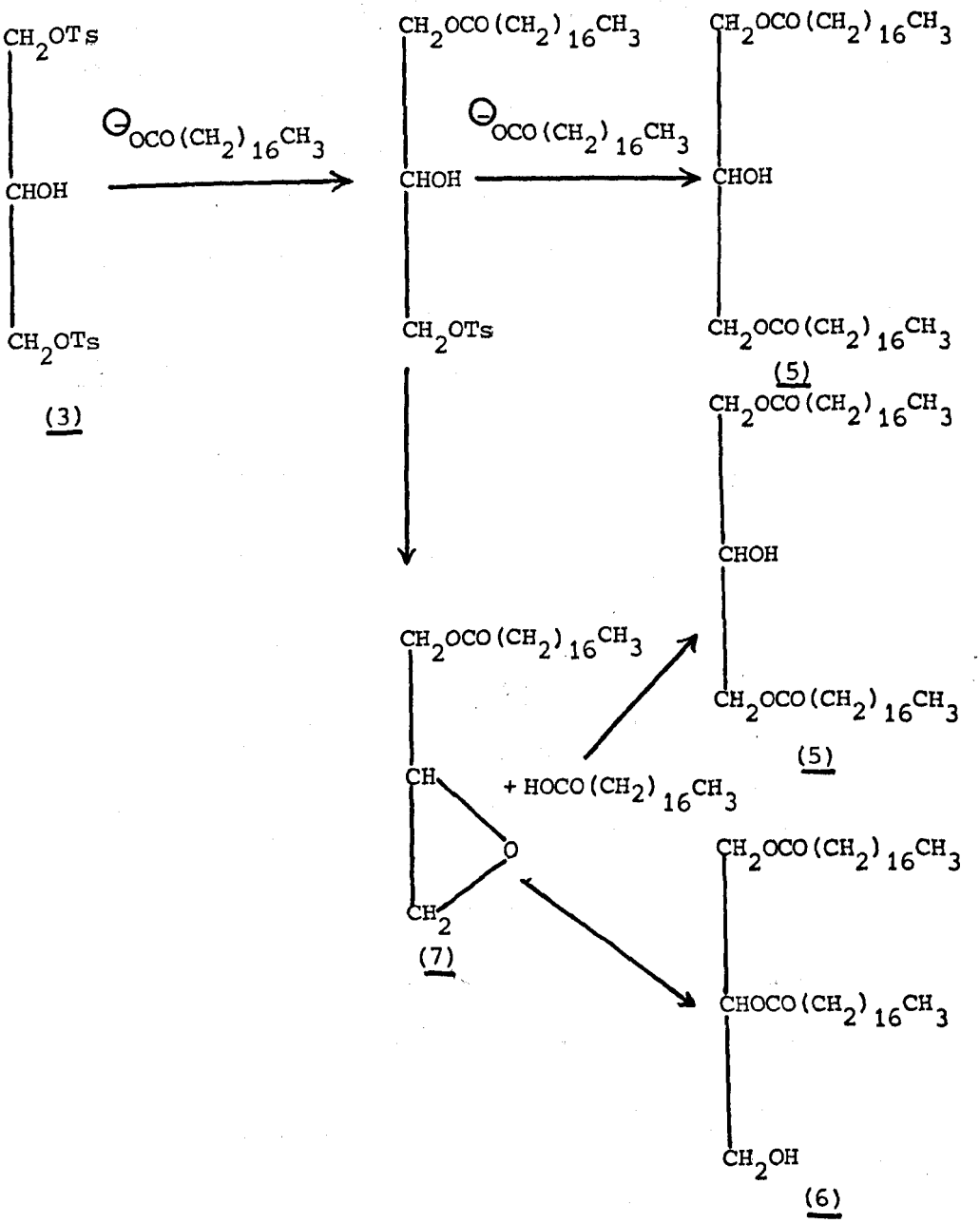
d. (3) + TCMAS $\xrightarrow{\text{n-hexane}}$ 90% (5) and 10% (6).

When using sodium salts, whether the solvent was n-hexane or DMSO, the reactions did not go to completion and the amount of by-products was high. When TCMAS was used all the substrate reacted and the highest amount of the desired product was obtained in n-hexane, a non-polar aprotic solvent. The alkali-metal salts of fatty acids are insoluble in most organic solvents and sparingly soluble in dipolar aprotic solvents such as DMSO. The partial reactivity of the substrate (tables 4 and 5) was due mainly to the shortage of carboxylate anions in solution. The most interesting results were obtained using quaternary ammonium cations as counter-ions e.g. TCMAS. Such quaternary ammonium cations are soluble in most organic solvents. As a result of the steric effects of the alkyl groups of the cation in preventing the close approach of the anion, the anions of the solubilized salts possess considerable reactivity (tables 6-9). An interesting feature of the reaction was the formation of a relatively high proportion of (6) in DMSO. It has been ascertained (see below) that (6) arose neither from (3) nor from (5). Thus (3) did not undergo any isomeri-



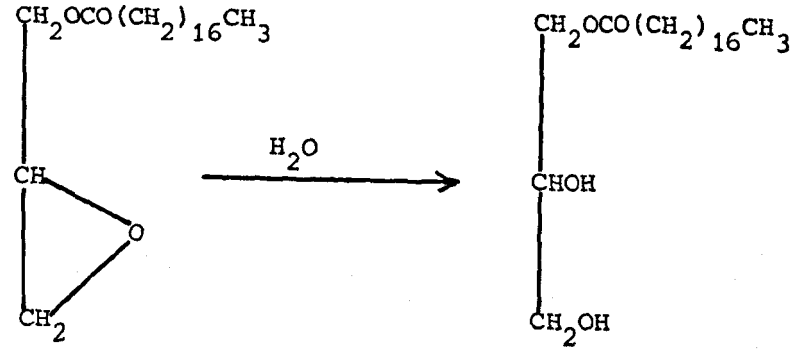
TCMAS

zation on heating in n-hexane or DMSO. Similarly (5), on heating with or without TCMAS in the above-mentioned solvents, did not change to (6). (6) in fact arose from 1-stearate-2,3-epoxypropane,(7),which was formed by elimination (Scheme: 25). (7) was the major product in the reaction of (3) with sodium stearate in DMSO (table 4). (7) was detected also by GLC (0.6% (w/w) of the products) in the reaction of (3) with TCMAS in n-hexane (table 6). Similar to (6), the glycerol monostearate, (4), was produced from (7) (Scheme: 26). Anion species involved in the reaction are very important in the determination of the way in which the reaction goes. In low polarity solvents such as n-hexane, ion pairs ($M^{\oplus}\,^{\ominus}OCOC_{17}H_{35}$) and aggregate ion pairs ($(M^{\oplus}\,^{\ominus}OCOC_{17}H_{35})_n$ represent the prevailing species[87,88] and they are termed "associated species". The quaternary ammonium complexes show maximum yields for products [(4),(6) and (7)] formed by elimination reactions. In DMSO, whether a quaternary ammonium cation or an alkali-metal cation is used, the solvent-separated ion pairs or free ion



Scheme: 25

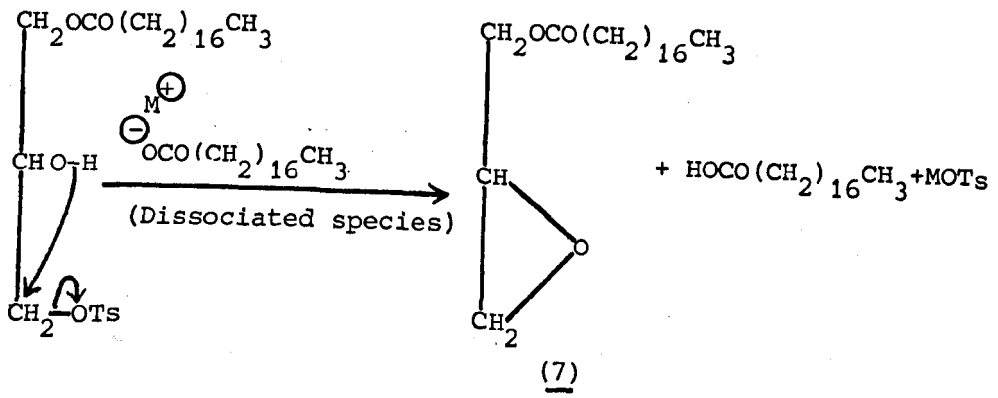
pairs ($M^{\oplus} + {}^{\ominus}OCOC_{17}H_{35}$) prevail and they are termed collectively "dissociated species". The proportion of elimination products, viz. (4), (6) and (7), was a maximum in DMSO (tables 5 and 6). The observed enhanced basicity of the carboxylate anion in DMSO can be attributed, in part, to solvent assistance in carbanion formation at C-2 in the substrate. Evidently the associated nucleophile found in non-



(7) (4)

Scheme: 26

polar aprotic solvents has a small tendency to take part in the elimination reaction compared with the dissociated species (Scheme: 27). This implies that the basicities of the associated and dissociated forms of the nucleophile are very different. The lowering of base strength induced by ionic association explains why the most favourable proportion of substitution products was found in the non-polar solvent, n-hexane. The attractive ion-dipole interactions between the counter-ion of the associated nucleophile and the leaving group also may play a part in determining the proportion of substitution to elimination products. Glycerol-1,2-distearate,(6),is not the product



Scheme: 27

of acyl migration which involves the five-membered ring of the dioxolenium ion intermediate,(1) (cf. 1.4). Instead a three membered ring was formed (7) as a result of an elimination reaction producing the epoxide (7) and the free fatty acid or toluene-p-sulphonic acid. In contact with the incoming carboxylate anions or the free fatty acid, (7), can open either way to give the 1,2- or the 1,3 isomer (Scheme: 25). However, because of steric hindrance, the substitution on the terminal carbon is much preferred.

Since the aim of the synthesis is to maximize the yield of the substitution and minimize any elimination products, the combination of TCMAS, as the source of carboxylate anions, and n-hexane as a solvent gives the best conditions for nucleophilic substitution on (3).

2.1.2.4 Preparation of symmetrical diacylglycerols from (3).

Using the conditions above, the tricaprylmethyl ammonium salts of stearic, palmitic and myritic acids were reacted with (3). The results

of the TLC analysis of the product mixture are given in tables 7, 8 and 9. The results are comparable in all three cases. The reaction using TCMAP gave the lowest amount of the 1,2-diacylglycerol isomers (7% (w/w)) while those using TCMAS and TCMAM gave higher but equal amounts of the 1,2-diacylglycerol isomers. The 1,3-diacylglycerol was separated from the reaction mixture by TLC using silica gel containing 5% boric acid (in order to prevent unwanted isomerization[10]) and 2.5% (v/v) methanol in dichloromethane as the elution system. For high melting point diacylglycerols large quantities can be separated by recrystallization.[10,89] Mixtures of the 1,3- and 1,2-isomers in a

R_f values (1% v/v CH_3OH in CH_2Cl_2)	Composition % (w/w)	Inference (cf. 2.1.3)
0.08	10	Glycerol-1,2-dimyristate
0.16	90	Glycerol-1,3-dimyristate

Table 8: products from reaction of (3) with TCMAM after 4 hours in refluxing n-hexane.

R_f values (1% v/v CH_3OH in CH_2Cl_2)	Composition % (w/w)	Inference (cf. 2.1.3)
0.11	7	Glycerol-1,2-dipalmitate
0.23	93	Glycerol-1,3-dipalmitate

Table 9: products from reaction of (3) with TCMAP after 4 hours in refluxing n-hexane.

solid state can be isomerised to 99% 1,3-isomer by heating.[10,12,89,90] Liquid 1,3- and 1,2-diacylglycerols are separated either by TLC or column chromatography or HPLC. Preparative amounts of 1,3- and 1,2-diacylglycerols from the mixtures above were separated by HPLC using the conditions described in Part A (3.2.2). The pure 1,3- and 1,2-diacylglycerols were used for subsequent synthetic and analytical purposes.

2.1.2.5 Preparation of symmetrical diacid triacylglycerols.

The final stage of the synthesis was the acylation of the pure 1,3-diacylglycerols with 10% molar excess of the appropriate acid chloride in dry chloroform containing pyridine as the HCl scavenger (Scheme: 23). The triacylglycerols were separated from the product mixture by TLC with silica gel, containing boric acid. The yield of triacylglycerols produced was very high (88.3% to 98.9%). Five symmetrical diacid triacylglycerols were prepared as listed:

1: glycerol-1,3-dimyristate-2-laurate.

2: glycerol-1,3-dimyristate-2-palmitate.

3: glycerol-1,3-dipalmitate-2-myristate.

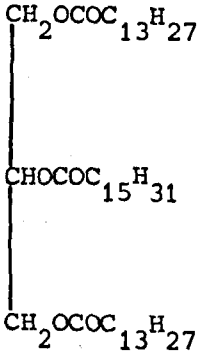
4: glycerol-1,3-distearate-2-myristate.

5: glycerol-1,3-distearate-2-oleate.

2.1.2.6 Determination of the purity of the triacylglycerols prepared.

The 5 symmetrical diacid triacylglycerols prepared were characterized by GLC for their molecular weight and by lipolysis[91] with pancreatic lipase for their structural purity. GLC analysis confirmed the molecular weight of the triacylglycerols prepared and showed no

contamination of any particular triacylglycerol with other triacylglycerol species of lower or higher molecular weights. The pancreatic lipase catalyses the hydrolysis of the primary ester linkages[92] only, leaving the secondary ester linkages intact. The 2-monoacylglycerols produced by lipolysis were analysed by GLC, as their methyl esters, after transesterification with sodium methoxide in dry methanol. The triacylglycerols had purities lying between 98.4% to 99.1% (w/w) (2.1.3.9). Theoretically the purities should be 100%, but the partial acylglycerols produced are liable to acyl migration so that acids present in the 1- or 3-positions may migrate to the 2-position. Also impurities of non-specific enzymes may produce 1-monoacylglycerols. Under ideal conditions, the results may be 99% or higher but when under the worst conditions the percentage impurity may be higher. Therefore the triacylglycerols prepared have an almost theoretical structural purity.



A typical symmetrical diacid triacylglycerol.

(Glycerol-1,3-dimyristate-2-palmitate)

2.1.3 EXPERIMENTAL.

All solvents were Analar or were purified and dried by standard methods. In the synthetic work solvents were distilled from the reaction mixture using a rotary thin-film evaporator. All fatty acids were obtained commercially and most of them were 99% pure (GLC). Anhydrous glycerol was distilled just before use: the first and last 15% were discarded. Melting points were determined on a Gallenkamp apparatus in open capillary tubes and were not corrected. Toluene-p-sulphonyl chloride was Analar grade and was stored under nitrogen. In tricaprylmethyl ammonium chloride (Aliquat 336) the alkyl groups are a mixture of C_8-C_{12} straight chains with an average chain length of 10 carbon atoms and a molecular weight of approximately 507. Anhydrous magnesium sulphate was used for drying solutions unless otherwise mentioned. PMR was performed on a Perkin-Elmer R32 (90 MHz) machine with tetramethylsilane (TMS) as the internal standard and deuterated chloroform ($CDCl_3$) as the solvent and the infrared data were obtained using a Pye-Unicam SP1000, unless otherwise stated.

2.1.3.1 Preparation of glycerol-1,3-ditosylate.

a. Method.

To a stirred mixture of anhydrous glycerol (4.97 g, 54 mmol) and dry pyridine (50 cm^3) in a 250 cm^3 round bottom (r.b.) flask, fitted with a pressure equalising funnel and a calcium chloride guard-tube, was added slowly at 0°, toluene-p-sulphonyl chloride (20.59 g, 108 mmol), dissolved in dry pyridine. The flask then was secured with a calcium chloride guard tube and the mixture was left to react for 44 hours in the fridge (0-3°). The heterogenous mixture was poured over crushed ice

and acidified with conc. HCl. The organic layer was separated and the aqueous layer washed with chloroform (2 x 100 cm^3). The combined organic extracts were washed successively with 1 mol dm^{-3} hydrochloric acid (2 x 20 cm^3), distilled water (20 cm^3), saturated sodium carbonate solution (2 x 20 cm^3) and distilled water (2 x 20 cm^3) and dried. When the chloroform was distilled off a colourless viscous product (21.73 g) was obtained.

b. Analysis of the crude mixture.

b.1 TLC analysis.

Plates of silica gel G 60 (20 cm x 20 cm and 0.5 mm thick) were prepared by a standard method[93] and activated at 100°C for 1 hour before use. A mixture of methanol and dichloromethane (5:95, v/v) was used as the eluting solvent system. Bands or spots were located by spraying the plates with 0.2% ethanolic solution of 2,7'-dichlorofluorescein and viewing under an UV light. For identification and quantification the spots were located and the silica gel of each area immediately scraped off the plate and washed with hot chloroform (3 x 10 cm^3). The solvent then was evaporated and the eluted compounds analysed.

Analytical TLC showed three spots of R_f values 0.70, 0.55 and 0.24. The R_f value for toluene-p-sulphonyl chloride was 0.66.

Preparative TLC showed that the crude product consisted mainly of three components, as follows.

Compound of R_f 0.70 formed 7.4% (w/w) of mixture.

Compound of R_f 0.55 formed 90% (w/w) of mixture.

Compound of R_f 0.24 formed 2.6% (w/w) of mixture.

b.2 HPLC.

b.2.1 Apparatus and conditions.

CE 210 liquid chromatograph, Cecil Instruments.

CE Variable wavelength UV monitor, Cecil Instruments.

Column, 25 cm x 4 mm (i.d.) packed with 5 μm silica.

Wavelength set at 254 nm.

Solvent system: 4% (v/v) methanol in dichloromethane (v/v).

Flow rate: 0.7 cm^3 mm^{-1}.

b.2.2 Analysis of the three main components from the preparative TLC.

If the products of R_f values 0.74, 0.55 and 0.24 were the tri-, di- and monotosylates respectively, then the intensity of absorption of the glycerol tritosylate should be three times as much as that of the mono, and that of the ditosylate twice as much as that of the monotosylate. The expected intensity ratios with the other results are given in Table 10.

Compound of R_f	Concentration/ mg cm^{-3}	Amount inj./μl	Retention time/min	Expected intensity ratios	Intensity ratios from peak areas
0.70	1.07	10	3.7	2.5	2.6
0.55	1.02	10	4.0	1.9	1.8
0.24	0.87	10	9.4	1.0	1.0

Table 10: separation and analysis of the glycerol tosylate products.

There is good agreement between the experimental results and the theoretical values. Therefore the product of R_f 0.55 was the glycerol ditosylate.

b.3 Separation of the glycerol distosylate by flash chromatography.

Apparatus and conditions.

Column: 50 cm x 2 cm (i.d.) glass column.

Silica gel 60: 230-400 mesh.

Solvent system: 2% (v/v) methanol in dichloromethane.

Flow rate: 15 cm^3 min^{-1}.

Nitrogen pressure employed 4-5 psi.

The method is a modified version of the procedure described by Clark.[86] Into the dry-packed column, two cotton wool plugs were introduced one at the top and one at the bottom. Chloroform was introduced into the column at a pressure of 10 psi until the silica gel was freed from air pockets (ca. 10 min). The flow rate was set (15cm^3 min^{-1}) and the sample (ca. 1 g) was introduced as a chloroform solution (2 cm^3) and forced through the column at the set flow rate without letting the solvent sink below the top of the cotton wool plug. The sample container was washed with more chloroform (2 cm^3) and the washing introduced into the column in the same way. The column was connected to a continuous supply of 2% (v/v) methanol in dichloromethane which was pumped at a regular flow rate of 15cm^3 min^{-1}. When the run was finished the silica gel was washed, dried and reused. Fractions of 20 cm^3 were collected and analysed by TLC. Fractions 6, 7, 8 and 9 were found to contain most of the pure glycerol-1,3-ditosylate (cf. 2.1.2.2).

b.4 Analytical data for (3).

PMR. The PMR was run on a Brucker 200 MHz instrument.

δ: 2.41 (s*, 6H, $-CH_3$), 2.72 (unresolved s, 5H, CH_2-CH-CH_2), 4.00

*s = singlet, d = doublet, t = triplet and m = multiplet.

(s, H, OH), 7.31 and 7.72 (2d, 8H, aromatic C-H).

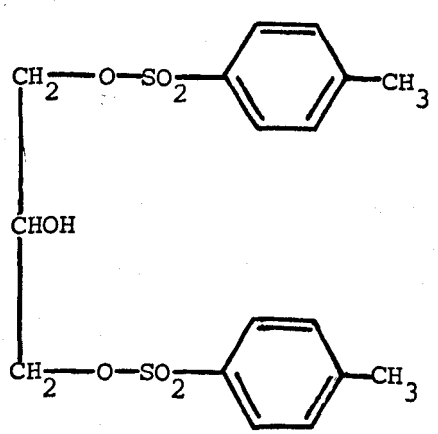
The PMR values agree with the structure of (3).

CI-MS. The chemical ionization spectra were obtained using isobutane as a reagent gas at a source ionizing pressure of ca.8 x 10^{-5} torr. The source was operated with a 100 eV electron beam, an electron current of 500 μA and a 2 kV accelerating voltage.

m/z (major and significant ions): 401 [M^++1], 229 ($C_{10}H_{12}O_4S+H)^+$, 215($C_9H_{10}O_4S+H)^+$; 173($C_7H_7O_3S+2H)^+$, 157($C_7H_7O_2S+2H)^+$ and 139($C_7H_6OS+H)^+$. The fragmentation is thus consistent with the structure of glycerol-1,3-ditosylate,(3). It should be noted that fragment ions from the isomeric glycerol-1,2-ditosylate are absent.

Infrared. $\bar{\nu}/cm^{-1}$ (Inferences): 1175 and 1355 (SO_2^-), 2900 and 2930 (CH aliphatic), 1600 and 3100 (CH aromatic).

The analytical data above confirm (3) as glycerol-1,3-ditosylate. (3) is an odourless and colourless viscous liquid ($n_D^{30°}$ = 1.5437, $n_D^{20°}$ = 1.5490 and $n_D^{10°}$ = 1.5502) which solidified on long-term standing (m.p: 45-46°).



(3)

2.1.3.2 Preparation of stearyl chloride.

Pure stearic acid (99% GLC) was treated with a 1.4 molar excess of redistilled thionyl chloride. The reaction was completed by refluxing for 2 hours with the exclusion of moisture. The excess thionyl chloride was removed under reduced pressure. The slightly yellow acid chloride was used without any further purifications.

Other acid chlorides (palmityl and myristyl) were prepared in the same way.

2.1.3.3 Preparation of oleoyl chloride.

Oleic acid was treated with 1.8 molar excess of oxalyl chloride and stirred for 3 days at room temperature, with the exclusion of moisture. The excess oxalyl chloride was removed and the oleoyl chloride purified by distillation under reduced pressure.

2.1.3.4 Preparation of sodium stearate and similar fatty acid salts.

Pure stearic acid (7.9 g, 0.03 mole) was dissolved in warm acetone (150 cm^3). 5 mol dm^{-3} sodium hydroxide solution (5.27 cm^3) was added dropwise over a 10 min period. The contents were stirred vigorously, with warming, for a further 2 hours. The sodium stearate was filtered under suction, washed with ice-cold water (2 x 30 cm^3) and air-dried. The course granular material was ground to a fine powder and extracted with diethyl ether using a soxhlet apparatus for 24 hours. The product was dried to constant weight over P_2O_5 under vacuum at a temperature of 45° and a pressure of 3.0 mm Hg. Analysis of the product by infrared showed it to be free of moisture and carboxylic acid.

2.1.3.5 Preparation of tricaprylmethyl ammonium stearate and similar salts of other fatty acids.

5 mol dm^{-3} methanolic potassium hydroxide (6 cm^3) was added to a solution of tricaprylmethyl ammonium chloride (15.2 g, 0.03 mol) in methanol (40 cm^3). The mixture was stirred for 15 min, cooled to 0° and stirred for a further 15 min. The chilled solution was filtered into a warm methanolic solution of pure stearic acid (8.5 g, 0.03 mol). The warm mixture was stirred vigorously for 30 min; the solvent then was removed to yield a viscous liquid. The product was dissolved in n-hexane (100 cm^3), dried, filtered and the solvent removed. The product was dried to constant weight over phosphorus pentoxide in a vacuum at a temperature of 45° and a pressure of 3.00 mm Hg.

The palmitate and myristate salts were prepared in the same way.

2.1.3.6 Reactions of glycerol-1,3-ditosylate.

a. Reaction of glycerol-1,3-ditosylate with sodium stearate in n-hexane.

a.1 Method.

A mixture of glycerol-1,3-ditosylate (0.25 g, 0.6 mmol) and sodium stearate (0.50 g, 1.6 mmol) in dry n-hexane (20 cm^3) was heated and stirred under anhydrous conditions for 4 hours at 79° (oil bath) in a 50 cm^3 r.b. flask fitted with a water cooled condenser and a calcium chloride guard-tube. The n-hexane was removed using a rotary evaporator. The reaction product was dissolved in chloroform (20 cm^3), the salts were filtered off and washed with chloroform (2 x 5 cm^3). The chloroform extracts were combined and analysed.

a.2 Analytical data.

TLC. For plate preparation see section 2.1.3.1 b. Two compounds were separated (R_f values of 0.00 and 0.15) when using 1% (v/v) methanol in dichloromethane (Table 4).

a.2.1 Compound (4) (R_f = 0.00 or R_f = 0.30 in 5% (v/v) methanol in dichloromethane) had R_f values similar to those of glycerol monostearate in the same solvent systems.

GLC. The gas chromatograph used was a Pye 104 Chromatograph with dual flame-ionization detectors. Two different glass columns were used, 1.85 m x 2.5 mm i.d. or 0.45 m x 2.5 mm i.d., both packed with 3% OV17 on Supelcoport (100-120 mesh). Samples other than the triacylglycerols were silylated using N-trimethylsilylimidazole.[94] The nitrogen flow rate was 40 ml min^{-1} for 1.85 m columns and 60 ml min^{-1} for the 0.45 m columns. The detector temperature was 360°. The retention time (r.t.) for compound (4) when silylated was 2.56 min (column temperature 240° for 20 min, then increased by 4° min^{-1} to 350°). Glycerol-monostearate r.t. under the same conditions was 2.56 min.

Infrared. $\bar{\nu}/cm^{-1}$ (Inferences): 1735 (C=O ester) and 3350 (OH).

The analysis confirms the compound as glycerol monostearate.

a.2.2 Compound (5) had an R_f value of 0.15 which is similar to that of glycerol-1,3-ditosylate under the same conditions.

Infrared. $\bar{\nu}/cm^{-1}$ (Inferences): 1355 and 1175 (S=O of SO_2), 1600 (C=C aromatic), 2930 and 2900 (C-H aliphatic) and 3010 (CH aromatic).

PMR. δ: 2.43 (s, 6H, CH_3); 2.72 (s, unresolved, 5H, CH_2-CH-CH_2),

4.03 (s, OH), 7.33 and 7.77 (d, 8H, aromatic C-H).

The analytical data above confirm compound(3) to be glycerol-1,3-ditosylate.

b. Reaction of glycerol-1,3-ditosylate with sodium stearate in DMSO.

b.1 Method.

A mixture of glycerol-1,3-ditosylate (0.18 g, 0.5 mmol) and sodium stearate (0.37 g, 1.2 mmol) in dried DMSO (20 cm^3) was heated and stirred under anhydrous conditions for 4 hours at 79° (oil bath) in a 50 cm^3 r.b. flask fitted with a water cooled condenser and a calcium chloride guard-tube. The reaction mixture was cooled to room temperature and poured into water (60 cm^3) to precipitate the products . Sodium chloride was added, with stirring, until the solvents was clear. The products were partitioned into warm n-hexane (3 x 20 cm^3) and the combined extracts washed with distilled water (2 x 10 cm^3), aqueous sodium carbonate (2 x 10 cm^3) and distilled water (2 x 10 cm^3) and dried. Chloroform was added to the n-hexane solution to ensure that the products remained in solution. The solution then was filtered off and analysed.

b.2 Analytical data.

6 compounds were separated by TLC (Table 5).

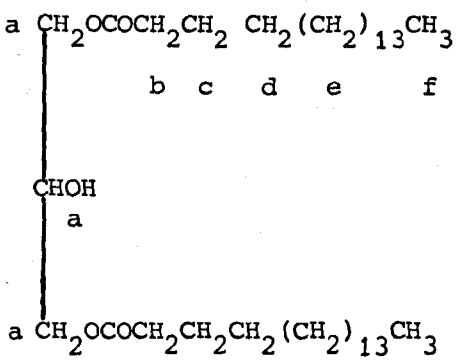
b.2.1 Compound(4) had the same analytical data as compound(4) (a.2 above) and therefore was identified as glycerol monostearate.

b.2.2 Compound(3) had the same analytical data as compound(3) (a.2 above). Therefore it was identified as glycerol-1,3-ditosylate.

b.2.3 Compound (5): R_f = 0.30 (solvent system: 1% (v/v) methanol in dichloromethane).

Infrared. $\bar{\nu}/cm^{-1}$ (Inferences): 1720 and 1740 (C=O of ester groups) and 3450 (OH).

PMR. δ; 4.17: (a); 2.36: (b); 1.98: (c); 1.64: (d); 1.29: (e); 0.9: (f).



GLC. A non-silylated sample run on a 0.45 m column gave a retention time of 5.2 min (304° column temperature, isothermic conditions and for other conditions refer to a.2 above). When silylated the retention time was 41.0 min (column length 1.85 m, 245° held for 20 min, then 4° min^{-1} to 350° (for other conditions refer to a.2 above)).

All these data agree with those of glycerol-1,3-distearate.

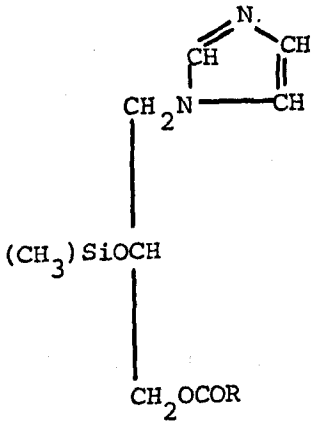
b.2.4 Compound (7): R_f = 0.59 (1% v/v methanol in dichloromethane).

Infrared. $\bar{\nu}/cm^{-1}$ (Inferences): 1755 (C=O of ester) and 3020 (C-H of epoxide).

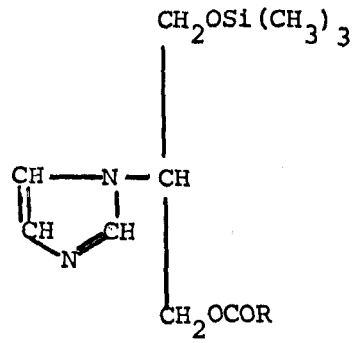
GLC. (Conditions similar to those of compound (5)). When silylated retention times of the addition products were 20.5 min and 23.4 min. When non-silylated the retention time was 2.6 min.

The data given above are similar to those for 1-stearate-2,3-epoxypropane . Hence compound (7) was 1-stearate-2,3-epoxypropane . .

The addition products formed on treatment with TMSI (retention times of 20.5 and 23.4 min) were as follows.



and



The rest of the compounds in the reaction mixture were not identified mainly because of the small amounts present in the mixture and the absence of reference compounds.

c. Reaction of glycerol-1,3-ditosylate with TCMAS in DMSO.

c.1 Method.

A mixture of glycerol-1,3-ditosylate (0.34 g, 0.8 mmol) and TCMAS (1.71 g, 2.3 mmol) in freshly-distilled DMSO (20 cm^3) was heated and stirred under anhydrous conditions for 4 hours at 79° (oil bath) in a 50 cm^3 r.b. flask, fitted with a water cooled condenser and a calcium chloride guard-tube. The reaction mixture was cooled to room temperature and poured into water (60 cm^3) to precipitate the products. Solid sodium chloride was added, with stirring, until the solvent was clear. The products were partitioned into warm hexane (3 x 20 cm^3) and the combined extracts washed with water (2 x 10 cm^3) and dried. Chloroform (20 cm^3) was added to the hexane solution to ensure that the products remained in solution. The solvents then were removed. The products were precipitated from the residue by adding methanol-water mixture (85:15 (v/v), 20 cm^3). The mixture was cooled to 0° and allowed to stand for 15 min. The products were filtered under suction and washed with further portions of the ice-cold methanol-water mixture (2 x 10 cm^3) Hydrolysis of the TCMAS might take place and removal of the free fatty acid was necessary. The products were dissolved in chloroform (20 cm^3), dried, filtered and analysed.

c.2 Analytical data.

6 compounds were separated by TLC (Table 6).

c.2.1 Compound (4) had the same analytical data as Compound (4) (a.2.1 above) and therefore was identified as glycerol monostearate.

c.2.2 Compound (6) had the same R_f value (0.16) as glycerol-1,2-distearate.

Infrared. $\bar{\nu}/cm^{-1}$ (Inferences): 1725 and 1740 (C=O ester groups) and 3400 (OH).

GLC. The retention time was 5.2 min using a 0.45 m column at 304°C under isothermic conditions. The retention time was similar to glycerol-1,2-distearate and glycerol-1,3-distearate standards.

When silylated (conditions given in a.2 above) the retention time was 39.8 min .

The analytical data agree with those of glycerol-1,2-distearate and confirm compound (6) as glycerol-1,2-distearate.

c.2.3 Compound (5): the analytical data identified compound (5) as glycerol-1,3-distearate.

The rest were unknowns. GLC on all unknowns showed that their molecular weights were lower that those of the corresponding diacylglycerols.

d. Reaction of glycerol-1,3-ditosylate with TCMAS in n-hexane.

d.1 Method.

A mixture of glycerol-1,3-ditosylate (0.23 g, 0.6 mmol) and TCMAS (1.10 g, 1.5 mmol) in dry n-hexane (20 cm^3) was heated and stirred under anhydrous conditions for 4 hours at 79° (oil bath) in a 50 cm^3 r.b. flask fitted with a water cooled condenser and a calcium chloride guard-tube. The n-hexane then was removed. The products were precipitated from the residue by adding methanol-water mixture (85:15 (v/v), 20 cm^3). The mixture was cooled to 0° and allowed to stand for 15 min. The products were filtered under suction and washed with further portions of the ice-cold methanol-water mixture (2 x 10 cm^3). Hydrolysis of the

TCMAS might take place and removal of the free fatty acids was necessary. The products were dissolved in chloroform (20 cm^3), dried, filtered and analysed.

d.2 Analytical data.

Only two compounds were separated by TLC (cf. Table 7). For other conditions refer to a.2.1 above .

Compound (6) was identified as glycerol-1,2-distearate.

Compound (5) had an R_f value of 0.29 and the same analytical data as compound (5), (6-23), and therefore was identified as glycerol-1,3-distearate.

2.1.3.7 Preparation of the symmetrical diacylglycerols.

a. Glycerol-1,3-distearate.

A mixture of glycerol-1,3-ditosylate (0.23 g, 0.6 mmol) and TCMAS (1.1 g, 1.5 mmol) was reacted in dry hexane as described in 2.1.3.6 d. The crude product, free from acid, contained 90% (w/w) glycerol-1,3-distearate and 10% (w/w) glycerol-1,2-distearate. The two isomers were separated by TLC on silica containing 5% boric acid (w/w) with 1% (v/v) methanol in dichloromethane. For other conditions refer to 2.1.3.1 b . The yield of glycerol distearate was 0.22 g or 61.2% (w/w) based on glycerol-1,3-ditosylate. The diacylglycerols were recrystallized from methanol as white powdery crystals before TLC separation.

Glycerol-1,3-distearate had a m.p. of 76.5-77.0°, (Lit. 79.1°[95], highest melting form, 76°[96]).

b. Glycerol-1,3-dipalmitate.

A mixture of glycerol-1,3-ditosylate (0.54 g, 1.3 mmol) and tricaprylmethyl ammonium palmitate (2.40 g, 3.4 mmol) was reacted and the products separated as in (a) above. The yield of glycerol dipalmitate was 0.51 g or 66.0% (w/w) based on glycerol-1,3-ditosylate. The

product was recrystallized from methanol. It contained 93% (w/w) of glycerol-1,3-dipalmitate and 7% (w/w) of glycerol-1,2-dipalmitate. The two isomers were separated by TLC (Table 9) in the way used for glycerol distearate ((a) above).

Glycerol-1,3-dipalmitate had a m.p. of 70-71°, (Lit. 74.1°[8], highest melting form, 76°[63]).

c. Glycerol-1,3-dimyristate.

A mixture of glycerol-1,3-ditosylate (0.54 g, 1.3 mmol) and tricaprylmethyl ammonium myristate (2.28 g, 3.4 mmol) were reacted and the products separated as in (a) above. The yield of glycerol dimyristate was 0.40 g or 56.1% (w/w) based on glycerol-1,3-ditosylate. The product was recrystallized from methanol as white powdery crystals. It contained 90% (w/w) of glycerol-1,3-dimyristate and 10% (w/w) of glycerol-1,2-dimyristate. The two isomers were separated by TLC (table 8) in the way used for glycerol distearate ((a) above).

Glycerol-1,3-dimyristate had a m.p. of 65.5°, (Lit. 65°[96], 64°[97]).

2.1.3.8 Preparation of triacylglycerols.

To a mixture of 1 mol 1,3-diacylglycerol, 1.3 mol dry pyridine and dry chloroform as a solvent, the appropriate acid chloride (1.2 mol) was added dropwise at 0° while stirring. The acid chloride was diluted in half dry chloroform out of the total volume of dry chloroform to be added to the reaction mixture. The reaction was allowed to proceed at room temperature for 72 hours. The chloroform then was removed and the product residue dissolved in ethyl acetate. The solution was washed with distilled water, 1.0 mol dm^{-3} HCl, water, aqueous sodium carbonate

and water, and dried. The solvent was removed and the products separated and purified by TLC.

Five symmetrical triacylglycerols were prepared using the procedure above with the yields being almost quantitative.

a. Glycerol-1,3-dimyristate-2-laurate.

Glycerol-1,3-dimyristate (0.02 g, 0.03 mmol) was reacted with lauryl chloride as described in 2.1.3.8 to obtain, after TLC separation, 0.03 g of glycerol-1,3-dimyristate-2-laurate (92.0% (w/w) yield). The GLC retention time on a 0.45 m column (260 to 350°, 6° min^{-1}), was 8.65 min. For other conditions refer to 2.1.3.6 a . GLC analysis of the product formed by lipase hydrolysis (2.1.3.9) gave 98.2% (w/w) $C_{12:0}$ and 1.8% (w/w) $C_{14:0}$.

b. Glycerol-1,3-dimyristate-2-palmitate.

Glycerol-1,3-dimyristate (0.04 g, 0.08 mmol) was reacted with palmityl chloride as described in 2.1.3.8 to obtain after TLC separation 0.04 g of glycerol-1,3-dimyristate-2-palmitate (88.3% (w/w) yield), The GLC retention time on a 0.45 m column (conditions similar to (a) above) was 11.35 min. GLC of the product formed by lipase hydrolysis (2.1.3.9) gave 99.1% (w/w) $C_{16:0}$ and 0.9% (w/w) $C_{14:0}$.

c. Glycerol-1,3-dipalmitate-2-myristate.

Glycerol-1,3-dipalmitate (0.03 g, 0.06 mmol) was reacted with myristyl chloride as described in 2.1.3.8 to obtain, after TLC separation 0.04 g of glycerol-1,3-dipalmitate-2-myristate (98.9% (w/w) yield). The GLC retention time on a 0.45 m column (conditions similar to (a) above) was 12.6 min. GLC of the product formed by lipase

hydrolysis (2.1.3.9) gave 98.8% (w/w) $C_{14:0}$ and 1.2% (w/w) $C_{16:0}$.

d. Glycerol-1,3-distearate-2-myristate.

Glycerol-1,3-distearate (0.02 g, 0.02 mmol) was reacted with myristyl chloride as described in 2.1.3.8 to obtain, after TLC separation, 0.03 g of glycerol-1,3-distearate-2-myristate (89.0% (w/w) yield). The GLC retention time on a 0.45 m column (conditions similar to (a) above) was 15.5 min. GLC of the product formed by lipase hydrolysis (2.1.3.9) gave 98.4% (w/w) $C_{14:0}$ and 1.6% (w/w) $C_{18:0}$.

e. Glycerol-1,3-distearate-2-oleate.

Glycerol-1,3-distearate (0.01 g, 0.02 mmol) was reacted with oleoyl chloride as described in 2.1.3.8 to obtain, after TLC separation, 0.01 g of glycerol-1,3-distearate-2-oleate (95.1% (w/w) yield). The GLC retention time on a 0.45 m column (conditions similar to (a) above) was 18.2 min. GLC of the product formed by lipase hydrolysis (2.1.3.9) gave 98.7% (w/w) $C_{18:1}$, 0.8% (w/w) $C_{18:0}$ and the rest were $C_{16:0}$ and $C_{14:0}$ impurities.

2.1.3.9 Lipolysis.

a. Reagents.

Pancreatin

Sodium tauroglycholate solution (0.05% (w/w)).

Buffer solution: 2-amino-2(hydroxymethyl)Propane-1,3-diol(tris) (1.2 mol, pH 8.0).

Calcium chloride: 22% solution (w/w).

Acetone (50 cm^3) was added to pancreatin (10 g) in a 200 cm^3 conical

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

b. Procedure[91] (hydrolysis of 5 to 10 mg of sample).

To the sample was added pancreatin (0.9 mg) as a freshly-made solution in the tris buffer (1 cm^3 solution of 9 mg pancreatin in 10 cm^3 of tris buffer). n-Hexane (0.25 ml) was added to the lipolysis medium to ensure a more homogeneous dispersion. Also added were 0.1 cm^3 of 22% calcium chloride solution and 0.25 ml of 0.05% bile salt solution. The vial and contents were warmed firstly on a water bath at 40° for 1 min without shaking. The cap then was tightly stoppered, secured with strips of plastic tape and the vial shaken for 10 min. The shaking speed was 300 shakes a minute. At the end of the reaction, the contents were acidified with 6.0 mol dm^{-3} HCl (0.5 ml) and extracted with diethyl ether (3 x 5 cm^3). The ether extracts were washed with water several times until the washings were neutral to Congo red paper, dried and the solvent evaporated. The lipolytic products were separated into mono-, di- and triacylglycerols and free fatty acids by TLC on 20 x 20 cm plates using methanol:dichloromethane:formic acid (5:94.5:0.5 v/v) as the elution system. The separated bands were visualized by spraying the plate with a 0.2% ethanolic solution of 2,7'-dichlorofluorescein and viewing under an UV light. To determine the fatty acid composition at the sn-2-position, the monoacylglycerols band was scraped off the plate and extracted with hot 10% (v/v) methanol in chloroform (3 x 10 cm^3). The solvent was removed under a stream of nitrogen to yield the monoacylglycerols. (If the hydrolysis is low, a monoacylglycerol

reference may be needed to locate the band of the product.).

c. Preparation of methyl esters.

1 mol dm^{-3} sodium methoxide solution (5 cm^3) was added to the monoacylglycerols fraction and the mixture refluxed for 5 min. The transesterified mixture was cooled and 0.5 mol dm^{-3} sulphuric acid added (5 cm^3). The esters were extracted with chloroform (2 x 10 cm^3), the chloroform extracts washed with water until neutral, dried and the volume reduced to 1.0 cm^3 under a stream of nitrogen.

d. Analysis of the methyl esters.

The gas chromatograph used was a Pye Unicam GCD Chromatograph with flame ionization detectors and a 1.85 m x 2.5 mm i.d. glass column containing 3% OV17 on Supelcoport for saturated methyl esters and 3% Apiezon on Supelcoport for unsaturated methyl esters. The carrier gas flow rate was 40 cm^3 min^{-1}. The methyl esters were identified by co-chromatography with reference compounds. For other GLC conditions refer to 2.1.3.6 a.

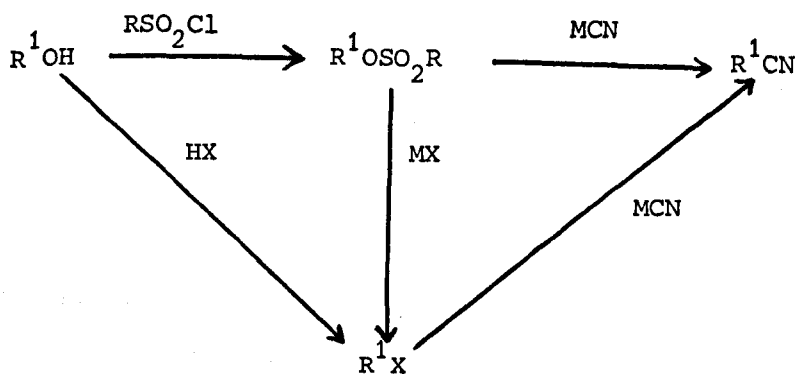
2.1.4 FINDINGS AND CONCLUSIONS.

The reaction of toluene-p-sulphonyl chloride with glycerol was very selective and only the 1,3-disubstituted derivative was obtained. The glycerol-1,3-ditosylate (5), produced in high yields, was isolated by flash chromatography and subjected to nucleophilic substitution reactions. Quaternary ammonium salts of fatty acids in n-hexane gave very good yields of the 1,3-diacylglycerols, there being only a little accompanying elimination. The substrate is very stable and, in contrast to the dihalide derivative (1.4), can be prepared easily. The method can be used for the preparation of symmetrical diacid triacylglycerols of high structural purity.

2.2 THE SILYL ETHER ROUTE

2.2.1 INTRODUCTION.

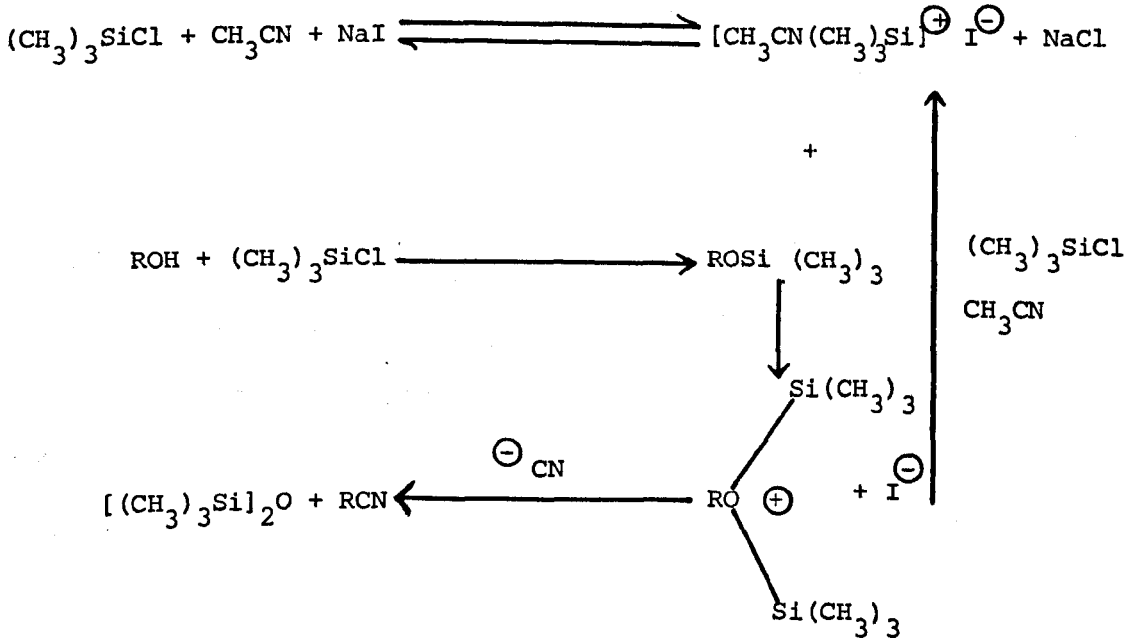
Silicon chemistry has seen important advances in the past decade. It is second only to carbon chemistry in the number of papers published every year.[98] Advances in the field have been reviewed.[99,100] Trialkylsilyl derivatives, in particular, are finding increasing utility as reagents in the synthesis of novel and complex organic molecules.[100,107,108] Nitriles are usually prepared via sulphonate esters or halide intermediates[101] (Scheme: 28) involving more than one step.



X = halide; M: alkali metal, R^1: alkyl group

Scheme: 28

In a recent publication[101] alcohols were reported to have been converted into nitriles in a one-step procedure. Nitriles were prepared by treating the alcohols with sodium cyanide and trimethylsilyl chloride in an acetonitrile/dimethyl formamide (DMF) solvent mixture, with sodium iodide serving as the catalyst. The mechanism suggested is outlined below (Scheme: 29). The trimethylsilyl chloride reacts with sodium iodide in acetonitrile to form a trimethylsilyl iodide complex



Scheme: 29

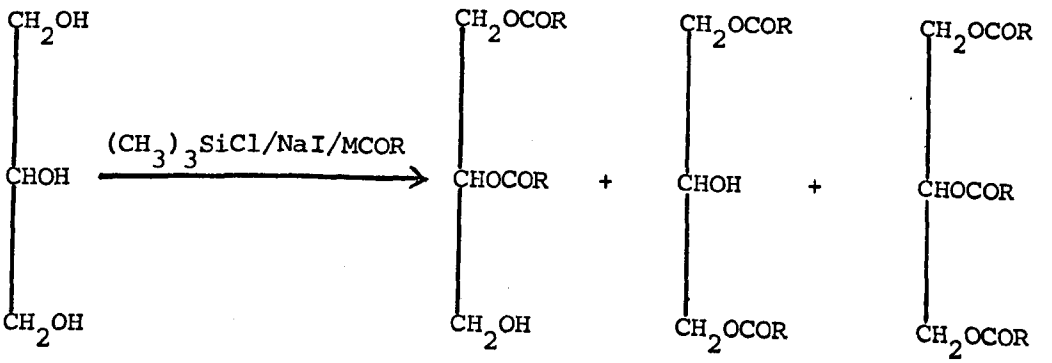
in situ which then reacts with the trimethylsilyl ether of the alcohol to form a bis-trimethylsilyl alkoxy iodide complex. The latter complex is unstable and disproportionates to form a nitrile and a bis-trimethylsilyl ether, the iodide complex being regenerated. The net result is nucleophilic substitution of a hydroxyl group by a cyanide group. It is proposed to adopt the same procedure to make symmetrical diacid triacylglycerols.

2.2.2 RESULTS AND DISCUSSION.

2.2.2.1 Acylation of glycerol via the trimethylsilyl ether derivative.

Anhydrous glycerol was reacted with trimethylsilyl chloride in acetonitrile/DMF solvent mixture using sodium iodide as a catalyst.

The carboxylate anion was introduced as an alkali-metal-fatty acid salt. Analysis of the product mixture revealed that it contained the diacylglycerols and the triacylglycerols (Scheme: 30). Two fatty acid



1,2-Diacyl-glycerol | 1,3-Diacyl-glycerol | Triacylglycerol

Scheme: 30

salts, sodium n-butyrate and sodium stearate were used. The glycerol di-n-butyrate and glycerol distearate were a mixture of both the 1,2- and 1,3-isomers. They constituted more than 70% (w/w) of the acylated products. The rest of the products were the corresponding triacyl-glycerols. The overall yields were low (10-15%). Increasing either the reaction time or reaction temperature did not improve the yields of the acylated products. Moreover the product mixture was not suitable for the preparation of specific triacylglycerols. Since the trimethyl-silyl ethers can be cleaved under the conditions mentioned above (Scheme: 29), it is better to prepare the 1,3-bis-trimethylsilyloxy-propan-2-ol (12) and then effect the nucleophilic substitution.

2.2.2.2 Trimethylsilyl ethers of glycerol.

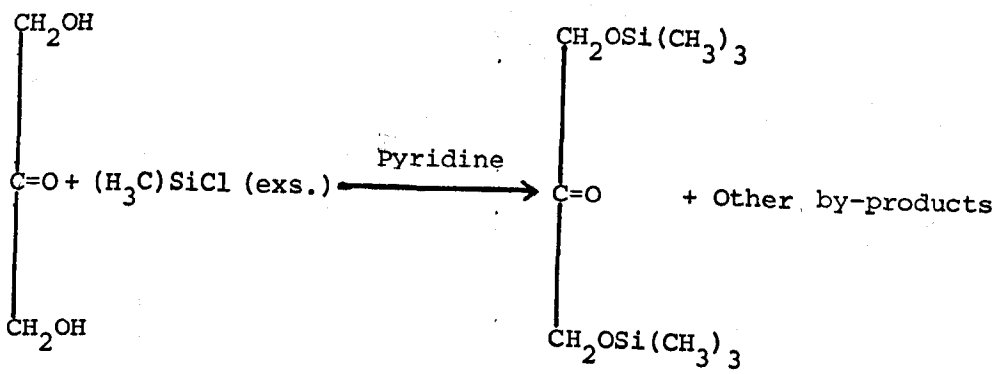
1 mol of glycerol was reacted with 2 mol of trimethylsilyl chloride in pyridine. After 24 hours, the product mixture was analysed. It contained mainly the trisilylated product (59.9% (w/w)) and two other components, the disilylated product (31.2% (w/w)) and the monosilylated product (8.9% w/w)). Although the yields of the trimethylsilyl ether derivatives were high (75.0%), in contrast to toluene-p-sulphonyl chloride (cf. route 2.1), trimethylsilyl chloride did not discriminate between the reactivities of the primary and secondary hydroxyl groups in the glycerol molecule. Further analysis showed that the disubstituted trimethylsilyl ethers of glycerols were a mixture of the 1,2- and 1,3-isomers. It was observed also that the trimethylsilyl ethers of glycerol are labile. On standing the percentage content of the trisilyl derivative of glycerol increased from 82% to 90% (GLC). An authentic sample of the disilylated derivative of glycerol was prepared from 1,3-dihydroxyacetone (1,3-DHA) in order to identify some of the components in the product of the glycerol/trimethylsilyl chloride reaction mixture. Since the reaction of the trimethylsilyl chloride was not selective, i.e. it did not discriminate between the primary and secondary alcohols, it is more convenient to prepare the disilyl derivative of glycerol (9) starting from 1,3-DHA.

2.2.2.3 Trimethylsilyl ethers of 1,3-DHA.

a. Reaction of 1,3-DHA with trimethylsilyl chloride.

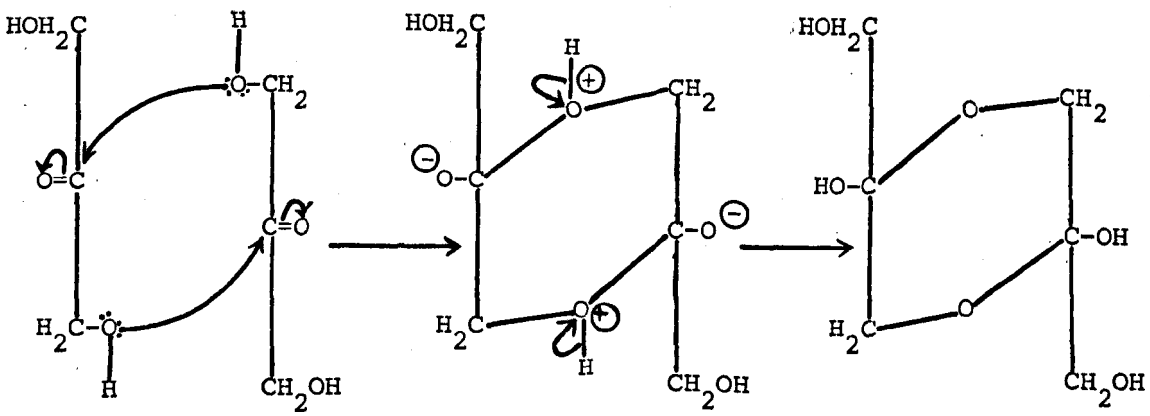
1,3-DHA was reacted with excess trimethylsilyl chloride for 24 hours in anhydrous pyridine. On analysis the product mixture showed four components instead of the expected disilylated product (9). They were identified as the mono- and disilylated monomer of 1,3-DHA and the tri-

and tetrasilylated derivatives of the dimer of 1,3-DHA. The amount of the disilylated monomer was only 28.5% (w/w) of the product mixture with



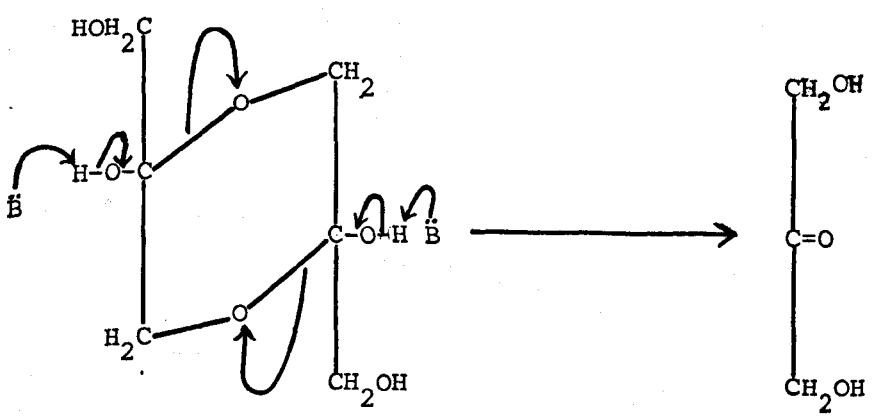
(8) (9)

the silylated derivatives of the dimer constituting 71.3% (w/w) of the products and the monosilylated monomer 0.2% (w/w). This unexpected observation was obviously due to the fact that commercial 1,3-DHA is supplied as a dimer, and that the reagent and conditions used in the present work were inadequate to convert the dimer (10) into the monomer (8). Therefore, the dissociation of the dimer (10) to the monomer (8) was studied. The monomeric form of 1,3-DHA (8) dimerizes slowly to form (10). Complete[104] dimerization takes 25-30 days (Scheme: 31). It is to be noted that in the preparation of 1,3-diacyl derivatives of 1,3-DHA, pyridine is used as co-reagent, and this must, therefore, be implicated in the depolymerization (Scheme: 32). The commercial 1,3-DHA was dissolved in anhydrous pyridine, and the mixture left to stand for different periods (table 11) before the addition of trimethylsilyl chloride. The product mixtures were analysed after 45 min of reaction time by GLC on a glass column (1.85 m x 2.5 mm i.d.) packed with 3% OV17.



(10)

Scheme: 31



(10) (8)

(B̈ = base)

Scheme: 32

Contact time of 1,3-DHA and pyridine before reaction / hours	% (w/w) Composition of product mixture after 45 min reaction			
	Monomer		Dimer	
	Monosilyl deriv.	Disilyl deriv.	Trisilyl deriv.	Tetrasilyl deriv.
0.25	0	0	68	32
1.00	0	18	40	42
1.50	0	17	44	39
2.00	3	37	41	19
3.00	6	62	20	13
4.00	4	73	17	6
5.00	6	77	13	4
6.00	9	85	5	1
7.50	9	90	1	0
24.00	7	92	0	1

Table 11: variation of the composition of 1,3-DHA silylated products with the dissociation time of the dimer of 1,3-DHA in pyridine.

When the first addition was made after 0.25 hour, there was hardly any mono or disilyl derivative of (8). The product mixture contained 68% and 32% (w/w)

of the tri- and tetrasilyl derivatives of (10) respectively. After 3 hours in pyridine, the product mixture contained 6% (w/w) of the monosilyl derivative of 1,3-DHA, 62% (w/w) of (9) and 20% and 13% (w/w) of the tri- and tetrasilyl derivatives of (10) respectively. After 7.5 hours the % (w/w) of (9) increased to 90%, while the silylated derivatives of (10) accounted for only 1% (w/w). When the time was increased further, the % of (9) did not change significantly (92% w/w, fig. 2). Usually a reaction mixture such as this (cf. 2.1.3) is neutralized and the products are extracted in diethyl ether or chloroform. Neither this procedure, nor heating the mixture, in order to distil out the products, was suitable to isolate (9). Both resulted in converting (9) into a mixture containing the trisilyl derivative of (10) and small amounts of the monosilyl derivative of 1,3-DHA and tetrasilyl derivative of (10). This unexpected change from the monomer form to the dimer form was thought to be the result of the catalytic action of pyridinium hydrochloride (11). To a distilled mixture containing the monosilylated monomer (49% w/w) and (9) (51% w/w) was added trimethylsilyl chloride in pyridine in order to fully silylate the 1,3-DHA monomer. After working up and analysing, the product mixture was found to contain 30% of silylated (10). Attempts to separate (9) by precipitating (11) at -50°C and extracting the product in a non-polar solvent such as n-hexane were not successful. The n-hexane extract contained pyridine only. Solvents such as chloroform dissolve (11), while ethylacetate in which (11) is sparingly soluble did not extract any product. Next bis-trimethylsilyl trifluoroacetamide, in place of trimethylsilyl chloride was investigated in order to avoid the formation of the by-product (11) which appeared to be the cause of the above mentioned problem.

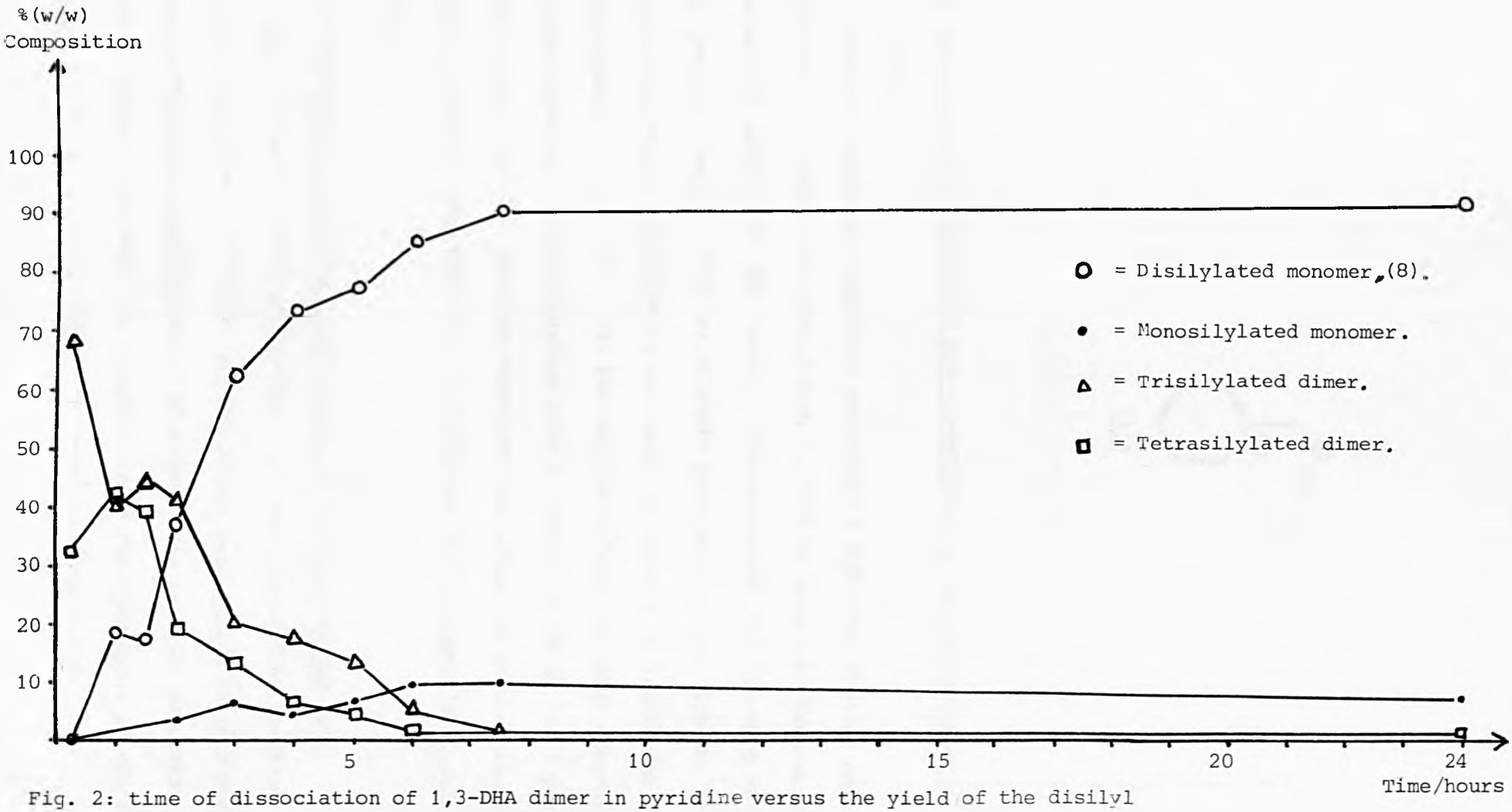
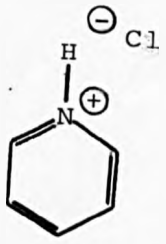


Fig. 2: time of dissociation of 1,3-DHA dimer in pyridine versus the yield of the disilyl derivative of the monomer.



(11)

b. Reaction of 1,3-DHA with bis-trimethylsilyl trifluoroacetamide (BSTFA).

Using the reaction conditions established above for getting maximum yield of (9), BSTFA was reacted with 1,3-DHA to give a product mixture containing mainly (9) (88% (w/w)). The silylated (10) was only 6% of the product mixture. The use of BSTFA gave by-products (Scheme: 33) which were relatively volatile and could be removed by fractional distillation (cf. 2.2.3). (9) was separated from the other components by distillation. Refractionation gave a product of 99.0% (w/w) purity (GLC trace: fig. 3). Now the substrate can either be reduced to the 1,3-disilyl ether of glycerol (12) or subjected to nucleophilic substitution.

2.2.2.4 Acylation of the silyl ethers of 1,3-DHA and glycerol.

The 1,3-disilyl ether of glycerol (12) was prepared by reacting the 1,3-disilyl ether of 1,3-DHA (9) with sodium borohydride in tetrahydrofuran (THF) and a small quantity of water. Two reaction products were formed, namely (12) and (13) (Scheme: 34). The hydrolysis of one of

(cont. p.145)

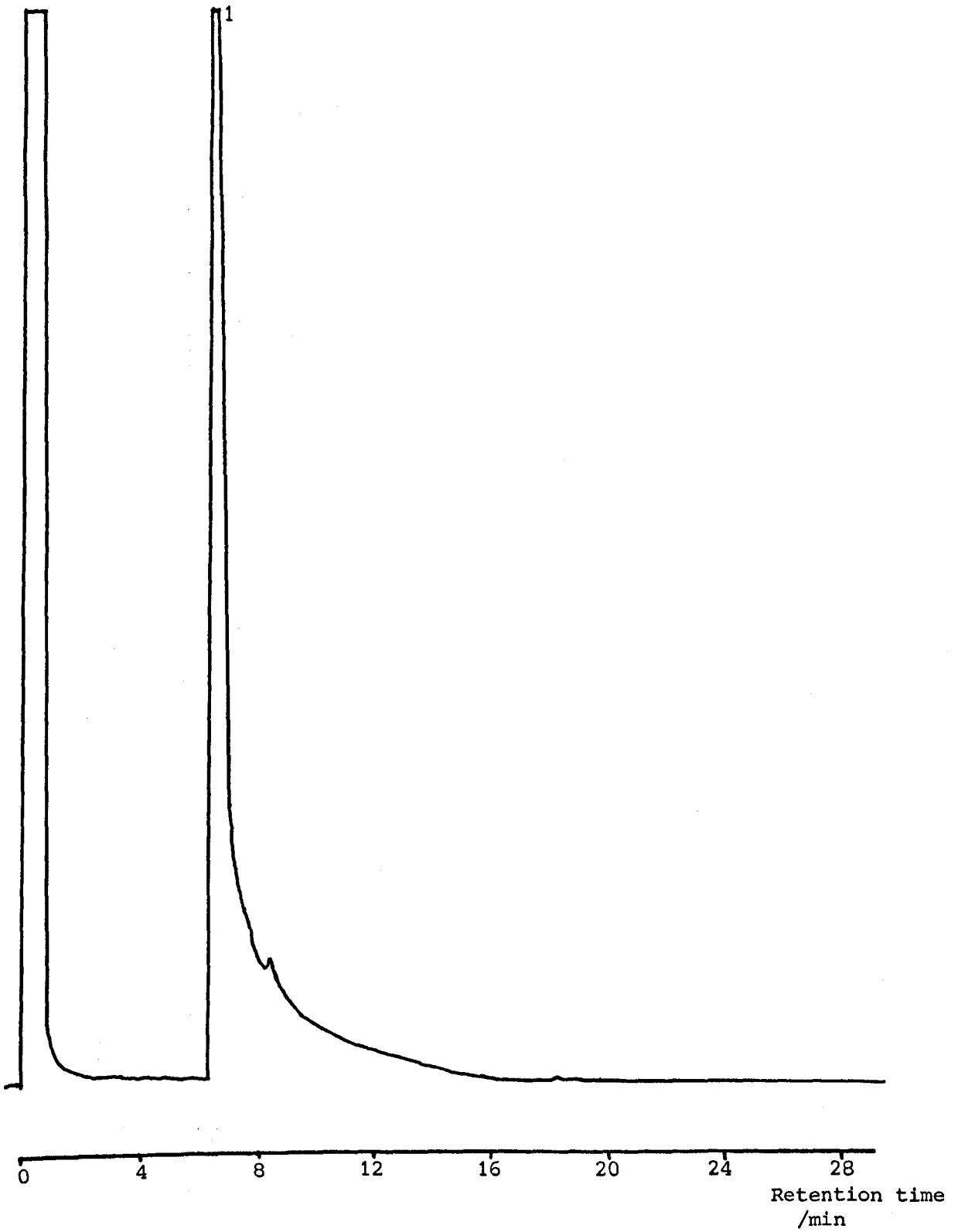
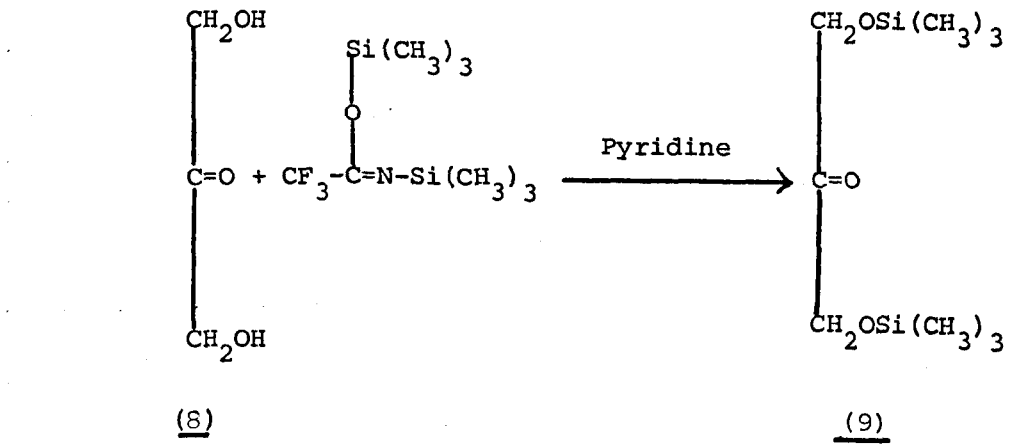
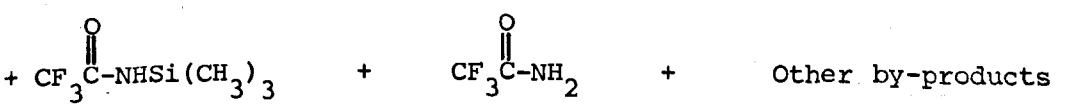


Fig. 3: 1,3-DHA + BSTFA (refractionated product).

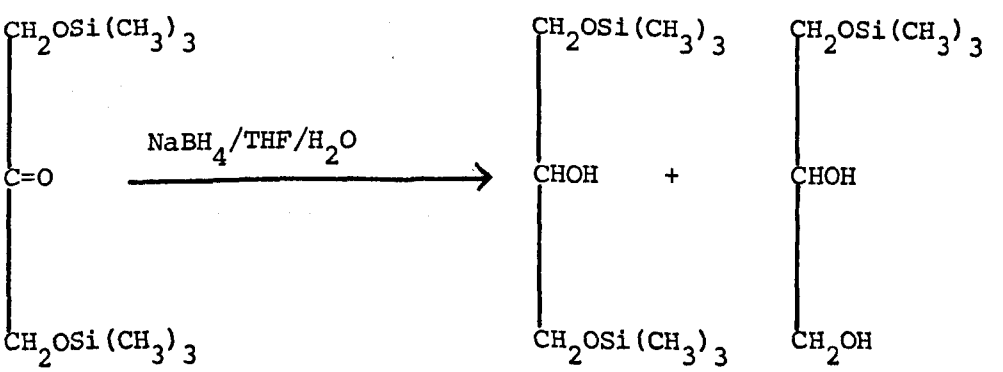
1 = disilyl derivative of the monomer of 1,3-DHA,(9),(99.06% pure). (cf. 2.2.3.3 for GLC conditions).



(8) (9)



Scheme: 33

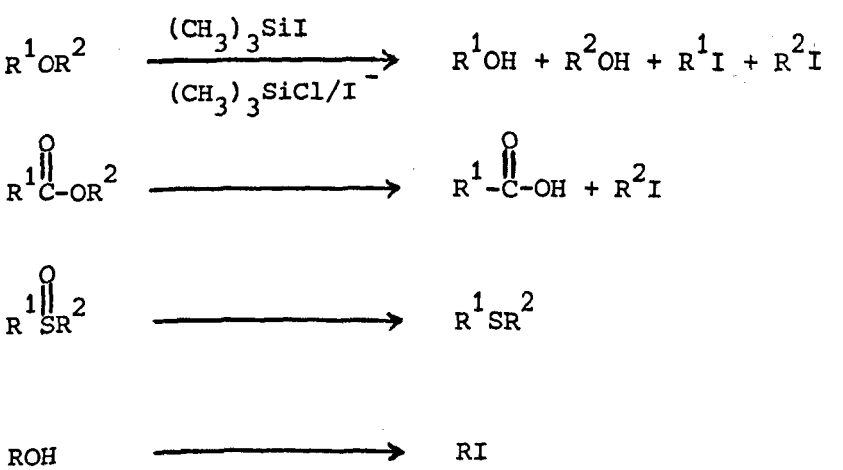


(9) (12) (13)

Scheme:34

the silyl groups must have occurred due to the basic conditions of the reaction mixture (NaOH). Other minor by-products were the silylated dimers of 1,3-DHA which were observed to form even under neutral aqueous conditions. Products (12) and (13) were separated by TLC (2.2.3).

Using the reaction conditions described for making nitriles (2.2.1), (12) and (9) were both reacted with sodium n-butyrate and trimethylsilyl chloride in acetonitrile/DMF solvent mixture with NaI serving as a catalyst, under anhydrous conditions. On analysis neither (12) nor (9) gave detectable amounts of the acylated products. The use of sodium stearate instead of sodium n-butyrate gave the same result. It appears that the reaction mechanism described in Scheme: 29 does not apply in this instance because of the difference in conditions i.e. silyl ethers were used here instead of the alcohols, although as shown in Scheme: 29, one of the initial steps was the formation of the silyl ether. Trimethylsilyl iodide, produced here *in situ*, has been used for mild, neutral, nonaqueous cleavage-hydrolysis reactions, deoxygenations, oxidations, halogenations etc.[103] Therefore the use of trimethylsilyl



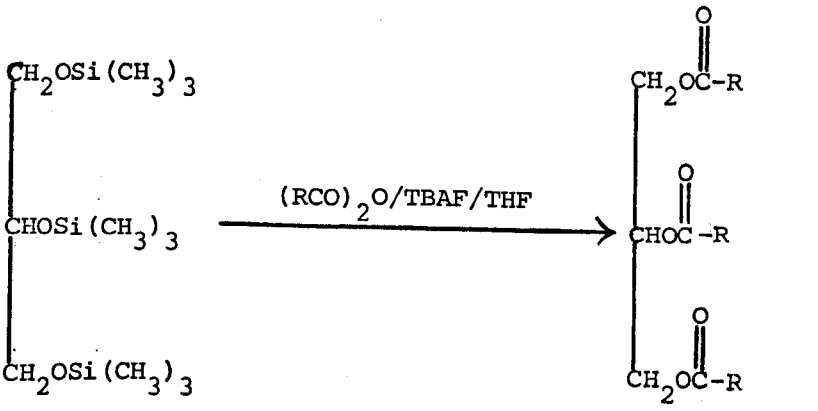
iodide can cleave ester groups under certain conditions.[108] Although the conditions here are not favourable, ester cleavage may be a side-reaction. The use of a more exposed nucleophile in the form of a tetralkyl ammonium salt such as tricaprylmethyl ammonium stearate (TCMAS) in refluxing n-hexane and in the reaction of (12) and (9) did not give any acylated products. Other conditions were next explored to substitute the trimethylsilyl group with a carboxylate anion.

2.2.2.5 Acylation with cleavage of the trimethylsilyl group using tetra-n-butyl ammonium fluoride (TBAF).

It has been reported[104] recently that selective acylation of an amide bond in a model dipeptide derivative has been achieved after treatment with trimethylsilyl chloride, triethyl amine and acetyl chloride. These conditions cannot be applied either to (12) or (9). However, TBAF is a powerful reagent for the cleavage of silyl ethers[105] and it was used in the acylation of sterically hindered hydroxyls and a direct replacement of silyl groups by acyl groups. Acylation was effected using TBAF and a fatty acid anhydride in THF.[106] These conditions were tried next.

a) Triacylglycerols from silyl ethers.

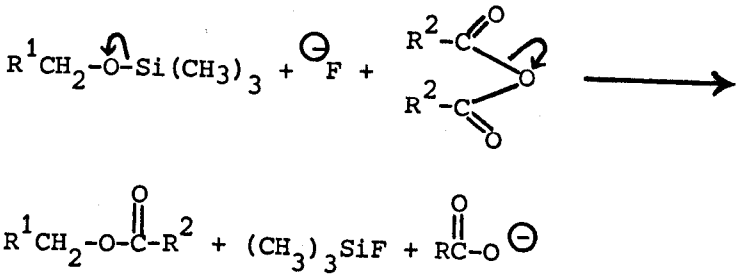
The trisilyl ether of glycerol (14) was reacted with a particular fatty acid anhydride in anhydrous THF using TBAF as a catalyst. The reaction mixture was refluxed for 20 hours under anhydrous conditions and the product(s) analysed. The major products were the triacylglycerols with the monoacylated and diacylated derivatives as the minor components (Scheme: 35).



(14) Major product

Scheme: 35

The reaction appears to take place as shown in Scheme: 36.



Scheme: 36

The following monoacid triacylglycerols were prepared.

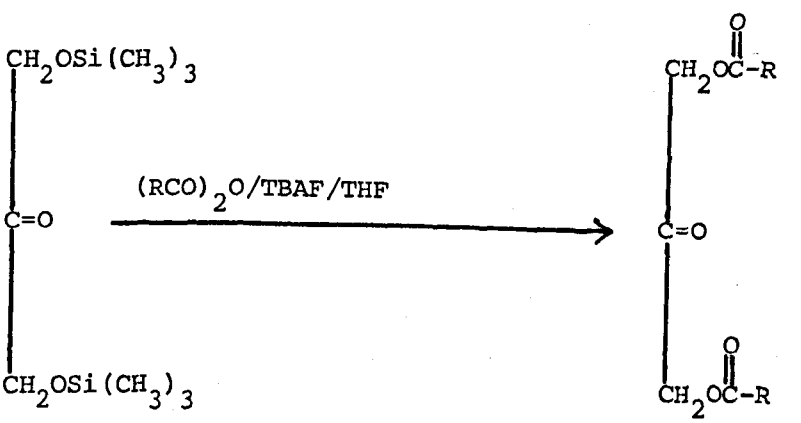
- Glycerol triacetate (yield: 72.5% (w/w))
- Glycerol tri-n-butyrate (yield: 88.0% (w/w))
- Glycerol tri-n-decanoate (yield: 67.0% (w/w))

Although a monoacid triacylglycerol can be prepared more easily by direct

reaction of glycerol with an acid anhydride in pyridine, the above experiment served to establish conditions for the preparation of diacid triacylglycerols.

b) Symmetrical diacylglycerols from the disilyl derivative of 1,3-DHA.

Symmetrical diacylglycerols cannot be prepared from the disilyl derivative of glycerol (12) under the conditions described in (a) above, since the reaction results in the formation of the monoacid triacylglycerols. However they can be prepared from the 1,3-disilyl derivative of 1,3-DHA (9). (9) was reacted with a given fatty acid anhydride in anhydrous THF using TBAF as a catalyst. The reaction mixture was refluxed for 20 hours under anhydrous conditions and the products analysed. Low yields of (15), the corresponding diacyl derivative of 1,3-DHA, were obtained (16-20%) (Scheme: 37).



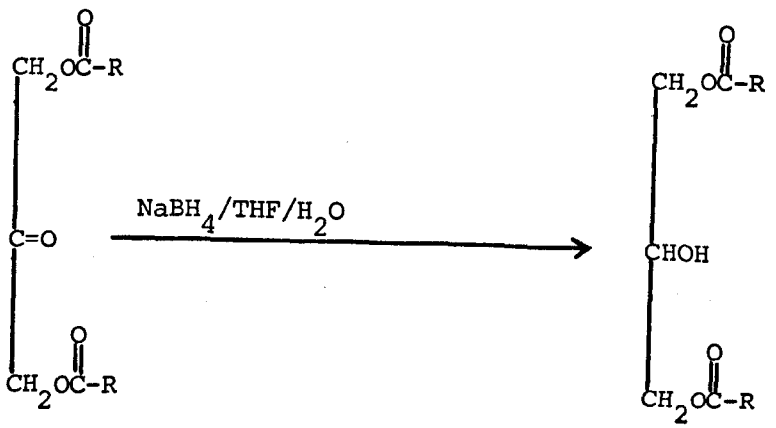
(9) (15)

Scheme: 37

Acetic, n-butyric and n-decanoic anhydrides were used to give the the following products:

- 1,3-diacetyloxy-propane-2-one (yield: 16% (w/w))
- 1,3-di-n-butyryloxy-propane-2-one (yield: 20% (w/w))
- 1,3-di-n-decanoyloxy-propane-2-one (yield: 17% (w/w)).

The above products were separated by TLC from the reaction mixture, and the ketone group was reduced with $NaBH_4$ in wet THF to give the corresponding diacylglycerols namely: glycerol-1,3-diacetate, glycerol-1,3-di-n-butyrate and glycerol-1,3-di-n-decanoate (Scheme: 38). The



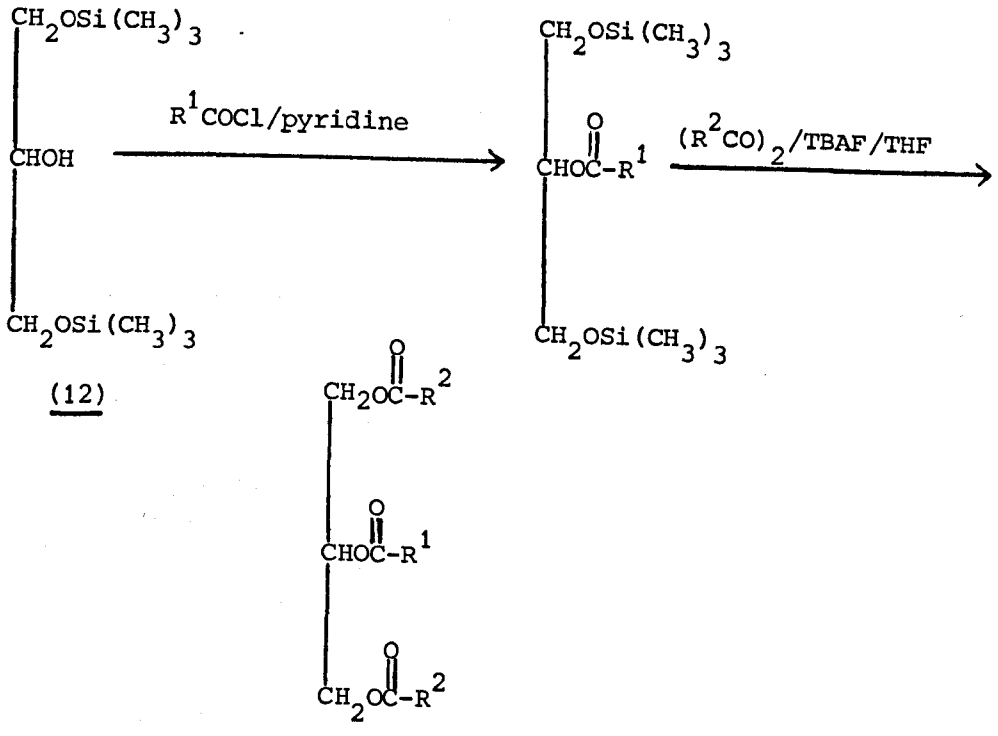
(15) (16)

Scheme: 38

symmetrical diacid triacylglycerols can be prepared by acylating the above symmetrical diacylglycerols (16) with an acid chloride (Scheme: 23).

It was previously mentioned that diacylglycerols cannot be prepared from (12) using the conditions above (2.2.2.5 b). Another alternative was to acylate the free hydroxyl of (12), with an acid chloride, then to apply the conditions described in (b), and this would result in symmetrical diacid triacylglycerols (Scheme: 39). Unfortunately

complications were encountered at the first stage.



Scheme: 39

2.2.3 EXPERIMENTAL.

Most of the experimental conditions in 2.1.3 apply. The trimethylsilyl chloride and bis-trimethylsilyl trifluoroacetamide (BSTFA), used, were 99% pure. Tetra-n-butyl ammonium fluoride trihydrate $[(CH_3(CH_2)_3)_4NF.3H_2O]$ was dried[107] by azeotropic distillation under reduced pressure using 1:1 toluene:acetonitrile, and final drying at 35° and 1.5 mm Hg for 24 hours. 1,3-Dihydroxyacetone was of GPR grade. The preparation of fatty acid salts was given in 2.1.3.4-5. Trimethyl silyl imidazole (TMSI) was used for GLC derivatizations and the acylglycerols used for identification purposes were 99% pure (GLC).

2.2.3.1 Acylglycerols from reaction of glycerol with trimethylsilyl chloride, NaI and a fatty acid salt.

a. Method.

In a 100 cm^3 r.b. flask fitted with a water condenser and a calcium chloride guard-tube, anhydrous glycerol (0.5 g, 5.4 mmol) was reacted with trimethylsilyl chloride (2.3 g, 21.2 mmol) and sodium stearate (6.7 g, 21.9 mmol) in a degassed mixture of 50% (v/v) acetonitrile in DMF (30 cm^3) using NaI (1 mg) as a catalyst. The mixture was stirred for 6 hours at 65° (oil bath). Distilled water was added then to the cooled mixture (120 cm^3). The reaction mixture was extracted with diethyl ether (2 x 50 cm^3), washed with brine (2 x 10 cm^3) and dried. The diethyl ether was distilled off and the products analysed.

The reaction procedure was repeated using sodium n-butyrate instead of sodium stearate.

b. Analysis of the product mixtures.

The products were analysed using GLC, infrared and TLC techniques and further identified using reference compounds when possible. The TLC plates were developed in 2% (v/v) methanol in dichloromethane and visualized with 2,7'-dichlorofluorescein and viewed under a UV light.

b.1 Analysis of product mixture from the sodium stearate reaction.

TLC analysis showed that the sodium stearate reaction mixture contained six components of R_f values: 0.1, 0.23, 0.42, 0.48, 0.60 and 0.78. The following compounds were identified.

b.1.1 Compound of R_f 0.42.

Infrared. $\bar{\nu}/cm^{-1}$ (Inferences): 1725 and 1740 (C=O ester groups) and 3400 (OH).

GLC. Retention time: 5.6 min on a 0.45 m column (304°C at isothermal conditions; for other conditions refer to 2.1.3.6 a). When silylated the retention time was 41.3 min.

The analytical data agree with the structure of glycerol-1,2-distearate and confirm the compound of R_f 0.42 as glycerol-1,2-distearate.

b.1.2 Compound of R_f 0.48.

Infrared. $\bar{\nu}/cm^{-1}$ (Inferences): 1730 and 1743 (C=O ester groups) and 3400 (OH).

GLC. Retention time: 5.6 min on a 0.45 m column (304°C isothermal conditions; for other conditions refer to 2.1.3.6 a). When silylated the retention time was 42.7 min.

The analytical data are similar to those of glycerol-1,2-distearate and therefore confirm the compound of R_f 0.48 as glycerol-1,3-distearate.

b.1.3 Compound of R_f 0.78.

Infrared. $\bar{\nu}/cm^{-1}$ (Inferences): 1740 (C=O ester group).

GLC. The retention time was 18.4 min on a 0.45 m column (for other conditions refer to 2.1.3.8 a).

The analytical data are similar to those of glycerol tristearate and therefore confirm the compound of R_f 0.78 as glycerol tristearate.

Preparative TLC showed that the % relative compositions of the 3 components were: glycerol-1,2-distearate (0.1003 g, 26.3% (w/w)), glycerol-1,3-distearate (0.1689 g, 44.1% (w/w)) and glycerol-tristearate (0.1120 g, 29.6% (w/w)). They constituted only 10.2% (w/w) of the amount of glycerol used initially.

b.2 Analysis of the product mixture from the sodium n-butyrate reaction.

The sodium n-butyrate reaction mixture was separated into four different components of R_f values: 0.12, 0.21, 0.40 and 0.48. The following components were identified.

b.2.1 Compound of R_f 0.21.

Infrared. $\bar{\nu}/cm^{-1}$ (Inferences): 1730 and 1740 (C=O, ester groups) and 3400 (OH).

GLC. Retention time: 4.35 min on a 1.85 m column (initial column temperature 110°C increased at a rate of 6° min^{-1} to 340°, for other conditions refer to 2.1.3.6 a).

The analytical data are similar to those of glycerol-1,2-di-n-butyrate and therefore confirm the compound of R_f 0.21 as glycerol-1,2-di-n-butyrate.

b.2.2 Compound of R_f 0.40.

The compound of R_f 0.40 had the infrared spectra and the GLC retention time similar to those of glycerol-1,2-di-n-butyrate above, but it had the same R_f value as glycerol-1,3-di-n-butyrate. Therefore the compound of R_f 0.40 was identified as glycerol-1,3-di-n-butyrate.

b.2.3 Compound of R_f 0.48.

Infrared. $\bar{\nu}/cm^{-1}$ (Inferences): 1745 (ester groups).

GLC. Retention time: 7.72 min (conditions similar to those used for the compound of R_f 0.21).

The analytical data above are similar to those of glycerol tri-n-butyrate and therefore confirm the compound of R_f 0.48 as glycerol tri-n-butyrate

Preparative TLC showed that the % (w/w) relative compositions of the 3 components identified above were: glycerol-1,2-tri-n-butyrate (31.6%), glycerol-1,3-di-n-butyrate (42.1%) and glycerol tri-n-butyrate (26.3%). They constituted 15.1% (w/w) of the amount of glycerol used initially.

2.2.3.2 Reaction of glycerol with trimethylsilyl chloride.

a. Method.

In a 100 cm^3 r.b. flask fitted with a pressure equalising funnel and calcium chloride guard-tube, anhydrous glycerol (3.0 g, 32.6 mmol) was reacted with trimethylsilyl chloride (7.1 g, 65.4 mmol) in anhydrous pyridine (7 cm^3) and anhydrous (ethanol free) chloroform (70 cm^3). Trimethylsilyl chloride was added slowly to the reaction mixture and the temperature was kept at 0°. The reaction was left to proceed for 24 hours at room temperature. The reaction mixture was washed with 1.0 mol dm^{-3} HCl (2 x 10 cm^3) and distilled water (2 x 10 cm^3) and dried. The weight of the crude product recovered was 6.4 g.

b. Analysis of product mixture.

TLC analysis showed that the product mixture contained four components of R_f 0.08, 0.19, 0.22 and 0.64 with the component of R_f 0.64 being the major constituent (82% w/w). 2% (v/v) methanol in dichloro-

methane was the elution system (for other conditions refer to 2.1.3.1 b).

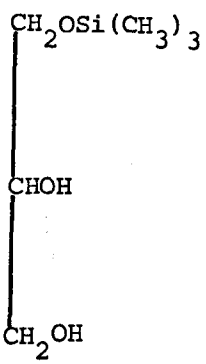
b.1 Compound of R_f 0.08.

Infrared. $\bar{\nu}/cm^{-1}$ (Inferences): 1240, 1140 and 1085 (Si-O and C-O ether groups); 2970, 2930 and 2880 (CH_2 and CH_3 groups) and 3400 (OH).

GLC. Retention time: 4.93 min on a 1.85 m column (70° isothermal conditions, for other conditions see 2.1.3.6 a).

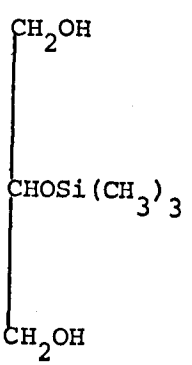
EI-MS. The mass spectra were determined using a KRATOS MSIT instrument with a GLC column packed with an OV17 stationary phase (65°, constant). m/z: (major and significant ions), 149 $[M^+-CH_3]$, 132 $[H_2CCH(OH)CH_2OSi(CH_3)_2]^+$, 117 $[H_2CCH(OH)CH_2OSiCH_3]^+$, 89 $[OSi(CH_3)_3]^+$, 73 $[Si(CH_3)_3]^+$, 71 $[CHCH_2OSi]^+$, 43 $[SiCH_3]^+$ and 41 $[CH_2CHCH_2]^+$.

The analytical data above confirm that the compound of R_f 0.08 was a mixture of the monosilyl derivatives of glycerol, i.e.



(13)

and



(18)

(MW: 164)

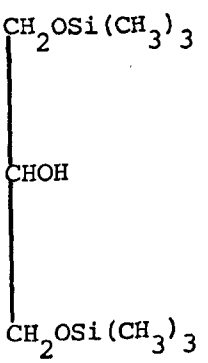
b.2 Compounds of R_f 0.19 and 0.23. (These two components had the same infrared absorbances and the same GLC retention time)

Infrared. $\bar{\nu}/cm^{-1}$ (Inferences): 1250, 1145 and 1070 (Si-O and C-O of ether groups); 2970, 2935 and 2885 (CH_2 and CH_3 groups) and 3400 (OH).

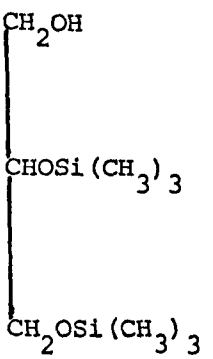
GLC. Retention time: 5.67 min, the conditions were similar to those applied to the above compound of R_f 0.19 and 0.23.

EI-MS. m/z (major and significant ions): 221 $[M^+-CH_3]$, 206 $[M^+-2(CH_3)]$, 205 $[M^+-CH_2OH]$, 191 $[M^+-3(CH_3)_3]$, 163 $[M^+-Si(CH_3)_3]$, 147 $[M^+-OSi(CH_3)_3]$, 133 $[M^+-CH_2OSi(CH_3)_3]$, 117 $[CH=CH_2OSi(CH_3)_3 + 1]^+$, 103 $[CH_2OSi(CH_3)_3]^+$, 89 $[OSi(CH_3)_3]^+$ and 73 $[Si(CH_3)_3]^+$. (For instrument and conditions refer to b.1 above).

The analytical data above confirm that the two compounds of R_f 0.19 and 0.23 were the disilyl derivatives of glycerol (the 2 isomers) i.e.



(12)



(17)

(MW: 236)

b.3 Compound of R_f 0.63.

Infrared. $\bar{\nu}/cm^{-1}$ (Inferences): 1245, 1145 and 1085 (Si-O and C-O of ether groups); 2970, 2935 and 2885 (CH_2 and CH_3 groups).

GLC. Retention time: 7.04 min, the conditions were similar to those applied to the above compounds of R_f 0.19 and 0.23.

EI-MS. m/z (major and significant ions): 293 $[M^+-CH_3]$, 219 $[M^+-OSi(CH_3)_3]$, 205 $[M^+-CH_2OSi(CH_3)_3]$, 190 $[(H_3C)_3SiOCH_2CHOSi(CH_3)_2]^+$, 175 $[(H_3C)_3SiOCH_2CHOSiCH_3]^+$, 132 $[(H_3C)_3SiOCH_2CHO]^+$, 117 $[(H_3C)_2SiOCH_2CHO]^+$, 103 $[(H_3C)_3SiOCH_2]^+$ and 73 $[Si(CH_3)_3]^+$. (For instrument and conditions refer to b.1 above).

The analytical data above confirm that the compound of R_f 0.63 was the trisilyl derivative of glycerol (14).

Quantitative analysis (GLC and TLC) showed that the percentage composition (w/w) of the product mixture in the mono, di- and trisilyl derivatives of glycerol were 5.2, 12.8 and 82.0 respectively. 69.1% (w/w) of the initial amount of glycerol reacted.

2.2.3.3 The silyl derivatives of 1,3-DHA.

a. Reaction of 1,3-DHA with trimethylsilyl chloride.

a.1 Method.

In a 100 cm^3 r.b. flask fitted with a pressure equalising funnel and a calcium chloride guard-tube, a pyridine solution (20 cm^3) of 1,3-DHA (0.5 g, 5.6 mmol) which was standing for 24 hours, was reacted at -3° with trimethylsilyl chloride (1.4 g, 12.9 mmol). The reagent was added in 5 portions in a period of 30 min. The reaction was left to proceed at room temperature for 4 hours and the products analysed.

a.2 Analysis.

TLC. The components in the reaction mixture were separated by TLC using 20% (v/v) diethyl ether in petroleum spirit as the elution system (for other conditions refer to 2.1.3.1 b). Two components of R_f 0.1 and 0.51 were present in the product mixture.

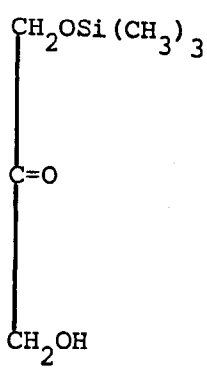
a.2.1 Compound of R_f 0.1.

Infrared. $\bar{\nu}/cm^{-1}$ (Inferences): 1245, 1135 and 1025 (Si-O and C-O of ether groups), 1740 (C=O); 2980, 2940 and 2860 (CH_2 and CH_3 groups) and 3400 (OH).

GLC. Retention time: 5.0 min. The column used was a 1.85 m glass column packed with 3% OV17. The initial column temperature was 70° increased at the rate of 4° min^{-1} to 340° (for other conditions see 2.1.3.6 a).

EI-MS. m/z (major and significant ions): 147 $[M^+-CH_3]$, 132 $[M^+-2(CH_3)]$, 117 $[M^+-3(CH_3)]$, 89 $[OSi(CH_3)_3]^+$ and 73 $[Si(CH_3)_3]^+$. (For instrument and conditions refer to 2.2.3.2 above).

The analytical data above confirm that the compound of R_f 0.1 was the monosilyl derivative of 1,3-DHA i.e.



Monosilyl derivative of 1,3-DHA (MW: 162)

a.2.2 Compound of R_f 0.51.

Infrared. $\bar{\nu}/cm^{-1}$ (Inferences): 1240, 1135 and 1020 (Si-O and C-O of ether groups), 1740 (C=O), 2960, 2900 and 2830 (CH_2 and CH_3 groups).

GLC. Retention time: 6.7 min.(The conditions are similar to those in a.2.1 above).

EI-MS. m/z (major and significant ions): 219 $[M^+-CH_3]$, 189 $[M^+-3(CH_3)]$, 129 $[H_2CCCH_2OSi(CH_3)_3]^+$, 103 $[CH_2OSi(CH_3)_3]^+$ and 73 $[Si(CH_3)_3]^+$. (For instrument and conditions refer to 2.2.3.2 above).

The analytical data above confirm that the compound of R_f 0.51 was the disilyl derivative of 1,3-DHA (9).

Because of the difficulties mentioned in 2.2.2.3, (9) could not be isolated by means other than TLC. The dimers which formed when the product mixture was worked up or heated were identified by GLC-MS. The major constituent was the trisilylated dimer of 1,3-DHA (retention time: 11.6 min; the conditions are similar to a.2.1 above). Its infrared data were similar to those of a.2.1 above. The EI-MS of the trisilyl derivative of the dimer of 1,3-DHA was as follows:

m/z (major and significant ions): 378 $[M^+-H_2O]$, 366 $[M^+-2(CH_3)]$, 307 $[M^+-OSi(CH_3)_3]$, 293 $[M^+-CH_2OSi(CH_3)_3]$, 275 $[M^+-H_2O-CH_2OSi(CH_3)_3]$, 217 $[M^+-(H_2O + 3CH_3 + CH + CH_2OSi(CH_3)_3]$, 189 $[M^+ - (H_2O + CH + Si(CH_3)_3 + CH_2OSi(CH_3)_3]$, 147 $[OCH_2CO(OSiCH_3)CH_2O]^+$, 131 $[CH_2CO(OSiCH_3)CH_2O]^+$, 129 $[H_2CCCH_2OSi(CH_3)_3]^+$, 103 $[CH_2OSi(CH_3)_3]^+$ and 73 $[Si(CH_3)_3]^+$. (For instrument and conditions refer to 2.2.3.2 above).

b. Reaction of 1,3-DHA with BSTFA - Preparation of the disilyl derivative of 1,3-DHA, (9).

b.1 Method.

In a 250 cm^3 r.b. flask fitted with a pressure equalising funnel, an anhydrous pyridine (100 cm^3) solution of 1,3-dihydroxyacetone (4.0 g, 44.4 mmol), which was standing for 24 hours, was reacted with bis-trimethylsilyl trifluoroacetamide (25.0 g, 97.1 mmol). The reagent was added slowly, under anhydrous conditions, in 5 portions in a period of 5 hours at -3°. The mixture was stirred continuously during the addition. The reaction was left to proceed for 24 hours at room temperature. The pyridine was distilled off at 13 mm Hg. (9) was separated from the product mixture at 70-2°, 2.3 mm Hg (oil bath 125°) to give a colourless product (6.6 g) which was analysed. (The by-products from the BSTFA reaction i.e. monotrimethylsilyl triofluoroacetamide and trifluoroacetamide distilled off at ca. 60°, 2.3 mm Hg).

b.2 Analysis.

GLC of the product distilled at 70° (2.3 mm Hg) showed it was 99% (w/w) pure (retention time 6.98 min, conditions as in a.1 above). The infrared GLC-MS analysis were similar to those of the compound of R_f 0.51 described in a.2 above i.e. the disilyl derivative of 1,3-DHA, (9). The amount of (9) isolated was 6.6 g or 63.5% (w/w) yield. The product mixture before distillation contained 88% (w/w) disilylated monomer 1,3-DHA, 8% (w/w) of the monosilylated monomer and 4% (w/w) of the silylated dimer of 1,3-DHA.

2.2.3.4 Reduction of (9).

a. Method.

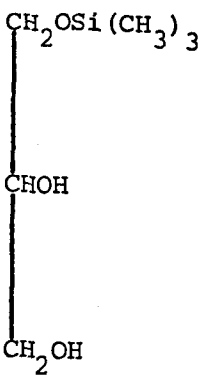
In a THF (5 cm^3) solution containing the disilyl derivative of 1,3-DHA, (9), (0.2942 g, 0.87 mmol) and distilled water (0.03 ml) was added slowly sodium borohydride (0.0087 g, 0.23 mmol) at 5°, under continuous stirring. The reaction was left to proceed for 30 min. The unreacted sodium borohydride was neutralized with acetic acid. The mixture was dissolved in chloroform (25 cm^3), washed with sodium carbonate (2 x 5 cm^3) and distilled water (2 x 5 cm^3) and dried.

b. Analysis.

TLC analysis showed that the product mixture contained mainly two products of R_f 0.08 and 0.23. The minor by-product at R_f 0.63 was identified as the trisilyated dimer of 1,3-DHA (2.2.3.3 a). The plates were eluted with 2% (v/v) methanol in chloroform (for other conditions refer to 2.1.3.1 b).

b.1 Compound of R_f 0.08.

This compound had the same analytical data as those given for the compound of R_f 0.09 described in 2.2.3.2 i.e. the monosilyl derivative of glycerol, (13).



(13)

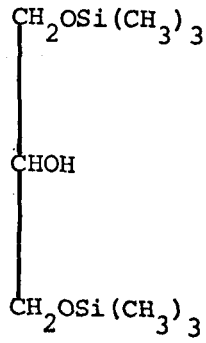
b.2 Compound of R_f 0.23.

Infrared. $\bar{\nu}/cm^{-1}$ (Inferences): 1240, 1130 and 1080 (Si-O and C-O of ether groups), 2980, 2935 and 2860 (CH_2 and CH_3 groups) and 3410 (OH).

GLC. Retention time: 5.63 min. The conditions are similar to those described in 2.2.3.2 b.

EI-MS. m/z (major and significant ions): 221 $[M^+-CH_3]$, 206 $[M^+-2(CH_3)]$, 191 $[M^+-3(CH_3)]$, 147 $[M^+-OSi(CH_3)_3]$, 133 $[M^+-CH_2OSi(CH_3)_3]$, 103 $[CH_2OSi(CH_3)_3]^+$, 89 $[OSi(CH_3)_3]^+$ and 73 $[Si(CH_3)_3]^+$.

The analytical data above confirm that the compound of R_f 0.23 was the 1,3-isomer of the disilyl derivative of glycerol,(12).



(12)

The amount of (9) reduced was almost quantitative. For preparative work, (12) was separated by TLC. The proportion of the monosilylated glycerol in the product mixture varied from 25% to 10% (w/w). There was no 1,2-disilyl derivative of glycerol.

2.2.3.5 Synthesis of monoacid triacylglycerol from the trisilyl ether of glycerol (14).

a. Glycerol triacetate.

a.1 Method.

In a 25 cm^3 r.b. flask fitted with a water cooled condenser and a calcium chloride guard-tube, the trisilyl derivative of glycerol (14) (0.026 g, 0.085 mmol) was reacted with acetic anhydride (0.270 g, 2.647 mmol) and tetra-n-butyl ammonium fluoride (TBAF) (0.136 g, 0.521 mmol) in anhydrous THF (10 cm^3). The reaction was refluxed for 16 hours. The THF was distilled off and the residue dissolved in diethyl ether (20 cm^3), washed with distilled water (2 x 5 cm^3) and dried. The product was separated by TLC after distilling off the diethyl ether.

a.2 Analysis.

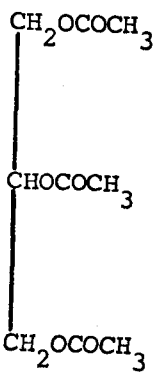
TLC. Glycerol triacetate was isolated from the product mixture by TLC using a reference compound. 2% (v/v) methanol in chloroform was the elution system (for other conditions refer to 2.1.3.1 b). Glycerol triacetate had an R_f value of 0.58.

Infrared. $\bar{\nu}/cm^{-1}$ (Inferences): 1385 and 1450 (CH_2 and CH_3 groups), 1755 (C=O of ester groups), 2880, 2940 and 2960 (CH_2 and CH_3 groups).

GLC. Retention time: 2.71 min. The column used was a 1.85 m column packed with 3% OV17. (For other conditions refer to 2.2.3.1 b).

PMR. δ: 5.28 (t, 1H, CH), 4.26 (m, 4H, CH_2), and 2.1 (s, 9H, CH_3).

The analytical data above confirm that the compound of R_f 0.58 was glycerol triacetate (yield: 72.5% (w/w)).



Glycerol triacetate

b. Glycerol tri-n-butyrate.

b.1 Method.

The trimethylsilyl ether of glycerol (14) (0.038 g, 0.123 mmol) was reacted with n-butyric anhydride (0.597 g, 3.778 mmol) and TBAF (0.192 g, 0.736 mmol) in anhydrous THF (10 cm^3) in the same way as a.1 above.

b.2 Analysis.

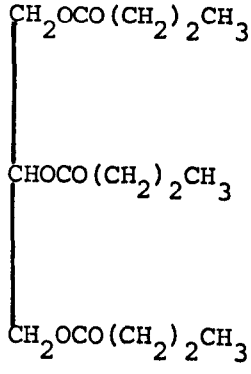
TLC. Glycerol tri-n-butyrate was separated from the product mixture by TLC using a reference compound. 2% (v/v) methanol in chloroform was the elution system (for other conditions refer to 2.1.3.1 b). Glycerol tri-n-butyrate had an R_f value of 0.65.

Infrared. $\bar{\nu}/cm^{-1}$ (Inferences): 1750 (C=O of ester groups), 2910 and 2980 (CH_2 and CH_3 groups).

GLC. Retention time: 7.93 min (for other conditions refer to a.2 above).

PMR. δ: 5.15 (t, 1H, CH), 4.18 (m, 4H, CH_2 of glycerol skeleton), 2.34 (t, 6H, α-CH_2 of the n-butyryl chain), 1.67 (m, 6H, β-CH_2 of the n-butyryl chain) and 0.97 (t, 9H, CH_3).

The analytical data above confirm that the compound of R_f 0.65 was glycerol tri-n-butyrate (yield: 88.0% (w/w)).



Glycerol tri-n-butyrate

c. Glycerol tri-n-decanoate.

c.1 Method.

c.1.1 Preparation of n-decanoic anhydride.

In a 100 cm^3 r.b. flask fitted with an efficient water cooled condenser n-decanoic acid (25.0 g, 0.145 mmol) and acetic anhydride (22.2 g, 0.218 mmol) were refluxed at 200° (oil bath) for 24 hours. n-Decanoic anhydride was separated from the reaction mixture at 186° (2.5 mm Hg) as a pure product from the free fatty acids, the mixed anhydride and acetic anhydride.

---

*s = singlet, d = doublet, t = triplet, m = multiplet.

c.1.2 Preparation of glycerol tri-n-decanoate.

The trimethylsilyl derivative of glycerol, (14), (0.039 g, 0.127 mmol) was reacted with n-decanoic anhydride (1.239 g, 3.800 mmol) and TBAF (0.201 g, 0.770 mmol) in anhydrous THF (10 cm^3) in the same way as in a.1 above.

c.2 Analysis.

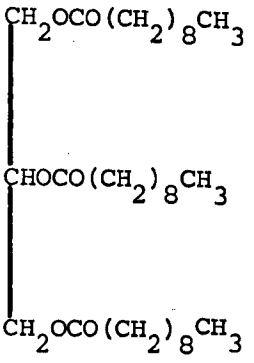
TLC. Glycerol tri-n-decanoate was separated from the product mixture by TLC using a reference compound. 2% (v/v) methanol in chloroform was the elution system (for other conditions refer to 2.1.3.1 b). Glycerol tri-n-decanoate had an R_f value of 0.82.

Infrared. $\bar{\nu}/cm^{-1}$ (Inferences): 1740 (C=O of ester groups); 2860, 2930 and 2970 (CH_2 and CH_3 groups).

GLC. Retention time: 3.12 min (for other conditions refer to 2.1.3.8 a).

PMR. δ: 5.2 (t, CH), 4.2 (m, CH_2 of glycerol skeleton), 2.36, 1.98 and 1.64 (m (α-, β- and γ-CH_2) of the n-decanoyl chain respectively), 1.29 (s, $-(CH_2)_5-$ of the n-decanoyl chain) and 0.9 (m, CH_3).

The analytical data above confirm that the compound of R_f 0.82 was glycerol tri-n-decanoate (yield: 67% (w/w)).



Glycerol tri-n-decanoate

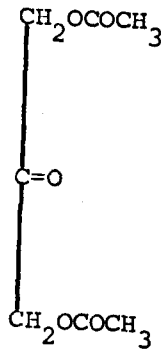
2.2.3.6 Synthesis of symmetrical diacylglycerols from the 1,3-disilyl derivative of dihydroxyacetone, (9).

a. Glycerol-1,3-diacetate.

a.1 Acylation of (9).

a.1.1 Method.

In a 50 cm^3 r.b. flask fitted with a water cooled condenser and a calcium chloride guard-tube, the silyl derivative of 1,3-DHA,(9), (0.112 g, 0.480 mmol) was reacted with acetic anhydride (0.988 g, 9.686 mmol) and TBAF (0.504 g, 1.930 mmol) in THF (30 cm^3). The reaction mixture was refluxed for 16 hours. The THF was distilled off and the residue was dissolved in diethyl ether (20 cm^3), washed with distilled water (2 x 5 cm^3) and dried. The product was separated by TLC after distilling off the diethyl ether (yield; 16.3% w/w based on (9)).



(19)

a.1.2 Analysis

TLC. The diacyl derivative of 1,3-DHA,(19), was separated from the product mixture by TLC using a reference compound. The elution system was 60:39.5:0.5 (v/v) hexane:diethyl ether:acetic acid respectively (for other conditions refer to 2.1.3.1 b). 1,3-diacetyloxy-propane-2-one had an R_f value of 0.38.

Infrared. $\bar{\nu}/cm^{-1}$ (Inferences): 1390 and 1415 (CH_2 and CH_3), 1750 and 1770 (C=O of ketones and esters), 2972 (C-H aliphatic).

PMR. δ: 4.77 (s, 2H, CH_2) and 2.16 (s, 3H, CH_3).

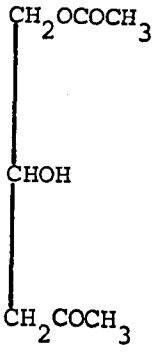
The analytical data agree with the configuration of (19) and confirm that the product isolated by TLC was 1,3-diacetyloxy-propane-2-one (19).

a.2 Preparation of glycerol-1,3-diacetate,(20).

a.2.1 Method.[16]

In a 25 cm^3 conical flask, a solution of 1,3-diacetyloxy-propane-2-one,(19), (0.011 g, 0.063 mmol) in THF (5 cm^3) and distilled

water (0.02 cm^3) was added sodium borohydride (0.001 g, 0.027 mmol) at 5°. The reaction was left to proceed for 30 min. The excess sodium borohydride was neutralized with acetic acid and the mixture dissolved in chloroform (30 cm^3), washed with distilled water (5 cm^3), aqueous sodium carbonate (2 x 5 cm^3) and distilled water (2 x 5 cm^3) and dried. The solvent was distilled off and glycerol-1,3-diacetate was separated by TLC (yield: 87.2% (w/w).



(20)

a.2.2 Analysis

TLC. (20) had an R_f value of 0.27 (for conditions refer to a.1.2 above).

Infrared. $\bar{\nu}/cm^{-1}$ (Inferences): 1363 and 1450 (CH_2 and CH_3 groups), 1745 and 1760 (C=O of ester groups), 2950 and 2985 (CH_2 and CH_3 groups) and 3420 (OH).

PMR. δ: 5.28 (t, 1H, CH), 4.26 (m, 2H, CH_2) and 2.1 (s, 3H, CH_3).

All the analytical data agree with the configuration of (20) and

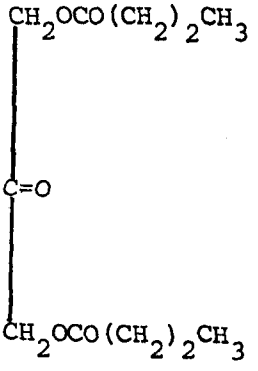
therefore confirm that the product of R_f 0.27 was glycerol-1,3-diacetate. After reduction, TLC analysis showed the presence of relatively small amounts of glycerol-1,2-diacetate (R_f 0.19) with glycerol-1,3-diacetate.

b. Glycerol-1,3-di-n-butyrate.

b.1 Acylation of (9).

b.1.1 Method.

In a 50 cm^3 r.b. flask fitted with a water cooled condenser and a calcium chloride guard-tube, the disilyl derivative of 1,3-DHA (9) (0.103 g, 0.440 mmol) was reacted with n-butyric anhydride (1.442 g, 8.901 mmol) and TBAF (0.480 g, 1.839 mmol) in dry THF (30 cm^3) in the same way as described in a.1.1 above. The product was separated by TLC after distilling off the diethyl ether (yield: 20.1% w/w based on (9)).



(21)

b.1.2 Analysis.

TLC. The R_f of the diacyl derivative was 0.43. For TLC experimental conditions refer to a.1.2 above.

Infrared. $\bar{\nu}/cm^{-1}$ (Inferences): 1400, 1420 and 1470 (CH_2 and CH_3 groups); 1740 and 1750 (C=O of esters and ketones), 2860, 2920 and 2960 (CH_2 and CH_3 groups).

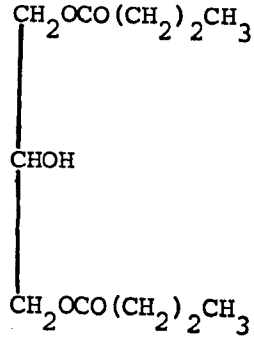
PMR. δ: 4.77 (s, 2H, CH_2 of glycerol skeleton); 2.42 (t, 2H, α-CH_2 of n-butyryl chain), 1.7 (m, 2H, β-CH_2 of n-butyryl chain) and 0.99 (t, 3H, CH_3).

The analytical data above agree with the configuration of (21) and confirm that the product isolated by TLC at R_f 0.43 was 1,3-di-n-butyryloxy-propane-2-one, (21).

b.2 Preparation of glycerol-1,3-di-n-butyrate, (22).

b.2.1 Method.

Glycerol-1,3-di-n-butyrate (22) was prepared from (21) in the same way as that described for glycerol-1,3-diacetate (a.2.1 above). (22) was isolated by TLC (yield: 86.8% (w/w)).



(22)

b.2.2 Analysis.

TLC. (22) had an R_f value of 0.31 (for TLC experimental conditions refer to a.1.2 above).

Infrared. $\bar{\nu}/cm^{-1}$ *(Inferences): 1430 and 1475 (CH_2 and CH_3 groups); 1745 and 1750 (C=O of ester groups), 2915 and 2980 (CH_2 and CH_3 groups)* and 3450 (OH).

PMR. δ: 5.15 (t, 1H, CH), 4.18 (m, 4H, CH_2 of glycerol skeleton), 2.34 (t, 4H, α-CH_2 of n-butyryl chain), 1.67 (m, 4H, β-CH_2 of n-butyryl chain) and 0.97 (m, 6H, CH_3)

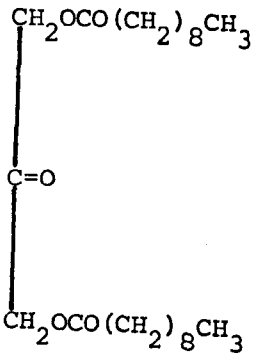
The analytical data above agree with the configuration of (22) and confirm that the product isolated by TLC at R_f 0.31 was glycerol-1,3-di-n-butyrate,(22). Here also relatively small amounts of the 1,2-isomer (glycerol-1,2-di-n-butyrate) were observed at R_f 0.22.

c. Glycerol-1,3-di-n-decanoate.

c.1 Acylation of (9).

c.1.1 Method.

(9) (0.097 g, 0.414 mmol) was reacted with n-decanoic anhydride (2.712 g, 8.320 mmol) and TBAF (0.429 g, 1.647 mmol) in dry THF (30 cm^3) as described in a.1.1 above. The product (23) was separated from the reaction mixture by TLC (R_f: 0.75) (yield: 17.2% w/w based on (9)).



(23)

c.1.2 Analysis.

TLC. (23) had an R_f value of 0.75 (for TLC experimental conditions refer to a.1.2 above).

Infrared. $\bar{\nu}/cm^{-1}$ (Inferences): 1425, 1435 and 1480 (CH_2 and CH_3 groups), 1745 and 1753 (C=O of esters and ketones), 2870, 2940 and 2980 (CH_2 and CH_3 groups).

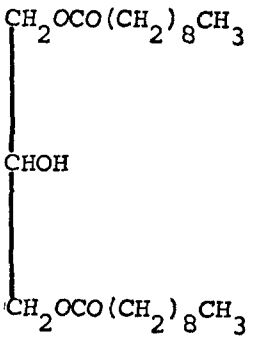
PMR. δ: 4.77 (s, 2H, CH_2 of glycerol skeleton), 2.44 (t, 2H, α-CH_2 of the n-decanoyl chain), 1.93 (m, 2H, β-CH_2 of the n-decanoyl chain); 1.6 (m, 2H, γ-CH_2 of the n-decanoyl chain), 1.39 (s, 10H, CH_2 of the n-decanoyl chain) and 0.92 (m, 3H, CH_3).

The analytical data above agree with the configuration of (23) and confirm the compound of R_f 0.75 as 1,3-di-n-decanoyloxy-propane-2-one.

c.2 Preparation of glycerol-1,3-di-n-decanoate, (24).

c.2.1 Method.

Glycerol-1,3-di-n-decanoate (24) was prepared from (23) in the same way as that described for glycerol-1,3-diacetate (a.2.1 above). (24) was isolated by TLC (yield: 93.4% (w/w)).



(24)

c.2.2 Analysis

TLC. (24) had an R_f value of 0.53 (for conditions refer to a.1.2 above).

Infrared. $\bar{\nu}/cm^{-1}$ (Inferences): 1400, 1430 and 1480 (CH_2 and CH_3 groups), 1720 and 1740 (C=O of ester groups), 2860, 2930 and 2970 (CH_2 and CH_3 groups) and 3500 (OH).

PMR. δ: 4.17 (m, CH_2 of glycerol skeleton), 2.36, 1.98 and 1.64 (α-, β- and γ-CH_2 of the n-decanoyl chain), 1.29 (s, (CH_2) of the n-decanoyl chain) and 0.9 (m, CH_3).

The analytical data above agree with the configuration of (24) and confirm that the product of R_f 0.53 was glycerol-1,3-di-n-decanoate (24). No 1,2-isomer was observed.

The symmetrical diacid triacylglycerols can be prepared from (20), (22) or (24) using a suitable acid chloride (2.1.3.8).

2.2.4 FINDINGS AND CONCLUSIONS

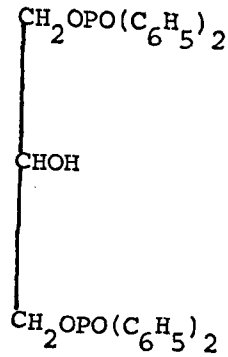
In the exploration of the feasibility of the silyl ether route for the preparation of symmetrical triacylglycerols, conditions were found to prepare the silylated monomer of 1,3-DHA i.e. (9) and consequently the 1,3-disilyl derivative of glycerol (12). Contrary to the tosyl group (cf. 2.1), the trimethylsilyl group, being a relatively smaller molecule, did not discriminate between the primary and secondary hydroxyl functions of the glycerol molecule. The silyl derivatives of 1,3-DHA and glycerol were labile under neutral conditions yet they were resistant to nucleophilic substitution by relatively strong nucleophiles such as carboxylate anions in the form of tricaprylmethyl ammonium salts. Monoacid triacylglycerols were prepared easily and in high yields from the trisilyl derivative of glycerol. However the yields of the symmetrical diacid triacylglycerols, prepared from the disilyl derivatives of 1,3-DHA were low. Therefore it is concluded that the silyl ether route does not offer any advantages over existing routes.

2.3 THE PHOSPHATE ROUTE

2.3.1 INTRODUCTION.

Biochemical research has unveiled an amazing variety of the manifestations and functions of organic phosphates in living processes.[109-111, 114] There is hardly anything that goes on in the cell in which esters of phorphoric acids, in one form or another, are not involved at some stage. A recent review[11] revealed that synthesis of triacylglycerols, using methods which assimilate processes which occur in plant and animal cells, has not been described. The method involves exploring the possibility of synthesising diacid triacylglycerols by nucleophilic substitution on the 1,3-diphosphate derivative of glycerol using different fatty acids. The diacylglycerols, synthesised, are reacted with the desired acid chloride to give the corresponding triacylglycerols as in route 2.1 (Scheme: 23). Phosphorylating reagents have been well covered in the literature for their reactivities and uses.[112-114] Diphenyl phosphoryl chloride (DPPC) is known to react selectively with a primary hydroxyl function in the presence of a secondary hydroxyl function.[115,116] The phenyl groups are removed only by alkaline treatment or vigorous platinum catalysed hydrogenation. Thus the reagent was chosen for preparing the substrate, i.e. glycerol-1,3-bis-diphenyl phosphate (25). Firstly some model reactions were conducted in order to find suitable conditions for the preparation of (25) and the subsequent nucleophilic substitution on it. The alcohols chosen were 1-propyl alcohol and 2-propyl alcohol with a primary and a secondary hydroxyl function respectively, similar to those in the glycerol molecule and ethylene glycol which has two

primary hydroxyl functions and would offer a good test for the reactivity of DPPC with vicinal hydroxyl functions.



(25)

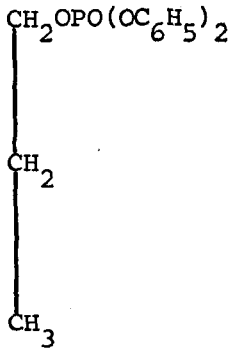
2.3.2 RESULTS AND DISCUSSION.

2.3.2.1 Propyl stearates from diphenyl phosphate intermediates.

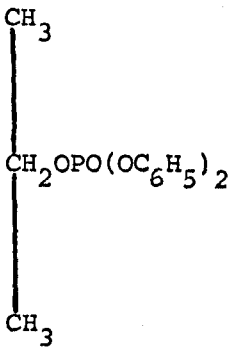
a. Reaction of DPPC with 1- and 2-propyl alcohols.

1-Propyl alcohol and 2-propyl alcohol were reacted with excess DPPC with pyridine serving both as a solvent and a nucleophilic catalyst. After a 16 hour reaction, the reaction mixtures were worked up and the products analysed by TLC (2.3.3.1). Both 1-propyl diphenyl phosphate and 2-propyl diphenyl phosphate reaction products had a one spot material. 1-Propyl diphenyl phosphate (26) had an R_f value of 0.48 and a percentage yield (w/w) of 96%. 2-Propyl diphenyl phosphate (27) had an R_f value of 0.52 and a percentage yield (w/w) of 92%. Diphenyl phosphoryl chloride reacted easily with the primary and secondary hydroxyl functions of the monopropyl alcohols and the yields were very high in both cases with the phosphate derivative of 1-propyl alcohol

being higher (4% higher). Probably this was due to steric hindrance.



(26)



(27)

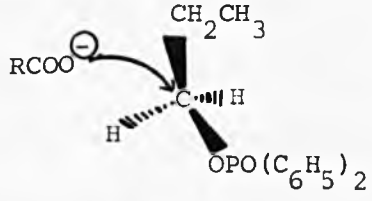
b. Preparation of the propyl stearate.

The phosphate derivatives of 1-propyl alcohol and 2-propyl alcohol ((26) and (27)) were reacted with tricaprylmethyl ammonium stearate, (TCMAS) (cf. 2.1.2), using different solvents to assess the reactivities of both substrates in different media. The effects of temperature and time were studied also. The 1- and 2-propyl stearates were separated from the product mixture by TLC using 40% (v/v) diethyl ether in petroleum spirit (60-80°) as the elution system. The results are given in tables 12 and 13. It may be observed from the results that 1-propyl diphenyl phosphate (26) was more susceptible to nucleophilic substitution than 2-propyl diphenyl phosphate (27). In all solvents (26) reacted much better giving high yields of 1-propyl stearate. The highest yield was obtained using n-hexane as a solvent (65.5% (w/w)). For 2-propyl stearate the highest yield was obtained using DMSO as a solvent (32.1% (w/w)). It was thus possible to displace the phosphate group by a carboxylate anion, but the displacement of the phosphate group took place more readily with the primary than with the secondary

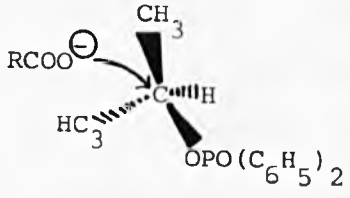
Solvent	Oil bath temperature/°C	Reaction time /hours	Yield of pure product % (w/w)
n-Hexane	85	4	65.5
Toluene	125	4	48.1
DMSO	85	4	56.7
50% (v/v) dioxane in water	105	4	27.0
Water	105	4	38.0
Water	105	8	47.2
Water pH 7.4	105	4	40.0

Table 12: preparation of 1-propyl stearate from 1-propyl diphenyl phosphate and TCMAS using different solvents.

phosphate derivative. The displacement or substitution in the reaction of (27) was presumably taking place through an S_N2 mechanism where steric hindrance has maximum effects on the incoming nucleophile. It would be easier for the nucleophile to approach (26) than (27) as shown below. The yield of 2-propyl stearate was higher in DMF than in



(26)



(27)

Solvent	Oil bath temperature/°C	Reaction time /hours	Yield of pure product % (w/w)
n-Hexane	85	4	12.0
n-Hexane	85	8	27.0
n-Hexane	85	16	29.0
n-Hexane	39	4	10.0
n-Hexane (TCMAS 1.5 molar excess)	85	4	14.5
DMF	85	5	29.0
Toluene	120	4	30.7
DMSO	85	16	32.1
50% (v/v) dioxane in water	105	4	8.4
Water	105	4	24.9
Water pH 7.4	105	4	26.5

Table 13: preparation of 2-propyl stearate from 2-propyl diphenyl phosphate and TCMAS using different solvents.

n-hexane (table 13). (27) is a neutral (i.e. it carries no charge) secondary phosphate derivative and depending on the medium, substitution could take place either by an S_N1 or an S_N2 mechanism. In DMF (a dipolar aprotic solvent), the reaction probably adopted an S_N1

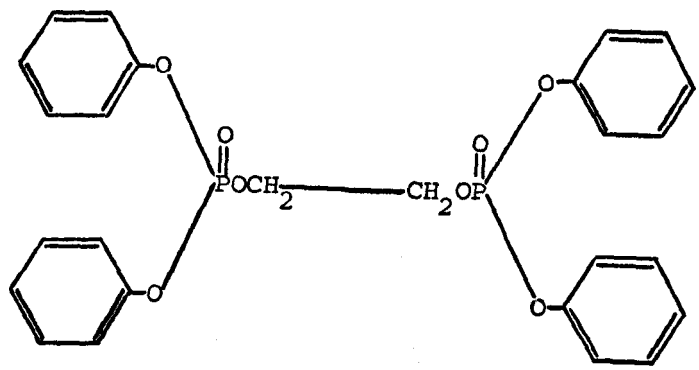
character due to the polarizing effects created by the solvent. These effects would facilitate the leaving of the phosphate group from the site it had occupied even before the nucleophile had made its approach, thus resulting in a much more effective substitution. Even in water, which is a polar aprotic solvent, the yield of 2-propyl stearate was higher than that obtained in n-hexane. In the latter substitution was relatively slow. The yield obtained in a 4 hour reaction using DMF was achieved in a 16 hour reaction when using n-hexane. The temperature affected the yields as well. In non-polar aprotic solvents, increasing the temperature increased the yield of the product e.g. in n-hexane the yield of 2-propyl stearate was increased from 10% to 14.5% (w/w) by increasing the temperature from 39° to the boiling point of n-hexane (ca. 80°). In toluene, a non-polar aprotic solvent, it was possible to increase the temperature to 115°. The yields obtained using toluene were similar to those obtained when DMF was used. It was concluded that there is not only selectivity in the phosphorylation but also in the substitution of the phosphate group. It was easier to phosphorylate and effect the substitution in 1-propyl alcohol than in 2-propyl alcohol. Since the preparation of (25) requires the phosphorylation of two primary hydroxyl functions, the use of n-hexane with TCMAS offered the best conditions for the preparation of 1,3-diacylglycerols. The conditions were applied next to an alcohol with two vicinal hydroxyl functions i.e. ethylene glycol.

2.3.2.2 Ethylene glycol distearate from the diphenyl phosphate derivative of ethylene glycol.

a. Reaction of DPPC with ethylene glycol.

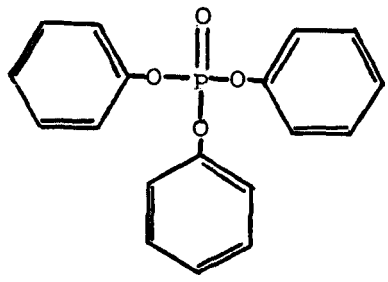
Anhydrous ethylene glycol was reacted with excess DPPC in anhydrous

pyridine and the reaction mixture worked up and the products analysed by TLC (2.3.3.1). Three components of R_f values 0.62, 0.49 and 0.37 were separated. They were identified as ethylene glycol bis-diphenyl phosphate, (28) (R_f 0.49), ethylene glycol diphenyl phosphate (R_f 0.37) and triphenyl phosphate, (29) (R_f 0.62). The percentage yield of the diphosphate derivative of ethylene glycol (28) was only 35.4% (w/w) based on the original amount of ethylene glycol used compared with ca. 90%



(28)

obtained with 1- and 2-propyl alcohols. Two side reactions were taking place. Firstly the formation of phenol and secondly the reaction of the phenol formed with DPPC to give triphenyl phosphate (29). Presumably all the phenol produced had reacted with DPPC since none of it was detected. It appears that the reaction of DPPC presents complications with polyhydric alcohols. In the work up of the reaction mixture, excess DPPC was neutralised by the addition of water which was thought to be the cause of the formation of the phenol and consequently the formation of (29). However it was not the case since the reaction was followed by TLC without working it up and was found to contain (29). (28) was isolated from the reaction mixture by flash



(29)

chromatography (2.3.3) and it was subjected to nucleophilic substitution.

b. Preparation of ethylene glycol distearate (30).

(29) was reacted with TCMAS in n-hexane and also in toluene. The various components in the reaction mixture were separated and analysed by TLC as in 2.3.2.1 b. Three components of R_f values 0.76, 0.64 and 0.40 were detected. They were identified (2.3.2.2) as ethylene glycol distearate, (30), of R_f 0.72, ethylene glycol monostearate of R_f 0.64 and ethylene glycol 1-stearate 2-diphenyl phosphate. (30) constituted 62.4% (w/w) of the product mixture or a percentage yield of 25.8% (w/w) when using n-hexane and 58.0% (w/w) of the product mixture or a percentage yield of 21.65% (w/w) when using toluene. The percentage yields were based on the original amount of (28) utilized. The substitution of the phosphate group certaintly was not as effective as the substitution which occurred in the reaction of n-propyl diphenyl phosphate (26) despite the fact that they both have primary phosphate groups. However the ethylene glycol derivative (28) has two phosphate

groups which are in close proximity to each other and consequently they were hindering the substitution which most probably took place through an S_N2 mechanism. While it was possible to synthesise (30) via the phosphate derivative, the yields with the diols were much lower than those obtained with the monoalcohols (2.3.2.1). Furthermore side reactions (e.g. formation of (29)) which occurred during the preparation of (28) could happen again with other polyhydric alcohols such as glycerol.

2.3.2.3 Glycerol-1,3-distearate from the diphenyl phosphate derivative of glycerol.

a. Reaction of DPPC with glycerol.

1 Mol of glycerol was reacted with 2 mol of DPPC in anhydrous pyridine and the reaction mixture worked up and analysed by TLC (2.3.3.3). Three main components were separated and identified. The product mixture contained the following: phenol (R_f 0.64, 17.4% (w/w)), triphenyl phosphate,(29), (R_f 0.62, 66.5% (w/w)),and glycerol-1,3-bis-diphenyl phosphate, (25), (R_f 0.40, 16.1% (w/w)). The % (w/w) yield of (25) was 15.5% based on the original amount of glycerol used. The yields of the phosphate derivatives decreased dramatically with the increasing number of hydroxyl functions i.e. in 1-propyl alcohol the yield was 96% (w/w), in ethylene glycol it was 35.4% (w/w) and in glycerol it was only 15.5% (w/w). The amount of phenol produced was higher than that of the desired product. In the reaction of DPPC with ethylene glycol phenol was not detected in the free state but was produced in relatively small amounts in the form of triphenyl phosphate,(29). (25) was separated by TLC from the reaction mixture which was freed from pyridine beforehand, and was subjected to

nucleophilic substitution.

b. Preparation of glycerol-1,3-distearate (5).

(25) was reacted with TCMAS in n-hexane using the conditions described in 2.3.2.1. (5) was separated from the reaction mixture by TLC using 1% (v/v) methanol in dichloromethane as the elution system. Two compounds of R_f 0.16 and 0.29 were separated. They constituted 17.3% (w/w) of the reaction mixture free from n-hexane or a yield of 22.4% (w/w) based on the original amount of (25) used. The percentage yield was similar to that of ethylene glycol distearate and it was probably low for the same reasons (2.3.2.2 b). The two compounds represent glycerol-1,3-distearate (5) (18.2% (w/w)) and glycerol-1,2-distearate, (6), (4.2% (w/w). (6) was formed via an intermediate and a mechanism most probably similar to those which resulted in the formation of (6) in the tosylate route (2.1.2.3). (5) can be obtained in a highly pure state (2.1.2.4) and reacted with a desired fatty acid chloride to give the corresponding symmetrical diacid triacylglycerol (2.1.3.8).

2.3.3 EXPERIMENTAL.

Most of the experimental conditions described in 2.1.3 apply. The diphenyl phosphoryl chloride was 95% pure and was redistilled. The propyl alcohols and ethylene glycol were also redistilled before use. For complementary TLC and GLC experimental conditions refer to 2.1.3.1 b and 2.1.3.6 a respectively. The GLC-MS spectra were obtained using a VG MM 12F mass spectrometer. In the electron impact (EI) mode it was operated at a resolution of ca. 1:800, an electron energy of 70 eV, an emission current of 200 μA and an accelerating

voltage of 2.5 kV.

2.3.3.1 Preparation of the propyl stearates.

a. Preparation of the propyl diphenyl phosphates.

a.1 Method.

To a 50 cm^3 r.b. flask fitted with a pressure equalising funnel and a calcium chloride guard-tube and containing a continuously stirred solution of 1-propyl alcohol (1.1 g, 18.3 mmol) and dry pyridine (10 cm^3) was added slowly diphenyl phosphoryl chloride (5.0 g, 18.6 mmol) at ca. -10°. The mixture was left to react for 16 hours at 3°. Water (1 cm^3) was added to neutralise the unreacted DPPC. Pyridine was distilled off and the reaction mixture dissolved in chloroform (100 cm^3), washed with 0.1 mol dm^{-3} hydrochloric acid (2 x 10 cm^3), distilled water (10 cm^3), saturated sodium carbonate (2 x 10 cm^3) and distilled water (2 x 10 cm^3) and dried. The chloroform was distilled off and the product weighed and analysed. The amount of product was 5.2 g (96% (w/w) based on 1-propyl alcohol).

2-Propyl diphenyl phosphate was prepared in the same way. The percentage yield was 92% (w/w) based on 2-propyl alcohol.

a.2 Analysis.

TLC. The TLC plates were run first half-way in ethyl acetate, air-dried and then chromatographed in 40% (v/v) diethyl ether in petroleum spirit (60-80°). The product from both the 1-propyl and 2-propyl alcohols showed a one spot material. For 1-propyl alcohol the product had an R_f value of 0.48 and for 2-propyl alcohol the product had an R_f value of 0.52.

Infrared. $\bar{\nu}/cm^{-1}$ (Inferences): 680 and 750 (C-H monosubstituted aromatic ring), 950 (P-O of POC aromatic), 1015 (P-O of POC aliphatic), 1290 (P=O of phosphates), 1480 and 1465 (CH of CH_2 and CH_3), 1600 (C=C aromatic), 2840, 2920 and 2970 (CH aliphatic) and 3050 (CH aromatic). For the product from 2-propyl alcohol there were two other bands at 1375 and 1390 (CH of $C(CH_3)_2$).

PMR. For the product from 1-propyl alcohol reaction, the δ values were as follows: 6.25 (s, 10H, C-H aromatic), 3.5 (m, 2H, α-CH_2), 3.2 (m, 2H, β-CH_2) and 0.8 (m, 3H, CH_3).

$$(PhO)_2\text{-}\overset{\overset{O}{\|}}{P}\text{-}O\text{-}\underset{\alpha}{CH_2}\text{-}\underset{\beta}{CH_2}\text{-}CH_3$$

(26)

For the product from 2-propyl alcohol reaction the δ values were as follows: 7.23 (s, 10H, CH aromatic), 4.8 (m, 1H, CH) and 1.32 (d, 6H, CH_3).

The analytical data indicate clearly that the products obtained in pure form were 1-propyl diphenyl phosphate (26) [$n_D^{30°}$ = 1.5212 and $n_D^{20°}$ = 1.522] and 2-propyl diphenyl phosphate (27) [$n_D^{30°}$ = 1.5159, $n_D^{20°}$ = 1.5199].

b. Preparation of the propyl stearates.

b.1 Method.

In a 50 cm^3 r.b. flask fitted with a water cooled condenser, 1-propyl diphenyl phosphate (1.0 g, 3.4 mmol) was reacted with TCMAS (3.1 g, 4.2 mmol) in refluxing n-hexane (30 cm^3) for 4 hours. The

ester product was separated by TLC using 40% (v/v) diethyl ether in petroleum spirit (60-80°). The percentage yield was 65.5% (w/w) based on the amount of (26) used.

2-propyl-stearate was prepared from (27) in the same way using toluene as a solvent. The percentage yield was 30.7% (w/w) based on the amount of (27) used.

b.2 Analysis

Infrared. $\bar{\nu}/cm^{-1}$ (Inferences): 1485, 1465, 2840, 2970 (CH aliphatic), 1745 cm^{-1} (C=O, ester group). For the product from (27) there were two other bands at 1380 and 1395 (CH of $C(CH_3)_2$).

GLC. The TLC separated products were chromatographed using a glass column (1.85 m x 2.5 mm) packed with 3% OV17. The initial column temperature was 180° and it was increased by 6° min^{-1} to 350°. The retention time of the product prepared from (26) was 6.26 min (1-propyl stearate had a retention time of 6.26 min) and the retention time of the product prepared from (27) was 5.41 min (the retention time of 2-propyl stearate was 5.41 min). They contained no impurities.

The analytical data above demonstrated that the products prepared from the reaction of (26) and (27) with TCMAS were 1-propyl stearate (m.p. 30.5-31.5° , Lit.[117] 28.8°) and 2-propyl stearate (m.p. 28.5-29.5°) respectively.

2.3.3.2 Preparation of ethylene glycol distearate.

a. Preparation of ethylene glycol bis-diphenyl phosphate.

a.1 Method.

Ethylene glycol (0.5 g, 8.1 mmol) was reacted with DPPC (5.4 g, 20.1 mmol) in pyridine and worked up as described for 1-propyl alcohol (2.3.3.1 a). The weight of the crude product isolated was 3.6 g.

a.2 Analysis.

TLC. Three components were separated with R_f values of 0.62, 0.49 and 0.37 (for TLC conditions refer to 2.3.3.1 a). The compound of R_f 0.49 constituted 46.9% (w/w) of the product mixture.

a.2.1 Compound of R_f 0.62.

Infrared. $\bar{\nu}/cm^{-1}$ (Inferences); 685 and 750 (CH monosubstituted aromatic ring), 950 (P-O of P-O-C aromatic), 1015 (P-O of P-O-C aliphatic), 1290 (P=O phosphates), 1590 (C=C aromatic) and 3055 (CH aromatic).

PMR. δ: 7.3 (m, 2H, CH aromatic), 7.2 (m, 3H, CH aromatic).

EI-MS. m/z (major and significant ions), 326 $[M^+]$, 249 $[M^+-C_6H_5]$, 233 $[M^+-OC_6H_5]$, 217 $[(H_5C_6O)_2P]^+$, 156 $[(H_5C_6O)OPO]^+$, 140 $[(H_5C_6O)PO]^+$ and 94 $[C_6H_5O+1]^+$.

The analytical data above confirm the structure of (29) i.e. triphenyl phosphate.

a.2.2 Compound of R_f 0.49.

Infrared. $\bar{\nu}/cm^{-1}$ (Inferences): 680 and 745 (CH monosubstituted aromatic ring), 950 (P-O of P-O-C aromatic), 1010 (P-O of P-O-C aliphatic), 1290 (P=O of phosphates), 1595 (C=C aromatic), 2940 (CH aliphatic) and 3050 (CH aromatic).

PMR. δ: 7.2 (m, 5H, CH aromatic) and 4.55 (s, 1H, CH_2).

The data above confirm the structure of the compound of R_f 0.49 as ethylene glycol bis-diphenyl phosphate (28). It was separated from the product mixture by flash chromatography using 50% (v/v) ethyl acetate in petroleum spirit (60-80°). For other experimental conditions refer to 2.1.3.1 b. Fractions 11 to 16 contained (28). The percentage recovery (w/w) was 92.1%. Once isolated (28) was subjected to nucleophilic substitution (b.1 below).

a.2.3 Compound of R_f 0.35.

Infrared. The compound of R_f 0.35 had the same infrared as in (a.2.2) above plus one broad band at 3450 cm^{-1} (OH).

PMR. δ: 7.2 (m, 5H, CH aromatic), 4.6 (t, 1H, CH_2) and 4.2 (t, 1H, CH_2).

The analytical data above confirm the structure of ethylene glycol diphenyl phosphate.

$$HOCH_2CH_2OPO(OC_6H_5)_2$$

b. Preparation of ethylene glycol distearate.

b.1 Method.

In a 100 cm^3 r.b. flask fitted with a water cooled condenser, ethylene glycol bis-diphenyl phosphate (1.0 g, 1.9 mmol) was reacted with TCMAS (3.7 g, 5.0 mmol) in refluxing n-hexane (50 cm^3). The mixture was left to react for 4 hours. The solvent was distilled off and the mixture analysed by TLC for its ester products.

The reaction was repeated using toluene as a solvent.

b.2 Analysis.

TLC. The reaction mixture was separated using 2% (v/v) methanol in dichloromethane as the elution system. Three components of R_f values 0.76, 0.64 and 0.40 were separated. (Ethylene glycol distearate had an R_f value of 0.76 and ethylene glycol monostearate had an R_f value of 0.61. They were prepared from ethylene glycol and stearyl chloride).

b.2.1 Compound of R_f 0.76.

Infrared. $\bar{\nu}/cm^{-1}$ (Inferences): 1480 and 1485 (CH aliphatic), 1745 (C=O of esters), 2880, 2970 and 2980 (CH aliphatic).

GLC. The compound was chromatographed using a glass column (1.85 m, 2.5 mm i.d.) packed with 3% OV17. The initial column temperature was 210° and it was increased by 6° min^{-1} to 350°. The compound had a retention time of 25.8 min similar to that of ethylene glycol distearate.

It is concluded therefore that the compound of R_f 0.76 was ethylene glycol distearate. It constituted 62.4% (w/w) of the reaction mixture

and it had a percentage yield of 25.8% (w/w) based on the original amount of (28) used. Ethylene glycol distearate had a m.p. of 75-76° (Lit.[83] 76°).

$$H_{35}C_{17}COOCH_2CH_2OCOC_{17}H_{35}$$

(30)

b.2.2 Compound of R_f 0.64.

Infrared. The compounds of R_f 0.64 had the same bands as b.2.1 plus a broad band at 3500 cm^{-1} (OH).

GLC. The retention time was 3.7 min similar to that of ethylene glycol monostearate (GLC conditions similar to those described in b.2.1).

The analytical data above confirm the compound of R_f 0.64 as ethylene glycol monostearate.

$$HOCH_2CH_2OCOC_{17}H_{35}$$

b.2.3 Compound of R_f 0.40.

Infrared. $\bar{\nu}/cm^{-1}$ (Inferences): 700 and 760 (CH monosubstituted aromatic ring), 970 (P-O of P-O-C aromatic), 1020 (P-O of P-O-C aliphatic), 1300 (P=O of phosphates), 1600 (C=C aromatic), 1740 (C=O of esters), 2880 and 2910 (CH of CH_2) and 2960 and 2990 (CH of CH_3).

GLC. The retention time was 7.4 min (GLC conditions similar to those described in b.2.1).

The analytical data above agrees with the structure of ethylene glycol 1-stearate-2-diphenyl phosphate.

$(C_6H_5O)_2POOCH_2CH_2OCOC_{17}H_{35}$

2.3.3.3 Preparation of glycerol-1,3-distearate.

a. Preparation of glycerol bis-1,3-diphenyl phosphate.

a.1 Method.

In a 50 cm^3 r.b. flask fitted with a pressure equalising funnel and a calcium chloride guard-tube, DPPC (6.2 g, 23.0 mmol) was added slowly to a continuously stirred solution of glycerol (1.0 g, 10.9 mmol) in dry pyridine (20 cm^3) at ca. -10°. The mixture was left to react for 16 hours at 3°. The pyridine was distilled off and the residue dissolved in ethyl acetate (30 cm^3), washed with ice-cold distilled water (2 x 10 cm^3) and dried.

a.2 Analysis.

TLC. Three components were separated by TLC. They had the R_f values of 0.64, 0.62 and 0.40. The components of R_f 0.64 and 0.62 were identified as phenol and triphenyl phosphate,(29) (cf. 2.3.3.2 a), respectively.

Infrared of the compound of R_f 0.40. $\bar{\nu}/cm^{-1}$ (Inferences): 680 and 750 (CH, monosubstituted aromatic ring), 770 and 1000 (P-O of P-O-C aliphatic), 950 and 1180 (P-O of P-O-C aromatic), 1290 (P=O of phosphate esters), 1455 (CH aliphatic), 1590 (C=C aromatic), 2840, 2910 and 2940 (CH aliphatic), 3060 (CH aromatic) and 3460 (OH).

PMR of the compound of R_f 0.40. δ: 7.2 (m, 5H, CH aromatic) and 4.4 (m, 1H, CH_2).

The analytical data above agree with the structure of glycerol bis-

1,3-diphenyl phosphate (25). It was separated from the product mixture by TLC and subjected to nucleophilic substitution.

b. Preparation of glycerol-1,3-distearate.

b.1 Method.

In a 25 cm^3 r.b. flask fitted with a water cooled condenser, glycerol bis-1,3-diphenyl phosphate (0.0236 g, 0.04 mmol) was reacted with TCMAS (0.076 g, 0.10 mmol) in refluxing n-hexane (15 cm^3) for 4 hours. The solvent was distilled off and the mixture separated by TLC and analysed.

b.2 Analysis.

The diacylglycerols were the only components separated and analysed.

TLC. Two components of R_f values 0.16 and 0.29 were separated using 1% (v/v) methanol in dichloromethane. The two components had the same infrared spectra and were not separated by GLC but they separated on silylation (2.1.3.6 a). No 2,3-epoxypropane-1-stearate (7) was detected.

Infrared. $\bar{\nu}/cm^{-1}$ (Inferences): 1745 (C=O of esters) and 3450 (OH).

GLC. Both compounds had a retention time of 5.2 min when run on the 0.45 m column. However when silylated (1.85 m column) the compound of R_f 0.16 had a retention time of 40.7 min and the compound of R_f 0.29 a retention time of 42.1 min (2.1.3.6 b).

EI-MS. m/z (major and significant ions): 623 $[M^+-1]$, 324 $[CH_2CHCH_2OCOC_{17}H_{35}]^+$ and 300 $[CH_2OCOC_{17}H_{35}+3]^+$.

The analytical data above confirm the compounds of R_f values 0.16 and 0.29 as glycerol-1,2-distearate (6) and glycerol-1,3-distearate (5) respectively. The total percentage yield of glycerol distearate was 22.4% (w/w), 81.2% of which were glycerol-1,3-distearate. (5) can be purified by TLC (2.1.3.7) and reacted with a given fatty acid chloride to give the corresponding symmetrical diacid triacylglycerol (2.1.3.8).

2.3.4 FINDINGS AND CONCLUSIONS.

The phosphate route is more hopeful than the silyl ether route in that the phosphate group can be substituted easily and under mild conditions by a carboxylate anion in a primary alkyl phosphate derivative. The substitution can take place in non-polar and polar protic and aprotic solvents. However the route using DPPC is not suitable for the preparation of symmetrical diacid triacylglycerols. The side-reactions which occurred during the preparation of the substrate were mainly caused by the participation of the phenyl groups in the formation of by-products when DPPC was reacted with polyhydric alcohols such as ethylene glycol and glycerol. A choice of a much more stable reagent which is also selective in its reactions with primary hydroxyl functions in the presence of secondary hydroxyl functions and which does not hinder its own substitution would be more satisfactory to the phosphate route.

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