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CHROMATOGRAPHIC ANALYSIS OF ALKOXY-LIPIDS

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CONTENTS

1. INTRODUCTION

The previous review[1] "Chromatographic analysis of plasmalogens" (alk-1′-enyl ether lipids) scarcely referred to their saturated analogs, alkyl ether lipids. In the last five years, the overwhelming evidence from biochemical studies on the bio-

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synthesis of alkoxy-lipids (1-alk-1′-enyl and alkyl glyceryl ether lipids) has clearly established the earlier postulate of Thompson[2] of a precursor product relationship between them. Hence tihs review, which summarizes the various chromatographic techniques employed in biochemical and chemical studies of alkoxy-lipids in the last five years, is titled "Chromatographic analysis of alkoxy-lipids". A small section on the chemistry, biochemistry and synthesis of alkoxy-lipids is also included for the benefit of the readers.

2. CHEMISTRY, BIOCHEMISTRY AND SYNTHESIS OF ALKOXY-LIPIDS

A. Chemistry of alkoxy-lipids

Polyol-lipids containing ether bonds are best classified as alkyl or 1-alk-1′-enyl ether lipids, where these terms with respect to unsaturation refer only to the α–β carbon linkage of the O-alkoxy moiety. The other carbon-to-carbon linkage may or may not be unsaturated.

CH_2–O–CH_2–$(CH_2)_n$–CH_3
|
CH_2–
alkyl ether bond

CH_2–O–CH=CH–$(CH_2)_m$–CH_3
|
CH_2–
alk-1′-enyl ether bond

Only the 1-alk-1′-enyl ether lipids have been referred to by other names like plasmalogens, vinyl ethers and enol ethers. Both types of ether lipids rarely occur free in nature and are usually found chemically linked to other moieties like acyl, phosphate, phosphonate, phosphorylated bases, etc. With the exception of 1,2-dialkyl-type lipids[3], the alkyl and 1-alk-1′-enyl groups are known to be linked to the C-1 of glycerol[4] (*Nomenclature of Lipids*, IUPAC-IUB Commission on Biochemical Nomenclature)[5]. In nature, to a large extent, the alkyl ethers are found as non-polar neutral lipids, while the 1-alk-1′-enyl ethers are found as polar phospholipids. Exceptions to this are: alkyl ether phospho- and phosphonolipids of *Tetrahymena pyriformis*[6] and alkyl ether phospholipids of beef red blood cells[7]. A detailed account on the distribution of these lipids in nature is available[4,8].

Physical methods have been used in the characterization of alkoxy-lipids. In the infrared (IR) studies, the alkyl ether lipids are typified by an absorption around 9 μ (ref. 9). This absorption is, however, masked in a phospholipid by phosphate absorption in that region. The 1-alk-1′-enyl lipids, on the other hand, are characterized by an absorption around 6 μ (ref. 10). Naturally occurring 1-alk-1′-enyl lipids possess *cis* configuration at the vinyl ether linkage. The derived alk-1′-enyl ether lipids possess both the *cis* and the *trans* configuration. They are easily distinguished by a singlet absorption at 1675 cm^{-1} for the former and a doublet absorption at 1680 cm^{-1} and 1665 cm^{-1} for the latter[11].

The technique of nuclear magnetic resonance (NMR) spectroscopy was used to distinguish the isomers of free saturated, monoenoic, and dienoic alkyl glyceryl ethers[9]. NMR studies were also done on naturally occurring alkoxy-lipids[12–15]. Although mass spectrometry (MS) is becoming a powerful tool in the field of lipid analysis[16], its application in the characterization of alkoxy-lipids has been limited[17–20].

The characterization of alkoxy-lipids often involves their chemical degradation

from their native state. The degradation products sometimes are further derivatized before their chromatographic analysis. Here only some of the important chemical degradation procedures will be described. Literature on derivatization will be cited in the Appendix.

Quantitative removal of acyl and phosphorylated base moieties from native alkoxy-lipids by reduction with lithium aluminium hydride[21] or Vitride[22], without disturbing the alkyl and 1-alk-1′-enyl ether linkage, is a very useful method (Table 6, No. 1).

The susceptibility of the alk-1′-enyl ether linkage to acidic conditions has been profitably used either in the liberation of free fatty aldehydes[23] or in their transformation to acetal derivatives[24]. These products are useful in the molecular species determination of 1-alk-1′-enyl ether chains[25].

The molecular species determination of alkyl ether chains is achieved after its cleavage to alkyl iodide by refluxing with hydriodic acid. The alkyl iodides can be converted to either alkanes by reductive dehalogenation or to alkenes by dehydrohalogenation[26]. All these derivatives are suitable for characterization by gas chromatography (GC).

Other chemical reactions, like saponification[27], deacylation with Grignard's reagent[28] for the stereospecific analysis of glycerides containing an ether bond, and lipolysis[29], have found limited use in the study of alkoxy-lipids.

The determination of the location of unsaturation in the hydrocarbon chains of ether lipids has necessitated the use of techniques like reductive ozoanalysis[30], permanganate–periodate oxidation[31] and chromic acid oxidation[32].

B. Biochemistry of alkoxy-lipids

(a) Biosynthesis of alkoxy-lipids

The earlier experimental evidence *in vivo*, implicating long-chain alcohols as the precursors of alkyl glyceryl ether lipids[33,34], has been confirmed by studies *in vitro*[35–38]. The initial belief that long-chain alcohols and dihydroxyacetone phosphate (DHAP) were the precursors of alkyl glyceryl ether lipids[35,36] had to be modified when it was established that DHAP had to be acylated to acyl DHAP prior to its transformation into alkyl DHAP[37,38]. During ether bond formation, the oxygen of the long-chain alcohol is retained[39] and the hydrogen attached to the non-phosphorus-containing carbon of DHAP is lost[40]. Alkyl DHAP is reduced to alkyl glycerophosphate in the presence of NADPH and subsequently acylated to alkyl acyl glycerophosphate[35,41]. A Mg^{2+}-requiring phosphatase (incidently, this enzyme is inhibited by F^-) produces alkyl acyl glycerol from alkyl acyl glycerophosphate. The alkyl acyl glycerol is subsequently converted into ethanolamine or choline phosphatide by reaction with CDP-ethanolamine or CDP-choline, respectively[42]. Enzyme systems catalysing such reactions have been detected in neoplastic cells[39,41–47] and normal animal tissues[35,37,48–51].

An alternate route for the synthesis of ether lipids has recently been detected. This involves alkyl glycerol instead of alkyl DHAP as the precursor[52]. The route appears to be essential for incorporation of alkyl glycerol derived from dietry sources or catabolic processes. Although this new pathway involves other reactions such as acylation, phosphorylation, etc., their order of sequence is not known at present. This

later finding thus supports some of the earlier observations[50,53,54] indicating non-involvement of DHAP in ether bond formation.

In recent years, a great deal of evidence in mammalian and non-mammalian systems has indicated alkyl glycerophospholipids to be the precursors of alk-1′-enyl glycerophospholipids[22,55–71]. Recent experiments *in vivo*[65,68] and *in vitro*[69–71] have established that intact 1-O-alkyl-2-acyl-*sn*-glycero-3-phosphorylethanolamine is the immediate precursor of structurally related 1-O-alk-1′-enyl-2-acyl-*sn*-glycero-3-phosphorylethanolamine. However, Debuch and coworkers[63,72] suggest 1-O-alkyl-*sn*-glycero-3-phosphorylethanolamine to be the likely precursor of 1-O-alk-1′-enyl-*sn*-glycero-3-phosphorylethanolamine, which is subsequently acylated to ethanolamine plasmalogen. Recent reports from the laboratories of Snyder *et al.*[73] and Paltauf and Holasek[74] suggest the desaturase involved in this conversion to be a mixed function oxidase similar to the one involved in the biosynthesis of monoenoic fatty acids[75,76]. The common features between these two reactions are: *cis* elimination of hydrogen during olefination, requirement of molecular oxygen and reduced pyridine nucleotide, and inhibition by cyanide but not by carbon monoxide.

(b) Biodegradation of alkoxy-lipids

A tetrahydropteridine hydrolase capable of splitting the ether bond of alkyl glycerols was reported by Tietz *et al.*[77]. An NADPH-linked reductase was essential to regenerate the tetrahydropteridine from dihydropteridine produced during the hydroxylation step. A hemi-acetal structure, with a hydroxyl group on the α-carbon of the O-alkyl moiety, is supposed to break spontaneously to glycerol and fatty aldehyde. In the presence of NAD^+ fatty acid is produced, while in the absence of NAD^+ fatty aldehyde[77,78] and fatty alcohol[78] are produced.

Enzymes capable of cleaving specifically 1-alk-1′-enyl-*sn*-glycero-3-phosphorylcholine[10] and 1-alk-1′-enyl-2-acyl-*sn*-glycero-3-phosphorylethanolamine[79] are known.

A survey of enzymes responsible for the cleavage of ester bonds in alkoxy-lipids has recently been published[80].

C. Chemical synthesis of alkoxy-lipids

(a) Alk-1′-enyl ether lipids

The basic requirement for the synthesis of alkoxy-lipids is the synthesis of an ether bond. It is always easy to hydrogenate 1-alk-1′-enyl ethers of polyols to the corresponding alkyl ethers, but the reverse chemical reaction is rather difficult to achieve. Hence, in this section we will consider the chemical synthesis of 1-alk-1′-enyl ethers of polyols and then that of alkyl ethers of polyols.

The chemical synthesis of alk-1′-enyl ether lipids is grouped into three categories for convenience, *viz.* (i) synthesis of 1-alk-1′-enyl ethers of polyols, (ii) acylation of (i) to neutral plasmalogens, and (iii) conversion of (i) to phosphatide plasmalogens.

(i) Synthesis of 1-alk-1′-enyl ethers of polyols. A number of methods are available for this purpose. The method developed by Craig and Hamon[81,82] involves the dehalogenation of the condensation product of 2-benzylglycerol and 2-bromo-1,1-dimethoxyalkane with lithium, to yield the *cis* and *trans* isomers of 1-alk-1′-enyl ether of glycerol. The isomers, after acetylation, were separated by preparative gas–liquid chromatography (GLC) (Table 8, No. 2). A similar transacetalation followed

by debromination approach was used in the synthesis of 1-alk-1′-enyl ether of ethanediol. The isomers were separated by argentation thin–layer chromatography (TLC)[83] (Table 6, No. 5).

Gigg and co-workers obtained symmetrical alkanal di(*sn*-glycerol-2,3-carbonate) acetal by transacetalating *sn*-glycerol-2,3-carbonate[84] and the corresponding alkanal dimethyl acetal. Reaction with acetyl chloride of the condensation product produced the corresponding 1′-chloroether. Subsequent elimination of hydrogen chloride by triethylamine and hydrolysis of the carbonate group by alkali gave predominantly the *cis* isomer[85,86].

Elimination of either hydrogen iodide or *p*-toluenesulfonic acid with potassium *tert*.-butoxide from either 1-(2′-iodo-*or*-tosyloxyalkyl)glycerol or 1-(2′-iodo-*or*-tosyloxyalkyl)-2,3-epoxypropane yielded 1-alk-1′-enyl glyceryl ether[88–92] or 1-alk-1′-enyl-2,3-epoxypropane[93,94].

(ii) Acylation of (i) to neutral plasmalogens[86,87,95]. The acylation of 1-alk-1′-enyl glyceryl ethers with long-chain acid chlorides in pyridine yielded neutral plasmalogens. A similar approach was used to synthesize diol (neutral) plasmalogens[96]. A semi-synthetic approach was reported by Viswanathan *et al.*[97]. The $LiAlH_4$ reduction products of phosphatide plasmalogens, after chromatographic isolation, were interesterified with reference fatty acid methyl esters in the presence of sodium methoxide as a catalyst.

Transacetalation of 1,2-diacylglycerol with either 1-alk-1′-enyl ethyl ether or diethyl acetal in the presence of *p*-toluenesulfonic acid followed by elimination of ethanol in the presence of sulfanilic acid yields directly neutral plasmalogens[98].

(iii) Conversion of (i) to phosphatide plasmalogens. 1-Alk-1′-enyl-2,3-epoxypropane[93,94] on acylation with fatty acid chloride or bromide yields 1-alk-1′-enyl-2-acylglycerol-3-halohydrin[99,100], which is converted to phosphatide plasmalogens by the classical pathways[101].

A method using enhanced reactivity of the primary compared to the secondary hydroxyl group of 1-alk-1′-enyl glyceryl ether involved selective tosylation of the primary hydroxy group and then acylation at the secondary hydroxy group. This product was converted to iodohydrin and then coupled with silver dibenzyl or di-*p*-nitrobenzyl phosphate, yielding a phosphotriester[102]. This was converted to dimethylethanolamine plasmalogen by well known procedures of phospholipid chemistry[103,104].

A semisynthetic approach involving conversion of beef heart phosphatide plasmalogens (choline and ethanolamine) by phospholipase C to 1-alk-1′-enyl-2-acylglycerol followed by its transformation to choline plasmalogen[105] and phosphatidic acid plasmalogen[106] has been reported.

(b) Alkyl ether lipids.

For the sake of convenience, the synthesis of alkyl ether lipids is considered under three sub-headings, *viz.*, (i) synthesis of alkyl ethers of polyols, (ii) alkoxydiglycerides, and (iii) alkoxy-phospholipids. In deriving any of these three products, one can hydrogenate the corresponding 1-alk-1′-enyl ether lipids. However, this approach would yield only saturated alkyl ether lipids while the naturally occurring alkyl ether lipids usually contain polyunsaturated acids and saturated or monosaturated ether chains. Hence some other synthetic approaches have been used and they are briefly summarized here.

(i) Alkyl glyceryl ethers. Alkyl halides[107-111], alkyl tosylates[112], and alkyl mesylates[113,114] on condensation with 1,2-O-isopropylidene and 1,3-benzylidene glycerol derivatives followed by acid hydrolysis and hydrogenolysis yield alkyl glyceryl-(1) and glyceryl-(2) ethers, respectively. The elaidization of unsaturation from the oleyl moiety of oleyl bromide during condensation[108] was avoided by condensing 1,2-isopropylidene-3-tosyl glycerol with sodium oleoxide[115]. Preferential tritylation of the primary hydroxy group of alkyl glyceryl ethers was used to prepare 1,2-dialkyl and subsequently trialkyl glyceryl ethers[116]. Additional advantage of the trityl derivative over benzyl and 1,3-benzylidene derivatives is its easy removal by acid hydrolysis. Thus trityl derivatives are useful in the preparation of unsaturated ethers[111].

Alkyl ethers of 1,2-ethanediols were prepared[117] by glycol cleavage of alkyl glyceryl-(1) ether and subsequent reduction of the resulting alkoxy acetaldehyde with $LiAlH_4$.

(ii) Alkyl diglycerides. These were prepared by acylation of alkyl ethers with acyl chloride in pyridine[111,117,118].

(iii) Alkyl phospholipids. Esterification of di-O-alkyl glycerol in the presence of triethylamine and 2-phthalimidoethylphosphonic acid via its monochloride and removal of the protective phthaloyl group by hydrazinolysis yield dialkyl phosphonates[119]. A similar approach, but with use of a phosphoric acid analog, yielded phosphatidylethanolamine[112]. The diether analog of phosphatidylcholine was prepared by phosphorylation of di-O-alkyl glycerol with monophenylphosphoryl dichloride and pyridine, followed by esterification of the condensation product with choline iodide and subsequent removal of the phenyl group by hydrogenolysis[120]. Interaction of alkyl acyl glycerol diiodide with silver 2-(benzyloxycarbonylamino)ethyl phosphate yielded alkylacylcephalin[121,122]. Recently, syntheses of optically active alkyl ether glycerides and phospholipids have been reported[123].

An important intermediate in the biosynthesis of alkoxy-phospholipids, 1-O-alkyl dihydroxyacetone phosphate[124,125] was recently synthesized from alkyl glyceryl-(1) ether. This compound after benzoylation was oxidized to the keto intermediate with dimethyl sulfoxide and dicyclohexyl carbodiimide in the presence of trifluoroacetic acid. The compound after ketalysis and alkaline hydrolysis was phosphorylated with diphenyl phosphorochloridate.

3. ANALYSIS OF ALKOXY-LIPIDS

A. Preparation of lipid material

This aspect was dealt with in an earlier review[1]. Since then, three new books have been added to the general methodology of lipids[126-128]. A recent modification[129] of the Bligh and Dyer method[130], as applied to materials containing large quantities of water, is a useful one.

B. Fractionation of lipids into classes

This aspect was also dealt with exhaustively in the previous review[1]. Useful details can be had in two of the recent publications[126,127]. The technique of ascending

dry column chromatography (CC), as applied to the fractionation of non-polar lipid classes[131], and its extension to the fractionation of polar lipids[132], both on a preparative scale, is very useful. A recent useful addition to this is the publication "Qualitative analysis of lipid classes by gradient elution absorption chromatography", by which 28 components in rat red blood cell lipids could be detected by a relatively fast single operation[133]. The chromatographic system consisted of a pressurized apparatus and involved the use of a continuous series of gradient changes of pentane, diethyl ether, chloroform and methanol containing 8% ammonia with a column packed with Corasil II (37–55 μ particle size) modified by treatment with ammonia.

Theoretical aspects involved in the fractionation of lipids by liquid CC and TLC and some practical suggestions for these fractionations have been recently discussed[134].

C. *Quantitation of alkoxy-lipids*

The available methods will be considered under three separate categories: (a) Estimation of 1-alk-1′-enyl ether lipids, (b) estimation of alkyl ether lipids, and (c) estimation of 1-alk-1′-enyl and alkyl ether lipids in the presence of one another.

TABLE I

TLC SYSTEMS USED IN THE QUANTITATION OF ALKOXY-LIPIDS

No.	*(a) Source of lipid* *(b) Method of estimation* *(c) Class estimated*	*Chromatographic system*	*Reference*
1	(a) Total lipids of animal tissues (b) Polar lipid classes separated in the first dimension free of non-polar lipids; chemical cleavage of the 1-alk-1′-enyl bond and separation in the second dimension between mono- and diacyl phospholipids; "P" determined (c) 1-Alk-1′-enyl content	Both dimensions (run up to 3/4 height of plate): silica gel G; chloroform–methanol–acetic acid–water (65:43:1:3) First dimension (run up to top of plate): petroleum ether (b.p. 40–60°)–diethyl ether–acetic acid (80:20:1) Second dimension: chloroform–methanol–water (60:35:8)	156
2	(a) Analogs of phosphatidylcholine or ethanolamine of animal origin (b) Chemical cleavage of the 1-alk-1′-enyl bond with 2,4-dinitrophenylhydrazine–phosphoric acid reagent, subsequent separation of the products and spectrophotometric estimation of hydrazones (c) 1-Alk-1′-enyl content	Silica gel G; chloroform–methanol–conc. ammonia (75:25:4)	137
3	(a) Phosphatidylethanolamine of animal origin (b) Formation of aldehydes and methyl esters by reaction chromatography and their separation followed by gravimetric estimation (c) 1-Alk-1′-enyl content	Silica gel G; toluene	153

(Continued on p. 136)

TABLE 1 *(continued)*

No.	*(a) Source of lipid* *(b) Method of estimation* *(c) Class estimated*	*Chromatographic system*	*Reference*
4	(a) 1-Alk-1′-enyl acyl and diacyl ethanolamine from beef heart lipids (b) After acid cleavage of 1-alk-1′-enyl linkage, separation of mono- and diacyl phospholipids and aldehydes in the first dimension. Subsequent methanolysis of acyl phospholipids on the plate and then separation in the second dimension. Estimation with internal standard (gas chromatographic analysis) (c) 1-Alk-1′-enyl content	First dimension: silica gel G/chloroform–methanol–conc. ammonia (70:30:5); second dimension: silica gel G/toluene	150
5	(a) Diglyceride acetates derived from analogs of phosphatidylcholine isolated from beef heart (b) After acid cleavage of 1-alk-1′-enyl linkage, aldehydes, monoacyl, alkyl acyl, and diacyl glyceride acetates separated in the first dimension. Subsequent methanolysis of all acyl moieties on the plate and separation in the second dimension. Estimation with internal standard (gas chromatographic analysis) (c) Alkyl and 1-alk-1′-enyl content	First dimension: silica gel G/petroleum ether–diethyl ether (88:12); second dimension: silica gel G/toluene	151
6	(a) $LiAlH_4$ reduction product of total alkoxy-lipids	Silica gel G/diethyl ether–30% aqueous ammonia (100:0.25) or silica gel G/diethyl ether–water (100:0.5)	149

(a) Estimation of 1-alk-1′-enyl ether lipids[1]

It may suffice to say that the plasmalogen content of a natural lipid mixture can be determined by reacting the mixture with an acidic carbonyl reagent like fuchsin–sulfurous acid[135,136] or 2,4-dinitrophenylhydrazine–phosphoric acid reagent[137] and then measuring spectrophotometrically the colour complexes of the liberated aldehyde. An alternate but sensitive method of Sigga and Edsberg[138], by which specific iodination of an α–β unsaturation in a vinyl ether can be achieved, was successfully used to estimate the plasmalogen content of lipids[139,140].

(b) Estimation of alkyl ether lipids

Alkyl ethers, when present as alkyl glyceryl ethers in glycerolipids, are isolated as free alkyl glyceryl ethers either by saponification (applicable only to non-phospho- and non-glycoglycerolipids) or by acetylosis[141] followed by saponification. These can be estimated by periodate oxidation[142–143], in which the vicinal hydroxyl groups of

alkyl glyceryl ethers produce formaldehyde that is estimated colorimetrically by reaction with chromotropic acid.

A semiquantitative method by infrared spectroscopy of free alkyl glyceryl ethers in chloroform by measurement of its optical density at 9.0 μ (a strong ether adsorption) has been reported[144].

Spectrophotometric methods utilising either ultraviolet absorbancies of alkyl iodides derived from alkyl glyceryl-(1) ethers at 257 nm[145] or cyclic thionocarbonate derivatives of 1(3)-alkyl glycerols at 235 nm[146] have been reported.

Gravimetric estimation of O-alkyl glycerols, after their isolation by CC procedures, has been feasible[147]. All the methods cited above can be applied to free 1-alk-1'-enyl glyceryl ethers after hydrogenation[148].

Alkyl diol ethers could be estimated by infrared spectroscopy[144], by alkyl iodide spectrophotometry[145], and by gravimetric measurement[147].

(c) Estimation of 1-alk-1'-enyl and alkyl ether lipids in the presence of one another

Two approaches have been used to achieve this, *viz.* (i) conversion of glycerolipids to free alkoxyglyceryl ethers followed by their chromatographic separation and quantitation[149] (Table 1, No. 6) and (ii) two-dimensional reaction TLC in the separation and quantitation of native phospholipids[150] or their modified derivative[151] (Table 1, Nos. 4 and 5, respectively).

(i) Conversion of glycerolipids to free alkyl and 1-alk-1'-enyl glyceryl ethers. This is achieved by saponification of neutral lipids, acetolysis of non-polar or polar glycerolipids[141] and by reduction with lithium aluminium hydride[21] or Vitride[152]. The resulting alkoxy-lipids are isolated by CC and estimated gravimetrically or separated by TLC and estimated by densitometry of the charred products[149].

(ii) Two-dimensional reaction thin-layer chromatography

Native phospholipids. The acid hydrolysis of alk-1'-enylacylethanolamine phosphatide[153] and the alkaline methanolysis[153,154] of 2-acyl- and diacylethanolamine phosphatide on TLC plates was shown to be quantitative (Table 1, No. 3). Application of this technique[153] to a two-dimensional TLC system[150] made it possible not only to determine the fatty acid composition of alkenylacyl- and diacyl- plus alkylacylethanolamine phosphatides but also the plasmalogen content of the phospholipid and its aldehyde composition. The plasmalogen content could be determined by alternate methods: either by phosphorus determination of the glycerylphosphorylethanolamine residues released by alkenylacyl and diacyl and alkylacyl analogs or by the use of a methyl ester internal standard.

Derivatives of phospholipids. A modified version of the above-described two-dimensional reaction TLC was developed by our group[151] which permitted a quantitative determination of the three analogs, their individual fatty acid compositions and fatty aldehyde composition of the alkenylacyl analog. The modification of the procedure involved the conversion of analogs of beef heart choline phosphatides into three families of diglyceride acetate by phospholipase C action followed by acetylation and, after TLC separation in the first dimension, interesterification of the diglyceride acetates by 2 *N* sodium methoxide in absolute methanol[155]. This procedure also helped detect the presence of dialkylcholine phosphatides in beef heart phosphatides.

A two-dimensional method for the estimation of the plasmalogen content of

a mixture of phospholipid classes[156] was described in the previous review[1] (Table 1, No. 1).

D. Isolation of alkoxy-lipids

TLC was used successfully in the isolation of pure alkyl diacyl glyceryl ethers[157] and dialkyl ethers of pentanediol[158] from total lipids of dogfish oil and jaw oil of porpoise (*Phocoena phocoena*), respectively. The TLC system silica gel G/hexane–diethyl ether–acetic acid (90:10:1) was used by Malins *et al.*[157] to separate diacyl glyceryl ethers of dogfish from the accompanying triacylglycerol (Table 2, No. 1). On the other hand, Varanasi and Malins[158], fractionating the total lipids of porpoise jaw oil by adsorption TLC using hexane–diethyl ether (80:20) as the developing solvent, isolated a fraction

TABLE 2

TLC SYSTEMS FOR THE ISOLATION OF NONPOLAR ALKOXY-LIPIDS

No.	*(a) Source of lipid mixture* *(b) Isolated fraction*	*Chromatographic system*	*Reference*
1	(a) Total non-polar lipids of dogfish (b) O-Alkyl diglyceride	Silica gel G/hexane–diethyl ether–acetic acid (90:10:1)	157
2	(a) Total non-polar lipids of human perinephric fat (b) O-Alkyl and O-alk-1′-enyl diglycerides	Silica gel G/hexane–diethyl ether (95:5), repeated chromatography	159
3	(a) Total non-polar lipids of porpoise jaw oil (b) O-Dialkyl pentanediol	Silica gel G/hexane–diethyl ether (80:20)	158
4	(a) Concentrates of O-alk-1′-enyl 2-acylethanediol (b) O-Alk-1′-enyl 2-acylethanediol	Grade IV alumina (thickness 2 mm)/hexane–diethyl ether (20:1)	
5	(a) Concentrates of O-alkyl 2-acylethanediol (b) O-Alkyl 2-acylethanediol	Silica gel (thickness 1.0 mm)/hexane–diethyl ether (85:15)	160
6	(a) Total non-polar lipids of the pink portion of the Harderian gland of the New Zealand White rabbit (b) Uncharacterized ether lipid containing esterified hydroxyalkyl glyceryl ether moiety	Silica gel G/benzene (two developments)	161, 162
7	(a) Synthetic mixture of diol-lipids (i) 1-alk-1′-enyl *trans*-2-acylethanol (ii) 1-alk-1′-enyl *cis*-2-acylethanol (iii) alkyl 2-acylethanol (iv) diacyl ethanol (b) —	Silica gel G/hexane–diethyl ether (95:5)	96

(R_F 0.6) which separated completely from the accompanying triacylglycerol (Table 2, No. 3). This fraction, on reduction with $LiAlH_4$, yielded two fractions (R_F 0.13 and 0.62, in the same TLC system). The fraction of lower mobility was found to be alkyl glyceryl-(1) ether and the other fraction was characterized by IR and NMR spectroscopy and MS as mainly dialkoxypentane having primarily two C_{18} units.

In an earlier review[1], it was indicated how repeated chromatography on 1-mm-thick layers of silica gel G resulted in the concentration of 1-alk-1′-enyl and alkyl diglycerides from human perinephric fat with hexane–diethyl ether (95:5) as the developing solvent. This ultimately yielded pure fractions of 1-alk-1′-enyl diglycerides as well as alkyl diglycerides[159] (Table 2, No. 2).

Recently alkyl and 1-alk-1′-enyl ethers of ethanediol, which constitute about 35% of the triglycerides, were isolated by combination of CC and TLC from the lipids of starfish, *Distolasterias nipon*[160]. The lipids were fractionated by elution from silica gel KSK columns with a mixture of hexane–diethyl ether (95:5). The first couple of 20-ml fractions from the column (40 × 3.5 cm) contained the 1-alk-1′-enyl ethers of monoacylated ethanediol and the subsequent couple of fractions contained alkyl ethers of monoacylated ethanediol. The triglycerides were eluted with a 4:1 mixture of hexane–diethyl ether. The fractions containing the 1-alk-1′-enyl ether of acylethanediol were purified by preparative TLC on grade IV alumina (alkaline) 2.0-mm thick plates with hexane–diethyl ether (20:1) as the developing solvent. The lipid fraction with an R_F of 0.60 was found to be mainly the octadeca-1′,9′-dienyl ether of stearoylethanediol. On the other hand, the fractions containing the alkyl ether of acylethanediol were further purified by preparative TLC on 1-mm-thick plates made from silica gel derived from sodium silicate using hexane–diethyl ether (85:15) as the developing solvent. The lipid was mainly a mixture of hexadecyl and octadecyl ethers of stearoylethanediol. In both cases the ether lipids were recovered from the silica gel layers by extraction with mixtures of chloroform–methanol (4:1) (Table 2, Nos. 4 and 5, respectively).

New types of alkoxy-lipids were recently isolated by Snyder's group[161,162] from the pink portion of the Harderian gland of the New Zealand white rabbit. The total lipids of this tissue contain two unusual alkoxy lipids with trace amounts of normal 1-alkyl-2,3-diacylglycerol. The minor component of the two unusual lipids had the usual 1-alkyl-2,3-diacylglycerol structure with a short-chain acid (isovaleric) remaining esterified specifically to the C-3 of glycerol[161]. The major component, as yet uncharacterized, on the other hand, had again the usual 1-alkyl-2,3-diacylglycerol structure, with an ether chain comprising either 16 or 18 carbon atoms having a hydroxyl group attached to C-10 or C-11 and C-11 or C-12, respectively[162]. In the native lipid, the hydroxyl group was found to be esterified. The two unusual alkoxy-lipids were separated from each other by preparative TLC. On silica gel G plates and with two developments in benzene, the minor component of the unusual alkoxy-lipid travelled ahead of the major component of the unusual alkoxy-lipid but still indicated slower mobility than the reference triacylglycerol. In this system, the normal 1-alkyl-2,3-diacylglycerol travelled ahead of triacylglycerol (Table 2, No. 6). In another TLC system, silica gel G/hexane–diethyl ether–acetic acid (90:10:1), the major unusual alkoxy-lipid had the same R_F value as the triacylglycerol but the minor unusual alkoxy-lipid and the normal 1-alkyl-2,3-diacylglycerol travelled ahead of triacylglycerol and also separated from one another[162].

A chromatographic system for the separation of synthetic alkoxy-diol lipids was reported[96]. This should be useful for the isolation of natural alkoxy-diol lipids (Table 2, No. 7).

(a) Isolation of alkoxy-phospholipids

A variety of alkoxy-phospholipids exist in nature and hence one can subdivide them into diol alkoxy-phospholipids and alkoxy glycerophospholipids. These can be further divided into 1-alk-1'-enyl phospholipids and alkyl phospholipids.

In the class of diol phospholipids, so far the presence of alk-1'-enyl ethers of ethanediolphosphorylcholine and 1,3-propanediolphosphorylcholine have been established but their isolation has not been achieved[163,164]. No alkyldiol phospholipids have been detected so far.

In an earlier review[1], applications of methods like partition chromatography[165], argentation chromatography[166,167], selective enzymatic hydrolysis[168,169], and alkaline hydrolysis[170,171] for the isolation of reasonably pure concentrates of phosphatide plasmalogens were reported. A modified base-catalysed selective methanolysis procedure was recently used to isolate ethanolamine plasmalogen from bovine white matter[172]. According to the latter authors, the method was based on the resistance of the acyl linkage of the plasmalogen to mild alkaline hydrolysis in the presence of methanol and in the absence of chloroform. The isolated ethanolamine plasmalogen, however, contained about 10% of the alkyl acyl analog. The acyl linkage of the latter component seemed to be equally resistant to mild alkaline hydrolysis.

The same technique of base-catalysed selective methanolysis was used in the isolation of alkyl acyl glycerophosphorylethanolamine from the phosphatidylethanolamine fraction of bovine red blood cells[173] and alkyl acyl 2-aminoethylphosphonoglyceride from phosphonoglycerides of *Tetrahymena pyriformis* W[174].

In the author's laboratory, concentration of ethanolamine plasmalogens from sheep brain lipids[175] and alkyl acyl glycerophosphorylcholine[176] as well as alkyl acyl 2-aminoethylphosphonoglyceride[132] from lipids of *Tetrahymena* was achieved by ascending dry CC. The total lipids were fractionated by ascending dry CC (silica gel G/chloroform–methanol–ammonia (65:35:5) and different sections of the column were analysed for the nature of the phospholipid. Among the various fractions of phosphatidylethanolamine isolated from sheep brain, the ones that were ahead were almost free of diacyl phosphatidylethanolamine. A similar behaviour of phosphatidylcholines isolated from *Tetrahymena pyriformis* W by ascending dry CC (silica gel G/chloroform–acetic acid–methanol–water, 75:25:5:1.5) was observed when re-run on a second column with chloroform–methanol–conc. ammonia (65:35:5) as the developing solvent.

E. Fractionation of alkoxy-lipids

In spite of the availability of various chromatography techniques, PC, CC, TLC, and GC, only the last two techniques have found wide application in the study of alkoxy-lipids. PC has been scarcely used and CC finds some limited use. Hence a discussion on the application of CC, TLC, and GC to the study of alkoxy-lipids will follow.

(a) Column chromatography

Since the availability of TLC, especially on a preparative scale with thicker layers, the technique of CC has fallen behind. Micro-techniques like coupled GC–MS[177] have further contributed to this. In spite of all this, CC has certain advantages.

Recently, applicaton of ascendiing dry CC[131] to the fractionation of polar lipids of the protozoan *Tetrahymena pyriformis* was reported from the author's laboratory[132]. With 200 g of TLC-grade silica gel G, 6 g of total lipids were successfully fractionated and success was achieved in obtaining substantial amounts of pure alkyl acyl 2-aminoethylphosphonoglycerides and pure diacyl 2-aminoethylphosphonoglycerides. Using the same technique, but on a smaller scale, it was possible to separate alkyl acyl glycerophosphorylcholine from diacyl glycerophosphorylcholine of *Tetrahymena pyriformis* and 1-alk-1′-enyl acyl glycerylphosphorylethanolamine from diacyl glycerylphosphorylethanolamine of sheep brain[178].

Preparative isolation of free glyceryl ethers, after acetolysis and saponification of fish oils, by silicic acid CC was achieved by elution with hexane–diethyl ether (7:3)[147].

Aluminium oxide (Brockman Grade I, pH 7.0) CC was used to separate the saturated from the unsaturated alkyl glyceryl ethers. They were fractionated as 2,3-O-isopropylidene glyceryl ether adducts of mercury acetate (acetoxymercurimethoxy derivatives). Petroleum ether (b.p. 40–60°) was used to elute the saturated alkyl glyceryl ethers. Diethyl ether containing 5% methanol eluted the monounsaturates. Methanol containing 1 part of 12 *N* hydrochloric acid was used to elute the polyunsaturated alkyl glyceryl ethers. The alkyl glyceryl ethers were generated from their adducts by treatment at room temperature for 45 min with methanol containing 10% hydrochloric acid and subsequently extracted in diethyl ether[173].

Separation by gel CC of naturally occurring phosphatidylcholine mixtures according to number of ethylenic linkages was reported by King and Clements[179]. They used a column of Sephadex LH-20 (an alkylated dextran) and eluted mercury acetate addition compounds of unsaturated phosphatidylcholines by the organic solvent system benzene–chloroform–methanol (30:30:40) with increasing amounts of glacial acetic acid or chloroform–methanol (50:50) with increasing amounts of glacial acetic acid, the latter varying from 0.01 to 0.1%). Their system resolved phosphatidylcholines, isolated from animal tissues, into at least four molecular species. In preliminary investigations a similar system was used in our laboratory for the fractionation of alkyl acyl glycerylphosphorylcholine from *Tetrahymena pyriformis*.

(b) Thin-layer chromatography

Fatty alcohols, aldehydes and acids, which are constituents of alkoxy-lipids and which are also used in the synthetic and biochemical studies of alkoxy-lipids, occur in nature as a variety of molecular species varying in chainlength (straight and branched), unsaturation (number and location), and substituent groups (structure, number, and location). Their separation into individual molecular species is achieved by a combination of TLC techniques like adsorption chromatography, argentation adsorption chromatography, and reversed-phase chromatography. Their derivatization (ozonides, bromides, and mercuric acetate adducts) followed by fractionation by adsorption chromatography further aids in the characterization of these molecular species. These methods were reviewed earlier[1,180].

Separation of specifically iodinated choline plasmalogen from the corresponding

non-iodinated diacylcholine phosphatide was achieved by preparative TLC on a silica gel G plate with chloroform–methanol–ammonia (70:30:5) as the developing solvent. This helped in the determination of the fatty acid composition of both the analogs separately[181]. TLC was also successfully used to isolate concentrates of choline and ethanolamine plasmalogens and to separate alkyl acyl and diacyl analogs of 2-aminoethylphosphonoglycerides[182] (Table 3).

TABLE 3

TLC SYSTEMS FOR THE SEPARATION OF ALKOXY-PHOSPHOLIPIDS FROM THE ACCOMPANYING DIACYL ANALOGS

No.	*Lipid components separated*	*Chromatographic system*	*Reference*
1	Alkyl acyl and diacyl 2-aminoethylphosphonoglycerides	Silica gel F_{254} chloroform–methanol–conc. ammonia (65:35:5) or silica gel F_{254} chloroform–acetic acid–methanol–water (75:25:5:1.5)	182
2	Concentration of 1-alk-1′-enyl acyl phosphatidylcholine from analogs of phosphatidylcholine of beef heart origin	Silver nitrate-impregnated silica gel G chloroform–methanol–water (70:25:3)	166
3	Concentration of 1-alk-1′-enyl acyl phosphatidylethanolamine from analogs of phosphatidylethanolamine of beef heart origin	Silver nitrate-impregnated silica gel G chloroform–methanol–water (70:30:4.5)	167
4	Specifically iodinated 1-alk-1′-enyl acyl phosphatidylcholine from the accompanying diacyl analog of beef heart origin	Silica gel G chloroform–methanol–ammonia (70:30:5)	181

At present no chromatographic system is available to achieve a quantitative separation between different analogs of a phospholipid class. This is only possible when these are transformed into non-polar derivatives such as family of diglyceride acetates[157,183], or dimethyl phosphatidates[184], or O-methylated N-dinitrophenylated derivatives[185]. Adsorption chromatographic systems on silica gel G plates have been described (Table 4). Thus the family of diglyceride acetates could be separated into three analogs by the use of petroleum ether (b.p. 40–60°)–diethyl ether (1:1) followed by toluene, the latter solvent being essential to separate the alkoxy analogs[183] (Table 4, No. 1). Degradation of 1-alk-1′-enyl diglyceride acetate with hydrochloric acid fumes followed by TLC separation with successive use of petroleum ether (b.p. 40–60°)–diethyl ether (88:12) and toluene resolved the family of glyceride acetates into aldehydes, dialkyl glyceride acetate, alkyl acyl glyceride acetate, diacyl glyceride acetate and monoacyl glyceride acetate in that order of decreasing mobility[157]. Successive use of hexane–chloroform (4:6) and toluene–chloroform (4:6) five times each resulted in the separation of O-methylated N-dinitrophenylated derivatives of analogs of phosphatidylethanolamine[185] (Table 4, No. 2). Seven successive developments with the same solvent system, hexane–chloroform (1:1), resolved dimethylphosphatidate derivatives of phosphatidylcholine analogs[184] (Table 4, No. 3).

The keto-intermediates O-alkyl dihydroxyacetone phosphate (O-alkyl DHAP)

TABLE 4

TLC SYSTEMS FOR THE SEPARATION OF NON-POLAR DERIVATIVES OF PHOSPHOLIPID ANALOGS

No.	*Non-polar derivatives separated*	*Chromatographic system*	*Reference*
1	Alkyl acyl, 1-alk-1′-enyl acyl and diacyl glyceride acetates	Silica gel G/petroleum ether(b.p. 40–60)–diethyl ether (1:1) followed by toluene	183
2	Alkyl acyl, 1-alk-1′-enyl acyl and diacyl phosphatidylethanolamine as O-methylated N-dinitrophenylated derivatives	Five successive developments on silica gel G plates with hexane–chloroform (4:6) followed by another five successive developments with toluene–chloroform (4:6)	185
3	Alkyl acyl, 1-alk-1′-enyl acyl and diacyl phosphatidylcholine as dimethylphosphatidate derivatives	Seven successive developments on silica gel G plates with hexane–chloroform (1:1) as the developing solvent	184

and O-alkyldihydroxyacetone (O-alkyl DHA) involved in the biosynthesis of O-alkyl-linked lipids were identified by TLC of derivatives and standards prepared by $LiAlH_4$ reduction, periodate oxidation before and after $LiAlH_4$ reduction, acid hydrolysis, alkaline hydrolysis, action of alkaline phosphatase, etc.[186]. Some of the acetylation products were also characterized. On a TLC plate of silica gel G with chloroform–methanol–acetic acid (98:2:1) as the developing solvent, O-alkyl DHAP, O-alkyl glycerol, fatty alcohol, O-alkyl DHA, and wax esters separated in that order of increasing mobility. On using an alternative solvent system, petroleum ether (b.p. 40–60)–diethyl ether–methanol–acetic acid (70:30:5:1), only fatty alcohol and O-alkyl DHA reversed their order of mobility. Conversion of O-alkyl DHA to O-alkyl glycerol with $LiAlH_4$ was tested in the chromatography system silica gel G/diethyl ether–water (200:1), in which as expected the former possessed higher mobility than the latter. O-Alkyl glycolic acid, fatty alcohol and O-alkyl glycolic aldehyde were separated in that order of increasing mobility on silica gel G plates using diethyl ether–acetic acid (99.5:0.5) as the developing solvent. Fatty alcohol acetate travelled ahead of O-alkyl ethylene glycol acetate in the TLC system silica gel G/hexane–diethyl ether (80:20) (Table 5, Nos. 1–4).

Three molecular species of alkyl glyceryl ethers —alkyl glyceryl-(1) ether, 2′-hydroxyalkyl glyceryl-(1) ether (synthetic product), and 9′(10′)-hydroxyalkyl glyceryl-(1) ether (from New Zealand white rabbit)— are separable on silica gel G plates using diethyl ether–water (100:0.5) as the developing solvent. The 9′(10′)-hydroxyalkyl glyceryl ether has a higher R_F value than the corresponding 2′-hydroxy lipid[162] (Table 6, No. 2).

The positional isomers of alkyl glyceryl-(1 or 2) ethers, which were synthesized chemically, could be easily separated from one another on silica gel G plates impregnated with either 10% sodium arsenite or 5% boric acid. With chloroform–methanol (98:2) as the developing solvent system, the 1-isomer had a higher mobility than the corresponding 2-isomer on arsenite-impregnated plates[9]. This position was just reversed on the boric acid-impregnated plates[9] (Table 6, No. 3).

Silver nitrate-impregnated silica gel G plates were used by Wood and

TABLE 5

TLC SYSTEMS USED FOR THE CHARACTERIZATION OF KETO-INTERMEDIATES INVOLVED IN THE BIOSYNTHESIS OF O-ALKYL LIPIDS, AND THEIR DERIVATIVES OBTAINED BY CHEMICAL AND ENZYMATIC REACTIONS

No.	*Lipid components separated*	*Chromatographic system*	*Reference*
1	O-Alkyl dihydroxyacetone phosphate, O-alkyl glycerol, O-alkyl dihydroxyacetone, fatty alcohol, and wax esters	Silica gel G/chloroform–methanol–acetic acid (98:2:1) Or silica gel G/petroleum ether–diethyl ether–methanol–acetic acid (70:30:5:1)	186
2	O-Alkyl glycerol and O-alkyl dihydroxyacetone	Silica gel G/diethyl ether–water (200:1)	
3	O-Alkyl glycolic acid, O-alkyl glycolic aldehyde, and fatty alcohol	Silica gel G/diethyl ether–acetic acid (99.5:0.5)	
4	O-Alkyl ethylene glycol acetate and fatty alcohol acetate	Silica gel G/hexane–diethyl ether (80:20)	

TABLE 6

TLC SYSTEMS FOR THE SEPARATION OF FREE UNESTERIFIED ALKOXY-LIPIDS AND CLOSELY RELATED PRODUCTS

No.	*Lipid components separated*	*Chromatographic system*	*Reference*
1	Alkyl- and 1-alk-1′enyl glyceryl ether and fatty alcohols obtained by $LiAlH_4$ reduction of alkoxy-lipids	Silica gel G/petroleum ether–diethyl ether–acetic acid (30:70:1)	21
2	Synthetic and natural alkyl glyceryl ethers: (a) hexadecyl-glycerol; (b) 1-[9′(10′)-hydroxy]hexadecyl-glycerol; (c) 1-(2′-hydroxy)hexadecyl-glycerol	Silica gel G/diethyl ether–water (100:0.5)	162
3	Synthetic 1- and 2-isomers of alkyl glyceryl ethers	10% sodium arsenite or 5% boric acid-impregnated silica gel G chloroform–methanol (98:2)	9
4	Vinylogues of alkyl glyceryl ethers	8% silver nitrate-impregnated silica gel G chloroform–ethanol (90:10)	187
5	Geometrical isomers of 2-alk-1′-enyloxy-ethanol	5% silver nitrate-impregnated silica gel G/hexane–diethyl ether (70:30)	83
6	Alkyl, 1-alk-1′-enyl and 1′-(methoxy)-alkyl glyceryl ethers derived by $LiAlH_4$ reduction of stored phosphatidyl-ethanolamine	Silica gel G/petroleum ether–diethyl ether–acetic acid (30:70:1)	97

Snyder[187] to separate vinylogues of alkyl glyceryl ethers with chloroform–ethanol (90:10) as the developing solvent (Table 6, No. 4).

9′(10′)-Hydroxyalkyl glyceryl ether, isolated from the pink portion of the Harderian gland of the New Zealand white rabbit, has been chromatographically characterized, after conversion to either acetate or isopropylidene or isopropylidene and acetate derivatives on silica gel G plates using either diethyl ether–30% aqueous am-

TABLE 7

TLC SYSTEMS FOR THE SEPARATION OF DERIVATIVES OF ALKOXY-LIPIDS

No.	*Lipid components separated*	*Chromatographic system*	*Reference*
1	Acetate, isopropylidene and acetate isopropylidene derivatives of unesterified hydroxyalkyl glyceryl ether	Silica gel G/hexane–diethyl ether (60:40)	162
2	Alkyl monoiodide and diiodide derived from alkyl glyceryl ether	Silica gel H/heptane	145
3	Nitrates of alkyl glyceryl ethers (1- or 2-isomers) and dialkyl glyceryl ethers	Silica gel G/hexane–diethyl ether (85:15)	190
4	Alkyl acyl, 1-alk-1'-enyl acyl, diacyl, dialkyl glycerols and monoethers and monoesters of glycerol and glycol	Silica gel G/hexane–diethyl ether–methanol (80:20:5)	188

monia (100:0.25) or diethyl ether–water (100:0.5). The mixed derivative had the highest R_F value and the isopropylidene derivative had the lowest one[162] (Table 7, No. 1).

The characterization of molecular species of alkoxyglyceryl ethers is often achieved by degradation analysis. The TLC techniques involved in the characterization of degradation products of alk-1-enyl glyceryl ethers such as aldehydes, dimethyl acetals, cyclic acetals, vinyl methyl ethers, have all been reviewed earlier[1,25].

Alkyl halides, which are the degradation products of alkyl glyceryl-(1) ethers, are often isolated as alkyl iodides for the GC characterization of alkyl chains. The use of hydriodic acid for this purpose usually yields alkyl monoiodides (from saturated glyceryl ethers) and alkyl diiodides (from monounsaturated glyceryl ethers). Although saturated and unsaturated alkyl glyceryl ethers can be separated by either argentation chromatography[188] or by adsorption chromatography of the regenerable mercuric acetate adducts (silica gel G/diethyl ther)[189], sometimes the alkyl glyceryl ethers obtained by $LiAlH_4$ reduction are directly refluxed with hydriodic acid. Under such circumstance it becomes necessary to separate the monoiodides from the diiodides by adsorption TLC. This is done on silica gel G plates with heptane as the developing solvent[145] (Table 7, No. 2).

TLC systems for the separation of alkyl and dialkyl glyceryl ethers as nitrates have been reported[190] (Table 7, No. 3).

Various synthetic neutral alkoxy-lipids of diols and triols were also separated by Mangold's group[188] (Table 7, No. 4).

(c) Gas chromatography

High-temperature GC of intact lipids, especially non-polar lipids, is a useful technique in the molecular species characterization of alkoxy-lipids. Thus naturally occurring glyceryl ether diesters and families of diglyceride acetates derived from analogs of phosphatidylcholine and phosphatidylethanolamine of tumor origin, after TLC fractionation and hydrogenation, were separated into various molecular species on the non-polar stationary phase OV-1 (ref. 28) (Table 8, No. 1).

Bergelson's group[8] used high-temperature preparative GC to separate ethylene glycol dipalmitate from accompanying triglycerides of regenerating rat liver (Table 8, Nos. 3 and 4). QF-1 silicone was used as a non-polar stationary phase. The same system

TABLE 8

EXPERIMENTAL CONDITIONS FOR GAS CHROMATOGRAPHY OF ALKOXY-LIPIDS

No.	*Lipid class investigated*	*Purpose*	*Chromatographic system*	*Reference*
1	Glyceryl ether diesters, diacyl acetates of glycerol, alkyl acyl acetates of glycerol, and 1-alk-1'-enyl acyl acetates of glycerol (after hydrogenation)	Molecular species separation	Glass column (70 cm × 4 mm) packed with Gas-Chrom Q (100–120 mesh) coated with 1% OV-1; temperature programmed between 200–335° at 5°/min at a helium flow-rate of 100 ml/min; injector temperature, 325°; detector temperature, 350°	28
2	*cis* and *trans* isomers of the 2,3-diacetate derivative of 1-alk-1'-enyl glyceryl ether (synthetic)	Separation	Column (5 ft. × 0.25 in.) packed with Celite coated with 30% QF-1; temperature, isothermal at 238°; helium flow at 15 p.s.i.	82
3	Diol lipids	Separation from triol lipids	Column (100 cm × 4 mm) packed with Chromosorb W (85–100 mesh) coated with 3% QF-1 silicone; temperature programmed between 210–320° at 3°/min; argon flow-rate, 60 ml/min	8
4	Diol lipids	Separation from triol lipids	Column (50 cm × 2 mm) packed with Chromosorb W coated with 2% SE-30; temperature programmed between 200–320° at 5°/min; helium flow-rate, 100–120 ml/min	8

should be able to separate naturally occurring alkyl and 1-alk-1'-enyl ethers of ethanediol from the accompanying non-polar triol-lipids.

Wood *et al.*[188] studied the GC behaviour of a series of synthetic 1,2-dialkyl glyceryl ethers, 1-alkyl-2-acyl glyceryl ethers and 1,2-diacyl glyceryl ethers either as TMS or as acetate derivatives. These compounds can be formed by phospholipase C degradation of naturally occurring phospholipids. The authors made the following significant observations on their separation characteristics, in addition to the fact that the method was found suitable for quantification (Table 9).

(1) Dialkyl, alkyl acyl and diacyl glyceryl ethers of the same carbon number are eluted in that order after conversion to either TMS or acetate derivatives. Interestingly,

TABLE 9

EXPERIMENTAL CONDITIONS FOR GC OF ALKOXY LIPIDS

No.	*Lipid class investigated*	*Purpose*	*Chromatographic system*	*Reference*
1	Dialkyl, alkyl acyl, and diacyl glyceryl ethers as TMS or acetate derivatives	Molecular species separation	Column (70 cm × 4 mm) packed with Gas-Chrom Q coated with 1% OV-1; temperature programmed between 150–275° at 5°/min; helium flow-rate, 100 ml/min	188

similar behaviour of acetate derivatives on silica gel G plates was observed by Viswanathan *et al.*[151].

(2) TMS and acetate derivatives of dialkyl, alkyl acyl and diacyl glyceryl ethers are retained longer than the triglycerides with the same carbon number; among the two types of derivatives, acetates are retained longer.

(3) A "critical pair"[180,191] between the TMS derivative of dialkyl glyceryl ether and that of diacyl glyceryl ether with two methylene groups fewer is formed.

(4) Acetates of dialkyl and diacyl glyceryl ethers are separated as a class and as individual components from each other.

(5) No resolution between either the TMS or the acetate derivatives of 1,2- and 1,3-diglycerides was possible. This has been achieved since[177].

Preparative GC was used by Craig and Hamon to resolve the synthetic *cis* and *trans* isomers of 1-alk-1'-enyl glyceryl ethers as diacetate derivatives. 30% QF-1 was used as a non-polar stationary phase to achieve this separation[82] (Table 8, No. 2). The *cis* isomer had a relatively smaller retention time. Viswanathan *et al.*, on the other hand, isolated a series of homologues, vinylogues and geometrical isomers of 1-alk-1'-enyl methyl ethers on a polar phase (20% EGS containing 2% phosphoric acid)[1].

Synthetic monoethers and monoesters of 1,2-ethanediol were subjected to GC separation as either acetates, trimethylsilyl ethers or trifluoroacetates on either a non-polar stationary phase (5% SE-30) or a polar stationary phase (15% ethyleneglycol succinate methyl silicone polymer)[192]. It was observed that, irrespective of the nature

TABLE 10

EXPERIMENTAL CONDITIONS FOR GC OF ALKOXY-LIPIDS (MODIFIED FREE ALKYL GLYCERYL ETHERS)

No.	*Lipid class investigated*	*Purpose*	*Chromatographic system*	*Reference*
1	Free hydroxyalkyl glyceryl ether as TMS, acetate of isopropylidene and TMS of isopropylidene and the methyl ester of keto-substituted alkylglycolic acid	Characterization of modified alkyl glyceryl ether moiety	Column (6 ft. × 1.8 in.) packed with Gas-Chrom P coated with 10% EGSS-X; isothermal at 200°; helium flow-rate, 35 ml/min	162
2	O-Alkyl dihydroxyacetone as O-alkyl ethylene glycol acetate	Characterization of modified alkyl glyceryl ether moiety	Column (6 ft. × 1.8 in.) packed with Gas-Chrom P coated with 10% EGSS-X; isothermal at 200°; helium flow-rate, 35 ml/min	18
3	Diol-lipids; monoethers and monoesters as TFA derivatives or TMS ethers	Separation	Stainless-steel column (5 ft. × 1.8 in.) packed with Chromosorb W (60–80 mesh) coated with 5% SE-30; temperature programmed between 150–235°; flash heater temp., 275°; detector temp., 250°; helium flow-rate, 40–60 ml/min Or stainless-steel column (1.5 m × 3 mm) packed with Gas-Chrom P coated with 15% EGSS-X; temperature programmed between 125–190°; helium flow-rate, 40–60 ml/min	192

TABLE II

EXPERIMENTAL CONDITIONS FOR GC OF ALKOXY LIPIDS (FREE ALKYL GLYCERYL-ETHER DERIVATIVES)

No.	*Lipid class investigated*	*Purpose*	*Chromatographic system*	*Reference*
1	Isopropylidene derivative of free alkyl glyceryl ether	Molecular species separation	Column (5 ft.) packed with Anachrom AB (60–70 mesh) coated with 15% EGSS-X; isothermal at 175°; argon flow, 12 lb./sq. in. at inlet	173
2	TFA derivative of free alkyl glyceryl ether	Molecular species separation	Column (5 ft. × 1/8 in.) packed with Gas-Chrom P (100–120 mesh) coated with 10.5% EGSS-X; isothermal at 170°; helium flow variable between 4–18 lb/sq. in.	187
3	TMS ethers of free alkyl glyceryl ether	Molecular species separation	Column (5 ft. × 1/8 in. or 6.75 ft. × 1/8 in.) packed with silylated Chromosorb W (60–80 mesh) coated with 5% Apiezon L; isothermal at 250°	193
4	Alkoxyacetaldehyde derived from free alkyl glyceryl ether	Molecular species separation	Column (1.5 m × 4 mm) packed with Gas-Chrom P (80–100 mesh) coated with 20% EGS; isothermal at 160°; helium flow at inlet, 2.5 kg/cm² Or column (1.6 m × 4 mm) packed with Gas-Chrom P (80–100 mesh) coated with 15% EGSS-X; temperature programmed between 150–185°; helium flow at inlet, 2.5 kg/cm²	193
5	Dimethoxy derivative of free alkyl glyceryl ether	Molecular species separation	U-shaped aluminium tube (2 m) packed with kieselguhr coated with 25% high-vacuum grease; isothermal at 265°; helium flow-rate, 70 ml/min Or U-shaped aluminium (3 m) tube packed with kieselguhr coated with 25% Reoplex 400; isothermal at 246°; helium flow-rate, 50 ml/min Or U-shaped aluminium tube (4 m) packed with kieselguhr coated with 25% DEGS; isothermal at 243°; helium flow-rate, 40 ml/min	32
6	Allyl alkyl ethers, isopropylidene ethers, cyclic carbonates, or cyclic thionocarbonates derived from free alkyl glyceryl ether	Molecular species separation and quantitation	Stainless-steel column (5 ft. × 3 mm) packed with Chromosorb W (60–80 mesh) coated with 5% SE-30	146

of the derivatives, or the type of stationary phase used, glycol ethers were always eluted ahead of their corresponding glycol esters. Similar behaviour was noted for glycerolipids also[187]. The authors also observed that, for the same compound, the elution order for different types of derivative was TMS, trifluoroacetate (TFA), and acetate on the polar phase and TFA, TMS, and acetate on the non-polar phase. With the exception of the results of GC separation obtained with TFA derivatives of glycol ethers and esters on the polar phase, the rest could be used for quantitation purposes (Table 10, No. 3).

Free alkyl glyceryl ether chains, obtained by $LiAlH_4$ reduction of non-polar lipids or glycerophospholipids and hydrogenation of alk-1-enyl glyceryl ethers, are converted to non-polar derivatives by masking their free hydroxyl groups with trifluoroacetyl[187], dimethoxy[32], acetyl, or isopropylidene groups[173] and then analysed by GC (Table 11, Nos. 1–4). Sometimes they can be converted to alkoxyacetaldehyde[193] or ethylene glycol acetate[186] and analysed by GC. Their separation characteristics

TABLE 12

EXPERIMENTAL CONDITIONS FOR GC OF ALKOXY LIPIDS (DEGRADATION PRODUCTS OF FREE ALKOXY GLYCERYL ETHERS)

No.	*Lipid class investigated*	*Lipid derivative investigated*	*Chromatographic system*	*Reference*
1	Saturated and unsaturated alkyl glyceryl-(1) ether	(a) Alkyl iodide	Stainless-steel column (10 ft. × 1/8 in.) packed with Gas-Chrom P (80–100 mesh) coated with 15% EGS; isothermal at 200°; injector port modified to prevent direct on-column injection	145
		(b) Alkanes (c) Alkenes	Stainless-steel column (10 ft. × 1/8 in.) packed with Gas-Chrom A (80–100 mesh) coated with 10% EGS	
2	Free alkyl glyceryl ether	Methyl ester of monocarboxylic acid formed by permanganate–periodate oxidation at the double bond	Column packed with Anakrom AB (60–70 mesh) coated with 15% EGS; isothermal at 140°; gas flow, 6–8 p.s.i. at inlet	173
3	Free alkyl glyceryl ether	Methyl ester of monocarboxylic acid formed by chromic acid oxidation	U-shaped aluminium column (3 m × 4 mm) packed with Kieselguhr (80–100 mesh) coated with 25% Dow Corning high-vacuum grease; isothermal at 183°; helium flow-rate, 50 ml/min Or U-shaped aluminium column (2 m × 4 mm) packed with Kieselguhr (80–100 mesh) coated with Reoplex 400; isothermal at 165°; helium flow-rate, 44 ml/min	32

were as expected. The only point of interest was that TFA derivatives of the 1- and 2-isomers of alkyl glyceryl ether could be separated on a stationary polar phase[187].

Successful attempts to locate the position of the double bond in the alkyl chain of glyceryl ethers by GC were made. Small-chain fatty acids produced by oxidation of the double bond with either permanganate–periodate reagent[173] or chromic acid reagent[32] were analysed by GC as their methyl esters (Table 12, Nos. 2 and 3).

Various derivatives of hydroxyalkyl glyceryl ether were the subject of a recent GC study[162] (Table 10, No. 1). The TMS, the acetate of isopropylidene, and the TMS of isopropylidene derivatives of hydroxyalkyl glyceryl ether and the methyl ester of keto-substituted alkylglycolic acid when chromatographed on a polar stationary phase were eluted in the sequence mentioned.

GC characterization of alkyl glyceryl ethers as allyl alkyl ethers, cyclic carbonates and cyclic thionocarbonates was reported on a non-polar stationary phase (5% SE-30)[146] (Table 11, No. 6). The authors indicated the potentiality of the cyclic thionocarbonate derivatives in GC analysis for two reasons, *viz.* (1) The derivatization procedure in an alkaline medium is well suited to prepare similar derivatives from 1-alk-1′-enyl glyceryl ethers. (2) Identification of these derivatives in nanogram amounts with either an electron capture detector or flame photometric method.

Alkyl monoiodides and diiodides which are synthesized from saturated and monounsaturated alkyl glyceryl-(1) ethers (also from fatty alcohols) can be quantitatively analysed by GC (Table 12, No. 1) on a polar stationary phase (ethylene glycol succinate) after suitable modification of the injector insert and suitable correction factors for weight response[145]. The usual injector insert is replaced with another tube which is constricted at the junction to the chromatographic column. This modification prevents direct on-column injection and thus helps completion of dehydrohalogenation, preventing tailing of peaks. Further, alkyl iodides can be analysed by GC after converting them to either alkanes by reductive dehalogenation or to alkenes by dehydrohalogenation. As diiodide or diene isomers do not separate during GC, they can be directly quantitated. The alkanes and alkenes do not need a correction factor for weight response.

In vitro formation of alkanediols from fatty acids was recently documented[194]. TLC separation of alkanediols and alkyl glyceryl ethers or their derivatives is not satisfactory and hence a possibility of wrong identification exists. This can be overcome by GC. Thus Snyder's group achieved GC separation of isopropylidene derivatives of 1,2-, 1,3-, 1,4- and 1-tetradecyl glyceryl ether on a stationary polar phase. The elution order of the isopropylidenes of the alkanediols was 1,4 > 1,2 > 1,3 for the hexadecane series greater than 1-tetradecyl glyceryl ether.

4. SUMMARY

Application of various chromatographic techniques, with special emphasis on thin-layer and gas chromatography, in the study of the chemistry and biochemistry of alkoxy-lipids is reported.

5. APPENDIX

PREPARATION OF DERIVATIVES FROM ALKOXY-LIPIDS FOR CHROMATOGRAPHIC ANALYSIS

No.	*Lipid derivatized*	*Type of derivative*	*Reference*
1	Alkyl glyceryl ether	(a) Isopropylidene	173
		(b) Dimethoxy	32
		(c) Diacetyl	28
		(d) Bis(trimethylsilyl)	193
		(e) Bis(trifluoroacetyl)	187
		(f) Allyl alkyl ethers	146
		(g) Cyclic carbonate	146
		(h) Cyclic thionocarbonate	146
		(i) Alkyl iodide	145
		(j) Alkane	145
		(k) Alkene	145
2	Hydroxyalkyl glyceryl ether	(a) Acyl	162
		(b) Acetyl	162
		(c) Keto-substituted alkylglycolic acid methyl ester	162
		(d) Hydroxyalkyl glycolaldehyde	162
		(e) Hydroxyalkyl glycol	162
3	1-Alk-1'-enyl glyceryl ether	(a) Aldehyde	1
		(b) Dimethyl acetal	1
		(c) Cyclic acetal	1
		(d) 2,4-Dinitrophenylhydrazone	137
		(e) Iodinated vinyl ether	181

REFERENCES

1 C. V. Viswanathan, *Chromatogr. Rev.*, 10 (1968) 18.
2 G. A. Thompson, Jr., *J. Biol. Chem.*, 240 (1965) 1912.
3 M. Kates, P. S. Sastry and L. S. Yengoyan, *Biochim. Biophys. Acta*, 70 (1963) 705.
4 F. Snyder, in R. T. Holman (Editor), *Progress in the Chemistry of Fats and Other Lipids*, Vol. 10, Pergamon, Oxford, 1969, p. 287.
5 IUPAC-IUB Commission on Biochemical Nomenclature, *Biochim. Biophys. Acta*, 152 (1968) 1.
6 G. A. Thompson, Jr., and Y. Nozawa, *Ann. Rev. Microbiol.*, 26 (1972) 249.
7 D. J. Hanahan and R. Watts, *J. Biol. Chem.*, 236 (1961) PC 59.
8 L. D. Bergelson, in R. T. Holman (Editor), *Progress in the Chemistry of Fats and Other Lipids*, Vol. 10, Pergamon, Oxford, 1969, p. 239.
9 R. Wood and F. Snyder, *Lipids*, 2 (1967) 161.
10 H. R. Warner and W. E. M. Lands, *J. Amer. Chem. Soc.*, 85 (1963) 60.
11 V. Mahadevan, F. Phillips and C. V. Viswanathan, *Chem. Phys. Lipids*, 2 (1968) 183.
12 H. H. O. Schmid, W. J. Baumann and H. K. Mangold, *J. Amer. Chem. Soc.*, 89 (1967) 4797.
13 J. C. Craig and D. P. G. Hamon, *J. Org. Chem.*, 30 (1965) 4168.
14 M. F. Frosolono and M. Marsh, *Chem. Phys. Lipids*, 10 (1973) 203.
15 C. V. Viswanathan, *J. Chromatogr.*, 87 (1973) 193.
16 C. V. Viswanathan, *J. Chromatogr.*, 98 (1974) 105.
17 B. Hallgren and G. Stallberg, *Acta Chem. Scand.*, 21 (1967) 1519.
18 K. Christiansen, V. Mahadevan, C. V. Viswanathan and R. T. Holman, *Lipids*, 4 (1969) 421.
19 C. V. Viswanathan, S. P. Hoevet, W. O. Lundberg, J. M. White and G. A. Muccini, *J. Chromatogr.*, 40 (1969) 225.
20 K. Kasama, W. T. Rainey, Jr., and F. Snyder, *Arch. Biochem. Biophys.*, 154 (1973) 648.
21 G. A. Thompson, Jr. and P. Lee, *Biochim. Biophys. Acta*, 98 (1965) 151.

22 F. Snyder, M. L. Blank and R. L. Sykle, *J. Biol. Chem.*, 246 (1971) 3639.
23 R. Feulgen and H. Rosenbeck, *Hoppe-Seyler's Z. Physiol. Chem.*, 135 (1924) 230.
24 C. V. Viswanathan and V. Mahadevan, *J. Amer. Oil Chem. Soc.*, 43 (1966) 348A.
25 C. V. Viswanathan, *Chromatogr. Rev.*, 11 (1969) 153.
26 R. V. Panganamala, C. F. Sievert and D. G. Cornwell, *Chem. Phys. Lipids*, 7 (1971) 336.
27 G. A. Thompson, Jr., *Biochemistry*, 6 (1967) 2015.
28 R. Wood and F. Snyder, *Arch. Biochem. Biophys.*, 131 (1969) 478.
29 A. J. Slotboom, G. H. de Haas and L. L. M. van Deenen, *Chem. Phys. Lipids*, 1 (1967) 192.
30 O. S. Privett and E. C. Nickell, *J. Amer. Oil Chem. Soc.*, 39 (1962) 414.
31 E. von Rudloff, *Can. J. Chem.*, 34 (1965) 1413.
32 B. Hallgren and S. Larsson, *J. Lipid Res.*, 3 (1962) 31.
33 S. J. Friedberg and R. C. Greene, *J. Biol. Chem.*, 242 (1967) 5709.
34 W. Stoffel, D. Le Kim and G. Heyn, *Hoppe-Seyler's Z. Physiol. Chem.*, 351 (1970) 875.
35 A. K. Hajra, *Biochem. Biophys. Res. Commun.*, 37 (1969) 486.
36 R. L. Wykle and F. Snyder, *Biochem. Biophys. Res. Commun.*, 37 (1969) 658.
37 A. K. Hajra, *Biochem. Biophys. Res. Commun.*, 39 (1970) 1037.
38 R. L. Wykle, C. Piantadosi and F. Snyder, *J. Biol. Chem.*, 247 (1972) 2944.
39 F. Snyder, W. T. Rainey, Jr., M. L. Blank and W. H. Christie, *J. Biol. Chem.*, 245 (1970) 5853.
40 S. J. Friedberg, A. Heifetz and R. C. Greene, *J. Biol. Chem.*, 246 (1971) 5822.
41 R. L. Wykle and F. Snyder, *J. Biol. Chem.*, 245 (1970) 3047.
42 F. Snyder, M. L. Blank and B. Malone, *J. Biol. Chem.*, 245 (1970) 4016.
43 F. Snyder, B. Malone and R. L. Wykle, *Biochem. Biophys. Res. Commun.*, 34 (1969) 40.
44 F. Snyder, R. L. Wykle and B. Malone, *Biochem. Biophys. Res. Commun.*, 34 (1969) 315.
45 F. Snyder, B. Malone and M. L. Blank, *J. Biol. Chem.*, 245 (1970) 1790.
46 F. Snyder, B. Malone and F. A. Goswitz, unpublished results.
47 F. Snyder, B. Malone and R. B. Cumming, *Can. J. Biochem.*, 48 (1970) 212.
48 F. Snyder, M. Hibbs and B. Malone, *Biochim. Biophys. Acta*, 231 (1971) 409.
49 F. Snyder, B. Malone and M. L. Blank, *Biochim. Biophys. Acta*, 187 (1969) 302.
50 V. M. Kapoulas and G. A. Thompson, Jr., *Biochim. Biophys. Acta*, 187 (1969) 594.
51 D. C. Malins and J. R. Sargent, *Biochemistry*, 10 (1971) 1107.
52 K. Chae, C. Piantadosi and F. Snyder, *Biochem. Biophys. Res. Commun.*, 51 (1973) 119.
53 E. E. Hill and W. E. M. Lands, *Biochim. Biophys. Acta*, 202 (1970) 209.
54 H. Okuyama and W. E. M. Lands, *Biochim. Biophys. Acta*, 218 (1970) 376.
55 G. A. Thompson, Jr., *Biochim. Biophys. Acta*, 152 (1968) 409.
56 L. A. Horrocks and G. B. Ansell, *Lipids*, 2 (1967) 329.
57 R. Bickerstaffe and J. F. Mead, *Lipids*, 3 (1968) 317.
58 M. L. Blank, R. L. Wykle, C. Piantadosi and F. Snyder, *Biochim. Biophys. Acta*, 210 (1970) 442.
59 R. Wood and K. Haely, *Biochem. Biophys. Res. Commun.*, 38 (1970) 205.
60 O. E. Bell, Jr., M. L. Blank and F. Snyder, *Biochim. Biophys. Acta*, 231 (1971) 579.
61 R. L. Wykle, M. L. Blank and F. Snyder, *Fed. Eur. Biochem. Soc. Lett.*, 12 (1970) 57.
62 W. Stoffel and D. Lekin, *Hoppe-Seyler's Z. Physiol. Chem.*, 352 (1971) 501.
63 H. Debuch, J. Muller and H. Furniss, *Hoppe-Seyler's Z. Physiol. Chem.*, 352 (1971) 984.
64 F. Paltauf, *Biochim. Biophys. Acta*, 239 (1971) 38.
65 F. Paltauf, *Fed. Eur. Biochem. Soc. Lett.*, 17 (1971) 118.
66 M. L. Blank, R. L. Wykle and F. Snyder, *Fed. Eur. Biochem. Soc. Lett.*, 18 (1971) 92.
67 F. Paltauf, *Fed. Eur. Biochem. Soc. Lett.*, 20 (1972) 79.
68 F. Paltauf, *Biochim. Biophys. Acta*, 260 (1972) 352.
69 F. Snyder, R. L. Wykle, M. L. Blank, R. H. Lumb, B. Malone and C. Piantadosi, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, 31 (1972) 454.
70 L. A. Horrocks and A. Radominska-Pyrek, *Fed. Eur. Biochem. Soc. Lett.*, 22 (1972) 190.
71 M. L. Blank, R. L. Wykle and F. Snyder, *Biochem. Biophys. Res. Commun.*, 47 (1972) 1203.
72 H. Furniss and H. Debuch, *Hoppe-Seyler's Z. Physiol. Chem.*, 353 (1972) 1377.
73 R. L. Wykle, M. L. Blank, B. Malone and F. Snyder, *J. Biol. Chem.*, 247 (1972) 5442.
74 F. Paltauf and A. Holasek, *J. Biol. Chem.*, 248 (1973) 1615.
75 N. Oshino, Y. Imai and R. Sato, *J. Biochem. (Tokyo)*, 69 (1971) 155.
76 R. R. Brenner, *Lipids*, 6 (1971) 567.
77 A. Tietz, M. Lindberg and E. P. Kennedy, *J. Biol. Chem.*, 239 (1964) 4081.

78 R. C. Pfleger, C. Piantadosi and F. Snyder, *Biochim. Biophys. Acta*, 144 (1967) 633.
79 G. B. Ansell and S. Spanner, *Biochem. J.*, 97 (1965) 375.
80 F. Snyder, *Advan. Lipid Res.*, 10 (1972) 233.
81 J. C. Craig and D. P. G. Hamon, *Chem. Ind. (London)*, (1965) 1559.
82 J. C. Craig and D. P. G. Hamon, *J. Org. Chem.*, 30 (1965) 4168.
83 J. K. G. Kramer and H. K. Mangold, *Chem. Phys. Lipids*, 3 (1969) 176.
84 J. Gigg and R. Gigg, *J. Chem. Soc. (C)*, (1967) 1865.
85 J. Cunningham and R. Gigg, *J. Chem. Soc.*, (1965) 2968.
86 J. Gigg and R. Gigg, *J. Chem. Soc. (C)*, (1968) 16.
87 J. Gigg and R. Gigg, *J. Chem. Soc. (C)*, (1968) 2030.
88 E. A. Parfenov, G. A. Serebrennikova and N. A. Preobrazhenskii, *Zh. Organ. Khim.*, 2 (1966) 629.
89 G. A. Serebrennikova, A.. E Parfenov, N. A. Perlova and N. A. Preobrazhenskii, *Zh. Organ. Khim.*, 2 (1966) 1570.
90 G. A Serebrennikova, T. I. Ryaplova, E. A. Parfenov and N. A. Preobrazhenskii, *Zh. Organ. Khim.*, 3 (1967) 1958.
91 G. A. Serebrennikova, E. A. Parfenov, N. I. Sergbryakova and N. A. Preobrazhenskii, *Khim. Prir. Soedin.* 2 (1966) 306; *C.A.*, 67 (1967) 21423.
92 G. A. Serebrennikova, B. A. Trubaichuk and N. A. Preobrazhenskii, *Zh. Organ. Khim.*, 4 (1968) 765.
93 E. A. Parfenov, G.A . Serebrennikova and N. A. Preobrazhenskii, *Zh. Organ. Khim.*, 3 (1967) 1566.
94 E. A. Parfenov, G. A. Serebrennikova and N. A. Preobrazhenskii, *Zh. Organ. Khim.*, 3 (1967) 1766.
95 E. A. Parfenov, G. A. Serebrennikova, S. Ya. Roitberg and N. A. Preobrazhenskii, *Khim. Prir. Soedin.* 2 (1966) 367; *C.A.*, 67 (1967) 2726.
96 J. K. G. Kramer and H. K. Mangold, *Chem. Phys. Lipids*, 4 (1970) 332.
97 C. V. Viswanathan, S. P. Hoevet, W. O. Lundberg, J. M. White and G. A. Muccini, *J. Chromatogr.*, 40 (1969) 225.
98 T. V. Serebryakova, E. N. Zvonkova, G. A. Serebrennikova and N. A. Preobrazhenskii, *Zh. Organ. Khim.*, 2 (1966) 2004.
99 E. A. Parfenov, G. A. Serebrennikova and N. A. Preobrazhenskii, *Zh. Organ. Khim.*, 3 (1967) 1951.
100 G. A. Serebrennikova, I. B. Vitorov, G. N. Fedorova and N. A. Preobrazhenskii, *Zh. Organ. Khim.*, 4 (1968) 603.
101 A. J. Slotboom and P. P. M. Bonsen, *Chem. Phys. Lipids*, 5 (1970) 301.
102 G. A. Serebrennikova, V. I. Titov and N. A. Preobrazhenskii, *Zh Organ. Khim.*, 5 (1969) 550.
103 G. A. Serebrennikova, P. L. Ovechkin, J. B. Vtorov and N. A. Preobrazhenskii, *Zh. Organ. Khim.*, 5 (1969) 546.
104 N. A. Preobrazhenskii, G. A. Serebrennikova, P. L. Ovechkin and I. B. Vtorov, *Otkryviya, Izobret. Prom. Obraztsy. Tovarnye Znaki*, 46, No. 21 (1969) 20; *C.A.*, 71 (1969) 101304.
105 A. J. Slotboom, G. H. de Haas and L. L. M. van Deenen, *Chem. Phys. Lipids*, 1 (1967) 192.
106 H. Eibl and W. E. M. Lands, *Biochemistry*, 9 (1970) 423.
107 E. Baer and H. O. L. Fischer, *J. Biol. Chem.*, 140 (1941) 397.
108 E. Baer, L. J. Rubin and H. O. L. Fischer, *J. Biol. Chem.*, 155 (1944) 447.
109 G. G. Davies, I. M. Heilbron and W. M. Owens, *J. Chem. Soc.*, (1934) 1232.
110 M. Kates, T. H. Chan and N. Z. Stanacev, *Biochemistry*, 2 (1963) 394.
111 B. Palameta and M. Kates, *Biochemistry*, 5 (1966) 618.
112 P. J. Thomas and J. H. Law, *J. Lipid Res.*, 7 (1966) 453.
113 W. J. Baumann and H. K. Mangold, *J. Org. Chem.*, 29 (1964) 3055.
114 W. J. Baumann, L. L. Jones, B. E. Barnum and H. K. Mangold, *Chem. Phys. Lipids*, 1 (1966) 63.
115 E. Baer and H. O. L. Fischer, *J. Biol. Chem.*, 170 (1947) 337.
116 W. J. Baumann and H. K. Mangold, *J. Org. Chem.*, 31 (1966) 498.
117 W. J. Baumann, H. H. O. Schmid, H. W. Ulshofer and H. K. Mangold, *Biochim. Biophys. Acta*, 144 (1967) 355.
118 W. J. Baumann and H. K. Mangold, *Biochim. Biophys. Acta*, 116 (1966) 570.
119 E. Baer and N. Z. Stanacev, *J. Biol. Chem.*, 240 (1965) 44.

120 N. Z. Stanacev, E. Baer and M. Kates, *J. Biol. Chem.*, 239 (1964) 410.
121 T. H. Bevan and T. Malkin, *J. Chem. Soc.*, (1958) 2962.
122 T. H. Bevan and T. Malkin, *J. Chem. Soc.*, (1960) 350.
123 G. K. Chacko and D. J. Hanahan, *Biochim. Biophys. Acta*, 164 (1968) 252.
124 C. Piantadosi, K. S. Ishaq and F. Snyder, *J. Pharm. Sci.*, 59 (1970) 1201.
125 C. Piantadosi, K. S. Ishaq, R. L. Wykle and F. Snyder, *Biochemistry*, 10 (1971) 1417.
126 J. M. Lowenstein (Editor), *Methods Enzymol.*, 14 (1969).
127 A. R. Johnson and J. B. Davenport (Editors), *Biochemistry and Methodology of Lipids*, Wiley-Interscience, New York, 1971.
128 M. Kates, in T. S. Work and E. Work (Editors), *Laboratory Techniques in Biochemistry and Molecular Biology*, Vol. 3, North-Holland, Amsterdam, 1972, p. 267.
129 D. J. van der Horst, A. H. van Gennip and P. A. Voogt, *Lipids*, 4 (1969) 300.
130 E. G. Bligh and W. J. Dyer, *Can. J. Biochem. Physiol.*, 37 (1959) 911.
131 G. Jacini and E. Fedeli, in W. L. Holmes, L. Carlson and R. Paoletti (Editors), Vol. 4, *Advances in Experimental Medicine and Biology*, Plenum Press, New York, 1969, p. 639.
132 C. V. Viswanathan and A. Nagabhushanam, *J. Chromatogr.*, 75 (1973) 227.
133 A. Stolyhwo and O. S. Privett, *J. Chromatogr. Sci.*, 11 (1973) 20.
134 G. Rouser, *J. Chromatogr. Sci.*, 11 (1973) 60.
135 R. Feulgen, W. Boguth and G. Anderson, *Hoppe-Seyler's Z. Physiol. Chem.*, 387 (1951) 90.
136 H. R. Warner and W. E. M. Lands, *J. Lipid Res.*, 4 (1963) 216.
137 K. S. Rhee, R. R. D. Rosario and L. R. Dugan, *Lipids*, 2 (1967) 334.
138 S. Sigga and R. L. Edsberg, *Anal. Chem.*, 20 (1948) 762.
139 E. L. Gottfried and M. M. Rapport, *J. Biol. Chem.*, 237 (1962) 329.
140 J. N. Williams, Jr., C. E. Anderson and A. D. Jasik, *J. Lipid Res.*, 3 (1962) 378.
141 D. J. Hanahan and R. Watts, *J. Biol. Chem.*, 236 (1961) PC 59.
142 D. J. Hanahan and J. N. Olley, *J. Biol. Chem.*, 231 (1958) 813.
143 M. L. Karnovsky and W. S. Rapson, *J. Soc. Chem. Inc.*, 65 (1946) 138.
144 G. A. Thompson and V. M. Kapoulas, *Methods Enzymol.*, 14 (1969) 668.
145 R. V. Panganamala, C. F. Sievert and D. G. Cornwell, *Chem. Phys. Lipids*, 7 (1971) 336.
146 S. Ramachandran, R. V. Panganamala and D. G. Cornwell, *J. Lipid Res.*, 10 (1969) 465.
147 B. Hallgren and S. Larsson, *J. Lipid Res.*, 3 (1962) 39.
148 L. J. Nutter and O. S. Privett, *J. Chromatogr.*, 35 (1968) 519.
149 R. Wood and F. Snyder, *Lipids*, 3 (1968) 129.
150 C. V. Viswanathan, F. Phillips and W. O. Lundberg, *J. Chromatogr.*, 35 (1968) 66.
151 C. V. Viswanathan, F. Phillips and W. O. Lundberg, *J. Chromatogr.*, 38 (1968) 267.
152 J. Vit, B. Casensky and J. Machacek, *Fr. Pat.*, 1,515,582 (March 1968).
153 C. V. Viswanathan, M. Basilio, S. P. Hoevet and W. O. Lundberg, *J. Chromatogr.*, 34 (1968) 241.
154 H. P. Kaufmann, S. S. Radwan and A. K. S. Ahmad, *Fette, Seifen, Anstrichm.*, 68 (1966) 261.
155 K. Oette and M. Doss, *J. Chromatogr.*, 32 (1968) 439.
156 K. Owens, *Biochem. J.*, 100 (1966) 354.
157 D. C. Malins, J. C. Wekell and C. R. Houle, *J. Lipid Res.*, 6 (1965) 100.
158 U. Varanasi and D. C. Malins, *Science*, 166 (1969) 158.
159 H. H. O. Schmid and H. K. Mangold, *Biochem. Z.*, 346 (1966) 13.
160 V. A. Vaver, N. A. Pisareva, B. V. Rozynov, A. N. Ushakov and L. D. Bergelson, *Chem. Phys. Lipids*, 7 (1971) 75.
161 M. L. Blank, K. Kasama and F. Snyder, *J. Lipid Res.*, 13 (1972) 390.
162 K. Kasama, W. T. Rainey, Jr. and F. Snyder, *Arch. Biochem. Biophys.*, 154 (1973) 648.
163 L. D. Bergelson, V. A. Vaver, N. V. Prokazova, A. N. Ushakov, B. V. Rozynov, K. Stefanov, L. I. Ilukhina and T. N. Simonova, *Biochim. Biophys. Acta*, 260 (1972) 571.
164 N. V. Prokazova, B. V. Rozynov, A. N. Ushakov, K. Stefanov, L. I. Ilukhina, T. N. Simonova and L. D. Bergelson, *Dokl. Biochem.*, 205 (1972) 477 (English Translation 252).
165 G. M. Gray and M. G. Macfarlane, *Biochem. J.*, 70 (1968) 409.
166 S. P. Hoevet, C. V. Viswanathan and W. O. Lundberg, *J. Chromatogr.*, 34 (1968) 195.
167 C. V. Viswanathan, S. P. Hoevet and W. O. Lundberg, *J. Chromatogr.*, 35 (1968) 113.
168 W. E. M. Lands and P. Hart, *Biochim. Biophys. Acta*, 98 (1965) 532.
169 E. L. Gottfried and M. M. Rapport, *J. Biol. Chem.*, 237 (1962) 329.
170 O. Renkonen, *Acta Chem. Scand.*, 17 (1963) 634.

171 G. B. Ansell and S. Spanner, *J. Neurochem.*, 10 (1963) 941.
172 M. F. Frosolono and M. Marsh, *Chem. Phys. Lipids*, 10 (1973) 203.
173 D. J. Hanahan, J. Ekholm and C. M. Jackson, *Biochemistry*, 2 (1963) 630.
174 H. Berger, P. Jones and D. J. Hanahan, *Biochim. Biophys. Acta*, 260 (1972) 617.
175 C. V. Viswanathan, unpublished observations.
176 C. V. Viswanathan, unpublished observations.
177 C. V. Viswanathan, *J. Chromatogr.*, 98 (1974) 105.
178 C. V. Viswanathan, unpublished experiments.
179 R. J. King and J. A. Clements, *J. Lipid Res.*, 11 (1970) 381.
180 C. V. Viswanathan, *Chromatogr. Rev.*, 11 (1969) 153.
181 C. V. Viswanathan, S. P. Hoevet and W. O. Lundberg, *Fette, Seifen, Anstrichm.*, 70 (1968) 858.
182 C. V. Viswanathan, *J. Chromatogr.*, 75 (1973) 141.
183 O. Renkonen, S. Liusvaara and A. Miettinen, *Ann. Med. Exp. Biol. Fennia (Helsinki)*, 43 (1965) 200.
184 O. Renkonen, *Biochim. Biophys. Acta*, 152 (1968) 114.
185 O. Renkonen, *J. Lipid Res.*, 9 (1968) 34.
186 F. Snyder, M. L. Blank, B. Malone and R. L. Wykle, *J. Biol. Chem.*, 245 (1970) 1800.
187 R. Wood and F. Snyder, *Lipids*, 1 (1966) 62.
188 R. Wood, W. J. Baumann, F. Snyder and H. K. Mangold, *J. Lipid Res.*, 10 (1969) 128.
189 S. Ramachandran, H. W. Sprecher and D. G. Cornwell, *Lipids*, 3 (1968) 511.
190 D. C. Malins, J. C. Wekell and C. R. Houle, *Anal. Chem.*, 36 (1964) 658.
191 C. V. Viswanathan, B. Meera Bai and U. Sitarama Acharya, *Chromatogr. Rev.*, 4 (1962) 160.
192 R. Wood and W. J. Baumann, *J. Lipid Res.*, 9 (1968) 733.
193 H. K. Mangold and W. J. Baumann, in G. V. Marinetti (Editor), *Lipid Chromatographic Analysis*, Vol. 1, Marcel Dekker, New York, 1967, p. 339.
194 M. L. Blank, E. A. Cress, N. Stephens and F. Snyder, *J. Lipid Res.*, 12 (1971) 638.