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COUPLED GAS CHROMATOGRAPHY–MASS SPECTROMETRY IN THE SEPARATION AND CHARACTERIZATION OF POLAR LIPIDS

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1. INTRODUCTION

Gas chromatography (GC) and mass spectrometry (MS) are both indispensable microtechniques in the field of lipid research. Their combination adds a new dimension to this field. Even when available in microgram quantities, a variety of classes of polar lipids and their innumerable molecular species can be characterized by this combined technique.

It is known that polar (ionized) lipids are not amenable either to vapour phase separations or MS characterization (exception is the direct inlet system). However, the volatility conferred on many of these substances by derivatization, especially with a trimethylsilyl (TMS) group, has made this method one of great importance in GC, and, more recently, in MS.

In the present review, for the sake of convenience and clarity, the polar lipids are categorized into two major groups, *viz.* (1) glycerolipids and (2) sphingolipids. The types of lipids so far investigated in the first group belong to the subgroup glycerophospholipids; glyceroglycolipids have not been investigated so far. The types of lipids investigated in the second group are divided into three categories, *viz.* (a) sphingosines and ceramides, (b) sphingophospholipids and sphingophosphonolipids, and (c) sphingoglycolipids.

Before discussing the observations made by many investigators in this field, it would be appropriate to discuss the salient features of the methodology adopted by them for the success of their investigations.

2. METHODS

A. Preparation of lipid samples

Procedures for the extraction of lipids from animal tissues, plant tissues and micro-organisms[1–4], and their purification from non-lipid contaminants were described[1,5,6]. Separation of lipids into individual classes by various chromatographic techniques was also described[3,7]. Chromatographic techniques used in the isolation of molecular species of lipids were recently reviewed[8,9].

B. Preparation of lipid derivatives

The importance of preparing volatile derivatives from polar lipids for GC–MS investigations was emphasized earlier. The derivatization procedure, however, depends on the chemical make-up of the individual class of polar lipids. In this respect a polar lipid classification on the following basis will be useful:

(1) Phospholipids (glycerophospholipids, sphingophospholipids and sphingophosphonolipids).

(2) Glycolipids (sphingoglycolipids).

Phospholipids, because of the thermal susceptibility of their phosphate ester bond to hydrolytic cleavage, are usually silylated after dephosphorylation. The dephosphorylation is achieved either chemically or enzymatically. On the other hand, the sphingoglycolipids are usually directly silylated. However, sometimes they are deglycosylated chemically and then silylated.

(a) Dephosphorylation

(i) Chemical dephosphorylation[10]. Glycerophospholipids and sphingophospholipids were dephosphorylated in phenyl ether at 250° for 15 min. The respective yields of diglycerides and ceramides could be increased by addition of trace amounts of water. In the former case the products formed were composed of a mixture of 1,2- and 1,3-diglycerides. In the latter case ceramides and anhydroceramides were formed and hence ceramides had to be isolated by preparative thin-layer chromatography (TLC) (silica gel G/chloroform) and then silylated.

(ii) Enzymatic dephosphorylation[9]. Although phospholipase *c* can be isolated from *Clostridium welchii* and *Bacillus cereus*, the enzyme from the latter organism is preferred because of its broader specificity with many glycerophospholipids. The reaction is best performed in diethyl ether. For optimal enzymatic activity, phospholipase *c* from *Clostridium welchii* requires Tris buffer (pH 7.3) containing 0.03 *M* $CaCl_2$ and 30° incubation temperature. The enzyme from *Bacillus cereus*, on the other hand, requires a phosphate buffer (pH 7.0) containing 0.001 *M* 2-mercaptoethanol and 0.004 *M* $ZnCl_2$ and 37° incubation temperature for its optimum activity.

(b) Deglycosidation

The technique of deglycosidation developed by Carter *et al.*[11] to transform cerebrosides into ceramides on a preparative scale was modified by Hammarström[12] for microgram amounts of sample. The removal of glycosyl residue from the cerebrosides was achieved in three steps: Firstly, the glycoside ring was opened by oxidation with potassium periodate, then the oxidized product was reduced by sodium borohydride and finally the alcohol formed was removed by mild acid hydrolysis. The resulting ceramide, if necessary, can be converted to the long-chain base by alkaline hydrolysis. The base so obtained retains its natural stereochemical configuration.

(c) Deacylation[13,14]

A mixture of glycerophospholipids can be analysed for its individual components by GC–MS. But before derivatization to TMS ethers, it is necessary to deacylate the mixture. The deacylation is achieved in a solution of chloroform–methanol (1:4) with 0.1 *N* aqueous sodium hydroxide. After neutralization with 0.1 *N* aqueous acetic acid, the solution is extracted with a mixture of chloroform–isobutanol–methanol–water (1.8:1:0.2:2). The separated aqueous layer contains the sodium salt of glycerophosphate esters, which are converted to the acids by passage through a cation-exchange resin (Dowex 50W, H^+ form). With the exception of glycerophosphorylcholine, the remaining glycerophosphate esters are eluted from the resin with water. If the former is required, it should be eluted from the resin with dilute acid. The aqueous solution of glycerophosphate esters is lyophilized and then silylated.

(d) Oxidation[15,16]

The location of unsaturation in a long-chain aliphatic moiety can be determined by the oxidative technique developed for polyunsaturated fatty acids[16] and sphingosine bases[15]. Oxidation with either potassium permanganate[16] or osmium tetroxide[15,16] yields a diol at the site of unsaturation which is subsequently converted to its TMS derivative and analysed by GC–MS.

(c) Silylation[17]

Silylation of hydroxyl or amino compounds is achieved using a combination of different solvents (pyridine, acetonitrile, etc.), different catalysts (trimethylchlorosilane (TMCS), water) and a variety of silylating reagents (hexamethyldisilazane (HMDS), N-trimethylsilylimidazole (TSIM), bis(trimethylsilyl)acetamide (BSA) and bis(trimethylsilyl)fluoroacetamide (BSTFA), etc.) in varying proportions. The silylation reaction is usually carried out overnight at room temperature or around 60° for *ca.* 2 h. However, the various silylating agents show some specificity. Thus TSIM in either pyridine or acetonitrile converts the phenolic and alcoholic hydroxyl groups to the TMS derivative in about 3 h at 60°, but N-silylation does not take place at this temperature even over a period of 48 h (ref. 18). On the other hand, HMDS[18], BSA[19-20] and BSTFA[19-20] can convert all types of hydroxyl groups and the primary amino group into TMS derivatives: the secondary amino group, however, remains unaffected[19-20]. The rate of reaction in all these instances could be considerably hastened by the catalytic amounts of TMCS and trace amounts of water[18]. The marked increase in the rate of reaction resulting from the addition of water is of considerable interest, since most discussions on silylation reactions point out the desirability of maintaining anhydrous conditions.

C. Coupling of GC–MS unit[21]

An initial attempt to characterize substances separated by GC by MS was by condensing the eluate emerging from the GC column on the inner surface of a glass tube, which was then transferred to the inlet system of the mass spectrometer. This method was rather time consuming, and the idea of directly combining these instruments through some kind of pressure reduction system arose. The pioneering attempt in this direction was made by Holmes and Morell[22]. A historical account of this has been published[21].

Although an excellent review on the subject of GC–MS coupling is available[21], a few important points are briefly summarized here.

(a) Proper operating conditions for GC

The main difference in the operation of an ordinary gas chromatograph as compared to the one linked to a mass spectrometer via a molecular separator interface is that the coupled instrument is more sensitive to the carrier gas flow-rate and to the liquid phase bleeding effect. The former problem is taken care of through installing a flow controller operating at 3 kg/cm^2 or 35 p.s.i. By this, even during temperature programming of the column, the flow-rate is kept constant. The problem of liquid phase bleeding has to be controlled by choosing such a phase (selective or non-selective) which will produce the desired separation effect but at the same time produce a minimum of column bleed. In more common use for this purpose are the non-selective type phases like SE-30 (methyl silicone), OV-1 (dimethyl silicone) or moderately selective type like OV-17 (methyl phenyl silicone), which are relatively stable at high temperatures. As the amount of bleed is proportional to the surface area of the coated support, concentration of liquid phase and the operating temperatures, best results are obtained with a liquid phase concentration of 0.5 to 5%.

(b) Proper operating conditions for MS

The scanning of a GC component by MS should be as rapid as possible to ensure the sample concentration remaining relatively constant during this period.

(c) Transfer of eluate and enrichment of sample

Transferring of the eluate at atmospheric pressure from the gas chromatograph to the mass spectrometer without exceeding the vacuum limit of the latter and increasing the sample to carrier gas ratio (enrichment of sample) when its input and output concentrations are considered, are problems, which have been solved by using a molecular separator interface as the connecting link between the gas chromatograph and the mass spectrometer. A number of such devices are available.

In a jet separator the carrier gas and the eluate from the GC column are fed under pressure and viscous flow conditions to a nozzle from which the gas emerges in a jet periphery. A sharp-edged collector nozzle effects a separation, since the lighter component of the gas is enriched in the peripheral portion, which is evacuated[23].

Another category of molecular separators exist, which work on the principle of different permeabilities for the carrier gas and heavier molecules. The devices, like porous glass separators, PTFE capillary separators, porous silver membrane separators, porous stainless-steel membrane separators and variable conductance separators, favour the permeability of the carrier gas. The carrier gas is preferentially separated from the solute by application of vacuum to the outer surface of the semi-permeable separator.

On the other hand, a third category of molecular separators, like silicone rubber membrane and silicone-coated rubber membrane, work on the principle of diffusion of the organic vapours through the membrane to the ion source of the mass spectrometer while the carrier gas is rejected because of its low solubility in the membrane. One point to be remembered in using any type of differential permeability separators is to silanize them before use. This is essential to avoid absorption of polar components on their larger active surface.

Recently a two-stage molecular separator (combined membrane and porous silver separator) has been developed[24].

D. Chromatography

(a) Thin-layer chromatography

The complexity of individual classes of polar lipids, especially that of sphingolipids, necessitated investigators to use certain specific prefractionation TLC techniques. These are summarized in Table 1.

(b) Gas chromatography

Experimental conditions for the GC separations of TMS ethers of glycerophosphate esters, of TMS ethers of glycerophospholipids, and of TMS ethers of sphingolipids for their characterization by a coupled GC–MS technique are summarized in Tables 2, 3, and 4, respectively.

TABLE I

PREFRACTIONATION TLC TECHNIQUES USED FOR THE CHARACTERIZATION OF MOLECULAR SPECIES OF SPHINGOLIPIDS BY GC–MS

No.	*Sphingolipids to be separated*	*Adsorbent used*	*Activation of adsorbent*	*Development solvent*	*Reference*
1	Ceramides with normal fatty acids from those with hydroxy fatty acids	Silica gel G	Activation at 120° for 45 min	Chloroform–methanol–water (95:5:0.5) or chloroform–methanol (93:7)	12
2	Ceramide acetates (vinylogues)	Silica gel G–$AgNO_3$ (15:1, by wt.)	Activation at 120° for 1 h after 2-h drying at room temperature	Chloroform–benzene–methanol (80:20:1)	25
3	Glucosyl ceramide from galactosyl ceramide	Silica gel G–borate (30 g gel: 65 ml 1% $Na_2B_2O_7 \cdot 10\ H_2O$: pH 9.2)	Activation at 125° for 1 h after drying overnight at room temperature	Chloroform–methanol–water–15 *N* ammonium hydroxide (280:70:6:1)	26
4	Glycosyl, galactosyl and xylosyl ceramides from each other	Silica gel G–$Na_2B_2O_7 \cdot 10\ H_2O$ (50:1, by wt.)	Activation at 120° for 30 min	Chloroform–methanol–water (65:25:4)	27
5	Cerebrosides with normal fatty acids from those with hydroxy fatty acids	Silica gel G	Activation at 110° for 30 min	For galactosyl ceramides: chloroform–methanol–ammonia (75:25:4)	28
				For glucosyl ceramides: chloroform–methanol–water (24:7:1)	26

3. INVESTIGATIONS OF POLAR LIPIDS BY GC–MS

It was pointed out in Introduction that the coupled GC–MS technique has been used for both the separation and characterization of classes of polar lipids as well as their innumerable molecular species. Now we will first consider the separation and characterization of polar lipids. This will be followed by separation and characterization of molecular species of glycerophospholipids, sphingolipids (sphingosine bases, ceramide), spingophospholipids, sphingophosphonolipids and sphingoglycolipids.

A. Class separation and characterization of polar lipids

Almost simultaneously, but independently, two research groups[13,14,29] approached this problem in identical manner. This method has so far been applied only to 1,2-diacyl-*sn*-glycero-3-phosphate esters. (It may be mentioned that the same method

TABLE 2

EXPERIMENTAL CONDITIONS FOR GC SEPARATION OF TMS ETHERS OF GLYCEROPHOSPHATE ESTERS FOR THE CHARACTERIZATION OF GLYCEROPHOSPHOLIPIDS BY GC–MS

No.	*Lipid class studied**	*Derivative of the lipid class investigated*	*Details about column packing material*	*Column dimensions and carrier gas flow-rate*	*Column temperature (°C)*	*Reference*
1	PA, PG, PI, PE, PDME, and PS	TMS ethers of glycerophosphate esters (after deacylation of individual class of glycerophospholipid)	1% OV-17 on Supelcoport, 80–100 mesh	6 ft. × 2 mm glass column; helium at 25 ml/min	Temperature programming from 150–250° at 5°/min	13
2	M-PI, D-PI, T-PI, and CL	TMS ethers of glycerophosphate esters	1% SE-30 on Gas-Chrom Q	2 ft. × 1/4 in. glass column; helium at 75 ml/min	Temperature programming from 180–240° at 4°/min	14, 29
3	PnE, PnAl, PE, and PS	TMS ethers of 1-phosphono-2-aminoethane, 2-amino-3-phosphonopropionic acid, O-phosphorylethanolamine, and O-phosphorylserine	3% OV-1 on Gas-Chrom Q, 100–120 mesh	6 ft. × 3 mm glass column; argon at 20 ml/min	Isothermal at 150	30

* Abbreviations: PA = phosphatidic acid; PG = phosphatidylglycerol; PS = phosphatidylserine; M-PI = monophosphoinositide; D-PI = diphosphoinositide; T-PI = triphosphoinositide; PE = phosphatidylethanolamine; PDME = phosphatidyldimethylethanolamine; CL = cardiolipin or diphosphatidylglycerol; PnE = glyceride 2-aminoethylphosphonate; and PnAl = glyceride phosphonoalanine.

can be applied to lyso(acyl)phospholipids as well as to 1-alk-1′-enyl-2-acyl-*sn*-glycero-3-phosphate esters.)

A mixture of diacylphospholipids were deacylated by mild alkaline hydrolysis and the resulting sodium salts of *sn*-glycero-3-phosphate esters were converted to free acids by ion-exchange chromatography and, after lyophilyzation, silylated and analysed by GC–MS. The conditions employed during the GC separation of TMS ethers of various *sn*-glycero-3-phosphate esters are given in Table 2. Their retention times are given in Table 5.

From Table 5 it is clear that eight classes of glycerophospholipids could be separated from each other as the TMS derivatives of glycerophosphate esters. Duncan *et al.*[13], however, pointed out that although the method possessed the capability of being used as a convenient qualitative method of analysis, its use as a quantitative analysis procedure was not yet realizable. The reasoning put forward for this was that varying amounts of TMS ethers of *sn*-glycero-3-phosphate and *sn*-glycero-2-phosphate were detected during GC of all glycerophosphate ester derivatives. On the other

BLE 3

PERIMENTAL CONDITIONS FOR GC SEPARATION OF TMS ETHERS OF GLYCEROPHOS-GC–MS PHOLIPIDS FOR THE CHARACTERIZATION OF THEIR MOLECULAR SPECIES

*Lipid class studied**	*Derivative of the lipid class investigated*	*Details about column packing material*	*Column dimensions and carrier gas flow-rate*	*Temperature (°C)*		*Reference*
				Column	*Injector, detector, etc.*	
PC, PE, and L-PC	Diacyl ester of propenediol (thermal cleavage product) Monoacyl ester of propenediol	5% SE-30 on acid-washed Chromosorb W, 60–80 mesh	2 ft. × 1/8 in. stainless-steel column: helium at 25 ml/min	Either isothermal at 280° or programming from 200–360° at 12°/min	Injector between 350° to 400° and detector at 360°	31
PC	Diacyl ester of propenediol	Either 3% JXR or 1% OV-17 on Gas-Chrom Q, 100–120 mesh	2 ft. × 1/8 in. stainless-steel column; nitrogen at 150 ml/min at 200°	Linearly programmed at 4–6°/min over the range 100–325°	Injector at 300°, detector at 340°, and detector line at 320°	32, 33
PS, PC, PE, PI, CL, PA, and SPH	TMS ether of original phospholipid yields by thermal cleavage TMS ether of diglycerides	1% SE-30 on acid-washed and silanized Gas-Chrom P, 100–120 mesh	9 ft. × 4 mm glass column; nitrogen at 26 p.s.i. (45 ml/min at 200°)	Temperature programmed from 270–300° at 2°/min followed by isothermal operation at 300°	Injector at 260° and detector at 300°	10, 34–36
Glycerophospholipids	TMS ethers of 1,2-diglyceride obtained by phospholipase C hydrolysis of glycerophospholipids	1.5% XE-60 on silanized Chromosorb W	15-in. glass column: helium at 45 ml/min	Temperature programmed from 255° at 3°/min	—	37

* Abbreviations: PC = phosphatidylcholine; L-PC = lyso-PC; SPH = sphingomyelin. See also footnote Table 2.

hand, Cicero and Sherman[14,29] used GC–MS as a method of measuring and identifying mono-, di-, and triphosphoinositides as well as diphosphatidylglycerol from rat brain extracts. For quantitation purposes they compared the chromatographic peak heights of their sample with standards run under identical conditions.

The ions used in the characterization of these compounds can be grouped under three categories. The first category, characterizing the molecular weight of the compound, represents ions formed by loss of a number of neutral fragments typical of TMS derivatives and these were confirmed by labelling the parent compound with TMS-d_9 groups[39]. The second category of ions are diagnostic for compounds with the terminal sequence $CH_2(OTMS)\cdot CH(OTMS)\cdot CH_2OPO(OTMS)_2$ and were confirmed upon labelling with the TMS-d_9 group by the corresponding mass shift. The first of

TABLE 4

EXPERIMENTAL CONDITIONS FOR GC SEPARATION OF TMS ETHERS OF SPHINGOLIPIDS AND THEIR OTHER DERIVATIVES FOR THE CHARACTERIZATION OF THEIR MOLECULAR SPECIES BY GC–MS

No.	*Lipid class studied*	*Derivative of the lipid class investigated*	*Details about column packing material*	*Column dimensions and carrier gas flow-rate*	*Column temperature (°C)*	*Reference*
1	2-Amino-1,3-diol alkanes	Di-O-TMS ether N-Acetyl di-O-TMS ether	2% SE-30 on Chromosorb W, 60–80 mesh	1 m × 3 mm glass column	190	38, 39
		Aldehyde Fatty acid methyl ester	15% diethylene-glycol succinate on Chromosorb W, 60–80 mesh	2 m × 3 mm glass column	190	38, 39
		N-acetyl poly-TMS ether	2% SE-30 on Chromosorb W, 60–80 mesh	1 m × 3 mm glass column	205	38, 39
		N-acetyl di-O-TMS ether	3% SE-30 or 3% OV-1 (non polar) 3% XE-60 or 3% OV-17 (polar) on acid-washed Gas-Chrom S, 100–120 mesh	6 ft. × 3 mm glass column	220	40
		Di-O-TMS ether	6% silicone on silanized Gas-Chrom P, 80–100 mesh	2 m × 3.5 mm glass column; helium, 1 kg/cm²	207	41
2	Ceramide with normal or hydroxy fatty acids	TMS ether	1% SE-30 on acid-washed and silanized Gas-Chrom P, 100–120 mesh	12 ft. × 3.5 mm glass column; nitrogen, 40–50 ml/min at 200°	Temperature programming from 230° up to 350° at 2°/min and from 270° up to 350° at 2°/min for ceramides of normal and hydroxy fatty acids, respectively	42
3	Ceramide with either sphingosine or sphinganine or phylosphingosine as base and either normal or hydroxy fatty acids	TMS ether	Variation between 1 to 2.5% OV-1 on Gas-Chrom Q, 60–80 mesh	Between 1 to 1.7 m × 3 mm U-shaped glass column; helium with an inlet pressure of 3 kg/cm²	Isothermal between 275° to 350°, depending on the components of ceramide	25, 43–46

(Continued on p. 114)

TABLE 4 *(continued)*

No.	*Lipid class studied*	*Derivative of the lipid class investigated*	*Details about column packing material*	*Column dimensions and carrier gas flow-rate*	*Column temperature (°C)*	*Reference*
4	Cerebrosides	TMS ether	1 to 2% OV-1 on Gas-Chrom Q, 100–120 mesh	1.4 m × 3 mm U-shaped glass column; helium, 90 ml/min	Isothermal at 320°	47, 48
5	Ceramide aminoethyl-phosphonate	TMS ether	1% OV-1 on acid-washed Chromosorb W, 60–80 mesh	2 m × 3 mm coiled glass; helium, 25 ml/min	Isothermal at 280°	39

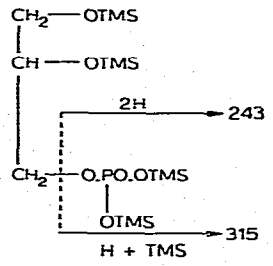
TABLE 5

RETENTION TIMES OF TMS ETHERS DERIVED FROM DIFFERENT *sn*-GLYCERO-3-PHOSPHATE ESTERS

No.	*Glycerophospholipid investigated*	*Nature of derivative formed*	*Investigators*	
			Duncan et al.[33]	*Cicero and Sherman*[29]
1	1,2-Diacyl-*sn*-glycero-3-phosphate	Tetrakis(TMS)-*sn*-glycero-3-phosphate	0.10	--
		Tetrakis(TMS)-*sn*-glycero-2-phosphate	0.07	--
2	1,2-Diacyl-*sn*-glycero-3-phosphorylglycerol	Pentakis(TMS)-*sn*-3-glycero-phosphorylglycerol	0.41	--
3	1,2-Diacyl-*sn*-glycero-3-phosphoryl-ethanolamine	Tris(TMS)-N,N-bis(TMS)-*sn*-glycero-3-phosphorylethanolamine	0.49	--
4*	1,2-Diacyl-*sn*-glycero-3-phosphorylserine	Tetrakis(TMS)-N-TMS-*sn*-glycero-3-phosphorylserine	0.52	--
5	1,2-Diacyl-*sn*-glycero-3-phosphorylinositol	Octakis(TMS)-*sn*-glycero-3-phosphorylinositol	1.00	1.00
6	Bis(1,2-diacyl-*sn*-glycero-3-phosphoryl)-1′,3′-*sn*-glycerol	Heptakis(TMS)-bis(*sn*-glycero-3-phosphoryl)-1′,3′-*sn*-glycerol	--	1.38
7	1,2-Diacyl-*sn*-glycero-3-phosphorylinositol monophosphate	Nonakis(TMS)-*sn*-glycero-3-phosphorylinositol monophosphate	--	1.62
8	1,2-Diacyl-*sn*-glycero-3-phosphorylinositol diphosphate	Decakis(TMS)-*sn*-glycero-3-phosphorylinositol diphosphate	--	2.12

* Only one proton of the serine amino group is silylated.

these ions from the second category results from cleavage of the carbon and phosphate ester oxygen bond with charge retention by the oxygen accompanied by a hydrogen and TMS back-transfer yielding a protonated phosphate ion. The second ion results from back-transfer of two hydrogens. The third ion of this category at $m/e = 299$ originates through loss of methane in a rearrangement ion at $m/e = 315$ ($C_9H_{28}O_4Si_3P$) (ref. 50) (Fig. 1). The third category of ions are the most important ones in the char-



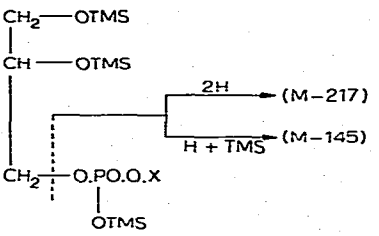
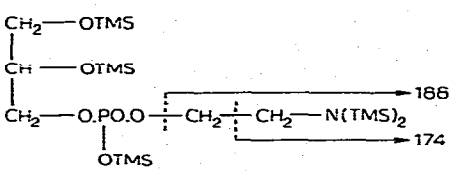


Fig. 1. MS fragmentation pattern of the TMS derivative of *sn*-glycero-3-phosphate. TMS = Trimethylsilyl.

Fig. 2. MS fragmentation pattern of the TMS derivative of *sn*-glycero-3-phosphate-X. X = Ethanolamine or serine or glycerol or inositol, etc.

acterization of the individual class of glycerophospholipid. In a phosphate diester like *sn*-glycero-3-phosphate-X, the cleavage between carbon and phosphate ester oxygen bond with charge retention by the oxygen accompanied by either double hydrogen or hydrogen and TMS back-transfer yielding a protonated phosphate ion can result in either loss of glycerol or the X group. The ions resulting from the loss of glycerol [(M — 217) double H-transfer; M — 145 (H and TMS transfer)] are diagnostic of the various X groups present in phosphate diesters (Fig. 2). In addition to these, there are certain ions which are typically characteristic for the individual class of phosphate diesters. Thus, for example, the TMS ether of *sn*-glycero-3-phosphorylserine yields abundant ions due to loss of the carbotrimethylsiloxy radical (–COOTMS) from the molecular ion at M — 117, a cleavage process noted previously for the TMS derivatives of amino acids[51]. In the case of ethanolamine or ethanolamine-derived glycerophospholipids, a simple single-bond cleavage between its carbon and phosphorus ester oxygen bond with charge retention on itself (N^+) yields structurally relevant information. Thus the TMS ether of *sn*-glycero-3-phosphoryl-N,N-bis (trimethylsilyl) ethanolamine will yield ions at m/e = 174 (M — 401) and m/e = 188 (M–387) (Fig. 3), while the TMS ether of *sn*-glycero-3-phosphoryldimethylethanolamine will yield m/e = 72 (M — 401) and m/e = 58 (M — 387) (Fig. 4). At low ionizing energy these simple cleavages predominate even to a greater extent.



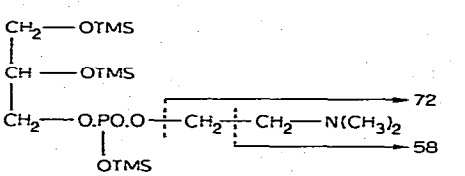


Fig. 3. MS fragmentation pattern of the TMS derivative of *sn*-glycero-3-phosphoryl-N,N-bis (trimethylsilyl)ethanolamine.

Fig. 4. MS fragmentation pattern of the TMS derivative of *sn*-glycero-3-phosphoryldimethylethanolamine.

Since the discovery of phosphonate-containing organic substances in nature[52], their existence as lipids is well documented[53]. Hence the necessity to differentiate chemically the phosphonates from the phosphates has been very essential. Although methods like paper chromatography[54], ion-exchange chromatography[55], electro-

phoresis[56], infrared[57] and nuclear magnetic resonance[56] spectroscopy, as well as knowledge of the stability of phosphonate phosphorus to prolonged acid hydrolysis[58,59] existed to distinguish phosphates from phosphonates, they required relatively large amounts of material and did not always provide conclusive evidence. On the other hand GC–MS could be used successfully for this purpose[30]. When using reference materials, 2-aminoethylphosphonate, O-phosphorylethanolamine, 2-amino-3-phosphonopropionic acid, and O-phosphorylserine were separated from each other as TMS ethers on a non-selective phase (Table 2). The separation was no doubt based on the molecular weight of the compounds but the "phosphono" components were always eluted ahead of their corresponding "phosphate" components. The distinguishing mass spectral features in these components were: (1) The "phosphono" derivatives had a more intense ion (M − 15) than the corresponding "phospho" derivatives. (2) The phosphate derivatives showed the characteristic ion $m/e = 299$ while the phosphonate derivatives possessed the ion $m/e = 298$ ($C_9H_{27}O_3Si_3P$).

Using this as his basis, Karlsson[30] identified 1-phosphono-2-aminoethane and small amounts of N-methyl-1-phosphono-2-aminoethane as the polar parts of the major sphingophosphonolipids of the sea-anemone, *Metridium senile*. The polar parts were released from this sphingophosphonolipid by phospholipase *c*.

B. Separation and characterization of molecular species of polar lipids

(a) Glycerophospholipids

Separation and characterization of molecular species of various glycerophospholipids were achieved by GC–MS using either direct pyrolysis products obtained by GC of native underivatized glycerophospholipids[31–33] or TMS ethers derived from 1,2-diglycerides obtained from glycerophospholipids by enzymatic hydrolysis[37] or TMS ethers derived from 1,2- and 1,3-diglycerides obtained by chemical degradation of glycerophospholipids[36] or TMS ethers of native glycerophospholipids[36]. The derivatives used had a higher temperature of volatilisation and hence it was necessary to use non-polar liquid phases and high temperatures during GC separations (Table 3). The use of high temperatures either during derivatization procedures or GC separations results in the partial isomerization of 1,2-diglycerides to 1,3-diglycerides. TMS derivatives of 1,2-diglyceride are always eluted ahead of the corresponding TMS derivatives of 1,3-diglyceride (Fig. 5), but the latter isomer is likely to overlap the 1,2-isomer of another diglyceride with a higher carbon number[10,37].

The MS characterization of the TMS ethers of 1,2- and 1,3-diglyceride showed a $[M - 15]^+$ fragment, indicating its molecular weight. The spectra also showed peaks due to acyl ions corresponding to each substituent fatty acid and peaks due to loss of acyloxy plus TMS minus hydrogen, loss of acyl plus TMS minus hydrogen, and loss of acyloxy. A readily distinguishable feature between TMS ethers of 1,3- and 1,2-diglyceride was that the former had an intense peak due to the loss from the molecular ion of an acyloxy group together with methylene (Fig. 6). The TMS ethers of pairs of mixed acid 1,2-diglyceride isomers can be readily distinguished from their mass spectra when introduced into the ion source on a direct insertion probe. In these spectra there is a preferential loss of the acyloxy group from the 2 position and an even more marked difference in the preferential loss of the complete acid from the 2 position[37].

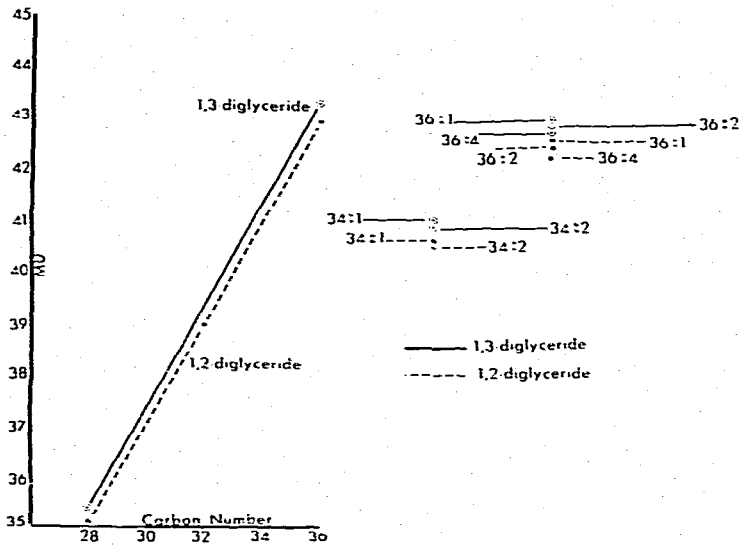


Fig. 5. Relationship of methylene unit (MU) values of TMS derivatives of 1,2- and 1,3-diglycerides to the sum of the fatty acid carbon number in the diglyceride[19]. 34:1 = Total thirty-four carbons with one double bond in the two fatty acids of diglyceride. 36:2 = Total thirty-six carbons with two double bonds in the two fatty acids of diglyceride.

(b) Sphingosines

Saturated and unsaturated, odd- and even-numbered, and straight and branched long-chain 2-aminopolyols (2-amino-1,3-diol; 2-amino-1,3,4-triol) are the basic constituents of sphingolipids. These bases are derived from the native lipids by either acid hydrolysis[38] or alkaline hydrolysis[40]. The free bases are characterized by GC–MS as: (1) long-chain aldehydes obtained by periodate[41] or lead tetraacetate oxidation; (2) di-O-TMS ethers of long-chain 2-amino-1,3-diols, and (3) N-acetyl-di-O-TMS ethers of long-chain 2-amino-1,3-diols. For locating the position of the double bonds in these compounds they are analysed by GC–MS as: (1) methyl esters of monocarboxylic acids derived by periodate–permanganate oxidation of the N-acetyl base and (2) the TMS ether of the polyhydroxylated N-acetyl long-chain base obtained by OsO_4 oxidation of the double bond. The GC conditions for various separations are summarized in Table 4. Although it should be possible to separate by argentation chromatography the fully acetylated C_{18}-saturated, monoene and diene 2-amino-1,3-diols, Polito *et al.*[60] used a combination of preparative GC on polar and non-polar phases to get pure analogs of C_{18} bases. On a non-polar phase (3% SE-30 or 3% OV-1) the mono-unsaturated base was eluted ahead of the saturated and di-unsaturated bases,

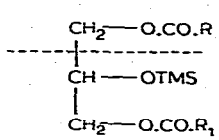


Fig. 6. Characteristic MS fragmentation pattern of the TMS derivative of 1,3-diglyceride. R and R_1 = Alkyl chains.

which were eluted together. The latter eluant, when put on a polar phase (3% XE-60 or 3% OV-17), eluted the saturated component ahead of the di-unsaturated component.

The MS characterization of long-chain 2-amino-1,3-diols as their di-O-TMS ethers depends on the detection of the following ions (Fig. 7): (1) M — 15, resulting from loss of the methyl group from one of the TMS groups; (2) M — 103, resulting

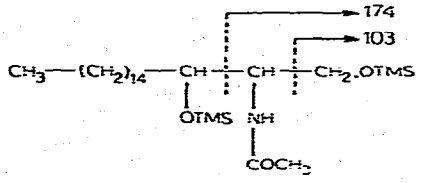


Fig. 7. MS fragmentation pattern of the TMS derivative of N-acetylsphinganine.

from cleavage between C_1 and C_2 of the base, and (3) M — 132 (or M — 174 when the amino group is acetylated), resulting from cleavage between C_2 and C_3. Thus, for a saturated C_{18} base one should look for peaks at m/e = 428, 340 and 311, for a mono-unsaturated C_{18} base at m/e = 426, 338 and 309, and for a di-unsaturated C_{18} base at m/e = 424, 336, and 307. When investigating polyhydroxylated O-TMS ethers derived from unsaturated bases, the positions of diols are easily located. This is because simple cleavage of the C–C bond between two carbons with O-TMS groups tends to produce O-TMS fragments[61] (Fig. 8). Thus, in a C_{18} diene base the location of unsaturation between C_4 and C_5 as well as C_8 and C_9 was determined on the basis of m/e = 461, m/e = 378, m/e = 371, and m/e = 288 for C_4 and C_5, and m/e = 229, m/e = 610, and m/e = 430 for C_8 and C_9 (ref. 62). Similarly, the characterization of C_{18} 2-amino-1,3,4-triol (phytosphingosine) was based on the fragments m/e = 401 $(M - 174)^+$ and m/e = 299 $(M - 276)^+$ produced by the N-acetyl-tri-O-TMS ether of this base. These two fragments indicated cleavage between C_2–C_3 and C_3–C_4, respectively[63].

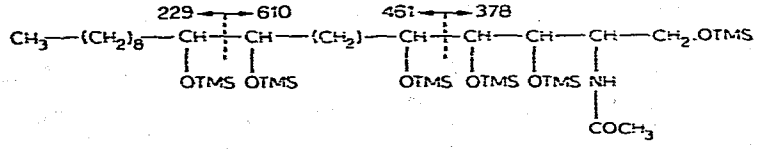


Fig. 8. MS fragmentation pattern of the TMS derivative of polyhydroxylated N-acetylsphinganine.

The aldehydes and methyl esters of monocarboxylic acid are characterized individually by their respective molecular peaks and M — 29 (cleavage between C_1–C_2 in the case of aldehydes) and M — 59 (cleavage between C_1–C_2 in the case of methyl esters)[62].

(c) Ceramides

The GC–MS technique has been successfully exploited for the characterization of molecular species of ceramides. The complex nature of ceramides, resulting from combinations between long-chain saturated or unsaturated 2-aminodiols or -triols

with normal or hydroxy fatty acids, necessitates use of certain prefractionation TLC techniques, which are briefly summarized in Table 1. The ceramides differing in chromatographic properties because of the presence of an additional hydroxyl group either in the long-chain aminopolyol or long-chain fatty acid are separated by plain adsorption chromatography. On the other hand, ceramides differing only in number of unsaturated sites in their molecules are separated by argentation chromatography. This is achieved by accentuating the polarity difference due to unsaturation alone, after acetylating the free hydroxyl groups of ceramide molecules. The resulting polyacetates are then separated by argentation chromatography, and after hydrolysing the O-acetyl bonds by mild alkaline hydrolysis, the ceramides are silylated and subjected to GC–MS analysis.

The GC data obtained by Casparrini *et al.*[42] on a 1% SE-30 liquid phase as methylene unit (MU) values of TMS derivatives of ceramides were plotted against the fatty acid carbon number. The 2-aminodiol component of all the ceramides was sphingosine (Fig. 9). The solid line represents ceramides with hydroxy fatty acids and

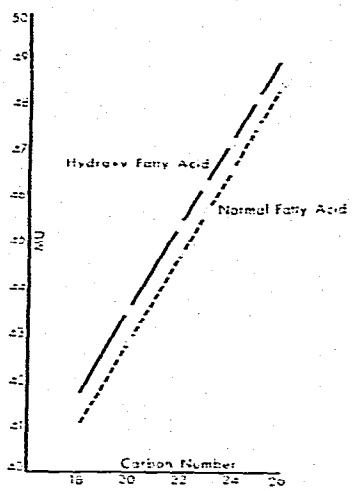


Fig. 9. Relationship of methylene unit (MU) values of TMS derivatives of ceramides (with normal or hydroxy fatty acids) to the carbon number of fatty acids in the ceramide[42]. The 2-aminodiol component of all the ceramides was sphingosine.

the dotted line represents ceramides with normal fatty acids. Similar data obtained by Samuelsson's group with ceramides derived from sphingosine and normal fatty acids[43], sphingosine with hydroxy fatty acids[45], dihydrosphingosine with normal fatty acids[43], dihydrosphingosine with hydroxy fatty acids[45], and phytosphingosine with normal fatty acids[45] are shown in Fig. 10.

The MS characterization of the TMS ethers of ceramides can be briefly summarized as follows. For details please refer to the original publications[42–45].

(1) Molecular weight fragments characterizing ceramides (all types) are those formed by elimination of a methyl group, trimethylsilanol group, and $-CH_2 \cdot OTMS$ group from the molecular ion.

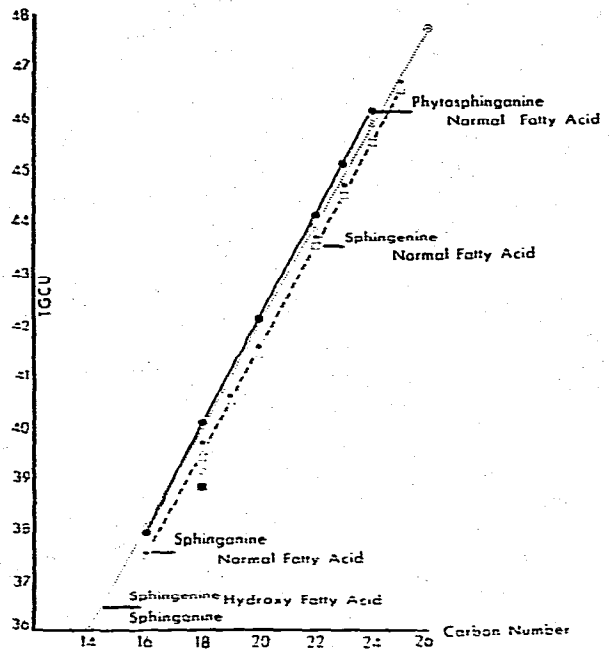


Fig. 10. Relationship of triglyceride carbon unit (TGCU) values of TMS derivatives of ceramides to the carbon number of fatty acids in the ceramide[43-46].

(2) The fragments resulting from cleavage between C_2 and C_3 of the long-chain aminopolyols of the ceramide (all types) yield information on long-chain base and fatty acids (Fig. 11).

(3) Information similar to that obtained in (2) is also derived due to cleavage of the amide structure and hydrogen transfer (exception is the ceramide derived from phytosphingosine) (Fig. 11).

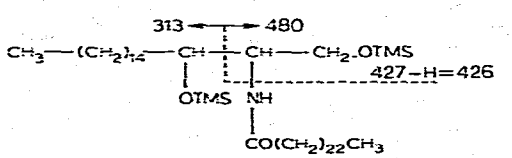


Fig. 11. MS fragmentation pattern for a TMS derivative of ceramide with normal fatty acid.

(4) In ceramides containing phytosphingosine as the long-chain base additional cleavage between C_3 and C_4 of the base gives additional ions characteristic of the base and fatty acid (Fig. 12).

(5) Ceramide containing normal fatty acid and a diol amino base has a special intense peak at $m/e = 85$, representing the two terminal carbons of the base and the amide derived structure remaining after a McLafferty cleavage of the long-chain acyl group[42] (Fig. 13).

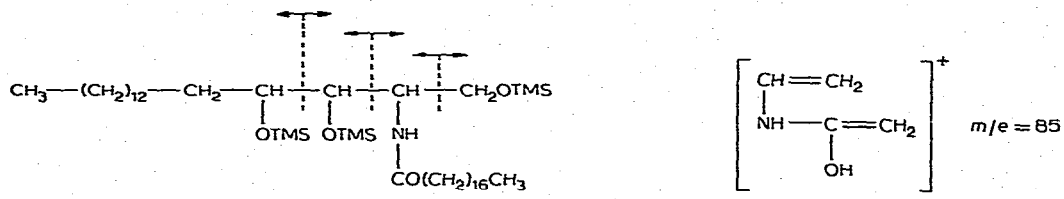


Fig. 12. MS fragmentation pattern of a TMS derivative of ceramide with normal fatty acid and phytosphingosine base.

Fig. 13. MS fragmentation ion typical for a TMS derivative of ceramide with normal fatty acid.

(6) Ceramides containing 2-hydroxy fatty acids yield a fatty acid-characterizing fragment due to cleavage between the carboxyl carbon and the hydroxyl carbon (Fig. 14).

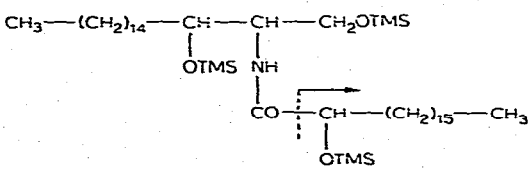


Fig. 14. MS fragmentation pattern for a TMS derivative of ceramide with 2-hydroxy fatty acid.

(d) Sphingophospholipids

Two types of sphingophospholipids, *viz.* (1) ceramide phosphorylcholine (sphingomyelin)[64] and (2) ceramide phosphorylethanolamine (sphingoethanolamine)[65], occur in nature. So far no attempts to analyse the molecular species of ceramide phosphorylethanolamine by GC–MS have been made. However, a number of different approaches have been used to characterize the molecular species of ceramide phosphorylcholine[10,25,36]. In one of the methods, phospholipase *c* is used to release the ceramide moiety of the lipid, which is subsequently analysed for its molecular species as the di-O-TMS ether derivative by GC–MS[25]. In the second method, silylated ceramide phosphorylcholine was directly analysed. Similar to glycerophospholipids, the phosphate ester moiety was eliminated, perhaps in a thermal elimination fashion equivalent to dehydration[36]. In the third method[10], the ceramide phosphorylcholine was thermally degraded in phenyl ether. This reaction yielded three products with R_F values of 0.2, 0.28, and 0.39 on a Chromar sheet with chloroform as the developing solvent. One of the products was ceramide, the other was anhydroceramide and the third remained uncharacterized. Water in trace amounts increased the yield of ceramide. These products, after preparative TLC, were analysed as their TMS ether by GC–MS.

(e) Sphingophosphonolipids

The following sphingophosphonolipids have been discovered so far: (1) ceramide 2-aminoethylphosphonate[66], (2) ceramide N-monomethyl-2-aminoethylphosphonate[54], (3) ceramide N-acyl-2-aminoethylphosphonate[68], and (4) ceramide N,N-acylmethyl-2-aminoethylphosphonate[68]. Recently Hayashi and Matsuura[69] re-

ported the occurrence of a new type of N-methyl sphingophosphonolipid containing 2-hydroxy fatty acid and phytosphingosine. The use of GC–MS has been made to characterize the molecular species of two types of ceramide 2-aminoethylphosphonate by Hayashi's group[39,69].

In analysing the molecular species of ceramide 2-aminoethylphosphonate Hayashi *et al.*[39,70] used two different approaches, *viz.* (1) GC–MS analysis of ceramides obtained from ceramide 2-aminoethylphosphonate by enzymatic (phospholipase *c*) hydrolysis and (2) analysis of intact ceramide 2-aminoethylphosphonate by GC–MS. In both cases they were converted to TMS ethers prior to analysis. To substantiate the results obtained by the above two techniques they independently analysed the fatty acids and long-chain bases derived by methanolysis of ceramide 2-aminoethylphosphonate and further identification of long-chain bases by GC analysis of their oxidation products. All the three analytical approaches yielded the same results: the presence of N-hexadecanoylhexadecasphing-4-eninyl-2-aminoethylphosphonate as the major component; the three minor components included N-heptadecanoylhexadecasphing-4-eninyl-, N-hexadecanoyloctadecasphing-4,8-dieninyl-, and N-hexadecanoyloctadecasphing-4-eninyl-2-aminoethylphosphonate. One interesting observation the authors made about their GC separation was that intact ceramide 2-aminoethylphosphonate could be separated according to the number of both carbon atom and double bond on a non-selective OV-1 liquid phase. Although intact ceramide 2-aminoethylphosphonate as TMS ether could be analysed directly by GC–MS, no information could be derived about the C–P moiety. This was done by the method of Karlsson[30].

Recently molecular species analysis by GC–MS of ceramide 2-N-methylaminoethylphosphonate containing 2-hydroxy fatty acid and phytosphingosine as components, was reported. The ceramides derived from this sphingophosphonolipid after silylation yielded at least nine different peaks on a non-selective 2% OV-1 liquid phase column which was run isothermally at 290°. The authors quantitated the nine different peaks and found as main components ceramide 2-N-methylaminoethylphosphonates containing 46% of C_{18} 2-hydroxyhexadecanoylphytosphingosine, 17% of C_{19} 2-hydroxyhexadecanoylphytosphingosine, and 27% of C_{22} 2-hydroxyhexadecanoyldehydrophytosphingosine. The MS characterization was based on the results obtained on reference ceramides by Samuelsson's group[43–46]. The authors[69] suggested that a collection of data on mass spectra of standard ceramides containing 2-hydroxy fatty acids and phytosphingosines would be useful.

(f) Sphingoglycolipids

Long-chain aminopolyols, fatty acids and carbohydrates constitute sphingoglycolipids. They are all derivatives of ceramides to which one or more carbohydrate units are linked glycosidically (mostly in β-configuration) via the hydroxyl group at the C-1 in the long-chain aminopolyol. So far GC–MS analysis has been exploited in the successful characterization of molecular species of ceramide monohexosides (ceramide galactosides[47] and ceramide glucosides[48]). Two approaches have been used. In the first one[12], the cerebrosides were chemically degraded by the procedure of Carter *et al.*[11] and the resulting ceramides were analysed by GC–MS as their di-O-TMS ethers. In the second approach, the cerebrosides were analysed by GC–MS directly after silylation[10,47,48].

In studying the molecular species composition of mouse brain glucosyl ceramides, it is essential to use some prefractionation TLC techniques. Use of borate-impregnated silica gel plates (Table 1) was made to free the glucosyl ceramides from the bulk of the galactosyl ceramides. The isolated pure glucosyl ceramides were then fractionated on plain silica gel plates into non-hydroxy fatty acid-containing and hydroxy fatty acid-containing glucosyl ceramides (Table 1). A point to remember is that the cerebrosides having either normal fatty acids or the hydroxy fatty acids with identical long-chain bases cannot be separated by GC. This effect is due to the polyhydroxy function in the hexose molecule. These were subsequently silylated and analysed by GC–MS[48].

The conditions employed to achieve GC separation are reported in Table 4 and the achieved GC separations are shown in Fig. 15. The MS characterizations of

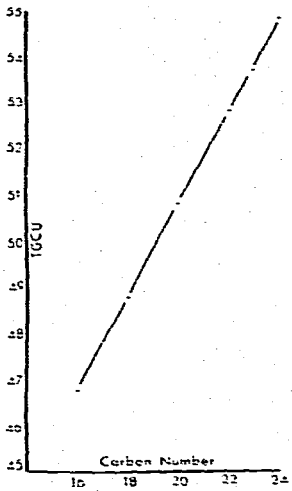
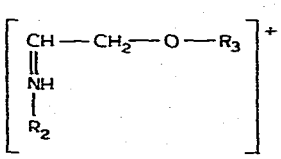


Fig. 15. Relationship of triglyceride carbon unit (TGCU) values of TMS derivatives of ceramide glucosides to the carbon number of fatty acids in the cerebroside.

TMS ethers of cerebrosides are, in many respects, similar to those of ceramides[10,47,48]. Thus a C_2–C_3 cleavage in the long-chain base produced ions characterizing the base as well as the fatty acid. The ion characterizing the fatty acid (Fig. 16) by further elimination of hexose produced another ion characteristic for fatty acids (Fig. 17). The ions formed by elimination of the whole hexose moiety and by elimination of



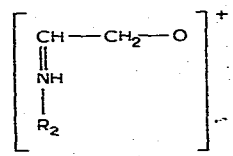


Fig. 16. Ion characterizing fatty acid chain in a cerebroside. R_2 = Acyl chain; R_3 = silylated hexose.

Fig. 17. Ion characterizing fatty acid chain in a cerebroside. R_2 = Acyl chain.

hexose plus TMS-OH from molecular ion yielded fragments characteristic of the ceramide. The distinguishing feature between the galactosyl ceramide and the glucosyl ceramide was the relatively higher abundance of the ion formed by the elimination of the glycosyl residue from the cerebroside[47].

4. APPLICATIONS

A. Class separation and characterization of polar lipids

A sensitive and reliable means of identifying and quantitatively assaying the tissue content of phosphoinositides (mono-, di- and triphosphoinositides)[14] and cardiolipin (diphosphatidylglycerol)[29] was developed. The values obtained for rat brain extracts were 2.42, 0.3, 0.19, and 0.62 μmoles per g of wet tissue for mono-, di-, triphosphoinositides and diphosphatidylglycerol, respectively.

Qualitative identification of phosphatidic acid, phosphatidylglycerol and phosphatidylinositol in the bronchopulmonary lavage from two patients with pulmonary alveolar proteinosis and in two lavages obtained *post mortem* from undiseased lungs[71] was achieved.

Confirmation of the base 1-phosphono-2-aminoethane and identification of a new base, N-methyl-1-phosphono-2-aminoethane, in the sphingolipids of the sea anemone, *Metridium senile*, was reported[30].

GC–MS was successfully used to characterize three molecular species of diolcholine plasmalogens[40]. The lipid was enzymatically dephosphorylated and analysed without prior derivatization. The three molecular species were identified as: (1) hexadec-1′-enyl-ethanediolphosphorylcholine, (2) hexadec-1′-enyl-propane-1,3-diolphosphorylcholine, and (3) octadec-1′-enyl-propane-1,3-diolphosphorylcholine.

B. Separation and characterization of molecular species of polar lipids

(a) Glycerophospholipids

In the bovine phosphatidylserine, 1-stearoyl-2-oleoyl- (and perhaps 1-oleoyl-2-stearoyl)*sn*-glycero-3-phosphorylserine was the major molecular species. Other species included: 1-stearoyl-2-docosahexaenoyl- and 1-stearoyl-2-eicosaenoyl-*sn*-glycero-3-phosphorylserine. These results agreed very well with the fatty acid composition of the phosphatidylserine determined by independent methods[34]. Molecular species of phosphatidylcholine (from egg, plant and bile), phosphatidylinositol, phosphatidylethanolamine and diphosphatidylglycerol from commercial sources were also reported[10,36].

(b) Sphingosines

A long-chain base from oyster glycolipid has been identified as sphinga-4,8-diene[62]. The unique occurrence of 2-amino-1,3-diol-4-*trans*-tetradecane and 2-amino-1,3-diol-4-*trans*-hexadecane was reported in *M. domestica* larvae and adults[72]. The presence of sphinga-4,14-diene in human plasmal sphingomyelin has also been reported[15,25]. Two branched phytosphingosine bases, 1,3,4-trihydroxy-2-amino-16-methyloctadecane and 1,3,4-trihydroxy-2-amino-16-methylnonadecane, were identified by Karlsson *et al.*[67] in the sulfatides of the salt gland of the spiny dogfish.

(c) Ceramides

Identification and quantitation of ceramides in human plasma have been reported[73]. Only ceramides with normal fatty acids (C_{20}–C_{24} chainlength) were found. Although sphingosine was the main long-chain base, the other bases detected were hexadeca-, heptadeca- and nonadecasphingosine, sphinganine and sphingadiene. Healthy subjects had a ceramide content of 5.64 ± 1.51 μg/ml of plasma.

A patient who had a special phenotype of lipogranulomatosis and a control were studied for ceramide content and its nature[74]. In a subcutaneous nodule (analysed one year before the death of the patient), ceramide had accumulated to the extent of 11.9 mg/g of wet weight. The main components were sphingosine base and palmitic, behenic and lignoceric acids. In the kidney of the dead, the accumulation of ceramides with hydroxy fatty acids was seven times higher than in that of the control (600 μg/g of wet weight).

Human platelets which had a free ceramide content of 1.31 μg/109 platelets or 0.47 μg/mg platelet protein contained as its major molecular species: N-palmitoylsphingosine, N-stearoylsphingosine, N-docosanoylsphingosine, and N-tetracosanoylsphingosine[75]. Minor amounts of ceramides containing sphinganine and sphingadiene as well as unsaturated fatty acids were also present. The quantitation of ceramides was achieved by use of radioactive ceramide as internal standard and synthetic crystalline ceramides for comparison of peak areas.

(d) Sphingophospholipids (sphingomyelin)

A combination of prefractionation TLC and GC–MS indicated the existence of at least 33 molecular species of sphingomyelin from human plasma and two of the GC fractions were pure molecular species of N-tetracosenoylsphingosine and N-tetracosenoylsphinga-4,14-diene[25].

(e) Sphingophosphonolipids

In the ceramide 2-aminoethyl phosphonate isolated from oyster adductor[39], N-hexadecanoylhexadecasphing-4-eninyl-2-aminoethylphosphonate was the major molecular species. The other species were: N-heptadecanoylhexadecasphing-4-eninyl-, N-hexadecanoylsphing-4,8-dieninyl-, and N-hexadecanoylsphing-4-eninyl-2-aminoethylphosphonate.

The molecular species of ceramide monomethylaminoethylphosphonate occurring in the viscera of *Turbo cornutus* were: C_{18} 2-hydroxyhexadecanoylphytosphingosine-, C_{19} 2-hydroxyhexadecanoylphytosphingosine-, and C_{22} 2-hydroxyhexadecanoyldehydrophytosphingosine-2-N-methylaminoethylphosphonate in the ratio of 46:17:27 (ref. 70).

(f) Sphingoglycolipids

About fifteen molecular species each of glucosyl ceramide[48] and galactosyl ceramide[12] have been identified. Mouse brain glucosyl ceramides contained 20% hydroxy fatty acids (mainly 2-hydroxystearic and -behenic acids), and the rest non-hydroxy (stearic, arachidic, lignoceric, and behenic acids, in that order of abundance). The major long-chain base was sphingosine and C_{20}-sphingenine comprised 20% of the total bases.

Cerebrosides from cerebrospinal fluid[69], containing sphingosine, normal and

hydroxy fatty acids, and galactose, resembled in composition the cerebrosides from brain.

5. DISCUSSION

It is clear from the information given above that GC–MS has been successfully exploited not only for the identification and sometimes quantitation of the molecular species of a variety of glycerophospholipids, long-chain 2-amino-1,3- or -1,3,4-polyols, ceramides, sphingophospholipids, sphingophosphonolipids, and sphingoglycolipids, but also for the identification and quantitation of a number of glycerophospholipid classes from samples of biological origin.

Recently some reports have appeared in the literature on identification of molecular species of complex lipids by MS alone. Thus Klein[76–78] has demonstrated the possibility to detect fragments related to the molecular species of phosphatidylcholines in naturally occurring mixtures and has tentatively identified eight different molecular species[78]. The author suggested the necessity to obtain correction factors for the commonly occurring species and, in certain cases, for the less readily available molecular species, if the tissue were rich in a particular fatty acid, and in case quantitative analysis of biological samples was sought. The molar correction factor derived showed that the greatest response was for those compounds containing the smallest number of carbon atoms and the introduction of one double bond increased the response approximately two-fold.

During their work on structure determination of pure sphingoglycolipids as their TMS derivatives by direct MS, Sweeley and Dawson[79] used a partially scanned mass spectrum (*m/e* 60 to *m/e* 800) to get the information regarding the positions of substitution of monosaccharide units, the nature of sphingolipid bases and the relative amounts of constituent fatty acids.

Stoffel and Hanfland[80] used GC–MS to characterize methylated alditol acetates and N-methylacetamidoalditol acetates in characterizing amino-sugar containing sphingoglycolipids.

A recently reported centrichromatography–mass spectrometry technique seems to have similar potential of analysing microgram quantities of multicomponent mixtures. This technique involves the application of centrifugal force to accelerate the migration and separation by solid–liquid chromatography of samples through columns of microparticulate silica and subsequently analyse them by MS[81].

6. ACKNOWLEDGEMENTS

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7. SUMMARY

The review discusses, in brief, the methodology involved in the coupled gas chromatography–mass spectrometry separation and characterization of some classes of polar lipids and their molecular species. Its application to the field of biological sciences has been indicated.

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