Diol Lipids of Rat Liver. Quantitation and Structural Characteristics of Neutral Lipids and Phospholipids Derived from Ethanediol, Propanediols, and Butanediols†

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ABSTRACT: Specific enzymatic and chemical degradation of neutral lipid and phospholipid fractions from rat liver revealed the presence of novel types of lipid metabolites bearing a short-chain diol backbone. Diol-derived lecithin and cephalin analogs were readily cleaved by phospholipase C (EC 3.1.4.3) from *Bacillus cereus*, although the cephalin analogs required "carrier" lecithin to sustain hydrolysis. The products of phospholipase hydrolyses as well as the neutral lipid fractions were subjected to alkaline and acidic methanolysis, and constituent short-chain diols were analyzed as long-chain cyclic acetals. Gas chromatography–mass spectrometry confirmed that 1,2-ethanediol, 1,2-propanediol, 1,3-propanediol, and 1,3-butanediol can form the polyol backbone of neutral lipids and phospholipids. [1,1,2,2-^{2}H]Ethanediol monohexadecanoate, dihexadecanoate, hexadecanoylphosphorylcholine, and hexadecanoylphosphorylethanolamine were synthesized chemically and served as internal standards to assure accurate quantitation of the low levels of diol lipids (350 μg/g of total lipid) present in rat liver.

Lipids containing a short-chain diol backbone, instead of glycerol, are common but minor constituents of many organisms, including mammalian tissues (for reviews, see Bergelson, 1969, 1973). We have previously shown that ethanediol can be incorporated directly into the lipids of mammalian systems, and that this route of diol lipid biosynthesis does not involve intermediary oxidation and reduction steps at the diol moiety (Lin and Baumann, 1974). Our data also suggested that phosphorylated diol-derived lipids serve as precursors of neutral diol lipid constituents. It appears that the low levels of diol lipids usually found in mammalian systems are maintained through the high turnover rates of these novel types of lipid metabolites.

Evidence for the occurrence of diol lipids in rat liver has been brought forward previously (Bergelson *et al.*, 1966, 1972; Vaver *et al.*, 1967). These pioneering studies drew attention to the fact that short-chain diols may replace glycerol as polyol function in triglycerides, phosphatidylcholines, and possibly also in the phosphatidylglycerols of this tissue. Progress in this field has been hampered by difficulties in the analysis and isolation of diol lipids. Difficulties arise from the chemical and physical similarity of diol- and glycerol-derived lipid constituents, from the generally low levels of diol lipids present in the bulk of glycerolipids, and through losses, contamination, and the formation of artifacts. The quantitation of diol lipids has not been accomplished so far.

Reliable identification and accurate quantitation of lipid-bound short-chain diols in complex lipid mixtures are prerequisites for studying the origin, metabolism, and biological function of diol lipids. Diol phospholipids have attracted considerable interest as membrane-active compounds. Diol-derived lecithin analogs possess strongly lytic activity, exceeding that of lysolecithins (Baer, 1953; Baer *et al.*, 1968; Reman *et al.*, 1969), and at the lower natural levels, diol phospholipids may function as membrane modifiers (Bergelson, 1973). Anticoagulant activity has been ascribed to diol-derived phosphatidylserine analogs (Turner *et al.*, 1972).

In the present communication, we report on the structures and amounts of neutral lipids and phospholipids derived from ethanediol, propanediols, and butanediols, as they occur in rat liver.

Experimental Procedures

Materials

Throughout the experiments, special care was taken to eliminate diol contamination from solvents, chemicals, glassware, and chromatographic adsorbents. Solvents used were reagent grade and were further purified by chemical treatment (hexane and benzene over P_2O_5; methanol over magnesium; anhydrous diethyl ether over $LiAlH_4$), followed by fractionation over a 2-m distillation column. Chloroform was purified by distillation only. Glassware was cleaned in chromic sulfuric acid. Silica Gel H (E. Merck, Darmstadt, Germany) was prewashed, and silica gel chromatoplates were predeveloped with chloroform–methanol, 1:1 (v/v) and reactivated.

Cyclic acetals used as standards were prepared by acid-catalyzed condensation of short-chain diols with hexadecanal (Schupp and Baumann, 1973). Internal lipid standards derived from perdeuterated ethanediol were synthesized as follows: [1,1,2,2-^{2}H]Ethanediol monohexadecanoate and [1,1,2,2,-^{2}H]ethanediol dihexadecanoate were prepared by acylation of [1,1,2,2-^{2}H]ethanediol (99.9%; Merck Co., Rahway, N.J.) with hexadecanoyl chloride (Baer, 1953; Baumann *et al.*, 1967). Hexadecanoyl[1,1,2,2-^{2}H]ethanediolphosphorylcholine was synthesized by condensation of the monoester with β-bromoethyl dichlorophosphate (Hirt and Berchtold, 1958), followed by hydrolysis of the

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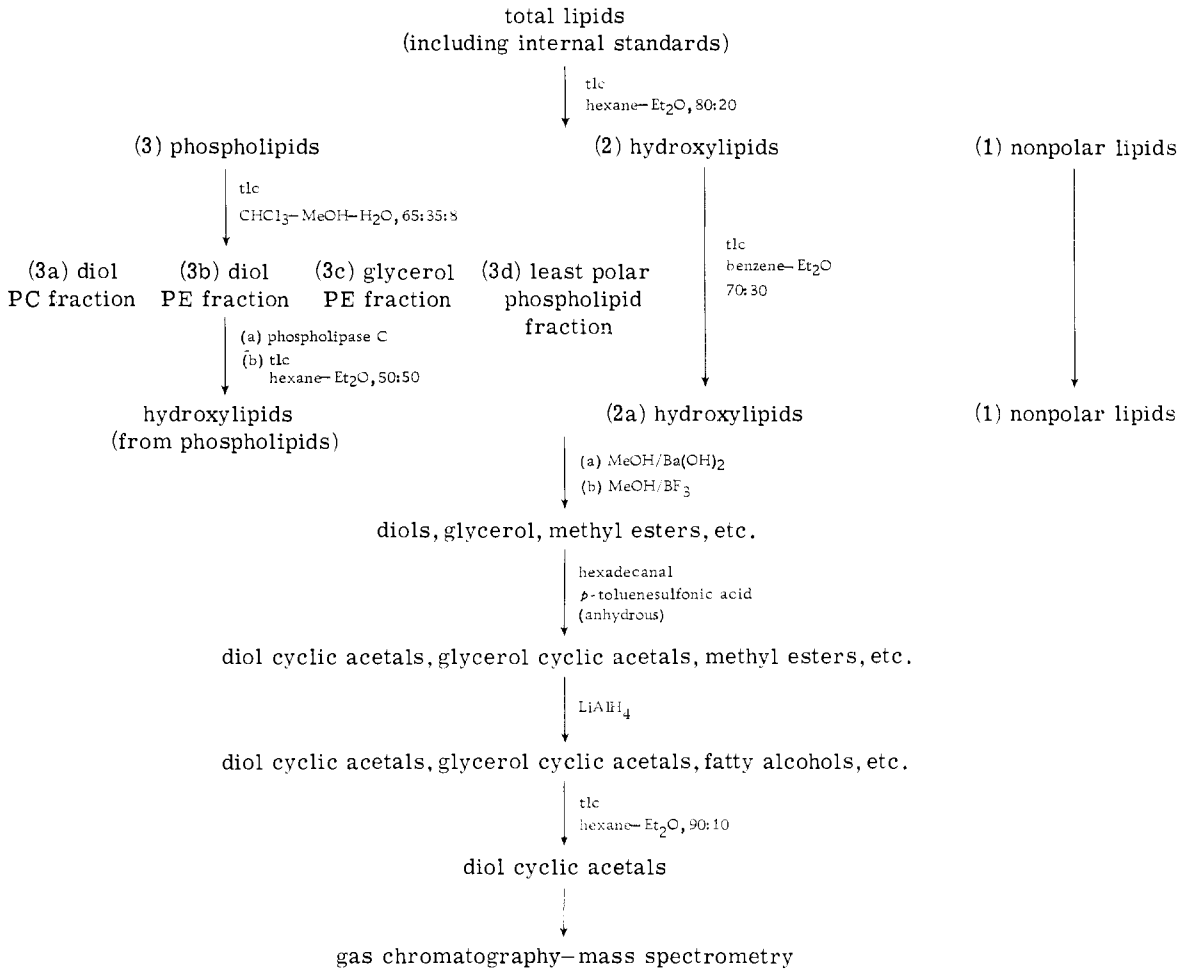
SCHEME I: Fractionation and Analysis of Diol Lipids.



chloride, reaction with trimethylamine, and removal of bromide with silver carbonate. Hexadecanoyl[1,1,2,2-^{2}H]ethanediolphosphorylethanolamine was prepared by condensation of monoester with phthalimidoethyl dichlorophosphate (Hirt and Berchtold, 1957), followed by hydrolysis of the chloride, and removal of the phthaloyl protecting group with hydrazine.

Methods

Preparative Thin-Layer Chromatography (tlc). Total lipids and phospholipids were applied in chloroform solution, neutral lipids in hexane, on layers of Silica Gel H, 0.3 or 3 mm thick (Schmid *et al.*, 1967). After chromatography in tanks lined with filter paper (unless noted otherwise), neutral lipid fractions were eluted from the adsorbent with water-saturated diethyl ether; phospholipids were eluted with chloroform–methanol–water, 50:40:10 (v/v).

Gas–Liquid Chromatography (glc). Diol cyclic acetals were analyzed on a Hewlett-Packard 5750 instrument with a flame ionization detector, using a glass column, 300 cm × 0.2 cm i.d., packed with 10% SP-1000 on Gas-Chrom P, 80–100 mesh (Supelco, Bellefonte, PA.). The column was operated at 210° with helium (20 ml/min) as carrier gas.

Gas–Liquid Chromatography–Mass Spectrometry (glc ms). The glc column (aluminum), which was operated at 210° at a flow rate of 10 ml/min, was connected to a Hitachi-Perkin-Elmer single-focusing mass spectrometer, RMU-6D, by means of a Watson-Biemann separator. Mass spectra were recorded at a source temperature of 240° and 70 eV ionization potential.

Lipid Extraction and Fractionation. Livers, 66.7 g, from female rats (180–195 g each) of the Sprague-Dawley strain (D. Rolfsmeyer, Madison, WI.) were homogenized and extracted with 20 parts (v/w) of chloroform–methanol, 2:1 (v/v). [1,1,2,2-^{2}H]Ethanediol dihexadecanoate (6 μg/g of tissue), [1,1,2,2-^{2}H]ethanediol monohexadecanoate (3 μg/g), hexadecanoyl[1,1,2,2-^{2}H]ethanediolphosphorylethanolamine (6 μg/g), and hexadecanoyl[1,1,2,2-^{2}H]ethanediolphosphorylcholine (6 μg/g) were added as internal standards, and stirring was continued for 2 hr. The tissue was filtered off and reextracted with 5 parts (0.35 l.) of fresh solvent mixture. The combined extracts were *not* partitioned with water. The solvents were removed on a rotary evaporator at 40°, and residual water was distilled off azeotropically with chloroform. The residue was taken up in 100 parts (v/w) of chloroform–methanol–water, 50:40:10 (v/v), 5 parts (w/w) of activated Silica Gel H was added, and the slurry was agitated. The adsorbent was filtered off and washed with a total of 50 parts (v/w) of fresh solvent mixture. After evaporation, 4.15 g of lipid material was recovered.

The total lipids were fractionated on 3-mm layers of Silica Gel H in unlined tanks; developing solvent, hexane–diethyl ether, 80:20 (v/v). Three fractions were taken (Scheme I): (1) the nonpolar lipid fraction (R_f 0.6–1.0), 0.45 g, containing triglycerides and less polar constituents; (2) the hydroxylipid fraction (R_F 0.05–0.6), 0.24 g, consisting of lipids which migrate off the origin, but are more

polar than triglycerides; and (3) the phospholipid fraction, 2.91 g, which was recovered from the origin.

The phospholipids (3) were rechromatographed on 0.3-mm layers of Silica Gel H; developing solvent chloroform–methanol–water, 65:35:8 (v/v). Four lipid fractions were taken: (3a) the diol PC[1] fraction (R_F 0.05–0.3), 0.91 g, containing constituents such as sphingomyelin and lysoglycerol PC migrating off the origin but being more polar than glycerol PC; (3b) the diol PE fraction (R_F 0.3–0.6), 1.14 g, comprising mostly glycerol PC; (3c) the glycerol PE fraction (R_F 0.6–0.8), 0.37 g; and a fraction (3d) of least polar phospholipids (R_F 0.8–1.0), 0.12 g, containing constituents less polar than glycerol PE.

Phospholipid fractions (3a–3d) and the nonpolar lipid fraction (1) were analyzed as such. The hydroxylipids (2) were rechromatographed on 0.3-mm layers in unlined tanks; developing solvent, benzene–diethyl ether, 70:30 (v/v). The fraction of purified hydroxylipids (2a) (R_F 0.25–1.0), 0.15 g, was isolated for further analysis (Scheme I); materials with an R_F value smaller than 50% of that of cholesterol, possibly including free diols, were discarded.

Enzymatic Degradation of Diol Phospholipid Fractions (3a–3d). Phospholipids, 80 mg, 10 ml of diethyl ether, 10 ml of Tris buffer (0.05 M, pH 7.2), 5 ml of $ZnCl_2$ solution (0.01%), and 2 mg of phospholipase C (EC 3.1.4.3) from *Bacillus cereus* (General Biochemicals, Chagrin Falls, Ohio) were incubated under nitrogen in a 50-ml flask under continuous shaking at 25° for 18 hr. The mixture was extracted twice with diethyl ether, then with 25 ml of chloroform–methanol, 2:1 (v/v), and finally with chloroform. The combined organic extracts were brought to dryness. The lipids were taken up in chloroform and purified on 0.3-mm layers of Silica Gel H; developing solvent, hexane–diethyl ether, 50:50 (v/v). The hydroxylipids (R_F 0.2–0.6), consisting largely of diglycerides, were thoroughly dried *in vacuo*.

Enzymatic hydrolysis of phospholipid fractions (3a–3d), 80 mg each, produced 7 mg of hydroxylipids from 3a, 41 mg from 3b, 43 mg from 3c, and 13 mg from 3d. These hydroxylipids derived from phospholipids, as well as the native *hydroxylipids* (2a) and *nonpolar lipids* (1), were subjected to methanolysis as follows:

Methanolysis. Neutral lipids, 50 mg or less, and 4 ml of anhydrous methanol were placed in a 25-ml three-necked flask equipped with nitrogen inlet tube, reflux condenser with drying tube, and magnetic stirrer. The mixture was heated to reflux temperature in an atmosphere of dry nitrogen. Anhydrous $Ba(OH)_2$, 15 mg, prepared from $Ba(OH)_2 \cdot 8H_2O$ (Fisher Scientific, Fairlawn, N.J.) by drying *in vacuo* at 100° to constant weight, was added, and the mixture was refluxed for 30 min. Addition of 2.5 ml of a solution (14%, w/v) of BF_3 (Matheson, East Rutherford, N.J.) in dry methanol to the hot mixture brought transesterification to completion within 2–3 min. After the mixture was cooled, 0.4 g of NaF (Merck, Rahway, N.J.) was added and stirring was continued for 10 min. Dilution with 20 ml of anhydrous ether and stirring for 30 min caused precipitation of most of the inorganic material which was filtered off on a sintered glass funnel and washed with 6 ml of diethyl ether–methanol, 3:1. The solvent was removed on a rotary evaporator at room temperature using a 50-ml three-necked flask suitable for the following acetalation step.

Acetalation. Dry benzene, 45 ml, and 25 mg of hexadecanal, prepared by dimethyl sulfoxide oxidation (Mahadevan *et al.*, 1966) of hexadecyl methanesulfonate (Baumann and Mangold, 1964), were added to the transesterified lipid mixture. The flask was equipped with nitrogen inlet tube, distillation head and condenser, and magnetic stirrer. The mixture was heated in an atmosphere of dry nitrogen for 20 min, while approximately 15 ml of benzene was distilled off. Upon cooling, 25 mg of anhydrous *p*-toluenesulfonic acid (commercial monohydrate, Eastman Kodak, Rochester, N.Y., was dried by azeotropic removal of water with benzene) in 0.5 ml of dry benzene was added, and stirring at room temperature was continued for 2 hr. The mixture was transferred into a separatory funnel containing an excess of cold aqueous 2% K_2CO_3 solution (30 ml) and extracted with ether (100 ml). The organic phase was washed with water, the solvent was evaporated, and the residue was dried *in vacuo*.

Lithium Aluminum Hydride Reduction. A mixture of $LiAlH_4$, 60 mg, and 30 ml of anhydrous diethyl ether was stirred and refluxed in a 50-ml flask under dry nitrogen. The products of acetalation in 10 ml of anhydrous ether were added. Excess $LiAlH_4$ was destroyed after 2 hr by addition of water-saturated ether, and inorganic hydroxide was precipitated with 1 ml of water. The ether solution was decanted, the hydroxide was washed with fresh ether, and the combined extracts were evaporated to dryness.

The residue dissolved in hexane was fractionated on 0.3-mm layers of Silica Gel H; developing solvent hexane–diethyl ether, 90:10 (v/v). The fraction of diol cyclic acetals (R_F 0.4–0.8) was eluted for glc and glc–ms analysis.

Identification and Quantitation. Control analyses involving phospholipase C hydrolysis and/or methanolysis, acetalation, and $LiAlH_4$ reduction were carried out on the actual amounts of reagents to account for small amounts of free diols in chemicals and solvents. The control analyses revealed formation of a long-chain cyclic acetal from the reagents which was tentatively identified as 2-pentadecyl-4,4,5-trimethyl-1,3-dioxolane (R_T 13.4 min). The origin of this artifact remained unknown. Other "background" diol acetals were less than 10% of the natural levels and were accounted for in each diol lipid analysis.

The C_{16}-acetals of diols were analyzed by glc and identified by comparison of their retention times with those of authentic standards. Definitive structural assignments were based on glc–ms data.

Individual diol lipids were quantified as the respective diol acetals by glc relative to the amount of 2-pentadecyl-1,3-[4,4,5,5-^{2}H]dioxolane present in the ethanediol acetal fraction. The ratio of deuterated (from internal standard) to nondeuterated ethanediol cyclic acetals was determined by glc–ms on the basis of the intensities of the prominent 1,3-dioxolane ions (65% of total ionization) at *m/e* 77 and *m/e* 73 for the deuterated and nondeuterated species, respectively (Schupp and Baumann, 1973; Baumann *et al.*, 1973). The amounts of individual acetals were finally corrected for small amounts of "background" acetals as determined through control analyses on reagents only. The recoveries of internal standards were usually 35–50% reflecting losses in the course of extraction, fractionation, and derivatization.

Results and Discussion

Reliable identification and quantitation of the small amounts of diol lipids commonly found in mammalian tissue required special precautions in the course of extraction,

[1] Abbreviations used are: PC, lipids containing phosphorylcholine; PE, lipids containing phosphorylethanolamine.

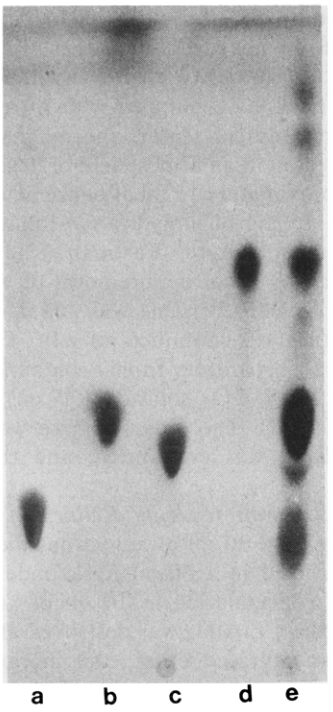


FIGURE 1: Thin-layer chromatogram of hexadecanoylethanediolphosphorylcholine (a), hexadecanoylethanediolphosphorylethanolamine (b), phosphatidylcholine (c), phosphatidylethanolamine (d), rat liver phospholipids consisting largely of phosphatidylcholine and phosphatidylethanolamine (e); developing solvent, chloroform–methanol–water, 65:35:8 (v/v).

fractionation, and analysis of the lipid-bound diols of rat liver. Lipids were extracted by a modified Folch procedure (Folch *et al.*, 1957) to preclude loss of water-soluble diol phospholipids and, in particular, of the diol-derived lecithin analogs (Baer, 1953; Baer *et al.*, 1968; Kozhukov *et al.*, 1969). Thus, the crude chloroform–methanol extracts were not partitioned with water, but were treated instead with activated silicic acid to remove nonlipid contaminants. In order to account for losses of diol lipids during fractionation and analysis, synthetic monoester, diester, acylphosphorylcholine, and acylphosphorylethanolamine of perdeuterated ethanediol were added as quantitative standards to the tissue homogenates. The lipids were fractionated by thin-layer chromatography (Scheme I) into (1) nonpolar lipids, containing [1,1,2,2-^{2}H]ethanediol dihexadecanoate as internal standard, (2) hydroxylipids, containing [1,1,2,2-^{2}H]ethanediol monohexadecanoate, and (3) phospholipids. Fraction 3 was further resolved into lipid fractions representative of diol PC (3a), which contained hexadecanoyl[1,1,2,2-^{2}H]ethanediolphosphorylcholine as internal standard, diol PE (3b), containing hexadecanoyl[1,1,2,2-^{2}H]ethanediolphosphorylethanolamine, diol phospholipids of unknown structure associated with the glycerol PE fraction (3c), and a fraction of least polar phospholipids (3d). As shown in Figure 1, ethanediol PC (a) is more polar than glycerol PC (c), and ethanediol PE (b) is significantly more polar than the glycerol analog (c). Ethanediol PE (b) shows a migration rate in tlc similar to that of glycerol PC (c).

The phospholipid fractions (3a–3d) were hydrolyzed with phospholipase C (EC 3.1.4.3) from *Bacillus cereus* (Chu, 1949). The enzyme preparation (General Biochemicals) was found to cleave effectively synthetic hexadecanoylethanediolphosphorylcholine at pH 7.2 with zinc ions present (Ottolenghi, 1965; Bergelson *et al.*, 1972). The less water-soluble hexadecanoylethanediolphosphorylethanolamine was hydrolyzed only when substantial quantities of natural "carrier" lecithin were added to the incubation mixture. However, with glycerol PC comprising the major portion of our diol PE fraction (3b), difficulties were not encountered in the hydrolysis of ethanediol PE as judged from the recoveries of internal deuterated standard. It had previously been shown that even phospholipase C from *Clostridium welchii* readily cleaves ethanediol PC (Baer and Robinson, 1968) and PC containing a long-chain 1,2-alkanediol backbone (Chang and Schmid, 1973). Saturated acylphosphorylcholines derived from nonvicinal diols appear to be more resistant to hydrolysis (Molotkovskii *et al.*, 1971), at least in the absence of a suitable carrier.

The hydroxylipids produced through phospholipase C hydrolysis of individual phospholipid fractions (3a–3d), the native hydroxylipids (2a), and the nonpolar lipid fraction (1) were degraded, and their constituent diols were derivatized, as shown in Scheme I.

Methanolysis in the presence of anhydrous barium hydroxide completely cleaves ester functions at polyhydric moieties, including those linked to tertiary hydroxy groups. Boron trifluoride accomplishes conversion of the barium salts of fatty acids to methyl esters and, at the same time, cleaves alk-1-enyl ethers without the formation of artifacts

Table I: Diol-Bound Lipids in Rat Liver.

	Diol Lipid[b] Derived from							
	1,2-Ethanediol		1,2-Propanediol		1,3-Propanediol		1,3-Butanediol	
Lipid Fraction[a]	[μg/g]	[nmol/g]	[μg/g]	[nmol/g]	[μg/g]	[nmol/g]	[μg/g]	[nmol/g]
Nonpolar lipids (1)[c]	72.7	6.38	1.7	0.15	8.4	0.73	23.1	1.93
Hydroxylipids (2a)	32.8	5.17	1.7	0.26	1.4	0.22	3.0	0.43
Glycerol PE (3c)[d]	8.1	0.90	27.2	2.93	11.4	1.24	1.3	0.13
Diol PE (3b)	36.4	4.07	11.3	1.21	8.8	0.94	3.7	0.38
Diol PC (3a)	78.2	7.94	8.7	0.86	3.3	0.33	2.5	0.24
Total diol lipids	228.2	24.46	50.6	5.41	33.3	3.46	33.6	3.11

[a] Lipid fractions as defined in the Experimental Section. [b] Diol lipid contents are expressed in μg of the diol hexadecanoate of the particular diol lipid class per g of total lipid and as nmol per g of wet tissue. [c] Traces of lipid-bound 2,3-butanediol are associated with the nonpolar lipid fraction. [d] Diol lipid contents of the glycerol PE fraction are expressed as they are diol PE.

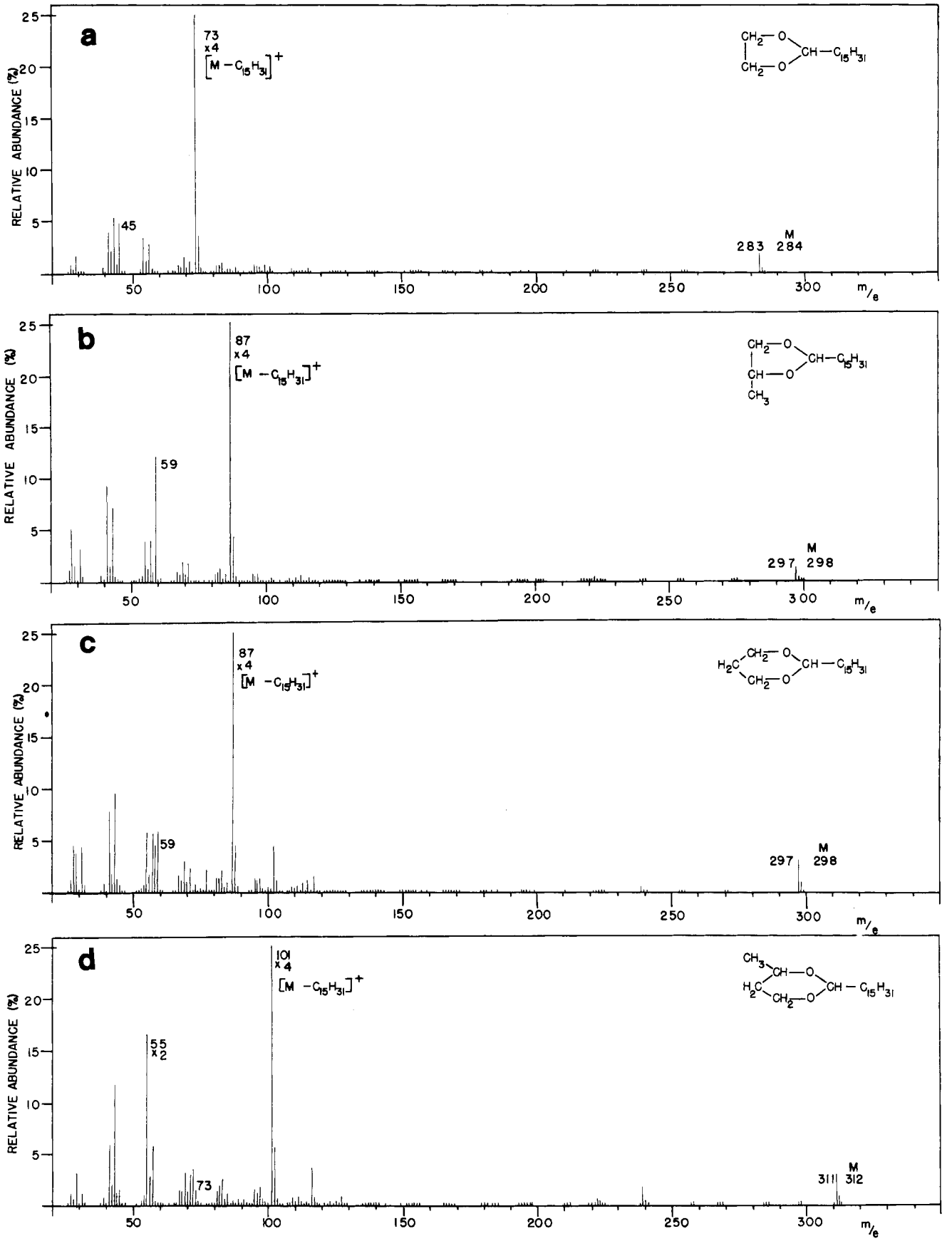


FIGURE 2: Mass spectra (70 eV) of the C_{16}-acetals of ethanediol (a), 1,2-propanediol (b), 1,3-propanediol (c), and 1,3-butanediol (d).

(Schupp and Baumann, 1973). Alkyl diol ethers would not be cleaved and accounted for using this procedure. Excess boron trifluoride is then precipitated from the reaction mixture by complexing it with sodium fluoride.

The procedure for the acetalation of polyhydric alcohols with long-chain aldehyde catalyzed by *p*-toluenesulfonic

acid under azeotropic removal of water with benzene, a method proven effective for synthetic mixtures (Schupp and Baumann, 1973), required some modification to assure reproducible analyses of diol lipids in natural lipid fractions, particularly in the presence of cholesterol.

Condensation of the polyols in the transmethylation mixture with hexadecanal is brought about at room temperature by addition of anhydrous *p*-toluenesulfonic acid. The latter not only acts as catalyst, but simultaneously shifts the equilibrium toward complete acetal formation by capturing the water formed as insoluble monohydrate. After acetalation, excess aldehyde and fatty acid methyl esters are converted to alcohols by lithium aluminum hydride reduction, without affecting the diol cyclic acetals, to facilitate the chromatographic separation of diol acetals from the bulk of polar materials including long-chain alcohols and glycerol acetals (Scheme I).

Gas chromatography of the mixtures of diol cyclic acetals derived from the nonpolar lipids (1), hydroxylipids (2a), and the diol PC (3a), diol PE (3b), and glycerol PE (3c) fractions from rat liver indicated the presence of lipid-bound short-chain diols in these fractions. Diol lipids were not found associated with the least polar phospholipids (3d). The cyclic acetals of hexadecanal and 1,2-propanediol (*cis*-2-pentadecyl-4-methyl-1,3-dioxolane, R_T 13.4 min; trans isomer, R_T 14.4 min), 1,2-ethanediol (R_T 15.7 min), 1,3-butanediol (R_T 18.1 min), and 1,3-propanediol (R_T 21.8 min) were produced from fractions 1, 2a, 3a, 3b, and 3c and were identified by comparison of their retention times (R_T) with those of authentic standards. Definitive structural assignments were based on mass spectral data obtained by glc–ms.

Under electron impact, C_{16}-acetal of ethanediol (Figure 2a) produces ion $[M - C_{15}H_{31}]^+$ at *m/e* 73 in extremely high abundance. The stability of such cyclic, resonance-stabilized 1,3-dioxolane ions (Baumann *et al.*, 1973) is also reflected in the prominence of $[M - H]^+$ at *m/e* 283. Both dioxolane ions, as well as $[M - C_{15}H_{31}CO]^+$ at *m/e* 45, are of diagnostic significance, as they contain the intact diol moiety. Corresponding ions are formed with similar intensities from the C_{16}-acetals of 1,2-propanediol (Figure 2b), 1,3-propanediol (Figure 2c), and 1,3-butanediol (Figure 2d). The spectra of the diol acetals derived from the natural lipid fractions were in excellent agreement with those of the synthetic standards.

These data clearly demonstrate that diol lipids are not associated only with the nonpolar lipid fraction (Bergelson *et al.*, 1966), the diol PC fraction (Bergelson *et al.*, 1972), and the glycerol PE fraction (Vaver *et al.*, 1967) of rat liver, but are also constituents of hydroxylipids and phosphorylethanolamines. The variety of diols functioning as lipid backbones is also greater than has been realized previously, although we cannot confirm the occurrence of lipid-bound 1,2- and 1,4-butanediols (Bergelson *et al.*, 1966, 1972).

The amounts of diol lipids associated with individual lipid fractions from rat liver are given in Table I. These data have been corrected for diol lipid losses that occurred during lipid isolation and analysis through use of internal perdeuterated ethanediol lipid standards. The internal standard was quantified by glc and ms as 2-pentadecyl-1,3-[4,4,5,5-^{2}H]dioxolane, based on the ratio of ions *m/e* 73 and *m/e* 77 produced from nondeuterated and deuterated ethanediol acetal species, respectively. The prominence of ions $[M - C_{15}H_{31}]^+$ facilitated quantitations of diol lipids with the aid of deuterated internal standards. Corrections were also made for potential diol contamination, as determined through simultaneous control analyses on reagents only.

It is evident from Table I that diol lipids derived from 1,2-ethanediol (228.2 μg/g of total lipids) are the most abundant representatives occurring at particularly high levels in the nonpolar lipid fraction (72.7 μg/g) and in the diol PC fraction (78.2 μg/g). Smaller quantities of neutral and polar lipids bearing 1,2-propanediol (50.6 μg/g), 1,3-propanediol (33.3 μg/g), and 1,3-butanediol (33.6 μg/g) as backbones are present in rat liver as well. At this point, assignments of diol lipids are solely based on chromatographic properties, as compared to the respective synthetic diol lipid standards, and on the fact that the diol lipids are cleaved by phospholipase C and/or methanolysis. It appears reasonable to assume that they occur as long-chain esters and alk-1-enyl ethers. Definitive structural correlations must await the isoation of intact diol lipids by gel permeation chromatography (Calderon and Baumann, 1970a,b), gas chromatography, and by other means.

It was the purpose of the present investigation to develop a general scheme for the reliable identification and accurate quantitation of diol lipids, and thus to provide the means and basis for studying diol lipid metabolism and function. Our data clearly show that 1,2-ethanediol, 1,2-propanediol, 1,3-propanediol, and 1,3-butanediol form the polyol backbone of a variety of neutral lipids and phospholipids of rat liver. These diol lipids occur at relatively low levels in normal mammalian tissue, a fact that can be rationalized on the basis of the strong biological activity of diol-derived phosphatidylcholine and phosphatidylserine analogs.

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Phase Separations in Phospholipid Membranes†

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ABSTRACT: Phase diagrams representing lateral phase separations in the plane of lipid bilayer membranes have been determined for binary mixtures containing dielaidoylphosphatidylcholine together with dimyristoylphosphatidylcholine, dipalmitoylphosphatidylcholine, distearoylphosphatidylcholine, dioleoylphosphatidylcholine, and dipalmitoylphosphatidylethanolamine. The phase diagrams were deduced from observations of the temperature dependence of the paramagnetic resonance spectra of low concentrations of spin-labels incorporated in these bilayer membranes. In one case, the binary mixture of dipalmitoylphosphatidylethanolamine and dielaidoylphosphatidylcholine, evidence has been obtained for fluid–fluid immiscibility, in specified temperature and composition ranges. This immiscibility could give a lateral phase separation into fluid domains in the plane of the membrane, and/or a transverse phase separation into an asymmetrical bilayer membrane, and/or possibly discontinuous bilayer membranes of different composition. An asymmetrical bilayer membrane can be expected on theoretical grounds to form a nonplanar membrane.

A large number of experimental studies of thermally induced fluid ⇌ gel phase transitions in phospholipid bilayers have been carried out, using a variety of physical techniques. For leading references to the earlier literature, see Shimshick and McConnell (1973a,b). Theoretical (statistical mechanical) studies of these transitions have also been made (Nagle, 1973). Recently, Shimshick and McConnell (1973a,b) have used spin-labels to derive phase diagrams for a number of binary mixtures of lipids in excess water. These diagrams represent lateral phase separations of lipids into domains of differing composition and fluidity. For certain binary mixtures of phosphatidylcholines the sizes and proportions of fluid and solid domains can be visualized directly using freeze fracture electron microscopy; the relative proportions of fluid and solid domains can be accounted for by the phase diagrams obtained from paramagnetic resonance data (Grant *et al.*, 1974a). This visualization can be facilitated by the incorporation of certain intrinsic membrane proteins into the binary lipid mixtures, since some of these proteins associate preferentially with fluid (F) rather than solid (S) lipid domains[1] (Kleemann *et al.*, 1974; Grant and McConnell, 1974b; Chen and Hubbell, 1973).

Aside from their inherent interest, phase diagrams for binary lipid mixtures are of biophysical and biochemical interest for at least three reasons. (i) In special fatty acid auxotrophs of *Escherichia coli* the fatty acid composition of the membranes can approximate that of a binary mixture of lipids; lateral phase separations in these membranes can be detected using spin-labels, and freeze-fracture electron microscopy, and can be correlated with the temperature dependence of the rates of sugar uptake into these cells (Linden *et al.*, 1973; Kleemann and McConnell, 1974). (ii) In view of the marked effect of solid phase–fluid phase equilib-

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[1] Abbreviations used are: F-phase lipids, a pure lipid, or a mixture of lipids, in the "fluid" state where there is a relatively high rate of lateral diffusion, and a high binding affinity for small hydrophobic molecules; S-phase lipids, a pure lipid, or a mixture of lipids, in the "solid" state where there is a relatively low rate of lateral diffusion, and a low binding affinity for small hydrophobic molecules; Tempo, 2,2,6,6-tetramethylpiperidine-1-oxyl; DPPC, *O*-(1,2-dipalmitoyl-*sn*-glycero-3-phosphoryl)choline; DPPE, *O*-(1,2-dipalmitoyl-*sn*-glycero-3-phosphoryl)ethanolamine; DMPC, *O*-(1,2-dimyristoyl-*sn*-glycero-3-phosphoryl)choline; DSPC, *O*-(1,2-distearoyl-*sn*-glycero-3-phosphoryl)-choline; DEPC, *O*-(1,2-dielaidoyl-*sn*-glycero-3-phosphoryl)choline; DOPC, *O*-(1,2-dioleoyl-*sn*-glycero-3-phosphoryl)choline.