Journal of Chromatography, 526 (1990) 303–318
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 5124

Simple method for the analysis of glycerol enol ethers derived from plasmalogens in complex lipid mixtures and subsequent determination of the aldehydic components by gas chromatography–mass spectrometry

WALTER KNÖRR and GERHARD SPITELLER*

Lehrstuhl für Organische Chemie I der Universitat Bayreuth, Postfach 101251, Universitätsstrasse 30, D-8580 Bayreuth (F.R G.)

(First received August 17th, 1989; revised manuscript received November 17th, 1989)

SUMMARY

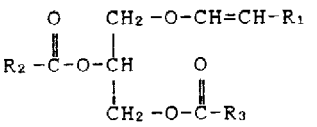
Glycerol enol ethers, obtained by the reduction of plasmalogens with lithium aluminum hydride, can be converted into glycerol alk-(1)-enyl ether bismethyl ethers with diazomethane in the presence of silica gel. Their mass spectra allow the position of the enol ether group in the glycerol unit to be deduced. Branches in the aldehydic components of the glycerol alk-(1)-enyl ether bismethyl ethers can be identified unequivocally by preparation of the 2-alkyl-1,3-dithiolanes, desulphurization with Raney nickel and deuterium to hydrocarbons and subsequent analysis by gas chromatography–mass spectrometry.

INTRODUCTION

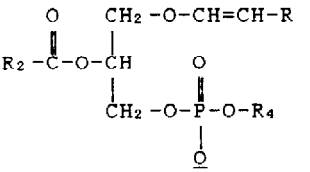
Plasmalogens **1a** and **1b** (Fig. 1) are lipids containing a fatty aldehyde bound as enol ether in position 1 to glycerol. They occur in all mammalian tissues and body fluids. Their high content in heart and brain [1–10] suggests that they play an important role in the structure [11] and function [12,13] of biological membranes. Their influence on regulation of elasticity [14,15] and permeability [16] of biological membranes has been examined in earlier studies.

The position of the aldehyde bound to the glycerol backbone of plasmalogens can be determined by lipid degradation with lithium aluminum hydride

0378-4347/90/$03.50



1a



1b

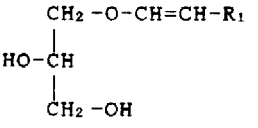
R_1, R_2, R_3 = alkyl

R_4 = ethanolamine, ch

Fig 1 General structure of plasmalogens.

PLASMALOGENS

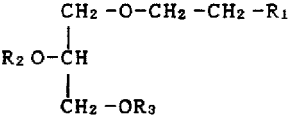
$LiAlH_4$



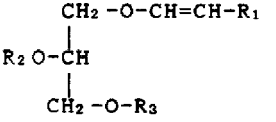
2

1) hydrogenation
2) derivatisation

1) derivatisation



7



3, 4, 5, 6

R_1	=	alkyl	
R_2, R_3	=	$-CH_3$	7/3
R_2, R_3	=	$-C(=O)-CH_3$	7/4
R_2, R_3	=	$-Si(CH_3)_3$	7/5
$R_2 + R_3$	=	$C(CH_3)_2$	7/6

Scheme 1.

($LiAlH_4$) to glycerol enol ethers (**2**) (Scheme 1) [17]. These are analysed either directly by gas chromatography–mass spectrometry (GC–MS) [18] or after derivatization. Various derivatization procedures have been used: acetic anhydride to diacetates (**4**) [17,19]; MSTFA (trimethylsilylation) to bistrimethylsilyl ethers (**5**) [19,20]; acetone to isopropylidene derivatives (**6**) [19,21]; hydrogenation to alkylglycerol derivatives (**7**) [20,22–27].

All these procedures suffer from a relatively low sensitivity for plasmalogen detection: although acetates (**4**) are separable by thin-layer chromatography (TLC), their mass spectra provide little information on their structure [19]. Trimethylsilyl (TMS) ethers (**5**) show informative mass spectra [19,20], but they decompose during the separation on TLC plates. The preparation of isopropylidene derivatives (**6**) from lyso compounds (**2**) in the presence of enol ether groups [21] is problematic [19]. The derivatives **4**, **5** and **6** all exhibit very long retention times on GC columns.

This paper describes the preparation of bismethyl ether derivatives **3**, which do not show these disadvantages. Since branched aldehydes are very abundant in plasmalogens [28–38], we developed a new method for the unequivocal determination of the branches, which was previously very difficult. Determination was usually achieved by comparison of refractive index (RI) values of synthetic aldehyde derivatives with those of authentic natural materials [28–38] on GC columns. Unfortunately, an MS identification of the branching sites in aldehydes and aldehyde derivatives was not possible. Consequently we converted the bismethyl ether derivatives (**3**) into dithiolanes (**8**), which were subsequently reduced to deuterated hydrocarbons (**11**) (Scheme 4). Their mass spectra allowed an unambiguous determination of the branching positions.

EXPERIMENTAL

Lipid extraction

Fresh bovine liver (100 g) was homogenized in 150 ml of methanol with 50 mg of bis-*tert.*-butylhydroxytoluene (BHT) and 100 mg of ethylenediaminetetraacetate (EDTA) in a precooled (−20°C) Braun M-32 mixer. After addition of 300 ml of dichloromethane, two layers were obtained and separated. The extraction procedure was repeated three times with 300 ml of the extraction medium dichloromethane–methanol (2:1, v/v). The combined organic layers were concentrated at 20°C using rotary evaporation, and the wet lipid residue was freeze-dried in the dark.

$LiAlH_4$ degradation of the lipid [17]

Freeze-dried lipid (7–8 g) was dissolved in 60 ml of dry benzene (for quantification purposes a synthetic plasmalogen standard was added). The lipid solution was slowly added to a suspension of 3 g of $LiAlH_4$ in 200 ml of diethyl ether. After stirring at room temperature for 3 h under nitrogen, 10 ml of sat-

urated aqueous sodium carbonate solution were added and the reaction mixture was stirred for 5 min. Subsequently, the organic layer was separated and the aqueous layer extracted three times with 30 ml of diethyl ether. The combined ether layers were carefully concentrated to dryness.

Chromatography of the reduction product

Depending on the sample size, the fatty alcohol contamination can be removed either by preparative TLC or by column chromatography with silica gel as the stationary phase and cyclohexane–ethyl acetate (1:1, v/v) as the eluent.

Preparative TLC

The separation was performed on laboratory-made silica gel TLC plates (20 cm × 20 cm, layer thickness 1 mm). Each plate may be loaded with up to 80 mg without impairing the separation quality. After development, the silica gel zone corresponding to the R_F values 0.1–0.7 was scraped off and eluted with ethyl acetate.

Column chromatography

Separation of the fatty alcohols from the glycerol enol ethers (**2**) (Scheme 1) was achieved by passing them through columns with a silica gel/substance ratio of 30:1. After elution of the fatty alcohols (TLC control) the polar fractions were eluted with ethyl acetate.

Methylation of the lysoplasmalogens according to the method of Ohno et al. [39]

The fraction eluted with ethyl acetate (ca. 100 mg) was evaporated to dryness. The residue was dissolved in 5 ml of benzene and adsorbed on silica gel [at least a ten-fold amount (weight) of silica gel was required]. Freshly prepared ethereal diazomethane solution was added under vigorous stirring until the yellow colour remained. This reaction mixture was stirred for 40 h under nitrogen. If necessary, an additional amount of diazomethane solution had to be added. Subsequently, excess diazomethane was removed under a stream of nitrogen, and the silica gel was filtered off and washed with diethyl ether. The combined organic layers were carefully evaporated to dryness.

Preparative TLC of the methylated samples

The samples were dissolved in benzene and deposited on preparative silica gel TLC plates. These were developed with cyclohexane–ethyl acetate (7:1, v/v). The glycerol enol ether bismethyl ethers (GEEBMEs) (**3**) were detected by spraying the plate margin with 10% methanolic phosphomolybdic acid solution and heating with warm air. The enol ethers appeared as dark spots at an R_F value of 0.69. Isolation of the relevant silica gel layer and elution with diethyl ether provided the GEEBMEs in high purity.

Preparation of dithioethyleneglycol acetals (DTEGAs) of fatty aldehydes from GEEBMEs

A 50-μl volume (ca. 50 μg) of the GEEBME GC sample (benzene solution) was treated with 2 μl of 1,2-dimercaptoethane and one drop of a 5% ethereal boron trifluoride solution for 2 h at room temperature. The reaction was stopped by addition of one drop of 10% methanolic KOH solution. The solvent and the excess 1,2-dimercaptoethane were removed by a stream of nitrogen, and the residue was extracted with diethyl ether.

Preparation of deuterated Raney nickel (RaNi) according to Protiva et al. [40]

To a suspension of 200 mg of Ni/Al alloy (50% Ni) in 2 ml of ^2H_2O, 2 ml of 40% sodium deuterooxide in ^2H_2O were added under stirring. When gas evolution ceased and the nickel sponge had precipitated, the liquid was decanted and the solid residue was washed twice with 3 ml of [^{2}H-hydroxy]ethanol. The Raney nickel (RaNi) was used as a suspension in [^{2}H-hydroxy]ethanol.

Desulphurization of DTEGAs [40]

The dry DTEGA sample was dissolved in 10 ml of [^{2}H-hydroxy]ethanol and stirred with freshly prepared deuterated RaNi for 2 h at 70 °C and subsequently overnight at room temperature. Filtration with ethyl acetate through 500 mg of silica gel 60 and very careful removal of the solvent (to prevent loss of volatile hydrocarbons) by a stream of nitrogen provided the fatty aldehyde corresponding mono- and dideuterohydrocarbons (**11**) in GC quality (Scheme 4).

*Synthesis of 1-O-(dec-1-enyl)-glycerol-2,3-diacetate as a standard for quantification of bovine liver plasmalogens (**9a/b, 9c/d**; Fig. 2)*

Decanal (0.1 mol, 15.6 g) was refluxed overnight with 50 ml of glycerol-1,3-diacetate and 5 g of *p*-toluenesulphonic acid in 100 ml of benzene [19]. The reaction mixture was extracted with 30 ml of a 10% aqueous $KHCO_3$ solution and, after evaporation of the organic layer, the residue was chromatographed on silica gel. The isomer mixture (13.6 g, 50 mmol) of acetylated glycerol acetals with 2-alkyl-4-hydroxymethyl-1,3-dioxolane and 2-alkyl-5-hydroxy-1,3-dioxane structures was treated at room temperature for five days with freshly distilled acetyl chloride [41]. Excess acetyl chloride was removed under water exclusion by rotary evaporation, and the residue was refluxed for 30 min with absolute triethylamine [41]. The residue obtained after evaporation of the solvent was filtered over 20 g of silica gel with diethyl ether and chromatographed on silica gel with an eluent of cyclohexane–ethyl acetate (4:1, v/v). The yield was 86% (27 g, 0.09 mol).

The pairs of geometric isomers and positional isomers (GC control of the fractions) could be separated in the following elution sequence: 1-O-*trans*-(dec-1-enyl)-glycerol-2,3-diacetate (1-O-*trans*-DG-2,3-DA) (**9a**) 1-O-*cis*-(dec-1-enyl)-glycerol-2,3-diacetate (1-O-*cis*-DG-2,3-DA) (**9b**), 2-O-*trans*-

(dec-1-enyl)-glycerol-1,3-diacetate (2-O-*trans*-DG-1,3-DA) (**9c**), 2-O-*cis*-(dec-1-enyl)-glycerol-1,3-diacetate (2-O-*cis*-DG-1,3-DA) (**9d**).

Proton nuclear magnetic resonance (^{1}H NMR) data were obtained for the four compounds **9a–d** in deuterochloroform.

1-O-*cis*-DG-2,3-DA (**9b**): 0.88 (t; J=6.7 Hz; 3H), 1.21–1.37 (m; 12H), 2.0–2.06 (m; 2H), 2.07 (s; 3H), 2.09 (s; 3H), 3.84 (dd; J_1=0.7 Hz; J_2=5.3 Hz; 2H), 4.17 (dd; J=6.0 Hz; J_2=12.0 Hz; 1H), 4.34 (dd; J_1=3.9 Hz; J_2=12.0 Hz; 1H), 4.40 (dt; J_1=6.1 Hz; J_2=7.4 Hz; 1H), 5.19 (dt; J_1=5.4 Hz; J_2=6.1 Hz; 1H), 5.89 (dt; J_1=1.4 Hz; J_2=6.1 Hz; 1H).

1-O-*trans*-DG-2,3-DA (**9a**): 0.88 (t; J=6.7 Hz; 3H), 1.21–1.38 (m; 1.86–1.94 (m; 2H), 2.08 (s; 3H), 2.10 (s; 3H), 3.78 (d; J=5.1 Hz; 2H), 4.18 (dd; J_1=6.1 Hz; J_2=12.0 Hz; 1H), 4.34 (dd; J_1=3.9 Hz; J_2=12.0 Hz; 1H), 4.79 (dt; J_1=7.3 Hz; J_2=12.6 Hz; 1H), 5.20–5.28 (m; 1H), 6.21 (dt; J_1=2.7 Hz; J_2=12.6 Hz; 1H).

2-O-*cis*-DG-1,3-DA (**9d**): 0.87 (t; J=6.7 Hz; 3H), 1.21–1.38 (m; 12H), 1.98–2.06 (m; 2H), 2.07 (s; 6H), 3.98 (tt; J_1=J_2=5.4 Hz; 1H), 4.17 (d; J=5.4 Hz; 4H), 4.43 (dt; J_1=6.2 Hz, J_2=6.7 Hz; 1H), 5.96 (dt; J_1=1.4 Hz; J_2=6.2 Hz; 1H).

2-O-*trans*-DG-1,3-DA (**9c**): 0.87 (t; J=6.7 Hz; 3H), 1.22–1.37 (m; 12H),

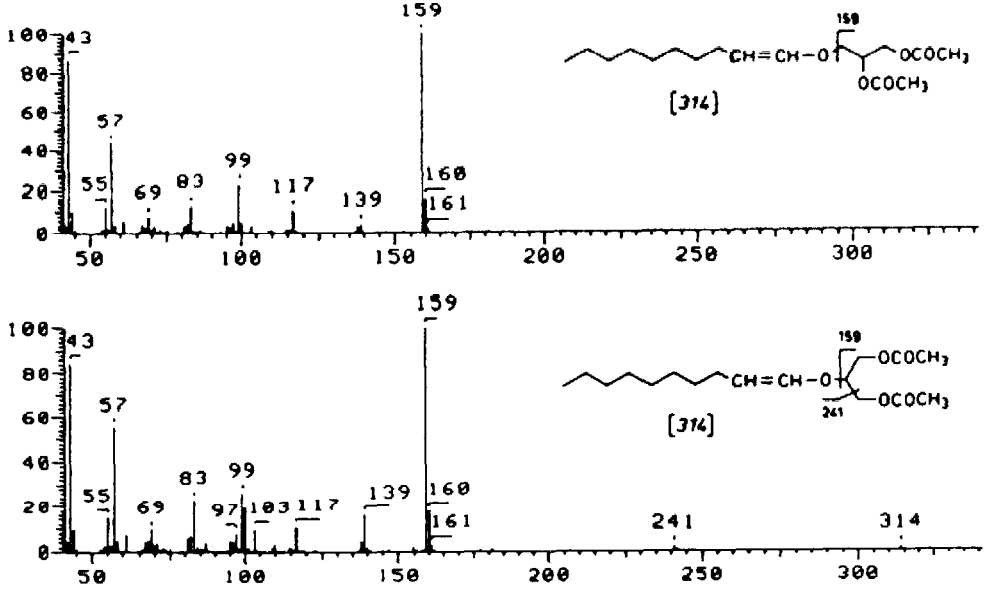


Fig. 2. EI mass spectra of 1-O-(dec-1-enyl)glycerol-2,3-diacetate (**9a/b**) (top) and 2-O-(dec-1-enyl)glycerol-1,3-diacetate (**9c/d**) (bottom).

1.85–1.93 (m; 2H), 2.08 (s; 6H), 4.05–4.10 (m; 1H), 4.17–4.21 (m; 4H), 4.99 (dt; $J_1 = 7.4$ Hz, $J_2 = 12.3$ Hz, 1H), 6.10 (dt; $J_1 = 1.4$ Hz; $J_2 = 12.3$ Hz; 1H).

Mass spectra of the pairs **9a/b** and **9c/d** are shown in Fig. 2.

*Synthesis of 1-O-(dodec-1-enyl)glycerol-2,3-bismethyl ethers (**10a/b**) and 2-O-(dodec-1-enyl)glycerol-1,3-bismethyl ethers (**10c/d**) as reference compounds for MS characterization of bismethyl ether derivatives (**3**) of native origin (Fig. 3)*

Synthesis of glycerol-2,3-bismethyl ether by a modified version of the method of Mazza and Malaguzzi-Valeri [42]. Acrylic acid (0.5 mol, 36 g) in 100 ml of methanol was added dropwise to a cooled solution (0°C) of 27 g (0.5 mol) of sodium methoxide in 500 ml of methanol. Subsequently, 80 g (0.5 mol) of bromine in 100 ml of methanol was added at room temperature over a period of 1 h. The reaction mixture was stirred for an additional hour. Then, 54 g (1 mol) of sodium methoxide in 200 ml of methanol was added under cooling (0°C). The mixture was refluxed overnight, then acidified with methanolic HCl solution to pH 1 and refluxed for 3 h with 53 g (0.5 mol) of trimethyl orthoformate. The methanol was removed by rotary evaporation and the residue, dissolved in diethyl ether, was filtered and concentrated. Distillation under reduced pressure yielded 46 g (0.31 mol) of methyl-2,3-dimethoxypropionate (b.p. 70°C at 12 Torr). Reduction with $LiAlH_4$ in diethyl ether provided 33.6 g (0.28 mol) of glycerol-2,3-bismethyl ether (G-2,3-BME) (b.p. 68°C at 12 Torr), a yield of 56% (33.6 g, 0.28 mol).

Synthesis of glycerol-1,3-bismethyl ether (G-1,3-BME) [43]. 1,3-Dichloro-2-propanol (0.2 mol, 25.8 g) in 100 ml of methanol was added dropwise to a solution of 24.3 g (0.45 mol) of sodium methoxide in 300 ml of methanol. The reaction mixture was refluxed overnight. The residue obtained after rotary evaporation of the methanol was dissolved in diethyl ether and, after removal of the salts by filtration, distilled under reduced pressure (b.p. 65°C at 12 Torr). The yield was 83% (20 g, 0.17 mol).

*Synthesis of the enol ethers **10a/b** and **10c/d** according to the method of Gigg and Gigg [44].* Dodecanal (50 mmol, 0.9 g) in 100 ml of benzene was refluxed overnight with 100 mg of *p*-toluenesulphonic acid and 20 ml of glycerolbismethyl ether (either G-2,3-BME or G-1,3-BME), using a water separator. The reaction was stopped with 0.5 ml of 10% methanolic KOH solution, and the solvent was removed by rotary evaporation. Excess glycerolbismethyl ether was distilled off. The evaporation residue was dissolved in diethyl ether, filtered and subsequently chromatographed on silica gel with an eluent of cyclohexane–ethyl acetate (2:1, v/v). The product was treated with 50 ml of freshly distilled acetyl chloride [41] under water exclusion for 60 h at room temperature. Excess acetyl chloride was subsequently removed under anhydrous conditions by rotary evaporation, and the residue was stirred under reflux for 30 min with 50 ml of anhydrous triethylamine [41]. The triethylamine was removed by rotary evaporation, and the residue was taken up in 20 ml of 10% aqueous sodium carbonate solution and extracted three times with 30 ml

of diethyl ether. After evaporation of the combined and dried (over Na_2SO_4) ether layers, the residue was chromatographed on silica gel with an eluent of cyclohexane–ethyl acetate (4:1, v/v).

The yield of 1-O-*cis*-(dodec-1-enyl)glycerol-2,3-bismethyl ether was 42% (6.0 g, 21 mmol), and the ^{1}H NMR data for the *cis–trans* mixture (in deuterochloroform) were: 0.88 (t; J=6.9 Hz; 3H), 1.13–1.46 (m; 16H), 1.86–1.94 (m; 2H), 3.38 (s; 3H), 3.48 (s; 3H), 3.43–3.56 (m; 3H), 3.73/3.80 (t; J=4.9 Hz; 2H), 4.36(*cis*)/4.79(*trans*) (dt; $J_{1cis} = J_{1trans} = 7.2$ Hz; $J_{2cis} = 7.1$ Hz; $J_{2trans} = 12.3$ Hz; 1H), 6.94(*cis*)/6.24(*trans*) (dt; $J_{1cis} = J_{1trans} = 3$ Hz; $J_{2cis} = 7.1$ Hz; $J_{2trans} = 12.3$ Hz; 1H).

The yield of 2-O-*cis*-(dodec-1-enyl)glycerol-1,3-bismethyl ether was 31% (4.4 g, 15.5 mmol), and the ^{1}H NMR data for the *cis–trans* mixture were: 0.88 (t; J=6.7 Hz; 3H), 1.13–1.46 (m; 16H), 1.85–1.94 (m; 2H), 3.37 (s; 6H), 3.51 (d; J=4.8 Hz; 4H), 3.86(*cis*)/3.93(*trans*) (tt; $J_1 = J_2 = 5.0$ Hz; 1H), 4.38(*cis*)/4.96(*trans*) (dt; $J_{1cis} = J_{2cis} = J_{1trans} = 6.2$ Hz; $J_{2trans} = 12.3$ Hz; 1H), 6.03(*cis*)/6.15(*trans*) (dt; $J_{1cis} = J_{1trans} = 1.4$ Hz; $J_{2cis} = 6.2$ Hz; $J_{2trans} = 12.3$ Hz; 1H).

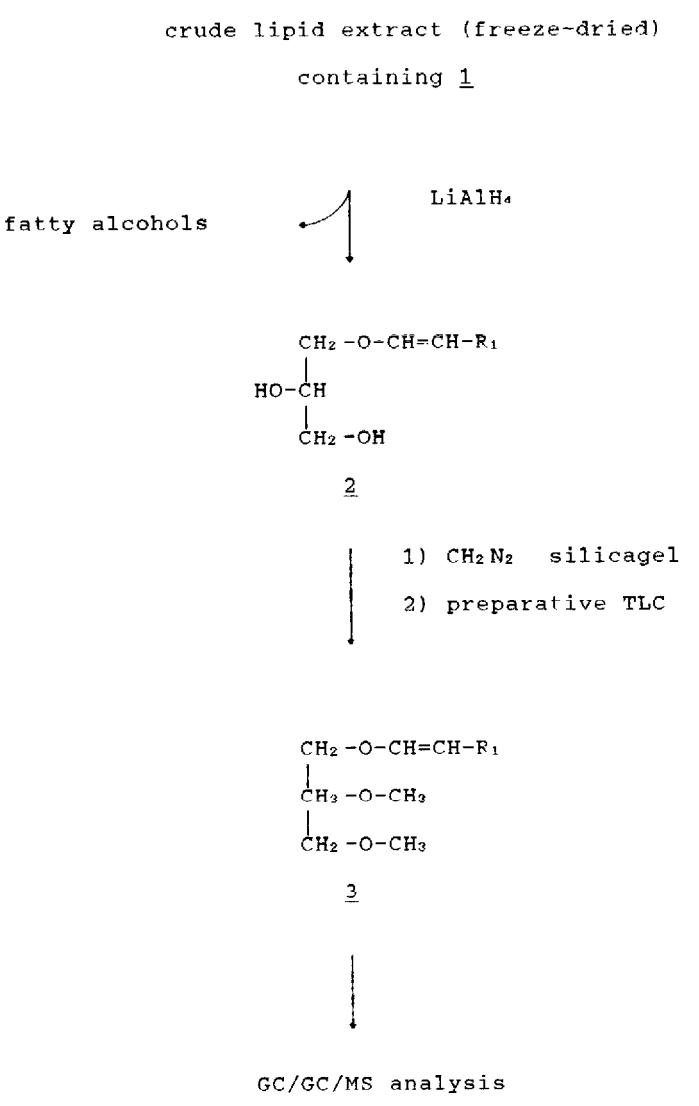
GC and GC–MS analysis

Gas chromatograms were obtained with a Packard 438 S gas chromatograph equipped with a flame ionization detector and a WCOT glass capillary column (30 m×0.3 mm I.D.) static coated [45] with OV 101. The carrier gas was hydrogen with a flow-rate of 0.5 kg/cm^2. The injector temperature was 270°C and the detector temperature 280°C. The temperature programme was 100°C for 3 min, rising to 280°C at 3°C/min. For calculating the Kovats indices [46] the hydrocarbon standards C_8, C_{10}, C_{12}, C_{14}, C_{16}, C_{18}, C_{20}, C_{22}, C_{24}, C_{26} were coinjected. The chromatograms were recorded with a Shimadzu C-R3A integrator.

Electron-impact (EI) mass spectra were obtained using a Varian 3700 gas chromatograph equipped with a WCOT glass capillary column (25 m×0.3 mm I.D.) static coated [45] with OV 101, and interfaced to a Varian MAT 312 mass spectrometer with MAT SS 300 data system. The ionization energy was 70 eV.

Work-up procedure

The freeze-dried lipid extract, obtained from bovine liver by the extraction method of Folch and co-workers [47,48], was degraded with $LiAlH_4$ to lysolipids (**2**) [17]. These compounds were extracted with diethyl ether and separated from large amounts of accompanying fatty alcohols by TLC or column chromatography (see above). The reaction with diazomethane in the presence of silica gel provided the corresponding bismethyl ethers (**3**). The lysoplasmalogen derivatives (**3**) were isolated in a second TLC step and analysed by GC–MS. The procedure is summarized in Scheme 2.



Scheme 2.

RESULTS AND DISCUSSION

Bismethyl ether derivatives of lysoplasmalogens (**3**) have shorter retention times than the other derivatives, **4**, **5**, and **6**. They do not decompose by separation on TLC plates and they are degraded in the mass spectrometer to structure-specific fragments. The only disadvantage is the long reaction time (40 h) required for methylation of lysoplasmalogens (**2**) with diazomethane.

Other methods of methylating hydroxyl groups, e.g. reaction with diazo-

methane–boron trifluoride [49] or with sodium methylsulphinylmethide (DMSO/CH_3I) [50], cannot be applied, since they cause a number of side-reactions. The alternative method, prior hydrogenation of the enol ethers to alkyl ethers [20,22–27], precludes a determination of the branching positions in the aldehydic moieties, because the alkyl ether linkage is much more stable to hydrolysis under comparable conditions.

A typical GC separation profile of plasmalogen derivatives (**3**) obtained from bovine liver is reproduced in Fig. 3.

Fig. 4 shows the EI mass spectrum of peak 10 from the gas chromatogram shown in Fig. 3. It corresponds to the lysoplasmalogen bismethyl ether derived from palmitic aldehyde.

Figs. 5 and 6 show the EI mass spectra of synthetic 1-O-*cis*-(dodec-1-enyl)glycerol-2,3-bismethyl ether **10a** and 2-O-*cis*-(dodec-1-enyl)glycerol-1,3-

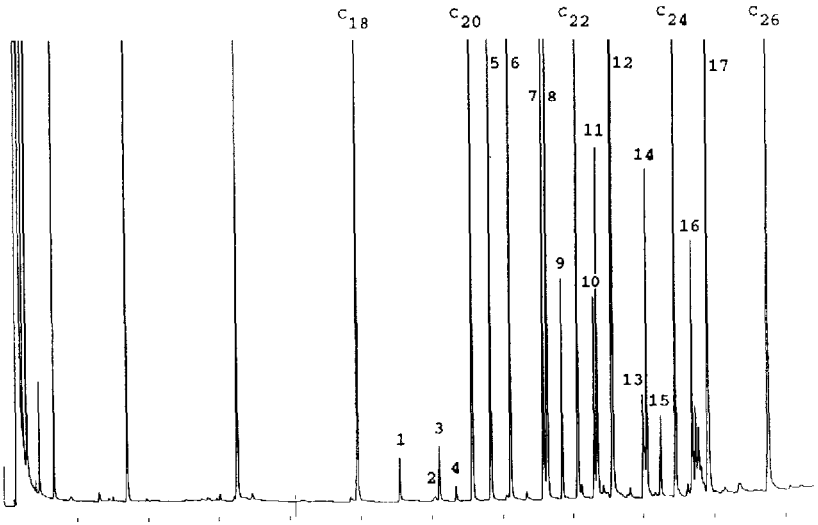


Fig. 3 GC separation profile of bovine liver plasmalogen derivatives (**3**).

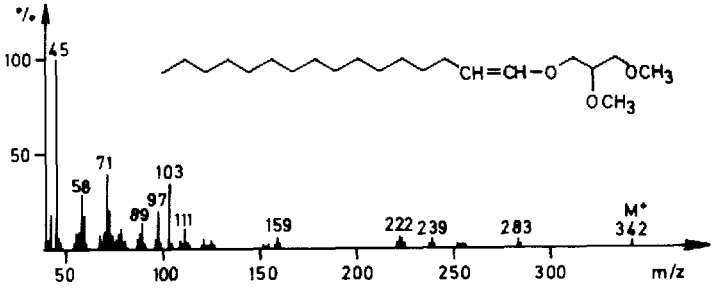


Fig 4. EI mass spectrum of peak 10 in Fig 3.

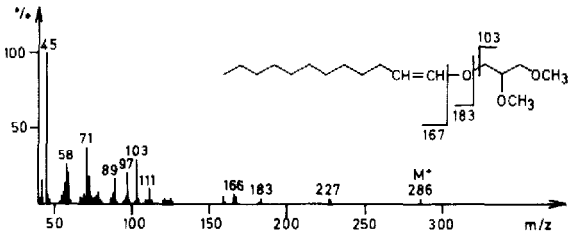


Fig. 5. EI mass spectrum of 1-O-*cis*-(dodec-1-enyl)glycerol-2,3-bismethyl ether (**10a**).

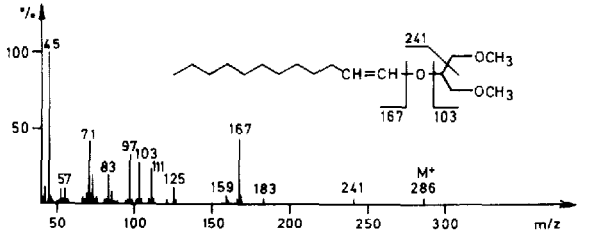
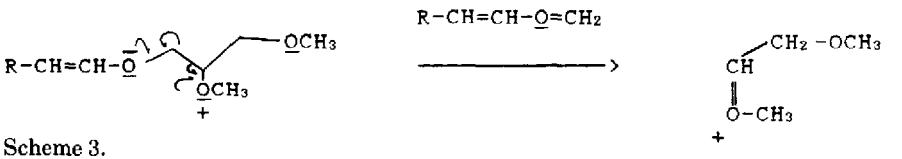


Fig. 6. EI mass spectrum of 2-O-*cis*-(dodec-1-enyl)glycerol-1,3-bismethyl ether (**10c**).

bismethyl ether **10c**. The mass spectra of the isomeric *trans* compounds (**10b**, **10d**) are identical with those of the *cis* isomers [19].

Studies on these model compounds demonstrated that 2-O-(alk-1-enyl)glycerol-2,3-bismethyl ethers are characterized by an intensive M−119 ion. This ion corresponds to the typical fragmentation of ethers with a long alkyl chain [51], and indicates the formal elimination of a 1,3-dimethoxypropane-2-oxy fragment. In fact such reactions require the migration of one or two hydrogens from the glycerol part to the ether oxygen, which is lost either together with the glycerol unit or as H_2O. The analogous fragmentation in 1-O-(alk-1-enyl)glycerol-1,3-bismethyl ethers is far less favoured. The alternative cleavage, migration of two hydrogens from the aldehydic part to the ether oxygen and loss of water, produces the other typical ion for alk-1-enylglycerol bismethyl ethers, which is a dimethoxypropyl fragment of mass 103. It decomposes to an ion of mass 71 by elimination of methanol. The base peak in the mass spectra of both enol ether isomers is the ion of mass 45, $CH_2{=}\overset{+}{O}CH_3$ (Figs. 5 and 6).

The 1-O-alkenyl compounds may produce an ion of mass 89 ($CH_3OCH_2CH{=}\overset{+}{O}CH_3$) by direct cleavage as shown in Scheme 3, which is absent in the spectra of the 2-O-isomers.



Scheme 3.

Typical for the 1-O-alkenyl compounds is the presence of an intense double peak at mass 58/59. A further difference in the degradation pattern of both isomers is the loss of a $^{\bullet}CH_2$–OCH_3 radical, which occurs only in 2-alkenyl compounds.

In contrast, the mass spectra of 1-O-alkenylglycerol derivatives show an M – 59 ion, probably corresponding to a loss of a $CH_3O^{\bullet}$ radical followed by a rearrangement with a hydrogen transfer and further loss of ethylene. According to their characteristic mass spectra, all detectable lysoplasmalogen derivatives (**3**) of natural origin were found to be 1-O-(alk-1-enyl)glycerol-2,3-bismethyl ethers, in agreement with earlier investigations [52–57].

The stereoisomers can be unequivocally identified in the GC profiles by correlation of the integrated heights with those in the ^1H NMR spectrum of the synthetic mixture of *cis* and *trans* isomers of O-(dodec-1-enyl)glycerolbismethyl ether (**10a/b** and **10c/d**): 1-O-derivatives: *cis* isomer **10a**, RI 1873; *trans* isomer **10b**, RI 1940; 2-O-derivatives: *cis* isomer **10c**, RI 1857; *trans* isomer **10d**, RI 1915.

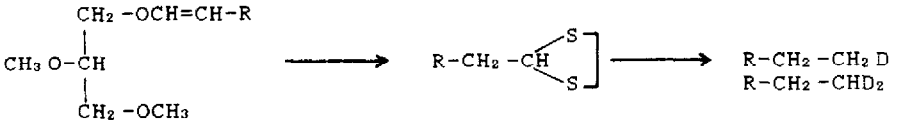
According to the RI values, obtained by extrapolation, the detected 1-O-(alk-1-enyl)glycerol-2,3-bismethyl ethers (**3**) derived from naturally occurring plasmalogens are *cis* isomers, as already shown in earlier investigations [57–59].

The structure elucidation of the aldehyde chain of plasmalogens was previously limited to the determination of GC retention times, requiring reference compounds [28,33]. EI-MS investigations of generated aldehyde derivatives are limited to the determination of the length of the aldehyde chains. Unfortunately the MS data usually do not allow a distinction between straight-chain and branched aldehydes [19].

Quantification of plasmalogens by release of the aldehydes, in most cases in form of dimethyl acetals, and subsequent GC analysis [60] are further impaired by incomplete conversion or recovery. Other quantification methods, e.g. photometric measurement [61–63] of the aldehyde content of plasmalogens by treating the carbonyl function with reagents containing a chromophore, e.g. reaction with *p*-nitrophenylhydrazine [64–66] or fuchsinsulphuric acid [67], or the enol ether-specific iodination method [68–71], suffer from interference by impurities [72–74] or poor reproducibility [75–78].

If the TLC fraction of the O-(alk-1-enyl)glycerolbismethyl ethers (**3**) is treated with 1,2-dimercaptoethane, the dithioethylglycol acetals (**8**) of the aldehydes are obtained. In the gas chromatograms the peaks of these derivatives are shifted 170 RI units to higher values compared with those of the O-(alk-1-enyl)glycerolbismethyl ethers (**3**). The 2-alkyl-1,3-dithiolanes (**8**) themselves are not suitable for a structure elucidation of the alkyl chain by MS, since ionization predominantly occurs at the sulphur atoms and thus typical ions are observed only for the unspecific dithiolane moiety, not for the hydrocarbon residue [19]. Only the number of carbon atoms can be deduced from

these mass spectra. The determination of the branching positions was achieved by preparation of the corresponding hydrocarbons (**11**), a method that we recently also used for the determination of branched fatty acids [79]. For that purpose the dithiolanes (**8**) were desulphurized with deuterated RaNi, as shown in Scheme 4. The original carbonyl function was converted into either a $^2HCH_2$



3 8 11

Scheme 4.

or a ^2H_2CH group. The generated deuterohydrocarbons **11** were investigated by GC–MS. All fragments containing deuterium atoms appear one or two mass units higher in the mass spectra.

In branched-chain hydrocarbons cleavage predominantly occurs at branching positions, which can be deduced from intense peaks in the mass spectra (Figs. 7 and 8).

The mass shift in the spectra of the deuterated compounds allowed an unambiguous location of the original aldehyde group.

This method is applicable even if these lipids occur only in trace amounts and even if they are accompanied by a variety of other fatty compounds present in large amounts.

The investigation of bovine liver plasmalogens proved the presence of a surprisingly high number of iso- and anteiso-branched compounds (Table I).

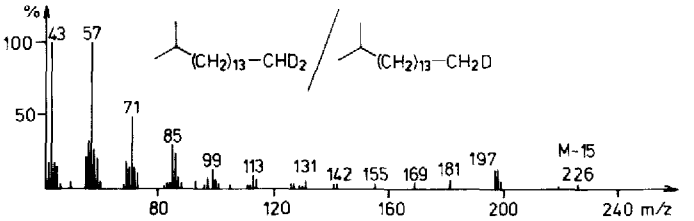


Fig. 7. EI mass spectrum of an iso C_{17}-deuterohydrocarbon of natural origin.

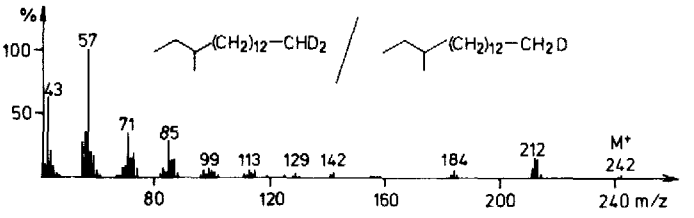


Fig. 8 EI mass spectrum of an anteiso C_{17}-deuterohydrocarbon of natural origin.

TABLE I

LIST OF THE 1-O-*cis*-(ALK-1-ENYL)-GLYCEROL-2,3-BISMETHYL ETHERS ISOLATED FROM BOVINE LIPID

Peak No	Alk-1-enyl ether chain	RI value	Amount per g lipid
1	n-12.0	1873	40 - 50 μg
2	iso-13·0	1934	5 - 15 μg
3	anteiso-13:0	1943	70 -160 μg
4	n-13:0	1972	20 - 70 μg
5	iso-14:0	2033	0 6 - 0.9 mg
6	n-14:0	2073	0.5 - 0.6 mg
7	iso-15:0	2134	0.5 - 0.9 mg
8	anteiso-15 0	2143	0.9 - 1.3 mg
9	n-15:0	2171	0 3 - 0.5 mg
10	iso-16·0	2234	0 2 - 0.3 mg
11	anteiso-16·0	2242	0.25- 0.3 mg
12	n-16 0	2271	2.2 - 2.3 mg
13	iso-17 0	2334	0.1 - 0.2 mg
14	anteiso-17 0	2343	0.3 - 0.35 mg
15	n-17 0	2371	0.3 - 0.12 mg
16	18.1	2437	0.2 - 0.3 mg
	18.1	2443	0.1 mg
	18·1	2450	60 - 70 μg
	18 1	2454	25 - 30 μg
17	n-18 0	2471	0.8 - 0.9 mg

As shown recently [80], vegetables and plants contain relatively large amounts of iso- and anteiso-branched fatty acids. It is reasonable to assume that these branched acids in plants are the source of the branched aldehyde derivatives [81–84] in bovine liver. Nevertheless, the high content of branched fatty aldehydes in bovine liver suggests an enrichment process compared with the unbranched aldehydes. Further studies are required to clarify this observation.

ACKNOWLEDGEMENTS

We thank the Deutsche Forschungsgemeinschaft and Fonds der Chemischen Industrie for financial support of this work. We are obliged to Michael Glaessner for running mass spectra and Dietrich Laatsch for providing the glass capillary columns.

REFERENCES

1 F. Leupold, Hoppe-Seyler's Z. Physiol. Chem., 285 (1950) 216.

2 H. Debusch, Hoppe Seyler's Z. Physiol. Chem , 311 (1958) 266.
3 E. Klenk, Hoppe Seyler's Z. Physiol. Chem., 282 (1945) 18.
4 T.W. Scott, B.P. Setchell and J.M. Bassett, Biochem. J., 104 (1967) 1040
5 R.M.C. Dawson, N. Hemington and J B.F. Davenport, Biochem. J , 64 (1962) 497.
6 E. Klenk, in D. Richter (Editor), Metabolism of the Nervous System, Pergamon Press, London, 1957, p 396.
7 J S. O'Brien, D.L. Fillerup and J.F. Mead, J. Lipid Res., 4 (1964) 329.
8 G.B. Ansell and J.N Hawthorne, Phospholipids - Chemistry Metabolism and Function, B.B.A Library, vol. 3, Elsevier, Amsterdam, 1964, p. 439.
9 G.B. Ansell and S. Spanner, Biochem. J., 108 (1968) 207.
10 K. Owens, Biochem. J., 100 (1966) 354.
11 L.A. Horrocks and M. Sharma, in J.N. Gawthorne and G.B Ansell (Editors), Phospholipids, Elsevier, Amsterdam, 1982, pp. 51-93.
12 L.A. Horrocks, in F Snyder (Editor), Ether Lipids: Chemistry and Biology, Academic Press, New York, 1972, pp. 172-272.
13 R.W. Gross, Biochemistry, 23 (1984) 158.
14 G.K. Khuller and H. Goldfine, J. Lipid Res., 15 (1974) 500.
15 B.I. Roots and P.V. Johnston, Comp. Biochem Physiol , 26 (1968) 553.
16 J.K. Selkirk, J.C Elwood and H.P. Morris, Cancer Res., 31 (1971) 27.
17 H.H.O. Schmid, W.J. Baumann and H.K. Mangold, Biochim. Biophys Acta, 144 (1967) 344.
18 J.C. Craig and D.P.G. Hamon, J. Org. Chem., 30 (1965) 4168.
19 W. Knörr, dissertation, Universität Bayreuth, Bayreuth, 1989.
20 J.J. Myher, L. Marai and A. Kuksis, J. Lipid Res., 15 (1974) 586
21 G Guella, I. Manchini and F. Pietra, Helv Chim. Acta, 70 (1987) 1050.
22 B. Hallgren and S. Larsson, J. Lipid Res., 3 (1962) 31.
23 T. Muramatsu and H.H.O. Schmid, Chem. Phys. Lipids, 9 (1972) 123.
24 B. Hallgren and G. Ställberg, Acta Chem. Scand., 21 (1967) 1519.
25 B. Hallgren, A. Niklasson, G. Stallberg and H. Thorin, Acta Chem. Scand., B28 (1974) 1035.
26 H.W. Mueller, J.T. O'Flaherty and R.L. Wykle, Lipids, 17 (1982) 72.
27 H.H.O. Schmid and H K. Mangold, Biochem. Z., 346 (1966) 13.
28 F. Spener and H.K. Mangold, J. Lipid Res., 10 (1969) 609.
29 I. Katz and M. Keeney, Biochim. Biophys. Acta, 84 (1964) 128.
30 H. Goldfine, J. Biol. Chem., 239 (1964) 2130.
31 H. Goldfine and C. Panos, J. Lipid Res., 12 (1971) 214.
32 J.W Farquahar, J. Lipid Res., 3 (1962) 21.
33 J.F. Radloff and W.J. Ferrell, Physiol. Chem. Phys., 2 (1970) 105.
34 J.C.M. Schogt, P. Haverkamp Begemann and J.H. Recourt, J. Lipid Res., 2 (1961) 142.
35 G.M. Gray, J. Chromatogr , 4 (1960) 52.
36 H.H.O. Schmid and T. Takahashi, Biochim. Biophys. Acta, 164 (1968) 141
37 J.R. Gilbertson, W.J. Ferrell and R.A. Gelman, J. Lipid Res., 8 (1967) 38.
38 G.M. Gray and M.G. MacFarlane, Biochem. J., 81 (1961) 480.
39 K. Ohno, H. Nishiyama and H. Nagase, Tetrahedron Lett., 45 (1979) 4405.
40 J. Protiva, R. Ocadlik, E. Klinotová, J. Klinot and A. Vystrcil, Coll Czech Chem Commun., 52 (1987) 501.
41 J. Cunningham and R. Gigg, J. Chem. Soc., 2 (1965) 2968.
42 F.P. Mazza and C. Malaguzzi-Valeri, Arch. Sci Biol., 25 (1939) 271.
43 H.R. Henze and B.G. Rogers, J. Am. Chem. Soc., 61 (1939) 433.
44 J. Gigg and R. Gigg, J. Chem. Soc C, 1 (1968) 16.
45 A Gossauer, F. Rössler and H. Zilch, Liebigs Ann. Chem., 9 (1979) 1309
46 E. Kovats, Helv. Chim. Acta, 41 (1958) 1915.

47 J. Folch, I. Ascoli, M. Lees, J A. Mearth and F.N. LeBaron, J. Biol. Chem., 191 (1951) 833.
48 J. Folch, M. Lees and G.H. Sloane Stanley, J. Biol. Chem., 226 (1957) 497
49 E. Müller and W. Rundel, Angew Chem., 70 (1958) 105
50 N. Hirai, D.G. Kingston, R.L Van Tassell and T.D. Wilkins, J. Nat. Prod., 48 (1985) 622
51 M. Spiteller and G. Spiteller, Massenspektrensammlung von Lösungsmitteln, Verunreinigungen, Säulenbelegmaterialien und einfachen aliphatischen Verbindungen, Springer-Verlag, Vienna, New York, 1973, pp 107–112.
52 G.V. Marinetti, J. Erbland and E. Stotz, J. Am. Chem. Soc., 80 (1958) 1624.
53 G.V. Marinetti, J. Erbland and E. Stotz, J. Am. Chem. Soc., 81 (1959) 861.
54 H. Debuch, Hoppe Seyler's Z. Physiol. Chem., 314 (1959) 49.
55 J. Eichberg, J.R. Gilbertson and M.L. Karnovsky, J. Biol. Chem., 236 (1961) PC15
56 J.R. Gilbertson and M.L. Karnovsky, J. Biol. Chem., 238 (1963) 893.
57 W.T. Norton, E.L. Gottfried and M.M. Rapport, J. Lipid Res , 3 (1962) 456.
58 H.R. Warner and W.E.M. Lands, J. Am. Chem. Soc., 85 (1963) 60.
59 J.C Craig and D.P.G. Hamon, J. Org. Chem., 30 (1965) 4168.
60 G.M. Gray, Biochem. J., 70 (1958) 425.
61 R Wood and F. Snyder, Lipids, 3 (1968) 129.
62 F. Snyder and R. Wood, Cancer Res., 28 (1968) 972.
63 S.S Levin, K.A. Snyder and J.C. Touchstone, in J.C. Touchstone and J. Sherma (Editors), Proceedings of the 3rd Biennial Symposium on Thin Layer Chromatography, 1982, Wiley, New York, 1985.
64 J.B. Wittenberg, S.R. Korey and F.H. Swenson, J. Biol. Chem., 219 (1956) 39.
65 I. Katz and M. Keeney, J. Lipid Res., 7 (1966) 170.
66 R.W. Keenan and B H. Marks, Biochim. Biophys. Acta, 39 (1960) 533.
67 M.L. Blank, E.A. Cress, C. Piantadosi and F. Snyder, Biochim. Biophys. Acta, 380 (1975) 208.
68 M.M. Rapport and R.E. Franzl, J Neurochem., 1 (1957) 303.
69 W.T. Norton, Biochim. Biophys. Acta, 38 (1960) 340.
70 J.N. Williams, C.E. Anderson and A.D. Jasik, J. Lipid Res., 3 (1962) 378.
71 M.M. Rapport and N.F. Alonzo, J. Biol. Chem., 235 (1960) 1953.
72 G. Ehrlich, H.E. Taylor and H Waelsch, J. Biol. Chem., 173 (1948) 547.
73 G. Camejo, M.M Rapport and G.A. Morrill, J. Lipid Res., 5 (1964) 75.
74 M.M. Rapport and B. Lerner, Biochim. Biophys. Acta, 33 (1959) 319.
75 J.C. Touchstone, K.A. Snyder and S.S. Levin, J. Liq Chromatogr., 7 (1984) 2725.
76 J.C. Nevenzel, A. Gibbs and A.A. Benson, Comp. Biochem. Physiol., 82B (1985) 293.
77 T. Huque, J.C. Brand, J.L. Rabinowitz and G.F. Fernandez, Comp. Biochem. Physiol., 86B (1987) 135.
78 M.M. Rapport and W.T. Norton, Ann. Rev. Biochem., 31 (1963) 103.
79 W. Link and G. Spiteller, Fat Sci. Technol., 1 (1990) 19.
80 H. Spreitzer and G. Spiteller, Monatsh. Chem., 120 (1989) 169
81 M.J. Allison, M.P. Bryant, M. Keeney and I. Katz, J. Diet. Sci., 44 (1961) 1203.
82 M.J. Allison, M.P. Bryant, I. Katz and M. Keeney, J. Bacteriol., 83 (1962) 1084.
83 G.H. Wegner and E.M. Foster, J. Bacteriol., 85 (1963) 53.
84 P.O. Hagen and H. Goldfine, J. Biol. Chem., 242 (1967) 5700.