DIOL LIPIDS

XV.* ISOLATION OF A NEUTRAL DIOL PLASMALOGEN FROM THE LIPIDS OF STARFISH

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We found neutral diol plasmalogens – acyl derivatives of alken-1-yl ethers of diols – some years ago in natural fats [1, 2]. However, it has not hitherto been possible to isolate this type of compounds, since their properties are close to those of the glycerol plasmalogens and their content in natural materials is generally low. In a search for suitable sources for the isolation of diol plasmalogens we have turned to a study of starfish which contain an exceptionally large amount of aldehydogenic lipids [3]. By means of methods developed previously [1, 2] we found that the neutral lipids of the Far-Eastern starfish Distolasterias nipon contain about 15% of bound ethylene glycol. Consequently, we assumed that D. nipon is a promising source for the isolation of individual diol plasmalogens.

The present paper describes the isolation of an ethylene glycol plasmalogen from the lipids of the starfish D. nipon and the determination of its structure.

The preliminary investigation of the lipids of the starfish D. nipon was carried out in the following way. The chromatography of the total lipid extract on silica gel under conditions not leading to an appreciable cleavage of the plasmalogens (see page 675) give six fractions of neutral lipids (Table 1 and Fig. 1) in which the contents of free and bound aldehydes were determined (Table 2). Then each fraction of the neutral lipids was subjected to acid methanolysis and the resulting methyl esters and aldehyde dimethyl acetals were separated from the polyols. The latter were acetylated and analyzed by gas-liquid chromatography (GLC). As can be seen from the chromatograms (Fig. 2), the methanolysis of the lipids of fractions 2 and 3 did not form glycerol. Furthermore, on the chromatograms of the acetates of the polyols (see Fig. 2), peaks of ethylene glycol diacetate and of an unidentified substance with a relative retention volume (V_R^{rel}) of 0.13 can be seen. Glycerol was detected only in the hydrolysate of fraction 7, corresponding in R_f value to triglycerides; fractions 5 and 6 (see Table 1), intermediate in polarity between the triglycerides and the diol lipids gave neither glycerol nor diols under the conditions of acid methanolysis. It is possible that they consist mainly of ether derivatives.

Of the seven neutral lipid fractions obtained by column chromatography (see Table 1), fraction 2 was selected for the isolation of the diol plasmalogens, since its saponifiable part consisted exclusively of diol derivatives, contained a high percentage of bound aldehydes, and under the conditions of thin-layer chromatography (TLC) on silica gel had a R_f value close to that of a synthetic ethylene glycol plasmalogen – 1-hexadec-1'-enyloxy-2-stearoyloxyethane [4]. The rechromatography of fraction 2 in a thin layer of alkaline alumina gave a substance the IR spectrum of which coincided almost completely with that of the synthetic ethylene glycol plasmalogen (Fig. 3). The only difference was the presence in the spectrum of the synthetic sample of a singlet at 965 cm^{-1} corresponding to the stretching vibrations of a trans-olefinic bond (Fig. 3b). This band was absent from the IR spectrum of the natural plasmalogen (Fig. 3a), but there was a weak band at 727 cm^{-1}. Consequently, the natural substance contains only cis double bonds.

*For Communication XIV, see [6].

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TABLE 1. Results of the Chromatography of the Neutral Lipids of D. nipon on Silica Gel

Fraction	Weight of lipids, mg	R_f^*
1	—	0,95
2	184,5	0,92; 0,82; 0,74
3	21,0	0,82; 0,74; 0,66
4	16,8	0,74
5	80,9	0,66
6	29,2	0,66; 0,60
7	435,0	0,49

*Plates a, hexane–ether–acetic acid (85:14:1) system.

TABLE 2. Content of Free and Bound Aldehydes in the Neutral Lipids of D. nippon

Fraction	Aldehyde content μmoles		Content of vinyl ethers, % of fraction weight
	free	bound	
2	0,000	0,060	5,60
3	0,025	0,120	10,00
4	0,020	0,160	13,55
5	0,008	0,071	6,00
6	0,006	0,120	10,00
7	0,020	0,048	4,00

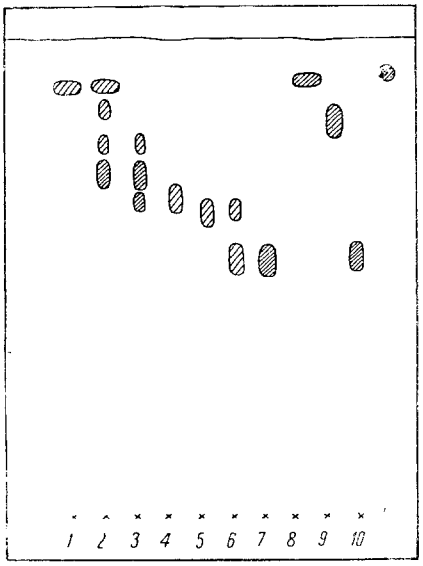
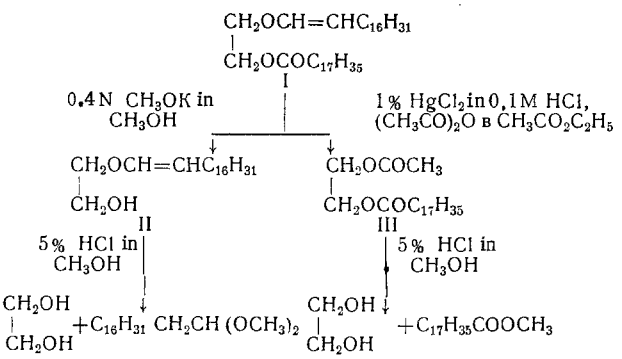


Fig. 1. Thin-layer chromatogram of the fractions of the neutral lipids of the starfish: 1-7) neutral lipid fractions obtained by column chromatography; 8) cholesterol stearate (standard); 9) 1-hexadec-1'-enyloxy-2-stearoyloxyethane (standard); 10) triolein (standard). For the conditions of chromatography, see Experimental.

The subsequent investigation of the diol plasmalogen (I) isolated was performed in accordance with the following scheme:



The plasmalogen (I) was subjected to mild acid hydrolysis, and the hydrolysis products were acetylated [5] and studied by GLC. It was found that the only high-boiling reaction product was an acetate (III) coinciding in its retention temperature (T_R 190°C) and mass spectrum (Fig. 4) with synthetic 1-acetoxy-2-stearoxyloxyethane [6]. The acid methanolysis of III gave (according to GLC) equimolecular amounts of ethylene glycol and stearic acid.

When the natural diol plasmalogen (I) was heated with methanolic potassium methoxide and the methanolysis products were chromatographed on nonfixed silica gel (see page 675) methyl esters and a comparatively polar aldehyde fraction (II) were isolated. Gas-chromatographic analysis of the methyl ester fraction showed that it consisted to the extent of 98% of methyl stearate. The aldehydogenic fraction (II) was identical in chromatographic behavior with synthetic 2-hexadec-1'-enyloxyethanol [4].

The acid methanolysis of this fraction gave only two substances: ethylene glycol and olealdehyde dimethyl acetals in a molar ratio of 1 : 1.

Thus, the combination of the results obtained has shown that the plasmalogen isolated from the starfish D. nipon is 1-cis, cis-octadeca-1',9'-dienyloxy-2-stearoyloxyethane (I). The high content of this substance in the neutral lipids of D. nipon (15% of the total amount of triglycerides) shows that in some organisms diol derivatives can be one of the main products of the biosynthesis of lipids.

EXPERIMENTAL

The solvents were purified by standard methods [7] and their purity was checked by GLC. For the extraction of the lipids, the digestive glands of Distolasterias nipon were homogenized with a mixture of chloroform and methanol (2 : 1) at the rate of 20 ml of the mixed solvent per gram of crude tissue [8]. The extract was filtered and mixed with 20% of water, and the chloroform layer was separated off and evaporated in vacuum (15-20 mm) at 40°C to constant weight. The IR spectra were recorded in a film on a "Carl Zeiss" (Jena, GDR) UR-10 instrument; the mass spectra of the acyl derivatives of ethylene glycol were recorded on a LKB-9000 chromato mass spectrometer. The substances were introduced through a column (3000 × 1.5 mm) containing 2.5% of fluorinated silicone QF-1 on Chromosorb W, 60-80 mesh. The temperature of the thermostat was 240°C and that of the separator 260°C, and the energy of the ionizing electrons was 70 eV.

Plates with dimensions of 13 × 18 cm (A) and 6 × 9 cm (B) coated with KSK silica gel (150-200 mesh, fixed with 5% of gypsum, layer thickness 0.5 mm) and also plates 6 × 9 cm with silica gel prepared from sodium silicate by a published method [9] (C) and plates with alumina (activity grade IV, layer thickness 0.5 mm) with dimensions of 13 × 18 cm (D) were used for analytical TLC. Preparative TLC was carried out on 13 × 18 cm plates coated with silica gel obtained from sodium silicate [9] with a layer thickness of 1.0 mm (E) or on plates of the same dimensions coated with alumina (activity grade IV, layer thickness 2.0 mm) (F).

Determination of the Stability of the Plasmalogens under the Conditions of Column Chromatography on KSK Silica Gel. A mixture consisting of 25.3 mg of synthetic tristearin and 0.1 mg of synthetic 1-hexadec-1'-enyloxy-2-stearoyloxyethane [4] was deposited on a 15 × 1.5 cm column containing 10 g of KSK silica gel (100-150 mesh). The lipids were eluted by means of the hexane–ether (85 : 15) system for 3 h. The eluate was evaporated and analyzed for its content of free and bound aldehydes (see below and [10]). The results of the analysis show that on column chromatography for 3 h not more than 4.5-4.7% of the initial plasmalogen is decomposed.

Determination of the Stability of the Plasmalogens under the Conditions of Thin-Layer Chromatography on KSK Silica Gel Fixed with Gypsum. A mixture of 24 mg of synthetic triolein and 0.1 mg of the synthetic ethylene glycol plasmalogen [4] was deposited on four plates A. The chromatograms were run in the hexane–ether (85 : 15) system, the zones with R_f 0.4-0.5 were cut out and eluted with chloroform, and the extracts were studied for their contents of free and bound aldehydes [10]. The results obtained show that under the given conditions of TLC about 32% of the plasmalogen deposited on the chromatograms decomposes.

Determination of the Stability of the Plasmalogens under the Conditions of Thin-Layer Chromatography on Nonfixed KSK Silica Gel. The preceding experiment was repeated on plates A (with a nonfixed layer of KSK silica gel). A determination of the free and bound aldehydes in the eluate from the zone with R_f 0.4-0.5 showed that when gypsum was absent from the KSK silica gel plates only 3.5% of the initial plasmalogen decomposed.

Chromatography of the Neutral Lipids. The total lipids of the starfish (4.5 g) were deposited on a column (40 × 3.5 cm) containing 200 g of KSK silica gel, 100-150 mesh. The neutral lipids were eluted with a mixture of hexane and ether (95 : 5), 20-ml fractions being obtained by means of a collector. These fractions were analyzed by TLC (see Table 1). When triglycerides began to be eluted from the column, to accelerate chromatography the system mentioned was replaced by the hexane–ether (4 : 1) system. The fractions containing substances with the same R_f values according to TLC were combined and evaporated.

Determination of the Free and Bound Aldehydes in the Fractions of the Neutral Lipids of the Starfish D. nipon. The fractions of neutral lipids obtained by column chromatography of the total lipid extract were dissolved in chloroform to give an approximate final concentration of 10 mg/ml. Two parallel samples were taken from the solution of each fraction, and in these samples the free and bound aldehydes were determined by the method of Warner and Lands [10]. The absorption of the colored solutions arising on the reaction of the aldehydes with fuchsine/sulfur dioxide were measured on an SF-4 spectrophotometer and also on a "Hitachi" (Japan) EPS-3T recording spectrophotometer at 550 nm. The standard straight-line curve for the quantitative determination of vinyl ethers was constructed from the results obtained with weighed samples of pure synthetic 1-O-(hexadec-1'-enyl)-2,3-di-O-stearoylglycerolobtained by a published method [11] (see Table 2).

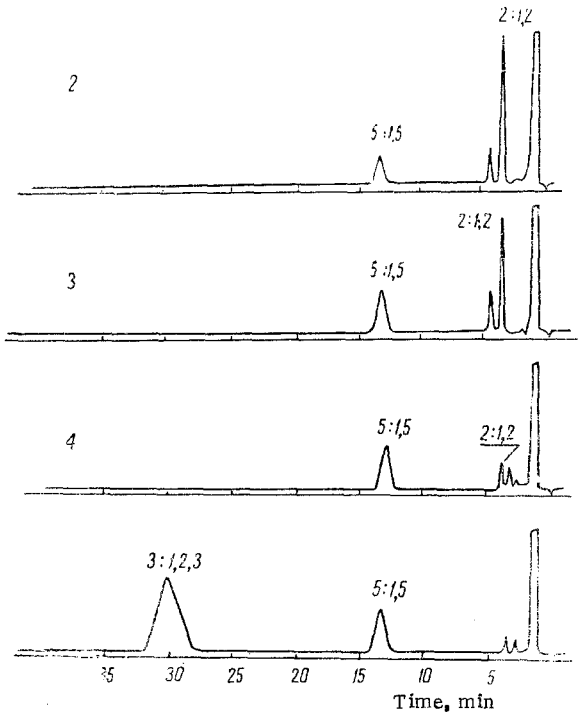


Fig. 2. Gas-liquid chromatograms of acetates of polyols obtained by the acid methanolysis of fractions 2, 3, 4, and 7 of the neutral lipids. Polyols: the first figure represents the number of carbon atoms in the molecule and the figure after the colon the positions of the hydroxyl groups. Pentane-1,5-diol (5:1.5) was used as an internal standard. For the conditions of chromatography, see [12].

Acid Methanolysis of the Neutral Lipids, and the Separation and Analysis of the Methanolysis Products. To each of a number of 15- to 60-mg samples of fractions 2-7 was added 2.5 ml of 2.5% hydrogen chloride in absolute methanol. The mixtures were boiled for 2 h, cooled, and neutralized with 0.4 N potassium methoxide in methanol. The precipitates of potassium chloride were separated by centrifuging and the methanolysis products were evaporated and deposited on plates E. The chromatograms were run in the hexane–ether (9 : 1) system, the zones of the separated substances being revealed with iodine vapor. The methyl esters and the aldehyde dimethyl acetals (zone with R_f 0.8) were eluted with chloroform and the starting zone (alcohols) with methanol. The latter were rechromatographed on a plate B in the chloroform–methanol (4 : 1) system. The zones corresponding to the diols and triols (R_f 0.4-0.2; revealing agent an ammoniacal solution of silver nitrate) were cut out and eluted with methanol (4 × 2 ml). The eluates were evaporated, acetylated [5], and analyzed by GLC [1, 2, 12].

Isolation of the Diol Plasmalogen. 125 mg of the lipids of fraction 2 was deposited on five plates F (25 mg on each plate). The chromatograms were run in the hexane–ether (20 : 1) system and were dried in the air. The edges of all the plates were treated with iodine vapor. The zones with R_f 0.60 were cut out and eluted with chloroform–methanol (4 : 1; 4 × 10 ml). Analysis by the TLC method on a plate D in the hexane–ether (20 : 1) system showed that the eluate contained a chromatographically homogeneous substance with R_f 0.60, identical with the R_f value of synthetic 1-hexadec-1'-enyloxy-2-stearoyloxyethane [4].

Mild Acid Hydrolysis of the Diol Plasmalogen (I). To a solution of 10 mg of I in 5 ml of a mixture of chloroform and methanol (1 : 1) was added 0.75 ml of a 1% solution of mercuric chloride in 0.1 M hydrochloric acid. The mixture was heated at 60°C for 3 h, the completeness of hydrolysis being checked by TLC on a plate C in the hexane–ether–acetic acid (85 : 14 : 1) system. At the end of hydrolysis, the reaction products were treated with 2 ml of chloroform, 0.5 ml of methanol, and 0.4 ml of water. The mixture was

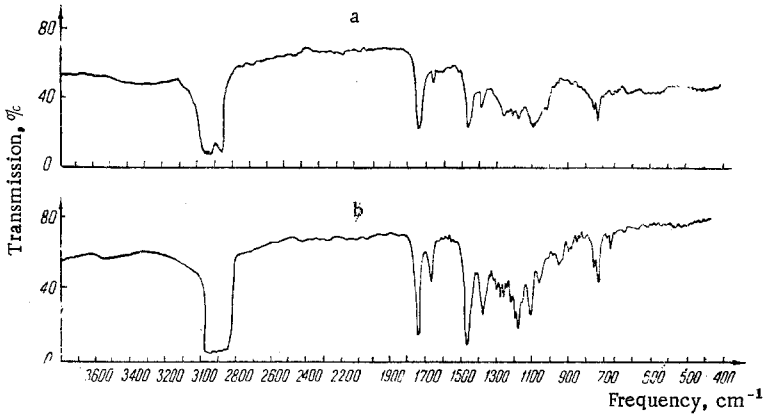


Fig. 3. IR spectra of the natural diol plasmalogen (a) and of synthetic 1-hexadec-1'-enyloxy-2-stearoyloxyethane (b).

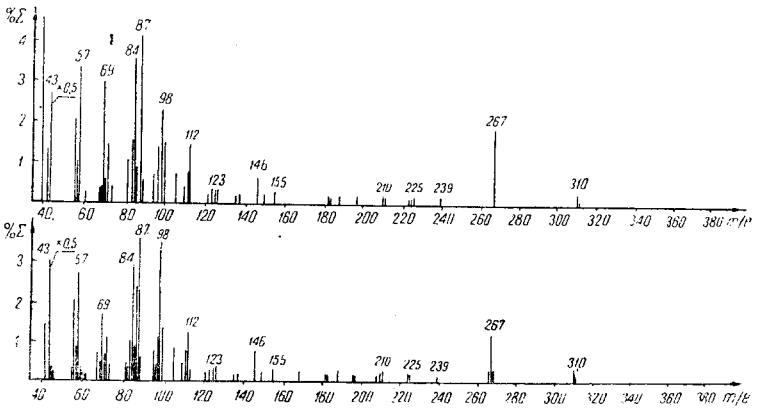


Fig. 4. Mass spectra of synthetic (a) and natural (b) 1-acetoxy-2-stearoyloxyethanes.

shaken vigorously, and the chloroform layer was separated off by centrifuging, washed with water (2 × 0.2 ml), and evaporated in vacuum. The residue was acetylated [5] and analyzed by high-temperature GLC [6].

Alkaline Methanolysis of the Diol Plasmalogen (I). To a solution of 10 mg of I in 0.5 ml of absolute benzene was added 1 ml of 0.4 N methanolic potassium methoxide. The reaction mixture was boiled for 1.5 h, the completeness of methanolysis being checked by TLC on a plate B in the hexane–ether (9 : 1) system. After the end of the reaction, the mixture was evaporated and separated by TLC on a plate B with a nonfixed layer of silica gel in the benzene–ether (4 : 1) system. The zones with R_f 0.8-0.9 (methyl ester fraction) and 0.20-0.25 [alk-1-enyl ether of ethylene glycol (II)] were cut out and eluted with chloroform and with a mixture of chloroform and methanol (4 : 1), respectively. The methyl esters were analyzed by GLC, and the aldehydogenic fraction was subjected to acid methanolysis (see below).

Acid Methanolysis of the alk-1-enyl Ether of Ethylene Glycol (II) and the Acetylated Monoester (III); Separation and Analysis of the Methanolysis Products. To a solution of 5 mg of II or III in 1 ml of benzene was added 2.5 ml of a 2.5% solution of hydrogen chloride in methanol. The mixture was boiled for 1.5 h, the completeness of methylation being checked by TLC on plates B in the hexane–ether (9 : 1) system. After cooling, the reaction mixture was neutralized with 0.4 N methanolic potassium methoxide. The precipitate of potassium chloride was separated off by centrifuging and the methanolysis products were separated by TLC on plates C in the hexane–ether (9 : 1) system. The zones of the methyl esters or of the dimethyl acetals (R_f 0.0) were cut out and eluted with chloroform and with methanol, respectively.

Gas-chromatographic analysis of the dimethyl acetals fraction obtained from the diol aldehydogenic fraction II showed that it consisted to the extent of 95% of olealdehyde dimethyl acetal; the methyl ester fraction formed in the methanolysis of III consisted of practically pure methyl stearate. The polyol fraction

was acetylated [5] and analyzed by GLC, and in both cases it was found that it contained only ethylene glycol, which was identified in the form of its acetate [12].

SUMMARY

1. An ethylene glycol plasmalogen has been isolated by column and thin-layer chromatography from the lipids of the starfish Distolasterias nipon.

2. It has been shown by thin-layer chromatography, IR spectroscopy, and stepwise hydrolysis of the plasmalogen isolated, and also by the gas-liquid chromatography and mass spectrometry of the hydrolysis products, that this substance is 1-cis, cis-octadeca-1',9'-dienyloxy-2-stearoyloxyethane.

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