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PREPARATION AND CHARACTERIZATION OF NEUTRAL DIOL PLASMALOGENS

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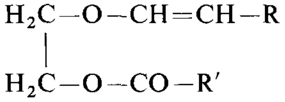
Neutral diol plasmalogens, (*cis* and *trans* 2-alk-1′-enyloxy-1-O-acyl-ethanols) were synthesized. These alk-1-enyl ether-esters of ethanediol were characterized by their chemical properties and by physical data; their chromatographic behavior was studied and compared to that of the alkyl ether-esters and diesters of ethanediol.

Introduction

Neutral diol plasmalogens, *i.e.*, 2-alk-1′-enyloxy-1-O-acyl-ethanols (I), have been shown to occur as minor constituents in rat liver, cod liver, sunflower oil, mutton fat, corn seeds, egg yolk and soil yeast (*Lipomyces* spec.)1–3), but they have not been isolated. The synthesis of the geometric isomers of alk-1-enyl ethers of ethanediol has been reported recently4), as well as a semisynthetic method for the preparation of the *cis* compounds from natural *cis*-1-alk-1′-enyl glycerol ethers5).



$$\begin{array}{l} H_2C-O-CH=CH-R \\ \;\;| \\ H_2C-O-CO-R' \end{array}$$

(I)

The present communication describes the preparation of neutral diol plasmalogens from alk-1-enyl ethers of ethanediol whose synthesis has been described previously4). *cis* and *trans* 2-hexadec-1′-enyloxy-ethanol and 2-octadec-1′-enyloxy-ethanol were acylated6) with tetradecanoyl, hexadecanoyl, octadecanoyl and 9-*cis*-octadecenoyl chlorides. The *cis* and *trans* isomers of the neutral diol plasmalogens were separated and isolated by adsorption and argentation chromatography. The physical and spectroscopic properties of these unusual lipids are reported, and compared to those of the closely related alkyl ether-esters and diesters of ethanediol with which the neutral diol plasmalogens often occur in nature3).

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Acid-catalyzed hydrolysis of the neutral diol plasmalogens yields aldehydes and monoacyl ethanediols as sole products. Catalytic hydrogenation of the geometric isomers of neutral diol plasmalogens leads to products which are identical to authentic 2-alkoxy-1-O-acyl-ethanols.

Experimental

Analytical methods

Melting points were determined on a Kofler hot stage.

The purity of reactants and products were checked by thin layer chromatography (TLC) on Silica Gel G. Thicker layers of Silica Gel H were used for preparative separations. Mixtures of the isomeric neutral diol plasmalogens were separated by argentation chromatography. Mixtures of hexane and diethyl ether served as developing solvents in adsorption as well as argentation chromatography. After chromatography, all organic material was visualized by spraying the plates with a solution of sodium dichromate in sulfuric acid, and charring. In preparative separations, fractions were often visible without the use of an indicator. However, when required, fractions were detected in UV light after spraying with a solution of 2′, 7′-dichlorofluorescein in ethanol. Products were eluted from the adsorbent with water-saturated diethyl ether and the eluates were filtered through sintered glass funnels.

For the separation-reaction-separation technique (SRS)[7], square plates, 20 × 20 cm, were used, coated either with layers of Silica Gel G or Silica Gel G impregnated with silver nitrate[8]). The sample was spotted 2 cm in from one corner of the plate. After developing in the first direction, the layer was exposed to fumes of HCl in order to hydrolyze the alk-1-enyl ethers. Following this reaction, the chromatogram was developed at a right angle to the first direction, thus separating the unreacted compounds from the products of acid-catalyzed hydrolysis. Aldehydes were detected by spraying the chromatograms with a 5% solution of 2,4-dinitrophenylhydrazine in ethanolic 0.1 N HCl, and all other organic material was made visible by charring with chromic-sulfuric acid solution.

Reversed-phase partition chromatography was carried out on layers of Silica Gel G rendered hydrophobic by placing the coated plates into unlined tanks containing a 5% solution (v/v) of liquid paraffin in hexane which was permitted to ascend the height of the plates. The plates were used after brief air drying. Chromatograms were developed twice with acetone-acetonitrile, 60/40, v/v[9]). Iodine vapor was used to visualize the neutral diol plasmalogens.

Gas chromatography was carried out with a Victoreen 4000 series instrument equipped with a hydrogen flame detector. The column used was 70 cm in length, 2.5 mm i.d. and packed with a stationary phase of 1% OV-1 Silicone on 100/200 Chromosorb W (HP). Helium flow rate was 70 ml/min at 80 psi. Temperature programming between 150 and 300 °C was carried out at heating rates of 5 °C per minute.

Infrared (IR) spectra were recorded on a Perkin-Elmer spectrometer, model 21, using carbon disulfide and tetrachloroethylene solutions.

Nuclear magnetic resonance (NMR) spectra were recorded on a Varian A-60 instrument using CCl_4 as solvent and tetramethylsilane (TMS) as the reference compound. The chemical shifts recorded as δ values, number of protons, nature of signal (bs = broad singlet, d = doublet, t = triplet, dt = double triplet, m = multiplet), splitting constants and their assignments are indicated in that order.

Mass spectra were measured at 15 and 70 eV with a Hitachi-Perkin Elmer RMU-6D single focusing, magnetic scanning mass spectrometer and recorded as m/e (relative intensity).

Elemental analyses were performed by Mikroanalytisches Laboratorium I. Beetz, Kronach, Ofr., Germany.

Synthetic procedures

2-Hexadec-1′-enyloxy-ethanol and 2-octadec-1′-enyloxy-ethanol were prepared as described previously[4]. Acylations were carried out in an atmosphere of nitrogen according to well established procedures[6]. The neutral diol plasmalogens (table 1) were prepared from the 2-alk-1′-enyloxy-ethanols as follows:

2-Octadec-1′-enyloxy-1-O-octadecanoyl-ethanol. 2-Octadec-1′-enyloxy-ethanol (1.0 g, 3.2 mmoles), freshly distilled dry benzene (50 ml) and pyridine (4 ml) were placed in a 100 ml three-necked flask, equipped with condenser, $CaCl_2$-tube, nitrogen inlet and dropping funnel. While the solution was stirred magnetically, octadecanoyl chloride (1.5 g, 5.0 mmoles) in 10 ml of dry benzene was added dropwise, over a period of 30 min. After stirring at room temperature for one hour, the reaction mixture was heated under reflux for an additional three hours. Upon cooling, 50 ml of ice water and 50 ml of ether were added, the ether phase was recovered, and the aqueous phase extracted with 25 ml of ether. The combined ether extract was washed consecutively with 3 × 50 ml portions of water and 2 × 50 ml portions of 2% aqueous K_2CO_3 solution, and dried over anhydrous Na_2SO_4. The ether was removed, the product was dissolved in hexane and purified on layers of Silica Gel G, 0.5 mm in thickness.

The 2-heptadecyl-1,3-dioxolane formed in this reaction as a by-product

TABLE 1

Neutral diol plasmalogens (*cis* and *trans* 2-alk-1′-enyloxy-1-O-acyl-ethanols)

2-Alk-1′-enyloxy	1-Acyl	Molecular formula	Molecular weight	Yield (%)	Melting point (°C)	Elemental analysis					
						Calculated			Found		
						C	H	O	C	H	O
cis-hexadec-1′-enyloxy	O-tetradecanoyl	$C_{32}H_{62}O_3$	494.85	62	36.0–38.0	77.67	12.63	9.70	77.00	12.45	10.47
trans-hexadec-1′-enyloxy	O-tetradecanoyl	$C_{32}H_{62}O_3$	494.85		44.5–46.5	77.67	12.63	9.70	78.18	12.75	9.11
cis-hexadec-1′-enyloxy[a]	O-hexadecanoyl	$C_{34}H_{66}O_3$	522.90	57	44.0–46.0	78.10	12.72	9.18	77.45	12.88	9.82
trans-hexadec 1′-enyloxy[a]	O-hexadecanoyl	$C_{34}H_{66}O_3$	522.90		48.5–50.0	78.10	12.72	9.18	78.35	12.63	8.93
cis-hexadec-1′-enyloxy[b]	O-octadecanoyl	$C_{36}H_{70}O_3$	550.96	61	48.0–49.0	78.48	12.81	8.71	78.10	12.63	9.43
trans-hexadec-1′-enyloxy[b]	O-octadecanoyl	$C_{36}H_{70}O_3$	550.96		53.5–54.5	78.48	12.81	8.71	78.70	12.83	9.36
cis-hexadec-1′-enyloxy[b]	O-*cis*-octadec-9-enoyl	$C_{36}H_{68}O_3$	548.94	57	15.0–16.5	78.77	12.49	8.76	78.52	12.20	9.36
trans-hexadec-1′-enyloxy[b]	O-*cis*-octadec-9-enoyl	$C_{36}H_{68}O_3$	548.94		17.5–19.0	78.77	12.49	8.76	79.32	12.83	8.04
cis-octadec-1′-enyloxy	O-tetradecanoyl	$C_{34}H_{66}O_3$	522.90	65	44.5–46.0	78.10	12.72	9.18	78.11	12.58	9.33
trans-octadec-1′-enyloxy	O-tetradecanoyl	$C_{34}H_{66}O_3$	522.90		48.5–50.0	78.10	12.72	9.18	78.08	12.46	9.30
cis-octadec-1′-enyloxy[c]	O-hexadecanoyl	$C_{36}H_{70}O_3$	550.96	52	48.0–49.0	78.48	12.81	8.71	78.92	12.73	8.54
trans-octadec-1′-enyloxy[c]	O-hexadecanoyl	$C_{36}H_{70}O_3$	550.96		54.0–55.5	78.48	12.81	8.71	78.68	12.55	8.82
cis-octadec-1′-enyloxy[d]	O-octadecanoyl	$C_{38}H_{74}O_3$	579.01	70	54.0–56.5	78.83	12.88	8.29	79.04	13.03	7.77
trans-octadec-1′-enyloxy[d]	O-octadecanoyl	$C_{38}H_{74}O_3$	579 01		58.0–59.0	78.83	12.88	8.29	79.18	12.64	8.17
cis-octadec-1′-enyloxy[d]	O-*cis*-octadec-9-enoyl	$C_{38}H_{72}O_3$	576.99	60	20.0–21.0	79.10	12.58	8.32	78.80	12.86	8.51
trans-octadec-1′-enyloxy[d]	O-*cis*-octadec-9-enoyl	$C_{38}H_{72}O_3$	576.99		24.5–26.0	79.10	12.58	8.32	78.99	12.80	8.17

[a] The product of hydrogenation (2-hexadecyloxy-1-O-hexadecanoyl-ethanol) had m.p. 48.5–50 °C [lit.[10]), m.p. 54–55 °C].

[b] The product of hydrogenation (2-hexadecyloxy-1-O-octadecanoyl-ethanol) had m.p. 50–51 °C [lit.[10]), m.p. 50.5–51.5 °C].

[c] The product of hydrogenation (2-octadecyloxy-1-O-hexadecanoyl-ethanol) had m.p. 53–54.5 °C [lit.[10]), m.p. 52.5–54 °C].

[d] The product of hydrogenation (2-octadecyloxy-1-O-octadecanoyl-ethanol) had m.p. 61–62 °C [lit.[10]), m.p. 61.5–62 °C].

was effectively separated from the isomeric neutral diol plasmalogens on adsorbent layers. The geometric isomers obtained in a yield of 1.3 g (70%) were resolved by argentation chromatography to give pure *cis*- and *trans*-2-octadec-1′-enyloxy-1-O-octadecanoyl-ethanols.

2-cis-Octadec-1′-enyloxy-1-O-octadecanoyl-ethanol, m.p. 55.0–56.5 °C; ν max. 1662 ($C{=}C$ stretching), 727 and 1390 (*cis* $C-H$ out-of-plane and in-plane bending, respectively), 1735 (ester stretching) and 1108 cm^{-1} (ether stretching); NMR showed signals at δ 5.78 (1, d, 6.1, $-CH_2-O-CH{=}CH-$), 4.25 (1, dt, 6.5 and 7.3, $-O-CH{=}CH-CH_2-$), 4.14 (2, m, 5.6, $-CO-O-CH_2-CH_2-O-$), 3.79 (2, m, 5.1, $-CH_2-CH_2-O-CH{=}$), 2.25 (2, unsym. t, 6.6, $-OC-CH_2-CH_2-$), 2.03 (2, apparent d, 7.2, $={CH}-CH_2-CH_2-$), 1.26 (58, bs, $-(CH_2)_n-$) and 0.89 (6, unsym. t, 5.0, $-CH_3$); mass spectrum 329 (1), 312 (24.6), 311 (100), 310 (2.2), 267 (3.8), 251 (1.5), 155 (2.2), 99 (9.1), 97 (5.5), 86 (3.2), 85 (4.4), 83 (6.4), 73 (2.6), 71 (7.2), 69 (7.9), 57 (15.4), 55 (13.1), 43 (19.2), 41 (8.8).

*2-*Trans*-octadec-1′-enyloxy-1-O-octadecanoyl-ethanol*, m.p. 58.0–59.0 °C; ν max. 1648 (s) and 1668 (m) ($C{=}C$ stretching), 929 (s) and 915 (m) (*trans* $C-H$ out-of-plane bending), 1733 (ester stretching), and 1160 cm^{-1} (ether stretching); NMR showed signals at δ 6.10 (1, d, 12.5, $-CH_2-O-CH{=}CH-$), 4.61 (1, dt, 12.4 and 6.9, $-O-CH{=}CH-CH_2-$), 4.16 (2, m, 4.6, $-CO-O-CH_2-CH_2-O-$), 3.69 (2, m, 4.6, $-CH_2-CH_2-O-CH{=}$), 2.24 (2, unsym. t, 6.4, $-OC-CH_2-CH_2-$), 1.87 (2, apparent d, 6.7, $={CH}-CH_2-CH_2-$), 1.24 (58, bs, $-(CH_2)_n-$) and 0.88 (6, unsym. t, 4.5, $-CH_3$); mass spectrum 329 (0.9), 312 (24.8), 311 (100), 310 (2.0), 267 (3.8), 251 (1.3), 155 (2.5), 99 (10), 97 (4.3), 86 (4.2), 85 (3.2), 83 (5.6), 73 (2.2), 71 (5.2), 69 (6.4), 57 (15.2), 55 (10.3), 43 (18.1), 41 (8.3).

Hydrogenation of cis- *and* trans-*2-alk-1′-enyloxy-1-O-acyl-ethanols*. Each of the geometric isomers of the neutral diol plasmalogens was hydrogenated in freshly distilled hexane with platinum dioxide as catalyst.

Results and discussions

Mixtures of *cis* and *trans* alk-1-enyl ethers of ethanediol were acylated in benzene in the presence of pyridine and obtained in yields of about 65%. The 2-alkyl dioxolanes, formed as a by-product in this reaction, were removed by adsorption chromatography. Then, the *cis* and *trans* 2-alk-1′-enyloxy-1-O-acyl-ethanols were isolated in pure form by argentation chromatography.

The *trans* alk-1-enyl ether-esters of ethanediol melt consistently higher than the corresponding *cis* compounds although the differences in the melting points for geometric isomers decrease with increasing molecular weight. Comparing the melting points of the neutral diol plasmalogens to the corresponding alkyl ether-esters and diesters of ethanediol[10]), we find that the geometric isomers usually melt slightly lower than the corresponding alkyl ether-ester, whereas, a larger difference exists between these two compounds and the diesters of ethanediol.

Catalytic hydrogenation of alk-1-enyl ether-esters of ethanediol gives a single product indistinguishable in its melting point and in its chromatographic behavior from authentic alkyl ether-esters of ethanediol[10]). The IR spectra of the hydrogenated products are identical to those of the alkyl ether-esters of ethanediol; ν max. 1735, 1170 (ester stretching) and 1120 cm^{-1} (ether stretching).

Chromatographic behavior

The *cis* and *trans* isomers of the neutral diol plasmalogens can be separated by adsorption chromatography (fig. 1), however, the separation is more pronounced in argentation chromatography. The *trans* isomer migrates ahead of the *cis* isomer in both cases. *cis* and *trans* Neutral diol plasmalogens can be resolved also by GLC on a nonpolar stationary phase of silicone, the *cis* isomer being eluted first (table 2). In reversed-phase partition chromatography a clear separation between the geometric isomers does not occur.

TABLE 2

Gas-chromatographic separation of neutral diol plasmalogens

2-Alk-1′-enyloxy	1-O-Acyl	Relative retention time
cis-hexadec-1′-enyloxy	O-tetradecanoyl	0.72
trans-hexadec-1′-enyloxy	O-tetradecanoyl	0.76
cis-octadec-1′-enyloxy	O-tetradecanoyl	0.80
trans-octadec-1′-enyloxy	O-tetradecanoyl	0.84
cis-hexadec-1′-enyloxy	O-octadecanoyl	0.88
trans-hexadec-1′-enyloxy	O-octadecanoyl	0.92
cis-octadec-1′-enyloxy	O-octadecanoyl	1.00
trans-octadec-1′-enyloxy	O-octadecanoyl	1.04

Temperature programming from 150–270°C at a rate of 5° C per minute. See text for further experimental conditions. The retention time of 2-*cis*-octadec-1′-enyloxy-1-O-octadecanoyl ethanol is taken as 1.00.

Neutral diol plasmalogens differing in molecular weight are not separated by adsorption nor by argentation chromatography. By GLC a mixture of a homologous series of neutral diol plasmalogens can be completely resolved, and to a lesser extent, the *cis* and *trans* isomers of each homologue (table 2).

In reversed-phase partition chromatography a mixture of a homologous series of neutral diol plasmalogens is well resolved; the lower the molecular weight, the greater the Rf value.

The chromatographic behavior of the *cis* isomers, the assumed naturally occurring neutral diol plasmalogens[5]), was compared to the corresponding alkyl ether-esters and diesters of ethanediol. In adsorption chromatography the alk-1-enyl ether-esters migrate ahead of the alkyl ether-esters and these travel ahead of the diesters (fig. 1). In argentation chromatography an alk-1-enyl ether-ester migrates between the alkyl ether-ester and the diester, the latter being again the most polar compound. In reversed-phase partition

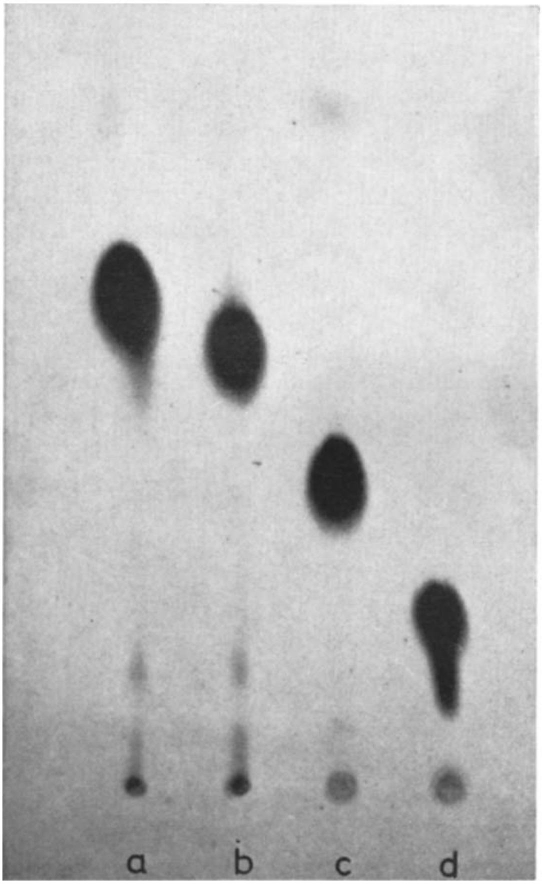


Fig. 1. Thin-layer chromatograms of neutral diol lipids. (a) 2-*trans*-octadec-1′-enyloxy-1-O-octadecanoyl-ethanol, (b) 2-*cis*-octadec-1′-enyloxy-1-O-octadecanoyl-ethanol, (c) 2-octadecyloxy-1-O-octadecanoyl-ethanol, (d) 1,2-di-O-octadecanoyl-ethanediol. Adsorbent: Silica Gel G. Solvent: hexane-diethyl ether (95/5, v/v).

chromatography the alk-1-enyl ether-ester migrates at the same rate as the alkyl ether-ester, but both lag far behind the diester of ethanediol. These three classes of compounds can be separated by GLC, the alk-1-enyl ether-ester is eluted first, followed closely by the alkyl ether-ester, and the diester emerges last (table 3).

TABLE 3

Gas-chromatographic separation of neutral diol lipids

2-position	1-position	Relative retention time
cis-hexadec-1′-enyloxy	O-hexadecanoyl	0.80
hexadecyloxy	O-hexadecanoyl	0.83
O-hexadecanoyl	O-hexadecanoyl	0.87
cis-octadec-1′-enyloxy	O-octadecanoyl	1.00
octadecyloxy	O-octadecanoyl	1.02
O-octadecanoyl	O-octadecanoyl	1.05

Temperature programming from 150–300°C at a rate of 5° C per minute. See text for further experimental conditions. The retention time of 2-*cis*-octadec-1′-enyloxy-1-O-octadecanoyl ethanol is taken as 1.00.

The SRS technique can be used to detect compounds containing the acid-labile alk-1-enyl ether bond[7]). The three lipid classes are separated by adsorption (fig. 2A) or argentation-TLC (fig. 2B), (horizontal), then the layer is exposed to HCl vapors. Eventually, the products of reaction and the unchanged compounds are separated in the second direction. The diesters (a) and alkyl ether-esters (b) are not hydrolyzed by HCl vapors, but *cis* or *trans* neutral diol plasmalogens are cleaved to an aldehyde (c) (which yields a positive 2,4-dinitrophenylhydrazine test) and a monoacyl ethanediol (d).

Infrared spectra

The IR spectra of *cis*- and *trans*-2-alk-1′-enyloxy-1-O-acyl-ethanols (fig. 3) exhibit uniquely different absorption bands associated with the C=C stretching, C—H bending and C—O—C stretching vibrations. The absorption band due to C=C stretching[11–14]) occurs at 1666 cm^{-1} for the *cis* isomer. The *trans* isomer has a doublet at 1650 (s) and 1670 cm^{-1} (m). The C—H bending vibration of the *cis* isomer occurs at 730 (out-of-plane) and 1390 cm^{-1} (in-plane). The *trans* isomer has a doublet at 930 (s) and 916 cm^{-1} (m). The absorption band at 3030 cm^{-1} is assigned to C—H stretching of the alk-1-enyl ether, while the absorption at 2990 cm^{-1} is attributed to the C—H stretching of unsaturation in the acyl side chain.

The C—O stretching frequency of the ether is more complicated. Two

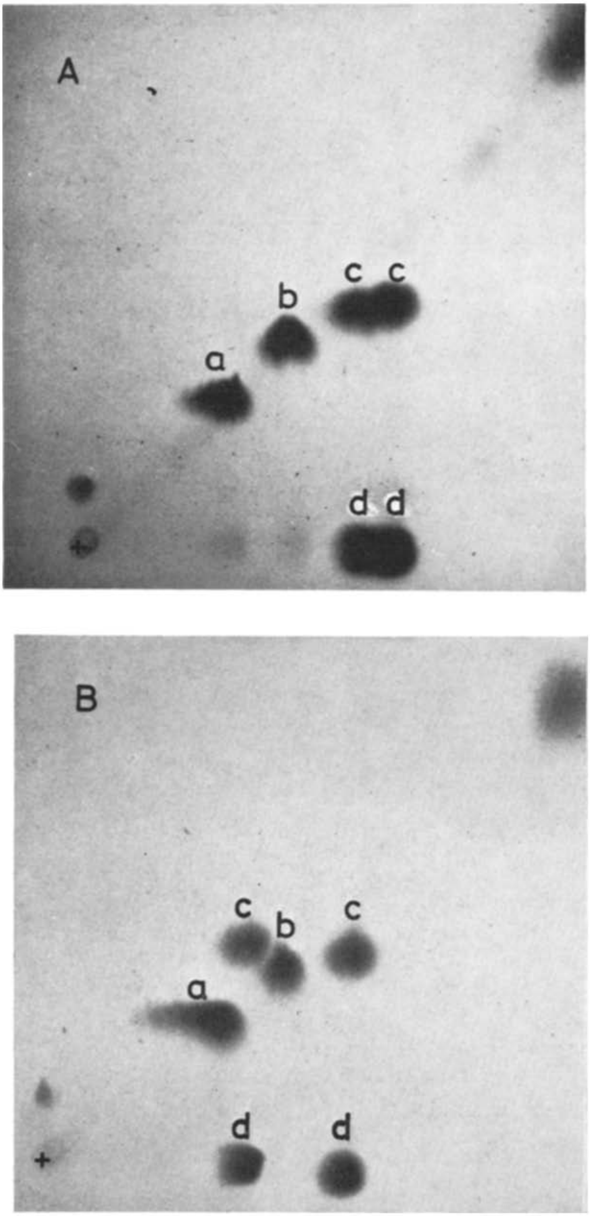


Fig. 2. Thin-layer chromatograms (SRS) of neutral diol lipids: (a) 1,2-di-O-octadecanoyl-ethanediol, (b) 2-octadecyloxy-1-O-octadecanoyl-ethanol, and *cis* and *trans* 2-octadec-1′-enyloxy-1-O-octadecanoyl-ethanol. Adsorbent: Silica Gel G (A) Silica Gel G impregnated with 5% silver nitrate. (B)Solvent: in the first (horizontal) and the second (vertical) direction of both chromatograms, hexane-diethyl ether (95/5, v/v). Reaction: After development in the first direction, chromatograms were exposed to fumes of HCl. Octadecanal, spot (c) showed up yellow after spraying with 2,4-dinitrophenylhydrazine. Spot (d) is 2-O-octadecanoyl-ethanediol.

absorption bands are expected[15]), one closely related to an aliphatic ether near 1110 cm^{-1}, and one similar to an aromatic ether near 1230 cm^{-1}. The *cis* isomer has a strong ether absorption at 1110 cm^{-1} and a relatively weak peak at 1168 cm^{-1}. The *trans* isomer shows exactly opposite absorption intensities, strong absorption at 1160 cm^{-1}, and weak absorption at 1110 cm^{-1}. After hydrogenation only a single band is observed at 1120 cm^{-1}.

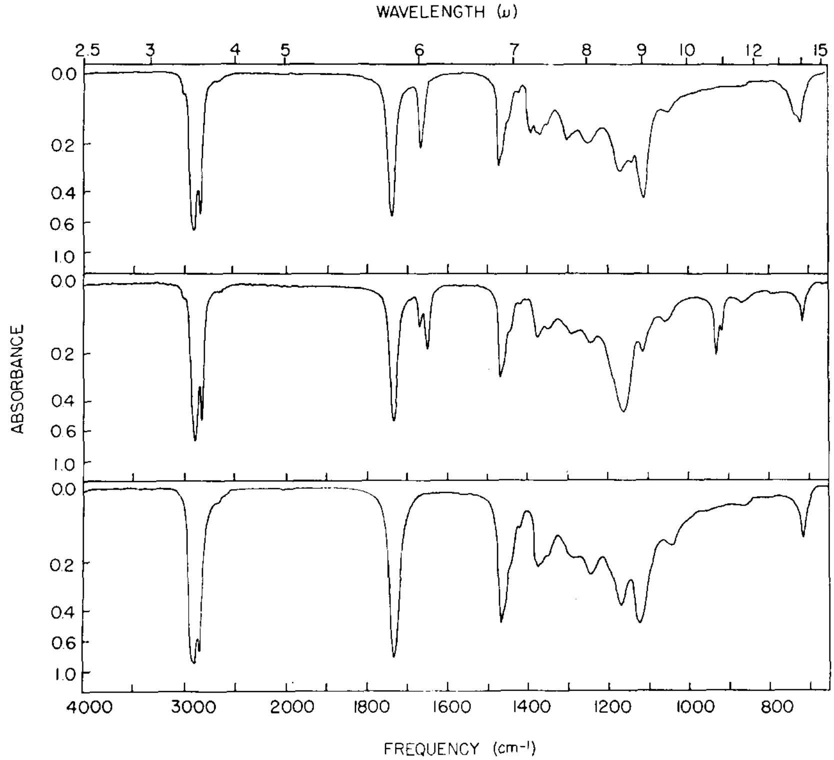


Fig. 3. Infrared spectra of 2-*cis*-octadec-1′-enyloxy-1-O-octadecanoyl-ethanol (top), 2-*trans*-octadec-1′-enyloxy-1-O-octadecanoyl-ethanol (center) and 2-octadecyloxy-1-O-octadecanoyl-ethanol (bottom).

Nuclear magnetic resonance spectra

The NMR spectra of enol ethers and, in particular, of alk-1-enyl ethers of glycerol[12, 14, 16, 17]) and ethanediol[4]) have been reported. The NMR spectra of the geometric isomers of 2-alk-1′-enyloxy-1-O-acyl-ethanols (fig. 4) have not been described.

The protons associated with C-1 and C-2 of the alkenyl chain are distinctly different in the *cis* and *trans* isomers and are readily differentiated by their chemical shifts and coupling constants. The proton of C-1 of the alkenyl chain gives rise in the *trans* isomer to a doublet at δ 6.10 ppm with a coupling constant of 12.5 cps and in the *cis* isomer to a doublet at δ 5.78 ppm with a coupling constant of 6.1 cps. The difference in the chemical shift for this proton in the *trans* and *cis* isomers is 0.32 ppm, which is in agreement with those reported for similar enol ethers [lit.[18]), 0.32 ppm]. The coupling constants are also in close agreement, J_{trans} 12.5 cps and J_{cis} 6.1 cps [lit.[18]), J_{trans} 12.7 cps, J_{cis} 6.3 cps]. The proton on C-2 of the alkenyl chain gives rise in the *trans* isomer to a five line pattern centered at δ 4.61 ppm which is interpreted as a double triplet with coupling constants of 12.4 and 6.9 cps. In the *cis* isomer a four line pattern appears centered at δ 4.25 ppm which is interpreted as a double triplet with coupling constants of 6.5 and 7.3 cps. The difference in the chemical shift for this proton in the geometric isomers is 0.36 ppm.

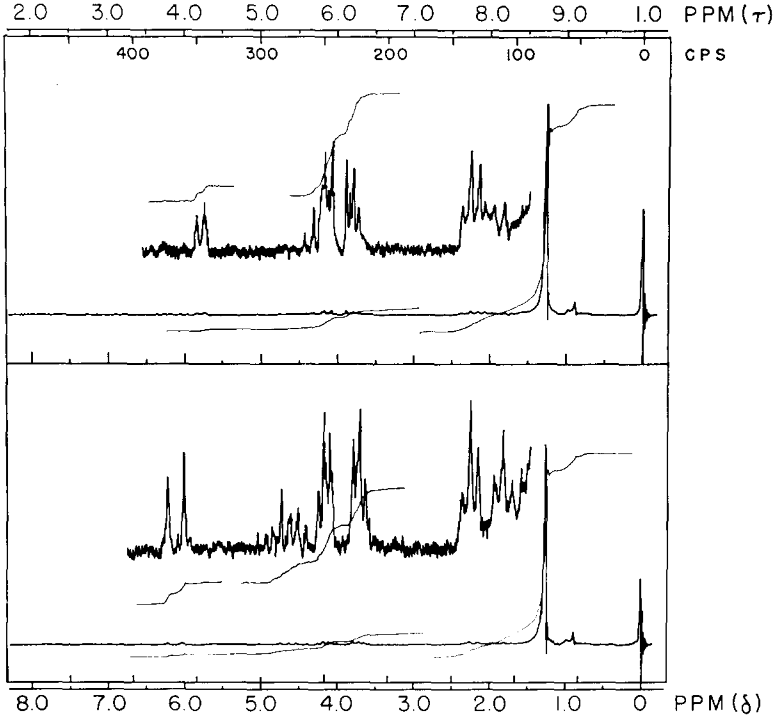


Fig. 4. NMR spectra of 2-*cis*-octadec-1′-enyloxy-1-O-octadecanoyl-ethanol (top) and 2-*trans*-octadec-1′-enyloxy-1-O-octadecanoyl-ethanol (bottom).

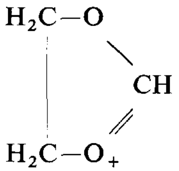
The other features of the spectra are similar for the isomers, two multiplets at δ 4.15 for the protons on C-1 of the ethanediol moiety, and at δ 3.79 (*cis*) and δ 3.69 (*trans*) for the protons on C-2 of ethanediol, an unsymmetrical triplet at δ 2.25 for the methylene protons adjacent to the carbonyl, an apparent doublet at δ 2.03 (*cis*) and δ 1.87 (*trans*) for the methylene protons adjacent to the enol ether, a broad singlet at δ 1.25 for the chain methylene protons and an unsymmetrical triplet at δ 0.89 for the terminal methyl protons.

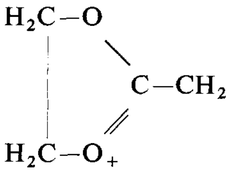
Mass spectrometry

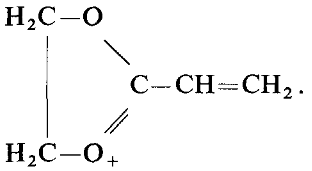
Mass spectra of alk-1-enyl ethers of glycerol14) have been reported, as well as of long chain mono- and diesters of ethanediol19). The mode of fragmentation of the alk-1-enyl ether-esters of ethanediol (I) was found not to be similar to that of the above mentioned compounds. To elucidate the mass spectra of the neutral diol plasmalogens, the mass spectra of the geometric isomers of 2-octadec-1′-enyloxy-1-O-acyl-ethanols were measured, in which the acyl chain was tetradecanoyl, hexadecanoyl, octadecanoyl and 9-*cis*-octadecenoyl.

In the spectrum of the neutral diol plasmalogens, the hydrocarbon ($m/e=29+14n$), olefinic ($m/e=27+14n$) and acetylenic peaks ($m/e=39+14n$) are abundant. However, the main mode of fragmentation occurs by the loss of the alkenyl side chain giving rise to the base peak at $M-(O-CH{=}CH-R)$. The alkenyl fragments $R-CH{=}CHO^+$ and $R-CH{=}CH^+$ are present at about 1–2%. The ion resulting from the loss of the acyl group, $M-OCR'$, is small, less than 1% of the base peak as observed when the acyl and alkenyl groups are not of the same chain length. Fragmentation according to the McLafferty rearrangement20) is not observed.

The characteristic fragments formed from the neutral diol plasmalogens involve the ethanediol moiety in a 1,3-dioxolane structure. The 2-alkyl side chain of the 1,3-dioxolane is that of the acyl rest. The ions appear at $m/e=73$ (2–3%) II, 86 (3–4%) III, 99 (9–11%) IV and $99+14n$. In this series two predominant ions occur: $m/e=155$ (2%) where $n=4$, and the parent member at $m/e=310$ (2%). The following structures are suggested for these ions:


(II)


(III)


(IV)

The ion at $m/e=99$, IV, is the result of the energetically favorable allylic homolytic cleavage21).

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