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1-*O*-(2-Hydroxyalkyl)glycerols Isolated from Greenland Shark Liver Oil

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2-Hydroxy-substituted glycerol ethers with 14, 16, and 18 carbon atoms in the long carbon chains have been found in the unsaponifiable fraction of Greenland shark liver oil. The mass spectrum of the isopropylidene derivative of the predominant 16:1 hydroxy compound from shark liver oil and that of synthetically prepared 1-*O*-(2-hydroxy-4-hexadecenyl)-2,3-*O*-isopropylideneglycerol were identical.

Methoxy-substituted glycerol ethers with 16:0 16:1, and 18:1 * as the predominating carbon chains have been isolated from shark liver oil.[1] They have also been found in trace quantities in the lipids from other marine animals [2] and from mammals including man.[3] In isolating larger quantities of methoxy-substituted glycerol ethers from other lipids in the unsaponifiable fraction of Greenland shark liver oil, the enrichment steps were checked by thin-layer chromatography (TLC). A small spot with an R_F-value lower than that of the methoxy glycerol ethers was observed. By a series of concentration steps the unknown compounds were obtained in milligram quantities. The R_F-values, compared with those of the unsubstituted and the methoxy-substituted glycerol ethers and their isopropylidene derivatives and later also with synthetically prepared 1-*O*-(2-hydroxyhexadecyl)glycerol [4] and 1-*O*-(2-hydroxyhexadecenyl)glycerol, indicated that the unknown compounds could be hydroxy-substituted glycerol ethers. This was also supported by the gas chromatographic – mass spectrometric (GLC–MS) analysis. The retention time and the mass spectrum of the compound, corresponding to the predominant peak of the gas chromatogram, were identical with the retention time and the mass spectrum of synthetically prepared 1-*O*-(2-hydroxy-4-hexadecenyl)-2,3-*O*-isopropylideneglycerol (Figs. 1 and 2). Hydroxy-substituted tetradecyl, tetradecenyl, hexadecyl, and octadecenyl glycerol ethers were also indicated. It is not likely that the position of the double bond differs from that in the methoxy-substituted glycerol ethers.[1] Therefore the compounds found would be 1-*O*-(2-hydroxytetradecyl)glycerol, 1-*O*-(2-hydroxy-4-tetradecenyl)glycerol, 1-*O*-(2-hydroxyhexadecyl)glycerol,[4,5] 1-*O*-(2-hydroxy-4-hexadecenyl)glycerol, and 1-*O*-(2-hydroxy-4-octadecenyl)glycerol.

In investigations on the biosyntheses of alk-1-enyl glycerol ethers from the corresponding alkyl compounds Blank *et al.*[6] and Snyder *et al.*[7] found an unidentified compound that behaved similarly to a β-hydroxy-*O*-alkylglycerol. It has been proposed [7] that a glycerol ether compound with a substituent such as hydroxy in the 2-position of the alkyl chain could be an intermediate in the biosynthesis of the alk-1-enyl lipids from the alkyl ones. However, in experiments by Muramatsu and Schmid,[8] labelled 1-*O*-(2-hydroxyheptadecyl)-glycerol, which had been formed in rat brain after administration of 1,2-heptadecanediol-2-^{14}C, did not form observable amounts of labelled *O*-alk-1-enyl ether.

* The first figure denotes the number of carbon atoms in the long carbon chain (the methoxy group excluded) and the figure after the colon the number of double bonds.

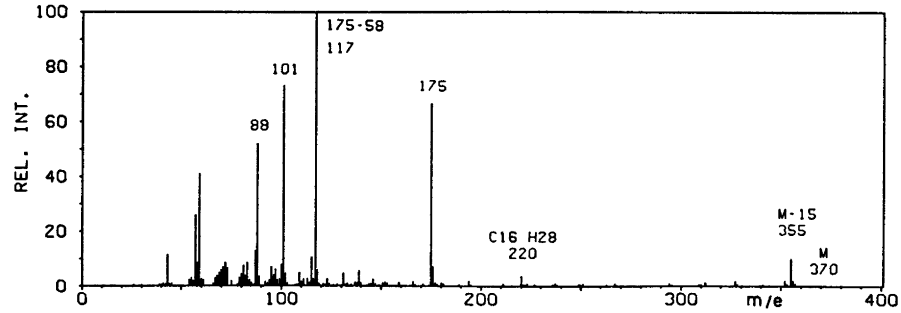


Fig. 1. Mass spectrum of the isopropylidene derivative of 1-*O*-(2-hydroxyhexadecenyl)glycerol, isolated from Greenland shark liver oil.

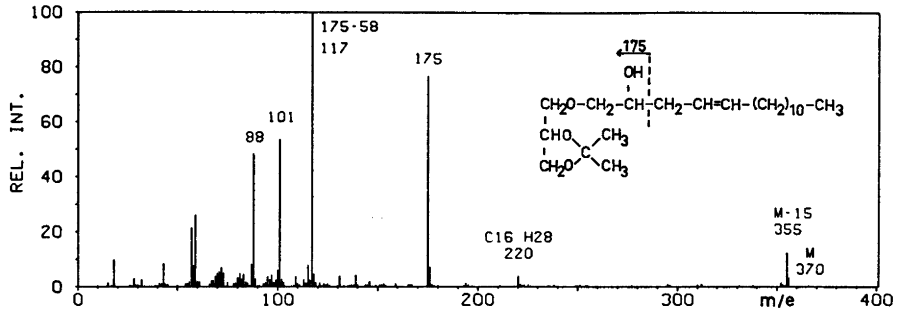


Fig. 2. Mass spectrum of synthetic 1-*O*-(2-hydroxy-4-*cis*-hexadecenyl)-2,3-*O*-isopropylideneglycerol.

EXPERIMENTAL

Enrichment of hydroxy-substituted glycerol ethers from the unsaponifiable fraction of the liver oil from Greenland shark * (*Somniosus microcephalus*) was performed by the following procedure. The unsaponifiable fraction (13 g), obtained after hydrolysis of the liver oil in 1 M ethanolic KOH, was freed from some less polar material by crystallization from acetone (120 ml) at −18 °C. The material (4.0 g) from the filtrate was dissolved in 81 % methanol (125 ml) and extracted continuously with light petroleum, b.p. 40−60 °C, (in an extractor with a sintered disc distributor). The lipids (2.3 g) obtained from the methanol−water phase were chromatographed on a silicic acid (75 g) column (Bio-Sil HA minus 325 mesh, Bio-Rad Laboratories, Richmond Calif). After eluting less polar components with ether, the more polar ones were eluted with methanol. TLC (silica gel G, Merck, developing solvent: trimethylpentane − ethyl acetate − methanol, 50:40:10) showed that the unknown compounds were enriched in the first fractions of the methanol eluate. This material (10 mg) was treated with acetone in the presence of 10^{-2} M $HClO_4$.[9] TLC (developing solvent: trimethylpentane−ethyl acetate, 60:30) indicated that some compounds in the mixture had been transformed to isopropylidene derivatives. Preparative TLC gave 1.4 mg of a material, which was subjected to gas chromatographic-mass spectrometric analysis.

The GLC−MS analysis was performed on a LKB 9000 combination instrument under the following operating conditions: electron energy 70 eV, ion accelerating voltage 3.5 kV, trap current 60 μA, and ion source temperature 270 °C. The gas chromatographic separation was carried out at 210 °C using a 3 m × 3 mm i.d. glass column packed with Gas Chrom Q 80−100 mesh, containing 1 % Apiezon L. The flow was 30 ml helium/min.

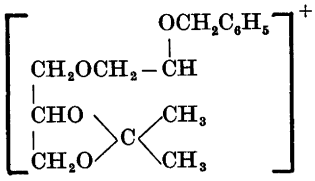
Synthesis of 1-*O*-(2-hydroxy-4-*cis*-hexadecenyl)-2,3-*O*-isopropylideneglycerol

2-Benzyloxy-4-hexadecynyl p-toluenesulfonate and methanesulfonate. Reduction of methyl 2-benzyloxy-4-hexadecynoate [10] (2.95 g) with lithium aluminium hydride gave 2.7 g of crude 2-benzyloxy-4-hexadecyn-1-ol, which by treat-

* The liver oil was supplied by A/S Johan C. Martens and Co., Bergen, Norway.

ment with *p*-toluenesulfonyl chloride (2.0 g) in the presence of dry pyridine [11] gave 3.9 g of almost pure (as shown by TLC) 2-benzyloxy-4-hexadecynyl *p*-toluenesulfonate. The corresponding methanesulfonate was obtained by treatment of the alcohol with methanesulfonyl chloride in the manner described by Baumann and Mangold.[12]

1-O-(2-Benzyloxy-4-hexadecynyl)-2,3-O-isopropylideneglycerol was prepared according to the procedure described by Gupta and Kummerow.[13] Potassium (0.45 g) was granulated by stirring in refluxing dry benzene (25 ml). Isopropylideneglycerol (4.0 g) was slowly added during 15 min. After 1 h, when no more potassium could be observed in the flask, a solution of 2-benzyloxy-4-hexadecynyl *p*-toluenesulfonate (3.9 g) in dry benzene (5 ml) was added. The mixture was refluxed for 5 h. After cooling, ether was added. The benzene–ether solution was washed with water and dried over anhydrous sodium sulfate. The crude product was purified by silicic acid chromatography, yielding 1.57 g (41 %) of almost pure (as shown by TLC) acetonide of 2-benzyloxyhexadecynylglycerol. (On storage the acetonide slowly decomposed into several compounds). The 1-*O*-(2-benzyloxyhexadecynyl)-2,3-*O*-isopropylideneglycerol was also prepared from the methanesulfonate. Tetrahydrofuran was used as solvent instead of benzene. The yield of purified acetonide was 56 % of the calculated amount. The mass spectrum of the acetonide [4] shows a molecular ion peak at $m/e = 458$. The base peak at $m/e = 91$ is probably due to tropylium ions, formed from the benzyl group. Prominent peaks at $m/e = 265$ and $m/e = 313$ correspond to the fragments



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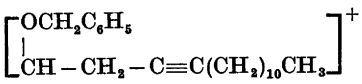

*1-O-(2-Hydroxy-4-*cis*-hexadecenyl)-2,3-O-isopropylideneglycerol.* Selective hydrogenation [14] of 1-*O*-(2-benzyloxy-4-hexadecynyl)-2,3-*O*-isopropylideneglycerol (660 mg) in pyridine solution (5 ml) and with 5 % palladium on barium sulfate (70 mg) as catalyst gave a mixture of benzyloxy- and hydroxy-substituted hexadecenyl compounds (in the proportions 9:1). Chromatography on a silicic acid column (eluent: light petroleum – ether, 9:1) gave 60 mg of 1-*O*-(2-hydroxy-4-hexadecenyl)-2,3-*O*-isopropylideneglycerol (MS in Fig. 2). Hydrolysis of the acetonide in a mixture of ether and concentrated hydrochloric acid [15] and purification by chromatography on a silicic acid column (eluent: ether-methanol, 99:1) gave the free glycerol ether (pure as shown by TLC) as a colourless transparent jelly, which after crystallization at −18 °C melted at about 34 °C (probably polymorphism). The IR spectra of the 1-*O*-(2-hydroxy-4-hexadecenyl)glycerol and of 1-*O*-(2-hydroxyhexadecyl)glycerol were almost identical except in the ranges of the double bond absorptions (≈3010, 1635 and 700 cm^{-1}). The *cis* form of the unsaturated compound was confirmed by the bands at 1635 and 700 cm^{-1}.

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