ACTA CHEMICA SCANDINAVICA 21 (1967) 1519–1529

# Methoxy-substituted Glycerol Ethers Isolated from Greenland Shark Liver Oil

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Glycerol ethers more polar than the ordinary ones were separated from Greenland shark liver oil. The molecular structure was determined by mass spectrometry, NMR, infrared spectroscopy and gas-liquid chromatography. The structure was confirmed by synthesis. The following three methoxy glycerol ethers were isolated from shark liver oil: 1-*O*-(2-methoxyhexadecyl)-glycerol, 1-*O*-(2-methoxy-4-hexadecenyl)-glycerol, and 1-*O*-(2-methoxy-4-octadecenyl)-glycerol.

In a previous study [1] the composition of the glycerol ethers in shark liver oils was determined by gas-liquid chromatography and mass spectrometry. Besides chimyl, batyl, and selachyl alcohols, compounds with saturated $C_{14}$ and monounsaturated $C_{16}$, $C_{20}$, and $C_{22}$ chains were found. The present investigation deals with the separation and identification of hitherto unknown glycerol ethers more polar than the ordinary ones.

## EXPERIMENTAL

*Material.* Liver oil from Greenland shark was freed from some volatile components by molecular distillation. About 10 % of the oil was distilled off. The residue was subjected to alkaline hydrolysis in 1 N ethanolic KOH by boiling under reflux for 1 h. The unsaponifiable material containing the glycerol ethers was extracted from the saponification mixture by diethyl ether.

*Chromatography on silicic acid columns.* Silicic acid specially prepared for the chromatography of lipids was obtained from Calbiochem and was used without further pretreatment. The nonsaponifiable material was dissolved in a mixture of 5 % diethyl ether in light petroleum (b.p. 60–80°C) and applied to columns in amounts of about 15 mg per g of silicic acid. A fraction mainly consisting of hydrocarbons and cholesterol was eluted by 5 % diethyl ether in light petroleum. The glycerol ethers were then eluted by diethyl ether. The effluents were collected in 20 ml fractions.

*Thin-layer chromatography.* The column chromatography was checked by thin-layer chromatography on silica gel G (E. Merck AG, Darmstadt, Germany). In some cases the plates were impregnated with silver nitrate. The plates were activated at 110°C for about 1 h and 40–50 μg of substance dissolved in dichloromethane was applied to each spot. The chromatograms were developed for about 60 min with a solvent mixture of

50 ml trimethylpentane, 40 ml ethyl acetate, and 7 ml methanol. The lipids were detected by charring after spraying with sulphuric acid or in the case of silver nitrate impregnated plates with phosphoric acid.

*Preparation of derivatives.* The isopropylidene derivatives of glycerol ethers were prepared by acetonation at room temperature in the presence of $10^{-2}$ M $HClO_4$ according to Hanahan *et al.*[2] The isopropylidene derivatives were separated from unreacted material by elution with 1 % diethyl ether in light petroleum from a silicic acid column.

Glycerol ethers were converted to their dimethoxy derivatives by treatment with boron trifluoride and diazomethane as described by Müller and Rundel.[3] The dimethoxy derivatives were separated from monomethoxy derivatives and unchanged glycerol ethers by chromatography on silicic acid columns. The dimethoxy derivatives were eluted by light petroleum or 5 % diethyl ether in light petroleum.

*Hydrogenation.* The dimethoxy or isopropylidene derivatives of the glycerol ethers were hydrogenated in ethanol solution with platinum as catalyst. The hydrogenation was performed at atmospheric pressure at room temperature. The samples were shaken for 1 h to achieve complete hydrogenation.

*Oxidation by sodium periodate and potassium permanganate.* In order to study the structure of the glycerol ethers they were subjected to oxidation by a mixture of periodate and permanganate according to the method of von Rudloff [4] in the modification of Hanahan *et al.*[2] The oxidation products dissolved in diethyl ether were esterified with diazomethane and analyzed by gas chromatography and mass spectrometry.

*Gas-liquid chromatography.* A Perkin-Elmer gas chromatograph model 116 with a flame ionization detector was used. Apiezon L, silicone polymer, and polyethylene glycol succinate were used as stationary phases. The columns were made of aluminium tubing with an inner diameter of 4 mm. The Apiezon column, 1.8 m, was of the low loaded type containing 1 % Apiezon L and 0.1 % polyethylene glycol M 20 000 by weight of the solid support, which consisted of Gas Chrom P, 100—200 mesh (Applied Science Laboratories Inc., State College, Pa.). The Gas Chrom P was treated with dimethyldichlorosilane. The silicone column was of 2 m length and contained 3 % silicone polymer SE-30 (General Electric Co.) on silazane-treated Gas Chrom P, 100—200 mesh. The succinate column, 1.8 m, contained 15 % polyethylene glycol succinate on Gas Chrom P, 100—120 mesh. Helium was used as carrier gas.

*Mass spectrometry.* The mass spectra were registered at the Laboratory for Mass Spectrometry, Karolinska Institutet, Stockholm, Sweden, using a Dempster type of instrument.[5]

*NMR spectroscopy.* The NMR investigations were performed by Dr. Carl Lagercrantz, Institute of Medical Physics, University of Gothenburg, Sweden. The NMR spectra were measured in carbon tetrachloride solution, with a Varian A 60 instrument. Tetramethylsilane was used as reference standard (0 ppm).

*Infrared spectroscopy.* The infrared spectra were registered with a Perkin-Elmer double-beam grating spectrophotometer model 237. The spectra were measured in carbon tetrachloride solution.

## Synthesis of 1-*O*-(2-methoxyhexadecyl)-glycerol

*Methyl 2-bromohexadecanoate* was prepared according to the method of Schwenk and Papa.[6] Palmitic acid (76.8 g, 0.30 mole) of high purity as checked by gas chromatography was converted to the acid chloride by refluxing with thionyl chloride (175 ml) for 3 h. Then bromine (51 g, 0.32 mole) was added dropwise during a period of 3 h. After refluxing for another hour excess bromine and thionyl chloride was distilled off, and the residue slowly poured into methanol *p.a.* (500 ml). After standing over night, water was added and the bromo ester was extracted with ether. The ether extract was washed with dilute sodium bicarbonate and water and dried over magnesium sulphate. After evaporation of the ether the residue was fractionated *in vacuo* and gave 84.6 g (81 % yield) of methyl 2-bromohexadecanoate b.p. 146—148° (0.1 mm). $n_D^{25}$ 1.4610.

*Methyl 2-methoxyhexadecanoate.* To a solution of methyl 2-bromohexadecanoate (26.5 g 0.076 mole) in methanol *p.a.* (25 ml) a solution of sodium methoxide, prepared from sodium (2 g, 0.087 mole) and methanol p.a. (25 ml), was added.[7] After refluxing for 3 h water was added and the product was extracted with ether. The ether solution

was dried over magnesium sulphate, the ether evaporated and the residue fractionated *in vacuo*. There was obtained 21.6 g (95 % yield) of methyl 2-methoxyhexadecanoate, b.p. 148—150° (0.1 mm), $n_D^{23}$ 1.4415. After solidification it melted at about 30°.

*2-Methoxyhexadecanol-1* was prepared by reduction of the methyl ester just described with lithium aluminium hydride. Methyl 2-methoxyhexadecanoate (18.7 g) dissolved in dry ether (80 ml) was added drop by drop to a solution of lithium aluminium hydride (2.3 g) in dry ether (50 ml) during continuous stirring. After 15 min firstly ethyl acetate and then water was added cautiously to destroy the excess of lithium aluminium hydride. During cooling in ice-water the solution was acidified with diluted sulphuric acid and then extracted with ether. There was obtained 16.5 g of a colourless liquid, which slowly solidified at room temperature. The product was recrystallized twice from a mixture of acetone and water (5:1). 12.4 g (73 % yield) of a white powder, m.p. 34.5—35.5°, was obtained.

*2-Methoxyhexadecyl* p-*toluenesulfonate* was prepared by treating 2-methoxyhexadecanol (1.9 g) with *p*-toluenesulfonylchloride in pyridine.[8] *p*-Toluenesulfonate (2.6 g) with a melting point of 38—39° was obtained. Thin-layer chromatography (trimethylpentane 70, ethyl acetate 20) showed that the esterification was complete.

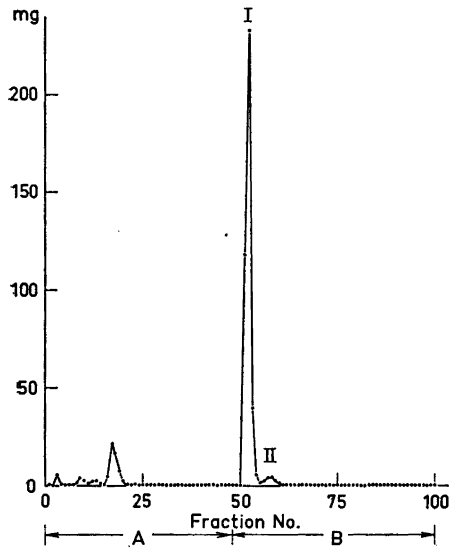


*Fig. 1.* Chromatography of non-saponifiable material (467 mg) from Greenland shark liver oil on a silicic acid column (35 g). Eluting solvents: 5 % diethyl ether in light petroleum, b.p. 60—80° (A) and diethyl ether (B), Fraction volume: 20 ml.

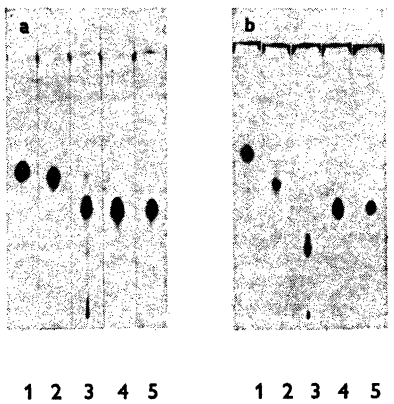


*Fig. 2a and 2b.* Thin-layer chromatograms on silica gel G (Fig. 2a) and on silver nitrate impregnated silica gel G (Fig. 2b) with trimethylpentane — ethyl acetate — methanol, 50:40:7, as solvent.

1. Batyl alcohol
2. Ordinary glycerol ethers isolated by silicic acid column chromatography (peak I, Fig. 1)
3. Glycerol ethers eluted after the ordinary ones during the silicic acid column chromatography (peak II, Fig. 1)
4. Ditto after hydrogenation
5. Synthetic 1-*O*-(2-methoxyhexadecyl)-glycerol.

*2,3-O-Isopropylidene-1-O-(2-methoxyhexadecyl)-glycerol* was prepared by condensing the methoxyhexadecyl *p*-toluenesulfonate (2.5 g) with 1,2-isopropylidene glycerol[9] (1.1 g) as described by Gupta and Kummerow.[10] The crude product was purified by silicic acid chromatography using light petroleum (b.p. 40—60°) as solvent. About 45 % of the crude product consisted of the 2,3-*O*-isopropylidene compound of the glycerol ether.

*1-O-(2-methoxyhexadecyl)-glycerol.* The isopropylidene compound (78 mg) was treated with 0.5 N HCl (50 µl) at 100° for 3 h during continuous shaking. The glycerol ether was extracted by ether and after evaporation of the ether a colourless liquid (72 mg) was obtained. Thin-layer chromatography showed that the splitting of the isopropylidene compound was nearly complete. The product was purified by silicic acid chromatography, the less polar contaminants were removed by elution with 5 % diethyl ether in light petroleum and the free glycerol ether was then eluted with ether. 83 % of the crude product consisted of free glycerol ether.

## RESULTS AND DISCUSSION

During the chromatography of the unsaponifiable material of shark liver oil on silicic acid columns the main part of the glycerol ethers was eluted as a sharp peak by diethyl ether (peak I, Fig. 1). Immediately after this peak a small one appeared (peak II, Fig. 1). After thin-layer chromatography the material from the small peak was obtained as a distinct spot with a lower $R_F$-value than that of the ordinary glycerol ethers (Fig. 2a). Thin-layer chro-

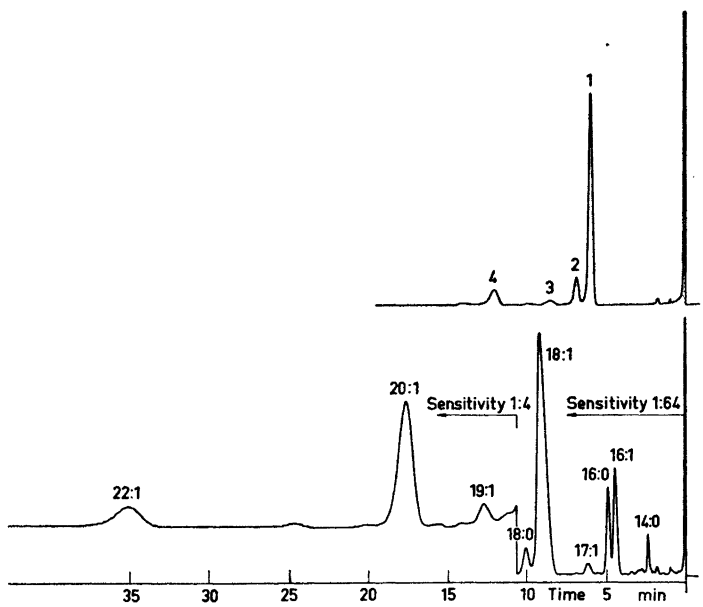


*Fig. 3.* Gas-liquid chromatograms of the isopropylidene derivatives of glycerol ethers. Lower curve: Ordinary glycerol ethers isolated by silicic acid column chromatography (peak I, Fig. 1). Upper curve: Glycerol ethers eluted after the ordinary ones (peak II, Fig. 1). Column: Apiezon L, 1 % and polyethylene glycol, 0.1 % by weight of the solid support, Gas Chrom P, 100—120 mesh. Temperature: 218°.

matography on silver nitrate impregnated plates showed that the main part consisted of unsaturated compounds (Fig. 2b). The isopropylidene, as well as the dimethoxy, derivatives of the glycerol ethers from peak I and peak II were subjected to gas-liquid chromatography both before and after hydrogenation. A comparison of the gas-liquid chromatograms of the isopropylidene derivatives of the ordinary glycerol ethers from peak I and the corresponding derivatives of the compounds in peak II on Apiezon L showed that the main compound in peak II had a retention time similar to that of a glycerol ether with heptadecenyl as long alkyl chain (Fig. 3). There were also compounds

*Table 1.* Retention times of the isopropylidene derivatives of the ordinary glycerol ethers (I) and those in the small peak (II) of the silicic acid chromatogram relative to the isopropylidene derivative of batyl alcohol.

| Glycerol ethers I * | Retention at 218° on Apiezon L (1 %) | | Retention at 194° on polyethylene glycol succinate (15 %) | |
|---|---|---|---|---|
| | Before hydrogenation | After hydrogenation | Before hydrogenation | After hydrogenation |
| 14:0 | 0.24 | 0.23 | 0.34 | 0.34 |
| 16:0 | 0.49 | 0.48 | 0.58 | 0.58 |
| 16:1 | 0.44 | 0.48 | 0.69 | 0.58 |
| 17:0 | 0.69 | 0.68 | 0.75 | 0.75 |
| 17:1 | 0.61 | 0.68 | 0.88 | 0.75 |
| 18:0 | 1.00 | 1.00 | 1.00 | 1.00 |
| 18:1 | 0.91 | 1.00 | 1.15 | 1.00 |
| 19:1 | 1.26 | 1.40 | 1.42 | 1.28 |
| 20:1 | 1.75 | 1.99 | 1.90 | 1.67 |
| 22:1 | 3.48 | 4.03 | 3.23 | 2.89 |
| Glycerol ethers II ** | | | | |
| 1 | 0.59 | 0.68 | 1.30 | 1.21 |
| 2 | 0.68 | 0.68 | 1.21 | 1.21 |
| 3 | 0.84 | 0.97 | 1.67 | 1.55 |
| 4 | 1.19 | 1.38 | 2.17 | 2.02 |

* The glycerol ethers are represented by the long alkyl chain of the molecule.

** The figures 1, 2, 3, and 4 refer to the components in the gas-liquid chromatogram on Apiezon L (Fig. 3, upper curve).

with retention times similar to those of glycerol ethers with a saturated $C_{17}$ alkyl chain and a mono-unsaturated $C_{19}$ chain. Traces of other homologues were also found. Gas-liquid chromatography on polyethylene glycol succinate of the isopropylidene derivatives of the compounds from peak II, before and after hydrogenation, indicated that the main part of the unsaturated components were mono-unsaturated (Table 1). Gas-liquid chromatography and mass spectrometry of the esterified oxidation products of the components in peak II showed the presence of methyl esters of mainly dodecanoic acid and tetradecanoic acid. This indicates that the double bond is situated between carbon atoms Nos. 12 and 13 calculated from the free end of the long hydro-

carbon chain in the mono-unsaturated glycerol ether with the lower molecular weight and between carbon atoms Nos. 14 and 15 in the higher mono-unsaturated homologue.

The retention times of the unknown saturated isopropylidene glycerol ethers derived from peak II relative to the saturated ordinary ones were longer on a polar phase such as polyethylene glycol succinate than on Apiezon and silicone polymer indicating that the compounds contained some polar group. The retention time relative to the isopropylidene derivative of batyl alcohol for the hydrogenated main component of peak II was 0.68 on Apiezon and 1.21 on polyethylene glycol succinate (Table 1). A linear relationship was found between the logarithms of the relative retention times and the number of carbon atoms in the long hydrocarbon chains for the saturated as well as the mono-unsaturated unknown isopropylidene glycerol ethers.

The NMR spectrum of the free glycerol ethers from peak II showed a peak at about 3.3 ppm which was absent in the spectrum of the ordinary glycerol ethers. This peak could be due to the presence of a methoxy group in the new compounds. The isopropylidene or dimethoxy derivatives of the unknown glycerol ethers were separated by gas-liquid chromatography on a silicone column before and after hydrogenation and the different components were collected and subjected to mass spectrometry. The mass spectra of the isopropylidene derivatives are shown in Figs. 4—7. The molecule ion of the main component had apparently *m/e* 384 and that of the higher homologue *m/e* 412, corresponding to molecular weights of 344 and 372 for the free glycerol ethers. After hydrogenation the molecular weights of the isopropylidene derivatives increased to 386 and 414, respectively. The base peaks of the mass spectra of the hydrogenated compounds had mass numbers of 241 and 269, respectively. The fragmentation patterns could be explained by a structure with a methoxy group attached to the $\beta$-carbon atom of the long alkyl chain. The base peaks of the mono-unsaturated compounds had mass numbers of 131 corresponding to a cleavage of the molecule at the glycerol ether bond.

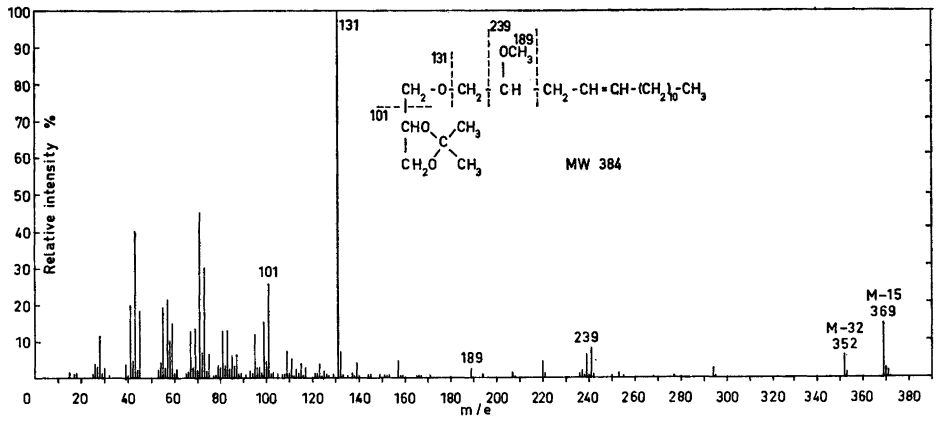


*Fig. 4.* Mass spectrum of 2,3-*O*-isopropylidene-1-*O*-(2-methoxy-4-hexadecenyl)-glycerol from Greenland shark liver oil.

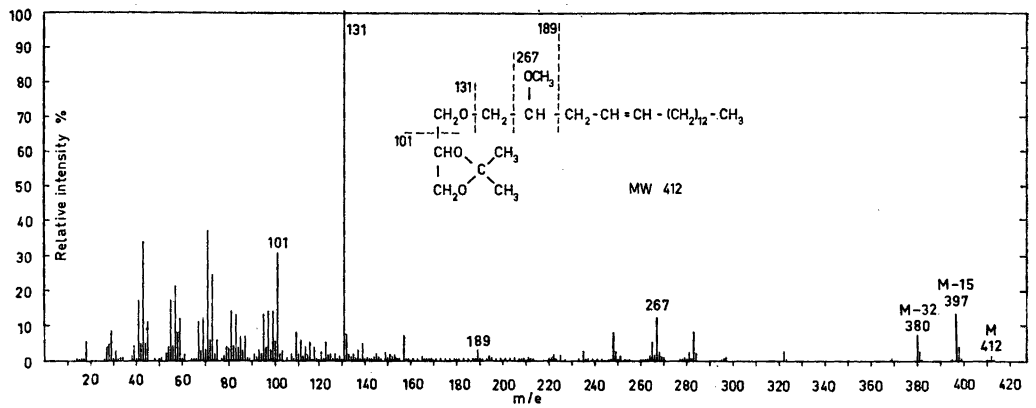


*Fig. 5.* Mass spectrum of 2,3-*O*-isopropylidene-1-*O*-(2-methoxy-4-octadecenyl)-glycerol from Greenland shark liver oil.

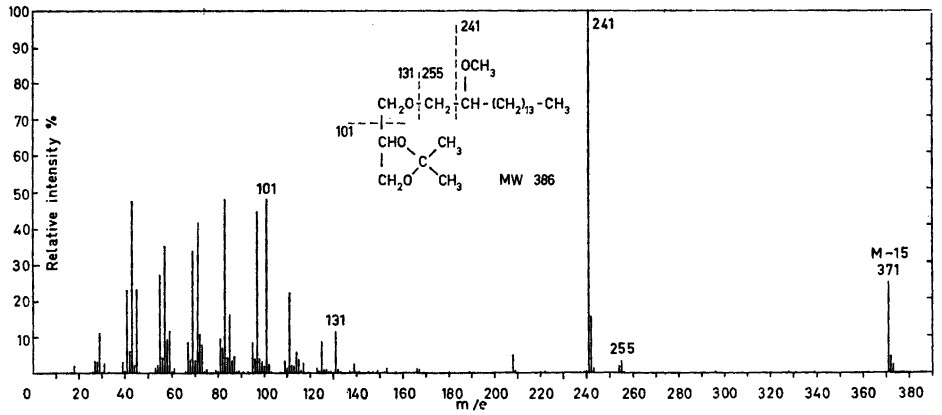


*Fig. 6.* Mass spectrum of 2,3-*O*-isopropylidene-1-*O*-(2-methoxyhexadecyl)-glycerol from Greenland shark liver oil.

The mass spectrum of the isopropylidene derivative of batyl alcohol (Fig. 9) showed a different fragmentation pattern with a base peak of mass number 101.

The mass spectrum of the dimethoxy derivative of the hydrogenated glycerol ether with the lower molecular weight (Fig. 10) had a base peak at *m/e* 241. The mass spectrum of the dimethoxy derivative supports the anticipated molecular structure.

In order to confirm the structure determined by mass spectrometry the saturated methoxy glycerol ether with the molecular weight of 346 and a methoxy group attached to the $\beta$-carbon atom of the alkyl chain was synthesized. The mass spectrum of the isopropylidene derivative of the synthesized methoxy glycerol ether (Fig. 8) was identical with the corresponding derivative of the hydrogenated natural product (Fig. 6).

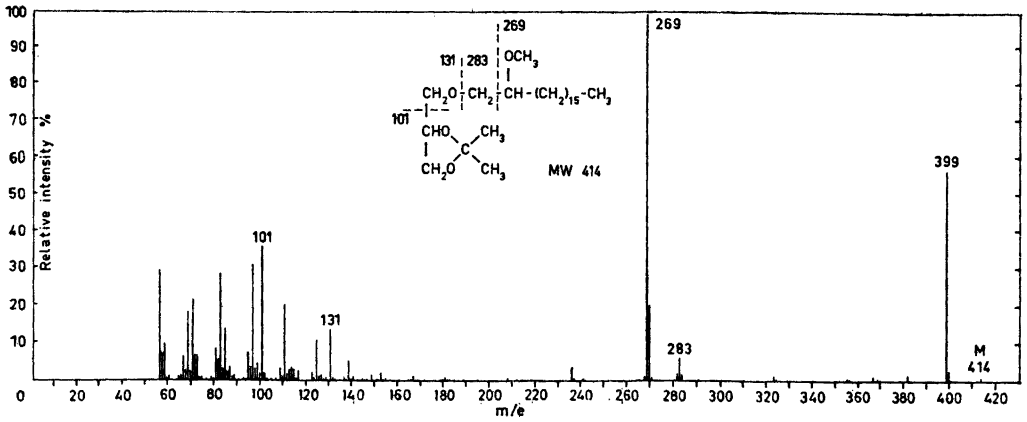


*Fig. 7.* Mass spectrum of 2,3-*O*-isopropylidene-1-*O*-(2-methoxyoctadecyl)-glycerol from Greenland shark liver oil.

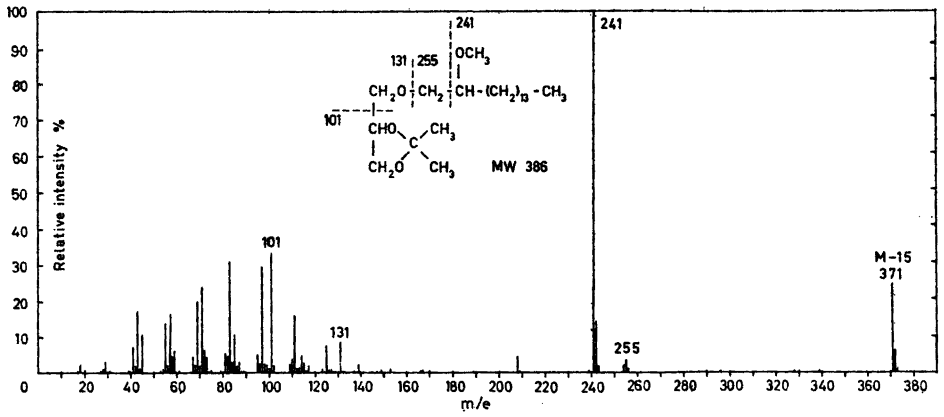


*Fig. 8.* Mass spectrum of synthetic 2,3-*O*-isopropylidene-1-*O*-(2-methoxyhexadecyl)-glycerol.

The NMR spectrum of the synthesized methoxy glycerol ether (Fig. 11) was very similar to that of the hydrogenated methoxy glycerol ether from the shark liver oil except for peaks due to the proton resonances of the hydroxyl groups, which are dependent on concentration and temperature. The spectra were run at 40° but at different concentrations. For the unhydrogenated glycerol ethers peaks were registered at about 5.2 ppm due to the presence of the double bond.

The infrared spectra of the methoxy glycerol ethers differ from those of the ordinary glycerol ethers in the ether bond absorption range, 1050—1150 $cm^{-1}$. At 2830 $cm^{-1}$ a slight bend could be observed in the spectra of the methoxy glycerol ethers. This absorption was not found in the spectra of the ordinary

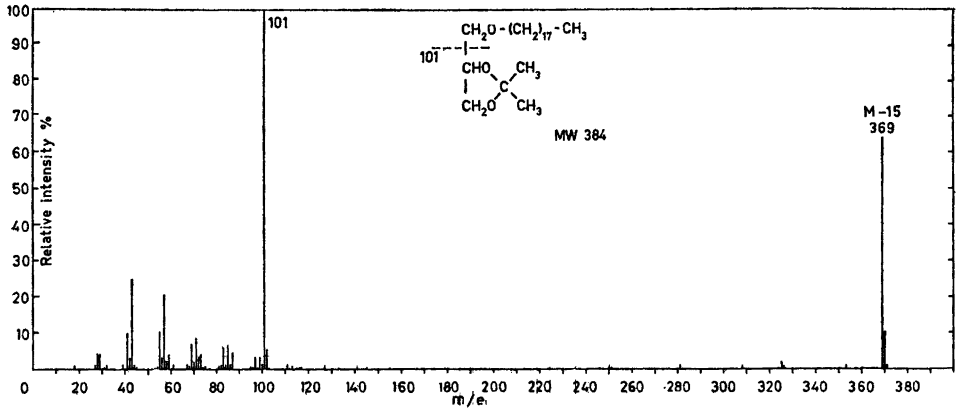


*Fig. 9.* Mass spectrum of 2,3-*O*-isopropylidene-1-*O*-octadecylglycerol (isopropylidene batyl alcohol).

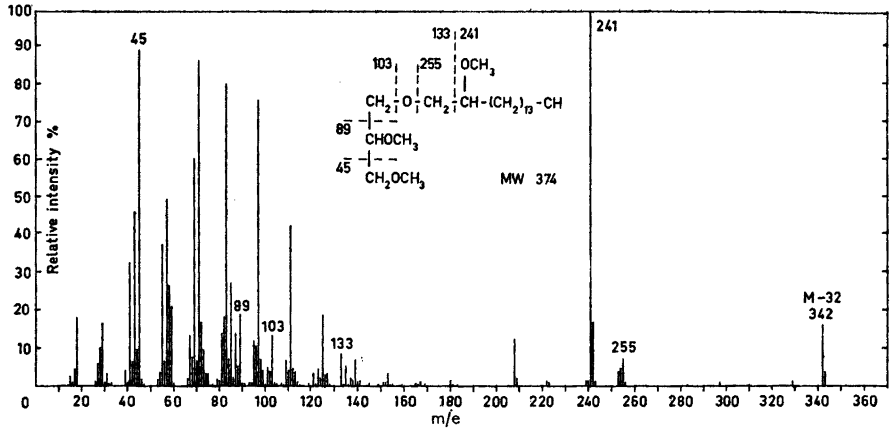


*Fig. 10.* Mass spectrum of 2,3-di-*O*-methyl-1-*O*-(2-methoxyhexadecyl)-glycerol from Greenland shark liver oil.

glycerol ethers and was most probably due to the methoxy group. The absorption at 1410 $cm^{-1}$ and possibly that at 1655 $cm^{-1}$ in the spectra of the mono-unsaturated compounds indicates a *cis*-form. The infrared spectrum of the hydrogenated methoxy glycerol ethers isolated from shark liver oil was identical with that of the synthesized compound (Fig. 12).

The gas-liquid chromatograms of the isopropylidene derivatives of the methoxy glycerol ethers in shark liver oils after hydrogenation were compared with those of the synthetic compound. The main component after hydrogenation had exactly the same retention time as the synthetic compound on several different stationary phases. The compounds were run on Apiezon L at 218°,

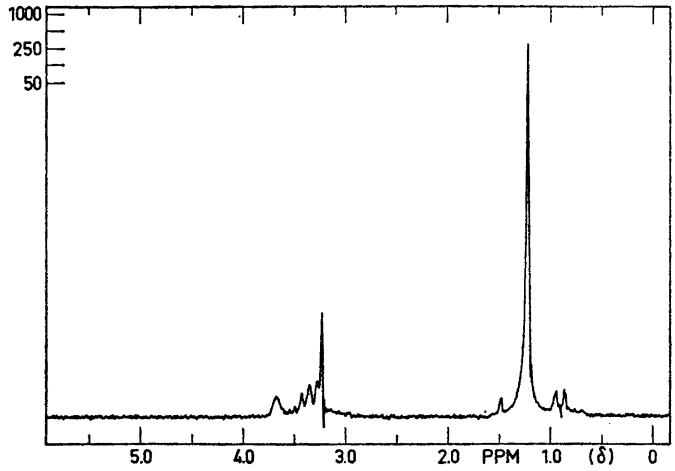


*Fig. 11.* NMR spectrum of synthetic 1-*O*-(2-methoxyhexadecyl)-glycerol. Sample: 46 mg per ml $CCl_4$.

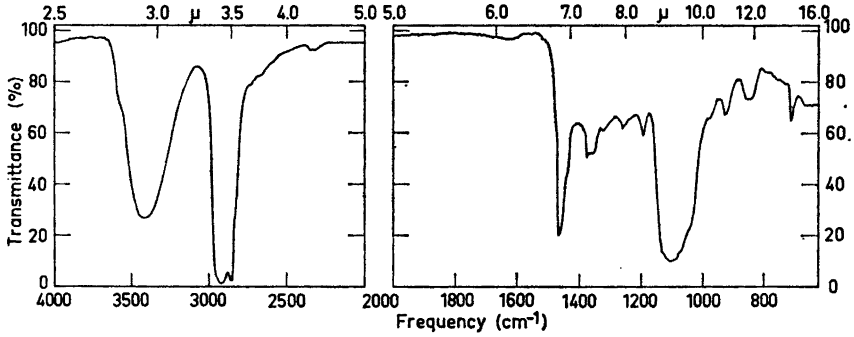


*Fig. 12.* Infrared spectrum of synthetic 1-*O*-(2-methoxyhexadecyl)-glycerol. Solvent $CCl_4$.

on silicone polymer at 227°, on fluorosilicone at 200° and 215°, on polyethylene glycol succinate at 194°, and on a mixture of polyethylene glycol succinate and adipate (1:3) at 190°. On all these phases the retention time of the synthetic compound was identical with that of the corresponding hydrogenated natural component.

The data from mass spectrometry, NMR and infrared spectroscopy, and gas-liquid chromatography on several stationary phases definitely show that the structure of the saturated natural methoxy glycerol ether with a molecular weight of 346 is identical with that of the synthetic 1-*O*-(2-methoxyhexadecyl)-glycerol. The mass spectra of the mono-unsaturated compounds as well as

those of their oxidation products separated by gas-liquid chromatography show the presence of 1-*O*-(2-methoxy-4-hexadecenyl)-glycerol and 1-*O*-(2-methoxy-4-octadecenyl)-glycerol.

As the new compounds contain two asymmetric carbon atoms four isomeric forms are possible. However, the configuration of the compounds has not yet been determined.

About 4 % of the glycerol ethers in the studied Greenland shark liver oils was found to be methoxy glycerol ethers. The quantitative distribution within the mixture of methoxy glycerol ethers was about 60 % 1-*O*-(2-methoxy-4-hexadecenyl)-glycerol, 15 % 1-*O*-(2-methoxyhexadecyl)-glycerol, and 20 % 1-*O*-(2-methoxy-4-octadecenyl)-glycerol.

*Acknowledgements.* The skilful technical assistance of Mrs. Ingela Strömer, Mrs. Anita Niklasson and Miss Berit Andersson is gratefully acknowledged.

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Received March 1, 1967.