

The Separation and Identification of a Methoxy-substituted Polyunsaturated Glycerol Ether From Greenland Shark Liver Oil

BO HALLGREN, GUNNEL STÄLLBERG and HANS THORIN

Research Laboratories, Astra Nutrition AB, Mölndal, Sweden

A polyunsaturated methoxy-substituted glycerol ether has been isolated from Greenland shark liver oil. The positions of the double bonds have been determined by converting the polyunsaturated ether to a polymethoxy-compound, which was analysed by gas chromatography-mass spectrometry. The structure of the glycerol ether was found to be 1-*O*-(2-methoxy-4,7,10,13,16,19-docosaheptaenyl)-glycerol.

Methoxy-substituted glycerol ethers have been isolated from Greenland shark liver oil.¹ In the mixture of glycerol ethers obtained from this oil the methoxy-substituted components amounted to about 4 % (w/w). Saturated and monounsaturated methoxy glycerol ethers with 14, 15, 16, 17, 18, 19, 20, and 22 carbon atoms in the long hydrocarbon chains (the methoxy groups not included) were identified. The ethers with 16 and 18 carbon atoms in the long alkyl chains constituted the main part, *i.e.* about 90 %. The present study describes the isolation and identification of a polyunsaturated methoxy-substituted glycerol ether. This polyunsaturated component amounted to 3-4 % of the methoxy glycerol ethers.

The mixture of saturated, mono- and polyunsaturated methoxy-substituted glycerol ethers was separated from the unsubstituted glycerol ethers and converted to isopropylidene derivatives as earlier described.¹ The isopropylidene derivative of the polyunsaturated methoxy-substituted glycerol ether was enriched by column chromatography, until the mixture contained about 85 % of the polyunsaturated component. Gas chromatography before and after hydrogenation of the mixture indicated a glycerol ether with 22 carbon atoms in the long alkyl chain.

Combined gas chromatography-mass spectrometry of the material before and after hydrogenation gave molecular weights of 458 and 470, respectively, indicating six double bonds. The mass spectrum of the hydrogenated compound shows the same characteristic fragmentation pattern as the spectra of 2,3-*O*-isopropylidene-1-*O*-(2-methoxyhexadecyl)-glycerol and 2,3-*O*-isopropylidene-1-*O*-(2-methoxyoctadecyl)-glycerol.¹

The UV spectrum of the material containing about 85 % polyunsaturated methoxy-substituted glycerol ether did not show any appreciable absorption at wavelengths characteristic of conjugated double bonds. The NMR spectrum of the same material had multiplets at about $\delta=2.1$, 2.8, and 5.3 ppm [$(\text{CH}_3)_4\text{Si}=0$ ppm], which indicate CH_2 -groups adjacent to one doubly bonded carbon atom, CH_2 -groups between two doubly bonded carbon atoms, and $\text{CH}=\text{CH}$ -groups adjacent to CH_2 -groups, respectively. The IR spectrum shows a weak band at 1640 cm^{-1} and a strong band at 700 cm^{-1} which indicate *cis* double bonds.

The positions of the double bonds were determined by converting the polyunsaturated glycerol ether to a polymethoxy compound, which was analysed by gas chromatography-mass spectrometry using the method developed by Niehaus and Ryhage³ for the determination of double bond positions in fatty acids. *cis*-Addition of osmium tetroxide to the six double bonds in the methoxy glycerol ether gives six osmate ester groups, which by reductive cleavage are split to six *vic*-diol groups. By methylation of these twelve hydroxy groups and the two hydroxy groups of the glycerol part of the molecule a compound with fifteen methoxy groups and with a molecular weight of 818 should be obtained. The mass spectrum (Fig. 1) of the main product obtained after the oxidation and methylation indicated the structure given in Fig. 2.

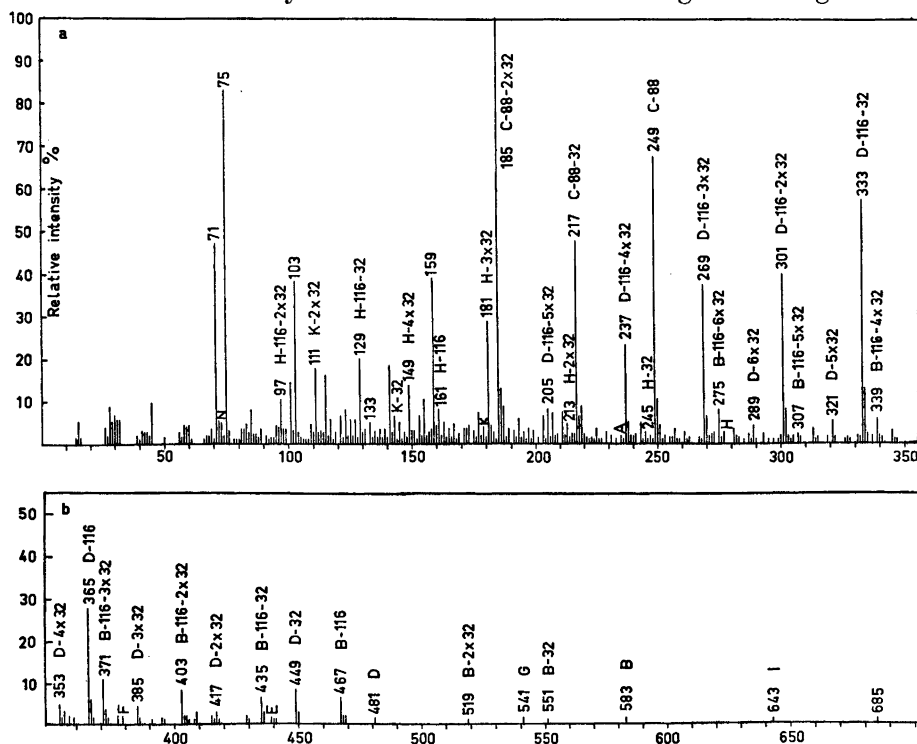
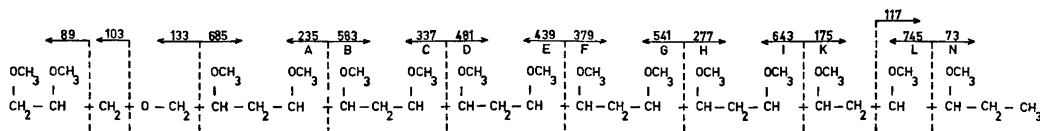


Fig. 1. Mass spectrum of the polymethoxy derivative obtained from 1-*O*-(2-methoxydocosaheptaenyl)-glycerol.

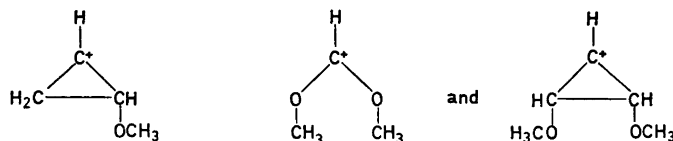


M-818

Fig. 2. Structure of the polymethoxy derivative obtained from 1-*O*-(2-methoxydocosaheptaenyl)-glycerol.

No molecular ion is seen. The principal fragmentation occurs between the second pair of methoxy-substituted carbon atoms numbered from the oxygen atom in the long chain, giving the ion fragments C ($m/e = 337$) and D ($m/e = 481$). These fragments further lose one or several elements of methanol, but more predominant are the losses of the end fragments $\text{CH}_2(\text{OCH}_3) - \text{CH}(\text{OCH}_3) -$ and $-\text{CH}(\text{OCH}_3) - \text{CH}(\text{OCH}_3) - \text{CH}_2 - \text{CH}_3$ with successive losses of methanol. The fragments arising from cleavages between the other pairs of methoxy-substituted carbon atoms would have m/e values of 73, 175, 235, 277, 379, 439, 541, 583, 643, and 745. All these fragments except $m/e = 745$ is also seen, but in this part of the spectrum the exact positions of the peaks are uncertain.

The fragmentation pattern is in accordance with those reported by Niehaus and Ryhage,² who found that the principal fragmentations in their methoxy-substituted esters occurred between the methoxy-substituted carbon atoms and also adjacent to the final pair of methoxy-substituted carbons. Another similarity with the spectra of Niehaus and Ryhage is the presence of peaks at $m/e = 71, 75,$ and 101 , for which these authors propose the structures



The mass spectrum of the polymethoxy compound shows that the double bonds of the polyunsaturated chain were at positions 4, 7, 10, 13, 16, and 19. The structure of the glycerol ether thus was 1-*O*-(2-methoxy-4,7,10,13,16,19-docosaheptaenyl)-glycerol.

This polyunsaturated glycerol ether was also found in trace quantities in the lipids of mackerel fillets, shrimps, cod liver oil,³ and human red blood cells.⁴

EXPERIMENTAL

Enrichment of polyunsaturated methoxy-substituted glycerol ether. The mixture of the isopropylidene derivatives of methoxy-substituted glycerol ethers was chromatographed on silicic acid columns, using silicic acid specifically prepared for lipid chromatography

(Bio-Sil HA minus 325 mesh, Bio-Rad Laboratories, Richmond, Calif.). 5% diethyl ether in light petroleum (b.p. 40–60°C) was used as eluent. The polyunsaturated compound was enriched in the last fractions. After repeated chromatography a mixture containing about 85% of the polyunsaturated component was obtained. The purification was controlled by thin-layer chromatography on silica gel G using trimethylpentane-ethyl acetate (70:20 v/v) as solvent. The R_F -value for the polyunsaturated component was 0.29 and for the saturated and monounsaturated components 0.35.

The free glycerol ether was obtained by treatment of the isopropylidene derivative (62 mg) with a mixture of diethyl ether (0.5 ml) and concentrated hydrochloric acid (0.5 ml) at 0°C during stirring for 1 h (cf. Ref. 5). Distilled water (4 ml) was added and the stirring at 0°C was continued for another hour. The product was taken up in ether. After evaporation of the ether the residue was purified by chromatography on a silicic acid column. A small amount of less polar material was eluted with 5% diethyl ether in light petroleum (b.p. 40–60°C) and then the glycerol ether was eluted with diethyl ether.

Osmium tetroxide oxidation and methylation. The mixture of methoxy-substituted glycerol ethers containing about 85% of the polyunsaturated component (9 mg) was treated^{2,6} with osmium tetroxide (100 mg) in dioxane (16 ml). Pyridine (2 ml) was added to catalyze the oxidation.⁷ After 80 min at room temperature a mixture of sodium sulphite (3.2 g), water (20 ml), and methanol (5 ml) was added. The mixture was kept at room temperature for 90 min and then filtered. The filtrate was evaporated to dryness. The residue was suspended in methanol and again filtered. The residue after evaporation of the methanol was extracted with ether and then with methanol. The ether extract, containing the less polar material was discarded.

The methanol extract containing the polyhydroxy compound was evaporated to dryness. A solution of dimethylsulphinyl carbanion in dimethylsulphoxide, prepared from sodium hydride (5 mg) and dimethylsulphoxide (1 ml) was added to the residue (nitrogen atmosphere). After 30 min methyl iodide in excess was added drop by drop. The mixture was left at room temperature for another 30 min. The methylated product, taken up in dichloromethane, was directly used for the gas chromatography-mass spectrometry analysis.

Gas-liquid chromatography. The gas chromatography was performed with a Perkin-Elmer F 11 instrument equipped with a flame ionization detector. A 180 cm × 2 mm i.d. stainless steel column, packed with Gas Chrom Q 80–100 mesh containing 1% Apiezon L was used. The column temperature was 220°C.

Gas-liquid chromatography-mass spectrometry. The GLC-MS was carried out with the LKB 9000 combination instrument. The operating conditions of the mass spectrometer were: electron energy 70 eV, ion source temperature 270°C, trap current 60 μ A and accelerating voltage 3.5 kV.

The gas chromatography on the combined instrument was carried out at 220°C using a 300 cm × 2 mm i.d. glass column packed with Gas Chrom Q 80–100 mesh, containing 1% Apiezon L.

Infrared spectroscopy. The IR spectrum was registered with a Perkin-Elmer double beam grating spectrophotometer model 237.

NMR spectroscopy. The NMR spectrum was recorded on a Varian A 60 spectrometer using tetramethylsilane as internal standard. The sample was dissolved in carbon tetrachloride.

REFERENCES

1. Hallgren, B. and Ställberg, G. *Acta Chem. Scand.* **21** (1967) 1519.
2. Niehaus, Jr., W. G. and Ryhage, R. *Anal. Chem.* **40** (1968) 1840.
3. Hallgren, B., Niklasson, A., Ställberg, G. and Thorin, H. *To be published.*
4. Hallgren, B., Niklasson, A., Ställberg, G. and Thorin, H. *To be published.*
5. Baer, E. and Fischer, H. O. L. *J. Am. Chem. Soc.* **67** (1945) 2031.
6. McCloskey, J. A. and McClelland, M. J. *J. Am. Chem. Soc.* **87** (1965) 5090.
7. Criegee, R., Marchand, B. and Wannowius, H. *Ann.* **550** (1942) 99.

Received March 25, 1971.