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The glyceryl ethers in the liver oils of elasmobranch fish*

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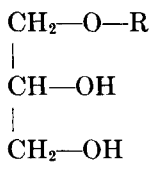
SUMMARY

The glyceryl ethers from the liver oils of the grey dogfish, the Greenland shark, and the ratfish were isolated by chromatography on alumina. These ethers were then converted into the corresponding dimethoxy derivatives, which were rechromatographed on alumina and then submitted to gas-liquid chromatography (GLC). By these means the presence of several new glyceryl ethers was demonstrated. Besides chimyl, batyl, and selachyl alcohols, compounds with saturated C_{14} and monounsaturated C_{16}, C_{20}, and C_{22} chains were found. Small amounts of a glyceryl ether with a C_{18} chain with two double bonds were also present. By oxidation with chromic acid, the position of the double bond in the hexadecenyl chain was found to be 9:10; in the eicosenyl and the docosenyl chains the double bond was in position 13:14. The molecular weight of the dimethoxy derivatives was determined by mass spectrometry. The mass spectrometric fragmentation pattern provided additional proof of the structure of the compounds.

The naturally occurring glyceryl ethers can be represented by the following formula:



Hitherto, three such compounds have been characterized: batyl alcohol with stearyl, selachyl alcohol with oleyl, and chimyl alcohol with palmityl as the long-chain part of the molecule. The glyceryl ethers are more widespread in marine animals than in land animals (1). In the liver oils of several elasmobranch fish, the glyceryl ethers constitute a large proportion of the lipids (2). In fish oils, these compounds seem to be present in the form of their fatty acid diesters.

The present study was undertaken to find a convenient method for the isolation and identification of the glyceryl ethers in different tissues (3). As these compounds are available in several fish oils, we studied the liver oils of the grey dogfish (*Squalus acanthias* L.), the Greenland shark (*Somniosus microcephalus* Schneid.), and the ratfish (*Chimaera monstrosa* L.). The starting material in the case of the grey dogfish was a fresh liver,[1] which was homogenized in a Turmix blender and extracted with chloroform-methanol, 2:1 (v/v). The fresh liver contained 59% lipids (wet weight). In the case of the Greenland shark[2] and the ratfish,[3] we started with the liver oils. The percentage of nonsaponifiable matter and its glyceryl ether composition are summarized in Table 1.

EXPERIMENTAL METHODS

Hydrolysis and Extraction of Nonsaponifiable Material. One volume of the oil was hydrolyzed in 10 volumes of 1 N methanolic KOH by boiling under reflux for 2 hours. About half the volume of the methanol was then distilled off. The remaining mixture was diluted with an equal volume of water and extracted four times with diethyl ether. The ether solution was washed with water, and the ether was evaporated under reduced pressure (maximum temperature 30°) to a small volume. Petroleum ether (b.p. 40° to 60°) was added and the solution was dried over sodium sulfate. After filtration, most of the solvent was distilled off under reduced pressure—the temperature not exceeding 40°. An aliquot of the remaining solution was taken for gravimetric determination.

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[1] The dogfish liver was a gift from Mr. M. Öberg, Gothenburg.
[2] Supplied by A. S. Johan Martens & Co., Bergen, Norway.
[3] Obtained from Dr. S. Brohult, Stockholm, Sweden.

Chromatography on Activated Alumina. The glyceryl ethers were separated from other nonsaponifiable material (e.g., cholesterol) and from contaminating soaps by chromatography on alumina pretreated as described by Asselineau (4).

One kilogram of aluminum oxide was suspended in 2 liters of water, and hydrochloric acid was added so that the pH was maintained at 2 for 10 minutes. Then the alumina was washed until the wash-water was neutral. The wet powder was dried in an oven at 110° for 24 hours. The particles passing a 120 U.S. mesh sieve were used for chromatography. The alumina was suspended in methylene chloride and transferred to glass columns of convenient size. The nonsaponifiable material was dissolved in methylene chloride and applied to the columns in amounts of 20 mg per g of the adsorbent. After elution by methylene chloride of the hydrocarbons, other substances of low polarity, and cholesterol, the glyceryl ethers were eluted by 10% methanol in methylene chloride.

The glyceryl ethers were converted to their dimethoxy derivatives by treatment with boron trifluoride and diazomethane according to the method described by Müller and Rundel (5). To 100 to 150 mg of glyceryl ethers was added 1 ml of a 1% solution of boron trifluoride dimethyl etherate in distilled diethyl ether. An ice-chilled solution of freshly distilled diazomethane in ether was added drop by drop until the yellow color of the sample persisted. The best yield of dimethoxy derivatives was obtained when there was a simultaneously abundant formation of polymethylene, indicated by the formation of large aggregates insoluble in ether. After 30 minutes at room temperature the excess diazomethane was evaporated, and the mixture was filtered. To remove the methyl esters of contaminating fatty acids, the ether solution was concentrated to about 1 ml, 6 ml of 1 N KOH solution in water was added, and the mixture was allowed to stand at room temperature for about 20 hours. The mixture was then extracted four times with diethyl ether. The combined ether extracts were washed to neutrality, then dried over $MgSO_4$ and filtered. The ether was evaporated almost to dryness and petroleum ether was added. Next, the dimethoxy derivatives of the glyceryl ethers were separated from the monomethoxy derivatives and unchanged glyceryl ethers by chromatography on alumina. The material dissolved in petroleum ether was applied to a column of alumina packed using the same solvent. The dimethoxy derivatives were eluted with 10% diethyl ether (dried over sodium) in petroleum ether.

Oxidation by Chromic Acid. The positions of double bonds in the alkyl chains were determined by oxidation with chromic acid. A mixture of 7.5 mg of the dimethoxy derivative of a glyceryl ether, 7.5 mg of chromium trioxide, 0.5 ml of acetic acid, and 0.1 ml of water was boiled on a steam bath under reflux for 1 hour. One milliliter of methanol was added to reduce the excess chromic acid. In order to convert the carboxylic acids into their methyl esters, 4 ml of 2% methanolic sulfuric acid was added and the mixture was boiled for 1½ hours under reflux on a steam bath. Five milliliters of water was added and the esters were extracted by diethyl ether. The ether solution was carefully concentrated at a temperature lower than 20° by blowing nitrogen over the surface and the residue was analyzed by gas-liquid chromatography (GLC).

TABLE 1. COMPOSITION OF THE NONSAPONIFIABLE MATTER IN FISH LIVER OILS

Species	Percentage of Nonsaponifiable Matter in the Oil	Composition of the Nonsaponifiable Matter
Chimaera monstrosa	33	Almost exclusively glyceryl ethers
Somniosus microcephalus	8	About 90% glyceryl ethers, 8% cholesterol
Squalus acanthias	7	84% glyceryl ethers, 13% cholesterol

Hydrogenation. The dimethoxy derivatives of the glyceryl ethers (57 mg) were dissolved in 2 ml of ethanol (99.5%) and the solution was added to 6.3 mg of platinum oxide (Adams' catalyst) in a dry flask. The hydrogenation was performed at atmospheric pressure and at room temperature. After shaking for about 30 minutes, hydrogenation was complete.

Gas-Liquid Chromatography. For the GLC analyses, we used a Perkin-Elmer apparatus (model 116) without thermostat. By use of a separate heater, the temperature of the injection block was increased to the desired value—about 40° above the oven temperature. Thermistor thermal-conductivity cells were used as detectors. Helium was the carrier-gas and the detector operated at 8 v. A Hewlett-Packard 425 A direct current amplifier was used to amplify the detector voltage before recording with a Speedomax (type G) recorder. The full-scale response of the system was 1 mv. The linearity of the detectors was tested by analyzing mixtures of weighed amounts of pure methyl esters of saturated *n*-monocarboxylic acids. The peak areas found were proportional to the weight percentages of the components (6).

In the initial stages of the work, silicone grease and Reoplex 400 (7) were used as stationary phases. A better separation of saturated and unsaturated com-

pounds can, however, be achieved on succinate diethylene glycol polyester (8, 9), and this stationary phase was therefore used in the latter part of the investigation. The separation factor between the dimethoxy derivatives of batyl and selachyl alcohols was 1.08 on Reoplex and 1.14 on the succinate polyester. A disadvantage of the succinate polyester is that the odd-numbered saturated long-chain compounds have about the same retention times as the monounsaturated compounds with one carbon atom less.

In all instances, kieselguhr (098–1504, Perkin-Elmer, Ueberlingen) was used as solid support; this material is white in color and very similar to Celite 545. The kieselguhr was treated with concentrated hydrochloric acid followed by 10% sodium hydroxide and then washed with water to neutrality. Dust and fine particles were removed by flotation. The wet powder was dried in an oven at 200° overnight. The dry powder was carefully sieved to get particles of uniform size; 60 to 80 U.S. mesh was used throughout this study. The supporting medium was impregnated with 25% by weight of the stationary phase. The material was packed into U-shaped aluminum tubings with i.d. 4 mm.

The outlet pressure of the helium was atmospheric. The outlet system of the fractometer was modified to permit collection of high boiling substances as described by Ryhage *et al.* (10).

The following three columns were used for the analyses of the dimethoxy derivatives of the glyceryl ethers:

Two-Meter Silicone Column: Dow Corning High Vacuum Grease, the column filling, was heated for several days at 330° in an oxygen-free stream of N_2 before use (10). The analyses were run with an inlet pressure of 26 psi and a flow rate of about 70 ml per minute at a temperature of 265°. The number of theoretical plates was 1440 for methyl stearate and 1780 for methyl behenate.

Three-Meter Reoplex Column: Reoplex 400 is a polar polyester supplied by The Geigy Company, Ltd. The inlet pressure was 19 psi and the flow rate 50 ml per minute at a temperature of 246°. The number of theoretical plates for methyl stearate was 2250 and for methyl behenate 2270.

Four-Meter Succinate Column: Diethylene glycol succinate polyester was synthesized by Dr. S. Stenhagen according to Craig and Murty (9). The inlet pressure was 18 psi and the flow rate 40 ml per minute at a temperature of 243°. The number of theoretical plates was 2500 for methyl stearate and 2820 for methyl behenate.

In the analyses of the methyl esters of the higher monocarboxylic acids, the following two columns were used.

Two-Meter Silicone Column: The inlet pressure was 19 psi, the flow rate 45 ml per minute, and the temperature 240°. The number of theoretical plates for methyl stearate was 1570.

Four-Meter Column with Reoplex 400: The inlet pressure was 26 psi, the flow rate 60 ml per minute, and the temperature 230°. The number of theoretical plates for methyl stearate was 3340.

The methyl esters of the short-chain monocarboxylic acids up to nonanoic acid formed by oxidation with chromic acid were analyzed on silicone and Reoplex columns as follows:

Three-Meter Silicone Column: The inlet pressure was 15 psi, the flow rate 50 ml per minute, and the temperature 183°. The retention time for methyl nonanoate was 2.8 minutes and the number of theoretical plates was 3050.

Two-Meter Reoplex Column: The inlet pressure was 5.9 psi, the flow rate 44 ml per minute, and the temperature 165°.

RESULTS

A flow sheet of the preparation of the dimethoxy derivatives of the glyceryl ethers from the liver oil of *Squalus acanthias* is shown in Figure 1. A typical chromatogram of the separation of glyceryl ethers and cholesterol is given in Figure 2. The glyceryl ethers

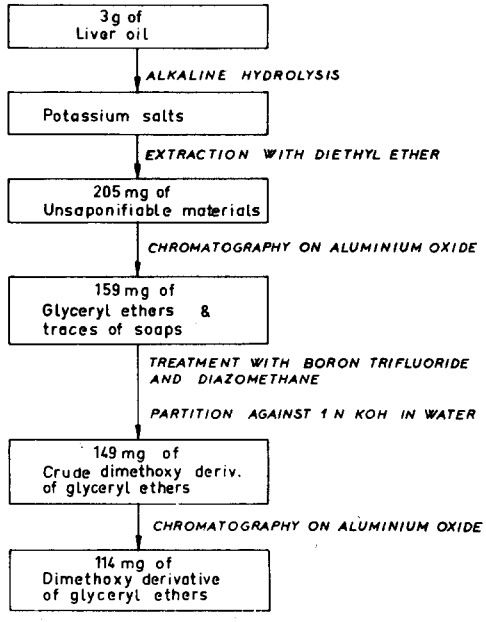

FIG. 1. Flow sheet demonstrating the isolation of the glyceryl ethers from the liver oil of *Squalus acanthias*.

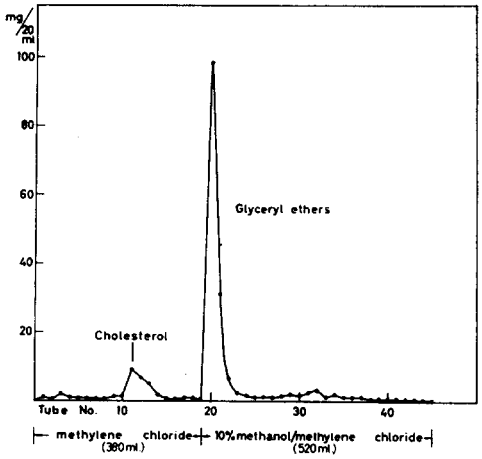

FIG. 2. Chromatographic separation on activated alumina of the nonsaponifiable matter from the liver oil of *Squalus acanthias*.

were eluted by 10% methanol in methylene chloride (v/v). Representative gas-liquid chromatograms on succinate diethylene glycol polyester of the dimethoxy derivatives before and after hydrogenation are given in Figures 3 and 4. In Figure 5 the logarithms of the retention times relative to the dimethoxy derivative of synthetic α-batyl alcohol in succinate polyester have been plotted against the number of carbon atoms. These values are those found for the dimethoxy derivatives of the glyceryl ethers from *Squalus acanthias* before and after hydrogenation. Compounds with branched alkyl chains normally have shorter retention times than those with straight chains with the same number of carbon atoms. In the GLC pattern of the hydrogenated dimethoxy derivatives of the glyceryl ethers, we observed components with shorter retention times than those of the unbranched compounds. These compounds are denoted in Figure 5 as branched (br). The retention times relative to methyl stearate are given in Table 2 for the dimethoxy derivatives of the glyceryl ethers before hydrogenation, on diethylene glycol succinate polyester; and after hydrogenation, on silicone grease. The retention times of some methyl esters of normal saturated fatty acids relative to methyl stearate are also included in Table 2. Blomstrand and Gürtler (11) have previously shown that it is possible to separate chimyl, batyl, and selachyl alcohols by GLC after acylation of the two hydroxyl groups.

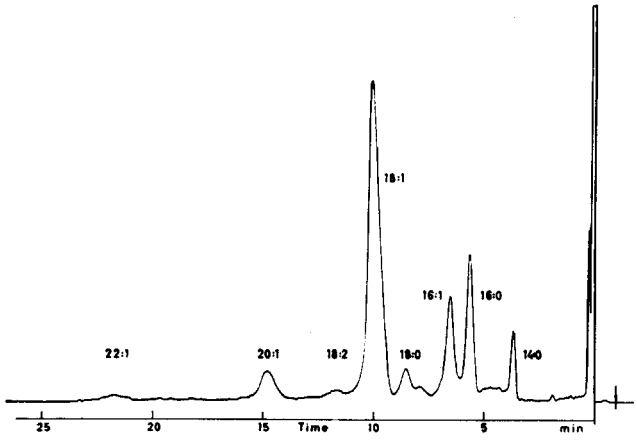

FIG. 3. Gas-liquid chromatogram of the dimethoxy derivatives of the glyceryl ethers in the liver oil of *Squalus acanthias* before hydrogenation. Stationary phase: Succinate diethylene glycol polyester. Temperature: 243°. Sample size: 1.1 μl of a concentrated solution. The figures beside the peaks refer to the long-chain component of the molecule. The figure to the left of the colon denotes the number of carbon atoms and the figure after the colon, the number of double bonds.

Having identified the dimethoxy glyceryl ether by GLC as described above, the quantitative composition of the mixture was determined by cutting out and weighing the peaks after they had been carefully redrawn on smooth, uniform white paper sheets. The percentage composition of the glyceryl ethers from the liver oils of *Squalus acanthias*, *Somniosus microcephalus*, and *Chimaera monstrosa* is given in Table 3. The chimyl, batyl, and selachyl alcohols together constituted only 64% of the glyceryl ethers in the oil of *Squalus*

TABLE 2. RETENTION TIMES RELATIVE TO METHYL STEARATE

Dimethoxy Derivatives of Glyceryl Ethers*		Methyl Esters of Saturated *n*-monocarboxylic Acids	
Succinate polyester at 243°			
Before hydrogenation			
14:0	1.05	18:0	1.00
16:0	1.59	19:0	1.24
16:1	1.87	20:0	1.51
17:1	2.25	21:0	1.86
18:0	2.43	22:0	2.30
18:1	2.76		
18:2	3.34		
20:1	4.22		
22:1	6.20		
Silicone at 260°			
After hydrogenation			
14:0	1.00	18:0	1.00
15:0	1.30	19:0	1.30
16:0	1.67	20:0	1.67
17:0	2.17	21:0	2.17
18:0	2.78	22:0	2.78
19:0	3.58		
20:0	4.62		
22:0	7.66		

* The number of carbon atoms refers to the long-chain component of the molecule. The number after the colon denotes the number of double bonds.

and 71% in the oils of *Somniosus* and *Chimaera*. The rest was composed of glyceryl ethers not previously described. In Table 4 the percentage composition of the fatty acids from the same liver oils is given.

In the case of the monounsaturated glyceryl ethers, the position of the double bond was determined by oxidation with chromic acid. The carboxylic acids formed were converted into their methyl esters and analyzed by GLC. The dimethoxy derivatives of the glyceryl ethers with hexadecenyl as the long-chain part of the molecule gave heptanoic acid as the main component, showing that the double bond was situated between carbon atoms 9 and 10. By oxidation of the dimethoxy derivative of the glyceryl ether with octadecenyl as the long-chain component, we confirmed that this component was selachyl alcohol with the double bond in position 9:10. Oxidation in this case gave nonanoic acid. The oxidation of the dimethoxy derivatives of the glyceryl ethers with eicosenyl and docosenyl as long alkyl chains gave heptanoic and nonanoic acids, respectively. In these glyceryl ethers, therefore, the double bonds were situated in position 13:14.

In all cases the predominant peak in the GLC spectra of the oxidation products of the monounsaturated glyceryl ethers was the methyl ester of either heptanoic or nonanoic acid. There were only small peaks of the homologs with one carbon atom less. For instance, the oxidation of the dimethoxy derivative of the docosenyl glyceryl ether produced only one-sixth as much octanoic acid as nonanoic acid. In the GLC spectra there were also oxidation products with longer retention times that originated from the glyceryl part of the molecule. These compounds have not yet been identified.

The infrared (IR) spectra of the dimethoxy derivatives of the different glyceryl ethers were very similar

TABLE 3. THE PERCENTAGE COMPOSITION (WEIGHT) OF THE GLYCERYL ETHERS FROM LIVER OILS*

Glyceryl Ether	Grey Dogfish (*Squalus acanthias*)	Greenland Shark (*Somniosus microcephalus*)	Ratfish (*Chimaera monstrosa*)
14:0	5.7	2.0	1.7
15†	1.9	0.7	1.1
16:0	13.2	9.1	10.4
16:1	10.6	10.8	9.1
17†	3.0	3.6	4.7
18:0	3.4	2.8	6.7
18:1	47.8	59.4	53.6
18:2	2.4	1.6	2.5
18:3	trace	?	?
19‡	1.2	1.5	2.4
20:1	8.0	6.2	6.4
22:1	2.7	2.2	1.0

* The glyceryl ethers are represented by the long-chain component of the molecule.

† The GLC spectra indicate that both branched and normal chain C_{15} and C_{17} glyceryl ethers are present. A monounsaturated C_{17} is also found.

‡ The GLC pattern of the hydrogenated mixture shows the presence of a C_{19} glyceryl ether.

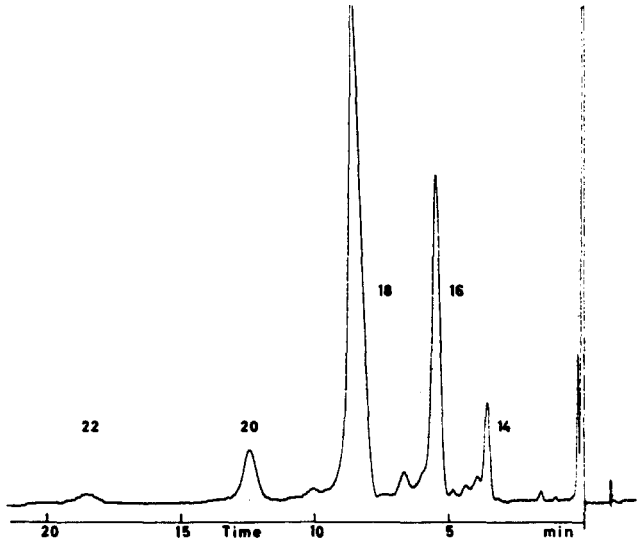

FIG. 4. Gas-liquid chromatogram of the dimethoxy derivatives of the glyceryl ethers in the liver oil of *Squalus acanthias* after hydrogenation. The column and the temperature were the same as in Figure 3. Sample size: 1.4 μl of a concentrated solution. The figures beside the peaks denote the number of carbon atoms in the long-chain component of the molecule.

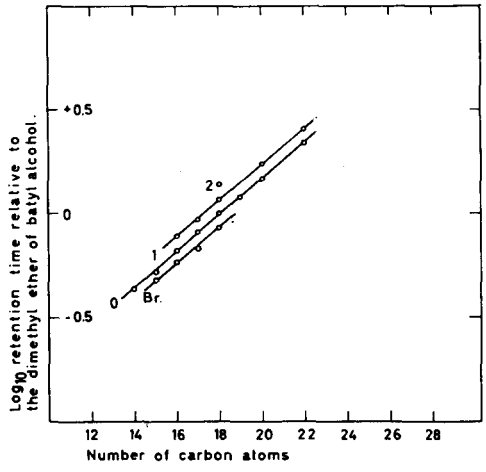

FIG. 5. Plot of $\log_{10}$ retention times of the dimethoxy derivatives relative to the dimethoxy derivative of batyl alcohol vs. the number of carbon atoms in the long-chain component of the molecule. Stationary phase: succinate diethylene glycol polyester. Temperature: 243°. The number of double bonds in the long chains are denoted by 0, 1, or 2. Br means components with branched chains. The values refer to glyceryl ethers isolated from *Squalus acanthias*.

TABLE 4. THE PERCENTAGE COMPOSITION (WEIGHT) OF THE TOTAL FATTY ACIDS FROM THE FISH LIVER OILS STUDIED

Fatty Acid	Grey Dogfish (*Squalus acanthias*)	Greenland Shark (*Somniosus microcephalus*)	Ratfish (*Chimaera monstrosa*)
14:0	2.9	0.6	1.0
15*	0.7	0.2	0.5
16:0	16.9	6.3	9.8
16:1	3.8	4.3	4.7
17*	2.1	1.1	2.5
18:0	3.1	1.7	3.8
18:1	21.2	32.1	45.6
18:2	2.0	1.0	2.0
18:3	0.9	?	?
19	0.4	0.4	1.0
20:1	13.4	27.8	11.6
20:5	2.4	1.0	1.0
22:1	23.6	18.9	12.1
22:6	4.1	1.6	0.9
24:1	2.2	3.2	3.0

* The GLC spectra indicate that the C_{15} and C_{17} fatty acids have both normal and branched chains. The C_{17} fatty acids also include a monounsaturated one.

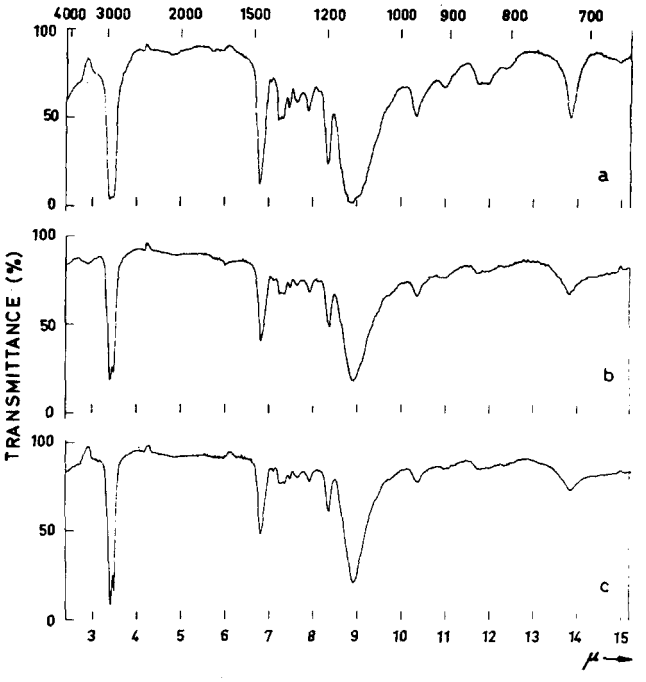

FIG. 6. Infrared spectra of dimethoxy derivatives of glyceryl ethers taken in the solid state with a Perkin-Elmer 21 Spectrophotometer (about 3 mg in 300 mg KBr). All substances were isolated by GLC. *a*. The dimethoxy derivative of synthetic α-batyl alcohol (a gift from Dr. S. Ställberg-Stenhagen). *b*. The dimethoxy derivative of selachyl alcohol from grey dogfish. *c*. The dimethoxy derivative of eicosenyl glyceryl ether from grey dogfish.

to each other. This is demonstrated in Figure 6 for synthetic batyl alcohol, selachyl alcohol from grey dogfish, and the glyceryl ether with eicosenyl as the long-chain component isolated from the same specimen. However, these spectra are useful in demonstrating that all components belong to the same type, viz., dimethoxy derivatives of α-alkyl glyceryl ethers. The IR spectrum of a dimethoxy derivative shows no band at 3 μ in contrast to the spectrum of an untreated glyceryl ether, which absorbs at this wavelength owing to the presence of free hydroxyl groups. The strong absorption band at 9 μ is caused by ether bonds. Debuch (12) has shown that the IR spectrum of a synthetic β-isomer of batyl alcohol is somewhat different from the naturally occurring α-isomer from shark liver oil. The IR spectra of the dimethoxy derivatives of the glyceryl ethers isolated by us were very similar to the IR spectrum of the dimethoxy derivative of synthetic α-batyl alcohol. The α-batyl alcohol was a gift from S. Stenhagen and had been synthesized by her from allyloctadecylether by treatment with silver benzoate and iodine (13). The dibenzoate of the α-batyl alcohol was then hydrolyzed to give free α-batyl alcohol.

The mass spectra of single components were of great value for identification of individual ethers. In Figure 7 the mass spectra of the dimethoxy derivatives of synthetic α-batyl alcohol, of selachyl alcohol, and of the glyceryl ether with eicosenyl as the long-chain component of the molecule are given. The selachyl alcohol and the eicosenyl glyceryl ether were both isolated from *Squalus acanthias*. The parent peaks corresponding to the unfragmented molecules gave the molecular weights of the compounds (372, 370, and 398, respectively). As can be seen in Figure 7, the fragmentation patterns are very characteristic. The details of this work will be published separately.[4]

The fatty acids found in the unfractionated liver oils of the three species of fishes included relatively large amounts of C_{20} and C_{22} monounsaturated components. Oxidation of the monounsaturated C_{20} fatty acid from *Squalus acanthias* gave nonanoic acid and a dicarboxylic acid with 11 carbon atoms, showing that the double bond was situated between carbon atoms 11 and 12. The oxidation of the monounsaturated C_{22} fatty acid gave nonanoic acid, undecanoic acid, and dicarboxylic acids with 11 and 13 carbon atoms. Thus, this fraction was a mixture of two monounsaturated C_{22} fatty acids with double bonds situated between carbon atoms 13 and 14 and between carbon atoms 11 and 12, respectively. There were also

[4] The mass spectrometric analyses were performed by Dr. R. Ryhage at the Mass Spectrometric Laboratory, Karolinska Institutet, Stockholm, Sweden.

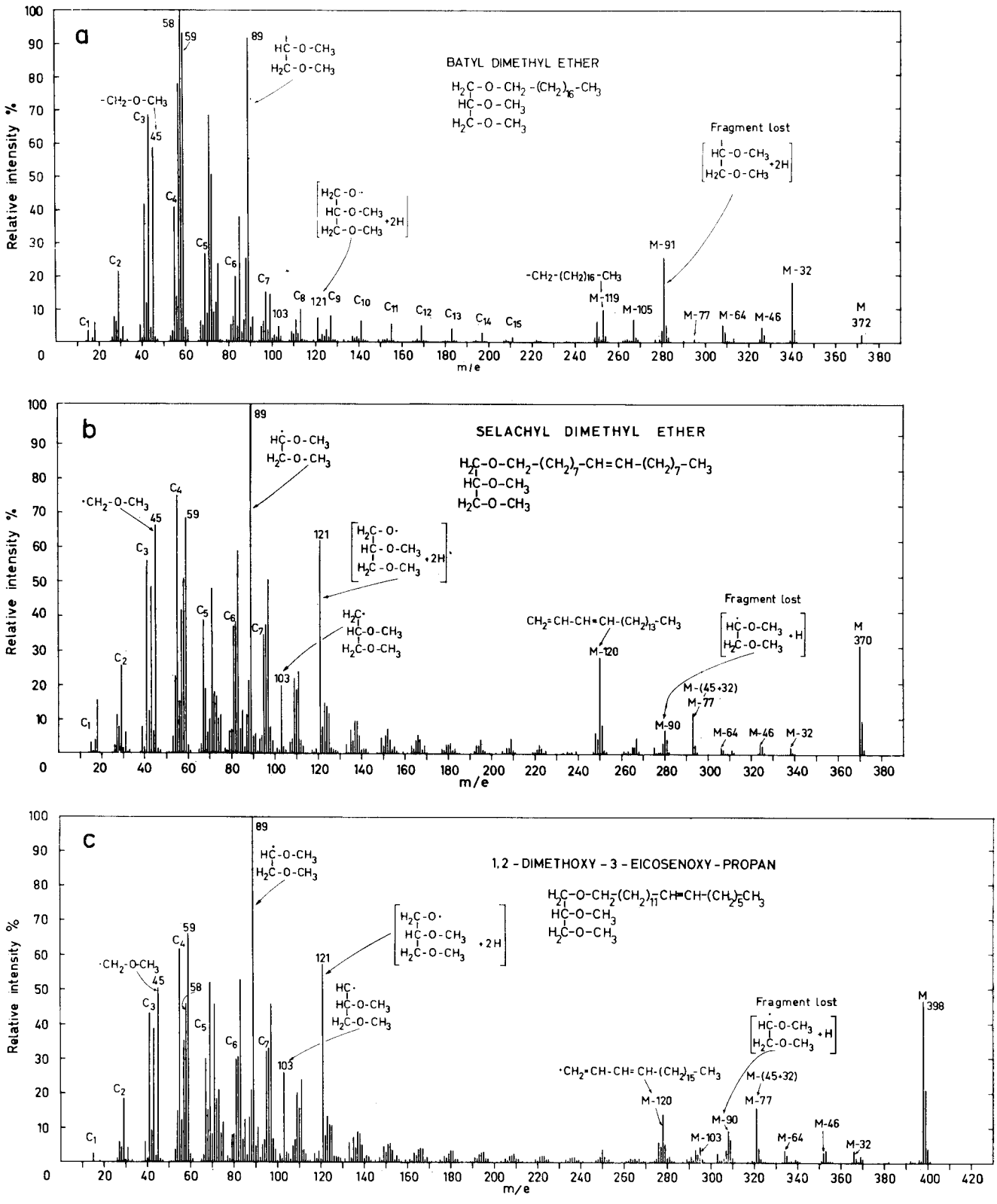

FIG. 7. Mass spectra of dimethoxy derivatives of glyceryl ethers. *a*. The dimethoxy derivative of synthetic α-batyl alcohol. *b*. The dimethoxy derivative of selachyl alcohol isolated from grey dogfish. *c*. The dimethoxy derivative of eicosenyl glyceryl ether isolated from grey dogfish.

small amounts of eicosapentaenoic and docosahexaenoic acids in the oils. Among the glyceryl ethers those with *n*-eicosenyl and *n*-docosenyl constituted a lower percentage than the percentage of eicosenoic and docosenoic acids in the mixture of fatty acids. No alkyl chains with 5 or 6 double bonds were found in the glyceryl ethers. On the whole, we can say that the alkyl chains in ether linkage are shorter and more saturated than the esterified chains.

The fatty acid diesters of glyceryl ethers constituted about 30% of the liver oil in the *Squalus* and *Somniosus* species. In *Chimaera monstrosa*, the liver fat was composed almost completely of fatty acid diesters of glyceryl ethers with only traces of triglycerides.

DISCUSSION

In the present study, we started with materials rich in glyceryl ethers. Swain (14) separated the nonsaponifiable matter from the oils of marine animals on activated alumina. The hydrocarbons were eluted by petroleum ether, cholesterol and vitamin A by benzene or methylene chloride, and glyceryl ethers by diethyl ether. We found that methylene chloride with 10% methanol gave a more rapid and complete elution of the glyceryl ethers. It is not desirable to have substances adsorbed on alumina for a long time in view of the transformations (hydrolysis, isomerization, salt-formation, etc.) shown by other workers to occur on alumina. The dimethoxy derivatives of the glyceryl ethers are extremely resistant to hydrolysis in contrast to the diacetates, which can be hydrolyzed even at room temperature in an alkaline water solution such as 1 N NaOH (12).

In our gas-liquid chromatograms, there were no components other than dimethoxy derivatives of glyceryl ethers. The possibility that the glyceryl ethers themselves were artifacts produced in the course of preparation is very unlikely. The alkyl groups now identified in glyceryl ethers all seem closely related to the fatty acids from animal sources. The GLC patterns obtained before and after hydrogenation were compared by plotting the retention times against the number of carbon atoms in the long alkyl chains of the glyceryl ethers. The saturated ethers fell on a straight line. If a polar polyester was used as the stationary phase, the monounsaturated components fell on a parallel line. Because of the polarity of the double bonds, the retention time of an unsaturated compound is longer than that of the corresponding saturated one. A synthetic α-batyl alcohol was also used as reference and had exactly the same retention time as the batyl alcohol isolated from the fish oils.

The IR spectra of the isolated components showed that they were of the same type, viz., dimethoxy derivatives of the α-glyceryl ethers. The components were also identified by their mass spectra, which showed their molecular weight and characteristic fragmentation patterns.

In summary, the chromatography on alumina, the etherification of the hydroxyl groups, and the subsequent purification eliminated contaminating substances. The GLC patterns and IR spectra showed that all components were of the same type, differing only with regard to the alkyl groups. By hydrogenation and by use of synthetic α-batyl alcohol as reference, all components could be identified. The molecular weights as well as the fragmentation patterns obtained from the mass spectra gave further proof of the identification of the new compounds.

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