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Studies on the composition of glyceryl ethers and their preparation from diacyl glyceryl ethers in liver oils*

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SUMMARY

Procedures are described for the preparation of alkyl iodides from fatty alcohols and glyceryl ethers. Alkyl iodides were separated directly by gas-liquid chromatography (GLC) on ethylene glycol succinate columns and do not require calibration factors in their analysis with a thermal conductivity detector. Iodide derivatives have absolute retention times on GLC similar to the corresponding fatty alcohol acetates. Alkyl iodides were converted to alcohol acetates with silver acetate or to hydrocarbons with lithium aluminum hydride and these derivatives were separated by GLC. The elimination of hydriodic acid from secondary iodides during GLC is described. The resulting monoene iodides are readily separated from saturated iodides by GLC. Alkoxy structures have been assigned by comparison of the retention time data of iodide derivatives with standard alcohol derivatives. Unsaturation was confirmed by catalytic hydrogenation. Branched components were identified as the alkanes.

Glyceryl ethers were prepared from diacyl glyceryl ethers by hydrogenolysis with lithium aluminum hydride. The glyceryl ether-fatty alcohol mixture was separated quantitatively by thin-layer chromatography (TLC) and sufficient material obtained from one TLC plate for the preparation of alkyl iodide derivatives.

Three procedures for the analysis of glyceryl ether mixtures have recently been described. The glyceryl ethers are converted either to glyceryl ether diacetates (1), dimethoxy glyceryl ethers (2, 3), or alkoxy glycolaldehydes (4), and these derivatives separated by gas-liquid chromatography (GLC). These procedures have several limitations. A large series of pure reference compounds are not readily available for peak identification and calibration. Only one derivative is usually prepared. Additional purification may be required to separate partial conversion products (3, 4). Long retention times, especially for dialkoxy glycerols (5, 6), are found with some derivatives.

In the present investigation, glyceryl ethers were converted to alkyl iodides with hydriodic acid. Alkyl iodides were analyzed directly by GLC and converted to alcohol acetates and hydrocarbons for further identification. Reference compounds were prepared from commercially available alcohols and from alcohols synthesized from fatty acid methyl esters. Alkyl iodide, alcohol acetate, and hydrocarbon derivatives of a glyceryl ether were first prepared by Heilbron and Owens (7), who investigated the structure of batyl alcohol.

Diacyl glyceryl ethers occur in high concentrations in some fish liver oils (8). These oils also contain glycer-

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ides, sterols, and hydrocarbons (8). Glyceryl ethers are usually isolated from the nonsaponifiable fraction of these oils by chromatography on alumina (3, 8, 9) or silicic acid (4, 10). In this study, fish liver oils were reduced with lithium aluminum hydride. The reduction mixture, an ethereal solution of glyceryl ethers, sterols, fatty alcohols, and hydrocarbons, was readily separated into component fractions by thin-layer chromatography (TLC). Sufficient material was separated on one thin-layer plate for the preparation of derivatives and their analysis by GLC.

MATERIALS AND METHODS

Materials. Dodecanol, tetradecanol, hexadecanol, and octadecanol were purchased from Eastman Organic Chemicals (Rochester, N. Y.). Analysis by GLC of the alcohol acetates showed that octadecanol contained 3.1 mole % hexadecanol. 9-Octadecenol, 1,12-octadecanediol, and 10-nonadecanol were prepared by the lithium aluminum hydride reduction (11) of oleic and 12-keto stearic acids purified in this laboratory, and 10-nonadecanone was kindly supplied by Dr. L. D. Metcalf, Armour Industrial Chemical Co. (Chicago, Ill.). 1-Octadecene was kindly supplied by Dr. E. L. Miller and Mr. G. E. Hinds, Continental Oil Co. (Houston, Texas). Tripalmitin was kindly supplied by Dr. F. Baur, Proctor and Gamble Co. (Cincinnati, Ohio). Chimyl alcohol, batyl alcohol, selachyl alcohol, diacetyl batyl alcohol, and *Chimera monstrosa* liver oil were kindly supplied by Dr. W. Chalmers, Western Chemical Industries Ltd. (Vancouver, Canada). *Squalus acanthias* liver oil, and crude glyceryl ethers from *Squalus acanthias* liver oil were kindly supplied by Dr. Neva Karrick, Bureau of Commercial Fisheries, U.S. Department of the Interior (Seattle, Wash.). Hydriodic acid (57%, stabilized with hypophosphorus acid) and silver acetate were purchased from Fisher Scientific Co. (Fair Lawn, N. J). Lithium aluminum hydride was purchased from Metal Hydrides Inc. (Beverly, Mass.). Platinum dioxide was purchased from Baker Catalysts Inc. (Newark, N. J.). Hexane (95 mole %) was purchased from Phillips Petroleum Co. (Bartlesville, Okla.).

Preparation of Alkyl Iodides. Glyceryl ethers or fatty alcohols, 50–150 mg, were weighed into a flask and 10 ml of hydriodic acid added. The mixture was refluxed for 24 hr and then transferred to a separatory funnel with 150 ml ether. This mixture was washed successively with 20 ml water and 10 ml saturated potassium bicarbonate to remove excess hydriodic acid. Free iodine was then removed with 10 ml of 40 to 50% sodium thiosulfate. The ethereal solution was partially dried by desiccation with 30 ml saturated sodium chloride and then filtered through anhydrous magnesium sulfate. Solvent was removed at 30° on a rotary vacuum evaporator.

Preparation of Acetates from Alkyl Iodides. Approximately 100 mg of alkyl iodide was weighed into a flask and a tenfold molar excess of silver acetate in 15 ml glacial acetic acid was added. The mixture was refluxed for 24 hr and then filtered into a separatory funnel with 100 ml ether. The ethereal solution was washed successively with 30 ml of water and 10 ml of saturated sodium chloride. The ethereal solution was then neutralized with 30 ml of saturated sodium bicarbonate and solid sodium bicarbonate, and washed with a second 15 ml of saturated sodium bicarbonate. The neutral ethereal solution was washed with 15 ml of 40 to 50% sodium thiosulfate, partially dried by desiccation with 30 ml saturated sodium chloride, and filtered through anhydrous magnesium sulfate. Solvent was evaporated on a rotary vacuum evaporator.

Preparation of Hydrocarbons from Alkyl Iodides. From 50 to 100 mg of alkyl iodide was dissolved in ether and added dropwise to a 50-fold molar excess of lithium aluminum hydride suspended in 30 ml ether. This mixture was refluxed 12–18 hr. Absolute ethanol was added until no visible reaction occurred, and the mixture was heated to boiling and then cooled. From 30 to 40 ml of 6 N hydrochloric acid was added, and the two phases were transferred to a separatory funnel. The aqueous layer was removed and washed with ether, and the ether layers were combined. The ethereal solution was neutralized with several 20-ml portions of saturated sodium bicarbonate and then washed successively with 20 ml of 2 M sodium thiosulfate, water, and 2 M sodium chloride. The solution was filtered through anhydrous magnesium sulfate and the solvent removed.

Hydrogenation of Unsaturated Alcohols and Glyceryl Ethers. Approximately 100 mg of lipid in 20 ml ether was placed in a hydrogenation flask and 50 mg platinum dioxide added. The hydrogen pressure was adjusted to 20 psi and the flask agitated for 2 hr. The mixture was then filtered and the solvent removed.

Hydrogenolysis of Diacyl Glyceryl Ether–Triglyceride Mixtures. Approximately 200 mg of lipid in 50 ml ether was placed in a flask and lithium aluminum hydride suspended in ether was added slowly until the cessation of visible reaction (11). A 100% excess of lithium aluminum hydride was added. The mixture was allowed to stand for 30 min and then refluxed for 90 min. Excess lithium aluminum hydride was decomposed with ethyl acetate and moist ether. Hydrochloric acid, 6 N, was added to dissolve the precipi-

tate, and the aqueous phase was extracted with several 20-ml portions of ether. The ether solutions were combined, washed with 10 ml saturated potassium bicarbonate and then with 20 ml saturated sodium chloride, and filtered through anhydrous magnesium sulfate. Solvent was removed under vacuum.

Thin-Layer Chromatography. Equipment and silica gel G were purchased from C. A. Brinkmann and Co. (Great Neck, N. Y.). All solvent mixtures were prepared v/v. Plates were prepared and developed as described by Mangold (12). Spots were visualized by charring after spraying with 50% sulfuric acid or by exposure to iodine vapor. Alkyl iodides were identified by the specific spray for organic iodides developed by Robbins (13) for paper chromatography.

Gas-Liquid Chromatography. Alkyl iodides and alcohol acetates were analyzed in an Aerograph A-350-B gas chromatograph.[1] These derivatives were separated on 10-ft stainless steel columns, 0.25 in. i.d., containing 10% polyethylene glycol succinate (EGS) on 60–80 mesh Gas Chrom P[2]. Hydrocarbon analyses were obtained with an Aerograph A-90-C[1] and a 5-ft stainless steel column, 0.25-in. i.d., containing 33% G.E. SF-96 silicone grease on Chromosorb.[1] The chromatographs were equipped with a Wheelco 1 Mv recorder and Wheelco Type A-2 electronic integrator.[3] Specific operating conditions are listed in Tables 2 and 3.

Additional chromatograms were obtained with a Barber-Colman Model 10 gas chromatograph equipped with a high-temperature flame detector, Wheelco 5 Mv recorder, and Wheelco Type A-2 electronic integrator.[3] Separations were made in 6-ft glass columns, 3-mm i.d., containing 10% ethylene glycol succinate–silicone copolymer (EGSS-X) on 100–120 mesh Gas Chrom P[2].

RESULTS

Separation and Identification of Derivatives. R_F values for different compound classes separated by TLC are reported in Table 1. This technique was used to establish reaction conditions and monitor products for the presence of reactants. Thus alcohols and glyceryl ethers were not detected in their alkyl iodide reaction products,[4] and alkyl iodides were not detected in their alcohol acetate reaction products. While alkyl iodides were not separated from hydrocarbons on TLC, their absence from hydrogenolysis products was shown when plates were sprayed for organic iodide. Alkyl diiodides, synthesized from unsaturated or dihydroxy alcohols and glyceryl ethers, were separated from monoiodides on TLC. These diiodides yielded several products upon acetolysis and hydrogenolysis. The identity and quantitative estimation of unsaturated alcohols and glyceryl ethers was therefore confirmed by chromatography of diiodides prepared before catalytic hydrogenation and monoiodides and alcohol acetates prepared after catalytic hydrogenation.

Relative retention times on GLC for iodide, acetate, and hydrocarbon derivatives synthesized from alcohol standards are reported in Table 2. Absolute retention times for the 18-carbon reference derivatives are included in this table. Alkyl iodides prepared from saturated and monoene 20 and 22 carbon alcohols had long retention times and in many cases gave unsymmetrical peaks with a broad leading edge. While

TABLE 1. R_F VALUES OF DIFFERENT CLASSES OF COMPOUNDS ON TLC

Compound	R_F Values				
	Solvent I*	Solvent II*	Solvent III*	Solvent IV*	Solvent V*
Glyceryl ether	0.10	0.00	0.00	0.00	0.38
Fatty alcohol	0.58	0.07	0.04	0.00	0.67
Cholesterol	0.42				0.67
Monoglyceride					0.33
Diglyceride					0.87
Triglyceride	0.96				
Alcohol acetate		0.52	0.22		
Diacyl glyceryl ether	0.98				
Alkyl iodide		0.96	0.94	0.85	
Alkyl diiodide				0.68	

* Solvents were: I, diethyl ether–hexane 50:50; II, diethyl ether–hexane 5:95; III, diethyl ether–hexane 1:99; IV, hexane; V, chloroform–methanol–acetic acid 96:2:2.

TABLE 2. RELATIVE RETENTION TIMES FOR STANDARD ALCOHOL DERIVATIVES

Alcohol	Iodide*	Acetate*	Hydrocarbon†
Dodecanol	0.19	0.20	0.08
Tetradecanol	0.32	0.32	0.18
Hexadecanol	0.56	0.56	0.30
Octadecanol	1.00	1.00	1.00
9-Octadecenol	1.16	1.12	—
1,12-Octadecanediol	1.17	6.6	—

* Iodides and acetates were separated on 10% EGS columns at 180°. At a helium flow of 45.5 ml/min, retention times were 16.0 min and 15.6 min for octadecyl iodide and octadecyl acetate, respectively.

† Hydrocarbons were separated on 33% G.E. SF-96 silicone grease at 160°. At 30 psi (helium flow 273 ml/min), the retention time was 11.8 min for octadecane.

[1] Wilkens Instrument and Research, Inc., Walnut Creek, California.

[2] Applied Science Laboratories, State College, Pennsylvania.

[3] Barber-Colman Co., Rockford, Illinois.

[4] The mean weight recovery of octadecyl iodide synthesized from octadecanol was 97% of the theoretical recovery.

TABLE 3. RELATIVE RETENTION TIMES FOR PRIMARY AND SECONDARY ALCOHOL DERIVATIVES*

Alcohol	Acetate	Iodide
1-Octadecanol	1.00	1.01
10-Nonadecanol	1.00	0.34

* Derivatives were analyzed on a 10% EGS column at 200°. At a helium flow of 19.0 ml/min, retention times were 19.3 min and 13.7 min for octadecyl acetate and 10-nonadecyl acetate, respectively.

chromatograms were improved by increasing column temperature and carrier gas flow, optimal conditions for separation of 20 and 22 derivatives varied for specific columns.

Alkyl iodides and alcohol acetates had similar relative and absolute retention times; however, alkyl diiodides, prepared from either 1,12-octadecanediol or 9-octadecenol, resembled 9-octadecenyl acetate more closely than the corresponding diacetate. This suggested that secondary iodides were unstable and decomposed with the elimination of hydriodic acid during GLC. The elimination of hydriodic acid was confirmed in a second experiment. 10-Nonadecanone was converted to the alcohol, acetate, and iodide derivatives and these derivatives were analyzed by TLC and GLC (Table 3). The relative retention time for the secondary alkyl iodide was much shorter than the corresponding secondary alcohol acetate while the primary alkyl iodide resembled the alcohol acetate. The retention time of 4.7 min obtained for the secondary alkyl iodide with 19 carbon atoms was similar to the retention time of 4.1 min obtained for a reference alkene, 1-octadecene, analyzed under the same conditions. Primary iodides were stable in GLC since alkene peaks arising from dehydroiodination were not observed. An alkyl triiodide synthesized from the diene 9,12-octadecadienol gave two peaks on GLC with closely similar retention times in the unsaturated alkyl iodide range. These peaks were not identified further.

Quantitative Analysis of Fatty Alcohol Mixtures. A weighed mixture was prepared containing the saturated alcohols dodecanol, tetradecanol, hexadecanol, and octadecanol. The composition of the mixture was confirmed by acetylation and GLC analysis (Table 4). Alkyl iodides were then synthesized and analyzed by GLC. One alkyl iodide preparation was converted to acetate and hydrocarbon derivatives. These derivative mixtures were analyzed by GLC. Comparable results were obtained with direct acetylation, hydriodolysis, and acetolysis of alkyl iodides (Table 4). While alcohol acetates were corrected for relative response (11, 15), response factors were very near unity and thus unnecessary in the alkyl iodide determinations reported in this paper. The hydrocarbon results did not agree with the known composition of the mixture as well as did the other analyses; however, pure hydrocarbons were not available as standards for calibration.

TABLE 4. GLC ANALYSIS* OF A SATURATED ALCOHOL MIXTURE

Sample	Alcohol† 12:0	14:0	16:0	18:0
	Composition in mole %‡			
Alcohol (known)	51.3	26.5	0.7	21.6
Acetate-A (7)§[11]	51.2 ± 1.8	28.4 ± 1.5	1.1 ± 0.2	19.3 ± 1.3
Acetate-B (8)	50.9 ± 3.0	27.5 ± 1.3	0.7 ± 0.5	20.9 ± 3.0
Iodide-A (6)	51.0 ± 0.8	27.8 ± 1.1	0.4 ± 0.5	20.8 ± 1.9
Iodide-B (7)	50.8 ± 1.3	27.3 ± 1.3	0.3 ± 0.4	21.5 ± 1.7
Acetate from iodide (8)	50.9 ± 1.6	26.8 ± 1.0	0.8 ± 0.6	21.6 ± 1.9
Hydrocarbon from iodide (6)	46.8 ± 0.4	27.0 ± 0.4	0.4 ± 0.4	25.9 ± 0.3

* Operating conditions are reported in Table 2.

† The shorthand system of Dole et al. (14) is used to designate carbon atoms and double bonds in alcohols and the alkoxy moieties of glyceryl ethers.

‡ Mean ± standard deviation of observation.

§ A and B were different samples. Figures in parentheses represent number of GLC tracings.

[11] Acetate data were corrected for relative mole response (11).

TABLE 5. GLC ANALYSIS OF A MIXTURE OF SATURATED AND UNSATURATED ALCOHOLS*

Sample	Alcohol 16:0	18:0	18:1
	Composition in mole %		
	Before Hydrogenation		
Alcohol (known)	49.0	32.7	18.4
Acetate (4)	50.7 ± 0.6	30.9 ± 0.9	18.4 ± 0.8
Iodide (6)	50.2 ± 1.5	33.7 ± 1.5	16.2 ± 0.7
	After Hydrogenation		
Alcohol (calculated)	49.0	51.1	
Acetate (5)	48.4 ± 1.7	51.6 ± 1.7	
Iodide-A (4)	49.1 ± 1.3	50.9 ± 1.3	
Iodide-B (6)	49.5 ± 0.4	50.5 ± 0.4	
Acetate from iodide (5)	48.7 ± 0.5	51.3 ± 0.5	
Hydrocarbon from iodide (5)	45.1 ± 0.3	54.9 ± 0.3	

* For a description of the data, see footnotes in Table 4.

TABLE 6. GLC ANALYSIS OF CHIMYL, BATYL, AND SELACHYL ALCOHOL CONCENTRATES*

Sample	Alkoxy Moiety 14:0	15	16:0	16:1	17	18:0	18:1	20:0	20:1
	Composition in mole %								
Chimyl alcohol									
Iodide (6)	2.6 ± 0.3	0.9 ± 0.4	79.1 ± 1.8	2.1 ± 0.8	1.9 ± 0.7	13.5 ± 0.7			
Batyl alcohol									
Iodide (2)			3.3		0.4	92.2		4.0	
Selachyl alcohol									
Iodide-A (8)	0.6 ± 0.3		2.7 ± 0.3	11.3 ± 1.1	0.5 ± 0.5		81.1 ± 1.2		3.8 ± 0.7
Iodide-B (6)	0.8 ± 0.1		2.8 ± 0.1	10.0 ± 0.5	0.8 ± 0.3		81.3 ± 0.6		4.3 ± 0.3

* For a description of the data, see footnotes in Table 4. Glyceryl ether concentrates were obtained from Western Chemical Industries.

Analysis by GLC of a mixture containing both saturated and unsaturated alcohols is reported in Table 5. The composition of this mixture was first determined with acetate and iodide derivatives. A part of the mixture was then hydrogenated and acetates and iodides were prepared. One iodide sample was also converted to acetate and hydrocarbon derivatives. Data obtained from direct acetylation and hydriodolysis before hydrogenation, and data obtained from acetylation, hydriodolysis, and acetolysis after hydrogenation, were in close agreement with the known composition of the mixture. As in the previous experiment, hydrocarbon derivatives gave less satisfactory GLC results.

The data reported in this study were obtained with a thermal conductivity detector. However, several alkyl iodide mixtures were also analyzed with a flame ionization detector. With the latter detector, data from iodide mixtures had much more variation than comparable data from fatty acid methyl ester mixtures. This problem is being investigated further.

Composition of Glyceryl Ether Concentrates. Alkyl iodides were prepared by hydriodolysis of chimyl, batyl, and selachyl alcohol concentrates obtained from Western Chemical Industries. Data on GLC are summarized in Table 6. Analyses of two alkyl iodide preparations from selachyl alcohol indicate the reproducibility of the method. The carbon number and unsaturation for each alkoxy moiety was assigned from the retention time. These structures were confirmed with retention time data for peaks obtained before and after catalytic hydrogenation (see Table 8).

Separation of Glyceryl Ethers and Fatty Alcohols by TLC. As much as 100 mg of a lipid mixture containing fatty alcohols and glyceryl ethers was applied to a TLC plate in a streak. Chromatographic separations were then achieved with 1:1 ether in hexane (Table 1) and bands visualized by brief exposure to iodine. These bands were scraped from the plate, transferred to 40-ml centrifuge tubes, and agitated with 6 ml chloroform–methanol 1:1 (v/v) using a Vortex Stirrer. Tubes were centrifuged, and the supernatant solution was recovered. The extraction process was repeated three times. The analysis of a mixture containing known amounts of hexadecanol, octadecanol, chimyl alcohol, and batyl alcohol is reported in Table 7. It is apparent that the original glyceryl ether and alcohol mixtures were separated by TLC.

The complete hydrogenolysis–TLC separation process was demonstrated by the conversion of a mixture

TABLE 7. GLC ANALYSIS OF GLYCERYL ETHER ALCOHOL MIXTURES SEPARATED BY TLC*

Sample	16:0	17:0	18:0	20:0
	Composition in mole %			
Mixture I†				
Alcohol				
Known	84.1		15.9	
Iodide (5)	84.3 ± 1.5		15.7 ± 1.5	
Glyceryl ether				
Known	33.3		66.7	
Iodide (6)	33.5 ± 1.4		66.5 ± 1.4	
Mixture II‡				
Alcohol				
Known	100			
Iodide (4)§	96.8 ± 1.0		3.2 ± 0.9	
Glyceryl ether‖				
Known	3.3	0.4	92.2	4.0
Iodide (4)	4.0 ± 0.1	0.7 ± 0.2	90.8	4.6 ± 1.0

* For a description of the data see footnotes in Table 4.

† Mixture I contained weighed amounts of hexadecanol, octadecanol, batyl alcohol, and chimyl alcohol. Alkoxy content was calculated from glyceryl ether composition (Table 6) ignoring minor alkoxy components.

‡ Mixture II, tripalmitin and diacetyl batyl alcohol, was reduced with lithium aluminum hydride before TLC.

§ Two minor peaks with shorter elution times are omitted.

‖ The alkoxy composition of batyl alcohol (Table 6) was assumed for diacetyl batyl alcohol.

TABLE 8. GLC ANALYSIS OF GLYCERYL ETHERS IN *Squalus acanthias* AND *Chimera monstrosa* LIVER OILS*

Sample†	14:0	15‡	16:0	16:1	17‡	18:0	18:1	19§	20:0	20:1	22:0	22:1
	Composition in mole %											
Squalus acanthias-I												
a. Before hydrogenation												
Iodide (6)	3.1 ± 0.2	1.0 ± 0.2	12.7 ± 0.3	12.8 ± 0.5	0.5 ± 0.1	2.1 ± 0.6	65.5 ± 0.9			2.2 ± 0.7		trace
Iodide after TLC (1)	2.7	1.0	12.9	13.0	0.7	1.8	65.4			2.6		
b. After hydrogenation												
Iodide (6)	3.5 ± 0.1	0.1 ± 0.2	29.0 ± 0.5		0.4 ± 0.4	64.6 ± 0.7		trace	2.4 ± 0.6		trace	
Acetate from iodide (7)	3.3 ± 0.7	0.2 ± 0.2	27.2 ± 0.2			67.4 ± 0.7			2.0 ± 0.5			
Squalus acanthias-II												
Iodide (4)	1.1 ± 0.1	0.4 ± 0.1	11.0 ± 0.8	8.5 ± 1.1	0.2 ± 0.1	4.9 ± 1.5	69.9 ± 1.4			3.9 ± 0.7		
Squalus acanthias-III												
Dimethoxy	6.6	2.1	13.8	11.2	3.0	3.3	46.3	1.1		7.8		2.4
Chimera monstrosa-I												
Iodide (4)	3.2 ± 0.6	0.5 ± 0.3	16.8 ± 1.6	11.5 ± 2.0		2.7 ± 1.4	63.2 ± 2.0			2.0 ± 0.2		
Chimera monstrosa-II												
Dimethoxy	2.0	1.2	11.1	9.7	4.8	6.5	52.5	2.2		6.4		0.9

* For a description of the data, see footnotes in Table 4.

† *Squalus acanthias* samples were: (I) glyceryl ether concentrate from nonsaponifiable fraction; (II) glyceryl ethers prepared from liver oil by hydrogenolysis and TLC purification; (III) calculated from weight % data of Hallgren and Larssen (3). *Chimera monstrosa* samples were: (I) glyceryl ethers prepared from liver oil by hydrogenolysis and TLC purification; (II) calculated from weight % data of Hallgren and Larssen (3).

‡ Several small peaks, which may include both branched and unsaturated alkoxy groups.

§ Masked by 18:1 in sample before hydrogenation.

of diacetyl batyl alcohol (Western Chemical Industries) and tripalmitin to a mixture of glyceryl ether and fatty alcohol with lithium aluminum hydride. Data on GLC obtained with alkyl iodide derivatives of the fatty alcohol and glyceryl ether fractions separated by TLC yielded results similar to the known composition of tripalmitin and diacetyl batyl alcohol (Table 7). Alkyl iodides prepared from the fatty alcohol fraction (tripalmitin) contained a small quantity of 18:0 and two minor peaks with retention times shorter than the 16:0 derivative. These peaks were also found in alkyl iodides prepared from the hydrogenolysis product of pure tripalmitin (97.7 and 2.3 mole % 16:0 and 18:0) even though the acetate derivative showed only a 16:0 alcohol.

Glyceryl Ether Composition of Fish Liver Oils. Alkyl iodides were prepared by hydriodolysis of a glyceryl ether concentrate from *Squalus acanthias* liver oil (glyceryl ethers supplied by Bureau of Commercial Fisheries) before and after catalytic hydrogenation. A part of the saturated alkyl iodide preparation was then converted to the acetate, and the iodide and acetate mixtures were analyzed by GLC. Alkyl iodides, prepared from the glyceryl ether concentrate after TLC purification, were also examined. Comparable results were obtained in the different experiments (Table 8). Catalytic hydrogenation not only confirmed alkoxy structures assigned from retention time data but also unmasked minor components such as the 19 derivative. Saturated alkyl iodides were also converted to hydrocarbons by hydrogenolysis. Qualitative GLC analyses of these hydrocarbons indicated that peaks 15 and 17 contained both branched and normal components while peak 19 contained only a normal component. Two small peaks, 21 and 22, were also observed.

The glyceryl ether concentrate and glyceryl ethers from *Squalus acanthias* liver oil (oil supplied by Bureau of Commercial Fisheries) prepared by hydrogenolysis with lithium aluminum hydride were similar in alkoxy composition (Table 8). Alkyl iodides and dimethoxy glyceryl ethers (3) gave somewhat different results for both *Squalus acanthias* and *Chimera monstrosa* liver oils. Alkyl iodides showed a lower 17 and 20:1 and a higher 18:1 content than dimethoxy ethers, which also contained small amounts of the 18:2 and possibly the 18:3 derivatives. Since the 22:1 alkyl iodide derivative gives a broad unsymmetrical peak, a quantitative analysis of the minor 22:1 component in glyceryl ethers was not obtained.

DISCUSSION

Hydriodolysis has several advantages in the investigation of glyceryl ethers. The alkyl iodide derivatives are synthesized in high yields and do not require extensive purification prior to their analysis by GLC.

The derivatives are separated on EGS columns at temperatures less than 200° and may be detected by thermal conductivity, flame ionization, or electron capture[5] (16). Alkyl iodides give a linear response over a wide concentration range with the thermal conductivity detector. Peak area percentage data may be converted to mole % composition without calibration when a thermal conductivity detector is employed.

Since alkyl iodides are synthesized from either alcohols or glyceryl ethers, a large series of reference compounds is readily available for peak identification and calibration. Alkyl iodides, prepared from several methyl ester test mixtures, should be analyzed in order to establish optimal conditions for a specific instrument and column. Two additional derivatives, alcohol acetates and hydrocarbons, may be prepared from saturated alkyl iodides in order to confirm the structure and composition of the original glyceryl ether mixture. Hydrogenolysis with lithium aluminum hydride facilitates the identification of long-chain alkoxy groups since their hydrocarbon derivatives have much shorter elution times on GLC. Furthermore, metal iodides formed during hydrogenolysis may be estimated quantitatively by cerate oxidimetry[6] (17).

When alkyl diiodides are prepared from monoene alcohols and glyceryl ethers, the secondary iodide is eliminated as hydriodic acid during GLC. Alkyl diiodides are therefore eluted with the shorter retention time of monoene iodides rather than the long retention time that would be postulated for diiodides. Alkyl triiodides prepared from dienes yield several peaks on GLC. Thus the small quantities of diene and triene alkoxy groups in glyceryl ethers (3, 4) are not identified and estimated by hydriodolysis.

Diacyl glyceryl ethers are quantitatively converted to glyceryl ethers and fatty alcohols by hydrogenolysis with lithium aluminum hydride. Hydrogenolysis replaced saponification and extraction, which is usually employed in the isolation of glyceryl ethers. Since the ethereal glyceryl ether–fatty alcohol mixture is concentrated and separated directly by TLC, difficulties are avoided in the quantitative extraction of glyceryl ethers from saponification emulsions. Sufficient material is separated on one TLC plate for the analysis of both glyceryl ethers and fatty alcohols as their alkyl iodide derivatives.

The quantitative hydrogenolysis of glyceryl ether phospholipids has not been investigated. Preliminary experiments suggest that the glycerol–phosphate linkage is only partially broken during hydrogenolysis. However, partial hydrolysis products would remain at the origin in the TLC systems used here and could be isolated together with glyceryl ethers for hydriodolysis. If plasmalogens react with hydriodic acid, acid hydrolysis and chromatography may be necessary before hydriodolysis is employed in the analysis of glyceryl ether phospholipids.

[5] We are indebted to Wilkens Instrument and Research, Inc., for preliminary electron capture data.

[6] T. D. Beardmore, K. E. Guyer, and D. G. Cornwell, in preparation.

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