Glycerolysis of Marine Oils and the Preparation of Acetylated Monoglycerides[1]

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THE α-MONOGLYCERIDES and acetylated monoglycerides from vegetable and from terrestrial animal sources are used as lubricating-oil additives (1), oil-modified alkyd resins (2), emulsifiers (3), and chemical intermediates for the synthesis of detergents (4, 5) and of epoxy type of plasticizers (6).

Relatively inexpensive α-monoglycerides and acetylated monoglycerides may be produced from the oils of marine animals. Furthermore the fatty acid constituents of marine oils generally have longer chain-lengths and a higher degree of unsaturation than do fatty acids not of marine origin. This distinction is maintained when derivatives are formed from marine oils, so these compounds might be expected to have certain chemical and physical properties different from such compounds produced from other sources. Accordingly marine-oil α-monoglycerides and acetylated monoglycerides merit investigation.

There are two commercially important methods for producing α-monoglycerides of long-chain fatty acids, the esterification of glycerol with fatty acids and the glycerolysis of triglycerides under conditions of ester interchange. The simplicity of the glycerolysis reaction makes it the more attractive one. Little has been reported however on the production of α-monoglycerides from marine oils by glycerolysis (6, 7). Rossi *et al.* (8) in their studies on the glycerolysis of linseed, olive, and coconut oils investigated the quantities of reagents, catalyst, and reaction temperatures. They found that the time of reaction varied, depending on the kind of oil observed. A modification of their procedure was used in the present work.

In the preparation of the acetoglycerides modifications had to be made in the usual acetylation procedure in order to insure quantitative conversions and minimum loss in unsaturation.

Owing to the fact that conventional methods of analysis of lipid mixtures were not applicable to acetoglycerides from marine oils, it was necessary to investigate other methods. Thin-layer silicic acid chromatography was considered to be suitable for this purpose (9, 10).

The objectives of the work reported in the present paper therefore are as follows: a) the determination of the optimum reaction time for the glycerolysis of marine oils in the production of α-monoglycerides, b) the development of a laboratory method for producing acetylated α-monoglycerides, and c) the investigation of the application of thin-layer silicic acid chromatography to the analysis of acetoglycerides.

α-Monoglycerides

Although the production of α-monoglycerides from oils of nonmarine origin had been studied rather extensively (8, 11–18), the question still remained as to whether a satisfactory yield could be obtained from marine oils. Furthermore an essential part of that question was whether this yield would be obtained at the expense of ethylenic bonds since the conditions for glycerolysis seemed to be somewhat drastic for an oil of high unsaturation. Optimum reaction time, in the present work, was therefore considered as the time required to produce the highest yield of α-monoglycerides with the minimum destruction of double bonds. The study of the optimum reaction time for glycerolysis of marine oils and the method for preparing α-monoglycerides is described.

Procedure. Menhaden, tuna, herring, and sardine oils were used since these are the principal marine oils of commerce. In addition, oil from the eggs of pink salmon was included because the amount of unsaturation in this oil is unusually high and the oil can be produced in commercial quantities if a suitable market develops. Data characterizing the particular lots of the various oils used are given in Table I.

The following method for determining optimum reaction time for producing α-monoglycerides by glycerolysis of menhaden oil is representative of that used with the other four oils.

Into a reaction flask equipped with electric heating mantle, mechanical stirrer, thermometer, reflux condenser, and gas inlet tube were placed 200 g. of alkali-refined menhaden oil and 90 g. of anhydrous glycerol. Oxygen-free nitrogen was bubbled through the reaction mixture at a rate such that none of the reactants were lost by volatilization. Vigorous stirring of the mixture throughout the reaction was essential since glycerol is only partially soluble in triglycerides (18, 19). The temperature was raised to 60°C. and was maintained for 30 min. in order to eliminate dissolved oxygen. The temperature was then rapidly raised to 220°C. When thermal equilibrium was established (220° ± 1°C.), 0.3 g. of KOH catalyst was added, which immediately dissolved. Samples were removed from the reaction mixture with a 25-ml., rapid-flowing pipette at specific intervals of time after the addition of the catalyst. Each 25-ml. sample was quickly added to 25 g. of crushed ice, extracted with 50 ml. of a 1:1 petroleum ether (b.p. 30°–60°C.)—ethyl ether solution, washed four times with 50-ml. portions of 20% sodium chloride solution, dried over anhydrous sodium sulfate, and filtered. Each sample was recovered by evaporation of the solvent over a water bath at 65°C. at reduced pressure and then was analyzed for percentage of α-monoglyceride by the periodic acid method (20) and for Hanus iodine value.

Results. Of the five oils studied, the optimum reaction time varied from 45 to 60 min. (Table II), depending on the kind of oil used. These results were reproducible. The relationship of the iodine value and percentage of α-monoglycerides as a function of reaction time for menhaden oil is shown in Figure 1.

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TABLE I

Analyses of the Marine-Oil Reactants

Marine oil Common name—Scientific name	Gardner number	Iodine value	Acid value	Saponification value	Peroxide value	Unsaponifiable matter
						%
Herring—*Clupea pallasi*	7	130.2	2.9	189.9	10	0.8
Sardine—*Sardinops caerulea*	12	178.4	0.6[a]	190.7	5	0.8
Menhaden—*Brevoortia tyrannus*	11	182.8	0.5[a]	192.6	4	1.2
Tuna—*Katsuwonus pelamis* and *Neothunnus macropterus*	12	182.2	1.2[a]	189.7	6	1.2
Pink-salmon eggs—*Oncorhynchus gorbuscha*	....[b]	228.1	1.0	183.1	5	2.0

[a] Alkali-refined oil.
[b] Reddish color of egg oil is not adaptable to Gardner color standards.

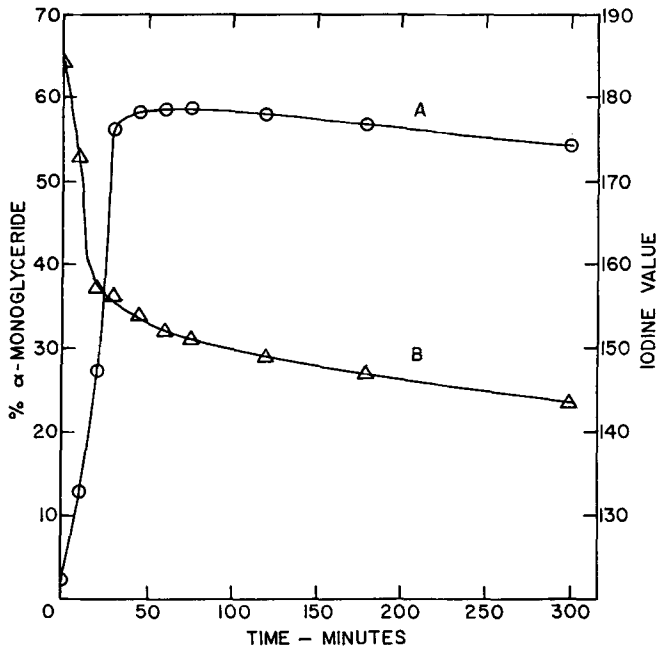

FIG. 1. Effect of reaction time on the α-monoglyceride content (curve A) and iodine value (curve B) of the equilibrium mixture from the glycerolysis of menhaden oil.

Extension of the reaction time beyond optimal resulted in loss of unsaturation, as reflected by lowering of the iodine value, and in destruction of α-monoglycerides. Although the change in α-monoglyceride content was small, loss of ethylenic bonds was substantial. With the highly unsaturated salmon-egg oil, for example, extending the reaction time from 60 min. to 180 min. lowered the ethylenic bonds by 7.2 mole-%.

After optimum reaction times had been determined, kilogram quantities of each oil were used in the preparation of α-monoglycerides by using proportionally larger quantities in the method of glycerolysis just described. Yields were in excellent agreement with those obtained by using smaller quantities.

Each of these large batches of crude glycerolysis mixtures was analyzed for iodine value, saponification equivalent, α-monoglycerides (20), total hydroxyl content (21), and unsaponifiable matter (Table II). Comparison of the observed iodine values with the theoretical values indicates that there was little destruction of ethylenic bonds. An ultraviolet-absorption analysis (22) of a sample of the tuna-oil glycerolysis mixture, for example, showed very little conjugation.

The glycerolysis mixtures were relatively free from odor and considerably improved in color as compared with the raw oils. Gardner numbers, for example, are lowered from about 11 for raw oils to about 6 for the crude glycerolysis mixtures. Herring oil, which was light oil to start with, gave exceptionally light products.

Acetylated α-Monoglycerides

A mild acetylation reaction was used, which gave quantitative yields and only slight loss of unsaturation. In the purification of the product it was anticipated that acetoglycerides would be amenable to molecular distillation. Consequently this approach was investigated.

Procedure. Crude glycerolysis mixtures from herring, menhaden, and pink-salmon-egg oils were chosen for this investigation because of the wide range of iodine values of the raw oils. The following procedure applied to the menhaden-oil glycerolysis mixture is typical of the method used in quantitatively acetylating the mixtures:

Into a reaction flask fitted with heating mantle, thermometer, mechanical stirrer, reflux condenser, and gas inlet tube were placed 1,000 g. of crude glycerolysis mixture (3.97 moles total OH) and 543 g. (5.26 moles) of 97% acetic anhydride. The reaction mixture was constantly stirred while oxygen-free nitrogen was moderately bubbled into it for 30 min. without the addition of heat. The temperature was rapidly raised to 140°C. and held at that temperature for 1 hr. The reaction mixture was poured into 1,000 ml. of cold oxygen-free water, and the organic layer

TABLE II

Analysis of the Glycerolysis Mixtures from Five Marine Oils

Marine oil	Optimum reaction time	Analysis									
		Saponification equivalent[a]		Iodine value[a]		Total OH	α-Monoglyceride	Diglyceride	Triglyceride	Unsapon. matter	Gardner no.
		Theor.	Obs.	Theor.	Obs.						
	min.					%	%	%	%	%	
Herring	45	330.3	343.2	115.3	110.9	6.36	54.1	42.0	3.2	0.7	5
Menhaden	60	325.8	336.0	159.2	155.9	6.74	57.5	40.3	1.1	1.1	7
Salmon-egg	45	335.2	347.4	200.6	190.6	6.33	56.5	37.5	4.2	1.8	6
Sardine	60	.....	341.0	.....	149.1	6.74	58.1	40.8	0.3	0.8	10[b]
Tuna	60	.....	342.8	.....	145.0	.....	57.8	.....	.....	1.1	11[b]

[a] All values are observed except where indicated as theoretical. Theoretical values are calculated from the analysis of the original starting oil. Observed values are corrected for the amount of unsaponifiable matter present in the oil. Acid values of all products were essentially zero.
[b] It is expected that improved color of original oil will result in monoglycerides of lower Gardner number.

was separated and washed five times with 1,000-ml. portions of oxygen-free water at 65°C. The organic layer was diluted with 300 ml. of a 1:1 ethyl ether-petroleum ether solution, washed twice with 200-ml. portions of 1% sodium carbonate solution, and then washed with cold oxygen-free water until the final washings were neutral. The ethereal solution was dried over anhydrous sodium sulfate and filtered, and the solvent was evaporated over a water bath at 60°C.

The crude acetylation mixtures were fractionated in a centrifugal type of molecular still[3] at a pressure of 0.1 μ.

Analysis of Acetoglycerides

It was desirable to investigate the composition of the distilled acetoglycerides by methods that were independent of chemical analysis. Chromatography, using a very thin layer of silicic acid on glass plates, has been developed recently (10) and has current application in lipid chemistry for the resolution of complex mixtures according to types of compounds (23).

Procedure. In the present work, silicic acid was spread evenly on glass plates and dried at 150°C. for 1½ hr. The glyceride mixture was then applied, and the chromatogram was developed to a height of 10 to 15 cm. The system of 8 volumes of ether to 92 volumes of Skellysolve F was found to be suitable for the resolution of monoaceto-, diaceto-, and marine-oil glycerides. The mixtures were resolved according to classes without any pronounced separation because of differences in the long-chain fatty acid moieties. The lipids were located by exposure to iodine vapors (24). The entire procedure took less than 2 hr.

The glycerides were chromatographed in amounts varying from 10 γ to 500 γ. Standards were analyzed on the same plate for visual estimation of the impurities in the mixtures. Resolution of the classes was best when each glyceride was less than 50 γ. Diacetoglyceride spots of 500 γ showed about 5 γ impurities of monoacetoglycerides. Consequently they were suitable as standards in the estimation of the purity of the monoacetoglycerides. The analysis of the distilled mono- and diacetoglycerides is presented in Table III, and a representative chromatogram of herring-oil glycerides is illustrated in Figure 2.

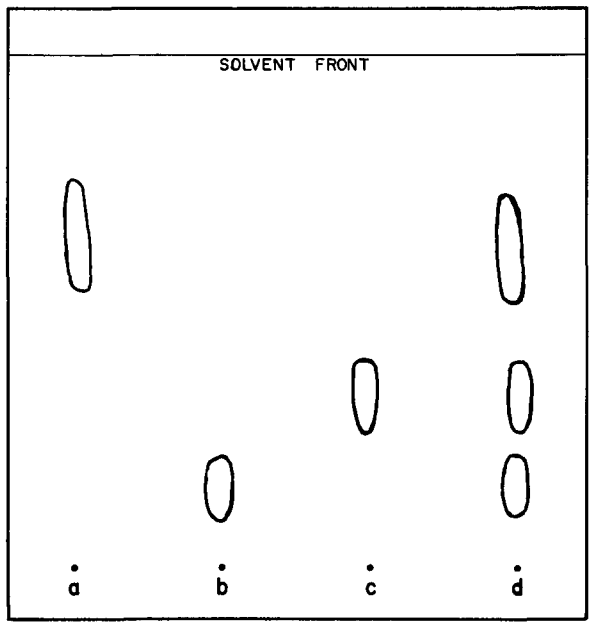

FIG. 2. Silicic acid chromatogram of herring-oil glycerides: a) triglycerides; b) diacetoglycerides; c) monoacetoglycerides; and d) equal-portioned mixture of diaceto-, monoaceto-, and triglycerides.

Conclusions

The glycerolysis reaction was applied to menhaden, tuna, herring, sardine, and salmon-egg oils. Optimum reaction time for the preparation of α-monoglycerides was found to vary, reproducibly, from 45 to 60 min., depending on the particular kind of marine oil employed. The method was suitable for the preparation of kilogram quantities.

A laboratory method for the preparation of kilogram quantities of highly pure acetylated α-monoglycerides was developed. Both the glycerolysis mixtures from marine oils and their acetylated products were light in color and substantially free of odor.

Thin-layer silicic acid chromatography, as described here, was found suitable for the analysis of acetoglycerides from marine oils. The work indicated that the method may have wide use for the separation and identification of other lipid classes as well.

Acknowledgments

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[3] Model CMS-5, Consolidated Electrodynamics Corporation, Rochester, N.Y.

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TABLE III

Analysis of Distillation Fractions of the Acetylated Marine-Oil Glycerolysis Mixtures

Acetylation product		Analysis								
		Saponification equivalent		Iodine value		Diaceto-glyceride	Mono-aceto-glyceride	Triglyc-eride	Unsap. matter	Gardner no.
Product	Marine oil	Theor.	Obs.	Theor.	Obs.					
						%	%	%	%	
Acetylated glycerolysis mixture[a]	Herring	177.3	173.5	99.7	94.1	57.9	38.8	2.7	0.6	....
	Menhaden	170.6	164.8	137.6	133.0	61.5	36.7	0.9	0.9	....
	Salmon-egg	179.1	169.7	176.6	160.5	60.2	34.6	4.0	1.2	....
Diacetoglycerides[b]	Herring	146.0	146.8	87.8	82.8	98.0	1.0	None	1.0	1
	Menhaden	144.2	145.4	123.0	116.6	98.0	1.0	None	1.0	3
	Salmon-egg	148.5	148.3	156.3	145.5	98.0	1.0	None	1.0	2
Monoacetoglycerides[b]	Herring	219.6	221.6	116.7	109.6	8.0	82.0	9.0	1.0	4
	Menhaden	216.1	219.9	164.3	160.8	3.0	86.0	10.0	1.0	6
	Salmon-egg	224.6	227.1	206.7	193.4	2.0	95.0	2.0	1.0	5

[a] Composition determined from the glycerolysis mixture. [b] Composition determined by thin-layer silicic acid chromatography.

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The Composition of the Difficultly Extractable Soybean Phosphatides

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THE MAJORITY of the papers dealing with soybean phosphatides have been concerned with the readily extractable soybean phosphatides, *i.e.*, that group of phosphatides which under normal industrial conditions are extracted with hexane or similar nonpolar solvents. They may be divided in two subgroups, *i.e.*, the hydratable phosphatides, which constitute the commercial product lecithin, and the nonhydratable phosphatides, which remain in the oil after the hydration. It has been shown that the hydratable phosphatides consist of phosphatidylcholine, -ethanolamine, and -serine as well as phosphoinositides (15, 19). In the latter group the presence of two types of inositides has been revealed, *i.e.*, phosphatidylinositol (13, 16) and another more complex inositide which contains carbohydrate (6, 7). In addition, the presence of lysolecithin (18), acetalphosphatides (9), and a phytoglycolipide (3), which contains phytosphingosine (2, 19), has been demonstrated.

The nonhydratable soybean phosphatides consist chiefly of a mixture of phosphatidic acids and lysophosphatidic acids (12).

So far no investigations have been published on the composition of the difficultly extractable soybean phosphatides, which is taken here to mean phosphatides that cannot be extracted with hexane but only with a mixture of hexane and alcohol.

Experimental

Difficultly extractable phosphatides were isolated from flaked soybeans by extraction with a mixture of 80% hexane and 20% absolute alcohol. The greater part of the lipides was removed previously by extraction with hexane at 60°C. The water content of the flaked soybeans was about 12%, and the oil content of the hexane-extracted flakes was about 0.7%. The difficultly extractable phosphatides amounted to 0.5% of the soybeans, a little less than the percentage of phosphatides extracted with hexane.

The phosphatides were fractionated by countercurrent distribution (15). For this purpose a 100-tube glass apparatus was used. The solvent system applied was carbon tetrachloride:chloroform:methylene chloride:methanol:water, 25:15:10:40:10 (4).

The content of phosphorus was determined by means of a colorimetric semi-micro method, depending on the formation of the yellow molybdivanadophosphoric acid (12) while nitrogen was determined according to the micro-Kjeldahl method. The individual nitrogen compounds were identified and determined by paper chromatographic methods previously described (12). Phosphoric acid esters were likewise identified by paper chromatography, and the same applies to glycerol and inositols (12), which were also determined quantitatively by oxidation with periodate after elution of the paper chromatograms. The carbohydrate-containing phosphatides were isolated by chromatographic separation on a column of cellulose powder, using the solvent system

n-propanol:conc. ammonia:water, 6:3:1.

Determination of the alkoxy group was performed according to Zeisel's method, as described in (10). Fatty acid esters were determined according to the method described in (8), which is based on the formation of hydroxamic acids.

Carbohydrates were determined according to the colorimetric method devised by Somogyi (17) and Nelson (11), following hydrolysis performed by refluxing for 7 hrs. with 0.6 N sulfuric acid.

Results and Discussion

Figure 1 shows the different distribution curves plotted by means of the above-mentioned analytical methods while Figure 2 illustrates the distribution of the nitrogen compounds.

The weight-distribution curve has four peaks and thus indicates the presence of four different groups of compounds.