life in indirect heated fryers because of their low film temp (8,9) require some reinvestigation.

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Synthesis of Triglycerides from Fish Oil Fatty Acids

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Abstract

The generally accepted methods for the synthesis of triglycerides are unsatisfactory when they are applied to highly unsaturated systems such as those present in fish oil. The methods either fail to give sufficiently high yields, or they are prohibitive in cost when applied to large scale production. Of the numerous reactions studied, the most feasible was the zinc-catalyzed esterification of fish oil fatty acids with glycerol. Thin-layer chromatography (TLC) showed that this reaction gave yields of 75–78% triglycerides. Gas-liquid chromatography (GLC) demonstrated the composition of the triglycerides was essentially the same as that of the original fatty acids.

Introduction

DURING RECENT YEARS, there has been an increasing demand for highly unsaturated glycerides. This demand has developed because of the growing interest in the effect of highly unsaturated lipids in nutritional and medical research. The fact that naturally occurring sources of triglycerides with a high degree of unsaturation are not available has limited the efforts of some researchers in these fields. Fish oils do contain appreciable amounts of highly unsaturated fatty acids; however, due to the random distribution of the total fatty acids on the glycerol molecules, a conc of highly unsaturated triglycerides is not readily obtainable.

The object of this research therefore was to develop a method whereby highly unsaturated triglycerides could be synthesized in large quantities from fish oil fatty acids.

Classical Methods of Preparation

There have been numerous reports in the literature on the preparation of mono- and diglycerides, but little has been reported on the synthesis of triglycerides. The reviews of Norris (1) and Malkin and Bevan (2) cover the majority of this work thoroughly, and consequently, no effort will be made to discuss in detail the syntheses presented by these authors. In general, the methods they describe are for the syntheses of specific glycerides, which usually are those containing single fatty acids with low or no unsaturation. This is especially true for the synthesis of triglycerides. Some papers have described the conversion of vegetable oil fatty acids to glycerides (3,4), but here again, there is little information regarding the more highly unsaturated compounds.

The acylation of glycerol with unsaturated acid chlorides has been successfully used to obtain good yields of triglycerides (5,6). However, the preparation of kg quantities of the acid chlorides from highly unsaturated fatty acids makes this method prohibitively expensive. For that reason, this reaction was not considered further.

Eckey (7,8), showed that the iodine value (I.V.) of fish oil can be substantially increased by base-catalyzed directed interesterification with simultaneous crystallization. The unprecipitated triglycerides obtained by this method still contain appreciable amounts of low unsaturated entities as well as a certain amount of saturates. Consequently, the I.V. of the product obtained from this process is not as high as would be desired. In addition, the process is lengthy.

Other methods for the synthesis of triglycerides appeared to be more applicable to the highly unsaturated systems found in fish oils. One of the more attractive of these is the base-catalyzed ester-ester interchange between triacetin and methyl esters (9,10). It was found that this method worked well in the preparation of triglycerides, such as tristearin or triolein, but it was not satisfactory for the preparation of mixed triglycerides containing the wide range of chain lengths and degrees of unsaturation found in fish oil methyl esters. Initially, the reaction proceeded quite rapidly at temp of 50–70C, but it then proceeded slowly even when the temp was raised as high as 125C. The amount of methyl acetate condensed and collected in a cold trap indicated yields of better than 90%. Analysis of the reaction products by TLC (11), however, showed that the yields of triglycerides were no greater than 35–40%, with mono- and diglycerides being the major by-products. Use of forcing conditions such as increased temp, change of pressure, varied molar ratios of the reactants, different types and concn of catalysts, and extended reaction times failed to give a more desirable yield.

Since neither directed interesterification or ester-ester interchange proved to be satisfactory for the preparation of highly unsaturated triglycerides, attention was turned toward acid- or base-catalyzed esterification of the highly unsaturated fish oil fatty acids with glycerol. It was found that mineral acid catalysts tended to attack the unsaturated fatty acids even under fairly mild conditions or gave poor yields (<5%). When a strong organic acid such as *p*-toluenesulfonic acid was used, yields up to 30% were obtained. Strong base catalysts proved to be no better than the mineral acids. A number of metal oxides and salts have also been used as catalysts for the esterification of vegetable oil fatty acids with

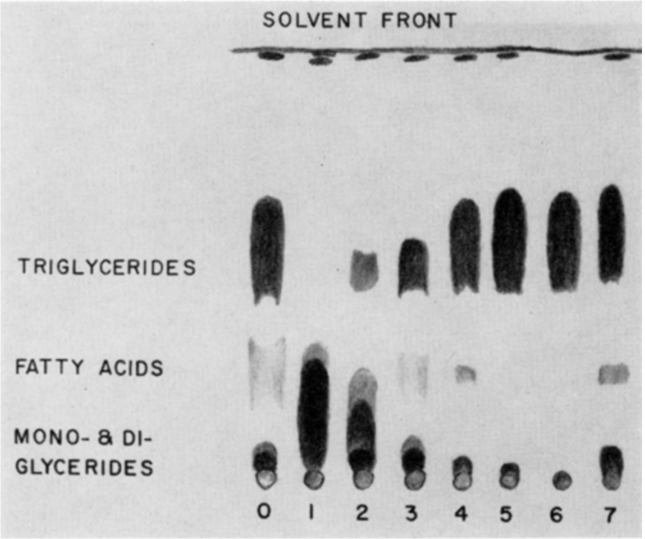

Fig. 1. Thin-layer silicic acid chromatogram of the distillation of crude synthetic triglycerides; 0, crude reaction mixture; 1–6, fractions from molecular distillation; 7, pot residue.
Solvent system: 90:10 petroleum ether (bp 30–60C) ethyl ether.
Development time: 30 min.
Indicator: iodine vapors.

glycerol (3). Of these compounds, stannous chloride appeared to be the best catalyst, but when used in a system with fish oil fatty acids, the yields of triglycerides were found to be no greater than 30% by TLC analysis.

Zinc Catalyzed Esterification

The most successful catalyst for direct esterification was found to be zinc dust (12). Yields of 75–78% triglycerides were consistently obtained from molecularly-distilled fractions of fish oil fatty acids. To obtain these yields, we found it necessary to run the reaction at 225C. Higher temp failed to increase the yield and resulted in further destruction of the unsaturated moieties. It was also found that it was necessary to add the zinc dust in several small portions rather than all at once, since the zinc dust tends to agglomerate and become inactive. When the zinc dust was added, the reaction mixture immediately became lighter in color. There was also a noticable amount of water that distilled along with a small amt of an unidentified white solid. When the evolution of water became negligible, another portion of catalyst was introduced into the system. Zinc dust in excess of 0.2% by wt did not increase the triglyceride yield and resulted in extreme emulsion problems during workup of the crude reaction mixture. Extending the reaction time beyond one hour had no effect on increasing the yields of triglycerides, and actually tended to result in large amt of polymeric material and in general destruction of the double bonds.

UV analysis showed that the increase in conjugated double bonds in the product was somewhat greater than would be desired. This result, however, was to be expected when lipids of a high degree of unsaturation are subjected to high reaction and distillation temp. IR analysis showed a slight increase in the amt of *trans* isomers (10.3 μ), but the increase was not sufficient to make *cis-trans* isomerization a serious problem.

Small amounts of relatively pure triglycerides can readily be obtained by various chromatographic methods (13). The larger quantities of triglycerides involved in this work, however, required a different method of purification. Molecular distillation provided a means of purifying kg quantities of crude material with a minimum of further destruction to the unsaturation. The equipment used was a centrifugal-type molecular still (Consolidated Vacuum Corporation, Model CMS-5), which has a short contact time (fraction of a sec) of the oil with the heated surface. Fractionation with this type of still is not clean cut, as can be seen in the TLC of the distilled fractions (Fig. 1), but careful manipulation of the equipment can yield a conc of relatively pure triglycerides.

TABLE I
Fatty Acid Composition of Menhaden Oil Fatty Acids and Synthetic Triglycerides

Fatty acid carbon atoms: double bonds	Content of fatty acids (% area)	
	Original fatty acids	Triglycerides
14:0	0.1	0.1
16:0	0.7	0.9
16:1	0.3	0.3
17:0	0.1	0.1
16:2	0.1	0.1
18:0	1.5	1.8
18:1	4.6	4.9
Unknown	0.2	0.3
18:2	0.6	0.6
Unknown	0.2	0.3
Unknown	0.1	0.2
20:0	0.5	0.7
18:3	0.8	0.8
20:1	4.0	4.1
18:4	1.3	1.0
Unknown	0.7	0.8
20:2 ?	0.4	0.5
Unknown	0.2	0.2
20:3	0.4	0.5
20:4	2.0	2.1
Unknown	1.2	1.9
Unknown	2.9	2.8
20:5	27.5	25.8
22:4	2.6	2.6
24:1	1.1	2.3
Unknown	1.3	2.0
22:5	7.9	7.6
22:6	37.9	34.9

If small amount of higher purity triglycerides are required, the conc from the molecular distillation could be freed of polymeric material by chromatographing over a suitable adsorbent.

Fatty Acid Analysis

It was of interest to determine whether the fatty acid composition of the synthetic triglycerides was the same as that of the starting fatty acids or whether there was some selectivity in the esterification reaction. This was not evident from a comparison of the chemical analysis of the starting material and fraction 6 of the distilled triglycerides. The saponification equivalent of the triglycerides (330) is essentially within experimental error of the theoretical value (332) calculated from the neutralization equivalent of the fatty acids. The observed I.V. of the triglycerides (308) is low when compared to the calculated theoretical value of 313, but this discrepancy can be accounted for by the increase in conjugated double bonds. Although these data appear to indicate no selectivity in the reaction, they are not conclusive, so it was necessary to use GLC as a final means of comparison.

Methyl esters of the starting fatty acids were prepared by the method of Schlenk and Gellerman (14). The methyl esters of the synthetic triglycerides were prepared by alcoholysis (15). These ester samples were chromatographed on a Research Specialities Model 600 Gas Chromatograph (beta ionization detector). A glass column 173 cm x 0.37 cm I.D. was packed with 7% DEGS on Anakrom ABS (70–80 mesh) and operated at 170C with an argon flow rate of 23 ml/min. The results of these analyses show in Table I. Only slight differences were found between the composition of the original fatty acids and

TABLE II
Molecular Distillation of Synthetic Triglycerides at 40 Microns Pressure

Fraction	Temp C	Wt in g
1	118–170	80.1
2	235	27.0
3	238	24.9
4	240	23.6
5	260	188.3
6	275	487.6
7	280–300	50.3
Residue	......	96.0

the synthetic triglycerides. The observed differences are within the generally accepted experimental error of the method and tend to support the conclusions from the chemical analysis that only slight selectivity occurred during the esterification reaction.

Preparation of Triglycerides

Into a reaction flask equipped with mechanical stirrer, thermometer and gas-bubbling tube were placed 1100 g (3.45 moles) molecularly distilled menhaden oil fatty acids (N.E. 319; I.V. 326; 1.66% conjugated diene and 0.03% conjugated triene; Gardner No. 6–7) and 108 g (1.11 moles) 95% glycerol. The fatty acid-glycerol mixture was vigorously stirred under an atmosphere of purified (99.99%) nitrogen and heated to 225C. The catalyst, 2.2 g (0.2% based on weight of fatty acids) zinc dust, was added in four equal portions at 15-min intervals. The reaction was allowed to proceed for 15 min after the final addition of zinc dust.

The hot reaction mixture was immediately poured onto one kg cracked ice to quench the reaction. This mixture was then transferred to a separatory funnel, and the crude product was extracted with petroleum ether (bp 30–60C). The petroleum ether extract was washed with equal volumes of water, 5% hydrochloric acid, 5% sodium bicarbonate, saturated sodium chloride solution, and water—in that order. Deoxygenated water was used throughout the washing procedure.

The petroleum ether extract was dried over anhydrous sodium sulfate, filtered, and the solvent was then removed under vacuum on a water bath. The crude product (1111 g) obtained was light yellow (Gardner No. 4).

Analysis of the crude product by TLC (Fig. 1) indicated approx 75% triglycerides. The remaining 25% consisted of unreacted fatty acids, mono- and diglycerides, and a small amt of polymeric material.

Molecular distillation was used to purify the crude reaction product. The results of the molecular distillation of 996 g crude product show in Table II. All fractions were analyzed by TLC (Fig. 1) and the principal fraction (Fraction 6) was found to be approximately 95% synthetic triglycerides (S.E. 330; I.V. 308; 3.55% conjugated diene, 2.13% conjugated triene, and no tetraene; Gardner No. 2).

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Spectrophotometric Determination of Small Amounts of 1-Monoglycerides in Fats

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Abstract

A method is described for the determination of small amounts of 1-monoglycerides in fats. The method consists of a periodic acid oxidation, the resulting glycol aldehyde fatty acid ester then being converted to the 2,4-dinitrophenylhydrazone derivative, which is determined spectrophotometrically. The colored solutions are stable and follow Beer's law in the range 0.0–1.0% 1-monoglycerides with a standard deviation of ±0.024%. Glycerol does not interfere.

Introduction

PERIODIC ACID oxidation has been widely employed for the quantitative determination of 1-monoglycerides (1–5, 9, 11, 12). Amounts of less than 1–3% 1-monoglycerides, however, cannot be accurately assessed by these methods. In 1959 Jensen et al. (6,7,8) published papers on the estimation of 0.1–1.0% 1-monoglycerides in milk. According to their method the formaldehyde produced by periodic acid oxidation is reacted with chromotropic acid and determined colorimetrically. Glycerol and carbohydrates interfere and have to be removed prior to the oxidation.

The present method assesses the fatty acid ester of glycol aldehyde produced by periodic acid oxidation after conversion to its 2,4-dinitrophenylhydrazone. Since the oxidation is quantitative, the 1-monoglyceride content of the sample is proportional to the increase in absorption of the dinitrophenylhydrazones obtained from the oxidised fat as compared with the original value. Glycerol does not interfere.

The oxidation step is based on the AOCS Method (1) for 1-monoglyceride determination, except that carbonyl-free benzene is used as a solvent instead of chloroform and the aqueous extraction before the oxidation is omitted. Excess periodic and iodic acids are removed by adding an aqueous potassium iodide solution and then slightly overtitrating with 0.1 *N* sodium thiosulfate. The aqueous phase is separated and re-extracted with benzene.

The method for assessing the carbonyl compounds is based on that of Lohman (10).

Experimental Procedures and Data

Reagents and Materials. Carbonyl-free benzene was prepared by refluxing 4 liters benzene for 3–4 hours