notes on methodology

Low temperature partial alcoholysis of triglycerides

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SUMMARY Egg triglycerides chromatographed on silicic acid are eluted in a skew curve in which fatty acid composition varies.

The triglycerides were cleaved by sodium methoxide in chloroform–methanol at 0°C to yield diglycerides (11.3%) and monoglycerides (17.5% of original triglycerides) after 3 min. Complete cleavage to fatty acid methyl esters and glycerol was obtained at 18 min at 0°C or 5 min at room temperature.

KEY WORDS alcoholysis · triglycerides · low temperature · partial methanolysis · asymmetric elution curves

IN A PREVIOUS paper (1) the methanolysis of lecithin by sodium methoxide was reported. It was shown that the controlled reaction at 0°C permitted the isolation of lysolecithin. In this paper the methanolysis of egg triglycerides by sodium methoxide is reported. The reaction occurs very rapidly at room temperature to yield glycerol and fatty acid methyl esters. However, at 0°C the methanolysis occurs less rapidly, so that the intermediate diglycerides and monoglycerides can be isolated. This study also includes an analysis of the fatty acids of egg triglycerides and their release with time by sodium methoxide.

Preparation of Egg Triglycerides. Egg yolk triglycerides (4 g) were prepared by column chromatography on silicic acid with the use of a gradient of ethyl ether in heptane (2). The column was monitored by paper chromatography (3).

A sample (180 mg) of the triglycerides was refractionated on 20 g of Unisil silicic acid (Clarkson Chemical Co., Williamsport, Pa.). Fifty 5-ml fractions were obtained by elution with 4% ethyl ether in *n*-heptane. Each fraction was analyzed for ester groups by the hydroxamate–ferric chloride reaction (4).

Sodium Methoxide Methanolysis of Egg Triglycerides. The triglycerides (160 mg = 174 μmoles based on an average molecular weight of 919) were dissolved in 40 ml of chloroform–methanol 2:1. The solution was placed in an ice bath, and 10 ml of ice-cold 0.5 N sodium methoxide in methanol was added. At 3, 6, 12, 24, and 36 min, aliquots (5 ml) were pipetted out into a flask containing 2 ml of 1.0 N HCl in methanol. The mixture was immediately poured into a separatory funnel containing water and diethyl ether. The upper (ether–chloroform) phase was washed with water. The water phases were combined and analyzed for glycerol by the periodate method (5). The ether phase was evaporated to dryness, the residue dissolved in *n*-heptane, and the fatty acid methyl esters, triglycerides, diglycerides, and monoglycerides were separated by column fractionation on silicic acid (Unisil) by sequential elution with 1, 4, and 25% ethyl ether in heptane, and then with ethyl ether. The amount of each lipid was determined by analysis for ester groups (4). This method failed to detect the monoglycerides, although they were shown to be present in the 100% ether eluate by weighing and by paper chromatography. Hence it was necessary to treat this fraction with sodium methoxide or methanolic HCl in order to convert what was presumed to be a cyclic form of the monoglyceride to fatty acid methyl esters, which were then analyzed by the hydroxamate procedure.

Gas Chromatographic Analysis of the Fatty Acid Methyl Esters. The fatty acid methyl esters liberated by the methoxide cleavage of egg triglycerides at the 3, 6, and 36 min intervals and the fatty acids in the isolated monoglycerides obtained after 3 minutes' reaction were subjected to gas chromatographic analysis under the same conditions as described previously (1) (see Table 1). The fatty acid analysis at the 36 min interval represents the total fatty acids of egg triglycerides with the exception of the 18:3 acids, which occurred in very small amount (see Fig. 1).

Results and Discussion. The column elution curve for egg triglycerides in silicic acid is shown in Fig. 1. The skewed curve gave evidence for a heterogeneous population of mixed triglycerides, a phenomenon observed by others for lecithin (6). This was confirmed by the fatty acid analysis of the triglycerides in tubes 15–20, 21–25, 26–30, 31–35, 36–40, and 41–50. These analyses are also shown in Fig. 1.

It can be seen that 18:0, 16:0, 18:1 acids decrease from tube 15 to tube 50 whereas the 16:1, 18:2, and 18:3 acids increase in relative proportion. The increase in proportion of 16:1 late in the elution curve is noteworthy and suggests that this acid is present in triglycerides containing 18:3 and (or) 18:2 acids rather than in triglycerides containing 16:0 and (or) 18:0 acids. The distribution curve shows that silicic acid can adsorb more strongly the more unsaturated species of a mixture of homologous lipids (6).

Fatty acids are designated by number of carbon atoms : number of double bonds.

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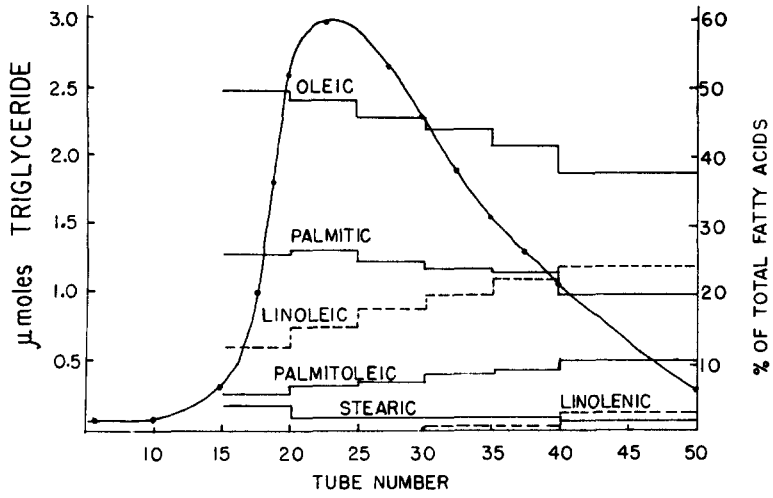

FIG. 1. Column fractionation and fatty acid analyses of egg triglycerides. The skew curve represents total triglycerides eluted from silicic acid. The percentage composition of the fatty acids in each fraction is given by the horizontal lines.

The time course of methanolysis of the mixed egg triglycerides at 0°C is shown in Fig. 2. The disappearance of the triglycerides, the appearance of glycerol, and the appearance and subsequent cleavage of the intermediate diglycerides and monoglycerides are illustrated in this figure. It can be seen that the maximum yields of mono- and diglycerides are attained after only 3 min reaction and represent 17.5 and 11.3% respectively of the starting triglycerides. The qualitative aspect of this reaction was easily and very effectively studied by applying spots of the reaction mixture, after different time intervals, to silicic acid-impregnated paper and developing chromatograms in *n*-heptane–diisobutylketone–acetic acid 96:6:0.5. This system gives a complete separation of the monoglycerides, diglycerides, triglycerides, and fatty acid methyl esters.

The monoglyceride fraction isolated by column chromatography showed an interesting phenomenon. The monoglycerides behaved like monoesters of glycerol with regard to their solubility and paper and column chromatographic properties but gave no hydroxamate–ferric chloride test for the ester group. Only after methanolysis with either methanolic HCl or sodium methoxide (both of

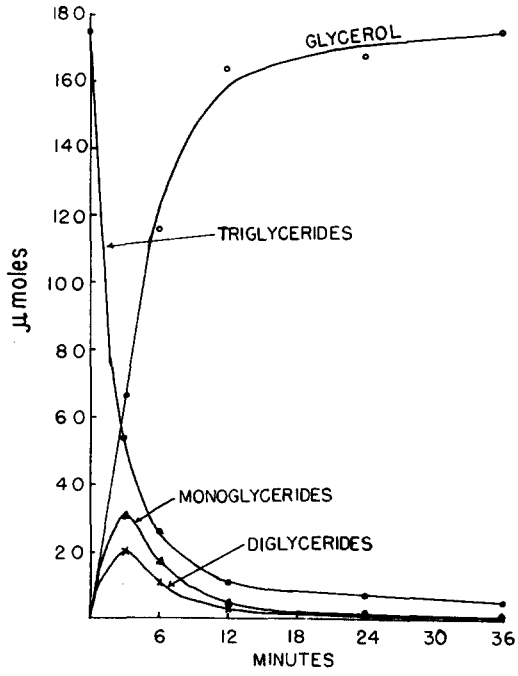

FIG. 2. Rate curves showing the sodium methoxide-catalyzed methanolysis of egg triglycerides at 0 °C.

TABLE 1 GAS CHROMATOGRAPHIC ANALYSIS OF FATTY ACID METHYL ESTERS RELEASED BY SODIUM METHOXIDE METHANOLYSIS OF EGG TRIGLYCERIDES AT 0 °C

Methanolysis Time	Per Cent of Total Fatty Acids*					
	14:0	16:0	16:1	18:0	18:1	18:2
min						
3	0.75	27.2	8.3	1.9	44.3	17.5
6	0.43	27.0	8.6	1.4	46.4	16.2
36	0.65	26.6	7.9	1.4	47.5	16.6
Monoglycerides obtained after 3 min	0.77	22.0	8.0	1.3	45.2	22.7

Linolenic acid is not included in this analysis since it occurred in very small amount (see Fig. 1).

* The analyses were carried out on a Perkin-Elmer Model 154D vapor fractometer, with ethylene glycol succinate polyester on Chromosorb (Perkin-Elmer P column). The temperature was 200 °C, helium pressure 25 psi; flame ionization detector.

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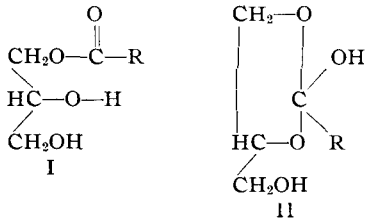
which yielded fatty acid methyl esters) did this fraction lend itself to ester analysis by the hydroxamate procedure. The infrared spectrum of this monoglyceride fraction also showed a lack of carbonyl absorption in the ester region 5.7–5.9 μ. These data suggest that during passage of monoglycerides (I) through silicic acid, ring closure of the ester group occurs to yield a cyclic derivative (II). Compound II differs from orthoesters such as ethyl orthoformate in that not all the hydroxyls are esterified. Compound II does have two hydroxyl groups as does compound I and hence has similar polarity and similar chromatographic properties. However, only compound I has a free ester carbonyl group. The structure of Compound II is provisional.



When the fatty acids released after 3 min are compared with those released after 36 min (Table I) it can be seen that the percentages of 14:0, 16:0, and 16:1 are similar, while those of 18:0 and 18:2 decrease, and that of 18:1 increases. The monoglycerides produced after 3 min contain lower percentages of 16:0 and 18:0 and more 18:2 than the fatty acids released at this time. These results are consistent with the idea that the methanolysis occurs more rapidly with the 16:0 and 18:0 fatty acids. If the saturated acids are linked primarily to the α,α'-positions and 18:2 to the β-position of triglycerides (7), the data suggest that methanolysis occurs slightly more rapidly at the α-position. This is in agreement with our finding with egg lecithin (1), that methanolysis cleaves the primary fatty acid ester faster than the secondary ester group. Admittedly one must also consider special effects of chain length, degree of unsaturation, and proximity of specific acids in each triglyceride molecule, but it is the author's opinion that the type of ester bond (pirmary or secondary) plays a more dominant role in the rate of methanolysis than these other factors.

The stepwise breakdown of triglycerides by methoxide anion provides a way for the production of mixed diglycerides and monoglycerides. We have found that under the experimental conditions given above alcoholysis also occurs rapidly with ethoxide, propoxide, butoxide, isopropoxide, isobutoxide, and isopentoxide in the corresponding alcohol, so that the fatty acid esters of these alcohols can easily be prepared at room temperature in quantitative yield. At room temperature the reaction is complete within 5 min, as demonstrated by paper chromatography.

The alcoholysis of glycerides, which has been extensively used (8), is usually accelerated by catalysts such as hydrochloric acid, sulfuric acid, benzenesulfonic acid, or alkaline hydroxides, but even then these reactions are relatively slow. Sodium and potassium methoxide or sodium ethoxide has been used for ester–ester interchange for the production of mixed glycerides (8, 9), but little information is available on the use of alkoxides at low temperatures to convert the glycerides completely to fatty acid esters of the alcohol component of the alkoxide. Stepwise hydrolysis of glycerides at 0°C may make it possible to obtain diglycerides enriched with the α, β-isomer and monoglycerides enriched with the β-isomer. The very mild conditions for quantitative conversion of triglyceride fatty acids to esters of various alcohols minimize alteration or degradation of highly labile unsaturated fatty acids. The use of chloroform–methanol mixtures also allows the reaction to occur in a single-phase system even at 0°C.

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