Studies on Methanolysis of Triglycerides on Thin Layer Chromatoplate

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ABSTRACT AND SUMMARY

Methanolysis of triglycerides on thin layer chromatography plate, for the preparation of corresponding fatty acid methyl esters suitable for gas liquid chromatography analysis, has been studied with synthetic mixtures of pure tricaprylin, trilaurin, tripalmitin, and triolein. The long chain saturated glyceride, tripalmitin, has been found to be least susceptible to methanolysis. Plate temperature of 65 C and KOMe-methanol reagent concentration of 1.0 N have been found to be optimal for samples containing large amount of trisaturated glyceride like tripalmitin without appreciable loss in short chain components by evaporation. For fats containing lower proportions of trisaturated glyceride like vegetable oils, a lower concentration, i.e., 0.5 N of the reagent can be used at the above temperature. Addition of lipid solvent like benzene in the reagent has little effect on the conversion. Long chain triglycerides containing one or more double bonds in the molecule respond more to this reaction than do trisaturated glycerides of the same carbon number. Fatty acid compositions of cottonseed and peanut oil triglycerides obtained by the GLC analysis of the methyl esters prepared by the present method and that of Luddy et al. [JAOCS 45:549 (1968)] have been found to agree well.

INTRODUCTION

Recently the authors (1) introduced a method for the preparation of methyl esters from the triglycerides of a few mg of a vegetable oil. The oil is first subjected to fractionation into lipid classes on a thin layer chromatography (TLC) plate. Methanolysis of the triglyceride band is then carried out on that plate at 60 C using a 0.5 N solution of potassium methoxide (KOMe) in methanol. The resulting methyl esters are extracted with suitable solvent and analyzed by gas liquid chromatography (GLC) for fatty acid composition of the triglycerides. The advantage of this method is that the separation of triglycerides from other lipid components and preparation of corresponding methyl esters can be carried out on the same TLC plate. During the development of this method it was found that at lower temperatures the amounts of methyl palmitate produced were always less than the expected values.

The present communication deals with the study of methanolysis by KOMe reagent on TLC plate of synthetic mixtures containing pure triglycerides like tricaprylin, trilaurin, tripalmitin, and triolein in order to find the optimum conditions.

EXPERIMENTAL PROCEDURES

Samples: Two solutions of known amounts of triglycerides (99+ % grade, Applied Science Laboratories, State College, PA) in chloroform were prepared by direct weighing on a microbalance. Mixture 1 contained tricaprylin, trilaurin, tripalmitin, and triolein; mixture 2 contained a greater proportion of tripalmitin than that in mixture 1 but no tricaprylin.

Reagents: For methanolysis: ca. 2 N solution of KOMe in methanol was prepared by reacting small pieces of freshly cut metallic potassium with absolute methanol and kept as stock reagent in cold. 0.5 N, 0.75 N, and 1 N solutions were prepared by diluting the stock reagent with absolute methanol before use. The solvents were purified, dried and distilled. All other reagents were of analar grade.

Procedure for Methanolysis on TLC Plate

Aliquot from the prepared mixtures containing about 10 mg of triglycerides was applied as a band on a 14 x 14 cm Silica Gel G (E. Merck, Darmstadt, Germany) plate of 0.5 mm thickness activated at 110 C for 1 hr. The plate was developed for a distance of 12 cm with a mixture containing benzene, ether, and acetic acid (100:10:1). The triglyceride band was located by exposing the air-dried chromatogram to iodine vapor and outlined with a needle. A metallic tray was heated to 65-70 C in an incubator. The chromatogram was then placed on the tray in the incubator and heated for 5 min. The tray with the plate was taken out and the triglyceride band was immediately soaked with the KOMe-methanol reagent from a capillary dropper. The plate with the coated face down was quickly cooled under a fan. The band was scraped off the plate into a small glass column (12 x 1 cm) provided with a sintered disc and a capillary tip. Lipid materials were extracted from the adsorbent with a 1:1 mixture of petroleum ether (40-60 C) and ether using 5 elutions of 2 ml each. Solvents from the pooled extract were evaporated under nitrogen and the fatty acid composition of the residue was determined by GLC. An "F & M Model 700R" dual column, dual flame ionization detector, gas chromatograph with "F & M Model 240" linear programmer was used. 6 ft x 0.25 in. stainless

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TABLE I

Methanolysis of Triglyceride Mixture 1 on Thin Layer Chromatography Plate

Composition of triglyceride				Fatty acid composition (% wt) with 0.5 N KOMe in methanol				Fatty acid composition (% wt) with 0.5 N KOMe in methanol benzene mixture (4:1)			
Component glycerides	% wt[a]	Component acids	% wt[a]	50 C	60 C	70 C	80 C	50 C	60 C	70 C	80 C
Tricaprylin	16.5	Caprylic ($C_{8:0}$)	16.0	18.5	17.3	15.4	10.3	16.9	23.3	13.5	7.8
Trilaurin	27.2	Lauric ($C_{12:0}$)	27.1	36.4	35.3	34.1	32.1	54.4	36.8	36.8	36.3
Tripalmitin	30.4	Palmitic ($C_{16:0}$)	30.6	17.9	19.3	22.0	24.1	19.4	28.2	29.8	34.2
Triolein	25.9	Oleic ($C_{18:1}$)	26.3	27.2	28.1	28.5	33.5	9.3	11.7	19.9	21.7

[a]Calculated values.

TABLE II

Methanolysis of Triglyceride Mixture 2 on Thin Layer Chromatography Plate at 70 C

Component glycerides	Component fatty acids	Calculated composition (% wt)	Fatty acid composition (% wt) KOMe concentration 0.5 N reagent	0.75 N reagent	1.0 N reagent
Trilaurin	$C_{12:0}$	32.3	42.9	31.6	30.7
Tripalmitin	$C_{16:0}$	36.4	28.9	35.6	35.8
Triolein	$C_{18:1}$	31.3	28.2	32.8	33.5

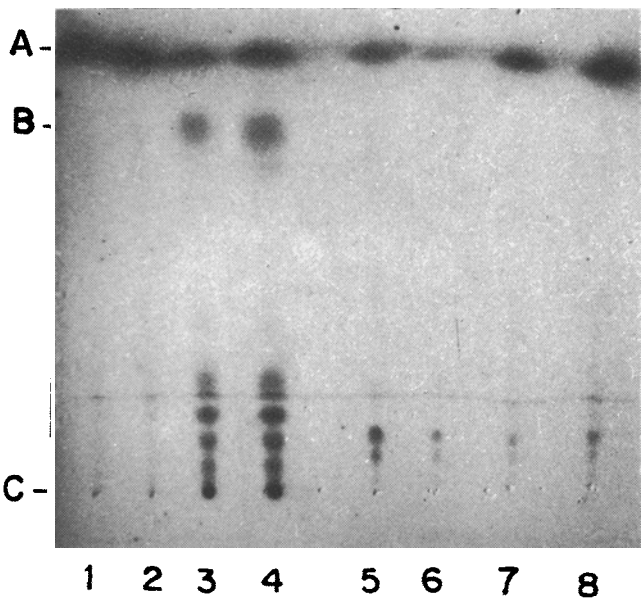

FIG. 1. Extent of methanolysis of simple and mixed triglycerides on thin layer chromatography plate at 70 C using 0.5 KOMe solution in methanol. Plate activation: 1 hr at 110 C; developing solvent; petroleum ether (40-60 C), ether and acetic acid (90:10:1), developed in S-Chamber up to 11 cm; materials applied were the products of methanolysis of: Triolein (1 & 2), mixture 1 (3 & 4), monooleodipalmitin (5 & 6), and dioleomonopalmitin (7 & 8). Visualization: by charring; spots: A, methyl esters; B, unreacted triglycerides, lower spots unidentified. C, line of application.

steel column was packed with 10% diethyleneglycol succinate on 60-80 mesh Gas-Chrom Z (Applied Science Laboratories). Nitrogen at flow-rate 40 ml/min was used as carrier gas. The column temperature was programmed between 90-160 C at different rates to obtain optimum separation.

Preparation of Mixed Glycerides Monooleodipalmitin and Dioleomonopalmitin

Ca. 0.3 g each of pure tripalmitin and triolein in 1.5 ml n-hexane were randomized (2) for 1 hr with sodium methoxide catalyst (0.4% of triglycerides). The product was acidified with 10% hydrochloric acid and extracted with ether. The ether extract was washed free of mineral acid and dried over anhydrous sodium sulphate. The randomized mixture was applied as a band on each of five $AgNO_3$ impregnated silica gel TLC plates and the component glycerides were separated according to Barrett et al. (3). The bands were marked after spraying with Rhodamine 6G and scraped off. Scrapings from similar bands were mixed and extracted with chloroform. An aliquot of each extract was subjected to methanolysis and the mixed methyl esters were analyzed by GLC to characterize the band. Monooleodipalmitin and dioleomonopalmitin thus isolated were further purified by rechromatography on $AgNO_3$-TLC plate, isolated and stored under nitrogen in cold.

RESULTS AND DISCUSSION

The effects of increase in temperature on the extent of on-plate methanolysis of the triglyceride mixture 1 using 0.5 N KOMe in methanol are presented in Table I. It is seen that at a temperature as low as 50 C, the percentages of $C_{8:0}$, $C_{12:0}$, and $C_{18:1}$ acids are higher than the theoretical values. The percentages of $C_{8:0}$ and $C_{12:0}$ acids decreased continuously throughout the temperature range and this decrease is greater in the case of former acid particularly between 70 C and 80 C. The percentage of $C_{18:1}$ acid has a tendency to increase with temperature. In the case of $C_{16:0}$ acid, the percentage increases steadily but does not reach the theoretical value even at 80 C. These results indicate that the conversions of tricaprylin, trilaurin, and triolein are high even at 50 C but that of tripalmitin remains incomplete even at 80 C. At higher temperatures of 70 C, 80 C, or more, some losses in $C_{8:0}$ and $C_{12:0}$ acids, particularly in the former, are indicated presumably due to evaporation of their esters. The higher percentages of $C_{18:1}$ acid at higher temperatures are due to contributions of (a) lower conversion of $C_{16:0}$, (b) evaporation loss of $C_{8:0}$ and $C_{12:0}$ esters.

Results of similar experiments using 0.5 N KOMe in a mixture of methanol and a lipid solvent like benzene (4:1) are also presented in the same table. It shows that the presence of benzene has not increased the conversion significantly.

In Table II are presented the results of on-plate methanolysis of triglyceride mixture 2 at 70 C with 0.5 N, 0.75 N, and 1 N solutions of KOMe. Mixture 2 contains a higher proportion of tripalmitin than that in mixture 1. From results in Table II, it appears that with reagents of greater KOMe concentrations, viz. 0.75 N and 1 N, conversions of tripalmitin are equivalent to that of trilaurin and triolein and the fatty acid compositions are comparable to the calculated one.

It has been found (Table I) that in spite of its higher molecular weight the conversion of triolein was more than that of the trisaturated glyceride tripalmitin. It was thought to be of interest to find the influence of the presence of one or more double bonds in the triglyceride molecule on the susceptibility of such glycerides towards on-plate methanolysis. In the TLC chromatogram presented in Figure 1, of the products isolated after on-plate methanolysis of mixture 1, which contained a high proportion of tripalmitin, monooleodipalmitin, dioleomonopalmitin, and triolein using 0.5 N KOMe at 70 C, spot due to unreacted triglyceride can be seen only in the chromatograms of mixture 1 (3,4). This shows that unsaturated triglycerides are more susceptible to on-plate methanolysis than the trisaturated ones. It may be due to the fact that unlike higher saturated glycerides unsaturated ones are comparatively low-melting (liquid at room temperature). Regarding the lower spots in the chromatogram of mixture 1 (3,4), the lowermost is due to monoglycerides and the second, third, and fourth in the ascending order are due to low molecular weight 1,2-diglycerides (e.g., 1,2-dicaprylin), mixture of low molecular weight 1,3-diglycerides and high molecular

TABLE III

Fatty Acid Compositions (Major Components) of Triglycerides of Cottonseed and Peanut Oils

Component fatty acids of triglycerides	Cottonseed oil		Peanut oil	
	Present method	Method of Luddy et al. (4)	Present method	Method of Luddy et al. (4)
$C_{14:0}$	1.08	0.9	0.3	0.8
$C_{16:0}$	24.1	24.7	12.1	11.7
$C_{18:0}$	3.0	3.3	3.1	4.0
$C_{18:1}$	18.6	19.6	54.7	55.1
$C_{18:2}$	52.4	51.1	28.1	26.1
$C_{18:3}$	0.9	0.4	0.8	1.1
$C_{20:0}$	-	-	0.9	1.2

weight 1,2-diglycerides (e.g., 1,3-dicaprylin and 1,2-dipalmitin), and high molecular weight 1,3-diglycerides (e.g., 1,3-dipalmitin). This identification is based on the TLC under similar conditions, of the products of on-plate methanolysis of authentic tricaprylin and tripalmitin. No spot corresponding to free fatty acid appeared in these chromatograms. The fifth spot could not be identified, but that this spot is not due to fatty acid can be inferred from its absence in the chromatograms of other products in Figure 1 as well, in those of the products of methanolysis of tricaprylin and tripalmitin. In the light of these results together with the fact that natural triglycerides, particularly those of vegetable origin, scarcely contain high proportion of higher trisaturated glycerides like tripalmitin, tristearin, etc., it can be concluded that on-plate methanolysis of these samples can be carried out with 0.5 N KOMe reagent at 65-70 C. For fats containing greater proportions of trisaturated glycerides higher concentration, i.e. 1 N of the reagents has to be used at the above temperature.

The close agreement between the fatty acid composition of cottonseed and peanut oil triglycerides, presented in Table III, obtained by the GLC analysis of the methyl esters prepared by the present method and that of Luddy et al. (4), supports the above conclusion.

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