Gas-Liquid Chromatographic Analysis of Lactoylated Monoglycerides[1]

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Abstract

This paper describes a method for analyzing gas-liquid chromatograms of silylated monoglycerides (1) and lactoylated monoglycerides. A simple method for lactoylating a monoglyceride for the determination of the products formed is also revealed. Resolution of glycerides containing as many as five lactoyl groups is obtained.

Introduction

THE BASIS OF THIS PAPER is that a lactoylated monoglyceride contains the same number of hydroxy equivalents as the parent monoglyceride. For this reason both the lactoylated and nonlactoylated monoglycerides are isolated in the same column chromatographic phase from diglycerides and triglycerides. Similarly, since both lactoylated and nonlactoylated monoglycerides contain two free hydroxy groups, their trimethylsilyl (TMS) ethers are easily formed. The TMS derivatives have a low polarity and are easily separated by GLC.

Experimental Procedures

Isolation of Monoglycerides and Monoglyceride Derivatives

Prior to gas chromatography, all monoglycerides and lactoylated monoglycerides were isolated from di- and triglycerides by column chromatography (2). Separation of glyceride classes was achieved by passing 1 g of sample dissolved in approximately 5 ml of chloroform through 30 g of a 5% to 6% hydrated silica gel G. A chromatographic grade of silicic acid ($SiO_2 \cdot xH_2O$) can be used in place of the hydrated silica gel G (SiO_2 anhydrous). Triglycerides were eluted with 250 ml of 100% benezene; diglycerides with 250 ml of 10% diethyl ether in benzene; and monoglycerides with 250 ml of 100% diethyl ether. The more hydrophylic substances such as glycerol and polyglycerols were eluted with 250 ml of 100% methanol. Solvents were removed by gently heating the sample to constant weight under a stream of nitrogen.

Silylation and Lactoylation

Twenty milligrams of the isolated mono- or lactoylated monoglyceride was dissolved in 1 ml of *p*-dioxane. To form the silyl ethers, 0.4 ml of 1,1,1,3,3,3-hexamethyldisilizane and 0.2 ml of trimethylchlorosilane were added to the monoglyceride solution. This mixture was shaken for 1 min and allowed to stand for 30 min. The TMS derivatives of the monoglycerides were then injected into the gas chromatograph. It is important to note that, if these silyl ethers are to be stored for future reference, they should be extracted with a nonpolar solvent from the *p*-dioxane and refrigerated (1).

To obtain the TMS derivatives of known lactoylated monoglycerides, 2 ml of 85% lactic acid was added directly to a silylated nonlactoylated monoglyceride. The acidified silylated glyceride solution was shaken, placed in a boiling water bath for 10 min, and cooled to room temperature. The partially lactoylated glycerides were consecutively extracted using 10 ml of *n*-hexane and 10 ml of water. Three to four extractions were usually sufficient to remove the water soluble esters and unreacted lactic acid (Fig. 1). Excess *n*-hexane was removed under a stream of nitrogen. The lactoylated monoglycerides were then dissolved in 1 ml *p*-dioxane and silylated again in the same manner as described above.

Gas Chromatography

A Wilkens, Model 600B gas chromatograph equipped with a flame ionization detector was used throughout the entire analysis. Samples were chromatographed primarily on a ¼ in. × 3 ft stainless steel column, packed with 2.0% SE 30 on Aeropack 30. The column was temperature programmed from 175–375 C at 6 C/min. A flow rate of nitrogen was maintained at 70 ml/min. Sample sizes injected varied from 2.0 to 3.0 μliters.

Identification of monoglyceride peaks was made by comparing the retention times of unknown with those for known monoglycerides. Identified peaks were quantitated by multiplying the peak height times the

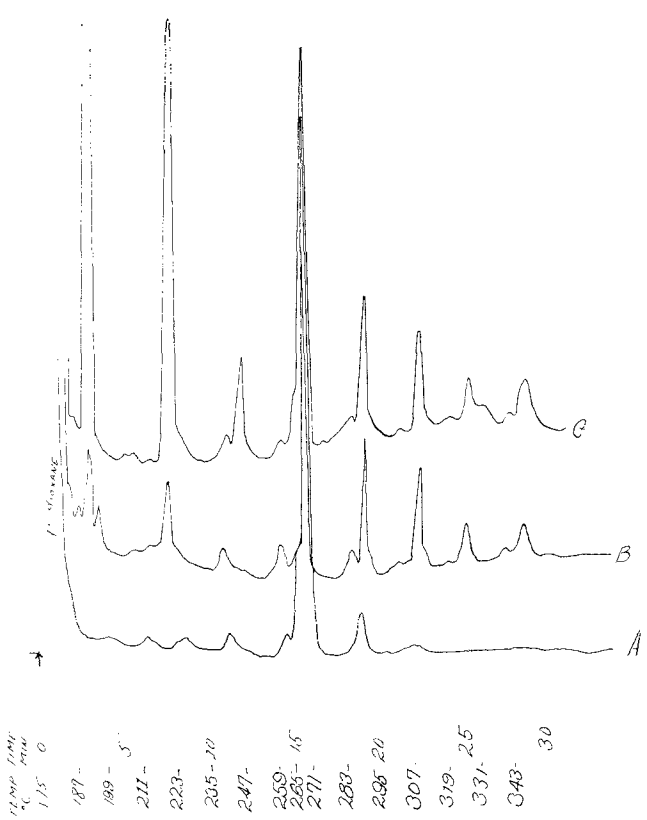

FIG. 1. Effects of lactoylation and washing of a lactoylated glycerol monostearin sample: A. Glycerol mono stearin; B. Lactoylated glycerol monostearin after washing; C. Lactoylated glycerol monostearin before washing.

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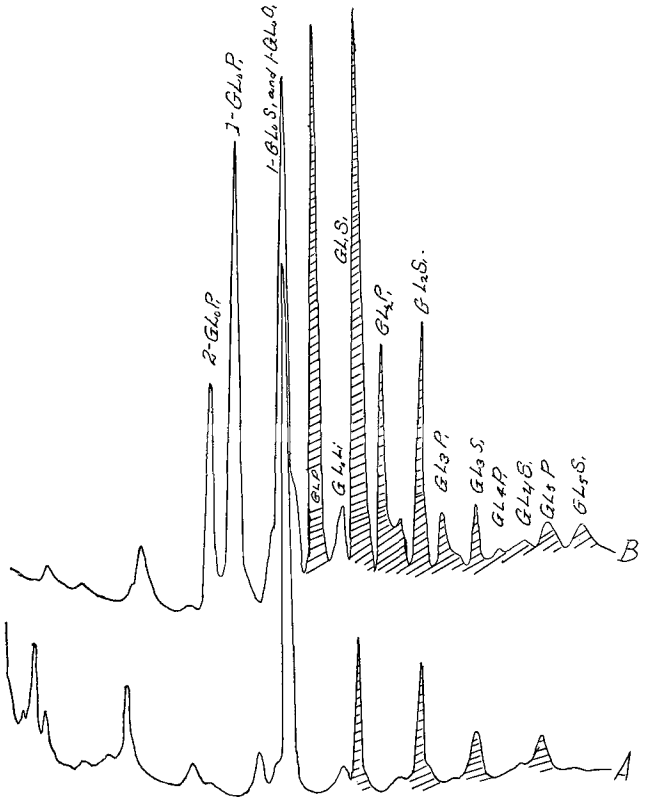

FIG. 2. A GLC comparison of known lactoylated monostearin (A) and a commercially lactoylated glycerol monostearin (B). The shaded areas indicate lactoylated monoglycerides. Abbreviations are: G—Glycerol; P—Palmitic; S—Stearin; Li—Linoleic; O—Oleic; and L—Lactic. The numerical subscripts indicate the equivalents of the particular acid.

peak width at one half peak height. Fatty acid ester distributions were obtained by esterification and subsequent gas chromatography of the same silylated sample. The method described by Metcalf, et al. (3) was used in the esterification of silylated monoglycerides. Comparison of calculated fatty acid esters with analyzed fatty acid distributions was used to confirm monoglyceride identifications made from the retention data.

Commercial lactoylated monoglycerides were identified by the comparison of their retention times with those of peaks of known monoglycerides following lactoylation. Comparison of calculated and analyzed fatty acid esters of the commercial samples confirmed the identifications. In calculating the fatty acid ester distributions from the lactoylated monoglyceride chromatograms, each new peak was assumed to be a lactoylated monoglyceride containing one equivalent weight of the fatty acid.

Discussion

Column Chromatography

In the column chromatographic method employed in this work, separation of the glycerides is achieved through the hydrophylic attractions of their respective hydroxy groups to hydrated silicic acid. The greater the number of free hydroxy groups in a particular glyceride the greater is the hydrophylic attraction. The individual glycerides are then eluted by solvents varying in polarity. Since lactic acid contains a secondary hydroxy group, lactoylation of a monoglyceride will not alter the number of free hydroxy groups. Where lactoyl groups polymerize, there are still hydroxy groups associated with the terminal lactoyl units. Therefore, the lactoylated and polylactoylated monoglycerides are eluted with monoglycerides in the 100% diethyl ether phase.

TABLE I

Calculation of the Fatty Acid Distributions From a Commercial Lactoylated Monopalmitin and Comparison With the Fatty Acid Ester Distribution Obtained by Direct Methyl Esterification

Pk No.	Composition	%	Fatty acids—mole fractions		
			C_{16}	C_{18}^{0}	$C_{18}^{=}$
1	Stearic acid	2.0		2.0	
2	2-GL_0P_1	5.5	4.2		
3	1-GL_0P_1	13.4	10.3		
4	$GL_0S_1 + GL_0O_1$	15.7		12.4	
5	GL_1P_1	15.2	9.7		
6	GL_0Li	3.0			2.4 not confirmed
7	GL_1S_1	17.2		11.4	
8	GL_2P_1	6.2	3.3		
9	GL_1Li	2.9			1.9 not confirmed
10	GL_2S_1	5.7		3.3	
11	GL_3P	2.9	1.4		
12	GL_3S	2.4		1.2	
13	GL_4P	Not quantitated			
14	GL_4S	1.8		0.8	
15	GL_5P	2.9	1.0		
16	GL_5S	3.2		1.3	
	Sums	100.0	29.9%	32.4%	4.3 = 66.6%
Fatty acid weight percent			44.8	48.8	6.4 = 100.0%
Fatty acids found percent			44.3 +	47.3 +	4.0 + 5.4 Oleic

Gas Chromatography

Comparison of the chromatograms of a glycerol monostearin before and after lactoylation revealed as many as five new peaks following lactoylation (Fig. 1). The peaks having retention times shorter than monostearin were considerably reduced or lost following water washing of the lactoylated monoglyceride. These peaks were regarded as polymerized lactic acid, or lactoylated glycerol. The peaks having retention times longer than monostearin were not effected by water washing. There also appeared to be a linear relationship between the increase of retention times of these new and longer retained peaks and a unit increase in molecular weight of the parent monoglyceride. The linear increase was assumed to be due to an increase of lactic acid units on the monoglyceride. These peaks were tentatively identified as lactoylated monostearin, and were matched to previously unidentified peaks in commercial lactoylated samples.

The fatty acid compositions were calculated from the lactoylated and nonlactoylated peaks identified in commercial lactoylated and monostearin samples. It was not uncommon to find from 50% to 60% of a particular fatty acid associated with the lactoylated monoglycerides. The same commercial lactoylated and silylated monoglycerides were methyl esterified and the fatty acid distributions compared with the calculated fatty acid distributions (Table II). The comparison of calculated with observed fatty acid distributions served to confirm the identifications made from the retention data.

At least two and sometimes four and five new peaks were observed following lactoylation of monomyristin and monopalmitin. As with the monostearin sample, the new peaks were in homologous series with the parent monoglyceride. The new peaks formed as a result of lactoylation were regarded as lactoylated monoglycerides.

How the lactoyl groups are arranged was not ascertained. There are (N + 1) possible isomers per lactoylated monoglyceride, where N is equal to the number of lactic acid groups. A more comprehensive

study of the specific structure of these lactoylated glycerides was made by Lauridsen, et al. (4) using both GLC and mass spectroscopy.

Several commercially lactoylated glycerides were analyzed by the method described. Comparison of calculated fatty acid content with fatty acid distributions showed them to be virtually identical. Good results were obtained on fat samples containing as low as 6% of commercially nonlactoylated monoglycerides and lactoylated monoglycerides. In the chromatograms of these samples, the nonlactoylated glyceride peaks were much larger than the lactoylated glyceride peaks. Therefore, the lactoylated glyceride peaks associated with more than two equivalents of lactic acid, were low and their calculated fatty acid values were insignificant in the actual fatty acid distributions.

REFERENCES

1. Wood, R. D., JAOCS *4*, 161–165 (1965).
2. Sahasrabudhe, M. R., J. J. Legari and W. P. McKinley, J. Assoc. Offic. Agr. Chemists *42*, 789–792 (1965).
3. Metcalfe, L. C., A. A. Schmitz and J. R. Pelka, Anal. Chem. *38*, 514 (1966).
4. Lauridsen, J. B., AOCS, 58th Meeting, New Orleans, May 1967.

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