THE SEPARATION OF GLYCERIDES OF MIXED FATTY ACID CHAIN LENGTH BY GLASS PAPER CHROMATOGRAPHY

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(Received July 4th, 1960)

INTRODUCTION

Acetoglycerides, or acetin fats as they are sometimes called, are synthetic mixed glycerides in which acetic acid is substituted for a portion of the long chain fatty acids. They are being considered as possible lubricants for food machinery, spreads, and as lower melting food fats[1,2]. There are presently no published reports on their separation and characterization by means of paper chromatography.

The following report describes a method which separates several glycerides of fatty acids of mixed chain length on glass paper impregnated with silicic acid.

MATERIALS

Glycerides

1,3-Distearin, diolein adipate, 1-mono-palmitin, 1-aceto-3-stearin, 1-butyro-3-palmitin, 1,3-diolein, 1-aceto-3-olein, 1,2-diaceto-3-stearin, 2-aceto-1,3-distearin, 1-monoölein, 1-monostearin, and 1,2-diaceto-3-olein were synthesized by first preparing acetone glycerol by the method of FISCHER AND PFAHLER[3], then acylating according to the method of AVERILL, ROCHE AND KING[4]. Triolein, tristearin, stearic acid, oleic acid, 1,2-dipalmitin, 1,3-dipalmitin, and palmitic acid were commercial products recrystallized to constant melting point. All compounds were shown by chemical methods to be 98–99 % pure.

Solvents

The developing solvents employed were 2:98 and 5:95 (v/v) ether in isoöctane.

METHODS

Preparation of paper

Glass fiber filter paper, type 934-AH (H. Reeve Angel and Co.)**, was cut into 4 × 7 in. sheets and heated in a 600° oven for one hour to remove the organic binder present.

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** Trade names are given as part of the exact experimental conditions and not as an endorsement of the products over those of other manufacturers.

The sheets were impregnated with silicic acid by the method of DIECKERT *et al.*[5] and stored in a dust-free container until used.

Chromatographic procedure

The chromatograms were prepared essentially by the technique developed by DIECKERT AND REISER[6]. About 5 μg of material per spot was applied to the sheets of paper as approximately 0.05 % solutions in commercial hexane (Skellysolve B)*. The chromatograms were developed for 18 min, dried, then sprayed with sulfuric acid, and heated until the chromatographed materials charred. The charred spots were outlined in pencil and the R_F values determined.

RESULTS AND DISCUSSION

For this investigation the following three classes of glycerides were selected for comparison:

1. Stearins in which the stearic acid is partially replaced by acetic or butyric acids.
2. Oleins in which oleic acid is similarly replaced by acetic acid.
3. Palmitins in which palmitic acid is similarly replaced by acetic or butyric acids.

Figs. 1 and 2 represent tracings from typical chromatograms obtained using the solvent system: 2 % ether in isoöctane. The results given in Table I (A, B, and C) have been calculated from such chromatograms and are reproducible averages of

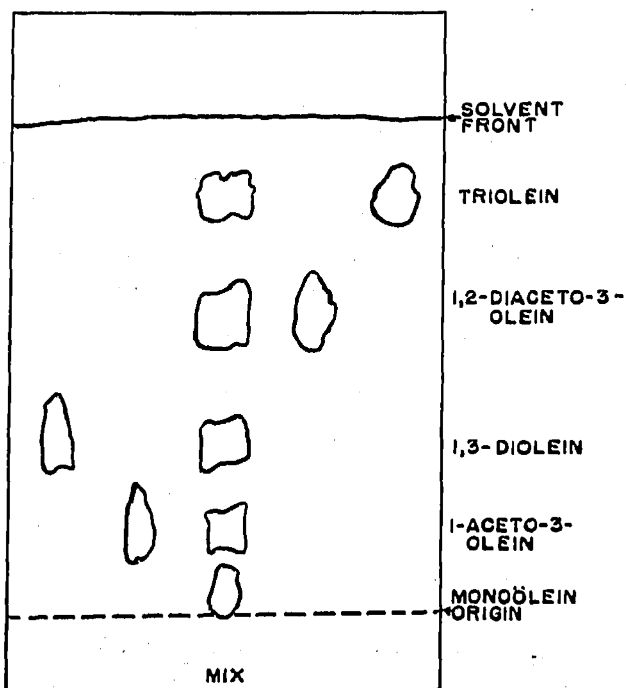

Fig. 1. Separation of oleins using 2 % ether–isoöctane as developing solvent.

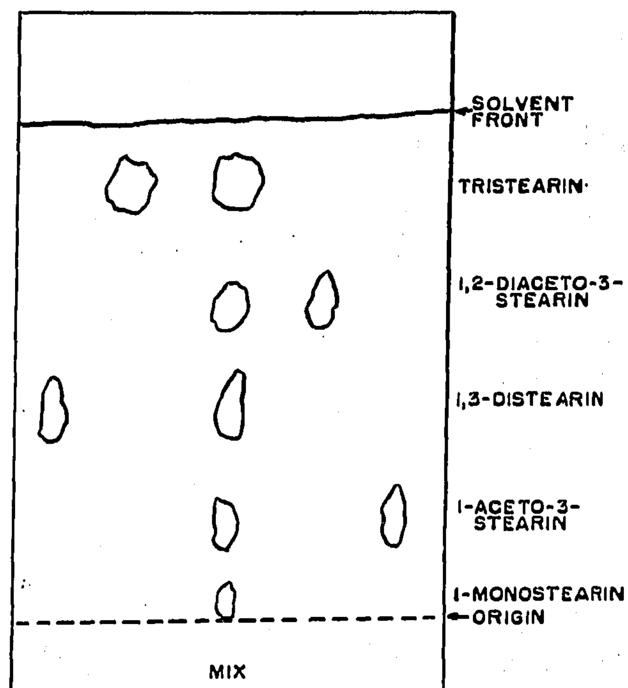

Fig. 2. Separation of stearins using 2 % ether–isoöctane as developing solvent.

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from 4–10 values each. Table IA lists the results obtained in the separation of fats containing stearic acid. With 5 % ether in isoöctane as the developing solvent, the triglycerides moved so close to the solvent front that differences in R_F were not evident when only one acetyl group replaced a higher acyl group. However, development with 2 % ether in isoöctane and/or replacement of a stearyl group with an

TABLE I

SEPARATION OF SUBSTITUTED GLYCERIDES IN ETHER–ISOÖCTANE SOLUTIONS

Glyceride	*Solvent, ether–isoöctane*	
	2% ether	*5% ether*
A. Stearins		
1-monostearin	0.05	0.14
1,3-distearin	0.33	0.65
tristearin	0.82	0.93
1,2-diaceto-3-stearin	0.58	0.85
2-aceto-1,3-distearin	0.76	0.94
1-aceto-3-stearin	0.21	0.40
stearic acid	0.77	0.90
1-butyro-3-stearin	0.29	0.55
B. Oleins		
1-monoölein	0.05	0.17
1,3-diolein	0.24	0.66
triolein	0.78	0.95
1,2-diaceto-3-olein	0.50	0.84
1-aceto-3-olein	0.18	0.41
oleic acid	0.67	0.87
diolein adipate*	0.08	0.21
C. Palmitins		
1-monopalmitin	0.03	0.15
1,2-dipalmitin	0.20	0.55
1,3-dipalmitin	0.26	0.68
1-aceto-3-palmitin	0.21	0.46
palmitic acid	0.75	0.89
1-butyro-3-palmitin	0.30	0.56

* The ester of 1,3-diolein and adipic acid.

acetyl group caused a decrease in the relative movement of the triglyceride. Substitution of two stearyl with acetyl groups lessened the movement still more, reducing the R_F from 0.82 to 0.76 to 0.58 for tristearin, 2-aceto-1,3-distearin, and 1,2-diaceto-3-stearin, respectively (Fig. 2). A similar separation of 1,2-diaceto-3-olein and triolein is shown in Fig. 1.

With the series of 1,3-diglycerides, the better separations were made with 5 % ether in isoöctane. When 1-aceto- and 1-butyro-substituted diglycerides were chromatographed concurrently with the long chain diglycerides (Table IA and C), R_F values increased with increasing chain length in the order aceto-, butyro-, and palmito-(stearo- or oleo-)glycerides. In this system, it would seem that as fatty acid chain length increases (within each class of glycerides), there is an increase in movement of the material up the chromatogram.

There is little or no difference between the R_F values of the monoglycerides of oleic, stearic, and palmitic acids, nor between their di- or triglycerides. However, ORY *et al.*[7] separated saturated and unsaturated fatty acids by bromination at the double bond, followed by methylation, and subsequent chromatography. Since the R_F values for mono-, di-, and triglycerides are notably different, as shown in Table I and by others[6,8,9], the mono-, di-, and triglycerides of oleic and stearic acids may be conveniently separated by developing a mixture in one direction, bromination directly on the chromatogram by exposure to bromine vapors in a closed jar, and redevelopment in a second direction. This was done and, as expected, the brominated glycerides moved slower than their saturated counterparts.

This technique rapidly separates the members of a mixture of mixed glycerides based on the different mobilities of the short and long chain fatty acid components. FRANC AND JOKL[10] showed that increasing the length of the chain by increments of a methylene group in some aliphatic mono- and dicarboxylic acids resulted in different mobilities on the chromatogram.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. J. W. DIECKERT for his helpful suggestions, and T. L. WARD, N. V. LOVEGREN, and A. T. GROS for some of the glycerides used here.

SUMMARY

A chromatographic separation on glass fiber paper of mixed glycerides of acetic, butyric, stearic, palmitic, and oleic acids in solvents consisting of 2 % and 5 % ether in isoöctane has been described. In general, glycerides containing fatty acids of increasing chain length tend to move further up the chromatograms than those of shorter chain length.

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