oleate, are reasonably constant over a temperature range of 50C. Although other parameters were changed, column temperature seems to be the most critical parameter for both linoleic and linolenic acids, and the temperature dependence would probably restrict the use of the PF values reported here to a narrow range, probably 190–200C.

Data developed in the present study support the conclusion that the precision of GLC analysis for C_{14}–C_{18} fatty acid methyl esters is comparable to conventional spectrophotometric methods. However the use of uncorrected area percent data can introduce significant absolute errors. It can be calculated from data presented here that the absolute weight error for each methyl ester is as follows: myristic, +10.6%; palmitic, +5.5%; stearic, 0%; oleic, +1.5%; linoleic, −7.8%; and linolenic, −16.9%.

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Quantitative Estimation of Isomeric Monoglycerides by Thin-Layer Chromatography[1]

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Abstract

A method has been developed for the analysis of isomeric monoglycerides by thin-layer adsorption chromatography. Isomeric monoglycerides are separated on silica gel impregnated with boric acid; a mixed solvent system is used. The interaction of hydroxy compounds with boric acid allows the separation of 1- and 2-monoglycerides which cannot be resolved on silica gel alone. In order to make the method quantitative, the resolved components are charred and their spot densities measured. Glycerol, fatty acids, diglycerides, and triglycerides do not interfere. The method does not induce isomerization.

Introduction

ALTHOUGH THE TECHNIQUE of thin-layer chromatography (TLC) has been known for a number of years, it was not until 1956 through the work of Stahl (1,2) that its use in the lipid field was established. Since that time the technique has undergone rapid development for analytical applications, especially for the direct separation of lipids without prior chemical modification.

Lipids are readily resolved into classes (3–7); mono-, di-, and triglycerides can be separated according to the number of hydroxyl groups in the molecule, irrespective of chain length and degree of unsaturation, by adsorption TLC on silica gel (4–8). Subfractionation within classes may be obtained by reversed phase partition TLC, e.g., on siliconized silica gel (5,6,9,10).

Saturated and various unsaturated members of an homologous-vinylogous series are separated according to their degree of unsaturation by TLC on silica gel-silver nitrate (6,11–14) or as their mercuric acetate addition compounds on silica gel (6). In addition, some *cis-trans* and positional isomers are resolved on silica gel-silver nitrate (6,11–13).

Isomeric diglycerides are easily resolved by adsorption TLC on silica gel (8,15).

Although compounds with very small differences in polarity can be separated by TLC, a satisfactory chromatographic method for the analysis of unmodified isomeric monoglycerides was not available until now.

The most successful previous attempt to resolve isomeric monoglycerides is that described by Hofmann (16), who attempted to resolve them by TLC on hydroxyl-apatite. Hofmann shows that at room temp the isomers are resolved; however, considerable isomerization occurs. At 10C the extent of isomerization is less, but resolution of the isomers is drastically reduced.

Boric acid or borate complexing of polyhydroxy compounds has long been employed in carbohydrate chemistry to effect separations (17) and to assign configurations (18). This technique has also been used to remove benzylidene or isopropylidene blocking groups without causing appreciable isomerization of partial glycerides to occur (19–21).

Silica gel-boric acid TLC has been employed to resolve sugars (22), and a similar system was used to resolve *threo* and *erythro* isomers of dihydroxy acids (23).

The present study describes the use of silica gel-boric acid adsorption TLC to separate isomeric monoglycerides. Results are quantified by means of transmittance densitometry.

Experimental

Thin-Layer Chromatography

The analyses were carried out on 8 × 8 in chromatoplates of silica gel impregnated with boric acid. A slurry was prepared from 50 ml of 0.4 M (24.7 g/liter) aqueous H_3BO_3 and 25 g of Silica Gel GF "Merck" from which layers approximately 0.3 mm thick were spread. Chromatoplates were dried at room temp and activated by heating for 2 hr at 110C in a convention-type oven. No special storage conditions were employed. However, the plates were generally used

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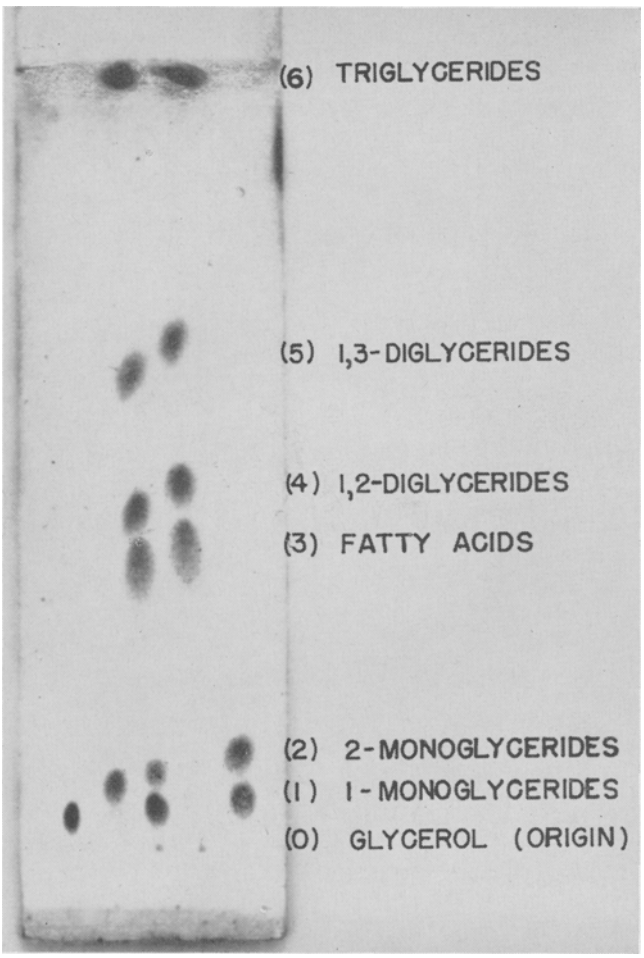


FIG. 1. Chromatoplate of isomeric monoglycerides and other lipids on silica gel-boric acid. Solvent: 96% chloroform, 4% acetone.

within two days of their preparation.

Samples were carefully dissolved in chloroform at room temp, and the dilute solutions of known concn were spotted. The chromatoplate was then developed with an appropriate solvent mixture. In order to visualize the resolved components, the chromatoplate was sprayed lightly with a saturated solution of CrO_3 in 70% by volume of aqueous sulfuric acid, and then heated for 25 min at 200C.

Densitometry

A Photovolt Densitometer-Photometer (Models 52C and 520-A) equipped with a synchronous motor-driven TLC stage (3 in/min) was used. A 1 × 15 mm slit was employed. Readings were plotted by means of a strip-chart recorder and the peak areas were determined by triangulation.

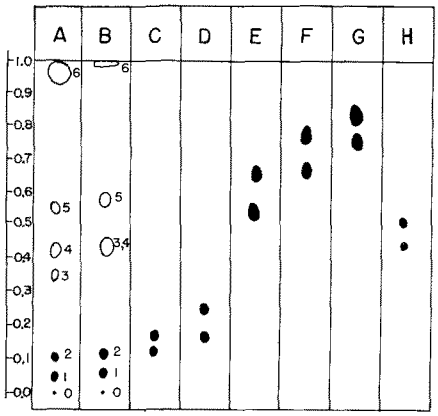
Results

The qualitative separation of isomeric monoglycerides on a boric acid-silica gel chromatoplate is illustrated in Figure 1.

In addition to the separation of isomeric monoglycerides: glycerol, fatty acids, 1,2-diglycerides, 1,3-diglycerides and triglycerides are also resolved with the solvent system employed, i.e., 96% chloroform and 4% acetone.

The separation of 1- and 2-monoglycerides is obtained readily with a variety of solvent combinations as seen in Figure 2.

Chromatoplate A, developed with 96% chloroform and 4% acetone as previously seen in Figure 1, shows



SOLVENT	CHLOROFORM	ACETONE	ACETIC ACID	METHANOL
A	96	4	–	–
B	95.5	4	0.5	–
C	92	8	–	–
D	88	12	–	–
E	71	25	–	4
F	50	50	–	–
G	57	37	–	6
H	72.5	25	0.5	2

FIG. 2. Chromatoplates of isomeric monoglycerides and other lipids on silica gel-boric acid obtained with several solvent systems.

the resolution of glycerol (origin), 1-monoglycerides, 2-monoglycerides, free fatty acids, 1,2-diglycerides, 1,3-diglycerides and triglycerides. It should be noted that the incorporation of boric acid in the chromatoplate causes the fatty acids to migrate further and in a more compact form than would be expected on a chromatoplate of silica gel alone.

Chromatoplate B was developed with a similar solvent system except that 0.5% acetic acid was incorporated. The presence of acetic acid in the solvent systems caused the fatty acids to migrate even further, and to coincide with the 1,2-diglycerides. In addition, the presence of acetic acid produced rounder more compact spots for the isomeric monoglycerides, especially at higher R_f values. Other combinations of chloroform and acetone (C, D, F) and chloroform, acetone, and methanol (E, G) produced the separations of isomeric monoglycerides illustrated on chromatoplates (C–G). For reasons which will be presented later, quantitative determination of isomeric monoglycerides is best accomplished with solvent system H, for which the separated monoglycerides have R_f values near 0.5. This solvent system consists of 72.5% chloroform, 25% acetone, 0.5% acetic acid, and 2% methanol.

Evidence that isomerization does not occur on thin-layers of boric acid-silica gel was obtained by two-dimensional chromatography. When pure monostearins were chromatographed individually, a single characteristic spot was obtained for each. Had iso-

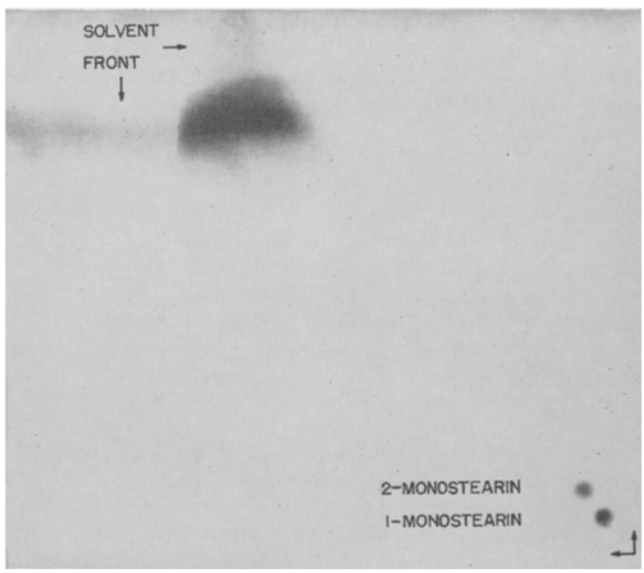


FIG. 3. Two-dimensional thin-layer chromatoplate of partially isomerized 2-monostearin.

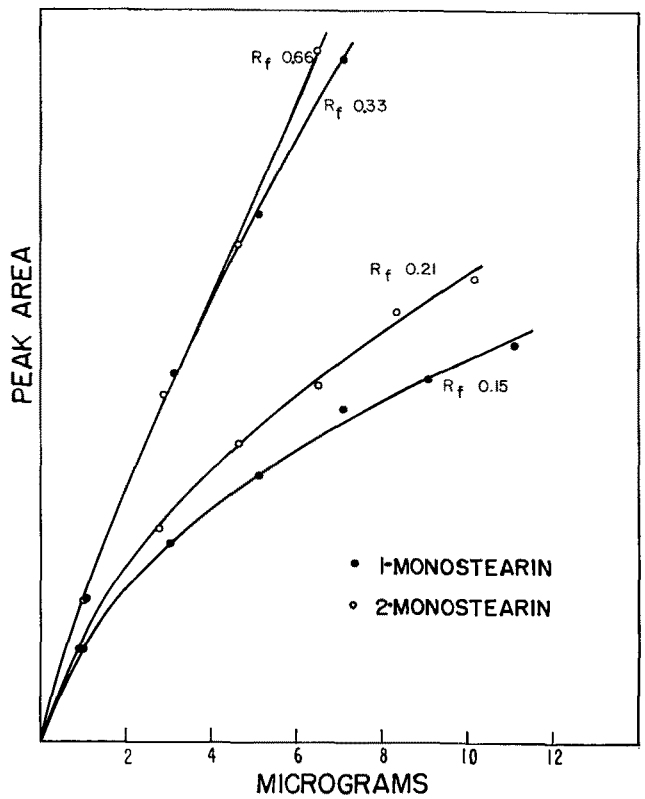


FIG. 4. Effect of R_f on peak area-conen relationship.

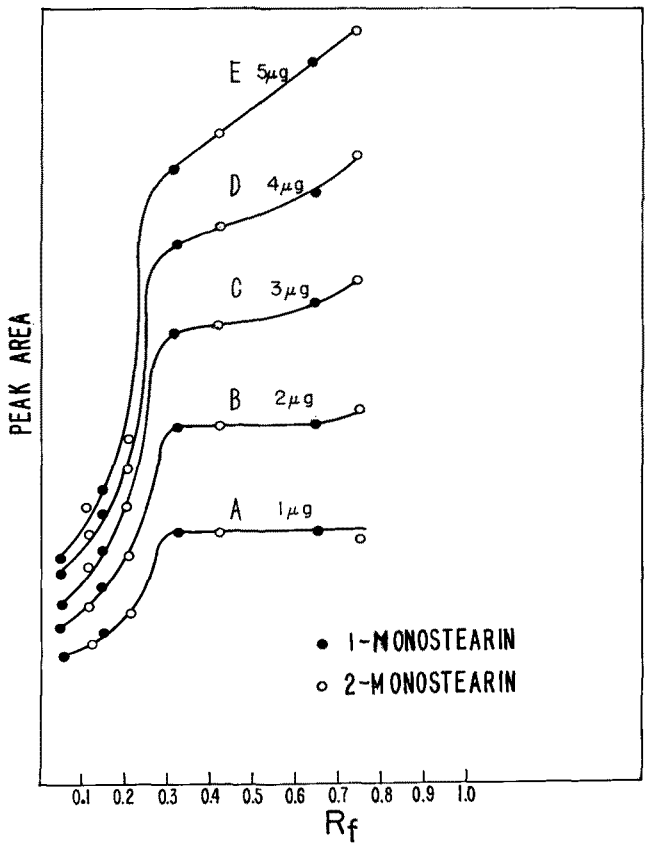


FIG. 5. Peak area-R_f relationship.

merization occurred on the chromatoplate, four spots should have been produced. Hofmann reported three spots for this attempted separation on hydroxyl-apatite (16). The fourth spot was apparently below the level of detection.

Crude 2-monostearin, purified by preparative TLC on boric acid-silica gel and by column chromatography on 100–200 mesh silica gel (Davidson Grade 923) containing about 5% by weight boric acid, did not isomerize as determined by this method. However, 2-monostearin chromatographed through a column of silica gel in the absence of boric acid, partially isomerized. Proof of isomerization is presented in Figure 3, which is a chromatoplate of the isolated monostearins obtained on silica gel-boric acid.

These data support the conclusion of other workers (21,25,26) that monoglycerides isomerize on silica gel, and in addition show that isomerization of monoglycerides does not occur on columns or thin-layers of boric acid-silica gel adsorbent. Similar results were obtained for diglycerides.

In addition to the obvious advantage of silica gel-boric acid adsorbent over hydroxyl-apatite with respect to isomerization, the former adsorbent will also resolve isomeric diglycerides under the conditions necessary to resolve isomeric monoglycerides.

Quantitation was achieved by transmission densitometry, employing techniques which have been described in the literature (8,15,27–29). Analyses of charred spots on chromatoplates containing isomeric monostearins show that the peak area vs. component concentration is influenced by the R_f of the spot as seen in Figure 4.

This R_f dependence causes an apparent difference in peak area for similar concentrations of isomeric monostearines at low R_f values as seen in the lower curves for 1-monostearin (R_f 0.21). The deviation in peak area at higher R_f values (1-monostearin, R_f 0.33 and 2-monostearin, R_f 0.66) is considerably reduced as seen in the top curves.

This R_f dependence is readily evident in a plot of peak area vs R_f as shown in Figure 5, e.g., curve A is a plot of the peak area for 1μg each of 1-monostearin and 2-monostearin vs R_f. There is a rapid increase in

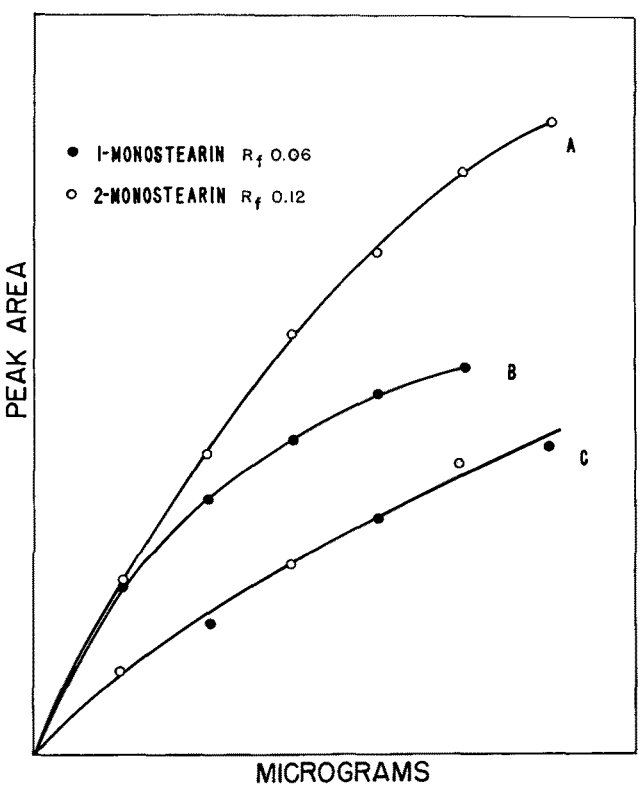


FIG. 6. Effect of light scattering on peak area-conen relationship of low R_f spots.

peak area between 0.06 and 0.3 R_f units. The peak area is essentially constant from an R_f of about 0.3 to 0.8. The peak area-R_f relationship is also somewhat dependent on sample size as shown in curves B–E. However, over an optimum range of R_f and sample size, isomeric monostearins exhibit identical responses. Direct analysis of monoglycerides without standards can only be accurately made at R_f values from about 0.3 to 0.8. A relationship similar to that shown by curve A was recently reported (28) for an unspecified weight of tripalmitin. Monoglycerides of other chain length and containing unsaturation would be expected to have a response in proportion to their carbon content as shown by Privett and Blank (24) for glycerides charred with chromic-sulfuric acids. Although at this time we do not have sufficient data to state the accuracy and precision of the method, one would expect absolute errors of about ±1.5% as previously reported (7) for the quantitative analysis of lipids by TLC and densitometry. There is some evidence which indicates that R_f dependence may be related to light scattering effects as shown in Figure 6. Curves A and B represent the peak area-concn relationships for 2-monostearin and 1-monostearin having R_f values of 0.12 and 0.06, respectively. The peak areas were obtained densitometrically as previously described. Curve C shows the peak area-concn relationship exhibited by monostearins on the same chromatoplate sprayed with mineral oil in ether to make the plate translucent and thus reduce light scattering. Linearity of the peak area-concn curves is considerably improved and both components exhibit a similar response over the range of sample size examined. However, the sensitivity of the measurement is considerably reduced. A more satisfactory method of eliminating the influence of refracted light without a corresponding decrease in sensitivity is to collimate the light beam by placing a narrow slit below as well as above the chromatoplate (7).

In view of the extreme ease with which monoglycerides isomerize, isolation and other handling prior to silica gel-boric acid chromatography must be carefully controlled. Procedures involving heat, acids or bases, and even chromatographic isolation in a noncomplexed form should be avoided.

Summary

We have demonstrated that isomeric monoglycerides may be resolved by thin-layer adsorption chromatography on boric acid-impregnated silica gel, and that under the conditions described, no isomerization occurs. We have further demonstrated that monoglycerides may be isolated by preparative TLC or on columns without causing isomerization.

Finally, we have shown that monoglyceride isomers may be quantitated by the densitometric measurement of charred spots over an optimum range of R_f and sample size.

ACKNOWLEDGMENT

Isomeric monostearin synthesis by H. J. Harwood.

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Lubricants. I. Preparation and Properties of Benzyl and Substituted Benzyl Esters of Dilinoleic Acid

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Abstract

Benzyl and substituted benzyl esters of dilinoleic acid and of hydrogenated dilinoleic acid have been prepared in good yield. Some of the chemical characteristics and physical properties of the resulting products have been measured including a study of their thermal stability by thermogravimetry. Also, they have been examined by several of the bench tests used in laboratory evaluation of lubricants. Several of them compare favorably with control materials used in the study.

THE SILICONE, SILICATE, hydrocarbon, fluorocarbon, ether and ester fluids are among the important classes of compounds being prepared and evaluated in the search for new lubricants, lubricant additives, and hydraulic fluids to meet the needs of the future. Klaus, Tewksbury and Fenske have estimated that the upper useful limit for most of these classes is between 370–427 (15).

This paper is primarily concerned with the preparation and properties of long-chain esters derived from fatty materials and benzyl alcohol and its derivatives. These alcohols were selected because they lacked a *beta* hydrogen; therefore their esters could not decompose by the usual cyclic mechanism (6), but by a free radical mechanism (1,13), which insures higher thermal stabilities. Durr, Meador and Thompson used a sim-

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