Chromatographic Analysis of Sorbitan Fatty Acid Esters[1]

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Abstract

A procedure is described for the separation, identification and quantitative estimation of mono-, di- and tri-fatty acid esters of sorbitol and of its anhydrides. Stearic, palmitic and oleic acid esters of sorbitol, 1,4-sorbitan and isosorbide were synthesized in the laboratory. Lipid classes were separated by liquid partition column chromatography and TLC. The individual mono- and di-fatty acid esters and the polyols were analyzed by GLC as trimethylsilyl ethers. Recoveries of known compounds in mixtures were in a range of 92% to 100%.

Introduction

In the food industry the fatty acid esters of sorbitol and its anhydrides are known as the sorbitan fatty acid esters (SFAE). They are used as emulsifiers and stabilizers in such foods as confectionery products, cake mixes, cake icings, whipped vegetable toppings and beverage bases at levels of 0.4% to 1%. Several compositions of SFAE are available to the food industry under trade names such as Span (Atlas Chemical Industries Inc.) and Glycomul (Glyco Chemicals Inc.).

Chemically, SFAE are complex mixtures of fatty acid esters of several polyols derived from sorbitol. Commercially, these compounds are prepared by heating sorbitol and fatty acids at 200–250 C in the presence of a catalyst such as phosphoric acid (1). Two of the known polyols other than sorbitol itself, are its anhydride, 1,4-sorbitan and the dianhydride, isosorbide. The polyols are esterified with fatty acids resulting in a complex mixture of mono-, di- and tri-fatty acid esters. The complexity of these compounds and the small quantities used in mixtures with other emulsifiers present considerable difficulty in developing methods of analysis for these products in foods. Very little information is available in the literature on the composition of commercial products.

Schrepfer and Egle (2) reported a qualitative method for the identification of sorbitan mono-fatty acid esters in some baking preparations. Gatewood and Graham (3) assayed these esters quantitatively by periodate oxidation of the polyols. Such procedures cannot be applied to foods which contain other diols and polyols such as in propylene glycol esters and polyglycerol esters.

More recently Wetterau et al. (4) reported a procedure for the quantitative estimation of sorbitan monostearate in cake mixes and baked cakes. The method is based on the assumption that Spans are fatty acid esters of a reproducible mixture of polyols derived from sorbitol. In their method, 1,4-sorbitan is isolated by paper chromatography and isosorbide by GLC. The amount of the ester is then calculated from the polyol content.

Our preliminary studies indicated that in addition to isosorbide and 1,4-sorbitan at least two other anhydride isomers occur in the commercial Span and that the polyols and their fatty acid esters could be separated and quantitatively estimated by liquid partition column chromatography (LPCC) and GLC. Earlier Cedras et al. (5) and Suffis et al. (6) have reported the separation of some of the components of Spans by TLC and GLC but none of the esters was identified.

This study was undertaken to determine the chemical composition of commercial SFAE, to incorporate the analysis of these products in our general scheme of analysis of emulsifiers. In keeping with the concept of a schematic procedure for emulsifiers, the techniques for lipid class separation by LPCC (7) and quantitative analysis by GLC were essentially the same as reported earlier for mono- and di-glycerides (8), propylene glycol esters (9) and polyglycerol esters (10).

This paper describes the identification and quantitative estimation of individual mono- and di-fatty acid esters (palmitic, stearic and oleic acids) of sorbitol, 1,4-sorbitan and isosorbide.

Materials and Methods

Samples

Commercial samples of sorbitan monostearate (Span 60, Glycomul-S), sorbitan monooleate (Span 80, Glycomul-O), sorbitan monopalmitate (Span 40) and sorbitan tristearate (Span 65) were obtained from the industry.

Preparation of Esters

Individual fatty acid esters were prepared in the laboratory by two procedures. The purpose of synthesizing individual esters was to obtain pure standards and no attempt was made to determine the optimum conditions for the best yield. Sorbitol and 1,4-sorbitan (arlitan) were esterified with fatty acid methyl esters by the Snell procedure as described by Lemieux and McInnes (11) (Method A). Under these conditions isosorbide distills off from the reaction medium and therefore esterification of isosorbide was carried out with fatty acid chlorides (12) (Method B).

All fatty acid methyl esters used were of 96–99% purity as determined by GLC. Fatty acid chlorides were commercial technical grade. The 1,4-sorbitan and isosorbide were recrystallized from isopropyl alcohol. As examples, methods for the preparation of stearic acid esters of 1,4-sorbitan and isosorbide are described.

Method A

1,4-Sorbitan (20 μmole) was dissolved in dimethyl formamide (DMF, 100 ml) and conditioned at 80 $\pm$ 5 C at 75 mm pressure for 30 min. The reaction mixture was stirred mechanically and had a fine stream of nitrogen passing through it. The methyl ester (20 μmole) in *n*-pentane (20 ml) was then added to the DMF and *n*-pentane was rapidly removed under reduced pressure. When the temperature of the reaction mixture reached 80 C, a 1 ml aliquot of the DMF solution was taken for analysis by GLC of 1,4-sorbitan and methyl ester (see below). Sodium carbonate (20 mg) was then added and the reaction continued for 4 hr. Another aliquot was then taken for the analysis of the polyol, the fatty acid methyl ester and the

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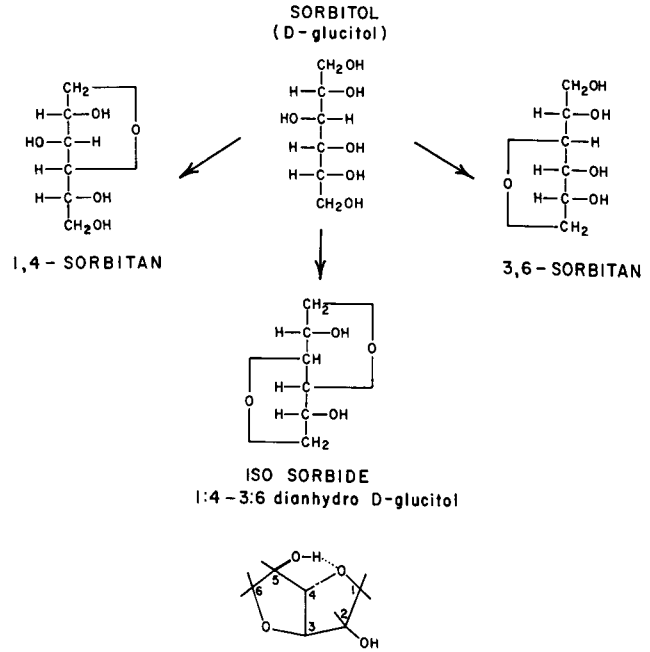

FIG. 1.

polyol esters. The reaction mixture was then evaporated to dryness at reduced pressure.

Composition. 1,4-Sorbitan monostearate, 34.9% (polyol-stearic acid found, 33.6:63.6; calculated 35.4:64.5); 1,4-sorbitan distearate 10.1% (polyol-stearic acid found 21.0:78.1; calculated 21.5:78.4) 1,4-sorbitan, 10.3%; methyl stearate 34.7%.

Method B

Isosorbide (0.1 mole) in DMF (40 ml) with dry pyridine (2 ml) and dry chloroform (30 ml) were cooled in an ice bath. Stearoyl chloride (0.1 mole) in chloroform (30 ml) was added dropwise with constant stirring and the reaction mixture was stored overnight at room temperature. The mixture was then dissolved in ether and successively washed with 0.5 M HCl and water. The solvent was then removed under reduced pressure.

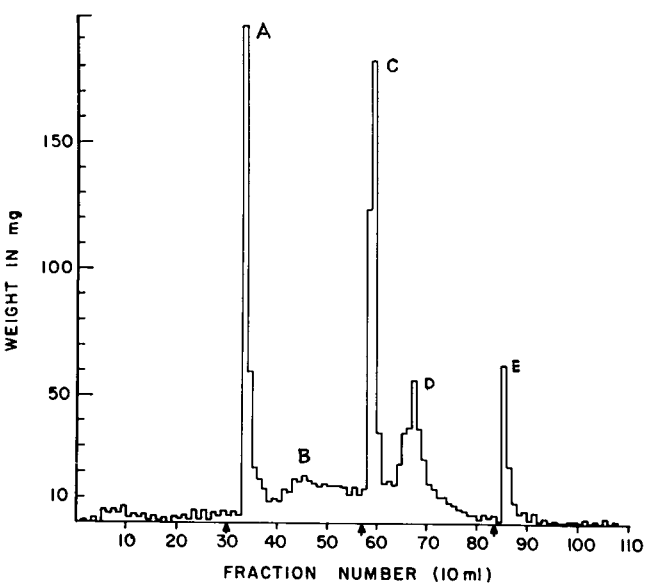

FIG. 2. LPCC fractionation of components of Span-60. Arrows at fraction numbers 30, 58 and 84 indicate solvent changes. A, sorbitan tri-fatty acid esters and free fatty acids; B, isosorbide mono-fatty acid esters; C, sorbitan di-fatty acid esters; D, sorbitan mono-fatty acid esters; E, free polyols.

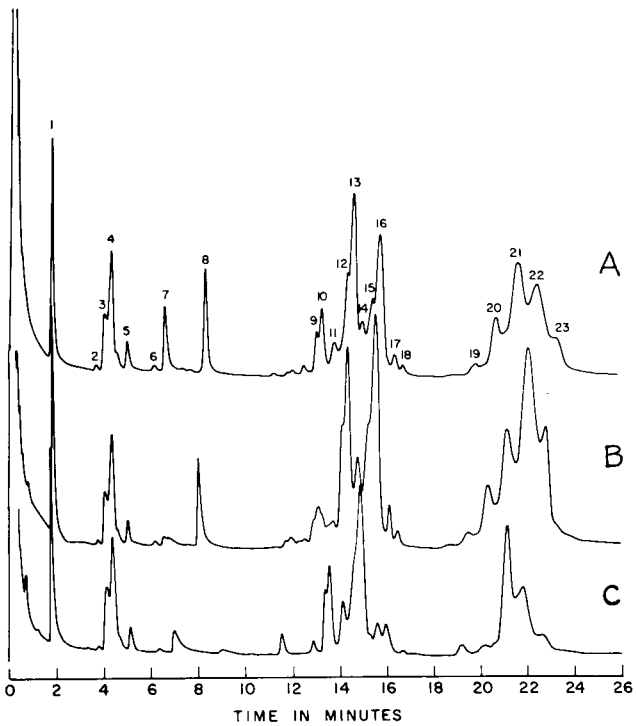

FIG. 3. GLC separation of polyols and their esters A, Span 60; B, Span 80; C, Span 40. 1, Isosorbide; 2, 3 and 5, unconfirmed sorbitol anhydrides; 4, 1,4-sorbitan; 6, sorbitol; 7, palmitic acid; 8, stearic acid; 9 and 10, isosorbide monopalmitates; 11, unknown; 12, isosorbide monostearate; 13, 1,4-sorbitan monopalmitate; 14, unknown; 15, 1,4-sorbitan mono-oleate and sorbitol monopalmitate; 16, 1,4-sorbitan monostearate; 17, sorbitol monostearate; 18, 19, unidentified; 20, sorbitan dipalmitate; 21, sorbitan palmitostearate; 22, sorbitan distearate; 23, unknown.

Composition. Isosorbide monostearate, 23.1% (polyol-stearic acid found, 31.1:66.8; calculated, 32.8:67.1); isosorbide distearate 27.9% (polyol-stearic acid found 18.9:80.1; calculated, 19.6:80.3); isosorbide, 1.1%; unidentified, 47.8%.

The fatty acid esters prepared by the above methods were fractionated by LPCC and by TLC and were checked for identity by their composition with respect to the polyol and fatty acid contents as described below. Only those compounds which showed a purity of 90+% as indicated by GLC were used as stan-

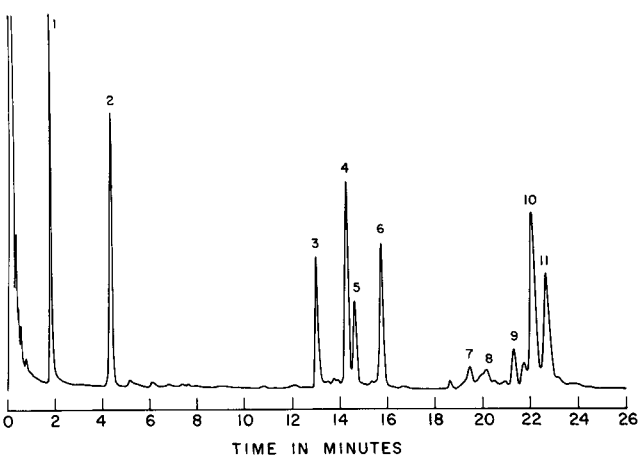

FIG. 4. GLC analysis of a known mixture of polyols and their esters. 1, Isosorbide; 2, 1,4-sorbitan; 3, isosorbide monopalmitate; 4, isosorbide monostearate; 5, 1,4-sorbitan monopalmitate; 6, 1,4-sorbitan monostearate; 7, isosorbide dipalmitate; 8, isosorbide distearate; 9, 1,4 sorbitan dipalmitate; 10 and 11, 1,4-sorbitan distearates.

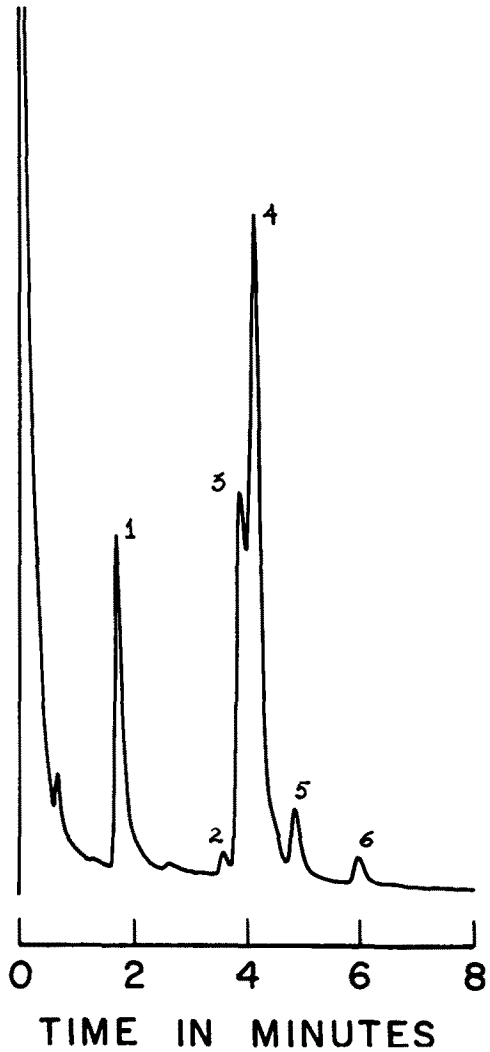

FIG. 5. GLC analysis of polyols. 1, Isosorbide; 2, 3 and 5, unconfirmed sorbitol anhydrides; 4, 1,4-sorbitan; 6, sorbitol.

dards. Several mixtures of the standard compounds were prepared and analyzed by GLC for establishing the relative response factors (8,9,10).

Liquid Partition Column Chromatography

Individual samples (0.3 to 1.5 g) were eluted from silicic acid columns successively with 300 ml each of benzene (I), benzene with 10% ethyl ether (II), ethyl ether (III) and ethyl alcohol (IV) as described earlier (5). Ten-milliliter fractions were collected on an automatic fraction collector (LKB—RadiRac, Sweden).

Gas-Liquid Chromatography

Commercial SFAE, individual fractions from LPCC and TLC, free polyols and synthetic esters were analyzed as trimethylsilyl ether (TMS) derivatives. The TMS derivatives were prepared by treating 50–60 mg samples in pyridine (0.5 ml) with hexamethydisilazane (0.3 ml) and trimethylchlorosilane (0.2 ml). The reaction mixture was shaken vigorously for 30 sec and allowed to stand for 10 min. Supernatant (1 μliter) was injected into the gas chromatograph. GLC can be performed on any instrument equipped for dual column, dual flame ionization detection. A Perkin Elmer model 800 gas chromatograph equipped with a differential, ceramic tipped hydrogen flame ionization detector was used in this study. Columns were 3 ft long, 1/8 in. OD

TABLE I

Analysis of Span 60[a]

	GLC	LPCC
Polyols		
Isosorbide	1.6 (1)[b]	
X,X Sorbitan	1.4 (3)	6.4 (E, IV)[c]
1,4 Sorbitan	1.9 (4)	
Other	0.2	
Free Fatty Acids		
Palmitic	2.0 (7)	2.5 (A, II)
Stearic	2.8 (8)	
Mono-Fatty Acid Esters		
Isosorbide	31.8 (9–18)	16.6 (B, II)
Sorbitan		18.8 (D, III)
Di-Fatty Acid Esters	46.1 (19–23)	24.6 (C, III)
Tri-Fatty Acid Esters		17.5 (A, II)
Unidentified	.....	3.8 (III)
	.....	4.7 (IV)
Total Recovery (%)	87.8	94.9

[a] All values are averages of three determinations.
[b] Figures in parentheses refer to GLC peak numbers (Fig. 3).
[c] Letters and roman numbers refer to LPCC fractions (Fig. 2).

stainless steel, packed with 3% JXR on Gas Chrom-Q 110–120 mesh (Applied Science Labs, Inc.). Helium flow was regulated at 37 ml/min at ambient temperature. The columns were programmed at 10 C/min from 125 to 325 C. The injection port temperature was 250 C. The detector temperature in this instrument is controlled by the programmer and ranged from an initial 150 C to 325 C. The fatty acids were analyzed as methyl esters (13) on 6 ft long, 1/8 in. OD stainless steel columns packed with 6% butane diolsuccinate (BDS) on Anakrom ABS, 80–90 mesh (Analabs Inc.), programmed from 125 to 225 C at 10 C/min.

Thin-Layer Chromatography

TLC was carried out on 250 μ layers of Adsorbosil I containing 4% boric acid coated on 20 × 20 cm glass plates. Coated plates were activated at 120 C overnight and stored in desiccation boxes (14). Samples were applied with disposable capillary Pasteur pipets as 10% solutions in ethyl alcohol (polyols), or 1:1 ethyl ether and alcohol (esters). The spots were dried under a stream of nitrogen. Polyols were developed with benzene-methanol (6:4) and the esters with benzene-ethyl ether-methanol (75:20:5). The plates were viewed under UV light following a spray of 2,6 dichlorofluorescein (1%) in ethanol.

Analysis of Polyols and Fatty Acids by Transesterification

SFAE (300 mg) was transesterified with H_2SO_4-methanol (2.5%; 40 ml) under reflux for 6 hr. The reaction mixture was then cooled and transferred to a separatory funnel. After the addition of water (100 ml) and *n*-pentane (20 ml) the two phases were separated. The *n*-pentane layer containing the methyl esters was washed with water and the washings were added to the aqueous polyol solution. The fatty acid methyl esters were estimated by GLC on BDS columns with methyl pentadecanoate as the internal standard (13).

TABLE II

Analysis of Span Polyols

	Range (15)	Average (15)
Isosorbide	14.1–22.0	18.9
Unknown I	0.6– 1.3	0.8
X,X-Sorbitan	27.8–32.5	31.0
1,4-Sorbitan	36.8–43.3	40.4
Unknown II	0.0– 5.0	3.7
Unknown III	3.2– 6.1	4.4
Sorbitol	0.0– 2.3	0.8
		100.0

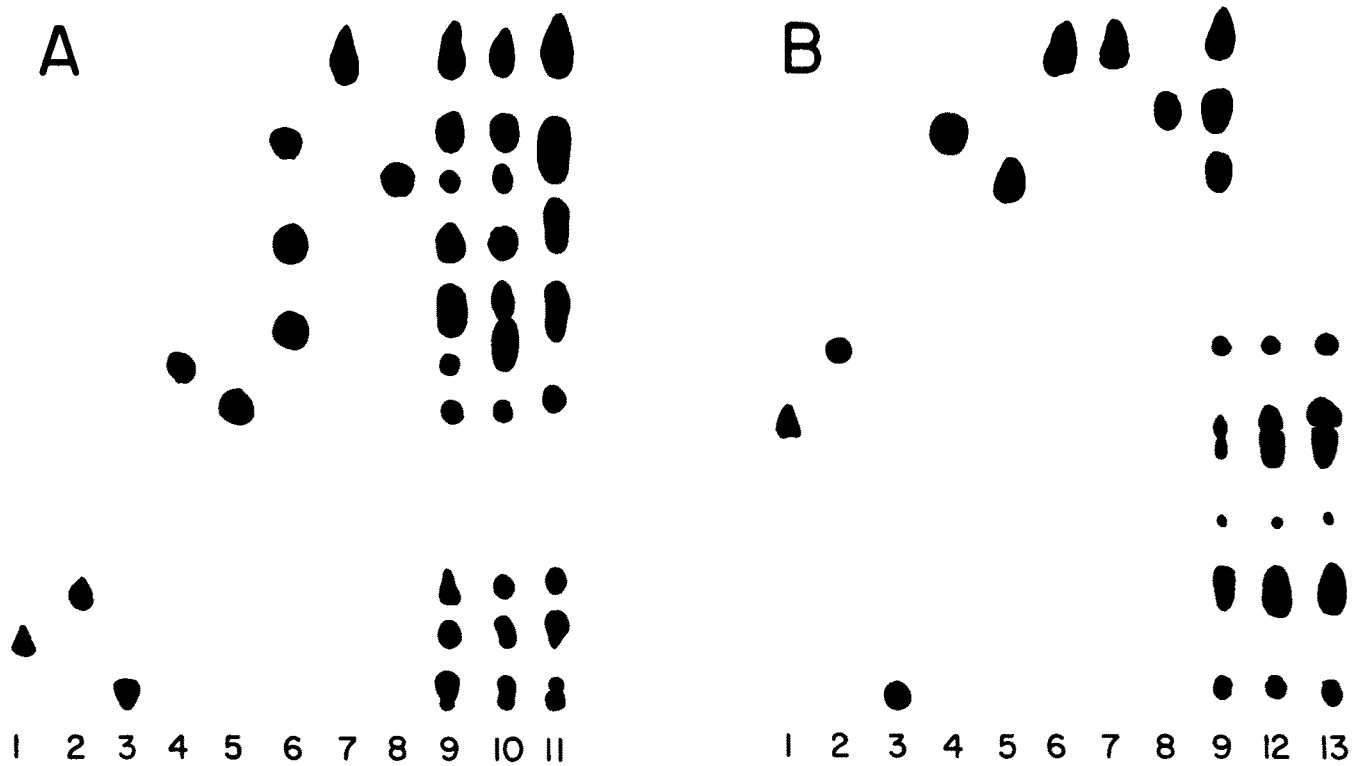

FIG. 6. TLC separation of polyols and their fatty acid esters on Adsorbosil I with 4% boric acid. Solvents: A, benzene-ethyl ether-methanol 75:20:5; B, benzene-methanol 6:4. 1, 1,4-Sorbitan; 2, isosorbide; 3, sorbitol; 4, isosorbide monopalmitate; 5, 1,4-sorbitan monopalmitate; 6, sorbitan di-fatty acid esters from Span 60; 7, sorbitan tri-fatty acid esters from Span 60; 8, palmitic acid; 9, Span 60; 10, Span 40; 11, Span 80; 12, polyols from Span 60; 13, polyols from Span 40.

For the analysis of polyols, sorbitol (40 mg) was added as the internal standard to the concentrated aqueous polyol solution which was then passed through a 30 cm × 18 mm OD ion exchange column (30 g Amberlite IRA-400, OH-form). The eluate, neutral to litmus, was evaporated to dryness on a water bath and the residue was dissolved in pyridine. An aliquot was trimethylsylilated and analyzed by GLC on JXR columns. The quantities of polyols were calculated from the peak areas in relation to sorbitol as an internal standard.

Results and Discussion

On being heated with acidic reagents, sorbitol (D-glucitol) readily loses one or two molecules of water to form anhydro sorbitol or di-anhydro sorbitol (isosorbide). The constitution of isosorbide has been shown to be 1:4–3:6 dianhydrosorbitol (15). Esterification of isosorbide has also been studied by several workers (15–18). Although both hydroxyls can be easily esterified, Lemieux and McInnes (17) have demonstrated that the hydrogen bonded *endo* 5-OH esterifies more rapidly than the *exo* 2-OH (Fig. 1). Thus esterification of isosorbide with stearic acid will result in a mixture of 5-stearyl, 2-stearyl and 2,5-distearyl 1:4–3:6 dianhydrosorbitol in which the 5-stearyl dianhydride will predominate.

It has also been shown that sorbitol is converted more readily to the 1,4-anhydride than the 3,6-anhydride (19); other anhydrides such as 1,5-sorbitan are also likely to occur in commercial production. Esterification of anhydrides derived from sorbitol thus results in variety of mono-, di- and tri-fatty acid esters.

Separation of lipid classes by LPCC on silicic acid (7,8) is based on the differences in polarity because of the free hydroxyl groups. Figure 2 shows the typical fractionation of Span 60 by LPCC. Several commercial and synthetic sorbitan fatty acid esters were chromatographed under similar conditions. The fractionation was reproducible within 2% and the recoveries ranged from 95% to 100%. The fractions were analyzed by GLC for total polyols and fatty acids and also by the conventional procedures for free fatty acids and hydroxyl values. Based on these observations the following elution pattern was established: Fraction II (benzene – ethyl ether 10%), free fatty acids (A), sorbitan tri-fatty acid esters (A), isosorbide di-fatty acid esters (A) and isosorbide mono-fatty acid esters (B); Fraction III (ethyl ether), sorbitan di-fatty acid esters (C) and sorbitan mono-fatty acid esters (D); Fraction IV (ethyl alcohol), free polyols (E). For routine analysis it was not necessary to collect individual 10 ml fractions and the eluates were collected in 500 ml flasks for each solvent.

Figure 3 shows the GLC resolution of free polyols, free fatty acids and fatty acid esters of sorbitol anhydrides. Each peak was identified with known standards prepared in the laboratory. Quantitative estimation of the individual components by GLC was achieved with the use of sorbitol as an internal standard. Figure 4 shows the resolution of a standard mixture of polyols and their fatty acid esters. Relative flame-response factors (8,9,10) were calculated from the GLC analysis of several mixtures of known compounds. With sorbitol as one unit, RRF used for calculations were as follows: isosorbide, 1.05; 1,4-sorbitan, 1.01; palmitic acid, 2.0; stearic acid, 1.95; isosorbide monopalmitate, 1.88; isosorbide monostearate, 1.88; 1,4-sorbitan monopalmitate, 1.90; 1,4-sorbitan monooleate, 1.90; 1,4-sorbitan monostearate, 1.88; sorbitol monopalmitate, 1.90; sorbitol monostearate, 1.91; 1,4-sorbitan dipalmitates, 2.96; and 1,4-sorbitan distearates, 3.00. The values are averages of 3 to 6 determinations. The peak response is de-

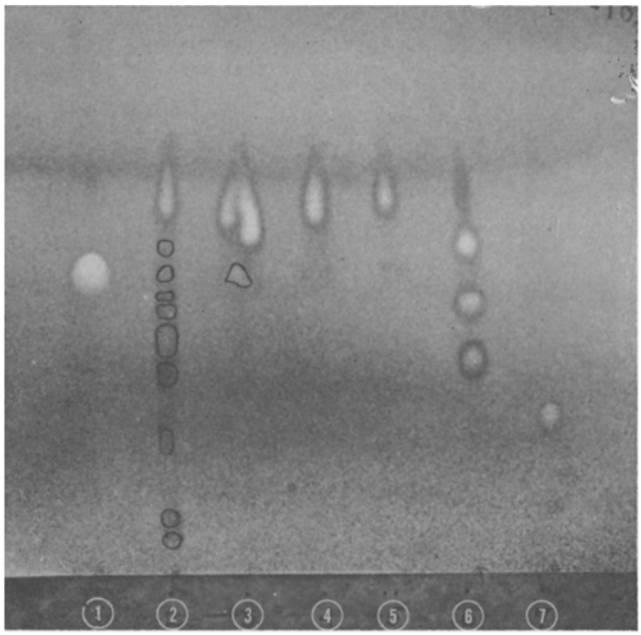

FIG. 7. TLC separation of synthetic 1,4-sorbitan mono-, di- and tristearates. Solvents: benzene-ethyl ether-methanol 75:20:5; Spray: 2,6 dichlorofluorescein. Photographed in U.V. light. 1, Stearic acid; 2, Span 60 + 1,4-sorbitan tristearate; 3, 1,4-sorbitan tristearate + stearic acid; 4, and 5, sorbitan tristearates; 6, 1,4-sorbitan distearates; 7, 1,4-sorbitan monostearate.

pendent on factors such as size of the molecule and the number of trimethylsilyl groups; within each class the responses were comparable. Table I shows the analysis by GLC and LPCC of a representative sample of Span 60. In GLC the low recovery (87.8%) is accounted for by the fact that no correction was made for moisture and the polymeric materials which were not detectable by GLC. In LPCC parts of this material were eluted in benzene (Fraction I), in ethyl ether (Fraction III) and in alcohol (Fraction IV).

As suggested by Wetterau et al. (4), commercial SFAE constitutes a reproducible mixture of polyols. Analysis of total polyols by GLC of 15 samples of Span is shown in Table II; these include six separate batch samples of Span 60 and three each of Span 65, Span 40 and Span 80. A typical GLC tracing of polyols is shown in Figure 5. Figures 6 and 7 show the TLC separation of polyols and their esters. Two separate solvent systems were developed for polyols and the fatty acid esters. Isosorbide moves faster than the mono-anhydrides and appears as a distinct pink colored spot when sprayed with 2,6-dichlorofluorescein and viewed in daylight. The 1,4-sorbitan with a 2,3 *trans* glycol group is believed to move faster than the 3,6-sorbitan which possesses a 4,5 *cis* glycol because of the complexing properties of boric acid (Fig. 6B). Polyols from commercial SFAE gave four distinct spots on TLC with benzene-methanol (6:4) which were reproducible in all brands of Spans studied. The fatty acid esters (palmitic and stearic) were developed in benzene-ethyl ether-methanol (75:20:5). The di-fatty acid esters of 1,4-sorbitan show three distinct spots (Fig. 6A and 7) suggesting at least as many isomers. Further studies are necessary for the identification of these isomers.

Fractionation of SFAE on silicic acid columns and quantitative analysis of individual components by GLC has provided a simple means to estimate the esters in foods and emulsifier compositions. The methods reported here have been incorporated in the schematic procedure for the analysis of mixtures of emulsifiers including mono- and diglycerides and propylene glycol fatty acid esters. The total lipid material is fractionated into four fractions by LPCC as described above and each fraction is analyzed by GLC. Details of this scheme will be reported later.

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