through the experimental points was calculated according to procedures developed for K = 4.5. The calculated line for K = 1 (equal reactivity of primary and secondary OH's) is also shown. Obviously the experimental data fit the curve favoring esterification of the primary hydroxyls. Although the preparations were made at 200°C. or higher temperatures where K is about 2, the fact that the points fit well on a curve for K = 4.5 shows that a substantial degree of intramolecular rearrangement has occurred on cooling, prior to analysis of the samples.

Summary

A method has been devised for determining the relative esterifiability of the primary and secondary hydroxyl groups of glycerol. Contrary to the theory previously advanced by Feuge and Bailey, the primary and secondary hydroxyls are not equally esterifiable. The equilibrium constant favoring esterification of primary hydroxyl over secondary is *ca.* 2.3 at reaction temperature (200°C.) and between 6 and 10 at room temperature. Since the equilibrium constant is substantially different at room temperature from that at reaction temperature, monoglycerides as customarily prepared are not at equilibrium at room temperature and undergo intramolecular migration of acyl groups from *beta* to *alpha* hydroxyl positions. The rate of migration depends on the physical form of the ester and is accelerated by basic catalysts. In the vicinity of room temperature intermolecular rearrangement occurs only over very prolonged periods. The method of calculating relative esterifiability of primary and secondary hydroxyls should be applicable to other polyols.

Acknowledgment

Grateful acknowledgment is made of the assistance of C. F. Smullin and Virginia Olsanski in analyzing these samples.

REFERENCES

1. Birkmeier, R. L., and Brandner, J. D., J. Agr. Food Chem., *6*, 471 (1958).
2. Brokaw, G. U., Perry E. S., and Lyman, W. C., J. Am. Oil Chemists' Soc., *32*, 194 (1955).
3. Crossley, A., Freeman, I. P., Hudson, B. J. F., and Pierce, J. H., J. Chem. Soc., 1959, 760.
4. Demarcq, M., La Revue Français des Corps Gras, *5*, 336 (1956).
5. Feuge, R. O., and Bailey, A. E., Oil and Soap,*23*, 259 (1946).
6. Martin, J. B., J. Am. Chem. Soc., *75*, 5483 (1953).
7. Pohle, W. D., and Mehlenbacher, V. C., J. Am. Oil Chemists' Soc., *27*, 54 (1950).
8. Quinlan, P., and Weiser, H. J., J. Am. Oil Chemists' Soc., *35*, 325 (1958).
9. Smullin, C. F., and Olsanski, V. L., paper presented at annual meeting, American Oil Chemists' Society, New Orleans, April 20–22, 1959.
10. Van Lohuizen, O. E., and Verkade, P. E., Rec. trav. chim., *79*, 133 (1960).
11. West, E. S., Hoagland, C. L., and Curtis, G. H., J. Biol. Chem., *104*, 627 (1934).

[Received December 28, 1959]

A General Method for the Chromatographic Analysis of Mono-, Di-, and Triglycerides and the Mono- and Diesters of Ethylene Glycol and Polyethylene Glycol

GERALD J. PAPARIELLO, SUNIS CHULKARATANA, and TAKERU HIGUCHI, School of Pharmacy, University of Wisconsin, Madison, and J. E. MARTIN and V. P. KUCESKI, The C. P. Hall Company of Illinois, Chicago, Illinois

IN THE COMMERCIAL manufacture of partial esters of polyhydric alcohol mixtures, the monoesters, diesters, and the triesters are usually obtained along with the acid and alcohol reactants. A direct, general method for the quantitative analysis of such complex mixtures has been lacking. Recently several specialized methods have been developed. Ravin, Meyer, and Higuchi (1) developed a chromatographic method of analysis of mixtures of glyceryl esters and mineral oil, and Quinlan and Weiser (2) also chromatographed a glyceryl system. Malkemus and Swan (3) developed a procedure for the analysis of polyethylene glycol ester mixtures, based on extraction.

This paper is concerned with the application of the chromatographic method of Ravin *et al.* (1) to esters of ethylene glycol, polyethylene glycol, and glycerol. Their method was based on the observation of Kaufman and Wolf (4) that silica gel will adsorb the most polar component of a mixture of glycerides to the greatest extent. Ravin *et al.* employed a mobile phase consisting of a series of solvents of increasing polarity to separate the components of mixtures of mono-, di-, and tristearin and mineral oil. It was hoped that this principle could be applied to any polyhydric alcohol and its esters.

Two solvent systems were developed. The first was a slight modification of the eluent system of Ravin *et al.* It separated the components of two types of mixtures, glyceryl esters and ethylene glycol esters. A second system was developed for the separation of polyethylene glycol ester mixtures.

The exact weight compositions of the ester mixtures chromatographed were determined by adding together the weights of the residues found in all fractions under each peak. The positive identification of the peak material was made by interpretation of infrared spectra and saponification values.

To determine whether this method of analysis was as general as it was originally hoped to be, the effects of such factors as unsaturation in the acid moiety of the glyceryl esters or a change in the molecular weight by 100 or 200 of the polyethylene glycol were investigated.

Since glyceryl ester mixtures which are to be ana-

lyzed often contain only a small amount of the monoglyceride, an extraction procedure prior to the usual chromatographic technique was investigated as a means of accurately analyzing concentrations as low as 1%.

Experimental

Apparatus. A 20-mm. borosilicate glass chromatographic column 45 cm. long was used. The column was packed by using a close-fitting glass plunger.

Aluminum moisture pans

Reagents. Benzene, thiophene-free

Methanol, anhydrous, reagent grade

Isopropyl ether, purified by washing with ferrous sulfate solution to remove peroxides, washing with water, then drying over calcium chloride and distilling.

Chloroform, reagent grade

Isooctane,[1] 99.5%

Anhydrous ethyl ether, reagent grade

Absolute ethanol, aldehyde-free

Silica gel, Davison No. 922,[2] through 200 mesh, used as supplied from the commercial source without further treatment

All commercial ester mixtures were used as supplied by the C. P. Hall Company.

Chromatographic Procedure. The only difference in procedure in the chromatographic analysis of ester mixtures of the three types of polyhydric alcohol is in the composition of the mobile phase and in the special extraction procedure used to determine the presence of small amounts of monoglyceride.

Twenty-five grams of silica gel, prewetted with benzene, were packed into the column in the usual manner. Benzene was allowed to percolate through, and a solvent head which gave a drop rate of approximately 15 to 25 drops per minute was established.

A 250-mg. sample was diluted to 10 ml. with benzene. This solution was then quantitatively transferred onto the chromatographic column.

The following eluent systems were used to obtain chromatographic resolution:

For glyceryl ester mixtures and ethylene glycol ester mixtures

Eluent	Volume, ml.	Composition, % (v/v)
A	60–90	40% isopropyl ether in iso-octane
B	60	70% ethyl ether in iso-octane
C	80	20% ethanol in isopropyl ether
For polyethylene glycol ester mixtures		
A	30	3% methanol in isopropyl ether
B	120	12% methanol in isopropyl ether
C	50	10% methanol in chloroform
D	120	35% methanol in chloroform

Ten-ml. fractions from the column were collected and transferred to tared aluminum moisture pans. The solvents were permitted to evaporate, and the pans were reweighed. Blank determinations were run simultaneously.

Extraction Procedure to Determine Small Amounts of Monoglyceride. A 5-g. sample is weighed, melted, and extracted with 5 ml. of absolute ethanol. The extract is removed from the residue by a decantation and filtration procedure. This procedure is repeated until 30 ml. of extract are collected. The extracting solvent is evaporated off at 50°C. The residue from this evaporation is dissolved in benzene, and the chromatographic procedure previously described for analysis of glyceryl ester mixtures is carried out. As a further precaution, this procedure is repeated with 20 ml. more of extract.

[1] Eastman Organic Chemicals, Distillation Products Industries, Rochester, N. Y.

[2] Davison Chemical Company, Baltimore, Md.

Results and Discussion

In the analysis of the mono-, di-, and triglycerides, resolution for the first peak, the tristearin peak, was not obtained when the suggested eluents of Ravin *et al.* were used (Figure 1). A decrease in the polarity of the first eluent by increasing the percentage of iso-octane present improved the resolution (Figure 2). The best resolution was obtained when 40% isopropyl ether in iso-octane was used.

To show the sensitivity of the method, chromatograms of cottonseed oil, a triglyceride, and of cotton-

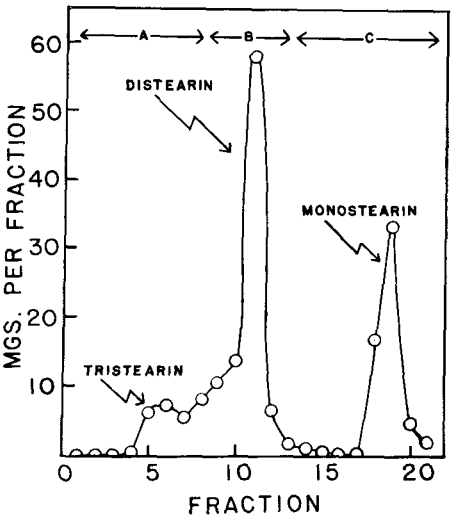

FIG. 1. Chromatogram of a glyceryl ester mixture. The eluting solutions were; A, 60% isopropyl ether in iso-octane; B, 70% ethyl ether in iso-octane; C, 20% absolute ethanol in isopropyl ether.

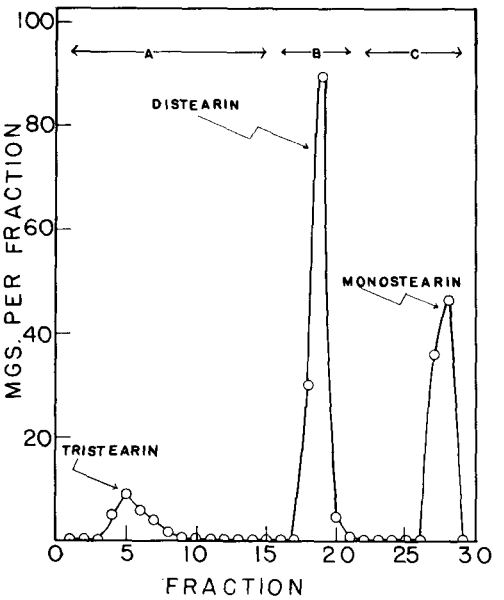

FIG. 2. Chromatogram of a glyceryl ester mixture. The following eluents were used: A, 40% isopropyl ether in iso-octane; B, 70% ethyl ether in iso-octane; C, 20% absolute ethanol in isopropyl ether.

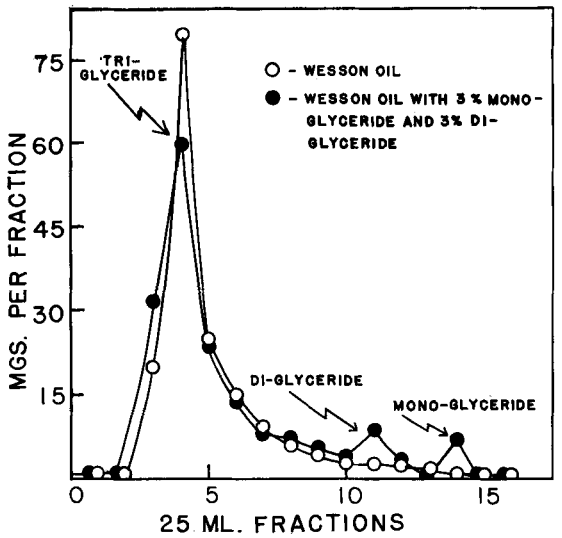

FIG. 3. Chromatograms of Wesson Oil and Wesson Oil with a small amount of mono- and diglyceride added to it.

seed oil containing small amounts of added mono- and diglyceride were made. Figure 3 shows the peaks are all well defined.

Although small amounts of monoglyceride and diglyceride can easily be detected by this method, it was thought that both the accuracy and the precision of the method could be greatly improved by concentrating the di- and monoglycerides by a preliminary extraction procedure and running a chromatogram on this extract. It was found that complete extraction of the diglyceride by use of ethanol or other extraction solvents was not possible. Nevertheless the monoglyceride was completely extracted with ethanol. To demonstrate the accuracy that can be obtained in determining small amounts of monoglyceride, analysis of glyceryl ester samples containing known amounts of added monoglyceride was performed.

From Table I it can be seen that an average recovery at 98% is obtainable with concentrations of monoglyceride as small as 1 to 2%.

Since an extraction procedure to determine small amounts of the diglyceride with greater accuracy

TABLE I

Recovery of Small Amounts of Added Monoglyceride Extraction Procedure

Added monoglyceride		Recovered monoglyceride	
mg.	%	*mg.*	%
503	0.980	490	97
755	1.45	738	98
		758	100
1015	1.91	959	95
Average			98

TABLE II

Recovery of Small Amounts of Added Diglyceride Normal Chromatographic Method

Added diglyceride	Total sample recovered	Diglyceride recovered
%	%	%
4.15	99.7	109
4.18	100	106
Average	99.9	108
8.21	98.7	94.7
8.20	99.8	99.5
Average	99.3	97.1
12.0	98.1	96.7
12.0	99.7	98.2
Average	98.9	97.5

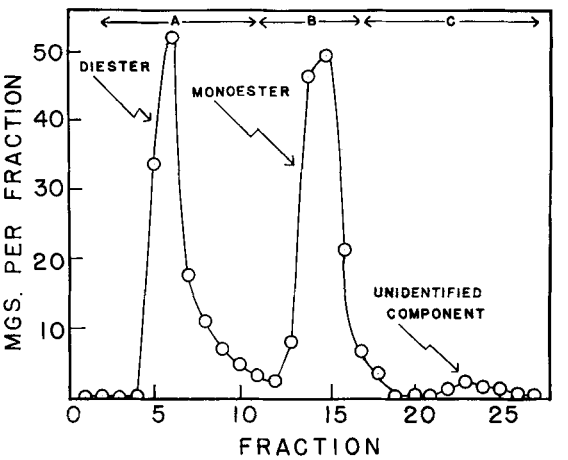

FIG. 4. Chromatogram of a commercial sample of ethylene glycol monostearate. The eluting solvents were: A, 40% isopropyl ether in iso-octane; B, 70% ethyl ether in iso-octane; C, 20% absolute ethanol in isopropyl ether.

could not be found, the normal chromatographic method was further investigated to see how accurately it alone could determine small known amounts of diglyceride. As can be seen from Table II, as little as 8% of diglyceride can be analyzed with a fair amount of accuracy.

A randomization procedure (5) with linseed oil was used to prepare a mixture of glyceryl esters containing unsaturation in their acid moieties. Chromatographic analysis of this mixture indicated that unsaturation has very little effect on the obtained chromatogram since no shifting of the peaks occurred.

Figure 4 shows the degree of separation obtained when the same solvent system used for the glyceryl ester mixtures is employed in the analysis of ethylene glycol ester mixtures. An infrared spectrum was obtained of the material contained in each peak as an identification of the material.

In Figure 5 are shown the results of analyzing a mixture of esters of polyethylene glycol 300. There are four peaks present. The compounds represented by these peaks were identified not only by their infrared spectra but also by saponification numbers. The component of the first peak was identified as oleic acid by its infrared spectrum and its acid value.

Since the sample placed on the column was rather small for ordinary saponification methods, a modification of the micro-saponification method of Marcali and Rieman (6) by Ketchum (7) was used. The double indicator method is accurate to within 5% of the theoretical value. Such accuracy is sufficient since the saponification number is determined only as a means of identification of the components. As an example, the micro-saponification method was carried out on the material from the second and third peaks in the chromatogram of a commercial sample of polyethylene glycol 400 distearate. The first peak was the free fatty acid. Micro-saponifications showed the second and third peaks to be the diester and the monoester, respectively.

Table III indicates the extent of total recovery obtainable from this adsorption chromatography procedure. The amount of material left on the column varies from 0.1% to 2% of the weight of the sample placed on the column.

A sample made up of commercial polyethylene glycol 400 monostearate and polyethylene glycol 600

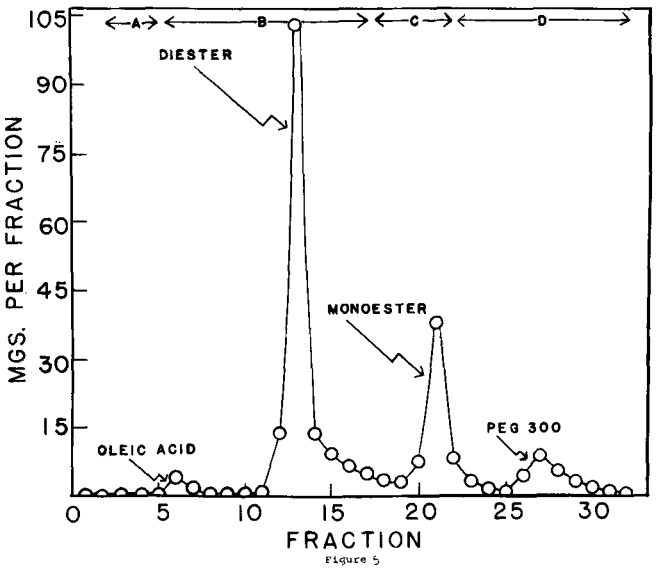

FIG. 5. Chromatogram of a commercial sample of polyethylene glycol 300 1½ oleate. The eluting solvents were: A, 3% methanol in isopropyl ether; B, 12% methanol in isopropyl ether; C, 10% methanol in chloroform; D, 35% methanol in chloroform.

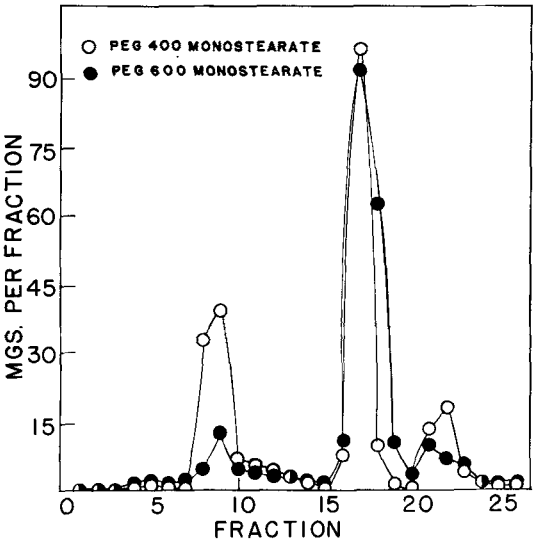

FIG. 6. Chromatogram of commercial samples of polyethylene glycol 400 monostearate and polyethylene glycol 600 monostearate.

monostearate was chromatographed, using the appropriate chromatographic system. Only three peaks showed up in the chromatogram. When these same two commercial preparations were run simultaneously on different columns, their peaks appeared at approximately the same fraction number (Figure 6). Thus, an increase in molecular weight of the polyethylene glycol chain does not affect, to any great extent, the chromatogram obtained by this method of analysis.

TABLE III

Analysis of Commercial Ester Products

Product	Sample weight	Weight recovered	Blank weight recovered	Total recovery
	mg.	*mg.*	*mg.*	*%*
Glycerol monostearate	192.0	195.0	4.1	99.9
Glycerol mono-oleate	150.4	154.2	.....	102.5
Ethylene glycol monostearate	232.7	248.7	12.4	101.4
PEG 300 1½ oleate	248.1	272.3	24.5	99.9
PEG 400 distearate	248.4	283.0	11.7	101.2

It is thought that this separation procedure could be applied to ester mixtures of many more polyhydroxyl alcohols than were tried here. The solvent system indicated for glyceryl esters should prove effective in the separation of ester mixtures of most straight-chain polyhydric alcohols. The solvent system used for analysis of ester mixtures of several different polyethylene glycols could probably be applied to any polyethylene glycol ester mixture.

Summary

A procedure for the chromatographic separation and determination of the ester mixtures of glycerin, ethylene glycol, and polyethylene glycol is presented. The adsorbent is silica gel, and the mobile phase is a series of solvents with increasing polarity. A procedure for quantitative determination of small amounts of monoglyceride present in a glyceryl ester mixture is presented.

A solvent system different from that used for glyceryl and ethylene glycol ester mixtures was developed for ester mixtures of polyethylene glycols. The esters of polyethylene glycols with average molecular weights of 300–600 were shown to give the same chromatogram.

REFERENCES

1. Ravin, L. V., Meyer, R. J., and Higuchi, T., J. Am. Oil Chemists' Soc., *34*, 261 (1957).
2. Quinlan, P., and Weiser, H. J., *ibid.*, *35*, 325 (1958).
3. Malkemus, J. D., and Swan, J. D., *ibid.*, *34*, 342 (1957).
4. Kaufmann, H. P., and Wolf, W., Fette und Seifen, *50*, 519 (1943).
5. Bailey, A. E., "Industrial Oil and Fat Products," Interscience Publishers Inc., New York, 1951, p. 820.
6. Marcali, K., and Rieman III, W., Ind. Eng. Chem., Anal. Ed., *18*, 144 (1946).
7. Ketchum, D., *ibid.*, *18*, 273 (1946).

[Received January 7, 1960]