Chromatography of Polar Triglycerides on Silicic Acid Columns[1]

C. D. EVANS, D. G. McCONNELL, R. L. HOFFMANN, and HELEN PETERS,
Northern Regional Research Laboratory,[2] Peoria, Illinois

Abstract

Triglycerides containing polar fatty acids are resolved by silicic acid chromatography into molecular species containing increased amounts of the polar acids. Natural fats like isano, oiticica, castor, or kamala seed oils with both nonpolar fatty acids and polar hydroxy or keto acids have been resolved into component glycerides according to the degree of polarity. Silicic acid chromatography offers a means of obtaining specific glycerides from fats for detailed studies on glyceride composition and structure.

Introduction

THE CHROMATOGRAPHIC separation of triglycerides, as a class from phospholipids, sterols, and partial glycerides has been the subject of many reports (7,9,16). Studies on the fractionation of triglycerides themselves have been less numerous (11,12,15). A wide variety of solvent systems has been suggested for these fractionations, usually with many different eluting solvents, each of which exhibits increased polarity. It has been shown that all glycerides from tristearin to triacetin can be eluted with a single methanol-benzene solvent system (1). The application and operational details of this liquid chromatography (LC) technique have been described in earlier studies on the separation of dimeric fat products (2), hydroperoxides (3), and hydroxy fatty acids (4). With this system one may choose in advance the elution position, where a large change in polarity of the column effluent (gradient) will take place. Furthermore one may select areas for any particular fractionation, where high resolution is desired, by adjusting the solvent polarity. For example, the small difference in polarity shown by a primary and a secondary hydroxyl group is sufficient for the resolution of 1,2- and 1,3-diglycerides. Thus it is possible to analyze for the content of these isomers in partial glycerides (1). Columns which show a decreasing polarity of eluting solvent (negative gradient), rather than an increasing polarity, and columns which show no change in the polarity of the eluting solvent are also possible.

Natural triglycerides which have polar fatty acids are easily resolved into molecular species that contain increased amounts of the polar acids. Samples of fats and seeds were obtained from reliable commercial sources so that correct identity could be assumed.

Figure 1, isano oil, shows the type of resolution achieved with natural fats which contain a high percentage of hydroxy acids. Isano oil is reported to be 40–45% hydroxy acid (8). A more complete separation of peaks I and II can be obtained by reducing the amount of methanol in the stationary phase, Figure 2. However peaks IV and V of Figure 2 are eluted together as a single peak IV, in Figure 3, because of the effect of the gradient. The five major fractions indicated in Figure 1 were isolated, esterified, and

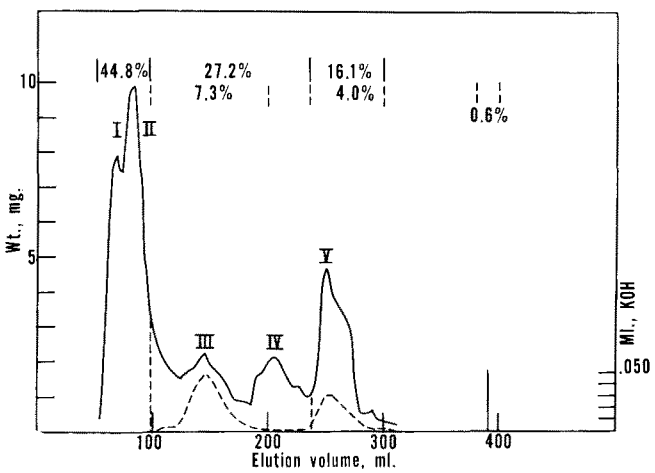

FIG. 1. Liquid chromatographic separation of the glycerides of isano oil, a fat which contains both highly unsaturated acids and unsaturated hydroxy fatty acids. Dotted line indicates free acid titration.

analyzed for hydroxy content by LC, gas-liquid chromatography (GLC), and infrared (IR), Table I. Fractions I, II, and IV contain 0, 1, and 2 moles of hydroxy respectively. Fraction III contains free fatty acids. Fraction V includes the tail of Fraction IV, free hydroxy fatty acids, trihydroxy glycerides, and other polar oxidation products. For optimum analytical results isano oil should be completely hydrogenated to saturate all -ynoic fatty acids.

Although fractionation of the oil is based on the polarity of the hydroxyl groups, ultraviolet absorption studies on these same fractions show that the polyene fatty acids are concentrated in peaks I and III. Absorption of simple poly-ynes $R-C\equiv C-R'$ show absorption peaks at 242, 255, 268, 284, and 300 mμ (5). These absorptions correspond to findings for isano oil, Figure 3, with the exception of a spectral band occurring at 228 and the absence of a band at 300 mμ. The specific absorption curves shown in Figure 4 were obtained by plotting the K values for the various wavelengths against the elution volumes. For the sake of clarity the absorption at 242 mμ is not shown since it is similar to the absorption at 254 mμ but of slightly less intensity. Since the polyene fatty acids are concentrated in the first and third peaks, the absorption spectra of these fractions are similar to the spectrum of the unfractionated isano

TABLE I

Composition of Polar Triglycerides

Me esters		Percentage OH			Percentage Keto	
		IR	LC	GLC	LC	GLC
Whole isano		48.2	34.6	31.7	.....	.....
Isano oil peak	I	1.8	0	0	.....	.....
	II	31.2	37.2	20.1	.....	.....
	IV	56.9	62.9	57.7	.....	.....
	V	62.9	58.0	57.3	.....	.....
Whole oiticica		.....	.....	.....	47.6	46.1
Oiticica peak	I	.....	.....	.....	.....	tr.
	II	.....	.....	.....	.....	25.8
	III	.....	.....	.....	.....	52.3
Whole kamala		.....	.....	69.3	.....	5.2(?)

[1] Presented at the AOCS Meeting, Cincinnati, October 1965.
[2] No. Utiliz. Res. Dev. Div., ARS, USDA.

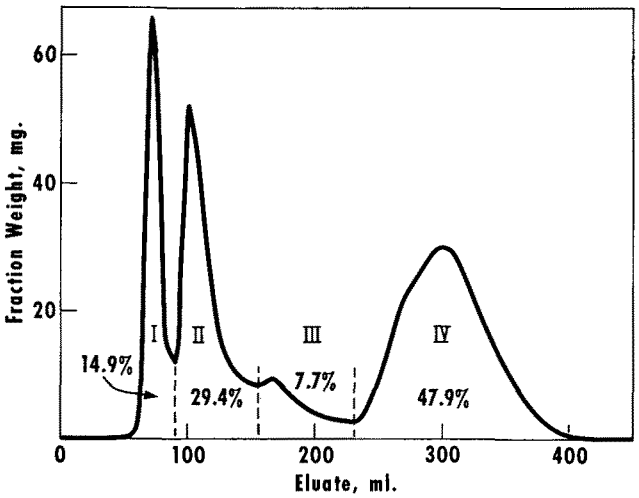

FIG. 2. Liquid chromatogram of the glycerides of isano oil, showing greater separations by reducing the amount of methanol in the stationary phase.

oil. Fraction III however shows a greater absorption at the lower wavelengths than does the whole oil.

Figure 5 shows the three major fractions of triglycerides, containing a keto fatty acid, that were separated by reducing the methanol in the stationary phase. GLC analysis reveals that mono-keto acid triglycerides and normal triglycerides are in fraction I; and di-keto acid triglycerides are in fraction II, Table I. Some highly polar components (15.9%) are eluted at the gradient position. Various compositions for oiticica oil have been reported (6); results indicate that this oil contained 46% licanic acid instead of the reported 70–80%.

A chromatogram of the diethyl ether-soluble fraction, obtained after kamala seed had been extracted with petroleum ether, is shown in Figure 6. Although Hilditch (6) reports this seed to contain 26–58% of hydroxy eleostearic acid, Puntambekar (10) indicates that an isomeric keto linoleic acid (25.7%) constitutes the polar acid in this fat.

The ether-soluble fraction contains 69% hydroxy acid and a 5% component that could be a keto acid (Table I). Complex polyester-linked glyceride structures have been suggested (6), and results tend to substantiate this.

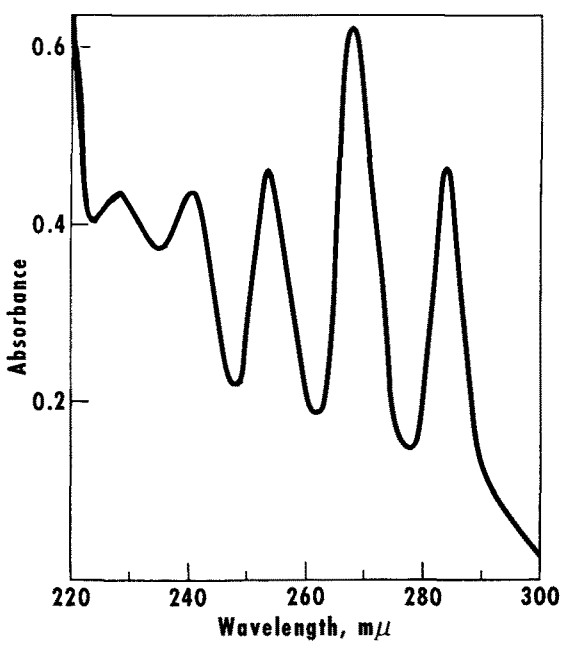

FIG. 3. Ultraviolet absorption spectrum of isano oil.

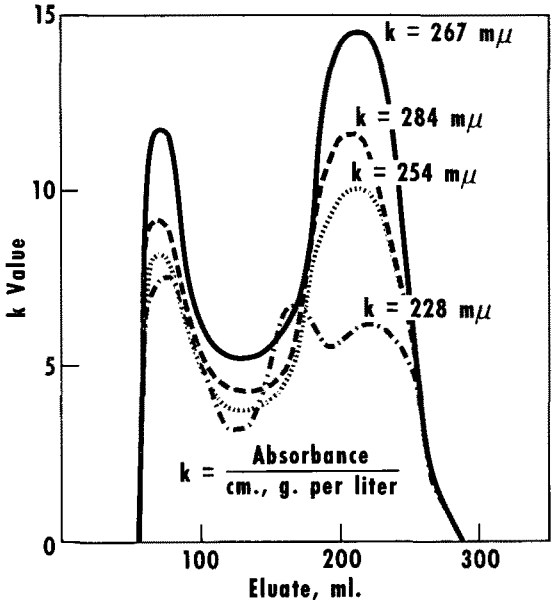

FIG. 4. Specific absorptions of isano oil at wavelengths of 228, 254, 284, and 267 mμ.

Figure 7 indicates that a number of polar glycerides exist in the seed of *Dimorphotheca aurantiaca*. Smith et al. (13) report that a conjugated hydroxy fatty acid constitutes 65% of the fatty acids in this seed. Results indicate at least eight glyceride fractions, the major portion of which comes over at the gradient position. A rather high diethyl ether eluate was observed with this particular sample. Polarity of the major peak, elution volume 260 ml, is the same as that for the major glyceride (82.8%) of castor oil.

Although the method is shown to work particularly well with triglycerides containing polar fatty acids,

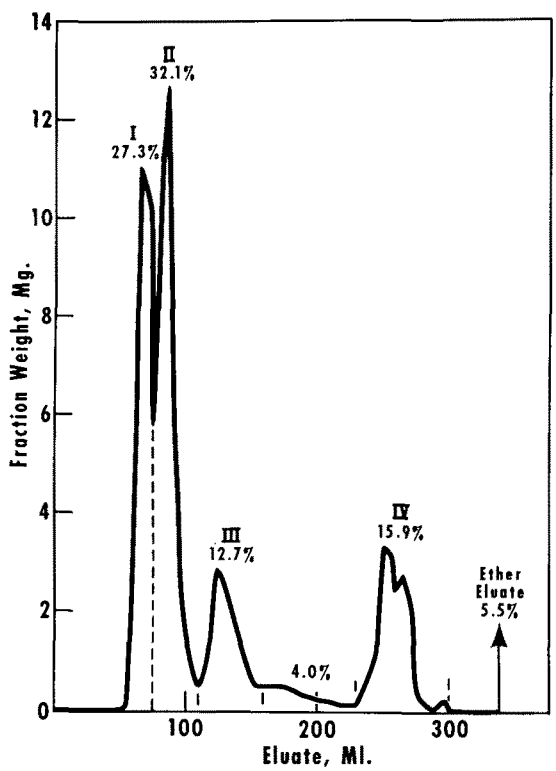

FIG. 5. Liquid chromatographic separation of the glycerides in the liquid portion of oiticica oil.

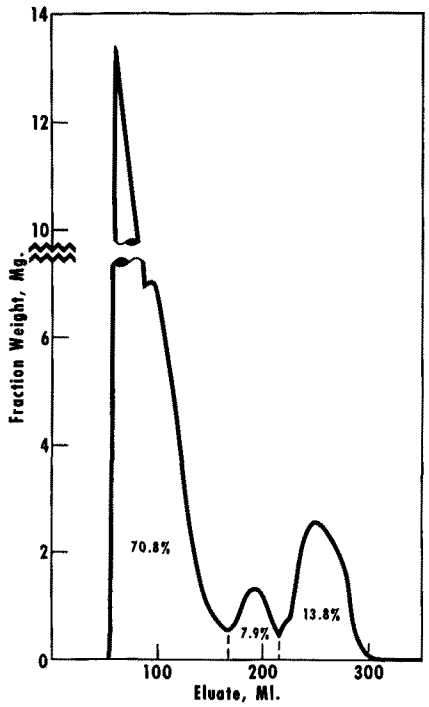

FIG. 6. Liquid chromatographic separation of the glycerides in the diethyl ether-soluble portion of kamala seed oil.

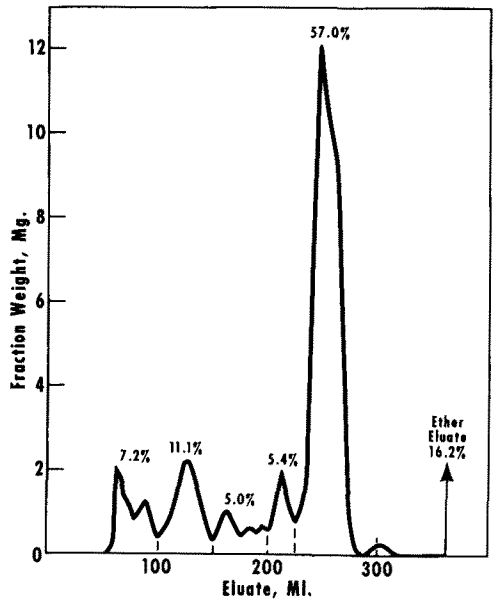

FIG. 7. Liquid chromatographic separation of the glycerides in the oil of *Dimorphotheca aurantiaca*.

many other solvent combinations need to be evaluated to obtain the optimum resolution of triglycerides containing shorter chain and unsaturated fatty acids. It would be expected that the presence of unsaturated linkages and the position of the hydroxyl group, relative to the other functional groups, would influence the polarity of the glycerides. With chromatographic columns using the same solvent system, the elution volumes are triolein 70 ml, triacetin 235 ml, diacetin monoolein 95 ml, and monoacetin monoolein 255 ml. The method obtains a rather complete picture of the types of glycerides present in a synthetic mixture. Similar fractionations can be made of natural and modified fats. When less than 1% of a material is originally present or when high resolution is desired, it may be necessary to run several chromatographic columns to obtain the desired amount of sample. Reproducibility of the method permits the use of the elution volume for identification purposes and allows cuts to be made on a volumetric basis without the use of a fraction collector or a drop counter.

The mildness of the procedure allows the isolation of natural triglycerides in unaltered form and offers another tool for characterization studies on glyceride structure. It is also a source of specific glycerides for standards in chemical or physical tests. Fractionations of oiticica, castor, dimorphotheca, isano, and kamala oils to obtain the component glycerides illustrate the potentialities of the method. Fractionation and analysis of ozonized, autoxidized, and modified fats and of synthetic triglycerides are currently being carried out in the laboratory. The method offers many applications for the analysis of epoxide and polyepoxidized fats and their derivatives because of the difference in polarity of epoxide, alkoxy, and hydroxy groups (14).

Simplicity, high degree of resolution, excellent reproducibility, and the wide range of polarity which can be attained with a single binary solvent system offer much to any lipid investigation that involves glyceride compositional studies. Many other applications of the method will be obvious to research workers in both the chemistry and nutrition of natural lipids and in the resolution of chemically modified and altered fats, and it is primarily for these reasons that this paper is offered.

ACKNOWLEDGMENT

The authors wish to thank the Pacific Vegetable Oil Company for supplying oiticica oil and kamala seed, the Southern Regional Research Laboratory for the samples of aceto:eins, and F. R. Earle for the sample of *"dimorphotheca"* oil.

REFERENCES

1. Evans, C. D., D. G. McConnell, E. N. Frankel and E. J. Panek, J. Am. Oil Chemists' Soc., Technical Program Abstract No. 27, Fall Meeting 1961.
2. Frankel, E. N., C. D. Evans, Helen A. Moser, D. G. McConnell and J. C. Cowan, JAOCS *38*, 130–134 (1961).
3. Frankel, E. N., C. D. Evans, D. G. McConnell and E. P. Jones, Ibid. *38*, 134–137 (1961).
4. Frankel, E. N., D. G. McConnell and C. D. Evans, Ibid. *39*, 297–301 (1962).
5. Gillam, A. E., and E. S. Stern, "Electronic Absorption Spectroscopy in Organic Chemistry," 2nd ed., Edward Arnold Ltd., London, 1960.
6. Hilditch, T. P., "The Chemical Composition of Natural Fats," John Wiley and Sons, New York, 1956.
7. Hirsch, J., and E. H. Ahrens, J. Biol. Chem. *233*, 311–320 (1958).
8. Kneeland, J. A., D. Kryiacou and R. H. Purdy, JAOCS *35*, 361–363 (1958).
9. Mangold, H. K., and D. C. Malins, Ibid. *37*, 383–385 (1960).
10. Puntambekar, S. V., Proc. Indian. Acad. Sci. *35A*, 57–68 (1952); C. A., *47*, 2511 (1953).
11. Reinbold, Catherine L., and H. J. Dutton, JAOCS *25*, 117–120 (1948).
12. Sahasrabudhe, M. R., and D. J. Chapman, Ibid. *38*, 88–92 (1961).
13. Smith, C. R., T. L. Wilson, E. H. Melvin and I. A. Wolff, J. Am. Chem. Soc. *82*, 1417–1421 (1960).
14. Staff Report, Chem. Eng. News *39*(35), 36 (1961).
15. Walker, F. T., and M. R. Mills, J. Soc. Chem. Ind. *61*, 125–128 (1942).
16. Wren, J. J., J. Chromatog. *4*, 173–195 (1960).

[Received January 18, 1963]