cottonseed or of soybean oils at 20% concn were prepared in which lecithin at 1% concn was the emulsifier, and a 2.5% solution of glycerol was the aqueous phase. The pH of these emulsions was adjusted to 6.8. For a comparison between the two emulsions, the number of dispersed oil particles of stated diameters from 1–10 μ were determined, and the volume of dispersed oil in the form of particles of stated diameter was calculated. These calculated volumes are relative rather than absolute, since particles of less than 1 μ in diam have not been taken into account. They do provide, however, a basis for the comparison of emulsions. The results of the particle size distributional analysis are plotted in Figure 1. In both emulsions, only 1% of the relative volume of dispersed oil was in the form of particles whose diam was more than 5 μ, and ca. 0.5% of the volume consisted of particles more than 7 μ in diam. The soybean oil emulsion appeared to have a somewhat larger volume of oil in the form of particles of 1–2 μ in diam than did the cottonseed emulsion, although both emulsions were desirably low in the volume of oil dispersed as particles of large diameters (7–10 μ).

Animal Testing. The described emulsion has been experimentally evaluated in animals only in a preliminary manner, and results are not conclusive as yet. Limitations to use of the emulsion in experimental animals only is strongly recommended.

Four rats received 4 ml of the emulsion/100 g of body wt, daily for 15 days, administered by intravenous injection through the tail vein. Results of these injections were as follows: tail necrosis, none; hemoglobin, −6.2%; red blood cells, −7.7%; body wt, +11.9%; food intake, −17%; physical activity, good; mortality, none. Complete results of more extensive testing will be reported by others at a later date.

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REFERENCES

1. Chain, E., and J. Kemp, Biochem. J. *28*, 2052 (1934).
2. Fischgold, H., and E. Chain, *Ibid. 28*, 2044 (1934).
3. Geyer, R. P., Physiol. Revs. *40*, 150 (1960).
4. Jukes, T. H., J. Biol. Chem. *107*, 783 (1934).
5. Schuberth, O., and A. Wretlind, Acta Chim. Scand. Suppl. *278*, 1–21 (1961).
6. Singleton, W. S., and M. L. Brown, "Particle Size Distribution in Fat Emulsions by Electronic Counting," in press.
7. Singleton, W. S., M. S. Gray, M. L. Brown and J. L White, *Ibid.* "Chromatographically Homogeneous Lecithin from Egg Phospholipids." in press.
8. Singleton W. S., J. L. White, L. L. diTrapani and M. L. Brown, *Ibid. 39*, 260 (1962).
9. Yeadon, D. A., L. A. Goldblatt and A. M. Altschul, *Ibid. 35*, 435 (1958).

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Determination of the Glyceride Structure of Fats: Anomalous Features of the Seed Fat of Bitter Gourd *(Momordica charantia)*[1]

M. R. SUBBARAM,[2] M. M. CHAKRABARTY,[3] C. G. YOUNGS and B. M. CRAIG, National Research Council of Canada, Prairie Regional Laboratory, Saskatoon, Saskatchewan, Canada

Abstract

Glyceride composition of the seed fat of *Momordica charantia* has been determined by gas liquid chromatography (GLC) of oxidized esterified glycerides. Stearodiunsaturated glycerides form ca. 80% of the total glycerides. Values obtained by the above method agree closely with those obtained by Youngs' (8) method. However, there is a wide variation between the present results and those calculated according to Vander-Wal (5) from lipase hydrolysis data. Possible reasons for this anomalous glyceride pattern are discussed.

Introduction

CHAKRABARTY ET AL. (1) studied the fatty acid composition of some seed fats of the Cucurbitaceae family, and distinguished two definite trends. Some members such as the melons and pumpkins had a simple fatty acid composition containing only oleic and linoleic acids as unsaturated components, while others such as snake gourds and bitter gourd (*Momordica charantia*) contained, in addition, considerable proportions of conjugated triene acids. Verma and Aggarwal (6) also reported the presence of ca. 50% α-eleostearic acid besides stearic, oleic and linoleic acids in the seed fat of *Momordica charantia.* During the course of an investigation on the glyceride structure of fats both by the methods of Youngs (8) and Youngs and Subbaram (9), we had occasion to examine the seed fat of *Momordica charantia.* In the present paper results of glyceride analysis of the fat are presented. The percentages of glyceride types and isomeric forms calculated according to Vander-Wal (5) from lipase hydrolysis data are also given and compared with the values obtained experimentally.

Experimental

Extraction of the Oil. The oil was extracted from the seeds by the method described by Troeng (4). The extract was filtered through a bed of celite, the residue washed with Skellysolve F and the solvent removed under reduced pressure. The yield of oil was 29.6%. The oil was preserved as a 0.1% solution in Skellysolve F in the dark.

Preparation of Methyl Esters. Ca. 20 mg of the oil was added to 5 ml of a solution containing 250 mg potassium hydroxide in 80% v/v ethanol. The mixture was kept at room temp for 12 hr after which it was diluted with 95 ml water. The soap solution was transferred to a separatory funnel and extracted twice with ethyl ether to remove unsaponifiable matter. It was then acidified with 5 *N* hydrochloric acid. After saturating the aqueous solution with NaCl, the fatty acids were extracted with three 50-ml portions of ethyl ether. Ether was removed under vacuum in a rotary evaporator. The residual fatty acids were esterified with an ethereal solution of diazomethane.

Determination of Fatty Acid Composition. The methyl esters were analyzed by GLC using both fluorinated silicone and polyester columns. An F & M, Model 500, temp programmed gas chromatographic unit with thermal conductivity detectors was used to analyze the esters on the silicone column. The methyl esters were separated into C_{16}, C_{18} and eleostearates

[1] Issued as N.R.C. No. 8051.
[2] National Research Council of Canada Postdoctorate Fellow, 1962–1963.
[3] Visiting Scientist. Present address: Department of Applied Chemistry, University College of Science and Technology, Calcutta, India.

TABLE I
Fatty Acid Composition of the Seed Fat of *Momordica charantia* (mole %)

	16:0	18:0	18:1	18:2	Eleostearic	Total unsaturated
Original	2.2	30.4	5.3	5.8	56.3	67.4
Calcd.[a]	2.6	31.0				66.4
1–3[b] .	2.6	43.0	9.6	4.3	40.5	54.4

[a] Values calculated from glyceride composition.
[b] Data obtained from lipase hydrolysis.

on a 6 ft x 1/4 in. column packed with Chromosorb W (40–60 mesh) coated with 16% by wt of QF-1 (commercial fluorinated silicone) and temp programming from 150–250C at 4°/min with an 80-ml/min helium flow rate. The polyester column (8 ft x 3/16 in. copper tube) was made up with *o*-phthalic ethylene glycol on Chromosorb W (40–60 mesh) in a ratio of 1:6 by wt. The unit was operated at 208C with injector at 250C and a flow rate of 40–60 ml helium/min. The degree of unsaturation and the chain length of the components were deduced from their emergence times. The proportion of stearic, oleic and linoleic acids in the C_{18} peak on the silicone solumn was determined by the data from the polyester column.

Oxidation of the Glycerides. Details of the permanganate-periodate method (7) of oxidation of glycerides were described earlier (9). In the case of *Momordica charantia* seed fat which contains considerable proportions of eleostearates, it was necessary to slightly alter the reaction conditions. The proportion of reagents used for the oxidation was adjusted according to the fatty acid composition. Eleostearate requires three times the amt of oxidant required for oleic acid. Oxidation was carried out at 75C for 4 hr. Completion of the oxidation was checked as follows: A portion of the oxidized glycerides was hydrolyzed by cold saponification with alcoholic alkali and the fatty acids were esterified with diazomethane and analyzed by GLC on a silicone column.

Analysis of Oxidized Glycerides. The analysis was carried out both by the method of Youngs (8) and Youngs and Subbaram (9). Thus a portion of the oxidized glycerides was separated on a liquid-liquid partition column into two fractions, the first containing glycerides having no dicarboxylic acid or one dicarboxylic acid and the second containing glycerides with two or three dicarboxylic acids. Determination of the fatty acid composition of these fractions by GLC, coupled with lipase hydrolysis allowed the calculation of six glyceride types. Another portion of the oxidized glycerides was esterified with diazomethane and the esterified oxidized glycerides analyzed directly by GLC on an F and M model 1609 temp programmed unit with a flame ionization detector. The column (4 ft x 3/16 in. stainless steel tubing) was packed with 2% S.E. 30 on Anakrom ABS and the helium flow rate was 100 ml/min. Temp programing was from 260–325C at 3°/min with an injector temp of 385C and detector temp of 355C. All compositions were calculated as mole percentages.

Results and Discussion

To get a sample of methyl esters with very little conversion of α-eletostearate to the β-form, it was necessary to carry out cold saponification of the seed fat. The amt of α- and β-eletostearates were determined from the results obtained on the silicone column, as the longer retention time on the polyester column, resulted in broad peaks which were difficult to measure. Fatty acid composition of the seed fat shows in Table I. The proportion of eleostearate thus obtained agreed with the values obtained by UV absorption measurements. Oxidation of glycerides containing conjugated acids requires a slightly higher temp and a longer reaction period than those with nonconjugated acids.

TABLE II
Glyceride Composition of the Seed Fat of *Momordica charantia* (mole %)

	Method of Youngs and Subbaram (9)		Method of Youngs (8)	
	Found[a]	Calcd.[b]	Found[c]	Calcd.[b]
U_3 .	7.4	27.7	UUU 11.3	27.7
PU_2	4.6	3.1	USU 3.5	1.9
SU_2	79.7	45.1	UUS 76.6	46.3
P_2U		0.1	SUS 6.3	19.2
PSU . .	3.3	2.8	USS 1.5	3.3
S_2U	5.0	19.6	SSS 0.8	1.6
P_3 .				
P_2S .				
PS_2 .		0.6		
S_3		1.0		

[a] U—unsaturated acids; P—palmitic acid and S—stearic acid.
[b] Calculated from pancreatic lipase hydrolysis data according to VanderWal (5).
[c] U—unsaturated acids and S—saturated acids.

The glyceride composition of the seed fat as obtained by direct GLC analysis of the esterified oxidized glycerides shows in Table II. Stearodiunsaturated glycerides account for nearly 80% of the total glycerides. This is in agreement with the value obtained by Youngs' method. The value calculated from lipase hydrolysis data according to VanderWal (5) is only 45%. Values obtained for U_3 and S_2U are much lower than the calculated values. Fatty acid composition calculated from the glyceride composition agreed closely with the original fatty acid composition (Table I).

The glyceride composition of other natural fats (3) agreed closely with the composition calculated from lipase hydrolysis data. The seed fat of *Momordica charantia* is the first instance where we have found a major difference between the values obtained by the GLC method and the calculated values according to VanderWal (5). The postulation of Kartha (2) that natural fats must remain fluid *in vivo,* would provide a possible explanation for this anomalous glyceride pattern of *Momordica charantia* seed fat. Eleostearic acid which has a relatively high melting point as compared to other unsaturated acids, could place a restriction on the formation of disaturated eleostearic glycerides. Such a restriction would then result in the formation of a higher proportion of monosaturated glycerides at the expense of some triunsaturated glycerides (U_3). Another possible explanation would be the disproportionation of stearic acid in the 1 and 3 positions. If stearic acid occupies predominantly either one or the other of these positions, this would also give the type of distribution found in the present investigation. At the moment, we have no method of distinguishing the fatty acids in the 1 and 3 positions. It is hoped that in future such methods will be devised which will make it possible to check the validity of such a postulation.

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REFERENCES

1. Chakrabarty, M. M., D. K. Chowdhury and B. K. Mukherji, Naturwissenschaften *42*, 344 (1955).
2. Kartha, A. R. S., "Studies on the Natural Fats," Vol. 1 published by the author, Ernakulam, India (1951).
3. Subbaram, M. R., and C. G. Youngs, JAOCS, in press.
4. Troeng, Sixten, *Ibid. 32*, 124 (1955).
5. VanderWal, R. J., *Ibid. 37*, 18 (1960).
6. Verma, J. P., and J. S. Aggarwal, J. Indian Chem. Soc. *33*, 357 (1956).
7. von Rudloff, E., Can. J. Chem. *34*, 1413 (1956).
8. Youngs, C. G., JAOCS *38*, 62 (1961).
9. Youngs, C. G., and M. R. Subbaram, *Ibid. 41*, 218 (1964).

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