COMPONENT GLYCERIDES OF VEGETABLE FATTY OILS. PART I. NIGER SEED OIL.

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The fatty oil extracted from the seed of *Guizotia abyssinica* (Niger seed) has been found to contain myristic acid (along with some capric, caprylic and lauric acids) (1·7%), palmitic acid (5%), stearic acid (2%), oleic acid (38·9%) and linolic acid (51·6%) along with traces of archidic, behenic and lignoceric acids.

The glyceride structure of the oil has been determined by brominating the neutral oil, resolving the brominated solid and liquid products into a number of simpler fractions and estimation of the fatty acid composition of the different fractions. The component glycerides of the oil have been found to be trilinolin (2%), oleo-di-linolin, (40%). di-oleo-linolin (30%), myristo-di-linolin (2%) myristo-oleo-linolin (3%). palmito-di-linolin (6%), palmito-oleo-linolin (11%). stearo-di-linolin (2%) and stearo-oleo-linolin (4%).

The methods adopted by Hilditch *et al.* (*J. Soc. Chem. Ind.*, 1938, **57**, 44) for the determination of glyceride structure of solid seed fats cannot be adopted for the liquid fats, because they do not contain any appreciable quantity of di- or tri-saturated glycerides. Up till now there has been no satisfactory method for the study of the fully unsaturated glycerides. With a view to explore a satisfactory method for the quantitative estimation of the triolein in natural and synthetic fats, Hilditch and Griffith (*J. Chem. Soc.*, 1932, 2315) converted oleic acid into elaidic acid. But they found that only 66% of the acid could be converted to elaidic acid when the equilibrium was attained and the process could not be used for any quantitative work.

Hilditch and Jones (*J. Soc. Chem. Ind.*, 1934, **53**, 131) examined the glyceride structure of a number of technically important fatty oils by the fractional crystallisation of the fully hydrogenated oils from different solvents. This method does not throw any light upon the association of the different unsaturated acids in the glycerides.

Suzuki and Yokoyama (*Proc. Imp. Acad. Tokyo*, 1927, **3**, 529) and Hashi (*J. Soc. Chem. Ind. Japan*, 1927, **30**, 849 ; 1928, **31**, 117) brominated a number of vegetable and fish oils and determined the glyceride composition of these oils by the fractional crystallisation of the solid bromo-glycerides from different solvents. They could not examine the liquid bromo-glycerides, which are the products of bromination of the glycerides containing mono- and diethylenic acids. Stein and Ulzer (*Chem. Zentrl.*, 1928, **II**, 1337) and Sahashrabudhi and Kale (*J. Univ. Bombay*, 1932, **2**, 37) have brominated Niger seed oil and they have reported some qualitative results.

The present work has been taken up with a view to find out a method for the quantitative estimation of the component glycerides of the fatty oils, and it has been found that the quantity of fully unsaturated and di-unsaturated glycerides containing mono- and diethylenic acids can be determined from a fatty oil by resolving the liquid bromo-glycerides into a number of simpler fractions by extraction with different solvents. The method has been tried upon the Niger seed oil and a number of other vegetable fatty oils is being studied by this process. The method is described here in brief.

The component fatty acids of the oil are determined by the usual method of lead salt separation, ester fractionation and hexabromide estimation. Then the neutral oil is dissolved in 6 times its weight of dry acetone and crystallised at 0°. Any fully saturated or disaturated glyceride will crystallise out. The quantity of fully saturated glyceride in the oil is determined by successive oxidation of the neutral oil in acetone solution (Hilditch and Lea, *J. Chem. Soc.*, 1927, 3106). The neutral oil or the glyceride, from which fully saturated and disaturated glycerides have been separated out, is dissolved in low boiling petroleum ether and brominated at 0°. The solid and liquid bromo-glycerides are further resolved into a number of fractions with benzene, acetone, alcohol and acetone and alcohol mixture (1 : 1). All the fractions are debrominated, the component fatty acids are determined and the amount of the various glycerides in the fractions is calculated.

Niger seed oil has been found to consist of the following glycerides : Tri-linolin (2%), myristo-di-linolin (2%), myristo-oleo-linolin (3%), palmito-di-linolin (6%), stearo-di-linolin (2%), palmito-oleo-linolin (11%), stearo-oleo-linolin (4%), di oleo-linolin (30%) and oleo-di-linolin (40%). Palmito-glycerides contain the small quantity of lauric, and other lower acids and stearo-glycerides contain the archidic, lignoceric and behenic acids.

The neutral oil was hydrogenated in presence of nickel catalyst suspended on kieselguhr by agitation process to two stages, one to the iodine number of 1·8 and the other to the iodine number of 27·2. The former one gave 75·3% of tristearin on crystallisation from ether at 0° and the latter one gave 51·2% tristearin in 76·2% of fully saturated glycerides, on making allowance for the non-saponifiables of 2·4% in the oil. The amount of C_{18} glycerides calculated in the oil is about 77·3% and the remaining 20·8% of the glycerides contain at least one acid having less than 18 carbon atoms in the chain. It agrees fairly well with the results obtained from the brominated glycerides.

The oleic and linolic acids, being the major component acids, have distributed themselves evenly in the glycerides.

EXPERIMENTAL.

Finely crushed seeds of *Guizotia abyssinica* (Niger seed, N.O. *Compositae*) were extracted with carbon tetrachloride. The oil, golden yellow in colour, was 40% on the weight of the seeds and had the following physical and chemical constants (*cf.* Crossby and Sueur, *J. Soc. Chem. Ind.*, 1898, **27**, 991 ; Bolton, "Oils, Fats and Fatty Foods," p. 299).

TABLE I.

Sp. gr. at 28°	0·9475
Refractive Index at 25°	1·472
Sapon. value	189·7
Iodine value	129·2
Acetyl value	19·8
Free fatty acids (% as oleic acid)	4·27
Non-saponifiables	3·65%

Component Fatty Acids of the Oil.—440 G. of the oil were saponified and the non-saponifiables of the oil were extracted from the soap. The mixed acids, liberated from the soap, were subjected to the usual procedure of lead salt, alcohol and ether separation. The resulting acid fractions were converted into methyl esters and fractionally distilled under a pressure of 0·2 mm. as given in the following tables.

TABLE II.

	Wt.	Percentage.	Saponification equivalent.	Iodine value.
Oil	440 g.	...	295·5	129·2
Mixed acids (free from non-sap.)	403	91·7	281·5	129·4
(A) Acids from alcohol-soluble lead salts	233	57·5	281·3	132·2
(E) Acids from ether-soluble lead salts	98·5	24·6	281·1	143·2
(S) Acids from insoluble lead salts	72·5	17·9	273·2	52·42
Esters of A			294·2	147·0
Esters of E			294·7	141·1
Esters of S			286·9	52·35

TABLE III.

Fractional distillation of esters A.

Fractions.		Stillhead.	Weight.	Saponification equivalent.	Iodine value.
A_1	A 11	60-120°	3·1 g.	186·6	54·68
	A 12	120-135°	4·6	230·0	80·23
	A 13	135-141°	6·8	287·0	124·8
	A 14	141-145°	4·6	292·4	147·9
	A 15 (Residue)	—	4·5	295·4	155·1
A_2		145-150°	47·1	294·3	150·7
A_3		152-155°	32·0	294·3	157·6
A_4		155-160°	16·6	294·7	150·2
A_5		160-190°	7·8	294·6	120·4
A_6	(Residue)	—	24·8	309·1	121·0

N.B. The residue, fraction A_6, contained 14% of non-saponifiables and the correctcd sap. equivalent is 289·1.

TABLE IV.

Fractional distillation of ester E.

E_1	72-145°	5·3 g.	292·5	127·9
E_2	145-147°	3·0	292·6	142·2
E_3	147-152°	10·5	292·8	151·0
E_4	152°	6·2	296·8	138·5
E_5 (Residue)	—	12·4	295·8	139·0

TABLE V.

Fractionation of ester S.

S_1	90-140°	5·6 g.	262·3	25·2
S_2	140-142°	12·1	280·5	35·12
S_3	142-143°	12·0	282·6	55·82
S_4	146-150°	12·4	291·7	66·19
S_5	150°	3·5	291·7	49·74
S_6 (Residue)	—	7·9	307·1	49·60

Lauric acid (m.p. 42°), myristic acid (m.p. 52°), palmitic acid (m.p. 62°) and stearic acid (m.p. 62°) were identified from the various fractions by their mixed m.p. with authentic samples. By the oxidation of the fractions of the liquid acids in alkaline permanganate (Lapworth and Mottram, *J. Chem. Soc.*, 1925, **127**, 1678), dihydroxystearic acid (m.p. 130°) and two tetrahydroxy acids, one melting at 154° and the other melting at 174°, were identified.

The residue S_6 from the solid esters gave three acids melting at 72-73°, 81-82°, and 75-76° having the saponification equivalents of 316·2, 342·2 and 366·6 respectively. The second one, melting at 81-82°, was identified to be behenic acid. The other two had lower melting point for straight chain archidic and lignoceric acids, though their equivalents are fairly correct. As these acids have been found to be present in vegetable fats in branched chain forms these have been considered to be branched chain archidic and lignoceric acids.

By steam distillation of the splitted up soap of the oil 0·7% of steam-volatile acids was obtained, which on estimation of barium from their barium salts were found to be a mixture of capric and caprylic acids. (Found : Ba, 62.8 and mean M. W., 163·1. Calc. for $C_{10}H_{20}O_2$: Ba, 54 per cent and $C_8H_{16}O_2$: Ba, 46 per cent. Equivalent, 162·1). On brominating the mixed acids of the freshly extracted oil in ether no hexabromide was obtained. Hence the oil does not contain any linolenic acid. From these observations the component fatty acids of the glyceride of Niger seed oil have been calculated as given in Table VI.

TABLE VI.

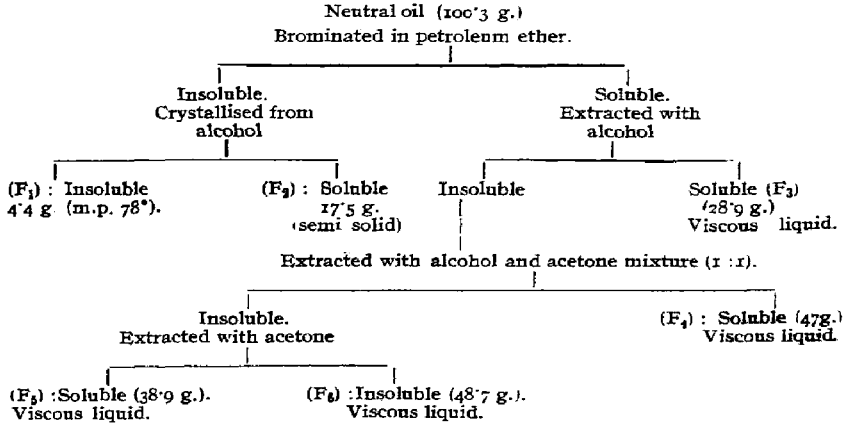
	In A.	In B.	In S.	% on mixed acids.	
Acids.	57·5%	24·6%	17·9%	%by wt.	Mol. %.
Lauric, capric, caprylic and myristic acids	0·8	nil	0·9	1·7	1·9
Palmitic acid	0·3	—	4·7	5·0	5·6
Stearic acid	—	—	2·1	2·1	2·0
Archidic, behenic and lignoceric acids	—	—	0·2	0·2	trace
Oleic acid	20·5	8·9	10·0	38·9	38·9
Linolic acid	35·9	15·7	—	51·6	51·6

Component Glycerides.—The oil from Niger seed was rendered neutral by treating with sodium carbonate and filtered through decolourising charcoal.

The oil (101·2 g.) was dissolved in 600 c.c. of dry acetone and left overnight at 0°. Nothing precipitated out and hence the oil does not contain any appreciable quantity of any disaturated glyceride. 490 C.c. more of acetone were added to the solution and it was oxidised thrice with powdered potassium permanganate. Finally it gave 1·2 g. of neutral product, which was found to be only non-saponifiables, and the oil was found to have no fully saturated glycerides.

The neutral oil (100·3 g.) was dissolved in one litre of petroleum ether and cooled down to −5°. Bromine was added slowly till the whole solution was permanently brown. It was left overnight at 0°, the precipitate was filtered off and washed with chilled petroleum ether. It was further resolved into two fractions by crystallisation from alcohol. From the filtrate the excess of bromine was washed off with sodium thiosulphate solution and on evaporating off the solvent a viscous liquid was obtained. It was successively extracted with alcohol, alcohol and acetone mixture (1:1) and acetone. Four fractions, three soluble in the solvent and one residue were obtained. The general scheme of separation is shown in Table VII.

TABLE VII.



All the fractions were debrominated by boiling with zinc dust and hydrogen chloride in methyl alcohol solution. The debrominated products

were saponified and acids were liberated after extracting the non-saponifiables. The quantity of individual acids was estimated in each fraction by determining their saponification equivalents, iodine values and thiocyanogen values. The quantity of saturated acids being too small in these fractions for estimating the amounts of individual acids, they were considered as one and their mean molecular weight was determined on extracting with petroleum ether the oxidation products of each fraction with alkaline potassium permanganate.

F_1 was obtained in a small quantity. Instead of subjecting it to the same procedure its bromine content was estimated according to the method of Bacons (*Chem. News*, 1903, **99**, 6). It was found to be a triglyceride of tetrabromo-linolic acid. (Found: Br, 52·02. Calc. for $C_{57}H_{98}O_6Br_{12}$: Br, 49.63 per cent). The analytical results are given in Table VIII.

TABLE VIII.

	F_1.	F_2.	F_3.	F_4.	F_5.	F_6.
Weights (in g.)	4·4	17·5	28·9	47·1	38·9	48·7
Saponification equivalent ...	—	276·7	278·2	282·0	281·7	281·9
Iodine value	—	121·6	92·7	121·7	147·4	147·6
Thiocyanogen value	—	61·3	59·0	90·1	90·0	90·1*
Weight of debrominated material (as glycerides and non-sap.) ...	2·1	10·0	17·5	26·4	44·8	
Weight of non-saponifiables ...	0·08	0·002	0·09	0·225	0·803	
% of glycerides (free from non-sap.)	2·0	10·0	17·4	26·2	44·4	
Saponification equivalents of the saturated acids	—	264·2	258·4	—	—	

* As the fractions 5 and 6 gave almost similar analytical figures, they were combined and considered as one fraction.

TABLE IX.

Mol. % of the acids in each fraction.

	F_1.	F_2.	F_3.	F_4.	F_5.	Mean.
Acids	2%	10%	17·4%	26·2%	44%	—
Saturated acids	—	32	33·5	—	—	9·2%
Oleic acid	—	2	30·4	65·6	37·4	39·1
Linolic acid	100	66	36·1	34·4	62·4	51·7

TABLE IX (*contd.*).

Mol. % of the acids in the fractions on total acids.

	F_1.	F_2.	F_3.	F_4.	F_5.	Mean.
Saturated	—	3·3%	5·86%	—	—	9·16%
Oleic acid	—	0·2	5·27	17·05%	16·6%	39·12
Linolic acid	2·0%	6·5	5·27	9·15	27·8	51·72

These figures agree within the experimental limits with the component fatty acids of the oil determined from the oil direct.

From the above figures the component glycerides of the Niger seed oil have been calculated as given in Table X.

TABLE X.

Glycerides in	F_1.	F_2.	F_3.	F_4.	F_5.	Total in the oil.
	2%	10%	17·4%	26·2%	44·4%	100%
1. Fully saturated glyceride	Nil	Nil	Nil	Nil	Nil	Nil
2. Disaturated glyceride	—	—	—	—	—	—
3. Monosaturated glyceride						
(*a*) Monosaturated dilinolin	—	9·3	1·5	—	—	10·8
(*b*) Monosaturated oleo-linolin	—	0·6	15·9	—	—	16·5
4. Tri-unsaturated glycerides						
(*a*) Dioleo-linolin	—	—	—	25·1	5·4	30·5
(*b*) Oleo-di-linolin	—	—	—	1·1	39·0	40·1
(*c*) Trilinolin	2·0	0·1	—	—	—	2·1

1. By the oxidation of the neutral oil with potassium permanganate in acetone.
2. By the crystallisation of the neutral oil from acetone at 0°
3 and 4. From the component fatty acids of the brominated fractions.

All the saturated acids have been considered as one acid in calculation of the glycerides. As all the saturated acids are combined in the glycerides of Niger seed oil as mono-saturated-diunsaturated glycerides, the assumption that the saturated acids are proportionately divided in mono-saturated-dilinolin and mono-saturated-oleo-linolin glycerides will not be incorrect. From the molecular percentage of the saturated acids in the oil, the component glycerides of Niger seed oil may be given in round figures as—trilinolin (2%), oleodilinolin (40%), dioleo-linolin (30%), myristo-dilinolin (2%), myristo-oleo-linolin (3%), palmito-di-linolin (6%), palmito-oleo-linolin (11%), stearo-

dilinolin (2%) and stearo-oleo-linolin (4%). The myristo-glycerides will contain small quantities of lauric, capric and caprylic acids which are about 2% and the stearo-glycerides will contain small quantity of archidic, behenic and lignoceric acids which will be less than 1%.

Glyceride Composition by the Progressive Hydrogenation of the Oil.— The neutral oil was hydrogenated in presence of nickel catalyst, suspended on kieselguhr by agitation at 140° till it was saturated (Iodine value 1·8). On crystallisation from ether at 0° it gave 75% of tristearin, melting at 71°, and had saponification equivalent of 297·6. Another portion of the oil was hydrogenated in the same way to an iodine value of 27·2. It gave 76% of fully saturated glyceride on successive oxidation with potassium permanganate in acetone. The fully saturated glyceride contained 2·4% of non-saponifiables. On crystallisation from ether the hydrogenated product gave 51·6% of tristearin. According to the observations of Hilditch and Bushell (*J. Chem. Soc.*, 1937, 1767) during the process of hydrogenation, the glycerides having one or more acids containing less than 18 carbon atoms are saturated before the glycerides containing acids with 18 or more than 18 carbon atoms. In accordance with this view the observation of the hydrogenated products of Niger seed oil shows that it must contain about 22·8% of the glycerides having at least one acid with less than 18 carbon atoms in the chain and the rest 77·2% of the glycerides must be only tri-C_{18} glycerides. This observation is in agreement with the glyceride composition obtained by bromination.

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