REFERENCES

1. Burns-Brown, W., J. Soc. Chem. Ind., *55*, 321 (1936).
2. Burns-Brown, W., J. Soc. Chem. Ind., *56*, 116 (1937).
3. Hollingsworth, R. L., and Waling, B. F., Am. Ind. Hyg. Assoc. Quart., *16*, 52 (1955).
4. Kerchow, W. F., Z. Anal. Chem., *108*, 240 (1937).
5. Lubatti, O. F., J. Soc. Chem. Ind., *51*, 361 (1932).
6. Lubatti, O. F., J. Soc. Chem. Ind., *54*, 424 (1935).
7. Lubatti, O. F., J. Soc. Chem. Ind., *63*, 133 (1944).
8. Phillips, C. R., and Kaye, S., Am. J. Hyg., *50*, 270 (1949).
9. Public Law No. 518, 83rd Congress.
10. Rankin, W. B., J. Agr. Food Chem., *4*, 214 (1956).

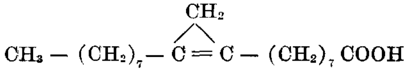
[Received January 22, 1957]

Composition of the Seed Oil of Sterculia foetida, Linn.

J. P. VARMA, SHARDA DASGUPTA, BHOLA NATH, and J. S. AGGARWAL,
National Chemical Laboratory, Poona, India

Sterculia foetida, Linn, which is called Java olives in English and Jangli Badam in Hindi *(N. O. Sterculiaceae),* is a large evergreen tree found usually in the western and southern parts of India, Burma, and Ceylon and occasionally (17, 7) in east tropical Africa, Borneo, Java, Sumatra, Indo-China, Malaya, and North Australia. The seed kernels contain 53–55% of a pale yellow oil which polymerizes rapidly at 240–250°C. and even to some extent at lower temperatures (14).

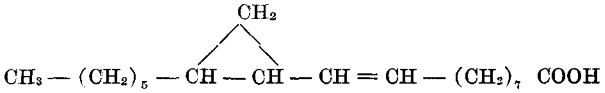
From the oxidation products of the mixed methyl esters of this oil Hilditch, Meara, and Zaky (6) deduced the presence of 70% of a new C_{19}-acid, 12-methyl-9:11 octadecadienoic acid. Later Nunn (9) fractionated the mixed acids with urea and isolated this acid, which he named sterculic acid and assigned the structure (I).



$$CH_3-(CH_2)_7-\overset{CH_2}{\overset{\wedge}{C=C}}-(CH_2)_7COOH$$

(I)

Recently pure sterculic acid has been isolated in this Laboratory from the total fatty acids of the oil by low-temperature, fractional crystallization. On the basis of physical constants, spectroscopic data, and degradative and synthetic studies, it has been given the structure (II) (15, 13, 16).



$$CH_3-(CH_2)_5-\overset{CH_2}{\overset{\wedge}{CH-CH}}-CH=CH-(CH_2)_7\ COOH$$

(II)

The availability of the pure acid prompted further studies on the composition of the oil. In the absence of a suitable method for the estimation of sterculic acid, an infrared technique has been evolved in this Laboratory. The 9.92μ band charcateristic of a cyclopropane ring (2) has been employed for the estimation of sterculic acid.

Extraction and Saponification of the Oil. The seeds of *Sterculia foetida* were obtained from the Government Botanical Gardens, Poona, and decorticated. The kernels were finely crushed and exhaustively extracted with petroleum ether (b.p. 40–60°C.) at 15°C. Oil was recovered from a portion of the extract and found to have the following characteristics:

Specific gravity (40°C.)	0.9239
refractive index (40°C.)	1.4662
acid value	5.7
saponification value	177.5
iodine value (Wijs)	74.0

In order to avoid polymerization of the oil, the remainder of the extract was hydrolyzed by being stirred with an excess of methanolic caustic potash for about 18 hrs. at 25–28°C. The solvent was distilled off under reduced pressure, and the soap was dissolved in water. After removal of the unsaponifiable matter, according to the method of the Society of Public Analysts (11), the total fatty acids were isolated and analyzed.

Estimation of Sterculic Acid. The presence of the 9.92μ absorption band, characteristic of a cyclopropane ring, has been used in these experiments for estimating sterculic acid. The measurements were made with a Grubb Parsons, single-beam, infrared spectrometer with a sodium chloride prism, a fixed slit width of 0.25 mm., and a 0.1-mm. sodium chloride cell. Optical densities at various concentrations of sterculic acid in carbon disulphide solution are given below:

Conc. g/liter	optical density
54	0.10
82	0.15
113	0.21
200	0.37
250	0.46
300	0.56

The "base line" technique (4) was adopted in measuring the absorption peaks. The specific extinction coefficient of sterculic acid was calculated according to the formula

$$A = Ect.$$

where A = optical density; C = Conc. in g. per liter; E = specific extinction coefficient; and t = thickness in cm. The mean specific extinction coefficient comes to be 0.185.

The accuracy of the method was tested by examining known mixtures of sterculic and linoleic acids. The results recorded below show a good agreement with the known weights of sterculic acid in the mixtures.

Added	Found
79.1	79.8
60.3	59.8
50.9	50.5
19.0	18.7

In six replicate analyses the sterculic acid content of sterculia oil was found to be between 71.5–72.0% (average 71.8%) of the total fatty acids.

Estimation of Polyethenoid Acids. When sterculic acid is isomerized according to the method recommended by Hilditch, Morton, and Riley (5) for linoleic acid, the $E^{1\%}_{1\,cm.}$ value at 234 mμ is 66.1.

After the mixed acids of sterculia oil were isomerized with a potassium hydroxide-glycol reagent at

TABLE I

Fractionation of Methyl Esters of Saturated Acids

Fraction No.	1	2	3	4	5	6
Boiling point °C. (3–3.4 mm. of Hg.)	154–158	158–162	162–165	165–168	168–172	Residue
Weight (g.)	1.28	4.95	11.65	2.89	4.29	0.81
Ref. index (27.5°C.)	1.4340	1.4388	1.4390	1.4392	1.4396	1.4499
Iodine value (Wijs)	1.5	1.8	2.0	2.3	3.1	8.5
Sap. value	236.1	224.1	216.7	206.7	202.2	199.2
Mean mol. wt.	237.1	250.3	258.8	271.3	277.3	281.2
Component esters (g.):						
Unsaturated esters	0.02	0.12	0.25	0.07	0.14	0.08
Methyl laurate	0.21	—	—	—	—	—
Methyl myristate	1.05	3.45	4.70	—	—	—
Methyl palmitate	—	1.38	6.70	2.75	3.14	0.28
Methyl stearate	—	—	—	0.07	1.01	0.45

180°C. for 60 min. (5) the $E^{1\%}_{1\,\mathrm{cm.}}$ value at 234 mμ was 64.7. Therefore, allowing for the contribution of sterculic acid under these conditons, the calculated percentage of linoleic acid was 1.9. Spectroscopic examination did not reveal the presence of linolenic acid in the oil. The residual fraction, after most of sterculic acid was removed by low-temperature, fractional crystallization gave a tetra-bromide which melted at 113–114°C. and did not depress the melting point of pure tetrabromostearic acid (m.p. 113.5–114°C.). Therefore it indicates that the dienoic acid is the ordinary 9,12-linoleic acid.

Estimation of saturated acids. Because the presence of a large quantity of sterculic acid in fatty acid mixtures always causes appreciable polymerization, the separation of saturated acids by permanganate oxidation procedure was impractical. The saturated acids were therefore separated with some unsaturated acids by crystallization of the mixed fatty acids in acetone (75.g/liter) at —30°C. This fraction was then subjected to permanganate oxidation by the modified Bertram method (1). The product, consisting chiefly of saturated acids (18.4%), was converted to methyl esters by reaction with methyl alcohol saturated with dry HCl gas. These esters were fractionally distilled through a micro fractionation unit under reduced pressure (3–4 mm. of Hg). The composition of each fraction was calculated from the analytical data recorded in Table I.

The composition of saturated acids therefore is as follow:

Acid	Percentage
Lauric	0.84
Myristic	36.50
Palmitic	57.19
Stearic	5.47

The acids from fractions 1 and 4 were isolated, and these on repeated crystallizations from ethyl acetate gave fairly pure samples of myristic acid (m.p. 53–55°C.) and palmitic acid (m.p. 63–64°C.), respectively.

Component Acids of Sterculia foetida *Oil.* From the above data the percentages of various acids in *Sterculia foetida* oil are as follows although that of oleic acid was found by difference:

Acids	Percentage
Sterculic	71.8
Linoleic	1.9
Oleic	8.2
Lauric	0.1
Myristic	6.6
Palmitic	10.5
Stearic	0.9

Glyceride Composition of the Oil. Sterculia oil was dissolved in acetone solution (1:7) and subjected to low-temperature, fractional crystallization. The characteristics of fractions collected at various temperatures, together with their fatty acid compositions, have been included in Table II.

TABLE II

Characteristics of the Fractions of Sterculia Oil

Fraction No.	1	2	3	4	5
Temp. of crystallization °C.	zero	—30	—40	—60	residue
Weight (g.)	1.6	72.3	63.5	48.6	18.3
Yield %	0.78	35.40	31.01	23.80	9.00
Iodine value (Wijs)	nil	65.1	95.1	93.7	95.9
Sap. value	191.3	199.8	185.0	187.0	187.8
Mean mol. wt.	879.9	842.2	910.0	900.0	896.0
Fatty acid composition %:					
Sterculic acid	—	55.89	94.86	79.91	37.22
Linoleic acid	—	—	—	3.91	11.99
Oleic acid	—	0.90	3.80	9.30	50.80
Saturated acids	100	43.1	1.3	6.9	—

From the above data the glyceride composition of the oil has been computed as follows:

Glyceride	%
GS_3	0.8
GS_2U	
dipalmitin monosterculin	8.9
dimyristin monosterculin	8.9
GSU_2	
monopalmitin disterculin	17.4
monomyristin disterculin	8.8
GU_3	
tristerculin	31.4
monoolein disterculin	11.5
monolinolein disterculin	3.5
diolein monosterculin	7.0
dilinolein monosterculin	1.8

Discussion

The *Sterculia foetida* seed kernel oil has been found to contain 71.8% of an unusual C-19 fatty acid, sterculic acid, which exhibited marked tendency towards polymerization (9, 14). This acid did not show any strong absorption in the ultraviolet region but gave an intense infrared absorption band at 9.92μ, which is characteristic of a cyclopropyl grouping (2). Because no method was known for the identification as well as estimation of the compounds containing a 3-carbon ring system, an infrared method was developed and successfully employed in determining quantitatively the amount of sterculic acid in *Sterculia foetida* oil. The accuracy of this technique was established by the analyses of mixtures containing known amounts of linoleic acid and sterculic acid or some similar synthetically prepared acids (13). Although the infrared technique had been used previously in the determination of iso-oleic acid (12), sterculic acid is possibly the first natural fatty acid to be successfully estimated by this means.

It may also be added that a cyclopropane ring behaves, more or less, like an olefinic double bond toward heat as well as some reagents (8). In Formula II for sterculic acid a cyclopropane ring is shown conjugated with a $C = C$ bond. This type of grouping is known to behave like a typical diene conjugated system (3, 10), and this may explain the striking gelation properties of *Sterculia foetida* oil.

Summary

Sterculia foetida oil has been found to contain 71.8% of sterculic acid and minor proportions of oleic, linoleic, and saturated acids. The saturated component consists mostly of myristic and palmitic acids.

The oil consists of traces of tristearin (0.8%) and a major quantity of tristerculin (31.4%) together with different amounts of the glycerides of the type GS_2U, GSU_2, and GU_3 of other fatty acids.

REFERENCES

1. Bertram, S. H., Z. untersuch. Lebensmit, *55*, 179 (1928).
2. Derfer, J. M., Pickett, E. E., and Boord, C. E., J. Am. Chem. Soc., *71*, 2482 (1949).
3. Gilman, H., "Organic Chemistry," Wiley, New York, p. 36 (1939).
4. Heigl, J. J., Bell, M. F., and White, J. U., Anal. Chem., *19*, 293 (1947).
5. Hilditch, T. P., Morton, R. A., and Riley, J. P., Analyst, *70*, 68 (1945).
6. Hilditch, T. P., Meara, M. L., and Zaky, Y. A. H., J. Soc. Chem. Ind., *60*, 198T (1941).
7. Jamieson, G. S., "Vegetable Fats and Oils," Reinhold Publishing Corp., 165 (1943).
8. Noller, C. R., "Organic Chemistry," Saunders, London, p. 559 (1951).
9. Nunn, J. R., J. Chem. Soc., 313 (1952).
10. Royals, E. E., "Organic Chemistry," Prentice-Hall, New York, pp. 213–219 (1954).
11. S.P.A. Method, Analyst, *58*, 203 (1933); J. Assoc. Official Agr. Chemists, *28*, 282 (1946).
12. Swern, Daniel, Knight, H. B., Shreve, O. D., and Heether, M. R., J. Am. Oil Chemists' Soc., *27*, 17 (1950).
13. Varma, J. P., Dasgupta, Sharda, Nath, Bhola, and Aggarwal, J. S., J. Ind. Chem. Soc., *33*, 111 (1956).
14. Varma, J. P., Nath, Bhola, and Aggarwal, J. S., J. Oil and Oilseeds, *7*, (No. 6), 10 (1954).
15. Varma, J. P., Nath, Bhola, and Aggarwal, J. S., Nature, *175*, 84 (1955).
16. Varma, J. P., Nath, Bhola, and Aggarwal, J. S., J. Chem. Soc., 2550 (1956).
17. Watts, G., "The Economic Products of India," Allen and Company, London (Pt. 3), 362 (1893).

[Received March 12, 1956]

Feed Value and Protein-Quality Determinations on Cottonseed Meals

EDWARD EAGLE and DONALD L. DAVIES, Research Laboratories,
Swift and Company, Chicago, Illinois

THE USE of cottonseed meal in rations for poultry and swine has been limited in the past by toxicological considerations. While most observers have directed their attention to the gossypol content of cottonseed meal as the criterion for its toxicity ever since the work of Withers and Carruth dating back to more than 40 years ago (1–4), several groups of investigators have questioned the analyzed gossypol content as a true indicator of cottonseed toxicity (5–12). Since practically all of these observations had been made on the rat, it was decided to determine if the conclusions reached were likewise applicable when some of the very same cottonseed meals used in our rat studies were appraised in practical feeding experiments on the chick. In addition, a series of nine coded cottonseed meals tested in a Collaborative Study[1] were evaluated for their protein quality in the rat and in the chick. The effect of incorporation of a constant gossypol level supplied by different samples of cottonseed pigment glands or pure gossypol was likewise determined in the rat.

Experimental

In the first six experiments day-old sexed chicks (breeds to be specified later) were fed a commercial broiler ration for a period of 24 to 48 hrs., after which they were weighed, inoculated with modified live virus Newcastle vaccine, wing-banded, separated into groups having similar average starting weights, and placed on test. The chicks were reared for three to four weeks in a back-warmer type, electrically-heated battery brooder and were then housed in wire-floored batteries. The number of chicks on a given diet in the various experiments varied from 11 to 24; the larger groups were divided into two groups at the time of transfer to the batteries so that no more than 12 birds were housed in a single battery section. All of the chick experiments were carried out in air-conditioned rooms. Feed and water were supplied *ad libitum,* and all birds were weighed weekly.

The composition of the basal mixture, which constituted 65% of each ration in the first four chick experiments (practical feeding tests), is given in Table I. The remaining 35% of each ration consisted of either 35% test cottonseed meal or 17.5% test cottonseed meal plus 17.5% control soybean meal; the control ration contained 35% soybean meal.

TABLE I

Composition of Basal Mixture Comprising 65% of Each Chick Ration[a] in Experiments 1, 2, 3, and 4

Ingredient	Percentage in ration
Ground milo	28.0
Ground yellow corn	25.5
Dehydrated alfalfa meal	3.0
Fish meal	2.0
Steamed bone meal	2.0
Dried whey	2.0
Ground oyster shell	1.5
Iodized salt	0.5
Aurofac	0.5
Total	65.0

[a] Each ration contained the following in mg. per kg. of ration: Vitamin A and D feeding oil (2250 I.U. A; 300 I.C. D_3 per gram) 4400, methionine 500, choline chloride 440, manganese sulfate 176, niacin 26, calcium pantothenate 11, riboflavin 4, and menadione 1.

The composition of the semi-synthetic rations used in the protein-quality evaluations in the chick (replicated experiments 5 and 6) is given in Table II. To obviate the picking, coprophagy, and cannibalism which inevitably occur in such an experiment because of the low level of protein fed, all birds within a given group were removed from the battery-brooder at the first sign of any of such complications and were individually caged even prior to the scheduled time of transfer.

In the protein-quality evaluations in the rat (rep-

---

[1] The results obtained in this Collaborative Study were reported by the participants at the Fourth Conference on Cottonseed Processing as Related to Nutritive Value of the Meal, Southern Regional Research Laboratory, U.S.D.A., New Orleans, La., Jan. 14–16, 1957.