

## Chemical Examination of the Seeds of *Caesalpinia Bonducella*, Flem. Part II. Fatty Oil.

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The seeds of *Caesalpinia Bonducella* being used to a considerable extent as a febrifuge, an attempt was made by one of us (M.C.T.K.) to isolate and study the nature of their active principle. In that connection the powdered kernel was extracted with cold alcohol by repeated percolation and the alcoholic extract used for further study. The residual powder when extracted with low-boiling petroleum ether yielded a pale yellow tasteless oil. Since nothing was known about the nature of this oil except some constants (Lewkowitach, "Chemical Technology and Analysis of Oils, Fats and Waxes," Vol. II (6th edition), p. 246 ; Bhaduri, *Proc. Chem. Soc.*, 1912, 28, 53) it was thought interesting to subject this to a detailed study. Very recently when the results of this investigation were being prepared for publication, a paper by Godbole, Paranjpe and Shrikhande (*J. Indian Chem. Soc.*, 1929, 6, 295) appeared dealing with this oil. They found pamic, stearic, oleic and linoleic acids and a crystalline sterol melting at 135°. In view of the fact that some additional compounds have been isolated from the oil we have examined, we thought it necessary to record our results here.

### EXPERIMENTAL.

#### *Physical and Chemical Constants of the Oil.*

Specific gravity	...	...	...	0.9260 at 30°/30°
Refractive index	...	...	...	1.4745 at 29°
Saponification value	...	...	...	197.9
Iodine value (Hanus)	...	...	...	111.0
Acetyl value	...	...	...	35.6
Hobner value	...	...	...	90.9
Unseaponifiable matter	...	...	...	1.1 per cent.
Acid value	...	..	...	8.5

*Chemical Constants of Mixed Fatty Acids.*

Mean molecular weight	...	..	282
Iodine value (Hanus)	...	...	117.0
Saturated acids (Twitchell's method)	..	...	13 per cent.
Unsaturated acids	..	..	87 per cent.

Crude fatty acids from 500 g. of the oil were treated with a slight excess of sodium hydroxide solution. The resulting soap was dried on the water-bath with filter-paper pulp and the dry powder extracted with ethyl ether in a large Soxhlet apparatus. The ether extract was examined for unsaponifiable matter in the manner described later.

*Examination of Fatty Acids.*

400 G. of the total fatty acids obtained from the residual soap were separated into saturated and unsaturated acids by Twitchell's method (*Ind. Eng. Chem.*, 1921, 13, 806). When the lead salts that were first precipitated, were dissolved in hot alcohol to be reprecipitated, a certain amount of a brown sticky material did not dissolve. This was examined separately.

*Unsaturated Acids.*

Methyl esters of the acids, obtained only from the first filtrate were prepared in the usual manner and 47.5 g. of these esters fractionally distilled under a pressure of 6 mm. About 85 per cent. of the distillate consisted of a fraction of iodine value (Hanus) 135.3 and mean molecular weight 291; the remaining was distributed into four fractions of considerably lower molecular weight and iodine value. The latter, however, were too small for further work.

A portion of the major part of the esters was saponified and the alkaline soap solution oxidised with dilute potassium permanganate solution. The crude hydroxy-acids thus obtained were first extracted with petroleum ether and then with ethyl ether in a Soxhlet extractor. The petroleum ether extract was found to contain unoxidised acids and decomposition products.

The ether extract after two crystallisations from alcohol yielded an acid of melting point 129°-130° and molecular weight 316.3. These properties correspond very closely with those of dihydroxy-stearic acid.

The residue after the ether extraction gave after four crystallisations from alcohol an acid of melting point 156°-160° and molecular

weight 948.5. This acid is no doubt a tetrahydroxystearic acid (sativic acid). Although the melting point is lower than that usually assigned to the latter (173°) it has been noted, however, by many investigators (Power and Barrowcliff, *J. Chem. Soc.*, 1903, 87, 899; Power and Moore, *ibid*, 1910, 97, 1103; Power and Salway, *J. Amer. Chem. Soc.*, 1910, 32, 350; Lewkowitsch. "Chemical Technology and Analysis of Oils, Fats and Waxes," Vol. I, (8th edition, pp. 237-238) that acids agreeing in composition and character with tetrahydroxystearic acid possessed melting points varying from 156° to 165° and it is therefore possible that these compounds represent isomeric acids of the formula  $C_{18}H_{36}O_6$ .

It might be mentioned here that no hexahydroxystearic acid was found in the aqueous portion of the oxidation products.

When the acids from another portion of the esters were brominated according to the method of Eibner and Muggenthaler (Lewkowitsch, "Chemical Technology and Analysis of Oils, Fats and Waxes," Vol. I (6th edition), p. 585) no hexabromides were obtained but only a pale yellow product of melting point 113°-114°, bromine content 53.4 per cent. by Stepanow method and therefore corresponding with tetrabromostearic acid was isolated.

Thus from the examination of the oxidation and bromination products, the presence of oleic and linoleic acids in the unsaturated acids has been established.

#### *Saturated Acids.*

Methyl esters of these acids were prepared in the usual manner and 36 g. of the crude esters were fractionally distilled under a pressure of 6 mm.

Fraction.	Boiling point.	Amount obtained.	Mean M.W. of acids from saponification value.
I	Below 165°	0.50 g.	265
II	165°-168°	2.60 "	261
III	168°-172°	13.48 "	263
IV	172°-174°	5.95 "	265
V	174°-177°	3.21 "	270
VI	177°-182°	3.49 "	274
VII	182°-185°	2.80 "	282
VIII	185°-196°	2.77 "	301
Residue and loss.	Above 195°	1.22 "	—
		Total 36.00 "	

The acids were liberated from each of these fractions and crystallised from various solvents to identify individual acids.

*Fraction I.* After three crystallisations from alcohol and acetone a product of m.p. 60°-62° and M.W. 263 was obtained. This seems to be impure palmitic acid.

*Fraction II.* The acids after four crystallisations from alcohol, acetone and ethyl acetate gave a colourless shining compound, m.p. 62°-63° and having a M.W. of 257. These properties correspond with those of palmitic acid. This view was confirmed by a mixed melting point with Kahlbaum's pure palmitic acid.

The filtrates from above yielded a mixture of acids of m.p. 55°-56° and M.W. 271-272.

*Fractions III, IV, and V.* No definite acid could be isolated in spite of the repeated crystallisations from various solvents; but a large amount of a substance of m.p. 55°-56° which remained constant after several crystallisations from various solvents and M.W. 270-272 was obtained in varying amounts.

*Fraction VI.* After six crystallisations from alcohol and ethyl acetate an acid of m.p. 68° and M.W. 285 was obtained. These properties very closely correspond with those of stearic acid. The identity of this acid was confirmed by a mixed melting point with Kahlbaum's recrystallised pure stearic acid.

*Fraction VII.* In this fraction also no definite acid was isolated; but an acid of m.p. 65° and M.W. 299 was obtained. The molecular weight indicates that this may be a mixture of stearic acid and some higher acids.

*Fraction VIII.* The acids after four crystallisations from alcohol and acetone yielded shining crystals of m. p. 78·5° and M. W. 369. These properties indicate that the acid is lignoceric. This view was confirmed by a mixed melting point with pure lignoceric acid kindly supplied by Mr. P. Ramaswami Ayyar.

*Residue.* The brown acid when crystallised three times from alcohol, acetone and ethyl acetate gave a white acid, m. p. 78°-78·5° This was apparently lignoceric acid as its melting point did not show any change when mixed with lignoceric acid of Fraction VIII.

The acid from the brown sticky material from the lead salts of saturated acids insoluble in hot alcohol gave after several crystallisations from alcohol a product melting at 76°-77°. This seemed to be impure lignoceric acid as there was no noticeable change in its melting point when mixed with the lignoceric acid from Fraction VIII.

The m. p. 55°-56° and the M. W. 270-272 of the product from Fractions II, III, IV, and V agree very closely with those of daturic acid ( $C_{17}H_{34}O_2$ ) as well as a mixture of palmitic and stearic acids in equimolecular proportions. In order to have some more information concerning this acid, the following additional tests were carried out with the limited amount of the material at hand.

1. About 0.5 g. of the material was dissolved in boiling alcohol and to this solution was added a boiling alcoholic solution of magnesium acetate containing just enough salt to precipitate only half the amount of the acid. The mixture on cooling deposited the magnesium salt of the acid. The melting point of the acid recovered from the precipitate was 54.5°-55.5° and that of the acid from the filtrate was 55°-56°.

2. No appreciable change was observed in the melting points of the acids from two fractions obtained by partially neutralising a certain amount of the original acid with sodium hydroxide solution and separating the acids subsequently.

3. Zinc salt of the acid was prepared in the usual manner. This had the m. p. 119°-121°. This on further crystallisation from benzene melted at 120°-121°.

4. A synthetic mixture of pure palmitic acid (1.28 g.) with pure stearic acid (1.42 g.) which had the M. W. 269 and m. p. 56°-57° was crystallised from 95 per cent. alcohol. The product obtained had a M. W. of 277 and m. p. 61°-63°. This on being crystallised twice from alcohol gave pure stearic acid of m. p. 68°-69° and M. W. 283. But attempts to isolate pure palmitic acid from the first filtrate were unsuccessful even after more than six crystallisations from alcohol. The melting points and the molecular weights of compounds obtained each time varied only between 55° and 58° and 271 and 274 respectively.

Although the first three tests indicate the homogeneity of the substance, and the existence of fatty acids of odd carbon atoms in vegetable kingdom has now been fairly well established, the great difficulty of separating fatty acids by mere fractional crystallisation specially from a mixture of palmitic and stearic acids of certain composition as shown by the fourth test, renders any definite conclusion with respect to this product very doubtful.

Thus from the examination of the total saturated acids the presence of palmitic, stearic and lignoceric acids has been definitely proved. No attempts are made to calculate the percentage of each

of these acids in the oil, as we do not know definitely the nature of the intermediate fractions we have obtained. Godbole, Paranjpe, and Shrikhande (*loc. cit.*) report the presence of only palmitic and stearic acids in the saturated acids of the oil they have examined. They have definitely isolated and identified only stearic acid; the presence of palmitic acid has been assumed from the molecular weight of a mixture which might have contained this acid. The absence of lignoceric acid is, we think, due to small amounts (6.58 g.) of the total saturated acids they have handled from the original oil and to the presence of an unusually large amount of stearic acid in the acids from the hydrogenated oil, rather than to the different source of the oil they have examined.

#### *Examination of Unsaponifiable Matter.*

When the viscous residue from the ether extract of the dry soaps was dissolved in hot alcohol, an appreciable amount of an oily material separated out. This was removed by filtration and the filtrate on cooling deposited fine colourless leaflets melting at 117°-120°. This product on being recrystallised twice from alcohol gave a product, m. p. 122°-123°. This substance gave the usual colour reactions of phytosterols and an acetate of m. p. 109°-111°.

The filtrate from above was concentrated to a thick syrup which on standing for nearly a week deposited some crystals. These on subsequent crystallisation from alcohol yielded colourless plates, m. p. 132°-133°. This compound also gave the common colour reactions of phytosterols and an acetate of melting point 119°-120°. The melting points of the compound and its derivative indicate its identity with sitosterol.

The oily material which separated out from the hot alcohol solution of the ether extract, solidified on cooling. This, when crystallised thrice from a comparatively large volume of alcohol, gave colourless microscopic needles melting at 58°-59°. The insolubility of this compound in concentrated sulphuric acid even on warming on the water-bath for some time indicates that it is probably a saturated hydrocarbon. The compound was analysed with the following results. (Found: C, 85.5; H, 15.3; M. W. [by Rast's method, *Ber.*, 1922, 55, 1051], 393.  $C_{27}H_{56}$  requires C, 85.3; H, 14.7 per cent. M. W., 380).

These properties correspond very closely with those of heptacosane and this compound no doubt seems to be identical with the similar one isolated by Power and Tutin (*Arch. Pharm.*, 1907, 245, 340) from the leaves and stems of *Lippia Scaberrima*.

*Summary.*

The oil from the kernels of the seeds of *Cassalpinia Bonducella* which were first extracted with alcohol to remove the bitter principle, has been found to contain the glycerides of palmitic, stearic, lignoceric, oleic and linoleic acids; two phytosterols, one melting at 122°-123° and the other identified as sitosterol, and a hydrocarbon of melting point 58°-59° which was proved to be identical with heptacosane.

In the absence of any hydroxy-acids in the oil, the high acetyl value may be accounted for by assuming the presence of some mono- or di-glycerides, which though uncommon in vegetable oils is not impossible.

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