

AN EXAMINATION OF AMOORA ROHITUKA

Part I.—Constituents of the Seed

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Constituents of the seed of the plant *Amoora Rohituka* have been investigated. An essential oil and a sterol have been obtained. The fixed oil after removal of the essential oil consisted of a mixture of saturated and unsaturated acids.

Introduction

The plant has been mentioned in Watt's Dictionary of Economic Products of India¹ as well as in Bengal Plants² and Flora of British India.³ It appears to be synonymous with *Amoora Tikta-raj* and *Andersonia Rohituka*, locally known as *Rohituka* or *Royna*. It grows wild in various parts of East Pakistan, and is a medium sized ever green tree with a large crown of branches, compound leaves are imparipinnate and leaflets oblique. Flowers are sessile, spicate; male spikes are branched, while the females are simple; calyx 5-partite. The fruits are about 1.5" across, subglobose, coriaceous, loculicidally 3-valved capsules. Seeds are with fleshy bright arillus. The outer seed coat is black and glossy. Only some rudimentary work appears to have been done on the seeds and seed contents.^{4,5,6,7} But these appear to be rather incomplete. There is no mention anywhere about the essential oil that has now been detected in it.

A sample quantity of seeds of this plant was obtained through the courtesy of the Conservator of Forests, Eastern Circle in East Pakistan and our preliminary work has been done with that sample.

The oil expressed from the seeds has been in use for a long time in East Bengal for burning purposes. It has also been sometimes used medicinally, as a massage oil in rheumatic pains. The seeds, however, do not seem to have any large scale industrial use at present. To explore the possibility of putting the oil to a more economic use, an investigation was undertaken.

The seeds on being extracted with petroleum ether yielded an oil, about forty eight per cent by weight. It contained an essential oil, which we have named *Amoorin*, that could be removed by distillation in steam. A sterol, has also been isolated after saponifying the oil⁸ as a non-saponifiable constituent, which melts at 135.5°C. and its fuller examination is in progress. The

fixed oil fraction that remained after the removal of the essential oil, has rather a high iodine value. It was expected, therefore, to contain highly unsaturated fatty acids and could have pronounced drying properties. Its examination revealed the fact that it consisted of about a quarter of its weight of saturated acids and three quarters of unsaturated acid mixture. The solid acid appears to be a mixture of stearic and palmitic acids, while the liquid fraction could be separated into oleic, linoleic and linolenic acids. The percentages of the acid constituents in the mixture have been shown in the following. The values are somewhat different from what have been recorded in the literature.⁵

From about 51 g. of the mixed acids obtained from the oil, the quantities of the constituent acids were approximately as follows:—

Palmitic Acid	4.64 g.	9.52%
Stearic Acid	7.4595 "	15.32%
Oleic Acid	8.1428 "	16.72%
Linoleic Acid	25.8681 "	53.11%
Linolenic Acid	2.5940 "	5.32%

Experimental

Constituents of the Seed.—100 g. of the seeds weighed 59.3 g. From these the kernels were separated consisting of 79.5% of the whole seed, while the seed coat consisted of 20.5% by weight. Results of analysis of the kernel are given below. These are very different from what Raoul Lecoq has recorded.⁸

Moisture	6.81%	Proteins	11.08%
Fats and oils	48.69%	Ash	2.74%
Carbohydrates	29.66%	Fibre	1.02%

Extraction of the Oil.—(a) The seed pulp was pressed in a local g'hani, and about 36 per cent of the weight of the seed was obtained as oil. This possessed a very disagreeable odour. When the oil was mixed with petroleum ether a colourless precipitate was formed in small quantity, which settled on standing. This was washed with petroleum ether several times and has been kept for examination.

(b) Second method of isolation of the oil consisted in extraction of the powdered pulp with a solvent. For this 2.5 kg. of the kernel powdered and extracted with petroleum ether at the ordinary temperature by percolation, till practically the whole of the oil came out. The solution was treated with animal charcoal and the solvent was removed by distillation, when a residual oil 1.2 kg. was obtained. This was steam distilled, when the fixed oil was freed from an essential oil, which went over with steam. The fixed oil thus purified was freed from solvent, dried and analysed. It has sp. gr. 0.9187 at 25°C.; $[n]_D^{25}$ 1.4786; saponification value 180.2; iodine value 122.1, and acid value 13.1. The residual pulp is under examination now and results will be communicated in due course.

Essential oil Amoorin.—One part of the crude oil (1.2 kg.), was distilled in a current of steam for 4 hours, when an essential oil (5.1 g.) with a rather characteristic odour came along. This was isolated in the usual way. It was distilled under reduced pressure, when it boiled at 95°–107°/8 mm. On redistillation, most of it came over at 105°C./8 mm. It is a light yellow coloured mobile liquid with a peculiar odour. It has $[d]_4^{31}$, 0.9037 and $[n]_D^{31.5}$, 1.4931. It analysed for $C_{26}H_{42}O$. C, 84.42% and H, 11.32% was found while $C_{26}H_{42}O$ required C, 84.32% and H, 11.35%. Its molecular weight (by Rast method) found 377.2, required 370.6. This oil has been named "amoorin" and work on its constitution is now in progress.

Composition of the Fixed Oil of Royna.—75 g. of the oil was saponified with alcoholic potash; the dry potassium soap was extracted with ether to remove unsaponifiable matter, and then the soap was decomposed with hydrochloric acid. 69.5 g. of mixed fatty acids was thus obtained. Saturated and unsaturated acids in the fatty acid portion were estimated by Twitchell's lead-alcohol method.⁹ Out of this 60 g. was dissolved in 300 cc. of 95% alcohol, and heated to boiling. To this, was added a boiling solution containing 42 g. of lead acetate in 300 cc. of 95% alcohol. The alcohol in both cases contained 1.5% of its weight of glacial acetic acid. The mixture was kept at

room temperature overnight. The precipitate was then filtered and washed with cold 95% alcohol until the washings were free from lead. The precipitated lead salts were then dissolved in the same solvent as above, and the solution was cooled overnight. The undissolved precipitate was removed, washed with the same solvent and decomposed with dilute hydrochloric acid. The ethereal solution containing the solid fatty acids was then washed with distilled water, until the washings no longer acid to methyl orange. The ether was then distilled off, the residue dried and weighed. Lead salt of the liquid fatty acid was recovered from alcoholic solution and treated in the same way. The mixed acid weighed 58.6 g. of which the saturated acid consisted of 14.78 g. or 25.2% and the unsaturated acid amounted to 43.92 g. or 74.8%.

The iodine value of the saturated acids was found to be 4, its molecular weight was 274.99, and it melted at 54.5° to 55.5°C. After several crystallisations from alcohol, the melting point of the saturated fatty acids gradually rose to 64.5°–65.5° when it had m.w. 281.45.

Examination of Unsaturated Acids.—The unsaturated acid was characterised through its bromine additive products as carried out by Eibner and Muggenthaler¹⁰ recommended by Jamieson and Baughman.¹¹ For this purpose 2 g. of the acid was dissolved in 50 cc. of dry ether cooled in ice to 0°C.; bromine was then slowly added to it, and the mixture further cooled and allowed to stand for two hours at about –10°C. At this stage, no precipitate, insoluble in ether, was obtained. This indicated that no appreciable quantity of linolenic acid was present in the mixed unsaturated acids. This would be converted into its hexabromoderivative, which is almost insoluble in ether. The excess of bromine was then removed from the ethereal solution by washing it with an aqueous solution of sodium thiosulphate. It was dried over anhydrous sodium sulphate and the solvent was removed. The residue was then taken up with 100 cc. of petroleum ether (b.p. 60–80°C.) and kept in a cooler overnight. A precipitate of linolenic tetrabromide was obtained which on recrystallisation from xylene melted sharply at 114°C., which is the melting point of tetrabromostearic acid.

*Separation of Fatty acids by the "Urea Adduct Method"*¹² used by Mehta and Dabhade¹³:—50.95 g. of mixed fatty acid was added to a solution of 100 g. of powdered urea in 450 cc. of rectified spirit. The mixture was heated and kept overnight at room temperature. The adduct obtained was filtered. The filtrate was further concentrated

TABLE I.—FRACTIONATION OF MIXED FATTY ACIDS OF ROYNA OIL WITH UREA—STARTING MATERIAL: 50.95 g. MIXED FATTY ACID, 100 g. UREA, 450 cc. ALCOHOL.

Fractions	F ₁	F ₂	F ₃	F ₄	F ₅	F ₆	F ₇	F ₈
Weight of fatty acid in each fraction	6.8745	5.0442	3.7411	3.3055	5.1560	4.5970	3.8821	16.1040
I. V.	6.75	14.29	53.86	104.70	154.61	174.70	184.92	195.00
M. w.	170.01	278.6	283.1	283.				
Saturated C ₁₆ (in g.)	3.53	1.04	0.07					
Saturated C ₁₈ (in g.)	2.8282	3.2022	1.4291					
Oleic	0.5163	0.8020	2.2420	2.7678	1.4952	0.3195		
Linoleic	—	—	—	0.5377	3.6602	4.2725	3.7192	13.6729
Linolenic	—	—	—	—	—	—	0.1629	2.4311

Result:—Palmitic Acid	— 4.64 g. or 9.52%.	Linoleic Acid	— 25.8681 „ „ 53.11%.
Stearic Acid	— 7.4595 „ „ 15.32%.	Linolenic Acid	— 2.5940 „ „ 5.32%.
Oleic Acid	— 8.2428 „ „ 16.72%.	Total	— 48.8044

under reduced pressure and was allowed to crystallise at room temperature. The precipitate obtained was treated similarly. This fractionation technique was repeated a number of times. Each of the fractions obtained was treated with acidulated distilled water and extracted with ether and washed free of mineral acid. The ether extract was dried over anhydrous sodium sulphate and the solvent was removed in the usual way. Each of these fractions was then examined for iodine and neutralization values.

The fatty acid composition of each fraction was calculated from the iodine and neutralization values by assuming the fractions to be binary mixtures. The mean molecular weight of the saturated acid in each fraction could be calculated from the neutralization values. The results are enumerated in Table I.

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