FATTY ACIDS OF NEEM OIL

By C. J. Dasa Rao and T. R. Seshadri

(From the Departments of Chemistry and Chemical Technology, Andhra University, Waltair)

Received November 25, 1941

In studying the chemical composition of Neem oil the substances responsible for the following three important properties demand attention: (1) The oil has an obnoxious odour and this seems to be due to a mixture of volatile compounds some of which contain sulphur. (2) It is highly and disagreeably bitter. In a paper by Murti, Rangaswamy and Seshadri[1] the nature of the bitter principles was discussed and the isolation and properties of two bitter substances described. They are not glycosides and have compositions represented by the empirical formulæ $C_5H_7O_2$ and $C_4H_7O_2$. When tested on earthworms and fish they are not found to possess marked toxic properties. (3) The oil is reputed to be an antiseptic and has been used as an anthelmintic and in the treatment of certain skin diseases. The position is not definite regarding the chemical components to which the oil owes these properties. For this purpose too, the volatile portion is probably the most useful. In view of the fact that in certain medicinal oils, such as the chaulmoogra oil, the fatty acids are responsible for the therapeutic action, the fatty acid composition of the Neem oil has also received considerable attention. In this paper a critical examination of the fatty acid components is made.

The results of a large number of workers who studied the fatty acid composition of the Neem oil are in general agreement and the most important of them are presented in Table I. The chief fatty acids are palmitic, stearic, oleic and linoleic acids. Within limits of experimental error they account for the entire composition of the oil. The claim of Chatterjee and Sen[2] to have obtained a special acid called margosic acid may be mentioned here. It was considered to occur to the extent of 24% of the oil and to be responsible for the medicinal properties. But this was disproved by Watson and co-workers[3] and subsequently by Roy and Dutt[4] and Child and Ramanathan.[5] The so-called margosic acid was shown to be a mixture of other known fatty acids. In spite of all previous work recently Qudrat-i-Khuda, Ghosh and Mukherjee[6] have put forward a claim for having isolated four new acids whose glycerides are said to form the main bulk of the oil. Two

TABLE I

	Roy and Dutt[4]	Child and Ramanathan[5]	Hilditch and Murthi[10]	Khuda and others[6]	Present sample
Sp. gr.	0·9162 at 10·5° C.	0·9159	..	0·9108 at 22·9° C.	0·9129 at 31° C.
Ref. Index	..	1·4620	..	1·46185 at 22·9° C.	1·4658 at 31° C.
Sap. Val.	197·0	201·0	197·0	198·8	195·6
Iod. Val.	74·3	71·5	67·9	69·56	69·2
Unsap.	1·2	0·7	1·7	..	1·9
Acid value	9·5	..	18·0	..	11·2
Sat. Acids					
Lower fatty acids ..	2·3	..	..	..	..
Palmitic	12·62	13·1	14·9	Acids	13·8
Stearic	21·38	18·5	14·4	A and B	18·2
Arachidic	0·74	2·3	1·3		1·8
Unsat. Acids					
Oleic	52·08	47·5	61·9	Acids	52·6
Linoleic	2·12	15·13	7·5	C and D	13·6

of these, A and B are saturated and the other two, C and D unsaturated. Their properties are presented in Table II.

TABLE II

	M.P.	M.Wt.	Carbon %	Hydrogen %	Formulæ
Acid A	67° C. ..	232·4	74·21	12·02	$C_{14}H_{28}O_2$ *iso*-tetradecoic
Acid B	55° ..	256·7	75·3	12·2	$C_{16}H_{32}O_2$ *iso*-palmitic
Acid C	47–48° ..	..	75·2	11·57	$C_{15}H_{28}O_2$ Oleic acid series
Acid D	31–33° ..	..	77·01	11·7	$C_{18}H_{32}O_2$ Cyclic series

Though the methods employed by these authors are not accepted standard ones and the characterisation of the acids is not satisfactory, still in view of the importance of the results and the possibility of explaining the important properties of the oil if the presence of any special acids could be proved, their work has been critically examined and repeated as far as possible. From the physical properties and the chemical contents reported by them (see Table I) it can be inferred that they were dealing with a genuine sample of Neem oil. Since none of the previous workers have detected any abnormal acids and they do not obviously seem to have missed any component and Qudrat-i-Khuda *et al.* find the oil to consist entirely of new acids and do not record the presence of any of the ordinary acids, it

becomes necessary to examine how far the proofs are convincing. C_{16} solid acid (B) according to them melts lower than the C_{14} acid (A) . Acid C seems to be rather unusual having an odd number of carbon atoms. Acid D said to belong to the cyclic series may be expected to exhibit optical activity and such support is lacking. It is to be noted that though C and D are unsaturated they are solids melting at 47–48° C. and 31–33° C. No support is given for the composition of the acids by the determination of Iodine value or neutralisation value. With reference to the claims for the isolation of acids with 14 and 15 carbon atoms may be mentioned the conclusions of Child and Ramanathan that no acid above C_{20} group and below C_{16} group exist in the oil.

With a view to exhaust all possibilities we have secured a genuine sample of Neem oil from the Ramnad District. Its physical and chemical constants (Table I) were quite normal. Following the method of Khuda *et al.* it was subjected to steam distillation and extraction with water and finally saponified. The total fatty acids were separated into two fractions by filtration under suction. Subsequently by crystallising the solid portion from petroleum ether two solid acid fractions were obtained as mentioned by the concerned authors, one melting at 69° and the other at 53° C. But the saponification equivalents were found to be much higher than those recorded for them by those authors and also indicated that they were mixtures. By careful fractionation using barium acetate, fraction A was found to consist of arachidic and stearic acids and fraction B stearic and palmitic acids. The remaining portion (liquid) which the previous workers called oil " X " was esterified and the methyl esters fractionated. From a careful study of the various fractions they were found to consist of mixtures of liquid (oleic and linolic) and solid (palmitic and stearic) fatty acids. None of them gave any solid acids corresponding to the description of acids " C " and " D ". The fractions obtained by us did not exhibit any optical activity. Consequently our experiments do not support the contention of Khuda, Ghosh and Mukherjee. On the other hand, when the same sample of oil was analysed using well-established technique of oil study and effecting the separation of the solid and liquid acids by the lead salt alcohol method, it was found to have the following percentage composition: palmitic 13·8, stearic 18·2, arachidic 1·8, oleic 52·6 and linoleic 13·6. Our results support the conclusions of Hilditch and Murthi[7] that except in palmitic acid content the seed fat of *Azadirachta indica* may be less constant in fatty acid composition than usually obtained in the same species of plants. They agree more closely with those of Child and Ramanathan. This may be due to the fact that the concerned samples were obtained from localities having almost similar climatic conditions.

Experimental

Search for the New Acids.—450 g. of Neem oil were subjected to steam distillation employing a water-bath to heat the flask in order to minimise decomposition. The oil was subsequently extracted with hot water five times and 400 g. of the purified oil were hydrolysed by heating on a gently boiling water-bath with 200 g. of potassium hydroxide and 1000 c.c. of alcohol for 10 hours. The soap was collected and decomposed by treatment with dilute sulphuric acid when a semi-solid mass of the mixture of fatty acids floated on the top. This was separated from the aqueous layer and cooled. After standing for some time the mixture of solid and liquid fatty acids was filtered under suction. The solid residue left on the filter was triturated with petroleum ether and cooled to about 25° and filtered. It was purified by recrystallising from petroleum ether a number of times. It then melted at 69° C. and had a sap. eq. of 287·0. This corresponded to acid "A". The petroleum mother-liquor when concentrated and cooled to 15° C. yielded another fraction which after recrystallisation from the same solvent was found to melt at 53° C. and had a sap. eq. of 264·0. This corresponded to acid "B". The two fractions were separately examined by dissolution in alcohol and fractional precipitation using barium acetate. Each fraction of the barium salt was separately decomposed and the solid acid obtained was examined for its melting point and sap. eq. From these data it was proved that "A" and "B" were mixtures and that they contained arachidic, stearic and palmitic acids (Table III).

TABLE III

Fraction	M.P.	Sap. Eq.	Acids identified
	°C.		
"A" 1	72·5	304·6	Arachidic and stearic
"A" 2	66·5	281·2	Stearic and palmitic
"A" 3	60·0	275·3	Stearic and palmitic
"B" 4	58·0	264·0	Stearic and palmitic
"B" 5	61·5	257·2	Palmitic

The liquid portion obtained by the filtration of the mixed fatty acids and corresponding to oil "X" was esterified with methyl alcohol and sulphuric acid. For this purpose 60 g. of the acids were mixed with 200 c.c. of methyl alcohol and 10 c.c. of concentrated sulphuric acid and heated on a water-bath for seven hours. The alcohol was then distilled off, the esters were taken up in ether, washed free from acid and recovered by distilling off the solvent. The liquid was found to have an I.V. of 76·3 and a mean

M.Wt. of 281·3. It was subjected to distillation at 2 mm. pressure and collected in five fractions. Each fraction was analysed for Iodine and Sap. values and the component fatty acids identified by oxidation of the fraction and separation of the acids obtained. The results are presented in Table IV. It is clear that considerable amounts of saturated acids are present in oil "X".

TABLE IV

Total Weight of Ester Distilled 57·0 *g.*

Temperature of still head	Wt. of Fraction	Sap. Eq.	I. V.	Acids identified
°C.	g.			
135–42	7·2	285·2	67·2	Palmitic and oleic
150–52	13·0	290·3	74·2	Stearic and oleic
152–54	15·0	293·6	81·4	Stearic, oleic and linoleic
154–55	12·0	293·4	98·7	Stearic, oleic and linoleic
Residue	9·8	296·7	72·2	Stearic, oleic and linoleic

Analysis of the Sample of Oil for Fatty Acid Composition.—450 g. of a fresh sample of oil was subjected to steam distillation and extraction with water. The resulting sample was saponified using standard conditions. 400 g of the oil were employed with 250 g. of potassium hydroxide and 2500 c.c. of 95% alcohol and saponified for a duration of six hours. After distilling off the alcohol the soap was dried and extracted with ether to remove the unsaponifiable matter. It was then decomposed with dilute sulphuric acid, the mixed fatty acids were taken up in ether and washed with water. After removing the solvent, 374·0 g. of the mixed fatty acids were obtained. The solid and liquid acids were separated by the lead salt alcohol method using the procedure of Hilditch.[8] Particulars of these fractions obtained from 260 g. of mixed fatty acids are given below.

	Wt.	% by Wt.	Sap. Eq.	I. V.
Solid acids ..	117·4	32·6	276·4	2·8
Liquid acids ..	242·6	67·4	283·1	103·6

100 g. of solid acids and 200 g. of liquid acids were esterified with methyl alcohol and sulphuric acid and the properties of the esters examined.

	Wt.	Sap. Eq.	I. V.
Esters of solid acids ..	98·6	288·9	3·4
Esters of liquid acids ..	196·5	296·3	102·6

The results obtained by the fractional distillation of the esters at 2 mm. pressure and by the analysis of the various fractions are presented in Table V.

TABLE V

Still head temperature		Wt. of fraction in g.	Saponification equivalent	Iodine Value	Acids identified and quantities obtained in g.		
					Palmitic	Oleic	Linoleic
	°C.						
1	140–150	7·3	292·1	83·9	1·1	5·6	0·6
2	150–152	11·4	295·8	99·6	nil	10·2	1·2
3	152	13·2	295·7	101·0	nil	11·6	1·6
4	152–154	13·6	295·6	108·7	nil	10·8	2·8
5	154–158	8·7	295·4	115·1	nil	6·3	2·4
6	Residue	5·8	294·8	144·9	nil	2·3	3·5
	Total weight ..	60·0	..	..	1·1	46·8	12·1
	Calculated on 67·4 g. of liquid acids	67·4	..	..	1·2	52·6	13·6

Still head temperature °C.		Wt. of fraction in g.	Saponification equivalent	Iodine Value	Acids identified and quantities obtained in g.		
					Palmitic	Stearic	Arachidic
1	105–140	4·5	272·7	2·1	4·1	0·4	nil
2	140–142	8·6	277·8	3·4	6·2	2·4	nil
3	145–150	11·3	284·4	4·6	5·5	5·8	nil
4	152	12·8	296·2	3·1	0·8	12·0	nil
5	Residue	5·8	309·7	2·9	nil	3·4	2·4
	Total ..	43·0	..	..	16·6	24·0	2·4
	Calculated for 32·6 g. of solid acids	32·6	..	..	12·6	18·2	1·8

The following is the summary of the data relating to the fatty acid composition of the Neem oil (Table VI).

TABLE VI

	Solid Acids (32·6%) by wt.	Liquid Acids (67·4%) by wt.	Component fatty acids of the oil (% by wt.)
Palmitic	12·6	1·2	13·8
Stearic	18·2	..	18·2
Arachidic	1·8	..	1·8
Oleic	..	52·6	52·6
Linoleic	..	13·6	13·6

Summary

The work of Khuda *et al.* relating to the isolation of new fatty acids from Neem oil has been examined and their experiments repeated as far as possible. No confirmation could be obtained for the discovery of new acids. Examination of the oil by the standard methods corroborates the results of former workers.

REFERENCES

1. Murti, Rangaswamy and Seshadri — *Ind. Jour. of Pharm.*, 1940, **2**, No. 4.
2. Chatterjee and Sen .. *Ind. J. Med. Res.*, 1920–21, **8**, 356.
3. Watson and co-workers .. *J. S. C. I.*, 1923, **42**, 387 T.
4. Roy and Dutt .. *Ibid.*, 1929, **48**, 333 T.
5. Child and Ramanathan .. *Ibid.*, 1936, **55**, 124 T.
6. Khuda and others .. *J. Ind. C. S.*, 1940, **17**, 189.
7. Hilditch and Murthi .. *J. S. C. I.*, 1939, **58**, 310 T.
8. Hilditch .. *The Chemical Constitution of Natural Fats.* 1940 Edn.