JOURNAL OF
THE
INDIAN INSTITUTE OF SCIENCE

Volume 47 OCTOBER 1965 Number 4

THE FATTY OIL FROM ELAEODENDRON PANICULATA SEEDS

BY B. K. MOHAN MURALI, S. K. BALASUBRAMANIAN AND B. H. IYER
(Department of Organic Chemistry, Indian Institute of Science, Bangalore-12)

(Received on May 14, 1965)

ABSTRACT

The composition of the fatty acids of the oil from the seeds of *Elaeodendron paniculata* has been determined by the ester fractionation method and also by the reversed phase circular paper chromatography and shown to contain stearic, palmitic, oleic and linoleic acids. The linoleic acid has also been estimated by the ultra violet absorption spectrophotometry. The unsaponifiable matter has been shown to be squalene.

INTRODUCTION

Elaeodendron paniculata, *E. glaucum* and *E. rhoxburgi* which belong to the natural order *Celastraceae* appear to be practically the same.[1,2] The common names for this plant are Ceylon Tea (English); Bakra, Jamrasi (Hindi); Nirija (Telugu); Seluppai (Tamil); Kannire (Canarese) and Bhutpal (Sanskrit). The plant is found throughout the hotter parts of India.[2] A literature survey indicated that the fatty oil from the seeds has not so far been investigated. As part of a programme on investigation of natural products, a supply of dry fruits of *E. paniculata* was obtained by the kind courtesy of Sri J. K. Gowd, Field Officer for the survey of the non-edible oils, Malleswaram, Bangalore-3 and it was thought interesting to do the chemical analysis of the fatty oil. The composition of the mixed acids from the oil has been determined by the ester fractionation method,[3] after Twitchell's lead salt separation[4] and also by the reversed phase circular paper chromatography.[5] Linoleic acid has been estimated in the total mixed acids by the ultra violet absorption spectrophotometric method.[6]

EXPERIMENTAL

Extraction of the oil: The seed kernels amounting to 14.8% of the fruits when extracted with petrol (b.p. 40-60°) in a glass soxhlet apparatus gave 57% of a light yellow oil on the weight of dry kernels. Large scale extraction was carried out in a soxhlet type apparatus suitably assembled with a 5-litre aspirator bottle.

Physical and chemical characteristics of the oil (Table I): These were determined according to the standard methods.[7]

TABLE I

Characteristics of the oil

No.	Characteristics	Values
1.	Colour	light yellow
2.	Refractive Index	n_D^{25} 1.4795
3.	Specific Gravity	d^{25}0.9430
4.	Acid Value	2.3
5.	Iodine Value (Hanus)	92.2
6.	Saponification Value	243.7
7.	Unsaponifiable Matter	0.7%

Preparation of mixed fatty acids: 100 g. of the oil was saponified by refluxing with alcoholic potash (35 g. in 300 cc. of distilled ethyl alcohol) for 6 hours. Alcohol was removed by distillation; the soap was then dissolved in excess of water and repeatedly extracted with ether to remove the unsaponifiable matter. The aqueous solution was then acidified with dilute sulfuric acid and the liberated free mixed acids were extracted with ether (yield 85 g.)

Twitchell's lead salt separation of mixed fatty acids[4]: — 100 g. of the mixed acids were dissolved in 500 cc. of 95% ethanol containing 5 cc. of glacial acetic acid and the solution was boiled. A boiling solution of lead acetate (70 g) dissolved in 500 cc. of 95% ethanol containing 5 cc. of glacial acetic acid was added to the former solution with stirring. The resulting mixture was boiled well, cooled and allowed to stand at 15°C for 24 hours. The insoluble lead salt was filtered and washed well with alcohol. This was decomposed by boiling with dilute hydrochloric acid and extracted with ether to get the soild acids (yield 24 g.). The liquid acids were obtained in a similar way from the filtrate after distilling off the alcohol (yield 76 g.) The characteristics of the solid and liquid acids are given in the Table II.

TABLE II

No.	Acids	% of Total Acids	Iodine Value	Neutralisation value
1.	Solid	24	4.19	206.40
2.	Liquid	76	122.20	204.00

Methyl esters of solid and liquid acids : – Solid and liquid acids were esterified separately by refluxing the acids with excess of absolute methanol containing 3% concentrated sulfuric acid for 6 hours. The excess of methanol was distilled off and the ester taken up in ether, washed free from acid and dried.

Fractionation of the methyl esters of solid and liquid acids : The methyl esters of solid and liquid acids were fractionated separately under reduced pressure. A long vacuum-jacketed column (14" indentured) and a Perkin receiver were used for efficient fractionation. The saponification and iodine values of all the fractions were determined and the percentage composition of the mixed acids computed from the fractionation data according to the method of Jamieson and Baughman.[8] The fractionation data of the methyl esters of solid and liquid acids are given in the Tables III and IV ;respectively.

TABLE III

Fractionation data of methyl esters of solid acids

Weight of ester distilled – 25 g. ; Saponification Value = 197.8 ;
Iodine Value = 3 46 ; Refractive Index = n_D^{25} 1.4400.

		Fractions				
		S_I	S_{II}	S_{III}	S_{IV}	S_V
Boiling Point (°C/2.5 mm)	.....	Upto 157	157-62	162-64	164-72	Residue
Weight in grams	.....	6.61	6.84	5.00	5.70	0.85
Refractive Index n_D^{25}	.....	1.4380	1.4400	1.4400	1.4480	.....
Saponification Value	.....	203.6	201.6	195.6	194.9	188.9
Iodine Value	.....	1.73	2.05	2.86	5.75	.....

TABLE IV

Fractionation data of methyl esters of liquid acids

Weight of ester distilled – 50 g. Iodine Value = 114.6 :

Saponification Value = 198.5 Refractive index – n_D^{25} 1.4580

	Fractions					
	L_I	L_{II}	L_{III}	L_{IV}	L_V	L_{VI}
Boiling Point °C	Upto 158/ 1.5 mm	158-64/ 1.5mm	164-67/ 2.5 mm	167/ 2.5 mm	167-70/ 2.5 mm	Residue
Weight in grams	3.30	9.30	5.72	15.50	11.61	4 57
Refractive Index n_D^{25}	1.4880	1.4530	1.4530	1.4550	1.4550	.....
Iodine Value	42.60	113.40	122.60	126.0	125.2	90.03
Saponification Value	187.4	187.1	188.7	191.7	190.6	195.6

The percentage composition of the mixed acids as estimated by the ester fractionation method is given in the Table V.

Reversed phase circular paper chromatography of the mixed acids[5] : The quantitative estimation of the fatty acids including the critical pairs was done according to the method of Buchanan[9] suitably modified by Viswanathan and Meera Bai[10]. Whatman No. 3 filter paper impregnated with liquid paraffin (by dipping in a 10% petrol solution of liquid paraffin and drying) has been used for the separation of fatty acids. The amount of mixed acids taken for spotting was about 100 μg. dissolved in absolute ethy alcohol. The chromatogram was developed with 90% aqueous acetic acid[11] for 5 hours and then air dried. The dried filter paper was then dipped in mercuric acetate solution (1 g. in 1 litre of water containing 0.5 cc. of acetic acid) for 15 minutes and washed with running water for 1 hour and dried and sprayed with a 0.2% alcoholic solution of s-diphenyl carbazide when blue areas appeared on the filter paper. The areas were cut separately and the colour complex extracted with 1:1 toluene-methanol mixture. The acids were then estimated colorimetrically in a "Spectronik analyser" at 530 $m\mu$.

In order to separate the critical pairs a second chromatogram was developed with 6:1:1 mixture of acetic acid, formic acid and hydrogen peroxide which analysed only for the saturated acids. The amount of unsaturated acids was then determined by calculation.

The percentage composition of the mixed acids obtained by the paper chromatographic method is given in the Table V and compared with the results obtained from the ester fractionation data.

TABLE V

Composition of the mixed acids of the oil

No.	Acid	Wt. % obtained in the paper chromatographic method	Wt. % obtained from the ester fractionation data
1.	Stearic	12.4	11.2
2.	Palmitic	14.6	15.8
3.	Oleic	41.5	44.2
4.	Linoleic	31.5	28.8

Linoleic acid estimation[6]: 0.1 *g.* of the total mixed acids was weighed in a small pyrex cup of 1 cc. capacity. 11 *g.* of ethylene glycol-caustic potash solution (11% by weight) were taken into a 9″ × 1″ pyrex tube suspended in an oil bath set at 180 ± .5°C. When the temperature of the solution reached 180°C (after 20 minutes of heating) the cup containing the fatty acid mixture was dropped into the tube and swirled gently. An empty pyrex cup was dropped into another reaction tube which serves as a reference blank. Twenty-five minutes after the sample was dropped the reaction tube was removed from the oil bath and rapidly cooled under the tap water and the contents were dissolved with gentle stirring in 20 cc. of spectroscopic ethyl alcohol. The solution was then transferred quantitatively to a 100 cc. volumetric flask and made up with spectroscopic ethanol. Optical density of this solution was measured on a Beckman model D.U. quartz spectrophotometer at a wavelength of 233 $m\mu$. The ethylene glycol-caustic potash blank solution (when the empty cup was dropped) was used in the blank cell. The entire alkali isomerisation of the fatty acid mixture was carried out in an atmosphere of nitrogen.

Linoleic acid content in the mixed acids as estimated by the alkali isomerisation U. V. absorption spectrophotometric method is 33.1%.

Unsaponifiable matter: The oil after saponification was poured into large excess of water and extracted with ether several times. The combined ether extract was washed well with water and dried. Removal of the solvent gave 1.2% of crude unsaponifiable matter. This was chromatographed over neutral alumina (1:30) using petrol (*b.p.* 40 – 60°) as eluant. The infrared spectrum[4] of the chromatographed product was found to be identical with the reported infrared spectrum for squalene[12]. On catalytic hydrogenation it absorbed 6 moles of hydrogen.

Found : C = 88.05%; H = 11.90%; n_D^{25} = 1.4935; Iodine Value = 364.5

$C_{30}H_{50}$ (squalene)[13] requires: C = 87.73%; H = 12.27%; n_D^{25} = 1.4965;
Iodine Value = 370.9

Liebermann-Burchard colour test for sterols was negative.

The differences observed in the analytical values obtained by the ester fractionation, reversed phase paper chromatography and spectroscopic methods are considered as experimental errors.

We thank Professor D. K. Banerjee for his kind interest in the investigation, Professor J. V. Bhat and Dr. S. Sridhara for their help in the chromatographic work, Mr. B. Seetharamia for the elemental microanalysis and Mr. J. K. Gowd for the supply of the seeds.

REFERENCES

1, Kirtikar and Basu .. .. *Indian Medicinal Plants*, 2nd Edition, 1933, Vol. I, 580.

2. Nadkarni .. .. *Indian Materia Medica*, 3rd Edition, Vol. I, 473.

3. Hilditch .. .. *The Chemical Constitution of Natural Fats*, 3rd Edition, 1956, 581.

4. Twitchell .. .. *J. Ind. Eng. Chem.*, 1921, 13, 806.

5. Viswanathan and Meera Bai .. .. *Chromatographic Reviews*, Edited by Micheal Lederer, 1962, 4, 160.

6. Brice and Swain .. .. *J. Am. Oil Chem. Soc.*, 1952, 29, 279.

7. .. .. *Official Methods of Analysis of the Association of official Agricultural Chemists*, 9th Edition, 1960, p. 358 and *Indian Standard Methods of Sampling and Test for Oils and Fats* (Revised) IS : 548 (1964).

8. Jamieson and Baughman .. .. *J. Am. Chem. Soc.*, 1920, 42, 152.

9. Buchanan .. .. *Anal. Chem.*, 1959, 31, 1616.

10. Viswanathan and Meera Bai .. *J. Chrom* , 1961, 6, 264.

11. Balance and Crombie .. .. *Biochem. J.*, 1958, 69, 632.

12. Isler *et al*, ... *Helv. Chim. Acta.*, 1956, 39, 897.

13. .. .. *Merck Index*, 8th Edition, 1960, 974.