

STUDIES ON *ABROMA AUGUSTA* LINN. PART I

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Abroma augusta Linn. (Sterculiaceae), called "olat kambal" in Urdu and "devil's-cotton" in English, grows throughout the hotter parts of India and is mentioned in Chopra's and subsequently in Nadkarni's Indian Materia medica. According to these authors the roots contain an alkaloid in very minute quantity (0.01%) besides a fixed oil, resins and water soluble bases. The leaves and stems are reported to be demulcent, the root bark emmenagogue.

Some time after having started the present investigation the authors came to know that Srivastava and Basu¹ had succeeded in isolating from the roots a hygroscopic water soluble alkaloid which they named "abromine" m.p. 283-85°C. (dec.). Its chlorhydrate showed no rotation in aqueous solution. Based on the analysis of the chlorhydrate (C, 42.56; H, 8.34; N, 8.0; Cl, 24.7) they put up the molecular formula $C_6H_{13}NO_2 \cdot HCl$. Besides this alkaloid, they isolated a phytosterol m.p. 153-57°C., to which they attributed the formula $C_{30}H_{52}O_2$. This sterol too did not have any optical activity in benzene solution. The values found were C, 81.21; H, 11.56. Its acetate melted at 123-128°C.

Because *Abroma augusta* is not easily available in the West Pakistan market, only one batch of 20 kg. could so far be investigated. The results obtained differ from those reported by Srivastava and Basu and are published as a preliminary account.

The coarsely powdered dry roots, the storage time of which could not be determined, were exhaustively extracted with petroleum ether b.p. 60-80°C. and this extract submitted to adsorption analysis on alumina according to Brockmann (E. Merck) with chloroform as eluate, fractions of 30 ml. each being collected. According to the behaviour of their residues, fractions 1-9 were combined into group I, while fractions 10-20 formed group II. A fairly pure, crystalline substance appeared with the fraction 21, and finally the fractions 22-50 were united into group IV. Group I contained as the main substance laths m.p. 247-50°C. (ethyl acetate) showing an $[\alpha]_D^{35.5} = -26^\circ$ in 1% chloroform solution. Found, C, 84.0; H, 11.67; O, 4.26 m.w. (Rast camphor), 376. Digitonin, Liebermann, Burchardt and Salkowsky tests were nega-

tive. The infrared spectrogram furnished a strong band at 1700 cm^{-1} indicating a carbonyl group. According to the analytical findings a formula within the range of $C_{25-30}H_{42-50}O$ had to be taken into consideration in which only the presence of one oxygen atom was certain as indicated by the carbonyl group. In agreement with this, an oxime m.p. 290-294°C. and a 2,4-dinitrophenylhydrazone could be obtained. The comparison of these data with those for the already known sterols, triterpenes, etc. show that the compound is friedelin, which was isolated by Chevreul,² Istrati et al.,³ and Friedel.⁴ In order to clearly identify the substance obtained from *Abroma augusta* with friedelin from cork, one kg. of cork stoppers were extracted according to the method of Ruzicka et al.⁵ and Brunn,⁶ and friedelin, which is more soluble in the common solvents and is present in bigger quantities, was isolated. The friedelin obtained from cork corresponded with that obtained from *Abroma augusta*, as shown in Table I.

TABLE I

	m.p.	$[\alpha]_D$	Shape of crystals
Friedelin (cork)	247-50°C.	- 22°	laths
Friedelin (<i>Abroma augusta</i>)	247-50°C.	- 26°	laths

The identity was finally confirmed by the infrared spectrogram in Fig. 1.

Further investigations are in hand to ascertain whether cerin is also present. Being sparingly soluble in chloroform it may occur in group IV.

Repeated recrystallisation of the combined fractions 20, group II, revealed that it consists of a mixture of several constituents, difficult to separate by crystallisation. Therefore the several parts received according to Table 2 were again submitted to adsorption analysis on alumina with benzene as eluate. At least two sterols could be isolated from the mixture. They may be named abromasterol A and B as long as it cannot be ascertained that they are identical with any known ones.

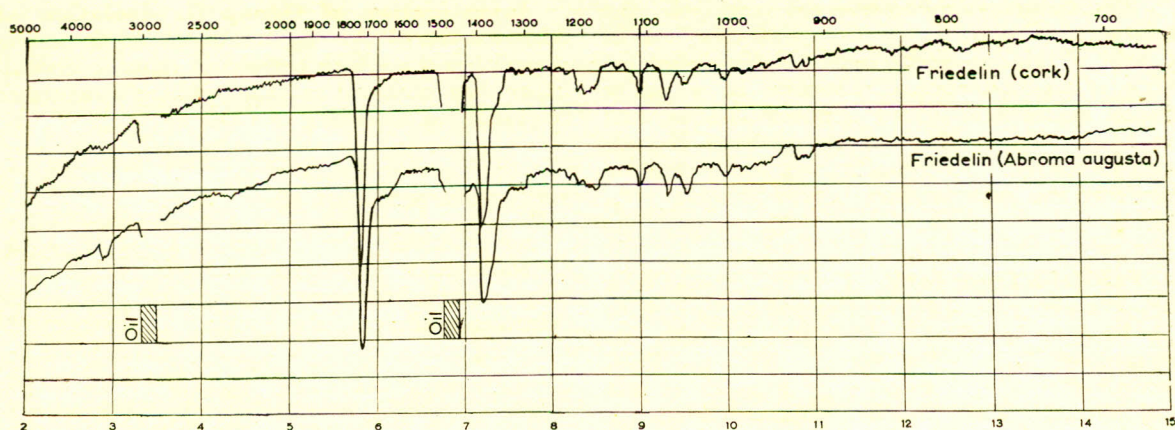


Fig. 1.—Infrared spectrograms.

Sterol A, m.p. 126-128°C. $[\alpha]_D^{35} -26^\circ$ in 1% chloroform solution, [found: C, 82.56; H, 11.98; O, 5.46, m.w. 304 (Rast, camphor)] fits into the range of $C_{19-22}H_{32-38}O$. A strong band at approximately 3500 cm^{-1} in the infrared spectrogram indicated a hydroxyl group. In agreement with this, an acetyl derivative could be prepared which melted at 110-112°C.

Sterol B could be clearly separated from sterol A due to the fact that it remained completely adsorbed on alumina during the elution with benzene, which brought down sterol A. Only after adding ether to the benzene could sterol B be eluted. However it was still mixed with another constituent, from which it could only be separated by high vacuum sublimation. It crystallises in needle shaped aggregates melting at 135-137°C. The benzoyl derivative melted at 138-140°C. Its melt on resolidification showed a play of colours, chiefly blue, which is suggestive of β -sitosterol benzoate

Experimental

Extract of *Abroma Augusta*.—20 kg. of the air-dried roots were shredded, coarsely powdered and exhaustively extracted with petroleum ether (b.p. 60-80°C.) by percolation at room temperature (30°C.). On removal of the solvent, a red oily mass (30.5 g.) remained, which on standing over-night deposited increasing amounts of crystalline material.

Notwithstanding the presence of these crystals, 15.4 g. of that crude mass was dissolved in chloroform (25 ml.) and passed through a column of alumina according to Brockmann (E. Merck) of 45 cm. length, 3.5 cm. breadth containing 470 g. Elution was done with chloroform, fractions

of 30 ml. each were collected and the solvent removed.

After preliminary examination, the fractions were combined in the following groups:

Group I, fractions	1-9	10.8 g.	73% yield
Group II, fractions	10-20	2.9 g.	19% yield
Group III, fractions	21	0.011 g.	—
Group IV, fractions	22-50	0.41 g.	2.7% yield

Investigation of Group I. Isolation of Friedelin.—On adding petroleum ether to the crude oily mass, a crystallisate was obtained (0.82 g.), which on repeated recrystallisation from ethyl acetate gave laths of constant m.p. 247-250°C. They were readily soluble in chloroform, moderately so in benzene, and sparingly soluble in petroleum ether, alcohol, ether and acetone. $[\alpha]_D^{35} = -26^\circ$ in 1% chloroform solution. Digitonin, Liebermann, Burchardt and Salkowsky tests were negative.

Calculated for $C_{30}H_{50}O$: C, 84.44; H, 11.81; O, 3.75; m.w., 426.7. Found: C, 84.00, H, 11.67; O, 4.26; m.w., 376 (by camphor).

Oxime.—To 0.8 g. of hydroxylamine hydrochloride dissolved in 5 ml. of alcohol, 0.7 g. of potassium hydroxide in 5 ml. of alcohol was added and this solution was refluxed together with 200 mg. of the substance of m.p. 247-50°C. for two hours. After cooling it was poured in water and acidified with hydrochloric acid. The substance coming out was filtered by suction, washed and dried. Yield, 140 mg. Recrystallised from ethyl acetate and benzene, m.p. 292°C.

2,4-Dinitrophenylhydrazone.—200 mg. of the substance of m.p. 247-50°C., was dissolved in 5 ml. of glacial acetic acid, the solution of 0.2 g. of 2,4-dinitrophenylhydrazine in 2 ml. of glacial acetic acid added and refluxed for half an hour. On cooling orange coloured crystals were deposited. Recrystallisation from benzene furnished the pure product melting at 296-297°C. (dec.).

Friedelin from cork.—One kilogram of cork stoppers were exhaustively extracted as such with ethyl acetate in a Soxhlet apparatus in order not to damage them. The extract was processed according to the description of Ruzicka et al.⁵ and Brunn.⁶ The finally obtained friedelin, m.p. 247-50°C., showed no difference in appearance and no depression when mixed with the main substance from the first group of *Abroma augusta*. The final confirmation gives Fig. 1 where the infrared spectrograms of both substances are to be seen side by side.

Investigation of Group II. Isolation of Abromasterol A.—By treatment of the crude material (2.9 g.) with petroleum ether, a crystallisate was obtained (1.53 g.), which was further recrystallised according to Table 2.

TABLE 2

1.53 g. from petroleum ether			
↓			
Crystallisate 1	→	mother liquor 1	}
0.946 g. again from petroleum ether			
↓			
Crystallisate 2	→	mother liquor 2	}
0.48 g. again from petroleum ether			
↓			
Crystallisate 3	→	mother liquor 3	
0.018 g., m.p. unsharp 140°C.		0.288 g.	

Combined
1.056 g.

TABLE 3

Fraction	Volume of fraction in ml.	Weight of residue in g.	m.p.°C. Kofler block	Remarks
1	11	0.063	Sublimes at approx. 180.	Needle shaped crystals solubilities similar to friedlin.
2	10	0.026	Partly subl. at 180. Rest melts at 190-200	Needle shaped crystals mixed with hexagonal ones
3	15	0.016	Oil	Yellow
4	10	0.016	Oil	Yellow
5	10	0.043	118-142	Light yellow wax containing clusters of needles
6	10	0.049	117-125	Colourless waxy substance containing clusters of needles.
7	10	0.061	123-127	"
8	10	0.075	123-127	"
9	10	0.074	125-128	"
10	10	0.072	125-128	"
11	10	0.098	126-130	"
12	10	0.086	127-129	"
13	10	0.051	120-130	"
14	15	0.010	118-130	"
		0.074		

The combined residues of mother liquors 1 and 2 (1.056 g.) were dissolved in 50 ml. of alcohol, filtered from an insoluble part, which turned out to be friedelin, and brought to dryness again. Its solution in 10 ml. of benzene was submitted to adsorption analysis again on an alumina column (30 g., 34 cm. length, 0.9 cm. breadth), the elution being done with benzene. The results are given in Table 3.

On repeated recrystallisation of the mixed residues of fractions 10, 11 and 12 (256 mg.) from alcohol, colourless plates (112 mg.) were obtained with the constant m.p. 126.5°C. (Kofler block).

They were readily soluble in chloroform, less so in alcohol, benzene, acetone, ether, ethyl acetate and petroleum ether. On drying at 100°C. over phosphorus pentoxide no loss in weight was observed. $[\alpha]_D^{35} = -26^\circ$ in 1% chloroform solution. It gave positive Liebermann, Burchardt, Salkowsky and digitonin tests.

Calculated for the range $C_{19-22}H_{32-38}O$; C, 82.54-82.95; H, 11.66-12.03; O, 5.79-5.02; m.w. 276.5-318.5. Found: C, 82.56; H, 11.38; O, 5.46, m.w., 304 (camphor).

Abromasterol A Acetate.—75 mg. of sterol A were kept standing for 14 hours at room temperature (30°C.) with acetic anhydride in the presence of a drop of pyridine. Poured in water and kept until the anhydride was hydrolysed, and finally filtered by suction. m.p. 110-112°C. from alcohol

Isolation of Abromasterol B.—Attempts to further purify the residue of mother liquor 3 in Table 2 (0.268 g.) by fractional crystallisation and adsorption analysis on alumina were unsuccessful. It was noted that this fraction was completely adsorbed by alumina from petroleum ether and benzene solutions as distinct from sterol A, which was eluted with benzene. This fraction, which could only be eluted by adding ether to the benzene, was found to consist mainly of two types of crystals: one needle shaped m.p. 135-137°C. (Kofler block), which showed some sublimation, the other plate or leaf type, in much smaller amounts. The whole fraction therefore

was subjected to sublimation in high vacuum (0.01 mm.), when 70 mg. of the needle shaped substance was obtained. As there was a considerable decomposition in spite of the use of high vacuum, the process was not repeated for further purification. Instead, on benzylation of the sublimate by treatment with benzoyl chloride and pyridine at room temperature colourless plates (25 mg.) m.p. 138-140°C. (Kofler block) could be obtained. They were sparingly soluble in alcohol, readily so in chloroform and ethyl acetate. The melt of the substance exhibited, play of colours, chiefly blue, on resolidification which is suggestive of β -sitosterol benzoate.

Group III fraction 21, consisting of 11 mg. showed a fairly sharp melting point at 178-184°C. but could not be purified further due to its small quantity.

The group IV fraction (0.41 g.) is under investigation.

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