CRystalline Chemical Components of the Bark of Wrightia Tinctoria, Br.

By S. Rangaswami, F.A.Sc. and M. Nageswara Rao

(Department of Pharmacy, Andhra University, Waltair)

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Wrightia tinctoria, Br. (fam.: Apocynaceae) is a small deciduous tree growing in several parts of India, particularly Central India, the western portions of the peninsula and isolated regions in the east coast. The bark and the leaves of the tree are reputed to have some medicinal uses. According to a recent report the bark of the tree contains α-amyrin as its acetate. No other information is available on the chemistry of this plant. In the present paper, the results of our examination of this bark which commenced much earlier than the above-mentioned report, are described.

The powdered bark was extracted with petroleum ether, ether, chloroform and alcohol in succession. No crystalline component could be obtained from the last three extracts. The petroleum ether extract contained a lot of rubbery material besides closely related triterpene compounds, so that the isolation of pure ingredients proved to be a matter of considerable difficulty. After elimination of rubbery substances by suitable technique one triterpene compound could be obtained by direct fractional crystallisation. Its properties do not agree with those recorded in the literature for any substance and we consider that it may be a new triterpene. From the mother liquors of this substance another crystalline substance was obtained in small yield by chromatography. This proved to be β-sitosterol.

Though considerable amounts of crystalline material remained in the mother liquors after the separation of the above two substances, no success could be achieved in the isolation of further pure components by resort to fractional crystallisation. Hence a portion of the mixture, representing mother liquor residues, was acetylated and the rest of it benzoylated. The mixed acetates on chromatography yie'ded a pure crystalline substance which could be recognised as β-amyrin acetate since it could be hydrolysed to free β-amyrin which in turn was benzoylated to give β-amyrin benzoate. In this chromatogram a small amount of the acetate of the new triterpene was also obtained.
Fractional crystallisation of the mixed benzoate prepared as mentioned above, yielded a pure crystalline substance which could be identified as lupeol benzoate. It gave lupeol on hydrolysis which was further characterised as lupeol acetate.

The identity of β-amyrin, lupeol and β-sitosterol and their derivatives, have been established by colour reactions, elementary analysis, optical rotation and by mixed melting point with authentic samples.

Thus the bark of Wrightia tinctoria contains the well-known triterpenes β-amyrin and lupeol besides a new triterpene and β-sitosterol. We could not isolate α-amyrin through any of the procedures adopted by us though in the work cited earlier the authors claim to have obtained α-amyrin as acetate.

The simultaneous occurrence of triterpenes and sterols in the same plant material has considerable interest from the point of view of the biogenesis of these groups of compounds. Though the common origin of both groups from isoprene was postulated some three decades ago, the exact pathways by which they are formed are not clearly known. The only laboratory interconversion that has been so far achieved is that of cholesterol into lanosterol (a triterpene). But this evidence touches only the fringe of the problem furnishing as it does only confirmation of certain configurational details. It is now believed that squalene, cholesterol and the triterpenes are all built up from mevalonic acid units. The pathway appears to be common up to the stage of squalene which may then give rise to triterpenes of the β-amyrin, α-amyrin or lupeol variety by passing through one mode of rearrangement and lanosterol through another mode of rearrangement; the last loses three carbons to give rise to cholesterol.

Co-occurrence of triterpenes and sterols in the same source can lend plausibility to the assumption of a common biogenetic origin for them, but the evidence in this direction is very limited. Breiskorn et al. reported the co-occurrence of oleanolic acid, ursolic acid and β-sitosterol in the leaves of Salvia triloba, and of ursolic acid and β-sitosterol in apple leaves, the sterol only in much smaller yield compared to the triterpenes. Other cases of co-occurrence of β-sitosterol and the above-mentioned two triterpenes are met with in Ocimum basilicum, Hysopus officinalis and Satureja hortensis, the sterol again being found only in comparatively minute proportions. Bersin and Müller found that the so-called crataegus acid obtained from Crataegus oxyacantha is a mixture of oleanolic acid, ursolic acid and β-sitosterol. Rangaswami and Rao found a similar co-occurrence of β-sitosterol and the triterpene alcohols α- and β-amyrins with similar quantitative
Crystalline Chemical Components of Bark of Wrightia tinctoria, Br. 117 relationship, in the bark of Plumeria alba.\textsuperscript{10} Evidently the high preponderance of the triterpenes and the very low proportion of sterols has something to do with the metabolic pathways in the plant. The finding recorded in the present paper is therefore a significant addition to the meagre evidence already available, which can eventually throw light on the biogenesis of the sterols and triterpenes.

**Experimental**

2·5 kg. of air-dried powdered stem bark was exhausted by cold percolation with petroleum ether. The percolate (10 l) was concentrated to 1 l and left in the icechest when a colourless crystalline solid (1 g.) m.p. 80–120–170° was deposited. No pure entity could be obtained from this by fractional crystallisation or by chromatography, and its further examination was therefore given up.

The mother liquor on concentration to 250 ml. deposited a very sticky and rubbery material (3 g.). The clear supernatant solution was decanted and evaporated to remove all solvent (residue 40 g.). This was saponified by refluxing on a boiling water-bath with benzene (250 ml.) and N/1. alc. potassium hydroxide (300 ml.) for 15 hours. The solvents were removed under reduced pressure and the residue stirred with a large amount of water when a bulky solid separated. This was filtered, washed with water and dried (unsaponifiable matter, 34 g.). The solid (1·1 g.) which separated on acidifying the alkaline filtrate could not be crystallised from any solvent or solvent mixtures and was discarded.

The unsaponifiable matter was crystallised alternately from petroleum ether and alcohol repeatedly and thus most of the sticky impurity was eliminated. The crystalline material (residue A, 15 g.) was extracted with boiling methanol in which it was only sparingly soluble. The methanol soluble portion after further repeated crystallisations from ethyl alcohol gave a pure crystalline substance (1 g., triterpene X). All the impure fractions of residue A (\textit{i.e.}, exclusive of triterpene X) were united yielding residue A\textsubscript{1}, whose further examination is described below.

The triterpene X crystallised from ethyl alcohol as colourless prisms m.p. 170–72°. With concentrated sulphuric acid it gave a pale yellow colour changing to orange and finally to orange brown. In the Liebermann-Burchard test it gave a brown colour changing to red and then to violet red. With thionyl chloride there was no colour, but with thionyl chloride containing 0·01% antimony trichloride it gave an orange-yellow colour changing to pale pink. Tetranitromethane gave a yellow colour. \([\alpha]_B^\theta = + 37·9°\)
\[ \pm 2^\circ \text{ (} c = 0.796 \text{ in chloroform). Found: } C, 83.8\%; H, 11.2\%; C_{30}H_{50}O \text{ requires } C, 84.5\%; H, 11.8\%. \]

The acetate of the above substance was prepared by the action of pyridine and acetic anhydride for 3 days in the cold. It crystallised from alcohol as colourless nodules and from petroleum ether as feathery needles, m.p. 160-62°. With concentrated sulphuric acid it gave a yellow colour changing to orange. In the Liebermann-Burchard test it gave brown colour changing through pink to purple. With thionyl chloride there was no colour, but with thionyl chloride containing antimony trichloride it gave an orange red colour changing to reddish violet. \[ [\alpha]_D^{19} = +60.2^\circ \pm 2^\circ \text{ (} c = 0.930 \text{ in chloroform). Found: } C, 81.2\%; H, 11.5\%; C_{32}H_{52}O_2 \text{ requires } C, 82.0\%; H, 11.2\%. \]

**Isolation of \( \beta \)-sitosterol.**—A portion of residue \( A_1 \) (4 g.) was chromatographed over alumina (120 g.) using pure petroleum ether and petroleum ether-benzene mixtures for elution. The residues from the first two petroleum ether eluates were resolved into several fractions by fractional crystallisation from alcohol. Fractions of similar m.p. and colour reactions were united and recrystallised from alcohol when colourless shining plates were obtained, m.p. 132-34° (\( \beta \)-sitosterol, 50 mg.). The substance gave the following colour reactions described in the literature for \( \beta \)-sitosterol: in the Sakowski reaction—red in the chloroform layer and yellow in the sulphuric acid layer; in the Liebermann-Burchard test a rose colour which changed to violet, then deep blue and finally deep green. A solution of the substance in 96% alcohol gave the following colours: with concentrated sulphuric acid a yellow orange ring with green fluorescence at the interphase; with a trace of benzaldehyde followed by concentrated sulphuric acid a dark orange ring changing to purple; and with a trace of furfural followed by concentrated sulphuric acid a dark blue ring. \[ [\alpha]_D^{19} = -36.0^\circ \pm 2^\circ \text{ (} c = 1.11 \text{ in chloroform). Found: } C, 82.9\%; H, 12.1\%; C_{32}H_{54}O \text{ requires } C, 84.0\%; H, 12.2\%. \text{ Mixed melting point with } \beta \text{-sitosterol obtained from Plumeria alba}^{10} \text{ was undepressed.} \]

The acetate prepared using pyridine and acetic anhydride in the cold, crystallised from alcohol as colourless fine needles, m.p. 120-24°. The mixed melting point with authentic \( \beta \)-sitosterol acetate\(^{10} \) was undepressed.

**Isolation of \( \beta \)-amyrin through its acetate.**—A portion of the residue \( A_1 \) (2 g.) was acetylated by the action of pyridine and acetic anhydride for 3 days in the cold. The crude acetate mixture (2.2 g.) was chromatographed over alumina (60 g.) using petroleum ether and petroleum ether-benzene mixtures for elution. From the first two petroleum ether eluates
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by fractional crystallisation from alcohol, feathery needles were obtained, m.p. 238–40° (β-amyrin acetate, 0·5 g.). It showed the colour reactions as described in the literature for β-amyrin acetate. \([\alpha]_D^{19} = +75·0^\circ \pm 2^\circ \) (c = 1·00 in chloroform). Found: C, 82·1; H, 11·5%; \(C_{32}H_{52}O_2\) requires C, 82·0; H, 11·2%.

This was deacetylated by boiling with N/2 potassium hydroxide in alcohol: benzene (10: 1). The product crystallised from alcohol as colourless needles, m.p. 188–92°. It showed the colour reactions described in the literature for β-amyrin with concentrated sulphuric acid, with thionyl chloride, with thionyl chloride containing antimony trichloride, with tetranitromethane and in the Liebermann-Burchard test. \([\alpha]_D^{19} = +79·5^\circ \pm 2^\circ \) (c = 1·23 in chloroform). Found: C, 83·9; H, 12·1%; \(C_{30}H_{50}O\) requires C, 84·5; H, 11·8%.

The benzoate was prepared by the action of pyridine and benzoyl chloride at 20° for 15 hours followed by heating at 100° for 2 hours. It crystallised from alcohol as shining plates, m.p. 230–31°. It answered the colour tests described in the literature for β-amyrin benzoate. Found: C, 82·9; H, 10·0%; \(C_{37}H_{54}O_2\) requires C, 83·7; H, 10·3%.

The free amyrin, its acetate and benzoate showed no depression in m.p. when mixed with authentic samples of β-amyrin, its acetate and benzoate obtained from Plumeria alba in this laboratory.\(^{10}\)

The petroleum ether: benzene (49: 1 and 19: 1) eluates in the above-mentioned chromatography of the crude acetate mixture yielded colourless feathery needles from petroleum ether, m.p. 160–62°, undepressed on admixture with the acetate of the triterpene X described earlier.

Isolation of Lupeol through its benzoate.—Residue \(A_1\) was benzoylated by the action of pyridine and benzoyl chloride at 20° for 15 hours followed by heating at 100° for 2 hours. The mixed benzoate was extracted with ethyl alcohol (3×20 ml.). The alcohol insoluble portion when repeatedly crystallised from alcohol-chloroform yielded shining flakes, m.p. 266–70° (lupeol benzoate, 0·5 g.). It answered the colour reactions described in the literature for lupeol benzoate. \([\alpha]_D^{19} = +61·0^\circ \pm 2^\circ \) (c = 1·00 in chloroform). Found: C, 82·7; H, 10·9%; \(C_{37}H_{54}O_2\) requires C, 83·7; H, 10·3%.

This benzoate was debenzoylated by refluxing with N/5 alc. potash and benzene for 2 hours; the resulting product crystallised from alcohol as colourless shining plates, m.p. 206–08° (lupeol). This
showed the following colour reactions described in the literature for lupeol: with concentrated sulphuric acid: brown changing to yellow, then orange-yellow and finally orange; in the Liebermann-Burchard test: violet colour changing to purple; with thionyl chloride containing 0.01% antimony trichloride: orange-red changing to reddish-violet, then deep pink and purple. $[\alpha]_{D}^{28} = +33.2^\circ \pm 2^\circ$ ($c = 0.934$ in chloroform). Found: C, 83.9; H, 11.5%; $C_{30}H_{50}O$ requires C, 84.5; H, 11.8%.

This substance was acetylated using pyridine and acetic anhydride in the cold; the acetate crystallised from alcohol as colourless shining plates, m.p. 198–202° (lupeol acetate). It answered the colour tests described in the literature for lupeol acetate.

The substance, its acetate and benzoate showed no depression in m.p. when mixed with authentic samples of lupeol, lupeol acetate and lupeol benzoate kindly supplied by Prof. Govindachari.

**SUMMARY**

The bark of *Wrightia tinctoria* has been found to contain triterpenes as the major components and $\beta$-sitosterol as a minor component. The former included $\beta$-amyrin, lupeol and another triterpene alcohol which seems to be new.

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**REFERENCES**