

Chemical Examination of *Nyctanthes Arbor-tristis* Linn.

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β -Amyrin, β -sitosterol, hentriacontane, and benzoic acid have been isolated from the leaves of *Nyctanthes arbor-tristis* Linn. in addition to mannitol. The presence of glycosides, free glucose, and fructose has been shown.

Nyctanthes arbor-tristis Linn. (Fam. Oleaceae; Hindi: Harsingar, Parajita) is a large shrub, 15-25 feet high and is cultivated as a garden plant throughout India; it is also found wild in the forests of Madhya Pradesh and the Sub-Himalayan tracts. The leaves are used in the Ayurvedic system of medicine for the treatment of a variety of diseases, such as fever, rheumatism, sciatica, and intestinal worm infections¹⁻². Although the presence of an alkaloidal material, an amorphous glucoside, and a peppermint-like oil had been reported earlier³, Lal and Dutta⁴ did not find any alkaloid, but obtained D-mannitol, an amorphous glucoside, m.p. 71°, and traces of an essential oil. Since the earlier work appeared to be of a rather preliminary nature, it was considered worthwhile to undertake a systematic re-examination of the leaves.

The ethanolic extract of the powdered leaves on concentration provided a crystalline substance, which was identified as D-mannitol. The residual concentrate was divided into a benzene-soluble and a benzene-insoluble fraction. Steam distillation of the former fraction furnished an essential oil, which yielded a crystalline substance, identified as benzoic acid.

The non-steam volatile fraction was saponified and the unsaponifiable material was chromatographed over Brockmann alumina, using light petroleum, benzene, and their mixtures as eluents. The light petroleum eluate yielded a crystalline substance (A), $C_{31}H_{64}$, m.p. 68°, identified as hentriacontane.

The light petroleum-benzene (3:1) eluate provided a substance (B), $C_{30}H_{50}O$, m.p. 198-99°, $[\alpha]_D^{25} + 89^\circ$ (chloroform). It formed a monoacetate, $C_{30}H_{48}O.COCH_3$, m.p. 235°, $[\alpha]_D^{25} + 82^\circ$ (chloroform). Formation of monoacetate indicates the presence of one hydroxyl group in the compound (B). Treatment of (B) with thionyl chloride in presence of tin (Noller reaction) shows the typical sequence of colours (yellow $\rightarrow$ orange $\rightarrow$ purple) for a triterpene. A yellow colour with tetranitromethane indicates presence of an ethylenic double bond. The molecular rotation difference⁵ ($M_D + 4.82^\circ$) and the observation that the substance (B) does not take up hydrogen in presence of Pd-C catalyst show that (B) belongs to the amyirin group of triterpenes. The physical constants of (B) suggest it to be β -amyrin. This has been confirmed by the mixed m.p. with an authentic sample of β -amyrin acetate.

1. Kirtikar and Basu, "Indian Medicinal Plant", vol. II, p. 1527.
2. Chopra, Nayar, and Chopra, "Glossary of Indian Medicinal Plants", C.S.I.R., p. 177.
3. Dymock, Warden, and Hooper, "Pharmacographia Indica", vol. II, p. 378.
4. Bull. Acad. Sci., U.P., 1933, 3, 83.
5. Larton and Jones, J. Chem. Soc., 1944, 659.

The crystalline substance (C), $C_{29}H_{50}O$, from the light petroleum-benzene (1:1) eluate, m.p. $137-8^{\circ}$, $[\alpha]_D^{30} -34.3$ (chloroform), showed positive colour reactions (Burchard-Lieberman and Salkowski) for sterols. It was identified as β -sitosterol.

The benzene-insoluble fraction was dissolved in water and the solution extracted with butanol. The residue from the butanol layer appeared to be glycosidic in nature, whereas glucose and fructose were detected in the aqueous layer by paper chromatography. The isolation of the pure glycosides is in progress.*

EXPERIMENTAL

The leaves of *N. arbor-tristis*, collected locally in December, were air-dried and powdered. The powder (4 kg.) was Soxhleted with hot ethanol. The total extract (10 litres) was concentrated under reduced pressure to a small volume (1 litre) and kept in the cold when a crystalline substance deposited. This was filtered and crystallised from aqueous methanol in needles, m.p. 166° . (Found: C, 39.42; H, 7.31. Calc. for $C_{26}H_{44}O_6$: C, 39.56; H, 7.69%). It did not depress the m.p. of an authentic specimen of D-mannitol.

The filtrate was further concentrated to remove practically all the ethanol and the viscous syrup was extracted with benzene (10×1 litre). The benzene extracts were combined and the solvent removed under reduced pressure. The deep green, viscous residue (230 g.) was distilled with steam, providing a steam-volatile oil and a non-volatile residue.

Benzoic Acid.—The steam-volatile oil was taken up in ether, the ethereal solution dried over sodium sulphate (anhydrous) and the solvent removed. The residual oil (2.1 ml; n_D 1.4555, sp.gr. 0.9134) had a fragrant smell. It was distilled at 8 mm, when a liquid (0.5 ml) came over. The residual solid sublimed on increasing the temperature. It was repeatedly crystallised from hexane when colorless flakes, m.p. $119-20^{\circ}$, were obtained. The substance dissolved in sodium bicarbonate with effervescence and its IR spectrum showed bands at 5.94 and 6.28 μ , indicating presence of carboxylic acid and a phenyl ring, respectively. (Found: C, 69.17; H, 5.02. Calc. for $C_7H_6O_2$: C, 68.85, H, 4.92%). Its m.p. remained undepressed on admixture with an authentic sample of benzoic acid; its identity was further confirmed by the superimposability of their IR. spectra.

An aliquot of the non-volatile residue (20 g.) was saponified with ethanolic KOH (10%) and was worked up in the usual manner for the unsaponifiable material (6 g.). This was chromatographed over alumina, using light petroleum ($40-60^{\circ}$), benzene, and their mixtures as eluents.

Hentriacontane.—The thick reddish oil, obtained as the residue from the light petroleum eluate was repeatedly crystallised from benzene as colorless needles (250 mg.), m.p. 68° . (Found: C, 85.55; H, 14.78. Calc. for $C_{31}H_{64}$: C, 85.33; H, 14.60%). It showed no characteristic UV absorption and its IR spectrum did not indicate presence of any functional groups. It did not absorb bromine. It was found to correspond to the hydrocarbon hentriacontane⁶ by mixed m.p. with an authentic sample and superimposability of their IR spectra.

6. Heilbron *et al.*, *J. Chem. Soc.*, 1934, 1573.

*Two of these glycosides have been isolated and characterised as 3-glucoside and 3-rhamnoglucoide of kaempferol. Details will be published later.

β-Amyrin.—The residue from the light petroleum-benzene (3:1) eluate was repeatedly crystallised from a mixture of chloroform-methanol (1:1) as colorless needles (95 mg.), m.p. 198-99°, $[\alpha]_D^{25} + 89^\circ$ (chloroform). (Found: C, 84.31; H, 11.82. Calc. for $C_{30}H_{50}O$: C, 84.50; H, 11.73%). The acetate, prepared in the usual manner, crystallised from a mixture of benzene and ethanol, m.p. 235°, $[\alpha]_D^{25} + 82^\circ$ (chloroform). (Found: C, 81.85; H, 10.85. Calc. for $C_{30}H_{48}O.COCH_3$: C, 82.05; H, 11.11%). On heating with benzoyl chloride and pyridine at 100° for 2 hours, it formed a benzoate, crystallising from acetone as shining flakes, m.p. 230°, $[\alpha]_D^{25} + 94^\circ$ (chloroform). (Found: C, 83.97; H, 10.82. Calc. for $C_{30}H_{48}O.COC_6H_5$: C, 83.77; H, 10.18%). Identity of the triterpene with *β*-amyrin was confirmed by mixed m.p. determination of its acetate with an authentic sample of *β*-amyrin acetate.

β-Sitosterol.—The crystalline residue from the light petroleum-benzene (1:1) eluate was recrystallised from methanol to provide the substance (C) (250 mg.), m.p. 137-38°, $[\alpha]_D^{30} - 34.3^\circ$ (chloroform). It gave a pink colour, changing to blue and then green in the Burchard-Lieberman test. On treatment with acetic anhydride and pyridine in the usual way it afforded an acetate, crystallising from methanol as plates, m.p. 128-29°, $[\alpha]_D^{30} - 38^\circ$ (chloroform). (Found: C, 81.42; H, 11.62. Calc. for $C_{27}H_{48}O_2$: C, 81.58; H, 11.40%). The benzoyl derivative of *β*-sitosterol was similarly prepared from *β*-sitosterol, benzoyl chloride, and pyridine. It crystallised in colorless flakes from a mixture of methanol and acetone, m.p. 144-45°. (Found: C, 83.42; H, 10.61. Calc. for $C_{36}H_{54}O_2$: C, 83.39; H, 10.42%).

β-Sitosterol acetate and benzoate did not show any change in their m.p. when admixed with their authentic samples.

Isolation of Crude Glycosides.—The benzene-insoluble fraction of the ethanol extractive (412 g.) was dissolved in water and the solution filtered from some insoluble material. The dark brown filtrate was extracted with *n*-butanol (6 × 500 ml); the extract was washed with water (3 × 100 ml) and the solvent removed completely under reduced pressure. The residue was taken up in the minimum amount of methanol and impurities precipitated by dilution with excess ether. The process was repeated several times when a brown powder (48 g.), m.p. 95-140°, was finally obtained. It reduced Fehling's solution after hydrolysis with H_2SO_4 (dil.).

The aqueous solution, after removal of glycosides, was purified and concentrated. The concentrate was subjected to ascending and descending paper chromatography, using *n*-butanol-acetic acid-water (4:1:5) as solvent. When the chromatograms were sprayed with aniline hydrogenphthalate, spots corresponding to glucose and fructose were observed.

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