CHEMICAL EXAMINATION OF THE LEAVES OF *RHODODENDRON CINNABARINUM* HOOK.

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The results of the chemical examination of the leaves of 5 species of Rhododendron have been reported in earlier communications from these laboratories.[1-5] *Rhododendron cinnabarinum* Hook. is a shrub 1·2 to 2·4 metres high, growing in the slopes of the Eastern Himalayas in Sikkim and Bhutan at altitudes of 3,000 to 3,500 metres. These shrubs are known to be highly poisonous to cattle and goats.[6] A toxic principle, andromedotoxin, has been formerly reported to be present.[7] No further report of chemical examination of this plant is available in the literature. The present communication describes the results of our detailed chemical study of this material.

Experimental

The air-dried, coarsely powdered leaf (2 kg.) was successively extracted in the cold with petroleum ether (4 ×3 l.), chloroform (4 ×3 l.) and methanol (4×2·5 l.).

Petroleum ether extract.—The united dark green extract was concentrated to 300 ml. and set aside for a few days when a waxy solid separated. The supernatant solution was decanted off and the solid treated with warm acetone, when most of it went into solution leaving behind a small quantity of colourless powder (Fraction A, 30 mg.). This was filtered. The filtrate was united with the supernatant mentioned above and the solvents were removed. The resulting dark green waxy residue (16·5 g.) was saponified with 10% sodium hydroxide in benzene-alcohol.[2] The unsaponifiable matter (10 g.), isolated in the usual manner, did not yield any crystalline material.

Fraction A, on crystallization from absolute alcohol and benzene, yielded colourless nodules, m.p. 238–42° (15 mg., minor component 1). No colour was obtained in the Liebermann-Burchard test.

Chloroform extract.—The dark green extract was evaporated and the resulting semi-solid residue (15 g.) was extracted with warm acetone (200 ml.), when the wax and colouring matter went into solution. The insoluble material (6 g.) was filtered and washed thoroughly with warm acetone. It was then dissolved in excess of hot alcohol (400 ml.), diluted with an equal volume of water and the resulting suspension cooled and extracted with ether (8 ×100 ml.). The ethereal extract was shaken with 5% sodium hydroxide solution when a precipitate separated at the interphase.

The precipitate was filtered and decomposed with acid.[2] The resulting solid (2·5 g.) answered the colour reactions for ursolic acid; but it did not crystallise well. There was no improvement even after purification through the sodium salt. A portion (0·25 g.) of it was methylated with diazomethane and the residue (0·3 g.) was chromatographed over alumina (15 g.) using petroleum ether and benzene as solvents for elution. The residues obtained from the petroleum ether-benzene (1 : 1) and benzene eluates (total 180 mg.) crystallized from petroleum ether as feathery needles, m.p. 110–12° $[\alpha]_D^{25}$ = + 60·5° (Pyridine). Found: C, 79·5; H, 10·4; $-OCH_3$, 7·1. $C_{31}H_{50}O_3$ requires: C, 79·1; H, 10·7; $-OCH_3$ (1), 6·6%. A portion of this was acetylated; the acetate crystallized from methanol as colourless plates, m.p. 246–48°. $[\alpha]_D^{25}$ = + 63·4° (Chloroform). Found: C, 76·8; H, 10·4. $C_{33}H_{52}O_4$ requires: C, 77·3; H, 10·2%. Mixed m.p. of these samples with authentic methyl ursolate and methyl acetyl ursolate respectively[1] were undepressed.

The ethereal layer, remaining after extraction with alkali, was washed neutral, dried and the solvent removed, when a light green semi-solid residue (2 g.) was obtained. Most of it melted below 100°, but a small portion melted at 210–20°. It was chromatographed over alumina (30 g.) using petroleum ether, benzene and chloroform as solvents. The benzene and benzene-chloroform (19 : 1) eluates yielded residues, which being similar were united and repeatedly crystallized from benzene, when colourless nodules, m.p. 218–21°, were obtained (0·2 g.) (minor component 2). In the Liebermann-Burchard test it gave an orange colour rapidly changing to pink.

The alkaline extract, obtained during alkali separation, gave a greenish precipitate on neutralization with acid. It could not be crystallized from any solvent or solvent mixture.

Methanol extract.—The combined extract was concentrated to about 500 ml., when a sticky precipitate separated. After decanting off the clear supernatant solution, the precipitate was treated with hot acetone when the

waxy and colouring matter went into solution leaving behind a solid. Crystallization of this solid (0·13 g.) twice from alcohol yielded a colourless powder melting at 260–70°. Colour reactions were those of ursolic acid. The methyl ester (diazomethane method) crystallized from petroleum ether as feathery needles, m.p. and mixed m.p. 110–13°.

The aqueous methanol layer, obtained by decantation above, gave a positive reaction for anthoxanthins. It was repeatedly extracted with ether (3 ×500 ml.) and the ethereal extract was shaken with 5% potassium hydroxide solution. The alkaline extract, on neutralization with acid, gave only a dark brown resin (12 g.).

To the aqueous methanol layer left after ether extraction sufficient sulphuric acid was added to give a 7% concentration of the acid and the liquid was boiled under reflux for 2 hours. It was cooled and extracted with ether (3 ×300 ml.). The residue obtained from the ether extract crystallized from alcohol as yellow needles, m.p. 308–12° (decomp.). It answered the colour reactions of quercetin. The acetate crystallized from methanol as colourless needles, m.p. and mixed m.p. 196–98°. Found: C, 58·8; H, 4·2. $C_{25}H_{20}O_{12}$ requires: C, 58·6, H, 3·9%.

Summary

The leaves of *Rhododendron cinnabarinum* Hook. have been examined for their chemical constituents. Ursolic acid and quercetin were isolated and characterized. In addition two minor components, which could not be identified or characterized, have also been obtained.

References

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