CHEMICAL EXAMINATION OF THE LEAVES OF *RHODODENDRON CAMPANULATUM* D.DON.

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PLANTS belonging to the genus *Rhododendron* are considered to be poisonous to cattle.[1] The results of the chemical examination of the leaves of *Rhododendron falconeri* Hook. and the leaves and flowers of *Rhododendron nilagiricum* Zenk. have been reported in earlier communications from these laboratories.[2–4] *Rhododendron campanulatum* D.Don. is an evergreen gregarious shrub and is found in the outer and inner ranges of the Alpine Himalayas from Kashmir to Bhutan at altitudes of 9,000 to 14,000 ft. The leaves of the plant are said to possess several medicinal and poisonous properties.[1, 5, 6] There is only a single reference recorded in the literature to past chemical work on this species.[7] It is stated therein that a specimen of the leaves, obtained from Kashmir, was analysed and a toxic substance was isolated, whose chemical and pharmacological properties resembled those of andromedotoxin. The results of a systematic chemcial examination of the leaves of this plant are described in the present paper.

The dried leaf powder was extracted successively with petroleum ether, ether and methanol. From the petroleum ether extract, by suitable manipulation as described in the experimental section, a new substance designated as "campanulin", epifriedelanol, minor component 1, m.p. 74–76°, and minor component 2, m.p. 168–70°, were obtained. The ether extract, on complete removal of the solvent, gave a dark green residue from which the colouring matter was removed by treating with petroleum ether and acetone. The acetone-insoluble residue, by fractional crystallization and chemical separation, could be separated into ursolic acid and minor components 3 and 4 having melting points 248–52° and 272–74°, respectively. Concentration of the methanol extract deposited greenish solids from which ursolic acid was again obtained. The mother liquor, on acid hydrolysis, gave quercetin.

We consider campanulin to be a new triterpenoid compound. Its chemical constitution will be discussed in a separate communication. The minor component, m.p. 198°, obtained from the leaves of *Rhododendron falconeri* Hook. (*see* p. 246 in ref. 2) has now been found to be identical with campanulin.

The identity of epifriedelanol obtained in the present investigation was deduced from the colour reactions, melting points, mixed melting points, optical rotations and analyses of the parent compound and its benzoate.

Ursolic acid and quercetin were identified through their properties and those of their acetates and by mixed melting points.

EXPERIMENTAL

The material was obtained from Darjeeling, N. India. The coarsely powdered leaf (3 kg.) was extracted with petroleum ether (4 × 12 l.) in the cold, then with ether (4 × 13 l.) in the cold and finally with methanol (3 × 12 l.) under reflux.

Petroleum ether extract. The united dark green extract, on concentration to about 250 ml. and leaving at room temperature for a number of days, deposited a good amount of crystalline material. This was filtered and washed with warm acetone till the washings were only slightly coloured (fraction 1, 4·1 g.). The filtrate was concentrated to a low volume and seeded with fraction 1 obtained above. On keeping aside for a day, a further quantity of crystalline material separated along with some waxy matter. As the solid could not be filtered under suction, the mixture was vigorously shaken with petroleum ether (50 ml.) when the waxy portion went into solution. The residual crystalline portion was filtered and washed with petroleum ether (fraction 2, 4·8 g.). The filtrate, on complete removal of the solvent, gave a dark green sticky residue (residue X, 90 g. *see* later).

Fraction 1. This was found to be a mixture of long prismatic rods and short cubes, which were separated mechanically. Recrystallization of the former (rods) twice from benzene-petroleum ether yielded colourless needles, m.p. 204° (campanulin, 2·1 g.).

When the m.p. of the other crystalline portion (cubes) (1·5 g.) was taken on a Koller block, a trace of the residue melted at 198°, but the major portion melted only at 270–74°. The crystals were dissolved in excess of hot chloroform, filtered and the filtrate was concentrated to a low volume and left aside. A heavy crystalline substance (plates, *see* below) deposited slowly. The turbid supernatant liquid was decanted, heated to get a clear solution, concentrated and set aside, when a small quantity of a crystalline powder separated. This was filtered and crystallized twice from chloroform-acetone when colourless nodules were obtained, m.p. 74–76° (minor component 1, 30 mg.). The mother liquor, on removal of the solvent, gave a residue which was crystallized from benzene-petroleum ether when colourless needles were obtained, m.p. 201–03° (0·3 g.). It was found to be identical with campanulin.

The heavy deposit of plates mentioned above, was crystallized twice from chloroform when glistening colourless plates were obtained, m.p. 281–82°. Further crystallizations from benzene did not improve the m.p. (epifriedelanol, 0·8 g.).

Minor component 1 was insoluble in cold acetone and only sparingly soluble in cold chloroform. No colour was obtained either in the Salkowski or in the Liebermann-Burchard test. Owing to insufficiency of the material it could not be examined further.

Fraction 2.—Crystallization of this fraction twice from benzene-petroleum ether yielded colourless needles, m.p. 200–02° (3·9 g.). This was also found to be identical with campanulin (mixed m.p. and colour reactions).

Campanulin.—The substance was almost insoluble in acetone, methanol and cold glacial acetic acid, sparingly soluble in pyridine, alcohol and hot glacial acetic acid, moderately soluble in benzene and petroleum ether and readily soluble in cold chloroform. In the Salkowski reaction the yellow colour immediately formed gradually changed to orange-yellow. In the Liebermann-Burchard test an immediate deep red colour was obtained which rapidly turned deep pink. In the Tschugaeff reaction, an immediate yellow colour was obtained. On warming the reaction mixture, the colour changed to orange-red and then to blood-red in about three minutes. A deep red colour was obtained when the substance was warmed with trichloroacetic acid; the solution exhibited a bright yellow fluorescence under U.V. light (Hirschsohn reaction[8]). $[\alpha]_D^{29} = +75{\cdot}2° \pm 2°$ ($c = 1{\cdot}085$ in chloroform). [Found: C, 84·8; H, 11·4. $C_{30}H_{50}O$ (probable formula of campanulin) requires: C, 84·5; H, 11·8%.]

Epifriedelanol.—The substance gave a yellow colour in the Salkowski reaction. In the Liebermann-Burchard test a red colour changing to deep pink was obtained. No colour was obtained with tetranitromethane in chloroform. $[\alpha]_D^{29} = +25{\cdot}6° \pm 3°$ ($c = 0{\cdot}978$ in chloroform). [Found: C, 84·2; H, 12·6. $C_{30}H_{52}O$ (epifriedelanol) requires: C, 84·1; H, 12·2%.) Mixed m.p. with an authentic sample of epifriedelanol, isolated from the leaves of *R. nilagiricum* Zenk.,[3] was undepressed.

The benzoate, prepared by heating the substance (0·15 g.) with pyridine (5 ml.) and benzoyl chloride (1 ml.) at 110° for 4 hours and working up in the usual way, crystallized from benzene-alcohol as shining colourless plates, m.p. 250–52°. $[\alpha]_D^{29} = +31{\cdot}3° \pm 4°$ ($c = 0{\cdot}748$ in chloroform). [Found: C, 83·9; H, 10·9. $C_{37}H_{56}O_2$ (epifriedelanol benzoate) requires: C, 83·4; H, 10·6%.] Mixed m.p. with an authentic sample of epifriedelanol benzoate[3] was undepressed.

Residue X (*see* para 2 of experimental).—This was saponified with 1 N alcoholic potash and the unsaponifiable matter was extracted in the usual way. It was dissolved in petroleum ether and set aside for a number of days when a minute quantity of a crystalline residue was obtained which was filtered. Recrystallization twice from petroleum ether yielded colourless needles, m.p. 168–70° (10 mg. minor component 2). This was not examined further.

Ether extract.—The extract, on complete removal of the solvent, gave a dark green syrupy residue (*ca.* 65 g.). It was boiled under reflux with petroleum ether (3×1 l.) and the extracts were filtered. The united dark green extract was concentrated to a low volume (200 ml.) and left in the ice-chest for a number of days. As no crystalline material deposited, it was not examined further.

The petroleum ether-insoluble residue still contained a considerable amount of the colouring matter. It was triturated with acetone (4×500 ml.) when the colouring matter went into solution. The suspension was filtered and the residue washed with acetone till the washings were only slightly coloured. Evaporation of the filtrate yielded a greenish sticky residue (25 g.) which was also not examined further.

The light green acetone-insoluble residue was dissolved in excess of benzene-methanol (1 : 1, 300 ml.) and filtered through a thin bed of charcoal. The clear filtrate, on partial removal of the solvents and allowing to stand, deposited colourless plates, m.p. 270–74° (fraction 3, 12·5 g.).

The mother liquor of fraction 3 was evaporated to a syrupy residue (8·2 g.). This was dissolved in hot benzene-methanol (1 : 1, 200 ml.), cooled and diluted with water (100 ml.) when two layers separated. The lower aqueous methanol layer (suspension) was tapped into another separating funnel, extracted with ether (4×200 ml.) and the ether extracts were united with the benzene phase. The resulting benzene-ether solution (900 ml.) was shaken with 5% sodium hydroxide solution (4×100 ml.) when a precipitate separated at the interphase. This was filtered from the alkaline layer and washed with water. The precipitate, the alkaline filtrate and the solvent layer were worked up separately as described below.

The precipitate was dissolved in methanol (100 ml.) and the solution was acidified with hydrochloric acid (1 : 1) to congo red when a colourless precipitate separated out. The resulting suspension was concentrated to 50 ml. and left in the ice-chest for a day. The solid that separated was filtered and washed with water (fraction 4, m.p. 278–80°, 6 g.).

The alkaline filtrate yielded only a small quantity (0·2 g.) of an amorphous residue on acidification and extraction with ether; it was not examined further.

The benzene-ether solution was washed neutral and dried and the solvents were removed when a waxy residue (0·95 g.) was obtained. This was chromatographed over alumina (30 g.). The waxy solid obtained from the initial petroleum ether eluate was treated with warm benzene when the waxy portion went into solution leaving behind a small quantity of a crystalline residue. Recrystallization from benzene-petroleum ether yielded colourless needles, m.p. 248–52° (10 mg., minor component 3). The residues obtained from the subsequent petroleum ether-benzene (9:1, 4:1 and 1:1) eluates were separately crystallized from benzene-petroleum ether. As the crystalline substances all melted between 268–74°, they were united and recrystallized from benzene-petroleum ether when colourless prisms were obtained, m.p. 272–74° (20 mg., minor component 4). Minor components 3 and 4 both gave a light yellow colour in the Salkowski reaction and a pink colour in the Liebermann-Burchard test.

Fractions 3 *and* 4.—As these two fractions gave identical colour reactions, they were united and crystallized from excess of alcohol when colourless needles were obtained, m.p. 282–83°. $[\alpha]_D^{29} = +66{\cdot}6° \pm 2°$ ($c = 1{\cdot}216$ in absolute alcohol). [Found: C, 78·5; H, 11·1. $C_{30}H_{48}O_3$ (ursolic acid) requires: C, 78·9; H, 10·6.%] In the Liebermann-Burchard test it gave a red colour, changing to violet, then to blue and finally to green. Mixed m.p. with an authentic sample of ursolic acid from *R. falconeri* Hook.[2] was undepressed.

The acetate crystallized from alcohol as colourless needles, m.p. 282–84°. $[\alpha]_D^{29} = +67{\cdot}8° \pm 3°$ ($c = 1{\cdot}127$ in chloroform). [Found: C, 77·5; H, 10·6. $C_{32}H_{50}O_4$ (ursolic acid acetate) requires: C, 77·1; H, 10·1%.] Mixed m.p. with an authentic sample of ursolic acid acetate[2] was undepressed.

Methanol extract.—The united extract (36 l.) was concentrated to about 1·5 l. and left overnight at room temperature when a greenish granular solid deposited (fraction 5, 10 g.) which was filtered. Further concentration of the filtrate to about 700 ml. deposited some more greenish solid (fraction 6, 4·8 g.) and a sticky mass (2 g.) which were collected separately. The final filtrate gave a weak but positive test for anthoxanthins. It was hydrolysed by adding concentrated sulphuric acid to give a 7% strength and refluxing the mixture for 2 hours. The hydrolysate was diluted with water (250 ml.), the alcohol was removed by distillation and the resulting brown suspension was extracted with ether (4×200 ml.). The united ethereal extract was washed

with water till neutral, dried and the solvent removed. Repeated crystallization of the reddish-yellow residue from dilute alcohol yielded fine yellow needles, m.p. 306–10° (decomp.) (0·2 g.). The substance gave a scarlet-red colour with magnesium and hydrochloric acid and an orange precipitate with neutral lead acetate solution. It gave a green colour with alcoholic ferric chloride [Found: C, 59·4; H, 3·7. $C_{15}H_{10}O_7$ (quercetin) requires: C, 59·6; H, 3·3%). The acetate crystallized from alcohol as colourless needles, m.p. 197–98°. [Found: C, 58·4; H, 4·2. $C_{25}H_{20}O_{12}$ (quercetin penta-acetate) requires: C, 58·6; H, 3·9%.] Mixed m.p. with an authentic sample of quercetin penta-acetate[2] was undepressed.

Fractions 5 *and* 6.—These were united and triturated with acetone (100 ml.) when much of it including the colouring matter went into soloution. The undissolved portion was filtered, washed with acetone and crystallized from methanol-benzene and then from alcohol when colourless needles were obtained, m.p. 280–82° (3 g.). It answered the colour reactions of ursolic acid $[\alpha]_D^{29} = +66{\cdot}9° \pm 2°$ ($c = 0{\cdot}942$ in absolute alcohol). The acetate crystallized from alcohol as colourless needles, m.p. 282–84°. $[\alpha]_D^{29} = +64{\cdot}9° \pm 3°$ ($c = 1{\cdot}204$ in chloroform). Mixed melting points of the acid and its acetate with the respective authentic samples were undepressed.

Summary

The chemical examination of the leaves of *Rhododendron campanulatum* D.Don. by extraction with solvents is described. Besides ursolic acid, quercetin and the saturated triterpenoid alcohol epifriedelanol, a new triterpenoid compound, designated as " campanulin ", has been isolated in reasonable yields. A few minor components have also been obtained.

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