CHEMICAL EXAMINATION OF THE LEAVES OF *RHODODENDRON GRANDE* WIGHT

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ABSTRACT

The leaves of *Rhododendron grande* Wight have been examined for their chemical constituents. Campanulin and friedelin were obtained from the petroleum ether extract, ursolic acid from the ether extract and quercetin and a minor component from the alcoholic extract.

PLANTS belonging to the genus *Rhododendron* are considered to be poisonous to cattle.[1] The results of the chemical examination of the leaves of seven species of *Rhododendron* have been reported by Rangaswami and others (*see* ref. 2 and earlier papers on *Rhododendron* species cited therein). *R. grande* is an evergreen tree of 30 ft. height, found in *Sikkim-Himalayas*, at altitudes of 7,000 to 11,000 ft.[3] The leaves are oblong-lanceolate, glabrous and silvery beneath, 9 inches long and 3 inches broad, tapering at the base, with primary nerves prominent and parallel, and with petiole $\frac{3}{4}$–$1\frac{1}{2}$ inches.

A search in the literature revealed that the plant has not been chemically examined so far. The present communication reports the results of our studies on the leaves of this plant.

The powdered leaf was extracted with petroleum ether, ether and alcohol in succession. The petroleum ether extract yielded a crystal mixture which could be separated into two components. One melting at 202–03° was identified as campanulin. The other melting at 256–58° was identified as friedelin.[4–6] The ether extract yielded ursolic acid. The alcoholic extract yielded quercetin and a minor component after hydrolysis with acid. The four principal substances, campanulin, friedelin, ursolic acid and quercetin were identified by their properties and those of some of their derivatives and by mixed melting points with authentic samples.

Campanulin has so far been found in nature only in plants of the genus *Rhododendron*, viz., *R. campanulatum*, *R. decipiens*, *R. barbatum*, *R. westlandii* and *R. falconeri*. Friedelin which has a wider distribution has so far

been found to occur in three *Rhododendron* species, *viz.*, *R. westlandii*, *R. cinnamomeum* and *R. decipiens*.

Experimental

All the melting points were determined on a Kofler block. The alumina used for chromatography was of activity I unless mentioned otherwise. Petroleum ether refers to the fraction with boiling range 60–80°. Paper used for chromatography was Whatmann No. 1 unless otherwise specified. Abbreviations used are: Pet = Petroleum ether; B = Benzene.

The leaves were collected from Darjeeling (West Bengal). The air-dried, coarsely powdered leaves (1 kg.) were extracted exhaustively under reflux with petroleum ether. The solvent-free powder was then extracted with ether and finally with hot alcohol.

Petroleum ether extract.—The combined dark green extract (4 l.) was concentrated to 200 ml. and left overnight at room temperature, when a large quantity of crystalline solid deposited. This was filtered and washed with petroleum ether (fraction A, 3 g.). The filtrate was concentrated, the syrupy residue taken up in hot acetone (100 ml.) and the clear solution was again left overnight at room temperature when some more crystalline solid deposited (fraction B, 1·5 g.). The mother liquor on evaporation left a dark green sticky residue (X, 52 g.).

Fractions A and B which were found to be mixtures were combined and chromatographed over alumina in 1 g. portions, employing 30 g. of alumina and 100 ml. of eluant per fraction. The course of the chromatogram is summarised in Table I.

Table I

Chromatography of crystallisate from petroleum ether extract

Fraction No.	Eluting solvent	Weight of residue in mg.	m.p. of the residue after one crystallisation
1	Pet	280	200–02°
2	Pet-B (19 : 1)	60	200–02°
3	Pet–B (9 : 1)	70	252–54°
4	Pet–B (3 : 1)	210	252–55°
5	Pet–B (1 : 1)	150	252–55°
6	B	20	252–55°

Fractions 1 and 2 crystallised from chloroform-acetone as colourless needles, m.p. 202–03° (crystallisate A, 280 mg.). Combined fractions 3–6 crystallised from chloroform-acetone as colourless needles, m.p. 256–57° (crystallisate B 370 mg.).

The residue X was saponified with 10% sodium hydroxide in benzene-alcohol (1 : 9). The unsaponifiable matter (8 g.) was taken up in hot acetone (100 ml.) and set aside for several days when a crystalline mass deposited. This on further recrystallisation from chloroform-acetone yielded colourless needles, m.p. 201–03° (50 mg., identical with crystallisate A).

Crystallisate A (Campanulin).—Colourless needles from chloroform acetone, m.p. 202–03°. In the Salkowski reaction it gave an yellow colour changing to orange and in the Liebermann-Burchard test a red colour changing rapidly to deep pink. No yellow colour was obtained with tetranitromethane in chloroform. $[\alpha]_D^{35} = +82{\cdot}1°$ ($c = 0{\cdot}99$ in chloroform). (Found: C, 84·8; H, 12·2%. $C_{30}H_{50}O$ requires: C, 84·5; H, 11·8%.) Mixed m.p. with authentic campanulin[7] was undepressed.

Isomerisation with mineral acid.—Crystallisate A (0·5 g.) dissolved in absolute alcohol (100 ml.) was refluxed with concentrated hydrochloric acid (4 ml.) for half-an-hour. The mixture was diluted with water (100 ml.) and the alcohol removed under reduced pressure. The resulting suspension was extracted with ether (4 × 50 ml.). The united ethereal extract was washed with water till neutral, dried over sodium sulphate and the solvent removed. Crystallisation of the residue twice from chloroform-acetone gave shining colourless plates, m.p. 241–43° (0·4 g.). A yellow colour was obtained with tetranitromethane in chloroform. $[\alpha]_D^{35} = -38{\cdot}8°$ ($c = 1{\cdot}44$ in chloroform). Mixed m.p. with campanulol was undepressed.

Oxidation of the isomerisation product.—A solution of the isomerisation product (0·3 g.) in benzene was treated with a slight excess of 2% solution of chromic acid in acetic acid at room temperature for two hours with occasional shaking. After removing the solvent under reduced pressure, the residue was taken up with dilute sulphuric acid and extracted with ether. The ethereal extracts were washed neutral, dried and the solvent was removed. Crystallisation of the residue twice from chloroform-acetone gave shining colourless plates, m.p. 251–53° (0·1 g.). It gave a yellow colour with tetranitromethane in chloroform. A pink colour was obtained in the Zimmermann test. $[\alpha]_D^{35} = -88{\cdot}9°$ ($c = 0{\cdot}90$ in chloroform). The mixed melting point with authentic campanulone was undepressed.

Crystallisate B (*Friedelin*).—Colourless needles from chloroform-acetone, m.p. 256–57°. It gave no colour reaction in Salkowski, and Liebermann-Burchard reactions or with tetranitromethane reagent. $[\alpha]_D^{35} = -27{\cdot}1°$ ($c = 0{\cdot}67$ in chloroform). (Found: C, 84·0; H, 12·3%. $C_{30}H_{50}O$ requires: C, 84·5; H, 11·8%.)

Reduction of crystallisate B.—The ketone (0·3 g.) was taken in methanol-dioxan (3: 1, 100 ml.) and sodium borohydride (0·4 g.) was added to it. There was vigorous reaction. The solution was kept at room temperature for 45 minutes. The solvents were removed after acidifying with dilute hydrochloric acid. The product was extracted with ether and the solvent evaporated; the pale yellow residue was chromatographed over acid-washed alumina (30 g.) employing 50 ml. portions of solvents and solvent mixtures for elution. The course of the chromatogram is given in Table II.

TABLE II

Chromatography of the reduction product of crystallisate B

Fraction No.	Eluting solvent	Weight of residue in mg.	m.p. of the residue after one crystallisation
1	Pet	50	278–81°
2	Pet–B (19: 1)	10	279–81°
3	Pet–B (9: 1)	..	...
4	Pet–B (3: 1)	150	295–299°
5	Pet–B (1: 1)	Traces	..
6	B	..	..

Fractions 1 and 2 above were united and recrystallised twice from chloroform when shining plates were obtained, m.p. 280–82° (35 mg.). Mixed m.p. with an authentic sample of epifriedelanol from *Rhododendron campanulatum*[7] was undepressed.

Fraction 4 crystallised from chloroform-acetone as shining plates, m.p. 298–301° (125 mg.). $[\alpha]_D^{35} = +21{\cdot}9°$ ($c = 0{\cdot}37$ in chloroform). The acetate prepared by the action of pyridine and acetic anhydride at 100° for 4 hours, and purified by passing through a small column of alumina

crystallised from benzene-acetone as colourless needles, m.p. 307–09°. $[\alpha]_D^{35} = +23{\cdot}1°$ ($c = 1{\cdot}30$ in chloroform). (Found: C, 81·1; H, 11·5%. $C_{32}H_{54}O_2$ (friedelanol acetate) requires: C, 81·6; H, 11·6%.) The mixed m.p. of the substance of m.p. 298–301° with an authentic sample of freidelanol obtained from *Rhododendron cinnamomeum*[5] was undepressed.

Ether extract.—The dark green extract was concentrated to 150 ml. when a large amount of pale green solid deposited. It was filtered and washed with acetone (3 g.) and the filtrate further concentrated and left in the ice-chest for a number of days. As no more solid material deposited from it, it was not examined further.

The above solid was dissolved in excess of hot methanol (200 ml.), diluted with an equal volume of water and the resulting suspension cooled and extracted with ether (5 × 50 ml.). The ethereal extract was shaken with 5% sodium hydroxide when a precipitate separated at the interphase.

The ethereal layer was washed, dried and the solvent removed, when a green sticky residue was left behind, from which no crystalline substance could be obtained. The lower alkaline layer gave a greenish precipitate on neutralisation with acid. It could not be crystallised from any solvent or solvent mixtures.

The precipitate formed at the interphase was filtered, dissolved in methanol and decomposed with mineral acid. The resulting solid (1·3 g.) answered the colour reactions for ursolic acid; but it did not cyrstallise well. There was no improvement even after a second purification through the sodium salt. A portion (0·3 g.) of the acid was suspended in methanol and treated with ethereal diazomethane and the product (0·32 g.) was chromatographed over alumina. The residues obtained from petroleum ether-benzene (1 : 1) and benzene eluates (total 200 mg.) crystallised from petroleum ether as feathery needles, m.p. 114–17° (methyl ursolate). $[\alpha]_D^{35} = +71{\cdot}4°$ ($c = 0{\cdot}62$ in chloroform (Found: C, 78·6; H, 10·5; $-OCH_3$ 6·2%. $C_{31}H_{50}O_3$ requires: C, 79·1; H, 10·7; $-OCH_3$ (1), 6·6%.) A portion of this ester was acetylated; the acetate crystallised from alcohol as colourless rectangular rods, m.p. 247–48°. $[\alpha]_D^{35} = +57{\cdot}9°$ ($c = 0{\cdot}93$ in chloroform). (Found: C, 76·9; H, 9·9%. $C_{33}H_{52}O_4$ (methyl acetyl ursolate) requires: C, 77·3; H, 10·2%.) Mixed m.p. of these samples with authentic methyl ursolate and methyl acetylursolate respectively were undepressed.

Alcohol extract.—The alcoholic extract was concentrated under reduced pressure to a low volume (200 ml.) with intermittent additions of water. The greenish mass sticking to the sides of the vessel was rejected. The decanted supernatant solution was heated to boiling and progressively diluted with water till no more resin separated. To the filtered clear red solution sulphuric aicd was added to give a 7% concentration of the acid and the mixture was refluxed for 2 hours. A large amount of brown resin slowly separated out. The suspension was cooled and extracted with ethyl acetate (4 × 100 ml.). The extract was washed, dried over sodium sulphate, concentrated to 10 ml. and diluted with dry benzene (15 ml.). The mixture was warmed to get a clear solution and left in ice-chest for a number of days when a pale yellow crystalline solid was obtained. This was filtered and washed with dry benzene (minor component, 100 mg.). The filtrate was concentrated when a yellow precipitate was obtained. This on recrystallisation from alcohol gave a yellow compound, m.p. 308–12° (decomp., 170 mg.). Colour reactions were as described in the literature for quercetin. The U.V. absorption taken in ethanol and the shifts with specific reagents were as below: Compound in ethanol: 256, 368–70; with sodium ethoxide: 325; with sodium acetate: 274; with sodium acetate and boric acid: 386–88; with aluminium chloride: 269, 425 mμ. Comparison with authentic quercetin on a descending paper chromatogram using butanol-acetic acid-water (4: 1: 5) system and including a mixed chromatogram confirmed its identity as quercetin and also the mixed melting point was undepressed.

Minor component.—This compound obtained from the alcohol extract as described earlier, crystallised from ethyl acetate-benzene as pale yellow plates, m.p. 204–10° (decomp.). It was neutral in character and did not give flavonoid or triterpenoid colour reactions. It was optically inactive and did not contain any methoxyl groups. (Found: C, 65·8; H, 5·7%.) The U.V. spectrum of the compound taken in ethanol and the shifts with reagents are as below:

Compound: $\lambda_{max.}$ 268 and 317·5; $\lambda_{min.}$ 243·5 and 296 mμ.

Compound with $AlCl_3$: $\lambda_{max.}$ 275 and 325; $\lambda_{min.}$ 245 and 300 mμ.

Compound with NaOAc: $\lambda_{max.}$ 266 and 312·5; $\lambda_{min.}$ 243·5 and 291 mμ.

Compound with NaOAc/Boric acid: $\lambda_{max.}$ 266 and 312·5; $\lambda_{min.}$ 243·5 and 291 mμ.

Main I.R. maxima (Nujol): 3472 (m), 1639 (S), 1595 (S), 1319 (S), 1258 (m), 1212 (s), 1175 (m), 1160 (m)., 1085 (m), 1031–1010 broad (w), 991 (s), 971 (w) 885 (m), 809 (s), 806 (w), 675 (m) and 651 (m) $cm.^{-1}$

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