

CRYSTALLINE CHEMICAL COMPONENTS OF THE FLOWERS OF *RHODODENDRON* *NILAGIRICUM* ZENK.

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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 nilagiricum* Zenk. (Syn. *R. arboreum* Sm. var. *nilagirica* Cl.) have been communicated recently from these laboratories.² *Rhododendron* flowers were found to possess insecticidal activity.³ According to Madden⁴ the flowers of *R. arboreum* Sm. are eaten by the local people in some parts of the Himalayas and they become intoxicated if they consume them in large quantities. There is no reference in the published literature to past chemical work on the flowers of *R. nilagiricum*. The results of the chemical examination of the flowers of this plant are described in the present paper.

The air-dried flowers were extracted with petroleum ether, ether and methanol in succession. The petroleum-ether extract yielded, besides a large amount of wax, a minute quantity of a crystalline substance. The ether extract yielded quercitrin and ursolic acid as the crystalline components. From the methanolic extract, besides a large amount of resinous material, a small quantity of quercetin could be obtained after hydrolysis with mineral acid; the presence of glucose in the aqueous portion after hydrolysis was shown by the preparation of its osazone and paper chromatography.

Quercitrin is quercetin-3-rhamnoside. Although its occurrence was reported in a wide variety of plants, its isolation has not so far been reported from the genus *Rhododendron*. The isolation of quercitrin from "lemon flavine" obtained from the bark of the black oak in high yield has been described by Booth and DeEds.⁵

The identity of the glycoside obtained in the present investigation as quercitrin has been established by its m.p., analysis, hydrolysis to quercetin and rhamnose and the hydrolysis of the methyl ether to 5:7:3':4'-O-tetramethyl quercetin (m.p. and mixed m.p. with an authentic sample).

Quercetin was characterized as its acetate (m.p. and mixed m.p.). The nature of the sugar in the original glycoside was established by descending paper chromatography.

Ursolic acid was identified through its physical properties and preparation of its acetate (m.p. and mixed m.p. with an authentic sample).

EXPERIMENTAL

The material used in this investigation was obtained from Ootacamund, Nilgiri Hills in S. India.

The air-dried flowers (500 g.) were extracted with petroleum ether (2×6 l.) in the cold, then with solvent ether (3×4 l.) in the cold and finally with methanol (3×4 l.) by boiling under reflux.

Petroleum ether extract.—On complete removal of the solvent a greenish syrupy residue (6 g.) was obtained which on keeping for two months deposited a small quantity of a crystalline material along with some waxy matter. On warming the residue, the waxy matter melted leaving behind the crystals. The melt was decanted off and the crystals washed with warm petroleum ether. Crystallization from chloroform-benzene gave colourless plates, m.p. 278–80° (Yield: *ca.* 10 mg.). In the Liebermann-Burchard test it gave a red colour slowly changing to pink. Dearth of material prevented further examination of this substance.

Ether extract.—The yellow residue (*ca.* 4 g.) obtained on complete removal of the solvent turned partly crystalline when left for a week. Ether (50 ml.) was added and thoroughly shaken when a portion of the residue went into solution. The ethereal layer was decanted (solution X, *see later*) and the remaining yellow granular powder was again treated with ether (25 ml.). The ethereal layer was decanted and united with the first portion.

The yellow granular residue (1.7 g.) answered the colour reactions for anthoxanthins. Crystallization from dilute alcohol, ethyl acetate-methanol, alcohol-chloroform and dilute alcohol in succession gave yellow needles, m.p. 180–82° (quercitrin) (yield: 0.8 g.) The different melting points reported in the literature for quercitrin vary from 168°⁶ to 182–85°⁷ [Found on sample dried under high vacuum at 110° for 6 hrs. over phosphorus pentoxide and weighed in a closed pig: C, 56.0; H, 4.3. C₂₁H₂₀O₁₁ (quercitrin) requires: C, 56.2; H, 4.5%]. An alcoholic solution gave a deep pink colour with magnesium and hydrochloric acid. A deep green colour was obtained with neutral alcoholic ferric chloride. Molisch's test was positive. A yellow precipitate was obtained with neutral lead acetate

solution. In the test with zirconium oxychloride and citric acid, as described by Hörhammer and Hänsel,⁸ a colourless solution was obtained at the end, indicating the absence of a free hydroxyl group in the 3-position.

Methylation of the glycoside.—This was carried out by repeatedly treating an ice-cold absolute alcohol solution of the glycoside with ice-cold ethereal diazomethane until the ferric reaction was negative. Crystallization from absolute alcohol-ether gave colourless needles, m.p. 180–81° [Found on sample dried under high vacuum at 110° for 6 hrs. over phosphorus pentoxide and weighed in a closed pig: C, 60.1; H, 6.2; —OCH₃, 23.9. C₂₅H₂₈O₁₁ (tetramethylether of quercitrin) requires: C, 59.5; H, 5.6; —OCH₃ (4), 24.6%].

Hydrolysis of the methylated glycoside.—The above methylated glycoside (40 mg.) was hydrolysed by boiling under reflux with 2% sulphuric acid in 50% alcohol for 1 hr. The reaction mixture was worked up in the usual way. On crystallizing the residue from alcohol yellow needles, m.p. 190–91° were obtained [Found: C, 63.8; H, 5.6. C₁₉H₁₈O₇ (quercetin tetramethyl ether) requires: C, 63.7; H, 5.1%]. Mixed m.p. with an authentic sample of 5:7:3':4'-O-tetramethyl quercetin, obtained from hyperoside² was undepressed.

Hydrolysis of the glycoside.—This was carried out by heating the glycoside (85 mg.) with 2% aqueous sulphuric acid (15 ml.) in a boiling water-bath for 1 hr. The resulting suspension was cooled in ice, the crystals that separated were filtered, washed and crystallized from dilute acetone when yellow needles, m.p. 318–20°, were obtained [Found: C, 60.6; H, 3.8. C₁₅H₁₀O₇ (quercetin) requires: C, 59.6; H, 3.3%]. It answered the colour reactions described for quercetin.

Identification of the aglycone by descending paper chromatography.—Whatman No. 1 filter-paper was used. Butanol-acetic acid-water (4:1:5 by volume) was employed as the developing solvent. The chromatograms were run for 18 hrs. Comparison was made with quercetin obtained from the leaves of *Rhododendron falconeri* Hook.⁹ Mixed chromatograms were also run. The spots were located by observing under ultraviolet light when they were seen as bright yellow spots with green fluorescence. The aglycone under question and the authentic sample moved through the same distance in individual chromatograms. No separation was observed in mixed chromatograms.

The acetate of the aglycone (prepared using acetic anhydride and sodium acetate) crystallized from alcohol as colourless needles, m.p. 197–98°

[Found: C, 59.1; H, 4.4. $C_{25}H_{20}O_{12}$ (quercetin penta-acetate) requires: C, 58.6; H, 3.9%]. Mixed m.p. with quercetin penta-acetate from the leaves of *Rhododendron falconeri* (*loc. cit.*) was undepressed.

Identification of the sugar.—The acidic filtrate obtained after removing the aglycone from the hydrolysis of the original glycoside was neutralized with freshly precipitated barium carbonate. The suspension was filtered and the filtrate was worked up in the usual manner to get the sugar (syrup) which was identified by descending paper chromatography. Whatman No. 1 filter-paper was used. Butanol-acetic acid-water (4: 1: 5 by volume) was employed as the irrigating solvent and aniline hydrogen phthalate as the spray reagent.¹⁰ Direct comparison of the sugar in question with authentic D-glucose, D-galactose and L-rhamnose showed that the sugar in question might be L-rhamnose. A mixed chromatogram of the unknown with L-rhamnose confirmed the identity.

Treatment of ether solubles of the residue from ether extract (referred to earlier as solution X).—Complete removal of the solvent gave an almost colourless residue which on crystallization from alcohol yielded a colourless powder, m.p. 210–25° (2 g.). The substance was dissolved in hot benzene-methanol (1: 1, 200 ml.) and diluted with water (100 ml.) resulting in the separation of two layers. The lower aqueous-methanol layer was separated, extracted with ether (3×100 ml.) and the extracts were united with the benzene phase. The benzene-ether solution was shaken with 2% sodium hydroxide solution when a precipitate separated at the interphase. This was filtered and washed with water. The precipitate, the filtered alkaline solution and the organic solvent layer were worked up separately as described below.

The precipitate was dissolved in hot methanol and decomposed with hydrochloric acid (1: 1). The resulting suspension was concentrated to a low volume, cooled and the precipitate filtered and washed. Crystallization twice from alcohol yielded colourless needles, m.p. 280–82° (ursolic acid) (yield: 0.8 g.). $[\alpha]_D^{29} = +67.2^\circ \pm 3^\circ$ ($c = 0.792$ in absolute alcohol) [Found: C, 79.0; H, 11.2. $C_{30}H_{48}O_3$ (ursolic acid) requires: C, 78.9; H, 10.6%]. In the Liebermann-Burchard reaction it gave a red colour changing to violet, then blue and finally green. Mixed m.p. with ursolic acid obtained from the leaves of *R. nilagiricum* Zenk. (*loc. cit.*) was undepressed.

The acetate crystallized from alcohol as colourless needles, m.p. 282–84°. $[\alpha]_D^{30} = +62.9^\circ \pm 3^\circ$ ($c = 0.983$ in chloroform). [Found: C, 76.9;

H, 10.5. $C_{32}H_{50}O_4$ (ursolic acid acetate) requires: C, 77.1; H, 10.1%]. Mixed m.p. with an authentic sample of ursolic acid acetate was undepressed.

No worthwhile residue was obtained by neutralizing the alkaline filtrate and extracting with ether.

The organic solvent layer was washed with water till neutral, dried and the solvents removed, when a colourless residue (0.22 g.) was obtained. This was found to be mostly waxy in nature. Chromatography over alumina yielded a small quantity (*ca.* 10 mg.) of a colourless substance (nodules), m.p. 255–60°, which was not examined further.

Methanol extract.—Concentration to 400 ml. and leaving in the ice-chest for a month led to the deposition of greenish sticky matter. The solution was decanted and filtered. The filtrate was concentrated under reduced pressure with occasional addition of water and the resulting solution left in the ice-chest. The clear deep red liquid (200 ml.) was decanted from the resin that had again deposited, diluted with an equal volume of alcohol and sufficient concentrated sulphuric acid was added to give a 7% concentration of the acid. The solution was boiled under reflux for 2 hours. Water was added and the alcohol was removed under reduced pressure. The resulting suspension was cooled and extracted with ether. The ethereal extract was washed with water till neutral, dried and the solvent removed. Repeated crystallization of the residue from dilute acetone yielded yellow needles, m.p. 300–06° (0.3 g.). The colour reactions were identical with those answered by quercetin. No separation was observed when mixed chromatograms were run with authentic quercetin as described under the aglycone of the glycoside.

The acetate (colourless needles from alcohol) melted at 192–94° and the mixed m.p. with authentic quercetin penta-acetate was undepressed.

The acidic aqueous suspension left after ether extraction was filtered. The brown residue that was obtained was washed and dried (15 g.). It did not melt below 350° and could not be crystallized from any solvent or solvent mixtures. The clear aqueous filtrate was neutralized with barium carbonate, filtered and the filtrate concentrated under reduced pressure when a large quantity of a syrupy residue was obtained (*ca.* 50 g.). A portion of the residue was dissolved in water, filtered through a bed of charcoal and the filtrate again reduced to a syrup. It yielded an osazone (yellow needles from dilute alcohol), m.p. 207–08°. Mixed m.p. with an authentic sample of D-glucosazone was undepressed.

The sugar was further identified as glucose by descending paper chromatography using butanol-acetic acid-water as the irrigating solvent and aniline hydrogen phthalate as the spray reagent.

SUMMARY

The flowers of *Rhododendron nilagiricum* Zenk. have been examined for their crystalline components by extraction with organic solvents and fractionation along usual lines. Quercitrin and ursolic acid could be isolated as such and quercetin after hydrolysis of one of the crude fractions.

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