

CHEMICAL EXAMINATION OF THE LEAVES 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 falconeri* Hook. have been reported in an earlier communication from these laboratories.² *Rhododendron nilagiricum* Zenk. (Syn. *R. arboreum* Sm. var. *nilagirica* Cl.) grows abundantly in the Nilgiri Hills.³ Wehmer reports that the species *R. arboreum* Sm. is poisonous and probably contains the glucoside ericolin⁴; but there is no reference in the published literature to past chemical work on the *nilagirica* variety. The results of the chemical examination of the leaves of this plant are described in the present paper.

The dried leaf material was subjected to two different extraction procedures in separate batches. In one procedure the leaf powder was extracted directly with alcohol. During concentration a large quantity of resinous matter separated, from which by suitable manipulation as described in the experimental portion, ursolic acid and epifriedelinol were obtained. From the resin-free alcoholic solution, hyperoside (3-D-galactoside of quercetin) was obtained along with some free quercetin.

In the second extraction procedure, the leaf powder was extracted first with ether and then with alcohol. Essentially the same crystalline components, viz., ursolic acid, epifriedelinol and quercetin, were obtained; the isolation of the glycoside was given up in this extraction procedure, in view of the tedious nature of the procedural details (see experimental).

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

Epifriedelinol is a triterpenoid compound of rather limited occurrence and has been reported to occur in *Ceratopetalum apetalum* D. Don.,^{5,7} *Cetraria nivalis*^{6,7} and *Prunus nepalensis* Koch.⁸ The identity of epifriedelinol obtained in the present investigation was deduced from the colour reactions and the m.p., optical rotation and the analysis of the parent compound, of its acetate and its benzoate.

The identity of quercetin was deduced from its m.p., colour reactions and its acetate (m.p. and mixed m.p. with an authentic sample).

Hyperin was the name given to a glycoside first isolated from *Hypericum perforatum* L.⁹ and reported to be the 3- α -D-galactoside of quercetin. The name hyperoside was suggested as more appropriate for it by Casparis *et al.*¹⁰ Hyperoside has been shown to occur in many species belonging to the plant families Betulaceæ and Polygonaceæ, in a few cases by preparative methods, but more largely by paper chromatographic methods.¹¹⁻¹⁴ Hörhammer *et al.* have summarised the available information on the occurrence of hyperoside and of the closely related glycosides quercitrin and rutin.^{13, 14} Quercetin-3-galactoside has also been reported to occur in the skins of Grimes Golden and Jonathan apples.¹⁵

The identity of the glycoside isolated in the present investigation as hyperoside has been established by its m.p., analysis and optical rotation, preparation of its acetate and its methyl ether, hydrolysis to quercetin and galactose 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). The nature of the sugar in the original glycoside was established by the preparation of its osazone and by paper chromatography.

EXPERIMENTAL

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

First extraction procedure

The air-dried leaf powder (4 kg.) was extracted with cold methylated spirit repeatedly. The total extract (70 litres) was concentrated under reduced pressure to 4 litres, the solid separating being removed from time to time. Dilution with water and further concentration yielded some more solid. The combined solids (180 g.) and the clear aqueous-alcoholic solution (referred to again as solution X) were examined separately.

The solid was dissolved in methanol-benzene (1:1) (3 litres), filtered and diluted with enough water to lead to the separation of two layers. After separation of the layers, the aqueous methanol layer was extracted with ether (3 \times 500 ml.) and the ether phase was added to the benzene phase. The combined benzene-ether solution (*ca.* 3 litres) was shaken with 5% aqueous sodium hydroxide solution (2 \times 500 ml.) when a copious solid separated at the interphase. The filtered alkaline solution, the organic solvent layer and the floating solid were examined separately as below.

The alkaline solution, on acidification and ether extraction yielded on promising residue.

The filtered solid was dissolved in hot methanol, cooled and decomposed with excess of mineral acid. The precipitate obtained was purified by crystallization from alcohol, giving colourless needles, m.p. 280–82° (ursolic acid).

The benzene-ether solution was evaporated to remove the solvents and the residue partitioned between 90% acetic acid and petroleum ether, employing 300 ml., 30 ml., 30 ml. and 30 ml. portions of the former and 1 litre, 100 ml., 100 ml. and 100 ml. portions of the latter solvent. The united petroleum ether solution was washed neutral, dried and concentrated to a low volume (250 ml.), when a greenish solid (0.9 g.) separated. This on two crystallizations from chloroform yielded stout needles, m.p. 280–81° (epifriedelinol). The mother liquor yielded a resinous solid (84 g.) on complete removal of the solvent; this was not examined further.

No worthwhile residue was obtained from the acetic acid layer on dilution with water and extraction with ether.

The residual aqueous-alcoholic extract of the original leaf (solution X, *see* earlier) was extracted with ether (10×400 ml.) containing 3% alcohol. The united ether solution was concentrated with occasional addition of water and the remaining aqueous solution was extracted with *iso*-amyl alcohol (5×200 ml.). The united amyl alcohol extract was concentrated to 100 ml. and poured with stirring into a large excess of petroleum ether. The precipitated solid was filtered (42 g.) and extracted with hot water repeatedly (10×200 ml.). The aqueous extract was concentrated under reduced pressure to a syrupy consistency and the residue was taken in absolute alcohol and left in the ice-chest when a fine yellowish powder separated after a week (0.6 g.) (hyperoside).

The alcoholic mother liquor of hyperoside was diluted with water in stages, removing the sticky matter that separated at every stage. The final clear aqueous-alcoholic solution, on almost complete removal of the solvents and leaving in the ice-chest for three weeks, deposited a small quantity of a yellow solid embedded in a large amount of a syrupy matrix. Crystallization from aqueous acetone twice yielded yellow needles, m.p. 312–16° (0.3 g.) answering the description of quercetin. It further yielded an acetate, m.p. 196–98°, undepressed by admixture with an authentic sample of quercetin penta-acetate.

The aqueous residue of solution X remaining after ether extraction was hydrolysed by the addition of sulphuric acid to give a concentration of 7%

and refluxing for 2 hours. A large amount of resinous matter separated, which was filtered, washed, dried and extracted with ether. The ether-insoluble part of the resin was not examined further. The acidic filtrate was extracted with ether and this ether solution was united with the ether extract of the resinous portion. The united ether solution, on removal of the solvent, yielded quercetin (0.5 g.), m.p. 310–14°; acetate, m.p. 194–96° (mixed m.p. undepressed). The acid aqueous layer exhibited strong reducing property towards Fehling's solution indicating the rich presence of sugars.

Second extraction procedure

Only a very brief description of this procedure is given here. The powdered leaf was exhausted with ether and then with alcohol. The ether extract (solution) when shaken with aqueous alkali led to the separation of ursolic acid as the sparingly soluble sodium salt. The filtered alkaline solution yielded no crystalline substance. The ether solution was evaporated and the residue partitioned between 90% acetic acid and petroleum ether. The petroleum ether phase yielded epifriedelinol, m.p. 279–81°; $[\alpha]_D^{25} = +25.6^\circ \pm 3^\circ$ ($c = 1.156$ in chloroform). From the acetic acid phase, by dilution with water and ether extraction a small quantity of an amorphous material was obtained, which could not be further purified.

The alcoholic extract of the leaf gave colour reactions for anthoxanthins and on hydrolysis with sulphuric acid yielded, besides a large quantity of resinous material, a crystalline substance identified as quercetin.

Ursolic acid

When crystallized twice from alcohol, it separated as colourless needles, m.p. 280–82°; $[\alpha]_D^{25} = +62.4^\circ \pm 3^\circ$ ($c = 1.027$ in absolute alcohol). [Found: C, 78.6; H, 10.1. $C_{30}H_{48}O_3$ (ursolic acid) requires: C, 78.9; H, 10.6%.] With concentrated sulphuric acid it gave a yellow colour slowly changing to violet, and in the Liebermann-Burchard test it gave a red colour changing to violet, then blue and finally green.

The acetate, prepared by heating the substance with acetic anhydride and fused sodium acetate at 140° for 4 hours crystallized from benzene-petroleum ether and then from alcohol as colourless needles, m.p. 282–84°; $[\alpha]_D^{25} = +68.8^\circ \pm 3^\circ$ ($c = 0.927$ in chloroform). [Found: C, 76.7; H, 10.6; $-\text{COCH}_3$, 9.3. $C_{32}H_{50}O_4$ (ursolic acid acetate) requires; C, 77.1; H, 10.1; $-\text{COCH}_3$ (1), 8.6%.] Mixed m.p. with an authentic sample of ursolic acid acetate was undepressed.

Epifriedelinol

When crystallized twice from chloroform, it separated as stout needles, m.p. 280–81°; $[\alpha]_D^{25} = +22.4^\circ \pm 3^\circ$ ($c = 0.996$ in chloroform). [Found;

C, 84.7; H, 12.5. $C_{30}H_{52}O$ (epifriedelinol) requires: C, 84.1; H, 12.2%.] With concentrated sulphuric acid it gave a pale yellow colour. In the Liebermann-Burchard test a light pink colour was obtained.

Derivatives of epifriedelinol

(a) *Acetate*.—It was prepared by heating the substance with acetic anhydride and pyridine in a paraffin bath at 120° for 6 hours and purified by chromatography over alumina. The acetate crystallized from benzene-acetone as colourless plates, m.p. $282-84^\circ$; $[\alpha]_D^{20} = +30.2^\circ \pm 2^\circ$ ($c = 0.685$ in chloroform). [Found: C, 82.3; H, 11.8. $C_{32}H_{54}O_2$ (epifriedelinol acetate) requires: C, 81.6; H, 11.6%.]

(b) *Benzoate*.—The substance (0.15 g.) was dissolved in hot absolute pyridine (5 ml.), freshly distilled benzoyl chloride (1.0 ml.) was added and the mixture heated on a paraffin bath at 115° for 3 hours. It was cooled, diluted with a mixture of chloroform and ether (1:3) (50 ml.), transferred to a separating funnel and washed successively with 2 N sulphuric acid (3×10 ml.), 2 N sodium carbonate (3×10 ml.) and finally with water till neutral. The solution was dried, the solvents were removed and the residue was repeatedly crystallized from benzene-alcohol, when colourless plates, m.p. $248-50^\circ$ were obtained. $[\alpha]_D^{20} = +36.8^\circ \pm 3^\circ$ ($c = 0.942$ in chloroform). [Found: C, 83.3; H, 11.1. $C_{37}H_{56}O_2$ (epifriedelinol benzoate) requires: C, 83.4; H, 10.6%.]

Hyperoside

When crystallized twice from water and twice from absolute alcohol, it separated as fine yellow needles, m.p. $238-40^\circ$; $[\alpha]_D^{20} = -65.8^\circ \pm 2^\circ$ ($c = 0.821$ in pyridine : alcohol 1:1). [Found on sample dried under vacuum at 110° for 10 hours and weighed in a closed pig: C, 53.2; H, 4.8. $C_{21}H_{20}O_{12}$ (hyperoside) requires C, 54.3; H, 4.3%.] It gave an orange-red colour with magnesium and hydrochloric acid and an olive-green colour with alcoholic ferric chloride. With neutral lead acetate solution a bright yellow precipitate was obtained. Molisch's test was positive.

Derivatives of hyperoside

(a) *Acetate*.—This was prepared by heating the substance with acetic anhydride and sodium acetate. It could not be crystallized from any solvent or solvent mixtures. By dissolving in benzene (charcoal) and adding excess of petroleum ether, it could be obtained as a micro-crystalline powder, m.p. $102-04^\circ$. [Found: C, 55.3; H, 4.9; $-\text{COCH}_3$, 44.0. $C_{37}H_{36}O_{20}$ requires: C, 55.5; H, 4.5; $-\text{COCH}_3$ (8), 43.0%.]

(b) *Methyl ether*.—This was prepared by repeated treatment of the glycoside in methanol solution with diazomethane in ether. The product was crystallized from methanol, when almost colourless needles separated, m.p. 224–26°. [Found on sample dried under vacuum at 110° for 10 hours and weighed in a closed pig: C, 56.4; H, 5.9; —OCH₃, 23.0. C₂₅H₂₈O₁₂ (hyperoside tetramethyl ether) requires: C, 57.7; H, 5.4; —OCH₃ (4), 23.8%.] It gave no colour with ferric chloride.

The low carbon values for the glycoside and its methyl ether are perhaps due to traces of difficultly removable impurities. It may be mentioned in passing that Casparis *et al.*¹⁰ also have reported for their sample of hyperoside carbon values about 1.1% lower than the theoretical.

Hydrolysis of hyperoside (quantitative)

The glycoside (weighed amount) was refluxed with N-sulphuric acid in 50% alcohol for 2 hours. The alcohol was removed under reduced pressure with occasional addition of small quantities of water. The resulting suspension was left in the ice-chest for a day. The precipitate that separated was filtered, washed with water, dried at 105° for 2 hours under vacuum and weighed. Aglycone obtained: 66.7%. Aglycone present in the glycoside as per calculations: 65.2%.

Hydrolysis of hyperoside (preparative)

The substance (0.3 g.) was refluxed with 7% sulphuric acid (100 ml.) in 50% alcohol for 2 hours. The solution was diluted with water, alcohol was removed and the aglycone that separated on standing was filtered and washed with water. Repeated crystallization from dilute alcohol (charcoal) gave fine yellow needles, m.p. 318–22°. [Found: C, 59.7; H, 3.8. C₁₅H₁₀O₇ (quercetin) requires: C, 59.6; H, 3.3%.] It gave a deep orange-red colour with magnesium and hydrochloric acid. With neutral lead acetate solution, an orange precipitate was obtained.

The acetate of this aglycone crystallized from alcohol as colourless needles, m.p. 197–98°. [Found: C, 59.4; H, 4.4. C₂₅H₂₀O₁₂ (quercetin penta-acetate) requires: C, 58.6; H, 3.9%.] Mixed m.p. with an authentic sample of quercetin penta-acetate was undepressed.

The acidic filtrate obtained after removing the aglycone was shaken with a little ether to remove the remaining aglycone and neutralized with excess of freshly precipitated barium carbonate. The suspension was filtered, washed with water and the filtrate and washings were united and concentrated to a syrupy residue under reduced pressure. The residue was

dissolved in a little water, filtered (charcoal) and the filtrate again reduced to a syrup.

The osazone prepared from a portion of the above sugar residue crystallized from dilute alcohol as characteristic yellow needles, m.p. 189–91° (decomp.). Mixed m.p. with an authentic sample of D-galactosazone was undepressed.

The identity of the sugar was further established by descending paper chromatography using butanol:acetic acid:water 4:1:5 (by volume) as the irrigating solvent and aniline hydrogen phthalate as the spray reagent.¹⁶ Direct comparison of the sugar in question was made with pure D-glucose, pure D-galactose and L-pure rhamnose. Mixed chromatograms of the unknown with glucose and galactose separately were also run for further confirmation.

Hydrolysis of the methyl ether of the glycoside

The substance (50 mg.) was refluxed with 7% sulphuric acid in 50% alcohol (25 ml.) for 2 hours. The resulting solution was diluted with water to 50 ml. and extracted with ether (4×50 ml.). The ethereal extract was washed with water till neutral, dried and the solvent removed. On crystallizing the residue from alcohol (charcoal) light yellow needles, m.p. 194°, were obtained. [Found: C, 64.2; H, 5.5; —OCH₃, 33.4. C₁₉H₁₈O₇ (quercetin tetramethyl ether) requires: C, 63.7; H, 5.1; —OCH₃ (4), 34.6%.] Mixed m.p. with an authentic sample of 5:7:3':4'-O-tetramethylquercetin (m.p. 194°), kindly supplied by Prof. T. R. Seshadri of the Delhi University, was undepressed.

SUMMARY

The examination of the leaves of *Rhododendron nilagiricum* Zenk. by extraction with solvents is described. Ursolic acid, epifriedelinol and hyperoside have been isolated and characterized.

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