CRYSTALLINE CHEMICAL COMPONENTS OF THE LEAVES OF *RHODODENDRON FALCONERI* HOOK.

BY S. RANGASWAMI, F.A.SC. AND K. SAMBAMURTHY

(Department of Pharmacy, Andhra University, Waltair)

Received June 24, 1957

RHODODENDRON leaves are generally considered to be poisonous to cattle and sheep. *Rhododendron falconeri* is reported by Plugge[1] to contain the toxic substance andromedotoxin. Chopra[2] mentions the plant as a fish poison. The plant grows in the Himalayas from Nepal to Bhutan at altitudes from 9,000 to 13,000 ft. and has been described in the monograph *Poisonous Plants of India* by Chopra, Badhwar and Ghosh.[3] There is no record of chemical work on this species other than what has been mentioned above. We have therefore examined the leaves with a view to isolate the crystalline components thereof.

The powdered leaves were extracted with petroleum ether, ether and alcohol in succession. The petroleum-ether extract yielded, besides a large amount of wax, three crystalline substances, A, B and C, all of them in very poor yield. The ether extract yielded ursolic acid as the crystalline component in fairly good yield. From the alcoholic extract quercetin was obtained both before and after hydrolysis with mineral acid indicating that the pigment may be present in the plant material both in the free form and as glycoside.

The three crystalline substances obtained from the petroleum-ether extract appear to be triterpenoid in nature. Substance A which seems to be isomeric with α- and β-amyrins gave neither an acetate nor a 2: 4-dinitrophenylhydrazone. But it underwent isomerization on treatment with mineral acids. Substance B, whose analysis corresponds to that of a hydrocarbon, seems to be a new entity, while Substance C agrees with skimmiol (isolated from *Skimmia japonica*[4]) in its melting point and analysis. Dearth of material prevented any work on these three substances.

The identity of ursolic acid was established by its conversion into the acetate, the methyl ester, the acetate of the methyl ester, the benzoate of the methyl ester and methyl ursonate. Their physical constants agreed with values described in the literature[5–7] except in the case of the methyl ester where our preparation has a lower melting point than described in the literature[8]; the optical rotation however agrees with the literature value.[9]

Quercetin was characterized as its penta-acetate and pentamethyl ether. The combustion values for the pigment obtained from the extract were not entirely satisfactory, but the preparation obtained by hydrolysing the purified acetate was quite satisfactory in every respect. The details are given in the experimental portion.

EXPERIMENTAL

The coarsely powdered leaves (650 g.) were extracted under reflux with petroleum ether (4×2 litres). The petroleum ether-free powder was next extracted with ether (5×2 litres) and then with alcohol (3×2 litres) under reflux.

Petroleum-ether extract.—The combined extract, on concentration and keeping in the ice-chest, deposited a large amount of wax. This was filtered and the filtrate evaporated to a thick consistency. When the residue was set aside for about two months, it deposited a small amount of crystalline solid. On treatment with warm acetone the crystals remained insoluble, but the amorphous portion went into solution (Solution X, see later). The crystalline portion was separated by fractional crystallization from benzene-acetone into two fractions, one melting at about 198° and the other at about 232°. The former fraction, on recrystallization from benzene, separated as long needles (Substance A: 300 mg.) melting at 198°; $[\alpha]_D^{29} = +71{\cdot}9° \pm 3°$ (c = 0·873 in chloroform) (Found on sample dried *in vacuo* for 2 hrs. at 105°: C, 84·7; H, 12·0. $C_{30}H_{48}O$ requires: C, 84·8; H, 11·4%). With conc. sulphuric acid the substance gave a deep yellow colour changing to light orange and then to light pink; in the Liebermann-Burchard test it showed a pink colour.

Isomerization.—To a solution of the substance (25 mg.) in absolute alcohol (5 c.c.), conc. hydrochloric acid (4 drops) was added. After refluxing for ½ hr. the solution was diluted with water (5 c.c.) when a precipitate separated. This was filtered, washed free from acid and dried in the desiccator. On crystallization from benzene-acetone it separated as thin lustrous plates (20 mg.), m.p. 248–50°; $[\alpha]_D^{30} = -30{\cdot}5° \pm 2°$ (c = 0·452 in chloroform) (Found on sample dried *in vacuo* for 2 hrs. at 105°: C, 84·3; H, 11·8. $C_{30}H_{48}O$ requires: C, 84·8; H, 11·4%). The colour reactions were identical with those of the parent substance. The same product was obtained when in the above experiment conc. hydrochloric acid was substituted by 60% sulphuric acid.

The fraction melting at about 232° on recrystallization from benzene-petroleum ether separated as fine needles (Substance B: 100 mg.) melting at

232–34° (Found: C, 87·7; H, 12·2. $C_{30}H_{48}$ requires: C, 88·2; H, 11·8%). It gave a yellow colour with conc. sulphuric acid and a pink colour in the Liebermann-Burchard test.

The wax that separated from the petroleum-ether concentrate was warmed with excess of petroleum ether when most of it went into solution (Solution Y) leaving behind a small quantity of granular powder. This on filtering and crystallizing from benzene-methanol and benzene melted at 279–80° (Substance C: 75 mg.) (Found: C, 85·3; H, 12·3. $C_{30}H_{50}O$ requires: C, 84·5; H, 11·8%). It gave the usual triterpenoid colour reactions.

Solutions X and Y were mixed and evaporated to a syrupy residue which was saponified with N alcoholic potash and the unsaponifiable matter was extracted in the usual way. This on crystallization from absolute alcohol yielded some more quantity (120 mg.) of Substance A.

Ether extract: ursolic acid.—The combined extract on concentration to 200 c.c. deposited a pale greenish solid. This was filtered and repeatedly crystallized from benzene-methanol and absolute alcohol, when it separated as colourless needles, m.p. 266–68° (2·6 g.). The ethereal filtrate was poured into excess of petroleum ether when a greenish solid separated. This on crystallization from benzene-methanol gave colourless plates (3·6 g.), m.p. 261–65°. A further quantity (0·2 g.) of the same substance was obtained on evaporating the petroleum-ether filtrate to a syrupy residue, extracting with hot methanol, treating the mixed methanolic extract with charcoal, filtering and concentrating the filtrate. All the above three fractions were identical (mixed melting point and colour reactions) and were therefore mixed and recrystallized from benzene-methanol, when colourless plates, m.p. 266–68°, were obtained; $[\alpha]_D^{29} = +64{\cdot}6° \pm 3°$ (c = 0·692 in absolute alcohol) [Found: C, 79·1; H, 11·0. $C_{30}H_{48}O_3$ (ursolic acid) requires: C, 78·9; H, 10·6%]. With conc. 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.

Derivatives of ursolic acid.—The acetate, prepared by heating the substance with acetic anhydride and fused sodium acetate, crystallized from alcohol as colourless needles, m.p. 281–83°; $[\alpha]_D^{28} = +64{\cdot}3° \pm 3°$ (c = 1·048 in chloroform) [Found: C, 77·5; H, 10·4. $C_{32}H_{50}O_4$ (ursolic acid acetate) requires: C, 77·1; H, 10·1%].

The methyl ester (diazomethane method) crystallized from petroleum ether as clusters of feathery needles, m.p. 110–12°; $[\alpha]_D^{28} = +60{\cdot}8° \pm 2°$

(c = 0·670 in pyridine) [Found on sample dried at 90° for 3 hrs. under vacuum: C, 79·6; H, 10·1; $-OCH_3$, 7·3. $C_{31}H_{50}O_3$ requires: C, 79·1; H, 10·7; $-OCH_3$ (1), 6·6%].

The acetate of the methyl ester, prepared from the methyl ester with acetic anhydride and sodium acetate, crystallized from alcohol as rectangular rods, m.p. 243–45°; $[\alpha]_D^{28} = +63·7° \pm 3°$ (c = 0·662 in chloroform) [Found: C, 76·6; H, 10·2; $-OCH_3$, 6·0. $C_{33}H_{52}O_4$ requires: C, 77·3; H, 10·2; $-OCH_3$ (1), 6·1%].

The benzoate of the methyl ester prepared from the methyl ester with pyridine and benzoyl chloride crystallized from chloroform-methanol as colourless plates, m.p. 213°; $[\alpha]_D^{28} = +72·8° \pm 2°$ (c = 0·893 in pyridine) (Found: C, 80·3; H, 9·8. $C_{38}H_{54}O_4$ requires: C, 79·4; H, 9·5%).

Methyl ursonate prepared by treating a glacial acetic acid solution of methyl ursolate with chromic acid and purified by chromatography over alumina crystallized from chloroform-acetone as prisms, m.p. 192°; $[\alpha]_D^{30} = +83·3° \pm 1°$ (c = 2·262 in pyridine) (Found: C, 80·0; H, 10·8. $C_{31}H_{48}O_3$ requires: C, 79·4; H, 10·3%).

Alcoholic extract.—The combined extract was concentrated to 200 c.c. and the concentrate poured into excess of ether (500 c.c.) whereby two immiscible layers were obtained.

The lower layer was diluted with water and hydrolysed by boiling with sulphuric acid (effective concentration 7%) for 2 hrs. The dark brown precipitate that separated on cooling (13 g.) could not be crystallized. The aqueous filtrate was extracted with ether, the extract washed neutral, dried and evaporated. On crystallizing the residue from alcohol yellow needles (1 g.), m.p. 296–98°, were obtained.

The ether layer was shaken with 5% potassium hydroxide solution and the separated alkaline solution neutralized with acid. On allowing to stand, the solution first deposited some sticky impurities and then a pale yellow substance which was identical with the substance obtained by the hydrolysis described above.

Penta-acetyl-quercetin prepared from the above crystallized from alcohol as woolly needles, m.p. 194° [Found: C, 58·7; H, 4·0; $-COCH_3$, 45·0. $C_{25}H_{20}O_{12}$ requires: C, 58·6; H, 3·9; $-COCH_3$ (5), 42·0%].

The acetate was deacetylated by dissolving the substance (60 mg.) in alcohol (5 c.c.), adding 20 c.c. of N sulphuric acid and refluxing for 18 hrs. The solution was cooled when the pigment precipitated out. It was filtered, washed and crystallized from aqueous methanol and aqueous acetone, when

it separated as fine needles, m.p. 318–22° (Found on sample dried *in vacuo* for 4 hrs. at 105°: C, 59·4; H, 3·8. $C_{15}H_{10}O_7$ requires: C, 59·6; H, 3·3%).

The pentamethyl ether of quercetin, prepared by the action of dimethyl sulphate and anhydrous potassium carbonate in acetone solution, crystallized from dilute alcohol as needles, m.p. 150–51° [Found: C, 64·7; H, 5·7; $-OCH_3$, 40·3. $C_{20}H_{20}O_7$ requires: C, 64·5; H, 5·4; $-OCH_3$ (5), 41·7%].

Summary

The leaves of *Rhododendron falconeri* Hook. have been examined for their crystalline components by extraction with organic solvents and fractionation along usual lines. Three crystalline substances which appear to be triterpenoid in nature were obtained from the petroleum-ether extract in very low yields. From the ether extract ursolic acid was obtained and from the alcoholic extract quercetin.

Acknowledgment

We thank Mr. E. Venkata Rao and Mr. M. Satyanarayana for the microanalysis.

References

1. Plugge, P. C. .. *Arch. Pharm.*, 1891, **229**, 552.
2. Chopra, R. N. .. *Indigenous Drugs of India*, Art Press, Calcutta, 1933, p. 523.
3. ———, Badhwar, R. L. and Ghosh, S. *Poisonous Plants of India*, Manager of Publications, Delhi, 1949, **1**, 613.
4. Takeda, K. .. *J. Phar. Soc.*, Japan, 1941, **61**, 117.
5. Ewen, E. S. and Spring, F. S. .. *J. Chem. Soc.*, 1943, 523.
6. Jacobs, W. A. and Fleck, E. E. *J. Biol. Chem.*, 1931, **92**, 487.
7. Kuwada, S. and Matsumoto, T. *J. Phar. Soc.*, Japan, 1933, **53**, 368.
8. Heilbron, I. and Bunbury, H. M. *Dictionary of Organic Compounds*, Eyre and Spottiswoode Ltd., London, 1953, **4**, 655.
9. Josephy, E. and Radt, F. .. *Elsevier's Encyclopædia of Organic Chemistry*, Elsevier Publishing Co., Inc., New York-Amsterdam, 1940, **14**, 566.

The Bangalore Press, Bangalore City, by C. Vasudeva Rao, Superintendent,
Published by The Indian Academy of Sciences, Bangalore.