

PART II. SWERTIA CHIRRETA.

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Swertia Chirreta (N.O., Gentianaceae) grows abundantly in the temperate Himalayas from Kashmir to Bhutan and the Khasia range at altitudes from 4000 to 10,000 feet. The vernacular names are:—Charayatah (Hindi); Nila-vembu (Tamil); Kiratatikta chunimba (Sanskrit); Chireta (Bengali); Chiravata (Gujarati). Numerous other varieties of this drug are found in almost every part of India, and it has long been used by Hindu physicians as a bitter tonic, stomachic, febrifuge, antiperiodic, antibilious and anthelmintic, and in large doses as an aperient, the varieties differing in their strength (K. M. Nadkarni, *Indian Materia Medica*, p. 390). Experiments at the School of Tropical Medicine (Chopra, Ghosh and Ratnagiriswaran, *Ind. Jour. Med. Res.*, 1929, **16**, 770) show that it can effectively replace the gentian of the pharmacopoeia.

More than a century ago Richard Ratly separated from the drug a free acid, a very bitter extract, some resinous material and a large proportion of gums with potassium and calcium chlorides. Later, Höhn (*Jahr. Fort. Chem.*, 1869, 771; *Arch. Pharm.*, ii, **129**, 213) found the plant to contain a yellow, bitter acid, called ophelic acid and to which he assigned the formula, $C_{13}H_{20}O_{10}$ and another bitter principle chiratin, $C_{26}H_{48}O_{15}$, resolved with dilute hydrochloric acid into ophelic acid and chiratogenin, $C_{13}H_{24}O_3$. He obtained also a tasteless, yellow, crystalline substance in quantity too small for identification, but he did not record any melting points.

Examination of the drug has been undertaken (a) to make a more complete analysis of the constituents and (b) to inquire into the chemical nature of the active principles. Besides the foregoing compounds of Höhn, the following have now been isolated:—Palmitic, oleic and stearic acids, a phytosterol, a monohydroxy-acid, two yellow, crystalline phenols melting at $179-80^\circ$ and $256-57^\circ$ respectively, a neutral, pale yellow crystalline compound, m.p. $196-98^\circ$ and a large proportion of resinous material. It is interesting to note that Höhn's chiratin does not appear to be a chemical individual, as two new substances melting respectively at 119° and $179-80^\circ$ and a non-crystallisable viscous mass have now been isolated from the supposed chiratin.

Höhn suggested the presence of three double bonds in his formula, $C_{13}H_{20}O_{10}$, for ophelic acid; but from the composition of the bromine additive compound now prepared ($C_{13}H_{20}O_{10}Br_4$) it appears to contain only two double bonds.

EXPERIMENTAL.

The material employed in this investigation consisted of leaves, roots and stems of the plant obtained from the local market.

The dry and powdered drug (25 g.) extracted with Prollius' fluid having indicated absence of alkaloids, the material (50 g.) dried at 100° was extracted with various solvents in a Soxhlet apparatus, giving the following amounts (grams) dried at 100°:— Petroleum ether (b.p. 50-60°), 2.0; ethyl ether, 0.7; chloroform, 1.6; ethyl acetate, 0.92; ethyl alcohol (91 per cent.), 7.5; water, 8.0; total, 20.72.

For detailed investigation the disintegrated material (25 kg.) was thoroughly extracted by continuous percolation with 91 per cent. hot alcohol. Removal of the solvent left a dark, pungent, bitter viscous mass (about 2.75 kg.), some (1700 g.) of which was submitted to a current of steam to remove all volatile products. The turbid, pungent distillate was acidic, with minute drops of brownish oil on the surface. In the distilling flask there remained a dark-brown liquid (A) and a dark insoluble residue (B).

The steam distillate.—This was saturated with sodium chloride and extracted with ether, the slightly brownish extract being shaken successively with 10 per cent. sodium bicarbonate, sodium carbonate and sodium hydroxide. No definite chemical individual could be isolated from the resinous precipitates obtained from the alkaline extracts on acidification. The ethereal solution then left isoamyl alcohol (b.p. 129-131°; about 1.5 g.) while the residual aqueous solution on evaporation deposited a dark brown resinous mass from which nothing definite could be isolated.

Water-insoluble resin, B.—After repeated washing with hot water the dark, amorphous mass (700 g.) was extracted successively in a Soxhlet apparatus with solvents, when the following amounts (grams) of extracts dried at 80° were obtained.

Petroleum ether (b.p. 50-60°)	145	20.7 per cent.
Ether	55	7.85 " "
Chloroform	39	5.57 " "
Ethyl acetate	37	5.28 " "
Alcohol	96	13.7 " "
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Total extract	372 g.	43.1 " "

The residue from the above extractions was an alkali-soluble greyish, resinous powder.

Petroleum ether extract.—The greenish semi-solid left after removal of the solvent was dissolved in excess of ether and successively extracted with 10 per cent. sodium bicarbonate, sodium carbonate and sodium hydroxide. No definite compound could be obtained from the first two, but the sodium hydroxide extract gave on acidification a greenish amorphous mass (about 1.4 g.) of which about 0.5 g. remained insoluble in 91 per cent. cold alcohol and crystallised from ethyl acetate in yellow needles with phenolic properties; yield, 0.3 g., m.p. 179-180° (Found: C, 61.9; H, 5.4. $(C_{13}H_{14}O_3)_x$ requires C, 62.4; H, 5.6 per cent.). The alcoholic solution on concentration and cooling gave a trace of the phenolic substance, but on complete evaporation, a brown resinous mass was obtained.

The washed ethereal solution after alkali extractions gave a viscous residue (about 142 g.) after removal of the solvent; this was saponified with an excess of 2*N* alcoholic potassium hydroxide and the unsaponifiable portion removed by ether extraction.

Saponification products.—The potassium salts left after ether extraction were dissolved in hot water and filtered from the filter paper pulp. The fatty acid mixture liberated from the aqueous solution on acidification was extracted with ether and the washed ethereal solution dehydrated with anhydrous sodium sulphate. After the removal of ether, the mixture of fatty acids was boiled in chloroform solution with animal charcoal for about two hours. The pale green filtrate gave a mixture of fatty acids (about 32 g.) from which the saturated (22 g.) and unsaturated (8 g.) acids were separated by Twitchell's method (*Ind. Eng. Chem.*, 1924, **13**, 806).

The unsaturated acids.—The yellowish oily acid after purification in the usual way through the sodium salt gave an iodine value, 90.5-91 and mean M.W. 281.3. It was oxidised with alkaline potassium permanganate (Le Sueur, *J. C. S.*, 1901, **79**, 1315) to a hydroxy-acid which on being repeatedly crystallised from alcohol formed colourless laminae, m.p. 126-127°, and was evidently dihydroxystearic acid. The unsaturated acid consisted, therefore, mainly of oleic acid (Found; C, 67.9; H, 11.8. $C_{18}H_{36}O_4$ requires C, 68.3; H, 11.4 per cent.).

The saturated acids.—The saturated mixture after purification gave a mean M.W. 298.3 and after being worked up according to the method of Kries and Hafner (*cf.* foregoing paper, p. 31) gave palmitic acid (m.p. 62.5°; mean M.W. 256.1), stearic acid (m.p. 69-69.5°; mean M.W. 284.4) and a third acid (m.p. 80-82°; mean M.W. 395.5) which appears to be cerotic acid. The identity of the first two acids with palmitic and stearic acids respectively has been confirmed by taking mixed melting points with authentic samples.

Unsaponifiable matter.—The ethereal extract of the saponified mass was washed with water, the residue from the solvent being then digested with 96 per cent. alcohol and filtered, but nothing definite could be isolated from the insoluble green viscous residue. The alcoholic solution after being boiled under reflux with animal charcoal for 3 hours was filtered hot and gradually treated with an excess of 1 per cent. hot solution of digitonin. After 12 hours in a cold chamber, the flocculent precipitate was boiled with xylene on a sand bath for 24 hours, when the filtered xylene was slowly evaporated to dryness. The residue, after several crystallisations from alcohol, formed colourless needles m.p. 128-130°; it responded to the tests for phytosterol and gave an acetate, m.p. 127-29°.

Ether extract.—This was filtered from the less soluble portion (*a*, about 25 g.) which gradually separated during extraction. The filtrate gave an amorphous green powder (*b*, about 30 g.) after removal of the solvent. The less soluble portion (*a*) appeared to contain resinic acids from which no definite compound could be isolated. The green product (*b*) was partly (20 g.) insoluble in 60 per cent. alcohol, which extracted a very small amount of substance, m.p. 272-275° (decomp.) and a very bitter principle (about 2 g.); the former could not be identified due to insufficiency of material, and the latter on hydrolysis gave an unsaturated acid identified as ophelic acid.

The fraction insoluble in 60 per cent. alcohol was digested with a large excess of boiling 91 per cent. alcohol which dissolved almost all; the filtrate on dilution with water after animal charcoal treatment gave a pale green precipitate which was repeatedly crystallised from alcohol, then dissolved in ether and shaken with a solution of sodium hydroxide, when the sparingly soluble sodium salt separated in the aqueous layer. The acid from the sodium salt crystallised from absolute alcohol in needles, m.p. 295°, is soluble in alcohol, ether, chloroform, and pyridine and burns without smoky flame (Found; C, 77.3; H, 11.09. M.W. by Walker, Lumsden and McCoy's method, 395. $C_{26}H_{44}OH.COOH$ requires C, 77.51; H, 11.00; M.W. 418). The *acetyl* derivative crystallised from ethyl acetate in needles, m.p. 250-251° (Found: C, 76.49; H, 10.5; M.W. 442. $C_{29}H_{48}O_4$ requires C, 75.65; H, 10.04; M.W. 460).

Chloroform extract.—The less soluble portion (19 g.) which separated during extraction was found to be readily soluble in caustic alkali, but ethyl acetate extracted only 2 g., the remainder being resinous. The ethyl acetate solution on concentration after animal charcoal treatment gave a phenolic substance (about 1 g.) which after repeated crystallisation from ethyl acetate formed yellow needles, m.p. 256-57°. The portion soluble in chloroform and remaining on

evaporation was a dull yellowish, amorphous powder (20 g.) of which the alkali-soluble fraction was indefinite, while that insoluble in alkali yielded to acetone a small amount of neutral material which after repeated crystallisation from benzene formed pale yellow needles, m.p. 197-98° (Found C, 59.1; H, 6.27. $(C_6H_8O_3)_x$ requires C, 58.9; H, 6.5 per cent.).

Ethyl acetate and alcohol extracts.—Ethyl acetate and alcohol extracted respectively 37 g. and 19 g. of brown, alkali-soluble amorphous products from which nothing definite could be isolated.

The water-soluble portion, A.—The dark brown liquid was concentrated to a small volume, mixed with purified sawdust, dried and extracted successively with petroleum ether (b.p. 50-60°), ether and chloroform.

Nothing definite could be isolated from the petrol and the ether extracts. The latter, however, gave a small amount of a bitter substance which was found to be a mixture of ophelic acid (readily soluble in water) and chiratin (sparingly soluble in water). The latter was easily decomposed into ophelic acid and a tar by boiling, moderately dilute hydrochloric acid (see below).

Chloroform extract.—The very bitter residue from the solvent partly dissolved in cold water. The concentrated aqueous solution after alkali treatment was extracted with chloroform which gave a bitter, brownish material decomposing into ophelic acid on treatment with dilute acid. The alkaline solution after acidification was extracted with chloroform, but the viscous brown product therefrom could not be crystallised. It decolorises a dilute solution of potassium permanganate and bromine water, reduces Fehling's solution and alkaline copper sulphate, is bitter and dissolves in sodium bicarbonate solution with the evolution of carbon dioxide. An aqueous solution with excess of bromine gave a viscous, brown insoluble mass which was purified by repeated precipitation with water from an alcoholic solution as a pale brown, crystalline powder, m.p. 114-115.5° (Found: Br, 47.85. $C_{13}H_{22}O_{10}Br_4$ requires Br, 48.63 per cent.).

The water-insoluble, brown, viscous mass solidified during several months in a vacuum. Though soluble in alcohol, acetone and pyridine it could not be crystallised from these solvents. It is bitter, very hygroscopic and is hydrolysed by dilute hydrochloric acid into ophelic acid and a tar. From these properties it seems to be chiratin, the active principle of the drug.

The bitter principle.—The powdered dry plant material (100 g.) was exhausted with 63 per cent. hot alcohol, when the filtered solution deposited on cooling an amorphous, yellowish powder (0.6 g.) which

after several crystallisations from alcohol lost its bitter taste and became colourless, m.p. $268-70^{\circ}$ (yield, 0.08 g.). The dry residue obtained from the cold filtrate on evaporation to dryness in presence of lead carbonate under reduced pressure was digested with cold water which dissolved ophelic acid. The chiratin was extracted by boiling alcohol from the residue as a dark yellowish, viscous material (1 g.) solidifying in a vacuum desiccator, representing 1 per cent. of the drug (Found: C, 50.99; H, 7.29. $C_{26}H_{48}O_{15}$ (Höhn) requires C, 50.00; H, 8.00 per cent.).

The dry material (Höhn's so-called chiratin) on digestion with ether partly dissolved leaving a mixture of a brown and pale yellow powder. The ether soluble portion on evaporation gave a greenish, viscous, bitter residue (0.4 g., i.e. 0.4 per cent. of the drug) and did not solidify even on keeping over sulphuric acid in a vacuum.

The ether insoluble residue partly dissolved in chloroform leaving a brown, crystalline powder which was washed thoroughly with chloroform and then dried in a vacuum desiccator; yield 0.4 g., i.e., 0.4 per cent of the drug. It begins to shrink at 145° and melts at $179-180^{\circ}$ with decomposition, is intensely bitter and is insoluble in ether, petrol, alcohol and acetone, but soluble slightly in pyridine (Found: C, 50.02; H, 7.21. $(C_8H_{14}O_5)_x$ requires C, 50.5; H, 7.34 per cent.).

The chloroform solution gave an intensely bitter, greenish-yellow powder which melts at $118-119^{\circ}$ and froths with decomposition at $121-122^{\circ}$; yield, about 0.1 g. i.e., 0.1 per cent. of the drug. It thus seems to be different from chiratin which according to Höhn (*loc. cit.*) is brown.

The cold aqueous solution was evaporated to dryness in a vacuum yielding a brown syrupy mass (1.2 g., i.e., 1.2 per cent of the drug). It gave the same bromo-compound, m.p. $114-116^{\circ}$, as ophelic acid described before.

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