Constituents of the leaves of Helinus ovatus / by John Augustus Goodson.

XVII.—*Constituents of the Leaves of* Helinus ovatus

By John Augustus Goodson.

Helinus ovatus, E. Meyer (Nat. Ord. *Rhamnaceae*), is a climbing shrub indigenous to South Africa, where it is found growing on the borders of woods and thickets. No species of this genus, which, according to Oliver ("Flora of Tropical Africa"), is confined to Africa and India, appears to have been examined previously, although the constituents of the genus Rhamnus, from which the order is named, are fairly well known, including as they do such well-known drugs as cascara sagrada and buckthorn.

The results of the present investigation show that *H. ovatus* contains aconitic acid, quercetin, a saponin, and scyllitol. The last-mentioned substance was first isolated from certain plagiostomous fishes, including the spur dogfish, but has since then been

found in a number of plants, such as the acorns of the common oak and the leaves of *Cocos plumosa* and *C. nucifera*.

The occurrence of scyllitol, the second of the two known *meso*-inositols, in these plants is of considerable biological interest in view of the suggestion made by Winterstein, Contardi, and others (compare Posternak, *Compt. rend.*, 1919, **169**, 37) that phytin, which is believed to be the usual organic phosphorus reserve constituent of plants, is a *meso*inositol hexaphosphate.

The material came from Komgha, Cape Province, and was supplied by Mr. I. B. Pole Evans, Chief of the Division of Botany, Union of South Africa, who stated that it is used medicinally by the natives, and is known locally as "soap-plant," since the leaves have the property of yielding a lather when rubbed in the hands with water.

Preliminary Examination.

The leaves contained 9·5 per cent. of moisture and 9·2 per cent. of ash, of which 20·2 per cent. was potash (K_2O), equivalent to 1·8 per cent. in the leaves.

No alkaloid or cyanogenetic glucoside could be detected by the usual reagents.

The finely ground leaves gave the following percentages of extract on exhaustion in a Soxhlet apparatus with solvents in the order named: petroleum (b. p. 35—60°), 2·2; ether, 2·3; chloroform, 2·2; alcohol, 23·0.

Isolation of Ceryl Alcohol.

The petroleum extract consisted of brown, waxy matter, of which about one-third remained undissolved when digested with ether. This was boiled with alcoholic potassium hydroxide solution to remove traces of oil and wax. The residue left after removal of the alcohol was crystallised from ethyl acetate, and then melted at 78°; a specimen of ceryl alcohol melted at 81° in the same bath, and a mixture of the two at 79°. (Found: C = 82·0; H = 14·0. Ceryl alcohol, $C_{26}H_{54}O$ [Henriques, *Ber.*, 1897, **30**, 1415], requires C = 81·6; H = 14·2 per cent.)

The remaining extracts were systematically examined, with results which showed that the quantity of plant available (650 grams) could best be dealt with by extraction with chloroform to remove wax and resinous matter, and then in succession with alcohol and water.

Examination of the Alcoholic Extract.

The bulk of the alcohol was removed, and the resulting syrup set aside for some days, when it deposited a considerable quantity of potassium chloride. The filtrate was poured into about four times its volume of water, and treated successively with lead acetate and basic lead acetate. The lead was removed from the two precipitates and from the filtrate by hydrogen sulphide in the usual manner.

Isolation of Quercetin.

The aqueous solution of the material recovered from the lead acetate precipitate contained a considerable amount of tannin. Extraction with ether removed a small quantity of a yellow substance, probably quercetin (see below). The liquor was then acidified with hydrochloric acid, boiled to hydrolyse glucosides, cooled, and again extracted with ether, the extract yielding a yellow substance crystallising in rosettes of needles. This was recrystallised from a mixture of alcohol and chloroform, and then melted at 309°. On acetylation, it formed matted, colourless needles melting at 195°, and this melting point was not depressed when the substance was mixed with penta-acetylquercetin. The yellow colouring matter is therefore quercetin.

The aqueous solution of the material recovered from the basic lead acetate precipitate also contained tannin, and a small amount of yellow colouring matter, which could not be obtained in a crystalline condition.

The filtrate, after removal of the lead as sulphide, was concentrated and extracted with butyl alcohol, which removed a saponin. The latter was purified by solution in water and precipitation with basic lead acetate, the lead precipitate being decomposed with hydrogen sulphide in the usual manner, and the filtrate evaporated to dryness under diminished pressure. The quantity of saponin obtained was so small that no further purification could be effected. The material frothed strongly in aqueous solution, gave no compound with cholesterol in alcoholic solution, did not reduce Fehling's solution, and was not hæmolytic. It was hydrolysed by boiling with dilute hydrochloric acid, the resulting solution yielding a small amount of apparently crystalline sapogenin on extraction with ether. The residual aqueous solution reduced Fehling's solution strongly, but did not give a crystalline phenylosazone.

The aqueous liquid, after extraction with butyl alcohol, was concentrated under diminished pressure and set aside, when a further

quantity of potassium chloride separated. The filtrate yielded *d*-phenylglucosazone on treatment with phenylhydrazine.

Examination of the Aqueous Extract.

The aqueous extract was treated successively with lead acetate and basic lead acetate, and the two precipitates were collected.

Isolation of Aconitic Acid.

The lead acetate precipitate was suspended in water and decomposed by hydrogen sulphide. The filtrate was concentrated under diminished pressure and extracted with ether, which removed 12·6 grams of a crystalline substance, corresponding with 1·9 per cent. in the leaves.

This, on recrystallisation from water, formed minute prisms melting at 191°, and gave all the reactions of aconitic acid, including that described by Taylor (T., 1919, **115**, 886). (Found: C = 41·3, 41·4; H = 3·9, 3·6. Aconitic acid, $C_6H_6O_6$, requires C = 41·4; H = 3·5 per cent.)

Isolation of Scyllitol.

The basic lead acetate precipitate was decomposed in the usual manner, and the filtrate concentrated under diminished pressure, when slightly brown crystals separated. Two crops of the crude substance, amounting to 3 grams and corresponding with 0·46 per cent. in the leaves, were obtained. The product was purified by recrystallisation from hot water, from which it separated in anhydrous, monoclinic rhombs. (Found: C = 39·7, 40·1; H = 6·8, 6·9. Scyllitol, $C_6H_{12}O_6$, requires C = 40·0; H = 6·7 per cent.)

The properties of the substance agreed closely with those recorded for scyllitol (J. Müller, *Ber.*, 1907, **40**, 1821, and H. Müller, T., 1907, **91**, 1767; 1912, **101**, 2383). When recrystallised slowly from cold water, it separated in transparent, hexagonal prisms, which, on removal from the solvent, became opaque and friable owing to loss of water of crystallisation. Crystals freed as rapidly as possible from adhering mother liquor lost, on exposure to air, 24·9 per cent. of water. $C_6H_{12}O_6,3H_2O$ requires $H_2O = 23·1$ per cent.

H. Müller noted this change in crystal habit and transparency, but did not establish the fact that it is due to loss of water of crystallisation (T., 1907, **91**, 1772).

When heated, the scyllitol obtained from *H. ovatus* leaves coloured slightly at 300°, darkened considerably at 320°, and melted and effervesced at 353°, as recorded by a mercury thermometer.

J. Müller (*loc. cit.*) gave the solubility of scyllitol in water as about 1 gram in 100 c.c. at 18°; the author finds a solubility of 1·03 grams in 100 grams at 18° for his specimen, whereas H. Müller (*loc. cit.*) gave it as 1·7 grams in 100 c.c. at 15°.

Its identity with scyllitol was confirmed by the preparation of the hexa-acetyl derivative, which melted at 291°. H. Müller (*loc. cit.*) gives 290—291° (corr.). (Found: C=50·1; H=5·6. Hexa-acetylscyllitol, $C_6H_6(C_2H_3O)_6O_6$ requires C=50·0; H=5·6 per cent.)

The filtrate from the lead precipitates, after removal of the lead, contained merely inorganic salts.

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