# THE ISOFLAVONE GLYCOSIDE OF PIPTANTHUS NANUS M. POP.

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7-Hydroxy-4'-methoxyisoflavone (formononetin) has been found by Hlasiwetz [1] in the roots of the restharrow Ononis spinosa L. in the form of the 7-O-D-glucoside of ononin, the structure of which was subsequently established by the work of numerous authors [2-5]. Subsequently, formononetin was found in some plants of the family Leguminosae -- Cicer arietinum L., Trifolium pratense L., T. incarnatum L., and T. subterraneum L.[6] -- but only in the form of the free aglycone.

In an investigation of the above-ground parts of the Central Asiatic plant Piptanthus nanus (family Leguminosae) we have isolated a small amount of a crystalline substance which has proved to be identical with Hlasiwetz' ononin. Thus, P. nanus is the second plant which contains formononetin in the form of its glucoside.

The large amount of resinous substance sparingly soluble in chloroform and other similar solvents in P. nanus made it difficult to isolate the glucoside. The sample of ononin for comparison with the substance that we isolated was obtained from a Central Asiatic strain of restharrow Ononis antiquorum L., the aerial parts of which that were at our disposal containing ononin but in still smaller amount than Piptanthus. It is interesting that the formononetin obtained by the hydrolysis of the glucoside isolated from Piptanthus had, even after several crystallizations, a lower melting point than that given in the literature. Only after its acetylation, and the recrystallization and deacetylation of the acetate, was a preparation obtained having the melting point of formononetin. The aglycone obtained by the hydrolysis of the glucoside obtained from the restharrow had the characteristic melting point without additional treatment.

## EXPERIMENTAL

The plant material used in our investigations was collected by the VNIKhFI (S. Ordzhonikidze Chemical and Pharmaceutical Institute) Central Asiatic expedition under the leadership of P. S. Massagetov (Piptanthus in Tien Shan in June 1960 and the restharrow in Southern Kazakhstan at the beginning of July 1962). The analyses were carried out in the microanalytical laboratory of the VNIKhFI under the direction of V. V. Kolpakova.

Extraction of Piptanthus and isolation of the glucoside. Five hundred grams of crushed air-dry plant material was extracted with methanol in a Jenatherm apparatus. After the methanol had been distilled off from the extract, the semiliquid residue (190 g) was treated with 790 ml of acetone (four volumes), which precipitated a viscous brown mass. The clear acetonic solution was decanted off, the acetone was distilled off, and 50 ml of chloroform and 100 ml of water were added to the residue with careful stirring. When the mixture was allowed to stand in the refrigerator, a yellowish crystalline precipitate formed above the chloroform layer and a brown resinous mass separated out on the bottom of the flask. The aqueous layer together with the chloroform and the precipitate were decanted off and the precipitate was collectected on a glass filter. The resin was treated with 20 ml of methanol, in which it dissolved, leaving a small crystalline residue. The latter was combined with the first residue, after which the material was washed on the filter with 10 ml of methanol. Yield 0.8 g (0.16%), mp 210-211° (twice from methanol), $[\alpha]_D^{25}$ -28.8° (c 1.04, pyridine); -44.5° (c 0.56, pyridine-alcohol, 1:1).

Found %: C 61.45; H 5.31; $CH_3O$ 7.07. $C_{22}H_{22}O_9$. Calculated %: C 61.39; H 5.15; $CH_3O$ 7.21.

The following figures are reported for synthetic ononin: mp 214° $[\alpha]_D^{23}$ -24.2° (pyridine) [5].

Hydrolysis of the glucoside, isolation of the aglycone, its acetylation, and the deacetylation of the acetate. Production of formononetin. A mixture of 0.1 g of the glucoside and 30 ml of 5% sulfuric acid was heated in the boiling water bath for 3 hr; the aglycone separated out in the form of characteristic curved plates [8]. After four recrystallizations from methanol and alcohol, fine needles with mp 252-254° deposited. On acetylation with acetic anhydride in pyridine (90°; 1.5 hr), fine needles with mp 167-169° (three times from methanol) were obtained; mp 170° is reported for formononetin acetate [8].

Found %: C 69.66; H 4.74; $C_{18}H_{14}O_5$. Calculated %: C 69.70; H 4.52.

Deacetylation of the acetate by heating it on a boiling water bath with conc. HCl led to the free aglycone, mp 257° (mp 258° is reported for formononetin [8]. UV spectrum (in alcohol): $\lambda_{max}$ 300 mμ (log ε 4.12); 250 mμ (log ε 4.50), corresponding to the spectrum of formononetin [6].

Found %: C 71.33; H 4.50; $CH_3O$ 11.52. $C_{16}H_{12}O_4$. Calculated %: C 71.64; H 4.51; $CH_3O$ 11.56.

Methylation of the aglycone. Production of 7, 4'-dimethoxyisoflavone. The aglycone was methylated with an excess of a solution of diazomethane in ether (18 hr, 5°), mp 161-162° (from dilute acetone); mp 162-163° [8].

Found %: C 72.27; H 5.00. $C_{17}H_{14}O_4$. Calculated %: C 72.33; H 4.99.

The aqueous solution obtained by the hydrolysis of the glucoside was neutralized with barium carbonate and the filtrate was evaporated in vacuum to small volume; paper chromatography in the n-butanol-alcohol-water (6:1:2) system showed only the spot of glucose.

The glucoside from the restharrow was isolated without the acetone treatment of the concentrated methanolic extract, since the plant contained a considerably smaller amount of extractable substances interfering with the crystallization of the glucoside. This deposited directly from the concentrated extract after treatment with water and chloroform and storage in the refrigerator. Yield 0.08%, mp 214° (from methanol), $[\alpha]_D^{20}$ -30.3° (C 1.07, pyridine). The elementary composition corresponded to the formula of ononin. In admixture with the substance isolated from Piptanthus, the glucoside gave no depression of the melting point. The same applies to the aglycone obtained from it.

SUMMARY

Ononin (7-D-glucosyloxy-4'-methoxyisoflavone) has been isolated from the aerial parts of the Central Asiatic plant Piptanthus nanus M. Pop.; consequently, P. nanus is the second species of the Leguminosae family containing 7-hydroxy-4'-methoxyisoflavone (formononetin) in the form of a glucoside. Ononin has also been found in the aerial parts of a Central Asiatic species of restharrow, Ononis antiquorum.

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