ORNITHOGALIN – A CARDENOLIDE GLYCOSIDE FROM Ornithogalum magnum

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UDC 547.92+615. 711.5

From the seed pods of Ornithogalum magnum Krasch et Schischk. (great star of Bethlehem), in addition to the rohdexin A [1] and ornithogaloside [2] isolated previously, we have obtained a new glycoside, which we have called ornithogalin. It is readily cleaved by the enzymes of the grape snail and of the fungus Aspergillus oryzae into D-glucose and an aglycone with the composition $C_{23}H_{32}O_4$ which, from its R_f value in the methyl ethyl ketone–xylene (1 : 1)/formamide system is comparatively close to digitoxigenin and uzarigenin. Both the glycoside and the aglycone have a smooth positive optical rotatory dispersion curve (Fig. 1) and are not reduced by sodium borohydride, which shows the absence of a carbonyl group in the steroid skeleton. They give a positive reaction characteristic for Δ^4 and Δ^5 bonds [3, 4].

From its molecular weight, elementary composition, and the presence of a double bond in position 4 or 5, the aglycone may be identified as canarigenin [4-7] or xysmalogenin [8]. On comparing the properties of the aglycone under investigation with the substances just mentioned, we came to the conclusion that it was identical with canarigenin. In confirmation of this conclusion, we obtained canarigenin from periplogenin [6, 8], and it proved to be identical with the aglycone of ornithogalin.

A β-glycosidic bond was found in ornithogalin according to Klyne's rule [9]. It is more resistant to hydrolysis with 0.05 N sulfuric acid than the glucofuranoside scorpioside [10] and is readily cleaved by emulsin. This shows the pyranose form of the glucose in the glucoside.

Thus, the structure of ornithogalin can be given as 3β-O-β-D-glucopyranosyl-14β-hydroxycard-4,20(22)-dienolide. Glycosides of canarigenin have been found previously in Digitalis canariensis L. [4, 6, 7], Acokanthera friesiorum Markgr., and A. oppositifolia (Lam.) Codd [5, 16].

EXPERIMENTAL

The silica gel for the column partition chromatography was prepared as described in a handbook [11]. The melting points were determined on a Kofler block. The substances for analysis were dried in vacuum over P_2O_5 at 110°C for 5 h. The analyses of all the compounds corresponded to the calculated figures.

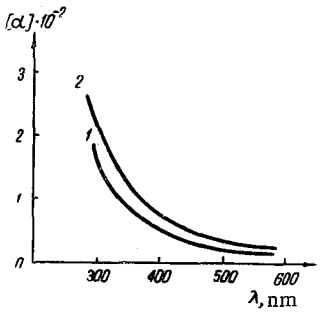

Fig. 1. Optical rotatory dispersion spectrum (c 0.1; ethanol) of ornithogalin (1) and the aglycone of ornithogalin (2).

Ornithogalin. In the preparation of rohdexin A [1] and ornithogaloside [2], fractions containing an unknown cardenolide were collected, and this was separated from the glycosides mentioned by partition chromatography on silica gel. Water was used as the stationary phase and benzene containing various proportions of methyl ethyl ketone as the mobile phase. The glycoside crystallized from water and methanol in the form of acicular crystals with mp 207-216°C, $[\alpha]_D^{20}$ +21° (c 0.1; ethanol). Its elementary composition was $C_{29}H_{42}O_9$.

With 84% sulfuric acid the substance forms a coloration changing with time: 0 min, brownish; 1-3 min,

Khar'kov Chemical and Pharmaceutical Scientific-Research Institute. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 38-40, January, 1971. Original article submitted November 10, 1970.

dirty violet; 4-60 min, violet. It gives positive Legal and Raymond reactions, which are characteristic for cardenolides [12]. Sodium borohydride reduction was carried out as described previously [13].

Hydrolysis of Ornithogalin by the Enzymes of the Grape Snail. The substance (60 mg) was hydrolyzed and the hydrolysis products were worked up by the method described in [14]. The crystalline form of the aglycone (27 mg) and the sugar component (9 mg) of the glycoside under investigation were isolated. The substance obtained by crystallization from ethanol melted at 160-163°C. A mixture of it with D-glucose gave no depression of the melting point. The paper chromatography of the sugar and its osazone [2] confirmed the identity of the carbohydrate component of ornithogalin as D-glucose.

The aglycone of ornithogalin was crystallized from acetone–ether; the crystals that deposited when the solvent slowly evaporated melted at 135-141°C (222-227°C), $[\alpha]_D^{18}$ +29° (c 0.1; ethanol). The elementary composition was $C_{23}H_{32}O_4$. With 84% sulfuric acid it formed a coloration changing with time: 0 min, reddish; 1-2 min, red-violet; 60 min, brownish violet.

Reaction for Δ^4 and Δ^5 Bonds [3, 4]. Reagent 1 (liquid phenol) and reagent 2 (1 g of ammonium molybdate and 2.5 ml of 60% perchloric acid in 100 ml of 0.1 N hydrogen chloride) were used.

Paper with a solution of ornithogalin or its aglycone deposited on it was sprayed successively with reagent 1 and then reagent 2. After each spraying, the paper was dried at 80°C. A bright blue spot appeared at the point where the substance had been deposited. Hyrcanogenin and pachygenin were used as reference samples [15].

Preparation of Canarigenin from Periplogenin. Over 5 h, 1.5 ml of a 2% solution of chromium trioxide in acetic acid was added to a solution of 80 mg of periplogenin in 1 ml of glacial acetic acid. The reaction mixture was left for 3 h, and then 1.5 ml of methanol was added and it was again left for 17 h. After this time, 7 ml of water was added to the green solution and the reaction product was extracted with 15 ml of chloroform. The extract was evaporated in vacuum, giving 66 mg of a faintly yellowish product; this was dissolved in 5 ml of glacial acetic acid and the solution was boiled for 6 min. The acid was evaporated in vacuum and the residue was recrystallized from a mixture of acetone and ether. The crystals that deposited (41 mg) melted at 243-248°C. The substance obtained was reduced with sodium borohydride in isopropanol just as described by Studer et al. [6]. On chromatographic analysis of the reaction mixture, it was found to contain two substances, one of them in large amount (canarigenin) and the second in the form of trace amounts (3-epicanarigenin) [6]. By the usual working-up procedure with subsequent separation by partition chromatography on silica gel (with formamide as the stationary phase and benzene containing various amounts of chloroform as the mobile phase), 18 mg of canarigenin was obtained; it gave no depression of the melting point with the aglycone under investigation and exhibited a similar coloration in 84% sulfuric acid.

SUMMARY

From the pods of Ornithogalum magnum Krasch. et Schischk. we have isolated a new cardenolide glycoside which has been called ornithogalin, the structure of which may be given as 3β-O-β-D-glucopyranosyl-14β-hydroxycard-4,20(22)-dienolide.

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