CARDENOLIDES OF THE PODS OF ORNITHOGALUM MAGNUM KRASCH. ET SCHISCHK

N. F. Komissarenko

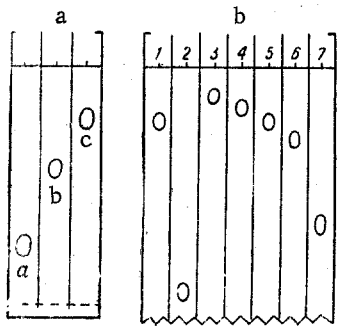
Khimiya Prirodnykh Soedinenii, Vol. 1, No. 3, pp. 156-160, 1965

Ornithogalum magnum (Liliaceae Hall.) (great star-of-Bethlehem) is an endemic bulbous plant distributed in Cis-Caucasia and eastern Trans-Caucasia, where it grows in woods, scrub, and vineyards [1].

Preliminary investigations have shown that the pods of this plant contain steroid glycosides belonging to the group of cardenolides.

The present paper reports the results of a study of the cardenolide composition of the pods of O. magnum.

To isolate this group of compounds, the comminuted pods were extracted with 80% alcohol. After the alcohol had been distilled off, the aqueous residue was purified from accompanying substances, and the total glycosides were then separated on a column of alumina. Some of the fractions yielded three individual substances provisionally denoted on the paper chromatogram by the letters a, b, and c (figure, a).



Paper chromatograms: a) toluene-butan-1-ol (3: 1)-water (35%) system, 3.5 hr, 19°; b) chloroform-formamide system, 3 hr, 23°; 1) aglycone investigated; 2) periplogenin; 3) cannogenol; 4) coroglaucigenin; 5) sarmentogenin; 6) digoxigenin; 7) gitoxigenin.

To establish the structure of substance A, acid hydrolysis was carried out [2], and this gave the sugar L-rhamnose and an aglycone with the empirical formula $C_{23}H_{34}O_5$. The UV spectrum of the latter has one maximum in the 218 mμ region (log ε 4.19) which is characteristic for a five-membered unsaturated lactone ring C_{17}. Three hydroxyl groups were found in the aglycone, two of which are capable of being acetylated. On paper chromatography, the genin under investigation (Figure, b, sample 1) exhibited a relatively high polarity. This indicates that, in addition to the hydroxyl groups at C_3 and C_{14} which are characteristic for the cardenolides, the third OH group may be a primary one (at C_{19}) or a secondary one in an equatorial position. In view of the high polarity of the aglycone, it is probably less subject to the influence of the lactone ring and the hydroxy groups mentioned. In this case, the formation of hydrogen bonds with the polar stationary phase takes place readily and leads to a lower R_f value (position 11α, 12β, or 19) [3].

The results of the oxidation of the aglycone with chromic oxide in glacial acetic acid [4, 5] indicate the absence of a primary hydroxyl group at C_{19} and it is therefore possible that this hydroxy group is secondary.

The position and configuration of the secondary hydroxyl group in the genin under study was established by comparing the difference in the molecular rotation due to an OH group in various positions of known aglycones of the general formula $C_{23}H_{34}O_5$ (see table).

Determination of the Position and Configuration of the Hydroxyl Group in the Aglycone Under Investigation by the Method of Molecular Rotation

Cardenolides	Position and configuration of the OH group	$[\alpha]_D$	$[M]_D$	$\Delta [M]_D$
Acovenosigenin [6]	1β	+ 2.3°	+ 9.0°	−62.5°
Digitoxigenin [7]	—	+19.1	+ 71.5	
7-Hydroxydigitoxigenin [8]	7β	+39.0	+152.3	+80.8
Digitoxigenin	—	+19.1	+ 71.5	
Sarmentogenin [9]	11α	+21.1	+ 82.4	+11.0
Digitoxigenin	—	+19.1	+ 71.5	
11-Episarmentogenin [10]	11β	+29.2	+114.0	+42.5
Digitoxigenin	—	+19.1	+ 71.5	
Digoxigenin [11]	12β	+27.0	+105.4	+34.0
Digitoxigenin	—	+19.1	+ 71.5	
Gitoxigenin [12]	16β	+33.0	+129	+57.5
Digitoxigenin	—	+19.1	+ 71.5	
Aglycone under investigation	11α	+21.4	+ 83.6	+12.0
Digitoxigenin	—	+19.1	+ 71.5	

It can be seen from the table that $\Delta[M]_D$ of the aglycone under investigation corresponds to an 11α OH group. In this case, its structure must be identical with that of sarmentogenin. The proposed structure was confirmed by paper chromatography (figure, b, samples 1 and 5) by the color reactions with 84% sulfuric acid and with antimony trichloride, and by the melting point of a mixture with an authentic sample of sarmentogenin.

Using Klyne's rule [13], an α-glycosidic bond was found in glycoside A. Its stability to acid hydrolysis [5, 14] gives grounds for assuming that the L-rhamnose in the glycoside under consideration is in the pyranose form.

Thus, substance a is 3β-(O-α-L-rhamnopyranosyl)-11α, 14β-dihydroxy-5β-card-20(22)-enolide. From its structure, it must be identical to rohdexin A isolated from the Japanese plant Rohdea japonica (Thunb.) Roth. [15].

Experimental

The adsorption chromatography was carried out on neutral alumina of the third activity group. For analysis, the substances were dried in vacuum (10^{-2} mm Hg) at 110-115° for 4-5 hr over P_2O_5. The melting point was determined on a Kofler apparatus. The UV spectra were taken on a SF-4 spectrophotometer.

Isolation of the glycosides. 1.5 kg of comminuted pods of O. magnum, collected in the period of ripening of the seeds, was treated with 80% ethanol until the cardenolides had been extracted completely. The resulting extract was evaporated to 300 ml and was washed with chloroform, and the aqueous residue was then filtered through a layer of inactive alumina (h = 10 cm, d = 4 cm) with subsequent elution of the glycosides with water. The aqueous filtrate (500 ml) was treated with mixtures of chloroform and alcohol (3: 1) and (2: 1).

After evaporation of the chloroformic alcoholic extract (3: 1), 11 g of dry residue was obtained, and this was dissolved in 100 ml of chloroform containing 5% of alcohol and transferred to a column of alumina (h = 35 cm, d = 4 cm). The column was first washed with chloroform and then with chloroform with a gradually increasing content of alcohol up to 15%. The glycosidic composition of the fractions obtained (300 ml each) was analyzed by means of paper chromatography. Fractions having a similar glycosidic composition were combined, evaporated, and subjected to crystallization. In all, three glycosides were isolated and were provisionally called substances a, b, and c (cf., figure, a).

Glycoside a (rohdexin A). Crystallized from alcohol and water in the form of colorless needles (340 mg) with mp 249-252°, $[\alpha]_D^{19}$ − 23° (c 1.0; methanol). Course of the color reaction with 84% sulfuric acid with time: 1 min – reddish brown, 10-15 min – bluish green, 30-35 min – greenish blue, 60 min – bright green, 90 min – greenish gray.

Found %: C 65.04, 65.36; H 8.73, 8.49; molecular weight 537.4.

Calculated for $C_{29}H_{44}O_9$: C 64.84; H 8.42%; molecular weight 536.6

Acid hydrolysis of glycoside a. A solution of 100 mg of glycoside A in 12 ml of anhydrous acetone was treated with 0.12 ml of concentrated hydrochloric acid, and the mixture was stirred and left at room temperature.

The completeness of the hydrolysis was followed by paper chromatography in the chloroform-formamide system. After five days, the initial substance could not be detected on the paper. The hydrolyzate was then worked up by the usual method [16].

Sugar component of glycoside a. The chloride ions were eliminated from the aqueous residue after the removal of the aglycone with chloroform by means of 1.5 g of AV-17 anion-exchanger. Then the anion-exchanger was filtered off, the filtrate was evaporated to give a syrupy residue, and this was crystallized from acetone containing a few drops of water.

The crystals obtained (18 mg) melted at 74-77° and on paper chromatography proved to be identical with L-rhamnose. The melting point of a mixed sample also corresponded to L-rhamnose.

The aglycone of glycoside a. The chloroformic extract containing the aglycone was evaporated to dryness and the residue was crystallized from methanol-ether. This gave 48 mg of needle-like crystals, which melted at 264-271°, $[\alpha]_D^{19}$ + 21.4° (c 0.92; in methanol). Course of the color reaction with 84% sulfuric acid with time: 1 min – yellowish, 10 min – faint brown with greenish blue border, 15 min – brown-green, 30 min – dark blue, 40-60 min – dark green, 90-120 min – bright green.

After the chromatogram had been sprayed with a 20% solution of antimony trichloride in chloroform and then heated for 5 min at 100-105°, the spot of the aglycone fluoresced light blue in UV light.

Found %: C 70.41, 70.59; H 8.81, 8.9; molecular weight 391.2.

Calculated for $C_{23}H_{34}O_5$: C 70.73; H 8.78%; molecular weight 390.5.

Paper chromatography (figure, b, samples 1 and 5) and a mixed-melting-point test showed the identity of the aglycone with sarmentogenin.

Acetyl derivative of the aglycone. A solution of 30 mg of the aglycone in 0.5 ml of absolute pyridine was treated with 0.5 ml of acetic anhydride and the reaction mixture was left for a day, the degree of acetylation being followed by paper chromatography. After the usual working up and crystallization from acetone-ether, 26 mg of crystals with mp 147-154° was obtained.

Found %: C 68.17; H 7.92; molecular weight 473.80.

Calculated for $C_{27}H_{38}O_7$: C 68.33; H 8.07%; molecular weight 474.56.

Two acetyl residues were found in the molecule of the acetate.

Oxidation of the aglycone. With stirring, 0.3 ml of a 2% solution of chromic oxide in glacial acetic acid was added to a solution of 12 mg of the aglycone in 0.5 ml of glacial acetic acid. After the mixture had stood at room temperature for 4 hr, 0.1 ml of methanol was added and it was allowed to stand for a further 1 hr. Then the reaction mixture was evaporated in vacuum to give a resinous residue. This was dissolved in 1 ml of chloroform and the chloroform solution was washed with 2 ml of 2 N sulfuric acid and with water and then with 2 ml of 2 N sodium carbonate solution. After being washed with chloroform, the sodium carbonate solution contained no substances of a cardenolide nature, which showed the absence from the aglycone of a carboxyl group at C_{10} forming water-soluble cardenolide salts.

The residue after the distillation of the chloroform was dissolved in a small amount of alcohol and filtered through a layer of alumina, the filtrate was evaporated, and the new dry residue obtained was crystallized from methanol-ether. The crystals melted at 235-237°. When the oxidized aglycone was developed on a paper chromatogram with a solution of alkali by Bush's method [17, 18], the spot of the substance became colored bright yellow, demonstrating the presence of a keto group in position 11 of the steroid skeleton.

The chromatography was carried out on chromatographic paper of the Leningrad "Goznak" mill.

The plant material was collected and identified by I. G. Zoz and N. A. Chernykh.

Summary

Steroid glycosides of the cardenolide group have been found in Ornithogalum magnum.

Three glycosides, provisionally called substances a, b, and c, have been isolated by adsorption chromatography on alumina.

Substance a is 3β-(O-α-L-rhamnopyranosyl)-11α, 14β-dihydroxy-5β-card-20(22)-enolide (rohdexin A).

REFERENCES

1. I. M. Krashennikov, "The genus Ornithogalum L. – Star-of-Bethlehem," Flora SSSR, 4, 380, 1935.
2. C. Mannich and G. Siewert, Ber., 75, 737, 1942.
3. I. M. Hais and K. Macek, Paper Chromatography [Russian translation], Moscow, 334, 366, 1962.
4. A. Katz, Pharm. Acta Helv., 1, 22, 1947.
5. A. Hunger and T. Reichstein, Helv. Chim. Acta, 35, 1073, 1952.
6. Ch. Tamm and T. Reichstein, Helv. Chim. Acta, 38, 1013, 1955.
7. A. Windaus and G. Stein, Ber., 61, 2436, 1928.
8. G. Juhasz and Ch. Tamm., Helv. Chim. Acta, 44, 1063, 1961.
9. A. Kats, Helv. Chim. Acta, 31, 993, 1948.
10. O. Schindler, Helv. Chim. Acta, 39, 375, 1956.
11. S. Smith, Soc., 508, 1930.
12. J. H. Russel, O. Schindler, and T. Reichstein, Helv. Chim. Acta, 43, 167, 1960.
13. W. Klyne, Biochem., 47, no. 4, 41, 1950.
14. T. Reichstein, Angew. Chem., 63, 412, 1951.
15. H. Nawa, J. Pharmac. Soc., Japan, 72, 404, 1952.
16. N. F. Komissarenko, V. T. Chernobai, and D. G. Kolesnikov, Med. prom. SSSR, no. 1, 12, 1961.
17. I. E. Bush, Biochem. J., 50, 370, 1952.
18. B. Fechtig, J. V. Euw, O. Schindler, and T. Reichstein, Helv. Chim. Acta, 43, 1570, 1960.

24 December 1964

Kharkov Scientific Research Chemical-Pharmaceutical Institute