SUMMARY

From the epigeal parts of Allium turcomanicum Rgl., in addition to the known spirostans yuccagenin, neoagigenin, neoagigenone, neoagigenin, and alliogenin, we have isolated a new steriod sapogenin the structure of which has been established as neoagigenin 6-O-benzoate.

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NEOCONVALLATOXOLOSIDE – A CARDENOLIDE GLYCOSIDE FROM Convallaria majalis

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The isolation from the seeds of Convallarium majalis L. (lily of the valley) collected in the Khar'kov oblast of polar glycosides – lokundjoside, convalloside, and convallatoxoloside – has been reported previously [1]. On investigating the leaves of this plant, Ya. Bochvarov isolated three polar cardenolides [2] which were provisionally denoted as substances (I), (II_1), and (II_2).

The present paper gives a proof of the structure of the glycoside (II_1), which we have called neoconvallatoxoloside (I).

The glycoside isolated was not reduced by sodium tetrahydroborate. Its UV spectrum showed only one maximum, in the 220 nm region (log ε 4.18), which is characteristic for the butenolide ring of a cardenolide; the optical rotatory dispersion (ORD) spectrum had the form of a smooth curve: 600 nm (−8°), 589 nm (−11°), 520 nm (−12°), 440 nm (−12°), 306 nm (−10°), 300 nm (+40°), and 294 nm (+120°). These facts show the absence of a carbonyl group in the steroid skeleton of the substance under investigation. On acid hydrolysis according to Mannich and Siewert [3], neoconvallatoxoloside (I) was cleaved into D-glucose (V), L-rhamnose (IV), and a number of products of aglycone nature, two (II and III) of which present in predominating amount.

To isolate these substances, the aglycone fraction of the hydrolyzate was separated by partition chromatography on silica gel using chloroform as the mobile phase and formamide as the stationary phase. As a result, strophanthidol (II) and a substance similar in its physicochemical properties to 5(6)-anhydrostrophanthidol (pachygenol) [4] was isolated in the crystalline state. To confirm its structure we obtained 5(6)-anhydrostrophanthidin (IX) from strophanthidin (VII) and reduced it with sodium tetrahydroborate to 5(6)-anhydrostrophanthidol (III).

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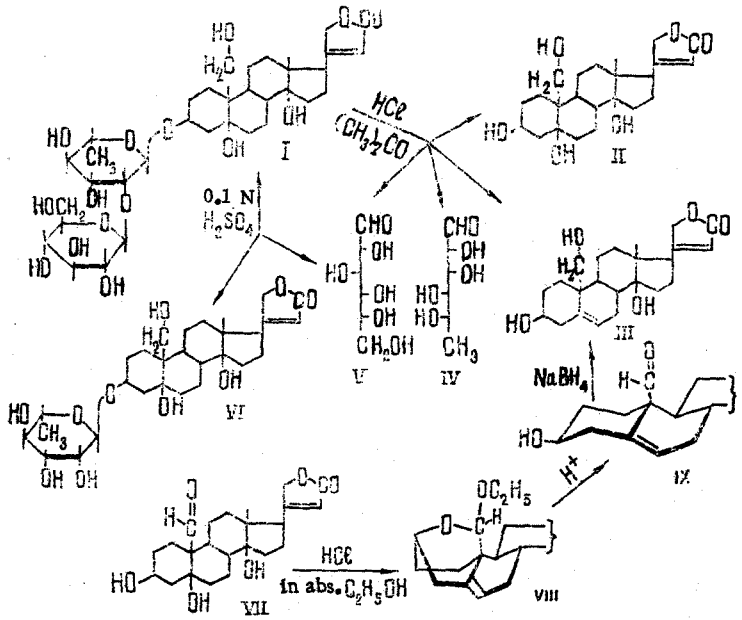

Chemical transformations of neoconvallatoxoloside

By its physicochemical properties, coloration with 84% sulfuric acid, R_f values in a series of solvent systems, and a mixed melting point, the substance isolated from the hydrolyzate was identified as pachygenol [4]. Convallatoxoloside has similar hydrolysis products. However, the properties of the two glycosides have fundamental differences. In contrast to convallatoxoloside, the glycoside (I) under investigation was not hydrolyzed by the enzymes of the grape snail and of the fungus Aspergillus oryzae.

The resistance of the glycoside (I) to the action of enzymes may be due to a number of factors: 1) the glucose forming the terminal sugar in the glycoside belongs to the L-series [5]; 2) this monosaccharide has a furanose oxide ring [6]; 3) there is an α-glycosidic bond between the carbohydrate residues [7]; 4) the sugars are linked by a 1 → 2 bond [8]; and 5) the L-rhamnose is the terminal sugar but is not attached to the D-glucose by a 1 → 6 bond.

To determine the sequence of addition of the sugars we carried out the stepwise hydrolysis of neoconvallatoxoloside with 0.1 N sulfuric acid, as a result of which convallatoxol (VI) [9] was isolated and D-glucose was identified chromatographically. We found no L-rhamnose in the products of periodate oxidation, which excludes a 1 → 3 bond between the sugars. In contrast to convallatoxoloside [1], the glycoside under investigation does not form an isopropylidene derivative. On the basis of the results obtained, we concluded that the D-glucoside in neoconvallatoxoloside is attached to the L-rhamnose by a 1 → 2 glycosidic bond.

In the hydrolysis products of the exhaustively methylated [6] glycoside (I) we found 2,3,4,6-tetramethyl-D-glucose and 3,4-dimethyl-L-rhamnose by paper chromatography.

It was found by the method of molecular rotation differences [10] that the D-glucose is attached to the L-rhamnose by a β glycosidic bond and the latter to the aglycone by an α glycosidic bond.

Thus, the structure of neoconvallatoxoloside can be represented as strophanthidol 3-O-[O-β-D-glucopyranosyl-(1 → 2)-α-L-rhamnopyranoside]. It must be noted that a 1 → 2 bond between the sugars is found rarely among known cardenolide glycosides [8].

EXPERIMENTAL

For analysis, the substances were dried over P_2O_5 in vacuum (10^{-2} mm Hg) at 100-115°C for 4-5 h. The melting points were determined on a Kofler block, and the UV spectra were taken on a EPS-3 spectrophotometer and the optical rotatory dispersion on a SPU-E instrument with a cell 1 dm long. The aglycone and its derivatives were chromatographed in the following systems: 1) chloroform–formamide; 2) benzene–methyl ethyl ketone (2 : 1)–water (35%); and 3) benzene–chloroform (7 : 3)–formamide.

The monosaccharides were determined in the following systems: 4) butan-1-ol–acetic acid–water (4 : 1 : 2); and 5) butan-ol–methyl ethyl ketone–borate buffer (1 : 1 : 2).

The process of isolating neoconvallatoxoloside from the leaves of lily of the valley collected in May in the environs of Bachkovo (Rodony, Bulgaria) has been described previously [2].

The glycoside melted at 161-164°C, $[\alpha]_D^{20}-11 \pm 2°$ (c 1.0; ethanol); elementary composition $C_{35}H_{54}O_{15}$.

With 84% sulfuric acid, the glycoside (I) formed colorations changing with time: 0-15 sec, brownish; 1-5 min, brown with a violet tinge; 20-30 min, cherry-red; 60-120 min, dirty violet.

Acid Hydrolysis of Neoconvallatoxoloside. The glycoside (180 mg) was suspended in 15 ml of acetone containing 1% of hydrogen chloride and the mixture was left for 5 days with a periodic check on the degree of hydrolysis by paper chromatography in system 1. The subsequent working up of the hydrolyzate was as described elsewhere [11]. This gave 79 mg of an aglycone fraction in which a number of substances were detected. Two of them were the main ones. The carbohydrate fraction was obtained in the form of a syrup (107 mg).

Isolation of the Aglycone (II) and its Degradation Product (III). A solution of 79 mg of the aglycone fraction in 3 ml of chloroform was transferred to a column of silica gel (25 × 1 cm) impregnated with formamide. The column was washed with chloroform. When cardenolides appeared in the eluates, the collection of 5-ml fractions was begun. The fractions containing similar compounds were combined, the solvent was evaporated off, and the residues were crystallized. Two substances were obtained. Substance (II) (19 mg) with mp 145-148°C/225-227°C, $[\alpha]_D^{20}$ + 37° (c 0.1; ethanol) was identified as strophanthidol. Substance (III) (14 mg) melted at 123-127°C/216-231°C; $[\alpha]_D^{20}$ + 10° (c 0.1; methanol). With 84% sulfuric acid compound (III) gave a coloration changing with time: 0 min, yellow; 1-5 min, orange with greenish edges; 20 min, red-brown with greenish edges 30-40 min, brown-grey; 180 min, green-brown with a red edge. The reaction for a $\Delta^{4(5)}$ bond was positive [12].

Preparation of 5(6)-Anhydrostrophanthidol (III) from Strophanthidin (VII). First, using the method of Jacobs and Collins [13], 100 mg of strophanthidin was converted into 89 mg of 3,19-oxido-5-anhydrostrophanthidin ethyl acetal (VIII), mp 227-230°C, $[\alpha]_D^{21}-48.0°$ (c 1.0; chloroform). Then it was hydrolyzed to 5(6)-anhydrostrophanthidin (68 mg), mp 119-121°/217-222°C, $[\alpha]_D^{20}-120.0°$ (c 1.0; chloroform).

Compound (IX) was reduced with 15 mg of sodium tetrahydroborate in 3 ml of methanol cooled to −(3-5)°C and was worked up by the method described above [4]. Recrystallization from methanol−diethyl ether gave 38 mg of acicular crystals of pachygenol (III) with mp 124-127°C/215-231°C, $[\alpha]_D^{20}$ + 10° (c 0.2; methanol), which showed no depression of the melting point in admixture with the substance isolated from the hydrolyzate of glycoside (I) and gave the same colorations with sulfuric acid.

The Sugar Component of Neoconvallatoxoloside. When the carbohydrate fraction of the hydrolyzate was chromatographed in systems 4-6, two monosaccharides were found − glucose and rhamnose. After fermentation with yeast only the rhamnose remained in solution, which enables it to be assigned to the L series, and the glucose, as a readily fermentable carbohydrate, to the D series.

The solution containing the L-rhamnose was evaporated to 2 ml, and 10 ml of ethanol and 0.5 g of activate carbon were added. The resulting mixture was boiled under reflux for 5 min and was filtered without cooling. The solvent was distilled off from the filtrate, and the syrup was crystallized from a mixture of methanol and ether. This gave 12 mg of crystals having the form of triangular prisms with mp 67-70°C. A mixture with an authentic sample of L-rhamnose gave no depression of the melting point.

Periodate Oxidation. The glycoside (I) (30 mg) was oxidized in 7 ml of 1% sodium metaperiodate solution at 5-7°C for 2 days. The excess of periodate was decomposed with ethylene glycol, the solution was evaporated to dryness, and the residue was treated with ethanol. The ethanolic extract was hydrolyzed with 5% sulfuric acid for 3 h. After neutralization with AV-17 anion-exchange resin (OH form), no sugars were detected in the hydrolyzate.

Stepwise Hydrolysis. A solution of 120 mg of glycoside (I) in 10 ml of 0.1 N sulfuric acid was heated at 100°C for 6 h. On paper-chromatographic analysis of the hydrolyzate in the toluene−butan-1-ol (2 : 1) −water (35%) system, five substances of cardenolide nature were detected in it (with R_f 0.09, 0.36, 0.5, 0.72, and 0.86), the substance with R_f 0.5 being present in considerable amount. The substance with R_f 0.09 was the initial glycoside.

To separate the feebly polar cardenolides (R_f 0.72, 0.86), the hydrolyzate was treated with 15 ml of chlor form. The evaporated chloroform extract yielded 5 mg of strophanthidol (II) (R_f 0.86), and in the mother solution 5(6)-anhydrostrophanthidin was identified chromatographically (system 1). Then the hydrolyzate was treat with a mixture of chloroform and ethanol (8 : 2). The extract contained mainly the substance with R_f 0.5, which was purified on a column (12 × 1 cm) of neutral alumina (activity grade (III)), using as eluent chloroform contai

ing 5-8% of ethanol. The eluates containing the individual substance were combined and evaporated, and the residue was crystallized from methanol–ether. The crystals obtained (29 mg) with mp 170-172°C, $[\alpha]_D^{20}$ −10° (c 0.1; methanol) were identified as convallatoxin [9].

Methylation of Neoconvallatoxoloside. The glycoside (I) (150 mg) was methylated as described previously [6]. A hydrolyzate of the methylated glycoside was found by paper chromatography in the systems described by Aspinall and Wood [15] to contain 2,3,4,6-tetramethyl-D-glucose and 3,4-dimethyl-L-rhamnose.

SUMMARY

A new cardinolide glycoside has been isolated from the leaves of Convallaria majalis L.; it has been called neoconvallatoxolisode and its structure has been established as strophanthidol 3-O-[O-β-D-glucopyranosyl-(1 → 2)-α-L-rhamnopyranoside].

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ALKALOIDS OF Sophora alopecuroides

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Previously, from Sophora alopecuroides collected on the banks of the R. Zeravshan in the period of incomplete ripening of the fruit, in addition to known alkaloids, we isolated two new bases 6 and 7 [1, 2]. Base 7 has the composition $C_{15}H_{22}O_2N_2$, mp 68-70°C (from ether), $[\alpha]_D^{25}$ + 27.5° (c 0.75; water). The present paper gives the results of a study of the structure of this alkaloid.

The UV spectrum of base 7 has adsorption in the 253 nm region corresponding to the presence of the chromophore (−CH=CH−CO−). The IR spectrum of the alkaloid lacks the absorption band of a trans-quinolizidine (2800-2700 cm^{-1}). Strong absorption is observed in the 1602 and 1658 cm^{-1} region (−CH=CH−CO−N<), and weak absorption at 970, 950, and 925 cm^{-1}, which is characteristic for a N-oxide group [3].

The mass spectrum of the bases has, in addition to the peak of the molecular ion (M^+ 262) confirming the composition of the alkaloid, the peaks of ions with m/e 246 (M−16; 86%), 245 (M−17; 100%), 217 (8%), 203 (12%), 177 (90%), 150 (72%), 138 (42%), 122 (19%), 96 (68%), which are characteristic for the matrine alkaloids [4-6].

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