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NEOCONVALLOSIDE – A CARDENOLIDE GLYCOSIDE FROM PLANTS OF THE GENUS Convallaria

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In a study of the epigeal part and seeds of Convallaria keiskei, C. majalis, and C. transcaucasica, in addition to lokundjoside, convalloside, convallotoxoloside, and neovallotoxoloside, we have isolated the previously unknown glycoside neoconvalloside, for which, on the basis of the physicochemical properties of the compound and of the products of its chemical transformations, the structure of strophanthidin 3-O-[O-β-D-glucopyranosyl-(1 → 2)-α-L-rhamnopyranoside has been established.

Continuing an investigation of the epigeal parts and seeds of the lilies of the valley Convallaria keiskei Miq., C. majalis L., and C. transcaucasica Utkin, in addition to lokundjoside, convalloside, convallotoxoloside, and neoconvallotoxoloside, we have isolated a cardenolide glycoside (I) with the empirical formula $C_{35}H_{52}O_{15}$. Its UV spectrum exhibits two absorption maxima, in the 220 and 303 nm regions (log ε 4.19 and 1.2, respectively), which are characteristic for the butenolide rings and aldehyde groups of cardenolides. The Mannich–Siewert hydrolysis of (I) (scheme) led to its cleavage into D-glucose (V), L-rhamnose (IV), and a number of products of aglycon nature, two of which had R_f values in various systems coinciding with those of strophanthidin (II) and of 5-anhydrostrophanthidin (III).

On the basis of the results obtained, it was possible to assume that the substance was convalloside [2]. However, in contrast to convalloside it was not hydrolyzed by the enzymes of the grape snail [3] or of Aspergillus oryzae [4], which may be connected with the order or position of attachment of the sugar residues in its molecule.

The stepwise hydrolysis of neoconvalloside with 0.1 N sulfuric acid led to the formation of convallotoxin (VI) and D-glucose (V). This showed that the D-glucose residue was the terminal residue.

In the products of the periodate oxidation of the glycoside (I) under investigation we detected L-rhamnose, which indicates the absence of a 1 → 3 bond between the sugar residues.

Thus, the most probable linkage of the D-glucose residue in neoconvalloside to the L-rhamnose residue is by a 1 → 2 glycosidic bond. To confirm this hypothesis we performed the reduction of the isolated glycoside (I) with sodium tetrahydroborate. As a result we

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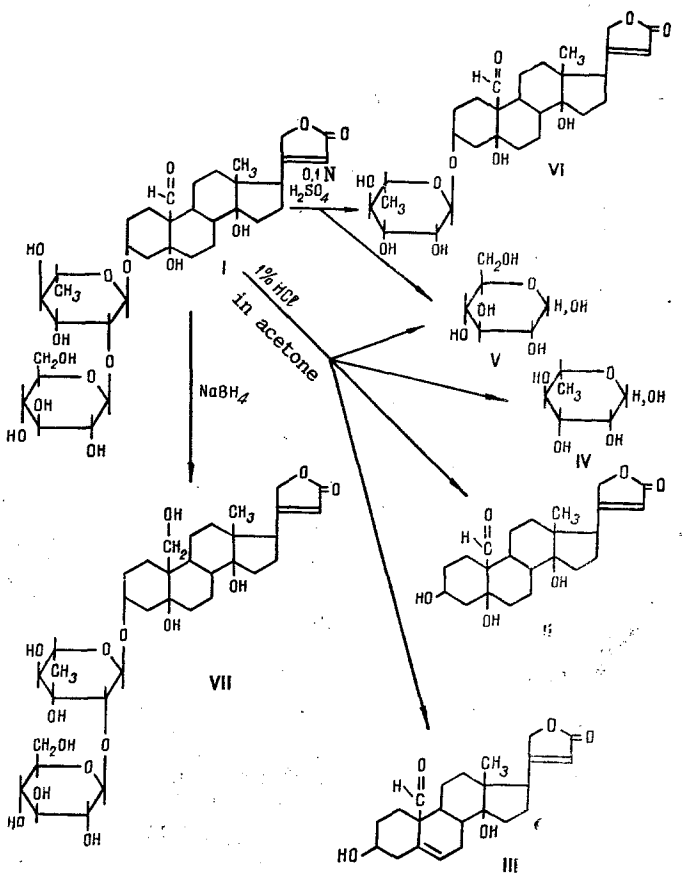

Scheme of the chemical transformations of reoconvalloside (I)

obtained neoconvallotoxoloside (VII), which has previously been isolated from lily of the valley leaves [5]. On the basis of the results obtained, the structure of neoconvalloside can be represented as stroph anthidin 3-O-[O-β-D-glucopyranosyl-(1 → 2)-α-L-rhamnopyranoside.

EXPERIMENTAL

For analysis, the substance was dried in vacuum (10^{-2} mm Hg) at 110-115°C over P_2O_5 for 5 h. Melting points were determined in a Kofler instrument, UV spectra were taken on an EPS spectrophotometer, and optical rotations were determined on a SPU-E instrument with a cell 1 dm long. The aglycon and its derivatives were chromatographed in the following systems: 1) chloroform–formamide; 2) benzene–methyl ethyl ketone (2:1) – water (35%). Glycosides were chromatographed in the following systems; 3) toluene–butan-1-ol (1:1) –water (35%); 4) chloroform–tetrahydrofuran (1:1)–formamide; and monosaccharides in the systems; 5) butan-1-ol–acetic acid–water (4:1:2); and 6) butan-1-ol–methyl ethyl ketone–borate buffer (1:1:2).

The isolation of neoconvalloside from the leaves of Convallaria keiskei, C. majalis, and C. transcaucasica collected in the flowering period and also from the seeds of these species was performed by the following scheme: the cardiac glycosides were extracted from 5 kg of comminuted leaves with 45 liters of 80% ethanol. The extract was evaporated in vacuum to an aqueous residue from which the precipitate of lipophilic substances that had deposited was filtered off, and the filtrate was treated with mixtures of chloroform and ethanol in ratios of 3:1 (7 liters) and 2:1 (10 liters). We studied the glycosidic composition of the chloroform–ethanol (2:1) extract. The cardenolides were extracted from the methylene-chloride-defatted seeds of C. keiskei by a method similar to that described above.

The chloroform–ethanol (2:1) fraction obtained was chromatographed on alumina. The residue after the evaporation of the solvent was deposited on a column (3 × 35 cm) of alumina

(activity grade III). The column was first washed with chloroform containing 10% of ethanol until cardenolides appeared in the eluate, and then the concentration of ethanol was increased to 15-20%. Fractions with a volume of 100 ml were collected, their cardenolide compositions being determined by paper chromatography in system 3.

Lokundjoside (37 mg), convalloside (42 mg), neoconvalloside (57 mg), convallotoxoloside (28 mg), and neoconvallotoxoloside (46 mg) were isolated. The lokundjoside, convalloside, convallotoxoloside, and neoconvallotoxoloside were identified on the basis of their physicochemical properties, their transformation products, comparative chromatography in various systems, and mixed melting points.

Neoconvalloside proved to be previously unknown. The glycoside melted at 162-170°C, $[\alpha]_D^{20}$ − 14°C (c, 0.2; methanol); elementary composition $C_{35}H_{52}O_{15}$. With 84% of sulfuric acid it gave colorations changing with time; 0 sec, reddish; 5-45 min, yellow-brown; 60 min, greenish brown; 75-90 min, dirty green.

Acetylation of Neoconvalloside. A solution of 20 mg of the substance under investigation in 3 ml of anhydrous pyridine was treated with the same amount of acetic anhydride and the mixture was left for a day, after which it was poured into 50 ml of ice water. The crystals that deposited in the form of colorless needles were filtered off and were washed with distilled water, followed by recrystallization from ethanol. This gave 21 mg of neoconvalloside acetate with the elementary composition $C_{47}H_{64}O_{21}$, mp 135-140°C, $[\alpha]_D^{20}$ −23.3° (c 0.7; ethanol). Six acetyl residues were determined in the acetate by the Kuhn–Roth method [6].

Acid Hydrolysis of Neoconvalloside. A suspension of 15 mg of the glycoside in 2 ml of acetone containing 1% of hydrogen chloride was left for 5 days with periodic checking of the degree of hydrolysis by paper chromatography in systems 1 and 2. The hydrolysis products were found to contain stroph anthidin (II), 5-anhydrostrophanthidin (III) [5], and a series of weakly polar products from the degradation of the aglycon. The further working up of the neoconvalloside hydrolysate was performed as described in [7]. On chromatography in systems 5 and 6, D-glucose and L-rhamnose were found as the sugar components.

The enzymatic hydrolysis of the glycoside under investigation was performed with separations of the grape snail as described in [3]. The initial substance was recovered.

Stepwise Hydrolysis. A solution of 15 mg of neoconvalloside in 2 ml of 0.1 N sulfuric acid was heated at 100°C for 6 h. When the hydrolysate was subjected to paper-chromatographic analysis in system 3, a number of substances of cardenolide nature was detected among which convallotoxin (VII) was present in considerable amount.

Periodate Oxidation. The glycoside under investigation (25 mg) was oxidized with 6 ml of a 1% solution of sodium metaperiodate 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 hydrolysate.

Preparation of Neoconvallotoxoloside from Neoconvalloside. A solution of 30 mg of neoconvalloside in 5 ml of methanol was treated with 10 mg of sodium tetrahydroborate and the reaction mixture was left for 3 h, the completeness of reduction being monitored by paper chromatography in system 3. The reduced glycoside was isolated by a procedure described previously [5]. Isopropanol gave 19 mg of crystals with the elementary composition $C_{35}H_{54}O_{15}$, mp 162-165°C, $[\alpha]_D^{20}$ −12° (c 0.2; ethanol). From its physicochemical properties its coloration with 84% sulfuric acid, its R_f value in system 3, and a mixed melting point, the reduced glycoside was shown to be identical with the neoconvallotoxoloside isolated from the lily of the valley species under investigation.

SUMMARY

From the epigeal parts of Convallaria keiskei Miq. C. majalis L., and C. transcaucasica Utkin and the seeds of these species – in addition to lokundjoside, convalloside, convallotoxoloside, and neoconvallotoxoloside – we have isolated the previously unknown glycoside neoconvalloside, and have established its structure as strophanthidin 3-O-[O-β-D-glucopyranosyl-(1 → 2)-α-L-rhamnopyranoside.

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ALKALOIDS OF FOUR SPECIES OF Argemone

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The alkaloid compositions of four species of plants of the genus Argemone (A. mexicana L., A. alba L., A. platyceras Link et Otto., and A. hybrida) collected in the flowering phase in the Tashkent Botanical Garden have been studied. They have yielded 16 alkaloids. Corydine and isocorydine have been detected in plants of this genus for the first time, and O-methylplatycerine has been found in nature for the first time.

Plants of the genus Argemone (family Papaveraceae) are widely distributed in North America [1]. These plants do not grow wild in the Soviet Union and Europe but they are widely cultivated. At the present time, about 40 alkaloids belonging to six groups of isoquinoline bases have been isolated from plants of the genus Argemone [1-22].

We have studied the alkaloid compositions of the epigeal parts of four species of Argemone: A. mexicana L., A. alba L., A. platyceras Link et Otto., and A. hybrida collected in the flowering phase in the Tashkent Botanical Garden. This has led to the isolation of 16 alkaloids, of which O-methylplatycerine has been isolated from a plant for the first time and corydine and isocorydine have been isolated from this genus for the first time; the total yields of alkaloids from the species mentioned as percentages of the weights of the air-dry raw material were 0.49, 0.3, 0.33, and 0.38%, respectively.

Alkaloid	A. mexicana	A. alba	A. platyceras	A. hybrida
Protopine	+	+	+	+
α-Allocryptopine	+		+	+
Scoulerine	+	+		+
Sanguinarine		+	+	+
Cheilanthifoline	+			+
Stylopine α-methiodine			+	
Chelerythrine		+		
Reticuline	+	+	+	+
Berberine	+	+	+	+
Corydine				+
Isocorydine	+			
Magnoflorine			+	
Munitagine			+	+
Platycerine			+	
O-Methylplatycerine			+	
Argemonine			+	

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