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CONVALLATOXOLOSIDE—A NEW CARDIAC GLYCOSIDE FROM SEEDS OF LILY OF THE VALLEY (*CONVALLARIA MAJALIS* L.)

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Until now, only convalloside had been isolated from seeds of lily of the valley ([1,2]). In the present work, results are reported from a study of the glycoside composition of the seeds of this species of lily of the valley,* in particular, the isolation and chemical investigation of a new glycoside—convallatoxoloside.

By paper chromatography of alcoholic extracts of lily-of-the-valley seeds, the presence in them of 9 substances of cardenolide character was established (Fig. 1, sample 1).

To isolate the glycosides, the comminuted and defatted raw material was extracted to exhaustion with boiling 96% ethanol (Legal test). The extract was evaporated, the resinous residue was treated with water, and the solution was filtered through a small layer of aluminum oxide. From the aqueous filtrate purified with chloroform, the glycosides were extracted with chloroform-alcohol mixtures (8.5 : 1.5) and (2 : 1). The qualitative glycoside composition of the resulting extracts was analyzed by paper chromatography (Fig. 1, samples 2 and 3). From the chloroform-alcohol extract (8.5 : 1.5), with the aid of column chromatography on Al_2O_3, there were isolated in the individual crystalline state and identified: desgluco-cheirotoxin (A), convallatoxin ( ), and convallatoxol ( ) (Fig. 1, sample 1).

Fig. 1. 1—Sum of glycosides of seeds of lily of the valley; 2—chloroform-alcohol extract (8.5 : 1.5); 3—chloroform-alcohol extract (2 : 1); 4—substance (convallatoxoloside); 5—monoglycoside of substance (convallatoxol); 6—convalloside restored at the aldehyde group. System: benzene—*n*-butanol (2 : 1); water (35%), time 4 hours, T° 19°. Leningrad "B" paper.

The resinous residue of the chloroform-alcohol extract (2 : 1) was crystallized from alcohol. The substance obtained proved identical with convalloside ( ). To isolate the other glycosides, the mother liquor (after crystallization of convalloside) was chromatographed on a column of Al_2O_3 previously impregnated with water. The column was developed with a mixture of benzene and *n*-butanol (2 : 1), saturated with water. In this way, three substances were isolated in the individual crystalline state: glycoside-VI (= locundioside), previously obtained

Scheme for proving the structure of glycoside : acid hydrolysis of glycoside (I) gives strophanthidol (II), L-rhamnose (III), and D-glucose (IV); enzymatic hydrolysis gives monoglycoside (V) and D-glucose (IV); reduction with NaBH4 gives compound (VI).

Figure 1: Scheme for proving the structure of glycoside : acid hydrolysis of glycoside (I) gives strophanthidol (II), L-rhamnose (III), and D-glucose (IV); enzymatic hydrolysis gives monoglycoside (V) and D-glucose (IV); reduction with NaBH4 gives compound (VI).

from the herb of Far Eastern lily of the valley (^{3}), an additional small amount of convalloside, and glycoside (Fig. 1, sample 1; , , and).

* The glycoside composition of seeds of *C. keiskei* Miq. and *C. transcaucasica* Utkin is similar to that studied.

Glycoside , by its R_f values in various systems, differs from the glycosides previously isolated from different species of lily of the valley. This substance was crystallized from a mixture of isobutyl alcohol and acetone. The crystals are readily soluble in water and alcohol, poorly soluble in acetone, and insoluble in chloroform. Glycoside gives reactions characteristic of cardiac glycosides with a five-membered lactone ring (Raymond, Kedde, and Legal). With 84% H_2SO_4 it forms changing colors: 15 sec—orange, 1 min—pink, 2 min—reddish brown, 5 min—brownish red, 10 min—cherry, 30 min—dark cherry, 1 hr—brown with a violet shade, 2 hr—gray. Melting point 234-236°; $[\alpha]_D^{19} = -16.4 \pm 2^\circ$ ($C = 0.80$ in CH_3OH).

For analysis the substance was dried in vacuo (10^{-2}) at a temperature of 115° for 6 hr over P_2O_5.

Found %:	C 59.21, 58.87;	H 7.72, 7.58
$C_{35}H_{54}O_{15}$(714.78). Calculated %:	C 58.8;	H 7.61

To elucidate the structure of glycoside (see scheme), acid hydrolysis was carried out according to Mannich and Siewert (4). Paper chromatography in various systems of the hydrolysis products established that its aglycone is strophanthidol (II), and the sugar components are *L*-rhamnose (III) and *D*-glucose (IV).

Scheme for proving the structure of glycoside

In order to establish the order of attachment of the sugar residues, the glycoside was subjected to stepwise hydrolysis by enzymes of the fungus *Aspergillus oryzae* (5), as a result of which the monoglycoside (V) was obtained, identical with convallatoxol in its principal properties, coloration with 84% H_2SO_4, R_f value, and IR spectra. The sugar isolated after enzymatic cleavage was identified by melting point and mixed test as *D*-glucose (IV).

The character of the glycosidic bonds was established according to Klyne (6): glycoside ($[M]^{\mathrm{I}}_D = -117.2$)–convallatoxol ($[M]^{\mathrm{V}}_D = -55.2$), convallatoxol ($[M]^{\mathrm{V}}_D = -55.2$)–strophanthidol ($[M]^{\mathrm{II}}_D = +150.4$).

To confirm the results obtained, the aldehyde group of convalloside (VI) was reduced with $NaBH_4$ in pyridine solution, and the substance obtained was identified with glycoside Zh.

Table 1

Determination of the form of glycosidic bonds in glycoside Zh

Name of sugars	$[M]_D$ of methyl glycopyranosides α	$[M]_D$ of methyl glycopyranosides β	$\Delta[M]_D$	Form of glycosidic bond
L-rhamnose ([8], [9])	−112	+170	$[M]^V_D - [M]^{III}_D = -205.6$	α
D-glucose ([7])	+309	−66	$[M]^I_D - [M]^V_D = -62$	β

Thus, it has been established that glycoside Zh is strophanthidol ([3])–α-*L*-rhamno-β-*D*-glucoside, to which, by analogy with other lily-of-the-valley glycosides, the trivial name convallatoxoloside has been assigned.

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Note: Figure translations are in progress. See original paper for figures.