CARDIAC GLYCOSIDES OF Cheiranthus allioni. IX*

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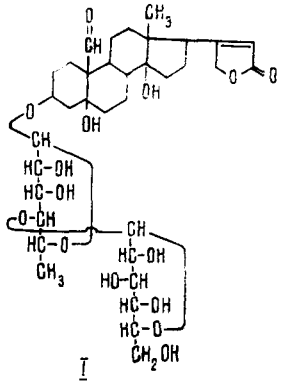
UDC 547.918+547.926+615.711.5

Continuing investigations of the cardenolides of the seeds of Cheiranthus allioni hort., we have isolated another three cardiac glycosides which have been provisionally designated M-87, M-91, and M-92. The method of isolation was similar to that described in preceding papers [2, 3].

Compound M-91 consists of a diglycoside with the composition $C_{35}H_{52}O_{15}$. Under the action of an enzyme preparation from the grape snail it hydrolyzes, forming D-glucose and a monoglycoside of desglucocheirotoxin. This composition corresponds to the known glycoside cheirotoxin obtained previously from another species of Cheiranthus [4]. The properties of these glycosides are also similar. The results of a direct comparison of glycoside M-91 with a sample of cheirotoxin kindly provided by Prof. T. Reichstein showed their identity. To determine the site of attachment of the D-glucose in cheirotoxin we used the method [5] of the selective synthesis of isopropylidene derivatives at cis-α-glycol groups. 2',3'-O-Isopropylidenecheirotoxin was obtained. The enzymatic hydrolysis of the latter split off D-glucose with the formation of 2',3'-O-isopropylidenedesglucocheirotoxin, which shows (for an explanation, see [5]) a 1→4 bond of the D-glucose with the D-gulomethylose.

The acid hydrolysis of cheirotoxin with 0.1 N sulfuric acid solution led to the formation of erycordinobiose – a disaccharide with an established structure [7] (in admixture with D-glucose and D-gulomethylose; identification by paper chromatography).

These results, in combination with the information of previous investigators on the structure of cheirotoxin [4], permit formula (I) to be proposed for it, which characterizes it as strophanthidin 3β-O-[4'-O-β-D-glucopyranosyl-β-D-gulomethylopyranoside]



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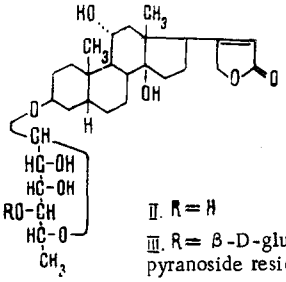

II. R = H

III. R = β-D-glucopyranoside residue

Compound M-87 is a diglycoside with mol.wt. 703 (determined by UV spectrometry [6]); calculated for the composition $C_{35}H_{54}O_{14}$, mol. wt. 698.8. This glycoside, which we have called gulosarmentoglucoside was obtained in small amount (22 mg), and therefore the experimental investigations on it were performed on the micro scale.

The glycoside was hydrolyzed by the Mannich–Siewert method for 2 days, and an aglycone was isolated in the crystalline state which was identified as sarmentogenin. Chromatography of the carbohydrate

*For Communication VIII see [1].

Khar'kov Scientific-Research Institute of Pharmaceutical Chemistry. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 607-611, September-October, 1974. Original article submitted May 8, 1973.

component showed the presence in it of D-glucose, gulomethylose, and erycordinobiose. Since the structure of erycordinobiose is known (4-O-β-D-glucopyranosyl-D-gulomethylose [7]), the structure of the carbohydrate moiety in M-87 is thereby practically determined. The glycoside is therefore sarmentogenin 3β-O-[4'-O-β-D-glucopyranosyl-β-D-gulomethylopyranoside] (III).

The enzymatic hydrolysis of the mother liquors from the gulosarmentoglucoside gave a monoglycoside (II) in the pure state which we have called sarmentogulomethyloside (M-92). This glycoside was also detected, although in very small amount, in the seeds of the plant studied. By chromatography on "medium-polar" alumina of part of the glycosides we isolated a fraction enriched with sarmentogulomethyloside. Then by preparative paper chromatography the monoglycoside was isolated in the individual crystalline state. The identity of the two samples was confirmed by the direct comparison of their properties.

EXPERIMENTAL

In the chromatography of the cardenolides on paper, the following solvent systems were used: 1) toluene–butan-1-ol (1:1.5)/water; 2) chloroform–tetrahydrofuran (1:1)/formamide; 3) methyl ethyl ketone–m-xylene (1:1)/formamide; and 4) benzene/formamide. The sugars were chromatographed in systems 5) butan-1-ol–acetic acid (4:1)/water and 6) butan-1-ol–methyl ethyl ketone–borate buffer (1:1:2) [8].

Cheirotoxin (M-91). The glycoside was crystallized from butanol–ether, mp 198-201°C, $[\alpha]_D^{22}$ −15.8 ± 3° (c 0.65; methanol). With concentrated H_2SO_4 it gave a coloration changing with time: 0 min – red; 1 min – yellow-brown; 180 min – green. $R_{cheirotoxin} = 1.00$ (system 1).

The glycoside (0.11 g) was hydrolyzed with an enzyme preparation from the grape snail (0.1 g, aqueous solution, pH 6.0) for 46 h. Then the enzymes were precipitated with hot ethanol and the solution was concentrated to an aqueous residue. The monoglycoside was extracted with a mixture of chloroform and ethanol (3:1). It was crystallized from water: mp 189-192°C, $[\alpha]_D^{21}$ −6.5 ±3° (c 0.59; methanol). A mixture with desglucocheirotoxin showed no depression of the melting point (189-192°C). $R_{desglucocheirotoxin} = 1.00$ (system 2).

Cheirotoxin (40 mg) was dissolved with heating in acetone, 0.3 g of anhydrous copper sulfate was added, and the mixture was heated in a sealed glass tube at 90°C for 5 min. The solution was rapidly filtered through a layer of kieselguhr and alumina (activity grade III), and the adsorbent was washed with a mixture of ethanol and chloroform (1:2). The filtrate was treated with water (4 × 5 ml) and evaporated. The 2',3'-O-isopropylidenecheirotoxin obtained consisted of an amorphous white powder, $[\alpha]_D^{23}$ −22.2 ± 4° (c 0.64; ethanol), $R_{strophanthidin} = 0.47$ (system 2).

The isopropylidenecheirotoxin (20 mg) was hydrolyzed with the enzymes of the grape snail in neutral aqueous solution by the usual method (see above). The results of a chromatographic analysis showed that the hydrolyzate consisted of D-glucose (system 5) and 2',3'-O-isopropylidenedesglucocheirotoxin (systems 2 and 3; in system 2, $R_{strophanthidin} = 1.14$). In the identification of the latter compound, it was compared with a sample of 2',3'-O-isopropylidenedesglucocheirotoxin obtained previously [5].

Gulosarmentoglucoside (M-87). Melting point 204-207°C (acetone), $[\alpha]_D^{23}$ −23.9 ± 4° (c 0.43; ethanol); molecular weight found 703 (for the method of determination, see [6]). With concentrated H_2SO_4 it formed the following colorations: 0 min – yellow-brown; 50 min – brown; 70 min – violet; 165 min – blue-violet.

The gulosarmentoglucoside (14 mg) was dissolved in 15 ml of a mixture of acetone and concentrated hydrochloric acid (99:1 by volume), and the solution was left at room temperature (21-22°C) for 44 h. Then 30 ml of water was added and the acetone was evaporated off in vacuum at 45-50°C. The residue was heated additionally at this temperature for 30 min. The aglycone part was extracted with a mixture of chloroform and ethanol (4:1; 3 × 40 ml). The ethanolic chloroform solution was washed with a 2 N solution of sodium carbonate (5 ml) and with water (4 × 5 ml) and was evaporated. The residue was chromatographed on 0.5 g of alumina (activity grade IV). For elution we used mixtures of chloroform and benzene (6:4 to 9:1), chloroform, and chloroform–ethanol (98:2). The aglycone was crystallized from ethanol–water, mp 262-265°C. A mixture with a sample of sarmentogenin melted at 262-266°C; $R_{sarmentogenin} = 1.00$ (system 3). The aqueous solution containing the carbohydrate components of the glycoside was neutralized with silver carbonate at 0-3°C and filtered. The filtrate was saturated with hydrogen sulfide, likewise with cooling, and was filtered through a layer of kieselguhr. On paper chromatography (systems 5 and 6) three reducing sugars were found at the levels of D-glucose, D-gulomethylose, and erycordinobiose.

Sarmentogulomethyloside (M-92). The dry residue from the mother solutions (15 mg) remaining after the crystallization of the gulosarmentoglucoside was hydrolyzed by the enzymes of the grape snail as for the enzymatic hydrolysis of cheirotoxin. The monoglycoside obtained was purified by chromatography on alumina (activity grade III), using as eluent mixtures of chloroform and ethanol (90:10 to 80:20). The sarmentogulomethyloside melted at 307-309°C; $[\alpha]_D^{22} -33.5 \pm 5°$ (c 0.26; ethanol), $R_{alliotoxin} = 0.78$ (system 2).

One of the fractions of "medium-polarity" cardenolides obtained previously [2, 3] (in the separation of the combined glycosides on alumina) consisted, according to paper chromatography, of sarmentogulomethyloside in admixture with a series of other monoglycosides. This fraction was separated preparatively in system 2. The formamide was driven off from sections of paper cut out at the level of the desired glycoside by heating to 100-110°C, and they were then extracted with ethanol–chloroform (1:3). The extracts were washed with 0.5 N sulfuric acid solution, with water, with 1 N sodium carbonate solution, and with water again, and were evaporated. The residue was crystallized from ethanol. The glycoside so obtained melted at 307-310°C; a mixture with a sample of sarmentogulomethyloside showed no depression of the melting point (307-310°C).

SUMMARY

From the seeds of Cheiranthus allioni hort. another three cardiac glycosides have been isolated. One of them has been identified as cheirotoxin. It has been established that in cheirotoxin the D-glucose is attached to C_4 of the D-gulomethylose and this glycoside therefore has the structure of strophanthidin 3β-O-[4'-O-β-D-glucopyranosyl-β-D-gulomethylopyranoside]. The two other glycosides, which have been named sarmentogulomethyloside and gulosarmentoglucoside are new and are, respectively, sarmentogenin-3β-O-β-D-gulomethylopyranoside and sarmentogenin 3β-O-[4'-O-β-D-glucopyranosyl-β-D-gulomethylopyranoside].

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