CARDIAC GLYCOSIDES OF Cheiranthus allioni. V

I. F. Makarevich

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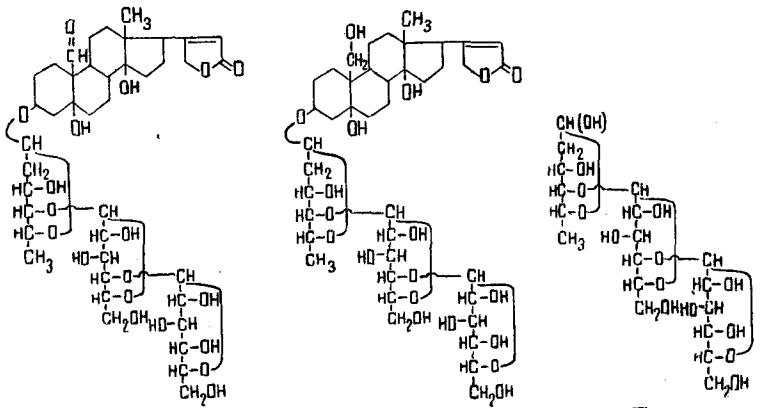
As already reported, we have obtained eleven cardiac glycosides provisionally ascribed to the group of moderately polar glycosides from the seeds of Cheiranthus allioni Hort. [= Erysimum marschalli (Stark) Boiss]. From the highly polar substances, by partition chromatography, we have isolated two additional cardiac glycosides which, after the determination of their structure, have been called "glucoerysimoside" and "glucoerysimosol."

Maksyutina [5, 6] obtained glucoerysimoside from plants of the family Cruciferae in the form of a complex compound. From the later paper [6] it is also known that it consists of strophanthidin 3-[O-β-D-digitoxopyranosyl-(4 → 1)-O-β-D-glucopyranosyl-(4 → 1)-β-D-glucopyranoside]. Glucoerysimoside was found even earlier by Kaiser et al. [7] in strophanthus seeds. However, no information on the preparation of glucoerysimoside in the pure state and its properties is given in the literature [5-7]. Nor is there any evidence in favor of the structure given, namely: the attachment position of the terminal D-glucose, the size of its oxide ring, and the configuration of the glycoside bond. Consequently, in this paper it is appropriate to give a description of the properties and those experimental facts which characterize the structure of glucoerysimoside.

Glucoerysimoside has the composition $C_{41}H_{62}O_{19}$, which corresponds to a steroid triglycoside. The substance gives positive Legal, Raymond, and Kedde reactions. With the Keller–Kiliani reagent it gives a green color. It has an absorption maximum in UV light at 218 nm (log ε 4.16), which is characteristic for a butenolide ring. The glycoside has a fairly high biological activity (0.175 mg/kg body weight of the cat, determined by L. Ya. Topchii). Under the action of an enzyme preparation obtained from the pancreatic juice of the grape snail, it hydrolyzes, forming the known monoglycoside erysimin and D-glucose.



I II III

In a study of the dynamics of this process, it was found that the initial triglycoside is hydrolyzed almost completely in 3 h with the formation of the final product (erysimin) and an intermediate cardenolide which appears on chromatograms at the level of erysimoside, and which is also gradually converted into erysimin. Thus, in order to isolate the assumed erysimoside from the triglycoside, the latter was subjected to partial enzymatic hydrolysis, and the mixture of resulting cardenolides was separated into its individual components by adsorption chromatography on alumina. The monoglycoside and diglycoside that were obtained in the

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TABLE 1

Substance	$[M]_D$, deg
Glucoerysimoside, mol. wt. 858.9, $[\alpha]_D$ +11.6 ± 3°	+99.6 ± 25.8
Diglycoside from glucoerysimoside (erysimoside), mol. wt. 696.8, $[\alpha]_D$ +19.2 ± 2°	+133.8 ± 13.9
Monoglycoside from glucoerysimoside (erysimin), mol. wt. 534.6, $[\alpha]_D$ +28.7 ± 3°	+153.5 ± 15.0
Terminal D-glucose moiety of glucoerysimoside	−34.2 ± 39.7
D-Glucose moiety of the diglycoside	−19.6 ± 32.9
Methyl β-D-glucopyranoside	−66.4 [10, 11]
Methyl α-D-glucopyranoside	+308.6 [10, 11]

pure state were identified as erysimin and erysimoside, respectively. As is well known [8], erysimoside is strophanthidin 3-[O-β-D-digitoxopyranosyl-(4 → 1)-β-D-glucopyranoside], i.e., its carbohydrate component is digilanidobiose.

An analysis of the molecular rotations of the mono-, di-, and triglycosides in accordance with Klyne's rule [9] shows that both molecules of D-glucose are attached by β-glycoside bonds. The figures for determining the configuration of the glycoside bonds in glucoerysimoside are given in Table 1.

To establish the attachment position of the terminal glucose residue and the size of its oxide ring, glucoerysimoside was subjected to exhaustive methylation by Kuhn's method, and the methylated compound was hydrolyzed. The following hydrolysis products were identified: 2,3,4,6-tetra-O-methyl-D-glucose; 2,3,6-tri-O-methyl-D-glucose; and D-cymarose (3-O-methyl-D-digitoxose). The substances mentioned were identified by paper chromatography in various solvent systems using developers which differentiate the methylated sugars to a considerable extent by the color of the spots (see Experimental). The results obtained permit the conclusion that the carbohydrate component of glucoerysimoside forms an unbranched chain in which the monosaccharide residues are present in the pyranose form and are connected to one another by 1 → 4 glycoside bonds.

On the basis of all that has been said, glucoerysimoside may be characterized as strophanthidin 3-[O-β-D-digitoxopyranosyl-(4 → 1)-O-β-D-glucopyranosyl-(4 → 1)-β-D-glucopyranoside] (I). This is in agreement with the structure proposed by Maksyutina [6].

The hydrolysis of glucoerysimoside with 0.05 N sulfuric acid at 80°C enables the trisaccharide to be split off smoothly. Under these conditions the glucose is not hydrolyzed off, which confirms its pyranose form in the glycoside. The trisaccharide, which has been called cellobiosyldigitoxose, was obtained in the pure crystalline state. Elementary analysis confirmed the expected composition $C_{18}H_{32}O_{14}$. On the basis of the transformations of the described glycoside, the structure of the trisaccharide may be represented by formula III and it may be characterized as O-β-D-glucopyranosyl-(1 → 4)-O-β-D-glucopyranosyl-(1 → 4)-D-digitoxose.

Glucoerysimosol has the composition $C_{41}H_{64}O_{19}$, which also corresponds to a steroid triglycoside. UV spectrum: $\lambda_{max}^{ethanol}$ 217 nm (log ε 4.16). Enzymatic hydrolysis gives helveticosol (strophanthidol 3-O-β-D-digitoxopyranoside) and D-glucose. Acid hydrolysis of glucoerysimosol carried out under mild conditions made it possible to obtain strophanthidol and an oligosaccharide identical with cellobiosyldigitoxose. Consequently, it was natural to assume that the described glycosides differ only by the substituents at C_{10} in the aglycone moiety. To confirm this hypothesis, glucoerysimoside was reduced with sodium borohydride. The resulting reduction product proved to be identical with glucoerysimosol. Thus, this glycoside may be considered as strophanthidol 3-[O-β-D-digitoxopyranosyl-(4 → 1)-O-β-D-glucopyranosyl-(4 → 1)-β-D-glucopyranoside] (formula II).

EXPERIMENTAL

The substances were analyzed after being dried over P_2O_5 at 80°C in vacuo (0.01 mm Hg) for 5 h. The sugars were revealed on paper with aniline phthalate [12], p-anisidine hydrochloride [13], aniline trichloroacetate [13], diphenylamine trichloroacetate [13], and the diphenylamine–urea and diphenylamine – p-anisidine phosphate reagents [14].

The combined polar glycosides were obtained as described previously [1] and were separated by partition column chromatography in a toluene–butan-1-ol (1 : 2)–water system. Silica gel was used as the support for the stationary phase. The ratio of the combined cardenolides to the silica gel (taken on the dry weight) was 1 : 300. The fractions obtained from the column were analyzed by paper chromatography in the same system. The glucoerysimoside and glucoerysimosol were crystallized from butan-1-ol.

Glucoerysimoside. The glycoside melts at 203-206°C, $[\alpha]_D^{18}$ +11.6 ± 3° (c 0.73, methanol). Its biological activity is 0.175 mg/kg body weight of the cat. With conc. H_2SO_4 it forms a color which changes with time: 0 min, green; 30 min, lemon-yellow; 140 min, light brown; and 350 min, brown. Found %: C 57.25; H 7.53. Mol. wt. 858.3 (spectroscopic method). $C_{41}H_{62}O_{19}$. Calculated %: C 57.33; H 7.27. mol. wt. 858.9.

Enzymatic Hydrolysis of Glucoerysimoside. The glycoside (0.3 g) and a dry enzyme preparation obtained from the pancreatic juice of the grape snail (0.4 g) were dissolved in 25 ml of water, and the solution was kept at 37-38°C for 40 h. The enzymes were precipitated with 150 ml of hot ethanol and separated by filtration. The filtrate was treated with chloroform. The ethanol–chloroform extract was evaporated and the residue, consisting of erysimin monoglycoside, was crystallized from methanol–benzene. The monoglycoside obtained gave a positive Keller–Kiliani reaction (blue color), mp 147-151°C, $[\alpha]_D^{19}$ +28.7 ± 3° (c 1.08, methanol). A mixture with authentic erysimin gave no depression of the melting point (147-152°C). On paper chromatography it had the same R_f value as erysimin.

After the elimination of erysimin, the aqueous solution was evaporated. The residue was dissolved in 90% ethanol, and the solution was purified with activated carbon and again evaporated. The monosaccharide was crystallized from ethanol–ether. The substance melted at 145-146°C, $[\alpha]_D^{19}$ +53.1 ± 5° (c 0.72, aqueous solution after 2 h). A mixture with an authentic sample of D-glucose gave no mp depression (145-146°C). The phenylosazone with mp 207-208°C was obtained.

Methylation of Glucoerysimoside. A solution of 0.2 g of glucoerysimoside in 8 ml of dimethylformamide was treated with 10 ml of tetrahydrofuran, 4 ml of methyl iodide, and 8 g of freshly prepared silver oxide. The reaction mixture was heated with constant stirring for 9 h. During the reaction, an additional 5 ml of methyl iodide and 7 g of silver oxide were added. The solid matter was filtered off and washed with chloroform, and the filtrate was evaporated in vacuo. The reaction product was remethylated in a similar manner.

Hydrolysis of Methylated Glucoerysimoside. The methylated glucoside was dissolved in 17.5 ml of acetic acid, and 27.5 ml of water and 5 ml of conc. HCl were added. The mixture was heated in a boiling water bath for 1 h and then neutralized with NH_3 and evaporated in vacuo. To separate the methylated sugars from the salts, the residue was treated with a mixture of chloroform and ethanol (2 : 1 : 70 ml) and filtered through a layer of kieselguhr and evaporated. The residue was again dissolved in 20 ml of ethanol–chloroform (1 : 2). The heated solution was treated with 1 g of alumina (activity grade III), stirred for 5 min, and filtered. The residue was washed with 15 ml of pure, hot solvent. The filtrate was evaporated, and the residue was investigated by paper chromatography in benzene–methyl ethyl ketone (1 : 1)/water and the butanol–acetic acid (4 : 1)/water systems.

Partial Enzymatic Hydrolysis of Glucoerysimoside. Isolation and Identification of Erysimoside. Glucoerysimoside (0.4 g) and an enzyme preparation from the grape snail (0.6 g) were dissolved successively in 30 ml of water, and the solution was kept at 23°C for 2 h. The reaction was stopped by precipitating the enzymes with 300 ml of hot ethanol. The precipitate was filtered off, and the solution was concentrated in vacuo to a volume of about 30 ml and treated with a mixture of chloroform and ethanol (2 : 1.5 × 70 ml). The extract was evaporated and the glycosides were separated by chromatography on 15 g of alumina. The column was eluted with ethanol–chloroform (5 : 95–25 : 75). The first eluates contained pure erysimin. Subsequent elution with chloroform–ethanol (80 : 20) gave pure erysimoside, which was crystallized from ethanol. It had mp 235-240°C, $[\alpha]_D^{18}$ +19.2 ± 2° (c 0.94, methanol). On being dissolved in conc. H_2SO_4, the glycoside formed a green color changing to green-brown after 3 min and to brown after another 3 min. A mixture with a sample of erysimoside gave no mp depression (235-241°C).

Cellobiosyldigitoxose and Strophanthidin. A solution of 0.31 g of alliotrioside in 50 ml of 0.05 N H_2SO_4 was heated at 80°C for 45 min. The solution was neutralized with barium carbonate and filtered through a layer of kieselguhr. The filtrate was treated with a mixture of chloroform and ethanol (9 : 1; 7 × 100 ml). The ethanol–chloroform extract was washed with 30 ml of water and evaporated. The residue, which consisted of the aglycone of glucoerysimoside, was crystallized from ethanol.

The aglycone had a double melting point, 140-145°C/228-232°C, $[\alpha]_D^{20}$ +44.3 ± 4° (c 0.73, methanol). On dissolution in conc. H_2SO_4, it formed the following colors which changed with time: 0 min, yellow-green; 4 min, green-orange; and 20 min, green. These results, and also those of a mixed mp and paper chromatography, show the identity of the aglycone with strophanthidin.

The aqueous solution containing the carbohydrate component was evaporated in vacuo. The residue was dissolved in 2 ml of water, 10 ml of ethanol was added, and the solution was filtered. The filtrate was concentrated in vacuo to a syrupy consistency. After the addition of ethanol, crystals of the trisaccharide deposited. They were separated off and recrystallized.

The cellobiosyldigitoxose melted at 195-199°C, $[\alpha]_D^{19}$ +18.5 ± 3° (c 1.00, aqueous solution after 2 h). On enzymatic hydrolysis, D-digitoxose and D-glucose were formed (identification by paper chromatography). The elementary analysis agreed with that calculated for $C_{18}H_{32}O_{14}$.

Glucoerysimosol. The melting point of the glycoside was 260-262°C, $[\alpha]_D^{20}$ +15.8 ± 4° (c 0.65, methanol). With conc. H_2SO_4 it formed a color which changed with time: 3 sec, yellow; 10 sec, green; 30 sec, brown; and 5 h, gray-green. The elementary analysis and molecular weight agreed with those calculated for $C_{41}H_{64}O_{19}$.

Enzymatic Hydrolysis of Glucoerysimosol. Glucoerysimosal (0.15 g) was hydrolyzed by means of an enzyme preparation from the grape snail, and the hydrolysis products were separated as described above for glucoerysimoside. The monoglycoside was crystallized from ethanol, mp 165-170°C, $[\alpha]_D^{18}$ +26.9 ± 5° (c 0.45, methanol). A mixture with a sample of helveticosol gave no melting point depression (165-171°C). On paper chromatography, these substances exhibited similar R_f values.

The monosaccharide posseses the same properties as the monosaccharide obtained by the enzymatic hydrolysis of glucoerysimoside, and was identified as D-glucose.

Acid Hydrolysis of Glucoerysimosol. Glucoerysimosol (0.12 g) was hydrolyzed in the same manner as glucoerysimoside in 0.05 N H_2SO_4. After the usual working up of the hydrolyzate, the aglycone and an oligosaccharide were obtained in the pure state. The aglycone melted at 138-143°C (acetone), $[\alpha]_D^{19}$ +37.1 ± 5° (c 0.45, methanol). A mixture with a sample of strophanthidol gave no melting point depression (138-143°C). Paper chromatography also showed the identity of the substance with strophanthidol.

On paper chromatography, the oligosaccharide had the same R_f value as cellobiosyldigitoxose. It melted at 194-198°C. A mixture of the substances gave no melting point depression (194-199°C).

Reduction of Glucoerysimoside. During a period of 25 min 130 mg of sodium borohydride (in small portions) was added to a solution of 0.1 g of glucoerysimoside in 10 ml of 80% dioxane. The solution was neutralized with 0.1 N H_2SO_4, and then 150 mg of mannitol and 30 ml of a saturated aqueous solution of sodium chloride were added and the mixture was treated with ethanol–chloroform (1:2, 5 × 50 ml). The ethanol–chloroform extract was dried over anhydrous Na_2SO_4, filtered, and evaporated. The residue was chromatographed on a column containing 30 g of cellulose, using a toluene–butan-1-ol (1:2)/water system. The reduced glycoside of glucoerysimosol had mp 259-262°C (butan-1-ol), $[\alpha]_D^{20}$ +14.5 ± 5° (c 0.53, methanol). A mixture with natural glucoerysimosol gave no melting point depression (259-262°C). On paper chromatography, the substances appeared at the same level.

CONCLUSIONS

Two additional cardiac glycosides, glucoerysimoside and glucoerysimosol, have been isolated from the seeds of Cheiranthus allioni Hort. Glucoerysimoside is strophanthidin 3-[O-β-D-digitoxopyranosyl-(4 → 1)-O-β-D-glucopyranosyl-(4 → 1)-β-D-glucopyranoside]. Glucoerysimosol is a new cardenolide having the same carbohydrate component, but its aglycone is strophanthidol. From these glycosides a new trisaccharide has been obtained which has been characterized as 4-O-β-cellobiosyl-D-digitoxose.

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