CARDIAC GLYCOSIDES OF Cheiranthus allioni. VII*

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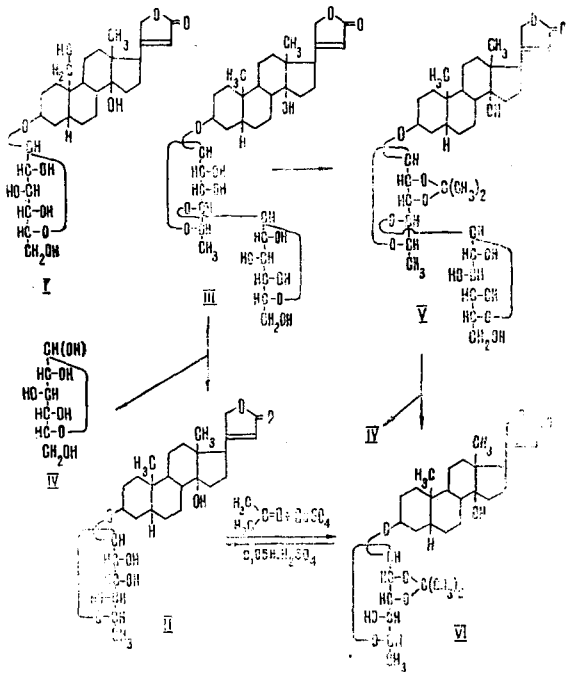
By the method described previously [2], from the seeds of Cheiranthus allioni hort. (plains erysimum) we have additionally isolated four cardiac glycosides which have provisionally been denoted by the symbols M-57, M-70, M-72, and M-73. They were all obtained from the "medium-polarity" cardenolide fraction (see [2]).

Glycoside M-70 has the composition $C_{29}H_{42}O_{11}$; on enzymatic hydrolysis, strophanthidin and D-glucose were formed. In composition and properties it corresponds to glucostrophanthidin [3]. As was assumed, the strophanthidin 3β-O-β-D-glucopyranoside that we synthesized (see Experimental) and M-70 proved to be identical.

Cardenolide M-72 is a monoglycoside, $C_{29}H_{44}O_8$. Its acid hydrolysis by the Mannich-Siewert method [4] gave the pure aglycone, which was identified as digitoxigenin, and L-rhamnose. The properties of M-72 are extremely close to those of the known glycoside evomonoside [5] – digitoxigenin 3β-O-α-L-rhamnopyranoside (II). The results of a direct comparison of M-72 with the evomonoside that we had obtained [6] from the spindle-tree [6] showed their identity. The IR spectra of the substances also coincided.

Glycoside M-73 has the composition $C_{29}H_{44}O_{10}$. Its UV spectrum is characterized by a single absorption maximum at 218 nm (log ε 4.16), which is due to the presence of a butenolide ring. Under the action of an enzyme preparation from the grape snail, glycoside M-73 is hydrolyzed to cannogenol and D-glucose.



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This compound is hydrolyzed by dilute mineral acids under fairly severe conditions, which shows the pyranose form of the D-glucose residue. Analysis of the molecular rotations of the glycoside and the aglycone in accordance with Klyne's rule [7] shows that the D-glucose is attached to the aglycone by a β-glycosidic bond (the molecular rotation of the sugar component in M-73 is $87 \pm 24°$). Thus, the cardenolide M-73 can be characterized as cannogenol 3β-O-β-D-glucopyranoside, and its structure can be represented by formula (I). The independent synthesis of the glycoside performed on the micro scale confirmed the proposed structure.

Glycoside M-57 has the composition $C_{35}H_{54}O_{13}$, corresponding to a steroid diglycoside. In its UV spectrum there is a single absorption maximum at 217 nm (log ε 4.16; butenolide ring). On enzymatic hydrolysis, M-57 forms a monoglycoside – evomonoside – and a monosaccharide – D-glucose. On this basis, it may be assumed that the glycoside is evobioside [8], or an isomer of it. In spite of the substantial difference in melting points (mp of evobioside 195-198°C [8] and that of glycoside M-57, 267-271°C), a sample of evobioside kindly given to us by Professor T. Reichstein proved to be identical with M-57 in its chromatographic behavior and its reactions with conc. sulfuric acid and antimony trichloride.

The glycoside was not hydrolyzed to an appreciable extent by 0.05 N sulfuric acid at 80°C in 30 min, which shows the pyranose form of the monosaccharide residues.

In view of the fact that the position of attachment of the D-glucose had not previously been determined for evobioside, we performed some transformations of the glycoside. First, it was subjected to periodate oxidation. Hydrolysis of the oxidized cardenolide and chromatographic analysis of the hydrolysate showed the complete absence of both D-glucose and L-rhamnose from it. According to this, a 1→3 linkage of the monosaccharide residues is excluded. We then used the method of the selective production of isopropylidene derivatives at cis-α-glycol groupings [9]. Evobioside M-57 readily reacts with acetone in the presence of anhydrous copper sulfate at room temperature with the formation of compound (V). On enzymatic hydrolysis, this compound gave D-glucose and isopropylideneevomonoside (VI). Substance (VI) was obtained in the pure form and shown to be identical with the 2',3'-O-isopropylideneevomonoside, obtained directly from evomonoside (II). The IR spectrum of (VI) confirmed the presence of an isopropylidene grouping in it (maxima at 1380 and 1375 cm^{-1}). On acid hydrolysis under mild conditions, the isopropylideneevomonoside (VI) was completely reconverted into evomonoside (II).

Thus, the formation of 2',3'-O-isopropylideneevomonoside (V) permits the unambiguous conclusion that in evobioside M-57 the cis-α-glycol grouping in the L-rhamnose residue is free and this residue is bound to the D-glucose by means of the C_4' hydroxy group.

EXPERIMENTAL

For analysis, the substances were dried in vacuum (0.01 mm Hg) over phosphorus pentoxide at 110°C. Paper chromatography was carried out with the following solvent systems: 1) tetrahydrofuran–chloroform (1:1)/formamide; 2) methyl ethyl ketone–m-xylene (1:1)/formamide; 3) benzene/formamide; 4) butan-1-ol–acetic acid (4:1)/water; and 5) methyl ethyl ketone–butan-1-ol–borate buffer (1:1:2). The molecular weights of the cardenolides were determined spectrophotometrically [10].

The combined glycosides of "medium" polarity were obtained as described previously [2] and were separated by partition column chromatography in the toluene–butan-1-ol (8:1 to 1:1)/water systems. The support for the stationary phase was silica gel or cellulose. The ratio of the combined cardenolides to the dry support was 1:300. The fractions were obtained by means of a collector and were analyzed by paper chromatography in solvent systems 1 and 2.

Glucostrophanthidin (M-70). The glycoside crystallized from isopropanol; mp 170-173°C $[\alpha]_D^{21}$ + 18.8 ± 2° (c 1.20; methanol). With conc. H_2SO_4 it gave the following time-dependent coloration: 0 min – red; 5 min – brown; 160 min – green. The results of elementary analysis corresponded to those calculated for the composition $C_{29}H_{42}O_{11}$.

The glycoside (60 mg) and a dry enzyme preparation (40 mg) obtained from the pancreatic juice of the grape snail were dissolved in 5 ml of water, and the solution was kept at 41-42°C for 30 h. The enzymes were precipitated with ethanol (90 ml) and were separated by filtration through a layer of kieselguhr. The filtrate was evaporated in vacuum. Then by the usual treatment with water and chloroform–ethanol (4:1), the hydrolysate was separated into aglycone and carbohydrate fractions. The aglycone fraction

yielded 26 mg of crystalline strophanthidin with mp 229-232°C (ethanol), which was shown to be the aglycone mentioned by direct comparison with an authentic sample. The aqueous fraction contained a sugar component identical with D-glucose according to paper chromatography (systems 4 and 5).

Synthesis of Strophanthidin 3β-O-β-D-Glucopyranoside. A solution of 1 g of anhydrous strophanthidin in 100 ml of dichloroethane was treated with 4 g of silver carbonate and 1 g of calcium oxide, and the reaction mixture was heated to the boil. Boiling was maintained and stirring was continued while 2 g of crystalline 2,3,4,6-tetra-O-acetyl-D-glucosyl bromide was added over 5 min. Then the mixture was boiled for another 7 min. The solution was filtered through a layer of kieselguhr under vacuum. The filtrate was evaporated, the residue was dissolved in 20 ml of methanol saturated with ammonia and the solution was left to stand for 18 h. Then it was evaporated in vacuum. The residue was dissolved in 200 ml of chloroform–ethanol (2 : 1) and 20 ml of water. The aqueous layer was separated off and the ethanolic-chloroformic layer was additionally treated with water (2 × 15 ml) and evaporated. To free the glycoside from contaminating strophanthidin, it was twice precipitated from concentrated acetone solution with hot benzene, and was then crystallized from isopropanol.

The glucostrophanthidin synthesized (0.52 g) had mp 170-174°C; $[\alpha]_D^{22}$ + 19.2 ± 2° (c 1.50; methanol). On enzymatic hydrolysis it gave strophanthidin and D-glucose. A direct comparison of the glycoside synthesized and M-70 by paper chromatography (system 1), by a mixed melting point (170-174°C), and by the reactions with conc. H_2SO_4 and with $SbCl_3$ showed their identity.

Evomonoside (M-72). The cardenolide crystallized from methanol; mp 239-242°C; $[\alpha]_D^{22}$ −28.9 ± 2° (c 1.00; ethanol). With conc. H_2SO_4 it formed the following colorations: 0 min – yellow; 45 min – pink; and 70 min – violet. The results of elementary analysis corresponded to the calculated figures. Found: mol. wt. 523.1. $C_{29}H_{44}O_8$. Calculated: mol. wt. 520.7.

The glycoside (110 mg) was dissolved in 20 ml of acetone, and then 0.2 ml of conc. hydrochloric acid was added and the mixture was left at 20-24°C for 10 days. After this, the solution was diluted with 40 ml of water and the acetone was driven off in vacuum. Then the reaction mixture was heated under reflux at 80°C for 20 min. The aglycone was extracted with chloroform (4 × 40 ml). The chloroform solution was treated with 2 N sodium acetate (5 ml) and with water (4 × 10 ml) and was evaporated. The residue was chromatographed on a column of alumina (3 g, activity grade III), elution being performed with chloroform–benzene (1 : 1). The crystalline aglycone (35 mg) with mp 250-255°C (methanol), $[\alpha]_D^{23}$ + 18.4 ± 2° (c 0.95; chloroform) deposited. When chromatographed on paper, the aglycone showed the same R_f values as digitoxigenin. A mixture with an authentic sample of digitoxigenin showed no depression of the melting point (250-256°C). From the aqueous part of the hydrolysate after the elimination of the acid with silver carbonate a monosaccharide was obtained; mp 90-95°C (acetone). On paper chromatography (systems 4 and 5) it was identified as L-rhamnose. Melting point of the phenylosazone, 185-188°C.

The IR spectra of glycoside M-72 and evomonoside, taken on a UR-10 instrument using crystalline forms in potassium bromide, were completely identical. The results of paper chromatography (systems 1 and 2) and a mixed melting point (mp 239-242°C) confirmed the identity of M-72 as evomonoside.

Glucocannogenol (M-73). The glycoside crystallized from acetone; mp 225-229°C; $[\alpha]_D^{22}$ + 7.0 ± 3° (c 0.92; ethanol). With conc. H_2SO_4 it gave the following colorations: 0 min – yellow; 60 min – lemon-yellow; 120 min – brown; 170 min – reddish-brown. The results of elementary analysis corresponded to the calculated figures. Found: mol. wt. 549.4. $C_{29}H_{44}O_{10}$. Calculated: mol. wt. 552.7.

The glycoside (50 mg) was hydrolyzed with an enzyme preparation from the grape snail as described above. This gave a crystalline aglycone with mp 235-239°C. When chromatographed on paper, it showed the same R_f values as cannogenol. A mixed melting point (mp 235-239°C) and the reaction with conc. H_2SO_4 also showed the identity of these substances. On chromatography in systems 4 and 5, the sugar component proved to be identical with D-glucose. The phenylosazone melted at 206-208°C. The results of a direct comparison of it with a sample of D-glucose phenylosazone by paper chromatography (systems 1 and 2) and a mixed melting point (206-208°C) showed their identity.

Glycoside M-73 was scarcely hydrolyzed by 0.05 N H_2SO_4 at 80°C for 30 min (analysis by paper chromatography).

The cannogenol (20 mg) was acetylated with acetic anhydride in pyridine at 22°C for 10 min. The 19-O-acetylcannogenol obtained, which was contaminated with cannogenol and with 3,19-di-O-acetylcannogenol,

was glycosylated with 2,3,4,6-tetra-O-acetyl-D-glucosyl bromide as in the experiment described above. After saponification with ammonia and the elimination of the D-glucose (treatment of an ethanolic-chloroformic solution with water), the reaction product, without further purification, was analyzed by paper chromatography. The glucocannogenol synthesized and glycoside M-73 proved to have identical R_f values.

Evobioside (M-57). The glycoside crystallized readily from acetone, butan-1-ol, and ethanol; mp 267-271°C (ethanol), $[\alpha]_D^{22}-24.3 \pm 2°$ (c 1.00; methanol). With conc. H_2SO_4 it gave the following colorations: 0 min – yellow; 60 min – light brown; 120 min – orange; 180 min – red; and 280 min – pink. The results of elementary analysis corresponded to the calculated figures. Found: mol. wt. 685.2. $C_{35}H_{54}O_{13}$. Calculated: mol. wt. 682.7.

The glycoside (120 mg) was dissolved in 0.5 ml of dimethylformamide, and 70 mg of an enzyme preparation from the grape snail dissolved in 20 ml of water was added. The solution was kept at 41-42°C for 5 days. Then the hydrolysate was treated as described above. A monoglycoside and a monosaccharide were obtained in the pure state. The monoglycoside had mp 239-242°C (methanol), $[\alpha]_D^{20}-28.3 \pm 2°$ (c 0.94; ethanol). On paper chromatography (systems 1 and 2) its R_f values were the same as for evomonoside. A mixture of these substances gave no depression of the melting point (239-242°C). The monosaccharide was identical with D-glucose with respect to its melting point (145-146°C, from ethanol–ether), a mixed melting point (145-146°C), and paper chromatography (systems 4 and 5).

The glycoside (3 mg) was dissolved in 0.4 ml of methanol, and then 0.4 ml of 0.1 N sulfuric acid was added and the solution was heated in a sealed glass tube at 80°C for 30 min. The acid was neutralized with barium carbonate. A chromatographic analysis of the hydrolysate showed that the glycoside suffered practically no change under this treatment.

Periodate Oxidation of Evobioside. A solution of 20 mg of evobioside in 100 ml of 50% acetone was treated with 0.1 ml of acetic acid and 0.15 g of sodium metaperiodate. The solution was kept at 20-22°C for 46 h and was then evaporated in vacuum to an aqueous residue. The oxidized cardenolide was extracted with a mixture of chloroform and ethanol (2 : 1). The ethanolic-chloroformic extract was evaporated, and the residue was hydrolyzed by the Mannich-Siewert method [4]. When the hydrolysate was analyzed by paper chromatography (in system 4), no D-glucose or L-rhamnose were detected.

2',3'-O-Isopropylideneevobioside. With heating, 80 mg of evobioside was dissolved in 300 ml of acetone, and then 2 g of finely ground anhydrous copper sulfate was added and the mixture was shaken in a shaking apparatus at 20-21°C for 3 h. After the solvent had been filtered off through a layer of kieselguhr and alumina (activity grade III), the solution was evaporated in vacuum (20-22°C). The residue was dissolved in chloroform–ethanol (3 : 1; 30 ml), and the solution was washed with water (3 × 3 ml), and reevaporated. On paper chromatography in solvent system 3, the resulting amorphous 2',3'-O-isopropylideneevobioside (containing a less polar cardenolide as impurity) had $R_{digitoxigenin} = 0.17$; $[\alpha]_D^{21}-16.0 \pm 4°$ (c 0.96; ethanol).

2',3'-O-Isopropylideneevomonoside. Evomonoside (40 mg) was subjected to reaction with acetone in a similar manner, except that the reaction was carried out for 5 h. For purification, the reaction product was chromatographed on alumina (2 g, activity grade III) and was eluted with a mixture of chloroform and benzene (2 : 3). The 2',3'-O-isopropylideneevomonoside crystallized from methanol–water; mp 129-132°C; $[\alpha]_D^{23}-12.1 \pm 4°$ (c 0.55; chloroform). The results of elementary analysis corresponded to the composition $C_{32}H_{48}O_8$. With conc. H_2SO_4 the substance gave the following colorations: 0 min – yellow; 3 min – yellow-brown; 20 min – orange; 40 min – red; 120 min – red-violet. In solvent system 3, $R_{digitoxigenin} = 1.70$.

Enzymatic Hydrolysis of 2',3'-O-Isopropylideneevobioside. A solution of 70 mg of isopropylideneevobioside (V) in 5 ml of ethanol was treated with 70 mg of an enzyme preparation (from the grape snail) dissolved in 50 ml of water, and the solution was left at 41-42°C for 6 days. During the reaction, two further portions of enzyme preparation (30 mg each) were added. Then, by the usual treatment (see above), the hydrolysate was separated into carbohydrate and cardenolide fractions. The cardenolide fraction, after purification on alumina, gave pure isopropylideneevomonoside, with mp 128-132°C; $[\alpha]_D^{21}-13.2 \pm 4°$ (c 0.67; chloroform). A mixture with a sample of 2',3'-O-isopropylideneevomonoside gave no depression of the melting point (128-132°C). The chromatographic results (systems 2 and 3) also showed the identity of the substances. The IR spectrum of a 2% chloroform solution taken in the 4000-700 cm^{-1} region had absorption bands due to an isopropylidene group (1380 and 1375 cm^{-1}), the carbonyl group of a butenolide ring (1785 and 1744 cm^{-1}), the C=C bond of a butenolide ring (1634 cm^{-1}), and hydroxy groups (3605 and 3470 cm^{-1}). The carbohydrate fraction of the hydrolysate contained D-glucose (paper chromatography).

Acid Hydrolysis of 2',3'-O-Isopropylideneevomonoside to Evomonoside. A solution of 10 mg of isopropylideneevomonoside in 0.3 ml of ethanol was treated with 0.3 ml of 0.1 N sulfuric acid and the mixture was heated in a sealed glass tube at 90°C for 20 min. After the acid had been neutralized with barium carbonate, the filtered hydrolysate was analyzed by paper chromatography (systems 1 and 2). It was found that the isopropylideneevomonoside had hydrolyzed completely. The more polar chromatographically individual cardenolide formed had the same R_f values as evomonoside.

SUMMARY

A further four cardiac glycosides have been isolated from the seeds of Cheiranthus allioni hort. Three of them have been identified as glucostrophanthidin (strophanthidin 3β-O-β-D-glucopyranoside), evomonoside (digitoxigenin 3β-O-α-L-rhamnopyranoside, and evobioside (glucoevomonoside). It has also been established that in evobioside the D-glucose is attached to C_4' of the L-rhamnose and the glycoside has the structure digitoxigenin 3β-[O-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranoside]. The fourth glycoside is new; it is cannogenol 3β-O-β-D-glucopyranoside.

LITERATURE CITED

1. I. F. Makarevich, Khim. Prirodn. Soedin., 40 (1971).
2. I. F. Makarevich, O. I. Klimenko, and D. G. Kolesnikov, Khim. Prirodn. Soedin., 188 (1969).
3. R. Mauli, C. Tamm, and T. Reichstein, Helv. Chim. Acta, 40, 305 (1957); F. C. Uhle and R. C. Elderfield, J. Org. Chem., 8, 162 (1943).
4. C. Mannich and G. Siewert, Ber., 75, 736 (1942).
5. H. Hauenstein, A. Hunger, and T. Reichstein, Helv. Chim. Acta, 36, 87 (1953).
6. S. G. Kislichenko, I. F. Makarevich, and D. G. Kolesnikov, Khim. Prirodn. Soedin., 440 (1966).
7. W. Klyne, Biochem. J., 47, No. 4 (1950).
8. A. Meyrat and T. Reichstein, Pharm. Acta Helv., 23, 135 (1948). F. Šantavy and T. Reichstein, Helv. Chim. Acta, 31, 1655 (1948).
9. I. F. Makarevich, Khim. Prirodn. Soedin., 267 (1969).
10. I. F. Makarevich and S. G. Kislichenko, Khim. Prirodn. Soedin., 125 (1972).