CARDIAC GLYCOSIDES OF Cheiranthus allioni.

XI*

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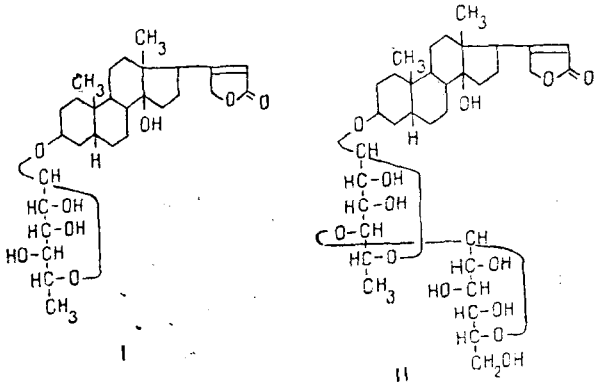
Another three cardiac glycosides have been isolated from the seeds of Cheiranthus allioni hort. (Erysium asperum; plains erysium) (family Cruciferae). One of them has been identified as digifucocellobioside – digitoxigenin 3β-O-[O-β-D-glucopyranosyl(1→ 4)-O-β-D-glucopyranosyl(1 → 4)-O-β-D-fucopyranoside].

The second cardenolide was first isolated after the enzymatic hydrolysis of the polar fractions of the glycosides of plains erysium. Having available a sample of this substance, we then found it as a native product in a mixture of erysimin, evomonoside, and other monoglycosides of the plant under investigation. An additional amount of the pure cardenolide was obtained from this mixture by preparative paper chromatography.

The results of elementary analysis and of a molecular-weight measurement (mol. wt. 520, mass spectrum) corresponded to the molecular formula $C_{29}H_{44}O_8$.

The hydrolysis of the cardenolide with acid yielded an aglycone and a monosaccharide, and those were identified as digitoxigenin and D-gulomethylose. In view of this composition of the substance, we have called it digitoxigenin gulomethyloside (I).

The mass spectrum of digitoxigenin gulomethyloside is characterized by the presence of a series of peaks belonging to the molecular ion of the glycoside (M^+ 520; 5.7%), to that of the aglycone (M^+ 374; 9.1%) and to their fragments: m/e 357 (aglycone – H_2O + 1; 77.5%); 339 (357 – H_2O; 55.9%); 246 (32%); 203 (100%; $C_{15}H_{23}$ [12]); 162 (20%); 147 (37.6%); 124 (22.8%); 121 (35.9%); 111 (42.2%); 107 (53.6%); 105 (44.5%); 95 (57%); 93 (58.1%); 91 (59.3%); 81 (58.1%); 79 (57%) and others.



The following functional groups have been observed in the PMR spectrum of digitoxigenin gulomethyloside (I) (ppm): 5.91, singlet of a CH group at the double bond of a butenolide ring (at C_{22}); 4.92, broadened signal of a methylene group of a butenolide ring (at C_{21}); 4.64, doublet of the anomeric proton of a sugar residue with J = 7.5 Hz, which corresponds to a 1',2'-axial–axial interaction of the protons, i.e., to an equatorial β-glycosidic bond; 1.29, doublet of a CH_3 group of the carbohydrate component with J = 6.5 Hz; 0.98 ppm, singlet of an angular methyl group (at C_{10}); and 0.92, singlet of an angular methyl group (at C_{13}).

Thus, digitoxigenin gulomethyloside is 3β-O-(β-D-gulomethylopyranosyl)-14-hydroxy-5β-card-20(22)-enolide (I).

*For Communication X, see [1].

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The properties of the digitoxigenin gulomethyloside that we isolated (mp 150-152°C, $[\alpha]_D$ −22° in methanol) and of the glycoside described previously under the same name (mp 225-230°C, $[\alpha]_D$ −9.9° in methanol [3]) differ considerably. It is possible that are different compounds. Such a hypothesis is confirmed further by the fact that the structure of the glycoside described previously [3] and, in particular, the identification of the carbohydrate component, was given provisionally, and the magnitude of the specific rotation (9.9°) does not agree well with the suggested structure.

The third glycoside, which, after having determined its structure, we called glucodigigulomethyloside (II) was isolated via the isopropylidene derivative. The use of this method was due to the necessity for separating a fraction consisting of two isomeric glycosides. One of them was the glycoside under investigation and the other glucodigifucoside [4, 5]. The two glycosides are very close in polarity, and their separation by adsorption or ion-exchange chromatography proved to be difficult.

Assuming that in the glycoside under investigation the carbohydrate component had the same structure as in erycordin and glucosarmentogulomethyloside, which have been isolated previously from the same plant [6-9], i.e., in the D-gulomethylose residue the cis-α-glycol grouping is free, we subjected the isomeric mixture to reaction with acetone under mild conditions (see Experimental). As was expected, under this treatment the desired glycoside formed a monoisopropylidene derivative (acetonide), and the isomeric glycoside, not having the glycol grouping mentioned, remained unchanged.

The polarities of the acetonide of the cardenolide under study and of glucodigifucoside differed markedly; consequently their separation presented no difficulty. By means of adsorption chromatography on alumina, the acetonide was obtained in the pure state.

On enzymatic hydrolysis, D-glucose was split off and a cardenolide of low polarity was formed – the acetonide of a monoglycoside. This test confirms that the isopropylidene grouping is present in the D-gulomethylose moiety.

In order to obtain the diglycoside (II) under investigation from the acetonide, the latter was hydrolyzed with dilute acetic acid, the course of the reaction being monitored by paper chromatography. After chromatography on alumina, the glucodigigulomethyloside was obtained in the pure crystalline state. The molecular weight of the cardenolide (II) determined by means of the UV spectrophotometric method [10] was 677; calculated for the composition $C_{35}H_{54}O_{13}$, 682.8.

The enzymatic hydrolysis of substance (II) led to the formation of D-glucose and the monoglycoside digitoxigenin gulomethyloside (I).

Analysis of the molecular rotation of the aglycone and of the mono- and diglycosides in accordance with Klyne's rule showed that both the D-gulomethylose and the D-glucose are bound by β-glycosidic bonds – the molecular rotation of the D-glucose moiety is minus 104 ± 30° and of the D-gulomethylose moiety minus 184 ± 18° (compare with the solutions of the methyl α- and β-glycosides of these sugars given in the literature [8]).

The fact that glucodigigulomethyloside (II) forms a monoacetonide shows a 1 – 4 bond of the monosaccharide units. To confirm this conclusion, partial acid hydrolysis was performed. Analysis of the hydrolyzate by paper chromatography showed the presence in it of erycordinobiose (together with D-glucose and D-gulomethylose. We have determined the structure of erycordinobiose previously [7]; it is 4-O-(β-D-glucopyranosyl)-D-gulomethylose.

The results obtained permit the cardenolide (II) to be characterized as 3β-O-(4'-O-β-D-glucopyranosyl-β-D-gulomethylopyranosyl)-14-hydroxy-5β-card-20(22)-enolide.

EXPERIMENTAL

The mass spectra were taken on a Varian-CH-8 instrument at a temperature of the internal inlet tube of 230°C and an energy of the ionizing electrons of 75 eV. The PMR spectrum was recorded on a R-20A spectrometer with a working frequency of 60 MHz at 34°C. The solvent was deuterochloroform. Tetramethylsilane was used as internal standard.

The glycosides were isolated by methods described previously (see [13, 14]). An exception was glucodigigulomethyloside (II), the isolation of which in the pure state was achieved via the isopropylidene derivative (see below).

Digifucocellobioside. The glycoside was crystallized from ethanol; mp 270-275°C; $[\alpha]_D^{21} = -6.4 \pm 3°$ (c 0.78; 85% ethanol).

The molecular weight, determined by the UV-spectrophotometric method [10], was 855. The results of elementary analysis corresponded to the composition $C_{41}H_{64}O_{18}$; calculated mol. wt. 845.

Enzymatic hydrolysis was performed by the usual method (see [4]). The aglycone so obtained melted at 248-252°C; $[\alpha]_D^{20}$ + 18.4 ±3° (c 0.64; methanol). A mixture with digitoxigenin gave no depression of the melting point (248-252°C); $R_{digitoxigenin}$ = 1.00. According to paper chromatography, the carbohydrate moiety consisted of two monosaccharides the R_f values of which corresponded to D-glucose and D-fucose.

Partial acid hydrolysis was performed with 0.1 N sulfuric acid at 100°C for 3 hours, and analysis of the hydrolyzate by paper chromatography showed the presence in it of cellobiose, in addition to other sugars.

Digititoxigenin Gulomethyloside. The glycoside had mp 150-152°C (60% methanol); $[\alpha]_D^{20}$ −22.2 ±2° (c 1.46; methanol). UV spectrum: $\lambda_{max}^{ethanol}$ 219 nm (log ε 4.17). With concentrated H_2SO_4 the substance formed the following colorations changing with time: 0 min, faint yellow; 20 min, lemon yellow; 35 min, brown; 90 min, red; 220 min, violet.

Its elementary analysis and molecular weight (see the mass spectrum) corresponded to the composition $C_{29}H_{44}O_8$.

The cardenolide was hydrolyzed with 0.1 N sulfuric acid for 2 hours at 100°C. The hydrolyzate was neutralized with barium carbonate and was separated by the usual treatment into carbohydrate and aglycone fractions. Chromatography of the aglycone fraction on alumina (activity grade III) using methylene chloride as eluent gave digitoxigenin and Δ^{14}-anhydrodigitoxigenin.

Digitoxigenin, mp 243-250°C; a mixture with a sample of the aglycone melted at 243-251°C; in paper chromatography, $R_{digitoxigenin}$ = 1.00.

The carbohydrate component had $R_{D\text{-gulomethylose}}$ = 1.00; its phenylosazone had mp 175-179°C a mixture with a sample of D-gulomethylose phenylosazone melted at 175-181°C.

Glucodigigulomethyloside. A mixture (0.56 g) of the two isomeric glycosides obtained by adsorption chromatography on alumina of the total cardenolides of plains erysium was dissolved in 50 ml of acetone, and 5 g of anhydrous copper sulfate was added; then the mixture was stirred for 20 h and 100 ml of a mixture of chloroform and ethanol (3 :1) was added. The precipitate was filtered off. The solution was evaporated and the residue was chromatographed on 40 g of alumina (activity grade III). Mixtures of methylene chloride and ethanol (98 :2-80 :20) were used as eluents. The fractions containing the less polar cardenolide yielded the amorphous but chromatographically individual 2',3'-O-isopropylideneglucodigigulomethyloside; $[\alpha]_D^{19}$ −37.8 + 5° (c 0.87; methanol).

The acetonide was hydrolyzed with 3.5% acetic acid in 80% ethanol at 70°C for 20 min. Then a double volume of chloroform was added and the solution was neutralized with sodium bicarbonate, washed with water, and evaporated. The residue after purification by alumina was crystallized from aqueous solution.

The glucodigigulomethyloside melted at 179-182/197-198°C; $[\alpha]_D^{20}$ = −32.1 ±3° (c 0.70; methanol). With concentrated H_2SO_4 it formed the following colorations: 0 min, pale yellow; 15 min, orange; 95 min, red; 190 min, violet.

The molecular weight determined by the UV-spectrophotometric method [10] was 677; calculated for the composition $C_{35}H_{54}O_{13}$, 682.8.

The glucodigigulomethyloside (40 mg) was hydrolyzed with an enzyme preparation from the grape snail. The enzyme was precipitated and the hydrolyzate was worked up in the usual way (see [11]). This gave a crystalline monoglycoside having mp 149-151°C, $R_{digitoxigenin\ gulomethyloside}$ = 1.00. A mixture with digitoxigenin gulomethyloside isolated from the plant gave no depression of the melting point (149-152°C).

SUMMARY

Another three cardiac glycosides have been isolated from the seeds of Cheiranthus allioni hort. One of them has been identified as digifucocellobioside (digitoxigenin 3β-O-[O-β-D-glycopyranosyl(1 → 4)-O-β-D-glucopyranosyl(1 → 4)-O-β-D-fucopyranoside]). The two other glycosides, called digitoxigenin gulomethyloside and glucodigigulomethyloside are, respectively, digitoxigenin 3β-O-β-D-gulomethyloside and digitoxigenin 3β-O-(4'-O-β-D-glucosyl-β-D-gulomethyloside).

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STRUCTURE AND ABSOLUTE CONFIGURATION OF COLLUTINE

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Continuing an investigation of the mixture of bases from the epigeal parts of Colchicum luteum Baker, we have isolated, in addition to substances known previously [1, 2], a new compound which we have called collutine. Collutine has the composition $C_{21}H_{25}O_5N$, mp 192-194°C, $[\alpha]_D$ −182° (c 1.7; chloroform), mol. wt. 371 (mass spectrometry). From the nature of its UV spectrum, with an adsorption maximum at 238 nm (log ε 4.3) and an inflection at 275 nm (log ε 2.81), this base resembles compounds of the homoproaporphine and homomorphinandienone series, which are biogenetically related to the tropolone alkaloids [3, 4]. The IR spectrum of the base (Fig. 1) shows the presence of an α, β-unsaturated carbonyl group and of an aromatic nucleus (1660, 1630, 1600, and 1560 cm^{-1}) and of hydroxy (3450 cm^{-1}) and methylene (2940, 1455 cm^{-1}) groups. In its mass spectrum, collutine differs sharply from the homoproaporphine alkaloids and is close to the homomorphinandienone compounds. Its mass spectrum has the main peaks of ions with m/e 371 (M^+, 100%), 356 (16%), 340 (10%), 328 (10%), and 210 (22%). From the specific color reaction with concentrated sulfuric acid [5], this base can also be assigned to the group of compounds of the type of androcymbine.

The NMR spectrum of collutine (Fig. 2) shows the signals of three methoxy groups (3.98, 3.80, and 3.59 ppm), a N-methyl group (2.35 ppm), and three aromatic protons (one-proton singlet at 6.77 ppm and two-proton singlet at 6.22 ppm).

On methylation with diazomethane, collutine formed a methyl ether which was chromatographically identical with O-methylandrocymbine [6]. This shows that the oxygen substituents in the homomorphinandienone skeleton of collutine are present in the same positions as in androcymbine.

On the basis of the results of a comparison of the spectral characteristics of collutine and known homomorphinandienone alkaloids, a structure with a hydroxy group at C_2, C_3, C_4, or C_6 may be proposed for this base. Structures with the hydroxy group at C_2 or C_3 are excluded for collutine, since they correspond to the alkaloid CC-10 [7] and to androcymbine [8]; the structure with the hydroxy group at C_6 can also be excluded, since the three-proton singlet at 3.59 ppm corresponds to an olefinic methoxy group in ring D [9]. Thus, the most probable structure for collutine is that with the hydroxy group in position C_4, i.e., the structure of 4-hydroxy-2,3,6-trimethoxyhomomorphinandienone: (See scheme on next page.)

On the basis of literature information [10], the one-proton singlet at 6.77 ppm in the NMR spectrum of collutine can be assigned to the C_5 position and the two-proton singlet at 6.22 ppm to C_1 and C_8.

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