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SCORPIOSIDE—A CARDENOLIDE GLYCOSIDE FROM CORONILLA SCORPIOIDES

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The isolation from the seeds of Coronilla scorpioides (L). Koch. (scorpion coronilla) of—in addition to corotoxigenin, frugoside, glucocorotoxigenin, and coronillobioside—a substance (IV) to which the name scorpioside was given, has been reported previously [1]. This glycoside (Fig. 1a, sample 1) has a maximum in the UV spectrum in the 218 mμ region (log ε 4.20), which is characteristic for a butenolide ring, and also a maximum at 303 mμ (log ε 1.50) relating to the absorption of an aldehyde group. The IR spectrum of the substance has absorption bands at 1787, 1735, and 1622 cm^{-1} (five-membered lactone ring) and also at 2755 and 1716 cm^{-1} (aldehyde group). The acetylation of the glycoside led to a tetraacetyl derivative. Scorpioside, in contrast to glucocorotoxigenin and coronillobioside, is not cleaved by emulsin [2] or by the enzymes of the seeds of C. scorpioides and of the fungus Aspergillus oryzae [3]. On acid hydrolysis, the glycoside is split into an aglycone (Fig. 1b, sample 3) and glucose (Fig. 1c, sample 9).

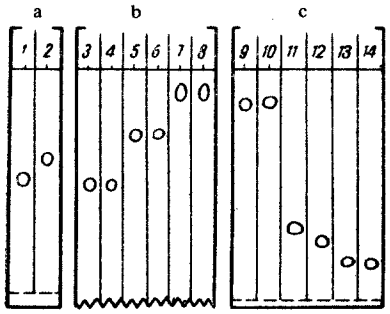

Fig. 1. Sketch of chromatograms of scorpioside and the products of its transformations: 1) scorpioside; 2) strophanthidin 3-O-β-D-glucopyranoside; 3) aglycone of scorpioside; 4) strophanthidin; 5) reduced aglycone of scorpioside; 6) strophanthidol; 7) phenylosazone of the sugar component of scorpioside; 8) phenylosazone of D-glucose; 9) sugar component of scorpioside; 10) D-glucose; 11) 2, 3, 6-trimethyl-D-glucose; 12) 2, 3, 4, 6-tetramethyl-D-glucose; 13) methylated sugar component of scorpioside; 14) 2, 3, 5, 6-tetramethyl-D-glucose.

In the molecule of the aglycone, three hydroxyl groups have been detected previously by IR spectroscopy [4], and of these only two are capable of undergoing acetylation. In the IR spectrum, an absorption band in the 1033 cm^{-1} region shows the axial arrangement of the hydroxyl group on a tertiary carbon atom. The optical rotatory dispersion (ORD) spectrum (Fig. 2) shows a curve with a negative Cotton effect. This gives grounds for assuming that rings A and B have a cis linkage. On the basis of the results obtained, the aglycone is apparently identical with strophanthidin. The results of a comparison of the physicochemical properties of an authentic sample of strophanthidin and the aglycone of scorpioside, their IR, UV, and optical rotatory dispersion spectra (see Fig. 2), their R_f values (see Fig. 1b, samples 3 and 4), and the R_f values of their reduction products (Fig. 1b, samples 5 and 6) show the complete identity of the substances mentioned.

Consequently, scorpioside is strophanthidin 3-O-glucoside. For a definite elucidation of its structure, it was necessary to determine whether the glucose belonged to the D or the L series and also to find the configuration of the glucosidic bond and the size of the oxide ring.

Scorpioside differs from strophanthidin 3-O-β-D-glucopyranoside [5] both in its R_f value (see Fig. 1a, samples 1 and 2) and in some physicochemical properties. The glycoside under investigation is not hydrolyzed by a number of enzymes which hydrolyze strophanthidin 3-O-β-D-glucopyranoside [5, 6]. Its resistance to enzymatic cleavage possibly depends on the structural features of the glucose, which may belong to the L series [7, 8] or have an α-glucosidic link, like scilliglaucoside [9], or a furanose oxide ring [2, 10]. The ease of fermentation of the glucose obtained and the positive optical rotation indicate that it belongs to the D series [7, 8]. On the basis of Klyne's rule [11], we have found a β-glucosidic linkage in the glucoside (table).

Thus, it must be assumed that the compound under investigation is strophanthidin β-D-glucofuranoside. This is confirmed by the resistance of the glucoside to cleavage with emulsin, which is characteristic for glucofuranosides [excluding the aryl β-D-glucofuranosides (II)] and was first observed for them as long ago as 1914 by Fischer [2, 10]. In addition to this, β-glucofuranosides generally have more negative optical rotations than β-glucopyranosides. This rule is obeyed by scorpioside (see table). In this case it may be expected that the optical rotatory dispersions spectrum of the glycoside under investigation should be similar to that of strophanthidin 3-O-β-D-glucopyranoside but shifted in the direction of the negative region. On a comparison of the ORD spectra of the compounds under consideration (Fig. 2), this assumption is completely confirmed.

It is known that furanosides are hydrolyzed by dilute mineral acids several times faster than pyranosides [13]. The acid hydrolysis of scorpioside by Mannich and Siewert's method [14] is complete in four days, and that of strophanthidin 3-O-β-D-glucopyranoside in 14-15 days, the latter substance being hydrolyzed by dilute solutions of acids with the destruction of the aglycone.

For a definitive proof of the size of the oxide ring of the glucose in scorpioside we performed its exhaustive methylation [15, 16]. After hydrolysis of the methylated product, 2, 3, 5, 6-tetra-O-methyl-D-glucose was identified (see Fig. 1c, sample 13).

Thus, the structure of scorpioside can be represented as 3β-(β-D-glucofuranosyloxy)-5, 14β-dihydroxy-19-oxo-5β-card-20(22)-enolide. This compound is the first cardiac glycoside with the carbohydrate component having a furanose oxide ring.

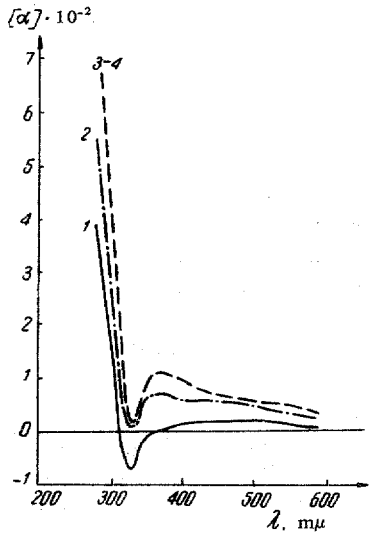

Fig. 2. Optical rotatory dispersion (ORD) spectra (c 0.1; methanol): 1) scorpioside; 2) strophanthidin 3-O-β-D-glucopyranoside; 3) aglycone of scorpioside; 4) strophanthidin.

Experimental

The substances for analysis were dried in high vacuum over P_2O_5 at 110–115° C for 5 hr. The melting points were determined on a Kofler block, the optical activities and optical rotatory dispersion (ORD) spectra on a SPU instrument, the UV spectra on EPS-3 instrument, and the IR spectra on a UR-10 spectrophotometer (tablets with potassium bromide); chromatography was carried out on paper of type FN-1, fast, in the following solvent systems: 1) benzene–butan-1-ol (1 : 1)/water (35%), 4 hr; 2) chloroform–formamide, 3 hr; 3) butanol–acetic acid–water (1 : 1 : 2), 16 hr.

Isolation of scorpioside. In the preparation of scorpioside, account was taken of its resistance to cleavage by enzymes. The other glycosides accompanying it (glucocorotoxigen, coronillobioside) were hydrolyzed to D-glucose and corotoxigenin and the latter and the frugoside were readily separated from the substance under investigation. The comminuted seeds (3 kg) were wetted with water and were then placed in a thermostat at 38–40° C for three days. After the fermentation, the cardenolides were extracted with 70% ethanol and the extract was concentrated to an aqueous residue which was treated with chlorchloroform and then with mixtures of chloroform and ethanol (8.5 : 1.5 and 3 : 1).

The chloroform extract yielded coumarins [17] and corotoxigenin, and the aqueous ethanolic (85 : 1.5) extract yielded frugoside. Paper chromatography showed the presence of a single substance (IV) in the chloroformic ethanol (3 : 1) extract. To isolate it, the extract was evaporated, the residue was dissolved in water, and the solution was filtered through a small layer of neutral alumina. The aqueous filtrate was treated with a mixture of chloroform and ethanol (2 : 1) until the cardenolides had passed into the organic phase completely, and the latter was then evaporated to a dry residue which was crystallized from ethanol. This gave 4.13 g of scorpioside with mp 267–269° C, $[\alpha]_D^{20}$ +8.0 (c 0.2; methanol). With 84% H_2SO_4 it forms a coloration changing with time, min: 0) yellow; 1–10) yellow-orange; 15–20) orange; 30) orange-red; 45) cherry red-orange; 60–90) reddish orange; 120–150) pink-gray; 180) gray precipitate.

Found, %: C 61.18; H 7.63. Calculated for $C_{29}H_{42}O_{11}$, %: C 61.47; H 7.47.

Tetra-O-acetylscorpioside. The glycoside (100 mg) was acetylated by the usual method [18]. This gave 79 mg of scorpioside acetate with mp 247–250° C (from ethanol), $[\alpha]_D^{20}$ +21.0° (c 0.2; chloroform).

Found, %: C 60.18; H 6.99. Calculated for $C_{37}H_{50}O_{15}$, %: C 60.48; H 6.86.

The optical rotatory dispersion of scorpioside acetate (methanol, c 0.08, 21° C: 589 mμ + 21.3°; 400 mμ + 56.2°; 350 mμ +118.7°; 307 mμ +66.2°; 300 mμ +67.5°; 285 mμ +112.5°.

Substance	Mol. wt	$[\alpha]_D$, deg.	$[M]_D$, deg.
Scorpioside	556.6	+ 8.0 (CH_3OH)	+ 45.3
Strophanthidin 3-O-β-D-glucopyranoside	556.6	+19.8 (CH_3OH)	+112.2
Strophanthidin	404.5	+42.0 (CH_3OH)	+169.9
Proportion of $[M]_D$ for the D-glucose of scorpioside	—	—	—124.6
Proportion of $[M]_D$ for the D-glucose of strophanthidin 3-O-β-D-glucopyranoside	—	—	— 57.7
Methyl α-D-glucofuranoside [10]	194.2	+118.0 (H_2O)	+229.2
Methyl β-D-glucofuranoside [10]	194.2	— 77.0 (H_2O)	—149.5
Methyl α-D-glucopyranoside [8]	194.2	+158.2 (H_2O)	+308.6
Methyl β-D-glucopyranoside [8]	194.2	— 34.2 (H_2O)	— 66.4

Acid hydrolysis of scorpioside. A) Hydrolysis by Mannich and Siewert's method [13]. To a solution of 600 mg of the glycoside in 50 ml of anhydrous acetone was added 0.5 ml of conc HCl, and the mixture was left in a dark place at room temperature. The hydrolysis process was monitored by paper chromatography in the chloroform–formamide system (3 hr).

Strophanthidin 3-β-D-glucopyranoside was hydrolyzed in parallel under similar conditions.

The hydrolysis of the scorpioside was complete in four days, and that of the strophanthidin 3-β-D-glucopyranoside in twelve days. The further treatment of the hydrolysate was carried out by the usual method [18]. An aglycone (159 mg) with the composition $C_{23}H_{32}O_6$, mp 135–141° C/222–224° C, $[\alpha]_D^{20}$ +42.0° (c 0.2; methanol) was isolated which formed a monoacetyl derivative $C_{25}H_{34}O_7$ with mp 247–250° C, $[\alpha]_D^{20}$ +56.0° (c 1.0; chloroform). A mixture of the genin of the substance studied with strophanthidin gave no depression of the melting point.

B) Hydrolysis with 0.05 N H_2SO_4. A solution of 20 mg of scorpioside in 3 ml of 0.05 N H_2SO_4 was heated at 100° C. The hydrolysis process was monitored by paper chromatography. The bulk of the glycoside was split to form the aglycone in 10 hr. When strophanthidin 3-O-β-D-glucopyranoside was hydrolyzed under similar conditions, only traces of the products of its hydrolysis were formed.

The reduction of the aglycone with sodium borohydride [4] gave strophanthidol, $C_{23}H_{34}O_6$, mp 220–222° C (methanol–ether), $[\alpha]_D^{20}$ +37.0° (c 0.8; ethanol).

Sugar component. After the extraction of the aglycone with chloroform, the aqueous extract was evaporated to small volume and the chloride ion was bound by freshly-precipitated silver carbonate. The clear filtrate was treated with a current of H_2S in the cold and the precipitate that deposited was filtered off. The solution was filtered through a layer of washed animal charcoal. The filtrate was evaporated to a syrupy residue, and to this was added a small amount of isopropanol. After 1.5 months, crystals of a sugar deposited (91 mg) with mp 161–164° C, $[\alpha]_D^{20}$ +91.5° (c 0.2; water, after 3 min), +58.8° C (after 5 hr). The IR spectrum of the sugar had absorption bands at 928, 866, 805, and 773 cm^{-1}, well agreeing with the type of absorption A, B, C, and D described for ethyl α-D-glucofuranoside [19], and also bands at 1065 and 1028 cm^{-1}, which are characteristic for tetrahydrofuran and its derivatives [20]. These results show that the D-glucose crystallizes from a syrup containing traces of isopropanol apparently in the furanose.

The fermentation of the sugar isolated with yeast took place rapidly. A mixture of the sugar component of scorpioside and D-glucose (mp 148–152° C) melted in the range 148–164° C, i.e., gave no depression of the melting point. Their R_f values in different systems were identical.

Phenylosazone of the sugar component of scorpioside. The phenylosazone was obtained by the usual method [8] from 300 mg of the sugar syrup separated after the hydrolysis of scorpioside. The brown-yellow crystals that deposited melted at 201–204° C. They showed no depression of the melting point with an authentic sample of D-glucose phenylosazone and their IR spectra were completely identical (for paper chromatography, see Fig. 1b, samples 7 and 8).

Methylation of scorpioside. A solution of 700 mg of the glycoside in 5 ml of methanol was treated with 10 ml of methyl iodide and 7 g of silver oxide. The reaction mixture was kept at a gentle boil for 3 days. Then the silver oxide was filtered off, the methanol and methyl iodide were evaporated off, and the operation was repeated similarly twice more. Final methylation was carried out in 10 ml of freshly-distilled dimethylformamide with 7 g of silver oxide, the reaction mixture being stirred and heated at 70° C for 3 hr, and then the mixture was left at room temperature for 48 hr. Chromatography of the methylated product in the benzene–formamide and chloroform–formamide systems revealed a single spot. The silver oxide was filtered off and the filtrate was evaporated in vacuum.

The dry residue of the methylated glycoside was hydrolyzed with a mixture consisting of 15 ml of methanol and 15 ml of 2 N HCl for 2 hr. The methanol was evaporated off and from the aqueous residue the aglycone was first extracted with chloroform and then the chloride ion was bound with 3 g of AV-17 anion-exchanger (OH form) (for paper chromatography, see Fig. 1c, sample 14). A syrup (212 mg) was obtained.

The sample of strophanthidin 3-O-β-D-glucopyranoside was kindly given to us by V. T. Chernobai.

Conclusions

From the seeds of Coronilla scorpioides (L.) Koch. a new cardenolide glycoside has been obtained which has been called scorpioside and which has the structure of 3-β-(β-D-glucofuranosyloxy)-5, 14β-dihydroxy-19-oxo-5β-card-20(22)-enolide.

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