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CARDENOLIDES OF THE SEEDS OF *Coronilla glauca* AND OF *C. scorpioides*. NEW GLYCOSIDES ALLOGLAUCOSIDE AND SCORPIOSIDOL

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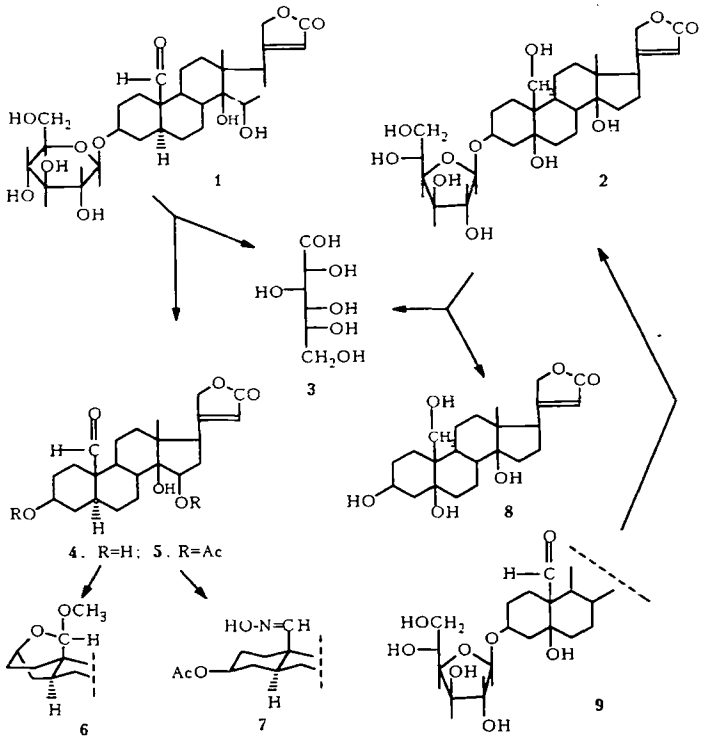
Two new glycosides have been isolated from the seeds of Coronilla glauca *L. and* C. scorpioides *Koch: 3β-(β-D-glucopyranosyloxy)-14β,15β-dihydroxy-19-oxo-5α-card-20(22)-enolide (alloglaucoside) and 3β-(β-D-glucofuranosyloxy)-5,14β,19-trihydroxy-5β-card-20(22)-enolide (scorpiosidol), together with a glycoside previously unknown for the* Coronilla *genus — 3β-(β-D-glucopyranosyloxy)-14β-hydroxy-5α-card-20(22)-enolide (desglucouzarin) and known aglycons (corotoxigenin, alloglaucotoxigenin, coroglaucigenin) and glycosides (glucocorotoxigenin, coronillobioside, glucocoroglaucigenin, coronillobiosidol and scorpioside).*

The isolation of a series of cardenolides from plants of the *Coronilla* genus (Fabaceae) has been reported previously [1, 2]. Continuing a study of the seeds of *Coronilla glauca* L. (honey coronilla) and *C. scorpioides* Koch (scorpion coronilla), we have obtained, in addition to those isolated previously, two new glycosides, which we have called alloglaucoside (**1**) and scorpiosidol (**2**). On the basis of reactions for functional groups [3] these were also assigned to the cardenolides, while their molecular masses and elementary compositions characterized them as monosides.



Scheme 1. Chemical transformations of alloglaucoside (**1**) and scorpiosidol (**2**)

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Alloglaucoside (1). In the UV spectrum of (1) there was a maximum in the 219 nm region (log ε 4.35), characteristic for a butenolide ring, and also a maximum at 305 nm (log ε 1.45), ascribed to the absorption of an aldehyde group.

On hydrolysis with grape snail enzymes [2], substance (1) split into *D*-glucose (3) and an aglycon (4) (scheme), which, on acetylation formed a diacetate (5), showing the presence of two OH groups capable of undergoing acylation. One of the hydroxyls is present at C-3 of the aglycon and is due to the biogenesis of the cardenolides [4], while with methanol in an acid medium the aldehyde group formed a methylcyclosemiacetal (6). Such a property of the aglycon is possible only if the aldehyde group is located at C-10 and a hydroxyl at C-3 and rings *A/B* of the steroid skeleton are *trans*-linked. The presence of an aldehyde group in aglycon (4) was also confirmed by the formation of the oxime (7) [4].

The properties described above are possessed by an aglycon obtained previously from the seeds of honey coronilla — alloglaucotoxigenin [5, 6], which is 3β,14β,15β-trihydroxy-19-oxo-5α-card-20(22)-enolide (4). It has been detected in the free state in fermented seeds of honey coronilla and of scorpion coronilla.

On the basis of Klyne's rule [7], a β-glycosidic bond was found in alloglaucoside (1). The ease of enzymatic splitting of glycoside (1) and its resistance to acid hydrolysis with 0.05 N H_2SO_4 gave grounds for assigning the β-*D*-glucose residue to the pyranose form [8]. From the experimental results, the structure of (1) can be represented as 3β-(β-*D*-glucopyranosyloxy)-14β,15β-dihydroxy-19-oxo-5α-card-20(22)-enolide.

Scorpiosidol (2). The UV spectrum of (2) revealed one maximum in the 220 nm region (log ε 4.2), characteristic for a butenolide ring. In contrast to alloglaucoside (1), substance (2) was hydrolyzed by 0.05 N H_2SO_4 — to the aglycon strophanthidol (8) [8] and *D*-glucose. Scorpiosidol was resistant to the action of enzymes. It was shown by Klyne's method [7] that the glucose residue was attached to the aglycon by a β-glycosidic bond.

Similar properties are possessed by the glucofuranoside scorpioside [8], and the most probable structure for the compound under study is therefore strophanthidol 3-O-β-*D*-glucofuranoside. To confirm this hypothesis we reduced scorpioside (9) at the aldehyde group with $NaBH_4$. The compound obtained proved to be identical with the substance under investigation, (2). Thus, the structure of (2) corresponds to the name 3β-(β-*D*-glucofuranosyloxy)-5,14β,19-trihydroxy-5β-card-20(22)-enolide.

In adition to the compounds (1) and (2) described above, we isolated uzarigenin 3-O-β-*D*-glucopyranoside (desglucouzarin) [9], not previously known for the *Coronilla* genus.

EXPERIMENTAL

For **general observations**, see [1, 2].

Isolation of the cardenolides from unfermented honey coronilla and scorpion coronilla seeds has been described in [1].

From both coronilla species we isolated the glycosides desglucouzarin $C_{29}H_{44}O_9$, mp 227-234°, $[\alpha]_D^{20}$ −43° (*c* 0.6; CH_3OH) [9]; glucocorotoxigenin $C_{29}H_{42}O_{10}$, mp 272-275°, $[\alpha]_D^{20}$ +6.5° (*c* 0.8; CH_3OH) [1]; glucocoroglaucigenin $C_{29}H_{44}O_{10}$, mp 178-184°, $[\alpha]_D^{20}$ −8° (*c* 0.5; CH_3OH) [1]; coronillobioside $C_{35}H_{54}O_{15}$, mp 195-201°, $[\alpha]_D^{22}$ +4.0° (*c* 1.0; CH_3OH) [1]; coronillobioside $C_{35}H_5O_{15}$, mp 232-240°, $[\alpha]_D^{21}$ −9.5° (*c* 0.7; CH_3OH) [2]; and scorpioside $C_{29}H_{42}O_{11}$, mp 267-269°, $[\alpha]_D^{22}$ +8.4° (*c* 0.8; CH_3OH) [1, 8]; and also the new compounds alloglaucoside (1) and scorpiosidol (2), the properties of which are given below.

Isolation of Cardenolides from Autofermented Seeds [8]. Honey coronilla seeds (100 g) were defatted with petroleum ether, dried, moistened with water, and left for a day in a thermostat at 37-40°C. After fermentation, only two polar substances of cardenolide nature, one of them, (2), in very small amount, were detected by paper chromatography in the solvent system toluene−*n*-butyl alcohol (2:1)−water (35%), while in the chloroform−formamide system no less than four aglycons were revealed. Then ethanol was added to the fermented raw material in an amount to give 80% ethanol.

The extract obtained was evaporated to 50 ml and was extracted with 100 ml of chloroform and 120 ml of chloroform−ethanol (2:1). After the solvent had been evaporated off, a solution of the chloroform−alcoholic-extract in 10 ml of distilled water was filtered through a layer of alumina (1.5 × 2 cm). The evaporated filtrate deposited 93 mg of scorpioside crystals. The glycosides in the mother liquor were separated on a column of alumna (1.5 × 10 cm) with elution by chloroform−alcohol (4:1). An additional 12 mg of scorpioside and 18 mg of scorpiosidol (2) was obtained.

We also isolated from the chloroform fraction the aglycons uzarigenin $C_{23}H_{34}O_4$, mp 251-257°, $[\alpha]_D^{21}$ +16.0° (*c* 0.6; CH_3OH) [9], corotoxigenin $C_{23}H_{32}O_5$, mp 220-223°, $[\alpha]_D^{20}$+43.0° (*c* 1.0; CH_3OH) [1], alloglaucotoxigenin $C_{23}H_{32}O_6$, mp 221-230°, $[\alpha]_D^{22}$+26.0° (*c* 0.7; CH_3OH) [5, 6], and coroglaucigenin $C_{23}H_{34}O_5$, mp 249-251°, $[\alpha]_D^{20}$+25.0° (*c* 0.8; CH_3OH) [1].

Alloglaucoside (1). The substance was obtained in an amorphous state — $C_{29}H_{42}O_{11}$, $[\alpha]_D^{20}$+7.1° (*c* 0.7; C_2H_5OH). The compound isolated gave characteristic reactions for cardenolides with *m*-dinitrobenzene (Raymond reaction) and with sodium nitroprusside (Legal reaction) [3].

Enzymatic hydrolysis of glycoside (**1**) was performed with an enzyme preparation from the grape snail as described in [2]. *D*-Glucose and aglycon (**4**) were obtained.

Alloglaucotoxigenin (4): mp 224-230°, $[\alpha]_D^{20}$+26.0° (*c* 0.8; CH_3OH). Found, %: C 68.27, H 8.03; $C_{23}H_{32}O_6$ (404.2). Calculation, %: C 68.30, H 7.97.

UV spectrum (EtOH, λ_{max}, nm): 218 (log ε 4.42).

Acetate (5). A solution of 100 mg of the aglycon in 3 ml of acetic anhydride with the addition of the same amount of anhydrous pyridine was left at room temperature for two days. Then the reaction mixture was poured into 15 ml of ice water and the resulting precipitate was filtered off and recrystallized from ethanol. The resulting acicular crystals (90 mg) had the composition $C_{27}H_{38}O_8$, mp 191-193°, $[\alpha]_D^{20}$−9.2° (*c* 0.5; CH_3OH).

Oxime (7). A solution of 50 mg of the aglycon acetate (**5**) and 25 mg of hydroxylamine hydrochloride in 1 ml of C_2H_5OH was treated with 50 mg of CH_3COONa in 0.5 ml of H_2O, and the reaction mixture was heated under reflux for 2 h. The solution was then concentrated in vacuum and diluted with water, and the crystals that deposited (49 mg) were separated off and recrystallized from methanol and hot water, $C_{27}H_{37}O_8$, mp 264-267°, $[\alpha]_D^{22}$+6.0° (*c* 0.5; CH_3OH).

Methylcyclosemiacetal (6) from Alloglaucotoxigenin (4). A solution of 40 mg of the aglycon (**4**) in 5 ml of dry methanol was treated with 0.25 ml of methanol saturated with 5% HCl. After a day the initial substance could no longer be detected in the reaction mixture. The methanol was evaporated off, the residue was dissolved in 3 ml of chloroform, and the solution was washed twice with water. After this, the chloroform phase was evaporated and the residue was crystallized from acetone−hexane, giving 32 mg of crystals of (**6**) with mp 209-212°C, $C_{24}H_{34}O_6$.

Scorpiosidol (2). This formed colorless acicular crystals with mp 191-196°C (from CH_3OH and diethyl ether), $[\alpha]_D^{22}$−5.0° (*c* 0.5; CH_3OH), $C_{29}H_{44}O_{10}$; UV spectrum (EtOH, λ_{max}, nm): 220 (lgε 4.12). Like scorpioside, compound (**2**) was resistant to enzymatic hydrolysis [8].

Acid Hydrolysis of (2). A solution of 15 mg of (**2**) in 3 ml of 0.05 N H_2SO_4 as heated for 10 h. Subsequent working up was carried out as described in [8]. Strophanthidol, $C_{23}H_{34}O_6$, mp 137-141°, $[\alpha]_D^{21}$+38.0° (*c* 0.1; CH_3OH) [10], and *D*-glucose were obtained and identified.

Reduction of Scorpioside (9) to Scorpiosidol (2). The reduction of 42 mg of (**9**) with $NaBH_4$ was carried out by a procedure described previously [1]. This gave 30 mg of substance (**2**), $C_{29}H_{44}O_{10}$, mp 142-197°, $[\alpha]_D^{20}$−5.1° (*c* 0.2; CH_3OH).

The scorpiosidol obtained by the reduction of scorpioside gave no depression of the melting point with the substance (**2**) isolated from coronilla seeds. They had the same physicochemical properties and the same R_f values in a series of solvent systems.

REFERENCES

1. V. N. Kovalev and A. N. Komissarenko, Khim. Prir. Soedin., 676 (1985).
2. A. N. Komissarenko and V. N. Kovalev, Khim. Prir. Soedin., 720 (1988).
3. N. P. Maksyutina, N. F. Komissarenko, A. P. Prokopenko, L. I. Pogodina, and G. N. Lipkan, Plant Medicinal Agents [in Russian], Kiev (1985).
4. L. Fieser and M. Fieser, Steroids, Reinhold, New York (1959).
5. A. Stoll, A. Pereira, and J. Renz, Helv. Chim. Acta, **32**, 203 (1949).
6. R. Brandt, W. Stocklin, and T. Reichstein, Helv. Chim. Acta, **44**, 1 (1966).

7. W. Klyne, Biochem. J., **47**, 41 (1950).
8. N. F. Komissarenko, Yu. N. Beletskii, I. P. Kovalev, and D. G. Kolesnikov, Khim. Prir. Soedin., 381 (1969).
9. B. T. Chernobai and N. F. Komissarenko, Khim. Prir. Soedin., 445 (1971).
10. A. A. Ponomarenko, N. F. Komissarenko, and K. L. Stukkei, Khim. Prir. Soedin., 848 (1971).