SUMMARY

The structure of a new triterpene bisdesmolide isolated from low meadow rue has been established. The bisdesmolide, which has been called thalicoside B, has the structure of oleanolic acid 28-O-β-D-glucopyranoside 3-O-[O-α-L-rhamnopyranosyl-(1 → 2)-O-β-D-glucopyranosyl-(1 → 3)-α-L-arabinopyranoside].

LITERATURE CITED

1. A. S. Gromova, V. I. Lutskii, E. V. Kostyleva, N. I. Dedkova, and A. A. Semenov, Izv. Sib. Otd. Akad. Nauk SSSR, Ser. Khim. Nauk, Series No. 4, issue No. 9, 129 (1981).
2. A. S. Gromova, V. I. Lutskii, A. A. Semenov, V. A. Denisenko, and V. V. Isakov, Khim. Prir. Soedin., 213 (1984).
3. D. M. Doddrell, P. W. Khong, and K. G. Lewis, Tetrahedron Lett., No. 27, 2381 (1974).
4. K. Tori, S. Seo, A. Shimaoko, and Y. Tomito, Tetrahedron Lett., No. 48, 4227 (1974).
5. S. Seo, Y. Tomito, K. Tori, Y. Yoshimura, J. Am. Chem. Soc., 100, 3331 (1978).
6. A. S. Shashkov and O. S. Chizhov, Bioorg. Khim., 2, 437 (1976).
7. J. H. Sloneker, in: Methods in Carbohydrate Chemistry, Vol. VI, R. L. Whistler and J. N. BeMiller, eds., Academic Press, New York (1972), pp. 20-24.
8. V- M. Easterwood and B. J. L. Huff, Svensk Papperst., No. 23, 768 (1968).

CARDENOLIDES OF *Coronilla glauca* AND *C. scorpioides*

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The cardenolide glycosides glucocorotoxigenin, scorpioside, and coronillobioside and a new compound which has been called glucocoroglaucigenin have been isolated from the seeds of *Coronilla glauca* L. for the first time. The new glycoside has the structure of 3β-(β-D-glucopyranosyloxy)-14,19-dihydroxy-5α-card-20(22)-enolide. This glycoside has also been isolated from the seeds of *C. scorpioides*.

The present paper gives the results of a study of the cardenolides of the seeds of *Coronilla glauca* L. (honey coronilla) and *Coronilla scorpioides* (L.) Koch. (scorpion coronilla) [1-4]. The glycosides were isolated from the comminuted seeds as described in [5].

From the seeds of honey coronilla we obtained and identified the aglycone corotoxigenin (I), the glycosides glucocorotoxigenin (III), scorpioside (V) and coronillobioside (VI), and a new substance which we have called glucocoroglaucigenin (IV). From scorpion coronilla, in addition to those known previously and mentioned above we have obtained for the first time coroglaucigenin and substance (IV). The UV spectrum of the substance exhibited a single maximum in the 219 nm region (log ε 4.12), which is characteristic for the butenolide ring of a cardenolide.

Compound (IV) was not reduced by sodium tetrahydroborate, which indicated the absence of carbonyl groups from the molecule. On enzymatic hydrolysis (scheme), the glycoside was cleaved into D-glucose (I) and an aglycone (II) which was identified by its physicochemical properties, color reactions with 84% sulfuric acid, Rf values in various solvent systems, and a mixed melting point as 3β,14β,19-trihydroxy-5α-card-20(22)-enolide (coroglaucigenin).

A β-glycosidic bond was found in the glycoside (IV) on the basis of Klyne's rule [4]. Difficulty in acid hydrolysis with 0.05 N sulfuric acid showed the pyranose form of the carbohydrate moeity of the substance being studied.

To confirm the results obtained, glucocorotoxigenin (III) was reduced with sodium tetrahydroborate. The substance obtained proved to be identical with the glucoside isolated.

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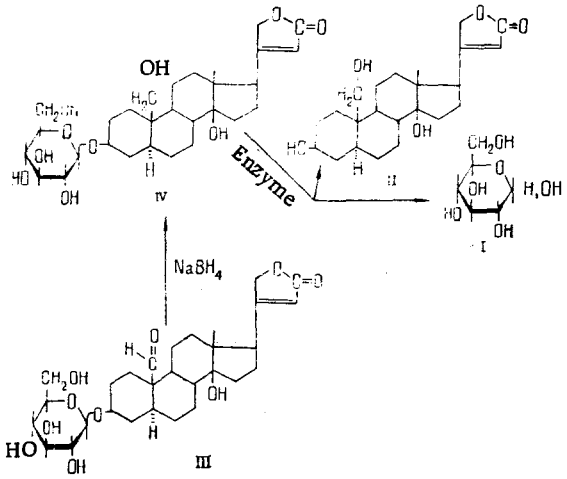

Scheme for proving the structure of glucocoroglaucigenin (IV).

Thus, the structure of glucocoroglaucigenin (IV) can be represented as 3β-(β-D-glucopyranosyloxy)-14β,19-dihydroxy-5α-card-20,22-enolide.

EXPERIMENTAL

The absorption chromatography of the cardenolides was performed on neutral alumina (activity grade III) and that of the coumarins on acidic alumina. For analysis, the substances were dried in vacuum (10^{-2} mm Hg) over P_2O_5 at 110-115°C for 4-5 h. Melting points were determined on a Kofler stage. UV spectra were recorded on SF-16 spectrophotometer.

Isolation of the Cardenolides. The comminuted seeds (0.1 kg) of honey coronilla were defatted with 200 ml of petroleum ether, after which they were extracted with a tenfold amount of 80% ethanol. The extract was evaporated to an aqueous residue (50 ml), and on standing in the cold this deposited crystals of daphnoretin, $C_{19}H_{12}O_7$, mp 254-256°C [5]. After the crystals had been removed, the aqueous residue was treated successively with chloroform (100 ml) and mixtures of chloroform and ethanol in ratios of 4:1 (150 ml) and 2:1 (150 ml). The extract obtained on the defatting of the seeds was evaporated to 40 ml and deposited on a column of acidic alumina [6] (10 × 2 cm) and was eluted first with petroleum ether (70 ml), and then with benzene (80 ml).

The benzene eluate yielded 53 mg of the furocoumarin psoralen, $C_{11}H_6O_3$, mp 161-163°C [5].

The chloroform extract was evaporated, and the residue (3.6 g) was separated with the aid of partition chromatograph on silica gel as described in [6]. As a result, the hydroxycoumarins umbelliferone, $C_9H_6O_3$, mp 231-233°C, and scopoletin, $C_{10}H_8O_4$, mp 200-202°C, and the cardenolides corotoxigenin (I) and coroglaucigenin (II) [2] were isolated and were crystallized from ethanol.

The residue (5.1 g) after the evaporation of the chloroform—ethanol (4:1) extract was deposited on a column of alumina (1.5 × 40 cm), which was washed with chloroform and then with mixtures of chloroform and ethanol. The amount of ethanol was increased from 2 to 7%. Fractions with a volume of 20 ml were collected and their qualitative cardenolide compositions were determined by paper chromatography in the solvent system benzene—butan-1-ol (2:1)—water. In this way, 12 mg of glucocorotoxigenin (III) and 15 mg of glucocoroglaucigenin (IV) were isolated.

By chromatography on alumina (40 × 2 cm) using the procedure described above (elution by chloroform containing from 4 to 15% of ethanol), the residue from the chloroform-alcohol (2:1) fraction (6.3 g) yielded 15 mg of scorpioside (V) and 13 mg of coronillobioside (VI).

In a similar manner, 1 kg of the seeds of scorpion coronilla yielded the same glucosides (II-VI), including 102 mg of substance (IV).

Thus, the following cardenolides were isolated from honey coronilla and scorpion coronilla: corotoxigenin ($C_{23}H_{32}O_5$, mp 220-223°C, from ethanol), $[\alpha]_D^0 +43°$ (c 0.5 ; methanol);

coroglaucigenin ($C_{23}H_{34}O_5$, mp 245-249°C, from ethanol), $[\alpha]_D^{20}$ +25° (*c* 0.5 ; methanol); glucocorotoxigenin ($C_{29}H_{42}O_{10}$, mp 273-276°C, from ethanol), $[\alpha]_D^{0}$ —15° (*c* 0.5; , methanol); scorpioside ($C_{29}H_{42}O_{11}$, mp 266-269°C, from ethanol), $[\alpha]_D^{20}$ + 8.1° (*c* 0.5;; methanol); and coronillobioside ($C_{35}H_{52}O_{15}$, mp 231-239°C, from propanol), $[\alpha]_D^{22}$ —9° (*c* 0.5 ; methanol), these being identified from their changes in coloration in time with 84% sulfuric acid, the physical properties of the substances themselves and of their acetyl derivatives, the products of enzymatic hydrolysis and of reduction, mixed melting points, and Rf values on parallel chromatography with authentic samples in various solvent systems.

Substance (IV) (glucocoroglaucigenin), $C_{29}H_{44}O_{10}$, consisted of colorless acicular druses with mp 178-184°C (from methanol), $[\alpha]_D^{20}$ +8° (*c* 0.8 methanol); $\lambda_{max}^{C_2H_5OH}$; 219 nm (log ε 4.12). With 84% sulfuric acid it formed a coloration changing with time: 0 min — yellow; 30 min — orange-violet; 60 min — light pink; 180 min — pink.

Enzymatic Hydrolysis. A solution of 30 mg of substance (IV) in 4 ml of water was treated with 20 ml of grape snail enzyme, the mixture was carefully stirred, and it was left in a thermostat at 38-40°C for a day, after which a fivefold amount of ethanol was added and it was heated to boiling. The precipitated enzyme was filtered off in vacuum through a filter packed with kieselguhr. The filtrate was evaporated, the residue was dissolved in 3 ml of water, and the solution was treated four times with 3-ml portions of chloroform. The residue after the evaporation of the chloroform extract was crystallized from acetone. This gave compound (II), $C_{23}H_{34}O_5$, with mp 246-249°C (from ethanol), $[\alpha]_D^{20}$ +26° (*c* 0.3 ; methanol). With 84% sulfuric acid the algycone formed colorations changing with time: 0 min — light yellow; 1-60 min — lemon yellow; about 120 min — orange yellow; 180 min — yellow.

From its melting point, coloration with 84% sulfuric acid, Rf values on paper chromatography in the benzene—chloroform (9:1)—formamide systems and on thin-layer chromatography on Silufol plates in the chloroform—methanol (9:1) system, substance (II) was found to be identical with an authentic sample of coroglaucigenin [2, 7].

Reduction of Glucocorotoxigenin (III) to Glucocoroglaucigenin (IV). A solution of 50 mg of substance (III) in 8 ml of ethanol was cooled in the refrigerator to 5-7°C, and 20 mg of sodium tetrahydroborate was added. The completeness of reduction was monitored by paper chromatography in the benzene—butan-1-ol (2:1)—water (35%) system. After 2 h, the initial substances were no longer detected on the chromatogram. The reaction mixture was evaporated and the residue was dissolved in 5 ml of distilled water, after which compound (IV) was extracted with a 3:1 mixture of chloroform and ethanol. The extract was evaporated and the residue was crystallized from acetone. This gave 39 mg of substance (IV), $C_{29}H_{44}O_{10}$, with mp 179-182°C, $[\alpha]_D^{22}$ +8.5° (*c* 0.5 ; methanol).

From its coloration with 84% sulfuric acid, mixed melting point, and Rf values in various solvent systems, the reduced substance was identical with the glycoside (IV) isolated.

Acid Hydrolysis of Substance (IV) with 0.05 N Sulfuric Acid. A solution of 10 mg of the glycoside (IV) in 2 ml of 0.05 N sulfuric acid was heated at 100°C. The hydrolysis reaction was monitored by paper chromatography. After 10 hours' heating only traces of hydrolysis products were detected in the reaction mixture.

SUMMARY

The cardenolide glycosides glucocorotoxigenin, scorpioside, and coronillobioside and a new compound which has been called glucocoroglaucigenin have been isolated from the seeds of *Coronilla glauca* L. for the first time. The structure of the new compound is represented by the name 3β-(β-D-glucopyranosyloxy)-14β,19-dihydroxy-5α-card-20(22)-enolide. This glycoside has also been isolated from the seeds of *Coronilla scorpioides* (L.) Koch.

LITERATURE CITED

1. A. Stoll, A. Pereira, and G. Renz, Helv. Chim. Acta, 32, 293 (1949).
2. R. Brandt, W. Stocklin, and T. Keichstein, Helv. Chim. Acta, 45, 1662 (1966).
3. N. F. Komissarenko and I. G. Zoz, Rast. Res., 6, 562 (1970).
4. N. F. Komissarenko, Yu. N. Beletskii, I. P. Kovalev, and D. G. Kolesnikov, Khim. Prir. Soedin., 381 (1969).
5. N. F. Komissarenko, J. G. Zoz, J. N. Beletzky, and W. S. Sokolov, Planta Medica, 17, 170 (1969).

6. D. G. Kolesnikov, N. F. Komissarenko, and V. T. Chernobai, Med. Promst. SSSR, 32 (1961).
7. I. F. Makarevich, E. P. Kemertelidze, G. Kislichenko, V. V. Zatula, A. A. Reznichenko, D. G. Kolesnikov, and I. P. Kovalev, Cardenolides and Bufadienolides [in Russian], Tbilisi (1975), p. 107.

STRUCTURE OF 22-DEOXOCUCURBITACINS ISOLATED FROM *Bryonia alba* AND *Ecbalium elaterium*

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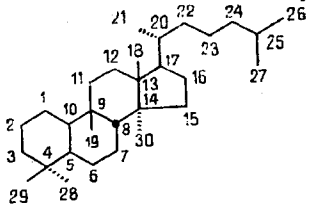
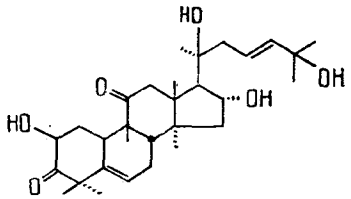
22-Deoxocucurbitacin D (II), dihydrocucurbitacin B, cucurbitacin L, and the previously unknown rhamnoglucosyl-22-deoxo-16,23-epoxycucurbitosides A (III) and B (IV) have been isolated from the roots of *Bryonia alba* and identified. Glucoside (III) has also been identified in the fruit of *Ecbalium elaterium* L.

It has been shown previously that the main components of an extract of the roots of *Bryonia alba* L., which are widely used in folk medicine for the treatment of various diseases are tetracyclic triterpenes — dihydrocucurbitacin D (Dh-D), 2- and 25-glucosyldihydrocucurbitacins D, and 2,25-diglucosyldihydrocucurbitacin D [1].

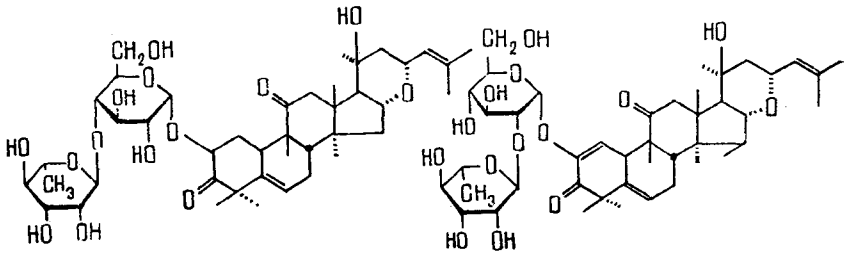
The cucurbitacins [2] exhibit a high cytotoxicity in cultures of tumor cells of the KB and Hela lines [3-6], show antitumoral [5, 6], antimicrobial [7], anthelminthic [8], purgative [9], antihepatic [10], and stimulating and tonic [11] actions, increase the permeability of capillaries and lower the blood pressure [11], inhibit the growth of plants [13], and are insect attractants [14]. It has been shown that the cucurbitacins inhibit the synthesis of DNA by selectively suppressing the inclusion of thymidine in the lymphocytes [15], and they inhibit the anaerobic glycolysis and respiration of tumor cells [16] and also the binding of [^{3}H]cortisol with the glucocorticosteroid receptors in HeLa cells [17]. The action of the cucurbitacins is also connected with their influence of the level of arachidonic acid in the tissues [18] and their inhibiting action on the liberation arachidonic acid and the biosynthesis of leukotrienes in human leukocytes [19].



I cucurbitane



II 22-Deoxocucurbitacin D



III—22-Deoxocucurbitoside A, IV—Deoxocucurbitoside B

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