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CARDENOLIDES AND COUMARINS OF CORONILLA VARIA, C. CRETICA, ETC.

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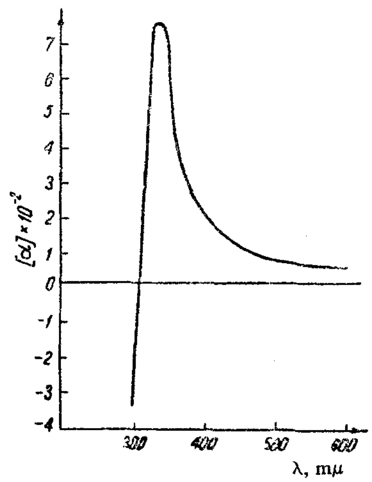
The present communication gives information on the cardenolide and coumarin composition of Coronilla varia L., (crownvetch coronilla) (family Leguminosae) and three related species: C. cretica L., C. hyrcana Prilipko, and C. orientalis Müll.

C. varia was investigated earlier by Z. V. Zova [1], who succeeded in isolating from a chloroform fraction a crystalline substance of cardenolide nature. The structure of this substance was not established. From the flowers of this species of plant, Polish workers [2] have obtained three flavonoid compounds: kaempferol, astragalin, and kaempferol 3-galactoside.

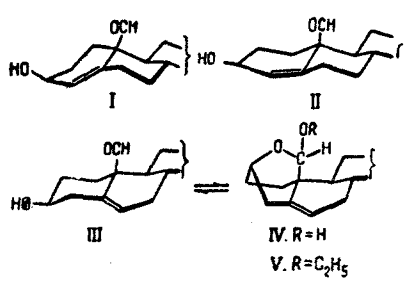
Paper chromatography of freshly-prepared ethanolic extracts of the seeds of C. varia and related species has shown the presence, mainly of one substance belonging to the cardenolides. When the extracts are allowed to stand, a series of substances of cardenolide nature appears. This has been found previously in a study of the cardiotonic substances of C. hyrcana [3]. In its physical properties, coloration with 84% sulfuric acid, R_f values in various systems, and melting point, the native glycoside obtained by the method described proved to be identical with hyrcanoside. As has been shown previously [3], hyrcanogenin is the Δ^4 isomer of pachygenin (Δ^5-strophanthidin) [4]. Because of the presence of this double bond, the substance is distinguished by certain characteristic features.

Thus, the double bond at C_4, in contrast to a double bond at C_5, imparts a high positive optical rotation to steroids [5a]. An analogous difference is observed in the optical rotatory dispersion. While in hyrcanogenin the optical rotatory dispersion curve has a positive Cotton effect (figure), in pachygenin the Cotton effect is negative [6]. In contrast to pachygenin, hyrcanogenin does not form an ethylal under ordinary conditions, which shows the spatial remoteness of the OH group at C_3 from the aldehyde group at C_{10} [7]. Under more severe conditions the formation of 3:4, 5:6-dianhydrostrophanthidin takes place. Such anhydride formation is characteristic for the Δ^4-cardenolides [8b, 9] and the bufadienolides [8a-11].



Optical rotatory dispersion curve of hyrcanogenin (c, 0.098%; l = 1 dm; SPU-E instrument).

It may be assumed that hyrcanogenin and the majority of Δ^4-steroids have a spatial arrangement of the A and B rings close to that of the A/B-cis series. Confirmation of this is given by the oxidation [12] and hydrogenation [13] reactions in a neutral medium at the Δ^4 bond, leading to the formation of substances with a cis linkage of rings A and B (because of the free approach of the reagent from the β side of the molecule) and also by the impossibility of forming an ethylal. This is feasible only when the steroid skeleton has conformation I [5b] and not II. The hypothesis of the nature of the oxidation reduction reactions at the double bond for hyrcanogenin requires experimental demonstration.



In pachygenin, the spatial arrangement of rings A and B, which is close to that of compounds of the A/B-trans series (III), favors the ready transition of ring A into the boat form with the production of a semiacetal (IV) or an acetal (V) under both mild and severe conditions. Δ^5-Steroids, and also compounds with a trans linkage of rings A and B, are most highly screened on the β side of the molecule [13]. This is also shown by the reduction of the double bond [6, 14], as a result of which cardanolides of the trans-A/B series are formed.

The chemical compositions of the plants C. cretica, C. hyrcana, and C. orientalis are extremely similar to that of C. varia. They all contain hyrcanoside. Only in C. orientalis is a small amount of another glycoside with more polar properties found, in addition to hyrcanoside.

The coumarin composition of the Coronilla species mentioned is represented in its full complexity by five coumarins, of which umbelliferone, scopoletin, and daphnoretin have been identified. Umbelliferone was isolated from C. hyrcana previously by R. B. Bagirov [15], while scopoletin and daphnoretin have been found in plants of the Coronilla genus for the first time. Until now, daphnoretin was known only from the genus Daphne L. (family Thymelaeaeaceae Adans.) [16].

Experimental

The comminuted seeds (0.5 g) of the Coronilla species mentioned were steeped in 15 ml of 96% ethanol for 24 hr and then the extract was filtered and the seeds were steeped again in 10 ml of ethanol and heated at 60° C for 2 hr. The extract obtained was filtered and the residue was washed on the filter with 2 ml of ethanol. The combined extracts were evaporated to 0.5 ml and deposited (in 0.04-ml portions) on chromatographic paper. The qualitative composition of the cardenolides was determined in the benzene–butan-1-ol (1 : 1)–water (35%) system and that of the coumarins in the chloroform–formamide system.

Isolation of the cardenolides and coumarins. The cardenolides and coumarins were extracted from 300 g of comminuted and petroleum-ether-defatted seeds with 3 *l* of 70% ethanol. The extract was evaporated until the ethanol had been completely eliminated, after which crystals of daphnoretin deposited from the aqueous residue. After the crystals had been separated off, the aqueous residue was extracted with mixtures of chloroform and ethanol in ratios of 8.5 : 1.5 (250 ml) and 2 : 1 (300 ml).

Mainly, coumarins were found in the first chloroform-ethanol extract (8.5 : 1.5). By partition chromatography on silica gel (35 × 2 cm; stationary phase, formamide; mobile phase, chloroform), daphnoretin, scopoletin, and umbelliferone were obtained. From the second chloroform-ethanol extract (2 : 1), 451 mg of hyrcanoside was isolated.

Cardenolides. Hyrcanoside, isolated from the Coronilla species studied, had mp 201–207° C, $[a]_D^{19}$ +7.0° (c 1.0; methanol).

Found, %: C 59.91; H 7.12; mol. wt. 679.2 (lactone titration). Calculated for $C_{34}H_{48}O_{14}$, %: C 59.99; H 7.11; mol. wt. 680.72.

Stepwise hydrolysis with the enzymes of the fungus Aspergillus oryzae, carried out by a procedure described previously [3], led to the formation of D-glucose and desglycohyrcanoside, $C_{28}H_{38}O_9$, with mp 197–199° C, $[\alpha]_D^{20}$ +25.0° (c 0.80; methanol–pyridine, 8 : 2). Enzymatic cleavage of the monoside yielded D-xylose and hyrcanogenin, $C_{23}H_{30}O_5$, with mp 227–235° C; $[\alpha]_D^{2}$ + 93° [c 0.9; chloroform–ethanol (3 : 2)].

Experiment on the production of an ethylal of hyrcanogenin under mild conditions: a solution of 100 mg of the substance in 15 ml of anhydrous ethanol was treated with 6 ml of glacial acetic acid. The further procedure was as described by Chernobai [7]. The initial substance was recovered.

Experiment on the production of an ethylal of hyrcanogenin under severe conditions: after 1 day, a solution of 70 mg of the substance in 10 ml of anhydrous ethanol saturated with 5% hydrogen chloride was evaporated in vacuum (without heating) to 3–4 ml, and the crystals that had deposited (51 mg) were filtered off. A substance with mp 186–191° C $[\alpha]_D^{20}$ –58° (c 0.5; chloroform) was obtained.

Found, %: C 75.00; H 7.61; mol. wt. 365.9. Calculated for $C_{23}H_{28}O_4$, %: C 74.96; H 7.65; mol. wt. 368.5.

The compound underwent reduction with sodium borohydride, which shows the presence of an aldehyde group in it. After attempted acetylation, the substance was recovered unchanged. This shows the absence of a hydroxy group capable of acetylation at C_3, which shows the possibility of the formation of 3,5-dianhydrostrophanthidin.

To confirm the structure of the compound obtained, it was oxidized with sodium permanganate in anhydrous acetone with subsequent esterification of the carboxy group with diazomethane [8b]. A substance $C_{24}H_{30}O_5$ with mp 220–228° C, $[\alpha]_D^{19}$ –104.4° (c 0.47; chloroform) was isolated, the physicochemical properties and color reactions of which showed it to be identical with methyl ester of 3,5-dianhydrostrophanthidinic acid [8b]. For comparison, 5,14-dianhydrostrophanthidin was also obtained [17].

Substance E, isolated earlier from the fermented total glycosides of C. hyrcana [3] is identical in its physicochemical properties, coloration with 84% sulfuric acid, R_f values in various systems, and mixed melting point with 3,5-dianhydrostrophanthidin.

5-Anhydrostrophanthidin (pachygenin). First, the ethylal of 3,19-cyclo-oxy-5-anhydrostrophanthidin was obtained from strophanthidin by the method of Jacobs and Collins [17,18], mp 227–230° C $[\alpha]_D^{21}$ –48.0° (c 1.0; chloroform). Then it was hydrolyzed to 5-anhydrostrophanthidin $C_{23}H_{30}O_5$ with $[\alpha]_D^{20}$ –120.0° (c 1.0; chloroform).

Ethylal of 5-anhydrostrophanthidin: the substance was obtained in an anhydrous ethanol–glacial acetic acid medium by a known method [7]. From 150 mg of pachygenin was obtained 115 mg of crystals identical with the ethylal of 5-anhydrostrophanthidin.

Coumarins. Daphnoretin, $C_{19}H_{12}O_7$, had mp 254–256° C (from chloroform); acetate, $C_{21}H_{14}O_8$ with mp 240–242° C; methyl derivative of daphnoretin, $C_{20}H_{14}O_7$, with mp 239–241° C (from acetone). The thermal decomposition of daphnoretin at 380–400° C in a current of hydrogen led to the formation of umbelliferone and scopoletin [18].

Umbelliferone, $C_9H_6O_3$, mp 223–224° C. The physicochemical properties of the coumarin isolated and a mixed melting point showed that it was identical with umbelliferone.

Scopoletin, $C_{10}H_8O_4$, mp 200–202° C. A comparison of the properties of the substance obtained and scopoletin shows that they were identical.

The optical rotatory dispersion spectrum was obtained by N. P. Kovalev. The Coronilla varia seeds were sent to us by Z. D. Zhakhov.

Conclusions

The cardenolide and coumarin composition of C. varia L. and the closely related species C. hyrcana Pril., C. cretica L., and C. orientalis Müll have been investigated. It has been shown that they all contain the cardiac glycoside hyrcanoside, with the exception of C. orientalis, in which another, unidentified, glycoside, has also been found.

A comparative study of some properties of hyrcanogenin (Δ^4-strophanthidin) and pachygenin (Δ^5-strophanthidin) connected with conformational features has been carried out.

In the species studied, no less than five coumarins have been found of which umbelliferone, scopoletin, and daphnoretin have been isolated.

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