GLYCOSIDES OF ERYSIMUM

VIII. CARDENOLIDES OF Erysimum cuspidatum

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In its anatomomorphological characteristics, E. cuspidatum (M. B.) DC differs from other species of Erysimum, and in the "Flora of the USSR" it is given in a separate section [1].

We have previously [2] reported the isolation from the seeds of this plant (var. dolichocarpum N. Busch.) of three glycosides – erysimin, erysimoside, and cuspidoside. This is the first time that the last-mentioned compound has been found in plants of the genus Erysimum. It has the composition $C_{29}H_{44}O_{10}$, does not show a positive Keller–Kiliani reaction, and is not hydrolyzed by 0.1 N H_2SO_4. Consequently, the sugar moiety of this glycoside is not a 2,6-dideoxyhexamethylose. When the substance was hydrolyzed by Mannich's method [3] we found L-rhamnose in the hydrolyzate, its presence also being shown by paper chromatography, gas–liquid chromatography of the trimethylsilyl derivative of the methyl glycoside [4], and by the preparation of the phenylosazone.

When the aglycone fraction of the hydrolyzate was separated preparatively in a thin layer of silica gel, we detected three chromatographically individual compounds. In the mass spectrum of the least polar of the compounds that we found (I), the intensity of the molecular ion, M^+ 406, was very low (about 1%). The recording of fragments with m/e 388 ($M-H_2O$), 370 ($M-2H_2O$), 352 ($M-3H_3O$), 334 ($M-4H_2O$) shows the presence of at least four hydroxy groups. The formation of fragments with m/e 242,* 227, and 199 permits the hypothesis that these groups are located in rings A, B, and C [5, 6]. The same fragments show the presence of an angular methyl group at C_{10}. The three hydroxy groups are apparently attached to the carbon atoms in positions 3, 5, and 14. The high intensity of the fragment with m/e 199 (60%) shows that the fourth OH group is at C_1 or C_{11}. Since the two other hydrolysis products have a smaller molecular weight, it is possible that compound (I) is the genin (see structure on the following page.)

A compound of medium polarity (II), obtained in the hydrolysis of the glycoside, is amorphous. In its mass spectrum, the molecular ion M^+ 388 differs from the molecular ion of the aglycone by one molecule of water (18 units). The remaining fragments are almost identical, but with different intensities. The formation of fragments with m/e 242, 227, and 199 shows that the hydroxy group is located at C_{14}. Compound (II) is undoubtedly a 5-anhydro derivative of the aglycone.

The third, least polar, compound (III) was obtained in the crystalline state. The mass spectrum of this substance differs somewhat from the mass spectra of the preceding two. The intensity of its molecular ion is extremely considerable (M^+ 370 – 70%). Under electron impact only two molecules of water were split out. Fragments with m/e 242, 227, and 199 were absent from the mass spectrum of this compound. A double bond between C_{14} and C_{15} excludes the possibility of the formation of these fragments. Substance (III) is apparently a 5,14-dianhydro derivative of the genin.

* The fragment with m/e 242 (ion of the c type) is given in Fayez' scheme [6]. As subsequent investigations with deuterium analogs have shown, type c ions in cardenolides do not contain a hydroxy group (see [12]).

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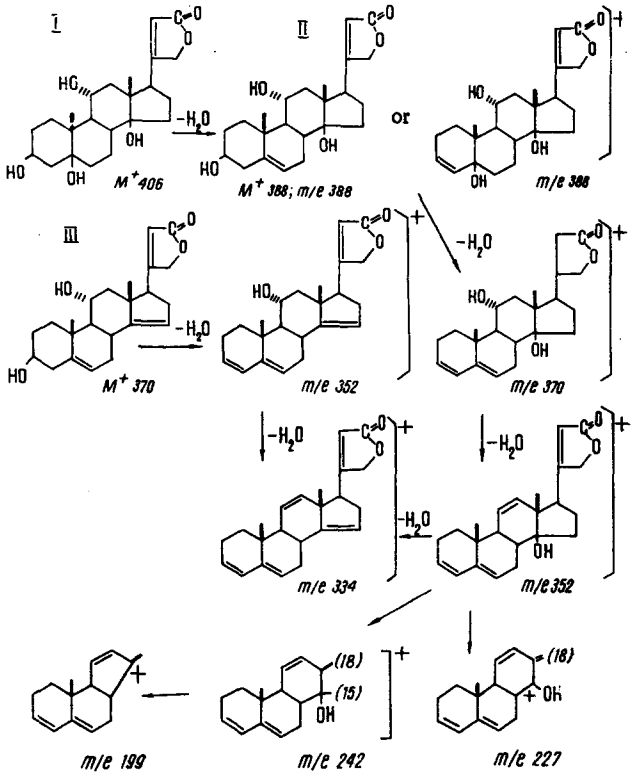

The analysis of the mass spectra that has been given permits the assumption that the aglycone (I) is identical with bipindogenin [7]. The results of a comparison of the physicochemical properties and IR spectra, and also a direct chromatographic comparison, confirmed this assumption.

Consequently, we have isolated from the seeds of the plant bipindogenin L-rhamnoside, i.e., locundeside, which was first found in Strophanthus tholloni Franch [8] and in some varieties of Strophanthus sarmentosus P. DC (family Apocynaceae) [9], and somewhat later in Convalleria keiskei Miq [10].

EXPERIMENTAL

Thin-layer chromatography (TLC) was carried out with KSK silica gel containing 5% of gypsum, and paper chromatography (PC) with paper of types "S" ("slow") and "M" ("medium") of the Leningrad No. 2 mill. The following solvent systems were used: 1) butan-1-ol–acetic acid–water (4 : 1 : 5), 2) butan-1-ol–methyl ethyl ketone–borate buffer (1 : 1 : 2) [11]; 3) tetrahydrofuran–chloroform–formamide (50 : 50 : 6.5); 4) benzene–methyl ethyl ketone (2 : 1) – 3.5% of water; 5) chloroform–benzene–methanol (5 : 5 : 2); 6) toluene–butanol–water (1 : 1 : 1); and 7) chloroform–dioxane–formamide (7 : 2 : 0.5).

The mass spectra were recorded on an MKh-1303 mass spectrometer at a temperature of the internal inlet tube of 150°C with an ionizing voltage of 40 V.

Isolation and Separation of the Cardenolides. The comminuted seeds of E. cuspidatum (6 kg) were defatted with petroleum ether until there was no more fatty oil in the extracts and were then extracted with 70% ethanol. The ethanolic extracts were evaporated in vacuum at 42-45°C. The residue, which consisted of a viscous mass, was filtered through a layer (6 cm) of inactive alumina (0.6 kg) filled into a Büchner funnel. The alumina was washed with distilled water until the Raymond reaction was negative. The cardenolides were extracted from the combined aqueous solution first with chloroform (20 × 1 liter) and then with ethanol–chloroform (1 : 2) (22 × 1 liter) and with butanol (8 × 1 liter). All three extracts were separately dried with sodium sulfate and evaporated in vacuum until the solvents has been completely eliminated. This gave 27 g of chloroformic, 61 g of ethanolic–chloroformic, and 63 g of butanolic fraction. The latter was not studied in detail.

TABLE 1

Fraction No.	Ratio of chloroform to ethanol, vol. %	Amt. of eluate, ml	Characteristics of the substance isolated	Spots on TLC
			Chloroformic fraction	
1–7	100:0	2100	Brown mass	
8–15	95:5	2400	Yellow crystals	Reaction for cardenolides negative
16–20	95:5	1500	Yellow viscous residue	
21–29	—	2700	Amorphous powder	Reaction for cardenolides positive
			Alcoholic–chloroformic fraction	
1–3	80:20	1500	Black viscous residue	Reaction for cardenolides negative
4–13	75:25	5000	The same	Traces of erysimin
14–15	70:30	1000	Brown viscous residue	Traces of erysimin and desglucocheirotoxin
16–25	60:40	5000	Yellow amorphous residue	Locundeside
26–31	60:40	3000	The same	Locundeside + erysimoside
32–40	60:40	4500	The same	Erysimoside

The chloroformic fraction (27 g) was mixed with 25 g of alumina and transferred to a column containing 945 g of alumina (activity grade III). The glycosides were eluted with chloroform containing gradually increasing amounts of ethanol. Fractions with a volume of 300 ml were collected, and after concentration, each was analyzed by TLC in system 5 (Table 1).

Fractions 8-15 yielded 300 mg of a crystalline substance with mp 120°C giving no reaction for sugars and cardenolides. The study of this substance is continuing. Fractions 21-29 gave 350 mg (0.06% of the weight of the raw material) of an individual substance in the amorphous state. After its crystallization from ethanol, mp 170-172°C, $[\alpha]_D^{24}$ + 30.1° (ethanol). A mixed melting point, a superposition of IR spectra, and a chromatographic comparison in systems 6 and 7 identified the substance as erysimin, $C_{29}H_{42}O_9$.

The ethanol–chloroformic (1 : 2) extract (61 g) was mixed with 50 g of alumina and transferred to a column containing 2135 g of alumina (activity grade III). The glycosides were eluted with 500-ml portions of mixtures of the same solvents with gradually increasing ethanol contents (see Table 1).

Fractions 14 and 15 yielded 15 mg of amorphous desglucocheirotoxin (chromatographic comparison).

Fractions 16-25 were rechromatographed on alumina. The column was washed with chloroform – isoamyl alcohol–water (1 : 1 : 1). Fractions with a volume of 100 ml were collected. Fractions 9-15 deposited 3.50 g (0.58% of the weight of the raw material) of a crystalline glycoside which, after two recrystallizations from ethanol,had mp 233-235°C, $[\alpha]_D^{24}$ −9.9° (c 2.43; methanol), $C_{29}H_{44}O_{10}$. The lethal dose in tests on cats was 0.104 mg/kg.

Fractions 32-40 yielded 4 g (0.66% of the weight of the raw material) of an individual cardenolide in the amorphous state. After reseparation on a column of alumina and repeated reprecipitation from ethanol–ether, the glycoside was obtained in the crystalline state with mp 234-236°C, $[\alpha]_D^{20}$ + 23.6° (in methanol). By a mixed melting point and a chromatographic comparison in systems 6 and 7, the substance was identified as erysimoside, $C_{35}H_{52}O_4$.

Hydrolysis of Locundeside. A solution of 200 mg of the glycoside in 8 ml of methanol was mixed with 72 ml of acetone and 0.8 ml of concentrated hydrochloric acid. The changes taking place on hydrolysis were checked by TLC in system 5. After three days, when the spot of the initial compound had disappeared, the solution was diluted with an equal amount of water and the acetone was evaporated off in vacuum. The aqueous residue was extracted first with chloroform and then with ethanol–chloroform (1 : 2). The combined chloroformic extracts and the combined ethanolic–chloroformic extracts were separately treated with 2 N sodium carbonate solution and were then washed with water to neutrality. The chloroformic extract, after the distillation of the solvent, gave a mixture of three substances. They were separated by preparative TLC in system 5. After the positions of the zones had been shown up by the appropriate reagents, the plate was separated into three zones. The lowest zone gave 60 mg of an aglycone with mp 256-258°C, $[\alpha]_D^{22}$ + 25.2° (c 1.84; methanol). In systems 6 and 7, this substance migrated at the same level as bi-

pindogenin. With 84% H_2SO_4 it gave a coloration changing with time: 0 min – yellow; 0.5 min – orange; 5 min – brown (violet edges); 20 min – violet; 1 h – lilac.

Mass spectrum: M^+ 406 (1%); m/e 388 ($M-H_2O$) – 8%; 370 ($M-2H_2O$) – 28%, 352 ($M-3H_2O$) – 100%; 337 ($M-3H_2O-CH_3$) – 19%, 334 ($M-4H_2O$) – 72%, 319 ($M-4H_2O-CH_3$) – 24%, 308 ($M-3H_2O-CO_2$) – 8%, 242 – 9%, 227 – 12%, 199 – 60%.

The aglycone (50 mg) was dissolved in a mixture of 1 ml of pyridine and 1 ml of acetic anhydride, and the solution was kept in the thermostat at 37°C for three days. After the usual working up, bipindogenin 3-O-acetate was obtained with mp 265-270°C (from ethanol).

The middle zone yielded 15 mg of an amorphous but chromatographically homogeneous compound with the mass spectrum: M^+ 388 – 4%, m/e 370 ($M-H_2O$) – 35%, 352 ($M-2H_2O$) – 100%, 337 ($M-2H_2O-CH_3$) – 24%, 334 ($M-3H_2O$) – 30%, 319 ($M-3H_2O-CH_3$) – 36%, 308 ($M-2H_2O-CO_2$) – 16%, 242 – 24%, 227 – 20%, 199 – 50%.

The top zone yielded 25 mg of 5,14-dianhydrobipindogenin with mp 170-172°C, $[\alpha]_D^{22}$ + 20.8° (c 1.63; methanol), mass spectrum: M^+ 370 – 70%, m/e 352 ($M-H_2O$) – 100%, 337 ($M-H_2O-CH_3$) – 3%, 334 ($M-2H_2O$) – 30%, 319 ($M-2H_2O-CH_3$) – 34%, 308 ($M-H_2O-CO_2$) – 10%.

The ethanolic–chloroformic extract, after the solvent had been distilled off, gave 25 mg of bipindogenin with mp 256-258°C.

The aqueous solution after the separation of the aglycone component was neutralized with silver carbonate, filtered, and evaporated to dryness. By means of GLC [4] and TLC (systems 1 and 2), the sugar was identified as L-rhamnose (revealing agent: a solution of 0.4 g of salicylic acid and 0.5 ml of o-toluidine in 10 ml of ethanol).

A mixture of 50 mg of the L-rhamnose obtained from locundeside, 100 mg of phenylhydrazine hydrochloride, 150 mg of sodium acetate, and 1 ml of water was boiled for 30 min in the boiling water bath. The yellow crystals that deposited had mp 180-182°C (from ethanol), and a mixture with the phenylosazone of an authentic sample of L-rhamnose showed no depression of the melting point. On chromatography in systems 3 and 4, the phenylosazones migrated at the same level (revealing agent: 6 g of KOH + 25 ml of H_2O + 45 ml of CH_3OH).

Samples of bipindogenin and locundeside were kindly given to us by N. F. Komissarenko [KhNIKhFI (Kharkov Chemical and Pharmaceutical Scientific-Research Institute)].

CONCLUSIONS

The cardenolide glycoside cuspidoside from Erysimum euspidatum (M. B.) DC is a bipindogenin L-rhamnoside and is identical with locundeside. In addition to locundeside, the plant has been found to contain erysimin, desglucocheirotoxin, and erysimoside. The mass spectra of bipindogenin and its anhydro derivatives have been studied.

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