CARDENOLIDES OF Erysimum suffruticosum

I. F. Makarevich

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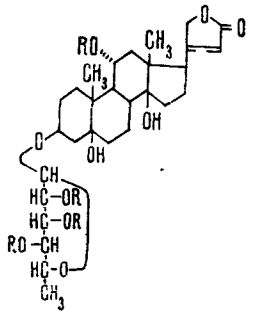
As reported previously [1], the seeds of Erysimum suffruticosum Spreng. contain not less than 12 cardenolides. After the enzymatic hydrolysis of the native compounds, canescein was isolated preparatively [2].

Continuing these investigations, we have isolated another six cardenolides, of which one proved to be new and has been named bipindogulomethyloside (1). The other cardenolides were identified as digitoxigenin, strophanthidin, erysimin (strophanthidin 3β-O-β-D-digitoxoside) [3], desglucoerycordin (cannogenol 3β-O-β-D-gulomethyloside) [4], and desglucocheirotoxin (strophanthidin 3β-O-β-D-gulomethyloside) [5].

The seeds of E. suffruticosum grown in an experimental field at the Khar'kov Scientific-Research Institute of Pharmaceutical Chemistry (I. G. Zoz, 1965-1967) were "autofermented" and extracted with ethanol, and, after the usual purification of the extract, a mixture of cardenolides (not less than 11) was obtained which was separated by adsorption chromatography (on alumina) and partition chromatography (on cellulose). The known substances were identified both by their properties and by direct comparison with authentic samples.

Bipindogulomethyloside (1) is a monoglycoside with the composition $C_{29}H_{44}O_{10}$. When chromatographed on paper, it scarcely differs from locundjoside (bipindogenin 3β-O-α-L-rhamnoside, Table 1). However, the other properties of these glycosides and, particularly, the properties of their acetates show that they are different compounds. After acid hydrolysis, performed by the Mannich–Siewert method [6], the aglycone bipindogenin and an amorphous monosaccharide were obtained in the pure state. The latter was identified as D-gulomethylose from the results of chromatographic analysis, the preparation of a crystalline phenylosazone, and a comparison of its properties with an authentic sample. Analysis of molecular rotations of the glycoside and the aglycone in accordance with Klyne's rule [7] showed that the D-gulomethylose is attached by a β-glycosidic bond. Thus, the new glycoside is bipindogenin 3β-O-β-D-gulomethyloside (bipindogulomethyloside), 1.



At the present time, only five cardiac glycosides the aglycone of which is bipindogenin have been isolated (Prof. T. Reichstein has kindly provided us with samples of three glycosides and their acetyl derivatives). The results of the comparison of the properties of the five glycosides of this group and their acetates are given in Table 1.

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TABLE 1

Glycosides and their molecular formulas; acetyl derivatives	Mp, °C	$[\alpha]_D$, deg and solvent	$R_{strophanthidin}$ in the chl–thf (1:1)/fmd system	Literature ref.
Alliside (α-L-glucomethyloside) $C_{29}H_{44}O_{10}$	180–183	–47,7 (met.)	0,184	[8]
Locundjoside (α-L-rhamnoside) $C_{29}H_{44}O_{10}$	184–187/277–279	–12,1 (met.)	0,170	[9]
Bipindaloside (β-D-digitaloside) $C_{35}H_{46}O_{10}$	167–169	+15,2 (met.)	0,260	[10]
Bipindoside (α-L-talomethyloside) $C_{29}H_{44}O_{10}$	163–166	–22,6 (met.)	0,360	[11]
Bipindogulomethyloside-(β-D-gulomethyloside)-$C_{29}H_{44}O_{10}$	150–153/168–170	–17,8 (met.)	0,174	Present work
			R_f in benzene/fmd system	
Tetra-O-acetylalliside	152–156	–65,0 (chl.)	0,60	Present work
Tetra-O-acetyllocundjoside	158–161	–24,3 (chl)	0,39	[9a]
Tri-O-acetylbipindaloside	286–289	+19,3 (chl)	0,33	[10]
Tetra-O-acetylbipindoside	153–156	–35,7 (chl)	0,24	[11]
Tetra-O-acetylbipindogulomethyloside	153–155	–3,1 (chl)	0,36	Present work

Note. chl–chlorform; met. – methanol; thf – tetrahydrofuran; fmd – formamide.

TABLE 2

Fraction No.	Solvent	Amount, ml	Composition
1	Chl	280	Oil
2	Chl–alc (99:1–98:2)	90	Digitoxigenin (I)
3	Chl–alc 98:2	120	Strophanthidin (II)
4	The same	50	—
5	Chl–alc (97:3)	100	Unknown cardenolide (III) + erysimin (IV)
6–9	The same	350	Erysimin (IV)
10–15	Chl–alc 96:4 –95:5	400	Erysimin (IV) + unknown cardenolides (V, VI)
16–22	" 94:6 –90:10	800	Desglucoerycordin (VII) + traces of VI
23–30	" 89:11–87:13	500	Desglucoerycordin (VII) + desglucocheirotoxin (VIII)
31–42	" 86:14–83:17	900	Desglucocheirotoxin (VIII) + (IX)
43–44	" 83:17	120	(IX) + unknown cardenolide (X)
45–47	" 82–18–81:19	350	—
48–56	" 80:20–77:23	550	Canescein (XI)
57–62	" 75–25–74–26	350	Canescein (XI, traces) + erysimoside (?, XII, traces)
63–80	" 73:27–70:30	1400	—

With concentrated H_2SO_4 they form extremely similar colors changing with time (see, for example, the reaction of bipindogulomethyloside in the experimental part). Consequently, this reaction can hardly be used as a criterion for distinguishing the bipindogenin glycosides from one another. However, it is possible to use it as one of the methods for the group identification of the glycosides.

The isolation from fermented seeds of digitoxigenin and strophanthidin, and also the almost complete absence of these compounds in the unfermented raw material shows the possible presence in E. suffruticosum of glycosides of the structure in which sugars capable of being split off under the action of enzymes are attached directly to the aglycones. The most probable sugars for Erysimum in this connection are D-glucose and D-fucose.

EXPERIMENTAL

The substances were analyzed after drying at 100°C in vacuum over phosphorus pentoxide. The cardenolides were chromatographed on paper in the Kaiser systems: methyl ethyl ketone–m-xylene (1:1)/formamide (I) and chloroform–tetrahydrofuran (1:1)/formamide (II).

The comminuted seeds (0.8 kg) were defatted with petroleum ether, dried in the air, uniformly wetted with water, and left in a thermostat at 40–42°C for 46 h. The cardenolides were extracted with ethanol until the raw material was free from bitter taste. The ethanolic extracts were concentrated to a volume of

about 1 liter, 1 liter of water was added, and concentration was repeated to an aqueous residue. The aqueous solution was purified by shaking with petroleum ether (3 × 500 ml) and with diethyl ether (100 ml). Then it was filtered through 200 g of alumina. The adsorbent was washed with water until the reaction of the wash-waters for cardenolides (Legal reaction) was negative. The filtrate was saturated with sodium sulfate and the glycosides were extracted with ethanol–chloroform (1:2) (6 × 0.7 liter). The combined ethanolic–chloroformic extracts were washed with water (2 × 70 ml), dried over anhydrous sodium sulfate, filtered, and evaporated. The residue consisted of 16 g of a viscous light brown mass solidifying on cooling.

The mixture of glycosides was chromatographed on 500 g of alumina (activity grade III). Elution was performed with chloroform and with chloroform–ethanol (99:1–70:30; Table 2).

The first separation of the glycosides gave chromatographically pure digitoxigenin (I), erysimin (IV), strophanthidin (II), and canescein (XI), and also desglucoerycordin (VII) contaminated with a small amount of an unknown cardenolide (VI). To improve crystallization, the digitoxigenin was rechromatographed on alumina, using for elution benzene and benzene–chloroform (9:1-3:7).

The mixture of bipindogulomethyloside (IX) and desglucocheirotoxin (VIII) from fractions 31-42 (0.7 g) was chromatographed on 350 g of cellulose in the chloroform–tetrahydrofuran (1:1)/formamide system. The formamide had previously been purified with alumina (activity grade II) and activated with carbon. For the deposition of the stationary phase the cellulose was impreganted with formamide–acetone (1:1), the excess of solvent was eliminated by filtration, and it was dried in the air (until it no longer smelled of acetone). The fractions were taken with the aid of an automatic collector. The separation of the glycosides was practically complete. The eluates containing the individual substances were evaporated and were dried (to free them from formamide) in a vacuum of 0.01 mm Hg. In order to eliminate impurities of a noncardenolide nature and to improve the crystallization, the glycosides were separatedly adsorbed on alumina (activity grade III), the alumina was washed with chloroform, and desorption was then performed with chloroform–ethanol (4:1).

Digitoxigenin (I) was crystallized from ethanol, mp 246-250°C, $[\alpha]_D^{22}$ +18.2 ± 2° (c 0.62; methanol). A mixture with an authentic sample melted at 246-252°C, $R_{digitoxigenin}$ = 1.00 (system 1, and also benzene/formamide).

Strophanthidin (II), mp 158-163/229-232°C. A mixture with an authentic sample gave no depression of the melting point; $R_{strophanthidin}$ = 1.00 (systems 1 and 2).

Erysimin (IV) [3] had mp 174-177°C (60% ethanol). A mixture with an authentic sample of erysimin melted at 174-178°C; $[\alpha]_D^{20}$ +24.8 ± 3° (c 1.09; methanol); $R_{erysimin}$ = 1.00 (systems 1 and 2).

Desglucoerycordin (VII). In spite of the presence in fractions 16-22 of a small amount of an unknown cardenolide, after two crystallizations they yielded pure desglucoerycordin with mp 161-164°C (acetone); mixture with an authentic sample mp 161-164°C; $[\alpha]_D^{22}$ −20.8 ± 2° (c 0.93; methanol); $R_{desglucoerycordin}$ = 1.00 (system 2).

Desglucocheirotoxin (VIII) [5] crystallized from aqueous solution with mp 189-192°C, $[\alpha]_D^{21}$ −7.1 ± 3° (c 1.00; methanol). A mixture with an authentic sample (kindly provided by N. F. Komissarenko) gave no depression of the melting point (189-192°C); $R_{desglucocheirotoxin}$ = 1.00 (system 2). The hydrolysis of the glycoside (performed as described for bipindogulomethyloside, see below) formed strophanthidin and D-gulomethylose (identified by paper chromatography).

Bipindogulomethyloside (XI) melted at 150-153/168-170°C (isopropanol–ether), $[\alpha]_D^{20}$ −17.76 ± 2° (c 1.10; methanol). The results of elementary analysis agreed with the figures calculated for the composition $C_{29}H_{44}O_{10}$. With concentrated H_2SO_4 it formed a coloration changing with time: 0-2 sec – yellow; 5 sec – orange; 5 min – brown; 75 min – violet; 500 min – blue-violet with a precipitate.

Tetra-O-acetylbipindogulomethyloside. The glycoside (45 mg) dissolved in absolute pyridine (1 ml) was acetylated with acetic anhydride (0.5 ml) at 21-23°C for 46 h. Then water and ice were added and the mixture was stirred in the cold for 1.5 h. The crystals that deposited were filtered off and washed with water. The residues of acetyl glycoside were extracted with chloroform (100 ml). The chloroform solution was treated with 5% sulfuric acid (2 × 10 ml) and with water (5 × 10 ml) and was evaporated. The product was crystallized from methanol–water at 3-5°C. The acetate melted at 153-155°C, $[\alpha]_D^{21}$ −3.1 ± 2° (c 1.57; chloroform).

Hydrolysis of Bipindogulomethyloside. A solution of 80 mg of the glycoside in 10 ml of a mixture of concentrated hydrochloric acid and acetone (1:99 by volume) was left to stand for 70 h. Then 25 ml of

water was added and the acetone was evaporated off in vacuum at 45-50°C. The mixture was heated further at the same temperature in a flask with a reflux condenser for 20 min. The aglycone fraction of the hydrolyzate was extracted with chloroform–ethanol (3 : 1) (5 × 40 ml).

The ethanolic-chloroformic solution was washed with a 2 N solution of sodium bicarbonate (5 ml) and with water (4 × 10 ml) and was evaporated. The residue was chromatographed on 1.6 g of alumina (activity grade III). Elution was performed with chloroform and with chloroform–ethanol (99 : 1–93 : 7). The aglycone crystallized from isopropanol in the form of square plates with mp 242-248/304-308°C, $[\alpha]_D^{20}$ + 29.6 ± 2° (c 1.22; methanol). A mixture with a sample of bipindogenin gave no depression of the melting point. With concentrated H_2SO_4 the aglycone obtained and bipindogenin gave similar colorations changing with time: 0 min – yellow; 1 min – yellow-orange; 10 min – brown; 120 min – reddish brown; 320 min – blue. The IR spectrum (taken by I. P. Kovalev) was also identical with that of bipindogenin.

The aqueous solution was neutralized with silver carbonate at 0-3°C and was filtered. The filtrate was saturated with hydrogen sulfide, likewise with cooling, and was filtered through a layer of kieselguhr. After evaporation, a monosaccharide was obtained in the form of a vitreous mass. By paper chromatography in the following solvent systems: butan-1-ol–acetic acid (4 : 1)/water and butan-1-ol–methyl ethyl ketone–borate buffer (1 : 1 : 2) [12], the monosaccharide was identified as D-gulomethylose. The phenylosazone of the monosaccharide had mp 179-181°C. A mixture with an authentic sample melted at 179-181°C. On paper chromatography, $R_{\text{D-gulomethylose phenylosasone}} = 1.00$ (system 1 and benzene/formamide).

Canescein (XI) [2, 13] crystallized from water, mp 193-195°C, $[\alpha]_D^{20}$ −23.6 ± 3° (c 0.62; methanol); $R_{\text{canescein}} = 1.00$ (system 2). A mixture with an authentic sample gave no depression of the melting point.

CONCLUSION

Seven cardenolides have been isolated from the seeds of Erysium suffruticosum Spreng.: digitoxigenin, strophanthidin, erysimin, desglucocheirotoxin, desglucoerycordin, canescein, and a new glycoside which we have called bipindogulomethyloside. The latter is bipindogenin 3β-O-β-D-gulomethyloside.

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