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GLYCOSIDES OF ERYSIMUM

VI. Cardenolides of E. altaicum, E. cuspidatum, E. diffusum, E. marschallianum, E. nuratensae, E. violascens, and Syrenia siliculosa

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In previous communications, we have given the results of a study of the cardenolides of Erysimum diffusum Ehrh. [1] and E. gypsaceum Botsch. et Vved. [2]. The present paper gives a summary of orienting studies of some other wild and cultivated species of Erysimum. The quantitative content of glycosides was determined photometrically. In view of the fact that erysimoside has been found in all the species of Erysimum previously studied [3], and is, apparently, the main glycoside for this plant genus, in individual cases in addition to the total content of cardenolides we made a rough determination of the amount of erysimoside (table).

The cardenolide composition of the seven plants described below was studied in more detail. The methods of isolating and separating the glycosides were roughly the same. The method used in studying the cardenolides of E. diffusum [1] was employed.

The total extractive substances removed by ethanol from the epigeal part of E. altaicum C.A.M. in 1961 were kindly placed at our disposal by P. M. Loshkarev (All-Union Scientific Research Institute for Medicinal Plants). In the chloroform – isoamyl alcohol (1:1) – water system, the presence in the plant of eight substances of a cardenolide nature was demonstrated. Glycosides of erysimin and erysimoside were obtained from them in the crystalline state in amounts of 0.009 and 0.088% of the dry weight of the plant, respectively.

E. cuspidatum (M. B.) DC is being introduced into the botanical Gardens, AS UzSSR. By paper chromatography, a methanolic extract of the defatted seeds of this plant was shown to contain seven cardenolides. Three glycosides were isolated in the crystalline state, erysimin, erysomide, and a new cardiac glycoside which we have called cuspidoside. The yields of erysimin and erysomide were approximately 0.03% each, and of cuspidoside 0.05% of the weight of the seeds.

E. diffusum Ehrh. (syn. E. canescens Roth.) is found in Central Asia in the wild form, but for our work we used raw material cultivated in the Botanical Gardens, AS UzSSR. The seeds of this plant were found to contain the glycosides described previously [1, 4], erysimin, erysimide, desglucocheirotoxin, and canescein, and also a new cardenolide which we have called erydiffuside. The content of erydiffuside in the seeds varied between 0.015 and 0.02%. The mutual positions of the spots of the individual compounds on the control paper chromatograms has been shown previously [1], spot A corresponding to erysimin, B to desglucocheirotoxin, C to canescein, D to erysimoside, and E to erydiffuside. Erysimin and erysimoside were found in the seeds of the cultivated E. marschallianum Andrz. [5] and in the epigeal part of the wild E. nuratensae M. Pop. From an extract of the flowers of the wild E. violascens M. Pop, in addition to erysimin and erysimoside, we isolated gypsobioside, which has previously been found in E. gypsaceum Botsch. et Vved. [2]. The seeds of Syrenia siliculosa (M. B.) Andrz. contain a complex mixture of glycosides including eight components. Erysimin and erysimoside were isolated in the pure state.

Experimental

Quantitative determination of the glycoside. One gram of homogeneously ground plant raw materials that had been passed through a sieve (with 1-mm openings) was covered with 100 ml of 70% ethanol and vigorously stirred for 2 hr on a vibration shaker. If the material consisted of seeds, it was previously defatted with petroleum ether (it was not necessary to free the extract from other foreign materials). One milliliter (accurate volume) of the filtered extract was transferred with a micropipet to a 10-ml flask and was mixed with 1.25 ml of a 0.075% solution of 2,4-dinitrodiphenyl sulfone [6] in alcohol. Simultaneously, a solution for the two control cells was prepared in another flask by mixing 2 ml of 70% ethanol, 2.5 ml of the 0.075% solution of 2,4-dinitrodiphenyl sulfone in alcohol, and 0.5 ml of a 0.15 N solution of caustic potash. The time of adding the alkali was recorded by a stopwatch. Readings were taken every half minute on the scale of the right drum of a FEK-M photocolorimeter fitted with a yellow filter (λ_{max} 595 mμ). The maximum value of the optical density, which was generally reached after 3.5–4.0 min and retained for 1.5–2.0 min, was recorded. The optical density of a standard solution prepared by dissolving 6–7 mg (accurately weighed) of erysimoside in 100 ml of 70% ethanol was determined in exactly the same way. The linear dependence between the concentration and the optical density makes it unnecessary to construct a special calibration curve. The content of glycoside in the raw material was calculated directly from the ratio of the optical densities of the standard solution and the extract.

Results of a Quantitative Determination of the Glycosides in some Species of Erysimum, % on the Air-Dry Weight of the Plant

Plant	Plant organ	Glycoside content	
		total	including erysimoside
E. altaicum C. A. M.	epigeal part	0.29	0.12
E. Badghysi (Korsh) Lipsky	flowers	0.29	—
E. clausioides Botsch. et Vved.	flowers	0.12	—
	leaves	0.06	—
E. crepidifolium Rchd.	seeds	1.00	—
	leaves	0.36	—
E. croceum M. Pop.	flowers	0.37	—
	leaves	0.17	—
E. cuspidatum DC (M. B.)	seeds	0.83	0.32
	flowers	0.35	—
	leaves	0.06	
E. diffusum Ehrh. (syn. E. canescens) Roth.	seeds	4.96	2.92
E. gypsaceum Botsch. et Vved.	seeds	1.24	0.46
	flowers	0.58	—
	leaves	0.16	—
E. humillimum (Ldb.) N. Busch.	flowers	0.29	—
	leaves	0.20	—
E. linifolium (Pers.) Gag	flowers	0.26	—
E. Marschallianum Andrz.	seeds	0.98	0.67
	flowers	0.26	—
	leaves	0.16	—
E. nuratensae M. Pop.	epigeal organs	0.56	0.27
E. pannonicum Crantz.	flowers	0.16	—
	leaves	0.04	—
E. Perowskianum Fisch. et Mey	flowers	0.70	—
	leaves	0.34	—
	stems	0.15	—
E. repandum L.	seeds	0.45	0.24
	flowers	0.05	—
	leaves	0.02	—
E. semperflorens (syn. Cheiranthus semperflorens Schnusb)	seeds	0.91	0.47
E. silvestris Scop.	seeds	4.30	2.42
	flowers	0.14	—
	leaves	0.11	—
E. sylveticum M. B.	flowers	0.20	—
E. suffruticosum Spr.	flowers	0.15	—
	leaves	0.08	—
E. violascens M. Pop.	flowers	0.50	0.21
	leaves	0.08	—
Syrenia angustifolia (Ehrh.) Rchb [syn. E. angustifolium (Ehrh.)]	seeds	0.48	0.28
Syrenia siliculosa (M. B.) Andrz. (syn. E. siliculosum DC)	seeds	0.88	0.39
	flowers	0.11	—
	leaves	0.17	—

To determine the erysimoside separately, we used a preparative chromatographic method similar to that proposed for the analysis of vegetable raw material containing k-strophanthin-β [7]. An alcoholic extract of the defatted seeds of the plant was deposited on a thin nonfixed layer of alumina and, after chromatographic separation, the zone corresponding to the erysimoside was scraped off and eluted with ethanol. The concentration of the glycoside in the eluate was determined colorimetrically.

The numerical data on the content of erysimoside given in the table are indicative in two respects. On chromatography, this zone contains not only erysimoside, but also erychroside, erycordin, and other biosides. However, on preparative isolation, which is unavoidably connected with certain losses, the erysimoside always proved to be present in larger amount than the other glycosides.

Isolation and separation of the glycosides. The ground raw material was defatted with petroleum ether and exhaustively extracted with methanol at room temperature. The combined extract was evaporated in vacuum at 35–40° C to a volume equal to the weight of the initial raw material and diluted with half a volume of water, after which it was treated with hydrated lead oxide and, after being freed from excess of lead ion, it was evaporated in vacuum to dryness.

The dry extract was mixed with an equal amount of alumina and transferred to a column of alumina containing 30–40 g of Al_2O_3 for each gram of extract. The column was eluted with a mixture of equal volumes of toluene and butanol or benzene and butanol that had previously been saturated with water. The fractions eluted were analyzed by paper chromatography in the chloroform – isoamyl alcohol (1 : 1) – water and toluene – butanol (1 : 1) – water systems. Fractions containing similar compounds were combined and, where it was impossible to crystallize them, were refractionated on a column of alumina, being eluted with chloroform with increasing amounts of ethanol or isoamyl alcohol. To obtain erydiffuside in the crystalline state from the seems of E. diffusum, preparative chromatography on cardboard in the chloroform–isoamyl alcohol (1 : 1) – water system was used, as well.

A description of the individual compounds is given below.

Erysimin, $C_{29}H_{42}O_9$, mp 174–175° C [from methanol – water (3 : 2)], $[\alpha]_D^{20}$ +32.2° (c 1.84; methanol). The other properties are similar to those of desglucoerysimoside [1].

Erysimoside, $C_{35}H_{52}O_{14}$, mp 236°–238° C (from propanol), $[\alpha]_D^{20}$ +20.4° (c 2.32; methanol). Hydrolysis with 0.1 N sulfuric acid gave digilanidobiose with mp 218°–220° C, $[\alpha]_D^{20}$ +28.2° (c 1.84; water) and strophanthidin [1].

Cuspidoside, $C_{29}H_{44}O_9$, mp 233°–235° C (from ethanol), $[\alpha]_D^{20}$ −9.9 ± 2° (c 2.43; methanol). Cardenolide reactions (Legal, Kedde, etc.) positive, Keller-Kiliani reaction negative. Color reaction with concentrated sulfuric acid: 0 min orange-yellow, 1 min yellow, 5 min yellow with pink edges, 10 min light brown with violet edges, 30 min violet, 1 hr dirty violet.

UV spectrum: λ_{max} (in ethanol) 218 mμ (log ε 4.20); λ_{max} (in conc. H_2SO_4) 232–234 (butenolide) [8], 328–330 (2-hydroxy sugar), 440–445, 504–506 (log ε 4.22, 3.82, 3.60, 3.80). IR spectrum: 3440 (OH group), 1880, 1740, 1625 cm^{-1} (butenolide ring). The nature of the UV and IR spectra show the absence of an angular carbonyl group. In experiments on enzymatic hydrolysis with the pancreatic juice of Helix plectotropis and on acid hydrolysis with 0.1 N H_2SO_4, the glycoside was unaffected. In its physicochemical constants and the color reaction in sulfuric acid, cuspidoside is extremely similar to desglucocheiroside [9], but in the toluene – 1-butanol (1 : 1) – water system it has a R_f value only half that of desglucocheiroside. On vigorous acid hydrolysis with 10% sulfuric acid the glycoside underwent degradation. A chromatogram of the aglycone moiety in the chloroform – ethanol (9 : 1) – formamide system showed the presence of a mixture of four compounds. In the 1-butanol – pyridine – benzene – water (5 : 1 : 3 : 3) system, the sugar component moved at the same level as L-rhamnose, but it is not excluded that it is another isomeric methylpentose.

Desglucocheirotoxin, $C_{29}H_{42}O_{10}$, mp 183°–185° C (from ethanol), $[\alpha]_D^{20}$ −10.2 ± 2° (c 1.96; methanol). By comparison of its physicochemical constants, color reaction, and chromatography in the chloroform – isoamyl alcohol (1 : 1) – water system, this cardenolide of E. diffusum was shown to be identical with an authentic sample of desglucocheirotoxin first found in Cheiranthus cheiri [9]. The sample of glycoside was kindly supplied by Prof. T. Reichstein (Switzerland).

Canescein, $C_{29}H_{42}O_{10}$, mp 193°–194°C (from ethanol), $[\alpha]_D^{20}$ +5.1 ± 1° (c 2.03; methanol). The Keller–Kiliani and Webb – Levy reactions were negative. On paper chromatography in the water – saturated butanol system, the glycoside gave a spot more polar than desglucocheirotoxin and less polar than erysimoside. On the basis of the results given it must be assumed that the substance that we had isolated was canescein, first described by Liu Yung-lung and Loshkarev [4].

Erydiffuside, $C_{29}H_{42}O_{10}$, mp 193°–195° C [from ethanol – ether (1 : 3)], $[\alpha]_D^{20}$ −15.2 ± 2° (c 1.30; methanol). Acetate mp 176°–178° C (from ethanol). The compound is readily soluble in ethanol and methanol, fairly readily in water, and sparingly in chloroform. Cardenolide reactions positive. Keller – Kiliani and Webb – Levy reactions negative. The color reaction with concentrated sulfuric acid was as follows: 0 min yellow, 1 min light brown, 5 min chocolate brown, 30 min brown with blue-green edges, 1 hr dirty green, 1 hr 30 min olive green. UV spectrum: λ_{max} (in ethanol) 220, 300 mμ (log ε 4.22, 1.48). IR spectrum: 3400 (OH group), 1740, 1630 (butenolide ring), 2700, 1730 cm^{-1} (C=O group). The UV and IR spectra showed the presence of an angular aldehyde group. A determination of the number of acetyl groups from the integral intensity for the ester band [10] in the IR spectrum of the acetate showed the presence of four acetylatable hydroxy groups in erydiffuside. A snail enzyme preparation and a 0.1 N solution of sulfuric acid did not hydrolyze the glycoside. Acid hydrolysis by the Mannich – Sivert method led to resinification. Hydrolysis with 5% sulfuric acid yielded crystals, but chromatographic analysis in the chloroform – alcohol (9 : 1) system showed that the reaction product was a mixture of three substances. The carbohydrate component was identified as D-gulomethylose in the 1-butanol – acetic acid – water (4 : 1 : 5), butanol – benzene – pyridine – water (5 : 1 : 3 : 3), and butanol – methyl ethyl ketone – borate buffer (1 : 1 : 2) systems. The borate buffer consisted of equal volumes of 0.1 M H_3BO_3 and 0.1 M $Na_2B_4O_7$ [11]. Thus, erydiffuside is the D-gulomethyloside of an aglycone of unknown structure containing two acetylatable hydroxy groups in addition to an aldehyde group.

Gypsobioside, $C_{34}H_{50}O_{14}$, mp 240°–241° C (from alcohol – ether) $[\alpha]_D^{20}$ +27.8 ± 1° (c 1.37; 80% methanol). By

chromatography in the toluene – butanol (1:1) – water system and by a comparison of physicochemical properties, color reactions and absorption spectra in sulfuric acid, it was shown to be identical with an authentic sample of gypsobioside from E. gypsaceum [2]. Hydrolysis with 0.1 N sulfuric acid gave strophadogenin with mp 144°–147° C and gypsobiose.

Summary

The cardenolides of six species of Erysimum and of Syrenia siliculosa have been studied. The cardiac glycosides erysimin and erysimoside have been found in E. altaicum, E. marschallianum, E. nuratensae, and S. siliculosa. In addition to these glycosides, E. diffusum has yielded desglucocheirotoxin, canescein, and a new cardenolide, erydiffuside, E. violascens has yielded gypsobioside, and E. cuspidatum has yielded a new glycoside cuspidoside.

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