Erysimum GLYCOSIDES

X. CARDENOLIDES OF Erysimum cuspidatum

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UDC 547.918:547.926+615.711.5

We have previously [1, 2] reported the isolation from the seeds of E. cuspidatum (M.B.) DC of four cardenolides – erysimin, desglucocheirotoxin, lokundjoside, and erysimoside.

When a chloroform fraction of an extract of the plant was separated on alumina, we again obtained a crystalline product giving no reactions for cardenolides and sugars [2]. Elementary analysis showed the empirical composition of the substance to be $C_5H_9O_2S_2N$. The UV spectrum of the compound in ethanol had a maximum at 244 nm with a fairly high extinction (log ε 4.83). This maximum which is extremely characteristic for a N=C=S group [3] shows that we had isolated an isothiocyanate.

As early as 1909, sulfur-containing glycosides were isolated from the seeds of Cheiranthus cheiri the aglycone of which was the isothiocyanate cheiroline $CH_3SO_2CH_2CH_2CH_2N{=}C{=}S$ [4].

The mass spectrum of the compound that we isolated has the peak of the molecular ion with M^+ 179, and fragments with m/e 121 (M – NCS), 79 (CH_3SO_2), and 72 (CH_2NCS). On the basis of these results, we may consider that the isothiocyanate from E. cuspidatum and cheiroline are identical.

From an ethanolic-chloroformic extract [2], after the separation of the erysimoside we eluted the combined polar glycosides, which, after rechromatography, were separated into three individual compounds. The first of them, obtained in the amorphous form, was not hydrolyzed by 0.1 N sulfuric acid. After the fermentation of the glycoside with snail gastric juice, D-glucose and desglucocheirotoxin were detected. Mannich hydrolysis [5] led to strophanthidin, D-gulomethylose and D-glucose. These properties correspond to cheirotoxin [6]. A chromatographic comparison of the substance that we isolated with an authentic sample of cheirotoxin confirmed their identity.

The glycoside more polar than cheirotoxin, after its structure had been established, was called glucolokundjoside. This substance is not hydrolyzed by 0.1 N sulfuric acid. Consequently, the aglycone in this compound is not bound to a 2-deoxysugar. On Mannich hydrolysis [5], we found in the hydrolyzate by thin-layer chromatography bipindogenin, D-glucose, and L-rhamnose. The presence of these sugars in a ratio of 1 : 1 was also shown by gas–liquid chromatography of the silylated methyl glycosides.

The position of attachment of the sugars was established provisionally on the basis of the mass spectra of the completely-acetylated glycoside and was confirmed by enzymatic hydrolysis. Under electron impact, the hexaacetate of glucolokundjoside (II) formed fragments with m/e 331 and 231, relating respectively, to ions derived from tetra-O-acetylglucose (M – 17) and di-O-acetylrhamnose (M – 17).

The action on the glycoside of snail pancreatic juice formed D-glucose and a monoglycoside which was identified as lokundjoside. The positions of attachment of the D-glucose and L-rhamnose were determined with the aid of periodate oxidation and methylation.

When the glycoside that had been oxidized by sodium periodate was hydrolyzed, no sugars were found, which indicates the absence of 1→3 bonds. The acid hydrolysis of the glycoside that had been methylated by Hakamori's method [7] yielded 2,3,4,6-tetra-O-methyl-D-glucose and a di-O-methyl-L-rhamnose. Since a 1→3 bond was excluded, of the three possible isomeric dimethylrhamnoses the compound that we obtained could be 2,3- or 3,4-di-O-methyl-L-rhamnose. The methylpentose was not oxidized by periodic acid,

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 166-170, March-April, 1975. Original article submitted December 18, 1973.

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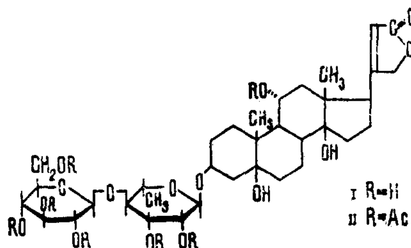
which means that it was 2,3-di-O-methyl-L-rhamnose. It follows from this fact that in glucolokundjoside the D-glucose can be attached to the L-rhamnose only by a 1 → 4 bond.

The configuration of the glycosidic bond was established from molecular rotation differences of glucolokundjoside and lokundjoside: $[M]_D$ for glucolokundjoside is 57.12, for lokundjoside 49.74. The proportion of the rotation due to D-glucose is 7.38, which shows a β-glucosidic bond. Consequently, the glycoside that we isolated from Erysimum cuspidatum has the structure of bipindogenin 3β-O-[4'-O-β-D-glucopyranosyl-α-L-rhamnopyranoside] (I):



The last compound eluted from the column was a crystalline glycoside which was hydrolyzed under the action of a snail enzyme preparation, forming erysimoside, erysimin, and D-glucose. The results obtained show that the glucoside is a compound of erysimoside with glucose. At the present time, two such compounds have been described: strophanthidin 3-O-[O-β-D-glucopyranosyl-(1 → 4)-O-β-D-glucopyranosyl-(1 → 4)-β-D-digitoxopyranoside] (mp 203-206°C, $[\alpha]_D$ +11.6 ± 3°, methanol), which has been called glucoerysimoside [8, 9, 10], and strophanthidin 3β-O-[O-β-D-glucopyranosyl-(1 → 6)-O-β-D-glucopyranosyl-(1 → 4)-β-D-digitoxopyranoside] (mp 195-196°C, $[\alpha]_D$ +8.9 ± 2°; ethanol), which has been called neoglucoerysimoside [11]. The physicochemical constants of the glycoside that we have isolated (mp 234-236°C, $[\alpha]_D$ +16 ± 2°; methanol) differ considerably from those of the two compounds mentioned. However, the hydrolysis of the glycoside from E. cuspidatum with 0.2 N sulfuric acid yielded D-digitoxose, D-glucose, and cellobiose. The presence of the latter disaccharide gave us grounds for assuming that the compound isolated is in fact, in spite of some difference in the constants, glucoerysimoside.

A direct chromatographic comparison with an authentic sample of glucoerysimoside confirmed our assumption.

EXPERIMENTAL METHOD

For thin-layer chromatography we used type KSK silica gel containing 5% of gypsum, and for paper chromatography type "M" ("slow") paper. 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); 3) butan-1-ol—methanol—water (5 : 3 : 1); 4) toluene—isobutanol—water (1 : 3 : 2); 5) benzene—chloroform—methanol (5 : 5 : 2); 6) butan-1-ol—toluene—water (1 : 1 : 1); 7) chloroform—dioxane—butanol (7 : 2 : 0.5)/formamide; 8) benzene—acetone (2 : 1); 9) chloroform—methanol (9 : 1); and 10) butan-1-ol—toluene—water (1 : 4 : 5).

The mass spectra were taken on an MKh-1303 mass spectrometer at a temperature of the internal inlet tube of 200°C and an ionizing voltage of 40 V. The UV spectra were taken on a Hitachi spectrophotometer.

The isolation and separation of the glycosides was performed as described previously [2]. On separation of the chloroform fraction on alumina, 300 mg of yellowish crystals of cheiroline with mp 120°C (from methanol) were obtained. Schneider [4], who was the first to describe cheiroline gave its melting point as 47-48°C. The large difference between the melting point of our compound and that given in the literature has not yet been explained.

From an ethanolic-chloroformic extract by chromatographic separation on a column of alumina on elution with chloroform containing increasing concentrations of ethanol (from 20 to 40%) we obtained the cardenolides erysimin, desglucocheirotoxin, lokundjoside, and erysimoside [2]. Then the column was washed with a mixture of toluene, butan-1-ol, and water in a ratio of 1 : 3 : 2 (organic phase). The eluates were dried with sodium sulfate and evaporated to dryness. The fractions corresponding to polar glycosides (checked by PC, system 4) were combined and were reseparated on a column of alumina. For this purpose, 5 g (corresponding to 2 kg of the seeds of Erysimum cuspidatum) of the combined polar glycosides were mixed with 5 g of alumina and deposited on a column containing 200 g of alumina of activity grade III. The glycosides were eluted with the organic phase of system 4. The results are given below:

Fraction No.	Amount of eluate ml	Characteristics of the compounds isolated	Paper chromatographic check
41-46	600	Dark brown mass	Reaction for cardenolides negative
47-52	600	"	Traces of erysimoside and cheirotoxin
53-55	300	Light brown amorphous powder	Cheirotoxin and glucolocundjoside
56-61	600	Light brown amorphous powder	Glucolokundjoside
62-62	200	Yellow powder	Glucolokundjoside and glucoerysimoside
64-71	800	"	Glucoerysimoside

By preparative separation on chromatographic paper (system 4), fractions 47-52 yielded 15 mg (0.0007% of the weight of the raw material) of cheirotoxin in the form of a white amorphous powder.

The residue after the evaporation of fraction 56-61 and additional purification by means of activated carbon gave 80 mg (0.004% of the weight of the raw material) of a powder of glucolokundjoside.

After recrystallization from methanol–ether, fractions 64-71 gave 60 mg (0.003%) of small white crystals of glucoerysimoside.

Cheirotoxin

$C_{35}H_{52}O_{15}$, $[\alpha]_D^{22} - 18 \pm 2°$ (c 1.4; methanol). Cheirotoxin (5 mg) in one drop of methanol was mixed with 2 mg of acetone containing 1% of hydrochloric acid. The course of hydrolysis was checked by TLC in system 5. After two days, the spot of the initial compound had disappeared. The solution was treated with 2 ml of water and was extracted with chloroform. The chloroform extract was washed with water, with 2% sodium bicarbonate solution, and again with water, and was dried with sodium sulfate and evaporated. The residue consisted of strophanthidin, which was identified by TLC in system 5 and PC in system 7. The acid aqueous solution was neutralized with freshly precipitated silver carbonate, filtered, evaporated, and analyzed in system 3 by the TLC method on plates prepared by the method of Ovodov et al., [12] and by PC in system 2. The presence of D-gulomethylose and D-glucose was established.

The glycoside (5 mg) was dissolved in 0.1 ml of ethanol, the solution was diluted with 2 ml of water, and one drop of the gastric juice of the grape snail was added. After a day, the solution was evaporated in vacuum to dryness. The residue was dissolved in 0.5 ml of ethanol, and the solution was filtered and was again evaporated. The residue consisted of desglucocheirotoxin, which was identified by means of PC in system 6 and by TLC in system 5 with an authentic sample.

Glucolokundjoside

$C_{35}H_{54}O_{15}$. $[\alpha]_D^{22} - 8 \pm 2°$ (c 2.1; methanol), $\lambda_{max}^{C_2H_5OH}$ 218 nm (log ε 4.11); with concentrated sulfuric acid gave a coloration changing with time: 0 min – orange; 5 – brown: 10 – brown with violet edges; 20 – violet; 1 h – lilac.

The glycoside (20 mg) was dissolved in 0.2 ml of methanol, and 10 ml of acetone containing 10% of hydrochloric acid was added. After three days, the solution was treated as described for cheirotoxin. From a chloroform extract we isolated 8 mg of bipindogenin with mp 254-256°C. The aglycone was shown to be identical with an authentic sample in systems 5 (TLC) and 7 (PC). In the acid aqueous solution after neutralization with Ag_2CO_3, L-rhamnose and D-glucose were found (system 3).

The glucolokundjoside (15 mg) was dissolved in 20 ml of water, 2-3 drops of a snail enzyme preparation was added, and the mixture was left at room temperature (25°C). The changes were followed by TLC in system 5 every 10 min. After 3 h, the fermentation had taken place completely. The reaction mixture was worked up as described for cheirotoxin. The residue was recrystallized from ethanol, giving 7 mg of crystals of lokundjoside with mp 230-233°C. It was identified in systems 6 (PC) and 3 (TLC).

A solution of 30 mg of glukolocundjoside in 3 ml of dry dimethyl sulfoxide was treated with 30 mg of sodium hydride, and the mixture was stirred for 4 h. Then, with continued stirring, 2 ml of CH_3I was added dropwise and stirring was continued for another 5 h. Then the mixture was poured into water and extracted with chloroform. The chloroform extract was washed with a solution of hyposulfite and water. The chloroform was evaporated off, the residue was dissolved in 10 ml of 5% methanolic sulfuric acid, and the mixture was heated on the boiling water bath for 2 h. Then 10 ml of water was added and the methanol was distilled off in vacuum. The aqueous solution was boiled for 2 h, neutralized with barium carbonate,

filtered, and evaporated. Analysis by the TLC method in system 8 showed the presence of 2,3,4,6-tetra-O-methyl-D-glucose and a di-O-methyl-L-rhamnose.

A solution of 30 mg of the glycoside in 2 ml of dry pyridine was treated with 2 ml of freshly distilled acetic anhydride, and the mixture was left at room temperature for three days. Then the solvent was distilled off and the residue was dissolved in 0.5 ml of methanol and poured into water. The precipitate that deposited was recrystallized from aqeuous methanol. The hepta-O-acetylglucolokundjoside ($C_{49}H_{68}O_{22}$) melted at 147-150°C $[\alpha]_D^{20}$ −17.3 ± 2 (c 2°; methanol). Mass spectrum: m/e 352-71%, 334-71%, 331 (4AcGlc−17)-100%, 319-35%, 289 (4AcGlc−17−CH_2CO)-40%, 271 (4AcGlC−17−AcOH)-16%, 231 (2AcRha−17)-95%, 229 (4AcGlc−17−CH_2CO−AcOH)-97%, 227-30%, 199-35%, 171 (2AcRha−AcOH−17)-100%. The formation of the fragments with m/e 352, 334, 319, 227, and 199 has been described previously [2].

Glucoerysimoside

$C_{41}H_{62}O_{19}$, mp 234-236°C, $[\alpha]_D^{23}$ +16 ± 2° (c 1.77; methanol); $\lambda_{max}^{C_2H_5OH}$ 218 nm (log ε 4.18). With concentrated sulfuric acid it gave a bright green coloration gradually changing to brown.

Glucoerysimoside (10 mg) was subjected to enzymatic cleavage as described for glucolokundjoside. The reaction mixture was checked at first every minute and then every five minutes by TLC in system 5. Only three minutes after the addition of the enzyme no more than traces of the initial compound remained and erysimoside had appeared. After five minutes, the initial compound had disappeared, and the erysimoside formed was gradually converted into erysimin.

A solution of 30 mg of the glycoside in 3 ml of 0.1 N methanolic sulfuric acid was heated in the boiling-water bath for 0.5 h. After the usual working up, 12 mg of crystalline strophanthidin was obtained with mp 172-174°C. It was identified in systems 7 (PC) and 5 (TLC).

A solution of 20 mg of glucoerysimoside in 3 ml of 0.2 N aqueous sulfuric acid was heated at 100°C for 0.5 h, and then the solution was filtered, neutralized with $BaCO_3$, and evaporated. D-Digitoxose was detected by TLC in system 9 and PC in system 10. D-Glucose and cellobiose were detected in system 3 (TLC) and system 1 (PC).

SUMMARY

In the separation of the combined polar glycosides from the seeds of Erysimum cuspidatum (M.B.) DC, in addition to cardenolides isolated previously we also obtained cheirotoxin, glucoerysimoside, and a new glycoside which we have called glucolokundjoside. It has been shown that glucolokundjoside has the structure of bipindogenin 3β-O-[O-β-D-glucosyl-(1 → 4)-α-L-rhamnopyranoside].

The isothiocyanate cheiroline has been isolated from the chloroform fraction.

A sample of glucoerysimoside was kindly given to us by I. F. Makarevich (Khar'kov), and a sample of cheirotoxin by T. Reichstein (Basle, Switzerland).

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