CARDIAC GLYCOSIDES OF Erysimum crepidifolium

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Erysimum crepidifolium Rchb. has been cultivated since 1962 in the experimental field of the Khar'kov Scientific-Research Institute of Pharmaceutical Chemistry. From the seeds of this plant, Reichstein and his colleagues [1] have isolated erysimin (= helveticoside) and other secondary cardenolides of undetermined structure. By paper chromatography, we [2] have shown that the leaves contain erysimoside, erycordin, and a number of other glycosides, in addition to erysimin. According to Soviet and foreign investigators [3], the total amount of cardiac glycosides in the seeds of E. crepidifolium is between 1 and 3.5%. The amounts of erysimin and erysimoside in various parts of the plant have been determined by Reuter et al. by thin-layer chromatography.

To study the native cardiac glycosides of E. crepidifolium we have used, as appropriate, extraction from the raw material with ethanol, purification with lead hydroxide and alumina, adsorption chromatography on alumina, and partition chromatography on cellulose. As a result, we have isolated four glycosides in the individual crystalline state, and we have denoted them provisionally by the symbols Ec2, Ec9, Ec12, and Ec14. The first three have been identified by direct comparison with authentic samples as erysimin, erysimoside, and erycordin.

Compound Ec14 is a new cardiac glycoside which we have named, after determining its structure, glucostrophalloside. Glucostrophalloside has the composition $C_{35}H_{52}O_{15}$. Its IR spectrum is characterized by absorption bands with frequencies of (cm^{-1}) 3440 (strong band due to an OH group), 2945 and 2885 (CH, CH_2, and CH_3 groups), 2760 (CH of an aldehyde group), 1740 (CO of a butenolide), 1715 (CO of an aldehyde group), and 1622 (C=C of a butenolide ring). On enzymatic hydrolysis it formed D-glucose and a monoglycoside the properties of which corresponded to those given in the literature for strophalloside [9]. The latter is strophanthidin 3β-O-β-D-allomethyloside. The acid hydrolysis of the monoglycoside, performed by the Mannich–Siewert method [5] gave the aglycone strophanthidin and the monosaccharide D-allomethylose.

Glucostrophalloside was subjected to periodate oxidation. The results of the hydrolysis of the oxidized product and of an analysis of the hydrolyzate showed that it contained no D-glucose or D-allomethylose. This excludes the possibility of a 1→3 bond in the glucoside.

Glucostrophalloside is hydrolyzed comparatively rapidly by an enzyme preparation from the grape snail, and also by a preparation from the fungus Aspergillus oryzae; after 24 h, practically only the monoglycoside strophalloside could be detected.

Since it is known that when a 1→2 bond is present the splitting off of D-glucose by the enzymes of the fungus Aspergillus oryzae either does not take place at all or proceeds very slowly (more than 30 days [6]), it may be considered that this bond is unlikely for glucostrophalloside.

The results obtained show that a 1→4 bond is the most likely for the glycoside studied.

This glycoside is scarcely hydrolyzed by 0.05 N sulfuric acid at 70°C in 30 min, which shows [7] the pyranose form of the monosaccharide residues. An analysis of molecular rotations in accordance with Klyne's rule [8] showed that the D-glucose and D-allomethylose were attached by β-glycosidic bonds.

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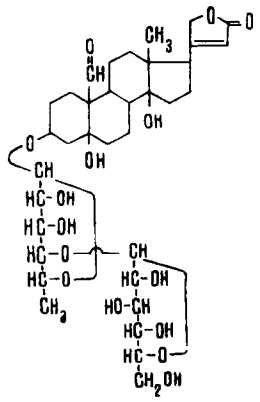
Glucostrophalloside can be characterized as strophanthidin 3β-O-[4'-O-β-D-glucopyranosyl-β-D-allomethylopyranoside].



EXPERIMENTAL

For analysis, the substances were dried in vacuum at 80°C over phosphorus pentoxide. Paper chromatography was performed with the following solvent systems: 1) toluene–butan-1-ol (1:1)/water; 2) chloroform–tetrahydrofuran (1:1)/formamide; 3) methyl ethyl ketone–m-xylene (1:1)/formamide; 4) butan-1-ol–acetic acid (4:1)/water; and 5) butan-1-ol–methyl ethyl ketone–borate buffer (1:1:2) [8].

The comminuted leaves and flowers (7 kg) of Erysimum crepidifolium were extracted with 96% ethanol until the bitter taste had disappeared. The extract was concentrated by evaporation in vacuum to a volume of about 2 liters, and then 5 liters of hot water were added with stirring and the mixture was again concentrated until the ethanol had been eliminated completely. The resinous precipitate was separated by centrifuging. Sodium chloride (12%) was added to the aqueous solution, and the cardenolides were extracted completely with chloroform–ethanol (2:1). The chloroform–ethanol extract was dried with anhydrous sodium sulfate and evaporated. The residue was dissolved in 4 liters of 40% ethanol and the solution was stirred with 0.5 kg of lead hydroxide for 25 min. The precipitate was separated by filtration. The lead ions present in the solution were precipitated with dilute sulfuric acid. After the addition of sodium chloride, the filtrate was extracted with ethanol–chloroform (1:2). The residue from the evaporation of the extracts was dissolved in ethanol–chloroform, and the residue of sodium chloride was filtered off. The cardenolides were transferred into aqueous solution. This solution was then purified with alumina (400 g). The adsorbent was washed with water until the filtrates no longer had a bitter taste.

The aqueous filtrate was saturated with sodium sulfate, and the cardenolides were extracted completely by treatment with mixtures of ethanol and chloroform. Alumina (activity grade III) was used to separate the glycosides.

The column was eluted with chloroform and then with chloroform–ethanol, the concentration of ethanol being increased by 1% for each liter of solvent passed. At a concentration of 5% of ethanol in the chloroform, fractions were obtained which contained only erysimin. The work of the column was stopped at a concentration of ethanol in the chloroform of 22%. Then the column of adsorbent was separated into 14 equal zones, and they were analyzed by paper chromatography (systems 1 and 2). The individual glycosides (erysimoside and erycordin) were desorbed with ethanol.

The polar glycosides desorbed from the other zones of the alumina were subjected to partition chromatography on cellulose (ratio of the combined substances to be separated to cellulose 1:300) in the solvent system toluene–butan-1-ol (1:1)/water. The fourth cardenolide – Ec14 – was isolated in the individual state.

Erysimin. The erysimin was crystallized from 50-60% ethanol, mp 176-178°C. The Keller–Kiliani reaction was positive. A mixture with authentic erysimin gave no depression of the melting point (176.5-178°C). $R_{erysimin} = 1.00$ (system 3).

Erysimoside. The glycoside melted at 235-240°C (ethanol); $[\alpha]_D$ +18.9 ± 2° (c 1.00; methanol). A mixture with an authentic sample of erysimoside showed no depression of the melting point (235-241°C). $R_{erysimoside} = 1.00$ (systems 1 and 2).

Erycordin. The glycoside was crystallized from acetone–water, mp 200-202.5°C. The mixture with an authentic sample of erycordin melted at the same temperature (200-202.5°C). $R_{erycordin} = 1.00$ (system 1). Enzymatic hydrolysis performed in the same way as the hydrolysis of glucostrophalloside (see below) gave the monoglycoside desglucoerycordin and D-glucose. Hydrolysis with hydrochloric acid by the Mannich–Siewert method [5] led to the formation of the aglycone cannogenol. The hydrolysis products were identified by paper chromatography.

Glucostrophalloside. The glycoside was crystallized from butan-1-ol–ether, mp 189-192°C, $[\alpha]_D$ +3.3 ± 2° (c 0.66; methanol). The results of elementary analysis corresponded to the figures calculated for the composition $C_{35}H_{52}O_{15}$. With concentrated sulfuric acid, the substance formed a coloration changing with time: 0 min – yellow; 50 min – light brown; 60 min – green-yellow; 95 min – green; 135 min – bluish green; 155 min – gray-green; and 200 min – brown.

Enzymatic Hydrolysis of Glucostrophalloside. A solution of 80 mg of the glycoside and 100 mg of an enzyme preparation from the grape snail in 5 ml of water was kept in a thermostat at 42°C for 2 days. Then 30 ml of hot ethanol was added and the resulting precipitate of enzymes was separated off by filtration. The filtrate was concentrated under vacuum to 10 ml, and 10 ml of water was added. The monoglycoside was extracted with ethanol–chloroform (1:3; 4 × 20 ml). The ethanol–chloroform extracts were washed with water (5 ml). The substance was crystallized from methanol–ether and was recrystallized from water. The strophalloside obtained melted at 163-166°C; $[\alpha]_D$ +5.0 ± 2° (c 1.0; methanol). The aqueous solution was concentrated to form a syrup. Ethanol was added and the mixture was left for crystallization. The melting point of the monosaccharide was 144-146°C. On paper chromatography it showed the same R_f values as D-glucose. The melting point of a mixture with an authentic sample of D-glucose was 144-146°C.

The monoglycoside was hydrolyzed with hydrochloric acid in acetone by the Mannich–Siewert method for 5 days. The usual treatment separated an aglycone fraction and a carbohydrate fraction. When the aglycone fraction was chromatographed on alumina [activity grade III; eluent chloroform and ethanol–chloroform (2:98)], an aglycone with mp 228-233°C (ethanol–benzene), $R_{strophanthidin} = 1.00$ (system 3) was obtained. A mixture with an authentic sample of strophanthidin gave no depression of the melting point (228-233°C).

The carbohydrate fraction consisted of a monosaccharide which was identical, according to paper chromatography (system 5), with D-allomethylose.

Periodate Oxidation of Glucostrophalloside. A solution of 10 mg of the substance, 500 mg of sodium metaperiodate, and 0.3 ml of acetic acid in 100 ml of water (pH of the solution ~5) was left for 24 h. Then it was saturated with sodium sulfate and treated with a mixture of ethanol and chloroform (1:2; 3 × 200 ml). The extract was washed with 20 ml of water and evaporated. The oxidized product was hydrolyzed with 0.35 N sulfuric acid at 100°C for 2 h, and the hydrolyzate was neutralized with barium carbonate, filtered through a layer of kieselguhr, and evaporated in vacuum. When the residue was chromatographed on paper and the chromatogram was treated with aniline phthalate reagent, no D-glucose or D-allomethylose was detected.

SUMMARY

Four cardiac glycosides have been isolated from the leaves of Erysimum crepidifolium Rchb. Three of them have been identified as erysimin, erysimoside, and erycordin. The fourth glycoside, which has been named glucostrophalloside, is new, and it consists of strophanthidin 3β-O-[4'-O-β-D-glucopyranosyl-β-D-allomethylopyranoside].

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