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CARDENOLIDE GLYCOSIDES OF *Cheiranthus allioni*. XVI. NEOEVONOSIDE AND CELLOBIOSYL-DIGIGULOMETHYLOSIDE

I. F. Makarevich, A. I. Alistrenko, D. A. Kobzin, Yu. A. Chernyaev, and T. V. Slyusarskaya

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Another three isomeric glycosides have been isolated from the seeds of Cheiranthus allioni *Hort., two of which are new: after their chemical structures were determined they were named neoevonoside and cellobiosyl-digigulomethyloside.*

As already reported [1, 2], 35 native cardenolide compounds have been isolated from the seeds of *Cheiranthus allioni* Hort.* Continuing an investigation of this plant, from the polar fraction we have isolated another two isomeric cardenolides, which, after determining their structures, we have named neoevonoside (**1**) and cellobiosyl-digigulomethyloside (**2**). We isolated a third glycoside from the same fraction and identified it as digifucocellobioside (**3**) [3].

Glycoside (**1**) had the composition $C_{41}H_{64}O_{18}$; i.e., it was a triglycoside. Under the action of an enzyme preparation obtained from the pancreatic juice of the grape snail, triglycoside (**1**) was hydrolyzed with the formation of a monoglycoside and a monosaccharide; these were isolated in the individual state, and from their properties and also by a direct comparison with authentic specimens were identified as evomonoside and *D*-glucose. As is known, evomonoside is 3β-(α-*L*-rhamnopyranosyloxy)-14-hydroxy-5β,14β-card-20(22)-enolide [4, 5].

When (**1**) was subjected to partial enzymatic hydrolysis the reaction mixture was shown by paper chromatography to contain evobioside, which is 3β-[O-*D*-glucopyranosyl-(1→4)-*L*-rhamnopyranosyloxy]-14-hydroxy-5β,14β-card-20(22)-enolide [5, 7].

In the light of these results, it was necessary to determine the position of attachment of the terminal *D*-glucose unit, the size of the oxide ring in it, and the configuration of the glycosidic bond. We carried out the following investigation for this purpose.

Glycoside (**1**) was comparatively resistant to acid hydrolysis: on heating in 0.5 N H_2SO_4 at 70°C for 30 min it did not change, which showed the pyranose form of the monosaccharide units within the molecule.

An analysis of the molecular rotations of triglycoside (**1**) and the diglycoside evobioside in accordance with Klyne's rule showed that the *D*-glucose ring was attached by a β-glycosidic bond ($\Delta[M]_{D\text{-glucose}} = -87°$).

The controlled partial acid hydrolysis of (**1**) formed digitoxigenin, evomonoside, *D*-glucose, *L*-rhamnose, evobioside, and cellobiose. The presence of the disaccharide cellobiose in the hydrolysate showed that the *D*-glucose units were linked to one another in the 1→4 position.

The results obtained permitted the structure of glycoside (**1**) to be characterized as 3β-[O-β-*D*-glucopyranosyl-(1→4)-O-β-*D*-glucopyranosyl-(1→4)-α-*L*-rhamnopyranosyloxy]-14-hydroxy-5β,14β-card-20(22)-enolide. We consider it desirable to give the new glycoside (**1**) the trivial name neoevonoside, since its difference from the known evonoside [7] consists only in the position of attachment of the terminal *D*-glucose unit, the bond between the *D*-glucoses in evonoside being 1→6 [6]. We had

*Usually identified as "plains erysimum", but see Chemistry of Natural Compounds, 305 (1992) — Translator.

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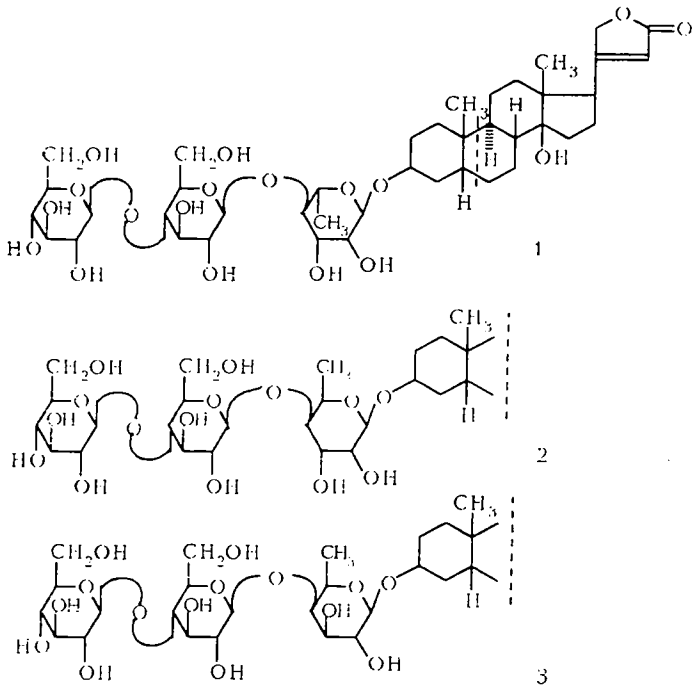
the possibility of a direct chromatographic comparison of glycoside (1) with a specimen of evonoside kindly provided for us by Prof. T. Reichstein. Their polarities were close but the difference was fairly clear.

Glycoside (2) had the same molecular formula as (1): $C_{41}H_{64}O_{18}$. Its enzymatic hydrolysis enabled us to obtain a monoglycoside and a monosaccharide, which were identified from their properties and by comparison with authentic specimens as digigulomethyloside and *D*-glucose. Digigulomethyloside is 3β-(β-*D*-gulomethylopyranosyloxy)-14-hydroxy-5β,14β-card-20(22)-enolide [7].



The controlled partial hydrolysis of (2) yielded a mixture of substances consisting of digitoxigenin, cellobiose, *D*-glucose, *D*-gulomethylose, and a trisaccharide. The presence of cellobiose in the glycosidic fragment showed that the terminal *D*-glucose was attached to the other *D*-glucose unit by a β-glycosidic bond in the 1→4 position and was present in the pyranose form. The test for resistance to acid hydrolysis was similar to that described above for (1) and likewise characterized the pyranose forms of the monosaccharide units in (2).

On interacting with acetone in the presence of anhydrous copper sulfate, glycoside (2) formed an isopropylene derivative with the composition $C_{47}H_{68}O_{18}$. On enzymatic hydrolysis the latter gave *D*-glucose and the isopropylidene derivative of a monoglycoside. These facts showed that glycoside (2) contained a free *cis*-α-glycol grouping, at which the reaction with acetone took place. There is such a grouping only in the *D*-gulomethylose unit: see the *cis*-position of the OH groups at C-2′ and C-3′. Consequently, the linkage of the *D*-glucose and the *D*-gulomethylose was in the 1→4 position.

An analysis of molecular rotations of the triglycoside (2) and of the monoglycoside digigulomethyloside by Klyne's rule showed that the cellobiose was attached by a glycosidic bond ($\Delta[M]_{D\text{-cellobiose}} = -227°$).

Consequently, glycoside (2) was 3β-[O-β-*D*-glucopyranosyl-(1→4)-O-β-*D*-glucopyranosyl-(1→4)-β-*D*-gulomethylopyranosyloxy]-14-hydroxy-5β,14β-card-20(22)-enolide or cellobiosyl-digigulomethyloside.

The third isomeric glycoside, identified from the properties of its hydrolysis products as digifucocellobioside, was 3β-[O-β-*D*-glucopyranosyl-(1→4)-O-β-*D*-glucopyranosyl-(1→4)-β-*D*-fucopyranosyloxy]-14-hydroxy-5β, 14β-card-20(22)-enolide (3) [3].

EXPERIMENTAL

The elementary analysis of the substances was conducted on an automatic C−H−N analyzer: the results corresponded to the calculated figures. Melting points were determined on a Kofler block. Paper chromatography was performed with the

following solvent systems: toluene–butan-1-ol (1:1)/water, chloroform–tetrahydrofuran (1:1)/formamide, and butan-1-ol–acetic acid–water (4:1:2).

The glycosides were isolated from the polar fraction obtained previously [8] by chromatography on a column of powdered cellulose. The ratio of the total amount of substances to be separated and cellulose was 1:200 and the eluent was toluene–butan-1-ol (2:1–1:1)/water. The fractions were obtained with an automatic collector.

Neoevonoside (1), $C_{41}H_{64}O_{18}$, mp 138-142°C (isopropanol), $[\alpha]_D$ 30.0 ± 2° (*c* 0.8; methanol).

Cellobiosyl-digigulomethyloside (2), $C_{41}H_{64}O_{18}$, $[\alpha]_D$ −33.0 ± 2° (*c* 0.7; methanol).

Digifucocellobioside (3), $C_{41}H_{64}O_{18}$, mp 270-275°C (ethanol), $[\alpha]_D$ −6.4 ± 2° (*c* 0.5; methanol).

The enzymatic hydrolysis of glycosides (1-3) and the working up of the hydrolysates were carried out as described in [9]. As a result, we obtained:

— from glycoside (1), the monoglycoside evomonoside, mp 243-246°C, $[\alpha]_D$ −25.3 ± 2° (*c* 0.45; methanol) and *D*-glucose;

— from glycoside (2), the monoglycoside digigulomethyloside, mp 226-230°C, $[\alpha]_D$ −10.02° [sic — ? −10.0 ± 2°?] (*c* 0.39; methanol) and *D*-glucose;

— from glycoside (3), the aglycon digitoxigenin, mp 252-256°C, $[\alpha]_D$ +18.4 ± 2° (*c* 0.55; chloroform), *D*-glucose, and *D*-fucose.

The cardenolide hydrolysis products were identified by their properties and the results of direct comparison with specimens of the substances; sugars were identified with the aid of paper chromatography.

Partial Acid Hydrolysis of Neoevonoside (1). A solution of 0.15 g of glycoside (1) in 2 ml of ethanol was treated with 3 ml of 0.1 N H_2SO_4. The mixture was sealed into a glass tube and heated at 70°C for 10 h. The hydrolysate was treated with barium carbonate and the precipitate was separated off. Cardenolides were extracted from the filtrate with chloroform and chloroform–alcohol (1:2). The alcoholic chloroform solution and the aqueous solution were concentrated and were analyzed by paper chromatography. The first of them contained digitoxigenin, evomonoside, and evobioside, in addition to the initial glycoside (1). The aqueous solution contained *D*-glucose, *L*-rhamnose, and cellobiose.

The partial acid hydrolysis of cellobiosyl-digigulomethyloside (2) was conducted similarly. Digitoxigenin was identified in the cardenolide fraction of the hydrolysate, and *D*-glucose, *D*-glulomethylose, and a presumed trisaccharide in the carbohydrate fraction.

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