# CARDIAC GLYCOSIDES OF Erysimum contractum. II

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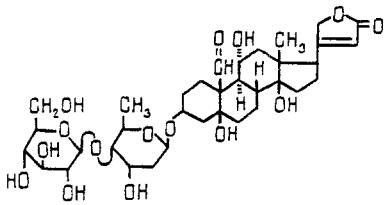
The structure of a new cardiac glycoside isolated from the seeds of Erysimum contractum has been established. The glycoside, which has been called nigrescigenin digilanidobioside is 3β,5,11α,14-tetrahydroxy-19-oxo-5β,14β-card-20(22)-enolide 3-O-(4-O-β-D-glucopyranosyl-β-D-digitoxopyranoside).

As already mentioned [1] six cardenolide glycosides have been isolated from the seeds of Erysium contractum Somm. et Lev. The structure of one of them, designated E. cn. 5 had not been established. Continuing its study, we have ascertained the following facts: E. cn. 5 (I) is a new highly polar diglycoside. The molecular formula of (I) is $C_{35}H_{52}O_{15}$. The glycoside contains a 2-deoxy sugar, as was shown by a positive Keller–Kiliani reaction. Its molecule includes an aldehyde group, the presence of which was shown by spectral characteristics: in the IR region of the spectrum there were clearly resolved bands at 2760 and 1720 $cm^{-1}$; the PMR spectrum included a one-proton signal in the 9.94 ppm region.

Under the action of an enzyme preparation obtained from the pancreatic juice of the grape snail, the glycoside was hydrolyzed with the formation of a monoglycoside and a monosaccharide, which were isolated in the individual state and from their properties and also on the basis of a direct comparison with samples were identified as nigrescigenin digitoxoside and D-glucose. Nigrescigenin digitoxoside was first obtained by us [1]. It is 3β,5,-11α,14-tetrahydroxy-19-oxo-5β,14β-card-20(22)-enolide 3-O-β-D-digitoxopyranoside. The results obtained reduced the further investigations to establishing the site of attachment of the D-glucose residue, the configuration of its glycosidic bond, and the size of its oxide ring. Having put forward the structure of the glycoside shown in formula (I) as the most probable, the following transformations were carried out to confirm it. On hydrolyzing (I) with 0.05 N sulfuric acid, the aglycon nigrescigenin and the disaccharide digilanidobiose were obtained. Both compounds were isolated in the individual state and were identified from their properties and by direct comparison with authentic samples. The structure of the disaccharide digilanidobiose is known – it is 4-O-β-D-glucopyranosyl-D-digitoxose [2, 3].

The experimental facts given permit the structure of glycoside (I) to be characterized unambiguously as 3β,5,11α,14-tetrahydroxy-19-oxo-5β,14β-card-20(22)-enolide 3-O-(4-O-β-D-glucopyranosyl-β-D-digitoxopyranoside). We suggest for it the following semitrivial name: nigrescigenin digilanidobioside.



## EXPERIMENTAL

The elementary analyses of the substances were carried out with the aid of an automatic C–H–N analyzer; the results of the analyses corresponded to the calculated figures. PMR spectra were taken on a Tesla BS-497 instrument (100 MHz). Melting points were determined

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on a Kofler block. Paper chromatography was conducted in the following systems: toluene–butan-1-ol (1:2)/water; chloroform–tetrahydrofuran (1:1)/formamide; and butan-1-ol–acetic acid–water (4:1:2).

Nigrescigenin digilanidobioside (I) $C_{35}H_{52}O_{15}$, mp 169-174°C (from methanol–ethyl ether) $[\alpha]_D^{20}$ −2.6 ± 2° (c 0.90; methanol).

Enzymatic Hydrolysis of Glycoside (I). A solution of 0.25 g of glycoside (I) in 3 ml of water was treated with 0.3 g of a dry enzyme preparation obtained from the pancreatic juice of the grape snail; the solution was kept in a thermostat at 40 ± 1°C for 20 h. A chromatographic test showed that after this time none of the initial glycoside remained in the solution. To precipitate the enzymes, 10 ml of ethyl alcohol was added, the mixture was heated to the boil, and the precipitate that had deposited was filtered off.

A monoglycoside was extracted from the solution first with chloroform (2 × 15 ml) and then with chloroform–ethanol (2:1; 4 × 10 ml). The alcoholic chloroform solutions were combined and evaporated, and the glycoside so obtained was crystallized from methanol–benzene. The monoglycoside had mp 141-145°C, $[\alpha]_D^{21}$ +16.1 ± 2° (c 0.70; methanol). From its properties and comparative chromatographic results it was identical with a sample of nigrescigenin digitoxoside.

The aqueous phase, containing a monosaccharide, was evaporated, and the residue was crystallized from ethanol. The crystals obtained had mp 145-146°C. Comparison with a sample of D-glucose, including a mixed melting point, showed their identity.

Acid Hydrolysis of Glycoside (I). A solution of 0.2 g of glycoside in 2 ml of 0.05 N sulfuric acid was sealed in a glass capsule and heated at 75 ± 2°C for 40 min. The acid was neutralized with barium carbonate, the precipitate of salts was separated off, and the aglycon was extracted from the solution with chloroform–ethanol (2:1; 3 × 4 ml). The combined ethanolic chloroform solutions containing the aglycon were evaporated, and the residue was crystallized from methanol–diethyl ether; mp 223-230°C; $[\alpha]_D^{21}$ +23.7 ± 2° (c 0.54; methanol). From its properties and comparative chromatographic results, the aglycon was identical with an authentic sample of nigrescigenin.

The aqueous solution after the separation of the aglycon was evaporated to dryness. The residue was crystallized from ethanol. The resulting disaccharide had mp 223-227°C. A mixed melting point and comparative chromatographic results showed its identity with digilanidobioside.

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