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STEROIDAL GLYCOSIDE-PROTODIOSCIN FROM *Digitalis ciliata*

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Digitalis ciliata Trautv. is endemic to the Caucuses and widely distributed in the flora of Georgia. It has been proposed as raw material for producing an effective cardiotonic preparation of acetyldigitoxin [1]. Triterpene glycosides [2, 3], carotinoids [4], and steroidal glycosides were detected in the remaining extracts, the mother liquors.

After exhaustive extraction of cardenolide glycosides, the remaining solution was condensed to a resinous condition and subjected (5 g) to total acid hydrolysis with HCl (2 N). Chromatography of the hydrolysate over a column of Al_2O_3 using ether, ether:benzene, and benzene:chloroform isolated three genins that were identified as tigogenin, diosgenin, and gitogenin by their physical constants and TLC in the presence of authentic samples.

The glycoside composition of *D. ciliata* was studied by dissolving the resinous extract in water with vigorous stirring. The insoluble solid that contained mainly spirostane glycosides was separated. The aqueous phase was extracted several times with *n*-butanol. The extracts were combined and evaporated to dryness. The solid was dissolved in ethanol. The glycosides were precipitated with acetone to afford an amorphous cream-colored powder (12.5 g), a portion (3.5 g) of which was chromatographed successively over columns of silica gel (KSK, 40-100 μm) and Sephadex G-75 using $CHCl_3$:CH_3OH:H_2O (65:35:8) to afford a mixture of glycosides **1** and **2**, which gave two spots on TLC with a small difference (ΔR_f 0.1) in mobility. Rechromatography over the columns isolated the pure compounds **1** (0.185 g) and **2** (0.076 g). Both glycosides gave a positive reaction with Ehrlich's reagent [5]. Their IR spectra lacked signals characteristic of the spiroketal group. These data indicated that the compounds were furostanes.

Glycoside **1**, white amorphous powder (ethanol), mp 189-192°C, $[\alpha]_D^{25}$ -80.5° ($CHCl_3$:CH_3OH, 1:1, *c* 0.5), lit. [6] mp 190-196°C, $[\alpha]_D^{25}$ -79.8° (pyridine, *c* 0.1). IR spectrum (KBr, ν, cm^{-1}): 3400 (OH), 1045, 930 (weak broad band), 915, 840. FAB MS (*m*/*z*, %): 1071 $[M + Na]^+$, 925 $[M + Na - deoxyhexose]^+$, 891 $[M + Na - hexose - H_2O]^+$.

The acid hydrolysate of **1** contained diosgenin. GC of the aldonitriles of the sugars identified glucose and rhamnose in a 1:1 ratio. Hakomori [7] methylation of **1** and subsequent methanolysis produced in the hydrolysate 2,3,4-tri-*O*-methyl-L-rhamnose, 2,3,4,6-tetra-*O*-methyl-D-glucose, and 3,6-di-*O*-methyl-D-glucose.

Enzymatic hydrolysis of **1** by β-glucosidase gave the prosapogenin that was identified using physical constants and mass and NMR spectra as dioscin [8]. The hydrolysate contained D-glucose. Considering these data, **1** was assigned the structure 3-*O*-{[α-L-rhamnopyranosyl-(1→4)]-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranosyl}-(25*R*)-furost-5-en-3β,22α,26-triol-26-β-D-glucopyranoside or protodioscin [9].

This glycoside was isolated from plants of the genus *Digitalis* for the first time.

Glycoside **2** had molecular weight *m*/*z* 1085 $[M + Na]^+$ according to FAB MS, 14 amu greater than that of **1**. The PMR spectrum had a 3H singlet at δ 3.26 (OMe). Thus, it was assumed that **2** was the 22-*O*-methyl ether of **1**, which was consistent with its PMR and ^{13}C NMR spectra and agreed well with the literature [10] (Table 1).

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TABLE 1. Chemical Shifts in PMR and ^{13}C NMR Spectra of **2** (δ, ppm, J/Hz, pyridine-d_5, 0 = HMDS)

C atom	δ_C	C atom	δ_C	Proton	δ_H
1	37.5	D-Glucose		Me-18	0.84 (s)
2	29.8	1′	101.4*	Me-19	1.04 (s)
3	77.6	2′	77.9	Me-21	1.17 (d, J = 7.4)
4	39.3	3′	74.7	Me-27	1.00 (d, J = 7.4)
5	140.9	4′	77.4	CH2-26	3.62 (br.d, J = 11.8)
6	121.9	5′	75.9	H-6	5.32 (br.d, J = 9.1)
7	32.2	6′	61.9	H-1′	4.88 (d, J = 7.7)
8	31.6	L-Rhamnose		H-1″	6.28 (br.s)
9	50.3	1″	99.3	H-1‴	5.80 (br.s)
10	36.9	2″	72.5	H-1⁗	4.78 (d, J = 7.1)
11	21.1	3″	71.4	Me″	1.71 (d, J = 6.0)
12	40.0	4″	75.9	Me‴	1.60 (d, J = 6.0)
13	40.7	5″	69.3	OMe	3.26 (s)
14	56.5	6″	18.5		
15	32.4	L-Rhamnose			
16	81.1	1‴	103.5*		
17	64.0	2‴	72.6		
18	16.3	3‴	71.6		
19	19.5	4‴	75.9		
20	41.8	5‴	68.6		
21	16.5	6‴	18.4		
22	112.8	D-Glucose			
23	30.5	1⁗	103.7		
24	28.1	2⁗	74.8		
25	33.7	3⁗	77.5		
26	74.8	4⁗	71.4		
27	17.3	5⁗	77.5		
		6⁗	63.6		

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