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DIGITOSIDE, A NOVEL TRITERPENE GLYCOSIDE FROM *DIGITALIS CILIATA*

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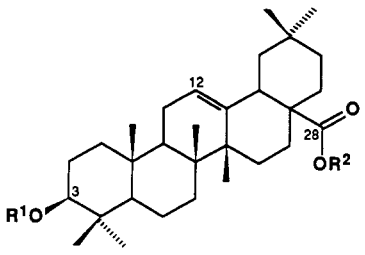
ABSTRACT.—A new pentacyclic triterpene glycoside, digitoside [**1**], has been isolated from the leaves of *Digitalis ciliata*. Its structure has been established as 3-*O*-[β-xylopyranosyl-(1↦4)-α-L-rhamnopyranosyl]-28-*O*-[β-D-glucopyranosyl-(1↦6)-β-D-glucopyranosyl]-oleanolic acid through chemical and spectral studies.

Digitalis ciliata Trautv. (Scrophulariaceae) is endemic to the Caucasus. Studies on the chemical constituents of different parts of the plant have led to the isolation of several cardenolides, steroid glycosides, and flavonoids (1–4). In the course of studies on the constituents of the leaves, several triterpenes have been isolated. The present paper deals with the isolation and structural elucidation of a new pentacyclic triterpenoid provisionally named as digitoside [**1**].

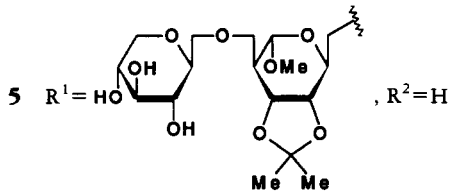
RESULTS AND DISCUSSION

The new glycoside **1** gave oleanolic acid [**3**] as the aglycone and L-rhamnose, D-xylose, and D-glucose as the sugar moieties on acid hydrolysis. The aglycone was identified as oleanolic acid by comparison (mp, ir, ^{1}H nmr, ^{13}C nmr) with the literature (5) and also by comparison with an authentic sample. Glc analysis of the acetylated sugars indicated that the glycoside **1** contained D-glucose, L-rhamnose, and D-xylose in the molar ratio 2:1:1, respectively.

The appearance of an ester adsorption band in its ir spectrum (1755 cm^{-1}) and a carboxyl carbon signal (δ 176.0) and one of the anomeric carbon signals (δ 95.1) at rather high field in its ^{13}C-nmr spectrum strongly indicated that one of the sugars was linked to the 28-carboxylic group of the genin in the ester form. Alkaline hydrolysis (6) of **1** gave the progenin **2** and D-glucose as the sugar moiety. A comparison of the ^{13}C-nmr spectrum of **2** with that of the glycoside **1** revealed a loss of 12 resonance signals. It could, therefore, be suggested that **1** had a disaccharide chain composed of two glucose units bonded to the C-28 carboxyl group by an ester linkage. Furthermore, the disaccharide moiety was considered to be 6-*O*-glucosylglucopyranoside (gentiobiose) from the fact that the C-6 resonance signal (δ 69.1) of a glucose molecule connected to the C-28 carboxyl group ex-



1 R^1=β-D-xyl(1↦4)-α-L-rha, R_2=β-D-glc(1↦6)-β-D-glc
2 R^1=β-D-xyl(1↦4)-α-L-rha, R^2=H
3 R^1=R^2=H
4 R^1=α-L-rha, R^2=H



hibited a characteristic low field shift due to alkyl substitution (7–9). The modes of linkage of both glucose units were regarded at β on the basis of the ^{13}C-^{1}H coupling constants ($J = 161$ and 162 Hz) of the anomeric carbon signals (δ 104.3 and 95.1) in the ^{13}C-nmr spectrum of glycoside **1**.

The progenin **2** on partial acid hydrolysis yielded the monoside **4** and D-xylose. The presence of L-rhamnose in the hydrolysate of **2** indicated undoubtedly that this sugar was attached to oleanolic acid at position C-3. Acetonization (10) of the progenin **2** gave compound **5**. In comparison of ^{1}H-nmr data of **5** with those of **2**, two resonance signals appeared at δ 1.06 and 1.10 (both 3H, s). The formation of the acetonated derivative **5** of the progenin **2** indicated that rhamnose had free hydroxyl groups at the 2 and 3 positions. Therefore, the attachment point of xylose was determined to be the 4 position of rhamnose. The anomeric carbon signals of the sugar moieties in **2** were found at δ 105.1 ($J = 162$ Hz) and 101.1 ($J = 169$ Hz) confirming the β-D- configuration for the xylose linkage and α-L configuration for the rhamnose linkage (11).

Based upon the above data, the structure of the new triterpenoid glycoside was established, and digitoside [**1**] consequently has the structure 3-*O*-[β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl]-28-*O*-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-oleanolic acid. To the best of our knowledge, the occurrence of **1** in nature has not been previously reported.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All mp's were determined on a Kofler hot stage and are uncorrected. Ir spectra were recorded on a UR-20 (K. Zeiss, Jena) spectrometer, and ^{1}H- and ^{13}C-nmr spectra for C_5D_5N solution (80°) were obtained on a BS-567.A (Tesla, Brno, 100 MHz) ft-spectrometer. Optical rotations were measured with an SU-2 polarimeter (Kiev, USSR). Analytical paper chromatography was carried out on a Filtrak FN-12 and Silufol UV-254 (ČSSR). The following solvent systems were used: $CHCl_3$-MeOH (10:1; 10:1.5; 10:2), $CHCl_3$-MeOH-H_2O (70:23.5:2), C_5H_5N–C_6H_6–*n*-BuOH–H_2O (3:5:1:3). Glc analysis was performed on a column of 5% silicone XE-60 on Chromaton-super (Chrom- 5 instrument, Laboratorni pristroje, Praha) under the following conditions: carrier gas He; flow rate 40 ml/min; column temperature 210°; injector temperature 230°; detector temperature 250°.

PLANT MATERIAL.—Leaves of flowering *D. ciliata* were collected from the Mestia region of Georgia, USSR. A voucher specimen is preserved at the herbarium of our Institute.

EXTRACTION AND ISOLATION.—Air-dried, finely cut, whole leaves (5.0 kg) were extracted with 70% MeOH. After evaporation of the MeOH, the liquor was extracted with Et_2O, $CHCl_3$, and EtOH-$CHCl_3$ (1:1). The last extraction gave a residue (110 g) which was chromatographed after purification by $Pb(OH)_2$. Cc was carried out on Sephadex G-25. The following solvent systems were used: C_6H_6-Et_2O (2:1; 1:1; 1:2; 1:4); Et_2O; Et_2O-EtOAc (1:1)/H_2O; EtOAc–*n*-BuOH (10:1, 1:1)/H_2O. The fraction (230 ml) eluted by EtOAc-*n*-BuOH (1:1), after usual workup, was chromatographed on a Si gel column using $CHCl_3$-MeOH (10:1) as eluent to give 0.83 g of the glycoside **1** as colorless needles: mp 229–231°; $[\alpha]^{20}D$ −1.54° [$c = 0.11$, $CHCl_3$-MeOH (1:1)]; ir (KBr) ν max cm^{-1} 3400 (OH), 1755 (C=O), 1650 (CH=C); R_f 0.36 [$CHCl_3$-MeOH (10:1)] and 0.43 [$CHCl_3$-MeOH-H_2O (70:23.5:2)]; ^{1}H nmr (100 MHz) δ 0.79 (9H, s), 0.92 (3H, s), 0.95 (3H, s), 1.08 (3H, s), 1.12 (3H, s), 1.45 (3H, d, $J = 6$ Hz, Me of rhamnose), 5.35 (1H, br s, H-12); ^{13}C nmr (25.142 MHz) see Table 1.

ACID METHANOLYSIS.—Glycoside **1** (150 mg) was dissolved in a mixture of MeOH (20 ml) and concentrated H_2SO_4 (0.05 ml) and heated under reflux for 3 h. The solution was diluted with H_2O and extracted with EtOAc after the MeOH was evaporated. The EtOAc extraction was washed with H_2O and evaporated to dryness, and the residue was chromatographed on a Si gel column (14 g) using $CHCl_3$-MeOH (10:1) as eluent to give 0.036 g of oleanolic acid [**3**] as colorless needles: mp 301–302°; ir (KBr) ν max cm^{-1} 3600 (OH), 1700 (C=O), 1645 (CH=C); ^{1}H nmr ($CDCl_3$) δ 0.73 (3H, s), .075 (3H, s), 0.90 (6H, s), 0.97 (3H, s), 1.11 (3H, s), 1.24 (3H, s), 3.20 (1H, t, $J = 7.0$ Hz, H-3), 5.26 (1H, br s, H-12); ^{13}C nmr see Table 1.

The H_2O-soluble portion of the hydrolysate was diluted with distilled H_2O and dried. This workup was repeated several times. The residue was dissolved in 50% MeOH (2 ml) and treated with $NaHBO_4$ (12 h room temperature) and with the ion exchanger KU-2 (OH^- form, Biochimreactiv, USSR). The residue obtained from the

TABLE 1. ^{13}C-nmr Spectral Data of Compounds **1–3**.

Carbon	Compound			Sugar moiety	Compound	
	1	**2**	**3**		**1**	**2**
C-1	39.0	38.9	38.4	rha-1′	101.1	101.1
C-2	26.1	26.1	27.4	rha-2′	71.9	71.9
C-3	88.0	87.9	77.7	rha-3′	71.5	71.5
C-4	38.7	38.5	38.6	rha-4′	78.1	78.2
C-5	55.8	55.6	55.4	rha-5′	69.1	69.0
C-6	18.1	18.1	17.9	rha-6′	17.9	17.8
C-7	32.7	32.5	32.5			
C-8	39.5	39.2	39.3	xyl-1″	104.9	105.1
C-9	47.7	47.5	47.6	xyl-2″	73.5	73.3
C-10	36.6	36.5	36.8	xyl-3″	77.5	77.5
C-11	23.2	23.3	23.2	xyl-4″	70.8	70.8
C-12	122.4	121.8	121.8	xyl-5″	65.9	66.0
C-13	143.7	144.4	144.2			
C-14	41.8	41.6	41.7	glc-1‴	95.1	
C-15	27.7	27.3	27.2	glc-2‴	73.5	
C-16	22.9	23.3	23.2	glc-3‴	78.0	
C-17	46.5	46.1	46.0	gc-4‴	70.8	
C-18	41.8	41.6	41.6	glc-5‴	77.9	
C-19	45.9	46.2	46.0	glc-6‴	69.1	
C-20	30.2	30.2	30.2			
C-21	33.6	33.8	33.7	glc-1⁗	104.3	
C-22	31.9	32.5	32.6	glc-2⁗	74.4	
C-23	27.7	27.7	28.0	glc-3⁗	77.9	
C-24	16.4	16.3	15.6	glc-4⁗	71.3	
C-25	15.1	14.9	14.8	glc-5⁗	78.0	
C-26	16.9	16.8	16.8	glc-6⁗	62.3	
C-27	25.4	25.4	25.5			
C-28	176.0	178.7	178.5			
C-29	32.5	32.5	32.8			
C-30	23.2	23.2	23.2			

filtered reaction mixture was treated with Ac_2O (2 ml) in C_5H_5N (2 ml) at room temperature for 12 h. Glc of the residue from $CHCl_3$ extract (12) was carried out.

ALKALINE HYDROLYSIS.—Compound **1** (110 mg) was heated in 1% KOH (5 ml) for 30 min at 100° (6). After cooling, the reaction mixture was acidified with HCl (pH = 7) and extracted with *n*-BuOH. The *n*-BuOH solution was evaporated to dryness under reduced pressure to give the progenin **2** (95 mg) as colorless needles: mp 268–270°; $[\alpha]^{20}D$ +5.1° (*c* = 1.0, EtOH); ^{1}H nmr δ 0.72 (3H, s), 0.80 (6H, s), 0.86 (3H, s), 1.00 (3H, s), 1.10 (3H, s), 1.14 (3H, s), 1.53 (3H, d, *J*=6.0 Hz), 5.31 (1H, br s); ^{13}C nmr see Table 1.

The H_2O-soluble portion of the hydrolysate was heated under reflux for 30 min with 0.5% H_2SO_4. The reaction mixture was neutralized with the ion exchanger KU-2 (OH^- form) and evaporated. Paper chromatography of the residue revealed D-glucose.

PARTIAL HYDROLYSIS OF PROGENIN **2**.—Progenin **2** (100 mg) in 0.5% H_2SO_4/MeOH was refluxed for 8 h. The reaction mixture was diluted with H_2O and extracted with *n*-BuOH to give **4** (70 mg) as colorless needles: mp 198–200°; $[\alpha]^{20}D$ −7.0° (*c* = 1.0, $CHCl_3$). The H_2O-soluble portion of the hydrolysate was neutralized with ion exchanger KU-2 (OH^- form) and evaporated. Paper chromatography of the residue revealed D-xylose.

ACETONIZATION OF PROGENIN **2**.—Progenin **2** (121.6 mg) was treated with 0.2% H_2SO_4 in dry Me_2CO (20 ml, 6 h, room temperature). The reaction mixture was diluted with H_2O (70 ml) and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, neutralized, and evaporated (10,13). The residue was chromatographed on a Si gel column using $CHCl_3$-MeOH (10:1) as eluent to give compound **5** (20 mg) as colorless needles: mp 185–186°, R_f 0.75 [$CHCl_3$-MeOH (10:1)].

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