TRITERPENE GLYCOSIDES OF THE LEAVES OF Digitalis ciliata

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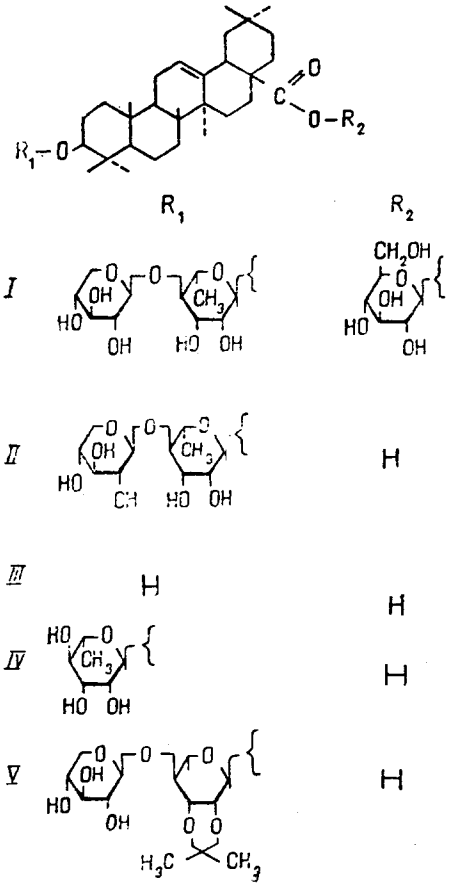
Two new terpene glycosides have been isolated from the leaves of the foxglove Digitalis ciliata Trautv.: β-D-glucopyranosyl oleanolate 3-O-[O-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranoside] and oleanolic acid 3-O-[O-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranoside].

Two new triterpene glycosides have been isolated from the leaves of the foxglove Digitalis ciliata Trautv., and their chemical structures have been established. Glycoside (I), which has been called digitoside A β-D-glucopoyranosyl oleanolate 3-O-[O-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranoside, and glycoside (II), which has been called digitoside B is oleanolic acid 3-O-[O-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranoside]. This is the first time that triterpene glycosides have been isolated from plants of the genus Digitalis and described.

Continuing an investigation of the leaves of Digitalis ciliata in alcohol-chloroform fractions of a methanolic extract we have found, together with cardenolides and flavonoids [1, 2], new glucosides of triterpene nature giving positive reactions with a 25% solution of tungstophorphoric acid [3]. In the present paper we consider the results of an investigation of glycosides (I) and (II) in order of decreasing polarity.



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The acid hydrolysis of glycosides (I) and (II) led to the aglycons which were both identified from their melting points, chromatographic mobilities, and IR, PMR, and ^{13}C NMR characteristics as oleanolic acid (III).

When the hydrolysates obtained were reduced and then acetylated, the following were detected in the reaction mixture by GLC: for glycoside (I) – acetates of the polyols L-rhamnitol, D-xylitol, and D-sorbitol in a ratio of 1:1:1; and for glycoside (II) – those of L-rhamnitol and D-xylitol in a ratio of 1:1.

On the alkaline hydrolysis [5] of glycoside (I), glucose was split out, and the resulting progenin coincided in its TLC characteristics with glycoside (II). Thus, in both the compounds under investigation the xylose and rhamnose residues formed a carbohydrate chain attached to the aglycon by a glycosidic bond, while in (I) the glucose residue was attached by an ester bond. The presence of an agyloside bond in glycoside (I) was also shown by an absorption band in its IR spectrum in the 1755 cm^{-1} region which had shifted in the progenin and in (II) into the 1700 cm^{-1} region that is characteristic for an unsubstituted carboxy group.

The results of GLC and TLC unambiguously confirmed the results of ^{13}C NMR spectroscopy. The SC value of δ 95.4 for the signal of the anomeric carbon atom of the glucose residue meant that it was attached to the aglycon by an ester bond. After alkaline hydrolysis, the C-28 signal (δ 176.0) in (I) had shifted downfield by 2.7 ppm, and after acid hydrolysis the C-signal in both glycosides had shifted upfield by 10 ppm, which unambiguously showed the position of attachment of the carbohydrate residues [5].

A signal from the C-4 atom in the region of δ (79-84) is characteristic for monosaccharides with furanose rings [6]. The only signal in the spectra of (I) and (II) close to this region (δ 88.0 and 87.9, respectively) related to the C-3 resonance of oleanolic acid. Consequently all the carbohydrate residues were in the pyranose form.

To establish the nature of the glycosidic bonds we recorded the spectra without suppression of carbon-proton interactions but with retention of the nuclear Overhauser effect. According to the literature [7], the $J_{C_1-H_1}$ constants (Table 1) indicated that the glucose and the xylose had β- and the rhamnose α-glycosidic bonds.

The stepwise acid hydrolysis of glycoside (II) gave the monoside (IV), and in the hydrolysate the terminal sugar residue (β-xylose) was detected. The acetonidation [8] of glycoside (II) gave a product (V), which showed the presence of a free α-diol grouping (lack of substitution at C-2' and C-3') in the rhamnosyl residue. Consequently, it was possible to characterize the biose at C-3 in both glycosides as O-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranose. This conclusion was not contradicted by ^{13}C NMR results for the carbohydrate moiety of glycoside (II). The assignment of the signals for the carbon atoms was made by comparing the CSs with those for methyl β-D-xylopyranoside [7], and for rhamnose on the basis of numerous ^{13}C NMR figures from [9].

Thus, glycoside (I) consisted of β-D-glucopyranosyl oleanolate 3-O-[O-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranoside], and glycoside (II) of oleanolic acid 3-O-[O-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranoside]. The first has been given the name of digitoside A, and the second that of digitoside B.

EXPERIMENTAL

To separate the total glycosides we used Sephadex G-25 and type L silica gel 40/100 (Czechoslovakia). For chromatography we used type M paper of domestic production and FN-12 paper and Silufol plates with a fixed layer of silica gel, and the following solvent systems: 1) chloroform–methanol (10:1); 2) chloroform–methanol–water (79:23.5:2); 3) ethyl acetate–methanol–water (100:16.5:13.5); and 4) pyridine–benzene–butanol–water (3:5:1:3). Triterpene glycosides and their aglycons were detected on the chromatograms with a 25% alcoholic solution of tungstophosphoric acid in ethanol [3], and the monosaccharides with o-toluidine salicylate [10].

GLC analysis was conducted on a Chrom-5 instrument (Czechoslovakia) using a glass column (4 mm × 1.5 m) filled the sorbent Chromaton-super impregnated with 5% of Silicone XE-60 at a column temperature of 210°C with an evaporator temperature of 230°C and a detector temperatrue of 250°C, the carrier gas being helium at a rate of flow of 50 ml/min. IR spectra were taken on a UR-spectrophotometer in KBr tablets, and NMR spectra on a Tesla BS-567 spectrometer with a resonance frequency of 100 MHz for protons and 25.14 MHz for ^{13}C in C_5D_5N with TMS as

TABLE 1. ^{13}C NMR Characteristics of Digitoside A (I), Digitoside B (II), and Oleanolic Acid (III), δ, ppm

Nuclei of the aglycon	Compound			Nuclei of the sugar residues	Compound	
	I	II	III		I	II
1	39,0	38,9	38,4	Rha -1′	101,1	101,1
2	26,1	26,1	27,4			
3	88,0	87,9	77,7	Rha -2′	71,9	71,9
4	38,7	38,5	38,6	Rha -3′	71,5	71 5
5	55,8	55,6	55,4	Rha -4′	78,1	78,2
6	18,1	17,9	17,9	Rha -5′	69,1	69,0
7	32,7	32,5	32,5	Rha -6′	17,9	17,9
8	39,4	39,2	39,3			
9	47,7	47,5	47,6	Xyl -1″	105,0	105,1
10	36 6	36,5	36,8			
11	23 3	23,2	23,2	Xyl -2″	73,4	73 5
12	122 3	121,8	121,7	Xyl -3″	77,4	77,5
13	143,7	144,3	144,3	Xyl -4″	70,8	70,8
14	4 ,8	41,6	41,7	Xyl -5″	66,1	66,0
15	27,7	27,5	27,2			
16	22,8	23,3	23 2	Glu1‴	95,4	
17	46,6	46,1	46,0			
18	41,8	41,6	41,6	Glu -2‴	73,8	
19	45 9	46,1	46,0	Glu -3‴	78,2	
20	30,2	30,2	30,2	Glu -1‴	71,0	
21	33,6	33,8	33,7	Glu -5‴	79,0	
22	31 9	32,5	32,6	Glu -6‴	62,1	
23	27,7	27,7	28,0			
24	16,4	16,3	15,6			
25	15,1	14,9	14,8			
26	16 9	16,8	16,8			
27	25,4	25,4	25,5			
28	176,0	178 7	178,5			
29	32,5	32,5	32,8			
30	23,2	23,2	23,2			

internal standard. Optical rotations were determined on a SU-2 polarimeter, and melting points on a Kofler block.

Isolation of the Triterpene Glycosides. Air-dry comminuted leaves of the foxglove Digitalis ciliata (5 kg) were extracted with 70% methanol, the solvent was distilled off from the extract, and the aqueous residue was treated successively with ether, chloroform, and alcohol–chloroform (1:2). The alcoholic chloroform extract was evaporated, and the residue was dissolved in 5% methanol and was purified with freshly prepared $Pb(OH)_2$; the methanol was evaporated off and the glycosides were again extracted with alcohol–chloroform (1:2), the extract was dried and the solvent was distilled off. The residue (33 g) was deposited on a column of Sephadex (40 g) that had been swollen in water. The column was washed successively with benzene-ethyl ether (2:1, 1:1, 1:2, and 1:4), ethyl ether–ethyl acetate (1:1)/water, and ethyl acetate–butanol (10:1 and 1:1)/water. The residue from the ethyl acetae–butanol (1:1)/water fraction (5 g) was dissolved in acetone and was reprecipitated with benzene. The precipitate (2.1 g) was chromatographed on a column of silica gel in system 1. This gave gllycosides (I) and (II) in amounts of 0.95 and 0.89 g, respectively.

Digitoside A: mp 220-221°C; M 896, $C_{47}H_{76}O_{16}$; $[\alpha]_D^{20}$ −3.0° (c 1.0; EtOH); R_f 0.21 in system 1. IR spectrum: ν_{max}^{KBr}, cm^{-1}: 3400 (–OH), 1755 (C=O), 1650 (C=C). PMR spectrum: 0.73 (3H, s)), 0.79 (3H, s), 0.82 (3H, s), 0.86 (3H, s), 1.00 (3H, s), 1.11 (3H, s), 1.14 (3H, s), 1.53 (3H, d, J = 6Hz, rhamnose Me), 5.33 (1H, Br.s; H-12).

Digitoside B: mp 268-270°C; M 734, $C_{41}H_{66}O_{11}$: $[\alpha]_D^{20}$ −5.1° (c 1.0; EtOH); R_f 0.41 in system 1. IR spectrum; ν_{max}^{KBr}, cm^{-1}: 3400 (–OH), 1700 (C=O), 1650 (C=C). PMR spectrum: 0.72 (3H, s), 0.80 (3H, s), 0.86 (3H, s), 1.10 (3H, s), 1.14 (3H, s), 1.52 (3H, d, J = 6 Hz, rhamnose Me), 5.31 (1H, br.s, H-12).

Acid Hydrolysis. Glycosides (I) and (II) (0.15 g) were each dissolved in 20 ml of methanol and, after the addition of 0.05 ml of concentrated sulfuric acid, were hydrolyzed by boiling for 3 h. The reaction mixtures were diluted with water, the alcohol was distilled off, the aglycons were extracted with ethyl acetate, and the extract was dried and evaporated. Each residue was purified on a column containing 14 g of silica gel using mixture 1 as eluent. In this way, glycoside (I) gave 29 mg, and glycoside (II) 32 mg, of aglycon: mp 301-302°C;

IR spectrum, ν_{max}^{KBr}, cm^{-1}: 3600 (—OH), 1700 (C=O) 1645 (C=C): PMR spectrum: ($CDCl_3$): 0.73 (3H, s), 0.75 (3H, s) 0.90 (6H, s), 0.97 (3H, s), 1.11 (3H, s), 1.24 (3H, s), 3.20 (1H, t, J = 7 Hz, H-3), 5.26 (1H, br.s, H-12). In the hydrolysate from (I), D-glucose, L-rhamnose, and D-xylose were detected by PC analysis, and in that from glycoside (II) D-xylose and L-rhamnose.

Alkaline Hydrolysis. A solution of 110 mg of digitoside A or B in 5 ml of a 1% solution of KOH was heated at 100°C for 30 min [5], and then the reaction mixture was neutralized with HCl and was extracted with n-BuOH. The organic phase was evaporated to dryness. In both cases, TLC analysis showed the presence of digitoside B. D-Glucose was detected by PC analysis in the hydrolysate from digitoside A.

Stepwise Hydrolysis. Digitoside B (100 mg) was hydrolyzed with a 0.5% aqueous solution of H_2SO_4 by boiling for 8 h [11], the reaction mixture was diluted with water, and the monoside that had been produced was extracted with n-BuOH. After the evaporation of the butanolic extract, 70 mg of monoside (IV) as obtained with mp 198-200°C, $[\alpha]_D^{20}$ −7.0° (c 1.0; $CHCl_3$). The acidity of the reaction mixture after the separation of the monoside was brought to pH 7, and the mixture was evaporated. D-Glucose was detected in the hydrolysate.

Preparation of an Acetonide. A solution of 103 mg of digitoside B in 20 ml of dry acetone [8] containing 0.2% of H_2SO_4 was stirred for 6 h, adn then 7 ml of water was added and the reaction products were extracted with chloroform. This gave 20 mg of digitoside B acetonide (V) with mp 185-186°C. As compared with that of digitoside B, the PMR spectrum showed two additional signals at δ 1.06 and δ 1.10 belonging to the methyl groups of a dioxolane ring.

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