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# STRUCTURE OF A STEROID SAPONIN FROM *Digitalis ciliata*

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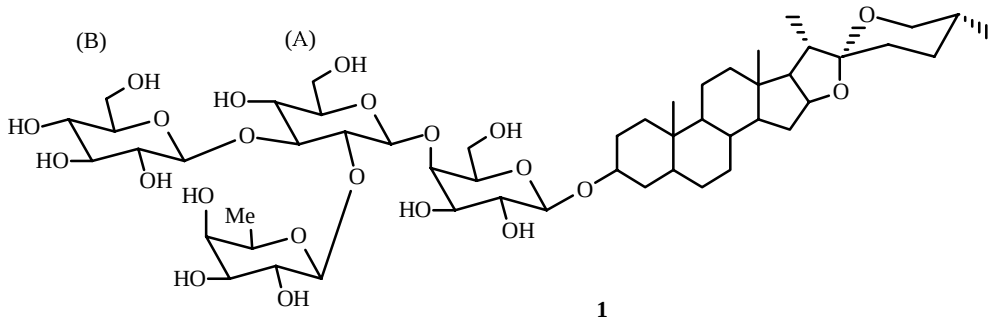
*A new steroid glycoside was isolated from leaves of* Digitalis ciliata *(Scrophulariaceae) by fractionation of the total extracted substances. Its structure was determined as (25*R*)-5*α*-spirostan-3*β*-ol 3*-O-*β*-D-glucopyranosyl-(1→3)[*β*-D-fucopyranosyl-(1→2)]-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside based on chemical transformations, physical constants, and spectral data.*

**Key words:** *Digitalis ciliata*, Scrophulariaceae, steroid glycoside, spirostane, tigogenin.

We have previously reported on steroid glycosides that are derivatives of gitogenin and diosgenin [1] and are isolated from leaves of *Digitalis ciliata* (Scrophlariaceae). Herein the structure of a new glycoside (**1**), a derivative of tigogenin, is proved.

Glycoside **1** had a positive Sannie—Lapin color reaction [2] and IR absorption characteristic of a spiroketal belonging to the 25*R*-spirostane series [3].




**1**

Acid hydrolysis of **1** produced the aglycon, which was identified by its physical chemical constants, spectral data, and comparison with an authentic sample of tigogenin [4].

Methanolysis of **1** and subsequent analysis of the sugars by GC [5] established that the carbohydrate part contained galactose, glucose, and fucose in a 1:2:1 ratio.

The FAB mass spectrum of **1** gave key peaks with relative mass *m*/*z* 1071 $[M + Na]^+$, 925 $[M + Na - \text{deoxyhexose}]^+$, 909 $[M + Na - \text{hexose}]^+$, and 439 $[M + Na - \text{tetraose}]^+$.

The $^{13}C$ NMR spectrum (Table 1) recorded with full decoupling of protons contained 51 lines, of which 27 belonged to the aglycon and, therefore, 24 to the sugars. Four lines were observed in the region of anomeric resonances. Five methyls appeared at strong field. Obviously, the fifth methyl could belong only to one of the sugars. Thus, a comparison of the FAB mass spectrum of tigogenin (*m*/*z* 439 $[M + Na]^+$) and its reported $^{13}C$ NMR spectrum [6] with those of **1** and GC data led to the conclusion that the carbohydrate part consisted of four sugars, one of which was a deoxyhexose.

Methylation of **1** by the Hakomori method [7] produced its permethylated derivative. The completeness of the methylation was monitored by the disappearance in the IR spectrum of hydroxyl absorptions and the presence in the FAB mass spectrum of a peak for the molecular ion with *m*/*z* 1239 $[M + Na]^+$.

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TABLE 1. $^{13}$C NMR Spectral Data for **1** and Tigogenin [5]

| C atom | Tigogenin, δ, ppm | **1**, δ, ppm | C atom | **1**, δ, ppm | $^{1}$J (CH)/Hz |
|---|---|---|---|---|---|
| 1 | 37.5 | 37.3 | Galactose | | |
| 2 | 32.6 | 30.0 | 1 | 103.0 | 159.2 |
| 3 | 70.5 | 77.5 | 2 | 73.4 | |
| 4 | 39.1 | 34.8 | 3 | 75.6 | |
| 5 | 45.1 | 44.8 | 4 | 79.9 | |
| 6 | 29.1 | 29.0 | 5 | 76.0 | |
| 7 | 32.5 | 32.4 | 6 | 61.0 | |
| 8 | 35.4 | 35.3 | Glucose (A) | | |
| 9 | 54.6 | 54.5 | 1 | 105.0 | 162.6 |
| 10 | 35.9 | 36.0 | 2 | 81.8 | |
| 11 | 21.3 | 21.3 | 3 | 87.2 | |
| 12 | 40.3 | 40.2 | 4 | 71.4 | |
| 13 | 40.8 | 40.8 | 5 | 78.1 | |
| 14 | 56.6 | 56.5 | 6 | 63.0 | |
| 15 | 32.1 | 32.2 | Glucose (B) | | |
| 16 | 81.1 | 81.1 | 1 | 104.8 | 162.0 |
| 17 | 63.1 | 63.1 | 2 | 75.4 | |
| 18 | 16.7 | 16.6 | 3 | 78.2 | |
| 19 | 12.5 | 12.4 | 4 | 71.7 | |
| 20 | 42.0 | 42.0 | 5 | 78.3 | |
| 21 | 15.0 | 15.0 | 6 | 62.8 | |
| 22 | 109.3 | 109.2 | Fucose | | |
| 23 | 31.9 | 31.8 | 1 | 106.0 | 161.4 |
| 24 | 29.3 | 29.3 | 2 | 71.9 | |
| 25 | 30.6 | 30.6 | 3 | 75.0 | |
| 26 | 66.9 | 66.9 | 4 | 72.9 | |
| 27 | 17.2 | 17.3 | 5 | 71.7 | |
| | | | 6 | 17.3 | |

TABLE 2. PMR Spectral Data for the Carbohydrate Part of **1**

| Atom | Chemical shift, ppm, J/Hz | Atom | Chemical shift, ppm, J/Hz |
|---|---|---|---|
| 3-*O*-Galactose | | Glucose (B) | |
| H-1 | 4.87, J(1-2) = 7.6 | H-1 | 5.34, J(1-2) = 7.8 |
| H-2 | 4.32, J(2-3) = 9.1 | H-2 | 4.04, J(2-3) = 8.8 |
| H-3 | 4.12, J(3-4) = 3.2 | H-3 | 4.15, J(3-4) = 8.8 |
| H-4 | 4.54, J(4-5) < 2 | H-4 | 4.26, J(4-5) = 9.0 |
| H-5 | 4.09, m | H-5 | 3.83, m |
| H-6 | * | H-6 | * |
| Glucose (A) | | Fucose | |
| H-1 | 5.14, J(1-2) = 7.6 | H-1 | 4.64, J(1-2) = 7.5 |
| H-2 | 4.38, J(2-3) = 8.6 | H-2 | 4.24, J(2-3) = 8.9 |
| H-3 | 4.22, J(3-4) = 8.6 | H-3 | 4.06, J(3-4) = 3.5 |
| H-4 | 3.74, J(4-5) = 8.6 | H-4 | 4.02, m |
| H-5 | 3.80, m | H-5 | 3.73, m |
| H-6 | * | Me-6 | 1.55 br.s |

*Signals could not be found on a 300 MHz spectrometer.

Tigogenin and the total methylated sugars were isolated from the reaction mixture after methanolysis of the permethylate. GC identified 2,3,4,6-tetra-*O*-methyl-D-glucopyranose (terminal), 2,3,4-tri-*O*-methyl-D-fucopyranose (terminal), 4,6-di-*O*-methyl-D-glucopyranose (disubstituted at C-2 and C-3), and 2,3,6-tri-*O*-methyl-D-galactopyranose (substituted at C-4).

The structure of the carbohydrate chain of **1** and its attachment site to the aglycon were established using PMR and $^{13}C$ NMR data (Tables 1 and 2).

The PMR spectrum of **1** was solved by a series of selective homonuclear double resonance experiments using the usual and difference versions. The SSCC that were found were consistent with the carbohydrate part of **1** containing D-glucose, D-galactose, and 6-deoxy-D-galactose (fucose). The values that were found for the anomeric protons were consistent with vicinal axial—axial couplings and indicated that all glycoside bonds of all sugars had the $\beta$-configuration [8].

The results of the GC analysis of the attachment sites in the carbohydrate agreed with experimental data of the observed nuclear Overhauser effect (NOE). Thus, pre-irradiation of anomeric proton H-1 ($\delta$ 5.34) of glucose (B) markedly increased the intensity of two signals, H-2 (4.04) of glucose (B) and H-3 (4.22) of glucose (A), by 5.9 and 7.2%, respectively. Pre-irradiation of H-1 of glucose (A) (5.14) caused an analogous reaction by H-2 of glucose (A) (4.38) and H-4 of galactose (4.54). Pre-irradiation of H-1 of fucose (4.64) caused a reaction of H-2 of fucose (4.24) and H-2 of glucose (A) (4.38). Thus, fucose and glucose (B) glycosylated glucose (A) at C-2 and C-3, respectively; glucose (A), galactose at C-4. This was consistent with the paramagnetic shift of the signals for the glycosylated centers in the $^{13}C$ NMR spectrum compared with those in the literature [6, 9] by $\Delta \sim 9.1$ ppm for galactose C-4 and by $\Delta \sim 5.8$ ppm and $\Delta \sim 9.0$ ppm for glucose (A) C-2 and C-3.

A comparison of the chemical shifts for **1** and tigogenin [5] showed that C-3 had the largest paramagnetic shift ($\Delta = 7.0$ ppm); C-2 and C-4, diamagnetic shifts of 2.6 and 4.3, respectively. This fact unambiguously indicated that C-3 was the attachment site of the carbohydrate.

Finally, geminal heteronuclear SSCC $^1J(CH)$ (Table 1) confirmed conclusively that the C-1 OR group of all sugars was equatorial, i.e., all glycosidic bonds had the $\beta$-configuration with the $^4C_1$-conformation for their rings [9].

Thus, **1** is (25*R*),5$\alpha$-spirostan-3$\beta$-ol 3-*O*-$\beta$-D-glucopyranosyl-(1→3)-[$\beta$-D-fucopyranosyl-(1→2)]-$\beta$-D-glucopyranosyl-(1→4)-$\beta$-D-galactopyranoside.

## EXPERIMENTAL

**General Comments.** Kieselgel $60F_{254}$ (Merck) and Silufol UV 254 plates were used for TLC. Column chromatography used silica gel (KSK, particle size <63 and 63-100 μm). The following solvent systems were used: 1) $CHCl_3$:$CH_3OH$:$H_2O$ (a, 65:15:2; b, 65:35:8), 2) $CHCl_3$:$CH_3OH$ (a, 10:1; b, 50:1).

GC was performed on a Chrom-5 instrument. Monosaccharides were chromatographed as trimethylsilyl esters of methylglycosides over a column (3 m × 4 mm) with Chromaton N-AW containing silicone SE-30 (5%), thermostat 190°C, He carrier gas, flow rate 45 mL/min.

Methylglycosides of methylated sugars were prepared by boiling (4 h) methyl esters in absolute methanol containing HCl (5%). The products were chromatographed over a column (1.2 m × 3 mm) with celite containing 1,4-polybutanediol succinate (20%), thermostat 160°C, He carrier gas, flow rate 50 mL/min.

PMR and $^{13}C$ NMR spectra were recorded on a AM-300 (Bruker) spectrometer at 300 MHz for $^1H$ and 75 MHz for $^{13}C$ in pyridine-$d_5$ with TMS internal standard.

Other data have been reported [1].

**Isolation of 1.** Preliminary processing of the total extracted substances has been described [1].

After extraction of diosgenin and gitogenin, fractions enriched with the studied glycoside were combined and chromatographed over silica-gel columns using systems 1a and 1b. Fractions containing chromatographically homogeneous **1** (0.48 g) were collected. The overall yield calculated per air-dried weight of the raw material was 0.01%.

**(25*R*),5$\alpha$-Spirostan-3$\beta$-ol 3-*O*-$\beta$-D-glucopyranosyl-(1→3)-[$\beta$-D-fucopyranosyl-(1→2)]-$\beta$-D-glucopyranosyl-(1→4)-$\beta$-D-galactopyranoside.** White crystalline powder, mp 266-269°C (ethanol), $[\alpha]_D^{20}$ -62.5° ($CHCl_3$ + MeOH, 1:1, *c* 0.8). FAB MS (*m*/*z*, %): 1071 (38) $[M + Na]^+$, 925 (100) $[M + Na - deoxyhexose]^+$, 909 (42) $[M + Na - hexose]^+$, 809 (12) $[M + 2Na - (deoxyhexose + hexose)]^+$, 439 (24) $[M + Na - tetrose]^+$. IR spectrum (KBr, $\nu$, $cm^{-1}$): 3420, 2900, 1440, 1350, 1045, 975, 915, 890, 695. PMR spectrum ($\delta$, J/Hz): 0.70 (3H, d, J = 5.5, Me-27), 0.82 (3H, s, Me-18), 0.87 (3H, s, Me-19), 1.12 (3H, d, J = 6.8, Me-21), 3.50 (1H, dd, J = 10.5, 10.5, $H_b$-26), 3.58 (1H, dd, J = 10.5, 2.9, $H_a$-26), 3.68 (1H, m, H-3).

**Acid Hydrolysis.** Glycoside **1** (50 mg) was dissolved in aqueous methanol (10 mL, 50%) containing conc. $H_2SO_4$ (0.4 mL) and boiled for 10 h. The resulting precipitate was filtered off and recrystallized from ethanol to afford the aglycon (22 mg), $C_{27}H_{44}O_3$, mp 200-202°C, $[\alpha]_D^{20}$ -69° ($CHCl_3$, *c* 0.5). IR spectrum (KBr, ν, $cm^{-1}$): 3400 (OH), 1045, 980, 961, 920, 901, 860. FAB MS (*m*/*z*): 439 $[M + Na]^+$. These data, the $^{13}C$ NMR spectrum, and TLC with an authentic sample identified the aglycon as tigogenin.

**Methanolysis of 1.** Glycoside **1** (10 mg) was dissolved in absolute methanol (4 mL) containing HCl (5%), boiled for 12 h, cooled, and treated with an equal volume of water. The resulting precipitate was filtered off and shown to be identical with tigogenin (TLC, system 2a). The solid was neutralized with $Ag_2CO_3$, filtered off, and evaporated to dryness. GC detected D-galactose, D-glucose, and D-fucose in a 1:2:1 ratio.

**Methylation of 1.** Glycoside **1** (100 mg) was dissolved in DMSO (10 mL), slowly treated with NaH (100 mg), stirred vigorously for 1 h, slowly treated with $CH_3I$ (1.5 mL), stirred another 3 h, poured into water (0.1 L), and extracted with $CHCl_3$ (5 × 10 mL). The combined $CHCl_3$ extracts were treated with sodium thiosulfate and evaporated to dryness. The solid was chromatographed over a silica-gel column (system 2b). Recrystallization from methanol produced the permethylated product (88 mg) which was subjected to acid hydrolysis as described above. The resulting precipitate of the aglycon was separated and identified as tigogenin.

An aqueous solution of the methylated sugars was boiled for 6 h, after which the reaction mixture was neutralized with anion-exchanger EDE-10P and taken to dryness. The methylglycosides that were described above in the discussion were identified by GC.

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