# STEROID GLYCOSIDES FROM THE SEEDS OF *Petunia hybrida*. STRUCTURES OF PETUNIOSIDES B, D, AND F

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*The seeds of the petunia* Petunia hybrida *L. have yielded three steroid glycosides of the spirostan series: petuniosides B, D, and F. The structures of the glycosides have been demonstrated on the basis of chemical transformations and spectral characteristics. Petuniosides B and D proved to be known compounds identical with capsicosides $A_1$ and $B_1$, respectively. Petunioside F is a new compound with the following structure: (25R)-5α-spirostan-2α,3β-diol 3-O-{[O-β-*D*-glucopyranosyl-(1 → 2)]-[O-β-*D*-glucopyranosyl-1 → 4)]-β-*D*-galactopyranoside}.*

We have previously reported the presence of steroid glycosides of the spirostan series in petunia seeds [1]. In the present paper we give a proof of the chemical structures of three gylcosides of the spirostan series isolated from the seeds of *Petunia hybrida* L.

As the result of repeated chromatographic separation of a methanolic extract from petunia seeds on a column of silica gel, we obtained three individual steriod glycosides, which we called petuniosides B (**1**), D (**2**), and F (**3**). Each of the compounds isolated gave a positive reaction with the Sannié reagent [2] and a negative reaction with the Ehrlich reagent [3], which showed their spirostanol nature. The IR spectra of each glycoside contained the absorption bands in the 900 > 920 region that are characteristic for a spiroketal chain of the (25R) series [4].

On the complete acid hydrolysis of the petuniosides, in each case we obtained a genin which was identified from its physicochemical constants and its $^{13}C$ NMR spectrum (Table 1) as gitogenin (**4**) [5], while in the monosaccharide fraction by the PC and GLC of the acetates of the aldononitrile derivatives we showed the presence of one galactose molecule in compound (**1**), galactose and glucose in a ratio of 1:1 in (**2**), and galactose and glucose in a ratio of 1:2 in (**3**). The results obtained were confirmed by the $^{13}C$ NMR spectra of the glycosides, where one signal (103.5 ppm) was observed in the region of anomeric atoms in the spectrum of (**1**), signals at 103.8 and 106.95 ppm in the spectrum of (**2**), and signals at 102.8, 105.4, and 106.7 ppm in that of (**3**).

The positions of attachment and the sizes of the oxide rings of the monosaccharides of each petunioside were determined by the Hakomori methylation of the neutral compounds, followed by the methanolysis of the permethylated products obtained. By GLC in the presence of markers, we identified Me 2,3,4,6-tetra-O-Me-*D*-galactopyranoside (**5**) for permethylated (**2**); Me 2,3,4,6-tetra-O-Me-*D*-glucopyranoside (**6**) and Me-2,3,6-tri-O-Me-*D*-galactopyranoside (**7**) for permethylated (**2**); and (**6**), (**7**), and Me 3,4,6-tri-O-Me-*D*-glucopyranoside (**8**) for permethylated (**3**)

The sequences of attachment of the monosaccharide residues in the petuniosides were determined with the aid of partial acid hydrolysis. In the case of (**2**), we obtained the progenin (**9**), which decomposed on hydrolysis into gitogenin and galactose. Consequently, in petunioside D the galactose was directly attached to the aglycon. The cleavage of (**3**) led to two progenins — (**10**) and (**11**). Progenin (**10**) contained gitogenin and galactose in a ratio of 1:1 and was identical with progenin (**9**). According to its physicochemical constants, progenin (**9**) was identical with petunioside B, and (**11**) with petunioside D.

In a comparison of the $^{13}C$ NMR spectra of gitogenin and the glycosides, it was seen that the signal of the C-3 atom of the genin was shifted downfield in each case, which is explained by the glycosylation effect. The chemical shifts of the other carbon atoms in the aglycon remained unchanged. The $^{1}H$ NMR spectra (Table 2) confirmed the presence in ring *A* of two hydroxyls in positions 2 and 3, and also the equatorial position of the methyl at C-25 of the genin.

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TABLE 1. Chemical Shifts of the $^{13}C$ Carbon Atoms of Gitogenin (**4**) and of Petuniosides B (**1**), D (**2**), and F (**3**), ($\delta$, ppm 0 — TMS, $C_5D_5N$)

| C-Atom | Compound | | | | C-Atom | Compound | | |
|---|---|---|---|---|---|---|---|---|
| | **4** | **1** | **2** | **3** | | **1** | **2** | **3** |
| 1 | 46.65 | 45.7 | 45.2 | 45.3 | Gal | | | |
| 2 | 73.25 | 71.9 | 72.0 | 71.8 | 1 | 103.8 | 103.65 | 102.8 |
| 3 | 76.8 | 85.15 | 86.0 | 84.9 | 2 | 73.1 | 72.9 | 73.35 |
| 4 | 37.1 | 34.3 | 34.5 | 34.6 | 3 | 75.9 | 75.7 | 75.4 |
| 5 | 45.5 | 45.05 | 45.1 | 45.15 | 4 | 70.3 | 79.85 | 80.6 |
| 6 | 28.5 | 28.3 | 28.3 | 28.35 | 5 | 75.9 | 75.4 | 75.75 |
| 7 | 32.55 | 32.45 | 32.4 | 32.6 | 6 | 61.2 | | |
| 8 | 34.95 | 34.9 | 34.85 | 34.9 | Glc (1) | | | |
| 9 | 54.95 | 54.8 | 54.85 | 54.9 | 1 | | 106.95 | 105.0 |
| 10 | 37.75 | 37.1 | 37.2 | 37.3 | 2 | | 75.3 | 85.6 |
| 11 | 21.7 | 21.6 | 21.6 | 21.6 | 3 | | 78.4 | 78.4 |
| 12 | 40.4 | 40.3 | 40.45 | 40.5 | 4 | | 70.7 | 70.9 |
| 13 | 41.0 | 41.0 | 41.1 | 41.15 | 5 | | 78.7 | 78.9 |
| 14 | 56.7 | 56.6 | 56.6 | 56.7 | 6 | | 63.3 | 63.3 |
| 15 | 32.25 | 32.25 | 32.2 | 32.2 | Glc (2) | | | |
| 16 | 81.3 | 81.3 | 81.4 | 81.35 | 1 | | | 106.7 |
| 17 | 63.3 | 63.3 | 63.4 | 63.45 | 2 | | | 76.6 |
| 18 | 16.6 | 16.65 | 16.55 | 16.6 | 3 | | | 77.8 |
| 19 | 13.8 | 13.5 | 13.7 | 13.6 | 4 | | | 72.0 |
| 20 | 42.2 | 42.2 | 42.3 | 42.2 | 5 | | | 78.1 |
| 21 | 14.9 | 14.9 | 14.9 | 14.95 | 6 | | | 62.2 |
| 22 | 109.4 | 109.4 | 109.0 | 109.1 | | | | |
| 23 | 32.05 | 32.1 | 32.1 | 32.1 | | | | |
| 24 | 29.4 | 29.4 | 29.3 | 29.3 | | | | |
| 25 | 30.7 | 30.7 | 30.6 | 30.7 | | | | |
| 26 | 67.1 | 67.1 | 67.15 | 67.2 | | | | |
| 27 | 17.3 | 17.3 | 17.1 | 17.2 | | | | |

TABLE 2. Chemical Shifts (ppm, 0 — TMS, $C_5D_5N$) and SSCCs (J, Hz) of the Protons of Petunioside A (**1**), D (**2**), and F (**3**)

| Protons of the aglycon and the sugars of (**1**) | | | Protons of the sugars of (**2**) | | | Protons of the sugars of (**3**) | | |
|---|---|---|---|---|---|---|---|---|
| Gitogenin | | | *D*-Galactose | | | *D*-Galactose | | |
| 1 | 2.19 | $J_{1,2}=4.7$ | 1 | 4.82 | $J_{1,2}=7.8$ | 1 | 4.86 | $J_{1,2}=7.8$ |
| 1 | 1.20 | $J_{1,1}=13.0$ | 2 | 4.33 | $J_{2,3}=10.0$ | 2 | 4.42 | $J_{2,3}=10.0$ |
| 2 | 3.95 | $J_{2,1}=9.1$ | 3 | 4.14 | $J_{3,4}=3.1$ | 3 | 4.05 | $J_{3,4}=4.4$ |
| 2 | – | $J_{2,3}=11.5$ | 4 | 4.56 | $J_{4,5}=2$ | 4 | 4.46 | $J_{4,5}=2$ |
| 3 | 3.76 | $J_{3,4}=8.9$ | 5 | 4.05 | $J_{5,6}=8.0$ | 5 | 4.00 | $J_{5,6}=9.0$ |
| 4 | 1.82 | $J_{4,4}=12.6$ | 6 | 4.49 | $J_{6,6}=11.8$ | 6 | 4.57 | $J_{6,6}=11.3$ |
| 4 | 1.62 | $J_{3,4}=5.2$ | 6 | 4.18 | $J_{5,6}=6.0$ | 6 | 4.17 | $J_{5,6}=5.7$ |
| 15 | 2.03 | $J_{15,16}=6.2$ | *D*-Glucose (1) | | | *D*-Glucose (1) | | |
| 15 | 1.40 | $J_{15,16}=8.2$ | 1 | 5.13 | $J_{1,2}=8.0$ | 1 | 5.02 | $J_{1,2}=7.8$ |
| 16 | 4.50 | $J_{16,17}=8.2$ | 2 | 3.98 | $J_{2,3}=9.2$ | 2 | 4.00 | $J_{2,3}=9.1$ |
| 17 | 1.81 | | 3 | 4.10 | $J_{3,4}=9.2$ | 3 | 4.15 | $J_{3,4}=9.1$ |
| 25 | 1.55 | $J_{25,26}=4.5$ | 4 | 3.93 | $J_{4,5}=9.2$ | 4 | 3.85 | $J_{4,5}=9.1$ |
| 26 | 3.56 | $J_{26,26}=10.1$ | 5 | 3.97 | $J_{5,6}=2.0$ | 5 | 3.60 | $J_{5,6}=2.0$ |
| 26 | 3.47 | $J_{25,26}=10.1$ | 6 | 4.45 | $J_{6,6}=12.6$ | 6 | 4.48 | $J_{6,6}=12.6$ |
| 27 | 0.70 | $J_{25,27}=6.0$ | 6 | 4.10 | $J_{5,6}=7.0$ | 6 | 4.01 | $J_{5,6}=7.0$ |
| *D*-Galactose | | | | | | *D*-Glucose (2) | | |
| 1 | 5.39 | $J_{1,2}=7.8$ | | | | 1 | 5.15 | $J_{1,2}=8.0$ |
| 2 | 4.41 | $J_{2,3}=7.5$ | | | | 2 | 3.94 | $J_{2,3}=9.5$ |
| 3 | 4.03 | $J_{3,4}=3.0$ | | | | 3 | 4.04 | $J_{3,4}=9.3$ |
| 4 | 4.37 | $J_{4,5}=3.5$ | | | | 4 | 4.06 | $J_{4,5}=9.3$ |
| 5 | 3.94 | | | | | 5 | 3.85 | $J_{5,6}=2.7$ |
| 6A | 4.29 | $J_{5,6A}=5.5$ | | | | 6 | 4.51 | $J_{6,6}=12.6$ |
| | | $J_{6A,6B}=12.5$ | | | | 6 | 4.29 | $J_{5,6}=4.0$ |
| 6B | 4.41 | $J_{5,6B}=5.3$ | | | | | | |

The nature of the splitting and spin–spin coupling constants (SSCCs) of all the skeletal protons of the sugar residues of the glycosides agreed completely with the galacto- and gluco- configurations of the monosaccharides in the pyranose forms, while the $\beta$- configuration of the glycoside centers of the glucose and galactose residues followed from the magnitudes of the SSCCs.

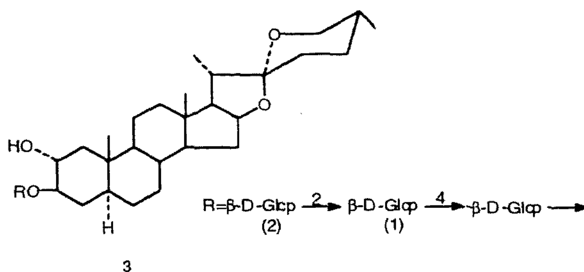
On analyzing the results obtained, we came to the conclusion that petunioside B was identical with capsicoside $A_1$ ((25R)-5$\alpha$-spirostan-2$\alpha$,3$\beta$-diol 3-O-$\beta$-*D*-galactopyranoside) and petunioside D with capsicoside $B_1$ ((25R)-5$\alpha$-spirostan-2$\alpha$,3$\beta$-diol 3-O-[O-$\beta$-*D*-glucopyranosyl-(1→4)-$\beta$-D-galactopyranoside]), which we had isolated previously from the roots of red pepper [7].

The proton spectrum of petunioside F (**3**) was interpreted with the aid of two-dimensional correlation spectroscopy, COSY, of resonance in the difference variant [8]. From the SSCCs of the sugar residues of (**3**) we identified both β-glucopyranose (two residues) and β-galactopyranose (see Table 2). The types of substitution in the residues and in the aglycon were revealed after recording the two-dimensional NOE spectrum in a rotating coordinate system (ROESY) [9].

In this spectrum, in addition to the contacts of the anomeric protons with the H-2, H-3, and H-5 protons of the same residue that are customary for pyranoses with the β-configuration of the glycosidic center, correlation peaks were seen in the coordinates of the chemical shifts of H-1 of galactopyranose and H-3 of gitogenin, H-1 of glucopyranose 1 and H-4 of galactopyranose, and H-1 of glucopyranose 2 and H-3 of glucopyranose 1. This clearly shows the sequence of linkage of the residues, while the type of substitution in the glucopyranose 1 residue (at the C-2 hydroxyl) was definitively established after the heteronuclear correlation $^{13}C/^{1}H$ COSY spectrum had been recorded.

The presence of a weak correlation peak in the ROESY spectrum in the coordinataes of the H-1(Glcp-2)/H-3(Glcp-1) chemical shifts in this case can be explained by the diffusion of spin density during spin-locking (0.2 s in this experiment). The two-dimensional heteronuclear COSY spectrum likewise confirmed the remaining types of substitution in the residues.

On the basis of the results obtained, the following structure is proposed for petunioside F:




## EXPERIMENTAL

For chromatography we used silica gels L 100/250, 100/160, and 40/100 μm, FN-3 paper, and Silufol plates with the following solvent systems: 1) chloroform–methanol (9:1), 2) chloroform–methanol (4:1), 3) benzene–ethanol (9:1), 4) benzene–diethyl ether (7:3), and 5) *n*-butyl alcohol–benzene–pyridine–water (5:1:3:3, upper layer).

Melting points were determined on a Boëtius stage. Specific rotations were measured on an SM polarimeter. IR spectra were taken on a Specord 74-IR instrument. Mass spectra were recorded on a MKh-1320 spectrometer with a system for the direct injection of the substance into the ion source, at an ionizing energy of 70 eV.

GLC analysis was conducted on a Chrom-5 instrument with a flame-ionization detector, using a 3 × 2400 mm glass column with the stationary phase 5% of XE-60 on Chromaton N-AW-HMDS. PMR spectra were recorded on Bruker AM-300 and WM-250 instruments for solutions in pyridine-$d_5$ at 70°C. $^{13}C$ NMR spectra were recorded on an AM-300 instrument with a working frequency of 75 MHz under similar conditions.

**Isolation of the Steroid Glycosides.** Air-dry petunia seeds (1 kg) were ground and defatted with chloroform and were then extracted exhaustively by heating with 70% aqueous ethyl alcohol. The extract obtained was concentrated in vacuum to a dry residue, which was dissolved in a small amount of methyl alcohol, the total steroid glycosides were precipitated with acetone, and the residue was filtered off and dried. This gave 29 g of product, which amounted to 2.9% of the weight of the initial raw material.

The total glycosides isolated were repeatedly chromatographed on a column of silica gel in solvent systems 1 and 2. Monitoring was carried out by TLC in the same solvent systems. This gave 320 mg of petunioside B (**1**), mp. 258°C, $[\alpha]_D^{20}$ −40°(*c* 1.2; $CH_3OH$), 280 mg, petunioside D (**2**), mp. 263°C, $[\alpha]_D^{20}$ −48° (*c* 1.0; $CH_3OH$) and 460 mg. petunioside F (**3**), mp 235°C, $[\alpha]_D^{20}$ −56° (*c* 1.0; $CH_3OH$). Absorption bands in the IR spectrum at 3500-3400, 987, 920, 900, 850 (900>920) $cm^{-1}$ were characteristic for each of the compounds isolated.

**Acid Hydrolysis of Petuniosides.** Each glycoside (50 mg) was hydrolyzed with 2.5% sulfuric acid at 110°C in a sealed tube for 8 h, after which the reaction mixture was diluted with water and the aglycons were extracted from the hydrolysates with chloroform (3 × 20 ml). After chromatographic purification on a column of silica gel in system 4, in each case gitogenin was obtained with mp 265°C, $[\alpha]_D^{20}$ −76° (*c* 1.0; $CHCl_3$), $[M]^+$ 432. IR spectrum: 1300, 862, 898>924, and 982 $cm^{-1}$.

By paper chromatography in system 5 and by the GLC of acetates of the aldononitrile derivatives of the sugars [10], galactose was identified in the hydrolysate of (**1**), galactose and glucose in a ratio of 1:1 in that of (**2**), and galactose and glucose in a ratio of 1:2 in that of (**3**).

**Methylation of the Petuniosides and the Progenins.** A solution of 20 mg of a glycoside or progenin in 5 ml of methylsulfonyl anion (prepared from 350 mg of NaH and 15 ml of DMSO) was stirred at 50°C in an atmosphere of argon for 1 h. Then 10 ml of $CH_3I$ was added to the reaction mixture and it was left at room temperature in the dark for 12 h. After this, it was diluted with water and extracted with chloroform. The chloroform extract obtained was washed with saturated $Na_2S_2O_3$ solution and with water and was concentrated in vacuum. The methylation product was purified by column chromatography on silica gel in system 3.

The methanolysis of the permethylates obtained was performed with 72% $HClO_4$ in methanol (1:10) at 100°C for 6 h. The reaction mixture was neutralized with an anion-exchange resin and evaporated. The methyl glycosides mentioned in the discussion were identified by GLC in the presence of markers.

**Partial Acid Hydrolysis of the Petuniosides.** Compounds (**2**) and (**3**) (150 mg of each, separately) were dissolved in 30 ml of 1% $H_2SO_4$ in methanol, and the solution was heated in the water bath for 1.5 h. The course of the reaction was followed every 30 min by TLC in systems 1 and 2. Each reaction mixture was diluted with water and extracted with butanol. The butanol extract was evaporated and chromatographed on a column of silica gel successively with systems 1 and 2. Compound (**2**) yielded gitogenin and progenin (**9**) (87 mg), mp 266-267°C, $[\alpha]_D^{20}$ −42° (*c* 1.0; ($CH_3OH$), and (**3**) yielded gitogenin and two progenins: (**10**) (52 mg) and (**11**) (40 mg). According to their physicochemical constants, progenin (**10**) was identical with (**9**) and (**11**) with (**2**).

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