Two New Triterpenoid Saponins from *Platycodon grandiflorum*

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Two new triterpenoid saponins, platycoside D [3-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-2β,3β,16α,23-tetrahydroxyolean-12-ene-28-oic acid 28-*O*-β-D-apiofuranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside] and platycoside E [3-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-2β,3β,16α,23,24-pentahydroxyolean-12-ene-28-oic acid 28-*O*-β-D-apiofuranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside] were isolated from the roots of *Platycodon grandiflorum*. Structural determination is based on spectral and chemical evidence.

Key words *Platycodon grandiflorum*; Campanulaceae; saponin; platycoside D; platycoside E

In a previous paper, we reported the isolation and structural elucidation of three new triterpenoid saponins from the roots of *Platycodon grandiflorum* A. DC.[1] Further investigation led to the isolation of two new triterpenoid saponins, platycosides D (**1**) and E (**2**).

Platycoside D (**1**), an amorphous powder, $[\alpha]_D$ −17.9°, possessed the molecular formula $C_{69}H_{112}O_{37}$, as determined by negative electron-spray ionization (ESI)-MS (at *m/z* 1531 $[M-H]^-$) and from ^{13}C and ^{13}C distortionless enhancement by polarization transfer (DEPT) NMR spectral data. The ^1H- and ^{13}C-NMR spectral data indicated that **1** had the same sapogenin, 2β,3β,16α,23-tetrahydroxyolean-12-ene-28-oic acid, as polygalacin D.[2,3] In the ^{13}C-NMR spectrum, seven anomeric carbons were observed at δ 93.5, 101.1, 105.1, 105.2, 105.5, 106.7, and 111.2. In the ^1H-NMR spectrum, one doublet methyl signal at δ 1.73 (J=6.1 Hz) belonging to rhamnose was observed. The component sugars (glucose, arabinose, rhamnose, xylose and apiose/3 : 1 : 1 : 1 : 1) were confirmed by GLC analysis of the trimethylsilyl (TMSi) derivatives of the acid hydrolysate. The nature of the monosaccharides and the sequence of the oligosaccharide chains were determined by a combination of ^1H–^1H correlated spectroscopy (COSY), homonuclear Hartmann-Hahn (HOHAHA), DEPT, ^1H–^{13}C heteronuclear correlation (HETCOR), heteronuclear multiple-bond connectivity (HMBC), and phase-sensitive nuclear Overhauser enhancement (NOESY) experiments. Starting from the anomeric protons of each sugar unit, all the hydrogens within each spin system were

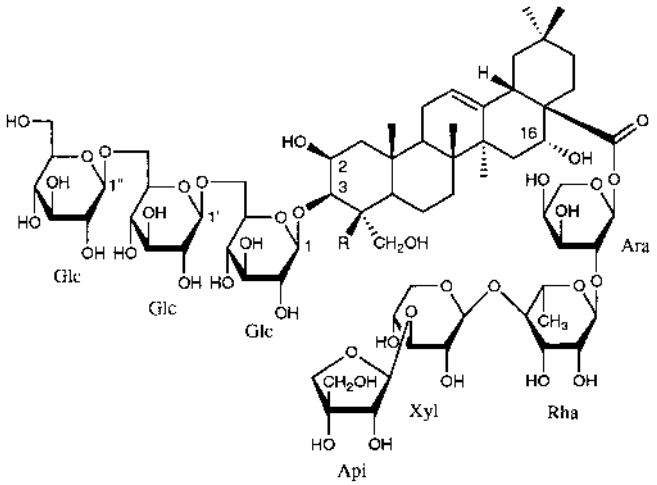

Chart 1

Table 1. ^{13}C NMR Data for Platycosides D (**1**)[a] and E (**2**)[b] in Pyridine-d_5

Carbon	1	2	Carbon	1	2
1	44.1	45.2	Glucose (center)		
2	69.4	68.6	1′	105.1	104.9
3	84.3	88.8	2′	75.2	75.3
4	42.8	48.1	3′	78.5	78.4
5	47.7	47.5	4′	71.5	71.3
6	18.4	19.5	5′	77.0	77.1
7	33.2	33.5	6′	70.1	70.2
8	40.2	40.5	Glucose (terminal)		
9	47.6	45.0	1″	105.2	105.6
10	37.0	37.9	2″	74.8	75.2
11	24.0	24.0	3″	78.6	78.6
12	123.1	123.1	4″	70.8	71.0
13	144.3	144.3	5″	77.7	77.7
14	42.2	42.4	6″	62.6	62.7
15	36.1	36.1	Arabinose		
16	74.1	73.9	1	93.5	93.6
17	49.6	49.7	2	75.3	75.2
18	41.3	41.6	3	71.4	71.3
19	47.1	47.1	4	66.0	66.4
20	30.9	30.9	5	62.9	63.1
21	36.0	36.0	Rhamnose		
22	32.2	32.1	1	101.1	101.1
23	66.1	63.6	2	72.1	71.9
24	15.3	67.1	3	72.7	72.7
25	17.6	19.1	4	83.7	83.9
26	17.5	17.6	5	68.6	68.5
27	27.2	27.0	6	18.4	18.3
28	176.0	176.0	Xylose		
29	33.3	33.2	1	106.7	106.8
30	24.8	24.7	2	75.2	76.0
Glucose (inner)			3	84.7	84.8
1	105.5	106.0	4	69.7	69.4
2	74.7	74.8	5	67.0	66.9
3	78.4	78.3	Apiose		
4	71.9	72.3	1	111.2	111.2
5	76.7	76.5	2	78.1	77.8
6	70.6	70.8	3	80.4	80.4
			4	75.2	75.2
			5	65.4	65.4

a) 100 MHz. *b*) 125 MHz.

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delineated using COSY with the aid of two dimensional (2D)-HOHAHA and NOESY spectra. On the basis of the assigned protons, the ^{13}C resonances of each sugar unit were identified by HETCOR and further confirmed by HMBC spectra. The HMBC correlations revealed two oligosaccharide sugar linkages as follows: from H-1 of a terminal glucose at δ 4.89 (d, J=7.7 Hz) to C-6 of center glucose at δ 70.1, from H-1 of center glucose at δ 4.76 (d, J=8.2 Hz) to C-6 of an inner glucose at δ 70.6, from H-1 of an inner glucose at δ 5.08 (d, J=7.6 Hz) to C-3 of sapogenin at δ 84.3 and from H-1 of terminal apiose at δ 6.25 (br s) to C-3 of xylose at δ 84.7, from H-1 of xylose at δ 5.08 (d, J=6.9 Hz) to C-4 of rhamnose at δ 83.7, from H-1 of rhamnose at δ 5.75 (br s) to C-2 of arabinose at δ 75.3, and from H-1 of arabinose at δ 6.49 (br s) to C-28 of the sapogenin at δ 176.0. Thus, platycoside D (**1**) was identified to be 3-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-2β,3β,16α,23-tetrahydroxyolean-12-ene-28-oic acid 28-*O*-β-D-apiofuranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside.

Platycoside E (**2**), an amorphous powder, $[\alpha]_D$ −26.9°, possessed the molecular formula $C_{69}H_{112}O_{38}$, as determined by negative ESI-MS (at *m/z* 1547 $[M-H]^-$) and from ^{13}C and DEPT NMR spectral data. On acid hydrolysis, **2** gave the same component sugars as **1**. The sapogenin obtained by the acid hydrolysis of **2** was identified as 2β,3β,16α,23,24-pentahydroxyolean-12-ene-28-oic acid.[1] The ^1H- and ^{13}C-NMR spectral data indicated that **2** had the same oligosaccharide moieties at C-3 and C-28. Thus, platycoside E (**2**) was identified to be 3-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-2β,3β,16α,23,24-pentahydroxyolean-12-ene-28-oic acid 28-*O*-β-D-apiofuranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside.

Experimental

IR spectra were determined using a JASCO 7300 FT-IR spectrometer. Optical rotations were measured using a JASCO DIP-370 digital polarimeter. ESI-MS were conducted using a Finnigan LCQ mass spectrometer. ^1H-NMR and ^{13}C-NMR spectra were recorded using a JEOL EX-400 (^1H at 400 MHz, ^{13}C at 100 MHz) or a JEOL α-500 (^1H at 500 MHz, ^{13}C at 125 MHz) NMR spectrometer. Chemical shifts are expressed in δ (ppm) downfield from tetramethylsilane as an internal standard. Diaion HP-20 (Mitsubishi Chemical) and silica gel (Silica gel 60, Merck) were used for column chromatography. Preparative HPLC was performed using an octadecyl silica (ODS) column (PEGASIL ODS, Senshu Pak, 10 mm i.d.×250 mm), detector: UV 210 nm. GLC: Shimadzu GC-7A. Column: Silicone OV-17 on Uniport HP 2%, 3 mm i.d.×2.1 m column temperature, initial 140 °C for 16 min and rising 2 °C/min to final 170 °C; carrier gas, N_2, flow rate, 25 ml/min.

Extraction and Isolation The roots of *Platycodon grandiflorum* (2.25 kg) were extracted with MeOH and the extract was evaporated to give a residue (469 g), which was partitioned between *n*-BuOH and water. The *n*-BuOH layer was evaporated to provide a residue (161 g), which was then chromatographed using Diaion HP-20 (solvent, MeOH : H_2O=40 : 60→MeOH). The fraction (20 g), obtained by elution with 60% MeOH,[1] was subjected to column chromatography on silica gel (solvent, $CHCl_3$: MeOH : H_2O=30 : 10 : 1→6 : 4 : 1) to give a crude saponin fraction. Further purification by medium pressure LC (Lichroprep RP-18 (Merck), solvent MeOH : H_2O=7 : 3, detector UV 210 nm) and finally by HPLC (MeOH : H_2O=65 : 35), afforded platycosides D (**1**, 8 mg) and E (**2**, 110 mg).

Platycoside D (**1**) : White amorphous powder, $[\alpha]_D^{24}$ −17.9° (c=0.16, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3402, 2927, 1739, 1637,1038. ESI-MS (negative ion mode): *m/z* 1531 $[M-H]^-$. ^1H-NMR (400 MHz, pyridine-d_5) δ: 1.01, 1.15, 1.17, 1.36, 1.55. 1.73 (each 3H, s, CH_3 of C-29, C-26, C-30, C-25, C-24, C-27), 1.73 (3H, d, J=6.1 Hz, CH_3 of Rha), 3.92, 4.51 (each 1H, m, H-23), 4.53 (1H, m, H-3), 4.55 (1H, m, H-2), 4.76 (1H, d, J=8.2 Hz, H-1 of center Glc), 4.89 (1H, d, J=7.7 Hz, H-1 of terminal Glc), 5.08 (1H, d, J=7.6 Hz, H-1 of inner Glc), 5.08 (1H, d, J=6.9 Hz, H-1 of Xyl), 5.25 (1H, br s, H-16), 5.56 (1H, br s, H-12), 5.75 (1H, br s, H-1 of Rha), 6.25 (1H, br s, H-1 of Api), 6.49 (1H, br s, H-1 of Ara). ^{13}C NMR data: Table 1.

Platycoside E (**2**): Amorphous powder, $[\alpha]_D^{26}$ −26.9° (c=0.20, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400, 2927, 1739, 1655, 1038. ESI-MS (negative ion mode): *m/z* 1547 $[M-H]^-$. ^1H-NMR (500 MHz, pyridine-d_5) δ: 1.02, 1.09, 1.13, 1.38, 1.70 (each 3H, s, CH_3 of C-29, C-26, C-30, C-25, C-27), 1.69 (1H, d, J=6.1 Hz, CH_3-6 of Rha), 3.91, 4.50 (each 1H, m, H-23), 4.07, 4.55 (each 1H, m, H-24), 4.54 (1H, m, H-3), 4.56 (1H, m, H-2), 4.76 (1H, d, J=8.2 Hz, H-1 of center Glc). 4.85 (1H, d, J=7.3 Hz, H-1 of inner Glc), 5.05 (1H, d, J=7.6 Hz, H-1 of terminal Glc), 5.05 (1H, d, J=6.9 Hz, H-1 of Xyl), 5.24 (1H, br s, H-16), 5.65 (1H, br s, H-12), 5.80 (1H, br s, H-1 of Rha), 6.20 (1H, br s, H-1 of Api), 6.41 (1H, br s, H-1 of Ara). ^{13}C-NMR data: Table 1.

Acid Hydrolysis Platycoside D (**1**, 3 mg) was heated in 1 ml of 1 M HCl (dioxane–H_2O, 1 : 1) at 80 °C for 3 h on a water bath. Dioxane was removed and the solution was extracted with EtOAc (1 ml×3 times). The monosaccharide portion was neutralized by passing it through an ion-exchange resin (Amberlite MB-3) column, and was then concentrated and treated with 1-trimethylsilylimidazole at room temperature for 1 h. After excess reagent was decomposed with water, the reaction product was extracted with *n*-hexane (1 ml×3 times). The TMSi derivatives of the monosaccharides were identified as glucose, arabinose, rhamnose, xylose, and apiose in a ratio of 3 : 1 : 1 : 1 : 1 by GLC. By the same method, platycoside E (**2**, 30 mg) afforded platycodigenin[1] and the sugars in **2** were identified to be glucose, arabinose, rhamnose, xylose, and apiose (3 : 1 : 1 : 1 : 1).

References

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