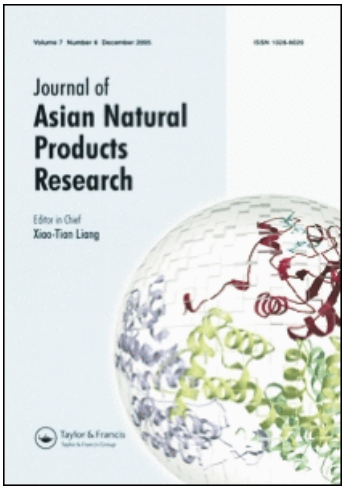

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Triterpenoid saponins from *Platycodon grandiflurum*

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A new bisdesmosidic saponin, named deapio-platycoside E (**1**), together with two known triterpenoid saponins (**2, 3**) were isolated from the roots of *Platycodon grandiflorum* (Jacq.) A. D.C. Their structures were elucidated by spectroscopic and chemical methods.

Keywords: *Platycodon grandiflorum*; Triterpenoid saponin; Deapio-platycoside E

1. Introduction

The roots of *Platycodon grandiflorum* (Jacq.) A. D.C. have been used widely in Chinese and Japanese traditional medicines as an antiphlogistic, antitussivic and expectorant agents [1]. Up to now more than 20 triterpenoid saponins have been isolated from the roots of the plant [2,3,5]. This paper deals with the isolation and the structural determination of three saponins from the roots of *P. grandiflorum*. Their structures were elucidated on the basis of their spectral data and chemical evidence as 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-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranoside (**1**), 3-*O*-β-D-glucopyranosyl-2β,3β,16α,23,24-pentahydroxyolean-12-ene-28-oic acid 28-*O*-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranoside (deapio-platycodin D) (**2**), and 3-*O*-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 (polygalacin D) (**3**) (figure 1).

Among them, **1** is a new compound, named deapio-platycoside E. The ^{1}H NMR and ^{13}C NMR spectral data of **2** and **3** in CD_3OD is first reported. In addition, prosapogenin (**1b**) afforded by alkaline hydrolysis of **1** is also a new compound.

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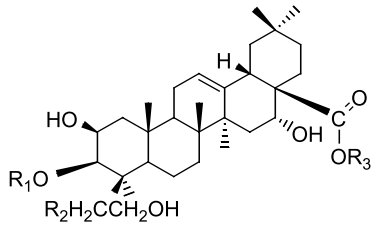

Figure 1. The structure of compounds **1–3**.

2. Results and discussion

Compound **1** was obtained as amorphous powder. $[\alpha]_D^{25}$ −15.05 (*c* 1.20 MeOH). The molecular formula of **1** was determined as $C_{64}H_{104}O_{34}$ by positive HRFAB-MS. The IR spectrum indicated the presence of a hydroxyl band at 3420 cm^{-1}, an olefinic band at 1634.9 cm^{-1} and esteric bands at 1738 and 1237 cm^{-1}. The ^{1}H NMR spectrum showed the signals at δ 0.77, 0.88, 0.97, 1.24 and 1.38, one trisubstituted olefinic proton signal at δ 5.38 (br s), and six anomeric proton signals at δ 4.30 (d, $J = 8.0$ Hz), 4.38 (d, $J = 7.5$ Hz) (2H), 4.51 (d, $J = 7.0$ Hz), 5.04 (br s), 5.61 (br s). The ^{13}C NMR and HMQC spectroscopic data revealed the presence of six quaternary carbons at δ 31.4, 38.3, 41.2, 43.0, 48.4 and 50.4, a pair of olefinic carbons at δ 123.9 and 144.7, six anomeric carbons at δ 94.0, 101.3, 104.8, 104.9, 105.6, 106.6, and an ester carbonyl at δ 177.1. The above data suggested that **1** contained six sugars and a triterpene aglycone. Acid hydrolysis of **1** afforded platycodigenin (**1a**), which was characterised as 2β,3β,16α,23,24-pentahydroxyolean-12-ene-28-oic acid by comparison of its physical and spectral properties with that reported in the literature [2]. The ^{13}C NMR signals due to the aglycone of **1** are identical to those of 3-*O*-β-D-glucopyranosyl-2β,3β,16α,23,24-pentahydroxyolean-12-ene-28-oic acid 28-*O*-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranoside (deapio-platycodin D) (**2**) [3], indicating that **1** is a bisdesmoside of platycodigenin with the same glycosidation sites at C-3 (δ 87.6) and C-28 (δ 177.1) as deapio-platycodin D (**2**) [3]. The sugar moieties (glucose, arabinose, rhamnose, xylose) were confirmed by PC with authentic samples after acid hydrolysis. The nature of the monosaccharides and the sequence of the oligosaccharide chains were determined by a combination of ^{1}H–^{1}H COSY, HOHAHA, ^{13}C–^{1}H COSY, HMBC and TOCSY experiments. Thus, **1** was characterised as a bisdesmosidic saponin, with one triglycoside moiety at C-3 of the aglycone via glycosidic linkage and another triglycoside moiety at C-28 of the aglycone through ester bond. Starting from the anomeric protons of each sugar unit, all the protons within each spin system were delineated using different COSY spectra. On the basis of the assigned protons, the ^{13}C resonances of each sugar unit were identified by ^{13}C–^{1}H COSY and further confirmed by HMBC spectra. The HMBC correlations (figure 2) revealed two oligosaccharide sugar linkages as follows: from H-1″ of glucose at δ 4.38 (d, $J = 7.5$ Hz) to C-6′ of glucose at δ 70.2, from H-1′ of glucose at δ 4.30 (d, $J = 8.0$ Hz) to C-6 of glucose at δ 70.8, from H-1 of glucose to C-3 of sapogenin at δ 87.6 and from H-1 of terminal xylose at δ 4.51 (d, $J = 7.0$) to C-4 of rhamnose at δ 83.4, from H-1 of rhamnose at δ 5.04 (br s) to C-2 of arabinose at δ 75.6, and from H-1 of arabinose at δ 5.61 (br s) to C-28 of the sapogenin at δ 177.1. All the monosaccharides were determined to be pyranose form from their ^{13}C NMR data. The β-anomeric configurations of the three glucoses and xylose were determined by the ^{13}CNMR

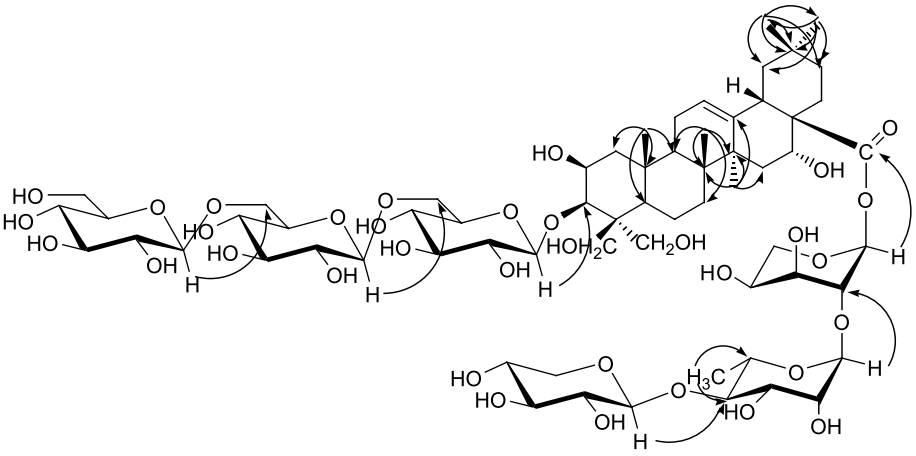

Figure 2. The key HMBC correlations of compound **1**.

data at δ 105.6, 104.9, 104.8 and 106.6 and the coupling constants of the anomeric protons δ 4.31 (1H, d, $J = 8.0$ Hz), 4.38 (2H, d, $J = 7.5$ Hz) and 4.51 (1H, d, $J = 7.0$ Hz). The broad singlet for H-1, ^{13}C chemical shifts at δ 94.0 and 101.3 of C-1 of arabinose and rhamnose indicated its glycosidic linkage presented α-configuration; the coupling constant of arabinose could be explained by the high conformational mobility of the arabinopyranosides between $^4C_1$ and $^1C_4$ with the predominant $^1C_4$ conformation [4]. In addition, alkaline hydrolysis of **1** afforded prosapogenin (**1b**), which was characterised as 3-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosylplatycodigenin on the basis of its spectral data and chemical evidence. Comparing the ^{13}C NMR data of glucose moieties in **1b** with those in the literature [3], the former shifted downfield at C-6 ($\Delta\delta + 8.4$) of glucose and C-6′ ($\Delta\delta + 7.2$) of glucose, indicating that C-6 of inner and centre glucose are glycosidated; the ^{13}C NMR data due to the sugar moiety of **1b** agreed well with those of sugar chain linked to C-3 of platycoside E [5]. Comparison of the ^{13}C NMR spectra of **1** and **1b** reveals that the signals due to the sugar chain linked to C-28 of the aglycone are identical to those of saponin **2** [3]. Thus, **1** 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-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranoside, named deapio-platycoside E.

Saponins **2** and **3** were identified by spectral data and by comparison of their physical and spectral properties with those reported in the literature [3].

3. Experimental

3.1 General experimental procedures

Optical rotations were performed with a Perkin–Elmer 241MC polarimeter. FAB-MS and HRFAB-MS spectra were recorded on a JEOL JMS-SX 102A mass spectrometer. IR spectra were measured with a Bruker IFS-55 infrared spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded with a JEOL α500 NMR spectrometer. Chemical shifts were reported in parts per million on the scale with TMS as an internal standard. Silica gel (Qingdao Haiyang

Chemical Co. Ltd., 200–300 mesh) and Lichroprep RP-18 (Merck) were used for silica gel column chromatography and MPLC. Preparative HPLC was performed using an octadecyl silica (ODS) column (Pegasil ODS, Senshu Pak, 10 mm i.d. × 250 mm) on a Hitachi LC system with a RI detector. Spots were visualised by spraying with ethanol/10% H_2SO_4 and heating (110°C, 5 min).

3.2 Plant material

The roots of *Platycodon grandiflorum* were collected from Shenyang city of Liaoning Province in China in 2001, and were taxonomically identified by Professor Sun Qishi of Shenyang Pharmaceutical University. A voucher specimen is deposited at the School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University.

3.3 Extraction and isolation

The air-dried roots (10 kg) were extracted with 75% ethanol for three times under reflux. The combined solutions were concentrated *in vacuo*, and subjected to macroporous resin D101 column chromatography, eluting with 60% EtOH. The solution was evaporated to dryness under vacuum to obtain a residue (180 g). The residue was chromatographed on silica gel with $CHCl_3$/MeOH (in gradient) to give six fractions. Fraction 2 (27.3 g) was further separated by silica gel column chromatography with a solvent system of EtOAC/EtOH/H_2O (8:2:1–15:5:4) to obtained six fractions. Fraction 2-6 (4.0 g) was repeatedly chromatographed on silica gel with $CHCl_3$/MeOH/H_2O (6:4:1) and separated with MPLC and preparative RP-18 HPLC (MeOH/H_2O 3: 2) to yield **1** (266.5 mg). Fraction 2-4 (4.0 g) separated with MPLC and preparative RP-18 HPLC (MeOH/H_2O, 55: 45) to give **2** (133.7 mg); Fraction 2-2 (1.6 g) was repeatedly chromatographed on silica gel with EtOAC/EtOH/H_2O (9:1:0.5–85:15:7.5) to obtained eight fractions. Fraction 2-2-4 (0.8 g) was separated with MPLC and preparative RP-18 HPLC (MeOH/H_2O 1:1) to yield **3** (5.1 mg).

3.3.1 Saponin 1. White amorphous powder; $[\alpha]_D^{25}$ −15.6 (MeOH, *c* 1.2); IR (KBr) cm^{-1}: 3420, 2925.4, 1738, 1634.9, 1237, 1043.1, 611.3; FAB^+-MS *m/z*:1439.8 $[M + Na]^+$, 1417.8 $[M + H]^+$; HRFAB-MS *m/z*: 1439.6260 $[M + Na]^+$ (calcd for $C_{64}H_{104}O_{34}Na$, 1439.6307); ^1H NMR (500 MHz, CD_3OD) δ: 0.77, 0.88, 0.97, 1.24, 1.38 (each 3H, s, CH_3 of C-26, C-29, C-30, C-25, C-27), 1.29 (3H, d, $J = 6.5$ Hz, CH_3 of Rha), 3.20, 3.83 (each 1H, m, H-24), 3.51, 4.26 (each 1H, m, H-23), 3.86 (1H, m, H-3), 4.11 (1H, m, H-2), 4.31 (1H, d, $J = 8.0$ Hz, H-1′ of Glc), 4.38 (2H, d, $J = 7.5$ Hz, H-1 of Glc), 4.48 (1H, br s, H-16), 4.51 (1H, d, $J = 7.0$ Hz, H-of Xyl), 5.04 (1H, br s, H-1 of Rha), 5.34 (1H, br s, H-12), 5.61 (1H, br s, H-1 of Ara). ^{13}C NMR data: see table 1.

3.3.2 Saponin 2. White amorphous powder; ^1H NMR (500 MHz, CD_3OD) δ: 0.77, 0.88, 0.97, 1.25, 1.37 (each 3H, s, CH_3 of C-26, C-29, C-30, C-25, C-27), 1.29 (3H, d, $J = 6.0$ Hz, CH_3 of Rha), 3.17, 3.85 (each 1H, m, H-24), 3.98, 4.33 (each 1H, m, H-23), 3.84 (1H, m, H-3), 4.18 (1H, m, H-2), 4.41 (1H, d, $J = 7.5$ Hz, H-1 of Glc), 4.49 (1H, br s, H-16), 4.52 (1H, d, $J = 7.0$ Hz, H-1 of Xyl), 5.04 (1H, br s, H-1 of Rha), 5.38 (1H, br s, H-12),

Table 1. ^{13}C NMR (125 MHz) spectral data for compounds **1** and **1b**.

No.	***1***[a]	***1b***[b]	*No.*	***1***[a]	***1b***[b]	*No.*	***1***[a]	***1b***[b]
1	45.1	45.2	25	18.9	19.0	4″	70.0	71.3
2	69.8	70.2	26	17.9	17.6	5″	77.5	77.7
3	87.6	87.5	27	27.3	27.1	6″	62.7	62.7
4	48.4	48.1	28	177.1	180.0	Ara		
5	48.4	48.1	29	33.4	33.3	1	94.0	
6	19.8	19.8	30	25.1	24.7	2	75.6	
7	34.1	33.6	Glc (inner)		3	70.0		
8	41.2	40.4	1	105.6	106.1	4	67.3	
9	48.4	47.5	2	75.0	74.8	5	63.6	
10	38.3	38.0	3	77.9	78.4	Rha		
11	24.7	24.0	4	72.2	72.3	1	101.3	
12	123.9	122.8	5	76.7	76.5	2	72.2	
13	144.7	145.0	6	71.1	70.8	3	72.4	
14	43.0	42.5	Glc (centre)		4	83.4		
15	36.3	36.0	1′	104.8	104.9	5	69.0	
16	74.6	74.7	2′	75.1	75.4	6	18.1	
17	50.4	49.0	3′	78.1	78.5	Xyl		
18	42.3	41.7	4′	71.6	71.6	1	106.6	
19	47.6	47.3	5′	77.0	77.1	2	76.0	
20	31.4	31.1	6′	70.0	70.2	3	78.1	
21	36.4	36.2	Glc (terminal)		4	71.1		
22	31.9	32.8	1″	104.9	105.7	5	67.1	
23	63.7	63.1	2″	75.0	75.2			
24	67.3	68.7	3″	78.3	78.7			

[a]In CD_3OD.
[b]In pyridine-d_5.

5.62 (1H, br s, H-1 of Ara). ^{13}C NMR (125 MHz, CD3OD) δ: 17.9 (C-26), 18.1 (Rha C-6), 18.3 (C-25), 19.7 (C-6), 24.8 (C-11), 25.1 (C-30), 27.3 (C-27), 31.4 (C-20), 31.9 (C-22), 33.4 (C-29), 34.1 (C-7), 36.3 (C-15), 36.4 (C-21), 37.9 (C-10), 41.0 (C-8), 42.2 (C-18), 43.0 (C-14), 45.0 (C-1), 47.6 (C-4), 47.6 (C-19), 48.3 (C-5), 48.3 (C-9), 50.4 (C-17), 62.4 (Glc C-6), 63.7 (Ara C-5), 63.8 (C-23), 67.0 (C-24), 67.3 (Xyl C-5), 67.5 (Ara C-4), 69.6 (Rha C-4), 70.5 (C-2), 71.2 (Xyl C-4), 71.3 (Ara C-3), 72.1 (Glc C-4), 72.1 (Rha C-2), 72.4 (Rha C-3), 74.6 (C-16), 75.2 (Glc C-2), 76.3 (Xyl C-2), 77.9 (Glc C-5), 78.1 (Xyl C-3), 78.3 (Glc C-3), 83.4 (Rha C-4), 86.5 (C-3), 94.2 (Ara C-1), 101.3 (Rha C-1), 105.9 (Glc C-1), 106.2 (Xyl C-1), 123.9 (C-12), 144.7 (C-13), 177.1 (C-28).

3.3.3 Saponin 3. White amorphous powder; ^{1}H NMR (500 MHz, CD_3OD) δ: 0.79, 0.88, 0.96, 0.97, 1.30, 1.38 (each 3H, s, CH_3 of C-26, C-29, C-24, C-30, C-25, C-27), 1.29 (3H, d, $J = 6.0$ Hz, CH_3 of Rha), 3.27, 3.63 (each 1H, m, H-23), 3.61 (1H, m, H-3), 4.33 (1H, m, H-2), 4.44 (1H, d, $J = 9.0$ Hz, H-1 of Glc), 4.49 (1H, br s, H-16), 4.54 (1H, d, $J = 6.5$ Hz, H-of Xyl), 5.06 (1H, br s, H-1 of Rha), 5.24 (1H, d, $J = 3.0$ Hz), 5.37 (1H, br s, H-12), 5.61 (1H, d, $J = 4.0$ Hz, H-1 of Ara). ^{13}C NMR (125 MHz, CD3OD) δ: 14.8 (C-24), 17.7 (C-26), 18.0 (Rha C-6), 18.2 (C-25), 18.8 (C-6), 24.7 (C-11), 25.1 (C-30), 27.4 (C-27), 31.4 (C-20), 32.0 (C-22), 33.4 (C-29), 33.7 (C-7), 36.4 (C-15), 36.5 (C-21), 37.6 (C-10), 40.9 (C-8), 42.1 (C-18), 43.0 (C-14), 43.2 (C-4), 44.5 (C-1), 47.7 (C-19), 48.5 (C-5), 48.5 (C-9), 50.4 (C-17), 62.3 (Glc C-6), 64.0 (Ara C-5), 65.1 (Api C-5), 65.8 (C-23), 66.8 (Ara C-4), 69.0 (Rha C-5), 70.0 (Xyl C-4), 71.2 (C-2), 71.2 (Ara C-3), 71.5 (Glc C-4), 72.1 (Rha C-2), 72.4 (Rha C-3), 74.6 (C-16), 75.1 (Xyl C-2), 75.4 (Glc C-2), 75.7 (Ara C-2), 75.7 (Api C-4), 77.8 (Api C-2), 77.9 (Glc C-5), 78.2 (Glc C-3), 80.6 (Api C-3), 83.7 (Rha C-4), 84.0 (C-3), 86.5 (Xyl C-3),

94.1 (Ara C-1), 101.4 (Rha C-1), 105.5 (Glc C-1), 106.6 (Xyl C-1), 111.3 (Api C-1), 123.8 (C-12), 144.8 (C-13), 177.1 (C-28).

3.4 Acid hydrolysis of 1

A solution of **1** (100 mg) in 7% H_2SO_4 alcohol/H_2O (15 ml) was heated at 100°C for 4 h. The alcohol was evaporated thoroughly *in vacuo,* and the remaining aqueous layer was extracted with EtOAc(10 ml × 3). The EtOAc layer was washed with H_2O (2 ml) and the solvent was distilled off to give a white powder (43 mg), which was purified by silica gel column chromatography (solvent system: $CHCl_3$/MeOH (3:2)) to give platycodigenin (**1a**, 18 mg), which is characterised on the basis of its physical and spectral properties [2]. The aqueous layer was neutralised to pH 7 with saturated $Ba(OH)_2$ solution. After filtration and concentration of the filtrate, glucose, xylose, rhamnose and arabinose were detected by PC.

3.5 Alkaline hydrolysis of 1

Compound **1** (100 mg) was heated in 20 ml of 0.8 M NaOH at 100°C for 3 h. After cooling, the reaction mixture was neutralised with 1 M HCl and then extracted with *n*-BuOH (10 ml × 3). The *n*-BuOH layer was washed with H_2O (10 ml), then the solvent was distilled off to give powder (61 mg) which was purified by silica gel CC (solvent system $CHCl_3$/MeOH/H_2O (6:4:1)) to give prosapogenin (**1b**, 36 mg). The aqueous layer was concentrated and then further hydrolysed with acid. Arabinose, rhamnose and xylose were detected from the resulting solution by PC.

3.6 Prosapogenin 1b

White amorphous powder. IR (KBr) cm^{-1}: 3401.8, 2925.9, 1694.1, 1382.4, 1053.9, 604.6; FAB^+-MS *m/z*: 1030 $[M + Na]^+$; HRFAB-MS *m/z*: 1029.4910 $[M + Na]^+$ (calcd for $C_{48}H_{78}O_{22}Na$, 1029.5240); ^1H NMR (pyridine-d_5, 500 MHz) δ: 1.05, 1.07, 1.19, 1.43, 1.76 (each 3H, s, CH_3 of C-29, C-26, C-24, C-30, C-25, C-27), 4.53 (1H, m, H-3), 4.60 (1H, m, H-2), 4.79 (1H, d, $J = 7.5$, H-1 of centre Glc), 4.89 (1H, d, $J = 8.0$, H-1 of inner Glc), 5.07 (1H, d, $J = 7.0$, H-1 of terminal Glc), 5.24 (1H, br s, 16H), 5.67 (1H, br s, 12-H). Its ^{13}C NMR data are shown in table 1.

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