New A-Ring Lactone Triterpenoid Saponins from the Roots of *Platycodon grandiflorum*

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Three new A-ring lactone triterpenoid saponins, platycoside M-1 [3-*O*-*β*-D-glucopyranosyl platycogenic acid A lactone], platycoside M-2 [3-*O*-*β*-D-glucopyranosyl platycogenic acid A lactone 28-*O*-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranoside], and platycoside M-3 [3-*O*-*β*-D-glucopyranosyl platycogenic acid A lactone 28-*O*-*β*-D-xylopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranoside], were isolated from the roots of *Platycodon grandiflorum* A. DC. Their chemical structures were elucidated on the basis of their spectral data and chemical evidence.

Key words *Platycodon grandiflorum*; saponin; platycogenic acid A lactone; platycoside M-1; platycoside M-2; platycoside M-3

Platycodi Radix, the roots of *Platycodon grandiflorum* A. DC. (Campanulaceae), has been used in traditional Oriental medicine as an expectorant and antitussive to treat coughs, colds, upper respiratory tract infections, sore throats, tonsillitis, and chest congestion.[1,2] In northern China and Korea, P. radix is also used as a food. Chemical investigation of P. radix revealed that triterpenoid saponins[3—7] were the main chemical components. In our previous papers, we reported the isolation and structural elucidation of sixteen triterpenoid saponins, including five new triterpenoid saponins, from the roots of *P. grandiflorum* A. DC.[8,9] Further investigation on the polar fractions of *P. grandiflorum* led to the isolation of three new triterpenoid saponins, platycosides M 1—3 (**1**—**3**). Platycosides M-1—3 seem to be unusual by containing *γ*-lactone in A-ring of pentacyclic triterpenoid saponins, which had been isolated only in the plant and reported in a few literature.[3,7] This paper reports the isolation and structure elucidation of compounds **1**—**3** by extensive NMR studies and chemical degradations.

Results and Discussion

The 75% EtOH extract from the roots of *Platycodon grandiflorum* were partitioned with aqueous EtOAc. The aqueous layer was separated by a macroreticular resin column to give the 60% EtOH eluates that upon drying afforded the total saponins. The total saponins were chromatographed on silica gel, a reversephase column, and finally on HPLC to afford three new compounds **1**—**3**.

Platycoside M-1 (**1**) was a white amorphous powder, and its molecular formula was assigned to be $C_{36}H_{54}O_{12}$ based on the high-resolution (HR)-FAB-MS spectrum. The spectral features and physicochemical properties revealed **1** to be a triterpenoid saponin. The IR spectrum exhibited absorptions at 3425 cm^{-1} (OH), 1758 cm^{-1} (*γ*-lactone carbonyl), and 1702 cm^{-1} (carbonyl). The five tertiary methyl groups (δ 1.02, 1.05, 1.18, 1.33, 1.75 ppm) and one trisubstituted olefinic proton (δ 5.62, br s) were observed in the ^{1}H-NMR spectrum. The ^{13}C-NMR spectrum showed five sp^3 carbons at δ 17.3, 18.0, 24.7, 27.3, and 33.3, two sp^2 olefinic carbons at δ 121.8 and 145.8, and four oxygenated methylene and methine carbons at δ 57.1, 74.6, 83.1, and 89.0 ppm (Table 2). The information on the ^{1}H-NMR spectrum coupled with the ^{13}C-NMR spectrum indicated that **1** has an olean-12-en skeleton. A 2D NMR study revealed that the aglycon was platycogenic acid A lactone (3*β*,16*α*,23-trihydroxyolean-12-en-2,24-lactone-28-oic acid).[7] The C-24 ester (or lactone) carbonyl group (δ_C 178.2) was connected to the C-4 quaternary carbon (δ_C 54.1), and the low-field resonances of the oxymethine proton and carbon on C-2 (δ_H 5.20, δ_C 83.1) and the correlation between H-2 and C-24 carbonyl group on the basis of HMBC spectrum implied that the C-2 oxymethine was connected with the C-24 carbonyl group to form *γ*-lactone ring. Until now, this kind of *γ*-lactone ring formation at ring A has been reported in a prosaponin isolated from the same plant only[3,7] (see Fig. 1). The ^{1}H- and ^{13}C-NMR spectra of **1** exhibited only one sugar anomeric proton at δ 5.35 (d, J=7.0 Hz), and carbon at δ 105.2 (see Tables 1, 2). Acid hydrolysis of **1** gave glucose only, which was analyzed by gas chromatography as glucitol acetate. The sugar unit was identified as one *β*-D-glucopyranose unit by its $^3J_{H1,H2}$ coupling constants (7.0 Hz) combined with its ^{1}H- and ^{13}C-NMR data (see Tables 1, 2). The sugar linkages were determined on the basis of the HMBC spectrum which showed correlation between a proton signal at δ 5.35 (glc-H-1) and a carbon signal at δ 89.0 due to C-3 of the aglycone moiety (see Fig. 1). On the basis of all the foregoing evidence, Platycoside M-1 (**1**) was identified to be 3-*O*-*β*-D-glucopyranosyl platycogenic acid A lactone, which had been reported as the prosapogenin methyl esters.[7]

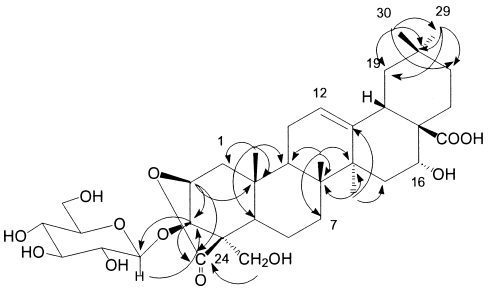

Fig. 1. The Structure and Selected HMBC Correlations of Platycoside M-1 (from H to C)

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Table 1. ^{1}H-NMR Spectroscopic Data (δ) for the Sugar Moieties of **1**—**3** (600 MHz in Pyridine-d_5)

H	**1**	**2**	**3**
C_3-Glc			
1	5.35 d (7.0)	5.33 d (7.0)	5.34 d (8.0)
2	4.05 t-like	4.04 t-like	4.02—4.06 m
3	3.92—3.96 m	3.90—4.00 m	3.92—3.98 m
4	4.25 t-like	4.15—4.30 m	4.15—4.25 m
5	4.19 t-like	4.15—4.25 m	4.15—4.25 m
6	4.36—4.40 m	4.34—4.40 m	4.25—4.35 m
	4.54 br d (10.5)	4.50—4.55 m	4.40—4.50 m
C_{28}-Ara			
1		6.52 br s	6.46 d (3.0)
2		4.50—4.60 m	4.50—4.60 m
3		4.50—4.60 m	4.50—4.55 m
4		4.40—4.50 m	4.35—4.45 m
5		3.90—4.00 m	3.92—3.98 m
		4.50—4.55 m	4.50—4.55 m
Rha			
1		5.81 br s	5.80 br s
2		4.50—4.60 m	4.50—4.61 m
3		4.50—4.60 m	4.50—4.61 m
4		4.22—4.32 m	4.35—4.45 m
5		4.54—4.60 m	4.35—4.45 m
6		1.68 d (6.5)	1.74 d (5.5)
Xyl			
1			5.17 d (8.0)
2			4.02—4.07 m
3			4.02—4.07 m
4			4.06—4.11 m
5			3.50 t-like
			4.15—4.25 m

The assignments are based upon DEPT, HMQC, and HMBC experiments. Overlapped signals are labeled with multiplicity (m). Coupling constants (J values in Hz) are shown in parentheses.

Table 2. ^{13}C-NMR Spectroscopic Data (δ) for the Compounds **1**—**3** (150 MHz in Pyridine-d_5)[a)]

Carbon	**1**	**2**	**3**	Carbon	**1**	**2**	**3**
1	41.0	41.1	41.4	Glc-			
2	83.1	83.1	82.8	1	105.2	105.2	105.4
3	89.0	89.1	89.8	2	75.3	75.3	75.5
4	54.1	54.0	53.9	3	78.7	78.7	78.9
5	51.7	51.8	52.0	4	71.4	71.4	71.6
6	19.3	19.3	19.4	5	78.4	78.4	78.8
7	33.4	33.4	33.5	6	62.6	62.6	62.8
8	40.2	40.4	40.6	Ara-			
9	48.1	48.1	48.3	1		93.6	93.8
10	37.6	37.5	37.8	2		75.2	75.4
11	24.5	24.5	24.6	3		70.1	70.5
12	121.8	122.1	122.1	4		66.1	66.4
13	145.8	145.1	145.1	5		63.1	63.5
14	42.3	42.3	42.3	Rha-			
15	36.0	36.0	36.2	1		101.5	101.4
16	74.6	73.9	74.1	2		72.4	72.2
17	48.8	49.5	49.4	3		72.6	72.9
18	41.5	41.3	41.5	4		73.8	84.0
19	47.2	47.1	47.0	5		70.5	68.8
20	31.0	30.9	30.8	6		18.6	18.6
21	36.2	36.0	36.2	Xyl-			
22	32.9	32.0	32.0	1			107.1
23	57.1	57.1	57.4	2			76.2
24	178.2	178.2	177.6	3			78.6
25	17.3	17.4	17.3	4			71.2
26	18.0	18.2	18.3	5			67.6
27	27.3	27.2	27.3				
28	179.9	175.9	177.6				
29	33.3	33.2	33.0				
30	24.7	24.8	24.9				

a) Assignments are based on HMQC, TOCSY, and HMBC experiments.

Platycoside M-2 (**2**) was shown to have the molecular formula $C_{47}H_{72}O_{20}$ based on its high-resolution (HR)-FAB-MS spectrum. A comparison of the ^{1}H- and ^{13}C-NMR spectra of **2** with those **1** clearly revealed that the signals of protons and carbons for the aglycon parts and the sugar chains at C-3 of the aglycon were superimposable, indicating that compound **2** possesses the same aglycon and the same glucose residue at C-3 as **1** (Tables 1, 2). The chemical shifts of C-3 (δ 89.1) and C-28 (δ 175.9) revealed that **2** was a bisdesmosidic glycoside. The ^{1}H- and ^{13}C-NMR spectra of **2** exhibited three sugar anomeric protons at δ 5.33 (d, J=7.0 Hz), 5.81 (br s), and 6.52 (br s) and carbons at δ 105.2, 101.5, and 93.6 (see Table 1). The methyl carbon signals at δ 18.6 coupling with the doublet methyl proton signal at δ 1.68 (3H, d, J=6.5 Hz) indicated the presence of one 6-deoxy sugar unit. Acid hydrolysis of **2** gave arabinose, rhamnose, and glucose, which were analyzed by gas chromatography as their alditol acetates in a ratio of 1 : 1 : 1. Their absolute configurations of sugars were shown to be L-arabinose, L-rhamnose, and D-glucose according to the method reported by Hara and coworkers.[10)] All the monosaccharides of **2** were in pyranose forms, as determined by their ^{1}H- and ^{13}C-NMR spectral data as well as 2D NMR experiments. After all of the proton and carbon signals were assigned, combined with the $^3J_{H1,H2}$ coupling constants (Tables 1, 2), the three sugar units were identified as one β-D-glucopyranose unit, one α-L-arabinopyranose unit predominantly in the $^1C_4$ form,[4,9)] and one α-L-rhamnopyranose unit.[4,9)] The sequence of the sugar chain at C-28 was established from the following HMBC correlations: H-1 (δ 6.52) of inner arabinose with C-28 (δ 175.9) of the aglycon, and H-1 (δ 5.81) of terminal rhamnose with C-2 (δ 75.2) of inner arabinose. Therefore, the structure of platycoside M-2 (**2**) was established as 3-*O*-β-D-glucopyranosyl platycogenic acid A lactone 28-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl ester.

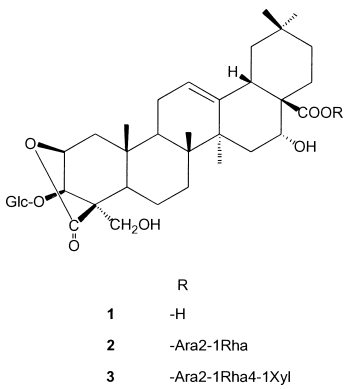

Fig. 2. Structures of Compounds

Platycoside M-3 (**3**), an amorphous powder, has a molecular formula $C_{52}H_{80}O_{24}$ based on its high-resolution (HR)-FAB-MS spectrum, 132 mass units larger than **2**. A comparison of the NMR spectra of **2** and **3** revealed that the signals of protons and carbons for the aglycon parts and the sugar chains at C-3 of the aglycon were very similar to each other, indicating that compound **3** possesses the same aglycon and the same glucose residue at C-3 as **2** (Tables 1, 2). The com-

position of the sugar moieties of **3** (Glc, Xyl, Ara, Rha, in a ratio of 1 : 1 : 1 : 1), as determined from acid hydrolysis and subsequent GLC analysis. The four sugar units were identified as one β-D-glucopyranose unit, one β-D-xylopyranose unit, one α-L-arabinopyranose unit predominantly in the $^1C_4$ form,[4,9] and one α-L-rhamnopyranose unit,[4,9] using the same protocol as described for **2**. The sequence of the sugar chain at C-28 was established from the following HMBC correlations: H-1 (δ 6.46) of Ara with C-28 (δ 177.6) of the aglycon, H-1 (δ 5.80) of Rha with C-2 (δ 75.4) of Ara, and H-1 (δ 5.17) of Xyl with C-4 (δ 84.0) of Rha. Therefore, platycoside M-3 (**3**) was identified as 3-*O*-β-D-glucopyranosyl platycogenic acid A lactone 28-*O*-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl ester. Platycoside M-1—3 were pentacyclic triterpenoid saponins, containing γ-lactone in A-ring, which had been isolated only in the plant until now.

Experimental

General Experimental Procedures FAB-MS and HR-FAB-MS spectral were recorded on a Jeol JMS-SX 102A mass spectrometer. IR spectra were measured with a Bruker IFS-55 infrared spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded with a JNM A 500 FT NMR spectrometer. Chemical shifts were reported in parts per million on the δ 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, 250 mm×10 mm i.d.) on a Hitachi liquid chromatography system with an RI detector. Gas liquid chromatography was carried out on a Shimadzu GC-7A under the following conditions: column, 3%ECNSS-M (2 m×0.3 mm); column temperature, 190 °C; injection temperature, 210 °C; carrier gas, N_2; and flow rate, 25 ml/min. Spots were visualized by spraying with ethanol–10% H_2SO_4 and heating (110 °C, 5 min).

Plant Material The roots of *P. grandiflorum* were collected from Shenyang, Liaoning Province, China, in 2003 and were taxonomically identified by Professor Sun Qishi of Shenyang Pharmaceutical University. A voucher specimen (No. 20030321) is deposited at the Shenyang Pharmaceutical University, School of Traditional Chinese Material Medica.

Extraction and Isolation The extraction procedure was the same as described previously.[9] Fraction C (18 g) was subjected to a silica gel column chromatography in a gradient manner (solvent, EtOAc : EtOH : H_2O = 9 : 1 : 0.5—85 : 15 : 7.5), then fractions 9—12 (Part I), fraction 24—26 (Part II), and fractions 27—28 (Part III) were obtained. Part I was chromatographed over a Lobar RP-C18 (Merck) column, and the 70% MeOH fraction gave **1** (6.0 mg) after HPLC purification (PEGASIL ODS, Senshu Pak column, 62% MeOH as the mobile phase). Part II and Part III were passed over a Lobar RP-C18 (Merck) column twice. Part II was subjected to HPLC (PEGASIL ODS column) with 55% MeOH to give **2** (6.7 mg). Part III was subjected to HPLC with 52% MeOH to give **3** (19.3 mg).

Platycoside M-1 (1) White amorphous powder; IR (KBr) cm^{-1}: 3425 (OH), 2948, 1758 (γ-lactone carbonyl), 1702 (carbonyl), 1383 (*gem*-dimethyl), 1078; HR-FAB-MS *m/z*: 701.3554 [M+Na]$^+$ (Calcd for $C_{36}H_{54}O_{12}Na$, 701.3513); ^{1}H-NMR (500 MHz, pyridine-d_5): aglycon δ 1.02 (3H, s, H-26), 1.05 (3H, s, H-29), 1.18 (3H, s, H-30), 1.33 (3H, s, H-25), 1.33—1.40 (4H, m, H-1a, 7a, 19a, 21a), 1.65 (2H, d-like, H-6a, 15a), 1.75 (3H, s, H-27), 1.79 (1H, m, H-7b), 1.92—1.96 (2H, m, H-11a, 1b), 2.02—2.04 (2H, m, H-9, 11b), 2.22—2.27 (3H, m, H-22a, H-5, 6b), 2.36 (1H, d-like, H-15b), 2.40—2.43 (2H, d-like, H-15b, 22b), 2.50 (1H, t-like, H-21b), 2.82 (1H, dd, H-19b), 3.60 (1H, d-like, H-18), 4.36—4.40 (1H, m, H-23a), 4.80 (1H, br s, H-3), 4.89 (1H, d, J=10.5 Hz, H-23b), 5.20 (1H, m, H-2), 5.22 (1H, br s, H-16), 5.62 (1H, br s, H-12). Other ^{1}H- and ^{13}C-NMR data: see Tables 1, 2.

Platycoside M-2 (2) White amorphous powder; IR (KBr) cm^{-1}: 3417 (OH), 2927, 1750 (γ-lactone carbonyl), 1629 (trisubstituted double bond), 1384 (*gem*-dimethyl), 1078; HR-FAB-MS *m/z*: 979.5174 [M+Na]$^+$ (Calcd for $C_{47}H_{72}O_{20}Na$, 979.5043); ^{1}H-NMR (500 MHz, pyridine-d_5): aglycon δ 1.01 (3H, s, H-29), 1.09 (3H, s, H-26), 1.16 (3H, s, H-30), 1.26—1.32 (1H, m, H-21a), 1.36 (3H, s, H-25), 1.35 (1H, t-like, H-19a), 1.37 (1H, t-like, H-1a), 1.47 (1H, t-like, H-7a), 1.65—1.70 (1H, m, H-6a), 1.71 (1H, m, H-15a), 1.71 (3H, s, H-27), 1.78 (1H, m, H-7b), 1.88—1.90 (2H, m, H-11a, 1b), 1.96—2.02 (2H, m, H-9, 11b), 2.17—2.20 (1H, m, H-22a), 2.22 (2H, d-like, H-5, 6b), 2.27—2.35 (2H, m, H-15b, 22b), 2.41 (1H, t-like, H-21b), 2.76 (1H, t-like, H-19b), 3.58 (1H, dd-like, H-18), 3.34—4.39 (1H, m, H-23a), 4.79 (1H, br s, H-3), 4.88 (1H, d, J=10.5 Hz, H-23b), 5.20 (1H, m, H-2), 5.24 (1H, br s, H-16), 5.61 (1H, br s, H-12). Other ^{1}H- and ^{13}C-NMR data: see Tables 1, 2.

Platycoside M-3 (3) White amorphous powder; IR (KBr) cm^{-1}: 3421 (OH), 2927, 1749 (γ-lactone carbonyl), 1634 (trisubstituted double bond), 1387 (*gem*-dimethyl), 1042; HR-FAB-MS *m/z*: 1111.4901 [M+Na]$^+$ (Calcd for $C_{52}H_{80}O_{24}Na$, 1111.4938); ^{1}H-NMR (500 MHz, pyridine-d_5): aglycon δ 1.00 (3H, s, H-29), 1.09 (3H, s, H-26), 1.15 (3H, s, H-30), 1.26—1.32 (1H, m, H-21a), 1.34 (3H, s, H-25), 1.28—1.40 (2H, m, H-1a, 19a), 1.49 (1H, d-like, H-7a), 1.59—1.67 (1H, m, H-6a), 1.70 (3H, s, H-27), 1.72—1.77 (1H, m, H-15a), 1.76—1.86 (1H, m, H-7b), 1.85—1.92 (1H, m, H-11a), 1.93—2.06 (2H, m, H-1b, 11b), 2.10—2.20 (1H, m, H-22a), 2.18—2.34 (2H, d-like, H-5, 6b), 2.24—2.35 (2H, m, H-15b, 22b), 2.38—2.43 (1H, m, H-21b), 2.75 (1H, t-like, H-19b), 3.57 (1H, dd, J_1=14.0 Hz, J_2=5.0 Hz, H-18), 4.24—4.36 (1H, m, H-23a), 4.79 (1H, br s, H-3), 4.87 (1H, d, J=10.5 Hz, H-23b), 5.20 (1H, m, H-2), 5.23 (1H, br s, H-16), 5.61 (1H, br s, H-12). Other ^{1}H- and ^{13}C-NMR data: see Tables 1, 2.

Acid Hydrolysis of 1—3 Compounds **1**—**3** (3 mg each) were heated in 1 ml of 1 M HCl (dioxane–H_2O, 1 : 1) at 90 °C for 3 h in a water bath. Dioxane was removed, the solution was extracted with EtOAc (1 ml×3), and the EtOAc was removed. The monosaccharide portions were analyzed by gas chromatography after conversion of the hydrolysates into corresponding alditol acetates. The arabinitol, glucitol, rhamnitol and xylitol acetates from compounds **2** and **3** were detected in a ratio of 1 : 1 : 1 : 0, and 1 : 1 : 1 : 1 respectively using gas chromatography analysis. The absolute configurations of the sugars were determined according to the method reported by Hara and coworkers[10] using gas chromatography with the following conditions; column, 3% ECNSS-M (2 m×0.3 mm); column temperature: 190 °C; injection temperature: 210 °C; and retention times (min), D-xylose (11.1), L-arabinose (12.0), L-rhamnose (12.1), and D-glucose (17.9). By the same method, compounds **1**, the monosaccharide was identified only glucitol acetate.

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