Platycoside N: A New Oleanane-Type Triterpenoid Saponin from the Roots of Platycodon grandiflorum

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Abstract: A new oleanane-type triterpenoid saponin, named platycoside N (1), together with six known saponins, was isolated from the roots of Platycodon grandiflorum. On the basis of acid hydrolysis, comprehensive spectroscopic data analyses and comparison with the spectral data of the known compounds, its structure was elucidated as 3-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-2β,3β,16α,23-tetrahydroxyolean-12-en-28-oic acid 28-O-β-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside. The six known compounds were platycodin D (2), deapioplatycodin D (3), platycodin D3 (4), deapioplatycodin D3 (5), platycoside E (6) and deapioplatycoside E (7).

Keywords: platycoside N; Platycodon grandiflorum; triterpenoid saponin
1. Introduction

The roots of *Platycodon grandiflorum* A. DC (Campanulaceae), Platycodi Radix, are traditionally used as food and a herbal medicine in the treatment of a wide range of diseases, including bronchial asthma, hepatic fibrosis, bone disorders [1-4], hypercholesterolemia and hyperlipidemia [5]. The principal bioactive constituents of this herb are triterpenoid saponins (platycosides), which exhibit a variety of pharmacological activities, such as anti-inflammatory [6,7], anti-obesity [8-11], anti-cancer [12-15] and hypoglycemic effects [16,17]. To date, more than 30 saponins have been isolated from this plant [18-25]. In order to find more bioactive compounds, we have now studied the chemical constituents of *P. platycodiflorum*, and in this paper, we report the presence in this species of a new oleanane-type triterpenoid saponin, named platycoside N, together with six known compounds, from the roots of *Platycodon grandiflorum*. (Figure 1)

**Figure 1.** Chemical structures of compounds 1-7.

2. Results and Discussion

Platycoside N (1) was a white amorphous powder, and its molecular formula C<sub>53</sub>H<sub>86</sub>O<sub>24</sub> was determined based on the HR-ESI-MS spectra. The oleanane-type triterpenoid saponin nature of compound 1 was revealed through analysis of its spectral features. The IR spectrum exhibited absorptions at 3,425 cm<sup>-1</sup> (OH), 1,647 cm<sup>-1</sup> (ester carbonyl), and 1,616 cm<sup>-1</sup> (double bond). Six methyl groups (δ 0.89 × 2, 0.98, 1.17, and 1.58 × 2) and one olefinic proton (δ 5.46, br s) of the aglycon were observed in the 1H-NMR spectrum. The 13C-NMR spectrum showed that the aglycon had six methyl carbons at δ 16.0, 18.6, 17.7, 27.2, 33.3, and 24.8, two olefinic carbons at δ 123.1 (CH) and 144.5 (C), one oxymethylene and three oxymethine carbons at δ 66.6, and 70.1, 74.2 and 83.1, respectively, and one carbonyl carbon at δ 176.0 (Table 1). The information of the 1H-NMR spectrum
coupled with the $^{13}$C-NMR spectrum indicated that 1 had $2\beta,3\beta,16\alpha,23$-tetrahydroxyolean-12-en-28-oic acid (polygalacic acid) as an aglycon [20]. The $^{13}$C-NMR spectrum showed 53 signals, of which 30 were assigned to a triterpenoid moiety and 23 to the saccharide portion. The downfield shift of C-3 ($\delta$ 83.1) and for the upfield shift of C-28 ($\delta$ 176.0), revealed that the sugar moieties were attached to the aglycon at these two positions. The $^1$H and $^{13}$C-NMR spectra of 1 exhibited four anomic protons at $\delta$ 5.10 $\times$ 2 (2H, d, $J=7.5$ Hz), 6.27(1H, d, $J=2.5$ Hz ), 5.68 (1H, br s) ppm and carbons at $\delta$ 106.7 $\times$ 2, 93.9, 101.5 (Table 1). In the $^1$H-NMR spectrum, one methyl signal at $\delta$ 1.59 (3H, d, $J=5.5$ Hz) belonging to rhamnose was observed. In addition, the monosaccharides were identified as glucose, rhamnose and arabinose by TLC and a combination of DEPT, HMQC and HMBC experiments. Acid hydrolysis of 1 also gave glucose, arabinose and rhamnose in a ratio of 2:1:1 respectively, as confirmed by GC analysis of the respective trimethylsilyl derivatives [20]. The $^1$H- and $^{13}$C-NMR and 2D-NMR analysis indicated that all the monosaccharides of 1 were in pyranose forms. The $\beta$-anomeric configurations of the D-gulose units were determined by its $^3$$J_{H1,H2}$ coupling constants (7.5 Hz). The $\alpha$-anomeric configurations of the L-arabinose and L-rhamnose were determined by the broad singlet of their anomeric protons [24]. The linkages between sugar moieties and C-3 of the aglycon were corroborated through HMBC experiments, i.e., H-1 ($\delta$ 5.10) of the terminal glucose correlated with C-6 ($\delta$ 70.8) of the inner glucose, and H-1 ($\delta$ 5.10) of the inner glucose correlated with C-3 ($\delta$ 83.1) of the sapogenin. The linkages of sugar moieties at C-28 were established based on HMBC correlations between H-1 ($\delta$ 5.68) of rhamnose and C-2 ($\delta$ 75.3) of arabinose, and H-1 ($\delta$ 6.27) of arabinose and C-28 ($\delta$ 176.0) of aglycone (Figure 2). On the basis of all the above evidence, platycoside N (1) was identified as 3-$O$$\beta$-D-glucopyranosyl-(1$\rightarrow$6)-$\beta$-D-glucopyranosyl-2$\beta,3\beta,16\alpha,23$-tertahydroxyolean-12-en-28-oic acid 28-$O$$\beta$-L-rhamnopyranosyl-(1$\rightarrow$2)-$\alpha$L-arabinopyranoside.

Table 1. $^{13}$C-NMR data of compound 1 in pyridine-$d_5$ ($\delta$ ppm).

| Position | $\delta_C$  | Position | $\delta_C$  |
|----------|-------------|----------|-------------|
| 1        | 46.8        | 3-O-Glc  | 106.7       |
| 2        | 70.1        | 1        | 75.3        |
| 3        | 83.1        | 2        | 78.7        |
| 4        | 42.4        | 3        | 72.1        |
| 5        | 47.5        | 4        | 76.0        |
| 6        | 20.5        | 5        | 70.8        |
| 7        | 33.6        | 6        |             |
| 8        | 40.2        | Glc      |             |
| 9        | 47.5        | 1$'$     | 106.7       |
| 10       | 37.2        | 2$'$     | 74.9        |
| 11       | 24.4        | 3$'$     | 78.7        |
| 12       | 123.1       | 4$'$     | 71.6        |
| 13       | 144.5       | 5$'$     | 78.7        |
| 14       | 42.4        | 6$'$     | 62.3        |
| 15       | 36.2        | Ara      |             |
| 16       | 74.2        | 1        | 93.9        |
| 17       | 48.6        | 2        | 75.3        |
| 18       | 41.5        | 3        | 70.8        |
Table 1. Cont.

|   |   |   |
|---|---|---|
| 19 | 47.2 | 4 |
| 20 | 31.0 | 5 |
| 21 | 36.2 | Rha |
| 22 | 32.2 | 1 |
| 23 | 66.6 | 2 |
| 24 | 16.0 | 3 |
| 25 | 18.6 | 4 |
| 26 | 17.7 | 5 |
| 27 | 27.2 | 6 |
| 28 | 176.0 | |
| 29 | 33.3 | |
| 30 | 24.8 | |

Figure 2. The key HMBC correlations of compound 1 (from H to C).

The six known saponins were identified as platycodin D (2), deapio platycodin D (3), platycodin D₃ (4), deapio platycodin D₃ (5), platycoside E (6) and deapio platycoside E (7) through comparison of their UV, IR, NMR and MS data with literature values [25,26].

3. Experimental

3.1. General

ESI-MS (negative mode) measurements were carried out on an Agilent 1100 series LC/MSD Trap SL mass spectrometer. HR-ESI-MS (positive and negative modes) was analyzed on a Bruker FT-ICRMS spectrometer. IR spectra were recorded on an IR-47 spectrometer. NMR spectra were recorded on a Bruker Avance DRX 400 NMR spectrometer using TMS as internal standard, and chemical shifts δ were given in ppm. Silica gel (200–300 mesh) for column chromatography and silica
molecules were purchased from Qingdao Marine Chemical Factory, Qingdao, China. AB-8 macroporous resin was purchased from Tianjin Nankai factory. Preparative HPLC was performed on a Waters 600 liquid chromatography instrument with a UV detector, monitored at 210 nm using a C18 column (Zorbax Eclipse XDB, 250 mm × 9 mm; 10 μm).

3.2. Plant material

The roots of *P. grandiflorum* were purchased at Changchun Guangfulu market in Changchun-city of Jilin province, China and identified by Prof. Yi-Nan Zheng, College of Chinese Material Medicine, Jilin Agricultural University. A voucher specimen (No.20050116) has been deposited in the herbarium of the same college.

3.3. Extraction and isolation

Dry and powdered roots of *P. grandiflorum* (2.0 Kg) were refluxed three times with 30 L of 70% methanol, 3 h each time. Extracts were concentrated, suspended in water and sequentially partitioned with ethyl acetate and n-butanol. The n-butanol fraction was subjected to macroporous resin AB-8 column and eluted sequentially with water, 30% ethanol and 70% ethanol. The 30% ethanol elution was repeatedly chromatographed on a reverse-phase column, and eluted with aqueous methanol, affording three fractions A-C. Fraction A was purified by HPLC to afford compounds 1 (23 mg), 2 (15 mg) and 3 (40 mg). Fraction B and C gave 4 (32 mg), 5 (22 mg), 6 (16 mg) and 7 (15 mg).

Platycoside N (1): White amorphous powder; IR (KBr) cm⁻¹: 3425, 2947, 1647, 1616, 1114; ESI-MS *m/z*: 1105 [M-H]⁻, HR-ESI-MS *m/z* 1105.5408 [M-H]⁻ (Calcd for C₅₃H₈₅O₂₄, 1105.5431). ¹H-NMR (400 MHz, pyridine- d₅) δ: 0.89 × 2, 0.98, 1.17, 1.58 × 2 (each 3H, s, CH₃ of C-26, C-29, C-30, C-24, C-25, C-27), 1.59 (3H, d, CH₃ of rhamnose), 4.36 (1H, d, J = 3.0Hz, H-3), 3.82, 4.60 (each 1H, d, H-23), 4.72(1H, m, H-2), 5.10 × 2 (each 1H, d, J = 7.5 Hz, H-1' and H-1of glucose), 5.68 (1H, br s, H-1 of rhamnose), 6.27 (1H, br s, H-1 of arabinose), 5.46 (1H, br s, H-12) 5.03 (1H, br s, H-16). ¹³C-NMR (100 MHz, pyridine- d₅) data: see Table 1.

3.4. Acid hydrolysis of 1

Compound 1 (2.0 mg) was refluxed with 4.0 M HCl (5.0 mL) for 1 h at 95 °C, and the reaction mixture was extracted with ethyl acetate. The aqueous layer was then adjusted to pH 7.0 with NaHCO₃. After evaporating to dryness, the sugar mixture was dissolved in pyridine and developed on silica gel TLC [CHCl₃-MeOH-H₂O (7:3:0.5, lower phase), n-BuOH-AcOH-H₂O (4:1:5, upper phase). Three spots were seen on the TLC after spraying with 4% α-naphthol-EtOH-5% H₂SO₄. Through comparison with authentic sugar standards (purchased from Sigma), it was found that compound 1 possessed D-glucose, L-rhamnose and L-arabinose units.

4. Conclusions

In summary, we have isolated a new oleanane-type triterpenoid saponin, named platycoside N (1), together with six known saponins from the roots of *Platycodon grandiflorum*.
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Sample Availability: Samples of the compounds are available from the authors (liwei7727@126.com).

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