Three new chlorinated phenolic glycosides from *Przewalskia tangutica*

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**A R T I C L E   I N F O**

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**A B S T R A C T**

Three new chlorinated phenolic glycosides, namely przewatangosides A-C (1-3), along with one known compound, globosumoside A (4), were isolated from the whole plants of *Przewalskia tangutica*. Their structures were unequivocally determined by extensive spectroscopic analysis and chemical method. The cytotoxic activities of the isolated phenolic glycosides (1-4) were evaluated against the five human cancer cell lines A549, MCF-7, SMMC-7721, HepG2 and HL-60. Przewatangoside A (1) exhibited weak cytotoxicity against SMMC-7721 with the IC50 value of 38.1 μM. All the tested compounds were inactive (IC50 > 50 μM) to the normal human hepatocyte cell line (L02).

1. Introduction

*Przewalskia tangutica* Maxim. is the only species in the endemic monotypic genus *Przewalskia* (Solanaceae). It is mainly distributed in sandy and gritty grasslands on the Qinghai-Tibetan Plateau at the altitudes ranging from 3000 to 5000 m (Zeng et al., 2015). The whole plant of *P. tangutica*, known as “Tang-Chong-Ga-Bao” in traditional Tibetan medicine has long been used to treat anthrax, diphtheria, and gastrointestinal spasm pain (Yang, 1991). Previous phytochemical investigations of *P. tangutica* led to the isolation of a series of tropane alkaloids, phenolic acids, sesquiterpenes, triterpenoids, and coumarins (Xiao and He, 1982; Zhu et al., 2011; Shao et al., 2015). In our continuing endeavor to discover structurally unique natural products with potent bioactivity from this traditional Tibetan medicine, three new chlorinated phenolic glycosides, namely przewatangosides A-C (1-3), along with one known compound, globosumoside A (4), were isolated from the whole plants of *P. tangutica*. These chlorinated compounds are discovered for the first time in the family of Solanaceae. Their structures (Fig. 1) were unequivocally elucidated by extensive spectroscopic analysis and chemical method. We herein report the isolation, structure elucidation and cytotoxicity evaluation of the isolated compounds.

2. Results and discussion

Compound 1 was obtained as a white amorphous powder. It displayed [M-H]− peaks at m/z 375.0844 and 377.0814 in a peak height ratio of 3:1 in the HRESIMS spectrum consistent with the molecular formula C16H21ClO8 (calcd. for C16H20O8Cl, 375.0847). The IR spectrum showed the presence of hydroxy group (3424 cm−1) and benzene moiety (1626, 1419, 1248, 1063 cm−1). The 1H, 13C NMR and HSQC spectrum of 1 showed one methyl group at δH 1.86 (3H, d, dd)/δC 18.7; one methoxy group at δH 3.86 (3H, s)/δC 61.7; two olefinic groups at δH 6.12 (1H, dq) and 6.92 (1H, dd), and their corresponding carbons at δC 127.5 and 127.2; the presence of a penta-substituted aromatic ring [δC 151.6, 151.2, 141.7, 133.3, 114.9, and δC 108.2/δH 6.81 (1H, s)], and a hexose unit (δC 106.3, 73.0, 74.8, 70.0, 76.8, and 62.0). The D-glucose unit was established by acid hydrolysis and GC analysis. The β-anomeric configuration of the glucose unit was deduced by its 1JH1,1H2 value (7.8 Hz) (Melek et al., 2014). Complete assignments of the 1H and 13C NMR signals of the glucose were achieved by extensive 2D NMR analysis. The gluconic chain (C1-C2-C3-C4-C5′-C6′) was determined by the 1H-1H COSY correlations of H-1′ (δH 4.76)/H-2′ (δH 3.80), H-2′ (δH 3.80)/H-3′ (δH 3.54), H-3′ (δH 3.54)/H-4′ (δH 3.87), H-4′ (δH 3.87)/H-5′ (δH 3.41) and H-5′ (δH 3.41)/H-6′ (δH 3.64, 3.71), and the NOESY correlations of H-1′ (δH 4.76)/H-2′ (δH 3.41), H-1′ (δH 4.76)/H-5′ (δH 3.41), H-1′ (δH 3.41)/H-2′ (δH 3.80)/H-4′ (δH 3.87) (Fig. 2). A 1-prostenyl side chain (C7-C9) was confirmed by the 1H-1H COSY correlations between δH 6.92 (H-7) and δH 6.12 (H-8), and between δH 6.12 (H-8) and δH 1.86 (H-9). The 1JH1,1H2,3 value (15.9 Hz) revealed an E-configuration for the C-7/C-8 double bond. The HMBC correlations from H-7 (δH 6.92)/H-8 (δH 6.12)/H-9 (δH 1.86) to C-6 (δC 133.3)
indicated that the 1-propenyl side chain was connected to C-6. The HMBC correlations from H-7 (δH 6.92)/H-8 (δH 6.12) to C-1 (δC 141.7), and from H-1′ (δH 4.76) to C-1 (δC 141.7), suggested that the glucose unit was connected to C-1 (Fig. 2). The HMBC correlations from H-7 (δH 6.92)/H-8 (δH 6.12) to C-5 (δC 108.2), and H-5 (δH 6.81) to C-7 (δC 127.2) suggested that C-5 was free. The HMBC correlation from H-7 (δH 6.92) to C-2 (δC 151.2), a methoxy group (δH 3.86) to C-2 (δC 151.2), and the NOESY correlation between 2-OCH3 (δH 3.86) and H-2′ (δH 3.80), suggested that the methoxy group was connected to C-2. In addition, a hydroxy group was connected to C-4, which was supported by the HMBC correlation from H-5 (δH 6.81) to C-4 (δC 151.6), and H-7 (δH 6.92) to C-4 (δC 151.6). The chemical value (δC 114.9) of the quaternary carbon C-3 suggested that a chlorine atom was connected to C-3, which was also supported by the HMBC correlation from H-5 (δH 6.81) to C-3 (δC 114.9). Based on the above evidence, the structure of 1 was identified as (E)-3-chloro-2-methoxy-6-(prop-1-enyl)-1,4-phenol-1-O-β-D-glucoside, and was named przewatangoside A.

Compound 2 was obtained as a white amorphous powder. Its molecular formula C16H20Cl2O8 was established according to the HRESIMS spectrum (m/z 409.0459, 411.0431, and 413.0416 [M-H]−, calcd. for C16H1935Cl2O8, 423.0613). The D-glucose unit was determined by acid hydrolysis and GC analysis. The 1H, 13C NMR and HSQC spectra of 2 revealed that 2 differed from 1 by the presence of an additional methoxy group at δH 3.82 (3H, s)/δC 150.8. The HMBC correlation from 4-OCH3 (δH 3.82) to C-4 (δC 150.8), and H-7 (δH 6.48) to C-4 (δC 150.8) suggested that the methoxy group was connected to C-4. Thus, the structure of 3 was identified as (E)-3,5-dichloro-2-methoxy-6-(prop-1-enyl)-1,4-phenol-1-O-β-D-glucoside, and was named przewatangoside B.

Compound 3 was obtained as a white amorphous powder. Its molecular formula C17H22Cl2O8 was established according to the HRESIMS spectrum (m/z 423.0615, 425.0587, and 427.0560 [M-H]−, calcd. for C17H2135Cl2O8, 423.0613). The D-glucose unit was determined by acid hydrolysis and GC analysis. The 1H, 13C NMR and HSQC spectra of 3 revealed that 3 differed from 2 by the presence of an additional methoxy group at δH 3.82 (3H, s)/δC 150.8. The HMBC correlation from 4-OCH3 (δH 3.82) to C-4 (δC 150.8), and H-7 (δH 6.48) to C-4 (δC 150.8) suggested that the methoxy group was connected to C-4. Thus, the structure of 3 was identified as (E)-3,5-dichloro-2,4-dimethoxy-6-(prop-1-enyl)-1-phenol-1-O-β-D-glucoside, and was named przewatangoside C.

Additionally, one known chlorinated phenolic glycoside was identified as globosumoside A (4) (Chen et al., 2012) by comparing its physicochemical and spectroscopic data with literature data. All of the isolated compounds (1-4) were evaluated for their cytotoxic activities against the five human cancer cell lines A549, MCF-7, SMMC-7721, HepG2 and HL-60, and the results were shown in Table 2. All the tested compounds 1-4 were inactive (IC50 > 50 μM) to the five human cancer cell lines A549, MCF-7, SMMC-7721, HepG2, and the normal human hepatocyte cell line (L02), and compound 1 was the only compound showed weak cytotoxicity against SMMC-7721 with the IC50 value of 38.1 μM.

Fig. 1. Structures of compounds 1-4 isolated in P. tangutica.
3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a Perkin Elmer Model 341 Polariometer. IR measurements were obtained by using a PerkinElmer one FT-IR spectrometer. NMR data were obtained on a Bruker Ascend 400 spectrometer in CD3OD with TMS as an internal standard. HRESIMS experiments were performed on a Bruker MicrOTOF QII mass spectrometer. Semi-preparative HPLC was performed on a LC 3000 liquid chromatography system (ChuangXingTongHeng Science And Technology Co., Beijing) using a Kromasil RP-C18 column (10 mm × 250 mm i.d., 5 μm) and a LC 3000 UV detector with detection wavelength of 254 nm. Column chromatography (CC) was performed with silica gel (200–300 mesh, Qingdao Marine Chemical Factory, China), and ODS (50 μm, YMC, Japan). TLC detection was using a precoated silica gel GF254 plate (Qingdao Marine Chemical Factory, China). All of the solvents used were of analytical grade.

3.2. Plant material

Whole plants of *P. tangutica* were collected from the Linzhou Country of the Tibet Autonomous Region, P. R. China, in September 2014. The plant material was identified by Dr. Zhuo-Ma Da-Wa (Tibet Autonomous Region Institute for Food and Drug control, Lhasa). A voucher specimen (No. CIB20150914) was deposited at Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, P. R. China.

3.3. Extraction and isolation

Air-dried and powdered plant material of *P. tangutica* (12 kg) was extracted with MeOH-H2O (80:20, v/v; 48 L × 3) at room temperature for three times (each for 7 days). The combined hydroalcoholic extracts were concentrated under vacuum to yield a crude extract (1.36 kg). The latter was suspended in H2O and then partitioned successively with R-0Ac and n-BuOH saturated with H2O. The n-BuOH extract (166 g) was subjected to silica gel CC (200–300 mesh, 8 × 100 cm, 2 kg) and eluted with a gradient of CHCl3-MeOH-H2O (50:1:0; 20:1:0.1; 10:1:0.1; 4:1:0.1; 7:3:0.3; 6:4:0.3; 1:1:0.1) to obtain fractions A-H. Fraction D (6.4 g) was subjected to CC (ODS, 50 μm, 2 × 30 cm, 200 g), and eluted with 30%, 40%, 50%, 70%, 90% and 100% MeOH-H2O to give five subfractions (a-e). Fraction D-c (240 mg) was purified by semi-preparative HPLC using MeOH-H2O (28:72, v/v; 3 mL/min) to yield compounds 1 (11 mg, tR 29.8 min) and 4 (8 mg, tR 31.2 min). Fraction D-d (180 mg) was purified by semi-preparative HPLC using MeOH-H2O (40:60, v/v; 4 mL/min) to yield compounds 2 (7 mg, tR 18.2 min) and 3 (9 mg, tR 16.4 min).

3.3.1. Przewatangoside A (I)

White amorphous powder; C14H21ClO6; [α]20 D +21 (c 0.5, CH3OH); IR (KBr) ν max 3424, 2925, 1626, 1419, 1248, 1063, 170

![Diagram](image-url)
for \(^{1}H\) NMR and \(^{13}C\) NMR spectroscopic data, see Table 1.

3.4. Sugar identification

Each compound (3 mg) was separately hydrolyzed with 2 mol/L CF\(_{3}\)COOH (5 mL) in a water bath (80 °C) for 4 h. After suspension with CHCl\(_{3}\), the aqueous phase was evaporated to dryness. The residue was dissolved in 0.1 mL anhydrous pyridine, and 0.1 mL 1-(trimethylsilyl)-imidazole was added. The mixture was stirred at 60 °C for 5 min and dried with a stream of N\(_2\). After partitioning between hexane and H\(_2\)O, the hexane layer was analyzed by GC using an L-Chirasil-Val column (De Marino et al., 2003). The retention time for standard sugar after being treated in the same way was detected at 14.72 min (D-glucose). The configuration of the sugar unit of 1-4 was identified as D-glucose by comparing the retention time of their trimethylsilylated derivatives with those of the standard sugars’ derivatives using GC analysis.

3.5. Cytotoxicity assay

The cytotoxic activities of compounds 1-4 against A549 (human alveolar basal epithelial cancer cell line), MCF-7 (human breast cancer cell line), SMMC-7721 (human hepatocellular carcinoma cell line), HepG2 (human liver hepatoma cancer cell line), and HL-60 (human promyelocytic leukemia cancer cell line) were determined by the MTT method as previously described (Zhao et al., 2016). The cancer cell lines were obtained from the American Type Culture Collection (ATCC).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytol.2017.04.037.

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3.3.2. Przewatangoside B (2)

White amorphous powder, C\(_{16}\)H\(_{20}\)Cl\(_{2}\)O\(_{8}\); \([\alpha]\)\(_{D}\) +13 (c 0.1, CH\(_{3}\)OH); IR (KBr) \(v\) max 3397, 2938, 1653, 1561, 1416, 1297, 1212, 1073, 1022, 964, 927, 754, 658 cm\(^{-1}\); HRESIMS: m/z 409.0459, 411.0431, and 413.0416 [M-H]\(^{-}\) (calcd. for C\(_{16}\)H\(_{20}\)Cl\(_{2}\)O\(_{8}\), 409.0457); for \(^{1}H\) NMR and \(^{13}C\) NMR spectroscopic data, see Table 1.

3.3.3. Przewatangoside C (3)

White amorphous powder, C\(_{17}\)H\(_{22}\)Cl\(_{2}\)O\(_{8}\); \([\alpha]\)\(_{D}\) +17 (c 0.1, CH\(_{3}\)OH); IR (KBr) \(v\) max 3444, 2925, 1699, 1602, 1453, 1356, 1243, 1120, 1089, 1046, 957, 770, 592 cm\(^{-1}\); HRESIMS: m/z 423.0615, 425.0587, and 427.0560 [M-H]\(^{-}\) (calcd. for C\(_{17}\)H\(_{21}\)Cl\(_{2}\)O\(_{8}\), 423.0613); for \(^{1}H\) NMR and \(^{13}C\) NMR spectroscopic data, see Table 1.

Table 1

\(\Delta^{13}C\) (100 MHz in CD\(_{3}\)OD) and \(^{1}H\) (400 MHz in CD\(_{3}\)OD) NMR spectroscopic data of compounds 1-3.

| Compounds | 1 | 2 | 3 |
|-----------|---|---|---|
| \(\delta_{C}\) | \(\delta_{H}\) (J in Hz) | \(\delta_{C}\) | \(\delta_{H}\) (J in Hz) | \(\delta_{C}\) | \(\delta_{H}\) (J in Hz) |
| 1 | 141.7 | - | 142.3 | - | 146.2 | - |
| 2 | 151.2 | - | 149.9 | - | 150.2 | - |
| 3 | 114.9 | - | 115.8 | - | 122.6 | - |
| 4 | 151.6 | - | 148.2 | - | 150.8 | - |
| 5 | 108.2 | 6.81 s | 117.2 | - | 124.2 | - |
| 6 | 133.3 | - | 131.9 | - | 132.7 | - |
| 7 | 127.2 | 6.92 dd (15.9, 1.7) | 124.5 | 6.47 dd (16.2, 1.0) | 124.2 | 6.48 dd (16.0, 1.2) |
| 8 | 127.5 | 6.12 dq (15.9, 6.6) | 135.2 | 6.39 dq (16.2, 1.6) | 135.8 | 6.42 dq (16.0, 6.2) |
| 9 | 18.7 | 1.86 d (6.6, 1.7) | 19.4 | 1.89 d (5.4) | 19.4 | 1.90 d (5.1) |

2-OCH\(_{3}\) 61.7 | 3.86 s | 61.8 | 3.87 s | 61.8 | 3.88 s

2-OCH\(_{3}\)/4-OCH\(_{3}\) 60.9 | 3.82 s

1-O-Glc 61.9 | 3.82 s

\(^a\) Overlapped with other signals.

Table 2

Cytotoxicity of compounds 1-4 against five cancer cell lines (IC\(_{50}\), \(\mu\)M).

| Compounds | A549 | MCF-7 | SMMC-7721 | HepG2 | HL-60 | L02 |
|-----------|------|------|-----------|------|------|------|
| 1         | > 50 | > 50 | > 31     | > 50 | > 50 | > 50 |
| 2         | > 50 | > 50 | > 50     | > 50 | > 50 | > 50 |
| 3         | > 50 | > 50 | > 31     | > 50 | > 50 | > 50 |
| 4         | > 50 | > 50 | > 31     | > 50 | > 50 | > 50 |

Positive control 12.6\(^a\) 18.1\(^b\) 9.8\(^c\) 0.21\(^d\) 0.42\(^e\) > 50\(^f\)

\(^a\) IC\(_{50}\) values are means from three independent experiments in which each compound concentration was tested in three replicate wells.

\(^b\) Cipiolatin as positive control.

\(^c\) Adramycin as positive control.

601 cm\(^{-1}\); HRESIMS: m/z 375.0844, 377.0814 [M-H]\(^{-}\) (calcd. for C\(_{17}\)H\(_{22}\)Cl\(_{2}\)O\(_{8}\), 375.0847); for \(^{1}H\) NMR and \(^{13}C\) NMR spectroscopic data, see Table 1.