Polygonflavanol A, a novel flavonostilbene glycoside from the roots of Polygonum multiflorum

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A B S T R A C T

Polygonflavanol A (1), a novel flavonostilbene glycoside with an unusual coupling pattern, together with five known stilbene glycosides (2–6), was isolated from the roots of Polygonum multiflorum. The structures were elucidated on the basis of extensive spectroscopic analysis and chemical evidence. The absolute configuration of 1 was further determined by CD analysis. Most of the compounds could inhibit nitric oxide (NO) secretion of RAW264.7 cells in respond to lipopolysaccharide (LPS) in a dose dependent manner. Among them, compound 2 displayed the highest inhibitory activity.

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1. Introduction

The plant Polygonum multiflorum Thunb. (Polygonaceae) is widely cultivated in southern China. The root of this plant, commonly known as “Heshouwu” in China, is a famous traditional Chinese medicine, which has been widely used for the treatment of hyperlipidemia, coronary heart disease, neurosis, and other diseases (Wu, 2009). Recent pharmacological studies demonstrated that the crude extract of the roots of P. multiflorum showed obvious anti-inflammatory and anti-atherosclerotic effects (Zhang et al., 2007), as well as the strongly suppressing oxidation property (Lv et al., 2007) and the ability to clean the active oxygen free radicals (Ryu et al., 2002) activities. Previous phytochemical investigation of this plant had led to the isolation of a number of compounds, such as anthraquinones, stilbene glycosides, flavonoids, and tannins (Zhang et al., 2008). Among them, stilbene glycosides are the major and characteristic composition of P. multiflorum, some of which have been reported to possess potent anti-inflammatory (Zhang et al., 2007), antioxidant (Chen et al., 1999a,b), anti-HIV (Lin et al., 2010), and liver protection (Wang et al., 2012) activities. As a part of our ongoing program to assess the chemical and biological diversities of medicinal plants in southern China, we carried out the phytochemical study on the roots of P. multiflorum. A novel flavonostilbene glycoside, polygonflavanol A (1), with an unusual coupling pattern between flavanone and stilbene moiety, together with five known stilbene glycosides (2–6) (Fig. 1) was isolated from the n-butanol soluble fraction. The structure of compound 1 was elucidated by means of HRESIMS, NMR, as well as CD analysis. Herein, we report the isolation and structural elucidation of the new compound, as well as the anti-inflammatory activity of the six isolated compounds.

2. Results and discussion

Compound 1 was isolated as brown oil with positive optical rotation value [α]D 19°: +9.46 (c 0.01, MeOH). The HR-ESI-MS showed a quasi-molecular ion at m/z 717.1789 [M+Na]+ (calcd for C35H32O13Na: 717.1790), which is consistent with a molecular formula of C35H32O15. The UV spectrum of 1 showed the absorptions maxima at 205 and 280 nm. The IR spectrum implied the presence of hydroxyl group (3444 cm⁻¹) and aromatic ring (1618, 1508 and 1461 cm⁻¹). The 1H NMR spectrum of 1 revealed
signals for a para-disubstituted benzene ring [δH 7.08 (2H, d, J = 8.5 Hz, H-10′ and H-14′) and 6.58 (2H, d, J = 8.5 Hz, H-11′ and H-13′)], a 1,3,4-trisubstituted benzene ring [δH 6.89 (1H, s, H-2′), 6.73 (1H, d, J = 8.5 Hz, H-6′) and 6.71 (1H, d, J = 8.5 Hz, H-5′)], two tetrasubstituted benzene rings [δH 6.97 (2H, d, J = 2.6 Hz, H-4”), 6.07 (1H, d, J = 2.6 Hz, H-6”), 6.01 (1H, d, J = 2.2 Hz, H-8) and 5.93 (1H, d, J = 2.2 Hz, H-6]), and five methines [δH 5.03 (1H, d, J = 10.4 Hz, H-8”), 4.97 (1H, d, J = 3.8 Hz, H-2), 4.30 (1H, t, J = 10.4 Hz, H-7”), 4.18 (1H, dd, J = 7.0, 3.8 Hz, H-3) and 3.02 (1H, dd, J = 11.3, 7.0 Hz, H-4)]. In addition, the 1H NMR spectrum of 1 displayed the signals due to an anomic proton [δH 4.46 (1H, d, J = 7.9 Hz, H-1”)] and a hydroxymethyl group [δH 3.79 (1H, d, J = 12.0 Hz, H-6”a)] and 3.72 (1H, dd, J = 12.0, 4.3 Hz, H-6”b)]. Acid hydrolysis of 1 afforded α-glucose, which was identified by GC analysis. The β-configuration of α-glucose was determined based on the J H,2 coupling constant of the anomic proton (J = 7.9 Hz).

The 13C NMR and DEPT-135 spectra of 1 revealed the presence of thirty-five carbon signals, including twenty-four aromatic carbons, three oxymethines and two alkyl-methines, as well as a β-α-glycopyranosyl moiety. All above information suggested that 1 possessed a flavonone moiety (1a) and a stilbene unit (1b). The 1H-1H COSY, HSQC, and HMBC spectra of 1 allowed the full assignment of all proton and carbon signals (Table 1).

The 1H and 13C NMR signals assigned to 1a were very similar to those of (-)-epi-catechin (Hwang et al., 2001), indicating that 1 possessed the same flavan-3-ol moiety. Moreover, a comparison of the NMR data assigned to 1b with those of 2,3,5,4’-tetrahydroxy-stilbene 2-O-β-α-glycopyranoside (2) (Chen et al., 1999a,b) suggested that they were very similar, except for the absence of trans-1,2-disubstituted vinyl unit and the presence of two methines in 1b. The 1H-1H COSY spectrum of 1 revealed the presence of spin systems in bold as shown in Fig. 2. The HMBC correlations between H-2 and C-4, between H-8 and C-4/C-10, as well as between H-7” and C-6” indicated that 1a and 1b were

**Table 1**

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<th>Position</th>
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</table>

* Assignments were established by interpretation of the 1H-1H COSY, HSQC, and HMBC spectra.

* Overlapped signals are reported without designating multiplicity.

Fig. 2. Key 1H-1H COSY and HMBC correlations of 1.
connected via C-4–C-7″ bond. Furthermore, according to the molecular formula information, as well as the obvious downfield chemical shift value of C-8′ (δ C 85.8) and upfield one of the C-5 (δ C 157.1), the remaining oxygen atom was assigned to bridge C-5 and C-8′ to form a dihydropyran moiety (Fig. 2). The linkage position of the β-o-glycopyranosyl moiety was confirmed by the HMBC correlation between H-1″ and C-2″.

The relative configuration of 1 was deduced by the vicinal coupling constants and the ROESY experiment (Fig. 3). The coupling constant (J = 3.8 Hz) between H-2 and H-3 indicated that the two protons were on the same side of the molecule plane (assigned as β-orientation), which was identical to that of (-)-epicatechin (Hwang et al., 2001). On the contrary, the greater coupling constant (J = 7.0 Hz) between H-3 and H-4 indicated that they were in the opposite orientation (Frieboes, 1993). Thus, H-4 was assigned to be α-orientation. Moreover, the large coupling constant (J = 10.4 Hz) between H-7″ and H-8′ suggested the opposite orientation of these two protons. The ROESY correlations between H-3 and H-7″ as well as between H-4 and H-8′ suggested that the orientations of H-7″ and H-8′ were β and α, respectively. The negative Cotton effects at λ max 286.7 and 245.8 nm in the CD spectrum indicated the presence of 2R and 3R in 1 (Slade et al., 2005). Thus, the absolute configurations of all chiral centers of the aglycone part in 1 were determined to be 2R, 3R, 4S, 7″R, and 8″S, respectively.

The absolute configuration of 1 was further confirmed by the quantum chemical CD calculation method (Li et al., 2010). By using MMFF94S force field, a conformational analysis was performed. The resulted global minima conformers were found and used as the basis for the B3LYP/6-311++G(2d,p)//B3LYP/6-31G(d) calculations to finally provide the theoretically predicted CD spectrum with PCM model in CH3CN solvent (Fig. 4) by using Gaussian 09 software. The calculated CD curve of 1 revealed a good agreement with the measured spectrum. Thus, the absolute configuration of 1 was further confirmed as 2R, 3R, 4S, 7″R, and 8″S.

The term flavonostilbene was first applied by Shirataki and coworkers in 1991 to describe the coupling of flavanones and stilbenes. Up to now, about twenty-three flavonostilbenes and one nor-flavonostilbene had been isolated from natural sources (Ayers et al., 2008; Iliya et al., 2003; Linuma et al., 1995, 1994, 1991; Shirataki et al., 1991; Steynberg et al., 1987; Wada et al., 2007). All skeletons of the reported flavonostilbenes were formed by a flavonone and a stilbene through one of the three ring system (rings A–C). It is noteworthy that 1 is the first example of flavonostilbene whose flavanone moiety is coupled with the stilbene moiety through both rings A and C. In addition, all the known flavonostilbenes exist naturally as aglycone form, and 1 represents the first example of flavonostilbene glycoside.

The five known stilbene glycosides were identified as (E)-2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucoside (2) (Chen et al., 1999a, b), (E)-2,3,5,4′-tetrahydroxystilbene-2-O-(6′-O-acetyl)-β-D-glucoside (3) (Chen et al., 2000a), (E)-2,3,5,4′-tetrahydroxystilbene-2,3-di-O-β-D-glucoside (4) (Zhou et al., 1994), (E)-2,3,5,4′-tetrahydroxystilbene-2,3-di-O-β-D-glucoside (5) (Chen et al., 2000b), and (Z)-2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucoside (6) (Xu et al., 2006), respectively, by comparison of their spectroscopic data with those reported in the literature.

The anti-inflammatory activities of compounds 1–6 were investigated. The results showed that compounds 1–3, 5 and 6 could inhibit NO secretion of RAW264.7 cells in response to LPS in a dose dependent manner. Compound 2 displayed the highest inhibitory activity with NO concentration value of 0.89 ± 0.06 μM. However, all the compounds showed weak inhibitory activity against the proliferation of cells.

3. Materials and methods

3.1. General experimental procedures

Optical rotation values were measured on a JASCO P-1020 polarimeter with a 0.01 dm length cell. UV spectra were recorded on a JASCO V-550 UV/VIS spectrophotometer. IR spectra were measured on a JASCO FT/IR-480 plus Fourier transform infrared spectrometer with KBr pellets. CD spectra were obtained on a JASCO J-810 spectropolarimeter at room temperature. 1D and 2D NMR spectra were recorded on a Bruker AV-400 spectrometer with TMS as internal standard. ESI-MS data were obtained on a Finnigan LCQ advantage spectrometer. HR-ESI-MS was conducted on an Agilent 6210 LC/MS/MS TOF mass spectrometer. GC–MS analyses were performed on a Shimadzu GCMS-QP2010 plus gas chromatograph–mass spectrometer. Column chromatographies were carried out on macroporous resin Diaion HP-20 (Mitsubishi Chemical Corporation), silica gel (200–300 mesh; Qingdao Marine Chemical Group Co. Ltd.), ODS (50 μm, 120 Å; YMC), or Sephadex LH-20 (Pharmacia Biotech AB). TLC analyses were performed on precoated silica gel GF254 plates (Yantai Chemical Industrial Institute, Yantai, China). Analytical high-performance liquid chromatography (HPLC) was carried out on an Agilent chromatograph equipped with a 1260 pump and a 1260 Dual λ absorbance detector with a Cosmosil SC18–MS-II reversed-phase column (4.6 mm × 250 mm, 5 μm, Nacalai Tesque, Kyoto, Japan). Preparative HPLC was carried out on an Agilent chromatograph equipped with a 1260 pump and a 1260 Dual λ absorbance detector with a Cosmosil SC18–MS-II reversed-phase column (4.6 mm × 250 mm, 5 μm, Nacalai Tesque, Kyoto, Japan).
a UV/VIS-1260 detector, with a Cosmostat 5 C18-MS-II reversed-phase column (20 mm × 250 mm, 5 μm, Nacalai Tesque, Kyoto, Japan).

3.2. Plant material

The roots of *P. multiflorum* were collected in Deqing county, Guangdong province of China, in October of 2010 and authenticated by Prof. Guang-Xiong Zhou (College of Pharmacy, Jinan University). A voucher specimen (No. 20101003) was deposited in the Institute of Traditional Chinese Medicine & Natural Products, Jinan University, Guangzhou, PR China.

3.3. Extraction and isolation

The air-dried roots of *P. multiflorum* (14.5 kg) were pulverized and extracted with 95% (v/v) EtOH for two times (2 × 25 L, 2 h each time) under reflux. The solution was concentrated under vacuum to yield a crude extract (1200 g). The suspension of the extract in water was partitioned successively with petroleum ether (b.p. 60–90°C), ethyl acetate, and n-butanol, respectively. After removing the solvent, the n-butanol soluble fraction (144 g) was re-dissolved in water and subjected to macroporous resin HP-20 column and eluted successively with water, 30% EtOH, 50% EtOH, 70% EtOH, and 95% EtOH. The 30% EtOH eluate (121 g) was subjected to silica gel column and eluted with gradient mixtures of CHCl3–MeOH (90:10 → 0:100, v/v) to afford ten fractions (Fr.1–Fr.10). Fr.4 (5.6 g) was separated by preparative HPLC on a reversed-phase C18 column using MeOH–H2O (35:65) to give compounds 2 (2 g), 3 (90 mg), and 6 (7 mg), respectively. Fr.6 (7.5 g) was separated by ODS column with MeOH–H2O (10:90 → 100:0) as eluent to give nine subfractions (Fr.6a–Fr.6i). Fr.6e (1.5 g) was further separated by ODS column using MeOH–H2O (15:85 → 45:55) as eluent to obtain compound 4 (16 mg) and a mixture. The mixture (1.1 g) was re-separated by Sephadex LH-20 column (MeOH) and preparative HPLC to yield compounds 1 (10 mg) and 5 (14 mg), respectively.

3.3.1. Polygonumflavan A (1)

Brown oil. [α]D20: -9.46 (c = 0.01, MeOH); IR (KBr): 3444, 1618, 1508, 1462 cm−1. UV λmax (MeOH) nm (log ε): 205 (3.68), 280 (2.77). CD λ (CH3CN) nm (Δε): 245.8 (-8.60), 286.7 (-5.07). 1H NMR (400 MHz, CD3OD) and 13C NMR (100 MHz, CD3OD); see Table 1. HR-ESI-MS: m/z 717.1789 [M+Na]+, calcd for C35H32O12Na: 717.1790.

3.4. Acid hydrolysis and GC analysis of 1

The solution of compound 1 (3 mg) in MeOH was heated in an ampoule with 1.5 mL of 2 N HCl at 80 °C for 4 h. The solution was evaporated with a stream of N2. The reaction mixture was dissolved in H2O and extracted with EtOAc. The aqueous layer was evaporated and then 2 mL of anhydrous pyridine and 3 mg of i-cysteine methyl ester hydrochloride were added to the residue, and the reaction mixture was heated at 60 °C for 2 h. The solution was concentrated to dryness with N2. Furthermore, N-(trimethylsilyl)imidazole (0.2 mL) was slowly added into the reaction mixture and then kept at 60 °C for 1 h. Finally, H2O (1 mL) was added to stop the reaction, and the aqueous layer was extracted with hexane (2 mL). The organic layer was analyzed using gas chromatography (GC) under the following conditions: column, HTSE-30 (0.32 mm × 30 m, 0.5 μm); detector, FID; column temperature, 250 °C; detector temperature, 270 °C; injector temperature, 270 °C; and carrier gas, N2. The standard o-glucose and l-glucose were treated by the same reaction and GC conditions. As a result, o-glucose [Ig (min): 18.866 (reference), 18.870 (sample)] was detected from the hydrolyzate of 1.

3.5. Bioassay of anti-inflammation activity

*In vitro* anti-inflammation activity was evaluated by determining the nitrite concentration in the medium and the proliferation of RAW264.7 cells. Briefly, the RAW264.7 cells (4 × 104 cells) were cultured in the medium with different concentration of samples (0, 12.5, 25, 50, 100 μM) for 48 h in the presence of LPS (200 ng/mL). One hundred microliters of each supernatant was mixed with 100 μL of Griess reagent and incubated at room temperature for 15 min. The absorbance was measured at 540 nm with a microplate reader (Thermo, USA). The cell proliferation was determined in fresh medium by MITT method.

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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.phyto.2012.08.007

References


