Phytochemistry Letters 5 (2012) 756–760

Contents lists available at SciVerse ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

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

Li-Li Chen [a,b,1], Xiao-Jun Huang [a,b,c,1], Man-Mei Li [a,b], Guo-Min Ou [a,b], Bing-Xin Zhao [a,b], Min-Feng Chen [a,b], Qing-Wen Zhang [c], Ying Wang [a,b,*], Wen-Cai Ye [a,b,**]

[a] Institute of Traditional Chinese Medicine and Natural Products, College of Pharmacy, Jinan University, Guangzhou 510632, PR China
[b] Guangdong Province Key Laboratory of Pharmacodynamic Constituents of TCM and New Drugs Research, Jinan University, Guangzhou 510632, PR China
[c] State Key Laboratory of Quality Research in Chinese Medicine (University of Macau) and Institute of Chinese Medical Sciences, University of Macau, Macao SAR, PR China

ARTICLE INFO

Article history:
Received 26 April 2012
Received in revised form 3 August 2012
Accepted 19 August 2012
Available online 30 August 2012

Keywords:
Polygonum multiflorum
Flavanostilbene
Stilbene glycoside
Polygonflavanol A
Anti-inflammatory

ABSTRACT

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.

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-inflammoatory 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-inflammoatory (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 $[\alpha]_D^{19}$: +9.46 (*c* 0.01, MeOH). The HR-ESI-MS showed a quasi-molecular ion at *m/z* 717.1789 $[M+Na]^+$ (calcd for $C_{35}H_{34}O_{15}Na$: 717.1790), which is consistent with a molecular formula of $C_{35}H_{34}O_{15}$. 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

* Corresponding author at: Institute of Traditional Chinese Medicine & Natural Products, College of Pharmacy, Jinan University, Guangzhou 510632, PR China.
** Corresponding author at: Institute of Traditional Chinese Medicine & Natural Products, College of Pharmacy, Jinan University, Guangzhou 510632, PR China. Tel.: +86 20 85220936; fax: +86 20 85221559.
E-mail address: chywc@yahoo.com.cn (W.-C. Ye).
[1] These authors contributed equally to this work.

1874-3900/$ – see front matter © 2012 Phytochemical Society of Europe. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.phytol.2012.08.007

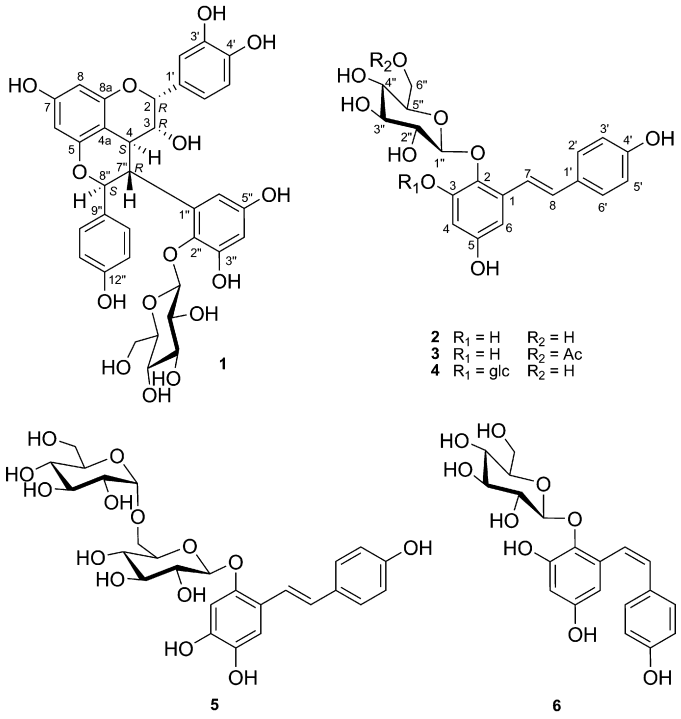

Fig. 1. Chemical structures of compounds **1–6**.

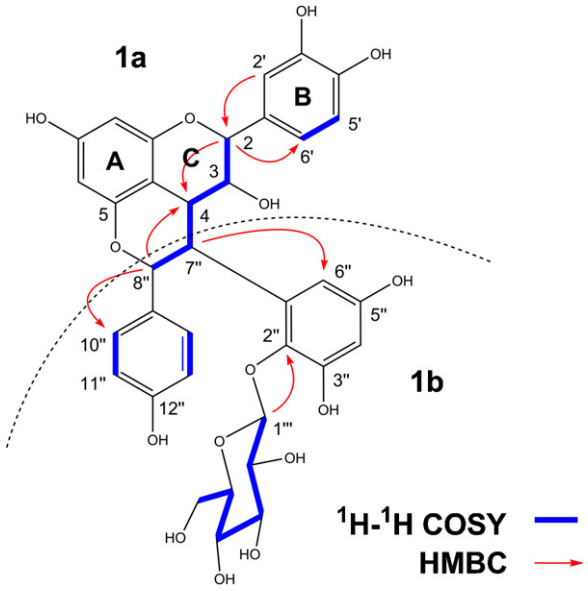

Fig. 2. Key ^{1}H–^{1}H COSY and HMBC correlations of **1**.

signals for a *para*-disubstituted benzene ring [δ_H 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 [δ_H 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 [δ_H 6.09 (1H, 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 [δ_H 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 ^{1}H NMR spectrum of **1** displayed the signals due to an anomeric proton [δ_H 4.46 (1H, d, J = 7.9 Hz, H-1‴)] and a hydroxymethyl group [δ_H 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 D-glucose, which was identified by GC analysis. The β-configuration of D-glucose was determined based on the $^{3}J_{H1,H2}$ coupling constant of the anomeric proton (J = 7.9 Hz). The ^{13}C NMR and DEPT-135 spectra of **1** revealed the presence of thirty-five carbon signals, including twenty-four aromatic carbons, three oxy-methines and two alkyl-methines, as well as a β-D-glucopyranosyl moiety. All above information suggested that **1** possessed a flavonone moiety (**1a**) and a stilbene unit (**1b**). The ^{1}H–^{1}H COSY, HSQC, and HMBC spectra of **1** allowed the full assignment of all proton and carbon signals (Table 1).

The ^{1}H and ^{13}C 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′-tetrahydroxystilbene 2-*O*-β-D-glucopyranoside (**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 ^{1}H–^{1}H 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
^{1}H and ^{13}C NMR spectral data of compound **1** (CD_3OD, J in Hz).[a,b]

Position	δ_C	δ_H	Position	δ_C	δ_H
2	80.9	4.97 d (3.8)	1″	136.8	–
3	71.9	4.18 dd (7.0, 3.8)	2″	139.1	–
4	41.3	3.02 dd (11.3, 7.0)	3″	151.7	–
5	157.1	–	4″	103.3	6.09 d (2.6)
6	96.8	5.93 d (2.2)	5″	155.9	–
7	158.9	–	6″	106.1	6.07 d (2.6)
8	96.4	6.01 d (2.2)	7″	40.5	4.30 t (10.4)
8a	156.9	–	8″	85.8	5.03 d (10.4)
4a	102.0	–	9″	132.0	–
1′	131.4	–	10″, 14″	130.6	7.08 d (8.5)
2′	115.7	6.89 s	11″, 13″	115.5	6.58 d (8.5)
3′	146.0	–	12″	158.0	–
4′	146.0	–	1‴	107.7	4.46 d (7.9)
5′	116.0	6.71 d (8.5)	2‴	75.4	3.46
6′	120.1	6.73 d (8.5)	3‴	78.3	3.34
			4‴	70.8	3.43
			5‴	78.5	3.37
			6‴	62.3	3.79 d (12.0) 3.72 dd (12.0, 4.3)

[a] Assignments were established by interpretation of the ^{1}H–^{1}H COSY, HSQC, and HMBC spectra.
[b] Overlapped signals are reported without designating multiplicity.

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-D-glucopyranosyl 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 (−)-*epi*-catechin (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 (Friebolin, 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 2*R* and 3*R* in **1** (Slade et al., 2005). Thus, the absolute configurations of all chiral centers of the aglycone part in **1** were determined to be 2*R*, 3*R*, 4*S*, 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 CH_3CN 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 2*R*, 3*R*, 4*S*, 7″*R*, and 8″*S*.

The term flavonostilbene was first applied by Shirataki and co-workers 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.

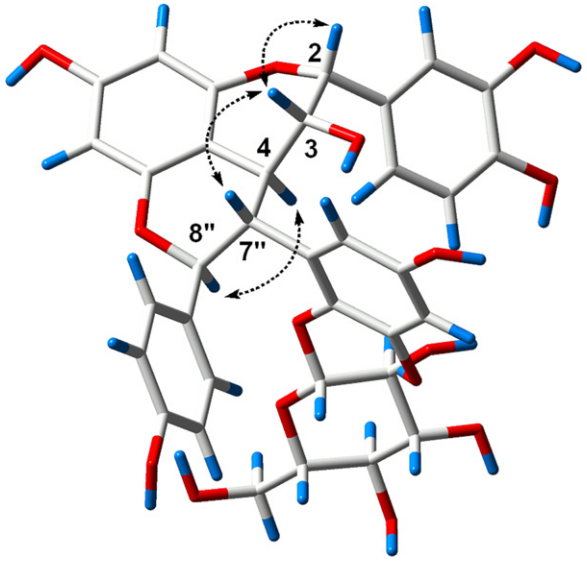

Fig. 3. Key ROESY correlations of **1**.

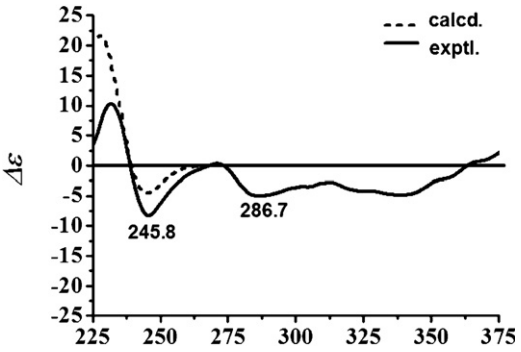

Fig. 4. Calculated and experimental CD spectra of **1**.

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-*O*-(6″-*O*-α-D-glucoside)-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 respond 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/MSD 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 pre-coated silica gel GF_{254} plates (Yantai Chemical Industrial Institute, Yantai, China). Analytical high-performance liquid chromatography (HPLC) was carried out on a Agilent chromatograph equipped with a 1260 pump and a 1260 Dual λ absorbance detector with a Cosmosil $5C_{18}$-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 UV/VIS-1260 detector, with a Cosmosil 5 C_{18}-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 macrosporous 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 $CHCl_3$–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 C_{18} column using MeOH–H_2O (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–H_2O (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–H_2O (15:85 → 45:55) as eluent to obtain compound **4** (16 mg) and a mixture. The mixture (1.1 g) was reseparated by Sephadex LH-20 column (MeOH) and preparative HPLC to yield compounds **1** (10 mg) and **5** (14 mg), respectively.

3.3.1. Polygonflavanol A (*1*)

Brown oil. $[\alpha]_D^{19}$: +9.46 (*c* = 0.01, MeOH). IR (KBr): 3444, 1618, 1508, 1462 cm^{-1}. UV λ_{max} (MeOH) nm (log ε): 205 (3.68), 280 (2.77). CD λ (CH_3CN) nm (Δε): 245.8 (−8.60), 286.7 (−5.07). ^1H NMR (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD): see Table 1. HR-ESI-MS: *m/z* 717.1789 $[M+Na]^+$, calcd for $C_{35}H_{34}O_{15}Na$: 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 N_2. The reaction mixture was dissolved in H_2O and extracted with EtOAc. The aqueous layer was evaporated and then 2 mL of anhydrous pyridine and 3 mg of L-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 N_2. Furthermore, *N*-(trimethylsilyl)imidazole (0.2 mL) was slowly added into the reaction mixture and then kept at 60 °C for 1 h. Finally, H_2O (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, HT-SE-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, N_2. The standard D-glucose and L-glucose were treated by the same reaction and GC conditions. As a result, D-glucose [t_R (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 (4×10^4 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 MTT method.

Acknowledgments

This work was supported financially by the Program for Changjiang Scholars and Innovative Research Team in the University (No. IRT0965), the Joint Fund of NSFC-Guangdong Province (U0932004), the Team Project of Natural Science Foundation of Guangdong Province (No. 8351063201000003), and the Science and Technology Development Fund of Macau Special Administrative Region (No. 013/2008/A1).

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.2012.08.007

References

Ayers, S., Zink, D.L., Brand, R., et al., 2008. Struthiolanone: a flavanone-resveratrol adduct from *Struthiola argentea*. Nat. Prod. Commun. 3, 189–192.
Chen, Y., Wang, M.F., Rosen, R.T., et al., 1999a. 2,2-Diphenyl-1-picrylhydrazyl radical-scavenging active components from *Polygonum multiflorum* Thunb. J. Agric. Food Chem. 47, 2226–2228.
Chen, W.S., Liu, W.Y., Yang, G.J., et al., 2000a. Structural elucidation of a new tetrahydroxystilbene of Radix *Polygoni Multiflori* Preparata and study on its cardiovascular activity. Acta Pharmacol. Sin. 35, 906–908.
Chen, W.S., Yang, G.J., Zhang, W.D., et al., 2000b. Two new compounds of Radix *Polygoni Multiflori Preparata*. Acta Pharmacol. Sin. 35, 273–276.
Chen, W.S., Fan, W., Yang, G.J., et al., 1999b. Studies on the chemical constituents of Radix *Polygoni multiflori Preparata*. Acad. J. Second Mil. Med. Univ. 20, 438–440.
Friebolin, H., 1993. Basic One- and Two-dimensional NMR Spectroscopy, 2nd ed. VCH, New York.
Hwang, Y.B., Kim, H.S., Lee, J.H., et al., 2001. Antioxidant benzoylated flavan-3-ol glycoside from *Celastrus orbiculatus*. J. Nat. Prod. 64, 82–84.
Iliya, I., Tanaka, T., Ali, Z., et al., 2003. Six flavonostilbenes from *Gnetum afircanum* and *Gnetum gnemon*. Heterocycles 60, 159–166.
Li, X.C., Ferreira, D., Ding, Y.Q., 2010. Determination of absolute configuration of natural products: theoretical calculation of electronic circular dichroism as a tool. Curr. Org. Chem. 14, 1678–1697.
Lin, H.W., Sun, M.X., Wang, Y.H., et al., 2010. Anti-HIV activities of the compounds isolated from *Polygonum cuspidatum* and *Polygonum multiflorum*. Planta Med. 76, 889–892.
Linuma, M., Ohyama, M., Tanaka, T., 1995. Six flavonostilbenes and a flavanone in roots of *Sophora Alopecuroides*. Phytochemistry 38, 519–525.
Linuma, M., Ohyama, M., Tanana, T., et al., 1994. A flavonostilbene and two stilbene oligomers in roots of *Sophora leachiana*. Phytochemistry 37, 1157–1159.
Linuma, M., Tanaka, T., Kawai, M., et al., 1991. Two complex flavanones from the roots of *Sophora leachiana*. Phytochemistry 30, 3773–3775.
Lv, L.S., Gu, X.H., Tang, J., et al., 2007. Antioxidant activity of stilbene glycoside from *Polygomum multiflorum* Thunb. *in vivo*. Food Chem. 104, 1678–1681.
Ryu, G., Ju, J.H., Park, Y.J., et al., 2002. The radical scavenging effects of stilbene glucosides from *Polygonum multiflorum*. Arch. Pharm. Res. 25, 636–639.
Shirataki, Y., Noguchi, M., Yokoe, I., Tomimori, T., et al., 1991. Sophoraflavanones H, I and J, flavnostilbenes from *Sophora moorcroftiana*. Chem. Pharm. Bull. 39, 1568–1572.
Slade, D., Ferreira, D., Marais, J.P.J., 2005. Circular dichroism, a powerful tool for the assessment of absolute configuration of flavonoids. Phytochemistry 66, 2177–2215.
Steynberg, J.P., Ferreira, D., Roux, D.G., 1987. Synthesis of condensed tannins. Part 18. Stilbenes as potent nucleophiles in regio- and stereo-specific condensations: novel guibourtinidol-stilbenes from *Guibourtia coleosperma*. J. Chem. Soc. Perkin Trans. 1: Org. Bio-Org. Chem. (1972–1999), 1705–1712.
Wada, S.I., Yasui, Y., Hitomi, T., et al., 2007. Structures and radical-scavenging activities of phenolic constituents from the bark of *Picea jezoensis* var. *jezoensis*. J. Nat. Prod. 70, 1605–1610.

Wang, M.J., Zhao, R.H., Wang, W.G., et al., 2012. Lipid regulation effects of *Polygoni Multiflori* Radix, its processed products and its major substances on steatosis human liver cell line L02. J. Ethnopharmacol. 139, 287–293.

Wu, X.Q., 2009. General review on chemical constituents and pharmacological effects of Radix *Polygoni multiflori*. LiShiZhen Med. Mater. Med. Res. 20, 146–147.

Xu, M.L., Zheng, M.S., Lee, Y.K., et al., 2006. A new stilbene glucoside from the roots of *Polygonum multiflorum* Thunb. Arch. Pharm. Res. 29, 946–951.

Zhang, Y.Z., Shen, J.F., Xiao, J.H., et al., 2007. Inhibitory effects of 2,3,5,4′-tetrahydroxystilbene-2-*O*-*β*-D-glucoside on experimental inflammation and cyclooxygenase 2 activity. J. Asian Nat. Prod. Res. 9, 355–363.

Zhang, Z.G., Lv, T.S., Yao, Q.Q., 2008. The research progress of *Polygonum multiflorum* Thunb. Pharm. J. Chin. 62–64 PLA 24.

Zhou, L.X., Lin, M., Li, J.B., et al., 1994. Chemical studies on the ethyl acetate insoluble fraction of the roots of *Polygonum mutifloum* Thunb. Acta Pharmacol. Sin. 29, 107–110.