New phenolic constituents obtained from *Polygonum multiflorum*

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

**Objective:** To isolate the phenolic compounds obtained from the dried roots of *Polygonum multiflorum* and investigate their pharmacological activities.

**Methods:** The chemical constituents were isolated and purified by combining them with a macroporous resin (DM-8), MCI gel, and Sephadex LH-20 and by performing ODS column chromatography. Their structures were elucidated by 1D and 2D NMR analyses, as well as mass spectrometry. The isolated compounds were evaluated to determine their hepatoprotective and α-glucosidase inhibitory activities in vitro.

**Results:** Two phenolic compounds, namely, polygonimitin E (1) and polygonimitin F (2), were isolated from the dried roots of *P. multiflorum*. Compound 2 (10 μmol/L) only showed moderate hepatoprotective activity against N-acetyl-p-aminophenol (APAP)-induced HepG2 cell damage. Unfortunately, these two compounds exhibited no α-glucosidase inhibitory activity.

**Conclusion:** Compounds 1 and 2 were new compounds. Compound 2 could be one of the potential hepatoprotective constituents of *P. multiflorum*.

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1. Introduction

*Polygonum multiflorum* Thunb. is a well-known traditional Chinese medicine (TCM), commonly used to treat a variety of different conditions, including coronary heart disease, hyperlipidemia, neurosis, hair loss prevention, and premature graying (The State Pharmacopoeia Commission, 2015). Several previous reports have shown that *P. multiflorum* contains many secondary metabolites, such as anthraquinones, naphthalenes, stilbenoids, flavonoids, phenolic acids (Lin et al., 2015; Zhang and Cui, 2016; Yuan, Gao, Yang, & Wang, 2017) and dianthrones (Yang et al., 2016, 2017, 2018a, 2019). Furthermore, extracts of *P. multiflorum*, which are rich in phenolic constituents, have been reported to exhibit hepatoprotective effects and a good inhibitory activity towards α-glucosidase (Lin et al., 2015; Yang, Zhao, Liu, Song, & Liu, 2014; Yang et al., 2017). As part of our ongoing research program towards the identification of bioactive constituents of *P. multiflorum*, we describe the isolation and structural elucidation of two new compounds, namely, polygonimitin E (1) and polygonimitin F (2), from a 70% ethanol extract of *P. multiflorum* (Fig. 1). The hepatoprotective and α-glucosidase inhibitory activity of these two new compounds was also evaluated and described in this paper.

2. Materials and methods

2.1. General experimental procedures

The optical rotations were observed with a Jasco P-2000 polarimeter (Jasco Inc., Tokyo, Japan). The UV data were recorded using a Jasco V-650 spectrophotometer (Jasco Inc.). The IR spectra were measured with a Nicolet iN 10 Micro FTIR spectrophotometer (Thermo Nicolet Inc., Waltham, MA, USA). The NMR spectra were recorded with Varian Inova-300, 500 and 600 spectrophotometers (Varian Inc., Palo Alto, CA, USA). The HR-ESI-MS data were obtained using an Agilent 1100 UPLC-Q-TOF mass spectrometer (Agilent Technologies Ltd., Santa Clara, CA, USA). Column chromatography was performed with MCI gel (70–150 μm; Mitsubishi Chemical Corp., Tokyo, Japan), Sephadex LH-20 resin (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) and a reversed-phase C18 silica gel (40–60 μm, Alltech, Deerfield, IL, USA). Preparative high-performance liquid chromatography (HPLC) separations were...
carried out using a Shimadzu LC-10 A system equipped with a YMC-Pack ODS-A column (250 × 20 mm, 5 μm; Kyoto, Japan) and a Shimadzu SPD-20 A detector (Shimadzu).

2.2. Plant material

The dried roots of *P. multiflorum* were collected from Deqing in Guangdong Province, China in October 2012 and were identified by associate Professor Ji Zhang (Research and Inspection Center of Traditional Chinese Medicine and Ethnomedicine, National Institutes for Food and Drug Control, State Food and Drug Administration). A voucher sample of the roots (No. 060104) was deposited at the Research and Inspection Center of Traditional Chinese Medicine and Ethnomedicine, National Institutes for Food and Drug Control, State Food and Drug Administration, Beijing, China.

2.3. Extraction and isolation

The dried roots of *P. multiflorum* (28.0 kg) were refluxed three times with 70% EtOH in water. The combined EtOH extracts were concentrated under reduced pressure below 50 °C to give a residue (4.0 kg), which was suspended in 1.5 L of H2O and extracted with CHCl3. The H2O fraction (3.5 kg) was purified over the macroporous resin (DM-8) by eluting with a gradient of water and EtOH (H2O, 25% EtOH, 40% EtOH, 55% EtOH and 95% EtOH) to give five fractions [A-E; fraction A (2.0 kg); fraction B (62.0 g); fraction C (12.0 mg); fraction D (38.0 g); fraction E (55.0 g)]. Fraction D was purified by column chromatography over an RP-18 silica gel using a stepwise gradient elution of methanol in water (from 10% to 100%) to give six fractions (fractions M1-M6). Fraction M2 (1.2 g) was separated over a Sephadex LH-20 column (200.0 g); fraction D (38.0 g); fraction E (55.0 g). Fraction D was purified by preparative HPLC (CH3CN/H2O, 25:75; 100% MeOH) to give five subfractions (M2S1-M2S5). M2S2 (145.0 mg) was purified by preparative HPLC (12.0 mg, 45.0 min). M2S4 (145.0 mg) was purified by preparative HPLC (CH3CN/H2O, 40:60; YMC column, 250 × 20 mm, particle size 5-5 μm; detection, 220 nm; flow rate, 6.0 mL/min) to yield compound 1 (12.0 mg, 45.0 min).

2.4. Acid hydrolysis and sugar analysis

The absolute configuration of sugars was determined according to the methods shown in previous reports (Tian et al., 2013; Ma, Li, Yang, Sun, & Zhang, 2017). The analysis of the acid hydrolysates revealed the presence of D-glucose and D-apiose, which was determined by comparing the retention times (tR) of their derivatives with those of the authentic sugars derivatized in the same way.

2.5. Hepatoprotective activity assay

Human HepG2 hepatoma cells were cultured in DMEM fortified with 100 U/mL penicillin, 10% fetal calf serum, and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere of 5%
CO₂ + 95% air. Then, the cells were passaged with 0.25% trypsin in 0.02% EDTA. An MTT assay was used to assess the cytotoxicity of the test samples. The cells were all seeded in 96-well plates. After overnight incubation at 37 °C with 5% CO₂, APAP (final concentration of 8 mmol/L) and 10 μmol/L test samples were added into the wells and incubated for another 48 h. Then, 100 μL of 0.5% CMC MT was added to each well after the withdrawal of the culture medium and incubated for an additional 4 h. The resulting formazan was dissolved in 150 μL of DMSO after the aspiration of the culture medium. The plates were placed on a plate shaker for 30 min and read immediately at 570 nm using a microplate reader (μquant Biotek, Biotek instruments, Inc., Winooski, VT, USA) to determine the OD value (Wang et al., 2016; Ma et al., 2017).

2.6. α-Glucosidase inhibition assay

The α-glucosidase inhibitory activities of the two compounds isolated in this study were determined according to previously reported methods (Kim, Nam, Kurihara, & Kim, 2008; Dang et al., 2015), with minor modifications. Then, 3 mmol/L PNPG (p-nitrophenyl-alpha-D-glucopyranoside) (20 μL), α-glucosidase (20 μL, 1 U/mL in PBS, pH 6.8), PBS (50 μL), and the sample solution (10 μL) were placed in a 96-well microplate and preincubated for 15 min at 37 °C. Next, 0.4 mol/L Na₂CO₃ (50 μL) was added, and the mixture was incubated additionally for 35 min. The absorbance of each well was measured at 400 nm with a microplate spectrophotometer.

3. Results

3.1. Characterization

Compound 1 was isolated as a yellow amorphous powder, and its molecular formula was determined to be C₃₀H₄₀O₁₃ by HR-ESI-MS (m/z 563.1755 [M + Na⁺], suggesting ten degrees of unsaturation. The IR spectrum of 1 showed the presence of hydroxyl (3392 cm⁻¹), carbonyl (1630 cm⁻¹) and benzene groups (1586 cm⁻¹). The ¹H NMR (Table 1) and ¹³C NMR (Fig. 2) spectra of 1 showed aromatic signals belonging to one set of meta-coupled aromatic protons (δ H 6.91 (1H, d, J = 1.8 Hz, H-5); 7.00 (1H, d, J = 1.8 Hz, H-7)); one set of uncoupled aromatic protons [δ H 7.10 (1H, s, H-4)]; two methyl groups [δ H 2.52 (3H, s, Me-12); 2.24 (3H, s, Me-13)]; and one methoxy group at δ H 3.84 (3H, s, OCH₃-6). The anomic signals at δ H 4.85 (1H, d, J = 3.0 Hz, H-1') and 5.06 (1H, d, J = 7.2 Hz, H-1’) suggested the existence of two glycosyl residues. One of residues was assumed to be an apiofuranosyl unit based on the chemical shift of its anomeric signal. We also observed two pairs of doublets at δ H 3.62 (1H, d, J = 9.6 Hz) and 3.92 (1H, d, J = 9.6 Hz) and at δ H 3.41 (1H, d, J = 9.6 Hz) and 3.39 (1H, d, J = 9.6 Hz), which were attributed to the two oxygenated methylene groups CH₂-4’ and CH₂-5’ respectively (Kanchanapoom, kasai, & Yamasaki, 2002). The ¹³C NMR and DEPT spectra of 1 showed a total of 25 carbon signals (Table 1). Ten carbon signals were assigned to the naphthalene ring, along with two methyl groups (δ C 32.7, 20.0), one methoxyl group (δ C 55.8) and one carboxyl group (δ C 204.9). The remaining 11 carbon signals were assigned to the two glycosyl moieties. In addition to one apiofuranosyl unit (δ C 109.9), the other sugar residue was determined to be a glucosyl moiety based on the chemical shift of its anomeric carbon (δ C 103.1). The composition of this sugar was further determined by the GC analysis of the acid hydrolysate of 1, which confirmed the presence of D-apiosyl and D-glucosyl residues. The nature of the connection between these units was established based on HMBC experiments (Fig. 2). The molecular weight and NMR spectroscopic data obtained for 1 were compared with those of torachrysone-8-O-D-glucoside (Li, Liu, Liao, Xu, & Wei, 2006), confirming the presence of a D-apiosyl residue in 1. The HMBC analysis revealed a correlation from H-1’ to C-6’, which indicated

![Fig. 2. Key ¹H-¹H COSY and HMBC correlations of compound 1.](image-url)
that the D-apioside residue was attached to the C-6' position of the glucopyranoside moiety. A further HMBC correlation from H-1' to C-8 suggested that the sugar moiety in 1 was attached to the C-8 position. The detailed analysis of the HSQC and HMBC data led to the structural determination of 1 as torachrysone-8-O-β-D-apiofuranosyl (1→6)-β-D-glucopyranoside, as shown in Fig. 1, which revealed the trivial name polygonitin E.

Compound 2 was isolated as a colorless amorphous powder, and its molecular formula was determined to be C54H82N2O12 by HR-ESI-MS in the positive ionization mode (m/z 936.3686, [M + H]+). The IR spectrum showed absorptions at 3244, 1654, 1612 and 1515 cm⁻¹, indicating the presence of hydroxyl and amide groups. The ¹H NMR (Table 2) and ¹H–¹H COSY spectra (Fig. 2) displayed resonance signals characteristic of three tyramine moieties [δH 8.21 (1H, t, J = 5.4 Hz, 𝛼-NH), 3.23–3.25, (2H, m, H-8'''), 2.62 (2H, t, J = 7.2 Hz, H-7'''); 7.74 (1H, t, J = 5.4 Hz, 𝛽-NH), 3.01–3.11, (2H, m, H-8'''), 2.39 (2H, t, J = 7.2 Hz, H-7'''); 7.75 (1H, d, J = 5.4 Hz, γ-NH)], 3.20–3.23, (2H, m, H-8'''), 2.45–2.50 (2H, m, H-8''').

Three para-substituted Ph groups [δH 6.84 (2H, d, J = 8.4 Hz, H-2''/6''); 6.54 (2H, d, J = 8.4 Hz, H-3''/5''); 6.97 (2H, d, J = 8.4 Hz, H-2''/6''); 6.66 (2H, d, J = 8.4 Hz, H-3''/5''); 6.82 (2H, d, J = 8.4 Hz, H-2''/6''); 6.62 (2H, d, J = 8.4 Hz, H-3''/5'')] were determined by the HMBC analysis, which revealed correlations between H-7 to C-1, C-7 and C-9 based on the HMBC correlations from H-8 to C-1, C-7 and C-9. Olefinic C-7 was determined to be linked to the C-1 of the 1,3,4,6-tetrasubstituted benzene ring based on the HMBC correlations from H-8 to C-2', C-6', C-8' and C-9'. C-8' was determined to be linked to C-8 and C-9' based on the HMBC correlations from H-8' to C-3', C-7' and C-9'. Olefinic carbon C-7' was found to be linked to C-1' of the 1,3,4-trisubstituted benzene ring based on the HMBC correlations from H-7' to C-2', C-6', C-9' and C-8'; and H-5' to C-3' and C-7'. Three OMe groups were determined to be linked to C-3, C-3' and C-3'', respectively, based on the HMBC correlations. The correlations between C-9, C-9' and C-9'' positions and the p-tyramine moieties were determined by the HMBC analysis, which revealed correlations between H-8''/C-9, H-8'''/C-9' and H-8'''/C-9''. Further 2D NMR experiments confirmed that compound 3 adopted a planar structure, which was similar to the structures of melogenamide D and thoreliamide C.

The relative structure of 2 was confirmed based on the results of a 1D NOESY experiment. Significant NOE enhancements (Fig. 3) were observed between H-7' and H-8'. The relative configuration of H-7' and H-8' was determined to be cis. The relative structure of 2 was therefore established as shown in Fig. 1 and characterized as (1R,2R)-1,2-dihydro-1-(4-hydroxy-3'-methoxyphenyl)-7-[((1Z)-2-(4-hydroxy-3-methoxyphenyl)-1-[[2-(4-hydroxyphenyl)ethyl]amino]carbonyl]ethenyl][oxy]-N,N,N'-bis[2-(4-hydroxyphenyl)ethyl]6-methoxy-2,3-naphthalenedicarboxamide, which was also given the trivial name polygonitin F.

3.2. Hepatoprotective effect of compounds 1 and 2

Compounds 1 and 2 were tested to determine their hepatoprotective effects against N-acetyl-p-aminophenol (APAP)-induced HepG2 cell damage (human hepatocellular liver carcinoma cell line) cells, and the hepatoprotective drug bicyclol was used as the positive control. At 10 μmol/L, the positive control bicyclol yielded a 41.27% (P < 0.05) survival rate. Compound 2 could reduce APAP-induced HepG2 cells damage by increasing the survival rate from 33.22% (P < 0.001) to 42.26% (P < 0.05) at 10 μmol/L. However, the survival rate of compound 1 was 30.56%.

3.3. α-Glucosidase inhibition activity of compounds 1 and 2

Acarbose was used as the positive control. At the concentration of 10 μmol/L, the inhibitions of compounds 1 and 2 were 13.8% and 12.3%, respectively.

4. Discussion

The traditional medicinal herb P. multiflorum has been widely used in East Asia since ancient times for the treatment of diseases and for its hair-blacking, anti-aging, anti-diabetic, anti-hyperlipidemia, antioxidant, anti-inflammatory, anticancer, hepatoprotective and immunomodulating effects. However, the toxicity of P. multiflorum, especially its adverse hepatic effect, was constantly reported in China, Korea, Australia and other countries (Li et al., 2019; Lin et al., 2015; Liu et al., 2018). Thus, it is necessary to elucidate the active and toxic components to avoid adverse reactions and improve the safety of P. multiflorum. It had been reported that the anthraquinones and dianthrones present in P. multiflorum may be related to the hepatotoxicity described in previous studies (Yang et al., 2018b; Wang et al., 2017). What are the active ingredients for protecting the liver and reducing blood sugar? In this paper, the results showed that compound 2 only showed a moderate hepatoprotective activity.
Unfortunately, these two compounds exhibited no α-glucosidase inhibitory activity.

5. Conclusion

According to the literature (Yang et al., 2017; Wang and Yao 2016), stilbenes may be potential α-glucosidase inhibitors. Accordingly, stilbenes could be given more attention to obtain α-glucosidase inhibitors from P. multiflorum. Phenolic compounds, such as compound 1, which belongs to a class of cinnamic acid amides and lignanamides, may be a potential hepatoprotective constituent according to the results in this paper. Therefore, we may need to concentrate on these phenolic compounds and isolate more similar compounds to systematically evaluate their hepatoprotective activity in the future. This will be conducive to further determining whether these phenolic compounds could be potential hepatoprotective ingredients in P. multiflorum.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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