Chinese Herbal Medicines 12 (2020) 342–346

Contents lists available at ScienceDirect

Chinese Herbal Medicines

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

Original Article

New phenolic constituents obtained from *Polygonum multiflorum*

Jian-bo Yang [a], Hua Sun [b], Jie Ma [b], Yun-fei Song [a], Yue Liu [a], Qi Wang [a], Shuang-cheng Ma [a,*], Xian-long Cheng [a], Feng Wei [a,*]

[a] *Research and Inspection Center of Traditional Chinese Medicine and Ethnomedicine, National Institutes for Food and Drug Control, State Food and Drug Administration, Beijing 100050, China*

[b] *State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China*

ARTICLE INFO

Article history:
Received 11 October 2019
Revised 10 January 2020
Accepted 24 February 2020
Available online 2 July 2020

Keywords:
α-glucosidase inhibition
hepatoprotective activity
phenolic compounds
polygonimitin E
polygonimitin F
Polygonum multiflorum Thunb

ABSTRACT

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*.

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 a-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 C_{18} silica gel (40–60 μm, Alltech, Deerfield, IL, USA). Preparative high-performance liquid chromatography (HPLC) separations were

* Corresponding authors.
E-mail addresses: masc@nifdc.org.cn (S.-c. Ma), weifeng@nifdc.org.cn (F. Wei).

https://doi.org/10.1016/j.chmed.2020.02.001
1674-6384/

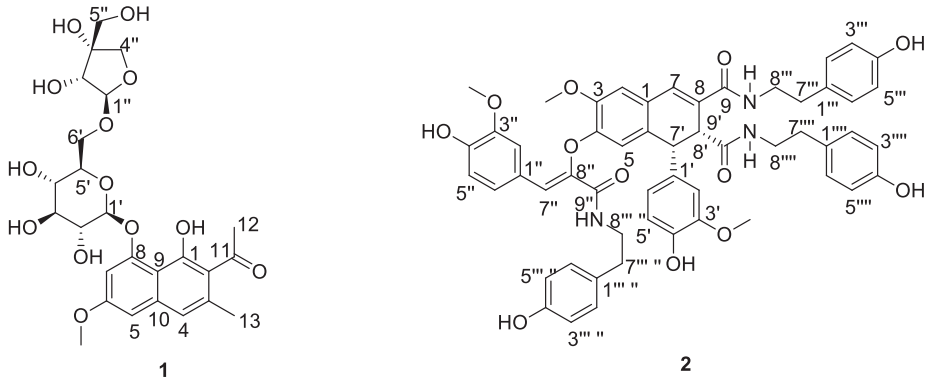

Fig. 1. Structures of compounds **1** and **2** from roots of *P. multiflorum*.

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 H_2O and extracted with CH_2Cl_2. The H_2O fraction (3.5 kg) was purified over the macroporous resin (DM-8) by eluting with a gradient of water and EtOH (H_2O, 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 (200.0 g); 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 (A_1, 0.5 g; A_2, 2.8 g; A_3, 15.8 g; A_4, 4.5 g; A_5, 7.7 g; A_6, 3.2 g). Fractions A_1-A_2 (3.3 g) were separated by column chromatography over MCI gel (2 × 20 cm, 500 g; MeOH/H_2O, 0:1 to 1:0) to give six fractions (fractions M1-M6). Fraction M2 (1.2 g) was separated over a Sephadex LH-20 column (100% MeOH) to give five subfractions (M2S1-M2S5). M2S2 (267 mg) was purified by preparative HPLC (CH_3CN/H_2O, 25:75; YMC column, 250 × 20 mm, particle size, S-5 μm; detection, 220 nm; flow rate, 6.0 mL/min) to yield compound **1** (12.0 mg, 45.0 min). M2S4 (145.0 mg) was purified by preparative HPLC (CH_3CN/H_2O, 40:60; YMC column, 250 × 20 mm, particle size S-5 μm; detection, 220 nm; flow rate, 6.0 mL/min) to yield compound **2** (4.0 mg, 2.5 min).

Polygonimitin E (**1**): Yellow powder; [α]25 D −3.5° (c = 1.0, MeOH); UV (MeOH) λmax (logε): 225 (3.34), 264 (3.04), 295 (2.92) nm; IR (KBr) ν_{max}: 3392, 2928, 1630, 1586, 1449, 1399, 1359, 1260, 1202, 1166, 1072 cm^{-1}; ^1H NMR (DMSO d_6, 600 MHz) and ^{13}C NMR (DMSO d_6, 150 MHz) data, see Table 1; HRESI-MS: *m/z* 563.1755 $[M + Na]^+$ (calcd for $C_{25}H_{32}NaO_{13}^+$, 563.1735, error of -3.56×10^{-6}).

Table 1
^1H NMR (600 MHz) and ^{13}C NMR (150 MHz) data for compound **1** were measured in DMSO d_6.

Positions	δ_H (*J*)	δ_C
1		151.5
2		123.7
3		134.1
4	7.10, s	119.3
5	6.91, d (1.8)	101.7
6		158.8
7	7.00, d (1.8)	103.5
8		155.8
9		109.0
10		137.3
11		204.9
12	2.52, s	32.7
13	2.24, s	20.0
6-OMe	3.84, s	55.8
1′	5.06, d (7.2)	103.1
2′	3.35–3.40, m	73.7
3′	3.35–3.40, m	76.7
4′	3.15–3.19, m	70.7
5′	3.64–3.68, m	76.4
6′	3.51, dd (11.4, 7.2); 3.92, m	68.1
1″	4.85, d (3.0)	109.9
2″	3.78, brs	76.6
3″		79.3
4″	3.62, d (9.6);3.92, d (9.6);	73.9
5″	3.41, d (9.6); 3.39, d (9.6)	64.0

Polygonimitin F (**2**): Colorless amorphous powder; [α]25 D 0° (c = 1.0, MeOH); UV (MeOH) λmax (logε): 226 (4.05), 286 (3.76), 324 (3.79) nm; IR (KBr) ν_{max}: 3244, 2938, 1655, 1612, 1515, 1453, 1271, 1132, 1026, 1003, 825 cm^{-1}; ^1H NMR (DMSO d_6, 600 MHz) and ^{13}C NMR (DMSO d_6, 150 MHz) data, see Table 2; HRESI-MS: *m/z* 936.3668 $[M + H]^+$ (calcd for $C_{54}H_{54}N_3O_{12}^+$, 936.3702, error of -4.7×10^{-6})

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 (t_R) 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%

Table 2
^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) data for compound **2** were measured in DMSO.

No.	δ_H (*J*)	δ_C	No.	δ_H (*J*)	δ_C	No.	δ_H (*J*)	δ_C
1		126.9	1′		134.6	1″		124.1
2	7.05, s	112.6	2′	6.53, d (1.8)	112.0	2″	7.20, d (1.8)	113.2
3		147.7	3′		147.8	3″		147.5
4		146.0	4′		145.3	4″		148.3
5	6.47, s	114.5	5′	6.43, d (8.4)	115.3	5″	6.68, d (8.4)	115.8
6		130.9	6′	6.10, dd (8.4, 1.8)	119.8	6″	6.99, dd (8.4,1.8)	124.6
7	7.26, s	131.4	7′	4.21, d (1.8)	45.1	7″	7.02, s	122.5
8		129.0	8′	3.68, d (1.8)	48.0	8″		141.3
9		167.8	9′		170.9	9″		162.8
3-OMe	3.90, s	56.2	3′-OMe	3.58, s	55.5	3″-OMe	3.52, s	55.8
1‴		130.0	1⁗		129.6	1⁗		129.8
2‴	6.84, d (8.4)	129.8	2⁗	6.97, d (8.4)	129.9	2⁗	6.82, d (8.4)	129.9
3‴	6.64, d (8.4)	115.5	3⁗	6.66, d (8.4)	115.6	3⁗	6.62, d (8.4)	115.6
4‴		156.0	4⁗		156.0	4⁗		156.1
5‴	6.64, d (8.4)	115.5	5⁗	6.66, d (8.4)	115.6	5⁗	6.62, d (8.4)	115.6
6‴	6.84, d (8.4)	129.8	6⁗	6.97, d (8.4)	129.9	6⁗	6.82, d (8.4)	129.9
7‴	2.62, t (7.2)	34.8	7⁗	2.39, t (7.2)	34.7	7⁗	2.45–2.50 m	34.7
8‴	3.23–3.25, m	41.2	8⁗	3.01–3.11, m	41.7	8⁗	3.20–3.23, m	41.3
NH (a)	8.21, t (5.4)		NH (β)	7.74, t (5.4)		NH (γ)	7.75, t (5.4)	

CO_2 + 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% CO_2, 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 mg/mL MTT 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 precultured for 15 min at 37 °C. Next, 0.4 mol/L Na_2CO_3 (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 power, and its molecular formula was determined to be $C_{25}H_{32}O_{13}$ 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 ^{1}H NMR (Table 1) and ^{1}H-^{1}H COSY (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, OCH_3-6). The anomeric 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 group 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 CH_2-4″ and CH_2-5″, respectively (Kanchanapoom, kasai, & Yamasaki, 2002). The ^{13}C 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 carbonyl group (δ_C 204.9). The remaining 11 carbon signals were assigned to the two glycosyl moieties. In addition to one apiosyl 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*-β-*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

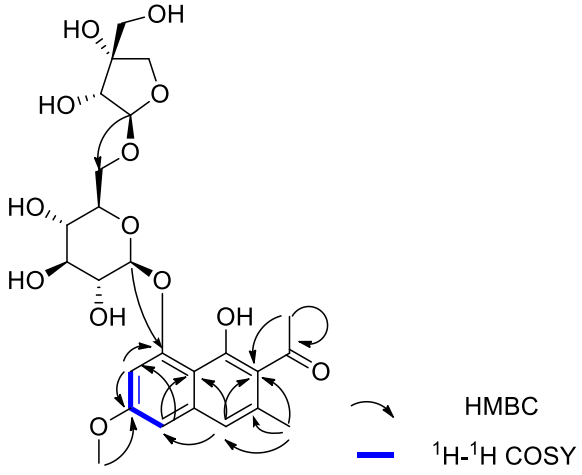

Fig. 2. Key ^{1}H–^{1}H COSY and HMBC correlations of compound **1.**

that the *D*-apiosyl 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 was given the trivial name polygonimitin E.

Compound **2** was isolated as a colorless amorphous powder, and its molecular formula was determined to be $C_{54}H_{53}N_3O_{12}$ by HR-ESI-MS in the positive ionization mode (*m*/*z* 936.3668, $[M + H]^+$). 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 [δ_H 8.21 (1H, t, *J* = 5.4 Hz, *a*-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-7⁗)]; three *para*-substituted Ph groups [δ_H 6.84 (2H, d, *J* = 8.4 Hz, H-2‴/6‴), 6.64 (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⁗)], two 1,3,4-trisubstituted benzene rings [δ_H 7.20 (1H, d, *J* = 1.8 Hz, H-2″), 6.99 (1H, dd, *J* = 8.4, 1.8 Hz, H-6″), 6.68 (1H, d, *J* = 8.4 Hz, H-5″); 6.53 (1H, d, *J* = 1.8 Hz, H-2′), 6.43 (1H, d, *J* = 8.4 Hz, H-5′), 6.10 (1H, dd, *J* = 8.4, 1.8 Hz, H-6′)], one 1,3,4,6-tetrasubstituted benzene ring [δ_H 7.05 (1H, s, H-2), 6.47 (1H, s, H-5)], two conjugated olefinic protons [δ_H 7.26 (1H, s, H-7), 7.02 (1H, s, H-7″)], two coupling methines [δ_H 4.21 (1H, d, *J* = 1.8 Hz, H-7′), 3.68 (1H, d, *J* = 1.8 Hz, H-8′)] and three methoxy groups [δ_H 3.90 (3H, s, OMe-3), 3.58 (3H, s, OMe-3′), 3.52 (3H, s, OMe-3″)]. The ^1H NMR, ^{13}C NMR, HSQC and DEPT data (Table 2) of **2** were very similar to those of melongenamide D (Sun et al., 2014) and thoreliamide C (Ge, Tang, & Ye, 2008). The HMBC analysis revealed correlations between H-7‴/C-2‴ (6‴), H-8/C-9 and C-1‴; H-7⁗/C-2⁗ (6⁗), H-8⁗/C-9′ and C-1⁗; H-7⁗/C-2⁗ (6⁗), H-8⁗/C-9′′ and C-1⁗, which confirmed the presence of three *p*-tyramine moieties in **2** (Fig. 3). C-7′ was determined to be linked to C-1′ and C-6 based on the HMBC correlations from H-7′ to C-5, C-2′, C-6′ 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-1, C-7 and C-9′. Olefinic carbon 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-7 to C-2, C-9 and C-8′; H-2 to C-4 and C-6; and H-5 to C-1 and C-3. Olefinic carbon C-7′′ was found to be linked to the 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-1′′. The 3-OMe, 3′-OMe and 3′′-OMe groups were determined to be linked to C-3, C-3′ and C-3′′, respectively, based on the HMBC correlations. The connections between the 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 melongenamide 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 (1*R*,2*R*)-1,2-dihydro-1-(4-hydroxy-3- methoxyphenyl)-7-[[(1*Z*)-2-(4-hydroxy-3-methoxyphenyl)-1-[[[2-(4-hydroxyphenyl) ethyl] amino] carbonyl]ethenyl]oxy]-N^2,N^3-bis[2-(4-hydroxyphenyl)ethyl]-6-methoxy-2,3-naphthalenedicarboxamide, which was also given the trivial name polygonimitin 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.

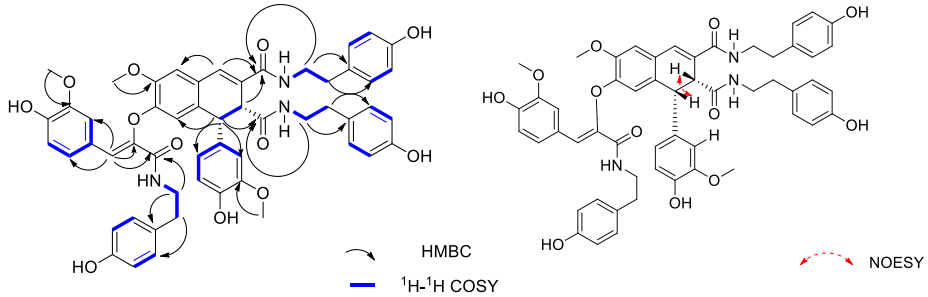

Fig. 3. Key ^1H–^1H COSY, HMBC, and significant NOESY correlations of compound **2**.

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.

Acknowledgments

The authors are grateful to the members of the analytical group of the State Key Laboratory of Bioactive Substances and Functions of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences (CAMS) and Peking Union Medical College, Beijing, China for the spectroscopic data measurements and bioactivity tests. This project was financially supported by the National Natural Science Foundation of China (Nos. 81703665, 81773874, 81973476 and 81903807) and the National Major Scientific and Technological Special Project for "Significant New Drugs Development" (2018ZX09735006).

References

Dang, P. H., Nguyen, N. T., Nguyen, H. X., Nguyen, L. B., Le, T. H., Do, T. N. V., et al. (2015). *a*-glucosidase inhibitors from the leaves of *Embelia ribes*. *Fitoterapia, 100*, 201–207.

Ge, F., Tang, C. P., & Ye, Y. (2008). Lignanamides and Sesquiterpenoids from Stems of *Mitrephora thorelii*. *Helvetica Chimica Acta, 91*(6), 1023–1030.

Kanchanapoom, T., kasai, R., & Yamasaki, K. (2002). Phenolic glycosides from *Barnettia kerrii*. *Phytochemistry, 59*(5), 565–570.

Kim, K. Y., Nam, K. A., Kurihara, H., & Kim, S. M. (2008). Potent *a*-glucosidase inhibitors purified from the red alga *Grateloupia elliptica*. *Phytochemistry, 69*(16), 2820–2825.

Li, C. P., Rao, T., Chen, X. P., Zou, Z. S., Wei, A. W., Tang, J. F., et al. (2019). HLA-B*35:01Allele is a potential biomarker for predicting *Polygonum multiflorum*-induced liver Injury in humans. *Hepatology, 70*(1), 346–357.

Li, X. E., Liu, J. Z., Liao, S. T., Xu, L. X., & Wei, X. Y. (2006). Chemical constituents from tubers from *Polygonum multiflorum* Thunb. *Journal of Tropical and Subtropical Botany, 17*(6), 617–620.

Lin, L. F., Ni, B. R., Lin, H. M., Zhang, M., Li, X. C., Yin, X. B., et al. (2015). Traditional usages, botany, phytochemistry, pharmacology and toxicology of *Polygonum multiflorum* Thunb.: A review. *Journal of Ethnopharmacology, 159*, 158–183.

Liu, Y., Wang, Q., Yang, J. B., Guo, X. H., Liu, W. X., Ma, S. C., et al. (2018). *Polygonum multiflorum* Thunb.: A review on chemical analysis, processing mechanism, quality evaluation, and hepatotoxicity. *Frontiers in Pharmacology, 9*, 364–379.

Ma, J., Li, C. J., Yang, J. Z., Sun, H., & Zhang, D. M. (2017). New phenylpropanoid and coumarin glycosides from the stems of Hydrangea paniculata Sieb. *Molecules*, 22(1), 133/1–133/7.

Sun, J., Gu, Y. F., Su, X. Q., Li, M. M., Huo, H. X., Zhang, J., et al. (2014). Anti-inflammatory lignanamides from the roots of *Solanum melongena* L. *Fitoterapia, 98*, 110–116.

The State Pharmacopoeia Commission (2015). *The People's Republic of China Pharmacopoeia* (pp. 175–176). Beijing: Chinese Medicine Science and Technology Press.

Tian, J., Ma, Q. G., Yang, J. B., Wang, A. G., Ji, T. F., Wang, Y. G., et al. (2013). Hepatoprotective phenolic glycosides from *gymnema tingens*. *Planta Medica, 79*(9), 761–767.

Wang, Q., Wang, Y. D., Li, Y., Wen, B. Y., Dai, Z., Ma, S. C., et al. (2017). Identification and characterization of the structure-activity relationships involved in UGT1A1 inhibition by anthraquinone and dianthrone constituents of *Polygonum multiflorum*. *Scientific Reports, 7*, 17952–17961.

Wang, X. F., & Yao, C. S. (2016). Naturally active oligostilbenes. *Journal of Asian Natural Products Research, 18*(4), 376–407.

Wang, Y. G., Ma, Q. G., Tian, J., Ren, J., Wang, A. G., Ji, T. F., et al. (2016). Hepatoprotective triperpenes from the gum resin of *Boswellia carterii*. *Fitoterapia, 109*, 226–273.

Yang, D., Zhao, J. D., Liu, S., Song, F. R., & Liu, Z. Q. (2014). The screening of potential α-glucosidase inhibitors from the *Polygonum multiflorum* extract using ultrafiltration combined with liquid chromatography-tandem mass spectrometry. *Analytical Methods, 6*(10), 3353–3359.

Yang, J. B., Li, L., Dai, Z., Wu, Y., Geng, X. C., Li, B., et al. (2016). Polygonumnolides C1–C4; minor dianthrone glycosides from the roots of *polygonum multiflorum* Thunb. *Journal of Asian Natural Products Research, 18*(9), 813–822.

Yang, J. B., Tian, J. Y., Dai, Z., Ye, F., Ma, S. C., & Wang, A. G. (2017). α-Glucosidase inhibitors extracted from the roots of *Polygonum multiflorum* Thunb. *Fitoterapia, 117*, 65–70.

Yang, J. B., Yan, Z., Ren, J., Dai, Z., Ma, S. C., Wang, A. G., et al. (2018a). Polygonumnolides A1–B3, Minor dianthrones derivatives from the roots of *Polygonum multiflorum* Thunb. *Archives of Pharmacal Research, 41*(6), 617–624.

Yang, J. B., Liu, Y., Wang, Q., Ma, S. C., Wang, A. G., Cheng, X. L., et al. (2019). Characterization and identification of the chemical constituents of *Polygonum multiflorum* Thunb. by high-performance liquidchromatography coupled with ultraviolet detection and linear iontrap FT-ICR hybrid mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis, 172*, 149–166.

Yang, J. B., Li, W. F., Liu, Y., Wang, Q., Cheng, X. L., Wei, F., et al. (2018b). Acute toxicity screening of different extractions, components and constituents of *Polygonum multiflorum* Thunb. on Zebrafish (Danio rerio) embryos *in vivo*. *Biomedicine & Pharmacotherapy, 99*, 205–213.

Yuan, W., Gao, Z. P., Yang, J. B., & Wang, A. G. (2017). Chemical constituents from *Polygonum multiflorum*. *Chinese Traditional and Herbal Drugs, 48*(4), 631–634.

Zhang, J. X., & Cui, Y. M. (2016). Chemical constituents from *Polygonum multiflorum*. *China Journal of Chinese Materia Medica, 41*(17), 3252–3255.