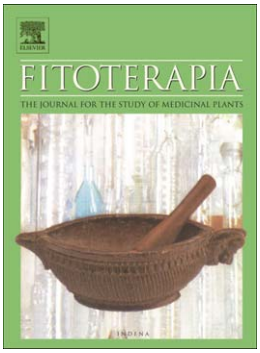

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a-Glucosidase inhibitors extracted from the roots of *Polygonum multiflorum* Thunb

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Abstract A novel stilbene glucoside, polygonumnolide D (**1**), and a novel dianthrone glycoside, polygonumnolide E (**2**), were isolated from a 70% EtOH extract of the dried roots of *Polygonum multiflorum* Thunb., together with six known compounds (**3-8**). Their structures were elucidated by 1D and 2D NMR as well as mass spectroscopy data. The isolated compounds were evaluated for their *a*-glucosidase inhibitory activities *in vitro*. Compounds **1**, **2** and **5** showed the inhibitory activity against *a*-glucosidase with the IC_{50} values of 2.4, 2.7 and 0.3 μM, respectively.

Keywords: *Polygonum multiflorum* Thunb.; stilbene glucoside; dianthrone glycoside; polygonumnolide D; polygonumnolide E; *a*-glucosidase inhibition

1. Introduction

The incidence of diabetes mellitus (DM), a complex chronic metabolic disorder, is increasing every year. Diabetes mellitus is associated with age and is characterized by hyperglycemia. *a*-Glucosidase is an enzyme that can reduce the absorption of carbohydrates from the digestive tract and decrease after-meal glucose levels [1, 2]. However, some *a*-glucosidase inhibitors on the market, such as voglibose and acarbose, often cause severe gastrointestinal side effects, such as flatulence and diarrhea [3]. Therefore, there is a great need for natural *a*-glucosidase inhibitors derived from Traditional Chinese Medicine (TMC).

Our preliminary screening study revealed that the 70% EtOH extraction of the roots of *Polygonum multiflorum* Thunb, exhibited significant *a*-glucosidase inhibitory activity with IC_{50} value of 3.1 μg/ml. *P. multiflorum* is among the most important and widely used TCM herbs, and has a long history as a traditional folk medicine for treating coronary heart disease, hyperlipidemia, neurosis, hair loss prevention and premature graying [4]. Phytochemical studies on *P. multiflorum* have led to the isolation of anthraquinones, stilbenes, phenolic acid, phospholipids, flavones [5] and dianthrones [6]. In this research, bioactivity-guided fractionation of the 70% EtOH extraction of *P. multiflorum* roots was carried out. A new stilbene glucoside (**1**) and a new dianthrone glycoside (**2**) were isolated together with six known compounds. We then determined the *a*-glucosidase inhibitory activity of the compounds.

2. Experimental

2.1. Plant material

The dried roots of *P. multiflorum* Thunb were collected at 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) has been 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.

2.2. Chemicals and instrumentation

a-Glucosidase (Sigma, St. Louis, MO, USA) and PNPG (*p*-nitrophenyl-alpha-D-glu copyranoside) (Sigma). Acarbose was purchased from the Research and Inspection Center of Traditional Chinese Medicine and Ethnomedicine, National Institutes for Food and Drug Control. Optical rotations were acquired on a Jasco P-2000 polarimeter (Jasco Inc., Tokyo, Japan). UV data were recorded using a Jasco V-650 spectrophotometer (Jasco Inc.). IR spectra were measured on a Nicolet iN 10 Micro FTIR spectrophotometer (Thermo Nicolet Inc., Waltham, MA, USA). NMR spectra were recorded on Varian Inova-300, 500 and 600 spectrophotometers (Varian Inc., Palo Alto, CA, USA). HR-ESI-MS were obtained using an Agilent 1100 UPLC-Q-TOF mass spectrometer (Agilent Technologies Ltd., Santa Clara, CA, USA). Column chromatography was performed with silica gel (200–300 mesh; Qingdao Marine Chemistry Company, Qingdao, China), Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) and reversed-phase C_{18} 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).

2.3. Extraction and isolation

The dried roots of *P. multiflorum* (28.0 kg) were refluxed three times with 70%

EtOH. The obtained EtOH extracts were concentrated under reduced pressure below 50 °C and gave a residue (4.0 kg), which was suspended in 1.5 L of H_2O and successively partitioned with CH_2Cl_2. The H_2O fraction (3.5 kg) was subjected to macroporous resin (DM-8) 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, IC_{50}>50 μg/ml); fraction B (62.0 g, IC_{50}: 10.9 μg/ml); fraction C (200.0 g, IC_{50}: 10.7 μg/ml); fraction D (38.0 g, IC_{50}: 2.1 μg/ml); fraction E (55.0 g, IC_{50}: 6.3 μg/ml)]. Fraction D was separated on a RP-18 silica gel column using a step gradient elution of methanol–water (from 10% to 100% v/v) 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). Fraction A_3 was purified on a Sephadex LH-20 column (100% MeOH) to give three fractions, B_1–B_3.

Fraction B_1 was recrystallized by methanol–water (20:80) and compound **3** (12.5 g) was obtained. Fraction B_3 (96 mg) was further separated and purified by preparative HPLC (MeOH/H2O, 30:70; YMC column, 250 × 20 nm, particle size S-5 μm; 220 nm; flow rate 6.0 mL/min) to yield **4** (8.0 mg, 36.2 min) and **5** (20.0 mg, 43.5 min). Fraction A_4 was purified on a Sephadex LH-20 column (100% MeOH) to give five fractions, C_1–C_5. Fraction C_3 (560 mg) was further separated and purified by preparative HPLC (MeOH/H2O, 80:20; YMC, 250 × 20 nm, S-5 μm; 220 nm; 6.0 mL/min) to yield **6** (50.0 mg, 49.0 min) and **1** (230.0 mg, 68.0 min). Fraction C_4 (860 mg) was further separated and purified by preparative HPLC (CH_3CN/H_2O, 40:60; YMC, 250 × 20 nm, S-5 μm; 220 nm; 6.0 mL/min) to yield **7** (125.0 mg, 45.0 min) and **8** (215.0 mg, 57.0 min). Fraction C_5 (380 mg) was further separated and purified by preparative HPLC (CH_3CN/H_2O, 50:50; YMC, 250 × 20 nm, S-5 μm; 220 nm; 6.0 mL/min) to yield **2** (8.0 mg, 35.0 min).

2.3.1 Polygonumnolide D (**1**)

Yellow powder; $[\alpha]_D^{25}$ + 24.28° (c = 0.6, MeOH); UV (MeOH) λmax (logε): 219 (3.50), 332 (5.66) nm; IR (KBr) ν_{max}: 3374, 2974, 2931, 1606, 1514, 1443, 1343, 1246, 1173, 1070, 838 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data, see Table 1; HRESI-MS: *m/z* 839.2734 $[M+H]^+$ (calcd for $C_{42}H_{47}O_{18}^+$, 839.2757, error of −2.3 ppm)

2.3.2 Polygonumnolide E (**2**)

Yellow powder; $[\alpha]_D^{25}$ −102° (c = 0.1, MeOH); UV (MeOH) λmax (logε): 209 (4.88), 278 (4.27), 350 (4.31) nm; IR (KBr) ν_{max}: 3373, 2975, 1620, 1603, 1566, 1490, 1462, 1358, 1336, 1294, 1259, 1219, 1176, 1075, 1038, 976, 898, 862 cm^{-1}; ^1H NMR (CD_3CO CD_3, 600 MHz) and ^{13}C NMR (CD_3CO CD_3, 150 MHz) data, see Table 2; HRESI-MS: *m/z* 685.1921 $[M-H]^-$ (calcd for $C_{37}H_{33}O_{13}^-$, 685.1927, error of −0.6 ppm).

2.4. Enzyme hydrolysis of compounds **1** and **2**

According to the methods shown in previous reports [6, 7], compound **1** (3.0 mg each) was enzymatically hydrolyzed by *β*-glucosidase (8.0 mg) from almonds (Sigma Aldrich) at 30 °C for 12 h. The reaction mixtures were extracted with EtOAC three times (3×10 mL). The aqueous layer was frozen for 12 h and a neutral residue was successfully obtained. The residue was dissolved in anhydrous pyridine (3 ml) and L-cysteine methyl ester hydrochloride (1.5 mg) was added. The mixtures were kept at 60 °C for 2 h, and were frozen to dryness. Trimethylsilylimidazole (1.0 mL) was then added to the reaction mixture and kept at 60 °C for another 2 h, and separated by H_20 (2 mL) and n-hexane (3 × 10 mL). The n-hexane extract was subjected to GC analysis, run on a Agilent 7890A gas chromatograph equipped with an Agilent 19091J-216 HP-5 capillary column (60 m × 0.32 mm × 1.0 μm) and an H_2 flame ionization detector with the following conditions: column temperature, 160–280 °C, increasing, 5 °C/min and maintained at 280 °C for about 20 min; carrier gas, N_2 (1 mL/min); injector and detector temperature, 300 °C; injection volume, 5 μL; and a split ratio of 1/30. From the enzymatic hydrolysate of **1**, D-glucose was confirmed by comparison of the retention time of the derivative with that of authentic sugar derivatized in a similar way. Both showed a retention time of 40.5 min. The constituent sugar of compound **2** was identified by the same method as with **1**.

2.5 *a*-Glucosidase inhibition assay

According to the modified methods of previous reports [8, 9], the inhibitory activity of *a*-glucosidase was determined. A total of 3 mM PNPG (*p*-Nitrophenyl-alpha-D-glucopyranoside) (20 μL) and *a*-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. 0.4 M 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. Acarbose was used as the positive control (see Table 3).

3. Results and discussion

In total, eight compounds (**1**-**8**), including two novel compounds (**1** and **2**), were isolated and identified based on NMR and mass spectra. By comparison of the spectral data with reported references, the structures of the known compounds were confirmed as (*E*)-2,3,5,4'-tetrahydroxylstilbene-2-*β*-D-glucopyranoside (**3**) [10], (*Z*)-2, 3,5,4'-tetrahydroxylstilbene-2-*β*-D-glucopyranoside (**4**) [11], rhaponticin (**5**) [12], (*E*)-2,3,5,4'-tetrahydroxylstilbene-2-*β*-D-(3″-O- monogalloylesters)-glucoside (**6**) [13], *N-trans*-feruloyl tyramine (**7**) [14], *N-trans*-feruloyl-3-methyldopamine (**8**) [14] based on the spectroscopic analysis and comparison with literature data (Fig. 1).

Compound **1** was obtained as a yellow amorphous powder. Its HR-ESI-MS exhibited a molecular ion peak at *m/z* 839.2734 $[M+H]^+$, corresponding to the molecular formula $C_{42}H_{46}O_{18}$. Its IR spectrum showed absorption bands at 3374 cm^{-1} (OH), and 1606, 1514 and 1443 cm^{-1} (phenyl). The ^1H NMR spectrum (CD_3OD, Table 1) and ^1H-^1H COSY spectra (Fig. 2) of **1** exhibited signals for two sets of AA′BB′ systems [δ_H 7.41 (4H, d, J = 8.4 Hz, H-2a/2b), 6.72 (4H, d, J = 8.4 Hz, H-3a/3b)], two sets of uncoupled aromatic protons [δ_H 6.69/6.67 (2H, s, H-14a/14b)], two *trans*-coupled vinylic protons [δ_H 7.64 (2H, d, J = 16.2 Hz, H-8a/8b), 6.89 (2H, d, J = 16.2 Hz, H-7a/7b)], one set of an AX_3 system [δ_H 5.13 (1H, q, J = 7.8 Hz, H-15a), 1.70 (3H, d, J = 7.8 Hz, H-16a)] and two *β*-glucopyranosyl anomeric protons [δ_H 4.51 (1H, d, J = 8.4 Hz, H-1a′ or 1b′) and 4.49 (1H, d, J = 7.8 Hz, H-1a′ or 1b′)].

The ^{13}C NMR (CD_3OD, Table 1) and HSQC spectra of **1** revealed signals for 42 carbons. Comparison of the 1D NMR spectrum of **1** with those of (*E*)-2,3,5,4'-tetrahydroxystilbene-2-*β*-D-glucopyranoside [10] suggested **1** could be a symmetrical stilbene glucoside dimer linked via a $CH(CH_3)$ bridge. Interpretation of HMBC data (Fig. 2) then led to the proposed structure of **1**. In the HMBC spectrum, long-range correlations were observed between H-7a/7b (δ_H 6.89) and C-9a/9b (δ_C 129.9), C-2a/2b (δ_C 127.7), C-6a/6b (δ_C 127.7), confirming that C-7a/7b were linked to C-1a/1b of rings A/A′. The observed HMBC correlations of H-8a/8b (δ_H 7.64) with

C-1a/1b (δ_C 129.4), C-10a/10b (δ_C 137.0/136.9) and C-14a/14b (δ_C 102.5) indicated C-8a/8b were linked to C-9a/9b of rings B/B′. The observed HMBC correlations of H-15a (δ_H 5.13) with C-16a (δ_C 16.8), C-11a/11b (δ_C 152.3/152.2), C-12a/12b (δ_C 117.7/117.3) and C-13a/13b (δ_C 148.1/147.8) indicated that C-15a was linked to C-13a/13b of rings B/B′, which was further confirmed by the observed HMBC correlations of H-16a (δ_H 1.70) with C-15a (δ_C 26.7) and C-12a/12b (δ_C 117.7/117.3). The HMBC correlations (Fig. 2) between H-1a′/1b′ (δ_H 4.51/4.49) and C-10a/10b (δ_C 136.9/137.0) suggested that the sugar moieties in **1** were attached at C-10a/10b.

The *β*-glucopyranosyl configurations were inferred from the coupling constant ($^3J_{H\text{-}1'',\ H\text{-}2''} > 7.5$ Hz) [7], while the D-glucosyl stereochemistry was determined on the basis of the enzymatic hydrolysis with *β*-glycoside hydrolase, followed by GC analysis of its corresponding trimethylsilylated L-cysteine adduct. Accordingly, the structure of **1** was clarified as shown (Fig. 1) and given the trivial name polygonumnolide D.

Compound **2** was obtained as a yellow powder. Its molecular formula, $C_{37}H_{34}O_{13}$, was deduced from HR-ESI-MS from the peak at *m/z* 685.1921 $[M-H]^-$, which corresponds to 21 indices of hydrogen deficiency. The IR spectrum showed strong absorption bands at 1620 and 1603 cm^{-1} that were assigned to carbonyl groups, a peak from hydroxyl groups at 3373 cm^{-1} and a peak from aromatic ring functionalities at 1490 cm^{-1}. The UV spectrum showed absorption maxima at 209, 278 and 350 nm, which were very close to those of previously reported dianthrone derivatives [6, 15, 16]. The ^1H NMR spectrum ($CD_3CO\ CD_3$, Table 2) and ^1H-^1H COSY spectra (Fig. 2) showed the signals of eight meta-coupled aromatic protons [δ_H 6.66 (1H, s, H-2), 6.20 (1H, br s, H-4), 6.60 (1H, br s, H-5), 7.08 (1H, d, *J* = 2.4 Hz, H-7), 6.69 (1H, s, H-2′), 6.45 (1H, br s, H-4′), 6.00 (1H, br s, H-5′), and 6.29 (1H, d, *J* = 2.4 Hz, H-7′)], two methyl groups [δ_H 2.26 (3H, s, Me-3) and 2.31 (3H, s, Me-3′)], one methoxy group at δ_H 3.89 (3H, s, O$\underline{CH_3}$-6), two vicinal methine protons [δ_H 4.67 (1H, d, *J* = 3.6 Hz, H-10) and 4.59 (1H, d, *J* = 3.6 Hz, H-10′)], and one *β*-glucopyranosyl anomeric proton at δ_H 4.74 (1H, d, *J* = 7.8 Hz, H-1″).

Comparison of the 1D NMR spectrum of **2** with those of physcion dianthrones, emodin dianthrones, and physcion-emodin dianthrones [17-19] suggested **2** could be a C-10/C-10′ isomer of a physcion-emodin dianthrone glycoside. The ^{13}C NMR and DEPT spectra (Table 2) exhibited 37 carbon signals, including 31 carbon signals of physcion-emodin dianthrones that were categorized by DEPT and HSQC techniques

into 18 quaternary carbons, including with two carbonyl groups [δ_C 191.1 (C-9′) and 188.3 (C-9)], 10 methine groups [δ_C 117.3 (C-2), 121.5 (C-4), 110.7 (C-5), 107.1 (C-7), 117.7 (C-2′), 122.3 (C-4′), 110.3 (C-5′), 102.9 (C-7′), 57.5 (C-10) and 56.4 (C-10′)], two methyl groups [δ_C 21.9 (Me-3) and 22.1 (Me-3′)], one methoxy group δ_C 56.5 (OMe-6)] and six carbon signals characteristic of glucose [δ_C 106.5, 75.0, 78.7, 71.5, 77.5, 62.9].

The HMBC spectrum (Fig. 2) of **2** showed long-range correlations from H-2 (δ_H 6.66) to C-1 (δ_C 162.4), C-4 (δ_C 121.5), and C-1a (δ_C 117.3), and from Me-3 (δ_H 2.26) to C-2 (δ_C 117.3), C-3 (δ_C 146.9), and C-4 (δ_C 121.5), indicating the presence of an A ring. The HMBC correlations between H-7 (δ_H 7.08) and C-5 (δ_C 110.7), C-6 (δ_C 165.0), C-8 (δ_C 161.8), and C-8a (δ_C 117.6), and from OMe-6 (δ_H 3.89) to C-6 (δ_C 165.0) indicated the presence of a B ring. Meanwhile, the HMBC correlations from H-2′ (δ_H 6.68) to C-1′ (δ_C 162.8), C-4′ (δ_C 122.3), and C-1a′ (δ_C 115.4), and from Me-3′ (δ_H 2.31) to C-2′ (δ_C 117.7), C-3′ (δ_C 147.8), and C-4′ (δ_C 122.3) indicated the presence of an A′ ring. The HMBC correlations from H-7′ (δ_H 6.30) to C-5′ (δ_C 110.3), C-6′ (δ_C 165.2), C-8′ (δ_C 165.6), and C-8a′ (δ_C 111.1) indicated the presence of an B′ ring. The configuration of the C-10/C-10′ junction of the two anthronyl moieties was deduced from the HMBC correlations observed between the proton at δ_H 4.67 (1H, d, J = 3.6 Hz, H-10) and C-1a (δ_C 117.3), C-4 (δ_C 121.5), C-5 (δ_C 110.7), C-8a (δ_C 117.6), and C-10′ (δ_C 56.4) and between the proton at δ_H 4.59 (1H, d, J = 3.6 Hz, H-10′) and C-1a′ (δ_C 115.4), C-4′ (δ_C 122.3), C-5′ (δ_C 110.3), C-8a′ (δ_C 111.1), and C-10 (δ_C 57.5). The HMBC correlations between δ_H 4.74 (1H, d, J = 7.8 Hz, H-1″) and C-8 (δ_C 161.8) suggested that the sugar moiety in **2** was attached at C-8. The *β*-glucopyranosyl configuration was inferred from the coupling constant ($^3J_{H\text{-}1'',\,H\text{-}2''}$ = 7.8 Hz) [7], while the D-glucosyl stereochemistry was determined on the basis of the enzymatic hydrolysis with *β*-glycoside hydrolase, followed by GC analysis of its corresponding trimethylsilylated L-cysteine adduct. Thus, the planar structure of **2** was assigned as physcion-emodin-8-O-*β*-D-glucopyranoside dianthrone.

The relative structure of dianthrone derivatives was unable to be confirmed by NOESY experiments and constants between H-10 and H-10′ [20-22]. According to the reports [6, 23, 24], the H-4 (δ_H 6.20) and H-5′ (δ_H 6.00) peaks of **2** are more upfield than the H-5 (δ_H 6.59) and H-4′ (δ_H 6.45) peaks because of the shielding effect from A′/B rings, suggesting the relative structure of **2** was confirmed as a *cis* H-10/10′ dianthrone. Accordingly, the structure of **2** was clarified as shown (Fig. 1) and given

the trivial name polygonumnolide E.

Meanwhile, dianthrone derivatives, such as polygonumnolides C1-C4, emodin dianthrones [6] and polygonumnolide E (**2**), were firstly isolated from *P. multiflorum* and anthraquinones were widely distributed in Polygonum species. Accordingly, postulated biogenetic relationship between dianthrones, including some possible new compounds, and anthraquinones was speculated as Scheme **1**.

The isolated compounds were also subjected to an evaluation of their *a*-glucosidase inhibitory activities in vitro. Compounds **1**-**6** showed the inhibitory activity against *a*-glucosidase with the IC_{50} values of 2.4, 2.7, 14.9, 8.6, 0.3 and 11.1 μM, respectively. Among them, compounds **1** and **3**-**6** belong to stilbenes. According to the literature [25], stilbenes showed diverse bioactivities, such as antioxidant, anti-inflammatory, anticancer and anti-diabetic activity. Thus, stilbenes and dianthrone derivatives may be given more attentions in order to obtain *a*-glucosidase inhibitors from *P. multiflorum*.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Spectroscopic data for the new compounds (**1** and **2**) including ^{1}H, ^{13}C and 2D NMR, IR and ESI-MS/MS data are provided in the supporting information.

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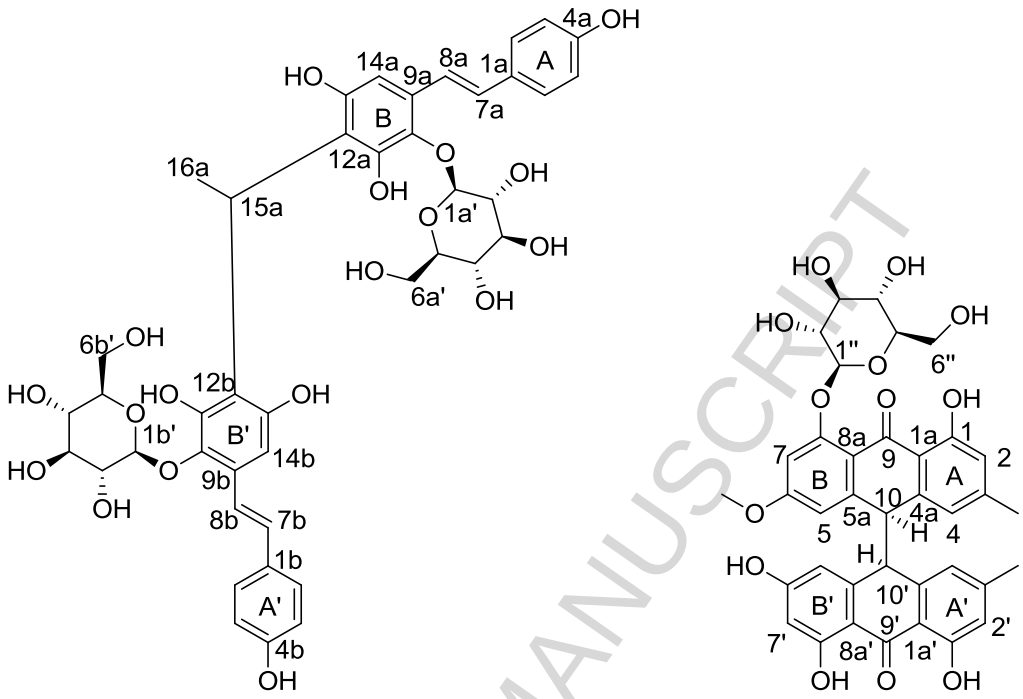


Fig. 1. Structures of compounds **1** and **2** from the roots of *Polygonum multiflorum* Thunb.

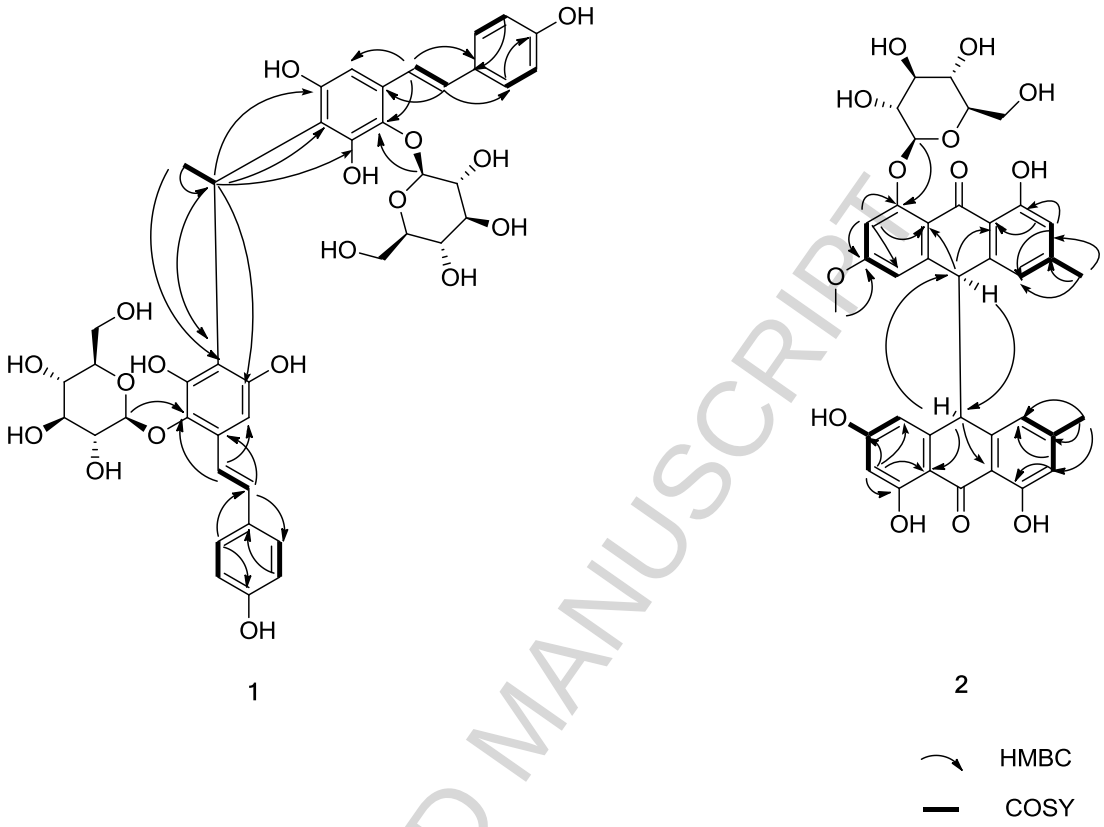
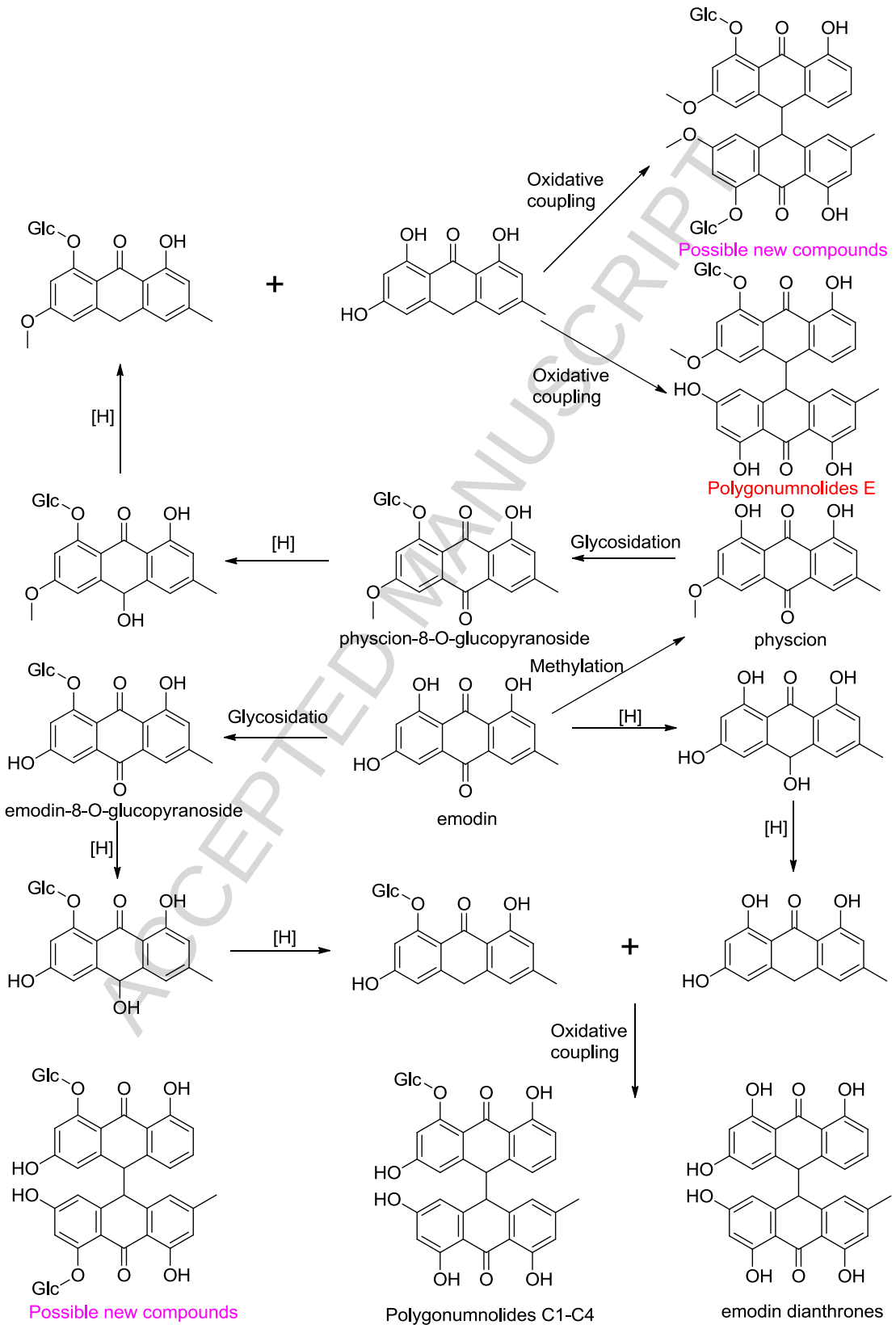


Fig 2. Key ^{1}H–^{1}H COSY and HMBC correlations of compounds **1** and **2**.



Scheme 1. Hypothetical Biogenetic Relationship between dianthrones and anthraquinones

Table 1
^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) data for **1** were measured in CD_3OD

Position	**1**	
	δ_H (*J* in Hz)	δ_C
1a/1b		129.4
2a/2b	7.41, d (8.4)	127.7
3a/3b	6.72, d (8.4)	115.0
4a/4b		156.9/156.8
5a/5b		115.0
6a/6b		127.7
7a/7b	6.89, d (16.2)	128.4/128.3
8a/8b	7.64, d (16.2)	119.9
9a/9b		129.9
10a/10b		137.0/136.9
11a/11b		152.3/152.2
12a/12b		117.7/117.3
13a/13b		148.1/147.8
14a/14b	6.69,s; 6.67,s	102.5
15a	5.13, q (7.8)	26.7
16a	1.70, d (7.8)	16.8
1a′/1b′	4.51, d (8.4); 4.49, d (7.8)	106.7
2a′/2b′	3.53-3.57, m	74.1
3a′/3b′	3.41, dd (8.4, 9.6)	76.4
4a′/4b′	3.48-3.51, m	69.4
5a′/5b′	3.22-3.25, m	76.8/76.7
6a′/6b′	3.81, dd (2.4, 12.0), 3.78, dd (2.4, 12.0), 3.74, dd (4.8, 12.0), 3.73, dd (4.2, 12.0)	60.8/60.7

Table 2
^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) data for **2** were measured in CD_3COCD_3

Position	**2**		Position	**2**	
	δ_C	δ_H (J in Hz)		δ_C	δ_H (J in Hz)
1	162.4		1′	162.8	
2	117.3	6.66, s	2′	117.7	6.68, s
3	146.9		3′	147.8	
4	121.5	6.20, br s	4′	122.3	6.45, br s
5	110.7	6.60, br s	5′	110.3	6.00, br s
6	165.0		6′	165.2	
7	107.1	7.08, d (2.4)	7′	102.9	6.30, d (2.4)
8	161.8		8′	165.6	
9	188.3		9′	191.1	
10	57.5	4.67, d (3.6)	10′	56.4	4.59, d (3.6)
1a	117.3		1a′	115.4	
4a	142.2		4a′	140.7	
5a	145.4		5a′	144.4	
8a	117.6		8a′	111.1	
3-Me	21.9	2.26 s	3′-Me	22.1	2.31 s
6-0Me	56.3	3.89, s	1″	106.5	4.74, d (7.8)
OH-1		12.15, s	2″	75.0	3.46-3.49, m
OH-8′		11.94, s	3″	78.7	3.56-3.59, m
OH-1′		11.79, s	4″	71.5	3.43-3.46, m
OH-6			5″	77.5	3.55-3.57, m
OH-6′		9.75, s	6″	62.9	3.98, dd (1.2, 12); 3.74, dd (6.0,12)

Table 3

a-glucosidase inhibitory activity of the isolated compounds (n=3)

Compound	IC_{50} [a] (μM)
Acarbose [b]	50.04
1	2.4
2	2.7
3	14.9
4	8.6
5	0.3
6	11.1
7	26.6 % inhibition at 10 μM
8	16.1 % inhibition at 10 μM

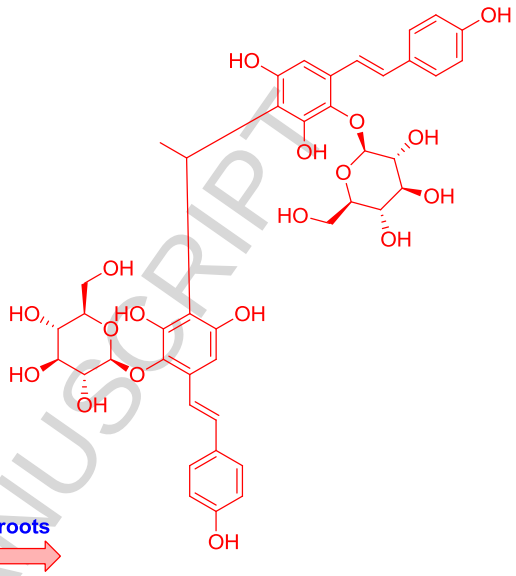
[a] IC_{50} is the concentration required to produce 50 % inhibition of the enzyme activity.

[b] Acarbose as a positive control

Graphical abstract

Polygonum multiflorum Thunb

Isolation from the roots



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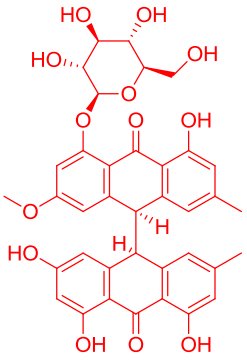

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