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Journal Name

ARTICLE

A novel adduct of ECG fused to piceid and four new dimeric stilbene glycosides from *Polygonum cuspidatum*†

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

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Polyflavanostilbene B (**1**), an unusual adduct of epicatechin-3-*O*-gallate fused to piceid through a carbon–carbon bond, four new dimeric stilbene glycosides (**2**–**5**), three new stilbene glucosides (**6**–**8**), one new flavan glucoside (**9**), and six known compounds were isolated from the rhizome of *polygonum cuspidatum*. The structures of these compounds were elucidated using spectroscopic data, including electronic circular dichroism (ECD) and $Rh_2(OCOCF_3)_4$-induced CD spectra. All of the compounds were screened for their inhibitory activity against α-glucosidase using acarbose as a positive control (IC_{50} = 385 μM), and strong inhibitory activity against α-glucosidase was observed for compound **8** (IC_{50} = 3.04 μM).

Introduction

The genus *polygonum* comprises more than 300 species around the world and approximately 120 species distributed in China. The rhizome of *polygonum cuspidatum*, named HuZhang in China, is a traditional Chinese medicine (TCM) that has been documented in the Chinese Pharmacopoeia (1977–2015 editions), and it is conventionally used for the treatment of diabetes, inflammation, hyperlipemia, and infection.[1,2] Modern pharmacological studies on *P. cuspidatum* have revealed significant biological activity, including antioxidant,[3] antiviral,[4] antibacterial,[5] antitumour,[6] and neuroprotective effects.[7] Various anthroquinones,[8] stilbenes,[9] flavonoids,[10] and phenols,[11] have been isolated from this plant. In particular, stilbenes (resveratrol and piceid) and anthraquinones are considered to be the major constituents of *P. cuspidatum* and are generally regarded as the index for quality assessment of this herb.[12]

Previously, we investigated the bioactive components of the rhizome of *P. cuspidatum*. This study revealed a novel adduct of epicatechin-3-*O*-gallate and piceid known as polyflavanostilbene A,[13] two novel naphthalene-fused piceid glycosides, and a series of naphthalene glucosides.[14] In the current study, we report the isolation of an unusual adduct of epicatechin-3-*O*-gallate fused to piceid through a carbon−carbon bond (**1**), four new dimeric stilbene glycosides (**2**−**5**), three new stilbene glucosides (**6**−**8**), and one new flavan glucoside (**9**), as well as six known compounds (**10**−**15**) (Fig. 1).

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† Electronic Supplementary Information (ESI) available: 1D NMR, 2D NMR HRMS, IR, ECD and $Rh_2(OCOCF_3)_4$ induced CD spectra. See DOI:

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The structures of these compounds were using spectroscopic data (1D and 2D NMR, UV, IR, ORD, HRESIMS, ECD, and $Rh_2(OCOCF_3)_4$-induced CD) and comparison to literature data. Additionally, the inhibitory activities of compounds **1**−**15** against α-glucosidase were examined. Compound **8** exhibited strong inhibitory activity against α-glucosidase with an IC_{50} value of 3.04 μM.

Results and discussion

Compound **1** was isolated as a white powder. Its molecular formula was determined as $C_{42}H_{40}O_{19}$ from the HRESIMS ion at *m/z* 847.2094 $[M - H]^-$ (calcd for $C_{42}H_{39}O_{19}$: 847.2091), indicating 23 degrees of unsaturation. IR absorptions revealed the presence of hydroxyl (3372 cm^{-1}) and carbonyl (1613 cm^{-1}) groups, in addition to aromatic rings (1513 and 1450 cm^{-1}).

The ^1H NMR spectrum (Table 1) of **1** displayed a set of AA′BB′ system aromatic protons at δ_H 7.04 (2H, d, J = 8.5 Hz) and 6.53 (2H, d, J = 8.5 Hz), three meta-coupled aromatic protons at δ_H 6.18 (1H, s), 6.15 (1H, s), and 6.12 (1H, s), three ABX system aromatic protons at δ_H 6.96 (1H, dd, J = 8.5, 1.5 Hz), 6.90 (1H, d, J = 1.5 Hz), and 6.68 (1H, d, J = 8.5 Hz), a singlet aromatic proton at δ_H 6.00 (1H, s), and two characteristic signals of a galloyl moiety at δ_H 6.88 (2H, s). In addition, four methine protons at δ_H 4.45 (1H, d, J = 10.5 Hz), 5.39 (1H, dd, J = 10.5, 4.0 Hz), 5.18 (1H, br s), and 5.36 (1H, br s) and two methylene protons at δ_H 2.76 (1H, dd, J = 17.5, 4.5 Hz) and 3.03 (1H, d, J = 17.5 Hz) were observed. Finally, a doublet at δ_H 4.61 (1H, d, J = 7.5 Hz) caused by an anomeric proton and the proton signals between δ_H 3.15 and 3.64 indicated the presence of a glycosyl group. After cellulose hydrolysis, the sugar unit of **1** was confirmed to be the D-configuration by GC analysis of its trimethylsilyl L-cysteine derivative.

The ^{13}C NMR spectrum (Table 2) of **1** revealed a total of 42 carbon signals, six of which were assigned to an *O*-glucose

unit; the remaining 36 carbons were assigned to a C6-C2-C6 unit and a flavan aglycone with an additional galloyl moiety. A careful comparison of the 1D NMR signals of **1** with the corresponding data of epicatechin-3-*O*-gallate (ECG),[15] suggested the presence of an ECG moiety in **1**, which was verified by HMBC correlations from H-3 to C-2, C-4, C-10, C-11, and C-17, and from H-19, H-23 to C-17. By considering the remaining eight degrees of unsaturation and the molecular formula, the C6-C2-C6 unit was elucidated as the 4′,8′,11′,13′-tetrahydroxyl diphenylethane moiety. The connectivity of these two moieties was established by analysing the HMBC spectrum (Fig. 2). Correlations from H-7′ to C-7, C-8, and C-9 were observed, suggesting that the C-7′ is connected to the C-8 of the ECG moiety by a C-C bond. The sugar moiety was confirmed to be at C-11′ position of the aglycone by the HMBC correlation of H-1″ with C-11′.

Examination of the ECD and $Rh_2(OCOCF_3)_4$-induced CD spectra of **1** allowed for determination of the absolute configurations of C-2, C-3, C-7′, and C-8′. Cellulase hydrolysis of **1** resulted in **1a** (the aglycone) and D-glucose. The 8′*S* configuration was supported by a positive Cotton effect at 357 nm in the $Rh_2(OCOCF_3)_4$-induced CD spectrum of **1a** (Fig. S7, Supporting Information).[16] The *threo* configuration between the two chiral centres at C-7′ and C-8′ was established using the coupling constant (10.5 Hz) between H-7′ and H-8′.[17] From the above analysis, the absolute configuration of C-7′ was determined to be *R*. In addition, the 2*R*,3*R* configurations were confirmed by the small coupling constant of H-2/H-3 and the negative Cotton effect at 280 nm in the ECD spectrum.[18] Based on these results, the structure of **1** was assigned as shown in Fig.1, and the compound was accorded the trivial name polyflavanostilbene B.

Compound **2** was obtained as a white amorphous powder, with a molecular formula of $C_{40}H_{44}O_{17}$ on the basis of HRESIMS (*m/z* 819.2478 $[M + Na]^+$, calcd for $C_{40}H_{44}O_{17}Na$: 819.2471). Its ^1H NMR spectrum exhibited two sets of AA′BB′ system aromatic protons at δ_H 7.34 (2H, d, J = 8.0 Hz), 6.89 (2H, d, J = 8.0 Hz), 6.79 (2H, d, J = 8.0 Hz), and 6.43 (2H, d, J = 8.0 Hz), three meta-coupled aromatic protons at δ_H 6.53 (1H, s), 6.40 (1H, s), and 6.23 (1H, s), one 1, 2, 3, 5-tetrasubstituted aromatic protons at δ_H 6.54 (1H, s) and 6.21 (1H, s), two trans-olefinic protons at δ_H 7.27 (1H, d, J = 16.0 Hz) and 6.70 (1H, d, J = 16.0 Hz), two aliphatic protons at δ_H 5.57 (1H, d, J = 10.0 Hz) and 4.37 (1H, d, J = 10.0 Hz), and two glucopyranosyl anomeric protons at 4.85 (1H, d, J = 7.0 Hz) and 4.66 (1H, d, J = 7.0 Hz). The ^{13}C NMR spectrum of **2** showed 40 carbon resonances (Table 2), consisting of 12 carbon signals of two glucose units and 28 skeleton carbon signals corresponding to two C6-C2-C6 units. In the HMBC spectrum (Fig. 2), the correlation peaks of H-8′ at δ_H 4.37 with C-1 (δ_C 139.7), C-2 (δ_C 120.4), and C-3 (δ_C 157.1) helped verify the linkage point between C-8′ and C-2. Furthermore, two glucose units were determined to be located at C-3 and C-3′ based on correlations between H-1″ (δ_H 4.85) with C-3 (δ_C 157.1) and H-1‴ (δ_H 4.66) with C-3′ (δ_C 158.1) in the HMBC spectrum. This spectroscopic data suggested that **2** had the same planar structure as dimeric stilbene glycoside 1,[17] which was previously isolated from *P. cuspidatum* but the absolute configurations were not assigned. The absolute configurations of C-7′ and C-8′ were identified by the same method as that used for **1**. The coupling constant ($J_{7',8'}$ = 10.0 Hz) confirmed the *threo* configuration of C-7′ and C-8′. Hydrolysis of **2** with cellulase yielded **2a** and D-glucose, which was confirmed by GC analysis of its trimethylsilyl L-cysteine derivative. The absolute configuration of C-7′ in **2a** was defined as *S* based on a positive Cotton effect at 350 nm in the $Rh_2(OCOCF_3)_4$-induced CD spectrum (Fig. S15, Supporting Information). C-8′ was then assigned the *R* absolute configuration, in accordance with the 7′,8′-*threo* conformation. Thus, the structure of **2** was established as shown (Fig. 1), and this compound was given the trivial name stilbenedimer A.

The molecular formula of **3** was determined to be the same as **2** ($C_{40}H_{44}O_{17}$), based on the positive HRESIMS ion observed at *m/z* 819.2464 $[M + Na]^+$. Careful analysis of the UV, IR, and NMR spectroscopic data revealed that **3** had the same planar structure as **2**. The coupling constant (3.0 Hz) of the two aliphatic protons H-7′ (δ_H 5.49) and H-8′ (δ_H 4.47) suggested that they were in the *erythro* conformation. Cellulase hydrolysis of **3** resulted in **3a** and D-glucose. In the $Rh_2(OCOCF_3)_4$-induced CD experiment (Fig. S23, Supporting Information), a negative Cotton effect at 361 nm indicated the 7′*R*,8′*R* configurations for **3a**. Thus, **3** was established as shown and was named stilbenedimer B.

Compound **4** had the same molecular formula as **2**, as indicated by the sodiated molecular ion peak observed at *m/z* 819.2463 $[M + Na]^+$ in the HRESIMS. A characteristic coupling constant ($J_{7,8}$ = 12.0 Hz) between H-7 and H-8 in the ^1H NMR spectrum (Table 1) suggested the presence of a *cis*-stilbene moiety in **4** instead of the *trans*-stilbene moiety observed in **2**. The presence of the aglycone (**4a**) was determined by the hydrolysis of **4** with cellulose. The *threo* configuration of C-7′ and C-8′ was verified by the coupling constant between H-7′ and H-8′ ($J_{7',8'}$ = 10.5 Hz). This result, combined with the positive Cotton effect at 358 nm in the $Rh_2(OCOCF_3)_4$-induced CD spectrum of **4a** (Fig. S31, Supporting Information), indicated the 7′*S*,8′*R* configuration of **4**. Consequently, **4** was elucidated as an isomer of **2** and named stilbenedimer C.

Compound **5**, a white amorphous powder, had the same molecular formula ($C_{40}H_{44}O_{17}Na$, *m/z* 819.2468) as **4**. The UV, IR, and NMR spectroscopy data of **5** indicated that it was an optical isomer of **4**. Cellulase hydrolysis of **5** resulted in **5a** and D-glucose. In the $Rh_2(OCOCF_3)_4$-induced CD spectrum, a negative Cotton effect at 356 nm indicated the 7′*R* configuration in **5a** (Fig. S39, Supporting Information). Meanwhile, the 8′*S* configuration was confirmed by the large coupling constant between H-7′ and H-8′ ($J_{7',8'}$ = 10.5 Hz). Based on the aforementioned information, **5** was identified as shown (Fig. 1) and named stilbenedimer D.

Compound **6** was obtained as a white amorphous powder and had a molecular formula of $C_{26}H_{32}O_{13}$, as evidenced by the protonated molecular ion peak at *m/z* 553.1920 $[M + H]^+$ (calcd for $C_{26}H_{33}O_{13}$: 553.1916) in the HRESIMS. The UV, IR, and NMR spectroscopic data of **6** were closely related to those of piceid,[18] a known compound that was isolated as the major

component from the rhizome of *P. cuspidatum*. Comparison of the NMR spectra with those of piceid demonstrated these compounds differed only by the presence of an additional glucose moiety in **6**. The ^{1}H NMR spectrum displayed two anomeric protons at δ_H 4.78 (1H, d, J = 7.5 Hz) and δ_H 4.69 (1H, d, J = 3.0 Hz), which signified the presence of two glucose units with one β-linkage and one α-linkage. In the ^{13}C NMR spectrum, the resonance for C-6′ (δ_C 66.3) of **6** was shifted significantly downfield compared to piceid. This suggested that the α-glucose was attached to C-6′ of the β-glucose, which was further supported by the HMBC correlation between H-1″ and C-6′. After acid hydrolysis, the glucose unit was confirmed to be D-configuration by GC analysis of its trimethylsilyl L-cysteine derivative. Using the data above, the structure of **3** was elucidated and determined to be piceid-6′-*O*-α-D-glucopyranoside.

Compound **7** exhibited the same molecular formula ($C_{26}H_{32}O_{13}$) as that of **6**. Comparison of the NMR data for **7** and **6** showed that the two compounds differed by the location of the α-D-glucose unit. The key HMBC correlation from H-1″ (δ_H 5.19) to C-2′ (δ_C 75.0) confirmed the location of the α-D-glucose unit at C-2′. All of the aforementioned data indicated that the structure of **7** was piceid-2′-*O*-α-D-glucopyranoside.

Compound **8** was obtained as a white amorphous powder. The molecular formula of **8** was $C_{27}H_{26}O_{15}S$, as confirmed by the negative HRESIMS ion observed at *m/z* 621.0929 $[M - H]^-$ (calcd for $C_{27}H_{25}O_{15}S$: 621.0920), and likely contains a sulfate group. A detailed analysis of the spectroscopic data revealed that **8** was a piceid derivative with a galloyl moiety. The HMBC correlation observed between H-2′ (δ_H 4.92) and C-7″ (δ_C 165.4), together with the downfield shift of H-2′ (δ_H 4.92) indicated that the galloyl moiety was linked at C-2′ of glucose. The unusual downfield shift of the H-6′ (δ_H 4.16, 3.81), C-6′ (δ_C 66.1) was an important indicator that the sulfate group was attached at C-6′ of glucose. After acid hydrolysis, the sugar unit was confirmed to be D-glucose by GC analysis of its trimethylsilyl L-cysteine derivative. On the basis of these data, the structure of **8** was determined to be piceid-2′-galloyl-6′-sulfate.

Compound **9** was isolated as a white amorphous powder, and the molecular formula $C_{28}H_{28}O_{15}$ was determined by the positive HRESIMS ion (*m/z* 627.1316 $[M + Na]^+$). The IR spectrum revealed absorption bands attributed to hydroxy groups (3363 cm^{-1}), carbonyl groups (1698 cm^{-1}), and aromatic rings (1610, 1528, 1504, and 1453 cm^{-1}). The ^{1}H NMR spectrum (Table 3) of **9** revealed a set of ABX system aromatic protons at δ_H 6.67 (1H, d, J = 1.5 Hz), 6.63 (1H, d, J = 8.0 Hz) and 6.52 (1H, dd, J = 8.0, 1.5 Hz), two meta-coupled aromatic protons at δ_H 6.11 (1H, d, J = 1.5 Hz) and 5.85 (1H, d, J = 1.5 Hz), and two characteristic signals of a galloyl moiety at δ_H 6.94 (2H, s) in the downfield region. Additionally, two oxygenated methylene protons at δ_H 4.33 (1H, d, J = 7.5 Hz) and 3.59 (1H, m), two methylene protons at δ_H 2.45 (1H, overlap), 2.26 (1H, dd, J = 16.5, 8.5 Hz), and one anomeric proton at δ_H 5.11 (1H, d, J = 8.0 Hz) were also observed. The ^{13}C NMR spectrum of **9** showed 28 carbon resonances (Table 2) corresponding to the glucose unit, galloyl moiety, and 3,5,7,3′,4′-pentahydroxyflavane. The HMBC correlation (Fig. 2) between H-2′ (δ_H 4.96) and C-7″ (δ_C 165.0) indicated that the galloyl moiety was attached to C-2′ of the glucose. Furthermore, the linkage point of the glucose was identified by the correlation between the anomeric proton H-1′ and C-5 in the HMBC experiment. Acid hydrolysis of **9** gave the 3,5,7,3′,4′-pentahydroxyflavane (**9a**), gallic acid, and glucose. Using the same method as described in **8**, the D-configuration of glucose was confirmed. The coupling constant of H-2 (J = 7.5 Hz) indicated that H-2/H-3 was in a *trans* configuration. This observation, along with the negative Cotton effect at 281 nm in the ECD spectrum of **9a** (Fig. S64, Supporting Information), permits assignment of the absolute configurations of C-2 and C-3 as 2*R*,3*S*.[19] Thus, **9** was defined as (+)-catechin-5-*O*-β-D-(2′-*O*-galloyl)-glucopyranoside.

Based on MS data, NMR data and comparison with literatures, known compounds (Fig. 1) were identified as 7-*O*-(β-D-glucopyranosyloxy)-5-hydroxy-1(3H)-isobenzofuranone (**10**),[20] 2,4,6-trihydroxyacetophenone-3-C-β-D-glucopyranoside (**11**),[21] 2,4,6-trihydroxy-acetophenone-4-*O*-β-D-glucopyranoside (**12**),[22] 5,7-dihydroxyphthalide (**13**),[20] altechromone A (**14**),[23] and 7-demethylsiderin (**15**).[24]

Experimental

General experimental procedures

The optical rotations were measured on a Jasco P-2000 polarimeter (JASCO, Easton, MD, U.S.A.). ECD spectra were recorded with a JASCO J-815 spectrometer. IR spectra were recorded on a Nicolet 5700 spectrometer (Thermo Scientific, Waltham, MA, U.S.A.) using an FT-IR microscope transmission method. ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), and 2D NMR spectra were run on Bruker 500 MHz NMR spectrometer (Bruker-Biospin, Billerica, MA, U.S.A.) using TMS as internal standard and the values are given in ppm. High-resolution electrospray ionization mass spectrometry (HRESIMS) was performed on an Agilent 1100 series LC/MSD ion trap mass spectrometer (Agilent Technologies, Waldbronn, Germany). HPLC-DAD analysis was performed using an Agilent 1200 series system with an Apollo C_{18} column (250 mm × 4.6 mm, 5 μm; Grace Davison). GC analysis was performed using an Agilent 7890A series system with a capillary column, HP-5 (30 m ×0.25 mm, with a 0.25 μm film, Dikma). Preparative HPLC was carried out on a Shimadzu LC-6AD instrument with an SPD-20A detector (Shimadzu Corp., Tokyo, Japan), using a YMC-Pack ODS-A column (250 mm × 20 mm, 5 μm; YMC Corp., Kyoto, Japan). Column chromatography was performed with macroporous resin (Diaion HP-20, Mitsubishi Chemical Corp., Tokyo, Japan), Rp-18 (50 μm, YMC Corp.), Sephadex LH-20 (Pharmacia Fine Chemicals, Uppsala, Sweden).

Plant material

The rhizome of *P. cuspidatum* was purchased from Beijing Pu Sheng Lin Pharmaceutical Co., Ltd. in Beijing, People's Republic of China, in Feb 2010. The plant material was

identified by L. Ma (Institute of Materia Medica, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing 100050, P R. China). A voucher specimen (ID number: ID-S-2593) was deposited at the Institute of Materia Medica, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing 100050, P R. China.

Extraction and isolation

Powdered *P. cuspidatum* (20 kg) was extracted with 80 % EtOH under reflux (3 × 2 h). The EtOH extract was concentrated under reduced pressure to give a residue (4.2 kg), which was suspended in H_2O (4 L) and partitioned with, consecutively, petroleum ether (PE) (3 × 4 L), EtOAc (3 × 4 L), and *n*-BuOH (3 × 4 L). After evaporation of the n-BuOH solvent under reduced pressure, the extract (1375 g) was suspended in H_2O (2 L) and subjected to column chromatography over macroporous resin, eluting successively with H_2O, 30 % EtOH, 50 % EtOH, 70 % EtOH, and 95 % EtOH (30 L each). The 30 % EtOH was removed under reduced pressure and the fraction (86.4 g) was subjected to chromatography over Sephadex LH-20 with H_2O-MeOH in gradient as the mobile phase to yield 25 fractions (Fr. A−Fr. Y) on the basis of HPLC-DAD analysis.

Fr. B was subjected to reversed-phase preparative HPLC, using MeOH-H_2O (24 : 76) as the mobile phase, to give **11** (25 mg) and **12** (22 mg). Compound **10** (12 mg) was obtained from Fr. C by recrystallization. Fr. D was purified by preparative RP-HPLC using MeOH-H_2O (28 : 72) as the mobile phase to yield **14** (53 mg). Fr. E was purified by preparative RP-HPLC using MeOH-H_2O (31:69) as the mobile phase to yield **13** (13 mg). Fr. I was subjected to Sephadex LH-20 column and eluted with MeOH-H_2O (from 20 : 80 to 60 : 40) to afford 20 fractions (Fr. I-1−I-20). Fr. I-13 was further purified using preparative RP-HPLC with MeOH-H_2O (50 : 50) as the mobile phase to yield **15** (8 mg). Fr. N was subjected to Sephadex LH-20 column and eluted with MeOH-H_2O (from 20 : 80 to 60 : 40) to afford 20 fractions (Fr. N-1−N-20). Fr. N-9 was further purified using preparative RP-HPLC with MeOH-H_2O (33 : 67) as the mobile phase to yield **6** (11 mg), **7** (10 mg), **3** (10 mg), and **5** (6 mg). Fr. N-10 was further purified using preparative RP-HPLC with MeOH-H_2O (40 : 60) as the mobile phase to yield **2** (13 mg). Fr. O was subjected to Sephadex LH-20 column and eluted with MeOH-H_2O (from 20 : 80 to 60 : 40) to afford 20 fractions (Fr. O-1−O-20). Fr. O-18 was further purified using preparative RP-HPLC with MeOH-H_2O (34 : 66) as the mobile phase to yield **4** (10 mg). Fr. Q was subjected to Sephadex LH-20 column and eluted with MeOH-H_2O (from 20 : 80 to 60 : 40) to afford 15 fractions (Fr. Q-1−Q-15). Fr. Q-12 was further purified using preparative RP-HPLC with MeOH-H_2O (19 : 81) as the mobile phase to yield **9** (11 mg). Fr. R was subjected to Sephadex LH-20 column and eluted with MeOH-H_2O (from 20 : 80 to 60 : 40) to afford 20 fractions (Fr. R-1−R-20). Fr. R-11 was further purified using preparative RP-HPLC with MeOH-H_2O (33 : 67) as the mobile phase to yield **8** (10 mg). Fr. U was subjected to Sephadex LH-20 column and eluted with MeOH-H_2O (from 20 : 80 to 60 : 40) to afford 30 fractions (Fr. U-1−U-30). Fr. U-25 was further purified using preparative RP-HPLC with MeOH-H_2O (38 : 62) as the mobile phase to yield **1** (24 mg).

Structure characterization

Compound (1). White amorphous powder; $[\alpha]_D^{25}$ –149.1 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 280 (0.26) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 285 (–3.41), 222 (–5.38) nm; IR (KBr) ν_{max}: 3372, 1613, 1513, 1450 cm^{-1}; HRESIMS *m/z* 847.2094 $[M - H]^-$ (calcd for $C_{42}H_{40}O_{19}$, 847.2091); ^1H and ^{13}C NMR data, see Tables 1 and 2.

Compound (2). White amorphous powder; $[\alpha]_D^{25}$ –32.3 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 283 (0.21), 316 (0.14) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 276 (+6.54), 234 (–27.99) nm; IR (KBr) ν_{max}: 3328, 1603, 1514, 1453 cm^{-1}; HRESIMS *m/z* 819.2478 $[M + Na]^+$ (calcd for $C_{40}H_{44}O_{17}Na$, 819.2471); ^1H and ^{13}C NMR data, see Tables 1 and 2.

Compound (3). White amorphous powder; $[\alpha]_D^{25}$ –122.4 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 283 (0.21), 323 (0.12) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 298 (–3.16), 238 (–3.33) nm; IR (KBr) ν_{max}: 3365, 1604, 1511, 1453 cm^{-1}; HRESIMS *m/z* 819.2464 $[M + Na]^+$ (calcd for $C_{40}H_{44}O_{17}Na$, 819.2471); ^1H and ^{13}C NMR data, see Tables 1 and 2.

Compound (4). White amorphous powder; $[\alpha]_D^{25}$ –109.3 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 284 (0.21), 320 (0.13) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 280 (–2.93), 214 (–19.37) nm; IR (KBr) ν_{max}: 3328, 1574, 1514, 1416 cm^{-1}; HRESIMS *m/z* 819.2463 $[M + Na]^+$ (calcd for $C_{40}H_{44}O_{17}Na$, 819.2471); ^1H and ^{13}C NMR data, see Tables 1 and 2.

Compound (5). White amorphous powder; $[\alpha]_D^{25}$ 170.7 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 84 (0.21), 321 (0.13) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 279 (+0.31), 248 (–1.34), 230 (+6.32) nm; IR (KBr) ν_{max}: 3340, 1604, 1513, 1452 cm^{-1}; HRESIMS *m/z* 819.2468 $[M + Na]^+$ (calcd for $C_{40}H_{44}O_{17}Na$, 819.2471); ^1H and ^{13}C NMR data, see Tables 1 and 2.

Compound (6). White amorphous powder; $[\alpha]_D^{25}$ –18.9 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 307 (0.43), 321 (0.42) nm; IR (KBr) ν_{max}: 3348, 1592, 1513, 1445 cm^{-1}; HRESIMS *m/z* 553.1920 $[M + H]^+$ (calcd for $C_{26}H_{33}O_{13}$, 553.1916); ^1H and ^{13}C NMR data, see Tables 1 and 3.

Compound (7). White amorphous powder; $[\alpha]_D^{25}$ –17.2 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 306 (0.43), 321 (0.42) nm; IR (KBr) ν_{max}: 3317, 1603, 1514, 1449 cm^{-1}; HRESIMS *m/z* 553.1911 $[M + H]^+$ (calcd for $C_{26}H_{33}O_{13}$, 553.1916); ^1H and ^{13}C NMR data, see Tables 1 and 3.

Compound (8). White amorphous powder; $[\alpha]_D^{25}$ –77.5 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 296 (0.51), 323 (0.41) nm; IR (KBr) ν_{max}: 3297, 1701, 1603, 1514, 1450, 1218, 1047 cm^{-1}; HRESIMS *m/z* 621.0929 $[M - H]^-$ (calcd for $C_{27}H_{25}O_{15}S$, 621.0920); ^1H and ^{13}C NMR data, see Tables 1 and 3.

Compound (9). White amorphous powder; $[\alpha]_D^{25}$ –33.6 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 280 (0.40) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 285 (–3.41), 236 (–5.38) nm; IR (KBr) ν_{max}: 3363, 1698, 1610, 1528, 1504, 1453 cm^{-1}; HRESIMS *m/z* 627.1316 $[M + Na]^+$ (calcd for $C_{28}H_{28}O_{15}Na$, 627.1320); ^1H and ^{13}C NMR data, see Tables 1 and 3.

Cellulose hydrolysis of 1−5.

A solution of **1** (2 mg) in 2 mL 0.1 M HOAc-NaOAc buffer (pH 4.5) was incubated at 40 °C with cellulose (2 mg) for 5 h. The reaction was monitored by HPLC-DAD analysis. After cooling, the reaction mixture was extracted with EtOAc (3 × 5 mL). The aqueous layer was evaporated under vacuum to furnish a crude sugar fraction. Hydrolysis of **2**−**5** was performed according to the procedure described for **1**.

Acid hydrolysis of 6−9.

Compound **6** (2 mg) was dissolved in 0.5 M HCl-H_2O (2 mL) and refluxed for 2 h. After cooling, the reaction mixture was extracted with EtOAc (3 × 5 mL). The aqueous layer was evaporated under vacuum to furnish a crude sugar fraction. Hydrolysis of **7**−**9** was performed according to the procedure described for **6**.

Gas chromatographic (GC) analysis of glucose.

The crude sugar fraction was dissolved in anhydrous pyridine (1 mL), to which 2 mg of L-cysteine methyl ester hydrochloride was added. The mixture was stirred at 60 °C for 2 h. After evaporation under reduced pressure, 0.2 mL of *N*-trimethylsilylimidazole was added, and the mixture was kept at 60 °C for another 2 h. The reaction mixture was partitioned between *n*-hexane and H_2O (2 mL each), and the *n*-hexane extract was analysed by GC under the following conditions: capillary column, HP-5 (30 m × 0.25 mm, with a 0.25 μm film, Dikma); Detection, FID; detector temperature, 280 °C; injection temperature, 250 °C; initial temperature 160 °C, then raised to 280 at 5 °C /min, final temperature maintained for 10 min; carrier, N_2 gas. D-glucose was confirmed by comparison of the retention time of its derivative with the authentic sugar derivatized in a similar way, which exhibited a retention time of 19.03 min.

Inhibitory activity of α-Glucosidase.

The inhibitory activity of compounds **1**−**15** on α-glucosidase was determined spectrophotometrically on a 96-well microplate reader. In total, 20 μL of 0.2 U/mL α-glucosidase was premixed with 10 μL of compounds at various concentrations in 50 μL of 100 mM phosphate buffer (pH 7.0) at 37 °C for 5 min. Then, 20 μL of 2.5 mM substrate *p*-nitrophenyl-α-D-glucopyranoside was added to the mixture to initiate the reaction. The reaction was incubated at 37 °C for 15 min and stopped by the addition of 50 μL of 0.4 M Na_2CO_3. α-Glucosidase activity was determined by measuring the release of *p*-nitrophenol from *p*-nitrophenyl-α-D-glucopyranoside at 400 nm. The sample contained the mixture and the test compound, while the control consisted of the mixture, including the solvent, but without test compound. The sample blank contained the mixture, including the test compound, but without α-glucosidase. Finally, the control blank was the mixture, including the solvent, but without α-glucosidase.

The inhibition (%) of sample on α-glucosidase was calculated by the following formula:

Inhibition (%) = $[(A_{(sample)}-A_{(sample\ blank)})/(A_{(control)}-A_{(control\ blank)})] \times 100$

Conclusions

Herein, we reported the isolation and structure elucidation of nine new compounds from the rhizome of *P. cuspidatum*, including an unusual adduct of ECG fused to piceid through a carbon−carbon bond (**1**), four new dimeric stilbene glycosides (**2**−**5**), three new stilbene glucosides (**6**−**8**), and one new flavan glucoside (**9**). In order to determine the absolute configurations for the chiral carbons, the $Rh_2(OCOCF_3)_4$-induced CD assay was combined with the characteristic coupling constant and ECD spectrum analysis. Further investigation of *in vitro* bioactivities of **1**–**15** revealed that compound **8** showed significantly stronger inhibition activity than the positive control acarbose. This result indicated that compound **8** might be useful as a glucosidase inhibitor agent for the treatment of type 2 diabetes.

Acknowledgements

This project was supported by the National Natural Science Foundation of China (No. 81274066) and Natural Science Foundation of Beijing Municipality (No. 7112094).

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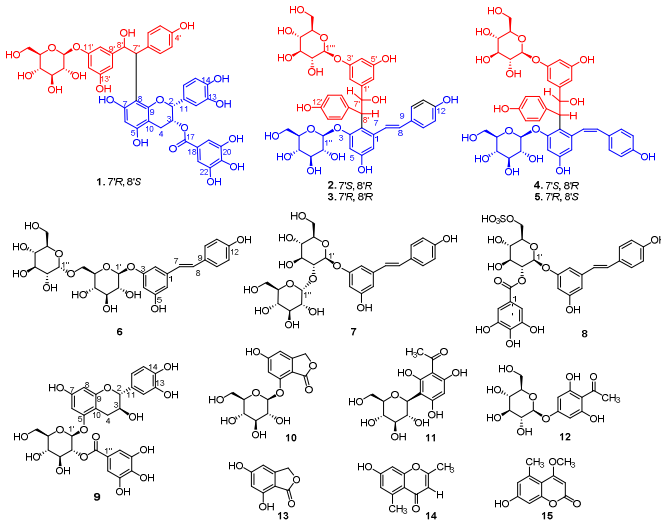

Fig. 1 Chemical structures of **1**–**15**.

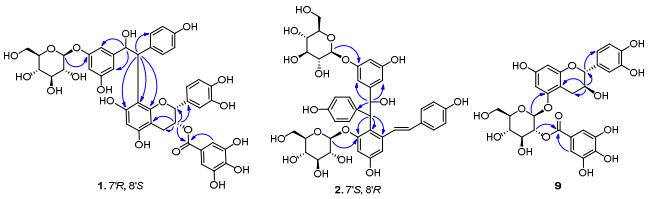

Fig. 2 Key HMBC correlations of **1**, **2**, and **9**.

Table 1 ^{1}H NMR Data of **1**–**5** in DMSO-d_6 (500 MHz, δ in ppm, J in Hz)

No.	1	2	3	4	5
2	5.18, br s				
3	5.36, br s				
4	3.03, d (17.5) 2.76, dd (17.5, 4.5)	6.21, s	6.13, s	6.44, s	6.48, s
6	6.00, s	6.54, s	6.27, s	6.06, s	6.06, s
7		6.70, d (16.0)	6.59, d (16.0)	6.60, d (12.0)	6.58, d (11.5)
8		7.27, d (16.0)	6.87, d (16.0)	6.51, d (12.0)	6.51, d (11.5)
9					
10		7.34, d (8.0)	7.35, d (8.5)	6.86, d (8.5)	6.87, d (8.5)
11		6.79, d (8.0)	7.01, d (8.5)	6.47, d (8.5)	6.46, d (8.5)
12	6.90, d (1.5)				
13		6.79, d (8.0)	7.01, d (8.5)	6.47, d (8.5)	6.46, d (8.5)
14		7.34, d (8.0)	7.35, d (8.5)	6.86, d (8.5)	6.87, d (8.5)
15	6.68, d (8.5)				
16	6.96, dd (8.5, 1.5)				
19	6.88, s				
23	6.88, s				
1′					
2′	7.04, d (8.5)	6.40, s	6.49, s	6.41, s	6.23, s
3′	6.53, d (8.5)				
4′		6.23, s	6.25, s	6.20, s	6.16, s
5′	6.53, d (8.5)				
6′	7.04, d (8.5)	6.53, s	6.40, s	6.27, s	6.32, s
7′	4.45, d (10.5)	5.57, d (10.0)	5.49, d (3.0)	5.61, d (10.5)	5.66, d (10.5)
8′	5.39, dd (10.5, 4.0)	4.37, d (10.0)	4.47, m	4.49, d (10.5)	4.29, d (10.5)
10′	6.15, s	6.89, d (8.0)	7.03, d (8.5)	6.94, d (8.0)	7.10, d (8.0)
11′		6.43, d (8.0)	6.54, d (8.5)	6.34, d (8.0)	6.33, d (8.0)
12′	6.18, s				
13′		6.43, d (8.0)	6.54, d (8.5)	6.34, d (8.0)	6.33, d (8.0)
14′	6.12, s	6.89, d (8.0)	7.03, d (8.5)	6.94, d (8.0)	7.10, d (8.0)
1″	4.61, d (7.5)	4.85, d (7.0)	4.87, d (7.5)	4.86, d (7.5)	5.02, d (7.0)
2″	3.15, m	3.46, m	3.19, m	3.46, m	3.50, m
3″	3.21, m	3.36, m	3.32, m	3.34, m	3.33, m
4″	3.25, m	3.27, m	3.17, m	3.27, m	3.23, m
5″	3.21, m	3.21, m	3.42, m	3.19, m	3.34, m
6″	3.55, m 3.64, m	3.52, m 3.75, d (11.0)	3.46, m 3.66, m	3.54, m 3.73, m	3.51, m 3.73, m
1‴		4.66, d (7.0)	4.57, d (6.0)	4.63, d (7.5)	4.57, d (7.5)
2‴		3.16, m	3.15, m	3.14, m	3.13, m
3‴		3.36, m	3.33, m	3.20, m	3.19, m
4‴		3.20, m	3.31, m	3.18, m	3.19, m
5‴		3.22, m	3.19, m	3.43, m	3.19, m
6‴		3.54, m 3.65, d (11.5)	3.46, m 3.66, m	3.50, m 3.61, d (11.5)	3.51, m 3.64, m

Table 2 ^{13}C NMR Data of **1**−**9** in DMSO-d_6 (125 MHz, δ in ppm)

No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1		139.7	140.0	141.0	140.6	139.4	139.4	140.1	
2	76.8	120.4	117.1	121.7	122.1	105.6	105.2	107.9	81.4
3	68.8	157.1	157.7	157.7	156.3	158.9	158.5	159.0	66.0
4	26.3	101.8	104.0	102.2	102.6	102.9	103.2	103.5	28.0
5	153.7	156.1	157.0	156.7	155.9	158.4	158.4	158.8	155.7
6	95.6	106.9	105.0	109.6	109.5	106.6	107.5	105.8	94.9
7	154.2	124.7	126.4	129.0	128.3	125.3	125.2	125.6	156.8
8	108.6	130.7	130.0	130.2	129.6	128.5	128.6	128.5	96.6
9	154.4	128.2	131.0	127.9	127.3	128.1	128.0	129.1	155.2
10	97.9	127.8	127.6	130.9	130.6	128.1	128.0	128.6	101.2
11	130.2	115.8	116.6	115.3	114.7	115.6	115.6	115.9	130.2
12	114.3	157.4	157.0	157.1	157.5	157.4	157.4	157.7	115.2
13	144.5	115.8	116.6	115.3	114.7	115.6	115.6	115.9	145.0
14	144.9	127.8	127.6	130.9	130.6	128.1	128.0	128.6	144.9
15	115.2								114.9
16	117.5								118.7
17	165.5								
18	119.8								
19	108.9								
20	145.6								
21	138.7								
22	145.6								
23	108.9								
1′	133.7	147.8	146.7	148.5	148.0	100.8	100.6	99.4	97.9
2′	129.6	106.8	105.9	107.2	106.8	73.2	75.0	74.0	73.5
3′	114.5	158.1	158.4	158.6	157.7	76.7	77.4	74.4	74.2
4′	154.8	102.0	102.0	102.1	101.5	69.7	69.9	70.8	69.7
5′	114.5	157.4	157.7	158.0	157.5	75.0	77.0	75.4	77.0
6′	129.6	108.6	107.4	109.5	107.9	66.3	60.5	66.1	60.4
7′	49.6	74.0	74.5	74.4	74.0				
8′	74.7	52.7	50.5	54.0	54.4				
9′	148.4	132.9	132.6	133.1	132.5				
10′	106.6	129.9	129.2	131.1	130.6				
11′	157.7	114.5	114.6	114.7	114.3				
12′	101.3	154.9	155.2	155.4	154.8				
13′	157.3	114.5	114.6	114.7	114.3				
14′	108.1	129.9	129.2	131.1	130.6				
1″	100.5	101.5	100.4	101.2	100.6	98.5	97.6	119.8	119.6
2″	73.3	74.4	73.3	74.0	73.3	72.1	72.1	109.2	108.9
3″	76.7	77.0	77.1	77.0	77.1	73.2	73.2	146.0	145.5
4″	69.2	69.6	69.8	69.6	69.8	70.2	70.1	139.0	138.5
5″	76.7	76.6	76.9	76.6	77.0	72.6	72.2	146.0	145.5
6″	60.3	60.6	60.5	60.3	60.8	60.8	60.7	109.2	108.9
7″								165.4	165.0
1‴		100.6	101.1	100.4	100.3				
2‴		73.2	73.3	73.1	73.1				
3‴		77.2	76.7	76.8	76.6				
4‴		69.4	69.5	69.3	69.3				
5‴		76.9	76.7	77.1	76.8				
6‴		60.5	60.8	60.6	60.3				

Table 3 ^{1}H NMR Data of **6**–**9** in DMSO-d_6 (500 MHz, δ in ppm, J in Hz)

No.	**6**	**7**	**8**	**9**
2	6.63, s	6.75, s	6.57, s	4.33, d (7.5)
3				3.59, m
4	6.40, s	6.33, s	6.17, s	2.26, dd (16.5, 8.5) 2.45, overlap
6	6.59, s	6.56, s	6.59, s	6.11, d (1.5)
7	6.87, d (16.5)	6.84, d (16.5)	6.83, d (16.5)	
8	6.99, d (16.5)	7.02, d (16.5)	6.93, d (16.5)	5.85, d (1.5)
10	7.41, d (8.5)	7.39, d (8.5)	7.42, d (8.5)	
11	6.74, d (8.5)	6.74, d (8.5)	6.71, d (8.5)	
12				6.67, d (1.5)
13	6.74, d (8.5)	6.74, d (8.5)	6.71, d (8.5)	
14	7.41, d (8.5)	7.39, d (8.5)	7.42, d (8.5)	
15				6.63, d (8.0)
16				6.52, dd (8.0, 1.5)
1′	4.78, d (7.5)	5.00, d (8.0)	5.01, d (8.0)	5.11, d (8.0)
2′	3.44, m	3.42, m	4.92, dd (8.0, 8.0)	4.96, dd (8.0, 8.0)
3′	3.27, m	3.50, m	3.57, m	3.58, m
4′	3.27, m	3.19, m	3.26, m	3.34, m
5′	3.52, m	3.35, m	3.70, m	3.43, m
6′	3.62, m 3.75, m	3.40, m 3.53, m	3.81, m 4.16, d (10.0)	3.56, m 3.72, d (11.0)
1″	4.69, d (3.0)	5.19, d (3.5)		
2″	3.21, m	3.90, m	6.97, s	6.94, s
3″	3.22, m	3.44, m		
4″	3.09, m	3.35, m		
5″	3.08, m	3.19, m		
6″	3.45, m 3.57, m	3.53, m 3.72, m	6.97, s	6.94, s