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Polyflavanostilbene A, a New Flavanol-Fused Stilbene Glycoside from *Polygonum cuspidatum*

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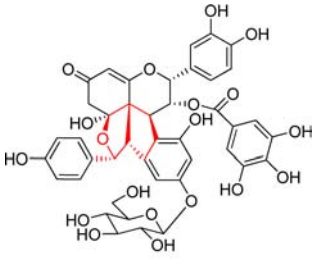
ABSTRACT



Polyflavanostilbene A (**1**)
2*R*,3*R*,4*S*,5*S*,7′*R*,8′*S*,10*S*

Polyflavanostilbene A, a new flavanol-fused stilbene glycoside, was isolated from the rhizome of *Polygonum cuspidatum*. Its unusual structure, including its absolute stereochemistry, was determined by UV, IR, HRESIMS, and 1D and 2D NMR data and by the comparison of experimental and calculated electronic circular dichroism (ECD) spectra. Polyflavanostilbene A has an unprecedented rearranged flavanol skeleton fused to stilbene via a hexahydrocyclopenta[*c*]furan moiety. Polyflavanostilbene A showed strong inhibitory activity against α-glucosidase with an IC_{50} value of 17.7 μM.

Polygonum cuspidatum Siebold & Zucc. (Polygonaceae), also called Huzhang in China, is a perennial herb and grows widely in Asia and North America. In China, the rhizome of *P. cuspidatum* is a known traditional Chinese medicine conventionally used for the treatment of suppurative dermatitis, gonorrhea, chronic bronchitis, jaundice, amenorrhea, hypertension, hyperlipemia and menopausal symptoms.[1] A recent study showed that its extract could be an inhibitor of phosphotyrosine phosphatase 1B, and some polyphenolic components have inhibitory activity against α-glucosidase.[2] Previous chemical investigations of this plant revealed the presence of flavonoids,[3] anthraquinones,[4] stilbenes (incuding dimeric stilbene),[5] and other phenols.[6] Among these compounds, anthraquinones and stilbenes (resveratrol and piceid) are high-content constituents[7] that are generally regarded as an index for quality control of this herb. As a continuation of our efforts to explore new structures with inhibitory activity against α-glucosidase, we performed a study on this plant and

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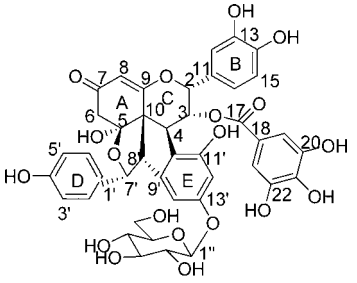
isolated a new adduct of epicatechin-3-*O*-gallate and piceid, named polyflavanostilbene A (**1**), which has an unprecedented rearranged flavanol skeleton fused to stilbene via a hexahydrocyclopenta[*c*]furan moiety. Polyflavanostilbene A was observed to exhibit strong inhibitory activity against α-glucosidase.



Polyflavanostilbene A (**1**)
2*R*,3*R*,4*S*,5*S*,7′*R*,8′*S*,10*S*

Polyflavanostilbene A (**1**) was isolated as a white powder, $[\alpha]_D^{20}$ 36.0 (*c* 0.08 MeOH). The molecular formula of **1** was determined as $C_{42}H_{38}O_{19}$ from the HRESIMS ion at *m*/*z* 845.1926 $[M - H]^-$ (calcd 845.1935), which indicated 24 degrees of unsaturation. IR absorptions indicated the existence of hydroxyl groups (3358 cm^{-1}) and of a vinylogous ester (1697 cm^{-1}).

The ^1H NMR spectrum (Table 1) of **1** displayed one set of *ortho*-coupled aromatic protons at δ_H 7.11 (2H, d, J = 8.5 Hz) and 6.65 (2H, d, J = 8.5 Hz); two *meta* protons at δ_H 6.26 (1H, s) and 5.01 (1H, s); three ABX system aromatic protons at δ_H 6.56 (1H, d, J = 8.0 Hz), 6.55 (1H, d, J = 1.5 Hz), and 6.48 (1H, dd, J = 8.0, 1.5 Hz); one olefinic proton at δ_H 5.84 (1H, s); and two characteristic signals of a galloyl moiety at δ_H 6.86 (2H, s). In addition, five methine protons at δ_H 5.50 (1H, s), 5.35 (1H, d, J = 8.0 Hz), 4.96 (1H, s), 4.52 (1H, d, J = 8.0 Hz), and 3.86 (1H, s) and two methylene protons at δ_H 2.71 (1H, d, J = 15.5 Hz) and 2.65 (1H, d, J = 15.5 Hz) were observed, while a doublet due to an anomeric proton at δ_H 4.10 (1H, d, J = 8.0 Hz), together with the signals between δ_H 3.06 and 3.56, indicated the presence of a glycosyl group. After cellulose hydrolysis, the sugar unit of **1** was confirmed to be D-glucose by GC analysis of its trimethylsilyl L-cysteine derivative.[8]

The ^{13}C NMR spectrum (Table 1) of **1** revealed a total of 42 carbon signals, including a galloyl and an *O*-glucose unit, and they were classified into 2 methylenes, 22 methines, and 18 quaternary carbons. Comparing the ^{13}C NMR data of **1** with the corresponding signals of epicatechin-3-*O*-gallate (ECG),[9] the chemical shift values for ring B and C-2, C-3 of ring C in **1** were similar to those of the corresponding data for ECG, while a significant difference was seen in the carbon chemical shifts for ring A in **1**. Careful analysis of the HMBC spectrum in **1** (Figure 1), which showed the cross-peaks of H-8 with C-6, C-9, and C-10, H-6 with C-5 and C-7, H-19 with C-17, H-3 with C-17, and H-2 with C-11 and C-12, revealed that ring A in **1** might be rearranged to an α,β-unsaturated cyclohexanone instead of an *O*-substituted aromatic ring in ECG. Besides the rearranged ECG (22 carbons) and one β-D-glucopyranosyl group (6 carbons), the remaining 14 carbons, including 12 aromatic and 2 aliphatic carbons, should be assigned to a 7′,8′-dihydro-resveratrol unit with one set of *ortho*-coupled protons at δ_H 7.11 (2H, d, J = 8.5 Hz), 6.65 (2H, d, J = 8.5 Hz), two aromatic protons (δ_H 6.26, 5.01) and two aliphatic protons (δ_H 5.35, J = 8.0 Hz; 4.52, J = 8.0 Hz) in the ^1H NMR spectrum of **1**. Furthermore, the connectivity of the rearranged ECG and the resveratrol moiety was established on the basis of the HMBC spectrum (Figure 1). In the HMBC experiment, the correlations of H-7′ with C-5 and C-2′, H-2′ with C-7′, and H-8′ with C-9, C-10, and C-14′ were observed, suggesting that the resveratrol unit was fused to ring A of **1** through a tetrahydrofuran moiety, while the HMBC correlations of H-3 with C-10′ and H-4 with C-8′, C-9′, C-10′, and C-10 indicated that the C-10′ of ring E in the resveratrol unit was connected with the C-4 of ring C. This signifies that an uncommon hexahydrocyclopenta[*c*]furan moiety was formed. The connectivity of the sugar moiety to the C-13′ position of the aglycon was confirmed by the HMBC correlations of H-1″ with C-13′.

The relative configuration of **1** was determined by a ROESY experiment (Figure 1). In the ROESY spectrum, the correlation of H-2 with H-3 indicated that they were on the same face of ring C, while the correlation of H-7′ with H-8′ suggested that they were also *cis*-oriented on the tetrahydrofuran ring, coupling with J = 8.0 Hz. The

Table 1. ^1H and ^{13}C NMR[a] Assignments for **1** in DMSO-d_6

no.	δ_H	δ_C	no.	δ_H	δ_C
1			1′		128.9
2	4.96 (s)	76.9	2′	7.11 (d, 8.5)	128.8
3	5.50 (s)	72.4	3′	6.65 (d, 8.5)	114.5
4	3.86 (s)	47.5	4′		156.7
5		103.9	5′	6.65 (d, 8.5)	114.5
6	2.65 (d, 15.5)	47.4	6′	7.11 (d, 8.5)	128.8
	2.71 (d, 15.5)				
7		194.2	7′	5.35 (d, 8.0)	81.9
8	5.84 (s)	106.0	8′	4.52 (d, 8.0)	61.4
9		174.6	9′		143.3
10		61.3	10′		121.0
11		127.5	11′		153.4
12	6.55 (d, 1.5)	113.9	12′	6.26 (s)	102.4
13		144.9	13′		157.7
14		145.1	14′	5.01 (s)	103.9
15	6.56 (d, 8.0)	115.2	1″	4.10 (d, 8.0)	100.2
16	6.48 (dd, 8.0, 1.5)	117.4	2″	3.06 (t, 8.0)	73.0
17		164.0	3″	3.21 (t, 8.0)	76.3
18		119.1	4″	3.18 (t, 8.0)	69.0
19	6.86 (s)	108.8	5″	3.08 (m)	76.1
20		145.5	6″	3.56 (m, 2H)	60.2
21		138.7			
22		145.5			
23	6.86 (s)	108.8			

[a] ^1H NMR (500 MHz) (δ in ppm, J in Hz), ^{13}C NMR (125 MHz).

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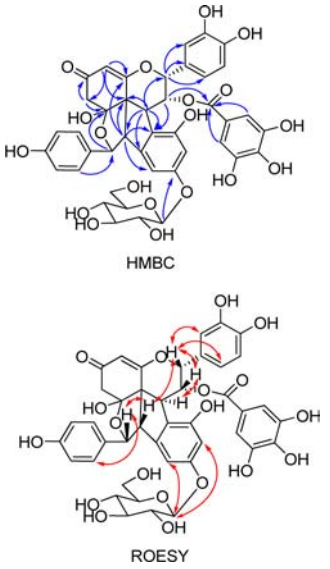

Figure 1. Key HMBC and ROESY correlations of **1**.

chemical shift of H-14′ at δ_H 5.01 (1H, s) has an unusual value for an aromatic proton, which also supported the *cis*-relationship between rings D and E on the tetrahydrofuran cycle. Furthermore, ROESY correlation of H-2 with H-8′ was observed, revealing that they were on the same face of the molecule and relatively close, and implied that H-2 and H-4 were in a *trans*-relationship.On the basis of the above correlations, only four diastereoisomers (**1a**, 2*R*,3*R*,4*S*,5*S*,7′*R*,8′*S*,10*S*; **1b**, 2*S*,3*S*,4*R*,5*R*,7′*S*,8′*R*,10*R*; **1c**, 2*R*,3*R*,4*S*,5*R*,7′*R*,8′*S*,10*S*; **1d**, 2*S*,3*S*,4*R*,5*S*,7′*S*,8′*R*,10*R*) remained. The absolute configuration of **1** was established by measurement of the electronic circular dichroism (ECD) spectrum and by comparison with calculated ECD data using the ZINDO method.[10] Due to at least 6.8 billion conformations stemming from its numerous single bonds, a simplified structure **2** (Figure S1 in Supporting Information), in which a methyl group instead of the glucosyl group in **1**, was used for ECD calculations. A systematic conformational analysis was performed for **2a** and **2c** corresponding to **1a** and **1c** using the MMFF94 molecular mechanics force field calculation (Supporting Information). The optimized conformations of **2a** and **2c** are shown in Figure 2. Considering the above-mentioned ROESY data, only a pair of enantiomers (**2a** and **2b**) were in good agreement with the ROESY data. The observations that there is no coupling between H-2 and H-3 and there is the correlation of H-3 with H-4 in the ROESY experiment are also in favor of a twist-boat conformation for ring C in the optimized conformations. ECD calculation was performed after optimization of the selected conformers using the ZINDO method. The overall pattern of calculated ECD spectrum of **2a** was in

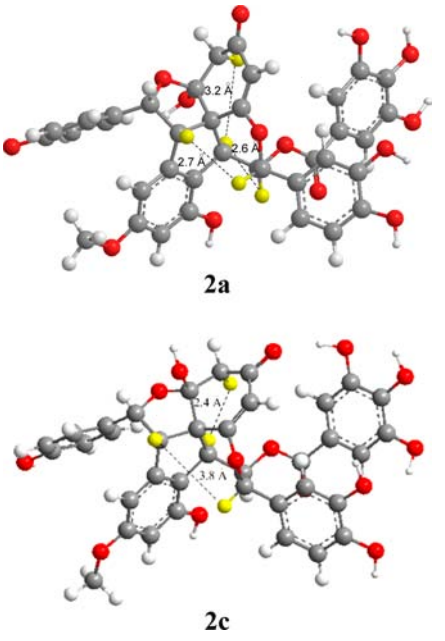

Figure 2. Optimized conformations of **2a** and **2c**.

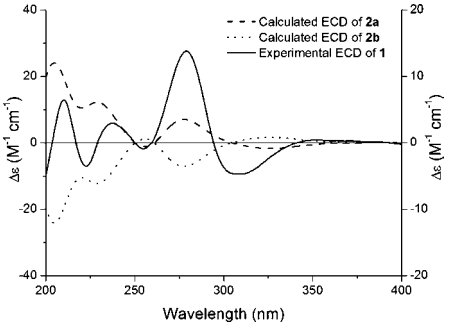

Figure 3. Experiment ECD spectrum of **1** and calculated ECD of **2a** and **2b** in MeOH.

accord with the experimental data of **1** (Figure 3). Therefore, the absolute configuration of **1** was established as 2*R*,3*R*,4*S*,5*S*,7′*R*,8′*S*,10*S*. The structure of **1** was elucidated as depicted and named polyflavanostilbene A.

Structurally, **1** has an unprecedented rearranged flavanol skeleton fused to stilbene. The most intriguing feature of **1** is the unusual hexahydrocyclopenta[*c*]furan moiety that includes an hemiketal group. Due to the reactivity of the double bond in stilbenes, they tend to form adducts with phenolic compounds. To the best of our knowledge, the main adducts isolated from natural products were formed by one C−C bond linkage or tetrahydrofuran moiety between the double bonded carbon in stilbenes and the aromatic ring in phenolic compounds.[11] Obviously, this finding reveals a

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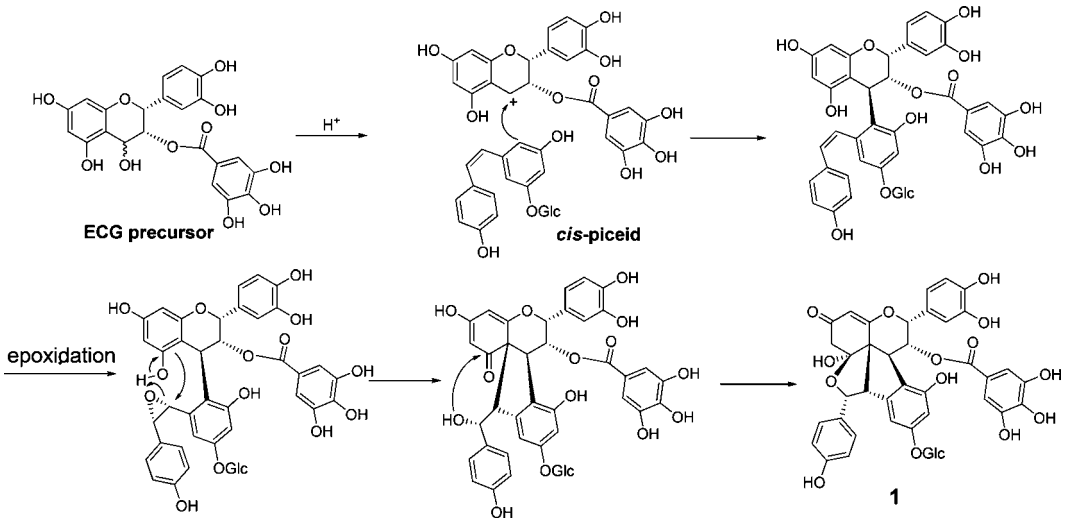
Scheme 1. Hypothetical Biogenetic Pathway of **1**



new type of linkage between a flavanol and a stilbene. A plausible biogenetic route of **1** is proposed in Scheme 1. The hexahydrocyclopenta[*c*]furan moiety in **1** is readily rationalized by a series of electrophilic substitution, oxidation, and nucleophilic additions. The electrophilic aromatic substitution between a flavan and piceid may be the key step, and the process of formation may be similar to that of the dimer of ECG.[12] The final product is then derived by an epoxidation of the stilbene moiety of *cis*-piceid and intramolecular nucleophilic additions.

In the *in vitro* bioactivity assays,[13] **1** exhibited a strong inhibitory activity against α-glucosidase with an IC50 value of 17.7 μM, using acarbose as a positive control (IC_{50} 385 μM) .

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Supporting Information Available. Detailed experimental procedures, physicochemical properties, 1D and 2D NMR, MS and IR spectra and related original ECD calculation data of compounds **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.