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Antioxidant phenolic glycosides from the roots of *Illicium dunnianum*

Jian Bai, Zhen-Feng Fang, Hui Chen, Shi-Shan Yu *, Dan Zhang, Huai-Ling Wei, Shuang-Gang Ma, Yong Li, Jing Qu, Song Xu, Jing-Hong Ren, Fen Zhao, Nan Zhao, Jun-Hong Liu

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, People's Republic of China

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ABSTRACT

Eight new phenolic glycosides, dunnianosides A–H (**1**–**8**), and nine known phenolic glycosides (**9**–**17**), were isolated from the roots of *Illicium dunnianum*. The structures of these new compounds were elucidated by spectroscopic methods including 1D and 2D NMR, HRESIMS, and chemical methods. Compounds **1**–**5**, **7**, and **9** exhibited potent antioxidant activities against Fe^{2+}-cystine-induced rat liver microsomal lipid peroxidation, with IC_{50} values ranging from 3.8 ± 0.6 to 23.0 ± 2.2 μM.

1. Introduction

Illicium dunnianum (Illiciaceae) is a toxic shrub distributed in Southern China and used as a folk medicine for relieving pain and treating rheumatism.[1] Studies on this genus showed its rich content of sesquiterpenes, prenylated C6–C3 compounds, and neolignans with extensive biological activities including cytotoxic, neurotrophic, anti-inflammatory, and antioxidant activities.[2–5] In our previous paper, six *allo*-cedrane sesquiterpenes, four *seco*-prezizaane-type sesquiterpenes, and two monocyclofarnesane sesquiterpenes with anti-inflammatory activities were isolated from the roots of this species.[6] Further investigation of bioactive constituents from this plant has led to the isolation and structural elucidation of 17 phenolic glycosides including eight new phenolic glycosides, dunnianosides A–H (**1**–**8**), and nine known ones (**9**–**17**) (Fig. 1), along with their antioxidant evaluation.

2. Results and discussion

The EtOAc fraction of the ethanol extract was subjected to column chromatography on silica gel, Sephadex LH-20, ODS, and HPLC, respectively, to afford eight new phenolic glycosides, dunnianosides A–H (**1**–**8**), together with nine known phenolic glycosides (**9**–**17**).

Compound **1** was isolated as a white amorphous powder. Its molecular formula was deduced to be $C_{23}H_{28}O_{11}$ by a positive HRESIMS ion at *m*/*z* 503.1533 $[M+Na]^+$ (calcd for $C_{23}H_{28}O_{11}Na$: 503.1524), which was supported by ^{13}C NMR spectroscopic data. The IR spectrum displayed absorptions ascribable to hydroxy (3462 cm^{-1}), carbonyl (1688 cm^{-1}), and aromatic (1614, 1518, and 1463 cm^{-1}) groups. The ^1H NMR spectroscopic data (Table 1) showed two aromatic protons attributed to a 1, 2, 4, 5-tetrasubstituted aromatic ring at δ_H 6.54 (1H, s, H-3) and 6.83 (1H, s, H-6), a singlet assignable to a symmetrical 1, 3, 4, 5-tetrasubstituted aromatic ring at δ_H 7.32 (2H, s, H-2″, 6″), two aromatic methoxy signals at δ_H 3.83 (6H, s, 3″, 5″-OCH_3), two methyl signals at δ_H 2.12 (3H, s, 2-CH_3) and 1.90 (3H, s, 5-CH_3), and an anomeric proton signal at δ_H 4.75 (1H, d, J = 7.5 Hz, H-1′). Acid hydrolysis of **1** afforded a glucose which was confirmed by TLC with an authentic sample of glucose, and the D-configuration was determined by GC analysis.[7] The β-anomeric configuration for the glucose was deduced from its large $^3J_{1',2'}$ coupling constant (7.5 Hz). The ^{13}C NMR spectrum showed a group of characteristic signals due to a syringoyl group at δ_C 121.3 (C-1″), 108.2 (C-2″, 6″), 148.3 (C-3″, 5″), 141.7 (C-4″), 166.5 (C-7″), 56.7 (3″, 5″-OCH_3) (Table 2). A series of HMBC correlations from the anomeric proton H-1′ (δ_H 4.75) to an aromatic carbon C-1 (δ_C 149.9), from 2-CH_3 (δ_H 2.12) to C-1 (δ_C 149.9), C-2 (δ_C 127.1), and C-3 (δ_C 117.2), from 5-CH_3 (δ_H 1.90) to C-4 (δ_C 151.1), C-5 (δ_C 122.5), and C-6 (δ_C 120.4), from H-3 (δ_H 6.54) to C-1 (δ_C 149.9) and C-5 (δ_C 122.5), and from H-6 (δ_H 6.83) to C-2 (δ_C 127.1) and C-4 (δ_C 151.1) indicated the 4-hydroxy-2, 5-dimethylphenyl 1-*O*-β-D-glucopyranoside moiety in **1** (Fig. 2). Furthermore, the deshielded 6′-methylene proton [δ_H 4.71 (1H, dd, J = 12.0, 1.5 Hz, H-6′a) and 4.42 (1H, dd, J = 12.0, 7.0 Hz, H-6′b)] of the glucose moiety due to the esterification correlated with the carbonyl carbon (δ_C 166.5, C-7″) of the syringoyl group, suggesting that the *O*-syringoyl group was attached to C-6′. Therefore, the

* Corresponding author. Tel.: +86 10 63165326; fax: +86 10 63017757.
E-mail address: yushishan@imm.ac.cn (S.-S. Yu).

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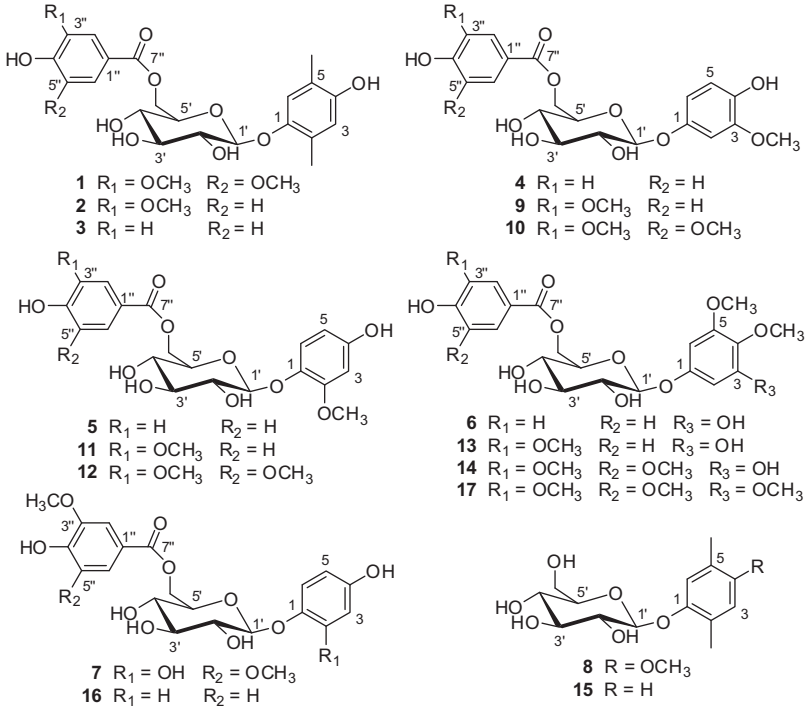

Figure 1. The structures of compounds **1**–**17**.

Table 1
^{1}H NMR spectroscopic data for compounds **1**–**8** (500 MHz, *J* in Hz)[a]

Position	1	2	3	4	5	6	7	8
2				6.68 br s		6.37 d (2.0)		
3	6.54 s	6.57 s	6.58 s		6.47 d (2.5)		6.28 d (3.0)	6.69 s
5				6.66 d (8.5)	6.34 dd (8.5, 2.5)		5.98 dd (9.0, 3.0)	
6	6.83 s	6.87 s	6.89 s	6.61 d (8.5)	7.06 d (8.5)	6.23 d (2.0)	6.92 d (9.0)	6.96 s
1′	4.75 d (7.5)	4.76 d (7.5)	4.77 d (7.5)	4.87 d (7.5)	4.73 d (7.5)	4.95 d (7.5)	4.60 d (8.0)	4.75 d (7.5)
2′	3.49 overlap	3.49 overlap	3.50 overlap	3.48 overlap	3.48 overlap	3.48 overlap	3.46 overlap	3.46 overlap
3′	3.53 overlap	3.53 overlap	3.54 overlap	3.55 overlap	3.54 overlap	3.58 overlap	3.48 overlap	3.47 overlap
4′	3.48 overlap	3.47 overlap	3.47 overlap	3.47 overlap	3.47 overlap	3.50 overlap	3.41 dd (9.0, 9.0)	3.44 overlap
5′	3.76 m	3.76 m	3.76 m	3.82 m	3.72 m	3.87 m	3.72 m	3.41 overlap
6′	4.71 dd (12.0, 1.5)	4.70 d (12.0)	4.70 dd (12.0, 2.0)	4.71 d (11.5)	4.71 dd (12.0, 2.0)	4.73 d (12.0)	4.73 dd (12.0, 2.0)	3.86 d (12.0)
	4.42 dd (12.0, 7.0)	4.33 dd (12.0, 7.0)	4.33 dd (12.0, 7.0)	4.34 dd (11.5, 7.0)	4.28 dd (12.0, 7.5)	4.29 dd (12.0, 7.0)	4.41 dd (12.0, 7.5)	3.69 dd (12.0, 8.5)
2″	7.32 s	7.55 s	7.91 d (8.5)	7.92 d (8.5)	7.91 d (9.0)	7.94 d (8.5)	7.37 s	
3″			6.93 d (8.5)	6.95 d (8.5)	7.02 d (9.0)	6.94 d (8.5)		
5″		6.91 d (8.0)	6.93 d (8.5)	6.95 d (8.5)	7.02 d (9.0)	6.94 d (8.5)		
6″	7.32 s	7.59 d (8.0)	7.91 d (8.5)	7.92 d (8.5)	7.91 d (9.0)	7.94 d (8.5)	7.37 s	
2-Me	2.12 s	2.13 s	2.13 s					2.22 s
5-Me	1.90 s	1.98 s	2.02 s					2.10 s
2-OMe					3.77 s			
3-OMe				3.73 s				
4-OMe						3.70 s		3.76 s
5-OMe						3.73 s		
3″-OMe	3.83 s	3.86 s					3.88 s	
5″-OMe	3.83 s						3.88 s	

[a] Measured in acetone-d_6 for compounds **1–6** and **8**, and in methanol-d_4 for compound **7**.

structure of **1** was elucidated as 4-hydroxy-2, 5-dimethylphenyl 1-*O*-β-D-(6′-*O*-syringoyl) glucopyranoside and named dunnianoside A (**1**).

Compound **2** was assigned the molecular formula $C_{22}H_{26}O_{10}$ by positive HRESIMS (*m*/*z* 473.1427 $[M+Na]^+$, calcd 473.1418). The UV, IR, and NMR spectra of **2** resembled those of **1**. However, the ^{1}H and ^{13}C NMR spectra of **2** (Tables 1 and 2) exhibited a group of characteristic signals assignable to a vanilloyl group [δ_H 7.55 (1H, s, H-2″), 6.91 (1H, d, *J* = 8.0 Hz, H-5″), 7.59 (1H, d, *J* = 8.0 Hz, H-6″); δ_C 122.7 (C-1″), 113.4 (C-2″), 148.2 (C-3″), 152.2 (C-4″), 115.6 (C-5″), 124.7 (C-6″), 166.5 (C-7″)], replacing signals due to the syringoyl group in the NMR spectra of **1**. This was confirmed by HSQC and HMBC experiments of **2**. In the HMBC spectrum, long-range correlations of H-2″ (δ_H 7.55) with C-1″ (δ_C 122.7), C-3″ (δ_C 148.2), C-4″ (δ_C 152.2), C-6″ (δ_C 124.7), and the carbonyl carbon C-7″ (δ_C 166.5), of H-5″ (δ_H 6.91) with C-1″ (δ_C 122.7), C-3″ (δ_C 148.2), and C-4″ (δ_C 152.2), of H-6″ (δ_H 7.59) with C-2″ (δ_C 113.4), C-4″ (δ_C 152.2), and C-7″ (δ_C 166.5), and of H_2-6′ (δ_H 4.70, 4.33) with C-7″ (δ_C 166.5) indicated the presence of the *O*-vanilloyl group linked at C-6′. Thus, **2** was determined to be 4-hydroxy-2, 5-dimethylphenyl 1-*O*-β-D-(6′-*O*-vanilloyl)glucopyranoside and designated as dunnianoside B (**2**).

Table 2
^{13}C NMR spectroscopic data for compounds **1**–**8** (125 MHz)[a]

Position	1	2	3	4	5	6	7	8
1	149.9	150.0	149.9	152.2	140.6	155.3	140.0	150.5
2	127.1	127.0	127.0	103.4	152.0	97.6	149.7	126.6
3	117.2	117.3	117.3	148.5	101.6	151.5	104.4	113.3
4	151.1	151.2	151.1	142.7	154.9	132.5	155.4	153.8
5	122.5	122.6	122.5	115.4	107.0	154.3	107.0	124.7
6	120.4	120.2	120.2	109.4	120.7	94.5	120.9	120.1
1′	104.1	104.0	104.0	103.0	104.0	102.1	105.8	103.8
2′	74.7	74.8	74.7	74.7	74.7	74.5	74.9	74.9
3′	77.8	78.0	77.8	77.8	77.7	77.7	77.5	78.1
4′	71.6	71.7	71.6	71.5	71.6	71.3	72.0	71.5
5′	75.1	75.0	75.0	75.1	75.2	75.1	75.9	77.6
6′	65.0	65.0	64.9	64.7	64.6	64.9	65.2	62.8
1″	121.3	122.7	122.4	122.4	122.2	122.3	121.3	
2″	108.2	113.4	132.5	132.5	132.5	132.6	108.4	
3″	148.3	148.2	116.0	116.0	116.1	116.1	149.0	
4″	141.7	152.2	162.6	162.8	163.0	162.8	142.1	
5″	148.3	115.6	116.0	116.0	116.1	116.1	149.0	
6″	108.2	124.7	132.5	132.5	132.5	132.6	108.4	
7″	166.5	166.5	166.5	166.4	166.4	166.6	167.8	
2-Me	16.2	16.0	16.2					16.2
5-Me	15.9	16.2	16.1					16.4
2-OMe					56.3			
3-OMe				56.2				
4-OMe						60.9		56.0
5-OMe						56.1		
3″-OMe	56.7	56.3					56.9	
5″-OMe	56.7						56.9	

[a] Measured in acetone-d_6 for compounds **1–6** and **8**, and in methanol-d_4 for compound **7**.

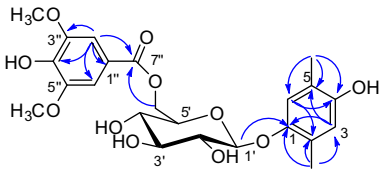

Figure 2. Key HMBC correlations (H→C) in compound **1**.

The molecular formula of compound **3** was determined as $C_{21}H_{24}O_9$ by positive HRESIMS (*m*/*z* 443.1334 $[M+Na]^+$, calcd 443.1313). The ^{1}H and ^{13}C NMR spectroscopic data (Tables 1 and 2) indicated the presence of *p*-hydroxybenzoyl group [δ_H 7.91 (2H, d, *J* = 8.5 Hz, H-2″, 6″), 6.93 (2H, d, *J* = 8.5 Hz, H-3″, 5″); δ_C 122.4 (C-1″), 132.5 (C-2″, 6″), 116.0 (C-3″, 5″), 162.6 (C-4″), 166.5 (C-7″)]. Comparison of the NMR spectroscopic data of **3** with those of **1** indicated that the structure of **3** was very close to that of **1**, except that the syringoyl group in **1** was substituted by the *p*-hydroxybenzoyl group in **3**. Moreover, the 2D NMR experiments resulted in the assignments of all NMR signals and the linkage of the structural units of **3**. Consequently, **3** was established as 4-hydroxy-2, 5-dimethylphenyl 1-*O*-β-D-[6′-*O*-(*p*-hydroxybenzoyl)] glucopyranoside and named dunnianoside C (**3**).

Compound **4** was assigned the molecular formula $C_{20}H_{22}O_{10}$ according to a positive HRESIMS ion at *m*/*z* 445.1112 $[M+Na]^+$ (calcd 445.1105). Comparison of the NMR spectroscopic data between **3** and **4** indicated that they shared the same 6′-*O*-(*p*-hydroxybenzoyl)-β-D-glucopyranosyl moiety. The remaining seven carbon resonances were identical to those of the aglycone of 6′-*O*-vanilloyltachioside (**9**),[8] suggesting that the aglycone of **4** was 4-hydroxy-3-methoxylphenoxy moiety. HMBC correlation between the anomeric proton H-1′ (δ_H 4.87) and C-1 (δ_C 152.2) indicated that the 6′-*O*-(*p*-hydroxybenzoyl)-β-D-glucopyranosyl moiety was attached to C-1. Therefore, **4** was elucidated as 4-hydroxy-3-methoxylphenyl 1-*O*-β-D-[6′-*O*-(*p*-hydroxybenzoyl)] glucopyranoside and named dunnianoside D (**4**).

Compound **5** possessed the same molecular formula as **4**, $C_{20}H_{22}O_{10}$, deduced from positive HRESIMS (*m*/*z* 445.1111 $[M+Na]^+$, calcd 445.1105). The IR, UV, and NMR spectroscopic data of **5** closely resembled those of **4**, except that a 1, 2, 4-trisubsituted aromatic ring moiety instead of a 1, 3, 4-trisubsituted one was located at the C-1′ position in **5**. This was confirmed by HMBC correlations from H-3 (δ_H 6.47) to C-1 (δ_C 140.6), C-2 (δ_C 152.0), C-4 (δ_C 142.7), and C-5 (δ_C 115.4), from H-5 (δ_H 6.34) to C-1 (δ_C 140.6), C-3 (δ_C 101.6), and C-4 (δ_C 142.7), from H-6 (δ_H 7.06) to C-1 (δ_C 140.6), C-2 (δ_C 152.0), and C-4 (δ_C 142.7), from a methoxy proton (δ_H 3.77) to C-2 (δ_C 152.0), and from H-1′ (δ_H 4.73) to C-1 (δ_C 140.6) (Fig. 3). Accordingly, the structure of **5** was determined to be 4-hydroxy-2-methoxylphenyl 1-*O*-β-D-[6′-*O*-(*p*-hydroxybenzoyl)]glucopyranoside and named dunnianoside E (**5**).

Compound **6** was assigned the molecular formula $C_{21}H_{24}O_{11}$ on the basis of its positive HRESIMS (*m*/*z* 475.1233 $[M+Na]^+$, calcd 475.1211). Comparison of the NMR spectroscopic data of **6** with those of **5** indicated that they possessed the same 6′-*O*-(*p*-hydroxybenzoyl)-β-D-glucopyranosyl moiety. In addition, the ^{1}H NMR spectrum showed two aromatic protons attributed to a 1, 3, 4, 5-tetrasubstituted aromatic ring at δ_H 6.37 (1H, d, *J* = 2.0 Hz, H-2) and 6.23 (1H, d, *J* = 2.0 Hz, H-6), and two aromatic methoxy signals at δ_H 3.70 (3H, s, 4-OCH_3) and 3.73 (3H, s, 5-OCH_3) (Table 1). HMBC correlations from H-1′ (δ_H 4.95) to C-1 (δ_C 155.3), from H-2 (δ_H 6.37) to C-1 (δ_C 155.3), C-3 (δ_C 151.5), C-4 (δ_C 132.5), and C-6 (δ_C 194.5), from H-6 (δ_H 6.23) to C-1 (δ_C 155.3), C-2 (δ_C 97.6), C-4 (δ_C 132.5), and C-5 (δ_C 154.3), from one methoxy proton (δ_H 3.70) to C-4 (δ_C 132.5), and from another methoxy proton (δ_H 3.73) to C-5 (δ_C 154.3) indicated that the aglycone of **6** was 3-hydroxy-4, 5-dimethoxyphenoxy unit and 6′-*O*-(*p*-hydroxybenzoyl)-β-D-glucopyranosyl moiety was located at C-1. Therefore, **6** was elucidated as 3-hydroxy-4, 5-dimethoxyphenyl 1-*O*-β-D-[6′-*O*-(*p*-hydroxybenzoyl)] glucopyranoside and named dunnianoside F (**6**).

The molecular formula of compound **7** was deduced to be $C_{21}H_{24}O_{12}$ by positive HRESIMS (*m*/*z* 491.1165 $[M+Na]^+$, calcd 491.1160). The NMR spectroscopic data (Tables 1 and 2) of **7** were closely related to those of **1** except for the signals attributed to the aglycone of **7**. Furthermore, the ^{1}H and ^{13}C NMR spectra showed a set of ABX coupling assignable to a 1, 2, 4-trisubstituted aromatic ring at δ_H 6.28 (1H, d, *J* = 3.0 Hz, H-3), 5.98 (1H, dd, *J* = 9.0, 3.0 Hz, H-5), and 6.92 (1H, d, *J* = 9.0 Hz, H-6), and six aromatic carbon signals at δ_C 140.0 (C-1), 149.7 (C-2), 104.4 (C-3), 155.4 (C-4), 107.0 (C-5), and 120.9 (C-6), indicating the presence of a 2,4-dihydroxyphenoxy moiety. This was confirmed by HMBC correlations of H-3 with C-1, C-2, C-4, and C-5, of H-5 with C-1 and C-3, and of H-6 with C-1, C-2, and C-4. Moreover, HMBC correlation between the anomeric proton H-1′ (δ_H 4.60) and C-1 suggested that 6′-*O*-syringoyl-β-D-glucopyranosyl moiety was attached to C-1. Thus, the structure of **7** was established as 2, 4-dihydroxyphenyl 1-*O*-β-D-(6′-*O*-syringoyl)glucopyranoside and named dunnianoside G (**7**).

Compound **8** was found to possess the molecular formula $C_{15}H_{22}O_7$ according to a positive HRESIMS ion at *m*/*z* 337.1260 $[M+Na]^+$ (calcd 337.1258). The IR spectrum showed the presence of hydroxy (3423 cm^{-1}) and aromatic (1518 and 1460 cm^{-1}) groups. The ^{1}H NMR spectrum showed the signals for a 1,2,4,5-tetrasubstituted aromatic ring at δ_H 6.69 (1H, s, H-3) and 6.96 (1H, s, H-6), an aromatic methoxy at δ_H 3.76 (3H, s, 4-OCH_3), two methyls

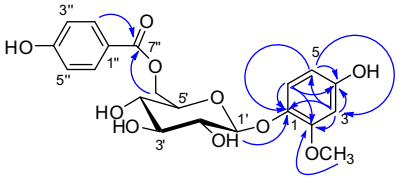

Figure 3. Key HMBC correlations (H→C) in compound **5**.

Table 3
Antioxidant activities of compounds **1**–**5**, **7**, and **9**[a]

Compound	IC_{50} (μM)
1	20.6 ± 2.0
2	6.9 ± 0.8
3	3.8 ± 0.6
4	5.4 ± 0.8
5	8.4 ± 1.3
7	15.2 ± 1.2
9	23.0 ± 2.2
Vitamin E[b]	23.4 ± 2.5

[a] Data are mean ± standard deviation (n = 3). The other compounds were inactive (IC_{50} >50 μM).
[b] Positive control.

at δ_H 2.22 (3H, s, 2-CH_3) and 2.10 (3H, s, 5-CH_3), and a anomeric proton at δ_H 4.75 (1H, d, J = 7.5 Hz, H-1′). The ^{13}C NMR spectrum exhibited signals for six aromatic carbons, one methoxy group (δ_C 56.0), two methyl groups (δ_C 16.2 and 16.4), and a glycosyl moiety (δ_C 103.8, 78.1, 77.6, 74.9, 71.5, and 62.8). Acid hydrolysis of **8** afforded a d-glucose which was compared with an authentic sample of D-glucose, and the D-configuration was confirmed by GC analysis.[7] The large coupling constant (7.5 Hz) of the anomeric proton at δ_H 4.75 (H-1′) indicated that the glucose was in the β-configuration. The NMR spectroscopic data (Tables 1 and 2) of **8** were very similar to those of 4-methoxy-2, 5-dimethylphenyl α-L-arabinofuranosyl-(1→6)-β-D-glucopyranoside,[9] except for the absence of signals due to α-L-arabinofuranosyl moiety in **8**. This was confirmed by the analysis of HSQC and HMBC experiments. Consequently, **8** was elucidated as 4-methoxy-2,5-dimethylphenyl 1-*O*-β-D-glucopyranoside and named dunnianoside H (**8**).

In addition, the absolute configuration of the glucoses in compounds **2**–**7** was also assigned as D by GC analysis.[7] The known compounds were identified as 6′-*O*-vanilloyltachioside (**9**),[8] 4′-hydroxy-3′-methoxyphenol-β-D-[6-*O*-(4″-hydroxy-3″,5″-dimethoxybenzoate)]glucopyranoside (**10**),[10] 6′-*O*-vanilloylisotachioside (**11**),[8] 4-hydroxy-2-methoxyphenyl-6-*O*-syringyl-β-D-glucopyranoside (**12**),[11] 1-*O*-3,4-dimethoxy-5-hydroxyphenyl-(6-*O*-vanilloyl)-β-D-glucopyranoside (**13**),[12] 1-*O*-3,4-dimethoxy-5-hydroxyphenyl-(6-*O*-3,5-dimethoxygalloyl)-β-D-glucopyranoside (**14**),[12] 2,5-dimethylphenyl 1-*O*-β-D-glucopyranoside (**15**),[13] 6′-*O*-vanilloylarbutin (**16**),[14] and oldhamioside (**17**),[15] respectively, by the comparison of their NMR spectroscopic data with those reported in literatures.

Compounds **1**–**16** were tested for their antioxidant activities against rat liver microsomal lipid peroxidation induced by Fe^{2+}-cystine in vitro. Vitamin E was used as the positive control. As shown in Table 3, compounds **1**–**5**, **7**, and **9** exhibited more significant antioxidant activities than the positive control Vitamin E (IC_{50} 23.4 ± 2.5 μM), with IC_{50} values ranging from 3.8 ± 0.6 to 23.0 ± 2.2 μM, while the other compounds were inactive (IC_{50} >50 μM). In compounds **1**–**3**, the antioxidant activity increased with the decreasing number of methoxy group substituted in the C-6′ benzoxy group. The same activity tendency was shown in compounds **4**, **9**, and **10**. These results suggested that the variation of the substituent units in the C-6′ benzoxy group and aglycone significantly influenced the activity and that the methoxy group substituted in the C-6′ benzoxy group could cause a considerable decrease in activity. In conclusion, the active phenolic glycosides showed potential as candidates of antioxidant agents useful for folk medicine application in treating rheumatism.

3. Experimental

3.1. General experimental procedures

Optical rotations were taken on a JASCO P-2000 automatic digital polarimeter. UV spectra were measured on a JASCO V650 spectrophotometer. IR spectra were recorded on a Nicolet 5700 FT-IR spectrometer. NMR spectra were recorded on an INOVA-500 spectrometer at 25 °C. ESIMS was measured on an Agilent 1100 Series LC/MSD ion trap mass spectrometer. HRESIMS data were recorded on an Agilent Technologies 6250 Accurate-Mass Q-TOF LC/MS spectrometer. Preparative HPLC was performed on a Shimadzu LC-6AD instrument with an SPD-10A detector, using a YMC-Pack ODS-A column (250 × 20 mm, 5 μm). GC data were recorded on an Agilent 7890A instrument with an FID detector. Sephadex LH-20 (Amersham Pharmacia Biotech AB, Sweden), ODS (45–70 μm, Merck), silica gel (160–200 mesh, Qingdao Marine Chemical Co., Ltd, China), and diatomite (Sinopharm Chemical Reagent Co., Ltd, China) were used for column chromatography (CC). TLC was carried out with glass precoated with silica gel GF_{254} plates (Qingdao Marine Chemical Co., Ltd, China). Spots were visualized under UV light or by spraying with 10% H_2SO_4 in EtOH–H_2O (95:5, v/v) followed by heating. Solvents [petroleum ether (60–90 °C), $CHCl_3$, EtOAc, MeOH, CH_2Cl_2, and EtOH] were of analytical grade and purchased from Beijing Chemical Company, Beijing, China.

3.2. Plant material

The roots of *I. dunnianum* were collected in Guangxi Province, China, in November 2009, and identified by Professor Song-Ji Wei of Guangxi College of Traditional Chinese Medicine. A voucher specimen (No. ID-S-2328) was deposited in the herbarium of the Department of Medicinal plants, Institute of Materia Medica, Chinese Academy of Medical Sciences.

3.3. Extraction and isolation

The roots of *I. dunnianum* (7.5 kg) was air-dried, ground, and extracted three times (2 h for each time) with EtOH–H_2O (3 × 80 L, 95:5, v/v) under conditions of reflux (90–95 °C), filtered, and the residue was refluxed with EtOH–H_2O (2 × 64 L, 70:30, v/v). The combined EtOH extract was evaporated to near dryness under reduced pressure to give the crude extract (850 g), which was absorbed by diatomite, and then successively extracted with petroleum ether (60–90 °C) (10 L), $CHCl_3$ (10 L), EtOAc (10 L), and MeOH (10 L). The EtOAc extract (110 g) was subjected to a silica gel CC (50 × 8 cm, 160–200 mesh) eluted with $CHCl_3$–MeOH (50:1, 20:1, 10:1, 5:1, 1:1, 0:1, v/v) to afford nine fractions E_1–E_9. Fraction E_4 (4.5 g) was applied to an ODS CC eluted with a gradient of MeOH–H_2O (10:90 → 100:0) to yield fractions $E_{4\text{-}1}$–$E_{4\text{-}9}$. Fraction $E_{4\text{-}4}$ (140 mg) was purified by preparative HPLC using CH_3CN–H_2O (20:80, 7 mL/min) to afford **17** (9.0 mg, *R*t 16.1 min). Fraction $E_{4\text{-}6}$ (250 mg) was submitted to a Sephadex LH-20 CC eluted with CH_2Cl_2–MeOH (50:50) to afford fractions $E_{4\text{-}6\text{-}1}$–$E_{4\text{-}6\text{-}5}$. Fraction $E_{4\text{-}6\text{-}2}$ (49 mg) was separated by preparative HPLC using CH_3CN–H_2O (20:80, 7 mL/min) to yield **8** (9.0 mg, *R*t 40.3 min) and **15** (5.0 mg, *R*t 42.6 min). Fraction $E_{4\text{-}6\text{-}3}$ (80 mg) was purified by preparative HPLC using MeOH–H_2O (39:61, 7 mL/min) to afford **9** (17.0 mg, *R*t 35.2 min) and **11** (7.2 mg, *R*t 38.7 min). Fraction $E_{4\text{-}6\text{-}4}$ (30 mg) was separated by preparative HPLC using MeOH–H_2O (41:59, 7 mL/min) to yield **10** (3.6 mg, *R*t 33.5 min) and **12** (12.5 mg, *R*t 36.1 min). Fraction $E_{4\text{-}7}$ (320 mg) was separated by preparative HPLC using CH_3CN–H_2O (15:85, 8 mL/min) to afford **14** (45.0 mg, *R*t 28.0 min), **13** (20.0 mg, *R*t 31.1 min), and **1** (45.0 mg, *R*t 33.2 min). Fraction $E_{4\text{-}8}$ (170 mg) was submitted to a Sephadex LH-20 CC eluted with CH_2Cl_2–MeOH (50:50) to afford fractions $E_{4\text{-}8\text{-}1}$–$E_{4\text{-}8\text{-}4}$. Fraction $E_{4\text{-}8\text{-}4}$ (67 mg) was separated by preparative HPLC using CH_3CN–H_2O (24:76, 7 mL/min) to yield **2** (8.5 mg, *R*t 23.4 min).

Fraction E_5 (8.4 g) was subjected to an ODS CC eluted with a gradient of MeOH–H_2O (10:90 → 100:0) to give eighteen fractions $E_{5\text{-}1}$–$E_{5\text{-}18}$. Fraction $E_{5\text{-}10}$ (3.1 g) was subjected to a Sephadex LH-20

CC eluted with a gradient of MeOH–H_2O (50:50 → 100:0) to afford fractions $E_{5\text{-}10\text{-}1}$–$E_{5\text{-}10\text{-}20}$. Fraction $E_{5\text{-}10\text{-}10}$ (50 mg) was separated by preparative HPLC using 0.1% TFA in CH_3CN–H_2O (21:79, 7 mL/min) to yield **7** (2.7 mg, *R*t 26.3 min), **16** (4.3 mg, *R*t 28.6 min), **6** (6.3 mg, *R*t 41.9 min), and a mixture $E_{5\text{-}10\text{-}10\text{-}1}$ (17.1 mg, *R*t 31.0 min). The mixture $E_{5\text{-}10\text{-}10\text{-}1}$ (17.1 mg) was purified by preparative HPLC using 0.1% TFA in MeOH–H_2O (42:58, 7 mL/min) to give **4** (7.9 mg, *R*t 31.1 min) and **5** (4.0 mg, *R*t 34.5 min). Fraction $E_{5\text{-}10\text{-}15}$ (30 mg) was separated by preparative HPLC using CH_3CN–H_2O (28:72, 7 mL/min) to yield **3** (5.0 mg, *R*t 14.0 min).

3.4. Identification

Dunnianoside A (**1**): White amorphous powder; $[\alpha]_D^{20}$ −42.3 (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ε) 202 (4.59), 218 (4.41), 280 (4.03) nm; IR (KBr) ν_{max} 3462, 3333, 2926, 1688, 1614, 1518, 1463, 1425, 1337, 1212, 1191, 1117, 1071, 867, 764 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) and ^{13}C NMR (125 MHz, acetone-d_6) data, see Tables 1 and 2; ESI-MS *m*/*z* 503 $[M+Na]^+$, 519 $[M+K]^+$, 479 [M−H], 515 [M+Cl]; HR ESI-MS *m*/*z* 503.1533 $[M+Na]^+$ (calcd for $C_{23}H_{28}O_{11}Na$, 503.1524).

Dunnianoside B (**2**): White amorphous powder; $[\alpha]_D^{20}$ −49.5 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 202 (4.62), 219 (4.38), 264 (4.03), 290 (3.89) nm; IR (KBr) ν_{max} 3382, 3268, 2974, 2926, 2893, 1716, 1688, 1601, 1522, 1467, 1410, 1383, 1285, 1202, 1085, 1067, 882, 872, 762 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) and ^{13}C NMR (125 MHz, acetone-d_6) data, see Tables 1 and 2; ESI-MS *m*/*z* 473 $[M+Na]^+$, 489 $[M+K]^+$, 449 [M−H], 485 [M+Cl]; HR ESI-MS *m*/*z* 473.1427 $[M+Na]^+$ (calcd for $C_{22}H_{26}O_{10}Na$, 473.1418).

Dunnianoside C (**3**): White amorphous powder; $[\alpha]_D^{20}$ −35.0 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.41), 258 (4.00) nm; IR (KBr) ν_{max} 3398, 2917, 1703, 1675, 1611, 1514, 1440, 1354, 1292, 1207, 1072, 969, 876, 847, 770, 616 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) and ^{13}C NMR (125 MHz, acetone-d_6) data, see Tables 1 and 2; ESI-MS *m*/*z* 443 $[M + Na]^+$, 459 $[M + K]^+$, 419 [M − H], 455 [M + Cl]; HR ESI-MS *m*/*z* 443.1334 $[M + Na]^+$ (calcd for $C_{21}H_{24}O_9Na$, 443.1313).

Dunnianoside D (**4**): White amorphous powder; $[\alpha]_D^{20}$ −37.3 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.42), 258 (4.10) nm; IR (KBr) ν_{max} 3402, 2923, 1693, 1610, 1516, 1451, 1281, 1200, 1170, 1075, 969, 848, 802, 770, 698, 614 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) and ^{13}C NMR (125 MHz, acetone-d_6) data, see Tables 1 and 2; ESI-MS *m*/*z* 423 $[M+H]^+$, 445 $[M+Na]^+$, 461 $[M+K]^+$, 421 [M−H], 457 [M+Cl]; HR ESI-MS *m*/*z* 445.1112 $[M+Na]^+$ (calcd for $C_{20}H_{22}O_{10}Na$, 445.1105).

Dunnianoside E (**5**): White amorphous powder; $[\alpha]_D^{20}$ −25.5 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.17), 258 (3.87) nm; IR (KBr) ν_{max} 3351, 2941, 1679, 1612, 1519, 1456, 1336, 1291, 1205, 1168, 1087, 977, 954, 849, 835, 802, 773, 616 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) and ^{13}C NMR (125 MHz, acetone-d_6) data, see Tables 1 and 2; ESI-MS *m*/*z* 423 $[M+H]^+$, 445 $[M+Na]^+$, 461 $[M+K]^+$, 421 [M−H], 457 [M+Cl]; HR ESI-MS *m*/*z* 445.1111 $[M+Na]^+$ (calcd for $C_{20}H_{22}O_{10}Na$, 445.1105).

Dunnianoside F (**6**): White amorphous powder; $[\alpha]_D^{20}$ −33.5 (*c* 0.27, MeOH); UV (MeOH) λ_{max} (log ε) 206 (4.88), 258 (4.33) nm; IR (KBr) ν_{max} 3392, 2943, 1686, 1608, 1509, 1438, 1318, 1281, 1204, 1169, 1105, 1073, 851, 773 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) and ^{13}C NMR (125 MHz, acetone-d_6) data, see Tables 1 and 2; ESI-MS *m*/*z* 453 $[M+H]^+$, 475 $[M+Na]^+$, 491 $[M+K]^+$, 451 [M−H], 487 [M+Cl]; HR ESI-MS *m*/*z* 475.1233 $[M+Na]^+$ (calcd for $C_{21}H_{24}O_{11}Na$, 475.1211).

Dunnianoside G (**7**): White amorphous powder; $[\alpha]_D^{20}$ −31.2 (*c* 0.049, MeOH); UV (MeOH) λ_{max} (log ε) 206 (4.17), 219 (4.10), 279 (3.69) nm; IR (KBr) ν_{max} 3387, 2921, 2851, 1679, 1617, 1516, 1466, 1428, 1338, 1206, 1119, 988, 845, 802, 767, 725 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) data, see Tables 1 and 2; ESI-MS *m*/*z* 469 $[M+H]^+$, 491 $[M+Na]^+$, 507 $[M+K]^+$, 467 [M−H], 503 [M+Cl]; HR ESI-MS *m*/*z* 491.1165 $[M+Na]^+$ (calcd for $C_{21}H_{24}O_{12}Na$, 491.1160).

Dunnianoside H (**8**): White amorphous powder; $[\alpha]_D^{20}$ −10.4 (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.17), 220 (3.73), 285 (3.25) nm; IR (KBr) ν_{max} 3424, 3325, 3254, 2940, 2881, 1514, 1460, 1380, 1211, 1082, 1044, 996, 898, 854 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) and ^{13}C NMR (125 MHz, acetone-d_6) data, see Tables 1 and 2; ESI-MS *m*/*z* 337 $[M+Na]^+$, 353 $[M+K]^+$, 651 $[2M+Na]^+$, 313 [M−H], 627 [2M−H]; HR ESI-MS *m*/*z* 337.1260 $[M+Na]^+$ (calcd for $C_{15}H_{22}O_7Na$, 337.1258).

3.5. Determination of the absolute configuration of the sugar moieties

According to the reported method,[7] each (2 mg) of the compounds **1**–**8** was hydrolyzed by 2 M HCl–H_2O at 95 °C for 10 h. After removal of HCl by evaporation and extraction with EtOAc, the H_2O extract was evaporated and dried in vacuo to give the monosaccharide residue. From the residue, glucose was detected by TLC [CH_2Cl_2: MeOH (5:1), *R*f 0.43] with authentic sample. The residue was dissolved in pyridine (1 mL) containing L-cysteine methyl ester hydrochloride (2 mg) and heated at 60 °C for 2 h, then evaporated under N_2 stream and dried in vacuo. The residue was dissolved in 0.2 mL of *N*-trimethylsilylimidazole and heated at 60 °C for 2 h. The reaction mixture was partitioned between *n*-hexane and H_2O (2 mL each), and the *n*-hexane extract was analyzed by GC (Agilent 7890A) under the following conditions: capillary column HP-5 (30 m × 0.32 mm × 0.25 μm); detector FID; carrier gas N_2, flow rate 1 mL/min; detector temperature 280 °C; injection temperature 250 °C; oven temperature gradient: 100 °C for 2 min, 100 °C → 280 °C (10 °C/min), 280 °C for 5 min. The same procedure was applied to authentic sample. By comparison with retention time of authentic sample ($t_{R\text{-}D\text{-}glucose}$ 19.851 min, $t_{R\text{-}L\text{-}glucose}$ 20.433 min), D-glucose (t_R 19.845 ~19.854 min) was identified in the acid hydrolysate of **1**–**8**.

3.6. Antioxidant activity assays

The antioxidant activities of compounds **1**–**16** were assessed by measuring the inhibitory ratios of malondialdehyde (MDA) in rat liver microsomal lipid peroxidation induced by Fe^{2+}-cystine as described previously.[16]

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