Tarennanosides A–H, Eight New Lignan Glucosides from *Tarenna attenuata* and Their Protective Effect on H_2O_2-Induced Impairment in PC12 Cells

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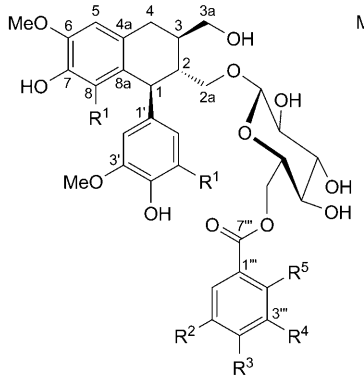
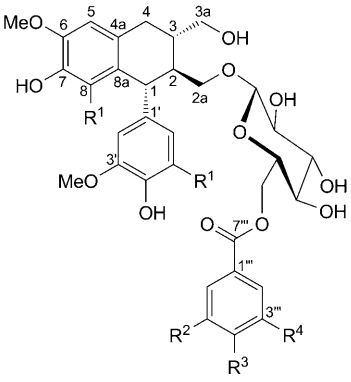
Eight new lignan glucosides, tarennanosides A–H (**1**–**8**, resp.), were isolated from the whole plant of *Tarenna attenuata*, together with three known compounds, fernandoside, (−)-lyoniresinol, and (−)-isolariciresinol. The planar structures of new compounds were elucidated mainly by analysis of physical and spectroscopic data, and the absolute configurations were determined by acid hydrolysis as well as CD spectroscopy. Compounds **1** and **2** exhibited potent antioxidant activities against H_2O_2-induced impairment in PC12 cells. Preliminary mechanism study by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method showed that these two compounds could act as radical scavengers.

Introduction. – *Tarenna attenuata* has been used as traditional antinociceptive and antipyretic in the Guangxi Province of P. R. China [1]. Previous studies on this plant showed that its EtOH extract had potent antioxidant effects against H_2O_2-induced impairment in PC12 cell [2]. However, ten iridoids (tarennin, tarenninosides A–G, ixoside, and 10-methylixoside) did not contribute to this activity [2]. Other compounds including two novel chalcones (tarennane and tarennone), 26 lignan and neolignans, and nine phenolics should be responsible for partial activities, but they are minor constituents [3][4]. Therefore, the principal bioactive agents of the extract still remain unknown. As such, further studies were carried out, which resulted in the isolation of eight new lignan glucosides, **1**–**8**, and three known ones. In this work, we describe the isolation, structural elucidation, and antioxidant activities of these eleven compounds.

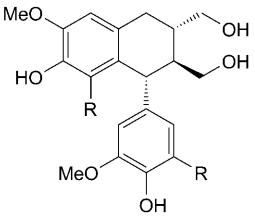
Results and Discussion. – 1. *Structure Elucidation*. Compound **1** had a molecular formula of $C_{37}H_{46}O_{17}$ as evidenced by the positive-ion HR-ESI-MS peak at *m*/*z* 785.2631 ($[M+Na]^+$). The IR spectrum indicated the presence of OH (3423 cm^{-1}) and CO (1706 cm^{-1}) groups, as well as aromatic rings (1610, 1516, and 1461 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectroscopic data of **1** (*Tables 1* and *2*) indicated the presence of three fragments, including a lyoniresinol [5] and a syringoyl group (δ(H) 7.26 (*s*, H−C(2‴,6‴)), 3.76 (*s*, MeO−C(3‴), MeO−C(5‴)); δ(C) 168.4 (C(7‴)), 118.6 (C(1‴)), 56.7 (MeO−C(3‴), MeO−C(5‴))), and a glucopyranose unit. Acid



	R^1	R^2	R^3	R^4	R^5
1	MeO	MeO	OH	MeO	H
3	H	H	OH	MeO	H
5	H	H	MeO	OH	H
7	H	MeO	OH	MeO	H
8	MeO	MeO	H	H	OH
9	MeO	MeO	OH	H	H



	R^1	R^2	R^3	R^4
2	MeO	MeO	OH	MeO
4	H	H	OH	MeO
6	H	H	MeO	OH



10 R = MeO
11 R = H

hydrolysis of **1** and chromatographic purification afforded D-glucose and (+)-lyoniresinol, which were detected by TLC. These were compared with authentic samples of D-glucose and (−)-lyoniresinol (**10**), and the configurations were determined by measurement of the optical rotations. The β-anomeric configuration for the glucose was deduced from its large ^3J(H−C(4),H−C(3)) coupling constant (J = 7.6 Hz). HMBC Correlations between H−C(2a)/C(1″) and H−C(1″)/C(2a) suggested that the β-D-glucopyranose unit was at the C(2a) of (+)-lyoniresinol. Compared with (+)-lyoniresinol 2a-*O*-β-D-glucopyranoside [5], the downfield shift of C(6″) in the glucopyranose unit from δ(C) 62.9 to 65.0 established the attachment of the syringoyl group at C(6″). The assumption was confirmed by an HMBC correlation of H−C(6″) (δ(H) 4.58 and 4.34, each 1 H) to the ester C(7‴)=O group (δ(C) 168.4) of the syringoyl group (*Fig. 1*). Thus, compound **1** was determined as (+)-lyoniresinol 2a-*O*-[6-*O*-(4-hydroxy-3,5-dimethoxy)benzoyl]-β-D-glucopyranoside, named tarennanoside A.

Compound **2** exhibited a $[M - H]^-$ ion peak at m/z 761.2656 in the negative-ion HR-ESI-MS, corresponding to the molecular formula $C_{37}H_{46}O_{17}$. Comparison of **2** with **1** revealed that they had not only the same molecular formula, but almost the same physical and NMR spectroscopic data, and differed mainly in their optical rotation values of −40.9 of compound **2** *vs.* +29.1 of compound **1**. The ^{13}C-NMR spectroscopic data of compound **2** showed some small but significant differences from **1** in the upfield 0.4-ppm resonance at C(1″) and the downfield 0.8-ppm signal at C(1). Such changes were also observed in other (+)- and (−)-lyoniresinol 2a-*O*-β-D-glucopyranosides [6][7], as well as for (+)- and (−)-2a-*O*-β-D-xylopyranosides [8]. Thus, compound **2** could be a (−)-lyoniresinol-type lignan. Acid hydrolysis of compound **2**, and further separation provided D-glucose and (−)-lyoniresinol, which were identified by TLC with

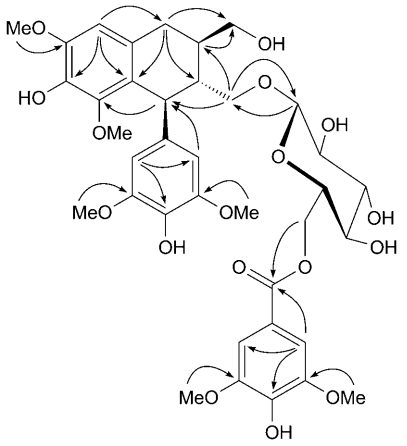


Fig. 1. *Key HMBC data of compound* **1**

authentic samples and measurement of their optical rotations. Further confirmation was found in the CD spectrum of **2** ($\Delta\varepsilon_{243}$ −162.6 and $\Delta\varepsilon_{272}$ −83.7), which indicated a (1*R*)-configuration at C(1) for **2** [6][9]. Therefore, compound **2** was unequivocally determined as (−)-lyoniresinol 2a-*O*-[6-*O*-(4-hydroxy-3,5-dimethoxy)benzoyl]-*β*-D-glucopyranoside and named tarennanoside B.

Compound **3** exhibited a $[M+Na]^+$ ion peak at *m*/*z* 695.2315 in the positive-ion HR-ESI-MS, corresponding to the molecular formula $C_{34}H_{40}O_{14}$. The IR spectrum indicated the presence of OH (3428 cm^{-1}) and CO (1703 cm^{-1}) groups, as well as aromatic rings (1611, 1516, and 1451 cm^{-1}). The ^{1}H- and ^{13}C-NMR-spectroscopic data of **3** (*Tables 1* and *2*) were very similar to those of (+)-isolariciresinol 2a-*O*-*β*-D-glucopyranoside [7]. In addition, the signals of a vanilloyl group were also observed (δ(H) 7.54 (*dd*, *J* = 8.3, 1.6, H−C(6‴)), 7.51 (*d*, *J* = 1.6, H−C(2‴)), 6.78 (*d*, *J* = 8.3, H−C(5‴)), 3.81 (*s*, MeO−C(3‴)); δ(C) 168.0 (C(7‴)), 122.4 (C(1‴)), 56.3 (MeO−C(3‴))). Compared with (+)-isolariciresinol 2a-*O*-*β*-D-glucopyranoside [7], the significant downfield shift of C(6″) from δ(C) 62.8 to 65.0, and upfield shift of C(5″) from δ(C) 78.5 to 75.4 in the glucopyranose unit established the attachment of the vanilloyl group at C(6″). This was confirmed by HMBC correlations from CH_2(6″) (δ(H) 4.58 and 4.36, each 1 H) of the glucosyl moiety to the ester CO at C(7‴) (δ(C) 168.0) of the vanilloyl group. Thus, compound **3** was determined as (+)-isolariciresinol 2a-*O*-[6-*O*-(4-hydroxy-3-methoxy)benzoyl]-*β*-D-glucopyranoside, named tarennanoside C.

Compound **4** had the same molecular formula, $C_{34}H_{40}O_{14}$, as **3**, deduced from the HR-ESI-MS (positive-ion mode) peak at *m*/*z* 695.2312 ($[M+Na]^+$), and exhibited physical and spectroscopic data very similar to those of **3**. Inspection of the ^{13}C-NMR data of **4** exhibited small but significantly different shifts as compared to the data of **3**: upfield shift of 1.3 ppm at C(1″), and the downfield shift of 0.7 ppm at C(3). Such changes were also observed for other (+)- and (−)-isolariciresinol 2a-*O*-*β*-D-glucopyranosides [7]. Thus, compound **4** could be a (−)-isolariciresinol type lignan

Table 1. *^{1}H-NMR Data* (δ [ppm]) *for* **1**–**8**. At 500 MHz in CD_3OD; *J* in Hz.

Position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	4.32–4.38 (*m*)	4.18 (*d*, *J*=7.1)	3.93–3.97 (*m*)	3.72–3.77 (*m*)	3.94–3.98 (*m*)	3.68–3.73 (*m*)	3.93–3.98 (*m*)	4.39–4.43 (*m*)
2	2.07–2.14 (*m*)	1.99–2.04 (*m*)	1.85–1.89 (*m*)	1.88–1.92 (*m*)	1.84–1.90 (*m*)	1.87–1.91 (*m*)	1.83–1.86 (*m*)	2.06–2.10 (*m*)
2a	3.83–3.89 (*m*), 3.41–3.47 (*m*)	3.88–3.94 (*m*), 3.51–3.59 (*m*)	3.91–3.98 (*m*), 3.26–3.32 (*m*)	3.80–3.86 (*m*), 3.60 (*dd*, *J*=10.2, 2.4)	4.00 (*d*, *J*=10.6), 3.25–3.30 (*m*)	4.01–4.06 (*m*), 3.58–3.63 (*m*)	3.93–3.98 (*m*), 3.27–3.32 (*m*)	3.84–3.89 (*m*), 3.25–3.29 (*m*)
3	1.59–1.66 (*m*)	1.70–1.76 (*m*)	1.98–2.03 (*m*)	1.96–2.01 (*m*)	2.01–2.04 (*m*)	1.94–1.99 (*m*)	2.01–2.07 (*m*)	1.67–1.71 (*m*)
3a	3.61 (*dd*, *J*=10.9, 4.4, H_a), 3.50 (*dd*, *J*=10.9, 6.6, H_b)	3.59–3.62 (*m*)	3.67–3.70 (*m*)	3.74–3.78 (*m*, H_a), 3.66–3.69 (*m*, H_b)	3.65–3.72 (*m*)	3.73–3.80 (*m*, H_a), 3.68–3.73 (*m*, H_b)	3.67–3.74 (*m*, H_a), 3.63–3.68 (*m*, H_b)	3.80–3.85 (*m*, H_a), 3.62–3.68 (*m*, H_b)
4	2.65 (*dd*, *J*=15.0, 4.6, H_a), 2.56 (*d*, *J*=15.0, H_b)	2.66–2.68 (*m*)	2.74–2.78 (*m*)	2.86 (*dd*, *J*=15.6, 4.3, H_a), 2.74 (*dd*, *J*=15.6, 4.3, H_b)	2.82–2.75 (*m*)	2.84 (*d*, *J*=15.7, H_a), 2.73 (*dd*, *J*=15.7, 4.3, H_b)	2.78–2.82 (*m*, H_a), 2.70–2.76 (*m*, H_b)	2.71 (*dd*, *J*=15.2, 4.6, H_a), 2.59 (*d*, *J*=15.2, H_b)
5	6.52 (*s*)	6.53 (*s*)	6.59 (*s*)	6.62 (*s*)	6.60 (*s*)	6.63 (*s*)	6.58 (*s*)	6.57 (*s*)
8			6.16 (*s*)	6.14 (*s*)	6.16 (*s*)	6.14 (*s*)	6.16 (*s*)	
2′	6.40 (*s*)	6.38 (*s*)	6.75 (*d*, *J*=1.7)	6.63 (*d*, *J*=1.6)	6.75 (*d*, *J*=1.6)	6.61 (*d*, *J*=1.5)	6.76 (*s*)	6.42 (*s*)
5′			6.72 (*d*, *J*=8.0)	6.65 (*d*, *J*=7.9)	6.76 (*d*, *J*=8.3)	6.76 (*d*, *J*=8.2)	6.72 (*d*, *J*=8.2)	
6′	6.40 (*s*)	6.38 (*s*)	6.61 (*dd*, *J*=8.0, 1.7)	6.60 (*dd*, *J*=7.9, 1.6)	6.62 (*dd*, *J*=8.3, 1.6)	6.58 (*dd*, *J*=8.2, 1.5)	6.60 (*d*, *J*=8.2)	6.42 (*s*)
1″	4.32 (*d*, *J*=7.6)	4.22 (*d*, *J*=7.8)	4.11 (*d*, *J*=7.8)	4.10 (*d*, *J*=7.8)	4.12 (*d*, *J*=7.7)	4.10 (*d*, *J*=7.8)	4.12 (*d*, *J*=8.0)	4.28 (*d*, *J*=7.8)
2″	3.25–3.31 (*m*)	3.22–3.26 (*m*)	3.20–3.25 (*m*)	3.18–3.23 (*m*)	3.19–3.23 (*m*)	3.18–3.23 (*m*)	3.20–3.26 (*m*)	3.20–3.24 (*m*)
3″	3.38–3.44 (*m*)	3.33–3.39 (*m*)	3.32–3.38 (*m*)	3.30–3.34 (*m*)	3.32–3.38 (*m*)	3.29–3.33 (*m*)	3.34–3.39 (*m*)	3.33–3.39 (*m*)
4″	3.36–3.44 (*m*)	3.37–3.43 (*m*)	3.32–3.36 (*m*)	3.34–3.39 (*m*)	3.33–3.37 (*m*)	3.38–3.42 (*m*)	3.33–3.40 (*m*)	3.42–3.46 (*m*)
5″	3.53–3.59 (*m*)	3.44–3.51 (*m*)	3.48–3.52 (*m*)	3.34–3.40 (*m*)	3.46–3.52 (*m*)	3.33–3.41 (*m*)	3.48–3.54 (*m*)	3.49–3.53 (*m*)
6″	4.58 (*d*, *J*=11.6, H_a), 4.34 (*dd*, *J*=11.6, 6.1, H_b)	4.64 (*d*, *J*=11.7, H_a), 4.37 (*dd*, *J*=11.7, 5.8, H_b)	4.58 (*dd*, *J*=11.8, 1.9, H_a), 4.36 (*dd*, *J*=11.8, 6.2, H_b)	4.55 (*dd*, *J*=11.8, 1.7, H_a), 4.38 (*dd*, *J*=11.8, 5.1, H_b)	4.58 (*dd*, *J*=11.9, 1.9, H_a), 4.35 (*dd*, *J*=11.8, 6.2, H_b)	4.52 (*dd*, *J*=11.8, 1.8, H_a), 4.37 (*dd*, *J*=11.8, 5.2, H_b)	4.56 (*d*, *J*=11.6, H_a), 4.36 (*dd*, *J*=11.6, 6.1, H_b)	3.59–3.65 (*m*, H_a), 3.52 (*dd*, *J*=10.9, 6.6, H_b)

Table 1 (cont.)

Position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
2‴	7.26 (*s*)	7.29 (*s*)	7.51 (*d*, *J* = 1.6)	7.52 (*s*)	7.50 (*d*, *J* = 1.5)	7.48 (*d*, *J* = 1.2)	7.25 (*s*)	
3‴								6.67 (*d*, *J* = 8.3)
4‴								7.47 (*dd*, *J* = 8.3, 1.9)
5‴			6.78 (*d*, *J* = 8.3)	6.82 (*d*, *J* = 8.2)	6.78 (*d*, *J* = 8.3)	6.65 (*d*, *J* = 8.1)		
6‴	7.26 (*s*)	7.29 (*s*)	7.54 (*dd*, *J* = 8.3, 1.6)	7.53 (*d*, *J* = 8.2)	7.53 (*dd*, *J* = 8.3, 1.5)	7.52 (*dd*, *J* = 8.1, 1.2)	7.25 (*s*)	7.42 (*d*, *J* = 1.9)
6-MeO	3.81 (*s*)	3.83 (*s*)	3.79 (*s*)	3.80 (*s*)	3.79 (*s*)	3.79 (*s*)	3.77 (*s*)	3.86 (*s*)
8-MeO	3.33 (*s*)	3.25 (*s*)						3.31 (*s*)
3′-MeO	3.71 (*s*)	3.69 (*s*)	3.76 (*s*)	3.74 (*s*)	3.76 (*s*)	3.70 (*s*)	3.76 (*s*)	3.73 (*s*)
5′-MeO	3.71 (*s*)	3.69 (*s*)						3.73 (*s*)
3‴-MeO	3.76 (*s*)	3.78 (*s*)	3.81 (*s*)	3.81 (*s*)			3.82 (*s*)	
4‴-MeO					3.81 (*s*)	3.85 (*s*)		
5‴-MeO	3.76 (*s*)	3.78 (*s*)					3.82 (*s*)	3.82 (*s*)

Table 2. *^{13}C-NMR Data* (δ [ppm]) *for* **1**–**9**. At 100 MHz; in CD_3OD.

Position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	42.8	43.6	48.0	48.7	48.0	48.5	47.9	42.8	42.7
2	46.4	46.8	45.7	45.6	45.7	45.6	45.8	46.4	46.3
2a	71.6	70.7	70.2	70.1	70.0	70.2	69.9	71.6	71.8
3	40.8	40.5	39.9	40.6	39.7	40.6	39.7	40.7	40.8
3a	66.2	65.9	65.1	65.5	65.1	65.5	65.1	64.2	66.1
4	33.7	34.0	33.7	33.7	33.7	33.7	33.7	33.8	33.6
4a	130.2	130.0	129.1	129.3	129.1	129.3	129.1	130.1	130.2
5	107.7	107.6	112.3	112.4	112.3	112.4	112.3	107.8	107.7
6	148.5	148.6	147.1	147.2	147.1	147.3	147.1	149.0	148.6
7	138.8	138.8	145.1	145.3	145.1	145.3	145.0	139.0	138.7
8	147.6	147.5	117.3	117.4	117.3	117.5	117.3	147.6	147.5
8a	126.2	126.3	134.1	133.8	134.1	133.8	134.1	126.3	126.1
1′	134.3	134.5	138.6	138.8	138.6	138.7	138.6	139.2	134.2
2′	106.8	107.0	114.2	113.9	114.2	113.9	114.2	106.8	106.7
3′	148.9	148.9	148.9	148.9	148.9	149.7	148.9	149.0	148.8
4′	139.2	139.7	145.8	146.0	145.8	146.0	145.8	134.9	139.2
5′	148.9	148.9	116.1	116.0	116.1	116.8	116.1	149.0	148.8
6′	106.8	107.0	123.1	123.5	123.1	123.6	123.0	106.8	106.7
1″	104.7	104.3	105.2	103.9	105.2	103.9	105.2	104.8	104.7
2″	75.1	75.1	75.1	75.1	75.1	75.1	75.1	75.1	75.4
3″	78.0	78.0	77.9	78.1	77.9	78.1	77.9	78.1	78.0
4″	71.9	71.9	71.9	71.8	71.9	71.8	72.0	71.9	71.8
5″	75.5	75.6	75.4	75.5	75.5	75.6	75.5	75.7	75.1
6″	65.0	65.0	65.0	64.8	64.7	64.6	64.9	66.2	65.0
1‴	118.6	120.8	122.4	122.2	125.2	121.0	117.3	117.4	122.3
2‴	108.3	108.2	113.6	113.8	113.1	113.5	108.4	159.7	113.5
3‴	149.5	149.1	148.7	148.9	155.1	155.9	149.8	118.5	148.5
4‴	145.0	142.8	152.9	152.9	148.9	149.0	147.1	126.5	152.8
5‴	149.5	149.1	116.0	115.9	116.1	116.0	149.8	151.7	115.9
6‴	108.3	108.2	125.2	125.3	125.9	125.7	108.4	112.8	125.1
7‴	168.4	168.0	168.0	168.1	168.8	168.5	168.6	169.5	168.0
6-MeO	56.6	56.6	56.4	56.5	56.3	56.4	56.3	56.5	56.5
8-MeO	60.2	60.2						60.2	60.2
3′-MeO	56.8	56.8	56.5	56.5	56.4	56.5	56.6	56.8	56.8
5′-MeO	56.8	56.8						56.8	56.8
3‴-MeO	56.7	56.7	56.3	56.5			56.5		56.4
4‴-MeO					56.1	56.3			
5‴-MeO	56.7	56.7					56.5	55.8	

glucoside. Further confirmation was obtained from its optical rotation value of -37.3. Therefore, compound **4** was determined as (−)-isolariciresinol 2a-*O*-[6-*O*-(4-hydroxy-3-methoxy)benzoyl]-β-D-glucopyranoside, named tarennanoside D.

Compound **5** had the same molecular formula, $C_{34}H_{40}O_{14}$, as **3**, as evidenced from the HR-ESI-MS (positive-ion mode) peak at *m*/*z* 695.2324 ($[M+H]^+$). It exhibited physical and spectroscopic data similar to those of **3**, except that an isovanilloyl group (δ(H) 7.53 (*dd*, $J=8.3$, 1.5, H−C(6‴)), 7.50 (*d*, $J=1.5$, H−C(2‴)), 6.78 (*d*, $J=8.3$, H−C(5‴)), 3.81 (*s*, MeO−C(4‴)); δ(C) 168.8 (C(7‴)), 155.1 (C(3‴)), 148.9 (C(4‴)),

125.2 (C(1‴)), 56.1 (MeO–C(4‴))) instead of a vanilloyl group was located at C(6″) in **5**. The assumption was confirmed by detailed analysis of the 2D-NMR spectroscopic data, including HSQC, HMBC, ^{1}H,^{1}H-COSY, and ROESY. Therefore, compound **5** was assigned to (+)-isolariciresinol 2a-*O*-[6-*O*-(3-hydroxy-4-methoxy)benzoyl]-*β*-D-glucopyranoside, named tarennanoside E.

Compound **6** had the same molecular formula, $C_{34}H_{40}O_{14}$, as **5**, as deduced from the HR-ESI-MS (negative-ion mode) peak at *m*/*z* 671.2322 ($[M-H]^-$). It also exhibited physical and spectroscopic data very similar to those of **5**. Inspection of the ^{13}C-NMR spectroscopic data of compound **6**, however, showed significant differences: an upfield shift of 1.3 ppm at C(1″), and a downfield shift of 0.9 ppm at C(3). Such phenomena were also observed for compounds **3** and **4**, and for other (+)- and (−)-isolariciresinol 2a-*O*-*β*-D-glucopyranosides [7]. Thus compound **6** could be a (−)-isolariciresinol-type lignan glucoside. Further confirmation was obtained from its optical rotation value of −44.2. Therefore, compound **6** was identified as (−)-isolariciresinol 2a-*O*-[6-*O*-(3-hydroxy-4-methoxy)benzoyl]-*β*-D-glucopyranoside, named tarennanoside F.

Compound **7** was found to possess the molecular formula $C_{35}H_{42}O_{15}$ as indicated by its HR-ESI-MS (negative-ion mode) peak at *m*/*z* 701.2448 ($[M-H]^-$). The UV, IR, and NMR data were also very similar to those of **3** except for the presence of a syringoyl group (δ(H) 7.25 (*s*, H–C(2‴), H–C(6‴)), 3.82 (*s*, MeO–C(3‴), MeO–C(5‴)); δ(C) 168.6 (C(7‴)), 117.3 (C(1‴)), 56.5 (*Me*O–C(3‴), *Me*O–C(5‴))) instead of a vanilloyl group at C(6″) of **7**. The assumption was confirmed by detailed analysis of the 2D-NMR spectroscopic data. Therefore, compound **7** was determined as (+)-isolariciresinol 2a-*O*-[6-*O*-(4-hydroxy-3,5-dimethoxy)benzoyl]-*β*-D-glucopyranoside, named tarennanoside G.

Compound **8** had a molecular formula of $C_{36}H_{44}O_{16}$ as indicated by its HR-ESI-MS (positive-ion mode) peak at *m*/*z* 755.2512 ($[M+Na]^+$). Inspection of the UV, IR, and NMR data of **8** showed considerable similarity to those of fernandoside (**9**), with the only difference being a 2-hydroxy-5-methoxybenzoyl group (δ(H) 7.47 (*dd*, *J*=8.3, 1.9, H–C(4‴)), 7.42 (*d*, *J*=1.9, H–C(6‴)), 6.67 (*d*, *J*=8.3, H–C(3‴)), 3.82 (*s*, MeO–C(5‴)); δ(C) 169.5 (C(7‴)), 117.4 (C(1‴)), 55.8 (*Me*O–C(5‴))) rather than a vanilloyl group at C(6″) in **8**. This was further confirmed by the HMBC correlation of H–C(6″) to the ester CO at C(7‴) (δ(C) 169.5) of the 2-hydroxy-5-methoxybenzoyl group. Thus, compound **8** was determined as (+)-lyoniresinol 2a-*O*-[6-*O*-(2-hydroxy-5-methoxy)benzoyl]-*β*-D-glucopyranoside, named tarennanoside H.

Three known lignan constituents were identified as fernandoside (**9**) [10], (−)-lyoniresinol (**10**) [6], and (−)-isolariciresinol (**11**) [7] by comparison of their spectroscopic data with literature values.

2. *Bioassay.* The antioxidant effects of **1**–**11** against H_2O_2-induced impairment in PC12 cells were evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium hydrobromide (MTT) method (*Table 3*). Edaravone (*Sigma Chemical Co.*; *Fig. 2*), the positive control, exhibited strong activity at the dose of 0.4 μM. Compounds **1** and **2** showed potent activities within the concentration range tested (0.4–50 μM). However, the activities are lower at 50 μM than those at 10 μM, which may be due to pro-oxidant effects. The absence of cytotoxicity of compound **1** on PC12 cells at concentrations up to 100 μM was demonstrated in a control experiment (data not shown). In a preliminary mechanism study, compounds **1** and **2** scavenged DPPH

Table 3. *Antioxidant Effects of Compounds* **1**–**11** *against* H_2O_2*-Induced Impairment in PC12 Cells* ($n=3$, $\bar{X}\pm SD$)

Groups	Concentration [μM]	Viability [%]
Control		100***[a])
Negative control[b])		47.1±2.5
Edaravone[c])	0.08	46.7±4.4 n.a.
	0.4	60.7±3.1**[a])
	2.0	54.7±3.8 n.a.
	10.0	43.1±4.2 n.a.
Compound **1**	0.4	51.4±4.1 n.a.
	2.0	56.1±4.2*[a])
	10.0	56.2±3.7*
	50.0	50.5±2.3 n.a.
Compound **2**	0.4	52.3±3.8 n.a.
	2.0	58.0±4.7*
	10.0	60.2±5.0**
	50.0	58.6±5.2*
Compounds **3**–**11**	2.0	n.a.
	10.0	n.a.
	50.0	n.a.

[a]) n.a.: No activity observed; *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$; compared to negative control. [b]) H_2O_2 alone. [c]) Positive control.

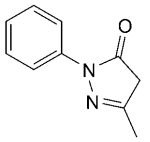


Fig. 2. *Chemical structure of edaravone*

radicals strongly with IC_{50} values of 36.8 and 34.4 μM (*Table 4*), respectively, implying that **1** and **2** could act as radical scavengers.

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh; *Qingdao Marine Chemical, Inc.*, Qingdao, P. R. China), silica gel *H* (10–40 μm; *Qingdao.*), *Sephadex LH-20* (40–70 μm; *Amersham Pharmacia Biotech AB*, S-Uppsala), and *Lichroprep RP-18* gel (40–63 μm; *Merck*, D-Darmstadt). Prep. TLC (1.0–1.5 mm): glass precoated silica gel GF_{254} (*Qingdao*). Visualization by exposure to UV at 254 nm. Optical rotations: *Horiba SEPA-300* or *JASCO DIP-370* digital polarimeters. CD Spectra: *JASCO J810* (*JASCO*, Tokyo, Japan) spectrometer. UV Spectra: *UV-2401PC* (*Shimadzu*, Tokyo, Japan) spectrometer. IR Spectra: *Bio-Rad FTS-135* spectrometer with KBr pellets. NMR Spectra (in CD_3OD

Table 4. *Radical Scavenging Activities of* **1**–**11** *against DPPH* ($n=3$, $\bar{X}\pm SD$)

Compound	IC_{50} [μM]
Edaravone[a])	26.8 ± 0.9
1	36.8 ± 2.1
2	34.4 ± 2.3
3	> 100
4	> 100
5	93.0 ± 3.6
6	> 100
7	72.2 ± 2.0
8	> 100
9	> 100
10	82.4 ± 2.3
11	56.4 ± 1.3

[a]) Positive control.

with TMS as internal standard): *Bruker AM-400* NMR spectrometer at 100 MHz for ^{13}C and a *DRX-500* NMR spectrometer at 500 MHz for ^{1}H. ESI-MS: *LCQ* ion trap mass spectrometer coupled to a *Waters Alliance* 2695 HPLC system. FAB-MS (glycerol as matrix) and HR-ESI-MS: *VG Auto Spec 3000* spectrometer.

Plant Material. Whole plant tissues of *T. attenuata* were collected from Xishuangbanna of Yunnan Province, P. R. China, in October 2004, and identified by Prof. *Jing-Yun Cui*, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences. A herbarium specimen was deposited with the Kunming Institute of Botany, Chinese Academy of Sciences with the specimen number of BN163.

Extraction and Isolation. The air-dried material (17 kg) was finely pulverized and extracted with EtOH/H_2O 95 : 5 (16 l) by heating under reflux, which was maintained for 4 h. This procedure was repeated three times. The combined extracts were filtered and concentrated to dryness under reduced pressure, and the resulting residue was suspended in H_2O (15 l) and partitioned, successively, with petroleum ether (PE; 15 l), AcOEt (20 l), and BuOH (15 l). The AcOEt extract (61 g) was separated into eight fractions (*Frs. 1–8*) by CC (10.0 cm × 1.2 m) on SiO_2 (200–300 mesh) using a $CHCl_3$/MeOH gradient (100 : 0 to 0 : 100). *Fr.* F_2 (7.5 g) was subjected to RP-MPLC (1.5 cm × 50 cm) eluted with MeOH/H_2O (40 : 60), and then to CC (2.0 × 80 cm) over silica gel *H* (10–40 μm) eluted with $CHCl_3$/MeOH 15 : 1 and AcOEt/MeOH 40 : 1, resp., to yield **10** (6 mg) and **11** (8 mg). *Fr. 8* (8.3 g) was divided into five subfractions (*Frs. S1–S5*) by CC (3.0 × 80 cm) over silica gel *H* (10–40 μm) eluted with AcOEt/MeOH 20 : 1. *Fr. S3* (1.3 g) was then subjected to RP-MPLC (1.5 × 50 cm) with H_2O/MeOH 25 : 75 as eluant. Further separations by repeated prep. TLC with AcOEt/MeOH 5 : 1 and CC over *Sephadex LH-20* with MeOH gave **3** (10 mg), **4** (3 mg), **5** (7 mg), **6** (6 mg), and **9** (43 mg). Compounds **1** (88 mg), **2** (11 mg), **7** (22 mg), and **8** (4 mg) were obtained from *Fr. S4* (1.7 g) by CC over a RP-MPLC (1.5 × 50 cm) with H_2O/MeOH 15 : 85 as eluant, and SiO_2 with $CHCl_3$/MeOH 5 : 1, followed by purification in *Sephadex LH-20* with MeOH.

Tarennanoside A (=*(+)-Lyoniresinol 2a*-O-*[6*-O-*(4-Hydroxy-3,5-dimethoxy)benzoyl]-β*-D-*glucopyranoside*; **1**). Amorphous powder. $[\alpha]_D^{25} = +29.1$ ($c = 0.52$, MeOH). UV (MeOH): 277 (4.03). CD (MeOH): $\Delta\varepsilon_{243}$ +188.8, $\Delta\varepsilon_{272}$ +82.1. IR: 3423, 2938, 1706, 1610, 1516, 1461, 1425, 1336, 1221, 1114, 1022, 765. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS: 785 ($[M+Na]^+$). HR-ESI-MS: 785.2631 ($[M+Na]^+$, $C_{37}H_{46}NaO_{17}^+$; calc. 785.2632).

Tarennanoside B (=*(−)-Lyoniresinol 2a*-O-*[6*-O-*(Hydroxy-3,5-dimethoxy)benzoyl]-β*-D-*glucopyranoside*; **2**). Amorphous powder. $[\alpha]_D^{20} = -40.9$ ($c = 0.75$, MeOH). UV (MeOH): 277 (4.05). CD (MeOH): $\Delta\varepsilon_{243}$ −162.6, $\Delta\varepsilon_{272}$ −83.7. IR: 3426, 2937, 1706, 1610, 1517, 1462, 1425, 1338, 1223, 1115, 1032, 766. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS: 761 ($[M-H]^-$). HR-ESI-MS: 761.2656 ($[M-H]^-$, $C_{37}H_{45}O_{17}^-$; calc. 761.2656).

Tarennanoside C (=*(+)-Isolariciresinol 2a*-O-*[6*-O-*(4-Hydroxy-3-methoxy)benzoyl]-β*-D-*glucopyranoside*; **3**). Amorphous powder. $[\alpha]_D^{20} = +15.7$ ($c = 0.89$, MeOH). UV (MeOH): 287 (3.93), 266 (3.94). IR: 3428, 2934, 1703, 1611, 1516, 1451, 1354, 1283, 1218, 1123, 1030, 765. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS: 695 ($[M+Na]^+$). HR-ESI-MS: 695.2315 ($[M+Na]^+$, $C_{34}H_{40}NaO_{14}^+$; calc. 695.2315).

Tarennanoside D (=*(−)-Isolariciresinol 2a*-O-*[6*-O-*(4-Hydroxy-3-methoxy)benzoyl]-β*-D-*glucopyranoside*; **4**). Amorphous powder. $[\alpha]_D^{19} = -37.3$ ($c = 0.25$, MeOH). UV (MeOH): 288 (3.69), 264 (3.70). IR: 3430, 2924, 2831, 1602, 1515, 1458, 1430, 1363, 1284, 1219, 1122, 1069, 1030, 765. ^{1}H- and ^{13}C-NMR, see *Tables 1* and *2*. ESI-MS: 695 ($[M+Na]^+$). HR-ESI-MS: 695.2312 ($[M+Na]^+$, $C_{34}H_{40}NaO_{14}^+$; calc. 695.2315).

Tarennanoside E (=*(+)-Isolariciresinol 2a*-O-*[6*-O-*(3-Hydroxy-4-methoxy)benzoyl]-β*-D-*glucopyranoside*; **5**). Amorphous powder. $[\alpha]_D^{19} = +9.5$ ($c = 0.35$, MeOH). UV (MeOH): 286 (4.00), 264 (4.02). IR: 3443, 2931, 1696, 1632, 1598, 1515, 1455, 1430, 1352, 1286, 1225, 1123, 1082, 1030, 765. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS: 671 ($[M-H]^-$). HR-ESI-MS: 695.2324 ($[M+Na]^+$, $C_{34}H_{40}NaO_{14}^+$; calc. 695.2315).

Tarennanoside F (=*(−)-Isolariciresinol 2a*-O-*[6*-O-*(3-Hydroxy-4-methoxy)benzoyl]-β*-D-*glucopyranoside*; **6**). Amorphous powder. $[\alpha]_D^{19} = -44.2$ ($c = 0.92$, MeOH). UV (MeOH): 287 (4.03), 265 (4.04). IR: 3431, 2934, 1691, 1601, 1515, 1452, 1350, 1285, 1220, 1122, 1032, 768. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS: 671 ($[M-H]^-$). HR-ESI-MS: 671.2322 ($[M-H]^-$, $C_{34}H_{39}O_{14}^-$; calc. 671.2339).

Tarennanoside G (=*(+)-Isolariciresinol 2a*-O-*[6*-O-*(4-Hydroxy-3,5-dimethoxy)benzoyl]-β*-D-*glucopyranoside*; **7**). Amorphous powder. $[\alpha]_D^{24} = +22.4$ ($c = 0.62$, MeOH). UV (MeOH): 282 (4.16). IR: 3421, 2936, 1700, 1608, 1515, 1463, 1427, 1338, 1281, 1227, 1119, 1029, 764. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS: 701 ($[M-H]^-$). HR-ESI-MS: 701.2448 ($[M-H]^-$, $C_{35}H_{41}O_{15}^-$; calc. 701.2445).

Tarennanoside H (=*(−)-Isolariciresinol 2a*-O-*[6*-O-*(2-Hydroxy-5-methoxy)benzoyl]-β*-D-*glucopyranoside*; **8**). Amorphous powder. $[\alpha]_D^{25} = +22.0$ ($c = 0.43$, MeOH). UV (MeOH): 328 (3.95), 263 (3.12). IR: 3424, 2934, 1706, 1589, 1503, 1460, 1421, 1384, 1293, 1220, 1118, 1086, 769. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS: 755 ($[M+Na]^+$). HR-ESI-MS: 755.2512 ($[M+Na]^+$, $C_{36}H_{44}NaO_{16}^+$; calc. 755.2527).

Acid Hydrolysis of **1**. Compound **1** (10.6 mg) was dissolved in 6% HCl (10 ml) and heated until reflux (80°), and these conditions were held for 3 h. The mixture was then cooled and extracted with AcOEt (10 ml). The aq. phase was concentrated under reduced pressure to yield D-glucose (2.1 mg), which was detected by TLC in $CHCl_3$/MeOH 1:1 and compared with an authentic sample. The optical rotation of the isolate was $[\alpha]_D^{20} = +79.6$ ($c = 0.52$, MeOH). The AcOEt phase was further submitted to prep. TLC with $CHCl_3$/MeOH 10:1 to give (+)-lyoniresinol (0.7 mg), which was detected by TLC with an authentic sample of (−)-lyoniresinol (**10**; $[\alpha]_D^{24} = +75.4$ ($c = 0.35$, MeOH)).

Acid Hydrolysis of **2**. Compound **2** (5.2 mg) was processed as described above, and D-glucose (1.1 mg) was obtained from the aq. phase ($[\alpha]_D^{26} = +49.0$ ($c = 1.04$, MeOH)). The AcOEt phase was further separated to give (−)-lyoniresinol (0.4 mg; $[\alpha]_D^{25} = -39.8$ ($c = 0.22$, MeOH)).

Antioxidant Assay against H_2O_2-Induced Impairment in PC12 Cells. PC12 Cells were seeded into 96-well plates in RPMI 1640 medium with 10% characterized Newborn Bovine Serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. After maintaining in a H_2O-sat. atmosphere of 5% CO_2 at 37° for 24 h, these eleven compounds of different concentrations and freshly prepared H_2O_2 (with final concentration of 0.2 mM) in phosphate-buffered saline (PBS) were added to continue incubation for 1 h. For neg. control groups, only H_2O_2 was added. The cell viability was evaluated by MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium hydrobromide) reduction according to the protocol reported in [4]. Briefly, MTT soln. (0.5 mg/ml) in PBS was added, and the incubation continued for 4 h. Finally, a soln. (100 μl) containing 5% i-BuOH, 10% SDS, and 0.004% HCl was added. The mixtures were kept overnight, and the index of cell viability (% of control) was calculated by measuring the optical density of the color produced by MTT dye reduction with a microplate reader at 570 nm.

DPPH Radical-Scavenging Activity Assay. According to the reported procedures [4], each sample (100 μl of five different concentrations) was added to the same volume of DPPH soln. (0.1 mM in EtOH). The absorbance was measured with a *Spectra MAX 340* microplate reader at 517 nm after 30 min of reaction at 37°.

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