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ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · MAY 2007

Impact Factor: 3.8 · DOI: 10.1021/np0603931 · Source: PubMed

8 AUTHORS, INCLUDING:

Xian Wen Yang
Third Institute of Oceanography China

Peiji Zhao
Chinese Academy of Sciences

Hong-Ping He
Chinese Academy of Sciences

Xiao-Jiang Hao
Chinese Academy of Sciences

Mixed Lignan−Neolignans from *Tarenna attenuata*

Xian-Wen Yang,[†,‡] Pei-Ji Zhao,[†] Yan-Lin Ma,[§] Hai-Tao Xiao,[⊥] Yi-Qing Zuo,[§] Hong-Ping He,[†] Ling Li,[§] and Xiao-Jiang Hao*,[†]

State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, People's Republic of China, Department of Natural Product Chemistry, School of Pharmacy, Second Military Medical University, Shanghai 200433, People's Republic of China, Yunnan Pharmacological Laboratories of Natural Products, Kunming Medical College, Kunming 650031, People's Republic of China, and School of Pharmacy, Guiyang Medical College, Guiyang 550004, People's Republic of China

Received August 10, 2006

Six new mixed lignan−neolignans and 20 known compounds were isolated from the whole plant of *Tarenna attenuata*. By analysis of physical and spectroscopic data, the structures of the new compounds were elucidated as (1*R*,5*R*,6*R*)-6-{4-*O*-[2-(1-(4-hydroxy-3-methoxyphenyl))glycerol]-3,5-dimethoxyphenyl}-3,7-dioxabicyclo[3.3.0]octan-2-one (**1**), 5″-methoxyhedyotisol A (**2**), 4″-*O*-(8-guaiacylglycerol)buddlenol A (**3**), 5″-methoxy-4″-*O*-(8-guaiacylglycerol)buddlenol A (**4**), 4,6-dimethoxy-5-hydroxy-3-hydroxymethyl-2-(3,4,5-trimethoxyphenyl)-2,3-dihydrobenzofuran (**5**), and 7-*O*-ethylguaiacylglycerol (**6**). Compounds **1**, **5**, **6**, and **8** showed potent antioxidant activities against H_2O_2-induced impairment in PC12 cells, and compounds **1**, **2**, **5**, and **7** scavenged DPPH radical strongly with IC_{50} values of 72, 87, 45, and 55 μM, respectively.

Tarenna attenuata (Voigt) Hutchins, a shrub or small tree of the Rubiaceae family, is widely distributed in India, Vietnam, Cambodia, and China.[1] It is used as an antinociceptive and antipyretic by native communities in the traditional medicinal system of Guangxi Province, People's Republic of China.[2] In our recent search for bioactive compounds from the crude extract of medicinal plants, we found that the ethanol extract of *T. attenuata* exhibited potent antioxidant effects against H_2O_2-induced impairment in PC12 cells. Previous studies have reported the isolation of 10 iridoids, but none of them showed antioxidant activities.[3] This paper deals with the isolation and structure elucidation of six new and 20 known lignan constituents, as well as the antioxidant activities of some.

Results and Discussion

The EtOAc-soluble fraction of the EtOH extract of the whole plants of *T. attenuata* was subjected to column chromatography on silica gel, RP-18, and Sephadex LH-20, as well as preparative TLC to afford six new (**1**−**6**) and 20 known lignans: hedyotisol A (**7**),[4,5] buddlenol A (**8**),[5,6] guaiacylglycerol (**9**),[7] buddlenols C−E,[5,6] fiscusesquilignan A,[5,8] (+)-pinoresinol,[9] (+)-syringaresinol,[9] 2,3-dihydro-2-(4-hydroxy-3-methoxyphenyl)-3-hydroxymethyl-5-(2-formylvinyl)-7-hydroxybenzofuran,[10] balanophonin,[11,12] glycosmisic acid,[13,14] ficusal,[8] ω-hydroxypropioguaiacone,[15] dihydrocubebin,[16] 4,4′,7-trihydroxy-3,3′-dimethoxy-9,9′-epoxylignan,[17,18] isoferulaldehyde,[19] sinapaldehyde,[20] cinnamic acid,[21] and ferulic acid.[22] Compound **1** had a molecular formula of $C_{24}H_{28}O_{10}$ from the positive HRESIMS (found 499.1571, calcd for $C_{24}H_{28}O_{10}Na$, 499.1580). Its IR spectrum showed absorptions of hydroxy (3439 cm^{-1}), ester carbonyl (1767 cm^{-1}), and aromatic moieties (1630, 1550, and 1462 cm^{-1}). The ^{1}H NMR data of **1** (Table 1) indicated the presence of a 1,3,4-trisubstituted benzene moiety [δ_H 6.91 (1H, s, H-2″), 6.77 (1H, d, J = 8.2 Hz, H-5″), 6.75 (1H, d, J = 8.2 Hz, H-6″)], a 1,3,4,5-tetrasubstituted benzene ring [(δ_H 6.62 (2H, s, H-2′,6′)], three oxymethines, three oxymethylenes, and two other aliphatic methines. From the ^{1}H−^{1}H COSY spectrum (Figure 1), two partial structures, [−OCHCH(OH)CH_2O−] and [−OCHCH(CH_2)$CHCH_2$O−], are feasible. In the HMBC spectrum (Figure 1), the correlations of H_2-4 (δ_H 4.41 and 4.24, each 1H) and H-1 (δ_H 3.49) to C-2 (δ_C 177.8), H-6 (δ_H 4.63) to C-2′,6′ (δ_C 102.8), and H-2′,6′ (δ_H 6.62) to C-6 (δ_C 86.0) suggested compound **1** could be a 3,7-dioxabicyclo[3.3.0]octane-type lignan. Moreover, the correlations of H-7″ (δ_H 4.99) to C-2″ (δ_C 108.2) and H-6″ (δ_H 6.73) to C-7″ (δ_C 72.5) indicated the presence of a guaiacylglycerol moiety. Compared to guaiacylglycerol (**9**), C-8″ in compound **1** was downshifted from δ_C 75.6 to 87.1 ppm, which implied the connection of C-8″ to C-4′. Since there was no correlation between H-8″ and C-4′ in the HMBC spectrum obtained using a standard Bruker HMBC pulse program, tandem MS was adopted to confirm the structure. According to the MS/MS spectrum, the parent ion at m/z 499.4 lost a guaiacylglycerol group to give the daughter ion at m/z 302.1, thus establishing the connection of C-8″ of the guaiacylglycerol moiety to C-4′. The absolute configuration at C-6 was considered to be *R*, as the coupling constant of H-6 was 7.2 Hz.[23] In the ROESY spectrum (Figure 1), the correlations of H-6/H-4a, H-4a/H-1, and H-1/H-5 suggested that C-1 and C-5 were both *R* configured. Therefore, compound **1** was defined as (1*R*,5*R*,6*R*)-6-{4-*O*-[2-(1-(4-hydroxyphenyl-3-methoxy))glycerol]-3,5-dimethoxyphenyl}-3,7-dioxabicyclo[3.3.0]octan-2-one.

Table 1. ^{1}H and ^{13}C NMR Data for Compound **1** in $CDCl_3$ (δ in ppm, J in Hz in parentheses)

position	δ_C[a]	δ_H[b]	position	δ_C[a]	δ_H[b]
1	46.0	3.49 m	1″	131.1	
2	177.8		2″	108.2	6.96 s
4	70.3	4.41 d (9.5)	3″	144.8	
		4.24 dd (9.5, 3.4)	4″	146.6	
5	48.6	3.13 m	5″	114.1	6.86 d (8.4)
6	86.0	4.63 d (7.2)	6″	118.6	6.73 d (8.4)
8	69.6	4.54 dd (9.8, 6.7)	7″	72.5	4.99 d (3.9)
		4.38 dd (9.8, 1.5)	8″	87.1	4.13 dd (6.6, 3.9)
1′	135.5		9″	60.5	3.92 m
2′,6′	102.8	6.62 s			3.51 m
3′,5′	153.6		3′,5′-OMe	56.3	3.90 s
4′	134.7		3″-OMe	56.0	3.90 s

[a] Recorded at 125 MHz. [b]Recorded at 500 MHz.

Compound **2** exhibited an [M + Na]$^+$ ion peak at m/z 863.3119 in the positive HRESIMS, corresponding to the molecular formula $C_{43}H_{52}O_{17}$. The IR spectrum displayed absorption bands for hydroxy (3441 cm^{-1}) and aromatic moieties (1618, 1505, and 1462 cm^{-1}). Its ^{1}H and ^{13}C NMR spectroscopic data were very closely related

* To whom correspondence should be addressed. Tel: +86-871-5223263. Fax: +86-871-5219684. E-mail: haoxj@mail.kib.ac.cn.
[†] Kunming Institute of Botany.
[‡] Second Military Medical University.
[§] Kunming Medical College.
[⊥] Guiyang Medical College.

10.1021/np0603931 CCC: $37.00
Published on Web 03/08/2007

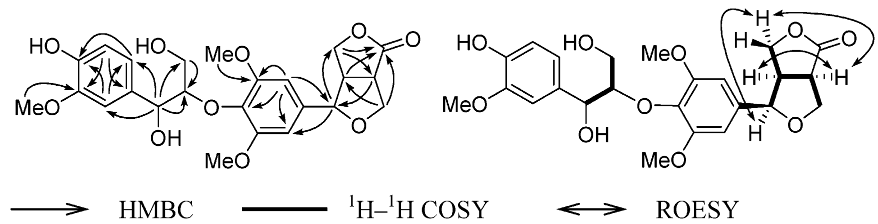
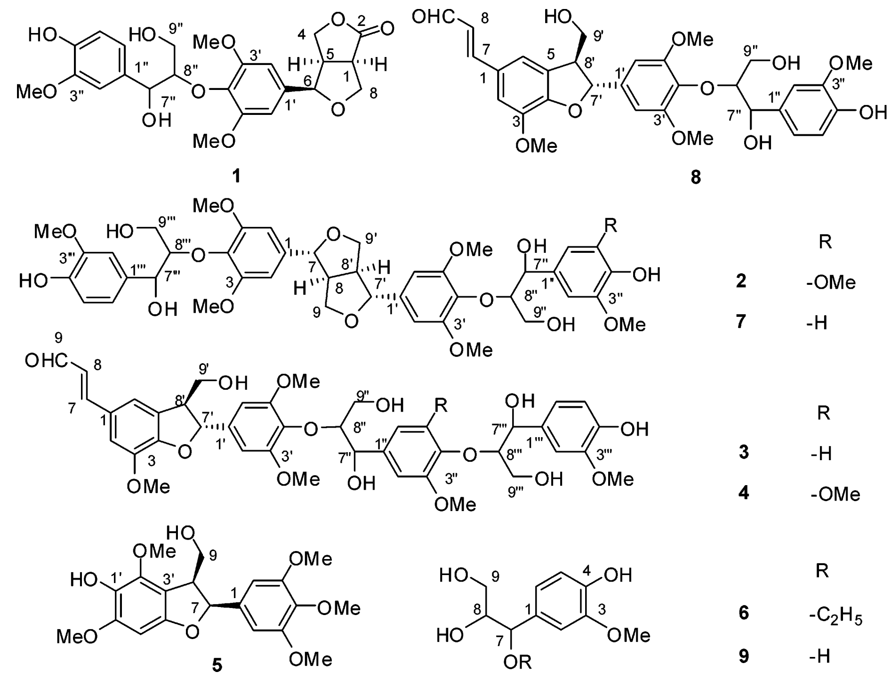


Figure 1. Key HMBC, $^1H-^1H$ COSY, and ROESY correlations of **1**.

Chart 1



to those of compound **7** (Table 2). Comparison of the ^{13}C NMR spectrum of these two compounds showed that compound **2** had an additional resonance at δ_C 104.4. In the HMBC spectrum, H-7″ (δ 4.85) correlated with this resonance. Therefore, the only difference between these two compounds is that H-5″ in compound **7** was substituted by a methoxy group in compound **2**. Further confirmation was found in the tandem MS spectrum of compound **2**, indicating that the parent ion at *m*/*z* 863.4 afforded daughter ions with loss of a guaiacylglycerol moiety at *m*/*z* 667.2, a syringoylglycerol moiety at *m*/*z* 637.2, and both guaiacylglycerol and syringoylglycerol moieties at *m*/*z* 439.2. Compound **2** was, therefore, defined as 5″-methoxyhedyotisol A.

Compound **3** was assigned the molecular formula $C_{41}H_{46}O_{15}$ from the positive HRESIMS. Its IR spectrum revealed absorption bands for hydroxy (3443 cm^{-1}) and aromatic moieties (1630, 1595, and 1464 cm^{-1}). The ^1H and ^{13}C NMR spectroscopic data were similar to those of compound **8**, except for the presence of a guaiacylglycerol [δ_H 7.00 (H, s, H-2‴), 6.83 (1H, m, H-6‴), 6.71 (1H, m, H-5‴), 4.88 (1H, m, H-7‴), 4.25 (1H, m, H-8‴); δ_C 120.6 (CH, C-6‴), 115.6 (CH, C-5‴), 111.6 (CH, C-2‴), 87.0 (CH, C-8‴), 73.9 (CH, C-7‴), 61.8 (CH_2, C-9‴), 56.4 (CH_3, 3‴-OMe)]. Compared to **8** and **9**, C-8‴ was downshifted from δ_C 75.6 to 87.0 and C-4″ was downshifted from δ_C 146.0 to 146.8, thus establishing the connection of C-8‴ to C-4″ via an ether linkage. This assumption was further confirmed by a tandem MS spectrum and an HMBC correlation of H-8‴ (δ_H 4.25, m) to C-4″ (δ_C 146.8). Therefore compound **3** was defined as 4″-*O*-(8-guaiacylglycerol)-buddlenol A.

Compound **4** had the molecular formula $C_{42}H_{48}O_{16}$ from the positive HRESIMS at *m*/*z* 831.2827. Its UV, IR, and ^1H and ^{13}C NMR spectroscopic data were similar to those of compound **3** except for the presence of a syringoylglycerol group and a

Table 2. ^1H and ^{13}C NMR Data for Compounds **2** and **7** in CD_3OD (δ in ppm, *J* in Hz in parentheses)

	2		7	
position	δ_C[a]	δ_H[b]	δ_C[a]	δ_H[b]
1,1′	132.7		135.7	
2,6,2′,6′	103.5	6.67 s	104.1	6.68 s
3,5,3′,5′	153.8		154.4	
4,4′	138.3		139.0	
7,7′	86.5	4.74 [f]	87.2	4.74 brs[f]
8,8′	55.0	3.11 brs	55.6	3.11 brs
9,9′	72.4	4.27 m[c], 3.91 m[d]	73.0	4.28 m[c], 3.92 m[d]
1″	133.1		133.8	
2″	104.4	6.62 s	111.2	6.95 s
3″	153.4		148.6	
4″	130.1		146.8	
5″	153.4		115.6	6.72 d (7.8)
6″	104.4	6.62 s	120.6	6.75 d (7.8)
7″	73.4	4.85 brs[f]	74.0	4.86 m[f]
8″	86.5	4.28 m[c]	87.1	4.26 m[c]
9″	60.8	3.90 m[d], 3.59 m	61.3	3.91 m[d], 3.60 d (11.9)
1‴	132.1		133.8	
2‴	110.6	6.94 s	111.2	6.95 s
3‴	148.2		148.6	
4‴	146.2		146.8	
5‴	115.0	6.73 m[e]	115.6	6.72 d (7.8)
6‴	120.0	6.74 m[e]	120.6	6.75 d (7.8)
7‴	73.6	4.84 brs[f]	74.0	4.86 m[f]
8‴	86.5	4.28 m[c]	87.1	4.26 m[c]
9‴	60.8	3.90 m[d], 3.59 m	61.3	3.91 m[d], 3.60 d (11.9)
3,5,3′,5′-OMe	56.0	3.82 s	56.6	3.85 s
3″-OMe	56.0	3.82 s	56.3	3.83 s
5″-OMe	56.0	3.82 s		
3‴-OMe	55.6	3.80 s	56.3	3.83 s

[a] Spectra were measured at 100 MHz. [b] Spectra were measured at 500 MHz. [c,d,e] Signals in the same column were overlapped. [f] Hidden in the D_2O signals.

Table 3. ^{1}H and ^{13}C NMR Data for Compounds **3**, **4**, and **8** in CD_3OD (δ in ppm, J in Hz in parentheses)

	3		**4**		**8**	
position	δ_C[a]	δ_H[b]	δ_C[a]	δ_H[b]	δ_C[a]	δ_H[b]
1	129.9		129.9s		129.9	
2	114.2	7.23 s	114.2d	7.23 s	114.5	7.20 s
3	146.0		145.9 s		146.0	
4	152.7		152.6s		152.8	
5	130.8		130.0s		130.9	
6	120.0	7.26 s	120.0d	7.26 s	120.0	7.24 s
7	156.1	7.61 d (15.7)	156.0d	7.60 d (15.7)	155.9	7.88 d (15.6)
8	127.2	6.67 m	127.2d	6.66 m	127.3	6.67 m
9	196.3	9.58 d (7.8)	196.2d	9.57 d (7.8)	196.1	9.50 d (7.8)
1′	138.9		139.0s		132.8	
2′,6′	103.8	6.68 s	103.8d	6.68 s	104.1	6.68 s
3′,5′	154.3		154.6s		154.7	
4′	136.4		136.4s		138.8	
7′	89.6	5.64 brs	89.6d	5.64 m	89.6	5.63 m
8′	54.9	3.50 m	55.0d	3.54 m	55.0	3.52 m
9′	64.6	3.88 m[d]	64.7t	3.87 m[d]	64.7	3.87 m[d]
		3.81 m		3.80 m		3.81 m
1″	133.7		139.0s		131.0	
2″	112.0	7.00 s	105.3d	6.70 m	111.4	6.94 s
3″	148.6		154.1s		149.2	
4″	146.8		136.4s		146.0	
5″	115.8	6.71 d (8.0)	154.1s		116.2	6.70 d (8.0)
6″	120.6	6.83 d (8.0)	105.3d	6.70 m	120.8	6.82 d (8.0)
7″	73.9	4.88 m[e]	74.0d	4.94 m[e]	74.3	4.88 m[e]
8″	87.0	4.25 m	87.5d	4.18 brs	87.2	4.25 m
9″	61.8	3.86 m[d]	61.3t	3.86 m[d]	61.8	3.86 m[d]
		3.45 m		3.60 m		3.45 m
1‴	133.7		133.8s			
2‴	111.6	7.00 s	111.2d	6.95 s		
3‴	148.8		148.7s			
4‴	146.6		146.8s			
5‴	115.6	6.71 d (8.0)	115.7d	6.72 m[c]		
6‴	120.6	6.83 d (8.0)	120.5d	6.73 m[c]		
7‴	73.9	4.88 m[e]	73.9d	4.93 m[e]		
8‴	87.0	4.25 m	86.8d	4.28 m		
9‴	61.8	3.86 m	61.6t	3.86 m[d]		
		3.45 m		3.54 m		
3-OMe	57.0	3.92 s	57.0	3.92 s	57.0	3.90 s
3′,5′-OMe	56.7	3.78 s	56.7	3.78 s	56.7	3.78 s
3″-OMe	56.6	3.80 s	56.6	3.80 s	56.4	3.80 s
5″-OMe			56.6	3.80 s		
3‴-OMe	56.4	3.79 s	56.4	3.79 s		

[a] Recorded in CD_3OD (100 MHz). [b] Recorded in CD_3OD (500 MHz). [c,d] Signals in the same column were overlapped. [e] Hidden in the D_2O signals.

guaiacylglycerol group instead of two guaiacylglycerol groups in **4**. Since there was no correlation of H-8″ and H-8‴ in the HMBC NMR spectrum, the tandem MS method was used to determine the structure of compound **4**. In the MS/MS spectrum, the parent ion at *m/z* 831.3 lost a guaiacylglycerol group to give the daughter ion at *m/z* 634.1, which indicated that the syringoylglycerol group was connected to C-4′, while the guaiacylglycerol moiety was attached to C-4″. On the basis of the above evidence, compound **4** was determined as 5″-methoxy-4″-*O*-(8-guaiacylglycerol)buddlenol A.

Compound **5** was assigned the molecular formula $C_{20}H_{24}O_8$ from the positive HRESIMS at *m/z* 415.1362. Its IR showed absorption bands for hydroxy (3441 cm^{-1}) and aromatic moieties (1619, 1517, and 1465 cm^{-1}). The ^{1}H NMR spectroscopic data (Table 4) indicated the presence of a benzofuran-type lignan moiety [δ_H 5.55 (1H, d, J = 4.4 Hz, H-7), 3.52 (1H, dd, J = 8.3, 4.4 Hz, H-8), 3.93 (1H, dd, J = 10.8, 3.8 Hz, H-9a), 3.66 (1H, dd, J = 10.8, 8.3 Hz, H-9b)], which was supported by resonances in the ^{13}C NMR spectrum [δ_C 89.0 (CH, C-7), 54.6 (CH, C-8), 64.1 (CH_2, C-9)]. Furthermore, its 1D NMR showed the presence of a 3,4,5-trimethoxylphenyl group [δ_H 6.62 (2H, s, H-2,6); δ_C 104.0 (CH, C-2,6), 61.5 (CH_3, 4-OMe), 56.8 (CH_3, 3,5-OMe)]. According to the HMBC spectrum, the above fragments were connected as shown in Figure 2. Since the coupling constant of H-7 was 4.4 Hz, the relative configuration of C-7 and C-8 was regarded as *cis*.[24] This configuration is opposite of that of the same protons in balanophonin,[11] for which J values (*trans*) are 7.0 Hz. Therefore compound **5** was determined as 4,6-dimethoxy-5-hydroxy-3-hydroxymethyl-2-(3,4,5-trimethoxyphenyl)-2,3-dihydrobenzofuran. However, a paucity of material prevented determination of the absolute configuration.

Compound **6** gave the molecular formula $C_{12}H_{18}O_5$ from the positive HRESIMS at *m/z* 265.1050. Its ^{1}H and ^{13}C NMR spectroscopic data (Table 4) gave two fragments, including a guaiacylglycerol group [δ_H 6.91 (1H, s, H-2), 6.77 (1H, d, J = 8.2 Hz, H-5), 6.75 (1H, d, J = 8.2 Hz, H-6); δ_C 121.3 (CH, C-6), 115.91 (CH, C-5), 111.7 (CH, C-2), 83.6 (CH, C-7), 77.0 (CH, C-8), 63.9 (CH_2, C-9), 56.3 (CH_3, 3-OMe)] and an ethoxy moiety [δ_H 3.39 (1H, d, J = 7.0 Hz, H-1′a), 3.34 (1H, d, J = 7.0 Hz, H-1′b) 1.16 (3H, t, J = 7.0 Hz, 2′-Me); δ_C 65.2 (CH_2, C-1′), 15.5 (CH_3, 2′-Me)]. Compared to guaicylglycerol (**9**), C-7 in compound **6** was downshifted from δ_C 75.6 to 83.6, which established the connection of the ethoxy at C-7 of the guaicylglycerol. This was confirmed by the HMBC correlation of H-7 (δ_H 4.19, d, J = 6.6 Hz) to C-1′ (δ_C 65.2). Therefore, compound **6** was elucidated as 7-*O*-ethylguaiacylglycerol. It has been reported that in the cases of syringoylglycerols and guaiacylglycerol derivatives, the coupling constant between H-7 and H-8 was about 5 Hz for the *erythro* isomer and 7 Hz for the *threo* isomer.[25] Thus, compound **6** was considered to be the *threo* isomer. To confirm whether compound **6** is artifactual because 95% EtOH was used as the solvent for extraction, another supply of the plant was obtained and extracted with MeOH and EtOH, respectively. By LC-MS, compound **6** was detected in the

Table 4. ^{1}H and ^{13}C NMR Data for Compounds **5**, **6**, and **9** in CD_3OD (δ in ppm, J in Hz in parentheses)

	5		**6**		**9**	
position	δ_C[a]	δ_H[c]	δ_C[b]	δ_H[c]	δ_C[b]	δ_H[c]
1	134.1		132.1		134.0	
2	104.0	6.62 s	111.7	6.91 s	111.6	6.96 s
3	149.4		149.0		149.9	
4	136.7		147.4		148.3	
5	149.4		115.9	6.77 d (8.2)	116.2	6.78 d (7.9)
6	104.0	6.62 s	121.3	6.75 d (8.2)	120.7	6.73 d (7.9)
7	89.0	5.55 d (4.4)	83.6	4.19 d (6.6)	77.6	4.48 d (3.4)
8	54.6	3.52 dd (8.3, 4.4)	77.0	3.65 m	75.6	3.64 m
9	64.1	3.93 dd (10.8, 3.8)	63.9	3.43 dd (11.1, 3.1)	64.3	3.45 m
		3.66 dd (10.8, 8.3)		3.33 m		
1′	136.8		65.2	3.39 d (7.0), 3.34 d (7.0)		
2′	152.5		15.5	1.16 t (7.0)		
3′	110.8					
4′	159.3					
5′	91.5	6.35 s				
6′	157.2					
3-OMe	56.8	3.80 s	56.3	3.84 s	56.4	3.84 s
4-OMe	61.5	3.73 s				
5-OMe	56.8	3.80 s				
2′-OMe	61.1	3.89 s				
6′-OMe	56.8	3.81 s				

[a] Recorded at 100 MHz. [b] Recorded at 125 MHz. [c] Recorded at 500 MHz.

Table 5. Antioxidant Effects of Compounds **1**−**8** by MTT and DPPH Assays

group	viability (%), concentration (μM)[a,b]				IC$_{50}$ (μM)[e]
	0.4	2.0	10.0	50.0	
control	100***				
model[c]	51.5 ± 2.7				
edaravone[d]	55.5 ±± 1.4*	53.1 ± 3.9	47.8 ± 2.2	25.6 ± 2.3	27
compound **1**	59.3 ± 2.8**	61.8 ± 2.5**	59.4 ± 1.9**	45.7 ± 4.4	72
compound **2**	51.9 ± 2.9	54.3 ± 3.9	54.7 ± 2.6	54.1 ± 3.6	87
compound **3**	52.8 ± 4.0	47.9 ± 8.9	64.4 ± 18.7	55.9 ± 6.4	201
compound **4**	50.3 ± 5.5	54.7 ± 4.2	55.2 ± 1.6*	51.0 ± 5.5	127
compound **5**	61.4 ± 3.1**	58.0 ± 2.7**	58.2 ± 1.5**	55.0 ± 3.0	45
compound **6**	54.1 ± 1.9	54.9 ± 1.8	56.8 ± 2.9*	57.3 ± 2.5*	132
compound **7**	52.2 ± 4.7	53.2 ± 3.5	54.3 ± 2.0	55.3 ± 5.1	55
compound **8**	56.9 ± 6.6	56.8 ± 5.2	62.6 ± 8.5*	64.8 ± 9.7*	147

[a] Effects of compounds **1**−**8** against H_2O_2-induced impairment in PC12 cells. [b] $n = 5$, $\bar{X} \pm$ SD. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs model. [c] Negative control. [d] Positive control. [e] Radical-scavenging activities of compounds **1**−**8** against DPPH.

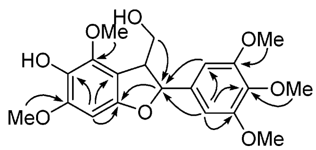


Figure 2. Key HMBC correlations of compound **5**.

EtOH extract (2.0 mg/mL), while it was not detected in the MeOH extract (2.0 mg/mL). Thus, compound **6** was established unequivocally as an artifact of extraction.

The antioxidant effects of compounds **1**−**8** were evaluated by both MTT and DPPH assays (Table 5). Compounds **1**, **5**, **6**, and **8** showed potent activities against H_2O_2-induced impairment in PC12 cells within the concentration range tested (0.4 to 50 μM), whereas compounds **1**, **2**, **5**, and **7** scavenged DPPH radical strongly, with IC$_{50}$ values of 74.5, 87.1, 45.4, and 55.0 μM, respectively.

T. attenuata has been used as an antinociceptive by native communities in the traditional medicinal system of Guangxi Province, People's Republic of China. Recent studies showed that the antinociceptive activity might be closely related to the free radical-scavenging effect.[26,27] Thus the result in our current study provides a possible mechanism to account for its use as a traditional antinociceptive.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Horiba SEPA-300 polarimeter or JASCO DIP-370 digital polarimeter. UV spectra were obtained using a Shimadzu UV-2401PC spectrometer. IR spectra were recorded on a Bio-Rad FTS-135 spectrometer with KBr pellets. ^{1}H and ^{13}C NMR experiments were performed on a Bruker AM-400 or DRX-500 NMR spectrometer with TMS as internal standard. LC-MS/MS and ESIMS were measured on a Waters 2695 HPLC-Thermo Finnigan LCQ Advantage ion trap mass spectrometer. EIMS and HRESIMS were taken on a VG Auto Spec 3000 spectrometer. Column chromatography was performed with silica gel (200−300 mesh; Qingdao Marine Chemical, Inc., Qingdao, People's Republic of China), silica gel H (10−40 μm; Qingdao), Sephadex LH-20 (40−70 μm; Amersham Pharmacia Biotech AB, Uppsala, Sweden), and Lichroprep RP-18 gel (40−63 μm; Merck, Darmstadt, Germany). Zones of preparative TLC plates (1.0−1.5 mm; Qingdao) and TLC plates (0.20−0.25 mm; Qingdao) were visualized under UV light or by spraying with 10% H_2SO_4 in 95% EtOH, followed by heating.

Plant Material. The whole plant of *T. attenuata*, collected in Xishuangbanna of Yunnan Province, People's Republic of China, in October 2004, was identified by Prof. Jing-Yun Cui, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences. A voucher specimen (BN163) was deposited in Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The dried and powdered (17 kg) sample of *T. attenuata* was extracted with 95% EtOH (16 L) under reflux for 3 × 4 h. The extract was concentrated to dryness under reduced pressure. The residue was suspended in H_2O and partitioned, sequentially, with petroleum ether, EtOAc, and *n*-BuOH. The EtOAc extract (61 g) was separated into eight fractions (F_1−F_8) by column chromatography on silica gel using a $CHCl_3$−MeOH gradient. Fraction F_1 was further separated by column chromatography on silica gel with petroleum ether−EtOAc (20:1) to give cinnamic acid (18 mg). Fraction F_2 was separated using reversed-phase MPLC by a gradient of H_2O−MeOH and preparative TLC with EtOAc−MeOH (100:1) to give **7** (32 mg) and **8** (3 mg). Repeated column chromatography of fraction F_3 over silica gel (petroleum ether−EtOAc, 1:1; $CHCl_3$−MeOH, 50:1) gave **1** (1.1 mg), **2** (3.7 mg), **3** (6.1 mg), and **4** (6.6 mg). Fraction F_4 was subjected to column chromatography on silical gel (EtOAc−MeOH, 50:1) and Sephadex LH-20 ($CHCl_3$−MeOH, 1:1) to give **6** (6 mg), buddlenol D (15 mg), and balanophonin (8 mg). Fraction F_5 was divided into six subfractions ($F_{5.1}$−$F_{5.6}$) by column chromatography over silica gel (EtOAc−MeOH, 50:1). Subfraction $F_{5.2}$ was then chromatographed on a silical gel column ($CHCl_3$−MeOH, 40:1) to give **9** (23 mg), 2,3-dihydro-2-(4-hydroxy-3-methoxyphenyl)-3-hydroxymethyl-5-(2-formylvinyl)-7-hydroxybenzofuran (3 mg), and glycosmisic acid (9 mg). Repeated chromatography of subfraction $F_{5.4}$ on silica gel eluted with $CHCl_3$−MeOH (30:1) afforded ω-hydroxypropioguaiacone (4 mg), 4,4′,7-trihydroxy-3,3′-dimethoxy-9,9′-epoxylignan (8 mg), isoferulaldehyde (11 mg), and sinapaldehyde (10 mg). Fraction F_6 was chromatographed on a silica gel column eluting with EtOAc−MeOH (20:1) to give seven subfractions ($F_{6.1}$−$F_{6.7}$). Subfraction $F_{6.2}$ was further chromatographed over a silica gel column ($CHCl_3$−MeOH, 10:1) to give **5** (0.6 mg) and buddlenol C (5 mg). Repeated chromatography of subfraction $F_{6.3}$ over silical gel ($CHCl_3$−MeOH, 10:1) afforded buddlenol E (2 mg), fiscusesquilignan A (1 mg), (+)-pinoresinol (2 mg), and (+)-syringaresinol (20 mg). Subfraction $F_{6.5}$ was separated by chromatography eluting with EtOAc−MeOH (8:1) and Sephadex LH-20 eluting with MeOH to give ficusal (6 mg), dihydrocubebin (9 mg), and ferulic acid (34 mg).

(1*R*,5*R*,6*R*)-6-{4-*O*-[2-(1-(4-Hydroxyphenyl-3-methoxy))glycerol]-3,5-dimethoxyphenyl}-3,7-dioxabicyclo[3.3.0]octan-2-one (1): amorphous powder; $[\alpha]_D^{20}$ −9.4 (*c* 0.60, MeOH); UV (MeOH) λ_{max} (log ϵ) 280 (3.81), 344 (3.17) nm; IR (KBr) ν_{max} 3439, 2932, 1767, 1630, 1550, 1462, 1425, 1382, 1333, 1273, 1221, 1125, 1033, 772 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Table 1; ESIMS (positive) *m/z* 499 $[M + Na]^+$; HRESIMS (positive) *m/z* 499.1571 $[M + Na]^+$ (calcd for $C_{24}H_{28}O_{10}Na$, 499.1580).

5″-Methoxyhedyotisol A (2): amorphous powder; $[\alpha]_D^{27}$ +6.5 (*c* 0.21, MeOH); UV (MeOH) λ_{max} (log ϵ) 205 (4.92), 280 (3.69), 357 (3.21) nm; IR (KBr) ν_{max} 3441, 2937, 1618, 1505, 1462, 1423, 1369, 1354, 1226, 1124, 1033, 774, 766 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Table 2; ESIMS (positive) *m/z* 863 $[M + Na]^+$; HRESIMS (positive) *m/z* 863.3119 $[M + Na]^+$ (calcd for $C_{43}H_{52}O_{17}Na$, 863.3102).

4″-*O*-(8-Guaiacylglycerol)buddlenol A (3): amorphous powder; $[\alpha]_D^{27}$ +5.4 (*c* 0.34, MeOH); UV (MeOH) λ_{max} (log ϵ) 204 (4.84), 281 (3.88), 335 (3.90) nm; IR (KBr) ν_{max} 3443, 2936, 1630, 1595, 1511, 1464, 1424, 1383, 1350, 1270, 1222, 1127, 1032, 822, 767 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Table 3; ESIMS (positive) *m/z* 801 $[M + Na]^+$; HRESIMS (positive) *m/z* 801.2753 $[M + Na]^+$ (calcd for $C_{41}H_{46}O_{15}Na$, 801.2734).

5″-Methoxy-4″-*O*-(8-guaiacylglycerol)buddlenol A (4): amorphous powder; $[\alpha]_D^{27}$ +5.9 (*c* 0.38, MeOH); UV (MeOH) λ_{max} (log ϵ) 205 (4.78), 279 (3.83), 334 (3.72) nm; IR (KBr) ν_{max} 3442, 2940, 2843, 1595, 1503, 1463, 1423, 1384, 1352, 1272, 1224, 1126, 1032, 831, 766 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Table 3; ESIMS (positive) *m/z* 831 [M + Na]$^+$; HRESIMS (positive) *m/z* 831.2827 [M + Na]$^+$ (calcd for $C_{42}H_{48}O_{16}Na$, 831.2840).

4,6-Dimethoxy-5-hydroxy-3-hydroxymethyl-2-(3,4,5-trimethoxyphenyl)-2,3-dihydrobenzofuran (5): amorphous powder; $[\alpha]_D^{19}$ +7.8 (*c* 0.35, MeOH); UV (MeOH) λ_{max} (log ϵ) 207 (4.76), 282 (3.67), 371 (3.02) nm; IR (KBr) ν_{max} 3441, 2933, 1619, 1517, 1465, 1429, 1372, 1275, 1201, 1167, 1118, 1033, 771 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Table 4; ESIMS (positive) *m/z* 415 [M + Na]$^+$, 807 [2M + Na]$^+$; HRESIMS (positive) *m/z* 415.1362 [M + Na]$^+$ (calcd for $C_{20}H_{24}O_8Na$, 415.1368).

7-*O*-Ethylguaiacylglycerol (6): amorphous powder; $[\alpha]_D^{27}$ +11.2 (*c* 0.27, MeOH); UV (MeOH) λ_{max} (log ϵ) 204 (4.17), 229 (3.49), 280 (3.06) nm; IR (KBr) ν_{max} 3421, 2929, 1631, 1612, 1518, 1454, 1432, 1372, 1356, 1279, 1156, 1122, 1094, 1035, 853, 773 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Table 4; ESIMS (positive) *m/z* 265 [M + Na]$^+$, 506 [2M + Na]$^+$; HRESIMS (positive) *m/z* 265.1050 [M + Na]$^+$ (calcd for $C_{12}H_{18}O_5Na$, 265.1051).

Antioxidant Assay against H_2O_2-Induced Impairment in PC12 Cells. PC12 cells were obtained from Kunming Institute of Zoology, Chinese Academy of Sciences, and maintained in a water-saturated atmosphere of 5% CO_2 at 37 °C. Cells were seeded into 96-well plates in RPMI 1640 medium (Invitrogen corporation, Grand Isband, NY) with 10% characterized newborn bovine serum (Lanzhou National Hyclone Bio-engineering Co. Ltd., Lanzhou, People's Republic of China). Experiments were carried out 24 h after cells were seeded according to the reported protocol.[28] Different concentrations of these eight compounds 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. The assay for cell viability was evaluated by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma] reduction.[29] Briefly, MTT solution (0.5 mg/mL) in PBS was added and the incubation continued for 4 h. Finally, a 100 μL solution containing 5% *i*-BuOH, 10% SDS (Sigma), 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 (Bio-Rad Model 680, Hercules, CA) at 570 nm.

DPPH Radical-Scavenging Activity Assay. The DPPH method[30] was used to determine the free radical-scavenging potential of each sample. Each compound (100 μL in five different concentrations ranging from 0.16 to 100.0 μM) was added to 100 μL of DPPH solution (0.1 mM in EtOH). The absorbance was measured with a Spectra MAX 340 microplate reader (Molecular Devices, Menlo Park, CA) at 517 nm after 30 min of reaction at 37 °C. The percentage of radical-scavenging activity (RSA %) was calculated using the following equation: RSA % = $[(A_C - A_S)/A_C] \times 100\%$, where A_C is the absorbance of the control and A_S is the absorbance of the samples at 517 nm. IC_{50} values denote the concentration of sample required to scavenge 50% DPPH free radicals.

Acknowledgment. We acknowledge the Department of Instrumental Analysis of our Institute for measuring the optical rotations, UV, IR, NMR, and mass spectra. This research was funded by National Natural Science Foundations of China (No. 30370957) and National Science Foundation of Yunnan Province (No. 2003C0061M).

Supporting Information Available: The ^{1}H and ^{13}C NMR spectra for compound **1**, tandem MS spectra for compounds **1**–**4**, and LC-MS spectra for compound **6**. This information is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Luo, X. R.; Gao, Y. Z.; Chen, W. Q.; Xu, X. H.; Wu, H. In *Flora Reipublicae Popularis Sinicae*; Wu, Z. Y., Ed.; Science Press: Beijing, 1999; Vol. 71, pp 382–383.
(2) Board of Health of Revolution Committee in Guangxi Zhuang Autonomous Region. *Guangxi Herbal Selections*; Guangxi People's Publishing House: Nanning, 1974; Vol. 2, pp 1798–1799.
(3) Yang, X. W.; Ma, Y. L.; He, H. P.; Wang, Y. H.; Di, Y. T.; Zhou, H.; Li, L.; Hao, X. J. *J. Nat. Prod.* **2006**, *69*, 971–974.
(4) Matsuda, S.; Kadota, S.; Tai, T.; Kikuchi, T. *Chem. Pharm. Bull.* **1984**, *32*, 5066–5069.
(5) Houghton, P. J. *Phytochemistry* **1985**, *24*, 819–826.
(6) Cui, Y. L.; Mu, Q.; Hu, C. Q. *Nat. Prod. Res. Dev.* **2003**, *15*, 277–283.
(7) Comte, G.; Vercauteren, J.; Chulia, A. J.; Allais, D. P.; Delage, C. *Phytochemistry* **1997**, *45*, 1679–1682.
(8) Li, Y. C.; Kuo, Y. H. *Chem. Pharm. Bull.* **2000**, *48*, 1862–1865.
(9) Abe, F.; Yamauchi, T. *Phytochemistry* **1988**, *27*, 575–577.
(10) Miki, K.; Ito, K.; Sasaya, T. *Mokuzai Gakkaishi* **1979**, *25*, 665–670.
(11) Sy, L. K.; Brown, G. D. *Phytochemistry* **1999**, *50*, 781–785.
(12) Haruna, M.; Koube, T.; Ito, K.; Murata, H. *Chem. Pharm. Bull.* **1982**, *30*, 1525–1527.
(13) Xiang, Y.; Wang, H.; Wen, Y. Y. *J. Trop. Subtrop. Bot.* **2002**, *10*, 69–73.
(14) Ohta, M.; Higuchi, T.; Iwahara, S. *Arch. Microbiol.* **1979**, *121*, 23–28.
(15) Achenbach, H.; Stöcker, M.; Constenla, M. A. *Phytochemistry* **1988**, *27*, 1835–1841.
(16) Dwuma-Badu, D.; Ayim, J. S. K.; Dabra, T. T. *J. Nat. Prod.* **1975**, *38*, 343–345.
(17) Estevez-Braun, A.; Estevez-Reyes, R.; Gonzalez-Perez, J. A.; Gonzalez, A. G. *J. Nat. Prod.* **1995**, *58*, 887–892.
(18) Braum, A. E.; Reyes, R. E.; Perez, J. A. G.; Gonzalez, A. G. *J. Nat. Prod.* **1995**, *58*, 887–892.
(19) Barakat, H. H.; Nawwar, M. A. M.; Buddrusa, J.; Linscheida, M. *Phytochemistry* **1987**, *26*, 1837–1838.
(20) Lin, J.; Hao, X. J.; Liang, G. Y. *Acta Pharm. Sin.* **1999**, *34*, 203–206.
(21) Li, S. S.; Tan, N. H.; Zhou, J.; Zhao, S. S. *Acta Bot. Yunn.* **2001**, *23*, 115–120.
(22) Luo, Y. M.; Zhang, J. H.; Pan, J. G.; Yao, S. L.; Huang, H. L.; Zhu, Y.; Li, Q. S. *Chin. Pharm. J.* **1994**, *29*, 714–716.
(23) Duan, H.; Takaishi, Y.; Momota, H.; Ohmoto, Y.; Taki, T. *Phytochemistry* **2002**, *59*, 85–90.
(24) Gongora, L.; Manez, S.; Giner, R. M.; Carmen Recio, M.; Gray, A. I.; Rios, J. L. *Phytochemistry* **2002**, *59*, 857–860.
(25) Kuima, K.; Otsuka, H.; Ide, T.; Ogimi, C.; Hirata, E.; Takushi, A.; Takeda, Y. *Phytochemistry* **1998**, *48*, 669–676.
(26) Ratnasooriya, W. D.; Deraniyagala, S. A.; Bathige, S. D.; Hettiarachchi, H. D. *J. Ethnopharmacol.* **2005**, *97*, 123–128.
(27) Zhao, Q.; Zhao, Y.; Wang, K. *J. Ethnopharmacol.* **2006**, *106*, 408–413.
(28) Wang, R.; Zhou, J.; Tang, X. C. *Mol. Brain Res.* **2002**, *107*, 1–8.
(29) Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 589–601.
(30) Blois, M. S. *Nature* **1958**, *181*, 1199–1200.

NP0603931