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A NEW NAPHTHOQUINONE AND A NEW NEOLIGNAN FROM *Ligularia vellerea* RHIZOMES

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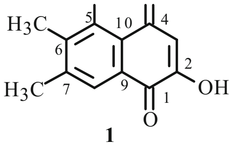
In the course of phytochemical investigations of Ligularia vellerea *rhizomes, a new naphthoquinone, 2,5-dihydroxy-6,7-dimethylnaphthoquinone (**1**), and a new neolignan, 4-[(3′,4′-dihydroxycinnamoyl)-oxy]-methyl cinnamate (**2**), have been isolated and characterized on the basis of spectroscopic methods (^{1}H NMR, ^{13}C NMR, 2D NMR, and MS).*

Keywords*:* *Ligularia vellerea*, naphthoquinone, neolignan, 2,5-dihydroxy-6,7-dimethylnaphthoquinone, 4-[(3′,4′-dihydroxycinnamoyl)-oxy]-methyl cinnamate.

The genus *Ligularia* Cass (Compositae) is represented in China by over 100 species [1]. A number of *Ligularia* species such as *L. fischeri*, *L. sibirica,* and *L. hodgsoni* are commonly used as herbal remedies to treat bronchitis, cough and asthma, phthisis and so on in southwest China and other areas [2]. *Ligularia vellerea* has been studied by M. Tori et al [3] and Y. S. Li [4]. We reinvestigated the plants and isolated two new compounds **1** and **2**: 2,5-dihydroxy-6,7-dimethylnaphthoquinone; 4-[(3′,4′-dihydroxycinnamoyl)-oxy]-methyl cinnamate. The structure elucidation was based on ^{1}H NMR, ^{13}C NMR, 2D NMR, and MS.

Compound **1**, yellow powder. Its molecular formula was determined as $C_{12}H_{10}O_4$ by NMR data and the quasi-molecular ion peak at *m/z* 219.2 $[M + H]^+$ in its positive APCI-MS. The ^{1}H NMR spectrum of **1** clearly displayed singlets for two methyls at δ_H 2.08 (3H, s) and 2.31 (3H, s) and two olefinic protons at δ_H 6.76 and 8.25. The ^{13}C NMR and HSQC spectrums of **1** showed eight quaternary carbons at δ_C 109.6, 117.0, 131.6, 145.8, 168.9, 169.7, 172.5, and 181.8, two methines at δ_C 100.0 and 128.6, and two methyls at δ_C 16.9 and 19.8. The above data revealed that **1** is a naphthoquinone with two methyls. The very downfield broad singlet at δ_H 12.08 (br.s, 1H) in the ^{1}H NMR spectrum showed that the structure of **1** has one α-hydroxyl (because of the deshielding effect from the carbonyl) located at the benzene ring. The downfield proton singlet at δ_H 8.25 (s, 1H) is ascribed to one α proton (because of the deshielding effect from the carbonyl) located at the benzene ring. The cross peaks in HMBC spectrum showed that the two protons at δ_H 6.76 and 8.25 were respectively located at C-3 and C-8, and the two methyls at C-6 and C-7. Thus compound **1** was identified as 2,5-dihydroxy-6,7-dimethylnaphthoquinone. The assignments of each signal in the NMR spectra based on HSQC and HMBC are shown in Table 1.



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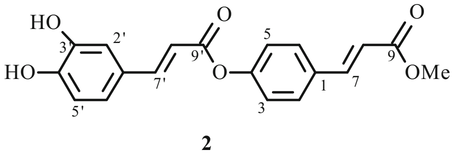

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TABLE 1. NMR Spectral Data of **1** in CD_3COCD_3 (400 MHz, δ, ppm, TMS)

C atom	δ_C	δ_H	HMBC	C atom	δ_C	δ_H	HMBC
1	172.5		H-8	7	145.8		CH_3-6, CH_3-7
2	169.7		H-3, 8	8	128.6	8.25 s	
3	100.0	6.76 s		9	117.0		H-3
4	181.8		H-8	10	109.6		H-3
5	168.9		H-8	CH_3-6	19.8	2.05 s	CH_3-7
6	131.6		CH_3-6, CH_3-7	CH_3-7	16.9	2.31 s	CH_3-6, H-3

TABLE 2. NMR Spectral Data of **2** in CD_3COCD_3 (400 MHz, δ, ppm, TMS, J/Hz)

C atom	δ_C	δ_H	HMBC	C atom	δ_C	δ_H	HMBC
1	125.9		H-3, 5, 7, 8	4′	147.6		H-2′, 5′, 6′
2, 6	129.8	7.55 (2H, d, J = 8.0)	H-6, 2, 7	5′	115.2	6.87 (1H, d, J = 8.0)	
3, 5	115.6	6.91 (2H, d, J = 8.0)	H-5, 3	6′	121.4	7.04 (1H, dd, J = 8.0, 2.0)	H-2′, 5′, 7′
4	159.4		H-2, 3, 5, 6	7′	144.6	7.53 (1H, d, J = 16.0)	H-2′, 6′
7	144.5	7.61 (1H, d, J = 16.0)	H-2, 6	8′	114.2	6.28 (1H, d, J = 16.0)	H-7′
8	114.6	6.34 (1H, d, J = 16.0)	H-7	9′	166.8		H-7′, 8′
9	167.2		H-7, 8, -OCH_3	OH		8.89	
1′	126.5		H-5′, 7′, 8′	OH		8.46	
2′	114.1	7.16 (1H, d, J = 2.0)	H-6′, 7′	OCH_3	50.4	3.71 (3H, s)	
3′	145.2		H-2′, 5′				

Compound **2**, pale yellow powder. Its molecular formula was determined as $C_{19}H_{16}O_6$ by NMR data and the quasi-molecular ion peak at *m/z* 341.4 $[M + H]^+$ in its positive APCI-MS. ^{1}H NMR data (Table 2) showed one methoxyl group (δ_H 3.71, s, 3H), two hydroxyls (δ_H 8.89, s, 1H; 8.46, s, 1H), two couples of olefinic protons δ_H 7.61 (d, J = 16.0 Hz); 7.53 (d, J = 16.0 Hz); 6.34 (d, J = 16.0 Hz); 6.28 (d, J = 16.0 Hz), three aromatic protons δ_H 7.16 (d, J = 2.0 Hz); 7.04 (dd, J = 8.0 Hz, 2.0 Hz); 6.87 (d, J = 8.0 Hz) in the ABX spin-spin system, and four aromatic protons δ_H 7.55 (d, J = 8.0 Hz, 2H); 6.89 (d, J = 8.6 Hz, 2H) in the AA′BB′ spin-spin system. The ^{13}C NMR and DEPT data (Table 2) showed one methoxyl group (δ_C 50.4), seven aromatic quaternary carbons (δ_C 125.9, 126.5, 145.2, 147.6, 159.4, 166.8, and 167.2), and eleven aromatic methine carbons (δ_C 114.1, 114.2, 114.6, 115.3, 115.6 (2C), 121.4, 129.8 (2C), 144.5 and 144.6), which suggested that the structure of **2** includes one *p*-oxygenated cinnamyl group and one 3,4-dioxygenated cinnamyl group. The assignments of each signals in the NMR spectra based on HSQC and HMBC. The HMBC spectra are shown in Table 2. The cross peaks (Table 2) in the HMBC spectrum showed that the two hydroxyls are located at C-3′ and C-4′, and OCH_3 at C-9. Correlations of the *p*-oxygenated cinnamoyl group and the 3,4-dioxygenated cinnamoyl group have not been observed in the HMBC spectrum, so acid hydrolysis was carried out in silica gel thin layer chromatography (TLC), and the spots of 3,4-dihydroxycinnamic acid and 4-hydroxycinnamoyl methyl ester were found by comparision with the authentic compounds. The result of acid hydrolysis showed that 3′,4′-dihydroxycinnamoyl occurred at C-4 through the ester bond. Thus, compound **2** was identified as 4-[(3′,4′-dihydroxycinnamoyl)-oxy]-methyl cinnamate. HSQCand HMBC correlations are shown in Table 2.

EXPERIMENTAL

General Methods. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR: Bruker DRX-400 spectrometer in acetone-d_6, values given in ppm; CC: Silica gel and Sephadex LH-20 using the solvent systems (1) petroleum ether–EtOAc–MeOH (3:1:0.1); (2) MeOH.

Plant Material. The plant material was collected from Lijiang, Guangxi Province, China, in August 2004. A voucher specimen has been deposited in the School of Pharmaceutical Sciences, Zhejiang University.

The dried roots (20 kg) were extracted with 95% EtOH at room temperature. The EtOH extract was evaporated at 45°C to yield a black residue (1.75 kg). The residue was suspended in H_2O and extracted with petroleum ether, EtOAc, and *n*-butanol. The EtOAc extract was subjected to silica gel column chromatography with $CHCl_3$–MeOH (gradiently) as eluent to give fractions I–VI. Fraction IV was purified by silica gel column chromatography (eluted with petroleum ether–EtOAc–MeOH 1:1:0.05, v/v/v) and then by Sephadex LH-20 column chromatography (eluted with MeOH) repeatedly to afford compound **1** (5 mg) and compound **2** (8 mg).

2,5-Dihydroxy-6,7-dimethylnaphthoquinone (1). Yellow powder; molecular weight $C_{12}H_{10}O_4$; positive APCI-MS *m/z* 219.2 $[M + H]^+$; ^1H NMR, ^{13}C NMR data and HMBC, see Table 1.

4-[(3′,4′-Dihydroxycinnamoyl)-oxy]-methyl cinnamate (2). Pale yellow powder; molecular weight $C_{19}H_{16}O_6$; positive APCI-MS *m/z* 341.4 $[M + H]^+$; ^1H NMR, ^{13}C NMR data and HMBC, see Table 2.

Acid Hydrolysis. Compound **2** was placed on a silica gel plate and acid hydrolysis carried out for 10 min using 12 N HCl in a sealed container. The plate was taken out and dried, then examined by chromatography using the solvent system $CHCl_3$–MeOH–HCOOH 10:1:0.02 and the results compared with authentic samples 3,4-dihydroxycinnamic acid and *p*-hydroxycinnamyl methyl ester.

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