Sesquiterpenoids from the Rhizome of *Ligularia virgaurea*

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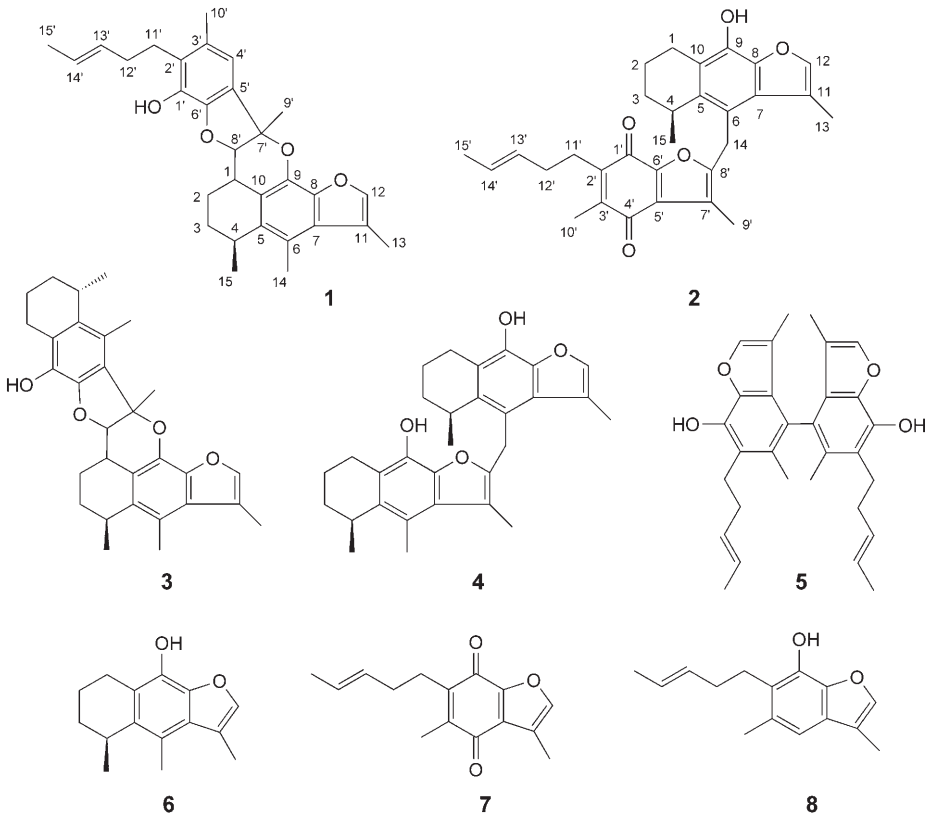
Two novel sesquiterpene dimers, compounds **1** and **2**, were isolated from the rhizome of *Ligularia virgaurea*, together with the six known sesquiterpenoids **3**–**8**. Their structures were established by physico-chemical and spectroscopic methods, especially by means of 1D- and 2D-NMR as well as HR-MS analyses. A mechanism based on a classical *Diels–Alder* cyclization is proposed for the formation of the dimer **1** from the precursors **8** and the quinone form of **6** (*Scheme*).

Introduction. – The genus *Ligularia* is an important source of sesquiterpenoids. A number of sesquiterpenoids, including a few unusual ones from *Ligularia* plants, have been reported in recent years [1]. During our search for new natural products, we investigated *Ligularia virgaurea*, a traditional herb used in folk medicine for the treatment of coughs and inflammation [2]. As a result, five dimeric sesquiterpenes, including the new compounds **1** and **2**, were isolated from the Et_2O/petroleum ether extract of this species. In this study, we describe the isolation and structural elucidation of the new compounds. In addition, we report the re-assigned, consistent ^{13}C-NMR data of the known isolates **3**–**8** [3–6].

Results and Discussion. – Compound **1** was isolated as a colorless powder. The quasi-molecular $[M+Na]^+$ ion peak at m/z 481.2350 (calc. 481.2349) in the HR-ESI mass spectrum indicated the molecular formula $C_{30}H_{34}O_4$, with 14 degrees of unsaturation. The IR spectrum of **1** exhibited strong absorption bands at 3395 (OH), 1698 (C=C), 1474 and 1449 (aromatic ring), and 1104 and 1087 cm^{-1} (C–O). Detailed analysis of the ^{1}H- and ^{13}C-NMR spectra of **1** (*Table 1*) enabled us to elucidate its structure as (5*S*)-5,6,7,7a,7b,12b-hexahydro-3,4,5,11,12b-pentamethyl-10-[(3*E*)-pent-3-en-1-yl]-furo[3″,2″:6′,7′]naphtho[1′,8′:4,5,6]pyrano[3,2-*b*]benzofuran-9-ol[1]).

The ^{13}C-NMR (DEPT) spectroscopic data of **1** (*Table 1*) indicated 30 C-atoms, including six Me, four CH_2, and seven CH groups, as well as 13 quaternary C-atoms, which suggested a sesquiterpene dimer. The ^{1}H-NMR spectrum showed the presence of a pent-3-enyl group (δ(H) 1.56 (*d*, J = 4.4 Hz, Me(15′)); 5.39–5.42 (*m*, H–C(13′), H–C(14′)); 2.04–2.07 (*m*, H–C(12′)); 2.55–2.63 (*m*, CH_2(11′))), an aromatic Me group (δ(H) 2.17 (*s*, Me(10′))), and an aromatic H-atom (δ(H) 6.75 (*s*, H–C(4′))). All

[1]) Systematic name. However, in the chemical formulae, arbitrary atom numbering is used throughout, based on the benzofuran sesquiterpene backbone, to facilitate data comparison.



these signals indicated that **1** had some structural characteristics similar to the known compound **8** [5].

The remaining ^{1}H-NMR signals of **1** indicated another structural fragment related to compound **6** [3]. These signals included a Me *doublet* (δ(H) 1.16 (*d*, $J = 6.4$ Hz, Me(15))), a Me group on a furan ring (δ(H) 2.26 (*s*, Me(13))), an aromatic Me group (δ(H) 2.44 (*s*, Me(14))), and a furan H-atom (δ(H) 7.27 (*s*, H–C(12))). The presence of the above-mentioned two fragments was further corroborated by HMBC experiments (*Fig. 1*, *Table 1*).

Upon comparison of the ^{1}H- and ^{13}C-NMR spectra of **1** with those of the known compounds **6** [3] and **8** [5], **1** was predicted to be a 'dimer' arising from them. The signals due to the C=C bond between H–C(8) (δ(H) 7.35; δ(C) 140.63) and C(7) (δ(C) 116.11) in **8** were changed into an oxymethine (δ(H) 5.05; δ(C) 95.31) and a quaternary C-atom (δ(C) 86.50), respectively, in **1**. In addition, the CH_2 group (δ(H) 3.00, 2.66; δ(C) 23.21) in **6** was replaced by a CH (δ(H) 3.11; δ(C) 30.42) in **1**. These observations suggested that **1** was a dimer of **8** and the quinone form of **6**, arising from a classical *Diels–Alder* reaction, as shown in the *Scheme*. This conclusion was supported by an HMBC correlation between H–C(8′) and C(10) (*Table 1*).

The configuration at C(4) in **1** was presumed to be (*S*), by analogy with the known configuration of **6** [6]; and the ring junction between C(7′) and C(8′) was *cis*, as

Table 1. *^{1}H- and ^{13}C-NMR as well as HMBC Data for* **1**. At 400/100 MHz, resp., in (D_6)acetone; δ in ppm, J in Hz.

Position[1])	δ(H)	δ(C) (DEPT)	HMBC
1	3.11 (*d*, *J* = 8.8)	30.42 (*d*)	C(5), C(9), C(10)
2	1.72 (br. *d*, *J* = 10.0), 2.49–2.53 (*m*)	20.64 (*t*)	C(1), C(4), C(10)
3	2.03–2.05 (*m*)	28.34 (*t*)	C(2)
4	3.17 (br. *s*)	29.01 (*d*)	
5		135.79 (*s*)	
6		121.16 (*s*)	
7		126.94 (*s*)	
8		143.51 (*s*)	
9		138.29 (*s*)	
10		118.98 (*s*)	
11		116.78 (*s*)	
12	7.27 (*s*)	141.46 (*d*)	C(7), C(8)
13	2.26 (*s*)	10.52 (*q*)	C(7), C(11), C(12)
14	2.44 (*s*)	13.17 (*q*)	C(5), C(6), C(7)
15	1.16 (*d*, *J* = 6.4)	19.30 (*q*)	C(3), C(4), C(5)
1′		138.42 (*s*)	
2′		125.79 (*s*)	
3′		129.06 (*s*)	
4′	6.75 (*s*)	115.68 (*d*)	C(3′), C(6′), C(7′), C(10′)
5′		127.73 (*s*)	
6′		146.27 (*s*)	
7′		86.50 (*s*)	
8′	5.05 (*s*)	95.31 (*d*)	C(9′), C(10)
9′	1.86 (*s*)	25.34 (*q*)	C(5′), C(7′), C(8′)
10′	2.17 (*s*)	18.86 (*q*)	C(3′), C(4′)
11′	2.55–2.63 (*m*)	26.89 (*t*)	C (1′), C(3′), C(12′)
12′	2.04–2.07 (*m*)	31.87 (*t*)	C(11′), C(13′), C(14′)
13′	5.39–5.42 (*m*)	131.46 (*d*)	C(12′), C(15′)
14′	5.39–5.42 (*m*)	124.59 (*d*)	C(12′), C(15′)
15′	1.56 (*d*, *J* = 4.4)	17.28 (*q*)	C(13′), C(14′)

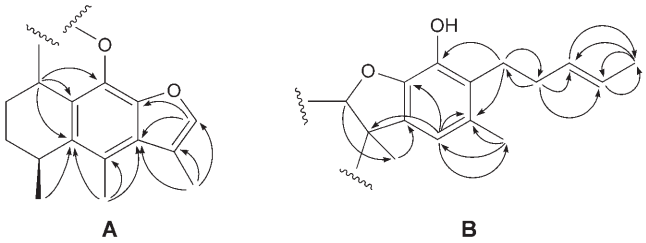
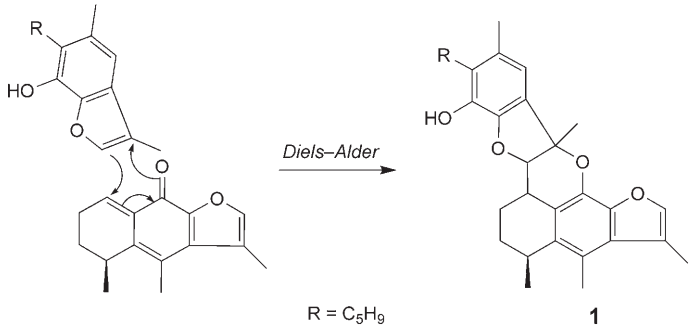


Fig. 1. *Partial structures of* **1** *based on 2D-NMR analysis*

determined on the basis of an NOE difference spectrum, in which the signal for Me(9′) was enhanced by 2.17% upon irradiation of H–C(8′). In addition, the C=C bond between C(13′) and C(14′) was deduced to be (*E*)-configured, as judged from the

Scheme



^{13}C-NMR chemical shift of C(15′) (δ(C) 17.28) and from an absorption band at 967 cm^{-1} in the fingerprint region of the IR spectrum of **1**.

Compound **2** was obtained as a colorless gum, showing a green spot on TLC when sprayed with 5% H_2SO_4 in EtOH, followed by heating on a hot plate. Its IR spectrum showed absorption bands at 3417 (OH), 1708 (C=O), 1656 and 966 ((*E*)-configured C=C), and 1628, 1583, 1544, and 1441 cm^{-1} (aromatic rings). The HR-ESI mass spectrum of **2** showed the quasi-molecular $[M+NH_4]^+$ ion peak at 490.2586 (calc. 490.2588), suggesting the molecular formula $C_{30}H_{32}O_5$, with 15 degrees of unsaturation. Analysis of the ^{1}H- and ^{13}C-NMR data (*Table 2*) established the structure of **2** as 2-{[(5*S*)-5,6,7,8-tetrahydro-9-hydroxy-3,5-dimethylnaphtho[2,3-*b*]furan-4-yl]methyl}-3,5-dimethyl-6-[(3*E*)-pent-3-en-1-yl]-1-benzofuran-4,7-dione[1]).

The EI mass spectrum of **2** exhibited the molecular-ion peak at *m*/*z* 472, and two fragments at *m*/*z* 229 ($C_{15}H_{17}O_2^+$) and 243 ($C_{15}H_{15}O_3^+$), suggesting a dimeric sesquiterpene. This was further confirmed by ^{13}C-NMR (DEPT) analysis (*Table 2*), which indicated the presence of 30 C-atoms, including five Me, six CH_2, and four CH groups, as well as 15 quaternary C-atoms. The ^{1}H-NMR spectrum of **2** showed two Me *doublet*s (δ(H) 1.18 (*d*, *J* = 7.2 Hz, Me(15)); 1.59 (*d*, *J* = 4.4 Hz, Me(15′))), two Me groups on furan rings (δ(H) 2.02 (*s*, Me(9′)); 2.27 (*s*, Me(13))), an aromatic Me group (δ(H) 2.02 (*s*, Me(10′))), a furan H-atom (δ(H) 7.46 (*s*, H–C(12))), two olefinic H-atoms (δ(H) 5.42–5.46 (*m*, H–C(13′), H–C(14′))), and a CH_2 group (δ(H) 4.50, 4.40 (2*d*, *J* = 17.2 Hz each, CH_2(14))) between two aromatic rings.

By comparison of the above signals with those of the known compounds **6** [3] and **7** [4], compound **2** was considered to be a 'dimer' arising from them. In the NMR spectra, H–C(8) (δ(H) 7.39; δ(C) 144.62) of **7** was replaced by a quaternary C-atom (δ(C) 158.00) in **2**, and the Me group on the aromatic ring (δ(H) 2.55; δ(C) 14.07) of **6** was changed into a CH_2 group (δ(H) 4.40, 4.50; δ(C) 26.12) in **2**, which supported the above assumption.

Extensive analysis of the HMBC data of **2** (*Table 2*, *Fig. 2*) led to the substructures **A** and **B**. Substructure **A** (similar as in **6**) was assembled on the basis of the HMBC correlations between H–C(13) and C(7), C(11) and C(12); between H–C(14) and C(5), C(6), and C(7); between H–C(1) and C(5), C(9), and C(10); and between H–C(15) and C(3), C(4), and C(5). Substructure **B** (resembling **7**) was assembled on

Table 2. *^{1}H- and ^{13}C-NMR as well as HMBC Data for* **2**. At 400/100 MHz, resp., in (D_6)acetone; δ in ppm, J in Hz.

Position[1])	δ(H)	δ(C) (DEPT)	HMBC
1	2.60–2.69 (*m*), 2.99 (*dd*, *J* = 17.6, 6.4)	23.44 (*t*)	C(2), C(3), C(5), C(9), C(10)
2	1.87–1.99 (*m*)	17.12 (*t*)	C(4), C(10)
3	1.70–1.79 (*m*)	30.45 (*t*)	C(1), C(2), C(5)
4	3.23 (br. *s*)	29.26 (*d*)	C(2), C(3), C(5), C(6), C(10), C(15)
5		137.48 (*s*)	
6		116.97 (*s*)	
7		127.97 (*s*)	
8		144.05 (*s*)	
9		139.63 (*s*)	
10		120.25 (*s*)	
11		117.23 (*s*)	
12	7.46 (*s*)	142.55 (*d*)	C(7), C(8), C(11)
13	2.27 (*s*)	10.61 (*q*)	C(7), C(11), C(12)
14	4.50 (*d*, *J* = 17.2), 4.40 (*d*, *J* = 17.2)	26.12 (*t*)	C(5), C(6), C(7), C(7′), C(8′)
15	1.18 (*d*, *J* = 7.2)	20.20 (*q*)	C(3), C(4), C(5)
1′		175.33 (*s*)	
2′		143.57 (*s*)	
3′		141.31 (*s*)	
4′		185.35 (*s*)	
5′		128.37 (*s*)	
6′		150.17 (*s*)	
7′		115.97 (*s*)	
8′		158.00 (*s*)	
9′	2.02 (*s*)	8.40 (*q*)	C(5′), C(7′), C(8′)
10′	2.02 (*s*)	12.06 (*q*)	C(2′), C(3′), C(4′)
11′	2.54 (*t*, *J* = 8.0)	26.89 (*t*)	C(1′), C(2′), C(3′), C(12′), C(13′)
12′	2.03–2.08 (*m*)	32.18 (*t*)	C(11′), C(13′), C(14′)
13′	5.42–5.46 (*m*)	130.98 (*d*)	C(12′), C(15′)
14′	5.42–5.46 (*m*)	126.35 (*d*)	C(12′), C(15′)
15′	1.59 (*d*, *J* = 4.4)	17.89 (*q*)	C(13′), C(14′)

the basis of the HMBC correlations between H–C(11′) and C(1′), C(2′), and C(3′); between H–C(10′) and C(2′), C(3′), and C(4′); and between H–C(9′) and C(5′), C(7′), and C(8′). The two moieties **A** and **B** were then connected to **2** based on the key correlations between H–C(14) and both C(7′) and C(8′). Finally, the absolute configuration at C(4) was presumed to be (*S*), in analogy with the known configuration of **6**.

The five known compounds were identified as adenositin B (**3**) [3], adenositin A (**4**) [3], virgaurin A (**5**) [4], cacalol (**6**) [3], 3,5-dimethyl-6-[(3*E*)-pent-3-en-1-yl]-1-benzofuran-4,7-dione (**7**) [5], and 3,5-dimethyl-6-[(3*E*)-pent-3-en-1-yl]-1-benzofuran-7-ol (**8**) [5], one the basis of physico-chemical and spectroscopic methods. Since there were some inconsistencies in the literature data, the ^{13}C-NMR spectroscopic data of **3**–**8** (*Table 3*) were unambiguously re-assigned on the basis of HMBC spectra.

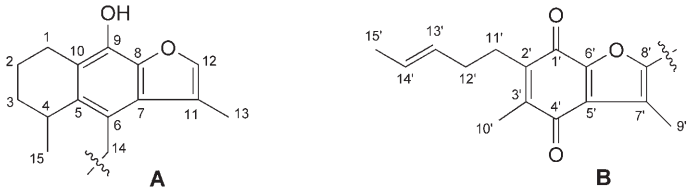


Fig. 2. *Partial structures of* **2** *based on 2D-NMR analysis*

Table 3. *Newly Assigned* ^{13}*C-NMR Data of the Known Compounds* **3**–**8**. At 100 MHz in (D_6)acetone (**3**–**5**) or $CDCl_3$ (**6**–**8**). Assignments were confirmed by HMBC analyses.

Position[a])	**3**	**4**	**5**	**6**	**7**	**8**
1 (1′)	30.46 (22.85)	22.87 (23.40)	138.86	23.21	175.61	138.42
2 (2′)	19.02 (16.29)	16.58 (16.98)	123.44	16.91	143.20	122.73
3 (3′)	28.76 (29.65)	29.85 (30.37)	130.22	30.34	141.31	131.73
4 (4′)	28.81 (28.47)	28.77 (28.99)	123.44	29.20	184.49	111.76
5 (5′)	134.69 (133.90)	136.72 (136.90)	126.92	135.81	126.54	127.56
6 (6′)	124.20 (124.46)	118.29 (118.61)	142.92	119.02	151.35	142.44
7 (7′)	127.07 (125.01)	127.45 (127.80)	117.03	126.37	120.84	116.11
8 (8′)	144.71 (144.71)	141.39 (143.24)	141.25	142.38	144.62	140.63
9 (9′)	135.52 (135.52)	138.54 (135.07)	7.90	136.54	8.55	7.89
10 (10′)	119.11 (121.57)	119.37 (119.37)	15.42	120.45	12.13	19.94
11 (11′)	116.29 (88.19)	116.96 (110.76)	26.92	117.39	26.45	26.57
12 (12′)	141.13 (96.41)	141.62 (151.50)	32.97	141.07	31.60	32.41
13 (13′)	11.26 (26.43)	10.20 (10.77)	131.51	11.58	129.73	131.12
14 (14′)	13.66 (12.50)	25.38 (13.35)	124.72	14.07	126.17	125.31
15 (15′)	20.86 (19.50)	21.20 (21.52)	17.47	21.63	17.81	17.89

[a]) Arbitrary atom numbering used in the literature.

This work was supported by the *National Natural Science Foundation of China* (20621091 QT Program).

Experimental Part

General. Column chromatography (CC): *Sephadex LH-20* (*Pharmacia*) or silica gel (200–300 mesh; *Qingdao Marine Chemical Factory*). Thin-layer chromatography (TLC): silica gel GF_{254} (10–40 μm; *Qingdao Marine Chemical Factory*); detection at 254 nm or by heating after spraying with 5% H_2SO_4 in EtOH. UV Spectra: *Shimadzu UV-260* spectrometer; λ_{max} (log ε) in nm. Optical rotations: *Perkin-Elmer-341* polarimeter. IR Spectra: *Nicolet NEXUS-670* FT-IR spectrometer; in cm^{-1}. NMR Spectra: *Varian Mercury-400BB* spectrometer; δ in ppm rel. to Me_4Si, *J* in Hz. EI-MS: *HP-5988A* GC/MS instrument; in *m/z* (rel. %). HR-ESI-MS: *Bruker APEX-II* mass spectrometer.

Plant Material. The rhizomes of *Ligularia virgaurea* were collected in Lintao County, Gansu Province, P. R. China, in August 2005. The plant was identified by Prof. *Guo-Liang Zhang*, Department of Life Science, Lanzhou University. A voucher specimen (No. 200508LV) was deposited at the Institute of Organic Chemistry, Lanzhou University, P. R. China.

Extraction and Isolation. The dried, milled rhizomes of *L. virgaurea* (2.0 kg) were extracted with petroleum ether (PE)/Et_2O 2 : 1 (3 × 4 l for 7 d each) at r.t. The extract was concentrated to afford a solid

residue (65.0 g), which was purified by CC (SiO_2; PE/acetone 30:1 → 0:1) to afford six crude fractions (*Fr. A–F*). *Fr. A* was subjected to CC (SiO_2; PE/AcOEt 100:1 → 20:1) to give six subfractions (*Fr. A.1–A.6*). *Fr. A.2* was re-subjected to CC (SiO_2; PE/acetone 80:1 → 0:1), which gave **8** (66 mg) after recrystallization from acetone. *Fr. A.3* was submitted to prep. TLC (SiO_2; PE/AcOEt 10:1) to yield **7** (26 mg). *Fr. B* was purified by CC (SiO_2; PE/acetone 80:1 → 1:1) to afford five subfractions (*Fr. B.1–B.5*). *Fr. B.1* was further separated by CC (SiO_2; PE/acetone 50:1 → 0:1) to afford **6** (98 mg) *Fr. B.2* was subjected to CC (*Sephadex LH-20*; $CHCl_3$/MeOH 2:1), followed by prep. TLC (SiO_2; PE/$CHCl_3$ 1:1) to provide **1** (3 mg) and **3** (8 mg). *Fr. D* was subjected to CC (SiO_2; PE/AcOEt 50:1 → 1:1) to give six subfractions (*Fr. D.1–D.6*). *Fr. D.3* was subjected to prep. TLC (SiO_2; PE/$CHCl_3$/AcOEt 60:20:1) to yield **5** (32 mg). *Fr. D.4* was purified by CC (*Sephadex LH-20*; $CHCl_3$/MeOH 2:1) to afford **2** (8 mg). *Fr. D.6* was further separated by CC (SiO_2; PE/acetone 5:1) to yield **4** (15 mg).

*(5*S*)-5,6,7,7a,7b,12b-Hexahydro-3,4,5,11,12b-pentamethyl-10-[(3*E*)-pent-3-en-1-yl]-furo[3″,2″:6′,7′]-naphtho[1′,8′:4,5,6]pyrano[3,2-*b*]benzofuran-9-ol* (**1**). Colorless powder. UV (MeOH): 223.6 (3.9), 255.0 (3.4), 264.4 (3.4). $[\alpha]_D^{20} = -48$ ($c = 0.15$, MeOH). IR (KBr): 3395, 2923, 1698, 1474, 1449, 1343, 1328, 1248, 1229, 1104, 1087, 967. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 481.2350 ($[M+Na]^+$, $C_{30}H_{34}NaO_4^+$; calc. 481.2349).

*2-{[(5*S*)-5,6,7,8-Tetrahydro-9-hydroxy-3,5-dimethylnaphtho[2,3-*b*]furan-4-yl]methyl}-3,5-dimethyl-6-[(3*E*)-pent-3-en-1-yl]-1-benzofuran-4,7-dione* (**2**). Colorless gum. UV (MeOH): 222.0 (4.6), 258.0 (4.3). $[\alpha]_D^{20} = 0$ ($c = 0.2$, MeOH). IR (KBr): 3417, 2930, 1708, 1656, 1628, 1583, 1544, 1441, 966. ^{1}H- and ^{13}C-NMR: see *Table 2*. EI-MS: 472 (32, M^+), 243 (5, $C_{15}H_{15}O_3^+$), 229 (5, $C_{15}H_{17}O_2^+$), 55 (100, $C_4H_7^+$). HR-ESI-MS: 490.2586 ($[M+NH_4]^+$, $C_{30}H_{36}NO_5^+$; calc. 490.2588).

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Received May 8, 2007