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PHENOL DERIVATIVES FROM *LIGULARIA INTERMEDIA*

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Key Word Index—*Ligularia intermedia*; Compositae; roots; phenol derivative; synapyl alcohols; chemotaxonomy.

Abstract—The roots of *Ligularia intermedia* yielded four benzofurans, a novel phenol derivative, 2-isopropenyl-6-acetyl-8-methoxy-1,3-benzodioxin-4-one, and three substituted geranyls, including two new hydroperoxide compounds, (*E*)-4-(6-hydroperoxy-3,7-dimethylocta-2,7-dienyloxy)-syringenin and (*E*,*E*)-4-(7-hydroperoxy-3,7-dimethylocta-2,5-dienyloxy)-syringenin. Structures were elucidated using chemical and spectroscopic methods. © 1997 Elsevier Science Ltd

INTRODUCTION

A recent review shows that *ca* 111 of the 130 species of *Ligularia* occur in China [1]. The roots of many of these have been used in folk medicine for the treatment of influenza, coughs, ulcers and tuberculosis since ancient times [2]. Therefore, there is an important applicable perspective for chemical research on these species. Previous chemical work has focused on the main metabolites, eremophilane derivatives and pyrolizidine alkaloids [3], while other metabolites have been ignored. In continuation of our research on *Ligularia* species, we now wish to report on a chemical and biogenic study on phenol derivative from roots of *L. intermedia* with consideration of prior work [4].

RESULTS AND DISCUSSION

The petrol (60–90°) diethyl ether methanol (1:1:1) extract of air-dried and finely powdered roots was subjected to column chromatography over silica gel and fractionated, then purified by repeated column chromatography and preparative TLC to yield four benzofurans, euparin (**1**) [5], 6-methoxyeuparin (**2**) [6], 2,5-diacetyl-6-methoxy-benzofuran (**3**) [7], 2-acetyl-5,6-dimethoxy-benzofuran (**4**) [8], as well as a novel phenol derivative 2-isopropenyl-6-acetyl-8-methoxy-1,3-benzodioxin-4-one (**5**) from the less polar fraction.

Compound **5** was obtained as colourless needles from acetane. The molecular formula $C_{14}H_{14}O_5$ was established on the basis of the strong $[M]^+$ peak at *m/z* 262 in the EI mass spectrum, together with the support of spectroscopic methods (^{1}H NMR (Table 1), ^{13}C NMR (Table 2) and DEPT). The IR showed the presence of an α,β-unsaturated ester carbonyl (1681 cm^{-1}) and a ketone carbonyl (1740 cm^{-1}). In the ^{1}H NMR spectrum, a pair of coupled protons at δ 5.49 (1H, *d*, *J* = 1.2 Hz) and δ 5.36 (1H, *d*, *J* = 1.2 Hz), and a methyl signal at δ 1.94 (3H, *s*), were due to an isopropenyl group, which corresponded with a tertiary carbon (δ 139.3), a methylene (δ 119.0) and a methyl (δ 16.3). In addition, there was an acetyl at δ_H 2.64 (3H, s), δ_C 196.2 (C=O) and δ_C 26.5 (CH_3) [8], as well as a methoxyl at δ_H 3.99 (3H, *s*) and δ_C 56.8 (CH_3).

Beside these signals, another pair of coupled doublets at lower field at δ 8.10 (1H, *d*, *J* = 1.7 Hz) and δ 7.80 (1H, *d*, *J* = 1.7 Hz) indicated that this compound was a tetra-substituted phenyl with two protons in a *meta*-position. The characteristic retro Diels–Alder (RDA) fragmentation ion at *m/z* 192 ($[M-C_4H_6O]^+$, base peak) showed it was a substituted 1,3-benzodioxin-4-one [9], supported by the IR and ^{13}C NMR, particularly the characteristic methine at δ 103.0 (O—CH—O). Furthermore, a series of important fragments in the EI mass spectrum confirmed the above deduction, *m/z* 177 (58, $[M-C_4H_6O-Me]^+$), 164 (98, $[M-C_4H_6-CO]^+$) and 43 (100, CH_3CO) The significant base peak at *m/z* 192 also suggested that the isopropenyl was connected at C-2 (Scheme 1) [9].

The remaining structural problem was the locations of the acetyl and methoxyl groups. As stated above the two groups should be located on the benzene ring with a *meta*-relationship. However, the special low-field proton at δ 8.10 (H-5) suggested that it should be deshielded by the ester carbonyl and the acetyl carbonyl, while the substituent effects on the ^{13}C

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Table 1. ^{1}H NMR data of compounds **3** and **5** (400 MHz, δ, coupling constants J in Hz)

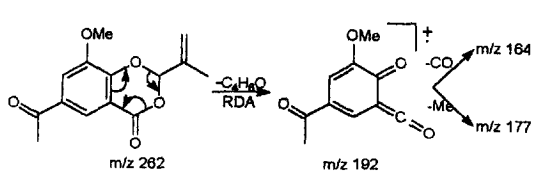
H	$(CD_3)_2CO$	**5** $CDCl_3$	**3** $(CD_3)_2CO$
2	6.27 *s*	5.99 *s*	
3			7.26 *s*
4			8.01 *s*
5	8.10 *d* (1.7)	8.17 *br s*	
7	7.80 *d* (1.7	7.79 *br s*	7.64 *s*
11			2.52 *s*
12	5.49 *d* (1.2), 5.36 *d* (1.2)	5.46 *br s*, 5.34 *br s*	
13	1.94 2s	1.99 *s*	2.56 *s*
15	2.64 *s*	2.65 *s*	
OMe	3.99 *s*	3.97 *s*	4.01 *s*

Table 2. ^{13}C NMR data of compounds **3** and **5** (100 MHz, $(CD_3)_2CO$)

C	**5**	DEPT	**3**	DEPT
2	103.0	CH	159.7	$C^{exchangeable}$
3			94.5	CH
4	161.6	C	125.0	CH
5	122.5	CH	126.8	C
6	133.2	C	158.3	C^e
7	116.7	CH	113.6	CH
8	149.4	C	152.7	C
9	152.4	C	119.9	C
10	115.6	C	186.5	C
11	139.3	C	25.3	CH_3
12	119.0	CH_2	197.7	C
13	16.3	CH_3	30.7	CH_3
14	196.2	C		
15	26.5	CH_3		
OMe	56.8	CH_3	55.4	CH_3

chemical shifts of the benzene gave the locations of the acetyl at C-6 and the methoxyl at C-8 [10]. Thus, the structure of compound **5** was finally established as 2-isopropenyl-6-acetyl-8-methoxy-1,3-benzodioxin-4-one.

Natural occurring benzofurans exhibiting a variety of biological activities, ranging from cytotoxicity to poisoning insects and livestocks. In this work, an important bioactive component was 6-methoxy-euparin (**2**), which exhibited insecticidal activity and feeding deterrency [11], being found in this genus for the first time. Benzofurans are noted for being a taxo-



Scheme 1. Significant fragmentations in the EI mass spectrum of compound **5**.

nomic character at the tribal and genetic level in the Senecioneae [12]. Previous work on *Ligularia* has reported only four chromenes in one species [13], while benzofurans are common. The ratio between chromenes and benzofurans reveals that it is trend to produce benzofurans rather than chromenes in *Ligularia*, which agrees with the findings of Proksch and Rodriguez [12]. In addition, based on biogenic thinking phenol (**5**) is probably an oxidation metabolite of benzofurans, as suggested by Bohlmann [14].

From the medium polar fraction, three compounds were obtained. Compound **6** was reported earlier by Bohlmann as geranyloxy synapyl alcohol [16]. The new compounds **7** and **8** were hydroperoxides. Compound **7** was obtained as a colorless oil. The KI–HOAc–starch reaction showed a blue colour, indicating it was a peroxide [15]. The highest fragment at m/z 378 gave the formula $C_{21}H_{30}O_6$, which was supported by the ^{1}H NMR, ^{13}C NMR and DEPT. The IR spectrum showed the presence of a trisubstituted double bond (3020, 1663 and 846 cm^{-1}), a benzene ring (1582 and 1503 cm^{-1}) and hydroperoxide group (3420 and 1010 cm^{-1}). The intense fragments at m/z 210 $[C_{11}H_{14}O_4]^+$ and m/z 167 $[C_{10}H_{15}O_2]^+$ indicated a sinapyl alcohol-type unit and an oxygenated geranyl-type unit, respectively.

In the ^{1}H NMR, two methoxyl signals at δ 3.85 (*s*, 6H), two aromatic protons at δ 6.59 (*s*, 2H), as well as an allylic alcohol group at δ 6.50 (*d*, 1H, J = 15.7 Hz), δ 6.26 (*dt*, 1H, J = 15.7 and 5.7 Hz) and δ 4.31 (*d*, 2H, J = 5.7 Hz), showed the presence of syringenin ((*E*)-1-(3-hydroxy-1-propenyl-3,3-dimethoxyphenyl, a synapyl alcohol) [16–18], which was further confirmed by ^{13}C NMR [19].

Beside the signals of syringenin, the remaining 10 carbons were attributed to a geranyloxy group (CH=C, C=CH_2, CH-O, CH_2O, 2 × CH_2, 2 × CH_3). For the C-4′ substituent of syringenin, typical signals were observed for O—CH_2—CH=C(Me) and CH_2—CH_2—CH—O spin systems in the ^{1}H NMR. The characteristic resonance of the only methine at δ

Table 3. ^{1}H NMR data of compounds **7** and **8** (400 MHz) δ, coupling constants J in Hz, $CDCl_3$)

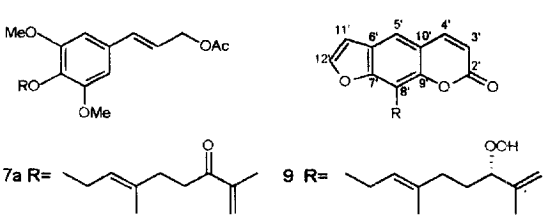
H	**7a**	**7**	**9**[20]	**8**
1	4.52 *d* (7.1)	4.53 *d* (7.1)	5.04 *d* (7.1)	4.53 *d* (7.1)
2	5.55 (*br t* (7.1)	5.58 *br t* (7.1)	5.65 *tq* (7.1, 1.3)	5.58 *br t* (7.1)
4	2.31 *t* (7.8)	2.05 *br t* (7.6)	2.07 *br t* (7.8)	2.75 *d* (6.5)
5	2.78 *t* (7.8)	1.53 *m*, 1.68 *m*	1.53 *m*, 1.65 *m*	5.64 *dt* (16.1, 6.5)
6		4.23 *t* (6.4)	4.24 *t* (6.7)	5.54 *d* (16.1)
8	5.76 *br s*, 5.94 *br s*	4.97 *br s*, 5.00 *br s*	4.95 *br s*, 5.00 *br s*	1.33 *s*
9	1.87 *s*	1.72 *s*	1.72 *s*	1.33 *s*
10	1.67 *s*	1.64 *s*	1.70 *s*	1.63 *s*
-OOH	2.09 *s* (CH_3CO)	7.97 *s*	7.98 *s*	8.55 *s*
2′	6.59 *s*	6.59 *s*		6.59 *s*
6′	6.59 *s*	6.59 *s*		6.59 *s*
7′	6.57 *d* (15.7)	6.50 *d* (15.7)		6.50 *d* (15.7)
8′	6.20 *dt* (15.7, 6.4)	6.26 *dt* (15.7, 5.7)		6.26 *dt* (15.7, 5.7)
9′	4.72 *d* (6.4)	4.31 *d* (5.7)		4.31 *d* (5.7)
MeO × 2	3.85 *s*	3.78 *s*		3.78 *s*

Table 4. ^{13}C NMR data of compounds **7** and **8** (100 MHz, $CDCl_3$)

C	**7a**	DEPT	**7**	DEPT	**9**[20]	DEPT	**8**	DEPT
1	69.2	CH_2	69.2	CH_2	70.3	CH_2	69.3	CH_2
2	120.6	CH	121.0	CH	120.4	CH	121.4	CH
3	140.2	C	140.5	C	142.4	C	139.8	C
4	35.8	CH_2	35.5	CH_2	35.6	CH_2	42.4	CH_2
5	33.7	CH_2	28.7	CH_2	28.8	CH_2	135.3	CH
6	201.3	CH	88.9	CH	89.1	CH	128.9	CH
7	144.4	C	143.8	C	144.1	C	82.0	C
8	122.5	CH_2	114.1	CH_2	114.6	CH_2	24.3	CH_3
9	17.5	CH_3	17.1	CH_3	17.3	CH_3	24.3	CH_3
10	16.5	CH_3	16.1	CH_3	16.7	CH_3	16.4	CH_3
1′	131.8	C	132.4	C	ommited		132.4	C
2′	103.6	CH	103.7	CH	o		103.6	CH
3′	153.6	C	153.7	C	o		153.7	C
4′	131.8	C	132.4	C	o		132.4	C
5′	153.6	C	153.7	C	o		153.7	C
6′	103.6	CH	103.7	CH	o		103.6	CH
7′	124.3	CH	127.9	CH	148.9	C	127.9	CH
8′	134.3	CH	131.2	CH	131.6	C	131.2	CH
9′	64.9	CH_2	63.6	CH_2	143.7	C	63.6	CH_2
MeOx2	56.0	CH_3	56.1	CH_3	o		56.1	CH_3

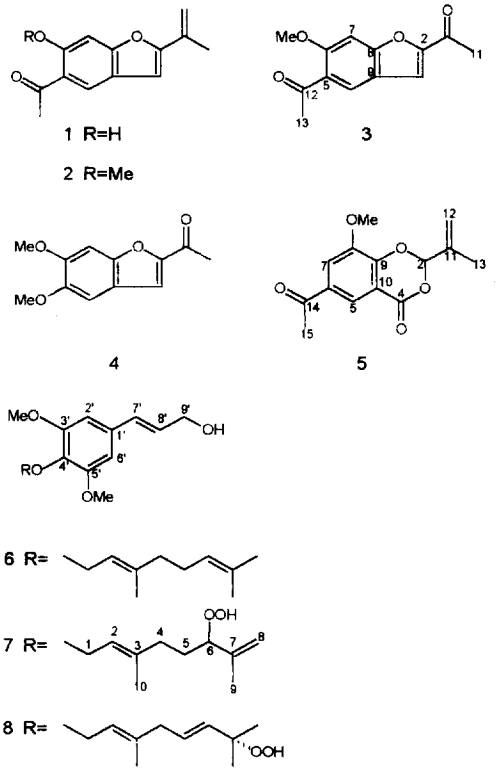
Acetyl of **7a**: δ170.8 (C = O) and δ 20.9 (CH_3).

88.9 showed that the geranyloxy moiety was 6-hydroperoxy-geranyl, which was identical to compound **9** [20] (Tables 3 and 4). The *E*-configuration of the 2,3-double bond could be derived with the aid of the chemical shift of the methyl group at C-3 [22]. Moreover, beside the reaction stated above, the presence of the hydroperoxide group was also indicated by the characteristic lowfield signal at δ 7.97 (*s*) in the high resolution ^{1}H NMR spectrum [21]. The EI mass spectral fragments *m/z* 360 $[M\text{-}H_2O]^+$ and 345 $[M\text{-}OOH]^+$ also supported this assumption. Recently we also obtained this compound from *L. duciformis*. Acetylation of **7** yielded a new derivative **7a**, which was analysed by ^{1}H NMR and ^{13}C NMR. Compared with



7, the marked low-field shift of H-8 confirmed that the hydroperoxide was located at C-6.

Compound **8** was a colorless oil. A positive reaction with the KI–HOAc–starch test showed it was also peroxide. The characteristic signal for —OOH



appeared at δ 8.55 (*s*). The spectra of compound **8** were similar to compound **7**, except for the change from a CH_2—CH_2—CH—O to a CH_2—CH=CH spin system; the 2-isopropenyl signal changed to two equivalent germinal methyl groups at δ 1.33 (*s*, 6H). Compared with **7**, the ^{13}C NMR showed no conjugation effects on the chemical shifts of C-2 and C-3 (Table 4), showing that the double bond is between C-5 and C-6 and that the two germinal methyls were at δ 24.2, indicating the presence of a $\Delta^{5,6}$-7-hydroperoxy-geranyloxy moiety [23]. The large coupling constants of H-5 and H-6 showed a *trans*-double bond.

EXPERIMENTAL

General. Mp are uncorr. IR spectra were recorded in KBr. 400 MHz ^1H NMR and ^{13}C NMR spectra were recorded on Bruker AM 400 FT-NMR with TMS as int. standard. EIMS were obtained at 70 eV (direct inlet). Silica gel (200–300 mesh) for CC and silica GF_{254} for TLC were supplied by the Qingdao Marine Chemical Factory.

Plant material. Roots of *L. intermedia* Nakai were collected in Shenlongjia, Hubei province, Peoples Republic of China, in August, 1994 and identified by Prof. Zi-En Zhao. A voucher specimen is deposited in the Herbarium of our Institute.

Extraction and isolation. Air-dried roots (3 kg) were finely powdered and extracted with petrol–Et_2O–MeOH (1:1:1) (7 days × 3) at room temp. The extract was concd to give a residue (130 g), which was subjected to CC over 1 kg of silica gel and eluted with petrol–Me_2CO (1:0–0:1); eight frs were collected. From the second fr. (petrol–Me_2CO, 20:1), **1** (100 mg) and **2** (20 mg) were obtained by repeated CC on silica gel with petrol–Et_2O (10:1), then purified by prep. TLC with benzene and petrol–EtOAc (15:1). The third fr. (petrol–Me_2CO 10:1) was eluted with petrol–EtOAc (5:1), then repeatedly purified with petrol–Et_2O (3:1) by CC and prep. TLC with petrol–CH_2Cl_2–Et_2O (2:1:1) and petrol–C_6H_6–Et_2O (1:1:1), finally giving **3** (23 mg), **4** (17 mg) and **5** (15 mg). The fifth fr. (petrol–Me_2CO, 3:) eluted with $CHCl_3$–Me_2CO (25:), then by repeated prep. TLC with petrol–Et_2O (1:2) gave **6** (15 mg) and a mixt. of **7** and **8** (25 mg), which was finally separated by $AgNO_3$ prep. TLC (petrol–EtOAc, 1:2).

2,5-Diacetyl-6-methoxy-benzofuran (**3**). Colourless needles, mp 139–140°. IR ν_{max}^{KBr} cm^{-1}: 3110, 3010, 2956, 2926, 2829, 1673, 1621, 1549, 1488, 1455, 1432, 1357, 1316, 1282, 1253, 1223, 1188, 1132, 1005, 971, 914, 876, 806, 773, 638, 542, EIMS *m/z* (rel. int.): 232 $[M]^+$ (68), 217 (100), 202 (20), 187 (8), 159 (13), 88 (8), 75 (7), 57 (10), 43 (60). ^1H NMR and ^{13}C NMR: Tables 1 and 2.

2-Isoprepenyl-6-acetyl-8-methoxy-1,3-benzodioxin-4-one (**5**). Colourless needles, mp 161–162°. $[\alpha]_D^{24}$ −62.6° (Me_2CO, *c* 0.25). IR ν_{max}^{KBr} cm^{-1}: 3089, 3024, 2986, 2847, 1740, 1681, 1612, 1589, 1499, 1451, 1429, 1369, 1305, 1274, 1222, 1181, 1149, 1069, 1041, 954, 884, 781, 594. EIMS *m/z* (rel. int.): 262 $[M]^-$ (30%), 192 (100), 177 (58), 164 (98), 134 (34), 121 (37), 106 (22), 93 (18), 79 (15), 65 (16), 50 (23), 43 (100). ^1H NMR and ^{13}C NMR: Tables 1 and 2.

(E)-4-(6-Hydroperoxy-3,7-dimethylocta-2,7-dienyl-oxy)-syringenin (**7**). Colourless oil. $[\alpha]_D^{24}$ −49.9° ($CHCl_3$, *c* 0.27). IR ν_{max}^{Film} cm^{-1}: 3420(-OOH), 3396(-OH), 3020, 3003, 2939, 2841, 1663 (C = C), 1582, 1508, 1457, 1419, 1241, 1126, 1010, 968, 846. EIMS *m/z* (rel. int.): 378 $[M]^+$ (1), 360 $[M\text{-}H_2O]^+$, 345 $[M\text{-}OOH]^+$, 322, 292, 251, 226, 210 (100), 167, 151, 135, 93, 69, 43. ^1H NMR and ^{13}C NMR: Tables 3 and 4.

Acetylation of **7**. Compound **7** (20 mg) was dissolved in Ac_2O–pyridine (1:1) and left for 24 h at room temp. Prep. TLC (petrol–EtOAc, 6:1) of the product afforded **7a** (10 mg). ^1H NMR and ^{13}C NMR: Tables 3 and 4.

(E,E)-4-(7-Hydroperoxy-3,7-dimethylocta-2,5-dienyloxy)-syringenin (**8**). Colourless oil. IR ν_{max}^{Film} cm^{-1}: 3421 (-OOH), 3398 (-OH), 3020, 3003, 2939, 2841, 1663 (C = C), 1582, 1508, 1457, 1419, 1241, 1126, 1010, 968, 842. EIMS *m/z* (rel. int.): 378 $[M]^-$ (2), 360 $[M\text{-}H_2O]^+$, 345 $[M\text{-}OOH]^+$, 322, 292, 251, 226, 210 (100), 167, 151, 135, 93, 69, 43. ^1H NMR and ^{13}C NMR: Tables 3 and 4.

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