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PHENOLIC NORSESQUITERPENES FROM *LIGULARIA DENTATA*

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Key Word Index—*Ligularia dentata*; compositae phenolic; norsesquiterpenes; ligudentatin A; ligudentatin B.

Abstract—Investigation of *Ligularia dentata* roots gave four known and two new phenolic norsesquiterpenes, ligudentatin A and ligudentatin B, as well as di-aromatic ether, 3-(4′-formylphenoxy)-4-methoxybenzaldehyde, which had been isolated from a natural source for the first time. © 1998 Elsevier Science Ltd. All rights reserved

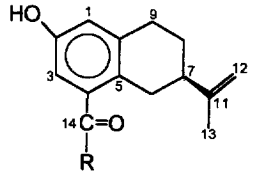
INTRODUCTION

Ligularia dentata Cass. has long been used as a medicinal herb for easing breathing, stimulating blood flow, reducing inflammation, alleviating pain, stopping coughs and getting rid of phlegm [1, 2]. We have now studied the roots of the plant grown in northwest of China.

RESULTS AND DISCUSSION

Seven compounds were isolated by repeated column chromatography and prep TLC from the petrol (60–90°)-diethyl ether—methanol (1:1:1) extract of the roots of *L. dentata*. They were identified as six phenolic norsesquiterpenes: ligudentatin A (**1**), ligudentatin B (**2**), liguhodgsonal (**3**), ligudentatol (**4**), ligujapone (**5**) and 2-hydroxplatyphyllide (**6**), and a di-aromatic ether: 3-(4′-formylphenoxy)-4-methoxybenzaldehyde (**7**). Compounds **1** and **2** are new; compound **7** has been found as a natural product for the first time. The antitumour activities of compounds **1**–**6** have been tested for their ability to inhibit the proliferation of Human Hepatoma (H-7402), Human Myelogenous Leukeamic (K562), and Mouse Melanin Carcinoma (B16). The IC_{50} values showed that liguhodgsonal (**3**) possesses strong antitumour activities.

Compound **1** was assigned the molecular formula of $C_{15}H_{18}O_3$ by El-mass spectroscopy {$[M]^+$ at *m/z* 246 (base peak)}, and elemental analysis (Found: C 73.01, H 7.12; requires: C 73.17, H 7.32%). The IR and UV spectra showed the presence of an ester carbonyl group conjugated with a phenyl ring. The ^1H and ^{13}C NMR spectra (Table 1) contained the signals for a methyl ester group, which was supported by the fragment peaks at *m/z* 231 $[M-Me]^+$, 215



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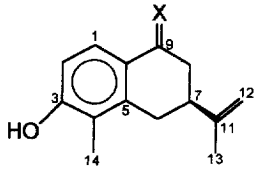
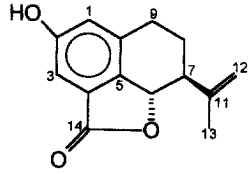
2 R = OCH_2CH_3
3 R = H



4 X = H_2
5 X = O



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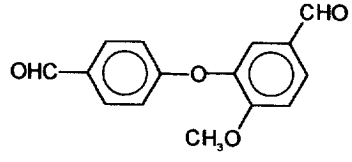

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Table 1. ^{1}H NMR and ^{13}C NMR spectral data for compounds **1** and **2** (400 MHz, $CDCl_3$, TMS, δ ppm)

δ_H (Hz)			δ_C			
H	**1**	**2**	C	**1**	**2**	DEPT
1	6.76 *d*(2.6)	6.78 *d*(2.7)	1	119.3	119.0	CH
3	7.18 *d*(2.6)	7.16 *d*(2.7)	2	152.7	153.1	C
6α	3.17 *dd*(17.3, 4.8)	3.20 *dd*(17.2, 5.0)	3	115.0	114.3	CH
6β	2.78 *dd*(17.3, 11.0)	2.79 *dd*(17.2, 11.0)	4	131.1	130.2	C
7α	2.26 *m*	2.27 *m*	5	130.4	130.1	C
8α	1.92 *m*	1.92 *m*	6	32.6	33.4	CH_2
8β	1.62 *m*	1.61 *m*	7	41.8	41.7	CH
9	2.84 *m*	2.84 *m*	8	27.2	27.3	CH_2
12	4.77 *brs*	4.78 *brs*	9	30.3	30.3	CH_2
13	1.80 *brs*	1.82 *brs*	10	139.4	139.4	C
			11	149.4	149.1	C
			12	109.3	109.5	CH_2
			13	20.7	20.8	CH_3
OH	5.12 *brs*	5.23 *brs*	14	168.2	168.4	C
OMe	3.87 *s*		OMe	51.9		CH_3
OEt		3.96 *q*(7.0)	OEt		62.4	CH_2
		1.30(7.0)			14.8	CH_3

$[M-OMe]^+$ and $[M-COOMe]^+$ in the El-mass spectrum. The remaining NMR signals indicated that the basic skeleton of **1** was that of a phenolic norsesquiterpenoid [3]. Furthermore, several other features confirmed the presence of a phenolic hydroxyl group: (i) a positive colour test with ethanolic ferric chloride; (ii) the mass spectrum of the acetylated product **1a** ($[M]^+ = m/z$ 288). Finally, a comparison of the ^{1}H and ^{13}C NMR spectral data of **1** with those of liguhodgsonal (**3**) [3, 4], showed that they had similar chemical shifts and nearly identical coupling constants, which implied that **1** had a similar structure and the same stereochemistry as **3**. Thus **1** was identified and named as ligudentatin A.

Compound **2** gave a positive colour test with ethanolic ferric chloride which indicated that like **1** it also contained a phenolic hydroxyl group. Its IR spectrum was very similar to that of compound **1**, and indicated that, besides a phenolic hydroxyl group, **2** also contained an ester carbonyl, a phenyl group and a double bond. The ^{1}H NMR and ^{13}C NMR spectra of **2** (Table 1) resembled those of **1** except that the ethyl ester group signal (δ_H 3.96, q, 2H; 1.30, t, 3H. δ_C 62.4, CH_2; 14.8, CH_3) in **2** was replaced by a methyl ester group signal in **1**. The $[M]^+$ peak at m/z 260 in the El-mass spectrum was 14 amu higher than that of **1**, which further confirmed the above proposal. Because the coupling constants of **2** were almost the same as those of **1**, the configuration of **2** was determined to be identical with that of **1**. Thus the structure of compound **2** was identified and named as ligudentatin B.

Compound **3** in its El-mass spectrum exhibited a $[M]^+$ at m/z 216 (base peak). The IR spectrum showed the absorption bands of hydroxy group at 3568 cm^{-1}, an aldehyde group at 2735 and 1680 cm^{-1} (α,β-unsaturated C=O) and a phenyl group and a double bond at 1644, 1594, 1572 and 1448 cm^{-1}. The ^{13}C NMR spectrum contained signals for 14 carbons, among which there was an aldehyde group (δ 192.6) conjugated with aromatic ring. In addition, an aldehyde group proton (δ 10.27, s) was observed in ^{1}H NMR spectrum. A further comparison of the ^{1}H NMR and ^{13}C NMR data of **3** with those of the known compound liguhodgsonal [3] showed that they were completely identical. So **3** was liguhodgsonal.

Compounds **4**, **5** and **6** were identified as ligudentatol, ligujapone and 2-hydroxplatyphyllide, respectively, by comparing their spectral data (MS, IR, ^{1}H NMR and ^{13}C NMR) and physical constants (mp., R_f) with those reported by Naya *et al.* [3].

Apart from one methoxy signal at δ 3.97 (*s*, 3H) and those for two formyl protons at δ 9.86 (*s*, 1H) and 9.83 (*s*, H), the ^{1}H NMR spectrum of compound **7** also contained the signals of three 1, 2, 4-tri-substituted aromatic protons at δ 7.45 (*dd*, 1H, $J = 8.0$ and 2.0 Hz), 7.43 (*d*, 1H, $J = 2.0$ Hz) and 7.06 (*d*, 1H, $J = 8.0$ Hz); and the signals of four 1,4-di-substituted aromatic protons at δ 7.82 (*d*, 2H, $J = 8.4$ Hz) and 7.00 (*d*, 2H, $J = 8.4$ Hz). The IR spectrum also indicated the presence of a formyl group (2745, 1680 cm^{-1}) and an aromatic ring (1584, 1512, 1451 cm^{-1}). A comparison of the ^{1}H NMR data of **7** with those of 3-hydroxy-4-methoxybenzaldehyde and 4-hydroxybenzaldehyde [5] led to the conclusion that **7** was a diaromatic ether resulting from the dehydration of the two above-mentioned hydroxybenzaldehydes and was named as 3-(4′-formylphenoxy)-4-methoxybenzaldehyde. A negative colour test with ethanolic ferric chloride and its ^{13}C NMR data supported the deduction [6]. The proposed structure was further confirmed by the El mass spectrum, which exhibited a $[M]^+$ at m/z 256 and significant fragments at m/z 151 and

121. This compound had been reported as a synthetic product [7, 8] but, as a natural product, it was obtained from *L. dentata* for the first time.

EXPERIMENTAL

General

^{1}H NMR, ^{13}C NMR and 2D-NMR: Bruker AM 400 FT-NMR spectrometer using TMS as int. standard; MS: VG-ZAB-HS spectrometer, 70 eV electron impact ion source (direct inlet); CC: Silica gel (200–300 mesh); TLC: silica GF_{254} (10–40 μ), Spots detected under UV lamp or by heating after spraying with 5% H_2SO_4.

Plant material

The plant material was collected in Zhang County, Gansu Province in 1992, and identified by Prof. R. N. Zhao of Department of Pharmacy, Lanzhou Medical College, 730000, P. R. China. A voucher specimen (No 929503) has been deposited in the herbarium of the College.

Extraction and isolation

The air-dried roots of *L. dentata* (6.0 kg) were powdered and extracted at room temp with petrol (60–90°)-Et_2O-MeOH (1:1:1) (7 days × 3). The resultant extract was concentrated, under reduced pressure, to give a residue (170 g) which was separated by CC over 1400 g silica gel (200–300 mesh) with a gradient of petrol–Me_2CO (50:1–1:1). Four crude fractions were collected. The second fraction was isolated by CC on silica gel (200–300 mesh) with petrol–EtOAc (10:1–1:1) to give crude **3**, **5**, **6** and a mixture of **1**, **2**, **4** and **7**. The crude components were crystallized in petrol–Me_2CO (4:1) to give 30 mg **3**, 38 mg **5** and 24 mg **6**. The mixture was separated by repeated (×3) prep TLC (silica GF_{254}) with $CHCl_3$–MeOH (50:1) to give 12 mg of **1**, 10 mg **2**, 20 mg **4** and crude **7** which was recrystallized from petrol to give 25 mg **7**.

Ligudentatin A (1). Gum, $[\alpha]_D^{14}$ +95.6° ($CHCl_3$; c 0.22). UV λ_{max}^{MeOH} nm (log ε): 307.4 (2.72), 283.6 (2.58), 218.6 (3.51); IR λ_{max}^{Film} (cm^{-1}): 3387, 3050, 2931, 1699, 1648, 1612, 1591, 1462, 1434, 1301, 1215, 1120, 1010, 888, 776; ^{1}H NMR and ^{13}C NMR: Table 1; EIMS *m/z* (rel. int.): 246(100), 231(91), 215(22), 203(48), 186(22), 178(60), 171(70), 163(31), 149(76), 131(15), 115(20), 91(16), 77(14), 65(11), 55(15), 41(21).

Ligudentatin B (2). Gum, $[\alpha]_D^{14}$ +94.8° ($CHCl_3$; c 0.42). IR γ_{max}^{Film} (cm^{-1}): 3386, 3073, 2930, 1698, 1610, 1592, 1462, 1434, 1348, 1250, 1171, 1010, 890; ^{1}H NMR and ^{13}C NMR: Table 1; EIMS *m/z* (rel. int.): 260(100), 231(80), 215(21), 186(18), 178(52), 171(68, 18) 163(30), 149(70), 115(22), 91(19), 77(12), 65(10), 55(15), 41(18).

3-(4′-formylphenoxy)-4-methoxybenzaldehyde (7). Needles, mp. 77–79° (petrol). IR γ_{max}^{Film} (cm^{-1}): 3010, 2967, 2825, 2745, 1680, 1584, 1512, 1451, 1322, 1227, 1214, 1156, 1029, 857; ^{1}H NMR ($CDCl_3$) δ: 7.43 (1H, *d*, J = 2.0 Hz, H-2), 7.06 (1H, *d*, J = 8.0, H-5), 7.45 (1H, *dd*, J = 8.0, 2.0 Hz, H-6), 7.00 (2H, *d*, J = 8.4 Hz, H-2′, H-6′), 7.82 (2H, *d*, J = 8.4 Hz, H-3′, H-5′), 9.86 (1H, *s*, CHO), 9.83 (1H, *s*, CHO), 3.97 (3H, *s*); ^{13}C NMR ($CDCl_3$) δ: 129.8 (C-1), 109.0 (C-2), 151.9 (C-3), 147.2 (C-4), 114.2 (C-5), 127.7 (C-6), 161.9 (C-1′), 116.0 (C-2′, C-6′), 132.5 (C-3′, C-5′), 129.8 (C-6′), 191.1 (CHO); EIMS *m/z* (rel. int.): 256(10), 151(85), 121(100), 105(12), 93(50), 83(23), 77(20), 65(60), 55(32).

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