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Two new compounds from *Ligularia dolichobotrys*

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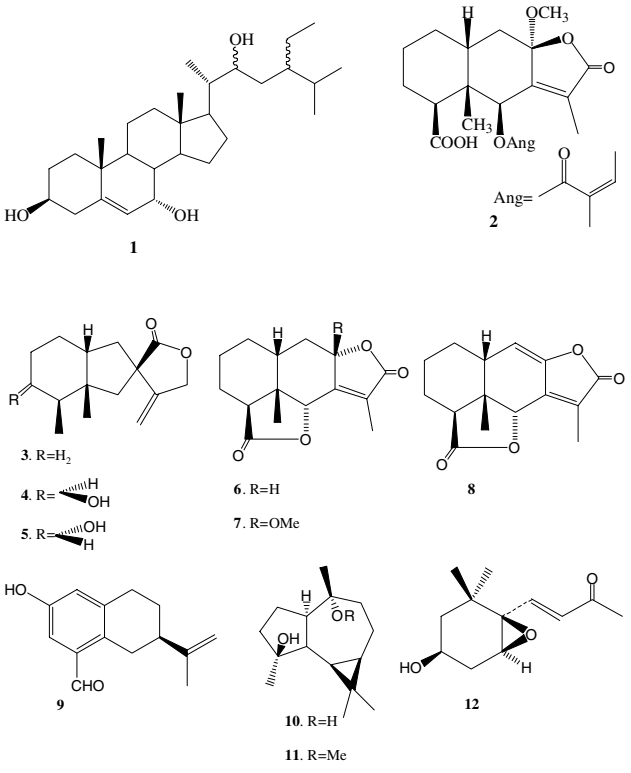
Two new compounds, a stigmasterol (**1**) and an eremophilenolide (**2**), were isolated from *Ligularia dolichobotrys* (Diels) together with ten known sesquiterpenoids, two known triterpenes and five known sterols. Their structures were elucidated by spectroscopic methods (IR, MS, ^1H, ^{13}C and 2D NMR). In addition, bakkenolide A (**3**) exhibited effective antitumor activity to human leukemia cells (HL-60), human hepatoma cells (Bel-7402) and human ovarian neoplasm cells (HO-8910).

1. Introduction

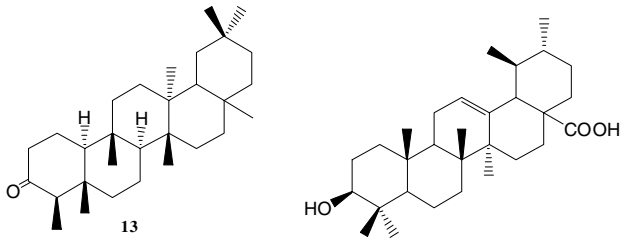
The genus *Ligularia* (Compositae) consists of about 150 species, with 100 distributed in China that mostly grow in the Northwestern, Southwestern and Northeastern areas (Hou 1982). Among them, about 27 species have long been used as traditional Chinese medicinal herbs for the treatment of fever and inflammation along with detoxication, invigorating the circulation of blood and soothing pain (Jiangsu College of New Medicine 1977; Chen et al. 1987). No phytochemical investigation of *L. dolichobotrys* has been reported up to now. In this paper, we describe the isolation and structural elucidation of the chemical constituents from the whole plant of this species and the antitumor activity of one of the compounds.

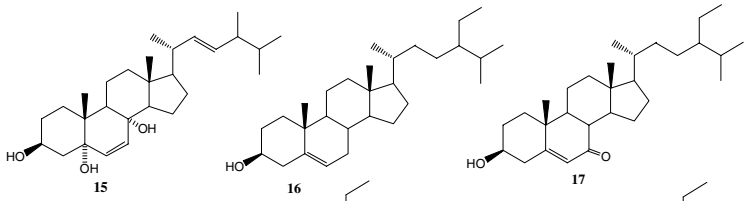
2. Investigations, results and discussion

The air-dried and powered whole plants of *L. dolichobotrys* were extracted three times at room temperature with petroleum ether (60–90 °C)-Et_2O–MeOH (1 : 1 : 1) (each time for 7 days). The residue was chromatographed on silica gel column with petroleum ether (60–90 °C)-EtOAc gradient in developing ratio. The results of the experiment yielded a new stigmasterol: 3β,7α,22-trihydroxy-stigmast-5-ene (**1**) and a new eremophilenolide: 8α-methoxy-6β-angeloyloxy-eremophil-7(11)-en-8β,12-olide-15-oic acid (**2**); ten known sesquiterpenoids: bakkenolide A (**3**) (Paul et al. 1991), 3β-hydroxy-bakkenolide A (**4**) (Fernando et al. 1989) which was reported as a natural product for the first time and its C-3 epimer 3α-hydroxy-bakkenolide A (**5**) (Fernando et al. 1989; Harmatha et al. 1976), eremophil-7(11)-en-6α,15;8α,12-diolide (**6**) (Moriyama et al. 1976), 8β-methoxy-eremophil-7(11)-en-6α,15;8α,12-diolide (**7**) (Zhao et al. 1995), eremophil-8(9), 7(11)-dien-6α,15;8,12-diolide (**8**) (Zhao et al. 1995), liguhodgsonal (**9**) (Bohlmann et al. 1977), aromadendranediol (**10**) (Ammanamanchi et al. 1995), 4β,10α-aromadendranediol-10-methylether (**11**) (Ammanamanchi et al. 1995) and a norsesquiterpene annuionone D (**12**) (Francisco et al. 1999); two known triterpenes friedelin (**13**) (Shashi et al. 1994) and ursolic acid (**14**) (Lee et al. 1993), and five known sterols: 3β,5α,8α-trihydroxy-campest-6,22-diene (**15**) (Gao et al. 1997), β-sitosterol (**16**) (Marina et al. 1990), 7-oxositosterol (**17**) (Marina et al. 1990), daucosterol (**18**) (Kuo et al. 1997) and sitoindoside I (**19**) (Luo et al. 2001). Their structures were determined by spectroscopic methods.



Compound **1** was obtained as colorless crystal from acetone, Its EIMS spectrum gave a molecular ion peak at m/z 446 and fragment ion peaks at m/z 428 $[M-H_2O]^+$, 410 $[M-2H_2O]^+$ and 395 $[M-2H_2O-Me]^+$, corresponding to a





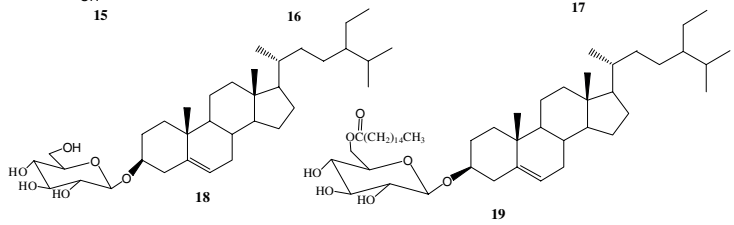


Table 1: ^{1}H NMR, ^{13}C NMR and DEPT data of compound 1

H	δ_H[a]	δ_H[b]	C	δ_C[a]	δ_C[b]	DEPT
			1	37.00	38.47	CH_2
			2	31.35	32.46	CH_2
3	3.59 (m)	3.76 (m)	3	71.30	71.01	CH
4α		2.66 (s)	4	41.99	43.71	CH_2
4β		2.64 (d, j = 4.44 Hz)				
			5	146.34	145.00	C
6	5.61 (d, J = 4.92 Hz)	5.87 (d, J = 5.13 z)	6	123.79	125.42	CH
7	3.86 (m)	4.08 (dd, J = 4.28, 4.35 Hz)	7	65.31	64.79	CH
			8	37.39	37.57	CH
			9	42.27	42.75	CH
			10	37.39	37.77	C
			11	20.69	21.22	CH_2
			12	39.16	39.88	CH_2
			13	42.48	42.75	C
			14	49.08	49.81	CH
			15	24.39	24.90	CH_2
			16	27.49	28.12	CH_2
			17	52.80	53.67	CH
18	0.72 (s)	1.05 (s)	18	11.62	11.99	CH_3
19	1.00 (s)	0.76 (s)	19	18.22	18.48	CH_3
			20	41.38	43.38	CH
21	0.79 (d, J = 6.68 Hz)	1.25 (d, J = 6.83 Hz)	21	12.28	13.09	CH_3
22	3.74 (brd, J=10.3 Hz)	4.03 (brdd, J = 10.2, 2.02 Hz)	22	71.26	70.25	CH
			23	29.87	30.31	CH_2
			24	42.48	41.70	CH
			25	28.73	29.40	CH
26	0.94 (d, J = 6.64 Hz)	0.98 (d, J = 6.80 Hz)	26	20.53	20.78	CH_3
27	0.90 (d, J = 6.64 Hz)	0.87 (d, J = 6.84 Hz)	27	17.53	18.17	CH_3
			28	23.58	23.90	CH_2
29	0.89 (t, J = 7.04 Hz)	0.90 (t, J = 7.39 Hz)	29	11.88	12.17	CH_3

^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz), TMS, δ/ppm
[a] measured in $CDCl_3$, [b] measured in pyridine-d_5

molecular formula $C_{29}H_{50}O_3$, which was supported by HRESIMS at m/z 429.3742 $[M-H_2O+H]^+$ (calcd. 429.3757) and 411.3618 $[M-2H_2O+H]^+$ (calcd. 411.3621). The IR spectrum revealed absorption bonds for −OH at 3400 cm^{-1} and C=C at 1665 cm^{-1}. The ^{1}H NMR, ^{13}C NMR and DEPT spectra of **1** (Table 1) exhibited signals for $6 \times CH_3$, $9 \times CH_2$, $11 \times CH$, $3 \times C$, which indicated that the structure of **1** was similar to a stigmastane skeleton with one double bond and three hydroxyl groups. Compared with the related compound 7α-hydroxysitosterol

Table 2: ^{1}H NMR, ^{13}C NMR and DEPT data of 2

H	δ_H*	C	δ_C*	DEPT
		1	20.9	CH_2
		2	24.5	CH_2
		3	27.8	CH_2
4α	2.46 (dd, J = 12.8, 4.2 Hz)	4	44.6	CH
		5	42.7	C
6	5.90 (q, J = 1.2 Hz)	6	70.3	CH
		7	154.2	C
		8	106.8	C
		9	38.4	CH_2
10β	2.85 (m)	10	36.0	CH
		11	126.3	C
		12	170.9	C
13	1.84 (d, J = 1.2 Hz)	13	8.1	CH_3
14	1.09 (s)	14	16.1	CH_3
		15	178.6	C
OMe	3.29 (s)	OMe	50.5	CH_3

^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz), $CDCl_3$, TMS, δ/ppm
* OAng: δ_H 6.33 ($H_{3'}$, qq, J = 7.2, 1.4 Hz), 2.10 ($H_{4'}$, dq, J = 7.2, 1.3 Hz), 2.01 ($H_{5'}$, dq, J = 1.4, 1.3)
δ_C 166.5 ($C_{1'}$, s), 126.7 ($C_{2'}$, s), 142.2 ($C_{3'}$, d), 20.6 ($C_{4'}$, q), 19.1 ($C_{5'}$, q)

(Marina et al. 1990), the side-chains of both were a little different. Compound **1** had a hydroxyl at C-22 ($\delta_{C\text{-}22}$ 71.26, $\delta_{H\text{-}22}$ 3.74 in $CDCl_3$) which can be confirmed by the cross signals between δ_H 1.25 (H-21) and δ_C 70.25 (C-22), δ_C 43.38 (C-20), δ_C 53.67 (C-17) in the HMBC spectrum (in pyridine-d_5). The configuration of the C-22 can't be determined only by comparing with the spectral data of similar compounds, although the absolute configurations of similar compounds were 22S (Satoshi et al. 1992). Thus compound **1** was deduced as 3β,7α,22-trihydroxy-stigmast-5-ene.

It needs to be said that the NMR spectra of **1** were firstly measured in $CDCl_3$, then in pyridine-d_5 in order to compare the results with literature data (Marina et al. 1990) (in $CDCl_3$) and the literature (Satoshi et al. 1992) (in pyridine-d_5).

Compound **2**, colorless gum, HRESIMS showed $[M + NH_4]^+$ at m/z 410.2164 (calcd. 410.2173), and EI-MS showed a molecular ion peak at m/z 392 in accordance with the molecular formula $C_{21}H_{28}O_7$ and the presence of 21 carbons was confirmed by its ^{13}C NMR and DEPT spectral data (Table 2). Its IR bands (1643, 1701, 1769 cm^{-1}) and UV absorption (225 nm) displayed a typical α,β-unsaturated γ-lactone. In the ^{1}H NMR spectral data, there was an angeloyloxy group and a methoxyl group signals. Except for the −OAng and the $-OCH_3$, the ^{13}C NMR and DEPT spectra showed 15 signal for $2 \times CH_3$ (one of which was tertiary methyl), $4 \times CH_2$, $3 \times CH$ (one of which was oxygenated) and $6 \times C$. Furthermore the signals of C-7 (δ 154.2, s), C-8 (δ 106.8, s), C-11 (δ 126.3, s), C-12 (δ 170.9, s) and C-13 (δ 8.1, q) showed the compound **2** was an eremophilane derivative with an α,β-unsaturated γ-lactone, a COOH-15 group (δ 178.6, s, C-15) (Zhao et al. 1995), a −OAng and a $-OCH_3$. The −OAng should be located at C-6 ($\delta_{C\text{-}6}$ 70.3, d), for $\delta_{C\text{-}6}$ must be about 80 ppm if the $-OCH_3$ was located at C-6 (Li et al. 2002; Mao et al. 2001; Zhang et al. 1998), thus the $-OCH_3$ located at C-8. Stereochemically, Me-14 and Me-15 are biogenetically β-orientations (Moriyama et al. 1976), so COOH-15 group should be in β-orientation. Besides, the presence of a homoallylic spin-coupling (J = 1.2 Hz) between H-6 and H-13 showed that the −OAng at C-6 was in β-orientation and the $-OCH_3$ at C-8 was in α-orientation (Moriyama et al. 1976; Naya et al. 1975). Therefore, the structure of compound **2** was determined as 8α-methoxy-6β-angeloyloxy-eremophil-7(11)-en-8β,12-olide-15-oic acid. Compound **3** exhibited strong activity against human leukemia cells (HL-60), human hepatoma cells (Bel-7402) and human ovarian neoplasm cells (HO-8910) (Table 3).

Table 3: IC_{50} (μg/ml) of compound 3

Compound	HL-60	Bel-7402	HO-8910
Vincristine	9.6 ± 0.98	25.9 ± 3.4	20.7 ± 1.9
3	25.5 ± 2.4	38.1 ± 4.1	56.7 ± 5.1

3. Experimental

3.1. Equipment

All optical rotations were measured on Perkin-Elmer M341 polarimeter. IR spectra were scanned on a Nicolet 170SX FT-IR spectrometer. ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) spectra and 2D NMR spectra were recorded on a Bruker AM 400FT-NMR spectrometer with TMS as internal reference. HRESI-MS and EI-MS were obtained on Bruker Daltonics APEX II 47e and HP-5988 AGCMS spectrometers respectively. Silica gel (200–300 mesh) was used for CC and silica GF_{254} for TLC. Spots were detected on TLC under UV light or by heating after spraying with 5% H_2SO_4 in C_2H_5OH.

3.2. Plant material

The whole plant was collected in August 2000, in Qinling Mountain, Shaanxi Province, People's Republic of China, and was identified by Prof. Y. J. Zhang, Department of Biology, Lanzhou University. A voucher specimen (No. 20000802) was deposited in College of Chemistry and Chemical Engineering, Lanzhou University.

3.3. Extraction and isolation

The air-dried and powered whole plants (1.0 kg) of *L. dolichobotrys* were extracted three times at room temperature with petroleum ether (60–90 °C)-Et_2O−MeOH (1 : 1 : 1) (each time for 7 days). The resultant extract was concentrated under reduced pressure to a residue (52 g), which was chromatographed on silica gel column with petroleum ether (60–90 °C)-EtOAc in developing gradient yielding ten crude fractions (Fr. 1– Fr. 10). The mixture of compounds **3** and **13** were deposited from Fr. 1 (petroleum ether-EtOAc 50 : 1) and after recrystalized in acetone, **3** (100 mg) and **13** (30 mg) were obtained. From Fr. 4 (petroleum ether-EtOAc 15 : 1), crude **16** was deposited and recrystalized in acetone, gave **16** (30 mg); **9** (17 mg) was purified by preparative TLC (petroleum ether-acetone 4 : 1); **15** (4 mg) was obtained by repeated silica gel column chromatography with petroleum ether-EtOAc (8 : 1). Fr. 5 (petroleum ether-EtOAc 10 : 1) was rechromatographed (petroleum ether-EtOAc 15 : 1) on silica gel column to give compounds **2** (5 mg), **7** (46 mg), **8** (38 mg), **11** (3 mg) and **14** (3 mg). Fr. 7 (petroleum ether-EtOAc 5 : 1) was separated by CC on silica gel with petroleum ether-acetone (10 : 1), crude compounds **5**, **6**, **17** were obtained and

then **6** was further purified by preparative TLC (petroleum ether-acetone 3 : 2) to afford **6** (34 mg); crude **5** and **17** were further chromatographed on silica gel column with petroleum ether-acetone (6 : 1) and gave **5** (8 mg) and **17** (6 mg). Fr. 8 (petroleum ether-EtOAc 3 : 1) was separated by CC on silica gel with petroleum ether-acetone (4 : 1) and then by preparative TLC ($CHCl_3$-acetone 5 : 1) to yield **4** (8 mg), **12** (2 mg) and **10** (3 mg). Compound **1** (11 mg) was afforded from Fr. 9 (petroleum ether-EtOAc 2 : 1) by CC on silica gel with $CHCl_3$-acetone (5 : 1) several times. Compound **18** (30 mg) was deposited and recrystalized in MeOH from Fr. 10 (petroleum ether-EtOAc 1 : 1). By CC on silica gel with $CHCl_3$−MeOH (10 : 1) and then preparative TLC ($CHCl_3$−MeOH 3 : 1), compound **19** (20 mg) was also afforded from Fr. 10.

3.4. 3β,7α,22-trihydroxy-stigmast-5-ene (**1**)

Colorless needle crystals (acetone); m.p. 122–123ºC; $[\alpha]_{23,D}$ −54º (c, 1.1, $CHCl_3$); IR (ν^{KBr}, cm^{-1}): 3400, 1665; EIMS m/z (rel int): 446 $[M]^+$ (2.2), 428 $[M-H_2O]^+$ (81), 410 $[M-2H_2O]^+$ (7.9), 300 (47.2), 176 (32.3), 158 (62.7), 105 (55.4), 91 (58.9), 81 (70.4), 69 (91.5), 55 (81.4), 43 (100); ^{1}H NMR, ^{13}C NMR and DEPT data (Table 1).

3.5. 8α-Methoxy-6β-angeloyloxy-eremophil-7(11)-en-8β,12-olide-15-oic acid (**2**)

Colorless needle crystals (acetone); m.p. 250–251 °C; $[\alpha]_D^{23}$ −86º (c, 0.5, $CHCl_3$); IR (ν^{KBr}, cm^{-1}): 3427, 1769, 1701, 1643, 1453, 1382, 1147, 1046; EIMS m/z (rel int): 392 $[M]^+$ (7), 360 (3), 310 (8), 292 (17), 260 (30), 232 (36), 203 (16), 171 (13), 83 (100); ^{1}H NMR, ^{13}C NMR and DEPT data (Table 2).

3.6. Antitumor assays

The antitumor activities of compounds **3**, **6**, **7** and **8** were measured in the Department of Biology of Lanzhou University by the SRB (Sulforhodamine B) method (Skehan et al. 1990). Only compound **3** exhibited strong antitumor activity to human leukemia cells (HL-60), human hepatoma cells (Bel-7402) and human ovarian neoplasm cells (HO-8910) (Table 3).

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