A Novel Benzofuran Derivative, a New Olean-Type Triterpene and Other Constituents from *Ligularia odontomanes*

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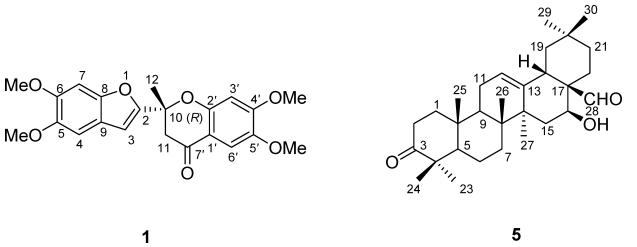
A novel benzofuran derivative (*R*)-(+)-Ligulaodonin A (1) and a new olean-12-ene triterpene (5), as well as 10 known compounds were isolated from the whole plant of *Ligularia odontomanes* Hand.-Mazz. The structures of the new compounds were determined by spectroscopic evidence, including IR, UV, EI-MS, positive HR-ESI-MS, 1D-NMR and 2D-NMR. The absolute configuration of 1 was elucidated on the basis of circular dichroism (CD) data.

Key words *Ligularia odontomanes*; Compositae; benzofuran derivative; triterpene

More than 20 *Ligularia* species are being used in Chinese folk medicines. Their roots, stems, leaves, and flowers are effective anti-inflammation agents, able to reduce phlegm and relieve cough and pain.[1)] As part of our program to systematically assess the chemical and biological diversity of *Ligularia* species in China, we have previously reported several new eremophilane-type sesquiterpenes, bisabolane-type sesquiterpenes and nortriperpenes obtained from some species of the genus *Ligularia*.[2—7)] Continuing our investigation into this genus, we report here the isolation and structural elucidation of a novel benzofuran derivative (*R*)-(+)-Ligulaodonin A (**1**) and a new olean-12-ene triterpene (**5**) along with 10 known compounds from the whole plant of *Ligularia odontomanes* Hand.-Mazz. No report has been found about the constituents of this plant to date. The known compounds were identified as 5,6-dimethoxy-2-isopropenylbenzofuran (**2**),[8)] euparin (**3**),[9)] 2-acetyl-5,6-dimethoxybenzofuran (**4**),[10)] gummosogenin (**6**),[11)] lupeol (**7**),[12)] Liguhodgsonal (**8**),[13)] Ligujapone (**9**),[13)] 1β,6α-dihydroxy-4(14)-eudesmene (**10**),[14)] 2-hydroxy-4,5-dimethoxybenzaldehyde (**11**),[15)] β-sitosterol (**12**)[16)] respectively, by comparison of their spectroscopic data with those previously described in the literature.

Results and Discussion

Compound **1**, $[\alpha]_D^{20}$ +10° (CH_3OH), was obtained as a yellow gum. The molecular formula was determined to be $C_{22}H_{22}O_7$ by positive HR-ESI-MS (*m*/*z* 421.1257, Calcd for $C_{22}H_{22}O_7$+Na 421.1258), indicating 12 degrees of unsaturation. The UV absorption maxima at 246sh, 277, 300 nm indicated the presence of a benzofuran ring.[17)] The ^{1}H-NMR spectrum of **1** showed four methoxyl singlets at δ 3.84, 3.87, 3.89, 3.90 (each 3H, s), one tertiary methyl at δ 1.89 (3H, s), a characteristic AB coupling system attributed to an isolated methylene group at δ 3.26 (1H, d, J=15.5 Hz) and 3.02 (1H, d, J=15.5 Hz) and five aromatic protons at δ 6.91, 6.53, 6.49, 7.01, 7.23 (each 1H, s). Four of five aromatic protons were the *para*-position protons of benzene rings, while a remaining proton had to be assigned to the furan ring. The ^{13}C-NMR and DEPT spectra showed 22 carbon signals including one methyl group, one methylene group, four methoxyl groups, one oxygenated quaternary carbon, one carbonyl, one benzofuran ring and one benzene ring, corresponding to 11 degrees of unsaturation. With seven oxygen atoms, four of them were assigned to four methoxyl groups, one to a benzofuran ring, and one to a carbonyl, therefore, the last one indicated an oxide ring was present, in accord with the remaining degree of unsaturation. The structure of **1** was finally concluded to be as shown in Fig. 1 by the HMBC spectrum, in which the two and three bond heteronuclear correlations from H_3-12 to C-2, C-10 and C-11, from H_2-11 to C-2, C-10 and C-7′, from H-3 to C-2, C-4 and C-9, from H-6′ to C-1′, C-4′, C-5′ and C-7′, and from H-3′ to C-1′, C-2′, C-4′ and C-5′ were observed and the correlations of OCH_3 at δ_H 3.84 with C-5 at δ_C 146.6, OCH_3 at δ_H 3.87 with C-6 at δ_C 148.3, OCH_3 at δ_H 3.89 with C-4′ at δ_C 155.8, OCH_3 at δ_H 3.90 with C-5′ at δ_C 156.3 established the locations of four methoxyl groups at C-5, C-6, C-4′, C-5′ of benzene rings. The absolute configuration of **1** was elucidated by CD excition chirality method.[17,18)] The sign of the first Cotton effect was negative [λ_{max} 244.1 nm ($\Delta\varepsilon$ −25.3)], while that of the second one [λ_{max} 240.5 nm ($\Delta\varepsilon$ +18.5)] was positive, indicating that the chirality between 5,6-dimethoxybenzofuran ring and benzene ring at C-10 should be unclockwise. Thus, the absolute configuration at C-10 was assigned as *R*. Ac-



1 **5**

Fig. 1. Structures of Compounds **1** and **5**

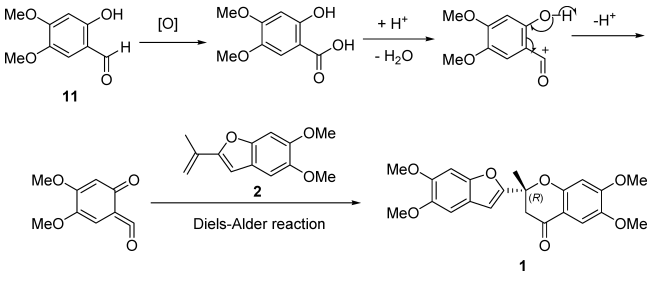


Chart 1. Proposed Biogenesis of Compound **1**

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cordingly, the structure of compound **1** was concluded and named as (*R*)-(+)-Ligulaodonin A. The carbon skeleton of **1** is very rare in nature and its possible biosynthetic pathway is proposed in Chart 1, in which compounds **2** and **11** obtained from the species are perhaps the precursor compounds of **1**.

Compound **5**, $[\alpha]_D^{20}$ +38° ($CHCl_3$), was also obtained as a yellow gum. The molecular formula of **5** was deduced to be $C_{30}H_{46}O_3$ by positive HR-ESI-MS that gave the quasi-molecular ion peak at *m/z* 477.3342 (Calcd for $C_{30}H_{46}O_3$+Na 477.3339) and the degree of unsaturation was 8. The IR spectrum showed the presence of hydroxyl (3398 cm^{-1}), carbonyl (1702 cm^{-1}) and double bond (1678 cm^{-1}) functionalities. In combination with thirty carbon signals in its ^{13}C-NMR spectrum (7×CH_3, 9×CH_2, 6×CH, 8×C) and seven methyl singlets in the highfield in the ^{1}H-NMR spectrum at δ 0.80, 0.90, 0.92, 1.00, 1.00, 1.05 and 1.17 (each 3H, s), this compound should be a pentacyclic triterpene. Furthermore, its ^{13}C-NMR spectrum showed the presence of one ketone carbonyl group at δ 217.6 (C), one aldehyde carbonyl group at δ 209.5 (CH), one trisubstituted double bond group at δ 123.6 (CH) and 141.8 (C) and one methine bearing a hydroxyl at δ 65.5 (CH). These evidence suggested that compound **5** was an olean-12-ene-28-al[11] with a hydroxyl and a ketone carbonyl group. Comparison of the NMR data of **5** with those of compound **6**[11] suggested that the hydroxyl group was connected at C-16 (δ_C 65.5), which was supported by the fragment ion peak at *m/z* 230 $[D/E–H_2O]^+$, 201 $[D/E–H_2O–CHO]^+$ in the EI-MS spectrum resulting from the RDA cleavage of the C-ring, and was confirmed by the HMBC correlations of H-16 with C-28, C-17, C-15 and C-14. The signal at δ 4.16 (1H, dd, J=10.2, 4.2 Hz) in the ^{1}H-NMR spectrum suggested that H-16 was α-axial orientation. In addition, the carbonyl signal at δ_C 217.6 (C) correlated with two methyls (Me-23, Me-24) and a methylene (H_2-2) in the HMBC spectrum, which led to the assignment of C-3 carbonyl. The characteristic RDA fragment peak at *m/z* 206 $[A/B]^+$ in the EI-MS spectrum supported this conclusion. Therefore, compound **5** was assigned as 3-oxo-16β-hydroxy-olean-12-ene-28-al.

Table 1. ^{13}C-NMR Data (75 MHz, $CDCl_3$) for Compound **1**

Position	δ_C	Position	δ_C
2	155.3 s	1′	144.4 s
3	104.9 d	2′	112.7 s
4	102.3 d	3′	100.4 d
5	146.6 s	4′	155.8 s
6	148.3 s	5′	156.3 s
7	95.3 d	6′	106.3 d
8	149.6 s	7′	189.9 s
9	119.3 s	MeO-5	56.1 q
10	78.7 s	MeO-6	56.2 q
11	45.9 t	MeO-4′	56.2 q
12	26.6 q	MeO-5′	56.3 q

Table 2. ^{13}C-NMR Data (75 MHz, $CDCl_3$) for Compound **5**

Position	δ_C	Position	δ_C
1	39.1 t	16	65.5 d
2	32.1 t	17	52.5 s
3	217.6 s	18	43.0 d
4	47.3 s	19	45.2 t
5	55.2 d	20	30.3 s
6	19.4 t	21	34.0 t
7	32.4 t	22	21.7 t
8	39.6 s	23	26.4 q
9	45.9 d	24	21.4 q
10	36.6 s	25	15.0 q
11	23.5 t	26	17.0 q
12	123.6 d	27	26.4 q
13	141.8 s	28	209.5 d
14	43.8 s	29	33.0 q
15	36.6 t	30	21.4 q

Experimental

General Experimental Procedures Optical rotations were measured on a Perkin Elmer 341 polarimeter. IR spectra were taken on a Nicolet NEXUS 670 FT-IR spectrometer. UV spectrum was measured using a Shimadzu UV-260 spectrophotometer. CD spectra were determined on a JASCO J-20C Recording spectropolarimeter. NMR spectra were recorded on a Varian Mercury-300BB NMR spectrometers with TMS as an internal standard. EI-MS data were obtained on an HP5988 GCMS spectrometer. HR-ESI-MS data were measured on a Bruker Daltonics APEX II 47e spectrometer. Silica gel (200—300 mesh) used for CC and silica gel GF_{254} (10—40 μ) used for TLC were supplied by the Qingdao Marine Chemical Factory, Qingdao, P. R. China. Spots were detected on TLC under UV light or by heating after spraying with 5% H_2SO_4 in C_2H_5OH (v/v).

Plant Material The whole plant of *Ligularia odontomanes* was collected from Muli County, Sichuan Province, People's Republic of China, in August 2004. The plant was identified by Prof. Guoliang Zhang, School of Life Sciences, Lanzhou University. A voucher specimen (No. 20040819) was deposited in College of Chemistry and Chemical Engineering, Lanzhou University.

Extraction and Isolation The air-dried whole plant of *L. odontomanes* (1.6 kg) was powdered and extracted with acetone (3×6 l, for 7 d each) at room temperature. The resultant extract (48 g) was subjected to a silica gel column chromatography (200—300 mesh, 400 g) with a gradient of petroleum ether–acetone (30 : 1, 15 : 1, 8 : 1, 4 : 1, 2 : 1, 1 : 1) as eluent. Six fractions were collected according to TLC analysis. From the fraction of petroleum ether–acetone (30 : 1), crude crystal of **2** was obtained, then recrystallized from acetone to give **2** (25 mg). The fraction of petroleum ether–acetone (15 : 1, 2.5 g) was subjected to a silica gel column chromatography (200—300 mesh, 50 g) and eluted with petroleum ether–AcOEt (30 : 1, 15 : 1, 8 : 1, 4 : 1) to give **3** (3 mg) and **7** (5 mg). The fraction of petroleum ether–acetone (8 : 1, 8.8 g) was subjected to a silica gel column chromatography (200—300 mesh, 100 g) and elueted with petroleum ether–AcOEt (7 : 1, 4 : 1, 2 : 1) to give three fractions (A, B, C). Fraction A (7 : 1, 0.9 g) was separated on a silica gel column chromatography (200—300 mesh, 30 g) with petroleum ether–AcOEt (8 : 1, 4 : 1) to give **12** (45 mg), **8** (1 mg), **4** (5 mg) and **5** (36 mg). Fraction B (4 : 1, 1.4 g) was subjected to a silica gel column chromatography (200—300 mesh, 40 g) with petroleum ether–AcOEt (8 : 1, 4 : 1) to give **9** (2 mg), **10** (1 mg) and **6** (8 mg). The fraction of petroleum ether–acetone (4 : 1, 7.0 g) was subjected to a silica gel column chromatography (200—300 mesh, 70 g) with petroleum ether–acetone (3 : 1) to give **11** (3 mg) and **1** (6 mg).

(*R*)-(+)-Ligulaodonin A (**1**): Yellow gum. $[\alpha]_D^{20}$ +10° (c=0.30, CH_3OH). UV λ_{max} (CH_3OH) nm: 246, 277, 300, 331. IR (KBr) cm^{-1}: 2926, 1733, 1653, 1611, 1505, 1457, 1375, 1072. ^{1}H-NMR (300 MHz in $CDCl_3$) δ: 7.23 (1H, s, H-6′), 7.01 (1H, s, H-7), 6.91 (1H, s, H-4), 6.53 (1H, s, H-3), 6.49 (1H, s, H-3′), 3.84 (3H, s, CH_3O-5), 3.87 (3H, s, CH_3O-6), 3.89 (3H, s, CH_3O-4′), 3.90 (3H, s, CH_3O-5′), 3.26 (1H, d, J=15.5 Hz, H-11a), 3.02 (1H, d, J=15.5 Hz, H-11b), 1.89 (3H, s H-12). ^{13}C-NMR (75 MHz in $CDCl_3$): see Table 1. EI-MS *m/z* (%): 398 $[M]^+$ (11), 383 (4), 370 (3), 355 (4), 218 (90), 69 (63), 43 (100). HR-ESI-MS *m/z*: 421.1257 $[M+Na]^+$ (Calcd for $C_{22}H_{22}O_7Na$ 421.1258).

3-Oxo-16β-hydroxy-olean-12-en-28-al (**5**): Yellow gum. $[\alpha]_D^{20}$ +38° (c=2.20, $CHCl_3$). IR (KBr) cm^{-1}: 3398, 2947, 1702, 1678, 1458, 1384, 754. ^{1}H-NMR (300 MHz in $CDCl_3$) δ: 9.47 (1H, s, H-28), 5.38 (1H, t, J=3.2 Hz, H-12), 4.16 (1H, dd, J=10.2, 4.2 Hz, H-16), 2.71 (1H, dd, J=13.5, 3.9 Hz, H-18), 1.17 (3H, s H-27), 1.05 (3H, s, H-23), 1.00 (3H, s, H-24), 1.00 (3H, s, H-25), 0.92 (3H, s, H-30), 0.90 (3H, s, H-29), 0.80 (3H, s, H-26). ^{13}C-NMR (75 MHz in $CDCl_3$): see Table 2. EI-MS *m/z* (%): 436 $[M-H_2O]^+$ (14), 407 (10), 246 (3), 230 (9), 218 (14), 206 (3), 205 (8), 201 (98), 84 (88), 55 (100). HR-ESI-MS *m/z*: 477.3342 $[M+Na]^+$ (Calcd for $C_{30}H_{46}O_3Na$ 477.3339).

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