Natural Product Research: Formerly Natural Product Letters

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gnpl20

Two new norsesquiterpenes from Ligularia Lapathifolia

Yun-Sen Li [a] , Zheng-Tao Wang [a] , Mian Zhang [a] , Ji-Jun Chen [b] & Shi-De Luo [b]

[a] Department of Pharmacognosy, China Pharmaceutical University, Nangjing 210038, P. R. China

[b] Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, P. R. China

Version of record first published: 15 Aug 2006

To cite this article: Yun-Sen Li, Zheng-Tao Wang, Mian Zhang, Ji-Jun Chen & Shi-De Luo (2004): Two new norsesquiterpenes from Ligularia Lapathifolia , Natural Product Research: Formerly Natural Product Letters, 18:2, 99-104

To link to this article: http://dx.doi.org/10.1080/1057563031000122149

Natural Product Research, Vol. 18, No. 2, April 2004, pp. 99–104

TWO NEW NORSESQUITERPENES FROM *LIGULARIA LAPATHIFOLIA*

YUN-SEN LI[a,*], ZHENG-TAO WANG[a], MIAN ZHANG[a], JI-JUN CHEN[b] and SHI-DE LUO[b]

[a]*Department of Pharmacognosy, China Pharmaceutical University, Nangjing 210038, P. R. China;* [b]*Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, P. R. China*

(Received 20 December 2002; In final form 4 April 2003)

Two new norsesquiterpenes were isolated from the extracts of the roots and rhizomes of *Ligularia lapathifolia* (Franch.) Hand.-Mazz. Their structures were identified as 2-acetyl-3a-methyl-5-(2-methyl-but-2-enoyloxy)-3a,4,5,6,7,7a-hexahydro-1H-indene-4-carboxylic acid (**1**) and 2-acetyl-8a-methyl-2-(2-methyl-but-2-enoyloxy)-6-oxo-1,2,3,4,4a,5,6,8a-octahydro-naphthalene-1-carboxylic acid (**2**), respectively, on the basis of spectral data and for **1** by single-crystal X-ray analysis.

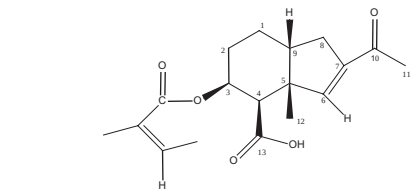
Keywords: *Ligularia lapathifolia*; Compositae; Norsesquiterpenes; X-ray analysis

INTRODUCTION

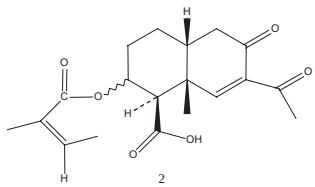
The Genus *Ligularia* belongs to the tribe senecioneae, Family Compositae, and comprises more than 110 species occurring in China, of which about 40 species have been used as Chinese traditional or folk herbs. *Ligularia lapathifolia* (Franch.) H.-M. distributes in southwest China. Its roots and rhizomes are used for treatment of cough and inflammation [1]. The presence of eremophilanes and pyrolizidine alkaloids in many *Ligularia* species is well documented. In continuation of our study on bioactive compounds from the *Ligularia* genus, this article reports the isolation and structural elucidation of 2-acetyl-3a-methyl-5-(2-methyl-but-2-enoyloxy)-3a,4,5,6,7,7a-hexahydro-1H-indene-4-carboxylic acid (**1**) and 2-acetyl-8a-methyl-2- (2-methyl-but-2-enoyloxy)-6-oxo-1,2,3,4,4a,5,6,8a-octahydro-naphthalene-1-carboxylic acid (**2**), two new norsesquiterpenes, from EtOH extracts of the roots and rhizomes of *L. lapathifolia* (Franch.) Hand.-Mazz.

*Corresponding author. E-mail: sinodlsia@yahoo.com

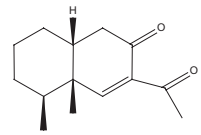
Natural Product Research
ISSN 1478-6419 print: ISSN 1029-2349 online © 2004 Taylor & Francis Ltd
http://www.tandf.co.uk/journals
DOI: 10.1080/1057563031000122149



1



2



2`

RESULTS AND DISCUSSION

Eighteen carbon signals could be observed in the ^{13}C-NMR spectrum of Compound **1**. Except for angeloyloxy signals at δ 5.83 (1H, dq), 1.81 (3H, d, $J = 5.89$ Hz), 1.72 (3H, s) and δ 167.4 (s), 137.8 (d), 128.8 (s), 22.9 (q), 16.0 (q) in the ^{1}H-NMR and ^{13}C-NMR spectra, respectively, of **1** (Tables I and II), only 13 skeletal carbon signals were apparent. The results of HR-MS at m/z 320.1640 allowed for a molecular formula of $C_{18}H_{24}O_5$. A COOH group was present as deduced from its IR absorption band at 3146 cm^{-1}, 1734 cm^{-1} and the ^{13}C-NMR resonance at δ 174.3 (see Table II). Furthermore, the presence of a keto group followed from the IR absorption band at 1646 cm^{-1} and ^{13}C-NMR resonance at δ 197.1 (Table II), while the presence of a double bond could be infered from the observed ^{13}C-NMR resonances at δ 143.1 (s) and δ 154.4 (d) (see Table II). The remaining two unsaturations could only be assigned to two rings. Considering that the ^{1}H-NMR spectrum exhibited only two methyl

TABLE I ^{1}H-NMR data and ^{1}H-^{1}H COSY of compound **1** (500 MHz, J values in Hz in parentheses)

Proton (s)	*Chemical shift* (δ)	*Coupling instant*	*^{1}H-^{1}H COSY*
1	1.54 m		
1′	1.54 m		
2	1.54 m		
2′	1.54 m		
3α	5.29 br.s		
4α	2.60 br.s		
6	6.83 br.s		
8	2.46 br.dd	15.7, 6.8	8H-9H, 8H-8′H, 8H-11H
8′	2.16 m		8′H-8H, 8′H-9H, 8′H-11H
9β	2.06 m		
11	2.16 s		11H-8H, 11H-8′H
12	1.24 s		

OAng: δ 5.83 (1H, dq, =CH–Me), 1.81 (3H, dq, =CH–Me), 1.72 (3H, br.s, MeCOO); Measured in C_5D_5N.

TABLE II ^{13}C-NMR data and HMBC correlations of compound **1** (125 MHz)

Carbon (s)	*Chemical shift* (δ)	*HMBC correlations*
1	25.4 (t)	
2	25.4 (t)	
3	70.4 (d)	
4	51.0 (d)	H-12,
5	49.7 (s)	H-8, H-9, H-12
6	154.4 (d)	H-8,H-9,H-11,H-12
7	143.1 (s)	H-8,H-9,H-11
8	34.5 (t)	H-9
9	45.0 (d)	H-8,H-12
10	197.1 (s)	H-8,H-11
11	26.6 (q)	
12	21.2 (q)	H-9
13	174.3 (s)	

Measured in C_5D_5N; OAng: δ 167.4 (s, COO), 137.8 (d, MeCH=), 128.8 (s, MeCH=), 22.9 (q, MeCH=), 16.0 (q, MeCCOO).

singlets at δ 1.24 and 2.16 (Table I), together with two methyl signals at δ 21.2 and at δ 26.6 (methyl ketone) in the ^{13}C-NMR spectrum of **1**, a 6, 5-fused AB ring system was assumed to be present, since only nine carbons remained to form the ring skeleton. The ^{13}C-NMR resonance of the keto carbonyl group at δ 197.1, the IR absorption band at 1646 cm^{-1}, and the olefinic hydrogen signal appearing as a br s at δ 6.83 (Table I) in the ^{1}H-NMR spectrum, suggested that a keto group was conjugated with the double bond. A pair of low-field methylene ^{1}H-NMR signals vicinal to a ketone group appeared at δ 2.46 (1H, br dd) and δ 2.16 (1H, m), and were assigned to H_2-8, which correlated with a methylene carbon signal at δ 34.5 (t), in a HMQC experiment. By a HMBC experiment, the important HMBC correlations of Compound **1** (see Table II) also eliminated the possibility of a 5,6-fused AB ring system, and supported the idea that **1** possessed an indene skeleton with a 6,7-en-10-oxo-moiety. From a biogenetic point of view, Compound **1**, apparently a noreremophilane derivative, should possess Me-12β and COOH-13β functionalities, since all the eremophilane derivatives isolated from plants in the Compositae have substituents (normally a Me, or a CH_2OH, or a COOH) at C-4 and C-5, both in β-configurations. Comparison of a model and the coupling constants of **1** suggested that the configuration of H-9 should be in the β-orientation. But 3-angeloyloxy could not be confirmed because the signal of correlation of H-3 and H-4 in a ^{1}H-^{1}H COSY experiment was not observed. Moreover, an X-ray experiment further confirmed position of angeloyloxy and was also in agreement with the structure proposed for this compound (Fig. 1).

Compound **2** was isolated as white gum. The molecular formula was determined to be $C_{19}H_{24}O_6$ by HR-EIMS at m/z 348.1608 and NMR data (Tables III and IV). The ^{1}H-NMR (Table III) and ^{13}C-NMR spectra (Table IV) showed signals due to a tertiary methyl group [δ_H 1.24 (3H, s, H-13), δ_C 24.5 (C-13)], an angeloyloxy group, an olefin group [δ_H 7.18 (1H, s, H-6), δ_C 160.1 (C-6), 138.1 (C-7)] and two carbonyl groups [δ_C 192.3 (C-8), δ_C 195.0 (C-11)]. In the ^{1}H-^{1}H COSY spectrum, correlations between H-1 and H-2, H-2 and H-3, H-3 and H-4, H-1 and H-10, and H-10 and H-9 were observed. The HMBC correlations of H-1, H-6, H-9 and H-10 to C-5 supported the structure of A ring. The HMBC correlations between H-9 and C-8, H-6 and C-11,

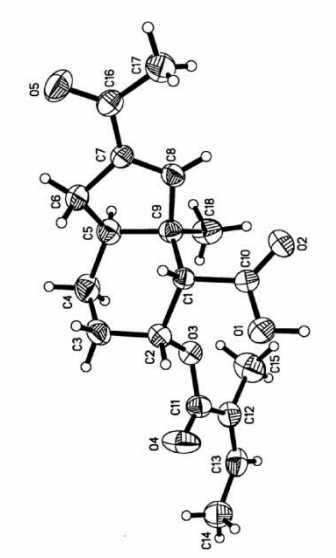


FIGURE 1 ORTEP representation of X-ray structure of compound **1**.

TABLE III ^{1}H-NMR of compound **2** (500MHz, *J* values in Hz in parentheses, measured in $CDCl_3$)

Proton (s)	*Chemical shift*	*Coupling constant*	*The important H-H COSY correlations*
1α	2.24m		H-1β and H-10β
1β	1.40 m		
2α	1.85m		H-2β and H-3
2β	2.08m		
3	4.86m		H-3 and H-2β, H-4
4α	3.12d	4.8	H-4 and H-3
6	7.18s		
9α	2.74m		H-9α and H-9β
9β	2.25m		
10β	2.52m		H-10β and H-1β
12	2.43s		
13	1.24s		
OAng	5.79dq	7.4, 1.4	
	1.96dq	7.4, 1.4	
	1.78br.s		

and H-6 and C-7 determined the structure of B ring, and correlations H-2, H-4, H-13 and H-4′ to C-3 and correlation of H-3 to C-14 established the position of the angeloyloxy group. Furthermore, the HMBC correlations of H-12, H-6 to C-11 and between H-12 and C-7 were observed, suggesting the presence of an acetyl group (H-12, C-11, C-12) at C-7. From a biogenetic point of view, Compound **2**, apparently a noreremophilane derivative, should possess Me-12β and COOH-13β functionalities, since all the eremophilane derivatives isolated from plants in the Compositae have substituents (normally a Me, or a CH_2OH, or a COOH) at C-4 and C-5, both in β-configurations. Comparison of Compound **2** [2] suggested that the configuration of H-9 should be

TABLE IV ^{13}C-NMR of compound **2** (125 MHz, measured in $CDCl_3$)

Carbon	*Chemical shift*	*DEPT*
1	26.4	CH_2
2	25.0	CH_2
3	70.6	CH
4	53.5	CH
5	40.9	C
6	160.1	CH
7	135.9	C
8	192.3	C
9	40.2	CH_2
10	36.0	CH
11	195.0	C
12	30.6	CH_3
13	24.5	CH_3
14	172.0	C
OAng	166.6	C
	138.9	CH
	127.4	C
	20.2	CH_3
	15.6	CH_3

in the β-orientation and was also in agreement with the structure proposed for this compound. Biogenetically, 3-angeloyloxy should be in the β-orientation.

EXPERIMENTAL

General

Column chromatography (CC): silica gel, 200–300 mesh. TLC: Precoated silica GF_{254} plates: detection at 254 nm, and by ceric sulfate reagent. IR spectra were obtained on a Bio-Rad FTS-135 spectrometer with KBr pellets. NMR spectra were recorded on a Bruker AM-400 or DRX-500 instrument with TMS as an internal standard and $CDCl_3$ or C_5D_5N as a solvent. ^{1}H-NMR, ^{1}H-^{1}H COSY spectra were measured at 400.13 or 500.13 MHz; ^{13}C-NMR and DEPT spectra were recorded at 100.6 MHz; HMBC spectrum was obtained at 500.13 MHz/125.8 MHz. ^{13}C-NMR assignments were determined by ^{13}C-^{1}H COSY and HMQC spectra. The EIMS were carried out on a VG Auto Spec-3000 spectrometer at 70 eV.

Plant Materials

The roots and rhizomes of *L. lapathifolia* were collected from Dong Mountain (altitude: 2500 m), Lijiang Prefecture of Yunnan Province, P.R. China, in July 1999, and authenticated by Dr. Main Zhang. A voucher specimen (No. 990005-Li) is deposited in the Herbarium of China Pharmaceutical University.

Isolation of Constituents

Air-dried and powdered roots and rhizomes of *L. lapathifolia* (1.0 kg) were extracted with EtOH (3×3 L, 7 days) at room temperature. After removal of solvent *in vacuo*,

an extract of 90.0 g was afforded. The extract was suspended in H_2O and partitioned with $CHCl_3$ and *n*-BuOH successively. 60.0 g of the evaporated $CHCl_3$ part were subjected to repeated chromatography on a silica gel column (200–300 mesh, 600.0 kg), eluted with $CHCl_3$–$(Me)_2CO$ gradient (50 : 1 to 0 : 1, each 500 mL). Three crude fractions were obtained after combining the eluates by TLC monitoring. The second fraction (6.0 g) was chromatographed over 100 g silica gel column (200–300 mesh) with petroleum ether-EtOAc (10 : 1 to 2 : 1, each: 100 mL) to furnish frs. 2.2 (110 mg) and frs. 2.3 (170 mg). After two times purification by CC over 10 g silica gel (400 mesh) with petroleum-EtOAc (5 : 1), frs. 2.2 and frs. 2.3 afforded **1** (50 mg, R_f : 0.41) and **2** (80 mg, R_f :0.32), respectively.

2-Acetyl-3a-methyl-5-(2-methyl-but-2-enoyloxy)-3a, 4, 5, 6, 7, 7a-hexahydro-1H-indene-4-carboxylic acid 1 White needles. $IR_{\nu max}$ (KBr): 3146, 2934, 2878, 1734, 1646, 1601, 1457, 1381, 1233, 1200, 1160, 1037, 979, 941, 918 cm^{-1}; EIMS *m/z* (%): 320$[M]^+$ (11), 302$[M-H_2O]^+$ (10), 238 (7), 220 (66), 205 (10), 177 (14), 161 (14), 161 (21), 149 (12), 135 (56), 122 (14), 107 (15), 100 (6), 91 (27), 83 (100), 65 (7), 55 (86); ^{1}H-NMR and ^{13}C-NMR (see Tables I and II).

X-ray crystallographic analysis of 1 Empirical formula = $C_{18}H_{24}O_5$; MW = 320.37; Orthorhombic, $P2_1\ 2_1\ 2_1$; $a = 8.9184(6)$ Å, $b = 13.5323(8)$ Å, $c = 14.4969(9)$ Å, $V = 1749.58(19)$ Å^3; $D_{calc} = 1.216$ mg/m^3, $Z = 4$; $T = 293$ K. A colorless crystal was used for data collection on a Bruker SMART CCD area detector, equipped with a graphite monochromated Mo-Kα radiation ($\lambda = 0.71073$ Å). The collected data were reduced using SAINT and empirical absorption correction being performed SADABS. A total of 10, 800 reflections were measured of which 4100 ($R_{int} = 0.1146$) reflections were unique. The structure was solved by a direct method and refined by full-matrix least squares against F^2 for all data using the program package SHELXTL.

2-Acetyl-8a-methyl-2-(2-methyl-but-2-enoyloxy)-6-oxo-1, 2, 3, 4, 4a, 5, 6, 8a-octahydro-naphthalene-1-carboxylic acid 2 White gum. $IR_{\nu max}$ (KBr): 2947, 1711, 1666, 1608, 1451, 1429, 1371, 1353, 1277, 1251, 1142, 990, 914 cm^{-1}. EIMS *m/z* (%): 348$[M]^+$ (43), 249$[M-angeloyloxy]^+$ (31), 234 (13), 221 (23), 204 (29), 189 (26), 177 (18), 163 (35), 151 (29), 135 (31), 121 (29), 111 (31), 100 [angelic acid]$^+$ (28), 91 (42), 83 (84), 69 (40) ; ^{1}H-NMR and ^{13}C-NMR (see Tables III and IV).

Acknowledgement

This research was financially supported by the Natural Science Foundation (NSFC) for outstanding young scientists, for Wang Z.T. (39825129).

References

[1] Z.Y. Wu (1990). *Xin Hua Ben Cao Gan Yao*, Vol. III. Shanghai Science and Technology press, Shanghai.
[2] Y. Goto, Y. Kojima and T. Nakayama (2001). *Phytochemistry*, **57**, 109–113.