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Two New Pyrrolizidines from *Ligularia lankongensis*

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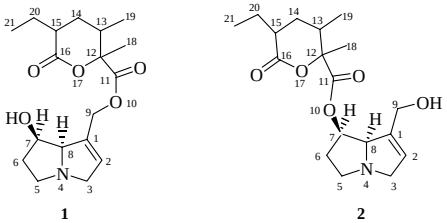
Abstract: Two new pyrrolizidines named lankongensisine A (**1**), B (**2**) were isolated from the roots of *Ligularia lankongensis* collected in Lijiang, Yunnan, and their structures were established by spectroscopic analysis.

Keywords: Pyrrolizidine, *Ligularia lankongensis* , lankongensisine A, lankongensisine B.

Pyrrolizidine alkaloids (PAs) have been found in a large number of plant species occurring throughout the world, specially in Compositae、 Boraginaceae and Leguminosae. Some of PAs are hepatotoxic to animals and human beings, called hepatotoxic pyrrolizidine alkaloids (HPAs). HPAs are esters of unsaturated necines (having a 1, 2 – double bond), which cause irreversible liver damage, and some of them showed a potential carcinogenic and mutagenic activity in some animinal feeding experiments[1]. Because of their high toxicity, the use of medicinal plants containing these alkaloids has been restricted in Germany and Australia, and WHO also published health and safety guide on pyrrolizidine alkaloids in 1989. *Ligularia lankongensis* is a herb distributed in the northwest and northeast of Yunnan, China. Roots and rhizome of this plant have been used as folk medicine for the treatment of antitussive and expectorant. The constituents of *Ligularia lankongensis* has not been studied up to now and this paper describes the isolation and structure elucidation of these two new pyrrolizidines: lankongensisine A (**1**) and lankongensisine B (**2**).

The air-dried and powered root (20 kg) of *L. lankongensis* was extracted with 90%

Figure 1 Chemical structure of two new pyrrolizidine alkaloids



1 **2**

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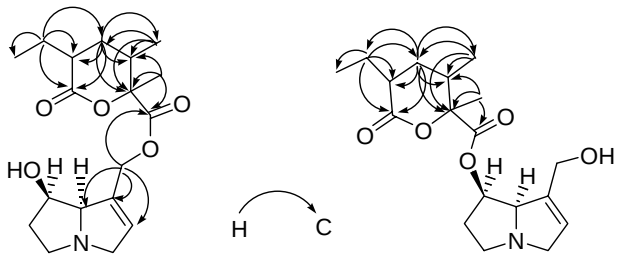
EtOH three times under reflux (each process lasting three hours). After removal of the solvent by evaporation, the residues were extracted with 0.8% H_2SO_4. The acid soluble fraction was defatted with $CHCl_3$, and then the acidic solution was reduced with zinc dust for five hours and filtered. The filtrate was made alkaline with ammonia and extracted with $CHCl_3$. The $CHCl_3$ solution was evaporated to give a crude alkaloidal mixture (15.0 g). The mixture was chromatographed over silica gel column using petroleum ether : acetone : diethylamine solvent system to give two new pyrrolizidine alkaloids: **1** (250 mg) and **2** (12 mg).

Compound **1**, yellow oil, $[\alpha]_D^{23.7}$ +48.44 (*c* 4.80, $CHCl_3$), The molecular formula was determined as $C_{18}H_{27}NO_5$ by HREIMS (at *m/z* 337.1892, calcd.: 337.1889), The EIMS had characteristic peaks at 80, 93, 94, 120, 136, 137, 138, this fragmentation indicated the presence of the unsaturated necine moiety[1], which was ascertained by the ^{1}H NMR sprectrum of **1** (**Table 1**): the three broad signals at 5.78, 4.21,4.07 ppm corresponded to one olefinic proton at C-2, and two methine protons at C-7 and C-8, respectively. The ^{13}C NMR and DEPT sprectra (**Table 1**) of **1** showed eighteen signals, including three methyls, six methylenes, five methines and four quaternary carbon atoms (two carbonyl carbons). The signals [δ_{H-7} 4.21 (br s), δ_{C-7} 70.9 (d); δ_{H-9} 4.79, 4.70 (d, 13.1), δ_{C-9} 62.8 (t)] showed the presence of a C-9 monoester structure which was supported by the characteristic intensities of the MS fragments at *m/z* (%) 136 (67), 137 (89), 138 (100) [2,3,4]. Furthermore, the ^{1}H - ^{13}C long - range correlation (**Figure 2**) between H-9 and C-1, C-2, C-8, C-11 suggested that the ester chain was at C-9, rather than at C-7. The IR spectrum of **1** also showed characteristic signals for a free hydroxyl group (7- OH) at 3353 cm^{-1}.

Figure 2 Selected HMBC correlations of **1** and **2**



1 **2**

The structure elucidation of the ester chain was performed on the basis of HMQC, HMBC and ^{1}H-^{1}H COSY experiments. The long-range correlation were observed between H-18 and C-11, C-12, C-13; H-19 and C-12, C-13, C-14; H-20 and C-14, C-15, C-16, C-21; H-14 and C-12, C-13, C-15, C-16, C-19, C-20, respectively. The quaternary carbon signals C-12 (86.6 ppm) showed that lactone ring connected at C-12. In the NOESY spectrum, correlations between H-7α and H-8α was observed. Based on above analysis, the structure of this compound was identified as **1**, named lankongensisine A.

Compound **2**, yellow oil, $[\alpha]_D^{20}$ +76.67 (*c* 1.20, $CHCl_3$), HREIMS gave the formula as $C_{18}H_{27}NO_5$ (at *m/z* 337.1890, calcd.: 337.1889). The ^{13}C NMR spectrum of **2** were similar to **1** except for C-1, C-2, C-7, C-9. The difference in the ^{13}C NMR spectrum was that: the chemical shifts were downfield shifted from δ_C 132.9 (C-1) and δ_C 70.9 (C-7) of **1** to δ_C 138.6 (C-1) and δ_C 75.8 (C-7) of **2**; the chemical shifts of δ_C 129.4 (C-2) and δ_C 62.8 (C-9) of **1** were upfield shifted to δ_C 124.3 (C-2) and δ_C 59.7 (C-9) of **2**,

respectively. Comparison of the ^{1}H NMR spectrum of **1**, the chemical shift of δ_H 4.21 (H-7) was downfield shifted to δ_H 5.38 (H-7), and δ_H 4.79, 4.70 (H-9) was upfield shifted to δ_H 4.15, 4.04 (H-9). All these changes indicated that the ester chain was at C-7, rather than at C-9. In the NOE spectrum, correlations between H-7α and H-8α was observed. Based on above analysis, the structure of this compound was identified as **2**, named lankongensisine B.

The configuration of the ester chain of these two new pyrrolizidine alkaloids remains to be determined. Further structure elucidation on the stereochemistry pertaining to C-12, C-13 and C-15 is in progress.

Table 1 The ^{1}H and ^{13}C NMR assignments for compounds **1** and **2** (400 MHz)*

No.	**1**		**2**	
	^{13}C	^{1}H	^{13}C	^{1}H
1	132.9 (s)	/	138.6 (s)	/
2	129.4 (d)	5.78 (br s)	124.3 (d)	5.61 (br s)
3	62.7 (t)	3.86 (dd, 15.5, 1.6)	63.0 (t)	3.91 (d, 14.8)
		3.37 (dd, 15.5, 1.8)		3.29 (d, 14.8)
5	53.7 (t)	3.19 (m)	53.4 (t)	3.35 (m)
		2.67 (m)		2.66 (m)
6	36.4 (t)	1.87 (m)	34.7 (t)	2.05 (m)
7	70.9 (d)	4.21 (br s)	75.8 (d)	5.38 (br s)
8	77.8 (d)	4.07 (br s)	76.1 (d)	4.33 (br s)
9	62.8 (t)	4.79 (d, 13.1)	59.7 (t)	4.15 (d,14.0)
		4.70 (d, 13.1)		4.04 (d, 14.0)
11	170.7 (s)	/	170.3 (s)	/
12	86.8 (s)	/	86.7 (s)	/
13	37.6 (d)	2.05 (m)	37.6 (d)	2.01 (m)
14	31.7 (t)	1.79 (m)	31.6 (t)	1.70 (m)
		1.34 (m)		1.36 (m)
15	42.0 (d)	2.40 (m)	42.3 (d)	2.30 (m)
16	173.0 (s)	/	173.0 (s)	/
18	23.6 (q)	1.58 (s)	23.5 (q)	1.48 (s)
19	16.2 (q)	1.02 (d, 6.9)	16.5 (q)	1.02 (d, 6.7)
20	24.1 (t)	1.90 (m)	24.1 (t)	2.03 (m)
		1.56 (m)		1.50 (m)
21	10.8 (q)	0.89 (t,7.4)	11.2 (q)	0.95 (t, 7.6)

*measured in $CDCl_3$, all values are in ppm, coupling constants in Hz.

Acknowledgment

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