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NEW PYRROLIZIDINE ALKALOIDS FROM *LIGULARIA TSANGCHANENSIS*

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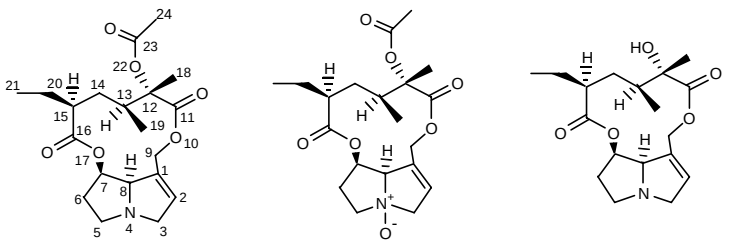
Abstract Two new pyrrolizidine alkaloids named *O*-acetylyamataimine (**1**) and *O*-acetylyamataimine *N*-oxide (**2**) along with a known one (**3**) were isolated from the roots of *Ligularia tsangchanensis*, and their structures were established by spectroscopic analysis.

INTRODUCTION

Ligularia tsangchanensis is distributed in southwest China and used as antitussive and expectorant agent in traditional Chinese medicine, but its chemical constituents have not been studied. This paper described the isolation and structure elucidation of two new pyrrolizidine alkaloids *O*-acetylyamataimine (**1**) and *O*-acetylyamataimine *N*-oxide (**2**), together with the assignment of spectral data of a known pyrrolizidine, yamataimine (**3**).



1 **2** **3**

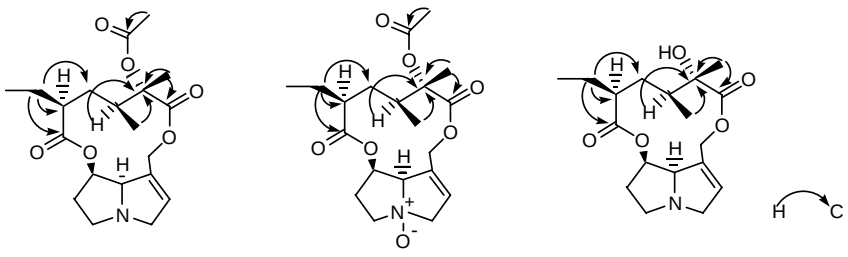
Figure 1 The structure of compounds (**1**-**3**)

RESULTS AND DISCUSSION

Compound (**1**) was determined to have the molecular formula of $C_{20}H_{29}NO_6$ by HREIMS (379.1995, calcd 379.1994). Twenty signals in the ^{13}C NMR (DEPT) spectra were recognized as (5 x C, 5 x CH, 6 x CH_2, 4 x CH_3), of which the signals at 173.8, 171.6 and 169.7 ppm indicated the presence of three ester carbonyl-carbons, the signals at 131.3, 136.0 ppm were assigned to the two olefinic carbons in the necine moiety. In the ^1H NMR spectrum of **1,** three broad singlets at 6.15, 4.94 and 4.17 ppm corresponded to one olefinic proton at C-2, and two methine protons at C-7 and C-8; the signals of geminal methylene protons at C-9 appeared as a pair of doublets at 5.30 and 3.97 ppm (J = 11.9 Hz). H-2 was observed at 6.15 ppm and the two H-9 signals had an appreciable differences of shift ($\delta_{H\text{-}9a}$- $\delta_{H\text{-}9b}$ = 1.23) and the coupling constant (J = 11.9 Hz), indicating that **1** was pyrrolizidine macrocylic diesters.[1, 2] The ^1H and ^{13}C NMR spectra of **1** were similar to yamataimine (**3**).[3, 4] Comparing with those of **3**, the ^{13}C NMR spectra of **1** displayed the presence of an acetoxyl group unit from the signals at δ_C 169.7 (C-23) and δ_C 20.9 (C-24) and the signal of C-12 of **1** shifted downfield to 83.5 ppm, which revealed that the acetoxyl group was present at C-12 in **1**. Furthermore, the presence of acetoxyl group was also supported by its EIMS (M^+ m/z 379). Hence, **1** was determined to be *O*-acetylyamataimine (**Figure 1**). Yamataimine was further converted to **1** by acetylation, so the absolute configuration of the asymmetric carbons at C-7, C-8, C-12, C-13, C-15 of **1** consisted with that of yamataimine (7*R*, 8*R*, 12*S*, 13*R*, 15*S*). The NMR spectral assignments of **1** were thoroughly carried out on the basis of 2D NMR experiments.

Compound (**2**) was determined to have the molecular formula of $C_{20}H_{29}NO_7$ based on HREIMS (395.1952, calcd 395.1994). The IR (KBr) spectrum suggested an *N*-oxide (broad band from 2900 to 3500cm^{-1}). The ^{13}C NMR spectrum of **2** was similar to that of **1** except that the signals at δ_C79.5 (t, C-3), δ_C69.7 (t, C-5), and δ_C 97.3 (d, C-8) were downfield shifted, which indicated **2** was *O*-acetylyamataimine *N*-oxide[5] (**Figure 1**). Subsequently, **2** was converted to **1** by reduction, so the absolute configuration of the asymmetric carbons at C-7, C-8, C-12, C-13, C-15 of **2** consist with that of **1** (7*R*, 8*R*, 12*S*, 13*R*, 15*S*). The NMR spectral assignments of **2** were thoroughly carried out on the basis of 2D NMR spectral experiments.

Compound (**3**) was identified as yamataimine by comparison with reported data,[3, 4] by means of HMBC (**Figure 2**), HMQC and ^1H-^1H COSY, the detail NMR spectral assignments are shown in **Table 1**.



1 **2** **3**

Figure 2 Selected HMBC of compounds (**1-3)**

Table 1 ^{1}H and ^{13}C NMR spectral Data for Compounds (**1-2)** in $CDCl_3$[a]

No.	**1**		**2**	
	^{1}H	^{13}C	^{1}H	^{13}C
1		131.3	/	128.5
2	6.15 (br s)	136.0	6.18 (br s)	130.9
3	3.94 (m)	63.3	4.55 (d, 16.4)	79.5
	3.32 (m)		4.40 (d, 16.4)	
5	3.28 (m)	53.0	3.95 (m)	69.7
	2.43 (m)		3.54 (m)	
6	2.25 (m)	34.9	2.85 (m)	33.0
	2.05 (m)		2.28 (m)	
7	4.94 (br s)	74.5	5.33 (br s)	73.2
8	4.17 (br s)	77.0	4.73(br s)	97.3
9	5.30 (d,11.9)	60.5	5.26 (d,12.2)	60.0
	3.97 (d,11.9)		4.08 (d,12.2)	
11	/	173.8	/	171.6
12	/	83.5	/	83.1
13	1.75 (m)	37.8	1.62 (m)	37.8
14	1.36 (m)	34.1	1.32 (m)	33.8
	1.06 (m)		1.07 (m)	
15	2.35 (m)	47.7	2.35 (m)	47.3
16	/	171.6	/	172.8
18	1.36 (s,3H)	14.5	1.29 (m)	14.4
19	1.06 (d, 6.4, 3H)	13.3	0.95 (d, 6.9, 3H)	13.2
20	1.42 (m, 2H)	26.4	1.40 (m, 2H)	26.2
21	0.86 (t, 6.8, 3H)	11.9	0.81 (t, 7.4, 3H)	11.8
23	/	169.7	/	169.6
24	2.02 (s, 3H)	20.9	1.94 (s, 3H)	20.8

a ^{1}H and ^{13}C NMR spectra were obtained at 400 and 100 MH_Z , respectively, at room temperature. Coupling constants were presented in Hz, unless otherwise indicated, all proton signals integrate to 1H .

EXPERIMENTAL

General Experimental Procedures. Melting points were determined using a Kofler micro-melting point apparatus and are uncorrected. Optical rotations were determined on Horiba SEPA-300 polarimeter. IR spectra were obtained on KBr pellets using a Bio-Rad FTS-135 spectrophotometer. 1D and 2D NMR spectra were recorded on a Bruker AM-400 and Bruker DRX-500 spectrometers, respectively. EIMS and HREIMS measurements were carried out on a VG Auto Spec-3000 spectrometers.

Plant Material. The *Ligularia tsangchanensis* was collected in Lijiang, Yunnan Province, China in July 2000. A voucher specimen has been deposited in the Herbarium of China Pharmaceutical University.

Extraction and Isolation. The air-dried roots of *L. tsangchanensis* (4 kg) were ground and refluxed with 95% EtOH (10 L x 3 in the sequence of 3, 3 and 2 h each time). After removal of the solvent by evaporation, the residue (400 g) was extracted with 0.8% H_2SO_4. The acid soluble fraction (38g) was washed with $CHCl_3$, and then the acidic solution was made alkaline with 25% ammonia and extracted with $CHCl_3$. The $CHCl_3$ solution was evaporated to give a crude alkaloidal mixture (2 g). The crude alkaloid was chromatographed on silica gel column using petroleum ether:acetone:diethylamine (2:2:0.1~2:2:1) to afford *O*-acetylyamataimine (**1**) (50 mg), *O*-acetylyamataimine *N*-oxide (**2**), (300 mg)

and yamataimine (**3**) (200 mg).

O-Acetylyamataimine (**1**), amorphous powder, mp128~129 °C (from Me_2CO), $[\alpha]_D^{20}$ +8.6° (c 10.4, $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 2961, 2934, 1738, 1736, 1681, 1371, 1272, 1254,1210, 1132, 753; EIMS (*m/z*, %): 379 (M^+, 36), 292 (100), 136 (80), 119 (89), 93 (68), 80 (39); HREIMS *m/z* 379.1996 (calcd for $C_{20}H_{29}NO_6$ 379.1995), ^1H and ^{13}C NMR spectrum see **Table 1**.

O-Acetylyamataimine *N*-oxide (**2**), yellow gum, $[\alpha]_D^{20}$ +15° (c 4.4, $CHCl_3$), IR ν_{max}^{KBr} cm^{-1}: 3372, 2961, 2933, 1739, 1737, 1734, 1615, 1270, 1254, 748; EIMS *(m/z*, %): 395 (M^+, 2), 377 (41), 292 (33), 155 (60), 134 (79), 119 (100), 90 (33), 83 (37), 55 (66); FAB$^+$MS *m/z* 396; HREIMS *m/z* 395.1952 (calcd for $C_{20}H_{29}NO_7$ 395.1994), ^1H and ^{13}C NMR see **Table 1**.

Acetylation of yamataimine: 20 mg of yamataimine (**3**) was treated with 6 mL of Ac_2O-pyridine (1:2) at 70 °C for 24 h. The reactive mixture was chromatographed on silica gel column (petroleum ether: acetone: diethylamine 2:2:0.1 ~ 2:2:1) to afford 14 mg of *O*-acetylyamataimine (**1**).

Reduction of **2** to **1**: 100 mg of zinc powder was added to the solution of 10 mg of **2** in 3 mL of 5% H_2SO_4. The resulting mixture was stirred at rt for 10 h. Finally the mixture was chromatographed on silica gel column (petroleum ether: acetone: diethylamine 2:2:0.1 ~ 2:2:1) to afford 5 mg of **1**.

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