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Two Phenanthroindolizidine Alkaloids from *Tylophora atrofolliculata*

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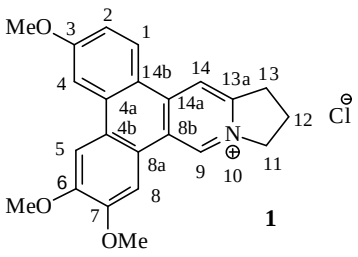
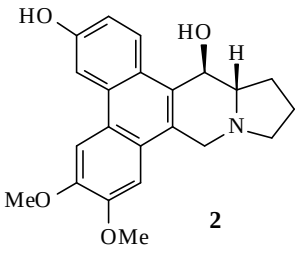
Abstract: Two alkaloids tylophoridicine D (**1**) and tylophoridicine E (**2**) have been isolated from *Tylophora atrofolliculata*. The structures of the compounds were elucidated on the basis of spect -roscopic analysis.

Keywords: *Tylophora atrofolliculata*, tylophoridicine D, tylophoridicine E, phenanthroindolizidine alkaloid.

In order to search for antitumor components of *Tylophora atrofolliculata*, we investigated this species, which was collected in Guangxi province. Two alkaloids tylophoridicine D (**1**) and tylophoridicine E (**2**) were isolated. In this paper, we present the structure elucidation of these alkaloids. Their structures were determined as 3, 6, 7-trimethoxy-9 (10), 13a (14)-dehydrophenanthroindolizidinium chloride and (13a S, 14R)-3, 14-dihydr-oxy-6, 7-dimethoxyphenanthroindolizidine by NMR, IR, UV, CD and MS spectra.



1



2

Tylophoridicine D (**1**), yellow amorphous powder, has a molecular formula of $C_{23}H_{22}NO_3^+Cl^-$, as determined by ^{1}H-NMR, ^{13}C-NMR, DEPT data (see **Table 1**) and $[M]^+$ at *m/z* 360 as well as silver chloride deposition reaction. The UV absorption at 260 (4.63), 286 (4.35) nm were similar to those of phenanthroindolizidine skeleton. The IR spectrum of **1** showed absorption of an $N^{\oplus}$ group at 1211 cm^{-1}. In ^{1}H-NMR spectrum, the signals at δ 4.05 (*s*, 3H), 4.04 (*s*, 3H), 4.03 (*s*, 3H) showed the presence of three methoxyl groups connected to an aromatic ring. The signals of four aromatic

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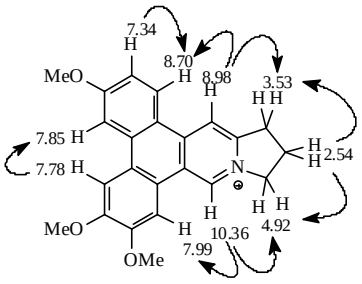
pro-tons at δ 10.36, 8.98, 7.99, 7.78 in the ^{1}H-NMR spectrum appeared as sharp singlets, while the other three signals at δ 8.70 (*d*, 1H, *J* = 9.2 Hz), 7.85 (*d*, 1H, *J* = 2.5 Hz), 7.34 (*dd*, 1H, *J* = 9.2, 2.5 Hz) formed an AMX system. The spectral data of **1** were similar to those of tylophorine[1] except for ring D, which showed to be an aromatic ring in **1**.

The foregoing evidence suggested that **1** possessed a skeleton of phenanthroindolizidine alkaloid with three methoxyls and an aromatic ring D. Positions of the methoxyl groups and the other protons were confirmed by NOESY (see **Figure 1**).

Figure 1 NOESY correlation of compound **1**



Assignment of ^{1}H-NMR and ^{13}C-NMR data was made by the aid of HMQC and HMBC spectra of **1** (see **Table 1**). On the basis of the above evidence, the structure of **1** was concluded to be 3, 6, 7-trimethoxy-9(10), 13a(14)-dehydrophenanthroindolizidini -um chloride. T. R. Govindachari has mentioned this alkaloid, but without spectroscopic identification of the structure [3].

Tylophoridicine E (**2**), brown amorphous solid, $[\alpha]_D^{16}$ +67.2 (*c* 0.55, $CHCl_3$) has a molecular formula $C_{22}H_{23}NO_4$, as determined by ^{1}H-NMR, ^{13}C-NMR data (see **Table 2**) and $[M]^+$ at *m/z* 365. The fragment peak at 296 $[M-69]^+$ was due to the loss of dihydropyrrole by RDA fission, and suggested the presence of tetrahydropyrrole ring. The IR spectrum of **2** showed absorption of hydroxyl group at 3427 cm^{-1}. The UV absorptions at 260 (4.26), 285 (4.10), 310 (sh, 3.88) nm were similar to those of phenanthroindolizidine skeleton. Addition of 1 mol/L NaOH solution, caused a bathchromic shift, typical for the phenolic hydroxyl group. In the ^{1}H-NMR spectrum of **2**, the signals at δ 3.98 (*s*, 3H) and 3.88 (*s*, 3H) showed the presence of two methoxyl groups connected to an aromatic ring, the signals at δ 4.34 (*d*, 1H, *J* = 14.5Hz) and 3.36 (*d*, 1H, *J* = 14.5Hz) were assigned to 9α and 9β protons. Five aromatic proton signals were observed in the ^{1}H-NMR spectrum, δ 7.87 (*s*, 1H), 7.03 (*s*, 1H) were sharp singlets, while the other three signals at δ 8.13 (*d*, 1H, *J* = 9.0Hz), 7.10 (*dd*, 1H, *J* = 9.0, 2.0Hz) and 7.90 (*d*, 1H, *J* = 2.0Hz) formed an AMX system. The spectral data of rings D and E of **2** were similar to those of tylophorinidine[1], which suggested that **2** has the same structure as tylophorinidine in rings D and E. The foregoing evidence suggested that compound **2** possessed a skeleton of phenanthroindolizidine alkaloid with two methoxyls, one phenolic hydroxyl and one aliphatic hydroxyl. The

positions of methoxyl and hydroxyl groups, and the other protons were confirmed by NOESY (see **Figure 2**).

Figure 2 NOESY correlation of compound **2**

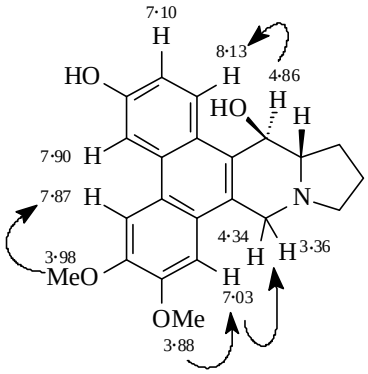


Table 1 The NMR data and the major correlations from the HMBC of tylophoridicine D (DMSO-d_6)

position.	^{1}H-NMR (δ ppm, *J* Hz)	^{13}C-NMR	HMBC (carbon)
1	8.70(1H, *d*, *J*=9.2)	127.95	3, 4a, 14a
2	7.34(1H, *dd*, *J*=9.2, 2.5)	115.87	4, 14b
3		162.29	
4	7.85(1H, *d*, *J*=2.5)	106.49	2, 3, 4b, 14b
4a		134.28	
4b		123.92	
5	7.78(1H, *s*)	105.34	6, 7, 4a, 8a
6		150.31	
7		150.64	
8	7.99(1H, *s*)	104.93	6, 7, 4b, 8a
8a		120.29	
8b		123.29	
9	10.36(1H, *s*)	138.28	8a, 8b, 11, 14a, 13a
11	4.92(2H, *m*)	57.99	9, 12
12	2.54(2H, *m*)	21.79	11, 13
13	3.53(2H, *m*)	31.01	12, 13a
13a		150.89	
14	8.98(1H, *s*)	115.87	8b, 13a, 13, 14b
14a		139.02	
14b		118.18	
MeO	4.05 (3H, *s*)	56.33	
MeO	4.04(3H, *s*)	56.02	
MeO	4.03(3H, *s*)	56.84	

Note: The assignment was based on DEPT, NOESY, HMQC and HMBC experiments. 500 MHz for ^{1}H-NMR, 125 MHz for ^{13}C-NMR, HMBC

The CD spectrum of **2** showed a negative Cotton effect, indicating an *S* absolute configuration for C-13a[2]. The ^{1}H-NMR line of 13a-H was significantly broadened upon addition of a small quantity of DCl, which suggested that the lone pair of nitrogen electrons is on the same side with 13a-H[4]; so the absolute configuration of C-14 is *R* since the dihedral angle between 13a and 14 protons is close to 90°, the H-14 signals appeared as a singlet. Therefore, the structure of **2** was established to be (13aS, 14R)-3, 14-dihydroxy-6, 7-dimethoxy-phenanthroindolizidine. This alkaloid was a new compound.

Table 2 ^{1}H, ^{13}C-NMR data of tylophoridicine E (DMSO-d_6)

position	^{1}H-NMR (δ ppm, *J* Hz)	^{13}C-NMR
1	8.13 (1H, *d*, *J*=9.0)	126.21
2	7.10 (1H, *dd*, *J*=9.0, 2.0)	116.04
3		155.08
4	7.90 (1H, *d*, *J*=2.0)	105.87
5	7.87 (1H, *s*)	103.92
6		149.04
7		148.51
8	7.03 (1H, *s*)	103.79
9	4.34 (1H, *d*, *J*=14.5)	53.34
	3.36 (1H, *d*, *J*=14.5)	
11	2.29 (1H, *m*), 3.25 (1H, *m*)	54.70
12	1.79(2H, *m*)	21.47
13	2.18 (1H, *m*), 1.79 (1H, *m*)	23.64
13a	2.34 (1H, *m*)	64.76
14	4.86 (1H, *s*)	63.41
6-MeO	3.98 (3H, *s*)	55.41
7-MeO	3.88 (3H, *s*)	55.41
C ring		130.47
		129.51
		125.18
		124.13
		123.89
		123.29

Note: The assignment was based upon DEPT, NOESY and ^{1}H-^{13}C COSY experiments. 500 MHz for ^{1}H-NMR, 125 MHz for ^{13}C-NMR.

Acknowledgments

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