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CRYSTAL STRUCTURE AND NMR ANALYSIS OF THE ALKALOID PROTOSTEMOTININE

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Key Word Index—*Stemona sessilifolia*; Stemonaceae; protostemotinine.

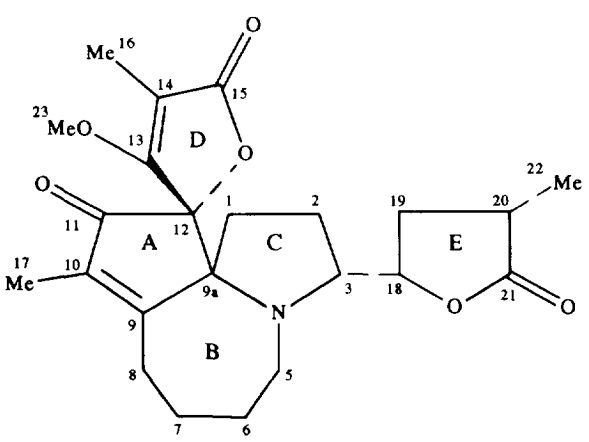
Abstract—A new alkaloid, protostemotinine, was isolated from the roots and rhizomes of *Stemona sessilifolia*. Its structure has been established by IR, MS, NMR and X-ray analysis.

INTRODUCTION

In a preliminary communication [1], Cong and Xu reported a new alkaloid, protostemotinine (**1**), from the roots and rhizomes of *Stemona sessilifolia* (Miq.) Miq. In this paper we present a full report of our chemical investigation, on the basis of which the assignment of the NMR data of **1** were elucidated using 2D NMR techniques, and the relative configuration of **1** was determined by X-ray analysis.

RESULTS AND DISCUSSION

The new compound was assigned the molecular formula $C_{23}H_{29}NO_6$ by EI-mass spectroscopy ($[M]^+$ = *m/z* 415.1993, calcd 415.1995). Its IR spectrum showed absorptions for a γ-lactone (1765 cm^{-1}), an α,β-unsaturated γ-lactone (1745 cm^{-1}) and an α,β-unsaturated five-membered cycloketone (1710 cm^{-1}) [2, 3].



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The ^1H NMR spectrum of **1** showed typical signals for the three methyls, one of them being alkanic (δ1.16, *d*) and two being alkenic (δ1.69, *s* and 1.92, *s*). The signal at δ3.91 was assigned to a methoxy group. The carbon chemical shift values for these four methyls were determined by direct measurement. The one-bond heteronuclear correlation experiment (HETCOR) aided in the assignment. The use of long-range heteronuclear correlation (COLOC), ^{13}C-DEPT, and proton–proton homonuclear correlation (COSY) led to the establishment of A, B, C, D and E rings in **1**.

In the COLOC spectrum a series of signals showed the correlation of groups in A, B and D rings. The C-16 methyl, resonating at δ1.92 (^1H) correlates with a carbonyl carbon (δ174.9, C-15) and two olefinic carbons (δ96.7, C-14; 172.5, C-13), and the C-13 correlates with the methoxy group protons (δ3.91), which show the presence of the α,β-unsaturated γ-lactone (D-ring) bearing the methyl at C-14 and the methoxy group at C-13. The proton signal of Me-17 (δ1.69) correlates with a carbonyl group (δ197.8, C-11), an olefinic carbon (δ136.2, C-10) and a quaternary olefinic carbon (δ173.3, C-9). These three groups, together with two quaternary carbons (C-12 and C-9a), complete the five-membered cyclic ketone (A-ring). The A/D rings formed a spiral structure with a spiro-atom (δ91.5, C-12). The signal of the methine proton at δ3.48 (H-5β) correlating with a methine carbon (δ25.4, C-7) and C-9a (δ79.2), and the signal at δ2.80 (H-8α) correlating with the C-6 methine (δ24.8) and C-9a, indicate a seven-membered heterocyclic ring (B-ring) including a nitrogen atom and C-9. The connective sequence of the four methines was confirmed by ^1H–^1H COSY. H-5β and H-8α are nearer at the side of the C_9–C_{10} double bond, with axial bonds. C-5 and C-9a are located next to the nitrogen. The methyl signal (δ1.16,

Table 1. NMR data for protostemotinine (1)

Carbon	δ (ppm)	Proton	δ (ppm)	J (Hz)
1	35.6	1	1.82	*m*
		1	2.00	*dd*, 11.7, 6.7
2	26.4	2	1.41	*m*
		2	1.82	*m*
		3α	3.30	*ddd*, 5.5, 7.6, 10.6
3	63.3	5β	3.48	*br d*, 16.3
		5α	2.80	*m*
5	47.1	6	1.75	*m*
		6	1.42	*m*
6	24.8	7β	1.93	*m*
7	25.4			
8	28.3	7α	1.30	*m*
9	173.3	8β	2.20	*m*
9a	79.2	8α	2.80	*m*
10	136.2			
11	197.8	16	1.92	*s*
		17	1.69	*s*
12	91.5	18β	3.78	*ddd*, 5.1, 7.7, 10.8
13	172.5	19β	1.43	*m*
14	96.7	19α	2.28	*m*
15	174.9	20β	2.49	*m*
16	8.8	22	1.16	*d*, 7
17	8.3	23	3.91	*s*
18	84.9			
19	34.2			
20	34.6			
21	179.6			
22	14.8			
23	58.7			

d, J = 7 Hz, Me-22) correlating with a methine (δ34.2, C-19), a methylene (δ34.6, C-20) and a carbonyl (δ179.6) in the COLOC spectrum, and the proton signal (δ3.78, H-18) coupling with H-19 (δ2.28, 1.43) in the ^{1}H–^{1}H COSY, show the presence of the γ-lactone (E-ring).

The COSY spectrum indicates that H-18 connects a methylene group (δ3.30, H-3) with the C-ring. Furthermore, the C-ring, composed of C-1, C-2, C-3, C-9a and the nitrogen atom, was elucidated. The substructure of the A, B, C, D ring was assumed as stemonamine [4]. All the NMR data for **1** were assigned as shown in Table 1 according to the methods mentioned above and comparison with the corresponding data of stemoninine [5]. The X-ray analysis confirms the basic structure of **1**, previously deduced from NMR data. The perspective view of the molecule is shown in Fig. 1. The B-ring assumes a twist-chain conformation with C-9a and N, the apex, and C-7, the foot. The A, C, D and E-rings have the envelope conformation. The A/D spiral rings are vertical to each other. The C-ring is under the plane of the B-ring and parallel to the E-ring.

EXPERIMENTAL

General. Mp uncorr. NMR spectral assignments were made on the basis of COSY, DEPT, HETCOR and COLOC spectra. $CDCl_3$ was used as a solvent for NMR. The NMR spectra were recorded at 300 and 75 MHz. The HREI mode was applied for mass spectra.

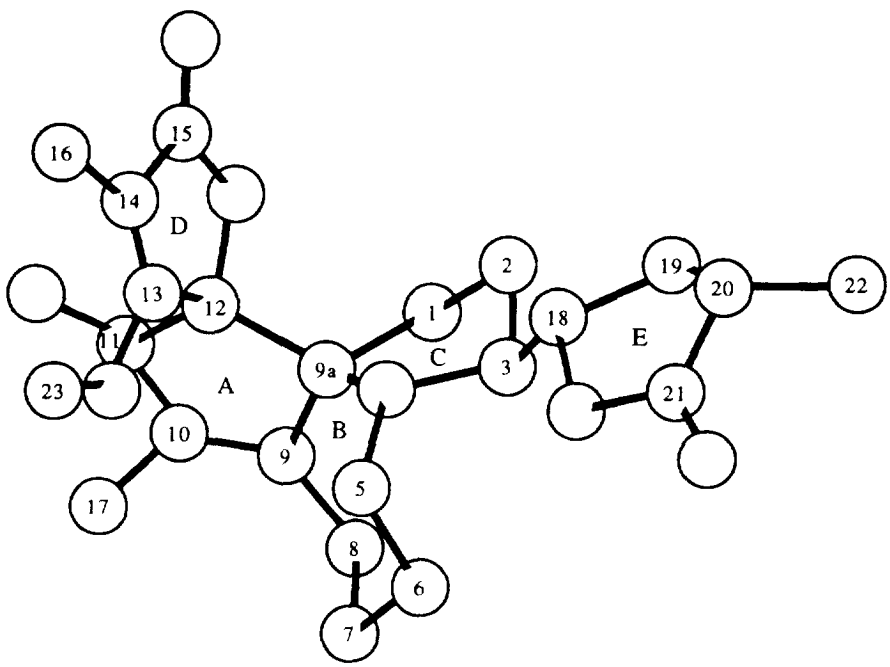

Fig. 1. Perspective view of protostemotinine.

Isolation of protostemotinine. Tuberous roots and rhizomes of *Stemona sessilifolia* (Miq.) Miq. were collected from east China (Anhui) in July 1978. The material was extracted with EtOH by percolation at room temp. The combined extracts were evapd *in vacuo*. The syrupy residue was agitated with H_2SO_4 soln (pH 1), allowed to stand and filtered. The clear filtrate was washed with $CHCl_3$. The aq. phase was adjusted to pH 10 with NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ extract was chromatographed on a silica gel column and the alkaloid eluted with increasing concns of ethyl acetate in petroleum and was crystallized from acetone. Protostemotinine (**1**). Mp 214–246°. NMR data, see Table 1.

X-Ray crystallographic analysis of **1**. $C_{23}H_{29}NO_6$, orthorhombic, space group P, $2_12_12_1$, $a = 9.415(2)$, $b = 13.874(3)$, $c = 16.556(5)$ Å, $V = 2162.74(1.85)$ mm^3 and $Z = 4$. Intensity data were measured on a R3m/E diffractometer with graphite monochromatized CuKα radiation by ω scan technique. The crystal structure was solved using the program SHELSX-86 by the direct method. Final $R = 0.0537$.

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