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Cochinchistemonine, a novel skeleton alkaloid from *Stemona cochinchinensis*

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Abstract—Cochinchistemonine (**1**), an alkaloid with a novel skeleton, was isolated from the roots of *Stemona cochinchinensis* collected from northern Vietnam. Its structure was established on the basis of one- and two-dimensional NMR and other spectroscopic studies. The relative configuration was confirmed by single crystal X-ray diffraction experiment.

The roots of *Stemona* plants have been used as insecticides and antitussive remedies in China, Japan, and Southeast Asian for some 2000 years.[1,2] Previous chemical and pharmacological investigations have revealed that alkaloids might represent the main bioactive constituents in these plants. Over 70 alkaloids have been isolated and structurally identified from the *Stemona* genus.[3] Most of them shared the same pyrrolo[1,2-α]azepine nucleus, while several alkaloids reported from Thai *Stemona* species were characterized with a pyrido[1,2-α]-azepine skeleton.[4]

Stemona cochinchinensis (Stemonaceae), widely distributed in Southeast Asia, was used to manage respiratory diseases, for example, bronchitis, tuberculosis, in local medicine.[4] A former study reported protostemonine- and stemofoline-type alkaloids from this plant.[4] In our systematic investigation on *Stemona* plants, a detailed study on the roots of *S. cochinchinensis* from the Sonla province of northern Vietnam was carried out. An unprecedented skeleton alkaloid, namely cochinchistemonine (**1**) was isolated and identified. The structure was elucidated by 1D- and 2D-NMR analyses together with other spectral studies. The relative configuration of **1** was confirmed by single crystal X-ray diffraction experiment. Herein we present the isolation and structural elucidation of cochinchistemonine (**1**).

The air-dried roots of *S. cochinchinensis* (1.52 kg) were ground into powder and extracted with 95% ethanol. After evaporation of the collected percolate, the crude extract was acidified with dilute HCl (4%) to pH 1–2 and partitioned between CH_2Cl_2 and water. The aqueous part was then basified with aqueous NH_3 to pH 9–10 and extracted with CH_2Cl_2 to afford 35.52 g of crude alkaloids. Crude alkaloids (15 g) were subjected to column chromatography over silica gel and eluted gradiently with petroleum ether–acetone, acetone, and then methanol. Twelve fractions were successively obtained. Fraction 10 was further separated on column chromatography over silica gel and then Sephadex LH-20 gel to yield **1** (21 mg).

Cochinchistemonine (**1**) was obtained as optically active colorless needles.[5] The HREIMS of **1** recording the molecular ion at *m*/*z* 409.2441 established the molecular formula as $C_{22}H_{35}NO_6$. Thus, six degrees of unsaturation were determined for **1**. The strong absorption at 3385 cm^{-1} was observed in the IR spectrum, indicating the presence of hydroxyl groups in the molecule. The IR band at 1740 cm^{-1} indicated the existence of an α,β-unsaturated-γ-lactone moiety, which was supported by quaternary carbon signals at δ_C 174.1, 171.2, and 99.3 in its ^{13}C NMR spectrum, as well as the typical maximum UV absorption at 232 nm.[6] EIMS base peak of **1** at *m*/*z* 350 suggested the presence of a hydroxy-

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propyl side chain ($[M-C_3H_7O]^+$).[4] Its ^1H NMR spectrum displayed three methyls at δ_H 2.01 (3H, s), 1.09 (3H, d, $J = 7.0$ Hz), and 1.08 (3H, t, $J = 7.7$ Hz), a methoxyl at δ_H 4.23 (3H, s) and a proton bearing an oxygen functionality at δ_H 4.67 (1H, d, $J = 10.8$ Hz). In addition to the above mentioned three sp^2 carbon signals, other 19 carbons were observed in the ^{13}C NMR spectrum, including two sp^2 quaternary carbons, six sp^3 tertiary carbons, seven sp^3 secondary carbons, and four sp^3 methyls. According to its ^{13}C NMR spectrum and molecular formula, three active protons were revealed in the molecule. Considering two from the six degrees of unsaturation were ascribed to the carbonyl group and the double bond, the remaining four were assumed for the presence of a tetracyclic system in **1**.

A 12-carbon aliphatic chain could be elucidated starting from the triplet methyl group (C-21) to the doublet methyl group (C-18) by using HSQC and ^1H–^1H COSY spectra (bold lines in Fig. 1a). Additionally a methylene chain could be also figured out (C-6 to C-8). The linkages of two aliphatic chains and the above mentioned α,β-unsaturated-γ-lactone were established by the following key HMBC correlations (arrows in Fig. 1a). The low-field chemical shifts of three carbons [C-4 (δ_H 3.40, t, $J = 9.6$ Hz; δ_C 69.6), C-6 (δ_H: α 3.68, dt, $J = 3.0$, 12.3 Hz, β 3.30, m; δ_C 42.9), and C-10a (δ_H 3.56, dd, $J = 4.7$, 9.1 Hz; δ_C 64.7)] suggested an attached nitrogen functionality (Table 1). An azepine ring was further constructed by HMBC correlations from H-10a to C-6, from H_2-8 to C-10, as well as from H-10a to C-9.[4] In addition, a hydroxypropyl side chain annexed to C-4 can be characterized from HMBC cross-peaks from H-19 to C-3, from H-4 to C-20. Thus the structural moiety of a pyrido[1,2-α]azepine ring with a 1-hydroxypropyl side chain at C-4 was constructed, which was the same as that of stemokerrin.[4] Furthermore, a five-membered ring fused at C-9 and C-10 can be formed by strong interactions from H_2-8 to C-13, from H-11 to C-10a, and C-13 in the HMBC spectrum. The existence of the α-methyl-β-methoxyl unsaturated γ-lactone was further confirmed by HMBC cross-peaks from H_3-17 to C-14 and C-16, and from H_3-22 to

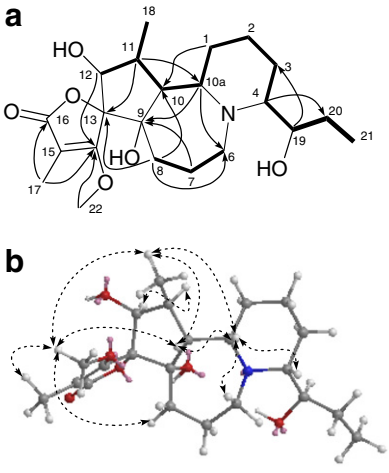


Figure 1. (a) Important ^1H–^1H COSY (bold lines) and HMBC (arrows) correlations of **1**; (b) key NOE correlations (broken arrows) and possible conformation of **1** generated by computer.

Table 1. The ^1H and ^{13}C NMR data of cochinchistemonine (**1**)

No.	δ_H (m, J, Hz)	δ_C (m)
1α	2.08 (m)	20.3 (CH_2)
1β	2.02 (m)	
2α	1.50 (m)	23.5 (CH_2)
2β	1.99 (m)	
3α	1.78 (m)	22.8 (CH_2)
3β	1.50 (m)	
4	3.40 (t, 9.6)	69.6 (CH)
6α	3.68 (dt, 3.0, 12.3)	42.9 (CH_2)
6β	3.30 (m)	
7α	2.26 (m)	20.8 (CH_2)
7β	1.92 (m)	
8α	1.96 (m)	29.8 (CH_2)
8β	1.61 (m)	
9		82.7 (C)
10	3.31 (m)	50.7 (CH)
10a	3.56 (dd, 4.7, 9.1)	64.7 (CH)
11	2.51 (m)	34.2 (CH)
12	4.67 (d, 10.8)	75.1 (CH)
13		96.6 (C)
14		171.2 (C)
15		99.3 (C)
16		174.1 (C)
17	2.01 (s)	8.6 (CH_3)
18	1.09 (d, 7.0)	12.1 (CH_3)
19	3.33 (m)	72.9 (CH)
20α	1.68 (m)	28.6 (CH_2)
20β	1.58 (m)	
21	1.08 (t, 7.7)	10.0 (CH_3)
22	4.23 (s)	59.9 (CH_3)

In $CDCl_3$; δ_H 400 MHz; δ_C 100 MHz.

C-14. The formation of a spiro unsaturated γ-lactone at C-13 could be confirmed by the HMBC correlation between H-12 and C-14. The low-field shift of C-13 (δ_C 96.6) supported such a conclusion. A hydroxyl group located at C-12 was indicated by its low-field chemical shift (δ_C 75.1). The remaining hydroxyl group was assigned at C-9 (δ_C 82.7) according to its ^{13}C NMR chemical shift. Thus, the construction of the planar structure of **1** was completed.

Taking its biogenesis into account, the relative configuration of **1** was fixed by ROESY spectrum (broken arrows in Fig. 1b). The cross-peaks observed between the proton pairs of H-4/H-10a, H-6β/H-10a, H-10/H-10a, H_3-18/H-10, and H_3-18/H10a indicated that H-4, H-6β, H-10, H-10a, and H_3-18 were β-oriented, the piperidine six-member ring in a chair conformation, and H-4 and H-10a standing axially, which were just

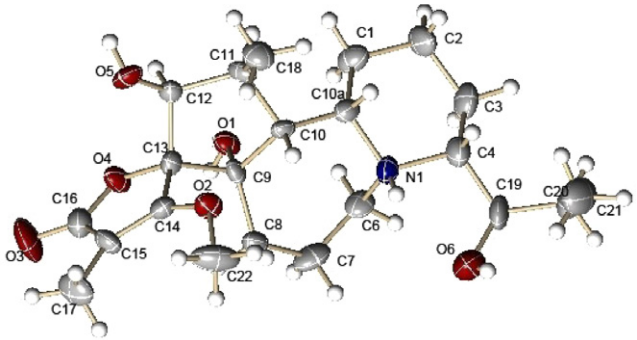
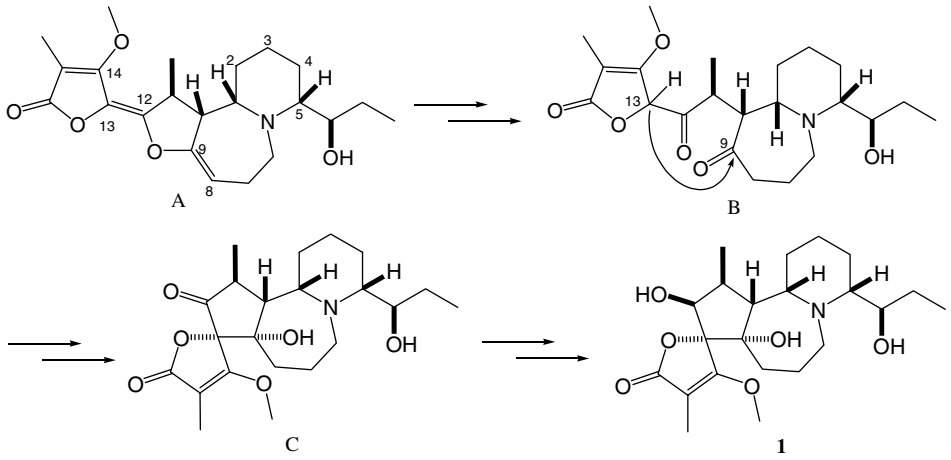


Figure 2. Perspective ORTEP drawing for compound **1**.



Scheme 1. Proposed biogenetic pathway for cochinchistemonine (**1**).

the same as those of stemokerrin.[4] The strong NOE correlation between H-11 and H-12 suggested that these protons were α-oriented. The relative configuration of C-13 was assigned to be R^* based upon the NOESY correlations between H_3-22 and H-10. The NOE cross-peak between H_3-22 and H-8β indicated that H-8β stood axially and the hydroxyl group at C-9 was α-oriented. Considering the biogenetic relationship between **1** and stemokerrin,[4] the orientation of its 1-hydroxypropyl side chain was proposed in the same way and thus C-19 was in R^*-configuration.

A computer modeled 3D structure (Fig. 1b) of **1** was generated by using the molecular modeling program CS Chem 3D Ultro Version 9.0, using MM2 force field calculations for energy minimization. The favorable conformation and relative stereochemistry of **1** offered by the computer modeling was consistent with that of **1** assigned by ROESY experiment. Ultimately the single crystal X-ray diffraction experiment confirmed unambiguously the entire stereochemistry of **1**, which was also in good agreement with the structure established by NOESY spectrum (Fig. 2).[7]

The biogenetic origin of cochinchistemonine (**1**) is proposed in Scheme 1. Stemokerrin (A),[4] the major alkaloid in *S. cochinchinensis*, was proposed as a starting material in this pathway. The ether bond in A might be hydrolyzed to form intermediate B which was subjected to an aldol condensation of the ketone groups at C-9 and C-13, followed by the functional transformation through reduction to give cochinchistemonine (**1**).

Cochinchistemonine (**1**) represents a novel cochinchistemonine-type *Stemona* alkaloid with a pyrido[1,2-α]-azepine nucleus and a spiro α,β-unsaturated-γ-lactone moiety at C-13. It is a new example of the structural diversity in *Stemona* alkaloids. So far there are rare reports about variations taking place in ring C among known *Stemona* alkaloids. Previously our group reported a compound stemodiol, in which the ether bond was hydrolyzed to two hydroxyl groups.[8] This compound may offer evidence for an unstable enol intermediate in the transformation from A to B.

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5. *Cochinchistemonine*: Colorless needles; mp 176–180 °C; $[\alpha]_D^{20}$ +9 (*c* 0.16, $CHCl_3$); CD (*c* 1.0×10^{-3} mg/mL, MeOH) Δε −0.29 (277.9), 0 (261.5), +3.11 (236.6), 0 (197.5); UV (MeOH) λ_{max} (log ε) 232 (6.95) nm; IR ν_{max} (KBr) 3385 (strong, broad), 1740 (strong, sharp), 1655, 1456, 1390, 1338, 1138, 1074, 758 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m/z* 409 ($[M]^+$), 380 ($[M-C_2H_5]^+$), 350 ($[M-C_3H_7O]^+$), 332, 194; ESIMS *m/z* 410.1 $[M+H]^+$, 841.3 $[2M+Na]^+$; HREIMS *m/z* 409.2441 ($C_{22}H_{35}NO_6$, calcd 409.2464); HRESIMS *m/z* 410.2531 ($C_{22}H_{36}NO_6$, calcd 410.2543).
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