Stemocurtisine, the First Pyrido[1,2-*a*]azapine *Stemona* Alkaloid

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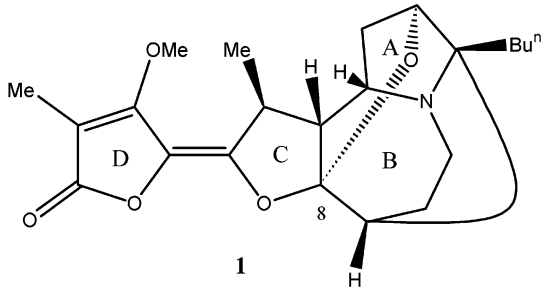
A new pentacyclic stemona alkaloid, stemocurtisine (**2**), with a novel pyrido[1,2-*a*]azapine A,B-ring system, has been isolated from a root extract of *Stemona curtisii*. The structure and relative stereochemistry was determined by spectral data interpretation and X-ray crystallography.

The stemona group of alkaloids includes more than 40 different natural products that have been structurally classified into five different groups.[1] The pyrrolo[1,2-*a*]-azapine (1-azabicyclo[5.3.0]decane) nucleus is common to all compounds in these groups. For example, the relatively complex *Stemona* alkaloid stemofoline (**1**) has the typical pyrrolo[1,2-*a*]azapine A,B-ring structure, characteristic of this class of alkaloids.[2] A few of these alkaloids do not fit these five structural groups and have a more complex bridged structure or ring structure that most likely arises from initial oxidative cleavage of the pyrrolo[1,2-*a*]azapine ring system.[1] Phytochemical studies on the Stemonacaeae family have been limited to eight of the recorded 30 or more species.[1−3] Extracts of the roots of *Stemona* species have been used in traditional Chinese medicine for the treatment of various respiratory diseases and as anthelmintic agents for domestic animals.[1,2,4−7] More recently, extracts and the pure alkaloids derived from the extracts of the leaves and roots of *Stemona collinsae* and *S. tuberosa* were shown to have insect toxicity, antifeedent, and repellent activities.[2] We report here the isolation and structure determination of a novel *Stemona* alkaloid (**2**) from the root extracts of *S. curtisii* Hook. f. growing in the southern region of Thailand. This plant is known in the Thai vernacular as "non tai yak" and has received no phytochemical and little biological attention apart from studies of its effect on the action potential of the frog sciatic nerve[8a] and its toxicity on house fly larvae (*Culex p.* fatigans and *Aedes aegypti*).[8b,c]

A crude ethanol extract of the roots of *S. curtisii* was partitioned between 5% hydrochloric acid solution and chloroform. The aqueous solution was made basic with aqueous ammonia and extracted with chloroform. The crude residue was subjected to flash column chromatography and preparative TLC to provide **2** in 0.5% yield (w/w) based on the dry weight of root material. Examination of the crude ethanol extract by TLC analysis showed the presence of **2**, indicating that this compound was not being produced via an acid-catalyzed reaction during the acid extraction process. We have named this compound stemocurtisine, on the basis of its botanical origin. Compound **2** was



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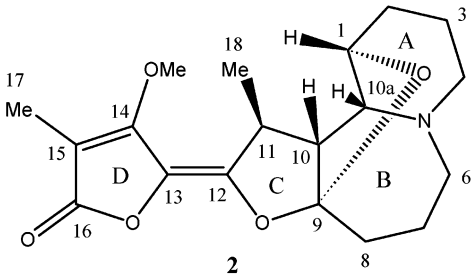

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obtained as colorless prismatic crystals (mp 149−151 °C) by careful and slow evaporation of a solution of **2** in ethyl acetate and diethyl ether. HRMS (CI +ve, *m*/*z* $[MH]^+$ 347.1727, calcd 347.1733) indicated that **2** has the molecular formula $C_{19}H_{25}NO_5$. The ^1H and ^{13}C NMR spectra of **2** indicated the presence of the C- and D-ring system that is typically found in the stemoamide group of alkaloids, including stemofoline (**1**).[1,6,9−17] The ^{13}C/DEPT NMR spectra of **2** indicated four methine carbons and six methylene carbons, and, unlike the other *Stemona* alkaloids, except **1** and its didehydro and 2′-hydroxy derivatives,[2] a quaternary carbon at δ 120.4 was apparent, indicative of a acetal-like structure (C-9). Indeed the corresponding acetal carbon (C-8) in **1** occurs further upfield at δ 112.7.[2] The X-ray structural analysis confirmed the molecular formula of **2** and revealed its connectivity and relative stereochemistry and showed that this alkaloid has a novel pentacyclic structure based on a unique pyrido[1,2-*a*]azapine A,B-ring system (that is, a 6,7-bicyclic A,B-ring system) and not the typical pyrrolo[1,2-*a*]azapine A,B-ring system (5,7-bicyclic A,B-ring system). This is the first *Stemona* alkaloid to have this type of base structure. The absolute configuration of **2** is not known but is assumed on the basis of the known configurations of *Stemona* alkaloids with similar C,D-ring structures.[6,9−17] The X-ray structure of **2** also showed that the piperidine A-ring adopts a chairlike conformation and

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Table 1. ^{13}C NMR (75 MHz) and ^{1}H NMR (500 Mz) Spectral Data of **2** in $CDCl_3$ Solution

position	δ_C (DEPT)	δ_H [mult., *J* (Hz)]	HMBC
1	75.5 (CH)	4.01 (s)	H-2β, H-3β, H-10, H-10a
2	26.9 (CH_2)	1.62 (m, β) 2.21 (d, 14.5, α)	H-3α, H-3β, H-4α, H-4β
3	18.9 (CH_2)	1.21 (d, 13.5, β) 1.82 (m, α)	H-1, H-2β, H-4α, H-4β
4	53.6 (CH_2)	2.87 (m, β) 3.02 (m, α)	H-3α, H-3β, H-6α, H-6β
6	53.0 (CH_2)	3.38 (t, 13, β) 2.96 (m, α)	H-4α, H-4β, H-7α, H-7β, H-8α, H-8β
7	27.0 (CH_2)	2.03 (m, α) 1.66 (m, β)	H-6α, H-6β, H-8α, H-8β
8	33.9 (CH_2)	2.36 (dd, 4.5, 13.5, α) 1.75 (m, β)	H-6α, H-6β, H-7α, H-7β, H-10, H-10a
9	120.4 (C)		H-7α, H-8α, H-8β, H-10, H-10a, H-11
10	57.0 (CH)	2.65 (d, 4.5)	H-8α, H-8β, H-10a, H-11, Me (18)
10a	62.0 (CH)	3.44 (s)	H-2α, H-6α, H-10, H-11
11	39.3 (CH)	3.07 (quin, 6.5)	H-10, H-10a, Me (18)
12	147.2 (C)		H-10, H-11, Me (17), Me (18)
13	124.9 (C)		H-11, Me (17)
14	162.7 (C)		OMe, Me (17)
15	97.3 (C)		Me (17)
16	169.8 (C)		Me (17)
17	9.2 (CH_3)	2.08 (s)	OMe
18	22.6 (CH_3)	1.37 (d, 7)	H-10, H-11
OMe	58.9 (CH_3)	4.15 (s)	

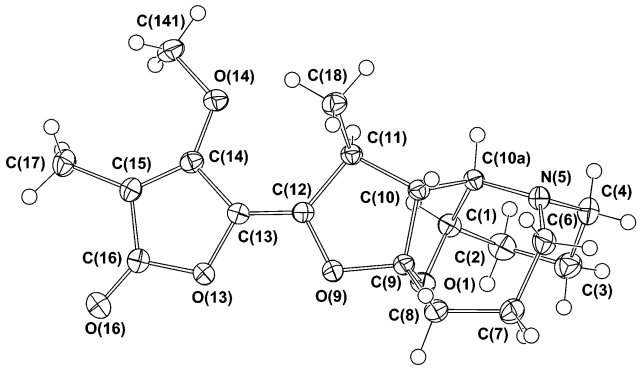

Figure 1. Molecular projection of stemocurtisine (**2**) showing 50% probability amplitude ellipsoids for C,N,O, hydrogen atoms having arbitrary radii of 0.1 Å.

is connected to the B- and C-rings through an ether bridge between C-1 and the quaternary acetal carbon C-9 readily identified in the ^{13}C NMR spectrum.

The full ^{1}H and ^{13}C NMR spectral assignments for **2**, based on extensive COSY, TOCSY, NOESY, HMQC, and HMBC experiments, are shown in Table 1. Of significance in the NOESY spectra of **2** were cross-peaks between H-10 and H-6β, H-6β and H-8β, H-7α and H-3α, H-3β and H-2β, and H-2β and H-4β (Figure 2). These cross-peaks allowed the unequivocal assignment of all methylene protons in the ^{1}H NMR spectrum of **2** (Table 1) and indicated that the solution structure of **2** is similar to the solid-state structure. That is, the A-ring adopts a chairlike conformation in which the axial proton H-3α is in close proximity to H-7α in the seven-membered ring on the concave face of the molecule (Figures 1 and 2).

In conclusion, a new pentacyclic stemona alkaloid, stemocurtisine (**2**), with a novel pyrido[1,2-*a*]azapine A,B-ring system has been isolated from a root extract of *S. curtisii*.

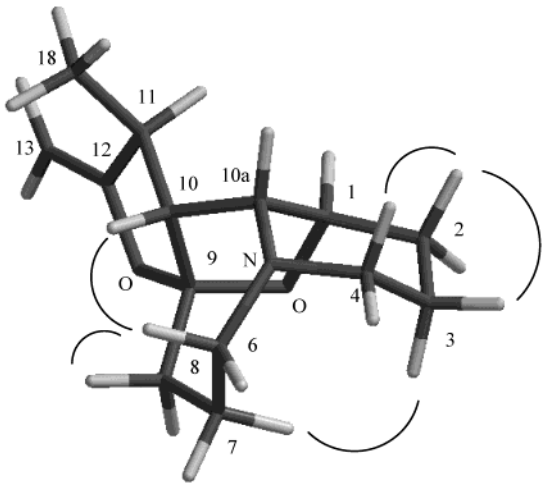

Figure 2. Molecular model (AM1, Spartan) of the A,B,C-ring substructure of **2** showing significant NOESY cross-peaks (curved lines).

Experimental Section

General Experimental Procedures. Melting points were determined by a Reichert hot-stage melting point apparatus and are uncorrected. Optical rotations were measured using a JASCO DIP-370 polarimeter. IR spectra were obtained on a Nicolet AVATAR 300 FTIR spectrophotometer. ^{1}H (500 MHz), ^{13}C (75 MHz), and 2D NMR spectra were recorded on Varian Unity 300 and 500 spectrometers. HRCIMS were recorded on a Fison/VG Autospec-TOF-oa mass spectrometer (70 eV).

Plant Material. The roots of *Stemona curtisii* were collected at Tumbol Kaunmao, Amphor Rasda, in the North of Trang Province, Thailand, in May 2002. The plant material was identified by Mr. James F. Maxwell from the Department of Biology, Chiang Mai University. A voucher specimen is deposited at the Herbarium (number 17581) of the Department of Biology, Chiang Mai University.

Extraction and Isolation. The dry ground root of *S. curtisii* (0.4 kg) was percolated with 95% ethanol (3 × 800 mL) over 3 days at room temperature. The ethanolic solution was evaporated to give a dark residue (50 g) that was partitioned between water and chloroform. The chloroform extract was extracted with 5% hydrochloric acid solution, and the aqueous solution was made basic with aqueous ammonia and extracted with chloroform to afford 0.9 g of crude alkaloid material. This material was chromatographed on silica gel (100 mL) using gradient elution from 100% dichloromethane to 50% methanol−dichloromethane containing 1% concentrated aqueous ammonia as eluent. A total of 1500 mL of eluent was collected in 25 mL test tubes. On the basis of TLC analysis these fractions were pooled to give 18 fractions. Fractions 12 (62.2 mg) and 13 (86.2 mg) were combined and rechromatographed by preparative TLC (dichloromethane−methanol−aqueous ammonia, 94:5:1) to give 20.8 mg of pure stemocurtisine (**2**).

Stemocurtisine (2): R_f 0.46 (Merck, silica gel 60 F254 TLC plates on aluminum backing using dichloromethane−methanol−aqueous ammonia, 94:5:1, as eluent) with detection using a UV lamp and/or iodine vapor; colorless prismatic crystals (from EtOAc−diethyl ether); mp 149−151 °C; $[\alpha]^{25}_D$ +334° (*c* 0.66, $CHCl_3$); IR (film) ν_{max} 2932, 1741, 1618, 1021 cm^{-1}; ^{1}H, ^{13}C, DEPT, and HMBC NMR data are presented in Table 1; HREIMS *m/z* 347.1727 $[M]^+$, calcd for $C_{19}H_{25}NO_5$ 347.1733.

X-ray Structure Determination: $C_{19}H_{25}NO_5$, M = 347.4; trigonal, space group $P3_1$ (C_3^2, No. 144), a = 9.0466(6) Å, c = 18.222(1) Å, V = 1291.5(2) Å^3. $D_c(Z = 3) = 1.34_0$ g cm^{-3}. μ_{Mo} = 0.97 cm^{-1}. Specimen: 0.48 × 0.43 × 0.38 mm; $T_{min/max}$ (multiscan absorption correction) = 0.95. $2\theta_{max}$ = 70°; N_{total} = 23 659, N_{unique} = 3751 (R_{int} = 0.037), N_{obs} ($F > 4\sigma(F)$) = 3294; R = 0.041, R_w = 0.042 (weights: $(\sigma^2(F) + 0.0002F^2)^{-1}$). $|\Delta\rho_{max}|$ = 0.31(3) e Å^{-3}. Bruker AXS instrument, ω-scans; monochromatic Mo Kα radiation, $\lambda = 0.7107_3$ Å; T ca. 153 K. Chirality adopted from chemical expectations, being indeterminate in

the present experiment, with $(x, y, z, U_{iso})_H$ refined throughout. CCDC 199384.

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Note Added after ASAP: One of the author's names was misspelled in the version published on the Web on June 11, 2003. The spelling of Damrat Supyen is correct in the version posted on June 12, 2003.

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