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# 6-Hydroxy-5,6-seco-stemocurtisine: a novel seco-stemocurtisine-type alkaloid

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**Keywords**
seco, 5, hydroxy, 6, alkaloid, novel, type, stemocurtisine, CMMB



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# 6-Hydroxy-5,6-seco-stemocurtisine: A novel seco-stemocurtisine-type alkaloid

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Keywords:

Stemona alkaloid

*Stemona curtisii*

pyrido[1,2-*a*]azepine

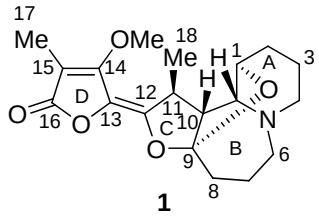
seco-alkaloid

ABSTRACT

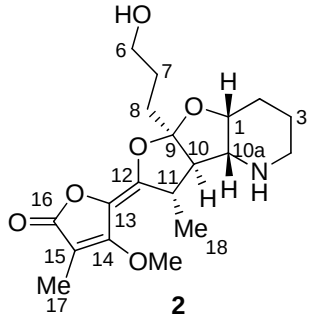
A novel seco-stemocurtisine-type alkaloid, 6-hydroxy-5,6-seco-stemocurtisine was isolated from the aerial parts of *Stemona curtisii* (Stemonaceae) collected from Trang Province in Thailand. The unprecedented 5,6-seco-pyrido[1,2-*a*]azepine structure was elucidated by 2D NMR analysis and a single crystal X-ray crystallographic analysis.

## 1. Introduction

The roots of the *Stemona* species of plants have been used as insecticides on agricultural pests and as anthelmintic agents for domestic animals. The root extracts have also been used in the treatment of various respiratory diseases and have been used as anticough agents in China and Japan [Greger, 2006; Pilli et al. 2000, 2005, 2010]. To date over 130 different *Stemona* alkaloids have been isolated with the majority of alkaloids having the pyrrolo[1,2-*a*]azepine base structure [Pilli et al. 2010]. In 2003, we reported the first pyrido[1,2-*a*]azepine based *Stemona* alkaloid, stemocurtisine **1** (Scheme 1), which was isolated from the roots of *Stemona curtisii* found in Trang Province, Thailand [Mungkornasawakul et al., 2003]. Other *Stemona* alkaloids with a pyrido[1,2-*a*]azepine structure were later reported by us [Mungkornasawakul et al., 2004; Pyne et al., 2007]], Greger [Kaltenegger et al., 2003] and Ye [Wang et al., 2007]. Pilli has classified these natural products as Stemocurtisine-type alkaloids [Pilli et al., 2010]. Here we reported the isolation and structure determination of the first seco-Stemocurtisine alkaloid, 6-hydroxy-5,6-seco-stemocurtisine **2** (Figure 1), from the aerial parts of *Stemona curtisii* (Stemonaceae) collected from Trang Province in Thailand.




**1**




**2**

Figure 1. The structures of stemocurtisine **1** and 6-hydroxy-5,6-seco-stemocurtisine **2** (compound numbering based on that of stemocurtisine)

## 2. Results and Discussion

6-Hydroxy-5,6-seco-stemocurtisine **2** was obtained as colorless plates (mp 168-170 ºC) by slow evaporation of a solution of **2** in MeOH. The HRMS (ESI +ve, *m/z* [M +$H^+$], 366.1917, calcd 366.1914) indicated that **2** has the molecular formula $C_{19}H_{27}NO_6$. The $^1H$ and $^{13}C$ NMR spectra of **2** (Table 1) were similar to those of **1** [Mungkornasawakul et al., 2003] and indicated the presence of the intact C- and D-ring systems of stemocurtisine. The $^{13}C$ NMR spectrum of **2**, like that of **1**, indicated six quaternary, four methine, six methylene, and three methyl carbons. When a comparison was made between the $^{13}C$ NMR spectra of **1** and **2**, significant differences were only noted in the chemical shifts for C-4 and C-6. The $^{13}C$ NMR chemical shift of C-6 in **2** was significantly (*ca* 10 ppm) downfield at δ 62.4 indicative of a C-6 primary hydroxyl group. A similar downfield shift was seen for the H-6 protons in the $^1H$ NMR spectrum of **2** when compared with that of **1**. While the $^{13}C$ NMR chemical shift of C-4 in **2**, was significantly upfield (*ca* -10 ppm) at δ 44.7, due to loss of the α-effect from the *N*-substituent (C-6). The full $^1H$ and $^{13}C$ NMR spectral assignments for **2** based on extensive 2D NMR experiments (HMBC and NOESY) are provided in Table 1. These data were fully consistent with the structure **2** shown in Figure 1. The structure of **2** was unequivocally confirmed by a single crystal X-ray crystallographic analysis (Figure 2) which established it as 6-hydroxy-5,6-seco-stemocurtisine, formed by formal cleavage of the C-6−N-5 bond of stemocurtisine **1**. This is the first Stemona alkaloid with a seco-pyrido[1,2-*a*]azepine structure. A relatively small number of Stemona alkaloids with a 1,9a- or 9,9a-seco-pyrrolo[1,2-*a*]azepine structure are known [Pilli et al., 2010] and only tuberocrooline has a seco-3,4-pyrrolo[1,2-*a*]azepine structure [Lin et al., 2008]. None of these alkaloids however, have the equivalent seco-4,5-pyrrolo[1,2-*a*]azepine structure to **2**.

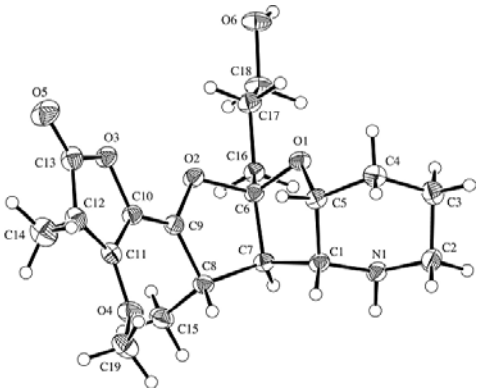



Figure 2. ORTEP plot of 6-hydroxy-5,6-seco-stemocurtisine **2** (compound numbering different to that used in Figure 1)

A proposed biosynthesis of **2** is provided in Scheme 1. Oxidation at C-6 of **1**, which would be assisted by N-5, would provide the aminal **3**, which could ring open to aldehyde **4**. A related chemical oxidation of a pyrrolo[1,2-*a*]azepine has been reported [Lindsay et al. 2004]. Reduction of the aldehyde group would then give 6-hydroxy-5,6-seco-stemocurtisine **2**.

Scheme 1. Proposed biosynthesis of **2** by oxidative cleavage of the 5-6 bond of stemocurtisine **1** and then reduction.

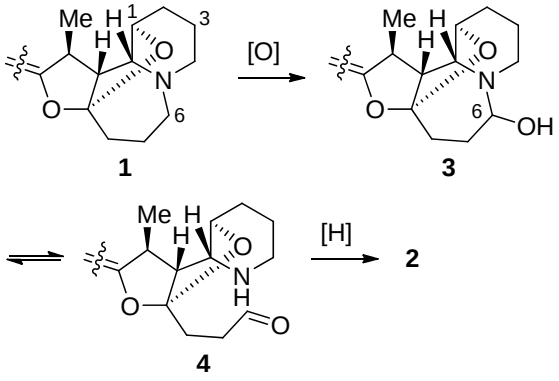


**Table 1** $^{13}C$ and $^{1}H$ NMR data of **2** in $CDCl_3$ (chemical shifts in ppm, *J* in Hz, compound numbering based on that of stemocurtisine)

| **Position** | **$^{13}C$** (125 MHz) | **$^{1}H$** (500 MHz) | **HMBC Correlations (from C to H)** | **NOESY (significant correlations)** |
|---|---|---|---|---|
| 1 | 74.4 (CH) | 3.91 (d, *J* = 2) | H-10 | H-10a |
| 2 | 25.1 ($CH_2$) | 2.18-2.09 (m) | - | - |
| 3 | 20.7 ($CH_2$) | 1.42-1.39 (m) | - | - |
| 4 | 44.7 ($CH_2$) | 2.96 (d, *J* = 13, α) 2.49 (dt, *J* = 2.5, 12.5, β) | - | H10-a |
| 6 | 62.4 ($CH_2$) | 3.76-3.70 (m) | H-7, H-8 | |
| 7 | 27.5 ($CH_2$) | 1.89-1.83 (p, *J* = 7) | H-8 | H-1, H-10 |
| 8 | 34.9 ($CH_2$) | 2.18-2.15 (m, α) | H-6, H-7 | - |
| | | 2.11-2.07 (m, β) | | - |
| 9 | 121.5 (C) | - | H-7, H-8, H-10a, H-11 | - |
| 10 | 60.7 (CH) | 2.13 (br, s) | H-18 | - |
| 10a | 63.4 (CH) | 3.08 (d, *J* = 2.5) | - | - |
| 11 | 40.6 (CH) | 3.25-3.23 (m) | H-18 | H-1, H-10a |
| 12 | 147.9 (C) | - | H-10, H-11, H-18 | - |
| 13 | 121.6 (C) | - | - | - |
| 14 | 163.4 (C) | - | H-17, OMe | - |
| 15 | 96.5 (C) | - | H-17 | - |
| 16 | 170.1 (C) | - | H-17 | - |
| 17 | 9.1 ($CH_3$) | 2.06 (s) | - | - |
| 18 | 22.4 ($CH_3$) | 1.29 (d, *J* = 7) | - | H-10 |
| OMe | 58.9 ($CH_3$) | 4.13 (s) | - | H-17 |

## 3. Experimental

### *3.1 General Experimental Procedure*

The IR spectrum was recorded on a MIRacle 10 Shimadzu Spectrometer and optical rotations on a Jasco P-2000 polarimeter. The ESIMS and HRESIMS were recorded on Micromass Platform LCZ and factory modified Waters QToF Ultima Mass Spectrometer (Wyntheshawe, UK). NMR spectra were recorded on Varian-500 MHz NMR spectrometer. Silica gel was used for column chromatography and TLC was carried out on silica gel 60 GF254 plates Merck HX1 15287. The TLC spots were viewed at 254 nm and visualized by Dragendorff's reagent.

### 3.2 *Plant material*

The aerial parts of *S. curtisii* were collected at Tambol Kaunmao, Amphor Rasda, in the North of Trang Province, Thailand, in May 2010. The plant material was identified by Mr. James Maxwell from the herbarium of the Department of Biology, Chiang Mai University, where a voucher specimen is deposited (number 17581).

*3.3 Extraction and Isolation*

The dry aerial parts of *Stemona curtisii* (2 kg) were extracted with 95% EtOH (3 x 1500 mL) over 3 days at room temperature. Evaporation of the EtOH solution under reduced pressure gave a green viscous oil (250 g). A portion of this oil (40 g) was partitioned between water and $CH_2Cl_2$. The $CH_2Cl_2$ fraction was extracted with 5% HCl solution and the aqueous solution was made basic (pH 9) with conc. aqueous $NH_3$ and extracted with $CH_2Cl_2$ to give 2.8 g of crude alkaloid material. This material was chromatographed on Sephadex LH20 (100 g) using isocratic elution with a mixture of MeOH and $CH_2Cl_2$ (v/v, 1:1) as eluent. On the basis of TLC analysis, two alkaloids fractions, fraction 1 (923.2 mg) and fraction 2 (453.0 mg), were obtained. These fractions were further purified by column chromatography. Fraction 1 was chromatographed on silica gel (100 mL) using gradient elution from 100% EtOAc to MeOH/EtOAc containing 1% conc. aqueous $NH_3$ as eluent. On the basis of TLC analysis, two alkaloid fractions, fraction 1-A (94.5 mg) and fraction 1-B (138 mg) were obtained. Fraction 1-A was re-chromatographed on silica gel (15 mL) using gradient elution from 100% $CH_2Cl_2$ to 1:1 MeOH/$CH_2Cl_2$ containing 1% conc. aqueous $NH_3$ as eluent. Three fractions were collected. Fraction 1-A2 (57.1 mg) was re-chromatograped by preparative TLC ($CH_2Cl_2$-MeOH-aqueous $NH_3$, 94:5:1) to give stemocurtisine **1** (11.2 mg). Fraction 1-B was re-chromatographed on silica gel (20 mL) using gradient elution from 100% $CH_2Cl_2$ to 1:1 MeOH/$CH_2Cl_2$ containing 1% conc. aqueous $NH_3$ as eluent. Four fractions were collected. Fraction 1-B3 (138.0 mg) was re-chromatographed by preparative TLC ($CH_2Cl_2$-MeOH-aqueous $NH_3$, 94:5:1) to give 40.5 mg of alkaloid **2**. Fraction 2 (453.0 mg) was rechromatographed on silica gel (60 mL) using gradient elution from 100% $CH_2Cl_2$/ to 1:1 MeOH/$CH_2Cl_2$ containing 1% conc.

aqueous $NH_3$ as eluent. The alkaloid fraction (54.2 mg) was re-chromatographed by preparative TLC ($CH_2Cl_2$-MeOH-aqueous $NH_3$, 94:5:1) to give stemocurtisine **1** (20.0 mg) and 6-hydroxy-5,6-seco-stemocurtisine **2** (40.5 mg):

*3.4 6-Hydroxy-5,6-seco-stemocurtisine **2***

White plates (MeOH), Mp: 168-170 $^oC$; $R_f$ 0.3 ($CH_2Cl_2$-MeOH-$NH_4OH$, 95:5:1); $[\alpha]^{23}_D$: +247.2 (*c* 0.84, MeOH); IR (film) 3256, 3105, 2945, 2822, 2163,2026, 1737, 1689, 1610, 1451, 1395, 1268, 1143, 1058, 1005, 916, 797 $cm^{-1}$. HRMS-ESI: *m/z* [M + $H^+$] calcd for $C_{19}H_{28}NO_6$: 366.1917; found: 366.1914.

## 4. X-ray crystallographic study

*4.1. Crystal data.*

Compound **2**. $C_{19}H_{27}NO_6$, $M$=365.43, $T$=200 K, trigonal, space group $P3_221$, $Z$=6, $a$=8.6115(2), $c$=43.6534(12) Å, $V$=2803.54(12) $Å^3$, $D_x$=1.299 g/$cm^3$, 30191 reflections measured (2θ=5 – 55°) merged to 2551 unique data, $R$=0.031 [for 2285 data with $I>2\sigma(I)$], $R_w$=0.080 [all data], $S$=0.99.

*4.2. Structure determination*. Images were measured on a Nonius Kappa CCD diffractometer (Mo *K*α radiation, graphite monochromator, λ=0.71073 Å) and data extracted using the DENZO package [Otwinowski and Minor, 1997]. Structure solution was by direct methods (SUPERFLIP) [Palatinus and Chapuis, 2007]. The structure was refined using the CRYSTALS program package [Betteridge et al., 2003]. Atomic coordinates, bond lengths and angles and displacement parameters have been deposited at the Cambridge Crystallographic Data Centre (CCDC no. CCDC 924878). These data can be obtained free-of-charge via www.ccdc.cam.ac.uk/data_requerst/cif, by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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Graphical Abstract

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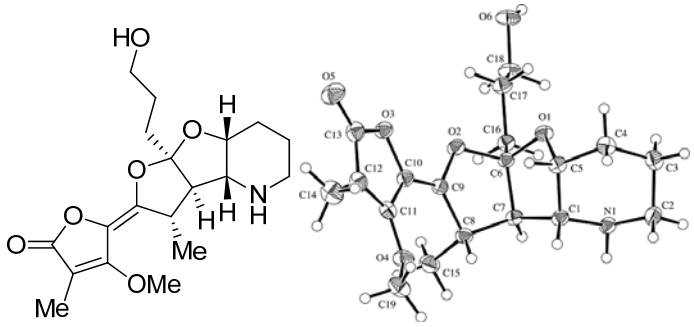


Highlights

► The first Stemona alkaloid with a seco-pyrido[1,2-*a*]azepine structure is reported ► The structure was elucidated using spectroscopic and X-ray crystallographic analysis ► Biosynthesis proposed by azepine ring cleavage of co-isolated stemocurtisine.