2004

Phytochemical and larvicidal studies on Stemona curtisii: Structure of a new pyriodo[1,2-a]azepine Stemona alkaloid

Pitchaya Mungkornasawakul
Chiang Mai University

Stephen G. Pyne
University of Wollongong, spyne@uow.edu.au

Araya Jatisatienr
Chiang Mai University

Damrat Supyen
Chiang Mai University

Chaiwat Jatisatienr
Chiang Mai University

See next page for additional authors

Phytochemical and larvicidal studies on Stemona curtisii: Structure of a new pyriodo[1,2-a]azepine Stemona alkaloid

Abstract

A new pentacyclic *Stemona* alkaloid, stemocurtisinol (**3**), with a pyrido[1,2-*a*]azepine A,B-ring system, and the known pyrrolo[1,2-*a*]azepine alkaloid oxyprotostemonine (**4**) have been isolated from a root extract of *S. curtisii*. The structure and relative stereochemistry of stemocurtisinol was determined by spectral data interpretation and X-ray crystallography. This compound is a diastereoisomer of oxystemokerrin and has the opposite configuration at C-4 and C-19. The individual alkaloid components showed significant larvicidal activity (IC_{50} 4–39 ppm) on mosquito larvae (*Anopheles minimus* HO).

Keywords

CMMB

Disciplines

Life Sciences | Physical Sciences and Mathematics | Social and Behavioral Sciences

Publication Details

Mungkornasawakul, P., Pyne, S. G., Jatisatienr, A., Supyen, D., Jatisatienr, C., Lie, W., Ung, A. T., Skelton, B. W. & White, A. H. (2004). Phytochemical and larvicidal studies on Stemona curtisii: Structure of a new pyriodo[1,2-a]azepine Stemona alkaloid. Journal of Natural Products, 67 675-677.

Authors

Pitchaya Mungkornasawakul, Stephen G. Pyne, Araya Jatisatienr, Damrat Supyen, Chaiwat Jatisatienr, Wilford Lie, Alison T. Ung, Brian W. Skelton, and Allan H. White

REVISED

Phytochemical and Larvicidal Studies on *Stemona curtisii*: Structure of a new Pyrido[1,2-*a*]azepine *Stemona* Alkaloid

Pitchaya Mungkornasawakul,[†] Stephen G. Pyne,*[,‡] Araya Jatisatienr,[§] Damrat Supyen,[⊥] Chaiwat Jatisatienr,[§] Wilford Lie,[‡] Alison T. Ung,[‡] Brian W. Skelton,[ll] and Allan H. White[ll]

Division of Environmental Sciences, Chiang Mai University, Chiang Mai 50202, Thailand, Department of Chemistry, University of Wollongong, Wollongong, New South Wales, 2522, Australia, Department of Biology, Chiang Mai University, Chiang Mai 50202, Thailand, Department of Chemistry, Chiang Mai University, Chiang Mai 50202, Thailand, and Department of Chemistry, University of Western Australia, Crawley, Western Australia, 6009, Australia

---

*To whom correspondence should be addressed. Tel: +61-24221-3511. Fax: +61-24221-4287. E-mail: spyne@uow.edu.au

[†]Division of Environmental Sciences, Chiang Mai University.

[‡]Department of Chemistry, University of Wollongong.

[§]Department of Biology, Chiang Mai University.

[⊥]Department of Chemistry, Chiang Mai University.

[ll]Department of Chemistry, University of Western Australia.

A new pentacyclic *Stemona* alkaloid, stemocurtisinol (**3**), with a pyrido[1,2-*a*]azepine A,B-ring system, and the known pyrrolo[1,2-*a*]azepine alkaloid oxyprotostemonine (**4**) have been isolated from a root extract of *S. curtisii*. The structure and relative stereochemistry of stemocurtisinol was determined by spectral data interpretation and X-ray crystallography. This compound is a diastereoisomer of oxystemokerrin and has the opposite configuration at C-4 and C-19. The individual alkaloid components showed significant larvicidal activity (IC_{50} 4-39 ppm) on mosquito larvae (*Anopheles minimus* HO).

The *Stemona* group of alkaloids includes more than forty different natural products that have been structurally classified into five different groups.[1] The pyrrolo[1,2-*a*]azepine (5,7-bicyclic A,B-ring system) nucleus is common to all compounds in these groups. In 2003 we reported the structure of stemocurtisine (**1**), the first example of a *Stemona* alkaloid with a pyrido[1,2-*a*]azepine A,B-ring system (that is, a 6,7-bicyclic A,B-ring system), isolated from the roots of *S. curtisii* Hook. f[2] Later in that year Hofer and Greger reported the isolation of five *Stemona* alkaloids with the pyrido[1,2-*a*]azepine A,B-ring system, including stemocurtsine (**1**), which they named pyridostemin, and oxystemokerrin (**2**) from an unidentified *Stemona* species (HG 915) and *S. kerrii*, respectively.[3] Interestingly, these workers also examined the phytochemicals from *S. curtisii*; the major and minor components were stemofoline, a known pyrrolo[1,2-*a*]azepine *Stemona* alkaloid, and 2′-hydroxystemofoline, respectively, with trace amounts of oxystemokerrin (**2**) also detected. Our root samples of *S. curtisii* were collected in Trang Province in Southern Thailand in May 2002, and a voucher specimen was deposited in the Herbarium at Chiang Mai University. In turn the *S. curtisii* plant samples of Hofer and Greger were collected from Sutun Province in Southern Thailand (date not disclosed). Unfortunately, no voucher plant specimen from the collection of Hofer and Greger was deposited in Thailand, making a botanical comparison difficult. However, we are confident that Mr. J. Maxwell, a taxonomist from the Herbarium at Chiang Mai University and an expert of tropical plants, has identified the correct plant species by comparing our specimen with the key to the genera (*Stemona*) of Duyfjes[4] and Prain.[5] We report here the isolation of two further *Stemona* alkaloids

from the roots of this plant and the larvicidal activity of the crude root extract of *S. curtisii*, and its three main alkaloid components, on mosquito larvae (*Anopheles minimus* HO).

Preparative TLC of the crude ethanol extracts of the dried root (400 g) of *S. cutisii* gave, stemocurtisine (**1**, 20.8 mg), stemocurtisinol (**3**, 12.7 mg) and oxyprotostemonine (**4**, 10.5 mg). The latter alkaloid was isolated in trace amounts from the roots of *S. kerrii* and *S. curtisii* by Hofer and Greger[3] and the ^{1}H and ^{13}C NMR and MS data of **4** were almost identical to that reported by these workers.

Compound **3** was obtained as colorless prismatic crystals (mp 209-211 ^{o}C) by careful and slow evaporation of a solution of **3** in ethyl acetate. HRMS (EI +ve, *m/z* [M^{+}] 405.2100, calcd 405.2151) indicated that **3** had the molecular formula $C_{22}H_{31}NO_6$. The ^{1}H and ^{13}C NMR specta of **3** indicated the presence of the ABCD-ring system of stemocurtisine (**1**), including the ether bridging structure between C-1 and C-9 and the C-4, 1-hydroxypropyl A-ring side chain. NOESY experiments showed significant cross peaks between H-19 and H-10a and H-19 and H-2β, revealing the β-configuration (axial orientation) of the C-4 substituent relative to the axial protons H-2β and H-10a (Figure 1). X-ray structural analysis confirmed the molecular formula of **3** and revealed its connectivity and relative stereochemistry (Figure 1). In the crystal, the hydroxyl hydrogen, H(19O), is hydrogen-bonded intramolecularly to N(5) at a distance of 2.04(3) Å (Figure 1). The absolute configuration of **3** was not established but is assumed, based on the known configurations of *Stemona* alkaloids with similar C,D-ring structures.[6,7] The NMR data of **2** and **3** were significantly different, especially in their ^{13}C NMR chemical shifts for the carbons near the C-4, 1-hydroxypropyl side chain, consistent with

these compounds being epimeric at C-4. Significant differences were observed in the chemical shifts for C-6 (δ 42.8 in **2**[3] and δ 54.8 in **3**) and C-19 (δ 70.5 in **2**[3] and δ 67.9 in **3**). The differences in the C-6 chemical shifts are consistent with the C-4 substituent having an equatorial disposition in **2** and an axial disposition in **3**. Indeed the ^{13}C NMR chemical shift for C-6 in **1**[2] and **3** were almost identical, whereas that in **2** is about 12 ppm upfield due to the γ-gauche effect[8] of the C-4 substituent on C-6. Compounds **2** and **3** also have opposite configurations at the carbinol carbon C-19.

The full ^{1}H and ^{13}C NMR spectral assignments for **3**, based on extensive COSY, TOCSY, NOESY, HMQC, and HMBC experiments, are shown in Table 1. As described earlier, NOESY experiments were used to determine the relative α or β orientation of the protons.[2]

The larvicidal activity of the crude root extract of *S. curtisii* and that of compounds **1**, **3** and **4** on mosquito larvae (*Anopheles minimus* HO), using the WHO method to determine the LC_{50},[9] are shown in Table 2. While the crude ethanol extract showed a LC_{50} of 81 ppm, the individual alkaloid components were significantly more potent (LC_{50} of 4-39 ppm). The most potent was oxyprotostemonine **4** having a LC_{50} of 4 ppm.

In conclusion, two further pentacyclic *Stemona* alkaloids, stemocurtisinol (**3**) and oxyprotostemonine (**4**) have been isolated from a root extract of *S. curtisii*. Stemocurtisinol (**3**) is a diastereoisomer of oxystemokerrin (**2**) and has the opposite configuration at C-4 and C-19. The individual alkaloid components showed significant larvicidal activity on mosquito larvae (*Anopheles minimus* HO). The differences between the phytochemical profiles of *S. curtisii* studied by us and by Hofer and Gerber may be

due in part to the fact that these plants were harvested from different geographic regions and possibly at different seasons.[10]

Experimental Section

General experimental procedures. As described previously.[2]

Plant Material. The plant material [a voucher specimen is deposited at the Herbarium (number 17581) of the Department of Biology, Chiang Mai University] was collected as described previously.[2]

Extraction and Isolation. The crude alkaloid material (0.9 g) that was described previously[2] 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 test tubes of 25 mL. 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 re-chromatographed by preparative TLC (dichloromethane-methanol-aqueous ammonia, 94:5:1) to give 20.8 mg of pure stemocurtisine (**1**). Fraction 10 (136.8 mg) was chromatographed on silica gel using gradient elution from 100% dichloromethane to 50% methanol/dichloromethane containing 1% concentrated aqueous ammonia as eluent, six fractions were collected. Fraction 2 (65.8 mg) and fraction 3 (30.6 mg) were re-chromatographed by preparative TLC (dichloromethane-methanol-ammonia, 96:4:1) to give 12.7 mg of pure stemocurtisinol (**3**) and 10.5 mg of pure oxyprotostemonine (**4**), respectively. The ^{1}H and ^{13}C NMR data of **4** were identical to that reported in the literature.[3]

Stemocurtisinol (3): pale yellow needles (ethyl acetate); mp 209-211 °C; $[\alpha]^{25}_{D}$ +233° (*c* 0.334, $CHCl_3$); IR (film) ν_{max} 2930, 1746, 1692, 1621, 1461, 1397, 1367, 1286, 1216, 1155, 1025, 994.62, 754 cm^{-1}; ^{1}H- and ^{13}C NMR, see Table 1; HREIMS *m/z* 405.2100 $[M]^+$, calcd for $C_{22}H_{31}NO_6$ 405.2151.

X-ray Structure Determination of 3: $C_{22}H_{31}NO_6$, *M* = 405.5. Orthorhombic, space group $P2_12_12_1$ (D_2^4, No. 19), *a* = 7.708(3), *b* = 14.177(6), *c* = 18.972(8) Å, *V* = 2073 $Å^3$. D_C (*Z* = 4) = 1.29$_9$ g cm^{-3}. μ_{Mo} = 0.09 mm^{-1}; specimen: 0.45 x 0.15 x 0.13 mm; $'T'_{min/max}$ (multiscan correction) = 0.90. $2\theta_{max}$ = 58°; N_{total} (full sphere) = 26220, merging to *N*(unique) = 3015 (R_{int} = 0.035), N_{obs} ($F>4\sigma(F)$) = 2631; *R* = 0.035, R_W = 0.041 (weights: $(\sigma^2(F) + 0.0003F^2)^{-1}$). $(x,y,z,U_{iso})_H$ refined. $|\Delta\rho_{max}|$ = 0.22(2) e $Å^{-3}$. Bruker AXS instrument, *T ca* 153 K; monochromatic Mo Kα radiation, λ = 0.7107$_3$ Å. CCDC # 226011.

Acknowledgment: We are grateful to the National Research Council of Thailand (NRCT), the National Center for Genetic Engineering and Biotechnology, Thailand (BIOTEC), and the University of Wollongong for supporting this project. We thank C.P.T. Wuttikorn Rodkvamtook for providing the plant material.

References and Notes

(1) Pilli, R. A.; Ferreira de Oliveira, M. C. *Nat. Prod. Rep*. **2000**, *17*, 117-127.

(2) Mungkornasawakul, P.; Pyne, S. G.; Jatisatienr, A.; Supyen, D.; Lie, W.; Ung, A. T.; Skelton, B. W.; White, A. H. *J. Nat. Prod*. **2003**, *66*, 980-982.

(3) Kaltenegger, E.; Brem, B.; Mereiter, K.; Kalchhauser, H.; Kahlig, H.; Hofer, O.; Vajrodaya, S.; Greger, H. *Phytochemistry* **2003**, *63*, 803-816.

(4) Duyfjes, B. E. E. *Flora Malesiana*. *Ser. I* **1993**, *11*, 399-409.

(5) Prain, D. *J. Asiatic Soc. Bengal*, **1905**, *73*, 39-44.

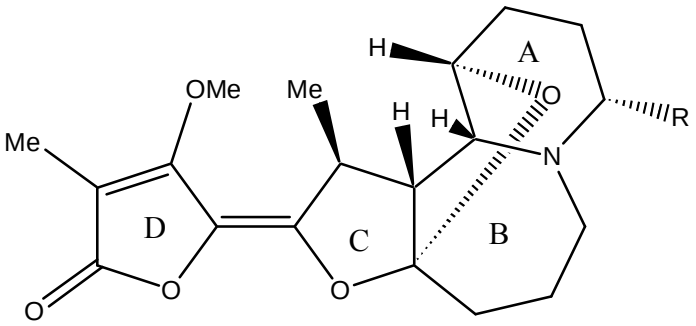
(6) Irie, H.; Masaki, N.; Ohno, K.; Osaki, K.; Taga, T.; Uyeo, S. *J. Chem. Soc., Sect. D* **1970**, 1066.

(7) Irie, H.; Harada, H.; Ohno, K.; Mizutani, T.; Uyeo, S. *J. Chem. Soc. Sect., D* **1970**, 268-269.

(8) Barfield, M. *J. Am. Chem. Soc*. **1995**, *117*, 2862-2876, and references cited therein.

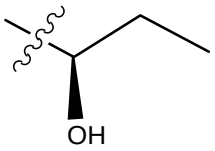
(9) Rahuman, A. A.; Gopalakrishnan, G.; Saleem, G. B. *Fitoterapia* **2000** *71*, 553-555. Refer to WHO Instruction for determining the susceptibility or resistance of mosquito larvae to insecticides. Mimeographed document WHO/VBC/1975,583.

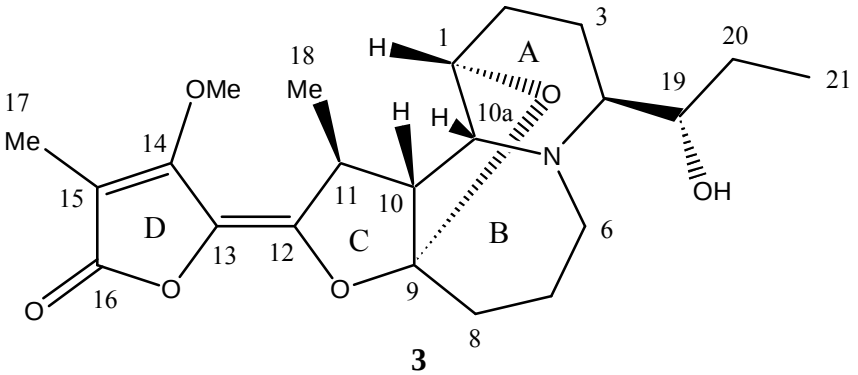
(10) Dong, T. T. X.; Cui, X. M.; Song, Z. H.; Zhao, K. J.; Ji, Z. N.; Lo, C. K.; Tsim, K. W. K. *J. Agric. Food Chem*. **2003**, *51*, 4617-4623.


H
A
OMe
Me
H
H
O
R
Me
N
D
C
B
O
O
O

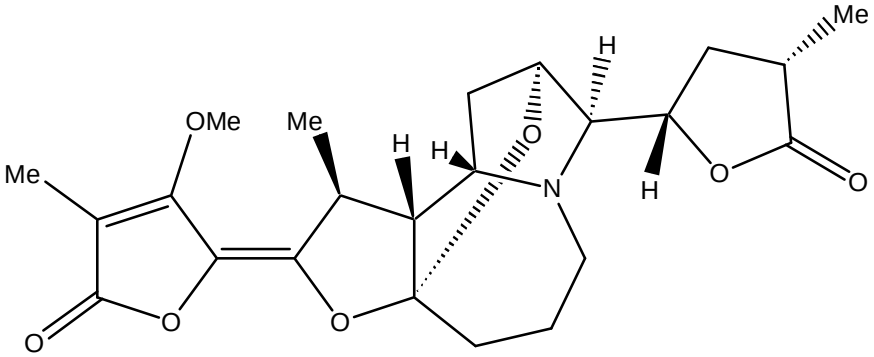
1, R = H

2, R =


OH


3
H
1
A
20
18
Me
OMe
19
21
17
O
H
H
10a
Me
N
14
OH
15
11
10
D
C
B
6
13
12
O
O
16
9
8

3


Me
H
OMe
Me
H
H
O
Me
N
H
O
O
O
O
O

4

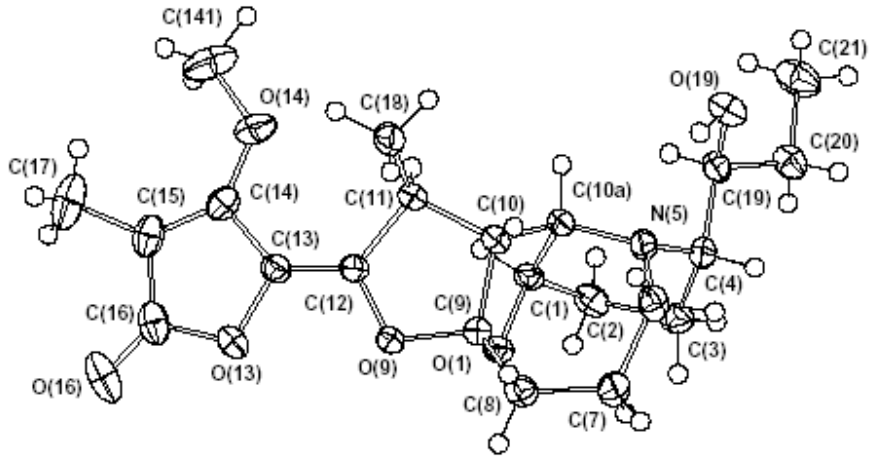


Figure 1. Molecular projection of **3**, showing 50% probability amplitude displacement ellipsoids, hydrogen atoms having arbitrary radii of 0.1 Å.

Table 1. ^{13}C NMR (75 MHz) and ^{1}H NMR (500 Mz) Spectral Data of **3** in $CDCl_3$ Solution.

position	δ_C (DEPT)	δ_H (mult., *J* (Hz), assign.)	HMBC
1	75.4 (CH)	4.05 (s)	H-2α, H-2β, H-3α, H-3β, H-10, H-10a
2	22.4 (CH_2)	1.73 (dd, 5.8, 12.3, β) 1.95 (m, α)	H-3α, H-4
3	18.4 (CH_2)	1.36 (m, β) 1.96 (m, α)	H-1, H-2β, H-4, H-19
4	65.5 (CH)	2.53 (m)	H-3α, H-3β, H-6α, H-6β, H–19
6	54.8 (CH_2)	2.92 (dd, 4.5, 15.5, α) 3.48 (m, β)	H-4, H-7α, H-7β, H-8α, H-8β, H–10a
7	25.8 (CH_2)	1.65 (m, β) 1.99 (m, α)	H-6α, H-6β, H-8α, H-8β
8	33.5 (CH_2)	1.76 (dd, 5.8, 13, β) 2.36 (dd, 4.1, 13, α)	H-6α, H-6β, H-7α, H-7β, H-10
9	120.1 (C)		H-7α, H-7β, H-8α, H-8β, H-10, H-10a, H-11
10	56.9 (CH)	2.70 (d, 4.7)	H-10a, H-11, Me (18)
10a	57.5 (CH)	3.40 (s)	H-2α, H-2β, H-10, H-11
11	39.3 (CH)	3.07 (quin, 6.1)	H-10, H-10a, Me (18)
12	146.8 (C)		H-10, H-11, Me (17), Me (18)
13	125.0 (C)		H-11, Me (17)
14	162.7 (C)		OMe, Me (17)
15	97.5 (C)		Me (17)
16	169.7 (C)		Me (17)
17	9.2 (CH_3)	2.07 (s)	OMe
18	22.6 (CH_3)	1.38 (d, 7)	H-10, H-11
19	67.9 (CH)	3.50 (m)	H-4, H-20, Me (21)

20	26.4 (CH_2)	1.25 (m) 1.60 (m)	H-4, H-19, Me (21)
21	10.3 (CH_3)	1.02 (t, 7.3)	H-19, H-20
OMe	58.9 (CH_3)	4.15 (s)	

Table 2. Larvicidal Activity of *S. curtisii* on Mosquito Larvae (*Anopheles minimus* HO) Using the WHO Method.[9]

extract/compound	LC_{50} (ppm)
ethanol crude extract	81
1	18
3	39
4	4