Phytochemical Studies on *Stemona burkillii* Prain: Two New Dihydrostemofoline Alkaloids

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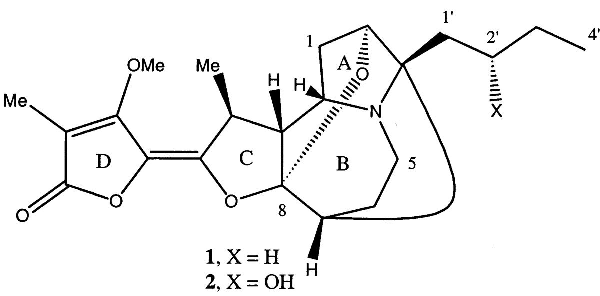
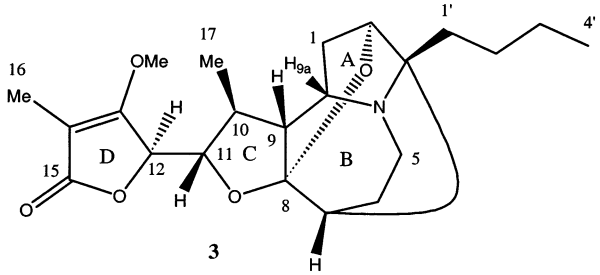
Two new dihydrostemofoline alkaloids, 11(*S*),12(*R*)-dihydrostemofoline (**3**) and stemoburkilline (**4**), along with stemofoline (**1**) and 2′-hydroxystemofoline (**2**) have been isolated from a root extract of *Stemona burkillii* Prain. The structure and relative configuration of **3** have been determined via spectroscopic data and from comparison with synthetic 11(*S*),12(*S*)-dihydrostemofoline (**5**). The configuration of the *exo*-cyclic alkene group in **4** is tentively assigned as *E* on the basis of mechanistic considerations.



1, X = H
2, X = OH



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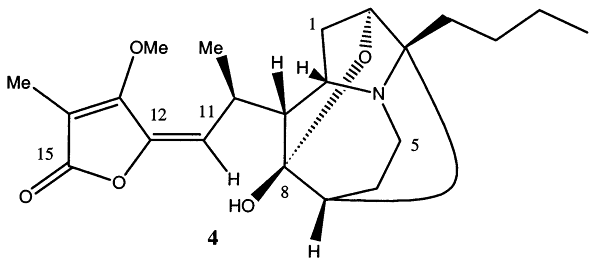

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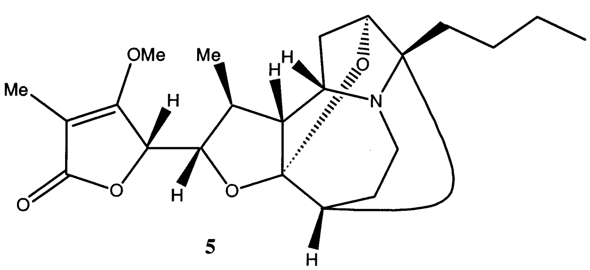

5

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*]-azepine (5,7-bicyclic A,B-ring system) nucleus is common to all compounds in these groups. In 2003 we[2] and then Hofer and Greger[3] reported the structures of *Stemona* alkaloids with a pyrido[1,2-*a*]azepine A,B-ring system (that is, a 6,7-bicyclic A,B-ring system), and in 2004 we disclosed the structure of another pyrido[1,2-*a*]azepine *Stemona* alkaloid.[4] These alkaloids comprise a new and sixth structural group. The pure alkaloids derived from the extracts of the leaves and roots of *Stemona* species have insect toxicity and antifeedent and repellent activities.[3−5] We report here the isolation and structure determination of two novel *Stemona* alkaloids, **3** and **4**, from the root extracts of *Stemona burkillii* Prain that were collected at Tambol Mae Hea, Amphur Meang, Chiang Mai, Thailand.

A crude ethanol extract (10.4 g) of the roots of *S. burkillii* was partitioned between 5% hydrochloric acid solution and dichloromethane. The aqueous solution was made basic with aqueous ammonia and extracted with dichloromethane to afford 0.224 g of crude alkaloid material. Successive purifications of this material by preparative TLC gave pure samples of stemofoline **1** (6.8 mg), 2′-hydroxystemofoline **2** (3.7 mg), 11(*S*),12(*R*)-dihydrostemofoline **3** (2.1 mg), and stemoburkilline (**4**) (1.5 mg). The former two known alkaloids were identified from a comparison of their spectroscopic/spectrometric data (NMR and MS) with those reported.[5,6] Compounds **3** and **4** are new compounds. We have named compound **4** stemoburkilline on the basis of its botanical origin. Examination of the crude ethanol extract by TLC and ^{1}H NMR analysis showed the presence of all four alkaloids, indicating that these compounds were not being produced via an acid-catalyzed reaction during the acid extraction process.

The HRMS (EI +ve, m/z [M]$^+$ 389.2202, calcd 389.2202) of **3** showed it had the molecular formula $C_{22}H_{31}NO_5$ and indicated that it was a dihydrostemofoline derivative. The ^{1}H and ^{13}C NMR specta of **3** indicated the presence of the A,B,C,D-ring system of stemofoline (**1**).[5,6] However a comparison of the ^{13}C/DEPT NMR spectra of **3** with that of **1** showed that **3** had two additional methine carbons (C-11 [δ 86.3] and C-12 [δ 76.5]) and was missing the two quaternary carbons at δ 148.4 and 127.9 and for C-11 and

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Table 1. ^{13}C NMR (125 Hz) and ^{1}H NMR (500 Mz) Spectra Data for Compounds **3**, **4**, and **5** in $CDCl_3$ Solution

	3			**4**			**5**		
position	δ_C (DEPT)	δ_H (mult., J (Hz), assign.	HMBC	δ_C (DEPT)	δ_H (mult., J (Hz), assign.	HMBC	δ_C (DEPT)	δ_H (mult., J (Hz), assign.	HMBC
1a	33.4 (CH_2)	1.99 (m)		32.9 (CH_2)	1.99 (m)		33.4 (CH_2)	1.97 (m)	
1b		1.63 (dd, $J_{1a,1b} = 3$, $J_{1b,2} = 7.5$)			1.80 (m)			1.62 (m)	
2	78.2 (CH)	4.22 (br s)	H-1a, H-1′	79.8 (CH)	4.38 (br s)	H-1a, H-1′a	78.6 (CH)	4.21 (br s)	H-1b
3	82.2 (C)		H-1a, H-1b, H-1′	82.9 (C)		H-1a, H-5b, H-1′a	82.1 (C)		H-1a, H-5b, H-6b, H-7, H-1′
5a	47.4 (CH_2)	3.14 (m) α		46.5 (CH_2)	3.27 (m) α	H-7	47.5 (CH_2)	3.09 (m) α	H-7
5b		3.01 (m) β			3.09 (m) β			2.96 (m) β	
6a	26.5 (CH_2)	1.82 (m)	H-7 (w), H-5b (w)	25.3 (CH_2)	1.93 (m)		26.8 (CH_2)	1.85 (m)	-
6b		1.72 (m)						1.73 (m)	
7	50.5 (CH)	2.45 (d, $J = 6$)		55.4 (CH)	2.34 (d, $J = 5.9$)	H-6	50.5 (CH)	2.47 (d, $J = 6$)	H-6b, H-1′ (w)
8	111.8 (C)		H-1b, H-2, H-7	105.7 (C)		H-6, H-7	112.8 (C)		H-2, H-6b, H-9, H-7, H-10, H-11
9	47.3 (CH)	1.64 (dd, $J_{9,9a} = 3$, $J_{9,10} = 12$)	H-7, H-10, H-17	44.2 (CH)	1.85 (m)	H-7, H-10, H-11, H-17	47.2 (CH)	1.60 (m)	H-10, H-17
9a	61.0 (CH)	3.44 (br s)	H-2, H-5b	63.4 (CH)	3.60 (br s)	H-2, H-5a, H-5b, H-9	61.1 (CH)	3.38 (br s)	H-2, H-5a, H-5b, H-9
10	33.1 (CH)	2.61 (m)	H-17	28.4 (CH)	3.18 (m)	H-9, H-17	35.1 (CH)	2.48 (m)	H-9, H-12, H-17
11	86.3 (CH)	3.79 (dd, $J_{10,11} = 9$, $J_{11,12} = 3$)	H-17	114.7 (CH)	5.5 (d, $J = 10$)	H-10, H-17	87.8 (CH)	3.69 (t, $J = 7$)	H-10, H-12, H-17
12	76.5 (CH)	4.60 (br s)		141.9 (C)		H-10, H-11, H-16	78.7 (CH)	4.75 (d, $J = 6.5$)	H-10, H-16
13	170.3 (C)		H-12, H-16, OMe	161.8 (C)		H-11, H-16, OMe	173.3 (C)		H-11, H-12, H-16, OMe
14	98.5 (C)		H-16	99.1 (C)		H-16	98.8 (C)		H-16
15	174.5 (C)		H-16	170.5 (C)		H-16	174.3 (C)		H-12, H-16
16	8.7 (CH_3)	2.01 (br s)		8.7 (CH_3)	2.07 (s)		8.2 (CH_3)	1.95 (br s)	
17	14.8 (CH_3)	1.08 (d, $J = 6.5$)		18.7 (CH_3)	1.08 (d, $J = 6.8$)	H-10, H-11	16.9 (CH_3)	1.12 (d, $J = 6.3$)	H-11
1′a	31.5 (CH_2)	1.56 (t, $J = 8$)	H-1′, H-7, H-2′b, H-3′, H-4′	30.2 (CH_2)	1.67 (m)	H-2′b	31.8 (CH_2)	1.54 (m)	H-7, H-3′, H-4′
b					1.60 (m)	H-4′			
2′a	27.2 (CH_2)	1.40 (m)	H-1′, H3′, H-4′	27.5 (CH_2)	1.39 (m)	H-1′b, H-3′	27.4 (CH_2)	1.40 (m)	H-3′, H-4′
b		1.23 (m)			1.29 (m)			1.23 (m)	
3′	23.2 (CH_2)	1.33 (m)	H-1′, H-2′a, H-2′b, H-4′	23.7 (CH_2)	1.36 (m)	H-2′a, H-2′b, H-4′	23.4 (CH_2)	1.35 (q, $J = 6.8$)	H-4′
4′	13.9 (CH_3)	0.87(t, $J = 7$)	H-1′, H-2′b	14.1 (CH_3)	0.92 (t, $J = 6.8$)	H-3′	14.1 (CH_3)	0.92 (t, $J = 6.8$)	H-3′
OMe	58.8 (CH_3)	4.11 (s)		59.3 (CH_3)	4.13 (s)		59.4 (CH_3)	4.10 (s)	

C-12, respectively, of stemofoline[5,6] (Table 1). Furthermore, the ^{1}H NMR spectrum of **3** showed two new signals at δ 3.79 (dd, $J = 3, 9$ Hz, H-11) and 4.60 (br s, H-12), indicating that compound **3** was an 11,12-dihydrostemofoline. NOESY experiments showed a significant cross-peak between the C-10 methyl protons (H-17) and H-11, indicating their *syn*-relationship. Thus, assuming that **3** had the same absolute configuration as stemofoline in the rings A–C, we have assigned the 11(*S*) configuration to compound **3**. Unfortunately, these experiments did not permit assignment of the configuration at C-12. In 2003, Velten[7] reported the synthesis of 11(*S*),12(*S*)-dihydrostemofoline **5** from the *syn*-hydrogenation of stemofoline. This compound also showed a significant cross-peak between the C-10 methyl protons (H-17) and H-11, consistent with the 11*S* configuration. Complete NMR data for **5** were, however, not reported. We have prepared compound **5** from hydrogenation of stemofoline **1**, and the NMR data for this compound are shown in Table 1.[7,8] The ^{1}H and ^{13}C NMR spectra of **3** and **5** are similar but not the same. Indeed, there is a significant difference in the chemical shifts and coupling constants for the signals for H-11 and H-12 in the ^{1}H NMR spectra of these two compounds, especially $J_{11,12}$, which was 3 Hz in **4** and 7 Hz in **5**. On the basis of these differences we have assigned the 12*R* configuration to **3**. The full ^{1}H and ^{13}C NMR spectra assignments for **3** and **5** based on extensive COSY, TOCSY, NOESY, HMQC, and HMBC experiments are shown in Table 1. A NOESY cross-peak between H-9 and H-5b permitted the unequivocal assignment of the H-5 protons (Table 1).

The HRMS (EI +ve, *m/z* [M]$^+$ 389.2194, calcd 389.2202) of stemoburkilline (**4**) showed that it also had the molecular formula $C_{22}H_{31}NO_5$. Its ^{1}H NMR spectrum indicated the presence of an olefinic proton (δ 5.5, 1H, d, $J = 10$ Hz, H-11) coupled to an adjacent CH group (H-10), while its ^{13}C/DEPT NMR spectrum, in comparison with that of **3**, showed the C-11 and C-12 methines in **3** had been replaced by two olefinic carbons (one quaternary and one methine). The full ^{1}H and ^{13}C NMR spectra assignments for **4** based on extensive COSY, TOCSY, NOESY, HMQC, and HMBC experiments are shown in Table 1 and indicated that **4** was formally the C-ring opened product of **3**. Our attempts to induce ring opening of **3** to produce **4** were not successful using either base (excess DBU, RT, 16 h) or acid (5% aqueous HCl, RT, 1 h) catalysis. The ^{13}C NMR spectrum of **4** showed that this compound existed in the hemiacetal form (δ 105.7, C-8, quaternary), and this was further supported from its IR spectrum (3382 cm^{-1}, br), which showed a hydroxyl group. NOESY experiments did permit assignment of the configuration of the C-11, C-12 alkene group. We have assigned the *E* configuration to **4** on the basis of the assumption that **4** arises from **3** via an *anti*-elimination process.

Antifungal studies were done on the crude ethanol extract and on pure samples of **1** and **2** and mixtures of **1** and **2** and of **3** and **4**. However these compounds and mixtures showed no significant activities ($EC_{50} > 219$ ppm) on inhibiting spore germination on *Cladosporium cladosporiodes* using the assay and procedures recommended in the literature.[9] Brine shrimp assays[10] on similar pure and mixed samples showed low to moderate toxicities ($LC_{50} > 33$ ppm).

In conclusion, two new dihydrosteomofoline alkaloids, 11(*S*),12(*R*)-dihydrostemofoline (**3**) and stemoburkilline (**4**), along with stemofoline (**1**) and 2′-hydroxystemofoline (**2**) have been isolated from a root extract of *S. burkillii*. The structure and relative configuration of **3** have been determined by spectroscopic data interpretation and by comparisons with synthetic 11(*S*),12(*S*)-dihydrostemofoline (**5**), which was prepared by *syn*-hydrogenation of stemofoline (**1**). The configuration of the *exo*-cyclic alkene group in **4** could not be unequivocally determined and is tentatively assigned as *E* on the basis of mechanistic considerations.

Experimental Section

General Experimental Procedures. These were as described previously.[2]

Plant Material. The roots of *S. burkillii* were collected at Tambol Mae Hea, Amphur Meang, Chiang Mai, Thailand, in December 2003. 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 17579) of the Department of Biology, Chiang Mai University.

Extraction and Isolation. The dry ground root of *S. burkillii* (1.8 kg) was extracted with 95% EtOH (3×2000 mL) over 3 days at room temperature. The EtOH solution was evaporated to give a dark residue (98 g). A portion of the extract (10.4 g) was partitioned between H_2O and CH_2Cl_2. The CH_2Cl_2 extract was extracted with 5% HCl solution, and the aqueous solution was made basic with aqueous ammonia and extracted with CH_2Cl_2 to afford 0.224 g of crude alkaloid material. This material was chromatographed on silica gel (100 mL) using gradient elution from 100% CH_2Cl_2 to 10% MeOH–CH_2Cl_2 containing 1% concentrated aqueous ammonia as eluent. A total of 900 mL of eluent was collected in test tubes of 20 mL. On the basis of TLC analysis these fractions were pooled to give two alkaloid fractions, fraction 1 (62 mg) and fraction 2 (88 mg). These fractions were further purified by preparative TLC. Separation of fraction 1 by three successive preparative TLC purifications (CH_2Cl_2–MeOH–aqueous ammonia, 97:3:1, then CH_2Cl_2–MeOH–aqueous ammonia, 98:2:1, and then (CH_2Cl_2–EtOAc–MeOH–Et_2NH, 70:30:5:1) gave pure samples of stemofoline (**1**) (6.8 mg) and 2′-hydroxystemofoline (**2**) (3.7 mg).

Separation of fraction 2 by three successive preparative TLC purifications (CH_2Cl_2–MeOH–aqueous ammonia, 95:5:1, and then CH_2Cl_2–MeOH–aqueous ammonia, 96:4:1, and on TLC plates that were impregnated with NH_4OAc using MeOH as eluent) gave pure samples of 11(*S*),12(*R*)-dihydrostemofoline (**3**) (2.1 mg) and stemoburkilline (**4**) (1.5 mg). The ^{1}H and ^{13}C NMR data of **1** and **2** were identical to that reported,[5,6] while those of **3**, **4**, and **5** are shown in Table 1.

Compound 3: yellow-brown gum; $[\alpha]^{26}_D$ +38.9° (*c* 0.35, $CHCl_3$); IR (film) ν_{max} 1753, 1671, 1458, 1389, 1339, 1233, 1216, 1079, 1031, 983 cm^{-1}; HREI *m/z* 389.2202 [M$^+$], calcd for $C_{22}H_{31}NO_5$ 389.2202.

Compound 4 (stemoburkilline): yellow-brown gum; $[\alpha]^{26}_D$ +37.5° (*c* 0.28, $CHCl_3$); IR (film) ν_{max} 3382 (br), 1754, 1634, 1455, 1262, 1032, 987, 802 cm^{-1}; HREI *m/z* 389.2195 [M$^+$], calcd for $C_{22}H_{31}NO_5$ 389.2202.

Hydrogenation of Stemofoline. To a solution of stemofoline (10.8 mg) in EtOH (1 mL) was added 10% Pd/C (3.7 mg). The mixture was left to stir under a hydrogen atmosphere (hydrogen balloon) at RT for 5 h, and then the reaction was filtered through a small pad of Celite and washed with more EtOH. The solvent was removed under reduced pressure, and the crude product was purified by preparative TLC (CH_2Cl_2–EtOAc–MeOH–Et_2NH, 70:30:5:1) to give compound **5** (5.3 mg) and an over-reduced product (2.5 mg).[7]

Compound 5: yellow gum; $[\alpha]^{26}_D$ +35.9 (*c* 0.22, $CHCl_3$); IR (film) ν_{max} 1754, 1668, 1636, 1458, 1391, 1339, 1061, 987 cm^{-1}; HREIMS *m/z* 389.2201 [M]$^+$, calcd for $C_{22}H_{31}NO_5$ 389.2202.

Note Added after ASAP: Structure **5** was incorrectly drawn in the version posted on August 28, 2004. The correct structure appears in the version posted on September 2, 2004.

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