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Kwankamol Sastraruji
University of Wollongong

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

Alison T. Ung
University of Wollongong, alison_ung@uow.edu.au

Pitchaya Mungkornawawakul
University of Wollongong

Wilford Lie
University of Wollongong, wilford@uow.edu.au

See next page for additional authors

Publication Details

Sastraruji, K., Pyne, S. G., Ung, A. T., Mungkornasawakul, P., Lie, W. & Jatisatienr, A. (2009). Structural revision of stemoburkilline from an E-alkene to a Z-alkene. Journal of Natural Products, 72 (2), 316-318.

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Structural revision of stemoburkilline from an E-Alkene to a Z-Alkene

Abstract
Semisynthesis studies starting from (11Z)-I',2'-didehydrostemofoline indicated that the known Stemona alkaloid stemoburkilline is the Z-isomer and not the E-isomer as initially reported. The semisynthesis involved conversion of (11Z)-1',2' -didehydrostemofoline to II(S), 12(S)-dihydrostemofoline followed by a stereoselective base-catalyzed ring-opening reaction to give (Z)-stemoburkilline. The same product was obtained using a similar synthetic protocol stariing from isostemofoline via a based-catalyzed ring-opening reaction of II (S), 12(R)-dihydrostemofoline. A re-examination of the crude root extracts of Stemona burkillii Prain and further NOE studies established stemoburkilline as the Z-isomer.

Keywords
CMMB

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

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Authors
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Structural Revision of Stemoburkilline from an *E*-Alkene to a *Z*-Alkene

Kwankamol Sastraruji,[†] Stephen G. Pyne,*[,†] Alison T. Ung,[†] Pitchaya Mungkornasawakul,[†,§] Wilford Lie,[†] and Araya Jatisatienr[‡]

School of Chemistry, University of Wollongong, Wollongong, New South Wales, 2522, Australia, Department of Chemistry, Chiang Mai University, Chiang Mai 50202, Thailand, and Department of Biology, Chiang Mai University, Chiang Mai 50202, Thailand

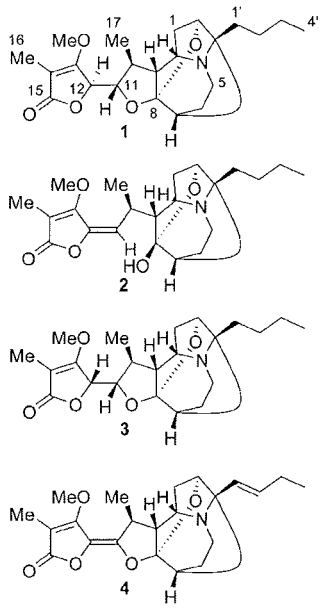
Received November 27, 2008

Semisynthesis studies starting from (11*Z*)-1′,2′-didehydrostemofoline (**4**) indicated that the known *Stemona* alkaloid stemoburkilline is the *Z*-isomer and not the *E*-isomer as initially reported. The semisynthesis involved conversion of (11*Z*)-1′,2′-didehydrostemofoline (**4**) to 11(*S*),12(*S*)-dihydrostemofoline (**3**) followed by a stereoselective base-catalyzed ring-opening reaction to give (*Z*)-stemoburkilline (**8**). The same product was obtained using a similar synthetic protocol starting from isostemofoline (**6**) via a based-catalyzed ring-opening reaction of 11(*S*),12(*R*)-dihydrostemofoline (**1**). A re-examination of the crude root extracts of *Stemona burkillii* Prain and further NOE studies established stemoburkilline as the *Z*-isomer (**8**).

Reports increase steadily each year on the isolation and biological activities of the natural products arising from extracts of plants of *Stemona* species. Over 100 *Stemona* alkaloids have been structurally characterized.[1] In 2004 we reported the isolation of two stemofoline alkaloids, 11(*S*),12(*R*)-dihydrostemofoline (**1**) and stemoburkilline (**2**), along with two known alkaloids from a root extract of *Stemona burkillii* Prain (Stemonaceae).[2] The structure and relative configuration of **1** were determined via interpretation of its spectroscopic data and from comparison with data from synthetic 11(*S*),12(*S*)-dihydrostemofoline (**3**). The configuration of the *exo*-cyclic alkene group in **2** was tentively assigned as *E* on the basis of mechanistic considerations. We had speculated that **2** arose from **1** via a ring-opening reaction involving an elimination process. We report here the synthesis of **1** and **3** from (11*Z*)-1′,2′-didehydrostemofoline (**4**)[3] and their base-catalyzed ring-opening reactions to give (*Z*)-stemoburkilline (**8**). A comparison of this synthetic compound (**8**) and the natural product found in the semipurified plant extracts (see Supporting Information) has allowed us to revise the structure of the natural product.

Hydrogenation of (11*Z*)-1′,2′-didehydrostemofoline (**4**) (isolated from the unidentified *Stemona* species reported earlier)[3] over Pd/C for 1 h gave stemofoline (**5**)[4] in 96% yield (Scheme 1). Photolysis of a solution of **5** in chloroform, in the presence of acetophenone, for 7 h gave a mixture of **5** and its isomer, isostemofoline (**6**),[5] in a ratio of approximately 9:11. This mixture was separated by column chromatography (CC) to give **6** in 41% yield and recovered **5** in 33% yield (Scheme 1). Controlled hydrogenation of **5** and **6** proved difficult and resulted in mixtures of the desired compounds **3** and **1**, respectively, plus the known over-reduced compound **7**.[6,7] The spectroscopic data of **1** and **3** were identical to those reported previously.[2]

Treatment of a solution of **3** with DBU (2 equiv) resulted in a 37:39:24 mixture of **1**, **3**, and **8**, respectively, that was difficult to separate (Scheme 2). These products most likely arise from interconversion reactions via a reversible base-catalyzed ring-opening of **3** and a reversible Michael addition reaction of the desired product **8** and/or base-catalyzed epimerization reactions between **1** and **3**. To circumvent this problem, we repeated the ring-opening reaction on **3** in the presence of TMSCl (2 equiv) to trap the intermediate ring-opened alkoxide product. Under these conditions clean formation of the TMS ether of **8** (**9**) was realized from

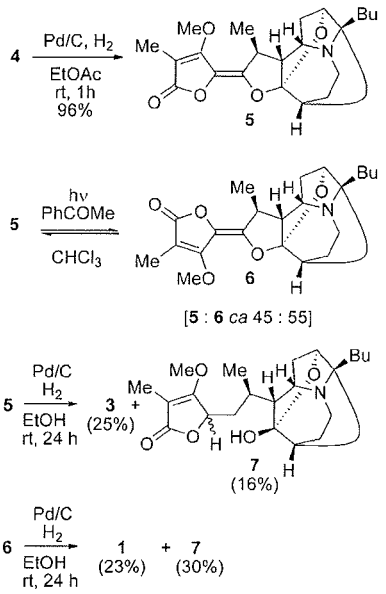


MS analysis of the crude reaction mixture. Removal of the TMS ether of **9** under acidic conditions then provided a pure sample of **8** in 61% yield after purification by CC. Under similar conditions compound **1** was converted to **8** in 69% yield (Scheme 2). The ^{1}H NMR spectrum of **8** was similar to that of stemoburkilline, which we had isolated earlier, but was not identical. The largest difference was observed for the chemical shift for H-9a, which occurred at δ 3.28 in **8** and was reported to be at δ 3.60 in the natural product.[2] An examination of the original ^{1}H NMR spectra of the original partially purified extracts of *Stemona burkillii* showed compounds **1**, **3**, and **8** to be present (42:11:47, respectively; see Supporting Information). In this mixture a signal at δ 3.32 was observed along with other resonances (e.g., δ 5.48 (d, *J* 10.0 Hz, 1H, H-11) and 4.30 (br s, 1H, H-2)) that were consistent with those of compound **8**; however no signal was seen at δ 3.60. Unfortunately we do not have the original sample of stemoburkilline to rerun its ^{1}H NMR spectrum under identical conditions to that of **8**. We re-examined the original crude extracts of *S. burkillii* that had been kept at −20 °C for 4.5 years. Partial purification of this extract by CC showed compounds **1**, **3**, and **8** to be present from ^{1}H NMR analysis (36:22:42, respectively; see Supporting Information). In this mixture a signal was observed at δ 3.27, along with those also corresponding to compound **8**. With compound **8** in hand we determined it to be

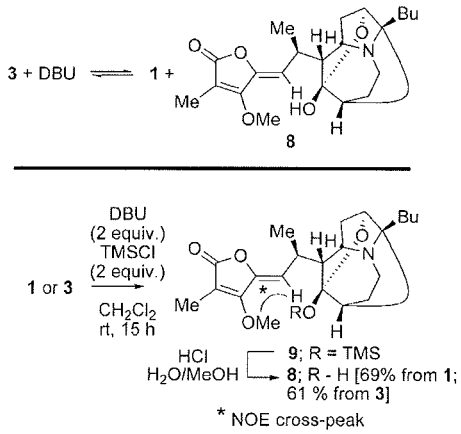
* To whom correspondence should be addressed. Tel: +61-24221-3511. Fax: +61-24221-4287. E-mail: spyne@uow.edu.au.
† School of Chemistry, University of Wollongong.
§ Department of Chemistry, Chiang Mai University.
‡ Department of Biology, Chiang Mai University.

10.1021/np800755p CCC: $40.75 © 2009 American Chemical Society and American Society of Pharmacognosy
Published on Web 01/30/2009

Scheme 1



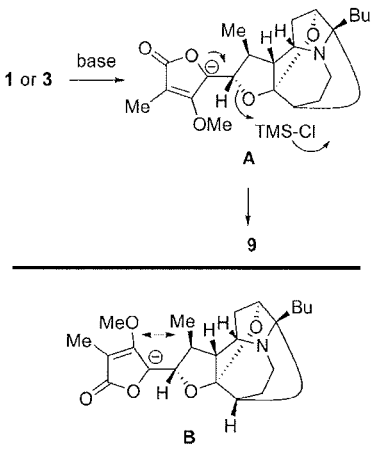
Scheme 2



the *Z*-isomer on the basis of a NOE cross-peak between the furanone methoxy group and the alkene proton. We suspect that our original NMR sample of stemoburkilline may have had traces of HCl in the solution (the $CDCl_3$ had not been base treated), resulting in a downfield shift of H-9a, although other protons near the protonated nitrogen atom would have also been expected to be observed significantly more downfield if this were the case. On the basis of these considerations and from the results of our ring-opening reactions we now reassign the structure of stemoburkilline to that of **8**, having the *Z*-configuration and not the *E*-configuration as we initially reported.[2] It is quite possible that some of compounds **1**, **3**, and **8** are artifacts, which have interconverted under nonenzymatic catalyzed reactions either in the plant or during the extraction/purification process. It has proven difficult however to analyze the crude extracts by NMR analysis to determine the ratio of these products due to their relative low abundance.

The stereochemical outcome of the base/TMSCl-initiated ring-opening reaction of **1** and **3** can be rationalized as occurring through an E1cB mechanism, as shown in Scheme 3. Deprotonation of **1** or **3** by DBU at the acidic γ-position of the lactone ring would result in the anionic intermediate **A**. TMSCl-assisted ring-opening would then give the *Z*-isomer **9**. Ring-opening via the anionic intermediate **B**, which would lead to (*E*)-stemoburkilline, would be less likely due to an unfavorable steric interaction between the methoxy and methyl groups in this intermediate (Scheme 3).

Scheme 3



Experimental Section

General Experimental Procedures. As described previously,[2,3] ^{1}H NMR assignments were achieved with the aid of gCOSY and, in some cases, NOESY experiments. ^{13}C NMR assignments were based upon DEPT, gHSQC, and gHMBC experiments. All compounds were homogeneous by TLC analysis and judged to be of >95% purity based upon ^{1}H NMR analysis.

Plant Material. The known starting material, (11*Z*)-1′,2′-didehydrostemofoline (**4**), was isolated from the unidentified *Stemona* species that we reported earlier.[3] The roots of this *Stemona* species were collected at Amphur Mae Moh, Lampang, Thailand, in November 2007. The plant material was identified by Mr. James Maxwell as the same species as we had previously studied.[3] A voucher specimen, number 25375, was deposited at the Herbarium of the Department of Biology, Chiang Mai University.

Extraction and Isolation. The dry, ground root of the *Stemona* species (935 g) was extracted with 95% EtOH (4 × 3000 mL) over 4 days at rt. The ethanolic solution was evaporated to give a dark brown residue (148 g). The extract was partitioned between $MeOH/H_2O$ (1:1) and CH_2Cl_2. The organic extract was dried over $MgSO_4$ and concentrated *in vacuo* to give a dark brown residue (20 g). A portion of this material (500 mg) was chromatographed on silica gel (100 mL) with gradient elution from CH_2Cl_2 to $CH_2Cl_2/MeOH/NH_3$ (95:5:1) to give (11*Z*)-1′,2′-didehydrostemofoline (**4**)[3] as a yellow-brown gum (242.8 mg, 48% w/w).

Stemofoline (5). To a solution of **4** (100.8 mg, 0.262 mmol) in EtOAc (4.0 mL) at rt was added Pd/C (10 mg, 10% w/w), and the flask was flushed with N_2 for 10 min before the solution was left to stir under a H_2 atmosphere for 1 h. The flask was flushed with N_2, and the solution was filtered through Celite and washed with EtOAc. The filtrate was dried over $MgSO_4$ and concentrated *in vacuo* to give **5** as a yellow-brown gum (98 mg, 0.253 mmol, 96% yield). The NMR data agreed with those reported for the natural product.[4]

Isostemofoline (6). To a large NMR tube (5 mm diameter) containing a solution of **5** (48.3 mg, 0.125 mmol) in $CHCl_3$ (2 mL) at rt was added acetophenone (50 μL). The mixture was irradiated with a 500 W lamp for 7 h to give a mixture of stemofoline (**5**) and isostemofoline (**6**) (ca. 9:11). The mixture was separated by CC using gradient elution from CH_2Cl_2 to $CH_2Cl_2/MeOH/NH_3$ (95:5:1) as eluent to give **6** as a white, amorphous solid (20 mg, 0.052 mmol, 41% yield) and **5** (15.8 mg, 0.041 mmol, 33% yield). The NMR data of **6** agreed with those reported for the natural product.[5]

11(*S*),12(*R*)-Dihydrostemofoline (1). To a solution of **6** (29.6 mg, 0.076 mmol) in EtOH (3.0 mL) at rt was added Pd/C (3.0 mg, 10% w/w), and the flask was flushed with N_2 for 10 min before the solution was left to stir under a H_2 atmosphere for 24 h. The flask was flushed with N_2, and the solution was filtered through Celite and washed with MeOH. The filtrate was concentrated *in vacuo*, and the residue was purified by column chromatography using gradient elution from CH_2Cl_2 to $CH_2Cl_2/MeOH/NH_3$ (95:5:1) to give **1** as a colorless gum (6.2 mg, 0.016 mmol, 21% yield) and the ring-open product **7** (8.9 mg, 0.023 mmol, 30% yield) as a brown gum. The NMR data of **1** agreed with those reported for the natural product.[2]

11(*S*),12(*S*)-Dihydrostemofoline (3). To a solution of **5** (83.1 mg, 0.214 mmol) in EtOH (3.0 mL) at rt was added Pd/C (8.3 mg, 10% w/w), and the flask was flushed with N_2 for 10 min before the solution was left to stir under a H_2 atmosphere for 24 h. The flask was flushed with N_2, and the solution was filtered through Celite and washed with MeOH. The filtrate was concentrated *in vacuo*, and the residue was purified by column chromatography using gradient elution from CH_2Cl_2 to CH_2Cl_2/MeOH/NH_3 (95:5:1) to give **3** as a colorless gum (20.9 mg, 0.054 mmol, 25% yield) and the ring-opened product **7** (13.5 mg, 0.035 mmol, 16% yield, dr = 72:28) as a brown gum. The NMR data of **3** agreed with that reported.[2] The ^{1}H NMR data of the major diastereomer of **7** agreed with those supplied to us by Dr. Velton.[6,7]

Compound 7: R_f = 0.10 in MeOH/EtOAc (1:4); $[\alpha]_D^{25}$ +6.3 (*c* 0.65, $CHCl_3$); ^{1}H NMR [major diastereomer, 500 MHz, $CDCl_3$] δ 4.72 (d, *J* 8.0 Hz, 1H, H-12); 4.28 (br s, 1H, H-2); 4.05 (s, 3H, O-Me); 3.24 (br s, 1H, H-9a); 3.02 (m, 1H, H-5a); 2.93 (m, 1H, H-5b); 2.48 (m, 1H, H-11a); 2.13 (m, 1H, H-10); 2.04 (d, *J* 3.5 Hz, 1H, H-7); 1.94 (s, 3H, H-16); 1.88 (m, 1H, H-1a); 1.85 (m, 2H, H-6a, H-6b); 1.66 (m, 1H, H-9); 1.62 (m, 1H, H-1b); 1.49 (m, 2H, H-1′a, H-1′b); 1.44 (m, 1H, H-11b); 1.40 (m, 1H, H-2′a); 1.33 (m, 2H, H-3′a, H-3′b); 1.22 (m, 1H, H-2′b); 1.00 (d, *J* 6.5 Hz, 3H, H-18); 0.91 (t, *J* 7.5 Hz, 3H, H-4′); ^{13}C NMR [125 MHz, $CDCl_3$] δ 175.0 (C-15); 174.6 (C-13); 107.0 (C-8); 97.6 (C-14); 82.1 (C-3); 80.1 (C-2); 78.1 (C-12); 63.5 (C-9a); 58.8 (O-CH_3); 57.2 (C-7); 47.5 (C-5); 43.6 (C-9); 40.8 (C-11); 34.3 (C-1); 31.8 (C-1′); 28.4 (C-10); 27.5 (C-2′); 26.6 (C-6); 23.3 (C-3′); 18.5 (C-17); 14.2 (C-4′); 8.3 (C-16). Minor diastereomer: ^{13}C NMR [125 MHz, $CDCl_3$] δ 175.0 (C-15); 174.3 (C-13); 106.7 (C-8); 97.4 (C-14); 82.1 (C-3); 80.2 (C-2); 76.6 (C-12); 63.7 (C-9a); 58.9 (O-CH_3); 57.0 (C-7); 47.5 (C-5); 43.3 (C-9); 40.2 (C-11); 34.4 (C-1); 31.7 (C-1'); 28.4 (C-10); 27.2 (C-2′); 26.7 (C-6); 23.3 (C-3′); 17.8 (C-17); 14.2 (C-4′); 8.5 (C-16); LRMS (ESI+) *m/z* 392.2 (100%) $[MH]^+$, 393.2 (46%), 394.2 (24%); HRMS (ESI+) *m/z* 392.2432 $[MH]^+$, calcd for $C_{22}H_{34}NO_5$ 392.2437.

(*Z*)-Stemoburkilline (8). To a solution of **3** (20.9 mg, 0.054 mmol) in dry CH_2Cl_2 (0.5 mL) at rt under a N_2 atmosphere were added DBU (16.0 μL, 0.107 mmol, 2.0 equiv) and TMSCl (13.7 μL, 0.107 mmol, 2.0 equiv), and the reaction mixture was left to stir at rt for 15 h until the reaction was complete by TLC analysis. The mixture was then diluted with CH_2Cl_2 (10 mL), and the solution was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo* to give the TMS-protected product **9** as a dark brown gum (LRMS (EI) *m/z* 461 ($M^{+\bullet}$, 100%)). The residue was dissolved in MeOH (1.5 mL), and then 10% w/v HCl (1.0 mL) was added and the solution was left to stir at rt under a N_2 atmosphere for 45 min. The solution was then evaporated under vacuum to give a white residue. A saturated $NaHCO_3$ solution (7 mL) was then added, and the mixture was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic extracts were washed with brine and dried over $MgSO_4$ before being concentrated *in vacuo*. The crude product was purified by column chromatography using gradient elution from CH_2Cl_2 to CH_2Cl_2/MeOH/NH_3 (95:5:1) to give (*Z*)-stemoburkilline (**8**) as a pale yellow, amorphous solid (12.9 mg, 0.033 mmol, 61% yield for the 2 steps): $[\alpha]_D^{24}$ +7.6 (*c* 0.64, $CHCl_3$); IR ν_{max} (cm^{-1}); 3751, 2933, 1750, 1635, 974, 756; ^{1}H NMR [500 MHz, $CDCl_3$] δ 5.48 (d, *J* 10.0 Hz, 1H, H-11); 4.30 (br s, 1H, H-2); 4.10 (s, 3H, O-Me); 3.28 (br s, 1H, H-9a); 3.13 (m, 1H, H-10); 3.05 (m, 1H, H-5a); 2.94 (m, 1H, H-5b); 2.15 (d, *J* 5.0 Hz, 1H, H-7); 2.05 (s, 3H, H-16); 1.91 (m, 1H, H-1a); 1.83 (m, 2H, H-6a, H-6b); 1.74 (m, 1H, H-9); 1.59 (m, 1H, H-1b); 1.48 (m, 2H, H-1′a, H-1′b); 1.38 (m, 1H, H-2′a); 1.32 (m, 2H, H-3′); 1.24 (m, 1H, H-2′b); 1.05 (d, *J* 6.5 Hz, 3H, H-17); 0.90 (t, *J* 7.5 Hz, 3H, H-4′); ^{13}C NMR [125 MHz, $CDCl_3$] δ 170.8 (C-15); 162.1 (C-13); 142.1 (C-12); 115.2 (C-11); 106.2 (C-8); 99.5 (C-14); 82.1 (C-3); 80.6 (C-2); 63.8 (C-9a); 59.1 (O-CH_3); 55.9 (C-7); 47.5 (C-5); 45.0 (C-9); 33.7(C-1); 31.7 (C-1′); 28.6 (C-10); 27.5 (C-2′); 26.7 (C-6); 23.4 (C-3′); 18.8 (C-17); 14.2 (C-4′); 8.8 (C-16); LRMS (EI) *m/z* 389 (33%) $[M]^+$; HRMS (EI) *m/z* 389.2213 $[M]^+$, calcd for $C_{22}H_{31}NO_5$ 389.2202.

The title compound **8** was also prepared from **1** (18.7 mg, 0.048 mmol) using the above procedure. The product was isolated as a pale yellow, amorphous solid (12.8 mg, 0.033 mmol, 69% yield for the 2 steps).

Acknowledgment. We thank the Australian Research Council, The Endeavour Fellowship Scheme (to P. M.), and the University of Wollongong for financial support.

Supporting Information Available: Copies of the ^{1}H NMR and ^{13}C NMR spectra of compound **8** and the ^{1}H NMR spectra of the originally partially purified fraction containing compounds **1**, **3**, and **8** and the recently partially purified fraction from the original crude extract containing compounds **1**, **3**, and **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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NP800755P