Phytochemical Investigations of *Stemona curtisii* and Synthetic Studies on Stemocurtisine Alkaloids

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The isolation of two new *Stemona* alkaloids, 1-hydroxyprotostemonine and stemocurtisine *N*-oxide, and a new benzofuran, stemofuran L, from the root extracts of *Stemona curtisii* is reported. The major known alkaloids from this plant extract, stemocurtisine, stemocurtisinol, and oxyprotostemonine, were also isolated along with oxystemokerrine *N*-oxide. The nonalkaloid components of this extract included a new benzofuran derivative, stemofuran L, the known stemofurans F, J, and K, dihydro-γ-tocopherol, and stigmasterol. Stemocurtisine and stemocurtisinol were converted to their respective *N*-oxides by oxidation. Stemocurtisine was converted to a tetracyclic derivative by oxidative cleavage of the γ-butyrolactone ring, while stemocurtisinol gave a novel lactam derivative by oxidative cleavage of the C-4 side chain under basic conditions. The acetylcholinesterase inhibitory activities of some known and new alkaloids and their derivatives are also reported. All were 10−20 times less active as acetylcholinesterase inhibitors than the pyrrolo[1,2-*a*]azepine *Stemona* alkaloids stemofoline and 1′,2′-didehydrostemofoline. None of the stemofuran compounds showed significant antibacterial or antifungal activities.

The *Stemona* family of alkaloids includes more than one hundred different natural products,[1,2] which have been structurally classified by Pilli into eight different groups.[1] The pyrrolo[1,2-*a*]azepine nucleus is common to six of these groups, while a pyrido[1,2-*a*]azepine ring system is found in the more recently discovered Stemocurtisine group of *Stemona* alkaloids.[1−10] A miscellaneous group comprising five *Stemona* alkaloids has also been identified.[1] Greger has recently classified the *Stemona* alkaloids into three skeletal types based on their proposed biosynthetic origins.[2]

In 2003 we reported the isolation of the first pyrido[1,2-*a*]azepine *Stemona* alkaloid, stemocurtisine (**1**), from the root extracts of *Stemona curtisii* found growing in Trang Province in Southern Thailand.[3] Subsequently Hofer and Greger reported the identification of five new pyrido[1,2-*a*]azepine *Stemona* alkaloids, namely, oxystemokerrin (**2**), oxystemokerrine *N*-oxide, stemokerrine (**4**), methoxystemokerrine *N*-oxide, and pyridostemine (**1**) (Figure 1).[4] The latter alkaloid was identical to stemocurtisine.[4] Stemokerrine and oxystemokerrin were the major and minor phytochemicals, respectively, found in root extracts of *S. kerrii*, whereas their sample of *S. curtisii* that was collected from Satun Province in Southern Thailand contained the pyrrolo[1,2-*a*]azepines stemofoline and 1′,2′-didehydrostemofoline as the major and minor components, respectively, along with a trace amount of oxystemokerrine.[4] In 2004, we reported the isolation of stemocurtisinol (**3**) (the C-4, C-1′ diepimer of oxystemokerrine) along with oxyprotostemonine (**5**) from the same crude extract of *S. curtisii* that had previously provided us with stemocurtisine.[5] More extensive phytochemical studies by Greger on the root extracts of different *Stemona* species growing in Thailand have shown the presence of pyrido[1,2-*a*]azepine *Stemona* alkaloids. This study included *S. curtisii* from Krabi Province, which contained significant amounts of oxystemokerrin and its *N*-oxide. One sample from Chumphon (HG 920) showed the presence of stemocurtisine, oxystemokerrin, the *N*-oxide of oxystemokerrin, and stemocurtisinol.[6] Ye et al.[9,10] have also found novel pyrido[1,2-*a*]azepine *Stemona* alkaloids from the extracts of *S. cochinchinensis*[9] and *S. saxorum*[10] plants growing in China and Vietnam, respectively.

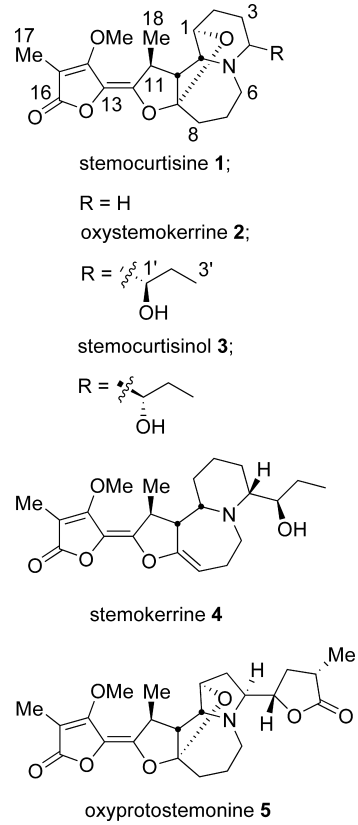

Figure 1. Previously reported alkaloids.

We report here an extension of our study on the extracts of the roots of *S. curtisii* that were collected in Trang Province in Thailand in May 2008 at the same location as our 2003 plant material. The main purpose of this investigation was to identify the minor alkaloid components and to examine the nonalkaloid phytochemicals. In

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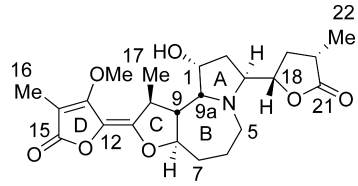
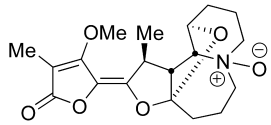


1-hydroxyprotostemonine **6**



7

Figure 2. New alkaloids **6** and **7**.

addition, we had planned to obtain sufficient quantities of stemocurtisine and stemocurtisinol to prepare analogues for biological testing.

Results and Discussion

Successive separations of the crude alkaloid material (2.6 g) from the roots of *S. curtisii* by column chromatography, and in some cases preparative TLC, gave the known alkaloids stemocurtisine[3] (269 mg), stemocurtisinol[5] (84 mg), oxyprotostemonine[4] (34 mg), and stemokerrine *N*-oxide (2 mg).[4] Two new alkaloids, 1-hydroxyprotostemonine (**6**) (1.1 mg) and stemocurtisine *N*-oxide (**7**) (13 mg), were also isolated (Figure 2). The ESI mass spectrum of **6** (*m*/*z* 434 $[M + H]^+$) indicated the possibility of a dihydro derivative of oxyprotostemonine. This was confirmed from a HRESIMS determination, which indicated the molecular formula of $C_{23}H_{31}NO_7$. The ^1H and ^{13}C NMR analysis of **6** indicated the presence of the two butyrolactone ring systems found in oxyprotostemonine, which ruled out the possibility of an 11,12-dihydro derivative of oxyprotostemonine. Such dihydro *Stemona* alkaloid derivatives are known in the case of the pyrrolo[1,2-*a*]azepine *Stemona* alkaloids.[9c,10a,11] The ^{13}C NMR analysis of **6** indicated that the quaternary resonance at 120.7 ppm associated with the C-8 acetal carbon in the ^{13}C NMR of oxyprotostemonine[4] was replaced with a methine resonance at 87.4 ppm in 1-hydroxyprotostemonine. The presence of the hydroxyl group in **6** at C-1 was verified by HMBC (correlations between C-1 and H-2 and H-9) and NOESY experiments (correlations between H-1 and H-2β, H-9a, and H-10). The latter experiments and molecular modeling studies confirmed the relative α-orientation of this hydroxyl group (Figure 3, see Table 1 of the Supporting Information). NOESY NMR experiments confirmed the relative configurations at C-3 (correlations between H-3 and H-2α) and H-18 (correlation between H-18 and H-2β) and the relative configuration of the C-3 butyrolactone ring (correlations between H-18 and H-19β, H-19β, and H-20) (Figure 3). The relative *trans*-configuration at the B−C ring junction in **6** was deduced from the observed NOESY correlations between H-8 and H-7β and H-6β (Figure 3). Correlations between H-9 and H-5β and H-5β and H-6β gave further support to our assignment of H-6β and thus the relative configuration at C-8 (Figure 3). The C-8 configuration was also evident from the multiplicity and the magnitude of the proton−proton coupling constants for H-8 when compared to those of H-8 in protostemonine, whose structure was unequivocally determined by a single-crystal X-ray analysis.[4] Thus 1-hydroxyprotostemonine may have arisen from an enzymatically catalyzed reductive ring-opening reaction of the C-1 to C-8 ether linkage of oxyprotostemonine or the direct hydroxylation at C-1 of protostemonine itself.

The ESI mass spectrum of **7** (*m*/*z* 364 $[M + H]^+$) suggested a structure arising from the addition of one oxygen atom to stemocurtisine. This was confirmed from a HRESIMS determination, which indicated the molecular formula of $C_{19}H_{25}NO_6$. This structure was further confirmed by its synthesis from stemocurtisine by oxidation with 30% H_2O_2/Na_2WO_4/MeOH at rt for 22 h. This reaction was very slow and resulted in a low isolated yield (25%) of **7** because of the presence of a significant amount of unreacted starting material due to the relatively hindered nature of the tertiary amino group. A comparison of the ^{13}C/DEPT NMR spectra of **7** with that of stemocurtisine showed that the main differences between them were the chemical shifts of the ^{13}C NMR signals for C-4, C-6, and C-10a. The chemical shifts of these carbons for stemocurtisine were δ 53.6, 53.0, and 62.0, respectively, whereas the chemical shifts for these carbons for **7** (δ 69.6 (C-4), 66.4 (C-6), and 82.3 (C-10a)) were significantly downfield and consistent with an *N*-oxide structure.[12]

A crude ethanol extract (100 g) of the roots of *S. curtisii* was partitioned between a mixture of water and petroleum ether. The petroleum ether solution was evaporated to provide 7.27 g of crude material. Successive separations of this material by column chromatography gave dihydro-γ-tocopherol[13] (16 mg) and stigmasterol[14] (16 mg). Hofer and Greger have identified dihydrotocopherols in a number of *Stemona* species.[13] Dihydro-γ-tocopherol was recognized as the major dihydrotocopherol in the root extracts of *S. curtisii* from Krabi Province in Thailand and was found to be a minor component of other *S. curtisii* plant extracts from other provinces. This compound was also found to be a minor component in the root extracts of *S. cochinchinensis*.[13] The aqueous phase was extracted with CH_2Cl_2, and the removal of the solvent provided 3.58 g of crude material. Successive separations of this material by column chromatography and in some cases preparative TLC (see Experimental Section for specific details) gave the known benzofurans stemofuran F (6 mg), J (4 mg), and K (3 mg)[15] and the new compound stemofuran L (**8**) (4 mg). The structure of **8**

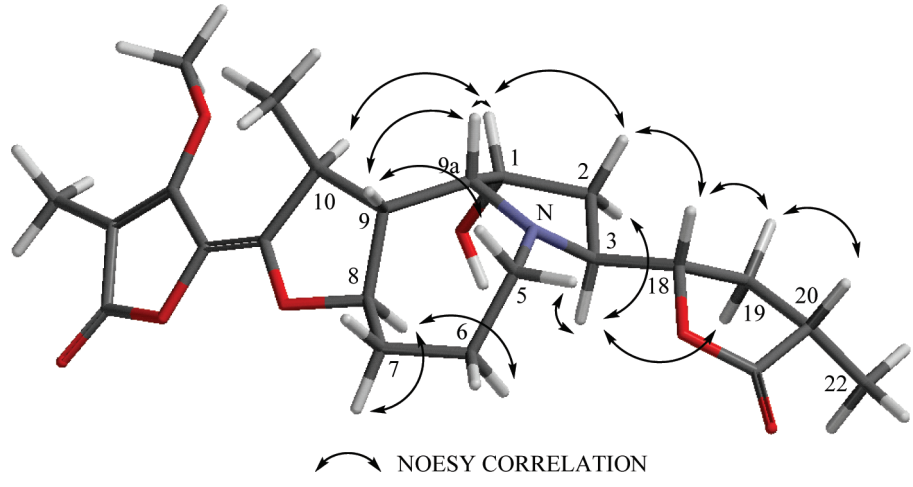
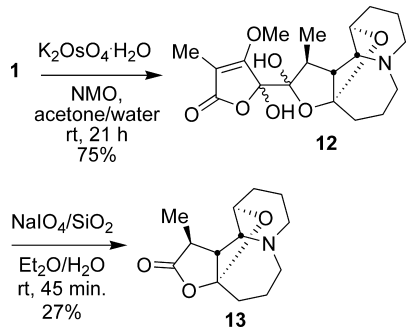
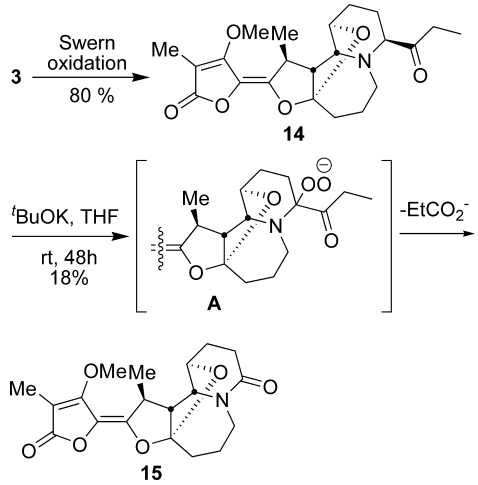


Figure 3. Spartan (AM1)-generated structure of 1-hydroxyprotostemonine (**6**) showing key NOESY correlations.

Scheme 1



Scheme 2

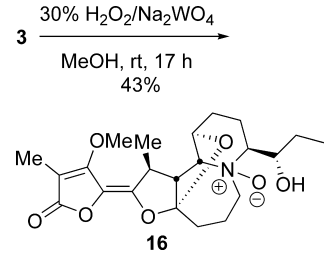


was deduced from its HREIMS (*m*/*z* 268.109537 $[M]^{\cdot+}$, calcd for $C_{17}H_{16}O_3$ 268.109945) and an interpretation of its relatively simple NMR spectra. The ^{1}H NMR showed resonances for five benzofuran protons at 7.62 (d, 1H, J = 7.5 Hz, H-2), 7.50 (d, 1H, J = 8.0, H-5), 7.30 (ddd, J = 8.0, 8.0, 1.5 Hz, 1H, H-4), 7.28 (ddd, J = 8.0, 8.0, 1.5 Hz, 1H, H-3), and 6.63 (s, 1H, H-1″) ppm. The substituted phenyl group showed a singlet aromatic proton signal at 6.51 (s, 1H, H-6′) ppm, a hydroxyl resonance at 5.20 (s, 1H, 3′-OH) ppm, and resonances for a methoxy group (3.78 (s, 3H, 5′-OCH_3) ppm) and two aromatic methyl groups (2.01 (s, 3H, 2′-CH_3) and 1.98 (s, 3H, 4′-CH_3) ppm). The positions of these substituents were established from NOESY and HMBC NMR experiments (see Table 2 of the Supporting Information for details). In particular the diagnostic NOESY correlations were between H-1″ and the 2′-CH_3, between H-6′ and the 5′-OMe, and between 4′-CH_3 and the 3′-OH and the 5′-OCH_3.

We have previously shown that the pyrrolo[1,2-*a*]azepine *Stemona* alkaloids stemofoline and 1′,2′-didehydrostemofoline have strong inhibitory activities against acetylcholinesterase (AChE) in a TLC bioautography assay.[16] These compounds had minimum inhibitory requirements of 10 and 5 ng, respectively, but were not as active as the positive control galanthamine, which had a minimum inhibitory requirement of 1 ng.[16] In order to examine the AChE activities of the natural pyrido[1,2-*a*]azepine *Stemona* alkaloids and their derivatives, the compounds **13**, **15**, and **16** were prepared as shown in Schemes 1−3. Stemocurtisine underwent an osmium-catalyzed *cis*-dihydroxylation reaction[17] to give the diol **12** as a single diastereomer in 75% yield (Scheme 1). The stereochemistry of this diol was not determined. Oxidative cleavage of the diol **12** using sodium periodate on silica gel[18] gave the lactone **13** in 17% yield (Scheme 1). The corresponding maleic anhydride byproduct was not isolatable.

Swern oxidation[19] of stemocurtisinol gave the ketone **14** in 80% yield. We next attempted to epimerize ketone **14** at C-4 to provide the more stable C-4 pseudoequatorial epimer by treatment with base

Scheme 3



(KO^{t}Bu) under a nitrogen atmosphere at rt for 48 h. TLC analysis showed starting compound **14** along with a new more polar product. After separation of this reaction mixture by column chromatography, the novel lactam **15** was isolated in 18% yield (Scheme 2). The structure of **15** was fully supported from its MS spectrometric and NMR spectroscopic data including a new lactam carbonyl resonance for C-4 at 173.6 ppm in the ^{13}C NMR spectrum. We assume that this compound arises from the presence of traces of oxygen (autoxidation), which results in the α-hydroperoxy ketone intermediate **A**, which further undergoes cleavage to lactam **15** (Scheme 2).[20]

Treatment of stemocurtisinol **3** with 30% H_2O_2/Na_2WO_4/MeOH at rt for 17 h gave the corresponding *N*-oxide **16** in 43% yield, the relatively low yield being due to a slow and incomplete reaction.

With the *N*-oxides **7** and **16** in hand we examined the crude root extract and the crude alkaloid extract for the presence of these compounds by TLC and ^{1}H NMR analysis. From these analyses we could not detect the presence of either of these compounds, suggesting that either they are artifacts or the method of detection was not sensitive enough.

The insecticidal activity shown by the root extracts of *Stemona* plants has been closely associated with the acetylcholinesterase inhibitory activities of their alkaloid components.[2,3,11,21] Compounds stemocurtisine, stemocurtisinol, oxyprotostemonine, **7**, **13**, **15**, and **16** were therefore screened by TLC bioautography for their AChE inhibitory activities using the method of Hostettmann et al.[22] and galanthamine and 1′,2′-didehydrostemofoline as positive controls. The results are shown in Table 1.

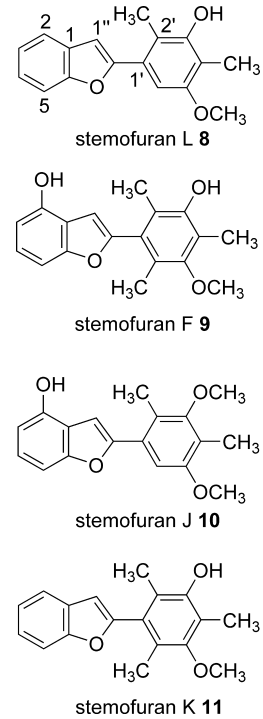


Figure 4. Stemofurans Showing Numbering According to ref 15.

Table 1. Minimum Amount of Sample Found to Inhibit AChE As Indicated by a White Zone of Inhibition

	minimum inhibitory requirement	
compound	ng	nmol
galanthamine	1	0.003
1′,2′-didehydrostemofoline	5	0.012
stemocurtisine	>1000	>2.88
stemocurtisinol	1000	2.47
oxyprotostemonine	>1000	>2.32
7	500	1.38
13	1000	2.77
15	500	2.11
16	100	0.24

In our earlier studies on the AChE inhibitory activities of several pyrrolo[1,2-*a*]azepine *Stemona* alkaloids and their analogues we found that 1′,2′-didehydrostemofoline and (1′*R*)-hydroxystemofoline were the most active compounds, with minimum inhibitory requirements of 5 ng.[16] These compounds, however, were not as active as the positive control galanthamine (minimum inhibitory requirement of 1 ng). In comparison, we found that the pyrido[1,2-*a*]azepine *Stemona* alkaloids and their analogues stemocurtisine, stemocurtisinol, oxyprotostemonine, **7**, **13**, **15**, and **16** were relatively inactive as AChE inhibitors, with minimum inhibitory requirements of 100−1000 ng (Table 1). These activities were about 10−20 times less than the reported pyrrolo[1,2-*a*]azepine *Stemona* alkaloids.[16]

The antimicrobial activities of dehydro-γ-tocopherol and stemofurans F, J, K, and L were tested against two Gram-negative bacteria, *Escherichia coli* and *Klebsiella pneumoniae,* and three Gram-positive bacteria, *Staphylococcus aureus*, methicillin-resistant *S. aureus*, and *Streptococcus pyogenes*, with gentamicin as the positive control. The antifungal activities of these compounds were tested against *Candida albicans* and *Cryptococcus neoformans* with amphotericin B as the positive control. These assays were carried out using known literature procedures.[23] The results indicated that these compounds are weakly active, with MIC values of 250 μg/mL.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a JASCO DIP-370 polarimeter. IR spectra were obtained on a Nicolet-Avatar 360 FTIR spectrophotometer. ^{1}H (500 MHz), ^{13}C (125 MHz), and 2D NMR spectra were recorded on a Varian Unity 500 spectrometer. ^{1}H NMR coupling constants (*J*) are given in Hz. High-resolution ESIMS were obtained with a Micromass QTOF 2 mass spectrometer using a cone voltage of 30 V and polyethyleneglycol as an internal reference. TLC analysis was performed on aluminum-backed Merck 60 GF_{254} silica gel, and bands were detected by UV light (λ 254 nm) and Dragendorff's reagent. Column chromatography was performed using Merck GF_{254} flash silica gel (40−63 μm). Acetylcholinesterase (906 U/mg, from electric eel) was purchased from Sigma-Aldrich.

Plant Materials. The roots of *S. curtisii* (Stemonaceae) were collected in Trang Province, Thailand, in May 2008. A voucher specimen (number 17581) was deposited at the herbarium of the Department of Biology, Chiang Mai University. Plant material was identified by Mr. James F. Maxwell from the Department of Biology, Chiang Mai University.

Isolation of Alkaloids 1, 3, 5, 7, and 8. Extraction and Isolation. The dry ground root of *S. curtisii* (2 kg) was extracted with 95% EtOH (3 × 1200 mL) over 3 days at rt. The solution was evaporated to give a dark residue (220 g). A 100 g sample of the ethanolic extract was partitioned between water (200 mL) and CH_2Cl_2 (400 mL). The CH_2Cl_2 solution was extracted with 5% aqueous HCl solution, and the aqueous solution was made basic with aqueous NH_3 and extracted with CH_2Cl_2 to afford 2.6 g of crude alkaloid material. This material (2.6 g) was purified by column chromatography on silica gel (200 mL) using gradient elution from 100% CH_2Cl_2 to MeOH/CH_2Cl_2/28% NH_3 (50:90:1) as eluent. On the basis of TLC analysis these fractions were pooled to give six fractions. Fraction 1 (204 mg) was repurified on silica gel using gradient elution from 100% CH_2Cl_2 to MeOH/CH_2Cl_2/28% NH_3 (1:99:1) as eluent to give stemocurtisinol (31.5 mg). Fraction 2 (270 mg) was repurified on silica gel using gradient elution from 100% EtOAc to MeOH/EtOAc/28% NH_3 (1:99:1) as eluent to give stemocurtisinol (52.2 mg) and oxyprotostemonine (33.6 mg). Fraction 5 (677 mg) was repurified on silica gel using gradient elution from 100% EtOAc to EtOAc/MeOH/28% NH_3 (5:95:1) and MeOH/CH_2Cl_2/28% NH_3 (5:95:1) to MeOH/CH_2Cl_2/28% NH_3 (20:80:1) as eluent. On the basis of TLC analysis these fractions were combined to give five fractions. Fraction 2 (15.7 mg) was purified by preparative TLC (EtOAc/MeOH/28% NH_3, 96:4:1) to give oxystemokerrine *N*-oxide (2.2 mg). Fraction 4 (403.9 mg) was repurified on silica gel using gradient elution from 100% CH_2Cl_2 to MeOH/CH_2Cl_2/28% NH_3 (20:80:1) to give two fractions based on TLC analysis. Fraction 1 (293 mg) was repurified on silica gel using gradient elution from 100% CH_2Cl_2 to MeOH/CH_2Cl_2/28% NH_3 (10:90:1) to give stemocurtisine (258.6 mg). Fraction 2 (30.5 mg) was repurified by preparative TLC (CH_2Cl_2/MeOH/28% NH_3, 10:90:1) to give stemocurtisine (10.2 mg), **6** (1.1 mg), and **7** (12.5 mg). The ^{1}H and ^{13}C NMR data of compounds stemocurtisine,[3] stemocurtisinol,[5] oxyprotostemonine,[4] and oxystemokerrine *N*-oxide[4] were identical to those reported.

1-Hydroxyprotostemonine (6): pale yellow gum; ^{1}H NMR (CD_3OD) δ 4.96 (dt, J = 3.8, 10.5 Hz, 1H, H-8), 4.41 (t, J = 3.9 Hz, 1H, H-1), 4.38−4.36 (m, 1H, H-18), 4.19 (br s, 3H, O−CH_3), 3.70−3.60 (m, 1H, H-9a), 3.70−3.60 (m, 1H, H-3), 3.70−3.60 (m, 1H, H-5β), 3.37−3.34 (m, 1H, H-10), 3.01 (dd, J = 6.5, 9.0, 1H, H-5α), 2.74−2.68 (m, 1H, H-20), 2.46−2.42 (m, 1H, H-19β), 2.29−2.26 (m, 1H, H-7α), 2.22−2.19 (m, 1H, H-9), 2.05 (s, 3H, H-16), 1.85 (dd, J = 6.0, 13.0 Hz, 1H, H-2α), 1.83−1.78 (m, 1H, H-6β), 1.83−1.78 (m, 1H, H-6α), 1.71−1.67 (m, 1H, H-2β), 1.61−1.51 (m, 1H, H-19α), 1.42 (d, J = 5.5 Hz, 1H, H-7β), 1.39 (d, J = 7.0 Hz, 3H, H-17), 1.21 (d, J = 7.0 Hz, 3H, H-22). ^{13}C NMR (CD_3OD) δ 181.7 (C-21), 173.0 (C-15), 165.8 (C-13), 153.0 (C-11), 125.6 (C-12), 97.3 (C-14), 87.4 (C-8), 83.3 (C-18), 71.9 (C-1), 63.9 (C-9a), 59.8 (O-CH_3), 48.7 (C-5), 40.9 (C-10), 37.5 (C-2), 35.9 (C-20), 35.5 (C-19), 35.0 (C-7), 21.9 (C-6), 20.8 (C-17), 14.9 (C-22), 8.9 (C-16); HRESIMS *m/z* 434.2194 $[M + H]^{\cdot+}$, calcd for $C_{23}H_{32}NO_7$ 434.2179.

Stemocurtisine *N*-oxide (7): pale yellow gum; $[\alpha]^{23}_D$ 264.0 (*c* 0.62, $CHCl_3$); IR ν_{max} 3441, 2924, 2366, 2330, 1744, 1685, 1618, 1454, 1214, 1067, 860 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.31 (br s, 1H, H-1), 4.13 (s, 3H, O-CH_3), 4.13−4.11 (m, 1H, H-6a), 3.44 (br s, 1H, H-4a), 3.43 (br s, 1H, H-10), 3.66 (br s, 1H, H-10a), 3.31−3.26 (m, 1H, H-4b), 3.03−2.99 (m, 1H, H-6b), 2.98−2.90 (m, 1H, H-11), 2.20−2.15 (m, 1H, H-8a), 2.13−2.12 (m, 1H, H-2a), 2.13−2.12 (m, 1H, H-3a), 1.90−1.80 (m, 2H, H-7), 2.06 (s, 3H, H-17), 2.02−2.00 (m, 1H, H-8b), 1.87−1.83 (m, 1H, H-3b), 1.79−1.72 (m, 1H, 2b), 1.42 (d, J = 7.0 Hz, 3H, H-18). ^{13}C NMR ($CDCl_3$) δ 169.6 (C-16), 162.6 (C-14), 146.9 (C-12), 124.5 (C-13), 119.2 (C-9), 97.7 (C-15), 82.3 (C-10a), 75.5 (C-1), 69.6 (C-4), 66.4 (C-6), 59.0 (O−CH_3), 51.0 (C-10), 38.9 (C-11), 32.0 (C-8), 22.4 (C-18), 21.8 (C-2), 19.9 (C-3), 18.9 (C-7), 9.1 (C-17); HRESIMS *m/z* 364.1748 $[M + H]^{\cdot+}$, calcd for $C_{19}H_{26}NO_6$ 364.1760.

Oxidation of Stemocurtisine (1) to Stemocurtisine *N*-oxide (7). To a solution of **1** (13.0 mg) and Na_2WO_4 (3.7 mg) in MeOH (1.0 mL) at 0 °C was added dropwise 30% H_2O_2 (0.2 mL).[8] The mixture was left to stir at rt for 22 h. Excess H_2O_2 was destroyed by the addition of MnO_2 (5.0 mg), and iodide paper was used to ensure the absence of H_2O_2 in the reaction mixture. The mixture was then filtered through a small pad of Celite and further washed with MeOH. The solvent was removed under reduced pressure, and the crude product was purified using gradient elution from 100% CH_2Cl_2 to MeOH/CH_2Cl_2/28% NH_3 (5:95:1) to give compound **7** as a pale yellow gum (3.3 mg, 25% yield). This material was identical by ^{1}H NMR and TLC analysis to stemocurtisine *N*-oxide **7** that was isolated from the root extract of *S. curtisii* as described above.

Isolation of Non-alkaloid Compounds. A portion of the ethanolic extract (100.0 g) was partitioned between petroleum spirits (2 × 200 mL) and water (300 mL) to yield 7.27 g of material after evaporation of the petroleum spirits. The aqueous phase was then extracted with $CHCl_3$. The $CHCl_3$ extracts were washed with 3% HCl solution and then evaporated to yield 3.58 g of the non-alkaloid fraction.

The petroleum spirits crude extract (7.12 g) was chromatographed on flash silica gel (600 mL) using gradient elution from 100% petroleum spirits to 100% CH_2Cl_2 and then CH_2Cl_2/MeOH (80:20) to give 13 fractions. Fraction 5 (105.8 mg) was purified by column chromatog-

raphy using gradient elution with petroleum spirits/CH_2Cl_2 (100:0 to 0:100) to yield 10 fractions (fractions 5.1−5.10). Further purification of fraction 5.7 (54.5 mg) by preparative TLC using EtOAc/petroleum spirits (5:95) as the eluent gave dihydro-γ-tocopherol[13] (16.3 mg). Fraction 9 (174.0 mg) was purified by recrystallization from ethanol to give stigmasterol (16 mg).[14] The ^{1}H and ^{13}C NMR data of dihydro-γ-tocopherol[13] and stigmasterol[14] were identical to those reported.

The $CHCl_3$ crude extract from above (3.22 g) was purified on flash silica gel (300 mL) using gradient elution from 100% CH_2Cl_2 to 50% MeOH/CH_2Cl_2 to give 15 fractions. Fraction 6 (23.0 mg) was separated by preparative TLC using 100% CH_2Cl_2 as the eluent to give stemofuran L (**8**) (4.0 mg) and stemofuran K (**11**) (3.0 mg). Fraction 8 (176.0 mg) was applied to a silica gel column using gradient elution with CH_2Cl_2/MeOH (100:0 to 97:3) to give six fractions (fractions 8.1−8.6). Further purification of fraction 8.5 (27.0 mg) by preparative TLC (100% CH_2Cl_2) gave stemofuran J (**10**) (4.0 mg). Fraction 12 (273.0 mg) was purified by column chromatography using gradient elution with CH_2Cl_2/MeOH (100:0 to 97:3) to yield six fractions (fractions 12.1− 12.6). Fraction 12.1 (45.0 mg) was purified by preparative TLC (100% CH_2Cl_2) to give stemofuran F **9** (4.0 mg). Fraction 13 (130.0 mg) was applied to a silica gel column using gradient elution with CH_2Cl_2/MeOH (100:0 to 95:5) to afford stemofuran F (**9**) (2.0 mg). The ^{1}H and ^{13}C NMR data of compounds **9**−**11** were identical to those reported.[15]

Stemofuran L (8): light brown gum; ^{1}H NMR ($CDCl_3$) δ 7.62 (d, J = 7.5 Hz, 1H, H-2), 7.50 (d, J = 8.0 Hz, 1H, H-5), 7.30 (ddd, J = 8.0, 8.0, 1.5 Hz, 1H, H-4), 7.28 (ddd, J = 8.0, 8.0, 1.5 Hz, 1H, H-3), 6.63 (s, 1H, H-1″), 6.51 (s, 1H, H-6′), 5.00 (br s, 3′-OH), 3.78 (s, 3H, 5′-O-CH_3), 2.01 (s, 3H, 2′-CH_3), 1.98 (s, 3H, 4′-CH_3); ^{13}C NMR ($CDCl_3$) δ 156.2 (C-5′), 154.7(C-2″), 154.5 (C-6), 152.2 (C-3′), 132.3 (C-1′), 128.7 (C-1), 123.8 (C-4), 122.6 (C-3), 120.8 (C-2), 119.5 (C-4′), 115.5 (C-2′), 111.2 (C-5), 106.3 (C-1″), 100.1 (C-6′), 55.7 (5′-O-CH_3), 12.9 (2′-CH_3), 12.6 (4′-CH_3); HREIMS m/z 268.1095 $[M]^{\cdot+}$, calcd for $C_{17}H_{16}O_3$ 268.1099.

Dihydroxylation of Stemocurtisine to Diol 12. To a stirred solution of stemocurtisine (47.7 mg, 0.137 mmol) in acetone (2.0 mL) and water (1.0 mL) were added $K_2OsO_4 \cdot H_2O$ (10.13 mg, 0.027 mmol) and NMO (64.19 mg, 0.55 mmol).[17] The mixture was stirred at rt for 21 h. Saturated Na_2SO_3 solution was added, the mixture was extracted with CH_2Cl_2 (4 × 20 mL), and the combined extracts were dried over Na_2SO_4. The solvent was removed under reduced pressure, and the crude product was purified by column chromatography using gradient elution from 100% CH_2Cl_2 to MeOH/CH_2Cl_2/28% NH_3 (6:94:1) to give **12** as a white gum (31.0 mg, 75% yield): $[\alpha]^{23}_D$ −35.0 (c 0.44, $CHCl_3$); IR ν_{max} 3395, 2929, 1751, 1670, 1460, 1329, 1142, 996 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.19 (br s, 1H, H-1), 4.11 (s, 3H, OMe), 3.34−3.28 (m, 1H, H-10a), 3.60−3.20 (m, 1H, H-6a), 3.34−3.20 (m, 1H, H-6b), 2.98−2.92(m, 1H, H-4a), 2.90−2.82 (m, 1H, H-4b), 2.73−2.70 (m, 1H, H-10), 2.67−2.60 (m, 1H, H-11), 2.30−2.10 (m, 1H, H-8a), 2.23−2.10 (m, 1H, H-7a), 1.99 (s, 3H, H-17), 1.98−1.90 (m, 1H, H-7b), 1.88−1.78 (m, 1H, H-3a), 1.70−1.62 (m, 1H, H-2b), 1.60−1.50 (m, 1H, H-2a), 1.60−1.50 (m, 1H, H-8b), 1.25−1.19 (m, 1H, H-3b), 1.10 (d, J = 6.5 Hz, 3H, H-18); ^{13}C NMR ($CDCl_3$) δ 172.0 (C-16), 168.0 (C-14), 167.0 (C-13), 117.0 (C-9), 107.0 (C-12), 101.0 (C-15), 74.2 (C-1), 60.4 (C-10a), 59.1 (O-CH_3), 56.9 (C-10), 53.4 (C-4), 52.8 (C-6), 39.6 (C-11), 36.4 (C-8), 26.8 (C-2), 26.7 (C-7), 18.7 (C-3), 14.0 (C-18), 8.5 (C-17); HRESIMS m/z 382.1868 $[M + H]^{\cdot+}$ calcd for $C_{19}H_{28}NO_7$ 382.1866.

Cleavage of Diol 12 to 13. To a stirred suspension of silica gel (95.7 mg) and diethyl ether (0.5 mL) was added a solution of $NaIO_4$ (9.40 mg, 0.0439 mmol) in water (0.1 mL).[18] A solution of **12** (12.9 mg, 0.0338 mmol) in CH_2Cl_2 (1.0 mL) was added to the above silica gel/$NaIO_4$ mixture, which was left to stir at rt for 45 min. The mixture was filtered, and the silica gel was washed with MeOH/CH_2Cl_2/28% NH_3 (5:95:1). The mixture was purified by column chromatography using 100% CH_2Cl_2 to MeOH/CH_2Cl_2/28% NH_3 (4:96:1) to give **13** as a pale yellow gum (2.2 mg, 27%): $[\alpha]^{23}_D$ −58.8 (c 0.16, $CHCl_3$); IR ν_{max} 2919, 2847, 1777, 1460, 1029, 983 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.20 (br s, 1H, H-1), 3.47−3.42 (m, 1H, H-10a), 3.41−3.35 (m, 2H, H-6), 3.09−2.90 (m, 1H, H-4a), 3.00−2.90 (m, 1H, H-4b), 2.81−2.79 (m, 1H, H-10), 2.70−2.62 (m, 1H, H-11), 2.37−2.30 (m, 1H, H-8a), 2.30−2.20 (m, 1H, H-7a), 2.10−2.00 (m, 1H, H-7b), 1.90−1.80 (m, 1H, H-3a), 1.75−1.65 (m, 2H, H-2), 1.70−1.60 (m, 1H, H-8b), 1.35 (d, J = 7, 3H, O-CH_3), 1.30−1.20 (m, 1H, H-3b); ^{13}C NMR ($CDCl_3$) δ 178.1 (C-12), 115.0 (C-9), 76.0 (C-1), 60.7 (C-10a), 54.8 (C-10), 53.5 (C-4), 52.9 (C-6), 37.8 (C-11), 34.8 (C-8), 26.9 (C-2), 26.8 (C-7), 18.8 (C-3), 16.0 (C-13); HRESIMS m/z 238.1445 $[M + H]^{\cdot+}$ calcd for $C_{13}H_{20}NO_3$ 238.1443.

Synthesis of Ketone 14. To a stirred solution of oxalyl chloride (0.2 mL) in dry CH_2Cl_2 (2.0 mL) at −78 °C was added DMSO (0.3 mL).[19] The mixture was left to stir for 10 min, and then a solution of **3** (75.3 mg, 0.186 mmol) in CH_2Cl_2 (1.0 mL) was added. The reaction was left to stir for 15 min, and then Et_3N (1.0 mL) was added at −78 °C. After 4 h, the reaction was warmed to −40 °C for 4 h and then warmed to 0 °C. After 12 h, the reaction was diluted with water (1.0 mL). The organic layer was separated, and the aqueous layer was extracted with ether (5 × 20 mL). The combined organic layers were washed with brine and dried over Na_2SO_4, and the solvent was removed under vacuum. The crude product was purified by column chromatography with EtOAc as eluent to give **14** as a colorless gum (46.1 mg, 80% yield): $[\alpha]^{23}_D$ 290.0 (c 0.47, $CHCl_3$); IR ν_{max} 2962, 2929, 2873, 1741, 1621, 1211, 1041, 1024 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.12 (s, 3H, O-CH_3), 3.96 (br s, 1H, H-1), 3.60−3.50 (m, 1H, H-6a), 3.45 (d, J = 3.5 Hz, 1H, H-4), 3.44 (br s, 1H, H-10a), 3.10−3.00 (m, 1H, H-11), 2.98−2.90 (m, 1H, H-6b), 2.70 (d, J = 4 Hz, 1H, H-10), 2.62−2.50 (m, 2H, H-20), 2.37 (dd, J = 13.5, 5 Hz, 1H, H-8a), 2.05 (s, 3H, H-17), 2.00−1.90 (m, 1H, H-2a), 2.00−1.90 (m, 1H, H-3a), 1.80−1.70 (m, 1H, H-2b), 1.70−1.60 (m, 1H, H-3b), 2.00−1.88(m, 1H, H-7a), 1.80−1.70 (m, 1H, H-7b), 1.80−1.70 (m, 1H, H-8b), 1.37 (d, J = 7.0 Hz, 3H, H-18), 1.05 (t, J = 7.5 Hz, 3H, H-21); ^{13}C NMR ($CDCl_3$) δ 212.9 (C-19), 169.9 (C-16) 162.9 (C-14), 147.0 (C-12), 125.0 (C-13), 120.4 (C-9), 97.5 (C-15), 75.8 (C-1), 68.6 (C-4), 59.0 (O-CH_3), 58.9 (C-10a), 56.9 (C-10), 54.2 (C-6), 39.1 (C-11), 33.3 (C-8), 32.5 (C-20), 25.7 (C-3), 23.1 (C-2), 22.6 (C-18), 18.6 (C-7), 9.1 (C-17), 8.1 (C-21); HRESIMS m/z 404.2057 $[M + H]^{\cdot+}$ calcd for $C_{22}H_{30}NO_6$ 404.2073.

Synthesis of Lactam 15. To a stirred solution of ketone **14** (12.3 mg, 0.030 mmol) in dry THF (1.0 mL) was added KO′Bu (6.85 mg, 0.061 mmol), and the mixture was left to stir at rt. After 48 h, the reaction mixture was diluted with water and extracted with CH_2Cl_2 (4 × 20 mL). The combined organic extracts were washed with brine (10.0 mL), dried (Na_2SO_4), and filtered, and the solvent was removed under vacuum. The crude product was purified by column chromatography using 100% EtOAc to EtOAc/MeOH (1:99) as eluent to give **15** (2.0 mg, 18% yield) as a pale yellow gum: $[\alpha]^{23}_D$ 73.0 (c 0.20, $CHCl_3$); IR ν_{max} 2981, 2924, 1735, 1650, 1618, 1396, 1216, 1048, 955 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.70−4.67 (m, 1H, H-6a), 4.66−4.62 (m, 1H, H-1), 4.17 (s, 3H, O-CH_3), 3.86 (br s, 1H, H-10a), 3.20−3.12 (m, 1H, H-11), 2.89−2.87 (m, 1H, H-6b), 2.84−2.80 (m, 1H, H-10), 2.36−2.34 (m, 1H, H-3b), 2.34−2.24 (m, 1H, H-2a), 2.40−2.37 (m, 1H, H-8a), 2.16−2.10 (m, 1H, H-3a), 2.08 (s, 3H, H-17), 1.84−1.76 (m,1H, H-7b), 1.84−1.76 (m, 1H, H-8b), 1.66−1.62 (m, 1H, H-2b), 1.57−1.51 (m, 1H, H-7a), 1.42 (d, J = 7 Hz, 3H, H-18); ^{13}C NMR ($CDCl_3$) δ 173.6 (C-4), 169.6 (C-16), 162.5 (C-14), 145.6 (C-12), 125.5 (C-13), 119.9 (C-9), 97.9 (C-15), 80.1 (C-1), 61.4 (C-10 a), 58.9 (O-CH_3), 55.6 (C-10), 38.7 (C-6), 38.6 (C-11), 31.9 (C-8), 31.2 (C-2), 22.6 (C-18), 25.3 (C-7), 24.8 (C-2), 9.1 (C-17); HRESIMS m/z 362.1617 $[M + H]^{\cdot+}$ calcd for $C_{19}H_{24}NO_6$ 362.1604.

Oxidation of Stemocurtisinol to Stemocurtisinol *N*-Oxide (16). To a solution of stemocurtisinol (6 mg) and Na_2WO_4 (1.5 mg) in MeOH (1.0 mL) at 0 °C was added dropwise 30% H_2O_2 (approximately 0.01 mL).[12] The mixture was left to stir at rt for 17 h. To the reaction was added MnO_2 (5.0 mg), and iodide paper was used to ensure the absence of H_2O_2 in the reaction mixture. The mixture was then filtered through a small pad of Celite and further washed with MeOH. The solvent was removed under reduced pressure, and the crude product was purified on silica gel using gradient elution from 100% CH_2Cl_2 to MeOH/CH_2Cl_2/28% NH_3 (4:96:1) to give compound **16** as a pale yellow gum (2.7 mg, 43% yield): $[\alpha]^{23}_D$ 362.0 (c 0.27, $CHCl_3$); IR ν_{max} 3462, 2965, 1741, 1682, 1629, 1449, 1340, 1315, 1287, 1224, 1032 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.36 (br s, 1H, H-1), 4.20 (br s, 1H, H-10a), 4.15 (s, 3H, O-CH_3), 4.13−4.12 (m, 1H, H-19), 4.12−4.10 (m, 1H, H-6b), 3.45 (d, J = 3.5, 1H, H-10), 3.22−3.16 (m, 1H, H-4), 3.04−2.96 (m, 1H, H-11), 2.34−2.26 (m, 1H, H-2a), 2.07 (s, 3H, H-17), 2.26−2.18 (m, 1H, H-8a), 2.06−2.02 (m, 1H, H-8b), 2.04−1.98 (m, 1H, H-3a), 1.88−1.82 (m, 1H, H-2b), 1.84−1.75 (m, 1H, H-3b), 1.67−1.61 (m, 1H, H-7a), 1.52−1.45 (m, 1H, H-7b), 1.43 (d, J = 7 Hz, 3H, H-18), 1.25 (br s, 2H, H-20), 1.04 (t, J = 7.5 Hz, 3H, H-21); ^{13}C NMR ($CDCl_3$) δ 169.6 (C-16), 162.6 (C-14), 146.7 (C-12), 124.7 (C-13), 119.1 (C-9), 97.8 (C-15), 76.3 (C-10a), 76.0 (C-4), 75.2 (C-1), 70.6 (C-19), 70.5 (C-6), 59.0 (O−CH_3), 51.2 (C-10), 39.1 (C-11), 31.8 (C-8), 29.6 (C-20), 28.6

(C-7), 22.5 (C-18), 21.1 (C-2), 19.4 (C-3), 9.1 (C-17), 8.8 (C-21); HRESIMS *m/z* 422. 2192 $[M + H]^{\cdot+}$, calcd for $C_{22}H_{32}NO_7$ 422.2179.

Bioautography Procedure. TLC bioautography was performed using the method described by Hostettmann et al.[22] and according to our previous publication.[16]

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Supporting Information Available: Tables of ^1H, ^{13}C, gCOSY, HMBC, and NOESY NMR data for compounds **6** and **8**. Copies of the ^1H NMR spectra of compounds **6**−**8** and **12**−**16**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Pilli, R. A.; Rosso, G. B. de Oliveira, M. C. F. *The Alkaloids*, Vol. 62; Cordell, G. A., Ed; Elsevier; San Diego, 2005; Chapter *2*, pp 77−173.
(2) Greger, H. *Planta Med.* **2006**, *72*, 99–113.
(3) 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.
(4) Kaltenegger, E.; Brem, B.; Mereiter, K.; Kalchhauser, H.; Kahlig, H.; Hofer, O.; Vajrodaya, S.; Greger, H. *Phytochemistry* **2003**, *63*, 803–816.
(5) Mungkornasawakul, P.; Pyne, S. G.; Jatisatienr, A.; Supyen, D.; Jatisatienr, C.; Lie, W.; Ung, A. T.; Skelton, B. W.; White, A. H. *J. Nat. Prod.* **2004**, *67*, 675–677.
(6) Schinnerl, J.; Brem, B.; But, P. P.-H.; Vajrodaya, S.; Hofer, O.; Greger, H. *Phytochemistry* **2007**, *68*, 1417–1427.
(7) Mungkornasawakul, P.; Matthews, H.; Ung, A. T.; Pyne, S. G.; Jatisatienr, A.; Lie, W.; Skelton, B. W.; White, A. H. *ACGC Chem. Res. Commun.* **2005**, *19*, 30–33.
(8) Pyne, S. G.; Ung, A. T.; Jatisatienr, A.; Mungkornasawakul, P. *Maejo Int. J. Sci. Technol.* **2007**, *1*, 157–165.
(9) (a) Lin, L.-G.; Dien, P.-H.; Tang, C.-P.; Ke, C.-Q.; Yang, X.-Z.; Ye, Y. *Helv. Chim. Acta* **2007**, *90*, 2167–2175. (b) Dien, P.-H.; Lin, L.-G.; Tang, C.-P.; Ke, C.-Q.; Ye, Y. *Nat. Prod. Res., Part A* **2008**, *22*, 915–920. (c) Peng, S.-Y.; Shi, T.; Wang, Y.-Z.; Lin, L.-G.; Yang, Y.-M.; Jiang, H.-L.; Ye, Y. *Rapid Commun. Mass Spectrom.* **2009**, *23*, 3621–3631.
(10) (a) Wang, Y.-Z.; Tang, C.-P.; Dien, P.-H.; Ye, Y. *J. Nat. Prod.* **2007**, *70*, 1356–1359. (b) Lin, L.-G.; Tang, C.-P.; Dien, P.-H.; Xu, R.-S.; Ye, Y. *Tetrahedron Lett.* **2007**, *48*, 1559–1561.
(11) Mungkornasawakul, P.; Pyne, S. G.; Jatisatienr, A.; Lie, W.; Ung, Al. T.; Issakul, K.; Sawatwanich, A.; Supyen, D.; Jatisatienr, C. *J. Nat. Prod.* **2004**, *67*, 1740–1743.
(12) Sastraruji, T.; Jatistienr, A.; Pyne, S. G.; Ung, A. T.; Lie, W.; Williams, M. C. *J. Nat. Prod.* **2005**, *68*, 1763–1767.
(13) Brem, B.; Seger, C.; Pacher, T.; Hartl, M.; Hadacek, F.; Hofer, O.; Vajrodaya, S.; Greger, H. *Phytochemistry* **2004**, *65*, 2719–2729.
(14) Forgo, P.; Kövér, K. E. *Steroids* **2004**, *69*, 43–50.
(15) Pacher, T.; Seger, C.; Engelmeier, D.; Vajrodaya, S.; Hofer, O.; Greger, H. *J. Nat. Prod.* **2002**, *65*, 820–827.
(16) (a) Baird, M. C.; Pyne, S. G.; Ung, A. T.; Lie, W.; Sastraruji, T.; Jatisatienr, A.; Jatisatienr, C.; Dheeranupattana, S.; Lowlam, J.; Boonchalermkit, S. *J. Nat. Prod.* **2009**, *72*, 679–684. (b) Sastraruji, K.; Sastraruji, T.; Pyne, S. G.; Ung, A. T.; Jatisatienr, A.; Lie, W. *J. Nat. Prod.* **2010**, *73*, 935–941.
(17) (a) Davis, A. S.; Pyne, S. G.; Skelton, B. W.; White, A. H. *J. Org. Chem.* **2004**, *69*, 3139–3143. (b) Ritthiwigrom, T.; Pyne, S. G. *Org. Lett.* **2008**, *10*, 2769–2771.
(18) Zhong, Y. L.; Shing, T. K. M. *J. Org. Chem.* **1997**, *62*, 2622–2624.
(19) Mancuso, A. J.; Swern, D. *Synthesis* **1981**, *165*, 185.
(20) (a) Sawaki, Y.; Ogata, Y. *J. Am. Chem. Soc.* **1975**, *97*, 6983–6989. (b) Sakurai, H.; Kamiya, I.; Kitahara, H.; Tsunoyama, H.; Tsukuda, T. *Synlett* **2009**, *245*, 248.
(21) Wang, P.; Liu, A.-L.; An, Z.; Li, Z.-H.; Du, G. -H.; Qin, H.-L. *Chem. Biodiversity* **2007**, *4*, 523–530.
(22) Marston, S.; Kissling, J.; Hostettmann, K. *Phytochem. Anal.* **2002**, *13*, 51–54.
(23) Washington, J. A. *Laboratory Procedures in Clinical Microbiology*, 2nd ed.; Springer-Verlag: New York, 1985.

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