Phytochemical Studies on *Stemona* Plants: Isolation of Stemofoline Alkaloids

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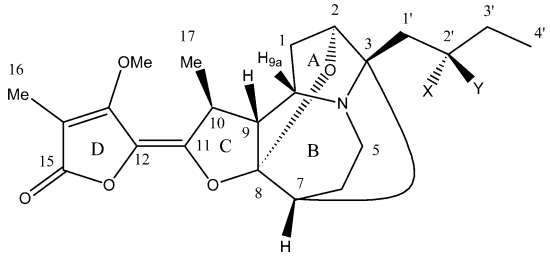
Six new stemofoline alkaloids, (2′*R*)-hydroxystemofoline (**5**), (3′*R*)-stemofolenol (**6**), (3′*S*)-stemofolenol (**7**), 1′,2′-didehydrostemofoline-*N*-oxide (**8**), the first C_{19} stemofoline alkaloid, methylstemofoline (**9**), and the first glycosidated *Stemona* alkaloid, stemofolinoside (**10**), and three known alkaloids, (2′*S*)-hydroxystemofoline (**2**), (11*Z*)-1′,2′-didehydrostemofoline (**3**), and (11*E*)-1′,2′-didehydrostemofoline (**4**), have been isolated from a root extract of an unidentified *Stemona* species. The structure and relative configuration of these new alkaloids have been determined by spectral data interpretation and from semisynthetic studies.

The *Stemona* family of alkaloids includes more than seventy different natural products that have been structurally classified into eight different groups.[1,2] The pyrrolo[1,2-*a*]azepine (5,7-bicyclic A,B-ring system) nucleus is common to all compounds in six of these groups, while a pyrido[1,2-*a*]azepine A,B-ring system (6,7-bicyclic A,B-ring system) is found in the more recently discovered stemoncurtisine group of *Stemona* alkaloids.[1,3−6] A miscellaneous group comprising five *Stemona* alkaloids has also been identified.[1] The pure alkaloids derived from extracts of the leaves and roots of *Stemona* species have insect toxicity, antifeedant and insect repellent activities,[1,4,5,7−9] and antitussive activities.[10] We report here the isolation and structure determination of six novel stemofoline alkaloids, as well as three known ones, from the root extracts of an unidentified *Stemona* species (*Stemona* sp.). The intact plants were collected at Amphur Chattrakarn, Phitsanulok, Thailand.

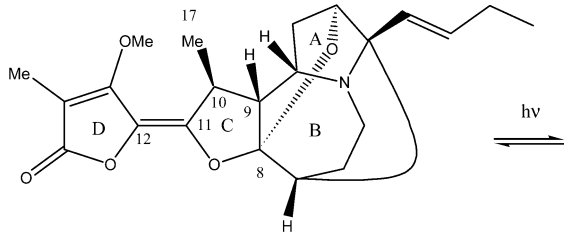
The stemofoline group of *Stemona* alkaloids comprises nine alkaloids,[1] including stemofoline (**1**),[1,4,7,8,11,12] (2′*S*)-hydroxystemofoline (**2**),[1,4,7,9,11] (11*Z*)-1′,2′-didehydrostemofoline (**3**),[4,7,8,11] and (11*E*)-1′,2′-didehydrostemofoline (**4**).[8] The absolute configurations of **1** and **2** have been determined by X-ray crystallographic studies on their HBr salt[12] and CH_2Cl_2 solvate,[11] respectively. The enantiomer (parvistemoninine)[13] and the 4′-hydroxy (oxystemofoline and its proposed enantiomer, parvistemoninol)[1,13] and the 4′-methoxy (methoxystemofoline) derivatives of **1**[1] have also been isolated in addition to two dihydrostemofolines, (11*S*,12*R*)-dihydrostemofoline and stemoburkilline.[9]

Result and Discussion

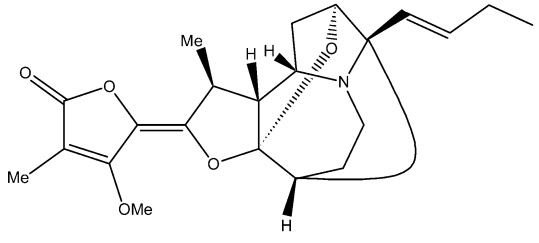
A crude ethanol extract (58.8 g) of the roots of *Stemona* sp. was partitioned between 5% hydrochloric acid solution and CH_2Cl_2. The aqueous solution was made basic with aqueous ammonia and extracted with CH_2Cl_2 to afford 0.732 g of crude alkaloid material. Successive purifications of this material by column chromatography and preparative TLC gave pure samples of (2′*S*)-hydroxystemofoline (**2**), (11*Z*)-1′,2′-didehydrostemofoline (**3**), (11*E*)-1′,2′-didehydrostemofoline (**4**, containing 10% of **3**), (2′*R*)-hydroxystemofoline (**5**), (3′*R*)-stemofolenol (**6**), (3′*S*)-stemofolenol (**7**), 1′,2′-didehydrostemofoline-*N*-oxide (**8**), methylstemofoline (**9**), and stemofolinoside (**10**).



1, X = H, Y = H
(2′*S*)-**2**, X = OH, Y = H



11(*Z*)-**3**



11(*E*)-**4**

^{1}H NMR analysis of these alkaloids readily identified them as stemofoline derivatives with characteristic methyl resonances in the range δ 4.07−4.15 (s) for the C-13 methoxy group, δ 2.00−2.08 (s) for C-16, and δ 1.30−1.39 (d) for C-17. Other diagnostic peaks were the doublet (J = 5−7 Hz) signal in the range δ 2.68−2.88 for H-7 and two broad singlets in the ranges δ 4.21−4.36 and 3.34−3.57 for H-2 and H-9a, respectively. All alkaloids had large and

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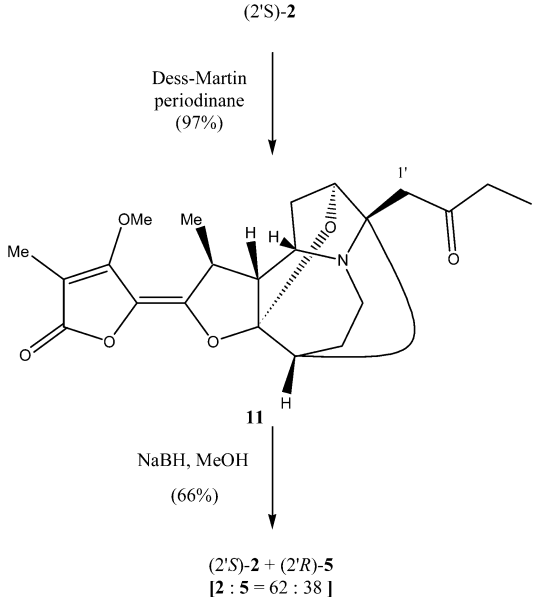
Scheme 1

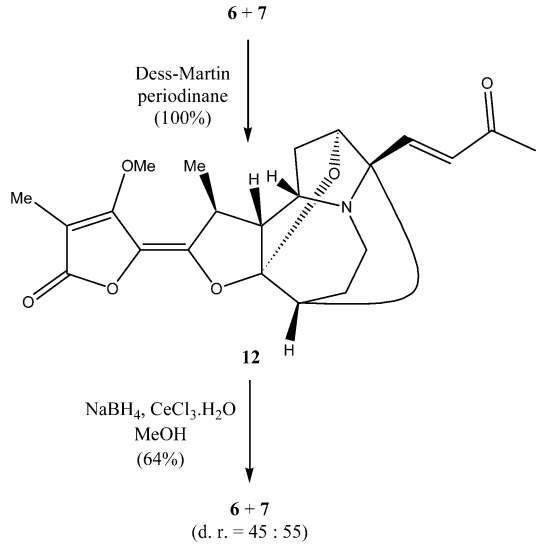


positive specific rotations that suggested that they all had the same absolute configuration about the common A,B,C-ring structure.

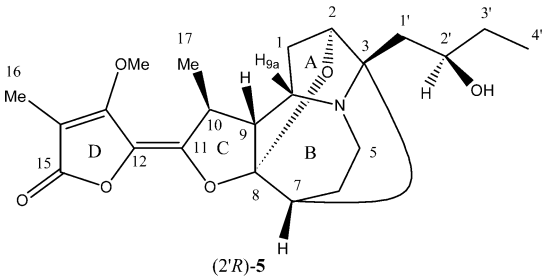
The major alkaloid isolated in this study was (11*Z*)-1′,2′-didehydrostemofoline (**3**), which had spectroscopic data identical to that reported.[4,7,8,11] The other known alkaloids isolated were (2′*S*)-hydroxystemofoline (**2**)[4,7,9,11] and (11*E*)-1′,2′-didehydrostemofoline (**4**).[8] The alkaloids **3** and **4** were isolated in pure form and as a 90:10 mixture of **4** and **3**, respectively. These isomeric compounds were isolated previously by Jiwajinda et al.,[8] who reported their very similar ^{1}H NMR chemical shifts, except for the H-17 methyl resonances for **3** (δ 1.38) and **4** (δ 1.46). We observed the same H-17 chemical shifts for these compounds; however smaller but significant differences in their other ^{1}H and ^{13}C NMR chemical shifts were also noticed, especially for the protons and carbons in the C- and D-rings (Supporting Information). A major difference was noted for the ^{13}C NMR chemical shifts for C-17 for **3** (δ 18.3) and **4** (δ 16.2). The earlier study did not report the ^{13}C NMR data for **4**, and this information is included in the Experimental Section and the Supporting Information. In the earlier study a 1:9 mixture of **3** and **4**, respectively, was exposed to fluorescent light for 120 h, which resulted in a 39:61 mixture of **3** and **4**, respectively.[8] We exposed pure **3** to irradiation from a 500 W sun lamp for 5 h, which also produced a mixture of **3** and **4** (**3**:**4** = 67:33), thus confirming the earlier suggestion[8] that **4** may be an artifact derived from **3** by photoisomerization.

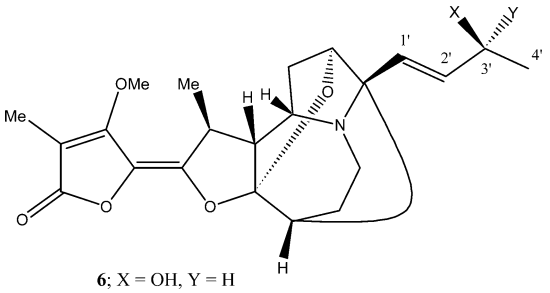
A new alkaloid, (2′*R*)-hydroxystemofoline (**5**), was also isolated, which had the same molecular formula as **2** (HREIMS, $C_{22}H_{29}NO_6$). These epimeric alkaloids had different mobilities on silica gel (**2** was more polar than **5**) and similar but different ^{1}H and ^{13}C NMR spectra (Experimental Section and Supporting Information). To unequivocally show that these compounds were C-2′ epimers, **2** was oxidized to the ketone **11** using the Dess−Martin periodinane reagent (Scheme 1).[14] Ketone **11** showed the expected downfield shifts for the ^{1}H and ^{13}C NMR resonances of the C-3 butyl side chain, especially for C-2′, which appeared at a typical aliphatic ketone carbonyl chemical shift (δ 208.3). Sodium borohydride reduction of **11** gave a 62:38 mixture of the epimeric carbinols **2** and **5**, respectively (Scheme 1).

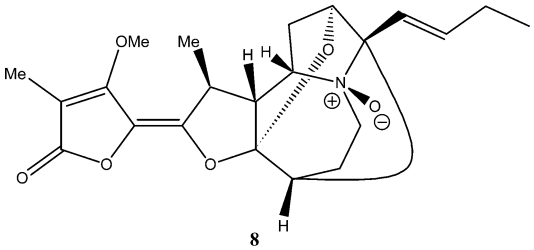
Scheme 2



The new *Stemona* alkaloids (3′*R*)-stemofolenol (**6**) and (3′*S*)-stemofolenol (**7**) were isolated as a 50:50 mixture that was homogeneous by TLC analysis. The HRMS (EI, *m/z* [M]$^+$ 401.1854, calcd 401.1838) showed the molecular formula $C_{22}H_{27}NO_6$ and indicated that **6** and **7** were didehydro-hydroxystemofoline derivatives. The ^{1}H NMR spectrum (500 MHz) of this mixture indicated an apparent single diastereomer with the C-3, 3′-hydroxy-(1′*E*)-butenyl side chain clearly evident (δ 5.87 (dd, *J* 15.9, 5.1 Hz, H-2′), 5.76 (d, *J* 15.9 Hz, H-1′), 4.36 (quin, *J* 6.5 Hz, H-3′), 1.28 (d, *J* 6.5 Hz, H-4′)). The ^{13}C NMR spectrum, however, showed 23 carbon signals, including two closely resolved



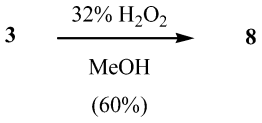




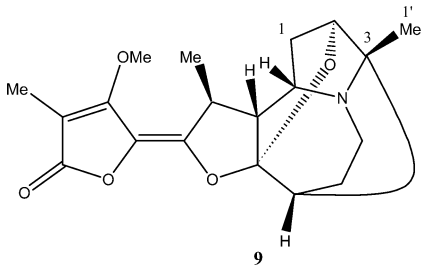
methyl resonances, in ca. a 50:50 ratio, at δ 23.41 and 23.35, that indicated two compounds epimeric at C-3′. This was further substantiated by treatment of the mixture of **6** and **7** with (*R*)-Mosher's acid chloride ((*R*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPACl)), which gave a 44:56 mixture of diastereomeric esters, as evident by the doubling up of all methyl resonances in the derivatives **6a** and **7a**. Further evidence that this sample was a mixture of C-3′ epimers was obtained from its oxidation with the Dess−Martin periodinane reagent[14] to give the ketone **12**, as a single diastereomer, which upon reduction with $NaBH_4-CeCl_3{\cdot}H_2O$,[15] provided a 45:55 mixture of **6** and **7**, respectively, as evidenced from ^{13}C NMR analysis (Scheme 2).

The very polar *N*-oxide **8** was isolated and was readily synthesized, as a single diastereomer, by oxidation of **3** with 32% H_2O_2 in MeOH (Scheme 3). The chemical shifts of the

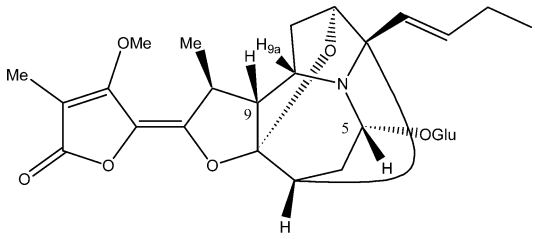
Scheme 3



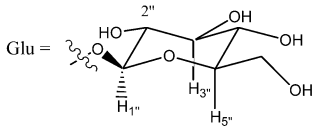
protons and carbons close to the *N*-oxide moiety in **8** were shifted significantly downfield relative to those in **3**.



9



10



Most interesting was the isolation of the first C_{19} stemofoline derivative, methylstemofoline (**9**), and the first glycosidated *Stemona* alkaloid, stemofolinoside (**10**). The HRMS of **9** showed it had the molecular formula $C_{19}H_{23}NO_5$, three carbons less than a normal stemofoline derivative. The ^1H NMR spectrum of **9** showed a methyl singlet at δ 1.35, for the C-3 methyl, while the ^{13}C/DEPT NMR spectra of **9** showed 19 carbons, four of which were methyl groups. The three methylenes of the C-3 butyl side chain of stemofoline were clearly absent. Furthermore, the new methyl resonance at δ 1.35 showed HMBC correlations to both C-5 and C-7, indicative of a C-3 methyl substituent. The full ^1H and ^{13}C NMR signal assignments for **9** are given in the Experimental Section.

The HRMS of **10** showed it had the molecular formula $C_{28}H_{37}NO_{11}$ (stemofoline + $C_6H_8O_6$), which indicated a glycosylated didehydrostemofoline derivative. This was further confirmed from ^{13}C NMR analysis that showed two alkene carbons (δ 129.1 (C-1′) and 134.4 (C-2′)), five new *C*HO (δ 102.4, 78.4, 77.6, 74.3, and 71.5) resonances, and a new *C*H_2O (δ 62.6) resonance for the glycoside. Furthermore, the typical C-5 methylene resonance of the stemofolines at ca. δ 48 had been replaced by a methine resonance at δ 92.2, which indicated hydroxylation had occurred at this position. The position of the glycoside at C-5 was evident from HMBC correlations between H-5 (δ 5.21) and the anomeric carbon of the glycoside (C-1″) at δ 102.4. In the ^1H NMR spectrum, the relatively large coupling constant $J_{1'',2''}$ of 8.5 Hz indicated the β-anomeric configuration of the glycoside.[16] NOESY NMR experiments showed cross-peaks between the anomeric proton (H-1″) and the glycoside H-3″ and H-5″ protons,[17] which indicated that the glycoside was either a β-glucopyranoside or a β-galatopyranoside.[16] This distinction however was readily made on the basis of the ^{13}C NMR chemical shifts of the glycoside that indicated a glucopyranoside, presumably of the D-configuration.[16] The full ^1H and ^{13}C NMR assignments for **10** based on extensive COSY, TOCSY, NOESY, HSQC, and HMBC experiments are given in the Experimental Section.

In conclusion, six new stemofoline alkaloids have been isolated from the root extract of an unidentified *Stemona* species, including several isomeric pairs of compounds, the first C_{19} stemofoline alkaloid, and the first glycosidated *Stemona* alkaloid.

Experimental Section

General Experimental Procedures. These were as described previously.[3] All column chromatography was performed on flash silica gel (0.040−0.063 mm) using gradient elution from 100% CH_2Cl_2 to 50% MeOH−CH_2Cl_2 containing 1% concentrated aqueous NH_3.

Plant Material. The intact plants of an unidentified *Stemona* species were bought at the market in Wat Phra Si Rattana Mahathat Temple, Amphur Muang, Phitsanulok, Thailand, in October 2004. These plants were growing naturally in Amphur Chattrakarn, Phitsanulok. The plant material was identified by Mr. James Maxwell, and a voucher specimen was deposited at the Herbarium (number 25375) of the Department of Biololgy, Chiang Mai University. Morphological traits of the plant were as follows: deciduous, perenial vine; both sides of the petal pale light yellow-cream, becoming more light green with maturity; filament, septum crest, connectives light green; anther locules pale light tan. The Supporting Information has photographs of this plant material.

Extraction and Isolation. The dry ground root of the unidentified *Stemona* species (1.1 kg) was extracted with 95% EtOH (4 × 3000 mL) over 4 days at RT. The ethanolic solution was evaporated to give a dark residue (233 g). A portion of the extract (58.8 g) was partitioned between H_2O and CH_2Cl_2. The organic extract was extracted with 5% HCl solution, and the aqueous solution was made basic with aqueous NH_3 and extracted with CH_2Cl_2 to afford 0.732 g of crude alkaloid material. This material was chromatographed on silica gel (100 mL). A total of 900 mL of eluent was collected in test tubes of 20 mL. These fractions were pooled on the basis of TLC analysis to give five alkaloid fractions: fraction 1 (490.6 mg), fraction 2 (28.0 mg), fraction 3 (32.3 mg), fraction 4 (84.3 mg), and fraction 5 (18.0 mg). These fractions were further purified as described below.

Separation of fraction 1 by column chromatography gave three alkaloid fractions, fraction 1.1, which was pure **3** (191.3 mg), fraction 1.2 (187.9 mg), and fraction 1.3 (15.8 mg). Separation of fraction 1.2 by column chromatography gave three alkaloid fractions, fraction 1.2.1, which gave additional

3 (44.6 mg), fraction 1.2.2 (85.7 mg), and fraction 1.2.3 (16.9 mg). Separation of fraction 1.2.2 by column chromatography gave pure **2** (45.1 mg). Separation of fraction 1.2.3 by preparative TLC (CH_2Cl_2−MeOH−aqueous NH_3, 100:2.5:1) gave additional **2** (13.3 mg). Separation of fraction 1.3 by preparative TLC (CH_2Cl_2−MeOH−aqueous NH_3, 100:2.5:1) gave **4** (2.2 mg) that contained 10% of **3**.

Separation of fraction 2 by two successive preparative TLC purifications (CH_2Cl_2−MeOH−aqueous NH_3, 100:5:1, and then CH_2Cl_2−MeOH−aqueous NH_3, 100:2.5:1) gave **9** (1.9 mg).

Separation of fraction 3 by column chromatography and then preparative TLC (CH_2Cl_2−MeOH−aqueous NH_3, 100:2.5:1) gave **5** (5.7 mg).

Separation of fraction 4 by column chromatography gave two alkaloid fractions, fraction 4.1 (39.5 mg) and fraction 4.2 (19.9 mg). Separation of fraction 4.1 by preparative TLC (CH_2Cl_2−MeOH−aqueous NH_3, 100:4:1) gave a 50:50 mixture of **6** and **7** (26.8 mg). Separation of fraction 4.2 by preparative TLC (CH_2Cl_2−MeOH−aqueous NH_3, 100:4:1) gave **10** (3.6 mg).

Separation of fraction 5 by preparative TLC (CH_2Cl_2−MeOH−aqueous NH_3, 100:5:1) gave **8** (2.3 mg).

(2′*S*)-Hydroxystemofoline (2): yellow-brown gum; $[\alpha]^{21}_D$ +249 (*c* 0.33, $CHCl_3$) [lit.[7] $[\alpha]^{20}_D$ +197 (*c* 0.5, MeOH)]; for the ^1H and ^{13}C NMR data, see the Supporting Information; HRMS (ESI) *m/z* $[M + H]^+$ 404.2046, calcd for $C_{22}H_{30}NO_6$, 404.2073.

(11*Z*)-1′,2′-Didehydrostemofoline (3): yellow-brown gum; $[\alpha]^{20}_D$ +195 (*c* 0.22, $CHCl_3$) [lit.[8] $[\alpha]^{18}_D$ +230 (*c* 0.74, MeOH)]; for the ^1H and ^{13}C NMR data, see the Supporting Information; HRMS (ESI) *m/z* 386.1963 $[M + H]^+$, calcd for $C_{22}H_{28}NO_5$ 386.1967.

(11*E*)-1′,2′-Didehydrostemofoline (4): yellow-brown gum; $[\alpha]^{21}_D$ +71 (*c* 0.11, $CHCl_3$); [lit.[8] $[\alpha]^{18}_D$ +130 (*c* 0.01, MeOH)]; ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.5 (C, C-15), 163.3 (C, C-13), 149.8 (C, C-11), 133.7 (CH, C-2′), 128.9 (C, C-12), 126.0 (CH, C-1′), 113.6 (C, C-8), 98.5 (C, C-14), 83.3 (C, C-3), 80.8 (CH, C-2), 60.9 (CH, C-9a), 59.4 (CH_3, OCH_3), 51.2 (CH, C-7), 47.9 (CH_2, C-5), 45.9 (CH, C-9), 36.2 (CH, C-10), 32.7 (CH_2, C-1), 26.7 (CH_2, C-6), 25.3 (CH_2, C-3′), 16.2 (CH_3, C-17), 13.4 (CH_3, C-4′), 8.8 (CH_3, C-16); HRMS (EI) *m/z* 385.1884 $[M^+]$, calcd for $C_{22}H_{27}NO_5$, 385.1889; for ^{1} H NMR data, see the Supporting Information.

(2′*R*)-Hydroxystemofoline (5): yellow-brown gum; $[\alpha]^{22}_D$ +168 (*c* 0.29, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 4.42 (1H, br s, H-2), 4.07 (3H, s, OMe), 3.69 (2H, m, H-2′), 3.43 (1H, br s, H-9a), 3.09 (1H, ddd, *J* = 14.3, 10.0, 5.0 Hz, H-5a), 3.02 (1H, dq, *J* = 9.0, 6.8 Hz, H-10), 2.95 (1H, ddd, *J* = 14.3, 8.8, 5.0 Hz, H-5b), 2.64 (1H, d, *J* = 6.0 Hz, H-7), 2.00 (3H, s, Me-16), 1.88 (1H, d, *J* = 12.5 Hz, H-1a), 1.88 (1H, m, H-6a), 1.77 (1H, m, H-6b), 1.81 (1H, m, H-1b), 1.76 (1H, dd, *J* = 15.2, 3.8 Hz, H-1′a), 1.72 (1H, dd, *J* = 9.0, 4.0 Hz, H-9), 1.70 (1H, dd, *J* = 15.2, 7.3 Hz, H-1′b), 1.43 (2H, quin, *J* = 7.5 Hz, H-3′), 1.30 (3H, d, *J* = 7.0 Hz, Me-17), 0.88 (3H, t, *J* = 7.5 Hz, Me-4′); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.7 (C, C-15), 162.8 (C, C-13), 148.3 (C, C-11), 127.8 (C, C-12), 111.8 (C, C-8), 98.4 (C, C-14), 81.9 (C, C-3), 79.8 (CH, C-2), 71.0 (CH, C-2′), 60.9 (CH, C-9a), 58.8 (CH_3, OCH_3), 51.8 (CH, C-7), 47.6 (CH_2, C-5), 47.3 (CH, C-9), 36.7 (CH_2, C-1′), 34.4 (CH, C-10), 32.8 (CH_2, C-1), 31.1 (CH_2, C-3′), 26.5 (CH_2, C-6), 18.2 (CH_3, C-17), 10.2 (CH_3, C-4′), 9.0 (CH_3, C-16); HRMS (EI) *m/z* 403.1993 $[M^+]$, calcd for $C_{22}H_{29}NO_6$ 403.1995.

(3′*R*)-Stemofolenol (6) and (3′*S*)-stemofolenol (7): ^1H NMR ($CDCl_3$, 300 MHz) δ 5.87 (1H, dd, *J* = 15.9, 5.1 Hz, H-2′), 5.76 (1H, d, *J* = 15.9 Hz, H-1′), 4.36 (1H, quin, *J* = 6.5 Hz, H-3′), 4.25 (1H, br s, H-2), 4.15 (3H, s, OMe), 3.57 (1H, br s, H-9a), 3.08 (1H, m, H-10), 3.05 (1H, m, H-5a), 3.01 (1H, m, H-5b), 2.89 (1H, d, *J* = 5.4 Hz, H-7), 2.07 (3H, s, Me-16), 1.98 (1H, dd, *J* = 12.0, 3.0 Hz, H-1a), 1.85 (1H, dd, *J* = 5.9, 3.0 Hz, H-9), 1.83 (2H, m, H-6), 1.80 (1H, m, H-1b), 1.39 (3H, d, *J* = 6.3 Hz, Me-17), 1.28 (3H, d, *J* = 6.5 Hz, Me-4′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.7 (C, C-15), 162.8 (C, C-13), 148.2 (C, C-11), 135.7 (CH, C-2′), 127.9 (C, C-12), 126.5 (CH, C-1′), 112.6 (C, C-8), 98.6 (C, C-14), 83.0 (C, C-3), 80.3 (CH, C-2), 67.9 (CH, C-3′), 60.9 (CH, C-9a), 58.8 (CH_3, OCH_3), 51.3 (CH, C-7), 47.9 (CH_2, C-5), 47.4 (CH, C-9), 34.5 (CH, C-10), 32.7 (CH_2, C-1), 26.6 (CH_2, C-6), 18.2 (CH_3, C-17), 23.41, 23.35 (CH_3, C-4′), 9.1 (CH_3, C-16); HRMS (EI) *m/z* 401.1854 $[M^+]$, calcd for $C_{22}H_{27}NO_6$ 401.1838.

1′,2′-Didehydrostemofoline-*N*-oxide (8): yellow-brown gum; $[\alpha]^{25}_D$ +99 (*c* 0.28, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 6.06 (1H, d, *J* = 16.0 Hz, H-1′), 5.83 (1H, dt, *J* = 16.0, 6.0 Hz, H-2′), 4.29 (1H, br s, H-2), 4.10 (3H, s, OMe), 4.08 (1H, br s, H-9a), 4.04 (1H, m, H-5a), 3.66 (1H, m, H-5b), 3.19 (1H, dq, *J* = 9.9, 6.5 Hz, H-10),3.13 (1H, d, *J* = 6.5 Hz, H-7), 2.98 (1H, d, *J* = 12.8 Hz, H-1a), 2.26 (1H, m, H-6a), 2.25 (1H, d, *J* = 9.9 Hz, H-9), 2.15 (2H, quin, *J* = 7.2 Hz, H-3′), 2.08 (1H, d, *J* = 12.8 Hz, H-1b), 2.02 (3H, s, Me-16), 1.84 (1H, m, H-6b), 1.39 (3H, d, *J* = 6.5 Hz, Me-17), 1.00 (3H, t, *J* = 7.2 Hz, Me-4′); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.3 (C, C-15), 162.3 (C, C-13), 147.1 (C, C-11), 137.1 (CH, C-2′), 128.2 (C, C-12),119.6 (CH, C-1′), 110.8 (C, C-8), 98.9 (C, C-14), 91.8 (C, C-3), 80.0 (CH, C-2), 77.0 (CH, C-9a), 63.5 (CH_2, C-5), 58.8 (CH_3, OCH_3), 48.5 (CH, C-9), 48.2 (CH, C-7), 34.7 (CH, C-10), 31.6 (CH_2, C-1), 25.7 (CH_2, C-3′), 21.9 (CH_2, C-6), 17.9 (CH_3, C-17), 12.9 (CH_3, C-4′), 9.0 (CH_3, C-16); HRMS (EI) *m/z* 401.1832 $[M^+]$, calcd for $C_{22}H_{27}NO_6$ 401.1838.

Methylstemofoline (9): yellow gum; $[\alpha]^{23}_D$ +125 (*c* 0.24, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 4.15 (1H, br s, H-2), 4.08 (3H, s, OMe), 3.55 (1H, br s, H-9a), 3.20 (1H, ddd, *J* = 14.0, 10.5, 5.0 Hz, H-5a), 3.03 (1H, dq, *J* = 9.8, 6.0 Hz, H-10), 3.03 (1H, m, H-5b), 2.69 (1H, d, *J* = 5.0 Hz, H-7), 2.00 (3H, s, Me-16), 1.97 (1H, m, H-6a), 1.93 (1H, d, *J* = 12.5 Hz, H-1a), 1.84 (1H, m, H-1b), 1.82 (1H, m, H-6b), 1.78 (1H, dd, *J* = 9.8, 3.5 Hz, H-9), 1.35 (3H, s, Me-1′), 1.32 (3H, d, *J* = 6.5 Hz, Me-17); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.6 (C, C-15), 162.7 (C, C-13), 148.0 (C, C-11), 127.9 (C, C-12), 112.3 (C, C-8), 98.5 (C, C-14), 80.1 (CH, C-2), 79.9 (C, C-3), 61.4 (CH, C-9a), 58.8 (CH_3, OCH_3), 51.4 (CH, C-7), 47.1 (CH_2, C-5), 46.6 (CH, C-9), 34.3 (CH, C-10), 32.6 (CH_2, C-1), 25.8 (CH_2, C-6), 18.6 (CH_3, C-1′), 18.1 (CH_3, C-17), 9.0 (CH_3, C-16); HRMS (EI) *m/z* 345.1561 $[M^+]$, calcd for $C_{19}H_{23}NO_5$ 345.1576.

Stemofolinoside (10): yellow-brown amorphous solid; $[\alpha]^{22}_D$ +138 (*c* 0.16, MeOH); ^1H NMR (CD_3OD, 500 MHz) δ 5.88 (1H, dt, *J* = 16.0, 6.0 Hz, H-2′), 5.79 (1H, d, *J* = 16.0 Hz, H-1′), 5.21 (1H, dd, *J* = 6.0, 3.0 Hz, H-5), 4.41 (1H, d, *J* = 8.5 Hz, H-1″), 4.28 (1H, br s, H-2), 4.21 (3H, s, OMe), 3.85 (1H, d, *J* = 11.8 Hz, H-6″a), 3.76 (1H, br s, H-9a), 3.68 (1H, dd, *J* = 11.8, 5.0 Hz, H-6″b), 3.37 (1H, m, H-5″), 3.32 (1H, m, H-4″), 3.31 (1H, m, H-3″), 3.29 (1H, m, H-2″), 3.10 (1H, m, H-10), 2.92 (1H, d, *J* = 5.5 Hz, H-7), 2.35 (1H, dd, *J* = 14.0, 6.0 Hz, H-6a), 2.15 (1H, m, H-6b), 2.10 (2H, quin, *J* = 7.3 Hz, H-3′), 2.06 (3H, s, Me-16), 2.00 (1H, d, *J* = 12.5 Hz, H-1a), 1.85 (1H, d, *J* = 10.0 Hz, H-9), 1.78 (1H, dd, *J* = 12.5, 3.5 Hz, H-1b), 1.40 (3H, d, *J* = 6.5 Hz, Me-17), 1.02 (3H, t, *J* = 7.3 Hz, Me-4′); ^{13}C NMR (125 MHz, CD_3OD) δ 172.3 (C, C-15), 165.1 (C, C-13), 150.9 (C, C-11), 133.4 (CH, C-2′), 129.1 (CH, C-1′), 129.0 (C, C-12), 113.3 (C, C-8), 102.4 (CH, C-1″), 99.2 (C, C-14), 92.2 (CH, C-5), 83.4 (C, C-3), 82.3 (CH, C-2), 78.4 (CH, C-2″), 77.6 (CH, C-5″), 74.3 (CH, C-3″), 71.5 (CH, C-4″), 62.6 (CH_2, C-6″), 59.9 (CH, C-9a), 59.9 (CH_3, OCH_3), 55.0 (CH, C-7), 49.8 (CH, C-9), 36.5 (CH_2, C-6), 36.2 (CH, C-10), 33.5 (CH_2, C-1), 26.6 (CH_2, C-3′), 18.0 (CH_3, C-17), 13.8 (CH_3, C-16), 9.0 (CH_3, C-4′); HRMS (ESI)+ve, *m/z* $[M + H]^+$ 564.2469, calcd for $C_{28}H_{38}NO_{11}$ 564.2445.

Oxidation of (2′S)-Hydroxystemofoline (2). To a solution of **2** (11.0 mg, 0.027 mmol) in CH_2Cl_2 (2 mL) were added $NaHCO_3$ (10.5 mg, 0.124 mmol) and Dess−Martin periodinane[15] (24.3 mg, 0.057 mmol). The mixture was left to stir under a nitrogen atmosphere at RT for 2 h, when complete reaction was shown by TLC analysis. Saturated aqueous $Na_2S_2O_3$ was added, and the mixture was extracted with CH_2Cl_2. The combined extracts were washed with saturated aqueous $NaHCO_3$ and then NaCl. The solvent was dried (K_2CO_3) and then removed under reduced pressure, and the crude product was purified by preparative TLC (CH_2Cl_2−MeOH−aqueous NH_3, 100:5:1) to give ketone **11** (10.6 mg, 97%) as a yellow-brown gum; $[\alpha]^{24}_D$ +143.8 (*c* 0.53, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 4.60 (1H, br s, H-2), 4.11 (3H, s, OMe), 3.56 (1H, br s, H-9a), 3.23 (1H, ddd, *J* = 14.8, 10.5, 5.5 Hz, H-5a), 3.14 (1H, m, H-5b), 3.10 (1H, dq, *J* = 9.7, 6.5 Hz, H-10), 2.94 (1H, d, *J* = 16.3 Hz, H-1′a), 2.73 (1H, d, *J* = 5.5 Hz, H-7),

2.65 (1H, d, J = 16.3 Hz, H-1′b), 2.46 (2H, m, H-3′), 2.05 (3H, s, Me-16), 2.01 (1H, d, J = 12.5 Hz, H-1a), 1.98 (1H, m, H-6a), 1.96 (1H, d, J = 12.5 Hz, H-1b), 1.87 (1H, m, H-6b), 1.78 (1H, dd, J = 9.7, 3.5 Hz, H-9), 1.35 (3H, d, J = 6.5 Hz, Me-17), 1.01 (3H, t, J = 7.3 Hz, Me-4′); ^{13}C NMR (125 MHz, $CDCl_3$) δ 208.3 (CH, C-2′), 169.6 (C, C-15), 162.7 (C, C-13), 148.0 (C, C-11), 128.0 (C, C-12), 111.7 (C, C-8), 98.7 (C, C-14), 80.9 (C, C-3), 78.9 (CH, C-2), 61.3 (CH, C-9a), 58.9 (CH_3, OCH_3), 51.2 (CH, C-7), 47.6 (CH_2, C-5), 47.2 (CH, C-9), 42.9 (CH_2, C-1′), 37.6 (CH_2, C-3′), 34.4 (CH, C-10), 32.7 (CH_2, C-1), 26.2 (CH_2, C-6), 18.2 (CH_3, C-17), 9.1 (CH_3, C-16), 7.3 (CH_3, C-4′); HRMS (EI) m/z 401.1839 [M$^+$], calcd for $C_{22}H_{27}NO_6$, 401.1838.

Reduction of 11. To a solution of the ketone **11** (6.0 mg, 0.015 mmol) in MeOH (2 mL) at 0 °C was added $NaBH_4$ (1.1 mg, 0.029 mmol). The mixture was left to stir under a nitrogen atmosphere at 0 °C for 1.5 h, when complete reaction was shown by TLC analysis. Water was added and the product was extracted into CH_2Cl_2. The combined extracts were dried ($MgSO_4$) and evaporated to give a mixture (62:38) of **2** and **5** (4.0 mg, 66%) as a yellow-brown gum.

Oxidation of (3′*R*)-Stemofolenol (6) and (3′*S*)-Stemofolenol (7). Oxidation of a mixture of **6** and **7** (10.4 mg, 0.026 mmol) was carried out as described above using CH_2Cl_2 (2 mL), $NaHCO_3$ (10.5 mg, 0.124 mmol), and Dess−Martin periodinane (24.3 mg, 0.057 mmol). The ketone **12** (10.4 mg, 100%) was obtained as a yellow gum; $[\alpha]^{22}_D$ +158 (c 0.52, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.82 (1H, d, J = 15.8 Hz, H-1′), 6.38 (1H, d, J = 15.8 Hz, H-2′), 4.32 (1H, br s, H-2), 4.15 (3H, s, OMe), 3.56 (1H, br s, H-9a), 3.12 (1H, m, H-10), 3.08 (2H, m, H-5), 2.97 (1H, t, J = 3.3 Hz, H-7), 2.28 (3H, s, Me-4′), 2.08 (3H, s, Me-16), 2.00 (1H, d, J = 12.0 Hz, H-1a), 1.87 (1H, dd, J = 6.6, 3.3 Hz, H-9), 1.86 (2H, m, H-6), 1.78 (1H, dt, J = 12.0 3.3 Hz, H-1b), 1.40 (3H, d, J = 6.6 Hz, Me-17); ^{13}C NMR (75 MHz, $CDCl_3$) δ 197.9 (CH, C-3′), 169.6 (C, C-15), 162.7 (C, C-13), 147.9 (C, C-11), 143.8 (CH, C-1′), 130.6 (CH, C-2′), 129.3 (C, C-12), 112.6 (C, C-8), 98.7 (C, C-14), 83.2 (C, C-3), 80.0 (CH, C-2), 61.0 (CH, C-9a), 58.9 (CH_3, OCH_3), 52.3 (CH, C-7), 48.2 (CH_2, C-5), 47.6 (CH, C-9), 34.5 (CH, C-10), 32.6 (CH_2, C-1), 27.7 (CH_3, C-4′),26.7 (CH_2, C-6), 18.3 (CH_3, C-17), 9.2 (CH_3, C-16); HRMS (ESI) +ve, m/z [M + H]$^+$ 400.1779, calcd for $C_{22}H_{26}NO_6$, 400.1760.

Reduction of 12. To a solution of ketone **12** (10.4 mg, 0.026 mmol) in MeOH (2 mL) was added $CeCl_3 \cdot 7H_2O$ (9.7 mg, 0.026 mmol) under a nitrogen atmosphere. $NaBH_4$ (1.0 mg, 0.026 mmol) was then added slowly to the mixture, which was stirred at RT for 15 min, when complete reaction was shown by TLC analysis. Water was added and the mixture was extracted with CH_2Cl_2. The combined extracts were dried ($MgSO_4$) and evaporated to give a 45:55 mixture of **6** and **7** (6.7 mg, 64%) as a yellow gum.

Oxidation of (11*Z*)-1′,2′-Didehydrostemofoline (3). To a solution of **3** (9.0 mg, 0.023 mmol) in MeOH (2 mL) was added H_2O_2 (32%, 0.2 mL). The mixture was left to stir under a nitrogen atmosphere at RT for 7 days, when complete reaction was shown by TLC analysis. MnO_2 was carefully added in small portions to destroy H_2O_2. The mixture was filtered through a small pad of Celite and washed with more MeOH. Water was added, and the mixture was extracted with CH_2Cl_2. The combined extracts were dried ($MgSO_4$), and the solvent was removed under reduced pressure. The crude product was purified by preparative TLC (CH_2Cl_2−MeOH−aqueous NH_3, 100:5:1) to give compound **8** (5.6 mg, 60%) as an oil.

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Supporting Information Available: Tables (S1−S6) of the ^{1}H and ^{13}C, DEPT, HMBC, and NOESY data for compounds **2**−**12** and photographs of the plant material. This material is available free of charge via the Internet at http://pubs.acs.org.

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