Phenanthroindolizidine Alkaloids from the Stems of *Ficus septica*

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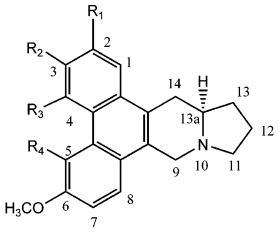
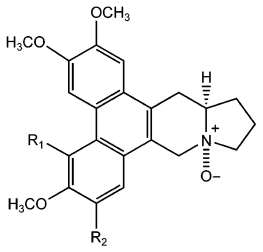
In addition to six known phenanthroindolizidine alkaloids, eight new alkaloids, namely, ficuseptines B−D (**1**−**3**), 10*R*,13a*R*-tylophorine *N*-oxide (**4**), 10*R*,13a*R*-tylocrebrine *N*-oxide (**5**), 10*S*,13a*R*-tylocrebrine *N*-oxide (**6**), 10*S*,13a*R*-isotylocrebrine *N*-oxide (**7**), and 10*S*,13a*S*-isotylocrebrine *N*-oxide (**8**), were isolated from a methanol extract of the stems of *Ficus septica*. The structures of the new compounds were elucidated by means of spectroscopic data interpretation. Cytotoxicity of some of these alkaloids was assessed in vitro using the HONE-1 and NUGC cell lines.

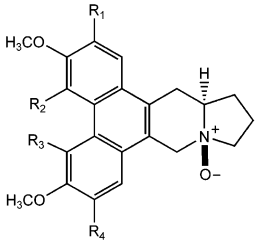
Ficus septica Burm. f. (Moraceae) is a subtropical tree, which occurs widely in low-altitude forests of Taiwan.[1] This species has been known for its detoxicant, purgative, and emetic effects.[1] The leaves of this plant have been used in folk medicine to treat colds, fever, and fungal and bacterial diseases.[2−4] Several phenanthroindolizidine alkaloids, triterpenoids, lignans, acetophenones, steroids, and long-chain aliphatic compounds have been reported earlier from the leaves and roots of *F. septica*.[5−10] Members of the phenanthroindolizidine alkaloid class are known to exhibit pronounced cytotoxicity and antiamebic, antifungal, antibacterial, and antiinflammatory activities and to also inhibit enzymes involved in the synthesis of DNA and proteins.[7,11−25] In our ongoing investigations on cytotoxic constituents from plants native to Taiwan, it was found that the methanol extract of the stem of *F. septica* exhibited potent cytotoxicity against the HONE-1 and NUGC cell lines. Purification of the alkaloidal fractions of the methanol extract by eluting over silica gel followed by HPLC afforded eight new phenanthroindolizidine alkaloids (**1**−**8**), along with six known analogues. In this paper, we report the isolation and characterization of these new phenanthroindolizidine alkaloids, which were found only as trace constituents of *F. septica* stems.



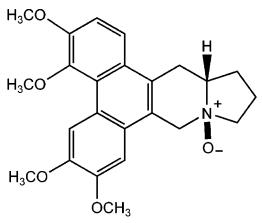
1 R_1, $R_2 = OCH_2O$, $R_3 = H$, $R_4 = OCH_3$
2 R_1, $R_2 = OCH_2O$, $R_3 = R_4 = H$
3 $R_1 = H$, $R_2 = R_3 = OCH_3$, $R_4 = H$



4 $R_1 = H$, $R_2 = OCH_3$
5 $R_1 = OCH_3$, $R_2 = H$



6 $R_1 = OCH_3$, $R_2 = H$, $R_3 = OCH_3$, $R_4 = H$
7 $R_1 = H$, $R_2 = OCH_3$, $R_3 = H$, $R_4 = OCH_3$



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Results and Discussion

Bioassay-guided fractionation and separation of methanolic extract of the stems of *F. septica* and subsequent HPLC purification gave eight new phenanthroindolizidine alkaloids (**1**−**8**), together with six known compounds, tylophorine,[26] tylocrebrine,[5] isotylocrebrine,[26] dehydrotylophorine,[27] 10*S*,13a*R*-tylophorine *N*-oxide,[26] and 10*S*,-13a*R*-antofine *N*-oxide.[16] Although a comparatively large quantity of plant material was collected for this investigation, these labile alkaloids were obtained in very low yields. Accordingly, it was not possible to obtain ^{13}C NMR spectroscopic data to aid in their structure elucidation in the normal manner.

Ficuseptine B (**1**), obtained as a colorless gum, showed a molecular ion peak at *m/z* 377.1630 in its HREIMS, consistent with the molecular formula $C_{23}H_{23}NO_4$. Its UV spectrum showed maxima at 255, 261, 281, 340, and 358 nm, indicating a substituted phenanthrene ring system.[28] A set of *ortho*-coupled doublets ($J = 9.2$ Hz) at δ 7.42 and 7.71 in ^1H NMR spectrum was assigned to H-7 and H-8, respectively, since the latter proton showed a NOE correlation with H-9β at δ 4.58. The two aromatic singlets at δ 9.18 and 7.45 were attributed to *para* positions, H-4 and H-1, respectively, of ring A, based on the NOE interaction between H-1 and H-14β (δ 3.28). The ^1H NMR spectrum of **1** also displayed signals corresponding to two methoxyl groups at δ 3.88 (3H, s) and 4.01 (3H, s) and one set of methylenedioxy protons at δ 6.15 (2H, s). An intense fragment ion peak was observed at *m/z* 308 in the EIMS, suggesting that these two methoxyls and the methylenedioxy group are present in the phenanthrene moiety. The placement of one of the methoxyl groups on C-6 was supported by the NOE cross-peak between H-7 and a methoxyl at δ 4.01. On the other hand, the methoxyl at δ 3.88 showed a NOE correlation between H-4 and OCH_3-6, suggesting that it is attached to C-5. Accordingly, the

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Table 1. ^{1}H NMR Data of Compounds **1**–**8**

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	7.45 s	7.43 s	7.83 d (8.4)	7.43 s	7.39 s	7.29 s	7.85 d (9.2)	7.87 d (9.4)
2			7.49 d (8.4)				7.45 d (9.2)	7.47 d (9.4)
4	9.18 s	8.18 s		8.03 s	9.28 s	9.26 s		
5		8.05 d (2.8)	9.31 d (2.8)	8.02 s			9.30 s	9.34 s
7	7.42 d (9.2)	7.21 dd (8.8, 2.8)	7.25 dd (7.2, 2.8)		7.45 d (7.8)	7.40 d (8.8)		
8	7.71 d (9.2)	7.86 d (8.8)	7.87 d (7.2)	7.28 s	7.69 d (7.8)	7.50 d (8.8)	6.98 s	7.25 s
9	4.58 d (14.8)	4.65 d (17.6)	4.58 d (14.6)	5.17 d (15.8)	5.13 d (16.0)	5.04 d (15.0)	5.03 d (15.2)	5.18 d (15.8)
9	3.57 d (14.8)	3.67 d (17.6)	3.57 d (14.6)	4.86 d (15.8)	4.81 d (16.0)	4.66 d (15.0)	4.65 d (15.2)	4.81 d (15.8)
11	3.35 td (9.0, 3.0)	3.59 td (14.4, 2.0)	3.25 td (14.8, 3.2)	3.80 td (9.0, 3.0)	3.72 br t (16.0)	3.85 td (18.0, 2.0)	3.87 td (19.0, 3.0)	3.80 td (19.2, 3.1)
11	2.45 q (9.0)	3.10 q (14.4)	2.41 q (14.8)	3.72 q (9.0)	3.65 q (16.0)	3.65 q (18.0)	3.68 q (19.0)	3.29 q (19.2)
12	2.05 m	2.07 m	2.01 m	2.14 m	1.88 m	2.37 m	2.35 m	1.84 m
12	1.91 m	1.89 m	1.82 m	2.33 m	2.60 m	2.11 m	2.13 m	2.59 m
13	2.21 m	2.28 m	2.20 m	1.89 m	2.14 m	2.31 m	2.28 m	2.16 m
13	1.69 m	1.71 m	1.70 m	2.95 m	2.30 m	2.19 m	2.18 m	2.32 m
13a	2.36 m	2.43 m	2.34 m	3.94 m	3.98 m	3.45 m	3.51 m	4.08 m
14	2.63 dd (16.0, 12.0)	3.30 dd (16.0, 5.2)	2.94 dd (15.0, 11.0)	3.57 dd (16.5, 12.0)	3.48 dd (16.0, 11.0)	3.52 dd (16.0, 3.0)	3.47 dd (14.0, 7.0)	3.53 dd (16.0, 8.0)
14	3.28 dd (16.0, 4.0)	3.35 dd (16.0, 2.0)	3.32 dd (15.0, 3.0)	3.37 dd (16.5, 4.0)	3.29 dd (16.0, 2.0)	3.15 dd (16.0, 11.0)	3.24 dd (14.0, 3.0)	3.29 dd (16.0, 2.0)
OCH_3								
2	6.15 s	6.15 s		4.02 s	4.01 s	3.99 s		
3			3.98 s	4.09 s	4.01s	3.93 s	4.02 s	4.02 s
4			4.05 s				4.01 s	4.02 s
5	3.88 s				3.90 s	4.02 s		
6	4.01 s	4.02 s	3.92 s	4.09 s	4.01 s	4.02 s	3.92 s	3.91 s
7				4.04 s			3.99 s	4.04 s

methylenedioxy group was placed at C-2 and C-3. The absolute stereochemistry of **1** was determined from the optical rotation and CD spectrum. Thus, phenanthroindolizidine alkaloids with the *R* configuration at C-13a have been found to exhibit a negative optical rotation and a positive Cotton effect around 260 nm.[29,30] Compound **1** was in agreement with this, as it showed a negative optical rotation measured at the sodium-D line and a positive Cotton effect at 259 nm in the CD spectrum. Thus, **1** possesses the *R* configuration at C-13a. The presence of a NOE between H-13a and H-9α suggested that the piperidine ring adopts a chair-like conformation.[31] On the basis of the foregoing spectroscopic studies, the structure of compound **1** was established as 5,6-dimethoxy-2,3-methylenedioxy-13a*R*-phenanthroindolizidine, for which the trivial name ficuseptine B was proposed, following a previous convention for these alkaloidal constituents of *F. septica*.[9]

Ficuseptine C (**2**) was obtained as colorless gum, and its UV absorption maxima were observed at 257, 282, and 340 nm, very similar to those of trioxygenated phenanthrene derivatives.[28] It exhibited a molecular ion peak at *m/z* 347.3513 in its HRFABMS, corresponding to the molecular formula $C_{22}H_{21}NO_3$, 30 amu less than that of **1**. The observation of an intense fragment ion at *m/z* 278 in the EIMS due to a phenanthrene moiety suggested the presence of one methoxyl group and one methylenedioxy group, instead of two methoxyl groups and one methylenedioxy group as in **1**. As expected, the ^{1}H NMR spectrum also displayed signals for methoxyl protons at δ 4.02 (3H, s) and methylenedioxy protons at δ 6.15 (2H, s). Since the proton signals of the B ring appeared as an ABX pattern at δ 8.05 (1H, d, J = 2.8 Hz, H-5), 7.86 (1H, d, J = 8.8 Hz, H-8), and 7.21 (1H, dd, J = 8.8, 2.8 Hz, H-7), and NOEs of H-8/H-9α (δ 4.65), H-5/H-4 (δ 8.18) and the methoxyl protons/H-5 and H-7 were observed, the methoxyl group was placed at C-6. The attachment of the methylenedioxy group at C-2 and C-3 was confirmed by the multiplicities for H-1 and H-4 of ring A as two aromatic singlets at δ 7.43 (1H, s, H-1) and 8.18 (1H, s, H-4) and NOE correlations of H-1/H-14β (δ 3.35) and H-4/H-5. The stereochemistry of C-13a was determined as having the *R* configuration on the basis of the negative optical rotation value and a positive Cotton effect at 257 nm in the CD spectrum, and thus H-13a was α oriented.[29,30] Finally, the NOE between H-13a and H-9α indicated the existence of a chair-like conformation for the piperidine ring.[31] Thus, the structure of **2** was elucidated as 6-methoxy-2,3-methylenedioxy-13a*R*-phenanthroindolizidine, and it has been designated as ficuseptine C.

Ficuseptine D (**3**) exhibited a molecular formula of $C_{23}H_{25}NO_3$, based on a molecular ion peak at *m/z* 363.1833 in its HREIMS. Its UV absorption maxima at 252, 260, 280, 342, and 351 nm suggested it to be a substituted phenanthrene derivative.[28] The ^{1}H NMR spectrum of **3** displayed signals for three methoxyl groups at δ 3.92, 3.98, and 4.05 (each 3H, s). Since **3** showed a similar pattern of ^{1}H NMR signals due to the indolizidine moiety as observed for **2** (Table 1), three methoxyl groups could be assigned in the phenanthrene moiety. This conclusion was supported by an intense fragment ion peak at *m/z* 294 in the EIMS. The *ortho*-coupled doublets appearing at δ 7.49 and 7.83 (each 1H, d, J = 8.4 Hz) were assigned to H-1 and H-2 of ring A, as a NOE correlation was observed between H-1 and H-14β (δ 3.32). A set of ABX pattern signals at δ 9.31 (1H, d, J = 2.8 Hz), 7.25 (1H, dd, J = 7.2, 2.8 Hz), and 7.87 (1H, d, J = 7.2 Hz) were attributed to H-5, H-7, and H-8 of ring B on the basis of the NOEs of H-8 with H-9α (δ 4.58) and H-7. The NOE correlation of the methoxyl protons at δ 3.92 with H-7 and H-5 was used to determine the location of the methoxyl group at C-6. The remaining two methoxyls at δ 3.98 and 4.05 were placed at C-3 and C-4, respectively, as the former gave a NOE with H-2 and the latter with H-5. The negative optical rotation value and positive Cotton effect at 259 nm in the CD spectrum of **3** supported the *R* configuration at C-13a.[29,30] In addition, a NOE correlation between H-13a and H-9α suggested a chair-like conformation for the piperidine ring.[31] Accordingly, the structure of **3** (ficuseptine D) was concluded to be 3,4,6-trimethoxy-13a*R*-phenanthroindolizidine.

Alkaloid **4**, obtained as yellow needles, was considered to have the same molecular formula, $C_{24}H_{27}NO_5$, as tylophorine *N*-oxide, on the basis of its HREIMS, suggesting it to be an isomer.[15] The ^{1}H NMR spectrum revealed the presence of four methoxyl group signals at δ 4.02, 4.04 (each 3H, s) and 4.09 (6H, s). Since the UV spectroscopic pattern and ^{1}H NMR signals and coupling patterns corresponding to the phenanthrene moiety were similar to those of tylophorine *N*-oxide,[15] the four methoxyl groups could be located at C-2, C-3, C-6, and C-7. This was supported by NOE cross-peaks between H-1/H-14α, H-14β, OCH_3-2; H-4/H-5, OCH_3-3; OCH_3-2/OCH_3-3; OCH_3-6/H-5, OCH_3-7; and H-8/H-9α, H-9β, OCH_3-7. Evidence for the presence of an *N*-oxide unit was also suggested by the downfield shifts of H-9α (δ 5.17), H-9β (δ 4.86), H-11α (δ 3.80), H-11β (δ 3.72), and H-13a (δ 3.94) and by the prominent $[M - 16]^+$ peak at *m/z* 393 in the mass spectrum of **4**. The negative optical rotation measured at the sodium-D line and positive Cotton effect at 256 nm in the CD spectrum for **4** inferred the C-13a *R* configuration.[29,30] Thus, H-13a was oriented in an α direction. The H-13a proton resonated at δ 3.94 and indicated a *cis*-fused ring junction of the indolizidine moiety.[32,33] Moreover, the strong deshielding of H-9α (δ 5.17) and H-11α (δ 3.80) by oxygen supported the α configuration of the *N*-oxide group.[16,33] The observation of NOE correlations between H-1 and H-14α, H-14β; H-8 and H-9α, H-9β; H-9α and H-11α; and H-14α and H-13α in the NOESY spectrum suggested that the indolizidine moiety adopted a boat-like conformation.[31] Hence, **4** was characterized as 10*R*,13a*R*-tylophorine *N*-oxide.

Alkaloid **5** was assigned a molecular formula of $C_{24}H_{27}NO_5$ from its HREIMS, one oxygen atom more than that of tylocrebrine, suggesting the presence of an *N*-oxide functionality. ^{1}H NMR multiplicities of the four aromatic proton signals and four methoxyl groups similar to those of tylocrebrine, and the fragment ion peak at *m/z* 324, due to the phenanthrene moiety in EIMS, allowed the four methoxyl groups to be fixed at C-2, C-3, C-5, and C-6, respectively. This was further supported by NOEs of H-1/H-14α, H-14β, OCH_3-2; H-4/OCH_3-3; OCH_3-5, OCH_3-6/H-7; and H-8/H-7, H-9α, H-9β. These assignments indicated that the extra oxygen must be present in the indolizidine moiety. Evidence for the presence of an *N*-oxide unit was also revealed by the significant $[M - 16]^+$ ion peak at *m/z* 393 and downfield shifts of H-9α (δ 5.13), H-9β (δ 4.81), H-11α (δ 3.72), H-11β (δ 3.65), and H-13a (δ 3.98) in the ^{1}H NMR spectrum. The negative optical rotation value and positive Cotton effect at 275 nm in the CD spectrum established the *R* stereochemistry at C-13a, with H-13a in an α orientation.[29,30] A strong deshielding of H-13a to δ 3.98 inferred a *cis*-fused ring junction of the indolizidine moiety.[32,33] The α orientation of the *N*-oxide group was also indicated by a strong deshielding of H-9α and H-11α by the oxygen atom.[16,33] Similar NOE correlations between H-1 and H-14α, H-14β; H-8 and H-9α, H-9β; H-9α and H-11α; and H-14α and H-13α as in **4** suggested a boat-like conformation for the indolizidine moiety.[31] Thus, **5** was concluded to be 10*R*,13a*R*-tylocrebrine *N*-oxide.

Alkaloid **6** was isolated as pale yellow needles. The molecular formula, $C_{24}H_{27}NO_5$, established from its HREIMS, was the same as that of **5**, indicating these alkaloids to be isomers. On the basis of the fact that **6** showed four aromatic proton signals and four methoxyl signals in the ^{1}H NMR spectrum similar to those of **5** (Table 1), the substituents were assigned to C-2, C-3, C-5, and C-6 as in **5**. These assignments were supported by NOESY correlations of H-1/H-14β, OCH_3-2; H-4/OCH_3-3, OCH_3-5; H-7/OCH_3-6; and H-8/H-7, H-9α. The positive CD curve at 261 nm and a negative optical rotation value inferred the *R* stereochemistry at C-13a and thus H-13a to be in an α orientation.[29,30] The resonance due to H-13a at δ 3.45 was indicative of a *trans*-fused indolizidine ring junction.[32,33] Moreover, the strong deshielding of H-9β (δ 4.66) and H-11β (δ 3.65) by oxygen led to the assignment of the β configuration of the *N*-oxide group.[16,33] Finally, the observation of a NOE between H-13a and H-9α indicated that the piperidine ring adopted a chair-like conformation as in **4**.[31] Consequently, **6** was identified as 10*S*,13a*R*-tylocrebrine *N*-oxide.

Alkaloid **7** was obtained as pale yellow needles. On the basis of the HREIMS data **7** was suggested to have the same molecular formula, $C_{24}H_{27}NO_5$, as isotylocrebrine *N*-oxide,[15] indicating these alkaloids to be isomers. Accordingly, two mutually coupled doublets ($J = 9.2$ Hz) at δ 7.85 and 7.45 and two singlets at δ 9.30 and 6.98 in its ^{1}H NMR spectrum were assigned to H-1, H-2, H-5, and H-8, respectively.[15] Thus, four methoxyl groups at δ 4.02, 4.01, 3.92, and 3.99 could be placed, in turn, at C-3, C-4, C-6, and C-7. These assignments were confirmed by the correlations of H-1/H-14β; H-2/OCH_3-3; H-5/OCH_3-4, OCH_3-6; and H-8/OCH_3-7, H-9α in a NOESY experiment. The positive Cotton effect at 279 nm in the CD spectrum of **7** and negative optical rotation inferred the *R* configuration at C-13a.[29,30] Hence, H-13a was located with an α orientation. The appearance of the H-13a signal at δ 3.51 led the ring junction configuration to be determined as *trans*[32,33] for the indolizidine unit. Moreover, the strong deshielding of H-9β (δ 4.65) and H-11β (δ 3.68) by oxygen also inferred the β configuration of the *N*-oxide group.[16,33] The appearance of NOE cross-peaks between H-13a and H-9α was indicative of a chair-like conformation for the piperidine ring.[31] The structure of **7** was, therefore, defined as 10*S*,13a*R*-isotylocrebrine *N*-oxide.

Alkaloid **8** was obtained as pale yellow needles. On the basis of the molecular formula, $C_{24}H_{27}NO_5$, as determined from the HREIMS, **8** was considered to be an isomer of **7**. Its UV spectrum showed absorptions at 245, 264, 285, 344, and 360 nm and was practically superimposable on that of **7**. In the ^{1}H NMR spectrum, four methoxyls and four aromatic proton signals were observed with the same multiplicities as those of **7**, but differed in their indolizidine protons (Table 1). The placement of four methoxyl groups at C-3, C-4, C-6, and C-7 was determined from NOEs between H-1/H-14β; H-2/OCH_3-3; H-5/OCH_3-4, OCH_3-6; and H-8/OCH_3-7, H-9α. The positive specific rotation and positive Cotton effect at 279 nm in the CD spectrum of **8** confirmed the *S* configuration of C-13a[16,26] and the β orientation of H-13a. The downfield shift of H-13a to δ 4.08 inferred that the indolizidine ring junction should be in the *cis*[32,33] configuration. Furthermore, the β configuration of the *N*-oxide group was also inferred from a strong deshielding of H-9β and H-11β by an oxygen atom.[16,33] The appearance of NOE correlations between H-1 and H-14α, H-14β; H-8 and H-9α, H-9β; H-9α and H-11α; and H-14α and H-13α in the NOESY spectrum indicated that the indolizidine moiety adopted a boat-like conformation.[31] Therefore, it was concluded that the **8** is 10*S*,13a*S*-isotylocrebrine *N*-oxide.

Compounds **6**, **7**, and tylophorine were tested in vitro for their cytotoxicity using HONE-1 and NUGC tumor cell lines.[9,34] All three compounds exhibited strong cytotoxicity against both HONE-1 and NUGC cell lines. The percentages of inhibition observed for **6**, **7**, and tylophorine at 10 μM concentration against HONE-1 cell lines were 92%,

87%, and 80%, respectively, whereas against NUGC cell lines they exhibited 94%, 93%, and 85% inhibition, respectively, at the same concentration. Compound quantities available did not permit the more formal determination of IC_{50} values.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a JASCO DIP-370 digital polarimeter. Circular dichroism (CD) and UV spectra were recorded at room temperature on a JASCO J-700 spectropolarimeter and on a Hitachi UV-3210 spectrophotometer, respectively. IR spectra were obtained with a Shimadzu FT-IR DR-8011 spectrophotometer. NMR spectra were recorded on Bruker AMX-400, AVANCE-300, and Varian Unity Plus 400 spectrometers. Chemical shifts are shown in δ values (ppm) with tetramethylsilane as an internal standard. Mass spectra were measured on a VG-70-250S spectrometer with EI or FAB ionization (positive-ion mode). Column chromatography was performed on silica gel (70−230 mesh, 230−400 mesh). Fractions were monitored by TLC (Merck precoated Si gel 60 F_{254} plates), using UV light and Dragendorff's reagent to visualize the spots. High-performance liquid chromatography (HPLC) was performed on a Shimadzu LC-10ATVP series pumping system equipped with a Shimadzu SPD-6AV UV−vis spectrophotometric detector at 275 nm, a Cosmosil packed column with 5C_{18}-AR-II Waters type (4.6 × 250 mm, 5 μm) and a Lichrospher 100 RP-8 column (4.6 × 250 mm, 5 μm), and a Rheodyne injector.

Plant Material. The stems of *F. septica* were collected in Tainan Hsien, Taiwan, Republic of China, in January 2000 and were authenticated by Prof. C. S. Kuoh, Department of Life Science, National Cheng Kung University. A voucher specimen (Wu 200000053) has been deposited in the Herbarium of National Cheng Kung University, Tainan, Taiwan.

Extraction and Isolation. Dried and powdered stem of *F. septica* (46.5 kg) was refluxed with methanol (7 × 10 L) and filtered. A large amount of precipitate, asparagine (260 g),[35] was formed during this process and filtered. The filtrate was concentrated and suspended in water. Then the water solubles were partitioned with chloroform and *n*-butanol, successively. The concentrated chloroform extract (80 g) was subjected to open column chromatography over silica gel by eluting with a stepwise gradient from 5% to 75% methanol in chloroform to afford 12 fractions. Fractions were monitored by TLC using Dragendorff's reagent to visualize the spots and tested for their cytotoxicity. Fractions 5−9 were considered to be active fractions, since they showed cytotoxicity against the HONE−1 and NUGC cell lines with the inhibition percentage values of 93% and 97%, 80% and 90%, 79% and 92%, 75% and 84%, and 85% and 96%, respectively at 250 μM concentration. Fraction 5 on repeated column chromatography over silica gel and further purification by HPLC using 1 mL/min of MeOH−H_2O−Et_2NH (75:24.5:0.5) gave compounds **1** (1.0 mg, 0.0012%), **2** (1.0 mg, 0.0012%), **3** (1.0 mg, 0.0012%), **4** (7.0 mg, 0.0087%), tylophorine (18.0 mg, 0.022%), tylocrebrine (1.0 mg, 0.0012%), isotylocrebrine (1.1 mg, 0.0013%), dehydrotylophorine (0.5 mg, 0.00062%), and 10*S*,13a*R*-tylophorine *N*-oxide (1.5 mg, 0.0018%). Similarly, repeated column chromatography and HPLC purification by using 1 mL/min of MeCN−H_2O−Et_2NH (20:79.5:0.5) of fraction 7 yielded compounds **6** (1.0 mg, 0.0012%) and **8** (0.5 mg, 0.00062%). Fraction 8 was subjected to repeated column chromatography separation and then HPLC purification using 1 mL/min of MeCN−H_2O−Et_2NH (25:75:0.5) to obtain compounds **4** (1.9 mg, 0.0023%), **5** (2.6 mg, 0.0032%), **6** (2.6 mg, 0.0032%), **7** (5.9 mg, 0.0073%), **8** (2.5 mg, 0.0031%), and 10*S*,13a*R*-antofine *N*-oxide (2.5 mg, 0.0031%). An attempt to work up cytotoxic fractions 6 and 9 did not lead to the isolation of any additional alkaloids due to the lability of these compounds.

Ficuseptine B (1): colorless gum; $[\alpha]_D^{25}$ −95.6° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 255 (3.80), 261 (3.91), 281 (2.59), 340 (3.24), 358 (3.18) nm; IR (KBr) ν_{max} 2924, 1745, 1711, 1683, 1511, 1467, 1424, 1396, 1286, 1010 cm^{-1}; CD (*c* 1.47 × 10^{-4}, MeOH) $[\theta]_{259}$ +4607; ^1H NMR (acetone-d_6, 400 MHz), see Table 1; EIMS *m/z* 377 $[M]^+$ (43), 308 (100), 293 (34), 277 (15), 129 (10), 96 (13), 69 (20); HREIMS *m/z* 377.1630 ($[M]^+$) (calcd for $C_{23}H_{23}NO_4$, 377.1627).

Ficuseptine C (2): colorless gum; $[\alpha]_D^{25}$ −79.9° (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 257 (3.46), 282 (3.42), 340 (2.68) nm; IR (KBr) ν_{max} 2925, 2853, 2375, 2311, 1715, 1710, 1683, 1548, 1514, 1454, 1412, 1391, 1371, 1212, 1035 cm^{-1}; CD (*c* 1.09 × 10^{-4}, MeOH) $[\theta]_{257}$ +4582; ^1H NMR (acetone-d_6, 400 MHz), see Table 1; EIMS *m/z* 347 $[M]^+$ (31), 278 (100), 264 (10), 248 (29), 175 (11), 153 (10), 129 (20), 95 (11), 69 (65); HREIMS *m/z* 347.3513 ($[M]^+$) (calcd for $C_{22}H_{21}NO_3$, 347.3515).

Ficuseptine D (3): colorless gum; $[\alpha]_D^{25}$ −75.6° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 252 (sh) (3.58), 260 (3.62), 280 (2.68), 342 (2.61), 351 (3.21) nm; IR (KBr) ν_{max} 2923, 1679, 1668, 1659, 1600, 1514, 1455, 1410, 1398, 1282, 1010 cm^{-1}; CD (*c* 1.40 × 10^{-5}, MeOH) $[\theta]_{259}$ +3177; ^1H NMR (acetone-d_6, 400 MHz), see Table 1; EIMS *m/z* 363 $[M]^+$ (40), 294 (100), 279 (18), 236 (10), 129 (11), 97 (15), 69 (21); HREIMS *m/z* 363.1833 ($[M]^+$) (calcd for $C_{23}H_{25}NO_3$, 363.1834).

10*R*,13a*R*-Tylophorine *N*-oxide (4): pale yellow needles; mp 210−220 °C (dec); $[\alpha]_D^{25}$ −77.7° (*c* 0.019, MeOH); UV (MeOH) λ_{max} (log ϵ) 241 (4.09), 258 (4.13), 287 (3.84), 303 (3.56), 340 (2.52) nm; IR (KBr) ν_{max} 2925, 1741, 1691, 1647, 1515, 1463, 1427, 1315, 1257, 1153, 1039 cm^{-1}; CD (*c* 4.55 × 10^{-5}, MeOH) $[\theta]_{256}$ +4934; ^1H NMR (CD_3OD, 300 MHz), see Table 1; EIMS *m/z* 409 $[M]^+$ (5), 393 (20), 392 (8), 337 (74), 319 (54), 176 (32), 154 (100), 136 (76), 86 (20); HRFABMS *m/z* 410.4748 ($[M + H]^+$) (calcd for $C_{24}H_{28}NO_5$, 410.4749).

10*R*,13a*R*-Tylocrebrine *N*-oxide (5): pale yellow needles; mp 205−212 °C (dec); $[\alpha]_D^{25}$ −66.6° (*c* 0.025, MeOH); UV (MeOH) λ_{max} (log ϵ) 244 (4.14), 265 (4.29), 276 (3.71), 310 (3.46) 320 (3.41) nm; IR (KBr) ν_{max} 2941, 1741, 1706, 1562, 1515, 1477, 1463, 1427, 1398, 1365, 1284, 1253, 1114, 1068, 1026 cm^{-1}; CD (*c* 6.36 × 10^{-5}, MeOH) $[\theta]_{275}$ +4611; ^1H NMR (CD_3OD, 400 MHz), see Table 1; EIMS *m/z* 409 $[M]^+$ (8), 393 (48), 392 (24), 337 (100), 324 (99), 176 (9), 154 (21), 86 (10); HRFABMS *m/z* 410.3916 ($[M + H]^+$) (calcd for $C_{24}H_{28}NO_5$, 410.3919).

10*S*,13a*R*-Tylocrebrine *N*-oxide (6): pale yellow needles; mp 215−218 °C (dec); $[\alpha]_D^{25}$ −61.4° (*c* 0.081, MeOH); UV (MeOH) λ_{max} (log ϵ) 245 (3.14), 264 (4.39), 279 (3.91), 285 (3.82), 307 (3.41), 319 (3.38) nm; IR (KBr) ν_{max} 2937, 1741, 1606, 1515, 1463, 1396, 1286, 1255, 1211, 1114, 1035, 1026 cm^{-1}; CD (*c* 6.45 × 10^{-5}, MeOH) $[\theta]_{261}$ +8604; ^1H NMR (CD_3OD, 300 MHz), see Table 1; EIMS *m/z* 409 $[M]^+$ (24), 393 (80), 337 (10), 324 (100), 154 (61), 136 (46), 86 (14); HRFABMS *m/z* 410.4416 ($[M + H]^+$) (calcd for $C_{24}H_{28}NO_5$, 410.4413).

10*S*,13a*R*-Isotylocrebrine *N*-oxide (7): pale yellow needles; mp 230−235 °C (dec); $[\alpha]_D^{25}$ −66.6° (*c* 0.025, MeOH); UV (MeOH) λ_{max} (log ϵ) 245 (3.24), 263 (5.39), 283 (3.12), 287 (3.08), 309 (2.41) 344 (2.24), 359 (2.19) nm; IR (KBr) ν_{max} 2943, 2842, 1691, 1635, 1521, 1458, 1413, 1267, 1114, 1028 cm^{-1}; CD (*c* 3.25 × 10^{-5}, MeOH) $[\theta]_{279}$ +1128; ^1H NMR (CD_3OD, 400 MHz), see Table 1; EIMS *m/z* 409 $[M]^+$ (14), 393 (80), 324 (100), 307 (10),176 (25), 154 (61), 136 (46), 86 (14); HRFABMS *m/z* 410.4961 ($[M + H]^+$) (calcd for $C_{24}H_{28}NO_5$, 410.4963).

10*S*,13a*S*-Isotylocrebrine *N*-oxide (8): pale yellow needles; mp 205−210 °C (dec); $[\alpha]_D^{25}$ +6.5° (*c* 0.025, MeOH); UV (MeOH) λ_{max} (log ϵ) 245 (3.21), 264 (5.41), 285 (3.17), 305 (2.89) 344 (2.21), 360 (2.14) nm; IR (KBr) ν_{max} 2941, 2851, 1696, 1645, 1562, 1463, 1255, 1114, 1024 cm^{-1}; CD (*c* 5.01 × 10^{-5}, MeOH) $[\theta]_{279}$ +1128; ^1H NMR (CD_3OD, 400 MHz), see Table 1; EIMS *m/z* 409 $[M]^+$ (17), 393 (30), 324 (74), 307 (25),176 (12), 154 (100), 136 (74), 118 (66); HRFABMS *m/z* 410.3446 ($[M + H]^+$) (calcd for $C_{24}H_{28}NO_5$, 410.3443).

Cytotoxicity Assay. Two human cancer cell lines, NUGC (gastric adenocarcinoma) and HONE-1 (nasopharyngeal carcinoma), were seeded in 96-well microliter plates at a density of 6000/well in 10 μL of culture medium (Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and nonessential amino acid) and maintained at 37 °C in a humidified incubator with 5% CO_2. After an overnight adaptation period, test compounds (final concentration, 50 μg/mL)

in serum-free medium were added to individual wells. Cells were treated with test compounds for 3 days. Cell viability was determined by the 5-(3-carboxymethoxyphenyl)-2-(4,5-dimethylthiazolyl)-3-(4-sulfophenyl)tetrazolium salt (MTS) reduction assay.[9,32] The 5 μM (final concentration) actinomycin D (showed 100% of inhibition at 10 μM) and 0.3% (final concentration) DMSO were used as positive and vehicle controls. Results were expressed as percent of DMSO control.

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