Phenanthroindolizidine Alkaloids from the Stems of \textit{Ficus septica}

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In addition to six known phenanthroindolizidine alkaloids, eight new alkaloids, namely, ficuseptines B–D (1–3), 10F,13αR-tylophorine N-oxide (4), 10F,13αR-tylocrebrine N-oxide (5), 10S,13αR-tylocrebrine N-oxide (6), 10S,13αR-isotylocrebrine N-oxide (7), and 10S,13αS-isotylocrebrine N-oxide (8), were isolated from a methanol extract of the stems of \textit{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.

\textit{Ficus septica} Burm. f. (Moraceae) is a subtropical tree, which occurs widely in low-altitude forests of Taiwan.\textsuperscript{1} This species has been known for its detoxicant, purgative, and emetic effects.\textsuperscript{1} The leaves of this plant have been used in folk medicine to treat colds, fever, and fungal and bacterial diseases.\textsuperscript{2–4} Several phenanthroindolizidine alkaloids, triterpenoids, lignans, acetophenones, steroids, and long-chain aliphatic compounds have been reported earlier from the leaves and roots of \textit{F. septica}.\textsuperscript{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.\textsuperscript{7,11–23} In our ongoing investigations on cytotoxic constituents from plants native to Taiwan, it was found that the methanol extract of the stem of \textit{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 \textit{F. septica} stems.

Results and Discussion

Bioassay-guided fractionation and separation of methanolic extracts of the stems of \textit{F. septica} and subsequent HPLC purification gave eight new phenanthroindolizidine alkaloids (1–8), together with six known compounds, tylophorine,\textsuperscript{24} tylocrebrine,\textsuperscript{5} and dehydrotylophorine,\textsuperscript{26} tylocrebrine,\textsuperscript{26} and 10S,13αR-antofine N-oxide.\textsuperscript{10} 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 \textsuperscript{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 \textit{m/z} 377.1630 in its HREIMS, consistent with the molecular formula \textit{C}_{22}\textit{H}_{24}\textit{N}_{2}\textit{O}_{4}. Its UV spectrum showed maxima at 255, 261, 281, 340, and 358 nm, indicating a substituted phenanthrene ring system.\textsuperscript{28} A set of ortho-coupled doublets (\textit{J} = 9.2 Hz) at δ 7.42 and 7.71 in \textit{1}\textsuperscript{H} NMR spectrum was assigned to H-7 and H-8, respectively, since the latter proton showed a NOE correlation with H-9/δ at δ 4.58. The two aromatic singlets at δ 9.18 and 7.45 were attributed to \textit{para} positions, H-4 and H-1, respectively, of ring A, based on the NOE interaction between H-1 and H-14/δ (δ 3.28). The \textit{1}\textsuperscript{H} NMR spectrum of 1 also displayed signals corresponding to two methoxyl groups at δ 3.88 (3H, s) and 4.01. On the other hand, the methoxyl at δ 3.28 showed a NOE correlation between H-4 and OCH\textsubscript{3}, suggesting that it is attached to C-5. Accordingly, the
derivatives. It exhibited a molecular ion peak at 347.3513 in its HRFABMS, corresponding to the molecular formula C_{22}H_{21}NO_{3}, 30 amu less than that of 363.1833 in its HREIMS, suggesting that the piperidine ring adopts a chair-like conformation. On the basis of the foregoing spectroscopic studies, the structure of compound 1 was established as 5,6-dimethoxy-2,3-methylenedioxy-13aR-phenanthroindolizidine, for which the trivial name ficuseptine B was proposed, following a previous convention for these alkaloidal constituents of *F. septica*.

Ficuseptine C (2) was obtained as colorless gum, and its UV absorption maxima were observed at 257, 260, 380, and 351 nm, similar to those of trioxigenated phenanthrene derivatives. 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 peak at *m/z* 294 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 1H NMR spectrum also 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 1H 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 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 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. Finally, the NOE between H-13a and H-9α indicated the existence of a chair-like conformation for the piperidine ring. Thus, the structure of 2 was elucidated as 6-methoxy-2,3-methylenedioxy-13αR-phenanthroindolizidine, and it has been designated as ficuseptine C.

Ficuseptine D (3) exhibited a molecular formula of C_{25}H_{26}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. The 1H 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 1H 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 3.35) 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. Finally, the NOE between H-13a and H-9α indicated the existence of a chair-like conformation for the piperidine ring. Thus, the structure of 2 was elucidated as 6-methoxy-2,3-methylenedioxy-13αR-phenanthroindolizidine, and it has been designated as ficuseptine C.

Table 1. 1H NMR Data of Compounds 1–8

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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. 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. On the basis of the foregoing spectroscopic studies, the structure of compound 1 was established as 5,6-dimethoxy-2,3-methylenedioxy-13αR-phenanthroindolizidine, for which the trivial name ficuseptine B was proposed, following a previous convention for these alkaloidal constituents of *F. septica*.
**Phenanthroindolizidine Alkaloids from Ficus**

Alkaloid 4, obtained as yellow needles, was considered to have the same molecular formula, C_{20}H_{25}NO_{5}, as tylophorine N-oxide, on the basis of its HREIMS, suggesting it to be an isomer.\textsuperscript{15} The \textsuperscript{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 \textsuperscript{1}H NMR signals and coupling patterns corresponding to the phenanthrene moiety were similar to those of tylophorine N-oxide,\textsuperscript{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-14a, H-14b, OCH_{3}-2; H-4/OCH_{3}-3, OCH_{3}-5; OCH_{3}-6/H-5, OCH_{3}-7; and H-8/H-9a, H-9b, OCH_{3}-7. Evidence for the presence of an N-oxide unit was also suggested by the downfield shifts of H-9a (δ 5.17), H-9b (δ 4.86), H-11α (δ 3.80), H-11β (δ 3.72), and H-13α (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 optical rotation measured at the sodium-D line and positive Cotton effect at 275 nm in the CD spectrum for the optical rotation inferred the \textsuperscript{R} configuration.\textsuperscript{29,30} Thus, H-13α was oriented in an α direction. The H-13α proton resonated at δ 3.94 and indicated a cis-fused ring junction of the indolizidine moiety.\textsuperscript{29,32} 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.\textsuperscript{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.\textsuperscript{31} Hence, 4 was characterized as 10R,13αR-tylophorine N-oxide.

Alkaloid 5 was assigned a molecular formula of C_{24}H_{27}NO_{5} from its HREIMS, allowing the same molecular formula, C_{24}H_{27}NO_{5}, as isotylocrebrine N-oxide,\textsuperscript{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 \textsuperscript{1}H NMR spectrum were assigned to H-1, H-2, H-5, and H-8, respectively.\textsuperscript{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 for 5 and negative optical rotation inferred the \textsuperscript{R} configuration at C-13α.\textsuperscript{29,30} Hence, H-13α was located with an α orientation. The appearance of the H-13α signal at δ 3.51 led the ring junction configuration to be determined as \textsuperscript{R} 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.\textsuperscript{16,33} The appearance of NOE cross-peaks between H-13α and H-9α was indicative of a chair-like conformation for the piperidine ring.\textsuperscript{31} The structure of 7 was, therefore, defined as 10S,13αS-tylocrebrine 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 \textsuperscript{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-13α,\textsuperscript{16,26} and the β orientation of H-13α. The downfield shift of H-13α to δ 4.08 inferred that the indolizidine ring junction should be in the cis configuration.\textsuperscript{29,32} 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.\textsuperscript{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 as in 4 suggested a boat-like conformation for the indolizidine moiety.\textsuperscript{31} Therefore, it was concluded that the 8 is 10S,13αS-isotylocrebrine N-oxide.

Compounds 6, 7, and tylophorine were tested in vitro for their cytotoxicity using HONE-1 and NUGC tumor cell lines.\textsuperscript{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 IC₅₀ 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-710 spectropolarimeter and on a Hitachi UV-3120 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. Mass spectra were measured in 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 F₂₅₄ 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 pump system equipped with a Shimadzu SPD-6AV UV–vis spectrophotometric detector at 275 nm, a Cosmosil packed column with SCX 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), was formed during this process and filtered. The filtrate was concentrated and suspended in water. The water soluble fractions 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 Et₂NH (20:79.5:0.5), and was 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.

**Isolation and Identification.** 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 Et₂NH (20:79.5:0.5), and was 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.
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-sulphophenyl)-tetrazolium salt (MTS) reduction assay.\(^5\)\(^2\) The 5 \(\mu\)M (final concentration) actinomycin D (showed 100\% of inhibition at 10 \(\mu\)M) DMSO were used as positive and vehicle control. Results were expressed as percent of DMSO control.

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**References and Notes**


