PHENANTHROINDOLIZIDINE ALKALOIDS AND THEIR CYTOTOXICITY FROM THE LEAVES OF *FICUS SEPTICA*

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Abstract – Phenanthroindolizidine \( \text{N-Oxide, ficuseptine-A (1), together with} \)
eighteen known compounds was isolated from the leaves of *Ficus septica*. The structures of these compounds were elucidated by spectroscopic analysis. Among them, phenanthroindolizidines, ficuseptine \((1)\), (+)-tylophorine \((4)\) and a mixture of (+)-tylocrebrine \((5)\) and (+)-isotylocrebrine \((6)\), exhibited strong cytotoxic activity against two human cancer cell lines, NUGC and HONE-1.

INTRODUCTION

*Ficus septica* (Moraceae) is a small evergreen tree growing in the tropical and subtropical region of the world.\(^1\) It is wildly distributed at low altitudes in Taiwan and used as a folk medicine to cure ulcer, cold, fever, fungal, bronchial asthma, allergic rhinitis and used as anti-tumor, anti-inflammatory and tonic medicament.\(^2\) Since the naturally occurring anti-tumor phenanthroindolizidine alkaloids were the constituents of *Ficus*, research in the field of *Ficus* dealing with isolation, structural elucidation and
pharmacological activity has been rapid increase. The phytochemical and pharmacological work on the leaves of *F. septica* therefore attracts our attention.

**RESULTS AND DISCUSSION**

The methanol extract of the leaves of *F. septica* was concentrated. The dark green syrup was suspended with water and partitioned with chloroform and *n*-butanol. Each layer was repeatedly separated by chromatography to give a new phenanthroindolizidine *N*-oxide, ficuseptine-A (1). In addition, eighteen compounds including seven phenanthroindolizidines: (+)-tylophorine *N*-oxide (2), 14α-hydroxyisotylocrebrine *N*-oxide (3), (+)-tylophorine (4), (+)-tylocrebrine (5), (+)-isotylocrebrine (6), (+)-antofine (7), and dehydrotylophorine (8); three steroids: β-sitosterol (9), stigmasterol (10) and β-sitosteryl-β-D-glucoside (11); two benzenoids: vanillic acid (12) and (5-acetyl-2-hydroxyphenyl)-β-D-glucopyranoside (13); two coumarins: umbelliferone (14) and esculin (15); one isoflavonoid: genistin (16); one flavonoid: kaempferitrin (17); one triterpenoid: squalene (18); and uracil (19) were also isolated and identified by comparing the physical data with those listed in the literature (Figure 1).

Ficuseptine-A (1) was isolated as pale yellow amorphous powder. The high resolution FABMS at *m/z* 456.2022 [M + H]+ established the molecular formula C25H30NO7. The UV spectrum (214, 263, 282, 359 and 405 nm) was likewise in accord with the phenanthrene chromophore. The 1H NMR spectrum exhibited similar pattern as that of phenanthroindolizidine alkaloids (2)–(8). A downfield proton signal at δ 5.26 (d, J = 2.6 Hz) which coupled with H-13α (δ 3.47) in the aliphatic region of the 1H NMR spectrum was assigned for H-14. The corresponding carbon signal at δ 64.7 (C-14) and the broad IR absorption at 3377 cm⁻¹ suggested a hydroxyl group on C-14. The chemical shifts for H-9 (δ 4.64 and 5.52), H-11 (δ 3.62 and 4.05), H-13α (δ 3.47) and C-9 (δ 66.5), C-11 (δ 70.3), C-13α (δ 70.8) were in lower field in comparison with those of 4 – 7, suggesting an *N*-oxide derivative of phenanthroindolizidine for 1. In the aromatic region, a singlet at δ 7.02 showing NOE with H-9α and a singlet at δ 7.61 showing NOE with H-14β assigned for H-8 and H-1, respectively (Figure 2).
The third aromatic proton at $\delta$ 9.24 was typical for H-4 or H-5. The latter was preferred because of the weak $J_{1H-1H}$ long range coupling between H-8 ($\delta$ 7.02) and the signal at $\delta$ 9.24 (H-5) in the COSY spectrum. Five methoxyl groups ($\delta$ 3.98, 4.04, 4.06, 4.07 and 4.09) apparently presented on C-2, 3, 4, 6, and 7. A phenanthroindolizidine N-oxide with 14-hydroxy group and 2,3,4,6,7-pentamethoxy substituents would be the structure of (1). The full assignments for $^1$H and $^{13}$C NMR signals were completed by the COSY, HMQC, HMBC and NOESY spectra.

The absolute configuration of 1 was determined as follows. A positive optical rotation under the sodium D line and a positive Cotton effect at 265 nm in the CD spectrum established the 13aS-$(+)$ configuration. Hence, H-13a located toward $\beta$-direction. Based on the small coupling constant and a strong NOE between H-14 and H-13a, the position of the hydroxyl group was determined to be trans with H-13a. Thus, the $\alpha$ configuration of 14-OH was obtained. The trans fused ring junction of indolizidine ring was determined by the chemical shift of H-13a at $\delta$ 3.47 which was closed to the reported chemical shift of H-13a at $\delta$ 3.34 for the trans-antofine N-oxide (20) instead of that at $\delta$ 4.22 for cis-antofine N-oxide (21). Furthermore, the strong deshielded H-9$\alpha$ ($\delta$ 5.52) and H-11$\alpha$ ($\delta$ 4.05) by
oxygen also inferred the $\alpha$ configuration of the $N$-oxide group.$^{20,21}$ The existence of NOE between H-13a and H-9$\beta$ ($\delta$ 4.64) suggested that the piperidine ring adopted a chair-like conformation (Figure 1).$^8$ Consequently, the alkaloid (1) was characterized to be (10$R$,13a$S$,14$S$)-14-hydroxy-2,3,4,6,7-pentamethoxyphenanthroindolizidine $N$-oxide and named as ficuseptine-A.

![Figure 2. The key NOE correlations of ficuseptine-A (1)](image)

Compounds (1), (4), mixture of 5 and 6, 8, 13, 15 and 16 were subjected to cytotoxicity evaluation (Table 1). Among them, phenanthroindolizidines (1), (4) and mixture of 5 and 6 exhibited strong cytotoxic activity against two human cancer cell lines including gastric carcinoma (NUGC) and nasopharyngeal carcinoma (HONE-1) even at 10 $\mu$M.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Growth ratio of cells (%)</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>NUGC 50 $\mu$M</td>
<td>9</td>
</tr>
<tr>
<td>10 $\mu$M</td>
<td>18</td>
</tr>
<tr>
<td>HONE-1 50 $\mu$M</td>
<td>10</td>
</tr>
<tr>
<td>10 $\mu$M</td>
<td>14</td>
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</tbody>
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$^a$ NUGC = human gastric carcinoma; HONE-1 = human nasopharyngeal carcinoma
EXPERIMENTAL

**General Experimental Method**  Melting points were recorded on a Yanaco MP-3 melting point apparatus and were not corrected. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. UV spectra were recorded on an Agilent 8453 spectrophotometer. IR spectra were measured on a Nicolet Magna FT-IR spectrophotometer as solid dispersion in KBr. NMR spectra were recorded on Bruker AC-200, AMX-300 and AMX-400 FT-NMR spectrometers; all chemical shifts were reported in ppm from tetramethylsilane as an internal standard. MS spectra were obtained on either Finnigan Trace or VG 70-250S spectrometer by a direct inlet system. CD spectra were determined on a JASCO J-720 spectropolarimeter.

**Plant Material**  The leaves of *Ficus septica* were collected from Tainan Hsien, Taiwan, Republic of China, in January 2000. It was verified by Professor C. S. Kuoh. A voucher specimen was deposited in the Herbarium of National Cheng Kung University, Tainan, Taiwan.

**Extraction and Isolation**  The air-dried fresh leaves of *Ficus septica* (3.6 kg) was powdered and extracted with CH$_3$OH (20 L x 6 times) under reflux for 8 h. The combined CH$_3$OH extract was concentrated under reduced pressure to give dark green syrup (500 g). The syrup was then suspended in H$_2$O and partitioned with CHCl$_3$ and n-C$_4$H$_9$OH. The concentrated CHCl$_3$ layer (150 g) was fractionated on a silica gel column chromatography eluted with a gradient solvent of hexane–CH$_3$CO$_2$C$_2$H$_5$–CH$_3$OH to obtain 5 fractions. Fractions 1 and 2 were combined and subjected to chromatographed on silica gel column eluting with a gradient of hexane–CH$_3$CO$_2$C$_2$H$_5$ to give 18 (5 mg), 9 (1.45 g) and 10 (1.32 g). Fraction 3 was chromatographed to give 14 (5 mg). Fraction 5 was repeated chromatography on silica gel column and eluted with a gradient of CHCl$_3$–CH$_3$OH to yield 4 (45 mg), a mixture of 5 and 6 (75 mg), 7 (2 mg), 8 (3 mg), 2 (5 mg), 3 (1 mg), 1 (3 mg), and 11 (1.08 g), successively. The concentrated n-C$_4$H$_9$OH layer (25 g) was subjected to column chromatography on Diaion LH-20 eluting with a gradient of H$_2$O–CH$_3$OH to give 6 fractions. Purification of fractions 1 and 6 on silica gel column eluting with a gradient of CH$_3$CO$_2$C$_2$H$_5$–CH$_3$OH gave 19 (2 mg) and 16 (26 mg), respectively. Further separation of fraction 5 on silica gel column eluting with a gradient of...
CH$_3$CO$_2$C$_2$H$_5$−CH$_3$OH yielded 13 (9 mg), 12 (2 mg), 15 (1 mg) and 17 (5 mg).

**Ficuseptine-A (1)** Pale yellow amorphous powder, mp 210ºC (decomp); [α]$_D$ +30.3° (c 0.033, CH$_3$OH); IR $\nu_{max}$ (KBr) 3377, 2952, 1633, 1514 cm$^{-1}$; UV $\lambda_{max}$ (CH$_3$OH) (log $\varepsilon$) 214 (4.35), 263 (4.19), 282 (4.00), 359 (3.11), 405 (2.79) nm; $^1$H NMR (CDCl$_3$) $\delta$ 2.25 (2H, m, H-12$\alpha$ and H-13$\beta$), 2.66 (1H, m, H-12$\beta$), 3.11 (1H, m, H-13$\alpha$), 3.47 (1H, m, H-13a), 3.62 (1H, m, H-11$\beta$), 4.05 (1H, m, H-11$\alpha$), 3.98 (3H, s 4-OCH$_3$), 4.04 (3H, s, 7-OCH$_3$), 4.06 and 4.07 (each 3H, s, 2- and 3-OCH$_3$), 4.09 (3H, s, 6-OCH$_3$), 4.64 (1H, d, J = 14.9 Hz, H-9$\beta$), 5.26 (1H, d, J = 2.6 Hz, H-14), 5.52 (1H, d, J = 14.9 Hz, H-9$\alpha$), 7.02 (1H, s, H-8), 7.61 (1H, s, H-1), 9.24 (1H, s, H-5); $^{13}$C NMR (CDCl$_3$) $\delta$ 20.3 (C-12), 22.4 (C-13), 55.7 (4-OCH$_3$), 56.0 (3- and 7-OCH$_3$), 60.6 (3-OCH$_3$), 61.4 (6-OCH$_3$), 64.7 (C-14), 66.5 (C-9), 70.3 (C-11), 70.8 (C-13a), 101.9 (C-1), 102.7 (C-8), 108.1 (C-5), 118.9 (C-4a), 121.0 (C-8b), 123.4(C-8a), 125.0 (C-4b), 127.7 (C-14a), 128.1 (C-14b), 142.9 (C-2), 148.3 (C-6), 148.9 (C-7), 151.4 (C-4), 152.3 (C-3); FABMS $m/z$ (rel. int.) 456 (100, [M + H]$^+$), 438 (40), 420 (35), 371 (30), 356 (48), 338 (76), 307 (25), 289 (28), 259 (20), 219 (31); HR-FABMS calcd for C$_{25}$H$_{30}$NO$_7$ $m/z$ 456.2022 [M + H]$^+$, found 456.2025; CD (MeOH, 7.3 x 10$^{-5}$ M) [θ]$_{209}^0$ +804º, [θ]$_{265}^0$ +446º.

**Cytotoxicity Assay** Human cancer cell lines, NUGC and HONE-1, were seeded in 96-well microliter plates at a density of 6000/well in 10 µL culture medium. After an overnight adaptation period. The 50 µg/mL (final concentration) of test compounds 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.$^{22}$ The 5 µM (final concentration) of actinomycin D and 0.3% (final concentration) of DMSO were used as positive and vehicle controls. Results were expressed as percent of DMSO control.

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