

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

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Abstract – Phenanthroindolizidine *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 widely 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.^{2,3} 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**),⁴ 14 α -hydroxyisotylocrebrine *N*-oxide (**3**),⁵ (+)-tylophorine (**4**),^{5,6} (+)-tylocrebrine (**5**),⁷ (+)-isotylocrebrine (**6**),⁵ (+)-antofine (**7**),^{2,8} and dehydrotylophorine (**8**),⁹ three steroids: β -sitosterol (**9**), stigmasterol (**10**) and β -sitosteryl- β -D-glucoside (**11**); two benzenoids: vanillic acid (**12**)¹⁰ and (5-acetyl-2-hydroxyphenyl)- β -D-glucopyranoside (**13**);¹¹ two coumarins: umbelliferone (**14**)¹² and esculin (**15**);¹³ one isoflavonoid: genistin (**16**);¹⁴ one flavonoid: kaempferitrin (**17**);¹⁵ one triterpenoid: squalene (**18**);¹⁶ and uracil (**19**)¹⁷ 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 $C_{25}H_{30}NO_7$. The UV spectrum (214, 263, 282, 359 and 405 nm) was likewise in accord with the phenanthrene chromophore.¹⁸ 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-13a (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-13a (δ 3.47) and C-9 (δ 66.5), C-11 (δ 70.3), C-13a (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-9 α and a singlet at δ 7.61 showing NOE with H-14 β assigned for H-8 and H-1, respectively (Figure 2).

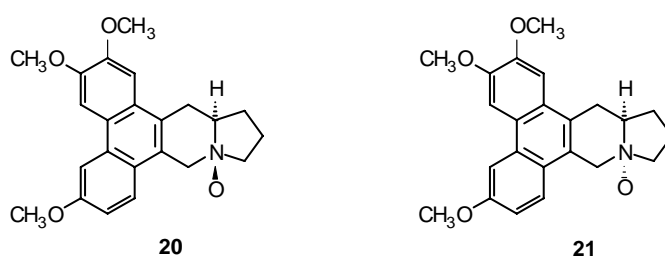
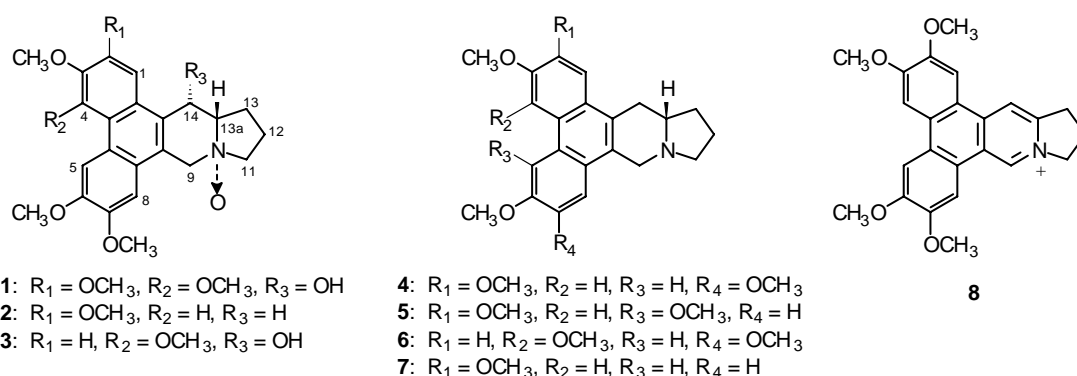


Figure 1. Structures of isolated compounds (1)–(8), (20), and (21)

The third aromatic proton at δ 9.24 was typical for H-4 or H-5.^{5,7} The latter was preferred because of the weak 5J ^1H – ^1H long range coupling between H-8 (δ 7.02) and the signal at δ 9.24 (H-5) in the COSY spectrum. Five methoxyl groups (δ 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 ^1H 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 13a*S*-(+) configuration.^{19,20} Hence, H-13a located toward β -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 α configuration of 14-OH was obtained. The *trans* fused ring junction of indolizidine ring was determined by the chemical shift of H-13a at δ 3.47 which was closed to the reported chemical shift of H-13a at δ 3.34 for the *trans*-antofine *N*-oxide (**20**) instead of that at δ 4.22 for *cis*-antofine *N*-oxide (**21**).²¹ Furthermore, the strong deshielded H-9 α (δ 5.52) and H-11 α (δ 4.05) by

oxygen also inferred the α configuration of the *N*-oxide group.^{20,21} The existence of NOE between H-13a and H-9 β (δ 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.

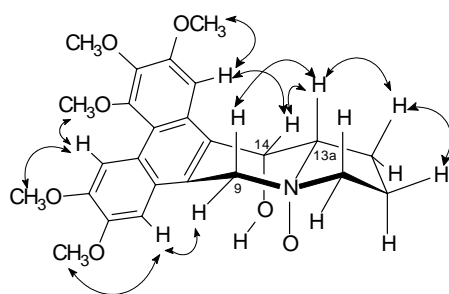


Figure 2. The key NOE correlations of ficuseptine-A (**1**)

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 μ M.

Table 1. Cytotoxicity of the Compounds (**1**), (**4**)–(**6**), (**8**), (**13**), (**15**), (**16**) from the Leaves of *Ficus septica* toward Two Human Cancer Lines^a

Cell line	Growth ratio of cells (%)						
	1	4	5 and 6	8	13	15	16
NUGC 50 μ M	9	3	2	64	100	99	88
10 μ M	18	11	10	101	102	102	94
HONE-1 50 μ M	10	3	18	67	97	96	83
10 μ M	14	9	17	91	104	103	89

^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 CH₃OH (20 L x 6 times) under reflux for 8 h. The combined CH₃OH extract was concentrated under reduced pressure to give dark green syrup (500 g). The syrup was then suspended in H₂O and partitioned with CHCl₃ and *n*-C₄H₉OH. The concentrated CHCl₃ layer (150 g) was fractionated on a silica gel column chromatography eluted with a gradient solvent of hexane–CH₃CO₂C₂H₅–CH₃OH to obtain 5 fractions. Fractions 1 and 2 were combined and subjected to chromatographed on silica gel column eluting with a gradient of hexane–CH₃CO₂C₂H₅ 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 CHCl₃–CH₃OH 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*-C₄H₉OH layer (25 g) was subjected to column chromatography on Diaion LH-20 eluting with a gradient of H₂O–CH₃OH to give 6 fractions. Purification of fractions 1 and 6 on silica gel column eluting with a gradient of CH₃CO₂C₂H₅–CH₃OH gave **19** (2 mg) and **16** (26 mg), respectively. Further separation of fraction 5 on silica gel column eluting with a gradient of

CH₃CO₂C₂H₅-CH₃OH yielded **13** (9 mg), **12** (2 mg), **15** (1 mg) and **17** (5 mg).

Ficuseptine-A (1) Pale yellow amorphous powder, mp 210°C (decomp); [α]_D +30.3° (c 0.033, CH₃OH); IR ν_{max} (KBr) 3377, 2952, 1633, 1514 cm⁻¹; UV λ_{max} (CH₃OH) (log ϵ) 214 (4.35), 263 (4.19), 282 (4.00), 359 (3.11), 405 (2.79) nm; ¹H NMR (CDCl₃) δ 2.25 (2H, m, H-12 α and H-13 β), 2.66 (1H, m, H-12 β), 3.11 (1H, m, H-13 α), 3.47 (1H, m, H-13a), 3.62 (1H, m, H-11 β), 4.05 (1H, m, H-11 α), 3.98 (3H, s, 4-OCH₃), 4.04 (3H, s, 7-OCH₃), 4.06 and 4.07 (each 3H, s, 2- and 3-OCH₃), 4.09 (3H, s, 6-OCH₃), 4.64 (1H, d, J = 14.9 Hz, H-9 β), 5.26 (1H, d, J = 2.6 Hz, H-14), 5.52 (1H, d, J = 14.9 Hz, H-9 α), 7.02 (1H, s, H-8), 7.61 (1H, s, H-1), 9.24 (1H, s, H-5); ¹³C NMR (CDCl₃) δ 20.3 (C-12), 22.4 (C-13), 55.7 (4-OCH₃), 56.0 (3- and 7-OCH₃), 60.6 (3-OCH₃), 61.4 (6-OCH₃), 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 C₂₅H₃₀NO₇ m/z 456.2022 [M + H]⁺, found 456.2025; CD (MeOH, 7.3 x 10⁻⁵ M) [θ]₂₀₉ +804°, [θ]₂₆₅ +446°.

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 μ L culture medium. After an overnight adaptation period. The 50 μ g/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-sulphophenyl) tetrazolium salt (MTS) reduction assay.²² The 5 μ M (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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