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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 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.[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] 14α-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 ^{1}H 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 ^{1}H 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-9α and a singlet at δ 7.61 showing NOE with H-14β assigned for H-8 and H-1, respectively (Figure 2).

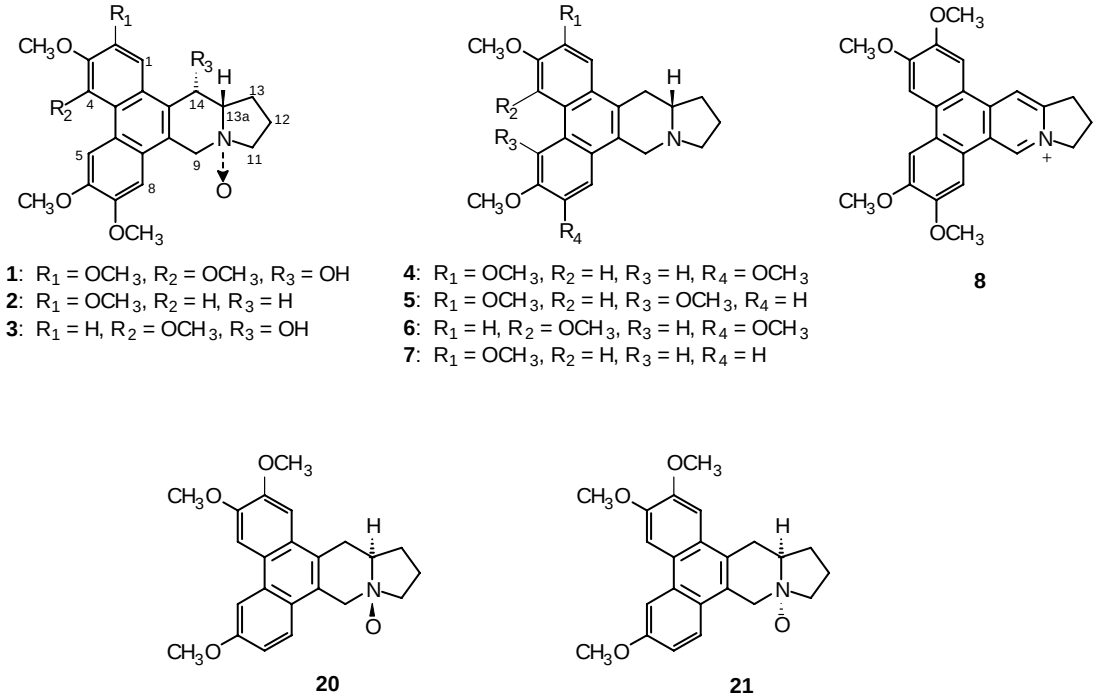

Figure 1. Structures of isolated compounds (**1**)–(**8**), (**20**), and (**21**)

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 ^{5}J ^{1}H–^{1}H 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 ^{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 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-9α (δ 5.52) and H-11α (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-9β (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.

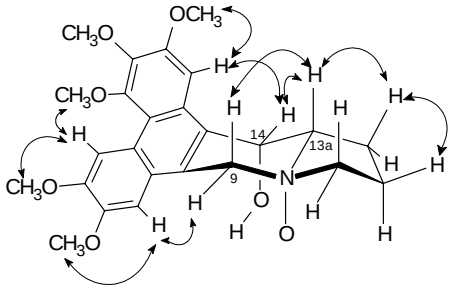

Figure 2. The key NOE correlations of ficuseptine-A **(1)**

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 μM.

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 μM	9	3	2	64	100	99	88
	10 μM	18	11	10	101	102	102	94
HONE-1	50 μM	10	3	18	67	97	96	83
	10 μM	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 CH_3OH (20 L x 6 times) under reflux for 8 h. The combined CH_3OH extract was concentrated under reduced pressure to give dark green syrup (500 g). The syrup was then suspended in H_2O and partitioned with $CHCl_3$ and *n*-C_4H_9OH. The concentrated $CHCl_3$ layer (150 g) was fractionated on a silica gel column chromatography eluted with a gradient solvent of hexane–$CH_3CO_2C_2H_5$–CH_3OH to obtain 5 fractions. Fractions 1 and 2 were combined and subjected to chromatographed on silica gel column eluting with a gradient of hexane–$CH_3CO_2C_2H_5$ 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_3OH 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_4H_9OH layer (25 g) was subjected to column chromatography on Diaion LH-20 eluting with a gradient of H_2O–CH_3OH to give 6 fractions. Purification of fractions 1 and 6 on silica gel column eluting with a gradient of $CH_3CO_2C_2H_5$–CH_3OH gave **19** (2 mg) and **16** (26 mg), respectively. Further separation of fraction 5 on silica gel column eluting with a gradient of

$CH_3CO_2C_2H_5$–CH_3OH yielded **13** (9 mg), **12** (2 mg), **15** (1 mg) and **17** (5 mg).

Ficuseptine-A (1) Pale yellow amorphous powder, mp 210ºC (decomp); $[\alpha]_D$ +30.3° (c 0.033, CH_3OH); IR ν_{max} (KBr) 3377, 2952, 1633, 1514 cm^{-1}; UV λ_{max} (CH_3OH) (log ε) 214 (4.35), 263 (4.19), 282 (4.00), 359 (3.11), 405 (2.79) nm; ^1H NMR ($CDCl_3$) δ 2.25 (2H, m, H-12α and H-13β), 2.66 (1H, m, H-12β), 3.11 (1H, m, H-13α), 3.47 (1H, m, H-13a), 3.62 (1H, m, H-11β), 4.05 (1H, m, H-11α), 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-9β), 5.26 (1H, d, J = 2.6 Hz, H-14), 5.52 (1H, d, J = 14.9 Hz, H-9α), 7.02 (1H, s, H-8), 7.61 (1H, s, H-1), 9.24 (1H, s, H-5); ^{13}C NMR ($CDCl_3$) δ 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) $[\theta]_{209}$ +804º, $[\theta]_{265}$ +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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