Aminocaprophenone- and Pyrrolidine-Type Alkaloids from the Leaves of *Ficus septica*

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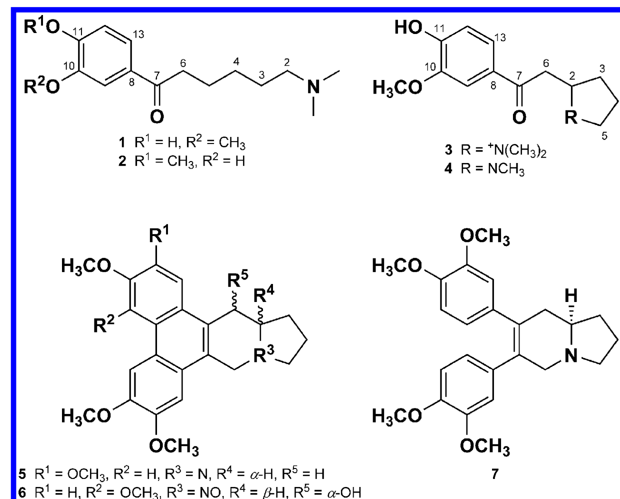
Two new aminocaprophenone alkaloids, ficuseptamines A (**1**) and B (**2**), and a new pyrrolidine alkaloid, ficuseptamine C (**3**), together with 12 known alkaloids and a known acetophenone derivative were isolated from a methanolic extract of the leaves of *Ficus septica*. The structures of **1**−**3** were determined on the basis of their spectroscopic data. The compounds obtained were evaluated for cytotoxicity against two cancer cell lines.

Ficus septica Burm. f.[1] is a small, arboreous, and evergreen plant belonging to the family Moraceae that is distributed widely throughout the tropical and subtropical regions of the Western Pacific area. This plant has been used as a folk medicine for treating colds, fevers, headaches, gastralgia, and microbial diseases[2–6] and has been reported to contain phenanthroindolizidine-type and indolizidine-type alkloids, triterpenoids, steroids, and phenolic compounds.[2–4] In the course of our chemical screening program for new compounds from natural resources, three new alkaloids, ficuseptamines A−C (**1**−**3**), were isolated from a MeOH extract of the leaves of this plant. We report here the isolation, structure elucidation, and cytotoxic activity of these new alkaloids.

An aqueous MeOH extract of *F. septica* leaves was divided into *n*-hexane-, acidic EtOAc-, basic EtOAc-, BuOH-, and H_2O-soluble fractions. The basic EtOAc- and BuOH-soluble fractions were separated using medium-pressure liquid chromatography (MPLC) and HPLC to yield two new caprophenone alkaloids (**1** and **2**), one new (**3**) and two known pyrrolidine alkaloids [phyllosterone[7] (**4**) and norruspoline[8]], eight known phenanthroindolizidine alkaloids [tylophorine[9] (**5**), 14α-hydroxyisocrebrine *N*-oxide[9] (**6**), ficuseptine A,[3] 14-hydroxy-3,4,6,7-tetramethoxyphenanthroindolizidine,[10] 14-hydroxy-2,3,4,6,7-pentamethoxyphenanthroindolizidine,[10] tylocrebrine,[11] isotylocrebrine,[9] and antofine[11,12]], and two known *seco*-phenanthroindolizidine alkaloids [septicine[13] (**7**) and secoantofine[12]], along with a known acetophenone glucoside (pungenin[14]). The structures of the new compounds **1**−**3** were elucidated mainly by spectroscopic methods, including 2D NMR techniques.



Ficuseptamines A (**1**) and B (**2**) were obtained as colorless amorphous solids, and their HRESIMS data (*m/z* 266.1730 and 266.1794, respectively) revealed both compounds to have the molecular formula $C_{15}H_{23}NO_3$. Absorptions assignable to carbonyl groups in **1** (ν_{max} 1662 cm^{-1}) and **2** (ν_{max} 1670 cm^{-1}) were observed in their IR spectra. Direct connectivities between protons and carbons were established from their HSQC spectra. The ^{13}C and ^{1}H NMR spectroscopic data for **1** and **2** are shown in Table 1. In the DQF-COSY spectrum of **1**, a spin coupling between H-12 (δ_H 6.89) and H-13 (δ_H 7.57) and a *meta*-coupling between H-9 (δ_H 7.54) and H-13 indicated the presence of a 1,2,4-trisubstituted benzene unit. The $^{1}H-^{13}C$ long-range couplings from H-9 to two aromatic carbons, C-11 (δ_C 152.1) and C-13 (δ_C 123.8), and a carbonyl carbon, C-7 (δ_C 198.6), at the *peri* position, from H-12 to two aromatic carbons, C-8 (δ_C 130.6) and C-10 (δ_C 148.3), and from H-13 to two aromatic carbons, C-9 (δ_C 111.5) and C-11, and the carbonyl carbon C-7 were used to establish the assignments of the benzene ring substituents. A long-range coupling from the methoxy proton (δ_H 3.89) to C-10 indicated that the methoxy group is substituted at the C-10 position, as shown in Figure 1A, whereas another low-field-shifted aromatic carbon, C-11, was found to bear a hydroxy group. Thus, a 4-hydroxy-3-methoxybenzoyl moiety was determined as constituting a partial structure of **1**, and the chromophore of this moiety was also supported by the UV spectrum (λ_{max} 227, 273, 298 nm).[8] In the DQF-COSY spectrum, a sequence from the methylene proton H_2-2 (δ_H 2.21) to the methylene proton H_2-6 (δ_H 2.93) through three methylene protons H_2-3 (δ_H 1.47), H_2-4 (δ_H 1.37), and H_2-5 (δ_H 1.68) was observed, as shown in Figure 1A. In the HMBC spectrum, the methylene proton H_2-6 was long-range coupled to the benzoyl carbonyl carbon C-7. In addition, an *N*,*N*-dimethyl proton (δ_C 2.13, δ_H 45.6) was coupled to the methylene carbon C-2 (δ_C 60.2). Thus, **1** was determined as a new aminocaprophenone derivative, 6-(dimethylamino)-1-(3-hydroxy-4-methoxyphenyl)hexanone.

As a result of analyzing the DQF-COSY and HMBC spectra of **2**, a 1,2,4-trisubstituted benzene ring and an *N*,*N*-dimethyl hexanoyl moiety were evident. $^{1}H-^{13}C$ NMR long-range couplings from the H-9 (δ_H 7.46) and H-13 (δ_H 7.52) aromatic protons and from the methoxy proton (δ_H 3.90) to the C-11 aromatic carbon (δ_C 152.4) were used to establish the substitution pattern of **2**. Thus, **2** was determined to be a second new aminocaprophenone derivative, 6-(dimethylamino)-1-(4-hydroxy-3-methoxyphenyl)hexanone.

Ficuseptamines C (**3**) was obtained as an amorphous solid and showed a molecular ion at *m/z* 264.1621 corresponding to $C_{15}H_{22}NO_3$ in the HRESIMS. The IR spectrum of **3** (ν_{max} 1677

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Table 1. ^{13}C and ^{1}H NMR Spectroscopic Data for **1−3**

	1[a]		**2**[a]		**3**[b]	
position	δ_C	δ_H (multiplicity, *J* in Hz)	δ_C	δ_H (multiplicity, *J* in Hz)	δ_C	δ_H (multiplicity, *J* in Hz)
2	60.2	2.21 (t, 7.2)	60.1	2.24 (t, 7.2)	73.4	4.14 (tdd, 10.5, 8.0, 3.0)
3	28.3	1.47 (quintet, 7.2)	28.1	1.48 (quintet, 7.2)	29.1	2.64 (m); 1.90 (m)
4	27.8	1.37 (quintet, 7.2)	27.7	1.37 (quintet, 7.2)	20.4	2.22 (m); 2.16 (m)
5	25.3	1.68 (quintet, 7.2)	25.2	1.66 (quintet, 7.2)	67.9	3.71 (ddd, 11.0, 8.0, 3.0); 3.61 (br q, 10.0)
6	38.4	2.93 (t, 7.2)	38.5	2.90 (t, 7.2)	37.9	3.75 (dd, 17.5, 3.0); 3.44 (dd, 17.5, 8.0)
7	198.6		198.9		195.7	
8	130.6		131.7		129.6	
9	111.5	7.54 (d, 2.0)	115.2	7.46 (d, 2.0)	111.8	7.57 (d, 2.0)
10	148.3		147.3		149.2	
11	152.1		152.4		153.9	
12	115.3	6.89 (d, 8.3)	111.5	7.01 (d, 8.5)	115.9	6.89 (d, 8.0)
13	123.8	7.57 (dd, 8.3, 2.0)	121.7	7.52 (dd, 8.5, 2.0)	124.8	7.65 (dd, 8.0, 2.0)
N-CH_3	45.6	2.13 (s)	45.5	2.15 (s)	51.5	3.22 (s)
					45.7	3.02 (s)
O-CH_3	56.2	3.89 (s)	56.3	3.90 (s)	56.5	3.91 (s)

[a] In acetone-d_6. [b] In methanol-d_4.

cm^{-1}) indicated the presence of a carbonyl group. The ^{13}C and ^{1}H NMR data for **3** are shown in Table 1. A sequence from the H_2-5 methylene protons (δ_H 3.71, 3.61) to the H_2-6 methylene protons (δ_H 3.75, 3.44) through the H_2-4 (δ_H 2.21, 2.16) and H_2-3 (δ_H 2.64, 1.90) methylene protons and the H-2 methine proton (δ_H 4.14) was established, as shown in Figure 1C. Long-range couplings from two nonequivalent *N*,*N*-dimethyl protons (δ_H 3.22, 3.02 and δ_C 51.5, 45.7, respectively) to C-2 (δ_C73.4) and C-5 (δ_C 67.9) and from the H_2-5 methylene protons to C-2 indicated a pyrrolidine moiety, as shown in Figure 1C. The ^{13}C NMR chemical shifts of the C-2 methine (δ_C 73.4) and C-5 methylene (δ_C 67.9) carbons also supported this substructure. The coupling pattern of three aromatic protons at H-9 (δ_H 7.57), H-12 (δ_H 6.89), and H-13 (δ_H 7.65) indicated the presence of a 1,2,4-trisubstituted benzene unit, as observed in **1**. The $^{1}H-^{13}C$ NMR long-range couplings from H-9 to the C-13 aromatic carbon (δ_C 124.8) and the C-7 carbonyl carbon C-7 (δ_C 195.7), from H-12 to the C-8 (δ_C 129.6) and C-10 (δ_C 149.2) aromatic carbons, and from H-13 to the C-9 (δ_C 111.8) and C-11 (δ_C 153.9) aromatic carbons and the C-7 carbonyl carbon, together with a long-range coupling from the methoxy protons (δ_H 3.91) to C-10, were used to establish the chromophore substructure of **3**, as shown in Figure 1C. Thus, **3** was determined to have a phenethylpyrrolidinium structure, as 1-(4-hydroxy-3-methoxyphenyl)-2-(*N*,*N*-dimethylpyrrolidinium-2-yl)ethanone.

To date, an aminocaprophenone alkaloid has been reported only from *Peripentadenia mearsii* (peripentamine),[15] while the phenethylpyrrolidines phyllosterone and norruspolone and their derivatives were isolated from *Ruspolia herpercrateriformis* and *Cryptocarpa phyllostemon* (of which the latter also contains phenanthoindolizidine alkaloids).[7,8]

The isolated caprophenone (**1** and **2**), pyrrolidine (**3** and **4**), phenanthroindolizidine (**5** and **6**), and the *seco*-phenanthroindolizidine (**7**) alkaloids were examined for cytotoxic activities against HeLa and ACC-MESO-1 cells (Table S1). The only active compounds were **5** and **6**, which showed IC_{50} values of 3.1 and 7.1 μM, respectively, against HeLa cells. Both **5** and **6** were inactive against ACC-MESO-1 cells (IC_{50} values >10 μM). All other alkaloids tested (**1−4**, **7**) were inactive to both HeLa and ACC-MESO-1 cells. Phenanthroindolizidine alkaloids are known as constituents of *F. septica*,[2−6] as well as other plants in the genus *Ficus*, and in species of the Asclepiadaceae (*Tylophora* spp., *Cynanchum* spp.)[9,10,13] and Lauraceae (*Cryptocarya* spp.),[7] and their cytotoxic activities have been reported.[3−5,13] The acetophenone glycoside pungenin was also tested against the two cancer cell lines, but was inactive. Further details on the biological activities of these compounds are now under examination.

Experimental Section

General Experimental Procedures. Optical rotations were carried out on a Horiba SEPA-300 polarimeter. The UV and IR spectra were measured by a DU730 Beckman Coulter UV/vis spectrophotometer and a Horiba FT-720 spectrophotometer, respectively. NMR spectra were measured on a Varian NMR System 500 NB CL. HRESIMS data were recorded on a Waters LCT-Premier XE mass spectrometer. Analytical TLC was carried out on precoated silica gel 60 F_{254} plates (0.25 mm thickness; Merck, Darmstadt, Germany). Reversed-phase MPLC was carried out on a Purif-Pack 100 μm ODS column (Moritex, Tokyo, Japan). Preparative reversed-phase HPLC was carried out on an XBridge Prep C_{18} column (5.0 μm OBD, 19 i.d. × 150 mm; Waters, Milford, MA; flow rate 10 mL/min), with detection using a Waters 2996 photodiode array detector and a Waters 3100 mass detector. The reagents and solvents used were of the highest grade available.

Plant Material. The leaves of *Ficus septica* were collected from Ishigaki Island, Okinawa Prefecture, Japan, in October 2008. The plant was identified by Dr. Y Kurihara (OP Bio Factory Co., Ltd.), and the voucher sample (ISG-081020-001A) is preserved at the laboratory of OP Bio Factory in Ishigaki Island.

Extraction and Isolation. The air-dried leaves (257 g) were extracted using MeOH−H_2O (80:20). The extract was concentrated in vacuo and successively partitioned between *n*-hexane and MeOH−H_2O (90:10), EtOAc and acidic H_2O (ca. pH 3), EtOAc and basic H_2O (ca. pH 12), and *n*-BuOH and H_2O. Each organic layer except for the BuOH layer was dried over anhydrous Na_2SO_4 and evaporated to dryness in vacuo to yield a hexane- (1.34 g), an acidic EtOAc- (2.24 g), and a basic EtOAc- (0.419 g) soluble fraction, while the BuOH layer was concentrated and evaporated to yield a BuOH-soluble fraction (2.61 g).

The basic EtOAc-soluble fraction was subjected to reversed-phase MPLC by stepwise elution with MeOH−H_2O [30%, 60−70% (linear gradient), 100% MeOH, successively]. The 30% MeOH eluate was purified by preparative HPLC with 10% MeOH containing 0.45% formic acid to yield norruspoline (1.3 mg, t_R = 27.3 min) and **4** (2.9 mg, t_R = 31.4 min). The 60% MeOH eluate was subjected to preparative HPLC with 25% MeOH containing 0.4% diethylamine to yield **4** (2.7 mg, t_R = 7.8 min), **1** (5.9 mg, t_R = 14.6 min), and **2** (8.9 mg, t_R = 29.5 min). The 60−65% MeOH eluate was separated by preparative HPLC with 40% MeOH containing 0.3% diethylamine to yield **6** (2.8 mg, t_R = 18.5 min) and ficuseptine A (1.7 mg, t_R = 26.5 min). The 65−70% MeOH eluate was purified by preparative HPLC with 60% MeOH containing 0.2% diethylamine to yield **7** (12.9 mg, t_R = 22.4 min), 14-hydroxy-3,4,6,7-tetramethoxyphenanthroindolizidine (2.5 mg, t_R = 29.7 min), **5** (8.7 mg, t_R = 30.7 min), 14-hydroxy-2,3,4,6,7-pentamethoxyphenanthroindolizidine (1.2 mg, t_R = 34.7 min), secoantofine (2.7 mg, t_R = 39.1 min), and a mixture of tylocrebrine and isotylocrebrine (ratio of 3:2, 5.2 mg, t_R = 46.2 min). The 100% MeOH eluate was subjected to preparative HPLC with 65% MeOH containing 0.2% diethylamine to yield **5** (5.3 mg, t_R = 17.8 min), a mixture of tylocrebrine and isotylocrebrine (ratio of 3:2, 2.3 mg, t_R = 26.1 min), and antofine (6.5 mg, t_R = 32.9 min).

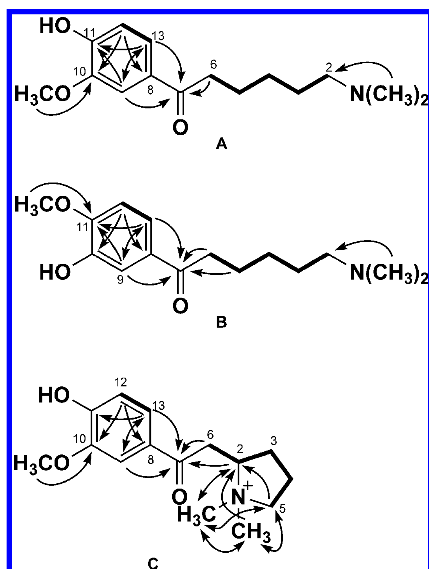

Figure 1. Key correlations observed in 2D NMR spectra of **1** (A), **2** (B), and **3** (C) (the bold lines show $^{1}H-^{1}H$ DQF-COSY results, and the arrows show CT-HMBC results).

The BuOH-soluble fraction was chromatographed with reversed-phase MPLC by stepwise elution with MeOH−H_2O [5%, 25%, 40−75% (linear gradient), 100% MeOH, successively]. The 5−25% MeOH eluate was separated by preparative HPLC with 15% MeOH containing 0.4% trifluoroacetic acid to yield **3** (9.4 mg, $t_R = 19.1$ min) and norruspoline (2.9 mg, $t_R = 23.1$ min). The 25% MeOH eluate was purified by preparative HPLC with 15% MeOH containing 0.4% trifluoroacetic acid to yield pungenin (6.9 mg, $t_R = 24.0$ min).

Ficuseptamine A (1): colorless, amorphous solid; UV (MeOH) λ_{max} (log ε) 227 (3.91), 273 (3.71), 298 (3.56, sh) nm; IR ($CHCl_3$) ν_{max} 1662 cm^{-1}; for ^{13}C and ^{1}H NMR data, see Table 1; HRESIMS *m/z* 266.1730 (calcd for $C_{15}H_{24}NO_3$ $[M + H]^+$, 266.1756).

Ficuseptamine B (2): colorless, amorphous solid; UV (MeOH) λ_{max} (log ε) 228 (3.89), 270 (3.73), 304 (3.51) nm; IR ($CHCl_3$) ν_{max} 1670 cm^{-1}; for ^{13}C and ^{1}H NMR data, see Table 1; HRESIMS *m/z* 266.1794 (calcd for $C_{15}H_{24}NO_3$ $[M + H]^+$, 266.1756).

Ficuseptamine C (3): colorless, amorphous solid; $[\alpha]^{27}_{D}$ +177.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 230 (3.80), 278 (3.65), 305 (3.61) nm; IR ($CHCl_3$) ν_{max} 1677 cm^{-1}; for ^{13}C and ^{1}H NMR data, see Table 1; HRESIMS *m/z* 264.1621 (calcd for $C_{15}H_{22}NO_3$ $[M]^+$, 264.1600).

Cytotoxic Activity. Cytotoxicity against human cervical carcinoma HeLa cells and human malignant pleural mesothelioma ACC-MESO-1 cells[16,17] was determined by a colorimetric assay using WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium monosodium salt]. Cells were cultured in DMEM medium (Wako Pure Chemical Industries, Tokyo, Japan) supplemented with 10% (v/v) fetal bovine serum (GIBCO, Carlsbad, CA), penicillin (100 units/mL), and streptomycin (100 μg/mL) at 37 °C in a humidified incubator under a 5% CO_2 atmosphere. The 384-well plates were seeded with aliquots of 20 μL of medium containing 1.0×10^3 cells per well and were incubated overnight before being treated with compounds at various concentrations for 48 h. Plates were incubated for 1 h at 37 °C after the addition of 2 μL of WST-8 reagent solution (Cell Counting Kit; Dojindo, Kumamoto, Japan) per well. The absorption of formazan dye formed was measured at 450 nm. The vehicle solvent (DMSO) was used as a negative control. Paclitaxel as a positive control showed cytotoxicity against HeLa and ACC-MESO-1 cells with IC_{50} values of 0.26 and 24 nM, respectively.

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Supporting Information Available: NMR spectra of copmpounds **1**−**3** and table of cytotoxic activities of **1**−**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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