Three New Pyridoacridine Type Alkaloids from a Singaporean Ascidian

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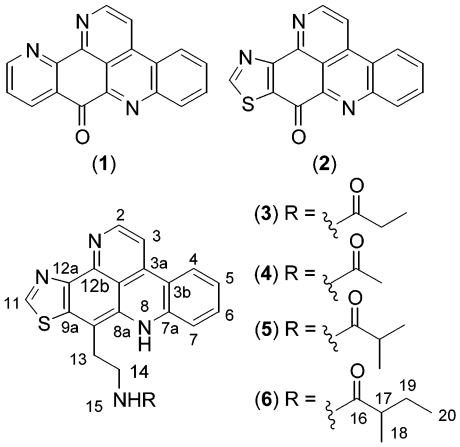
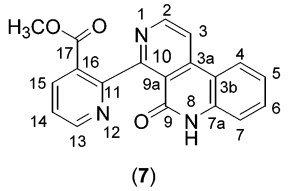
Two new pyridoacridine alkaloids, kuanoniamines E and F, and a new ring-opened pyridoacridine alkaloid, subarine, were isolated from a Singaporean ascidian. Also isolated were known pyridoacridine alkaloids ascididemin and kuanoniamines A and D. The structures of the alkaloids were determined by spectroscopic methods.

Pyridoacridine alkaloids have been isolated from sponges, ascidians, anemones, and a prosobranch.[1−3] These alkaloid pigments in their free base form are usually yellow to deep red in color but can be vivid blues and purples depending on their pH. There has been considerable interest in the synthesis and biological activity of these compounds, which includes anti-HIV, topoisomerase II and Ca^{2+} release, intercalation of DNA, insecticidal, and antifouling activity.[1−4]

A 1:1 CH_2Cl_2/MeOH extract of the ascidian was partitioned using a modified Kupchan procedure.[5] The CH_2Cl_2 fraction was chromatographed on silica gel and C18 HPLC to afford the known alkaloids ascididemin (**1**)[6] and kuanoniamines A (**2**), C (**3**), and D (**4**),[7] identified by comparison of their spectroscopic data with published data, and three new alkaloids, kuanoniamine E (**5**), kuanoniamine F (**6**), and subarine (**7**).





Examination of the spectroscopic data of kuanoniamines E (**5**) and F (**6**) indicated that these alkaloids were 15-*N*-isobutryamide and 15-*N*-2-methylbutyramide derivatives of the parent alkaloid *N*-deacetylkuanoniamine C (**8**).[7,8]

The molecular formula of kuanoniamine E (**5**), $C_{22}H_{20}N_4OS$, was established by a positive ion HRESIMS on the $[M + H]^+$ mass ion peak at *m*/*z* 389. The ^{1}H NMR spectrum of **5** displayed signals at δ 2.44 (1H, qq, J = 6.9, 6.9 Hz, H-17) and 1.17 (6H, d, J = 6.9 Hz, H-18 and H-19) associated with an isopropyl group. Positioning of the isopropyl group was confirmed by HMBC correlations from NH-15 and H-17 to C-16. The stereochemistry at C-17 remained unassigned.

The ^{1}H NMR spectrum of kuanoniamine F (**6**), molecular formula of $C_{23}H_{22}N_4OS$, showed the presence of one methine (δ 2.26, 1H, dd, J = 6.9, 13.8 Hz, H-17), one methylene (δ 1.56, 1H, dd, J = 7.4, 13.8 Hz, H-19a and 1.78, 1H, dd, J = 7.4, 13.8 Hz, H-19b), a primary methyl (δ 0.98, 3H, t, J = 7.4 Hz, H-20), and a secondary methyl group (δ 1.24, 3H, d, J = 6.9 Hz, H-18), which formed an isobutyl group. Connection of the isobutyl group to the C-16 amide carbonyl was confirmed by HMBC correlations from NH-15 and H-17 to C-16.

Compound **7** was obtained as a pale yellow gum. A molecular formula of $C_{19}H_{13}O_3N_3$ was established by a high-resolution mass measurement on the $[M]^+$ mass ion in the EI mass spectrum. Compound **7** showed a UV spectrum (λ_{max} 233, 267, and 335 nm) and IR absorptions (ν_{max} 1669 and 1651 cm^{-1}) indicative of an aromatic compound that contained an extended chromophore. Examination of the ^{1}H and ^{13}C NMR spectra recorded in $CDCl_3$ (Table 1) showed the presence of a 2,3-disubstituted pyridine ring [δ 8.83 (1H, dd, J = 4.8, 7.9 Hz, H-13), 8.48 (1H, d, J = 7.9 Hz, H-15), and 7.54 (1H, dd, J = 4.8, 7.9 Hz, H-14)]; δ 152.0 (d, C-13), 138.1 (d, C-15), and 122.3 (d, C-14)] and a methyl ester group [δ 3.62, 3H, s; δ 52.2 (q) and 165.6 (s)]. HMBC correlations from H-15 and 17-OCH_3 to the C-17 methyl ester carbonyl placed the carbomethoxy group at C-16. Therefore, the structural subunit from C-11 to C-17 of **7** was secured with an attachment point at C-11. Examination of the remaining ^{1}H, ^{13}C NMR, HMQC, and COSY NMR data of **7** (Table 1) suggested the presence of α- and β-pyridine protons at H-2 and H-3, respectively [δ 8.92 (1H, d, J = 5.5 Hz, H-2) and 8.15 (1H, d, J = 5.5 Hz, H-3); δ 150.7 (d, C-2) and 115.4 (d, C-3)], four contiguous aromatic protons from H-4 to H-7 [δ 8.25 (1H, d, J = 7.9 Hz, H-4), 7.50 (1H, dd, J = 7.9, 7.9 Hz, H-6), 7.33 (1H, dd, J = 7.9, 7.9 Hz, H-5), 6.91 (1H, d, J = 7.9 Hz, H-7); δ 131.7 (d, C-6), 124.0 (d, C-4), 123.1 (d, C-5), and 116.3 (d, C-7)], and an amide moiety [δ 10.12 (1H, br s, H-8); δ 161.0 (s, C-9)]. The ^{13}C NMR spectrum also contained seven quaternary aromatic carbons, three of which were attached to

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Table 1. NMR Data of Subarine (**7**) in $CDCl_3$

position	^{13}C (δ)	^{1}H (δ, mult., J in Hz)	COSY	HMBC
2	150.7	8.92, d, 5.5	H-3	C-3, C-3a, C-9a, C-10
3	115.4	8.15, d, 5.5	H-2	C-2, C-3b, C-9a
3a	142.4			
3b	116.7			
4	124.0	8.25, d, 7.9	H-5	C-3a, C-6, C-7a
5	123.1	7.33, dd, 7.9, 7.9	H-4, H-6	C-3b, C-6
6	131.7	7.50, dd, 7.9, 7.9	H-4, H-5, H-7	C-4, C-5, C-7a
7	116.3	6.91, d, 7.9	H-5, H-6	C-3b, C-5
7a	137.5			
8-NH		10.12, br s		C-3b, C-9a
9	161.0			
9a	119.0			
10	161.8			
11	161.4			
13	152.0	8.83, dd, 4.8, 7.9	H-14, H-15	C-3, C-11, C-14, C-15
14	122.3	7.54, d, 4.8, 7.9	H-13, H-15	C-13, C-15, C-16
15	138.1	8.48, d, 7.9	H-13, H-14	C-11, C-13, C-17
16	124.5			
17	165.6			
17-OCH_3	52.2	3.62, s		C-17

nitrogen. The connectivity between the amide moiety and pyridine and benzene rings was established by cross-peaks from H-3 to C-3b, from H-4 to C-3a, and from NH-8 amide proton to C-9a and C-3b. Therefore, the structure of the N-1 to C-10 subunit was established and joined to the C-11 to C-17 subunit to give the structure of the new alkaloid **7**, which was assigned a trivial name subarine after the island of collection. An EI mass spectral fragment ion at *m/z* 272, corresponding to loss of the CH_3COO- group from the molecular ion, and the fragment ion at *m/z* 136, corresponding to $C_7H_6O_2N$, supported this assignment. The structure of subarine (**7**) is interesting, as it could be a significant biosynthetic link in the biosynthesis of ascididemin (**1**) and other related pentacyclic pyridoacridines.

Experimental Section

General Experimental Procedures. General experimental procedures have been reported elsewhere.[9]

Animal Material. The ascidian sample was collected in November 1997 from a reef slope at 10 m off Pulau Subar Laut, Singapore, and kept frozen until extracted. The colony had an encrusting growth form and was dark brown/black underwater. The animal may belong to one of three families (Pseudodistomidae, Polyclinidae, or Polycitoridae), but the zooids were almost unrecognizable and confident identification could not be made. An underwater photograph of the ascidian is shown in Figure 1.

Extraction and Isolation. Freeze-dried animal (600 g) was homogenized in a blender and extracted with 1:1 CH_2Cl_2/MeOH (6 × 500 mL) to give 2.9 g of crude extract after evaporation of the solvent. The crude extract was partitioned according to a modified Kupchan scheme.[5] The CH_2Cl_2-soluble partition fraction (304 mg) was chromatographed on a silica gel column ($CHCl_3$/MeOH gradient) to afford nine fractions. Fraction 4 (4.6 mg) was purified using C18 HPLC (linear gradient from 100% H_2O to 100% CH_3CN in 30 min) to give ascididemin (**1**) (2.2 mg). Fraction 5 (30.5 mg) was chromatographed on a silica gel VLC (CH_2Cl_2/2-propanol gradient) and then C18 HPLC (linear gradient from 100% H_2O to 100% CH_3CN in 30 min) to afford kuanoniamine A (**2**) (1.4 mg), kuanoniamine C (**3**) (4.2 mg), kuanoniamine D (**4**) (10.5 mg), kuanoniamine E (**5**) (3.3 mg), kuanoniamine F (**6**) (2.3 mg), and subarine (**7**) (1.2 mg).

Figure 1. Underwater photograph of the ascidian.

Kuanoniamine E (5): yellow gum; UV (CH_3OH) λ_{max} nm (log ϵ) 204 (4.27), 241 (4.52), 265 (4.30), 296 (sh, 3.97), 346 (4.07), 362 (4.08), 452 (3.67); IR (KBr) ν_{max} cm^{-1} 3271, 2969, 1649, 1558, 1469, 1414, 1256, 758; ^1H NMR (500 MHz, $CDCl_3$) δ 8.72 (1H, s, H-11), 8.63 (1H, d, J = 5.0 Hz, H-2), 7.84 (1H, d, J = 8.0 Hz, H-4), 7.42 (1H, d, J = 5.0 Hz, H-3), 7.41 (1H, d, J = 8.0 Hz, H-7), 7.38 (1H, t, J = 8.0 Hz, H-6), 6.99 (1H, t, J = 8.0 Hz, H-5), 3.32 (2H, br t, J = 8.0 Hz, H_2-14), 3.02 (2H, br t, J = 8.0 Hz, H_2-13), 2.44 (1H, qq, J = 6.9, 6.9 Hz, H-17), 1.17 (6H, d, J = 6.9 Hz, H_3-18 and H_3-19); ^1H NMR (500 MHz, DMSO-d_6) δ 10.25 (1H, br s, H-8), 9.10 (1H, s, H-11), 8.69 (1H, d, J = 4.8 Hz, H-2), 8.42 (1H, br s, H-15), 8.08 (1H, d, J = 7.9 Hz, H-4), 7.70 (1H, d, J = 4.8 Hz, H-3), 7.51 (1H, br t, J = 5.2 Hz, H-7), 7.46 (1H, br t, J = 5.2 Hz, H-6), 7.05 (1H, br t, J = 5.2 Hz, H-5), 3.35 (2H, br t, J = 7.2 Hz, H_2-14), 3.09 (2H, br t, J = 7.2 Hz, H_2-13), 2.36 (1H, qq, J = 6.9, 6.9 Hz, H-17), 1.00 (6H, d, J = 6.9 Hz, H_3-18 and H_3-19); ^{13}C NMR (125 MHz, DMSO-d_6) δ 176.9 (s, C-16), 150.4 (d, C-2), 148.4 (d, C-11), 143.1 (s, C-12b), 140.1 (s, C-12a), 139.5 (s, C-7a), 139.1 (s, C-9a), 139.0 (s, C-3a), 131.3 (d, C-6), 123.4 (d, C-4), 120.4 (d, C-5), 117.4 (s, C-12c), 115.8 (d, C-7), 115.5 (s, C-3b), 107.9 (d, C-3), 104.3 (s, C-9), 36.0 (t, C-14), 33.6 (d, C-17), 30.6 (t, C-13), 19.1 (q, C-18), 19.1 (q, C-19); (+)-ESI-MS *m/z* 389 $[M + H]^+$, 387 $[M - H]^+$; (+)-HRESIMS *m/z* 389.1444 (calcd for $C_{22}H_{21}N_4OS$, 389.1436).

Kuanoniamine F (6): yellow gum; $[\alpha]_D$ +105° (*c* 0.18, CH_3OH); UV (CH_3OH) λ_{max} nm (log ϵ) 206 (4.26), 242 (4.49), 262 (4.29), 296 (sh, 3.98), 346 (4.04), 363 (4.05), 455 (3.38); IR (KBr) ν_{max} cm^{-1} 3271, 2966, 1647, 1573, 1469, 1413, 1260, 831, 758; ^1H NMR (500 MHz, $CDCl_3$) δ 10.20 (1H, br s, H-8), 8.81 (1H, d, J = 4.7 Hz, H-2), 8.77 (1H, s, H-11), 7.93 (1H, d, J = 8.0 Hz, H-4), 7.50 (1H, d, J = 4.7 Hz, H-3), 7.50 (1H, br d, J = 6.6 Hz, H-7), 7.42 (1H, t, J = 7.5 Hz, H-6), 7.05 (1H, t, J = 7.5 Hz, H-5), 6.22 (1H, br s, H-15), 3.42 (2H, dd, J = 5.2 and 6.8 Hz, H_2-14), 3.13 (2H, dd, J = 5.2, 6.8 Hz, H_2-13), 2.26 (1H, dd, J = 6.9, 13.8 Hz, H-17), 1.78 (1H, dd, J = 7.4, 13.8 Hz, H-19), 1.56 (1H, dd, J = 7.4, 13.8 Hz, H-19), 1.24 (3H, d, J = 6.9 Hz, H_3-18), 0.98 (3H, t, J = 7.4 Hz, H_3-20); ^1H NMR (500 MHz, DMSO-d_6) δ 10.24 (1H, br s, H-8), 9.11 (1H, s, H-11), 8.70 (1H, d, J = 4.8 Hz, H-2), 8.38 (1H, br s, H-15), 8.10 (1H, d, J = 7.9 Hz, H-4), 7.72 (1H, d, J = 4.8 Hz, H-3), 7.49 (1H, br t, J = 5.2 Hz, H-7), 7.46 (1H, br t, J = 5.2 Hz, H-6), 7.06 (1H, br t, J = 5.2 Hz, H-5), 3.36 (2H, t, J = 7.2 Hz, H_2-14), 3.09 (2H, br t, J = 7.2 Hz, H_2-13), 2.16 (1H, ddd, J = 6.9, 13.8 Hz, H-17), 1.52 (1H, dd, J = 6.9 and 7.4 Hz, H-19), 1.31 (1H, dd, J = 6.9, 7.4 Hz, H-19), 0.99 (3H, d, J = 6.9 Hz, H_3-18), 0.76 (3H, t, J = 7.4 Hz, H_3-20); ^{13}C NMR (125 MHz, DMSO-d_6) δ 176.8 (s, C-16), 150.6 (d, C-2), 148.6 (d, C-11), 143.4 (s, C-12b), 140.5 (s, C-12a), 139.7 (s, C-7a), 139.3 (s, C-9a), 139.3 (s, C-3a), 133.3 (s, C-8a), 131.5 (d, C-6), 123.7 (d, C-4), 120.7 (d, C-5), 117.6 (s, C-12c), 116.0 (d, C-7), 115.7 (s, C-3b), 108.2 (d, C-3), 104.5 (s, C-9),

41.1 (q, C-17), 36.2 (t, C-14), 30.9 (t, C-13), 26.5 (t, C-19), 17.1 (q, C-18), 11.5 (q, C-20); (+)-ESIMS *m/z* 403 $[M + H]^+$, 401 $[M - H]^+$; (+)-HRESIMS *m/z* 403.1598 (calcd for $C_{23}H_{23}N_4OS$, 403.1593).

Subarine (7): pale yellow gum; UV (CH_3OH) λ_{max} nm (log ϵ) 233 (4.45), 267 (4.14), 335 (3.72); IR (KBr) ν_{max} cm^{-1} 1669, 1651, 1290, 1152, 1026, 999, 825, 765; ^1H (300 MHz) and ^{13}C NMR (75 MHz) data, see Table 1; (+)-ESIMS *m/z* 332 $[M + H]^+$, 330 $[M - H]^+$; EIMS *m/z* 331 ($[M]^+$, 7%), 316 (3), 272 (100), 245 (32), 216 (15), 190 (11), 136 (32), 43 (28). HREIMS *m/z* 331.0932 (calcd for $C_{19}H_{13}O_3N_3$, 331.0957).

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References and Notes

(1) Molinski, T. F. *Chem. Rev.* **1993**, *93*, 1825−1838.
(2) Ding, Q.; Chichak, K.; Lown, J. W. *Curr. Opin. Chem.* **1999**, *6*, 1−27.
(3) Bowden, B. F. In *Studies in Natural Products Chemistry* (Bioactive Natural Products (Part D); Atta-Ur-Rahman, Ed.; Elsevier: New York, 2000; Vol. 23, Chapter 6, pp 233−283.
(4) Echavarren, A. M. *Adv. Nitrogen Heterocycl.* **1996**, *2*, 211−250.
(5) (a) Pettit, G. R.; Kamano, Y.; Aoyagi, R.; Herald, C. L.; Doubek, D. L.; Schmidt, J. M.; Rudloe, J. J. *Tetrahedron* **1985**, *41*, 985−994. (b) Kupchan, S. M.; Britton, R. W.; Ziegler, M. F.; Siegel, C. W. *J. Org. Chem.* **1973**, 38, 178−179.
(6) Kobayashi, J.; Cheng, J. F.; Nakamura, H.; Ohizumi, Y.; Hirata, Y.; Sasaki, T.; Ohta, T.; Nozoe, S. *Tetrahedron Lett.* **1988**, *29*, 1177−1180.
(7) Carroll, A. R.; Scheuer, P. J. *J. Org. Chem.* **1990**, 55, 4426−4431.
(8) Eder, C.; Schupp, P.; Proksch, P.; Wray, V.; Steube, K.; Müller, C. E.; Frobenius, W.; Herderich, M.; van Soest, R. W. M. *J. Nat. Prod.* **1998**, *61*, 301−305.
(9) Guo B. Y.; Dai J. R.; Ng S. B.; Huang Y. C.; Leong C. Y.; Ong W. C.; Carté B. K. *J. Nat. Prod.* **2000**, *63*, 602−604.

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