(dd, 1 H, J = 4.0, 16.8 Hz), 4.58 (1 H, m), 4.71 (1 H, d, J = 4.7 Hz), 5.99 (1 H, dd, J = 3.5, 3.8 Hz), 7.60 (1 H, bd, J = 3.8 Hz), 7.71, 8.04 (AA'BB', 4 H, J = 8.4 Hz), 8.39 (bs, 1 H); ^{13}C NMR (100.6 MHz, acetone-d_6) δ 192.6, 169.9, 165.7, 135.1, 132.6, 132.4, 130.3, 128.5, 121.5, 72.4, 67.8, 42.9, 24.3; CD (CH_3OH) $\Delta\epsilon$(315 nm) = -0.97, $\Delta\epsilon$(274 nm) = +1.46, $\Delta\epsilon$(240 nm) = -1.70; low-resolution mass spectrum (EI) 202.0, 200.0, 185.0, 183.0 (100), 167.0, 157.0, 155.0, 125.0, 43.0.

p-Bromobenzoate of 9 (11). To a cold (0 °C) stirred solution of **10** (28.0 mg, 0.167 mmol) in CH_2Cl_2 (5.0 mL) were added triethylamine (18.9 mg, 0.20 mmol), p-bromobenzoyl chloride (43.98 mg, 0.20 mmol) in THF (1.0 mL), and DMAP (1.0 mg). The reaction solution was allowed to warm to room temperature. After 30 h, the reaction mixture was cooled and quenched by adding crushed ice. The organic layer was separated, and the remaining aqueous solution was extracted with CH_2Cl_2 (3 × 2.0 mL). The combined organic solution was washed with H_2O (3 × 1.0 mL) and dried over Na_2SO_4. The dried extract was concentrated in vacuo to afford 64.0 mg of a solid, which was further purified on a silica gel 60 column (10 × 1.5 cm), eluting with 50% hexane/EtOAc to give 24.0 mg of partially pure (**11**). This material was chromatographed again with 25% hexane/EtOAc as eluting solvent to afford 12.0 mg (20% yield) of a colorless solid: mp 96–99 °C; IR (KBr) 3349, 1718, 1684, 1667, 1591, 1508, 1323, 1282, 1289, 1245, 1118, 1105, 897, 847, 757 cm^{-1}; UV max (ϵ) 240 (30600), 246.8 nm (24800); ^{1}H NMR (400 MHz, acetone-d_6) δ 2.11 (s, 3 H), 2.2 (m, 1 H), 2.5 (m, 1 H), 2.64 (ddd, J = 4.7, 9.9, 17.3 Hz), 2.80 (ddd, 1 H, J = 4.8, 7.0, 17.2 Hz), 5.92 (ddd, J = 3.8, 4.2, 7.6 Hz), 7.78 (d, 1 H, J = 3.7 Hz), 7.72, 7.98 (AA'BB', 4 H, J = 8.3 Hz), 8.39 (bs, 1 H); ^{13}C NMR (100.6 MHz, acetone-d_6) δ 193.4, 169.9, 165.5, 134.8, 132.7, 132.2, 130.3, 128.5, 124.9, 69.7, 34.3, 34.0, 28.7; CD (CH_3OH) $\Delta\epsilon$(280 nm) = +0.55, $\Delta\epsilon$(252 nm) = -3.79, $\Delta\epsilon$(220 nm) = +2.01; high-resolution mass spectrum (EI) calcd 351.01062, 353.00862, found 351.01040, 353.00860; low-resolution mass spectrum (EI) 353.0, 351.0, 311.0, 309.0, 202.0, 200.0, 185.0 (100), 183.0, 167.8, 157.0, 155.0, 126.0, 110.0, 109.0, 43.0.

(S)-(-)-α-Methylbenzylurethane of 2 (7). To a dry 10.0-mL two-necked round-bottomed flask, equipped with a condenser and a septum cap, a solution of **2** (44 mg, 0.24 mmol) in THF (4.0 mL) and (S)-(-)-α-methylbenzyl isocyanate (35.4 mg, 0.24 mmol) were introduced via the septum cap. The resulting solution was heated at reflux under a nitrogen atmosphere for 24 h. The wine-colored reaction mixture was diluted with CH_2Cl_2 (20.0 mL) and decolorized with active charcoal. Filtration and concentration in vacuo yielded a residue that was recrystallized from CH_2Cl_2 to give white fine needles of **7** (76 mg, 96%): mp 173–175 °C; IR (KBr) 3339, 3320, 1692, 1534, 1373, 1243, 1063, 875, 702 cm^{-1}; UV max (ϵ) 274 (43000), 218 nm (72000); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.9 (1 H, s, exch), 7.2–7.4 (6 H, m), 6.0 (1 H, d, J = 7.4 Hz, exch), 5.8 (1 H, m), 4.9 (1 H, d, J = 7.2 Hz), 4.0 (1 H, m), 3.6 (1 H, d, J = 3.8 Hz), 2.1 (3 H, s), 1.5 (3 H, d, J = 6.8 Hz); ^{13}C NMR δ (100.6 MHz, $CDCl_3$) 188.3, 168.0, 154.3, 142.9, 129.2, 128.8, 127.6, 125.9, 121.3, 66.5, 51.6, 51.4, 51.1, 24.6, 22.4; $[\alpha]^{20}_D$ = -125.6° (c 0.05, in MeOH). Anal. Calcd: C, 61.81; H, 5.49; N, 8.48. Found: C, 61.56; H, 5.44; N, 8.18.

X-ray Work on $C_{17}NO_5H_{17}$ (7). A crystal of dimensions 0.20 × 0.10 × 0.05 mm was used for collection of data. Unit cell parameters were refined from a least-squares analysis of the angle settings of 13 reflections in the range 22° < 27° < 35°. Intensity data were collected with the ω-2θ scan technique and a scan speed of 32° min^{-1} in ω. The intensities of three standard reflections monitored throughout the data collection exhibited an average fluctuation of 2.1%. From 1802 reflections measured to (sin $\theta/\lambda)_{max}$ = 0.5947 Å^{-1} with the range of indices $0 \le h \le 8$, $0 \le k \le 43$, and $0 \le l \le 5$, 1235 unique data having $F_o^2 \ge 3\sigma(F_o^2)$ were obtained.

All calculations were performed on a μVAX II computer with programs from the TEXRAY crystallographic software package.[18] Atomic positions for all non-hydrogen atoms were derived from the direct methods program MITHRIL.[19] Hydrogen atoms attached to C atoms were placed in calculated positions (C-H = 0.95 Å) and of the attached C atom. Following two cycles of least-squares refinement, the remaining hydrogen atoms, H(8) and H(11), were located from a difference electron density map; the positional parameters and an isotropic thermal parameter were subsequently refined for each of these atoms. Final refinement of F_o with 224 variable, 1235 observations, and $F_o^2 > 3\sigma(F_o^2)$ affords the residuals R = 0.038 and R_w = 0.049, where the weights are derived from counting statistics and a value of p = 0.05. In the final cycle Δ/σ - 0.01 and the maximum excursion in the final difference electron density map = 0.32 e Å^{-3}. The data were not corrected for absorption.

Acknowledgment. This work was supported by U.S. Public Health Service Grant GM 31715 and NSF Grant CHE-8711102 to S.J.G. Dr. Arnold Brossi (NIH) is thanked for suggesting the chiral methane derivatization. Professor W. C. Johnson (OSU) is thanked for use of the CD spectrometer, and J. Riazance is thanked for assistance in its operation. Professor Koji Nakanishi is thanked for helpful discussions. Dr. Donald Borders (Lederle Laboratories, American Cyanamid Co.) is thanked for a strain of *Streptomyces* LL-C10037 and a sample of LL-C10037α. The multinuclear Bruker AM 400 NMR spectrometer was purchased in part through grants from the National Science Foundation (CHE-8216190) and from the M. J. Murdock Charitable Trust to Oregon State University.

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Kuanoniamines A, B, C, and D: Pentacyclic Alkaloids from a Tunicate and Its Prosobranch Mollusk Predator *Chelynotus semperi*

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From a Micronesian tunicate and its predator, a prosobranch mollusk *Chelynotus semperi*, we have isolated five alkaloids, the known shermilamine B (**1**) and four new pentacyclic compounds, kuanoniamines A–D (**2–5**). The structures were established by extensive NMR analysis and correlations. Cytotoxicity (IC_{50}) against KB cells ranged from >10 μg/mL for **3** to 5 μg/mL for **1** and **5** to 1 μg/mL for **2**.

The sequestering of selective metabolites by opisthobranch mollusks from dietary sources has been an extensively studied area of chemical marine ecology.[1] Similar studies of mollusks from other gastropod subclasses are,

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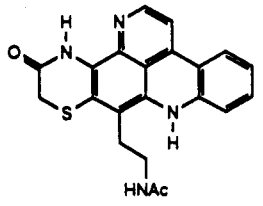
however, rare. Prosobranch mollusks of the family Lamellariidae have been described as specific predators of colonial ascidians.[2] These mollusks resemble opisthobranchs since they possess reduced shells covered completely or in part by a fleshy mantle. It has been suggested that this trend toward a reduced shell may be correlated with the presence of bioactive secondary metabolites.[3] The isolation of the lamellarin alkaloids from both a colonial tunicate, *Didemnum chartacium*,[3] and a Lamellariidae mollusk, *Lamellaria*, sp.,[4] has lent support to this idea. We report here another example of the chemical relationship between Lamellariidae mollusks and their ascidian prey.

Five pentacyclic polyaromatic alkaloids, shermilamine B (1)[5] and kuanoniamine[6] A (2), B (3), C (4), and D (5), were isolated from both the lamellariid mollusk *Chelynotus semperi*[7] and an unidentified purple colonial tunicate,[8] on which it was found grazing. Both animals were collected by SCUBA (-25 m) from Mante Channel, Pohnpei.



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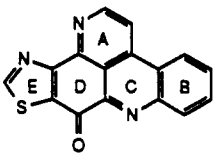

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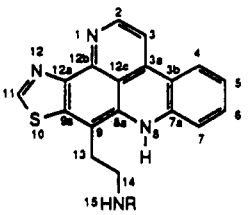
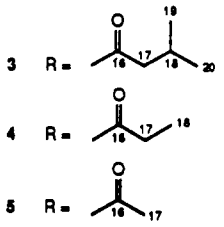




The frozen tunicates were extracted exhaustively with methanol and then with chloroform/methanol/ammonium hydroxide. The combined extracts were initially separated into basic and nonbasic fractions by partitioning between chloroform and hydrochloric acid. Chromatography of the basic extract on silica Bond Elut (chloroform/methanol, 80:20) and then HPLC on an amino bonded phase (chloroform/methanol, 98:2) yielded kuanoniamine A (2, 0.09%), shermilamine B (1, 0.10%), kuanoniamine B (3, 0.13%), C (4, 0.02%), and D (5, 0.07%), respectively. Kuanoniamine A was further purified by HPLC on silica (dichloromethane/ethyl acetate, 60:40). Extraction of a single specimen of *Chelynotus semperi* using the same protocol yielded kuanoniamine A (2, 0.22%), shermilamine B (1, 0.23%), kuanoniamine B (3, 0.22%), C (4, 0.09%), and D (5, 0.19%).

Table I. ^{13}C NMR Data for Kuanoniamine A, B, and D[a] (DMSO-d_6)

carbon no.	kuanoniamine A (2)	kuanoniamine B (3)	kuanoniamine D (5)
2	149.04	150.70	151.01
3	117.28	108.26	108.57
3a	137.21	139.13	139.33
3b	123.07	115.61	115.78
4	124.08	123.66	124.01
5	131.02	120.80	121.07
6	132.02	131.57	131.92
7	132.02	116.14	116.34
7a	144.87	139.17	139.33
8a	147.27	133.39	133.49
9	176.17	104.37	104.56
9a	135.21	139.70	139.59
11	162.72	148.50	149.01
12a	157.84	140.59	139.86
12b	147.12	143.42	143.56
12c	116.45	117.58	117.74
13		30.94	31.08
14		36.39	36.74
16		172.91	170.96
17		44.44	22.52
18		25.17	
19, 20		22.01	

[a] ^{13}C NMR spectra were recorded at 75 MHz. Assignments for kuanoniamine A and B were aided by DEPT sequence experiments, and $^1J_{C-H}$ and $^{2-3}J_{C-H}$ correlation experiments. Assignment for kuanoniamine D were based on a comparison of ^{13}C NMR assignments for kuanoniamine B. Chemical shifts are given in δ units (downfield of TMS).

Kuanoniamine A (2) was obtained as yellow needles, mp 255–258 °C, from chloroform. The molecular formula $C_{16}H_7N_3OS$, implying 15 elements of unsaturation, was determined by mass spectral data. A very stable structure was indicated from the EIMS since only a few fragment ions were observed, corresponding to successive losses of CO, HCN, and CS from the molecular ion. Extended conjugation was apparent from the UV spectrum of 2 [(MeOH): λ_{max} 214 (log ϵ 4.26), 224 (4.28), 250 (4.20), 258 (4.19), 295 (3.8), 354 (3.76), 394 nm (3.61)], and a bathochromic shift was observed upon addiition of acid. An intense absorption band at 1680 cm^{-1} in the IR spectrum in conjunction with a ^{13}C NMR signal at δ 176.17 indicated the presence of a conjugated ketone carbonyl group in 2. Lack of absorption in the region above 3100 cm^{-1} indicated that all three nitrogen atoms were tertiary and that the sulfur atom was disubstituted. The ^{13}C NMR spectrum of 2 (Table I) confirmed the presence of 16 carbons and indicated a high degree of unsaturation since all carbon resonances were downfield of 100 ppm. The nature of the carbons was revealed by a DEPT[9] experiment, which contained seven methine carbons. The ^1H NMR spectrum of 2 (Table II) contained seven aromatic protons. Proton homonuclear decoupling confirmed that protons on carbons 4-7 were on contiguous atoms of an aromatic ring, and that protons at C2 and C3 constituted an isolated vicinal pair which could be assigned to α- and β-pyridine protons from their homonuclear coupling constant (J = 6 Hz), proton chemical shifts, H2 (δ 8.69), H3 (δ 8.45), the chemical shifts of the attached carbons,[10] C2 (δ 149.04), C3 (δ 117.28) and their heteronuclear $^1J_{C-H}$ coupling constants (C2, $^1J_{C-H}$ = 182 Hz) and (C3, $^1J_{C-H}$ = 165 Hz). The remaining proton H11, a singlet at δ 9.28, appeared to be on a carbon situated between two heteroatoms from its ^{13}C

(1) Karuso, P. In *Bioorganic Marine Chemistry*; Scheuer, P. J., Ed.; Springer-Verlag; New York 1987; Vol. 1, pp 31–60.
(2) (a) Behrens, D. W. *Veliger* **1980**, *22*, 323. (b) Lambert, G. *Veliger* **1980**, *22*, 340.
(3) Lindquist, N.; Fenical, W.; Van Duyne, G.; Clardy, J. *J. Org. Chem.* **1988**, *53*, 4570–4574.
(4) Andersen, R. J.; Faulkner, D. J.; Cun-heng, H.; Van Duyne, G.; Clardy, J. *J. Am. Chem. Soc.* **1985**, *107*, 5492–5495.
(5) Carroll, A. R.; Cooray, N. M.; Poiner, A.; Scheuer, P. J. *J. Org. Chem.* **1989**, *54*, 4231–4232.
(6) The name kuanoniamine is derived from the Hawaiian word *kuanoni* meaning change when pertaining to color. This name seems appropriate since the colors of these compounds are extremely sensitive to changes in pH; in neutral and basic solution they are yellow, while in acidic solution they change to brilliant purple.
(7) The mollusk was identified as *Chelynotus semperi* (Bergh) by Dr. Anthony Poiner.
(8) The tunicate remains unidentified, but we are endeavoring to obtain a specimen of suitable quality to make identification feasible.
(9) Doddrell, D. M.; Pegg, O. T.; Bendall, M. R. *J. Magn. Reson.* **1982**, *48*, 323–327.
(10) Assignments for all protonated carbons were provided by a ^1H-detected heteronuclear one bond ^1H–^{13}H correlation experiment (HMQC).[19]

Table II. ^{1}H NMR Data for Kuanoniamines A–D[a] (DMSO-d_6)

atom	kuanoniamine A (2)	kuanoniamine B (3)	kuanoniamine C (4)	kuanoniamine D (5)
2	8.69 (d, 6.0, 1 H)	8.69 (d, 5.0, 1 H)	8.68 (d, 5.0, 1 H)	8.70 (d, 5.1, 1 H)
3	8.45 (d, 6.0, 1 H)	7.72 (d, 5.0, 1 H)	7.71 (d, 5.0, 1 H)	7.73 (d, 5.1, 1 H)
4	8.58 (d, 8.1, 1 H)	8.09 (d, 7.8, 1 H)	8.08 (d, 7.7, 1 H)	8.11 (d, 8.1, 1 H)
5	7.60 (dd, 8.1, 7.6, 1 H)	7.05 (ddd, 7.8, 7.2, 0.6, 1 H)	7.05 (dt, 7.7, 4.0, 1 H)	7.06 (dt, 8.1, 3.9, 1 H)
6	7.66 (dd, 7.8, 7.6, 1 H)	7.51 (dd, 8.1, 7.2, 1 H)	7.43 (d, 4.0, 1 H)	7.45 (d, 3.9, 1 H)
7	8.01 (d, 7.8, 1 H)	7.45 (dd, 8.1, 0.6, 1 H)	7.43 (d, 4.0, 1 H)	7.45 (d, 3.9, 1 H)
8		10.25 (s, 1 H)	10.10 (s, 1 H)	10.15 (s, 1 H)
11	9.28 (s, 1 H)	9.09 (s, 1 H)	9.07 (s, 1 H)	9.09 (s, 1 H)
13		3.10 (t, 7.2, 2 H)	3.08 (t, 7.2, 2 H)	3.07 (t, 7.2, 2 H)
14		3.34 (td, 7.2, 5.2, 2 H)	3.30 (td, 7.2, 5.2, 2 H)	3.27 (td, 7.2, 5.2, 2 H)
15		8.40 (t, 5.2, 1 H)	8.27 (t, 5.2, 1 H)	8.39 (t, 5.2, 1 H)
17		2.00 (m, 2 H)	2.06 (q, 7.2, 2 H)	1.87 (s, 3 H)
18		2.00 (m, 1 H)	0.96 (t, 7.2, 3 H)	
19, 20		0.83 (d, 7.0, 6 H)		

[a] ^{1}H NMR spectra were recorded at 300 MHz. Assignments for kuanoniamine A and B were aided by spin decoupling experiments, DEPT sequence experiments, and $^1J_{C-H}$ and $^{2-3}J_{C-H}$ correlation experiments. Assignments for kuanoniamine C and D were based on a comparison of ^{1}H NMR spectral data for kuanoniamine B. J values are reported in hertz, and chemical shifts are given in δ units (downfield of TMS).

Table III. $^{2-4}J_{C-H}$ Correlations from HMBC Experiments for Kuanoniamine A (2) and B (3) (DMSO-d_6)

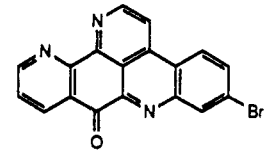
proton no.	long-range correlations to carbon no. kuanoniamine A (2)	kuanoniamine B (3)
H-2	C3, C3a, C12b, C12c	C3, C3a, C12b
H-3	C2, C3b, C12c, C12b, C3a	C2, C3b, C12c
H-4	C3a, C6, C7a, C3b	C3a, C6, C7a
H-5	C3b, C7	C3b, C7
H-6	C4, C7a	C4, C7a
H-7	C3b, C5	C3b, C5
H-8		C3b, C7, C9, C12c
H-11	C9a, C12a, C12b, C9	C9a, C12a
H_2-13		C8a, C98 C9a
H_2-14		C9, C16, C13
H-15		C13, C17
H_2-17		C19, C20
H-18		C16
H_3-19, 20		C17

chemical shift (δ 162.72) and $^1J_{C-H}$ coupling constant ($^1J_{C-H}$ = 217 Hz).

Since more than half of the carbons in **2** were nonprotonated, further information regarding the skeletal framework was sought from multiple bond proton–carbon couplings, which were identified by a ^{1}H-detected heteronuclear multiple bond ^{1}H–^{13}C correlation experiment (HMBC)[11] optimized for observing 5-Hz J_{C-H} coupling (Table III).

The presence of a benzene ring (C3b–C7a) was confirmed by cross peaks of H4 to C6 and C7a, H5 to C3b and C7, H6 to C4 and C7a, and H7 to C5 and C3b. The downfield chemical shift of C7a (δ 144.87) indicated tat it was bonded to one of the three aromatic nitrogens. The presence of a pyridine ring (N1–C3a, C12b–C12c) was deduced by cross peaks of H2 to C12b and C3a and H3 to C12c. Insertion of a nitrogen atom between C2 and C12b was based on observations of the lower field resonances of C2 (δ 149.04) and C12b (δ 147.12). The connectivity of C3a and C3b was confirmed by cross peaks between H3 and C3b and H4 and C3a. A strong NOE enhancement between H3 and H4 also supported their close spatial proximity. Cross peaks to C9a (δ 135.21) and C12a (δ 157.84) were observed only from the proton on the diheterosubstituted aromatic carbon C11, thereby indicating an isolated system in which C9a and C12a were at least four bonds away from any other protons. A thiazole ring was suggested for this isolated system. Two additional low intensity ($^4J_{C-H}$) cross peaks from H11 to the carbonyl carbon C9 (δ 176.17) and C12b (δ 147.12) were also observed. The large difference in chemical shift between C12a and C9a could therefore be rationalized if C12a was β and C9a was α to the conjugated carbonyl C9.

The remaining carbon resonance, an imino group from its chemical shift, δ 147.27, could logically be placed only at C8a since it showed no correlations in the HMBC experiment and was therefore at least four bonds away from any protons. This assignment was supported by comparison of the carbon chemical shifts of C12c (δ 116.45), C8a (δ 147.27), and C7a (δ 144.87) with the corresponding resonances of bromoleptoclinidinone (**6**)[12] (δ 117.9, 146.7, and 146.3) and ascididemin (**7**)[13] (δ 118.24, 145.94, and 145.75). The skeletal framework shown in **8** was therefore suggested.



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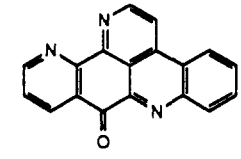

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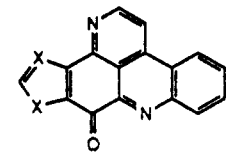

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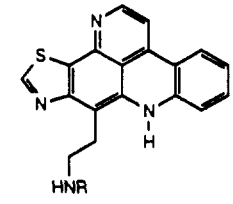

9 R = CH_3
10 R = $COCH_2CH_3$

The question of regiochemistry in the thiazole ring still needed to be addressed. In the structurally similar compounds, dercitamine (**9**) and dercitamide (**10**) isolated from the deep water sponge *Stelletta* sp.[14] the difference in chemical shifts of C9a and C12a was used as a basis for assigning S12 and N10 regiochemistry in the thiazole ring. The large difference in the chemical shift observed between C9a and C12a in kuanoniamine A can be explained solely on the basis of the effect of the attached α,β-unsaturated

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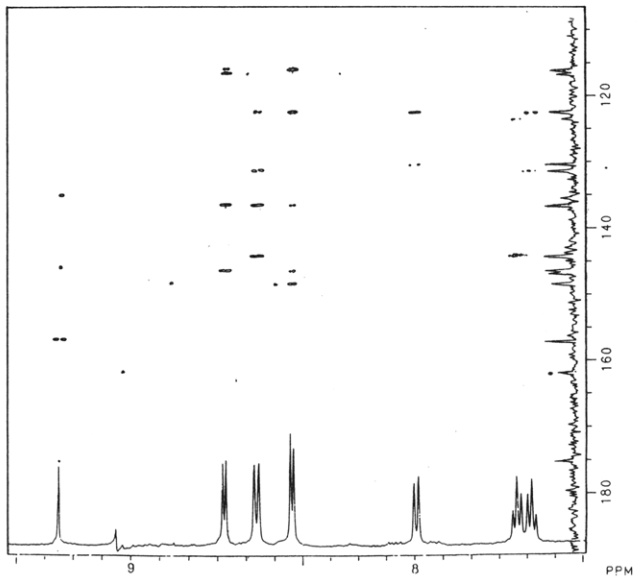


Figure 1. HMBC spectrum of kuanoniamine A (2) in DMSO-d_6.

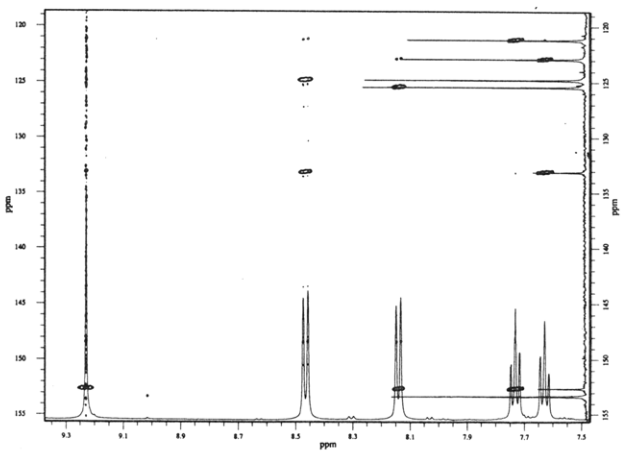


Figure 2. HMBC spectrum of benzothiazole ($CDCl_3$).

carbonyl C9. Fortuitously, the HMBC experiment (Figure 1) provided additional information regarding $^nJ_{C-H}$ couplings. Since the experiment was performed without ^{13}C decoupling, the magnitues of long-range couplings were measurable in the proton dimension. In the case of the two intense cross peaks from H11, one (δ 157.84) was a doublet ($^3J_{C-H}$ = 13.8 Hz), while the other (δ 135.21) was an unresolved singlet (indicating a coupling of <5 Hz). This observation is analogous to that for the model compound benzothiazole. In HMBC experiments performed in this laboratory on benzothiazole in deuteriochloroform the H2–C9 cross peak appears as a doublet in the proton dimension ($^3J_{C-H}$ = 14.0 Hz), while the H2–C8 cross peak is an unresolved singlet (Figure 2). Similarly, Faure et al.[15] measured $^3J_{C-H}$ couplings for a number of thiazole derivatives and found that in all cases the $^3J_{C4-H2}$ coupling (e.g. $^3J_{C4-H2}$ = 15.2 Hz for thiazole and 13.7 Hz for 3-phenylthiazole) is significantly larger than the $^3J_{C5-H2}$ coupling ($^3J_{C5-H2}$ was not resolved in any of the derivatives which they measured). Faure et al.[15] attributed this large difference in coupling between $^3J_{C4-H2}$ and $^3J_{C5-H2}$ in thiazole derivatives to electron delocalization and conjugation across the H2–C2–N3–C4 bonds, which was lacking across the H2–C2–S1–C5 bonds. By analogy, C12a (δ 157.84, $^3J_{C12a-H11}$ = 13.8 Hz) in kuanoniamine A must therefore be attached to nitrogen N12 and C9a (δ 135.21, $^3J_{C9a-H11}$ < 5 Hz) attached to sulfur S10. Kuanoniamine A was concluded to have structure **2**.

Kuanoniamine B (**3**) was obtained as an amorphous yellow powder, mp >300 °C from chloroform. Its molecular formular $C_{23}H_{22}N_4OS$ was established by high-resolution EIMS. An IR band at 1640 cm^{-1} and a ^{13}C NMR signal at δ 172.6 indicated the presence of an amide group in **3**. The UV spectrum exhibited absorptions compatible with a system containing an extended chromophore, and a distinctive color change from yellow in neutral solution to deep purple in acidic media was observed. The ^1H NMR spectrum (Table II) possessed many similarities to that of **2**, including four aromatic protons attached to contiguous carbons, a pair of α- and β-pyridine protons, and a low-field one-proton singlet, which could be attributed to an α-thiazole proton ($^1J_{C-H}$ = 217 Hz). In addition, the ^1H NMR spectrum contained two downfield exchangeable protons, upfield signals attributed to two methylenes, a three-proton multiplet, and two coincident methyl doublets, thereby accounting for all 22 hydrogens.

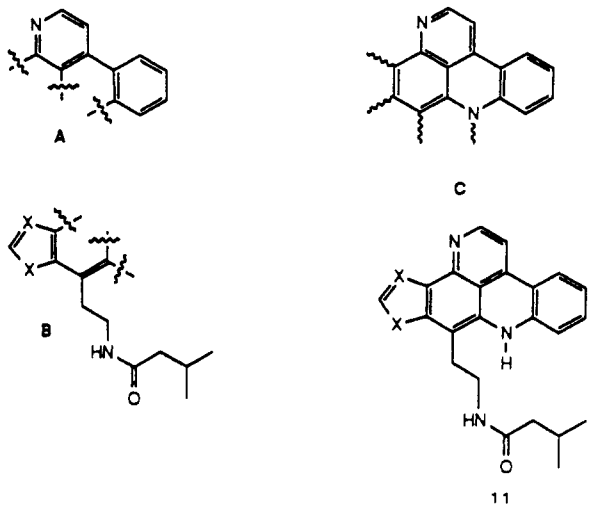
The ^{13}C NMR spectrum of **3** (Table I) contained 22 signals. The upfield region contained one methyl (two equivalent methyls), three methylene, and one methine signal, while the downfield region contained seven methine and 10 quarternary signals,[16] confirming the presence of 23 carbons. Definitive assignments for all protonated carbons was provided by a ^1H-detected heteronuclear one bond ^1H–^{13}C correlation experiment (HMQC)[17] optimized for observing 185-Hz J_{H-H} coupling. In particular, this experiment revealed that the three-proton multiplet at δ 2.1 could be assigned to a methylene C17 (δ 44.44) adjacent to a carbonyl and a methine, C18 α to that methylene. Proton homonuclear decoupling indicated that the methine H18 was vicinal to the two methyl groups. These connectivities constituted an isopentoyl amide unit. An EI mass spectral fragment at m/z 314, corresponding to loss of $C_5H_{12}O$ from the molecular ion, supported this assignment. Proton homonuclear decoupling also indicated that the methylene H_2-14 was vicinal to the methylene H_2-13 and the exchangeable proton H15.

To gain more skeletal information, in particular of the aromatic portion of the molecule, an HMBC experiment was performed (Table III). Delineation of the side chain C13–C20 was established by cross peaks of H15 to C17 and H_2-17 to C16. As was the case with **2**, the three-bond correlations from each of the four contiguous aromatic protons H4, H5, H6, and H7 established a benzene ring in the molecule, while three-bond correlations from the two vicinal pyridine protons H2 and H3 established a pyridine ring. Cross peaks between H3 and C3b and H4 and C3a confirmed the connectivity between C3a and C3b (partial structure A); a strong NOE between H3 and H4 was in agreement with this assignment. The protons of C13 showed two- and three-bond correlations to C8a, C9, and C9a; H11 of the thiazole showed cross peaks to C9a and C12a, thus establishing partial structure B. The farthest downfield exchangeable proton H8 (δ 10.25) showed three-bond correlations to C3b, C7, C9, and C12c, allowing the two partial structures A and B to be combined. An NOE between H8 and H7 and H_2-13 confirmed their close spatial proximity. Assumption of a bond between C12a and C12b established the skeletal framework of kuanoniamine B (**11**). The chemical shift of the two carbons C9a (δ 139.70) and C12a (δ 140.59) gave no clue as to the

(15) Faure, R.; Galy, J.-P.; Elguero, J.; Vincent, E. *Can. J. Chem.* **1978**, *56*, 46–55.

(16) These ^{13}C NMR assignments were based on information provided by broad-band decoupled and DEPT sequence experiments.

(17) Klenar, V. S.; Bax, A. *J. Magn. Reson.* **1987**, *71*, 379–383.



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regiochemistry of the sulfur and nitrogen in the thiazole ring; however, the HMBC experiment again provided useful information regarding the magnitude of $^3J_{C-H}$ couplings in the molecule. The H11, C12a cross peak was split into a doublet ($^3J_{C12a-H11}$ = 13.9 Hz), while the H11, C9a cross peak was an unresolved singlet, thus indicating that C12a was attached to nitrogen N12 and C9a was attached to sulfur S10. Therefore the structure of kuanoniamine B was concluded to be **3**.

Kuanoniamine C (**4**) was obtained as an amorphous yellow powder from chloroform mp >300 °C. It differed from kuanoniamine B (**3**) only in the acyl side chain, as indicated by the close similarities in the downfield region of the ^{1}H NMR spectra. It was clear by interpretation of the upfield region of the ^{1}H NMR spectrum (Table II) that the isopentoyl group was replaced by a propionyl group since a methyl triplet and a methylene quartet had replaced the resonances of the isopentoyl protons. The molecular formula $C_{21}H_{18}N_4OS$ from HREIMS corroborated this assignment for kuanoniamine C and hence structure **4**.

Kuanoniamine D (**5**) was also obtained as an amorphous yellow powder from chloroform mp >300 °C. The molecular formula $C_{20}H_{16}N_4OS$ was derived from HREIMS. The UV spectrum indicated the same chromophore as in kuanoniamine B (**3**). The ^{1}H NMR spectrum (Table II) and the ^{13}C NMR spectrum (Table I) contained characteristic signals for the aromatic portion of the molecule and differed from **3** only in the replacement of the isopentoyl resonances by an acetyl resonance ^{1}H/^{13}C: δ 1.87/22.52). Kuanoniamine D therefore had structure **5**.

Over the past two years a broad range of sponge and ascidian derived polyaromatic alkaloids each possessing the tetracyclic substructure C have appeared in the literature.[5,12-14,18] The presence of these similar polyaromatic skeletons in unrelated animal phyla is remarkable. Similar observations have been made previously,[18b] and a valid explanation would be that these compounds are produced by symbionts. The pentacyclic skeleton of the kuanoniamines although remarkably similar to that of the *Dercitus* sp.[18c] and *Stelleta* sp.[14] alkaloids differs in the regiochemistry of the thiazole E ring. This basic difference suggests a divergent biosynthetic origin for the sponge and ascidian alkaloids. The kuanoniamines and the shermilamines are presumbly derived from a common precursor. Recently, two further examples of S10-substituted polyaromatic ascidian derived alkaloids, the varamines,[18f] and diplamine[18g] have been described. Sequestering of the kuanoniamines from a tunicate prey may be doubly beneficial to the mollusk *Chelynotus semperi*. Firstly, the biological properties of these compounds indicate that they may provide chemical protection for this otherwise vulnerable animal. Secondly, concentration of these pigments for use in camouflage may be an important adaptation since the animal is cryptic when feeding on its host tunicate.

Cytotoxicity (IC_{50}) against KB cells was found to be 5 μg/mL for **1** and **5**, 1 μg/mL for **2**, and >10 μg/mL for **3**.

Experimental Section

General Procedures. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer Model 1420 spectrometer and ultraviolet spectra on a Hewlett-Packard Model 8452A diode array spectrophotometer. Mass spectra were measured on a VG-70SE instrument and NMR spectra on a General Electric QE-300 instrument at 300 MHz (^{1}H) and 75 MHz (^{13}C), respectively, and on a General Electric GN OMEGA 500 instrument for HMQC and HMBC experiments. Solvents were freshly distilled before use.

Isolation. Animals were collected by SCUBA (-25 m) in October 1987 from Mante Channel, Pohnpei, and were frozen until examined. The frozen tunicates (11.21 g dry weight after extraction) were extracted first with methanol, followed by repeated extraction with chloroform/methanol (1:1) containing 1% of a 30% ammonium hydroxide solution. The extracts were combined and concentrated. The aqueous residue was acidified with 1 M hydrochloric acid and partitioned against chloroform. The aqueous layer was basified with 10% ammonium hydroxide and partitioned against chloroform. The basic chloroform extract was concentrated, yielding an orange/yellow solid (0.12 g). This residue was chromatographed on silica gel (Bond Elut, elution with chloroform/methanol, 80:20) and then subjected to HPLC on an amino bonded phase (Brownlee Labs Lichrosorb NH_2, 10 μm) with chloroform/methanol (98:2), resulting in a crude kuanoniamine A fraction and pure shermilamine B (**1**, 11.3 mg, 0.10% dry weight), kuanoniamine B (**3**, 14.5 mg, 0.13% dry weight), kuanoniamine C (**4**, 2.2 mg, 0.02% dry weight), and kuanoniamine D (**5**, 8.6 mg, 0.07% dry weight), respectively. The crude kuanoniamine A fraction was further purified by HPLC on silica (dichloromethane/ethyl acetate, 60:40), yielding pure kuanoniamine A (**2**, 10.1 mg, 0.09% dry weight).

Extraction of a single specimen of the Prosobranch mollusk *Chelynotus semperi* (0.125 g dry weight after extraction) using the same protocol as described above yielded shermilamine B (**1**, 1.03 mg, 0.23% dry weight), kuanoniamine A (**2**, 1.00 mg, 0.22% dry weight), kuanoniamine B (**3**, 1.00 mg, 0.22% dry weight), kuanoniamine C (**4**, 0.41 mg, 0.09% dry weight), and kuanoniamine D (**5**, 0.87 mg, 0.19% dry weight).

Kuanoniamine A (2): yellow needles from chloroform; mp 255-258 °C dec; UV (MeOH) λ_{max} 214 (log ϵ 4.26), 224 (4.28), 250 (4.20), 258 (4.19), 295 (3.80), 354 (3.76), 394 nm (3.61); UV ($MeOH_2^+$) λ_{max} 208 (log ϵ 4.21), 226 (4.25), 244 sh (4.17), 292 (4.17), 382 nm (3.71); IR (solution in chloroform) 3020, 1680, 1590, 1290, 1240, 1220, 1050, 800, 750 cm^{-1}; HREIMS *m/z* 289.0307 ($C_{16}H_7N_3OS$ requires 289.0305), 261.0352 ($C_{15}H_7N_3S$ requires

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(19) The observation of $^4J_{CH}$ correlations, although unusual, is in accordance with the magnitude of $^4J_{CH}$ couplings reported for pyridine systems ($^4J_{C3-H6}$ = -1.7 Hz, $^4J_{C2-H5}$ = -0.9 Hz) and benzene systems ($^4J_{CH}$ = 1.3 Hz)[20] and is indicative of the greater sensitivity of the HMBC experiment over its detected counterpart, the COLOC experiment. Both HMBC experiments were optimized for detecting 5-Hz coupling.

(20) Kalinowski, H. O.; Berger, S.; Braun, S. *^{13}C NMR Spektroskopie*; Thieme: Stuttgart, 1984.

261.0344), 234.0249 ($C_{14}H_6N_2S$ requires 234.0246); EIMS m/z (38), 261 (42), 234 (14), 190 (16), 117 (13), 69 (18), 58 (100).

Kuanoniamine B (3): yellow amorphous powder from chloroform; mp >300 °C; UV (MeOH) λ_{max} 204 (log ϵ 4.24), 240 (4.32), 264 (4.18), 294 sh (3.89), 344 (3.87), 360 (3.87), 450 nm (3.44); UV ($MeOH_2^+$) λ_{max} 204 (log ϵ 4.17), 238 (4.21), 270 (4.08), 306 (4.27), 344 (3.65), 360 (3.73), 530 nm (3.41); IR (solution in chloroform) 3620, 1640, 1450, 1220, 1110, 1040 cm^{-1}; HREIMS m/z 402.1520 ($C_{23}H_{22}N_4OS$ requires 402.1526); EIMS m/z 402 (19), 314 (20), 301 (21), 289 (72), 261 (78), 234 (23), 190 (22).

Kuanoniamine C (4): yellow amorphous powder from chloroform; mp >300 °C; UV (MeOH) λ_{max} 206 (log ϵ 4.24), 240 (4.32), 264 (4.20), 294 sh (3.90), 344 (3.88), 359 (3.86), 450 nm (3.30). UV ($MeOH_2^+$) λ_{max} 206 (log ϵ 4.16), 240 (4.22), 270 (4.07), 306 (4.27), 360 (3.75), 526 nm (3.40); IR (solution in chloroform) 3580, 3150, 1645, 1590, 1370, 1310, 1140 cm^{-1}; HREIMS m/z 374.1270 ($C_{21}H_{18}N_4OS$ requires 374.1339), 289.0311 ($C_{16}H_7N_3OS$ requires 289.0313); EIMS m/z 374 (5%), 370 (10), 313 (15), 299 (46), 289 (52), 278 (47), 261 (61), 234 (11), 190 (13), 169 (12), 149 (49), 86 (77), 69 (100).

Kuanoniamine D (5): yellow amorphous powder from chloroform; mp >300 °C; UV (MeOH) λ_{max} 206 (log ϵ 4.24), 240 (4.32), 264 (4.20), 344 (3.88), 358 (3.87), 452 nm (3.38); UV ($MeOH_2^+$) λ_{max} 206 (log ϵ 4.15), 240 (4.22), 270 (4.07), 306 (4.27), 360 (3.75), 526 (3.39); IR (solution in chloroform) 3610, 3000, 2940, 2840, 1640, 1590, 1460, 1240, 1210, 1100, 1060, 1000 cm^{-1}; HREIMS m/z 360.1007 ($C_{20}H_{16}N_4OS$ requires 360.1045), 288.0590 ($C_{17}H_{10}N_3S$ requires 288.0595); EIMS m/z 360 (40), 356 (18), 314 (26), 301 (31), 299 (30), 288 (100).

Benzothiazole (Aldrich) HMBC spectrum (Figure 2) was measured at 500 MHz. Long-range couplings are optimized for J = 10 Hz. Final data set 2K × 1K.

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Isonitriles from the Blue-Green Alga *Scytonema mirabile*

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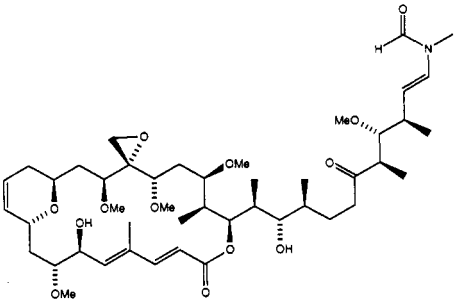
An aerial form of *Scytonema mirabile* (Dillwyn) Bornet, designated strain number BY-8-1, has been found to contain six novel isonitriles, viz. mirabilene isonitriles A-F (**7-12**), which are mildly cytotoxic and antimicrobial. The gross structures of **7-12** were determined by detailed spectral analysis. The relative and absolute stereochemistry of **7-12** were solved by chemical degradation and direct comparison of degradation products with synthetic samples; mirabilene-A isonitrile (**7**), for example, was degraded to methyl (3*R*,5*R*,7*S*,9*S*)-3,5,7,9-tetramethoxy-10-oxoundecanal (**15**) and isopropyl (*S*)-3-trifluoroacetamidobutyrate (**14**), which indicated that the absolute configurations of C-4, C-6, C-8, C-10, and C-16 in **7** were all *S*.

The major cytotoxic, fungicidal agent in the cultured terrestrial blue-green alga *Scytonema mirabile* (isolate BY-8-1) is tolytoxin (**1**).[1] Polymethoxyalkenes **2-3**, which are related to **4-6** from another tolytoxin-producing cyanophyte *Tolypothrix conglutinata* var. *colorata*,[2] are also present in *S. mirabile*.[3] Upon examining the extract of *S. mirabile*, another member of the Scytonemataceae, for other cytotoxins and fungicides, a new group of isonitriles was found, viz. the mirabilene isonitriles **7-12**, which are mildly cytotoxic and antimicrobial and obviously related to compounds **2** and **3**.[4] Hitherto isonitriles (e.g. hapalindoles) had been found only in blue-green algae belonging to the Stigonemataceae.[5,6] In this paper we describe the total structures of the mirabilene isonitriles.



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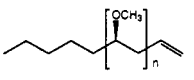

2 n = 5

3 n = 6

4 n = 8

5 n = 9

6 n = 10

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(4) The mirabilene isonitriles are cytotoxic to LoVo (a human colon adenocarcinoma cell line) at 5 μg/mL and KB (a human nasopharyngeal carcinoma cell line) at 1-10 μg/mL (**7** being the most active). None of the compounds, however, exhibit selective cytotoxicity toward murine or human solid tumor cell lines over L1210 leukemia in the Corbett assay [Corbett, T. H.; Polin, L.; Wozniak, A. J.; Bissery, M.; LoRusso, P. M.; Valeriote, F. A.; Baker, L. H. *Proc. Am. Assoc. Cancer Res.* **1988**, *29*, 533] or toward leukemia cell lines over CFU-GM (a normal bone marrow cell line) in the Valeriote assay. All of the mirabilene isonitriles exhibited weak antimicrobial activity (zones of 10-15 mm with 10 μg/disc) against Gram-positive bacteria and filamentous fungi (*Aspergillus oryzae* and *Penicillium notatum*). The details of the bioactivity data will be reported elsewhere.

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