by recrystallization or by column chromatography. The melting points of the products and purifying solvents are presented in Table III.

3-(1-(4-(*N*,*N*-Dimethylamino)phenyl)ethyl)indole (10a): ^{1}H NMR δ 1.65 (d, 3 H, *J* = 7), 2.87 (s, 6 H), 4.28 (q, 1 H, *J* = 7), 6.67 (d, 2 H, *J* = 8.5), 6.89–7.41 (m, 7 H), 7.82 (s, br, 1 H); ^{13}C NMR δ 22.5, 35.8, 40.8, 110.9, 112.9, 119.0, 119.8, 120.9, 121.7, 122.1, 126.9, 128.0, 135.1, 136.6, 148.9. Anal. Calcd for $C_{18}H_{20}N_2$: C, 81.78; H, 7.63; N, 10.60. Found: C, 81.54; H, 7.66; N, 10.75.

4-(Benzyl(indol-3-yl)methyl)-*N*,*N*-dimethylaniline (10b): ^{1}H NMR δ 2.82 (s, 6 H), 3.24 (dd, 1 H, *J* = 8.4, 13.6), 3.46 (dd, 1 H, *J* = 13.6, 6.8), 4.39 (dd, 1 H, *J* = 8.4, 6.8), 6.59 (d, 2 H, *J* = 8.5), 6.86–7.43 (m, 12 H), 7.71 (s, br, 1 H); ^{13}C NMR δ 40.7, 42.6, 43.7, 110.9, 112.7, 119.0, 119.6, 120.2, 121.4, 121.7, 125.6, 126.9, 127.9, 128.6, 129.0, 132.7, 136.4, 141.0, 148.9. Anal. Calcd for $C_{24}H_{24}N_2$: C, 84.67; H, 7.11; N, 8.23. Found: C, 84.65; H, 7.17; N, 8.00.

1-((4-(*N*,*N*-Dimethylamino)phenyl)(indol-3-yl)methyl)-cyclohexan-1-ol (10c): ^{1}H NMR δ 1.20–1.72 (m, 10 H), 2.86 (s, 6 H), 4.21 (s, 1 H), 6.65 (d, 2 H, *J* = 8.5), 7.03–7.43 (m, 7 H), 7.62 (d, 1 H, *J* = 8), 8.09 (s, br, 1 H); ^{13}C NMR δ 22.3, 25.8, 36.7, 37.0, 40.7, 51.3, 73.9, 110.8, 112.5, 116.5, 118.9, 119.1, 121.6, 122.4, 128.4, 129.9, 130.1, 135.4, 149.1. Anal. Calcd for $C_{23}H_{28}N_2O$: C, 79.27; H, 8.10; N, 8.04. Found: C, 79.27; H, 8.07; N, 8.39.

1-(4-Tolyl)-2-(4-(*N*,*N*-dimethylamino)phenyl)-2-(indol-3-yl)ethanol (10d): ^{1}H NMR δ 2.26 (s, 3 H), 2.45 (s, br, 1 H), 2.81 (s, 6 H), 4.49 (d, 1 H, *J* = 8), 5.25 (d, 1 H, *J* = 8), 6.52 (d, 2 H, *J* = 8.8), 6.9–7.3 (m, 10 H), 7.47 (d, 1 H, *J* = 8), 8.10 (s, br, 1 H); ^{13}C NMR δ 21.1, 40.6, 50.7, 77.5, 111.0, 112.5, 115.8, 119.3, 119.5, 122.0, 122.4, 126.7, 127.6, 128.5, 129.2, 130.0, 136.3, 136.5, 139.7, 149.0. Anal. Calcd for $C_{25}H_{26}N_2O$: C, 81.05; H, 7.07; N, 7.56. Found: C, 80.81; H, 7.13; N, 7.43.

1-(Pyrid-4-yl)-2-(4-(*N*,*N*-dimethylamino)phenyl)-2-(indol-3-yl)ethanol (10e): ^{1}H NMR δ 2.72 (s, br, 1 H), 2.86 (s, 6 H), 4.47 (d, 1 H, *J* = 7), 5.28 (d, 1 H, *J* = 7), 6.56 (d, 2 H, *J* = 8.8), 7.01–7.33 (m, 10 H), 7.43 (d, 1 H, *J* = 7.6), 8.28 (s, br, 1 H), 8.40 (d, 2 H, *J* = 6); ^{13}C NMR δ 40.6, 50.5, 76.5, 111.1, 112.6, 114.7, 119.3, 119.7, 121.7, 122.5, 127.4, 128.6, 129.2, 136.3, 149.2, 149.4, 151.7. Anal. Calcd for $C_{23}H_{23}N_3O$: C, 77.28; H, 6.49; N, 11.76. Found: C, 77.04; H, 6.64; N, 11.63.

2-(4-(*N*,*N*-Dimethylamino)phenyl)-2-(indol-3-yl)-1,1-diphenylethanol (10f): ^{1}H NMR δ 2.74 (s, 6 H), 3.03 (s, 1 H), 5.43 (s, 1 H), 6.38 (d, 2 H, *J* = 8.6), 6.77–7.32 (m, 16 H), 7.49 (d, 1 H, H, *J* = 7.4), 7.73 (s, br, 1 H); ^{13}C NMR δ 40.4, 50.6, 81.0, 110.7, 112.1, 115.3, 118.9, 119.0, 121.4, 125.0, 125.7, 125.9, 126.0, 126.3, 127.3, 127.6, 127.7, 130.6, 135.4, 146.5, 147.6, 149.0. Anal. Calcd for $C_{30}H_{28}N_2O$: C, 83.30; H, 6.52; N, 6.48. Found: C, 83.04; H, 6.62; N, 6.29.

1-(4-(*N*,*N*-Dimethylamino)phenyl)-1-(4-(*N*,*N*-diethylamino)phenyl)ethane (12): ^{1}H NMR δ 1.12 (t, 6 H, *J* = 7), 1.55 (d, 3 H, *J* = 7), 2.88 (s, 6 H), 3.29 (q, 4 H, *J* = 7), 3.96 (q, 1 H, *J* = 7), 6.60 (d, 2 H, *J* = 8.8), 6.67 (d, 2 H, *J* = 8.8), 7.05 (d, 2 H, *J* = 8.8), 7.10 (d, 2 H, *J* = 8.8); ^{13}C NMR δ 12.6, 22.3, 40.9, 42.7, 44.3, 111.9, 112.8, 128.1, 128.2, 134.1, 135.7, 146.0, 148.9. Anal. Calcd for $C_{20}H_{28}N_2$: C, 81.03; H, 9.52. Found: C, 81.44; H, 9.74.

2-Phenyl-2-(4-(*N*,*N*-dimethylamino)phenyl)acetophenone (14): ^{1}H NMR δ 2.90 (s, 6 H), 5.93 (s, 1 H), 6.68 (d, 2 H, *J* = 8.8), 7.1–7.5 (m, 10 H), 8.00–8.02 (m, 2 H); ^{13}C NMR δ 40.5, 58.6, 112.8, 126.5, 126.8, 128.48, 128.5, 128.9, 129.1, 129.8, 132.7, 137.1, 140.0, 149.6, 198.7. Anal. Calcd for $C_{22}H_{21}NO$: C, 83.78; H, 6.71; N, 4.44. Found: C, 83.41; H, 6.86; N, 4.35.

2-(4-(*N*,*N*-Dimethylamino)phenyl)-2-methoxy-1,1-diphenylethanol (15): ^{1}H NMR δ 2.86 (s, 6 H), 3.16 (s, 1 H), 3.26 (s, 3 H), 4.95 (s, 1 H), 6.50 (d, 2 H, *J* = 8), 6.86 (d, 2 H, *J* = 8), 7.0–7.4 (m, 8 H), 7.5–7.6 (m, 2 H); ^{13}C NMR δ 40.4, 56.5, 80.7, 86.5, 111.4, 123.6, 126.3, 126.4, 126.7, 127.1, 127.4, 127.8, 129.7, 144.1, 146.2, 149.9. Anal. Calcd for $C_{23}H_{25}NO_2$: C, 79.51; H, 7.25; N, 4.03. Found: C, 79.11; H, 7.36; N, 3.92.

1,1-Diphenyl-2-(indol-3-yl)-2-(4-(*N*,*N*-dimethylamino)-phenyl)ethylene (16): ^{1}H NMR δ 2.81 (s, 6 H), 6.4–7.3 (m, 17 H), 7.6–7.8 (m, 2 H), 8.0 (s, br, 1 H); ^{13}C NMR δ 40.3, 110.7, 111.5, 119.2, 119.6, 121.0, 121.4, 125.5, 126.6, 127.4, 127.5, 127.6, 128.0, 130.8, 131.1, 131.6, 132.0, 134.0, 135.7, 137.6, 144.7, 145.8, 148.8. Anal. Calcd for $C_{30}H_{26}N_2$: C, 86.92; H, 6.32; N, 6.76. Found: C, 86.52; H, 6.32; N, 6.57.

1,1-Dibenzoyl-2-(4-(*N*,*N*-dimethylamino)phenyl)ethane (9): A mixture of 4-(benzotriazol-1-ylmethyl)-*N*,*N*-dimethylaniline (**1b**) (0.63 g, 2.5 mmol), dibenzoylmethane (0.56 g, 2.5 mmol), and anhydrous zinc bromide (0.84 g, 3.75 mmol) in dry toluene was heated under reflux for 27 h, cooled, poured into aqueous NaOH solution (10%, 30 mL), extracted with ether, and dried over $MgSO_4$. The solvent was evaporated to give an oil, which upon flash column chromatography on silica gel using petroleum ether/EtOAc (15:1) as eluate gave the desired product (0.22 g, 25%): ^{1}H NMR δ 2.84 (s, 6 H), 3.37 (d, 2 H, *J* = 6.6), 5.51 (t, 1 H, *J* = 6.6), 6.60 (d, 2 H, *J* = 8.8), 7.11 (d, 2 H, *J* = 8.8), 7.3–7.5 (m, 6 H), 7.89–7.91 (m, 4 H); ^{13}C NMR δ 34.2, 40.6, 59.4, 112.8, 126.8, 128.5, 128.7, 129.5, 133.3, 136.0, 149.3, 195.5.

Aromatic Alkaloids from the Marine Sponge *Chelonaplysilla* sp.

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Four novel alkaloids derived from tryptophan and tyrosine subunits have been isolated from the marine sponge *Chelonaplysilla* sp. collected from a marine lake in Palau. The structures of chelonin A (**3**), chelonin B (**4**), bromochelonin B (**5**), and chelonin C (**6**) were determined by interpretation of spectral data and chemical conversions. Chelonin A (**3**) and C (**6**) are the first natural products incorporating a 2,6-disubstituted morpholine ring. Chelonin A (**3**), chelonin B (**4**), and bromochelonin B (**5**) exhibited antimicrobial activity against *Bacillus subtilis*, while chelonin A (**3**) showed in vivo antiinflammatory activity.

We have previously reported[1] the isolation of several diterpenes from a sponge of the genus *Dendrilla* collected from a marine lake in Palau. This sponge has now been reclassified as a member of the genus *Chelonaplysilla*.[2] The diterpenes isolated from this *Chelonaplysilla* sp. include 1-bromo-8-ketoambliol A acetate (**1**) and related compounds and rearranged spongian diterpenes exemplified by dendrillolide A (**2**). Chemical studies of the same *Chelonaplysilla* species collected in Pohnpei resulted in

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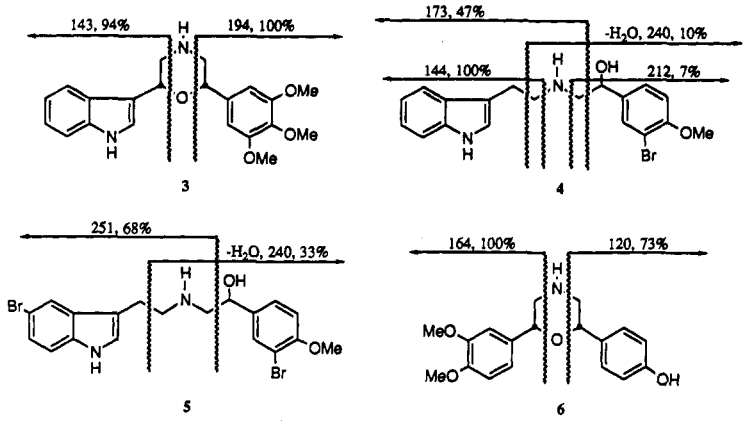
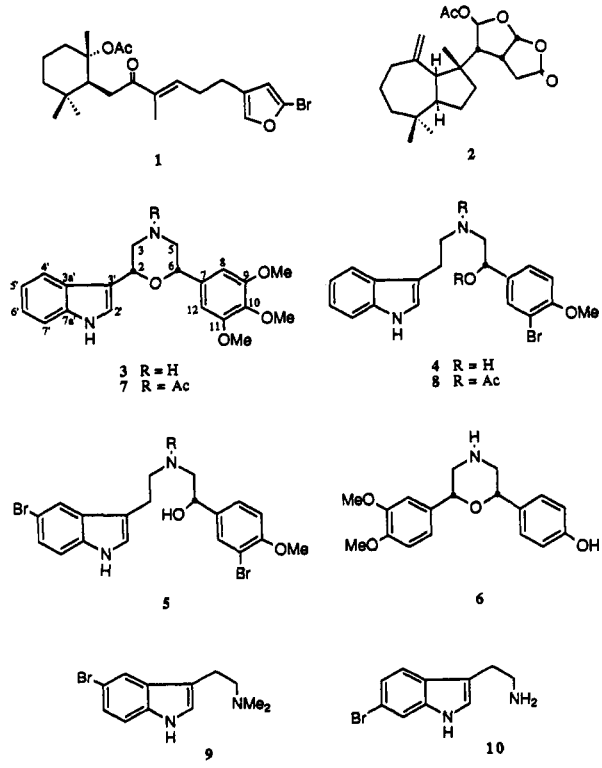
Scheme I. Prominent Fragment Ions in the Electron Impact Mass Spectra (70 eV, m/z, and Percent Base Peak) of Chelonin A (3), Chelonin B (4), Bromochelonin B (5), and Chelonin C (6)



the isolation of many of the same compounds, along with three novel spongian diterpenes.[3]

Chemical investigation of the more polar constituents of the Palauan sample of *Chelonaplysilla* sp. has resulted in the isolation and characterization of four aromatic alkaloids: chelonin A (3), chelonin B (4), bromochelonin B (5), and chelonin C (6). The chelonins appear to be derived biosynthetically from tryptophan and tyrosine subunits. The structures of chelonins A (3) and C (6) are constructed from these subunits in a manner that forms a 2,6-disubstituted morpholine ring, a structural fragment that has not previously been reported from a natural source.



The purple dendritic sponge *Chelonaplysilla* sp. was collected from a marine lake on Kaibaku Island, Iwayama Bay, Palau. The methanol extract of the freeze-dried sponge was triturated with dichloromethane to obtain a yellow oil that exhibited antimicrobial activity against *Bacillus subtilis* and *Staphylococcus aureus*. This triturate was separated on Sephadex LH-20 followed by flash chromatography on silica to yield several fractions that appeared to consist of mixtures of aromatic compounds as judged by ^{1}H NMR spectroscopy. This mixtures were further separated by centrifugal counter current chromatography and reversed-phase HPLC to obtain the novel aromatic alkaloids chelonin A (3, 0.064% dry wt), chelonin B (4, 0.009% dry wt), bromochelonin B (5, 0.006% dry wt), and chelonin C (6, 0.015% dry wt).

Chelonin A (3) was isolated as white crystals, mp 182 °C, which exhibited a molecular ion at m/z = 368.1713 ($C_{21}H_{24}N_2O_4$) in the high-resolution mass spectrum. Prominent fragment ions at m/z = 194.0938 ($C_{11}H_{14}O_3$, 100%) and 143.0726 ($C_{10}H_9N$, 94%) were identified as (trimethoxyphenyl)ethylene and indolylethylene units, respectively (Scheme I). The presence of a 3,4,5-trimethoxyphenyl subunit was indicated by the ^{13}C NMR signals at δ 154.4 (s, 2 C), 138.1 (s), 137.8 (s), 104.4 (d, 2 C), 61.1 (q), and 56.5 (q, 2 C) and the ^{1}H NMR signals at δ 6.74 (s, 2 H), 3.82 (s, 6 H), and 3.73 (s, 3 H). The indole subunit was responsible for the presence of eight additional downfield signals in the ^{13}C NMR spectrum at δ 138.4 (s), 127.3 (s), 123.3 (d), 122.7 (d), 120.5 (d), 120.1 (d), 115.3 (s), and 112.6 (d) and five additional downfield signals in the ^{1}H NMR spectrum at δ 7.77 (dd, 1 H, J = 6.9, 1.0 Hz), 7.35 (dd, 1 H, J = 7.0, 1.2 Hz), 7.25 (s, 1 H), 7.10 (ddd, 1 H, J = 7.0, 6.9, 1.0 Hz), and 7.01 (ddd, 1 H, J = 6.9, 6.9, 1.2 Hz). The remaining resonances in the ^{1}H NMR spectrum composed two ABX spin systems that were both assigned to $-NCH_2CHRO-$ fragments by analysis of ^{1}H–^{1}H NMR decoupling experiments. The molecular formula of chelonin A (3) requires 11 degrees of unsaturation in the molecule, indicating the existence of one nonaromatic ring. These data were consistent with a 2,6-disubstituted morpholine ring possessing 3,4,5-trimethoxyphenyl and indole substituents. Each of the ^{1}H and ^{13}C NMR resonances for chelonin A (3) was assigned by analysis of nuclear Overhauser effect difference spectroscopy (NOEDS) and ^{1}H–^{13}C direct (XHCORR) and long-range (COLOC) correlation experiments (Table I). The cis orientation of the substituents on the morpholine ring was determined by irradiation of the ^{1}H NMR signal at δ 5.00 (br t, 1 H, J = 6.8 Hz, H-2) to obtain an enhancement (16.1%) of the signal at 4.70 (dd, 1 H, J = 10.6, 2.4 Hz, H-6). Acetylation of chelonin A (3) produced an acetamide 7 that exhibited spectral data consistent with the proposed structure for chelonin A (3).

Chelonin B (4) was isolated as a white solid, 260 °C dec, and was determined to have a molecular formula of $C_{19}H_{21}N_2O_2Br$ by high-resolution mass measurement of the fragment ion at m/z = 370.0681 (M – H_2O)$^+$. The ^{1}H NMR, ^{13}C NMR, and mass spectra all indicated the

(3) Bobzin, S. C.; Faulkner, D. J. *J. Nat. Prod.*, in press.

Table I. ^{13}C NMR (50 MHz, Chemical Shift (δ), and Multiplicity) and ^1H NMR (200 MHz, Chemical Shift (δ), Multiplicity, Number of Hydrogens, and Coupling Constants (Hz)) Data for Chelonin A (3) in CD_3OD Solution

carbon no.[a]	^{13}C	^1H
2′	123.3 (d)	7.25 (s, 1 H)
3′	115.3 (s)	
3a′	127.3 (s)	
4′	120.5 (d)	7.77 (dd, 1 H, 6.9, 1.0)
5′	120.1 (d)	7.01 (ddd, 1 H, 6.9, 6.9, 1.2)
6′	122.7 (d)	7.10 (ddd, 1 H, 7.0, 6.9, 1.0)
7′	112.6 (d)	7.35 (dd, 1 H, 7.0, 1.2)
7a′	138.4 (s)	
2	75.1 (d)	5.00 (br t, 1 H, 6.8)
3	51.1 (t)	3.11 (br d, 2 H, 6.8)
5	52.8 (t)	2.74 (dd, 1 H, 12.8, 10.6)
		3.08 (dd, 1 H, 12.8, 2.4)
6	80.0 (d)	4.70 (dd, 1 H, 10.6, 2.4)
7	137.8 (s)	
8	104.4 (d)	6.74 (s, 2 H)
9	154.4 (s)	
10	138.1 (s)	
11	154.4 (s)	
12	104.4 (d)	6.74 (s, 2 H)
9-OMe	56.5 (q)	3.82 (s, 6 H)
10-OMe	61.1 (q)	3.73 (s, 3 H)
11-OMe	56.5 (q)	3.82 (s, 6 H)

[a] Assignments for chelonin A (3) were made by the analysis of ^1H–^{13}C 2D NMR shift correlation experiments. The delays were optimized for one-bond couplings of J = 135 Hz (XHCORR) or for long-range couplings of J = 7 or 10 Hz (COLOC). Carbon multiplicities were determined by the analysis of DEPT experiments.

presence of an indole subunit and a benzene subunit similar to those found in chelonin A (3; Scheme I), although the substitution pattern about the benzene ring in chelonin B (4) was obviously different. ^1H NMR signals at δ 7.48 (d, 1 H, J = 2.0 Hz, H-12), 7.14 (dd, 1 H, J = 8.5, 2.0 Hz, H-8), and 6.86 (d, 1 H, J = 8.5 Hz, H-9) indicated that a 1,2,4-trisubstituted benzene ring was present in chelonin B (4). A fragment ion at m/z = 211.9857 (C_9H_9OBr, 7.5%) in the high-resolution mass spectrum suggested that these substituents were bromine, methoxy, and alkyl groups. The arrangement of these substituents around the benzene ring was determined by comparison of the ^{13}C and ^1H NMR chemical shifts of chelonin B (4) to calculated values.[4] The molecular formula of chelonin B (4) requires one less degree of unsaturation than in chelonin A (3), suggesting that the morpholine ring of chelonin A was not present in chelonin B. The absence of one of the carbon–oxygen bonds was confirmed by the observation of a methylene carbon signal at δ 26.0 (t, C-2) and a proton signal at 2.96 (br s, 4 H, CH_2-2, CH_2-3). The position of the hydroxyl group was determined by the observation of the dehydrated fragment ion in the high-resolution mass spectrum at m/z = 240.0000 ($C_{10}H_{11}NOBr$, 10%; Scheme I). This was verified by acetylation of chelonin B (4) to produce the *N,O*-diacetate 8. The carbinol proton at δ 4.66 (dd, 1 H, J = 7.3, 5.7 Hz, H-6) in chelonin B was shifted downfield to 5.96 (dd, ~0.6 H, J = 7.6, 5.5 Hz, H-6) and 5.72 (dd, ~0.4 H, J = 8.4, 4.9 Hz, H-6) in 8. The majority of the signals in the ^1H NMR spectrum of 8 were doubled in a manner similar to that of H-6, presumably due to the presence of the syn and anti isomers of the acetamide in 8. The remainder of the spectral data for 8 was consistent with the proposed structure for chelonin B (4).

Bromochelonin B (5) was isolated as a white gum that was determined to have a molecular formula of $C_{19}H_{20}N_2O_2Br_2$ from high-resolution mass measurement of the molecular ion at m/z = 465.9891. Comparison of the molecular formula and the ^1H and ^{13}C NMR data of bromochelonin B (5) with those of chelonin B (4) indicated that 5 was simply at brominated analogue of 4. A prominant fragment ion in the high-resolution mass spectrum at m/z = 251.0117 ($C_{11}H_{12}N_2Br$, 68%) indicated a brominated indole subunit, while a fragment ion at m/z = 239.9997 ($C_{10}H_{11}NOBr$, 33%) supported the presence of a benzene subunit bearing methoxy and bromine substituents similar to that found in chelonin B (Scheme I). The location of the alcohol group and the substitution pattern of the benzene ring in bromochelonin B (5) were found to be the same as that in chelonin B (4) by analysis of NOEDS experiments. Irradiation of the resonance assigned to the carbinol proton at δ 4.55 (br t, 1 H, J = 5.9 Hz, H-6) produced enhancements of the signals at δ 7.48 (d, 1 H, J = 1.7 Hz, H-12, 2.7%) and 7.23 (dd, 1 H, J = 8.4, 1.7 Hz, H-8, 8.1%), indicating that these two aromatic protons were ortho to the side chain. Irradiation of the methoxyl signal at δ 3.81 produced an enhancement (13.1%) of the resonance at δ 6.98 (d, 1 H, J = 8.4 Hz, H-9), supporting the para position of the methoxy group. The coupling patterns of the remaining aromatic proton resonances indicated that the indole subunit possessed a bromine atom at either C-5′ or C-6′. The additional bromine in bromochelonin B (5) was placed at C-5′ by comparison of the ^1H NMR data to those reported for 5-bromo-*N,N*′-dimethyltryptamine (9)[5] and 6-bromotryptamine (10;[6] Table II).

Table II. Selected ^1H NMR Data (Chemical Shift (δ), Multiplicity, Number of Hydrogens, and Coupling Constants (Hz)) for Bromochelonin B (5), 5-Bromo-*N,N*′-dimethyltryptamine (9),[5] and 6-Bromotryptamine (10)[6]

H no.	5[a]	9[b]	10[a]
4′	7.66 (d, 1 H, 1.6)	7.70 (s, 1 H)	7.48 (d, 1 H, 8.5)
5′			7.09 (dd, 1 H, 8.5, 1.6)
6′	7.13 (dd, 1 H, 8.5, 1.6)	7.13 (d, 1 H, 7)	
7′	7.28 (d, 1 H, 8.5)	7.25 (d, 1 H, 7)	7.53 (d, 1 H, 1.6)

[a] Spectra recorded at 200 MHz in DMSO-d_6. [b] Spectrum recorded at 220 MHz in acetone-d_6.

Chelonin C (6) was isolated as a white solid, 238 °C dec, that exhibited a molecular ion at m/z = 315.1469 ($C_{18}H_{21}NO_4$) in the high-resolution mass spectrum. The presence of only 12 aromatic carbons in the ^{13}C NMR spectrum indicated that chelonin C (6) possessed two phenyl rings. Prominent fragment ions in the high-resolution mass spectrum at m/z = 164.0838 ($C_{10}H_{12}O_2$, 100%) and 120.0567 (C_8H_8O, 73%) were identified as (dimethoxyphenyl)ethylene and (hydroxyphenyl)ethylene subunits, respectively (Scheme I). The presence of a para substituted phenol subunit was indicated by the ^1H NMR resonances at δ 7.23 (d, 2 H, J = 8.5 Hz, H-8 and -12) and 6.75 (d, 2 H, J = 8.5 Hz, H-9 and -11) and the ^{13}C NMR signals at 158.3 (s), 132.6 (s), 128.6 (d, 2 C), and 116.1 (d, 2 C). A 1,2,4-trisubstituted benzene subunit possessing two methoxy substituents was indicated by the ^1H NMR signals at δ 6.99 (d, 1 H, J = 1.7 Hz, H-2′), 6.94 (dd, 1 H, J = 8.2, 1.7 Hz, H-6′), and 6.89 (d, 1 H, J = 8.2 Hz, H-5′) and ^{13}C NMR signals at 150.4 (s), 150.1 (s), 134.7 (s), 119.8 (d), 112.8 (d), and 111.3 (d). The placement of the

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methoxy substituents at C-3′ and C-4′ was determined by a NOEDS experiment in which both of the resonances assigned to the methoxyl groups at δ 3.80 and 3.83 were irradiated, resulting in enhancements of the signals at 6.99 (H-2′, 9.1%) and 6.89 (H-5′, 13.8%). This assignment was supported by the observation of an enhancement of the signals at δ 6.94 (H-6′, 4.6%) and 6.99 (H-2′, 4.3%) upon irradiation at 4.59 (dd, 1 H, J = 10.5, 4.9 Hz, H-2). The molecular formula of chelonin C (6) requires nine degrees of unsaturation, indicating that the structure must have one nonaromatic ring. The remaining resonances in the ^{1}H NMR spectrum composed two nearly overlapping ABX spin systems, which were both assigned to $-NCH_2CHRO-$ fragments of a 2,6-disubstituted morpholine ring. The orientation of the substituents on the morpholine ring was determined to be cis diequatorial by analysis of the coupling constants of H-2 and H-6: the large coupling constants ($J_{2,3ax}$ = 10.5 Hz and $J_{5ax,6}$ = 10.4 Hz) indicated that both H-2 and H-6 were axially oriented.

Chelonin A (3), chelonin B (4), and bromochelonin B (5) exhibited antimicrobial activity in the standard disk assay against *B. subtilis* at a concentration of 100 μg/disk. Chelonin A (3) exhibited a 60% inhibition of PMA-induced inflammation of the mouse ear at a concentration of 50 μg/ear, but showed no in vitro inhibition of bee venom phosopholipase A_2 at a concentration of 1.6 μg/mL. Synthetic compounds possessing 2,6-disubstituted morpholine rings have been reported to exhibit antifungal,[7] antiarhythmic,[8] antitumor,[9] anticonvulsant, hypnotic, narcotic, and sedative activities.[10] Further pharmacological screening of the chelonins is currently being undertaken.

There have been more than 20 reports of spongian diterpenes from dendroceratid sponges and the dorid nudibranchs that feed upon them,[11] yet there is only one previous report of a cooccurring polar nitrogenous metabolite, that being isolated from *D. membranosa*.[12] It appears that chemists have been so fascinated by the structural intracacy of the diterpenes that they have overlooked the more polar metabolites. Our initial studies indicate that the polar nitrogenous metabolites have much greater biological activity than the diterpenes and may possibly contribute to the chemical defense mechanisms of the dendroceratid sponges and dorid nudibranchs.

Experimental Section

Extraction and Chromatography. The purple dendritic sponge *Chelonaplysilla* sp. was collected by hand at a depth of 1–5 m in a marine lake on Kaibaku Island, Iwayama Bay, Palau. The sponge was stored at −10 °C for approximately 2 weeks and then freeze-dried. The lyophilized sponge tissue (109.5 g) was extracted with dichloromethane (4 × 1 L) and methanol (3 × 1 L). The methanol extract (7.1 g) of the freeze-dried sponge tissue was triturated with dichloromethane (3 × 100 mL) to obtain a brown solid (1.3 g) that exhibited antimicrobrial activity against *B. subtilis* and *S. aureus*. This mixture was separated on Sephadex LH-20 (column size 95 × 3 cm) using dichloromethane/methanol (1:1) to elute the material. The major fraction that exhibited antimicrobial activity (629 mg) was separated by flash chromatography on silica (Kieselgel 60, 230–400 mesh, column size 34 × 2.5 cm) using a solvent gradient from ethyl acetate/methanol (9:1) to methanol to yield a number of bioactive fractions that appeared to contain mixtures of aromatic compounds as judged by ^{1}H NMR spectroscopy. One of these fractions (173 mg) was subjected to centrifugal counter current chromatography (CCC) using a solvent system of hexane/ethyl acetate/methanol/water (3:7:5:5, upper phase stationary) to yield chelonin A (3, 69.8 mg, 0.064% dry weight).

A second bioactive fraction (160 mg) from the silica flash chromatography was separated by CCC in hexane/ethyl acetate/methanol/water (3:7:5:5, upper phase stationary) to yield two mixtures that exhibited antimicrobial activity. The first fraction (71 mg) was separated by successive CCC in hexane/ethyl acetate/methanol/water (3:7:5:5 upper phase stationary once, lower phase stationary once) and ethyl acetate/95% ethanol/water (2:1:2, lower phase stationary) to yield a mixture of indole alkaloids. This mixture (57 mg) was separated by reversed-phase HPLC on μ-Partisil C_{18} silica using methanol/water/diethylamine (75:25:1) as eluant followed by reversed-phase HPLC on a Dynamax C_{18} silica column using methanol/water (85:15) as eluant to yield chelonin B (4, 9.5 mg, 0.009% dry weight). The second bioactive fraction from the first CCC separation (32 mg) was separated by HPLC on a Selectosil NH_2 silica column using dichloromethane/methanol (97:3) as eluant to yield bromochelonin B (5, 6.5 mg, 0.006% dry weight). A third bioactive fraction (75 mg) from the original flash chromatography step was separated by HPLC on Selectosil NH_2 silica using dichloromethane/methanol (96:4) as eluant to yield more chelonin A (3) and an inactive compound identified as chelonin C (6, 16.7 mg, 0.015% dry weight).

Chelonin A (3): white crystals from MeOH; mp 182 °C; $[\alpha]_D$ −11.7° (c = 0.32, $CHCl_3$); IR ($CHCl_3$) 3480, 1595, 1510, 1505, 1465, 1455, 1420, 1335, 1130, 1095 cm^{-1}; UV (MeOH) 288 (ε 5100), 278 (ε 6400), 272 (ε 6300), 213 nm (ε 37 100); ^{1}H NMR (see Table I); ^{13}C NMR (see Table I); HREIMS, obsd m/z = 368.1713 (M^+), $C_{21}H_{24}N_2O_4$ requires 368.1736.

Chelonin B (4): white solid; 260 °C dec; IR ($CHCl_3$) 3470, 3330 (br), 1605, 1500, 1460, 1445, 1420, 1285, 1260, 1055 cm^{-1}; UV (MeOH) 288 (sh), 280 (ε 6400), 221 (ε 30 600), 208 nm (ε 29 000); ^{1}H NMR (CD_3OD) δ 2.75 (d, 1 H, J = 5.7 Hz), 2.76 (d, 1 H, J = 7.3 Hz), 2.96 (br s, 4 H), 3.82 (s, 3 H), 4.66 (dd, 1 H, J = 7.3, 5.7 Hz), 6.86 (d, 1 H, J = 8.5 Hz), 6.98 (ddd, 1 H, J = 7.3, 7.1, 1.0 Hz), 7.04 (s, 1 H), 7.04 (ddd, 1 H, J = 7.7, 7.3, 1.1 Hz), 7.14 (dd, 1 H, J = 8.5, 2.0 Hz), 7.32 (dd, 1 H, J = 7.7, 1.1 Hz), 7.48 (d, 1 H, J = 2.0 Hz), 7.52 (dd, 1 H, J = 7.1, 1.1 Hz); ^{13}C NMR (CD_3OD) δ 26.0 (t), 50.5 (t), 56.7 (q), 57.5 (t), 72.0 (d), 112.3 (d), 112.4 (s), 113.0 (s), 113.0 (d), 119.2 (d), 119.6 (d), 122.4 (d), 123.6 (d), 127.4 (d), 128.6 (s), 131.8 (d), 138.0 (s), 138.2 (s), 156.7 (s); HREIMS obsd m/z = 370.0681 $(M - H_2O)^+$, $C_{19}H_{19}N_2OBr$ requires 370.0681.

Bromochelonin B (5): white gum; $[\alpha]_D$ +3.7° (c = 0.27, DMSO); IR (KBr) 3470, 3330 (br), 1605, 1500, 1460, 1445, 1420, 1285, 1260, 1055 cm^{-1}; UV (DMSO) 299 (sh), 287 (ε 2500), 279 (sh), 221 (ε 30 600), 208 nm (ε 29 000); ^{1}H NMR (DMSO-d_6) δ 2.63 (br d, 2 H, J = 5.9 Hz), 2.79 (br s, 4 H), 3.81 (s, 3 H), 4.55 (br t, 1 H, J = 5.9 Hz), 5.25 (br, 1 H), 6.98 (d, 1 H, J = 8.4 Hz), 7.13 (dd, 1 H, J = 8.5, 1.6 Hz), 7.14 (br s, 1 H), 7.23 (dd, 1 H, J = 8.4, 1.7 Hz), 7.28 (d, 1 H, J = 8.5 Hz), 7.48 (d, 1 H, J = 1.7 Hz), 7.66 (d, 1 H, J = 1.6 Hz), 11.04 (br s, 1 H); ^{13}C NMR (DMSO-d_6) δ 24.7 (t), 49.4 (t), 56.1 (q), 56.7 (t), 69.9 (d), 110.2 (s), 110.9 (s), 112.0 (s), 112.1 (d), 113.3 (d), 120.6 (d), 123.2 (d), 124.4 (d), 126.4 (d), 129.1 (s), 130.3 (d), 134.9 (s), 137.9 (s), 154.2 (s); HREIMS obsd m/z = 465.9891 (M^+), $C_{19}H_{20}N_2O_2Br_2$ requires 465.9893.

Chelonin C (6): white solid; 238 °C dec; $[\alpha]_D$ +5.8° (c = 0.53, $CHCl_3$); IR ($CHCl_3$) 3295 (br), 1615, 1595, 1520, 1465, 1455, 1440, 1420, 1260, 1160, 1140, 1030 cm^{-1}; UV (MeOH) 277 (ε 3600), 227 (ε 13 800), 208 (ε 15 100); (MeOH + OH) 295 (sh), 279 (ε 3800), 236 (ε 12 900), 210 nm (ε 18 200); ^{1}H NMR (CD_3OD) δ 2.69 (dd, 1 H, J = 12.7, 10.4 Hz), 2.70 (dd, 1 H, J = 12.5, 10.5 Hz), 2.96 (dd, 1 H, J = 12.7, 4.7 Hz), 2.98 (dd, 1 H, J = 12.5, 4.9 Hz), 3.80 (s, 3 H), 3.83 (s, 3 H), 4.58 (dd, 1 H, J = 10.4, 4.7 Hz), 4.59 (dd, 1 H, J = 10.5, 4.9 Hz), 6.75 (d, 2 H, J = 8.5 Hz), 6.89 (d, 1 H, J = 8.2 Hz), 6.94 (dd, 1 H, J = 8.2, 1.7 Hz), 6.99 (d, 1 H, J = 1.7 Hz), 7.23 (d, 2 H, J = 8.5 Hz); ^{13}C NMR (CD_3OD) δ 52.8 (t, 2 C), 56.5 (q, 2 C), 80.3 (d, 2 C), 111.3 (d), 112.8 (d), 116.1 (d, 2 C), 119.8 (d), 128.6 (d, 2 C), 132.6 (s), 134.7 (s), 150.1 (s), 150.4 (s), 158.3 (s); HREIMS obsd m/z = 315.1469 (M^+), $C_{18}H_{21}NO_4$ requires 315.1470.

Acetylation of Chelonin A (3). Acetic anhydride (0.1 mL) was added to a stirred solution of chelonin A (3, 1.1 mg) in pyridine

(7) (a) German Patent DE 2825961, 1980. (b) German Patent DE 3633520 A1, 1988.
(8) U.S. Patent US 4241059, 1980.
(9) Japanese Patent JP 62/135472 A2 [87/135472], 1987.
(10) German Patent DE 2441350, 1976.
(11) Faulkner, D. J. *Nat. Prod. Rep.* **1984**, *1*, 551; **1986**, *3*, 1; **1987**, *4*, 539; **1988**, *5*, 613; **1990**, *7*, 269.
(12) Molinski, T. F.; Faulkner, D. J. *Tetrahedron Lett.* **1989**, *29*, 2137.

(1.0 mL), and the reaction was allowed to proceed for 17 h at room temperature. The solvents were removed from the reaction mixture in vacuo and the products separated on a silica Sep-pak (Waters) using ethyl acetate/methanol (94:6) as eluant. The product appeared as a mixture by ^{1}H NMR spectroscopy, and separation was attempted by HPLC on μ-Partisil using ethyl acetate as eluant. This yielded a single peak, which was determined to be a 6:4 mixture of syn and anti isomers of the acetamide 7 (0.9 mg, 73% yield).

Chelonin A acetamide (7): clear oil; IR ($CHCl_3$) 3480, 1640, 1630, 1595, 1510, 1460, 1420, 1130 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.18 (s, ~1.8 H), 2.19 (s, ~1.2 H), 2.74 (dd, 1 H, J = 13.5, 10.8 Hz), 3.05 (dd, 1 H, J = 13.0, 11.0 Hz), 3.29 (dd, 1 H, J = 13.5, 11.6 Hz), 3.54 (dd, 1 H, J = 13.0, 10.9 Hz), 3.80 (s, 3 H), 3.84 (s, 3 H), 3.87 (s, 3 H), 4.84 (dd, 1 H, J = 11.6, 10.9 Hz), 4.95 (dd, 1 H, J = 11.0, 10.8 Hz), 6.68 (s, 2 H), 7.18 (m, 3 H), 7.37 (d, ~0.4 H, J = 6.8 Hz), 7.40 (d, ~0.6 H, J = 7.2 Hz), 7.79 (d, ~0.4 H, J = 7.4 Hz), 7.83 (d, ~0.6 H, J = 7.2 Hz), 8.15 (br s, ~0.4 H), 8.20 (br s, ~0.6 H); LREIMS m/z = 410 (12%, M$^+$).

Acetylation of Chelonin B (4). Acetic anhydride (0.1 mL) was added to a stirred solution of chelonin B (4, 1.7 mg) in pyridine (1.0 mL), and the reaction was allowed to proceed for 21 h at room temperature. The solvents were removed from the reaction mixture in vacuo and the products separated on a silica Sep-Pak (Waters) in ethyl acetate. The product was purified by HPLC on μ-Partisil using hexane/ethyl acetate (1:9) as eluant to obtain a single peak, which was determined to be a mixture of conformational isomers of the N,O-diacetate 8 (1.7 mg, 84% yield).

Chelonin B N,O-diacetate (8): clear oil; IR ($CHCl_3$) 3475, 1745, 1635, 1605, 1500, 1455, 1440, 1420, 1375, 1260, 1225, 1055, 905 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.93 (s, ~2 H), 2.06 (s, 3 H), 2.09 (s, ~1 H), 2.99 (m, ~3 H), 3.14 (dd, ~0.3 H, J = 14.9, 4.6 Hz), 3.57 (m, ~3 H), 3.87 (s, 3 H), 5.72 (dd, ~0.4 H, J = 8.4, 4.8 Hz), 5.96 (dd, ~0.6 H, J = 7.6, 5.5 Hz), 6.81 (d, ~0.4 H, J = 8.2 Hz), 6.84 (d, ~0.6 H, J = 8.3 Hz), 6.97 (d, ~0.6 H, J = 2.3 Hz), 7.02 (d, ~0.4 H, J = 2.0 Hz), 7.21 (m, 3 H), 7.38 (m, ~1.4 H), 7.51 (d, ~0.6 H, J = 2.0 Hz), 7.56 (br d, ~0.6 H, J = 8.4 Hz), 7.67 (br d, ~0.4 H, J = 7.2 Hz), 8.00 (br s, ~0.4 H), 8.07 (br s, 0.6 H).

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Supplementary Material Available: ^{1}H NMR spectra of compounds 3–8 and ^{13}C NMR spectra of compounds 3–6 (10 pages). Ordering information is given on any current masthead page.

Vilsmeier Reactions of Porphyrins and Chlorins with 3-(Dimethylamino)acrolein To Give *meso*-(2-Formylvinyl)porphyrins: New Syntheses of Benzochlorins, Benzoisobacteriochlorins, and Benzobacteriochlorins and Reductive Coupling of Porphyrins and Chlorins Using Low-Valent Titanium Complexes

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Vilsmeier reactions between nickel(II) or copper(II) porphyrins or chlorins and 3-(dimethylamino)-acrolein/phosphoryl chloride (3-DMA/$POCl_3$) are described. For example, copper(II) octaethylporphyrin **5** affords the *meso*-(2-formylvinyl) derivative **7**, which cyclizes in strong acid to give the benzochlorin **10**. Treatment of the nickel(II) benzochlorin **9** with 3-DMA/$POCl_3$ gives the *meso*-(2-formylvinyl) derivative **14**, which is cyclized in acid to give the dibenzoisobacteriochlorin **16**. Prolonged treatment of nickel(II) octaethylporphyrin (**4**) with 3-DMA/$POCl_3$ gives the disubstituted compound **17** in which the acrolein substituents are on adjacent rather than opposite meso positions. Acid-promoted cyclization of **17** afforded the monocyclized product **15** as well as the dibenzobacteriochlorin **18**. The reaction is extended to obtain spiro benzochlorins (**37**, **38**) from (tetrabutano- and -pentanoporphyrinato)nickel(II) complexes, as well as benzoisobacteriochlorins (e.g., **28**, **29**) from nickel(II) mesochlorin e_6 trimethyl ester (**19**) and nickel(II) octaethylchlorin (**26**), respectively. Nickel(II) deuteroporphyrin IX dimethyl ester (**11**) also affords benzochlorins (**12**, **13**) resulting from the corresponding *meso*-(2-formylvinyl)porphyrin. A centrally chelated metal is shown to be essential in order to accomplish cyclization of the 2-formylvinyl substituents to afford benzo derivatives. Porphyrin and chlorin dimers joined by one or three carbon–carbon double bond linkages are formed in good yields via reductive coupling by low valent titanium complexes of nickel(II) or copper(II) porphyrins or chlorins containing a formyl or an acrolein side chain. For example, nickel(II) α-(formylvinyl)octaethylporphyrin (**6**) reacts with the active titanium reagent to produce dimer **48** in 96% yield. When two different porphyrins or chlorins are cross-reacted under the same conditions, a mixture of products is obtained. The acrolein group seems to be more reactive in reductive coupling reactions than does the corresponding formyl substituent.

Introduction

The Vilsmeier formylation reaction was first introduced into porphyrin chemistry, in 1966, by Inhoffen and coworkers.[1] Since that time it has been routinely exploited as a highly efficient means for introduction of substituents into the meso positions of numerous copper(II) and nickel(II) porphyrins and chlorins. An interesting vinylogous Vilsmeier formylation of pyrrole **1** [with 3-(dimethylamino)acrolein and phosphoryl chloride (3-DMA/$POCl_3$)] to give an acrolein-substituted analogue **2** was reported by Gosmann and Franck[2] in 1986. In this paper we describe

(1) Inhoffen, H. H.; Fuhrhop, J.-H.; Voigt, H.; Brockmann, H., Jr. *Justus Liebigs Ann. Chem.* **1966**, *695*, 133.

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