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Pseudoceratins A and B, Antifungal Bicyclic Bromotyrosine-Derived Metabolites from the Marine Sponge *Pseudoceratina purpurea*

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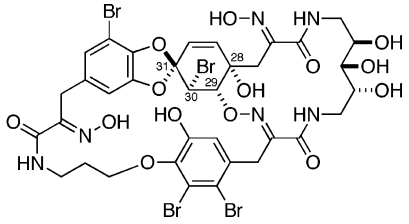
Received September 29, 2006

Pseudoceratin A
(can be epimeric at C-28-C-31)



Pseudoceratins A (**1**) and B (**2**) have been isolated from the marine sponge *Pseudoceratina purpurea*. Pseudoceratins are unique among the large class of bromotyrosine-derived sponge metabolites with respect to the substitution pattern of aromatic rings and the presence of spiroacetal and oxime ether functional groups as well as an 1,5-diamino-1,5-dideoxy-D-arabitol tether. Pseudoceratins A (**1**) and B (**2**) are epimeric differing in the orientation of the tether. Their structures were determined by analysis of spectral data. The rigidity of the compounds arising from their bridged structure gave ROESY/NOESY spectra with many transannular cross-peaks, which allowed the assignment of the relative stereochemistry of **1** and **2** to be established. The D-configuration of the 1,5-diamino-1,5-dideoxyarabitol unit was determined by converting the fragment isolated from the acid hydrolysate to the Mosher's ester and compared the ^{1}H NMR spectrum with those of standard samples. Pseudoceratins exhibited significant antifungal activity against *Candida albicans*.

Introduction

Even in the post-genomics era and chemical genetics, the importance of the discovery of bioactive molecules with novel structural features from nature has not been diminished.[1−3] We have searched for compounds active against the *erg6* mutant of *Saccharomyces cerevisiae*, which has an increased sensitivity to small molecules due to an altered sterol composition.[4] By using this yeast strain, we expected to detect antifungal and cytotoxic compounds with high sensitivity. In the screening of the extracts of a variety of Japanese marine invertebrates, we found that the marine sponge *Pseudoceratina purpurea* collected at Oshima-shinsone in southern Japan exhibited significant activity. Bioassay-guided fractionation of the extract afforded pseudoceratins A (**1**) and B (**2**), bromotyrosine-derived alkaloids of a new structural class. The isolation and structure elucidation of these two metabolites are described below.

Results and Discussion

The MeOH extract of the sponge was concentrated and partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ layer was partitioned between *n*-hexane and 90% MeOH, and the 90% MeOH layer was further partitioned between 60% MeOH and $CHCl_3$. The resulting $CHCl_3$ layer was purified by ODS flash chromatography followed by ODS HPLC to afford pseudoceratins A (**1**) and B (**2**). Pseudoceratin A (**1**) exhibited a cluster of molecular ion peaks at *m*/*z* 1080, 1082, 1084, 1086, 1088 in a ratio of 1:4:6:4:1 in the FABMS, indicating the presence of four bromine atoms. The molecular formula of $C_{35}H_{36}Br_4N_6O_{14}$

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(1) Edwards, A.; Berman, J.; Sundstrom, M. *Ann. Rep. Med. Chem.* **2005**, *40*, 350−369.
(2) Bode, H. B.; Müller, R. *Angew. Chem., Int. Ed.* **2005**, *44*, 6828−6846.
(3) Butler, M. S. *Nat. Prod. Rep.* **2005**, *22*, 162−195.
(4) Emter, R.; Heese-Peck, A.; Kralli, A. *FEBS Lett.* **2002**, *521*, 57−61.

10.1021/jo062010+ CCC: $37.00
Published on Web 01/26/2007

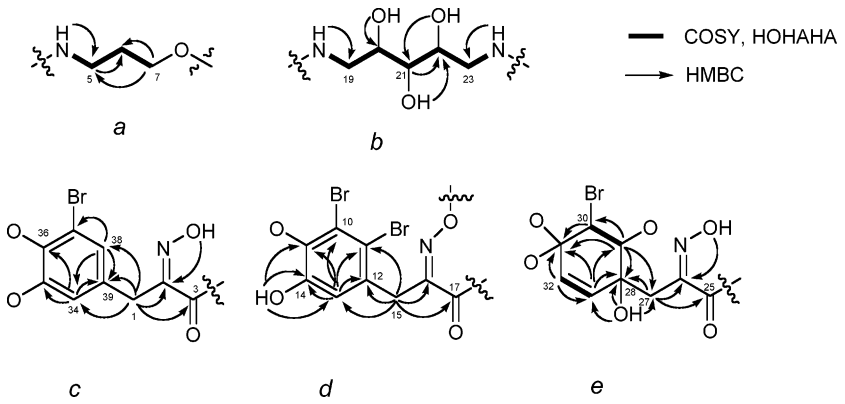


FIGURE 1. Partial structures and selected 2D-NMR correlations of pseudoceratin A (**1**).

was determined on the basis of a combination of the HRESIMS and NMR data (Table 1). The ^{13}C NMR spectrum of **1** in conjunction with the DEPT experiment exhibited 35 carbons; three carbonyl carbons, 12 non-protonated sp^2 carbons, five protonated sp^2 carbons, two quaternary carbons, five methine carbons, and eight methylene carbons (Table 1). The HSQC data allowed us to correlate the ^1H and ^{13}C signals of the methine and methylene carbons described above (Table 1). Additionally, 10 exchangeable ^1H signals were observed in DMSO-d_6: two oxime hydroxyl protons (δ 11.77 and 12.08), three amide protons (δ 8.02, 7.81, and 7.32), one phenolic hydroxyl proton (δ 9.33), and four hydroxyl protons (δ 5.30, 4.64, 4.55, and 4.44). Interpretation of the 2D-NMR data including COSY, TOCSY, HSQC, and HMBC spectra enabled the construction of units *a*, *b*, *c*, *d*, and *e* (Figure 1).

Unit *a* consists of three contiguous methylenes as revealed by the COSY data. The H_2-5 was coupled to an amide proton (δ 8.02, NH-4), whereas C-7 was suggested to be oxygenated on the basis of the carbon chemical shift of 70.2 ppm. The ^1H NMR spin system in unit *b* was deduced from the COSY data. Three contiguous oxymethines were flanked by nitrogenous methylenes. H_2-19 and H_2-23 were coupled to amide protons, NH-18 and NH-24, respectively.

Unit *c* contained a pair of meta-coupled aromatic protons [δ 6.26 (d, $J = 1.7$ Hz; H-34) and 6.93 (d, $J = 1.7$ Hz; H-38)] and a pair of isolated methylene protons [δ 3.53 (d, $J = 13.5$ Hz) and 3.73 (d, $J = 13.5$ Hz); H_2-1]. In the HMBC spectrum, H_2-1 were correlated with C-2 (δ 151.4), C-3 (δ 163.7), C-34 (δ 107.1), C-38 (δ 125.2), and C-39 (δ 131.9). The chemical shifts of C-2 (δ 151.4) and C-3 (δ 163.7) were reminiscent of oxime and carbonyl carbon, respectively.[5] The assignment of an oxime was confirmed by an HMBC correlation of C-2 with an exchangeable proton at δ 11.77. In the HMBC spectrum, H-34 exhibited large couplings with C-36 (δ 142.9) and C-38 as evidenced by intense cross-peaks and exhibited small couplings with C-35 (δ 148.2), C-37 (δ 98.4), and C-39, as suggested by weak cross-peaks. On the other hand, H-38 exhibits large couplings with C-34 and C-36 and small couplings with C-35, C-37, and C-39. The large couplings were assigned as $^3J_{CH}$ in a benzene ring.[6] C-35 was assigned as oxygenated because C-34 was shielded. C-36 was oxygenated and C-37 was brominated on the basis of chemical shift arguments.[7]

TABLE 1. NMR Spectral Data for Pseudoceratins in DMSO-d_6

	pseudoceratin A (**1**)		pseudoceratin B (**2**)	
position	δ_C	δ_H, m, *J* (Hz)	δ_C	δ_H, m, *J* (Hz)
1	28.7	3.53, d, 13.5 3.73, d, 13.5	28.4	3.55, d, 13.4 3.70, d, 13.4
2	151.4		151.0	
2-NOH		11.77, s		11.77, s
3	163.7		163.7	
4-NH		8.02, t, 6.0	105.5	8.08, t, 6.0
5	35.4	3.14, m 3.54, m	35.5	3.17, m 3.52, m
6	29.1	1.83, m	29.3	1.86, m
7	70.2	3.67, m 3.93, m	70.3	3.73, m 3.94, m
9	143.5		143.5	
10	120.2		120.2	
11	114.5		114.5	
12	132.5		132.6	
13	117.0	6.75, s	117.1	6.72, s
14	149.6		149.5	
14-OH		9.33, s		9.34, s
15	31.8	3.87, s	31.5	3.84, d, 15.2 3.95, d, 15.2
16	151.8		151.3	
17	162.7		162.4	
18-NH		7.32, dd, 3.0, 8.6	99.2	7.62, dd, 3.9, 9.4
19	42.1	3.21, ddd, 2.0, 3.0, 13.4 3.59, ddd, 2.6, 8.6, 13.4	40.6	2.82, ddd, 3.9, 10.3, 13.1 3.58, 6.0, 9.4, 13.1
20	68.2	3.68, ddt, 4.1, 11.7, 2.4	66.5	3.65, m
20-OH		4.55, d, 4.1		4.58, brs
21	67.4	3.40, ddd, 1.8, 6.9, 11.0	68.5	3.34, 1.4, 9.6
21-OH		4.64, d, 6.9		4.64, brs
22	66.2	3.83, dddd, 1.5, 5.3, 6.8, 10.1	69.2	3.60, dt, 9.6, 2.7
22-OH		4.44, d, 6.8		4.75, brs
23	41.0	3.05, ddd, 6.4, 10.4, 13.1 3.39, m	42.1	3.39, ddd, 2.7, 6.4, 13.7 3.67, m
24-NH		7.81, t, 6.4	100.6	7.73, t, 6.4
25	164.1		165.8	
26	151.2		151.0	
26-NOH		12.08, s		12.24, s
27	32.2	2.85, d, 13.3 2.92, d, 13.3	32.7	2.87, d, 13.5 2.95, d, 13.5
28	72.8		73.1	
28-OH		5.30,		5.62, s
29	87.9	4.62, d, 3.0	87.7	4.60, d, 3.0
30	51.8	5.17, d, 3.0	51.9	5.14, d, 3.0
31	111.3		111.1	
32	121.1	5.91, d, 10.3	121.3	5.95, s
33	138.8	5.94, d, 10.3	138.4	5.95, s
34	107.1	6.26, d, 1.7	106.8	6.24, d, 1.7
35	148.2		146.0	
36	142.9		142.9	
37	98.4		98.2	
38	125.2	6.93, d, 1.7	125.0	6.93, d, 1.7
39	131.9		132.0	

Unit *d* was assigned similarly. The isolated methylene protons [δ 3.87 (2H, s); H_2-15] were correlated to an oxime (δ 151.8;

(5) Peng, J.; Li, J.; Hamann, M. T. *Alkaloids* **2005**, *61*, 59−262.
(6) Breitmaier, E.; Voelter, W. *Carbon-13 NMR Spectroscopy*, 3rd ed.; VCH: Weinheim, Germany; 1989; p 145.
(7) Reference 6; pp 260−261.

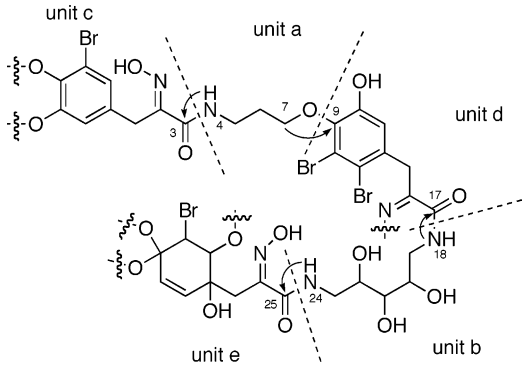


FIGURE 2. Inter-residual HMBC correlations of pseudoceratin A (**1**).

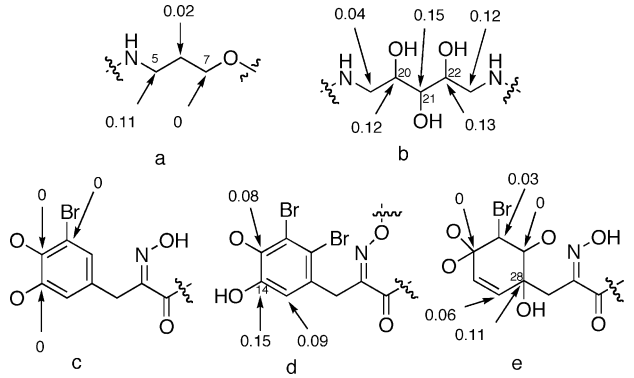


FIGURE 3. Deuterium-induced isotope shifts in the ^{13}C NMR spectrum of pseudoceratin A (**1**). $\Delta\delta = [\delta_C$ (H_2O/DMSO-d_6) $- \delta_C$ (D_2O/DMSO-d_6)].

C-16), an amide carbonyl carbon (δ 162.7; C-17), C-11 (δ 114.5), C-12 (δ 132.5), and C-13 (δ 117.0) in the HMBC spectrum. On the other hand, H-13 (δ 6.75 s) exhibited intense HMBC correlations with C-9 and C-11 and less intense correlations with C-10, C-11, and C-14. C-9, C-13, and C-14 were further correlated with an exchangeable proton (δ 9.33; OH-14) in the HMBC spectrum, indicating that C-14 was hydroxylated. Therefore, C-10 was placed para to C-13. Chemical shifts for C-10 and C-11 indicated that they were brominated,[7] whereas the chemical shift for C-9 indicated that it was oxygenated.[8] It must be noted that C-16 was not coupled to any of the oxime hydroxyl protons.

Unit *e* was composed of the remaining signals including two methines [δ 4.62 (d, $J = 3.0$ Hz; H-29) and 5.17 (d, $J = 3.0$ Hz; H-30)], two quaternary carbons [δ 72.8 (C-28) and 111.3 (C-31)], and 1,2-disubstituted *Z*-olefin [δ 5.91 (d, $J = 10.3$ Hz; H-32) and 5.94 (d, $J = 10.3$ Hz; H-33)] in addition to an isolated methylene [δ 2.85 (d, $J = 13.3$ Hz) and 2.92 (d, $J = 13.3$ Hz); H_2-27)], an oxime (δ 151.2; C-26), and a carbonyl carbon (δ 164.1; C-25). H_2-27 signals were coupled to C-25, C-26, C-28, C-29 (δ 87.9), and C-33 (δ 138.8). C-28 was assigned as a tertiary alcohol on the basis of its carbon chemical shift and an HMBC coupling to an exchangeable proton (δ 5.30 s; 28-OH), which was further coupled to C-27 (δ 32.2), C-29, and C-33. H-29 and H-30 were coupled to each other by 3.0 Hz, and H-29 was coupled to C-27, C-28, C-30 (δ 51.8), C-31, and C-33. Therefore, C-31 should be attached to C-30, whereas C-32 (δ 121.1) should be placed between C-31 and C-33 to form a six-membered ring. C-31 was assigned as an acetal, C-30 as brominated methine,[9] and C-29 as oxygenated methine, which were all based on chemical shift arguments.

Units *a*−*e* were connected to each other on the basis of the following HMBC correlations: NH-4/C-3, H_2-7/C-9, NH-18/C-17, NH-24/C-25 (Figure 2). In order to assign the locations of hydroxyl groups in **1**, deuterium-induced shifts were determined in the ^{13}C NMR spectrum.[10] Significant shifts were observed for C-14, C-20, C-21, C-22, and C-28 (Figure 3). Therefore, the remaining six oxygen atoms in units *c*, *d*, and *e* [C-9, C-16-NO, C-31 (×2), C-35, and C-36] should form three ether linkages among each other to satisfy the molecular formula. There are five possibilities in the modes of ether-bond formation (modes 1−5, Figure 4).

In modes 1 and 2, one of the phenolic hydroxyl groups is part of an oxime ether group. As a consequence, another phenolic hydroxyl group is connected to the acetal carbon (C-31), and the linkage between the remaining oxygen atoms on C-29 and C-31 should result in the formation of an oxetane ring. Because of the highly strained nature of an oxetane ring, vicinal coupling constants for the cis- and trans-protons are both expected to be larger than 6 Hz,[11] which contradicts the coupling constant of 3.0 Hz between H-29 and H-30 in pseudoceratin A (**1**). Modes 3 and 4 comprise the 1,3-dioxygenated cyclohexene whose oxygen atoms are each connected to the 1,2-positions of a benzene ring. Such a tricyclic system is unprecedented in the literature.[12] Because of the required coplanarity of the

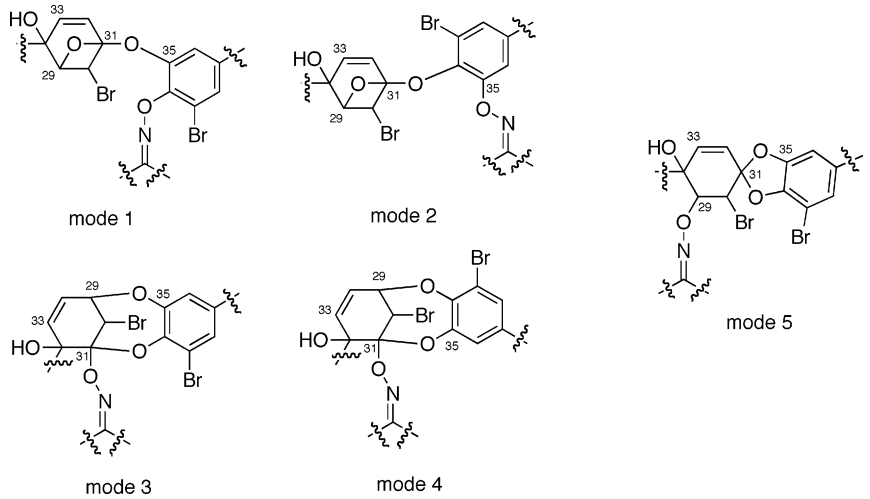
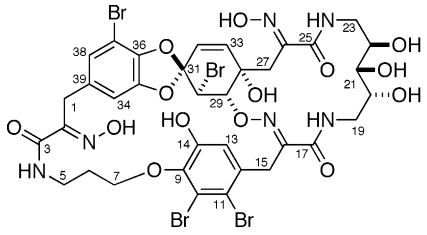
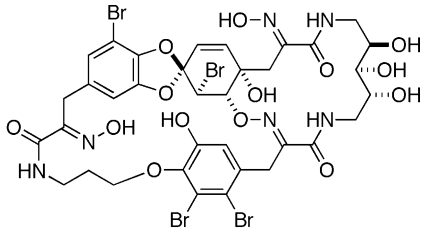


FIGURE 4. Five possible modes of incorporating three oxygen atoms in pseudoceratin A (**1**).

CHART 1. Structures of Pseudoceratins A (1) and B (2)



1



2

benzene ring and the two oxygen atoms both of which should occupy the 1,3-axial positions of the cyclohexene ring, this ring system is highly strained. In spite of the high improbability of these ring systems, rejection of modes 3 and 4 on the basis of spectral evidence was not straightforward due to the lack of crucial HMBC cross-peaks. Because of the absence of literature precedence for the same ring system, it is not possible to predict the chemical shift values of compounds formed by modes 3 and 4 to compare with those of **1**. The only evidence to refute modes 3 and 4 came from the ROESY data. The ROESY cross-peak, 26-NOH/H-30, implied that C-27 and C-30 are on the same face of the 6,9-dihydro-5*H*-[1,4]dioxonine ring (displayed in bold lines in Figure 5). With this constellation it is impossible

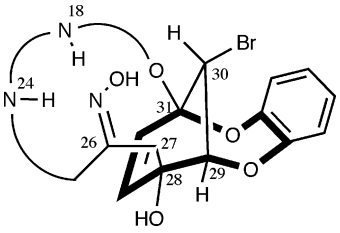


FIGURE 5. Structure of the tricyclic core formed with modes 3 and 4.

to detect a ROESY cross-peak, 28-OH/18-NH (Figure 5). In mode 5, catechol oxygens are involved in the formation of spiroacetal. The resulting structure not only satisfied the ROESY data (vide infra) but also accounted for the lower field chemical shifts for C-29 and C-31: the substituent shift of oxime ether is significantly larger than that of the hydroxyl group,[13,14] and five-membered spiroacetal carbons are deshielded.[15] From these analyses, the gross structure of pseudoceratin A (**1**) was assigned as shown in Chart 1.

Further analysis of the ROESY data and $^1H-^1H$ coupling constant data allowed the relative stereochemistry of pseudoceratin A (**1**) to be established. A small coupling constant between H-29 and H-30 and ROESY cross-peaks H-29/H-30 and H_2-27/H-30 showed that C-27, H-29, and H-30 should be placed on the same face of the cyclohexene ring. The ROESY cross-peaks between H_2-27 and H-30 required that C-27 and H-30 were both axially oriented. ROESY cross-peaks observed for the OH signals of the oxime groups, 2-NOH/H_2-1, 2-NOH/H-38, and 26-NOH/H-30, demonstrated the *E*-geometry of the oximes on C-2 and C-26. ROESY cross-peaks 28-OH/18-NH, H-21/18-NH, and H-21/24-NH showed that the right wing (Figure 6) was conformationally fixed. ROESY cross-peaks 18-

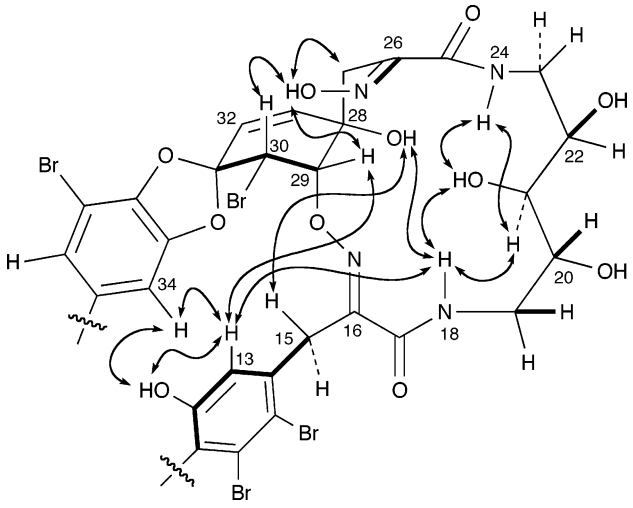


FIGURE 6. ROESY correlations in **1**. The cyclohexene ring is arbitrarily drawn as the 28*S*,29*S*,30*S*,31*S* form. All of the correlations can also be accounted for by the 28*R*,29*R*,30*R*,31*R* isomer.

NH/H-21 and 24-NH/H-21 indicated that N-18 and C-21 were in gauche positions with respect to the C-19−C-20 bond and that C-21 and N-24 were in gauche positions with respect to the C-22−C-23 bond. A large coupling constant between H-20 and H-21 and a small coupling constant between H-21 and H-22 implied that H-20 and H-21 were anti-periplanar, whereas H-21 and H-22 were gauche.[16] Therefore, the stereochemistry of the 1,5-diamino-1,5-dideoxypentritol unit is comparable to that of arabitol. There were additional trans-annular ROESY cross-

(8) Compounds with the same substitution pattern have been isolated not from sponges[5] but from the red alga: Fan, X.; Xu, N.-J.; Shi, J.-G. *J. Nat. Prod.* **2003**, *66*, 455−458.

(9) Reference 6; p 199.

(10) Newmark, R. A.; Hill, J. R. *Org. Magn. Reson.* **1980**, *13*, 40−44.

(11) Pretsch, E.; Bühlmann, P.; Sfflolter, C. *Structure Determination of Organic compounds*; Springer-Verlag, Berlin, Heidelberg, Germany, 2000; p 205.

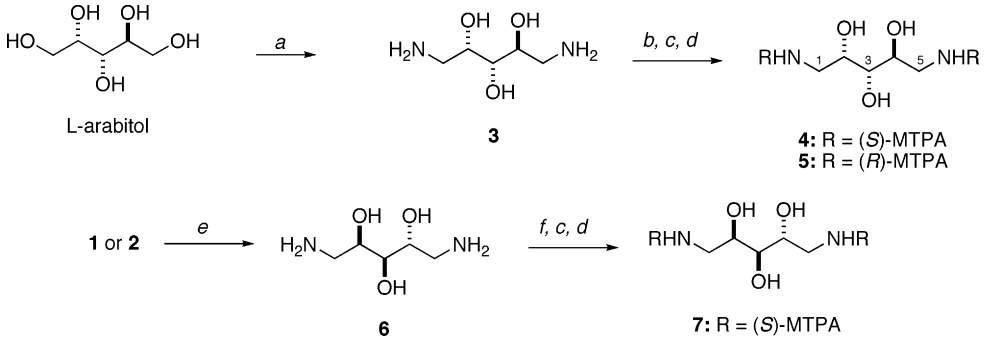
(12) A substructural search of either 2,5-dioxabicyclo[4.3.1]dec-3-ene or 2,5-dioxabicyclo[4.3.1]deca-3,7-diene through SciFinder Scholar afforded no hits.

(13) Carbon chemical shifts of inositols are between 67 and 75 ppm (ref 6; p 401), whereas the methine carbon in a cyclohexene ring attached to the oxygen atom of an oxime ether group resonated at 84 ppm in trichodermamides: Caro, E.; Starks, C. M.; Jensen, P. R.; Fenical, W.; Lobkovsky, E.; Clardy, J. *J. Nat. Prod.* **2003**, *66*, 423−426.

(14) Compounds with a similar substitution pattern were isolated from the marine sponge *Aplysina laevis*. The oxymethine carbons substituted by a hydroxyl group in the cyclohexenone ring resonated at 78.4 and 78.9 ppm. (Capon, R. J.; MacLeod, J. K. *Aust. J. Chem.* **1987**, *40*, 341−346.)

(15) The carbon chemical shifts of the acetal carbons of 1,1-dimethoxycyclohexane, 2-cyclohexene-1-one dimethyl acetal, and cyclohexanone ethylene acetal are 100.0, 97.6, and 109.0, respectively (Spectral Database for Organic Compounds: http://www.aist.go.jp/RIODB/SDBS/cgi-bin/direct_frame_top.cgi (accessed September 2006)). The structurally related acetal carbon in botryoxanthin A resonated at 107 ppm (Okada, S.; Matsuda, H.; Murakami, M.; Yamaguchi, K. *Tetrahedron Lett.* **1996**, *37*, 1065−1068).

SCHEME 1. Preparation of 4, 5, and 7[a]



[a] Conditions: (a) refs 17 and 18; (b) (*S*)- or (*R*)-MTPACl, pyridine; (c) K_2CO_3, MeOH; (d) SiO_2 column chromatography; (e) 6 N HCl, 110 °C, 4 h; (f) (*R*)-MTPACl, pyridine.

peaks, H-13/18-NH, H-13/H-29, H-13/H-34, 14-OH/H-34, and H_2-15/28-OH, which allowed the orientation of the tetrasubstituted benzene ring, i.e., relative stereochemistry at C-31, and the geometry of the oxime at C-16 to be determined as shown in Chart 1 (Figure 6).

The absolute stereochemistry of the 1,5-diamino-1,5-dideoxyarabitol unit in **1** was determined by chemical degradation (Figure 6). A standard sample of diamine **3** was prepared from L-arabitol as described in the literature[17,18] and converted to the *N*,*N*′-bis-(*R*)-MTPA amide **4** and the *N*,*N*′-bis-(*S*)-MTPA amide **5** (Scheme 1). Pseudoceratin A (**1**) was subjected to acid hydrolysis, and the product was partitioned between H_2O and EtOAc. The ^{1}H NMR analysis indicated that the aqueous phase was mostly composed of 1,5-diamino-1,5-dideoxyarabitol **6**, which was converted to the *N*,*N*′-bis-(*S*)-MTPA amide **7**. The ^{1}H NMR spectrum of **7** was identical with that of **5**. Therefore, the stereochemistry of the 1,5-diamino-1,5-dideoxyarabitol unit in pseudoceratin A (**1**) is comparable to that of D-arabitol. It was not possible to correlate the stereochemisty of the triol portion to that of the cyclohexene unit on the basis of the ROESY data. Therefore, the stereochemistry of pseudoceratin A is 20*R*,21*S*,22*R*,28*S**,29*S**,30*S**,31*S**.

Pseudoceratin B (**2**) was isomeric to **1**.[19] The ^{1}H and ^{13}C NMR spectra of **2** were similar to those of **1** (Table 1). Interpretation of the 2D NMR data of **2** showed that the gross structure of **2** was identical with that of **1**. Significant differences were observed for the coupling constants in the triol portion (Table 1). The NMR data showed that H-20 and H-21 were gauche and H-21 and H-22 were anti in pseudoceratin B (**2**). From this information, it was expected that **2** was the C-21 epimer of **1**.[21] The ROESY data of **2** were almost identical with that of **1** in the central and left portions of the molecule but significantly different in the right wing: instead of cross-peaks, 18-NH/21-OH and 24-NH/21-OH, in **1**, cross-peaks, 18-NH/H-29 and 24-NH/H-29 were observed in **2**, indicating that the shapes of the right macrocyclic rings were different for the two compounds. In order to determine the absolute stereochemistry of the 1,5-diamino-1,5-dideoxyarabitol portion, it was isolated from the acid hydrolysate of **2** and converted to the *N*,*N*′-bis-(*S*)-MTPA amide derivative as described above. The ^{1}H NMR spectrum of the bisamide was identical with the one prepared from **1**.[20] Therefore, the absolute stereochemistry of the triol portion in **2** is identical with that in **1**, confirming that **2** was the 21-*epi*-**1**, that is to say, differing from **1** in the orientation of the 1,5-diamino-1,5-dideoxyarabitol portion. Therefore, stereochemistry of pseudoceratin B (**2**) is 20*R*,21*R*,22*R*,28*S**,29*S**,30*S**,31*S**.

TABLE 2. Growth Inhibitory Activities of Pseudoceratin A (1) and Pseudoceratin B (2)

	pseudoceratin A (**1**)		pseudoceratin B (**2**)	
strain	10 μg/disk	50 μg/disk	10 μg/disk	50 μg/disk
Saccharomyces cerevisiae (W303-1B, wild type)	inactive	inactive	inactive	inactive
S. cerevisiae (YAT2285, *erg6* mutant)	6.5[a]	7.0	6.5	6.5
Penicillium chrysogenum	inactive	inactive	inactive	inactive
Mortierella ramanniana	inactive	inactive	inactive	inactive
Candida albicans	8.0	11.0	6.5	9.0
Escherichia coli	7.0	8.5	8.0	12.0
Bacillus subtilis	7.0	8.5	8.0	12.5
Staphylococcus aureus	6.5	7.5	7.0	10.0

[a] Diameter of inhibitory zone in mm.

Pseudoceratin A (**1**) and B (**2**) showed growth inhibitory activity against the *erg6* mutant of *S. cerevisiae*, but they did not show the activity against the wild type of *S. cerevisiae*. Interestingly, pseudoceratins exhibited potent antifungal activity against *Candida albicans*, but did not show activity against other fungi such as *Penicillium chrysogenum* and *Mortierella ramanniana*. Additionally, pseudoceratins showed moderate antibacterial activity against both Gram-positive and Gram-negative bacteria (Table 2).

Many bromotyrosine-derived metabolites with diverse structural features have been isolated from marine sponges.[5] They

(16) ^{1}H,^{1}H-coupling constants for the H_2-19−H_2-23 portion were determined by measuring the ^{1}H NMR spectrum in DMSO-d_6−CD_3OD (95:5); the ^{1}H signals were broad in DMSO-d_6 due to coupling with exchangeable protons.

(17) Glaçon, V.; Benazza, M.; Anzi, A. E.; Beaupère, D.; Demailly, G. *J. Carbohydr. Chem.* **2004**, *23*, 95−110.

(18) Glaçon, V.; Meslouti, A. E.; Uzan, R.; Demailly, G.; Beaupère, D. *Tetrahedron Lett.* **1996**, *37*, 3683−3686.

(19) Pseudoceratin B (**2**) was contaminated by 10% of inseparable related compound, which has not been characterized.

(20) Even though the purity of the compound **7** prepared from **2** was not high, the chemical shift and shape of diagnostic signals, especially the one at δ 3.26, matched with those of **5**.

(21) Another difference in the NMR parameter was the non-equivalence of H_2-15, which were coupled to each other by 15.2 Hz. This value was significantly different from those of H_2-1 and H_2-27, indicating the differences in the local environment of this particular methylene carbon from the others.[21] The corresponding value of 19.7 Hz for the methylene protons adjacent to the oxime carbon of trichodermamides[13] is noteworthy.

(22) Contreras, R. H.; Peralta, J. E. *Prog. Nucl. Magn. Reson. Spectrosc.* **2000**, *37*, 321−425.

can be classified according to the mode of peptide bond formation within the molecules, i.e., monomeric, dimeric, or trimeric, the last of which contain two bromotyrosine-derived units linked through a diamine, e.g., aerothionin.[23,24] The peptide-like units can be further modified by ether linkages to other unit(s). The enormous variety of this class of metabolites is generated by the different mode of modifications in the aromatic ring, the presence or absence of cyclization in the oxime group, the incorporation of a variety of amino acid precursors through amide bonds, and the combination of a variety of structural units.[5] Pseudoceratins can be considered as modifications of aerothionin,[24] because two bromotyrosine-derived units (C-9 to C-17 and C-25 to C-33) are connected through a diamine (C-19 to C-23); two of the indigenous aromatic rings are further connected to a bromotyrosine-derived dipeptide (C-1 to C-7 and C-34 to C-39). Pseudoceratins are the first examples of aerothionin-type of molecules to be further extended.[5] The mode of substitution in the pentasubstituted benzene ring (C-9 to C-14) is unprecedented among sponge metabolites[8] and the spiroketal ring formation between the cyclohexenone and the catechol, both of which are apparently derived from bromotyrosine, is extraordinary.[25] Additionally pseudoceratins are the first sponge metabolites that contain an oxime-ether group except for the commonly found spiro-isoxazoline derivatives.[5] The 1,5-diamino-1,5-dideoxyarabitol tether is first observed in natural products.[26]

Experimental Section

Extraction and Isolation. The sponge was collected at a depth of 150 m at Oshima-shinsone (28° 52′ N; 129° 33′ E) and identified as *Pseudoceratina purpurea*. A voucher specimen was deposited at the Zoologisch Museum, University of Amsterdam, with a reference number of ZMAPOR19094. The specimen was kept frozen at −20 °C until extraction. The sponge (1 kg) was homogenized and extracted with MeOH (4 × 3 L). The combined extracts were evaporated and partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ layer was partitioned between *n*-hexane and MeOH/H_2O (9:1). The MeOH concentration of the latter layer was adjusted to 60% by dilution with H_2O and extracted with $CHCl_3$. The $CHCl_3$ layer was subjected to ODS flash chromatography using a stepwise elution of mixtures of MeOH/H_2O, $CHCl_3$/MeOH (1:1), and $CHCl_3$/MeOH/H_2O (6:4:1). The fraction eluted with MeOH/H_2O (6:4) was separated by ODS HPLC (1.0 × 25 cm) with MeOH/H_2O (6:4), followed by ODS HPLC with MeCN/H_2O/TFA (40:60:0.01) to yield pseudoceratin A (**1**; 26 mg) and pseudoceratin B (**2**; 57 mg).

Pseudoceratin A (1): white solid; $[\alpha]^{28}_D$ +11.7 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 207 (5.64); IR (KBr) ν_{max} 3412, 1656, 1560, 1484, 1257, 1127, 1024 cm^{-1}; ^1H and ^{13}C NMR data in DMSO-d_6, see Table 1; HRESIMS *m*/*z* 1086.8896 $(M + H)^+$ ($C_{35}H_{37}{}^{79}Br^{81}Br_3N_6O_{14}$, calcd 1086.9040).

Pseudoceratin B (2): white solid; $[\alpha]^{28}_D$ −11.6 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 207 (5.64); IR (KBr) ν_{max} 3421, 1734, 1627, 1458, 1275, 1123, 1025 cm^{-1}; ^1H and ^{13}C NMR data in DMSO-d_6, see Table 1; HRESIMS *m*/*z* 1106.8798 $(M + Na)^+$ ($C_{35}H_{36}{}^{79}Br_2{}^{81}Br_2N_6O_{14}Na$, calcd 1106.8880).

(2*S*,4*S*)-1,5-Diaminopentane-2,3,4-triol (3).[17,18] To a solution of L-arabitol (153 mg) in THF (2 mL) was added a 0.75 M solution of diimidazolethionyl (4 mL) at −20 °C, and the mixture was stirred for 30 min at −20 °C. The solution was evaporated, diluted with water, and extracted with CH_2Cl_2. The organic phase was evaporated to afford di-*O*-sulfinyl-D-arabitol (100 mg).[17] NaN_3 (300 mg) was added to a solution of di-*O*-sulfinyl-D-arabitol (50 mg) in DMF (1 mL) and stirred at 80 °C for 16 h. The mixture was centrifuged, and the supernatant was evaporated and subjected to silica gel column chromatography to afford (2*S*,4*S*)-1,5-diazidopentane-2,3,4-triol[17] (13 mg). To a solution of (2*S*,4*S*)-1,5-diazidopentane-2,3,4-triol (13 mg) and ammonium formate (16 mg) in MeOH (1 mL) was added 10% Pd−C (18 mg), and the mixture was stirred at 60 °C for 5 min. The reaction mixture was filtered through Celite and evaporated. The residue was redissolved in H_2O (1 mL) and passed through a column of Dowex 1 × 8 (100−200 mesh, OH^- form). The unadsorbed material was collected to afford **3** (6 mg):[18] ^1H NMR (300 MHz, D_2O) δ 3.05 (dd, J = 9, 13 Hz), 3.18 (2H, d, J = 6 Hz), 3.42 (dd, J = 3, 13 Hz), 3.54 (d, J = 8 Hz), 3.97 (dt, J = 3, 7 Hz), 4.18 (t, J = 7 Hz).

Preparation of Bis-MTPA Amide Derivatives. (*R*)-MTPA chloride (7 μL) was added to a solution of **3** (1 mg) in pyridine (50 μL), and the solution was kept at rt for 10 min. The mixture was diluted with 1 M $NaHCO_3$ and extracted with EtOAc. The EtOAc extract was dried over $MgSO_4$ and passed through a silica gel column with $CHCl_3$−MeOH (95:5). The eluent was collected and evaporated. To a solution of the residue in MeOH (1 mL) was added K_2CO_3 (5 mg) and the mixture stirred at rt for 1 h. The residue was evaporated and purified by silica gel column chromatography eluting with mixtures of $CHCl_3$ and MeOH. The fractions eluted with $CHCl_3$−MeOH (95:5) afforded bis-(*S*)-MTPA-amide (**4**): ^1H NMR (500 MHz, CD_3OD) δ 3.22 (dd, J = 1.8 ,8.6 Hz; H-3), 3.35 (dd, J = 7.3, 14 Hz; H-1a), 3.37 (dd, J = 6.7, 13.7 Hz; H-5a), 3.45 (dd, J = 5.6, 14 Hz; H-1b), 3.64 (dd, J = 3.7, 13.7 Hz; H-5b), 3.75 (ddd, 3.7, 6.7, 8.6 Hz; H-4), 3.98 (ddd, J = 1.8, 5.6, 7.3 Hz; H-2). Bis-(*R*)-MTPA amide (**5**) was prepared in the same manner. **5**: ^1H NMR (500 MHz, CD_3OD) δ 3.26 (dd, J = 1.8 ,8.6 Hz; H-3), 3.39 (dd, J = 7.3, 13.7 Hz; H-1a), 3.39 (dd, J = 7.0, 13.7 Hz; H-5a), 3.46 (dd, J = 5.5, 13.7 Hz; H-1b), 3.62 (dd, J = 3.7, 13.7 Hz; H-5b), 3.74 (ddd, 3.7, 7.0, 8.6 Hz; H-4), 3.96 (ddd, J = 1.8, 5.5, 7.3 Hz; H-2).

Acid Hydrolysis of 1 and 2 and Preparation of Bis-MTPA Amide Derivatives. Compound **1** (0.5 mg) was dissolved in 0.5 mL of 6 N HCl containing 10 mM HCO_2H and kept at 110 °C for 4 h. The reaction mixture was evaporated and partitioned between H_2O and EtOAc. The ^1H NMR spectrum of the aqueous phase demonstrated that its major constituent was **3** or its enantiomer. Compound **2** was treated in the same manner to afford the aqueous fraction that gave an essentially identical ^1H NMR spectrum with the corresponding fraction prepared from **1**. These aqueous fractions were separately converted to the bis-(*S*)-MTPA amide derivative **7** whose ^1H NMR spectra were both indistinguishable from that of **5**.

Antimicrobial Assay. The antimicrobial activity was determined by the paper disk method. YAPD agar, composed of yeast extract (10 g/L), peptone (20 g/L), glucose (20 g/L), agar (20 g/L), and adenine (0.4 g/L), was used as the medium for growing *S. cerevisiae* W303-1B (wild type) and *S. cerevisiae* YAT2285 (*erg6*). YD agar, composed of yeast extract (2 g/L), glucose (10 g/L), and agar (20 g/L), was used for growing *P. chrysogenum*, *M. ramanniana*, and *C. albicans*. Nutrient broth agar was used for *E. coli*, *B. subtilis*, and *S. aureus*. Each sample was applied to a paper disk (6 mm) at doses of 10 μg/disk and 50 μg/disk, and the paper disks were air-dried. The disks were placed on the surface of agar plates seeded

(23) An exception to this generalization has recently been reported. Itampolins are biogentically considered as derived from homodetic tripeptides: Sorek, H.; Rudi, A.; Aknin, M.; Gaydou, E.; Kashman, Y. *Tetrahedron Lett.* **2006**, *47*, 7237−7239.

(24) Moody, K.; Thomson, R. H.; Fattorusso, E.; Minale, L.; Sodano, G. *J. Chem. Soc. Perkin Trans. 1*. **1972**, 18−24.

(25) Several dimeric diterpenes from the plants of the genus *Plectranthus* (Matloubi-Moghadam, F.; Rüedi, P.; Eugster, C. H. *Helv. Chim. Acta* **1987**, *70*, 975−983 and references cited therein) and dimers of triterpene and tetraterpene from the green alga *Botryococcus braunii* (Okada, S.; Tonegawa, I.; Matsuda, H.; Murakami, M.; Yamaguchi, K. *Phytochemistry* **1998**, *47*, 1111−1115) connected through a intermolecular spiroacetal ring have been reported.

(26) Peptides tethered by the related diamines have been synthesized: Zajaczkowski, I.; Stepinski, J.; Temeriusz, A.; Tam, S. W. *Z. Naturforsch. B: Chem. Sci.* **1995**, *50*, 1329−1334.

with one of the microbial strains. Growth inhibition zones were measured after 24 and 48 h of incubation at 26 °C.

Acknowledgment. We dedicate this paper to the memory of Takahiro Yamashita. We thank the crew of R/V Toyoshio-maru for assistance in collecting the sponge and H. Kobayashi for the measurement of HRESIMS. This work was partially supported by Grants-in-Aid from MEXT (Area B 10460091 and Priority Area 16073207).

Supporting Information Available: 1D and 2D NMR spectra of **1** and **2** and the ^{1}H NMR spectra of the bis-MTPA amides. This material is available free of charge via the Internet at http://pubs.acs.org.

JO062010+