Stereostructures of Geodiamolides A and B, Novel Cyclodepsipeptides from the Marine Sponge *Geodia sp.*

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Two novel cyclodepsipeptides, geodiamolides A (1) and B (2), were isolated from the marine sponge *Geodia sp.* and their stereostructures determined by X-ray crystallography. The geodiamolides contain a tripeptide unit of two (*S*)-alanines and a (*R*)-3-halotyrosine joined to a polypropionate unit in an 18-membered ring; the halogens are iodine and bromine in 1 and 2, respectively. 1 and 2 showed some activity against the fungus *Candida albicans*.

Although members of the genus *Geodia* (Class Demospongiae, Family Geodidae) have provided important insight into the mechanism of cell aggregation in the sponges (Porifera),[1] their secondary metabolites have received limited attention.[2] Previous studies on *Geodia* have yielded inositol, taurine, taurobetaine, agmatine, histamine, and herbipoline from *G. gigas*,[3] a series of sterols from *G. megastrella*,[4] and the polypeptides geodiastatins-1 and -2 from *G. mesotriaena*.[5] Polypeptides have also been isolated from other sponges[2c] including *Tedania ignis*,[6] *Cliona cilata*,[7] and *Discodermia kiiensis*.[8]

In this article we report the isolation and structural elucidation of two novel cyclodepsipeptides, geodiamolides A (1) and B (2), whose structures suggest a mixed peptide–polypropionate biogenesis. As will be apparent from the sequel, the geodiamolides are composed of a tripeptide unit (two (*S*)-alanines and a (*R*)-3-halotyrosine) joined to a 11-carbon polypropionate unit in an 18-membered ring. The geodiamolides were isolated from an acetone extract of the marine sponge *Geodia sp.* collected at Rusts Bay, Trinidad, and were obtained as stable, crystalline substances after purification by reversed-phase high-pressure liquid chromatography.

Geodiamolide A (1), $C_{28}H_{40}IN_3O_6$, mp 217–218 °C, had IR ($CHCl_3$, cm^{-1}) absorptions indicative of lactone (1725 cm^{-1}) and amide (1670, 1655, 1630 cm^{-1}) groups; these were confirmed by ^{13}C NMR singlets at 168.8, 170.9, 175.14, and 174.7 ppm (see Table I). Acid hydrolysis (6 N HCl, 120 °C/20 h), followed by treatment with trifluoroacetic acid and diazomethane and analysis of the product by gas liquid chromatography, revealed the presence of alanine and another amino acid. That there were two alanine residues present was evident from the ^1H NMR spectrum (see Table II) using decoupling techniques. Thus, irradiation of a methine signal at δ 4.46 (dq, J = 8, 7 Hz) led to the collapse of an NH doublet at δ 6.59 (J = 8 Hz) and a methyl doublet at δ 1.34 (J = 7 Hz), while irradiation of a second methine resonance at δ 4.75 (dq, J = 8, 6.5 Hz) resulted in the collapse of the other amide doublet at δ 6.52 (J = 8 Hz) and a methyl doublet at δ 1.02 (J = 6.5 Hz). The third amino acid residue was deduced to be 3-iodotyrosine on the basis of UV absorptions at 219, 284, and 292 nm (ϵ 13 800, 3200, 3000) and by ^1H and ^{13}C NMR spectroscopy and mass spectrometry. One proton signals in the ^1H NMR spectrum at δ 6.87 (d, J = 9.0 Hz), 7.05 (dd, J = 9.0, 2.0 Hz), and 7.29 (d, J = 2.0 Hz) revealed the presence of a 1,2,4-trisubstituted aromatic system; while a methine resonance at δ 5.21 (dd, J = 9.0, 8.0 Hz) and two

Table I. ^{13}C NMR Data[a]

C	1	2
1	168.8 (s)	168.7 (s)
2	49.0 (d)	49.0 (d)
3	175.1 (s)	175.1 (s)
4	56.7 (d)	56.7 (d)
5	174.4 (s)	174.4 (s)
6	45.9 (d)	45.8 (d)
7	170.9 (s)	170.7 (s)
8	42.4 (d)	42.4 (d)
9	43.3 (t)	43.4 (t)
10	129.6 (s)	129.9 (s)
11	131.6 (d)	131.7 (d)
12	29.0 (d)	29.0 (d)
13	43.7 (t)	43.8 (t)
14	71.0 (d)	71.0 (d)
15	18.7 (q)	18.8 (q)
16	30.7 (q)	30.6 (q)
17	18.8 (q)	18.8 (q)
18	20.4 (q)	20.4 (q)
19	17.7 (q)	17.7 (q)
20	18.2 (q)	18.2 (q)
21	20.6 (q)	20.5 (q)
22	32.7 (t)	32.5 (t)
23	133.0 (s)	133.1 (s)
24	132.2 (d)	132.1 (d)
25	85.1 (s)	110.0 (s)
26	154.5 (s)	151.4 (s)
27	116.1 (d)	116.0 (d)
28	129.4 (d)	129.5 (d)

[a] Determined at 50.4 MHz in $CDCl_3$. Chemical shifts are in parts per million with Me_4Si as an internal standard. [b] Assignments are based on chemical shifts, off-resonance, and DEPT[12] spectra and are tentative.

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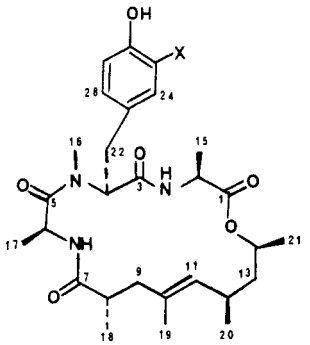
Table II. ^{1}H NMR Data[a]

^{1}H	1	2
CH-2	4.75 (dq, J = 8, 6.5)	4.73 (dq, J = 8, 6.5)
NH	6.52 (d, J = 8)	6.50 (d, J = 8)
CH-4	5.21 (dd, J = 9, 8)	5.20 (dd, J = 9, 8)
CH-6	4.46 (dq, J = 8, 7)	4.47 (dq, J = 8, 7)
NH	6.59 (d, J = 8)	6.59 (d, J = 8)
CH-8	2.32 (m)	2.32 (m)
CH_2-9	2.04 (dd, J = 15, 4)	2.03 (dd, J = 14, 4)
	2.16 (d, J = 15)	2.16 (d, J = 14)
CH-11	4.93 (d, J = 8)	4.94 (d, J = 8)
CH-12	2.16 (m)	2.16 (m)
CH_2-13	1.34 (m)	1.35 (m)
	1.59 (m)	1.60 (m)
CH-14	4.91 (m)	4.88 (m)
CH_3-15	1.02 (d, J = 6.5)	1.02 (d, J = 6.5)
CH_3-16	2.97 (s)	2.96 (s)
CH_3-17	1.34 (d, J = 7)	1.33 (d, J = 7)
CH_3-18	1.14 (d, J = 6.5)	1.13 (d, J = 6.5)
CH_3-19	1.49 (s)	1.49 (s)
CH_3-20	0.86 (d, J = 6.5)	0.84 (d, J = 6.5)
CH_3-21	1.24 (d, J = 6.5)	1.22 (d, J = 6)
CH_2-22	2.95 (dd, J = 15, 9)	2.92 (dd, J = 15, 9)
	3.15 (dd, J = 15, 8)	3.18 (dd, J = 15, 8)
CH-24	7.29 (d, J = 2)	7.30 (d, J = 2.0)
CH-27	6.87 (d, J = 9)	6.95 (d, J = 9)
CH-28	7.05 (dd, J = 9, 2)	7.07 (dd, J = 9, 2)
OH	6.27 (s)	6.29 (s)

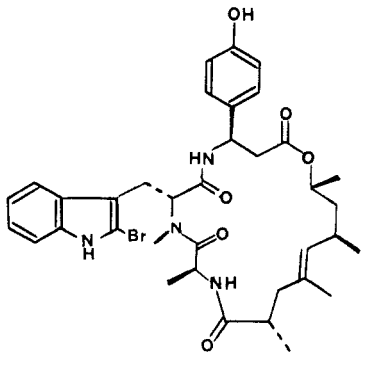
[a] Determined in $CDCl_3$ at 200 MHz. Chemical shifts are given in δ values relative to Me_3Si and coupling constants J are given in Hz with s = singlet, d = doublet, t = triplet, q = quartet, and m = multiplet.

geminal protons with signals at δ 2.95 (dd, J = 15.0, 9.0 Hz) and 3.15 (dd, J = 15.0, 8.0 Hz) were assigned to a β-substituted alanine residue.



1, X=I

2, X=Br



3

The 11-carbon polypropionate residue in 1 was identified primarily on the basis of NMR spectroscopy. Thus ^{1}H NMR signals at δ 0.86 (3 H, d, J = 6 Hz), 1.14 (3 H, d, J = 7 Hz), and 1.24 (3 H, d, J = 6 Hz) are ascribed to three secondary methyl groups, while absorptions at δ 1.49 (3 H, br s) and 4.93 are assigned to a vinylic methyl and an olefinic proton, respectively. Irradiation of the peak at δ 4.93 led to a sharpening of the absorption at δ 1.49. This result, taken in conjunction with ^{13}C NMR absorptions at 129.6 (s) and 131.6 (d) ppm, confirmed the presence of a trisubstituted double bond in geodiamolide A. In addition, absorption due to two methylene groups were discernible at 43.3 (t) and 43.7 (t) ppm (C-9 and C-13, respectively) in the ^{13}C NMR spectrum of geodiamolide A.

The preceding evidence did not lead to an unambiguous structural formulation for geodiamolide A; consequently an X-ray crystallographic analysis of this substance was undertaken. Pertinent X-ray crystallographic data are summarized in Table III, and a perspective drawing of 1 as obtained from the analysis is displayed in Figure 1. This drawing also represents the absolute stereochemistry of 1 as determined from the X-ray crystallographic analysis. The chiral centers in 1 are defined as 2*S*,4*R*,6*S*,8*S*,12*R*,14*S*. Of particular interest is the *R* configuration for 3-iodotyrosine, which to the best of our knowledge is unique; indeed, this is probably the first reported natural occurrence of 3-iodotyrosine. The double bond in 1 has the *E* configuration.

Geodiamolide B (2), $C_{28}H_{40}BrN_3O_6$, mp 203–204 °C, had spectral properties virtually identical with those of 1 except for the difference in the ^{13}C chemical shift of C-25, which appeared at 85.1 ppm in 1 but at 110.0 ppm in 2. In view of the unusual *R* configuration found for 3-iodotyrosine in geodiamolide A, we decided to determine the absolute stereochemistry of geodiamolide B by X-ray crystallographic analysis. The result of such an analysis (see Table III) revealed that geodiamolide B has the same absolute stereochemistry as 1, with the presence of the unique (*R*)-3-bromotyrosine.

The geodiamolides join jaspamide[9] (jasplakinolide,[10] 3), recently isolated from *Jaspis sp*, as the only depsipeptides to be isolated from sponges. A curious structural feature of these compounds, which may be of some taxonomic significance, is the presence of the same 11-carbon polypropionate unit. As in the case of jaspamide, the geodiamolides were devoid of activity against a variety of gram-positive and gram-negative bacteria but were active against the fungus *Candida albicans*.[11] Whether or not 1–3 are metabolites of the sponges or of microorganisms inhabiting the sponges has yet to be determined.

Experimental Section

General Procedure. Melting points were determined in capillaries on a Thomas-Hoover melting point apparatus and are uncorrected. Unless otherwise indicated, infrared (IR) and nuclear magnetic resonance spectra (NMR) were determined in $CHCl_3$ and $CDCl_3$, respectively, and ^{1}H and ^{13}C NMR spectra were recorded at 200 and 50.4 MHz, respectively. Chemical shifts are expressed in parts per million (ppm) relative to tetramethylsilane,

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Table III. X-ray Crystallographic Data and Experimental Details

	1	2
formula	$C_{28}H_{40}IN_3O_6$ (641.55)	$C_{28}H_{40}BrN_3O_6$ (594.54)
space group	$P2_1$	$P2_1$
a, Å	9.001 (4)	9.656 (1)
b, Å	17.139 (4)	17.118 (2)
c, Å	9.699 (2)	9.007 (2)
β, deg	95.16 (3)	95.37 (1)
unit-cell volume, Å^3	1490.1	1482.3
Z	2	2
d_{calcd}, g cm^{-3}	1.430	1.332
μ(Cu Kα), cm^{-1}	88.70	24.5
crystal size, mm	0.13 × 0.20 × 0.90	0.20 × 0.30 × 0.55
max θ, deg	75	70
no. of reflections	3178	2843
no. of obsd reflections	2897 [$I > 6.0\sigma(I)$]	2495 [$I > 2.5\sigma(I)$]
absorption correction	yes	yes
least-square refinement	full matrix	full matrix
heavier atoms	anisotropic	anisotropic
hydrogen atoms	iso (fixed)	iso (fixed)
final R	0.0416	0.0430
final R for enantiomer	0.0632	0.0477
final R_w	0.0516	0.0531
final R_w for enantiomer	0.0908	0.0578
final difference map largest peak, e Å^{-3}	<1.0	<0.6

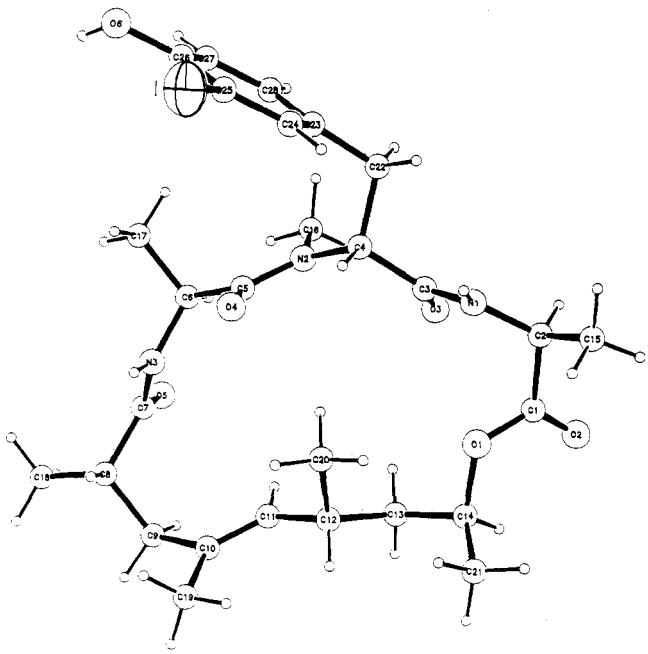

Figure 1. An ORTEP perspective drawing of geodiamolide A (1, absolute stereochemistry).

and coupling constants (J) are expressed in hertz (s = singlet, d = doublet, t = triplet, m = multiplet). Mass spectra (MS) were determined with a direct inlet system with ionization energy of 70 eV; m/z values are given with relative intensities in parentheses. Thin-layer chromatograms (TLC) were prepared from Merck (Darmstadt) silica gel G; spots were visible under short wavelength UV light or made visible by spraying with 10% phosphomolybdic acid in ethanol and heating the plates to 100 °C.

Isolation of Geodiamolides A (1) and B (2). *Geodia sp.* (8 kg, dry weight) was collected at a depth of ca. 25 m, at Rusts Bay, Trinidad and Tobago (West Indies), stored in acetone, and then blended. The acetone extract (20 L) was concentrated to ca. 6 L and was extracted, in portions, with ethyl acetate (3 × 200 mL). Evaporation gave a dark brown oil (11 g, 0.14%), which was dissolved in 10% aqueous methanol (150 mL) and extracted with light petroleum (4 × 150 mL) to remove lipids. The aqueous methanol phase was diluted with water (200 mL), extracted with ethyl acetate (3 × 200 mL), and evaporated to give a brown gum (3.0 g). The latter was applied to a short column of Florisil (100–200 mesh), eluted with a 50:45:05 mixture of light petroleum–ethyl acetate–methanol, and evaporated to give a gum (1.5 g). Preparative-scale HPLC on a C_{18} reversed-phase column (4.6 × 200 mm) with aqueous methanol (35:65) as eluant at a flow rate of 3 mL/min) gave geodiamolide A (152 mg, t_R 17.4 min) and geodiamolide B (93 mg, t_R 15.2 min). Geodiamolide A (1) crystallized from CH_3CN–CH_2Cl_2 as colorless prisms: mp 217–218 °C; $[\alpha]^{25}_D$ + 53° (c 0.04, $CHCl_3$); UV λ_{max} (CH_3OH), 219, 284, and 292 nm (ϵ 13 800, 3200, and 3000, respectively); IR ν_{max} ($CHCl_3$) 3420, 1725, 1670, 1655, and 1630 cm^{-1}; ^{13}C and ^1H NMR spectra, see Tables I and II; MS (FAB), m/z 642 (MH^+, 100), 516 (50), 393 (15), 276 (30), 217 (50), 150 (50). Anal. Calcd for $C_{28}H_{40}IN_3O_6$: C, 52.42; H, 6.28; I, 19.18; N, 6.55. Found: C, 52.01; H, 5.98; I, 19.62; N, 6.45.

Geodiamolide B (2) Crystallized from CH_3CN–CH_2Cl_2 as colorless crystals: mp 203–204 °C; $[\alpha]^{22}_D$ +101° (c 0.04, $CHCl_3$); UV λ_{max} (CH_3OH) 214, 281, and 290 nm (ϵ 15 400, 2700, and 2500, respectively); IR ν_{max} ($CHCl_3$) 3510, 3410, 1725, 1670, 1655 (sh), and 1630 cm^{-1}; ^{13}C and ^1H NMR, see Tables I and II; MS (FAB), m/z 594 (MH^+, 95), 516 (60), 345 (30), 267 (30), 228 (80), 150 (100). Anal. Calcd for $C_{28}H_{40}BrN_3O_6$: C, 56.57; H, 6.78; Br, 13.44; N, 7.07. Found: C, 56.05; H, 6.83; Br, 13.40; N, 6.99.

Acid Hydrolysis of Geodiamolides A (1) and B (2). Samples of 1 (1 mg) and 2 (1 mg) were hydrolyzed separately with 6 N hydrochloric acid in sealed, evacuated tubes at 110 °C for 20 h. The hydrolysates were treated with ethereal diazomethane followed by trifluoroacetic acid (ca. 1 mL) and trifluoroacetic anydride (ca. 1 mL). The mixtures were evaporated, and the residues were dissolved in acetone and analyzed by GLC (60–65 °C). This revealed the presence of alanine and another amino acid. Examination of the hydrolysates by paper chromatography [Whatman No. 1; n-BuOH/CH_3CO_2H/H_2O (13:4:5), ascending] revealed two ninhydrin-positive spots in each hydrolysate, one of which was identified as alanine (R_f 0.42) by comparison with an authentic sample. The other spot in the geodiamolide A hydrolysate had R_f 0.58, while that from geodiamolide B had R_f 0.60.

X-ray Crystallography. Crystallographic data for 1 and 2 were collected on a Enraf-Nonius CAD4 diffractometer (graphite-monochromated Cu Kα radiation, ω–2θ scans) and on a Hilger-Watts diffractometer (Ni-filtered, Cu Kα radiation, ω–2θ scans) and are summarized in Table III. The structures were solved by a multiple-solution procedure[13] and were refined by full-matrix least squares. In the final refinements, anisotropic thermal parameters were used for non-hydrogen atoms and isotropic temperature factors were used for hydrogen atoms. Hydrogen atoms were included in the structure-factor calculations but their parameters were not refined. The major peaks of the final difference maps are near the halogen atoms. The absolute configuration of 1 and 2 are based on the anomalous scattering of iodine and bromine, respectively. Both structures were found to have the same configuration. The final weighted R values (see Table III) are lower for the stereochemistry indicated than for those of the corresponding antipodes, in accordance with Hamilton's test.[14]

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Supplementary Material Available: Listings of final atomic parameters, final anisotropic thermal parameters, bond lengths, bond angles, and torsion angles (Tables IV, V, VI, VII, and VIII, respectively) for 1 and (Tables IX, X, XI, XII, and XIII) for 2 (12 pages). Ordering information is given on any current masthead page.

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