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Geodiamolides H and I, Further Cyclodepsipeptides from the Marine Sponge *Geodia* sp.

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Abstract: Two new cyclodepsipeptides, geodiamolide H (**3**) and I (**4**), were isolated from the marine sponge *Geodia* sp. The structures of **3** and **4** were determined by 2D NMR spectroscopy while the absolute stereochemistry of **4** was established by X-ray crystallography. Geodiamolide H **3** was cytotoxic to some human cancer cell lines.

Marine sponges have proven to be a rich source of unusual cyclodepsipeptides that possess a common twelve carbon polypropionate hydroxy acid linked to three variable amino acids.[1] The first example of these compounds was jaspamide/jaspaklinolide isolated from Fijian and Palauan *Jaspis* sp. and shown to have antifungal, insecticidal and antihelminthic bioactivity.[2,3] Jaspamide was subsequently isolated from *Auletta* cf. *constricta* collected in Papua New Guinea.[4] Two closely related cyclodepsipeptides, geodiamolides A (**1**) and B (**2**) were isolated from Caribbean *Geodia* sp. and were active against the fungus *Candida albicans*,[5,6] while geodiamolides A to F were isolated from a Papua New Guinean sponge of the genus *Pseudaxinyssa*.[7] More recently, geodiamolide TA was isolated from South African *Hemiasterella minor*,[8] neosiphoniamolide from a New Caledonian *Neosiphonia superstes*[9] and geodiamolide G the first such cyclodepsipeptide containing a modified polypropionate from Papua New Guinean *Cymbastela* sp.[10]

Neosiphoniamolide was shown to possess antifungal activity[10] and jaspamide and the geodiamolides exhibited cytotoxic activity against a number of human cancer cell lines.[4,7-9,11] The potent biological activities displayed by these cyclodepsipeptides have led to a considerable amount of synthetic activity, where a number of these compounds have been prepared in stereochemically pure form.[12-23] We have further investigated the sponge *Geodia* sp. from Trinidad, and report here, the isolation of (**1**) and (**2**), along with two new cyclodepsipeptides, geodiamolides H (**3**) and I (**4**).

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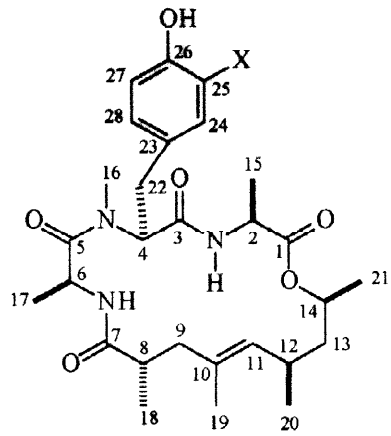
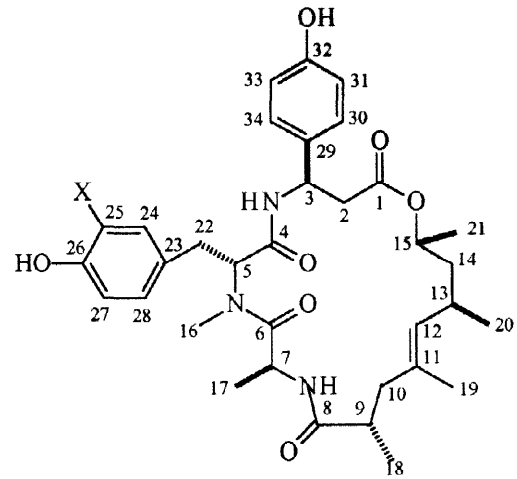


(1) X = I

(2) X = Br



(3) X = I

(4) X = Br

A detailed analysis of the NMR data of geodiamolides A (**1**) and B (**2**) using COSY, HMQC,[24] HMBC[25] and NOESY experiments, revealed that the ^{13}C assignments for C-1, C-3, C-7, C-10 and C-23 need revision. [6,7] In this regard, HMBC correlations were observed between H_3-15 and C-1 and C-2, between the H-22 protons and C-3, C-4 and C-23 and also between H_3-18 and C-7 and C-8, while the H_3-19 olefinic methyl showed correlations with C-10 and C-11. The revised ^{13}C assignments for **1** and **2** are summarized in Table 1.

Table 1. ^{13}C NMR Assignments for Geodiamolides A (**1**) and B (**2**) in $CDCl_3$ (125 MHz).[a]

C#	1	2	C#	1	2
1	171.0	170.8	15	17.6	18.2
2	48.9	49.0	16	30.4	30.5
3	169.2	168.7	17	18.1	18.7
4	56.2	56.6	18	18.5	18.6
5	173.9	174.6	19	17.1	17.6
6	45.4	45.8	20	19.9	20.4
7	174.8	175.2	21	20.4	20.6
8	41.8	42.3	22	32.7	32.6
9	43.0	43.3	23	129.1	129.9
10	132.4	133.3	24	138.4	132.1
11	131.9	131.6	25	84.1	110.1
12	28.4	28.9	26	155.1	151.4
13	43.5	43.6	27	114.8	116.2
14	70.2	71.0	28	129.6	129.6

[a]Assignments are based on COSY, HMQC and HMBC experiments.

Geodiamolide H (**3**), had the molecular formula $C_{34}H_{44}O_7N_3I$, while geodiamolide I (**4**), had the molecular formula, $C_{34}H_{44}O_7N_3Br$. Both compounds had IR absorptions due to hydroxy (3495 cm^{-1}), ester (1724 cm^{-1}) and amide (1670 cm^{-1}) functionalities. The ^{13}C and ^1H NMR chemical shifts for **3** and **4** were assigned from the usual combination of COSY, HMQC and HMBC spectra. The only unusual feature involved the assignment of the unsubstituted tyrosine residue as β-tyrosine rather than α-tyrosine. This was based on the observation that the side chain methine proton of the tyrosine residue (H-3, δ 5.25) showed HMBC cross-peaks with both the *ipso* (C-29, δ 130.8) and the *ortho* (C-30, δ 127.1) carbons of the tyrosine aromatic ring with a cross-peak also being observed between the *ortho* protons (H-30/H-34, δ 6.99) and the methine carbon (C-3, δ 48.8). By contrast, the methylene protons of the β-tyrosine residue (H-2, δ 2.59 and δ 2.68) only showed a cross-peak with the *ipso* carbon and no cross-peaks were observed between the tyrosine aromatic protons and the methylene carbon (C-2, δ 40.2). This pattern is only consistent with β-tyrosine since α-tyrosine should show cross-peaks between methylene protons/carbons and *ortho* carbons/protons, with the methine proton only showing a cross-peak to the *ipso* carbon.

Assigned ^1H and ^{13}C chemical shifts for **3** and **4**, along with measurable ^1H-^1H coupling constants are given in Table 2. With the exception of the carbons and protons of the halotyrosine ring, all other ^1H and ^{13}C chemical shifts for **3** and **4** are essentially identical. Similarly, the ^1H-^1H coupling constants for **4** are identical, within ±0.2 Hz with those for **3**. The crystal structure, including absolute stereochemistry was only determined for **4** (Fig. 1), however, the

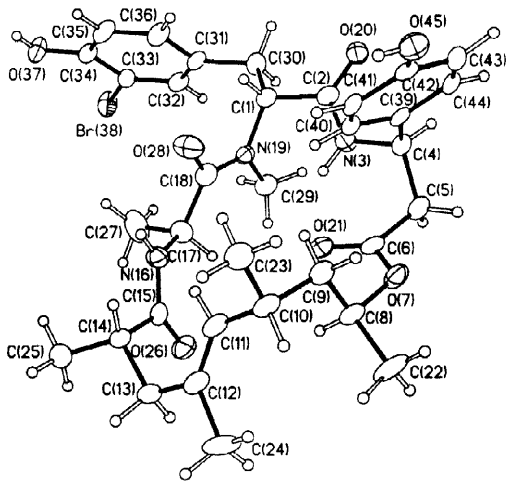


Figure 1. ORTEP Drawing of Geodiamolide I (4).

Table 2. ^{13}C and ^{1}H NMR Chemical shifts for **3** and **4** and ^{1}H-^{1}H coupling constants for **3**.

Carbon	δ_C[a] 3	δ_C[a] 4	δ_H[a] 3	δ_H[a] 4
1	170.5	170.5	----	----
2	40.2	40.0	2.68 (15.5, 4.5),[b] 2.59 (15.5, 6.6)	2.69, 2.60
3	48.8	48.8	5.25 (m)	5.24
4	168.4	168.4	----	----
5	56.8	56.7	5.38 (9.8, 6.7)	5.40
6	174.1	174.1	----	----
7	46.0	45.0	4.75 (m)[c]	4.24
8	175.0	174.9	----	----
9	40.1	40.1	2.55 (m)	2.56
10	40.9	40.9	2.36(15.2, 11.4), 1.95 (15.2, 2.0)	2.35, 1.94
11	133.6	133.6	----	----
12	128.4	128.3	4.82 (9.6)	4.82
13	29.2	29.2	2.28 (m)	2.28
14	43.4	43.4	1.39 (13.6, 11.1, 4.7), 1.18 (13.5, 9.4, 4.5)	1.38, 1.19
15	70.4	70.4	4.64 (m)	4.64
16	30.3	30.3	2.94 (s)	2.92
17	18.2	18.1	1.08 (7.0)	1.06
18	20.2	20.2	1.16 (7.0)	1.16
19	18.5	18.5	1.60 (1.0)	1.60
20	21.9	21.9	0.86 (7.0)	0.86
21	19.1	19.1	1.09 (7.0)	1.08
22	31.7	31.9	3.18 (14.5, 5.7), 2.86 (14.5, 9.9)	3.19, 2.88
23	129.6	129.2	----	----
24	138.7	132.7	7.48 (2.3)	7.26
25	84.2	109.7	----	----
26	155.2	152.6	----	----
27	115.1	116.4	6.79 (8.5)	6.85
28	129.8	128.8	6.96 (8.5, 2.3)	6.93
29	130.8	130.7	----	----
30,34	127.1	127.1	7.00 (8.5)	7.01
31,33	115.6	115.6	6.77 (8.5)	6.87
32	156.6	156.6	----	----
NH_1	----	----	7.46 (8.3)	7.47
NH_2	----	----	6.78 (8.5)	6.77
OH_1	----	----	9.12 (s)	8.85
OH_2	----	----	8.68 (s)	8.69

[a]Chemical shifts on a δ scale in $CDCl_3$ with *ca* 5% DMSO-d_6 added to promote solubility and slow exchange of NH and OH protons.

[b]Coupling constants (in Hz) in brackets.

[c](m) ≡ multiplet, (s) ≡ singlet.

identical chemical shifts and coupling constants for **3** and **4** demonstrate that they have identical stereochemistry. Furthermore, a detailed consideration of the magnitudes of vicinal ^1H-^1H couplings indicates that **3** and **4** have essentially the same solution conformation as the crystal structure for **4**. Evidence for this includes *anti* couplings between NH-1 and H-3, NH-2 and H-7 as well as between H-12 and H-13. In addition the δ 2.35 methylene proton (H-10) shows an *anti* coupling to H-9 while the other H-10 methylene proton at δ 1.94 a small coupling suggestive of a *gauche*-orientation with H-9. By contrast both H-2 methylene protons shows *gauche*-like couplings to H-3, suggested by the crystal structure. Finally, each of the H-14 methylene protons show one *gauche* and one *anti* vicinal coupling, as expected since H-13β is *anti* relative to H-12 and *gauche* with respect to H-14 in the crystal structure while H-13α is *gauche* with respect to H-12 and *anti* with respect to H-14. Presumably, the presence of three amide linkages, a *trans* double bond and the ester functionality in the macrocycle restricts its conformational mobility to the extent that only one conformation is significantly populated both in solution and the solid-state. In contrast, jaspamide/jaspaklinolide was shown to have two major conformations on the basis of molecular mechanics and dynamics calculations.[26,27]

Geodiamolide H **3** showed *in vitro* cytotoxicities, measured as Total Growth Inhibition (TGI),[28] against a number of human cancer cell lines: non small cell lung cancer, HOP 92 (1.18×10^{-7}M); cental nervous system, SF-268 (1.53×10^{-7}M); ovarian cancer, OV Car-4 (1.86×10^{-8}M); renal cancer, A498 (9.48×10^{-8}M) and UO-31 (1.85×10^{-7}M); and breast cancer MDA-MB-231/ATCC (4.33×10^{-7}M) and HS 578T (2.45×10^{-7}M). Suprisingly, geodiamolide I **4** was completely devoid of activity. Very recently four new cyclodepsipeptides, chondramides A-D were isolated as cytostatic and antifungal agents from the myxobacteria *Chondromyces crocatus*.[29] The chondramides, which represent the first examples of these compounds from a microorganism, has a modified hydroxy fatty acid in which a methylene group is absent.

EXPERIMENTAL

General Exerimental Procedures:

Melting points were determined on a Kofler hotstage and are uncorrected. IR spectra were obtained on a Nicolet 3DX FTIR spectrometer in $CHCl_3$ solutions. UV spectra were recorded on a Hewlet-Packard 8452A diode array UV spectrometer in EtOH solutions. A Perkin-Elmer 243B polarimeter was used to obtain optical rotations. NMR spectra were recorded on a Varian Unity 500 spectrometer with TMS as the internal standard. A VG-70-250S mass spectrometer was used to obtain MS data.

Extraction and Isolation:

Geodia sp. was collected at a depth of 20 m at Macqueripe Bay, Trinidad in July, 1996 and stored in acetone. The sponge (8 Kg, dried weight) was blended and soaked in acetone (32 L) and the solvent concentrated to a small volume, extracted with ethyl acetate and the solvent evaporated to give a brown oil (29.8 g). The crude extract was dissolved in 10% aqueous MeOH (200 mL) and extracted with hexane (3 x 200 mL). The aqueous MeOH fraction was diluted with water (100 mL) and extracted with ethyl acetate (3 x 200 mL). The solvent was removed from the ethyl acetate fraction to give a brown gum (7.6 g).

The ethyl acetate extract was flash chromatographed on silica gel using CH_2Cl_2/MeOH (96:4) to give six major fractions. Fraction #5 was rechromatographed on silica gel using hexane/acetone (3:2) to give two major fractions, 5A (327 mg) and 5B (584 mg). Fraction 5A was separated by reversed phase HPLC using MeOH/H_2O (65:35) to give **1** (60.7 mg) and **2** (33.6 mg). Fraction 5B was similarly separated to give **3** (16.9 mg, 2.1 x 10^{-4} %, dried weight) and **4** (20.2 mg, 2.5 x 10^{-4} %, dried weight).

Geodiamolide H **3**. Mp 186-189°C; $[\alpha]_D$ +19.1° (*c* 0.17, $CHCl_3$); UV λ_{max} 215, 280 (12,500, 2800) ; IR ν_{max} ($CHCl_3$) 3495, 1724, 1670 cm^{-1}; EIMS *m/z* 733 $[M]^+$ (48), 706 (10), 608 (15), 552 (5), 460 (54), 413 (17), 321 (24), 276 (100), 250 (19), 162 (73), 109 (46); HREIMS 733.2199 calcd for $C_{34}H_{44}O_7N_3I$ 733.2224.

Geodiamolide I **4**. Mp 168-170°C; $[\alpha]_D$ +39.3° (*c* 0.14, $CHCl_3$); UV λ_{max} 214, 280 (14,000, 3000); IR ν_{max} ($CHCl_3$) 3500, 1725, 1670 cm^{-1}; EIMS *m/z* 685 $[M]^+$ (38), 657 (10), 460 (52), 413 (24), 273 (26), 228 (100), 162 (87), 109 (59); HREIMS 685.2374 calcd for $C_{34}H_{44}O_7N_3Br$ 685.2363.

X-ray data:

Colorless crystal, monoclinic, $P2_1$, a = 10.005(2), b = 10.9523(8), c = 16.0394(11) Å, β = 100.145(6)°, V = 1730.1(4) $Å^3$, D_c = 1.318 mg m^{-3} for Z = 2. A total of 3380 reflections were measured using graphite monochromated Mo Kα radiation; 3189 unique reflections (R_{int} = 0.0208) were used in the structure solution and refinement. The structure was solved by direct methods and refined by full-matrix least-squares on F^2. Refinement including 415 parameters converged to give R = 0.0318 and wR = 0.0748. Maximum electron density in the final difference map was in the range -0.565 to 0.432 e$Å^3$. Atomic coordinates, bond lengths and angles and thermal parameters have been deposited at the Cambridge Crystallograhpic Data Centre.

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