Westiellamide, a Bistratamide-Related Cyclic Peptide from the Blue-Green Alga Westiellopsis prolifica

Michele R. Prinsep, Richard E. Moore, Ira A. Levine, and Gregory M. L. Patterson

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WESTIELLAMIDE, A BISTRATAMIDE-RELATED CYCLIC PEPTIDE FROM THE BLUE-GREEN ALGA *WESTIELLOPSIS PROLIFICA*

MICHELE R. PRINSEP, RICHARD E. MOORE,* IRA A. LEVINE, and GREGORY M.L. PATTERSON

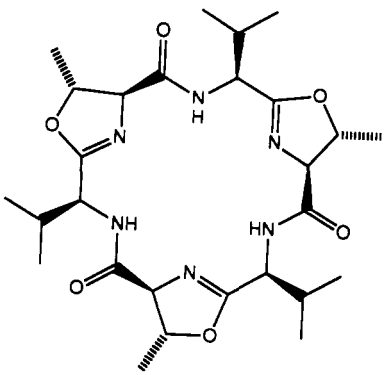
Department of Chemistry, University of Hawaii, Honolulu, Hawaii 96822

ABSTRACT.—The isolation and structural elucidation of westiellamide [**1**] from the terrestrial blue-green alga *Westiellopsis prolifica* is described. This moderately cytotoxic cyclic peptide appears to be identical with a bistratamide-type marine natural product from the aplousobranch ascidian *Lissoclinum bistratum*.

In aplousobranch ascidians belonging to the genus *Lissoclinum*, cyclic peptides such as patellamides and lissoclinamides from *Lissoclinum patella* (1) and bistratamides from *Lissoclinum bistratum* (2) appear to be located in the cells of the obligate prokaryotic algal symbionts (*Prochloron* spp.), strongly suggesting that the symbiotic prochlorophytes in the tunicates are involved in the biosynthesis of these secondary metabolites. Prochlorophytes resemble cyanophytes (blue-green algae) but differ in that they lack accessory phycobiliprotein pigments and possess both chlorophylls a and b (3). We report here the isolation and structural elucidation of westiellamide [**1**] from a terrestrial cyanophyte *Westiellopsis prolifica* Janet (Stigonematales, Stigonemataceae). Compound **1** is a moderately cytotoxic cyclic peptide which appears to be identical with a bistratamide-type compound that Hawkins *et al.* (4) have isolated from *L. bistratum* and inappropriately named trisoxazoline in a recent patent.

Trisoxazoline is a generic term that had been used in an earlier unrelated patent (5). Westiellamide was cytotoxic against KB and LoVo cell lines at 2 μg/ml, but was not solid tumor selective in the Corbett assay (6). Our finding of **1** in a cyanophyte provides additional circumstantial evidence that the *Prochloron* sp. in *L. bistratum* is responsible for the production of the bistratamides.

The blue-green alga was collected from a mud sample on the island of Oahu, Hawaii, and identified as *W. prolifica*. By repeated subculture, a clonal non-axenic culture of the cyanophyte, assigned strain number EN-3-1, was produced. Mass cultivation of the alga was carried out in a liquid medium (BG-11) using a general procedure that we have described elsewhere (7). The freeze-dried alga was extracted with 70% EtOH and the extract subjected to nor-



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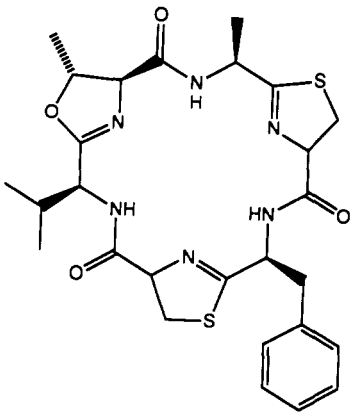

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mal and reversed-phase chromatography to give westiellamide [**1**] in 0.06% yield.

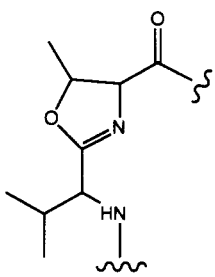
The structure of **1** was deduced by spectral analysis and chemical degradation. Hrms established the molecular formula $C_{27}H_{42}N_6O_6$. The nmr spectra, however, indicated that **1** was a trimer of a unit having the composition $C_9H_{14}N_2O_2$. Only nine signals could be observed in the ^{13}C-nmr spectrum: three methyl, four methine, and two quaternary carbon signals. The ^1H-nmr spectrum of **1** exhibited eight signals, seven of which correlated with the seven protonated-carbon signals in a HMQC experiment. The eighth signal was assigned to an exchangeable amide NH proton. ^1H-^1H COSY, and ^1H-^{13}C HMBC experiments strongly suggested that the repeating unit in **1** was a valylthreonyl unit that had lost the elements of H_2O. We considered two structures **a** and **b** for this unit, but **a** fit the data better. The ^1H and ^{13}C chemical shifts and coupling constants were very similar to those reported for the same **a** unit in bistratamide A [**2**] (2) and patellamide C (8). Furthermore, mild acid hydrolysis (2 N HCl/MeOH, 2 h reflux) led to a complex mixture of products, one of which appeared to be cyclo[Thr-Val-Thr-Val-Thr-Val], since a peak was observed at *m/z* 600 in the eims of the acid hydrolyzate. If westiellamide had been a trimer of **b**, we would have expected the imidate linkages to cleave readily on even milder acid hydrolysis to give 3-(1-hydroxyethyl)-6-isopropyl-2,5-piperazinedione as the major product. Westiellamide was therefore a trimer of **a**. Complete acid hydrolysis of westiellamide led to L-valine and L-threonine, which established the absolute stereochemistry as depicted in **1**.

EXPERIMENTAL

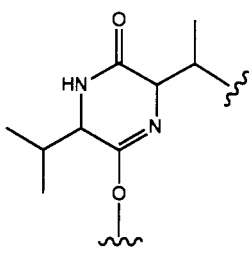
GENERAL PROCEDURES.—^1H- and ^{13}C-nmr chemical shifts are referenced to solvent peaks: δ_H 7.26 (residual $CHCl_3$) and δ_C 77.0 for $CDCl_3$. Homonuclear ^1H connectivities were determined with the COSY experiment, and heteronuclear ^1H-^{13}C connectivities were determined by HMQC (9) and HMBC (10) experiments.

CULTURE CONDITIONS.—A clonal isolate, designated UH strain EN-3-1 and identified as *W. prolifica*, was obtained from a freshwater mud sample on the island of Oahu, Hawaii. Repeated subculture on a solidified medium was used to purify the alga. Unialgal, nonaxenic cultures of EN-3-1 were grown on BG-11 medium in 20-liter glass bottles as described elsewhere (7). After 24 to 30 days, the alga was harvested by filtration and freeze-dried. Yields of lyophilized alga averaged 0.098 g/liter.

ISOLATION.—The freeze-dried alga (17.3 g) was macerated in a blender and extracted with 70% EtOH (700 ml) three times. The combined extract was filtered and the solvent removed in vacuo. The crude extract (3.5 g) was fractionated by reversed-phase flash chromatography on a column of C18 silica (YMC gel ODS 120 Å, 50 g), using a steep-stepped gradient from H_2O to MeOH to CH_2Cl_2. Repeated cc on Si gel (Davisil 200–425 mesh, 60 Å) using a hexane/EtOAc gradient, followed by further reversed-phase flash column chromatography with an H_2O/MeOH gradient, yielded pure westiellamide [**1**] (9.6 mg) as an amorphous solid: $[\alpha]_D$ +130° (MeOH, $c = 0.1$); ^1H nmr (500 MHz, $CDCl_3$) δ 7.77 (d, $J = 7.5$ Hz, N-H), 4.81 (m, $J = 8.6$, 5.9 Hz, H-3), 4.65 (dt, $J = 7.5$ and 2.7 Hz, H-6), 4.22 (dd, $J = 8.6$ and 2.2 Hz, H-2), 2.29 (m, $J = 7.0$, 6.5,



a



b

2.7 Hz, H-7), 1.58 (d, $J=5.9$ Hz, H-4), 0.89 (d, $J=7.0$ Hz, H_3-8), 0.82 (d, $J=6.5$ Hz, H_3-9); ^{13}C nmr (125 MHz, $CDCl_3$) δ 170.54 (C-1), 168.46 (C-5), 82.66 (C-3), 73.76 (C-2), 52.32 (C-6), 31.43 (C-7), 21.92 (C-4), 18.60 (C-8), 16.83 (C-9); eims *m/z* (rel. int.) $[M]^+$ 546 (43), $[M-Me]^+$ 531 (5), $[M-C_3H_7]^+$ 503 (43), $[M-C_4H_8]^+$ 490 (28), $[M-C_4H_8O-CO]^+$ 462 (41), 363 (7), 308 (12), 280 (22), 252 (10), 169 (35), 138 (50), 69 (100); hreims *m/z* 546.3182 (calcd for $C_{27}H_{42}N_6O_6$, −1.6 mmu error), 531.2959 (calcd for $C_{26}H_{39}N_6O_6$, −2.8 mmu error), 308.1615 (calcd for $C_{15}H_{22}N_3O_4$, −0.5 mmu error), 280.1663 (calcd for $C_{14}H_{22}N_3O_3$, −0.2 mmu error), 252.1711 (calcd for $C_{13}H_{22}N_3O_2$, +0.1 mmu error).

HYDROLYSIS AND AMINO ACID ANALYSIS.—Westiellamide (2 mg) in 6 N methanolic HCl was heated at 110° for 36 h. The amino acid hydrolyzate (2 mg) was heated with acetyl chloride (1.25 ml) and iPrOH (5 ml) at 100° for 45 min. The mixture was evaporated to dryness, and the residue was treated with pentafluoropropionic anhydride (2 ml) in CH_2Cl_2 (3 ml) at 100° for 15 min. After cooling, excess reagent was evaporated with a stream of N_2. The mixture of derivatized amino acids in MeOH was analyzed by gc-ms using an Alltech Chirasil-Val column (25 m × 0.25 mm) and the following conditions: column temperature 60⟶110° at 2°/min and a 12 psi head pressure (flow rate estimated to be about 0.6 ml/min). Using selective ion monitoring, the retention times for the *N*-pentafluoropropionyl isopropyl ester (PFP-IPA) derivatives of the amino acids from the westiellamide hydrolyzate were found to be 14.37 (L-valine) and 14.68 min (L-threonine). Retention times for the PFP-IPA derivatives of authentic amino acids were found to be 12.85 (D-valine), 13.52 (D-threonine), 14.27 (L-valine), 14.52 (L-threonine), 19.77 (D-allo-threonine), and 21.13 min (L-allo-threonine).

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NOTE ADDED IN PROOF: The Hawkins group has renamed **1** from *L. bistratum* cycloxazoline. T.W. Hambley, C.J. Hawkins, M.F. Lavin, A. van der Brenk, and D.J. Watters, *Tetrahedron*, in press.