activity to the 330-nm T3 transition. Further, the EPR spectrum of this trimer would not resemble that of mononuclear copper(II). Trimer g values result from the projection of the individual copper g tensors onto the trimer coordinate system with the spin on the central copper opposing the other two spins (see Figure 15 of ref 1c). Noncolinear g tensors would give g values less than 2.0 and provide an explanation of the helium temperature EPR signal. Structure A, with no interaction between the $O^{\bullet-}$ and a reduced T2 copper, would have the oxygen radical directly overlapping with the T3 Cu_α^{2+}, resulting in strong antiferromagnetic coupling between their spins. The magnetic model for such a trimer ($J_{O3\alpha} \gg J_{3\alpha 3\beta}$, where $J_{O3\alpha}$ is the $O^{\bullet-}$–T3 Cu_α^{2+} coupling; ref 1c) would have the spin localized predominantly on the T3 Cu_β^{2+} resulting in an EPR spectrum resembling mononuclear copper(II), which is not observed. These results suggest that the 4-electron reduction of dioxygen to water by laccase may proceed via two, 2-electron steps to a product (structure B in Scheme I) with different spectral properties from the resting enzyme. Subsequent loss of this T2–T3 hydroxide bridge would result in a T2 EPR signal and a diamagnetic T3 binuclear site as found in resting laccase. Experiments are underway to quantitate the nature of the ground-state wavefunction associated with this species (i.e., the relative contributions of structures A and B) in order to provide a complete description of its electronic structure.

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Revised Structure of Bistramide A (Bistratene A): Application of a New Program for the Automated Analysis of 2D INADEQUATE Spectra[1]

Mark P. Foster,[†] Charles L. Mayne,[§] Reinhard Dunkel,[§] Ronald J. Pugmire,[∥] David M. Grant,[§] Jean-Michel Kornprobst,[⊥] Jean-François Verbist,[⊥] Jean-François Biard,[⊥] and Chris M. Ireland*[,†,2]

Departments of Medicinal Chemistry, Chemistry, and Fuels Engineering, University of Utah Salt Lake City, Utah 84112
SMAB, Faculté de Pharmacie, Université de Nantes 44035 Nantes Cedex 01, France

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Didemnid ascidians (tunicates) are excellent sources of novel biologically active compounds of varied biosynthetic origin;[3] *Lissoclinum* spp., in particular, produce peptides[4] (e.g., lissoclinum peptides[5] and patellins[6]), macrolides (e.g., patellazoles),[7] and alkaloids (e.g., varamines).[8] *Lissoclinum bistratum* contains the cytotoxic cyclic peptides, bistratamides A and B, and the macrocyclic ether bistramide A (a.k.a. bistratene A).[9] This latter compound has demonstrated activity in a variety of systems: cytotoxicity toward MRC5CV1 fibroblasts and T24 bladder carcinoma,[10] P388 murine leukemia, KB, and human endothelial

Table I. Bistramide A NMR Data (500 MHz, $CDCl_3$)

C no.	^{13}C (mult)[a]	^1H (J_{HH}, hertz)[b]	CC (J_{CC}, hertz)[c]
1	18.33 (q)	1.88 (dd, 6.8, 1.4)	2 (41.7)
2	144.38 (d)	6.85 (dq, 15.7, 6.8)	1 (41.7)
3	132.10 (d)	6.10 (dq, 15.7, 1.5)	4 (53.8)
4	198.86 (s)		3 (53.8), 5 (41.0)
5	45.22 (t)	2.86 (dd, 17.0, 8.9), 2.49 (dd, 17.0, 3.0)	4 (41.0), 6 (41.8)
6	64.84 (d)	4.15 (m)	5 (41.8), 7 (36.5)
7	30.70 (t)	1.61 (m), 1.34 (m)	6 (36.5), 8 (32.5)
8	26.48 (t)	1.58 (m), 1.27 (m)	7 (32.5), 9 (33.1)
9	33.26 (d)	1.89 (m)	8 (33.1), 10 (35.4), 11 (36.2)
10	17.03 (q)	0.82 (d, 7.0)	9 (35.4)
11	74.73 (d)	4.02 (dd, 10.9, 4.6)	9 (36.2), 12 (35.8)
12	32.38 (t)	2.71 (dd, 15.1, 11.7), 2.10 (dd, 14.9, 1.4)	11 (35.8), 13 (51.0)
13	173.36 (s)		12 (51.0)
14	44.61 (t)	3.46 (dt, 14.0, 5.8), 3.19 (dt, 14.0, 5.7)	15 (39.8)
15	73.76 (d)	3.67 (dt, 10.3, 5.1)	14 (39.8), 16 (36.2)
16	43.32 (d)	2.34 (dq, 5.0, 7.0)	15 (36.2), 17 (34.5), 18 (49.0)
17	15.49 (q)	1.21 (d, 7.0)	16 (34.5)
18	175.12 (s)		16 (49.0)
19	39.46 (t)	3.26 (dt, 12.7, 6.6)[d]	20 (36.1)
20	25.80 (t)	1.77 (m), 1.50 (m)	19 (36.1), 21 (35.2)
21	30.41 (t)	1.67 (m), 1.30 (m)	20 (35.2), 22 (40.7)
22	74.22 (d)	3.11 (dt, 9.6, 1.8)	21 (40.7), 23 (36.7)
23	34.82 (d)	1.24 (m)	22 (36.7), 24 (35.4), 25 (33.0)
24	17.94 (q)	0.76 (d, 6.6)	23 (35.4)
25	27.87 (t)	1.52 (m), 1.42 (m)	23 (33.0), 26 (32.7)
26	36.06 (t)	1.57 (m), 1.40 (m)	25 (32.7), 27 (45.2)
27	95.41 (s)		26 (45.2), 28 (45.6)
28	35.44 (t)	1.52 (m), 1.32 (m)	27 (45.6), 29 (33.0)
29	19.17 (t)	1.79 (m), 1.48 (m)	28 (33.0), 30 (32.6)
30	31.30 (t)	1.48 (m), 1.08 (m)	29 (32.6), 31 (36.9)
31	69.02 (d)	3.40 (m)	30 (36.9), 32 (40.5)
32	34.05 (t)	1.33 (m), 1.26 (m)	31 (40.5), 33 (35.5)
33	33.43 (t)	1.33 (m), 1.27 (m)	32 (35.5), 34 (34.5)
34	31.82 (d)	2.31 (m)	33 (34.5), 35 (34.8), 36 (43.7)
35	20.90 (q)	0.90 (d, 6.8)	34 (34.8)
36	131.32 (d)	5.15 (d, 9.2)	34 (43.7)
37	137.16 (s)		38 (43.5), 39 (45.7)
38	11.79 (q)	1.58 (fd, 1.3)	37 (43.5)
39	73.23 (d)	4.16 (m)	37 (45.7), 40 (38.7)
40	21.74 (q)	1.20 (d, 6.3)	39 (38.7)
NH1		7.27 (bt, 5.8)	
NH2		6.93 (bt, 5.5)	
OH1		4.58 (d, 5.3)	
OH2		2.76 (broad)[e]	

[a] Determined from DEPT spectrum. [b] Assigned from HMQC, J_{CH} = 140 Hz; ^1H–^1H couplings measured from 1D ^1H spectrum. [c] C–C bonds determined from 2D INADEQUATE, J_{CC} = 40 Hz. [d] Degenerate methylene protons. [e] Observed in the benzene-d_6 spectrum of bistramide A.

[†] Department of Medicinal Chemistry, University of Utah.
[§] Department of Chemistry, University of Utah.
[∥] Department of Fuels Engineering, University of Utah.
[⊥] Faculté de Pharmacie, Université de Nantes.

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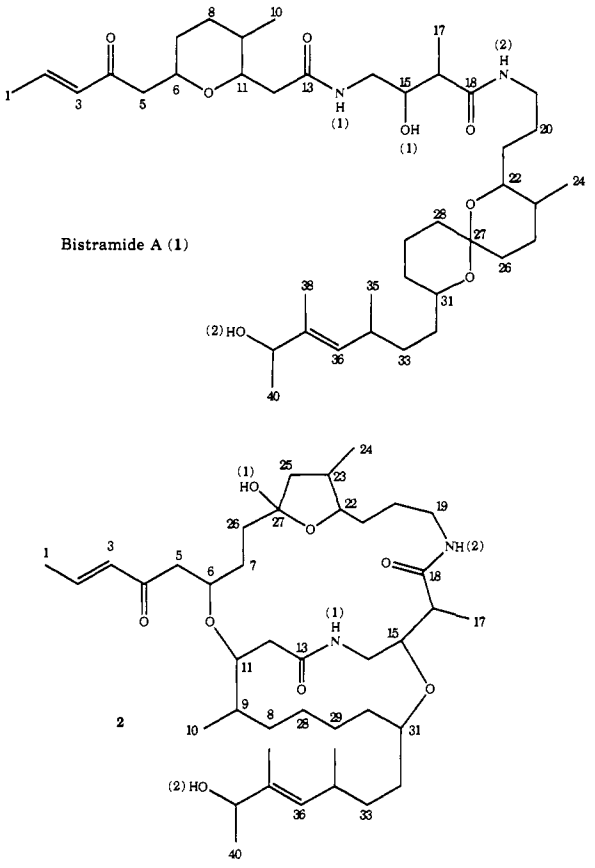
Chart I



cell lines (IC_{50}'s 0.01–0.1 μg/mL),[11] induction of differentiation in HL-60 cells, enhancement of the phospholipid-dependent activity of type II protein kinase C,[10] and induction of blockade in the G1 phase of the cell cycle while causing polyploidy in asynchronous cells of the NSCLCN-L16 line.[12]

We recently isolated from a Fijian *Lissoclinum* sp. a compound (**1**) that possessed the same molecular formula[13] and very similar spectral data to that reported previously for bistramide A (**2**);[9] it was also cytotoxic in vitro against the human colon tumor HCT116 and murine leukemia L1210 cell lines with an IC_{50} of 0.1 μg/mL. Due to severe overlap in the proton NMR spectrum, unambiguous assignment of the structure based on proton-correlation methods (e.g., PS-DQF-COSY,[14] HMQC,[15] HMBC[16]) was not possible, though several partial structures could be composed.

To elucidate the carbon backbone of the molecule, a 2D INADEQUATE[17,18a] experiment optimized for sp^3–sp^3 couplings was undertaken. The resulting data set was analyzed by a new automated procedure using the program CCBond.[18] The method is based on a parametric model of the spectral response of an AB spin system, with a convolution of Lorentzian and sinc line shapes, and determines the presence or absence of a bond between a pair of ^{13}C nuclei by a combination of response surface mapping and nonlinear regression analysis. Details are provided in the supplementary material.

This analysis allowed us to unambiguously identidy all carbon–carbon bonds in the molecule except the two sp^2–sp^2 bonds, C2–C3 and C36–C37 (Table I), which could easily be deduced from COSY (H2–H3, 6.85–6.10 ppm) and HMBC (C38–H36, 11.79–5.15 ppm) correlations. These data allowed construction of three fragments with contiguous carbon skeletons (C1–C13, C14–C18, C19–C40), which could be connected on the basis of HMBC correlations from the C13 amide carbonyl (173.36) to the amide proton at 7.27 ppm (NH1) and from C18 (175.12) to NH2 (6.93 ppm).

What remained was determining the site of ether linkages to satisfy the degrees of unsaturation in the molecule. The ether linkage between C6 and C11 was evident from HMBC correlations from C6 (64.84) to H11 (4.02 ppm) and from C11 (74.73) to H6 (4.15 ppm). The spiro-ketal functionality was deduced on the basis of results from HMBC and INAPT[19] experiments. An HMBC correlation from C27 (95.4) to H22 (3.11 ppm) established the first ether linkage; however, an analogous correlation from C27 to H31 was not observed. This linkage was established by an INAPT experiment (optimized for J_{CH} = 8 Hz) upon selective excitation of H31 (3.40 ppm).

The positions of the hydroxyls were confirmed by COSY correlations from the α protons to the exchangeable protons: H15 (3.67 ppm) to OH1 at 4.58 ppm (confirmed by an HMBC correlation from C15 at 73.76 to OH1); H39 (4.15) to OH2 (2.76 ppm).[20] This finalizes the study and allows us to report the structure of our metabolite as **1**. Comparison of this metabolite to bistramide A from New Caledonia reveals that they are identical, indicating the structure must be revised to **1**.

The proposal of structure **2** was apparently due in part to severe proton overlap for several methylenes. Specifically, C26 and C8 possess overlapping proton signals at 1.57/1.58 ppm, resulting in the proposal of C7–C26 and C8–C28 bonds based on a long-range correlation from protons at that chemical shift to C7 and C28. A partially exchanged OH1 was probably responsible for the proposal of an ether linkage from C15 to C31, placing the hydroxyl on C27.

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Supplementary Material Available: Details on 2D INADEQUATE processing, selected correlations from CCBond analysis, CCBond parameter listing, isolation procedures, key traces from HMBC spectrum, and INAPT, PS-DQF-COSY, HMQC, and 1D ^1H and ^{13}C spectra (27 pages). Ordering information is given on any current masthead page.

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(20) The OH2 signal was broad in the benzene-d_6 ^1H spectrum and not observed in deuteriochloroform, though OH1 was a clear doublet in both solvents. In addition, the T_1 for the C38 methyl was greater than 6 s. These observations may result from a high degree of motion in this region of the molecule.