ORGANIC LETTERS

2005
Vol. 7, No. 13
2731−2734

Sporolides A and B: Structurally Unprecedented Halogenated Macrolides from the Marine Actinomycete *Salinispora tropica*

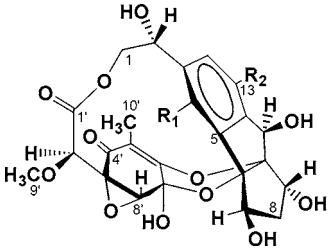
Greg O. Buchanan, Philip G. Williams, Robert H. Feling, Christopher A. Kauffman, Paul R. Jensen, and William Fenical*

Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California-San Diego, La Jolla, California 92093-0204

wfenical@ucsd.edu

Received April 22, 2005

ABSTRACT



1 R_1= Cl R_2 = H
2 R_1= H R_2 = Cl

Analysis of the fermentation broth of a strain of the marine actinomycete *Salinispora tropica* has led to the isolation of two unprecedented macrolides, sporolides A (1) and B (2). The structures and absolute stereochemistries of both metabolites were elucidated using a combination of NMR spectroscopy and X-ray crystallography.

After more then 50 years of intense scrutiny, pharmaceutical research into natural products from terrestrial actinomycetes has experienced a slow decline.[1] If these biomedically important microorganisms are to continue to provide new secondary metabolites that have medicinal relevance, then new strategies that lead to the isolation of genetically novel strains must be found.[2] Traditional microbial drug discovery strategies have focused on soil-derived strains,[3] but recent evidence indicates unequivocally that taxonomically diverse populations of actinomycetes reside in the marine environment.[4]

In 1991, we cultivated an unusual group of actinomycetes that required seawater for growth, which suggested that they existed only in the oceans.[5] Examination of strains of this new actinomycete genus *Salinispora*[6,7] showed that greater than 80% of these organisms produced culture extracts that inhibited the in vitro growth of human colon carcinoma HCT-116.

Initial fractionation of the culture broth of the strain designated CNB-392 (later assigned as *S. tropica*)[7] led to the isolation of salinosporamide A, a molecule that possessed an unusual fused γ-lactam-β-lactone ring structure.[8,9] The

(1) (a) Koehn, F. E.; Carter, G. T. *Nat. Rev. Drug Discovery* **2005**, *4*, 206−220. (b) Bérdy, J. *J. Antibiot.* **2005**, *58*, 1−26. (c) Bull, A. T.; Ward, A. C.; Goodfellow, M. *Microbiol. Mol. Bol. Rev.* **2000**, *64*, 573−604.

(2) Frantz, S. *Nat. Rev. Drug Discovery* **2004**, *3*, 900.

(3) Okami, Y.; Hotta, K. In *Actinomycetes in Biotechnology*; Goodfellow, M., Williams, S. T., Mordarski, M., Eds.; Academic Press: New York, 1988; pp 33−67.

(4) (a) Jensen, P. R.; Gontang, E.; Mafnas, C.; Mincer, T. J.; Fenical, W. *Environ. Microbiol.* **2005**, *7*, 1039−1048. (b) Maldonado, L. A.; Stach, J. E. M.; Pathom-aree, W.; Ward, A. C.; Bull, A. T.; Goodfellow, M. *Antonie van Leeuwenhoek* **2005**, *87*, 11−18. (c) Montalvo, N. F.; Mohamed, N. M.; Enticknap, J. J.; Hill, R. T. *Antonie van Leeuwenhoek* **2005**, *87*, 27−36.

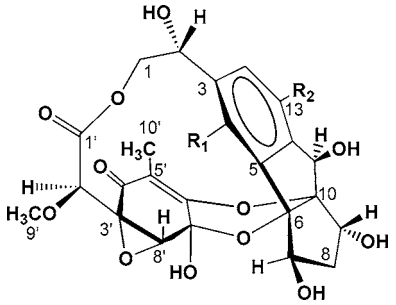
(5) Jensen, P. R.; Dwight, R.; Fenical, W. *Appl. Environ. Microbiol.* **1991**, *57*, 1102−1108.

(6) Mincer, T. J.; Jensen, P. R.; Kauffman, C. A.; Fenical, W. *Appl. Environ. Microbiol.* **2002**, *68*, 5005−5011.

(7) Maldonado, L. A.; Fenical, W.; Jensen, P. R.; Kauffman, C. A.; Mincer, T. J.; Ward, A. C.; Bull, A. T.; Goodfellow, M. *Int. J. Syst. Evol. Microbiol.* In press.

10.1021/ol050901i CCC: $30.25
Published on Web 06/04/2005

unusual chemical structure of salinosporamide A translated into potent biological activity, as salinosporamide A was shown in vivo to inhibit the proteolytic activity of the 20S subunit of the proteasome.[10] On the basis of this promising in vivo activity, salinosporamide A is anticipated to enter clinical trials for the treatment of cancer in late 2005.



1 R_1= Cl R_2 = H
2 R_1= H R_2 = Cl

As part of our continuing investigation into the chemistry of these unique marine microbes, a detailed time course study was carried out on the strain[11] that produced salinosporamide A. Monitoring this microbial fermentation by LC-MS indicated that the metabolite profile varied significantly over a 15-day period (see Figure S12 in Supporting Information for representative LC-MS traces for days 7 and 11). Optimization of the culture conditions and solid-phase extraction of the fermentation broth with Amberlite XAD-16 resin led to 4.18 g of crude organic extract after elution of the resin with acetone. This residue was then purified by silica gel flash column chromatography using increasing concentrations of acetone in dichloromethane. The fractions eluting with 3:1 and 1:1 acetone−dichloromethane were combined and subjected to further purification by reversed-phase HPLC, using a linear gradient of 10−100% acetonitrile in water over 30 min. This afforded two macrolides with unprecedented structures, sporolides A (**1**) and B (**2**).

Final purification of the more polar metabolite, sporolide A (**1**),[12] was obtained by reversed-phase HPLC. Low resolution mass spectral analysis provided two pseudo-molecular ion peaks at 539.1 $[MH]^+$ and 541.1 $[MH + 2]^+$ atomic mass units in a ratio of 3:1, which indicated **1** contained a chlorine atom. The high-resolution mass spectral data confirmed the presence of a chlorine by giving a molecular formula of $C_{24}H_{23}ClO_{12}$ for **1**. Also evident from the MS data was the presence of at least two free hydroxyl functionalities based on daughter ion peaks at 521 $[MH - H_2O]^+$ and 503 $[MH - 2H_2O]^+$. This was corroborated by IR absorptions characteristic of hydroxyl, ester, and conjugated ketone functionalities at 3284, 1737, and 1649 cm^{-1}, respectively. The latter two functional groups accounted for 3 of the 13 degrees of unsaturation required by the molecular formula. Three other degrees of unsaturation could be ascribed to double bonds (δ_C 146.5, 143.3, 138.2, 130.7, 129.0, and 125.1), which implied that **1** contained seven rings.

Table 1. NMR Spectral Data for **1** at 300 MHz in Pyridine-d_5

C/H no.	δ_H mult (*J* in Hz; integration)	δ_C, mult	COSY	HMBC
1a	3.55, t (10.2; 1H)	70.3, CH_2	1b, 2	2, 3, 1′
1b	5.22, dd (6.0, 10.2; 1H)		1a, 2	2, 3, 1′
2	5.91, dd (6.0, 10.2; 1H)	65.4, CH	1a/b	1, 3, 14
3		143.3, C		
4		146.5, C		
5		138.2, C		
6		95.7, C		
7	5.40, dd (4.2, 7.2; 1H)	68.3, CH	8a/b	6
8a	3.04, dd (7.8, 15.2; 1H)	47.0, CH_2	7, 8b	9, 10
8b	3.20, ddd (4.2, 7.2, 15.2; 1H)		7, 8a, 9	6, 9, 10
9	4.94, d (6.6; 1H)	74.1, CH	8b	6, 7, 10
10		102.7, C		
11	5.27, s (1H)	79.8, CH		5, 6, 10
12		130.7, C		
13	7.56, d (7.8; 1H)	125.1, CH	14	3, 5, 11, 14
14	7.93, d (7.8; 1H)	129.0, CH	13	2, 4, 12
1′		168.0, C		
2′	4.95, s (1H)	77.4, CH	8′	1′, 3′, 9′
3′		59.1, C		
4′		190.5, C		
5′		116.6, C		
6′		161.7, C		
7′		90.3, C		
8′	4.59, s (1H)	59.3, CH	2′	3′, 6′, 7′
9′	3.54, s (3H)	61.2, CH_3		2′
10′	1.48, s (3H)	7.8, CH_3		4′, 5′, 6′

Several substructures were determined by analyses of the 2D $^1H-^1H$ COSY, $^1H-^{13}C$ HMBC, and HMQC NMR spectral data. Substructure A was elucidated starting from the vinylic methyl (C-10′) group (Figure 1) that showed a series of HMBC correlations to carbons at 190.5, 161.7, and 116.6 ppm (C-4′, C-6′, and C-5′, respectively). These carbon chemical shifts were assigned to the aforementioned α,β-unsaturated ketone in which the β-position was an enol ether (substructure A). A second fragment could be assembled starting from the methoxy singlet (C-9′). Proton H-9′ showed a strong $^3J_{CH}$ correlation to an oxygenated methine carbon (C-2′), while the proton attached to this carbon showed a HMBC cross-peak to the ester carbon and to a relatively upfield oxygenated quaternary carbon (δ_C 59.1). On the basis of its chemical shift, this quaternary carbon was assigned to

(8) Feling, R. H.; Buchanan, G. O.; Mincer, T. J.; Kauffman, C. A.; Jensen, P. R.; Fenical, W. *Angew. Chem., Int. Ed.* **2003**, *42*, 355−357.

(9) Williams, P. G.; Buchanan, G. O.; Feling, R. H.; Kauffman, C. A.; Jensen, P. R.; Fenical, W. *J. Org. Chem.* Accepted for publication.

(10) (a) Macherla, V. R.; Mitchell, S. S.; Manam, R. R.; Reed, K. A.; Chao, T.-H.; Nicholson, B.; Deyanat-Yazdi, G.; Mai, B.; Jensen, P. R.; Fenical, W.; Neuteboom, S. T. C.; Lam, K. S.; Palladino, M. A.; Potts, B. C. M. *J. Med. Chem.* **2005**, *48*, 3684−3687. (b) Palladino, M.; Neuteboom, S.; Theodora, C.; Macherla, V. R. R.; Potts, B. C. Patent WO 02005002572, 2005.

(11) *Salinispora tropica*, strain CNB-392, was isolated in 1989 from marine sediments (−1 m) near Chub Cay, Bahamas. The strain was cultured in 40 × 1 L seawater-based media (filtered seawater, chitosan, kelp powder, menhaden meal, hydro solubles, and starch) and shaken for 10 days at room temperature under aerobic conditions before extraction.

(12) **Sporolide A** (**1**, 5.3 mg): amorphous crystals, mp 230−233 °C decomposed; $[\alpha]_D$ +80.7 (*c* 0.22, MeOH); UV (MeOH) λ_{max} (log ϵ) 268 (3.9), 206 (4.5) nm; IR (NaCl) λ_{max} 3284, 2931, 1737, 1654, 1649, 1420, 1184, 1102, 1020 cm^{-1}; NMR spectral data, see Table 1; ESIMS *m*/*z* 561.1 $[MNa]^+$, 539.1 $[MH]^+$; HRMALDI-FTMS *m*/*z* 561.0788 (calcd for $C_{24}H_{23}ClO_{12}Na$, $[MNa]^+$, 561.0776).

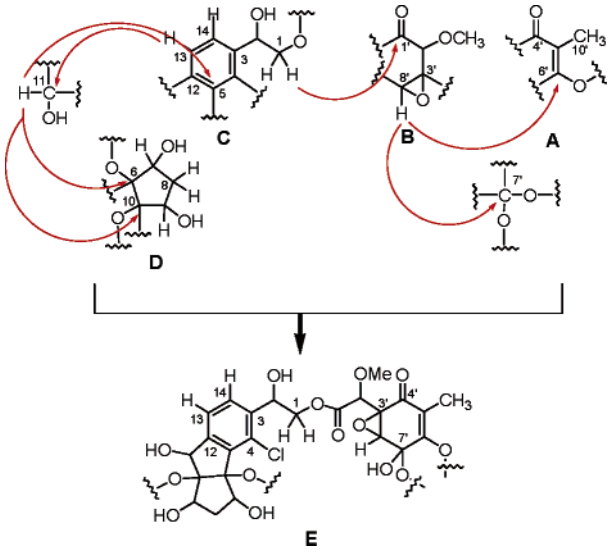


Figure 1. Partial structures and HMBC correlations used to establish the gross structure of **1**.

one of the carbon atoms of an epoxide ring; the other carbon atom in the epoxide was evidently a methine based on its relatively upfield carbon chemical shift of δ_C 59.3. Together this gave substructure B. Analysis of the COSY spectrum led to two small fragments (H-1/H-2 and H-13/H-14) that were joined on the basis of an HMBC correlation (H-2 to C-14) and then further expanded into a tetrasubstituted benzene ring to give substructure C. The final fragment was assembled on the basis of a network of COSY cross-peaks between H-7, H-8, and H-9 that defined a three-carbon chain containing a diol. This unit was then extended into a five-membered ring (substructure D) on the basis of a series of HMBC correlations to the oxygenated quaternary carbons C-6 and C-10 from all of these three proton signals.[13] These substructures (A−D) accounted for all but two (C-11 and C-7′) of the 24 carbons in the molecular formula of **1**.

HMBC correlations to these last two carbon signals (C-11 and C-7′) were crucial in assembling a larger portion of the gross structure of sporolide A (**1**). Briefly, cross-peaks to C-7′ and C-6′ from H-8′ connected substructures A and B, while HMBC correlations from H-11 to C-5, C-6, and C-10 linked fragments C and D together (Figure 1). The last connectivity that could be gleaned from the HMBC NMR spectrum was an ester linkage between C-1 and C-1′ (HMBC correlation from H-1 to C-1′) that connected substructures B and C.

Two other connectivities could be postulated on the basis of the available data. First, the chemical shift of C-4′ (δ_C 190.5) defined this carbonyl as a ketone and thus C-4′ had to be bonded to one of the two available carbons, either C-4 or C-3′. The remaining chlorine atom, mandated by the molecular formula of **1**, also had to be bonded to one of these two carbons. On the basis of carbon chemical shift considerations and the UV spectrum of **1**, the chlorine was attached to C-4 and the ketone joined to C-3′ to form substructure E, which contained substituted chlorobenzene and cyclohexenone rings.

Although substructure E (Figure 1) contained all of the necessary carbons and oxygens, two more ether rings were required by the molecular formula. Clearly, because of its carbon chemical shift that was indicative of a ketal, C-7′ was involved in one of these ether linkages, but since none of the alcohol proton signals were visible in the NMR spectra, several structures differing in the position of the ether linkages could be postulated.

In the end, to discriminate between these possibile structures, it became necessary to crystallize **1** from a mixture of isooctane−acetone. This resulted in orthorhombic crystals that were suitable for X-ray analysis. These crystals, which diffracted as a *P*2(1)2(1)2(1) space group, revealed the final connectivities needed to assign the structure of **1** (Figure 2)

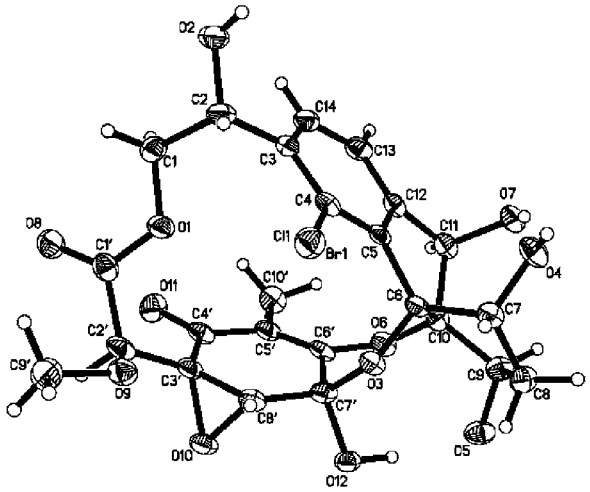


Figure 2. X-ray crystal structure of **1**.

and allowed the absolute configuration of **1** to be assigned as depicted on the basis of the diffraction anisotropy of the chlorine atom. X-ray analysis of these crystals indicated that they were a mixture of **1** and the corresponding C-4 bromo derivative. On the basis of this diffraction data, the latter was present in only trace amounts (6%) as compared to **1**. Attempts to isolate this analogue were unsucccessful, as only a trace amount of this metabolite was present in the bacterial extract.

The molecular formula of the less polar compound, sporolide B (**2**),[14] was deduced to be $C_{24}H_{23}ClO_{12}$, which

(13) The carbon chemical shifts of C-6 and C-10 are downfield of a typical oxygenated quaternary carbon. The constrained conformation imposed by the three-dimensional ring strucutre of **1** and **2** may place these carbons into the deshielding region of the anisotropic intramolecular magnetic field of the aromatic ring or of the adjacent oxygens.

(14) **Sporolide B** (**2**, 5.3 mg): colorless gum, $[\alpha]_D$ +8.6 (*c* 0.17, MeOH); UV (MeOH) λ_{max} (log ϵ) 269 (3.9), 206 (4.5) nm; IR (NaCl) λ_{max} 3424, 1737, 1648, 1420, 1190, 1108, 1020 cm^{-1}; ^{1}H and ^{13}C NMR see Table S1; ESIMS *m*/*z* 561.1 $[MNa]^+$, 539.1 $[MH]^+$; HRMALDI-FTMS *m*/*z* 561.0748 (calcd for $C_{24}H_{23}ClO_{12}Na$, $[MNa]^+$, 561.0776).

indicated it was isomeric with **1**. The ^{1}H and ^{13}C NMR spectra of sporolide B (**2**) (Table S1 in Supporting Information) displayed most of the key resonances seen in the spectra of sporolide A (**1**). The most noticeable difference in the proton NMR spectrum of **2** was the two aromatic proton signals that now appeared as broad weakly coupled singlets rather than doublets. Inspection of the COSY and HMBC data of **2** established that these aromatic protons were now meta to each other and that the chlorine atom was now substituted at C-13. Further analysis of the NMR data allowed the carbon framework of **2** to be defined as shown, and the positions of the ether linkages were confirmed by peracetylation of **2**. This yielded 2,7,9,11,7′-penta-acetyl-**2** as determined by analysis of the NMR and MS spectral data (not shown).

Unfortunately, the sporolides were inactive when screened in our standard assays. They displayed no activity against human colon carcinoma HCT-116, methicillin-resistant *Stapyhlococcus aureus*, and vancomycin-resistant *Enterococcus faecium*. The highest concentration tested against HCT-116 was 78 μg/mL and in the antibacterial assay the upper limit was 250 μg/mL.

The structures of sporolides A are B are unique for several reasons. First, while both compounds appear to be polyketides and therefore derived from acetate units, the number of oxidized carbons is amazing, with 23 of 24 carbons either oxygenated or sp^2 hybridized! This, in part, contributes to the highly unusual structures of the sporolides. The only compounds reported in the literature that have any structural resemblance to any portion of the sporolides are the cyclohexenone vertinoid polyketides, from the marine-derived fungus *Trichodema*.[15] These bear a slight resemblance to the lower cyclohexenone-half of the sporolides, but the upper portion of the sporolides and the overall structures are completely unprecedented.

The sporolides are also of interest because they are only the second class of compounds isolated from the recently described marine actinomycete genus *Salinispora*. The unique carbon skeletons of the sporolide and salinosporamide structural classes from this genus provide a clear indication of the tremendous potential of marine actinomycetes as a source of novel secondary metabolites.

Acknowledgment. We thank the National Cancer Institute for providing support through grant CA44848. The upgrades to the NMR spectrometers used in this research were funded by the NIH Shared Resources Program under grant S10RR017768. High-resolution mass spectral analyses were carried out by the UCR Mass Spectrometry Facility, Department of Chemistry, University of California. We thank Sara Kelly for the bioassay data and P. Gantzel, UCSD, for the X-ray crystallographic data.

Supporting Information Available: Tabulated NMR data for sporolide B (**2**); ^{1}H, ^{13}C, gCOSY, gHMQC, and gHMBC spectra of **1** and **2**; and LC-MS traces of the EtOAc extract of the fermentation broth on day 7 and day 11. This material is available free of charge via the Internet at http://pubs.acs.org.

OL050901I

(15) Sperry, S.; Samuels, G. J.; Crews, P. *J. Org. Chem.* **1998**, *63*, 10011−10014.