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Spirastrellolide A, An Antimitotic Macrolide Isolated from the Caribbean Marine Sponge *Spirastrella coccinea*

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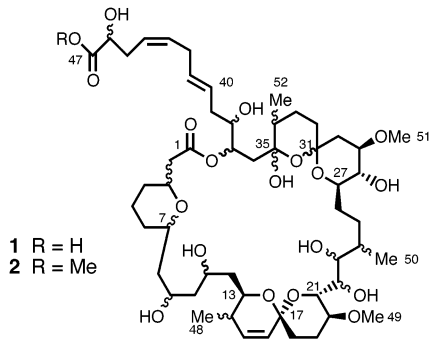
Antimitotic drugs based on natural products in the taxane (paclitaxel and docetaxel) and Vinca alkaloid (vincristine, vinblastine, vinorelbine) families are widely used in the treatment of cancer. The effectiveness of these drugs has stimulated the search for other natural products that arrest cancer cell-cycle progression in mitosis.[1] Marine invertebrates have been a particularly rich source of antimitotic secondary metabolites. Included in this group are discodermolide, dolastatin-10, laulimalide, eleutherobin, halichondrin B, peloruside, vitilevuamide, spongistatin, and hemiasterlin.[1,2a−c] As part of a program to discover new antimitotic natural products,[2] we have screened extracts of marine invertebrates in a cell-based assay that detects mitotic arrest.[3] Extracts of the Caribbean marine sponge *Spirastrella coccinea* (Duchassaing and Michelotti, 1864) showed potent activity in the assay. The major antimitotic component of the extract is the novel macrolide spirastrellolide A (**1**), whose structure is reported below.



Specimens of *S. coccinea* (2.6 kg) were collected on reef walls at a depth of 2−5 m off Capucin, Dominica, and extracted exhaustively with MeOH. Fractionation of the MeOH soluble materials by solvent partitioning (EtOAc/H_2O followed by hexanes/4:1 MeOH/H_2O) and Sephadex LH 20 chromatography (4:1 MeOH/CH_2Cl_2) gave a partially purified antimitotic mixture containing **1** as the major component, which was methylated by reaction with trimethylsilyldiazomethane to facilitate the final purification. The methylated material was subjected, in sequence, to normal and reversed phase flash chromatographies and finally reversed phase HPLC (CSC-Inertsil 150A/ODS2, 3:1 MeOH/H_2O) to give pure methylspirastrellolide A (**2**) (6.2 mg) and several very minor analogues.

Methyl ester **2** was obtained as an optically active oil ($[\alpha]^{25}$ +27° (*c* 0.16, CH_2Cl_2)) which gave a $[M + H]^+$ ion at *m*/*z* 1027.5805 in the HRCIMS appropriate for a molecular formula of $C_{53}H_{86}O_{19}$ (calcd for [M + H], 1027.5843) requiring 11 sites of unsaturation. The ^{13}C NMR spectrum (200 MHz, C_6D_6) of **2** showed only 52 resolved resonances (Supporting Information). Analysis of the HSQC data revealed that a signal at δ 131.8 represented two overlapping resonances, thereby accounting for all of the 53 carbon atoms indicated by the HRCIMS measurement. Six olefinic methine (δ 124.4, 126.1, 129.0, 131.8, 131.8, 135.2) and two carbonyl (δ 174.8, 169.2) resonances in the ^{13}C spectrum accounted for five sites of unsaturation. The remaining six sites of unsaturation required by the molecular formula had to be accounted for by rings. HSQC data showed that 78 of the 86 hydrogen atoms were attached to carbons (6 × CH_3, 18 × CH_2, 24 × CH), and, therefore, methyl ester **2** had to have 8 OH's.

The ^1H NMR spectrum of **2** acquired in C_6D_6 at 800 MHz was well dispersed, which facilitated the straightforward identification of the major features of the structure. Figure 1 shows five major fragments **A**−**E** of **2**. Each fragment contains an isolated ^1H spin system that could be identified directly from the COSY and HSQC data, along with OMe substituents and terminal nonprotonated carbons that were linked to the fragment via HMBC correlations (see Supporting Information for complete 1D and 2D data). Joining the fragments through their common nonprotonated carbons linked **A** and **B** through the ketal carbon with δ 108.7 (C-35), **B** and **C** through the ketal carbon with δ 97.6 (C-31), and **D** and **E** through the ketal carbon with δ 93.8 (C-17). HMBC correlations from the fragment **A** resonances at δ 1.98 (H-36α) and 2.29 (H-36β) to the fragment **B** resonance at δ 38.7 (C-34) confirmed the **A**/**B** junction at C-35, and a correlation between the fragment **D** resonance at δ 1.45 (H-18) and the fragment **E** resonance at δ 129.0 (C-16) supported the **D**/**E** junction at C-17. A four-bond HMBC correlation between the fragment **C** resonance at δ 1.36 (H-30) and the fragment **B** resonance at δ 108.7 (C-35) confirmed the **B**/**C** junction at C-31 and identified the C-31 to C-35 ether bond, which was further supported by four-bond correlations observed between δ 1.98 (H-36)/2.29 (H-36) and δ 97.6 (C-31).

HMBC correlations from the fragment **C** methyl resonance at δ 1.23 (Me-50) to the fragment **C** resonances at δ 34.0 (C-24) and 26.3 (C-25) and the fragment **D** resonance at δ 75.1 (C-23) identified a **C**/**D** connection at C-23/C-24, and a correlation observed between the fragment **A** resonance at δ 5.53 (H-37) and the fragment **E** resonance at δ 169.2 (C-1) established an ester linkage between C-1 and C-37, completing the macrocyclic ring in **2**. An HMBC correlation between δ 4.37 (H-21) and δ 93.8 (C-17) established the C-17 to C-21 ether linkage in fragment **D**, and correlations between δ 3.94 (H-13) and δ 93.8 (C-17) and between δ 3.67 (H-7) and δ 74.3 (C-3) identified the C-13 to C-17 and C-3 to C-7 ether linkages in fragment **E**. A final cyclic ether

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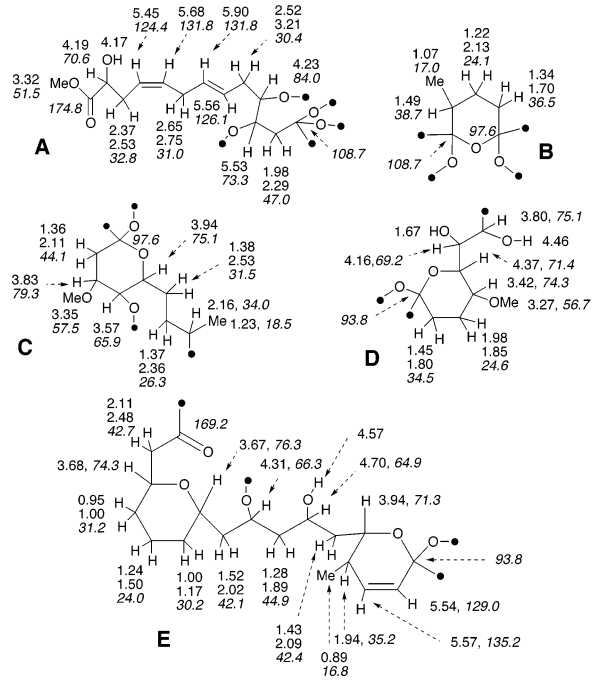

Figure 1. Proton and carbon (italics) NMR assignments for the fragments **A**, **B**, **C**, **D**, and **E**.

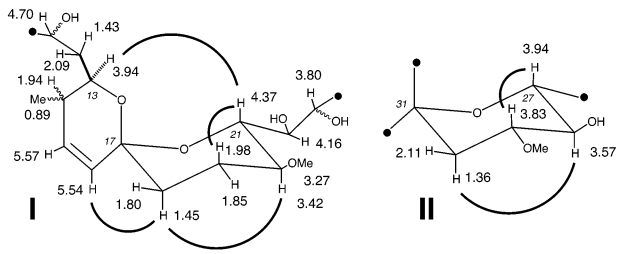

Figure 2. Relative stereochemistry of the substructures **I** (C-13 to C-21) and **II** (C-27 to C-31) in **2**. Arcs show ROESY correlations.

was required to generate the remaining site of unsaturation and to limit the number of OH's to 8. An HMBC correlation between δ 3.94 (H-27) and δ 97.6 (C-31) showed the presence of an ether linkage between C-27 and C-31 in fragment **C**, requiring a hemiketal at C-35 as shown in **2**.

Irradiation of the H-43 resonance (δ 5.68) in a 1D NOESY experiment gave a NOE in the H-44 resonance (δ 5.45), demonstrating that the $\Delta^{43,44}$ olefin has the *Z* configuration. The $\Delta^{40,41}$ olefin was assigned the *E* configuration on the basis of a 15.2 Hz H-40/H-41 vicinal coupling constant. Analysis of ROESY and coupling constant data identified partial relative configurations in each of the spiroketal substructures as shown in Figure 2. ROESY correlations were observed between δ 4.37 (H-21) and 1.98 (H-19_{ax}) and between 3.42 (H-20) and 1.45 (H-18_{ax}), demonstrating that OMe-49 and C-22 were equatorial substituents in a chair conformation. A ROESY correlation between δ 1.45 (H-18_{ax}) and 5.54 (H-16) established the relative configuration about the C-17 spiroketal center, while ROESY correlations between δ 3.94 (H-13) and 4.37 (H-21) established the relative configuration at C-13 as shown in **I**. Similarly, ROESY correlations between δ 3.94 (H-27) and 3.83 (H-29) and between δ 1.36 (H-30_{ax}) and 3.57 (H-28) demonstrated that OMe-29, OH-28, and C-26 were also equatorial substituents in a chair conformation as shown in **II**. It was not possible to unambiguously define the relative configurations of the remaining chiral centers in **2** from spectroscopic analysis. These stereochemical relationships are currently under investigation.

Spirastrelloide A (**1**) represents the first member of a new family of antimitotic sponge macrolides. It has a 47-carbon linear polyketide backbone incorporated into a highly functionalized 38-membered lactone containing a tetrahydropyran and two spiro bispyran substructures embedded in the macrocycle and a side chain terminating in a carboxylic acid. Spirastrellolide A methyl ester **2** exhibits potent activity (IC_{50} 100 ng/mL) in a cell-based antimitotic assay.[3] However, unlike antimitotic sponge macrolides in the spongistatin,[4] laulimalide,[1e] peloruside,[1d] and halichondrin[1f] families, ester **2** does not affect tubulin polymerization in vitro. In addition, it accelerates the entry of cells into mitosis from other cell-cycle stages prior to arresting them in mitosis.[5] These unusual biological effects and its novel structural features, including the polyketide chain length, macrolide ring size, and side chain functionality, set spirastrellolide A (**1**) apart from known antimitotic sponge macrolides.[1,4]

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Supporting Information Available: Experimental details, 1D and 2D NMR data for ester **2**, and tables of NMR assignments for **2** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(5) Complete details of the biological activity will be reported elsewhere.

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