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Spirastrellolide A: Revised Structure, Progress toward the Relative Configuration, and Inhibition of Protein Phosphatase 2A

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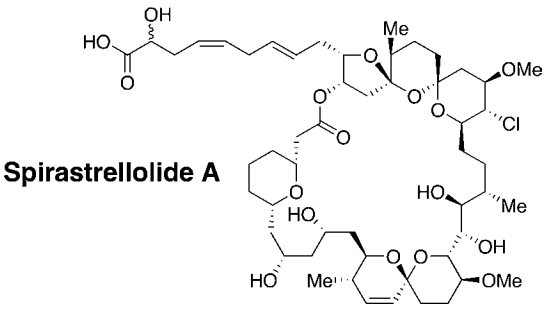
ABSTRACT



The structure of spirastrellolide A, a novel macrolide isolated from the marine sponge *Spirastrella coccinea*, has been revised to accommodate new MS and chemical transformation data. Relative configurations of three major fragments of the molecule have been elucidated from ROESY and coupling constant data. Spirastrellolide A has been shown to be a potent and fairly selective inhibitor of protein phosphatase 2A.

We recently reported the proposed constitution and partial relative stereochemistry **1** for the novel sponge macrolide spirastrellolide A isolated from extracts of the Caribbean marine sponge *Spirastrella coccinea*.[1] The spectroscopic analysis leading to the proposed structure **1** was carried out on spirastrellolide A methyl ester. Both the crude *S. coccinea* extract and pure methyl ester showed potent activity in a cell-based assay for antimitotic activity.[2] Although it was able to effectively arrest cells in mitosis, spirastrellolide A methyl ester did not interact with purified tubulin. It had the further interesting biological property of being able to drive cells directly from the S phase into mitosis before causing mitotic arrest.

The proposed structure **1** has 21 stereogenic centers, which makes complete assignment of the relative and absolute stereochemistry for the compound a major challenge. Detailed analysis of the NMR data for spirastrellolide A methyl ester provided some information about the relative stereochemistry in the C-13−C-21 and C-27−C-35 spiroketal fragments as initially reported, but it did not provide definitive information regarding the relative configurations at C-3 and C-7 of the tetrahydropyran ring or at the C-9, C-11, C-22, C-23, C-24, or C-46 stereogenic centers on the acyclic linkers and side chain. We envisioned that preparation of a variety of chemical derivatives, such as the OH-9/OH-

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(1) Williams, D. E.; Roberge, M.; Van Soest, R.; Andersen, R. J. *J. Am. Chem. Soc.* **2003**, *125*, 5296−5297.
(2) Roberge, M.; Cinel, B.; Anderson, H. J.; Lim, L.; Jiang, X.; Xu, L.; Kelly, M. T.; Andersen, R. J. *Cancer Res.* **2000**, *60*, 5052−5058.

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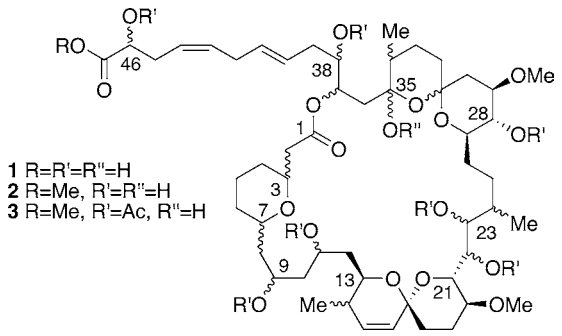
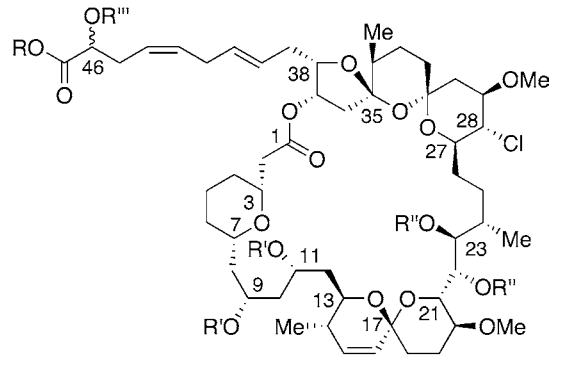
11 and OH-22/OH-23 bisacetonide, would provide additional stereochemical information. However, the initial collection of *S. coccinea* yielded only small quantities of spirastrellolide A methyl ester, which severely limited the range of chemical transformations that could be explored.



A subsequent larger collection of *S. coccinea* provided ~45 mg of spirastrellolide A methyl ester as starting material for chemical transformations. Acetylation of the ester with acetic anhydride and pyridine in the presence of a catalytic amount of DMAP at rt for 16 h gave a mixture of acetylation products. Careful HPLC fractionation of the mixture gave a pentaacetate as the major product of the reaction. NMR data obtained for the pentatacetate (Supporting Information) was consistent with acetylation at C-9 (H-9; δ 5.48), C-11 (H-11; δ 5.46), C-22 (H-22; δ 5.90), C-23 (H-23; δ 5.55), and C-46 (H-46; δ 5.13) of the proposed methyl ester structure **2**. Examination of the minor products from the acetylation reaction failed to identify the expected heptaacetate product **3**, or any partial acetylation products containing more than five acetates or having acetates at C-28 or C-38. The apparent lack of acetylation at C-28 and C-38 in spirastrellolide A methyl ester raised the possibility that the proposed structure **2** was incorrect.

In the initial structural analysis of spirastrellolide A methyl ester, no molecular ion was observed via electrospray MS despite trying many conditions. Consequently, the molecular weight and proposed molecular formula of the methyl ester were originally obtained from a CIHRMS measurement combined with a count of carbon atoms from the ^{13}C NMR and a count of protons attached to carbon from the HSQC data. Four of the exchangeable protons were observed in the ^{1}H NMR of the methyl ester (OH-11, OH-22, OH-23, OH-46), but a definitive count of the total number was not possible. With additional compound in hand from the sponge recollection, it became possible to expend more material on developing ESMS conditions, which ultimately led to the finding that using a relatively nonpolar solvent system ($MeOH/CH_2Cl_2$ (1:1) with 0.1% TFA) reproducibly gave a strong $[M + Na]^+$ ion at *m/z* 1049 for the methyl ester in the ESLRMS. Repeating the experiment with MeOD in place of MeOH gave a $[M + Na]^+$ ion at *m/z* 1054, demonstrating the presence of only five exchangeable protons instead of the expected eight, which was in agreement with the pentaacetylation results (see the Supporting Information).



4 R=Me R'=R''=R'''=H

5 R=Me, R'''=H, R'=R''= 

6 R=R'=R''=R'''=H

Identification of exactly five exchangeable protons from the deuterium-exchange ESLRMS experiment, combined with the already established carbon (C_{53}) and carbon-bound proton (H_{78}) counts, narrowed the number of possible molecular formulas that were within the acceptable mass deviation for the accurate mass measured in the ES-HRTOFMS experiment ($[M + Na]^+$ *m/z* 1049.5260). It was also apparent that an electronegative substituent other than OH had to be present at C-28 to account for the observed lack of acetylation. The ^{1}H and ^{13}C NMR data could also accommodate a chlorine atom instead of an oxygen at C-28 (H-28 δ 3.57: C-28 δ 65.9). Taken together, this information indicated that the correct molecular formula for spirastrellolide A methyl ester was $C_{53}H_{83}O_{17}Cl$ (calcd for $C_{53}H_{83}O_{17}$-ClNa 1049.5211). Simulation of the isotope pattern for this molecular formula gave a good match with the observed pattern from the ESHRTOFMS measurement. Independent confirmation of the correct formula was obtained via a ESHRFTMS analysis ($[M + Na]^+$ *m/z* 1049.52026 (calcd for $C_{53}H_{83}O_{17}ClNa$, 1049.52110)), which also involved comparison of the observed isotope pattern with a simulation (Supporting Information).

The revised molecular formula for spirastrellolide A methyl ester required one additional site of unsaturation (12 in total instead of 11), and it differed from the original proposed formula by the loss of H_3O_2 and the gain of Cl. It was clear from the acetylation and deuterium exchange results that the modifications in the structure had to eliminate the OH groups at C-28, C-35, and C-38. At the same time, the chemical shifts observed for C-38 (δ 84.0) and C-35 (δ 108.7) still required oxygen substitution at C-38 and a ketal at C-35. Formation of an additional cyclic ketal between the C-38 OH and the C-35 hemiketal, and replacement the C-28 OH in **2** with a chlorine atom, gave a revised constitution **4** that accounted for all of the data.

Analysis of ROESY and coupling constant data obtained for spirastrellolide A methyl ester (**4**) at 800 MHz in C_6D_6 led to a complete assignment of the relative stereochemistry in the two separate C-13−C-21 and C-27−C-38 spiroketal fragments as shown in Figures 1 and 2, respectively. The H-20 methine resonance at δ 3.42 appeared as a ddd with J = 10.4, 10.1, 4.8 Hz indicating that H-20 was axial, the tetrahydropyran ring was in a chair conformation, and H-21

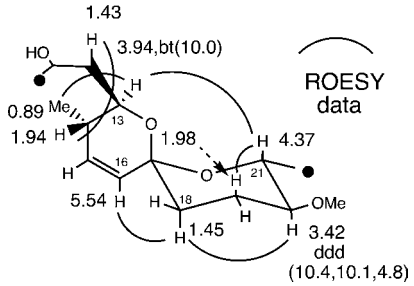


Figure 1. ROESY and coupling constant data for the C-13−C-21 segment of spirastrellolide A methyl ester (**4**).

(δ 4.37, d, J = 10.4 Hz) was also axial (Figure 1). ROESY correlations observed between δ 4.37 (H-21) and 1.98 (H-19_{ax}), and between δ 3.42 (H-20) and 1.45 (H-18_{ax}), confirmed that the tetrahydropyranose was in a chair conformation. A ROESY correlation observed between δ 1.45 (H-18_{ax}) and 5.54 (H-16) demonstrated that the C-16 olefinic methine carbon was equatorial at C-17, thereby defining the relative configuration at the spiroketal carbon (C-17) as shown in Figure 1. ROESY correlations observed between resonances at δ 1.94 (H-14) and 1.43 (H-12), and between 0.89 (H-48) and 3.94 (H-13), in conjunction with the coupling pattern observed for H-13 (t, J = 10 Hz) revealed that Me-48 and C-12 were trans to each other and both were pseudoequatorial.

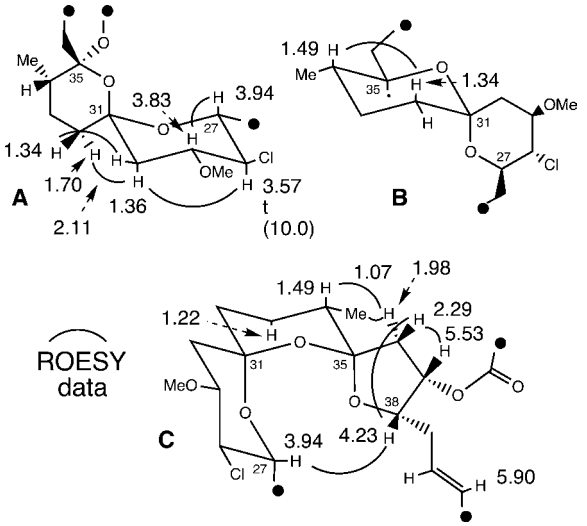


Figure 2. ROESY and coupling constant data for the C-27−C-38 segment of spirastrellolide A methyl ester (**4**).

The H-28 resonance at δ 3.57 appeared as a triplet with J = 10.0 Hz indicating that the tetrahydropyranose ring encompassing C-27−C-31 was in a chair conformation with H-27, H-28, and H-29 all axial as shown in Figure 2A. A ROESY correlation between δ 3.57 (H-28) and 1.36 identified the H-30_{ax} resonance, and ROESY correlations between δ 1.36 (H-30_{ax}) and 1.70 (H-32_{eq}), and between 2.11 (H-30_{eq}) and 1.34 (H-32_{ax}), established that C-32 was equatorial and trans to the C-28 Cl substituent (Figure 2A). Observation of a ROESY correlation between δ 1.34 (H-32_{ax}) and 1.49 (H-34_{ax}) showed that the tetrahydropyranose ring containing C-32−C-35 was also in a chair conformation and Me-52 was trans to C-30 (Figure 2B).

The relative configuration at the C-35 spiroketal was revealed by the ROESY correlations observed between δ 1.98 (H-36) and both of 1.49 (H-34_{ax}) and 1.07 (Me-52), which required that C-36 was equatorial and, therefore, trans to Me-52 (Figure 2C). ROESY correlations between δ 4.23 (H-38) and 3.94 (H-27), and between 5.53 (H-37) and 2.29 (H-36), showed that both C-39 and the ester oxygen on C-37 were cis to C-34 on the tetrahydrofuran ring (Figure 2C). The C-37 and C-38 assignments were supported by the observation of ROESY correlations between δ 5.90 (H-41) and 1.22 (H33_{ax}) and between 1.07 (Me-52) and 3.68 (H-3/H-7), which come from long range NOEs that are only possible with the relative configurations shown.

Reaction of methyl ester **4** with 2,2-dimethoxypropane and PPTS at room temperature followed by HPLC purification gave the bisacetonide **5** (see the Supporting Information for MS and NMR assignments). A ROESY correlation observed between the H-3 (δ 3.82) and H-7 (δ 3.59) resonances in **5** showed that the C-2 and C-8 substituents were cis and equatorial. The geminal methyl carbons (Me-55 and Me-56) in the C-9/C-11 acetonide had chemical shifts of δ 25.6 and 27.9 demonstrating that the C-9 and C-11 alcohols in **4** were anti.[3] ROESY correlations and coupling constant information shown in Figure 3 for the bisacetonide **5**

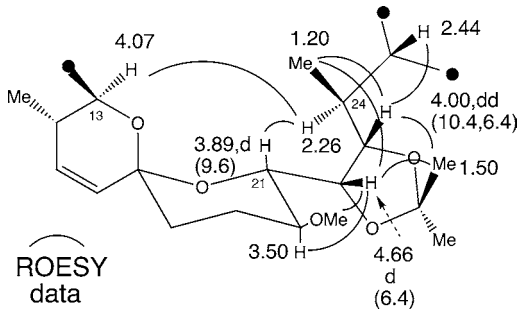


Figure 3. ROESY and coupling constant data for the C-21−C-24 segment of the bisacetonide **5**.

established the relative configurations from C-21 to C-24. The acetonide methyl resonance at δ 1.50 showed ROESY correlations to δ 4.66 (H-22) and 4.00 (H-23) indicating that H-22 and H-23 were cis. A coupling constant of 10.4 Hz observed between H-23 (δ 4.00) and H-24 (δ 2.26 showed that they were anti, and ROESY correlations between δ 1.20 and both 4.00 (H-23) and 4.66 (H-22), and between 2.44

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(H-25) and 4.00 (H-23), indicated that Me-50 was anti to the oxygen atom on C-23 and C-25 was anti to C-22. The absence of observable coupling between H-21 (δ 3.89) and H-22 (δ 4.66) required a dihedral angle of near 90°, and ROESY correlations observed between δ 4.66 (H-22) and 3.50 (H-20), and between 2.26 (H-24) and both 4.07 (H-13) and 3.89 (H-20), were only consistent with the C-21/C-22 relative configurations shown in Figure 3.

Finally, the results of a series of decoupling experiments and the ROESY data for the methyl ester **4** yielded the relative configurations at C-11 and C-13 as shown in Figure 4. A 10.0 Hz coupling between H-13 (δ 3.94) and the H-12

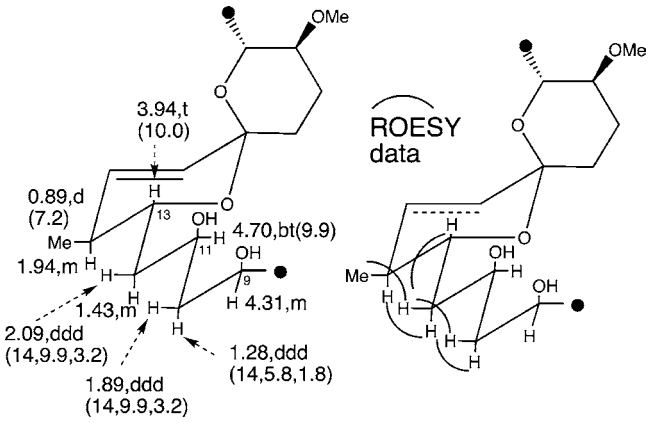

Figure 4. ROESY and coupling constant data for the C-9−C-14 segment of spirastrellolide A methyl ester (**4**).

proton with a resonance at δ 1.43 required that these protons were anti. Similarly, a 9.9 Hz coupling between the other H-12 proton (δ 2.09) and the H-11 methine (δ 4.70) required them to be anti. ROESY correlations observed between δ 0.89 (Me-48) and 2.09 (H-12), between 1.94 (H-14_{ax}) and 1.43 (H-12), between 2.09 (H-12) and 1.89 (H-10), and between 1.43 (H-12) and 1.28 (H-10) required the extended zigzag conformation and the relative configurations for the carbon chain extending from C-9 to C-13 as shown (Figure 4).

In summary, the revised constitution for spirastrellolide A is as represented in **6**. The relative configurations are as shown for the three independent segments, C-3−C-7, C-9−C-24, and C-27−C-38. The stereochemical relationships between the three segments and the isolated stereogenic center at C-46 remain to be determined. Chemical degradation efforts designed to provide this information are ongoing in our laboratory.

Spirastrellolide A causes premature entry into mitosis and mitotic arrest, biological properties reminiscent of those of the Ser/Thr phosphatase inhibitors fostriecin, okadaic acid, and calyculin A.[4] Spirastrellolide A inhibited the activity of protein phosphatase PP2A potently (IC_{50} = 1 nM), PP1 much less potently (IC_{50} = 50 nM) and PP2C not at all (Figure 5). Since spirastrellolide A exerts its cellular effects at submicromolar concentrations, its cellular targets are most likely protein phosphatases.

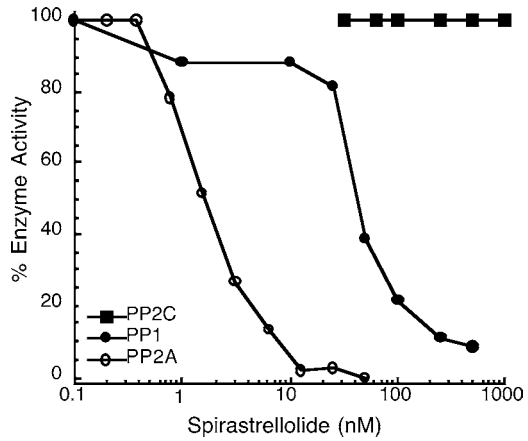

Figure 5. In vitro inhibition of PP2A and PP1 by **6**.

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Supporting Information Available: Experimental section, ^{1}H NMR spectra, and tables of ^{1}H and ^{13}C NMR data for **4**, **5**, and the pentaacetate of **4**; ESMS data for **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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