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Spirastrellolide B Reveals the Absolute Configuration of the Spirastrellolide Macrolide Core

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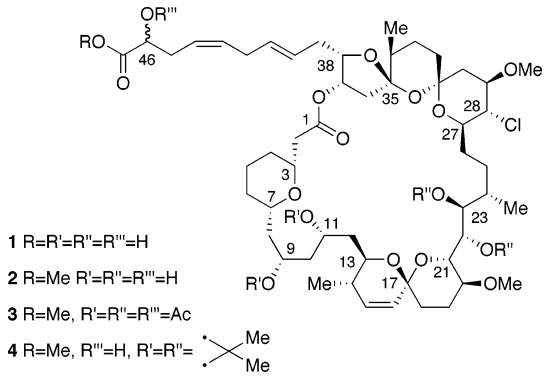
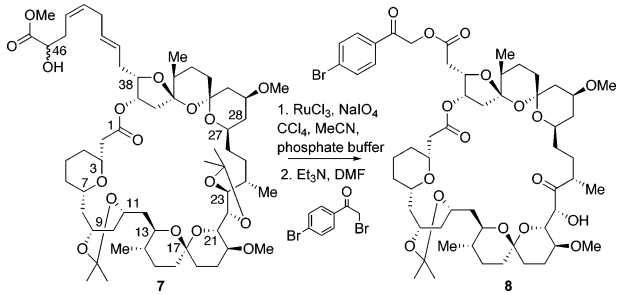
Spirastrellolide A (**1**) represents the first example of a new family of biologically active macrolides obtained from extracts of the marine sponge *Spirastrella coccinea* collected in Dominica.[1] It was initially isolated by our group because it showed strong activity and an unusual phenotypic response in a cell-based assay used to discover new natural product antimitotic agents.[1a,2] Subsequently, spirastrellolide A (**1**) was found to be a potent ($IC_{50} \approx 1$ nM) and selective inhibitor of protein phosphatase 2A.[1b] The structure of spirastrellolide A was elucidated by analysis of NMR and MS data obtained for the methyl ester **2**, peracetate **3**, and bisacetonide **4**.[1] Several groups have prepared analogues of major fragments of spirastrellolide A[3], and comparison of the NMR data obtained for these synthetic fragments with the data for the methyl ester **2** has provided further support for the constitution of **1** and our proposed relative configurations[1b] for the C-3 to C-7, C-9 to C-24, and C-27 to C-38 regions of the natural product.



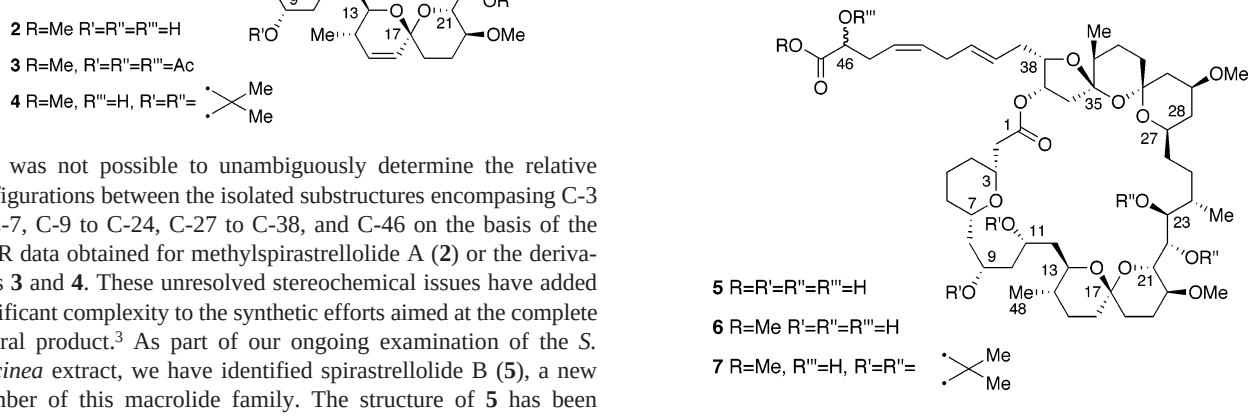
It was not possible to unambiguously determine the relative configurations between the isolated substructures encompasing C-3 to C-7, C-9 to C-24, C-27 to C-38, and C-46 on the basis of the NMR data obtained for methylspirastrellolide A (**2**) or the derivatives **3** and **4**. These unresolved stereochemical issues have added significant complexity to the synthetic efforts aimed at the complete natural product.[3] As part of our ongoing examination of the *S. coccinea* extract, we have identified spirastrellolide B (**5**), a new member of this macrolide family. The structure of **5** has been elucidated via detailed analysis of the spectroscopic data obtained for its methyl ester **6** in combination with a single-crystal X-ray diffraction analysis of the chemical transformation product **8** (Scheme 1). The X-ray analysis of **8** has also revealed the absolute configuration of the spirastrellolide macrolide core.

Spirastrellolide B methyl ester **6** was isolated from the crude *S. coccinea* MeOH extract following the procedure previously reported

Scheme 1. Chemical Transformation of Bisacetonide **7** to Crystalline Derivative **8**



for the isolation of spirastrellolide A methyl ester **2** (Supporting Information).[1] The NMR data recorded for **6** showed a strong resemblance to the data previously obtained for **2** indicating that spirastrellolides A and B were closely related. Methyl ester **6** gave a $[M + Na]^+$ ion at *m/z* 1017.5762 in the HR-ESI-MS consistent with a molecular formula of $C_{53}H_{86}O_{17}$ (calcd for $C_{53}H_{86}O_{17}Na$, 1017.5763) requiring 11 sites of unsaturation, which is one less than spirastrellolide A (**1**), The HR-ESI-MS data also showed that spirastrellolide B was missing the chlorine atom found in **1**. Only four olefinic methines were observed in the ^{13}C NMR spectrum of **6**, suggesting that the missing site of unsaturation relative to **2** was due to reduction of one of the double bonds present in **2**.



Analysis of the HMQC, COSY, HOHAHA, and HMBC data recorded for **6** (Supporting Information) showed that spirastrellolide B (**5**) contained a C-27 to C-47 fragment identical in all respects to that found in spirastrellolide A (**1**), except that it was missing the chlorine substituent found at C-28 in **1**. Thus, in the COSY spectrum of **6**, a resonance at δ 3.76 assigned to H-29 showed correlations to a pair of geminal methylene proton resonances at δ 1.19 and 1.94, assigned to H-28 and H-28′. These were in turn correlated to a resonance at δ 3.71, assigned to H-27, demonstrating the absence of a substituent at C-28. The 2D NMR data for **6** also

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confirmed that spirastrellolide B contained the C-26 to C-17 fragment found in spirastrellolide A, and the similarity in ^{13}C and ^{1}H chemical shift assignments and coupling constants for this region of the two molecules (Supporting Information) indicated that the relative configurations of the stereogenic centers in this fragment were identical in **2** and **6**.

The absence of ^{13}C resonances that could be assigned to a third olefin in **6** suggested that spirastrellolide B lacked the $\Delta^{15,16}$ double bond found in spirastrellolide A. This was confirmed by the HMBC data which showed correlations from a methyl resonance at δ 0.82, assigned to Me-48, to methine carbon resonances at δ 73.4 and 35.0 assigned to C-13 and C-14, respectively, and to a methylene carbon resonance at δ 29.3, assigned to C-15. HMQC correlations were observed from the C-15 resonance (δ 29.6) to proton resonances at δ 1.40 and 1.55. Further 2D NMR analysis showed that **6** contained a linear C-12 to C-9 fragment including the presence of hydroxyl functionalities at C-11 and C-9, a C-3 to C-7 tetrahydropyran fragment, and a C-1 to C-37 macrolide linkage identical to those found in **2**.

Previous attempts in our laboratory to form heavy-atom derivatives of spirastrellolide A methyl ester **2** or the bisacetonide **4** all failed to generate single crystals suitable for X-ray diffraction analysis. Spirastrellolide B (**5**) is missing the $\Delta^{15,16}$ olefin found in **1**, and this opened up the possibility of making derivatives having a side chain truncated at C-40. Our expectation was that the macrolide core of **5** should be quite rigid and, therefore, derivatives missing the conformational flexibility present in the side chain might be more highly crystalline. To test this possibility, methyl ester **6** was first converted in good yield to the bisacetonide **7** by reaction with 2,2-dimethoxypropane and PPTS. Oxidative cleavage of the side chain olefins was carried out using $RuCl_3/NaIO_4$ and, without workup, the putative C-40 carboxylic acid was reacted with *p*-bromophenacylbromide and Et_3N in DMF to give the ester **8** (Scheme 1).

^{1}H NMR and HR-ESI-MS analysis of **8** (Supporting Information) suggested that one of the acetonides had been cleaved and one of the liberated alcohols had been oxidized to a ketone during the transformation of bisacetonide **7** to the ester **8**. As anticipated, ester **8** gave suitable crystals for X-ray diffraction analysis. The structure was solved by direct methods (Supporting Information)[4], and all non-hydrogen atoms were refined with anisotropic thermal parameters, while all hydrogen atoms were placed in calculated positions and not refined. Despite having a weakly diffracting crystal, the presence of the bromine atom (with its large degree of anomalous dispersion) was enough to unambiguously determine the absolute configurations of the stereocentres as defined based on the refined Flack parameter[5] value of 0.004(8).

Examination of the ORTEP diagram for **8** (Figure 1) revealed that the C-22/C-23 acetonide present in **7** was missing in **8** and the C-23 alcohol had been oxidized to a ketone. NMR analysis of **6** had identified an alcohol at C-23 and showed that the C-22/C-23/C-24 relative configurations in spirastrellolide B (**5**) were identical to the relative configurations for the same three centers in spirastrellolide A (**1**). Therefore, the X-ray diffraction analysis of **8** confirmed the constitution of the macrolide core of spirastrellolide B (**5**) that was assigned from NMR analysis of **6** and comparison with the proposed structure for spirastrellolide A (**1**) (vide supra). Relative configurations within the individual C-3 to C-7, C-9 to C-24, and C-27 to C-38 fragments in **8** were identical to those we had previously assigned for **1** via NMR analysis. The absolute configurations of the stereogenic centers in the macrolide core as

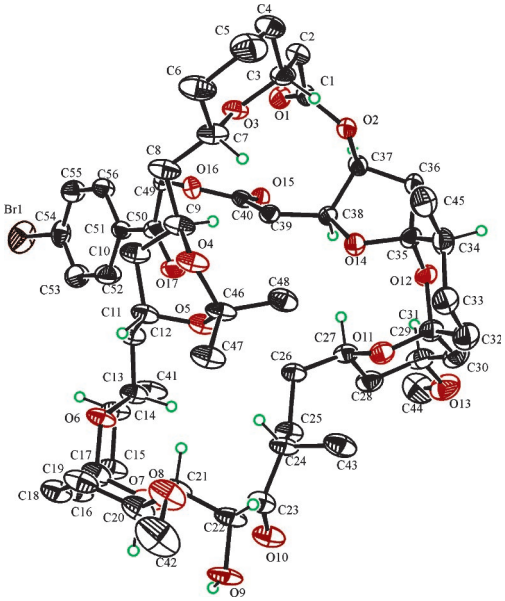

Figure 1. ORTEP diagram (33% ellipsoids) for the transformation product **8**.

revealed via X-ray diffraction analysis of **8** are 3*R*,7*S*,9*S*,11*S*,13*R*,14*S*,17*S*,20*S*,21*R*,22*S*,23*S*,24*S*,27*R*,29*S*, 31*R*,34*S*,35*R*,37*S*,38*S* for spirastrellolide B (**5**) and 3*R*,7*S*,9*S*, 11*S*,13*R*,14*S*,17*R*,20*S*,21*R*,22*S*,23*S*,24*S*,27*R*,28*S*,29*R*,31*R*,34*S*, 35*R*,37*S*,38*S* for spirastrellolide A (**1**). All that remains to complete the structures of the spirastrellolides is to determine the absolute configuration at C-46, which is currently under investigation in our laboratory. Knowledge of the absolute configuration of the spirastrellolide core should facilitate the ongoing efforts toward the total synthesis of this challenging natural product target.[3]

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Supporting Information Available: Experimental details, NMR data for **6**−**8**, and X-ray diffraction data for **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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