ChemInform Abstract: Bioactive Marine Metabolites. Part 94. Relative and Absolute Stereochemistry of Mycalolides, Bioactive Macrolides from the Marine Sponge Mycale magellanica

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Relative and Absolute Stereochemistry of Mycalolides, Bioactive Macrolides from the Marine Sponge *Mycale magellanica*[1]

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The mycalolides are macrocyclic lactones belonging to an emerging class of marine natural products.[1,2] These molecules contain an unusual trisoxazole moiety, which in turn is joined to an 11-carbon, stereochemically complex acyclic chain, lactonized at the C24 position. The mycalolides were isolated from marine sponges of the genus *Mycale* and the hard coral *Tubastrea faulkneri*.[3−5] Mycalolides A−C (**1**−**3**) were isolated from *Mycale* sp. as potent cytotoxins possessing inhibitory activity against B-16 melanoma cells[3] and were found to be potent actin-depolymerizing agents.[6] Other related natural products exhibiting the same mechanism of action include latrunculins,[7] scytophycins,[8] swinholides,[9] and aplyronins.[10] Subsequently, we isolated three new mycalolides, 30-hydroxymycalolide (**4**), 32-hydroxymycalolide A (**5**), and 38-hydroxymycalolide B (**6**) from *Mycale magellanica*, and disclosed that **1**−**6** possess the same relative stereochemistry at all stereogenic centers except that of the pendant esters (Figure 1).[5] The structural similarities among the mycalolides, ulapualides, halichondramides, and kabiramides, whose absolute stereochemical configurations have not been determined, provide circumstantial evidence for a common or central absolute stereochemical assignment consistent with that found for ulapualides A and B.[11] This prediction has not been confirmed experimentally, but it is likely that the mycalolides possess the same stereochemistry as the halichondramides, ulapualides, and kabiramides. Though the stereochemistry of other actin-depolymerizing macrolides has been determined by either X-ray crystallography or a combination of chemical degradation and synthesis, the stereochemistry of the trisoxazole-containing macrolides has not been determined.[2] In this communication we describe the asymmetric synthesis of key degradation products and the assignment of the relative and absolute stereochemistry of the mycalolides.

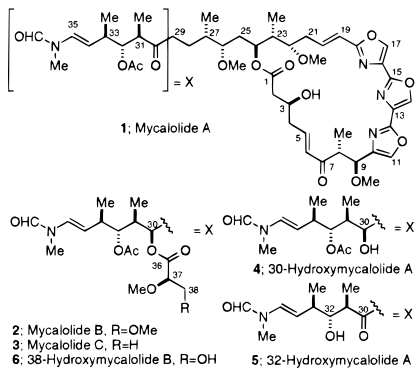

Figure 1. Mycalolides **1**−**6**.

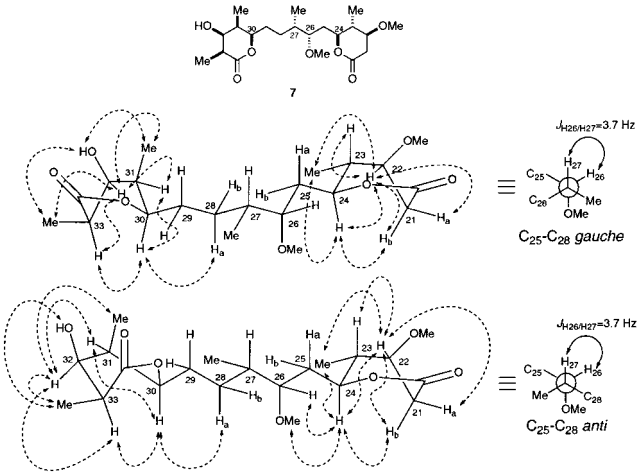

Figure 2. NOESY cross-peaks observed for **7**.

Our initial attempts at elucidating the relative stereochemistry of the C20−C34 fragment by extensive 2D-NMR experiments were hampered by the presence of multiple conformers. This was evident from the presence of an excess of NOESY cross-peaks in the region between H-19 and H-27 which could not be ascribed to a single conformer. Therefore, mycalolide B (**2**) was converted to a derivative in which continuous stereocenters are part of a conformationally biased six-membered lactone. Mycalolide B was oxidized with RuO_4[12] followed by methanolysis and lactonization to afford bislactone **7**. Analysis of **7** revealed intense NOESY cross-peaks between H-21b and H-24 and H-30 and H-33, suggesting that both lactone rings adopted a boatlike conformation (Figure 2).[13] With this working hypothesis in mind, analysis of the three-bond $^1H-^1H$ coupling constants and NOESY data led to assignment of the relative stereochemistry around the two rings. H-23 is anti to H-24 on the basis of a coupling constant of 9.4 Hz. Despite a small coupling constant for H-23 and H-22 (J = 5.0 Hz), a NOESY correlation between H-22 and H-24 indicated that H-22 is trans to H-23. Coupling constants of 5−6 Hz for H-30 and H-31 and for H-32 and H-33 indicated that these pairs of protons were in a syn relationship. This was supported by the

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(1) Bioactive Marine Metabolites. 94. Part 93: Fusetani, N.; Warabi, K.; Nogata, Y.; Nakao, Y.; Matsunaga, S. *Tetrahedron Lett.* In press.

(2) Other trisoxazole-containing macrolides are (a) kabiramides A−E (Matsunaga, S.; Fusetani, N.; Hashimoto, K.; Koseki, K.; Noma, M.; Noguchi, H.; Sankawa, U. *J. Org. Chem*. **1989**, *54*, 1360−1363), (b) ulapualides A and B (Roesener, J. A.; Scheuer, P. J. *J. Am. Chem. Soc*. **1986**, *108*, 846−847), (c) halichondramides (Kernan, M. R.; Molinski, T. F.; Faulkner, J. D. *J. Org. Chem*. **1988**, *53*, 5014−5020), and (d) jaspisamides A−C (Kobayashi, J.; Murata, O.; Shigemori, H. *J. Nat. Prod*. **1993**, *56*, 787−791).

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(12) Carlsen, P. H. J.; Katsuki, T.; Martin, V. S.; Sharpless, K. B. *J. Org. Chem*. **1981**, *46*, 3936−3938.

(13) For other examples of lactones adopting a boat conformation see: Walaszek, Z.; Horton, D.; Ekiel, I. *Carbohydr. Res.* **1982**, *106*, 193−201. For the ^1H NMR data of **7**, see Table 1 of the Supporting Information.

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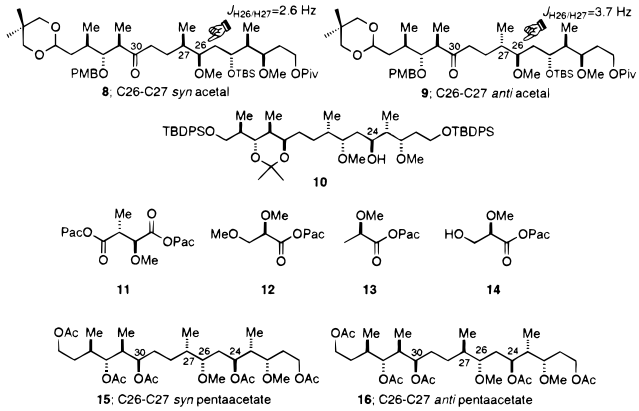


Figure 3. Structures **8**−**16**.

NOESY cross-peaks OH-32/Me-31 and OH-31/Me-33. Measurement of the *J* values for H-24, H_2-25, and H-26 and NOESY data indicated an anti relationship between H-24 and H-26. However, a coupling constant of 3.7 Hz for H-26 and H-27 and numerous NOESY cross-peaks among protons at C26 and C27 suggested that this section of the molecule adopted two conformations: one with an extended carbon chain and the other with a skewed conformation where C25−C28 adopted a gauche relationship (Figure 2). Assignment of the relative stereochemistry of C26 and C27 remained ambiguous even after comparison of NMR data with two model compounds, **8** and **9** (Figure 3). The $J_{26,27}$ value for the 26,27-syn derivative **8** was 2.6 Hz, whereas that of the 26,27-anti derivative **9** was 3.7 Hz. Since the difference of 1 Hz is not large enough to be conclusive, we found it necessary to synthesize the C20−C35 pentaacetate fragments **15** and **16** in a stereoselective manner for comparison with the fragment obtained through degradation of mycalolide (vide infra). In this manner we were able to assign the relative stereochemistry of this portion of the natural product.

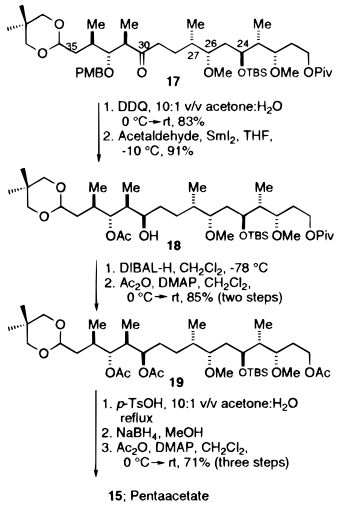
To assign the absolute stereochemistry, we relied on the synthesis and analysis of Mosher derivatives of the secondary hydroxyl groups at C3, C30, and C32. The absolute stereochemistry at C3 was established to be *S* by the advanced Mosher analysis of mycalolide B.[14] The stereochemistry of C30 in 30-hydroxymycalolide A (**4**) and C32 in 32-hydroxymycalolide A (**5**) was similarly assigned to be 30*R* and 32*R*, respectively. Advanced Mosher analysis of the derivative possessing a secondary hydroxyl group at C24, **10**,[15] led to the assignment of the 24*S* stereochemistry.

The C8−C9 stereochemistry was assigned from 38-hydroxymycalolide B (**6**). This material was oxidized with RuO_4 followed by conversion to a bis-*p*-bromophenacyl ester, **11**. A coupling constant of 7.0 Hz for H-8 and H-9 secured the anti relationship, while the 8*S*,9*R*-stereochemistry was determined by HPLC analysis using a chiral stationary phase (Chiralcel OJ).

Saponification products of mycalolide B (**2**) and C (**3**) and 38-hydroxymycalolide B (**6**) were esterified with *p*-bromophenacyl bromide followed by chromatographic separation to furnish *p*-bromophenacyl 2,3-*O*,*O*′-dimethylglycerate (**12**), *p*-bromophenacyl *O*-methyllactate (**13**), and *p*-bromophenacyl 2-*O*-methylglycerate (**14**), each of which was analyzed by HPLC on a chiral stationary phase (Chiralcel AD with EtOH in the case of **12** and **13** and Chiralcel OJ with EtOH in the case of **14**), thus assigning the D configuration for all the pendant esters.

Unexpectedly, we have determined the C22−C26 region of mycalolides is enantiomeric to the corresponding subunit of scytophycins and aplyronins, despite the stereochemical continuity in the C30−C35 portion of the side chain. To establish the stereochemistry at C27, the pentaacetate of the C20−C35 fragment **15**[15] was prepared from 38-hydroxymycalolide B (**6**) for comparison with synthetic pentaacetates **15** and **16**. The chemical synthesis commences with removal of the PMB ether

Scheme 1



of acetal **17**[16] with DDQ in 83% yield (Scheme 1), followed by a Tishchenko-like reduction to afford **18** in 91% yield.[17] Removal of the pivaloate protecting group and acetylation with Ac_2O/DMAP yielded **19** in 85% yield (two steps). Hydrolysis of the acetal **19** also effected removal of the C24 TBS ether. The derived hydroxy aldehyde was reduced with $NaBH_4$. Peracylation with Ac_2O/DMAP provided pentaacetate **15** in 71% yield for three steps (46% yield overall). Pentaacetate **16**, bearing an anti stereochemical relationship at C26−C27, was synthesized in a similar manner. ^{1}H NMR data of the synthetic pentaacetates **15** and **16** differed significantly in the chemical shifts for one of the *O*-methyl protons and for the higher field resonance of the C28 methylene protons. Further comparison of the ^{1}H NMR spectrum of the synthetic pentaacetates **15** and **16** with that of the pentaacetate derived from 38-hydroxymycalolide B revealed a 26,27-syn relationship, as the spectrum of **15** can be superimposed on that of the natural product derivative.

In closing, we have shown, through the combined use of chemical synthesis, degradation, and careful analysis of 1D and 2D ^{1}H NMR data, the stereochemistry of mycalolides has been unambiguously established as 3*S*,8*R*,9*S*,22*S*,23*R*,24*S*,26*S*,27*S*,-30*R*,31*R*,32*R*,33*R*,37*R*. Importantly, we have also learned that ulapualide B has the same stereochemistry as the mycalolides through comparison of a C20−C35 pentaacetate derived from the natural product with pentaacetate **15**.[18,19] It is noteworthy that the stereochemistry of the C30−C33 region is identical with that of scytophycins, aplyronins, and swinholides, while enantiomeric in the C22−C26 region.

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Supporting Information Available: Experimental procedures and ^{1}H NMR and ^{13}C NMR data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(19) Chattopadhyay and Pattenden reported the first total synthesis of ulapualide A, a related trisoxazole-containing macrolide.[11] As a result of the current degradation studies, a discrepancy in the stereochemistry has arisen.