Marine Natural Products. XXIV.[1] The Absolute Stereostructure of Misakinolide A, a Potent Cytotoxic Dimeric Macrolide from an Okinawan Marine Sponge *Theonella* sp.

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The absolute stereostructure of a potent cytotoxic dimeric macrolide misakinolide A (5) (= bistheonellide A), which was isolated from an Okinawan marine sponge of *Theonella* sp., was determined by chemical correlation with swinholide A (1). The stereochemistry of misakinolide A was shown to be identical with that of another cytotoxic dimeric macrolide, swinholide A (1), which was isolated from the Okinawan marine sponge *Theonella swinhoei* and the stereostructure of which was recently determined.

Keywords misakinolide A; bistheonellide A; swinholide A; marine sponge; *Theonella* sp.; *Theonella swinhoei*; cytotoxicity; dimeric macrolide

During the course of our search for new biologically active substances from marine organisms,[1,2] we have isolated from the Okinawan marine sponge *Theonella swinhoei* five tridecapeptide lactones, theonellapeptolides Ia—Ie,[3-5] and four cytotoxic dimeric macrolides, swinholides A (**1**),[6-8] B (**2**)[1] and C (**3**),[1] and isoswinholide A (**4**).[1] We have elucidated the absolute stereostructures of these constituents, among which swinholide A (**1**) was first isolated from the marine sponge *Theonella swinhoei* (collected from the Red Sea) by Kashman and co-workers and reported to be a monomeric macrolide having antifungal activity.[9,10] We have found that swinholide A (**1**) is a potent cytotoxin against various human carcinoma cells[11] and we concluded that swinholide A (**1**) has a 44-membered dilactone moiety. The absolute stereostructure of **1** was determined by a combination of X-ray crystallographic analysis and chemical degradation studies.[6-8] Furthermore, the congeneric dimeric macrolides, swinholides B (**2**) and C (**3**) and isoswinholide A (**4**), were also isolated from the Okinawan sponge specimen, and their absolute stereostructures have been determined by means of chemical correlations.[1]

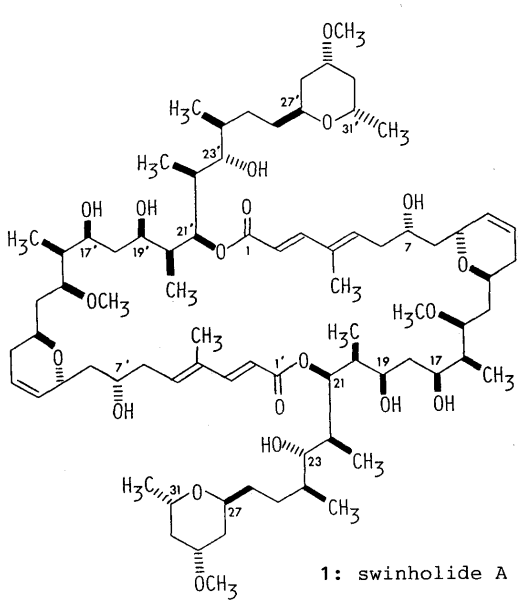
On the other hand, in 1986 the University of the Ryukyus group isolated another cytotoxic macrolide named misakinolide A from another Okinawan marine sponge species of *Theonella* and elucidated its gross structure as **5** (except for the stereochemistry), which possesses a 40-membered dimeric macrolide structure.[12,13] The carbon skeleton together with the oxygen functions in **5** are identical with those of swinholide A (**1**) except for the additional conjugated double bonds in **1**. Detailed comparison of the proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) data for swinholide A (**1**) and misakinolide A (**5**) and also for their monomeric methyl esters **6** and **7**, which were obtained by methanolysis of **1** and **5** respectively, has shown that **1** and **5** may have identical stereostructure.[6,7,14] These observations prompted us to clarify the absolute stereostructure of misakinolide A (**5**).

As described in the previous papers,[6-8] ozonolysis at -78 °C of the monomeric methyl ester **6**, which was obtained by methanolysis of swinholide A (**1**), and subsequent treatment of the product with semicarbazide, furnished a trisemicarbazone (**8**). Since the trisemicarbazone **8** thus prepared retains all 15 chiral centers of **6**, and thus those of swinholide A (**1**), we considered that it might be possible to chemically correlate **1** with misakinolide A (**5**) by preparation of **8** from **5**.

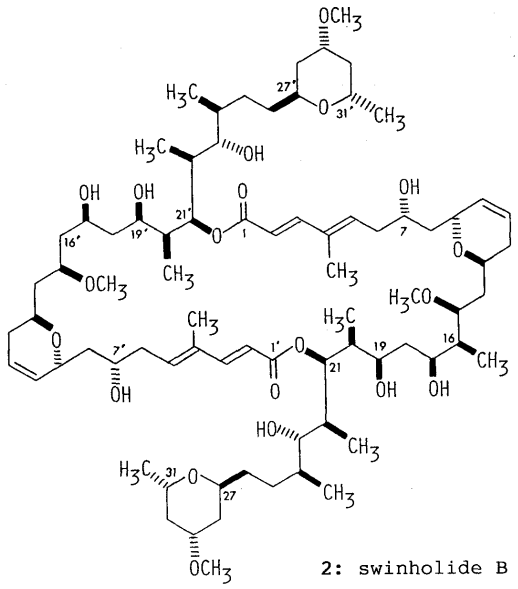
Treatment of misakinolide A (**5**) with 28% methanolic sodium methoxide liberated a monomeric methyl ester **7**, which gave identical NMR spectral data with those reported previously.[14] The methyl ester **7** in methanol–pyridine solution was treated with ozone at -78 °C and subsequently with dimethyl sulfide. The resulting trialdehyde was then treated with semicarbazide and sodium acetate in aqueous methanol to provide a trisemicarbazone as a single product. The trisemicarbazone gave an $(M+Na)^+$ ion peak at m/z 856 in its fast atom bombardment mass spectrum (FABMS). The ^{1}H-NMR spectrum of the trisemicarbazone showed three olefinic proton signals at δ 7.30 (1H, t, $J=5.6$ Hz), δ 7.25 (1H, t, $J=5.5$ Hz), and δ 7.12 (1H, d, $J=7.3$ Hz), respectively assignable to 11-H, 5-H and 10-H. Finally, it was confirmed that the trisemicarbazone obtained from misakinolide A (**5**) is identical with the trisemicarbazone **8** described above from swinholide A (**1**) in terms of infrared (IR), ultraviolet (UV), ^{1}H- and ^{13}C-NMR spectral comparisons, as well as optical rotation and high performance liquid chromatography (HPLC) behavior.

Consequently, the absolute configurations of misakinolide A (**5**) have been determined to be 5*S*, 5′*S*, 7*R*, 7′*R*, 11*S*, 11′*S*, 13*S*, 13′*S*, 14*S*, 14′*S*, 15*S*, 15′*S*, 17*R*, 17′*R*, 18*S*, 18′*S*, 19*S*, 19′*S*, 20*S*, 20′*S*, 21*S*, 21′*S*, 22*S*, 22′*S*, 25*S*, 25′*S*, 27*R*, 27′*R*, 29*S*, and 29′*S*, as shown.

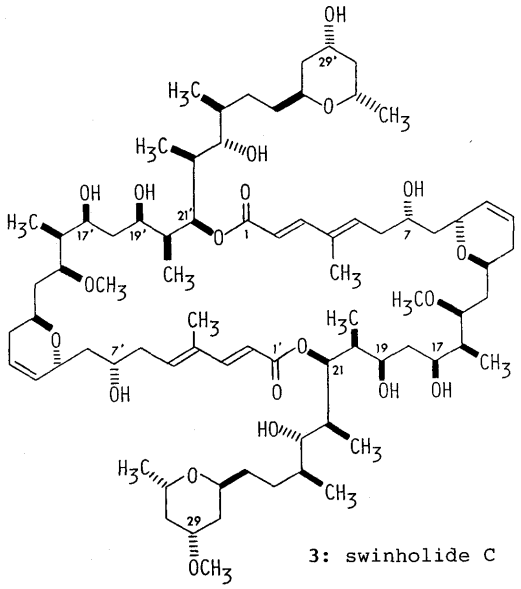
The stereostructure of misakinolide A (**5**) is therefore the same as that of swinholide A (**1**). In this connection, it is interesting to note that both **1** and **5** exhibit potent cytotoxic activities against human carcinoma cells.[7,13] In 1986, a cytotoxic monomeric macrolide, scytophycin C (**9**), was isolated from the cultured terrestrial blue-green alga *Scytonema pseudohofmanni* by Moore and co-workers.[15] The atomic arrays in the monomeric halves of swinholide A (**1**) and misakinolide A (**5**) are very similar to that in scytophycin C (**9**). Furthermore, the configurations at the corresponding asymmetric carbons of **1** and **5** (from C_5 to C_{27}) are identical with those of **9**. So, as was suggested by Moore and his group,[15] the true producers of these dimeric macrolides, swinholide A (**1**) and misakinolide A (**5**), may be symbiotic blue-green algae which are parasitic and/or symbiotic in their host marine sponges.



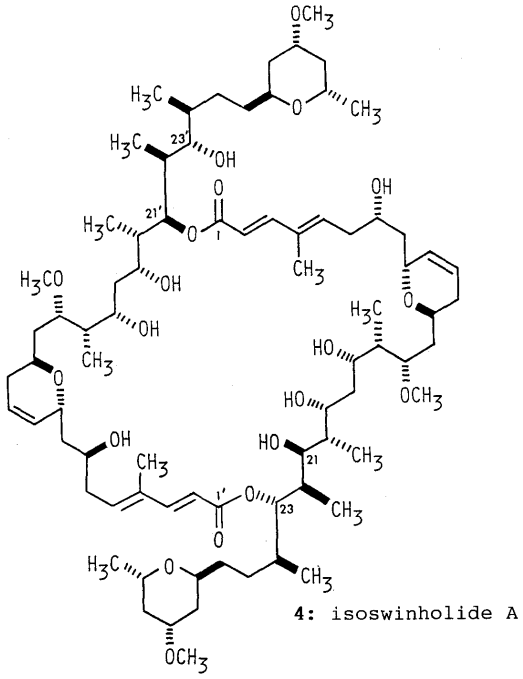
1: swinholide A



2: swinholide B



3: swinholide C



4: isoswinholide A

Chart 1

Experimental

The experimental conditions used to obtain physical data and for chromatography were the same as those reported in the previous papers.[1,6]

Isolation of Misakinolide A (5) Fresh sponges of *Theonella* sp. (9 kg, wet) collected from the coral reef of Kume Island were steeped in methanol (9 l). After removal of the organic solvent under reduced pressure, the resulting suspension was extracted with chloroform. The chloroform solution was evaporated under reduced pressure to give the extract (27 g). A portion (3 g) of the extract was chromatographed on a silica gel column eluting with a mixture of ethyl acetate and methanol (10 : 1), and subsequently subjected to HPLC [Hibar RP-8, MeOH–H_2O (7 : 1)] to afford misakinolide A (**5**) (67 mg)[12] as a white amorphous powder.

Methanolysis of Misakinolide A (5) A solution (0.5 ml) of 28% sodium methoxide in methanol was added dropwise to a solution of misakinolide A (**5**) (80 mg) in methanol (2 ml). The reaction mixture was stirred at room temperature for 2 h, then partitioned into ethyl acetate and water, and the ethyl acetate layer was taken and dried over $MgSO_4$. After evaporation of the solvent under reduced pressure, the crude product (82 mg) was purified by HPLC [Cosmosil $5C_{18}$, MeOH–H_2O (10 : 1)] to furnish the methyl ester **7** (49 mg, 58%). The methyl ester **7**: A white amorphous powder, $[\alpha]_D^{26}$ −25° (c=1.2, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3420, 2940, 1705, 1645, 1455, 1435, 1380, 1080, 970. UV λ_{max}^{MeOH} nm (ε): 218 (8800). The ^{1}H-NMR (500 MHz, C_6D_6) and ^{13}C-NMR (125 MHz, C_6D_6) data for **7** were identical with those reported.[13,14]

Ozonolysis of Methyl Ester 7 and Preparation of Trisemicarbazone 8 A solution of the methyl ester **7** (47 mg) in methanol (10 ml) and pyridine (0.5 ml) was bubbled with a stream of ozonated O_2 at −78 °C for 1 min. After the removal of excess ozone by flushing with nitrogen, dimethyl sulfide (3 ml) was added to the solution, and the reaction mixture was stirred for 1 h while warming up to room temperature. A solution of semicarbazide hydrochloride (60 mg) and sodium acetate trihydrate (73 mg) in methanol–water (1 : 1, 1 ml) was added to the reaction mixture and the whole was stirred at room temperature for 2 h, then partitioned into a mixture of ethyl acetate and butanol (1 : 1) and water. The organic layer was separated and dried over $MgSO_4$, then the solvent was evaporated off under reduced pressure to yield the crude product (40 mg), which was purified by HPLC [Cosmosil $5C_{18}$, MeOH–H_2O (1 : 1)] to give the trisemicarbazone **8** (25 mg, 46%). The trisemicarbazone **8**: A white amorphous powder, $[\alpha]_D^{26}$ +6.5° (c=2.3, MeOH). IR ν_{max}^{neat} cm^{-1}: 3360, 2930, 1675, 1580, 1425, 1380, 975. UV λ_{max}^{MeOH} nm (ε): 231 (31500). ^{1}H-NMR (500 MHz, CD_3OD) δ: 7.30 (1H, t, J=5.6 Hz, H_{11}), 7.25 (1H, t, J=5.5 Hz, H_5), 7.12 (1H, d, J=7.3 Hz, H_{10}), 4.23 (1H, m, H_9), 4.18

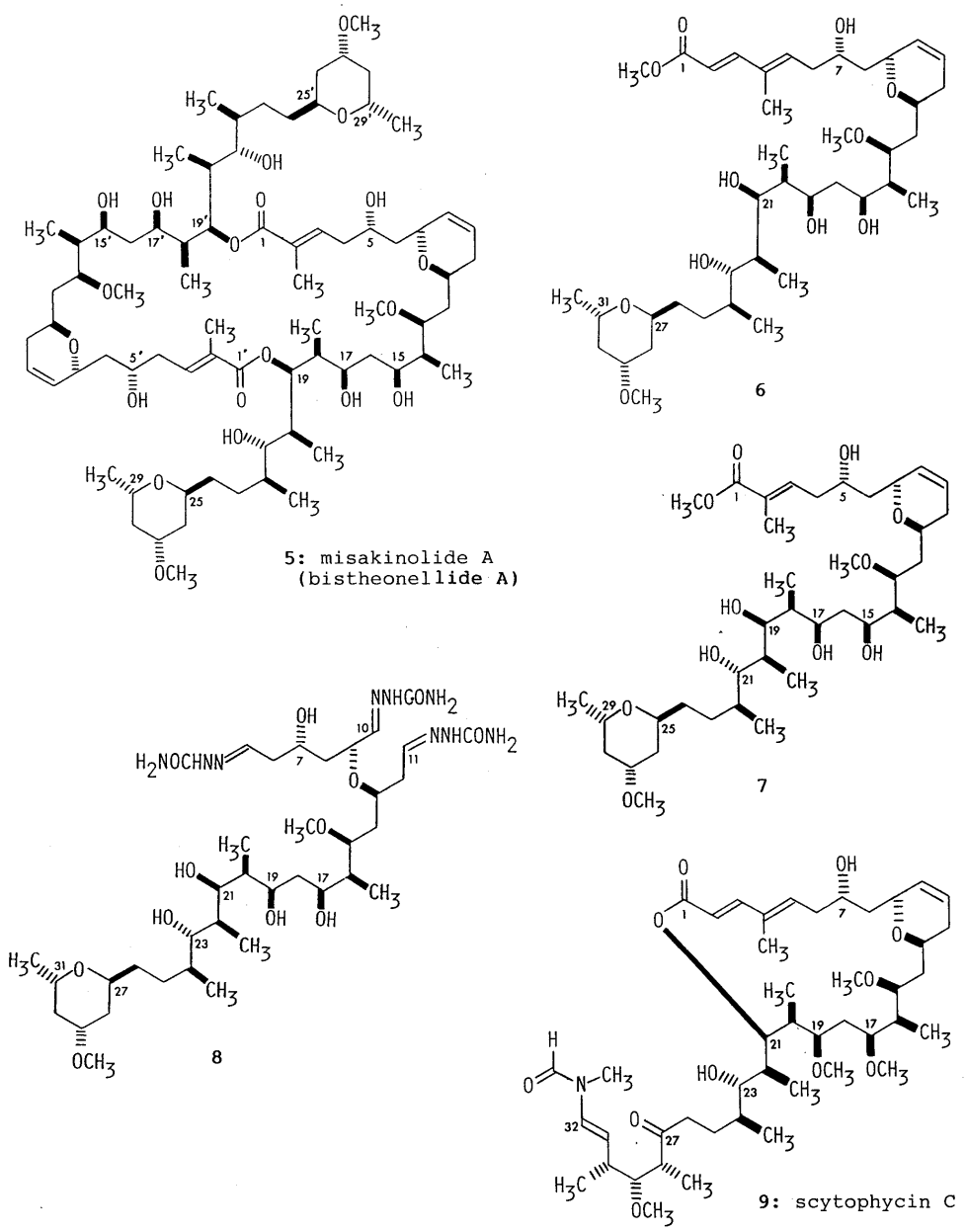


Chart 2

(1H, m), 4.06 (1H, d, J=9.8 Hz, H_{21}), 4.04 (1H, m, H_7), 3.97 (1H, m), 3.77 (2H, m), 3.70 (2H, m), 3.61 (1H, dddd, J=10.2, 10.2, 4.5, 4.5 Hz, H_{29}), 3.36 (1H, m), 3.34 (6H, s, $H_{15,29\text{-OMe}}$), 2.60 (1H, ddd, J=14.7, 5.6, 5.6 Hz, H_{12a}), 2.49 (1H, ddd, J=14.7, 5.8, 5.8 Hz, H_{12b}), 2.41 (2H, m, H_6), 2.01 (1H, br d, J=12.5 Hz), 1.18 (3H, d, J=6.1 Hz, $H_{31\text{-Me}}$), 1.10 (1H, ddd, J=12.5, 10.1, 10.1 Hz, H_{30a}), 0.95 (3H, d, J=6.7 Hz, $H_{16\text{-Me}}$), 0.92 (3H, d, J=7.0 Hz, $H_{24\text{-Me}}$), 0.79 (3H, d, J=7.0 Hz, $H_{22\text{-Me}}$), 0.78 (3H, d, J=7.0 Hz, $H_{20\text{-Me}}$). ^{13}C-NMR (125 MHz, CD_3OD) δ_C: 160.1; 160.1; 160.0 (each s, C=O), 146.8 (d, C_5 or C_{10} or C_{11}), 144.6 (d, C_5 or C_{10} or C_{11}), 144.2 (d, C_5 or C_{10} or C_{11}), 80.0 (d, C_{23}), 78.5 (d), 75.1 (d), 74.5 (d, C_{29}), 74.3 (d), 73.5 (d, C_{27}), 73.4 (d), 73.1 (d), 72.7 (d, C_{21}), 66.3 (d, C_7), 66.1 (d, C_{31}), 57.7 (q, $C_{15\text{-OMe}}$), 55.6 (q, $C_{29\text{-OMe}}$), 42.7 (d, C_{16}), 42.0 (t), 41.9 (t), 41.6 (d, C_{20}), 39.9 (t, C_{30}), 39.0 (t, C_{18}), 37.5 (t), 37.0 (d, C_{22}), 37.0 (t), 36.1 (d, C_{24}), 35.9 (t, C_{28}), 30.2 (t, C_{26}), 28.1 (t, C_{25}), 22.0 (q, $C_{31\text{-Me}}$), 17.3 (q, $C_{24\text{-Me}}$), 10.8 (q), 10.2 (q), 10.1 (q). FABMS: m/z 856 $(M+Na)^+$. HRFABMS: Calcd for $C_{37}H_{71}NaN_9O_{12}$: 856.512. Obsd: 856.513. The trisemicarbazone **8** was found to be identical with that obtained above from swinholide A (**1**) by IR, UV, ^{1}H-NMR, ^{13}C-NMR and HPLC [Cosmosil 5C_{18}, MeOH–H_2O (1 : 1)] comparisons.

Acknowledgments The authors wish to thank Prof. T. Momose and Dr. O. Muraoka, Kinki University, for the measurement of FABMS. They are also indebted to the Ministry of Education, Science, and Culture of Japan (Grant-in-Aid for Cancer Research No. 01010039) and Yamada Science Foundation for financial support.

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