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Misakinolide-A, an Antitumor Macrolide from the Marine Sponge *Theonella* Sp.

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A highly active antitumor macrolide, misakinolide-A has been isolated from the sponge *Theonella* sp. The gross structure was determined by extensive NMR studies including homo- and heteronuclear COSY experiments.

In our screening of marine organisms for antitumor agents, a crude extract of the sponge *Theonella* sp. showed significant activity against P388 mouse leukemia cells. Separation of the extract gave a macrolide named misakinolide-A (**1**) as an active constituent. We herein report the isolation and structure elucidation of **1**.

A fresh sample (320 g) of the sponge *Theonella* sp., collected at Maeda-misaki, Okinawa in June, 1985, was extracted by steeping in acetone. The ethyl acetate soluble oil (0.5 g) of the concentrated extract was chromatographed on polystyrene gel (methanol-water 20:1). P388-active fractions were combined and further separated on silica gel by eluting with ethyl acetate and then with dichloromethane-methanol (5:1). The latter eluate was purified by HPLC using a Merck Hibar RP-18 column and methanol as solvent to give 24 mg of misakinolide-A (**1**) as a colorless oil,[1] $[\alpha]_D^{20}$ -21.4° (c 5.6, $CHCl_3$).

The molecular formula $C_{37}H_{64}O_{10}$ was established by high resolution FABMS (M+1 m/z 669.4551, Δ -2.7 mmu) and by combustion analysis.[2] The ^1H and ^{13}C (PND and DEPT) NMR spectra revealed the presence of 5 CH_3 (d's), 1 CH_3 (s, vinylic), 2 OCH_3, 9 CH_2 , 4 CH, 11 CH-O, 3 =CH, 1 =C< , and 1 COO- group. These account for 37 carbons and 60 protons. The remaining four protons were those of hydroxyl groups as confirmed by addition of trichloroacetyl isocyanate (TAI) to the $CDCl_3$ solution of **1**. This addition caused the appearance of four NH signals at δ9.26, 8.98, 8.66, and 8.53 and also the downfield shifts of four CH-O resonances, namely protons at C-5, C-15, C-17, and C-21 to δ5.41, 5.14, 5.02, and 4.68. Since four hydroxyl, two methoxyl, and one ester function account for eight out of ten oxygen atoms and seven out of 11 methinoxy groups, each of the remaining two oxygens must be in an ethereal linkage occupying two methinoxy carbons. Evidences, *vide infra*, suggested that the ether and ester functions form three rings which fulfils the unsaturation requirement of the molecule. Analysis of the 360 MHz ^1H NMR spectra of **1** in

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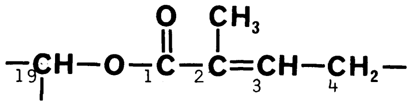
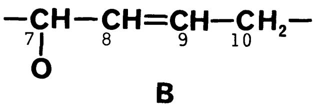


$-\underset{19}{\overset{|}{C}}H-O-\underset{1}{\overset{\overset{O}{\|}}{C}}-\underset{2}{\overset{\overset{CH_3}{|}}{C}}=\underset{3}{CH}-\underset{4}{CH_2}-$

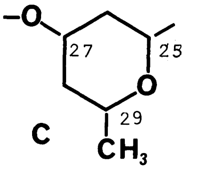
A



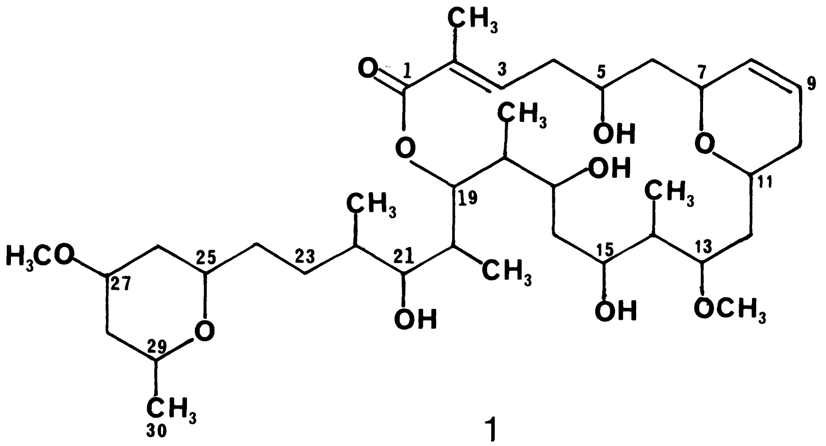
$-\underset{\underset{O}{|}}{\underset{7}{CH}}-\underset{8}{CH}=\underset{9}{CH}-\underset{10}{CH_2}-$

B

C



$CDCl_3$ and in C_6D_6 suggested the partial structures A, B, C, and several -CHCH(O)CH_2- segments. The connectivity of these segments was elucidated by 2D-NMR experiments. The C-H correlation spectra revealed all the one-bond carbon-proton connectivities including all nine geminal methylene proton pairs (see Table 1). Complete H-H correlation was obtained by recording COSY 45 and long range COSY 90 spectra with emphasis on 7, 4, and 2 Hz couplings (Table 1). The spectra taken in C_6D_6 after addition of D_2O were most informative in the CH-O region. Better resolution was obtained by virtue of the cancellation of CH-OH couplings, removal of the overlapping OH signals and better separation of H-5 from H-17 and of H-15 from H-25. Correlations apparent in the COSY spectra allowed the relation of subunits A, B, and C to the connecting segments of the molecule (C_4-C_7, C_{10}-C_{19}, and the side chain C_{19}-C_{25}) providing the planar structure of misakinolide-A (1). It is now evident that 1 is closely related to swinholide-A (2), another macrolide isolated recently by one of us from the Red Sea sponge *Theonella swinhoei*.[3]



1

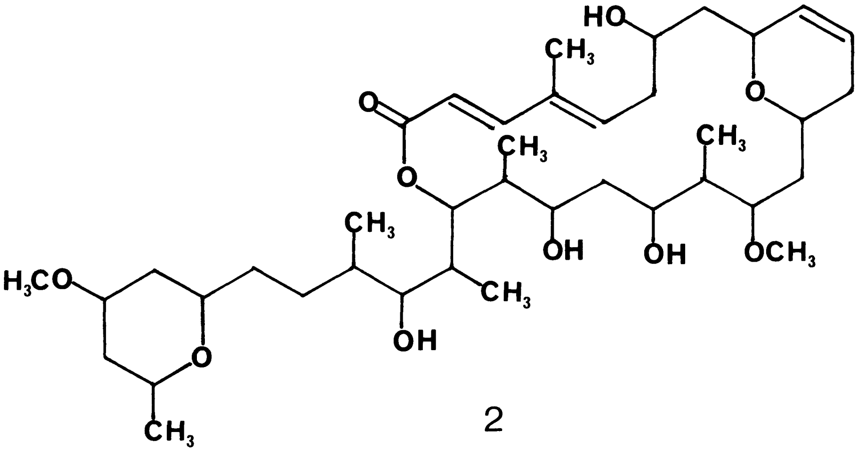


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Table 1. NMR data for misakinolide-A (**1**) in $CDCl_3$ and in C_6D_6 + D_2O

C No.	δ($CDCl_3$)		δ(C_6D_6+ D_2O)		Connectivity			
	^{13}C	^1H	^{13}C	^1H	^2J	^3J	^4J	^5J
1	170.5 s		171.0					
2	128.3 s		129.0					
2-Me	12.7 q	1.85 s	13.0	1.96			3	4,4'
3	142.8 d	7.00 brt	142.0	7.30		4,4'	2-Me	
4	37.4 t	2.22 ddd 2.37 brdt	38.2	2.18 2.34	4' 4	3,5 3,5		2-Me 2-Me
5	66.0 d	4.13 tt	66.8	4.23		4,4',6,6'		
6	41.3 t	1.58 m 1.68 m	41.6	1.41 1.65	6' 6	5,7 5,7		
7	66.5 d	4.51 dq	68.1	4.70		6,6',8	9	10
8	123.2 d	5.75 dq	123.6	5.64		7,9	10	
9	129.8 d	5.65 brdd	130.6	5.59		8,10	7	
10	30.4 t	1.82 m 1.98 m	31.5	1.81	10' 10	9,11	8	7
11	65.1 d	3.80 m	64.3	3.67		10,12,12'		
12	33.8 t	1.50 m 2.09 ddd	35.2	1.54 2.00	12' 12	11,13 11,13		
13	76.7 d	3.83 m	77.7	3.87		12,12',14	13-OMe	
13-OMe	55.2 q	3.18 s	55.0	3.20			13	
14	41.0 d	1.68 m	41.6	1.83		13,14-Me,15		
14-Me	9.0 q[a)]	0.82 d	9.5[b)]	1.04		14		
15	73.9 d	3.75 m	74.9	4.04		14,16,16'		
16	38.3 t	1.60 m	39.0	1.70 1.80	16' 16	15,17 15.17		
17	70.4 d	3.80 m	71.2	4.17		16,16',18		
18	40.3 d	1.78 m	41.1	1.89		17,18-Me,19		
18-Me	9.1 q[a)]	0.92 d	9.5[b)]	1.01		18		
19	75.3 d	5.30 brd	76.3	5.69		18,20		
20	37.1 d	1.90 m	37.7	2.18		19, 20-Me		
20-Me	9.2 q[a)]	0.85 d	9.6[b)]	0.95		20		
21	76.2 d	3.05 dd	76.9	3.28		20, 22		
22	33.2 d	1.67 m	33.4	1.76		21, 22-Me, 23,23'		
22-Me	17.7 q	0.98 m	18.2	1.09		22		
23	24.0 t	1.24 m 1.38 m	24.6		23' 23	22,24,24' 22,24,24'		
24	29.2 t	1.22 m 1.89 m	28.6	1.06 1.85	24' 24	23,23',25 23,23',25		
25	71.5 d	4.00 m	70.7	3.98		24,24',26,26'		
26	35.0 t	1.60 m 1.82 m	35.7	1.65	26' 26	25,27 25,27		
27	73.3 d	3.55 tt	73.6	3.33		26,26',28,28'		
27-OMe	57.1 q	3.18 s	56.8	3.40				
28	38.7 t	1.17 m 1.99 m	39.2	1.19 1.76	28' 28	27,29 27,29		
29	64.6 d	3.70 ddq	64.7	3.53		28,28',29-Me		
29-Me	21.8 q	1.20 d	21.6	1.19		29		

Table 1 (Continued)

$J_{H,H}$ (Hz): 3,4=6.5; 3,4'=7.5; 4,4'=15.5; 4,5=4; 4',5=9.5; 5,6=9.5, 5,6'=2.5; 6,7=9.3; 6',7≈2; 7,9=1; 7,10=1; 8,9=10; 8,10≈2; 8,10'≈2; 9,10=2; 11,12'=3.5; 12,12'=14; 12',13=9; 14,14-Me=7.0; 18,18-Me=6.8; 18,19=9.5; 19,20=1; 20,21=9.7; 20,20-Me=6.4; 21,22=2; 22,22-Me=6.6; 25,26≈3; 26,27=4.2; 26',27=10; 27,28=10; 27,28'=4; 28,29=9; 28',29=3; 29,29-Me=6.0; 7,8≈2; 25,26'=6.

a),b) Interchangeable.

The E-configuration of the C_2-C_3 double bond was established by the absence of NOE between the C_2-methyl and H-3 and by observing a small NOE between the methyl and one of the C-4 protons (δ2.22). Comparison of the ^{1}H-NMR data ($CDCl_3$) of **1** and **2** enabled assignment of the relative configuration of the tetrahydropyrane ring, i.e., of H-25eq., H-27ax., and H-29ax. As in the case of **2**, location of a methoxy group at C-27 was confirmed by EIMS(1) which exhibited a fragment ion at m/z 129 resulting from the cleavage of the bond (C_{24}-C_{25}) α to the ethereal linkage. The second methoxy group was located at the C-13 by COSY LR experiment (emphasis on 2 Hz) that showed a correlation between H-13 and the methoxy protons. The location of the second ethereal bridge was determined to be between C-7 and C-11, constructing a dihydropyrane ring, on the basis of the fact that the ^{1}H-NMR signals for H-7 and H-11 were unaffected by the addition of TAI, *vide supra*, and that allylic and homoallylic couplings were observed between H-7 and H-9 and between H-7 and H-10, respectively.

Misakinolide-A (**1**) possesses a 20-membered macrolide having essentially the same substitution pattern as **2**. The only structural difference is the presence of an additional ethene (CH=CH) unit in **2**, 22-membered macrolide. The oxidation patterns of these macrolides suggest polyketide biogenesis. All the methyl groups in **1** are in positions appropriate for propionate precursors. Other related macrolides are scytophycins isolated from the blue green alga *Scytonema pseudohofmanni*.(4) Misakinolide-A (**1**) showed *in vitro* antitumor activity at the levels of IC_{50} 0.01 μg/ml against P388 and 0.0005-0.005 μg/ml against human tumor cells (HCT-8, A549, MDA-MB-231). Antifungal activity against *Candida albicans* was MIC 5 μg/ml.

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References

1) For **1**: λ_{max} (MeOH) 220 nm (ε 5000); IR (film) 3430, 2970, 2940, 1680, 1640, 1455, 1380, 1260, 1195, 1150, 1125, and 1080 cm^{-1}; EIMS m/z 223 (3.7), 215 (3.7), 183 (10), 165 (8), 155 (24), 142 (13), 129 (100), 113 (19), 97 (45), 87 (97), 85 (52), 81 (28), 71 (37), 69 (50), 67 (36), 59 (89), 58 (54), 57 (63), and 55 (60%).

2) Found: C 65.67, H 9.77%. $C_{37}H_{64}O_{10}\cdot\frac{1}{2}H_2O$ requires C 65.56, H 9.66%.

3) S. Carmely and Y. Kashman, Tetrahedron Lett., 26, 511 (1985).

4) R. E. Moore, G. M. L. Patterson, J. S. Mynderse, and J. Barchi, Jr., Pure Appl. Chem., 58, 263 (1986).

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