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STRUCTURE OF SWINHOLIDE A, A POTENT CYTOTOXIC MACROLIDE FROM THE OKINAWAN MARINE SPONGE *THEONELLA SWINHOEI*

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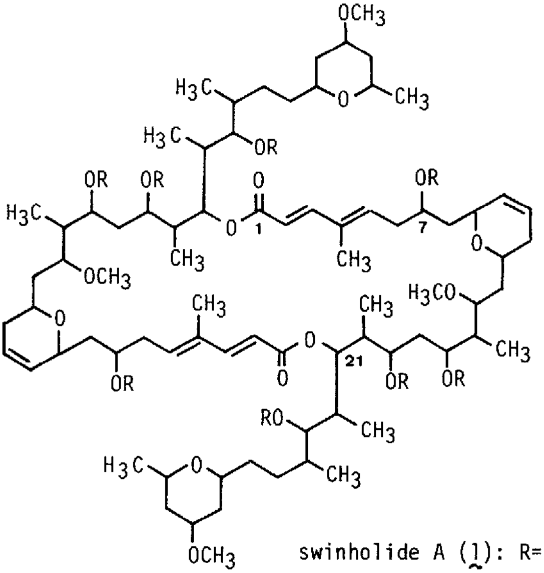
SUMMARY: A potent cytotoxic macrolide, isolated from the Okinawan marine sponge *Theonella swinhoei*, was found identical with swinholide A, which was previously isolated from the Red Sea marine sponge of the same species. The structure of swinholide A has been elucidated to be 1 having a 44-membered dilactone ring.

In search of new biologically active substances from marine organisms,1) we have isolated a macrolide and five tridecapeptide lactones named theonellapeptolides Ia, Ib, Ic, Id, and Ie from the marine sponge *Theonella swinhoei*2), which was collected in July in the coral reef of Zamami-jima, Okinawa Prefecture, and we have elucidated the absolute stereostructure of those tridecapeptide lactones.3) The physicochemical properties of the macrolide4) and its formyl derivative5) obtained by HCOOH treatment were noticed quite similar to those of swinholide A (3), a macrolide polyketide isolated from the Red Sea marine sponge *Theonella swinhoei* by Carmely and Kashman, and its tetraformate 4.6) In fact, our macrolide was found identical with swinholide A by comparison in detail of ^{1}H and ^{13}C NMR spectra.3a) Afterwards, however, we have found that positive and negative FABMS of our macrolide 1 showed ion peaks at m/z 1411 $(M+Na)^+$ and m/z 1388 M^-, respectively, while only 39 carbon signals were observed in the ^{13}C NMR spectrum. It has been presumed therefore that swinholide A may have a dimeric dilactone structure rather than previously proposed structure 3.6) This paper deals with our re-investigation on the chemical structure of swinholide A leading to the structure 1.

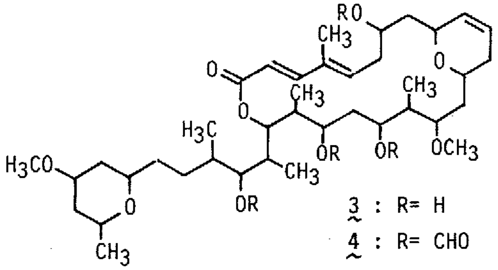
The acetone extract of the fresh marine sponge (2 kg) was partitioned into an AcOEt-H_2O mixture and the AcOEt soluble portion was subjected to silica gel column chromatography ($CHCl_3$-MeOH) to furnish swinholide A (1)4) (84 mg) and the oligopeptide fraction.3) Swinholide A (1) was converted to a diacetonide 5 with 2,2-dimethoxypropane and p-TsOH. On treatment with p-bromobenzoyl chloride in pyridine at 70°C for 2 h, 5 provided a di-p-Br-benzoate (6)7), a tri-p-Br-benzoate (7)8), and a tetra-p-Br-benzoate (8)9). FABMS of 6, 7, and 8 showed $(M+Na)^+$ ion peaks at m/z 1858, 2041, and 2224, respectively. Since 7 has an asymmetrical structure, it gave a complicated ^{1}H NMR spectrum as compared with the spectra of 6 and 8, both having a symmetrical structure. When swinholide A (1) was treated with NaOMe in MeOH, it furnished a monomeric methyl ester 910) which gave an $(M+H)^+$ ion peak at m/z 727 in its FABMS. ^{1}H and ^{13}C NMR analysis including homo and hetero COSY of 9 revealed the partial structures from C_1 to C_{23} and C_{27} to C_{31}-Me in 1.

In order to clarify the connection from C-23 to C-27, the methyl ester 9 was oxidized with O_3 in MeOH-pyridine at -78°C and then treated with dimethyl sulfide. The resulting tri-aldehyde was converted to a trisemicarbazone [δ 7.29 (1H, t, J=5.6 Hz), 7.24 (1H, t, J=5.6 Hz), 7.11 (1H, d, J=7.3 Hz)] with semicarbazide and NaOAc in MeOH-H_2O and then the product was



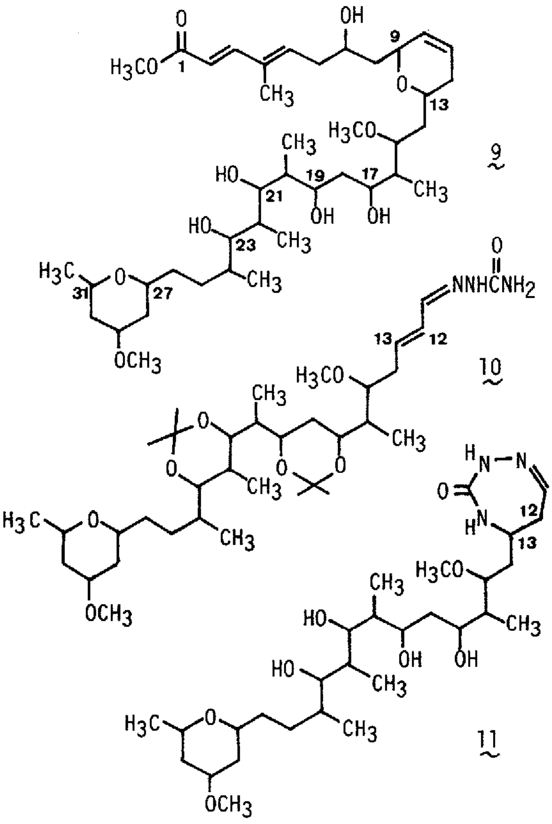
swinholide A (1): R= H

2 : R= CHO



3 : R= H

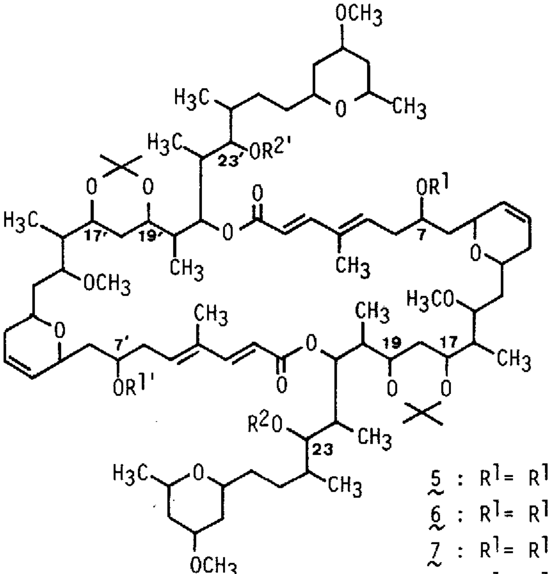
4 : R= CHO



9

10

11



5 : $R^1 = R^{1'} = R^2 = R^{2'} =$ H

6 : $R^1 = R^{1'} =$ p-BrBz , $R^2 = R^{2'} =$ H

7 : $R^1 = R^{1'} = R^2 =$ p-BrBz , $R^{2'} =$ H

8 : $R^1 = R^{1'} = R^2 = R^{2'} =$ p-BrBz

tion of the 17,19;21,23-diacetonide of 9 under the same reaction conditions provided a 12,13-unsaturated semicarbazone (10),[11] which was further converted in good yield to 11 by 80% aq. AcOH treatment (80°C, 30 min). COSY 45 of 11 showed six geminal proton cross peaks (at $C_{14,18,25,26,28,30}$), thus the connectivity from C_{23} to C_{27} has been clarified.

Furthermore, the location of the lactone linkage in swinholide A (1) has been clarified at C_{21} (and also $C_{21'}$) by comparison of the ^{1}H NMR spectra of 1 and 9. Thus, the signal

Table I. ^{13}C and ^{1}H NMR Data for Swinholide A (1), Monomeric methyl ester (9) and 11

	swinholide A (1)[a]		monomeric methyl ester (9)[a]		11[a]	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	169.6 s	----	168.1 s	----		
2	113.3 d	5.79 d	115.9 d	5.89 d		
3	152.5 d	7.58 d	150.3 d	7.62 d		
4	133.9 s	----	134.7 s	----		
4-Me	12.0 q	1.83 s	12.6 q	1.53 s		
5	141.2 d	6.08 dd	139.4 d	5.99 dd		
6	37.4 t	2.18 brd 2.46 ddd	38.1 t	2.22 ddd 2.31 ddd		
7	66.6 d	4.14 brdd	67.4 d	4.12 brddd		
8	40.4 t	1.58 m 1.63 m	40.8 t	1.38 m 1.65 m		
9	66.7 d	4.51 brd	68.9 d	4.63 d		
10	129.7 d	5.69 brdd	130.7 d	5.52 d		
11	123.1 d	5.78 brd	124.0 d	5.63 brd	144.6 d	7.30 dd
12	30.2 t	1.82 m 2.27 brd	31.1 t	1.73 m 1.85 m	39.7 t	2.42 ddd 2.43 ddd
13	65.1 d	3.86 m	65.3 d	3.69 m	74.8 d[b]	3.60 m
14	34.6 t	1.46 ddd 2.14 ddd	35.4 t	1.45 m 2.08 ddd	37.4 t	1.15 m 2.19 ddd
15	75.6 d	4.01 m	78.3 d	3.91 brddd	83.1 d	2.99 ddd
15-OMe	56.9 q	3.35 s	57.3 q[b]	3.24 s[b]	56.5 q[c]	3.36 s[b]
16	41.4 d[b]	1.68 m	41.5 d	1.70 m	43.5 d	1.30 m
16-Me	9.0 q[c]	0.81 d	10.8 q	0.83 d	13.2 q	0.95 d
17	73.5 d	3.83 dd	75.7 d	3.97 m	82.3 d	3.19 ddd
18	38.1 t	1.62 m 1.69 m	37.1 t	1.69 m 1.82 m	38.4 t	1.65 m 1.83 m
19	70.9 d	3.98 m	72.9 d	4.21 d	70.9 d	4.25 ddd
20	40.7 d[b]	1.75 dq	41.0 d	2.01 m	41.2 d	1.64 m
20-Me	8.9 q[c]	0.97 d	12.0 q	0.72 d	9.2 q	0.77 d
21	74.1 d	5.36 d	75.4 d	4.30 d	72.5 d	4.03 dd
22	37.2 d	1.95 m	36.0 d	1.77 m	36.8 d	1.80 m
22-Me	8.8 q[c]	0.84 d	17.0 q	0.86 d	10.2 q	0.89 d
23	75.8 d	3.12 d	80.7 d	3.39 m	80.2 d	3.35 m
24	32.9 d	1.65 m	35.6 d	1.84 m	36.3 d	1.73 m
24-Me	17.4 q	0.99 d	11.1 q	1.14 d	17.2 q	0.83 d
25	23.7 t	1.27 m 1.38 m	28.8 t	1.40 m 1.73 m	28.2 t	1.21 m 1.61 m
26	29.0 t	1.30 m 1.90 m	29.7 t	1.12 m 1.75 m	30.2 t	1.33 m 1.93 m
27	70.9 d	4.02 m	71.7 d	3.97 m	73.6 d	3.98 dddd
28	34.6 t	1.60 m 1.82 m	35.6 t	1.65 m 1.68 m	35.9 t	1.52 ddd 1.88 m
29	72.9 d	3.53 dddd	73.7 d	3.30 dddd	74.5 d[b]	3.60 m
29-OMe	54.8 q	3.33 s	55.3 q[b]	3.11 s[b]	55.6 q[c]	3.33 s[b]
30	38.3 t	1.18 ddd 1.96 m	38.9 t	1.23 m 1.74 m	40.0 t	1.10 ddd 2.02 dddd
31	64.3 d	3.69 ddq	65.2 d	3.58 m	66.1 d	3.76 ddq
31-Me	21.4 q	1.20 d	22.1 q	1.22 d	22.0 q	1.18 d

a Measured at 125 MHz (^{13}C NMR) and 500 MHz (^{1}H NMR) in $CDCl_3$ for 1, in C_6D_6 for 9, and in CD_3OD for 11. b, c Assignments may be interchangeable in the same column.

assignable to 21-H [δ4.30 (d, J=10.1 Hz) in C_6D_6] geminal to 21-OH in 9 was observed at higher field than that [δ 5.86 (d, J=11.0 Hz) in C_6D_6] in 1.

Consequently, the structures of swinholide A (1) and its octaformate (2) have been determined as shown having a 44-membered dilactone structure.[12)] Swinholide A (1) exhibited potent cytotoxic activity (IC_{50} 0.04 μg/ml) for KB cell. The stereostructure of 1 is under investigation.

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1) The recent papers: a) I. Kitagawa, M. Kobayashi, M. Hori, and Y. Kyogoku, Chem. Pharm. Bull., 37, 61 (1989); b) I. Kitagawa, Yakugaku Zasshi, 108, 398 (1988).

2) Recently identified by Prof. P. R. Bergquist, the University of Auckland, New Zealand, to whom the authors are grateful.

3) a) Theonellapeptolide Id: I. Kitagawa, M. Kobayashi, N. K. Lee, H. Shibuya, Y. Kawata, and F. Sakiyama, Chem. Pharm. Bull., 34, 2664 (1986); b) Ie: I. Kitagawa, N. K. Lee, M. Kobayashi, and H. Shibuya, Chem. Pharm. Bull., 35, 2129 (1987); c) Ia, Ib, and Ic: M. Kobayashi, N. K. Lee, H. Shibuya, I. Kitagawa, and T. Momose, presented at the 108th Annual Meeting of the Pharmaceutical Society of Japan held at Hiroshima, April 1988, Abstract paper p. 309.

4) **1**: amorphous solid, $[\alpha]_D$ +16° ($CHCl_3$), UV (MeOH, max): 270 nm (ε=41400), IR ($CHCl_3$): 3440, 1675, 1615 cm^{-1}, Anal. Calcd for: $C_{78}H_{132}O_{20}\cdot 2H_2O$, C, 65.70; H, 19.61. Found: C, 66.00; H, 19.64.

5) **2**: $[\alpha]_D$ -7.5° ($CHCl_3$), UV (MeOH): 266 nm (ε=40500), ^1H NMR (500MHz, C_6D_6, δ): 8.54, 7.96, 7.80, 7.63 (each 2H, s), 5.49 (4H, m, 7,7',17,17'-H), 5.21 (2H, m, 19,19'-H), 4.96 (2H, m, 23,23'-H).

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

7) **6**: $[\alpha]_D$ -93.3° ($CHCl_3$), UV (MeOH): 250 nm (ε=65900), 267 nm (sh)(ε=60100), IR ($CHCl_3$): 3490, 1720, 1620 cm^{-1}, ^1H NMR (500MHz, $CDCl_3$, δ): 5.34 (2H, dddd, 7,7'-H), 3.79 (2H, m, 17, 17'-H), 3.88 (2H, m, 19,19'-H), 5.41 (2H, d, 21,21'-H), 2.95 (2H, brd, 23,23'-H).

8) **7**: $[\alpha]_D$ -66.5° ($CHCl_3$), UV (MeOH): 247 nm (ε=88900), 270 nm (sh)(ε=63300), IR ($CHCl_3$): 3480, 1710, 1620 cm^{-1}, ^1H NMR (500 MHz, $CDCl_3$, δ): 5.37 (2H, m, 7,7'-H), 5.40 (2H, d, 21, 21'-H), 4.97 (1H, dd, 23-H), 2.94 (1H, d, 23'-H), 7.17, 7.35 (both 1H, d, 3,3'-H), 5.87 (1H, m, 5-H), 6.00 (1H, dd, 5'-H), 5.62 (1H, d, 2-H), 5.84 (1H, d, 2'-H).

9) **8**: $[\alpha]_D$ -47° ($CHCl_3$), UV (MeOH): 247 nm (ε=100000), 270 nm (sh)(ε=56000), IR ($CHCl_3$): 1710, 1620 cm^{-1}, ^1H NMR (500 MHz, $CDCl_3$, δ): 5.38 (2H, m, 7,7'-H), 5.42 (2H, d, 21,21'-H), 4.98 (2H, dd, 23,23'-H).

10) **9**: $[\alpha]_D$ -30.7° ($CHCl_3$), UV (MeOH): 268 nm (ε= 19000), IR($CHCl_3$): 3430, 1710, 1620 cm^{-1}.

11) **10**: FABMS: m/z 654 $(M+H)^+$, UV (MeOH): 266 nm (ε=24000), ^1H NMR(500 MHz, d_6-acetone,δ): 7.59 (1H, d, J=9.0 Hz, 11-H), 6.21 (1H, dd, J=15.6,9.0 Hz, 12-H), 6.02 (1H, ddd, J=15.6, 7.6, 7.6 Hz, 13-H), 2.56 (1H, ddd, J=14.1,7.6,6.4 Hz, 14-H), 2.27 (1H, ddd, J=14.1,7.6,7.1 Hz, 14-H). **11**: $[\alpha]_D$ +1.3° ($CHCl_3$), SIMS: Obsd. m/z 574.397 $(M+H)^+$, Calcd for $C_{29}H_{56}N_3O_8$= m/z 574.406, UV (MeOH): 227 nm (ε=9800), IR ($CHCl_3$): 3425 (br), 1685 cm^{-1}. Anal. Calcd for $C_{29}H_{55}N_3O_8$: C, 60.70; H, 9.66; N, 7.32. Found: C, 60.51; H, 9.85; N, 7.05.

12) Bistheonellides A and B, dimeric macrodiolides with a 40-membered ring were isolated from a marine sponge of Theonella sp.[13)]

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