Theonezolide A: A Novel Polyketide Natural Product from the Okinawan Marine Sponge *Theonella* sp.

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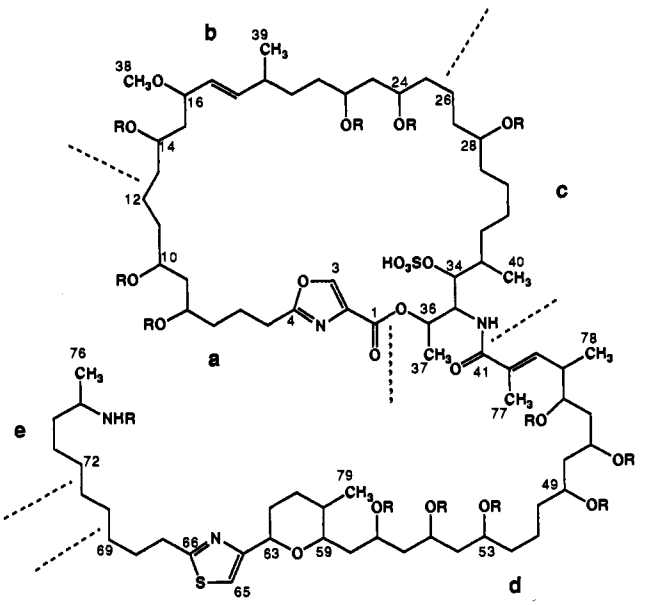
Abstract: Theonezolide A (**1**), a novel macrolide, has been isolated from the Okinawan marine sponge *Theonella* sp. and the planar structure elucidated on the basis of extensive spectroscopic analyses of **1** and its four ozonolysis products. Recent 2D NMR techniques of gradient-enhanced HMBC and HSQC-HOHAHA along with the FABMS/MS experiment were applied and proved to be quite efficient for structural study of this long aliphatic molecule. Theonezolide A (**1**), $C_{79}H_{140}N_4O_{22}S_2$, is the first member of a new class of polyketide natural products consisting of two principal fatty acid chains with various functionalities such as a sulfate ester, an oxazole, and a thiazole group, constituting a 37-membered macrocyclic lactone ring bearing a long side chain attached through an amide linkage.

Marine sponges of the genus *Theonella* frequently afford a variety of interesting secondary metabolites including polyoxygenated aliphatic compounds[2] as well as unusual cyclic peptides,[3] most of which exhibit significant biological activities. During our studies on bioactive substances from Okinawan marine organisms,[4] we examined extracts of *Theonella* sponges of several collections and recently isolated a novel tetrahydroprotoberberine alkaloid, theoneberine,[5] from a *Theonella* sponge collected off Ie Island. Further investigation on bioactive constituents from another *Theonella* sponge has now led to isolation of a novel 37-membered macrolide, theonezolide A (**1**), with unique structural features, belonging to a new class of polyketide natural products. Here we describe the isolation and structure elucidation of **1**.



1 R=H
2 R=Ac

Results and Discussion

The sponge *Theonella* sp., collected off Ie Island, Okinawa, was extracted with MeOH, and the extract was partitioned between EtOAc and H_2O. The aqueous phase was further extracted with *n*-BuOH, and the *n*-BuOH-soluble fraction was subjected to silica gel flash chromatography ($CHCl_3$/MeOH, 8:2), followed by gel filtration on Sephadex LH-20 (MeOH) and reversed-phase HPLC (ODS, 75% MeOH) to give theonezolide A (**1**, 0.04% yield, wet weight) as colorless needles.

The molecular formula of **1** was suggested as $C_{79}H_{140}N_4O_{22}S_2$ by negative HRFABMS [*m/z* 1559.9292 $(M - H)^-$, calcd for $C_{79}H_{139}N_4O_{22}S_2$, Δ −3.0 mmu] and combustion analytical data. The IR absorptions of **1** implied the presence of hydroxyl (3390 cm^{-1}), ester (1720 cm^{-1}), amide (1620 cm^{-1}), and sulfate (1220

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Table I. ^1H and ^{13}C NMR Data of Theonezolide A (1) in DMSO-d_6

position	C	H (J, Hz)	HMBC (^1H)	position	C	H (J, Hz)	HMBC (^1H)
1	159.9		36	41	168.2		NH, 43, 77
2	132.5		3	42	129.7		43, 44, 77
3	145.3	8.67 s		43	138.9	6.14 dd (9.9, 1.1)	44, 77, 78
4	165.0		3, 5, 6	44	39.2	2.36 ddq (9.9, 6.6, 6.6)	43, 78
5	27.2	2.75 t (7.5)	6	45	70.50	3.53 m	43, 44, 78
6	22.4	1.78 m, 1.69 m	5, 7, 8	46	42.8	1.35 m, 1.24 m	44
7	36.3	1.41 m, 1.33 m	5, 6	47	64.1	3.86 m	46, 48
8	68.5	3.61 m	6, 7, 9, 10	48	45.6	1.35 m, 1.25 m	
9	44.1	1.38 m		49	66.8	3.60 m	50
10	69.0	3.54 m	8, 9	50	38.2	1.27 m	
11	37.4	1.22 m		51	21.6	1.44 m	49, 50, 52, 53
12	21.3	1.21 m	11	52	38.1	1.27 m	
13	37.8	1.27 m	15	52	66.6	3.61 m	52, 54, 55
14	66.9	3.37 m	15, 16	54	45.0	1.32 m	56
15	43.2	1.58 m, 1.35 m	16, 17	55	65.9	3.81 m	53, 54, 56, 57
16	79.8	3.61 m	15, 17, 18, 38	56	45.1	1.45 m	54
17	128.9	5.08 dd (15.3, 8.4)	15, 19	57	66.4	3.75 m	56, 58, 59
18	139.9	5.41 dd (15.3, 8.3)	16, 19, 20, 39	58	40.3	1.51 m, 1.47 m	56, 59
19	36.2	2.09 m	17, 18, 39	59	77.4	3.76 m	58, 61, 63, 79
20	32.1	1.29 m	17, 18, 19, 21, 22, 39	60	29.2	1.72 m	79
21	35.0	1.29 m, 1.24 m	19, 20	61	30.5	1.84 m, 1.66 m	59, 60, 63, 79
22	68.8	3.57 m		62	25.9	1.67 m	63
23	44.3	1.36 m		63	76.5	4.41 dd (8.6, 4.8)	59, 61, 65
24	69.2	3.54 m		64	157.3		63, 65, 67
25	37.5	1.22 m		65	113.2	7.22 s	63, 67
26	21.4	1.21 m	24	66	170.0		65, 67, 68
27	37.7	1.31 m		67	32.6	2.90 t (7.6)	65, 68, 69
28	69.7	3.31 m		68	29.4	1.66 m	67, 69
29	37.2	1.22 m		69	28.3	1.30 m	67, 68
30	25.4	1.27 m, 1.14 m		70	28.6	1.25 m	
31	26.7	1.25 m, 1.18 m		71	28.5	1.30 m	73
32	32.9	1.44 m, 1.06 m	34, 40	72	28.6	1.25 m	74
33	34.6	1.77 m	34, 40	73	24.6	1.27 m	74, 75
34	81.9	4.06 dd (6.7, 4.4)	33, 40	74	34.1	1.52 m, 1.38 m	72, 73, 75, 76
35	51.8	4.24 dt (9.1, 3.8)	34, 37	75	46.9	3.12 qt (7.6, 6.3)	73, 74, 76
36	70.54	5.26 qd (6.5, 3.1)	34, 37	76	18.2	1.13 d (6.6)	74, 75
37	17.4	1.22 d (6.5)	35, 36	77	12.8	1.76 d (1.1)	43
38	54.8	3.09 s	16	78	15.6	0.92 d (5.7)	43, 44, 45, 77
39	21.3	0.95 d (6.7)	17, 18, 19	79	11.6	0.91 d (6.9)	59, 61
40	15.3	0.83 d (6.6)	33, 34	CONH		7.95 d (9.1)	

cm^{-1})[6,7] functionalities. ^1H and ^{13}C NMR spectra of **1** revealed signals due to one di- and one trisubstituted olefin, 17 oxymethines, two nitrogen-bearing methines, one vinyl and six secondary methyls, and many sp^3 methylenes. Acetylation of **1** afforded a tridecaacetate [**2**, negative FABMS m/z 2105 $(M - H)^-$]. Spectral comparison of **1** and **2** indicated the presence of 12 secondary hydroxyl groups and one amino group for **1**. The remaining five oxymethines were ascribed to those bearing a methoxy, an ester, a sulfate, and an ether oxygen forming a tetrahydropyran ring, whose ^1H resonances did not show a downfield shift by acetylation. By extensive analyses of the 2D NMR data of **1** including DQF-COSY, HOHAHA, ROESY, HSQC, and the recent techniques of gradient-enhanced[8] HMBC and HSQC-HOHAHA[9] spectra in DMSO-d_6, the following five partial structures were deduced: C-1∼C-12 (**a**), C-13∼C-25 (**b**), C-26∼C-37 (**c**), C-41∼C-69 (**d**), and C-72∼C-76 (**e**). Analyzing the DQF-COSY spectrum of **1** suggested the absence of 1,2-diols, while the presence of at least four 1,3-diols was inferred from the formation of acetonides[10] in which four acetone molecules were incorporated. It was revealed that the ^{13}C NMR signals for sp^3 methylene carbons located between two hydroxy-bearing methines (viz., 2-position of 1,3-diol) were observed at δ_C 43∼45 ppm, whereas sp^3 methylene carbons between a hydroxy-bearing methine and another sp^3 methylene resonated at δ_C 35∼38 ppm. The locations of secondary hydroxyls, a methoxy, and secondary methyl groups were elucidated mainly by the HMBC and HSQC-HOHAHA correlations. Applying the new technique of gradient-enhanced HMBC afforded data of high sensitivity with almost no noise (see supplementary material), while the HSQC-HOHAHA spectrum provided ^1H–^{13}C connectivity data through five or six bonds. All ^1H and ^{13}C NMR chemical shifts together with long-range ^1H–^{13}C correlations observed in the HMBC spectrum for **1** are presented in Table I, and the HSQC-HOHAHA correlation data are given in Table II.

The presence of a disubstituted 1,3-oxazole conjugated to an ester carbonyl carbon (C-1∼C-4 moiety) was revealed by characteristic NMR signals [δ_H 8.67 (s, H-3); δ_C 159.9 (C-1), 132.5 (C-2), 145.3 (C-3), and 165.0 (C-4)][11] and $^1J_{C-H}$ value for C-3 ($^1J_{C-H}$ = 213 Hz).[12] The C-4 signal showed HMBC correlation with methylene protons resonating at δ_H 2.75 (2H, t, J = 7.5 Hz; H_2-5), which was also correlated with two sp^3 methylenes (C-6 and C-7) in the HMBC spectrum. A 1,3-diol was shown to be placed at C-8∼C-10 by DQF-COSY (H-7/H-8, H-8/H-9, and H-9/H-10) and HMBC correlations (Table

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(7) The presence of a sulfate group was further confirmed by a negative FABMS/MS experiment (parent ion m/z 1559), which exhibited intense daughter ions at m/z 97 and 80, assignable to HSO_4^- and SO_3^- ions, respectively.

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(10) The acetonides were obtained by treatment of **1** with 2,2-dimethoxypropane in DMF in the presence of *p*-toluenesulfonic acid and found to be a mixture of four components with equal molecular weight based on HPLC and FABMS analysis [positive, m/z 1722 $(M + H)^+$; negative, m/z 1720 $(M - H)^-$]. This result was coincident with the presence of two 1,3-diols and two 1,3,5-triols in **1**.

(11) These chemical shifts were consistent with those of the corresponding portion of bistratamide C [δ_H 8.20 (s, H-21); δ_C 159.1 (C-22), 135.4 (C-20), 141.4 (C-21), and 163.7 (C-19)]: Foster, M. P.; Concepción, G. P.; Caraan, G. B.; Ireland, C. M. *J. Org. Chem.* **1992**, *57*, 6671–6675.

(12) ^{13}C–^1H one-bond coupling constants for oxazole [C-2, $^1J_{C-H}$ = 231 Hz; C-4, $^1J_{C-H}$ = 195 Hz; C-5, $^1J_{C-H}$ = 209 Hz]; the C-3 of **1** corresponds to the C-5 of oxazole: Hiemstra, H.; Houwing, H. A.; van Leusen, A. M. *Can. J. Chem.* **1979**, *57*, 3168–3170.

Table II. HSQC-HOHAHA Correlation Data of Theonezolide A (1)

H	C	H	C
1		41	
2		42	
3		43	44, 45, 46, 77, 78
4		44	43, 45, 46, 78
5	6, 7, 8, 9	45	43, 44, 46, 47, 48
6	5, 7, 8	46	
7	5, 6, 8, 9	47	45, 46, 48, 49
8	5, 6, 7, 9, 10	48	47, 49
9		49	47, 48, 50, 51
10	8, 9, 11, 12	50	
11		51	50, 52
12		52	
13		53	51, 52, 54, 55
14	12, 13, 15, 16, 17, 18, 19, 39	54	53, 55
15	13, 14, 16, 17, 18	55	54, 56, 57, 58
16	14, 15, 17, 18, 19, 39	56	54, 55
17	14, 15, 16, 18, 19, 20, 21, 39	57	55, 56, 58, 59
18	14, 15, 16, 17, 19, 20, 21, 39	58	56, 57, 59
19	15, 16, 17, 18, 20, 21, 22, 39	59	57, 58, 79
20	19, 21, 22, 23	60	79
21	20, 22, 23	61	62, 79
22	20, 21, 23, 24	62	63
23	20, 21, 22	63	60, 61, 62, 79
24	23, 25	64	
25		65	63
26		66	
27		67	68, 69
28	26, 27, 29, 30, 31	68	67, 69
29		69	67, 68
30	31, 32	70	
31	30, 32	71	
32	28, 29, 30, 31, 33, 40	72	73, 74, 75, 76
33	31, 32, 34, 40	73	72, 74, 75, 76
34	31, 32, 33, 35, 40	74	72, 73, 75, 76
35	33, 34, 36, 37, 40	75	72, 73, 74, 76
36	34, 35, 37	76	72, 73, 74, 75
37	35, 36	77	43
38		78	43, 44, 45
39	15, 16, 17, 18, 19, 20, 21	79	60, 61, 62, 63
40	29, 30, 31, 32, 33, 34, 35	NH	34, 35

I). In the HSQC-HOHAHA spectrum the oxymethine proton for H-10 showed connectivity to two sp^3 methylenes (C-11 and C-12, Table II). The partial structure for C-1~C-12 (**a**) was thus deduced. Interpreting the DQF-COSY spectrum easily revealed proton connectivities from H_2-13 to H_2-20. A methoxy group was located on C-16 by the HMBC cross peak for H_3-38/C-16. The Δ^{17}-double bond was shown to be *E* by the ^1H coupling constant ($J_{17,18}$ = 15.4 Hz). C-20 was shown to be connected to the C-21~C-25 unit containing a 1,3-diol unit by HSQC-HOHAHA correlations (Table II; *e.g.*, H-19/C-21, H-19/C-22, H-22/C-20, H-22/C-21, H-22/C-23, H-22/C-24, H-24/C-23, and H-24/C-25), thus giving rise to the partial structure **b** (C-13~C-25 moiety). For the partial structure **c** (C-26~C-37 part), the connectivities from H_2-32 to H_3-37 were clearly revealed by the DQF-COSY spectrum. The C-26~C-31 portion was shown to be connected to C-32 by the HSQC-HOHAHA correlations (Table II). Particularly, the methylene protons for H_2-32 markedly showed diagnostic cross peaks with C-28, C-29, C-30, C-31, C-33, and C-40; each of these cross peaks was observed as a pair since the two protons for H_2-32 resonated unequivalently at δ_H 1.44 and 1.06. The sulfate ester was inferred to be attached to C-34 by its ^1H and ^{13}C chemical shifts (δ_H 4.04 and δ_C 82.1),[13] while an amide group was placed on C-35 on the basis of the DQF-COSY cross peak between H-35 (δ_H 4.24) and NH (δ_H 7.95; D_2O-exchangeable). The oxymethine (C-36) bears a secondary methyl group (C-37), which proved to be a terminal of one polyketide chain contained in the molecule of **1**.

(13) The sulfate-bearing position (C-9) of maitotoxin (δ_H 4.27 and δ_C 82.6): Murata, M.; Iwashita, T.; Yokoyama, A.; Sasaki, M.; Yasumoto, T. *J. Am. Chem. Soc.* **1992**, *114*, 6594–6596.

The second polyketide aliphatic chain starts with an α,β-unsaturated ester or amide group [δ_C 168.2 (C-41), 129.7 (C-42), and 138.9 (C-43); δ_H 6.14 (H-43)].[14] The ^{13}C chemical shift of the methyl group on C-42 (C-77, δ_C 12.8) implied the *E*-configuration of the Δ^{42}-double bond.[14] In the DQF-COSY spectrum H-43 showed a cross peak with an sp^3 methine proton (δ_H 2.36, H-44), which in turn was coupled with a doublet methyl signal (δ_H 0.92, H_3-78). To this C-44 position, two 1,3,5-triol units linked via three sp^3 methylenes (C-45~C-57 unit) were connected as follows: the ^1H connectivities for the two 1,3,5-triol systems were evident from the DQF-COSY spectrum (from H-44 to H-49; from H-53 to H-57), and the presence of three sp^3 methylenes (C-50~C-52) between them was shown by the HMBC correlations for H-49/C-51, H_2-50/C-49, H_2-52/C-53, and H-53/C-51. The DQF-COSY spectrum showed ^1H connectivity from the oxymethine (H-57) to an sp^3 methine (H-60) bearing a secondary methyl group (H_3-79). Two oxymethine protons (H-59 and H-63) were observed at nearly the same chemical shifts in **1** (δ_H 3.76 and 4.41, respectively) and the acetate (**2**; δ_H 3.64 and 4.38, respectively), which implied the presence of an ether ring. Though the proton connectivities from H-60 to H_2-62 were not clearly observed in the DQF-COSY spectrum, the presence of a tetrahydropyran ring was suggested by the HMBC correlations (H-59/C-61, H-59/C-63, and H-63/C-61). The ROESY spectrum of **1** showed a cross peak for H-59/H-63, indicating that these protons on the tetrahydropyran ring were axially oriented. The tetrahydropyran ring was shown to be adjacent to a disubstituted 1,3-thiazole ring system by DQF-COSY (H-63/H-65) and HMBC (H-63/C-64 and H-63/C-65) correlations. The ^1H and ^{13}C NMR chemical shifts [δ_H 7.22 (s, H-65); δ_C 157.3 (C-64), 113.3 (C-65), and 170.0 (C-66)][15] as well as the $^1J_{C-H}$ value for C-65 ($^1J_{C-H}$ = 191 Hz)[16] argue well for the presence of the thiazole moiety. The ^1H NMR signal at δ_H 2.90 (2H, t) was assigned to H_2-67, which showed HMBC correlation to C-66. The H_2-67 signal was also correlated with two sp^3 methylene carbons (C-68 and C-69) in the HMBC spectrum,[17] indicating that an alkyl chain was attached to the C-66 position of the thiazole ring. The partial structure **d** (C-41~C-69 moiety) was thus suggested and further verified by the negative FABMS/MS experiment of **1** [parent ion *m/z* 1559 $(M - H)^-$], which showed characteristic daughter ions generated by fissions at α and β positions to OH groups.[18] The presence of the sulfate group proved to be desirable for the negative ion FABMS/MS analysis. Key daughter ions and fragmentation patterns are depicted in Chart I and are fully consistent with the partial structure **d**. The partial structure **e**, viz., the end of the molecule (C-72~C-76 moiety), was revealed by the following observations. A primary amino group is present at the terminal (C-75), which was indicated by the COSY cross peaks (N*H*Ac/H-75 and H-75/H_3-76) for the acetate (**2**). The HMBC spectrum of **1** afforded correlation data (Table I), implying that more than three methylene units are attached linearly to C-75.

The five partial structures **a**~**e** were thus elucidated, and connection of these partial structures remains to be interpreted. The HMBC correlations of **1** for H-36/C-1, H-35/C-41, and

(14) The ^1H and ^{13}C chemical shifts of the C-41~C-43 part of **1** corresponded well with those of the α,β-unsaturated ester moiety (C-1~C-3) of amphidinolide B [δ_C 167.7 (C-1), 128.3 (C-2), 139.9 (C-3), and 12.4 (Me on C-2); δ_H 6.77 (H-3)]: Ishibashi, M.; Ohizumi, Y.; Hamashima, M.; Nakamura, H.; Hirata, Y.; Sasaki, T.; Kobayashi, J. *J. Chem. Soc., Chem. Commun.* **1987**, 1127–1129.

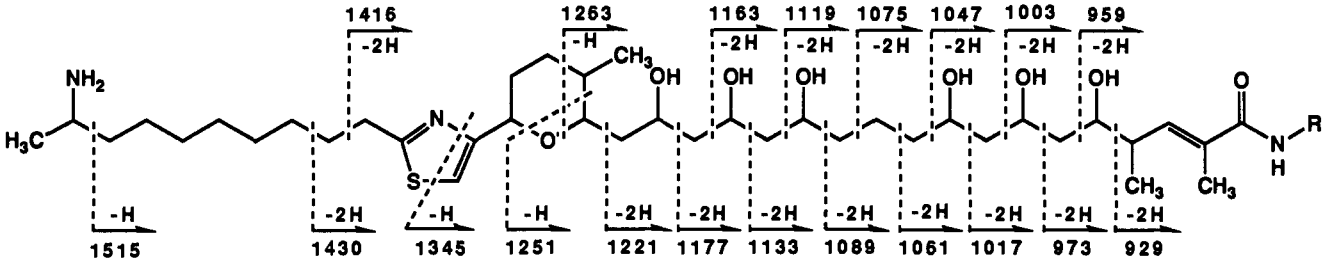
(15) The ^1H and ^{13}C NMR chemical shifts for the thiazole unit of mycothiazole [δ_H 6.73 (H-11); δ_C 154.3 (C-12), 112.7 (C-11), and 177.7 (C-10)]: Crews, P.; Kakou, Y.; Quiñoà, E. *J. Am. Chem. Soc.* **1988**, *110*, 4365–4368.

(16) ^{13}C–^1H one-bond coupling constants for thiazoles [C-2, $^1J_{C-H}$ = 213 Hz; C-4, $^1J_{C-H}$ = 187 Hz; C-5, $^1J_{C-H}$ = 191 Hz]; the C-65 of **1** corresponds to the C-5 of thiazole: Faure, R.; Galy, J.-P.; Vincent, E.-J.; Elguero, J. *Can. J. Chem.* **1978**, *56*, 46–55.

(17) The HMBC spectrum of **1** showed correlations via four bonds for H_2-67/C-64 and H_2-67/C-65.

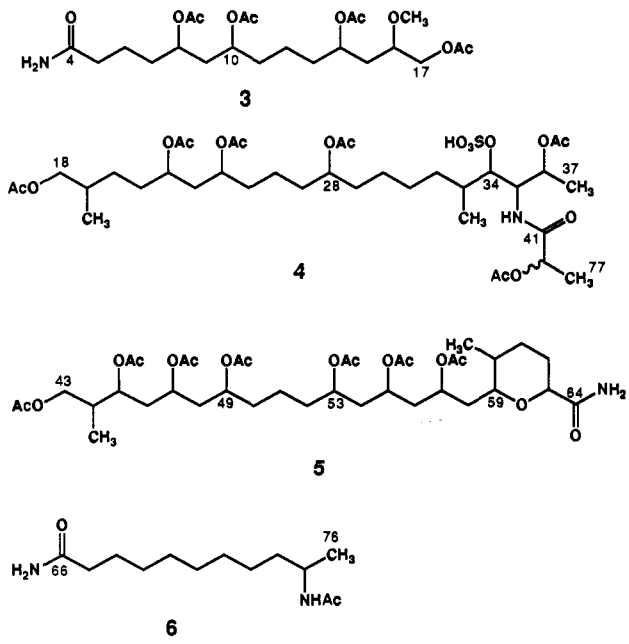
(18) Satake, M.; Murata, M.; Yasumoto, T.; Fujita, T.; Naoki, H. *J. Am. Chem. Soc.* **1991**, *113*, 9859–9861.

Chart I. Key Daughter Ions and Fragmentation Pattern Observed in the Negative FABMS/MS (Parent Ion, m/z 1559) of Theonezolide A (1)



NH-35/C-41 suggested that the partial structure c was connected with the partial structures **a** and **d** through ester and amide linkages, respectively. The remaining partial structures had to be connected between sp^3 methylene carbons. It was, however, extremely difficult to obtain unambiguous evidence for connection through sp^3 methylenes by spectral means because of the heavy overlapping of the NMR signals. The following chemical degradation experiments were therefore carried out to solve this problem.

Treatment of the acetate (**2**) with ozone followed by $NaBH_4$ reduction and acetylation afforded a complex mixture, which was purified by reversed-phase HPLC (ODS) to give four useful products (**3**~**6**), corresponding to C-4~C-17, C-18~C-37, C-43~C-64, and C-66~C-76 units of **1**, respectively. Thus the



connections for partial structures (**a**/**b** and **b**/**c**) as well as the numbers of sp^3 methylene carbons between the partial structures **d** and **e** were clearly revealed by the molecular weights of these ozonolysis products (**3**~**6**) [FABMS: **3**, m/z 490 $(M + H)^+$; **4**, m/z 824 $(M - H)^-$; **5**, m/z 788 $(M + H)^+$; **6**, m/z 243 $(M + H)^+$]. The ^1H and ^{13}C NMR data facilitated by the ^1H–^1H COSY spectra of degradation products **3**~**6** provided additional proof corroborating the total structure of **1**. From all of these data the whole structure of theonezolide A was concluded as **1**.

Theonezolide A (**1**) is the first member of an unprecedented class of polyketide natural products consisting of two principal fatty acid chains bearing several structural features of interest from the biogenetical viewpoint. The oxazole unit could be assumed to be derived from an amino acid, serine, whereas the origin of the thiazole ring is problematical. There are a number of oxazole- and thiazole-containing metabolites reported from marine origins,[19] and in most cases cysteine is suggested as a precursor of the thiazole functionality.[20] It might, however, be possible that the thiazole ring of theonezolide A (**1**) was generated via backbone rearrangement from a nitrogen-involved polyketide intermediate as proposed[21] for ulapualides[19g] or kabiramides.[19h] Theonezolide A (**1**) exhibited cytotoxicity against murine lymphoma L1210 and human epidermoid carcinoma KB cells in vitro both with IC_{50} values of 0.75 μg/mL.

Experimental Section

General Methods. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. UV and IR spectra were obtained on JASCO Ubest-35 and JASCO IR Report-100 spectrometers, respectively. ^1H and ^{13}C NMR spectra were recorded on Bruker AMX-500 and JEOL GSX-400 spectrometers; spectra were referenced to residual undeuteriated solvent (^1H) or to solvent signals (^{13}C). FAB mass spectra were obtained on a JEOL HX-110 spectrometer. Wako gel C-300, Wako Pure Chemical, was used for silica gel glass column chromatography, and Sephadex LH-20, Pharmacia Fine Chemicals, was used for gel filtration chromatography.

Isolation. The sponge *Theonella* sp. was collected off Ie Island, Okinawa, and kept frozen until used. The sponge (0.8 kg, wet weight) was extracted with MeOH (0.8 L × 3). After evaporation under reduced pressure, the residue (61 g) was partitioned between EtOAc (400 mL × 3) and a 1 M NaCl aqueous solution (400 mL), and the aqueous portion was subsequently extracted with *n*-BuOH (400 mL × 3). The *n*-BuOH-soluble material (6.7 g) was subjected to silica gel column chromatography (4.4 × 35 cm) with $CHCl_3$/MeOH (80:20). The fraction eluting from 630 to 1360 mL was further separated by a Sephadex LH-20 column (2 × 95 cm) with MeOH. The fraction eluting from 90 to 130 mL was finally purified by reversed-phase HPLC (Develosil Lop ODS 24S, Nomura Chemical, 24 × 360 mm, 30 μm; flow rate, 6.0 mL/min; UV detection at 254 nm; eluent, 75% MeOH) to give theonezolide A (**1**, t_R 42.4 min, 307 mg, 0.04% wet weight).

Theonezolide A (1): colorless needles; mp 123 °C; $[\alpha]^{28}_D$ –8.1° (c 1.5, MeOH); UV λ_{max} (MeOH) 210 nm (ϵ 22 000); IR ν_{max} (KBr) 3390, 1720, 1620, 1220, and 1110 cm^{-1}; ^1H and ^{13}C NMR (Table I); FABMS (negative ion; 3-nitrobenzyl alcohol as a matrix) m/z 1559 $(M - H)^-$; HRFABMS m/z 1559.9292 (M – H; calcd for $C_{79}H_{139}N_4O_{22}S_2$, 1559.9322). Anal. Calcd for $C_{79}H_{140}N_4O_{22}S_2 \cdot 3H_2O$: C, 58.71; H, 9.11; N, 3.47; S, 3.97. Found: C, 58.87; H, 9.23; N, 3.60; S, 4.31.

Tridecaacetate 2. Theonezolide A (**1**, 14 mg) was treated with 2 mL of acetic anhydride and 2 mL of pyridine at room temperature for 18 h. After the usual workup, purification by silica gel column chromatography (5 × 50 mm) eluted with $CHCl_3$/MeOH (95:5) afforded the tridecaacetate (**2**, 15 mg): colorless oil; $[\alpha]^{27}_D$ –6.0° (c 2.0, MeOH); IR ν_{max} (KBr) 1730, 1640, 1240, and 1100 cm^{-1}; ^1H NMR (400 MHz in DMSO-d_6) δ 8.69 (1H, s), 8.10 (1H, d, J = 8.8 Hz), 7.58 (1H, d, J = 7.8 Hz), 7.22

(19) For example: (a) Ichiba, T.; Yoshida, W. Y.; Scheuer, P. J.; Higa, T.; Gravalos, D. G. *J. Am. Chem. Soc.* **1991**, *113*, 3173–3174. (b) Lindquist, N.; Fenical, W.; Van Duyne, G. D.; Clardy, J. *J. Am. Chem. Soc.* **1991**, *113*, 2303–2304. (c) Zabriskie, T. M.; Mayne, C. L.; Ireland, C. M. *J. Am. Chem. Soc.* **1988**, *110*, 7919–7920. (d) Corley, D. G.; Moore, R. E.; Paul, V. J. *J. Am. Chem. Soc.* **1988**, *110*, 7920–7922. (e) Arabshahi, L.; Schmitz, F. J. *Tetrahedron Lett.* **1988**, *29*, 1099–1102. (f) Kato, Y.; Fusetani, N.; Matsunaga, S.; Hashimoto, K.; Fujita, S.; Furuya, T. *J. Am. Chem. Soc.* **1986**, *108*, 2780–2781. (g) Roesener, J. A.; Scheuer, P. J. *J. Am. Chem. Soc.* **1986**, *108*, 846–847. (h) Matsunaga, S.; Fusetani, N.; Hashimoto, K.; Koseki, K.; Noma, M. *J. Am. Chem. Soc.* **1986**, *108*, 847–849.

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(1H, s), 6.07 (1H, d, J = 10.3 Hz), 5.38 (1H, dd, J = 15.6 and 8.3 Hz), 5.27 (1H, qd, J = 6.6 and 3.7 Hz), 5.12 (1H, dd, J = 15.1 and 8.3 Hz), 4.95-4.68 (12H, m), 4.38 (1H, dd, J = 7.8 and 5.4 Hz), 4.25 (1H, dt, J = 8.8 and 4.4 Hz), 4.02 (1H, dd, J = 7.6 and 3.7 Hz), 3.69 (1H, m), 3.64 (1H, m), 3.47 (1H, m), 3.09 (3H, s), 2.91 (2H, t, J = 7.6 Hz), 2.76 (2H, t, J = 7.1 Hz), 2.70 (1H, m), 2.08 (1H, m), 1.99 (3H, s), 1.97 (12H, s), 1.96 (9H, s), 1.93 (3H, s), 1.92 (6H, s), 1.91 (3H, s), 1.89 (3H, s), 1.76 (3H, s), 1.24 (3H, d, J = 6.4 Hz), 0.98 (3H, d, J = 6.8 Hz), 0.94 (3H, d, J = 6.8 Hz), 0.90 (3H, d, J = 6.4 Hz), 0.88 (3H, d, J = 5.9 Hz), and 0.84 (3H, d, J = 6.8 Hz); ^{13}C NMR (100 MHz in DMSO-d_6) δ 170.04, 170.00, 169.9, 169.8, 169.7, 168.1, 164.4, 159.9, 157.2, 145.4, 140.0, 135.2, 132.6, 131.9, 128.4, 113.3, 82.0, 78.9, 76.6, 76.4, 73.1, 72.7, 70.6, 70.4, 69.3, 69.0, 68.7, 67.2, 66.1, 54.9, 51.8, 44.0, 39.1, 38.3, 37.9, 37.7, 37.1, 36.1, 36.1, 35.8, 35.4, 34.6, 33.8, 33.5, 33.4, 33.3, 33.2, 32.8, 32.5, 31.3, 31.2, 30.4, 29.9, 29.4, 28.9, 28.8, 28.6, 28.3, 26.7, 26.3, 25.8, 25.6, 24.8, 22.7, 21.8, 21.0, 20.8, 20.7, 20.3, 20.1, 20.0, 17.5, 16.1, 15.3, 12.8, and 11.4; FABMS (negative ion; diethanolamine as a matrix) m/z 2105 $(M - H)^-$.

Ozonolysis of 2. A solution of the acetate (**2**, 39 mg) in 1 mL of MeOH was bubbled with O_3 at −78 °C for 10 min. After the removal of excess ozone by bubbling argon, a solution of $NaBH_4$ (52 mg) in 0.5 mL of MeOH was added, and the whole mixture was stirred for 1 h at 0 °C. After addition of 2 mL of 1 M potassium phosphate buffer (pH 7.0), the reaction mixture was partitioned between ethyl acetate and brine. Evaporation of the organic layer afforded the crude product (35 mg), which was treated with 1 mL of acetic anhydride and 1 mL of pyridine for 12 h at room temperature. After evaporation of the reagent, the mixture was separated on a silica gel column (13 × 170 mm) with $CHCl_3$/MeOH (95:5 and 90:10) to give **4** (10.2 mg) and a mixture of other products, which was purified by reversed-phase HPLC (Develosil ODS-5, 10 × 250 mm, 5 μm; flow rate, 1.0 mL/min; refractive index detection; eluent, 50, 60, and 70% MeOH) to afford **3** (1.6 mg, t_R 13.6 min, 60% MeOH), **5** (1.8 mg, t_R 24.8 min, 70% MeOH), and **6** (0.5 mg, t_R 22.6 min, 50% MeOH).

Compound 3: colorless oil; $[\alpha]^{21}_D$ −4° (c 0.25, EtOH); ^{1}H NMR (400 MHz in C_5D_5N) δ 8.05 (1H, brs), 5.29 (1H, m), 5.23 (1H, m), 5.19 (1H, m), 4.39 (1H, dd, J = 11.7 and 3.9 Hz), 4.24 (1H, dd, J = 11.7 and 5.4 Hz), 3.61 (1H, m), 3.36 (3H, s), 2.45 (2H, td, J = 7.3 and 2.0 Hz), 2.07 (3H, s), 2.05 (3H, s), and 2.02 (6H, s); ^{13}C NMR (100 MHz in C_5D_5N) δ 174.9, 170.7, 170.5, 76.5, 71.3, 71.2, 65.1, 56.9, 38.8, 36.1, 35.7, 34.2, 21.7, 21.2, 21.1, 21.0, and 20.7; FABMS (positive ion; 3-nitrobenzyl alcohol as a matrix) m/z 490 $(M + H)^+$; HRFABMS m/z 490.2670 (M + H; calcd for $C_{23}H_{40}NO_{10}$, 490.2653).

Compound 4 (a 1:1 diastereomeric mixture at C-42): colorless oil; $[\alpha]^{27}_D$ −1.3° (c 0.80, MeOH); ^{1}H NMR (400 MHz in C_5D_5N) δ 9.25 and 9.11 (1H, each brs), 5.82 (1H, m), 5.50 (1H, qd, J = 6.8 and 2.9 Hz), 5.21 (2H, m), 5.12 (1H, ddd, J = 14.2, 8.3, and 3.4 Hz), 5.02 (1H, m), 4.80 (1H, ddd, J = 16.1, 7.8, and 1.0 Hz), 4.03 (1H, dd, J = 10.7 and 5.9 Hz), 3.93 (1H, dd, J = 10.7 and 6.8 Hz), 2.12, 2.10, 2.09, 2.08, 2.06, 2.05, 2.02, and 1.87 (each s), 1.67 (3H, d, J = 6.8 Hz), 1.46 and 1.39 (3H, d, J = 6.4 and 6.3 Hz), 1.31 and 1.28 (3H, d, J = 6.3 and 6.8 Hz), and 0.89 (1H, d, J = 6.8 Hz); ^{13}C NMR (100 MHz in C_5D_5N) δ 171.9, 171.7, 171.3, 170.8, 170.7, 170.6, 170.4, 81.7, 81.6, 74.0, 73.9, 71.7, 71.3, 71.1, 71.0, 69.0, 53.7, 38.8, 35.4, 35.1, 34.6, 34.5, 34.4, 34.3, 32.7, 31.8, 29.1, 27.80, 27.77, 26.0, 21.5, 21.3, 21.2, 21.1, 20.8, 20.7, 18.1, 18.0, 17.7, 16.9, 14.9, and 14.8; FABMS (negative ion; 3-nitrobenzyl alcohol as a matrix) m/z 824 $(M - H)^-$; HRFABMS m/z 824.3715 (M − H; calcd for $C_{37}H_{62}NO_{17}S$, 824.3738).

Compound 5: colorless oil; $[\alpha]^{21}_D$ −22° (c 0.5, MeOH); ^{1}H NMR (400 MHz in C_5D_5N) δ 8.16 (1H, brs), 7.43 (1H, brs), 5.40 (1H, m), 5.35 (1H, m), 5.33 (1H, m), 5.31 (1H, m), 5.23 (1H, m), 5.17 (1H, m), 4.22 (1H, dd, J = 11.0 and 6.6 Hz), 4.04 (1H, dd, J = 7.4 and 4.9 Hz), 4.02 (1H, dd, J = 11.0 and 6.6 Hz), 3.77 (1H, ddd, J = 8.3, 5.9, and 2.0 Hz), 2.11 (3H, s), 2.09 (3H, s), 2.08 (6H, s), 2.07 (6H, s), 2.06 (3H, s), 2.03 (3H, s), 0.98 (3H, d, J = 7.3 Hz), and 0.89 (3H, d, J = 6.8 Hz); ^{13}C NMR (100 MHz in C_5D_5N) δ 174.6, 170.6, 170.5, 78.9, 77.4, 70.4, 70.0, 69.7, 69.4, 67.8, 67.0, 66.1, 39.5, 39.4, 38.9, 38.4, 36.9, 36.7, 34.9, 34.8, 31.0, 30.9, 24.0, 21.2, 21.2, 21.1, 21.0, 20.9, 20.7, 11.8, and 11.4; FABMS (positive ion; 3-nitrobenzyl alcohol as a matrix) m/z 788 $(M + H)^+$; HRFABMS m/z 788.4109 (M + H; calcd for $C_{38}H_{62}NO_{16}$, 788.4068).

Compound 6: colorless solid; $[\alpha]^{22}_D$ + 12° (c 0.2, $CHCl_3$); ^{1}H NMR (400 MHz in $CDCl_3$) δ 5.50 (1H, brs), 5.20 (2H, brs), 3.96 (1H, tq, J = 6.8 and 6.8 Hz), 2.22 (2H, t, J = 7.6 Hz), 1.96 (3H, s), and 1.11 (3H, d, J = 6.8 Hz); ^{13}C NMR (100 MHz in $CDCl_3$) δ 175.4, 45.3, 36.9, 35.9, 29.2, 29.1, 29.0, 25.9, 25.4, 23.6, and 21.0; FABMS (positive ion; 3-nitrobenzyl alcohol as a matrix) m/z 243 $(M + H)^+$; HRFABMS m/z 243.2058 (M + H; calcd for $C_{13}H_{27}N_2O_2$, 243.2073).

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Supplementary Material Available: Selected 2D NMR and other spectra of compound **1** (33 pages). Ordering information is given on any current masthead page.