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MYCALOLIDES **A** - **C**, HYBRID MACROLIDES OF ULAPUALIDES AND HALICHONDRAMIDE, FROM A SPONGE OF THE GENUS MYCALE [1]

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Abstract: Three cytotoxic macrolides, mycalolides **A** - **C** have been isolated from a sponge Mycale sp., and their structures elucidated to be hybrids of ulapualides and halichondramide mainly by analyses of 2D NMR spectra as well as by comparison of spectral data.

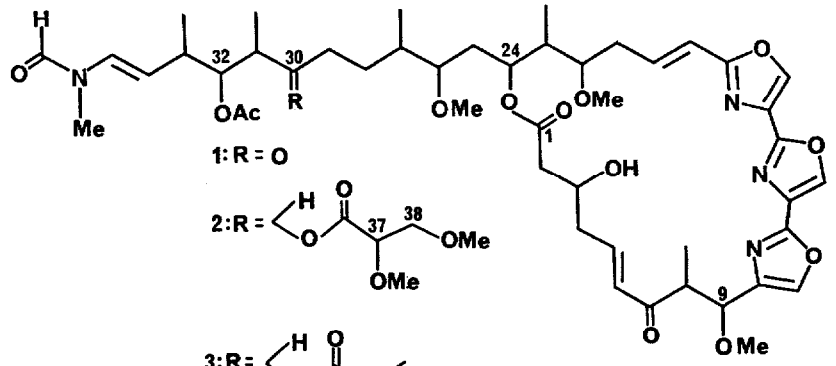
Marine sponges have proved to be a rich source of macrolides possessing potent antitumor or cytotoxic activity,[2] e.g. tedanolide,[3] halichondrins,[4] swinholide A,[5] bistheonellides A and B,[6] and laulimalides.[7] In the course of our search for bioactive metabolites from Japanese marine invertebrates, we encountered a sponge of the genus Mycale collected from shallow waters (-2 - -5 m) in Gokasho Bay of the Kii Peninsula, which showed marked activity in antifungal and starfish egg assays. We have isolated from this sponge three new cytotoxic macrolides, named mycalolides **A**, **B**, and **C**, related to kabiramides,[8,9] ulapualides,[10] and halichondramides.[11,12] In this paper, we describe the isolation and structure elucidation of these macrolides.

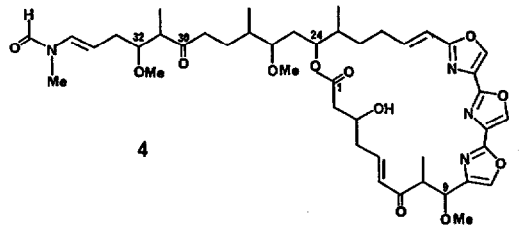
The ether-soluble materials from a methanol extract of the frozen sponges (1.8 kg) were subjected to vacuum flash chromatography on Kieselgel 60H (E. Merck) with $CHCl_3$/MeOH. The eluates were monitored by bioautography on Kieselgel 60 (E. Merck) TLC ($CHCl_3$/MeOH, 95 : 5) using the fungus Mortierella ramannianus. The active fractions were partitioned between n-hexane and 90 % aq. MeOH; the latter was then gel-filtered on Toyopearl HW 40SF (Toso Co., Ltd.) with $CHCl_3$/MeOH (1 : 1). The final purification was done by HPLC on YMC-SH-043 Sil (Yamamura Chem. Lab. Co., Ltd.) with benzene/i-PrOH (19 : 1) followed by Capcell Pak C_{18} (Shiseido Co., Ltd.) with 70% aq. MeOH to obtain mycalolides **A** (48 mg), **B** (89 mg), and **C** (38 mg), each as a yellowish gum.

Mycalolide **A** (**1**), $[\alpha]_D$ = -60.3° (c = 0.5 $CHCl_3$), showed UV [λ_{max} (MeOH) 230 nm (ε = 30,000)], IR [ν_{max} (film) 3350, 3150, 1720, 1700, 1680, 1650, 1550, 1450, 1370, 1230, 1080, 910 and 760 cm^{-1}], and ^1H and ^{13}C NMR spectra (Table 1), reminiscent of the trisoxazole containing macrolides, which are characterized by three aromatic singlet protons, duplicated formamide proton, and 1 : 2 doubled ^{13}C signals.[8-12] A molecular formula of $C_{47}H_{64}N_4O_{14}$ was established by FABMS [m/z 909 $(M + H)^+$] and ^1H and ^{13}C NMR data. Comparing ^1H-COSY and 2D C-H correlation spectra with those of halichondramide (**4**), it was clear that **1** had additional secondary methyl [δ_C 18.8 q; δ_H 1.05 (3H,d)], methoxyl [δ_C 58.0 q; δ_H 3.32 (3H,s)], and acetoxyl [δ_C 20.8 q, 170.0 s; δ_H 2.01 (3H,s)] signals instead of the C-32 methoxyl signal [δ_C 57.4, 57.2; δ_H 3.30], while otherwise the spectra were identical. The

positions of these functionalities were verified by a COSY spectrum, which revealed partial structure from C-30 to C-35; a methyl signal at δ 1.05 was coupled to a methine proton at δ 2.50 (H-33), which was in turn correlated with an oxymethine at δ 5.14 (H-32). Thus, the methyl group was placed at C-33, while the acetoxyl moiety was attached to C-32 in place of the methoxyl group in halichondramide (**4**). Similarly, the remaining methoxyl group was positioned at C-22, which experienced a downfield shift from δ_C 31.3 in **4** to 79.8 in **1**. The C-1 through C-9 portion of mycalolide **A** exhibited identical ^1H and ^{13}C NMR data with those of halichondramide, while the C-19 - C-35 portion was the same as that of ulapualide **A** (**5**). Therefore, mycalolide **A** can be considered a hybrid of halichondramide and ulapualide **A**.

Mycalolide **B** (**2**),[13] showing UV, IR, and NMR spectra similar to those of mycalolide **A** (**1**), had a molecular formula of $C_{52}H_{74}N_4O_{17}$, which was determined by FABMS [m/z 1027 $(M + H)^+$] and ^{13}C NMR spectra. ^1H and ^{13}C NMR spectra of **2** were quite similar to those of mycalolide **A** (**1**), except for the C-30 ketone (δ_C 211.6) which was replaced by series of signals in ^1H [δ 3.86 (m,1H), 3.58 (m,2H), 3.41 (s,3H), 3.32 (s,3H)] and ^{13}C NMR spectra [δ_C 170.3 s, 80.7 d, 73.1 t, 58.6 q, 59.3 q], which are assignable to a 2,3-di-O-methylglycerate constellation as in the case of ulapualide **B** (**6**).[10] This acid must be substituted at C-30 [δ_C 73.2; δ_H 5.05 (1H,m)], as confirmed by a COLOC experiment,[14] which revealed cross peaks between the C-36 carbonyl (δ_C 170.3) and a methine at δ 4.69 (H-32) and an acetoxymethyl at δ 2.00; thus the acetoxyl group was placed at C-32 and the dimethylglyceric ester at C-30. Accordingly, the following long range couplings were also observed: δ 7.60 (H-11) to 139.5 (C-10), 155.6 (C-12) and 131.0 (C-13); 8.06 (H-14) to 131.0 (C-13) and 156.5 (C-15); 8.03 (H-17) to 156.5 (C-15), 130.0 (C-16) and 162.8 (C-18). These spectral data along with COSY





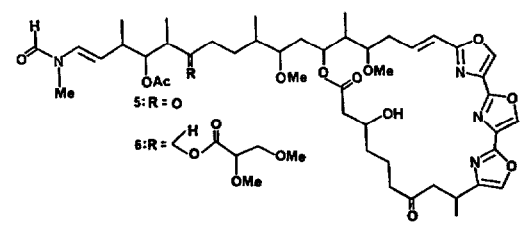


Table 1: ^{13}C[a] and ^{1}H[b] NMR data[c] for mycalolides A - C

	mycalolide A		mycalolide B		mycalolide C	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	172.1		172.0		172.2[h]	
2	42.8	2.50,2.63	42.8	2.42,2.54	42.8	2.46,2.58
3	67.6	4.47	67.6	4.35	67.7	4.42
3-OH		—		—		—
4	41.0	2.50	41.0	2.44	41.1	2.46
5	146.0	7.29	145.6	7.17	146.2	7.23
6	133.2	6.25	133.3	6.17	133.3	6.22
7	202.5		202.5		202.6	
8	43.9	4.00	44.1	3.92	44.0	3.97
8-Me	13.1	0.93	13.2	0.85	13.2	0.90
9	78.1	4.34	77.5	4.29	77.6	4.32
9-OMe	56.8[d]	3.19[e]	56.6	3.12	56.7[f]	3.16[g]
10	139.4		139.5		139.4	
11	137.0	7.66	137.1	7.60	137.2	7.63
12	155.6		155.6		155.6	
13	131.0		131.0		131.0	
14	137.2	8.11	137.4	8.06	137.3	8.09
15	156.4		156.5		156.5	
16	130.0		130.0		130.0	
17	137.3	8.08	137.5	8.03	137.5	8.06
18	162.9		162.8		162.9	
19	116.3	6.36	116.4	6.27	116.3	6.32
20	140.7	7.16	140.6	7.03	140.7	7.08
21	35.1	2.50,2.69	35.0	2.37,2.60	35.3	2.64,2.40
22	79.8	3.46	79.8	3.39	79.6	3.43
22-OMe	58.0[d]	3.32[e]	58.2	3.28	58.3[f]	3.32[g]
23	40.7	1.92	40.7	1.80	40.8	1.86
23-Me	9.0	0.94	9.0	0.83	9.1	0.88
24	73.2	5.29	73.3	5.19	73.2	5.22
25	32.0	1.55,1.63	32.1 (32.0)	1.42,1.50	31.9 (31.7)	1.45,1.54
26	81.8	2.99	81.7	2.90	81.7	2.93
26-OMe	58.2[d]	3.36[e]	57.9	3.24	58.0(57.9)[f]	3.27[g]
27	34.4	1.74	34.8	1.68	34.7(34.8)	1.72
27-Me	15.5	0.84	15.6	0.84	15.7	0.82
28	25.0	1.25,1.74	27.1(26.9)	1.37	30.4	1.52
29	39.5(39.8)	2.47	30.5(30.3)	1.50	30.6(30.4)	1.54
30	211.6		73.2	5.05	72.6(72.7)	5.07
31	48.9(48.6)	2.77	37.7(37.5)	1.76	37.6(37.5)	1.80
31-Me	13.3	1.07	9.9(9.7)	0.89	9.9(9.7)	0.93
32	77.5	5.14	76.5	4.69	76.5	4.74
32-OAc	170.0		170.7		170.6[h]	
	20.8	2.01	21.0	2.00	21.0	2.05
33	36.9(37.0)	2.50	37.0(37.1)	2.48	37.1(37.2)	2.50
33-Me	18.8	1.05	19.4(19.5)	0.93	19.4(19.5)	0.98
34	110.5	4.97	110.4	4.90	110.6	4.94
	(112.2)	(5.00)	(112.1)	(4.92)	(112.2)	(4.96)
35	129.5	6.50	129.5	6.44	129.5	6.46
	(125.5)	(7.16)	(125.5)	(7.08)	(125.5)	(7.13)
35-NMe	27.6	3.03	27.6	2.96	33.1	3.04
	(33.0)	(3.08)	(33.1)	(2.99)	(27.6)	(2.99)
35-NCHO	162.1	8.30	162.2	8.22	162.2	8.26
	(161.0)	(8.08)	(161.0)	(8.00)	(161.0)	(8.04)
36			170.3		172.8[h]	
37			80.7	3.86	76.7	3.80
37-OMe			59.3	3.32	57.7[f]	3.37[g]
38			73.1	3.58	18.5	1.38
38-OMe			58.6	3.41		

a. Recorded at 125 MHz in $CDCl_3$
b. Recorded at 500 MHz in $CDCl_3$
c. The data for the minor geometrical isomers are in parentheses.
d-h. Assignments may be interchanged.

spectra led to the gross structure of mycalolide **B** (**2**), a hybrid of ulapualide **B** and halichondramide.

Mycalolide **C** (**3**),[15] was closely related to mycalolide **B** (**2**) in spectral data, except for a molecular formula of $C_{51}H_{72}N_4O_{16}$ established by FABMS [m/z 997 $(M + H)^+$] and ^1H and ^{13}C NMR spectra, suggesting the loss of one methoxyl group from **2**. This was supported by the presence of a methyl signal in ^1H [δ 1.38 (3H,d)] and ^{13}C NMR spectra (δ 18.5 q), while methoxmethyl signals [δ_H 3.58 (2H,m), δ_C 73.1 t; 3.32 (3H,s), 59.3 q] in **2** were missing. These data together with COSY spectra led us to assign the gross structure of mycalolide **C** (**3**), which is characterized by an O-methyllactate group on C-30.

Mycalolides are antifungal against many pathogenic fungi and cytotoxic against B-16 melanoma cells with IC_{50}s' of 0.5 - 1.0 ng/mL. However, promissing life spans have not been obtained in in vivo experiments due to high toxicity. As reported by the Faulkner group,[11,12] macrolides embracing a trisoxazole unit found in nudibranchs seem to originate from sponges. However, we never encountered nudibranchs feeding on Mycale sp. It is of interest that mycalolides have hybrid structures of halichondramide and ulapualides. Stereochemistry of these compounds remains to be determined.

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13. Mycalolide **B** (**2**): $[\alpha]_D$ = -53.4° (c = 1.3, $CHCl_3$), UV [λ_{max} (MeOH) 230 nm (ε = 31000)]; IR [ν_{max} (film) 3350, 3150, 1720, 1700, 1680, 1650, 1550, 1450, 1370, 1230, 1080, 910, 760 cm^{-1}].
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