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Callystatin A, a Potent Cytotoxic Polyketide from the Marine Sponge, *Callyspongia truncata*

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Abstract: Callystatin A (**1**) has been isolated from the marine sponge, *Callyspongia truncata*, and the plane structure, including parts of the absolute configurations, is elucidated. Callystatin A (**1**) is a novel polyketide with a terminal α,β-unsaturated δ-lactone and exhibited potent cytotoxicity against KB cells at IC_{50} 0.01 ng/ml.

In our continuing search for new bioactive substances from marine organisms,[1] we have isolated a very potent cytotoxic polyketide named callystatin A (**1**) from the marine sponge, *Callyspongia truncata*. This paper reports the elucidation of the plane structure and parts of the absolute configurations of callystatin A (**1**).

An acetone extract of the titled fresh sponge (1.0 kg collected in August at Goto Islands, Nagasaki Prefecture), which exhibited cytotoxicity [98 % inhibition at 10 μg/ml (KB cells)], was subjected to bioassay-guided separation (cytotoxicity against KB cells). The extract was partitioned into a water-AcOEt mixture to provide the cytotoxic AcOEt soluble portion (10 g). Repeated SiO_2 column chromatography (*n*-hexane-AcOEt, *n*-hexane-Et_2O) of the AcOEt soluble portion furnished the active fraction (30 mg) [86 % inhibition at 0.01 μg/ml (KB)], which was further separated by HPLC (Cosmosil 5SL, *n*-hexane-Et_2O-CH_2Cl_2 ; Cosmosil 5C_{18}-AR, MeOH-H_2O) to provide callystatin A (**1**) (1.0 mg) (1.0 x 10^{-2} % from the AcOEt soluble portion). Callystatin A (**1**) exhibited extremely potent cytotoxicity against KB cells (IC_{50} 0.01 ng/ml).

Callystatin A (**1**) was obtained as a colorless oil: $[\alpha]_D$ -107° (*c*= 0.1, MeOH). The UV spectrum of **1** showed the absorption maxima at 243 nm (ε= 30900) and 300 nm (1300) ascribable to an α,β-unsaturated carbonyl and diene chromophores. The IR spectrum of **1** showed the absorption bands due to α,β-unsaturated δ-lactone (1730 cm^{-1}) and ketone (1709 cm^{-1}) groups. The FAB MS of **1** showed a quasimolecular $(M+H)^+$ ion peak at *m/z* 457 and the molecular formula was determined as $C_{29}H_{44}O_4$ by HR-FAB MS and NMR analysis.

The ^1H- and ^{13}C-NMR data of **1** indicated the presence of four secondary methyls, two ethyls, one olefinic methyl, and two oxymethine groups together with ten olefinic carbons and two carbonyl carbons. The COSY spectrum of **1** revealed the presence of four partial structures (fragment A: C-1∼C-7, fragment B: C-9∼C-13 together with a 25-methyl group, fragment C: C-14∼C-16 together with 26- and 27-methyl groups, and

Table 1. ^{1}H- and ^{13}C-NMR Data for Callystatin A (**1**). (500 MHz in $CDCl_3$)

No.	^{13}C δc	^{1}H δ (mult., *J*, (Hz))	HMBC (^{13}C)[a]	No.	^{13}C δc	^{1}H δ (mult., *J*, (Hz))	HMBC (^{13}C)[a]
1	164.1 (s)	-		16	45.6 (d)	3.66 dq (9.8, 6.6)	17
2	121.7 (d)	6.06 dd (9.6, 1.7)	4	17	216.4 (s)	-	
3	144.7 (d)	6.90 dt-like (9.6, 4.2)		18	45.7 (d)	2.85 qd (7.1, 4.3)	
4	30.1 (t)	2.47 m	2, 3	19	74.4 (d)	3.57 dd (6.6, 4.3)	20, 28, 29
5	78.9 (d)	4.98 dt-like (14.2, 6.8)		20	36.7 (d)	1.32 m	22, 29
6	124.7 (d)	5.76 dd (16.0, 6.8)		21	25.8 (t)	1.05 m	
7	129.9 (d)	6.63 d (16.0)	5, 23	22	10.9 (q)	0.84 t (7.3)	20, 21
8	135.3 (s)	-		23	26.4 (t)	2.18 q (6.9)	
9	137.1 (d)	5.24 d (9.6)	7, 8, 23	24	13.4 (q)	1.04 t (6.9)	23
10	32.1 (d)	2.67 m	25	25	20.7 (q)	0.96 d (7.6)	9, 10, 11
11	40.8 (t)	2.08 t-like (7.4)	10	26	13.0 (q)	1.82 d (1.2)	13, 15
12	127.7 (d)	5.57 dt (15.4, 7.4)	13	27	16.1 (q)	1.13 d (6.6)	16
13	135.4 (d)	6.01 d (15.4)	11, 12	28	11.2 (q)	1.11 d (7.1)	17, 18
14	136.2 (s)	-		29	14.2 (q)	0.88 d (6.4)	19, 21
15	128.3 (d)	5.13 dq (9.8, 1.2)	13, 16				

a) C coupled with H.

fragment D: C-18 ~ C-22 together with 28- and 29-methyl groups) as shown in Fig. 1. The connectivity between the 26-methyl group and C-14 was clarified by allylic coupling between H-15 and H_3-26. The presence of these partial structures has also been substantiated by the HOHAHA experiment of **1**. The connectivities of these four partial structures have been figured out on the basis of the following HMBC correlations: 1) adjacency of fragments A and B and ethyl group: cross peaks between H-9 and C-7, 8, 23; H-7 and C-23, 2) adjacency of fragments B and C: cross peaks between H-15, H_3-26 and C-13, 3)

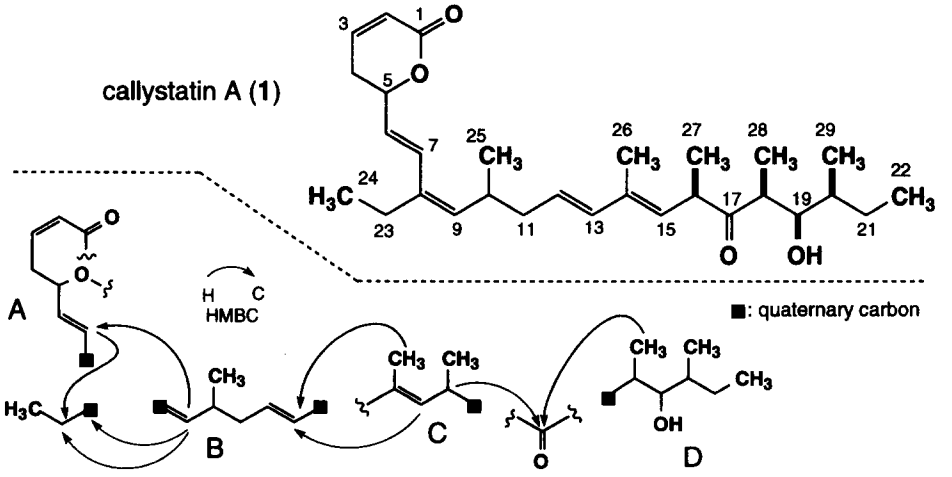


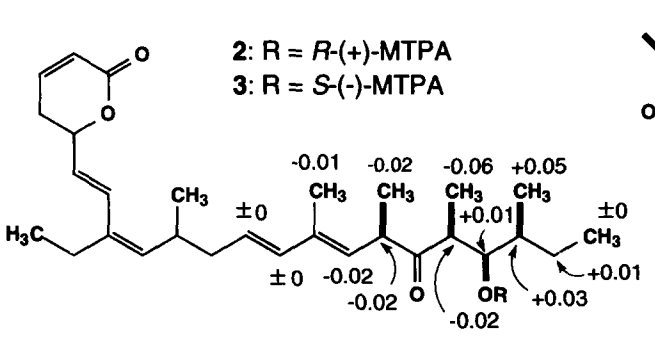
Fig. 1. HMBC correlations among four partial structures

adjacency of fragments C and D through the ketone carbonyl: cross peaks between H-16, H_3-28 and C-17. The presence of terminal δ-lactone was supported by the chemical shift of H-5 (δ 4.98 ppm in $CDCl_3$). The geometries of Δ-6 and Δ-12 olefins were determined as 6*E* and 12*E* by the coupling constants $J_{6,7}$ (16.0 Hz) and $J_{12,13}$ (15.4 Hz), respectively. The geometries of Δ-8 and Δ-14 olefins were confirmed as 8*Z* and 14*E* by the NOEs between H-6, H-9 and 23-methylene protons; between 26-methyl protons and H-12, H-16, respectively. Based on the accumulated evidence, the plane structure of callystatin A has been elucidated as **1** (Fig. 1).

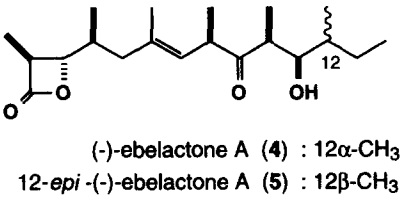
Next, we tried to elucidate the absolute stereostructure at C-19 in callystatin A (**1**) by application of modified Mosher's method.[2] Thus, **1** was treated with *R*-(+)- or *S*-(-)-2-methoxy-2-phenyl-2-trifluoromethylacetic acid (MTPA), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, and *N,N*-dimethylamino-pyridine in CH_2Cl_2 at room temperature to furnish the 19-*O*-*R*-(+)-MTPA ester **2**[3] and 19-*O*-*S*-(-)-MTPA ester **3**[4], respectively. All proton signals of both **2** and **3** were assigned and the absolute configuration at C-19 was determined as *R* by the analysis of Δδ values (Fig. 2).

As for stereochemistry at C-16, C-18, and C-20 methyls in **1**, we analyzed the NMR data by comparison with those of (-)-ebelactone A (**4**)[5,6], 12-*epi*-(-)-ebelactone A (**5**),[6] and the related polyketides[7] having a similar partial structure. The proton and carbon chemical shifts and coupling constants (*e.g.* $J_{18,19}$= 4.3 Hz, $J_{19,20}$= 6.6 Hz) of the structure from C-16 to C-22 including 27, 28, 29-methyls in **1** were very similar to those of **5**.[8] These findings led us to presume that the absolute configurations from C-16 to C-20 in callystatin A (**1**) were 16*R*, 18*S*, 19*R*, and 20*S*, respectively.

So far, several groups have isolated the related antitumor antibiotics, *e.g.*, leptomycin,[9,10] kazusamycin,[11] anguinomycin[12] and leptofuranin,[13] from the cultured broth of several strains of *Streptomyces* sp. and elucidated their plane structures. We have also isolated callystatin A (**1**) from a marine sponge of *Stelletta* sp. and an unidentified marine tunicate, which were collected at the same collection site as the titled sponge. These facts may indicate the participation of a presumable symbiotic microorganism in the biosynthesis of callystatin A (**1**) in the marine sponge *Callyspongia truncata*. The absolute stereostructures of C-5 and C-10 in callystatin A (**1**) are currently under investigation by synthetic methods.



Δδ = δ*S*-(-)-MTPA ester **3**− δ*R*-(+)-MTPA ester **2**



(-)-ebelactone A (**4**) : 12α-CH_3
12-*epi*-(-)-ebelactone A (**5**) : 12β-CH_3

Fig. 2. Application of modified Mosher's method to **2** and **3**

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3) HR-FAB MS: Obsd; *m/z* 673.3740. Calcd for $C_{39}H_{52}O_6F_3$; *m/z* 673.3716 $(M+H)^+$. IR (KBr); 1740, 1719, 1709, 1647 cm^{-1}. ^{1}H-NMR ($CDCl_3$, δ); 5.13 (br d, *J*=9.9 Hz, H-15), 3.55 (dq, *J*=9.9, 6.6, H-16), 3.00 (qd, *J*=7.1, 4.3, H-18), 5.49 (dd, *J*=6.6, 4.3, H-19), 1.43 (m, H-20), 1.27 (m, H-21), 0.80 (t, *J*=7.3, H-22), 1.82 (d, *J*=1.2, H-26), 1.13 (d, *J*=6.6, H-27), 1.01 (d, *J*=7.1, H-28), 0.69 (d, *J*=6.4, H-29).

4) HR-FAB MS: Obsd; *m/z* 673.3723. Calcd for $C_{39}H_{52}O_6F_3$; *m/z* 673.3716 $(M+H)^+$. IR (KBr); 1740, 1719, 1709, 1647 cm^{-1}. ^{1}H-NMR ($CDCl_3$, δ); 5.11 (br d, *J*=9.9 Hz, H-15), 3.53 (dq, *J*=9.9, 6.6, H-16), 2.98 (qd, *J*=7.1, 4.3, H-18), 5.50 (dd, *J*=6.6, 4.3, H-19), 1.46 (m, H-20), 1.28 (m, H-21), 0.80 (t, *J*=7.3, H-22), 1.81 (d, *J*=1.2, H-26), 1.11 (d, *J*=6.6, H-27), 0.95 (d, *J*=7.1, H-28), 0.74 (d, *J*=6.4, H-29).

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8) Reported NMR data[6] of **4** and **5** are assigned as follows. **4**: δ 2.84 (qd, *J*= 7.3, 2.3 Hz, 10-H), 3.49 (dt, *J*= 8.9, 2.3 Hz, 11-H), 1.47-1.40 (m, 12-H); δc 217.7 (9-C), 44.9 (10-C). **5**: δ 2.86 (qd, *J*= 7.1, 4.1 Hz, 10-H), 3.59-3.53 (m, 11-H), 1.44-1.32 (2H, m, 12-H); δc 216.9 (9-C), 45.7 (10-C).

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