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Cortistatins A, B, C, and D, Anti-angiogenic Steroidal Alkaloids, from the Marine Sponge *Corticium simplex*

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Angiogenesis is the process of generating new capillary blood vessels, which is responsible for the progression of solid tumors, diabetic retinopathy, psoriasis, and rheumatoid arthritis.[1] Especially, tumor growth and metastasis are highly dependent on angiogenesis. Therefore, specific inhibitors of angiogenesis are expected to be promising antitumor agents.[2]

In the course of our study of bioactive substances from marine organisms, we focused on a search for anti-angiogenic substances and isolated bastadin 6 and related compounds, spongean brominated-tyrosine tetramers, as selective inhibitors of proliferation of human umbilical vein endothelial cells (HUVECs).[3] Bastadin 6 also inhibited VEGF- or bFGF-induced tubular formation and VEGF-induced migration of HUVECs. Moreover, bastadin 6 almost completely blocked VEGF- or bFGF-induced in vivo neovascularization in mice corneal assay and suppressed the growth of subcutaneously inoculated A431 solid tumors in nude mice.[4] Recently, we have isolated four novel steroidal alkaloids named cortistatins A (**1**), B (**2**), C (**3**), and D (**4**), which exhibited highly selective anti-proliferative activity against HUVECs, from the marine sponge *Corticium simplex*. In this paper, the structure determination of these steroidal alkaloids is presented.

The MeOH extract of the titled dried sponge (1.5 kg), which showed selective anti-proliferative activity against HUVECs, was subjected to bioassay-guided separation. After solvent partition, the active alkaloid fraction was subjected to LH-20 column chromatography (eluted with MeOH) and HPLC (YMC-pak NH_2, $CH_3CN-CHCl_3-H_2O$) to isolate four novel steroidal alkaloids named cortistatins A (**1**, 22 mg), B (**2**, 7 mg), C (**3**, 25 mg), and D (**4**, 28 mg).

Cortistatin A (**1**) was obtained as a colorless powder. The ESI-TOF MS of **1** gave a molecular ion [$(M + H)^+$] peak at *m*/*z* 473, and the molecular formula was determined as $C_{30}H_{36}N_2O_3$ by HR ESI-TOF MS. All of the proton and carbon signals of **1** were assigned by 2D NMR (COSY, HMQC, and HMBC) analysis, and the planar structure consisting of a 9(10−19)-*abeo*-androstane and isoquinoline skeleton was clarified by HMBC analysis (Figures 1 and 2).

The relative stereostructure of **1** was elucidated on the basis of NOESY correlations and $^3J_{HH}$ coupling constants (Figure 2). Thus, the correlations between H-1 and H-3 and the large coupling constants (dd, J = 9.6, 9.6 Hz) of the H-2 revealed the axial orientations for H-1, -2, and -3 protons. The correlation between H-1 and H-6a (δ 2.19) also disclosed the geometry of the oxygenated bridge in the seven-membered ring B. The correlation between H-14 and Hax-12, H-17; H-7a (δ 1.78) and 18-CH_3 revealed the trans-axial orientation for H-14 and 18-CH_3. The β

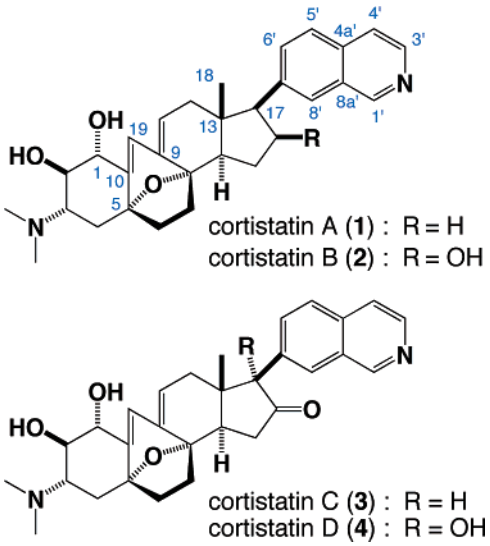

Figure 1. Chemical structures of cortistatins.

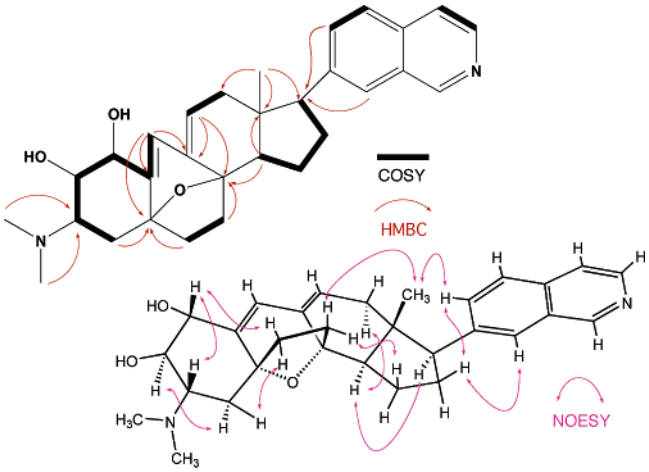

Figure 2. Key HMBC and NOESY correlations in cortistatin A (**1**).

orientation of the isoquinoline unit at the C-17 position was deduced from the correlation between 18-CH_3 and H-6′, H-8′ in the isoquinoline unit. The crystal of **1** obtained from acetone was subjected to X-ray crystallographic analysis to confirm the relative stereostructure of **1** (Figure 3).

To determine the absolute structure of cortistatin A (**1**), we applied the circular dichroism (CD) exciton chirality method[5] to **1**. Compound **1** showed split CD maxima ($\Delta\epsilon$ −17 at 237 nm and $\Delta\epsilon$ +35.0 at 217 nm), which came from the exciton coupling between the 9(11),10(19)-diene and the isoquinoline chromophores.

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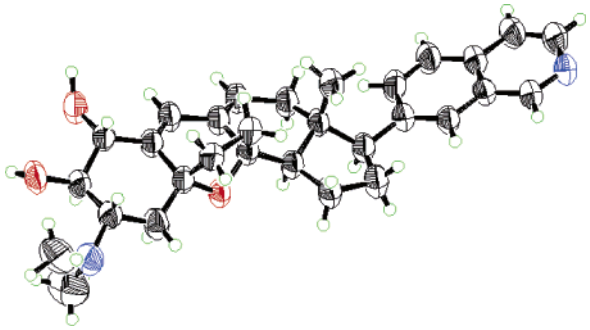

Figure 3. Perspective drawing of cortistatin A (**1**) obtained from X-ray crystallographic analysis.

As a result, the absolute configuration at the C-17 position in **1** was determined as *S*, and the absolute structure of cortistatin A (**1**) was confirmed as shown in Figure 1.

Cortistatin B (**2**, $C_{30}H_{36}N_2O_4$) showed closely similar ^{1}H and ^{13}C NMR spectra to those of **1**, except for additional signals ascribable to the oxymethine (δ 4.68, δc 73.8). On the basis of the 2D NMR analysis of **2**, cortistatin B was deduced to be a 16-hydroxyl analogue of **1**. The β configuration of the hydroxyl group at C-16 was disclosed from the NOESY correlation between H-16 and H-14, and the structure of cortistatin B (**2**) was determined as shown in Figure 1.

Cortistatin C (**3**, $C_{30}H_{34}N_2O_4$) also showed closely similar ^{1}H and ^{13}C NMR spectra to those of **1**, except for the H-17 signal (δ 3.64, s) and an additional ketocarbonyl signal (δc 214.4). On the basis of the IR absorption (1740 cm^{-1}) due to the five-membered ketone and the HMBC correlation between H-15ab, H-17 and ketocarbonyl, the chemical structure of cortistatin C (**3**) was determined to be the 16-keto analogue of **1**.

Cortistatin D (**4**, $C_{30}H_{34}N_2O_5$) showed a closely similar ^{1}H NMR spectrum to that of **3**, except for lacking the H-17 signal. In the ^{13}C NMR spectrum of **4**, an additional oxygenated quaternary carbon signal (δc 83.5) was observed, and the locations of the hydroxyl groups in **4** were confirmed by the deuterium shifts observed in the ^{13}C NMR spectra of **4** taken in CD_3OH and CD_3-OD.[6] On the basis of the HMBC and NOESY correlations of **4**, cortistatin D was confirmed to be the 17α-hydroxyl analogue of **3**.

Cortistatin A (**1**) showed cytostatic anti-proliferative activity against HUVECs at 100 pM to 1 μM, in which the selective index was more than 3000-fold in comparison with that of normal human dermal fibroblast (NHDF) and several tumor cells (KB epidermoid carcinoma cells (KB3-1), human chronic myelogenous leukemia cells (K562), and murine neuroblastoma cells (Neuro2A)) (Table 1). In contrast, doxorubicin showed weak anti-proliferative activity against HUVECs and no selectivity between HUVECs and the other cells (IC_{50} = 5.1 nM against HUVECs; selective index: 4.1, 3.5, 5.5, and 1.9 for KB3-1, K562, Neuro2A, and NHDF, respectively). The anti-angiogenic property of cortistatin A (**1**) was examined by the migration assay using a chemotactic chamber and the Matrigel tubular formation assay. Cortistatin A also inhibited migration and tubular formation of HUVECs induced by VEGF or bFGF at 2 nM concentration, respectively.

Table 1. Selective Growth Inhibition of Cortistatins against HUVECs[a]

	1		2		3		4	
cell line	IC_{50}	S.I.	IC_{50}	S.I.	IC_{50}	S.I.	IC_{50}	S.I.
HUVECs	0.0018	1	1.1	1	0.019	1	0.15	1
KB3−1	7.0	3900	120	110	150	7900	55	460
Neuro2A	6.0	3300	160	150	180	9500	>300	nd
K562	7.0	3900	200	180	>300	nd	>300	nd
NHDF	6.0	3300	>300	nd	>300	nd	>300	nd

[a] IC_{50} = μM; nd = not determined; S.I. = selective index: IC_{50} against testing cells/IC_{50} against HUVECs.

Cortistatins are a unique 9(10−19)-*abeo*-androstane-type steroidal alkaloids having oxabicyclo[3.2.1]octene and isoquinoline units. With a similar steroidal alkaloid[7] having a 9(10−19)-*abeo*-androstane skeleton and several steroidal alkaloids having amino groups at C-3, 29 positions have been found from marine sponges of *Corticium* sp. Interestingly, several steroidal alkaloids having a 9(10−19)-*abeo*-androstane skeleton have been also found from medicinal plants of *Buxus* and *Cimicifuga* sp.[8−11] Detailed evaluation of the anti-angiogenic effects of cortistatins is under investigation.

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Supporting Information Available: ^{1}H and ^{13}C NMR data, crystallographic data, and experimental procedure. This material is available free of charge via the Internet at http://pubs.acs.org.

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