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Tetrahedron Letters
Tetrahedron Letters 48 (2007) 4485–4488

Cortistatins J, K, L, novel abeo-9(10-19)-androstane-type steroidal alkaloids with isoquinoline unit, from marine sponge *Corticium simplex*

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Received 4 April 2007; revised 27 April 2007; accepted 1 May 2007
Available online 5 May 2007

Abstract—Three novel anti-angiogenic steroidal alkaloids, cortistatins J (**1**), K (**2**), L (**3**), have been isolated from the Indonesian marine sponge *Corticium simplex*. The chemical structures of cortistatins J (**1**), K (**2**), and L (**3**) were determined by 2D-NMR analysis to be unique abeo-9(10-19)-androstane-type steroidal alkaloids having isoquinoline unit instead of the side chain part, respectively. Cortistatin J (**1**) showed cytostatic anti-proliferative activity against human umbilical vein endothelial cells (HUVECs) at 8 nM, in which the selective index was 300–1000-fold in comparison with other cell lines.

Angiogenesis, the formation of new blood capillaries from the preexisting blood vessels, is a highly regulated and essential process for embryogenesis. In contrast, many pathological symptoms including cancer, rheumatoid arthritis, diabetic retinopathy, and various inflammatory diseases involve undesirable neovascularization.[1] In particular, angiogenesis is now widely recognized to be a necessary event for growth of tumor and metastasis. Therefore, specific inhibitors of angiogenesis are expected as promising antitumor agents.[2]

In the course of our study of bioactive substances from marine organisms, we focused on a search for selective inhibitors of proliferation of human umbilical vein endothelial cells (HUVECs), as anti-angiogenic substances.[3,4] On the basis of bioassay-guided separation, we have isolated four anti-angiogenic abeo-9(10-19)-androstane-type steroidal alkaloids named cortistatins A (**4**)–D[5] together with four inactive derivatives named cortistatins E (**5**)–H[6] from the Indonesian marine sponge *Corticium simplex*. Cortistatins A (**4**)–D were the unique steroidal alkaloids having isoquinoline unit instead of the side chain part, while cortistatins E (**5**)–H were the steroidal alkaloids having *N*-methyl piperidine or 3-methylpyridine unit in the side chain part. Cortistatin A (**4**) showed highly selective anti-proliferative activity against HUVECs and also inhibited migration and tubular formation of HUVECs induced by VEGF or bFGF. Further detailed examination of the extract of the same marine sponge led us to isolate three novel active derivatives having isoquinoline unit, named cortistatins J (**1**), K (**2**), and L (**3**). The structure elucidation and the anti-angiogenic property of these cortistatins are described.

The MeOH extract of the titled dried sponge (560 g), which showed selective anti-proliferative activity against HUVECs, was subjected to bioassay-guided separation. After solvent partition, the active alkaloids fraction was subjected to LH-20 column chromatography (eluted with MeOH) and HPLC (ODS column, eluted with MeOH–H_2O containing 0.1% Et_3N; 5-NH_2 column, eluted with CH_3CN–$CHCl_3$–H_2O) to isolate three novel steroidal alkaloids named cortistatins J (**1**, 3 mg), K (**2**, 4 mg), and L (**3**, 3 mg).

Cortistatin J (**1**)[7] was obtained as a colorless powder. The ESI-TOFMS of **1** gave a molecular ion [$(M+H)^+$]

Keywords: Cortistatins; Steroidal alkaloid; Anti-angiogenesis; HUVECs.
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doi:10.1016/j.tetlet.2007.05.003

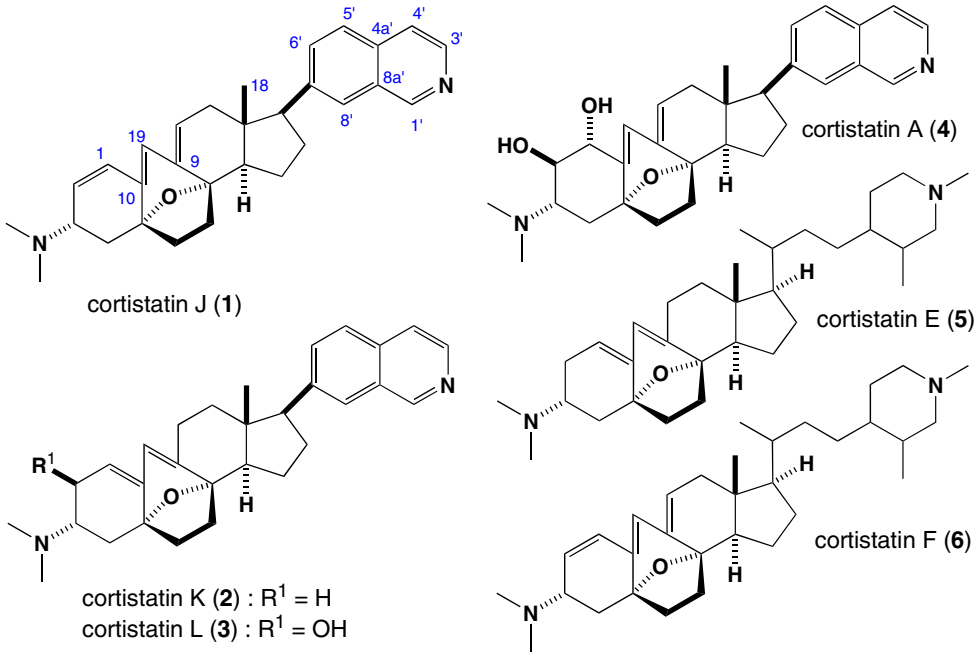

Figure 1. Chemical structures of cortistatin J (**1**), K (**2**), and L (**3**).

peak at *m*/*z* 439, and the molecular formula was determined as $C_{30}H_{34}N_2O$ by HR-ESI-TOFMS in conjunction with NMR analysis. The ^1H and ^{13}C NMR spectra of **1** (Tables 1 and 2) showed closely similar signals assignable to the abeo-9(10-19)-androstane-type steroidal skeleton with the oxabicyclo[3.2.1]octene (δ_C 82.3, 78.8) unit and the conjugated triene system (δ_C 141.1, 139.6, 131.2, 127.7, 122.0, 121.3) observed in cortistatin F (**6**) and the isoquinoline unit (δ 9.23 (s-like), 8.49 (br d, $J = 5.5$ Hz), 7.80 (s-like), 7.76 (br d, $J = 8.6$ Hz), 7.62 (d-like, $J = 5.5$ Hz), 7.59 (dd, $J = 1.6$, 8.6 Hz), δ_C 152.3, 142.4, 140.0, 134.7, 132.0, 128.5, 126.3, 125.8, 120.0) observed in cortistatin A (**4**). The UV absorption at 293, 280, and 269 nm also supported the presence of triene system in **1**. From the detailed 2D-NMR (COSY, HMQC and HMBC) analysis, cortistatin J (**1**) was deduced to be an analogue of cortistatin F (**6**) having the isoquinoline unit (Fig. 2). The relative stereostructure of cortistatin J (**1**) was determined on the basis of the NOESY correlations (Fig. 2). Thus, the correlations between H-3 and H-6a, H-6b revealed the β axial orientation for the H-3 proton and the geometry of the oxygenated bridge in the seven-membered ring B. The correlations between H-14 and H-12_{ax}, H-17; H-7a and 18-CH_3 revealed the trans–axial orientation for H-14 and 18-CH_3. The β orientation of the isoquinoline unit at the C-17 position was deduced from the correlation between 18-CH_3 and H-6′ or H-8′ in the isoquinoline unit.

Cortistatin K (**2**)[8] gave a molecular ion [$(M+H)^+$] peak at *m*/*z* 441, and the molecular formula was determined as $C_{30}H_{36}N_2O$ by HR-ESI-TOFMS. The ^1H and ^{13}C NMR spectra of **2** showed closely similar signals assignable to the abeo-9(10-19)-androstane-type steroidal skeleton having the oxabicyclo[3.2.1]octene and the 1(10),9(19)-diene system (δ_C 144.9, 140.2, 119.7, 117.7) of cortistatin E (**5**) and the isoquinoline unit of cortistatin A (**4**) (Tables 1 and 2). The detailed analysis of the HMBC correlations and NOESY correlations of **2** revealed the assignment of each ^1H and ^{13}C signals (Tables 1 and 2) and the chemical structure of cortistatin K (**2**) as shown in Figure 1.

Cortistatin L (**3**)[9] ($C_{30}H_{36}N_2O_2$) showed closely similar ^1H and ^{13}C NMR spectra to those of **2**, except for an additional signal ascribable to the secondary hydroxyl group [δ 4.24 (d-like, $J = 8.8$ Hz), δ_C 67.7]. The position of the secondary hydroxyl group was assigned at C-2 on the basis of the detailed 2D-NMR analysis of **3** (Fig. 3). The relative stereostructure of cortistatin L (**3**) including the β-equatorial orientation for the C-2 hydroxyl group was deduced from the NOESY correlations (Fig. 3) and the $^3J_{HH}$ coupling constant ($^3J_{HH} = 8.8$ Hz) between H-2 and H-3. Consequently, the chemical structure of cortistatin L was elucidated to be the C-2β hydroxyl analogue (**3**) of cortistatin K (**2**).

To confirm the absolute stereostructure of cortistatins J (**1**), K (**2**), and L (**3**), we have applied the circular dichroism (CD) exciton chirality method.[10] Compound **1** showed the characteristic split CD maxima ($\Delta\varepsilon$ −8.4 at 279 nm and $\Delta\varepsilon$ +22.6 at 224 nm), which came from the exciton coupling between the 1,9(11),10(19)-triene and the isoquinoline chromophores. Then, the absolute configuration at the C-17 position in **1** was determined as *S*, and the absolute structure of cortistatin J (**1**) was confirmed as shown in Figure 1.[8] While, compounds **2** and **3** did not show the split CD maxima, which were expected to induce the exciton coupling between the 1(10),9(19)-diene and the isoquinoline chromophores. This difference was presumed to come from the difference of the spatial direction of the transition moment between the conjugated triene and the conjugated diene

Table 1. ^{1}H NMR data for cortistatins J (**1**), K (**2**), and L (**3**) (at 600 MHz in $CDCl_3$, *J* values in Hz)

No.	**1**	**2**	**3**
1	6.11 (dd, 2.8, 9.9)	5.27 (dd, 2.0, 4.7)	5.34 (s-like)
2_{eq}	5.81 (d-like, 9.9)	2.29 (m)	—
2_{ax}	—	2.16 (m)	4.24 (d-like, 8.8)
3	3.51 (m)	2.67 (br s)	2.61 (dd-like, 8.8, 11.6)
4_{eq}	1.94 (dd, 4.2, 11.6)	2.02 (m)	1.92 (d-like, 11.8)
4_{ax}	2.00 (m)	1.89 (m)	1.83 (m)
6a	2.06 (m)	2.02 (m)	2.07 (m)
6b	1.68 (m)	1.87 (m)	1.87 (m)
7b	2.28 (m)	2.09 (dd-like, 5.2, 11.3)	2.08 (m)
7a	1.77 (m)	1.84 (m)	1.83 (m)
11_{ax}	5.43 (dd, 2.8, 5.2)	2.36 (m)	2.37 (m)
11_{eq}	—	2.29 (m)	2.30 (m)
12_{eq}	1.98 (m)	1.61 (ddd, 1.9, 5.2, 12.9)	1.62 (dd-like, 5.1,12.5)
12_{ax}	2.40 (d-like, 17.9)	1.51 (ddd, 4.9, 12.9, 12.9)	1.52 (ddd, 5.0, 12.5, 12.5)
14	2.56 (dd, 8.5, 11.5)	2.26 (m)	2.26 (m)
15b	2.06 (m)	2.00 (m)	1.98 (m)
15a	1.87 (dd-like, 5.0, 12.1)	1.81 (m)	1.80 (m)
16b	2.34 (m)	2.32 (m)	2.31 (m)
16a	2.19 (m)	2.14 (m)	2.14 (m)
17	3.16 (dd, 9.4, 10.7)	3.00 (dd, 9.6, 9.9)	3.00 (dd, 9.6, 9.9)
18	0.57 (3H, s)	0.60 (3H, s)	0.60 (3H, s)
19	5.84 (s-like)	5.74 (d, 2.0)	5.75 (s-like)
1′	9.23 (s-like)	9.22 (s-like)	9.22 (s-like)
3′	8.49 (br d, 5.5)	8.48 (br d, 5.8)	8.49 (br d, 5.5)
4′	7.62 (d-like, 5.5)	7.63 (d-like, 5.8)	7.62 (d-like, 5.5)
5′	7.76 (br d, 8.6)	7.75 (d-like, 8.3)	7.75 (d-like, 8.5)
6′	7.59 (dd, 1.6, 8.6)	7.57 (dd, 1.6, 8.3)	7.57 (d-like, 8.5)
8′	7.80 (s-like)	7.79 (s-like)	7.78 (s-like)
N-$(CH_3)_2$	2.34 (6H, s)	2.33 (6H, s)	2.31 (6H, s)

Table 2. ^{13}C NMR data for cortistatins J (**1**), K (**2**), and L (**3**) (at 150 MHz in $CDCl_3$)

No.	**1**	HMBC (^{1}H)	**2**	HMBC (^{1}H)	**3**	HMBC (^{1}H)
1	127.7	19	117.7	2_{ax}, 19	120.2	2, 19
2	131.2	4_{ax}	28.3	4_{ax}	67.7	3, 4_{eq}, 4_{ax}
3	60.3	1, 4_{eq}, 4_{ax}, *N*-$(CH_3)_2$	58.8	1, 2_{ax}, 4_{eq}, 4_{ax}, *N*-$(CH_3)_2$	66.1	1, 4_{eq}, 4_{ax}, *N*-$(CH_3)_2$
4	30.9	2	36.6	6b	30.2	6b
5	78.8	1, 4_{eq}, 4_{ax}, 6b, 7b, 19	79.2	1, 4_{eq}, 4_{ax}, 19	79.6	1, 4_{eq}, 4_{ax}, 19
6	38.0	4_{eq}, 4_{ax}, 7a, 7b	38.8	4_{ax}, 7b	38.5	4_{eq}, 7a, 7b
7	30.5	6b, 14	33.0	6b, 14	32.5	6a, 6b, 14
8	82.3	6a, 7b, 11, 14, 15a, 19	83.2	7a, 7b, 11_{eq}, 19	83.7	6b, 7b, 11_{eq}, 14, 15a, 19
9	141.1	7a, 7b, 12_{eq}, 12_{ax}, 19	144.9	7b, 12_{eq}	146.8	7b, 11_{eq}, 12_{eq}
10	139.6	1, 2, 4_{ax}, 6b	140.2	2_{eq}, 2_{ax}, 4_{eq}, 4_{ax}, 19	140.8	2, 4_{eq}, 4_{ax}, 6a, 6b, 19
11	122.0	12_{eq}, 12_{ax}, 19	28.4	12_{eq}, 12_{ax}, 19	28.5	12_{ax}, 19
12	40.3	11, 17, 18	37.2	17, 18	37.1	11_{eq}, 14, 17, 18
13	44.8	11, 12_{eq}, 12_{ax}, 14, 17, 18	45.3	12_{eq}, 12_{ax}, 14, 16, 17, 18	45.2	11_{eq}, 12_{eq}, 12_{ax}, 14, 15b, 16a, 17, 18
14	51.7	7b, 12_{ax}, 15a, 18	53.5	7b, 12_{eq}, 15a, 16a, 18	53.4	7b, 12_{eq}, 15a, 16a, 16b, 18
15	20.6	14, 16b	20.7	14	20.6	14
16	26.4	15a, 15b, 17	25.9	17	25.9	15a, 17
17	56.9	12_{eq}, 18, 6′, 8′	57.5	18, 6′, 8′	57.5	18, 6′, 8′
18	15.4	12_{eq}, 12_{ax}, 14, 17	12.8	12_{ax}, 14, 17	12.8	12_{ax}, 14, 17
19	121.3	1, 11	119.7	1	119.4	1, 11_{eq}
1′	152.3	3′, 8′	152.4	3′, 8′	152.3	3′, 8′
3′	142.4	1′, 4′	142.5	1′, 4′	142.5	1′, 4′
4′	120.0	3′, 5′	120.1	3′, 5′	120.1	3′, 5′
4a′	134.7	1′, 3′, 4′, 6′, 8′	134.6	1′, 3′, 5′, 6′, 8′	134.7	1′,3′, 5′, 6′, 8′
5′	125.8	4′	125.6	4′	125.7	4′
6′	132.0	17, 8′	132.3	17, 8′	132.3	17, 8′
7′	140.0	17, 5′	140.0	17, 5′	139.8	17, 5′
8′	126.3	17, 1′, 6′	126.4	17, 1′, 6′	126.4	17, 1′, 6′
8a′	128.5	1′, 4′, 5′	128.7	1′, 4′, 5′	128.5	1′, 4′, 5′
N-$(CH_3)_2$	40.2	*N*-$(CH_3)_2$	41.1	*N*-$(CH_3)_2$	40.4	3, *N*-$(CH_3)_2$

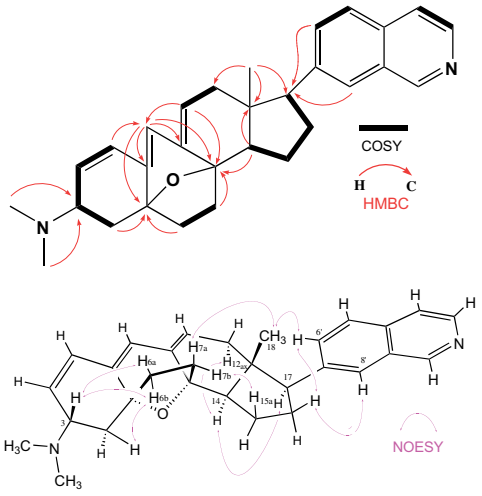

Figure 2. Key HMBC and NOESY correlations of cortistatin J (**1**).

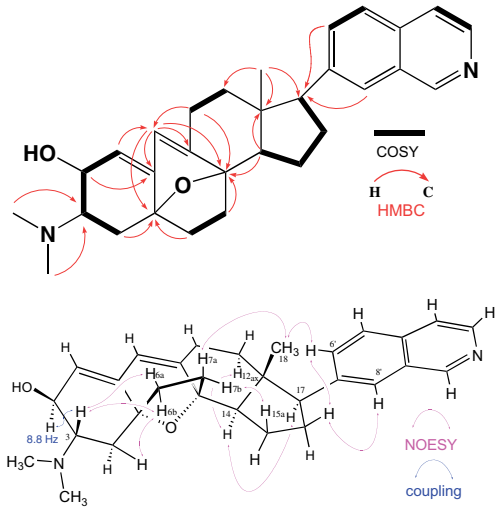

Figure 3. Key HMBC and NOESY correlations of cortistatin L (**3**).

chromophores. In view of the similarity of the chemical structures of cortistatins, the absolute stereostructures of cortistatins K (**2**) and L (**3**) were also presumed to be the same as cortistatins A (**4**) and J (**1**).

Cortistatin J (**1**) showed cytostatic anti-proliferative activity against HUVECs ($IC_{50} = 8$ nM), in which the selective index was 300–1100-fold higher in comparison with those of normal human dermal fibroblast (NHDF) and several tumor cell lines [KB epidermoid carcinoma cells (KB3-1), human chronic myelogenous leukemia cells (K562), and murine neuroblastoma cells (Neuro2A)]. While, cortistatins K (**2**) and L (**3**) showed less selective anti-proliferative activity against HUVECs ($IC_{50} = 40$ and 23 nM, respectively) with the 60–610-fold selective index. The mechanistic study and the structure–activity relationship study of cortistatins are now in progress.

Acknowledgment

This study was financially supported by Grant-in-Aid for scientific research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

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7. Cortistatin J (**1**): $[\alpha]_D^{20}$ −54.0 (*c* 0.26, $CHCl_3$). UV λ_{max} (MeOH) nm: 293 (ε 21200), 280 (ε 8500), 269 (ε 3000), 223 (ε 46200). HR-ESI-TOFMS: Obsd; *m/z* 439.2749. Calcd for $C_{30}H_{35}N_2O$; *m/z* 439.2749 $(M+H)^+$. IR (KBr): 1377, 1456 cm^{-1}. ^1H and ^{13}C NMR spectra: as shown in Tables 1 and 2.
8. Cortistatin K (**2**): $[\alpha]_D^{20}$ −47.1 (*c* 0.32, $CHCl_3$). UV λ_{max} (MeOH) nm: 225 (ε 35200). ESI-TOFMS: *m/z* 441 $(M+H)^+$. HR-ESI MS: Obsd; *m/z* 441.2910. Calcd for $C_{30}H_{37}N_2O$; *m/z* 441.2906 $(M+H)^+$. IR (KBr): 1377, 1454 cm^{-1}. ^1H and ^{13}C NMR spectra: as shown in Tables 1 and 2.
9. Cortistatin L (**3**): $[\alpha]_D^{20}$ −28.9 (*c* 0.20, $CHCl_3$). UV λ_{max} (MeOH) nm: 224 (ε 55900). ESI-TOFMS: *m/z* 457 $(M+H)^+$. HR-ESI MS: Obsd; *m/z* 457.2860. Calcd for $C_{30}H_{37}N_2O_2$; *m/z* 457.2855 $(M+H)^+$. IR (KBr): 1375, 1454, 3344 cm^{-1}. ^1H and ^{13}C NMR spectra: as shown in Tables 1 and 2.
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