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Orostanal, a novel abeo-sterol inducing apoptosis in leukemia cell from a marine sponge, *Stelletta hiwasaensis*

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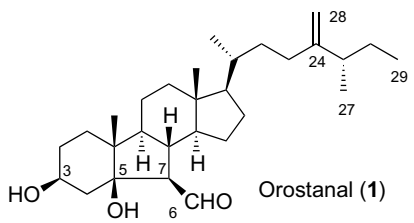
Abstract—A sterol derivative, orostanal, was obtained from a marine sponge of *Stelletta hiwasaensis*. Spectroscopic analysis and synthetic study revealed its structure as a novel 5(6→7)abeo-sterol. Orostanal and its synthetic analog induce apoptosis in human acute promyelotic leukemia cell.

Apoptosis is defined as a physiological and programmed cell death, which is distinguished from necrosis.[1] Apoptosis plays an important role not only in the development but also in the maintenance of homeostasis. Abnormal control of apoptosis may play a role in the development and survival of cancer as well as other diseases. It is well known that natural anti-cancer drugs, etoposide and camptothecin, induce apoptosis in cancer cells, so that the substance which induces apoptosis must be useful for human cancer chemotherapy.[2]

While searching for biologically active substances from marine invertebrates, we isolated a novel sterol named orostanal (**1**) as an active constituent inducing apoptosis, from a Japanese marine sponge, *Stelletta hiwasaensis*. In this paper, we will report on the isolation and structural determination of **1**.

S. hiwasaensis (wet weight 2.0 kg) was collected by hand at depths of 10 to 15 m off Oro Island, Fukuoka Prefecture, Japan, in 1997.[3] The *n*-hexane soluble fraction (7.86 g) of the acetone extract obtained from *S. hiwasaensis* showed chromatin condensation and DNA fragmentation at 33 μg/mL against human acute promyelotic leukemia cell (HL-60).[4] Bioassay-guided separation of the active fraction by silica-gel chromatography ($CHCl_3$:MeOH/10:0→1:1), followed by reversed-phase column chromatography (95% MeOH/H_2O), and reversed-phase HPLC (100% MeOH) gave an active compound (**1**) (3.6 mg, 1.8×10^{-4}% yield).



Orostanal (**1**)

Orostanal (**1**)[5] was obtained as an amorphous solid. The IR spectrum revealed absorption bands due to hydroxyl (3502 cm^{-1}) and carbonyl (1715 cm^{-1}). The ^1H, ^{13}C NMR and HSQC spectral data suggested the presence of one primary methyl, two secondary methyls, two tertiary methyls, ten methylenes, seven methines, two quaternary carbons, one oxygenated methine, one oxygenated quaternary carbon, one *exo*-methylene, and one aldehyde (Table 1). The HR-EI MS of **1** showed a molecular ion peak at *m/z* 444.3607 (Δ +0.4 mmu) corresponding to the molecular formula of $C_{29}H_{48}O_3$. The ^1H–^1H COSY, TOCSY, and HSQC–TOCSY spectra of **1** afforded three partial structures, A [C1–C4], B[C6–C12, C21, C23] and C[C25–C27, C29]. These three partial structures and three quaternary carbones, two tertiary methyls, and *exo*-methylene were merged by the aid of the HMBC experiment as shown in Fig. 1.

The relative stereochemistry except C-25 of **1** was assigned on the basis of the NOESY correlations, and the stereochemistry of C-25 was elucidated by comparison of the ^1H NMR data with those of (25*S*)- and (25*R*)-24(28)-dehydroaplysterols as shown in Fig. 2.[6]

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Table 1. ^{1}H and ^{13}C NMR spectral data of **1** and **2**

No.	**1** δ_C	δ_H	HSQC-TOCSY[a]	**2** δ_C
1	26.8 (t)	1.62 (m), 1.31 (m)	2, 3, 4	26.8 (t)
2	28.0 (t)	1.54 (m), 1.60 (m)	1, 3, 4	28.0 (t)
3	67.4 (d)	4.05 (quintet, 3.2)	1, 2, 4	67.4 (d)
4	44.3 (t)	1.66 (dd, 3.1, 14.9), 2.08 (brd)	3	44.3 (t)
5	84.2 (s)	–		84.3 (s)
6	204.6 (d)	9.63 (d, 2.8)	7	204.6 (d)
7	63.9 (d)	2.17 (dd, 2.8, 9.0)	6, 8, 9, 11, 12, 14	64.0 (d)
8	40.0 (d)	2.04 (m)	7, 9, 11, 12, 14	40.1 (d)
9	50.5 (d)	1.22 (m)	7, 8, 11, 12	50.5 (d)
10	45.5 (s)	–		45.5 (s)
11	21.6 (t)	1.32 (m), 1.38 (m)	7, 9, 11, 12	21.6 (t)
12	39.7 (t)	1.06 (m), 2.00 (m)	9, 11	39.7 (t)
13	44.8 (s)	–		44.8 (s)
14	56.2 (d)	1.12 (m)	7, 8, 15, 16	56.2 (d)
15	24.6 (t)	1.04 (m), 1.40 (m)	7, 9, 15, 16, 17	24.6 (t)
16	28.3 (t)	1.22 (m), 1.80 (m)	15, 17, 20, 21	28.3 (t)
17	55.6 (d)	1.08 (m)	15, 21, 22	55.7 (d)
18	12.5 (q)	0.65 (3H, s)	–	12.5 (q)
19	18.4 (q)	0.86 (3H, s)	–	18.4 (q)
20	35.6 (d)	1.36 (m)	15, 21, 22, 23	35.6 (d)
21	18.8 (q)	0.87 (3H, d, 6.6)	16, 17, 20, 22, 23	18.7 (q)
22	34.6 (t)	1.08 (m), 1.47 (m)	17, 20, 21, 23	36.2 (t)
23	30.4 (t)	1.74 (m), 1.98 (m)	20, 21, 22	23.8 (t)
24	155.2 (s)	–	–	39.5 (t)
25	41.7 (d)	1.93 (m)	26, 27, 29	28.0 (d)
26	28.3 (t)	1.25 (m), 1.37 (m)	25, 27, 29	22.5 (q)
27	19.8 (q)	0.92 (3H, d, 6.6)	25, 26, 29	22.8 (q)
28	107.2 (t)	4.61 (2H, brs)	–	–
29	12.0 (q)	0.76 (3H, t, 7.3)	25, 26, 27	–

[a] The mixing time was set to 90 ms.

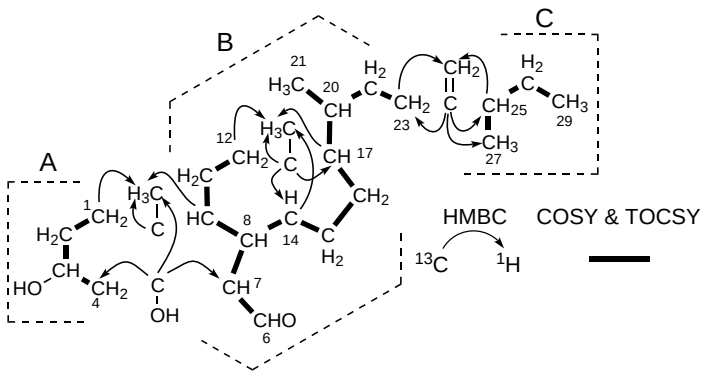

Figure 1. ^{1}H–^{1}H COSY, TOCSY and HMBC correlations of **1**.

The absolute configuration of **1** was determined by comparison of the CD spectrum with that of KPN-2001 (**2**), which is a synthetic analog of **1** prepared from cholesterol (Scheme 1).[7] The negative Cotton effects were observed at λ_{ext} 301 nm ($\Delta\varepsilon$ −34.0) in **1** and λ_{ext} 302 nm ($\Delta\varepsilon$ −20.3) in **2**. Since the absolute configuration is **1**, the same as that of natural cholesterol, accordingly, the final structure of **1** was determined.

Orostanal (**1**) and KPN-2001 (**2**) induced apoptosis in HL-60 cells at 10 μg/mL, and inhibited 50% cell growth at 1.7 and 2.2 μM, respectively. Further work is in progress on the mode of action for apoptosis caused by **1** and **2** and will be reported at a later date. To our knowledge, 6-5-6-5 fused rings sterol was only found in taiwaniasterols, which were isolated from the leaves of *Taiwania cryptomerioides*.[8]

Acknowledgements

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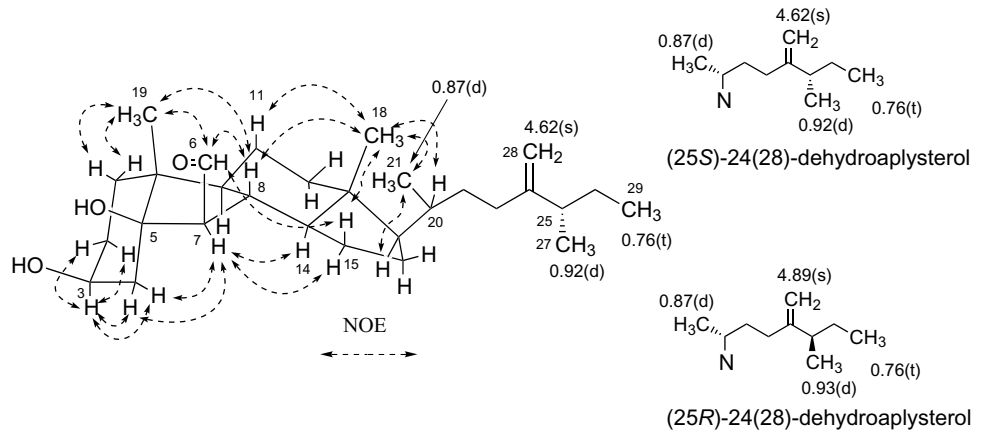
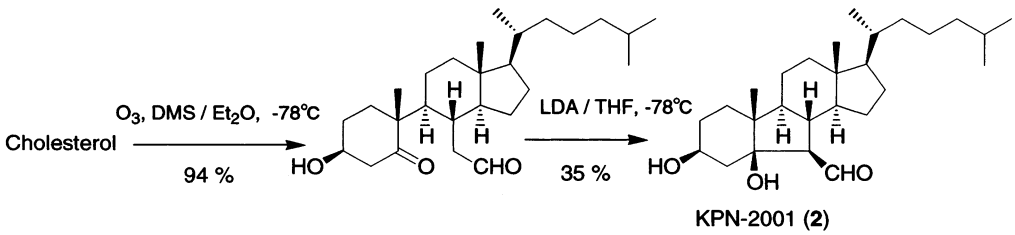


Figure 2. Relative stereochemistry of **1**.



Scheme 1.

References

1. Kerr, J. F.; Wyllie, A. H.; Currie, A. R. *Br. J. Cancer* **1972**, *26*, 239.
2. Kaufmann, S. H. *Cancer Res.* **1989**, *49*, 5870–5878.
3. *Stelletta hiwasaensis*; class Demospongiae, order Astrophorida, family Ancorinidae. A voucher specimen was deposited in the Zoological Museum in University of Amsterdam (ZMA Por 16382).
4. Duvall, E.; Wyllie, A. H. *Immunol. Today* **1986**, *7*, 115–119.
5. Orostanal (**1**): amorphous solid, $[\alpha]_D = +\ 50.6°$ ($c = 0.30$, $CHCl_3$), IR ($CHCl_3$, cm^{-1}) 3502, 2930, 2871, 1715, 1638, 1459, 1378, 893, EIMS (m/z) 444 (M^+), 426, 411, 398, 383, 300, 285, 231, CD (*n*-hexane, λ_{ext}) 301 nm ($\Delta\varepsilon$ −34.0), ^1H (600 MHz) and ^{13}C (150 MHz) NMR in $CDCl_3$, see Table 1.
6. Stoilov, L. I.; Thompson, E. J.; Cho, J.-H.; Djerassi, C. *J. Am. Chem. Soc.* **1986**, *108*, 8235–8241.
7. Cholesterol was ozonolyzed for 3 h at −78°C in Et_2O and the solution was treated with dimethylsulfide for 24 h to give an ozonolyzed product (94% yield). The product was added dropwise to LDA/THF solution at −78°C, and stirred for 10 h at room temperature to give **2** (35% yield). Compound **2**: amorphous solid, $[\alpha]_D = +29.6°$ ($c = 0.30$, $CHCl_3$), IR ($CHCl_3$, cm^{-1}) 3502, 2930, 2871, 1715, EIMS (m/z) 416, 398, 372, 357, 313, 126, CD (*n*-hexane, λ_{ext}) 302 nm ($\Delta\varepsilon$ −20.3), ^1H NMR ($CDCl_3$, 600 MHz) 0.72 (3H, s, Me-18), 0.86 (6H, each d, $J = 6.5$, 6.8, Me-26 and Me-27), 0.91 (3H, d, $J = 6.5$, Me-21), 0.93 (3H, s, Me-19), 2.23 (1H, dd, $J = 3.1$, 9.0, H-6), 4.12 (1H, m, H-3), 9.70 (1H, d, $J = 3.1$, H-7), ^{13}C NMR (600 MHz), see Table 1.
8. Lin, W.-H.; Fang, J.-M.; Cheng, Y.-S. *Phytochemistry* **1998**, *48*, 1392–1397.