Organic LETTERS

Letter

pubs.acs.org/OrgLett

Cinanthrenol A, an Estrogenic Steroid Containing Phenanthrene Nucleus, from a Marine Sponge *Cinachyrella* sp.

Koshi Machida,[†] Takahiro Abe,[†] Daisuke Arai,[†] Mayumi Okamoto,[‡] Isao Shimizu,[†,‡] Nicole J. de Voogd,[§] Nobuhiro Fusetani,[‡,∥] and Yoichi Nakao*[,†,‡]

[†]School of Advanced Science and Engineering, Waseda University, Shinjuku-ku, Tokyo 169-8555, Japan

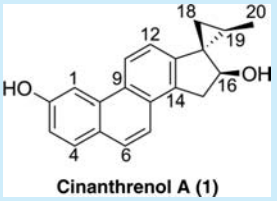
[‡]Research Institute for Science and Enginnering, Waseda University, Shinjuku-ku, Tokyo 169-8555, Japan

[§]Netherlands Centre for Biodiversity Naturalis, Leiden 2333 CR, The Netherlands

[∥]Fisheries and Oceans Hakodate, Hakodate, Hokkaido 041-8611, Japan

Supporting Information

ABSTRACT: Cinanthrenol A (**1**), a new steroid composed of a phenanthrene and a spiro[2,4]heptane system, was isolated from the marine sponge *Cinachyrella* sp. It is the first phenathrene-containing steroid with estrogen activity.



Cinanthrenol A (1)

Among more than 20 000 marine natural products, 2% of them were discovered from deep-water marine organisms.[1] Deep-water organisms are expected to produce metabolites unique in structures with interesting bioactivities.[2] In fact, such important bioactive compounds as discodermolide,[3] calyculins,[4] and halichondrins[5] were isolated from the sponges collected at deeper than 80 m. Dredging or trawling is an effective method to collect sessile organisms inhabiting such depth zone.[6] However, such fishing methods produce considerable amounts of crashed fragments of organisms during collection, and these fragmented samples are often discarded without being examined. To utilize such neglected marine resources, we have been trying to discover bioactive substances from these chunks of samples. During these efforts, we encountered a unique steroidal metabolite named cinanthrenol A (**1**). Subsequently, we could trace its producer to a sponge *Cinachyrella* sp. by using the characteristic fluorescence of cinanthrenol A. This communication deals with the isolation, structural elucidation, and biological activities of cinanthrenol A, as well as identification of the cinanthrenol-producing sponge.

Frozen fragments of marine organisms (6.5 kg) collected by dredging at Oshima-shinsone (140−160 m deep, 28.52.99 N, 129.33.05 E) were extracted with MeOH, and the extract was partitioned between H_2O and $CHCl_3$. The hydrophobic layer was subjected to Kupchan procedure[7] to yield *n*-hexane, $CHCl_3$, and aqueous MeOH layers. Bioassay-guided fractionation of the $CHCl_3$ layer by ODS flash chromatography followed by ODS HPLC yielded 3.5 mg of cinanthrenol A (**1**) as a brownish amorphous solid (5.4×10^{-5} % yield based on wet weight).

To identify the organism containing compound **1**, we used its characteristic fluorescence (ex 270, em 380 nm); extracts prepared from the 39-sorted samples collected at the same time were screened by the fluorescence detecting HPLC. As the result, only the aqueous extract of a marine sponge *Cinachyrella* sp. (70 g, wet weight) showed the similar fluorescent peak. This peak was collected and submitted to NMR and MS analyses, which led to the identification of cinanthrenol A (**1**) (0.4 mg, 5.7×10^{-4} % based on wet weight).

Cinanthrenol A (**1**) was obtained as a brownish amorphous solid with optical rotation of $[\alpha]_D^{20}$ −11.6 (*c* 0.16, MeOH). The molecular formula of **1** was established as $C_{20}H_{18}O_2$ by HRESIMS measurements ($[M - H]^-$ *m*/*z* 289.1235, calcd for $C_{20}H_{17}O_2$ 289.1229, Δ +0.6 mmu) requiring 12 sites of unsaturation. The IR absorption at 3392 cm^{-1} indicated the presence of hydroxyl groups. The UV and fluorescence spectra (ex 270, em 380 nm) were reminiscent of phenanthrene.[8] The ^1H NMR spectrum (Table 1) measured in pyridine-d_5 contained 16 proton signals, an sp^3 aliphatic methyl (δ_H 1.32), an oxymethine (δ_H 4.89), seven sp^2 methines (δ_H 7.01, 7.55, 7.68, 7.87, 7.97, 8.59, and 8.67), an sp^3 methine (δ_H 1.40), and two endocylic sp^3 methylene protons (δ_H 1.19, 1.49, 3.64, and 3.90) assigned by HMQC data, as well as two exchangeable protons (δ_H 6.54 and 12.04). The ^{13}C NMR spectrum (Table 1) revealed a total of 20 resonances in agreement with the molecular formula based on HRMS. ^{13}C NMR resonances were attributable to a methyl (δ_C 14.4), two sp^3 methylenes (δ_C 19.6, 41.3), an sp^3 methine (δ_C 22.0), an oxymethine (δ_C 71.8), an sp^3 quaternary carbon (δ_C 38.5), seven sp^2 methines (δ_C 107.5, 117.8, 117.9, 120.4, 122.8, 127.6, and 130.8), six sp^2 quaternary carbons (δ_C 120.8, 128.6, 129.3, 133.5, 137.1, and 146.0), and an sp^2 quaternary oxy-carbon (δ_C 158.2), accounting for seven

Received: January 1, 2014
Published: March 12, 2014

dx.doi.org/10.1021/ol5000023 | *Org. Lett.* 2014, 16, 1539−1541

Table 1. ^{1}H and ^{13}C NMR Data of Cinanthrenenol A (1) in Pyridine-d_5

position	δ_H mult (J in Hz)	δ_C	COSY	HMBC	NOESY
1	8.59 d (2.3)	107.5	H-3	C-2, C-3, C-5, C-9	H-11
2		158.2			
2-OH	12.04 s				
3	7.55 dd (8.6, 2.3)	117.8	H-4, H-1	C-1, C-5	H-4
4	7.97 d (8.6)	130.8	H-3	C-1, C-2, C-6, C-10	H-3, H-6
5		125.8			
6	7.87 d (8.9)	127.6	H-7	C-5, C-7, C-8, C-10	H-4, H-7
7	7.68 d (8.9)	120.4	H-6	C-5, C-6, C-8, C-9, C-14	H-6, H-15α
8		129.3			
9		128.6			
10		133.5			
11	8.67 d (8.5)	122.8	H-12	C-8, C-10, C-13	H-1, H-12
12	7.01 d (8.5)	117.9	H-11	C-9, C-11, C-14, C-17	H-11, H-18b, H-19
13		146.0			
14		137.1			
15α	3.90 dd (16.6, 7.6)	41.3	H-15β, H-16	C-13, C-14, C-16, C-17	H-7, H-15β, H-16
15β	3.64 dd (16.6, 3.8)		H-15α, H-16	C-13, C-14, C-16, C-17	H-15α
16	4.89 m (3.8, 6.7, 7.6)	71.8	H-15α, H-15β, OH-16		
16-OH	6.54 d (6.7)		H-16		
17		38.5			
18a	1.49 dd (6.0, 4.6)	19.6	H-18b, H-20	C-13, C-16, C-17, C-19, C-20	H-18b, H-19
18b	1.19 dd (8.4, 4.6)		H-18a, H-19	C-13, C-17, C-20	H-12, H-18a, H-19
19	1.40 dd (8.4, 6.0)	22.0	H-18b		H-12, H-18b
20	1.32 s	14.4	H-18a, H-18b	C-17, C-18, C-19	H-16, H-18a, H-18b

of the 12 sites of unsaturation, thus suggesting the presence of five rings.

Interpretation of the COSY and HMQC spectra led to the assignment of five isolated spin systems (A–E, Figure 1).

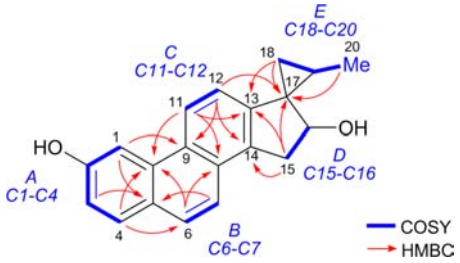


Figure 1. COSY and HMBC correlations of **1**.

Connectivities of these spin systems were established on the basis of HMBC analysis. HMBC correlations observed for H-1/C-5 and C-9, H-3/C-5, H-4/C-6 and C-10, H-6/C-8 and C-10, H-7/C-5, H-11/C-8, C-10, and C-13, and H-12/C-9 and C-14 indicated that spin systems A–C were linked via four quaternary carbons C-5, C-8, C-9, and C-10 to construct a phenanthrene unit.[8] This was supported by the UV absorption [λ_{max} (MeOH) 230.2, 263.2, 271.4, 311.2, 328.4, and 344.2 nm].[9]

The remaining two degrees of unsaturation were of two rings. Spin systems D and E were connected by HMBC correlations, H-15, H-18, and H-20/C-17, to afford a methylated cyclopropyl unit. HMBC correlations, H-18/C-13 and H-12/C-17, connected C-13 and C-17, while cross peaks of H-15/C-13 and C-14 completed a five-membered ring, which explained the whole unsaturations. Thus, the planar structure of cinanthrenol A (**1**) is composed of 2-hydroxy phenanthrene and 16-hydroxy, 19-methyl spiro[2,4]heptane units.

The relative stereochemistry of three stereogenic center in **1** was assigned on the basis of the NOESY data (Figure 2). H-18b

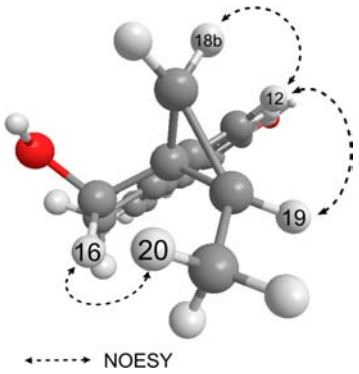


Figure 2. NOESY correlations of **1**.

and H-19 were both correlated with H-12, indicating that these protons located at the same face of the cyclopropane ring system. On the other hand, an NOE between H-16 and H-20 indicated that these protons located at the opposite side of the cyclopropane ring. In conjunction with the absence of a correlation between H-12 and H-20, we assigned the relative configuration of cinanthrenol A as 16S*, 17S*, 19S* (Figure 2).

Absolute configuration of **1** could be established by the modified Mosher's method.[10] Esterification of **1** with R-(−)- and S-(+)-MTPA chlorides led to an S- and R-MTPA esters of **1**, respectively. The $\Delta\delta$ values obtained from the proton signals assigned for these esters clearly indicated the absolute configuration at C-16 was S. Thus, the absolute configuration of **1** was established as 16S, 17S, 19S (Figure 3).

Cinanthrenol A (**1**) was cytotoxic against P-388 and HeLa cells with IC_{50} 4.5 and 0.4 μg/mL, respectively. The structural similarity between **1** and estradiol (E2) raised the possibility

dx.doi.org/10.1021/ol5000023 | Org. Lett. 2014, 16, 1539–1541

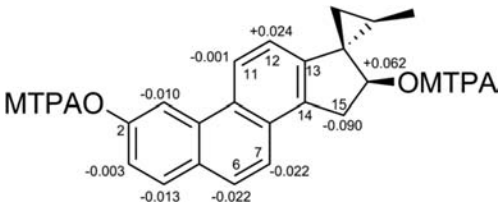


Figure 3. The $\Delta\delta$ values for *S*-/*R*-MTPA esters of **1**.

that **1** could act on estrogen receptor (ER). Estrogen such as estrone (E1), estradiol (E2), and estriol (E3) are known to allow cancer cells to proliferate with higher expression levels of ER, especially for breast cancer.[11] Estrogenic activity of **1** was evaluated by the relative binding assay. As the result, **1** bound to ER in a competitive manner against E2 with an IC_{50} value of 10 nM (Figure 4). Moreover, **1** changed gene expression level

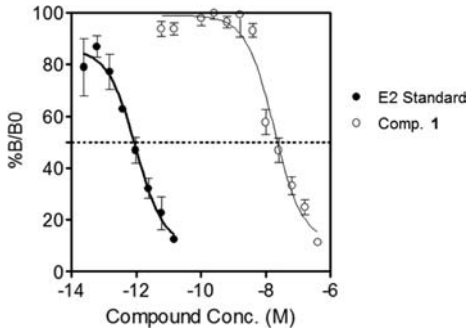


Figure 4. Competition between cinanthrenol A and 17β-estradiol for binding to the ER α.

of ER responsive genes *A-MYB* and *SMAD3* in MCF-7 cells in a similar manner to E2. These results indicated that cinanthrenol A has the estrogenic activity.

Cinanthrenol A (**1**) is a novel aromatic steroid, containing a phenanthrene and a spiro[2,4]heptane system. Until quite recently when a hexahydrophenanthrene sulfate named petromyzonin[12] was isolated from a sea lamprey, natural phenanthrene derivatives were known only from plants.[13−15] The biosynthetic pathway of cinanthrenol A could be similar to these of parathiosteroid A[16] or sokodoside B.[17]

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and spectral data of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: ayocha@waseda.jp

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We appreciate Prof. K. Hori and the crews of R/V Toyoshiomaru of Hiroshima University. This paper is a part of the outcome of research performed under a Waseda University Grant-in-Aid for Scientific Research, the Strategic Research Platforms for Private University, a Matching Fund Subsidy from the Ministry of Education, Science, Sports, and Technology (MEXT), Japan, and JSPS KAKENHI Grant Numbers 24651246, 25560408, and 25303008.

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