Supporting Information

Citrinadin A, A Novel Pentacyclic Alkaloid from Marine-derived Fungus *Penicillium citrinum*

Masashi Tsuda,[†] Yuu Kasai,[†] Kazusei Komatsu,[†] Teruo Sone,[‡] Michiko Tanaka,[‡] Yuzuru Mikami,[§] and Jun'ichi Kobayashi^{†,*}

Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan, Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan, and Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Chiba 260-0856, Japan

S2 Experimental section

S3 ~ S4 Figures S1 ~ S2. ¹H and ¹³C NMR spectra of the bis-salt of citrinadin A (**1**).

Experimental Section

General Experimental Procedures. ^1H and ^{13}C NMR spectra were recorded on a 600 MHz spectrometer using 2.5 mm micro cells for CDCl_3 (Shigemi Co., Ltd.). FAB mass spectrum were obtained on a JEOL HX-110 spectrometer using nitrobenzyl alcohol as a matrix.

Fungal Material and Fermentation. The fungus *Penicillium citrinum* (N059) was separated from a red alga *Actinotrichia fragilis*, which was collected at Hedo Cape, Okinawa Island. Subcultures of the fungus are deposited at Graduate School of Pharmaceutical Sciences, Hokkaido University. The fungus was grown in PYG seawater medium (peptone, 1 %; D-glucose, 2 %; yeast extract, 0.5 %; pH 7.5) for 14 days at 25 °C. The cultured broth (12 L) was filtered.

Extraction and Separation. The mycelium (435 g) of the cultured broth was extracted with MeOH (1 L x 2). The MeOH extracts were partitioned between 90% aqueous MeOH (1 L) and hexane (1 L x 3), and then the aqueous layer was extracted with *n*-BuOH (1 L x 3). The *n*-BuOH-soluble portions (5.78 g, wet weight) were subjected to LH-20 ($\text{CHCl}_3/\text{MeOH}$, 1:1) and then SiO_2 ($\text{CHCl}_3/\text{MeOH}$, 100:0 $\rightarrow$ 85:15) column chromatographies to afford the bis-salt of citrinadin A (**1**, 6.5 mg, 0.0015 %, wet weight).

Chiral HPLC Analysis of *N,N*-Dimethylvaline. Citrinadin A (**1**, 0.1 mg) was treated with 1N HCl aq. (50 μL) at rt for 14 h. After neutralization with 1N NaOH aq., the mixture was extracted with CHCl_3 . The aqueous layer was submitted to chiral HPLC analysis (SUMICHIRAL OA-5000, Sumitomo Chemical Industry, 4 x 150 mm, eluent: 1 mM CuSO_4 aq., flow rate: 0.2 mL/min, UV detection at 254 nm). Retention times (min) of authentic L- and D-*N,N*-dimethylvaline were as follows: L (10.3) and D (13.5). The retention time of *N,N*-dimethylvaline in the aqueous layer of hydrolysate of **1** was 10.3.

Figure S1. ^1H NMR spectrum of the bis-salt of citrinadin A (**1**) in CDCl_3 .

Figure S2. ^{13}C NMR spectrum of the bis-salt of citrinadin A (**1**) in CDCl_3 .