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# Acremostrictin, a Highly Oxygenated Metabolite from the Marine Fungus *Acremonium strictum*

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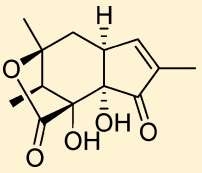
Supporting Information

**ABSTRACT:** The novel natural product acremostrictin (**1**) was isolated from the culture broth of *Acremonium strictum*, a marine fungus collected from a Choristida sponge off the coast of Korea. Structurally, acremostrictin is a tricyclic lactone of an unprecedented skeletal class based on combined spectroscopic and X-ray crystallographic analyses. The new compound exhibited weak antibacterial and moderate antioxidant activities.



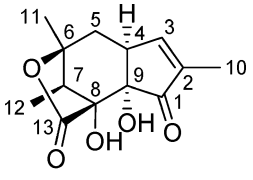
Marine microorganisms, and in particular actinomycete bacteria and fungi, produce a wide variety of biologically active and structurally unique metabolites.[1−6] Hundreds of novel compounds are isolated from these organisms annually, and they have become an emerging source of novel natural products.[7]

In our search for bioactive compounds from marine fungi, we isolated a strain of *Acremonium strictum* from an unidentified Choristida sponge collected from Korean waters whose organic extract significantly inhibited ($IC_{50}$ 25−50 $\mu$g/mL) the growth of diverse bacterial strains. The present report details the isolation and structural characterization of acremostrictin (**1**), a highly oxygenated, tricyclic metabolite of a new structural class.

A large-scale culture broth of *A. strictum* was extracted with EtOAc and repeatedly separated by reversed-phase HPLC to yield several common diketopiperazines and compound **1** as a colorless solid. The molecular formula of this compound, $C_{13}H_{16}O_5$, was determined by HRFABMS analysis. The $^{13}C$ NMR spectrum of this compound contained shifts corresponding to ketone and ester carbonyl carbon atoms at $\delta$ 207.4 and 176.8, respectively (Table 1). The strong absorption bands at 1769 and 1696 $cm^{-1}$ in its IR spectrum indicated that one of the carbonyls, possibly the ketone, was conjugated to a double bond. This interpretation coincided well with the olefinic carbon signals at $\delta$ 158.4 (CH) and 140.0 (C) in the $^{13}C$ NMR data, a downfield proton signal at $\delta$ 6.99 (1 H, br s) in the $^1H$ NMR data, and an absorption maximum at 233 nm in the UV spectrum. Thus, compound **1**, in conjunction with the six degrees of unsaturation inherent in the molecular formula, must be a tricyclic compound. The $^{13}C$ NMR spectrum also contained signals corresponding to three methyl carbon atoms at $\delta$ 22.4, 10.7, and 9.7 and oxygen-bearing quaternary carbon atoms at $\delta$ 86.5, 84.2, and 83.9 (Table 1), suggesting a deficiency of ring protons.

**Table 1. $^1H$ and $^{13}C$ NMR Assignments (500 MHz, pyridine-$d_5$) for Compound 1**

| position | $\delta_C$, type | $\delta_H$ ($J$ in Hz) | HMBC (H to C) |
|---|---|---|---|
| 1 | 207.4, C | | |
| 2 | 140.0, C | | |
| 3 | 158.4, CH | 6.99, s | 1, 2, 4, 5, 9, 10 |
| 4 | 44.4, CH | 3.34, br d (9.1) | 2, 3, 5, 8, 9 |
| 5 | 39.0, $CH_2$ | 2.18, dd (14.6, 9.4) | 3, 4, 7 |
| | | 2.08, d (14.6) | 3, 4, 6, 7, 11 |
| 6 | 86.5, C | | |
| 7 | 44.6, CH | 2.96, q (7.1) | 5, 8, 9, 12, 13 |
| 8 | 84.2, C | | |
| 9 | 83.9, C | | |
| 10 | 10.7, $CH_3$ | 1.73, s | 1, 2, 3 |
| 11 | 22.4, $CH_3$ | 1.30, s | 5, 6, 7 |
| 12 | 9.7, $CH_3$ | 1.09, d (7.1) | 6, 7, 8 |
| 13 | 176.8, C | | |



**1**

Long-range carbon−proton correlations derived from *g*HMBC data were particularly helpful in elucidating the structure of this proton-deficient compound. The chemical shifts of a methyl group ($\delta_C$ 10.7, $\delta_H$ 1.73) were indicative of a

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vinyl methyl group. Long-range correlations of these protons with carbon atoms at $\delta$ 207.4, 158.4, and 140.0 (C-1−C-3) revealed the presence of an $\alpha$-methyl-$\alpha,\beta$-unsaturated ketone, which was further supported by the same type of correlations between the olefinic proton at $\delta$ 6.99 and other carbon atoms

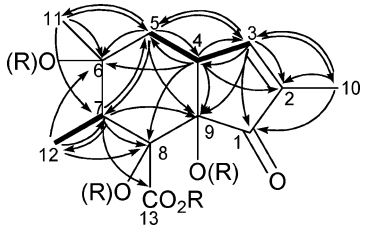


**Figure 1.** Key HMBC (arrows) and COSY (bold lines) correlations of compound **1**.

within the partial structure (Figure 1). $^1$H COSY data revealed a linear association between the olefinic proton (H-3) and the methylene protons at $\delta$ 2.18 and 2.08 (H-5) via a methine proton at $\delta$ 3.34 (H-4). This conclusion was supported by the HMBC correlations of these protons with neighboring carbon atoms. In this manner, the carbon framework was extended to include carbon signals at $\delta$ 86.5 and 44.6, placing these at C-6 and C-7, respectively. Long-range correlations of the methyl protons at $\delta$ 1.30 with carbon atoms at C-5−C-7 supported this interpretation and placed the methyl carbon signal at $\delta$ 22.4 at C-11. The H-7 proton at $\delta$ 2.96 showed a direct coupling with the methyl proton at $\delta$ 1.09 (H-12). Long-range correlations of the former proton with carbon atoms at $\delta$ 84.2 and 176.8 placed these at C-8 and C-13, respectively.

The H-7 methine proton at $\delta$ 2.96 exhibited an additional long-range coupling with the oxy-quaternary carbon atom at $\delta$ 83.9, placing it at C-9. Further correlations of this carbon atom with the H-3 and H-4 protons established a six-membered ring consisting of C-4−C-9 (Table 1). At this stage in the analysis, the carbon framework of **1** had open ends at C-1 ($\delta$ 207.4) and C-9 ($\delta$ 83.9). Despite the lack of decisive carbon−proton correlations in the *g*HMBC data, the only plausible connection was the direct linkage of these ends to form a bicyclic carbon framework.

Chemical shifts in the $^{13}$C NMR data in conjunction with the HRMS data revealed four oxygen atoms attached to C-6, C-8, C-9, and C-13 (carbonyl) in addition to the ketone at C-1 to form an additional oxycyclic ring such as an epoxide, ether, or lactone. Multiple structures for **1** were possible. However, compound **1** contained five asymmetric carbon centers: C-4, C-6, C-7, C-8, and C-9. Several of these centers lacked protons, which severely hindered the NMR-based configurational assignments. These problems were solved by X-ray crystallographic analysis, and the resulting ORTEP drawing is presented in Figure 2. Acremostrictin (**1**) is a highly oxygenated, tricyclic compound with a previously undocumented skeletal class. Based on the crystallographic analysis, the relative configurations of the carbon centers are 4*S**, 6*S**, 7*S**, 8*R**, and 9*S**. The carbon skeleton of **1** is reminiscent of a recently reported protoilludene-type sesquiterpene, 5-hydroxydichomitol, derived from the soil fungus *Dichomitus squalens* (Basidiomycete). This similarity may provide information on the biogenetic origin of this compound.[8]

Compound **1** exhibited weak activity against the bacteria *Micrococcus luteus* (IFO 12708), *Salmonella typhimurium*

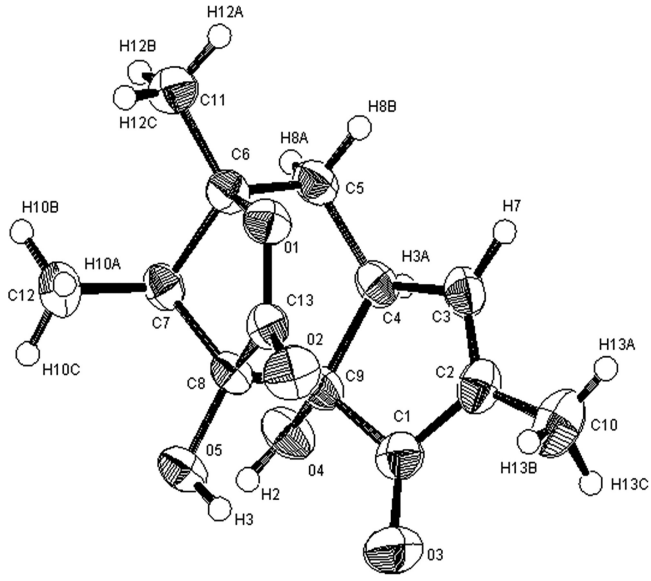


**Figure 2.** Crystal structure of compound **1** by ORTEP diagram.

(ATCC 14028), and *Proteus vulgaris* (ATCC 3851), with MIC values of 50, 50, and 12.5 $\mu$g/mL, respectively. Conversely, **1** was inactive against *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538p), and *Escherichia coli* (ATCC 35270), with MIC values higher than 100 $\mu$g/mL. This result suggests that the significant antibacterial activity observed for the extract may be due to common diketopiperazines such as the cyclo-(Trp-Leu) and cyclo-(Trp-Phe) that were isolated from this fungus. Compound **1** was also inactive against the leukemia cell line K562.

Moderate, concentration-dependent, antioxidant-related DPPH radical-scavenging activity was also observed with an $IC_{50}$ of 2.1 mM. This antioxidant activity was further correlated with the protection of oxidative stress-induced cell death. In these studies, compound **1** inhibited the $H_2O_2$-induced death of human keratinocyte HaCaT cells in a concentration-dependent manner (see Supporting Information).

## EXPERIMENTAL SECTION

**General Experimental Procedures.** The melting point was determined on a Büchi B-540 melting point apparatus. The optical rotation was measured on a JASCO P1020 polarimeter using a 1 cm path length cell. UV absorption spectra were recorded on a Hitachi U-3010 spectrophotometer. IR spectra were recorded on a JASCO FT/IR 4200. $^1$H and $^{13}$C NMR spectra were recorded in a pyridine-$d_5$ solution on Bruker Avance-500 spectrometers at 500 MHz for $^1$H and 125 MHz for $^{13}$C NMR. High-resolution fast-atom bombardment mass spectrometry (HRFABMS) was performed at the Daegu Branch of the Korea Basic Science Institute on a Jeol JMS 700 mass spectrometer. Semipreparative HPLC was performed with a SpectraSYSTEM RI-150 equipped with a SpectraSYSTEM P1000 pump and coupled to a Thermo RI-150 photodiode array detector. X-ray measurements were made on a Rigaku R-AXIS RAPID diffractometer using graphite-monochromated Mo K$\alpha$ radiation ($\lambda$ = 0.71075 Å). All solvents were spectral grade or distilled from glass prior to use.

**Fungal Material.** The fungal strain *Acremonium strictum* was isolated from an unidentified marine sponge of the class Choristida collected from Gagu-do, Korea, in October 2008. The isolate (strain number MB05005) was deposited at the Laboratory of Microbial Biotechnology, Department of Agricultural and Biotechnology, College of Agriculture & Life Science, Seoul National University. The isolate was cultured on slants of marine broth agar (37.4 g Difco


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marine broth 2216, 20.0 g glycerol, 18.0 g agar in 1 L purified $H_2O$) at 25 °C for 10 days. Spore solutions prepared from the slants ($10^7$ spores/mL) were inoculated into 250 mL Erlenmeyer flasks, 120 flasks in all, each containing 100 mL of media (37.4 g marine broth 2216 and 20.0 g glycerol in 1 L purified $H_2O$). Flask cultures were incubated at 28 °C on a rotary shaker at 150 rpm for 3 weeks.

**Identification of the Fungal Strain.** The fungal strain (MB05005) was identified using standard molecular biological protocols by DNA amplification and sequencing of the ITS region. Genomic DNA extraction was performed using Intron's i-genomic BYF DNA extraction mini kit according to the manufacturer's protocol. The nucleotide sequence of MB05005 has been deposited in the GenBank database under the accession number JF901805. The 18S rDNA sequence of this strain showed 100% identity with *A. strictum* Kw441-2010 (GenBank accession number FR694874).

**Extraction and Isolation.** The mycelia and culture broth of *A. strictum* were separated by filtration, and the broth (12 L) was extracted with EtOAc (12 L × 3). The solvent was evaporated to obtain an organic extract (700 mg). Because the LCMS of the extract showed a simple separation profile, the extract was separated directly by reversed-phase HPLC (YMC ODS-A column, 10 × 250 mm; $H_2O$–MeOH, 55:45) to yield 21 peaks. The major peak, peak 5, was further purified by reversed-phase HPLC ($H_2O$–MeOH, 85:15) to yield compound **1**; 30.6 mg.

*Acremostrictin (**1**):* colorless solid (MeOH–THF); mp 186.5–189.0 °C; $[\alpha]^{23}_{D}$ −14 (*c* 0.3, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 233 nm (3.74); IR (ZnSe) $\nu_{max}$ 3500 (br), 1769, 1696, 1626, 1442, 1314 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data, see Table 1; HRFABMS *m/z* 253.1283 $[M + H]^+$ (calcd for $C_{13}H_{17}O_5$, 253.1287).

*Crystallographic Data of Acremostrictin (**1**):* colorless crystal, $C_{13}H_{16}O_5$, $M_r$ = 252.27, monoclinic, space group $P2_1/c$, $a$ = 10.2400(6) Å, $b$ = 10.5343(8) Å, $c$ = 11.9796(8) Å, $\beta$ = 112.033(2)°, $V$ = 1197.9(2) Å$^3$, $T$ = 18.0 °C, $2\theta_{max}$ = 54.9°, $Z$ = 4, $D_{calcd}$ = 1.399 g $cm^{-3}$, crystal dimensions = 0.300 × 0.100 × 0.100 mm, $F(000)$ = 536.00. The final $R_1$ value is 0.0380 ($wR_2$ = 0.1108) for 2721 reflections [$I > 2.00\sigma(I)$].

Crystallographic data for the structure **1** reported in this paper have been deposited with the Cambridge Crystallographic Data Centre (deposition number: CCDC 842390). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

**Biological Assays.** Antibacterial and cytotoxicity assays were performed in accordance with previous protocols.[9−11] DPPH free radical scavenging and the viability assay in human keratinocyte cells were also carried out in accordance with the primary literature.[12,13]

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

$^1H$ NMR, $^{13}C$ NMR, 2D NMR spectra, a stereo-ORTEP drawing, a packing pattern with H-bonds of the crystal structure, the results of antioxidant assays, and a CIF file of X-ray crystallographic data for compound **1** are available free of charge via the Internet at http://pubs.acs.org.

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