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 (IC50 25–50 μg/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, C13H16O5, was determined by HRFABMS analysis. The 13C NMR spectrum of this compound contained shifts corresponding to ketone and ester carbonyl carbon atoms at δ 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 δ 158.4 (CH) and 140.0 (C) in the 13C NMR data, a downfield proton signal at δ 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 13C NMR spectrum also contained signals corresponding to three methyl carbon atoms at δ 22.4, 10.7, and 9.7 and oxygen-bearing quaternary carbon atoms at δ 86.5, 84.2, and 83.9 (Table 1), suggesting a deficiency of ring protons.

Table 1. 1H and 13C NMR Assignments (500 MHz, pyridine-d5) for Compound 1

<table>
<thead>
<tr>
<th>position</th>
<th>δC, type</th>
<th>δH (J in Hz)</th>
<th>HMBC (H to C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>207.4, C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>140.0, C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>158.4, CH</td>
<td>6.99, s</td>
<td>1, 2, 4, 5, 9, 10</td>
</tr>
<tr>
<td>4</td>
<td>44.4, CH</td>
<td>3.34, br d (9.1)</td>
<td>2, 3, 5, 8, 9</td>
</tr>
<tr>
<td>5</td>
<td>39.0, CH2</td>
<td>2.18, dd (14.6, 9.4)</td>
<td>3, 4, 7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.08, d (14.6)</td>
<td>3, 4, 6, 7, 11</td>
</tr>
<tr>
<td>6</td>
<td>86.5, C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>44.6, CH</td>
<td>2.96, q (7.1)</td>
<td>5, 8, 9, 12, 13</td>
</tr>
<tr>
<td>8</td>
<td>84.2, C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>83.9, C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>10.7, CH3</td>
<td>1.73, s</td>
<td>1, 2, 3</td>
</tr>
<tr>
<td>11</td>
<td>22.4, CH3</td>
<td>1.30, s</td>
<td>5, 6, 7</td>
</tr>
<tr>
<td>12</td>
<td>9.7, CH3</td>
<td>1.09, d (7.1)</td>
<td>6, 7, 8</td>
</tr>
<tr>
<td>13</td>
<td>176.8, C</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Long-range carbon–proton correlations derived from gHMBC data were particularly helpful in elucidating the structure of this proton-deficient compound. The chemical shifts of a methyl group (δC 10.7, δH 1.73) were indicative of a
vinyl methyl group. Long-range correlations of these protons with carbon atoms at δ 207.4, 158.4, and 140.0 (C-1–C-3) revealed the presence of an α-methyl-α,β-unsaturated ketone, which was further supported by the same type of correlations between the olefinic proton at δ 6.99 and other carbon atoms within the partial structure (Figure 1). 1H COSY data revealed a linear association between the olefinic proton (H-3) and the methylene protons at δ 2.18 and 2.08 (H-5) via a methine proton at δ 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 δ 86.5 and 44.6, placing these at C-6 and C-7, respectively. Long-range correlations of the methyl protons at δ 1.30 with carbon atoms at C-5–C-7 supported this interpretation and placed the methyl carbon signal at δ 22.4 at C-11. The H-7 proton at δ 2.96 showed a direct coupling with the methyl proton at δ 1.09 (H-12). Long-range correlations of the former proton with carbon atoms at δ 84.2 and 176.8 placed these at C-8 and C-13, respectively.

The H-7 methine proton at δ 2.96 exhibited an additional long-range coupling with the oxy-quaternary carbon atom at δ 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-7 (Table 1). At this stage in the analysis, the carbon framework of 1 had open ends at C-1 (δ 207.4) and C-9 (δ 83.9). Despite the lack of decisive carbon–proton correlations in the gHMBC data, the only plausible connection was the direct linkage of these ends to form a bicyclic carbon framework.

Chemical shifts in the 13C 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. Acremostricin (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 4S*, 6S*, 7S*, 8R*, and 9S*. The carbon skeleton of 1 is reminiscent of a recently reported protoellulene-type sesquiterpene, 5-hydroxyvichomitol, derived from the soil fungus Dichomitus squalens (Basidiomycete). This similarity may provide information on the biogenetic origin of this compound.

Compound 1 exhibited weak activity against the bacteria Micrococcus luteus (IFO 12708), Salmonella typhimurium (ATCC 14028), and Proteus vulgaris (ATCC 3851), with MIC values of 50, 50, and 12.5 μg/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 μg/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 IC50 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 H2O2-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. 1H and 13C NMR spectra were recorded in a pyridine-d5 solution on Bruker Avance-500 spectrometers at 500 MHz for 1H and 125 MHz for 13C NMR. High-resolution fast-atom bombardment mass spectrometry (HRFABMS) was performed at the Daejeon 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 Kα radiation (λ = 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 Gaju-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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**Figure 1.** Key HMBC (arrows) and COSY (bold lines) correlations of compound 1.

**Figure 2.** Crystal structure of compound 1 by ORTEP diagram.
marine broth 2216, 20.0 g glycerol, 18.0 g agar in 1 L purified H2O) at 
25 °C for 10 days. Spore solutions prepared from the slants (10³ 
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 H2O). Flask cultures were incubated at 
26 °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 
final 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 by reversed-phase HPLC (YMC ODS-A column, 10 
× 250 mm; MeOH–H2O = 70:30) to yield 700 mg. Because the LCMS of the extract 
showed a simple separation profile, the extract was separated directly 
and the solvent was evaporated to yield compound 1; 30.6 mg.

Acremostrictin (1): colorless solid (MeOH–THF); mp 186.5– 
189.0 °C [α]D 19, 14 = −14 (c 0.3, MeOH); UV (MeOH) λmax (log ε) 233 
nm (3.74); IR (ZnSe) νmax 3500 (br), 1769, 1696, 1626, 1442, 1314 
cm−1; 1H and 13C NMR data, see Table 1; HRFABMS 
[M + H]+ (calcd for C13H17O5, 253.1283) = 253.1283 

Crystallographic Data of Acremostrictin (1): colorless crystal, 
C13H17O5, Mw = 252.27, monoclinic, space group P21/c, a = 
10.4400(6) Å, β = 10.5343(8) Å, c = 11.9796(8) Å, β = 
112.03(2)°, V = 1979.9(2) Å³, T = 18.0 °C, βmax = 54.9°, Z = 4, 
Dcalc = 1.399 g cm−3; crystal dimensions = 0.300 × 0.100 × 0.100 mm, 
F(000) = 356.00. The final R1 value is 0.0380 (wR1 = 0.1108) for 2721 
reflections [I > 2σ(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.10–12 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, 13C 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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REFERENCES

(3) Bhakuni, D. S.; Rawat, D. S. Bioactive Marine Natural Products; 
109, 3012–3043.
(7) Blunt, J. W.; Copp, B. R.; Munro, M. H. G.; Northcote, P. T.; 
94, 868–874.
(9) Oh, K.-B.; Lee, J. H.; Chung, S.-C.; Shin, J.; Shin, H. J.; Kim, H.- 
(11) Ulukaya, E.; Ozdikicioglu, F.; Oral, A. Y.; Demirci, M. Toxicology 
(12) Lee, S. K.; Mbwambo, Z.; Chung, H.; Luyengi, L.; Gamez, E. J. 
C.; Mehta, R. G.; Kinghorn, A. D.; Pezzuto, J. M. Comb. Chem. High 
Throughput Screening 1998, 1, 35–46.
(13) Huang, C. C.; Fang, J. Y.; Wu, W. B.; Ching, H. S.; Wei, Y. J.; 