Notes

07H239-A, a New Cytotoxic Eremophilane Sesquiterpene from the Marine-Derived Xylariaceous Fungus LL-07H239

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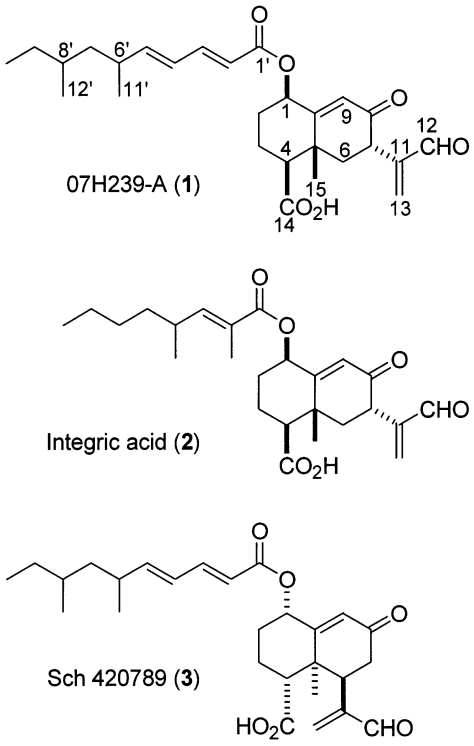
07H239-A (**1**), a new eremophilane sesquiterpene from a marine-derived xylariaceous fungus, was isolated, characterized, and shown to be cytotoxic toward a variety of cancer cell lines, with some selectivity for a CCRFCEM leukemia line (IC_{50} = 0.9 μg/mL).

As part of our ongoing effort to find bioactive natural products with therapeutic potential, we have discovered a new sesquiterpene, a decalin derivative, from the cell extract of the marine-derived xylariaceous[1] fungus LL-07H239. The crude fungal extract showed in vitro activity in a 25-cell-line cytotoxicity assay (mean IC_{50} = 3.2 μg/mL) modeled on that developed at the National Cancer Institute.[2] Guided by cytotoxicity against this diverse panel of tumor cell lines, our search for antitumor agents yielded the eremophilane sesquiterpene 07H239-A (**1**). The compound was purified from the crude extract by reversed-phase HPLC. NMR spectroscopy and mass spectrometry established the structure of 07H239-A unambiguously as the acyl eremophilane sesquiterpene **1**. Among the various biosynthetically related natural products, integric acid (**2**)[3] (Scheme 1) and its derivatives have reportedly shown HIV-1 integrase inhibitory activity.[4] Sch 420789 (**3**), whose spectroscopic properties are very similar to those of **1**, reportedly showed phospholipase D inhibitory activity.[5] Other related compounds have reportedly shown NPY-receptor antagonistic,[6] carcinostatic,[7] phytotoxic,[8] antifouling,[9] and plant growth regulatory[10] activities. We report here the structure and activity of **1**.

Compound **1** was recovered from the cell extract of LL-07H239, a marine-derived fungus of the family Xylariaceae, by reversed-phase HPLC chromatography on C_{18} support. The mass spectrum showed a protonated molecular ion at *m*/*z* 457.2. High-resolution ESI FTMS provided the exact mass of the ion (*m*/*z* 457.25859 $[M + H]^+$), from which the molecular composition $C_{27}H_{36}O_6$ (Δ = 0.12 mmu) was calculated. Positive ion electrospray MS^2 fragmentation of **1** resulted in loss of the 6′,8′-dimethyldeca-2′,4′-dienoic acid and carboxylic acid moieties. The assignment of a conjugated dienoate system was supported by the UV absorption at 264 nm.

One- and two-dimensional NMR data sets allowed the construction of a number of substructures from the 27 observed carbon resonances and the 35 protons detected by NMR. These data supported the presence of a conjugated ketone, an aldehyde, a carboxylic acid, an exomethylene, an angular methyl, a branched C_{12} unsaturated fatty acid, a contiguous C_4 aliphatic unit, and a C_2 aliphatic unit. HMBC data were used to assemble the partial structures into the proposed structure **1**. HMBC correlations between the angular methyl group protons (H15) and C4, C5, C6, and C10 established the relative position of the COSY-defined contiguous C_4 (C1−C4) and C_2 (C6−C7) aliphatic units and the conjugated ketone (C8−C10). Significantly, both H6ax and H6eq show reciprocal HMBC correlations to C15. Despite their close chemical shifts, the δ 44.5 (C6) and 45.0 (C7) resonances are easily distinguishable in the HMBC spectrum (Figure 1). APT, DEPT, and HMQC data show that the 44.5 ppm resonance is due to a methylene carbon attached to protons at δ 2.39 and 2.09 ppm, while the δ 45.0 ppm resonance is from a methine carbon attached to a proton at δ 3.72 ppm. Three-bond correlations from H9 to C1, C5, and C7 firmly established an unsaturated decalin ring with the conjugated carbonyl at C8.

Scheme 1. Structures of Some Eremophilane Sesquiterpenes



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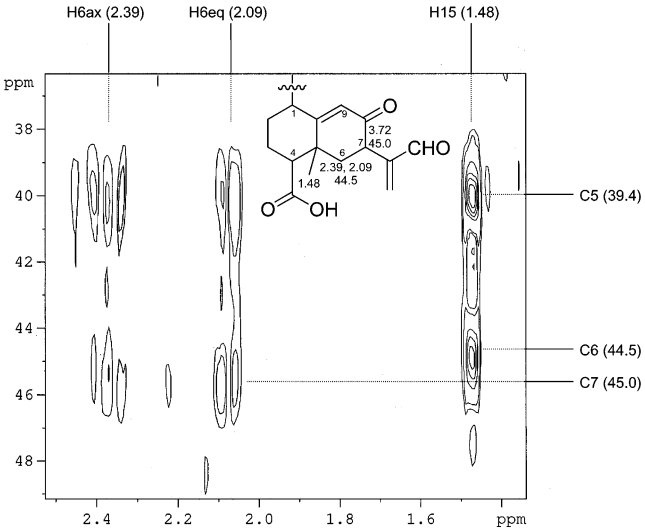

Figure 1. Portion of the HMBC spectrum of **1** in MeOH-d_4 showing important correlations to carbons 6 and 7.

HMBC correlations between both H13 protons and C12 defined the conjugated three-carbon aldehyde unit whose attachment at C7 was based on H12−C7 and H13−C7 HMBC correlations. The H4 proton shows an HMBC correlation to the C14 carbon, placing the carboxylic acid at the C4 position of the decalin ring. Protons H1, H2′, and H3′ correlate the ester carbonyl carbon, placing the fatty acid at the C1 position of the decalin.

The relative configuration of **1** was based on $^3J_{HH}$ values and ROESY correlations. The configurations of both double bonds of the 6′,8′-dimethyldeca-2′,4′-dienoic acid moiety were determined to be *E* on the basis of the large *trans* coupling constants (15.2 Hz) and the observed H2′−H4′ and H3′−H5′ ROEs. The small couplings exhibited by H1 suggested an equatorial position that was further corroborated by a strong ROESY correlation between the axial H15 protons and the H2′ proton of the axially oriented fatty acid. A significant 1,3-diaxial ROE between H7 and H15 places these protons on the same face of the molecule. The H7 methine proton must be axial due to strong (14.4 Hz) coupling to H6ax. Additional ROE correlations between H15 and H6eq, H4 and H2ax, and H2eq and H3ax established a chair conformation for the C1−C6 ring. The relative configuration of the 6′ and 8′ carbons could not be reliably determined by NMR.

The structure assigned for 07H239-A is completely consistent with MS and NMR data. Compound **1** is structurally related to the integric acid class and bears close resemblance to Sch 420789 (**3**), whose reported spectroscopic properties are virtually identical to those for **1**. 07H239-A apparently has the same fatty acid as compound **3** but with the three-carbon propenal unit attached at the C7 position. Although the eremophilane sesquiterpene core with the propenal (or other three-carbon) group attached at C7 is well represented in the literature, **3** is the sole reported sesquiterpene with the propenal unit (C11−C13) attached at the C6 position of the decalin ring and the only other natural product with a 6′,8′-dimethyldeca-2′,4′-dienoic acid reported.

The cytotoxic activity profile of **1** against a panel of diverse cancer cell lines indicates that the compound is mildly cytotoxic (mean $IC_{50} = 3.2$ μg/mL) and shows some selectivity for the CCRFCEM cell line ($IC_{50} = 0.9$ μg/mL), a human peripheral blood T lymphoblast line. Although active and slightly selective, the low potency of **1** may limit its potential as an anticancer agent.

Experimental Section

General Experimental Procedures. NMR data were acquired on a Bruker AMX 300, DRX 400, or DRX 500 spectrometer at proton and carbon frequencies of 300 and 75 MHz, 400 and 100 MHz, or 500 and 125 MHz, respectively. A typical data set included proton, carbon, or APT, DEPT, COSY, TOCSY, ROESY, HMQC, and HMBC spectra. For data acquired in MeOH-d_4, TMS (0 ppm) was used as a reference for proton data, while the residual solvent resonance at 49.0 ppm was used as a reference for carbon data. Similarly, for data acquired in acetone-d_6, TMS (0 ppm) was used as a reference for proton data, while the residual solvent resonance at 206.0 ppm was used as a reference for carbon data. Positive ion ESIMS and MS^2 experiments were performed on a VG Quattro triple quadruple instrument (Micromass, Beverly, MA). Nitrogen was used as both nebulizing and drying gas. Mass spectra were acquired over a range of 100−1500 Da in 5 s/scan using electrospray ionization in the positive ion mode with cone voltage set to 30 V. Sample was infused in 1:1 MeCN−H_2O with 0.1% formic acid. For MS^2 analysis on the Quattro, the gas pressure was set to 5.8×10^{-5} and the CE was set to 40 eV. Direct flow negative ion MS and MS^n analyses were carried out on a Finnigan DECA ion trap mass spectrometer fitted with an electrospray ionization (ESI) probe. The sample was dissolved in 1:1 MeCN−H_2O with 0.025% HCO_2H and infused via syringe. The mass spectrometer was operated in negative ion MS or MS^n scan (150−700 mass units) mode. The spray needle voltage was set to 5.0 kV, the capillary voltage was set at −32, and the capillary temperature was set to 300 °C. Nitrogen was used as the sheath and auxiliary gases, which were set to 80 and 20 units, respectively. For MS^n analyses on the DECA, the molecular or fragment ion was isolated and dissociated in the ion trap with a relative collision energy of ∼30 for each MS stage. High-resolution FTMS data were acquired using a Bruker-Daltonics APEX II Fourier transform mass spectrometer, equipped with a 9.4 T passively shielded superconducting magnet and an external ESI ion source. Optical rotation measurement was performed on a JASCO DIP-370 digital polarimeter. Ultraviolet spectrum was acquired using an HP 8453 UV−visible spectrophotometer.

Organism. Genomic DNA was isolated from mycelia of an agar grown culture of LL-07H239 by phenol/$CHCl_3$ extraction. The internally transcribed spacer region 1, 5.8S rDNA, and the internally transcribed spacer region 2 (ITS1−5.8S−ITS2) were PCR-amplified. The purified PCR product was sequenced directly on an Applied Biosystems ABI 3700 sequencer. A nucleotide Blast search (NCBI 2.2) was performed to compare the ITS region of LL-07H239 to other sequences in the GenBank database. The analysis revealed that LL-07H239 is a member of the Xylariaceae. LL-07H239 was plated onto Bennett's agar and incubated at room temperature (between 22 and 25 °C) with a natural light cycle. Growth was somewhat restricted. Colonies had a felt-like appearance at the centers, becoming thinner toward the margins, which were feathery and partially submerged. Surface colony color was white, and the reverse was cream. No fruiting structures or spores were formed.

Fermentation. Culture LL-07H239 was plated on Bennett's agar medium [10 g/L dextrose (Sigma), 1 g/L beef extract (Difco), 1 g/L yeast extract (Difco), 2 g/L N-Z-Amine A (Quest International), 15 g/L agar (Difco)] from a frozen 25% glycerol stock and incubated at 22 °C. A small agar slice bearing mycelial growth was inoculated into 10 mL of potato dextrose broth (PDB, Difco), pH 7.0, in a 25 × 150 mm test tube and incubated at 22 °C, 160 rpm, for 7 days. A second-stage seed was prepared by transferring 10 mL of culture broth to a 250 mL Erlenmeyer flask containing 50 mL of PDB, which was incubated at 22 °C, 200 rpm, for 4 days. Production fermentation was preformed in a 2.8 L Fernbach flask containing 1 L of PDB. Each flask was inoculated with 50 mL of second-stage seed and incubated at 22 °C, 200 rpm, for 7 days.

Compound 1. 07H239-A (**1**) was purified from one-half the acetone extract of the cell pellet from a 1-L fermentation by reversed-phase HPLC using a mobile phase comprised of MeOH−H_2O (80:20) at a flow of 10 mL/min to yield 24.1 mg of a white solid: $[\alpha]^{25}_D$ −71° ± 4 (*c* 0.258, MeOH); UV (MeOH) λ_{max} (log ϵ) 216 (4.61), 264 (4.86) nm; IR (KBr) ν_{max} 3489(br), 2961, 2926, 2875, 1707(s), 1683(s), 1642, 1458, 1312, 1243, 1139, 1004 cm^{-1}; ^1H NMR (MeOH-d_4, 400 MHz) δ 9.50 (1H, s, H-12), 6.05 (1H, dd, J = 15.2, 8.4 Hz, H-5′), 7.28 (1H, dd, J = 15.2, 10.8 Hz, H-3′), 6.43 (1H, s, H-13), 6.30 (1H, s, H-13), 6.03 (1H, s, H-9), 6.25 (1H, dd, J = 15.2, 10.8 Hz, H-4′), 5.85 (1H, d, J = 15.2 Hz, H-2′), 5.50 (1H, bs, H-1), 2.41 (1H, obs, H-4), 1.37 (1H, obs, H-7′), 1.11 (1H, obs, H-7′), 3.72 (1H, dd, J = 14.4, 4.5 Hz, H-7), 2.39 (1H, obs, H-6ax), 2.09 (1H, obs, H-6eq), 2.40 (1H, obs, H-6′), 1.34 (1H, obs, H-8′), 1.34 (1H, obs, H-9′), 1.15 (1H, obs, H-9′), 2.12 (1H, obs, H-2eq), 1.79 (1H, obs, H-2ax), 2.32 (1H, obs, H-3eq), 1.83 (1H, obs, H-3ax), 1.03 (3H, d, 6.6 H-11′), 1.48 (3H, s, H-15), 0.85 (3H, obs, H-12′), 0.87 (3H, obs, H-10′); ^{13}C NMR (MeOH-d_4, 100 MHz) δ 199.4 (C, C-8); 194.8 (CH, C-12); 176.1 (C, C-14); 167.4 (C, C-1′); 162.0 (C, C-10); 152.5 (CH, C-5′); 149.9 (C, C-11); 147.5 (CH, C-3′); 137.6 (CH_2, C-13); 130.3 (CH, C-9); 128.1 (CH, C-4′); 119.9 (CH, C-2′); 74.5 (CH, C-1); 54.7 (CH, C-4); 45.1 (CH_2, C-7′); 45.0 (CH, C-7); 44.5 (CH_2, C-6); 39.4 (C, C-5); 36.3 (CH, C-6′); 33.4 (CH, C-8′); 31.0 (CH_2, C-9′); 30.9 (CH_2, C-2); 21.4 (CH_2, C-3); 21.3 (CH_3, C-11′); 20.2 (CH_3, C-15); 19.3 (CH_3, C-12′); 11.6 (CH_3, C-10′); ^1H NMR (acetone-d_6, 500 MHz) δ 9.55 (1H, s, H-12), 6.11 (1H, dd, J = 15.2, 8.4 Hz, H-5′), 7.29 (1H, dd, J = 15.2, 10.8 Hz, H-3′), 6.43 (1H, s, H-13), 6.31 (1H, s, H-13), 5.99 (1H, s, H-9), 6.30 (1H, dd, J = 15.2, 10.8 Hz, H-4′), 5.90 (1H, d, J = 15.2 Hz, H-2′), 5.50 (1H, bs, H-1), 2.49 (1H, obs, H-4), 1.39 (1H, obs, H-7′), 1.10 (1H, obs, H-7′), 3.77 (1H, dd, J = 14.4, 4.3 Hz, H-7), 2.40 (1H, obs, H-6ax), 2.11 (1H, obs, H-6eq), 2.43 (1H, obs, H-6′), 1.33 (1H, obs, H-8′), 1.31 (1H, obs, H-9′), 1.13 (1H, obs, H-9′), 2.11 (1H, obs, H-2eq), 1.85 (1H, obs, H-2ax), 2.31 (1H, obs, H-3eq), 1.83 (1H, obs, H-3ax), 1.02 (3H, d, J = 6.6 Hz, H-11′), 1.51 (3H, s, H-15), 0.85 (3H, obs, H-12′), 0.85 (3H, obs, H-10′); ^{13}C NMR (acetone-d_6, 125 MHz) δ 197.1 (C, C-8); 193.9 (CH, C-12); 174.1 (C, C-14); 166.0 (C, C-1′); 160.3 (C, C-10); 151.5 (CH, C-5′); 149.6 (CH, C-11); 146.5 (CH, C-3′); 136.8 (CH_2, C-13); 130.0 (CH, C-9); 127.7 (CH, C-4′); 120.0 (CH, C-2′); 73.7 (CH, C-1); 53.9 (CH, C-4); 44.6 (CH_2, C-7′); 44.5 (CH, C-7); 43.9 (CH_2, C-6); 38.9 (C, C-5); 35.7 (CH, C-6′); 32.8 (CH, C-8′); 30.4 (CH_2, C-9′); 30.4 (CH_2, C-2); 21.1 (CH_2, C-3); 21.3 (CH_3, C-11′); 20.0 (CH_3, C-15); 19.2 (CH_3, C-12′); 11.5 (CH_3, C-10′) (obs = multiplicity obscured); negative ion ESIMS *m*/*z* 455.3 $[M + H]^-$; negative ion ESIMS/MS of *m*/*z* 455: *m*/*z* 455, 411, 277, 259, 215, 195; positive ion ESIMS *m*/*z* 457.2 $[M + H]^+$; positive ion ESIMS/MS of *m*/*z* 457: *m*/*z* 457, 261, 215, 197, 179, 81, 55; HR ESI^-FTMS *m*/*z* 457.25859 (calcd for $C_{27}H_{36}O_6$, 457.25847).

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Supporting Information Available: A table of NMR assignments for **1** in MeOH-d_4 and acetone-d_6 with significant HMBC correlations, proton, APT, COSY, ROESY, HMQC, and HMBC NMR spectra, MS, MS^2, and MS^3 spectra, mean bar graph of log IC_{50} data, structure with significant ROESY correlations, and structure with proposed MS fragmentation. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) The microorganism, isolated from a *Nypa* (mangrove palm) frond in Kuala Selangor, Malaysia, was supplied by Dr. Gareth Jones. Subsequent comparison of the ITS region with GenBank sequences revealed that LL-07H239 is a member of the Xylariaceae. Both the xylarenals (ref 3) and integric acid (ref 6) were reported from *Xylaria* sp. fungi.
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