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Antineoplastic Agents. 556. Isolation and Structure of Coprinastatin 1 from *Coprinus cinereus*¹

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Abstract

Cancer cell line bioassay-guided separation of an ethyl acetate extract prepared from a plant-associated fungus, *Coprinus cinereus*, led to the isolation of three new sesquiterpenes, coprinastatin 1 (**1**), coprinol (**2**), and the epimer (**4a**) of the known sesquiterpene triol **4b**. The previously described sesquiterpene **3** and oxazolinone **5** were also isolated. The structure and relative configuration of coprinastatin 1 (**1**) was determined by HRMS and by 1D- and 2D-NMR spectroscopic analyses. The structure of terpene **2** was elucidated by single-crystal X-ray diffraction experiments. The remaining structures were similarly determined, structure **3** by spectroscopic analyses and both **4a** and **5** by X-ray crystal structure determination. Coprinastatin 1 (**1**) was found to inhibit growth of the murine P388 lymphocytic leukemia cell line and the pathogenic bacterium *Neisseria gonorrhoeae*.

While pursuing new microorganisms with antineoplastic constituents,² we collected a small plant in the Shasta-Trinity National Forest, CA, that proved to be a useful source of an inky cap fungus, the multicellular basidiomycete *Coprinus cinereus* (*Coprinopsis cinerea*, *Hormographiella aspergillata*).³ *Coprinus cinereus* has been increasingly viewed as a useful source of a peroxidase (CiP) that can be used, for example, for polymerization of bisphenol A, 4a-c biodegradation of compounds isoelectronic to dibenzo-*p*-dioxin,^{4d} oxidative polymerization of cardanol to polycardanol,^{4e} and removal of phenols from aqueous waste streams.^{4f} The production of the extracellular enzymes (the secretome) by *C. cinereus* (including its peroxidase, metalloproteinases,^{5a} and the copper-containing laccases^{5b,c}) and by other saprophytic fungi has been reviewed.^{5d,e} Although there has been extensive genetic and molecular analysis of *C. cinereus*,^{6a-c} there appears to be only one report^{6d} on its small-molecule components, namely the sesquiterpenes lagopodins A and B and hydroxylagopodin B, which had been previously isolated from other *Coprinus* species^{6e} and are related to the antimicrobial enokipodins from *Flammulina velutipes*.^{6f} Seven biologically active diterpenoids were recently isolated from the related *C. heptenerus*, the first secondary metabolites to be reported from that species.^{6g,h} A number of sesquiterpenes have been identified in the culture broths from other *Coprinus* species, including the coprinolones⁶ⁱ and the biologically active illudins,^{6j,k} and a nematocidal piperidine derivative was recently isolated from *C. xanthothrix*.^{6l}

¹Dedicated to the late Dr. John W. Daly of NIDDK, NIH, Bethesda, Maryland and to the late Dr. Richard E. Moore of the University of Hawaii at Manoa for their pioneering work on bioactive natural products.

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Supporting Information Available: NMR spectra of compounds **1**, **2**, and **4a** and cif files of the X-ray data¹⁹ for compounds **2**, **4a**, and **5**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Results and Discussion

We found that an extract of the *C. cinereus* fermentation broth inhibited (ED_{50} 0.052 $\mu\text{g/mL}$) growth of the murine lymphocytic leukemia cell line (P388 system). By employing bioassay- (PS) guided separation of the *C. cinereus* broth extract, we isolated a P388 cell line active (ED_{50} 3.6 $\mu\text{g/mL}$) constituent designated coprinastatin 1 (**1**) and four other small molecule constituents (**2–5**). Herein we report the isolation and identification of substances **1–5**. An ethyl acetate extract of *C. cinereus* fermentation broth was partitioned between 9:1 $\text{CH}_3\text{OH-H}_2\text{O}$ and hexane, followed by dilution of the aqueous layer to 3:2 $\text{CH}_3\text{OH-H}_2\text{O}$ and extraction with CH_2Cl_2 . Bioassay-guided separation of the CH_2Cl_2 phase using a series of gel permeation and partition separations on Sephadex LH-20, followed by final purification via HPLC or by recrystallization, resulted in three new sesquiterpene compounds, coprinastatin 1 (**1**, 7.0×10^{-2} % yield), coprinol **2** (1.4×10^{-2} %), and sesquiterpene **4a** (6.0×10^{-2} %), as well as the previously reported sesquiterpene **3**⁷ (5.2×10^{-2} %) and the known oxazolinone **5** (1.7×10^{-2} %).^{8,9}

The molecular formula of coprinastatin 1 (**1**, colorless oil) was assigned as $\text{C}_{15}\text{H}_{22}\text{O}_2$ on the basis of high-resolution APCI⁺ mass spectroscopy, as well as interpretation of the 2D HMQC and HMBC NMR spectra (Table 1). The ^{13}C NMR (APT) spectrum (Table 1) of coprinastatin 1 (**1**) exhibited 15 carbon signals that included two aliphatic methyl groups, six methylenes (five aliphatic including one oxygenated and one olefinic), two aliphatic methines (one oxygenated), and five quaternary carbons (two aliphatic and three olefinic). In the ^1H NMR spectrum (Table 1), four high-field multiplets at δ 0.33 (1H, ddd, 10.8, 6.8, 3.6 Hz), 1.05 (1H, ddd, 10.0, 6.8, 3.6 Hz), 0.92 (1H, ddd, 10.8, 6.8, 5.2 Hz) and 1.19 (1H, ddd, 10.0, 6.8, 5.2 Hz), whose corresponding carbon signals were found at δ 18.27 and δ 10.53, were assigned to H-2' α , H-2' β , H-3' α and H-3' β , respectively. These NMR values were all attributed to characteristic resonances of the protons from a cyclopropane group.^{6j,7} The two olefinic proton signals appearing at δ 4.56 (1H, brs) and 4.95 (1H, dd, 2.0, 0.8) in the ^1H NMR spectrum, which corresponded to the carbon signal at δ 99.32, are the typical resonances of two protons from a terminal double bond.

The ^1H - ^1H COSY spectrum of **1** (Table 1) provided the correlation of H-7a to the protons on C-1 and C-7 and thus the C-1–C-7a–C-7 connection. Other connections were easily deduced by analysis of the HMBC spectrum (Table 1). The bonding of the 11-methyl group to the quaternary C-4 was confirmed by the observed correlations of the 11-methyl protons to C-4 and C-3a. The C-4–C-5–C-2'–C-3' segment was established by the correlations observed from the C-11 methyl protons and from H-2' and H-3' to C-4 and C-5. The C-5–C-6–C-7 segment was determined by the correlations of H-2' and H-3' to C-6, H-8 to C-5 and C-7, and H-7 to C-6 and C-8. Similarly, the C-3–C-3a–C-7a–C-1 unit was revealed by the correlations observed from H-3 to C-3a and C-7a, H-1 β to C-3a, and H-7 to C-1. Bonding of C-10, C-9, C-3 and C-1 to the quaternary C-2 was deduced by the correlations observed from H-3, H-1, H-10 and H-9 to C-2. Thus the structure of coprinastatin 1 (**1**) was unambiguously assigned as an illudane-type sesquiterpene.

The relative configuration of the three chiral carbons in coprinastatin 1 (**1**) at C-2, C-7a and C-7 was established by analysis of the NOESY spectrum (Table 1) and use of molecular models. The strong NOEs observed from H-7a to H-10, H-1 β and H-3 β showed that the 10-methyl, H-1 β , H-3 β and H-7a are all above the five-carbon planar ring. Thus, the relative configuration of C-2 could be assigned as *S*, and C-7a as *R*. There were no NOEs observed from H-7a to H-7, but H-7 exhibited a strong NOE signal to H-1 α . That observation confirmed that H-7a and H-7 were on opposite sides of the ring plane, thereby establishing the relative configuration of C-7 as *S*. These experiments confirmed the structure and relative configuration of coprinastatin 1 (**1**).

The new indene (**2**), herein named coprinol, was isolated as colorless needles. The molecular formula was assigned as $C_{15}H_{22}O_2$ on the basis of high-resolution APCI⁺ mass spectroscopy ($[M + H]^+$ at m/z 235) measurements. The structure of coprinol was unambiguously established as indene **2** (see Figure 1) by single crystal X-ray diffraction analysis. The molecular formula for sesquiterpene **3**, isolated as colorless oil, corresponded to $C_{15}H_{22}O_2$, based on analysis of the high-resolution APCI⁺ mass spectrum. The structure and relative configuration was determined in a manner analogous to that described above for coprinastatin 1 (**1**). By comparison of the data, sesquiterpene **3** was determined to be a structural isomer of **1** and identical to a compound previously isolated from another basidiomycete, a *Bovista* species.⁷

On the basis of spectroscopic (NMR, MS) analyses, compound **4a** (colorless needles from CH_3OH-H_2O) was found to have an additional oxygen atom in comparison to **2**, corresponding to a molecular formula of $C_{15}H_{22}O_3$. Single crystal X-ray diffraction revealed structure **4a** (Figure 2), a hydroxy analog of coprinol (**2**) with apparent *R* configuration at the single chiral center in this molecule. Assignment was based on the value of the refined Flack parameter (0.00001, esd 0.25693 for *R* enantiomer refinement vs 1.18670, esd 0.25686 for *S* enantiomer refinement). Surprisingly, the *R* configuration for compound **4a** appears to be in direct contrast to that previously reported for the natural product **4b**,^{10,11} which exhibits *S* configuration at that same chiral center. Measurement of the optical rotation of **4a** ($[\alpha]_D^{26} -56.25$) confirmed that it is not identical to the known compound (**4b**, lit¹⁰ $[\alpha]_D^{20} +2.5$, lit¹¹ $[\alpha]_D^{26} +1.2$).

The last component (**5**) was isolated as colorless needles following re-crystallization, with the molecular formula $C_9H_7NO_3$ on the basis of EI mass spectroscopy. The structure was assigned as 4-acetylbenzoxazolin-2(3*H*)-one (4-ABOA),^{8,9} again by X-ray crystal structure determination (Figure 3).

Biological evaluation of compounds **1–5** was conducted using the murine P388 lymphocytic leukemia cell line and a selection of human tumor cell lines. Only coprinastatin 1 (**1**) showed cancer cell growth inhibition, with an ED_{50} of 5.3 $\mu g/mL$ against the P388 lymphocytic leukemia line. Constituents **2–5** were found to be inactive against both the murine P388 and a panel (six) of human cancer cell lines. Compounds **1–5** were isolated in sufficient quantity for initial antimicrobial evaluations. In broth microdilution susceptibility assays,^{12,13} coprinastatin **1** had activity against the pathogenic bacterium *Neisseria gonorrhoeae* (minimum inhibitory concentration = 32–64 $\mu g/mL$).

Experimental Section

General Methods

All chromatographic solvents were redistilled. Sephadex LH-20 used for partition column chromatography was obtained from Pharmacia Fine Chemicals AB. Melting points were measured on a Fisher Scientific electrothermal melting point apparatus and are uncorrected. Analytical HPLC was conducted with a Hewlett-Packard Model 1050 unit coupled with a Hewlett-Packard diode array detector. Semipreparative HPLC was performed with a Waters 600 HPLC coupled with a Waters 2487 dual wavelength absorbance detector. Optical rotation measurements were recorded with a Perkin-Elmer 241 polarimeter. UV spectra were collected with a Perkin-Elmer Lambda 3B UV/vis spectrometer. NMR spectra were recorded using a Varian XL-400 or a Varian UNITY INOVA-500 spectrometer with tetramethylsilane (TMS) as an internal reference. High-resolution mass spectra were obtained using a JEOL LCMate magnetic sector instrument by APCI (positive ion mode) with a polyethylene glycol reference. EI mass spectra were obtained using a JEOL GCMate magnetic sector instrument. Single crystal X-ray diffraction experiments were performed using a Bruker 6000 X-ray diffractometer.

Specimen Collection and Fermentation

Small plants were collected (GRP) in the Shasta-Trinity National Forest, CA, in clean plastic bags. The plants were surface-disinfected with multiple passes through sterile H₂O, followed by 70% EtOH. After drying, the plant material was ground with a sterile mortar and pestle, and then dilution-plated. A fungus was selected on half-strength Thamnostylum agar. Scale-up fermentation to 380 L was performed in half-strength Thamnostylum broth at rt for seven days, with aeration. The fungus was identified by 18S rRNA gene sequence similarity (Accugenix, Newark, DE). Results from the MicroSeq database indicated that the fungus is *Coprinus cinereus* (% difference = 3.15; confidence level to species).

Extraction and Solvent Partition of *Coprinus cinereus*

The fermentation broth was extracted (3×) with EtOAc, and the combined solvent phase was dried in vacuo. A solution of the residue in 1:1 EtOAc–H₂O (300 mL) was extracted with EtOAc (5 × 150 mL). The combined EtOAc extracts were dried (in vacuo), and the residue (19.5 g) was dissolved in 9:1 CH₃OH–H₂O. Following extraction with hexane (3×), the aqueous layer was adjusted to 3:2 CH₃OH–H₂O and extracted with CH₂Cl₂ (3×) to afford 4.4 g of a hexane fraction and 13.4 g of a CH₂Cl₂ fraction. The solvent partitioning sequence was a modification of the original procedure of Bligh and Dyer.¹⁴

Isolation of Compounds 1–5

The CH₂Cl₂ extract (13.4 g) was dissolved in 1:1 CH₂Cl₂–CH₃OH (20 mL) and eluted through a column of Sephadex LH-20 to give five fractions that inhibited P388 cancer cell growth. One of these fractions (3.9 g) was subjected to partition chromatography on LH-20 in hexane–CH₃OH–2-propanol (3:1:1), resulting in 15 cancer cell inhibitory fractions. Three of these active fractions (A–C, in order of elution) were further separated.

Fraction B (90.3 mg) was separated by HPLC on a semipreparative Luna 5 μm C18(2) column, with a gradient mobile phase (20–50% CH₃CN in H₂O for 60 min, followed by 100% CH₃CN for 20 min), to give nine P388-active fractions and one inactive. One major active fraction (32.6 mg, ED₅₀ 2.5 μg/mL) was further separated by semipreparative HPLC on the same column in 30% CH₃CN/H₂O to give compound **1** (13.6 mg) as a colorless oil. The inactive fraction (16.8 mg) was taken up in CH₃OH/H₂O, and coprinol (**2**, 2.7 mg) crystallized in colorless needles.

Active fraction A (492 mg) from the LH-20 separation was subjected to HPLC on the same Luna column, with a gradient mobile phase (20–60% CH₃CN in H₂O for 60 min, followed by 100% CH₃CN for 20 min), to give 13 P388-active fractions. One major fraction (38.7 mg, ED₅₀ 0.31 μg/mL) was further separated on the Luna column in 38% CH₃CN/H₂O to give terpene **3** (10.1 mg) as a colorless oil.

Active fraction C (323 mg) from the LH-20 separation was subjected to HPLC on the Luna 5 μm C18(2) column, with a gradient mobile phase (20–50% CH₃CN in H₂O for 60 min, followed by 100% CH₃CN for 20 min), to give seven fractions. One of these (21.9 mg) was further separated by semipreparative HPLC on the same column in 19% CH₃CN/H₂O to give two fractions, one of which provided indene **4a** (11.6 mg) by crystallization from CH₃OH–H₂O; crystallization of the other fraction from CH₃OH yielded oxazole **5** (3.3 mg).

Coprinastatin 1 (1)—colorless oil; [α]_D²⁶ –37.7 (*c* 0.13, CH₃OH); UV (CH₃OH) λ_{max} (log ϵ) 202 (4.7) nm; ¹H and ¹³C NMR, see Table 1; HRMS (APCI+) *m/z* 235.1684 [M + H]⁺ (calcd for C₁₅H₂₃O₂, 235.1698).

Coprinol (2)—colorless needles from CH₃OH–H₂O; mp 159–160 °C; UV (CH₃OH) λ_{max} (log ϵ) 204 (4.3) nm; ¹H NMR (CD₃OD, 400 MHz) δ 1.13 (6H, s, H-9 and H-10), 2.11 (3H, s, H-8), 2.17 (3H, s, H-11), 2.61 (2H, s, H-3), 2.62 (2H, s, H-1), 2.87 (2H, t, 7.6, H-12), 3.53 (2H, t, 7.6, H-13); ¹³C NMR (CD₃OD, 100 MHz) δ 12.1 (C-11), 15.8 (C-8), 29.7 (C-9 and C-10), 34.3 (C-12), 40.3 (C-2), 45.6 (C-1), 48.8 (C-3), 62.3 (C-13), 122.6 (C-6), 124.9 (C-4), 127.7 (C-7a), 134.7 (C-5), 141.9 (C-3a), 150.2 (C-7); HRMS (APCI+) m/z 235.1725 [M + H]⁺ (calcd for C₁₅H₂₃O₂, 235.1698).

Coprinol (2) X-ray Crystal Structure Determination

A thin, colorless, plate-shaped crystal (~0.58 × 0.19 × 0.13 mm), grown from a CH₃OH–H₂O solution, was mounted on the tip of a glass fiber. Cell parameter measurements and data collection were performed at 123 ± 2 K on a Bruker SMART 6000 diffractometer. Final cell constants were calculated from a set of 3641 reflections from the actual data collection. Frames of data were collected in the θ range of 5.34 to 69.55° ($-37 \leq h \leq 36$, $-10 \leq k \leq 10$, $-11 \leq l \leq 11$) using 0.396° steps in ω such that a comprehensive coverage of the sphere of reflections was performed. After data collection, an empirical absorption correction was applied with the program SADABS.¹⁵ Subsequent statistical analysis of the complete reflection set using the XPREP¹⁶ program indicated the monoclinic space group *C2/c*.

Crystal data—C₁₅H₂₂O₂, $a = 31.0432(8)$ Å, $b = 8.6025(2)$ Å, $c = 9.5501(2)$ Å, $\beta = 92.677(2)^\circ$, $V = 2547.56(10)$ Å³, $\lambda = (\text{Cu K}\alpha) = 1.54178$ Å, $\mu = 0.690$ mm⁻¹, $\rho_c = 1.1131$ g/cm³ for $Z = 8$ and $fw = 234.33$, $F(000) = 1024$.

A total of 8891 reflections was collected, of which 2343 were unique ($R(\text{int}) = 0.0446$) and considered observed ($I_o > 2\sigma(I_o)$). These were used in the subsequent structure solution and refinement with SHELXTL.¹⁶ All non-hydrogen atoms for **2** were located using the default settings of that program. Hydrogen atoms were placed in calculated positions, assigned thermal parameters equal to either 1.2 or 1.5 (depending upon chemical type) of the U_{iso} value of the atom to which they were attached, then both coordinates and thermal values were forced to ride that atom during final cycles of refinement. All non-hydrogen atoms were refined anisotropically in a full-matrix least-squares refinement process. The final standard residual R_1 value for the model shown in Figure 1 converged to 0.0575 (for observed data) and 0.0640 (for all data). The corresponding Sheldrick R values were wR_2 of 0.1471 and 0.1549, respectively, and the GOF = 1.063 for all data. The difference Fourier map showed small residual electron density, the largest difference peak and hole being +0.438 and -0.332 e/Å³, respectively. Final bond distances and angles were all within acceptable limits.

Compound 4a—colorless crystals from CH₃OH–H₂O; $[\alpha]_D^{26} -56.25$ (c 0.56, CH₃OH) [(2*S*)-epimer: lit¹⁰ $[\alpha]_D^{26} +2.5$ (c 0.3, CH₃OH), lit¹¹ $[\alpha]_D^{26} +1.2$ (c 1.14, CH₃OH)]; UV (CH₃OH) λ_{max} (log ϵ) 256 (4.17) nm.

Compound 4a X-ray Crystal Structure Determination

A thin, colorless plate-shaped crystal (~0.48 × 0.13 × 0.06 mm), grown from a CH₃OH–H₂O solution, was mounted on the tip of a glass fiber. Cell parameter measurements and data collection were performed at 123 ± 2 K on a Bruker SMART 6000 diffractometer. Final cell constants were calculated from a set of 2189 reflections from the actual data collection. Frames of data were collected in the θ range of 5.27 to 69.17° ($-8 \leq h \leq 7$, $-7 \leq k \leq 7$, $-10 \leq l \leq 9$) using 0.396° steps in ω such that a comprehensive coverage of the sphere of reflections was performed. After data collection, an empirical absorption correction was applied with the program SADABS.¹⁵ Subsequent statistical analysis of the complete reflection set using the XPREP¹⁶ program indicated the triclinic space group *P1*.

Crystal data— $\text{C}_{15}\text{H}_{22}\text{O}_3$, $a = 6.7943(2) \text{ \AA}$, $b = 6.0697(2) \text{ \AA}$, $c = 8.6532(3) \text{ \AA}$, $\alpha = 90.358(2)^\circ$, $\beta = 104.1850(10)^\circ$, $\gamma = 90.0690(10)^\circ$, $V = 345.964(19) \text{ \AA}^3$, $\lambda = (\text{Cu K}\alpha) = 1.54178 \text{ \AA}$, $\mu = 0.658 \text{ mm}^{-1}$, $\rho_c = 1.202 \text{ g/cm}^3$ for $Z = 1$ and $\text{fw} = 250.33$, $F(000) = 136$.

A total of 2576 reflections was collected, of which 1624 were unique ($R(\text{int}) = 0.0251$) and considered observed ($I_o > 2\sigma(I_o)$). These were used in the subsequent structure solution and refinement with SHELXT.¹⁶ All non-hydrogen atoms for **4a** were located using the default settings of that program. Hydrogen atoms were placed in calculated positions, assigned thermal parameters equal to either 1.2 or 1.5 (depending upon chemical type) of the U_{iso} value of the atom to which they were attached, then both coordinates and thermal values were forced to ride that atom during final cycles of refinement. All non-hydrogen atoms were refined anisotropically in a full-matrix least-squares refinement process. The final standard residual R_1 value for the model shown in Figure 2 converged to 0.0384 (for observed data) and 0.0391 (for all data). The corresponding Sheldrick R values were wR_2 of 0.1033 and 0.1042, respectively, and the GOF = 1.064 for all data. The difference Fourier map for **4a** showed minimal residual electron density, the largest difference peak and hole being +0.236 and −0.232 $\text{e/\AA}^3$, respectively. Final bond distances and angles were all within acceptable limits.

The single crystal X-ray diffraction revealed structure **4a** (Figure 2), a hydroxy analog of coprinol with apparent R configuration at the single chiral center in this molecule. This assignment was based on the value of the refined Flack parameter obtained for refinement as the R configuration of 0.00001 (esd 0.25693). Refinement of the data assuming the S configuration resulted in a Flack value of 1.18670 (esd 0.25686). Surprisingly, the R configuration for compound **4a** is in direct contrast to that previously reported for the natural product **4b**,^{10,11} which exhibits S configuration at that same chiral center.

Oxazole 5 X-ray Crystal Structure Determination

A thin, colorless plate-shaped crystal ($\sim 0.64 \times 0.10 \times 0.06 \text{ mm}$), grown from a CH_3OH solution, was mounted on the tip of a glass fiber. Cell parameter measurements and data collection were performed at $123 \pm 2 \text{ K}$ on a Bruker SMART 6000 diffractometer. Final cell constants were calculated from a set of 5195 reflections from the actual data collection. Frames of data were collected in the θ range of 4.92 to 69.57° ($-10 \leq h \leq 10$, $-12 \leq k \leq 12$, $-20 \leq l \leq 19$) using 0.396° steps in ω such that a comprehensive coverage of the sphere of reflections was performed. After data collection, an empirical absorption correction was applied with the program SADABS.¹⁵ Subsequent statistical analysis of the complete reflection set using the XPREP¹⁶ program indicated the triclinic space group $P2_1/c$ for compound **5**.

Crystal data— $\text{C}_9\text{H}_7\text{O}_3$, $a = 8.7251(2) \text{ \AA}$, $b = 10.7734(3) \text{ \AA}$, $c = 16.5954(4) \text{ \AA}$, $\beta = 100.8540(10)^\circ$, $V = 1532.04(7) \text{ \AA}^3$, $\lambda = (\text{Cu K}\alpha) = 1.54178 \text{ \AA}$, $\mu = 0.991 \text{ mm}^{-1}$, $\rho_c = 1.536 \text{ g/cm}^3$ for $Z = 8$ and $\text{fw} = 177.16$, $F(000) = 736$.

A total of 11007 reflections was collected, of which 2841 were unique ($R(\text{int}) = 0.0291$), and considered observed ($I_o > 2\sigma(I_o)$). These were used in the subsequent structure solution and refinement with SHELXTL.¹⁶ All non-hydrogen atoms for **5** were located using the default settings of that program. Hydrogen atoms were placed in calculated positions, assigned thermal parameters equal to either 1.2 or 1.5 (depending upon chemical type) of the U_{iso} value of the atom to which they were attached, then both coordinates and thermal values were forced to ride that atom during final cycles of refinement. All non-hydrogen atoms were refined anisotropically in a full-matrix least-squares refinement process. The final standard residual R_1 value for the model shown in Figure 3 converged to 0.0332 (for observed data) and 0.0373 (for all data). Two independent molecules of the parent compound were contained in the asymmetric cell unit. The corresponding Sheldrick R values were wR_2 of 0.0861 and 0.0887, respectively, and the GOF = 1.048 for all data. The difference Fourier map for **5** showed

minimal residual electron density; the largest difference peak and hole being +0.263 and −0.184 e/Å³, respectively. Final bond distances and angles were all within acceptable limits.

Cancer Cell Line Bioassay Procedures

The National Cancer Institute's standard sulforhodamine B assay was used to assess inhibition of human cancer cell growth as previously described.¹⁷ The P388 lymphocytic leukemia cell line results were obtained as described previously.¹⁸

Antimicrobial Susceptibility Testing

Compounds **1–5** were screened against the bacteria *Stenotrophomonas maltophilia* ATCC 13637, *Micrococcus luteus* Presque Isle 456, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Enterobacter cloacae* ATCC 13047, *Enterococcus faecalis* ATCC 29212, *Streptococcus pneumoniae* ATCC 6303, and *Neisseria gonorrhoeae* ATCC 49226 and the fungi *Candida albicans* ATCC 90028 and *Cryptococcus neoformans* ATCC 90112, according to established broth microdilution susceptibility assays.^{12,13} Each substance was reconstituted in a small volume of sterile DMSO and diluted in the appropriate media immediately prior to susceptibility experiments. The minimum inhibitory concentration was defined as the lowest concentration of compound that inhibited all visible growth of the test organism (optically clear). Assays were repeated on separate days.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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19. CCDC 734016 (**2**), 734017 (**4a**), and 734018 (**5**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Figure 1.
X-ray crystal structure of coprinol (**2**). Thermal ellipsoids are shown at 50% probability.

Figure 2.
X-ray crystal structure of indene 4. Thermal ellipsoids are displayed at 50% probabilities.

Figure 3.
X-ray crystal structure of the benzoxazol-2-one **5** displaying the two independent molecules in the asymmetric cell unit. Thermal parameters are shown at 50% probabilities.

Table 1

The ^1H and ^{13}C NMR Assignments for Coprinastatin 1 (I) in CD_3OD^a

position	$\delta^1\text{H}$ (J in Hz)	^1H - ^1H COSY	NOESY	$\delta^{13}\text{C}$	HMBC ^b
1 α	1.35 (t, 11.6)	H-7 α	H-7, H-9	42.6	H-10, H-3 β , H-9, H-7
1 β	1.79 (dd, 11.6, 6.8)	H-7 α	H-7 α , H-10 H-3 β (w)		
2				44.0	H-10, H-1 α , H-1 β , H-3 α , H-3 β , H-9
3 α	2.31 (d, 16.8)		H-9, H-1 α	41.3	H-10, H-1 β , H-1 α (w), H-9
3 β	1.95 (d, 16.8)		H-10, H-1 β (w), H-7 α		
3 α				135.8	H-11, H-1 β , H-3 α , H-3 β , H-7 α (w)
4				126.3	H-11, H-3 α , H-3 β , H-2 α (w), H-2 β , H-3' α , H-3' β ,
5				27.2	H-8 α , H-8 β , H-11, H-2' α (w), H-2' β , H-3' α , H-3' β
6				156.1	H-2' α , H-2' β , H-3' α , H-3' β , H-7, H-8 α , H-8 β
7	3.92 (d, 10.0)	H-7 α	H-1 α	76.9	H-1 α , H-7 α (w), H-8 α , H-8 β
7 α	2.55 (m)	H-7, H-1 α , H-1 β , H-3 β	H-10, H-1 β	49.5	H-1 α , H-1 β , H-3 α , H-3 β , H-7
8 α	4.95 (brs)			99.3	H-7
8 β	4.56 (brs)		H-3 β		
9	3.38 (s)		H-1 α , H-3 α	71.9	H-10, H-1 α , H-3 α
10	1.00 (s)		H-1 β , H-3 β , H-7 α	24.6	H-9, H-3 α , H-3 β , H-1 α , H-1 β
11	1.29 (d, 1.2)		H-3' α , H-2' β	13.0	
2 α	0.33 (ddd, 10.8, 6.8, 3.6)	H-2' β , H-3' α , H-3' β	H-3' α	18.3	H-8 α , H-8 β , H-3' α , H-3' β
2 β	1.05 (ddd, 10.0, 6.8, 3.6)	H-2' α , H-3' α , H-3' β	H-3' β		
3' α	0.92 (ddd, 10.8, 6.8, 5.2)	H-2' α , H-2' β , H-3' β	H-2' α	10.5	H-2' α , H-2' β
3' β	1.19 (ddd, 10.0, 6.8, 5.2)	H-2' α , H-2' β , H-3' α	H-8 β		

^a Measured at 400 MHz (^1H) and at 100 MHz (^{13}C).

^b w = weak.