mixture was confirmed by ^1H NMR.[15]

Methylation of Robustadials. Isolation of Dimethylrobustadial A (10) and Dimethylrobustadial B (11). To a solution of the robustadial mixture (100 mg) in anhydrous acetone (20 mL) were added CH_3I (150 mg, 4 molar equiv based on 8) and K_2CO_3 (500 mg). The reaction mixture was refluxed for 8 h with further addition of CH_3I (150 mg) every 2 h. After cooling, the mixture was filtered and the solvent removed in vacuo. The residue was chromatographed [flash silica gel; 5:1 petroleum ether (bp 30–60 °C)/EtOAc] to obtain partially purified fractions of **10**, **11**, and the dimethyl ethers of **4** and **5**. Final purification of **10** and **11** was by normal-phase HPLC [Microsorb (5 μm), Rainin, 4.6 × 250 mm; flow rate, 1.5 mL/min; UV detection, 254 nm; 0.03% *i*-PrOH in *n*-heptane].

Dimethylrobustadial A (10): colorless oil; HRMS, *m/e* 414.2416 [M$^+$], calcd for $C_{25}H_{34}O_5$ 414.2406; UV λ_{max} (MeOH) 261 (ε 11 500), 280 (9200), 320 nm (sh, 2880); CD (MeOH)[4] 235 (Δε +1.96), 259 (+3.86), 275 (+2.29), 320 (+1.81), 354 nm (−0.59); IR (NaCl, film) 2957, 2928, 2860, 1686, 1566, 1462, 1383, 1121, 1070 cm^{-1}; ^1H and ^{13}C NMR, see Table I; low-resolution MS, *m/z* (relative intensity) 414 (M$^+$, 0.8), 399 (4), 371 (2), 357 (11), 279 (21), 223 (100), 136 (2), 91 (45).

Dimethylrobustadial B (11): colorless oil; UV λ_{max} (MeOH) 261 (ε 11 500), 280 (9200), 320 nm (sh, 2880); CD (MeOH)[4] 227 (Δε −2.54), 257 (−2.50), 292 (+1.15), 330 (+0.47), 354 nm (+0.19); IR (NaCl, film) 2953, 2928, 2868, 1686, 1564, 1461, 1385, 1126, 1103 cm^{-1}; ^1H and ^{13}C NMR, see Table I; low-resolution MS, *m/z* (relative intensity) 414 (M$^+$, 3), 399 (6), 371 (10), 357 (19), 279 (55), 223 (100), 136 (3), 91 (47).

Acknowledgment. We are grateful to the Department of Pharmacy, the Second Military Medical College of Shanghai, for providing the crude material, and to Jie-zhi Wang for performing the initial extraction of the *E. robusta* leaves. We also thank the Research Corporation, the donors of the Petroleum Research Fund, administered by the American Chemical Society, and the Camille and Henry Dreyfus Foundation for financial support.

Supplementary Material Available: DQCOSY, DEPT, and HETCOR spectra for **10** and HETCOR spectrum for **11** (11 pages). Ordering information is given on any current masthead page.

(15) Small amounts of the parent phenols, **8** and **9**, were separated and their high-resolution mass spectra reported in ref 4.

(16) While this manuscript was in press, the syntheses of robustadials A and B with the correct structures **4** and **5** were reported. Salomon, R. G.; Lal, K.; Mazza, S. M.; Zarate, E. A.; Youngs, W. J. *J. Am. Chem. Soc.* **1988**, *110*, 5213.

Podosporin A: A Novel Antifungal Metabolite from the Coprophilous Fungus *Podospora decipiens* (Wint.) Niessl

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Podosporin A (1), a new tetracyclic sesquiterpene quinone exhibiting antifungal activity, antibacterial activity, and brine shrimp toxicity, has been isolated from the coprophilous fungus *Podospora decipiens* (NRRL 6461), a colonist of cattle dung. Podosporin A was obtained from ethyl acetate extracts of liquid cultures of *P. decipiens* by silica gel chromatography and reversed-phase HPLC, and its structure was assigned by single-crystal X-ray diffraction analysis. The isolation process was guided by in vitro bioassays for antifungal antagonism toward other coprophilous fungi.

The phenomenon of interference competition among fungi from competitive ecological niches is commonly observed,[1-6] but the chemistry associated with these interactions remains largely unexplored. We have undertaken studies of such systems in order to obtain insight into the chemical ecology of interspecies competition, and to explore the potential value of these organisms as sources of natural antifungal agents.[1,2] During examination of the chemical basis for interspecies competition among coprophilous (dung-colonizing) fungi, we have encountered a novel antifungal metabolite produced by the late successional fungus *Podospora decipiens* (NRRL 6461), an ascomycete that exhibits antagonistic activity toward other coprophilous fungi in vitro. We now report details of the isolation, structure, and biological activity of this compound, which we have named podosporin A.

Podosporin A (1) was obtained from ethyl acetate extracts of liquid cultures of *P. decipiens* by silica gel chromatography and reversed-phase HPLC. The isolation of this metabolite was guided by bioassays for antifungal activity toward the early successional dung fungi *Sordaria*

Table I. Proton NMR Assignments for Podosporin A (1)[a]

position		position	
2	6.47 (d, 1.2 Hz)	16	2.05 (br ddd, 13, 12, 4.2)
7	2.83 (d, 19)		1.42 (br dd, 13, 3.4)
	1.92 (d, 19)	17	1.74 (m)
10	2.21 (ddd, 14, 13,	18	1.08 (d, 7.3)
	3.9)	19	0.92 (s)[b]
	1.72 (m)	20	4.12 (dq, 7.1, 1.2)
11	2.50 (m)	22	6.16 (dq, 16, 1.7)
	1.63 (m)	23	6.94 (dq, 16, 6.8)
12	3.52 (br s)	24	1.88 (dd, 6.8, 1.7)
14	1.73 (m)	25	1.26 (d, 7.1)
15	1.88 (m)	26	0.87 (s)[b]
	1.32 (m)	27	0.90 (s)[b]

[a] Spectrum recorded at 360 MHz in $CDCl_3$. [b] Assignments are interchangeable.

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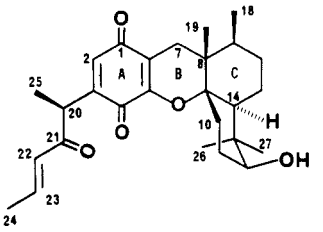

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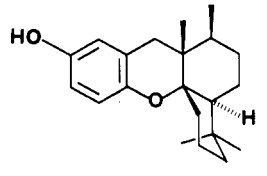
fimicola (NRRL 6459) and *Ascobolus furfuraceus* (NRRL 6460).[1-3] Analysis of podosporin A by EIMS, HRFABMS, and ^{13}C NMR spectroscopy suggested the molecular formula $C_{27}H_{36}O_5$. The ^{13}C NMR, ^1H NMR, UV, and IR spectra indicated the presence of trisubstituted quinone, hydroxyl, and α,β-unsaturated ketone moieties, and the lack of further sp^2 carbons suggested the presence of three additional rings. ^{13}C NMR chemical shifts and multiplicities indicated that the quinone ring was oxygen-substituted and that there was only one proton not bound to carbon. The ^1H NMR spectrum (Table I) contained resonances for four aliphatic methyl groups and one vinylic methyl group, along with a number of overlapping upfield signals corresponding to aliphatic methylene and methine protons. The complexity of the upfield region of the ^1H NMR spectrum prevented conclusive assignment of the connectivity of podosporin A from preliminary data. At this point, suitable crystals of the metabolite were obtained from methanol, thereby permitting assignment of the structure by X-ray diffraction analysis.

Figure 1 is a computer-generated perspective drawing of the structure of podosporin A as determined by X-ray crystallography. Hydrogens are omitted for clarity, and no absolute configuration is implied. All hydrogens were located, and all bond distances and angles agree well with accepted values. The final X-ray model indicates that podosporin A is a tetracyclic sesquiterpenoid quinone with an unusual ring system. The B,C and C,D ring junctures both have cis orientations, while the B and D rings are joined in spiro fashion at carbon 9. The double bond of the side chain possesses the *E* geometry. The B ring adopts a slightly distorted half-chair conformation in the solid state, while the C and D rings assume chair conformations.

The unusual benzo[*d*]xanthene ring system found in podosporin A has been previously encountered in nature only as a structural unit of aureol (2), a product of the



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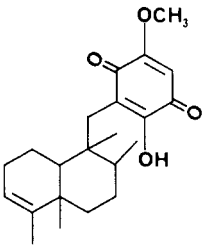
marine sponge *Smenospongia aurea*,[7] though the sites of oxygenation and degree of functionalization differ significantly. A compound containing the ring system possessed by podosporin A has been reported as a rearrangement product obtained when isospongiaquinone (3), a metabolite from the sponge *Stelospongia conulata*, is treated with concentrated acid.[8] Direct HPLC analysis of the fresh culture filtrate of *P. decipiens* confirmed that 1 is present



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as a major component and is not an artifact of the isolation process.

Podosporin A displays potent activity against the early successional coprophilous fungi *S. fimicola* and *A. furfuraceus*, exhibiting respective MIC values of 175 and 50 μg/mL as determined by using agar dilution techniques. Fungal growth was slowed appreciably at much lower concentrations. The growth rates of *S. fimicola* and *A. furfuraceus* were decreased by 50% relative to controls when challenged with podosporin A at concentrations of 25 and 10 μg/mL, respectively. Podosporin A also exhibits activity in a variety of other assays. Respective inhibitory zones of 15, 13, and 18 mm were observed in standard disk assays against *Bacillus subtilis* (ATCC 6051), *Staphylococcus aureus* (ATCC 25923), and *Candida albicans* (ATCC 14053) at 50 μg/disk, but no antagonistic activity toward *Aspergillus flavus* (NRRL 13461) was observed at 250 μg/disk. Podosporin A also displays potent brine shrimp toxicity[9] (LC_{50} = 10 μg/mL).

These results lend support to the concept that late successional, relatively slow growing coprophilous fungi may have a tendency to produce metabolites that inhibit the growth of early successional competitors.[1-3] Similar studies of other coprophilous fungi that display antagonism toward fungal competitors in vitro have also led us to the isolation of antifungal metabolites.[1,2] These findings suggest that this relatively unexplored class of organisms may be a potentially valuable source of antifungal agents. Further chemical and biological studies of coprophilous fungi that show antagonism toward competitors are under way in our laboratory.

Experimental Section

General Procedures. General experimental procedures employed in this work have been described previously.[10]

Cultivation of *P. decipiens*. A culture of *P. decipiens* (NRRL 6461), originally isolated from cattle dung collected in the Pawnee National Grassland in northeastern Colorado, was provided by Dr. D. T. Wicklow of the Agricultural Research Service Culture Collection, USDA Northern Regional Research Center. Five 2-L Erlenmeyer flasks, each containing 400 mL of potato dextrose broth (Difco), were inoculated with several 1-cm^2 agar plugs taken from 3-day-old Petri dish cultures of *P. decipiens* (potato dextrose agar). Flask cultures were incubated at 25–28 °C and aerated by agitation on an orbital shaker at 200 rpm. The antagonistic activity of the culture filtrate toward early successional coprophilous fungi reached a maximum after 12 days.

Assays for Antagonism toward Fungal Competitors. *A. furfuraceus* (NRRL 6460) and *S. fimicola* (NRRL 6459), two widely distributed early colonists of herbivore dung, were employed as test organisms. Rapid semiquantitative assays of extracts and fractions for antagonism against these test organisms were carried out using methods described previously.[1] MIC values were determined utilizing a modification of agar dilution tech-

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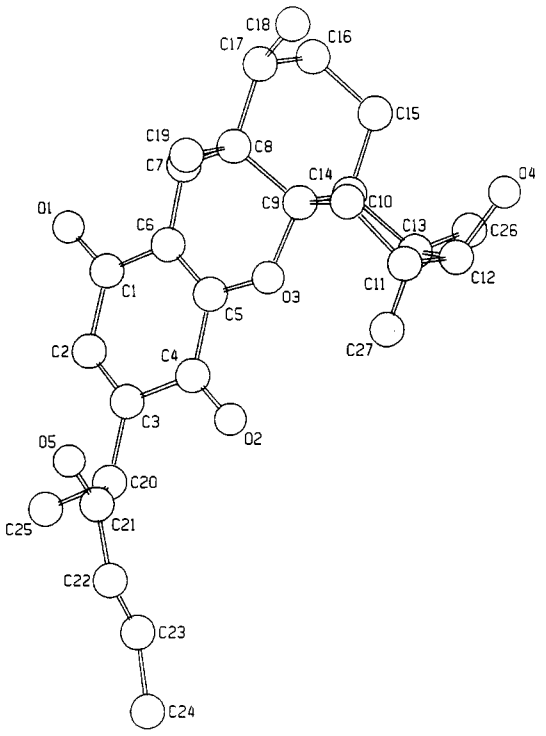

Figure 1. Final X-ray crystallographic model of podosporin A.

niques described by Mitscher et al.[11] Individual Petri plates were streaked with spore suspensions of a test organism and incubated at 26–28 °C. All control plates were covered with fungal growth after 5 days. MIC values are reported as the minimum concentrations in the medium that permitted no discernible fungal growth for the duration of this period.

Isolation and Characterization of Podosporin A (1). The filtered culture broth (2000 mL) was extracted with EtOAc (4 × 600 mL), and the organic phase was dried ($MgSO_4$) and evaporated to afford 550 mg of an orange oil, which accounted for most of the bioactivity. The oil was chromatographed on a silica gel column (4 × 65 cm) with a stepwise gradient from 0 to 6% (v/v) MeOH in $CHCl_3$, and 7-mL fractions were collected. Fractions of similar composition as determined by TLC analysis were pooled and bioassayed. Combined fractions eluting at 1% MeOH exhibited antifungal activity and were purified further by reversed-phase semipreparative HPLC (90:10 MeOH–H_2O) to afford 20.1 mg of podosporin A (1) as a yellow solid, which crystallized from MeOH upon standing at −10 °C. Podosporin A has the following properties: mp 176–178 °C; $[\alpha]_D$ +131.1° (*c* 0.16, $CHCl_3$); HPLC retention time 9.21 min under the above conditions; UV (MeOH) 254 (ε 8200), 222 nm (12 400); IR (neat) 3684, 3626, 3021, 2967, 2938, 2877, 1695, 1672, 1654, 1634, 1609, 1521, 1213 cm^{-1}; EIMS ions at *m/z* 440 (M^+, 0.4), 422 (0.3), 383 (0.2), 236 (16), 207 (100), 189 (58), 175 (10), 163 (11), 147 (13), 135 (26), 121 (11), 119 (22), 107 (18), 105 (14), 93 (12), 91 (14), 69 (96); ^1H NMR ($CDCl_3$), see Table I; ^{13}C NMR ($CDCl_3$) 198.1 (s), 186.8 (s), 180.9 (s), 150.0 (s), 145.2 (s), 144.5 (d), 133.5 (d), 129.8 (d), 117.8 (s), 87.5 (s), 74.1 (d), 45.0 (d), 41.9 (d), 39.1 (d), 37.90 (s), 37.86 (s), 30.5 (t), 30.3 (q), 28.0 (t), 26.5 (q), 25.8 (t), 24.2 (t), 23.4 (t), 20.3 (q), 18.4 (q), 16.9 (q), 15.2 ppm (q). The HRFAB mass spectrum gave an M + 2H ion of slightly larger intensity than the M + H ion, as is sometimes observed for quinones: obsd, 442.2745 (M + 2H); calcd for $C_{27}H_{36}O_5$ + 2H, 442.2718.

Single-Crystal X-ray Diffraction Analysis of Podosporin A (1). Crystals of podosporin A suitable for X-ray diffraction analysis were obtained from methanol by slow evaporation. Cell dimensions were determined from a least-squares fit to 15 diffractometer-measured values of reflections with 2θ between 20° and 25° to be a = 11.380 (2) Å, b = 9.453 (2) Å, c = 11.551 (2) Å, β = 100.79 (2)°, and V = 1220.6 Å^3. The systematic absences corresponded to space group $P2_1$. With two units of $C_{27}H_{36}O_5$ per cell, the calculated density is 1.19 g/cm^3. A half-sphere of diffraction intensity data (3078 total measurements) was collected with from 2° to 40° (Mo Kα radiation, λ = 0.710 73 Å) using an Enraf-Nonius four-circle automated diffractometer with variable scan speed (1.4° to 4°/min) and ω-dependent scan range (minimum range = 0.7°). The ratio of peak scan to background times was 2/1. The 3708 measurements yielded 3465 observed reflections, which averaged to 1223 unique reflections (internal agreement = 1.6% on F_o), of which 1137 (93%) had values greater than 3σ. Data were corrected for Lorentz and polarization effects, but not for absorption (negligible) or decay (<0.5%). The structure was solved by MULTAN and refined by full-matrix least squares.[12] All H-atom positions were found from difference maps, but they were put in ideal fixed positions and not refined. The final refinement, based on 1137 reflections, 153 parameters (oxygen atoms anisotropic, others isotropic), gave R_1 = 0.051, R_2 = 0.086, standard deviation of an observation of unit weight = 1.31, maximum parameter shift error = 0.07, and residual electron density maximum value = 0.27 e/Å^3. Additional crystallographic information is available and is described in the paragraph entitled Supplementary Material Available at the end of this paper.

Acknowledgment. We thank Dr. D. T. Wicklow of the ARS Culture Collection, USDA Northern Regional Research Center, for providing the fungal cultures employed in this work. Support for the purchase of the X-ray diffractometer was provided in part by a grant from the National Science Foundation.

Supplementary Material Available: Tables of fractional coordinates, thermal parameters, interatomic distances, and bond angles for podosporin A (1) (9 pages). Ordering information is given on any current masthead page.

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