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Curvicollides A—C: New Polyketide-Derived Lactones from a Sclerotium-Colonizing Isolate of *Podospora curvicolla* (NRRL 25778)

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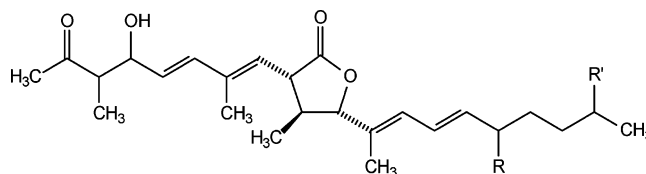
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ABSTRACT

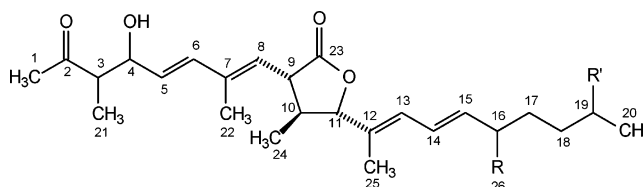


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| 1 | R = CH ₂ OH | R' = H |
| 2 | R = CH ₃ | R' = OH |
| 3 | R = CH ₃ | R' = H |

Curvicollides A–C (1–3) have been obtained from cultures of an isolate of *Podospora curvicolla* (NRRL 25778) that colonized a sclerotium of *Aspergillus flavus*. The structures of these compounds were elucidated by analysis of one- and two-dimensional NMR data. The lead compound (1) showed antifungal activity against *A. flavus* and *Fusarium verticillioides*.

Our ongoing targeted investigations of fungicolous and mycoparasitic fungi have afforded a variety of new bioactive natural products.^{1–4} Part of our work in this area has focused specifically on colonists of *Aspergillus* sclerotia as potential sources of anti-*Aspergillus* agents.⁴ During the course of this project, an isolate of *Podospora curvicolla* (NRRL 25778)⁵ was obtained from the surface of a sclerotium of *Aspergillus flavus* that had been buried in soil in an Illinois cornfield

for an extended period. An organic extract of solid-substrate fermentation cultures of this isolate was found to display significant antifungal activity against *A. flavus* (NRRL 6541) and *Fusarium verticillioides* (NRRL 25457). Bioassay-guided fractionation of this extract led to the isolation of three new highly modified γ -lactones, which we named curvicollides A–C (1–3). This report describes the production, isolation, and structure elucidation of these compounds.



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|---|------------------------|---------|
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Table 1. NMR Data for Curvicollides A (**1**) and B (**2**) in CDCl₃

position	curvicollide A (1)			curvicollide B (2)	
	δ_{H}^a (mult J_{HH})	δ_{C}^b	HMBC ^c (H→C#)	δ_{H}^d (mult J_{HH})	δ_{C}^b
1	2.21 (s)	29.8	2, 3	2.20 (s)	29.8
2		213.2			213.2
3	2.67 (dq, 7.2, 7.8)	52.3	2, 4, 5, 21	2.67 (dq, 7.2, 7.8)	52.3
4	4.23 (br t, 7.8)	75.1	2, 3, 5, 6, 21	4.23 (td, 7.2, 0.6)	75.1
5	5.62 (dd, 16, 7.8)	129.0	3, 4, 7	5.64 (dd, 16, 7.8)	129.0
6	6.30 (d, 16)	136.3	4, 7, 8, 22	6.30 (d, 16)	136.4
7		138.5			130.2
8	5.36 (br d, 9.6)	126.0	6, 9, 10, 22, 23	5.36 (br d, 9.0)	126.1
9	3.24 (dd, 12, 9.6)	48.4	7, 8, 10, 23, 24	3.26 (dd, 12, 9.0)	48.5
10	2.16 (m)	42.6	8, 9, 12, 24	2.16 (m)	42.8
11	4.34 (d, 9.4)	90.3	10, 13, 24, 25	4.35 (d, 9.6)	90.6
12		130.3			138.5
13	6.08 (br d, 11)	129.5	11, 14, 15, 25	6.04 (br d, 11)	129.2
14	6.33 (br dd, 15, 11)	127.4	12, 15, 16	6.20 (ddd, 16, 11, 1.2)	123.8
15	5.55 (dd, 15, 9.0)	138.2	13, 16, 17, 26	5.62 (dd, 16, 7.8)	143.0
16	2.28 (m)	46.2	15	2.20 (m)	37.2
17	1.23 (m); 1.42 (m)	30.7	18, 19	1.25–1.30 (m)	29.8
18	1.25 (m)	29.3		1.42 (m)	37.0
19	1.29 (m)	22.8		3.76 (m)	68.3
20	0.87 (t, 7.2)	14.0	18, 19	1.17 (d, 6.0)	23.5
21	1.08 (d, 7.2)	13.9	2, 3, 4	1.08 (d, 7.2)	13.9
22	1.81 (br d, 1.2)	13.2	6, 7, 8	1.81 (br d, 1.2)	13.2
23		176.2			176.2
24	1.04 (d, 6.6)	14.8	9, 10, 11	1.03 (d, 7.2)	14.8
25	1.75 (d, 0.6)	11.7	11, 12, 13	1.73 (br s)	11.5
26a	3.43 (dd, 11, 7.8)	65.9	15, 17	1.02 (d, 6.6)	20.4
26b	3.58 (dd, 11, 5.4)		15, 17		

^a Recorded at 600 MHz. ^b Recorded at 75.5 MHz. ^c Recorded at 600 MHz. ^d Recorded at 300 MHz.

The EtOAc extract from cultures of *P. curvicolla* was fractionated by silica gel VLC, followed by Sephadex LH-20 chromatography and/or reversed-phase HPLC, to afford curvicollides A–C (**1**–**3**).⁶ HRFABMS and NMR data revealed that **1** and **2** have the same molecular formula

(5) *Podospora curvicolla* (Wint.) Niessl (NRRL 25778) was isolated by DTW from a sclerotium of *A. flavus* buried in a corn field plot for three years following procedures described in: Wicklow, D. T.; Wilson, D. M.; Nelson, T. C. *Phytopathology* **1993**, 83, 1141–1147. *P. curvicolla* was cultured on slants of potato dextrose agar (PDA), from which a spore inoculum was prepared to give a final spore/cell suspension of 1×10^6 /mL. Fermentation was carried out on sterile rice incubated at 25 °C for 40 days.

(6) EtOAc extraction of the fermentation mixture yielded 3.3 g of crude extract, which was fractionated by silica gel VLC (hexane–CH₂Cl₂–MeOH gradient; 200 mL fractions). The fraction that eluted with 99:1 CH₂Cl₂–MeOH (44 mg) was further separated by semipreparative reversed-phase HPLC (Alltech HS Hyperprep 100 BDS C₁₈; 10 × 250 mm; flow rate, 2 mL/min; 35 to 50% CH₃CN in H₂O over 15 min, then 50% to 53% over 35 min) to afford curvicollides A (**1**, 6.5 mg, t_{R} = 48 min) and B (**2**, 2.1 mg, t_{R} = 40 min). The fraction that eluted from the silica gel VLC column with 100% CH₂Cl₂ (84 mg) was further separated by Sephadex LH-20 column chromatography using hexane–CH₂Cl₂ (1:4) as the eluent. Two subfractions were combined (38 mg) and partitioned between hexane and CH₃CN. The CH₃CN layer (7.4 mg) was further purified by HPLC (same column as above; 65 to 80% CH₃CN in H₂O over 20 min, isocratic at 80% for 5 min, and then 80 to 100% over 3 min) to afford curvicollide C (**3**, 3.0 mg, t_{R} = 24.8 min). Curvicollide A (**1**): colorless oil; $[\alpha]_{\text{D}} -9.0^\circ$ (c 0.083, CH₂Cl₂); UV (CH₂Cl₂) λ_{max} 253 (ε 5700); IR (CH₂Cl₂) ν_{max} 3606, 2960, 2931, 2857, 1771, 1714, 1607, 1459, 1380, 1360, 1269, 1166, 977 cm⁻¹; ¹H NMR, ¹³C NMR, and HMBC data, see Table 1; FABMS (3-NBA/NaI) obsd m/z 455 ([M + Na]⁺; rel int 3), 433 ([M + H]⁺; (4), 415 (14), 397 (9), 385 (12), 343 (6); 273 (17), 258 (13), 242 (15), 229 (21), 208 (20), 207 (16), 189 (17); HRFABMS (3-NBA/NaI) obsd m/z 455.2768 (M +

(C₂₆H₄₀O₅; seven degrees of unsaturation). Analysis of ¹H, ¹³C, and DEPT NMR data for curvicollide A (**1**; Table 1) revealed the presence of a methyl ketone unit (δ_{C} 29.8/213.2; δ_{H} 2.21), along with five other methyl groups, four methylene carbons (one oxygenated), eight olefinic carbons (six of which are protonated), six sp³ methine carbons, and one carboxyl carbon. These data accounted for all but two exchangeable protons and required curvicollide A to be monocyclic. Analysis of COSY results led to the identification of three isolated proton spin-systems corresponding to the C-3–C-6, C-8–C-11, and C13–C20 subunits of structure **1**. HMBC correlations of H₃-25 with C-11, C-12, and C-13 led to connection of the latter two spin-systems to C-12.

Na)⁺, calcd for C₂₆H₄₀O₅Na, 455.2773. Curvicollide B (**2**): colorless oil; $[\alpha]_{\text{D}} -21^\circ$ (c 0.1, CH₂Cl₂); UV (CH₂Cl₂) λ_{max} 253 (ε 4400); IR (CH₂Cl₂) ν_{max} 3602, 2963, 2932, 2858, 1775, 1708, 1461, 1376, 1265, 1163, 971 cm⁻¹; ¹H and ¹³C NMR data, see Table 1; FABMS (3-NBA) obsd m/z 455 ([M + Na]⁺; rel int 6), 415 (16), 273 (21), 207 (56), 178 (18). Curvicollide C (**3**): colorless oil; $[\alpha]_{\text{D}} -13^\circ$ (c 0.11, CH₂Cl₂); UV (CH₂Cl₂) λ_{max} 253 (ε 5400); IR (CH₂Cl₂) ν_{max} 3605, 1770, 1708 cm⁻¹; ¹H NMR (CDCl₃, 600 MHz) δ 6.30 (d, 15 Hz, H-6), 6.18 (br dd, 15, 12 Hz, H-14), 6.04 (br d, 12 Hz, H-13), 5.65 (dd, 15, 7.2 Hz, H-5), 5.63 (dd, 15, 9 Hz, H-15), 5.36 (br d, 9.6 Hz, H-8), 4.34 (d, 10 Hz, H-11), 4.23 (br t, 7.8 Hz, H-4), 3.24 (dd, 12, 9.6, H-9), 2.67 (dq, 7.2, 7.8 Hz, H-3), 2.20 (s, H₃-1), 2.16 (m, H-10), 1.81 (br d, 1.2 Hz, H₃-22), 1.73 (br s, H₃-26), 1.19–1.33 (br m, 6H, H₂-17, 18, and 19), 1.08 (d, 7.2 Hz, H₃-21), 1.03 (d, 6.6 Hz, H₃-25), 0.90 (d, 6.6 Hz, H₃-24), 0.87 (t, 7.2 Hz, H₃-20); ¹³C NMR data (CDCl₃, 75.5 MHz) δ 11.4, 13.2, 13.9, 14.1, 14.8, 20.4, 22.8, 29.8, 29.81, 36.6, 37.1, 42.6, 48.5, 52.3, 75.1, 90.7, 123.3, 126.1, 128.8, 128.9, 130.4, 136.4, 138.4, 143.7, 176.3, 213.2; HRESIMS (in the presence of NH₄OAc) obsd m/z 434.3249 ([M + NH₄]⁺, calcd for C₂₆H₄₀O₄NH₄, 434.3244).

Correlations of H₃-22 with C-6, C-7, and C-8 revealed that C-22 and the C3–C6 and C8–C11 units were joined at C-7. The methyl ketone unit was attached to C-3 on the basis of HMBC correlations from both H₃-1 and H₃-21 to C-2 and C-3. HMBC cross-peaks of H-8 and H-9 to carboxyl carbon C-23 (δ_C 176.3) revealed the connection of C-23 to C-9. At this point, the location of the required ring remained to be determined. Connection of C-23 to C-11 to form a γ -lactone ring was consistent with observation of an IR absorption at 1771 cm⁻¹, but no additional evidence for this linkage was provided by the HMBC data or by selective INEPT irradiation of H-11 (optimizing for J_{CH} = 4 or 7 Hz). Ultimately, the presence of a γ -lactone ring was confirmed by the results of an acetylation experiment. Treatment of curvicolliide A (**1**) with acetic anhydride resulted in formation of a diacetate.⁷ The ¹H NMR spectrum of the product revealed two new acetate methyl singlets at δ_H 2.00 and 2.05. The signals corresponding to H-4 (δ_H 4.23) and H₂-26 (δ_H 3.43, 3.58) in **1** were shifted downfield in the spectrum of the diacetate to δ_H 5.40 and 3.99 (2H, d), respectively, indicating that C-4 and C-26 bear free hydroxy groups in compound **1**. With both of the exchangeable protons accounted for, this result required that carboxyl carbon C-23 acylate the C-11 oxygen to form a γ -lactone. On the basis of these data, the structure of curvicolliide A was established as depicted in **1**.

The ¹H and ¹³C NMR data for curvicolliide B (**2**) were nearly identical to those observed for curvicolliide A, suggesting a very similar structure. Signals for the CH₂OH group and one of the aliphatic methylene units in **1** were replaced by resonances for a new methyl group (δ_H 1.03 d; δ_C 23.5) and an oxygenated methine unit (δ_H 3.76 m; δ_C 68.3) in the spectra of **2**. In addition, the H₃-20 signal was shifted slightly downfield (δ 1.17) and was present as a doublet, rather than a triplet. COSY data were fully consistent with the placement of a methyl group at C-16 and a hydroxy group at C-19, leading to the assignment of structure **2** for curvicolliide B.

The elemental composition for curvicolliide C (**3**) was established as C₂₆H₄₀O₄ on the basis of HRESIMS and NMR data. The ¹H NMR spectrum of curvicolliide C was very similar to that of curvicolliide B, except that the C-19 oxygenated methine unit (δ_H 3.76 m; δ_C 68.3) was replaced by a methylene unit (δ_H 1.19–1.33; δ_C 22.8), and the H₃-20 signal was shifted slightly upfield (δ_H 0.87) and appeared as a triplet. On the basis of these observations, the structure of curvicolliide C was proposed as shown in **3** and confirmed by analysis of COSY and HMBC data.

The C5–C6 and C14–C15 double bonds in curvicolliides A–C were each assigned the *E*-geometry on the basis of the coupling constants for the olefinic protons (15–16 Hz). The C7–C8 and C12–C13 double bonds were also assigned *E*-configurations on the basis of the upfield chemical shifts of C-22 and C-25 (δ 13.2 and 11.7, respectively, in the spectrum of **1**). The relative stereochemistry of the lactone ring in **1** was proposed by analysis of NOESY data. NOESY correlations of H-9 with H-11 and H₃-24 suggested that these protons are all on the same face of the five-membered ring. An additional NOESY correlation of H-10 with H₃-25 supported this assignment. *J*-values observed for H-9, H-10, and H-11 are consistent with those reported for previously described trisubstituted γ -lactones with the analogous relative stereochemistry.⁸ The similarity of these NMR data to those obtained for curvicolliides B (**2**) and C (**3**) led to analogous stereochemical assignments at the ring positions of **2** and **3**. NOESY correlations were not useful in assigning relative stereochemistry in the acyclic portions of the molecule. An effort was made to assign the relative stereochemistry at C-3 and C-4 on the basis of the H-3/H-4 coupling constant (7.8 Hz) and the ¹³C NMR chemical shifts for C-3, C-4, and C-21 (δ 52.3, 75.1, and 13.9, respectively).^{9,10} The δ -values for C-3 and C-4 lie in ranges that would fit either an anti or syn configuration. Both the C-21 shift and the J_{H3-H4} value appear to fit best for an anti isomer, although they did not enable a completely unambiguous assignment. In any event, the distance between the various segments of the molecule that contain stereochemical features, together with the absence of relevant NOESY correlations, would not permit the relative stereochemistry in one region to be related to that of either of the others. Thus, while the relative configurations of the ring stereocenters are shown, and the C-3/C-4 subunit most likely has an anti stereochemistry, the relative configurations at C-3, C-4, C-16 (in **1–3**), and C-19 (in **2**) have not been fully established.

Curvicolliide A (**1**) showed antifungal activity in disk assays¹¹ against *A. flavus* (NRRL 6541) and *F. verticillioides* (NRRL 25457) at 200 μ g/disk, producing a 24 mm inhibitory zone in each case. Comparable results in these assays were obtained with a standard of nystatin at 25 μ g/disk. Curvicolliides B (**2**) and C (**3**) were not tested in these assays due to sample limitations. Although γ -lactones are commonly encountered as bioactive metabolites of fungi and plants,^{8,12–14} curvicolliides A–C differ significantly from naturally occurring precedents in the identity and complexity of the side-chains and in the substitution pattern of the lactone ring.

(7) A solution of curvicolliide A (0.5 mg), 4-*N,N*-(dimethylamino)pyridine (catalytic amount), and acetic anhydride (0.5 mL) was stirred at room temperature for 24 h. The resulting solution was extracted with CH₂Cl₂, and the organic phase was dried under N₂ to afford the diacetate (0.5 mg, 85% yield): ¹H NMR δ 6.39 (d, 15 Hz, H-6), 6.27 (br dd, 15, 11 Hz, H-14), 6.05 (dd, 12, 1.2 Hz, H-13), 5.55 (dd, 15, 8.4 Hz, H-15), 5.45 (dd, 15, 8.4 Hz, H-5), 5.41 (br t, 7.8 Hz, H-4), 5.40 (br d, 9.0 Hz, H-8), 4.34 (d, 10 Hz, H-11), 3.99 (d, 6.6 Hz, H₂-26), 3.23 (dd, 11, 9.0 Hz, H-9), 2.84 (dq, 8.4, 7.2 Hz, H-3), 2.17 (s, H₃-1), 2.30 (m, H-16), 2.16 (m, H-10), 2.03 (s, CH₃COO–), 1.99 (s, CH₃COO–), 1.79 (br d, 1.2 Hz, H₃-22), 1.74 (d, 1.2 Hz, H₃-25), 1.60 (m, H-17a), 1.26 (m, H-17b), 1.24 (overlapping m, H₂-18 and H₂-19), 1.04 (d, 6.6 Hz, H₃-21), 1.03 (d, 7.2 Hz, H₃-24), 0.87 (t, 7.2 Hz, H₃-20); FABMS (3-NBA) obsd *m/z* 539 ([M + Na]⁺; rel int 15), 457 (69), 415 (26), 397 (48), 355 (42), 325 (28), 249 (18), 207 (30), 189 (73), 165 (100).

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Moreover, on the basis of the patterns of oxidation and methylation, curvicollides A–C appear to be derived from condensation of two polyketide units rather than from a single polyketide precursor. The occurrence of dimeric, pseudodimeric, or heterodimeric polyketide fungal metabolites is not unusual, although most such fusions involve two aromatic subunits¹⁴ or, in some instances, an ester linkage.¹⁵ In the case of the curvicollides, two connections between the putative polyketide chains would be required, one of which is a carbon–carbon bond. Relevant precedents would include alternaric acid¹⁶ and the xanthoquinodins,¹⁷ as well as compounds of mixed biogenetic origin.¹⁴

A variety of bioactive compounds such as podosporin A,¹⁸ appenolides A–C,¹⁹ decipenin A, and decipenolides A and

B²⁰ have been reported from other *Podospora* spp., but to our knowledge, curvicollides A–C are the first secondary metabolites to be reported from *P. curvicolla*.

Acknowledgment. Support for this work from the National Science Foundation (CHE-0079141 and CHE-0315591) is gratefully acknowledged.

Supporting Information Available: ¹H and ¹³C NMR spectra for curvicollide A (**1**) and ¹H NMR spectra for curvicollide B (**2**) and curvicollide C (**3**). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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