Communiols E−H: New Polyketide Metabolites from the Coprophilous Fungus *Podospora communis*

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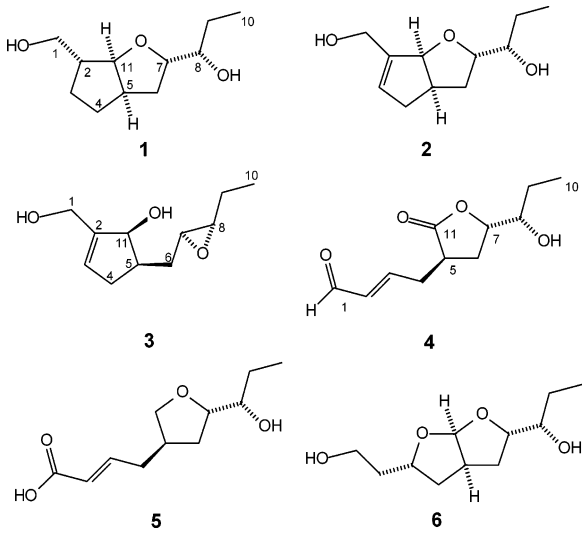
Communiols E−H (**1**−**4**), four new polyketide-derived natural products containing furanocyclopentane, furanocyclopentene, cyclopentene, or γ-lactone moieties, have been isolated from two geographically distinct isolates of the coprophilous fungus *Podospora communis*. The structures of these compounds were determined by analysis of NMR and MS data.

As part of our ongoing search for new bioactive metabolites from coprophilous fungi,[1−3] a culture of *Podospora communis* (Speg.) Niessl (JS 161) originally obtained from horse dung showed antagonistic activity toward the competitor fungus *A. furfuraceus* and was subjected to chemical studies, leading to a report of four new polyketide metabolites called communiols A−D.[1] Further studies of this isolate and a second culture of *P. communis* obtained from a different location have led to the isolation of four additional related compounds (communiols E−H; **1**−**4**). These compounds contain structural features significantly different from those of communiols A−D, but all eight metabolites appear to share similar biogenetic origins.

The EtOAc extract of the filtered culture broth obtained from fermentation of a subculture of *P. communis* JS 161, originally obtained from a sample of horse dung collected in northern California, was fractionated by Sephadex LH-20 column chromatography and reversed-phase HPLC to afford communiols E−H (**1**−**4**). Parallel studies of another isolate of *P. communis* (JS 349) obtained from a sample of horse dung collected in Ecuador led to the isolation of previously reported communiols B (**5**) and D (**6**), as well as communiol G (**3**).

The elemental composition of communiol E (**1**) was determined to be $C_{11}H_{20}O_3$ (two degrees of unsaturation) on the basis of HRFABMS and NMR data. Analysis of ^{1}H NMR (Table 1), ^{13}C NMR (Table 2), and DEPT data revealed the presence of one methyl group, five methylene units (one of which is oxygenated), and five sp^3 methine carbons (three of which are oxygenated). No carbonyl or olefinic carbon signals were observed in the ^{13}C NMR spectrum. These data, together with two exchangeable protons indirectly required by the DEPT results, indicated that communiol E (**1**) is bicyclic and must contain two hydroxy groups and one ether moiety. The presence of the same C6−C10 partial structure found in communiols A−D (e.g., in **5** and **6**) was evident on the basis of NMR comparisons and homonuclear decoupling experiments, but other signals were more distinctive. Some of the methylene proton signals showed significant overlap, leading to caution in assignment of the entire skeleton by decoupling experiments alone. However, the structure of the remaining C1−C5/C11 (cyclopentane) subunit was also established by



decoupling results, and the interconnectivity of the two subunits was confirmed by selective INEPT correlations. For example, the relatively well-resolved C-6 methylene proton signal at δ 1.49 was only weakly coupled to H-5 of the C1−C5/C11 subunit, but showed strong selective INEPT correlations to C-4, C-5, and C-11, confirming connection of C-5 to C-6. Observation of a key correlation from H-11 (δ 4.31) to C-7 (δ 80.8) again established the presence of a tetrahydrofuran ring in compound **1**, as in **5** and **6**. In contrast to the results for **5** and **6**, however, both H-11 and C-11 are significantly further upfield in **1**, and H-2 and H-11 are mutually coupled (J = 4.2 Hz), revealing that C-2 is directly connected to C-11 to form a cyclopentane ring that is fused to the tetrahydrofuran ring in communiol E (**1**).

The relative stereochemistry of communiol E (**1**) was assigned on the basis of NOESY data and coupling constants. A key, strong NOESY correlation of H-2 to H-7 required that these protons be on the same face of the bicyclic system, and that the ring fusion must be *cis* with the bridgehead protons oriented on the opposite face. A strong NOESY correlation of H-5 to H-11 supported the proposed *cis* relationship between these two protons, as did the vicinal coupling constant of 7.5 Hz between H-5 and H-11, which matches previously reported values for analogous *cis* protons in structures that incorporate similar ring fusions.[5−7] As in the case of **6**, the relative stereochemistry at C-7 and C-8 could not be assigned by application of Born's rule.[1,8,9] However, it was presumed to have the same

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Table 1. ^{1}H NMR Data for Communiols E−H (**1**−**4**) in $CDCl_3$

	δ_H (mult., J in Hz)			
position	communiol E (**1**)[a]	communiol F (**2**)[b]	communiol G (**3**)[b]	communiol H (**4**)[a]
1	3.61 (dd, 11, 6.3)	4.255 (br d, 12)	4.270 (br d, 12)	9.52 (d, 8.1)
	3.54 (dd, 11, 7.5)	4.253 (br d, 12)	4.268 (br d, 12)	
2	2.02 (m)			6.17 (ddt, 16, 8.1, 1.5)
3	1.80 (ddt, 15, 3.6, 6.6)	5.78 (br s)	5.68 (br s)	6.82 (dt, 16, 7.2)
	1.23 (m)			
4	1.94 (m)	2.65 (ddd, 17, 9.3, 2.4)	2.58 (br dd, 16, 8.1)	2.83 (m)
	1.38 (ddd, 14, 7.2, 6.6)	2.13 (br d, 17)	1.95 (ddt, 16, 6.6, 2.1)	2.50 (m)
5	2.65 (br pentet, 7)	2.97 (m)	2.25 (m)	2.97 (ddt, 9.6, 5.4, 8.2)
6	1.94 (m)	2.02 (dt, 13, 9.9)	1.85 (ddd, 14, 6.0, 3.9)	2.46 (m)
	1.49 (br dd, 13, 5.4)	1.49 (ddd, 13, 5.7, 1.5)	1.56 (m)	1.88 (dt, 13, 8.7)
7	3.90 (ddd, 10, 5.4, 3.3)	3.74 (m)	2.77 (ddd, 7.2, 3.9, 2.4)	4.42 (ddd, 8.4, 3.6, 3.6)
8	3.74 (dt, 3.3, 6.6)	3.74 (m)	2.73 (td, 5.4, 2.4)	3.82 (ddd, 7.8, 5.4, 3.6)
9	1.38 (pentet, 7.5)	1.40 (pentet, 7.4)	1.56 (m)	1.46 (m)
10	0.90 (t, 7.5)	0.96 (t, 7.4)	0.98 (t, 7.4)	1.00 (t, 7.4)
11	4.31 (dd, 7.2, 4.2)	5.14 (dd, 7.5, 1.8)	4.57 (br d, 5.7)	

[a] Recorded at 600 MHz. [b] Recorded at 300 MHz.

Table 2. ^{13}C NMR Data for Communiols E−H (**1**−**4**) in $CDCl_3$

	δ_C			
position	communiol E (**1**)	communiol F (**2**)	communiol G (**3**)	communiol H (**4**)
1	65.0	61.0	60.8	193.4
2	49.8	149.9	144.2	134.8
3	28.5	130.4	127.9	153.2
4	31.6	39.4	36.2[a]	34.6
5	42.9	39.8	47.2	38.5
6	31.0	33.2	36.1[a]	27.2
7	80.8	79.4	57.9	80.5
8	72.6	72.5	60.9	73.6
9	25.7	26.1	25.0	25.5
10	10.4	9.8	9.8	10.0
11	87.9	89.2	83.6	178.0

[a] These assignments are interchangeable.

relative stereochemistry as communiols A−D at these positions on the basis of the similarity of the H7−H8 coupling constant (J_{H-H} = 3.6 Hz) to that observed for other communiols.[1]

The molecular formula of communiol F (**2**) was determined to be $C_{11}H_{18}O_3$ (three degrees of unsaturation) on the basis of FABMS and NMR data. Analysis of ^{1}H and ^{13}C NMR data for **2** (Tables 1 and 2) revealed the presence of the same structural features as in compound **1**, except that the CH_2−CH unit corresponding to C-2 and C-3 of **1** was replaced by a trisubstituted olefin moiety, resulting in significantly less overlap of the ^{1}H NMR signals. The structure of communiol F (**2**) was assigned by comparison of these data with those of compound **1**, and the relative stereochemistry for **2** was deduced to be the same as that of **1** on the basis of the close structural similarities as well as relevant NMR coupling constant values (e.g., J_{H5-H11} = 7.5 Hz)[10] and chemical shift similarities with the C5−C10 subunit of **1**.

The molecular formula of communiol G (**3**) was established as $C_{11}H_{18}O_3$ (three degrees of unsaturation) on the basis of FABMS and NMR data. Analysis of ^{1}H and ^{13}C NMR data for **3** (Tables 1 and 2) revealed the presence of one methyl group, four sp^3 methylene carbons (one oxygenated), four sp^3 methine carbons (three oxygenated), and a trisubstituted olefin unit. These data, together with two exchangeable protons, accounted for the molecular formula and required communiol G (**3**) to be bicyclic. The continuous proton spin-system in **3** corresponding to C3−C11 was identified through homonuclear decoupling experiments and supported by data from selective INEPT experiments. Selective INEPT correlation of the broad, barely resolved oxymethylene AB pattern signals at δ 4.268 and 4.270 (H_2-1) to olefinic carbons C-2 and C-3 and oxymethine carbon C-11, along with correlations of the broad oxymethine doublet at δ 4.57 (H-11) to C-2 and C-3, indicated that C-1 and C-11 are both directly attached to the trisubstituted olefin unit at C-2 to form a cyclopentene unit. The ^{1}H NMR chemical shifts and J-values for H-8 (δ 2.73) and H-7 (δ 2.77), as well as the corresponding ^{13}C NMR shifts (δ 57.9 and 60.9), were characteristic of signals for an epoxide unit incorporating C-7 and C-8. Since two exchangeable protons are present in the molecule, two free hydroxy groups must be attached to C-1 and C-11, respectively, leading to assignment of the gross structure as shown in **3**, a ring-opened and epoxidized analogue of **2**. The J_{H5-H11} value of 5.7 Hz was consistent with a *cis* relationship of the two substituents on the cyclopentene ring,[11,12] as would be expected by analogy to **2**. The J_{H7-H8} value of 2.4 Hz suggested a *trans* relationship of the two protons on the epoxide moiety (literature for *cis*: 4.5 Hz; for *trans*: 3.1 Hz).[13] The relative stereochemistry of the epoxide with respect to the other stereocenters in the molecule could not be independently established based on NMR data, but is presumed to be as shown based on structural similarity to communiol F (**2**).

The molecular formula of the final compound, communiol H (**4**), was determined to be $C_{11}H_{16}O_4$ (four degrees of unsaturation) on the basis of FABMS and NMR data. Analysis of ^{1}H, ^{13}C, and DEPT NMR data (Tables 1 and 2) for **4** indicated the presence of one methyl group, three methylene units, three sp^3 methine units (two oxygenated), a *trans*-disubstituted olefin, one carboxyl carbon, and one aldehyde carbon, requiring this metabolite to be monocyclic. An α,β-unsaturated aldehyde unit was revealed by the presence of a ^{13}C NMR doublet at δ 193.4 and a ^{1}H NMR

doublet at δ 9.52 that was vicinally coupled to an olefinic proton at δ 6.17 (J = 8.1 Hz). The continuous proton spin-system corresponding to the C1−C10 structural subunit of **4** was established by analysis of COSY data, and ^{13}C NMR signal position assignments were confirmed by HMQC data. HMBC correlations of the methine multiplet at δ 2.97 (H-5) and the methylene multiplets at δ 2.83 and 2.50 (H_2-4) to the carboxyl carbon C-11 (δ178.0) indicated that C-5 is connected to C-11. Observation of an HMBC correlation of oxymethine H-7 (δ 4.42) to C-11 enabled completion of a γ-lactone ring. The C2−C3 double bond was assigned the *E*-geometry on the basis of the coupling constant for the olefinic protons (16 Hz).

The relative stereochemistry at C-7 and C-8 was again presumed to be *threo* by comparison of the coupling constant between H-7 and H-8 with those in communiols A−E. The two substituents on the five-membered lactone ring were assigned a *trans* relative stereochemistry due to the clear structural and biosynthetic relationship with communiol B (**5**). The absolute stereochemistry of **1**−**4** is presumed to be as shown by analogy to the assignment made previously for communiol A[1] using the modified Mosher NMR method.

Although communiols A−C exhibited antibacterial activity, communiols E−H (**1**−**4**) were inactive against *Bacillus subtilis* (ATCC 6051), *Staphylococcus aureus* (ATCC 29213), and *Candida albicans* (ATCC 90029) in disk assays at 200 μg/disk using standard protocols.[14,15] The reduced furanocyclopentanoid ring system found in **1** and **2** is relatively rare among natural products and is not precedented among fungal metabolites to our knowledge, although molecules with more extensive ring systems that incorporate such a unit are relatively common.[5−7] Despite the structural diversity among these compounds, it is likely that they all derive from common or similar biogenetic intermediates, presumably as part of a polyketide origin.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO model DIP-1000 digital polarimeter. IR spectra were obtained with a Mattson Cygnus 25 FT spectrophotometer. NMR spectra were recorded using $CDCl_3$ solvent, and chemical shifts were referenced to the residual solvent signals (δ_H 7.24/δ_C 77.0). ^1H NMR data were recorded at 300 MHz (Bruker AC-300) or 600 MHz (Bruker AMX-600), ^{13}C NMR data were recorded at 75 MHz (AC-300), and selective INEPT data were recorded at 90 MHz (Bruker WM-360). All 2D NMR data were recorded at 600 MHz (^1H dimension). EIMS data were obtained with a VG Trio-1 quadrupole mass spectrometer operating at 70 eV using a direct inlet probe. FABMS and HRFABMS data were recorded using a VG ZAB-HF double-focusing mass spectrometer. HPLC separations employed a semipreparative Alltech HS Hyperprep 100 BDS C_{18} column (250 × 10 mm) at a flow rate of 2 mL/min with UV monitoring at 215 nm.

Fungal Material. The two isolates of *P. communis* (Speg.) Niessl (Lasiosphaeriaceae) employed in this study were assigned the accession numbers JS 161 and JS 349 in the D. Malloch culture collection at the University of Toronto and were obtained from samples of horse dung collected by one of the authors, D.M., in Santa Cruz, California (JS 161), and in Ecuador (JS 349). Since only communiols B (**5**), D (**6**), and G (**3**) were obtained from JS 349, the protocols for metabolite production and purification of communiols E−H described here are based on work carried out with the JS 161 isolate. Six 2 L Erlenmeyer flasks, each containing 400 mL of potato dextrose broth (Difco), were each inoculated with two 0.5 cm^2 agar plugs taken from stock cultures of *P. communis*. Flask cultures were incubated at room temperature on an orbital shaker at 150 rpm for 25 days.

Extraction and Isolation. The culture broth was separated from the mycelium by filtration. The filtered broth (2.4 L) was extracted with EtOAc (4 × 400 mL), and the organic phase was dried over $MgSO_4$ and concentrated to afford 280 mg of brown oil. The crude extract was subjected to Sephadex LH-20 column chromatography using hexane−CH_2Cl_2−acetone gradient elution to afford a total of 20 fractions. Fraction 8 (8.4 mg), which was eluted with 1:4 hexane−CH_2Cl_2, was essentially pure communiol E (**1**) and was used for structure elucidation without further purification. Fraction 7 (21 mg), which was also eluted with 1:4 hexane−CH_2Cl_2, was further separated by reversed-phase HPLC (15−20% CH_3CN in H_2O over 45 min) to afford communiol F (**2**; 4.5 mg; t_R 15 min). Fraction 9 (15 mg), which was eluted with 4:1 CH_2Cl_2−acetone, was purified by reversed-phase HPLC (15−15.5% CH_3CN in H_2O over 35 min) to afford communiol G (**3**; 2.8 mg; t_R 24 min). Fraction 6 (41 mg), which was eluted with 1:4 hexane−CH_2Cl_2, was separated by reversed-phase HPLC (25−25.5% CH_3CN in H_2O over 50 min) to afford communiol H (**4**; 1.8 mg; t_R 28 min). Protocols for isolation of **5** and **6** from this extract have been described previously.[1]

Communiol E (1): colorless oil; $[\alpha]_D$ +129° (*c* 0.075, CH_2Cl_2); IR (CH_2Cl_2) ν_{max} 3600 (br), 2962, 2934, 2879, 1458, 1073, 1038 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; NOESY data ($CDCl_3$, H-# ↔ H-#) H-2 ↔ H-7, H-5 ↔ H-11, H-11 ↔ H_2-1, H-11 ↔ H-2; selective INEPT data ($CDCl_3$, H-# → C-#) H_3-10 → C-8 and C-9; H_2-9 → C-7, C-8, and C-10; H-8 → C-6, C-9, and C-10; H-6b → C-4, C-5, and C-11; H-5 → C-4 and C-6; H-3a → C-1, C-4, C-5, and C-11; H-2 → C-1; H_2-1 → C-3; H-11 → C-1, C-3, C-4, and C-7; EIMS (70 eV) *m/z* 171 ([M − Et]$^+$; rel int 3), 157 (9), 141 (89), 123 (97), 95 (99), 79 (100), 67 (86), 55 (33); HRFABMS (3-NBA/NaI) obsd *m/z* 223.1305 (M + Na)$^+$, calcd for $C_{11}H_{20}O_3Na$, 223.1310.

Communiol F (2): colorless oil; $[\alpha]_D$ +137° (*c* 0.058, CH_2Cl_2); ^1H and ^{13}C NMR data, see Tables 1 and 2; FABMS (thioglycerol) *m/z* 199 ([M + H]$^+$).

Communiol G (3): colorless oil; $[\alpha]_D$ −36° (*c* 0.15, CH_2Cl_2); ^1H and ^{13}C NMR data, see Tables 1 and 2; selective INEPT data ($CDCl_3$, H-# → C-#) H-11 → C-2, C-3, and C-4 (and/or overlapping signal C-6), H_3-10 → C-8 and C-9; H-4a → C-2, C-3, and C-11; H-5 → C-11; H-3 → C-1, C-2, and C-3; H_2-1 → C-2, C-3, and C-11; FABMS (DTT/DTE) *m/z* 221 ([M + Na]$^+$), 199 ([M + H]$^+$; HRFABMS (3-NBA/NaI) obsd *m/z* 221.1138 (M + Na)$^+$, calcd for $C_{11}H_{18}O_3Na$, 223.1154.

Communiol H (4): colorless oil; $[\alpha]_D$ −70° (*c* 0.1 CH_2Cl_2); IR (CH_2Cl_2) ν_{max} 3500 (br), 2950 (sh), 2931, 2855, 1770, 1700 (sh), 1460, 1178 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HMBC data ($CDCl_3$, H-# → C-#) H_3-10 → C-8 and C-9; H_2-9 → C-7, C-8, and C-10; H-8 → C-6, C-7, and C-10; H-7 → C-11; H-6a → C-4, C-7, and C-8; H-6b → C-4, C-5, C-7, C-8, and C-11; H-5 → C-3, C-4, C-6, and C-11; H_2-4 → C-2, C-3, C-5, C-6, and C-11; H-3 → C-1, C-4, and C-5, H-2 → C-1 and C-3; H-1 → C-2 and C-3; FABMS (3-NBA) *m/z* 213 ([M + H]$^+$).

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Supporting Information Available: ^1H and ^{13}C NMR spectra of representative communiols (**1**, **3**, **5**, and **6**). This material is available free of charge via the Internet at http://pubs.acs.org.

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