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Communiols A–D: new mono- and bis-tetrahydrofuran derivatives from the coprophilous fungus *Podospora communis*

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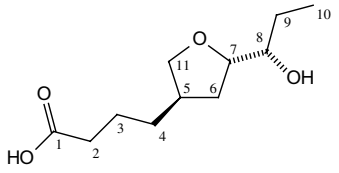
Abstract—Communiols A–D (**1**–**4**), new tetrahydrofuran and bis-tetrahydrofuran derivatives, have been isolated from cultures of the coprophilous fungus *Podospora communis*, and identified by spectroscopic methods.

During our ongoing search for new bioactive natural products from coprophilous (dung-colonizing) fungi,[1,2] an isolate of *Podospora communis* (Speg.) Niessl (JS 161)[3] obtained from horse dung was subjected to chemical studies. A subculture of this isolate was grown in liquid culture, and the EtOAc extract of the culture broth exhibited antibacterial activity. Fractionation of this extract by Sephadex LH-20 column chromatography and reversed-phase HPLC afforded four new tetrahydrofuran and bis-tetrahydrofuran derivatives that we called communiols A–D (**1**–**4**).[4] Details of the structures and bioactivities of these compounds are reported here.



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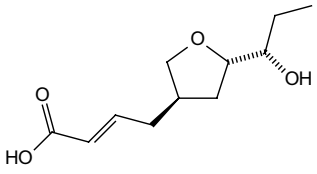

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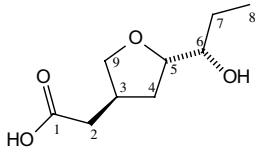

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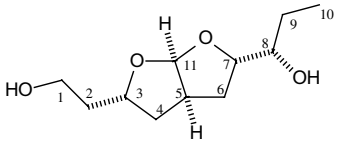

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The molecular formula of communiol A (**1**) was determined to be $C_{11}H_{20}O_4$ (two unsaturations) on the basis of HRFABMS and NMR data (Tables 1 and 2). Analysis of ^1H and ^{13}C NMR data for **1** revealed the presence of one methyl group, six methylene units (one of which is oxygenated), three sp^3 methine carbons (two oxygenated), and one carboxyl carbon. These signals, together with two exchangeable protons, accounted for the formula, and required **1** to be monocyclic. The identity of the sole proton spin system in **1** was established in part by COSY and decoupling experiments. The presence of units corresponding to C2–C4, C5–C8/C11, and C9–C10 in **1** was unambiguous, but assembly of these substructures required additional data due to signal overlap in the upfield region. Selective INEPT correlations of H-11a with C-4, C-6, and C-7, and of H-7 to C-11 provided most of the necessary information, and established the presence of a tetrahydrofuran ring. The signal for H-8 showed correlations to C-6, C-7, C-9,

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Table 1. ^{1}H NMR data for Communiols A–D (**1**–**4**) in $CDCl_3$[a]

#	Communiol A (**1**)[b]	Communiol B (**2**)[c]	Communiol C (**3**)[b]	Communiol D (**4**)[b]
1				3.75 (t, 6.0)
2	2.33 (t, 7.4)	5.84 (dt, 16, 1.5)	2.42 (dd, 7.8, 16) 2.44 (dd, 7.2, 16)	1.78 (m) 1.69 (ddt, 14, 8.4, 6.0)
3	1.62 (m)	6.99 (dt, 16, 7.2)	2.63 (br septet, 7)	4.25 (m)
4a	1.40 (m)	2.28 (m)	2.08 (ddd, 13, 8.4, 6.6)	1.85 (ddd, 13, 6.0, 2.1)
4b			1.52 (ddd, 13, 7.8, 6.0)	1.78 (m)
5	2.17 (br septet, 7)	2.35 (m)	3.90 (ddd, 10, 7.8, 3.6)	2.93 (m)
6a	2.03 (ddd, 12, 8.4, 6.9)	2.06 (dt, 13, 7)	3.68 (ddd, 8.4, 4.8, 3.6)	2.11 (dt, 13, 9.6)
6b	1.43 (m)	1.50 (ddd, 13, 9.0, 7)		1.58 (ddd, 13, 6.3, 2.4)
7	3.89 (ddd, 10, 6.9, 3.6)	3.88 (dt, 3.3, 7)	1.40 (m)	4.09 (ddd, 9.9, 6.3, 3.6)
8	3.66 (ddd, 9.0, 5.7, 3.6)	3.66 (ddd, 8.7, 5.4, 3.3)	0.96 (t, 7.4)	3.72 (ddd, 7.8, 4.8, 3.6)
9	1.40 (m)	1.39 (m)	4.09 (br t, 7.2) 3.41 (br t, 7.2)	1.37 (m)
10	0.96 (t, 7.4)	0.95 (t, 7.4)		0.96 (t, 7.4)
11a	4.02 (dd, 8.1, 6.6)	4.02 (dd, 8.4, 6.9)		5.74 (d, 5.4)
11b	3.32 (dd, 8.1, 7.5)	3.32 (dd, 8.4, 7.0)		

[a] δ_H (mult., J in hertz).
[b] Recorded at 600 MHz.
[c] Recorded at 300 MHz.

Table 2. ^{13}C NMR data (δ_C) for **1**–**4** in $CDCl_3$

#	**1**	**2**	**3**	**4**
1	178.3	170.8	176.3	60.8
2	33.9	122.5	37.2	37.7
3	23.4	148.5	35.5	78.9
4	32.5	35.6	30.7	39.3
5	39.2	38.2	81.3	42.6
6	30.9	30.6	73.7	31.2
7	81.5	81.5	25.6	82.6
8	73.7	73.7	10.3	72.4
9	25.6	25.6	73.7	25.5
10	10.3	10.3		10.3
11	73.6	73.2		108.8

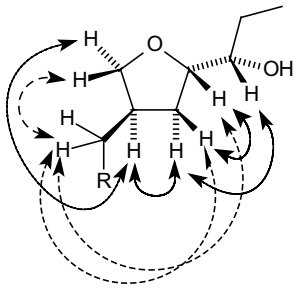

Figure 1. Key NOESY correlations for **1** (↔) and additional relevant correlations observed for **3** (↞↠).

and C-10. Irradiation of H_2-2 resulted in polarization transfer to carboxyl carbon C-1, as well as to C-3 and C-4, indicating that the carboxyl carbon is attached to C-2. The two exchangeable protons must be present as a free hydroxy group at C-8 and a free carboxylic acid at C-1. The presence of the free acid group was confirmed by formation of a methyl ester upon treatment of **1** with $TMSCHN_2$. These data enabled assignment of the gross structure of **1** as shown.

The substituents at C-5 and C-7 were assigned a *trans* relative orientation on the basis of NOESY data. For example, the signals for H-5 and H-7 showed no mutual correlation, but each showed a strong correlation to a different C-6 methylene proton, placing them on opposite faces of the ring (Fig. 1). NOESY experiments for other compounds in the series (see below) gave analogous results, suggesting retention of this relative stereochemistry in all four compounds. The C-7/C-8 relative stereochemistry could not be unambiguously established by analysis of NOESY data or ^{1}H NMR J-values, but was proposed on the basis of ^{13}C NMR shift data.[5–7] During investigations of acetogenins and model compounds, Born et al. proposed a method using ^{13}C NMR shift values to determine the relative configuration of an hydroxymethine carbon attached to a tetrahydrofuran ring at a position α to the ring oxygen atom.[5] Studies of further synthetic model compounds by Fujimuto et al. provided evidence in support of this approach.[7] The ^{13}C NMR shift for the hydroxymethine carbon is normally ca. δ 74 for a *threo* configuration, and δ 72 for an *erythro* relationship. This rule has been subsequently applied by other researchers for various THF-containing acetogenins, although these are generally (*cis*- or *trans*-)2,5-disubstituted analogs.[5,6] The near identity of the relevant NMR shifts and coupling constant data for **1**–**3** (Tables 1 and 2) suggested that these compounds all have the same relative stereochemistry at these positions, and the hydroxymethine chemical shift (δ 73.7 in each case) suggested a *threo* (S^*,S^*) relationship.

The NMR data for communiol B (**2**; $C_{11}H_{18}O_4$; three unsaturations) were similar to those of **1**, except that two of the side-chain methylene units (C2–C3) were clearly replaced by a *trans*-disubstituted olefin unit to give **2**. The ^{1}H and ^{13}C NMR data for communiol C (**3**; $C_9H_{16}O_4$) were also similar to those of **1**, except for the absence of two methylene units of the side chain. Both structures were assigned by NMR comparisons with **1** and by analysis of homonuclear decoupling experiments. In the case of **3**, additional NOESY correlations beyond those described for **1** were observed, which lent further support to the proposed ring stereo-

chemistry (Fig. 1). In the spectrum of **1**, the signal(s) for the side-chain methylene H_2-4 overlapped with the signal(s) for methylene H_2-9 at δ 1.40, rendering correlations to that signal position ambiguous. However, in the data for **3**, the corresponding methylene (numbered as H_2-2 in **3**) appeared as a distinctive, somewhat downfield-shifted AB pair that showed correlations to H-5 and to the H-4 and H-11 methylene proton signals on the same face, thereby providing further support for the relative stereochemistry at the ring positions as shown.

Analysis of ^{1}H and ^{13}C NMR data for communiol D (**4**) revealed the presence of one methyl group, five methylene units (one oxygenated), and five sp^3 methine units (four oxygenated). NMR and HRFABMS data indicated that **4** is an isomer of **1**, but that it lacks olefinic or carbonyl carbons, and must therefore be bicyclic. Comparison of the DEPT and ^{1}H NMR data again indicated two exchangeable protons. The single, continuous proton spin system in the molecule was established based on decoupling experiments, and these and other connections were verified by selective INEPT data. The chemical shifts of H-11 (δ_H 5.74) and C-11 (δ_C 108.8) required linkage of two oxygen atoms to C-11. The bis-THF skeleton was established by the observation of selective INEPT correlations of H-11 to both C-3 and C-7. The two exchangeable protons were assigned to free OH groups attached to C-1 and C-8. A key, strong NOESY correlation of H-3 with H-7 in **4** essentially sets the relative stereochemistry at all four centers of the ring system, since it indicates that these two protons are on the same face of the molecule, and also requires **4** to have a *cis* ring fusion, with the bridgehead protons on the opposite face of the ring system relative to H-3 and H-7. A NOESY correlation of H-5 with H-11 and a $J_{H\text{-}5\text{–}H\text{-}11}$ value of 5.4 Hz[8] were both consistent with a *cis* relationship. This relative stereochemistry is consistent with that assigned for compounds **1**–**3**. The relative stereochemistry at C-7 and C-8 was not proposed using Born's rule in this instance because the model compounds used in its development are monocyclic, and the C-8 shift in **4** (shifted slightly upfield to δ_C 72.4) could be affected by the presence of the second THF ring. Although detailed coupling information has not been systematically reported for model compounds of this type, the $J_{H\text{-}7\text{–}H\text{-}8}$ value of 3.6 Hz in **4** closely matched the corresponding values for **1**–**3**. This observation, together with the biogenetic similarity, suggested that **4** likely has a *threo* relationship at C-7 and C-8.

Although the *threo* assignments in **1**–**4** were not considered completely conclusive, an effort was made to propose the absolute stereochemistry at C-8 in **1** by application of the modified Mosher NMR method.[9] Treatment of communiol A (**1**) with (*S*)-MTPACl or (*R*)-MTPACl in the presence of DMAP afforded the (*R*)-MTPA ester or the (*S*)-MTPA ester, respectively. Formation of the esters was confirmed by a significant downfield shift of the signal for H-8, and by the appearance of the expected new aromatic and methoxy signals in the ^{1}H NMR spectra. Upon comparison of the ^{1}H NMR chemical shifts for the two products ($\Delta\delta$ values shown in Fig. 2), the shifts of the nearby methine (H-7) and methyl (H_3-10) signals were considered to be of greatest value in making the assignment, as the H_2-9 and H_2-6 methylene proton signals overlapped with others. The signals for H_2-11 also showed significant shift differences. All of the corresponding $\Delta\delta$ values (Fig. 2) were consistent with assignment of the *S* absolute configuration at C-8, leading to proposal of the overall 5*R*,7*S*,8*S* absolute stereochemistry in **1** as shown. Communiols B–D (**2**–**4**) were assumed to possess analogous absolute stereochemistry.

Communiols A–C (**1**–**3**) afforded 8- to 16-mm zones of inhibition against *Bacillus subtilis* (ATCC 6051) and *Staphylococcus aureus* (ATCC 29213) in standard disk assays at 200 μg/disk. Communiol D was inactive in these assays. A gentamicin sulfate standard (Sigma Chemical Co.) showed ca. 25-mm zones of inhibition in both assays at 50 μg/disk. None of the compounds showed activity against *C. albicans* (ATCC 90029).

Most of the known naturally occurring mono- and bis-tetrahydrofurans have been reported from plants, and include annonaceous acetogenins and clerodane diterpenoids. However, there are examples from fungal sources, such as aureonitol, from *Chaetomium*[10] and *Helichrysum* spp.,[11] and asteltoxin, a bis-THF derivative from *Aspergillus stellatus*[12] Although other fungal metabolites contain fused furanoid rings, to our knowledge, asteltoxin is the only other fungal metabolite that possesses an isolated bis-THF (hexahydrofuro[2,3-*b*]furan) unit like that found in communiol D (**4**). Communiols A–D (**1**–**4**) are likely to be derived from the polyketide pathway, although the pattern of oxygenation is unusual. The closest precedent for **1**–**4** appears to be aureonitol and its epimer(s), which possess butadienyl and pentadienyl substituents at the 2- and 4-ring positions.[10,11,13] Stable isotope incorporation experiments

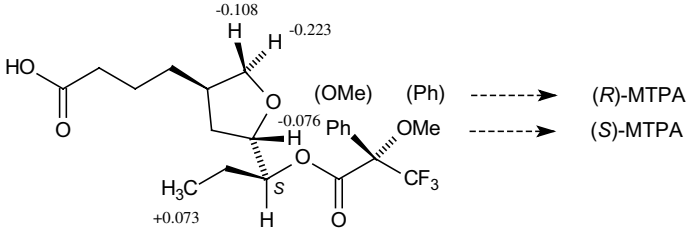

Figure 2. Observed chemical shift differences ($\Delta\delta = \delta_R - \delta_S$ in parts per million at 300 MHz) for selected protons of the *R*- and *S*-MTPA esters of communiol A (**1**).

reported for aureonitol confirmed a polyketide origin, with a decarboxylation step and an epoxide rearrangement invoked to rationalize the labeling pattern.[13] However, **1**–**4** lack the 3-OH group present in aureonitol, and also have ring closure at a different point along the chain. Compounds **1**–**4** are the first compounds to be reported from *P. communis*, and are among the few metabolites reported from members of the relatively common coprophilous genus *Podospora*.

Acknowledgements

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References and notes

1. Gloer, J. B. In *The Mycota IV*; Wicklow, D. T., Söderström, B., Eds.; Springers: Heidelberg, 1997; pp 249–268.
2. Hein, S. M.; Gloer, J. B.; Koster, B.; Malloch, D. *J. Nat. Prod.* **2001**, *64*, 809–812.
3. The isolate of *P. communis* (Speg.) Niessl was obtained from a sample of horse dung collected by D. Malloch in Santa Cruz, California on May 11, 1990. This isolate was identified by J. A. Scott and assigned the accession number JS 161 in the Malloch culture collection at the University of Toronto.
4. 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. The filtered culture 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 a crude extract. The extract was subjected to Sephadex LH-20 column chromatography using hexane–CH_2Cl_2–acetone gradient elution. Communiols A (**1**; 20 mg) and C (**3**; 12 mg) were eluted with 4:1 CH_2Cl_2–acetone. Another fraction (33 mg) obtained using the same eluent was subjected to reversed-phase HPLC (Alltech HS Hyperprep 100 BDS C_{18}; 10 × 250 mm; 15–16% CH_3CN in H_2O over 30 min) to afford communiol B (**2**; 2.5 mg; t_R 16 min). A 20-mg fraction eluted with 4:1 CH_2Cl_2–hexane was subjected to reversed-phase HPLC (15–20% CH_3CN in H_2O over 45 min) to afford communiol D (**4**; 3.0 mg; t_R 20.5 min). All four compounds were obtained as colorless oils. Communiol A (**1**): $[\alpha]_D$ −1.6 (*c* 0.25, CH_2Cl_2); IR (CH_2Cl_2) ν_{max} 3488, 2966, 2931, 1713, 1456, 1072 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; selective INEPT data ($CDCl_3$, H-# → C-#) H-8 → C-6, C-7, C-9, and C-10; H-6a → C-4, C-5, C-7, C-8, and C-11; H-6b → C-8; H-2 → C-1, C-3, and C-4; H-11a → C-4, C-6, and C-7; H-11b → C-4; EIMS (70 eV) *m/z* 199 ($[M-OH]^+$; rel int 7), 171 (8), 157 (88), 139 (93), 121 (46), 83 (76), 69 (100), 55 (84); HRFABMS (3-NBA) obsd *m/z* 239.1261 $(M+Na)^+$, calcd for $C_{11}H_{20}O_4Na$, 239.1259. Communiol B (**2**): $[\alpha]_D$ −95 (*c* 0.075, CH_2Cl_2); IR (CH_2 Cl_2) ν_{max} 3504, 2970, 2931, 2874, 2854, 1702, 1656, 1268 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS (70 eV) *m/z* 214 (M^+; rel int 0.2), 197 (1), 155 (48), 137 (41), 109 (38), 81 (62), 69 (100); FABMS (3-NBA) obsd *m/z* 215 ($[M+H]^+$). Communiol C (**3**): $[\alpha]_D$−3.4 (*c*0.142, CH_2Cl_2); IR (CH_2Cl_2) ν_{max} 3604, 2968, 2930, 1713, 1275, 1074 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS (70 eV) *m/z* 171 ($[M-OH]^+$; rel int 6), 155 (68), 137 (55), 125 (34), 109 (67), 95 (65), 81 (87), 69 (100), 55 (78); FABMS (3-NBA) obsd *m/z* 189 ($[M+H]^+$). Communiol D (**4**): $[\alpha]_D$ +2.7 (*c*0.3, CH_2Cl_2); IR (CH_2Cl_2) ν_{max} 3614, 2970, 2937, 2880, 1264, 1007 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 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-7, C-9, and C-10; H-7 → C-8; H-6a → C-4, C-5, C-7, and C-8; H-6b → C-5; H-5 → C-3, C-4, C-6, and C-7; H-4b → C-5 and C-6; H-3 → C-1; H-11 → C-3, C-4, C-5, and C-7; EIMS (70 eV) *m/z* 199 ($[M-OH]^+$; rel int 11), 171 (39), 157 (97), 139 (77), 113 (87), 95 (83), 69 (90), 55 (100); HRFABMS (3-NBA) obsd *m/z* 239.1260 $(M+Na)^+$, calcd for $C_{11}H_{20}O_4Na$, 239.1259.
5. Born, L.; Lieb, F.; Lorentzen, J. P.; Moeschler, H.; Nonfon, M.; Söllner, R.; Wendisch, D. *Planta Med.* **1990**, *56*, 312–316.
6. Chávez, D.; Acevedo, L. A.; Mata, R. *J. Nat. Prod.* **1998**, *61*, 419–421.
7. Fujimuto, Y.; Murasaki, C.; Shimada, H.; Nishioka, S.; Kakinuma, K.; Singh, S.; Singh, M.; Gupta, Y. K.; Sahai, M. *Chem. Pharm. Bull.* **1994**, *42*, 1175–1184.
8. Lin, Y. L.; Kuo, Y. H.; Chen, Y. L. *Heterocycles* **1989**, *29*, 1489–1495.
9. Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.
10. Abraham, W. R.; Arfmann, H. A. *Phytochemistry* **1992**, *31*, 2405–2408.
11. Bohlmann, F.; Ziesche, J. *Phytochemistry* **1979**, *18*, 664–665.
12. Kruger, G. J.; Steyn, P. S.; Vleggaer, R.; Rabie, C. J. *J. Chem. Soc., Chem. Commun.*, **1979**, 441–442.
13. Saito, M.; Sato, H.; Yonehara, H. *Agric. Biol. Chem.* **1983**, *47*, 2935–2937.