Petriellin A: A Novel Antifungal Depsipeptide from the Coprophilous Fungus *Petriella Sordida*

Kit K. Lee and James B. Gloer*

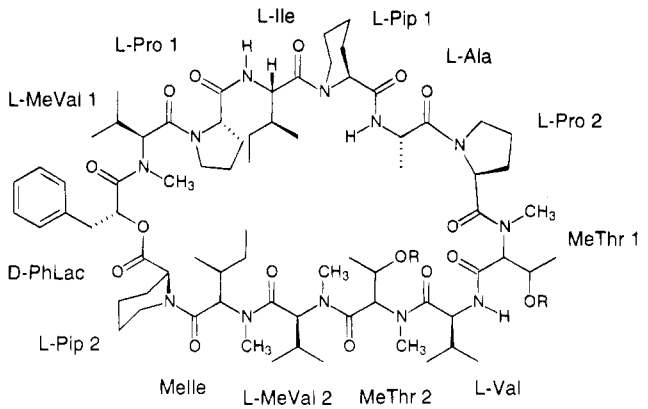
Department of Chemistry, University of Iowa, Iowa City, Iowa 52242

James A. Scott and David Malloch

Department of Botany, University of Toronto, Toronto, Ontario M5S 1A1, Canada

Received May 30, 1995

Antagonism between species of fungi has been frequently observed in studies of natural fungal communities.[1,2] The mechanism of antagonism often involves the production by one species of antifungal agents that inhibit the growth of competitors. Our chemical investigations of antagonistic coprophilous (dung-colonizing) fungi have demonstrated that such studies can lead to discovery of novel antifungal compounds, such as the preussomerins.[3,4] During our continuing investigations of these fungi, we found that organic extracts from cultures of *Petriella sordida* (UAMH 7493) exhibited potent antifungal activity. In this paper, we report the isolation and structure determination of petriellin A (**1**), a novel antifungal cyclic depsipeptide from *P. sordida*.



1 R = H
2 R = Ac

Petriellin A was isolated from the EtOAc extract of the culture broth[5] and crystallized from $CHCl_3$–acetone, producing fine colorless needles: mp 190–200 °C dec; $[\alpha]_D$ −140° (*c* 1.0 mg/mL, MeOH). The molecular formula for **1** was determined to be $C_{74}H_{118}N_{12}O_{16}$ by HRFABMS [*m*/*z* 1431.8920 $(M + H)^+$; Δ −5.3 mmu], and the ^1H and ^{13}C NMR spectra (Table 1) were indicative of a peptide. Petriellin A was ninhydrin-negative, indicating a cyclic peptide or blockage of the *N*-terminus. Extensive analysis of COSY, HOHAHA, HMQC, and HMBC data revealed spin systems consistent with the presence of β-phenyllactic acid (PhLac) and 12 amino acid residues: 1 equiv each of alanine (Ala), isoleucine (Ile), *N*-methylisoleucine (MeIle), and valine (Val) and 2 equiv each of *N*-methylvaline (MeVal), *N*-methylthreonine (MeThr), pipecolic acid (Pip), and proline (Pro). These units account for all but one degree of unsaturation for the molecule, suggesting that petriellin A is a cyclic depsipeptide. The amino acid composition of **1** was verified by GCMS analysis of the *N*-(trifluoroacetyl) *n*-butyl ester derivatives[6] of the amino acids present in the total acid hydrolyzate. Chiral GC of the *N*-(pentafluoropropionyl) isopropyl ester derivatives[7] on a Chirasil-Val column revealed that all of the common amino acids and the MeVal units have the L-configuration. The PhLac unit was assigned the D-configuration based on the optical rotation of β-phenyllactic acid isolated from the acid hydrolyzate of **1** by solvent extraction. The configurations of the MeThr and MeIle units are under investigation.

The amino acid sequence of petriellin A was determined by analysis of HMBC and selective INEPT data (Figure 1) and by FABMS-MS analysis of a ring-opened product of base hydrolysis. The methyl proton signals of all of the *N*-methylamino acids showed HMBC correlations to the carbonyl carbon signals of the adjacent amino acids. HMBC and selective INEPT results showed additional correlations of α- and β-proton signals that enabled assembly of a partial structure, as shown in Figure 1. However, the entire amino acid sequence of **1** could not be determined solely on the basis of heteronuclear NMR correlation data because of extensive NMR signal overlap, as well as the absence of certain key correlations in HMBC experiments.

FABMS analysis of the intact natural product provided little information about the amino acid sequence. However, basic methanolysis of the ester linkage of petriellin A, followed by treatment of the product with acetic anhydride, produced a triacetyl methyl ester of the expected linear peptide. FABMS–MS analysis of this product provided useful sequencing information that complemented the results obtained by NMR. Specifically, MS–MS analysis of the ion at *m*/*z* 625 revealed the sequence of an additional portion of the molecule. Fragmentation of the ion at *m*/*z* 625 $(AcPhLac\text{-}MeVal\text{-}Pro\text{-}Ile\text{-}Pip)^-$ afforded major ions at *m*/*z* 514 $(AcPhLac\text{-}MeVal\text{-}Pro\text{-}Ile)^+$, 401 $(AcPhLac\text{-}MeVal\text{-}Pro)^+$, 304 $(AcPhLac\text{-}MeVal)^+$, and 191 $(AcPhLac)^+$. The location of Ile in this sequence rather than MeVal (an isomeric unit) was based on NMR evidence unequivocally locating both MeVal residues at other positions. These data, together with the partial sequence depicted in Figure 1, accounted for all of the amino acid residues except Ala. Overlap of the PhLac-MeVal-1 segment shown in Figure 1 with the corresponding segment observed in the MS-defined fragment demonstrated that there was only one possible position for the Ala residue: between Pip-1 and Pro-2. These results led to assignment of the gross structure of petriellin A as **1**.

HMBC and selective INEPT experiments on the diacetate **2**, obtained upon treatment of petriellin A with acetic anhydride, afforded sequence-relevant correlations not observed among the data for **1**. These results included correlations of the Pro-1 α-H with the MeVal-1 CO, the Ile α-H with the Pro-1 CO, the Pip-1 α-H with

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(5) *P. sordida* was grown in liquid shake culture using potato dextrose broth (Difco) for 30 days. The filtered culture broth (8.4 L) was partitioned with 4 × 1.4 L EtOAc to afford 722 mg of crude extract as a yellow oil. A portion of the extract (425 mg) was subjected to silica gel column chromatography with 5%, 15%, and 50% hexanes in EtOAc and then EtOAc and MeOH as eluants. Petriellin A (45.3 mg) was obtained as a white powder by reversed-phase HPLC of the MeOH fractions (Rainin Dynamax-60A C_{18}, 8 μm, 250 × 21.4 mm, 88:12 MeOH–H_2O at 10.0 mL/min).
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0022-3263/95/1960-5384$09.00/0

Table 1. ^{1}H and ^{13}C NMR data for Petriellin A (1) in 1:4 $CDCl_3$/Acetone-d_6

position	^{13}C[a]	^{1}H (mult, J in Hz)[b]	position	^{13}C[a]	^{1}H (mult, J in Hz)[b]
β-PhLac CO	168.9		MeThr-1 CO	169.5	
α	72.1	5.84 (dd, 8.1, 5.9)	α	66.0	4.84 (d, 8.8)
β	37.5	3.10 (dd, 13.7, 5.8); 2.95 (m)	β	64.8	4.18 (m)
1	137.3		γ	20.3	1.17 (d, 6.5)
2, 6	130.5	7.24 (m)	*N*-Me	30.5	2.83 (s)
3, 5	128.9	7.24 (m)	Val CO	172.4	
4	127.2	7.16 (m)	α	53.8	5.07 (dd, 9.2, 2.7)
MeVal-1 CO	170.4		β	31.4	1.95 (m)
α	61.1	4.77 (d, 11.9)	γ	20.9	0.94 (d, 6.8)
β	28.2	2.18 (m)	γ	16.8	0.86 (ov)
γ	20.1	0.85 (ov)[c]	*N*-H		8.61 (d, 9.2)
γ	19.0	1.08 (d, 6.2)	MeThr-2 CO	171.8	
N-Me	31.2	3.04 (s)	α	58.7	5.42 (d, 9.3)
Pro-1 CO	172.6		β	65.0	4.28 (m)
α	60.7	4.67 (br t, 6.9)	γ	20.4	1.19 (d, 6.5)
β	31.3	2.13 (m); 1.88[d] (m)	*N*-Me	31.1	3.23 (s)
γ	25.8	2.10 (m); 1.90[d] (m)	MeVal-2 CO	171.2	
δ	48.4	4.34 (m); 3.63 (m)	α	58.0	5.24 (d, 10.9)
Ile CO	172.9		β	27.8	2.70 (m)
α	52.6	4.83 (br t, 10)	γ	19.5	0.88 (ov)
β	37.2	1.95 (m)	γ	18.5	1.85 (d, 6.5)
γ-Me	16.3	0.83 (d, 6.8)	*N*-Me	31.8	3.27 (s)
γ-CH_2	24.8	1.72 (m); 1.18[d] (m)	MeIle CO	169.3	
δ	11.4	0.85 (ov)	α	58.6	4.93 (d, 10.8)
N-H		7.57 (d, 10.5)	β	34.3	2.17 (m)
Pip-1 CO	171.3		γ-Me	16.8	0.86 (ov)
α	52.6	4.98 (m)	γ-CH_2	24.2	1.75 (m); 1.22[d] (m)
β	28.2	2.07 (m); 1.48[d] (m)	δ	11.8	0.83 (t, 7.5)
γ	20.7	1.72 (m); 1.58[d] (m)	*N*-Me	30.9	3.06 (s)
δ	26.4	1.68 (m); 1.35[d] (m)	Pip-2 CO	169.8	
ε	43.7	4.05 (m); 3.41 (ddd, 14, 14, 3.1)	α	52.7	5.36 (br d, 4.4)
Ala CO	171.4		β	26.5	2.18 (m); 1.40[d] (m)
α	47.2	4.62 (pentet, 6.7)	γ	21.6	1.62 (m); 1.20[d] (m)
β	17.9	1.21 (d, 6.8)	δ	26.1	1.58 (m); 1.18[d] (m)
N-H		6.96 (d, 7.1)	ε	43.9	3.88 (m); 2.90 (m)
Pro-2 CO	174.7				
α	56.4	5.00 (m)			
β	30.5[c]	2.14 (m); 2.06[d] (m)			
γ	26.2	2.33 (m); 1.95[d] (m)			
δ	48.1	3.93 (m); 3.68 (m)			

[a] At 100 MHz, acetone signal reference set at 29.8 ppm. [b] At 600 MHz, acetone signal reference set at 2.04 ppm. [c] The abbreviation ov indicates that the expected multiplicity could not be observed due to signal overlap. [d] These ^{1}H NMR signals overlapped and were assigned by analysis of HOHAHA data.

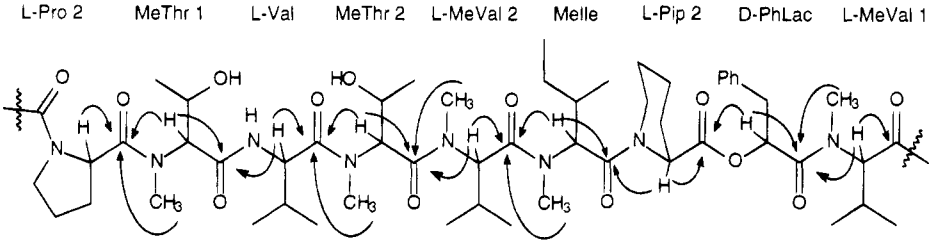

Figure 1. Key HMBC and selective INEPT correlations for petriellin A (**1**).

the Ile CO, and the Ala α-H with the Pip-1 CO. These correlations support the amino acid sequence proposed for petriellin A.

To our knowledge, petriellin A is only the second natural product reported from a member of the genus *Petriella*,[8] and occurrences of *N*-MeThr,[9,10] MeIle, and PhLac as peptide or depsipeptide subunits are relatively rare. Petriellin A (**1**) displays antifungal activity against the early successional coprophilous fungi *Ascobolus furfuraceus* (NRRL 6460) and *Sordaria fimicola* (NRRL 6459), with MIC values of 5 μg/mL and ≤2 μg/mL, respectively. However, no activity was observed in disk assays against *Candida albicans* (ATCC 90029) at 100 μg/disk. Compound **1** displays an approximate IC_{50} value of 10 μg/mL against human fibroblast MRC5 cells, but preliminary assays show the diacetate **2** to be significantly more cytotoxic (IC_{50} < 1 μg/mL). More detailed studies of the stereochemistry and bioactivities of these compounds, along with structural data for related components of the *P. sordida* extract, will be reported in due course.

Acknowledgment. We thank the University of Iowa Central MS and NMR Facilities for spectral data and Myco Pharmaceuticals, Inc., for cytotoxicity results. This work was supported by the National Institutes of Health (R01 AI 27436 and K04 CA 01571) and by an Alfred P. Sloan Foundation Fellowship.

Supporting Information Available: ^{1}H, ^{13}C, HMQC, and HMBC NMR data for compound **1** (4 pages).

JO950965E

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