

A Cyclopeptide from the Insect Pathogenic Fungus *Cordyceps* sp. BCC 1788

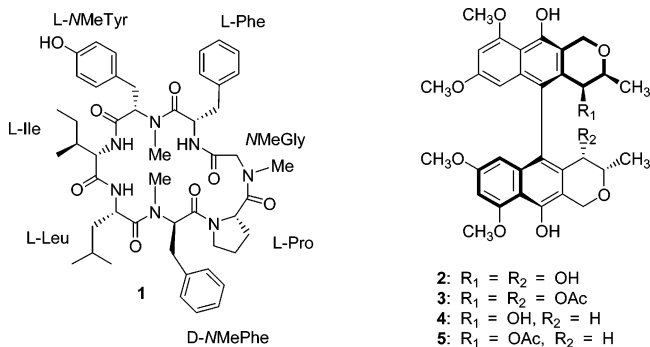
Vatcharin Rukachaisirikul,^{*,†} Sirinya Chantaruk,[†] Chittreeya Tansakul,[†] Saowanit Saithong,[†] Laksamee Chaichareernwimonkoon,[†] Chaveng Pakawatthai,[†] Masahiko Isaka,[‡] and Kamolphan Intereya[‡]

Department of Chemistry, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, 90112, Thailand, and National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand Science Park, Klong Luang, Pathumthani, 12120, Thailand

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A new cyclopeptide, cordyheptapeptide A (**1**), was isolated from the insect pathogenic fungus *Cordyceps* sp. BCC 1788 along with four known bioanthracenes (**2–5**). The structure was elucidated by spectroscopic data. The absolute configuration of amino acid residues was determined by HPLC and X-ray diffraction analyses.

Entomopathogenic fungi of the genus *Cordyceps* are sources of polysaccharides¹ and nucleosides^{1,2} that possess antitumor,^{1,2} antioxidant,^{1,3} antiinflammatory,⁴ antibacterial,⁵ and antifungal⁶ activities. However, there have been only two reports on the isolation of cyclic peptides from *C. militaris*⁷ and *C. sinensis*.⁸ Chemical investigation of the strain *Cordyceps* sp. BCC 1788 led to the isolation of a new cyclopeptide, named cordyheptapeptide A (**1**), along with four known bioanthracenes (**2–5**).^{9–11} The structure elucidation of **1** is described below.



Cordyheptapeptide A (**1**) was obtained as colorless crystals. The HREIMS of **1** showed a molecular ion at m/z 879.4850, corresponding to the molecular formula C₄₉H₆₅N₇O₈. The IR spectrum gave a characteristic amide absorption band at 1630 cm⁻¹. In the ¹³C NMR and DEPT spectra, the presence of seven amide carbonyl carbons (δ 174.2, 172.3, 170.9, 170.4, 170.3, 168.4, and 168.1), six α -methine carbons of amino acid residues (δ 69.2, 58.3, 57.8, 54.5, 50.1, and 47.6), one α -methylene carbon (δ 50.8), and three *N*-methyl carbons (δ 40.4, 35.5, and 30.1) identified **1** as a heptapeptide with three *N*-methyl amino acid residues. In addition, the ¹H NMR spectrum displayed typical signals of peptides: three amide protons (δ 8.60, 8.20, and 5.88), six α -methine protons (δ 5.56, 5.36, 4.93, 4.45, 4.39, and 3.40), and two nonequivalent α -methylene protons (δ 5.42 and 3.34). The amino acid residues were established on the basis of COSY, TOCSY, HMQC, and HMBC data as leucine (Leu), isoleucine (Ile), proline (Pro), phenylalanine (Phe), *N*-methylphenylalanine (*N*MePhe), *N*-methyltyrosine (*N*MeTyr), and *N*-methylglycine (*N*MeGly). The amino acid sequence was established by analysis of HMBC data. HMBC correlations of the *N*MePhe α -H (δ 5.56) and NMe (δ 3.04) with the Leu CO (δ 174.2), along with that of the Leu NH (δ 8.20) with the Ile CO (δ 170.9), constructed the amide linkages of the

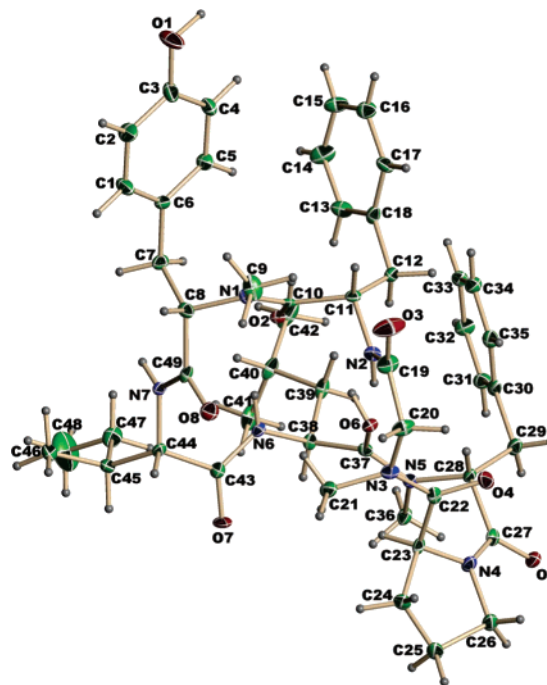


Figure 1. ORTEP view of **1**.

*N*MePhe *N*/Leu CO and the Leu *N*/Ile CO. The Ile *N* further linked with the *N*MeTyr CO according to HMBC cross-peaks of the Ile α -H (δ 4.45) and NH (δ 5.88) with the *N*MeTyr CO (δ 170.3). Additional HMBC correlations of the *N*MeTyr α -H (δ 3.40) and *N*Me (δ 2.61)/Phe CO (δ 170.4) and those of the Phe α -H (δ 5.36) and NH (δ 8.60)/*N*MeGly CO (δ 168.1) established the amide linkages of the *N*MeTyr *N* and Phe *N* with the CO groups of Phe and *N*MeGly, respectively. The *N*MeGly *N* was further connected with the Pro CO according to a ³*J* correlation between the *N*MeGly *N*Me (δ 2.91) and the Pro CO (δ 172.3). These results established a heptapeptide comprising *N*MePhe-Leu-Ile-*N*MeTyr-Phe-*N*MeGly-Pro. Compound **1** showed 21 degrees of unsaturation on the basis of the molecular formula. Since the heptapeptide has 20 degrees of unsaturation, **1** was identified as a cyclopeptide by formation of an amide bond between the Pro *N* and the *N*MePhe CO. An X-ray diffraction analysis (Figure 1) confirmed the assigned structure. The absolute configuration of the amino acid residues in **1** was addressed by HPLC analysis^{12,13} of the acid hydrolysate using a ligand-exchange-type chiral column (Sumichiral OA-5000). Co-injection using standard amino acids, L-Ile, D-Ile, L-Phe, and D-Phe, revealed that the hydrolysate of **1** contains L-Ile and L-Phe. Taken together with the relative configuration, established by X-ray crystallography, all amino acid residues, except for the *N*MePhe,

* To whom correspondence should be addressed. Tel: +66-74-288-435. Fax: +66-74-212-918. E-mail: vatcharin.r@psu.ac.th.

[†] Prince of Songkla University.

[‡] BIOTEC.

Table 1. ¹H and ¹³C NMR and Selected HMBC Data of Cordyheptapeptide A (1)

	δ_{H}	δ_{C}	HMBC		δ_{H}	δ_{C}	HMBC
NMePhe				NMeTyr			
α	5.56 (dd, 11.7, 4.5)	54.5	NMePhe: C=O, β -C, γ -C, N-Me Leu: C=O	α	3.40 (m)	69.2	NMeTyr: C=O Phe: C=O
β	3.33 (dd, 12.5, 11.7) 3.05 (dd, 12.5, 4.5)	35.2	NMePhe: α -C, γ -C, δ -C	β	3.14 (m) 2.72 (m)	32.4	NMeTyr: α -C, γ -C, δ -C
γ		136.8		γ		127.2	
δ	7.15 (m) ^a	129.7 ^b		δ	6.22 (d, 8.1)	129.8	
ϵ	7.35 (m) ^a	129.8 ^b		ϵ	6.53 (d, 8.1)	115.7	
ξ	7.15 (m) ^a	126.8		ξ		155.3	
NMe	3.04 (s)	30.1	NMePhe: α -C Leu: C=O	CO		170.3 ^c	
CO		168.4		NMe	2.61 (s)	40.4	NMeTyr: α -C Phe: C=O
Leu				Phe			
α	4.93 (t br, 10.8)	47.6		α	5.36 (m)	50.1	Phe: C=O NMeGly: C=O
β	1.39 (t br, 12.0) 0.12 (t br, 12.0)	39.9	Leu: δ -C, γ -Me	β	3.05 (dd, 12.3, 11.7) 2.82 (dd, 12.3, 3.0)	38.1	Phe: C=O, γ -C, δ -C
γ	1.55 (m)	24.8	Leu: δ -C	γ		137.3	
$\delta_{\text{A-Me}}$	0.91 (d, 6.3)	23.7	Leu: β -C, $\delta_{\text{B-Me}}$	δ	7.15 (m) ^a	130.1	
$\delta_{\text{B-Me}}$	0.84 (d, 6.9)	20.8	Leu: β -C, $\delta_{\text{A-Me}}$	ϵ	7.35 (m) ^a	129.8 ^b	
NH	8.20 (d, 9.6)		Ile: C=O	ξ	7.15 (m) ^a	126.8	
CO		174.2		CO		170.4 ^c	
Ile				NH	8.60 (d, 9.5)		NMeGly: C=O
α	4.45 (dd, 9.6, 2.7)	58.3	Ile: C=O, β -C, γ -C, β -Me NMeTyr: C=O	NMeGly			
β	2.33 (m)	35.4		α	5.42 (d, m) 3.34 (m)	50.8	NMeGly: C=O, NMe Pro: C=O
γ	1.35 (m) 1.00 (m)	24.2		NMe	2.91 (s)	35.5	Pro: C=O NMeGly: α -C
δ -Me	0.93 (t, 6.6)	12.1	Ile: γ -C	CO		168.1	
β -CH ₃	0.89 (d, 6.9)	16.3	Ile: α -C, β -C, γ -C NMeTyr: C=O	Pro			
NH	5.88 (d, 9.6)			α	4.39 (dd, 9.0, 2.4)	57.8	Pro: C=O, β -C, γ -C
CO		170.9		β	2.42 (m)	31.4	
				γ	2.03 (m) 1.85 (m)	22.0	
				δ	3.77 (m) 3.60 (m)	48.4	Pro: β -C, γ -C
				CO		172.3	

^{a,b,c} Chemical shifts with the same index may be interchanged.

had the L configuration. Thus, **1** was determined as *cyclo*-(D-NMePhe-L-Leu-L-Ile-L-NMeTyr-L-Phe-NMeGly-L-Pro).

Cordyheptapeptide A (**1**) exhibited antimalarial activity against *Plasmodium falciparum* K1 and cytotoxicity to Vero cell lines with 50% inhibitory concentration (IC₅₀) values of 5.35 and >56.88 μ M, respectively. The antimalarial and cytotoxic activities of known bioxanthracenes (**2–5**) were previously reported.⁹

Experimental Section

General Experimental Procedures. Ultraviolet spectra (UV) were measured with a UV-160A spectrophotometer (Shimadzu). Infrared spectra (IR) were obtained on a FTS 165 FT-IR spectrophotometer. ¹H and ¹³C NMR spectra were recorded on a Bruker Avance FT-NMR 300 MHz machine using tetramethylsilane (TMS) as internal standard. Optical rotations were measured at the sodium D-line (589 nm) on an automatic polarimeter (JASCO P-1020). EI and HREI mass spectra were measured on a Thermofinnigan MAT 95 XL spectrometer. Precoated thin-layer chromatography (TLC) was performed on silica gel 60 GF₂₅₄ (Merck). Column chromatography was done on silica gel (Merck) type 100 (70–230 mesh ASTM) eluted with a gradient system of MeOH/CH₂Cl₂, on Sephadex LH-20 with MeOH, or as otherwise stated. The solvents for extraction and chromatography were distilled at their boiling point ranges prior to use except for EtOAc, which was analytical grade reagent.

Fungal Material. *Cordyceps* sp. was collected on elaterid larva, identified and isolated by Dr. Nigel L. Hywel-Jones. This fungus was deposited at the Thailand BIOTEC Culture Collection as BCC 1788 on October 14, 1997.

Fermentation and Isolation. *Cordyceps* sp. BCC 1788 was maintained on potato dextrose agar at 25 °C, which was cut into pieces (1 × 1 cm), and inoculated into 2 × 250 mL Erlenmeyer flasks

containing 25 mL of Difco potato dextrose broth (PDB; composition, potato starch 4.0 g, dextrose 20.0 g, per liter) (10 pieces for each flask). After incubation at 25 °C for 6 days on a rotary shaker (200 rpm), each primary culture was transferred into a 1000 mL Erlenmeyer flask containing 250 mL of the same liquid medium (PDB) and incubated at 25 °C for 6 days on a rotary shaker (200 rpm). Each 25 mL portion of the secondary cultures (in 2 flasks) was transferred into 20 × 1000 mL Erlenmeyer flasks each containing 250 mL of PDB, and static fermentation was carried out at 25 °C for 20 days.

The cultures were separated by filtration into supernatant and mycelial cake. Wet mycelia was extracted with 0.5 L of MeOH, H₂O (25 mL) was added, and the mixture was washed with hexane (500 mL). The aqueous MeOH layer was separated and concentrated under reduced pressure. The residue was dissolved in EtOAc and washed with H₂O (100 mL). The organic layer was dried over MgSO₄ and concentrated under reduced pressure to obtain a brown gum (1.76 g). The crude mycelial extract was dissolved in MeOH to afford MeOH-soluble and MeOH-insoluble fractions. The MeOH-soluble fraction (1.40 g) was passed through a Sephadex LH-20 column to yield four fractions. Fraction 2 (36.9 mg) was further purified on a Sephadex LH-20 column, eluted with 50% MeOH/CH₂Cl₂, to obtain **1** (27.2 mg). Fraction 3 (186.9 mg) was subjected to silica gel column chromatography to afford three subfractions. The first subfraction (9.9 mg) was further separated by PLC with 20% EtOAc/petroleum ether to yield **4** (7.4 mg) and **5** (2.0 mg). The second subfraction (19.8 mg) was subjected to column chromatography over silica gel, eluted with 80% EtOAc/petroleum ether, to obtain **2** (7.1 mg). The last subfraction (24.0 mg) was fractionated on silica gel column chromatography to yield **3** (4.3 mg).

Cordyheptapeptide A (1): colorless crystals (MeOH/CH₂Cl₂); mp 179.6–179.8 °C; [α]_D²⁶ –68.5 (c 0.56, CHCl₃); UV (MeOH) λ_{max} (log ϵ) 214 (4.68), 279 (3.98), 350 (3.63); IR ν_{max} (neat) cm⁻¹ 3432, 1630;

^1H NMR (CDCl_3 , 300 MHz), see Table 1; ^{13}C NMR (CDCl_3 , 75 MHz), see Table 1; LR-EIMS m/z 879 $[\text{M}]^+$ (30), 878 (55), 150 (58), 134 (100), 86 (42), 40 (35); HR-EIMS m/z 879.4850 $[\text{M}]^+$ (calcd for $\text{C}_{49}\text{H}_{65}\text{N}_7\text{O}_8$, 879.4895).

X-ray Structure Determination of 1. X-ray diffraction data collection was carried out on a Bruker APEX CCD diffractometer. Crystal data of **1**: $[\text{C}_{49}\text{H}_{65}\text{N}_7\text{O}_8] \cdot 1.2\text{H}_2\text{O}$, MW 901.83, monoclinic, $P2_12_12_1$ (No. 19), $a = 12.1394(7)$ Å, $b = 17.7221(11)$ Å, $c = 23.0899(14)$ Å, $V = 4967.5(5)$ Å³, $D_x = 1.236$ g/cm³, $Z = 4$. A total of 35 960 reflections, of which 8760 were unique (6045 observed, $F > 4\sigma(F)$), were measured at 100 K from a $0.493 \times 0.263 \times 0.173$ mm³ colorless crystal using graphite-monochromated Mo K α ($\lambda = 0.71073$ Å) radiation on a Bruker APEX CCD diffractometer. The crystal structure was solved by direct methods using SHELXTL-NT, and all atoms except hydrogen and oxygen atoms of H_2O were refined anisotropically by full-matrix least-squares method on F using the XTAL3.7 program system to give a final R -factor of 0.10231 ($R_w = 0.12266$). The H atoms were localized by Fourier difference or calculated and constrained to ride on the respective parent atoms (except hydrogen atoms of H_2O , not included in the structure). Four oxygen atoms of H_2O were located by Fourier difference, were refined isotropically, and are disordered with the site occupancy of 0.45, 0.30, 0.25, and 0.20, respectively. Thus, the number of H_2O molecules in the structure is 1.2.

HPLC Analysis. A portion of compound **1** (1 mg) was hydrolyzed with 6 N HCl (0.6 mL) at 110–120 °C for 18 h. After concentration to dryness, the residue was dissolved in MeOH (100 μL) and subjected to HPLC analysis (column: Sumichiral OA-5000, 4.6×150 mm; mobile phase 20% MeOH in 2 mM aqueous CuSO_4 ; flow rate 1 mL/min; UV 238 nm). Co-injection using standard amino acids, L-Ile, D-Ile, L-Phe, and D-Phe, revealed that the peaks at t_R 13.5 and 33 min in the chromatogram of the hydrolysate were those of L-Ile and L-Phe, respectively.

Biological Assays. Antimalarial activity was evaluated against the parasite *Plasmodium falciparum* (K1, multidrug-resistant strain), using the microculture radioisotope technique based on the method described by Desjardins et al.¹⁴ The inhibitory concentration (IC_{50}) represents the concentration that causes 50% reduction in parasite growth as indicated by the in vitro uptake of [³H]-hypoxanthine by *P. falciparum*. An antimalarial drug, dihydroartemisinin, was used as positive control (IC_{50} 0.006 μM). Cytotoxic assay against African green monkey kidney fibroblast (Vero) cells was performed employing the calorimetric method as described by Skehan and co-workers.¹⁵ A standard compound, ellipticine, showed an IC_{50} value of 2.44 μM in the cytotoxicity assay.

Note Added after ASAP Publication. In the version of this paper that was published on the web Jan 6, 2006, there were two incorrect entries in column 8 of Table 1. The version that now appears is correct.

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- (16) Crystallographic data of compound **1** have been deposited at the Cambridge Crystallographic Data Center under the reference number CCDC 287376. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (e-mail: deposit@ccdc.cam.ac.uk).

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