An Antimycobacterial Cyclodepsipeptide from the Entomopathogenic Fungus *Ophiocordyceps communis* BCC 16475

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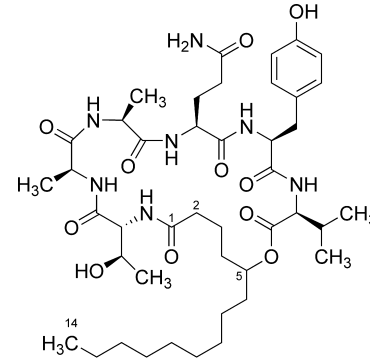
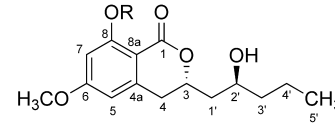
A novel cyclodepsipeptide, cordycommunin (**1**), and two dihydroisocoumarins (**2** and **3**) were isolated from the insect pathogenic fungus *Ophiocordyceps communis* BCC 16475. The absolute configurations of the amino acid residues of **1** were addressed by application of Marfey's method. Cordycommunin (**1**) showed growth inhibition of *Mycobacterium tuberculosis* H37Ra with an MIC value of 15 μM. This compound also exhibited weak cytotoxicity to KB cells with an IC_{50} of 45 μM, while it was inactive against BC, NCI-H187, and Vero cell lines at a concentration of 88 μM (50 μg/mL).

Insect pathogenic fungi belonging to the genus *Cordyceps* have been the source of a wide range of bioactive compounds.[1,2] Examples of unique secondary metabolites from this genus are cordycepin (3′-deoxyadenosine) from *C. militaris*,[3] cordypyridones (antimalarial *N*-hydroxypyridones) from *C. nipponica* BCC 1389,[4] cordyanhydrides (maleic anhydrides) from *C. pseudomilitaris* BCC 1620,[5] cordytropolone (a cytotoxic tropolone) from *Cordyceps* sp. BCC 1681,[6] and cicadapeptins (peptaibol-type peptides) from *C. heteropoda* ARSEF #1880.[7] Recently, reclassification of the mega genus *Cordyceps*, comprising over 400 species and previously classified in the family Clavicipitaceae (order Hypocreales), was proposed on the basis of a multigene phylogeny.[8] Most of the previously described *Cordyceps* species were reassigned and placed in two new families, Cordycipitaceae and Ophiocordycipitaceae. For example, the well-known Chinese traditional medicine *Cordyceps sinensis* was renamed as *Ophiocordyceps sinensis* (Ophiocordycipitaceae). In our research on novel bioactive metabolites of insect pathogenic fungi, we investigated the constituents of a recently described species, *Ophiocordyceps communis* Hywel-Jones & Samson,[8] strain BCC 16475, isolated from a termite (Isoptera), as extracts from a culture of this strain displayed a unique ^{1}H NMR spectroscopic profile. Chemical studies on BCC 16475 led to the isolation of a novel cyclodepsipeptide, cordycommunin (**1**), and two dihydroisocoumarin derivatives (**2** and **3**).



1



2 R = H
3 R = CH_3

Cordycommunin (**1**) was isolated as a colorless solid. The molecular formula of **1** was established by HRMS (ESI-TOF) as $C_{43}H_{69}N_7O_{11}$. The IR spectrum showed intense and broad absorption bands of amides at ν_{max} 3324 (NH) and 1655 (carbonyl) cm^{-1} and an ester band at 1731 cm^{-1}. The ^{1}H and ^{13}C NMR spectroscopic data in DMSO-d_6 were suggestive of a depsipeptide structure, showing eight carbonyl carbon signals in a range of δ_C 174.0−171.1, six amide NH protons, and ^{1}H and ^{13}C resonances of a linear alkyl chain. Six amino acid residues were assigned by interpretation of 2D NMR spectroscopic data. Thus, COSY and HMQC data indicated that six amide NHs that resonated at δ_H 8.40 (d, J = 7.0 Hz), 8.14 (d, J = 7.0 Hz), 8.00 (d, J = 5.2 Hz), 7.97 (d, J = 8.1 Hz), 7.66 (d, J = 8.1 Hz), and 7.44 (d, J = 8.3 Hz) were connected respectively to C-2 of amino acids at δ_C 59.8 (δ_H 3.82, t, J = 7.0 Hz), δ_C 49.1 (δ_H 4.17, m), δ_C 49.5 (δ_H 4.14, m), δ_C 53.5 (δ_H 4.01, m), δ_C 59.1 (δ_H 4.15, m), and δ_C 54.4 (δ_H 4.59, m). Further connections from the C-2 carbons to amino acid side chains were established on the basis of COSY and HMBC correlations. These amino acids were assigned respectively as valine (Val), alanine (Ala(1)), alanine (Ala(2)), glutamine (Gln), threonine (Thr), and tyrosine (Tyr). The ^{1}H NMR spectrum exhibited OH resonances (exchangeable with D_2O) of Tyr (δ_H 9.12, s) and Thr (δ_H 5.22, d, J = 5.0 Hz) and the carboxyamide NH_2 of Gln (δ_H 7.15, br s; and 6.72, br s; exchangeable with D_2O), which indicated that these functional groups are not involved in the peptide or ester linkages. The rest of the ^{1}H and ^{13}C resonances were assigned to those of a hydroxytetradecanoic acid on the basis of COSY and HMBC correlations. Thus, the diastereotopic methylene protons at δ_H 2.19 and 1.98 (H_2-2) showed HMBC correlations to the amide carbonyl (C-1), the unusually upfield shifted methylene carbon at δ_C 20.5 (C-3), and the downfield shifted methylene carbon at δ_C 33.1 (C-4). These chemical shifts strongly suggested the attachment of the oxymethine (δ_C 73.5, C-5) to C-4, and the other downfield shifted methylene at δ_C 34.0 should be assignable as C-6. Tulloch reported the ^{13}C NMR spectra of all the isomeric methyl hydroxy- and acetoxyoctadecanoates.[9] The carbon chemical shifts assigned for **1** (Table 1) showed excellent correlation to those of methyl 5-acetoxyoctadecanoate (δ_C C-2 33.73, C-3 20.71, C-4 33.41, C-5 73.66, C-6 34.02, C-7 25.28, C-8 29.49, C-9 29.51, C-10 29.55), but significantly differed from those of any other isomers such as methyl 6-acetoxyoctadecanoate (δ_C C-2 33.88, C-3 24.80, C-4 24.86, C-5 33.73, C-6 73.94, C-7 34.09, C-8 25.28, C-9 29.49, C-10 29.49) and methyl 7-acetoxyoctadecanoate (δ_C C-2 33.89, C-3

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Table 1. NMR Spectroscopic Data (500 MHz, DMSO-d_6) for Cordycommunin (**1**)

position	δ_C, mult,	δ_H, mult. (*J* in Hz)	position	δ_C, mult.	δ_H, mult. (*J* in Hz)	position	δ_C, mult.	δ_H, mult. (*J* in Hz)
	5-hydroxytetradecanoic acid		L-Tyr			L-Ala(1)		
1	173.1, qC		1	172.8, qC		1	172.2, qC	
2	35.3, CH_2	2.19, m; 1.98, m	2	54.4, CH	4.59, m	2	49.1, CH	4.17, m
3	20.5, CH_2	1.47−1.33,[b] m	3	38.2, CH_2	2.76, dd (13.6, 5.1)	3	17.8, CH_3	1.25, d (7.4)
4	33.1, CH_2	1.47−1.33,[b] m			2.66, dd (13.6, 9.6)	N*H*		8.14, d (7.0)
5	73.5, CH	4.80, m	4	127.9, qC		L-Ala(2)		
6	34.0, CH_2	1.47−1.33,[b] m	5, 9	130.6, CH	7.00, d (8.4)	1	172.9, qC	
7	25.3, CH_2	1.26−1.16,[c] m	6, 8	115.3, CH	6.59, d (8.4)	2	49.5, CH	4.14, m
8	29.4,[a] CH_2	1.26−1.16,[c] m	7	156.3, qC		3	17.4, CH_3	1.23, d (7.4)
9	29.3,[a] CH_2	1.26−1.16,[c] m	7-O*H*		9.12, s	N*H*		8.00, d (5.2)
10	29.2,[a] CH_2	1.26−1.16,[c] m	N*H*		7.44, d (8.3)	D-allo-Thr		
11	29.1,[a] CH_2	1.26−1.16,[c] m	L-Gln			1	171.1, qC	
12	31.7, CH_2	1.22,[c] m	1	171.2, qC		2	59.1, CH	4.15, m
13	22.5, CH_2	1.22,[c] m	2	53.5, CH	4.01, m	3	66.8, CH	3.98, m
14	14.4, CH_3	0.84, t (6.8)	3	27.4, CH_2	1.87, m; 1.72, m	4	20.4, CH_3	1.09, d (6.2)
L-Val			4	32.2, CH_2	2.02, m; 1.95, m	3-O*H*		5.22, d (5.0)
1	171.4, qC		*C*ONH_2	173.9, qC	7.15, br s; 6.72, br s	N*H*		7.66, d (8.1)
2	59.8, CH	3.82, t (7.0)	N*H*		7.97, d (8.1)			
3	29.7, CH	2.01, m						
4	19.5, CH_3	0.83, d (6.4)						
4′	19.0, CH_3	0.83, d (6.4)						
N*H*		8.40, d (7.0)						

[a] Carbon chemical shifts may be interchanged. [b] The proton resonances are overlapped. [c] The proton resonances are overlapped.

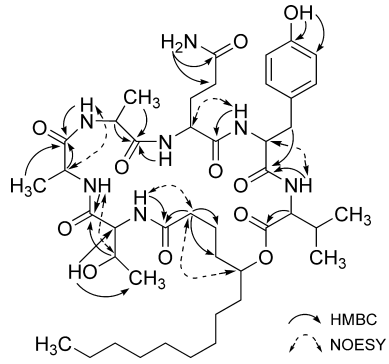


Figure 1. Selected HMBC and NOESY correlations for **1**.

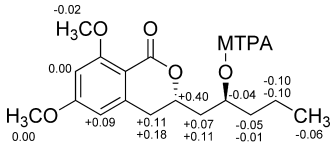
24.77, C-4 28.94, C-5 24.93, C-6 33.89, C-7 74.10, C-8 34.11, C-9 25.27, C-10 29.47). The downfield shift of the oxymethine proton H-5 (δ_H 4.80) suggested an ester linkage at this position.

The sequence of six amino acids and 5-hydroxytetradecanoic acid was addressed by analysis of HMBC correlations (Figure 1). Thus, amide protons of Thr, Ala(2), Ala(1), Gln, Tyr, and Val were correlated respectively to the carbonyl carbons of 5-hydroxytetradecanoic acid, Thr, Ala(2), Ala(1), Gln, and Tyr. This sequence was further supported by the NOESY correlations: NH of Thr and H-2 of 5-hydroxytetradecanoic acid; NH of Ala(2) and H-3 of Thr; NH of Ala(1) and H-2 of Ala(2); NH of Tyr to H-2 of Gln; and H-2 of Val to H-2 of Tyr. Although the key HMBC correlation from H-5 of 5-hydroxytetradecanoic acid to Val carbonyl (C-1) was not observed, the molecular formula of **1** (HRMS) required the ester linkage to form a cyclodepsipeptide.

The absolute configurations of the amino acid residues in **1** were determined by application of Marfey's method.[10,11] The hydrolysate of **1** was derivatized with N_α-(2,4-dinitro-5-fluorophenyl)-L-alaninamide (FDAA) and analyzed by HPLC using an ODS column. The HPLC chromatogram (UV 340 nm) of the FDAA-derivatized hydrolysate exhibited D-*allo*-Thr, L-Glu (derived from L-Gln), L-Ala × 2, L-Tyr, and L-Val. These peak assignments were confirmed by co-injections using Marfey derivatives of standard L- and D-amino acids (see Experimental Section). Attempts to isolate 5-hydroxytetradecanoic acid by acidic hydrolysis of **1** and the selective ester cleavage by basic hydrolysis or $LiBH_4$ reduction were all unsuccessful; therefore, the absolute configuration of the oxymethine (C-5) of 5-hydroxytetradecanoic acid remains undefined. The structure of **1** is related to the antifungal cyclodepsipeptides WA493 A and B, possessing a (3*S*,4*R*)-3-hydroxy-4-methyltetradecanoic acid residue, previously isolated from a *Fusarium* sp.[12] Interestingly, the six amino acid residues of WA493 A and the sequence are the same as those of **1**, but it contains three D-isomers (D-Tyr, D-Ala(1), and D-*allo*-Thr).

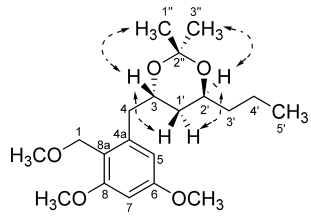
The molecular formula of compound **2** was determined by HRESIMS as $C_{15}H_{20}O_5$. Inspection of ^{1}H and ^{13}C NMR, DEPT135, and HMQC spectroscopic data revealed that **2** contained five sp^2 quaternary carbons resonating at δ_C 169.8, 165.9, 164.6, 141.1, and 110.3, a pair of *meta*-coupled sp^2 methines at δ_C 106.3 (δ_H 6.26, d, J = 2.3 Hz) and δ_C 99.5 (δ_H 6.37, d, J = 2.3 Hz), two oxygenated methines at δ_C 76.3 and 67.2, a methoxy group at δ_C 55.6, four methylenes at δ_C 42.4, 40.3, 33.8, and 18.7, and a methyl group at δ_C 14.1. The ^{1}H NMR spectroscopic data indicated the presence of a hydrogen-bonded OH (δ_H 11.21, s). A tetrasubstituted benzene was deduced from HMBC correlations from the hydrogen-bonded OH (8-O*H*) to C-7, C-8, and C-8a, from H-7 to C-5, C-6, C-8, and C-8a, from H-5 to C-7 and C-8a, and from the methoxy protons to C-6. HMBC correlations from diastereotopic methylene protons (H_2-4) at δ_H 2.93 and 2.88 to C-8a, C-4a, and C-5 indicated the connection of this methylene (C-4) to C-4a. The connection of C-4 to an oxymethine (δ_C 76.3, C-3) and a further linkage to a 2-hydroxypentyl group (C-1′−C-5′) was established on the basis of the COSY correlations. The hydrogen-bonded OH suggested the presence of a carbonyl (C-1, δ_C 169.8) attached to C-8a. Although a key HMBC correlation from H-3 to C-1 was not observed, the molecular formula (HRMS) required the δ-lactone form to constitute a 3,4-dihydroisocoumarin. This proposed structure was consistent with the downfield shift (δ_H 4.86) of H-3. The diastereotopic methylene protons (H_2-4) at δ_H 2.93 and 2.88 were vicinally coupled with H-3 with respective J values of 11.0 and 3.8 Hz, which indicated that the former proton was pseudoaxial and the latter was assignable to a pseudoequatorial position. Consequently, the gross structure of **2** was established to be 3,4-dihydro-8-hydroxy-3-(2-hydroxypentyl)-6-methoxyisocoumarin.

The ^{1}H and ^{13}C NMR spectra of compound **3** were similar to those of **2**. The significant differences were the absence of a hydrogen-bonded OH and, instead, the presence of two methoxy groups. The molecular formula of **3** was established as $C_{16}H_{22}O_5$ by HRESIMS. Detailed analysis of 2D NMR data, in a similar manner to that described above for compound **2**, confirmed that **3** is the 8-*O*-methylated analogue of **2**. Treatment of **2** with MeI and



4a (*S*)-MTPA ester
4b (*R*)-MTPA ester

Figure 2. $\Delta\delta$-Values ($\delta_S - \delta_R$) of the Mosher esters **4a** and **4b**.



5

Figure 3. Selected NOESY correlations for the acetonide derivative **5**.

K_2CO_3 in DMF gave a methylated analogue, whose ^{1}H NMR and MS data were identical to those of **3**.

Compound **2** has stereogenic centers at C-3 and C-2′. The absolute configuration of C-2′ was addressed by application of the modified Mosher method.[13,14] Compound **3**, obtained by methylation of **2**, was reacted with (−)-(*R*)-MTPACl and (+)-(*S*)-MTPACl in pyridine to afford (*S*)-MTPA ester **4a** and (*R*)-MTPA ester **4b**, respectively. The $\Delta\delta$ ($\delta_S - \delta_R$) values of the Mosher esters indicated the 2′*S* configuration (Figure 2). The reaction of **3** with $LiAlH_4$ in THF followed by treatment with *p*-TsOH in 2,2-dimethoxypropane gave the acetonide derivative **5** as a major product. It should be noted that the benzylic position, corresponding to the lactone carbonyl (C-1) of **3**, formed a methyl ether. The incorporation of the methoxy group should have occurred in the second step. One of the methylene protons in the 1,3-dioxane at δ_H 2.88 (Ha-1′) was coupled with H-3 and H-2′ with respective *J* values of 9.5 and 6.5 Hz, which indicated that the acetonide ring was not adopting a chair conformation. Key NOESY correlations for **5** (Figure 3) indicated an *anti* relation. On the basis of these results, the 3*S*,2′*S* configuration of compound **2** was unambiguously established.

Dihydroisocoumarins **2** and **3** are 7-desmethoxy analogues of fusarentin methyl ethers, which also possess a 3*S*,2′*S* configuration.[15] Simpson and co-workers reported[16] the synthesis of fusarentin methyl ethers and their 7-desmethoxy analogues (compounds **2** and **3**); however, NMR spectroscopic data and other physicochemical data of the synthetic samples of **2** and **3** have not been presented. This current report represents the first isolation of **2** and **3** as natural products.

Cordycommunin (**1**) exhibited activity against *Mycobacterium tuberculosis* H37Ra with an MIC of 15 μM, but it was inactive in an antimalarial (*Plasmodium falciparum* K1) and antifungal (*Candida albicans* and *Magnaporthe grisea*) assays. This compound also showed weak cytotoxicity to KB (human oral cavity carcinoma) cells with an IC_{50} of 45 μM, but it was inactive against MCF-7 (human breast cancer) and NCI-H187 (human small-cell lung cancer) cell lines and noncancerous Vero cells (African green monkey kidney fibroblasts) at a concentration of 88 μM (50 μg/mL). Compound **2** displayed weak cytotoxicity against MCF-7 cells with an IC_{50} of 62 μM, while it was inactive against other cell lines at 178 μM (50 μg/mL). Compound **3** was inactive in these assays. WA493 A and B are reported to exhibit antifungal activity against *Venturia inaequalis*, *Molinia mali*, and *Cochlibolus miyabeanus* (MIC 1−10 μg/mL).[12]

Experimental Section

General Experimental Procedures. Melting points were measured with an Electrothermal IA9100 digital melting point apparatus. Optical rotations were measured with a JASCO P-1030 digital polarimeter. UV spectra were recorded on a GBS Cintra 404 spectrophotometer. FTIR spectra were taken on a Bruker VECTOR 22 spectrometer. NMR spectra were recorded on Bruker DRX400 and AV500D spectrometers. ESI-TOF mass spectra were measured with Micromass LCT and Bruker micrOTOF mass spectrometers.

Fungal Material. The fungus used in this study was isolated from a termite (*Isoptera*) in Khao Yai National Park, Nakhon Nayok Province, Thailand, by one of the authors (K.T.), and a living culture was deposited in the BIOTEC Culture Collection as BCC 16475 on October 7, 2004. This strain was originally recorded as a *Cordyceps* sp. and was later renamed as *Ophiocordyceps communis* by Dr. Nigel L. Hywel-Jones, BIOTEC.[8]

Fermentation and Isolation. The fungus BCC 16475 was maintained on potato dextrose agar at 25 °C. The agar was cut into small plugs and inoculated into 4 × 250 mL Erlenmeyer flasks containing 25 mL of potato dextrose broth (PDB; potato starch 4.0 g, dextrose 20.0 g, per liter). After incubation at 25 °C for 7 days on a rotary shaker (200 rpm), each primary culture was transferred into a 1 L Erlenmeyer flask containing 250 mL of the same liquid medium (PDB) and incubated at 25 °C for 7 days on a rotary shaker (200 rpm). Each 25 mL portion of the secondary cultures (from 4 flasks) was transferred into 40 × 1 L Erlenmeyer flasks each containing 250 mL of PDB, and final fermentation was carried out at 25 °C for 109 days under static conditions. The culture was filtered to separate the residue (mycelia) and the filtrate (broth). The broth was extracted with EtOAc (4 × 10 L) and concentrated to a brown gum (6.0 g; extract A). The mycelium was macerated in MeOH (1.5 L, rt, 3 days) and filtered. The filtrate was partially concentrated to ca. 700 mL and then defatted with hexane (2 × 500 mL). The MeOH phase was evaporated, and the residue was diluted with EtOAc (2.4 L), washed with H_2O (100 mL), and concentrated under reduced pressure to leave a brown gum (640 g, extract B). Extract A was subjected to Sephadex LH-20 column chromatography (4.5 × 60 cm, MeOH) to obtain 10 fractions. Fraction 4 (2.18 g) was repeatedly chromatographed on Si gel using a step gradient elution with hexane/EtOAc (from 100:0 to 60:40) and then with CH_2Cl_2/MeOH (from 100:0 to 70:30) to afford **2** (88 mg) and a fraction containing **3** (33 mg). The latter fraction was purified by preparative HPLC using a reversed-phase column (Phenomenex Luna 10u C18(2) 100A, 21.2 × 250 mm, 10 μm; mobile phase MeCN/H_2O, 25:75, flow rate 8 mL/min) to furnish **3** (1.6 mg, t_R 8 min). Extract B was passed through a column on Sephadex LH-20 (4.5 × 60 cm), eluting with MeOH, to obtain nine fractions. Fraction 2 (214 mg) was subjected to column chromatography on Si gel (3.0 × 15 cm) using CH_2Cl_2/MeOH as eluent (step gradient from 100:0 to 60:40) and further purified by preparative HPLC (MeOH/H_2O, 35:65) to furnish **1** (8.1 mg, t_R 30 min).

Cordycommunin (1): colorless solid; mp 152−153 °C; $[\alpha]^{25}_D$ −26 (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ε) 225 (4.01) nm; IR (KBr, disk) ν_{max} 3324, 1731, 1655 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m*/*z* 860.5128 $[M + H]^+$ (calcd for $C_{43}H_{70}N_7O_{11}$, 860.5133).

(3,4-Dihydro-8-hydroxy-3-(2-hydroxypentyl)-6-methoxyisocoumarin (2): colorless solid; mp 62−63 °C; $[\alpha]^{26}_D$ −14 (*c* 0.13, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 216 (3.83), 266 (3.71), 302 (3.38) nm; IR (KBr, disk) ν_{max} 3443, 1661, 1628 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 11.21 (1H, s, 8-O*H*), 6.37 (1H, d, *J* = 2.3 Hz, H-7), 6.26 (1H, d, *J* = 2.3 Hz, H-5), 4.86 (1H, m, H-3), 4.07 (1H, m, H-2′), 3.83 (3H, s, 6-OCH_3), 2.93 (1H, dd, *J* = 16.3, 11.0 Hz, Ha-4), 2.88 (1H, dd, *J* = 16.3, 3.8 Hz, Hb-4), 1.97 (1H, ddd, *J* = 14.5, 9.7, 2.2 Hz, Ha-1′), 1.88 (1H, br d, *J* = 4.8 Hz, 2′-O*H*), 1.71 (1H, ddd, *J* = 14.5, 10.3, 2.9 Hz, Hb-1′), 1.52−1.47 (3H, m, Ha-3′, Hb-3′, and Ha-4′), 1.40 (1H, m, Hb-4′), 0.96 (3H, t, *J* = 6.9 Hz, H-5′); ^{13}C NMR ($CDCl_3$, 125 MHz) 169.8 (C, C-1), 165.9 (C, C-6), 164.6 (C, C-8), 141.1 (C, C-4a), 106.3 (CH, C-5), 101.8 (C, C-8a), 99.5 (CH, C-7), 76.3 (CH, C-3), 67.2 (CH, C-2′), 55.6 (CH_3, 6-OCH_3), 42.4 (CH_2, C-1′), 40.3 (CH_2, C-3′), 33.8 (CH_2, C-4), 18.7 (CH_2, C-4′), 14.1 (CH_3, C-5′); HRESIMS *m*/*z* 281.1387 $[M + H]^+$ (calcd for $C_{15}H_{21}O_5$, 281.1384).

3,4-Dihydro-6,8-dimethoxxy-3-(2-hydroxypentyl)isocoumarin (3): colorless, amorphous solid; $[\alpha]^{26}_D$ −45 (*c* 0.13, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 214 (4.22), 263 (3.97), 298 (3.66) nm; IR (KBr, disk) ν_{max} 3442, 1702, 1605 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) δ 6.54 (1H,

d, $J = 2.2$ Hz, H-7), 6.47 (1H, d, $J = 2.2$ Hz, H-5), 4.58 (1H, m, H-3), 3.88 (3H, s, 6-OCH_3), 3.87 (1H, m, H-2′), 3.86 (3H, s, 8-OCH_3), 3.78 (1H, d, $J = 5.5$ Hz, 2′-O*H*), 2.90−2.84 (2H, m, Ha-4 and Hb-4), 1.82 (1H, ddd, $J = 14.1$, 9.5, 2.3 Hz, Ha-1′), 1.61 (1H, ddd, $J = 14.1$, 10.1, 3.1 Hz, Hb-1′), 1.48 (1H, m, Ha-4′), 1.45−1.39 (3H, m, Ha-3′, Hb-3′, and Hb-4′), 0.91 (3H, t, $J = 7.0$ Hz, H-5′); ^{13}C NMR (acetone-d_6, 125 MHz) 164.3 (C, C-8), 163.0 (C, C-6), 160.7 (C, C-1), 144.4 (C, C-4a), 107.2 (C, C-8a), 104.1 (CH, C-5), 97.7 (CH, C-7), 74.1 (CH, C-3), 66.1 (CH, C-2′), 55.3 (CH_3, 8-OCH_3), 55.1 (CH_3, 6-OCH_3), 42.8 (CH_2, C-1′), 40.4 (CH_2, C-3′), 35.3 (CH_2, C-4), 18.6 (CH_2, C-4′), 13.5 (CH_3, C-5′); HRESIMS *m/z* 317.1361 $[M + Na]^+$ (calcd for $C_{16}H_{22}O_5Na$, 317.1359).

Preparation and Analysis of Marfey Derivatives. Cordycommunin (**1**, 1.0 mg) was hydrolyzed by heating in 6 M HCl (1 mL) at 110 °C for 15 h. After cooling, the solution was evaporated and dried in vacuo, and redissolved in H_2O (70 μL). To the mixture were added FDAA (Marfey's reagent, N_α-(2,4-dinitro-5-fluorophenyl)-L-alaninamide, 0.5 mg) in acetone (100 μL) and 1 M $NaHCO_3$ solution (20 μL), and the mixture was incubated at 40 °C for 1 h. The reaction was quenched by addition of 1 M HCl (20 μL), and the resulting homogeneous solution was diluted with MeOH (0.3 mL) and subjected to HPLC analysis. Standard L- and D-amino acids were also derivatized with FDAA in the same manner as that of the hydrolysate of **1**. HPLC analysis was performed with the following conditions: NovaPak C_{18} (3.9 × 150 mm, 4 μm), mobile phase MeCN/(0.05% TFA in H_2O), 25:75, flow rate 0.5 mL/min, UV detection at 340 nm. The glutamine (Gln) residue of **1** should be converted by acid hydrolysis to glutamic acid (Glu). Retention times (min) of the FDAA amino acid derivatives used as standards were as follows: L-Thr (6.5), D-Thr (8.6), L-Glu (7.4), D-Glu (11.8), L-Ala (10.4), D-Ala (15.0), L-Tyr (15.6), D-Tyr (21.6), L-Val (25.0), and D-Val (52.4). The HPLC chromatogram of the derivatized hydrolyzsate of **1** contained peaks of L-Glu, L-Ala × 2, L-Tyr, and L-Val; however, the remaining lowest retention time peak of a Thr isomer, eluted before L-Glu, was different from those of L-Thr or D-Thr. Assignment of this remaining residue was later examined using four possible threonine isomers as standard samples. The retention times of the standard Marfey derivatives using the same elution solvent system were larger than the earlier experiments: L-Thr (9.3), D-Thr (14.5), L-*allo*-Thr (9.3), D-*allo*-Thr (11.1). The earliest eluting peak of the derivatized hydrolysate of **1** was assigned as that of D-*allo*-Thr, which was confirmed by co-injection.

Methylation of 2. To a solution of **2** (5.0 mg) in DMF (0.2 mL) were added MeI (20 μL) and K_2CO_3 (20 mg), and the mixture was stirred at room temperature for 16 h. The mixture was diluted with EtOAc and washed with H_2O, and the organic layer was concentrated under reduced pressure to obtain a methylated product (4.3 mg), whose ^{1}H NMR and MS data were identical to those of **3**.

Preparation of Mosher Esters of 3. Compound **3** (2.0 mg, prepared by methylation of **2**) was treated with (−)-(*R*)-MTPACl (20 μL) in pyridine (0.2 mL) at room temperature for 16 h. The mixture was diluted with EtOAc and washed with H_2O, and the organic layer was concentrated under reduced pressure to furnish (*S*)-MTPA ester **4a**. Similarly, (*R*)-MTPA ester **4b** was prepared from **3** and (+)-(*S*)-MTPACl.

(*S*)-MTPA Ester 4a: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.48 (2H, br d, $J = 7.4$ Hz, phenyl of MTPA), 7.35−7.28 (3H, m, phenyl of MTPA), 6.41 (1H, d, $J = 2.1$ Hz, H-7), 6.24 (1H, br s), 5.36 (1H, m, H-2′), 4.24 (1H, m, H-3), 3.94 (3H, s, 8-OCH_3), 3.85 (3H, s, 6-OCH_3), 3.47 (3H, br s, −OCH_3 of MTPA), 2.86 (1H, dd, $J = 15.8$, 11.7 Hz, Ha-4), 2.68 (1H, dd, $J = 15.8$, 2.7 Hz,. Hb-4), 2.08 (1H, m, Ha-1′), 1.93 (1H, m, Hb-1′), 1.71 (1H, m, Ha-3′), 1.64 (1H, m, Hb-3′), 1.30 (1H, m, Ha-4′), 1.27 (1H, m, Hb-4′), 0.89 (3H, t, $J = 7.3$ Hz, H-5′); HRESIMS *m/z* 533.1755 $[M + Na]^+$ (calcd for $C_{26}H_{29}F_3O_7Na$, 533.1758).

(*R*)-MTPA Ester 4b: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.48 (2H, br d, $J = 7.9$ Hz, phenyl of MTPA), 7.25 (2H, m, phenyl of MTPA), 7.11 (1H, t, $J = 7.4$ Hz, phenyl of MTPA), 6.41 (1H, d, $J = 2.1$ Hz, H-7), 6.15 (1H, br s), 5.40 (1H, m, H-2′), 3.94 (3H, s, 8-OCH_3), 3.85 (3H, s, 6-OCH_3), 3.84 (1H, m, H-3), 3.59 (3H, br s, −OCH_3 of MTPA), 2.75 (1H, dd, $J = 15.8$, 11.9 Hz, Ha-4), 2.50 (1H, dd, $J = 15.8$, 2.5 Hz,. Hb-4), 2.01 (1H, m, Ha-1′), 1.82 (1H, m, Hb-1′), 1.76 (1H, m, Ha-3′), 1.65 (1H, m, Hb-3′), 1.40 (1H, m, Ha-4′), 1.37 (1H, m, Hb-4′), 0.95 (3H, t, $J = 7.3$ Hz, H-5′); HRESIMS *m/z* 533.1759 $[M + Na]^+$ (calcd for $C_{26}H_{29}F_3O_7Na$, 533.1758).

Synthesis of Acetonide Derivative 5. To a solution of **3** (2.0 mg; prepared from **2** using the same procedure as described above) in THF (0.3 mL) on an ice−water bath was added $LiAlH_4$ (2 mg), and the mixture was stirred for 30 min. The reaction was terminated by addition of H_2O (1 mL) and extracted with EtOAc (3 × 2 mL). The combined organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. To the residue was added 2,2-dimethoxypropane (0.3 mL) and *p*-TsOH·H_2O (ca. 1 mg), and the mixture was stirred at room temperature for 3 h. The reaction was quenched by addition of 1 M aqueous $NaHCO_3$ (1 mL) and extracted twice with EtOAc. The combined organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was subjected to preparative HPLC (MeCN/H_2O, 65:35) to furnish an acetonide derivative **5** (0.8 mg).

Acetonide Derivative 5: colorless gum; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.43 (1H, d, $J = 2.3$ Hz, H-5), 6.34 (1H, d, $J = 2.3$ Hz, H-7), 4.48−4.47 (2H, m, H-1), 4.09 (1H, dq, $J = 9.5$, 6.4 Hz, H-3), 3.80 (3H, s, 8-OCH_3), 3.79 (3H, s, 6-OCH_3), 3.74 (1H, m, H-2′), 3.35 (3H, s, 1-OCH_3), 2.96 (1H, dd, $J = 14.2$, 6.9 Hz, Ha-4), 2.75 (1H, dd, $J = 14.2$, 6.4 Hz, Hb-4), 1.69 (1H, ddd, $J = 12.8$, 9.5, 6.1 Hz, Ha-1′), 1.53 (1H, m, Hb-1′), 1.45−1.38 (2H, m, H-3′), 1.36 (3H, s, H-1″), 1.35−1.30 (2H, m, H-4′), 1.30 (3H, s, H-3″), 0.91 (3H, t, $J = 7.1$ Hz, H-5′); ESIMS *m/z* 375.21 $[M + Na]^+$.

Biological Assays. Growth inhibitory activity against *Mycobacterium tuberculosis* H37Ra and cytotoxicity to Vero cells were assessed using the green fluorescent protein microplate assay (GFPMA).[17] The MIC values of the standard antituberculosis compounds, isoniazid and rifampicin, were 0.17−0.34 and 0.036−0.015 μM, respectively. Cytotoxicity against cancer cell lines (KB, MCF-7, and NCI-H187) was evaluated using the resazurin microplate assay.[18] The IC_{50} values of the standard compound doxorubicin hydrochloride were 0.25 μM for KB cells, 1.6 μM for MCF-7 cells, and 0.10 μM for NCI-H187 cells.

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Supporting Information Available: NMR spectra of **1**−**3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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