New Diphenyl Ethers from the Insect Pathogenic Fungus *Cordyceps* sp. BCC 1861

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Two novel diphenyl ether glycosides and a new diphenyl ether, cordyol A—C (1—3), were isolated from the insect pathogenic fungus *Cordyceps* sp. BCC 1861. Structures of these compounds were elucidated by NMR and MS spectral analyses. Cordyol C (3) exhibited significant anti-HSV-1 activity with an IC_{50} value of 1.3 μg/ml, and cytotoxic activity against BC and NCI-H187 cancer cell lines with IC_{50} values of 8.65 and 3.72 μg/ml, respectively. Cordyol A (1) displayed weak antimycobacterial activity with a MIC value of 100 μg/ml.

Key words *Cordyceps* sp.; diphenyl ether; phenyl ether glycoside

Insect pathogenic fungi in the genus *Cordyceps* are known as potential sources for secondary metabolites with diverse chemical structures and biological activities,[1] for example, antimalarial cordypyridones from *Cordyceps nipponica*[2]; an antioxidant polysaccharide,[3] antitumor sterols[4] and diketopiperazines[5] from *Cordyceps sinensis*; a bioactive cyclic peptide (cordyheptapeptide A)[6] and a tropolone (cordytropolone)[7] from *Cordyceps* species; an antiviral deoxynucleoside, cordycepin,[8,9] and 10-membered lactones[10] from *Cordyceps militaris*. As part of our ongoing research program on bioactive constituents of insect pathogenic fungi,[1] we describe herein the isolation of two novel diphenyl ether glycosides, cordyols A (**1**) and B (**2**), a new diphenyl ether, cordyol C (**3**), together with three known diphenyl ethers, diorcinol (**4**),[11,12] violaceol-I (**5**) and violaceol-II (**6**),[13,14] from *Cordyceps* sp. BCC 1861.

Results and Discussion

The culture broth extract of *Cordyceps* sp. BCC 1861 was subjected to Sephadex LH-20 and silica gel column chromatography in order to isolate compound **1**. The mycelial extract was purified by Sephadex LH-20 chromatography to obtain 10 fractions. Fraction 6 was chromatographed on a silica gel column to yield compound **2**. Fraction 8 was separated by silica gel column chromatography to afford pure compounds **3**, **4**, and a mixture of **5** and **6**.

Cordyol A (**1**) has a molecular formula of $C_{21}H_{26}O_8$ as established by HR-MS (ESI-TOF). The IR spectrum of **1** showed broad absorption bands at 3389 and 3300 cm^{-1} (OH). The ^{1}H-, ^{13}C- and 2D-NMR data (including COSY, NOESY, HMQC, and HMBC) indicated that this compound possessed 4-*O*-methyl-β-glucopyranose unit attached to a 3,3′-dihydroxy-5,5′-dimethyl diphenyl ether (diorcinol).[11,12] The aglycone composed of six aromatic methine carbons, six aromatic quaternary carbons and two methyl carbons as observed from ^{13}C-NMR/DEPT spectra. The ^{1}H–^{1}H COSY cross signals were detected between H-2/H-4, H-2/H-6, H-4/H-7, H-6/H-7, H-2′/H-4′, H-2′/H-6′, H-4′/H-7′, and H-6′/H-7′. The connectivities between protons and carbons were confirmed by HMBC data in which correlations were observed from H-2 to C-3, C-6; H-4 to C-2, C-3, C-6, C-7; H-6 to C-1, C-2, C-4, C-7; H-7 to C-4, C-5, C-6; H-4′ to C-2′; H-6′ to C-4′; and H-7′ to C-4′, C-5′, C-6′ (Fig. 2). For the sugar moiety, five methine carbons, a methylene carbon and a methoxy carbon were detected from ^{13}C-NMR/DEPT spectra. The connectivities from H-1″ to H-6″ were determined from ^{1}H-NMR, COSY, and HMBC data. The vicinal coupling constants ($J_{1'',2''}$=7.9 Hz, $J_{2'',3''}$=9.2 Hz, $J_{3'',4''}$=9.1 Hz, $J_{4'',5''}$=9.6 Hz) revealed that H-1″ to H-5″ were all oriented in the axial positions of a pyranose ring. The attach-

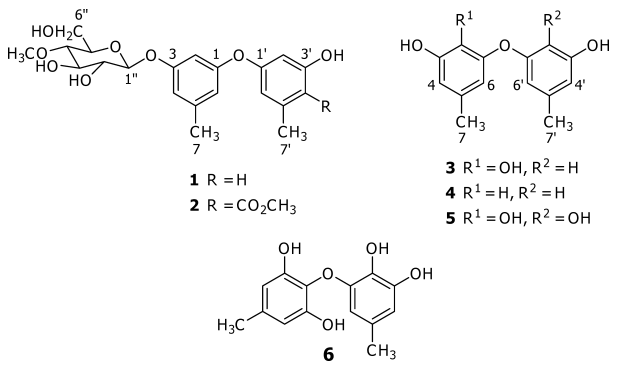

Fig. 1. Structures of Compounds **1**—**6** from BCC 1861

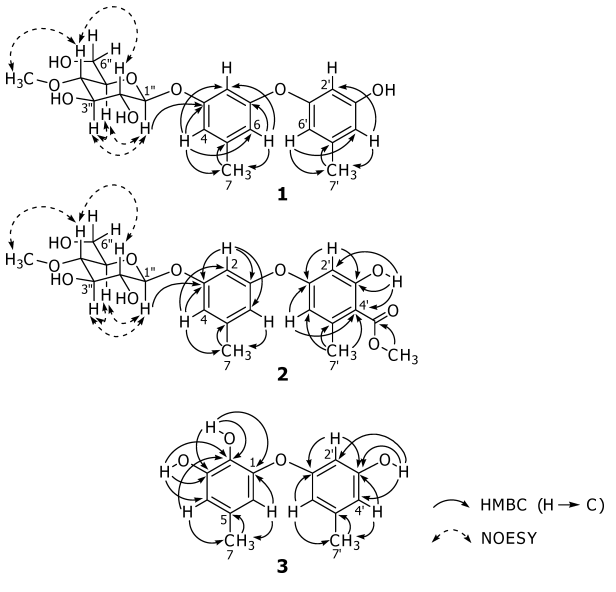

Fig. 2. Selected NOESY and HMBC Correlations for Compounds **1**—**3**

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Table 1. ^{1}H- and ^{13}C-NMR Data for **1** in Acetone-d_6–D_2O (88 : 12) and **2** in $CDCl_3$

	1		**2**	
	δ_H (mult., J in Hz)	δ_C	δ_H (mult., J in Hz)	δ_C
Diphenyl ether				
1		158.1		155.8
2	6.45 (1H, dd, 2.1, 1.8)	104.2	6.56 (1H, br s)	106.2
3		158.7		157.8
4	6.63 (1H, br s)	111.9	6.68 (1H, br s)	113.6
5		140.6		141.3
6	6.40 (1H, br s)	112.8	6.60 (1H, br s)	115.7
7	2.21 (3H, s)	20.7	2.33 (3H, s)	21.7
1′		158.6[c]		162.2
2′	6.25 (1H, m)[a]	103.5	6.34 (1H, d, 2.4)	103.2
3′		157.8[c]		165.1
3′-OH			11.69 (1H, s)	
4′	6. 24 (1H, m)[a,b]	110.6[d]		107.2
5′		140.5		143.8
6′	6.42 (1H, br s)[b]	111.5[d]	6.39 (1H, d, 2.3)	113.0
7′	2.17 (3H, s)	20.6	2.52 (3H, s)	24.3
C=O				172.0
OCH_3			3.96 (3H, s)	52.1
4-*O*-Methyl-β-glucopyranose				
1″	4.86 (1H, d, 7.9)	100.5	4.93 (1H, d, 7.7)	100.1
2″	3.41 (1H, dd, 9.2, 7.9)	73.6	3.65 (1H, dd, 9.2, 7.8)	73.7
3″	3.58 (1H, t, 9.1)	76.5	3.74 (1H, t, 9.1)	76.3
4″	3.19 (1H, dd, 9.6, 9.1)	79.1	3.33 (1H, dd, 9.3, 9.1)	78.8
4″-OCH_3	3.52 (3H, s)	59.8	3.63 (3H, s)	60.9
5″	3.40 (1H, ddd, 9.5, 4.6, 2.1)	75.8	3.45 (1H, ddd, 9.6, 4.1, 2.7)	75.6
6″	3.75 (1H, dd, 12.1, 2.1), 3.64 (1H, dd, 12.1, 4.7)	60.7	3.93 (1H, dd, 12.2, 2.5), 3.78 (1H, dd, 12.2, 4.3)	61.9

a) The proton signals were overlapped. *b*) Assignment of protons may be interchangeable. *c*, *d*) Assignment of carbons may be interchangeable.

ment of a methoxyl at C-4″ was assigned from HMBC spectrum in which correlations were observed from methoxyl protons (δ_H 3.52, 3H, s) to C-4″ (δ_C 79.1) and from H-4″ (δ_H 3.19, 1H, dd, J=9.6, 9.1 Hz) to methoxyl carbon (δ_C 59.8). These results indicated that the sugar unit was 4-*O*-methyl-β-glucopyranose. The connectivity between 4-*O*-methyl-β-glucopyranose moiety and aglycone was deduced by HMBC correlation from H-1″ (δ_H 4.86, 1H, d, J=7.9 Hz) to C-3 (δ_C 158.7). The assignment of D-configuration of 4-*O*-methylglucopyranose unit was performed by comparing the specific optical rotation of the aqueous layer of its acid hydrolysate ($[\alpha]_D^{25}$ +74.9°, c=1.0, MeOH) with $[\alpha]_D^{20}$ +80° (c=1.3, MeOH) of 4-*O*-methyl-D-glucopyranose reported in the literature.[15] The aglycone, obtained by acid hydrolysis of **1**, was spectroscopically identical to diorcinol (**4**),[11,12] which was also isolated as a co-metabolite from the fungus BCC 1861.

Cordyol B (**2**) was isolated as an off-white solid with a molecular formula of $C_{23}H_{28}O_{10}$ as established by HR-MS (ESI-TOF). The IR spectrum showed absorption bands at 3443 cm^{-1} (OH) and 1655 cm^{-1} (C=O). Proton and carbon signals in aglycone moiety of compound **2** were similar to those of compound **1** except for the lack of H-4′ signal and the presence of additional methoxycarbonyl signals in **2**. The downfield shift of hydroxyl proton (3′-O<u>H</u>, δ_H 11.69) indicated the presence of hydrogen bonding with the methoxycarbonyl group (δ_C 172.0, C=O; δ_C 52.1 and δ_H 3.96, OCH_3). The ^{1}H-, ^{13}C- and 2D-NMR data (including COSY, NOESY, HMQC, and HMBC) suggested that compound **2** possessed the same sugar unit (4-*O*-methyl-β-glucopyranose) as **1**. However, acid hydrolysis of compound **2** was not performed due to its small quantity. Selected NOESY cross signals and HMBC correlations for compound **2** are shown in Fig. 2.

Cordyol C (**3**) has a molecular formula of $C_{14}H_{14}O_4$ as established by HR-MS (ESI-TOF). The IR spectrum of **3** showed a broad absorption band at 3384 cm^{-1} (OH). The ^{1}H-, ^{13}C- and 2D-NMR data (including COSY, NOESY, HMQC, and HMBC) indicated that this compound consisted of 3,4,5-trihydroxytoluene unit connected to 3,5-dihydroxytoluene residue. Aromatic carbon signals at δ_C 159.3, 101.3, 158.4 and 140.0 were assigned to C-1′, C-2′, C-3′ and C-5′ in the dihydroxytoluene residue. With additional hydroxyl group, aromatic carbons in the trihydroxytoluene unit were observed at δ_C 143.4, 134.9, 146.5 and 128.7 (C-1, C-2, C-3 and C-5, respectively). Chemical shifts of the remaining protons and carbons at positions 4′, 6′ and 7′ were close to those of positions 4, 6 and 7, respectively. Selected NOESY cross signals and HMBC correlations of this compound are presented in Fig. 2.

Three known compounds, diorcinol (**4**),[11,12] violaceol-I (**5**) and violaceol-II (**6**),[13,14] were also isolated from *Cordyceps* sp. BCC 1861. Compounds **5** and **6** with the same molecular formula, $C_{14}H_{14}O_5$, were isolated as an inseparable mixture from this fungus. These two isomers were also reported as an inseparable mixture from *Emericella violacea*[13] and as single compounds from spore-derived lichen mycobionts of *Graphis scripta* var. *serpentina* and *G. rikuzensis*.[14] Interestingly, it was described in the latter report that, after standing of pure compound **5** in MeOH, the isomerization took place to yield a mixture of compounds **5** and **6** in a ratio of 18 : 11.[14]

All isolated compounds were tested for biological activi-

Table 2. Biological Activities of Compounds **1**—**6**

Compound	Anti-malaria[a] IC_{50} (μg/ml)	Anti-TB[b] MIC (μg/ml)	Anti-HSV-1[c] IC_{50} (μg/ml)	Cytotoxicity,[d] IC_{50} (μg/ml)			
				KB-cells	BC-cells	NCI-H187 cells	Vero cells
1	>10	100	>50	>20	>20	>20	>50
2	>10	>200	>50	>20	>20	>20	>50
3	>10	200	1.3	>20	8.65	3.72	13.1
4	>10	50	>50	>20	13.46	>20	18.6
5/**6**	3.38	200	>50	6.36	5.50	3.70	1.3

a) *In vitro* antimalarial activity against *Plasmodium falciparum* K1. *b*) *In vitro* antituberculous activity against *Mycobacterium tuberculosis* H_{37}Ra. *c*) *In vitro* anti-herpes simplex virus type 1 activity. *d*) Cytotoxic activities against human oral epidermoid carcinoma (KB), human breast cancer (BC), human small-cell lung cancer (NCI-H187), and African green monkey kidney fibroblast (Vero) cells.

ties (Table 2). Cordyol C (**3**) exhibited significant anti-HSV-1 activity with an IC_{50} value of 1.3 μg/ml, and it also showed moderate cytotoxic activity against BC and NCI-H187 cell lines with IC_{50} values of 8.65 and 3.72 μg/ml, respectively. Compounds **1** and **3**—**6** displayed weak growth inhibitory activity against *Mycobacterium tuberculosis* H_{37}Ra with MIC values of 50—200 μg/ml. A mixture of **5**/**6** showed antimalarial activity with an IC_{50} of 3.38 μg/ml and moderate cytotoxic activities.

Experimental

General Procedures Melting points measurements were conducted by using an Electrothermal IA9100 digital melting point apparatus. Optical rotations were recorded on a JASCO P-1030 digital polarimeter. UV and IR spectra were measured on a Varian Cary 1E UV–Vis spectrophotometer and a Bruker VECTOR 22 spectrometer, respectively. NMR spectra (^{1}H, ^{13}C, DEPT, ^{1}H–^{1}H COSY, NOESY, HMQC and HMBC) were taken on a Bruker AV500D spectrometer. ESI-TOF mass spectra were recorded on a Micromass LCT spectrometer.

Fungal Material The fungus *Cordyceps* sp. was collected on a Homoptera-cicada nymph from Khao Laem National Park, Kanchanaburi Province, isolated and identified by Dr. Nigel L. Hywel-Jones. This fungus was deposited at the BIOTEC Culture Collection as BCC 1861 on November 28, 2001.

Fermentation and Isolation BCC 1861 was cultured in potato dextrose broth (5 l) under stationary condition for 55 d at 25 °C, and then the culture was filtered. The filtrate was extracted with an equal volume of EtOAc. The organic layer was concentrated under reduced pressure to obtain a dark brown gum (2.2 g). This crude extract was subjected to Sephadex LH-20 column chromatography (100% MeOH) and silica gel column chromatography (0—10% MeOH/CH_2Cl_2) to yield **1** (95.2 mg).

The mycelial cakes were extracted with MeOH (1.0 l) and filtered. To the filtrate was added H_2O (100 ml) and washed with hexane (800 ml). The aqueous MeOH layer was concentrated under reduced pressure. The residue was dissolved in EtOAc (700 ml), washed with H_2O (200 ml). The organic layer was concentrated under reduced pressure to provide a dark brown gum (1.2 g). This crude mycelial extract was separated by a Sephadex LH-20 column (100% MeOH) to provide ten fractions. Fraction 6 was subjected to a silica gel column (0—10% MeOH/CH_2Cl_2) to yield **2** (3.4 mg). Fraction 8 was purified by silica gel column chromatography (0—20% Acetone/CH_2Cl_2) to yield **3** (6.2 mg) and **4** (98.5 mg) and a mixture of **5** and **6** (26.0 mg).

Cordyol A (**1**): Off-white solid. mp 172—174 °C. $[\alpha]_D^{25}$ −40.3° (c=0.20, MeOH). UV λ_{max} (MeOH) nm (log ε): 204 (5.22), 275 (3.96). IR ($CHCl_3$) cm^{-1}: 3389, 3300, 2920, 1605, 1588, 1467, 1300, 1155, 1077, 829, 687. HR-MS (ESI-TOF) m/z: 429.1533 ([M+Na]$^+$, Calcd for $C_{21}H_{26}O_8Na$: 429.1525). ^{1}H- and ^{13}C-NMR data in acetone-d_6–D_2O (88 : 12), see Table 1.

Cordyol B (**2**): Off-white solid. mp 137—139 °C. $[\alpha]_D^{27}$ −18.6° (c=0.21, MeOH). UV λ_{max} (MeOH) nm (log ε): 203 (4.82), 214 (4.66), 261 (4.09), 300 (3.70). IR ($CHCl_3$) cm^{-1}: 3443, 2924, 1655, 1619, 1452, 1321, 1261, 1162, 1081, 756. HR-MS (ESI-TOF) m/z: 487.1585 ([M+Na]$^+$, Calcd for $C_{23}H_{28}O_{10}Na$: 487.1580). ^{1}H- and ^{13}C-NMR data in $CDCl_3$, see Table 1.

Cordyol C (**3**): Amorphous powder. UV λ_{max} (MeOH) nm (log ε): 204 (5.33), 277 (4.17). IR ($CHCl_3$) cm^{-1}: 3384, 1599, 1514, 1464, 1322, 1213, 1145, 1050, 834. HR-MS (ESI-TOF) m/z: 269.0796 ([M+Na]$^+$, Calcd for $C_{14}H_{14}O_4Na$: 269.0790). ^{1}H-NMR (500 MHz, acetone-d_6) δ: 8.29 (1H, br s, 3′-OH), 7.85 (1H, br s, 3-OH), 7.70 (1H, br s, 2-OH), 6.53 (1H, d, J=1.6 Hz, H-4), 6.35 (1H, br s, H-4′), 6.28 (1H, d, J=1.8 Hz, H-6), 6.22 (1H, br s, H-6′), 6.15 (1H, dd, J=2.2, 2.1 Hz, H-2′), 2.19 (3H, s, H-7′), 2.16 (3H, s, H-7). ^{13}C-NMR (125 MHz, acetone-d_6) δ: 159.3 (s, C-1′), 158.4 (s, C-3′), 146.5 (s, C-3), 143.4 (s, C-1), 140.0 (s, C-5′), 134.9 (s, C-2), 128.7 (s, C-5), 112.6 (d, C-6), 112.2 (d, C-4), 110.1 (d, C-4′), 108.8 (d, C-6′), 101.3 (d, C-2′), 20.6 (q, C-7′), 20.0 (q, C-7).

Hydrolysis of Cordyol A (1) Compound **1** (70 mg) was hydrolyzed with 5% HCl (2 ml) at 90 °C for 12 h. The reaction mixture was then diluted with H_2O (3 ml), and extracted with EtOAc (3×5 ml). The aqueous layer was concentrated *in vacuo* to yield an anomeric mixture of 4-*O*-methyl-D-glucopyranose (22.6 mg; $[\alpha]_D^{25}$ +74.9°, c=1.0, MeOH). The organic layer was evaporated to dryness to obtain aglycone (37.5 mg) whose ^{1}H-NMR data was identical to the isolate **4** as well as literature data of diorcinol.[11,12]

Biological Assays Antimalarial activity against *Plasmodium falciparum* K1 was conducted by using microculture radioisotope technique.[16] The IC_{50} values of a standard antimalarial compound, dihydroartemisinin, were 3.1—4.3 ng/ml. Growth inhibition against *Mycobacterium tuberculosis* H_{37}Ra was determined using Microplate Alamar-Blue Assay (MABA).[17] The MIC value of a standard antitubercular drug, isoniazid, was 0.05 μg/ml. Cytotoxicity assays against human oral epidermoid carcinoma (KB) cells, human breast cancer (BC) cells, human small-cell lung cancer (NCI-H187) cells, and African green monkey kidney fibroblast (Vero) cells were carried out using a colorimetric method.[18,19] The standard compound, ellipticine, exhibited cytotoxic activities against KB, BC, NCI-H187 and Vero cell lines with IC_{50} values of 0.21—0.49, 0.11—0.35, 0.25—0.51 and 0.3—0.6 μg/ml, respectively. Anti-herpes simplex virus type 1 (HSV-1) assay was determined using a colorimetric method.[18] The IC_{50} value of a standard anti-HSV-1 compound, acyclovir, was 2.0 μg/ml.

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