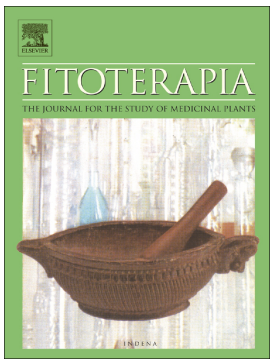

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Cordycepamides A–E and cordyglycoside A, new alkaloidal and glycoside metabolites from the entomopathogenic fungus *Cordyceps* sp.

Wenwen Fan[a,b,c,1], Erwei Li[c,1], Jinwei Ren[c], Wenzhao Wang[c], Xingzhong Liu[c,d,*], Yongjie Zhang[a,*]

[a] School of Life Science, Shanxi University, Taiyuan 030006, China.

[b] Institute of Biotechnology, Shanxi University, Taiyuan 030006, China.

[c] State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China.

[d] Department of Microbiology, College of Life Sciences, Nankai University, Tianjin 300071, China.

Corresponding authors.

E-mail addresses: liuxz@im.ac.cn (X. Liu), zhangyj2008@sxu.edu.cn (Y. Zhang).

[1] Wenwen Fan and Erwei Li contributed equally.

ABSTRACT

Five new alkaloidal metabolites cordycepamides A−E (**1**−**5**), and one glycoside metabolite cordyglycoside A (**6**), together with six known compounds (**7**−**12**) were isolated from the entomopathogenic fungus *Cordyceps* sp. (LB1.18060004) from unidentified insect collected in Baoshan City, Yunnan Province, People's Republic of China. The structures were characterized by NMR and HRESIMS spectroscopic analyses. Cordycepamides A and B (**1** and **2**) were mixtures of two isomers in 5:4 ratio by integration of ^{1}H NMR spectra. In additional, the structure of cordycepamide A (**1**) was further confirmed by X-ray crystallography as a pair of enantiomers. Absolute configurations of sugar moiety of cordyglycoside A (**6**) was confirmed by the acid hydrolysis and subsequent HPLC analysis. The isolated metabolites were evaluated for antimicrobial, cytotoxicity, and the DPPH scavenging assay, only **4** showed modest antioxidant effects in the DPPH scavenging assay (IC_{50} = 51.42±3.08 μM).

Keywords: *Cordyceps* sp.; entomopathogenic fungus; alkaloidal metabolite; enantiomer

1. Introduction

Entomopathogenic fungi such as species from the genera *Cordyceps* have been frequently used as foods and medicines for the treatment of various diseases. The diversity and complexity of the living environment and metabolic type of entomogenous fungi lead to them possessing the capability to produce structurally diverse and biologically active secondary metabolites [1]. One of the well-known metabolites was ISP-I, isolated from entomogenous *Isaria sinclairii*, while investigating structure-activity relationship of its derivatives, fingolimod (FTY720, Gilenya) was the first US Food and Drug Administration–approved oral therapy for relapsing forms of multiple sclerosis [2]. Notable other examples include beauvericins isolated from *Cordyceps cicadae* with cytotoxic activities [3], cordyformamide isolated from *Cordyceps brunneautbra* with antimalarial activity [4], cordypyridones A−D isolated from *Cordyceps nipponica* with antimalarial activity [5], dimethylguanosine isolated from *Cordyceps militaris* with antioxidant and HIV-1 protease inhibitory activity [6], cicadapeptins I and II isolated from *Cordyceps heteropoda* with antibacterial and antifungal activity [7]. Therefore, entomogenous fungi are considered to be important potential sources of natural medicines [8].

During an ongoing search for new bioactive natural products from entomogenous and related fungi [9-11], a strain of entomopathogenic fungus *Cordyceps* sp. from unidentified insect collected in Baoshan City, Yunnan Province, People's Republic of China in 2018, was subjected to chemical investigation. Fractionation of an EtOAc extract from a solid-substrate fermentation culture afforded five alkaloidal metabolites

cordycepamides A−E (**1**−**5**) and one glycoside metabolite cordyglycosides A (**6**), together with six known compounds, cordytakaoamide B (**7**) [12], beauvericin (**8**) [13], beauvericin A (**9**) [14], beauvericin E (**10**) [15], 1,4-dimethoxy-2, 4', 4''-trihydroxy-*p*-terphenyl (**11**) [16] and cyclo(L-phenylalaninyl-L-leucinyl) (**12**) [17]. Details of the isolation, structure elucidation, and biological activities of these compounds are reported herein.

2. Experimental

2.1 General experimental procedures

Optical rotations were measured on an Anton Paar MCP200 polarimeter (Graz, Austria). ^{1}H and ^{13}C NMR data were acquired with Bruker Avance-500 spectrometers (Rheinstetten, Germany). The HSQC and HMBC experiments were optimized for 125.0 and 8.0 Hz, respectively. HRESIMS data were recorded on an Agilent Accurate-Mass-Q-TOF LC/MS 6520 instrument (Santa Clara, CA, USA) in positive ion mode. HPLC data were obtained with a Waters 2695 instrument (Milford, MA, USA). Preparative HPLC was performed on an Agilent 1200 HPLC system using a C18 column (Reprosil Pur Basic C18 column; 5 µm; 10×250 mm; detector: UV) with a flow rate of 2.0 mL/min. The absorbance of contents in the 96-well clear plate was detected by a SpectraMax Paradigm microplate reader (Sunnyvale, CA, USA).

2.2 Fungal material

The strain of *Cordyceps* sp. (LB1.18060004) was isolated from unidentified insect collected in Baoshan City, Yunnan Province, People's Republic of China, in July 2018. The isolated strain was identified by sequence analysis (Genbank

Accession Number MN606111) of the rDNA internal transcribed spacer (ITS) region. The strain was firstly cultivated on culture dish of potato-dextrose agar at 25 °C for 10 days. Then the agar was cut into grain size to inoculate in two conical flasks (500 mL) that each containing 100 mL autoclave sterilized potato-dextrose broth. The flasks containing inoculated potato-dextrose broth were cultivated at 25 °C on a rotary shaker at 200 rpm for 6 days. Forty 500 mL Fernbach flasks, each containing 80 g of rice and 120 ml of distilled water, were autoclaved at 120 °C for 30 min, in which the fermentation proceeded. After cooling to room temperature, each of the flasks was inoculated with 10 ml of the spore inoculum and cultured at 25 °C for 40 days.

2.3 Extraction and isolation

The ferment culture was extracted with EtOAc (three times, each 6 L) and vacuum-dried to afford the crude extract (15 g). The extract was fractionated by silica gel vacuum liquid chromatography (VLC) in petroleum ether-dichloromethane-MeOH gradient elution to afford eighteen primary fractions (Frs.1−18). The fraction Fr.10 (2.3 g) was loaded on Sephadex LH-20 column chromatography eluted with MeOH to afford 43 subfractions Frs.10.1−10.43. Fr.10.14 was purified by RP HPLC (Reprosil-Pur Basic C18 column; 5 μm; 10×250 mm; 74% MeOH in H_2O with 0.1% HCOOH for 50 min; 2.0 mL/min) to afford **1** (9.3 mg, t_R 32.5 min). The subfraction Fr.10.15 was purified by RP HPLC (Reprosil-Pur Basic C18 column; 5 μm; 10×250 mm; 76% MeOH in H_2O with 0.1% HCOOH for 50 min; 2.0 mL/min) to afford **2** (3.9 mg, t_R 34.1 min). The subfraction Fr.10.16 was purified by RP HPLC (Reprosil-Pur Basic C18 column; 5 μm; 10×250 mm; 83% MeOH in

H_2O with 0.1% HCOOH for 60 min; 2.0 mL/min) to afford **8** (14.3 mg, t_R 35.3 min) and **9** (4.5 mg, t_R 44.5 min). The subfraction Fr.10.18 was purified by RP HPLC (Reprosil-Pur Basic C18 column; 5 μm; 10×250 mm; 83% MeOH in H_2O with 0.1% HCOOH for 60 min; 2.0 mL/min) to afford **10** (3.0 mg, t_R 31.2 min). The subfractions Frs.10.25−10.43 were combined to give 1.3 g was loaded on a reverse silica gel column chromatography (ODS) eluted with 50%-100% MeOH to afford seven tertiary fractions (Frs.10.25.1−10.25.7). Fr.10.25.4 was purified by RP HPLC (Reprosil-Pur Basic C18 column; 5 μm; 10×250 mm; 45%MeOH in H_2O with 0.1% HCOOH for 50 min; 2.0 mL/min) to afford **5** (5.8 mg, t_R 31.1 min). Fr.10.25.5 after elution was purified by RP HPLC (Reprosil-Pur Basic C18 column; 5 μm; 10×250 mm; 52%MeOH in H_2O with 0.1% HCOOH for 60 min; 2.0 mL/min) to afford **11** (3.8 mg, t_R 42.4 min). Fr.10.25.7 was purified by RP HPLC (Reprosil-Pur Basic C18 column; 5 μm; 10×250 mm; 52%MeOH in H_2O with 0.1% HCOOH for 60 min; 2.0 mL/min) to afford **3** (2.3 mg, t_R 52.2 min). The fractions Frs.11−13 were combined to give 5.4 g was loaded on Sephadex LH-20 column chromatography eluted with MeOH to afford 26 subfraction Frs.11.1−11.26. The subfraction Fr.11.8 was purified by RP HPLC (Reprosil-Pur Basic C18 column; 5 μm; 10×250 mm; 47% MeOH in H_2O with 0.1% HCOOH for 60 min; 2.0 mL/min) to afford **12** (2.5 mg, t_R 48.8 min). The subfraction Fr.11.13 was purified by RP HPLC (Reprosil-Pur Basic C18 column; 5 μm; 10×250 mm; 49% MeOH in H_2O with 0.1% HCOOH for 70 min; 2.0 mL/min) to afford **7** (4.3 mg, t_R 64.3 min). The subfraction Fr.11.14 was purified by RP HPLC (Reprosil-Pur Basic C18 column; 5 μm; 10×250 mm; 56% MeOH in H_2O with 0.1% HCOOH for

50 min; 2.0 mL/min) to afford **4** (5.9 mg, t_R 38 min).The subfraction Fr.11.26 was purified by RP HPLC (Reprosil-Pur Basic C18 column; 5 µm; 10×250 mm; 69% MeOH in H_2O with 0.1% HCOOH for 50 min; 2.0 mL/min) to afford **6** (4.0 mg, t_R 49.5 min).

*2.3.1 Cordycepamide A (**1**)*

White powder; $[\alpha]_D^{25}$ +13.57 (*c* 0.28, MeOH); UV (MeOH) λ_{max} (log ε): 224 (2.84) nm; IR (neat) ν_{max} 3307, 2954, 2926, 2855, 1666, 1622, 1538, 1478, 1350, 1279, 1248, 1201, 1066, 1032, 976, 726 cm^{-1}; ^1H and ^{13}C NMR data (500 MHz, $CDCl_3$) see Table 1; HMBC correlations (500 MHz, $CDCl_3$): H-1 → C-9; H-2 → C-3, 4; H_2-3 → C-1, 2, 4, 9; H-5 → C-4, 9; H-6 → C-5; H-7 → C-6, 8, 9; H-11 → C-10, 13; H-12 → C-10, 13, 14; H_2-13 → C-11, 12; H_2-14 → C-15; H_2-15 → C-13; H_2-16 → C-18; H_2-17 → C-18; H_2-18 → C-19; H_3-19 → C-18; HRESIMS: *m/z* 356.1841 $[M+Na]^+$ (calcd for $C_{19}H_{27}NO_4Na$ 356.1838).

*2.3.1.1 X-ray Crystallographic Analysis of **1***

Upon crystallization from MeOH–H_2O (3:1) using the vapor diffusion method, colorless crystals were obtained for **1**. A crystal (0.32 × 0.02 × 0.31 mm) was separated from the sample and mounted on a glass fiber, and data were collected using the Agilent CrysAlisPro software (Version 1.171.40.39) with graphite-monochromated Cu Kα radiation, λ = 1.54184 Å at 107.0(10) K. Crystal data: $C_{19}H_{27}NO_4$, *M* = 333.42, space group orthorhombic, *P*-1; unit cell dimensions *a* = 9.0892(2) Å, *b* = 13.7368(3) Å, *c* = 15.4522(3) Å, *V* = 1812.42(7) $Å^3$, *Z* = 4, D_{calcd} = 1.222 mg/m^3, μ = 0.687 mm^{-1}, *F*(000) = 720.0. The structure was solved by direct

methods using SHELXL-97 [18] and refined by using full-matrix least-squares difference Fourier techniques. All non-hydrogen atoms were refined with anisotropic displacement parameters, and all hydrogen atoms were placed in idealized positions and refined as riding atoms with the relative isotropic parameters. Absorption corrections were performed using spherical harmonics, implemented in SCALE3 ABSPACK scaling algorithm [19]. The 6318 measurements yielded 5750 independent reflections after equivalent data were averaged, and Lorentz and polarization corrections were applied. The final refinement gave R_1 = 0.0484 and wR_2 = 0.1425 [$I > 2\sigma(I)$]. Crystallographic data for **1** has been deposited with the Cambridge Crystallographic Data Centre as supplementary publications Nos. CCDC 1973842. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

*2.3.2. Cordycepamide B (**2**)*

White powder; $[\alpha]_D^{25}$ -4.37 (*c* 0.39, MeOH); UV (MeOH) λ_{max} (log ε): 219 (2.87) nm; IR (neat) ν_{max} 3307, 2927, 2855, 1666, 1629, 1545, 1457, 1221, 1074, 980, 751 cm^{-1}; ^1H and ^{13}C NMR data (500 MHz, $CDCl_3$) see Table 1; HMBC correlations (500 MHz, $CDCl_3$): H-1 → C-3, 9; H-2 → C-1, 3, 4; H_2-3 → C-1, 2, 4, 5, 9; H-5 → C-3, 7, 9; H-6 → C-7; H-7 → C-5, 9; H-8 → C-4, 7, 9; H-11 → C-10, 13; H-12 → C-10, 13; H_2-13 → C-11, 12, 14; H_2-14 → C-12, 16; H_2-15 → C-13; H_2-16 → C-17; H_2-17 → C-18; H_2-18 → C-19; H_3-19 → C-18; HRESIMS: *m/z* 318.2066 $[M+H]^+$ (calcd for $C_{19}H_{28}NO_3$ 318.2069).

*2.3.3. Cordycepamide C (**3**)*

Yellow oil; $[\alpha]_D^{25}$ -39.56 (*c* 0.23, MeOH); UV (MeOH) λ_{max} (log ε) 215 (2.69), 274 (0.41) nm; IR (neat) ν_{max} 3296, 2934, 1737, 1667, 1622, 1539, 1456, 1365, 1242, 1034, 755 cm^{-1}; ^1H and ^{13}C NMR data (500 MHz, acetone-d_6) see Table 1; HMBC correlations (500 MHz, acetone-d_6): H-1 → C-2, 3; H-2 → C-1, 3; H_2-3 → C-1, 2, 4, 5, 9; H-6 → C-4, 8; H-7 → C-5; H-8 → C-6, 9; H-9 → C-3, 5, 7; H-11 → C-10, 13; H-12 → C-10, 13; H_2-13 → C-11, 12, 14, 15; H_2-14 → C-12, 13, 15; H_2-16 → C-14, 15, 17; H_2-17 → C-15, 1'; H_3-2' → C-1'; NH → C-10; HRESIMS: *m/z* 350.1971 $[M+H]^+$ (calcd for $C_{19}H_{28}NO_5$ 350.1967).

*2.3.4. Cordycepamide D (**4**)*

Yellow oil; $[\alpha]_D^{25}$ -27.28 (*c* 0.59, MeOH); UV (MeOH) λ_{max} (log ε): 212 (2.36), 296 (0.38) nm; IR (neat) ν_{max} 3306, 2931, 2856, 1716, 1667, 1621, 1543, 1506, 1455, 1366, 1251, 1204, 1149, 1045, 978 cm^{-1}; ^1H and ^{13}C NMR data (500 MHz, DMSO-d_6) see Table 1; HMBC correlations (500 MHz, DMSO-d_6): H-1 → C-2, 3; H_2-3 → C-1, 2, 4, 5, 9; H-6 → C-4, 7, 8; H-7 → C-5, 9; H-9 → C-3; H-11 → C-10, 13; H-12 → C-10, 13, 14; H_2-13 → C-11, 12, 14, 15; H_2-14 → C-12, 13, 16; H_2-17 → C-16, 19; H_2-18 → C-17, 19; H_2-19 → C-17, 18, 1'; H_3-2' → C-1'; OH-1 → C-1, 2; OH-5 → C-4, 5, 6; OH-8 → C-7, 8, 9; NH → C-2, 10; HRESIMS: *m/z* 416.2046$[M+Na]^+$ (calcd for $C_{21}H_{31}NO_6Na$ 416.2049).

*2.3.5. Cordycepamide E (**5**)*

White powder; $[\alpha]_D^{25}$ +173.01 (*c* 0.56, MeOH); UV (MeOH) λ_{max} (log ε): 229 (3.61), 276 (0.91) nm; IR (neat) ν_{max} 3368, 2969, 2933, 1746, 1648, 1614, 1516, 1446, 1368, 1340, 1259, 1231, 1205, 1172, 1128, 1036, 826, 599, 576 cm^{-1}; ^1H and ^{13}C NMR

data (500 MHz, $CDCl_3$) see Table 2; HMBC correlations (500 MHz, $CDCl_3$): H-3 → C-2, 5, 10, 11, 17; H-6 → C-2, 5, 7, 8, 9; H-7 → C-5, 8, 9; H_3-8 → C-6, 7, 9; H_3-9 → C-6, 7, 8; H_2-10 → C-2, 3, 11, 16; H-12 → C-10, 13, 14; H-13 → C-11, 14; H_3-17 → C-3, 5; HRESIMS: *m/z* 278.1391 $[M+H]^+$ (calcd for $C_{15}H_{20}NO_4$ 278.1392).

*2.3.6. Cordyglycoside A (**6**)*

Yellow oil; $[\alpha]_D^{25}$ -7.50 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε): 229 (1.24) nm; IR (neat) ν_{max} 3391, 2929, 2857, 1714, 1456, 1374, 1198, 1109, 1080 cm^{-1}; ^1H and ^{13}C NMR data (500 MHz, DMSO-d_6) see Table 3; HMBC correlations (500 MHz, DMSO-d_6): H_2-2 → C-1, 3; H_2-6 → C-7, 8; H-7 → C-5, 9; H-8 → C-6, 10; H-9 → C-7, 8, 11; H-10 → C-8, 11; H-11 → C-9, 12, 1', H_2-12 → C-14; H_3-18 → C-17; H-1' → C-11; H-2' → C-1', 3', H-3' → C-2', 4'; H-4' → C-5', 4'-OCH_3; H-5' → C-4'; H_2-6a' → C-4', 5'; 4'-OCH_3 → C-4'; HRESIMS *m/z* 495.2937 $[M+Na]^+$ (calcd for $C_{25}H_{44}O_8Na$ 495.2934).

2.3.6.1 Acid hydrolysis and determination of the absolute configuration of glucose

Compound **6** (2.0 mg) was added with hydrochloric acid (3 M, 3 mL), and reacted in a water bath at 90 °C for 2 h. After the reaction was completed, an equal volume of ethyl acetate was added for extraction twice, and the aqueous layer was evaporated to dryness to obtain a sugar residue. The sugar residue was added with L-cysteine methyl ester hydrochloride (2.5 mg) and pyridine (1 mL), and then heated to 60 °C for 1 h. σ-Tolylisothiocyanate (10 μL) was added to the mixture, and heating was continued for an additional 1 h. The reaction mixture was directly analyzed using HPLC (10% to 100% MeCN gradient with 0.1% formic acid over 40 min) [20].

2.4 Cell Culture

Human breast cancer cells MCF-7, human liver cancer cells HepG-2, human colon cancer cells HT29, and human kidney normal cells 293t were purchased from Shanghai Institute of Biological Sciences (SIBS), Chinese Academy of Sciences. The cell line was added to RPMI-1640 medium (Hyclone) containing 10% fetal bovine serum, and all cells were cultured in a 5% CO_2, 37°C incubator.

2.5 Cytotoxicity assay

The cytotoxicity evaluations of **3**–**5** and **7** against normal human breast cancer cells MCF-7, human liver cancer cells HepG-2, human colon cancer cells HT29, and human kidney normal cells 293t were carried out using the 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Doxorubicin was used as positive control drug and deionized H_2O with the same DMSO concentration was used as parallel control. All experiments were repeated three times [21].

2.6 Antimicrobial assays

This experiment mainly tested the antibacterial activity of compounds **3**–**7** against three pathogenic bacteria, Staphylococcus aureus, Bacillus subtilis and E. coli. Each pathogen was cultured on a LA plate at 37°C overnight. A single bacterial colony was picked and suspended in LB medium to about 1×10^4 cfu/mL. Vancomycin and ampicillin were used as positive controls, and DMSO was used as a negative control. Prepare two-fold serial dilutions of each test compound (20 to 1.25 μg/mL in DMSO) and add an aliquot (2 μL) of each dilution to a 96-well containing 98 μL LB

medium In the board. An aliquot (100 µL) of the bacterial suspension was then added to each well (to make the final compound concentration of 200 to 12.5 µg/mL) and incubated at 37 °C for 12 h. Finally, the optical density of each well at 600 nm was measured with an EnVision 2103 Multilabel Plate Reader. MIC values were defined as the minimum concentration of compound that inhibited visible bacterial growth. All experiments were repeated three times.

2.7 DPPH Scavenging assay

The mixture containing 50 µL of each compound in DMSO (final concentrations of 6.25, 12.5, 25, 50, 100 µM) and 50 µL of DPPH (sigma) radicals in ethanolic solution (final concentration of 100 µM) were placed in a 96-well microplate and incubate in the dark for 30 minutes, and measurement of absorbance at 517 nm using a Spectra Max 190 microplate reader (Molecular Devices Inc.). The scavenging ability was calculated as follows: scavenging ability (%)= $[(A_{517\ of\ control} - A_{517\ of\ sample})/A_{517\ of\ control}] \times 100$. Ascorbic acid was used as positive control. All experiments were repeated three times.

2. Results and discussion

Cordycepamide A (**1**) was obtained as a mixture of two isomers (**1a** and **1b**) in a ratio of approximately 4:5 by analysis of its ^{1}H NMR spectrum (Fig. S1). Although the two isomers were well-resolved using a chiral HPLC column (Fig. S2), ^{1}H NMR spectra of each collected peak showed the presence of both isomers (Fig. S3), suggesting the occurrence of a spontaneous equilibration. Therefore, the structure was elucidated on the equilibrium mixture. Compound **1** has a molecular formula of

$C_{19}H_{27}NO_4$ (seven degrees of unsaturation) on the basis of its HRESIMS pseudomolecular ion peak ($[M+Na]^+$ at *m/z* 356.1841) and NMR data (Table 1). The ^1H and ^{13}C NMR data (Table 1) of the major isomer **1b** showed the presence of one carboxylic carbon (δ_C 167.8), eight aromatic/olefinic carbons with five protonated, one hemiacetal carbon ($\delta_{C/H}$ 93.5/5.52), one sp^3 methine carbon ($\delta_{C/H}$ 47.1/4.51), seven sp^3 methylene carbons, one methyl. According to its NMR data and the requirement of unsaturation degree, **1b** required to be bicyclic structure. Interpretation of the ^1H–^1H COSY NMR data led to the identification of three isolated proton spin-systems corresponding to the C-1–C-3, C-5–C-7 and C-11–C-19 fragments. HMBC correlations from H-5 to C-4 and C-9, from H-7 to C-8 and C-9, from H-1 to C-9, and from H_2-3 to C-4 and C-9 established one chromane moiety with two hydroxyl groups attached to C-1 and C-8, respectively. HMBC correlations from H-11 and H-12 to C-10, and the large coupling constant (J = 15.2 Hz) observed between H-11 and H-12 established (11*E*)-dec-2-enoic acid subunit. Consideration of the chemical shifts of C-10 (δ_C 167.8) and C-2 ($\delta_{C/H}$ 47.1/4.51), and the requirement of molecular formula, the chromane moiety and (11*E*)-dec-2-enoic acid subunit was linked together by amide bond. On the basis of these data, the gross structure of cordycepamide A was established as **1** (Fig. 1).

The proposed structure for **1** was confirmed by single-crystal X-ray diffraction analysis (Fig. 3). Compound **1** was found to crystallize as a mixture of *enantiomers* (1*R*, 2*S* and 1*S*, 2*R*). In additional, the presence of internolecular hydrogen bond and shielding or deshielding effects of benzene ring by observing X-ray diffraction

explained the phenomena that the 1D NMR spectra of **1a** and **1b** as a pair of enantiomers were not overlap.

Cordycepamide B (**2**) was also obtained as a mixture of two isomers (**2a** and **2b**) in a ratio of approximately 5:4 by analysis of its ^{1}H NMR spectrum (Fig. S8). Compound **2** has a molecular formula of $C_{19}H_{27}NO_3$ (seven degrees of unsaturation) on the basis of its HRESIMS pseudomolecular ion peak ($[M+H]^+$ at *m/z* 318.2066). Compared to that of **1**, the less 16 mass units suggested the absence of an oxygen atom. The ^{1}H and ^{13}C NMR data (Table 1) of **2** revealed the presence of structural features similar to those found in **1**, except that the oxygenated aromatic carbon (δ_C 145.1) was replaced by a protonated aromatic carbon ($\delta_{H/C}$ 6.83, 117.0) in the NMR spectra of **2**, and this observation was supported by HMBC correlations from the newly observed H-8 to C-4, C-7 and C-9. Therefore, the gross structure of cordycepamide B was proposed as **2**.

Cordycepamide C (**3**) was obtained as a yellow oil. It has a molecular formula of $C_{19}H_{27}NO_5$ (seven degrees of unsaturation) on the basis of its HRESIMS pseudomolecular ion peak ($[M+H]^+$ at *m/z* 350.1971). Its ^{1}H and ^{13}C NMR data (Table 1) revealed the presence of two carboxylic carbons (δ_C 167.6, 171.1, respectively), eight aromatic/olefinic carbons with six protonated, one sp^3 methine carbon ($\delta_{C/H}$ 54.3/3.87), seven sp^3 methylene carbons with two oxygenated, and one methyl. Analysis of its NMR data ($\delta_{C/H}$ 131.9, 157.2, 120.2/6.74, 128.8/7.06, 116.9/6.84, 125.5/7.09, respectively) and the requirement of unsaturation degree, **3** contained an *ortho*-disubstituted benzene ring moiety. Analysis of the ^{1}H–^{1}H COSY NMR data

led to the identification of three isolated proton spin-systems corresponding to the C-1–C-3, C-6–C-9 and C-11–C-17 fragments. HMBC correlations from H-3 to C-4, C-5 and C-9, from H-6 to C-4, and from H-9 to C-3 and C-5 established one 3-phenylpropan-1-ol subunit with two hydroxyl groups attached to C-1 and C-5, respectively. HMBC correlations from H-11 and H-12 to C-10, from H_2-17 and H_3-2' to C-1' (δ_C 171.1) and the large coupling constant (J = 15.3 Hz) observed between H-11 and H-12 established (11*E*)-8-hydroxyoct-2-enoic acid subunit with an acetyl attached to the terminal of the unsaturated fatty acid. Consideration of the chemical shifts of C-10 (δ_C 167.6) and C-2 ($\delta_{C/H}$ 54.3/3.87) and the requirement of molecular formula, the 3-phenylpropan-1-ol moiety and (11*E*)-8-hydroxyoct-2-enoic acid subunit was linked together by amide bond. On the basis of these data, the gross structure of cordycepamide C was established as **3** (Fig. 1). **3** possessed structural similarity to those of cordytakaoamide B, an alkaloidal metabolite from entomopathogenic fungus, *Cordyceps takaomontana* NBRC 101754 [12]. The negative optical rotation ($[\alpha]_D$ -39.56, MeOH) of **3** was agreement with those of cordytakaoamide B ($[\alpha]_D$ -23.22, MeOH), indicating that the absolute configuration of C-2 is the same to cordytakaoamide B as 2*S*.

Cordycepamide D (**4**) was obtained as a yellow oil. It has a molecular formula of $C_{21}H_{31}NO_6$ (seven degrees of unsaturation) on the basis of its HRESIMS pseudomolecular ion peak ($[M+Na]^+$ at *m/z* 416.2046). Its ^1H and ^{13}C NMR data (Table 1) revealed the presence of two carboxylic carbons (δ_C 164.8, 170.4, respectively), eight aromatic/olefinic carbons with five protonated, one sp^3 methine

carbon ($\delta_{C/H}$ 51.5/3.93), nine sp^3 methylene carbons with two oxygenated, one methyl carbon. Its ^1H NMR spectrum showed the presence of an ABX coupling system of benzene ring (δ_H 6.57, d, J = 8.5 Hz; 6.40, dd, J = 8.5, 2.9 Hz; 6.50, d, J = 2.9 Hz), indicating that **4** contained a 1,2,4-trisubstituted benzene ring moiety. Compared NMR data with **3**, **4** possessed structural similarity to **3** except extra an hydroxyl group attached at C-8, these confirmed by the HMBC correlations from OH-8 to C-7, C-8 and C-9. In addition, two more sp^3 methylenes than **3** was further confirmed by its ^1H–^1H COSY and HMBC correlations. The negative optical rotation ($[\alpha]_D$ -27.28, MeOH) of **4** was agreement with those of **3** ($[\alpha]_D$ -39.56, MeOH) and cordytakaoamide B ($[\alpha]_D$ -23.22, MeOH), indicating that the absolute configuration of C-2 is the same to **3** and cordytakaoamide B as 2*S*.

Cordycepamide E (**5**) was obtained as a white powder. It has a molecular formula of $C_{15}H_{19}NO_4$ (seven degrees of unsaturation) on the basis of its HRESIMS pseudomolecular ion peak ($[M+H]^+$ at m/z 278.1391). Its ^1H and ^{13}C NMR data (Table 2) revealed the presence of two carboxylic carbons (δ_C 167.7 and 166.7, respectively), six aromatic carbons with four protonated, one sp^3 methylene, three sp^3 methines with one oxygenated and one nitrogenated, three methyls including one *N*-methyl ($\delta_{H/C}$ 3.06/33.0). These accounted for all the NMR resonances of **5** except for one exchangeable proton (OH-14), and required **5** to be a monocyclic compound. One *p*-substituted benzene ring was confirmed by analysis of its ^1H NMR spectrum (δ_H 6.77, 2H, br d, J = 8.5 Hz; 6.97, 2H, br d, J = 8.5 Hz). HMBC correlations from H-3 to C-2, C-5, C-10 and C-11, from H-6 to C-2, C-5, C-7, C-8, and C-9, from H-7 to

C-5 established morpholine-2,5-dione moiety with one 4-methenylphenol and one isopropyl attached to C-3 and C-6, respectively. The *N*-methyl ($\delta_{H/C}$ 3.06/33.0) was attached to nitrogen atom by HMBC correlations from H_3-17 to C-3 and C-5. On the basis of these data, the gross structure of cordycepamide E was established as **5** (Fig. 1). **5** possessed structural similarity to those of bassiatin, an anticoagulant metabolite from soil fungus *Beauveria bassiana* K-717 [22]. Bassiatin and its stereoisomers have been synthesized [23]. Thus, compared the optical rotation of **5** ($[\alpha]_D$ +173.01, MeOH) to those of reported compounds (natural original bassiatin: +181.05, $CHCl_3$; synthetic bassiatin: +166, $CHCl_3$, +165.9, CH_2Cl_2) [22, 23] the absolute configurations of C-3 and C-6 were assigned as 3*S*, 6*R*.

Cordyglycoside A (**6**) was obtained as a yellow powder. It has a molecular formula of $C_{25}H_{44}O_8$ (four degrees of unsaturation) on the basis of its HRESIMS pseudomolecular ion peak ($[M+Na]^+$ at *m/z* 495.2937). Its ^1H and ^{13}C NMR data (Table 3) revealed the presence of one carboxylic carbon (δ_C 174.8), four protonated olefinic carbons, one hemiacetal carbon ($\delta_{C/H}$ 99.4/4.06), five sp^3 oxygenated methine carbons, 12 sp^3 methylene carbons with one oxygenated, one methoxyl ($\delta_{C/H}$ 59.5/3.40) and a methyl. Its ^1H and ^{13}C NMR spectral data showed the presence of a glucopyranose moiety ($\delta_{C/H}$ 99.4/4.06, 73.7/2.94, 76.6/3.22, 79.5/2.90, 75.5/2.99, 60.7/3.47, 3.59, respectively). Analysis of the ^1H–^1H COSY NMR data led to the identification of two isolated proton spin-systems corresponding to the C-2–C-18 and C-1'–C-6' fragments. HMBC correlations from H_2-2 to C-1, from H_2-6 to C-7 and C-8, from H-11 to C-9, and the large coupling constant (J = 15.3 Hz) observed between

H-7 and H-8, H-9 and H-10 established (7*E*, 9*E*)-octadeca-7, 9-dienoic acid subunit. HMBC correlations from H-11 to C-1' and from H-1' to C-11 indicated that glucopyranose moiety attached to C-11. The methoxyl was located at C-4' of the sugar by the HMBC correlation of OCH_3 to C-4'. The coupling constant (J = 7.8 Hz) of the anomeric proton (δ_H 4.06) indicated that C-1' of glucopyranose was β-configuration. According the reported procedure [20], the absolute configuration of glucose was determined by acid hydrolysis followed by chemical derivatization and comparison with authentic D- and L-glucose samples using HPLC (Fig. S32), the glucose in **6** was assigned as D-glucose. Due to few amount of its hydrolysis products, the configuration of C-11 were not determined by using the modified Mosher's method. Thus, the configuration of C-11 in **6** still remains unknown.

The bioactivities of isolated metabolites were evaluated for antimicrobial, cytotoxicity, and the DPPH scavenging assay. Compounds **3**–**5** and **7** showed no cytotoxic activity against the human breast cancer cells MCF-7, human liver cancer cells HepG-2, human colon cancer cells HT29, and human kidney normal cells 293t at the concentration of 20 μM. Compounds **3**–**7** also showed no antibacterial activity against *Bacillus subtilis*, *E. coli*, and *Staphylococcus aureus* at the concentration of 200 μg/mL. In the DPPH assay, compound **4** showed moderate free radical scavenging activity with IC_{50} = 51.42±3.08 μM, using ascorbic acid as a positive control (IC_{50} = 30.75±0.10 μM).

4. Conclusion

In summary, chemical investigation of solid-substrate fermentation of

entomopathogenic fungus, *Cordyceps* sp. led to the isolation of five new alkaloidal metabolites, cordycepamides A−E (**1**−**5**) and one glycoside metabolite cordyglycoside A (**6**). Cordycepamides A and B (**1** and **2**) are a class of chromane analogs with (11*E*)-dec-2-enoic acid moiety linked C-2 through an amide bond. Cordycepamides C and D (**3** and **4**) possess similar structural feature to **1** and **2**, except the tetrahydro-2*H*-pyran ring cleaved and the terminal of the long chain unsaturated fatty acids acetylated. Biogenetically, compounds **1**−**4** could be derived from phenylalanine and dec-2-enoic acid/oct-2-enoic acid [12]. The carboxyl group of phenylalanine has been reduced to aldehyde and alcohol, then though various degrees of oxygenation, dehydration, and cyclisation, compounds **1**−**4** could been product. Cordycepamide E (**5**) is a hydroxylated analogue of bassiatin, and cordyglycoside A (**6**) is a glycosylated derivate at C-11 of (7*E*, 9*E*)-octadeca-7, 9-dienoic acid. In the DPPH assay, compound **4** showed moderate free radical scavenging activity.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data (1D, 2D NMR spectra of compounds **1**–**6**, the chiral HPLC

chromatogram of **1** and **2**, and the HPLC chromatography of sugar residue of **6**) to this article can be found online at https://.

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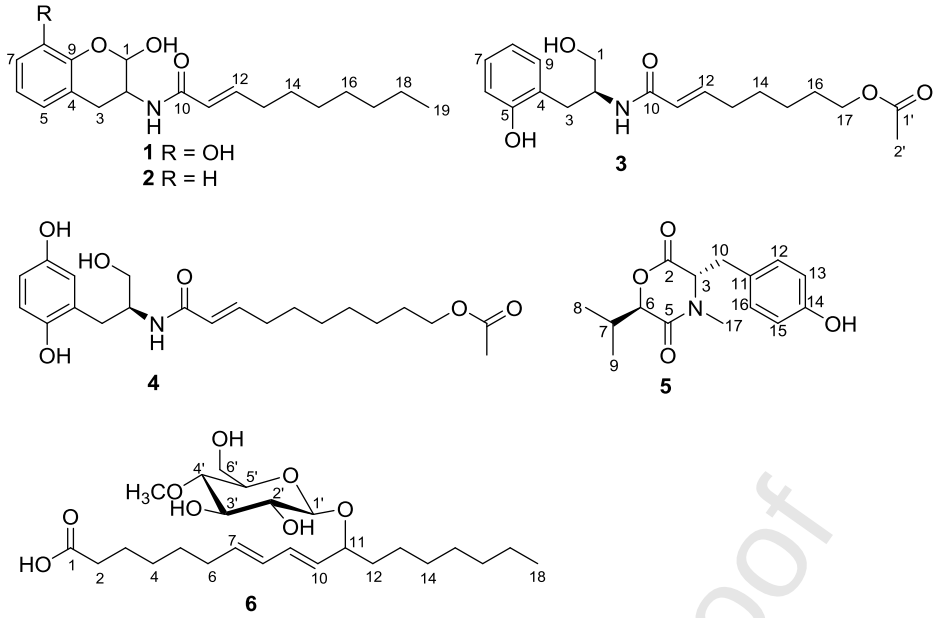


Figure 1. Structures of compounds **1**–**6**.

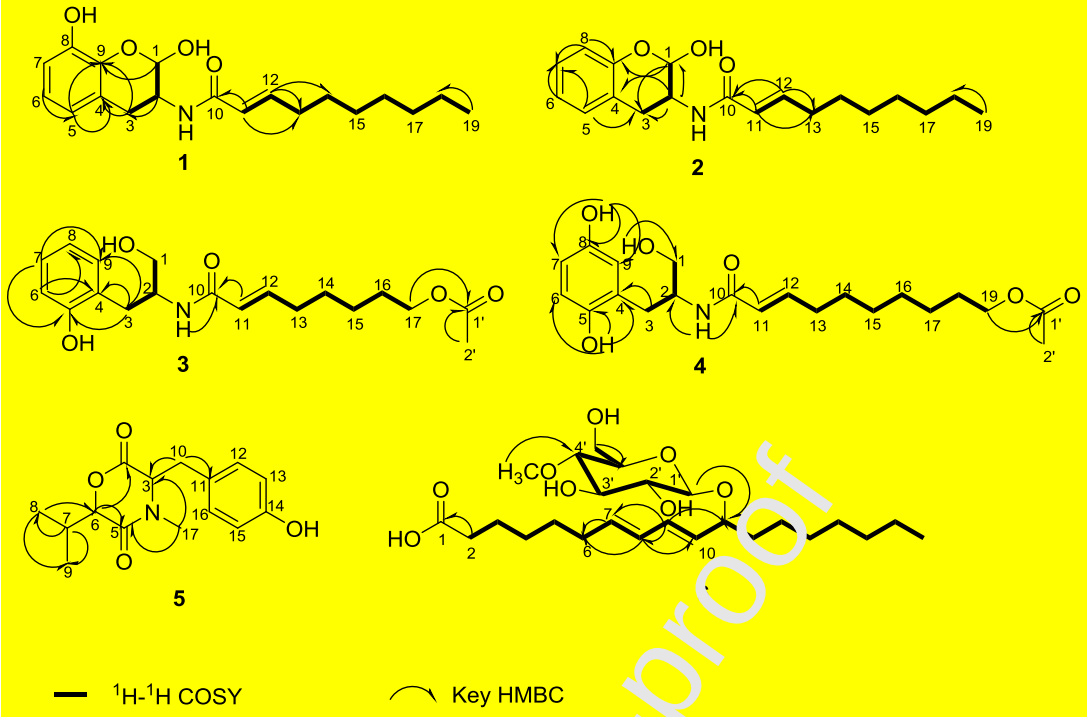


Figure 2. Selected ^{1}H- ^{1}H COSY and key HMBC correlations of compounds **1**−**6**.

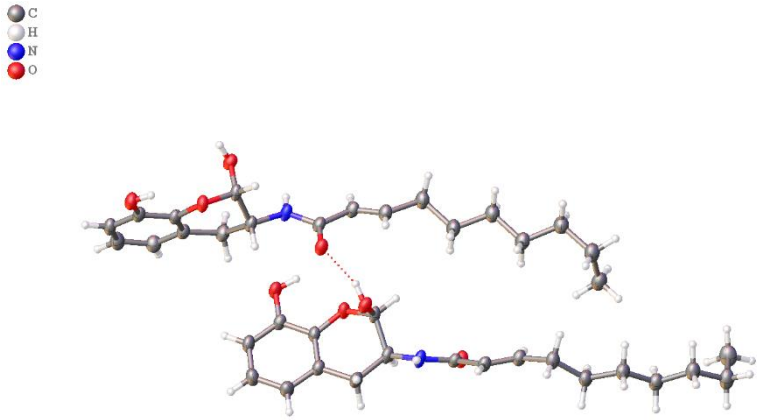


Figure 3. Thermal ellipsoid representation of **1**.

Table 1. NMR Data for **1**−**4**.

No	**1b**		**2a**		**3**		**4**	
	δ_C[a], mult.	δ_H[b] (*J* in Hz)	δ_C[a], mult.	δ_H[b] (*J* in Hz)	δ_C[a], mult.	δ_H[e] (*J* in Hz)	δ_C[f], mult.	δ_H[g] (*J* in Hz)
1	93.5, CH	5.52, m[c]	92.2, CH	5.47, d (2.0)	63.0, CH_2	3.61, qd (11.3,4.6)	62.6, CH_2	3.33, d
2	47.1, CH	4.51, m[c]	46.1, CH	4.52, m[c]	54.3, CH	3.87, tdd (9.9,8.0,6.3,4.3)	51.5, CH	3.93, q (7.6,7.2)
3a	29.4, CH_2	2.85, dd (16.4, 7.8)	28.3, CH_2	2.88, dd (16.1, 8.9)	33.0, CH_2	2.77, dd (13.9,8.7)	31.4, CH_2	2.47, s
3b		3.09, dd (16.4, 5.5)		3.06, dd (16.1, 5.7)		2.91, dd (13.8,4.1)		2.69, dd (13.7,6.5)
4	119.4, qC		119.1, qC		131.9, qC		125.8, qC	
5	113.7, CH	6.80-6.83, m[c]	129.4, CH	7.07, m[c]	157.2, qC		147.8, qC	
6	120.5, CH	6.59-6.63, m[c]	121.3, CH	6.83-6.95, m[c]	120.2, CH	6.74, d (7.[illegible]2.3)	115.3, CH	6.57, d (8.5)
7	122.5, CH	6.80-6.83, m[c]	127.9, CH	7.14, m[c]	128.8, CH	7.06, m	113.3, CH	6.40, dd (8.5,2.9)
8	145.1, qC		117.0, CH	6.83-6.95, m[c]	116.2, CH	6.84, m	149.5, qC	
9	139.2, qC		151.2, qC		125.[illegible] CH	7.09, m	117.2, CH	6.50, d (2.9)
10	167.8, qC		166.8, qC		147.6, qC		164.8, qC	
11	121.8, CH	5.80, d (15.2)	122.5, CH	5.78, dt (15.1, 1.4)	125.0, CH	6.06, d (15.3)	124.7, CH	5.89, d (15.4)
12	147.5, CH	6.92, m[c]	146.6, CH	6.83-6.95, m[c]	144.7, CH	6.81, m	142.1, CH	6.53, m
13	32.3, CH_2	2.16, m[c]	32.0, CH_2	2.18, m[c]	32.5, CH_2	2.21, m	31.2, CH_2	2.10, m
14	29.2, CH_2	1.42, m[c]	28.2, CH_2	1.43, m[c]	28.8, CH_2	1.50, p (7.3)	28.1, CH_2	1.38, p (7.0)
15	31.9, CH_2	1.25-1.28, m[c]	29.1, CH_2	1.24-1.29, m[c]	26.3, CH_2	1.39, p (7.4)	27.8, CH_2	1.25, m[c]
16	28.5, CH_2	1.25-1.28, m[c]	29.0, CH_2	1.24-1.29, m[c]	29.3, CH_2	1.63, p (6.9)	28.4, CH_2	1.27, m[c]
17	28.2, CH_2	1.25-1.28, m[c]	28.[illegible], CH_2	1.24-1.29, m[c]	64.7, CH_2	4.03, t (6.7)	25.3, CH_2	1.26, m[c]
18	22.8, CH_2	1.25-1.28, m[c]	22.6, CH_2	1.24-1.29, m[c]			28.5, CH_2	1.54, q (6.8)
19	14.2, CH_3	0.87, m[c]	13.0, CH_3	0.87, m[c]			63.8, CH_2	3.97, t (6.7)
1'					171.1, qC		170.4, qC	
2'					20.9, CH_3	1.98, s	20.7, CH_3	1.99 s
OH-1								4.70
OH-5								8.63
OH-8								8.53
NH						7,52, d (6.3)		7.73, d (8.0)

[a] Recorded at 125 MHz in $CDCl_3$. [b] Recorded at 500 MHz in $CDCl_3$. [c] Signals overlapped. [d] Recorded at 125 MHz in acetone-d_6. [e] Recorded at 500 MHz in acetone-d_6. [f] Recorded at 125 MHz in DMSO-d_6. [g] Recorded at 500 MHz in DMSO-d_6.

Table 2. NMR Data for **5**.

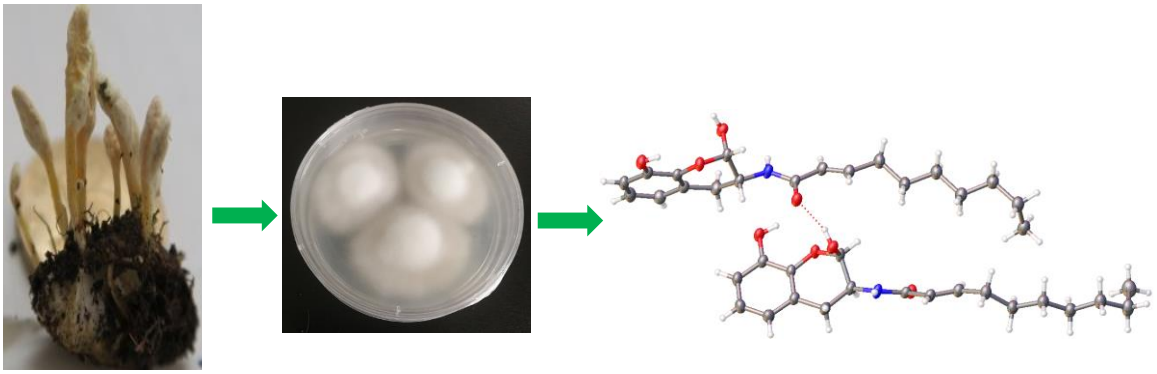
No	**5**	
	δ_C[a], mult.	δ_H[b] (*J* in Hz)
2	167.7, qC	
3	63.2, CH	4.39, t (4.1)
5	166.7, qC	
6	81.8, CH	3.15, m
7	30.4, CH	2.32, m
8	19.0, CH_3	0.87, d (7.0)
9	15.5, CH_3	0.77, d (6.7)
10a	36.6, CH_2	3.13, m
10b		3.25, dd (14.3, 3.7)
11	125.5, qC	
12	131.3, CH	6.97, brd (8.5)
13	116.5, CH	6.77, brd (8.5)
14	156.9, qC	
15	116.5, CH	6.77, brd (8.5)
16	131.1, CH	6.97, brd (8.5)
17	33.0, CH_3	3.06, s

[a] Recorded at 125 MHz in $CDCl_3$. [b] Recorded at 500 MHz in $CDCl_3$.

Table 3. NMR Data for **6**.

No	**6**	
	δ_C[a], mult.	δ_H[a] (*J* in Hz)
1	174.8, qC	
2	33.9, CH_2	2.16, t (7.4)
3	24.8, CH_2	1.47, t (6.9)
4	28.9, CH_2	1.23-1.30, m[c]
5	28.6, CH_2	1.34, m[c]
6	32.0, CH_2	2.04, t (7.4)
7	134.8, CH	5.68, m
8	129.7, CH	6.03, dd (15.2, 10.5)
9	132.6, CH	6.18, dd (15.3, 10.4)
10	131.1, CH	5.37, dd (15.3, 7.8)
11	76.7, CH	4.13, q (7.1)
12a	35.4, CH_2	1.52, m
12b		1.40, m
13	30.9, CH_2	1.23-1.30, m[c]
14	28.4, CH_2	1.23-1.30, m[c]
15	28.8, CH_2	1.23-1.30, m[c]
16	24.6, CH_2	1.23-1.30, m[c]
17	22.0, CH_2	1.23-1.30, m[c]
18	13.9, CH_3	0.86, t (6.9)
1’	99.4, CH	4.06, d (7.8)
2’	73.7, CH	2.94, m
3’	76.6, CH	3.22, t (8.9)
4’	79.5, CH	2.90, t (9.0)
5’	75.5, CH	2.99, m
6a’	60.7, CH_2	3.59, d (10.8)
6b’		3.47, d (5.3)
4'-OCH_3	59.5, CH_3	3.40, s

[a] Recorded at 125 MHz in DMSO-d_6. [b] Recorded at 500 MHz in DMSO-d_6. [c] Signals overlapped.



Unidentified insect *Cordyceps* sp. Thermal ellipsoid representation of **1**

Graphical abstract