Unusual Spirodecane Sesquiterpenes and a Fumagillol Analogue from *Cordyceps ophioglossoides*

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Investigation of the cultured mycelia of *Cordyceps ophioglossoides* resulted in the isolation and characterization of three new unusual spiro[4.5]decane sesquiterpenes, cordycepol A (**1**), cordycepol B (**2**), and cordycepol C (**3**), and a new fumagillol analogue, cordycol (**4**). Their structures were established by spectroscopic means. The cytotoxic activities were also evaluated, compounds **3** and **4** showing their IC_{50} values in the range of 12–33 μg/ml against HeLa and HepG2 (*Table 3*). In addition, **3** and **4** were not obviously harmful towards normal liver cell lines LO2, showing IC_{50} values above 80 μg/ml.

Introduction. – *Cordyceps*, such as *Cordyceps sinensis*, are rich sources of novel biologically active substances with diverse structural architecture [1]. *Cordyceps ophioglossoides*, a species of fungicolous fungi colonizing other fungal species, has a unique tiny niche in the kingdom Fungi which encompasses more than 80000 species [2][3]. *C.* ophioglossoides is a parasite of certain types of *Elaphomyces* and has been used as traditional Chinese medicine for hundreds of years as a tonic for human [1][4]. A variety of new secondary metabolites was isolated by *Wicklow* and co-workers from the fungicolous fungi [3][5]. In addition, previous investigations showed that the culture of *C. ophioglossoides* possessed the potent protein kinase C inhibitor balanol, estrogenic activities and a protecting effect towards *Alzheimer*'s dementia [6–10]. However, owing to human over-exploitation, the wild resource of *C. ophioglossoides* faced extinction. Therefore, producing the anamorphic forms in bioreactors became a necessity. Our lab purified and identified this fungus from fruit body of *C. ophioglossoides* and studied the optimal medium composition and culture conditions for submerged culture on the basis of ecological considerations. The productivity of *C. ophioglossoides* reached 20.2 g l^{-1}, and a large-scale fermentation became feasible to meet needs of human consumption [10]. After artifical cultivation of this fungus, we carried out some research. This paper deals with the investigation of chemical constituents and their bioactivities from the cultured mycelia of *C. ophioglossoides*. Three new unusual spiro[4.5]decane sesquiterpenes, cordycepol A–C[1]) (**1–3**) and a

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*

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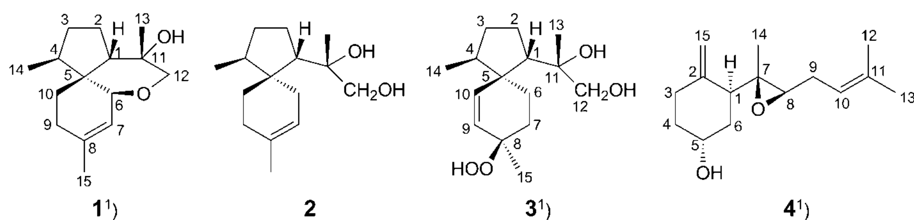
new fumagillol analogue, cordycol[1]) (**4**), were isolated and their structures were elucidated (fumagillol = (3*R*,4*S*,5*S*,6*R*)-5-methoxy-4-[(2*R*,3*R*)-2-methyl-3-(3-methylbut-2-en-1-yl)oxiran-2-yl]-1-oxaspiro[2.5]octan-6-ol). The antitumor activities of **3** and **4** against human cancer cell lines HeLa, A549, HepG2, and MCF-7 were also evaluated.



Results and Discussion. – The EtOH extracts of the cultured mycelia of *C. ophioglossoides* were subjected to repeated column chromatography on silica gel and *Sephadex LH-20* and to prep. HPLC (*C18*), to afford the four new sesquiterpenes **1**–**4**.

Compound **1** was isolated as optically active, colorless needles. The molecular formula was determined to be $C_{15}H_{24}O_2$ by analysis of the HR-ESI-MS ion peak at *m/z* 237.1838 ($[M+H]^+$). The IR spectrum suggested the presence of an OH group (3428 cm^{-1}) and a cyclic ether moiety (1072 cm^{-1}). The ^{13}C-NMR spectrum (*Table 1*) displayed 15 signals, comprising three Me groups (δ(C) 25.1, 16.3, and 23.3), four CH_2 groups (δ(C) 17.6, 29.4, 30.0, and 19.2), an OCH_2 group (δ(C) 77.9), two CH groups (δ(C) 56.2 and 43.7), an OCH group (δ(C) 81.3), a quaternary C-atom (δ(C) 42.5), an oxygenated quaternary C-atom (δ(C) 71.3), and a disubstituted C=C bond (δ(C) 120.1 and 142.1). The skeleton of a sesquiterpene was substantiated further by the molecular formula $C_{15}H_{24}O_2$. The ^{1}H-NMR spectrum of **1** (*Table 1*) displayed the general features of spiro[4.5]decane sesquiterpenes [11][12], which are considered as biogenetic precursors for the tricyclic cedrane sesquiterpenes [11]. In the COSY plot of **1**, the OCH moiety at δ(H) 3.33 (*d*, *J* = 4.5 Hz, H–C(6)) was coupled with an H-atom at δ(H) 5.69 (*d*, *J* = 4.5 Hz, H–C(7)), and the CH_2 groups at δ(H) 2.10–2.15 and 2.02–2.06 (2 *m*, CH_2(9)) and at δ(H) 2.42–2.48 and 1.43–1.47 (2 *m*, CH_2(10)) were coupled to each other. Another sequence, Me(14)/H–C(4)/CH_2(3)/CH_2(2)/H–C(1), was also observed in the COSY plot (*Fig. 1*). Analysis of the 1D- and 2D-NMR data and comparing with those of α- and β-acorenol led to the identification of the basic framework of **1** as a spiro[4.5]decane sesesquiterpene with an additional six-membered ether ring [11]. The ether ring was deduced to be formed between C(6) and C(12) *via*

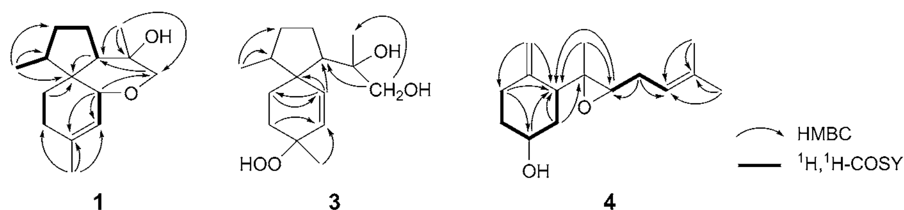


Fig. 1. *Key ^{1}H,^{1}H-COSY and HMBC features of compounds* **1**, **3**, *and* **4**

Table 1. *NMR Data* ($CDCl_3$) *of Compounds* **1**–**3**. δ in ppm, J in Hz.

Position	**1**		**2**		**3**	
	δ(H)	δ(C)[a])[b])	δ(H)	δ(C)[b])[c])	δ(H)	δ(C)[a])[b])
H–C(1)	1.35 (*dd*, J = 13.4, 7.1)	56.2 (CH)	1.84–1.87 (*overlap*)	56.0 (CH)	1.91–1.96 (*m*)	55.0 (CH)
CH_2(2)	1.65–1.69 (*m*), 1.56–1.60 (*m*)	19.2 (CH_2)	1.79–1.84 (*overlap*), 1.28–1.32 (*m*)	30.6 (CH_2)	1.83–1.88 (*overlap*), 1.32–1.37 (*m*)	29.3 (CH_2)
CH_2(3)	1.90 (*ddd*, J = 15.0, 9.2, 6.8), 1.37–1.41 (*m*)	30.0 (CH_2)	1.81–1.85 (*overlap*)	26.0 (CH_2)	1.83–1.88 (*overlap*)	23.5 (CH_2)
H–C(4)	1.52–1.57 (*m*)	43.7 (CH)	1.77–1.82 (*m*)	42.5 (CH)	1.67–1.72 (*m*)	46.6 (CH)
C(5)		42.5 (C)		44.6 (C)		47.4 (C)
H–C(6) or CH_2(6)	3.33 (*d*, J = 4.5)	81.3 (CH)	1.97–2.01 (*overlap*)	40.4 (CH_2)	1.86–1.90 (*m*), 1.62–1.67 (*m*)	18.0 (CH_2)
H–C(7) or CH_2(7)	5.69 (*d*, J = 4.5)	120.1 (CH)	5.35 (*s*)	121.3 (CH)	2.07–2.12 (*m*), 1.77–1.82 (*m*)	29.6 (CH_2)
C(8)		142.1 (C)		134.3 (C)		80.1 (C)
CH_2(9) or H–C(9)	2.10–2.15 (*m*), 2.02–2.06 (*m*)	29.4 (CH_2)	1.97–2.01 (*overlap*)	28.9 (CH_2)	5.75 (*d*, J = 12.6)	130.8 (CH)
CH_2(10) or H–C(10)	2.42–2.48 (*m*), 1.43–1.47 (*m*)	17.6 (CH_2)	1.87–1.92 (*overlap*)	24.2 (CH_2)	5.51 (*d*, J = 12.6)	141.9 (CH)
C(11)		71.3 (C)		75.5 (C)		75.8 (C)
CH_2(12)	3.71, 3.26 (2*d*, each J = 12.0)	77.9 (CH_2)	3.42, 3.30 (2*d*, each J = 10.8)	70.7 (CH_2)	3.54, 3.29 (2*d*, each J = 13.7)	69.8 (CH_2)
Me(13)	1.09 (*s*)	25.1 (Me)	1.23 (*s*)	21.9 (Me)	1.21 (*s*)	23.6 (Me)
Me(14)	0.99 (*d*, J = 7.0)	16.3 (Me)	0.91 (*d*, J = 6.8)	15.5 (Me)	0.87 (*d*, J = 8.6)	14.4 (Me)
Me(15)	1.69 (*s*)	23.3 (Me)	1.64 (*s*)	23.2 (Me)	1.36 (*s*)	23.8 (Me)

[a]) Recorded at 125 MHz. [b]) Multiplicities inferred from DEPT and HMQC experiments. [c]) Recorded at 100 MHz.

an O-atom bridge based on the HMBC cross-peak δ(H) 3.33 (H–C(6))/δ(C) 77.9 (C(12)). The C=C bond was assigned to C(7) and C(8) from analysis of the HMBC cross-peaks H–C(6)/C(7) and C(8) and Me(15)/C(7) and C(8). The relative configuration of the spiro[4.5]decane framework was shown to be the same as that of the known compound α-acorenol (=(1*R*,4*R*,5*S*)-α,α,4,8-tetramethylspiro[4.5]dec-7-ene-1-methanol) after detailed analysis of the NOESY data of **1** (*Fig. 2*) [11][12]. The H–C(1) at δ(H) 1.35 (*dd*, *J* = 13.4, 7.1 Hz) showed a NOESY correlation with Me(14) at δ(H) 0.99 (*d*, *J* = 7.0 Hz), thus suggesting a β-oriented Me(14) and an α-oriented disubstituted isopropyl unit at C(1). NOESY Cross-peaks Me(14)/H–C(9) and Me(15)/H–C(1) indicated that the partial unit –CO(–)–CH=C has α orientation. The relative configuration of the pyran ring was determined to be a chair with Me(13) in a β-equatorial orientation, based on the NOESY correlations Me(13)/$H_β$–C(12) and $H_α$–C(12) (*Fig. 2*). The new sesquiterpene was given the trivial name cordycepol A, the first sesquiterpene to be isolated from a natural source containing a spiro[4.5]decane C-atom framework with an additional ether bridge. The absolute configuration of **1** (and of **2**–**4**) remains to be determined.

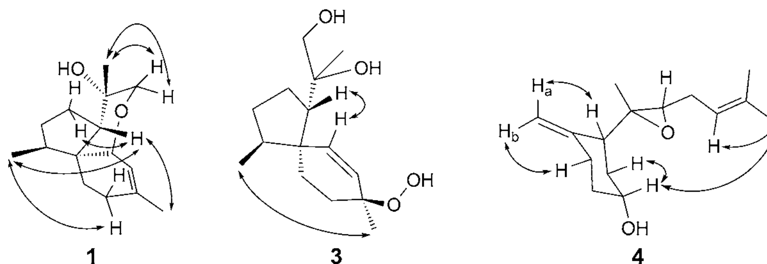


Fig. 2. *Key NOESY or ROESY correlations of compounds* **1**, **3**, *and* **4**

Compound **2** was obtained as colorless needles. The HR-ESI-MS exhibited a molecular-ion peak at *m/z* 261.1824 ([*M* + Na]$^+$), corresponding to the molecular formula $C_{15}H_{26}O_2$. The UV and IR spectra of **2** exhibited similar general patterns as those of **1**. The NMR spectra ($CDCl_3$) of **2** (*Table 1*) also possessed signals characteristic of spiro[4.5]decane-type sesquiterpenes [11–13] and showed similar chemical shifts and the same multiplicities as most C-atoms of **1**, except for an additional CH_2 group in **2** in place of the OCH unit in **1**, indicating that **2** is the ether ring opened derivative of **1**. This inference was further confirmed by detailed analysis of 1D- and 2D-NMR data. The complete ^{1}H- and ^{13}C-NMR signal assignments are listed in *Table 1*. The relative configuration of compound **2** was also assigned as drawn, based on the results of compound **1** and their similar biological origin. Compound **2** was named cordycepol B.

Compound **3** was obtained as colorless oil. The HR-ESI-MS exhibited a molecular-ion peak at *m/z* 293.1737 ([*M* + Na]$^+$), corresponding to the molecular formula $C_{15}H_{26}O_4$. The IR spectrum exhibited a broad OH absorption band at 3433 cm^{-1} and olefin bands at 1645 and 1463 cm^{-1}. When comparing the NMR data of **3** (*Table 1*) with those of **1** and **2** isolated from the same plant, compound **3** was deduced to possess the same substructure of a 2-(3-methylcyclopentyl)propane-1,2-diol as in **2**. The disub-

stituted C=C bond was positioned between C(9) and C(10) from the observation of HMBC cross-peaks H–C(10)/C(1) and C(5), and Me(15)/C(9) (*Fig. 1*). The broad signal at δ(H) 7.39 in the ^{1}H-NMR spectrum of **3** suggested the presence of a hydroperoxy group in the molecule, which was further confirmed by the molecular formula $C_{15}H_{26}O_4$. Since compounds **3** and **2** had the same biosynthetic origin, and the NMR data of the 2-(3-methylcyclopentyl)propane-1,2-diol unit of **3** were in close agreement with those of **2**, the hydroperoxy group must located at C(8) and not at C(11). The relative configuration of **3** was confirmed by a ROESY experiment (*Fig. 2*). The ROESY correlation of the olefin H-atom at δ(H) 5.75 (*d*, $J = 12.6$, H–C(9)) with H_β–C(1) at δ(H) 1.91–1.96 (*m*) indicated that the double bond was on the β-side of the spiro[4.5]decane molecule. Me(15) was deduced to be in an α orientation based on the ROESY cross-peak Me(14)/Me(15). Therefore, the structure of this isolate was elucidated as a new spiro[4.5]decane sesquiterpene hydroperoxide, and was given the trivial name cordycepol C.

Compound **4** was obtained as colorless oil. The TOF-EI-MS of **4** exhibited a molecular ion at *m/z* 236.1778 (M^+), which was in accordance with the molecular formula $C_{15}H_{24}O_2$. The IR spectrum showed OH and C=C bond absorptions at 3423, 1644, and 1442 cm^{-1}. The ^{1}H- and ^{13}C-NMR spectra indicated that compound **4** was a 5-demethoxyfumagillol derivative (*Table 2*) [14]. The NMR data of the unique pendant in the structure of **4**, *i.e.*, of the 2-methyl-3-(3-methylbut-2-en-1-yl)oxiranyl substituent, were very similar to those of the fumagillol derivatives (*Table 2*) [14–16]. This substructure was further confirmed by detailed ^{1}H,^{1}H-COSY, HMBC, and NOESY experiments (*Fig. 1*). The structure of the substituted cyclohexanol unit in **4** was different from that of fumagillol and its analogues since the characteristic OCH_2 signals of the latter at δ(H) 2.86 (*d*, $J = 4.4$ Hz) due to the spiro-linked epoxide moiety were missing [14–16] and replaced by the signals of an exocyclic CH_2=C moiety at δ(H) 4.81 (*s*) and 4.58 (*d*, $J = 1.5$ Hz). The exocyclic CH_2=C bond was located at C(2) based on the long-range correlations of CH_2(15) with C(1) and C(3). The HMBC cross-peaks CH_2(3)/C(5) and H–C(5)/C(1) indicated that C(5) was hydroxylated. The ^{1}H-NMR data of H–C(5) of **4** was in close agreement with that of the known demethoxyfumagillol with a 5α-positioned OH group [11]. This inference was further supported by

Table 2. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.; $CDCl_3$) *of Compound* **4**. δ in ppm, J in Hz.

Position	δ(H)	δ(C)[a]	Position	δ(H)	δ(C)[a]
H–C(1)	2.17–2.22 (*m*)	45.9 (CH)	H–C(8)	2.79 (*t*, $J = 6.4$)	63.2 (CH)
C(2)		148.0 (C)	H_a–C(9)	3.74–3.78 (*m*)	27.8 (CH_2)
H_α–C(3)	2.43 (*dd*, $J = 13.8$, 4.7)	30.2 (CH_2)	H_b–C(9)	2.17–2.22 (*m*)	
H_β–C(3)	2.14 (*dt*, $J = 13.8$, 4.5)		H–C(10)	5.20 (*t*, $J = 7.3$)	118.7 (CH)
H_α–C(4)	1.61–1.65 (*m*)	34.0 (CH_2)	C(11)		134.3 (C)
H_β–C(4)	1.77 (*dtd*, $J = 13.8$, 4.5, 2.0)		Me(12)	1.65 (*s*)	17.9 (Me)
H_β–C(5)β	4.18–4.23 (*m*)	65.8 (CH)	Me(13)	1.76 (*s*)	25.7 (Me)
H_α–C(6)	1.69–1.73 (*m*)	35.7 (CH_2)	Me(14)	1.26 (*s*)	13.7 (Me)
H_β–C(6)	1.90–1.94 (*m*)		H_a–C(15)	4.81 (*s*)	107.9 (CH_2)
C(7)		61.5 (C)	H_b–C(15)	4.58 (*d*, $J = 1.5$)	

[a]) Multiplicities inferred from DEPT and HMQC experiments.

the NOESY correlation of H–C(5) with H–C(6) in a β-equatorial orientation (*Fig. 2*). Me(13) in the pendant unit showed a NOESY cross-peak with H_β–C(5), indicating a β-oriented pendant as in the known compounds fumagillol and fumagillin [14–16]. Thus, Compound **4** was identified as a new sesquiterpene, and was given the trivial name cordycol.

The *in vitro* cytotoxicity of compounds **1**, **3**, and **4** were evaluated by the MTT (=2-(4,5-dimethylthiazol-2-yl)-3,5-diphenyl-2*H*-tetrazolium bromide) assay, in an attempt to find compounds with any potential values for clinical applications [17][18]. The A549, HepG2, MCF-7, and HeLa cell lines are all common human-tumor cell lines in the laboratory and are representing four tumor entities, *i.e.*, lung carcinoma (A549), hepatic carcinoma (HepG2), breast carcinoma (MCF-7), and cervical carcinoma (HeLa) [19]. Human liver cell lines LO2 were also used to measure selectively the cytotoxticity of the compounds. Cells were cultured with compounds **1**, **3**, and **4** at concentrations of 5, 10, 20, 40 or 80 μg/ml for 24 and 48 h. Compound **2** was too inactive to suggest further tests. The antiproliferation activities (see IC_{50}) of compounds **3** and **4** in a dose- and time-dependent manner were identified and summarized in *Table 3*. Thus compound **3** exhibited a selective cytotoxicity against human cancer cell lines. On the other hand, **3** and **4** were minimally harmful to the normal liver cell line LO_2 even at a concentration of 80 μg/ml. Compound **1** was barely toxic to these 5 cell lines, and its 50% maximal inhibitory concentration (IC_{50}) was above 80 μg/ml.

Table 3. *Growth-Inhibition Effects of Compounds* **3** *and* **4**

Cell line	IC_{50} [μg/ml] of **3**		IC_{50} [μg/ml] of **4**	
	24 h	48 h	24 h	48 h
MCF-7	39.5 ± 2.0	30.8 ± 3.0	> 80	> 80
A549	66.4 ± 5.2	44.8 ± 2.4	40.2 ± 2.44	26.4 ± 3.1
HepG2	37.9 ± 5.1	33.0 ± 3.9	36.2 ± 4.0	30.4 ± 1.4
HeLa	18.9 ± 3.2	12.0 ± 1.8	21.1 ± 7.1	15 ± 0.28
LO2	> 80	> 80	> 80	> 80

IC_{50} (24 h, 48 h) of positive control doxorubicin < 2 μg/ml

Cordyceps showed its prospects in clinical therapy as a popular and effective folk medicine [1][20]. Nucleosides, polysaccharides, cyclopeptides, and other secondary metabolites such as alkaloids, *p*-terphenyl derivatives, and epipolythiodioxopiperazines were found in *Cordyceps* with antitumour, antioxidation, antiinflammatory, antimicrobial, and immunopotentiation activities [20–26]. But, to the best of our knowledge, no sesquiterpenes were found in this genus. After a systematic chemical investigation, we now found three new unusual spiro[4.5]decane sesquiterpenes and a new fumagillol analogue. So far, these spirocyclic sesquiterpenes have always been found in plants; it is a novelty to be isolated from fungi [27]. Compound **3**, a hydroperoxide having the same C-atom skeleton as **2**, showed selective cytotoxic activities. Since **2** was not harmful to cell lines, this suggested that the hydroperoxy group may play an important role in the activities. The O–O bond is relatively weak and usually forms radicals of the form RO• [17], which are highly reactive and unstable. They will start a chain reaction beginning with lipid peroxidation or oxidative DNA damage that eventually leads to the cell's

death [28]. Tumor cells, which are always low in manganese superoxide dismutase activity, the copper and zinc superoxide dismutase activity and catalase activity, are easier to be damaged by an additional radical stress [18][28]. This may be the reason why compound **3** exhibited selective antitumor activities. Cordycol (**4**), a fumagillol analogue, also showed selective cytotoxic activites, further investigation should be carried out to identify its potential angiogenesis effects [29].

In conclusion, we identified three new unusual spiro[4.5]decane sesquiterpenes, cordycepol A (**1**), cordycepol B (**2**), cordycepol C (**3**), and a new fumagillol analogue, cordycol (**4**), from cultured mycelia of *C. ophioglossoides.* The activities of **3** and **4** suggest that they could be promising lead compounds to treat human hepatic carcinoma.

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Experimental Part

General. Column chromatography (CC): silica gel (200–300 mesh; *Qingdao Marine Chemical Group*) and *Sephadex LH-20* (*Amersham*). TLC: precoated plates silica gel *60 F_{254}* of 0.25 mm thickness (SiO_2; *Merck*). Prep. HPLC: *Agilent-1100* system equipped with a *Venusil MP-C_{18}* column (10 mm × 250 mm, *Agela Technologies*). M.p.: *Reichert* apparatus. UV Spectra: *Jasco-UV-2200* UV/VIS spectrophotometer; λ_{max} (log ε) in nm. Optical rotations: *Perkin-Elmer-341* polarimeter. IR Spectra: *Nicolet-Avatar-360* FT-IR spectrometer; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker-AM-400* or *-DRX-500* NMR spectrometer; at 500 or 400 (^{1}H) and 125 or 100 MHz (^{13}C), and at 25°; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-FT-ICR-MS: *Bruker-Apex-III* spectrometer; in *m/z*. ESI-MS: *Bruker-Esquire-3000plus* spectrometer; in *m/z*.

Fungal Material and Cultivation Conditions. A strain of *Cordyceps ophioglossoides*, named *C. ophioglossoides* L2, was isolated from Xi Shuang Ban Na in Yunnan Province and identified by its ITS-5.8s rDNA sequences (GenBank accession No. EU586043). It is now stored in the China General Microbiological Culture Collection Center (CGMCC), with the strain number 1146 [6]. The culture medium consisted of (g l^{-1}) sucrose (66.0 g), yeast extract (10.0 g), silkworm chrysalis (30.0 g), $MgSO_4 \cdot 7\,H_2O$ (0.4 g), and KH_2PO_4 (0.4 g). The fermentation was carried out first in a 15 l fermentor with two six-bladed disc impellers (*Biostar*, Shanghai GuoQiang, P. R. China) for 48 h and then subcultured to a 100 l fermentor for 72 h.

Extraction and Isolation. The whole culture broth of *C. ophioglossoides* (300 l) was initially filtered, and the air-dried mycelium (3.27 kg) was soaked at r.t. with 50% EtOH (4 × 100 l, each soaking for 2 d). The EtOH extract was reduced to a convenient volume (1 l) *in vacuo* and extracted with AcOEt (5 l) to give a concentrated residue (64 g). The latter was subjected to CC (SiO_2), gradient $CHCl_3$/MeOH 1:0 → 1:1): *Fractions A–F. Fr. B* (eluted by 2% MeOH) was further separated by CC (SiO_2, petroleum ether/acetone 50:1 → 1:1): *Frs. B_1–B_5. Fr. B_2* (eluted by 10% acetone) was further purified by CC (*Sephadex LH-20*, $CHCl_3$/MeOH 1:1); then repeatedly (SiO_2, CH_2Cl_2/acetone 20:1) to afford **4** (11.6 mg) and a rest that was purified by prep. HPLC ($MeCN/H_2O$ 60:40 → 85:15 within 25 min, flow rate 3 ml/min) to afford **1** (2.3 mg) and **2** (12.2 mg). *Fr. C* (eluted by 10% MeOH) was subjected to CC (*Sephadex LH-20*, $CHCl_3$/MeOH 1:1) and then prep. HPLC ($MeCN/H_2O$ 40:60 → 60:40 within 20 min, flow rate 3 ml/min): **3** (8.6 mg).

Cordycepol A (= rel-*(4a*R*,7*R*,7a*R*,10*R*,10a*S*)-2,4a,6,7,7a,8,9,10-Octahydro-3,7,10-trimethyl-1*H*-benzo[*b*]cyclopenta[*c*]pyran-7-ol*; **1**): Colorless needles from MeOH. M.p. 98–100°. $[\alpha]_D^{24} = -129.1$ ($c = 0.08$, MeOH). UV (MeOH): 204 (4.11). IR: 3428, 2955, 2932, 1463, 1377, 1193, 1072, 988, 806. ^{1}H- and ^{13}C-NMR: *Table 1*. ESI-MS: 236 ([M + H]$^+$). HR-FT-ICR-MS: 237.1838 ([M + H]$^+$, $C_{15}H_{25}O_2^+$; calc. 237.1849).

Cordycepol B (= rel-*(2*R*)-2-[(1*R*,4*R*,5*S*)-4,8-Dimethylspiro[4.5]dec-7-en-1-yl]propane-1,2-diol*; **2**): Colorless needles from MeOH. M.p. 48 – 50°. $[\alpha]_D^{24} = -48.6$ ($c = 0.25$, $CHCl_3$). UV (MeOH): 203 (4.07). IR: 3407, 3014, 2956, 2923, 2877, 2836, 1657, 1460, 1439, 1377, 1161, 1142, 1053, 1042, 967, 892, 802, 593. ^{1}H- and ^{13}C-NMR: *Table 1*. ESI-MS: 261 ($[M + Na]^+$). HR-FT-ICR-MS: 261.1824 ($[M + Na]^+$, $C_{15}H_{26}NaO_2^+$; calc. 261.1830).

Cordycepol C (= rel-*(2*R*)-2-[(1*R*,4*R*,5*S*,8*S*)-8-Hydroperoxy-4,8-dimethylspiro[4.5]dec-8-en-1-yl]-propane-1,2-diol*; **3**): Colorless oil. $[\alpha]_D^{24} = -38.6$ ($c = 0.28$, $CHCl_3$). UV (MeOH): 204 (3.98). IR: 3433, 2956, 2925, 2870, 1711, 1645, 1463, 1377, 1273, 1037, 933, 795, 548. ^{1}H- and ^{13}C-NMR: *Table 1*. ESI-MS: 293 ($[M + Na]^+$). HR-FT-ICR-MS: 293.1737 ($[M + Na]^+$, $C_{15}H_{26}NaO_4^+$; calc. 293.1728).

Cordycol (= rel-*(1*R*,3*S*)-4-Methylene-3-[(2*R*,3*R*)-2-methyl-3-(3-methylbut-2-en-1-yl)oxiran-2-yl]-cyclohexanol*; **4**): Colorless oil. $[\alpha]_D^{24} = -67.3$ ($c = 0.08$, MeOH). UV (MeOH): 208 (4.01). IR: 3423, 2932, 2857, 1644, 1442, 1383, 1328, 1261, 1225, 1169, 1077, 1030, 998, 895, 837, 743. ^{1}H- and ^{13}C-NMR: *Table 2*. EI-MS: 236 (M^+). TOF-EI-MS: 236.1778 (M^+, $C_{15}H_{24}O_2^+$; calc. 236.1776).

Cell Viability Assay. Human lung carcinoma A549, human hepatic carcinoma HepG2, human breast carcinoma MCF-7, human cervical carcinoma HeLa and human liver cell line LO2 were obtained from the Institute of Biochemistry and Cell Biology (Chinese Academy of Sciences). The cell viability was measured by the MTT method. Briefly, cells were seeded in 96-well microtiter plates at a density of $5 \cdot 10^3$ cells/well for 24 h. After drug treatment for the indicated times, cells were incubated with MTT (0.5 mg/ml) for 4 h. The formazan precipitate was dissolved in 150 μl of DMSO, and the absorbance was detected at 490 nm with a *Sunrise* microplate reader (*Tecan Group Ltd.*). Compounds were dissolved in DMSO and diluted to the proper concentrations before use, with the concentration of DMSO kept below 0.1% in all assays. DMSO (0.1%) was used as negative control, and doxorubicin (2 μg/ml) was used as the positive control for all assays. Each test was performed in triplicate.

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