JOURNAL OF NATURAL PRODUCTS

NOTE

pubs.acs.org/jnp

Anti-inflammatory Principles from *Cordyceps sinensis*

Mei-Lin Yang,[†,§] Ping-Chung Kuo,[‡,§] Tsong-Long Hwang,[⊥] and Tian-Shung Wu[*,†,||]

[†]Department of Chemistry, National Cheng Kung University, Tainan 701, Taiwan, Republic of China

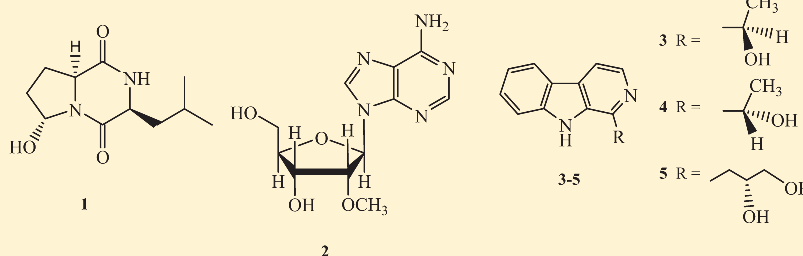
[‡]Department of Biotechnology, National Formosa University, Yunlin 632, Taiwan, Republic of China

[⊥]Graduate Institute of Natural Products, College of Medicine, Chang Gung University, Taoyuan 333, Taiwan, Republic of China

[||]Department of Pharmacy and Chinese Medicine Research and Development Center, China Medical University, Taichung 401, Taiwan, Republic of China

S Supporting Information

ABSTRACT:



In order to explore the anti-inflammatory principles of the mycelia of *Cordyceps sinensis*, the crude extract and partially purified fractions were examined for their inhibition of superoxide anion generation and elastase release. Further chemical investigation of the bioactive fractions has resulted in the identification of 50 compounds, including five constituents, cordysinins A−E (**1**−**5**), reported from a natural source for the first time. In addition, compounds were examined for their anti-inflammatory activity. 1-(5-Hydroxymethyl-2-furyl)-β-carboline displayed the most significant inhibition of superoxide anion generation and elastase release with IC_{50} values of 0.45 ± 0.15 and 1.68 ± 0.32 μM, respectively.

The *Cordyceps* genus, including *C. sinensis*, *C. militaris*, *C. pruinosa*, and *C. ophioglossoides* and more than 700 other species, constitutes a valuable fungal medicinal source in traditional Chinese medicine.[1−3] Among these species, *Cordyceps sinensis* is a parasitic fungus growing on the larvae of Lepidoptera, and it has been reported to exhibit various pharmacological activities.[4] It has been used for treating coughs, for disorders of the lungs, kidneys, and stomach, and also as a tonic food for invigoration or nutritional supplementation.[5] Previous research results provided evidence that *Cordyceps* species possess antioxidant,[6] anti-inflammatory,[7,8] antitumor,[9,10] immunomodulatory,[1,11] and kidney-protective bioactivities.[12] However, until now there have been no reports of the anti-inflammatory compounds from *C. sinensis*. In the present paper the crude extracts and partial purified fractions of mycelia of *C. sinensis* were examined for their inhibitory effects on superoxide anion generation and elastase release by human neutrophils in response to *N*-formyl-methionyl-leucyl phenylalanine/cytochalasin B (FMLP/CB). Several fractions exhibited significant inhibition of superoxide anion generation and elastase release, and these fractions were investigated for their chemical constituents and bioactivities.

Dried mycelia of *C. sinensis* were extracted with ethanol, and the concentrated extracts (CSE) were suspended in H_2O and partitioned with *n*-hexane to afford *n*-hexane-soluble (CSEH) and water-soluble fractions, respectively. The *n*-hexane fraction was partitioned between *n*-hexane and MeOH−H_2O (70:30) to yield a *n*-hexane/*n*-hexane fraction (CSEHH) and a *n*-hexane/MeOH−H_2O fraction (CSEHM). The water fraction was further extracted with ethyl acetate to an afford an ethyl acetate fraction (CSEE) and a water-soluble fraction (CSEW), respectively. The crude extract and the partially purified fractions were evaluated for their effects on superoxide anion generation and elastase release. At the tested concentration (10 μg/mL), CSEHH, CSEHM, and CSEE fractions displayed significant inhibition of superoxide anion generation and elastase release (44.02% and 70.76%; 94.99% and 97.10%; 82.73% and 97.49%, respectively). Thus these fractions were subjected to further purification to afford five compounds, cordysinins A−E (**1**−**5**), reported from the natural source for the first time, along with 45 known compounds, **6**−**50**. The structures of these constituents were established on the basis of 1D and 2D NMR and mass spectroscopic analyses and also by chemical transformation.

Compound **1** was isolated as an optically active, colorless powder and by HR-FABMS showed a pseudomolecular formula of $C_{11}H_{19}O_3N_2$. The IR absorption bands at 3402, 3236, and

Received: June 23, 2010
Published: August 17, 2011

ACS Publications Copyright © 2011 American Chemical Society and American Society of Pharmacognosy

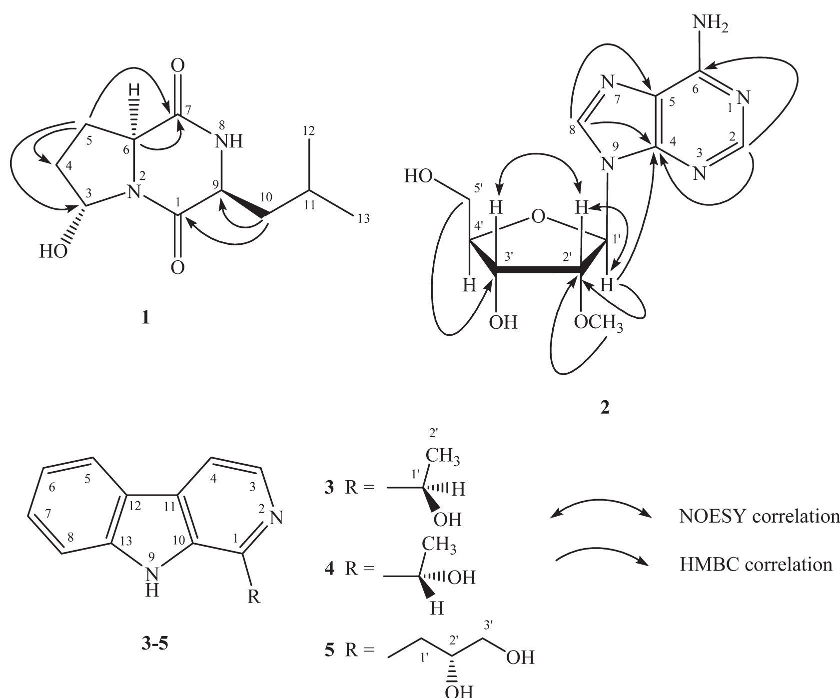


Figure 1. Chemical structures and significant NOESY and HMBC correlations of **1**−**5**.

1658 cm^{-1} indicated the presences of the amine, hydroxyl, and amide carbonyl groups. In the ^{1}H NMR spectrum, the proton signals of **1** were very similar with those of cyclo(L-Leu-L-Pro).[13] The only spectral differences were the appearance of one oxygenated methine group at δ 4.44 (1H) in **1** and the disappearance of one methylene group. This indicated that the basic skeleton of **1** was the same as cyclo(L-Leu-L-Pro) and one of the methylene carbons in **1** was hydroxylated. In addition, the HMBC experiment also displayed long-range correlations between δ 3.38 (H-4) and 68.8 (C-3) and between δ 2.20 (H-5) and 68.8 (C-3), which suggested the C-3 was hydroxylated. The absolute configuration at C-3 was further confirmed as *R* by Mosher's method.[14] Therefore the structure of **1** was established as cyclo(L-leucine-L-hydroxyproline) (Figure 1). It is a new compound and named trivially as cordysinin A.

The molecular formula of compound **2** was established as $C_{11}H_{15}N_5O_4$ by the HR-ESIMS analysis. The UV absorption maxima at 262 and 207 nm and the IR absorption bands at 3337, 2929, 1645, and 1600 cm^{-1} corresponded to the presence of an adenosine derivative.[15] The ^{1}H NMR and ^{13}C NMR spectra suggested the presence of adenosine,[15] and the only difference between adenosine and **2** is one more methoxyl singlet at δ 3.42 (3H), which displayed ^3J-HMBC correlation with the carbon signal at δ 84.6 (C-2′), indicating that the methoxyl substitution was located at C-2′. The stereochemistry of the sugar moiety of **2** was determined by the coupling constants and NOESY correlation spectrum. Thus the chemical structure of **2** was established as shown in Figure 1. Although this compound had already been reported in the previous synthesis work,[16] this is the first report from a natural source, and it was trivially named cordysinin B (**2**).

The mixture of enatiomers **3** and **4** was purified through the assistance of reversed-phase HPLC, and the molecular formula was established as $C_{13}H_{12}N_2O$ using HR-ESIMS. The UV absorption maxima characteristic of a β-carboline chromophore at 349, 302, 289, and 251 nm[17] and the infrared absorption bands at 3268, 1620, and 1424 cm^{-1} assignable to hydroxyl, carbon−carbon double-bond, and carbon−nitrogen double-bond functionalities suggested a mixture of enatiomers of a β-carboline derivative. In the ^{1}H NMR spectrum, two mutually coupled doublets with a small coupling constant (*J* = 5.4 Hz) at δ 8.20 and 7.99 were indicative of H-4 and H-3 protons of a β-carboline alkaloid skeleton. A set of four mutually coupled aromatic protons at δ 8.17, 7.64, 7.54, and 7.25 suggested that the aromatic ring A of β-carboline was unsubstituted. In addition, two mutually coupled proton signals at δ 5.33 and 1.65 indicated the presence of a CH_3CH- fragment. On the basis of the above spectroscopic evidence, this enantiomeric mixture was concluded to be 1-(9*H*-β-carbolin-1-yl)ethanol, which had been previously reported as a synthetic product,[18] but no enantiomeric purity was determined. Thus with the aid of HPLC equipped with a chiral column the relative composition of this mixture was analyzed and the enantiomers were purified as cordysinins C (**3**) and D (**4**), in which the area percentages of **3** and **4** were 49.5% and 50.5%, respectively. To determine the stereochemistry at C-1′, Mosher's reagents were utilized to prepare the (*S*)- and (*R*)-MTPA esters of **3** and **4**.[14] The absolute configuration at C-1′ in **3** was concluded as *R*, and that of **4** was determined as *S* (Figure 1).

The molecular formula of cordysinin E (**5**) was determined as $C_{14}H_{14}N_2O_2$. The UV and IR spectra of **5** exhibited characteristic absorption maxima and bands of a β-carboline chromophore. In the ^{1}H NMR spectrum, two *ortho*-coupled doublets at δ 8.46 and 8.30 with a small coupling constant (*J* = 5.0 Hz) and a set of four mutually coupled symmetrical-type aromatic protons were similar to those of β-carboline. Moreover, five mutually coupled protons at δ 3.75 (1H, m), 3.55 (2H, m), 2.06 (1H, dd, *J* = 13.5, 4.8 Hz), and 1.87 (1H, dd, *J* = 13.5, 8.6 Hz) in the ^{1}H NMR spectrum along with the carbon signals at δ 72.8, 67.4, and 29.0 in the ^{13}C NMR spectrum suggested the presence of a $-CH_2CHOHCH_2OH$ substituent. In the HMBC experiment, the ^2J- and ^3J-correlations constructed the 1-substituted

β-carboline basic skeleton. The absolute configuration at C-2′ was determined by comparison of the sign of optical rotation and the CD spectrum of **5** with those of cordysinin C (**3**) and cordysinin D (**4**). Compound **5** displayed a positive optical rotation that was the same sign as that of cordysinin D (**4**). In addition, the CD measurement of **3** displayed a positive Cotton effect in the 360−380 nm regions, and in contrast compound **4** exhibited a negative Cotton effect in the same regions. Comparatively, the CD spectrum of **5** showed a negative Cotton effect, which suggested that the spatial arrangement of the functional group at C-2′ was the same as that of cordysinin D (**4**).[19] Therefore, the chemical structure of new compound **5** was characterized as shown.

Forty-five known compounds were identified by comparison of their spectral and physical data (see Supporting Information). Those purified compounds isolated in sufficient quantity were evaluated for inhibition of $O_2^{\bullet-}$ generation and elastase release by human neutrophils in response to FMLP/CB (Table 1).[20] Compounds ergosteryl-3-*O*-β-D-glucopyranoside (**39**) and perlolyrine (**43**) inhibited superoxide anion generation and elastase release in FMLP/CB-activated human neutrophils in a concentration-dependent manner. The compound perlolyrine demonstrated the most significant inhibition toward superoxide anion generation and elastase release with IC_{50} values of 0.45 ± 0.15 and 1.68 ± 0.32 μM, respectively, compared with the reference compound indomethacin (IC_{50} of 38.32 ± 5.38 and 31.98 ± 6.49 μM, respectively). The formation of $O_2^{\bullet-}$ in neutrophils can be inhibited by modulating cellular signaling pathways, but also by direct radical scavenging. Therefore the purified constituents were also subjected to the DPPH radical scavenging assay in cell-free systems (data not shown).[21] Among the tested compounds, only 3′,4′,7-trihydroxyisoflavone displayed significant scavenging of DPPH free radicals with IC_{50} values of 31.97 μM, respectively, compared with the reference compound vitamin C (IC_{50} of 38.63 μM). None of the other constituents significantly inhibited free radical scavenging at 500 μM.

Table 1. Inhibitory Effects of Purified Samples on Superoxide Anion Generation and Elastase Release by Human Neutrophils in Response to FMLP/CB

	IC_{50} (μg/mL)[a] or (Inh %)[b]	
compound	superoxide anion generation	elastase release
1	(11.34 ± 4.95)	(13.02 ± 3.20)
16	(28.25 ± 2.73)[d]	(41.59 ± 6.25)[d]
17	6.19 ± 1.25	(24.22 ± 6.57)[c]
25	(5.59 ± 3.26)	(7.03 ± 3.76)
28	(6.59 ± 2.11)	(2.79 ± 3.64)
30	(12.83 ± 6.94)	(10.37 ± 0.55)
31	(5.65 ± 1.64)	(11.72 ± 5.92)
32	(9.61 ± 5.51)	(11.06 ± 6.14)
39	5.42 ± 0.50	5.62 ± 0.37
40	(21.62 ± 6.28)	(7.13 ± 5.55)
43	0.45 ± 0.15	1.68 ± 0.32
48	(6.43 ± 3.72)	(6.83 ± 2.46)
indomethacin	38.32 ± 5.38	31.98 ± 6.49

[a] Concentration necessary for 50% inhibition. [b] Percentage of inhibition (Inh %) at 10 μg/mL concentration. Results are presented as mean ± SEM (n = 3−4). [c] $p < 0.05$ compared with the control value. [d] $p < 0.001$ compared with the control value.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined using a Yanagimoto MP-S3 apparatus. Optical rotations were measured using a JASCO DIP-370 polarimeter. The UV spectra were obtained on a Hitachi UV-3210 spectrophotometer, and IR spectra were recorded on a Shimadzu FTIR-8501 spectrophotometer. NMR spectra were obtained on Bruker AMX-400 and AV-500 NMR spectrometers, with tetramethylsilane (TMS) as internal standard, and the chemical shifts are reported in δ values (ppm). The low- and high-resolution FAB and ESI mass spectra were obtained on JEOL JMS-700 and Bruker APEX II mass spectrometers, respectively. High-performance liquid chromatography (HPLC) was performed on a Shimadzu LC-10AT$_{VP}$ series pumping system equipped with a Shimadzu SPD-M10A$_{VP}$ diode array detector. CD spectra were recorded with a JASCO J-720 spectropolarimeter.

Mycelia. *C. sinensis* mycelia from fermentation in germinated soybean were provided by Taiwan Sugar Company (TSC) on January 2002. A voucher specimen (TSWu_TSC_200201) was deposited in the Department of Chemistry, National Cheng Kung University, Tainan, Taiwan.

Extraction and Isolation. The mycelia of *C. sinensis* were obtained from the medium by centrifugation and dried at room temperature. Mycelia of *C. sinensis* (10 kg) were extracted with ethanol (7 × 50 L) under reflux for 8 h and concentrated to give a brown syrup (2.0 kg, CSE). The extract was suspended in H_2O and partitioned to afford a *n*-hexane/*n*-hexane fraction (650 g, CSEHH), a *n*-hexane/MeOH−H_2O fraction (150 g, CSEHM), an ethyl acetate fraction (110 g, CSEE), and a water-soluble fraction (1090 g, CSEW), respectively.

The CSEHH fraction was purified with silica gel column chromatography eluted by a step gradient of *n*-hexane/ethyl acetate (100:1 to 1:1) to afford seven subfractions. Only subfractions 5 and 6 displayed significant spots, so they were further purified. Subfraction 5 (15.2 g) was column chromatographed over silica gel using a stepwise gradient of *n*-hexane/EtOAc (10:1 to 1:1) to afford **6** (120.0 mg), **7** (4.2 mg), **8** (3.1 mg), **9** (4.0 mg), and **10** (3.0 mg), respectively. Further purification of subfraction 6 (35.0 g) by repeated column chromatography with *n*-hexane/EtOAc gradient mixtures (from 5:1 to 1:1) and preparative TLC purification gave a mixture of **11** and **12** (360.4 mg), a mixture of **13** and **14** (3.8 mg), **15** (45.0 mg), and **16** (10.5 mg), respectively. The CSEHM fraction was separated by column chromatography over silica gel with gradient mixtures of chloroform and methanol (100:1, 75:1, 50:1, 25:1, 10:1, 5:1, 3:1, 1:1, and 0:1) to yield eight subfractions. The second subfraction (4.3 g) was purified with silica gel column chromatography eluted by chloroform/methanol (50:1) and recrystallized with methanol to yield **17** (254.2 mg). Subraction 3 (5.6 g) was further isolated by repeated column chromatography with gradient mixtures of chloroform/methanol (from 50:1 to 1:1) and preparative TLC purification with solvent mixtures of chloroform/methanol (10:1) to afford **18** (100.9 mg) and **19** (8.2 mg). Silica gel column chromatography of subfraction 4 (3.5 g) by a mixture of chloroform/methanol (20:1) and preparative TLC purification with the solvent pair of chloroform/methanol (10:1) yielded **20** (4.0 mg). The fifth subfraction (10.2 g) rechromatographed by a silica gel open column using step gradient mixtures of chloroform/methanol (from 20:1 to 1:1) and further recrystallization with chloroform/acetone afforded **21** (632.5 mg). Repeated silica gel column chromatography of subfraction 6 (13.2 g) with solvent mixtures of chloroform/methanol (10:1) and further recrystallization with chloroform/acetone gave **22** (15.3 mg) and **23** (524.0 mg), respectively.

The ethyl acetate-soluble CSEE fraction was subjected to silica gel column chromatography with step gradient mixtures of chloroform and methanol (20:1, 15:1, 10:1, 5:1, 3:1, 2:1, 1:1, and 0:1), yielding 10

subfractions. Subfraction 2 (3.6 g) was purified with silica gel column chromatography using a stepwise gradient of chloroform/methanol (from 20:1 to 1:1) and successive preparative TLC purification to obtain **24** (5.4 mg), **25** (110.0 mg), **26** (5.1 mg), and **27** (15.3 mg). The third subfraction (3.4 g) was rechromatographed with silica gel by mixing eluents of chloroform/methanol (20:1), and then the resulting fractions were recrystallized with chloroform/methanol to afford **28** (152.5 mg), **29** (5.7 mg), and **30** (70.6 mg), respectively. Column chromatography over silica gel of the subfraction 4 (5.1 g) by a stepwise gradient of chloroform/methanol (from 10:1 to 1:1) and recrystallization of the afforded fractions with chloroform/methanol yielded **31** (220.2 mg) and **32** (65.6 mg), respectively. Further preparative TLC purification of the minor fractions eluting with chloroform/methanol (10:1) afforded **33** (6.4 mg), **34** (3.1 mg), and **35** (4.6 mg), respectively. The fifth subfraction (8.4 g) was purified with open silica gel column chromatography eluting by step gradient mixtures of chloroform−methanol (from 5:1 to 1:1) to afford six minor fractions (5.1−5.6). Fraction 5.1 was recrystallized with acetone to yield **1** (120.0 mg), and preparative TLC purification of the residues gave **36** (4.3 mg). Rechromatography of fraction 5.2 and recrystallization of the resulting minor fractions afforded **37** (48.7 mg) and **38** (35.1 mg). Silica gel column chromatography of fraction 5.3 and recrystallization with chloroform/methanol yielded a white solid, **39** (134.7 mg), and further preparative TLC purification gave the minor constituents **40** (5.2 mg) and **41** (5.0 mg). Fraction 5.4 was rechromatographed with silica gel and further recrystallized with acetone to afford **42** (36.3 mg). The residual soluble was purified by reversed-phase HPLC with a Supelco Discovery HS C-18 (250 × 4.6 mm, 5 μm) column eluted at 0.5 mL/min with MeOH/H_2O (70:30) to give **5** (4.1 mg). Fraction 5.5 was purified by repeated column chromatography and preparative TLC to yield **43** (8.3 mg) and **44** (5.1 mg). Fraction 5.6 was subjected to column chromatography eluted with a gradient of chloroform/methanol (from 50:1 to 5:1) to yield **45** (8.1 mg). Subfraction 7 (3.6 g) of CSEE was divided into five fractions (7.1−7.5) by silica gel column chromatography using a stepwise gradient of chloroform/methanol (3:1 to 1:1). Fractions 7.1 and 7.2 were subjected to preparative TLC to afford **46** (3.5 mg) and **47** (5.2 mg), and **48** (10.1 mg), respectively. Fraction 7.3 was rechromatographed with silica gel open column and preparative TLC to give **49** (3.4 mg). Fraction 7.4 was purified by repeated column chromatography and preparative TLC with chloroform/methanol (5:1) to afford **2** (5.2 mg). With the aid of preparative TLC by mixing eluents of chloroform and methanol (5:1) the enatiomeric mixture of **3** and **4** was isolated from fraction 7.5, and further resolution of this mixture was achieved by reversed-phase HPLC equipped with Chiralcel OD-H (250 × 4.6 mm, 5 μm) at 0.5 mL/min with hexanes/2-propanol (90:10) as eluents to yield **3** (2.6 mg, t_R 14.8 min) and **4** (2.4 mg, t_R 21.4 min), respectively. The subfraction 8 (5.2 g) was rechromatographed with silica gel using a stepwise gradient of chloroform−methanol (2:1 to 1:1), and the resulting fractions were recrystallized with methanol to give **50** (12.3 mg).

*Cordysinin A (**1**):* colorless powder; mp 179−181 °C (acetone); $[\alpha]^{25}_D$ −37.8 (c 0.05, MeOH); IR (neat) ν_{max} 3402, 3236, 1658, 1429, 1308, 1217, 1101 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) δ 6.91 (1H, br s, D_2O exchangeable, NH-8), 4.46 (1H, m, H-6), 4.44 (1H, m, H-3), 4.21 (1H, br s, D_2O exchangeable, OH-3), 4.11 (1H, m, H-9), 3.62 (1H, dd, J = 12.4, 4.4 Hz, H-4), 3.38 (1H, d, J = 12.4 Hz, H-4), 2.20 (1H, m, H-5), 2.08 (1H, m, H-5), 1.97 (2H, m, H-10 and -11), 1.49 (1H, qd, J = 8.7, 8.7 Hz, H-10), 0.95 (6H, m, CH_3-12 and -13); ^{13}C NMR (acetone-d_6, 125 MHz): δ 171.3 (C-7), 167.3 (C-1), 68.8 (C-3), 58.2 (C-6), 54.8 (C-4), 54.0 (C-9), 39.4 (C-10), 38.2 (C-5), 25.4 (C-11), 23.3 (C-13), 22.2 (C-12); FABMS m/z 227 ([M + H]$^+$); HR-FABMS m/z 227.1395 ([M + H]$^+$, calcd for $C_{11}H_{19}O_3N_2$, 227.1396).

*Cordysinin B (**2**):* light yellow needles; mp 220 °C (dec) (MeOH); $[\alpha]^{25}_D$ −47.6 (c 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 262 (4.01), 207 (4.18) nm; IR (neat) ν_{max} 3337, 2929, 1645, 1600, 1464, 1321, 1222, 1094 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) δ 8.33 (1H, s, H-2), 8.19 (1H, s, H-8), 6.06 (1H, d, J = 6.1 Hz, H-1′), 4.49 (1H, dd, J = 5.0, 2.8 Hz, H-3′), 4.43 (1H, dd, J = 6.1, 5.0 Hz, H-2′), 4.16 (1H, ddd, J = 2.8, 2.8, 2.5 Hz, H-4′), 3.89 (1H, dd, J = 12.5, 2.5 Hz, H-5′), 3.76 (1H, dd, J = 12.5, 2.8 Hz, H-5′), 3.42 (3H, s, OCH_3-2′); ^{13}C NMR (CD_3OD, 125 MHz) δ 157.6 (C-5), 153.7 (C-8), 150.1 (C-4), 141.8 (C-2), 121.0 (C-6), 89.2 (C-1′), 88.4 (C-4′), 84.6 (C-2′), 70.9 (C-3′), 63.2 (C-5′), 58.8 (OCH_3-2′); ESIMS m/z 304 ([M + Na]$^+$, 100), 283 (14), 282 (20); HR-ESIMS m/z 304.1023 ([M + Na]$^+$, calcd for $C_{11}H_{15}N_5O_4Na$, 304.1022).

*Cordysinin C (**3**):* light yellow powder; mp 169−171 °C (EtOAc/MeOH); $[\alpha]^{25}_D$ −57.0 (c 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 349 (3.76), 302 (4.09), 289 (4.29), 251 (4.57, sh), 241 (4.63), 236 (4.63), 208 (4.48) nm; IR (neat) ν_{max} 3268, 3072, 2828, 1620, 1578, 1489, 1424, 1310, 1229 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 8.20 (1H, d, J = 5.4 Hz, H-4), 8.17 (1H, d, J = 8.0 Hz, H-5), 7.99 (1H, d, J = 5.4 Hz, H-3), 7.64 (1H, dd, J = 8.2, 0.9 Hz, H-8), 7.54 (1H, ddd, J = 8.2, 8.0, 0.9 Hz, H-7), 7.25 (1H, t, J = 8.0 Hz, H-6), 5.33 (1H, q, J = 6.6 Hz, H-1′), 1.65 (3H, d, J = 6.6 Hz, CH_3-2′); ESIMS m/z 213 ([M + H]$^+$, 86), 195 (100); HR-ESIMS m/z 213.1029 [M + H]$^+$ (calcd for $C_{13}H_{13}N_2O$, 213.1028); CD (MeOH, c 0.00157) $[\theta]_{378}$ +47, $[\theta]_{356}$ −93, $[\theta]_{336}$ +25, $[\theta]_{318}$ +20, $[\theta]_{306}$ −20, $[\theta]_{284}$ +451, $[\theta]_{226}$ +395.

*Cordysinin D (**4**):* yellow powder (EtOAc/MeOH); mp 168−170 °C (EtOAc/MeOH); $[\alpha]^{25}_D$ +60.4 (c 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 350 (3.98), 303 (4.23), 289 (4.49), 251 (4.74, sh), 241 (4.78), 236 (4.82), 209 (4.66) nm; IR (neat) ν_{max} 3268, 3072, 2828, 1620, 1578, 1489, 1424, 1310, 1229 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 8.20 (1H, d, J = 5.4 Hz, H-4), 8.16 (1H, d, J = 7.7 Hz, H-5), 7.99 (1H, d, J = 5.4 Hz, H-3), 7.64 (1H, dd, J = 8.2, 0.9 Hz, H-8), 7.54 (1H, ddd, J = 8.2, 7.8, 0.9 Hz, H-7), 7.25 (1H, t, J = 7.7 Hz, H-6), 5.33 (1H, q, J = 6.6 Hz, H-1′), 1.65 (3H, d, J = 6.6 Hz, CH_3-2′); ESIMS m/z 213 ([M + H]$^+$, 100), 195 (96); HR-ESIMS m/z 213.1029 ([M + H]$^+$, calcd for $C_{13}H_{13}N_2O$, 213.1028); CD (MeOH, c 0.00236) $[\theta]_{383}$ +23, $[\theta]_{349}$ +226, $[\theta]_{312}$ +129, $[\theta]_{301}$ −34, $[\theta]_{281}$ +434, $[\theta]_{252}$ +286, $[\theta]_{217}$ +228.

*Cordysinin E (**5**):* light yellow powder; mp 210 °C (dec) (MeOH); $[\alpha]^{25}_D$ +45.5 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 379 (2.79), 312 (2.84), 283 (3.22), 217 (3.62) nm; IR (neat) ν_{max} 3286, 2911, 1593, 1369, 1265, 1201 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) δ 8.46 (1H, d, J = 5.0 Hz, H-4), 8.30 (1H, d, J = 5.0 Hz, H-3), 8.22 (1H, d, J = 7.9 Hz, H-5), 7.71 (1H, d, J = 8.0 Hz, H-8), 7.59 (1H, t, J = 8.0 Hz, H-7), 7.31 (1H, t, J = 7.9 Hz, H-6), 3.75 (1H, m, H-2′), 3.55 (2H, m, H-3′), 2.06 (1H, dd, J = 13.5, 4.8 Hz, H-1′), 1.87 (1H, dd, J = 13.5, 8.6 Hz, H-1′); ^{13}C NMR (CD_3OD, 125 MHz) δ 143.5 (C-13), 138.6 (C-4), 137.5 (C-10), 136.9 (C-1), 133.3 (C-11), 130.3 (C-7), 122.7 (C-5), 121.7 (C-12), 121.6 (C-6), 120.1 (C-3), 113.5 (C-8), 72.8 (C-2′), 67.4 (C-3′), 29.0 (C-1′); FABMS m/z 243 ([M + H]$^+$, 1), 217 (3), 192 (8), 176 (9), 154 (100); HR-FABMS m/z 243.1135 [M + H]$^+$ (calcd for $C_{14}H_{15}N_2O_2$, 243.1134); CD (MeOH, c 0.00206) $[\theta]_{380}$ +24, $[\theta]_{338}$ +65, $[\theta]_{318}$ −42, $[\theta]_{290}$ −37, $[\theta]_{267}$ +8, $[\theta]_{245}$ −37, $[\theta]_{226}$ −58, $[\theta]_{206}$ +122.

Measurement of $O_2^{\bullet-}$ Generation and Elastase Release. The $O_2^{\bullet-}$ generation and elastase release assays were based on the reported method.[20]

Diphenyl Picrylhydrazyl (DPPH) Assay. The DPPH free radical scavenging assay was based on the reported method.[21]

Statistical Analysis. Results were expressed as mean ± SEM. Computation of 50% inhibitory concentration (IC_{50}) was computer-assisted (PHARM/PCS v.4.2). Statistical comparisons were made between groups using Student's t test. Values of p less than 0.05 were considered to be statistically significant.

■ ASSOCIATED CONTENT

Supporting Information. Extraction and partial purification of the mycelia of *C. sinensis*, the known compounds, and their

references, and the preparation of MTPA esters of **1**, **3**, and **4**, along with the NMR spectra for **1**−**5** are provided. This information is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author
*Tel: 886-6-2757575, ext 65333. Fax: 886-6-2740552. E-mail: tswu@mail.ncku.edu.tw.

Author Contributions
§Both authors provided equal contributions to this work.

ACKNOWLEDGMENT

The authors are thankful for the financial support from the National Science Council, Taiwan, ROC, awarded to T.S.W. This study is supported in part by Taiwan Department of Health Clinical Trial and Research Center of Excellence (DOH 100-TD-B-11-004).

REFERENCES

(1) Ng, T. B.; Wang, H. X. *J. Pharm. Pharmacol.* **2005**, *57*, 1509–1520.
(2) Zhong, J. J.; Xiao, J. H. *Adv. Biochem. Eng. Biotechnol.* **2009**, *113*, 79–150.
(3) Russell, R.; Paterson, M. *Phytochemistry* **2008**, *69*, 1469–1495.
(4) Xiao, J. H.; Chen, D. X.; Liu, J. W.; Liu, Z. L.; Wan, W. H.; Fang, N.; Xiao, Y.; Qi, Y.; Liang, Z. Q. *J. Appl. Microbiol.* **2004**, *96*, 1105–1116.
(5) Ji, D. B.; Ye1, J.; Li, C. L.; Wang, Y. H.; Zhao, J. *Phytother. Res.* **2009**, *23*, 116–122.
(6) Li, S. P.; Zhang, G. H.; Zeng, Q.; Huang, Z. G.; Wang, Y. T.; Dong, T. T.; Tsim, K. W. *Phytomedicine* **2006**, *13*, 428–433.
(7) Won, S. Y.; Park, E. H. *J. Ethnopharmacol.* **2005**, *96*, 555–561.
(8) Kim, H. G.; Shrestha, B.; Lim, S. Y.; Yoon, D. H.; Chang, W. C.; Shin, D. J.; Han, S. K.; Park, S. M.; Park, J. H.; Park, H. I.; Sung, J. M.; Jang, Y.; Chung, N.; Hwang, K. C.; Kim, T. W. *Eur. J. Pharmacol.* **2006**, *545*, 192–199.
(9) Park, C.; Hong, S. H.; Lee, J. Y.; Kim, G. Y.; Choi, B. T.; Lee, Y. T.; Park, D. I.; Park, Y. M.; Jeong, Y. K.; Choi, Y. H. *Oncol. Rep.* **2005**, *13*, 1211–1216.
(10) Shen, Y. D.; Shao, X. T.; Ni, Y. D.; Xu, H.; Tong, X. M. *Zhejiang Da Xue Xue Bao Yi Xue Ban* **2009**, *38*, 158–162.
(11) Han, J. Y.; Im, J.; Choi, J. N.; Lee, C. H.; Park, H. J.; Park, D. K.; Yun, C. H.; Han, S. H. *J. Ethnopharmacol.* **2010**, *127*, 55–61.
(12) Wang, Y.; Yin, H.; Lv, X.; Wang, Y.; Gao, H.; Wang, M. *Fitoterapia* **2010**, *81*, 397–402.
(13) Gautschi, M.; Schmid, J. P. *J. Agric. Food Chem.* **1997**, *45*, 3183–3189.
(14) Li, C. Y.; Wu, T. S. *J. Nat. Prod.* **2002**, *65*, 1452–1456.
(15) Wu, T. S.; Leu, Y. L.; Chan, Y. Y. *J. Chin. Chem. Soc.* **2000**, *47*, 221–226.
(16) Ekiel, I.; Darzynkiewicz, E.; Dudycz, L.; Shugar, D. *Biochemistry* **1978**, *17*, 1530–1536.
(17) Kuo, P. C.; Shi, L. S.; Damu, A. G.; Su, C. R.; Huang, C. H.; Ke, C. H.; Wu, J. B.; Lin, A. J.; Bastow, K. F.; Lee, K. H.; Wu, T. S. *J. Nat. Prod.* **2003**, *66*, 1324–1327.
(18) Yang, M. L.; Kuo, P. C.; Damu, A. G.; Chang, R. J.; Chiou, W. F.; Wu, T. S. *Tetrahedron* **2006**, *62*, 10900–10906.
(19) Wu, T. S.; Chan, Y. Y.; Leu, Y. L.; Wu, P. L.; Li, C. Y.; Mori, Y. *J. Nat. Prod.* **1999**, *62*, 348–351.
(20) Yang, M. L.; Kuo, P. C.; Hwang, T. L.; Chiou, W. F.; Qian, K.; Lai, C. Y.; Lee, K. H.; Wu, T. S. *Bioorg. Med. Chem.* **2011**, *19*, 1674–1682.
(21) Shimada, K.; Fujikawa, K.; Yahara, K.; Nakamura, T. *J. Agric. Food Chem.* **1992**, *40*, 945–948.