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New alkaloidal metabolites from cultures of entomopathogenic fungus *Cordyceps takaomontana* NBRC 101754

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ABSTRACT

Two new alkaloidal metabolites, cordytakaoamides A (**1**) and B (**2**), as well as, 2-[(2-hydroxyethyl) amino] benzoic acid (**3**) and 2*E*-decenamide (**4**), and three known compounds (**5–7**) were isolated from ethyl acetate and *n*-butanol soluble portions of the entomopathogenic fungus, *Cordyceps takaomontana* NBRC 101754. Compounds **3** and **4** were isolated here for first time from natural resources. The chemical structures were established depending upon spectroscopic techniques such as 1D, 2D NMR, and HRMS. The absolute configuration of **1** and **2** was elucidated *via* the total synthesis of **1** as well as the experimental circular dichroism. Compound **3** was confirmed by a signal crystal X-ray analysis.

1. Introduction

The entomopathogenic fungi were reported as promising resources of several novel and bioactive metabolites especially nitrogenous compounds and alkaloids [1]. Recently, several scientists and researchers pay attention to entomopathogenic fungal resources of novel bioactive compounds [2].

The *Ophiocordyceps sinensis* (synonym: *Cordyceps sinensis*) that belonging to the *Cordyceps* genus (Ascomycota) is an important medicinal kind of entomopathogenic fungi and widely used in traditional Chinese herb from ancient times in treatment of heart, liver, kidney and lung diseases, hyperlipidemia, hyperglycemia, renal dysfunction and failure, and arrhythmias [2,3]. About 400 species belonging to *Cordyceps* were documented [2]. The fungi belonging to genus *Cordyceps* are very rich resources for rare and novel secondary metabolites such as cordycepin and cyclodepsipeptides, polyketide [2,4–6], in addition to cerebrosides, steroids, mannitol, adenosine and polysaccharides [7,8]. Several bioactivities were reported such as antiapoptotic [7], anticancer [7], cardiovascular [7,8], anti-inflammatory [8], immunologic [9–11], antitrypanosomal [5], multiple sclerosis diseases of the nervous systems [2,3], hepatoprotection, nephroprotective, and antioxidant activities [10,11].

Herein, we reported (i) the isolation and identification of chemical constituents of the ethyl acetate and *n*-butanol soluble portions of entomopathogenic fungi, *Cordyceps takaomontana* NBRC 101754 for the first time (ii) identification of rare types of alkaloids depending upon NMR techniques, (iii) confirmation of the absolute configuration of **1** and **2** by total synthesis of **1**, as well as circular dichroism, respectively, and (iv) isolation of two nitrogenated compounds (**3** and **4**) for the first time from natural resource.

2. Experimental

2.1. General experimental procedures

Optical rotations were recorded on a JASCO P-2300 polarimeter (Tokyo, Japan). IR spectra were measured on a Shimazu FTIR-8400S instrument (Columbia MD 21046, USA). 1D and 2D NMR spectra were recorded on a Bruker 600 and or 500 NMR spectrometer (MA, USA). The chemical shifts were given in δ (ppm), and coupling constants were reported in Hz. HR-MS spectra were obtained on a JEOL JMS-700 instrument (Tokyo, Japan). Electronic circular dichroism (ECD) was displayed using CD Spectra: JASCO 810 spectropolarimeter. Silica gel 60 (Merck, 230–400 mesh, Merck, Darmstadt, Germany) was used for column chromatography. Precoated silica gel plates (Merck, Kieselgel 60 F_{254}, 0.25 mm, Merck, Darmstadt, Germany) was used for TLC

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analysis. Isolera-one flash chromatography (Biotage; Suite C Charlotte, NC; USA) was used for flash chromatography and purification. High-performance liquid chromatography (HPLC) was performed on a Jasco PU-980 pump intelligent HPLC pump equipped with a Jasco UV-970 intelligent UV/VIS detector at 210 nm. A semi preparative reversed-phase column (Cosmosil C18 column 250 × 10 mm, 5 μm) was used for HPLC.

2.2. Fungal material

The entomopathogenic fungus *C. takaomontana* NBRC 101754 was derived from single-ascospore of stroma that occurring on an unidentified pupa. The voucher strain and specimen (NBRC 101754) was deposited at the Biological Resource Center, NITE (NBRC), Kazusa Kamatari 2-5-8, Kizarashi, Chiba, Japan.

2.3. Fungal culture, fermentation, extraction and isolation

The fungus *C. takaomontana* NBRC 101754 was cultured in potato sucrose medium (at 25 °C, 21 days). Mycelia were separated from the broth by filtration and subsequently extracted with MeOH (2 times, 60 min) in an ultrasonic bath. After concentrating under vacuum, the MeOH crude extract (13.2 g) was then dissolved in distilled H_2O and fractionated with EtOAc and then *n*-butanol (500 ml × 3 times). The EtOAc and *n*-butanol soluble portions were concentrated under vacuum to afford a black gum (6.8 g and 3.7 g, respectively). The EtOAc extract was subjected to silica gel flash CC by using a gradient mobile phase, *n*-hexan-$CHCl_3$-MeOH, to yield 12 fractions that collected to 5 major fractions (CTE-1:CTE-5) after TLC examination. Fraction CTE-2 (1.27 g) was subjected to Sephadex LH-20 CC using $CHCl_3$-MeOH (1:1, *v*/v) as a solvent system and followed by RP-18 HPLC (MeOH − H_2O, 7:3) afforded **1** (9.4 mg) and **7** (5.3 mg). Fraction CTE-3 (869 mg) was subjected to the same steps of chromatography with RP-18 HPLC solvent systems (MeOH − H_2O, 1:1) afforded, **5** (3.2 mg) and **6** (4.7 mg). The *n*-butanol soluble portion was further chromatographed and fractionated *via* silica gel CC by using a *n*-hexan-$CHCl_3$-MeOH as elution system step gradient afforded 4 major fractions (CTB-1: CTB-4) after TLC examination. Fraction CTB-2 (931.2 mg) was eluted by $CHCl_3$-MeOH (1:1, *v*/v) over Sephadex LH-20 CC and followed by RP-18 HPLC (MeOH − H_2O, 3:2) afforded **4** (4.1 mg). By the same, the two sub-fractions (CTB-3A and CTB-3B) were obtained from chromatography of fraction CTB-3 (1.12 g) over Sephadex LH-20 CC eluted with $CHCl_3$-MeOH (1:1, *v*/v). Sub-fraction CTB-3A (142.3 mg) was subjected to RP-18 HPLC (MeOH − H_2O, 3:2) afforded **2** (2.1 mg) and **3** (3.3 mg).

2.4. Spectroscopic data of *1–4*

2.4.1. (S,E)-8-((1-hydroxy-3-phenylpropan-2-yl) amino)-8-oxooct-6-enoic acid (cordytakaoamide A; *1*)

White powder, $[\alpha]_D^{25}$ – 15.38° (*c* 0.1, MeOH); FT-IR: 1511, 1669, and 3397 cm^{-1}; ^1H and ^{13}C NMR (see Table 1); HR-CIMS, *m/z* 306.1668 $[(M + H)^+$; $C_{17}H_{24}NO_4$; calcd; 306.1705], Positive-TOF-ESIMS, *m/z* 328.1527 $[(M + Na)^+$; $C_{17}H_{23}NO_4Na$; calcd; 328.1525], and Negative-TOF-ESIMS, *m/z* 304.1517 $[(M-H)^-$;$C_{17}H_{22}NO_4$; calcd; 304.1549].

2.4.2. (S,E)-10-((1-hydroxy-3-(2-hydroxyphenyl)propan-2-yl) amino)-10-oxodec-8- enoic acid (cordytakaoamide B; *2*)

White powder, $[\alpha]_D^{25}$ – 23.22° (*c* 0.1, MeOH); FT-IR: 1593, 1661, and 3402 cm^{-1}; ^1H and ^{13}C NMR (see Table 1); HR-EIMS, *m/z* 349.1883 $[(M)^+$; $C_{19}H_{27}NO_5$; calcd; 349.1889].

2.4.3. 2-((2-hydroxyethyl)amino)benzoic acid (*3*): FT-IR: 1735 cm^{-1}, 3404 cm^{-1}; ^1H and ^{13}C NMR (see Table 2)

White crystals, HR-EIMS, *m/z* 181.0769 $(M)^+$; $C_9H_{11}NO_3$; calcd; 181.0793). X-ray Crystallographic Procedure: Single crystals of **3**, recrystallized from a *n*-butanol solution of **3**, were selected and fitted onto a glass fiber and measured at −173 °C with a Bruker Apex II ultra-diffractometer using MoKα radiation. Data correction and reduction were performed with the crystallographic package Apex3. The structure was solved and refined with the Bruker SHELXTL software package. The final anisotropic full-matrix least-squares refinement on F^2 with 124 variables converged at R1 = 7.49%, for the observed data and w*R*2 = 21.27% for all data. The ORTEP plot was obtained by the program PLATON (A. L. Spek, 2009). Crystal data: $C_9H_{11}NO_3$, MW = 181, Monoclinic, space group *P* 1 $2_1/n$ 1, *Z* = 2, a = 8.566(8) Å, b = 4.857(4) Å, c = 21.575(19) Å, β = 97.314(13)°, volume = 890.3(14) $Å^3$, GOF = 1.068.

Crystallographic data of **3** have been deposited at the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC1904434. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ UK (Fax: +44(0)-1223–336,033 or e-mail: deposit@ccdc.cam.ac.uk).

2.4.4. E-dec-2-enamide (*4*)

White powder, FT-IR: 1619, 3378 cm^{-1}; ^1H and ^{13}C NMR (see Table 2); HR-EIMS, *m/z* 169.1485 $[(M)^+$; $C_{10}H_{19}NO$; calcd; 169.1467].

2.5. Total synthesis of *1S*

2.5.1. Synthesis of (N-acryloyl)-L-phenylalaninol (*1a*, Scheme 1)

To a suspension of $NaBH_4$ (1.17 g, 30.88 mmol), L-phenylalanine (1.99 g, 12.05 mmol) in THF (40 ml) was slowly added a solution of I_2 (3.15 g, 12.41 mmol) in THF (7 ml) at 0 °C. The mixture was warmed to room temperature, stirred for 30 min, and then heated to reflux for 21 h before adding MeOH cautiously at room temperature. The organic solvent was removed under reduced pressure and the resulting white solid was dissolved with $CHCl_3$. The organic layer was washed with 20% aq KOH, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting white solid was recrystallized from toluene to obtain L-phenylalaninol (1.06 g, 7.01 mmol, 58%) as colorless needles. The spectral data was identical with the literature data [12]. To a stirred solution of L-phenylalaninol (50.3 mg, 0.332 mmol) in THF/H_2O (4:1, 2.0 ml) was added magnesium oxide (68.0 mg, 1.687 mmol), acryloyl chloride (60 ml, 0.742 mmol) at room temperature. After stirring for 4 h, the suspension was filtered through celite and washed with EtOAc. The filtrate was washed successively with saturated aqueous NH_4Cl and saturated aqueous NaCl, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to furnish (*N*-acryloyl)-L-phenylalaninol (**1a,** 60.7 mg, 0.296 mmol, 89%) as a white solid: $[\alpha]_D^{25}$ −23.5 (*c* 1.05, $CHCl_3$); IR (film) 3294, 3067, 3027, 2930, 2862, 1659, 1624, 1541 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.31–7.19 (m, 5H), 6.29 (br d, *J* = 8.3 Hz, 1H), 6.23 (dd, *J* = 17.0, 1.3 Hz, 1H), 6.06 (dd, *J* = 17.0, 10.3 Hz, 1H), 5.61 (dd, *J* = 10.3, 1.3 Hz, 1H), 4.23 (m, 1H), 3.71–3.55 (m, 2H), 3.46 (m, 1H), 2.91 (d, *J* = 7.2 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.0, 137.6, 130.6, 129.2 (2C), 128.6 (2C), 126.9, 126.6, 63.5, 52.8, 36.8; HRMS (CI) calcd for $C_{12}H_{16}NO_2$ $[(M + H)^+]$ 206.1176, found 206.1184.

2.5.2. Synthesis of 6-heptenoic acid (*1b*, Scheme 1)

To a solution of LDA (prepared *in situ* from *i*Pr_2NH (0.90 ml, 6.404 mmol) and *n*BuLi (1.6 M in hexane, 3.60 ml)) in THF (17.0 ml) were added AcOH (0.16 ml, 2.790 mmol) and DMPU (8.5 ml) at 0 °C. After cooling to −78 °C, 5-bromo-1-pentene (0.3 ml, 2.536 mmol) was added and then the solution was warmed gently to room temperature. The solution was stirred for 5 h before addition of 1 M aqueous HCl. The organic layer was extracted with Et_2O, dried over $MgSO_4$, filtered through celite, concentrated under reduced pressure. Purification of the residue by column chromatography (silica gel, 0% to 30% EtOAc/hexane) gave 6-heptenoic acid (**1b**, 64.8 mg, 0.506 mmol, 20%) as a colorless oil: The spectral data was identical with the literature data [13].

Table 1
^{1}H and ^{13}C NMR (MeOH-d_4, 600 Hz) of cordytakaoamides A (**1**) and B (**2**).

No	Cordytakaoamide A (**1**)				Cordytakaoamide A (**2**)	
	^{1}H NMR (*J* Hz)		^{13}C NMR		^{1}H NMR (*J* Hz)	^{13}C NMR
	1	**1*S***	**1**	**1*S***		
1	–	–	178.1, s	177.4, s	–	178.1, s
2	2.31 m	2.29 t (7.4)	31.6, t	34.6, t	2.26 t (7.4)	35.2, t
3	1.61 m	1.61 m	25.0, t	25.5, t	1.60 m	26.1, t
4	1.34 m	1.48 m	28.9, t	28.8, t	1.34 m	29.8, t
5	2.19 m[a]	2.19 m	32.7, t	32.6, t	1.34 m	30.0, t
6	6.70 dt (15.1, 7.3)	6.69 dt (15.4, 7.0)	145.2, d	145.1, d	1.45 m	29.2, t
7	5.90 br d (15.1)	5.90 dt (15.4, 1.5)	125.0, d	125.1, d	2.17 m	32.9, t
8	–	–	167.8, s	168.5, s	6.70 dt (15.4, 7.0)	145.5, d
9	–	–	–	–	5.89 dt (15.4, 1.4)	124.9, d
10	–	–	–	–	–	168.7, s
1′	4.15 m	4.15 m	54.2, d	54.2, d	4.16 m	53.7, d
2′a	3.52 m	3.52 m	64.2, t	64.1, t	3.57 dd (11.3, 4.8)	64.3, t
2′b					3.54 dd (11.3, 5.4)	
1″	–		139.8, s	139.8, s	–	125.9, s
2″	7.21–7.26 m[a]	7.21–7.26 m[a]	130.3, d	130.3, d	–	156.6, s
3″	7.21–7.26 m[a]	7.21–7.26 m[a]	129.3, d	129.3, d	6.75 dd (8.1, 1.0)	116.0, d
4″	7.16 m	7.16 m	127.3, d	127.3, d	7.01 ddd (8.1, 8.1, 1.6)	128.7, d
5″	7.21–7.26 m[a]	7.21–7.26 m[a]	130.3, d	129.3, d	6.73 ddd (8.1, 7.4, 1.0)	120.6, d
6″	7.21–7.26 m[a]	7.21–7.26 m[a]	129.3, d	127.3, d	7.08 dd (7.4, 1.6)	132.2, d
7″	2.74 dd (13.7, 8.0)	2.74 dd (13.9, 8.2)	38.0, t	38.0, t	2.77 dd (13.6, 7.7)	32.4, t
	2.92 dd (13.7, 6.3)	2.92 dd (13.9, 6.3)			2.89 dd (13.6, 6.6)	

[a] overlapped; s, quaternary, d, methine, and t, methylene; Multiplicity of carbons was determined by DEPT and HMQC experiments.

Table 2
^{1}H and ^{13}C NMR of **3** (MeOH-d4, 600 Hz) and **4** ($CDCl_3$, 500 Hz).

No	**3**		No	**4**	
	^{1}H NMR (*J* Hz)	^{13}C		^{1}H NMR (*J* Hz)	^{13}C
1	–	110.2, s	1	–	168.8, s
2	–	151.3, s	2	5.85 d (15.6)	122.5, d
3	6.74 d (8.5)	110.9, d	3	6.84 m	146.5, d
4	7.34 m	134.2, d	4	2.18 m	31.9, t
5	6.57 t (14.9)	114.2, d	5	1.44 m	28.0, d
6	7.90 d (7.9)	131.9, d	6	1.31 m[a]	28.9, d
7	–	170.6, s	7	1.31 m[a]	29.0. d
1′	3.34 t (11.4)	44.5, t	8	1.31 m[a]	31.6, d
2′	3.78 t (11.4)	60.1, t	9	1.31 m[a]	22.5, d
			10	0.88 t (7.2)	13.9, q

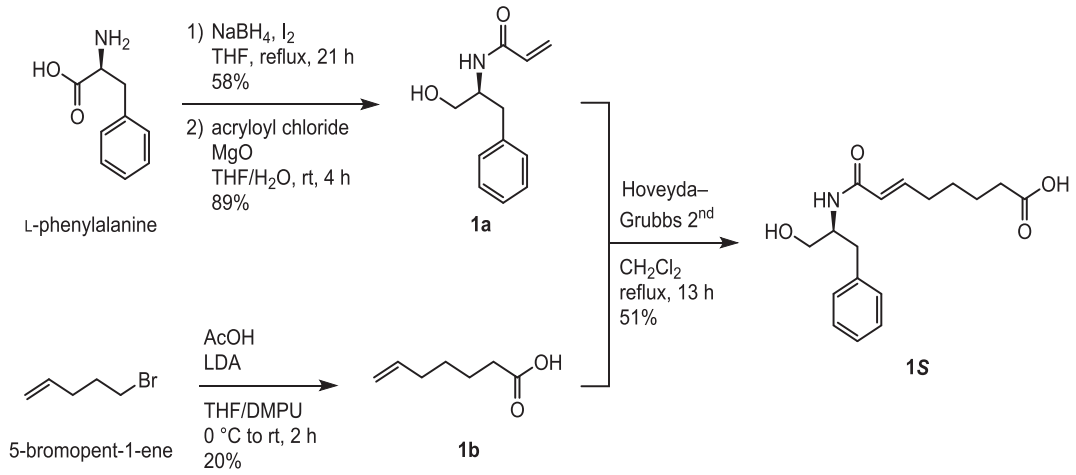
[a] overlapped; s, quaternary, d, methine, t, methylene, and q, methyl; Multiplicity of carbons was determined by DEPT and HMQC experiments.

2.5.3. Synthesis of cordytakaoamide A (*1S*, Scheme 1)

A solution of (*N*-acryloyl)-L-phenylalaninol **1a** (26.0 mg, 0.127 mmol), 6-heptenoic acid **1b** (15.7 mg, 0.122 mmol) and Hoveyda–Grubbs 2nd (5.2 mg, 0.00829 mmol) in CH_2Cl_2 (1.2 ml) was refluxed for 13 h before removal of solvent under reduced pressure. Purification of the residue by column chromatography (silica gel, 1st: $CHCl_3$/acetone = 4/1 to 2/1, 2nd: $CHCl_3$/MeOH = 1/0 to 10/1) gave cordytakaoamide A (**1*S***, 19.1 mg, 0.0625 mmol, 51%) as a colorless oil: $[\alpha]_D^{25}$ −31.2 (*c* 0.85, MeOH); IR (film) 3430, 3028, 2944, 2860, 1734, 1671, 1541, 1458 cm^{-1}; ^{1}H and ^{13}C NMR (600 MHz, CD_3OD); see Table 1; HRCIMS: *m/z* 306.1696; (calcd: 306.1700 for $C_{17}H_{24}NO_4$ $[(M + H)^+]$.

3. Results and discussion

The chemical characterization of the EtOAc and *n*-butanol soluble portions of fungus, *C. takaomontana* NBRC 101754, afforded two new alkaloidal compounds namely, cordytakaoamide A (**1**) and B (**2**), as well as two compounds isolated here for the first time from natural



Scheme 1. Total synthesis of cordytakaoamide A (**1*S***).

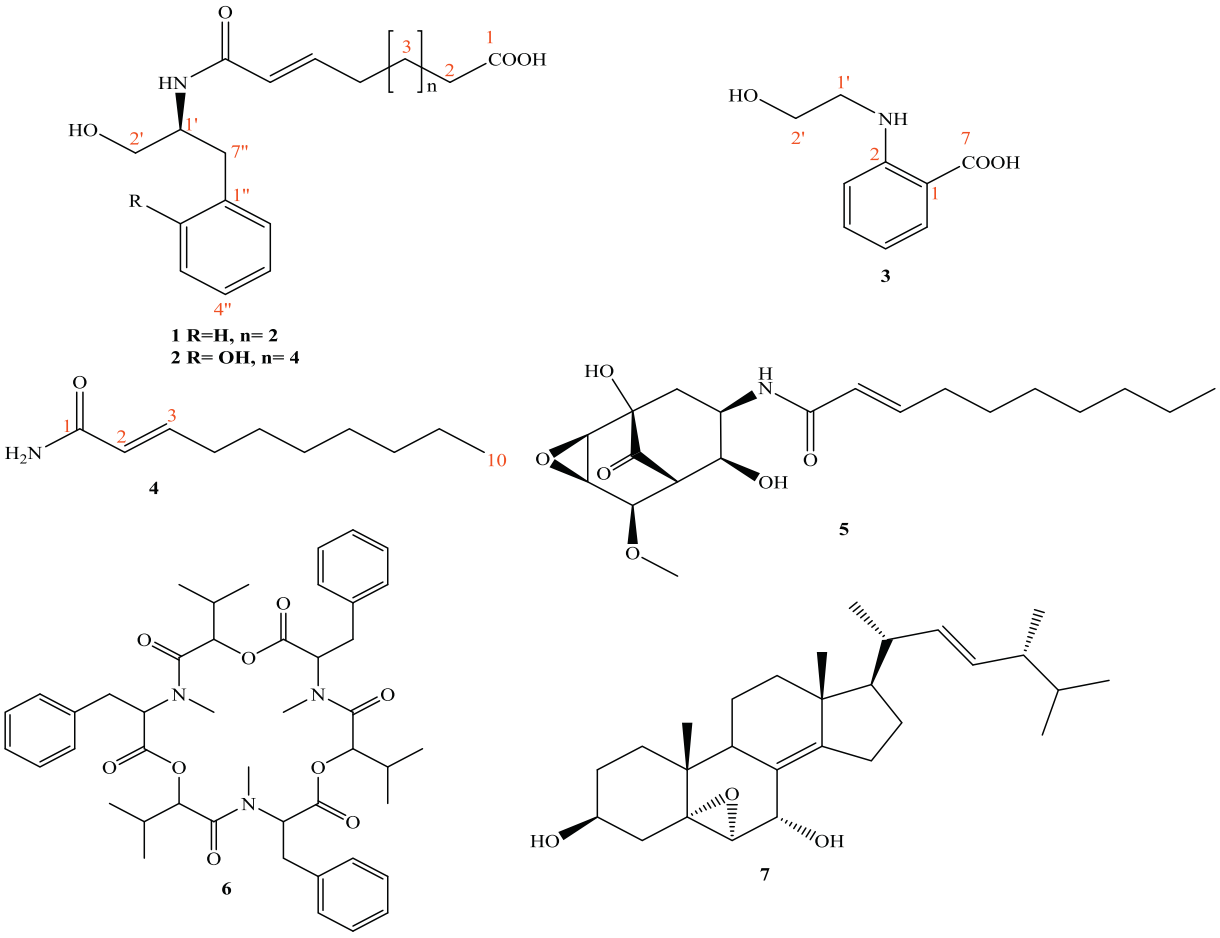


Fig. 1. Isolated compounds from *C. takaomontana*.

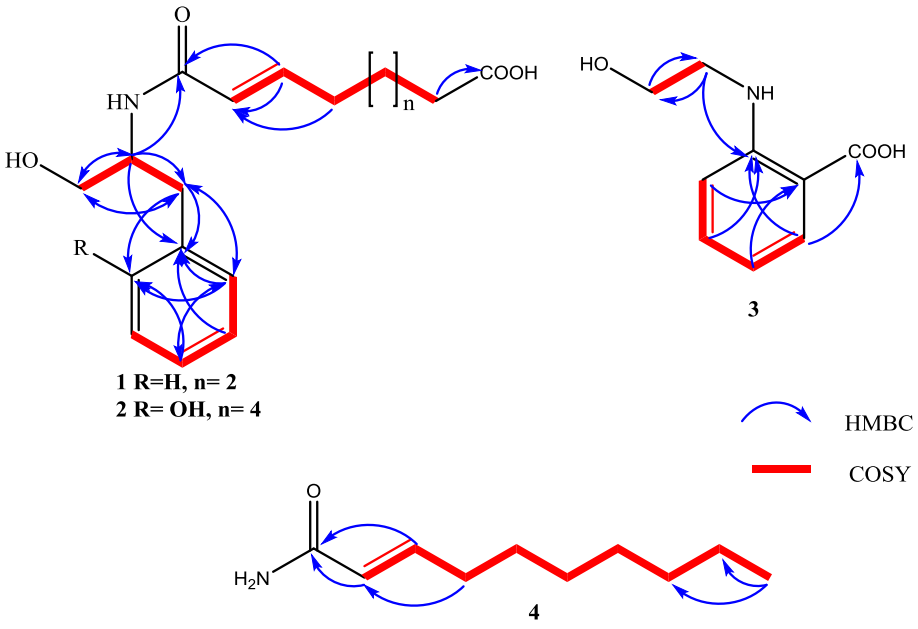


Fig. 2. Selected ^{1}H-^{1}H COSY and key HMBC of **1–4.**

resource, 2-((2-hydroxyethyl)amino)benzoic acid (**3**) and *E*-dec-2-en-amide (**4**), and three known compound, TK-57-164B (**5**) [14], one depsipeptide, beauvericin (**6**) [15,16] and one steroid, 5*α*,6*α*-epoxyergosta-8(14),22-diene-3*β*,7*α*-diol (**7**) [17,18] (Fig. 1).

Cordytakaoamide A (**1**, Fig. 1) was isolated as a white solid with a negative optical rotation in methanol ($[\alpha]_D^{25}$ – 15.38°). The HR-CIMS and TOF-ESIMS exhibited molecular ion peak at *m/z* HR-CIMS, *m/z* 306.1668 [$(M + H)^+$; $C_{17}H_{24}NO_4$; calcd; 306.1705], positive-TOF-

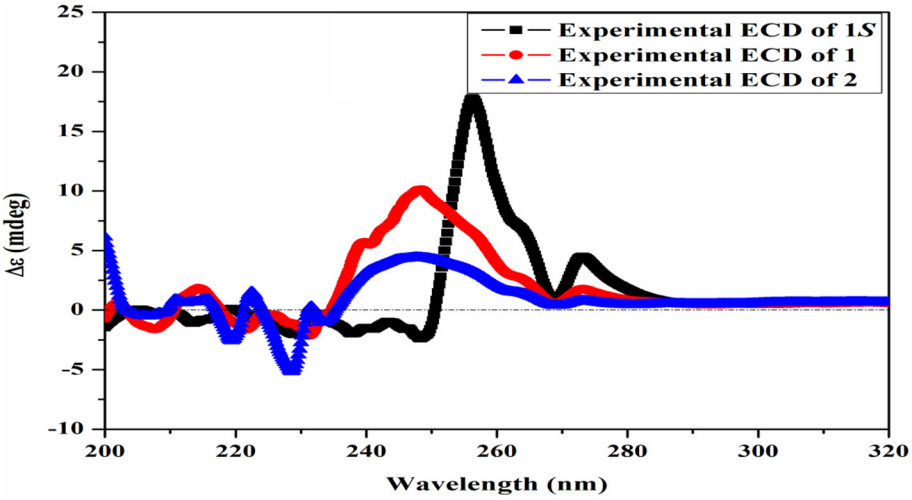


Fig. 3. Experimental CD of **1*S***, **1**, and **2.**

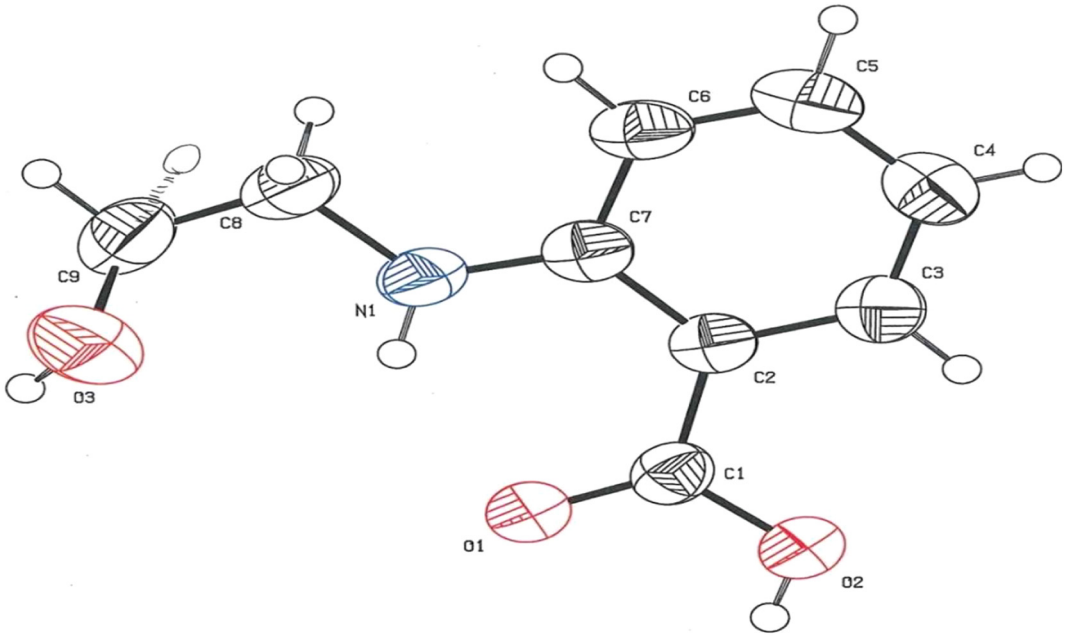


Fig. 4. X-ray crystallographic structure of **3.**

ESIMS, *m/z* 328.1527 $[(M + Na)^+$; $C_{17}H_{23}NO_4Na$; calcd; 328.1525], and negative-TOF-ESIMS, *m/z* 304.1517 $[(M\text{-}H)^-$; $C_{17}H_{22}NO_4$; calcd; 304.1549], respectively displaying seven degrees of unsaturation. The FT-IR of **1** exhibited absorption bands at 3397, 1669, and 1511 cm^{-1} corresponding to hydroxy and/or nitrogen, amide carbonyl and olefinic groups, respectively. The ^1H NMR spectra data (Table 1) of **1** exhibited five aromatic olefinic protons [at δ_H 7.16, 7.21–7.26 m], two aliphatic olefinic protons [at δ_H 5.90 br d (J = 15.1 Hz) and δ_H 6.70 dt (J = 15.1, 7.3 Hz)], one hydroxylated methylene protons [at δ_H 3.52 m], one methine proton attached with nitrogen [at δ_H 4.15 m] in addition to five aliphatic methylenes [at δ_H 1.34 m, δ_H 1.61 m, δ_H 2.19 m, δ_H 2.31 m, and δ_H 2.74 dd (J = 13.7, 8.0 Hz); 2.92 dd (J = 13.7, 6.3 Hz)]. Seventeen carbon resonances were assigned by ^{13}C NMR spectrum that characterized with DEPT and HMQC to, three quaternary carbons (including one carboxylic carbon at δ_C 178.1, one amide carbonyl carbon at δ_C 167.3, and one aromatic carbon at δ_C 139.8), five aromatic methines (at δ_C 127.3, 129.3 (2 x C) and 130.3 (2 x C)), two olefinic methines at δ_C 125.0, and 145.2, one methine attached to nitrogen at δ_C 54.2, alongside six aliphatic methylenes at δ_C 25.0, 28.9, 31.6, 32.7, 38.0, and oxyhenated one at δ_C 64.2. The complete analysis of 1D NMR followed by 2D NMR especially $^1H\text{-}^1H$ COSY and HMBC revealed that **1** include three internal structural part (i) monosubstituted benzene ring at the quaternary carbon at δ_C 139.8, (ii) CH_2-CH(NHCOR)-CH_2OH, and (iii) R-CH = CH-$(CH_2)_4$-COOH. The monosubstituted benzene ring was established *via* $^1H\text{-}^1H$ COSY correlations (Fig. 2) of the aromatic protons, H-2″ and/or H-6″ (δ_H 7.21–7.26 m)/H-3″ and/or H-5″ (δ_H 7.21–7.26 m), and H-3"/H-4″ (δ_H 7.16 m), as well as HMBC correlations (Fig. 2) of H-2″ and/or H-6"/C-4″ (δ_C 127.3, J^3), alongside H-3″ and/or 5″/ C-1″ (δ_C 139.8, J^3). The $^1H\text{-}^1H$ COSY correlations (Fig. 2) of H-7"/H-1′, H-1'/H-2′, in addition to HMBC correlations of H-1'/H-8 (amide carbonyl carbon at δ_C 167.8, J^3), H-7"/H-2′ (J^3), and H-1'/H-2′ (J^2) deduced the 2nd internal structural part. The continuous of $^1H\text{-}^1H$ COSY sequence of H-2/H-3 until H-5/H-6 (δ_H 6.70 dt (J = 15.1, 7.3 Hz)) and H-6/H-7 (5.90 br d, J = 15.1 Hz) alongside the HMBC correlations of H-3/C-1 (carboxyl group at δ_C 178.1, J^3), and H-5/C-7 (at δ_C 125.0, J^3) deduced the 3th structural part of **1**. The strong HMBC correlations of H-7″/C-2″ and/or C-6″ (at δ_C 130.3, J^3), and H-1″/C-1″ (at δ_C 139.8, J^3) assigned the connectivity of partial structure (ii) to the

monosubstituted benzene ring (i) *via* the quaternary C-1″ constructing phenylalaninol moiety. By the same, the clear HMBC correlations of H-6/C-8 (amide carbonyl carbon at δ_C 167.8, J^3), and H-7/C-8 (J^2) confirmed the connectivity of partial structure (ii) and (iii) *via* secondary amide linkage (-NHCO-). From above described 1D and 2D NMR data, **1** was established as 8-((1-hydroxy-3-phenyl-propan-2-yl)-amino)-8-oxooct-6-enoic acid. Compound **1** was characterized here with very rare alkaloidal skeleton that may be biosynthesized from the enzymatic reaction of L-phenylalaninol and 2-octenedioic acid. Recently, only one compound with similar alkaloidal skeleton namely, farinosone C, was reported from *Paecilomyces farinosus* [1].

In out trying for establishment of the absolute configuration at C-1′, **1*S*** was totally synthesized using L-phenylalanine as the starting material. The optically active **1*S*** synthesized with olefin metathesis as a key step exhibited a positive cotton effect at 258 and this is totally agreement with naturally isolated one with a positive cotton effect at 251 (Fig. 3). Thus, from above mentioned data, **1** was established as (*S*, *E*)-8-((1-hydroxy-3-phenyl-propan-2-yl)-amino)-8-oxooct-6-enoic acid (cordytakaoamide A; **1**).

Cordytakaoamide B (**2**, Fig. 1) was isolated as a white solid with a negative optical rotation in methanol ($[\alpha]_D^{25}$ – 23.22°). The HR-EIMS exhibited a molecular ion peak at *m/z* 349.1883 $(M)^+$, indicating the molecular formula $C_{19}H_{27}NO_5$ (calcd; 349.1889) revealing seven degrees of unsaturation. Absorption bands at 3402, 1661, and 1593 cm^{-1} were identified in FT-IR of **2** corresponding to hydroxy and/or nitrogen, amide carbonyl and olefinic groups, respectively. 1D NMR (Table 1) analysis deduced that **2** have very closed structure to the alkaloidal compound **1**, with exceptions of the (i) presence of hydroxylated aromatic quaternary carbon at δ_C 156.6 in the aromatic ring, and (ii) the difference of the numbers of methylenes in aliphatic chain. The HMBC correlations (Fig. 2) of H-7″ [at δ_H 2.77 dd (J = 13.6, 7.7 Hz); 2.89 dd (J = 13.6, 6.6 Hz)]/C-2″ (δ_C 156.6, J^3), H-4″ [at δ_H 7.01 ddd (J = 8.1, 8.1, 1.6 Hz)]/C-2″ (J^3), and H-6″ [at δ_H 7.08 dd, J = 7.4, 1.6 Hz]/C-2″ (J^3), confirmed the hydroxylation of C-2″. Also, the ^{1}H-^{1}H COSY of H-2 [δ_H 2.26 t (J = 7.4 Hz)]/H-3 [δ_H 1.60 m] until H-8 [δ_H 6.70 dt, J = 15.4, 7.0 Hz]/H-9 [δ_H 5.89 dt, J = 15.4, 1.4 Hz)] alongside the HMBC of H-3/C-1 (δ_C 178.1, J^2) deduced that the aliphatic chain is $C_{10}H_{16}NO_3$ in **2** instead of $C_8H_{12}NO_3$ in **1**. Compound **2** exhibited a positive cotton effect at 249 that completely agreement with **1** and **1*S*** (Fig. 3). Thus, from above mentioned data, **2** was assigned (*S*,*E*)-10-((1-hydroxy-3-(2-hydroxyphenyl)-propan-2-yl)-amino)-10-oxodec-8-enoic acid (cordytakaoamide B). The biosynthesis of this compound might be performed *via* two main steps, (i) the enzymatic hydroxylation of L-phenylalaninol by involvement of the enzyme P450 that afforded 2-hydroxy-phenylalaninol, followed by (ii) the 2-hydroxy- phenylalaninol then enzymatically react with 2-decenedioic acid.

2-[(2-Hydroxyethyl)-amino] benzoic acid (**3**, Fig. 1) was isolated as white crystals. The HR-EIMS exhibited a molecular ion peak at *m/z* 181.0769 $(M)^+$ indicating the molecular formula $C_9H_{11}NO_3$ (calcd; 181.0739). FT-IR of **3** exhibited absorption bands corresponding to hydroxy and/or nitrogen, and carboxylic carbonyl at 3404, and 1735 cm^{-1}, respectively. ^{1}H NMR (Table 2) exhibited four aromatic protons at δ_H 6.74 d (J = 8.5 Hz), δ_H 7.34 m, δ_H 6.57 t (J = 14.9 Hz), and δ_H 7.90 d (J = 7.9 Hz), in addition to one nitrogenated methylene at δ_H 3.34 (t, J = 11.4 Hz), as well as one oxygenated methylene at δ_H 3.78 (t, J = 11.4 Hz). ^{13}C NMR (Table 2) exhibited nine carbon signals that categorized depending upon DEPT-135 and HSQC to, three quintenary carbons (one carboxylic carbonyl at δ_C 170.6, nitrogenated aromatic one at δ_C 151.1 and aromatic at δ_C 110.2), two alphatic methylenes (one hydroxylated at δ_C 60.1 and one nitrogenated at δ_C 44.5), and four aromatic methines (at δ_C 110.9, 114.2, 131.9 and 134.2). The complete analysis of 1D (Table 2) and 2D NMR (Fig. 2) suggested that **3** is O-disubstituted benzene ring with carnoxylic acid and aminoethane-2-ol moiety. This structure was deduced by ^{1}H-^{1}H COSY (Fig. 2) sequence of the aromatic ring, H-3 (at δ_H 6.74 d, J = 8.5 Hz)/H-4 (at δ_H 7.34 m), H-4/H-5 (at δ_H 6.57 t, J = 14.9 Hz), and H-5/H-6 (at δ_H 7.90 d, J = 7.9 Hz), as well as H_2–2′ (at δ_H 3.78 t, J = 11.4 Hz)/H_2–1′ (at δ_H 3.34 t, J = 11.4 Hz) in addition to HMBC (Fig. 2) correlations of H_2–1′/C-2 (at δ_C 151.3, J^3) and H-6/C-7 (Carboxylic acid at δ_C 170.6, J^3). Thus **3** was established as 2-[(2-hydroxyethyl)-amino] benzoic acid. **3** was already known synthetic compound [19], but it was reported here for the first time from natural resource. The structure of **3** was confirmed by a single-crystal X-ray analysis (Fig. 4).

E-Dec-2-enamide (**4**, Fig. 1) was isolated as white solid. HR-EIMS of **4** showed a molecular ion peak at *m/z* 169.1485 $[(M)^+$ as $C_{10}H_{19}NO$ (calcd;169.1467]. FT-IR of **4** displayed nitrogen at 3378 cm^{-1} and an amide carbonyl group at 1619 cm^{-1}. The 1D NMR of **4** (Table 2) exhibited one methyl at C-10, at [δ_H 0.88 (t, J = 7.2); δ_C 13.9)], two olefinic carbons at C-2 and C-3 [at δ_H 5.85 (d, J = 15.6); δ_C 122.5 and δ_H 6.84 m; δ_C 146.5; respectively], and six aliphatic methylenes [at δ_H 1.31 m, 1.44 m, 2.18 m; and at δ_C 22.5–31.9]. ^{1}H-^{1}H COSY sequence (Fig. 2) of H-2/H-3, H-3/H-4, until H-9/H-10 as well as HMBC correlations (Fig. 2) of H-3/C-1 (amide carbonyl, δ_C 168.8, J^3), and H-4/C-2 (δ_C 146.5, J^3) were clearly observed. The *E*-geometry of $\Delta^{2,3}$ was determined *via* the *vicinal* coupling constant J = 15.6 Hz (Concellon et al., 2010). From above described data, **4** was constructed as *E*-dec-2-enamide. Although this compound was described before as a synthetic compound [20], but herein is the first time for isolation and identification of it from natural resource.

4. Conclusion

From the entomopathogenic fungus, *C. takaomontana* NBRC 101754, seven compounds were isolated and identified including two new rare alkaloidal metabolites, cordytakaoamides A (**1**) and B (**2**), as well as two compounds 2-[(2-hydroxyethyl) amino] benzoic acid (**3**) and 2*E*-decenamide (**4**), isolated for the first from natural resources. The absolute stereochemistry of **1** and **2** was established by the total synthesis of **1** in addition to experimental CD. **3** was confirmed by a signal crystal X-ray analysis.

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Declaration of Competing Interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

LR and HR-MS,^{1}H, ^{13}C NMR, DEPT-135, HSQC, HMBC, ^{1}H ^{1}H COSY and NOESY spectral data of **1–4** (**S1–S47**) are found in supplementary data. Supplementary data to this article can be found online. Supplementary data to this article can be found online at https://doi.org/10.1016/j.fitote.2019.104364.

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