Two New Octahydronaphthalene Derivatives from *Trichoderma spirale*, an Endophytic Fungus Derived from *Aquilaria sinensis*

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Two new octahydronaphthalene derivatives, trichodermic acid A (**1**) and trichodermic acid B (**2**), along with a known analog, trichodermic acid (**3**), and a known modified dipeptide, trichodermamide A (**4**), were isolated from the extract of *Trichoderma spirale*, an endophytic fungus present in the medicinal plant *Aquilaria sinensis*. The structures of these compounds were established by extensive analysis of their spectroscopic data including 1D- and 2D-NMR and MS data.

Introduction. – Endophytic fungi have been shown to be a well-established source for structurally diverse and biologically active secondary metabolites [1][2]. However, the traditional method of a single medium culture restricts the metabolic pathways of microorganisms and as a result, many metabolites cannot be produced. Recently, the use of various techniques to activate those metabolic pathways restricted by the traditional method has attracted much attention [3][4]. The OSMAC (One Strain – Many Compounds) approach is a simple and effective approach for activating metabolic pathways and has been successfully applied to explore nature's chemical diversity of microorganisms [3]. Several reports have already demonstrated that it is a useful tool to optimize the chemical diversity of fungi by variation of culture parameters [5–7].

In our chemical investigation of the endophytic fungus *Trichoderma spirale* that was isolated from *Aquilaria sinensis* (LOUR.) GILG (Thymelaeaceae), a famous traditional Chinese medicinal plant, an octahydronaphthalene derivative, trichodermic acid (**3**), a modified dipeptide, trichodermamide A (**4**) and tyrosol (=4-hydroxybenzeneethanol) were obtained from the extract of the fungus grown in liquid PD (potato/dextrose) medium, previously [8][9]. By application of the OSMAC approach to release the chemical diversity of *T. spirale*, two new octahydronaphthalene derivatives, trichodermic acid A[1]) (**1**) and trichodermic acid B[1]) (**2**), along with **3** and **4**, were isolated from the extract of *T. spirale* grown on solid rice medium, and the structures of the new compounds **1** and **2** were elucidated.

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

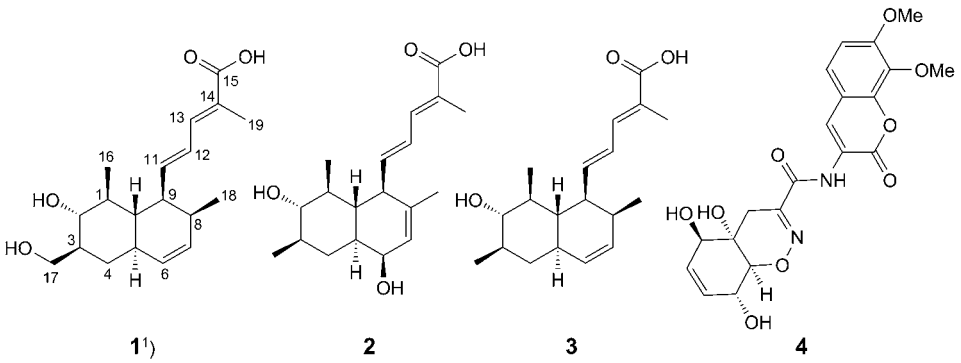

Fig. 1. *Compounds* **1**–**4**, *isolated from* Trichoderma spirale

Results and Discussion. – *Structure Elucidations.* Compound **1** was obtained as a colorless oil. Its IR spectrum indicated the presence of OH (3433 cm^{-1}), C=O (1675 cm^{-1}), and C=C (1640 cm^{-1}) groups. The molecular formula was determined as $C_{19}H_{28}O_4$ on the basis of the positive-ion-mode HR-ESI-MS (*m*/*z* 343.1884 ($[M+Na]^+$, $C_{19}H_{28}NaO_4^+$), which was in agreement with the ^{1}H- and ^{13}C-NMR data (*Table*). Detailed analysis of the 1D- and 2D-NMR spectra revealed that **1** was an octahydronaphthalene derivative, very similar to trichodermic acid (**3**) [8][10].

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

	1 (in CD_3OD)		**2** (in (D_6)acetone)	
	δ(H)	δ(C)	δ(H)	δ(C)
H–C(1)	1.31–1.39 (*m*)	44.5 (*d*)	1.28–1.31 (*m*)	48.4 (*d*)
H–C(2)	2.94 (*t*, *J* = 9.9)	78.4 (*d*)	2.61 (*t*, *J* = 9.5)	81.5 (*d*)
H–C(3)	1.56–1.64 (*m*)	47.1 (*d*)	1.40–1.46 (*m*)	40.4 (*d*)
CH_2(4)	0.96–1.00 (*m*), 1.85–1.87 (*m*)	34.4 (*t*)	1.33–1.36 (*m*), 1.52–1.54 (*m*)	37.5 (*t*)
H–C(5)	1.88–1.92 (*m*)	42.3 (*d*)	1.24–1.27 (*m*)	43.9 (*d*)
H–C(6)	5.50 (*dt*, *J* = 9.5, 1.8)	132.1 (*d*)	3.81 (*dd*, *J* = 6.4, 2.4)	67.5 (*d*)
H–C(7)	5.63 (*ddd*, *J* = 9.5, 4.3, 2.8)	133.4 (*d*)	5.83 (*dq*, *J* = 6.4, 1.4)	128.0 (*d*)
H–C(8) or C(8)	2.20–2.25 (*m*)	37.4 (*d*)		138.6 (*s*)
H–C(9)	2.52–2.57 (*m*)	50.6 (*d*)	2.68 (*t*, *J* = 8.9)	51.8 (*d*)
H–C(10)	1.03–1.08 (*m*)	46.5 (*d*)	1.54–1.57 (*m*)	43.6 (*d*)
H–C(11)	6.19 (*dd*, *J* = 14.9, 10.5)	148.6 (*d*)	5.99 (*dd*, *J* = 15.2, 8.9)	149.4 (*d*)
H–C(12)	6.36 (*dd*, *J* = 14.9, 11.2)	125.3 (*d*)	6.57 (*dd*, *J* = 15.2, 11.4)	128.3 (*d*)
H–C(13)	7.19 (*d*, *J* = 11.2)	139.2 (*d*)	7.19 (*dq*, *J* = 11.4, 1.2)	139.6 (*d*)
C(14)		126.5 (*s*)		126.2 (*s*)
C(15)		172.4 (*s*)		169.9 (*s*)
Me(16)	1.10 (*d*, *J* = 6.3)	17.8 (*q*)	1.09 (*d*, *J* = 6.5)	18.3 (*q*)
CH_2(17) or Me(17)	3.76 (*dd*, *J* = 10.6, 4.8), 3.55 (*dd*, *J* = 10.6, 6.2)	65.6 (*t*)	1.02 (*d*, *J* = 6.2)	20.0 (*q*)
Me(18)	0.99 (*d*, *J* = 7.1)	16.4 (*q*)	1.61 (br. *s*)	22.7 (*q*)
Me(19)	1.91 (*s*)	12.3 (*q*)	1.92 (*d*, *J* = 1.2)	13.1 (*q*)

However, the Me(17) signal at δ(H) 1.03 of **3** was missing in the ^{1}H-NMR spectrum of **1**. Instead, two *dd* of a CH_2–O group at δ(H) 3.76 and 3.55 (CH_2(17)) were observed. Accordingly, in the ^{13}C-NMR spectrum of **1**, a CH_2–O signal was observed at δ(C) 65.6 (C(17)) in place of the Me(17) signal of **3** at δ(C) 19.6. These observations along with the molecular formula indicated that a Me group of **3** was replaced by a CH_2OH group in **1**. The ^3J long-range correlations from CH_2(17) to C(2), C(3), and C(4) in the HMBC spectrum of **1** (*Fig. 2*) confirmed that the CH_2OH group was attached to C(3). The relative configuration of **1** was determined by the analysis of the J(H,H) coupling constants and the NOESY data (*Fig. 3*). The J(H–C(11),H–C(12)) of 14.9 Hz and the J(H–C(6),H–C(7)) of 9.5 Hz suggested (*E*)-geometry for C(11)=C(12) and (*Z*)-geometry for C(6)=C(7). The NOE H–C(12)/Me(19) indicated (*E*)-geometry for C(13)=C(14). H–C(2) was axially oriented because it appeared as a *t* with $J = 9.9$ Hz, the vicinal H–C(1) and H–C(3) also assuming an axial configuration. The NOE H–C(2)/H–C(10) suggested that H–C(10) was also axially oriented. The NOEs H–C(1)/H–C(3), H–C(5), and H–C(9), and H–C(9)/H–C(5) and H–C(8) indicated that H–C(1), H–C(3), H–C(5), H–C(8), and H–C(9) were on the same side of the

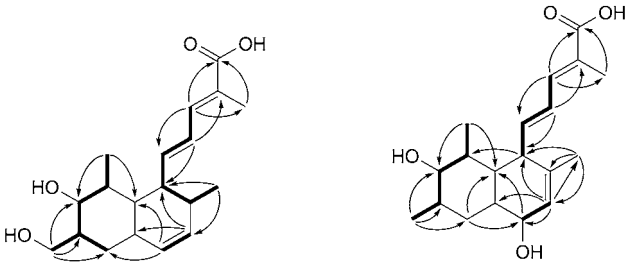

Fig. 2. *^{1}H,^{1}H-COSY* (—) *and key HMBC* (H → C) *data of* **1** *and* **2**

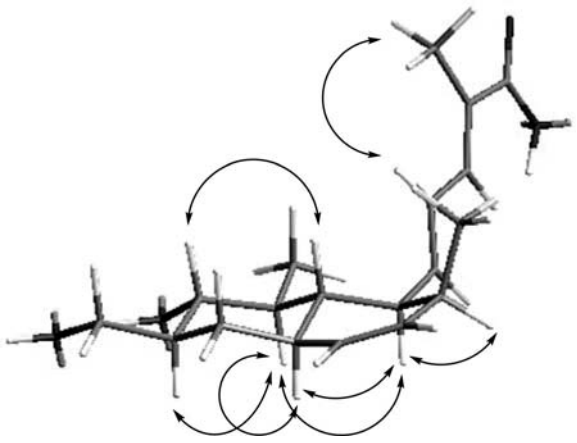

Fig. 3. *Selected NOESY correlations* (H ↔ H) *of compound* **1**

molecule. In conclusion, **1** had the same relative configuration as the parent compound **3** [10]. The absolute configuration of **1** was determined to be the same as that of **3** by comparison of their optical rotation ($[\alpha]_D^{25} = +50.9$ for **1** *vs.* $+58.7$ for **3**, both determined in MeOH). Based on the above spectral evidence, the structure of **1** was established as (2*E*,4*E*)-2-methyl-5-[(1*S*,2*S*,4a*R*,6*S*,7*R*,8*S*,8a*S*)-1,2,4a,5,6,7,8,8a-octahydro-7-hydroxy-6-(hydroxymethyl)-2,8-dimethylnaphthalen-1-yl]penta-2,4-dienoic acid, which was named trichodermic acid A.

Compound **2** was obtained as a colorless oil. Its IR spectrum showed absorption bands for OH (3410 cm^{-1}), C=O (1682 cm^{-1}), and C=C (1633 cm^{-1}) groups. The molecular formula was determined as $C_{19}H_{28}O_4$ on the basis of the positive-ion-mode HR-ESI-MS (*m*/*z* 343.1868 ($[M+Na]^+$, $C_{19}H_{28}O_4Na^+$), which indicated that **2** was an isomer of **1** and was in agreement with the ^{1}H- and ^{13}C-NMR data (*Table*). Detailed analysis of the 1D- and 2D-NMR spectra revealed that **2** was also an octahydronaphthalene derivative, as its companions **1** and **3**. The ^{1}H-NMR spectrum exhibited signals attributed to four Me groups at δ(H) 1.02 (Me(17)), 1.09 (Me(16)), 1.61 (Me(18)), and 1.92 (Me(19)), suggesting that Me(16), Me(17), and Me(19) of **2** were the same as those in **3**. However, the signal of Me(18) of **2** was shifted downfield and was a *s* suggesting that Me(18) was attached to a quaternary C-atom of a C=C bond rather than to an sp^3 C-atom as in **3**. The HMBC cross-peaks Me(18)/C(7) (δ(C) 128.0), C(8) (δ(C) 138.6), and C(9) (δ(C) 51.8), and H–C(7) (δ(H) 5.83)/C(9) and Me(18) confirmed that Me(18) was attached to a C=C bond positioned between C(7) and C(8) (*Fig. 2*). The HMBCs from H–C(6) (δ(H) 3.81) to C(10), and from H–C(4) and H–C(7) to C(6) (δ(C) 67.5) suggested the presence of an OH group at C(6) (*Fig. 2*). Thus the constitutional formula of **2** was deduced as shown in *Fig. 1*. The relative configuration of **2** was determined by analysis of the *J*(H,H) coupling constants and its NOESY plot. Similarly to **1**, the *J*(H–C(11),H–C(12)) of 15.2 Hz suggested (*E*)-geometry for C(11)=C(12), and the NOE H–C(12)/Me(19) indicated (*E*)-geometry for the C(13)=C(14). The coupling constant of the *t* of H–C(2) ($J = 9.5$ Hz) indicated that H–C(2) and H–C(1), as well as H–C(2) and H–C(3), were *trans*-axially arranged. H–C(9) was axially oriented because it appeared as a *t* with $J = 8.9$ Hz; so the vicinal H–C(10) was in axial orientation too, which was further corroborated by the NOE H–C(2)/H–C(10). The $J = 6.4$ and 2.4 Hz for the coupling of H–C(6) with H–C(7), and H–C(5) corroborated the equatorial orientation of H–C(6). The NOE H–C(5)/H–C(9) established the axial orientation of H–C(5). However, the absolute configuration of **2** remained unknown due to insufficient pure material. Based on the above spectral evidence, the structure of **2** was established as rel-(2*E*,4*E*)-2-methyl-5-((1*R*,4*S*,4a*S*,6*R*,7*S*,8*S*,8a*S*)-1,4,4a,5,6,7, 8,8a-octahydro-4,7-dihydroxy-2,6,8-trimethylnaphthalen-1-yl]penta-2,4-dienoic acid, which was named trichodermic acid B.

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2, 200–300 mesh; *Qingdao Haiyang Chemical Group Co.*), *Chromatorex ODS* (40–75 μm; *Fuji Silysia*), and/or *Sephadex LH-20* (*Amersham Biosciences*). TLC: precoated silica gel plates GF_{254} (SiO_2; *Merck*). Optical rotation: *Atago-AP-300*

automatic polarimeter. UV Spectra: *Biochrom-Ultrospec-6300pro* UV/VIS spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Bruker-Equinox-55* spectrophotometer; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker-Avance-500* spectrometer; at 500 and 125 MHz, resp.; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-ESI-MS: *Waters-Acquity-UPLC-Q-Tof-Micro* mass spectrometer; in *m*/*z*.

Fungal Material. The endophytic fungal strain *Trichoderma spirale* A17 was isolated from the root of *A. sinensis* (*ca.* 30 years old), which was collected at Xinyi, Guangdong Province, P. R. China, in November, 2007. The strain was identified by sequence analysis of the rDNA-ITS (internal transcribed spacer) region. The sequence of the ITS region of A17 has been submitted to the GenBank (Accession No. EU781674). By using BLAST (nucleotide sequence comparison program) to search the GenBank database, A17 has 100% similarity to *Trichoderma spirale* (GenBank Accession No. AY154933). The strain is preserved with the Guangdong Provincial Key Laboratory of Microbial Culture Collection and Application, Guangdong Institute of Microbiology, with the ID number A17.

Extraction and Isolation. For chemical investigations, the fungal strain was cultured on solid rice medium (100 ml of distilled H_2O and 0.6 g of peptone was added to 100 g of commercially available rice, then kept overnight prior to autoclaving, 50 500-ml flasks) at r.t. under stationary conditions for 30 d. The fermented rice substrate was extracted three times with AcOEt and the org. solvent evaporated to afford the crude extract (140 g). The extract was submitted to CC (SiO_2, petroleum ether/AcOEt 1:0 → 0:1, then $CHCl_3$/MeOH 10:1, 5:1, and 0:1): *Fractions 1–8* (by TLC analysis). *Fr. 3* was further fractionated by CC (*Sephadex LH-20*, $CHCl_3$/MeOH 1:1) and the product purified by recrystallization from MeOH: **3** (1.2 g). *Fr. 5* was subjected to CC (*Sephadex LH-20*, $CHCl_3$/MeOH 1:1), followed by CC (reversed-phase SiO_2 C_{18}, MeOH): *Frs. 5.1* and *5.2*. *Fr. 5.1* was separated by prep. TLC ($CHCl_3$/MeOH 10:1) and CC (*Sephadex LH-20*, $CHCl_3$/MeOH 1:1): **1** (43.9 mg). *Fr. 5.2* was further subjected to CC (reversed-phase SiO_2 C_{18}, MeOH/H_2O 7:3), followed by prep. TLC ($CHCl_3$/MeOH 10:1): **2** (3.6 mg). *Fr. 6* was fractionated by CC (SiO_2, $CHCl_3$/MeOH 10:1): *Frs. 6.1–6.3*. *Fr. 6.2* was further fractionated by CC (SiO_2, $CHCl_3$/MeOH 10:1), and the product purified by recrystallization from MeOH: **4** (232.7 mg).

Trichodermic Acid A (= *(2*E*,4*E*)-2-Methyl-5-[(1*S*,2*S*,4a*R*,6*S*,7*R*,8*S*,8a*S*)-1,2,4a,5,6,7,8,8a-octahydro-7-hydroxy-6-(hydroxymethyl)-2,8-dimethylnaphthalen-1-yl]penta-2,4-dienoic Acid*; **1**): Colorless oil. $[\alpha]_D^{25} = +50.9$ ($c = 0.67$, MeOH). UV (MeOH): 268 (4.46). IR (KBr): 3433, 2933, 2868, 1675, 1640, 1379, 1259, 1025, 989. ^{1}H- and ^{13}C-NMR: *Table*. HR-ESI-MS: 343.1884 ($[M+Na]^+$, $C_{19}H_{28}NaO_4^+$; calc. 343.1885).

Trichodermic Acid B (= rel-*(2*E*,4*E*)-2-Methyl-5-[(1*R*,4*S*,4a*S*,6*R*,7*S*,8*S*,8a*S*)-1,4,4a,5,6,7,8,8a-octahydro-4,7-dihydroxy-2,6,8-trimethylnaphthalen-1-yl]penta-2,4-dienoic Acid*; **2**): Colorless oil. $[\alpha]_D^{25} = +166.7$ ($c = 0.06$, MeOH). UV (MeOH): 268 (4.36). IR (KBr): 3410, 2923, 2877, 1682, 1633, 1384, 1248, 1051, 978. ^{1}H- and ^{13}C-NMR: *Table*. HR-ESI-MS: 343.1868 ($[M+Na]^+$, $C_{19}H_{28}NaO_4^+$; calc. 343.1885).

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