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Trichodermatides A–D, Novel Polyketides from the Marine-Derived Fungus *Trichoderma reesei*

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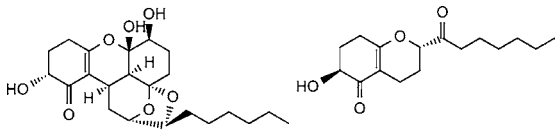
ABSTRACT



Trichodermatide A (1) Trichodermatide B (2)

Four new polyketide derivatives, Trichodermatides A–D (1–4) were isolated from the marine-derived fungus *Trichoderma reesei*. Trichodermatide A (1) is an unprecedented example of a polyketide with a ketal-containing pentacyclic skeleton. The chemical structures and absolute configurations of compounds 1–4 were elucidated by extensive spectroscopic methods, especially 2D NMR and CD spectral analysis, and supported by their proposed biosynthesis pathway. The cytotoxicity of 1–4 was evaluated against A375-S2 human melanoma cell line.

Marine-derived fungi are increasingly of interest as a potential source of new metabolites with a wide range of biological activities.[1] Early chemical investigation of the fungus *Trichoderma* spp. led to the isolation of several bioactive metabolites,[2] and terrestrial *Trichoderma* spp. have demonstrated utility as biocontrol agents.[3] Furthermore, the special metabolites of the genus *Trichoderma*, octaketide derivatives, display significant antibiotic activity.[4] In our continuing search for novel and bioactive substances from marine-derived fungi, we isolated four new polyketides, trichodermatides A, B, C, and D (**1**−**4**), from the fungus *Trichoderma reesei*. Trichodermatide A (**1**) is an unprecedented polyketide with a ketal-containing pentacyclic skeleton. In this contribution, we describe the structural elucidation, a plausible biogenetic pathway, and cytotoxic evaluation of these four metabolites.

The fungus *T. reesei* was obtained from marine mud in the tideland of Lianyungang, China. Its mycelial mass and fermentation broth were extracted with acetone and EtOAc, respectively. Both crude extracts (14.6 g) were isolated by normal-phase silica gel column and reversed-phase HPLC chromatography, yielding four metabolites Trichodermatide A (**1**, 3.6 mg), B (**2**, 4.5 mg), C (**3**, 2.0 mg), and D (**4**, 1.5 mg).

Trichodermatide A (**1**)[5] was obtained as a colorless oil with the molecular formula $C_{22}H_{32}O_7$ as determined by HREIMS (*m*/*z* 408.21465 $[M]^+$; calcd 408.21480), indicating seven degrees of unsaturation. Its UV spectrum (λ_{max} 257 nm) and IR spectrum (ν_{max} 1650 and 1620 cm^{-1}) suggested

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(5) Trichodermatide A (**1**), Colorless oil. $[\alpha]^{20}_D$ −62.5° (*c* 0.04, MeOH). UV (MeOH) λ_{max} 257 nm. CD (MeOH) $\Delta\epsilon_{296}$ −0.8601, $\Delta\epsilon_{253}$ +1.9531. IR (KBr) ν_{max} 3450, 2924, 1650, 1620, 1170, 1132 cm^{-1}. ^1H and ^{13}C NMR data, see Table 1. EIMS *m*/*z* 408.2 $[M]^+$.

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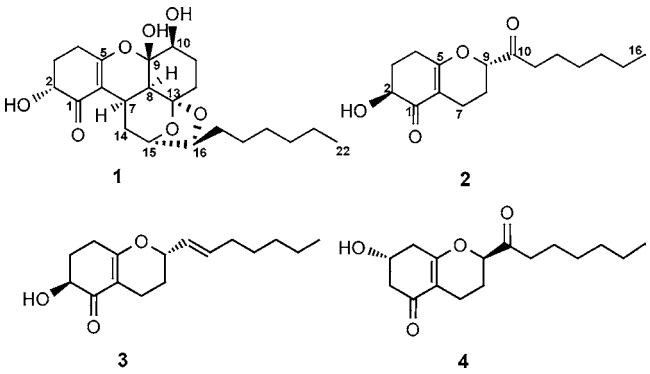


1 2 3 4

the presence of an α,β-unsaturated cyclohexenone group. The resonances in the ^{1}H NMR (Table 1) spectrum revealed five

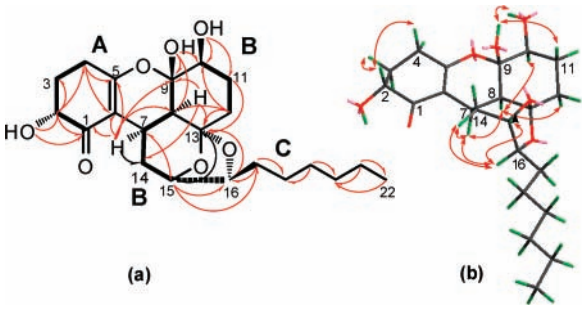
Table 1. ^{1}H and ^{13}C NMR Data for Trichodermatide A (**1**)[a,b]

position	δ_C	δ_H (multi, J in Hz)	position	δ_C	δ_H (multi, J in Hz)
1	197.7		15	77.1	4.11 (br d, 3.3)
2	70.7	3.91 (dt, 9.7, 3.8)	16	77.5	3.94 (dt, 4.8, 2.0)
3	29.4	2.06 (m), 1.64 (m)	17	35.4	1.36 (m)
4	27.4	2.45 (m), 2.32 (m)	18	28.4	1.23 (m)
5	167.9		19	28.8	1.23 (m)
6	111.7		20	31.4	1.23 (m)
7	21.7	2.85 (ddd, 7.8, 6.0, 2.9)	21	22.1	1.23 (m)
8	38.1	1.94 (d, 7.8)	22	14.1	0.84 (t, 7.0)
9	100.0		OH-2		4.85 (d, 3.6)
10	68.2	3.68 (dt, 8.1, 4.0)	OH-9		6.06 (s)
11	26.5	1.85 (m), 1.61 (m)	OH-10		5.06 (d, 3.7)
12	24.8	1.39 (m), 1.23 (m)			
13	106.2				
14	29.5	1.68 (ddd, 15.2, 3.3, 2.9) 1.83 (ddd, 15.2, 6.0, 3.3)			

[a] Data recorded in DMSO-d_6. [b] ^{1}H was recorded at 600 MHz, and ^{13}C at 150 MHz.

methylenes at δ_H 1.36 (m, H-17), 1.23 (m, H-18−21), and a methyl at δ_H 0.85 (t, J = 7.0 Hz, H-22). The corresponding resonances in the ^{13}C NMR spectrum (Table 1) were readily assigned to an aliphatic chain by analysis of the 2D NMR correlations. Comparison of the ^{13}C NMR and DEPT spectra revealed the presence of a methyl, ten methylenes, two methines, four oxymethines, and five quaternary carbons (including a carbonyl group, two olefinic carbons, and two hemiketal or ketal groups). Moreover, the ^{13}C NMR chemical shift of a downfield olefinic carbon (δ_C 167.9, C-5) indicated the presence of an oxygen substituent. The above functionalities accounted for two of the seven degrees of unsaturation in the molecule, revealing a pentacyclic structure for **1**. The gross structure of **1** was established with the assistance of HSQC, HMBC, and ^{1}H−^{1}H COSY experiments (Figure 1a).

Substructures A−C (Figure 1a) were connected using HMBC and ^{1}H−^{1}H COSY correlations. Substructure A was elucidated by starting with a series of HMBC correlations from H-2 (δ_H 3.91) to C-1 (δ_C 197.7), C-3 (δ_C 29.4), and C-4 (δ_C 27.4); and from H-4 (δ_H 2.45 and 2.32) to C-5 (δ_C 167.9), C-6 (δ_C 111.7). These carbon signals (C-1−C-6) were assigned to the aforementioned α,β-unsaturated cyclohexenone ring in which a hydroxyl was located at the α-position of C-1. Moreover, proton H-7 (δ_H 2.85) showed CH-correlations with C-5, C-6, C-8 (δ_C 38.1), and C-9 (δ_C 100.0), connecting C-9 and the olefinic carbon C-5 through an ether linkage to yield a pyran ring on the basis of the chemical shifts of C-5 and C-9. The additional correlations in the HMBC from H-8 (δ_H 1.94), OH-9 (δ_H 6.06) to C-9 suggested the presence of a hemiketal group at C-9. Analysis of the ^{1}H−^{1}H COSY spectrum indicated the presence of a spin system (H-7/H-8, H-14; and H-15/H-14, H-16) that was assembled into another pyran ring. Further HMBC correlations from H-8 to C-13 (δ_C 106.2) and C-14 (δ_C 29.5), from H-15 (δ_H 4.11) to C-13, C-16 (δ_C 77.5), and the chemical shift of C-13 indicated the presence of a ketal group connected to the pyran ring. Additional COSY correlations showed a spin system (H-10/H-11, H-12) to complete the substructure B, which was also supported by the HMBC correlations from OH-9 to C-10 (δ_C 68.2) and from H-12 to C-8, C-13. Finally, HMBC correlations from H-15 to C-17 (δ_C 35.4) and from H-17 (δ_H 1.36) to C-16, C-18 (δ_C 28.4) connected substructure B with the aliphatic side chain (substructure C) at C-16 to complete the structure of **1**.



(a) (b)

Figure 1. (a) Key HMBC correlations (H→C) and ^{1}H−^{1}H COSY (—) for connecting substructures A−C to the gross structure of **1**. (b) Key NOESY correlations of **1**.

The relative configuration of **1** was determined by NOESY experiment (Figure 1b) and ^{1}H NMR J values. In the ^{1}H NMR, a large coupling constant (J = 9.7 Hz) was observed for H-2, which required a *trans*-diaxial relationship between the oxymethine proton (H-2) and H-3α (δ_H 2.06). In addition, a NOESY correlation between H-2 and H-4β (δ_H 2.45) indicated that these two protons were in axial orientations and *cis* to one another. Correlations between H-16 (δ_H 3.94) and H-14α (δ_H 1.83), and especially between H-16 and H-7 and between H-8 and H-7, H-10 and H-12α (δ_H 1.39), suggested that these protons were on the same side and were assigned as the α-orientation. The correlation of H-8/H-10 indicated that H-8 and H-10 were at the axial positions. Further NOESY correlations of OH-9 with H-11β (δ_H 1.85) suggested that OH-9 was at the axial position and β-oriented but on the opposite side of H-7, H-8, and H-10. Thus, the observed NOESY correlations for **1** were fully consistent with its relative configuration.

Trichodermatide B (**2**)[6] had the molecular formula $C_{16}H_{24}O_4$, as derived from HREIMS data, indicating five degrees of unsaturation. Evidence for the presence of an α,β-unsaturated cyclohexenone was obtained from the UV and IR absorptions, which were similar to those of **1**. The ^{13}C NMR spectrum of **2** contained signals for four sp^2 carbons assigned to two carbonyl groups (δ_C 207.8 and 194.7) and two olefinic carbons (δ_C 176.1 and 110.7); thus, compound **2** was bicyclic. Comparison of the ^1H and ^{13}C NMR spectra suggested the presence of two oxymethines (δ_H 3.94, H-2/δ_C 70.7 and δ_H 5.14, H-9/δ_C 81.7) and an aliphatic chain (δ_H 2.42, 1.45, 1.26 and 0.85, H-11−16/δ_C 42.3, 22.9, 28.8, 31.1, 22.0, and 14.0).

The HMBC spectrum of **2** (Figure 2a) established the correlations from H-2 (δ_H 3.94), OH-2 (δ_H 5.08) to C-1 (δ_C 194.7), C-3 (δ_C 30.1) and from H-3 (δ_H 2.08, 1.78) to C-1, C-4 (δ_C 21.9) and C-5 (δ_C 176.1), which illustrated the presence of the α,β-unsaturated cyclohexenone ring, as was seen for **1**. A β-oxy substituent of an enol ether group was indicated by the low-field resonance of the tetrasubstituted olefinic carbon C-5 and the HMBC correlations. The methylene proton H-7 (δ_H 2.81 and 2.36) showed the same correlations as those of **1**, indicating that a pyran ring adjoined with the α,β-unsaturated cyclohexenone. Further HMBC correlations from H-9 (δ_H 5.14) to C-10 (δ_C 207.7) and from H-11 (δ_H 2.42) to C-10, C-12 (δ_C 22.9), and C-13 (δ_C 28.8) suggested that the carbonyl group linked the alphatic side chain to the furan ring at C-9. Analysis of NOESY data (Figure 2b) and *J* values established the relative configuration of **2**. Two strong cross-peaks of H-2/H-4α (δ_H 2.48) and H-9/H-7β (δ_H 2.81), along with the large vicinal couplings (9.7 and 9.3 Hz) observed for H-2 and H-9, supported assignment of the axial positions for H-2 and H-9.

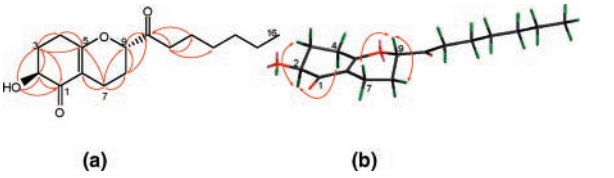


Figure 2. (a) Key HMBC correlations for **2**. (b) Key NOESY correlations for **2**.

The molecular formula of trichodermatide C (**3**)[7] was $C_{16}H_{24}O_3$ as deduced from HREIMS data. The NMR spectral data for **3** were similar to those of **2**. In the ^1H and ^{13}C NMR, the presence of a *trans*-double bond was revealed by two proton signals at δ_H 5.58 (dd, J = 15.4, 7.6 Hz, H-10) and 5.78 (dt, J = 15.4, 7.4 Hz, H-11), along with the corresponding carbon signals at δ_C 128.6 (C-10) and 134.6 (C-11). The HMBC correlations from H-9 (δ_H 5.29) to C-8 (δ_C 28.2), C-10 and C-11 placed the double bond at C-10 and C-11 and connected the pyran ring with the aliphatic side chain at C-9, as was seen in **2**. Additional HMBC correlations from the D_2O exchangeable proton (δ_H 5.06, OH-2) to C-1 (δ_C 194.6) and C-2 (δ_C 70.7) confirmed that the oxymethine was α to the carbonyl group. The NOESY cross-peaks of H-2/H-4α (δ_H 2.53) and H-9/H-7β (δ_H 2.83) and the coupling constants (9.4 and 9.5 Hz) of H-2 and H-9 were consistent with the relative configuration of **2** as depicted.

The molecular formula of **4**[8] was deduced from HREIMS as $C_{16}H_{24}O_4$. A comparison of the ^1H and ^{13}C NMR data of **2** and **4** showed that they differed in the position of the hydroxyl. The presence of the hydroxyl in the NMR of **4** was confirmed by the oxygenated carbon at δ_C 61.7 (C-3) and by the corresponding proton at δ_H 4.36 (tt, J = 3.9, 3.9 Hz, H-3), which revealed that the hydroxyl was at the β-position of the carbonyl group and was axial. The NOESY cross-peak of H-9 (δ_H 5.15) with H-7α (δ_H 2.81) and a large coupling constant of H-9 (J = 9.8 Hz) confirmed that these two protons were cofacial and were at the axial positions.

The absolute configurations of compounds **1**−**4** were determined on the basis of the CD spectral analysis (Figure 3). The n→π* and π→π* transitions in the CD curves clearly related to the helicity rule of the α,β-unsaturated ketone.[9] All the CD data for **1**−**4** were entirely compatible with the view that the hecility of enone moiety established the sign of the Cotton effect (CE) as a dominant factor. Furthermore, the enone helicity was determined by the conformation of cyclohexenone ring.[10] Thus, the ring conformation (with

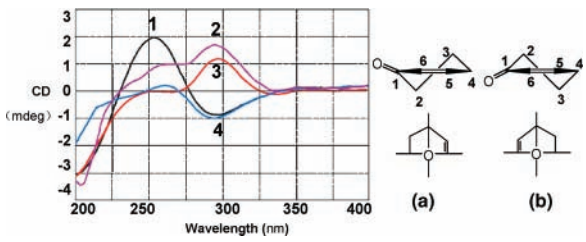


Figure 3. CD spectra of **1**−**4**. (a) Standard conformation of cyclohexenone ring showing the positive CE and its application of the octant rule. (b) Opposite conformation of (a) showing the negative CE and its application of the octant rule.

(6) Trichodermatide B (**2**). Colorless needles (MeOH); mp. 65−68 °C; $[\alpha]^{20}_D$ +64.0° (*c* 0.05, MeOH); UV (MeOH) λ_{max} 255 nm; IR (KBr) υ_{max} 3443, 2927, 1714, 1651, 1621 cm^{-1}; CD (MeOH) $\Delta\epsilon_{295}$ +3.3949, $\Delta\epsilon_{263}$ +2.4531; ^1H and ^{13}C NMR data, see Supporting information. EIMS *m/z* 280.2 $[M]^+$; HREIMS *m/z* 280.16800 $[M]^+$ (calcd for $C_{16}H_{24}O_4$, 280.16744).

(7) Trichodermatide C (**3**). Colorless oil. $[\alpha]^{20}_D$ +80.0° (*c* 0.02, MeOH); UV (MeOH) λ_{max} 253 nm; CD (MeOH) $\Delta\epsilon_{298}$ +1.205; ^1H and ^{13}C NMR data, see Supporting information. EIMS *m/z* 264.2 $[M]^+$; HREIMS *m/z* 264.17257 $[M]^+$, (calcd for $C_{16}H_{24}O_3$, 264.17253).

(8) Trichodermatide D (**4**). Colorless oil. $[\alpha]^{20}_D$ +22.5° (*c* 0.01, MeOH). CD (MeOH) $\Delta\epsilon_{295}$ −1.0281, $\Delta\epsilon_{261}$ +0.1880. ^1H and ^{13}C NMR data, see Supporting information. EIMS *m/z* 280.1 $[M]^+$; HREIMS *m/z* 280.16731 $[M]^+$ (calcd for $C_{16}H_{24}O_4$, 280.16744).

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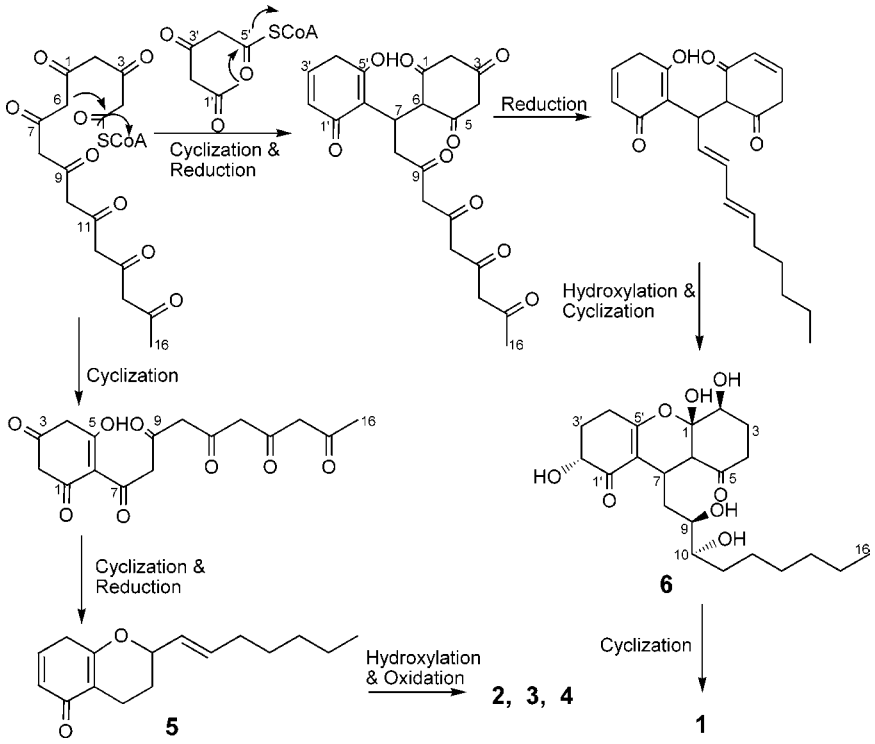
Scheme 1. Plausible Biosynthesis Route of **1**−**4**



positive ring chirality) in Figure 3a gave rise to a positive CE for the n→π* band, whereas the opposite conformation (Figure 3b) defined a negative CE. Therefore, the absolute configuration of **1** was assigned as 2*R*, 7*R*, 8*S*, 9*S*, 10*S*, 13*R*, 15*S*, and 16*S* according to the observed negative Cotton effect at 296 nm ($\Delta\epsilon_{296}$ −0.8601). Moreover, both the C-3 and C-9 of **4** could be assigned as *R*-configuration, and conformation of its cyclohexenone ring was identified to be that of **1**. The CD curves of both **2** and **3** showed similar positive Cotton effects at 295 nm ($\Delta\epsilon_{295}$ +3.395) and 298 nm ($\Delta\epsilon_{298}$ +1.205), indicating the same absolute configuration of 2*S*, 9*S*.

Polyketide derivatives with different skeletons have been isolated from several fungi and originated via similar biosynthesis pathways.[2,11] Scheme 1 shows a plausible biosynthesis pathway for polyketides **1**−**4** that is based on the linear octaketide-folding chain.[12] Initial Claisen condensation between C-5/6 (via an oxygen atom) followed by cyclization from C-5 to C-9 and reduction of the carbonyl groups (except for C-1) would yield octaketide-derived intermediate **5**. A subsequent oxidation of **5** at C-10 and hydroxylation at C-2 or C-3 would yield **2**, **3**, and **4**, respectively. When the octaketide-folding chain is condensed with a triketide unit (C-1′−C-6′), it would produce **1**. The cyclizations between the octaketide and triketide would proceed by further Claisen condensations. Subsequent steps would involve reduction and hydroxylation to yield **6**. Then the nucleophilic OH-9 and OH-10 would attack the carbonyl at C-5 in turn to form the ketal group in **1**.

The cytotoxicity of compounds **1**−**4** was evaluated against the A375-S2 melanoma cell line using the MTT assay. **1**−**4** showed weak cytotoxicity with IC_{50} values at 102.2, 187.3, 38.8, and 222.0 μg/mL, respectively.

Trichodermatide A (**1**) is the first example of a polyketide with a katal-containing pentacyclic skeleton. Octaketide derivatives with the unique bicyclic fusion of an α,β-unsaturated cyclohexenone and a pyran ring are produced only by fungi of the genus *Trichoderma*; this skeleton has been reported for about 10 metabolites to date.[4,13] The new octaketide derivatives Trichodermatides B−D (**2**−**4**) contain some structural features that differ from those of compounds previously reported; they contain a rare keto group or a pair of double bonds at C-10, instead of the hydroxyl.

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Supporting Information Available: Experimental details and 1D, 2D NMR, and MS data. This material is available free of charge via the Internet at http://pubs.acs.org.

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