Trichodermaketones A−D and 7-*O*-Methylkoninginin D from the Marine Fungus *Trichoderma koningii*

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Received October 11, 2009

Five new polyketide derivatives, 7-*O*-methylkoninginin D (**1**) and trichodermaketones A−D (**2**−**5**), together with four known compounds, koninginins A, D, E, and F, were isolated from the marine-derived fungus *Trichoderma koningii*. Trichodermaketones A (**2**) and B (**3**) are unprecedented polyketides with a bistetrafuran-containing tricyclic skeleton. The chemical structures and absolute configurations of compounds **1**−**5** were elucidated by comparing with literature data and extensive spectroscopic methods, including 2D NMR and CD spectroscopic analysis. Compounds **1**−**5** were evaluated for action against bacteria and fungi and for synergistic antifungal activity. Compound **2** showed synergistic antifungal activity against *Candida albicans* with 0.05 μg/mL ketoconazole.

Marine microorganisms have evolved novel physiological and chemical capabilities to survive in the marine environment, which can have extremes of pH, salinity, pressure, or temperature. These adaptations have provided marine microbes with the ability to produce secondary metabolites not found in terrestrial microorganisms.[1,2] The potencial for discovery of new compounds from marine microorganisms has been suggested to be far greater than from terrestrial sources.[3−6] In our search for bioactive compounds from the marine environment, a marine microbial extract library was constructed and screened for various biological activities.[7] The antifungal potentiator beauvericin has already been identified from this particular library,[8] demonstrating that potentially more bioactive compounds could be found.

The fungal *Trichoderma* species are present in a diverse array of habitats, including soil, marine sediments, marine sponges, and mangroves. Some species of *Trichoderma* have been developed as biocontrol agents against fungal diseases in plants and produce a wide array of enzymes and bioactive peptaibols.[9−14] Chemical investigations of other *Trichoderma* species have afforded a variety of bioactive natural products.[15−19] Using high-throughput synergistic screening methods[8] the current study identified an extract from a strain of *Trichoderma koningii* that showed synergistic antifungal activity against *Candida albicans* (SC5314). Using bioassay-guided fractionation, five novel polyketides, named 7-*O*-methylkoninginin D (**1**) and trichodermaketones A, B, C, and D (**2**−**5**), were isolated from this marine-derived fungus. Herein, we report the isolation, structure elucidation, and bioactivities of these polyketides.

Results and Discussion

Compound **1** was obtained as a white, amorphous powder. The molecular formula of **1** was determined to be $C_{17}H_{28}O_5$ (four degrees of unsaturation) by analysis of its HRESIMS spectrum (*m*/*z* 335.1829 $[M + Na]^+$) and NMR data (Table 1). The ^1H, ^{13}C, DEPT, and HSQC spectra of **1** showed 17 carbon signals for one methyl group, one methoxy group, eight methylenes, four oxymethines, two olefinic quaternary carbons, and one α,β-conjugated carbonyl carbon (δ_C 196.0). These data accounted for all ^1H and ^{13}C NMR resonances and required the compound to be bicyclic. Analysis of the $^1H-^1H$ COSY NMR data led to the identification of two fragments of **1**, one for C-2 to C-4, the other for C-7 to C-16. In the HMBC spectrum (Figure 1), correlations from H-7 to C-5 and C-6 revealed that C-1, C-5, and C-7 were attached to C-6. HMBC correlations from one exchangeable proton (δ_H 4.61) to C-3 and C-4 and the second exchangeable proton (δ_H 3.87) to C-11 indicated that C-4 and C-10 were attached to free hydroxy groups. Meanwhile, correlations from H-7 to C-17 and from H_3-17 to C-7 demonstrated that the methoxy group was located at C-7. Considering the chemical shifts of C-5 (δ_C 172.6) and C-9 (δ_C 77.5), as well as the unsaturation requirements for **1**, C-5 and C-9 have to be connected to the same oxygen atom to form a dihydropyran moiety. On the basis of these data, the structure of compound **1** was established.

Except for the methoxy group signals of H-17 and C-17 for **1**, the NMR data were very similar to those of koninginin D (Table 1).[20] From a biogenetic perspective, the configuration of **1** should be identical to that of the co-occurring koninginin D, of which the absolute configuration has been determined by a combination of enantioselective synthesis and X-ray crystallography.[21] Therefore, the absolute configuration of **1** is 4*R*, 7*R*, 9*S*, and 10*S*.

High-resolution ESIMS(−) analysis of trichodermaketone A (**2**) revealed a pseudomolecular ion at *m*/*z* 295.1582 $[M - H]^-$, consistent with the molecular formula $C_{16}H_{24}O_5$ and corresponding to five degrees of unsaturation. Its UV spectrum (λ_{max} 259 nm) and IR spectrum (ν_{max} 1650 cm^{-1}) suggested the presence of an α,β-unsaturated ketone group. The methylene proton signals at δ_H 1.27−1.32 (m, H-12−15), 1.51, and 1.59 (m H-11) and the methyl signal at δ_H 0.86 (t, J = 7.0 Hz, H-16) together with their corresponding carbon signals (Table 1) were assigned to an aliphatic chain by analysis of the 2D NMR correlations. The other carbon signals were assigned to two methylenes at δ_C 35.6 (C-2) and 32.2 (C-3), five oxymethines at δ_C 89.8 (C-8), 82.0 (C-10), 80.2 (C-7), 73.0 (C-9), and 63.9 (C-4), and an α,β-unsaturated enone with a β-substituted oxygen at δ_C 193.4 (C-1), 179.7 (C-5), and 115.9 (C-6). The α,β-unsaturated enone group accounted for two of the five degrees of unsaturation in the molecule, revealing a tricyclic structure for **2**. The planar tricyclic carbon skeleton of **2** was

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10.1021/np900642p
Published on Web 04/12/2010

Chart 1

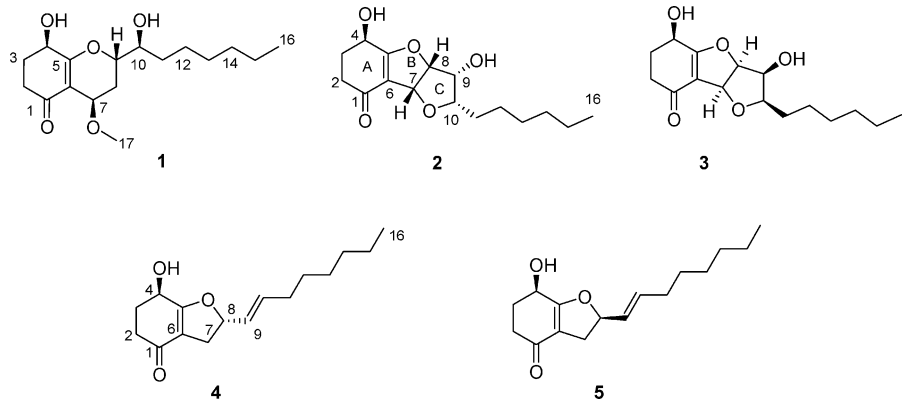

Table 1. NMR Spectroscopic Data for Koninginin D and 7-*O*-Methylkoninginin D (**1**) in Acetone-d_6

	koninginin D	7-*O*-methylkoninginin D (**1**)		
position	δ_C	δ_C	δ_H (*J* in Hz)	HMBC[a]
1	195.8, C	196.0, C		
2a	33.3, CH_2	33.9, CH_2	2.20, m	1, 3, 4
2b			2.54, ddd (16.0, 7.5, 4.5)	
3a	28.8, CH_2	30.1, CH_2	1.92, m	1, 2, 4, 5
3b			2.11, m	
4	65.7, CH	66.6, CH	4.37, dt (6.5, 4.0)	
5	171.5, C	172.6, C		
6	113.9, C	112.4, C		
7	57.1, CH	65.9, CH	4.21, t (2.5)	1, 5, 6, 9, 17
8	31.7, CH_2	28.6, CH_2	1.55, td (13.0, 2.5)	6, 7, 9, 10
			2.04, dt (13.0, 2.5)	
9	77.7, CH	77.5, CH	4.07, ddd (13.0, 4.5, 2.5)	7
10	73.2, CH	72.9, CH	3.67, dt (6.0, 4.5)	
11	32.4, CH_2	33.5, CH_2	1.62, m	9, 10, 12, 13
12	25.1, CH_2	26.4, CH_2	1.42, 1.56, m	
13	29.2, CH_2	30.2, CH_2	1.34, m	
14	32.2, CH_2	32.6, CH_2	1.32, m	
15	22.6, CH_2	23.3, CH_2	1.32, m	
16	14.0, CH_3	14.3, CH_3	0.88, t (7.0)	14, 15
17		56.5, CH_3	3.32, s	7
10-OH			3.87, d (6.0)	11
4-OH			4.61, d (4.0)	3, 4

[a] HMBC correlations, optimized for 8 Hz, are from proton(s) stated to the indicated carbon.

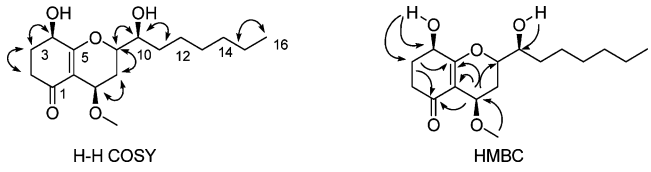

Figure 1. $^1H-^1H$ COSY and key HMBC correlations for compound **1**.

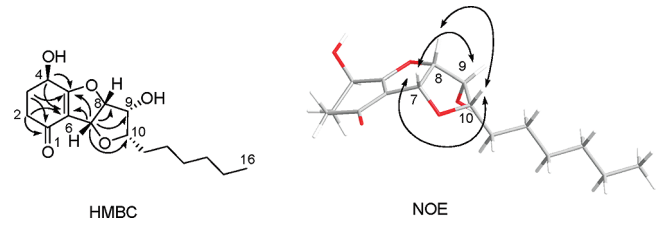

Figure 2. Key HMBC and NOE correlations for trichodermaketone A.

established by 2D NMR experiments. In the $^1H-^1H$ COSY spectrum, the correlations from H-7 through H-8, H-9, and H-10 to H-11, and from H-2 through H-3 to H-4 revealed the two subunits from C-7 to C-11 and from C-2 to C-4 of compound **2**. The HMBC correlations from H-2 to C-1 (δ_C 193.4) and C-6 (δ_C 115.9), from H-3 to C-1 and C-5 (δ_C 179.7), and from H-4 to C-5 and C-6 indicated the connections from C-4 through C-5 and C-6 to C-1. The HMBC correlations from H-7 to C-10 (δ_C 82.0) revealed the connection from C-7 to C-10 by an oxygen atom. The correlations from H-7 to C-5 and H-8 to C-6 indicated the connections of C-6 and C-7 directly from C-5 to C-8 through an oxygen atom. Therefore the structure of **2** was assigned as trichodermaketone A.

In the ^1H NMR spectrum, two intermediate coupling constants (J = 7.0, 5.0 Hz) were observed for H-4, which confirmed a pseudoequatorial position for H-4. From a biogenetic perspective, the octant rule for the cyclohexenones,[22] and a related report,[17] a negative Cotton effect at 292 nm ($\Delta\varepsilon$ −1.347) for the n→π* transition in the CD spectrum of **2** suggested that the absolute configuration of C-4 was 4*R*. In the NOE difference experiments (Figure 2), an irradiation of H-10 resulted in the enhancement of H-7, H-8, and H-9, and the irradiation of H-9 resulted in the enhancement of H-7, H-8, and H-10, revealing that H-7, H-8, H-9, and H-10 were on the same face of the C ring. Although the relative configuration for the protons on ring C was assigned, the absence of NOE correlations between the protons on rings A and C prevented an overall relative configuration assignment.

High-resolution ESIMS(+) analysis of trichodermaketone B (**3**) revealed a pseudomolecular ion at *m/z* 319.1521 $[M + Na]^+$ consistent with the molecular formula $C_{16}H_{24}O_5$, the same as for trichodermaketone A (**2**). It showed almost the same UV spectrum (λ_{max} 259 nm), IR spectrum (ν_{max} 1640 cm^{-1}), and NMR data as those of **2**, which suggested that it possessed the same planar

Table 2. NMR Spectroscopic Data for Trichodermaketones A (**2**) and B (**3**) in Acetone-d_6

	trichodermaketone A (**2**)			trichodermaketone B (**3**)	
position	δ_C	δ_H (J in Hz)	HMBC[a]	δ_C	δ_H[a] (J in Hz)
1	193.4, C			193.7, C	
2a	35.6, CH_2	2.26, dt (17.0, 5.0)	1, 3, 4, 6	35.6, CH_2	2.24, dt (17.0, 5.0)
2b		2.40 ddd (17.0, 7.5, 4.5)			2.42, ddd (17.0, 7.5, 4.5)
3a	32.2, CH_2	2.23, m	1, 2, 4, 5	32.2, CH_2	2.23, m
3b		1.94, m			1.96, m
4	63.9, CH	4.60, dd (7.0, 5.0)	2, 3, 5, 6	63.6, CH	4.53, dd (7.0, 5.5)
5	179.7, C			179.7, C	
6	115.9, C			113.8, C	
7	80.2, CH	5.12, d (7.0)	5, 6, 8, 9, 10	79.7, CH	5.40, d (6.5)
8	89.8, CH	5.21, dd (7.0, 6.0)	5, 6, 7, 9, 10	94.0, CH	4.98, d (6.0)
9	73.0, CH	4.33, dd (6.0, 4.0)	7	75.2, CH	4.13, d (2.0)
10	82.0, CH	3.64, ddd (8.5, 7.0, 4.0)		79.3, CH	3.46, td (7.0, 2.5)
11	29.2, CH_2	1.51, 1.59, m	10, 12, 13	28.3, CH_2	1.66, 1.61, m
12	27.0, CH_2	1.28, m		26.9, CH_2	1.32, m
13	30.3, CH_2	1.28, m		30.2, CH_2	1.31, m
14	32.5, CH_2	1.27, m		32.5, CH_2	1.28, m
15	23.2, CH_2	1.27, m		23.2, CH_2	1.30, m
16	14.3, CH_3	0.86, t (7.0)	14, 15	14.3, CH_3	0.86, t (7.0)

[a] HMBC correlations, optimized for 8 Hz, are from proton(s) stated to the indicated carbon.

Table 3. NMR Spectroscopic Data of Trichodermaketones C (**4**) and D (**5**) in Acetone-d_6

	trichodermaketone C (**4**)			trichodermaketone D (**5**)	
position	δ_C	δ_H[a] (J in Hz)	HMBC[a]	δ_C	δ_H (J in Hz)
1	194.0, C			194.0, C	
2a	35.0, CH_2	2.41, td (12.0, 7.0)	1, 3, 4	35.1, CH_2	2.41, m
2b		2.24, dt (12.0, 5.0)			2.24, dt (12.0, 5.0)
3a	32.3, CH_2	2.21, m	1, 2, 4, 5	32.3, CH_2	2.21, m
3b		1.94, m			1.94, m
4	63.6, CH	4.50, br d (5.0)		63.7, CH	4.51, br d (5.0)
5	176.5, C			176.5, C	
6	113.0, C			113.0, C	
7a	33.2, CH_2	2.89, ddd (14.0, 10.0, 1.5)	5, 6, 8, 9	33.2, CH_2	2.91, dd (14.5, 10.0)
7b		2.45, dd (14.0, 7.5)			2.45,ddd (14.5, 7.5, 2.0)
8	86.6, CH	5.23, dt (10.0, 7.5)	5, 6, 7, 9, 10	86.8, CH	5.22, dt (10.0, 7.5)
9	129.8, CH	5.60, dd (15.5, 7.5)	8, 11	129.7, CH	5.61, dd (15.0, 7.5)
10	135.1, CH	5.83, dt (15.5, 6.0)	8, 11, 12	135.5, CH	5.83, dt (15.0, 6.0)
11	32.7, CH_2	2.08, m	9, 10, 12	32.7, CH_2	2.08, m
12	29.6, CH_2	1.40, m	10	29.6, CH_2	1.41, m
13	32.4, CH_2	1.29, m		32.4, CH_2	1.29, m
14	29.5, CH_2	1.28, m		29.5, CH_2	1.28, m
15	23.2, CH_2	1.29, m		23.2, CH_2	1.29, m
16	14.3, CH_3	0.87, t (7.0)	14, 15	14.3, CH_3	0.88, t (7.0)
4-OH		4.62 d (5.0)	3, 4, 5		4.61, d (5.0)

[a] HMBC correlations, optimized for 8 Hz, are from proton(s) stated to the indicated carbon.

structure as trichodermaketone A. Its planar structure was confirmed by 2D NMR analysis. The main differences between the two compounds are proton and carbon signals at positions 8, 9, and 10 (Table 2). The H-9 of trichodermaketone A shows a doublet of doublet pattern at δ_H 4.33 with coupling constants of 6.0 and 4.0 Hz, but appears as a doublet peak with a small 1.5 Hz coupling constant in trichodermaketone B. In the NOE difference experiments, an irradiation of H-9 resulted in the enhancement of H-8 and H-10, and the irradiation of H-8 resulted in the enhancement of H-7 and H-9, revealing that H-7, H-8, H-9, and H-10 were on the same face of the C ring. In the CD spectrum, a negative Cotton effect for the n→π* transition was observed at 287 nm ($\Delta\varepsilon$ −0.882). This suggested a 4*R* absolute configuration for **3**. Because **3** and **2** have the same 4*R* configuration, the relative configuration for **3** between C-4 and ring C should be inverted compared to **2**. As with compound **2**, no NOE correlations were observed between protons on rings A and C, so the overall relative configuration remains tentative. Attempts to prepare the Mosher's ester derivatives to determine the absolute configuration of C-9 were unsuccessful.

High-resolution ESIMS(+) analysis of trichodermaketone C (**4**) revealed a pseudomolecular ion at m/z 287.1595 $[M + Na]^+$, consistent with the molecular formula $C_{16}H_{24}O_3$, indicating five degrees of unsaturation. Its UV spectrum (λ_{max} 272 nm) and IR spectrum (ν_{max} 1623 cm^{-1}) suggested the presence of an α,β-unsaturated enone group. In the ^1H NMR and ^{13}C NMR spectra, it showed the same α,β-unsaturated cyclohexenone moiety as trichodermaketones A and B, and the presence of a *trans*-double bond was revealed by proton signals at δ_H 5.60 (dd, J = 15.5, 7.5 Hz, H-9) and 5.83 (dt, J = 15.5, 7.5 Hz, H-10), along with the corresponding carbon signals at δ_C 129.8 (C-9) and 135.1 (C-10). In the $^1H-^1H$ COSY spectrum the correlations indicated the connections from C-7 to C-8, through C-9 to C-10, then to the aliphatic chain. The HMBC correlations from H-8 to C-5 revealed the connection from C-8 to C-5 through an oxygen atom. Further HMBC correlations from H-7 to C-5 and C-6 indicated the attachment of C-7 to C-6. On the basis of this data, the gross structure of trichodermaketone C was established as **4**.

The molecular formula of **5**, deduced by HRESIMS, was the same as that determined for **4** ($C_{16}H_{24}O_3$). Compound **5** showed the same maximum UV absorption (λ_{max} 272 nm) and had almost the same proton and carbon NMR data as **4** (Table 3) except for minor variations near C-8. This suggested that the two compounds were isomers, varying only in the configuration at C-8.

The H-4 proton signal in the ^1H NMR spectrum for both **4** and **5** appeared as a broad doublet (J = 5.0 Hz), revealing a pseudoequatorial orientation for the hydrogen at C-4. The negative

Cotton effects for **4** at 274 nm ($\Delta\varepsilon$ −2.582) and for **5** at 297 nm ($\Delta\varepsilon$ −1.436), correlating to the n→π* transition in the CD spectra, suggested the absolute configuration of C-4 is 4*R* in both molecules. The side chain with the double bond at C-8, corresponding to the back region relative to the carbonyl, resulted in the significant differences in the wavelengths of the Cotton effects for **4** and **5**. Because NOE correlations between rings A and B in **4** and **5** were absent, the configuration of C-8 was not assigned. Compounds **4** and **5** are epimers at C-8, with the α and β orientations at C-8 depicted arbitrarily

7-*O*-Methylkoninginin D (**1**) and trichodermaketones A−D (**2**−**5**) were evaluated for activity against methicillin-resistant *Staphylococcus aureus* (MRSA, clinical isolates, Beijing Chao-yang Hospital) and *C. albicans* (SC5314). None of the compounds showed anti-MRSA activity (MIC > 100 μg/mL). Trichodermaketone A had no antifungal activity against *C. albicans* (MIC > 125 μg/mL), but it showed synergistic antifungal activity against *C. albicans* SC5314 at 125 μg/mL with 0.05 μg/mL ketoconazole.

Many compounds with different bioactivities have been isolated from the genus *Trichoderma*.[15−17,19,23] Some hexahydrobenzopyran-5-one derivatives have been obtained from *T. reesei*, named the trichodermatides,[17] and koninginins have been isolated from *T. koningii*[24−27] and *T. harzzianum*.[28,29] The trichodermaketones A (**2**) and B (**3**), found in this study, are new members of the tricyclic polyketides with novel carbon skeletons, which should be of interest to natural product and synthetic chemists. This is the first report of tetrahydrobenzofuran-4-one derivatives obtained from a *Trichoderma* fungus. A plausible biosynthesis route to the trichodermatides has recently been reported,[17] and it may be that different cyclases generate the varied metabolites.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 241MC polarimeter. UV data were recorded on a Mariner System 5304 instrument. The CD spectra were recorded on a JASCO J-815 spectropolarimeter, using CH_3OH as a solvent. IR spectra were recorded on a Nicolet 5700 FT-IR microscope spectrometer (FT-IR Microscope Transmission). NMR spectra were recorded on a Varian Inova 500 MHz spectrometer at 500.103 MHz for ^{1}H and 125.762 MHz for ^{13}C in acetone-d_6 using solvent signals (acetone-d_6; δ_H 2.05/δ_C 29.8, 206.1) as reference; the coupling constants are in Hz. HRESIMS spectra were recorded with a Q-trap LC-MSMS system using a Turbo ionspray source. Column chromatography was performed with silica gel (200−300 mesh, Qingdao Haiyang Chemical Factory) and Sephadex LH-20 (Pharmacia Co.) columns. TLC was carried out using silica gel GF254 (Qingdao Haiyang Chemical Factory) plates. HPLC was performed using an Agilent Chromatorex C18 (10 μm) semipreparative column (10 × 250 mm). ODs were read by an Envision 2103 multilabel reader (PerkinElmer, USA).

Fungal Material and Cultivation. The fungus *Trichoderma koningii* was obtained from marine mud of the South China Sea, identified by analysis of the internal transcribed spacer (ITS) regions (GenBank accession number GU244589) and morphology, and assigned the accession number MF349 in the culture collection at the Institute of Microbiology, Chinese Academy of Sciences, Beijing. The culture medium of the strain contained 18 g of rice and 30 mL of artificial seawater in a 250 mL flask. It was cultured without shaking at 25 °C for 20 days before use.

Extraction and Isolation. The fermentation product was exhaustively extracted with EtOAc−MeOH−AcOH (80:15:5) to yield an extract (5 g). The residue was suspended in H_2O and then partitioned with EtOAc. The EtOAc fraction was chromatographed on a reduced pressure silica gel column using a gradient of $CHCl_3$ in MeOH to afford 27 fractions. The eighth fraction was subjected to normal-phase silica gel chromatography and eluted with 10% petroleum ether in acetone to yield (+)-koningingn D (32 mg) and compound **1** (18 mg). The seventh fraction was subjected to a Sephadex LH-20 column [petroleum ether−$CHCl_3$−MeOH (5:5:1)] to give five subfractions. The third subfraction was further purified by reversed-phase HPLC to afford trichodermaketones A (**2**, 5.1 mg) and B (**3**, 3.2 mg). The fourth fraction was again subjected to the Sephadex LH-20 column [petroleum ether−$CHCl_3$−MeOH (5:5:1)] to give five subfractions. The second subfraction was further purified by reversed-phase HPLC to yield trichodermaketones C (**4**, 2.3 mg) and D (**5**, 4.2 mg).

(+)-Koninginin D: white wax; $[\alpha]_D^{20}$ +159 (*c* 0.30, $CHCl_3$), lit. +166.9 (*c* 0.30, $CHCl_3$);[20] UV (MeOH) λ_{max} 252 nm; ^{13}C NMR data, see Table 1; ESIMS *m/z* 297 $[M - H]^-$.

7-*O*-Methylkoninginin D (1): white, amorphous powder; $[\alpha]_D^{20}$ +96.3 (*c* 0.17, MeOH); UV (MeOH) λ_{max} 252 nm; IR ν_{max} 3417, 2919, 2857, 2820, 1624, 1593, 1468, 1421, 1370, 1335, 1242, 1180, 1147, 1099, 1083, 1061, 1006, 952, 925, 915 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 311 $[M - H]^-$; HRESIMS *m/z* 335.1829 $[M + Na]^+$ (calcd for $C_{17}H_{28}O_5$, 335.1834).

Trichodermaketone A (2): colorless oil; $[\alpha]_D^{20}$ +73.0 (*c* 0.17, MeOH); UV (MeOH) λ_{max} 259 nm; CD (MeOH) $\Delta\varepsilon_{292}$ nm −1.347, $\Delta\varepsilon_{258}$ nm +1.067; IR ν_{max} 3391, 2949, 2923, 2864, 1675, 1650, 1596, 1433, 1415, 1335, 1296, 1234, 1188, 1141, 1078 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 295 $[M - H]^-$; HRESIMS *m/z* 295.1582 $[M - H]^-$ (calcd for $C_{16}H_{23}O_5$, 295.1546).

Trichodermaketone B (3): colorless oil; $[\alpha]_D^{20}$ −36.7 (*c* 0.17, MeOH); UV (MeOH) λ_{max} 259 nm; CD (MeOH) $\Delta\varepsilon_{287}$ nm −0.822, $\Delta\varepsilon_{259}$ nm −1.079; IR ν_{max} 3385, 2955, 2929, 2859, 1640, 1454, 1406, 1335, 1222, 1203, 1134, 1077, 1029 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 295 $[M - H]^-$; HRESIMS *m/z* 319.1521 $[M + Na]^+$ (calcd for $C_{16}H_{24}O_5Na$, 319.1521).

Trichodermaketone C (4): colorless oil; $[\alpha]_D^{20}$ −92.5 (*c* 0.17, MeOH); UV (MeOH); λ_{max} 272 nm; CD (MeOH) $\Delta\varepsilon_{274}$ nm −2.582, $\Delta\varepsilon_{235}$ nm −0.612; IR ν_{max} 3374, 2954, 2927, 2856, 1722, 1623, 1453, 1404, 1358, 1231, 1171, 1072 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 287 $[M + Na]^+$; HRESIMS *m/z* 287.1595 $[M + Na]^+$ (calcd for $C_{16}H_{24}O_3Na$, 287.1623).

Trichodermaketone D (5): colorless oil; $[\alpha]_D^{20}$ +96.8 (*c* 0.17, MeOH); UV (MeOH) λ_{max} 272 nm; CD (MeOH) $\Delta\varepsilon_{297}$ nm −1.436, $\Delta\varepsilon_{266}$ nm +1.610; IR ν_{max} 3375, 2954, 2927, 2856, 1720, 1619, 1454, 1403, 1360, 1230, 1190, 1171, 1071 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 287 $[M + Na]^+$; HRESIMS *m/z* 287.1604 $[M + Na]^+$ (calcd for $C_{16}H_{24}O_3Na$, 287.1623).

Antimicrobial Assay. Fresh Mueller-Hinton broth medium (40 μL) was added to each well of a sterilized 96-well microtiter plate, 2 μL of the samples to be tested was added to the test wells, and then 40 μL of the test strain solutions was added to every well. The plate was incubated at 37 °C overnight. Anti-MRSA activity of samples was checked by measuring and comparing the optical diversities of the blank control and tested wells.

Antifungal and Synergistic Antifungal Assay. *Candida albicans* SC5314 was used as a test strain for antifungal and synergistic antifungal bioassay according to a previous paper.[8] The antifungal activity and MICs were determined by the presence and absence of a subclinical concentration of 0.05 μg/mL ketoconazole in flat-bottom 96-well microtiter plates (VWR, West Chester, PA), with a total volume of 0.08 mL/well by using a broth microdilution protocol modified from the Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards) M-38A and M-27A2 methods. The cell densities of overnight cultures were determined, and dilutions prepared so that ∼1 × 10^4 cells were inoculated into each well in RPMI 1640 medium with 2 μL of samples and 8% Alamar blue (BioSource International, Camarillo, CA). Plates were then incubated overnight at 35 °C with 80% humidity and 5% CO_2.[6] The fluorescence, to determine the percentage of remaining viable cells, was measured at excitation wavelength 544 nm and emission wavelength 590 nm by using an Envision 2103 multilabel reader (PerkinElmer, USA). For the synergistic antifungal assay, the MIC of ketoconazole was first tested; then according to FICI, 1/4 MIC of ketoconazole was added into RPMI medium 1640, and the other protocols were the same as for the antifungal assay.

Acknowledgment. We thank E. J. Ashforth for her critical reading of the manuscript and helpful discussions. Part of this work was performed under a research collaboration between Global Alliance for TB Drug Development (TB Alliance) and the Institute of Microbiology of the Chinese Academy of Sciences (IMCAS). We acknowledge Drs. Z. Ma, A. Upton, and C. B. Cooper from the TB Alliance for their scientific input during the performance of this work. This work was also supported in part by grants from the National Natural Science Foundation of China (30901849, 30700015), MOST Key International Project 2007DFB31620 and 863 Hi-Tech Research and Development

Program of China (Grants 2006AA09Z402, 2007AA09Z443), Guangdong Province's 2006A50103001, and Chinese Academy of Sciences Innovation Project (KSCXZ-YW-G-013, KSCX2-YW-R-164). This work was also supported in part by the National Science & Technology Pillar Program (No. 200703295000-02) and Important National Science & Technology Specific Projects (No. 2008ZX09401-005). The authors gratefully acknowledge the support of the K.C. Wong Education Foundation, Hong Kong. L. X. Zhang was provided an award from the Hundred Talents Program.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra of 7-*O*-methylkoninginin D and trichodermaketones A−D. This material is available free of charge via the Internet at http://pubs.acs.org.

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NP900642P