Potent Phytotoxic Harziane Diterpenes from a Soft Coral-Derived Strain of the Fungus *Trichoderma harzianum* XS-20090075

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Two new harziane diterpene lactones, possessing a 6/5/7/5-fused carbocyclic core containing a lactone ring system, harzialactones A and B (1 and 2), and five new harziane diterpenes, harzianones A–D (3–6) and harziane (7), were isolated from the soft coral-derived fungus *Trichoderma harzianum* XS-20090075. Their structures were determined by extensive NMR spectroscopic data, ECD and OR calculations, as well as X-ray diffraction. The isolated compounds exhibited potent phytotoxicity against seedling growth of amaranth and lettuce. Harziane diterpenes were rarely reported for their remarkably bioactivities, and it was the first report to study the phytotoxicity of harziane diterpenes, which provide a new application of such compounds in agriculture for future research.

Increasing concerns for the management of weeds have been caused by scientists, as they can bring out greater reduction in crop yields than plant diseases and pests1. Nowadays more than half of the pesticides used are herbicides1,2. With the increasing attention to food safety and environmental protection, it is desirable to develop new types of bio-herbicides with high efficiency and low toxicity.

*Trichoderma* spp. are one of the most commonly disseminated fungi in nature, and are distributed around the world ranging from the tundra to the tropics. They have been widely used as biocontrol agents (*T. harzianum*, *T. atroviride*, and *T. asperellum*), and commercially marketed as biopesticides, due to their capacity to parasitize in other fungi and to compete with deleterious plant microorganisms3. However, there are few *Trichoderma* spp. products sold in the commercial market, and limited studies are focused on the phytotoxicity of compounds from *Trichoderma* spp4.

Marine fungi have gained more and more attention over the past decades, as the recognition that they are a quite diverse group and an excellent source of natural products, possessing prominent bioactivities, including antibacterial, antifungal, antiviral, anti-inflammatory, antitumor, and insecticidal5. Marine-derived *Trichoderma* spp. have been reported to represent a potential source for producing compounds with novel structures and remarkable bioactivities, such as trichodermanamides A and B6, dithioaspergillazine A7, tandyukisins E and F8, as well as harzianone9. Therefore, it has huge potential to find new phytotoxic compounds from marine-derived *Trichoderma* spp.

During our efforts to find novel bioactive compounds from coral-derived fungi in the South China Sea10–13, a *T. harzianum* XS-20090075 strain attracted our attention because the fingerprint for the extract of the fungal culture on HPLC showed abundant peaks with interesting UV absorption spectra at around 250 nm, and the
fungal extracts showed obvious phytotoxicity. Further chemical examination on the EtOAc extract resulted in the discovery of two new harziane diterpene lactones, harzianelactones A and B (1 and 2), and five new harziane diterpenes, harzianones A–D (3–6), and harziane (7) (Fig. 1). Herein, we describe the isolation, structure elucidation, and phytotoxicity of these harziane diterpenes.

Results and Discussion

Harzianelactone A (1) was obtained as a colorless oil with the molecular formula of C_{20}H_{30}O_{3} by HRESIMS, requiring six degrees of unsaturation. The 1H NMR spectrum (Table 1) showed three protons on oxygenated carbons at δ_H 3.89 (d, J = 9.0 Hz), 3.80 (d, J = 8.0 Hz), and 3.73 (d, J = 8.0 Hz), four methyl singlets at δ_H 2.33 (s), 1.43 (s), 0.85 (s), and 0.84 (s), as well as one methyl doublet at δ_H 1.15 (d, J = 8.0 Hz). The 13C NMR (Table 2) and DEPT spectra in combination with HMQC data revealed one lactone carbonyl (δ_C 171.3), one oxymethylene carbon (δ_C 78.4), one oxymethine carbon (δ_C 73.7), four methylenes (δ_C 50.5, 41.3, and 40.0), five methyl groups (δ_C 24.9, 22.9, 22.6, 21.2, 20.7), and five nonprotonated carbons (δ_C 155.9, 131.0, 49.7, 46.3, 44.9) including two quaternary olefinic ones. The aforementioned data corresponded to two degrees of unsaturation, and the remaining four degrees of unsaturation suggested the existence of four rings. The planar structure of 1 was elucidated on the basis of COSY and HMBC experiments (Fig. 2). The spin systems of H-14/H-15/H-2/H-3/H-4/H-5/H-18 in the COSY cross peaks and the correlations from H-3 to C-1, C-5, and C-15, from H-4 to C-2, C-6 and C-18, from H-5 to C-1 and C-14, from H-16 and H-17 to C-2 and C-6, and from H-18 to C-6 in the HMBC spectrum, led to the construction of a five-membered ring B and a six-membered ring A with a hydroxy and a methyl group anchored to C-4 and C-5, respectively. The seven-membered ring C with two methyl groups connected to C-9 and C-13 was further constructed according to the HMBC correlations from H-7 to C-9 and C-14, from H-8 to C-6 and C-10, from H-19 to C-10 and C-14, and from H-20 to C-8 and C-10. The lactone carbonyl (δ_C 171.3), in addition to the HMBC correlations from H-12 to C-10, C-11, C-14, and C-19, and from H-15 to C-13 indicated the existence of a five-membered lactone ring D connected to ring C. Finally, the connection of ring B and ring C was confirmed by the HMBC correlations from H-7 and H-14 to C-1, from H-7 to C-5, and from H-15 to C-13. Therefore, the planar structure of 1 was determined.

The NOESY correlations of H-3-16 with H-2 and H-14 indicated a cis-relationship of them (Fig. 3). Hence, ring D was oriented on the opposite face of ring C relative to these protons. The NOESY correlation of H-4/H-3-18 indicated an anti-relationship of 4-OH and H-3-18. The vicinity of H-5 and C-19 was deduced by the correlation of H-5/H-3-19 in the NOESY spectrum.

The absolute configuration of 1 was established by comparison of its calculated and observed ECD and optical rotations (OR) data (see Supplementary Information). The predicted ECD for (2R, 4S, 5S, 6S, 13S, 14R)-1 was in agreement with the experimental result of 1 (λ_max (Δε) 200 (−1.55), 239 (+7.03) nm) (Fig. 4). The computed ORs in the gas phase were −38.8 for (2S, 4R, 5R, 6R, 13R, 14S)-1, and +38.8 for (2R, 4S, 5S, 6S, 13S, 14R)-1, respectively, and the experimental value was +34.0. Based on both of ECD and OR calculations, the absolute configuration of 1 was assigned as 2R, 4S, 5S, 6S, 13S, 14R.

Harziane diterpenes are a unique class of terpenes, and only 16 such skeletons have been reported. The cyclization mechanism of these unique diterpenes was illuminated by studies of selectively 13C- and 2H-labeled synthetic mevalonolactone isotopologues. Distinguishing 1 from classic harziane diterpenes such as harzianone and harzianidione was the D ring, which is a product of a Baeeyer-Villiger monooxygenase catalyzed oxidation of a
6/5/7/4 fused tetra-cyclic skeleton; only two such harziane diterpene lactones have been discovered\cite{17,22}. Moreover, only one report has appeared on the absolute configuration of such a harziane diterpene lactone (harzialelactone) by comparison its optical rotation data with that of the classic harziane diterpene, harzianone\cite{17}. This report is the first to determine the absolute configuration of a harziane diterpene lactone by comparison of calculated and observed ECD spectra.

Harzialelactone B (2) was also isolated as a colorless oil and was assigned the same molecular formula C$_{20}$H$_{30}$O$_{3}$ as 1 by HRESIMS results. Extensive analysis of the 1D and 2D NMR data indicated that 2 was also a harziane diterpene lactone, possessing a 6/5/7/5 fused tetra-cyclic ring scaffold like 1. Compared to 1, the disappearance of the oxymethylene signals in the $^1$H ($\delta_{H}$ 3.80, 3.73) and $^{13}$C NMR ($\delta_{C}$ 78.4) spectra, and its replacement with ketomethylene signals at $\delta_{H}$ 2.46, 2.33, 2.33, and $\delta_{C}$ 73.5, and the significant downfield shift of C-10, as well as the different UV absorption ($\lambda$$_{max}$ = 220 nm; 1: $\lambda$$_{max}$ = 237 nm), in combination with biogenetic considerations, suggested that C-10 is connected to the O-atom of the ester carbonyl. Detailed analysis of the HMBC correlations from H-8 and H$_3$-20 to C-10, from H-12 to C-11, from H-14 to C-12, and from H-15 to C-13 (Fig. 2) also confirmed the structure. The relative configuration of 2 was deduced to be identical to that of 1 from the assignments of the cross-peaks in its NOESY spectrum (Fig. 3). The computed OR was +21.7 for (2R, 4S, 5S, 6S, 13S, 14R)-2, and the experimental value was +18.9 (see Supplementary Information). Therefore, the absolute configuration of 2 was determined as 2R, 4S, 5S, 6S, 13S, 14R. This is the first report of the absolute configuration of harziane diterpene lactones with the acyloxy group connected to C-10, such as 2.

Harzianone A (3) was obtained as a colorless oil. Its molecular formula of C$_{20}$H$_{30}$O$_{2}$ (six degrees of unsaturation) was determined by HRESIMS and NMR data (Tables 1, 2). The UV absorption and the IR spectrum as well as the ECD spectrum in combination with biogenetic considerations, revealed five ones. Compared

| Position | 1  | 2  | 3  | 4  | 5  | 6  | 7  |
|----------|----|----|----|----|----|----|----|
| 2        | 1.66, m | 1.65, m | 1.53–1.58, m | 1.59–1.65, m | 1.89–2.01, m | 1.73, m | 1.58–1.60, m |
| 3        | 2.39, ddd (13.5, 9.0, 4.0) | 2.30–2.41, m | 2.27–2.36, m | 2.38, ddd (14.5, 8.5, 4.0) | 2.73, brd (18.0) | 4.27, m | 2.38, ddd (14.0, 9.0, 4.5) |
| 4        | 2.14–1.45, m | 2.14–1.47, m | 1.43, d (15.0) | 1.48, d (14.5) | 2.55, d (16.0) | 1.58–1.60, m | 1.43, d (14.4) |
| 5        | 3.89, d (9.0) | 3.86, d (8.5) | 3.82, d (8.5) | 3.87, d (8.5) | 2.28–2.41, m | 1.62–1.82, m | 3.83, d (9.0) |
| 6        | 2.55, q (8.0) | 2.49, q (8.0) | 2.54, q (8.0) | 2.57, q (8.0) | 3.13, q (8.0) | 2.54, q (8.0) | 2.49, brq (8.0) |
| 7        | 1.83, dd (13.0, 7.0) | 1.76, dd (13.0, 6.5) | 1.85, dd (13.0, 7.0) | 2.28–2.41, m | 1.77–1.82, m | 1.75–1.82, m |
| 8        | 2.48, t (14.5) | 2.33, t (14.0) | 2.27–2.36, m | 2.30, t (13.0) | 2.28–2.41, m | 2.36, t (14.0) | 2.27, t (14.0) |
| 9        | 2.10, d (14.5, 7.0) | 1.87, dd (14.0, 6.5) | 1.82–1.90, m | 1.98, dd (13.0, 7.0) | 1.89–2.01, m | 1.93, dd (14.0, 5.0) | 1.75–1.82, m |
| 10       | 4.66, d (7.0) |
| 11       | 3.80, d (8.0) | 2.46, d (17.0) | 2.46, d (16.5) | 2.58, d (17.0) | 2.51, d (16.5) | 2.55, d (16.0) | 1.88–1.93, m |
| 12       | 3.73, d (8.0) | 2.33, d (17.0) | 2.33, d (16.5) | 2.47, d (17.0) | 2.38, d (16.5) | 2.39, d (16.0) | 1.58–1.60, m |
| 13       | 2.04, t (10.5) | 2.15, t (11.0) | 2.00, t (10.5) | 2.01, t (10.5) | 2.25–2.41, m | 2.19, t (11.0) | 1.91, t (10.5) |
| 14       | 1.79–1.86, m | 1.82–1.89, m | 1.82–1.90, m | 1.90–1.94, m | 1.17–1.23, m | 1.62–1.69, m | 1.82–1.86, m |
| 15       | 1.49–1.54, m | 1.57, t (12.0) | 1.53–1.58, m | 1.59–1.65, m | 1.37–1.45, m |
| 16       | 0.84, s | 0.85, s | 0.79, s | 0.83, s | 1.13, s | 0.89, s | 0.82, s |
| 17       | 0.85, s | 0.86, s | 0.80, s | 0.84, s | 1.01, s | 1.04, s | 0.83, s |
| 18       | 1.15, d (8.0) | 1.14, d (8.0) | 1.09, d (8.0) | 1.14, d (8.0) | 1.23, d (8.0) | 1.05, d (8.0) | 1.13, d (8.0) |
| 19       | 1.43, s | 1.43, s | 1.47, s | 1.56, s | 1.33, s | 1.48, s | 1.58, s |
| 20       | 2.33, s | 1.75, s | 2.02, s | 4.36, d (18.5) | 2.09, s | 2.08, s | 1.72, s |

Table 1. 1H NMR Data of 1–7 (500 MHz, CDCl$_3$, $\delta$ in ppm, $J$ in Hz).
to 3, the C-20 methyl group was replaced by an oxymethylene in 4, which was defined by the HMBC correlations from H-20 to C-8, C-9, and C-10. As expected, subsequent analyses of the coupling constants, NOESY correlations, and experimental ECD data (Fig. 5) indicated that 4 has the same absolute configuration (2R, 4S, 5S, 6S, 13S, 14S) as that of 3.

Harzianone C (5) was isolated as colorless crystal needles. The molecular formula, C20H30O2, was assigned to be the same as that of 3 by its HRESIMS. The 1H and 13C NMR spectra of 5 showed similar characteristic signals to 3 (Tables 1, 2), except for the chemical shifts around the oxymethine group. In the 1H NMR spectrum, the oxymethine proton appeared as a multiplet, which was different from the doublets for 1–4, and indicated the position of the hydroxy group was changed in 5. In the HMBC spectrum, the correlations of H-4 with C-6, of H-15 with C-3, and of H3-18 with C-4 indicated the hydroxyl group was attached to C-3. The NOESY correlations from H-3 to H3-18 suggested that 3-OH and H3-18 are on the opposite sites of ring A. The relative configurations of the other chiral centers were confirmed to be the same as those of 3. The ECD spectrum of 5 showed the same pattern as those of 3 and 4 (Fig. 5), suggesting that their chirality centers have the same absolute configurations. In addition,

Table 2. 13C NMR Data of 1–7 (125 MHz, CDCl3, δ in ppm). *Signals are exchangeable.

| Position | 1   | 2   | 3   | 4   | 5   | 6   | 7   |
|----------|-----|-----|-----|-----|-----|-----|-----|
| 1        | 44.9, C | 45.2, C | 45.2, C | 45.3, C | 46.2, C | 47.6, C | 45.2, C |
| 2        | 41.3, CH3 | 41.2, CH3 | 41.0, CH3 | 41.0, CH3 | 41.0, CH3 | 49.8, CH3 | 41.3, CH3 |
| 3        | 39.8, CH3 | 39.5, CH3 | 39.5, CH3 | 39.6, CH3 | 46.4, CH3 | 67.5, CH3 | 40.1, CH3 |
| 4        | 73.7, CH3 | 73.6, CH3 | 73.5, CH3 | 73.7, CH3 | 216.9, C | 35.4, CH3 | 74.1, CH3 |
| 5        | 40.0, CH3 | 39.8, CH3 | 39.8, CH3 | 39.8, CH3 | 46.1, CH3 | 29.9, CH3 | 40.3, CH3 |
| 6        | 49.7, C | 49.8, C | 50.2, C | 50.0, C | 51.1, C | 50.4, C | 50.1, C |
| 7        | 28.0, CH3 | 29.3, CH3 | 29.0, CH3 | 29.6, CH3 | 29.1, CH3 | 29.4, CH3 | 29.3, CH3 |
| 8        | 32.1, CH3 | 26.8, CH3 | 29.4, CH3 | 24.4, CH3 | 28.9, CH3 | 29.6, CH3 | 29.4, CH3 |
| 9        | 155.9, C | 112.4, C | 146.7, C | 153.4, C | 145.9, C | 146.2, C | 134.7, C |
| 10       | 131.0, C | 153.1, C | 150.5, C | 149.2, C | 149.9, C | 149.8, C | 143.7, C |
| 11       | 171.3, C | 174.4, C | 200.0, C | 200.1, C | 198.0, C | 199.0, C | 67.8, CH3 |
| 12       | 78.4, CH3 | 47.3, CH3 | 59.8, CH3 | 58.7, CH3 | 60.0, CH3 | 59.8, CH3 | 45.8, CH3 |
| 13       | 46.3, C | 44.9, C | 39.4, C | 38.9, C | 39.9, C | 40.6, C | 45.4, C |
| 14       | 50.5, CH3 | 51.3, CH3 | 51.5, CH3 | 51.2, CH3 | 53.3, CH3 | 51.9, CH3 | 52.9, CH3 |
| 15       | 28.2, CH3 | 29.2, CH3 | 28.5, CH3 | 28.4, CH3 | 29.7, CH3 | 21.9, CH3 | 28.0, CH3 |
| 16       | 24.9, CH3 | 24.9, CH3 | 24.9, CH3 | 24.8, CH3 | 26.1, CH3 | 24.9, CH3 |
| 17       | 22.9, CH3 | 23.0, CH3 | 22.7, CH3 | 22.8, CH3 | 22.4, CH3 | 22.0, CH3 | 23.3, CH3 |
| 18       | 21.2, CH3 | 21.0, CH3 | 21.2, CH3 | 21.2, CH3 | 17.0, CH3 | 20.8, CH3 | 21.4, CH3 |
| 19       | 20.7, CH3 | 22.1, CH3 | 21.8, CH3 | 22.0, CH3 | 21.7, CH3 | 21.3, CH3 | 23.3, CH3 |
| 20       | 22.6, CH3 | 19.5, CH3 | 21.7, CH3 | 66.8, CH3 | 22.4, CH3 | 22.5, CH3 | 19.8, CH3 |

Figure 2. COSY and key HMBC correlations of 1 and 2.
Figure 3. Selected NOESY correlations of 1 and 2.

Figure 4. Experimental ECD spectra of 1 and calculated ECD spectra for (2S, 4R, 5R, 6R, 13R, 14S)-1 and (2R, 4S, 5S, 6S, 13S, 14R)-1.

Figure 5. Experimental ECD spectra of 3–6.
Trichoderma phytoxicity of crude extracts of have stronger toxicity on the growth of root growth than hypocotyl. Although there are three reports on the at 200 ppm. It seemed that the isolated compounds caused weaker inhibition to lettuce than to amaranth, and no compound was found to inhibit the root growth of lettuce against amaranth at 200 mL), compared to the positive control glyphosate. No compound was found to inhibit the root growth of lettuce

Figure 6. X-ray ORTEP diagrams of compounds 5 and 7.

an X-ray crystallographic study (Fig. 6) was performed to confirm unambiguously the structure and determined the absolute configuration of 5 as 2S, 4S, 5S, 6S, 11R, 13S, 14S.

Harzianone D (6) was obtained as a colorless oil. The NMR spectral features suggested that 6 was closely related to 3. The additional carbonyl group (δ 216.9) and the disappearance of the oxymethine group (δ 73.5; δ 14 3.82 in 3) in 6 indicated that the hydroxy group at C-4 in 3 was replaced by a carbonyl group in 6, which was confirmed by the HMBC correlations from H-2, H-3, H-5, and H-18 to C-4. The relative configuration was determined as the same as 1−5 through NOESY spectrum. Similar cotton effects observed for 6 (Δε 223 + 4.16, Δε 280 −1.36) to 3 and 4 in their ECD spectra (Fig. 5) indicated that they shared the same absolute configurations of (2R, 5S, 6S, 13S, 14S).

Harziane (7) was obtained as colorless crystals. Its molecular formula, C20H16O4, was deduced from its HRESIMS data with five indices of hydrogen deficiency, one fewer than those of 1−5. The 1H and 13C NMR data (Tables 1, 2) of 7 and 3 were very similar with each other except for those in the vicinity of C-11. In the 13C NMR spectra, the signals for α,β-unsaturated ketone (δc 200.0) in 3 was disappeared and one more oxymethine (δc 67.8) was emerged in 7. These evidences as well as the unsaturation degrees of these two compounds indicated that the ketone carbonyl group at C-11 in 3 was replaced by an oxymethine group in 7, which was confirmed by the HMBC correlations from H-11 to C-9, C-10, and C-13. The relative configuration of all chiral centers but C-11 was determined by the NOESY spectrum of 7 like 1−6, and the correlation of H-11 with H3-18 indicated they were cis-oriented. To clarify its absolute stereochemistry, 7 was recrystallized in a dichloromethane/methanol (20:1) mixture to yield crystals. The low-temperature X-ray diffraction (CuKα) of the single crystals (Fig. 6) revealed that 7 had a (2R, 4S, 5S, 6S, 11R, 13S, 14S)-configuration.

Harziane diterpenes have rarely been reported to have significant bioactivities. In the present study, compounds 1−5 and 7 were evaluated for their phytotoxic and antibacterial activities. All the tested compounds showed obvious phytotoxicity against the seedling growth of amaranth and lettuce at a concentration of 200 ppm (Table 3). Compounds 1, 3, 4, and 5 were more effective as they could completely inhibit seed germination against amaranth at 200μg/mL, and this strong phytotoxicity was still evident at lower concentrations (50μg/mL), compared to the positive control glyphosate. No compound was found to inhibit the root growth of lettuce at 200 ppm. It seemed that the isolated compounds caused weaker inhibition to lettuce than to amaranth, and have stronger toxicity on the growth of root growth than hypocotyl. Although there are three reports on the phytotoxicity of crude extracts of Trichoderma spp., no one had studied the phytotoxicity of compounds from Trichoderma spp. Thus, this is the first report of the phytotoxic compounds from Trichoderma spp., and the phytotoxicity of harziane diterpenes is also reported for the first time. None of the isolated compounds exhibited antibacterial activities.

Conclusions
In summary, the present chemical investigation on the soft coral-derived T. harzianum XS-20090075 resulted in the discovery of a series of harziane diterpenes (1−7). Compounds 1 and 2 represent a unique type of harziane diterpene lactone derived from harziane diterpenes though Baeyer-Villiger monooxygenase catalyzed oxidations. Harziane diterpenes have rarely been studied, and only 18 such compounds have been reported, including two harziane diterpene lactones. In this study, the structures of harziane diterpenes were determined by NMR spectroscopic data, ECD and OR calculations, together with X-ray diffraction. The phytotoxicity of compounds from Trichoderma sp. was evaluated for the first time, and the isolated compounds exhibited potent phytotoxicity towards amaranth and lettuce.
**Harzianelactone A (1).** colorless oil; \([\alpha]_D^{20} + 33.8 (c 0.42, MeOH); UV (MeOH) \(\lambda_{\text{max}} (\log \varepsilon) 237 (3.81) \text{ nm}; ECD (1.57 \text{ mM, MeOH}) \lambda_{\text{max}} (\Delta \varepsilon) 200 (1.55), 239 (7.03) \text{ nm}; IR (KBr) \nu_{\text{max}} 3425, 2933, 2360, 1738, 1653, 1029 \text{ cm}^{-1}; ^1\text{H} \text{ and } ^{13}\text{C} \text{ NMR data, Tables 1, 2}; \text{HRESIMS } m/z 319.2263 \text{ [M} + \text{H}]^+ \text{ (calcd for C}_{20}\text{H}_{31}\text{O}_{3}, 319.2268).}

**Harzianelactone B (2).** colorless oil; \([\alpha]_D^{20} + 18.9 (c 0.42, MeOH); UV (MeOH) \(\lambda_{\text{max}} (\log \varepsilon) 204 (3.62) \text{ nm}; IR (KBr) \nu_{\text{max}} 3398, 2931, 2362, 2340, 1779, 1703, 1029 \text{ cm}^{-1}; ^1\text{H} \text{ and } ^{13}\text{C} \text{ NMR data, Tables 1, 2}; \text{ESIMS } m/z 319.3 \text{ [M} + \text{H}]^+ \text{, 341.3 [M} + \text{Na}]^+ \text{, 637.4 [2 M} + \text{H}]^+ \text{, 659.5 [2 M} + \text{Na}]^+ \text{; HRESIMS } m/z 319.2271 \text{ [M} + \text{H}]^+ \text{ (calcd for C}_{20}\text{H}_{31}\text{O}_{3}, 319.2268).}

**Harzianone A (3).** colorless oil; \([\alpha]_D^{20} + 72.1 (c 0.42, MeOH); UV (MeOH) \(\lambda_{\text{max}} (\log \varepsilon) 259 (3.98) \text{ nm}; ECD (1.65 \text{ mM, MeOH}) \lambda_{\text{max}} (\Delta \varepsilon) 251 (3.11), 340 (40.09) \text{ nm}; IR (KBr) \nu_{\text{max}} 3400, 2932, 2361, 1735, 1669, 1441, 1260, 1150, 1027 \text{ cm}^{-1}; ^1\text{H} \text{ and } ^{13}\text{C} \text{ NMR data, Tables 1, 2}; \text{HRESIMS } m/z 303.2316 \text{ [M} + \text{H}]^+ \text{ (calcd for C}_{20}\text{H}_{31}\text{O}_{3}, 303.2319).}

**Harzianone B (4).** colorless oil; \([\alpha]_D^{20} + 32.2 (c 0.41, MeOH); UV (MeOH) \(\lambda_{\text{max}} (\log \varepsilon) 203 (3.49), 256 (3.64) \text{ nm}; ECD (1.57 \text{ mM, MeOH}) \lambda_{\text{max}} (\Delta \varepsilon) 248 (6.98), 345 (7.02) \text{ nm}; IR (KBr) \nu_{\text{max}} 3425, 2935, 2363, 1722, 1689, 1029 \text{ cm}^{-1}; ^1\text{H} \text{ and } ^{13}\text{C} \text{ NMR data, Tables 1, 2}; \text{HRESIMS } m/z 319.2262 \text{ [M} + \text{H}]^+ \text{ (calcd for C}_{20}\text{H}_{31}\text{O}_{3}, 319.2268).
Harzianone C (5). — colorless crystals; mp 168–169 °C; [α]20 D +14.7 (c 0.46, MeOH); UV (MeOH) λmax (log ε) 256 (3.38) nm; ECD (1.65 mM, MeOH) λmax (Δε) 254 (−6.21), 337 (−4.66) nm; IR (KBr) νmax 3398, 2932, 2363, 1737, 1659, 1444, 1382, 1020 cm−1; 1H and 13C NMR data, Tables 1, 2; HRESIMS m/z 303.2318 [M + H]+ (calcld for C20H31O2, 303.2319).

Harzianone D (6). — colorless oil; [α]20 D +52.6 (c 0.28, MeOH); UV (MeOH) λmax (log ε) 255 (2.72) nm; ECD (1.67 mM, MeOH) λmax (Δε) 255 (−2.83), 338 (+3.02) nm; IR (KBr) νmax 2948, 2356, 1728, 1655, 1438, 1260, 1022 cm−1; 1H and 13C NMR data, Tables 1, 2; HRESIMS m/z 301.2161 [M + H]+ (calcld for C20H32O2, 301.2162).

Harziane (7). — colorless crystals; mp 214–215 °C; [α]20 D +5.1 (c 0.48, MeOH); UV (MeOH) λmax (log ε) 206 (3.58) nm; IR (KBr) νmax 3404, 2929, 2362, 1653, 1382, 1033 cm−1; 1H and 13C NMR data, Tables 1, 2; HRESIMS m/z 287.2364 [M + H − H2O]+ (calcld for C20H18O2, 287.2369).

X-ray Crystallographic Analysis of 5 and 7. — The single-crystal X-ray diffraction data were recorded on an Xcalibur, Atlas, Gemini ultra diffractometer at 120 K. Crystallographic data for 5 (deposition NO. CCDC 1573734) and 7 (deposition NO. CCDC 1573693) have been deposited in the Cambridge Crystallographic Data Centre. Copies of the data can be free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (0)-1223-363033 or e-mail: deposit@ccdc.cam.ac.uk].

Crystal data for 5. C20H30O2, Mw = 302.44, monoclinic, a = 7.13670 (10) Å, b = 13.7978 (3) Å, c = 8.4352 (2) Å, α = 90.00°, β = 94.516 (2)°, γ = 90.00°, V = 828.04 (3) Å3, space group P21, Z = 2, Dc = 1.213 mg/m3, μ = 0.586 mm−1, and F (000) = 332. Crystal size: 0.42 × 0.20 × 0.13 mm3. Reflections collected/unique = 8011/2950 [R(int) = 0.0230]. The final indices were R1 = 0.0295, wR2 = 0.0733 (I > 2σ(I)). Flack parameter = 0.13 (19).

Crystal data for 7. C20H32O2, Mw = 304.46, monoclinic, a = 18.9468 (16) Å, b = 8.3433 (2) Å, c = 13.245 (4) Å, α = 90.00°, β = 124.739 (8)°, γ = 90.00°, V = 1720.6 (5) Å3, space group C2, Z = 4, Dc = 1.175 mg/m3, μ = 0.564 mm−1, and F (000) = 672. Crystal size: 0.14 × 0.20 × 0.19 mm3. Reflections collected/unique = 9303/3064 [R(int) = 0.0261]. The final indices were R1 = 0.0307, wR2 = 0.0748 (I > 2σ(I)). Flack parameter = 0.13 (19).

Phytotoxicity bioassays. — Phytotoxicity against seeding growth of amaranth (Amaranthus retroflexus L.) and lettuce (Lactuca sativa) was assayed by the method reported previously26. Glyphosate was used as the positive control.

Antibacterial assays. — The antibacterial activity was evaluated by the conventional broth dilution assay27. Five pathogenic bacterial strains, including Gram-positive Kocuria rhizhophila (ATCC 9341), Staphylococcus aureus (ATCC 27154), and Gram-negative Escherichia coli (ATCC 25922),Ralstonia solanacearum, Vibrio anguillarum (ATCC 19019), and V. Parahemolyticus (ATCC 17802) were used, and ciprofloxacin and streptomycin sulfate were used as positive controls.

References
1. Dayan, V. E., Cantrell, C. L. & Duke, S. O. Natural products in crop protection. Bioorg. Med. Chem. 17, 4022–4034 (2009).
2. Cantrell, C. L., Dayan, F. E. & Duke, S. O. New natural products as sources for new pesticides. J. Nat. Prod. 75, 1231–1242 (2012).
3. Silva, R. N., Steindorff, A. S. & Monteiro V. N. Biotechnology and biology of Trichoderma (ed. Gupta, V. K., Schmoll, M., Herrera-Estrella, A., Upadhyay, R. S., Druzhinina, I. & Tuohy, M. G.) 363–367 (Elsevier, 2014).
4. Javid, A. & Ali, S. Herbicidal activity of culture filtrates of Trichoderma spp. against two problematic weeds of wheat. Nat. Prod. Res. 25, 730–740 (2011).
5. Blunt, J. W., Copp, B. R., Keyzers, R. A., Munro, M. H. G. & Prinsep, M. R. Marine natural products. Nat. Prod. Rep. 35, 8–53 (2018).
6. Iwano, B., Kikutani, T., Tanaka, H. & Morita, Y. Novel antiviral and antibacterial compounds from marine-derived Trichoderma sp. J. Nat. Prod. 81, 409–417 (2016).
7. Liu, Q. A. et al. Antiinflammatory and fungicidal resorcylic acid lactones from the sea anemone-derived fungus Cochliobolus lunatus. J. Agric. Food Chem. 62, 3183–3191 (2014).
8. Zhao, D. L. et al. Azaphilone and diphenyl ether derivatives from a gorgonian-derived strain of the fungus Penicillium pinophilum. J. Nat. Prod. 78, 2310–2314 (2015).
9. Iida, K. et al. (+)- and (−)-Pestaloziana A, a pair of antiviral enantiomeric alkaloid dimers with a symmetric spiro[oxazinane-piperazinedione] skeleton from Pestalotiopsis sp. Org. Lett. 17, 4216–4219 (2015).
10. Chen, M., Zhang, W., Shao, C. L., Chi, Z. M. & Wang, C. Y. DNA methyltransferase inhibitor induced fungal biosynthetic products: diethyleneglycol phthalate ester oligomers from the marine-derived fungus Cochliobolus lunatus. Mar. Biotechnol. 18, 409–417 (2016).
11. Ghislalti, E. L., Hockless, D. C. R., Rowland, C. & White, A. H. Harzianione, a new class of diterpene from Trichoderma harzianum. J. Nat. Prod. 55, 1690–1694 (1992).
12. Manna, L. et al. A new fungal growth inhibitor from Trichoderma viride. Tetrahedron 53, 3135–3144 (1997).
13. Adelin, E. et al. Bicyclic and tetracyclic diterpenes from a Trichoderma symbiont of Taxus baccata. Phytochemistry 97, 55–61 (2014).
14. Zhang, M. et al. Two new diterpenoids from the endophytic fungus Trichoderma sp. Xy24 isolated from mangrove plant Xylocarpus granatum. Chem. Chin. Lett. 27, 957–960 (2016).
15. Zhang, M. et al. Two furanharzianiones with 3/7/5/6/5 ring system from microbial transformation of harzianione. Org. Lett. 19, 1168–1171 (2017).
16. Zhang, M. et al. Microbial oxidation of harzianione by Bacillus sp. IMM-006. Tetrahedron 73, 7195–7199 (2017).
17. Song, Y. P., Fang, S. T., Miao, F. P., Yin, X. L. & Ji, N. Y. Diterpenes and sesquiterpenes from the marine algicoline fungus Trichoderma harzianum X-5. J. Nat. Prod. 81, 2553–2559 (2018).
21. Barra, L. & Dickschat, J. S. Harzianone biosynthesis by the biocontrol fungus Trichoderma. ChemBioChem 18, 2358–2365 (2017).
22. Xie, Z. L. et al. Trichodermaerin, a new diterpenoid lactone from the marine fungus Trichoderma erinaceum associated with the sea star Acanthaster planci. Nat. Prod. Commun. 8, 67–68 (2013).
23. Kuang, W. F., Wang, C. F. & Mao, W. L. Screening and evaluation of herbicidal metabolites produced by Trichoderma spp. Afr. J. Microbiol. Res. 10, 866–872 (2016).
24. Javaid, A., Shafique, G., Ali, S. & Shoaib, A. Effect of culture medium on herbicidal potential of metabolites of Trichoderma species against Parthenium hysterophorus. Int. J. Agric. Biol. 15, 119–124 (2013).
25. Zhang, Q. et al. Potential alelopathic indole diketopiperazines produced by the plant endophytic Aspergillus fumigatus using the one strain—many compounds method. J. Agric. Food Chem. 61, 11447–11452 (2013).
26. Zhu, A. et al. new anti-vibrio prenylxanthones from the marine-derived fungus Aspergillus sp. ZA-01. Mar. Drugs 16, 312 (2018).

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Author Contributions

D.L.Z. contributed to identification of the compounds and manuscript preparation. T.S. and C.Y.W. (Chao-Yi Wang) contributed to fungi identification, fermentation, extraction and isolation of the compounds. L.J.Y. contributed to bioassays of the compounds. C.L.S. and C.Y.W. (Chang-Yun Wang) conceived and designed research. All authors reviewed the manuscript.

Additional Information

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