Open access • Journal Article • DOI:10.1021/ACS.JNATPROD.8B00714

Diterpenes and Sesquiterpenes from the Marine Algicolous Fungus Trichoderma harzianum X-5. — Source link

Yin-Ping Song, Sheng-Tao Fang, Feng-Ping Miao, Xiu-Li Yin ...+1 more authors

Institutions: Chinese Academy of Sciences

Published on: 23 Oct 2018 - Journal of Natural Products (American Chemical Society and American Society of Pharmacognosy)

Topics: Trichoderma harzianum, Sesquiterpene, Diterpene and Terpene

JOURNAL OF NATURAL PRODUCTS

Article

Cite This: J. Nat. Prod. 2018, 81, 2553−2559

pubs.acs.org/jnp

Diterpenes and Sesquiterpenes from the Marine Algicolous Fungus *Trichoderma harzianum* X-5

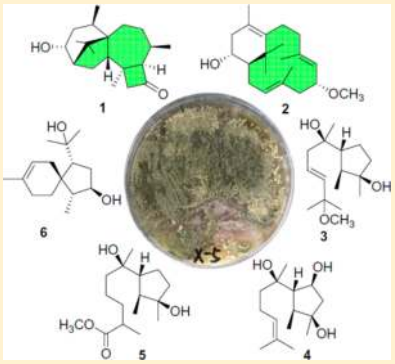
Yin-Ping Song,[†,‡] Sheng-Tao Fang,[†] Feng-Ping Miao,[†] Xiu-Li Yin,[†] and Nai-Yun Ji*[,†]

[†]Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, People's Republic of China

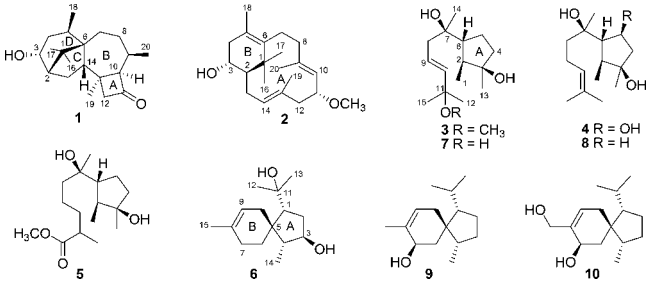
[‡]University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China

Supporting Information

ABSTRACT: Six new terpenes, including one harziane diterpene, 3*R*-hydroxy-9*R*,10*R*-dihydroharzianone (**1**), one proharziane diterpene, 11*R*-methoxy-5,9,13-proharzitrien-3-ol (**2**), three cyclonerane sesquiterpenes, 11-methoxy-9-cycloneren-3,7-diol (**3**), 10-cycloneren-3,5,7-triol (**4**), and methyl 3,7-dihydroxy-15-cycloneranate (**5**), and one acorane sesquiterpene, 8-acoren-3,11-diol (**6**), were isolated from the culture of *Trichoderma harzianum* X-5, an endophytic fungus obtained from the marine brown alga *Laminaria japonica*. Their structures and relative configurations were established by analysis of 1D/2D NMR, HREIMS, and IR data, and the absolute configurations were assigned on the basis of ECD curves or biogenetic considerations. These terpenes possess four different carbon skeletons, and compound **2**, with a rarely occurring bicyclic framework, represents a possible precursor of tetracyclic harzianes. Compounds **1**−**6** exhibited growth inhibition of some marine phytoplankton species.



More than 250 *Trichoderma* species have been discovered so far,[1] and some of them are used as effective biocontrol agents in agriculture, such as *T. harzianum*, *T. virens*, and *T. viride*.[2] Among those, *T. harzianum* is a well-known species for its antagonism against a wide range of phytopathogenic fungi and for production of secondary metabolites with various bioactivities.[2] To date, a number of antibiotic metabolites, such as sesquiterpenes, diterpenes, pyrones, oxazoles, and anthraquinones, have been characterized from terrestrial strains.[3−6] Although marine-derived fungi have proven to be a large reservoir of structurally unique and biologically active compounds,[7] only a few new structures have been contributed by the marine isolates of *T. harzianum*.[8,9] Moreover, their antagonistic potential against marine organisms has rarely been examined. In our ongoing search for new and bioactive secondary metabolites from marine-derived *Trichoderma* spp.,[10−13] an endophytic strain (X-5) of *T. harzianum* obtained from the marine brown alga *Laminaria japonica* was investigated. As a result, two new structurally related diterpenes, 3*R*-hydroxy-9*R*,10*R*-dihydroharzianone (**1**) and 11*R*-methoxy-5,9,13-proharzitrien-3-ol (**2**), and four new sesquiterpenes, 11-methoxy-9-cycloneren-3,7-diol (**3**), 10-cycloneren-3,5,7-triol (**4**), methyl 3,7-dihydroxy-15-cycloneranate (**5**), and 8-acoren-3,11-diol (**6**), as well as four known sesquiterpenes, including 9-cycloneren-3,7,11-triol (**7**),[10] (−)-cyclonerodiol (**8**),[14,15] trichoacorenol (**9**),[16] and trichoacorenol B (**10**),[17] were isolated and identified. Herein, the details of isolation, structure elucidation, and bioactivities of these compounds against marine phytoplankton and bacteria are described.



RESULTS AND DISCUSSION

EtOAc extracts of the mycelia and broth were combined, fractionated, and purified by repeated column chromatography (CC) on silica gel, RP-18, and Sephadex LH-20 as well as preparative thin-layer chromatography (TLC) to afford compounds **1**−**10**. Among them, **7**−**10** were identified to be 9-cycloneren-3,7,11-triol,[10] (−)-cyclonerodiol,[14,15] trichoacorenol,[16] and trichoacorenol B,[17] respectively, by comparison of their spectroscopic data with literature data.

Compound **1** was isolated as a colorless oil. A molecular formula of $C_{20}H_{32}O_2$ was assigned by interpretation of HREIMS data, requiring five degrees of unsaturation. Its IR spectrum displayed absorption bands at 3455 and 1772 cm^{-1}, corresponding to a hydroxy group and a nonconjugated cyclobutanone moiety,[18,19] respectively. In combination with HSQC data, the ^1H NMR spectrum (Table 1) showed three methyl singlets, two methyl doublets, two deshielded doublets

Received: August 23, 2018
Published: October 23, 2018

DOI: 10.1021/acs.jnatprod.8b00714
J. Nat. Prod. 2018, 81, 2553−2559

Table 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Data for 1 and 2

	1 (in CD_3OD)		2 (in $CDCl_3$)	
pos	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1	48.6, C		40.4, C	
2	52.1, CH	1.71, m	50.0, CH	1.72, m
3	68.4, CH	4.20, ddd (10.3, 7.1, 3.0)	67.5, CH	4.32, ddd (10.7, 7.2, 5.1)
4a	36.3, CH_2	1.88, ddd (14.4, 10.3, 9.8)	40.8, CH_2	2.21, m
4b		1.60, m		
5	32.5, CH	2.19, dq (9.8, 7.5)	124.7, C	
6	52.0, C		136.3, C	
7a	26.6, CH_2	1.71, m	26.0, CH_2	2.18, m
7b		1.61, m		
8a	30.3, CH_2	1.63, m	38.7, CH_2	2.56, ddd (13.0, 13.0, 5.1)
8b				2.02, ddd (12.8, 3.8, 3.8)
9	33.7, CH	2.25, m	138.8, C	
10	75.3, CH	3.05, ddd (6.6, 5.7, 2.0)	129.0, CH	4.68, br d (10.0)
11	215.0, C		75.6, CH	3.87, ddd (11.1, 10.0, 3.4)
12a	64.3, CH_2	2.73, br d (16.7)	46.8, CH_2	2.30, ddd (11.9, 2.5, 2.5)
12b		2.61, dd (16.7, 5.7)		1.97, dd (11.9, 11.1)
13	36.9, C		129.3, C	
14	52.4, CH	2.72, dd (11.0, 7.8)	129.0,CH	5.15, br d (11.7)
15a	23.1, CH_2	1.72, m	23.7, CH_2	2.39, br d (16.0)
15b				2.24, m
16	26.9, CH_3	1.01, s	24.8, CH_3	0.89, s
17	22.5, CH_3	1.05, s	32.5, CH_3	0.99, s
18	21.3, CH_3	1.11, d (7.6)	21.7, CH_3	1.71, s
19	27.2, CH_3	1.46, s	16.8, CH_3	1.57, t (1.5)
20	17.6, CH_3	1.10, d (7.4)	17.2, CH_3	1.64, d (1.2)
CH_3O			55.6, CH_3	3.24, s

of double doublets ascribable to two methines, and a series of signals at δ_H 1.5−2.8 for five methylenes and four methines. The ^{13}C NMR spectrum (Table 1) along with DEPT experiments demonstrated the presence of five methyls, five methylenes, six methines, and four nonprotonated carbons. An analysis of the above NMR data revealed that **1** differed from harzianone mainly at positions C-3, C-9, and C-10.[11] In view of the deshielded NMR signals of C-3 and H-3, a hydroxy group was attached to C-3 in **1**, which was supported by the COSY correlations of H-3 with H-2 and H-4. Replacing the double bond in harzianone, two methine groups were situated at C-9 and C-10 as seen from the COSY correlations of H_2-7/H_2-8/H-9/H-10 and HMBC correlations from H_3-20 to C-8, C-9, and C-10, from H_3-19 to C-10, C-12, C-13, and C-14, and from H-10 and H_2-12 to C-11. Thus, **1** was deduced to be 3-hydroxy-9,10-dihydroharzianone, validated by other HMBC and COSY correlations (Figure 1).

The relative configuration of **1** was confirmed by analysis of coupling constants and NOE correlations (Figure 2). The large coupling constant (10.3 Hz) between H-3 and H-4a indicated a diaxial-like relationship, and the former was *syn* to CH_3-17 based on its NOE correlation with H_3-17. H-14, CH_3-16, and CH_3-20 were located on the same face of the molecule by the NOE correlations of H-14 with H_3-16 and H_3-20, while CH_3-19 was *syn* to H-4a, H-5, H-10, and H-12b as seen from the NOE correlations of H_3-19 with these protons. Additionally, the same orientation of H-9 and H-10 was suggested by their NOE correlation. To ascertain the absolute configuration of **1**, its electronic circular dichroism (ECD) spectrum was determined in MeOH and simulated at the B3LYP/6-31G(d) level after conformational optimization at the same level via Gaussian 09 software.[20] The Boltzmann-weighted

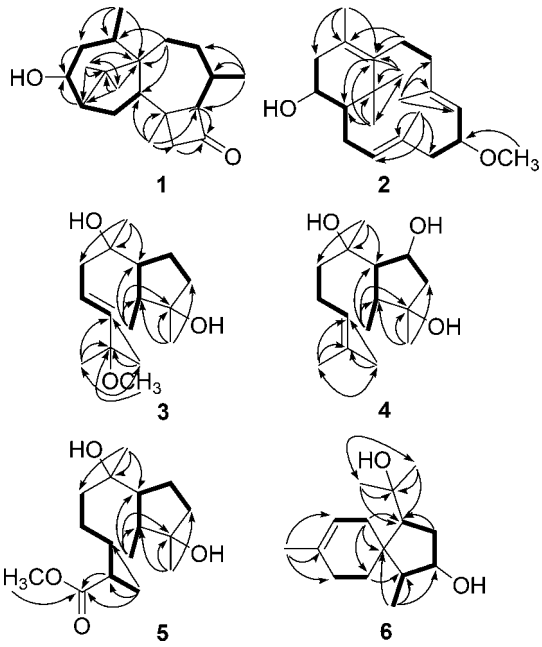


Figure 1. Key HMBC (arrows) and COSY (bold lines) correlations of **1**−**6**.

ECD curve agreed well with the experimental one (Figure 3), and the absolute configuration was assigned to be 2*S*, 3*R*, 5*R*, 6*R*, 9*R*, 10*R*, 13*S*, and 14*S*.

Compound **2** was obtained as a colorless oil. Its molecular formula was established as $C_{21}H_{34}O_2$ on the basis of HREIMS data, indicating five degrees of unsaturation. The IR absorption band at 3418 cm^{-1} suggested the presence of a hydroxy group.

DOI: 10.1021/acs.jnatprod.8b00714
J. Nat. Prod. 2018, 81, 2553−2559

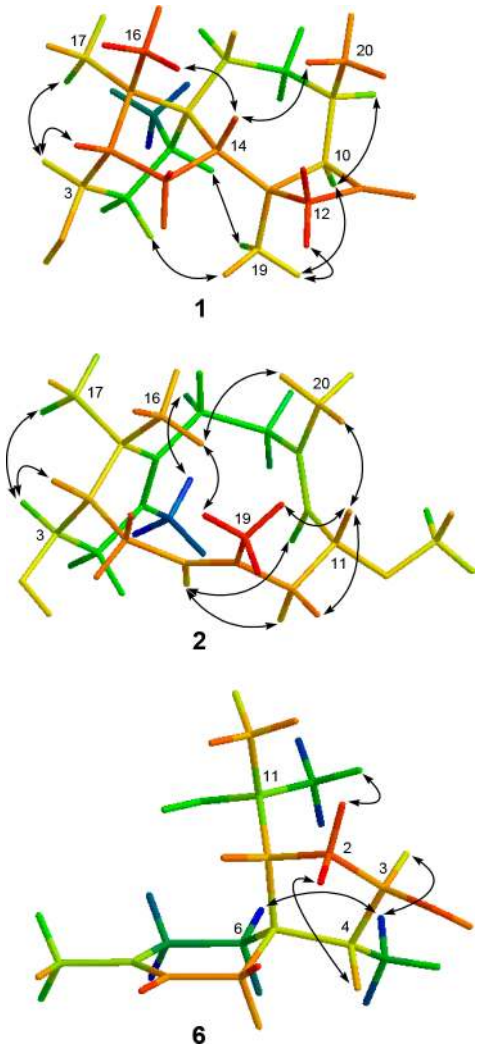


Figure 2. Dominant conformers (colored by red, orange, yellow, green, and blue from front to back) of **1** in MeOH, **2** in $CHCl_3$, and **6** in $CHCl_3$ and key NOE correlations.

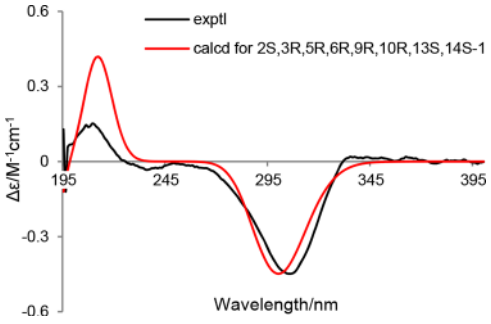


Figure 3. Experimental and calculated ECD spectra of **1**.

The ^{1}H NMR spectrum (Table 1) along with HSQC data exhibited notable signals including three methyl singlets, one methyl triplet, one methyl doublet, one methoxy singlet, two doublets of double doublets assignable to two oxymethines, and two broad doublets attributable to two olefinic protons. Aided by DEPT experiments, 21 resonances in the ^{13}C NMR spectrum (Table 1) were classified as six methyls, five methylenes, five methines, and five nonprotonated carbons. HMBC correlations (Figure 1) from H_3-16 to C-1, C-2, C-6, and C-17 and from H_3-17 to C-1, C-2, C-6, and C-16 suggested that CH_3-16 and CH_3-17 were *gem*-dimethyls attached at C-1, which was flanked by C-2 and C-6. This structural unit was connected to C-3, C-4, and C-5 to form ring B based on the COSY correlations of H-3 with H-2 and H-4 and HMBC correlations from H_3-18 to C-4, C-5, and C-6. Moreover, C-7 was bonded to C-6 according to the HMBC correlation from H_2-7 to C-6, and a continuous chain extended to C-2 to form ring A in view of the COSY correlations of H_2-7 with H_2-8, of H-11 with H-10 and H_2-12, and of H_2-15 with H-2 and H-14 and HMBC correlations from H_3-20 to C-8, C-9, and C-10 and from H_3-19 to C-12, C-13, and C-14. The methoxy group indicated by the ^{1}H NMR spectrum was linked to C-11 as seen from their HMBC correlation. The above spectroscopic data evidenced **2** to be 11-methoxy-5,9,13-proharzitrien-3-ol.

The relative configuration of **2** was established by interpretation of coupling constants and NOE correlations (Figure 2). The double bonds at C-9 and C-13 were deduced to feature *E* configurations by the NOE correlations between H-11 and H_3-20 and between H-12b and H-14, whereas a *Z* configuration was assigned to the double bond at C-5 by the NOE correlation between H-7 and H_3-18. The cofacial property of CH_3-16, CH_3-19, and CH_3-20 was suggested by the NOE correlations of H_3-16 with H_3-19 and H_3-20, which was further supported by the NOE correlation between H-10 and H-14. Additionally, H-11 was *syn* to CH_3-19 and CH_3-20 on the basis of its NOE correlations with H_3-19 and H_3-20, while H-3 and CH_3-17 were positioned on the same face by the NOE correlation between H-3 and H_3-17. The above NOE correlations also indicated H-11 to be opposite H-10 and H-12b, verified by the large coupling constants between H-10 and H-11 and between H-11 and H-12b. Although **2** has a 12-membered ring (ring A), only one conformer (Figure 2) appeared dominant and matched the above NOE correlations during quantum chemical calculations. The experimental and calculated ECD spectra for 2*R*,3*R*,11*R*-**2** in MeOH did not match well, but both exhibited a positive Cotton effect at 222 nm, suggesting this was the correct absolute configuration (Figure 4). The 2*R* and 3*R* absolute configurations are identical with those at C-2 and C-3 of **1**.

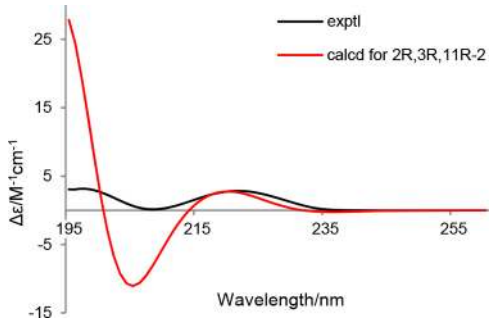


Figure 4. Experimental and calculated ECD spectra of **2**.

Compound **3** was purified as a colorless oil. A molecular formula of $C_{16}H_{30}O_3$ with two degrees of unsaturation was determined by HREIMS. The ^{1}H NMR spectrum (Table 2) displayed one methyl doublet, four methyl singlets, one methoxy singlet, two doublets of doublets ascribable to a methylene, one doublet and one doublet of double doublets attributable to two olefinic protons, and a range of multiplets at

DOI: 10.1021/acs.jnatprod.8b00714
J. Nat. Prod. 2018, 81, 2553−2559

Table 2. ^{1}H NMR Data for 3−6 (500 MHz, $CDCl_3$)

	3	4	5	6
pos	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)
1	1.05, d (6.8)	1.11, d (6.8)	1.03, d (6.8)	1.49, m
2a	1.63, m	1.58, m	1.60, m	2.08, m
2b				1.05, ddd (11.7, 11.7, 10.7)
3				3.33, ddd (10.7, 10.7, 4.5)
4a	1.69, m	1.88, d (14.0)	1.68, m	1.27, m
4b	1.58, m	1.73, dd (14.0, 5.9)	1.55, m	
5a	1.87, m	4.26, br d (5.9)	1.84, m	
5b	1.59, m		1.54, m	
6a	1.84, m	1.83, d (8.9)	1.83, m	1.79, ddd (13.4, 9.5, 4.6)
6b				1.44, ddd (13.4, 7.4, 3.7)
7a				2.28, m
7b				2.20, m
8a	2.26, dd (14.0, 7.1)	1.56, m	1.44, m	
8b	2.22, dd (14.0, 7.3)			
9	5.64, ddd (15.8, 7.3, 7.1)	2.11, td (7.6, 7.1)	1.34, m	4.91, br s
10a	5.52, d (15.8)	5.13, br t (7.1)	1.67, m	1.49, m
10b			1.40, m	1.15, d (13.5)
11			2.46, m	
12	1.27, s	1.69, s	1.16, d (7.0)	1.17, s
13	1.27, s	1.25, s	1.26, s	1.17, s
14	1.15, s	1.14, s	1.14, s	0.92, d (6.7)
15	1.27, s	1.63, s		1.71, br s
CH_3O	3.16, s		3.67, s	

δ_H 1.5−2.0 due to two methylenes and two methines. In accordance with the molecular formula, the ^{13}C NMR spectrum (Table 3) displayed 16 resonances, sorted into six

Table 3. ^{13}C NMR Data for 3−6 (125 MHz, $CDCl_3$)

	3	4	5	6
pos	δ_C, type	δ_C, type	δ_C, type	δ_C, type
1	14.7, CH_3	14.6, CH_3	14.7, CH_3	43.5, CH
2	44.5, CH	45.1, CH	44.4, CH	36.9, CH_2
3	81.4, C	82.1, C	81.4, C	74.2, CH
4	40.5, CH_2	48.7, CH_2	40.5, CH_2	46.8, CH
5	24.5, CH_2	74.8, CH	24.4, CH_2	54.1, C
6	54.5, CH	65.3, CH	54.3, CH	29.6, CH_2
7	74.6, C	74.4, C	74.9, C	37.5, CH_2
8	43.8, CH_2	41.4, CH_2	40.7, CH_2	138.9, C
9	125.4, CH	22.6, CH_2	21.7, CH_2	133.6, CH
10	139.8, CH	124.4, CH	34.5, CH_2	40.2, CH_2
11	75.0, C	132.3, C	39.6, CH	72.5, C
12	26.0, CH_3	25.9, CH_3	17.3, CH_3	27.1, CH_3
13	26.3, CH_3	25.3, CH_3	26.2, CH_3	27.4, CH_3
14	25.6, CH_3	24.5, CH_3	25.3, CH_3	11.9, CH_3
15	26.1, CH_3	17.9, CH_3	177.5, C	16.5, CH_3
CH_3O	50.5, CH_3		51.7, CH_3	

methyls, three methylenes, four methines, and three nonprotonated carbons by the DEPT and HSQC data. The above NMR signals closely resembled those of 9-cycloneren-3,7,11-triol (**7**),[10] except for the presence of a methoxy group. It was attached to C-11 based on their HMBC correlation. Thus, **3** was assigned as an *O*-methylated derivative of **7** at C-11, corroborated by HMBC and COSY correlations (Figure 1). The relative configuration was further supported by the NOE correlations between H_3-1 and H-6 and between H-2 and H_3-13, and the configuration of the double bond at C-9 was assigned as *trans* by the large coupling constant between H-9 and H-10. Based on biogenetic considerations, the absolute configurations at C-2, C-3, C-6, and C-7 were proposed to be the same as those of **7**, which were also supported by the similar specific rotation value to **7**.[10]

Compound **4** was obtained as a colorless oil, and HREIMS analysis gave the molecular formula $C_{15}H_{28}O_3$, one more oxygen atom than for (−)-cyclonerodiol (**8**).[14,15] Its ^{1}H and ^{13}C NMR spectra (Tables 2 and 3) exhibited high similarities to those of **8**, except for the presence of signals for an oxymethine group and the lack of signals for a methylene group. Thus, **4** was speculated to be a hydroxylated derivative of **8**, and the hydroxy group was attached to C-5 based on the COSY correlations of H-5 with H_2-4 and H-6 (Figure 1). COSY correlations of H-2 with H_3-1 and H-6 and of H_2-9 with H_2-8 and H-10 and HMBC correlations from H_3-1 to C-2, C-3, and C-6, from H_3-13 to C-2, C-3, and C-4, from H_3-14 to C-6, C-7, and C-8, and from H_3-12 and H_3-15 to C-10 and C-11 further evidenced **4** to be 10-cycloneren-3,5,7-triol. The hydroxy group at C-5 was *syn* to H-6 as seen from the NOE correlation between H-5 and H_3-14. The absolute configurations at C-2, C-3, C-6, and C-7 were speculated to be identical to those of **8** on the basis of biogenetic considerations, and the absolute configuration at C-5 was assigned to be *S*.

Compound **5** was isolated as a colorless oil. Its molecular formula was deduced to be $C_{16}H_{30}O_4$ by interpretation of HREIMS data, suggesting two degrees of unsaturation. The ^{1}H and ^{13}C NMR data (Tables 2 and 3) partially resembled those of **3**, which indicated the presence of ring A and its affiliated groups. In addition, COSY correlations of H_2-8/H_2-9/H_2-10/H-11/H_3-12 demonstrated the presence of a substituted pentyl group attached to C-7 as seen from the HMBC correlations from H_3-14 to C-6, C-7, and C-8. The remaining NMR data corresponded to a methoxycarbonyl group, and its attachment

DOI: 10.1021/acs.jnatprod.8b00714
J. Nat. Prod. 2018, 81, 2553−2559

Table 4. Antimicroalgal and Antibacterial Activities of 1−6

	IC_{50} (μg/mL)				inhibitory zone diameter (mm) at 20 μg/disk			
	Chattonella marina	*Heterosigma akashiwo*	*Karlodinium veneficum*	*Prorocentrum donghaiense*	*Vibrio anguillarum*	*V. harveyi*	*V. parahemolyticus*	*V. splendidus*
1	7.0	42	24	70	6.0	0	0	6.2
2	1.2	1.3	3.2	4.3	0	6.8	0	0
3	0.66	23	2.2	37	6.1	0	0	0
4	9.9	75	14	66	6.1	0	0	0
5	12	68	41	55	6.2	0	0	0
6	2.8	56	54	54	7.2	0	0	6.1
$K_2Cr_2O_7$	0.46	0.98	0.89	1.9				
chloramphenicol					18	18	20	19

to C-11 was established by the HMBC correlations from H-11 and H_3-12 to C-15. Thus, **5** was deduced to be methyl 3,7-dihydroxy-15-cycloneranate, confirmed by HMBC and COSY correlations (Figure 1). The absolute configurations at C-2, C-3, C-6, and C-7 were proposed to be the same as those of **7** and **8** in view of biogenetic considerations, but that at C-11 remains unassigned.

Compound **6** was separated as a colorless oil, and the molecular formula $C_{15}H_{26}O_2$ was determined by HREIMS, implying three degrees of unsaturation. Its IR spectrum showed an absorption band at 3452 cm^{-1}, suggesting the presence of a hydroxy group(s). The ^1H NMR spectrum (Table 2) alongside HSQC data displayed one methyl doublet, three methyl singlets, one doublet of double doublets for an oxymethine, and one broad singlet for an olefinic proton among others. The ^{13}C NMR spectrum (Table 3) exhibited 15 resonances, corresponding to four methyls, four methylenes, four methines, and three nonprotonated carbons by analysis of DEPT experiments. A detailed comparison of NMR data revealed that **6** differed from trichoacorenol B (**10**) mainly at the positions of hydroxy groups.[17] HMBC correlations (Figure 1) from H_3-12 and H_3-13 to C-1 and C-11 and from H_3-14 to C-3 and C-4 along with the deshielded NMR signals of C-3 and C-11 located the two hydroxy groups at C-3 and C-11, respectively, rather than C-7 and C-15 in **10**, which was further supported by the COSY correlations of H-1/H_2-2/H-3/H-4/H_3-14. Additionally, HMBC and COSY correlations established cyclohexene ring B (Figure 1). Moreover, the connectivity at C-5 was confirmed by the HMBC correlations from H-6a to C-4, from H-10b to C-1, and from H_3-14 to C-5. The above spectroscopic data evidenced **6** to be 8-acoren-3,11-diol, and its relative configuration was established by the NOE correlations of H_3-14 with H-3 and H-6a, of H-2a with H_3-12 and/or H_3-13, and of H-2b with H-4 (Figure 2). The identical configurations at C-1, C-4, and C-5 of **6**, **9**, and **10** seemed reasonable based on a shared biogenesis, and therefore the absolute configuration of **6** was tentatively assigned to be 1*R*, 3*R*, 4*R*, and 5*R*.

Compounds **1**−**6** comprise four different terpene skeletons, including harziane, proharziane, cyclonerane, and acorane, which further demonstrates the chemical diversity of *T. harzianum*. Biosynthetically, **2**, **3**, and **5** might be formed by *O*-methylation after the scaffolds are constructed during the fermentation. Compound **5** could be an oxidative degradation product formed from a precursor derived from **8**. It is also worth mentioning that the bicyclic framework of **2** represents a possible precursor of the tetracyclic harziane diterpenes,[21] of which the first member was also obtained from a *T. harzianum* strain.[3] An analogue of **2** was previously isolated from a tree-endophytic strain of *T. atroviridae*, but its relative and absolute configurations were not determined.[22]

The new isolates **1**−**6** were evaluated for inhibition of four marine phytoplankton species (*Chattonella marina*, *Heterosigma akashiwo*, *Karlodinium veneficum*, and *Prorocentrum donghaiense*) and four marine-derived pathogenic bacteria (*Vibrio anguillarum*, *V. harveyi*, *V. parahemolyticus*, and *V. splendidus*).[11,23] As shown in Table 4, they all exhibited growth inhibition of the four phytoplankton species tested. Although **3** was more active against *C. marina* and *K. veneficum* than **2**, the latter also exhibited potent inhibition of *H. akashiwo* and *P. donghaiense* with IC_{50} values in the low μg/mL range. On the other hand, none of the compounds displayed strong activity against the marine *Vibrio* strains when tested at 20 μg/disk. The tertiary allylic alcohol in the side chain of the cyclonerane sesquiterpene **3** may play a role in its increased potency against *C. marina* and *K. veneficum* relative to the related **4** and **5**.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were acquired on an SGW-3 polarimeter with a 2 mL (length 10 cm) cell (Shanghai Shenguang Instrument Co., Ltd.). ECD spectra were measured on a Chirascan CD spectrometer (Applied Photophysics Ltd.). IR spectra were recorded on a JASCO FT/IR-4100 spectrometer. 1D and 2D NMR spectra were determined on a Bruker Avance III 500 NMR spectrometer. Low- and high-resolution EI mass spectra were obtained on an Autospec Premier P776 mass spectrometer at 70 eV (Waters Corp.). CC was performed with silica gel (200−300 mesh, Qingdao Haiyang Chemical Co.), RP-18 (AAG12S50, YMC Co., Ltd.), and Sephadex LH-20 (GE Healthcare). TLC was operated with precoated silica gel plates (GF-254, Qingdao Haiyang Chemical Co.). Quantum chemical calculations were run with Gaussian 09 software (IA32W-G09RevC.01).

Fungal Material and Fermentation. *Trichoderma harzianum* X-5 was isolated from the inner tissue of the surface-sterilized brown alga *Laminaria japonica* collected from Chang Islands, China, in July 2016. The species was identified by morphology and by analysis of the ITS regions of its rDNA, whose sequence data have been deposited in GenBank with the accession number MH290366. Its fermentation was carried out statically at room temperature for 30 days in 400 × 1 L Erlenmeyer flasks, each containing 50 g of rice, 0.6 g of peptone, 50 mL of pure H_2O, and 50 mL of natural seawater from the coast of Yantai, China.

Extraction and Isolation. The mycelia were separated from the culture broth by filtration, and then they were dried in the shade and exhaustively extracted with CH_2Cl_2 and MeOH (1:1, v/v). After removing organic solvents by evaporation under vacuum, the residue was partitioned between EtOAc and H_2O to give an EtOAc-soluble extract (90.2 g). The filtrate was directly extracted with EtOAc and then concentrated to afford an extract (13.2 g). In view of the identical TLC profiles, these two parts were combined and then subjected to silica gel CC with step-gradient solvent systems

DOI: 10.1021/acs.jnatprod.8b00714
J. Nat. Prod. 2018, 81, 2553−2559

consisting of petroleum ether (PE)/EtOAc to yield six fractions (Frs. 1−6). Fr. One eluted with PE/EtOAc (20:1) and was further purified by CC on RP-18 (MeOH/H_2O, 3:1) and Sephadex LH-20 (MeOH) and preparative TLC (PE/EtOAc, 10:1) to obtain **9** (27.3 mg). Fr. 3 eluted with PE/EtOAc (5:1) and was further purified by CC on RP-18 (MeOH/H_2O, 7:3) and Sephadex LH-20 (MeOH) and preparative TLC (PE/EtOAc, 2:1) to yield **1** (3.0 mg), **8** (9.5 mg), and **10** (3.8 mg). Fr. 4 eluted with PE/EtOAc (2:1) and was further purified by CC on RP-18 (MeOH/H_2O, 9:1) and silica gel (PE/EtOAc, 10:1 to 5:1) to afford **2** (2.8 mg). Fr. 5 eluted with PE/EtOAc (1:1) and was further purified by RP-18 CC (MeOH/H_2O, 1:1) and preparative TLC (EtOAc) to give **4** (8.5 mg), **5** (2.0 mg), and **6** (2.0 mg). Fr. 6 eluted with EtOAc and was further purified by CC on RP-18 (MeOH/H_2O, 1:1) and Sephadex LH-20 (MeOH) and preparative TLC (CH_2Cl_2/MeOH, 15:1) to obtain **3** (3.3 mg) and **7** (3.2 mg).

*3R-Hydroxy-9R,10R-dihydroharzianone (**1**):* colorless oil; $[\alpha]^{20}_D$ −21 (*c* 0.12, MeOH); IR (KBr) ν_{max} 3455, 2964, 2871, 1772, 1636, 1452, 1384, 1288, 1042 cm^{-1}; ^1H and ^{13}C NMR data, Table 1; EIMS *m*/*z* (%) 304 $[M]^+$ (1), 289 (23), 262 (100), 244 (95), 229 (25), 119 (40), 42 (59); HREIMS *m*/*z* 304.2403 $[M]^+$ (calcd for $C_{20}H_{32}O_2$, 304.2402).

*11R-Methoxy-5,9,13-proharzitrien-3-ol (**2**):* colorless oil; $[\alpha]^{20}_D$ +38 (*c* 0.10, MeOH); IR (KBr) ν_{max} 3418, 2923, 2855, 1639, 1448, 1384, 1329, 1088, 1058, 947, 824 cm^{-1}; ^1H and ^{13}C NMR data, Table 1; EIMS *m*/*z* (%) 318 $[M]^+$ (3), 205 (20), 187 (30), 134 (50), 121 (62), 119 (70), 98 (100); HREIMS *m*/*z* 318.2558 $[M]^+$ (calcd for $C_{21}H_{34}O_2$, 318.2559).

*11-Methoxy-9-cycloneren-3,7-diol (**3**):* colorless oil; $[\alpha]^{20}_D$ −15 (*c* 0.12, MeOH); IR (KBr) ν_{max} 3424, 2960, 2855, 1638, 1455, 1384, 1075 cm^{-1}; ^1H and ^{13}C NMR data, Tables 2 and 3; EIMS *m*/*z* (%) 270 $[M]^+$ (2), 244 (23), 159 (19), 125 (23), 95 (40), 56 (73), 44 (100); HREIMS *m*/*z* 270.2199 $[M]^+$ (calcd for $C_{16}H_{30}O_3$, 270.2195).

*10-Cycloneren-3,5,7-triol (**4**):* colorless oil; $[\alpha]^{20}_D$ −11 (*c* 0.32, MeOH); IR (KBr) ν_{max} 3418, 2968, 2931, 2867, 1651, 1610, 1455, 1378, 1142, 1018, 925, 839, 737 cm^{-1}; ^1H and ^{13}C NMR data, Tables 2 and 3; EIMS *m*/*z* (%) 256 $[M]^+$ (0.6), 220 (42), 205 (12), 155 (20), 137 (50), 109 (100), 95 (65), 69 (79); HREIMS *m*/*z* 256.2045 $[M]^+$ (calcd for $C_{15}H_{28}O_3$, 256.2038).

*Methyl 3,7-dihydroxy-15-cycloneranate (**5**):* colorless oil; $[\alpha]^{20}_D$ −22 (*c* 0.072, MeOH); IR (KBr) ν_{max} 3454, 2930, 1723, 1639, 1462, 1383, 1206, 1157, 921, 885 cm^{-1}; ^1H and ^{13}C NMR data, Tables 2 and 3; EIMS *m*/*z* (%) 286 $[M]^+$ (0.2), 173 (40), 141 (96), 139 (95), 113 (45), 96 (42), 81 (45), 44 (100); HREIMS *m*/*z* 286.2151 $[M]^+$ (calcd for $C_{16}H_{30}O_4$, 286.2144).

*8-Acoren-3,11-diol (**6**):* colorless oil; $[\alpha]^{20}_D$ −16 (*c* 0.072, $CHCl_3$); IR (KBr) ν_{max} 3452, 2968, 2927, 2863, 1636, 1451, 1384, 1044, 922, 788 cm^{-1}; ^1H and ^{13}C NMR data, Tables 2 and 3; EIMS *m*/*z* (%) 238 $[M]^+$ (4), 194 (19), 180 (32), 162 (50), 133 (52), 107 (72), 94 (62), 59 (100); HREIMS *m*/*z* 238.1930 $[M]^+$ (calcd for $C_{15}H_{26}O_2$, 238.1933).

*9-Cycloneren-3,7,11-triol (**7**):* $[\alpha]^{20}_D$ −22 (*c* 0.040, MeOH); lit. $[\alpha]^{20}_D$ −22 (*c* 0.040, MeOH).[10]

*(−)-Cyclonerodiol (**8**):* $[\alpha]^{20}_D$ −21 (*c* 0.10, MeOH); lit. $[\alpha]^{20}_D$ −21 (*c* 0.10, MeOH).[10]

*Trichoacorenol (**9**):* $[\alpha]^{20}_D$ −5.8 (*c* 0.12, $CHCl_3$); lit. $[\alpha]_D$ −5.2 (*c* 0.12, $CHCl_3$).[16]

*Trichoacorenol B (**10**):* $[\alpha]^{20}_D$ −8.7 (*c* 0.14, $CHCl_3$); lit. $[\alpha]_D$ −7.4 (*c* 0.14, $CHCl_3$).[17]

Bioassay. The antimicroalgal activities against *Chattonella marina*, *Heterosigma akashiwo*, *Karlodinium veneficum*, and *Prorocentrum donghaiense* and the antibacterial activity against *Vibrio anguillarum*, *V. harveyi*, *V. parahemolyticus*, and *V. splendidus* were assayed as described previously,[11,23] with $K_2Cr_2O_7$ and chloramphenicol being used as positive controls, respectively.

Computational Details. Regardless of the rotations of the hydroxy, methoxy, and methyl groups, the energy-minimized conformers of compounds **1**, **2**, and **6** (Figures S43−S46) without vibrational imaginary frequencies were obtained after conformational optimization at the B3LYP/6-31G(d) level in MeOH or $CHCl_3$ via Gaussian 09 software.[20] Subsequently, the ECD spectrum of each conformer (**1a**, **1b**, or **1c** of **1** and **2a** of **2**) within a 3 kcal/mol energy threshold from the global minimum was simulated at the same level in MeOH through the time-dependent density functional theory method and then drawn by SpecDis software with sigma = 0.25 (UV-shift = 0 nm for **1** and 10 nm for **2**).[24] The overall calculated ECD curve of each compound was generated by Boltzmann weighting and magnified according to the experimental data. All of the above calculations were performed with the integral equation formalism variant of the polarizable continuum model as implemented in Gaussian 09.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.8b00714.

1D/2D NMR and HREIMS spectra (Figures S1−S42) of **1**−**6**, energy-minimized conformers (Figures S43−S46), and Cartesian coordinates (Tables S1−S4) (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: nyji@yic.ac.cn. Phone: +86-535-2109176. Fax: +86-535-2109000.

ORCID

Nai-Yun Ji: 0000-0002-6526-4731

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Financial support from the National Natural Science Foundation of China (31670355), the Natural Science Foundation for Distinguished Young Scholars of Shandong Province (JQ201712), the Open Fund of Key Laboratory of Experimental Marine Biology, CAS (KF2017NO4), the Youth Innovation Promotion Association of CAS (2013138), and the Self-Fund from Yantai Institute of Coastal Zone Research, CAS (Y755031012), is gratefully acknowledged.

■ REFERENCES

(1) Bissett, J.; Gams, W.; Jaklitsch, W.; Samuels, G. J. *IMA Fungus* **2015**, *6*, 263−295.

(2) Benítez, T.; Rincón, A. M.; Limón, M. C.; Codón, A. C. *Int. Microbiol.* **2004**, *7*, 249−260.

(3) Ghisalberti, E. L.; Hockless, D. C. R.; Rowland, C.; White, A. H. *J. Nat. Prod.* **1992**, *55*, 1690−1694.

(4) Ghisalberti, E. L.; Rowland, C. *J. Nat. Prod.* **1993**, *56*, 1799−1804.

(5) Lee, C.; Chung, M.; Lee, H.; Kho, Y. *J. Nat. Prod.* **1995**, *58*, 1605−1607.

(6) Vinale, F.; Flematti, G.; Sivasithamparam, K.; Lorito, M.; Marra, R.; Skelton, B. W.; Ghisalberti, E. L. *J. Nat. Prod.* **2009**, *72*, 2032−2035.

(7) Blunt, J. W.; Carroll, A. R.; Copp, B. R.; Davis, R. A.; Keyzers, R. A.; Prinsep, M. R. *Nat. Prod. Rep.* **2018**, *35*, 8−53.

(8) Yamada, T.; Umebayashi, Y.; Kawashima, M.; Sugiura, Y.; Kikuchi, T.; Tanaka, R. *Mar. Drugs* **2015**, *13*, 3231−3240.

(9) Yamada, T.; Suzue, M.; Arai, T.; Kikuchi, T.; Tanaka, R. *Mar. Drugs* **2017**, *15*, 169.

(10) Song, Y. P.; Liu, X. H.; Shi, Z. Z.; Miao, F. P.; Fang, S. T.; Ji, N. Y. *Phytochemistry* **2018**, *152*, 45−52.

(11) Miao, F. P.; Liang, X. R.; Yin, X. L.; Wang, G.; Ji, N. Y. *Org. Lett.* **2012**, *14*, 3815−3817.

DOI: 10.1021/acs.jnatprod.8b00714
J. Nat. Prod. 2018, 81, 2553−2559

(12) Shi, Z. Z.; Miao, F. P.; Fang, S. T.; Yin, X. L.; Ji, N. Y. *J. Nat. Prod.* **2018**, *81*, 1121−1124.
(13) Liang, X. R.; Miao, F. P.; Song, Y. P.; Liu, X. H.; Ji, N. Y. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 5029−5031.
(14) Laurent, D.; Goasdoue, N.; Kohler, F.; Pellegrin, F.; Platzer, N. *Magn. Reson. Chem.* **1990**, *28*, 662−664.
(15) Langhanki, J.; Rudolph, K.; Erkel, G.; Opatz, T. *Org. Biomol. Chem.* **2014**, *12*, 9707−9715.
(16) Huang, Q.; Tezuka, Y.; Hatanaka, Y.; Kikuchi, T.; Nishi, A.; Tubaki, K. *Chem. Pharm. Bull.* **1995**, *43*, 1035−1038.
(17) Zhang, M.; Zhao, J. L.; Liu, J. M.; Chen, R. D.; Xie, K. B.; Chen, D. W.; Feng, K. P.; Zhang, D.; Dai, J. G. *J. Asian Nat. Prod. Res.* **2017**, *19*, 651−658.
(18) Smith, A. B., III; Agosta, W. C. *J. Am. Chem. Soc.* **1973**, *95*, 1961−1968.
(19) Zhang, M.; Liu, J. M.; Zhao, J. L.; Li, N.; Chen, R. D.; Xie, K. B.; Zhang, W. J.; Feng, K. P.; Yan, Z.; Wang, N.; Dai, J. G. *Chin. Chem. Lett.* **2016**, *27*, 957−960.
(20) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Keith, T.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, O.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. *Gaussian 09*, Revision C.01; Gaussian, Inc.: Wallingford, CT, 2010.
(21) Barra, L.; Dickschat, J. S. *ChemBioChem* **2017**, *18*, 2358−2365.
(22) Adelin, E.; Servy, C.; Martin, M. T.; Arcile, G.; Iorga, B. I.; Retailleau, P.; Bonfill, M.; Ouazzani, J. *Phytochemistry* **2014**, *97*, 55−61.
(23) Shi, Z. Z.; Miao, F. P.; Fang, S. T.; Yin, X. L.; Ji, N. Y. *Phytochem. Lett.* **2018**, *27*, 101−104.
(24) Bruhn, T.; Hemberger, Y.; Schaumlöffel, A.; Bringmann, G. *SpecDis*, Version 1.51; University of Wuerzburg: Germany, 2011.

DOI: 10.1021/acs.jnatprod.8b00714
J. Nat. Prod. 2018, 81, 2553−2559