

Article

Trichoderols B-G, Six New Lipids from the Marine Algicolous Fungus *Trichoderma* sp. Z43

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Abstract: Six new lipids, trichoderols B-G (1–6), along with a known one, triharzianin B (7), were isolated from the culture of *Trichoderma* sp. Z43 obtained from the surface of the marine brown alga *Dictyopteris divaricata*. Their structures and relative configurations were identified by interpretation of 1D/2D NMR and MS data. Compounds 1–7 were assayed for inhibiting the growth of three phytopathogenic fungi (*Fusarium graminearum*, *Gaeumannomyces graminis*, and *Glomerella cingulata*), four marine phytoplankton species (*Amphidinium carterae*, *Heterocapsa circularisquama*, *Heterosigma akashiwo*, and *Prorocentrum donghaiense*), and one marine zooplankton (*Artemia salina*). Compounds 1, 4, and 7 exhibited weak antifungal activities against three phytopathogenic fungi tested with MIC \geq 64 μ g/mL. All compounds displayed moderate antimicrobial activity with IC₅₀ \geq 15 μ g/mL and low toxicity to the brine shrimp *Artemia salina*.

Keywords: *Trichoderma*; lipid; antifungal activity; antimicrobial activity



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1. Introduction

Crop diseases caused by pathogenic fungus have seriously restricted healthy development of agriculture in the world. *Fusarium* head blight of wheat, take-all disease, and plant anthracnose caused by *Fusarium graminearum*, *Gaeumannomyces graminis*, and *Glomerella cingulata*, respectively, lead to huge economic losses in agriculture every year [1–5]. In addition, some marine phytoplankton species including *Amphidinium carterae*, *Heterocapsa circularisquama*, *Heterosigma akashiwo*, and *Prorocentrum donghaiense* can induce red tides that harm aquaculture industry [6–9]. Thus, it is imperative to search for natural antifungal and antimicrobial drugs with high activity and safety. On the other hand, *Trichoderma* species are known for producing various metabolites with novel structures and intriguing bioactivities [10–12]. In recent years, natural products originated from marine-derived *Trichoderma* species have gained the attention of researchers [13], and a large number of new compounds with significant activities have been found, such as polyketides [14], terpenoids [15–17], and steroids [18]. Although *Trichoderma* species have proven to be a treasure-house of new natural products, only a few short-chain lipids have been isolated from this species. For example, triharzianins A-D were purified from *T. harzianum* obtained from mushroom *Tricholoma matsutake* [19], harzianumols A-H were separated from *T. harzianum* obtained from sponge *Petrospongia nigra* [20], and trichoderol A was acquired from *Trichoderma* sp. obtained from soil [21]. Moreover, these lipids displayed multifarious bioactivities such as feeding attractants and antimicrobial activities [19,21]. During our investigation of the chemical diversity and biological activity of marine-derived *Trichoderma*, the epiphytic *Trichoderma* sp. Z43 obtained from the marine brown alga *Dictyopteris divaricata* was examined. Its aerial mycelia grew rapidly. Most of them were white on the PDA medium at 25 °C, and some parts of the plate were yellow. Alternate permutation of the mycelia was observed through a common microscope. As a result, six new lipids, trichoderols B-G (1–6), along

with a known one, triharzianin B (7), were isolated and identified. Herein, the details of isolation, structure elucidation, and bioactivities of these compounds are described.

2. Results and Discussion

The organic extracts of marine-derived *Trichoderma* sp. Z43 isolated from the marine brown alga *Dictyopteris divaricata* were subjected to a series of column chromatography processes to produce six new lipids, namely, trichoderols B-G (1–6), along with a known one, triharzianin B (7) (Figure 1). Compound 7 was unambiguously identified by comparing its NMR data and specific rotation value with those reported in the literature [19].

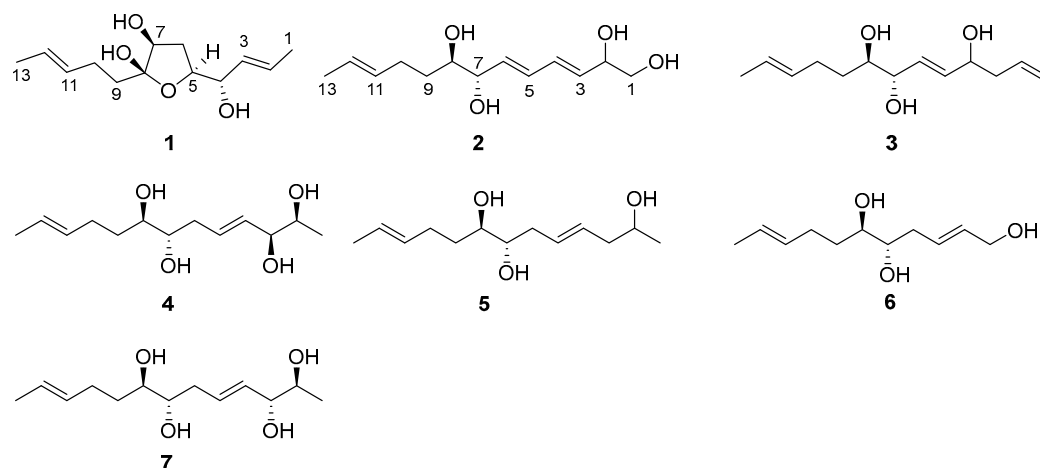


Figure 1. Chemical structures of 1–7.

2.1. Structural Elucidation

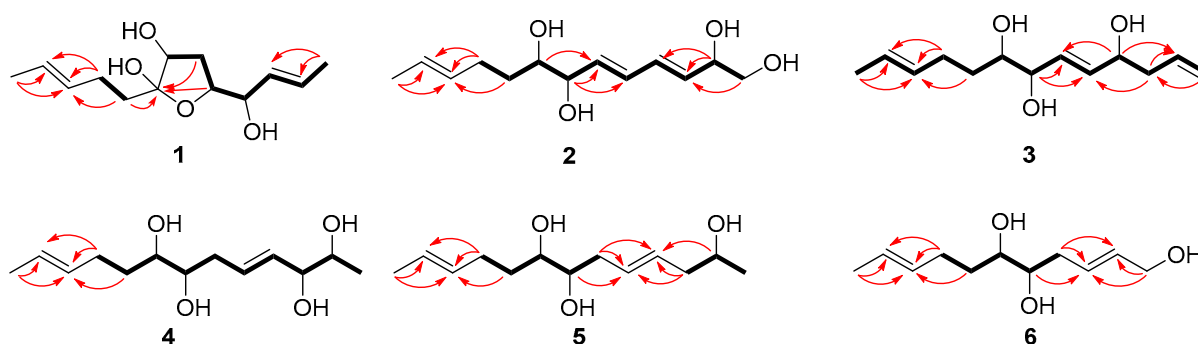
Compound 1 was isolated as a colorless oil with a molecular formula of $C_{13}H_{22}O_4$ established by HRESIMS (m/z 265.1420 $[M + Na]^+$), implying three degrees of unsaturation. The 1H NMR spectrum (Table 1) showed two methyl doublets, one double quartet, one double doublet, and two multiplets assignable to four olefinic protons and one double multiplet and two double doublets attributable to three oxymethines. The ^{13}C NMR and DEPT spectra (Table 2) displayed the presence of two methyls, three methylenes, seven methines, and one nonprotonated carbon. COSY correlations of H-1/H-2/H-3/H-4/H-5/H-6/H-7 established the linkage of C-1 to C-7 (Figure 2). A pentenyl group was confirmed by the COSY correlation of H-9 with H-10 and the HMBC correlations from H-10/H-13 to C-11 and C-12 and from H-9 to C-11, which was then elongated to C-7 via C-8 by the HMBC correlations from H-6/H-9 to C-8. To satisfy the molecular formula, an ether linkage was situated between C-5 and C-8, which was verified by the HMBC correlation from H-5 to C-8 (Figure 2). Thus, the planar structure of 1 was validated. The double bond at C-2 was attributed to be *trans* by the large coupling constant ($J = 15.2$) between H-2 and H-3. The chemical shifts of other two olefinic carbon atoms (C-11, δ_C 130.9; C-12, δ_C 125.5) were highly similar to those of triharzianin B (7) (C-11, δ_C 130.9; C-12, δ_C 124.6) [19], suggesting a *trans* configuration of the C-11 double bond, which was further verified by the IR absorption at 969 cm^{-1} . The relative configurations of H-5, OH-7, and OH-8 were confirmed by NOESY correlations of H-7 with H-5 and H-9 (Figure 3), and the relationship of H-4 and H-5 was deduced to be *threo* due to NMR data that were similar to those of (-)-(*S*)-1-[(2*S*,5*S*)-5-[2-propenyl]tetrahydrofuran-2-yl]prop-2-en-1-ol [22].

Table 1. ^1H NMR data for 1–6 (δ in ppm, J in Hz).

Pos	1 (CDCl ₃)	2 (CD ₃ OD)	3 (CDCl ₃)	4 (CD ₃ OD)	5 (CD ₃ OD)	6 (CD ₃ OD)
1a	1.72, d (6.6)	3.48, dd (11.1, 5.1)	5.17, brs	1.14, d (6.4)	1.14, d (6.2)	4.02, d (5.2)
1b		3.46, dd (11.2, 6.8)	5.14, brs			
2	5.81, dq (15.2, 6.6)	4.15, dt (6.3, 5.2)	5.80, m	3.65, qd (6.4, 6.1)	3.75, sext (6.2)	5.69, dt (15.4, 5.2)
3a	5.28, dd (15.2, 7.4)	5.71, dd (13.1, 6.3)	2.37, dddt (13.9, 6.6, 5.3, 1.2)	3.84, dd (7.1, 6.1)	2.18, m	5.72, dt (15.4, 6.7)
3b			2.28, dddt (13.9, 7.4, 6.6, 1.0)		2.14, m	
4a	4.20, dm (7.4)	6.32, dd (13.1, 10.6)	4.24, dt (6.1, 5.5)	5.60, ddm (15.5, 7.1)	5.54, dt (15.4, 6.4)	2.31, m
4b						2.19, m
5	4.52 dd (5.5, 3.1)	6.29, dd (13.1, 10.6)	5.84, ddd (15.5, 5.6, 0.9)	5.75, m	5.56, dt (15.4, 6.4)	3.44, m
6a	2.44, dd (13.5, 6.9)	5.73, dd (13.1, 5.7)	5.73, ddd (15.5, 6.3, 1.1)	2.33, m	2.35, dm (13.8)	3.43, m
6b	1.45, dddd (13.5, 5.5, 2.6, 1.2)			2.22, dd (14.2, 7.3)	2.12, m	
7	3.88, dd (6.9, 2.6)	3.93, dd (6.5, 5.7)	3.96, dd (6.3, 5.9)	3.47, m	3.40, m	1.52, m
8a		3.42, ddd (9.4, 5.7, 3.2)	3.49, ddd (9.3, 5.9, 3.5)	3.44, m	3.39, m	2.15, m
8b						2.04, m
9a	1.99, m	1.54, m	1.58, m	1.53, m	1.67, m	5.45, m
9b		1.38, m	1.49, m		1.41, m	
10a	2.24, m	2.16, m	2.18, m	2.16, m	2.19, m	5.45, m
10b		2.03, m	2.09, m	2.04, m	2.02, m	
11	5.49, m	5.44, m	5.44, m	5.46, m	5.46, m	1.64, d (4.7)
12	5.50, m	5.43, m	5.47, m	5.46, m	5.46, m	
13	1.64, d (4.6)	1.63, d (5.2)	1.65, d (6.0)	1.64, d (3.9)	1.64, d (3.9)	

Table 2. ^{13}C NMR data for 1–6 (δ in ppm).

Pos	1 (CDCl ₃)	2 (CD ₃ OD)	3 (CDCl ₃)	4 (CD ₃ OD)	5 (CD ₃ OD)	6 (CD ₃ OD)
1	18.1, CH ₃	67.2, CH ₂	118.9, CH ₂	18.7, CH ₃	22.9, CH ₃	63.7, CH ₂
2	131.4, CH	73.9, CH	134.0, CH	71.6, CH	68.5, CH	132.7, CH
3	126.5, CH	134.3, CH	42.0, CH ₂	77.9, CH	43.6, CH ₂	129.9, CH
4	80.0, CH	132.2, CH	70.8, CH	132.9, CH	130.1, CH	37.1, CH ₂
5	78.2, CH	132.6, CH	135.4, CH	131.1, CH	130.7, CH	75.0, CH
6	36.1, CH ₂	134.3, CH	130.0, CH	37.3, CH ₂	37.3, CH ₂	73.9, CH
7	74.2, CH	76.7, CH	75.4, CH	75.0, CH	75.9, CH	33.9, CH ₂
8	111.3, C	75.1, CH	74.2, CH	73.9, CH	74.7, CH	30.0, CH ₂
9	28.5, CH ₂	33.7, CH ₂	32.8, CH ₂	33.9, CH ₂	33.6, CH ₂	132.2, CH
10	26.5, CH ₂	29.9, CH ₂	28.9, CH ₂	30.0, CH ₂	29.9, CH ₂	126.0, CH
11	130.9, CH	132.2, CH	130.7, CH	132.2, CH	132.3, CH	18.1, CH ₃
12	125.5, CH	126.0, CH	125.9, CH	126.0, CH	126.0, CH	
13	18.1, CH ₃	18.1, CH ₃	18.1, CH ₃	18.1, CH ₃	18.1, CH ₃	

**Figure 2.** Key COSY and HMBC correlations of 1–6 (bold lines for COSY and arrows for HMBC).

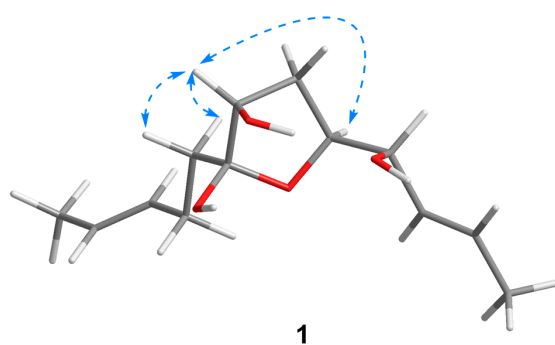


Figure 3. Key NOESY correlations of **1**.

Compound **2** was purified as a colorless oil and assigned a molecular formula of $C_{13}H_{22}O_4$ by interpretation of HRESIMS (m/z 265.1420 $[M + Na]^+$) data. In conjunction with HSQC data, the 1H NMR spectrum (Table 1) revealed notable signals including one methyl doublet, one double triplet, one double doublet, and one doublet of double doublets ascribable to three oxygenated methines, two double doublets attributable to an oxygenated methylene, and four double doublets and two multiplets assignable to six olefinic protons. DEPT experiments displayed 13 resonances in the ^{13}C NMR spectrum, which were assigned to one methyl, three methylenes, and nine methines. COSY correlations of H-1/H-2/H-3/H-4/H-5/H-6/H-7/H-8/H-9/H-10 established the linkage of C-1 to C-10, which was further confirmed by the HMBC correlations from H-1 to C-3, from H-2 to C-3 and C-4, from H-7 to C-5 and C-6, and from H-8 to C-6 (Figure 2). A propenyl group was located at C-10, verified by the HMBC correlations from H-9 to C-11 and from H-10/H-13 to C-11 and C-12 (Figure 2). Thus, the planar structure of **2** was affirmed. The double bonds at C-3 and C-5 were determined to be *trans* by the large coupling constants ($J = 13.1$), and the identical ^{13}C NMR data of **2** (C-11, δ_C 132.2; C-12, δ_C 126.0) with those of triharzianin C (C-11, δ_C 132.2; C-12, δ_C 126.0) [19] suggested the double bond at C-11 to be *trans*. The relationship of OH-7 and OH-8 was assigned to be *erythro* by comparison of NMR data with those for triharzianin C [19]. Although the chemical shifts of C-1, C-2, and C-3 in **2** were the same as those of (*S*)-but-3-ene-1,2-diol [23], the relative configuration of OH-2 could not be confirmed due to the existence of only one chiral carbon atom (C-2) in this moiety. Thus, compound **2** was named (*3E,5E,11E*)-trideca-3,5,11-trien-1,2,7,8-tetraol, and its structural formula was $HOCH_2CH(OH)CH=CHCH=CHCH(OH)CH(OH)CH_2CH_2CH=CHCH_3$.

Compound **3** was acquired as a colorless oil, and its molecular formula was determined to be $C_{13}H_{22}O_3$ by HRESIMS (249.1448 $[M + Na]^+$) data. The 1H NMR spectrum, along with HSQC data, exhibited notable signals including one methyl doublet, one double triplet, one double doublet, one doublet of double doublets assignable to three oxygenated methines, two broad singlets attributable to a terminal double bond, and three multiplets and two doublets of double doublets ascribable to five olefinic protons. The ^{13}C NMR spectrum displayed 13 resonances, sorted into one methyl, four methylenes, and eight methines. The 1H and ^{13}C NMR data (Tables 1 and 2) revealed the presence of a similar skeleton to **2**. COSY correlations of H-1/H-2/H-3/H-4/H-5/H-6/H-7/H-8/H-9/H-10/H-11/H-12/H-13 and HMBC correlations from H-1 to C-3, from H-3 to C-2 and C-5, from H-4 to C-2 and C-6, from H-7 to C-5 and C-6, from H-10/H-13 to C-11 and C-12, and from H-9 to C-11 (Figure 2) confirmed the planar structure of **3**. The double bonds at C-5 and C-11 were deduced to be *trans* by the large coupling constant ($J = 15.5$) between H-5 and H-6 and by the identical NMR data of C-11 and C-12 with those of **2**. The relative configurations of OH-7 and OH-8 were determined to be the same as those of **2** on the basis of highly similar chemical shifts and coupling constants of them. Despite the similar NMR data of C-1 to C-5 in **3** with those of 12(*S*)-hydroxy-5(*Z*),8(*Z*),10(*E*),14(*E*)-eicosatetraenoic acid [24], the relative configuration of OH-4 was still unsolved. Compound **3** was named (*5E,11E*)-trideca-1,5,11-trien-4,7,8-triol, and its structural formula was $CH_2=CHCH_2CH(OH)CH=CHCH(OH)CH(OH)CH_2CH_2CH=CHCH_3$.

Compound **4** was obtained as a colorless oil and given a molecular formula of $C_{13}H_{24}O_4$ by interpretation of HRESIMS data (m/z 243.1558 $[M]^+$), requiring two degrees of unsaturation. Its NMR data (Tables 1 and 2) were highly similar to those of triharzianin B (**7**) [19], except for chemical shifts of C-2 and C-3 (C-2, δ_C 70.2; C-3, δ_C 76.6 for triharzianin B; C-2, δ_C 71.6; C-3, δ_C 77.9 for **4**), which indicated that **4** and triharzianin B possessed the same planar structure. COSY correlations of H-1/H-2/H-3/H-4/H-5/H-6/H-7/H-8/H-9/H-10 and HMBC correlations from H-9 to C-11 and from H-10/H-13 to C-11 and C-12 (Figure 2) further afforded to this structure. The geometry of two double bonds at C-2 and C-11 and the relative configurations of OH-7 and OH-8 were deduced to be the same as those of triharzianin B (**7**) [19] on the basis of their identical NMR data. The relationship of H-2 and H-3 was *threo* by analysis of NMR data with those for separacenes A and B [25]. Therefore, compound **4** was named (4*E*,11*E*)-trideca-4,11-diene-2,3,7,8-tetraol, and its structural formula was $CH_3CH(OH)CH(OH)CH=CHCH_2CH(OH)CH(OH)CH_2CH_2CH=CHCH_3$.

Compound **5** was isolated as a colorless oil. HRESIMS analysis gave the molecular formula of $C_{13}H_{24}O_3$, consistent with two degrees of unsaturation. Its NMR data (Tables 1 and 2) resembled those of **4**, except for the presence of signals for a methylene and the lack of signals for a hydroxymethine group. COSY correlations of H-1/H-2/H-3 confirmed the linkage from C-1 to C-3, which was then elongated to C-10 by the HMBC correlations from H-2 to C-4, from H-3/H-6 to C-4 and C-5, and from H-7 to C-5 and the COSY correlations of H-6/H-7/H-8/H-9/H-10. A propenyl group was situated at C-10 by the HMBC correlations from H-9 to C-11 and from H-10/H-13 to C-11 and C-12 (Figure 2). Thus, the planar structure of **5** was confirmed. The configurations of OH-7, OH-8, and the double bond at C-11 were the same as those of **4** due to their similar NMR data, and the geometry of double bond at C-4 was deduced to be *trans* by the large coupling constant ($J = 15.4$) between H-4 and H-5. The relative configuration of OH-2 was uncertain in spite of comparing the NMR data of **5** with those of (*S*)-2-hexanol and (*R*)-octan-2-ol carefully [26]. Thus, compound **5** was named (4*E*,11*E*)-trideca-4,11-dien-2,7,8-triol, and its structural formula was $CH_3CH(OH)CH_2CH=CHCH_2CH(OH)CH(OH)CH_2CH_2CH=CHCH_3$.

Compound **6** was obtained as a colorless oil and was given a molecular formula of $C_{11}H_{20}O_3$ by analysis of HRESIMS data, requiring two degrees of unsaturation. Its NMR data exhibited high similarities to those of **4** except for the presence of signals for a hydroxymethylene group and lack of signals for a methyl and two hydroxymethine groups. COSY correlations of H-1/H-2/H-3/H-4/H-5/H-6/H-7/H-8 determined the linkage of C-1 to C-8, and a propenyl group was located at C-8, confirmed by the HMBC correlations from H-7 to C-9 and from H-8/H-11 to C-9 and C-10 (Figure 2). Other HMBC correlations further verified the structure of **6** (Figure 2). The large coupling constant ($J = 15.4$) between H-2 and H-3 demonstrated that the double bond at C-2 was *trans*. The identical NMR data of the spin system in **6** (from C-2 to C-11) and **4** (from C-4 to C-13) (Tables 1 and 2) ascertained the *trans* configuration of a double bond at C-9 and the *erythro* relationship of the vicinal diol (OH-5 and OH-6). Compound **6** was named (2*E*,9*E*)-undeca-2,9-dien-1,5,6-triol, and its structural formula was $HOCH_2CH=CHCH_2CH(OH)CH(OH)CH_2CH_2CH=CHCH_3$.

2.2. Bioactivity of Isolated Compounds

Compounds **1–7** were assayed for antifungal activity against *Fusarium graminearum*, *Gaeumannomyces graminis*, and *Glomerella cingulata*. The result showed that compounds **1**, **4**, and **7** displayed weak antifungal activity (Table 3). Compound **1** could inhibit the three phytopathogenic fungi tested with MIC values ranging from 64 to 256 $\mu\text{g}/\text{mL}$. Compounds **4** and **7** possessed identical inhibition against *Glomerella cingulata* and *Gaeumannomyces graminis*, with MIC values of 128 and 256 $\mu\text{g}/\text{mL}$, respectively. In addition, all the isolates were evaluated for antimicrobial activity against *Amphidinium carterae*, *Heterocapsa circularisquama*, *Heterosigma akashiwo*, and *Prorocentrum donghaiense* (Table 4). It was worth noting that only compound **1** was active against all the phytoplankton species tested with IC_{50} values ranging from 15 to 28 $\mu\text{g}/\text{mL}$. Moreover, the antimicrobial activity of compounds **2–7** ($IC_{50} \geq 30 \mu\text{g}/\text{mL}$) was weaker than that of compound **1**. The above results suggested

that the tetrahydrofuran ring could improve antifungal and antimicrobial activity of these lipids by analysis of their structure–activity relationships. In addition, the brine shrimp lethality of 1–7 was also estimated, with the lethal rates against *Artemia salina* of these compounds being less than 10% at 100 µg/mL (Table 4). All isolates showed low toxicity to the brine shrimp *Artemia salina*, which demonstrated the safety of their further exploitation. An in-depth study, such as chemical modification, should be conducted to promote the bioactivity of these compounds, increasing their prospective use in the development of antifungal and antimicrobial agents.

Table 3. Inhibition of three phytopathogenic fungi by 1–7.

Compounds	MIC (µg/mL)		
	<i>Fusarium graminearum</i>	<i>Gaeumannomyces graminis</i>	<i>Glomerella cingulata</i>
1	256	256	64
2	-	-	-
3	-	-	-
4	-	256	128
5	-	-	-
6	-	-	-
7	-	256	128
amphotericin B	2.0	2.0	1.0

-: no inhibition effect at 256 µg/mL.

Table 4. Inhibition of phytoplankton species by 1–7.

Compounds	IC ₅₀ (µg/mL)				Lethal Rate (at 100 µg/mL)
	<i>Amphidinium carterae</i>	<i>Heterocapsa circularisquama</i>	<i>Heterosigma akashiwo</i>	<i>Prorocentrum donghaiense</i>	<i>Artemia salina</i>
1	15	24	28	22	9.6%
2	61	-	43	37	5.2%
3	66	78	-	-	4.6%
4	-	-	53	42	6.1%
5	50	68	-	-	8.4%
6	44	-	82	-	3.2%
7	-	-	49	30	5.4%
K ₂ Cr ₂ O ₇	1.2	1.0	0.8	1.4	100%

-: no inhibition effect at 100 µg/mL.

3. Materials and Methods

3.1. General Experimental Procedures

NMR spectra were obtained on a Bruker Avance III 500 NMR spectrometer (500 and 125 MHz for ¹H and ¹³C, respectively) using tetramethylsilane (TMS) as an internal standard (Bruker Corp., Billerica, MA, USA). Chemical shifts are reported in parts per million (δ) in CDCl₃/CD₃OD (δ_H reported referred to CDCl₃/CD₃OD at 7.26/3.31 ppm; δ_C reported referred to CDCl₃/CD₃OD at 77.16/49.00 ppm) and coupling constants (J) in Hz. Optical rotations were measured on an SGW-3 polarimeter (Shanghai Shengguang Instrument Co., Ltd., Shanghai, China). IR spectra were recorded on a Nicolet iS50 FT-IR spectrometer (Thermo Fisher Scientific, Waltham, MA, USA); peaks are reported in cm⁻¹. High-resolution ESI mass spectra were determined on a Xevo G2-XS QToF mass spectrometer (Water Crop., Milford, MA, USA). Column chromatography (CC) was carried out with silica gel (200–300 mesh, Qingdao Haiyang Chemical Co., Qingdao, China), RP-18 (AAG12S50, YMC Co., Ltd., Kyoto, Japan), and Sephadex LH-20 (GE Healthcare, Uppsala, Sweden). Thin-layer chromatography (TLC) was performed with precoated silica gel plates (GF-254, Qingdao Haiyang Chemical Co., Qingdao, China).

3.2. Fungal Material and Fermentation

The fungal strain *Trichoderma* sp. Z43 was isolated from the surface of marine brown alga *Dictyopteris divaricata* collected from Zhoushan, China, in July 2018. The species was identified according to morphological characteristics and analysis of ITS regions of its rDNA, deposited at GenBank (OR196112). Mass fermentation was performed statically at room temperature for 30 days in 200 × 1 L Erlenmeyer flasks, each containing 300 mL of media, by adding 40.0 g glucose, 10.0 g peptone, and 7.0 g yeast extract powder into 1000 mL purified water.

3.3. Extraction and Isolation

At the end of fermentation, the mycelia of cultures were obtained by filtration, which were then dried at room temperature, smashed, and extracted with CH₂Cl₂ and MeOH (1:1, v/v). After removing organic solvents under reduced pressure, the residue was partitioned between ethyl acetate (EtOAc) and H₂O to afford an EtOAc-soluble extract (25.6 g). The filtrate was extracted with EtOAc and then dried to give an extract (10.0 g). The two parts were merged based on the similarity of TLC profiles and subjected to silica gel CC with step-gradient solvent systems of petroleum ether (PE)/EtOAc and CH₂Cl₂/MeOH to afford 11 fractions (Frs. 1–11). Fr. 8 eluted with CH₂Cl₂/MeOH (20:1) and was further purified by RP-18 CC to afford Fr. 8-1 (MeOH/H₂O, 3:7), Fr. 8-2 (MeOH/H₂O, 2:3), and Fr. 8-3 (MeOH/H₂O, 9:11). Fr. 8-1 was further purified by preparative TLC (CH₂Cl₂/MeOH, 20:1) and Sephadex LH-20 CC (MeOH) to give compound 7 (2.6 mg). Compound 1 (4.2 mg) was isolated from Fr. 8-2 by preparative TLC (CH₂Cl₂/MeOH, 20:1) and Sephadex LH-20 CC (MeOH). Compounds 3 (4.0 mg), 5 (4.5 mg), and 6 (2.7 mg) were purified from Fr. 8-3 by TLC (CH₂Cl₂/MeOH, 10:1) and Sephadex LH-20 CC (MeOH). Fr. 9 eluted with CH₂Cl₂/MeOH (10:1) and was further purified by RP-18 CC (MeOH/H₂O, 1:1) and preparative TLC (CH₂Cl₂/MeOH, 10:1) as well as Sephadex LH-20 CC (MeOH) to afford compounds 2 (3.1 mg) and 4 (3.8 mg).

3.4. Spectral and Physical Data of Compounds 1–6

Trichoderol B (1): colorless oil; $[\alpha]_D^{20} - 10.2$ (c 0.14, CH₃OH); IR (KBr) ν_{\max} 3443, 2923, 2854, 1633, 1453, 1384, 969 cm⁻¹; ¹H and ¹³C NMR data, Tables 1 and 2; HRESIMS m/z 265.1420 [M + Na]⁺ (calcd for C₁₃H₂₂NaO₄, 265.1416).

Trichoderol C (2): colorless oil; $[\alpha]_D^{20} - 4.2$ (c 0.10, CH₃OH); IR (KBr) ν_{\max} 3430, 2926, 2858, 1633, 1554, 1394, 1030 cm⁻¹; ¹H and ¹³C NMR data, Tables 1 and 2; HRESIMS m/z 265.1420 [M + Na]⁺ (calcd for C₁₃H₂₂NaO₄, 265.1416).

Trichoderol D (3): colorless oil; $[\alpha]_D^{20} + 7.9$ (c 0.13, CH₃OH); IR (KBr) ν_{\max} 3422, 2924, 2856, 1661, 1633, 1538, 1394, 1025 cm⁻¹; ¹H and ¹³C NMR data, Tables 1 and 2; HRESIMS m/z 249.1448 [M + Na]⁺ (calcd for C₁₃H₂₂NaO₃, 249.1467).

Trichoderol E (4): colorless oil; $[\alpha]_D^{20} - 12.2$ (c 0.13, CH₃OH); IR (KBr) ν_{\max} 3415, 2924, 1634, 1538, 1385, 1023 cm⁻¹; ¹H and ¹³C NMR data, Tables 1 and 2; HRESIMS m/z 243.1588 [M]⁻ (calcd for C₁₃H₂₃O₄, 243.1598).

Trichoderol F (5): colorless oil; $[\alpha]_D^{20} - 4.1$ (c 0.15, CH₃OH); IR (KBr) ν_{\max} 3417, 2924, 2855, 1632, 1554, 1384, 966 cm⁻¹; ¹H and ¹³C NMR data, Tables 1 and 2; HRESIMS m/z 251.1614 [M + Na]⁺ (calcd for C₁₃H₂₄NaO₃, 251.1623).

Trichoderol G (6): colorless oil; $[\alpha]_D^{20} - 5.7$ (c 0.09, CH₃OH); IR (KBr) ν_{\max} 3441, 2923, 2853, 1633, 1539, 1385, 985 cm⁻¹; ¹H and ¹³C NMR data, Tables 1 and 2; HRESIMS m/z 223.1301 [M + Na]⁺ (calcd for C₁₁H₂₀NaO₃, 223.1310).

3.5. Assay for Antifungal Activity

Antifungal activity against *Fusarium graminearum*, *Gaeumannomyces graminis*, and *Glomerella cingulata* was performed using the microdilution method in a 96-well plate, as described previously [17]. Briefly, a stock solution of each fungus tested was diluted in potato dextrose broth (PDB) to 5 × 10⁵ cfu/mL. Each sample was prepared in dimethyl sulfoxide (DMSO) and was diluted to final concentrations of 5120, 2560, 1280, 640, 320, 160, 80, 40, 20,

10, and 5 µg/mL in a set of capped test tubes by two-fold serial dilution. An amount of 5 µL diluent was added into each well of a 96-well flat-bottom microtiter plate containing 95 µL fungal suspension (the final sample concentrations were 256 to 0.25 µg/mL), and the fungi were cultivated at 28 °C for 48 h. The MIC value for each sample was defined as the minimum concentration of the compound with invisible microbial growth. Amphotericin B and DMSO were chosen as positive and negative controls, respectively.

3.6. Assay for Antimicroalgal Activity

The inhibition of four marine phytoplankton species (*Amphidinium carterae*, *Heterocapsa circularisquama*, *Heterosigma akashiwo*, and *Prorocentrum donghaiense*) was assayed using our previously reported method [17]. In brief, each microalga inoculum was cultured for 5 days using the sterilized f/2 medium in an incubator (20 °C, 14:10 light–dark cycle, 2000 lx light) and reached the exponential growth phase. The microalga suspension was diluted to $4\text{--}5 \times 10^4$ cells/mL and then added into a 96-well flat-bottom microtiter plate with 195 µL in each well. An amount of 5 µL sample solution (in DMSO) was pipetted into each well (the final sample concentrations were 100 to 0.125 µg/mL) and mixed uniformly. After 24 h inoculation, the live cells were counted using hemocytometer, and the inhibition rate was calculated as follows. Inhibition rate (IR) = $(N_{CK} - N_T)/N_{CK} \times 100\%$ (N_{CK} : the number of live algal cells under negative control, N_T : the number of live algal cells under treatment). DMSO and $K_2Cr_2O_7$ were taken as negative and positive controls, respectively.

3.7. Assay for Brine Shrimp Lethal Activity

The inhibition of the brine shrimp *Artemia salina* was assayed according to the procedures described in our previous report [15]. In brief, brine shrimp eggs were left to hatch in sea water for 48 h at 25 °C under natural light. About 10 brine shrimp were placed in a 96-well flat-bottom microtiter plate with a volume of 195 µL sea water in each well. An amount of 5 µL sample solution (in DMSO) was added into each well (the final sample concentrations were 100 to 0.125 µg/mL) and mixed uniformly. The lethality was observed after 24 h of cultivation. DMSO and $K_2Cr_2O_7$ served as negative and positive controls, respectively.

4. Conclusions

Chemical investigation towards the marine algicolous fungus *Trichoderma* sp. Z43 resulted in the isolation of seven lipids, including six new ones (trichoderols B–G (1–6)) and a known one, triharzianin B (7). The C_{13} and C_{11} lipids are rarely found in nature, especially in *Trichoderma* species, and these new compounds greatly enrich the chemical diversity of marine-derived natural products. Finding and stimulating silent biosynthetic gene clusters may be an effective means to excavate this kind of metabolite. These isolations were evaluated for inhibition against three phytopathogenic fungi and four marine phytoplankton species. Several of them exhibited inhibition of one or more fungi/plankton species tested, and the tetrahydrofuran ring could improve antifungal and antimicroalgal activity of these lipids by analysis of their structure–activity relationships. Moreover, all isolates exhibited low toxicity to the brine shrimp *Artemia salina*, suggesting the security for their further exploitation.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/md21080453/s1>, Figures S1–S42: 1D/2D NMR, HRESIMS spectra of 1–6.

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