

Article

# Cyclonerane Derivatives from the Algicolous Endophytic Fungus *Trichoderma asperellum* A-YMD-9-2

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Received: 9 March 2019; Accepted: 26 April 2019; Published: 28 April 2019



**Abstract:** Seven previously unreported cyclonerane derivatives, namely, 3,7,11-trihydroxycycloneran-10-one, cycloneran-3,7,10,11-tetraol, cycloneran-3,7,11-triol, 11,12,15-trinorcycloneran-3,7,10-triol, 7,10S-epoxycycloneran-3,15-diol, 7,10R-epoxycycloneran-3,15-diol, and (10Z)-15-acetoxy-10-cycloneran-3,7-diol, were isolated in addition to the known (10Z)-cyclonerotriol, (10E)-cyclonerotriol, catenioblin C, and chokol E from the culture of *Trichoderma asperellum* A-YMD-9-2, an endophytic fungus obtained from the marine red alga *Gracilaria verrucosa*. The structures of previously unreported compounds were established by spectroscopic techniques, including 1D/2D NMR, MS, and IR. The isolation of these new cyclonerane derivatives greatly adds to the structural diversity of unusual cyclonerane sesquiterpenes, and several isolates exhibit potent inhibition against some marine phytoplankton species.

**Keywords:** *Trichoderma asperellum*; sesquiterpene; cyclonerane

## 1. Introduction

*Trichoderma* species have proven to be prolific sources of diterpenes and sesquiterpenes, especially some unusual terpene skeletons, such as harziane, proharziane, wickerane, citrinovirin, trichaspin, and cyclonerane [1–8]. Of the cyclonerane-type sesquiterpenes, cyclonerodiol and its analogs have been isolated from several species of *Trichoderma* [4–6,9–14]. To date, around 30 cyclonerane sesquiterpenes have been characterized, and almost half of them occurred in *Trichoderma* species. In our ongoing search for new and bioactive metabolites from marine algicolous fungi [15], an endophytic strain (A-YMD-9-2) of *Trichoderma asperellum*, isolated from the marine red alga *Gracilaria verrucosa*, was chemically examined. As a result, seven new cyclonerane derivatives, namely, 3,7,11-trihydroxycycloneran-10-one (1), cycloneran-3,7,10,11-tetraol (2), cycloneran-3,7,11-triol (3), 11,12,15-trinorcycloneran-3,7,10-triol (4), 7,10S-epoxycycloneran-3,15-diol (5), 7,10R-epoxycycloneran-3,15-diol (6), and (10Z)-15-acetoxy-10-cycloneran-3,7-diol (7), together with four known compounds, namely, (10Z)-cyclonerotriol (8) [16], (10E)-cyclonerotriol (9) [16,17], catenioblin C (10) [18], and chokol E (11) [19], were isolated and identified (Figure 1). Herein, the isolation, structure elucidation, and bioactivity of these compounds are described in detail.

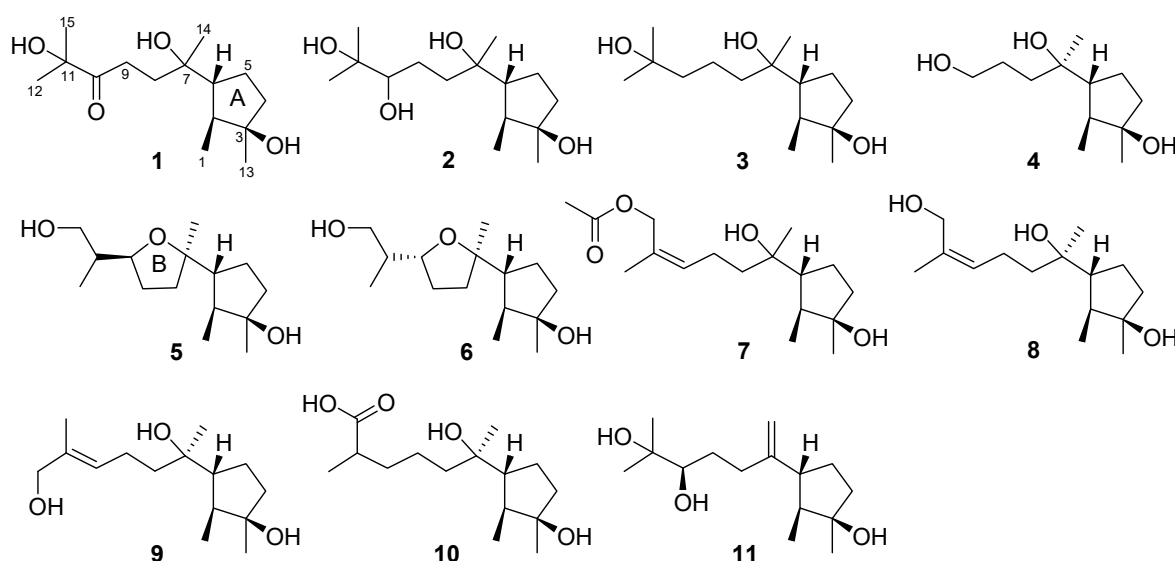


Figure 1. Structures of 1–11 (the stereochemistry in 1–8 and 10 only represents relative configuration).

## 2. Results and Discussion

Compound **1** was obtained as a colorless oil. The molecular formula  $C_{15}H_{28}O_4$  was determined by HREIMS  $m/z$  272.1991  $[M]^+$  (calculated for  $C_{15}H_{28}O_4$ , 272.1988), requiring two degrees of unsaturation. Its IR absorption bands at 3440 and  $1713\text{ cm}^{-1}$  suggested the presence of hydroxy and carbonyl groups. In combination with HSQC data, the  $^1\text{H}$  NMR spectrum (Table 1) showed one methyl doublet, four methyl singlets, and an array of signals at  $\delta_{\text{H}}$  1.5–2.6 for four methylenes and two methines. Aided by the  $90^\circ$  and  $135^\circ$  DEPT experiments, 15 resonances in the  $^{13}\text{C}$  NMR spectrum (Table 2) were assigned to five methyls, four methylene  $\text{sp}^3$ , two methine  $\text{sp}^3$ , three quaternary  $\text{sp}^3$  and one quaternary  $\text{sp}^2$  carbons. The  $^{13}\text{C}$  NMR spectrum of **1** exhibited the signals at  $\delta_{\text{C}}$  79.4 and 215.1, attributed to an oxygenated quaternary  $\text{sp}^3$  carbon and a ketone carbonyl group, respectively, instead of the signals for the vinyl group in cyclonerodiol [20]. HMBC correlations from  $\text{H}_3$ -12 ( $\delta_{\text{H}}$  1.30) to C-10 ( $\delta_{\text{C}}$  215.1), C-11 ( $\delta_{\text{C}}$  79.4), and C-15 ( $\delta_{\text{C}}$  28.0) and from  $\text{H}_3$ -15 ( $\delta_{\text{H}}$  1.29) to C-10, C-11, and C-12 ( $\delta_{\text{C}}$  27.8) established the connectivity between C-10 and C-11, which then extended to C-8 ( $\delta_{\text{C}}$  31.5) as corroborated by the HMBC correlation from  $\text{H}_2$ -9 ( $\delta_{\text{H}}$  2.51) to C-10 as well as by the COSY correlation from  $\text{H}_2$ -8 ( $\delta_{\text{H}}$  2.12 and 1.89) to  $\text{H}_2$ -9. The connectivity around ring A was deduced to be the same as those of cyclonerodiol based on their identical NMR data, and this was confirmed by the COSY correlations from  $\text{H}_3$ -1 ( $\delta_{\text{H}}$  1.03)/ $\text{H}_2$  ( $\delta_{\text{H}}$  1.56)/ $\text{H}_6$  ( $\delta_{\text{H}}$  1.97)/ $\text{H}_2$ -5 ( $\delta_{\text{H}}$  1.88 and 1.63)/ $\text{H}_2$ -4 ( $\delta_{\text{H}}$  1.67 and 1.56) and HMBC correlations from  $\text{H}_3$ -1 to C-2 ( $\delta_{\text{C}}$  44.9), C-3 ( $\delta_{\text{C}}$  81.4), and C-6 ( $\delta_{\text{C}}$  55.1), from  $\text{H}_3$ -13 ( $\delta_{\text{H}}$  1.25) to C-2, C-3, and C-4 ( $\delta_{\text{C}}$  40.4), and from  $\text{H}_3$ -14 ( $\delta_{\text{H}}$  1.19) to C-6, C-7 ( $\delta_{\text{C}}$  76.0), and C-8 ( $\delta_{\text{C}}$  31.5) (Figure 2). The NOESY correlation from  $\text{H}_3$ -1 to H-6 further verified that Me-1 was *syn* to H-6, while the NOESY correlations from  $\text{H}_3$ -1 to H-5a ( $\delta_{\text{H}}$  1.88) and from  $\text{H}_3$ -13 to H-5b ( $\delta_{\text{H}}$  1.63) suggested Me-1 to be opposite to Me-13. The above information evidenced **1** to be 3,7,11-trihydroxycycloneran-10-one.

Table 1.  $^1\text{H}$  NMR Data for 1–7 (500 MHz, in  $\text{CDCl}_3$ ,  $\delta$  in ppm,  $J$  in Hz).

Position	1	2	3	4	5	6	7
1 ( $\beta$ )	1.03, d (6.8)	1.03, d (6.8)	1.03, d (6.8)	1.05, d (6.8)	1.02, d (6.8)	1.02, d (6.8)	1.04, d (6.8)
2 ( $\alpha$ )	1.56, m	1.55, m	1.59, m	1.60, m	1.50, m	1.54, m	1.60, m
4a	1.67, m	1.69, m	1.67, m	1.69, m	1.68, m	1.68, m	1.68, m
4b	1.56, m	1.56, m	1.55, m	1.56, m	1.59, m	1.58, m	1.55, m
5a	1.88, m	1.88, m	1.85, m	1.88, m	1.89, m	1.89, m	1.85, m
5b	1.63, m	1.56, m	1.54, m	1.55, m	1.49, m	1.40, m	1.55, m

Table 1. Cont.

Position	1	2	3	4	5	6	7
6 ( $\beta$ )	1.97, m	1.89, m	1.85, m	1.87, m	1.95, m	2.00, m	1.84, m
8a	2.12, m	1.73, m	1.44, m	1.57, m	1.75, m	1.86, m	1.50, t (8.3)
8b	1.89, m	1.59, m			1.64, m	1.69, m	
9a	2.51, m	1.61, m	1.44, m	1.68, m	1.92, m	1.83, m	2.16, m
9b		1.40, m			1.71, m	1.79, m	
10		3.38, br d (10.3) <sup>a</sup>	1.45, m	3.68, m	4.07, ddd (7.6, 6.9, 4.1)	4.04, ddd (9.8, 5.6, 4.3)	5.41, t (7.3)
11					1.93, m	2.00, m	
12	1.30, s	1.16, s	1.22, s		0.93, d (7.0)	0.90, d (7.1)	1.74, br s
13 ( $\alpha$ )	1.25, s	1.25, s	1.25, s	1.26, s	1.24, s	1.25, s	1.25, s
14	1.19, s	1.15, s	1.16, s	1.17, s	1.14, s	1.17, s	1.16, s
15a	1.29, s	1.21, s	1.22, s		3.65, d (10.8, 6.1)	3.69, dd (10.8, 6.9)	4.62, d (11.9)
15b					3.61, d (10.8, 4.1)	3.58, dd (10.8, 3.7)	4.57, d (11.9)
CH <sub>3</sub> CO							2.06, s

<sup>a</sup> H-10 is coupled with H-9a and H-9b, but one of the couplings only results in broad resonance.

Table 2. <sup>13</sup>C NMR data for 1–7 (125 MHz, in CDCl<sub>3</sub>,  $\delta$  in ppm).

Position	$\delta_C$ , Type						
	1	2	3	4	5	6	7
1	14.4, CH <sub>3</sub>	14.5, CH <sub>3</sub>	14.7, CH <sub>3</sub>	14.6, CH <sub>3</sub>	14.0, CH <sub>3</sub>	13.8, CH <sub>3</sub>	14.7, CH <sub>3</sub>
2	44.9, CH	44.7, CH	44.4, CH	44.5, CH	45.4, CH	45.3, CH	44.4, CH
3	81.4, C	81.5, C	81.5, C	81.4, C	81.3, C	81.4, C	81.4, C
4	40.4, CH <sub>2</sub>	40.4, CH <sub>2</sub>	40.5, CH <sub>2</sub>	40.5, CH <sub>2</sub>	40.5, CH <sub>2</sub>	40.4, CH <sub>2</sub>	40.5, CH <sub>2</sub>
5	24.8, CH <sub>2</sub>	24.5, CH <sub>2</sub>	24.4, CH <sub>2</sub>	24.6, CH <sub>2</sub>	25.1, CH <sub>2</sub>	25.4, CH <sub>2</sub>	24.5, CH <sub>2</sub>
6	55.1, CH	54.5, CH	54.3, CH	54.9, CH	54.2, CH	54.1, CH	54.6, CH
7	76.0, C	75.1, C	75.0, C	74.8, C	85.7, C	86.0, C	74.8, C
8	31.5, CH <sub>2</sub>	37.0, CH <sub>2</sub>	41.1, CH <sub>2</sub>	36.8, CH <sub>2</sub>	35.7, CH <sub>2</sub>	34.8, CH <sub>2</sub>	40.5, CH <sub>2</sub>
9	32.8, CH <sub>2</sub>	25.8, CH <sub>2</sub>	18.7, CH <sub>2</sub>	27.2, CH <sub>2</sub>	28.1, CH <sub>2</sub>	27.4, CH <sub>2</sub>	22.5, CH <sub>2</sub>
10	215.1, C	79.0, CH	44.5, CH <sub>2</sub>	63.6, CH <sub>2</sub>	80.3, CH	84.2, CH	131.1, CH
11	79.4, C	73.4, C	71.2, C		38.2, CH	37.4, CH	130.0, C
12	27.8, CH <sub>3</sub>	23.5, CH <sub>3</sub>	29.4, CH <sub>3</sub>		11.8, CH <sub>3</sub>	12.0, CH <sub>3</sub>	21.6, CH <sub>3</sub>
13	26.3, CH <sub>3</sub>	26.2, CH <sub>3</sub>	26.2, CH <sub>3</sub>	26.2, CH <sub>3</sub>	26.2, CH <sub>3</sub>	26.2, CH <sub>3</sub>	26.2, CH <sub>3</sub>
14	23.5, CH <sub>3</sub>	25.0, CH <sub>3</sub>	25.2, CH <sub>3</sub>	25.2, CH <sub>3</sub>	23.1, CH <sub>3</sub>	26.4, CH <sub>3</sub>	25.0, CH <sub>3</sub>
15	28.0, CH <sub>3</sub>	26.7, CH <sub>3</sub>	29.5, CH <sub>3</sub>		66.9, CH <sub>2</sub>	66.7, CH <sub>2</sub>	63.3, CH <sub>2</sub>
CH <sub>3</sub> CO							171.3, C
CH <sub>3</sub> CO							21.1, CH <sub>3</sub>

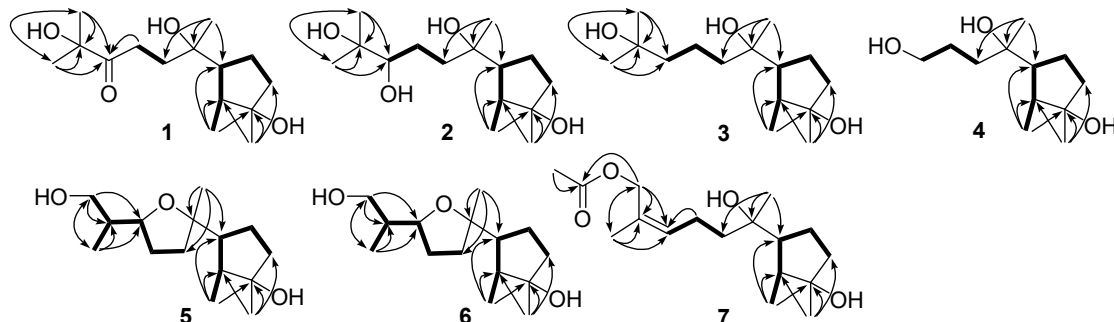


Figure 2. Key COSY (bold lines) and HMBC (arrows) correlations of 1–7.

Compound **2** was isolated as a colorless oil, and its molecular formula was established as  $C_{15}H_{30}O_4$ , which has two more hydrogen atoms than that of **1**, based on the (+)-HREIMS  $m/z$  297.2037  $[M + Na]^+$  (calculated for  $C_{15}H_{30}O_4Na$ , 297.2042). A detailed comparison of its NMR signals (Tables 1 and 2) with those of **1** revealed the presence of ring A and its affiliated groups in **2**. In addition, this compound exhibited a deshielded broad doublet ( $J = 10.3$  Hz) at  $\delta_H$  3.38 in the  $^1H$  NMR spectrum. Combined with the HSQC correlation of this proton signal to the carbon signal at  $\delta_C$  79.0, this signal was attributed to the oxymethine proton on C-10. This was supported by the HMBC correlations from oxymethine  $sp^3$  carbon at  $\delta_C$  79.0 (C-10) to H<sub>3</sub>-12 ( $\delta_H$  1.16) and H<sub>3</sub>-15 ( $\delta_H$  1.21) as well as a COSY correlation from H-10 to H<sub>2</sub>-9 ( $\delta_H$  1.61 and 1.40) (Figure 2). Thus, **2** was deduced to be cycloneran-3,7,10,11-tetraol. Previously, cyclonerodiol B from the mangrove-derived endophytic fungus *Trichoderma* sp. Xy24 was assigned the same structure as **2**, but it should be revised to epicyclonerodiol oxide in view of their identical NMR data [9,13]. To confirm the absolute configuration at C-10, Mosher's reactions were performed under the conditions described previously [21]. Unfortunately, no esterified products were obtained after the purification via preparative thin-layer chromatography (TLC).

Compound **3** was purified as a colorless oil, and its HREIMS  $m/z$  258.2198  $[M]^+$  (calculated for  $C_{15}H_{30}O_3$ , 258.2195) produced the molecular formula  $C_{15}H_{30}O_3$ , which has one oxygen atom less than that of **2**. Its  $^{13}C$  NMR spectrum (Table 2) showed a close similarity to that of **2**, except for the lack of the signal for an oxymethine group and the presence of the signal for a methylene group of C-10 ( $\delta_C$  44.5), which was supported by its HMBC correlations to H<sub>3</sub>-12 ( $\delta_H$  1.22) and/or H<sub>3</sub>-15 ( $\delta_H$  1.22). Other HMBC and COSY correlations, as shown in Figure 2, further confirmed **3** to be cycloneran-3,7,11-triol, a 10-deoxy derivative of **2**.

Compound **4** was also isolated as a colorless oil. A molecular formula  $C_{12}H_{24}O_3$  was assigned by HREIMS  $m/z$  216.1724  $[M]^+$  (calculated for  $C_{12}H_{24}O_3$ , 216.1725), implying one degree of unsaturation. Its  $^1H$  and  $^{13}C$  NMR data (Tables 1 and 2) resembled those of **2**, except for the lack of signals for a 2-hydroxy-2-propyl group ( $\delta_H$  1.16 and 1.21,  $\delta_C$  23.5, 26.7, and 73.4) and the presence of signals for a hydroxymethylene group ( $\delta_H$  3.68 and  $\delta_C$  63.6). This group was deduced to be situated at the side chain terminus on the basis of HMBC correlations from H<sub>3</sub>-14 ( $\delta_H$  1.17) to C-6 ( $\delta_C$  54.9), C-7 ( $\delta_C$  74.8), and C-8 ( $\delta_C$  36.8) and COSY correlations from H<sub>2</sub>-10 ( $\delta_H$  3.68) to H<sub>2</sub>-9 ( $\delta_H$  1.68) and from H<sub>2</sub>-9 to H<sub>2</sub>-8 ( $\delta_H$  1.57). HMBC correlations (Figure 2) from H<sub>3</sub>-1 ( $\delta_H$  1.05) to C-2 ( $\delta_C$  44.5), C-3 ( $\delta_C$  81.4), and C-6, and from H<sub>3</sub>-13 ( $\delta_H$  1.26) to C-2, C-3, and C-4 ( $\delta_C$  40.5), and COSY correlations from H<sub>3</sub>-1 to H<sub>2</sub>-4 ( $\delta_H$  1.69 and 1.56) via H-2 ( $\delta_H$  1.60), H-6 ( $\delta_H$  1.87), and H<sub>2</sub>-5 ( $\delta_H$  1.88 and 1.55), further verified the connectivity of ring A. Thus, **4** was proposed to be 11,12,15-trinorcycloneran-3,7,10-triol, possibly a degradation product of **1** or **2**. To ascertain the relative configuration at C-7, the energy-minimized conformers, regardless of the rotation of the methyl and hydroxy groups as well as the side chain, of 7 $\alpha$ - and 7 $\beta$ -methyl isomers optimized at the B3LYP/6-31G(d) level in chloroform, were subjected to the  $^{13}C$  NMR calculations using the gauge-independent atomic orbital (GIAO) method at the B3LYP/6-31+G(d,p) level via Gaussian 09 software [22]. Both experimental and calculated shifts were input into Sarotti's DP4+ sheet (see <https://sarotti-nmr.weebly.com>) [23], and the calculated data for 7 $\alpha$ - and 7 $\beta$ -methyl isomers displayed 73.74% and 26.26% DP4+ probabilities, respectively. Thus, the methyl group at C-7 was tentatively assigned as  $\alpha$  orientation.

Compound **5** was obtained as a colorless oil, and the HREIMS spectrum afforded the molecular formula  $C_{15}H_{28}O_3$ , suggesting two degrees of unsaturation. However, examination of the  $^1H$  and  $^{13}C$  NMR data (Tables 1 and 2) indicated the absence of any unsaturated bond. Thus, this compound was suggested to possess a bicyclic skeleton. As the NMR data of **5** resembled those of 7,10-epoxycycloneran-3,11,12-triol, the presence of a 7,10-epoxycyclonerane framework was proposed [5]. However, the signal for a methine group appeared in the  $^{13}C$  NMR spectrum of **5** instead of that for an oxygenated quaternary carbon in the  $^{13}C$  NMR spectrum of 7,10-epoxycycloneran-3,11,12-triol. The fact that the methine group was located at C-11 ( $\delta_C$  38.2) of **5** was corroborated by its HMBC correlations with H<sub>3</sub>-12 ( $\delta_H$  0.93) and H<sub>2</sub>-15 ( $\delta_H$  3.65 and 3.61). HMBC correlations (Figure 2) from H<sub>3</sub>-12 and H<sub>2</sub>-15 to C-10 ( $\delta_C$  80.3), from H<sub>3</sub>-1 ( $\delta_H$  1.02) to C-2 ( $\delta_C$  45.4), C-3

( $\delta_C$  81.3), and C-6 ( $\delta_C$  54.2), from H<sub>3</sub>-13 ( $\delta_H$  1.24) to C-2, C-3, and C-4 ( $\delta_C$  40.5), and from H<sub>3</sub>-14 ( $\delta_H$  1.14) to C-6, C-7 ( $\delta_C$  85.7), and C-8 ( $\delta_C$  35.7) further confirmed the structure of **5** as 11-deoxy derivative of 7,10-epoxycycloneran-3,11,12-triol. Additionally, H-10 ( $\delta_H$  4.07) and Me-14 ( $\delta_H$  1.14) were on the same face of ring B by their NOESY correlation (Figure 3). The NOESY correlations from H<sub>3</sub>-1 to H-5a ( $\delta_H$  1.89) and H-6 ( $\delta_H$  1.95) and from H<sub>3</sub>-13 to H-5b ( $\delta_H$  1.49) validated the relative configurations around ring A. However, the relative configuration at C-11 remains unsolved.

Compound **6** was isolated as a colorless oil and has the same molecular formula as that of **5** by HREIMS  $m/z$  256.2028 [M]<sup>+</sup> (calcd for C<sub>15</sub>H<sub>28</sub>O<sub>3</sub>, 256.2038). Its <sup>1</sup>H and <sup>13</sup>C NMR data (Tables 1 and 2) also resembled those of **5**, except for the deshielded signal for C-10 ( $\delta_C$  84.2). Thus, **6** was deduced to be a C-10 epimer of **5**, as supported by the NOESY correlation from H<sub>3</sub>-12 ( $\delta_H$  0.90) to H<sub>3</sub>-14 ( $\delta_H$  1.17). The similar chemical shifts of C-7 ( $\delta_C$  86.0) and C-10 ( $\delta_C$  84.2) to those of epicyclonerodiol oxide further evidenced the opposite orientation of H-10 and C-14 [9]. The relative configurations around ring A and at C-7 were deduced to be the same as those of **5** based on their identical <sup>1</sup>H and <sup>13</sup>C NMR data. Moreover, because both C-6 and C-7 are chiral centers, the rotation of the C-6–C-7 single bond is restricted to some extent. Thus, the relative configuration at C-7 could be locked by the NOESY correlations from H<sub>3</sub>-14 to H-2 ( $\delta_H$  1.54) and H-5b ( $\delta_H$  1.40) and from H-8a ( $\delta_H$  1.86) to H<sub>3</sub>-1 ( $\delta_H$  1.02) and H-2 (Figure 3). As is the case for **5**, the relative configuration at C-11 ( $\delta_C$  37.4) is not determined.

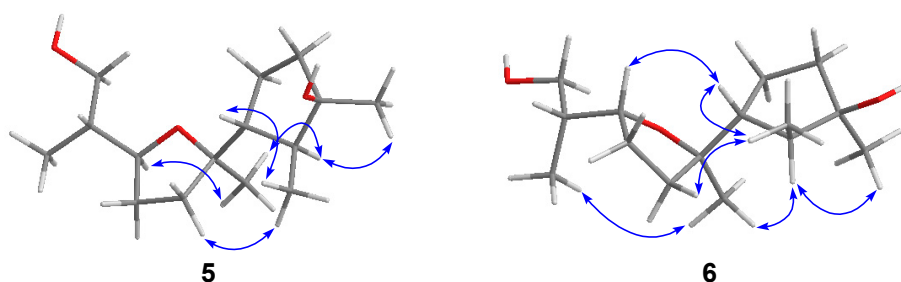


Figure 3. Key NOESY correlations of **5** and **6**.

Compound **7** was obtained as a colorless oil. Its molecular formula was determined to be C<sub>17</sub>H<sub>30</sub>O<sub>4</sub> by HREIMS ( $m/z$  298.2138), corresponding to three degrees of unsaturation. The carbonyl signal at  $\delta_C$  171.3 and the methyl signals at  $\delta_H$  2.06 and  $\delta_C$  21.1 as well as their HMBC correlation indicated the presence of an acetyl group. The remaining NMR data (Tables 1 and 2) showed a close similarity to those of (10Z)-cyclonerotriol (**8**) [16]. Thus, **7** was speculated to be an acetylated derivative of **8**, and the acetoxy group was attached to C-15, as evidenced from the HMBC correlation from H<sub>2</sub>-15 ( $\delta_H$  4.62 and 4.57) to the carbonyl carbon ( $\delta_C$  171.3). Other HMBC and COSY correlations (Figure 2) further validated the structure of **7**, and the Z geometry was corroborated by the NOESY correlations between H<sub>2</sub>-15 and H<sub>2</sub>-9 ( $\delta_H$  2.16) and between H<sub>3</sub>-12 ( $\delta_H$  1.74) and H-10 ( $\delta_H$  5.41).

To develop new inhibitors against harmful microalgae that seriously threaten marine aquaculture, **1–11** were evaluated for their growth inhibition against four marine phytoplankton species (*Chattonella marina*, *Heterosigma akashiwo*, *Karlodinium veneficum*, and *Prorocentrum donghaiense*) [24]. The results (Table 3) show that **10**, with IC<sub>50</sub> values ranging from 1.6 to 2.0  $\mu\text{g/mL}$ , is the most potent to inhibit the four phytoplankton species tested. An analysis of the structure–activity relationship suggested that the carboxyl group at C-15 may greatly contribute to the inhibitory ability of cyclonerane sesquiterpenes. Moreover, the toxicity of **10** to the marine zooplankton *Artemia salina* was also assayed [7], but it does not exhibit any lethal effect at the concentration of 100  $\mu\text{g/mL}$ .

**Table 3.** Inhibition against four marine phytoplankton species by 1–11.

Compound	IC <sub>50</sub> (µg/mL)			
	<i>Chattonella marina</i>	<i>Heterosigma akashiwo</i>	<i>Karlodinium veneficum</i>	<i>Prorocentrum donghaiense</i>
1	5.2	8.0	10	9.9
2	8.8	21	76	6.5
3	61	73	71	40
4	13	73	6.3	34
5	2.4	26	3.9	20
6	5.8	37	5.5	15
7	59	14	35	7.3
8	42	50	12	3.4
9	15	53	43	1.1
10	1.6	1.8	1.6	2.0
11	62	9.4	13	6.0

### 3. Materials and Methods

#### 3.1. General Experimental Procedures

Optical rotations and ECD curves were determined on a Chirascan CD spectrometer (Applied Photophysics Ltd., Surrey, UK). IR spectra were obtained on a Nicolet iS10 FT-IR spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). NMR spectra were recorded on a Bruker Avance III 500 NMR spectrometer (Bruker Corp., Billerica, MA, USA). Low- and high-resolution EI and ESI<sup>+</sup> mass spectra were measured on an Autospec Premier P776 mass spectrometer (Waters Corp., Milford, MA, USA) and an Agilent G6230 TOF mass spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA), respectively. 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). Quantum chemical calculations were run with Gaussian 09 software (Gaussian, Inc., Wallingford, CT, USA).

#### 3.2. Fungal Material and Fermentation

The fungal strain *Trichoderma asperellum* A-YMD-9-2 (Moniliaceae) was obtained from the inner tissue of the marine red alga *Gracilaria verrucosa* collected from Yangma Island, Yantai, China in August 2016. The fungus was identified by morphological observation and by analysis of the ITS regions of its rDNA, whose sequence data were deposited at GenBank with the accession number MH819724. The fermentation was performed statically at room temperature for 40 days in 200 × 1 L Erlenmeyer flasks, each containing 50 g rice, 0.6 g peptone, 50 mL pure water, and 50 mL natural seawater from the coast of Yantai, China.

#### 3.3. Extraction and Isolation

The mycelia and broth were separated by filtration at the end of the fermentation, and the former were dried in the shade and exhaustively extracted with CH<sub>2</sub>Cl<sub>2</sub> and MeOH (1:1, v/v). After removing organic solvents under a vacuum, the residue was partitioned between EtOAc and H<sub>2</sub>O to afford an EtOAc-soluble extract (202.1 g). The broth was directly extracted with EtOAc and then concentrated to give an extract (10.3 g). Based on the similar TLC profiles, these two parts were combined and subjected to silica gel CC with step-gradient solvent systems consisting of petroleum ether (PE)/EtOAc (50:1 to 0:1) and then CH<sub>2</sub>Cl<sub>2</sub>/MeOH (10:1 to 0:1) to give eight fractions (Frs. 1–8). Fr. 3 eluted with PE/EtOAc (2:1) and was further purified by CC on RP-18 (MeOH/H<sub>2</sub>O, 3:2) and silica gel (PE/EtOAc, 6:1) to obtain 1 (11.3 mg), 5 (5.2 mg), and 6 (5.9 mg). Fr. 4 eluted with PE/EtOAc (1:1) and was further purified by CC on RP-18 (MeOH/H<sub>2</sub>O, 1:1 to 3:2) and Sephadex LH-20 (MeOH) and preparative TLC

(CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 15:1) to yield **7** (8.1 mg), **8** (33.8 mg), **10** (10.1 mg), and **11** (3.6 mg). Fr. 5 eluted with EtOAc and was further purified by CC on RP-18 (MeOH/H<sub>2</sub>O, 2:3 to 3:2) and Sephadex LH-20 (MeOH) and preparative TLC (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 10:1) to give **2** (26.0 mg), **4** (1.7 mg), and **9** (10.0 mg). Fr. 6 eluted with CH<sub>2</sub>Cl<sub>2</sub>/MeOH (10:1) and was further purified by CC on RP-18 (MeOH/H<sub>2</sub>O, 1:1) and Sephadex LH-20 (MeOH) and preparative TLC (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 7:1) to produce **3** (12.0 mg).

3,7,11-Trihydroxycycloneran-10-one (**1**): Colorless oil; [ $\alpha$ ]<sub>D</sub><sup>20</sup> − 24 (c 0.44, MeOH); IR (KBr)  $\nu_{\max}$  3440, 2960, 2932, 1713, 1460, 1663, 1374, 1239, 1125, 1013, 921 cm<sup>−1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data, Tables 1 and 2 (Figures S1 and S2); 2D NMR data, Figures S3–S6; EIMS  $m/z$  (%) 272 [M]<sup>+</sup> (<1), 196 (12), 178 (45), 141 (95), 136 (44), 121 (39), 95 (39), 71 (40), 56 (40), 44(100); HREIMS  $m/z$  272.1991 [M]<sup>+</sup> (calcd for C<sub>15</sub>H<sub>28</sub>O<sub>4</sub>, 272.1988) (Figure S7).

Cycloneran-3,7,10,11-tetraol (**2**): Colorless oil; [ $\alpha$ ]<sub>D</sub><sup>20</sup> − 30 (c 1.0, MeOH); IR (KBr)  $\nu_{\max}$  3416, 2967, 1654, 1460, 1378, 1266, 1162, 1074, 1032, 921, 885 cm<sup>−1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data, Tables 1 and 2 (Figures S8 and S9); 2D NMR data, Figures S10–S13; ESI<sup>+</sup>MS  $m/z$  297 [M + Na]<sup>+</sup>; HRESI<sup>+</sup>MS  $m/z$  297.2037 [M + Na]<sup>+</sup> (calcd for C<sub>15</sub>H<sub>30</sub>O<sub>4</sub>Na, 297.2042) (Figure S14).

Cycloneran-3,7,11-triol (**3**): Colorless oil; [ $\alpha$ ]<sub>D</sub><sup>20</sup> − 18 (c 0.62, MeOH); IR (KBr)  $\nu_{\max}$  3406, 2965, 1654, 1460, 1377, 1164, 920, 885 cm<sup>−1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data, Tables 1 and 2 (Figures S15 and S16); 2D NMR data, Figures S17–S20; EIMS  $m/z$  (%) 258 [M]<sup>+</sup> (<1), 207 (25), 157 (28), 139 (81), 127 (70), 109 (81), 95 (38), 81(45), 44(100); HREIMS  $m/z$  258.2198 [M]<sup>+</sup> (calcd for C<sub>15</sub>H<sub>30</sub>O<sub>3</sub>, 258.2195) (Figure S21).

11,12,15-Trinorcycloneran-3,7,10-triol (**4**): Colorless oil; [ $\alpha$ ]<sub>D</sub><sup>20</sup> − 39 (c 0.062, MeOH); IR (KBr)  $\nu_{\max}$  3426, 2928, 1720, 1635, 1460, 1383, 1054, 920 cm<sup>−1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data, Tables 1 and 2 (Figures S22 and S23); 2D NMR data, Figures S24–S27; EIMS  $m/z$  (%) 216 [M]<sup>+</sup> (<1), 165 (11), 157 (11), 139 (79), 103 (31), 96 (40), 85 (98), 81(58), 44(100); HREIMS  $m/z$  216.1724 [M]<sup>+</sup> (calcd for C<sub>12</sub>H<sub>24</sub>O<sub>3</sub>, 216.1725) (Figure S28).

7,10S-epoxycycloneran-3,15-diol (**5**): Colorless oil; [ $\alpha$ ]<sub>D</sub><sup>20</sup> +11 (c 0.20, MeOH); IR (KBr)  $\nu_{\max}$  3426, 2965, 2931, 2876, 1654, 1460, 1376, 1205, 1092, 1024, 919 cm<sup>−1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data, Tables 1 and 2 (Figures S29 and S30); 2D NMR data, Figures S31–S34; EIMS  $m/z$  (%) 256 [M]<sup>+</sup> (<1), 183 (20), 143 (100), 139 (32), 125 (35), 113 (30), 59 (42), 44(100); HREIMS  $m/z$  256.2034 [M]<sup>+</sup> (calcd for C<sub>15</sub>H<sub>28</sub>O<sub>3</sub>, 256.2038) (Figure S35).

7,10R-epoxycycloneran-3,15-diol (**6**): Colorless oil; [ $\alpha$ ]<sub>D</sub><sup>20</sup> +6.5 (c 0.31, MeOH); IR (KBr)  $\nu_{\max}$  3426, 2965, 2931, 2868, 1635, 1460, 1376, 1206, 1152, 1093, 1024, 919 cm<sup>−1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data, Tables 1 and 2 (Figures S36 and S37); 2D NMR data, Figures S38–S41; EIMS  $m/z$  (%) 256 [M]<sup>+</sup> (<1), 237 (8), 179 (10), 143 (90), 125 (32), 95 (28), 56 (41), 44(100); HREIMS  $m/z$  256.2028 [M]<sup>+</sup> (calcd for C<sub>15</sub>H<sub>28</sub>O<sub>3</sub>, 256.2038) (Figure S42).

(10Z)-15-Acetoxy-10-cycloneran-3,7-diol (**7**): Colorless oil; [ $\alpha$ ]<sub>D</sub><sup>20</sup> − 11 (c 0.31, MeOH); IR (KBr)  $\nu_{\max}$  3452, 2963, 2932, 1735, 1654, 1460, 1370, 1240, 1025, 920, 885 cm<sup>−1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data, Tables 1 and 2 (Figures S43 and S44); 2D NMR data, Figures S45–S48; EIMS  $m/z$  (%) 256 [M]<sup>+</sup> (<1), 139 (33), 125 (98), 107 (28), 81 (27), 44(100); HREIMS  $m/z$  298.2138 [M]<sup>+</sup> (calcd for C<sub>17</sub>H<sub>30</sub>O<sub>4</sub>, 298.2144) (Figure S49).

#### 4. Conclusions

Chemical examination of the marine-alga-endophytic fungus *Trichoderma asperellum* A-YMD-9-2 led to the isolation and identification of seven new (**1–7**) and four known (**8–11**) cyclonerane derivatives, including an unprecedented trinorcyclonerane (**4**). The discovery of these new isolates greatly diversifies the unusual cyclonerane sesquiterpenes. Among the isolates, **10** displays significant inhibition of the four phytoplankton species and features no toxicity to any of the zooplankton tested.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/1660-3397/17/5/252/s1>, Figures S1–S49: 1D/2D NMR and HRMS spectra of compounds **1–7**.

**Author Contributions:** Conceptualization, N.-Y.J.; methodology, Y.-P.S., F.-P.M., and X.-L.Y.; software, Y.-P.S. and X.-L.Y.; validation, N.-Y.J.; formal analysis, Y.-P.S. and N.-Y.J.; investigation, Y.-P.S. and X.-H.L.; resources, X.-H.L.;

data curation, Y.-P.S. and N.-Y.J.; writing—original draft preparation, Y.-P.S.; writing—review and editing, N.-Y.J.; visualization, N.-Y.J.; supervision, N.-Y.J.; project administration, N.-Y.J. and F.-P.M.; funding acquisition, N.-Y.J. and F.-P.M.

**Funding:** This research was funded by the National Natural Science Foundation of China, grant number 31670355; the Natural Science Foundation for Distinguished Young Scholars of Shandong Province, grant number JQ201712; Key Cutting-Edge Research Program of the CAS, grant number QYZDB-SSW-DQC013; the Science and Technology Service Network Initiative of the CAS, grant number KFJ-STS-ZDTP-023; and the Youth Innovation Promotion Association of the CAS, grant number 2013138.

**Conflicts of Interest:** The authors declare no conflict of interest.

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