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Bisabolane, cyclonerane, and harziane derivatives from the marine-alga-endophytic fungus *Trichoderma asperellum* cf44-2

Yin-Ping Song [a, b], Xiang-Hong Liu [a], Zhen-Zhen Shi [a, b], Feng-Ping Miao [a], Sheng-Tao Fang [a], Nai-Yun Ji [a, *]

[a] Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China

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ABSTRACT

Three undescribed bisabolane derivatives, trichaspin, trichaspsides A and B, three undescribed cyclonerane sesquiterpenes, 9-cycloneren-3,7,11-triol, 11-cycloneren-3,7,10-triol, and 7,10-epoxycycloneran-3,11,12-triol, and one undescribed harziane diterpene, 11-hydroxy-9-harzien-3-one, were obtained from the culture of *Trichoderma asperellum* cf44-2, an endophyte of the marine brown alga *Sargassum* sp. Their structures and relative configurations were assigned by analysis of 1D/2D NMR and MS data, and their absolute configurations were established by ECD or specific optical rotation data. Trichaspin features an unprecedented ethylated bisabolane skeleton, while trichaspsides A and B represent the first amino-glycosides of bisabolane and norbisabolane sesquiterpenes, respectively. Nine of the compounds were evaluated for inhibition of five marine-derived pathogenic bacteria and toxicity to a marine zooplankton.

1. Introduction

Among the multifarious filamentous fungi, *Trichoderma* Pers. (Moniliaceae) species have been regarded as the most potential biocontrol agents in agriculture, and hundreds of specialised metabolites with various bioactivities, such as antifungal, antibacterial, weedicidal, and cytotoxic properties, have been discovered from them so far (Reino et al., 2008; Keswani et al., 2014). Although *Trichoderma* is commonly considered as a terrestrial genus, halotolerant strains have been continuously reported from marine sediments, invertebrates, and algae (Zhu et al., 2015). Moreover, marine-derived *Trichoderma* strains have already contributed more than 60 undescribed compounds, involving terpenes, polyketides, alkaloids, and peptides (Zhu et al., 2015; Blunt et al., 2017). Of those, only several (less than ten) were obtained from the marine algicolous strains of *Trichoderma* (Ji and Wang, 2016; Miao et al., 2012; Liang et al., 2016a, 2016b; Yamazaki et al., 2015, 2016), but they exhibited the high novelty due to cyclization and substitution and then encouraged our further investigation towards them. As a result, three undescribed bisabolane derivatives, trichaspin (**1**), trichaspsides A (**2**) and B (**3**), three undescribed cyclonerane sesquiterpenes, 9-cycloneren-3,7,11-triol (**6**), 11-cycloneren-3,7,10-triol (**7**), and 7,10-epoxycycloneran-3,11,12-triol (**8**), and one undescribed harziane diterpene, 11-hydroxy-9-harzien-3-one (**9**), together with the known (3*S*,6R,7*S*)-zingiberenol (**4**) (Terhune et al., 1975; Khrimian et al., 2014), cyclonerodiol (**5**) (Laurent et al., 1990; Langhanki et al., 2014), and harziandione (**10**) (Miao et al., 2012; Adelin et al., 2014) were isolated and identified from *Trichoderma asperellum* Samuels, Lieckfeldt & Nirenberg cf44-2 (Fig. 1), an endophyte of the marine brown alga *Sargassum* sp. (Sargassaceae). Herein, the isolation, structure elucidation, and bioactivity of these compounds are described in detail.

2. Results and discussion

Compound **1** was obtained as a white powder, and its molecular ion peak appeared at *m/z* 294 in the EI mass spectrum. A molecular formula of $C_{17}H_{26}O_4$ was determined by HREIMS (*m/z* 294.1838 $[M]^+$), requiring five degrees of unsaturation. The ^1H NMR spectrum (in $CDCl_3$, Table 1) alongside HSQC data displayed one methyl doublet, two methyl singlets, four double doublets assignable to two methylenes, one broad doublet due to a hydroxy proton, one

* Corresponding author.
E-mail address: nyji@yic.ac.cn (N.-Y. Ji).

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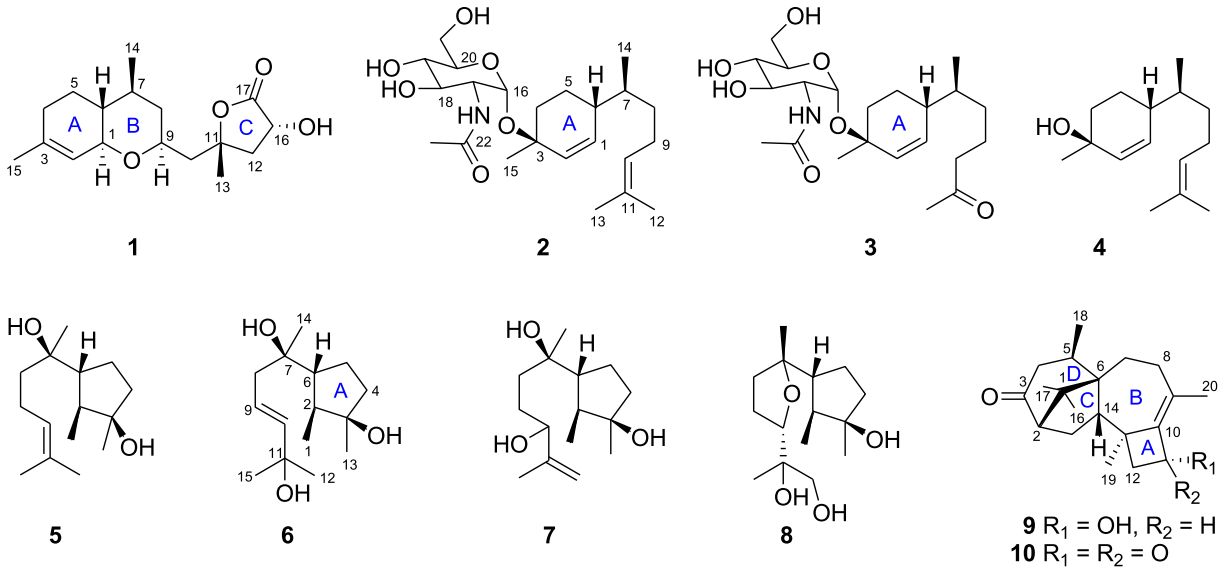

Fig. 1. Chemical structures of compounds **1**–**10**.

Table 1
^{1}H NMR Data for **1**–**3** (500 MHz, δ_H in ppm, *J* in Hz).

pos	**1**		**2**		**3**	
	in $CDCl_3$	in acetone-d_6	in $CDCl_3$	in CD_3OD	in $CDCl_3$	in CD_3OD
1	3.60, br d (9.1)	3.56, br d (9.1)	5.63, br d (10.9)	5.63, br d (10.3)	5.62, br d (10.7)	5.64, br d (10.3)
2	5.32, br s	5.31, br s	5.41, br d (10.2)	5.44, br d (10.4)	5.41, br d (10.3)	5.45, br d (10.3)
4a	2.03, m	2.03, m	1.95, m	2.08, td (13.4, 3.1)	1.95, td (13.0, 2.5)	2.08, td (13.2, 3.2)
4b	1.94, m	1.95, m	1.72, br d (12.7)	1.76, br d (13.0)	1.74, br d (12.8)	1.76, br d (13.0)
5a	1.94, m	1.97, m	1.63, m	1.67, m	1.64, m	1.63,
5b	1.13, m	1.13, dddd (12.6, 12.6, 10.5, 5.8)	1.33, m	1.39, m	1.33, m	1.38, m
6	0.91, m	0.86, dddd (12.9, 10.4, 9.1, 2.7)	2.11, m	2.13, m	2.11, m	2.13, m
7	1.39, m	1.40, m	1.48, m	1.50, m	1.47, m	1.50, m
8a	1.59, ddd (13.2, 3.8, 2.3)	1.63, ddd (13.1, 3.9, 2.3)	1.32, m	1.37, m	1.29, m	1.33, m
8b	1.13, m	1.06, ddd (13.0, 11.5, 11.5)	1.14, dtd (12.8, 9.1, 5.7)	1.17, dtd (13.3, 8.8, 5.9)	1.11, dddd (12.8, 10.4, 8.5, 5.0)	1.14, dddd (13.3, 10.5, 8.4, 5.2)
9a	3.68, ddt (11.3, 9.0, 2.2)	3.64, ddt (11.1, 8.6, 2.4)	1.99, m	2.01, m	1.61, m	1.60, m
9b			1.91, m	1.97, m	1.51, m	1.51, m
10a	1.97, dd (14.9, 9.1)	1.88, dd (14.6, 8.6)	5.08, br t (7.1)	5.10, br t (7.2)	2.41, t (7.4)	2.47, t (7.2)
10b	1.83, dd (15.0, 2.1)	1.78, dd (14.6, 2.5)				
12a	2.58, dd (13.0, 9.9)	2.42, d (9.4)	1.60, s	1.60, s		
12b	2.43, dd (13.0, 8.8)	2.42, d (9.4)				
13	1.44, s	1.41, s	1.68, s	1.68, s	2.13, s	2.13, s
14	0.92, d (6.5)	0.92, d (6.5)	0.80, d (6.8)	0.83, d (6.8)	0.80, d (6.8)	0.83, d (6.8)
15	1.65, br s	1.63, br s	1.27, s	1.29, s	1.27, s	1.29, s
16	4.61, td (9.7, 2.6)	4.64, br t (9.4)	5.06, d (3.7)	5.09, d (3.6)	5.06, d (3.6)	5.09, d (3.6)
17			4.00, td (9.8, 3.5)	3.79, dd (10.9, 3.6)	3.99, td (9.8, 3.5)	3.79, dd (10.8, 3.6)
18			3.72, t (10.2)	3.68, dd (10.8, 8.7)	3.70, t (9.7)	3.67, dd (10.8, 8.7)
19			3.64, t (9.1)	3.35, t (9.1)	3.62, t (9.1)	3.35, t (9.2)
20			3.77, dt (9.9, 3.0)	3.76, ddd (9.7, 5.2, 2.5)	3.77, dt (9.8, 3.0)	3.76, ddd (9.7, 5.5, 2.5)
21a			3.89, dd (11.5, 2.8)	3.76, dd (12.5, 2.4)	3.87, br d (10.5)	3.76, dd (12.6, 2.5)
21b			3.73 dd (11.5, 3.3)	3.69, dd (12.1, 5.9)	3.75, br d (10.9)	3.69, dd (12.4, 5.7)
23			2.04, s	1.99, s	2.04, s	1.99, s
OH	2.67, br d (3.0)	4.82, br d (4.7)				
NH			6.32, br d (8.4)		6.18, br d (8.8)	

broad doublet/a batch of triplets of double doublet/one double triplet ascribable to three oxygenated methines, and one broad singlet attributable to an olefinic proton. The ^{13}C NMR spectrum (Table 2) exhibited 17 resonances, sorted into three methyls, five methylenes, six methines, and three nonprotonated carbons by DEPT experiments. COSY correlations of H-12/H-16/OH-16 indicated the presence of a 1,2-disubstituted ethanol unit, which was flanked by C-11 and C-17 on the basis of HMBC correlations from H-12 to C-11 and C-17 and from H-13 to C-11 and C-12. Furthermore, C-10 was attached to C-11 by HMBC correlations from H-10 to C-11 and from H-13 to C-10, which was then extended to C-2, C-4, and C-14 by analysis of COSY correlations (Fig. 2). The connectivity at C-3 was established by HMBC correlations from H-15 to C-2, C-3, and C-4, and an ether linkage between C-1 and C-9 was suggested by comparison of NMR data with those reported for (2*S*,4R,6*S*)-6-methyl-2,4-diphenyltetrahydropyran (Fries et al., 2014). C-11 and

Table 2
^{13}C NMR Data for **1**–**3** (125 MHz, δ_C in ppm).

pos	**1**		**2**		**3**	
	in $CDCl_3$	in acetone-d_6	in $CDCl_3$	in CD_3OD	in $CDCl_3$	in CD_3OD
1	79.1, CH	79.6, CH	135.2, CH	135.7, CH	135.1, CH	135.6, CH
2	123.4, CH	125.3, CH	132.1, CH	133.8, CH	132.3, CH	133.9, CH
3	137.2, C	135.9, C	77.6, C	78.2, C	77.5, C	78.2, C
4	30.9, CH_2	31.3, CH_2	34.8, CH_2	35.8, CH_2	34.8, CH_2	35.8, CH_2
5	23.6, CH_2	24.3, CH_2	22.4, CH_2	23.4, CH_2	22.3, CH_2	23.4, CH_2
6	45.4, CH	46.2, CH	40.3, CH	41.5, CH	40.1, CH	41.4, CH
7	34.5, CH	35.2, CH	36.4, CH	37.5, CH	36.7, CH	37.9, CH
8	42.3, CH_2	43.1, CH_2	34.2, CH_2	35.3, CH_2	33.6, CH_2	34.6, CH_2
9	73.3, CH	74.1, CH	26.1, CH_2	27.0, CH_2	21.9, CH_2	22.8, CH_2
10	46.8, CH_2	47.9, CH_2	124.7, CH	125.7, CH	44.0, CH_2	44.4, CH_2
11	83.7, C	82.6, C	131.5, C	132.2, C	209.2, C	212.0, C
12	40.3, CH_2	41.4, CH_2	17.8, CH_3	17.7, CH_3		
13	28.4, CH_3	27.9, CH_3	25.9, CH_3	25.9, CH_3	30.1, CH_3	29.8, CH_3
14	18.8, CH_3	19.0, CH_3	15.8, CH_3	16.0, CH_3	15.8, CH_3	16.0, CH_3
15	23.0, CH_3	23.0, CH_3	27.3, CH_3	27.5, CH_3	27.3, CH_3	27.5, CH_3
16	68.8, CH	68.9, CH	92.9, CH	93.6, CH	92.8, CH	93.6, CH
17	177.1, C	176.9, C	54.0, CH	55.9, CH	54.0, CH	55.9, CH
18			73.1, CH	72.5, CH	73.3, CH	72.5, CH
19			70.8, CH	72.5, CH	71.1, CH	72.5, CH
20			71.4, CH	73.4, CH	71.3, CH	73.4, CH
21			61.8, CH_2	62.7, CH_2	62.0, CH_2	62.7, CH_2
22			171.9, C	173.5, C	171.8, C	173.5, C
23			23.5, CH_3	22.6, CH_3	23.5, CH_3	22.6, CH_3

C-17 were linked through an oxygen atom to form a γ-lactone ring to satisfy the unsaturation requirement, which was also supported by the deshielded signals (δ_C 83.7 in $CDCl_3$ and 82.6 in acetone-d_6) of C-11 (Zhang et al., 2014). Other HMBC correlations (Fig. 2) further verified the planar structure of **1**.

The relative configuration of **1** was established by analysis of coupling constants and NOE correlations. H-1 and H-9 were oriented to be axial by their respective constants, which were *syn* to H-7 based on their NOE correlations (Fig. 3). H-6 was axial and opposite to H-1, H-5b, and H-7 on the basis of its splitting pattern and large coupling constants (in acetone-d_6), and it was placed to be *syn* to H-4a and H-8b by its NOE correlations with them. NOE correlations between H-1 and H-5b and between H-8b and H-10a further corroborated the relative configurations of rings A and B, while those of H-13 with H-12b and H-16 located them on the same face of ring C. Although the different coupling constants of H-10a and H-10b suggested the bond rotation at C-10 to be restricted, the relative stereochemistry between rings B and C could not be resolved by the observed NOE correlation between H-9 and H-12a. The conformers with 1R, 6R, 7*S*, 9*S*, 11R, and 16R and 1R, 6R, 7*S*, 9*S*, 11*S*, and 16*S* configurations matching the above NOE correlations were subjected to Gaussian 09 software for the computation of ECD spectra using the time-dependent density function theory (TD-DFT) method at the B3LYP/6-31G(d) level in MeOH with the integral equation formalism variant (IEF) of the polarizable continuum model (PCM) (Frisch et al., 2010), and the results were depicted by SpecDis software with sigma = 0.2 (Bruhn et al., 2011). Based on the comparison of experimental and calculated ECD spectra (Fig. 4), the absolute configuration of **1**, trivially named trichaspin, was assigned to be 1R, 6R, 7*S*, 9*S*, 11R, and 16R.

Compound **2** was isolated as a colorless oil with a molecular formula of $C_{23}H_{39}NO_6$ given by HREIMS (*m/z* 425.2785 $[M]^+$), implying five degrees of unsaturation. The ^{1}H NMR spectrum (in $CDCl_3$, Table 1) showed one methyl doublet, four methyl singlets, one doublet ascribable to an oxygenated methine, one broad triplet and two broad doublets attributable to three olefinic protons, and one broad doublet due to an exchangeable proton, with the exception of six signals at δ_H 3.6–4.1 for four methines and one methylene. The ^{13}C NMR and DEPT spectra (Table 2) demonstrated the presence of five methyls, five methylenes, ten methines, and three nonprotonated carbons. HMBC correlations from H-12 and H-13 to C-10 and C-11 established the connectivity at C-11, which was elongated to C-2, C-4, and C-14 by COSY correlations (Fig. 2). A 1,10-bisaboladiene moiety oxygenated at C-3 was proposed by the deshielded signals (δ_C 77.6 in $CDCl_3$ and 78.2 in CD_3OD) of C-3 and HMBC correlations from H-15 to C-2, C-3, and C-4, which was confirmed by comparison of NMR data with those of zingiberenol (Terhune et al., 1975; Khrimian et al., 2014). In addition, an analysis of the other NMR chemical shifts and coupling constants indicated the presence of a 2-acetamido-2-deoxy-α-glucopyranosyl residue (Yamaoka et al., 1974; Afiyatullov et al., 2007), and its attachment to C-3 via a glycosidic linkage was supported by the observed HMBC correlation from H-16 to C-3. An acidic hydrolysis of **2** produced (3*S*,6R,7*S*)-zingiberenol and 2-N-acetylglucosamine, validated by their identical specific optical rotation values ($[\alpha]_D^{20}$ −46 and $[\alpha]_D^{22}$ +43, respectively) with those reported ($[\alpha]_D^{20}$ −37.7 and $[\alpha]_D^{22}$ +41.4, respectively) (Fondy and Emlich, 1981; Khrimian et al.,

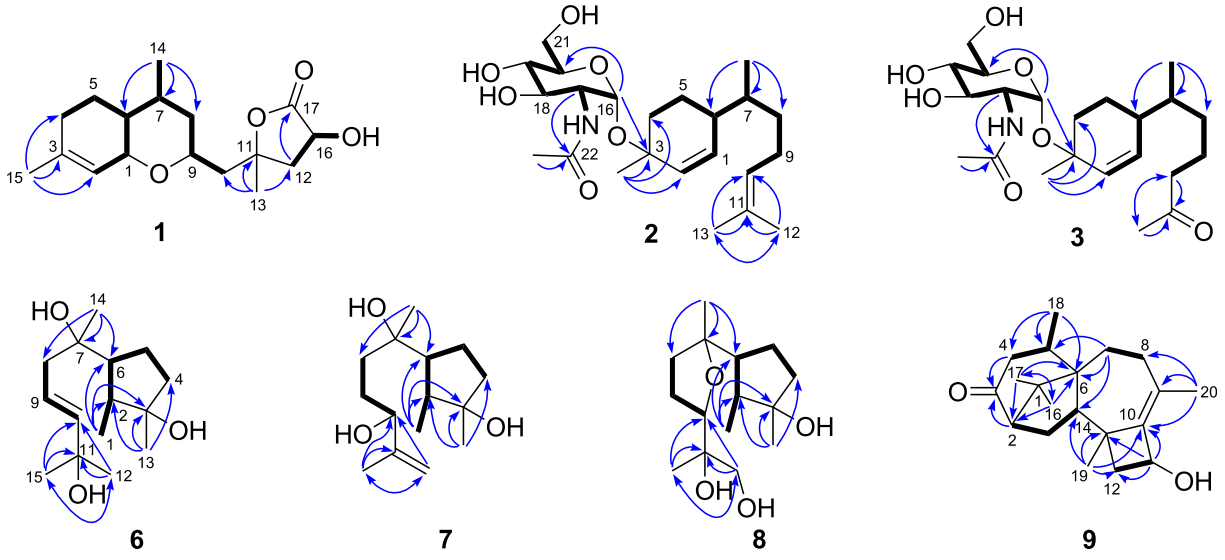

Fig. 2. Key COSY (bold lines) and HMBC (arrows) correlations of **1**–**3** and **6**–**9**.

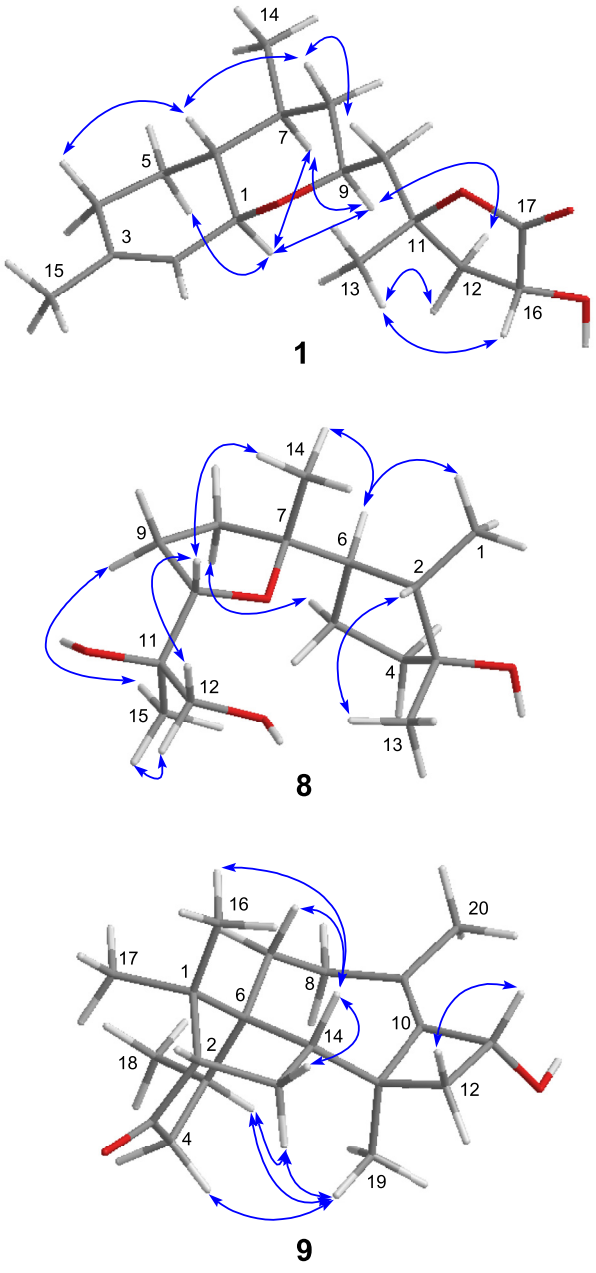

Fig. 3. Key NOE correlations of **1**, **8**, and **9**.

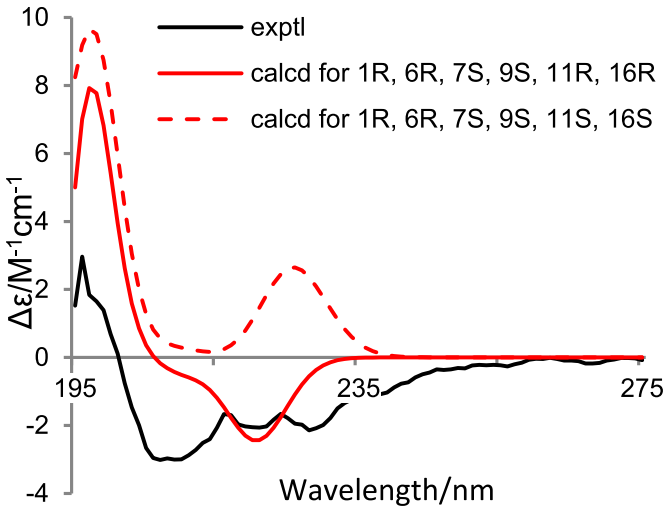

Fig. 4. Experimental and calculated ECD spectra of **1**.

2014). Thus, the absolute configuration of **2**, trivially named trichaspside A, was established to be 3*S*, 6R, 7*S*, 16R, 17R, 18R, 19*S*, and 20R.

Compound **3** was purified as a colorless oil and assigned a molecular formula of $C_{22}H_{37}NO_7$ by interpretation of HREIMS (*m*/*z* 427.2568 $[M]^+$), resembling that of **2** except for the presence of one additional oxygen atom and the lack of one carbon atom and two protons. In its ^1H and ^{13}C NMR spectra (Tables 1 and 2), the signals for a carbonyl group and a methylene group appeared, replacing those for a methyl group and a trisubstituted vinyl group at the side chain terminus of **2**. HMBC correlations from H-10 to C-11 and C-13 and from H-13 to C-10 and C-11 and COSY correlations of H-8/H-9/H-10 indicated the presence of a pentan-4-onyl group, which was bonded to C-7 by HMBC correlations from H-14 to C-6, C-7, and C-8. Other HMBC and COSY correlations (Fig. 2) further confirmed the planar structure. The absolute configuration of **3**, trivially named trichaspside B, was assigned as that of **2** based on the biogenic consideration and the same specific optical rotation data.

As a possible precursor of compounds **1**–**3**, (3*S*,6R,7*S*)-zingiberenol (**4**) was also obtained as a colorless oil. Its structure and absolute configuration were speculated by comparison of NMR and specific optical rotation data with those reported (Terhune et al., 1975; Khrimian et al., 2014). This sesquiterpene has previously been found in some terrestrial plants and animals (as a sex pheromone) but never been reported from fungi (Borges et al., 2006; Terhune et al., 1975; Khrimian et al., 2015). It is also worth to mention that **2** and **3** represent the first aminoglycosides of bisabolane and norbisabolane sesquiterpenes, respectively, especially for the presence of an α-glycosidic linkage. As for the skeleton of **1**, it may be an ethylated sesquiterpene or a trinorditerpene, but no related diterpenes were found herein.

Compounds **5** and **6** were purified as colorless oils, respectively, and the former was identified to be cyclonerodiol by the identical NMR and specific optical rotation data ($[\alpha]_D^{20}$ −21 (*c* 0.10, MeOH or $CHCl_3$) for **5**) (Laurent et al., 1990; Langhanki et al., 2014). The molecular formula of **6** was deduced to be $C_{15}H_{28}O_3$ by analysis of HREIMS (*m*/*z* 256.2041 $[M]^+$), implying two degrees of unsaturation. The ^1H NMR spectrum (Table 3) exhibited one methyl doublet, four methyl singlets, one doublet ascribable to a methylene, and one doublet and one triple doublet attributable two olefinic protons. The ^{13}C NMR spectrum (Table 4) displayed 15 resonances, classified into five methyls, three methylenes, four methines, and three nonprotonated carbons by DEPT and HSQC data. An analysis of the above NMR data revealed that **6** differed from **5** mainly at the side chain moiety (Laurent et al., 1990; Langhanki et al., 2014). Furthermore, HMBC correlations from Me-12 and Me-15 to C-10 and C-11 and COSY correlations of H-8/H-9/H-10 confirmed the presence of 4-hydroxy-4-methylpent-2-enyl unit, which was supported by the similar NMR data with those of asporyzin C (Qiao et al., 2010). Its attachment to ring A was indicated by HMBC correlations from Me-14 to C-6, C-7, and C-8. Thus, **6** was identified to be 9-cycloneren-3,7,11-triol, corroborated by the other HMBC and COSY correlations (Fig. 2). The geometry of double bond at C-9 was allowed to be *trans* by the large coupling constant between H-9 and H-10, and the absolute configurations at C-2, C-3, C-6, and C-7 were proposed to be the same as those of **5** based on the identical NMR and specific optical rotation data as well as the biogenic consideration.

Table 3
^{1}H NMR data for **6**–**9** (500 MHz, δ_H in ppm, *J* in Hz).

pos	**6** (in CD_3OD)	**7** (in CD_3OD)	**8** (in CD_3OD)	**9** (in $CDCl_3$)
1	1.02, d (6.8)	1.02, d (6.8)	1.01, d (6.8)	
2	1.64, m	1.60, m	1.53, m	2.20, d (7.9)
4a	1.63, m	1.63, m	1.66, m	2.85, m
4b	1.54, m	1.53, m	1.57, m	2.02, d (15.9)
5a	1.82, m	1.81, m	1.88, m	2.83, m
5b	1.62, m	1.59, m	1.50, m	
6	1.81, m	1.81, m	1.96, m	
7a				1.82, ddd (12.8, 6.4, 1.0)
7b				1.27, t (12.4)
8a	2.20, d (6.6)	1.52, m	1.71, m	2.34, t (13.7)
8b	2.20, d (6.6)	1.40, ddd (12.1, 11.2, 4.0)	1.67, m	1.86, m
9a	5.68, dt (15.6, 6.8)	1.62, m	1.98, m	
9b		1.56, m	1.93, m	
10	5.62, d (15.6)	3.97, t (6.4)	3.94, t (7.3)	
11				4.70, br d (6.8)
12a	1.27, s	4.91, br s	3.48, d (11.1)	1.88, m
12b		4.81, br s	3.43, d (11.1)	1.64, dd (12.2, 1.4)
13	1.23, s	1.22, s	1.22, s	
14	1.12, s	1.12, s	1.14, s	2.27, dd (11.3, 9.4)
15a	1.27, s	1.72, br s	1.10, s	1.91, m
15b				1.39, dd (14.4, 9.8)
16				0.97, s
17				0.94, s
18				1.08, d (7.3)
19				1.59, s
20				1.75, s

Table 4
^{13}C NMR data for **6**–**9** (125 MHz, δ_C in ppm).

pos	**6** (in CD_3OD)	**7** (in CD_3OD)	**8** (in CD_3OD)	**9** (in $CDCl_3$)
1	15.4, CH_3	15.4, CH_3	14.7, CH_3	49.5, C
2	45.4, CH	45.5, CH	46.4, CH	59.7, CH
3	82.0, C	82.1, C	81.9, C	215.3, C
4	41.4, CH_2	41.4, CH_2	41.4, CH_2	42.9, CH_2
5	25.1, CH_2	25.2, CH_2	26.2, CH_2	30.1, CH
6	55.4, CH	55.7, CH	55.5, CH	51.4, C
7	75.7, C	75.4, C	87.3, C	30.2, CH_2
8	45.1, CH_2	37.9, CH_2	35.8, CH_2	28.0, CH_2
9	123.9, CH	30.2, CH_2	26.7, CH_2	134.8, C
10	142.2, CH	77.4, CH	81.0 CH	142.3, C
11	71.2, C	148.8, C	74.8, C	68.7, CH
12	29.9, CH_3	111.6, CH_2	69.2, CH_2	45.9, CH_2
13	26.1, CH_3	26.1, CH_3	26.1, CH_3	46.1, C
14	25.3, CH_3	24.8, CH_3	23.2, CH_3	54.3, CH
15	29.9, CH_3	17.4, CH_3	19.2, CH_3	25.9, CH_2
16				23.5, CH_3
17				25.2, CH_3
18				21.0, CH_3
19				21.7, CH_3
20				20.8, CH_3

Compound **7** was isolated as a colorless oil. Its molecular formula was assigned to be $C_{15}H_{28}O_3$, the same as for **6**, by HREIMS (*m*/*z* 256.2029 $[M]^+$), suggesting two degrees of unsaturation. The ^{1}H and ^{13}C NMR data (Tables 3 and 4) closely resembled those of **5**, except for signals for the side chain terminus (Laurent et al., 1990; Langhanki et al., 2014). HMBC correlations from Me-15 to C-10, C-11, and C-12 suggested the connectivity at C-11, which was extended to C-8 by COSY correlations of H-8/H-9/H-10 and then attached to ring A via C-7 by HMBC correlations from H-14 to C-6, C-7, and C-8. The established side chain was also supported by its identical NMR data with chilianoside H (Jiang et al., 1999). Other HMBC and COSY correlations (Fig. 2) further validated **5** to be 11-cycloneren-3,7,10-triol, and its absolute configurations at C-2, C-3, C-6, and C-7 were also proposed to be the same as those of **5** and **6** based on the biogenic consideration. Unfortunately, the absolute configuration at C-10 was still unresolved due to the failure in preparing Mosher's esters and single crystals.

Compound **8** was obtained as a colorless oil with a molecular formula of $C_{15}H_{28}O_4$ given by HREIMS (*m*/*z* 272.1981 $[M]^+$), consistent with two degrees of unsaturation. Its ^{1}H and ^{13}C NMR data (Tables 3 and 4) showed high similarities to those of cyclonerodiol oxide (Fujita et al., 1984), except for the presence of signals for an oxymethylene group and the lack of signals for a methyl group. HMBC correlations from the oxymethylene to C-10, C-11, and Me-15 indicated its attachment to C-11. Thus, **8** was identified to be 7,10-epoxycycloneran-3,11,12-triol, which was further verified by the other HMBC and COSY correlations (Fig. 2). Me-1 was *syn* to H-6 by their NOE correlation, while Me-13 was *syn* to H-2 by their NOE correlation (Fig. 3). Additionally, Me-14 and H-10 were located on the same face by their NOE correlation. The absolute configurations of chiral centers except for C-11 were assigned as those of cyclonerodiol oxide by the similar specific optical rotation data (Fujita et al., 1984).

Compounds **9** and **10** were purified as a colorless oil and a white powder, respectively, and the latter was identified to be harziandione by its spectroscopic data (Miao et al., 2012; Adelin et al., 2014). As for **9**, a molecular formula of $C_{20}H_{30}O_2$ was established by interpretation of HREIMS (*m*/*z* 302.2241 $[M]^+$), requiring six degrees of unsaturation. The ^{1}H NMR spectrum (Table 3) in combination with HSQC data showed four methyl singlets, one methyl doublet, and one broad doublet due to an oxymethine, while the ^{13}C NMR and DEPT spectra (Table 4) demonstrated the presence of five methyls, five methylenes, four methines, and six quaternary carbons. A detailed comparison of NMR data with those of **10** revealed that their differences were mainly situated around C-11. Replacing the conjugated carbonyl group of **10**, a hydroxy group appeared at C-11, and its connectivity was confirmed by HMBC correlations from H-11 to C-10, C-12, and C-13, from H-19 to C-10, C-12, C-13, and C-14, and from H-20 to C-8, C-9, and C-10. HMBC correlations from H-2 and H-4 to C-3, from H-7 to C-5, C-6, and C-14, from H-16 and H-17 to C-1, C-2, and C-6, and from H-18 to C-4, C-5, and C-6 and COSY correlations of H-4/H-5/H-18, H-7/H-8, H-11/H-12, and H-14/H-15/H-2 (Fig. 2) further verified **9** to be 11-hydroxy-9-harzien-3-one. The relative configurations around

rings C and D were deduced to be the same as those of **10** on the basis of their identical NMR data (Adelin et al., 2014), which were supported by NOE correlations of H-14 with H-7b, H-15a, and Me-16 and of H-5 with H-15b (Fig. 3). Me-19 was *syn* to H-4a, H-5, and H-15b by its NOE correlations with them, while the relative configuration of H-11 was oriented by its different splitting pattern and chemical shift from those of 9-harzien-11-ol (Adelin et al., 2014). The ECD spectrum displayed a positive Cotton effect at 291 nm, which was then computed with the TD-DFT method at the gas-phase B3LYP/6-31G(d) level in Gaussian 09 software (Frisch et al., 2010). The result was drawn by SpecDis software with sigma = 0.2 (Bruhn et al., 2011), and it agreed well with the experimental one (Fig. 5). Thus, the absolute configuration of **9** was assigned to be 2*S*, 5R, 6R, 11R, 13*S*, and 15*S*.

In order to develop new inhibitors against pathogenic bacteria that greatly threatened marine aquaculture, compounds **1**–**4** were assayed for inhibition of five aquatic pathogens (*Vibrio parahaemolyticus*, *V. anguillarum*, *V. harveyi*, *V. splendidus*, and *Pseudoalteromonas citrea*) using the disk diffusion method at 20 μg/disk (Miao et al., 2012), and chloramphenicol with inhibitory zone diameters of 19.7, 18.2, 17.9, 18.7, and 19.7 mm, respectively, was taken as a positive control. Among them, only **2** and **3** showed potent inhibition (6.1–6.4 mm zones) of the four *Vibrio* bacteria tested (Table S1), which might correlate with the 2-acetamido-2-deoxy-α-D-glucopyranosyl group. Compounds **5**–**9** were assayed for inhibition of *V. parahaemolyticus* and *P. citrea*, and only **8** and **9** showed inhibition of *V. parahaemolyticus*, each with a 6.2 mm zone. Additionally, compounds **1**–**9** were evaluated for toxicity to the zooplankton *Artemia salina* using K_2CrO_7 as a positive control (100% lethal rate), but they exhibited only 52.2–78.7% lethal rates at 100 μg/mL.

3. Conclusion

Chemical investigation towards *Trichoderma asperellum* cf44-2, an endophyte of the marine brown alga *Sargassum* sp., resulted in the isolation and identification of ten terpenes, comprising three undescribed bisabolane derivatives (**1**–**3**), three undescribed cyclonerane sesquiterpenes (**6**–**8**), and one undescribed harziane diterpene (**9**). Among them, compound **1** possesses an undescribed ethylated bisabolane framework, while **2** and **3** represent the first aminoglycosides of bisabolane and norbisabolane sesquiterpenes, respectively. The bioassay results showed that **2**, **3**, **8**, and **9** could inhibit some marine-derived *Vibrio* species, and the effects of **2** and **3** might relate to their aminoglycoside moiety.

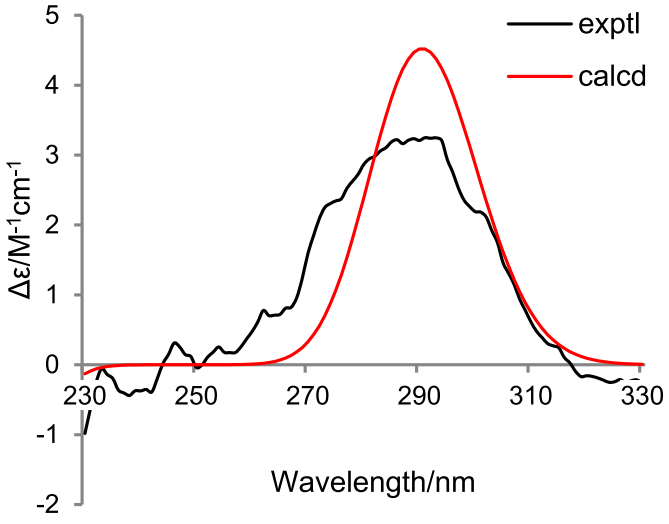

Fig. 5. Experimental and calculated ECD spectra of **9**.

4. Experimental section

4.1. General experimental procedures

Optical rotations were determined on a JASCO P-1020 polarimeter and an SGW-3 polarimeter. ECD spectra were measured on a Chirascan CD spectrometer. IR spectra were obtained on a JASCO FT/IR-4100 spectrometer. NMR spectra were recorded on a Bruker Avance III 500 NMR spectrometer (500 and 125 MHz for ^1H and ^{13}C, respectively) using tetramethylsilane (TMS) as an internal standard. Low and high resolution EI mass spectra were acquired on an Autospec Premier P776 mass spectrometer with a double-focusing magnetic sector mass analyzer. HPLC separation was operated on an Agilent HPLC system (1260 infinity quaternary pump, 1260 infinity diode-array detector) using an Eclipse SB-C18 (5 μm, 9.4 × 250 mm) column. Column chromatography (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). Thin-layer chromatography (TLC) was carried out with precoated silica gel plates (GF-254, Qingdao Haiyang Chemical Co.). Quantum chemical calculations were run with Gaussian 09 software (IA32W-G09RevC.01).

4.2. Fungal material and fermentation

Following a previous procedure (Wang et al., 2006), *Trichoderma asperellum* Samuels, Lieckfeldt & Nirenberg cf44-2 (Moniliaceae) as an endophyte was isolated from the fresh tissue of the surface-sterilized brown alga *Sargassum* sp. (Sargassaceae) collected from Zhoushan Islands (N30°01′20″, E122°05′14″) of China in August 2010. The species was identified by morphological taxonomy and by analysis of the ITS regions of its rDNA, deposited at GenBank (accession no. MG696741). Its fermentation was performed statically at room temperature for 30 days in 200 × 1 L Erlenmeyer flasks, each containing 300 mL of media prepared by addition of 500 mL potato (200 g) broth, 20 g glucose, 5 g peptone, and 5 g yeast extract powder into 500 mL natural seawater from the coast of Yantai.

4.3. Extraction and isolation

The mycelia were collected by filtration, which were then 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 (52.4 g). The filtrate was directly extracted with EtOAc and then concentrated to afford an extract (31.3 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 consisting of petroleum ether (PE)/EtOAc and CH_2Cl_2/MeOH to yield 12 fractions (Frs. 1–12). Fr. 3 eluted with PE/EtOAc (5:1) and was further purified by CC on RP-18 (MeOH/H_2O, 3:1) and Sephadex LH-20 (MeOH) and preparative TLC (PE/EtOAc, 2:1) to produce **4** (2.6 mg). Fr. 4 eluted with PE/EtOAc (2:1) and was further purified by CC on RP-18 (MeOH/H_2O, 7:3) and Sephadex LH-20 (MeOH) and preparative TLC (PE/EtOAc, 1:1) to yield **10** (12.8 mg). Fr. 7 eluted with PE/EtOAc (1:1) and was further purified by RP-18 CC (MeOH/H_2O, 7:3) and preparative TLC (CH_2Cl_2/MeOH, 30:1) as well as semipreparative HPLC (MeOH/H_2O, 3:7 to 4:1) to afford **1** (1.0 mg), **5** (4.8 mg), and **9** (3.4 mg). Fr. 9 eluted with EtOAc and was further purified by RP-18 CC (MeOH/H_2O, 3:7 to 2:3) and preparative TLC (EtOAc) as well as semipreparative HPLC (MeOH/

H_2O, 3:17 to 1:1) to give **6** (1.2 mg), **7** (1.5 mg), and **8** (1.5 mg). Fr. 12 eluted with MeOH and was further purified by CC on RP-18 (MeOH/H_2O, 7:3) and Sephadex LH-20 (MeOH) and preparative TLC (CH_2Cl_2/MeOH, 4:1) to obtain **2** (8.6 mg) and **3** (3.6 mg).

*4.3.1. Trichaspin (**1**)*

White powder; $[\alpha]_D^{20}$ +36 (*c* 0.060, MeOH); IR (KBr) ν_{max} 3406, 2920, 2858, 1774, 1631, 1446, 1381, 1304, 1180, 1134, 1072, 941 cm^{-1}; ^1H and ^{13}C NMR data, Tables 1 and 2; EIMS *m/z* (%) 294 $[M]^+$ (80); HREIMS *m/z* 294.1838 $[M]^+$ (calcd for $C_{17}H_{26}O_4$, 294.1831).

*4.3.2. Trichaspside A (**2**)*

Colorless oil; $[\alpha]_D^{20}$ +50 (c 0.26, CH_2Cl_2); IR (KBr) ν_{max} 3402, 2962, 2931, 2881, 1655, 1547, 1439, 1377, 1099, 1030, 737 cm^{-1}; ^1H and ^{13}C NMR data, Tables 1 and 2; HREIMS *m/z* 425.2785 $[M]^+$ (calcd for $C_{23}H_{39}NO_6$, 425.2777).

*4.3.3. Trichaspside B (**3**)*

Colorless oil; $[\alpha]_D^{20}$ +50 (*c* 0.13, CH_2Cl_2); IR (KBr) ν_{max} 3367, 2931, 2870, 1709, 1655, 1547, 1442, 1373, 1107, 1030, 741 cm^{-1}; ^1H and ^{13}C NMR data, Tables 1 and 2; HREIMS *m/z* 427.2568 $[M]^+$ (calcd for $C_{22}H_{37}NO_7$, 427.2570).

*4.3.4. (3S,6R,7S)-zingiberenol (**4**)*

Colorless oil; $[\alpha]_D^{20}$ −43 (*c* 0.10, CH_2Cl_2).

*4.3.5. 9-Cycloneren-3,7,11-triol (**6**)*

Colorless oil; $[\alpha]_D^{20}$ −22 (*c* 0.040, MeOH); IR (KBr) ν_{max} 3410, 2970, 1655, 1458, 1377, 1273, 1149, 980, 922, 887, 741 cm^{-1}; ^1H and ^{13}C NMR data, Tables 3 and 4; EIMS *m/z* (%) 256 $[M]^+$ (6), 223 (30), 221 (71), 220 (30), 205 (55), 139 (75), 133 (88), 125 (23), 95 (32), 82 (100), 67 (23); HREIMS *m/z* 256.2041 $[M]^+$ (calcd for $C_{15}H_{28}O_3$, 256.2038).

*4.3.6. 11-Cycloneren-3,7,10-triol (**7**)*

Colorless oil; $[\alpha]_D^{20}$ −24 (*c* 0.050, MeOH); IR (KBr) ν_{max} 3398, 2962, 1651, 1450, 1377, 1296, 1065, 1022, 895 cm^{-1}; ^1H and ^{13}C NMR data, Tables 3 and 4; EIMS *m/z* (%) 256 $[M]^+$ (29), 241 (15), 239 (25), 223 (49), 157 (63), 150 (45), 125 (100), 107 (30), 81 (49); HREIMS *m/z* 256.2029 $[M]^+$ (calcd for $C_{15}H_{28}O_3$, 256.2038).

*4.3.7. 7,10-Epoxycycloneran-3,11,12-triol (**8**)*

Colorless oil; $[\alpha]_D^{20}$ −21 (*c* 0.050, MeOH); IR (KBr) ν_{max} 3406, 2966, 2881, 1458, 1377, 1296, 1203, 1061, 922, 737 cm^{-1}; ^1H and ^{13}C NMR data, Tables 3 and 4; EIMS *m/z* (%) 272 $[M]^+$ (12), 194 (15), 179 (17), 159 (15), 135 (22), 109 (100), 108 (25), 59 (32); HREIMS *m/z* 272.1981 $[M]^+$ (calcd for $C_{15}H_{28}O_4$, 272.1988).

*4.3.8. 11-Hydroxy-9-harzien-3-one (**9**)*

Colorless oil; $[\alpha]_D^{20}$ +115 (*c* 0.10, MeOH); IR (KBr) ν_{max} 3436, 2931, 1697, 1628, 1443, 1385, 1300, 1261, 1126, 1068, 995 cm^{-1}; ^1H and ^{13}C NMR data, Tables 3 and 4; EIMS *m/z* (%) 302 $[M]^+$ (42), 287 (35), 274 (15), 258 (80), 217 (52), 173 (69), 133 (88), 107 (84), 91 (99), 83 (100), 69 (82); HREIMS *m/z* 302.2241 $[M]^+$ (calcd for $C_{20}H_{30}O_2$, 302.2246).

4.4. Acidic hydrolysis

According to an approach described previously (Afiyatullov et al., 2007), compound **2** (4.0 mg) was hydrolyzed using 2 N HCl (1 mL) in a stoppered vial at 100 °C for 2 h. At the end of this reaction, the residue was obtained after evaporation and then subjected to RP-18 CC (MeOH/H_2O, 1:19 to 1:0) to give (3*S*,6R,7*S*)-zingiberenol (1.1 mg), identified by the same NMR data as those of compound **4** (Khrimian et al., 2014), and 2-N-acetylglucosamine (1.2 mg). Their specific optical rotation values were determined to be $[\alpha]_D^{20}$ −46 (*c* 0.044, CH_2Cl_2) and $[\alpha]_D^{22}$ +43 (*c* 0.048, H_2O), respectively.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.phytochem.2018.04.017.

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