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

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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

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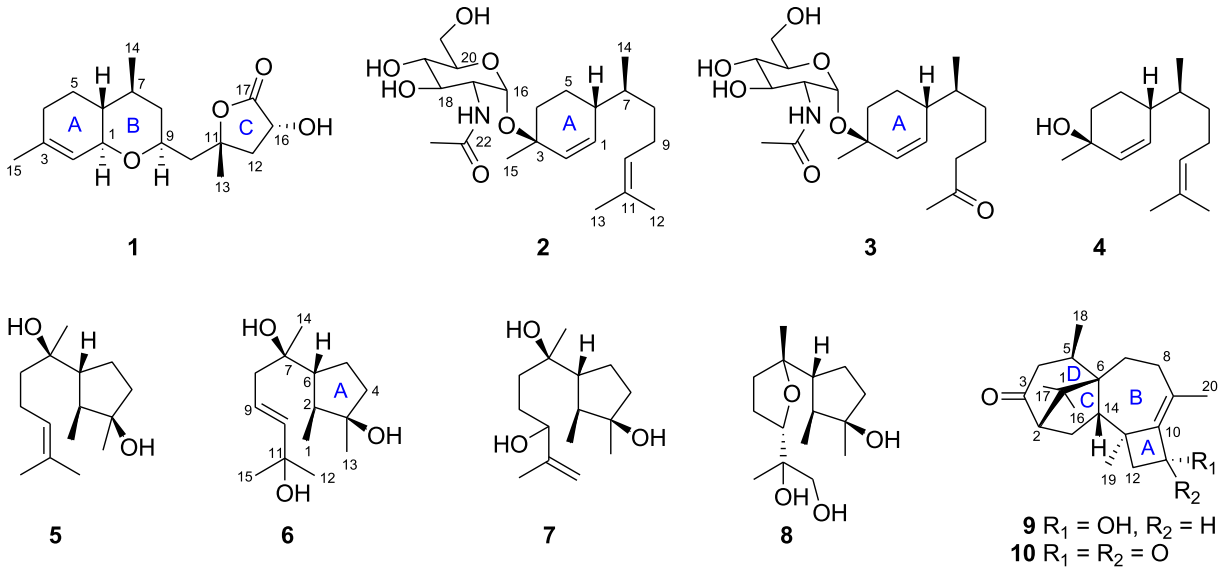

Fig. 1. Chemical structures of compounds **1**–**10**.

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