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Three novel phomactin-type diterpenes from a marine-derived fungus

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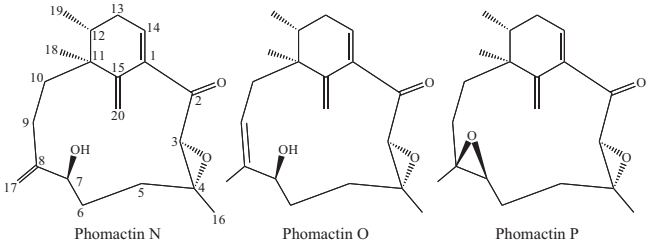
ABSTRACT

Three novel diterpenes, phomactins N (**1**), O (**2**), and P (**3**), were isolated from cultures of an unidentified marine-derived fungus. The structures of **1**–**3** were elucidated from spectroscopic data (NMR, MS, IR) and the absolute configurations of **1**–**3** were determined by X-ray diffraction analysis.

Introduction

Marine-derived fungi produce secondary metabolites with a variety of structures and some of these compounds are pharmacologically effective. Consequently, there has been increasing interest in the study of marine-derived fungi,[1] most of which have been collected from sponges and algae. We previously reported novel phomactin derivatives isolated from marine-derived fungi found in brown algae.[2–4]

Here, we describe the isolation and structural elucidation of three novel phomactins: N (**1**), O (**2**), and P (**3**). An unidentified fungus (MPUC 046) was isolated from the surface of the marine brown alga *Ishige okamurae*, collected at Tateishi, Kanagawa Prefecture, Japan, in September 2000. The D1/D2 26S rDNA and internal transcribed spacer regions, including the 5.8S rDNA in the rRNA gene of the isolate, were directly sequenced using PCR. The sequence data (approximately 1200 bp long) were searched using the BLAST algorithm (http://www.ncbi.nlm.nih.gov/BLAST/) against the sequences in the GenBank DNA database. The isolate was not assignable to any known species, but phylogenetically the isolate belongs to Dothideales. Furthermore, MPUC046 was not closely related to *Phoma* sp., which produce phomactins,[5–9] when compared using a molecular phylogenetic tree.



Results and discussion

Phomactin N (**1**) was obtained as a white powder and was recrystallized as colorless blocks.[10] The molecular formula of **1** was determined to be $C_{20}H_{28}O_3$ (seven degrees of unsaturation) by HR-EIMS analysis (316.2041: M^+ calcd for 316.2038). The IR spectrum of **1** indicated the presence of hydroxyl (3492 cm^{-1}) and carbonyl (1683 cm^{-1}) groups.

The ^{13}C NMR and DEPT data of **1** (Table 1) indicated the presence of a ketone (δ_C 199.6, C-2), six olefinic carbons (δ_C 152.9, C-8; 144.7, C-15; 141.1, C-1; 133.0, C-14; 116.3, C-20; 108.4, C-17), two singlet methyl carbons (δ_C 20.2, C-18; 19.2, C-16), a doublet methyl carbon (δ_C 16.9, C-19), five methylene carbons (δ_C 36.1, C-10; 34.5, C-6; 32.6, C-5; 31.6, C-13; 29.4, C-9), two oxymethine carbons (δ_C 69.0, C-7; 61.9, C-3), a methine carbon (δ_C 37.9,

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C-12), a quaternary oxycarbon (δ_C 64.2, C-4), and a quaternary carbon (δ_C 42.6, C-11). The ^{1}H NMR, HMQC and DQF-COSY data of **1** (Tables 1 and 2) showed the presence of three fragments: C6–C7, C12–C19, and C13–C14. Further information regarding the skeletal framework was obtained from HMBC correlations (Table 3). The connections between C11–C12–C13–C14–C1 were confirmed by the HMBC correlations between H-19/C-11, C-12, C-13, and H-13/C-1, C-19. The HMBC correlations between H-14/C-2, C-15, H-18/C-11, C-15, and H20/C-1, C-11, C15 showed a cyclohexene ring comprising C11–C12–C13–C14–C1–C15 and C-2 attached at C-1. Furthermore, the HMBC correlations between H-17/C-7, C-8, C-9, H-9/C-10, C-11, and H-10/C-11, C-12, C-15 indicated connections between C6–C7–C8–C9–C10–C11. On the other hand, the HMBC correlations between H-16/C-3, C-4, C5, and H-3/C-2 showed connections between C2–C3–C4–C5. The remaining bond, C5–C6, was inferred from the molecular formula. These data established the skeletal framework of **1** as consisting of a cyclohexene ring and a cyclododecane ring, suggesting that **1** is a phomactin derivative.[2–9] Comparison of the NMR data of **1** with those of phomactin M revealed that **1** contains an epoxy group (C-3/C-4) and a hydroxyl group (C-7).[4] Single-crystal X-ray diffraction analysis was conducted to determine the absolute configuration (Fig. 1).[11] The absolute configurations of C3, C4, C7, C11, and C12 were determined to be *S*, *R*, *S*, *S*, and *R*, respectively, deduced from the Flack parameter, −0.07 (3), and refined using 1310 Friedel pairs.[12]

Phomactin O (**2**) was obtained as a white powder and was recrystallized as colorless blocks.[13] The molecular formula of **2** was determined to be $C_{20}H_{28}O_3$ (seven degrees of unsaturation) by HR-EIMS analysis (316.2035: M^+ calcd for 316.2038). The IR spectrum of **2** indicated the presence of hydroxyl (3505 cm^{-1}) and carbonyl (1699 cm^{-1}) groups. The NMR data of **2** (Tables 1 and 2) showed that **2** was a phomactin derivative similar to **1** except at C8, C9, and C17. Single-crystal X-ray diffraction analysis was conducted to determine the absolute configuration (Fig. 2).[14] The absolute configurations of C3, C4, C7, C11, and C12 were determined to be *S*, *R*, *S*, *S*, and *R*, respectively, deduced from the Flack parameter, 0.03 (15), and refined using 1508 Friedel pairs.[12]

Table 2
DQF-COSY correlations of **1**, **2**, and **3** in $CDCl_3$

Position	1	2	3
H-5	Overlapped	H-6	H-6
H-6	H-7	H-5, 7	H-5, 7
H-7	H-6	H-6	H-6
H-9	Overlapped	H-10	H-10
H-10	Overlapped	H-9	H-9
H-12	H-19	H-19	Overlapped
H-13	H-14	H-14	H-14
H-14	H-13	H-13	H-13
H-19	H-12	H-12	Overlapped

Table 3
HMBC correlations of **1**, **2**, and **3** in $CDCl_3$

Position	1	2	3
H-3	C-2, 4, 5	C-2, 4, 5	C-1, 2, 4, 5
H-5	Overlapped	C-4, 6, 16	C-4, 6, 7
H-6	C-4	C-4, 5, 7, 8	C-5, 7
H-7	C-6, 8, 9, 17	C-6, 8, 9, 17	C-6
H-9	C-7, 8, 10, 11, 17	C-7, 17	C-7, 8, 10, 17
H-10	C-9, 11, 12, 15	C-8, 9, 11, 12, 15, 18	C-8, 9, 11, 12, 15, 18
H-12	Overlapped	Overlapped	Overlapped
H-13	C-1, 11, 12, 14, 19	C-1, 11, 12, 14, 19	C-1, 11, 12, 14, 19
H-14	C-2, 12, 15	C-2, 12, 15	C-2, 12, 15
H-16	C-3, 4, 5	C-4, 5	C-4, 5
H-17	C-7, 8, 9	C-7, 8, 9	C-7, 8, 9
H-18	C-11, 15	C-10, 11, 12, 15	C-10, 11, 12, 15
H-19	C-11, 12, 13	C-11, 12, 13	C-11, 12, 13
H-20	C-1, 11, 15	C-1, 11	C-1, 11

Phomactin P (**3**) was obtained as a white powder and was recrystallized as colorless blocks.[15] The molecular formula of **3** was determined to be $C_{20}H_{28}O_3$ (seven degrees of unsaturation) by HR-EIMS analysis (316.2040: M^+ calcd for 316.2038). The IR spectrum of **3** indicated the presence of carbonyl (1693 cm^{-1}) groups. The NMR data of **3** (Tables 1 and 2) showed that **3** was a phomactin derivative similar to phomactin F except at C15 and

Table 1
^{13}C and ^{1}H NMR spectra of **1**, **2**, and **3** in $CDCl_3$ (δ ppm)

Position	1				2				3			
	δ ^{13}C		δ ^{1}H		δ ^{13}C		δ ^{1}H		δ ^{13}C		δ ^{1}H	
1	141.1	s			141.4	s			141.7	s		
2	199.6	s			199.7	s			199.5	s		
3	61.9	d	4.33	(1H, s)	64.6	d	3.98	(1H, s)	64.5	d	3.87	(1H, s)
4	64.2	s			62.9	s			62.8	s		
5	32.6	t	1.63	(1H, m)	34.2	t	1.12	(1H, m)	35.6	t	1.09	(1H, m)
			2.29	(1H, m)			2.14	(1H, m)			2.25	(1H, ddd, 13.4, 4.4, 3.3)
6	34.5	t	1.40	(2H, m)	30.1	t	1.83	(2H, m)	24.3	t	1.48	(1H, m)
											2.13	(1H, m)
7	69.0	d	4.00	(1H, d, 6.9)	65.9	d	4.47	(1H, dd, 9.9, 5.7)	55.7	d	2.87	(1H, dd, 11.0, 3.9)
8	152.9	s			137.4	s			59.3	s		
9	29.4	t	1.84	(1H, m)	128.2	d	5.61	(1H, dd, 12.2, 1.7)	29.1	t	1.65	(1H, m)
			2.29	(1H, m)							2.01	(1H, m)
10	36.1	t	1.31	(1H, m)	35.1	t	1.70	(1H, m)	26.8	t	1.08	(1H, m)
			1.71	(1H, m)			2.95	(1H, m)			2.01	(1H, m)
11	42.6	s			42.1	s			42.2	s		
12	37.9	d	1.65	(1H, m)	37.5	d	1.73	(1H, m)	37.6	d	1.65	(1H, m)
13	31.6	t	1.97	(1H, dd, 20.7, 4.8)	31.5	t	2.02	(1H, dd, 20.7, 4.8)	31.4	t	1.96	(1H, dd, 20.6, 4.7)
			2.65	(1H, ddd, 20.7, 6.0, 2.6)			2.75	(1H, ddd, 20.6, 6.1, 2.9)			2.70	(1H, ddd, 20.7, 6.1, 2.7)
14	133.0	d	6.11	(1H, br s)	131.1	d	6.02	(1H, m)	131.3	d	5.95	(1H, br s)
15	144.7	s			144.5	s			144.2	s		
16	19.2	q	1.29	(3H, s)	14.4	q	1.24	(3H, s)	13.6	q	1.28	(3H, s)
17	108.4	t	4.90	(1H, s)	18.3	q	1.75	(3H, s)	19.0	q	1.27	(3H, s)
			5.07	(1H, br s)								
18	20.2	q	1.08	(3H, s)	20.3	q	1.22	(3H, s)	21.1	q	1.03	(3H, s)
19	16.9	q	0.82	(3H, d, 7.1)	16.9	q	0.83	(3H, d, 6.8)	17.0	q	0.83	(3H, d,7.1)
20	116.3	t	5.23	(1H, s)	115.2	t	5.06	(1H, br s)	116.4	t	5.18	(2H, br s)
			5.30	(1H, s)			5.11	(1H, br s)				