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Meroterpenoids from the Alga-Derived Fungi *Penicillium thomii* Maire and *Penicillium lividum* Westling

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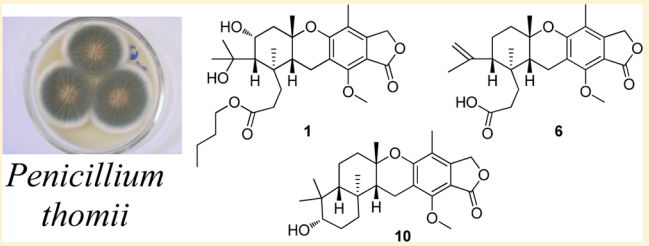
Supporting Information

ABSTRACT: Ten new austalide meroterpenoids (**1**−**10**) were isolated from the alga-derived fungi *Penicillium thomii* KMM 4645 and *Penicillium lividum* KMM 4663. Their structures were elucidated by extensive spectroscopic analysis and by comparison with related known compounds. The absolute configurations of some of the metabolites were assigned by the modified Mosher's method and CD data. Compounds **1**, **2**, **8**, and **9** were able to inhibit AP-1-dependent transcriptional activity in JB6 Cl41 cell lines at noncytotoxic concentrations. Austalides **1**−**5**, **8**, and **9** exhibited significant inhibitory activity against *endo*-1,3-β-D-glucanase from a crystalline stalk of the marine mollusk *Pseudocardium sachalinensis*.



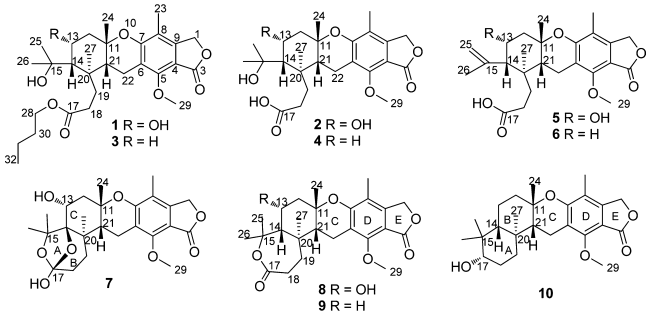
The austalides are a family of related meroterpenoid metabolites that were isolated for the first time from maize cultures of *Aspergillus ustus*.[1] It was shown that the austalide skeleton is biosynthesized after subsequent cyclization and oxidative modification of 6-[(2*E*,6*E*)farnesyl]-5,7-dihydroxy-4-methylphthalide.[2−4] The absolute configurations of the austalides A to L were assigned by X-ray structure analysis, which was carried out for a single crystal of the dicamphanate ester derivative of austalide D and by synthesis of austalide B.[4,5] Recently, five new austalides (M−Q) were isolated from a sponge-derived fungus *Aspergillus* sp. The absolute configurations of austalides M and P were elucidated by electronic circular dichroism calculations.[6]

In our search for fungal secondary metabolites with novel chemical structures and/or cytotoxic activity we have investigated the strains *Penicillium thomii* KMM 4645 and *Penicillium lividum* KMM 4663 associated with the marine brown alga *Sargassum miyabei*. We report herein the isolation, structure determination, and biological assay results of the new austalides (**1**−**10**) produced by these fungi.

RESULTS AND DISCUSSION

The fungi were cultured for 21 days on specially modified rice medium.[7] The EtOAc extracts of the mycelia were purified by a combination of Si gel column chromatography and reversed-phase HPLC to yield compounds **1**−**5**, **8**, and **9** from *P. thomii* and **2**−**10** from *P. lividum* as amorphous solids.

The molecular formula of **1** was determined to be $C_{29}H_{42}O_6$ by HRESIMS and was in accordance with the ^{13}C NMR data. The UV spectrum showed λ_{max} at 216 and 267 nm, consistent



with the substituted phthalide moiety[1a] in structure **1**. A comparison of the ^1H and ^{13}C NMR data (Tables 1 and 2) with those for the meroterpenoid austalide H showed a close similarity.[1a] The key differences were the lack of a methyl ester in **1** and the presence of three methylenes and an extra aliphatic methyl compared to austalide H.

The correlations observed in the COSY-45 spectrum and the HMBC correlations H_3-32/C-30 (δ_C 30.7), H_2-31/C-28 (δ_C 64.7), and H_2-28/C-17 (δ_C 173.6) revealed the location of a butyl ester at C-17 in **1**. Thus, compound **1** has a butyl ester at C-17 instead of a methyl ester in austalide H,[1a] and it was named austalide H acid butyl ester.

The NOE correlations H-14/H-13, H-13/H-24, H-24/H-21, H-22b (δ_H 2.81), and H_3-27/H-22a (δ_H 3.10) determined the relative configuration of **1**. The absolute configuration of **1** was

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Table 1. ^{1}H NMR Spectroscopic Data (δ, J in Hz) for Austalides 1, 5, 7, 8, and 10

position	1[a]	5[b]	7[c]	8[a]	10[b]
1	5.13, s	5.12, s	5.20, s	5.13, s	5.11, s
12	a: 2.53, dd (2.3, 15.3)	a: 2.51, dd (15.5; 2.5)	a:.31, dd (2.1, 15.6)	a: 2.55, dd (2.7, 15.7)	a: 2.27, m
	b: 1.81, dd (3.3, 15.3)	b: 1.93, dd (19.0; 4.1)	b: 2.19, dd (4.4, 15.6)	b: 1.98, dd (3.8, 15.7)	b: 2.24, m
13	4.68, m	4.08, brs	4.09, m	4.41, brs	α: 1.64, m
					β: 1.60, m
14	1.35, d (2.5)	2.04, brs		1.66, d (1.8)	0.93, d (11.5)
17					3.24, dd (4.5, 11.7)
18	a: 2.34, m	2.36, m	a: 1.87, d (8.6)	a: 2.82, dd (8.3, 16.5)	α: 1.57, m
	b: 2.25, m		b: 1.64, d (8.6)	b: 2.70, dd (12.3, 16.5)	β: 1.68, m
19	a: 2.29, m	a: 1.80, m	a: 1.93, d (7.4)	a: 2.06, m	a: 1.91, m
	b: 1.96, m	b: 1.70, m	b: 1.89, d (11.3)	b: 1.50, m	b: 0.88, m
21	1.68, d (8.0)	1.68, d (7.8)	2.46, d (7.9)	1.61, d (8.1)	1.39, d (8.8)
22	a: 3.10, d (18.0)	a: 2.96, d (18.8)	a: 2.96, d (18.5)	a: 3.05, d (18.7)	a: 2.91, d (18.6)
	b: 2.81, dd (8.0, 18.0)	b: 2.84, m	b: 2.90, dd (7.9, 18.5)	b: 2.88, dd (8.2, 18.7)	b: 2.73, dd (8.3, 18.6)
23	2.05, s	2.05, s	2.07, s	2.05, s	2.03, s
24	1.26, s	1.26, s	1.24, s	1.27, s	1.16, s
25	1.46, s	a: 5.06, s	1.56, s	1.75, s	0.78, s
		b: 5.01, s			
26	1.47, s	1.92, s	1.43, s	1.59, s	1.03, s
27	0.98, s	0.85, s	0.99, s	1.04, s	0.61, s
29	4.15, s	4.14, s	4.04, s	4.16, s	4.10, s
28	4.10, dt (1.2, 7.0)				
30	1.63, m				
31	1.40, m				
32	0.96, t (7.4)				

[a]Chemical shifts referenced to $CDCl_3$ at 700 MHz. [b]Chemical shifts referenced to $CDCl_3$ at 500 MHz. [c]Chemical shifts referenced to CD_3OD at 700 MHz.

established by the modified Mosher's method.[8] Esterification of **1** with (*R*)- and (*S*)-MTPA chloride occurred at the C-13 hydroxy group to give the (*S*)- and (*R*)-MTPA esters **1a** and **1b**, respectively. The observed chemical shift differences $\Delta\delta$ ($\delta_S - \delta_R$) (Supporting Information S73, 74) revealed the 13*R* configuration, and hence the absolute configuration of **1** was determined as 11*S*, 13*R*, 14*S*, 20*R*, 21*R*, which is consistent with the absolute configurations for known austalides G, H, and I.

The molecular formula of **2** was determined to be $C_{25}H_{34}O_8$ from HRESIMS and ^{13}C NMR data (Table 2). The structure of the substituted tetracyclic moiety of **2** was found by extensive NMR spectroscopy (^{1}H and ^{13}C NMR, COSY, HSQC, and HMBC, Supporting Information S11−S17) as for compound **1**. The key difference was that the structure had a free acid rather than a butyl ester.

Compound **2** showed characteristic Cotton effects (CEs) at λ_{270} +0.08, λ_{230} +0.42, and λ_{211} −0.36 in the CD spectrum in MeOH. The two CEs of the high-energy region were in good agreement with those for **1**, which allowed us to determine the absolute configuration of **2** as 11*S*, 13*R*, 14*S*, 20*R*, 21*R*. Compound **2** was named austalide H acid.

The molecular formula of **3** was determined to be $C_{29}H_{42}O_7$ from HRESIMS and was in accordance with the ^{13}C NMR data. The general features of the ^{1}H and ^{13}C NMR spectra (Table 2, Supporting Information S18, S20, S73) of **3** resembled those of **1** with the exception of the C-11−C-14 and C-25 proton and carbon signals. The correlations observed in the COSY and HSQC spectra (Supporting Information S19, S22) of **3** together with the molecular mass difference of 16 mass units between **1** and **3** indicated the absence of a hydroxy group at C-13 in **3**.

Compound **3** showed characteristic Cotton effects at λ_{269} +2.63, λ_{230} +4.69, and λ_{209} −4.59 in the CD spectrum in MeOH. The two strong CEs of the high-energy region reproduced well those for austalides P,[6] which confirmed the absolute configuration of **3** as 11*S*, 14*R*, 20*S*, 21*R*, and **3** was named austalide P acid butyl ester.

The molecular formula of compound **4** was determined to be $C_{25}H_{34}O_7$ from HRESIMS and ^{13}C NMR data. The ^{1}H and ^{13}C data observed for the tetracyclic core of **4** (Table 2, Supporting Information S25, S27, S73) matched those reported for compound **3**. The structure and location of the butyl ester at C-17 were established as for **2**.

Compound **4** exhibited a nearly identical CD spectrum to that of austalide P,[6] establishing its absolute configuration as 11*S*, 14*R*, 20*S*, 21*R*. Compound **4** was named austalide P acid.

The molecular formula of compound **5** was determined to be $C_{25}H_{32}O_7$ from HRESIMS and ^{13}C NMR data. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) observed for the substituted tetracyclic backbone of **5** closely resembled those of **1** with the exception of the proton and carbon signals at positions 13 and 14.

The HMBC correlations from methylene proton H-25a (δ_H 5.06) to the olefinic quaternary carbon signal detected at δ_C 145.0 (C-15) and from H_3-26 to C-15 and C-25 (δ_C 116.0) revealed the presence of the exomethylene moiety in **5**. The COSY-45 data and HMBC correlations from H_3-24 to C-12 (δ_C 44.6) and C-13 (δ_C 71.2), from H-14 to C-13, from H_2-25 to C-14 (δ_C 52.9), and from H_2-19 to C-14, C-20 (δ_C 39.6), C-17 (δ_C 177.3), and C-18 (δ_C 28.6) indicated the location of the hydroxy group and propenyl and carboxyethyl units at C-13, C-14, and C-20, respectively, and established the structure of **5**.

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Table 2. ^{13}C NMR Spectroscopic Data (δ in ppm) for Austalides 1−10

pos.	1[a]	2[b]	3[a]	4[c]	5[c]	6[a]	7[d]	8[a]	9[c]	10[c]
1	68.2	70.4	68.2	68.2	68.2	68.2	70.5	68.2	68.3	68.3
3	169.3	172.3	169.4	169.4	169.1	169.5	172.5	169.1	169.7	169.9
4	108.7	109.1	107.2	107.4	108.3	107.3	108.9	108.3	107.6	107.1
5	155.5	157.2	155.4	155.4	155.6	155.5	157.1	155.4	155.5	155.4
6	116.3	118.0	115.5	115.4	115.5	115.3	118.3	115.7	115.3	115.8
7	156.5	160.3	158.7	158.6	157.0	158.6	160.5	156.9	158.4	158.9
8	114.4	116.5	114.2	114.2	114.2	114.4	116.7	114.2	114.2	114.1
9	146.0	148.0	145.4	145.5	145.7	145.5	148.0	145.9	145.9	145.4
11	78.7	79.0	76.6	76.4	78.5	76.5	78.4	77.8	76.1	76.5
12	43.9	46.5	39.5	39.8	44.6	39.1	43.9	45.3	39.5	40.4
13	70.1	70.7	21.8	21.8	71.2	23.8	70.7	68.7	22.3	17.9
14	49.3	52.3	50.5	50.6	52.9	50.2	88.9	57.1	54.0	54.2
15	75.7	77.5	75.4	76.1	145.0	146.7	86.9	86.6	85.9	38.8
17	173.6	178.6	174.8	176.8	177.3	178.2	119.3	174.9	175.2	78.9
18	29.8	30.8	29.5	29.2	28.6	28.3	32.2	31.5	31.8	27.1
19	34.9	36.6	33.6	33.7	34.6	33.0	32.9	37.7	37.4	38.2
20	40.9	42.7	41.8	41.7	39.6	40.1	41.5	40.7	40.6	38.1
21	40.3	42.1	40.4	40.7	40.1	39.6	38.2	46.7	47.5	48.1
22	18.5	19.2	17.9	17.8	18.3	18.1	19.6	18.8	18.7	18.0
23	10.7	11.3	10.6	10.7	10.8	10.7	11.3	10.7	10.7	10.6
24	27.5	28.7	27.5	27.5	27.3	27.4	28.8	27.3	27.3	27.3
25	33.6	32.7	27.8	27.8	116.0	114.2	26.7	27.0	25.8	15.7
26	31.5	33.3	34.1	34.6	26.0	23.5	30.1	34.2	32.5	28.5
27	21.5	22.3	18.9	18.9	20.2	18.1	19.5	17.0	16.8	14.3
29	62.2	62.8	61.9	62.0	61.2	62.0	62.8	62.2	62.2	61.8
28	64.7		64.5							
30	30.7		30.6							
31	19.2		19.2							
32	13.7		13.7							

[a]Chemical shifts referenced to $CDCl_3$ at 176 MHz. [b]Chemical shifts referenced to CD_3OD at 176 MHz. [c]Chemical shifts referenced to $CDCl_3$ at 125 MHz. [d]Chemical shifts referenced to CD_3OD at 125 MHz.

The relative configuration of **5** was elucidated based on the observed NOE correlations. Compound **5** exhibited a nearly identical CD spectrum to that of austalide Q.[6] Thus, the absolute configuration of **5** was established as 11*S*, 13*R*, 14*S*, 20*S*, 21*R*. Compound **5** was named austalide Q acid.

The molecular formula of compound **6** was determined to be $C_{25}H_{32}O_6$ by HRESIMS and was in accordance with the ^{13}C NMR data (Table 2). The ^{13}C NMR data for this compound were very similar to those obtained for austalide Q acid (**5**) with the exception of the C-11−C-15 and C-25 carbon signals. The correlations observed in the COSY and HSQC spectra (Supporting Information S38, S41) of **6** together with the molecular mass difference of 16 mass units between **5** and **6** indicated the absence of a hydroxy group at C-13 in **6**.

The CD spectrum of **6** showed characteristic Cotton effects at λ_{297} −0.10, λ_{267} −0.02, λ_{231} +1.36, and λ_{212} −2.51, which were in good agreement with those for austalide P.[6] Thus, the absolute configuration of **6** was established as 11*S*, 14*S*, 20*S*, 21*R*. Compound **6** was named 13-deoxyaustalide Q acid.

The molecular formula of compound **7** was determined to be $C_{25}H_{32}O_8$ from HRESIMS and ^{13}C NMR data. The general features of the ^{13}C NMR spectrum (Table 2) of **7** resembled those of austalides M−O[6] with the exception of the C-6 and C-20−C-22 carbon signals. The key HMBC correlations (Supporting Information S48) confirmed the structure of rings A, B, and C. The long-range correlations H_2-1/C-3 (δ_C 172.5), C-4 (δ_C 108.9), C-8 (δ_C 116.7), and C-9 (δ_C 148.0); H_3-23/C-7 (δ_C 160.5), C-8, and C-9; H_3-29/C-5 (δ_C 157.1); and H_2-22/C-5, C-6 (δ_C 118.3), C-7, C-20, and C-21 (δ_C 38.2) established the structure of the substituted pyranophthalide moiety and its linkage with ring C in **7**. The main structural difference between the new austalide **7** and the previously reported austalide B is the presence of a hydroxy group at C-17 in **7** instead of a methoxy group in austalide B. Thus, compound **7** was named 17-*O*-demethylaustalide B. The relative configurations of the C-11, C-13, C-14, C-17, C-20, and C-21 stereogenic centers in **7** were the same as in austalide M[6] based on NOE correlations.

The molecular formula of compound **8** was determined to be $C_{25}H_{32}O_7$ from HRESIMS and ^{13}C NMR data. The general features of ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) of the C, D, and E rings of **8** closely resembled those of austalide I.[1a] The structure of the substituted pyranophthalide moiety in **8** was determined based on HMBC correlations as for compound **7**. The molecular mass difference of 42 mass units between austalide I and **8** and the upfield chemical shift of H-13 (δ_H 4.41) indicated replacement of an acetoxy group at C-13 by a hydroxy group. The relative configuration of **8** was elucidated based on NOE correlations as for **1**. Compound **8** was named 13-*O*-deacetylaustalide I.

The molecular formula of **9** was determined to be $C_{25}H_{32}O_6$ by HRESIMS and was in accordance with the ^{13}C NMR data. The ^{13}C NMR spectrum (Table 2) of **9** showed a close similarity of carbon chemical shifts to those of 13-*O*-deacetylaustalide I (**8**) with the exception of the C-11−C-14 and C-25 carbon signals. The COSY-45 data and HSQC

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spectra of **9** revealed the connectivity sequence of the protons ($-CH_2(12)-CH_2(13)-CH(14)<$). This information and the molecular mass difference of 16 mass units between **8** and **9** indicated the absence of a hydroxy group at C-13 in **9**. Compound **9** was named 13-deacetoxyaustalide I.

The CD spectrum of **9** showed characteristic Cotton effects at λ_{300} −0.16, λ_{260} −0.59, λ_{228} +1.02, and λ_{213} −1.54, which were in good agreement with those for austalide P.[6] Thus, the absolute configuration of **9** was established as 11*S*, 14*R*, 20*S*, 21*R*.

The molecular formula of compound **10** was determined to be $C_{25}H_{34}O_5$ from HRESIMS and ^{13}C NMR data. The ^{13}C NMR data (Table 2) observed for the substituted tetracyclic backbone (rings B−E) of **10** matched those for **9** with the exception of the C-13 and C-20 carbon signals. The HMBC correlations (Supporting Information S77) established the structure of the A ring as well as the linkage of this part of the molecule with the tetracyclic moiety. The HMBC correlations and the molecular mass difference of two mass units between **10** and the previously reported austalide K[1a] together with the absence of a ketone carbonyl in the ^{13}C NMR spectrum of **10** suggested the presence of a hydroxy group at C-17 in **10** instead of a ketone.

Observed NOE correlations between H-14 and H-13β (δ_H 1.60), H-17, and H-21, between H_3-27 and H-13α (δ_H 1.64), H-18α (δ_H 1.57), and H-22b (δ_H 2.91), between H-17 and H-18β (δ_H 1.68), and between H-24 and H-21 and H-22a (δ_H 2.73) indicated a *trans*-ring fusion of the A and B rings, as well as the β-orientation of the C-24 methyl group and α-orientations of the C-27 methyl and C-17 hydroxy groups in **10**.

The absolute configuration of **10** was established by the modified Mosher's method.[8] Esterification of **10** with (*R*)- and (*S*)-MTPA chloride occurred at the C-17 hydroxy group to give the (*S*)- and (*R*) MTPA esters **10a** and **10b**, respectively. The observed chemical shift differences $\Delta\delta$ ($\delta_S - \delta_R$) (Supporting Information S75) revealed the 17*S* configuration, and hence the absolute configuration of **10** was determined as 11*S*, 14*R*, 17*S*, 20*S*, 21*R*. Compound **10** was named 17*S*-dihydroaustalide K.

The austalides **1**, **2**, **6**, **8**, and **9** were assayed for their cytotoxic activity against MDA-MB-231 and JB6 Cl41 cell lines. None of the compounds exhibited cytotoxicity (IC_{50} < 10 μM).

The effect of compounds **1**, **2**, **6**, **8**, and **9** on the basal AP-1-dependent transcriptional activity was also studied using JB6 Cl41 cells stably expressing a luciferase reporter gene controlled by an AP-1-DNA binding sequence.[9−12] We have found that compounds **1**, **2**, **8**, and **9** are able to inhibit the transcriptional activity of the oncogenic nuclear factor AP-1 at noncytotoxic concentrations after 12 h of treatment. At a concentration of 6.25 μM these substances showed significant inhibition of the activity (Table 3), while no reduction of cell viability was observed up to 100 μM.

The austalides **1** to **9** were assayed for their inhibitory effects on an *endo*-1,3-β-D-glucanase from a crystalline stalk of the marine mollusk *Pseudocardium sachalinensis*. It was shown that compounds **1**−**5**, **8**, and **9** displayed strong inhibitory activities against this enzyme (Table 4).

Table 3. Inhibition of the Basal AP-1-Dependent Transcriptional Activity in JB6 Cl41 AP-1 Cells Treated with 6.25 μM of the Substances for 12 h[a]

compound	AP-1 transcriptional activity inhibition in % (at 6.25 μM)
control	0 ± 12.5
1	36.1 ± 9.5
3	34.0 ± 9.2
8	27.6 ± 5.9
9	36.3 ± 1.8

[a]All experiments were performed in triplicate. For all the values presented $p < 0.05$ (Student's *t*-test).

Table 4. Inhibition of *endo*-1,3-β-D-Glucanase from a Crystalline Stalk of the Marine Mollusk *Pseudocardium sachalinensis*

compound	IC_{50}, μM
1	0.2
2	1.0
3	0.1
4	0.01
5	0.12
6	1000
7	250
8	2.0
9	0.01
halistanolsulfate	130

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a PerkinElmer 343 polarimeter. UV spectra were recorded on a Shimadzu UV-1601PC spectrometer in MeOH. CD spectra were measured with a Chirascan-Plus CD spectrometer (United Kingdom). IR spectra were determined on a Bruker OPUS Vector-22 infrared spectrophotometer in $CHCl_3$. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$, MeOH-d_4, and pyridine-d_5 on a Bruker Avance-500 and Avance III-700 spectrometers operating at 500.13 and 125.77 MHz and 700.13 and 176.04 MHz, respectively, using TMS as an internal standard. HRESIMS spectra were measured on an Agilent 6510 Q-TOF LC mass spectrometer.

Low-pressure liquid column chromatography was performed using Si gel L (40/100 μm, Sorbpolimer, Russia). Glass plates (4.5 × 6.0 cm) precoated with Si gel (5−17 μm, Sorbfil) were used for thin-layer chromatography. Preparative HPLC was carried out on a Beckman-Altex chromatograph, using a Supelco Discovery C_{18} (5 μm, 4.6 × 250 mm) column with an RIDK−122 refractometer.

Fungal Strain. The strains of the fungi *Penicillium lividum* and *Penicillium thomii* were isolated from superficial mycobiota of the brown alga *Sargassum miyabei* (Lazurnaya Bay, the Sea of Japan) and were identified on the basis of morphological evaluation by one of the authors (N.N.K.). Strains are stored at the Collection of Marine Microorganisms, PIBOC, Vladivostok, Russia, with the codes KMM 4663 and KMM 4645, respectively.

Cultivation of *P. thomii* and *P. lividum*. The fungi were grown stationary at 22 °C for 21 days in 20 Erlenmeyer flasks (500 mL) (for each strain), each flask containing 20 g of rice, 20 mg of yeast extract, 10 mg of KH_2PO_4, and 40 mL of natural seawater (Marine Experimental Station of G.B. Elyakov Pacific Institute of Bioorganic Chemistry, Troitsa (Trinity) Bay, Sea of Japan).[7]

Extraction and Isolation. At the end of the incubation period, the mycelia and medium were homogenized and extracted with EtOAc (2 L). The extract of each fungus was concentrated to dryness. The residues were dissolved in 20% MeOH−H_2O (1 L) and were extracted with *n*-hexane (0.2 L × 3) and EtOAc (0.2 L × 3). After evaporation of the EtOAc layer, the residual materials (1.5 g, *P. thomii*; 1.3 g, *P. lividum*) were passed over silica columns (4 × 20 cm), which were eluted first with *n*-hexane (1 L) followed by a step gradient from 5% to 100% EtOAc in *n*-hexane (total volume 7 L). Fractions of 200 mL were collected and combined on the basis of TLC (Si gel, toluene−2-propanol, 6:1, v/v).

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Isolation Metabolites from P. thomii. The *n*-hexane−EtOAc (3:1, 0.4 L) eluate (35 mg) was purified by RP HPLC on a Supelco Discovery C_{18} column eluting with MeOH−H_2O (80:20) to yield **3** (4.5 mg). The *n*-hexane−EtOAc (7:3, 0.8 L) eluate (127 mg) gave **4** (5 mg) and **5** (1.9 mg) eluting with MeOH−H_2O (65:35). The *n*-hexane−EtOAc (3:2, 1.5 L) eluate (315 mg) yielded **1** (6.5 mg), **2** (11 mg), and **9** (3.8 mg) using MeOH−H_2O (65:35) and **8** (8 mg) after purification by HPLC (MeOH−H_2O, 50:50).

Isolation Metabolites from P. lividum. The *n*-hexane−EtOAc (5:1, 1.4 L) eluate (250 mg) was purified by RP HPLC on a Supelco Discovery C_{18} column eluting with MeOH−H_2O (80:20) to yield **6** (7 mg) and **10** (1.5 mg) and MeOH−H_2O (65:35) to yield **7** (1.5 mg) and **9** (2.7 mg). The *n*-hexane−EtOAc (1:1, 3.0 L) eluate (200 mg) gave **2** (4.5 mg), **4** (12 mg), **5** (3.8 mg), and **8** (2.5 mg) after purification by HPLC (MeOH−H_2O, 65:35).

*Austalide H acid butyl ester (**1**):* white powder, $[\alpha]^{20}_D$ −28 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 216 (4.36), 267 (3.96) nm; CD (*c* 0.05 mg/mL, MeOH) λ_{max} ($\Delta\varepsilon$) 211 (−1.32), 228 (+0.21), 262 (−1.21), 299 (−0.50) nm; IR ($CHCl_3$) ν_{max} 3610, 3526, 3440, 2961, 2875, 1748, 1712, 1610, 1457, 1438, 1373, 1340, 1310, 1140, 1103, 1071 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 519.2949 $[M + H]^+$ (calcd for $C_{25}H_{43}O_6$, 519.2952); 541.2767 $[M + Na]^+$ (calcd for $C_{29}H_{42}O_6Na$, 541.2772).

*Austalide H acid (**2**):* white powder, $[\alpha]^{20}_D$ −35 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 222 (3.74), 268 (3.47) nm; CD (*c* 0.05 mg/mL, MeOH) λ_{max} ($\Delta\varepsilon$) 211 (−0.36), 230 (+0.42), 270 (−0.08) nm; IR ($CHCl_3$) ν_{max} 3610, 3517, 3460, 2982, 1750, 1710, 1677, 1614, 1439, 1412, 1375, 1339, 1305, 1139, 1070, 1035, 1013 cm^{-1}; ^{13}C NMR data, see Tables 2; HRESIMS *m*/*z* 461.2196 $[M - H]^-$ (calcd for $C_{25}H_{33}O_8$, 461.2181); 485.2155 $[M + Na]^+$ (calcd for $C_{25}H_{34}O_8Na$, 485.2146).

*Austalide P acid butyl ester (**3**):* white powder, $[\alpha]^{20}_D$ −22 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 221 (3.47), 268 (3.19) nm; CD (*c* 0.05 mg/mL, MeOH) λ_{max} ($\Delta\varepsilon$) 209 (−4.58), 230 (+4.68), 269 (+2.63) nm; IR ($CHCl_3$) ν_{max} 3610, 3578, 3500, 3004, 2962, 2934, 2875, 1748, 1725, 1610, 1478, 1437, 1414, 1371, 1330, 1309, 1146, 1104, 1079, 1048 cm^{-1}; ^{13}C NMR data, see Tables 2; HRESIMS *m*/*z* 503.3000 $[M + H]^+$ (calcd for $C_{29}H_{43}O_7$, 503.3003); 525.2820 $[M + Na]^+$ (calcd for $C_{29}H_{42}O_7Na$, 525.2823).

*Austalide P acid (**4**):* white powder, $[\alpha]^{20}_D$ −35 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 223 (3.78), 269 (3.55) nm; CD (*c* 0.05 mg/mL, MeOH) λ_{max} ($\Delta\varepsilon$) 210 (−0.63), 234 (+0.12), 268 (−0.11), 296 (−0.11) nm; IR ($CHCl_3$) ν_{max} 3590, 3515, 2977, 1751, 1708, 1676, 1612, 1476, 1439, 1412, 1373, 1337, 1303, 1268, 1144, 1070, 1035, 1013 cm^{-1}; ^{13}C NMR data, see Tables 2; HRESIMS *m*/*z* 447.2386 $[M + H]^+$ (calcd for $C_{25}H_{35}O_7$, 447.2377); 469.2203 $[M + Na]^+$ (calcd for $C_{25}H_{34}O_7Na$, 469.2197).

*Austalide Q acid (**5**):* white powder, $[\alpha]^{20}_D$ −36 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 223 (3.79), 267 (3.47) nm; CD (*c* 0.05 mg/mL, MeOH) λ_{max} ($\Delta\varepsilon$) 210 (−0.73), 230 (+0.86), 263 (−0.09) nm; IR ($CHCl_3$) ν_{max} 3550, 3515, 2935, 1750, 1711, 1654, 1612, 1476, 1419, 1372, 1340, 1141, 1073, 1050 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 467.2044 $[M + Na]^+$ (calcd for $C_{25}H_{32}O_7Na$, 467.2040); 445.2219 $[M + H]^+$ (calcd for $C_{25}H_{33}O_7$, 445.2221); 443.2080 $[M - H]^-$ (calcd for $C_{25}H_{31}O_7$, 444.2075).

*13-Deoxyaustalide Q acid (**6**):* white powder, $[\alpha]^{20}_D$ −23 (*c* 0.58, MeOH); UV (MeOH) λ_{max} (log ε) 223 (4.31), 268 (4.04) nm; CD (*c* 0.05 mg/mL, MeOH) λ_{max} ($\Delta\varepsilon$) 212 (−2.51), 231 (+1.36), 267 (−0.02), 297 (−0.10) nm; ^{13}C NMR data, see Tables 2; HRESIMS *m*/*z* 451.2093 $[M + Na]^+$ (calcd for $C_{25}H_{32}O_6Na$, 451.2091); 427.2136 $[M - H]^-$ (calcd for $C_{25}H_{31}O_6$, 427.2126).

*17-O-Demethylaustalide B (**7**):* white powder, $[\alpha]^{20}_D$ −27 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ε) 223 (3.83), 263 (3.59) nm; CD (*c* 0.24 mg/mL, MeOH) λ_{max} ($\Delta\varepsilon$) 220 (+0.30), 230 (+0.09), 284 (+0.06) nm; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 483.1988 $[M + Na]^+$ (calcd for $C_{25}H_{32}O_8Na$, 483.1989).

*13-O-Deacetylaustalide I (**8**):* white powder, $[\alpha]^{20}_D$ −30 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.32), 267 (4.06) nm; IR ($CHCl_3$) ν_{max} 3610, 3573, 2928, 2856, 1753, 1712, 1611, 1479, 1438, 1414, 1374, 1338, 1310, 1287, 1140, 1071 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 445.2205 $[M + H]^+$ (calcd for $C_{25}H_{33}O_7$, 445.2221); 467.2025 $[M + Na]^+$ (calcd for $C_{25}H_{32}O_7Na$, 467.2040); 443.2082 $[M - H]^-$ (calcd for $C_{25}H_{31}O_7$, 443.2075).

*13-Deacetoxyaustalide I (**9**):* white powder, $[\alpha]^{20}_D$ −27 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 221 (4.09), 267 (3.79) nm; CD (*c* 0.05 mg/mL, MeOH) λ_{max} ($\Delta\varepsilon$) 213 (−1.54), 228 (+1.02), 260 (−0.59), 299 (−0.16) nm; IR ($CHCl_3$) ν_{max} 3610, 3570, 3510, 3005, 2934, 1748, 1720, 1611, 1609, 1478, 1456, 1438, 1414, 1373, 1337, 1309, 1283, 1247, 1145, 1112, 1081, 1050 cm^{-1}; ^{13}C NMR data, see Tables 2; HRESIMS *m*/*z* 429.2270 $[M + H]^+$ (calcd for $C_{25}H_{33}O_6$, 429.2272).

*17*S*-Dihydroaustalide K (**10**):* white powder, $[\alpha]^{20}_D$ −46 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 223 (4.17), 266 (3.85) nm; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 437.2287 $[M + Na]^+$ (calcd for $C_{25}H_{34}O_5Na$, 437.2298).

Preparation of (*S*)-MTPA and (*R*)-MTPA Esters of Austalide H Acid Butyl Ester (1). To a solution of **1** (2.0 mg) in pyridine were added 4-dimethylaminopyridine (a few crystals) and (*R*)-MTPACl (20 μL) at room temperature (25 °C) and stirred for 24 h. After evaporation of the solvent, the residue was purified by RP HPLC on a Supelco Discovery C_{18} column eluting with MeOH−H_2O (75:25) to afford the (*S*)-MTPA ester (**1a**). The (*R*)-MTPA ester (**1b**) was prepared in a similar manner using (*S*)-MTPACl. For ^1H, COSY, and MS data, see Supporting Information S8−10, S78.

Preparation of (*S*)-MTPA and (*R*)-MTPA Esters of 17*S*-Dihydroaustalide K (10). To a solution of austalidol K (0.8 mg) in pyridine-d_5 (0.6 mL) was added (*R*)-MTPACl (10 μL). The reaction mixture was shaken for 24 h at 25 °C to yield the (*S*)-MTPA ester of **10** (**10a**). The (*R*)-MTPA ester (**10b**) was prepared in a similar manner using (*S*)-MTPACl. For ^1H, COSY, and MS data, see Supporting Information S69−72, S78.

Enzyme Activity Assay and Protein Determination. Polysaccharide (laminaran) was isolated from the brown alga *Laminaria cichorioides* (Phaeophyceae) by the method described.[13] *endo*-1,3-β-D-Glucanase was isolated from the marine mollusk *P. sachalinensis*.

A standard reaction mixture contained 0.1% substrate solution (200 μL) and an enzyme solution (50 μL) in 0.025 M sodium acetate buffer, pH 5.2. The incubation was carried out for 20 min at 37 °C. The enzymatic hydrolysis of polysaccharides was determined from an increase in the amount of reducing sugars by the method of Nelson using glucose as a standard.[14] A unit of enzyme activity was defined as that amount of the enzyme required to catalyze the formation of 1 μM glucose in 1 min under standard conditions. The specific activity was expressed as unit/mg protein. The protein concentration in solution was determined by the method of Bradford.[15]

Inhibition Analysis Assay. Compounds were dissolved in EtOH (1 mg/mL). To 20 μL of an EtOH solution of the substances was added 20 μL of succinate buffer (0.025 M, pH 5.2). An equimolar mixture of EtOH and buffer was used as a negative control. Enzyme (10 μL) was added to all samples. Inhibitor preincubation time was 15 min. Enzyme activity was determined by the procedure described above. Halistanolsulfate was used as a reference substance.

Cytotoxicity Assay. The effect of the compounds on the cells' viability was evaluated using the MTS test, which is based on the reduction of MTS into its formazan product by live cells (Supporting Information S78).[16,17]

Determination of the Effects of Compounds on the Basal Transcriptional Activity of AP-1. The effects of the compounds on the basal transcriptional activities of AP-1 were evaluated using the JB6 Cl41 cell line stably expressing a luciferase reporter gene controlled by an AP-1-DNA binding sequence.[9] The experiment was performed as reported before[17a] with slight modifications (Supporting Information S78).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^1H, ^{13}C, COSY-45, HSQC, HMBC, and NOESY spectra of the new compounds **1**−**10**; DEPT spectra of **1**−**3**, **6**, **8**; ^1H, COSY

spectra of **1a**, **10a**, **10b**; ^{1}H spectra of **1b**; experimental details. These materials are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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