Natural Products

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Omaezallene from Red Alga *Laurencia* sp.: Structure Elucidation, Total Synthesis, and Antifouling Activity**

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Abstract: *Natural antifouling products have been the subject of considerable attention. We screened marine algae for antifouling activity and discovered omaezallenes, the new bromoallene-containing natural products isolated from the red alga Laurencia sp. Described is the isolation, structure elucidation, and total syntheses of omaezallenes. The relative and absolute configurations of natural omaezallenes were unambiguously established through total synthesis. The antifouling activities and ecotoxicity of omaezallenes were also evaluated.*

Antifouling coatings are needed to prevent organisms from fouling manmade submerged structures. Since the IMO (International Maritime Organization) treaty banned the use of tributyltin (TBT) on ships in 2008, natural antifouling products have been the subject of considerable attention. In particular, several natural marine antifouling products have been reported in the past decade as a result of the pursuit of nontoxic and environmentally benign paints.[1] These include isocyano compounds, which are well-known examples of natural antifouling products.[2] Currently, the mode of action of isocyanide is being explored using synthesized compounds.[3,4] However, a synthetic method is required to develop commercial antifouling agents and understand their mode of action. In recent work, we screened marine algae for antifouling activity and discovered omaezallenes, which were extracted from a red alga, *Laurencia* sp., and achieved the first total syntheses of the omaezallenes and 9-*epi*-omaezallenes. Herein, we report the isolation, structure elucidation, total synthesis, antifouling activity, and ecotoxicity of omaezallene (**1**; Figure 1) and its congeners.

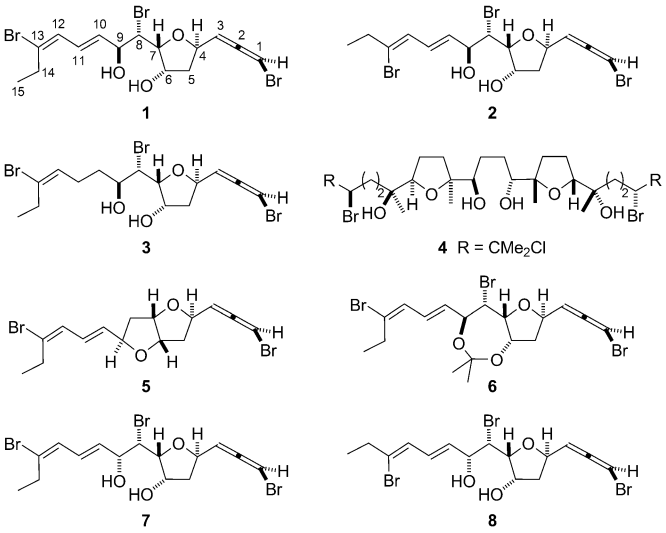

Figure 1. Brominated natural products (**1**–**5**) from red alga *Laurencia* sp., and unnatural omaezallenes (**6**–**8**).

Omaezallene (**1**) was isolated as a colorless oil ($[\alpha]_D^{21} = -127$ ($c = 0.41$ $CHCl_3$)) from a red alga, *Laurencia* sp., which was collected in Omaezaki, Japan. Voucher specimens were deposited in the herbarium of the Graduate School of Science, Hokkaido University. Taxonomic analysis of the species is in progress. A dried algal sample (250 g) was soaked in MeOH, and the MeOH extract was partitioned between EtOAc and H_2O. The lipophilic fraction was separated by silica gel chromatography and ODS-HPLC to give **1** (22.6 mg), **2** (1.6 mg), **3** (1.5 mg), and **4** (9.2 mg), and by preparative TLC to give **5** (9.7 mg). The NMR data for the bromoallene **5** was identical to those of the literature values.[5] The compound **4** was identified as intricatetraol.[6,7]

Based on HR-FABMS data, the molecular formula of **1** was assumed to be $C_{15}H_{19}Br_3O_3$ (*m*/*z* 482.8802, calcd for $C_{15}H_{18}{}^{79}Br_3O_3$, 482.8806 $[M-H]^-$). The existence of a bromoallene moiety was suggested by an IR absorption at 1954 cm^{-1}, ^{13}C NMR chemical shifts ($\delta = 201.3$, 101.1, 74.2 ppm), and ^1H NMR chemical shifts (Table 1). The ^1H

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Table 1: ^{1}H and ^{13}C NMR data for **1**.[a]

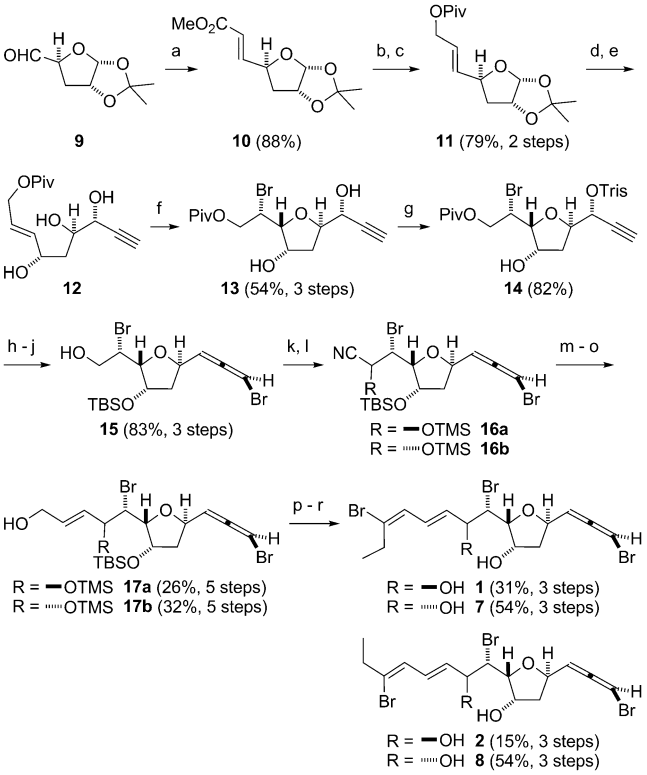
C	$\delta(^{13}C)$[b]	$\delta(^{1}H)$	Multiplicity	J [Hz]
1	74.2	6.10	dd	1.6, 5.7
2	201.3			
3	101.1	5.40	dd	5.7, 6.1
4	76.2	4.96	dddd	1.4, 6.0, 6.1, 9.6
5	40.4	2.27	ddd	1.0, 6.0, 14.0
		1.99	ddd	4.7, 9.6, 14.0
6	73.3	4.61	br s[c]	
7	83.9	4.05	dd	3.0, 10.5
8	55.7	4.24	dd	5.0, 10.5
9	73.1	4.48	br s[d]	
10	131.5	5.79	br dd	6.7, 13.5
11	127.5	6.43	ddd	1.0, 11.1, 13.5
12	130.2	6.48	d	11.1
13	132.6			
14	29.9	2.60	dq	1.2, 7.4
15	13.6	1.14	t	7.4
6-OH		2.10		
9-OH		2.92		

[a] Measured in [D_1]chloroform. [b] Assigned by the HMQC spectrum. [c] In D_2O; dd, $J = 1.0$, 3.0 (Hz). [d] In D_2O dd; $J = 5.0$, 6.1 (Hz).

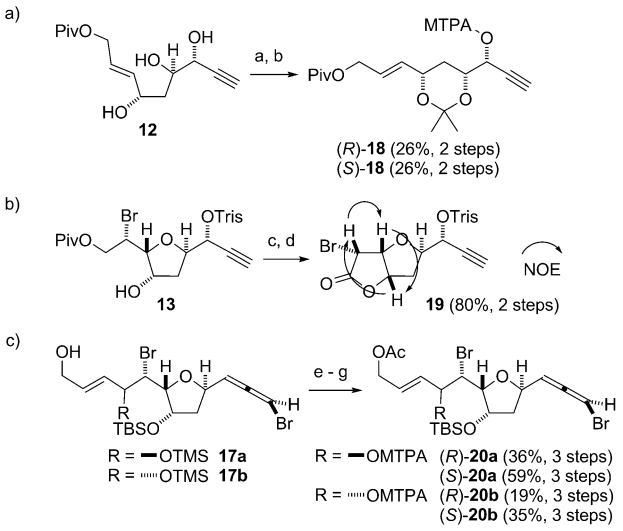
and ^{13}C NMR data for **1** were similar to those for **5**. However, the C8 methylene of **5** ($\delta_H = 1.73$ and 2.26 ppm, $\delta_C =$ 41.4 ppm) disappeared in the spectrum of **1**, and a brominated methine observed instead ($\delta_H = 4.24$ ppm, $\delta_C = 55.7$ ppm). The other notable differences in the ^{13}C NMR spectrum were observed at C6 and C9. In addition, two hydroxy protons were observed in **1**. Replacement of the ether bond of **5** with a diol at C6 and C9, as well as bromination at C8, accounted for the molecular formula of **1**. These interpretations together with COSY data revealed that **1** had a planar structure. The geometry of the double bond at C10 and C11 was determined to be *E* based on the coupling constant between H10 and H11 ($J = 13.5$ Hz). NOE contacts between H11 and H14 showed that the geometry of C12 and C13 was also *E*. Acetonide formaiton of **1** yielded **6**, thus allowing determination of the relative configurations from C4 to C8. NOESY experiments on **6** showed correlations between H4/H5a, H5b/H6, H6/H7, and H7/H8, which indicated the relative configurations of C4 to C8. The configuration at C9 was not determined at this point. The absolute configuration of the bromoallene moiety was assumed to be *R* based on its negative optical rotation and on Lowe's rule,[8] which has been applied to several acetogenins from *Laurencia*.

The compounds **2** and **3** are analogues of **1**. Notable differences between **1** and its isomer **2** were the chemical shifts of H11, H12, and C15. The NOESY peak between H12 and H14 of **2** revealed that **2** was the 12*Z* isomer of **1**. The molecular formula of **3** was determined to be $C_{15}H_{21}Br_3O_3$ based on ESI-TOFMS data (*m/z* 508.8893 (calcd for $C_{15}H_{22}{}^{79}Br_3O_3$, 508.8933 $[M+H]^+$)). Comparison of the ^{1}H NMR spectra of **1** and **3** indicated that the sp^2-carbon atoms at C10 and C11 in **1** did not exist in **3**, but instead there were two methylenes in **3**. Based on these data, together with analysis of the COSY spectrum, **3** was determined to be 10,11-dihydroomaezallene. The configurations of **2** and **3** were assumed to be identical to those of **1**.

To determine the absolute configuration of **1**, a total synthesis was carried out starting from the aldehyde **9**, prepared from D-glucose according to a known procedure,[9] which was converted into the pivaloate **11** via the unsaturated ester **10**[10] in three steps (Scheme 1). Removal of the acetonide group and addition of acetylide to the resultant hemiacetal gave the propargyl alcohol **12** with good diastereoselectivity (d.r. = 5:1). The desired diastereomer **12** for stereospecific installation[11] of the *R*-bromoallene moiety was formed as the major stereoisomer, as confirmed by the



Scheme 1. Total synthesis of omaezallenes. Reagents and conditions: a) $MeO_2C(H)C{=}PnBu_3$ (2.0 equiv), toluene, 90 °C, 30 min, 88%; b) DIBAL (3.0 equiv), CH_2Cl_2, 0 °C, 30 min; c) PivCl (3.5 equiv), Et_3N (3.5 equiv), CH_2Cl_2, RT, 3 h, 79% (2 steps); d) $TsOH{\cdot}H_2O$ (3.0 equiv), $MeCN/H_2O$ (10:1), 60 °C, 6 h; e) HC≡CMgCl (5.5 equiv), THF, 0 °C, 24 h; f) NBS (1.2 equiv), MeCN, 0 °C, 20 min, 54% (3 steps); g) Tris-Cl (1.2 equiv), DMAP (1.8 equiv), CH_2Cl_2, RT, 30 min, 82%; h) TBSOTf (1.5 equiv), 2,6-lutidine (2.0 equiv), CH_2Cl_2, 0 °C to RT, 2 h; i) LiBr (5.0 equiv), CuBr (5.0 equiv), THF, 60 °C, 4 h; j) DIBAL (2.4 equiv), CH_2Cl_2, 0 °C, 20 min, 83%, (3 steps); k) Dess–Martin periodinane (1.3 equiv), CH_2Cl_2, RT, 20 min; l) TMS-CN (2.0 equiv), Et_3N (0.2 equiv), CH_2Cl_2, RT, 90 min; m) DIBAL (1.5 equiv), Et_2O, 0 °C, 10 min; n) $EtO_2CCH_2P(O)(OEt)_2$ (1.5 equiv), NaHMDS (1.5 equiv), THF, −78 °C, 30 min; o) DIBAL (3.0 equiv), CH_2Cl_2, −78 °C, 10 min, 26% for **17a**, 32% for **17b** (5 steps); p) Dess–Martin periodinane (1.3 equiv), CH_2Cl_2, RT, 30 min; q) *n*-$PrPPh_3$-I (9.0 equiv), *n*BuLi, (9.0 equiv + 9.0 equiv), $BrCl_2CCCl_2Br$ (9.0 equiv), THF, −78 °C, 30 min, 88% (2 steps) from **17a**, 76% (2 steps) from **17b**; r) HF·Py, THF, 60 °C, 22 h, 36% for **1**, 17% for **2**, 72% for **7** + **8**. DIBAL = diisobutylaluminum hydride, DMAP = 4-(dimethylamino)pyridine, HMDS = hexamethyldisilazide, NBS = *N*-bromosuccinimide, Piv = pivaloyl, Py = pyridine, TBS = *tert*-butyldimethylsilyl, TMS = trimethylsilyl, Tf = trifluoromethanesulfonyl, Tris = 2,4,6-triisopropylbenzenesulfonyl, Ts = *para*-toluenesulfonyl.



Scheme 2. Determination of stereostructures. Reagents and conditions: a) $Me_2C(OMe)_2$ (10 equiv), CSA (catalytic), toluene, RT, 4 h, 35%; b) (*R*)- or (*S*)-MTPA-OH (1.5 equiv), EDCI (2.0 equiv), DMAP (catalytic), CH_2Cl_2, RT, 16 h, 75% for (*R*)-**18**, 74% for (*S*)-**18**; c) DIBAL (3.5 equiv), CH_2Cl_2, 0°C, 10 min; d) TEMPO (0.2 equiv), $PhI(OAc)_2$ (3.0 equiv), CH_2Cl_2, RT, 18 h, 80% (2 steps); e) Ac_2O (1.3 equiv), DMAP (1.3 equiv), CH_2Cl_2, RT, 15 min; f) HF·Py, THF, RT, 1.5 h; g) (*R*) or (*S*)-MTPA-Cl (3.0 equiv), Py (6.0 equiv), $(ClH_2C)_2$, 80°C, 18 h, 36% for (*R*)-**20a**, 59% for (*S*)-**20a** from the more polar epimer **17a** (3 steps), 19% for (*R*)-**20b**, 35% for (*S*)-**20b** from less polar epimer **17b** (3 steps). CSA = 10-camphorsulfonic acid, MTPA = 2-methoxy-2-trifluoromethylphenylacetyl, EDCI = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, TEMPO = 2,2,6,6-tetramethylpiperidine 1-oxyl.

modified Mosher method[12] with the acetonides (*R*)-**18** and (*S*)-**18** prepared from **12** (Scheme 2a). Intramolecular bromoetherification took place in a highly diastereoselective manner (d.r. = 10:1) upon treatment of **12** with NBS in acetonitrile to give the tetrahydrofuran **13**.[13] The resultant stereochemistries with regard to the contiguous stereocenters were unambiguously verified by an NOE experiment on the lactone **19**, which was derived from **13** in two steps (Scheme 2b). After conversion of **13** into the propargyl sulfonate **14**, stereospecific construction of *R*-bromoallene[14] with $LiCuBr_2$ and subsequent protecting-group manipulation afforded the alcohol **15**.

The stage was set for construction of the bromodiene moiety. The alcohol **15** was first transformed into the cyanohydrin **16** as a mixture of diastereomers (d.r. = 1:1; Scheme 1).[15] Reduction of the cyano group to an aldehyde through the use of DIBAL, a Horner–Wadsworth–Emmons reaction, and DIBAL reduction yielded a separable mixture of diastereomeric alcohols, **17a** and **17b**. The absolute configurations of **17a** (more polar) and **17b** (less polar) at C9 were assigned as *R* and *S*, respectively, by using the modified Mosher method with the MTPA esters (*R*)-**20a** and (*S*)-**20a** synthesized from **17a** and MTPA esters (*R*)-**20b** and (*S*)-**20b** synthesized from **17b** (Scheme 2c). The more-polar epimer **17a** was successively subjected to Dess–Martin oxidation, a modified Wittig reaction, as reported by Smith,[16] and removal of the silyl ether with a HF·pyridine complex to produce the omaezallenes **1** and **2** as a mixture of geometric isomers. After separation of the geometric isomers by silica gel column chromatography and subsequent HPLC, it was found that the ^1H and ^{13}C NMR spectra of **1** and **2** were completely identical to those of the natural *E*- and *Z*-omaezallene, respectively. The specific rotation values of synthetic **1** ($[\alpha]_D^{22} = -88.2$ ($c = 0.91$, $CHCl_3$)) and **2** ($[\alpha]_D^{22} = -72.5$ ($c = 0.71$, $CHCl_3$)) were similar to those of the natural products *E* omaezallene ($[\alpha]_D^{22} = -127$ ($c = 0.41$, $CHCl_3$)) and *Z* omaezallene ($[\alpha]_D^{22} = -44.3$ ($c = 0.0053$, $CHCl_3$)), respectively, thus indicating that the natural and synthetic omaezallenes were same enantiomers. Therefore, the absolute configurations of the natural omaezallenes **1** and **2** were undoubtedly established, as shown in Scheme 1. In addition, the less-polar C9 epimer **17b** was transformed into the C9 epimers **7** and **8** through the same synthetic scheme. Remarkably, the specific rotation values of 9-*epi*-*E*-omaezallene (**7**; $[\alpha]_D^{22} = +108.0$ ($c = 0.23$, $CHCl_3$)) and 9-*epi*-*Z*-omaezallene (**8**; $[\alpha]_D^{22} = +219.4$ ($c = 0.12$, $CHCl_3$)) are opposite in sign to those of **1** and **2**. The stereochemistry of the bromoallene moiety is often assumed based on Lowe's rule, which predicts the absolute configuration of allenes from the sign of their optical rotation. However, in **1** and **2** and **7** and **8**, the signs of the optical rotation values are not dependent on the stereochemistry of the C1 chiral axis. The results of a more detailed investigation will be reported in the near future.

The antifouling activities of natural and synthetic compounds against the cypris larvae of the barnacle *Amphibalanus amphitrite* were tested as described previously.[17] The antifouling activity of natural **1** ($EC_{50} = 0.22\ \mu g\,mL^{-1}$) was very potent (Table 2), while synthetic **1** showed a similar

Table 2: Biological activity value ($\mu g\,mL^{-1}$) with omaezallenes.

Compound	EC_{50}[a]	LC_{50}[a]	Ecotoxicity[b]
Natural **1**	0.22	4.8	2.5
Synthetic **1**	0.46	9.7	–
2	0.30	7.9	–
7	1.1	>10	–
8	1.2	>10	–

[a] Toward the cypris larvae of the barnacle *Amphibalanus amphitrite*. [b] LC_{50} toward the marine crustacean *Tigriopus japonicus*.

EC_{50} value ($0.46\ \mu g\,mL^{-1}$). The compound **1** showed only weak toxicity for barnacle larvae ($LC_{50} = 4.8\ \mu g\,mL^{-1}$). The natural **2** and synthetic **2** showed similar activities (EC_{50} = 0.50 and $0.30\ \mu g\,mL^{-1}$, $LC_{50} = 7.5$ and $7.9\ \mu g\,mL^{-1}$, respectively). The other congeners showed moderate activities (EC_{50} of natural **3** = 1.5; natural **5** = 5.6; synthetic **7** = 1.1; **8** = $1.2\ \mu g\,mL^{-1}$). These results show that the configuration at C9 is important in determining the activity of these compounds. The activity of **4** was also weak ($EC_{50} > 2.2\ \mu g\,mL^{-1}$).

In the development of environmentally benign antifouling agents, low ecotoxicity is essential. One representative ecotoxicity assay is toxicity to the freshwater crustacean *Daphnia magna*. Since antifouling agents are applied in the marine environment, we developed a toxicity assay based on the marine crustacean *Tigriopus japonicus* (for details see the Supporting Information). Commercially available antifouling

agents such as copper pyrithione and Sea-Nine 211 showed potent toxicities (48 h $LC_{50} = 0.03$ and $0.10\ \mu g\,mL^{-1}$, respectively) while the toxicity of **1** was low (48 h $LC_{50} = 2.5\ \mu g\,mL^{-1}$).

In summary, we achieved total syntheses, elucidated the structures, and evaluated the antifouling activities and ecotoxicity of omaezallenes. The absolute configuration of omaezallene was determined to be (1*R*, 4*R*, 6*S*, 7*R*, 8*S*, 9*S*) through total synthesis and NMR experiments with natural omaezallene. Investigations of structure–activity relationships with regard to antifouling activity in omaezallenes are currently underway.

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