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Antibacterial Quinone Metabolites from the Brown Alga, *Sargassum sagamianum*

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Antibacterial sargaquinoic acid derivatives were isolated from the brown alga *Sargassum sagamianum*. The structures of these quinone metabolites were confirmed by NMR and mass spectroscopy, and by comparison with published data. The relationship between their structures and antibacterial activities are briefly discussed.

Plastoquinones in plants are widely known to have many biological activities such as antioxidant activity, insect growth inhibition, cytotoxicity, and electron transport in the photosystem.[1–7] From the family of Sargassaceae algae, often visible about the coast of Japan, Japanese researchers have obtained plastoquinones such as sargaquinoic acid (**6**) and hydroxysargaquinone, and their derived chromenes such as sargachromenol (**7**) and hydroxysargaol (sargadiol-I).[8–12] Recently, plastoquinones and chromenes having antioxidant and antiviral activities were obtained from the same species.[13,14] These plastoquinones were easily converted into the corresponding chromenes.[3,8]

In our investigation of antibacterial compounds from the alga *Sargassum sagamianum*, five new sargaquinoic acid derivatives **1**–**5** were isolated. We describe herein the structures of these metabolites and discuss plausible pathways for their formation (Figure 1).

The MeOH/$CHCl_3$ (1:1 v/v) extract of the algae was subjected to silica gel column chromatography using a stepwise gradient of EtOAc/hexane. An antibacterial fraction of the EtOAc/hexane extracts was separated repeatedly by silica gel PLC and HPLC to afford five new sargaquinoic acid derivatives **1**–**5** together with known compounds, including sargaquinoic acid (**6**), sargachromenol (**7**), yezoquinolide, and 2-methyl-6-phytyl-1,4-benzoquinone, which were identified by comparison with NMR and MS spectra in the literature.[8,9]

The EIMS spectrum of **1** showed a characteristic base ion peak at m/z 175 $[M - C_{16}H_{25}O_3]^+$ indicating the plastoquinone skeleton, and a molecular ion peak at m/z 440.2537 ($C_{27}H_{36}O_5$, $[M]^+$ Δ −2.6 mmu). The ^{1}H and ^{13}C NMR spectra of **1** were very similar to the corresponding spectra of **6**, except for the ^{13}C chemical shifts were at δ_c 85.8 (C-14′) and 71.3 (C-15′) in **1** instead of the corresponding shifts at 123.4 and 132.1 in **6**, and the ^{1}H chemical shift was at δ 4.03 (H-14′) instead of δ 5.09. It was revealed to be the product (**1** was named 15′-hydroxysargaquinolide) formed by the lactonization via oxidation of the end double bond of isoprene units in **6**.

The HREIMS spectrum of **2** showed the same characteristic peaks at m/z 175 and 440 (m/z 440.2552: $C_{27}H_{36}O_5$, $[M]^+$ Δ −1.1 mmu) as **1**. The ^{1}H NMR spectrum showed four olefinic protons at δ 5.57 (H-3), 6.25 (H-4), 6.32 (H-5), and 6.48 (H-7), resembling those of the chromenol skeleton in **7**, and two methyl protons at δ 1.22 (H-14′) and 1.28 (H-13′) similar to the terminal isoprene unit of **1** (H-17′ and H-16′). Thus, **2** proved to be a chromenol derivative produced by the cyclization of **1**. Actually **1** was more unstable than **2**, and **1** was easily isomerized in pyridine at room temperature for 8 h to afford **2** in 30% yield together with starting material **1** (20%).[13]

Compound **3** showed a molecular formula of $C_{27}H_{34}O_4$ (m/z 422.2460 $[M]^+$ Δ +0.3 mmu) by HREIMS which indicates a dehydration compound from **1** or **2**. Also, a peak characteristic of a fragment of the quinone skeleton (m/z 175) was detected and the ^{1}H NMR spectrum showed end methylene signals at δ 4.95 and 5.04 (H-16′, d, J = 1.2 Hz). Compound **3** proved to be 15′-methylenesargaquinolide, which was formed by dehydration between the 15′-hydroxy group and a neighboring methyl hydrogen in **1**.

The HREIMS spectrum of **4** gave $C_{27}H_{34}O_6$, which indicates the molecular ion of **1** or **2** plus an oxygen atom. ^{1}H and ^{13}C NMR spectra revealed the presence of a lactone ring in the side chain and quinone skeleton as in **1** with the exception of having δ 1.67 and 1.89 as methylene signals next to the chromenol skeleton as in **2**, instead of the corresponding signal at δ 3.13 as those of the quinone skeleton in **1**. It also showed a signal at δ_c 82.9 of a methine carbon neighboring an oxygen like the chromenol skeleton in **2**. Detailed NMR analysis revealed that the skeleton of **4** was chromenquinone and that its side chain was a tetraprenyl group containing a lactone ring as in **1** and **2**; **4** was thus named chromequinolide.[15] Compound **4** might be postulated to be formed by the epoxidation of the 3′-position of the side chain followed by cyclization and subsequent oxidation.

The MS spectrum of **5** showed a molecular ion peak at m/z 258.1269 ($C_{16}H_{18}O_3$, $[M]^+$, Δ +1.3 mmu) and a base ion peak at m/z 175, which is characteristic of the quinone skeleton. ^{1}H and ^{13}C NMR spectra showed characteristic signals of a broad doublet at δ 2.94 (H-4′, J = 6.9 Hz, δ_c 42.4), and two double triplets at δ 6.75 (H-5′, J = 16.0, 6.9 Hz, δ_c 145.0) and at δ 6.09 (H-6′, J = 16.0, 1.4 Hz, δ_c 132.5), which were connected by COSY analysis. Further analysis of the HMBC spectrum

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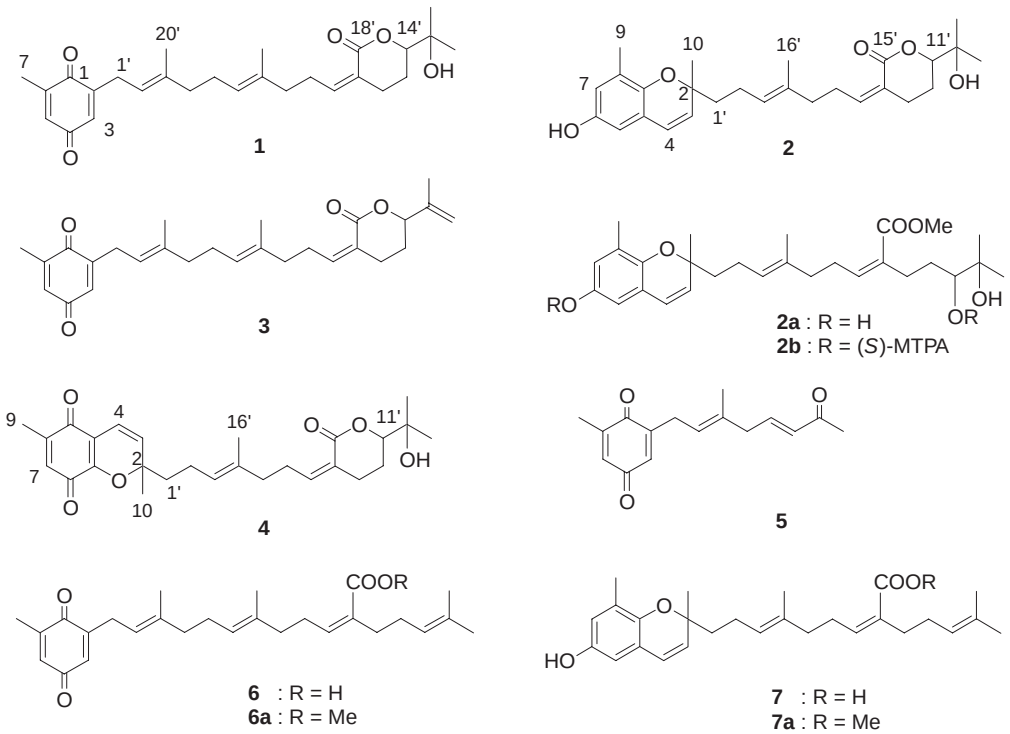

Figure 1. Sargaquinoic acid derivatives from *Sargassum sagamianum*.

connected C-5′ and C-7′ (cross peaks between H-5′/C-7′), and C-4′ and C-3′ (cross peaks between H-4′/C3′, H-4′/C-9′). These data revealed that **5** was (2′*E*,5′*E*)-2-methyl-6-(7′-oxo-3′-methylocta-2′,5′-dienyl)-1,4-benzoquinone, which was formed by oxidative cleavage of the 7′–8′ position of a quinone derivative such as **1** or **3** (Tables 1 and 2).

The chromenes such as **2** and **4** have a chiral center at C-2. They were considered to be artifacts from the corresponding plastoquinones because of their lack of optical activity.[3] Compound **2** has another chiral carbon at the C-11′ position besides C-2. After methanolysis of the lactone moiety with sodium methoxide, attempts at MTPA esterification of the product afforded diastereomers. Additionally, **1** and **2** have the same small optical rotation value; therefore it seems that the chiral carbon of the 11′-position in **2** exists in a racemic mixture as for kuhistanol.[16] Stereochemistry of the corresponding positions in **3** and **4** could not be determined because complex mixtures were obtained by the process of MTPA esterification.

Compounds **1**, **2**, and **6** show about 30% inhibition against *Bacillus subtilis* and *Staphylococcus aureus* as compared to a standard sample of vancomycin.[17] In contrast, **5** shows a strong inhibition of 80%. Thus, we conducted a detailed examination between the relationship of the compounds' structures and antibacterial activities by MIC measurements (Table 3). These compounds (especially **5**) proved to have strong activities against *S. aureus*. Compared to known compounds **6** and **7** with corresponding methyl esters **6a** and **7a**, the free carboxylic groups of **6** and **7** indicate very strong activity. However, **2** shows a reverse tendency. Additionally, it could be considered that the action mechanism of **7** is different from that of the others by MBC measurement results.

Compound **5** exhibits cytotoxicity against Hela S_3 cells with an IC_{50} of 4.0 μg mL^{-1}, and **1** and **2** show an IC_{50} of 10 μg mL^{-1}.[18] The activity of these compounds is about the same as that of chromene derivatives from plastquinone,[13] but weaker than that of cyclic depsipeptides reported in our laboratory.[19]

The brown algae, *Sargassaceae*, possess a bonanza of quinone metabolites, and the investigation of other bioactivities of these compounds and the detection of other new quinone derivatives from these algae are now in progress. Furthermore, the total synthesis of **5** is being attempted because of its detailed activities.

Experimental

General Experimental Procedures. NMR spectra were measured on a JEOL ECP-500 spectrometer operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C in $CDCl_3$. Optical rotation was determined on a Perkin-Elmer 341 digital polarimeter. Mass spectra were obtained with a JEOL MS-700 mass spectrometer. HPLC was performed on a Shimadzu LC-10 equipped with an RI or UV detector using a GL Science column (Inertsil prep-SIL, Inertsil prep-ODS, 10 × 250 or 20 × 250 mm^2). The solvents were distilled prior to use.

Plant Material. *Sargassum sagamianum* Yendo was collected in July 2005 at Manazuru in Kanagawa prefecture. The alga is often seen on the coast of Japan and was identified by Mr. A. Takahashi. A voucher specimen has been deposited at the Department of Chemistry and Biological Science, Aoyama Gakuin University.

Extraction and Isolation. An air-dried sample (320 g) was extracted with MeOH/$CHCl_3$ (1:1, 3 × 3 L) for 1 day. The combined extracts were concentrated and partitioned between $CHCl_3$ and H_2O (650 mL each). The $CHCl_3$ extract (6.1 g) was subjected to silica gel column chromatography (Fuji Silysia, BW-300 7.0 × 47.0 cm^2) using a stepwise gradient of EtOAc/hexane to afford roughly ten fractions. Checking against characteristic

Table 1. NMR Data for **1**, **3**, and **5**

#	**1**				**3**				**5**			
	C	H	J/Hz	HMBC H→C	C	H	J/Hz	HMBC H→C	C	H	J/Hz	HMBC H→C
1	188.0				188.0				187.8			
2	148.5				148.5				147.8			
3	132.3	6.46	dt, 2.7, 1.8	4	132.3	6.46	dt, 2.7, 1.8		132.4	6.47	m	4, 5, 1′
4	188.0				188.0				187.8			
5	133.2	6.55	dq, 2.7, 1.4	4	133.2	6.54	dq, 2.7, 1.4	4	133.2	6.57	dq, 1.8, 1.4	3, 4
6	145.9				145.9				146.0			
7	16.0	2.06	d, 1.4	1, 5, 6	16.0	2.06	d, 1.4	1, 5, 6	16.0	2.07	d, 1.4	1, 5, 6
1′	27.5	3.13	d, 7.8	2, 3, 2′, 3′	27.5	3.13	d, 7.3	2, 2′, 3′	27.8	3.16	br d, 7.3	1, 2, 3, 2′, 3′
2′	118.0	5.15	tq, 7.3, 1.3	1′	118.0	5.15	m		121.2	5.24	tq, 7.3, 1.4	1′
3′	139.8				139.8				136.2			
4′	39.6	2.10	m	5′	38.8	2.06	m	5′	42.4	2.94	br d, 6.9	2′, 3′, 5′, 6′, 9′
5′	26.4	2.10	m	4′	26.4	2.11	t, 7.1		145.0	6.75	dt, 16.0, 6.9	7′
6′	124.6	5.12	tq, 6.9, 1.3		124.6	5.13	m		132.5	6.09	dt, 16.0, 1.4	
7′	134.6				134.6				198.3			
8′	38.8	2.10	m	9′	39.6	2.11	t, 7.1	9′	27.1	2.26	s	7′
9′	28.1	2.70	q, 7.3	10′	28.0	2.72	br q, 6.8		16.4	1.67	br s	2′, 3′, 4′
10′	147.8	6.06	tt, 7.3, 1.8		147.6	6.03	tt, 7.3, 1.8					
11′	124.6				124.3							
12′	28.7	2.56	m		28.4	2.55	m					
13′	23.5	1.70	ddt, 13.7, 11.9, 6.4		27.5	1.83	dddd, 13.7, 10.1, 9.6, 6.9					
		1.98	ddt, 13.7, 5.5, 2.8			1.99	ddt, 13.7, 5.0, 3.2					
14′	85.8	4.03	dd, 11.9, 2.8		82.0	4.65	dd, 10.1, 3.2					
15′	71.3				142.6							
16′	25.7	1.28	br s		112.9	5.04	d, 1.2					
						4.95	d, 1.2					
17′	24.2	1.23	br s		18.1	1.78	s					
18′	165.5											
19′	15.9	1.60	br s		15.9	1.60	br s					
20′	16.1	1.62	br s		16.1	1.62	br s					

Table 2. NMR Data for **2**, **2a**, and **4**

#	**2**				**2a**			**4**			
	C	H	J/Hz	HMBC H→C	C	H	J/Hz	C	H	J/Hz	HMBC H→C
2	77.8				77.8			82.9			
3	130.7	5.57	d, 10.1	1′	130.7	5.52	d, 9.6	128.6	5.55	d, 10.1	2, 4
4	122.9	6.25	d, 10.1	8a	122.9	6.25	d, 9.6	115.7	6.50	d, 10.1	2
4a	121.3				121.3			115.5			
5	110.2	6.32	d, 3.2	8a	110.3	6.32	d, 3.2	184.5			
6	148.5				148.6			145.4			
7	117.0	6.48	br d, 3.2	5, 6, 8a	117.1	6.48	d, 3.2	131.3	6.47	q, 1.4	6, 8a
8	126.3				126.3			181.3			
8a	144.8				145.0			150.8			
9	15.5	2.14	s	7, 8, 8a	15.5	2.14	s	15.9	2.05	d, 1.4	7, 8, 8a
10	25.7	1.36	s	2, 3, 1′	26.0	1.36	s	27.4	1.46	s	2, 3, 1′
1′	40.7	1.66	m	2′	40.7	1.66	m	41.4	1.67	m	2′
									1.89	ddd, 14.2, 9.6, 6.4	
2′	22.6	2.08	m		22.6	2.12	m	22.5	2.03–2.14	m	
3′	125.0	5.12	br t, 7.3	2′	125.0	5.12	br t, 7.3	124.1	5.11	br t, 7.3	2′
4′	134.2				134.3			135.1			
5′	38.8	2.08	m	6′	39.1	2.05	t, 7.3	38.7	2.03–2.14	m	6′
6′	27.9	2.68	dt, 7.3, 7.2		28.0	2.58	dt, 7.3, 7.3	27.9	2.69	dt, 7.3, 7.2	5′
7′	147.9	6.01	tt, 7.3, 1.8		143.0	5.92	t, 7.3	147.5	6.03	tq, 7.3, 1.3	
8′	124.2				131.2			124.5			
9′	28.7	2.52	m		31.5	2.47	m	28.7	2.55	m	
						2.30	ddt, 16.0, 8.2, 2.8				
10′	23.5	1.66	m		31.4	1.42	dddd, 13.3, 10.6, 8.2, 5.0	23.5	1.69	m	
		1.97	ddt, 13.7, 6.0, 2.7			1.59	m		1.98	ddt, 13.7, 5.5, 2.7	
11′	85.7	4.01	ddd, 11.9, 2.7, 0.9		77.5	3.33	dd, 10.5, 1.8	85.7	4.02	dd, 11.9, 2.7	
12′	71.3				73.0			71.4			
13′	25.9	1.28	s	11′, 12′, 14′	26.4	1.19	s	25.7	1.28	s	11′, 12′, 14′
14′	24.1	1.22	s	11′, 12′, 13′	23.3	1.14	s	24.3	1.23	s	11′, 12′, 13′
15′	165.5				168.8						
–OCH_3					51.4	3.73	s				
16′	15.7	1.57	s	3′, 4′, 5′	15.7	1.56	s	15.9	1.56	s	3′, 4′, 5′

Table 3. Antibacterial Activity against *S. aureus*

Compound	MIC /μg mL^{-1}	MBC /μg mL^{-1}	Compound	MIC /μg mL^{-1}
1	16	128		
2	128	>256	**2a**	32
5	2	64		
6	32	256	**6a**	>256
7	8	8	**7a**	>256
Vancomycin	1	1		

^{1}H NMR signals (around δ 6.2–6.5) and/or antibacterial activities, the sixth and seventh fractions (60–80% EtOAc/hexane, 3.3 g) were re-chromatographed by silica gel column and/or PLC, and new quinone metabolites **1**–**5** were purified by repeated HPLC using EtOAc/hexane; **1** (5.5 mg, 1.7×10^{-3}%), **2** (15.8 mg, 4.9×10^{-3}%), **3** (0.7 mg, 2.3×10^{-4}%), **4** (4.7 mg, 1.5×10^{-3}%), and **5** (2.4 mg, 7.6×10^{-4}%), together with known **6** (28.4 mg, 8.9×10^{-3}%), **7** (510 mg, 1.6×10^{-1}%), yezoquinolide (1.1 mg, 3.5×10^{-4}%), and 2-methyl-6-phytyl-1,4-benzoquinone (0.9 mg, 2.9×10^{-4}%).

Compound 1 (15′-Hydroxysargaquinolide): Yellowish oil $[\alpha]_D^{20} = -5^\circ$ (*c* 0.025, $CHCl_3$); IR (KBr) ν_{max} 3460, 2926, 1716, 1653, 1457, 1381, 1294, 1180, 914, 680 cm^{-1}; HREIMS *m/z* 440.2537 [M]$^+$ (calcd for $C_{27}H_{36}O_5$, 440.2563); ^{1}H and ^{13}C NMR, see Table 1.

Compound 2 (11′-Hydroxysargachromelide): Yellowish oil $[\alpha]_D^{20} = -5^\circ$ (*c* 0.042, $CHCl_3$); IR (KBr) ν_{max} 3425, 2926, 1715, 1700, 1636, 1385, 1250, 1097, 758 cm^{-1}; HREIMS *m/z* 440.2552 [M]$^+$ (calcd for $C_{27}H_{36}O_5$, 440.2563); ^{1}H and ^{13}C NMR, see Table 2.

Compound 3 (15′-Methylenesargaquinolide): Yellowish oil $[\alpha]_D^{20} = +10^\circ$ (*c* 0.037, $CHCl_3$); IR (KBr) ν_{max} 3425, 2926, 1716, 1653, 1380, 1294, 1192, 908 cm^{-1}; HREIMS *m/z* 422.2460 [M]$^+$ (calcd for $C_{27}H_{34}O_4$, 422.2457); ^{1}H and ^{13}C NMR, see Table 1.

Compound 4 (Chromequinolide): Reddish oil $[\alpha]_D^{20} = +3^\circ$ (*c* 0.104, $CHCl_3$); IR (KBr) ν_{max} 3425, 1722, 1714, 1668, 1651, 1471, 1385, 1192, 921, 756 cm^{-1}; HREIMS *m/z* 454.2368 [M]$^+$ (calcd for $C_{27}H_{34}O_6$, 454.2350); ^{1}H and ^{13}C NMR, see Table 2.

Compound 5 [(2′*E*,5′*E*)-2-Methyl-6-(7′-oxo-3′-methylocta-2′,5′-dienyl)-1,4-benzoquinone]: Yellowish oil; IR (KBr) ν_{max} 3425, 2924, 1714, 1659, 1651, 1456, 1361, 1294, 1255, 979, 914, 756 cm^{-1}; HREIMS *m/z* 258.1269 [M]$^+$ (calcd for $C_{16}H_{18}O_3$, 258.1256);^{1}H and ^{13}C NMR, see Table 1.

Methanolysis of 2 (2a): To a solution of **2** (4.1 mg, 9.3 μmol) in methanol (0.2 mL), 0.3 M sodium methoxide solution (0.40 mL) was added and stirred constantly at room temperature. After 2.5 h, the solution was neutralized by several drops of saturated aqueous NH_4Cl and was passed through an ODS-cartridge. The extract was purified by SIL-HPLC (60% EtOAc in hexane) to afford the desired product **2a** in a 82% yield.

2a: Colorless oil $[\alpha]_D^{20} = +3^\circ$ (*c* 0.172, $CHCl_3$); HREIMS *m/z* 472.2851 [M]$^+$ (calcd for $C_{28}H_{40}O_6$, 472.2826); ^{1}H and ^{13}C NMR, see Table 2.

(*S*)-MTPA Esterification of 2a (2b): A solution of **2a** (4.1 mg, 9.3 μmol), (*R*)-MTPA-Cl (50 μmol), Et_3N (16 μL), and a catalytic amount of DMAP in CH_2Cl_2 (0.30 mL) was stirred for 16 h at room temperature. The reaction mixture was added to *N*,*N*-dimethyl-1,3-propanediamine and was blown down with nitrogen. The residue was subjected to SIL-HPLC (30% EtOAc in hexane) to give two (*S*)-MTPA diesters **2b**. **2b** (rt 18.0 min): 1.13 (s, 13′-CH_3), 1.19 (s, 14′-CH_3), 2.50 (dt, J = 7.5, 7.5 Hz, 9′-H), 3.56 and 3.68 (s, –OCH_3), 3.70 (s, –$COOCH_3$), 4.98 (dd, J = 7.5, 2.5 Hz, 11′-H), 5.79 (t, J = 7.4 Hz, 7′-H): **2b′** (rt 18.8 min): 1.11 (s, 13′-CH_3), 1.14 (s, 14′-CH_3), 2.50 (dt, J = 7.5, 7.5 Hz, 9′-H), 3.59 and 3.68 (s, –OCH_3), 3.71 (s, –$COOCH_3$), 4.98 (dd, J = 7.5, 2.5 Hz, 11′-H), 5.86 (t, J = 7.4 Hz, 7′-H).

Methylation of 6 to 6a: To a solution of **6** (6.1 mg, 14 μmol) in methanol (0.3 mL) and hexane (0.4 mL) was added $TMSCH_2N_2$ (144 μmol) in hexane (72 μL) at room temperature. After the reaction mixture was stirred for 10 min at room temperature and blown down with nitrogen, $TMSCH_2N_2$ was evaporated under reduced pressure. The oily residue was purified by passing over a small plug of silica gel (10% EtOAc in hexane), followed by SIL-HPLC separation (7% EtOAc in hexane) to afford **6a** (1.5 mg, 24% yield) as a pale yellowish oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.57 (s, H-16′), 1.60 (s, H-19′), 1.62 (s, H-20′), 1.67 (s, H-17′), 2.03–2.13 (m, H-4′, 5′, 8′, 13′), 2.06 (d, J = 1.4 Hz, H-7), 2.24 (t, J = 7.3 Hz, H-12′), 2.51 (dt, J = 7.3, 7.3 Hz, H-9′), 3.12 (br d, J = 7.3 Hz, H-1′), 3.73 (s, 18′-OMe), 5.08 (m, H-14′), 5.12 (m, H-6′), 5.15 (m, H-2′), 5.85 (br t, J = 7.3 Hz, H-10′), 6.46 (dt, J = 1.8, 2.8 Hz, H-3), 6.54 (dq, J = 2.8, 1.4 Hz, H-5). ^{13}C NMR (125 MHz, $CDCl_3$): δ 15.9 (q, C-19′), 16.0 (q, C-7), 16.2 (q, C-20′), 17.7 (q, C-16′), 25.7 (q, C-17′), 26.5 (t, C-5′), 27.6 (t, C-1′), 27.9 (t, C-13′), 28.0 (t, C-9′), 34.7 (t, C-12′), 39.2 (t, C-8′), 39.6 (t, C-4′), 51.1 (q, 18′-OMe), 118.0 (d, C-2′), 123.5 (d, C-14′), 124.5 (d, C-6′), 131.5 (s, C-11′), 132.1 (s, C-15′), 132.3 (d, C-3), 133.2 (d, C-5), 134.7 (s, C-7′), 139.9 (s, C-3′), 142.1 (d, C-10′), 145.9 (s, C-6), 148.5 (s, C-2), 168.5 (s, C-18′), 188.0 (s, C-1, 4). HREIMS *m/z* 438.2782 [M]$^+$ (calcd for $C_{28}H_{38}O_4$: 438.2771).

Methylation of 7 to 7a: Similar to the synthesis of **6a**, **7a** was obtained in a 79% yield as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.35 (s, H-10), 1.57 (s, H-16′), 1.58 (s, H-13′), 1.65 (m, H-1′), 1.67 (s, H-14′), 2.04 (t, J = 7.3 Hz, H-5′), 2.07 (dt, J = 7.3, 7.3 Hz, H-10′), 2.08 (m, H-2′), 2.14 (s, H-9), 2.24 (t, J = 7.4 Hz, H-9′), 2.50 (dt, J = 7.3, 7.3 Hz, H-6′), 3.73 (s, 15′-OMe), 5.08 (br t, J = 7.3 Hz, H-11′), 5.13 (br t, J = 7.3 Hz, H-3′), 5.58 (d, J = 9.6 Hz, H-3), 5.83 (br t, J = 7.3 Hz, H-7′), 6.24 (d, J = 10.1 Hz, H-4), 6.32 (d, J = 3.2 Hz, H-5), 6.48 (d, J = 3.2 Hz, H-7). ^{13}C NMR (125 MHz, $CDCl_3$): δ 15.5 (q, C-9), 15.7 (q, C-16′), 17.7 (q, C-13′), 22.6 (t, C-2′), 25.7 (q, C-10), 25.7 (q, C-14′), 27.8 (t, C-10′), 28.0 (t, C-6′), 34.7 (t, C-9′), 39.1 (t, C-5′), 40.8 (t, C-1′), 51.1 (q, 15′-OMe), 77.8 (s, C-2), 110.3 (d, C-5), 117.0 (d, C-7), 121.3 (s, C-4a), 122.9 (d, C-4), 123.5 (d, C-11′), 124.8 (d, C-3′), 126.3 (s, C-8), 130.7 (d, C-3), 131.4 (s, C-8′), 132.1 (s, C-12′), 134.4 (s, C-4′), 142.1 (d, C-7′), 144.9, (s, C-8a) 148.6 (s, C-6), 168.6 (s, C-15′). HREIMS *m/z* 438.2784 [M]$^+$ (calcd for $C_{28}H_{38}O_4$: 438.2771).

Antibacterial Assay.[20,21] The microdilution method was employed as an antibacterial activity test against the microorganism, *Staphylococcus aureus* (ATCC 6538P) obtained from RIKEN BioResource Center, Saitama, Japan). The specimen was suspended in aqueous DMSO at 512 μg mL^{-1} final concentration, and the suspension was transferred to a 96-well microplate (100 μL well^{-1} except for the first line which contained 200 μL well^{-1}). Two-fold dilutions were then prepared in a concentration range from 0.5 to 256 μg mL^{-1} (for positive control, the vancomycin concentration range was 0.125–64 μg mL^{-1}) diluted in 100 μL of brain heart infusion (BHI) broth. A 5-μL volume, which contained approximately 10^7 cfu mL^{-1} of *S. aureus*, was added. Following incubation of the plates for 24 h at 37 °C in ambient air, the minimum inhibitory concentration (MIC) values were determined by the lowest concentration at which observable growth was inhibited. Minimum bactericidal concentrations (MBC) were determined

by plating out aliquots of incubation BHI on agar plates in 1 μL drops (undiluted) of MIC testing mixtures that showed no growth in the BHI microplates after 24 h incubation. MBC values were recorded as the lowest concentrations at which no colony of *S. aureus* was observed. Experiments were carried out three times.

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References

1 D. H. S. Silva, F. C. Pereira, M. V. B. Zanoni, M. Yoshida, *Phytochemistry* **2001**, *57*, 437.

2 C. L. Céspedes, P. Torres, J. C. Marín, A. Arciniegas, A. R. de Vivar, A. L. Pérez-Castorena, E. Aranda, *Phytochemistry* **2004**, *65*, 1963.

3 A. Numata, S. Kanbara, C. Takahashi, R. Fujiki, M. Yoneda, Y. Usami, E. Fujita, *Phytochemistry* **1992**, *31*, 1209.

4 J. Kruk, K. Burda, G. H. Schmid, A. Radunz, K. Strzalka, *Photosynth. Res.* **1998**, *58*, 203.

5 A. Numata, S. Kanbara, C. Takahashi, R. Fujiki, M. Yoneda, E. Fujita, Y. Nabeshima, *Chem. Pharm. Bull.* **1991**, *39*, 2129.

6 A. L. Pérez-Castorena, A. Arciniegas, M. T. R. Apan, J. L. Villaseñor, A. R. de Vivar, *Planta Med.* **2002**, *68*, 645.

7 W. H. Gerwick, W. Fenical, *J. Org. Chem.* **1981**, *46*, 22.

8 T. Kusumi, Y. Shibata, M. Ishitsuka, T. Kinoshita, H. Kakisawa, *Chem. Lett.* **1979**, 277.

9 M. Segawa, H. Shirahama, *Chem. Lett.* **1987**, 1365.

10 T. Kato, A. S. Kumanireng, I. Ichinose, Y. Kitahara, Y. Kakinuma, Y. Kato, *Chem. Lett.* **1975**, 335.

11 M. Ishitsuka, T. Kusumi, Y. Nomura, T. Konno, H. Kakisawa, *Chem. Lett.* **1979**, 1269.

12 T. Kikuchi, Y. Mori, T. Yokoi, S. Nakazawa, H. Kuroda, Y. Masada, K. Kitamura, Y. Kuriyama, *Chem. Pharm. Bull.* **1983**, *31*, 106.

13 M. Iwashima, J. Mori, X. Ting, T. Matsunaga, K. Hayashi, D. Shinoda, H. Saito, U. Sankawa, T. Hayashi, *Biol. Pharm. Bull.* **2005**, *28*, 374.

14 K. H. Jang, B. H. Lee, B. W. Choi, H.-S. Lee, J. Shin, *J. Nat. Prod.* **2005**, *68*, 716.

15 P. E. Brown, R. A. Lewis, M. A. Waring, *J. Chem. Soc., Perkin Trans. 1* **1990**, 2979.

16 B. Chen. K. Kawazoe, Y. Takaishi, G. Honda, M. Itoh, Y. Takeda, O. K. Kodzhimatov, O. Ashurmetov, *J. Nat. Prod.* **2000**, *63*, 362.

17 K. Kousaka, N. Ogi, Y. Akazawa, M. Fujieda, Y. Yamamoto, Y. Takada, J. Kimura, *J. Nat. Prod.* **2003**, *66*, 1318.

18 Y. Nakao, S. Yoshida, S. Matsunaga, N. Fusetani, *J. Nat. Prod.* **2003**, *66*, 524.

19 Y. Takada, M. Umehara, Y. Nakao, J. Kimura, *Tetrahedron Lett.* **2008**, *49*, 1163.

20 W.-J. Syu, C.-C. Shen, J.-J. Lu, G.-H. Lee, C.-M. Sun, *Chem. Biodiversity* **2004**, *1*, 530.

21 S. Boonsri, C. Karalai, C. Ponglimanont, A. Kanjana-opas, K. Chantrapromma, *Phytochemistry* **2006**, *67*, 723.