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Phenanthrene Derivatives from *Cymbidium* Great Flower Marie Laurencin and Their Biological Activities

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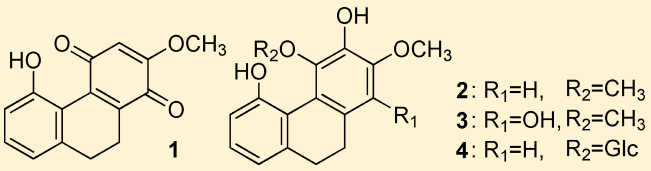
Supporting Information

ABSTRACT: A new phenanthrendione, ephemeranthoquinone B (**1**), two phenanthrenes, marylaurencinols A (**2**) and B (**3**), and a phenanthrene glucoside, marylaurencinoside A (**4**), were isolated from the roots of *Cymbidium* Great Flower Marie Laurencin, along with six known phenanthrenes, **5**−**10**. The structures of these compounds were established by a combination of extensive NMR spectroscopy and/or X-ray crystallographic analysis and chemical degradation. The compounds were tested for antibacterial activities against *Bacillus subtilis* and *Klebsiella pneumoniae* and for cytotoxic activity against the human promyelocytic leukemia (HL-60) cell line. Compounds **1**, **3**, and **6** showed antibacterial activities with minimum inhibitory concentration (MIC) values in the range of 4.88 to 65.10 μM. Notably, ephemeranthoquinone B (**1**) had a strong antibacterial effect on *B. subtilis*. Furthermore, **1** exhibited moderate cytotoxic activity (IC_{50} 2.8 μM) against HL-60 cells. Compounds **4**−**9** also showed weak cytotoxic activity against the HL-60 cell line with IC_{50} values of 19.3−52.4 μM.



Phenanthrenes are tricyclic aromatic hydrocarbons, probably biosynthesized by the oxidative coupling of the aromatic rings of stilbene precursors in higher plants.[1] A large number of phenanthrenes have been isolated from the Orchidaceae family, *Cymbidium*,[2] *Dendrobium*,[3] *Bulbophyllum*,[4] *Maxillaria*,[5] *Bletilla*,[6] *Ephemerantha*,[7] and *Eria*.[8] The Orchidaceae family consists of more than 35 000 species in approximately 750 genera of the flowering plants, which are widely distributed in temperate and tropical regions. Since ancient times, the Orchidaceae have been used not only for ornamental purposes but also as medicinal plants, to treat paralysis, cholera, diarrhea, and sores.[9] Therefore, the Orchidaceae may be a prodigious source of potential new drugs. Phenanthrenes exhibit various biological activities such as anti-inflammatory,[9] antiallergic,[9] antimicrobial,[10] cytotoxic,[11,12] antiplatelet aggregation inhibitory,[13] phytotoxic,[14] antifungal,[15] spasmolytic,[16] antifibrotic,[17] and inhibitory effects on NO production.[9]

In the course of our study of bioactive substances from the Orchidaceae, we started a search for novel antimicrobial metabolites from *Cymbidium* Great Flower Marie Laurencin. Each extract of this plant was tested for antimicrobial activities against *Bacillus subtilis* and *Klebsiella pneumoniae*. From the bioassay-guided separation of the EtOAc-soluble extract, a new phenanthrendione, ephemeranthoquinone B (**1**), two new phenanthrenes, marylaurencinols A (**2**) and B (**3**), and a phenanthrene glycoside, marylaurencinoside A (**4**), together with known phenanthrenes (**5**−**10**[16−18]) were found. This paper describes the isolation, structural elucidation, and biological activities of these compounds.

RESULTS AND DISCUSSION

Fresh roots of *C.* Great Flower Marie Laurencin were extracted with MeOH at room temperature. The MeOH extract was partitioned into an EtOAc−H_2O mixture to afford EtOAc- and H_2O-soluble portions. The EtOAc extract showed antimicrobial activity against *B. subtilis* at 18.75 μg/mL. The EtOAc extract was subjected to bioassay-guided separation, fractionated by silica gel column chromatography, and further purified by RP-HPLC to give a new phenanthrendione, ephemeranthoquinone B (**1**), two new phenanthrenes, marylaurencinols A (**2**) and B (**3**), and a phenanthrene glucoside, marylaurencinoside A (**4**), together with known phenanthrenes **5**−**10**.

Ephemeranthoquinone B (**1**) was obtained as red needles. The EIMS indicated a molecular ion at *m*/*z* 256 $[M]^+$, and the molecular formula was determined as $C_{15}H_{12}O_4$ by HREIMS in conjunction with NMR data analysis. The IR spectrum of **1** revealed strong absorption bands due to an aromatic ring (1595, 1452 cm^{-1}), conjugated carbonyl groups (1670, 1639 cm^{-1}), and hydroxy groups (3063 cm^{-1}). The UV spectrum supported the presence of the aromatic ring (λ_{max} 217.6, 223.4, 242.0, 276.2, 321.2 nm). In the ^1H NMR spectrum, four

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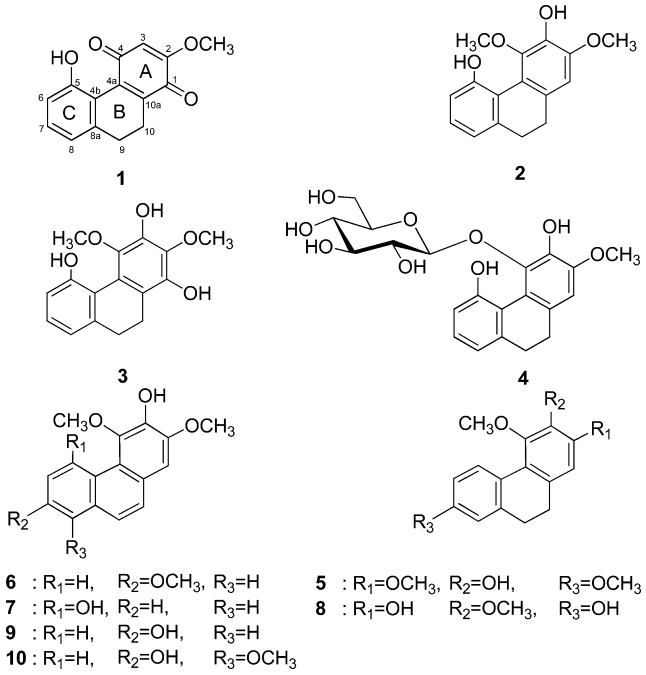

aromatic proton signals at δ_H 7.23 (t, J = 8.2 Hz), 6.91 (dd, J = 8.2, 1.4 Hz), 6.78 (dd, J = 8.2, 1.4 Hz), and 6.04 (s) were observed. The last three signals indicated the presence of a 1,2,3-trisubstituted phenyl group. Two methylene signals at δ_H 2.71 and 2.66 and one methoxy signal at δ_H 3.90 were also observed. The ^{13}C NMR spectrum also showed the presence of two carbonyl carbons (δ_C 191.5, 180.8), two methylene carbons (δ_C 28.5, 21.3), and 10 sp^2 hybridized carbons (δ_C 158.6, 155.3, 143.2, 140.6, 139.0, 132.3, 120.3, 119.3, 117.5, 108.2). The ^{1}H−^{1}H COSY spectrum indicated two partial structures indicated by thick lines in Figure 1. The long-range ^{1}H−^{13}C

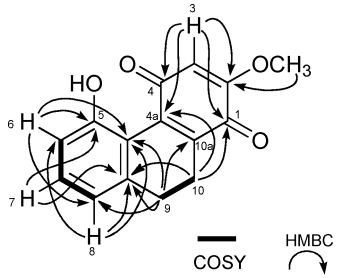

Figure 1. COSY and HMBC correlations of **1**.

correlations were analyzed via an HMBC spectrum that showed correlations of H-3 to C-1, C-2, C-4, and C-4a; H-6 to C-4b, C-5, and C-8; H-7 to C-5 and C-8a; H-8 to C-4b, C-6, and C-8a; H-9 to C-4b, C-8, C-8a, and C-10a; H-10 to C-1, C-4a, C-8a, and C-10a; and 2-OCH$_3$ to C-2 (Figure 1). Furthermore, NOE correlations were observed between H-3 (δ_H 6.04) and 2-OCH$_3$ (δ_H 3.90) and between H-8 (δ_H 6.78) and H-9 (δ_H 2.71). On the basis of these results, the structure of **1** was determined as 5-hydroxy-2-methoxy-9,10- dihydrophenanthrene-1,4-dione. X-ray crystallographic analysis confirmed the structure of **1** (Figure 2).

Marylaurencinol A (**2**) was obtained as a white, amorphous powder. The EIMS revealed a molecular ion at m/z 272 [M]$^+$, and the molecular formula was determined as $C_{16}H_{16}O_4$ by HREIMS. The IR spectrum of **2** exhibited strong absorption bands at 3348, 3215, 1616, 1582, 1501, 1452, 1329, 1236, and

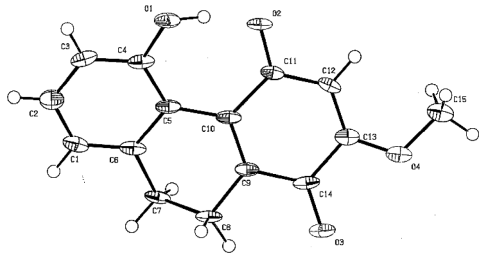

Figure 2. ORTEP drawing for the X-ray crystal structure of **1**.

1094 cm^{-1}, indicating the absence of carbonyl groups comparable to those in **1**. The ^{1}H NMR spectrum showed the presence of two methoxy groups (δ_H 3.91, 3.74), two methylene protons (δ_H 2.71, 2.64), and four aromatic protons (δ_H 7.15, 6.97, 6.84, 6.70). The ^{13}C NMR spectrum revealed the presence of two methoxy carbons (δ_C 61.8, 56.2), two methylene carbons (δ_C 31.2, 30.4), and 12 aromatic carbons and the absence of carbonyl carbons. Therefore, **2** was deduced to have a 9,10-dihydrophenanthrene skeleton. The ^{1}H−^{1}H COSY and HMQC spectra clarified the presence of two partial structures and two methoxy groups as shown in Figure 3.

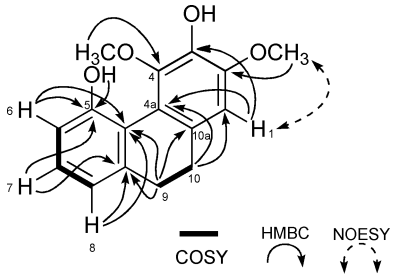

Figure 3. COSY, HMBC, and key NOESY correlations of **2**.

The connectivity of these partial structures and the other carbons was also defined on the basis of the HMBC and NOESY correlations shown in Figure 3. The structure of **2** was elucidated to be 3,5-dihydroxy-2,4-dimethoxy-9,10-dihydrophenanthrene.

Marylaurencinol B (**3**) was obtained as brown needles. The EIMS showed an [M]$^+$ ion at m/z 288, and the molecular formula was determined as $C_{16}H_{16}O_5$ by HREIMS in conjunction with NMR analysis. The IR spectrum of **3** contained bands for hydroxy groups (3232 cm^{-1}). The ^{1}H and ^{13}C NMR spectra showed differences in the C-1−4, 4a, 10, and 10a resonances compared to those of **2** (Table 2). Analysis of the ^{1}H NMR spectrum showed the presence of two methoxy groups (δ_H 3.75, 3.51), three aromatic protons (δ_H 7.11, 6.83, 6.81), and two methylene protons (δ_H 2.57, 2.51). The ^{13}C NMR and HMQC spectra further revealed the presence of two methoxy carbons (δ_C 61.8, 60.1), two methylene carbons (δ_C 30.3, 21.7), and nine quaternary aromatic carbons. However, the connectivity between these partial structures, as shown in Figure 4, could not be established on the basis of the ^{1}H−^{1}H COSY and HMBC correlations. Therefore, a NOESY experiment in DMSO-d_6 was conducted. The NOE correlations between 2-OCH$_3$ and 1-OH, 3-OH; 4-OCH$_3$ and 3-OH, 5-OH; and 5-OH and 4-OCH$_3$, H-6 confirmed the connectivities of the hydroxy, methoxy groups, and aromatic protons (Figure 4). On the basis of the above evidence, the structure of **3** was

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Table 1. ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) Spectroscopic Data for Compounds 1 and 2 in $CDCl_3$

	1		2	
position	δ_C mult.	δ_H (J in Hz)	δ_C mult.	δ_H (J in Hz)
1	180.8, qC		107.8, CH	6.70, s
2	158.6, qC		146.3, qC	
3	108.2, CH	6.04, s	137.1, qC	
4	191.5, qC		142.5, qC	
4a	139.0, qC		118.9, qC	
4b	117.5, qC		119.9, qC	
5	155.3, qC		153.4, qC	
6	119.3, CH	6.91, dd (8.2, 1.4)	117.9, CH	6.97, d (8.0)
7	132.3, CH	7.23, t (8.2)	128.2, CH	7.15, t (8.0)
8	120.3, CH	6.78, dd (8.2, 1.4)	119.8, CH	6.84, d (8.0)
8a	140.6, qC		140.5, qC	
9	28.5, CH_2	2.71, m	31.2, CH_2	2.71, m
		2.71, m		2.71, m
10	21.3, CH_2	2.66, m	30.4, CH_2	2.64, m
		2.66, m		2.64, m
10a	143.2, qC		132.0, qC	
2-OCH_3	56.6, CH_3	3.90, s	56.2, CH_3	3.91, s
4-OCH_3			61.8, CH_3	3.74, s
5-OH				8.67, s

established as 1,3,5-trihydroxy-2,4-dimethoxy-9,10-dihydrophenanthrene. The structure was confirmed by X-ray crystallographic analysis as shown in Figure 5.

Table 2. ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) Spectroscopic Data for Compounds 3 and 4

	3[a]		4[b]	
position	δ_C, mult.	δ_H (J in Hz)	δ_C, mult.	δ_H (J in Hz)
1	143.4, qC		101.4, CH	6.54, s
2	136.1, qC		153.1, qC	
3	141.5, qC		151.6, qC	
4	137.1, qC		137.2, qC	
4a	120.7, qC		115.3, qC	
4b	120.0, qC		122.3, qC	
5	153.3, qC		153.2, qC	
6	117.1, CH	6.81, d (8.1)	116.8, CH	6.82, dd (8.1, 1.2)
7	128.1, CH	7.11, t (8.1)	128.5, CH	7.07, t (8.0)
8	119.4, CH	6.83, d (8.1)	121.0, CH	6.84, dd (8.1, 1.2)
8a	140.8, qC		142.7, qC	
9	30.3, CH_2	2.51, m	31.5, CH_2	2.63, m
		2.51, m		2.56, m
10	21.7, CH_2	2.66, m	25.4, CH_2	2.89, m
		2.66, m		2.81, m
10a	116.8, qC		137.3, qC	
2-OCH_3	60.1, CH_3	3.75, s	56.3, CH_3	3.86, s
4-OCH_3	61.8, CH_3	3.51, s		
1-OH		8.68, s		
3-OH		8.96, s		
5-OH		8.86, s		
Glc-1′			106.0, CH	4.69, d (7.7)
Glc-2′			75.8, CH	3.46, dd (9.1, 7.7)
Glc-3′			78.0, CH	3.42, dd (9.1, 8.8)
Glc-4′			71.6, CH	3.37, dd (9.3, 8.8)
Glc-5′			78.1, CH	3.15, m
Glc-6′			62.8, CH_2	3.75, dd (11.8, 2.3)
				3.63, dd (11.8, 5.2)

[a]Recorded in DMSO-d_6. [b]Recorded in methanol-d_4.

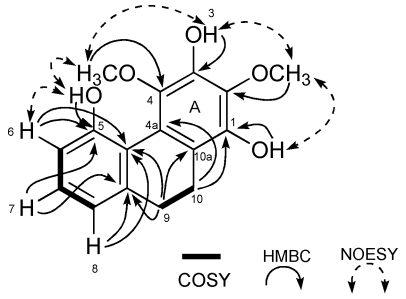

Figure 4. COSY, HMBC, and key NOESY correlations of **3**.

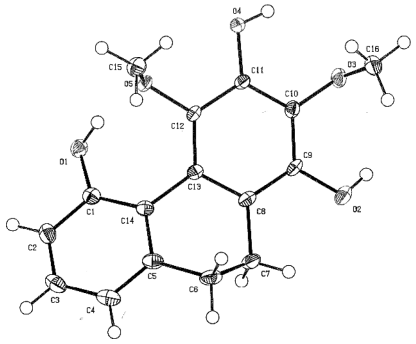

Figure 5. ORTEP drawing for the X-ray crystal structure of **3**.

Marylaurencinoside A (**4**) was obtained as a white, amorphous powder. The FABMS showed a quasimolecular ion at m/z 419 $[M - H]^-$. The molecular formula was determined as $C_{21}H_{24}O_9$ by HRFABMS. The IR spectrum revealed strong absorption bands due to hydroxy groups (3230 cm^{-1}). The ^{1}H and ^{13}C NMR data were similar to those for **2** and **3** except for the signals assignable to a sugar moiety (Table 2). The ^{1}H NMR spectrum of **4** showed the presence of a methoxy group (δ_H 3.86), four aromatic protons (δ_H 7.07, 6.84, 6.82, 6.54), four aliphatic protons (δ_H 2.89, 2.81, 2.63, 2.56), an anomeric proton (δ_H 4.69), two oxymethylene protons (δ_H 3.75, 3.63), and four oxymethine protons (δ_H 3.46, 3.42, 3.37, 3.15). Analyses of the ^{13}C NMR and HMQC spectra revealed the presence of a methoxy carbon (δ_C 56.3), four aromatic carbons (δ_C 128.5, 121.0, 116.8, 101.4), two methylene carbons (δ_C 35.4, 31.5), an anomeric carbon (δ_C 106.0), an oxymethylene carbon (δ_C 62.8), four oxymethine carbons (δ_C 78.1, 78.0, 75.8, 71.6), and eight quaternary aromatic carbons. Analysis of the ^{1}H−^{1}H COSY spectrum revealed a sugar moiety and two partial structures indicated by thick lines in Figure 6.

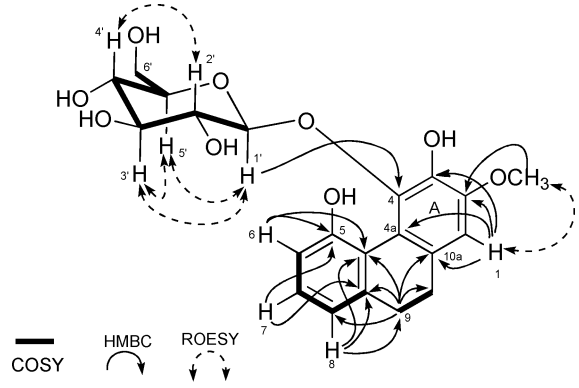

Figure 6. COSY, key HMBC, and key ROESY correlations of **4**.

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Analysis of the HMBC spectrum of **4** further clarified the presence of a hexose skeleton (based on the correlations between H-1′ and C-3′, C-5′; H-2′ and C-1′, C-3′, C-4′) and a 9,10-dihydrophenanthrene aglycone skeleton (based on the correlations between H-1 and C-2, C-3, C-4a, C-10a; 2-OCH_3 and C-2; H-6 and C-4b, C-5; H-7 and C-5, C-8a; H-8 and C-4b, C8a, C-9; H-9 and C4b, C8a, C10, C10a), respectively (Figure 6). Furthermore, the HMBC correlation between C-4 (δ_C 137.2) of the A-ring and H-1′ (δ_H 4.69) of the hexose moiety indicated that the sugar moiety was linked to the 4-hydroxy group of the A-ring. On the basis of this evidence, the molecular structure of marylaurencinoside A (**4**) was determined as shown. The relative configuration of the hexose moiety was elucidated by analysis of the ^{1}H NMR and ROESY spectra of **4**. The coupling constant of 7.7 Hz between H-1′ and H-2′ indicated a diaxial relationship between these two protons. In the ROESY spectrum of the hexose moiety, correlations between H-1′ and H-3′, H-5′; H-3′ and H-1′, H-5′; and H-2′ and H-4′ (Figure 6) indicated the presence of a β-glucopyranose unit. Hydrolysis of **4** in 2.5% H_2SO_4 gave D-glucose, which was identified by HPLC using an optical rotation detector.[19] Thus, the structure of **4** was determined as 3,4,5-trihydroxy-2-methoxy-9,10-dihydrophenanthrene-4-*O*-β-D-glucopyranoside.

The isolated phenanthrene derivatives (**1**−**10**) were tested for antimicrobial activity against *B. subtilis* and *K. pneumoniae*. Phenanthrenes **1**, **3**, and **6** exhibited moderate to insignificant antimicrobial activity against *B. subtilis* with MIC values of 4.88, 65.10, and 52.82 μM, respectively. Notably, the new phenanthrendione, ephemeranthoquinone B (**1**), showed moderate antibacterial activity against *B. subtilis*, with an MIC value of 4.88 μM. However, none of them were active against *K. pneumoniae*. The cytotoxic activities of pure compounds **1**−**10** were evaluated against a HL-60 cell line. The results revealed weak activity of **4**−**9** against the HL-60 cell line, with IC_{50} values of 52.4, 31.8, 19.3, 52.4, 44.6, and 34.2 μM, respectively.

Table 3. Antibacterial Activity of Isolated Compounds

compd	*Bacillus subtilis*	*Klebsiella pneumoniae*
extract[a]	18.75[b]	
1	4.88[c]	
3	65.10[c]	
6	52.82[c]	
7	185.19[c]	185.19[c]
9	185.19[c]	
10	250.00[c]	
ampicillin	0.36[c]	107.45[c]

[a]EtOAc-soluble portion of MeOH extract of roots of *Cymbidium* Great Flower Marie Laurencin. [b]MIC values, in μg/mL. [c]MIC values, in μM.

In contrast, ephemeranthoquinone B (**1**) show weak cytotoxic activity against HL-60 cells with an IC_{50} value of 2.8 μM (Table 4). A large number of phenanthrene-producing plants have been used in traditional medicine throughout the world, and phenanthrenes have been identified as their active constituents from phytochemical−pharmacological investigations. According to our studies, ephemeranthoquinone B (**1**) is a promising natural bioactive product.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined with a Yanagimoto micro melting point apparatus. Optical rotation was measured using a JASCO DIP-1000 polarimeter. The UV spectra were recorded using a Shimadzu UV-6000 spectrophotometer. IR spectra were recorded on a JASCO FT/IR-5300. NMR spectra were recorded on a Varian Unity 600 spectrometer. NMR experiments included COSY, HMQC, HMBC, NOESY, and ROESY. Coupling constants (*J* values) are given in Hz. HREIMS and HRFABMS were measured on a JEOL JMS-700 MS station. HPLC separation was performed on a JASCO PU1580 pump with a JASCO UV-970 detector. For HPLC, COSMOSIL 5C18-AR-II (Nacalai Tesque, Japan, 20 mm i.d. × 250 mm) was used. TLC was performed on precoated silica gel $60F_{254}$. Spots were detected by examining plates sprayed with the *p*-anisaldehyde/H_2SO_4/MeOH reagent followed by heating on a hot plate.

Table 4. Cytotoxic Activity of Compounds 1−10 against HL-60 Cells

compd	IC_{50}[a]	compd	IC_{50}[a]
1	2.8	**7**	52.4
2	96.8	**8**	44.6
3	173.5	**9**	34.2
4	52.4	**10**	103.3
5	31.8	MMC[b]	0.1
6	19.3		

[a]IC_{50} values, in μM. [b]MMC is mitomycin C.

Plant Material. Fresh roots of *C.* Great Flower Marie Laurencin (Ministry of Agriculture, Forestry and Fisheries of Japan, seed registration no. 2841) were cultivated and harvested in November 2008 at Kawano Mericlone Co., Ltd. (Tokushima Prefecture, Japan) and were identified by one of the authors (S.K.). A voucher specimen (TB 5430) has been deposited in the Herbarium of Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima, Japan.

Extraction and Isolation. The fresh roots (8.0 kg) were extracted with MeOH at room temperature for one month and concentrated *in vacuo*. The MeOH extract was partitioned into an EtOAc−H_2O mixture to afford EtOAc- and H_2O-soluble portions. The EtOAc extract (42 g) was subjected to Si gel CC with *n*-hexane−EtOAc−MeOH (10:1:0−0:1:10) to afford eight fractions (Fr. 1−8). Fr. 2 (0.58 g) was further purified using RP-HPLC (70% aq MeOH) to give **5** (8 mg). Fr. 3 (5.69 g) was chromatographed further over Si gel using *n*-hexane−EtOAc (7:3) to afford five subfractions (Fr. 3-A−E). Fr. 3-C was purified by RP-HPLC (60% aq MeOH) to yield **2** (390 mg) and **6** (80 mg). Fr. 3-D was chromatographed further over Si gel using *n*-hexane−EtOAc (6:4) and purified by RP-HPLC (40-65% aq MeOH) to afford **1** (13.6 mg), **3** (23 mg), **7** (38 mg), and **8** (52 mg). Fr. 4 (4.49 g) was further subjected to Si gel CC with *n*-hexane−EtOAc (6:4) and reseparated using RP-HPLC (85% aq MeOH) to furnish **1** (80 mg), **9** (70 mg), and **10** (117 mg). Fr. 7 (0.78 g) was also purified using RP-HPLC (45% aq MeOH) to give **4** (6.5 mg).

*Ephemeranthoquinone B (**1**):* red needles (MeOH); mp 148−150 °C; UV (MeOH) λ_{max} (log ε) 217.6 (3.67), 223.4 (3.67), 242 (3.46), 276.2 (3.38), 321.2 (3.38) nm; FT-IR (film) ν_{max} 3063, 2993, 1670, 1639, 1595, 1452, 1246 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 256 [M]$^+$ (100), 213 (61), 171 (46.5), 127 (38), 115 (81), 69 (33); HREIMS *m*/*z* 256.0709 (calcd for $C_{15}H_{12}O_4$, 256.0736).

*Marylaurencinol A (**2**):* white, amorphous powder; UV (MeOH) λ_{max} (log ε) 225.4 (3.89), 273.4 (3.80), 306.2 (3.81) nm; FT-IR (film) ν_{max} 3348, 3215, 1616, 1582, 1501, 1452, 1329, 1236, 1094 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 272 [M]$^+$ (100), 257 (32), 225 (32), 197 (74), 169 (42), 157 (31), 141 (29), 139 (40), 128 (32), 115 (33); HREIMS *m*/*z* 272.1040 (calcd for $C_{16}H_{16}O_4$, 272.1049).

*Marylaurencinol B (**3**):* brown needles (MeOH); mp 195.8−196.8 °C; UV (MeOH) λ_{max} (log ε) 227 (3.99), 270 (3.99), 297 (3.88) nm; FT-IR (film) ν_{max} 3232, 1610, 1458, 1252, 1097, 1047 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; EIMS *m*/*z* 288 [M]$^+$ (100), 273 (52), 241 (9.8), 213 (9.2), 185 (5.4), 127 (6.4), 115 (6.1), 70 (3.3), 43 (2.3); HREIMS *m*/*z* 288.1003 (calcd for $C_{16}H_{16}O_5$[M]$^+$, 288.0998).

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Marylaurencinoside A (4): brown, amorphous powder; $[\alpha]_D$ +6.3 (*c* 0.7, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.11), 268 (3.89), 301 (3.75) nm; FT-IR (film) ν_{max} 3230, 1610, 1450, 1221, 1188, 1095 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRFABMS *m/z* 419.1346 (calcd for $C_{21}H_{23}O_9$, 419.1343).

X-ray Crystallographic Data for Ephemeranthoquinone B (1) and Marylaurencinol B (3) (ref 20). Single crystals of **1**, obtained by slow evaporation of MeOH, were selected, fitted onto a glass fiber, and measured at −173 °C with a Bruker Apex II Ultra diffractometer using Mo Kα radiation. Data correction and reduction were performed with the crystallographic package Apex II. The structures were solved by direct methods using SHELXS-97 (Sheldrick, 1990) and refined using full matrix least-squares based on F^2 with SHELXL-97 (Sheldrick, 1997). All non-hydrogen atoms were refined anisotropically, and hydrogen atoms were positioned geometrically. A total of 205 parameters were considered. Final disagreement indices were R1 = 0.0770 and wR2 = 0.1830 ($I > 2\sigma(I)$). The ORTEP plot was obtained by the program PLATON (A. L. Spek, 2009). Crystal data: $C_{15}H_{12}O_4$, MW = 256.25, orthorhombic, space group $P2_12_12_1$, Z = 4, a = 7.0616(16) Å, b = 8.2285(19) Å, c = 19.953(5) Å, V = 1159.4(5) Å^3. X-ray analysis of **3** was conducted in the same way as described for **1**. A total of 195 parameters were considered. Final disagreement indices were R1 = 0.0284 and wR2 = 0.0789 ($I > 2\sigma(I)$). The ORTEP plot was obtained with the program PLATON (A. L. Spek, 2009). Crystal data: $C_{16}H_{14}O_5$, MW = 286.27, monoclinic, space group *Pn*, Z = 2, a = 7.4378(11) Å, b = 9.5159(14) Å, c = 10.0687(15) Å, β = 95.705(2)°. V = 709.18(18) Å^3.

Acid Hydrolysis of Marylaurencinoside A (4). A solution of **4** (1 mg) in 5% H_2SO_4−dioxane (1:1) was heated at 100 °C for 2 h. The reaction mixture was diluted with H_2O, neutralized with Amberlite IRA-35, and evaporated dry *in vacuo*. The identification of D-(+)-glucose was determined using RI detection (Shimadzu RID-10A) and chiral detection (Shodex OR-1) by HPLC (Shodex RSpak NH_2P-50 4D, CH_3CN−H_2O−H_3PO_4, 95:5:1, 1 mL/min, 47 °C), by comparison with an authentic D-glucose sample. The sugar portion gave a peak of D-(+)-glucose at 20.7 min.

Antimicrobial Assay. *B. subtilis* NBRC 3134 and *K. pneumoniae* NBRC 3512 were used for testing antimicrobial activity. These strains were tested by using microdilution assays, and MIC values were determined. Bacterial strains were inoculated on YP agar plates [1% polypeptone (Nihon Pharmaceutical, Japan), 0.2% yeast extract (Difco, USA), 0.1% $MgSO_4$−$7H_2O$, and 6% agar (Nacalai Tesque, Japan)] and incubated at 37 °C (*B. subtilis*) and 27 °C (*K. pneumoniae*) for 12 h. A stock solution of crude extracts or samples was prepared at 10 mg/mL in DMSO and further diluted to varying concentrations in 96-well plates that contained microbial strains incubated in YP medium for the bacterial strains. Each plate was further incubated at 37 °C overnight, and ampicillin (Nacalai Tesque, Japan) was used as a reference reagent for the bacterial strains.

Cytotoxic Assay. The antiproliferative potential of the test compounds was evaluated by the WST-8 assay (Promega, USA)[21] against the HL-60 cell line (DS Pharma Biomedical, Japan). HL-60 cells were maintained in RPMI-1640 medium (Nacalai Tesque, Japan) supplemented with 10% fetal bovine serum (Invitrogen, USA) at 37 °C in a humidified atmosphere of 5% CO_2. Cell growth was quantified by the ability of living cells to reduce WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium monosodium) to a highly water-soluble formazan. Briefly, cells were seeded in 96-well plates at a density of 5 × 10^3 cells/well (100 μL/well) and incubated for 2 h. Then, 100 μL of medium containing test compounds was added, and the cells were incubated for 72 h. After incubation, 20 μL of WST-8 solution was added and the cells were incubated for an additional 3 h. The optical density at 450 nm was measured by using a microplate reader (SpectraMax 340PC, Molecular Devices, Japan).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

NMR spectra of compounds **1**−**4** 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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(20) Crystallographic data (excluding structural factors) for the structures of **1** and **3** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 848411 and CCDC 848412, respectively. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ UK (fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk.
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