Dibenzyl Bromophenols with Diverse Dimerization Patterns from the Brown Alga *Leathesia nana*

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Six novel dibenzyl bromophenols (**1**−**6**) with different dimerization patterns and two propyl bromophenol derivatives (**7** and **8**), together with 11 known bromophenol derivatives, were isolated from the ethanolic extract of the brown alga *Leathesia nana*. On the basis of spectroscopic methods the structures of the new compounds were determined as 5,6′-diethyloxymethyl-3,4,2′-tribromo-2,3′,4′-trihydroxydiphenyl ether (**1**), 2-(2,3-dibromo-4,5-dihydroxybenzyl)-3,5-dihydroxy-4-methoxybenzyl alcohol (**2**), 6-(2,3-dibromo-4,5-dihydroxybenzyl)-2,3-dibromo-4,5-dihydroxy benzyl methyl ether (**3**), 9,10-dihydro-9,10-dimethoxy-3,4,7,8-tetrabromo-1,2,5,6-tetrahydroxyanthracene (**4**), (+)-3-(2,3-dibromo-4,5-dihydroxyphenyl)-4-bromo-5,6-dihydroxy-1,3-dihydroisobenzofuran (**5**), *rel*-(4a*S**,10a*R**)-(±)-6,7-dibromo-4a-hydroxy-3,8-dihydroxymethyl-10a-methoxy-1,4,4a,10a-tetrahydrodibenzo[*b*,*e*][1,4]dioxin-1-one (**6**), (*E*)-2-methyl-3-(2,3-dibromo-4,5-dihydroxyphenyl)propenal (**7**), and 2-methyl-3-(2,3-dibromo-4,5-dihydroxyphenyl)-1-propanol (**8**). Some compounds including **3** showed in vitro selective cytotoxicity against several human cancer cell lines. This is the first brown alga to be reported containing bromophenols.

As part of our recently initiated program to assess systematically the chemical and biological diversities of seaweeds distributed in the gulf of the Yellow Sea, China,[1−3] we have investigated the brown alga *Leathesia nana* S. et G. belonging to the Leathesiaceae family. Although the ethanolic extract of this organism was inactive in our primary cytotoxic assay against several tumor cell lines (IC_{50} > 50 μg/mL), thin-layer chromatography indicated the presence of compounds positive to ferric chloride spray reagent. Subsequent separation of the extract by a variety of chromatographic techniques over normal-phase silica gel, Sephadex LH-20, and reversed-phase C-18 silica gel afforded six novel dibenzyl bromophenols with diverse dimarization patterns (**1**−**6**) and two propyl bromophenol derivatives (**7** and **8**), together with 11 known bromophenols. On the basis of spectral evidence and comparison of their physical and spectral data with those in the literature, structures of the known bromophenols were identified as 2,2′,3,3′-tetrabromo-4,4′,5,5′-tetrahydroxydiphenylmethane (**9**),[4] 2,2′,3-tribromo-3′,4,4′,5-tetrahydroxy-6′-ethyloxymethyldiphenylmethane (**10**),[5] bis(2,3-dibromo-4,5-dihydroxybenzyl) ether (**11**),[6,7] 3-bromo-4-(2,3-dibromo-4,5-dihydroxybenzyl)-5-methoxymethylpyrocatechol (**12**),[6,8] 2,3-dibromo-4,5-dihydroxybenzyl alcohol (**13**),[9] 2,3-dibromo-4,5-dihydroxybenzyl methyl ether (**14**),[6,10,11] 2-bromo-4,5-dihydroxybenzaldehyde (**15**),[12] 3-bromo-4-hydroxybenzoic acid (**16**),[13] 2,3-dibromo-4,5-dihydroxybenzylaldehyde (**17**),[11,10,14] 3-bromo-4,5-dihydroxybenzaldehyde (**18**),[12] and 2,3-dibromo-4,5-dihydroxybenzyl ethyl ether (**19**).[14] In previous short communications we reported the structures of compounds **7** and **8**.[15] This paper deals with the structural elucidation of **1**−**6**. A variety of bromophenols have been isolated from marine red algae,[1−10] a green alga,[11] two brown alga,[16,17] sponges,[12−14,18,19] and ascidians,[20−23] and many of these compounds are reported to exhibit antimicrobial,[3,4,14,18,19] feeding deterrent,[10] α-glucosidase inhibitory,[5] antioxidant,[7] enzymatic inhibitory,[12] and cytotoxic[14,17] activities. All of these compounds were evaluated against several human cancer cell lines, and compounds **3** and **9**−**12** showed moderate selective cytotoxicities.

Results and Discussion

The EtOH extract of the brown alga was evaporated to dryness, then suspended in water and partitioned with EtOAc. The EtOAc-soluble extract was subjected to column chromatography over silica gel eluting with a gradient of increasing Me_2CO (0−100%) in petroleum ether (60−90 °C). The subsequent fractions were further purified by a variety of chromatographic techniques including reversed-phase preparative HPLC to yield the new compounds **1**−**6** and other compounds. All of these compounds gave positive reaction to $FeCl_3$ reagent and similar broadened strong IR absorption bands around 3350 cm^{-1} for phenolic hydroxyl and 1500 and 1600 cm^{-1} for aromatic rings.

The EIMS of **1** exhibited a characteristic tribrominated molecular ion peak cluster at *m*/*z* 568/570/572/574 (1: 3: 3: 1) $[M]^+$, and the HREIMS at *m*/*z* 567.8731 $[M]^+$ established the molecular formula $C_{18}H_{19}Br_3O_6$. Besides two pairs of characteristic proton spin-coupling systems attributed to two ethoxyl units at δ 3.42 (2H, q, J = 7.0 Hz, H-8), 3.37 (2H, q, J = 7.0 Hz, H-8′), and 1.03 (6H, t, J = 7.0 Hz, overlapped H-9 and H-9′), the ^{1}H NMR spectrum showed two pairs of singlets at δ 7.04 (1H, s, H-6′) and 6.60 (1H, s, H-6), and 4.37 (2H, s, H-7) and 4.30 (2H, s, H-7′), which were assignable to the aromatic protons and methylene protons of two pentasubstituted benzyl moieties, respectively. These data suggested a dimeric pentasubstitued benzyl ethyl ether structure for **1**. This suggestion was confirmed by the ^{13}C NMR spectral data of **1** (see Table 1). In combination with the chemical shift values of the protons and carbons, in the HMBC spectrum of **1** (see Figure 1) three-bond correlations from H-6 to C-2, C-4, and C-7, from H-7 to C-2 and C-6, and from H-8 to C-7 revealed that the left moiety is 4,5-dioxygenated 2,3-dibromobenzyl

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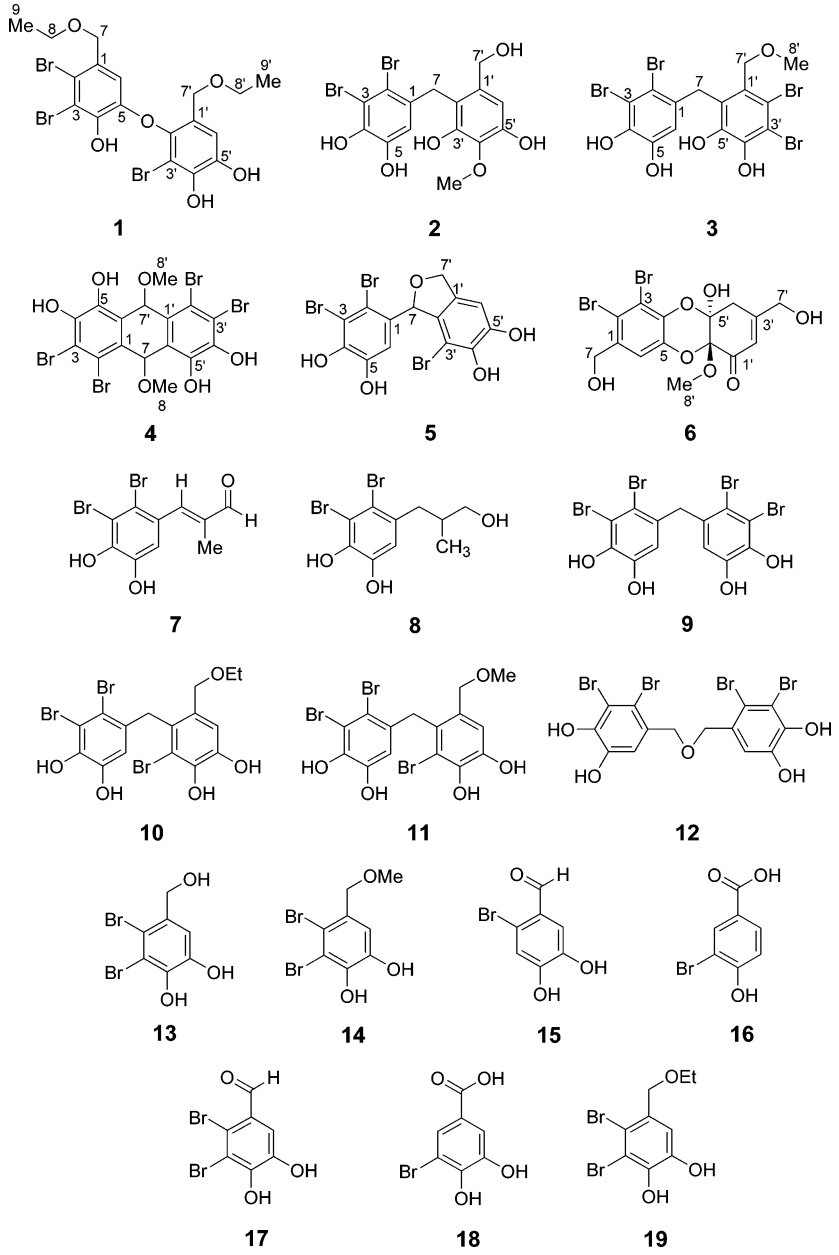
Chart 1. Structures of Compounds **1**−**19**



ethyl ether, while correlations from H-6′ to C-2′, C-4′, and C-7′, from H-7′ to C-2′ and C-6′, and from H-8′ to C-7′ indicated that the right moiety is 2,4,5-trioxygenated 3-bromobenzyl ethyl ether. In consideration of the molecular composition, the dimerization pattern of **1** must be a diaryl ether with one hydroxyl on the left aryl moiety and two hydroxyls on the right aryl moiety. Comparison of the NMR data assigned to the left moiety of **1** with those of **19** and literature data[14] indicated that H-6 and C-6 of **1** shifted upfield by 0.47 and 1.7 ppm, respectively, and C-2 and C-5 of **1** downfield by 3.1 and 0.9 ppm, respectively. These shifts demonstrated that the C-5 of the left aryl moiety is etherified with the right moiety. In addition, the upfield chemical shift values of H-7′ and C-7′ demonstrated that they are strongly shielded by the aromatic ring of the left moiety, indicating that the C-2′ of the right moiety is etherified with the left moiety. Therefore, the structure of **1** is 5,6′-diethyloxymethyl-3,4,2′-tribromo-2,3′,4′-trihydroxydiphenyl ether.

Compound **2** gave a dibrominated molecular ion peak cluster at *m*/*z* 452.0/450.0/448.0 (1:2:1) $[M]^+$ in its EIMS. The HREIMS at *m*/*z* 447.9165 $[M]^+$ established the molecular formula $C_{15}H_{14}Br_2O_6$ for **2**. In the ^1H NMR spectrum of **2** two pairs of singlets attributed to aromatic protons at δ 6.66 (1H, s, H-6′) and 6.22 (1H, s, H-6) and benzylic methylene protons at δ 4.35 (2H, s, H-7′) and 3.94 (2H, s, H-7) revealed that there are two tetrasubstituted benzyl moieties in the structure of **2**. In addition, a singlet at δ 3.80 (3H, s, OCH_3) indicated the presence of a methoxyl on one of the benzyl moieties. The ^{13}C NMR and DEPT spectral data of **2** (see Table 1) confirmed a dibenzyl dibromophenol structure for **2**. The substitution and connection patterns of the two benzyl moieties in **2** were determined by HMQC and HMBC experiments. The protonated carbons and their corresponding protons were unambiguously assigned by the HMQC experiment. In combination with chemical shift values of the protons and carbons, in the HMBC spectrum of **2** (see Figure 1) three-bond correlations from H-6 to C-2, C-4, and C-7 and from H-7 to C-2 and C-6 demonstrated that one of the benzyl moieties is 2,3-dibromo-4,5-dihydroxybenzyl, while correlations from H-6′ to C-2′, C-4′, and C-7′, from H-7′ to C-2′

Table 1. ^{13}C NMR Data for Compounds **1**−**6**

no.	1	2	3	4	5	6
1	131.4 qC	133.6 qC	132.4 qC	120.3 qC	133.5 qC	137.4 qC
2	117.4 qC	116.1 qC	116.3 qC	114.5 qC	117.5 qC	116.3 qC
3	114.0 qC	113.3 qC	113.6 qC	114.7 qC	113.8 qC	114.7 qC
4	145.1qC	143.2 qC	143.5 qC	146.3 qC	145.3 qC	139.4 qC
5	146.3qC	145.3 qC	145.3 qC	135.8 qC	145.4 qC	141.1 qC
6	113.5 CH	115.0 CH	114.9 CH	116.7 qC	115.2 CH	116.8 CH
7	72.9 CH_2	32.7 CH_2	34.4 CH_2	100.7 CH	87.9 CH	64.8 CH_2
8	66.2 CH_2			57.0 CH_3		
9	15.3 CH_3					
1′	125.0 qC	137.6 qC	130.9 qC	120.3 qC	133.2 qC	188.3 qC
2′	142.4 qC	115.1 qC	118.9 qC	114.5 qC	132.8 qC	120.1 CH
3′	105.7 qC	149.4 qC	113.0 qC	114.7 qC	105.0 qC	162.1 qC
4′	144.6 qC	134.9 qC	144.8 qC	146.3 qC	143.5 qC	37.5 CH_2
5′	144.1 qC	149.4 qC	145.1 qC	135.8 qC	147.0 qC	96.1 qC
6′	115.1 CH	107.5 CH	128.2 qC	116.7 qC	107.5 CH	94.5 qC
7′	67.6 CH_2	62.1 CH_2	73.2 CH_2	100.7 CH	73.5 CH_2	64.4 CH_2
8′	66.2 CH_2	60.7 CH_3	58.3 CH_3	57.0 CH_3		51.5 CH_3
9′	15.3 CH_3					

[a] The data were measured in acetone-d_6 at 500 MHz and at 125 MHz. The assignments were based on DEPT, HMQC, and HMBC experiments.

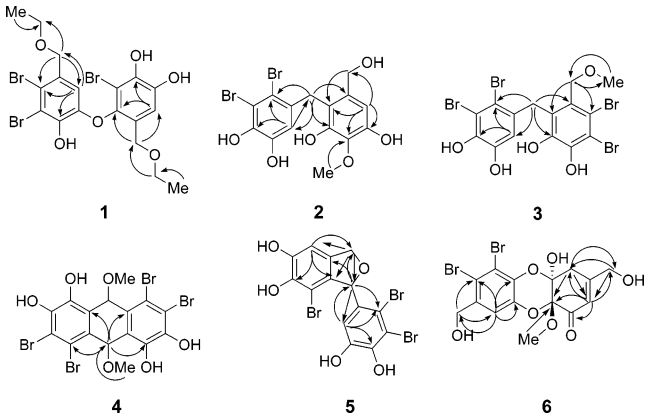


Figure 1. Key HMBC correlations of compounds **1**−**6**.

and C-6′, and from the methoxy protons to C-4′ indicated that the remaining benzyl moiety is 3′,5′-dihydoxy-4′-methoxybenzyl. In addition, three-bond correlations from H-7 to C-2, C-6, C-1′, and C-3′ unequivocally revealed that the two moieties were connected through a carbon bond between C-7 and C-2′. In consideration of the atom composition of the molecule and the chemical shift values of C-7′, a hydroxyl group was assigned at C-7′. Therefore, the structure of **2** was determined as 2-(2,3-dibromo-4,5-dihydroxybenzyl)-3,5-dihydroxy-4-methoxybenzyl alcohol.

The EIMS of **3** did not give the molecular ion peak; instead it gave a characteristic tetrabrominated fragment ion peak cluster at *m*/*z* 556/558/560/562/564 (1:4:6:4:1) [M − MeOH]$^+$. The molecular formula $C_{15}H_{14}Br_2O_6$ was established by the HREIMS at *m*/*z* 555.7149 [M − MeOH]$^+$ in combination with the NMR spectral data of **3** (see Table 1). The ^1H NMR spectrum showed four singlets at δ 6.18 (1H, s, H-6), 4.42 (2H, s, H-7′), 4.15 (2H, s, H-7), and 3.28 (3H, s, H-OCH_3) attributed to an aromatic proton and protons of two benzylic methylenes and one methoxyl, respectively. The ^{13}C NMR and DEPT spectra showed 15 carbon signals consisting of one methoxyl, two methylenes (one oxygenated), one sp^2 methine, and 11 sp^2 quaternary carbons. The protonated carbons were assigned by the HMQC experiment of **3**, and the oxygenated quaternary carbons were recognized by their chemical shifts (δ >143 ppm). All of the above data revealed that **3** is a dibenzyl tetrabromophenol consisting of one tetrasubstituted and one pentasubstituted benzyl moiety. In combination with the chemical shift values of the protons and carbons, the HMBC spectrum (see Figure 1) indicated the presence of the 2,3-dibromo-4,5-dihydroxybenzyl moiety in **3**, which is identical to the left moiety of **2**. Meanwhile, the HMBC correlations from H-7′ to C-1′, C-2′, C-6′. and the methoxyl carbon and from the methoxyl protons to C-7′ suggested that the pentasubstituted benzyl moiety is 2′,3′-dibromo-4′,5′-dihydroxybenzyl methyl ether. In addition, the HMBC correlations from H-7 to C-1′, C-5′, and C-6′ established the connectivity between C-7 and C-6′ of the two moieties. The substitution patterns of the two benzyl moieties were confirmed by carefully comparing the NMR data of **3** with those of the related compounds in the literature.[7,13,14] Consequently, the structure of **3** was determined as 6-(2,3-dibromo-4,5-dihydroxybenzyl)-2,3-dibromo-4,5-dihydroxy benzyl methyl ether.

The EIMS of **4** did not show the molecular ion cluster; instead it showed a strong tetrabrominated fragment ion peak cluster at *m*/*z* 558/556/554/552/550 (1:4:6:4:1) [M − 2MeOH − 2H]$^+$. The molecular formula $C_{16}H_{12}Br_4O_6$ was determined on the basis of the HREIMS at *m*/*z* 549.6679 in combination with the NMR spectral data (see Table 1 and Experimental Section), which showed half the signals expected from the molecular formula. The ^1H NMR spectrum of **4** showed only two singlets at δ 6.27 and 3.62 with integration ratio of 1:3, and the ^{13}C NMR and DEPT spectra showed eight signals attributed to six quaternary aromatic, one oxymethine, and one methoxyl carbon. The HMBC experiment (see Figure 1) demonstrated that the methine proton (H-7) correlated with the methoxyl carbon; in turn the methoxyl protons correlated only with the methine carbon, indicating the presence of a methoxymethine unit in the half structure. In addition the methine proton correlated with four aromatic carbons, which revealed that the structure of **4** is a dimer of 7-methoxydibromodihydroxybenzyl connected between C-6 and C-7′ as well as between C-7 and C-6′. The substitution pattern of bromine atoms and hydroxyl groups on half of the benzyl moiety was proposed to be identical to that of the right moiety of **3** on the basis of biogenetic reasons. To use NOE experiments to confirm the substitution pattern of the functional groups, we tried to prepare the methyl ether of **4** by using the dimethyl sulfate reagent, but it failed for the complex reaction products and the limitation of the available amount of **4**. Therefore, the structure of **4** was tentatively determined as 9,10-dihydro-9,10-dimethoxy-3,4,7,8-tetrabromo-1,2,5,6-tetrahydroxyanthracene.

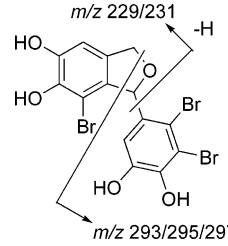


Figure 2. Key EIMS fragmentations of compound **5**.

Compound **5** displayed a tribrominated molecular ion peak cluster at *m*/*z* 494/496/498/500 $[M]^+$ (1/3/3/1) in the EIMS, and the HREIMS at *m*/*z* 493.7971 $[M]^+$ established the molecular formula $C_{14}H_9Br_3O_5$. The ^1H NMR spectrum showed three singlets at δ 6.88 (1H, s, H-6), 6.45 (1H, br s, H-7′), and 6.31 (1H, br s, H-6′) and an AB spin coupling system at δ 5.03 (1H, d, J = 12.0 Hz, H-7a) and 4.94 (1H, d, J = 12.0 Hz, H-7b). The ^{13}C NMR and DEPT spectra showed 14 carbon signals including one oxymethylene, two sp^2 methines, one sp^3 oxymethine, and 10 sp^2 quaternary carbons (four oxygenated, δ_C >143 ppm) (see Table 1). The proton signals and corresponding protonated carbon signals were unambiguously assigned by the HMQC experiment. All of the above data suggested that **5** is another dibenzyl tribromophenol. However, one benzylic methylene was replaced by the oxymethine, and two protons of another benzylic methylene appeared as an AB spin-coupling system instead of a singlet. The HMBC experiment (see Figure 1) confirmed that the two benzyl moieties in the structure of **5** are 2,3-dibromo-4,5-dihydroxybenzyl and C-2 substituted 3-bromo-4,5-dihydroxybenzyl. In the HMBC spectrum, correlations from H-7 to C-1′, C-2′, and C-3′ clearly revealed the connectivity between C-7 and C-2′ of the two moieties. In addition, the correlations from H-7 to C-7′ and from H-7′ to C-7 indicated that the two moieties were further connected between C-7 and C-7′ through an oxygen to form a dihydrofuran ring, which is consistent with the AB spin-coupling system of the methylene protons (H_2-7) in the H NMR. In the EIMS of **5** the base fragment ion peak cluster at *m*/*z* 229/231 (1:1) and the strong fragment ion peak clusters at *m*/*z* 293/295/297 (1:2:1) evidenced the connectivity between the benzyl moieties also (see Figure 2). Therefore, the structure of **5** was determined as (+)-3-(2,3-dibromo-4,5-dihydroxyphenyl)-4-bromo-5,6-dihydroxy-1,3-dihydroisobenzofuran. The stereochemistry of **5** has not been determined yet.

The EIMS of **6** showed a dibrominated molecular ion cluster at *m*/*z* 468/466/464 (1:2:1) $[M]^+$, and the HREIMS at *m*/*z* 463.9133 $[M]^+$ established the molecular formula $C_{15}H_{14}Br_2O_7$. The ^1H NMR spectrum of **6** showed two pairs of AB spin-coupling systems attributed to protons of two isolated methylenes at δ 4.33 (1H, d, J = 18.0 Hz, H-7′a), 4.30 (1H, d, J = 18.0 Hz, H-7′b), 3.15 (1H, d, J = 18.0 Hz, H-4a), and 2.94 (1H, d, J = 18.0 Hz, H-4b), in addition to four singlets at δ 7.37 (1H, s, H-6), 6.18 (1H, s, H-2), 4.63 (2H, s, H-7), and 3.19 (3H, s, H-OCH_3) assignable to protons of an aromatic methine, an olefinic methine, an oxymethylene, and a methoxyl, respectively. Besides the protonated carbon signals, the ^{13}C NMR and DEPT spectra of **6** showed nine quaternary carbons (see Table 1). The protonated carbons and the corresponding protons were unambiguously assigned by the HMQC experiment of **6**. In the EIMS the fragment ion peak clusters at *m*/*z* 296/298/300 (1:2:1) and 217/219 (1:1) strongly suggested the presence of a dioxygenated dibromobenzyl alcohol moiety[18−20] in the structure of **6**. In the HMBC spectrum (see Figure 1) correlations from H-7 to C-1, C-2, and C-6 and from H-6 to C-2, C-4, and C-7 in combination with the chemical shift values of these carbons and protons indicated that the dioxygenated dibromobenzyl alcohol moiety is 2,3-dibromo-4,5-dihydroxybenzyl alcohol. Meanwhile, the HMBC correlations from H-2′ to C-1′, C-4′, C-6′, and C-7′, from H-4′ to C-2′, C-3′, C-5′, C-6′, and C-7′, from H-7′ to C-2′, C-3′, and C-4′, and from the methoxyl protons to C-6′ revealed that there is another moiety, 5,5,6-trisubstituted 6-methoxy-3-oxymethylcyclohex-2-enone, in the structure of **6**. The chemical shift values of the quaternary carbons C-5′ and C-6′ demonstrated that they are ketal or semiketal carbons. In consideration of the atom composition of the molecule and the eight degrees of unsaturation, the two moieties must share two oxygen atoms to form a 1,4-dioxin structure. Thus, the plane structure of **6** was elucidated as one of the two isomers (see Figure 3). Fortunately, a single crystal of **6** suitable for X-ray diffraction analysis was obtained from the acetone solution, and the structure and relative stereochemistry of **6** were determined by the X-ray crystallographic analysis. The ORTEP drawing, with the atom-numbering scheme indicated, is shown in Figure 4. In addition, the space group $P\bar{1}$ of the crystal obtained from the X-ray crystallographic analysis indicated that compound **6** is a racemate, which was consistent with its optical inactivity. Thus, the structure of **6** was determined as the isomer I, *rel*-(4a*S**,10a*R**)-(±)-6,7-dibromo-4a-hydroxy-3,8-dihydroxymethyl-10a-methoxy-1,4,4a,10a-tetrahydrodibenzo-[*b*,*e*][1,4]dioxin-1-one.

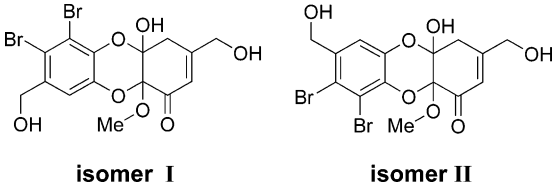


Figure 3. Two possible isomers of compound **6**.

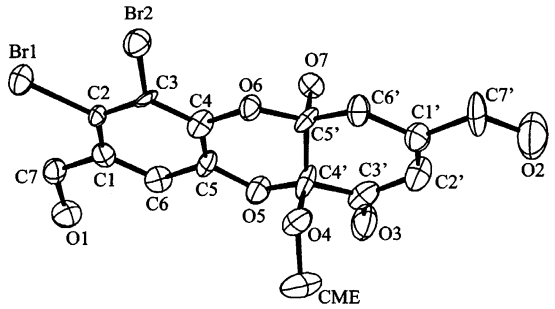


Figure 4. ORTEP diagram of compound **6**.

Compounds **1**, **10**, and **19** may be artifacts formed during the extraction procedure. Although compounds **1**−**19** were isolated not following a specific bioassay-guided separation protocol, follow-up in vitro biological screening indicated that **3** and **9**−**12** were cytotoxic against several human cancer cell lines including lung adenocarcinoma (A549), stomach cancer (BGC-823), breast cancer (MCF-7), hepatoma (Bel7402), and human colon cancer (HCT-8) cell lines (see Table 2).

Experimental Section.

General Experimental Procedures. Melting points (uncorrected) were determined on an XT-4 micro melting point apparatus. Optical rotations were measured on a P-E 241 MC automatic polarimeter. IR spectra were recorded as KBr disks on a Nicolet Impact 400 FT-IR spectrophotometer. NMR spectra were recorded on a Varian Inova 500 MHz spectrometer at 500.103 MHz for ^1H and 125.762 MHz for ^{13}C in

Table 2. Cytotoxicity of Compounds **3** and **9**−**12**

	IC_{50} value (μM/mL)				
compd	A549	BGC823	MCF-7	Bel7402	HCT-8
3	0.0025	0.0088	0.0027	0.0048	>0.0168
9	0.0018	0.0038	0.0027	>0.0182	0.0022
10	>0.0190	0.0046	0.0034	0.0055	0.0028
11	>0.0195	0.0086	0.0214	>0.0019	0.0207
12	0.0054	0.0180	0.0046	0.0074	0.0059

acetone-d_6 with TMS as internal standard. EIMS and HREIMS data were measured with a Micromass Autospec-Ultima ETOF spectrometer. Column chromatography was performed with silica gel (160−200 mesh, Qingdao Marine Chemical Inc. China) and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala Sweden). HPLC separation was performed on a chromatograph consisting of a Waters 600 Controller, a Waters 600 pump, and a Waters 2487 dual λ absorbance detector with an Alltima 250 cm × 2.2 cm i.d. preparative column packed with C_{18} (10 μm). TLC was carried out with glass precoated silica gel GF254 plates. Spots were visualized under UV light and by spraying with 2% $FeCl_3$ in 95% EtOH. All solvents used were either analytical grade or spectral grade or were distilled prior to use. X-ray diffraction intensity data of **6** were collected on a MAC DIP-2030K diffractometer with graphite-monochromated Mo Kα radiation ($\lambda = 0.71073$ Å) by the ω scan technique [scan width 0−180°, $2\theta \leq 50°$] and were corrected by Lorentz and polarization. Altogether 2069 reflections were collected, of which 1952 with $|F|^2 \geq 8\sigma|F|^2$ were observed. The structure was solved by direct methods and refined by block-matrix least-squares procedure to $R = 0.064$, $R_w = 0.066$ [$w = 1/\sigma|F|^2$]. Hydrogen positions were found from difference Fourier maps and geometric calculations. All calculations were carried out on a PC by using the NOMCSDP program system.

Material. *Leathesia nana* S. et G. was collected in Weihai of Shandong Province, China, in May 2002. The specimen identification was verified by Dr. Kui-Shuang Shao (Institute of Oceanology, Chinese Academy of Sciences, Qingdao, 266071, China). A voucher specimen (No. 200216) was deposited at the Herbarium of the Institute of Oceanology, Chinese Academy of Sciences, Qingdao, 266071, China.

Extraction and Isolation. The brown alga *L. nana* (8.25 kg) was extracted with 95% EtOH at room temperature for 3 × 72 h. After the solvent was removed under reduced pressure at <40 °C, a dark residue (710 g) was obtained. The residue was suspended in water and then partitioned with EtOAc. The EtOAc-soluble fraction (125 g) was chromatographed over silica gel eluting with a gradient of increasing Me_2CO (0−100%) in petroleum ether. The fraction eluted by 30% Me_2CO in petroleum ether was decolored by column chromatography over Bio-Beads SX3 using $CHCl_3$−EtOAc (1: 2) and rechromatographed over Sephadex LH-20 using petroleum ether−$CHCl_3$−MeOH (5:5:1) to afford three subfractions. The second fraction was further separated by reversed-phase preparative HPLC using MeOH−H_2O−AcOH (70: 30: 0.1) as mobile phase to yield compounds **1** (9 mg), **2** (13 mg), **3** (15 mg), **4** (17 mg), **5** (35 mg), and **6** (87 mg). Separation of the third fraction by reversed-phase preparative HPLC using MeOH−H_2O−AcOH (75: 25: 0.1) as mobile phase yielded compounds **7** (62 mg), **8** (146 mg), **9** (27 mg), **10** (249 mg), **11** (46 mg), **12** (52 mg), and **13** (75 mg).

5,6′-Diethyloxymethyl-3,4,2′-tribromo-2,3′,4′-trihydroxydiphenyl ether (1): brown powder (Me_2CO); mp 153−155 °C; IR (KBr) ν_{max} 3369, 2983, 2915, 2868, 1599, 1581, 1497, 1479, 1431, 1398, 1273, 1155, 1068, 999, 916, 858 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) δ 7.04 (1H, s, H-6′), 6.60 (1H, s, H-6), 4.37 (2H, s, H-7), 4.30 (2H, s, H-7′), 3.42 (2H, q, H-8), 3.37 (2H, q, H-8′), 1.03 (3H, t, H-9), 1.03 (3H, t, H-9′); ^{13}C NMR (acetone-d_6, 125 MHz), see Table 1; EIMS *m*/*z* (%) 574 (4), 572 (13), 570 (14), 568 (5) [M]$^+$, 528 (34), 526 (100), 524 (99), 522 (32) [M-OEt]$^+$, 511 (12), 509 (38), 507 (36), 505 (13) [M − OEt − OH]$^+$, 482 (9), 480 (28), 478 (30), 476 (10) [M − 2×OEt]$^+$, 469 (11), 467 (32), 465 (33), 463 (12) [M − 2×OEt − OH]$^+$, 447 (35), 445 (71), 443 (37) [M − OEt − Br]$^+$, 403 (6), 401 (16), 399 (18), 397 (7), 283 (21), 281 (64), 279 (23), 201; HREIMS *m*/*z* 567.8731 [M]$^+$ (calcd for $C_{18}H_{19}{}^{79}Br_3O_6$ 567.8732).

2-(2,3-Dibromo-4,5-dihydroxybenzyl)-3,5-dihydroxy-4-methoxybenzyl alcohol (2): brown powder (Me_2CO); mp 114−116 °C; IR (KBr) ν_{max} 3330, 2918, 1600, 1587, 1501, 1462, 1385, 1348, 1279, 1171, 1097 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) δ 6.66 (1H, s, H-6′), 6.22 (1H, s, H-6), 4.35 (2H, s, H-7′), 3.94 (2H, s, H-7), 3.80 (3H, s, H-OMe); ^{13}C NMR (acetone-d_6, 125 MHz), see Table 1; EIMS *m*/*z* (%) 452 (10), 450 (30), 448 (11) [M]$^+$, 434 (5), 432 (15), 430 (5) [M − CH_4]$^+$, 417 (21), 415 (38), 413 (18) [M − CH_4 − OH]$^+$, 368 (92), 366 (84) [M − HBr]$^+$, 352, 350 [M − HBr − CH_4]$^+$, 337 (60), 335 (55) [M − HBr − OMe]$^+$, 323 (16), 321 (21), 309 (10), 307 (17), 305 (9), 288 (100) [M − Br_2]$^+$, 273 (53) [M − Br_2 − Me]$^+$, 257 (35) [M − OMe]$^+$, 241 (27), 229 (25), 182 (46), 181 (53), 82 (100), 80 (100); HREIMS *m*/*z* 447.9165 [M]$^+$ (calcd for $C_{15}H_{14}{}^{79}Br_2O_6$ 447.9157).

6-(2,3-Dibromo-4,5-dihydroxybenzyl)-2,3-dibromo-4,5-dihydroxybenzyl methyl ether (3): brown powder (Me_2CO); mp 171−172 °C (dec); IR (KBr) ν_{max} 3620, 3458, 3329, 2920, 1597, 1566, 1501, 1496, 1456, 1420, 1381, 1281, 1174, 1082, 1063, 1010, 999, 947, 858, 812 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) δ 6.18 (1H, s, H-6), 4.42 (2H, s, H-7′), 4.15 (2H, s, H-7), 3.28 (3H, s, H−OCH_3); ^{13}C NMR (acetone-d_6, 125 MHz), see Table 1; EIMS *m*/*z* (%) 564 (6), 562 (22), 560 (35), 558 (19), 556 (5) [M]$^+$, 547 (3), 545 (10), 543 (17), 541 (8), 539 (4) [M − OH]$^+$, 484 (6), 483 (13), 482 (30), 481 (42), 480 (55), 479 (42), 478 (38), 477 (13), 476 (9), 467 (1), 466 (2), 465 (8), 464 (7), 463 (16), 462 (8), 461 (7), 460 (1), 459 (1), 437 (3), 435 (9), 433 (12), 431 (4), 402 (35), 400 (67), 398 (31), 322 (22), 320 (20), 296 (5), 294 (10), 292 (5), 267 (10), 242 (31), 239 (15), 200 (13), 82 (100), 80 (87); HREIMS *m*/*z* 555.7149 [M − MeOH]$^+$ (calcd for $C_{14}H_8{}^{79}Br_4O_4$ 555.7156).

9,10-Dihydro-9,10-dimethoxy-3,4,7,8-tetrabromo-1,2,5,6-tetrahydroxyanthracene (4): brown powder (Me_2CO); mp 117−119 °C (dec); $[\alpha]_D^{20}$ 0° (MeOH, *c* 0.16); IR (KBr) ν_{max} 3330, 2929, 2833, 1608, 1597, 1560, 1501, 1414, 1350, 1311, 1227, 1192, 1103, 1076, 1028, 935, 868 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) δ 6.27 (2H, s, H-7 and 7′), 3.62 (6H, s, 2×OCH_3); ^{13}C NMR (acetone-d_6, 125 MHz), see Table 1; EIMS *m*/*z* (%) 558 (8), 556 (28), 554 (46), 552 (27), 550 (6) [M − 2×MeOH − 2H]$^+$, 480 (1), 478 (4), 476 (6), 474 (1), 449 (2), 447 (6), 445 (5), 443 (2), 421 (2), 419 (4), 417 (4), 415 (1), 284 (6), 282 (4), 239 (5), 82 (100), 80 (93); HREIMS *m*/*z* 549.6679 [M]$^+$ (calcd for $C_{14}H_2Br_4O_4$ 549.6686).

(+)-3-(2,3-Dibromo-4,5-dihydroxyphenyl)-4-bromo-5,6-dihydroxy-1,3-dihydroisobenzofuran (5): brown powder (Me_2CO); mp 132−134 °C; $[\alpha]_D^{20}$ +6° (MeOH, *c* 0.16); IR (KBr) ν_{max} 3386, 2866, 1604, 1595, 1500, 1464, 1408, 1358, 1308, 1277, 1163, 1092, 1024, 858, 812 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) δ 6.88 (1H, s, H-6), 6.45 (1H, br s, H-7′), 6.31 (1H, br s, H-6′), 5.03 (1H, d, J = 12.0 Hz, H-7a), 4.94 (1H, d, J = 12.0 Hz, H-7b); ^{13}C NMR (acetone-d_6, 125 MHz), see Table 1; EIMS *m*/*z* (%) 500 (10), 498 (34), 496 (32), 494 (12) [M]$^+$, 418 (1), 416 (3), 414 (1) [M − HBr]$^+$, 338 (5), 336 (4) [M − Br_2]$^+$, 309 (7), 307 (10), 297 (36), 295 (72), 293 (32), 242 (8), 231 (98), 229 (100), 200 (10), 152 (13), 82 (18), 80 (20); HREIMS *m*/*z* 493.7971 [M]$^+$ (calcd for $C_{14}H_9{}^{79}Br_3O_5$ 493.8000).

***rel*-(4a*S**,10a*R**)-(±)-6,7-Dibromo-4a-hydroxy-3,8-dihydroxymethyl-10a-methoxy-1,4,4a,10a-tetrahydrodibenzo-[*b*,*e*][1,4]dioxin-1-one (6):** brown powder (Me_2CO); mp 193−195 °C; $[\alpha]_D^{20}$ 0° (MeOH, *c* 0.10); IR (KBr) ν_{max} 3415, 3251, 2920,1699, 1631, 1601, 1570, 1504, 1452, 1437, 1406, 1358, 1288, 1163, 1063, 999, 910, 876 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) δ 7.37 (1H, s, H-6), 6.18 (1H, s, H-2), 4.63 (2H, s, H-7), 4.33 (1H, d, J = 18.0 Hz, H-7′a), 4.30 (1H, d, J = 18.0 Hz, H-7′b), 3.19 (3H, s, H-OCH_3), 3.15 (1H, d, J = 18.0 Hz, H-4a), 2.94 (1H, d, J = 18.0 Hz, H-4b); ^{13}C NMR (acetone-d_6, 125 MHz), see Table 1; EIMS *m*/*z* (%) 468 (5), 466 (9), 464 (5) [M]$^+$, 356 (2), 355 (3), 354 (12), 353 (7), 352 (8), 351 (7), 350 (2), 359 (3), 341 (11), 339 (24), 337 (13), 300 (24), 298 (51), 296 (31), 284 (2), 283 (6), 282 (8), 281 (14), 280 (14), 279, (8), 278 (6), 277 (1), 254 (3), 252 (6), 250 (3), 219 (20), 217 (23), 190

(16), 188 (16), 170 (100), 153 (18), 127 (25), 110 (67), 81 (25), 58 (62); HREIMS *m/z* 463.9133 $[M]^+$ (calcd for $C_{15}H_{14}{}^{79}Br_2O_7$ 463.9106).

Crystal data of 6: $C_{15}H_{14}O_7Br_2$, M_r 466.08, triclinic, space group $P\bar{1}$, $a = 16.389(3)$ Å, $b = 7.402(1)$ Å, $c = 7.287(1)$ Å, $\alpha = 78.24(1)°$, $\beta = 78.51(1)°$, $\gamma = 94.74(1)°$; $V = 841.09$ (23) Å^3, $Z = 2$, $D_c = 1.880$ g cm^{-3}, $F(000) = 470$, μ(Mo Kα) = 2.57 cm^{-1}; crystal dimensions 0.04 × 0.04 × 0.10 mm.

Cells and Culture Conditions. Human lung adenocarcinoma (A549), human stomach cancer (BGC-823), human breast cancer (MCF-7), human hepatoma (Bel7402), and human colon cancer (HCT-8) cell lines were obtained from ATCC. Cells were maintained in RRMI1640 supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin, and 100 μg/mL streptomycin. Cultures were incubated at 37 °C in a humidified 5% CO_2 atmosphere.

Cell Proliferation Assay. A549, BGC823, MCF-7, Bel7402, and HCT-8 cells were seeded in 96-well microtiter plates at 1200 cells/well. After 24 h, the compounds were added to the cells. After 96 h of drug treatment, cell viability was determined by measuring the metabolic conversion of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) into purple formazan crystals by active cells.[24,25] MTT assay results were read using a MK 3 Wellscan (Labsystem Drogon) plate reader at 570 nm. All compounds were tested in five concentrations and were dissolved in 100% DMSO with a final DMSO concentration of 0.1% in each well. Each concentration of the compounds was tested in three parallel wells. IC_{50} values were calculated using Microsoft Excel software.

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Supporting Information Available: MS, 1D and 2D NMR spectra of compounds **1**−**6**; X-ray data of compound **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Katsui, N.; Suzuki, Y.; Kitamura, Y.; Irie, T. *Tetrahedron* **1967**, *23*, 1185−1188.
(2) Suzuki, M.; Kowata, N.; Kurosawa, E. *Bull. Chem. Soc. Jpn.* **1980**, *53*, 2099−2100.
(3) Kurata, K.; Amiya, T. *Phytochemistry* **1980**, *19*, 141−142.
(4) Glombitza, K.-W.; Sukopp, I.; Wiedenfeld, H. *Planta Med.* **1985**, *36*, 437−440.
(5) Kurihara, H.; Mitani, T.; Kawabata, J.; Takahashi, K. *J. Nat. Prod.* **1999**, *62*, 882−884.
(6) Flodin, C.; Whitfield, F. B. *Phytochemistry* **2000**, *53*, 77−80.
(7) Choi, J. S.; Park, H. J.; Jung, H. A.; Chung, H. Y.; Jung, J. H.; Choi, W. C. *J. Nat. Prod.* **2000**, *63*, 1705−1706.
(8) Yoo, H.-D.; Ketchun, S. O.; France, D.; Bair, K.; Gerwick, W. H. *J. Nat. Prod.* **2002**, *65*, 51−53.
(9) Fan, X.; Xu, N.-J.; Shi, J.-G. *J. Nat. Prod.* **2003**, *66*, 455−458.
(10) Kurata, K.; Taniguchii, K.; Takashima, K.; Hayashi, I.; Suzuki, M. *Phytochemistry* **1997**, *45* (3), 485−487.
(11) Sun, H. H.; Paul, V. J.; Fenical, W. *Phytochemistry* **1983**, *22*, 743−745.
(12) Fu, X.; Schmitz, F. J.; Govindan, M.; Abbas, S. A.; Hanson, K. M.; Horton, P. A.; Crews, P.; Laney, M.; Schatzman, R. C. *J. Nat. Prod.* **1995**, *58*, 1384−1391.
(13) Fu, X.; Schmitz, F. J. *J. Nat. Prod.* **1996**, *59*, 1102−1103.
(14) Handayani, D.; Edrada, R. A.; Proksch, P.; Wray, V.; Witte, L.; Van Soest, R. W. M.; Kunzmann, A.; Soedarsono, J. *J. Nat. Prod.* **1997**, *60*, 1313−1316.
(15) Xu, X.-L.; Fan, X.; Song, F.-H.; Zhao, J.-L.; Han, L.-J.; Shi, J.-G. *J. Asia Nat. Prod. Res.* (in press)
(16) Koch, M.; Gregson, R. P. *Phytochemistry* **1984**, *23*, 2633−2637.
(17) Green, D.; Kashman, Y.; Miroz Ahron. C. *J. Nat. Prod.* **1993**, *56*, 1201−1202.
(18) Ciminiello, P.; Dell'Aversano, C.; Fattorusso, E.; Magno, S.; Pansini, M. *J. Nat. Prod.* **2000**, *63*, 263−266.
(19) Utkina, N. K.; Denisenko, V. A.; Scholokova, O. V.; Virovaya, M. V. *J. Nat. Prod.* **2001**, *64*, 151−153.
(20) Lindsay, B. S.; Battershill, C. N.; Copp, B. R. *J. Nat. Prod.* **1998**, *61*, 857−858.
(21) Miao, S.; Andersen, R. J. *J. Org. Chem.* **1991**, *56*, 6275−6280.
(22) Carroll, A. R.; Healy, P. C.; Quinn, R. J.; Tranter, C. J. *J. Org. Chem.* **1999**, *64*, 2680−2682.
(23) Rudi, A.; Evan, T.; Aknin, M.; Kashman, Y. *J. Nat. Prod.* **2000**, *63*, 832−833.
(24) Mosumann, T. *J. Immunol. Methods* **1983**, *65*, 55−63.
(25) Carmichael, J.; DeGraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. *Cancer Res.* **1987**, *47*, 936−942.

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