Bromophenol Derivatives from the Red Alga *Rhodomela confervoides*

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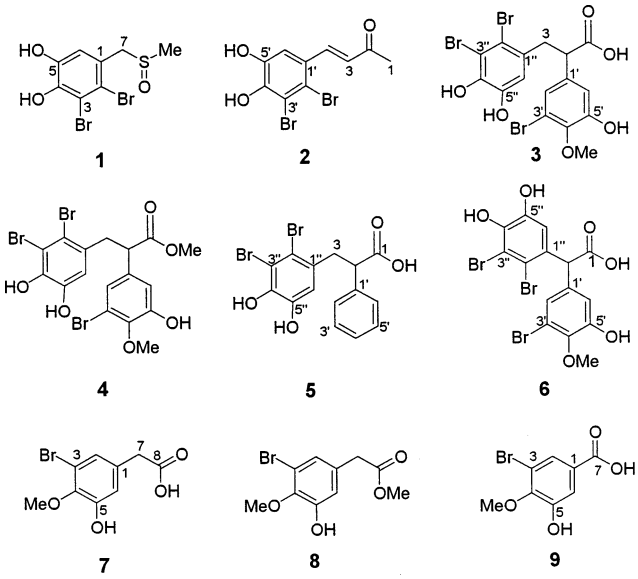
Received December 31, 2003

Eight new bromophenol derivatives, 2,3-dibromo-4,5-dihydroxybenzyl methyl sulfoxide (**1**), 4-(2,3-dibromo-4,5-dihydroxyphenyl)-3-butene-2-one (**2**), 2-(3-bromo-5-hydroxy-4-methoxyphenyl)-3-(2,3-dibromo-4,5-dihydroxyphenyl)propionic acid (**3**), 2-(3-bromo-5-hydroxy-4-methoxyphenyl)-3-(2,3-dibromo-4,5-dihydroxyphenyl)propionic acid methyl ester (**4**), 2-phenyl-3-(2,3-dibromo-4,5-dihydroxyphenyl)propionic acid (**5**), 4′-methoxy-2″,3′,3″-tribromo-4″,5′,5″-trihydroxydiphenylacetic acid (**6**), and 3-bromo-5-hydroxy-4-methoxyphenylacetic acid (**7**) and its methyl ester (**8**), together with a known bromophenol, 3-bromo-5-hydroxy-4-methoxybenzoic acid (**9**), were isolated from the red alga *Rhodomela confervoides*. Their structures were elucidated by spectroscopic methods including IR, EIMS, FABMS, ESIMS, HRFABMS, HRESIMS, 1D and 2D NMR, and single-crystal X-ray structure analysis. Compounds **1**−**4**, **8**, and **9** were found inactive against several human cancer cell lines and microorganisms.

The red marine algae of the Rhodomelaceae family are rich sources of bromophenols. Previous chemical studies on some species of this family have resulted in the characterization of more than 30 monoaryl and diaryl bromophenols.[1−12] Some of them showed feeding deterrent,[8] α-glucosidase inhibitory,[11] and growth stimulatory[13] activities. As part of our recently initiated program to assess systematically the chemical and biological diversity of seaweeds distributed in the gulf of the Yellow Sea, China, 14 bromophenols including monoaryl, diaryl, and triaryl structural types have been previously reported from a sample of *Rhodomela confervoides* collected at the coast of Qingdao, China.[14,15] Continuing our investigation on the same material, we report here the isolation and structure elucidation of nine bromophenol derivatives (**1**−**9**). Among them, compound **1** possesses a unique sulfoxide structural feature. Compound **7** was previously synthesized as an inhibitor of protein kinase C,[16,17] and compound **9** was synthesized and evaluated as an inhibitor of liver catechol *O*-methyltransferase[18] and human renal thiopurinemethyltransferase.[19] Compounds **1**−**4**, **8**, and **9** were evaluated, but found inactive, against several human cancer cell lines (IC_{50} >10 μg/mL) including lung adenocarcinoma (A549), stomach cancer (BGC-823), breast cancer (MCF-7), hepatoma (Bel7402), and human colon cancer (HCT-8) cell lines, as well as three microorganisms (MIC_{50} > 100 μg/mL) including *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*.



Results and Discussion

The air-dried and ground red alga *Rhodomela confervoides* was extracted with 95% EtOH, and the concentrated extract was suspended in water and then partitioned with EtOAc. The EtOAc extract was chromatographed over silica gel eluting with a gradient of increasing MeOH (0−100%) in $CHCl_3$. Further purification of the resulting fractions by a variety of chromatographic techniques yielded bromophenols **1**−**9**.

Compound **1** was obtained as colorless crystals, mp 168−170 °C. The IR spectrum showed an absorption band for hydroxyl groups at 3438 cm^{-1} as well as the characteristic absorption bands for aromatic rings at 1599 and 1504 cm^{-1}. The FABMS exhibited a characteristic quasi-molecular ion peak cluster at *m/z* 343/345/347 (1:2:1) $[M + H]^+$ suggesting the presence of two bromine atoms, and the molecular formula $C_8H_8O_3Br_2S$ was determined by HRFABMS. The ^1H NMR spectrum of **1** showed an aromatic singlet at *δ* 6.89 (1H, s, H-6), a methyl singlet at *δ* 2.55 (3H, s, $SOCH_3$), and a typical AB spin coupling system attributed to an isolated methylene at *δ* 4.19 (1H, d, *J* = 13.5 Hz, H-7a) and 4.02 (1H, d, *J* = 13.5 Hz, H-7b), as well as two exchangeable broad singlets of phenolic hydroxyl protons at *δ* 9.72 (1H, s, OH-4) and 10.10 (1H, s, OH-5). The ^{13}C NMR and DEPT spectra of **1** displayed eight carbon signals for a penta-substituted benzene ring, a methyl, and a methylene. A strong fragment cluster at *m/z* 279/281/283 (1:2:1) in the FABMS of **1** and the similarity between the NMR data of **1** and 2,3-dibromo-4,5-dihydroxybenzyl alcohol[11] suggested the presence of a 2,3-dibromo-4,5-dihydroxybenzyl moiety in **1**. This was unambiguously confirmed by long-range correlations from H-6 to C-1, C-2, C-4, C-5, and C-7, from H_2-7 to C-1, C-2, and C-6, and from the

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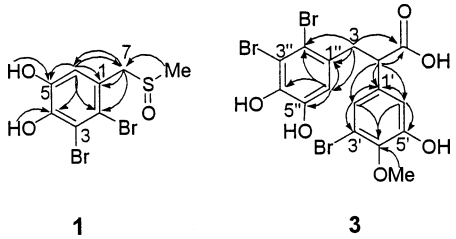
10.1021/np030546+ CCC: $27.50
Published on Web 06/25/2004



1 **3**

Figure 1. Key HMBC correlations of compound **1** and **3**.

exchangeable phenolic protons to C-4 and C-5 in the HMBC spectrum of **1**. In combination with the chemical shift values of the methyl group (δ_H 2.55 and δ_C 43.0) and the molecular composition of **1** ($C_8H_8O_3Br_2S$), the strong HMBC correlation from the methyl protons to the methylene carbon (C-7) unequivocally revealed that the 2,3-dibromo-4,5-dihydroxybenzyl moiety was connected with the methyl through a sulfoxide group. Therefore, the structure of **1** was determined as 2,3-dibromo-4,5-dihydroxybenzyl methyl sulfoxide. It is considered to be a racemate since no optical rotation was observed for **1**. The X-ray structure analysis confirmed the above structural assignment of **1**. The PLUTO drawing, with the atom-numbering scheme indicated, is shown in the Supporting Information. The second space group $P2_1/c$ of the crystal indicated that **1** is optically inactive in the crystal state.

Compound **2** was obtained as brown needles, mp 165−166 °C. The IR spectrum (KBr) showed absorption bands for hydroxyl (3431 cm^{-1}) and conjugated carbonyl (1712 cm^{-1}) groups and aromatic rings (1595, 1464 cm^{-1}). Its negative mode ESIMS exhibited dibrominated molecular ion peaks at *m/z* 333/335/337 (1: 2: 1) $[M - H]^-$. In combination with the NMR data, the molecular formula of **2** was established as $C_{10}H_8O_3Br_2$. The ^1H NMR spectrum of **2** showed an aromatic singlet at δ 7.32 (1H, s, H-6′), two *trans* coupled olefinic proton doublets (J = 16.0 Hz) at δ 6.53 (H-3) and 7.86 (H-4), and an acetyl singlet at δ 2.31 (3H). Besides the acetyl carbons (δ 197.4 and 26.3) and the two olefinic carbons (δ 142.6 and 113.7), the ^{13}C NMR spectra of **2** displayed six carbon signals attributed to a 2,3-dibromo-4,5-dihydroxyphenyl moiety. Therefore, the structure of **2** was elucidated as 4-(2,3-dibromo-4,5-dihydroxyphenyl)-3-butene-2-one, which was further confirmed by single-crystal X-ray structure analysis. The PLUTO drawing, with the atom-numbering scheme indicated, is shown in Supporting Information.

Compound **3** was obtained as a brown gum and showed IR absorptions for hydroxyl (3400 cm^{-1}) and carbonyl (1705 cm^{-1}) groups and aromatic rings (1568 and 1485 cm^{-1}). The negative mode ESIMS of **3** gave a characteristic tribromonated quasi-molecular ion peak cluster at 537/539/541/543 (1:3:3:1) $[M - H]^-$, and the molecular formula $C_{16}H_{13}O_6Br_3$ was determined by HRESIMS at *m/z* 560.8152 $[M + Na]^+$ (calcd for $C_{16}H_{13}O_6{}^{79}Br_3Na$ 560.8160). Three aromatic signals at δ 7.05 (1H, d, J = 1.5 Hz, H-2′), 6.93 (1H, d, J = 1.5 Hz, H-6′), and 6.79 (1H, s, H-6″) in the ^1H NMR spectrum and 12 sp^2 carbon signals including three methines and nine quaternary carbons (four oxygenated, δ > 143 ppm) in the ^{13}C NMR and DEPT spectra (see Experimental Section) indicated the presence of two benzene rings with 1,2,3,4,5-penta and 1,3,4,5-tetra substitution patterns. Meanwhile, an ABX spin coupling system at δ 3.85 (1H, dd, J = 9.5, 6.0 Hz, H-2), 3.34 (1H, dd, J = 14.0, 9.5 Hz, H-3a), and 3.02 (1H, dd, J = 14.0, 6.0 Hz, H-3b) in the ^1H NMR spectrum and three carbon signals at δ 173.8 (s, C-1), 51.0 (d, C-2), and 41.6 (t, C-3) in the ^{13}C NMR and DEPT spectra demonstrated the presence of a 2,3-disubstituted propionic acid unit in **3**. In addition, the NMR spectra showed that there is one methoxyl group at δ_H 3.79 (3H, s, O*CH*$_3$) and δ_C 60.7 (q). The unambiguous assignments of the proton and carbon signals and the establishment of the structure were based on the HMQC and HMBC experiments. In combination with the chemical shift values of the two benzene rings, long-range correlations of C-1′, C-3′, C-4′, and C-5′ with both H-2′ and H-6′, and C-4′ with the methoxyl protons in the HMBC spectrum, revealed that the 1,3,4,5-tetra-substituted benzene ring is a 3-bromo-5-hydroxy-4-methoxyphenyl, while correlations from H-6″ to C-1″, C-2″, C-4″, and C-5″ established that the 1,2,3,4,5-penta-substituted benzene ring is a 2,3-dibromo-4,5-dihydroxyphenyl. In addition, correlations of the carboxyl carbon (C-1) with H-2, H-3a, and H-3b confirmed the presence of the 2,3-disubstituted propionic acid unit, while correlations from H-2 to C-1′, C-2′, and C-6′ and from both H-3a and H-3b to C-1″, C-2″, and C-6″ unambiguously located the 3-bromo-5-hydroxy-4-methoxyphenyl and 2,3-dibromo-4,5-dihydroxyphenyl at C-2 and C-3 of the propionic acid unit, respectively. Therefore, the structure of **3** was determined as 2-(3-bromo-5-hydroxy-4-methoxyphenyl)-3-(2,3-dibromo-4,5-dihydroxyphenyl)propionic acid.

Compound **4** was obtained as a brown gum. Its negative mode ESIMS gave a tribrominated quasi-molecular ion peak cluster at *m/z* 551/553/555/557 (1:3:3:1) $[M - H]^-$, which is 14 mass units more than that of **3**. The IR and NMR spectra of **4** (see Experimental Section) showed resemblance to those of **3** except the appearance of an additional methoxyl signal at δ_H 3.58 (3H, s) and δ_C 52.2 (q, O*CH*$_3$) in the NMR spectra of **4**, suggesting that **4** is a methyl ester of **3**. This was confirmed by the HMBC experiment of **4** and the acidic hydrolysis of **4** yielding **3**. Consequently, **4** was unequivocally assigned as 2-(3-bromo-5-hydroxy-4-methoxyphenyl)-3-(2,3-dibromo-4,5-dihydroxyphenyl)propionic acid methyl ester.

Compound **5** was obtained as a brown gum, and the IR spectrum (KBr) showed absorption bands for hydroxyl (3330 cm^{-1}) and conjugated carbonyl (1712 cm^{-1}) groups and aromatic rings (1601, 1468 cm^{-1}). The negative FABMS spectrum showed a dibrominated quasi-molecular ion peak cluster at 413/415/417 (1:2:1) $[M - H]^-$. In combination with the NMR data, the molecular formula of **5** was established as $C_{15}H_{12}O_4Br_2$. In the NMR spectra, signals at δ_H 7.26 (1H, t, J = 7.5 Hz, H-4′), 7.32 (2H, t, J = 7.5 Hz, H-3′, 5′), and 7.38 (2H, d, J = 7.5 Hz, H-2′, 6′) and δ_C 128.1 (C-4′), 129.5 (C-3′ and C-5′), 128.8 (C-2′ and C-6′), and 140.1 (C-1′) revealed the presence of a phenyl unit in the structure of **5**. Meanwhile, signals at δ_H 6.78 (1H, s, H-6″) and δ_C 132.1 (s, C-1″), 116.7 (s, C-2″), 113.8 (s, C-3″), 144.0 (s, C-4″), 145.3 (s C-5″), and 117.8 (d, C-6″) demonstrated the presence of a 2,3-dibromo-4,5-dihydroxyphenyl moiety in **5**. In addition, a 2,3-disubstituted propionic acid unit was indicated by an ABX coupling system at δ_H 3.97 (1H, dd, J = 6.0, 9.0 Hz, H-2), 3.43 (1H, dd, J = 9.0, 14.0 Hz, H-3a), and 3.05 (1H, dd, J = 6.0, 14.0 Hz, H-3b) in the ^1H NMR spectrum and carbon signals at δ_C 174.3 (s, C-1), 52.1 (d, C-2), and 41.9 (t, C-3) in the ^{13}C NMR spectrum. The existence of the above three moieties was confirmed by the HMQC and HMBC experiments of **5**. In the HMBC spectrum, correlations from H-2 to C-2′ and C-6′ and from H-3 to C-2″ and C-6″ unequivocally established that the phenyl and 2,3-dibromo-4,5-dihydroxyphenyl were located at C-2 and C-3, respectively, of the 2,3-disubstituted propionic acid unit. Accordingly, the structure of **5** was determined as 2-phenyl-3-(2,3-dibromo-4,5-dihydroxyphenyl)propionic acid.

Compound **6** was obtained as a brown gum, and its negative mode ESIMS exhibited tribrominated molecular ion peaks at *m/z* 523/525/527/529 (1: 3:3: 1) $[M - H]^-$. In combination with NMR data, the molecular formula of **6** was established as $C_{15}H_{11}O_6Br_3$. The NMR spectra of **6** were in close agreement with those of **3**, except for the absence of signals for the methylene, suggesting that the 2,3-disubstituted propionic acid unit of **3** was replaced by a disubstituted acetic acid unit of **6**. The NMR signal assignments and the structure of **6** were confirmed by HMQC and HMBC experiments of **6**. In the HMBC spectrum of **6**, long-range correlations from H-2 to C-1, C-2′, C-6′, C-2″, and C-6″ confirmed the diarylacetic acid structure of **6**. Thus, the structure of **6** was determined as 4′-methoxy-2″,3′,3″-tribromo-4″,5′,5″-trihydroxydiphenylacetic acid. Compounds **3**−**6** did not show any optical rotation, suggesting these to be racemic mixtures.

The IR, MS, and NMR spectra of **7** and **9** were in good agreement with those reported for 3-bromo-5-hydroxy-4-methoxyphenylacetic acid and 3-bromo-5-hydroxy-4-methoxybenzoic acid, respectively. **7** has been synthesized as a protein kinase C inhibitor,[16,17] and **9** was synthesized as an inhibitor of catechol *O*-methyltransferase (COMT) and human renal thiopurinemethyltransferase.[18,19] However, **7** and **9** were obtained as natural products for the first time, and their ^{13}C NMR data assigned by HMQC and HMBC experiments were included (see Experimental Section) since these data did not appear in the previous literature.

The EIMS of **8** gave a molecular ion peak cluster with 14 mass units more than that of **7**, and the IR and ^1H NMR spectra of **8** were very similar to those of **7** except for the appearance of signals for an additional methoxyl at δ 3.63 (3H, s, COO*CH*$_3$) in the ^1H NMR spectrum of **8**, suggesting that **8** is 3-bromo-5-hydroxy-4-methoxyphenylacetic acid methyl ester, which was further confirmed by ^{13}C NMR, HMQC, and HMBC experiments and the acidic hydrolysis of **8** producing **7**. Compounds **4** and **8** were considered not to be artifacts from methylation of **3** and **7**, respectively, since the methylation did not occur in the simulated isolation conditions by heating the methanol solutions of **3** and **7** either with or without silica gel at 45 °C for 48 h.

Compounds **1**, **3**, **8**, and **9** were tested for their antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* and showed no growth inhibition at 100 μg/mL. Cytotoxicities of compounds **1**, **2**, **3**, **4**, and **8** were evaluated using the MTT method[20,21] 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, but were found to be inactive at 10 μg/mL.

Experimental Section

General Experimental Procedures. Melting points were determined on an XT-4 micro melting point apparatus and are uncorrected. IR spectra were recorded as KBr disks on a Nicolet Impact 400 FT-IR spectrophotometer. 1D- and 2D-NMR spectra were obtained at 500 and 125 MHz for ^1H and ^{13}C, respectively, on an Inova 500 MHz spectrometer in DMSO and acetone-d_6 with solvent peaks as references. EIMS, FABMS, and HRFABMS data were measured with a Micromass Autospec-Ultima ETOF spectrometer; ESIMS and HRESIMS data were measured with an Applied Biosystems Qtrap spectrometer and a Bruker AOEXIII 7.0 TESLA FTMS, respectively. Column chromatography was performed with silica gel (200−300 mesh), Bio-Beads SX3 (200−400 mesh), RP-18 reverse-phase silica gel (43−60 μm), and Sephadex LH-20. TLC was carried out with glass precoated silica gel GF_{254} plates. Spots were visualized under UV light or by spraying with 3% $FeCl_3$ in EtOH. HPLC was performed using an Alltima C18 10 μm preparative column (22 × 250 mm).

X-ray diffraction intensity data of **1** and **2** were collected on a Rigaku R-AXIS RAPID diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.71067 Å) by the ω scan technique (scan width 0−220°, $2\theta \le 55°$ for **1** and 0−180°, $2\theta \le 50°$ for **2**) and were corrected by Lorentz and polarization. A total of 2276 and 1582 reflections were collected, of which 1913 and 1396 with $|F|^2 \geqslant 3\sigma|F|^2$ were observed for **1** and **2**, respectively. The structures of **1** and **2** were solved by direct methods and refined by block-matrix least-squares procedures to $R_w = 0.079$ [$w = 1/\sigma|F|^2$], $R = 0.060$ of **1** and $R_w = 0.089$ [$w = 1/\sigma|F|^2$], $R = 0.087$ of **2**, respectively. Hydrogen positions were found from difference Fourier maps and geometric calculations. All calculations were carried out on a PC computer by using the NOMCSDP program system.

Material. The red alga *Rhodomela confervoides* were collected at the coast of Qingdao, China, in May 2001 and identified by Professor B.-M Xia. A voucher specimen (No. 200102) was deposited at the Department of Marine Algae Chemistry, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071.

Extraction and Isolation. Air-dried *R. confervoides* (14.4 kg) was extracted with EtOH at room temperature for 3 × 48 h. After the solvent was removed under reduced pressure at <40 °C, a dark residue was obtained. The residue was suspended in water and then partitioned with EtOAc. The EtOAc fraction (594.6 g) was chromatographed over silica gel (1200 g) eluting with a gradient of increasing MeOH (0−100%) in $CHCl_3$ and separated into 24 fractions (I−XXIV) on the basis of TLC analyses. Fraction XI (13 g) was chromatographed over Sephadex LH-20 eluting with petroleum ether−$CHCl_3$−MeOH (5: 5: 1), decolored by column chromatography over Bio-Beads SX3 using $CHCl_3$−EtOAc (1: 1), and then separated by flash chromatography to yield compound **1** (15 mg) and reverse-phase preparative HPLC using MeOH−H_2O−AcOH (60:40:0.1) as mobile phase to yield **2** (6 mg) and **5** (4 mg). Fraction XII (21 g) was chromatographed over reverse-phase MPLC eluting with a gradient of increasing MeOH in H_2O and separated into three subfractions. Each subfraction was decolored by Sephadex LH-20 eluting with $CHCl_3$−MeOH (1:1) to give corresponding decolored subfractions. The first fraction was then separated by reverse-phase preparative HPLC using MeOH−H_2O−AcOH (60:40:0.1) as mobile phase to yield **7** (15 mg) and **9** (10 mg). The second subfraction was purified by normal-phase preparative HPLC using hexane−2-propanol (50:50) as mobile phase to yield **6** (14 mg) and **8** (153 mg). The last subfraction was separated by reverse-phase preparative HPLC using MeOH−H_2O−AcOH (70:30:0.1) as mobile phase to yield **3** (213 mg) and **4** (27 mg).

2,3-Dibromo-4,5-dihydroxybenzyl methyl sulfoxide (1): colorless crystals (water−methanol, 1:1), mp 168−170 °C; IR (KBr) ν_{max} 3438, 3005, 2700, 1599, 1504, 1410, 1346, 1281, 1182, 1005, 966, 887, 856, 727, 648 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 10.10 (1H, br, s, OH-5), 9.72 (1H, br, s, OH-4), 6.89 (1H, s, H-6), 4.19 (1H, d, J = 13.5 MHz, H-7a), 4.02 (1H, d, J = 13.5 MHz, H-7b), 2.55 (3H, s, H-8); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 128.0 (s, C-1), 120.9 (s, C-2), 118.5 (s, C-3), 149.9 (s, C-4), 150.2 (s, C-5), 122.9 (d, C-6), 65.8 (t, C-7), 43.0 (q, C-8); FABMS *m/z* 343, 345, 347 $[M + H]^+$ (13, 26, 14), 283 (21), 281 (42), 279 (22), 185 (58), 133 (33), 93 (100), 75 (50), 57 (40); HRFABMS *m/z* 342.8641 (calcd for $C_8H_9O_3{}^{79}Br_2S$ 342.8639).

Crystal data of 1: $C_8H_8O_3Br_2S$, M_r 344.02, monoclinic, space group $P2_1/c$, a = 12.506(1) Å, b = 4.473(1) Å, c = 19.156 (2) Å, β = 91.89(1)°; V = 1071.0(2) Å^3, Z = 4, D_c = 2.134 g cm^{-3}; crystal dimensions 0.14 × 0.12 × 0.20 mm; see Supporting Information for more details.

4-(2,3-Dibromo-4,5-dihydroxyphenyl)-3-butene-2-one (2): brown crystals (MeOH−H_2O), mp 165−166 °C; IR (KBr) ν_{max} 3431, 2962, 2918, 2850, 1712, 1595, 1464, 1398, 1261, 1097, 1026, 968, 802, 698 cm^{-1}; H NMR (acetone-d_6, 500 MHz) δ 7.86 (1H, d, J = 16.0 Hz, H-4), 6.53 (1H, d, J = 16.0 Hz,

H-3), 2.31 (3H, s, H-1), 7.32 (1H, s, H-6′); ^{13}C NMR (acetone-d_6, 125 MHz) δ 26.3 (q, C-1), 197.4 (s, C-2), 113.7 (d, C-3), 142.6 (d, C-4), 130.7 (s, C-1′), 118.8 (s, C-2′), 114.4 (s, C-3′), 146.0 (s, C-4′), 147.7 (s, C-5′), 128.0 (d, C-6′); ESIMS *m*/*z* 333, 335, 337 $[M - H]^-$.

Crystal data of 2: $C_{10}H_8O_3Br_2$, M_r 335.98, orthorhombic, space group $P2_1/a$, a = 7.371(1) Å, b = 12.271(1) Å, c = 12.234(1) Å, β = 83.12(1)°; V = 1098.58(19) Å^3, Z = 4, D_c = 2.019 g cm^{-3}; crystal dimensions 0.05 × 0.05 × 0.20 mm; see Supporting Information for more details.

2-(3-Bromo-5-hydroxy-4-methoxyphenyl)-3-(2,3-dibromo-4,5-dihydroxyphenyl)propionic acid (3): brown gum; IR (KBr) ν_{max} 3400, 2937, 1705, 1568, 1485, 1427, 1406, 1275, 1232, 1182, 989, 926, 860, 820, 754, 652 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) δ 3.85 (1H, dd, J = 9.5, 6.0 Hz, H-2), 3.34 (1H, dd, J = 14.0, 9.5 Hz, H-3a), 3.02 (1H, dd, J = 14.0, 6.0 Hz, H-3b), 6.79 (1H, s, H-6″), 7.05 (1H, d, J = 1.5 Hz, H-2′), 6.93 (1H, d, J = 1.5 Hz, H-6′), 3.79 (3H, s, OCH_3); ^{13}C NMR (acetone-d_6, 125 MHz) δ 173.8 (s, C-1), 51.0 (d, C-2), 41.6 (t, C-3), 131.5 (s, C-1″), 116.6 (s, C-2″), 113.7 (s, C-3″), 145.3 (s, C-4″), 144.1 (s, C-5″), 117.7 (d, C-6″), 137.4 (s, C-1′), 123.5 (d, C-2′), 117.4 (s, C-3′), 145.3 (s, C-4′), 152.0 (s, C-5′), 116.6 (d, C-6′), 60.7 (q, OCH_3); ESIMS *m*/*z* 537, 539, 541, 543 $[M - H]^-$; HRESIMS *m*/*z* 560.8152 $[M + Na]^+$ (calcd for $C_{16}H_{13}O_6{}^{79}Br_3Na$ 560.8160).

2-(3-Bromo-5-hydroxy-4-methoxyphenyl)-3-(2,3-dibromo-4,5-dihydroxyphenyl)propionic acid methyl ester (4): brown gum; IR (KBr) ν_{max} 3406, 2927, 2852, 1724, 1568, 1485, 1429, 1275, 1169, 1128, 991, 926, 860, 802, 762, 652 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) δ 7.01 (1H, s, H-2′), 6.91(1H, s, H-6′), 6.75 (1H, s, H-6″), 3.86 (1H, dd, J = 9.0, 6.5 Hz, H-2), 3.34 (1H, dd, J = 14.0, 9.0 Hz, H-3a), 3.04 (1H, dd, J = 14.0, 6.5 Hz, H-3b), 3.58 (3H, s, COOCH_3), 3.79 (3H, s, OCH_3); ^{13}C NMR (acetone-d_6, 125 MHz) δ 173.4 (s, C-1), 51.2 (d, C-2), 41.7 (t, C-3), 130.9 (s, C-1″), 116.3 (s, C-2″), 113.7 (s, C-3″), 145.2 (s, C-4″), 144.5 (s, C-5″), 117.5 (d, C-6″), 137.0 (s, C-1′), 123.4 (d, C-2′), 117.4 (s, C-3′), 145.5 (s, C-4′), 152.0 (s, C-5′), 116.4 (d, C-6′), 60.6 (q, OCH_3), 52.2 (q, COOCH_3); ESIMS *m*/*z* 551, 553, 555, 557 $[M - H]^-$.

Acidic Hydrolysis of 4. A solution of **4** (2 mg) in 2 N HCl (2 mL) was stirred at 50 °C for 4 h and then evaporated to dryness under reduced pressure. The residue was dissolved with acetone (0.5 mL) and analyzed by TLC and reversed-phase HPLC together with **3**, indicating that the hydrolysis product of **4** is identical to **3**. The developing solvent system was $CHCl_3$−MeOH−AcOH (6:1:0.1) for TLC. The HPLC analysis was carried out with a YMC-Pack ODS-AM column (3 μm, 100 × 4.6 mm) and a DAD detector (254 nm) using methanol−water (7:3) as mobile phase (0.5 mL/min).

2-Phenyl-3-(2,3-dibromo-4,5-dihydroxyphenyl)propionic acid (5): brown gum; IR (KBr) ν_{max}3330, 2918, 2850, 1712, 1660, 1601, 1468, 1401, 1385, 1275, 1180, 860, 783, 698 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) δ 3.97 (1H, dd, J = 6.0, 9.0 Hz, H-2), 3.43 (1H, dd, J = 9.0, 13.5 Hz, H-3a), 3.05 (1H, dd, J = 6.0, 13.5 Hz, H-3b), 7.38 (2H, d, J = 7.5 Hz, H-2′, 6′), 7.32 (2H, t, J = 7.5 Hz, H-3′, 5′), 7.26 (1H, t, J = 7.5 Hz, H-4′); 6.78 (1H, s, H-6″); ^{13}C NMR (acetone-d_6, 125 MHz) δ 174.3 (s, C-1), 52.1 (d, C-2), 41.9 (t, C-3), 140.1 (s, C-1′), 128.8 (d, C-2′, 6′), 129.5 (d, C-3′, 5′), 128.1 (d, C-4′), 132.1 (s, C-1″), 116.7 (s, C-2″), 113.8 (s, C-3″), 144.0 (s, C-4″), 145.3 (s, C-5″), 117.8 (d, C-6″); FABMS *m*/*z* 413, 415, 417 $[M - H]^-$ (15, 27, 17), 339 (79), 325 (61), 311 (28), 293 (14), 291 (21), 289 (14), 265 (29), 263 (20), 183 (84), 173 (53), 171 (60), 151 (22), 91 (100), 71 (51).

4′-Methoxy-2″,3′,3″-tribromo-4″,5′,5″-trihydroxydiphenylacetic acid (6): brown gum; IR (KBr) ν_{max}3386, 2935, 1699, 1570, 1483, 1404, 1275, 1167, 1124, 991, 860 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) δ 7.00 (1H, s, H-2′), 6.85 (1H, s, H-6′), 6.89 (1H, s, H-6″), 5.32 (1H, s, H-2), 3.80 (3H, s, OCH_3); ^{13}C NMR (acetone-d_6, 125 MHz) δ 172.9 (s, C-1), 57.2 (d, C-2), 136.6 (s, C-1′), 124.7 (s, C-2′), 117.5 (s, C-3′), 145.3 (s, C-4′), 151.9 (s, C-5′), 117.7 (d, C-6′), 131.8 (s, C-1″), 117.4 (s, C-2″), 113.9 (s, C-3″), 144.7 (s, C-4″), 145.1 (s, C-5″), 116.4 (d, C-6″), 60.7 (q, OCH_3); ESIMS *m*/*z* 523, 525, 527, 529 $[M - H]^-$.

3-Bromo-5-hydroxy-4-methoxyphenylacetic acid (7): ^{13}C NMR (acetone-d_6, 125 MHz) δ 173.1 (s, C-8), 40.7 (t, C-7), 133.8 (s, C-1), 125. 1 (d, C-2), 117.0 (s, C-3), 144.6 (s, C-4), 151.6 (s, C-5), 118.0 (d, C-6), 60.6 (q, OCH_3); HREIMS *m*/*z* 259.9679 (calcd for $C_9H_9O_4{}^{79}Br$ 259.9684).

3-Bromo-5-hydroxy-4-methoxyphenylacetic acid methyl ester (8): brown gum; IR (KBr) ν_{max} 3402, 2951, 2835, 1732, 1570, 1489, 1429, 1290, 1234, 1165, 991, 837, 607 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) δ 6.98 (1H, d, J = 2.0 Hz, H-2), 6.85 (1H, d, J = 2.0 Hz, H-6), 3.54 (2H, s, H-7), 3.79 (3H, s, OCH_3), 3.63 (3H, s, COOCH_3); ^{13}C NMR (acetone-d_6, 125 MHz) δ 171.9 (s, C-8), 40.2 (t, C-7), 133.1 (s, C-1), 125.2 (d, C-2), 117.2 (s, C-3), 145.0 (s, C-4), 151.9 (s, C-5), 118.1 (d, C-6), 60.7 (q, OCH_3), 52.1 (q, COOCH_3); EIMS *m*/*z* 274, 276 $[M]^+$ (68, 70), 217 (99), 215 (100), 202 (8), 200 (8), 174 (5), 172 (5), 121 (13); HREIMS *m*/*z* 273.9824 (calcd for $C_{10}H_{11}O_4{}^{79}Br$ 273.9841).

Acidic Hydrolysis of 8. A solution of **8** (4 mg) in 2 N HCl (2 mL) was stirred at 50 °C for 4 h and then evaporated to dryness under reduced pressure. The residue was dissolved with acetone (0.5 mL) and analyzed by TLC and reversed-phase HPLC together with **7**, indicating that the hydrolysis product of **8** is identical to **7**. The developing solvent system was $CHCl_3$−MeOH−AcOH (6:1:0.1) for TLC. The HPLC analysis was carried out with a YMC-Pack ODS-AM column (3 μm, 100 × 4.6 mm) and a DAD detector (254 nm) using methanol−water (7:3) as mobile phase (0.5 mL/min).

3-Bromo-5-hydroxy-4-methoxybenzoic acid (9): ^{13}C NMR (acetone-d_6, 125 MHz) δ 129.0 (s, C-1), 125. 9 (s, C-2), 117.3 (s, C-3), 149.5 (s, C-4), 151.7 (s, C-5), 118.1 (d, C-6), 60.7 (q, OCH_3), 164.1 (s, C-7); FABMS *m*/*z* 245, 247 $[M - H]^-$ (100, 98), 173 (29), 171 (31), 167 (14), 81 (10), 79 (10).

Acknowledgment. The authors are grateful to A. Zeper for mass spectra measurements. Financial support is from the NSF (Grant No.99-929-01-26) and National “863” program (Grant No. 2001AA620403 and No. 2001AA234021).

Supporting Information Available: Details of X-ray diffraction of compounds **1** and **2** and MS and 1D and 2D NMR spectra of compound **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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NP030546+