Sulfur-Containing Polybromoindoles from the Formosan Red Alga *Laurencia brongniartii*

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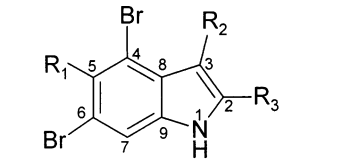
Five new sulfur-containing polybromoindoles, 2-methylsulfinyl-3-methylthio-4,5,6-tribromoindole (**1**), 3-methylsulfinyl-2,4,6-tribromoindole (**2**), 4,6-dibromo-2,3-di(methylsulfinyl)indole (**3**), 3,3′-bis(2′-methylsulfinyl-2-methylthio-4,6,4′,6′-tetrabromo)indole (**4**), and 3,3-bis(4,6-dibromo-2-methylsulfinyl)indole (**5**), as well as seven known sulfur-containing polybromoindoles, 3-methylthio-2,4,6-tribromoindole (**6**), 3-methylthio-2,4,5,6-tetrabromoindole (**7**), 4,6-dibromo-2,3-di(methylthio)indole (**8**), 2,3-di(methylthio)-4,5,6-tribromoindole (**9**), 4,6-dibromo-2-methylsulfinyl-3-(methylthio)indole (**10**), 4,6-dibromo-2-(methylthio)indole (**11**), and 3,3-bis(4,6-dibromo-2-methylthio)indole (**12**), have been isolated from the Formosan red alga *Laurencia brongniartii.* The structures were elucidated by extensive spectral analysis, and their cytotoxicity against selected cancer cells was measured in vitro.

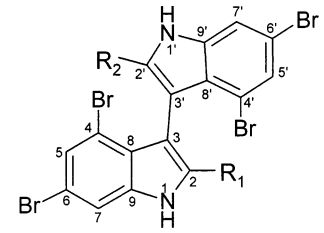
The red alga *Laurencia brongniartii* J. Agardh was reported to be a source of polybrominated and sulfur-containing indoles.[1−4] As part of our search for bioactive substances from marine organisms, Formosan red alga *L. brongniartii* was studied because EtOAc extracts showed significant cytotoxicity to A549 (human lung adenocarcinoma), HT-29 (human colon adenocarcinoma), NUGC-3 (human gastric adenocarcinoma), HONE-1 (human nasopharyngeal carcinoma), and P-388 (mouse lymphocytic leukemia) cell cultures as determined by standard procedures.[5,6] Bioassay-guided fractionation resulted in the isolation of five new sulfur-containing polybromoindoles, 2-methylsulfinyl-3-methylthio-4,5,6-tribromoindole (**1**), 3-methylsulfinyl-2,4,6-tribromoindole (**2**), 4,6-dibromo-2,3-di(methylsulfinyl)indole (**3**), 3,3′-bis(2′-methylsulfinyl-2-methylthio-4,6,4′,6′-tetrabromo)indole (**4**), and 3,3-bis(4,6-dibromo-2-methylsulfinyl)indole (**5**), as well as seven known sulfur-containing polybromoindoles, 3-methylthio-2,4,6-tribromoindole (**6**),[7] 3-methylthio-2,4,5,6-tetrabromoindole (**7**),[7] 4,6-dibromo-2,3-di(methylthio)indole (**8**),[7] 2,3-di(methylthio)-4,5,6-tribromoindole (**9**),[7] 4,6-dibromo-2-methylsulfinyl-3-(methylthio)indole (**10**),[2,4] 4,6-dibromo-2-(methylthio)indole (**11**),[2,4] and 3,3-bis(4,6-dibromo-2-methylthio)indole (**12**).[4] Compounds **4** and **5** exhibited cytotoxicity against selected cancer cell lines.



1 R_1 = Br, R_2 = SMe, R_3 = S(=O)-Me

2 R_1 = H, R_2 = S(=O)Me, R_3 = Br

3 R_1 = H, R_2 = R_3 = S(=O)-Me



4 R_1 = SMe, R_2 = S(=O)-Me

5 R_1 = R_2 = S(=O)-Me

The EIMS spectrum of **1** exhibited molecular ion quartets (*m/z* 465, 463, 461, and 459) characteristic of three bromine atoms, and its molecular formula was assigned as $C_{10}H_8Br_3NOS_2$ by HREIMS. The ^{1}H NMR spectrum of compound **1** was very similar to those of 2,3-di(methylthio)-4,5,6-tribromoindole (**9**), which was previously isolated from the same species collected in Taiwan.[7] The only difference is the replacement of the methylthio by a methylsulfinyl group (δ_H 3.09, δ_C 42.8). The location of the methylsulfinyl group was proved to be at C-2 by NOESY correlation between N*H* (δ 10.95) and the methylsulfinyl group (δ 3.09). The NOESY correlation between H-7 (δ 7.83) and N*H* (δ 10.95) also helped ascertain the substituted pattern of ring A. ^{13}C NMR data of **1** confirmed the presence of a methylsulfinyl at δ 42.8, methylthio at δ 22.9, aromatic methine at δ 116.7, three bromo-bearing quaternary carbons at δ 120.5, 122.0, and 117.8, and four other quaternary carbons. The above spectral findings confirmed the structure of compound **1** to be 2-methylsulfinyl-3-methylthio-4,5,6-tribromoindole.

Compound **2** also bears three bromine atoms as attested by the characteristic isotope peaks (M^+ at *m/z* 419, 417, 415, and 413) in the EIMS, and its molecular formula was determined as $C_9H_6Br_3NOS$ by HREIMS. The ^{1}H NMR spectrum of compound **2** showed the presence of a pair of *meta* aromatic proton signals at δ 7.50 (d, J = 1.4 Hz), 7.45 (d, J = 1.4 Hz), a N*H* at δ 9.25, and a methylsulfinyl group

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at δ 3.15 (3H, s). The ^{1}H NMR spectrum of compound **2** was very similar to those of 3-methylthio-2,4,6-tribromoindole, which was previously isolated from the same species collected in Taiwan.[5] The only difference is the replacement of the methylthio group by methylsulfinyl (δ_H 3.15, δ_C 40.9). The EIMS displayed significant isotopic peaks at *m/z* 398, 400, 402, and 404 for [$M^+ - CH_3$] and isotope clusters at *m/z* 351, 353, 355, and 357, indicating the loss of a methylsulfinyl group. Reduction of **2** with $LiAlH_4$ in THF gave 3-methylthio-2,4,6-tribromoindole (**6**). The structure of **2** was therefore determined as 3-methylsulfinyl-2,4,6-tribromoindole.

The EIMS spectrum of **3** showed molecular ion triplets (*m/z* 401, 399, and 397) characteristic of two bromine atoms, and its molecular formula was assigned as $C_{10}H_9Br_2NO_2S_2$ by HREIMS. NMR data of compound **3**, like that of **2**, showed a pair of aromatic *meta*-coupled protons (δ 7.56 and 7.72; δ_C 115.2 and 128.3), two methylsulfinyl groups (δ_H 3.07, 3.26; δ_C 45.4, 46.0) instead of one, and a N*H* at δ_H 10.85 ppm. EIMS showed significant isotopic peaks at *m/z* 386, 384, and 382 [$M^+ - CH_3$], as well as *m/z* 370, 368, and 366 [$M^+ - CH_3 - O$]. The above spectral data confirmed the structure of **3** to be 4,6-dibromo-2,3-di-(methylsulfinyl)indole.

The molecular formula of compound **4** was suggested to be $C_{18}H_{12}Br_4N_2OS_2$ from HREIMS and the presence of the molecular ion cluster at *m/z* 660, 658, 656, 654, and 652. The ^{1}H NMR spectral data exhibited the presence of two pairs of *meta* aromatic protons (δ 7.40, 7.53 and δ 7.42, 7.67). Each pair was confirmed coupled with each other by the COSY experiment. In addition ^{1}H NMR showed two N*H* at δ 8.62 and 11.20 and two methyl singlets at δ 2.32 and 2.90 assignable to methylthio and methylsulfinyl groups, respectively. ^{13}C NMR and DEPT experiment revealed the presence of a methylthio, a methylsulfinyl, four aromatic methines, and 12 aromatic quaternary carbons. These data indicated that compound **4** was an unsymmetrical bisindole. EIMS also displayed significant quintets indicating loss of an oxygen, a methylthio group, a methylsulfinyl group, and each indole monomer from M^+. NOESY experiment showed cross-peaks between N*H* at δ 8.62 and a methyl at δ 2.32 as well as between N*H* at δ 11.20 and a methyl at δ 2.90. These findings suggested substitutions of methylsulfinyl and methylthio groups should be at C-2 and C-2′, respectively. Therefore, the two halves of the molecule must be linked at C-3 and C-3′ but not at C-2 and C-2′. Compound **5** was therefore determined as 3,3′-bis(2′-methylsulfinyl-2-methylthio-4,6,4′,6′-tetrabromo)indole.

Compound **5** also bears four bromine atoms, as shown by the characteristic isotope peaks (M^+ at *m/z* 676, 674, 672, 670, and 668) in the EIMS. This information and the molecular formula $C_{18}H_{12}Br_4N_2O_2S_2$ deduced from HREIMS suggest that it is a bisindole. The ^{1}H NMR spectrum exhibited only two *meta*-coupled aromatic protons [δ 7.47 (d, J = 1.3 Hz), 7.72 (d, J = 1.3 Hz)], a methylsulfinyl singlet (δ 2.92), and a highly deshielded proton (δ 11.20), assigned to N*H*. ^{13}C NMR and DEPT experiment showed a methylsulfinyl signal, two aromatic methine signals, and six aromatic quaternary carbon signals. These data indicated the symmetrical nature of the bisindole. EIMS also displayed significant quintets indicating loss of an oxygen atom, a methylsulfinyl group, two methylsulfinyl groups, and an indole monomer from M^+. NOESY correlations between N*H* (δ 11.20) and methylsulfinyl groups (δ 2.92) indicated that methylsulfinyl groups should be placed at C-2/2′ and two monomers are connected at C-3/3′. Compound **5** was therefore determined as 3,3-bis(4,6-dibromo-2-methylsulfinyl)indole.

Bisindole **4** exhibited cytotoxicity against HT-29 and P-388 cell lines. Bisindole **5** exhibited cytotoxicity against the P-388 cell line. The other isolates were not cytotoxic against HT-29 and P-388 cell lines as evaluated by standard protocols.[5]

Experimental Section

General Experimental Procedures. Melting points were determined using a Yanagimoto micromelting point apparatus and are reported uncorrected. Optical rotations were determined on a JASCO DIP-181 polarimeter. UV spectra were obtained on a Shimadzu UV-160A spectrophotometer, and IR spectra were recorded on a Hitachi 26-30 spectrophotometer. The NMR spectra were recorded on a Varian Inova 500 or a Bruker Avance 300 spectrometer. The chemical shifts are given in δ (ppm) and coupling constants in Hz. EIMS spectra were obtained with a JEOL JMS-SX/SX 102A mass spectrometer at 70 eV. Silica gel 60 (Merck, 230−400 mesh) was used for column chromatography; precoated silica gel plates (Merck, Kieselgel 60 F_{254}, 0.25 mm) were used for TLC analysis.

Algal Material. The red alga *L. brongniartii* was collected at Ken-Ting National Park, south tip of Taiwan, in November 2000, and the wet red alga samples were kept in a refrigerator until extraction. A voucher specimen, KT-023, was deposited in the Department of Biology, National Changhua University of Education, Changhua, Taiwan.

Extraction and Isolation. Wet red alga *L. brongniartii* (5.0 kg) was extracted by ethyl alcohol (3 × 2 L). After removal of solvent in vacuo, the residue (30 g) was chromatographed over silica gel 60 using *n*-hexane/EtOAc and MeOH/EtOAc mixtures as eluting solvents. Elution by *n*-hexane/EtOAc (12:1) afforded fractions containing **6** and **7**. Elution by *n*-hexane/EtOAc (10:1) afforded fractions containing **8**−**12**. Elution by *n*-hexane/EtOAc (7:1) afforded fractions containing **4**. Elution by *n*-hexane/EtOAc (5:1) afforded fractions containing **1**. Elution by *n*-hexane/EtOAc (1:1) afforded fractions containing **2**. Elution by *n*-hexane/EtOAc (1:2) afforded fractions containing **3** and **5**. Compound **1** (5 mg) was further purified by Si gel column chromatography, eluting with *n*-hexane/acetone (3:1). Compound **2** (10 mg, t_R 61.2 min) was further purified by HPLC (LiChrosorb RP-18, 7 μm, 25 × 250 mm, 4 mL/min), eluting with MeOH/H_2O (7:3). Compound **3** (5 mg, t_R 49.5 min) was further purified by HPLC (LiChrosorb RP-18, 7 μm, 25 × 250 mm, 4 mL/min), eluting with MeOH/H_2O (7:3). Compound **4** (5 mg, t_R 63.0 min) was further purified by HPLC (LiChrosorb RP-18, 7 μm, 25 × 250 mm, 4 mL/min), by eluting with MeOH/H_2O (75:25). Compound **5** (4 mg, t_R 71.7 min) was further purified by HPLC (LiChrosorb RP-18, 7 μm, 25 × 250 mm, 4 mL/min), by eluting with MeOH/H_2O (65:35). Compounds **6** (104 mg) and **7** (64 mg) were further purified by silica gel column chromatography, eluting with *n*-hexane/acetone (15:1). Compounds **11** (50 mg), **8** (105 mg), **9** (42 mg), **12** (24 mg), and **10** (12 mg) were further purified by silica gel column chromatography, eluting with *n*-hexane/EtOAc (9:1), *n*-hexane/EtOAc (8:1), *n*-hexane/EtOAc (6:1), *n*-hexane/EtOAc (5:1), and *n*-hexane/EtOAc (3:1), respectively.

2-Methylsulfinyl-3-methylthio-4,5,6-tribromoindole (1): mp 142−144 °C; UV (MeOH) λ_{max} (log ϵ) 234 (4.6), 315 (4.2) nm; IR ($CHCl_3$) 3224, 1610, 1552, 1509, 1440, 1411, 1103, and 1033 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.42 (3H, s), 3.09 (3H, s), 7.83 (1H, s), 10.95 (1H, br s, N*H*); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 22.9 (q), 42.8 (q), 108.0 (s), 116.7 (d), 117.8 (s), 120.5 (s), 122.0 (s), 128.5 (s), 136.8 (s), 144.1 (s); EIMS *m/z* 465 (4), 463 (8), 461 (7), 459 (3), 449 (4), 447 (10), 445 (11), 443 (4), 402 (5), 400 (18), 398 (15), 396 (4); HREIMS *m/z* 462.7552 (calcd for $C_{10}H_8{}^{79}Br^{81}Br_2NOS_2$).

3-Methylsulfinyl-2,4,6-tribromoindole (2): mp 102−104 °C; UV (MeOH) λ_{max} (log ϵ) 236 (4.7) nm, 312 (4.1); IR ($CHCl_3$) 2917, 2888, 2383, 1560, 1449, 1398, 1116, and 1039 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.15 (3H, s), 7.45 (1H,

d, J = 1.4 Hz), 7.50 (1H, d, J = 1.4 Hz), 9.25 (1H, br s, N*H*); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 40.9 (q), 111.9 (s), 113.9 (d), 115.9 (s), 116.4 (s), 124.6 (s), 128.8 (d), 137.1 (s), 138.2 (s); EIMS *m/z* 419 (5), 417 (14), 415 (13), 413 (4), 404 (25), 402 (70), 400 (68), 398 (27), 357 (21), 355 (65), 353 (63), 351(22); HREIMS *m/z* 416.7662 (calcd for $C_9H_6{}^{79}Br^{81}Br_2NOS$).

4,6-Dibromo-2,3-di(methylsulfinyl)indole (3): mp 132−134 °C; UV (MeOH) λ_{max} (log ϵ) 230 (4.4), 310 (4.0) nm; IR ($CHCl_3$) 2980, 2726, 2364, 1544, 1172, 1027, and 1032 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.07 (3H, s), 3.26 (3H, s), 7.56 (1H, d, J = 1.4 Hz), 7.72 (1H, d, J = 1.4 Hz), 10.85 (1H, br s, N*H*); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 45.4 (q), 46.0 (q), 112.6 (s), 115.2 (d), 117.8 (s), 118.3 (s), 124.4 (s), 128.4 (d), 136.5 (s), 138.0 (s); EIMS *m/z* 401 (20), 399 (34), 397(17), 386 (12), 384 (20), 382 (11), 370(5), 368 (12), 366 (5), 338 (44), 336 (80), 334 (40), 322 (11), 320 (18), 318 (9); HREIMS *m/z* 398.8421 (calcd for $C_{10}H_9{}^{79}Br^{81}BrNO_2S_2$).

3,3′-Bis(2′-methylsulfinyl-2-methylthio-4,6,4′,6′-tetrabromo)indole (4): mp 187−189 °C; $[\alpha]^{25}_D$ +36° (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 238 (4.7), 312 (4.1) nm; IR ($CHCl_3$): 3110, 2345, 2335, 1544, 1500, 1350, 1150, and 1100 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.32 (3H, s), 2.90 (3H, s), 7.40 (2H, d, J = 1.4 Hz), 7.42 (2H, d, J = 1.4 Hz), 7.53 (2H, d, J = 1.4 Hz), 7.67 (2H, d, J = 1.4 Hz), 8.62 (1H, br s, N*H*), 11.20 (1H, br s, N*H*); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 18.5 (q), 41.2 (q), 110.5 (s), 110.8 (s), 113.2 (d), 114.6 (d), 114.7 (s), 115.9 (s), 116.0 (s), 117.5 (s), 124.3 (s), 127.4 (d), 127.8 (d), 132.8 (s), 133.0 (s), 136.9 (s), 137.5 (s), 137.9 (s); EIMS *m/z* 660 (15), 658 (50), 656 (70), 654 (48), 652 (12), 644 (5), 642 (20), 640 (25), 638 (16), 636 (4), 582 (22), 580 65), 578 (100), 576 (65), 574 (17), 550 (3), 548 (10), 546 (16), 544 (11), 542 (3); HREIMS *m/z* 655.7028 (calcd for $C_{18}H_{12}{}^{79}Br_2{}^{81}Br_2N_2OS_2$).

3,3-Bis(4,6-dibromo-2-methylsulfinyl)indole (5): mp 191−193 °C; $[\alpha]^{25}_D$ 0° (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 233 (4.5), 309 (308) nm; IR ($CHCl_3$) 3150, 2383, 2354, 1544, 1500, 1378, 1095, and 1032 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.92 (6H, s), 7.47 (2H, d, J = 1.3 Hz), 7.72 (2H, d, J = 1.3 Hz), 11.20 (2H, br s, N*H*); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 41.3 (q), 112.5 (s), 113.9 (d), 115.3 (s), 117.8 (s), 127.2 (d), 132.5 (s), 136.2 (s), 139.9 (s); EIMS *m/z* 676 (3), 674 (8), 672 (13), 670 (7), 668 (s), 660 (5), 658 (11), 656 (83), 654 (12), 652 (4), 613 (4), 611 (16), 609 (21), 607 (15), 605 (4), 550 (2), 548 (4), 546 (6), 544 (5), 542 (2); HREIMS *m/z* 671.6988 (calcd for $C_{18}H_{12}{}^{79}Br_2{}^{81}Br_2N_2O_2S_2$).

Reduction of 3-Methylsulfinyl-2,4,6-tribromoindole (2) with $LiAlH_4$. To a suspension of $LiAlH_4$ (30 mg) in THF (3 mL) was added a solution of **2** (4 mg) in the same solvent (2 mL), and the mixture was stirred for 1 h. After quenching by adding a dilute hydrochloric acid solution, the mixture was extracted with EtOAc. The organic layer was dried and purified by HPLC (LiChrosorb RP-18, 7 μm, 25 × 250 mm), eluting with MeOH/H_2O (78:22) to give 3-methylthio-2,4,6-tribromoindole (**6**) (0.5 mg).

Cytotoxicity Testing. P-388 cells were supplied by J. M. Pezzuto, Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago; A549 and HT-29 were purchased from the American Type Culture Collection. Cytotoxic assays were carried out according to the procedure described previously.[5,6]

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