Antioxidative Bromoindole Derivatives from the Mid-Intestinal Gland of the Muricid Gastropod *Drupella fragum*

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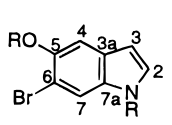
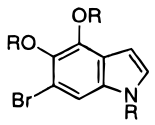
Three new bromoindoles, 6-bromo-5-hydroxyindole (**1**), 6-bromo-4,5-dihydroxyindole (**2**), and 6-bromo-4,7-dihydroxyindole (**3**), were isolated from the midintestinal gland of the muricid gastropod *Drupella fragum*. The structures of **2** and **3** were elucidated mainly by NMR spectroscopic analyses of their acetyl derivatives, whereas the structure of **1** was established by spectroscopic methods and total synthesis. Antioxidative activity for compounds **1**−**3** was evaluated by the POV method, and compound **1** was found to have as strong an antioxidative potency as BHT.

The muricid gastropod *Drupella fragum* (Muricacea) is well known to be a predator on Madreporaria corals, and a population explosion of the gastropod may cause widespread destruction of corals, resulting in an ecological imbalance.[1,2] No effective way for exterminating muricid predation on corals has been proposed to data. Chemical studies on the midintestinal gland of *D. fragum* may allow the isolation of metabolites from previously uninvestigated Madreporaria corals and may also lead to the discovery of new biologically active substances.[3−5]

D. fragum was collected from the shallow waters along the Ohtsuki coast, Kochi, Japan. The midintestinal gland of *D. fragum* was extracted with methanol and the methanol extract was partitioned sequentially between water and hexane, dichloromethane, and *n*-butanol. Compound **1** was isolated from the dichloromethane soluble portion, whereas compounds **2** and **3** were obtained from the *n*-butanol layer.



1 R = H
1a R = Me



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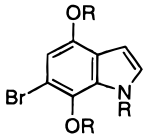
2a R = Ac



3 R = H
3a R = Ac

Compound **1** was obtained as a colorless amorphous solid. Its EIMS exhibited molecular ion peaks at *m*/*z* 213 and 211 in a 1:1 ratio, suggesting the presence of one bromine atom in the molecule. The molecular formula, C_8H_6BrON, consistent with six unsaturation units, was secured by HREIMS measurements at *m*/*z* 213 and 211. Absorption bands in the IR (3570 and 3520 cm^{-1}) and UV (218, 282, 296, and 312 nm) spectra suggested that **1** was a hydroxyindole derivative. The ^{13}C NMR data of **1** (Table 1) indicated that all the carbons were sp^2 and comprised four methines and four quaternary carbons. The ^1H NMR spectrum contained two aromatic singlet signals at δ_H 7.24 and 7.48 and a set of doublet signals at δ_H 6.42 and 7.15 (J = 2.9 Hz) assignable to H-3 and H-2 on the indole nucleus. Treatment of **1** with NaH−MeI in DMF yielded the dimethyl derivative **1a** [δ_H 3.75 (3H, s, NMe), 3.92 (3H, s, OMe)], confirming the presence of a hydroxy group

Table 1. NMR Spectra Data of Compounds **1**, **2a**, and **3a** in $CDCl_3$ (400 MHz for ^1H NMR, 100 MHz for ^{13}C NMR)

	1		**2a**		**3a**	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2	7.15 (t, 2.9)	126.0	7.36 (d, 3.7)	127.5	7.42 (d, 3.7)	126.7
3	6.42 (d, 2.9)	102.4	6.55 (d, 3.7)	105.3	6.49 (d, 3.7)	105.7
3a		128.8		126.4		124.5
4	7.48 (s)	105.7		140.6		136.4
5		145.9		134.2	8.69 (s)	118.5
6		106.3		113.9		113.8
7	7.24 (s)	113.8	7.38 (s)	121.0		135.4
7a		131.5		129.1		134.2
OH	5.19 (s)					
OAc Me			2.37 (s)	20.9	2.380 (s)	20.4
			2.42 (s)	24.9	2.382 (s)	20.5
			2.60 (s)		2.63 (s)	23.8
OAc C=O				167.0		167.7
				168.6		167.9
				168.7		168.4

Table 2. Antioxidative Activities of Compounds **1**−**3** Evaluated by the POV Method

sample	POVs[a]/POVc[b](mequiv/kg)
1	0.036
2	0.194
3	0.106
α-tocopherol	0.062
BHT[c]	0.031

[a] Peroxide value with the sample. [b] Peroxide value without the sample (control). [c] Butylated hydroxytolune.

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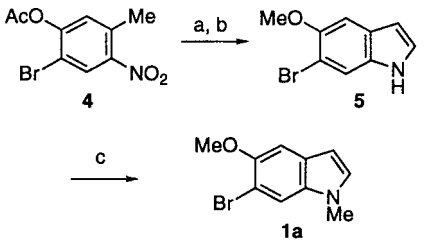
Scheme 1[a]



[a] Key: (a) pyrrolidine, $Me_2NH(OMe)_2$, DMF, 100 °C; (b) $TiCl_3$, NH_4OAc, DMF, rt; (c) NaH, DMF, MeI 0 °C → rt, 47%.

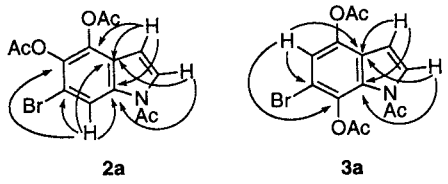


Figure 1. Significant $^1H-^{13}C$ couplings observed for **2a** and **3a** in HMBC (8.1 Hz) experiments.

and an indole moiety. Although the locations of the bromine atom and the hydroxy group were deduced from comparison with the spectral data of the previously known eudistomin C[6] to be on the C-6 and C-5 positions, respectively, the need to confirm the proposed structure prompted us to synthesize the dimethyl derivative **1a** (Scheme 1).

5-Acetoxy-4-bromo-2-nitrotoluene (**4**) was condensed with dimethylformamide dimethyl acetal in the presence of pyrrolidine followed by reduction of the nitro group into the amine with titanous chloride in a 4 M ammonium acetate buffer to give 5-bromo-6-methoxyindole (**5**).[7] Treatment of **5** with MeI−NaH in DMF furnished the *N*-methylindole in 47% yield, which was identical in all respects with **1a** derived from **1**. Thus, the structure of **1** was established as 5-bromo-6-hydroxyindole.

Compounds **2** and **3** gradually decomposed during NMR measurements, and the IR spectra indicated the presence of a hydroxy group. The stable triacetyl derivatives **2a** and **3a** were thus prepared from **2** and **3** by acetylation. The same molecular formula, $C_{14}H_{12}BrO_5N$, for **2a** and **3a** was established by HREIMS measurements at *m/z* 353, indicating again the presence of one bromine atom. Their UV absorption, analogous to that of **1**, suggested the presence of an indole nucleus (see the Experimental Section). The ^1H NMR spectrum of **2a** (Table 1) showed a singlet aromatic signal at δ_H 7.38 and a set of signals coupled to each other at δ_H 6.55 (J = 3.2 Hz, H-3) and 7.36 (J = 3.2 Hz, H-2) in addition to three acetoxyl signals at δ_H 2.37, 2.42, and 2.60. The NMR data of **3a** (Table 1) were similar to those of **2a**. These spectral data indicated that **2a** and **3a** are regioisomers in regard to the two hydroxy groups on the benzene ring of the indole nucleus. The positions of the acetoxy groups for **2a** and **3a** were clarified by HMBC experiments, and the results are summarized in Figure 1. The singlet carbon resonances in the spectrum of **2a** at δ_C 126.4 and 129.1, which were assigned to C-3a and C-7a by HMBC correlation from H-2 and H-3, respectively, showed further long-range couplings to the singlet proton signal at δ_H 7.38. The sole aromatic proton was thus placed on the C-7 position, and **2a** is *N*-acetoxy-6-bromo-4,5-diacetoxyindole. On the other hand, the sole singlet proton signal at δ_H 8.69 in **3a** was assigned to H-5 by the observation of its HMBC correlation to the C-3a resonance at δ_C 124.50, but not to the C-7a resonance at δ_C 134.18. Thus, **3a** was formulated as *N*-acetoxy-6-bromo-4,7-diacetoxyindole. Compounds **2** and **3** were thus assigned as 6-bromo-4,5-dihydroxyindole and 6-bromo-4,7-dihydroxyindole, respectively.

The bromoindoles **1**−**3** isolated from *D. fragum* were tested for antioxidant activity by the peroxide value (POV) method.[8] Among compounds **1**−**3**, **1** exhibited the most potent antioxidative activity, higher than that of α-tocopherol and almost equal to that of BHT. It should be noted that the promising antioxidant **1** is stable under the isolation procedures and the conditions of spectral measurements, while the same is not true for compounds **2** and **3**.

Experimental Section

General Experimental Procedures. Melting points were determined with a Mitamura hot-stage microscope and are uncorrected. IR and UV spectra were obtained using a JASCO FTIR-5300 and a JASCO UVIDEC 670 spectrophotometer, respectively. NMR spectra were recorded on a JEOL GX-400 and a Varian Unity-200, with TMS as the internal standard. The HMBC, HMQC, and ROESY were run on a JEOL at 400 MHz. MS spectra were measured with a JEOL JMS-D300 and a JEOL JMS HX-100. HRMS were recorded on a JMS HX-100. HPLC was carried out on a Waters Model 6000A with detection at 254 nm. Si gel 60 (Merck) and Wako gel C-300 (Wako) were used for column chromatography. Analytical TLC were carried out on silica gel plates (Kieselgel 60, Merck) and visualized by 5% $CeSO_4$ in H_2SO_4 followed by heating.

Collection, Extraction, and Isolation. The gastropod *D. fragum* was collected by scuba in November 1992, from Ohtsuki coast, Kochi, Japan. The gastropod was freeze-dried upon arrival and kept frozen until extraction. A voucher specimen has been stored at the Chemistry Department of Kochi University. After the samples (26.7 kg) were defrosted, the midintestinal gland (1.5 kg) of *D. fragum* was blended with MeOH, and after filtration the crude extract (20 L) was evaporated under vacuum to yield a residue (250 g) that was partitioned between hexane and H_2O. The aqueous portion was extracted with CH_2Cl_2, and then the aqueous layer was extracted with *n*-BuOH. The CH_2Cl_2 extract was concentrated to give a residue (6.2 g), which was loaded onto a size-exclusion column (Sephadex LH-20) and eluted with MeOH to yield 10 fractions. Fraction 9 (313 mg) was purified successively by column chromatography on Si gel (60 g) with Me_2CO−hexane (3:7) and HPLC [TSK-GEL LS-410K] with MeOH−H_2O (3:7) to give 6-bromo-5-hydroxyindole (**1**) (47 mg). The *n*-BuOH extract (12 g) was subjected to Sephadex LH-20 column chromatography eluted with MeOH, yielding seven fractions. Fraction 3 (100 mg) was chromatographed over a TOYOPEARL HW-40 with MeOH to yield 6-bromo-4,5-dihydroxyindole (**2**), and fraction 5 (112 mg) was purified by column chromatography on Sephadex LH-20 with MeOH to give 6-bromo-4,7-dihydroxyindole (**3**).

6-Bromo-5-hydroxyindole (1): colorless amorphous solid; IR ($CHCl_3$) 5570, 3520, 1580, 1460, 1355, 1220,

1150, 990 cm^{-1}; UV λ_{max} (EtOH) 312 (ϵ 4480), 296 (ϵ 6300), 282 (ϵ 7800), 218 (ϵ 24 300) nm; ^1H and ^{13}C NMR, see Table 1; EIMS *m/z* 213 (99), 211 (100); HREIMS *m/z* 212.9623 [M^+] (calcd for $C_8H_6{}^{81}BrON$, 212.9612), 210.9633 [M^+] (calcd for $C_8H_6{}^{79}BrON$, 210.9632).

6-Bromo-4,5-dihydroxyindole (2): colorless amorphous solid; IR (neat) 3430, 1630, 1590, 1500, 1260 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 6.55 (1H, br s), 7.07 (1H, br s), 7.11 (1H, br s).

6-Bromo-4,7-dihydroxyindole (3): colorless amorphous solid; IR (neat) 3420, 1630, 1580, 1480, 1350, 1270 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 6.50 (1H, d, J = 3.1 Hz), 7.07 (1H, d, J = 3.1 Hz), 7.15 (1H, br s).

Methylation of 1 (1a). To a suspension of NaH (50% dispersion in oil, 8 mg) in DMF (1 mL) was added a solution of compound **1** (10 mg) in DMF (0.2 mL) at 0 °C under an argon atmosphere, and the reaction mixture was allowed to stir at room temperature for 1 h. MeI (0.6 mL) was added to this solution. After being stirred for 1 h, the reaction mixture was diluted with Et_2O, washed with H_2O and brine, and then dried. The solvent was removed in vacuo to give the crude oil, which was chromatographed on Si gel with EtOAc to yield dimethyl derivative **1a** (5 mg) as colorless prisms: mp 113−114 °C; IR (KBr) 3441, 1630, 1483, 1244, 1154, 1042 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.75 (3H, s, OMe), 3.92 (3H, s, OMe), 6.38 (1H, dd, J = 3.2. 0.7 Hz, H-3), 7.02 (1H, d, J = 3.2 Hz, H-2), 7.17 (1H, s, H-7), 7.52 (1H, d, J = 0.7 Hz, H-4); EIMS *m/z* 241 (100), 239 (99); HREIMS *m/z* 238.9941[M^+] (calcd for $C_{10}H_{10}{}^{79}Br$ ON, 238.9946).

Acetate derivative of 2 (2a). Compound **2** (9.7 mg) was acetylated with Ac_2O (0.1 mL) + pyridine (0.1 mL), affording triacetate **2a** (5.7 mg) as colorless prisms: mp 156−156.5 °C; IR (Nujol) 1760, 1470, 1370, 1200 cm^{-1}; UV λ_{max} (EtOH) 308 (ϵ 9000), 298 (ϵ 8000), 270 (ϵ 18 000), 263 (ϵ 19 000), 236 (ϵ 38 000), 208 (ϵ 36 000); ^1H and ^{13}C NMR, see Table 1; HREIMS *m/z* 352.9882 [M^+] (calcd for $C_{14}H_{12}BrO_5N$, 352.9899).

Acetate Derivative of 3 (3a). Compound **3** (10 mg) was acetylated with Ac_2O (0.1 mL) + pyridine (0.1 mL), affording triacetate **3a** (2 mg). **3a**: colorless needles; mp 142.5−143.5 °C; IR (Nujol) 1770, 1720, 1400, 1380, 1200 cm^{-1}; UV λ_{max} (EtOH) 304 (ϵ 9300), 293 (ϵ 8900), 274 (ϵ 17 200), 268 (ϵ 16 800), 240 (ϵ 30 800), 204 (ϵ 32 000); ^1H and ^{13}C NMR, see Table 1; HREIMS *m/z* 352.9872 [M^+] (calcd for $C_{14}H_{12}BrO_5N$, 352.9899).

6-Bromo-5-methoxyindole (5). A solution of 5-acetoxy-4-bromo-2-nitrotoluene (200 mg, 0.73 mmol), dimethylformamide dimethyl acetal (0.3 mL), and pyrrolidine (0.1 mL) was stirred in DMF (2 mL) under an argon atmosphere and heated at 100−110 °C for 2.5 h. The red solution was cooled to room temperature, and a solution of 4 M ammonium acetate (2.5 mL) in DMF (2.5 mL) was added dropwise. The red solution was stirred for 25 min while 20% (w/v) $TiCl_3$ (2.8 mL) was added dropwise. The resulting gray suspension was extracted with Et_2O, washed with H_2O and brine, and then dried. The solvent was removed in vacuo to give the crude residue, which was chromatographed on Si gel with hexanes−EtOAc (6:1) to yield compound **5** (42 mg, 25%) as an oil: IR (neat) 3397, 2963, 1740, 1308, 1209, 1155, 1040 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 3.92 (3H, s, OMe), 6.48 (1H, d, J = 2.4 Hz, H-3), 7.13 (1H, s, H-4), 7.17 (1H, t, J = 2.4 Hz, H-2), 7.59 (1H, s, H-7), 8.03 (1H, br d, J = 2.4 Hz, NH); EIMS *m/z* 227 (100), 225 (99); HREIMS *m/z* 224.9803 [M^+] (calcd for C_9H_8BrON, 224.9790).

***N*-Methyl-6-bromo-5-methoxyindole (1a).** To a suspension of NaH (50% dispersion in oil, 4 mg) in DMF (0.5 mL) was added a solution of compound **1** (4.0 mg) at 0 °C under an argon atmosphere, and the reaction mixture was allowed to stir at room temperature for 1 h. MeI (0.05 mL) was added to this solution. After being stirred for 1 h, the reaction mixture was diluted with Et_2O, washed with H_2O and brine, and then dried. The solvent was removed in vacuo to give the crude oil, which was chromatographed on Si gel with EtOAc to yield 6-bromo-5-methoxyindole (2.0 mg), the ^1H NMR, IR and MS spectra of which were identical with those of dimethyl derivative **1a** converted from **1**.

Bioassay. POV (peroxide value) was measured according to the standard method[8] recommended by the Japanese Society of Fat and Oil.

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