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Antimicrobial Indolequinones from the Mid-Intestinal Gland of the Muricid Gastropod *Drupella fragum*

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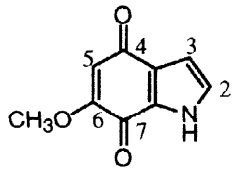
Abstract: Three new indolequinones, 6-methoxyindole-4,7-quinone (**1**), 5-methoxyindole-4,7-quinone (**2**) and 5-methylindole-4,7-quinone (**3**) were isolated from the mid-intestinal gland of the muricid gastropod *Drupella fragum*. The structures of **1** and **2** were established by spectroscopic methods and total synthesis, whereas the structure of **3** was elucidated mainly by NMR spectroscopic analyses. Compounds **1** ~ **3** exhibited moderate antimicrobial activities against *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli* with MIC = 6.25 ~ 50 μg/mL.

Population explosion of the muricid gastropod *Drupella fragum* , which is a predator on the Madreporaria corals, often causes the widespread destruction of corals in the sea near Miyake and Hachijyojima, Japan, resulting in an ecological unblance.[1, 2] No effective way for exterminating muricid predation on corals has been proposed to data. It is intriguing to investigate chemical components in the mid-intestinal gland of *D. fragum*, since there may be a chance not only to isolate metabolites from the Scleractinia corals which have not been allowed to directly investigate, but also to find out new type of biologically active substances.[3-5] In this paper, we report the structure elucidation of three new indole-4,7-quinones **1** ~ **3** with antimicrobial



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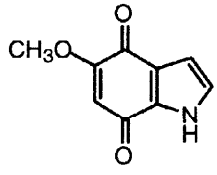

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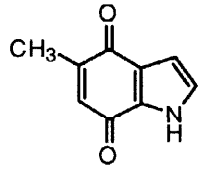

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activities and total syntheses of **1** and **2** attempted for their structure confirmation.

D. fragum was collected from the shallow waters along Ohtsuki coast, Kochi, Japan. The mid-intestinal gland of *D. fragum* was extracted with methanol and the methanol extract was partitioned sequentially between water and hexane, dichloromethane, and *n*-butanol. Compounds **1** ~ **3** were isolated from the dichloromethane soluble portion.

Compounds **1** and **2** were found to have the same molecular formula $C_9H_7NO_3$ established by the measurement of HREIMS at *m/z* 177 [M^+]. Their IR and UV spectra exhibited similar absorptions (see experimental part) to each other. The ^1H NMR spectra of **1** and **2** contained a set of doublet signals at δ_H 6.49 (d, J = 2.5 Hz) and 7.23 (d J = 2.5 Hz), and δ_H 6.50 (d, J = 2.5 Hz) and 7.12 (d, J = 2.5 Hz) assignable to H-3 and H-2 on the indole nucleus, respectively, a singlet olefinic signal at δ_H 5.80 (for **1**) and 5.79 (for **2**), and a methoxy signal at δ_H 3.76 (for **1**) and 3.77 (for **2**). On the other hand, their ^{13}C NMR spectra indicated the presence of two carbonyl groups (**1**: δ_C 170.17 and 182.83; **2**: δ_C 176.58 and 177.70). These spectral data suggest that compounds **1** and **2** belong to indolequinone derivatives. Treatment of **1** and **2** with 1,2-phenylenediamine in AcOH,[7,8] however, yielded no condensation product but totally the unchanged starting quinones. These results excluded an *o*-quinone-type from two *o*- and *p*- indolequinoid structures, and thereby **1** and **2** turn out to be indole-*p*-quinone with a methoxy group on different position, respectively. In order to clarify this ambiguity HMBC spectra were measured. On the basis of analyses of the HMBC as shown in Fig. 1, the structures for **1** and **2** were elucidated as 6-methoxyindole-4,7-quinone and 5-methoxyindole-4,7-quinone, respectively. Although 6-methoxyindole-4,7-quinone (**1**) was already synthesized[9-11] as a key-intermediate leading to several naturally occurring indole derivatives,[12] it should be noted that **1** was isolated as a natural product for the first time.

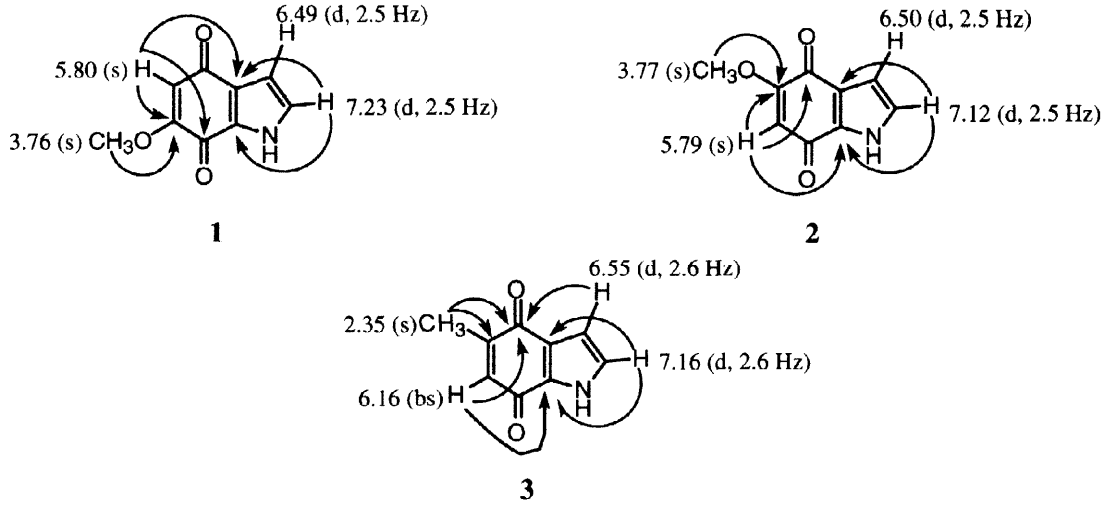
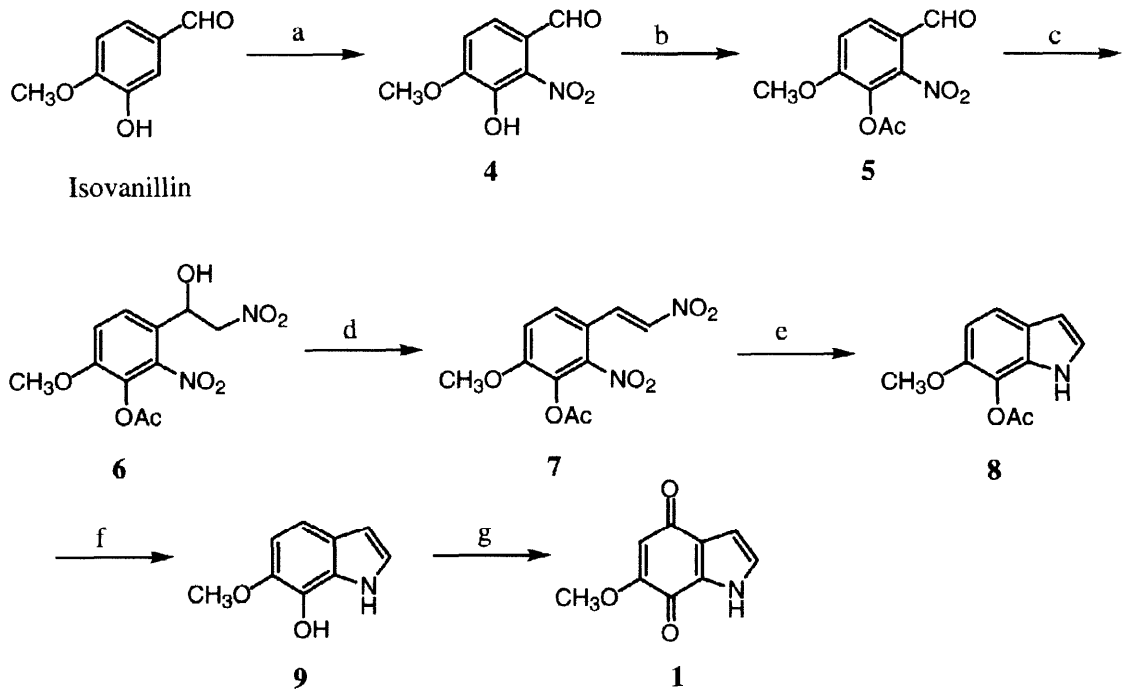


Figure 1. ^1H NMR data (400 MHz, DMSO-d_6); arrows denote significant ^1H-^{13}C long-range couplings for **1**, **2** and **3** observed in HMBC (8.1 Hz) experiments.

Compound **3** was suggested to be a indolequinone structurally resemble to **1** and **2** from its spectra data similar to those of **1** and **2** (see experimental part). The NMR spectra of **3** indicated the presence of a methyl group [δ_H 2.35 (3H, s); δ_C 13.30] instead of the methoxy resonance existing in **1** and **2**. In HMBC (Fig. 1) the methyl signal showed a cross peak to one of the carbonyl resonance at δ_C 178.96, which further correlated to H-3 at δ_H 6.55 as well as to H-2 at δ_H 7.16. Therefore, the structure of **3** was determined to be 5-methylindole-4,7-quinone.

Next, we have concentrated on synthetic works of **1** and **2** aiming not only at their structural confirmation but also at securing quantity enough to evaluate their biological activities. We have chosen Delgado's conventional procedure[9] to complete syntheses of **1** and **2** in a short-term.

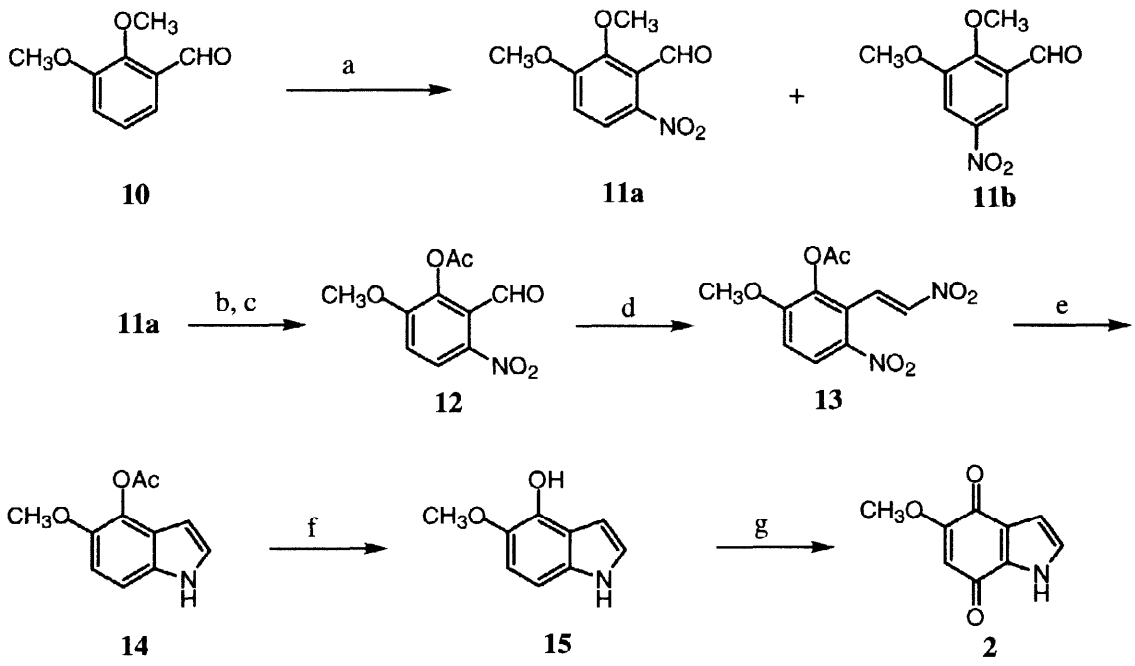
Nitration[13] of isovanillin afforded 2-nitro derivative **5** after acetylation. Condensation of **5** with nitromethane by using $KN(TMS)_2$ as base gave **6**, which was dehydrated with MsCl giving rise to dinitrostyrene **7** in high yield. Cyclization of **7** into an indole **8** was realized by silica gel-assisted reduction of the nitro group with Fe in AcOH. 7-Hydroxyindole **9** obtained by hydrolysis of the acetate of **8** with NaOH-$Na_2S_2O_4$ was subjected to an air-oxidation in the presence of salcomine[14] giving rise to the requisite indolequinone **1** in 60% yield, the ^1H NMR spectrum of which was superimposable to that of the natural one.



Scheme 1. Synthesis of **1**. Reaction conditions: a) NO_2BF_4, CH_3NO_2, CH_2Cl_2, –40°C, 46%; b) NaOAc, Ac_2O, 60°C, 87%; c) $KN(TMS)_2$, CH_3NO_2, THF, –78°C → rt, 92%; d) MsCl, CH_2Cl_2, 0°C → rt; e) Fe, SiO_2, AcOH, toluene, 90°C, 29% from **5**; f) NaOH, $Na_2S_2O_4$, DMF, 5°C, 98%; g) O_2, salcomine, rt, 60%.

5-Methoxyindole-4,7-quinone (**2**) was synthesized by essentially similar procedure used for the preparation of **1** as shown in Scheme 2. Nitration of 2,3-dimethoxybenzaldehyde (**10**) affored a mixture of 5- and 6-nitro derivatives, which were readily separated by chromatography on silica gel. Selective monodemethylation of 6-nitro isomer (**11a**) with BBr_3 gave 2-hydroxy derivative, which after acetylation afforded **12** in 74% yield. The benzaldehyde **12** was condensed with nitromethane followed by acetylation to afford nitrostyrene **13** in 70% yield. Silica gel-assisted reductive cyclization of **13** in the presence of Fe and AcOH afforded an idole **14** in low yield. Hydrolysis of the acetate of **14** followed by an air-oxidation yielded 5-methoxyindole-4,7-quinone, the ^1H NMR of which was in complete accordance with that of the natural one **2**. Thus, the structures of **1** and **2** were unambiguously established.

The three indolequinones **1** – **3** were tested against several microorganisms. Most active compound was **1**, whose MIC (minimum inhibitory concentration) was 7.5 μg/mL, 12.5 μg/mL, 50 μg/mL, and 6.25 μg/mL against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudanonas aeruginosa*, respectively. On the other hand, compounds **2** and **3** exhibited weakly antimicrobial activity against *B. subtilis* (**1**: MIC = 12.5 μg/mL; **2**: MIC = 50 μg/mL) and *S. aureus* (**1**: MIC = 50 μg/mL; **2**: MIC = 50 μg/mL).



Scheme 2. Synthesis of **2**. Reaction conditions: a) HNO_3, AcOH, rt, 79%; b) BBr_3, CH_2Cl_2, -78° C → rt, 95%; c) NaOAc, Ac_2O, 60°C, 76%; d) CH_3NO_2, KF, 18-crown-6, N-methylmorpoline, 10°C → rt, then NaOAc, Ac_2O, 60°C, 70%; e) Fe, SiO_2, AcOH, toluene, 90°C, 96%; f) NaOH, $Na_2S_2O_4$, DMF, rt, 84%; g) O_2, salcomine, 5°C, 58%.

EXPERIMENTAL

General Melting points were determined on a Mitamura hot-stage micro melting point apparatus without correction. IR and UV spectra were recorded on a JASCO 5300 FTIR and a JASCO UVIDEC 670 spectrophtometer, respectively. ^{1}H- and ^{13}C-NMR spectra were taken on Varian unity-200 or JEOL GX-400 spectrometer. Chemical shifts are expressed in δ units (part per million downfield from Me_4Si). The HMBC, HMQC, and ROESY were run on a JEOL at 400 MHz. Mass spectra (MS) were recorded on a JEOL AX-500. Air- and moisture-sensitive reagents were transferred via syringe or cannula, and reactions involving these materials were carried out in oven-dried flasks under a positive pressure of argon. Silica gel (Wako, C-300) was used for column chromatography. Analytical thin-layer chromatographies (TLC) were performed with Merck precoated TLC plates (Kiselgel 60 F_{254}, 0.25 mm), and spots were visualized with ultraviolet light and 5% $CeSO_4$ in H_2SO_4 by heating.

Collection, Extraction, and Isolation: The gastropod *Drupella 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) being defrosted, the mid-intestinal gland of *D. fragum* was blended with MeOH, and after filtration the crude extract was evaporated under vacuum to yield a residue 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 on a size exclusion column (Sephadex LH-20) and eluted with MeOH to divide into 10 fractions. Fraction 6 (150 mg) was purified successively by column chromatography on silica gel with Me_2CO–hexane (3 : 7) and HPLC (TSK-GEL LS-410K) with MeOH–H_2O (3 : 17) to give 6-methoxyindole-4,7-quinone (**1**) (6.5 mg) and 5-methoxyindole-4,7-quinone (**2**) (13.2 mg). Fraction 8 (99 mg) was chromatographed on silica gel with benzene-ethyl acetate (9 : 1 → 100%) to divide into 6 factions (fr. 11 – 16). Fraction 13 (7.2 mg) was purified by column chromatography on silica gel with hexane–ethyl acetate (4 : 1) to give 5-methylindole-4,7-quinone (**3**) (5.8 mg).

6-Methoxyindole-4,7-quinone (1): Orange prisms, mp 188-190°C; IR (KBr): 3337, 3202, 2925, 1672, 1643, 1595, 1545, 1496, 1406, 1254, 1098 cm^{-1}; UV λ_{max} (EtOH) 424 (ε 880), 326 (ε 3800), 282 (ε 16000), 222 (ε 17300) nm; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 3.76 (3H, s, OMe), 5.80 (1H, s, H-5), 6.49 (1H, *J* = 2.5 Hz, H-3), 7.23 (1H, d, *J* = 2.5 Hz, H-2); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 56.35 (OMe), 106.79 (C-5), 107.08 (C-3), 126.12 (C-3a), 127.48 (C-2), 129.20 (C-7a), 159.78 (C-6), 170.71 (C-7), 182.83 (C-4); HREIMS *m/z* 177.0433 [M$^+$] (calcd 177.0426 for $C_9H_7NO_3$).

5-Methoxyindole-4,7-quinone (2): Orange needles, mp 198-200°C; IR (KBr): 3368, 3237, 2924, 1680, 1647, 1593, 1547, 1408, 1250, 1114, 1086 cm^{-1}; UV λ_{max} (EtOH) 432 (ε 1120), 324 (ε 2540), 281 (ε 19400),

222 (ε 18200) nm; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 3.77 (3H, s, OMe), 5.79 (1H, s, H-6), 6.50 (1H, J = 2.5 Hz, H-3), 7.12 (1H, d, J = 2.5 Hz, H-2); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 56.42 (OMe), 106.00 (C-6), 107.13 (C-3), 122.87 (C-3a), 125.45 (C-2), 131.32 (C-7a), 160.57 (C-5), 176.58 (C-7), 177.70 (C-4); HREIMS *m/z* 177.0422 [M$^+$] (calcd 177.0426 for $C_9H_7NO_3$).

5-Methylindole-4,7-quinone (3): Orange prisms, mp 202-204°C; IR (KBr): 3266, 1663, 1636, 1537, 1495, 1203, 1120 cm^{-1}; UV λ_{max} (EtOH) 470 (ε 790), 316 (ε 4180), 250 (ε 2010), 228 (ε 8060) nm; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 2.35 (3H, s, Me), 6.16 (1H, brs, H-6), 6.55 (1H, J = 2.5 Hz, H-3), 7.16 (1H, d, J = 2.5 Hz, H-2); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 13.30 (Me), 107.67 (C-3), 123.74 (C-6), 123.98 (C-3a), 125.69 (C-2), 131.45 (C-7a), 154.58 (C-5), 174.26 (C-7), 178.96 (C-4).

4-Methoxy-2-nitro-3-hydroxybenzaldehyde (4): To a solution of isovanillin (300 mg, 2.0 mmol) in CH_2Cl_2 (10 mL) and nitromethane (5 mL) was added nitronium trifluroborate (320 mg) at –40°C. After being stirred at –40°C for 4 h, the reaction was terminated by the addition of water and the mixture was extracted with ether (3 times). The combined organic layers were washed with water and saturated aqueous NaCl, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel [hexane-ethyl acetate (2 : 1)] to afford compound **4** (178 mg, 46%) and 4-methoxy-6-nitro-3-hydroxybenzaldehyde (35 mg, 9%) as crystals, respectively. **4**: mp 147–150°C (lit.[15] mp 143–145°C); IR (film) 3233, 2924, 2853, 1682, 1532, 1508, 1285 cm^{-1}; ^{1}H-NMR (200 MHz, $CDCl_3$,) δ 4.04 (3H, s), 7.12 (1H, d, J = 8.5 Hz), 7.48 (1H, d, J = 8.5 Hz), 8.82 (1H, s), 10.08 (1H, s); HREIMS *m/z* 197.0329 [M$^+$] (calcd 197.0324 for $C_8H_7NO_5$).

3-Acetoxy-4-methoxy-2-nitrobenzaldehyde (5): A mixture of compound **4** (545 mg, 2.77 mmol), sodium acetate (42 mg) and acetic anhydride (5.5 mL) was refluxed for 2.5 h. After being cooled to room temperature, water was added and the mixture was extracted with ether. The combined organic layers were washed with water and saturated aqueous NaCl, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was chromatographed on silica gel [hexane-ethyl acetate (2 : 1)] to afford **5** (575 mg, 87%) as crystals. mp 115–118°C; ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 2.32 (3H, s), 3.98 (3H, s), 7.20 (1H, d, J = 8.7 Hz), 7.83 (1H, d, J = 8.7 Hz), 9.83 (1H, s); EIMS *m/z* (rel. int) 239 [M$^+$] (100).

3-Acetoxy- 4-methoxy-2,β-dinitrostyrene (7): To a solution of nitromethane (0.66 mL, 12 mmol) in THF (5 mL) was added dropwise 6.0 mL (3 mmol) of potassium bis(trimethylsilyl)amide (0.5 M solution in toluene) under an argon atmosphere at -78°C. To this solution was added a solution of compound **5** (600 mg, 3 mmol) in THF (4 mL), and the reaction mixture was allowed to stir at room temperature. After being stirred at room temperature for 2 h, water was added and the mixture was extracted with ether (4 times). The combined organic layers were washed with water and saturated NaCl solution, dried over $MgSO_4$, and concentrated *in vacuo* to give compound **6** (500 mg), which was dissolved in CH_2Cl_2 (5 mL) containing

triethylamine (0.25 mL) and was cooled to 0°C. To this solution was added methanesulfonyl chloride (0.15 mL). After being stirred at room temperature for 2 h, water was added to the mixture and extracted with ether. The combined organic layers were washed with water and saturated NaCl solution, dried over $MgSO_4$, and concentrated *in vacuo* to give compound **7** (470 mg) as an oil. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 2.29 (3H, s), 3.95 (3H, s), 7.15 (1H, d, J = 8.3 Hz), 7.46 (1H, d, J = 13.5 Hz), 7.53 (1H, d, J = 8.3 Hz), 7.88 (1H, d, J = 13.5 Hz); HREIMS *m/z* 282.0475 [M$^+$] (calcd 282.0488 for $C_{11}H_{10}N_2O_7$).

7-Acetoxy-6-methoxyindole (8): A mixture of compound **7** (100 mg, 0.35 mmol), silica gel (0.9 g), reduced iron powder (340 mg), glacial AcOH (1.9 mL), and toluene (4.0 mL) was heated to 90°C under an argon atmosphere with efficient mechanical stirring. After 10 min, the mixture was filtered, and the solids were washed with EtOAc (3 x 50 mL). The combined organic extracts were washed with water and saturated NaCl solution, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was chromatographed on silica gel [hexane-ethyl acetate (2 : 1)] to afford **8** (50 mg, 70%) as crystals. mp 141–143°C; IR (film) 3368, 2936, 2839, 1759, 1512, 1411 cm^{-1}; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 2.39 (3H, s), 3.88 (3H, s), 6.48 (1H, dd, J = 3.1, 2.1 Hz), 6.88 (1H, d, J = 8.7 Hz), 7.08 (1H, dd, J = 8.7, 2.1 Hz), 7.42 (1H, d, J = 3.1 Hz), 8.05 (1H, brs); HREIMS *m/z* 205.0739 [M$^+$] (calcd 205.0704 for $C_{11}H_{11}NO_3$).

7-Hydroxy-6-methxoyindole (9): To a stirred solution of **8** (50 mg, 0.31 mmol) in DMF (12 mL) containing 1.15 M $Na_2S_2O_4$ (3 mL) was added 3 mL of 1.25 M NaOH solution at 5°C. After being stirred for 40 min, the mixture was poured over ice water and then extracted with ether, and concentrated *in vacuo* to give **9** (39 mg, 98%) as an oil. IR (film) 3420, 2932, 2839, 1949, 1588, 1507, 1451 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 3.93 (3H, s), 6.46 (1H, dd, J = 3.2, 2.1 Hz), 6.84 (1H, d, J = 8.5 Hz), 7.13 (2H, m), 8.25 (1H, brs); HREIMS *m/z* 163.0613 [M$^+$] (calcd 163.0593 for $C_9H_9NO_2$).

Synthesis of 6-methoxyindole-4,7-quinone (1): A mixture of compound **9** (40 mg, 0.25 mmol), salcomine (20 mg), and DMF (2mL) was stirred under an oxygen atmosphere at room temperature overnight. The mixture was poured over ice-water and then extracted with ether. The organic layer was washed with water and saturated NaCl solution, dried over $MgSO_4$. Evaporation of the solvent left the residue, which was chromatographed on silica gel [hexane-ethyl acetate (2 : 1)] to give an indolequinone (26.1 mg, 60%) as orange needles, mp 210–212°C (lit.[10] mp 210–212°C), the ^{1}H NMR data of which were identical with those of natural compound **1** and those cited in the literature.[11] Anal. Cacld for $C_9H_7NO_3 \cdot 1/4H_2O$ (181.636): C 59.51 %, H 3.88 %, N 7.71 %; found C 59.32 %, H 3.89 %, N 7.73 %.

2,3-Dimethoxy-6-nitrobenzaldehyde (11a): A solution of 2,3-dimethoxybenzaldehyde (**10**) (4.0 g, 24.1 mmol) in glacial AcOH (80 mL) was cooled down to 14°C and treated with fuming HNO_3 (19 mL). After being stirred at room temperature for 45 min, the mixture was poured over ice and kept cold until an abundant yellow precipitate formed. Filtering and water washings gave the solids, which was chromatographed on

silica gel [hexane-ethyl acetate (2 : 1)] to afford **11a** (2.5 g, 49%) and **11b** (1.5g, 30%) as yellow crystals. **11a**: mp 105–107°C (lit.[16] mp 108–109°C); IR (film) 1699, 1574, 1480, 1329, 1285, 1244 cm^{-1}; ^{1}H-NMR (200 MHz, $CDCl_3$,) δ 3.90 (3H, s), 4.00 (3H, s), 7.05 (1H, d, *J* = 9.2 Hz), 7.97 (1H, d, *J* = 9.2 Hz), 10.37 (1H, s); HREIMS *m/z* 211.0479 [M^+] (calcd for 211.0481 for $C_9H_9NO_5$).

2-Acetoxy-3-methoxy-6-nitrobenzaldehyde (12): To a solution of **11a** (1.7 g, 8.0 mmol) in CH_2Cl_2 (65 mL) was added 8 mL of boron tribromide (1.0 M solution in CH_2Cl_2) under argon at -78°C. After being stirred at room temperature overnight, water was added to the mixture and extracted with ether. The combined organic extracts were washed with water and saturated NaCl solution, dried over $MgSO_4$, and concentrated *in vacuo* to give 2-hydroxy-3-methoxy-6-nitrobenzaldehyde (1.5 g, 95%) as crystals. mp 97–100°C (lit.[17] mp 92.5–93.5°C); IR (film) 3368, 2926, 1651, 1514, 1439, 1393, 1275 cm^{-1}; ^{1}H-NMR (200 MHz, $CDCl_3$,) δ 4.02 (3H, s), 7.05 (1H, d, *J* = 8.8 Hz), 7.76 (1H, d, *J* = 8.8 Hz), 10.51 (1H, s), 12.58 (1H, s); HREIMS *m/z* 197.0300 [M^+] (calcd 197.0324 for $C_8H_7O_5N$). This compound (1.4 g, 7.1 mmol) was dissolved in acetic anhydride (11 mL) and treated with NaOAc (5.7 mg). The mixture was heated at 70°C for 1.5 h. Water was added to the mixture and the formed precipitate was filtered, washed well with water, and dried to afford **12** (1.29 g, 76%) as crystals. mp 130–133°C (lit.[17] mp 138–139°C); IR (film) 1779, 1711, 1582, 1520, 1337, 1287, 1182 cm^{-1}; ^{1}H-NMR (200 MHz, $CDCl_3$,) δ 2.32 (3H, s), 3.98 (3H, s), 7.13 (1H, d, *J* = 8.8 Hz), 8.18 (1H, d, J = 8.8 Hz), 10.22 (1H, s); HREIMS *m/z* 239.0458 [M^+] (calcd 239.0429 for $C_{10}H_9NO_6$).

2-Acetoxy- 3-methoxy-6,β-dinitrostyrene (13): To a solution of **12** (1.0 g, 4.2 mmol) in N-methylmorpholine (23 mL) were added KF (150 mg), 18-crown-6 (100 mg), and nitromethane (1.3 mL) under argon at 10 °C. After being stirred for 21 h, The mixture was poured into acetic anhydride (13 mL) containing sodium acetate (270 mg) and warmed to 60°C. After 1h, the reaction mixture was poured over ice and stirred until a fine powder resulted. The solid was filtered and then taken up in EtOAc. The organic extracts were washed with water and saturated aqueous NaCl, dried over $MgSO_4$. After evaporation of the solvent, the crude product was chromatographed on silica gel eluted with hexane-EtOAc (2 : 1) to afford **13** (830 mg, 70 %) as yellow needles. mp 170-172°C; IR (film) 1775, 1530, 1348, 1323, 1283, 1177, 1088 cm^{-1}; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 2.38 (3H, s), 3.98 (3H, s), 7.11 (1H, d, *J* = 8.8 Hz), 7.25 (1H, d, *J* = 13.8 Hz), 8.16 (1H, d, *J* = 13.5 Hz), 8.23 (1H, d, *J* = 8.8 Hz); HREIMS *m/z* 282.0481 [M^+] (calcd 282.0488 for $C_{11}H_{10}N_2O_7$).

4-Acetoxy-5-methoxyindole (14): A mixture of compound **13** (257 mg. 0.91 mmol), silica gel (2.3 g), reduced iron powder (870 mg), glacial AcOH (5 mL), and toluene (10 mL) was heated at 90°C under argon with efficient mechanical stirring. After 30 min, the mixture was filtered, and the solids were washed with EtOAc. The combined organic extracts were washed with water and saturated aqueous NaCl, dried over $MgSO_4$. After evaporation of the solvent, the crude product was chromatographed on silica gel eluted with hexane-EtOAc (2 : 1) to afford **14** (179 mg, 96 %) as an oil. IR (film) 3405, 1759, 1497, 1246, 1227, 1202, 1092 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.41 (3H, s), 3.86 (3H, s), 6.39 (1H, s, *J* = 2.5 Hz), 6.97 (1H, d, *J* =

8.7 Hz), 7.15 (1H, d, J = 2.5 Hz), 7.20 (1H, d , J = 8.7 Hz), 8.13 (1H, brs); HREIMS m/z 205.0724 [M^+] (calcd 205.0739 for $C_{11}H_{11}NO_3$).

4-Hydroxy-5-methoxyindole (15): To a stirred solution of **14** (330 mg, 1.6 mmol) in DMF (9 mL) and 1.15 M $Na_2S_2O_4$ (2.3 mL) was added 2.3 mL of 1.25 M NaOH solution at 5°C. After being stirred for 20 min, the mixture was poured over ice-water and then extracted with ether, and concentrated *in vacuo* to give the residue, which was chromatographed on silica gel eluted with hexane-CH_2Cl_2 (2 : 1) to afford **15** (140 mg, 54 %) as crystals. mp 129–132°C; IR (film) 3453, 3383, 1499, 1464 1370, 1246, 1088 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 3.91 (3H, s), 5.92 (1H, s), 6.33 (1H, d, J = 2.3 Hz), 6.87 (1H, d, J = 8.0 Hz), 6.91 (1H, d, J = 8.0 Hz), 7.12 (1H, d, J = 2.3 Hz)), 8.01 (1H, brs); HREIMS m/z 163.0642 [M^+] (calcd 163.0633 for $C_9H_9NO_2$).

Synthesis of 5-methoxyindole-4,7-quinone (2): A mixture of compound **15** (246 mg, 1.51 mmol), salcomine (40 mg), and DMF (10 mL) was stirred under an oxygen atmosphere at room temperature overnight. The mixture was poured over ice-water and then extracted with ether. The organic layer was washed with water, saturated NaCl solution, and dried over $MgSO_4$. Evaporation of the solvent left the residue, which was chromatographed on silica gel [hexane-ethyl acetate (2 : 1)] to give an indolequinone (155 mg, 58%) as orange needles, mp 205–208°C; HREIMS m/z 177.0418 [M^+] (calcd 177.0426 for $C_9H_7NO_3$). The ^{1}H NMR data were identical with those of natural product **2**. Anal. Cacld for $C_9H_7NO_3$•1/4H_2O (181.636): C 59.51 %, H 3.88 %, N 7.71 %; found C 59.56 %, H 4.01 %, N 7.69 %.

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