

Column Diamonsil C₁₈ (200 mm × 4.6 mm, 5 μm). The mobile phase was methanol-water (80:20). Detection wavelength was 323 ± 1 nm. The flow rate was set at 1.0 ml/min. The retention time was of 8.1 min for osthol.

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Chemical constituents from the marine sponge *Iotrochoto birotulata*

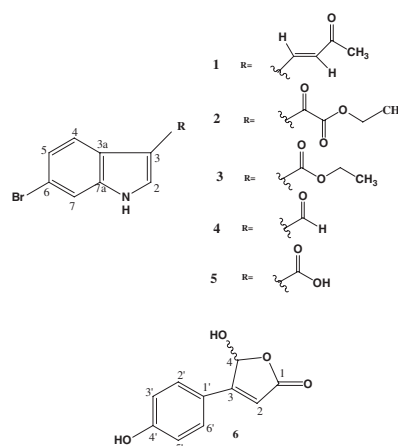
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In continuation to investigate bioactive secondary metabolites from Chinese marine organisms, the sponge *Iotrochoto birotulata* [1–5] was collected from Southern China sea, a tropical area in southern Hainan island. The MeOH extract of *I. birotulata* was partitioned between H₂O and EtOAc, and the EtOAc extract was concentrated and subjected to column chromatography repeatedly to afford six compounds (**1**–**6**). The basic structural pattern of **1** to **5** was that of 6-bromoindole analogues, and **2** to **5** were identified as (6-bromo-indol-3-yl)-oxo-acetic acid ethyl ester (**2**) [6], bromoester (**3**) [7–8], 6-bromoindole-3-carboxylic acid (**4**) [9] and 6-bromoindole-3-carbaldehyde (**5**) [9], by comparison of the physical and chemical properties as well as the spectral data with those reported in the literature. Compound **2** was obtained previously as synthesized product [6], but the ¹H and ¹³C NMR data have not been concluded before we elucidated them by extensive 2D NMR spectroscopy. Compound **6** was identical to hydroxybutenolide [10] due to an extensive 2D NMR spectral analysis. This is the first report to reveal the species *I. birotulata* containing brominated indole derivatives.



Compound **1** was isolated as a white powder. Its positive ESI-MS spectrum showed the molecular ion peak at *m/z* 264 and 266 with integration 1:1, suggesting the presence of a bromine element. The molecular formula C₁₂H₁₀NOBr was established by the negative HRESIMS spectrometry data (*m/z* 261.9850, calcd. for 261.9872). The IR spectrum exhibited absorptions at 3160, 1707,

Table: Spectroscopic data of compounds **1**, **2**, **6**

Compd.	Description	UV (MeOH) $\lambda_{\max}$ (nm)	IR (KBr) $\nu_{\max}$	^1H NMR δ (DMSO- d_6 , ppm)	HRESIMS (neg.) m/z :
2	Pale yellow amorphous solid	270, 320	3424, 2925, 1732, 1627, 1505, 1407 cm^{-1}	12.46 (br), 8.47 (d, $J = 3.0$ Hz), 8.10 (d, $J = 8.0$ Hz), 7.43 (dd, $J = 8.0, 1.5$ Hz), 7.75 (d, $J = 1.5$ Hz), 1.33 (t, $J = 7.0$ Hz), 4.37 (q, $J = 7.0$ Hz) ^{13}C NMR δ (DMSO- d_6 , ppm): 139.82 (d), 116.32 (s), 125.43 (s), 123.65 (d), 126.59 (d), 117.17 (s), 116.32 (d), 138.50 (s), 180.01 (s), 163.80 (s), 14.79 (q), 62.61 (t).	293.9752, 295.9721 ($\text{M}^+ - 1$, 1 : 1; calcd. for 293.9844)
6	pale white powder, m.p. 205–207 °C	310	3356, 3194, 2940, 1734, 1613, 1518, 1450, 1128	10.20 (s, OH), 7.89 (d, $J = 8.5$ Hz, OH), 7.65 (d, $J = 8.5$ Hz, H-2', 6'), 6.86 (d, $J = 8.5$, H-3', 5'), 6.52 (d, $J = 8.5$ Hz, H-4), 6.50 (s, H-2) ^{13}C NMR δ (CDCl_3 , ppm): 172.10 (s, CO), 164.25 (s, C-3), 161.24 (s, C-4'), 131.03 (d, C-2', 4'), 121.00 (s, C-1'), 116.55 (d, C-3', 5'), 111.86 (d, C-2), 98.65 (d, C-4)	ESI-MS (neg.) m/z : 191 ($\text{M}^+ - 1$); HRFABMS m/z : 191.0359 (calcd. for 191.0344)
1	pale white powder, m.p. 145–148 °C	270, 290, 340	3160, 2919, 1707, 1637, 1614, 1523, 1243 cm^{-1}	^1H NMR δ (CDCl_3 , ppm): 8.59 (br), 7.54 (s), 7.81 (d, $J = 8.5$ Hz), 7.38 (dd, $J = 8.5, 2.0$ Hz), 7.62 (d, $J = 2.0$ Hz), 6.80 (d, $J = 16.5$ Hz), 7.77 (d, $J = 16.5$ Hz), 2.41 (s) ^{13}C NMR δ (CDCl_3 , ppm): 129.66 (d), 114.00 (s), 124.00 (s, C-3a), 122.03 (d, C-4), 126.82 (d, C-5), 117.70 (s, C-6), 115.23 (d, C-7), 138.30 (s, C-7a) were assigned for indole unit, and four [δ 199.00 (s), 135.81 (d), 123.72 (s) and 27.85 (q)] were attributed to a side chain. The HMQC spectrum assigned the signals of protonated carbons as well as their associated protons. In HMBC spectrum, the olefinic proton at δ 6.80 correlated to carbonyl carbon (δ 199.00, s), C-3 (δ 114.00) and methyl carbon (δ 27.85, q), and the other olefinic proton at δ 7.77 correlated with C-3a (δ 124.00) and carbonyl carbon respectively, indicating a but-1-en-3-one moiety annexed to position C-3 of indole ring. A bromine element was considered to be substituted to C-6 by comparison of the ^1H and ^{13}C NMR data of 1 with those of known 6-bromoindoles 3 , 4 and 5 [9, 11] as well as HMBC correlation. Consequently, the structure of 1 was established as 3-(but-1- <i>E</i> -en-3-one)-6-bromoindole.	261.9850, 263.9848 ($\text{M}^+ - 1$, 1 : 1, calcd. for 261.9872). ESI-MS (positive) m/z : 264, 266 ($\text{M}^+ + 1$, 1 : 1); 222, 224 (1 : 1)

1637 and 1614 cm^{-1} , indicating the presence of an active proton (NH) and a conjugated carbonyl group as well as an aromatic group. The ^1H NMR spectrum showed an ABX coupled system at δ 7.81 (d, $J = 8.5$ Hz, H-4), 7.38 (dd, $J = 8.5, 2.0$ Hz, H-5) and 7.62 (d, $J = 2.0$ Hz, H-7), as well as a singlet at δ 7.54 (s, H-2) and a broadening signal at δ 8.59 (br, NH), in association with ^{13}C NMR data, **1** was characteristic of 3,6-disubstituted indole unit corresponding to that of **3**, **4** and **5**. Additionally, there were two olefinic protons at δ 6.80 (d, $J = 16.5$ Hz) and 7.77 (d, $J = 16.5$ Hz) with coupling constant $J = 16.5$ Hz for a trans geometry, and a singlet at δ 2.41 (s, 3H) was due to a methyl group. The ^{13}C NMR spectral data of **1** displayed 12 carbon signals, of which eight δ 129.66 (d, C-2), 114.00 (s, C-3), 124.00 (s, C-3a), 122.03 (d, C-4), 126.82 (d, C-5), 117.70 (s, C-6), 115.23 (d, C-7), 138.30 (s, C-7a) were assigned for indole unit, and four [δ 199.00 (s), 135.81 (d), 123.72 (s) and 27.85 (q)] were attributed to a side chain. The HMQC spectrum assigned the signals of protonated carbons as well as their associated protons. In HMBC spectrum, the olefinic proton at δ 6.80 correlated to carbonyl carbon (δ 199.00, s), C-3 (δ 114.00) and methyl carbon (δ 27.85, q), and the other olefinic proton at δ 7.77 correlated with C-3a (δ 124.00) and carbonyl carbon respectively, indicating a but-1-en-3-one moiety annexed to position C-3 of indole ring. A bromine element was considered to be substituted to C-6 by comparison of the ^1H and ^{13}C NMR data of **1** with those of known 6-bromoindoles **3**, **4** and **5** [9, 11] as well as HMBC correlation. Consequently, the structure of **1** was established as 3-(but-1-*E*-en-3-one)-6-bromoindole.

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