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Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
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To cite this Article Huang, Xing-Ping , Deng, Zhi-Wei , van Soest, Rob W. M. and Lin, Wen-Han(2008) 'Brominated derivatives from the Chinese sponge *Pseudoceratina* sp.', Journal of Asian Natural Products Research, 10: 3, 239 — 242
To link to this Article: DOI: 10.1080/10286020701604862
URL: http://dx.doi.org/10.1080/10286020701604862

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Journal of Asian Natural Products Research
Vol. 10, No. 3, March 2008, 239–242

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Brominated derivatives from the Chinese sponge *Pseudoceratina* sp.

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(*Received 9 November 2006; final version received 28 March 2007*)

Chemical examination of the marine sponge *Pseudoceratina* sp. resulted in the isolation and characterization of two new dibromotyrosine-derived metabolites (**1**–**2**), and a new histamine-derived alkaloid (**3**), along with eight known dibromotyrosine based products. Their structures were elucidated on the basis of IR, 1D and 2D NMR, and MS spectral data analyses.

Keywords: sponge; *Pseudoceratina* sp; brominated derivatives; alkaloid

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1. Introduction

The sponge genus *Pseudoceratina* (Aplysinellidae) includes at least six species (*P. crass*, *P. purpurea*, *P. rhax*, *P. verrucosa*, *P. durissima*, *P. clavata*), and is widely distributed in the Caribbean region and the Asian ocean region from Australia to Japan. *Pseudoceratina* and the genera of Verongida family (*Aplysina*, *Lanthella*, *Psammaplysilla*, and *Verongula*) were chemotaxonomically characterized by the presence of bromotyronsine-derived products as the principal metabolites. Previous chemical examination of genus *Pseudoceratina* resulted in the isolation of more than 56 brominated metabolites.[1–15] Part of the metabolites possessed various biological activities, such as antifouling, cytotoxic, antibacterial, and ion channel inhibitory activities.[16–17] In our continuation to discover the chemical diversity from marine organisms, a marine sponge belonging to genus *Pseudoceratina* was collected from Hainan Island in the South China Sea. A chemical examination of the EtOH extract resulted in the isolation of two new brominated metabolites (**1**–**2**), and a new histamine-derived alkaloid (**3**), together with eight known brominated derivatives. This paper reports the structural elucidation of the new compounds from this sponge.

2. Results and discussion

Compound **1** had a molecular formula of $C_9H_{12}O_4NBr_2$ as determined by HRFABMS data (*m/z* 355.9125 $[M + H]^+$). The ^1H and ^{13}C NMR spectral data of **1** were closely compatible to those of aeroplysinin-1,[18,23] as characterized by proton resonances for an oxymethine at δ 3.93 (1H, s, H-2), an olefinic singlet at δ 6.47 (1H, s, H-6), an isolated methylene at δ 2.42 (1H, d, $J = 14.5$ Hz, H-7a) and 2.44 (1H, d, $J = 14.5$ Hz, H-7b), and a methoxy group at δ 3.61 (3H, s) in the ^1H NMR spectrum, along with nine carbon resonances including four olefinic carbons at δ 147.1 (s, C-4), 135.4 (d, C-6), 117.8 (s, C-5), and 113.8 (s, C-3), and two oxygenated carbons at δ 78.1 (d, C-2) and 74.6 (s, C-1) in the ^{13}C NMR spectrum. These NMR spectral data were attributable to the 3,5-dibromo-1,2-dihydroxy-4-methoxy-3,5-cyclohexadiene nucleus. A methylene signal was determined to be a substitution at C-1 on the basis of HMBC correlation between H-6 and C-7 (δ 40.9, t). Compound **1** differed from aeroplysinin-1 due to the presence of the carbonyl signal of **1** at δ 173.5 (s, C-8) instead of the nitril of the latter compound. The presence of two amide protons at δ 7.65 (1H, br s, NH-a) and 7.19 (1H, br s, NH-b) which showed HMBC correlations with C-8 and C-7 indicated the existence of an acetamide at C-1. The relative configurations of hydroxy groups at C-1 and C-2 were in agreement with those of aeroplysinin-1 due to the positive optical rotation of both compounds, and no NOE correlation between H-2 and H_2-7, as well as the hypothesis of aeroplysinin-1 and **1** to be derived from the same intermediate (**11**) (Figure 2).

The molecular formula of **2** was established as $C_{10}H_{11}O_3NBr_2$ on the basis of HRESIMS (*m/z* 373.9007 $[M + Na]^+$) and NMR spectral data. The ^1H and ^{13}C NMR spectral data of **2** closely resembled those of 1-acetamide-3,5-dibromo-1-hydroxy-4-dimethoxy-2,5-cyclohexadiene, a metabolite originated from sponges *Verongia fistularis* and *P. purpurea*,[2,19] except for an acetonitrile unit at C-1 of **2** instead of an acetamide of the

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ISSN 1028-6020 print/ISSN 1477-2213 online
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DOI: 10.1080/10286020701604862
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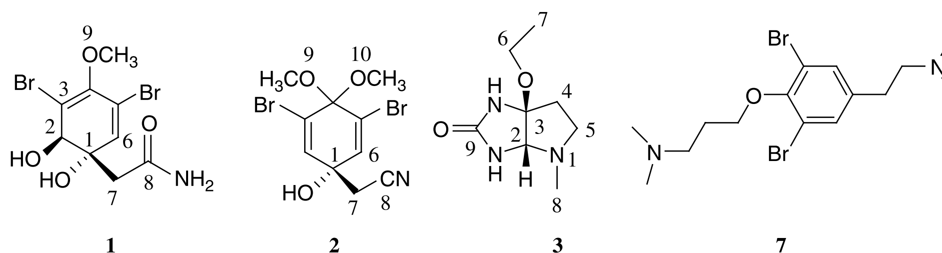

Figure 1. Structures of compounds **1**–**3** and **7**.

latter. The C-1 substitution was confirmed by a typical nitril signal at δ 117.2 (s, C-8) and which showed a HMBC correlation with the methylene protons at δ 2.98 (2H, s, H-7). The ^{1}H and ^{13}C NMR spectral data of **2** were entirely assigned by interpretation of HMQC and HMBC spectra.

The molecular formula of **3** was determined to be $C_8H_{15}O_2N_3$ by HRFABMS (*m/z* 186.1236 $[M + H]^+$). IR absorption bands at 3353, 3237, and 1709 cm^{-1} were diagnostic of amide, hydroxy, and carbonyl groups. The ^{13}C NMR and DEPT spectra exhibited two methyls at δ 36.1 (q) and 15.8 (q), three methylenes at δ 38.3 (t, C-4), 50.8 (t, C-5), and 58.1 (t, C-6), a methine at δ 77.7 (d, C-2), and two quaternary carbons at δ 97.7 (s, C-3) and 160.9 (s, C-9). With the help of HMQC spectrum all protons were assigned to their corresponding carbons. The ^{1}H-^{1}H COSY spectrum provided the cross peaks between protons at δ 1.10 (3H, t, $J = 7.0$ Hz, H-7) and 3.38 (1H, dq, $J = 7.0$, 12.0 Hz, H-6a), 3.42 (1H, dq, $J = 7.0$, 12.0 Hz, H-6b), suggesting the presence of an ethoxy group. The ^{1}H NMR spectrum displayed two exchangeable protons at δ 7.00 (br, NH) and 7.22 (br, NH), and the former showed a weak COSY correlation with a proton at δ 4.29 (1H, br s, H-2). An interpretation of HMBC spectrum revealed that both NH protons correlated with carbons of C-9, C-2, and C-3, indicating the presence of an 1,3-imidazolidin-2-one subunit. In addition, the presence of a pyrrolidine ring was evidenced by the HMBC correlations from H-2 to C-3, C-4, C-5, and C-9 in association with the COSY correlation between H_2-4 (δ 1.94, m; 1.88, m) and H_2-5 (δ 2.63, m; 2.44, m). Obviously, this subunit fused with imidazolidin-2-one at C-2 and C-3 to form an octahydropyrollo[2,3-d]imidazol-2-one nucleus. The ethoxy group was attached at C-3 due to the HMBC correlation between the oxymethylene protons H_2-6 and C-3, while a methyl group was positioned at N-1 according to the HMBC correlation between the methyl singlet at δ 2.20 (3H, s) and C-2 and C-5. A *cis* ring junction was supposed on the basis of NOE

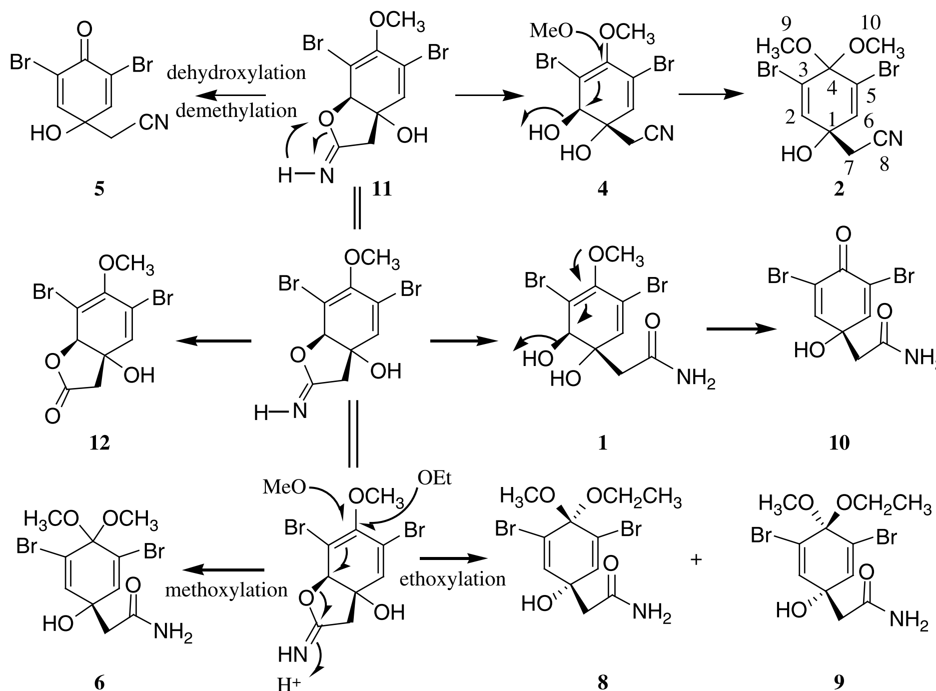

Figure 2. Proposed inter-transformation of the isolated brominated derivatives.

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correlation between H-2 and H_2-6. Thus, the structure of **3** was elucidated as 3-ethoxy-1N-methyl-octahydropyrollo[2,3-d] imidazol-9-one.

The known brominated metabolites were identified as aeroplysinin-1 (**4**),[18,23] 3,5-dibromo-1-hydroxy-4-oxo-2,5-cyclohexadien-1-acetonitrile (**5**),[20] 3,5-dibromo-1-hydroxy-4-dimethoxy-2,5-cyclohexadien-1-acetonitrile (**6**),[19,21] desalted aplysamine-1 (**7**),[12,18] 3,5-dibromo-4-ethoxy-1-hydroxy-4-methoxy-2,5-cyclohexadien-1-acetamide (**8**)[14] and its 4-epimer (**9**),[18,20,22] 3,5-dibromo-1-acetamide-1-hydroxy-2,5-cyclohexadien- 4-one (**10**),[19,21,23] and aeroplysinin-2 (**12**),[19] by comparison of their spectral data with those reported in literature.

It is noted that aeroplysinin-1 could be partially converted to 3,5-dibromo-1,2-dihydroxy-4-oxo-5-cyclohexene-1-acetonitrile [20] in $CHCl_3$. Thus, the latter compound was regarded as an artifact rather than a natural product as previously reported from sponge *Aplysina laevis*.[20] Compounds **8** and **9** were suggested to be artifacts generated during extraction by EtOH.[20]

The sponge genus *Pseudoceratina* is rich in brominated metabolites with two or more bromotyrosine residues such as isofistularin-3, in accompaniment with mono- bromotyrosine derivatives representing aeroplysinin-1 and dienone as frequently being demonstrated in Verongia order. The latters were derived from isoxazoline alkaloids through enzymatic conversion in the purpose of growth inhibitory and repellent activity toward marine bacteria, algae and gastropods to suppress fouling organisms, or when the sponges were wounded.[24] The high content of aeroplysinin-1 in the present sponge implied its severe ecological settlement. Detection of the EtOH extract by ESIMS spectrum revealed the presence of dibromo-derivatives in this sponge rather than tri- or tetra-brominated metabolites as frequently found from *Pseudoceratina* sponges growing in other marine zone. This evidence conducted to suppose the ecological environment of *Pseudoceratina* sponges in South China Sea somehow differed from the same genus growing in other marine regions. The desalted aplysamine-1 (**7**) from our specimen also occurring in Caribbean *Pseudoceratina* sp. with rich abundance[12] could be considered as chemotaxonomic marker since it has not yet been isolated from other *Pseudoceratina* species. The existence of aeroplysinin-2 (**12**) implied that an intermediate of imine-ether (**11**) as supposed by Faulkner[19] is a possible precursor to derive the isolated brominated derivatives (Figure 2).

3. Experimental

3.1 General experimental procedures

Optical rotations were measured with a Perkin-Elmer 243B Polarimeter using a sodium lamp. The IR spectra were recorded on a Thermo Nicolet Nexus 470 FT-IR spectrometer. The ^{1}H- and ^{13}C-NMR, as well as 2D NMR spectra were taken on a Bruker Avance-500 FT and an Varian INOVA 500 NMR spectrometers using TMS as internal standard. HRFABMS spectra were obtained on a Bruker FTICR APEXII mass spectrometer, and HRESIMS spectra were performed on a APEX IV FT-MS mass spectrometer. Column chromatography (CC) was carried out on silica gel (200–300 mesh, Qingdao Marine Chemistry Co. Ltd., Qingdao, China), and the HF_{254} silica gel for TLC was provided by Sigma Co. Ltd. Sephadex LH-20 (18–110 μm) was obtained from Pharmacia Co. High pressure liquid chromatography (HPLC) was performed on an Alltech-426 apparatus using a Kromasil prepacked column (ODS, 10×250 mm, for reversed phase) and monitored by UV detection (254 nm).

3.2 Sponge material

Sponge *Pseudoceratina* sp. was collected by SCUBA in inner reef of Hainan Island, in December 2005. A voucher specimen (HSG-05) is deposited in National Key Laboratory of Natural and Biomimetic Drugs, Peking University. The sponge species was authorized by Prof. R.W.M van Soest from University of Amsterdam, Netherlands.

3.3 Extraction and isolation

The sponge (1.87 kg, wt.) was homogenized and then extracted with EtOH. The EtOH extract was concentrated under reduced pressure, and the residue (156 g) was partitioned between H_2O and petroleum ether, EtOAc, and *n*-BuOH, successively. The EtOAc fraction (1.8 g) was subjected to silica gel column chromatography eluting with a gradient of petroleum ether-acetone (10:1 to 1:1) to obtain 7 fractions (F1–F7) according to TLC detection. F3 (40 mg, 10:1) was separated on Sephadex LH-20 column with MeOH as eluent to yield **1** (5.0 mg) and **2** (2.0 mg). F4 (500 mg, 8:1) was further purified by repeated silica gel column to obtain **4** (80.0 mg), and **12** (30.0 mg). F5 (300 mg) was subjected to a silica gel column with $CHCl_3$-MeOH (15:1) and then reversed-phase HPLC (MeOH/H_2O, 20/80) to afford **8** (20 mg) and **9** (15 mg). F6 (240 mg) was separated on Sephadex LH-20 column with MeOH as an eluant to collect a portion (180 mg) showing a main spot on TLC, and this portion was subsequently subjected to silica gel column eluting with Petroleum ether-EtOAc (2:1) to afford **5** (13.0 mg), **6** (2.0 mg), **7** (150.0 mg) and **10** (44.0 mg). F7 was purified on reverse phase HPLC (MeOH/H_2O, 10/90) to afford **3** (8.5 mg).

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*3.3.1 Compound **1***

Colorless amorphous solid, $[\alpha]_D^{25} + 12.5$ (c 0.3, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3454, 3282, 3196, 2930, 2851, 1779, 1660, 1451, 1301, 1024, 568; ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 7.65 (br s, NH-a), 7.19 (br s, NH-b), 6.47 (1H, s, H-6), 3.93 (1H, s, H-2), 3.61 (3H, s, H-9), 2.42 (1H, d, $J = 14.5$ Hz, H-7a), 2.44 (1H, d, $J = 14.5$ Hz, H-7b); ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 173.5 (s, C-8), 147.1 (s, C-4), 135.4 (d, C-6), 117.8 (s, C-5), 113.8 (s, C-3), 78.1 (d, C-2), 74.6 (s, C-1), 59.8 (q, C-9), 40.9 (t, C-7); HRFABMS m/z 355.9125 $[M + H]^+$ (calcd for $C_9H_{12}O_4NBr_2$, 355.9127).

*3.3.2 Compound **2***

Colorless amorphous solid, $[\alpha]_D^{25} + 7.5$ (c 0.26, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3386, 2926, 2854, 1675, 1448, 1228, 1099. 874, 705, 411; ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 6.81 (2H, s, H-2,6), 3.06 (3H, s, H-10), 3.05 (3H, s, H-9), 2.98 (2H, s, H-7); ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 141.16 (s, C-2, 6), 123.4 (s, C-3, 5), 117.2 (s, C-8), 96.9 (s, C-4), 51.5 (q, C-9), 51.4 (q, C-10), 29.6 (t, C-7); HRESIMS m/z 373.9007 $[M + Na]^+$ (calcd for $C_{10}H_{11}O_4NBr_2Na$, 373.8998).

*3.3.3 Compound **3***

Colorless amosphous solid, $[\alpha]_D^{25} + 10.0$ (c 0.4, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3353, 3237, 2926, 2851, 1709, 1461, 1211, 1097, 1050, 739; ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 7.22 (br, NH), 7.00 (br, NH), 4.29 (1H, brs, H-2), 3.38 (1H, dq, $J = 7.0$, 12.0 Hz, H-6a), 3.42 (1H, dq, $J = 7.0$, 12.0 Hz, H-6b), 2.63 (1H, m, H-5a), 2.44 (1H, m, H-5b), 2.20 (3H, s, H-8), 1.94 (1H, m, H-4a), 1.88 (1H, m, H-4b), 1.10 (3H, t, $J = 7.0$ Hz, H-7); ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 160.9 (s, C-9), 97.7 (s, C-3), 77.7 (d, C-2), 58.1 (t, C-6), 50.8 (t, C-5), 38.3 (t, C-4), 36.1 (q, C-8), 15.8 (q, C-7); HRFABMS m/z 186.1236 $[M + H]^+$ (calcd for $C_8H_{16}O_2N_3$, 186.1236).

Acknowledgements

This work was supported by grants from NSFC (No. 30672607), the National Hi-Tech Projects (2006AA09Z446, 2006DFA31100), China Uni-PhD Base Project (20060001149), and the International Cooperation Projects of “2 + 2” BMBF-MOST.

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