Urupocidin A: A New, Inducing iNOS Expression Bicyclic Guanidine Alkaloid from the Marine Sponge *Monanchora pulchra*


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**Supporting Information**

**ABSTRACT:** Urupocidins A and B (1 and 2), bisguanidine alkaloids with an unprecedented skeleton system, derived from polyketide precursors and containing an unusual N-alkyl-N-hydroxyguanidine moiety, have been isolated from the sponge *Monanchora pulchra*. The structures of 1 and 2, including absolute configuration, were established using the detailed analysis of 1D and 2D NMR, CD, and mass spectra as well as chemical transformations. Compound 1 increases nitric oxide production in murine macrophages via inducing iNOS expression.

The discovery that NO is responsible for an astonishing range of physiological processes in humans presents one of the most exciting findings in biological chemistry.1 The biosynthesis of NO is catalyzed by nitric oxide synthase (NOS), which is classified into three isoforms: inducible NOS (iNOS), endothelial NOS (eNOS), and neuronal NOS (nNOS). Both the endothelial NOS (eNOS) and the neuronal NOS (nNOS) are constitutive enzymes; however, the third isoform is inducible, produced by macrophages as part of the immune response. Therefore, the search for new natural modulators of iNOS may have therapeutic significance at the search for immunomodulatory compounds.5

Guanidine alkaloids are known to be characteristic metabolites of some marine sponges.6,7 These alkaloids demonstrate a broad spectrum of biological activities, including potent cytotoxic effects, induction of cellular apoptosis, and inhibition of the TRPV1 receptor.6-13 During our search for bioactive substances from marine organisms, we have previously isolated some guanidine alkaloids with unusual cyclic skeletons8-10,12 and rare acyclic alkaloids11,12 from sponges of the genus *Monanchora*. Herein, we describe the isolation and structure elucidation of urupocidins A (1) and B (2), possessing a new trisubstituted bicyclic skeleton system with an unprecedented N-alkyl-N-hydroxyguanidine fragment in one of the side chains, from a new collection of the sponge *Monanchora pulchra* (Figure 1). The induction of endogenous NO in Raw 264.7 cells and iNOS expression in macrophages by 1 is shown (Supporting Information). Urupocidins A and B (1 and 2) were named after one of the biggest Kuril Islands known as Urup Island, near which the sponge was collected.

The alkaloids were isolated from the frozen sponge (0.09% and 0.0024% of dry weight, respectively) by extraction with EtOH, evaporation, and partition between H₂O and n-BuOH, followed by the partition of the BuOH-soluble materials on Sephadex LH-20 (EtOH) and HPLC on an YMC-ODS-A column (65% EtOH/0.1% aqueous TFA). Urupocidin A (1), a colorless glass, has molecular formula C₂₀H₂₀N₆O₄ established by HRESIMS measurement of the [M + H]⁺ ion peak at m/z 547.3956. The peak at m/z 274.2015 (calcld for C₁₀H₁₄N₂O₂ 274.2020) in the HRESIMS of 1 corresponds to a doubly charged [M + 2H]²⁺ ion like similar peaks in MS of the earlier reported monanchomycalins A–C10,13 and two-headed sphingolipids.14

NMR data (DMSO-d₆; Table 1) of 1 revealed the presence of two guanidine groups (δH 8.35 (2H), 9.39 (1H) and δC 157.8), two methyl groups (δH 0.86, 0.85 and δC 13.57, 13.78), two hydroxy groups (δH 10.61, 10.86, 10.85 and δC 13.57, 13.78), one oxygenated methylene (δH 4.05, 4.00 and δC 43.41, 43.35), one oxygenated methine (δH 5.17 and δC 97.77), one nitrogenated methyl (δH 2.62 and δC 19.00), and one olefinic proton (δH 5.52 and δC 128.42). The structure of 1a, a bisguanidine triacetate, was supported by 1D and 2D NMR experiments (Figure 1).

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Table 1. $^1$H (700 MHz) and $^{13}$C (175 MHz) NMR Data of Urupocidins A (1) and B (2)

<table>
<thead>
<tr>
<th>position</th>
<th>δ$_C$</th>
<th>δ$_H$ mult (J in Hz)</th>
<th>position</th>
<th>δ$_C$</th>
<th>δ$_H$ mult (J in Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13.64*</td>
<td>0.86*, t (7.3)</td>
<td>16b</td>
<td>1.50, m</td>
<td>1.47, m</td>
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<tr>
<td>2</td>
<td>22.3*</td>
<td>1.33, sext (7.3)</td>
<td>17a</td>
<td>1.93, m</td>
<td>29.1, 1.25, m</td>
</tr>
<tr>
<td>3</td>
<td>28.74*</td>
<td>1.97, m</td>
<td>17b</td>
<td>2.07, m</td>
<td></td>
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<tr>
<td>4</td>
<td>129.5</td>
<td>5.33, m</td>
<td>18</td>
<td>128.5</td>
<td>5.36, m  30.8, 1.24, m</td>
</tr>
<tr>
<td>5</td>
<td>129.6</td>
<td>5.33, m</td>
<td>19</td>
<td>130.2</td>
<td>5.36, m  22.0, 1.27, m</td>
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<td>6a</td>
<td>23.0</td>
<td>2.00, m</td>
<td>20</td>
<td>28.68*</td>
<td>1.95, m  13.77*, 0.85*, t (7.3)</td>
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<td>6b</td>
<td>2.09, m</td>
<td>2.08, m</td>
<td>21</td>
<td>22.2*</td>
<td>1.31*, sext (7.3)</td>
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<td>13.57*</td>
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<td>8</td>
<td>66.8</td>
<td>3.51, m</td>
<td>23</td>
<td>150.6</td>
<td>150.5</td>
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<td>8.21, brs</td>
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<td>9a</td>
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<td>23b-NH</td>
<td>9.39, d (3.7)</td>
<td>9.17, d (3.9)</td>
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<tr>
<td>9b</td>
<td>1.68, m</td>
<td>1.66, m</td>
<td>24</td>
<td>164.2</td>
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<td>10</td>
<td>57.5</td>
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<td>25a</td>
<td>63.8</td>
<td>4.11, m  63.7, 4.10, m</td>
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<td>11a</td>
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<td>1.96, m</td>
<td>25b</td>
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<td>4.15, m</td>
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<td>11b</td>
<td>2.16, td (8.4; 4.0; 18.6)</td>
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<tr>
<td>12a</td>
<td>29.2</td>
<td>3.05, m</td>
<td>27</td>
<td>22.7</td>
<td>1.67, m  22.6, 1.66, m</td>
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<td>12b</td>
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<td>50.4</td>
<td>3.55, brt</td>
<td>50.4, 3.55, brt (6.6)</td>
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<td>29</td>
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<td>101.0</td>
<td>101.4</td>
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<tr>
<td>15</td>
<td>48.9</td>
<td>4.30, dd (4.1; 3.8)</td>
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<td>7.52, brs</td>
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<tr>
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<td>1.47, m</td>
<td>29a-N-OH</td>
<td>10.61, s</td>
<td>10.58, s</td>
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</tbody>
</table>

$^{13}$C NMR assignments supported by HSQC and HMBC data. *Overlapping signals.

4.70), two dissubstituted double bonds ($\delta_H$ 5.33 (2H) and $\delta_C$ 129.5, 129.6; $\delta_H$ 5.36 (2H) and $\delta_C$ 128.5, 130.2), one tetrastubstituted double bond ($\delta_C$ 151.5, 101.0), two N-substituted CH groups ($\delta_H$ 4.51, 4.30 and $\delta_C$ 57.5, 48.9), one N-substituted CH$_2$ group ($\delta_H$ 3.55 and $\delta_C$ 50.4), one oxymethylene ($\delta_H$ 3.51 and $\delta_C$ 66.8), one carbonyl-linked oxymethylene group ($\delta_H$ 4.11, 4.14 and $\delta_C$ 63.8), and 13 other methylene groups (Table 1). A quaternary carbon signal at $\delta_C$ 164.2 was assigned to the carbonyl group of an ester, which should be conjugated with a double bond according to the IR spectrum data.

Substructures a–d were established by COSY, HSQC, and HMBC experiments (Figure 2). Fragment a had never been previously seen in guanidine alkaloids$^{15}$ isolated from marine invertebrates. It was revealed starting from the signals of CH$_2$-12 group ($\delta_H$ 3.05, 3.13 and $\delta_C$ 29.2) and sequentially extended up to end methyl group that showed a higher field methyl triplet CH$_3$-1 in the $^1$H NMR spectrum ($\delta_H$ 0.86 and $\delta_C$ 13.64).

Interpretation NMR data, starting from the low-field NH-23b doublet ($\delta_H$ 9.39) cross-over signals of $\Delta^{18}$-olefin ($\delta_H$ 5.36 (2H) and $\delta_C$ 128.5, 130.2) and extending until to the high field CH$_2$-22 triplet ($\delta_H$ 0.85 and $\delta_C$ 13.57), indicated the substructure b (Figure 2).

Detailed analysis of NMR data concerning the substructure “c” led to the identification of four methylene groups of an open hydrocarbon chain, linked by one side to an oxygen atom ($\delta_H$ 4.11, 4.14 and $\delta_C$ 63.8) and by another side to a tertiary nitrogen atom of guanidine group ($\delta_H$ 3.55 and $\delta_C$ 50.4). Diagnostic ROESY correlations between the resonances of 29a-N-OH ($\delta_H$ 10.61) and 2H-27 ($\delta_H$ 1.67), 2H-28 ($\delta_H$ 3.55), 29b-NH, 29c-NH$_2$, indicated as the $^1$H–$^{13}$N HMBC correlations between 29b-NH, 29c-NH$_2$, 2H-28, and 29a-N indicated the position of N-OH group in substructure c. Moreover, it was confirmed by a downfield shift of proton and carbon signals of CH$_2$-28 group when compared with the corresponding signals in relative guanidine compounds.$^{15,16}$

Substructure d and its connectivity with a–c was assigned by HMBC experiments, which indicated that the H-10 proton at $\delta_H$ 4.51 correlated to C-11 ($\delta_C$ 26.4), C-12 ($\delta_C$ 29.2), C-14 ($\delta_C$ 101.0), and C-23 ($\delta_C$ 150.6) signals. In addition, the H-15 proton at $\delta_H$ 4.30 correlated to C-23 ($\delta_C$ 150.6), C-13 ($\delta_C$ 151.5), C-14 ($\delta_C$ 101.0), C-24 ($\delta_C$ 164.2), C-16 ($\delta_C$ 36.1), and C-17 ($\delta_C$ 21.5), and protons CH$_3$–25 group was also correlated to C-24 ($\delta_C$ 164.2). Moreover, the $^1$H–$^{13}$N HMBC correlations between 23b-NH, 16a-b-2H and 23b-NH, 11a,b-2H, 9a,b-2H, and 23c-N indicated that a bicyclic moiety with two N atoms at positions 23b, 23c and three substitutions at position C-10, C-15, and C-14 presented in the compound 1 (Figure 1). The Z-geometry of double bonds was assigned using the NMR signals of allylic carbons (Table 1).$^{17,18}$

![Figure 2. Partial structures of 1 with selected COSY, HMBC, and key ROESY correlations.](image-url)
When reacted with Ac₂O, urupocidin A gave the triacetate 1a, the NMR spectrum of which showed, along with the expected signals of the urupocidin skeleton system, the characteristic singlets at δ_H 2.07 (3H) (δ_C 171.0), δ_H 2.17 (3H) (δ_C 181.5), and δ_H 2.20 (3H) (δ_C 168.7) attributable to two O-acetyl and one acetamide groups, respectively (Supporting Information, Table S2).

A determination of a relative stereochemistry of C-10 and C-15 by NOE experiments using 1 was unsuccessful as a consequence of the overlapping of 2H-9, 2H-26, and 2H-27 signals in the 1H NMR spectrum of which showed, along with the expected signals of the urupocidin skeleton system, the diagnostic NOEs correlations between the resonances of H-9a (δ_H 1.68, ddd, 2.4, 9.4, 12.4), H-15 (δ_H 4.41, dd, 4.1, 7.1), and H-11a (δ_H 2.04, dddd, 3.8, 5.1, 8.2, 13.0) were indicative of the 10R* and 15S* relative configurations in 1 (Figure 3). Absolute 15S* configuration was proposed using CD (n → π* transition of the enone) and comparison with crambescin A (Figure 4). The 1H NMR data (Table 1) of 1 closely coincided with those of urupocidin A (1), maintaining the δ_H values for the left-hand protons and negative δ_H values for the most right-hand protons were observed in the 1H NMR spectra of 4 and 5 (Figure 4). These data established the absolute configuration at C-8 as R. Therefore, the 8R,10R,15S absolute configuration in 1 was assigned.

Urupocidin B (2), as a colorless glass, has the molecular formula of C_{27}H_{48}N_{6}O_{4} established by HRESIMS measurement of the [M + H]^+ ion peak at m/z 521.3816. The 1H NMR data (Table 1) of 2 closely coincided with those of urupocidin A (1), maintaining the δ_H values in addition, MS data showed the molecular mass of 2 to be 26 amu less than that of 1, suggesting that the hydrocarbon chain at C-15 is shortened by two CH₂ groups in 2. Comparison of the 1H and 13C NMR, and CD spectra and optical rotation data of urupocidin B (2) with those of urupocidin A (1) and their complete coincidence suggest the same absolute configurations of 2 as for 1.

The structures of urupocidins A and B (1 and 2) possess uncommon structural features, including a trisubstituted bicyclic system and an N-alkyl-N-hydroxyguanidine moiety unprecedented in comparison with other marine guanidine alkaloids. So far, natural N-alkyl-N-hydroxyguanidines have been detected in some terrestrial microorganisms only.22–27

Compound 1 induced an expression of iNOS with increase in NO production in macrophages at 10.0 and 1.0 μM concentrations, respectively (see the Supporting Information). Urupocidin A is the first marine alkaloid stimulator of NO production. To date, a few natural products have been reported to up-regulate NO level in cells; examples include polysaccharides from green algae Capniosiphon fulvescens and Strongylocentrotus nudus eggs and triterpene glycosides from Panax ginseng.30

### ASSOCIATED CONTENT

#### Supporting Information

Full experimental details, copies of 1D and 2D NMR, CD spectra for compounds 1 and 2, tabulated NMR data for compounds 1a, 1b, 3, 4, and 5, and bioassay results. This material is available free via the Internet at http://pubs.acs.org.

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**Notes**

The authors declare no competing financial interest.

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