3,7-Dimethylguanine, a New Purine from a Philippine Sponge Zyzzya fuliginosa

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A new purine 3,7-dimethylguanine (1) has been isolated from the marine sponge Zyzzya fuliginosa, along with the known metabolites, makaluvamines A, C, K (2—4), 4-hydroxyphenylacetic acid (5), methyl ester of 4-hydroxyphenylacetic acid (6), 4-hydroxyphenethyl alcohol (7), L-phenylalanine (8) and L-tryptophan (9). The structure of 3,7-dimethylguanine (1) was elucidated by analysis of 1D and 2D (one- and two-dimensional) NMR [HMBC (heteronuclear multiple bond connectivity), gHMBC (heteronuclear multiple bond connectivity), 1H-15N gHMBC] data, mass spectroscopy data, and by comparison with 3,7-dimethylisoguanine (10).

Key words marine sponge; Zyzzya fuliginosa; Poecilosclerida; 3,7-dimethylguanine; modified purine; makaluvamine A, C, K

Marine organisms have proven to be a valuable source of modified purine bases and nucleosides. A number of methylated guanine or isoguanine derivatives have been reported from marine sponges1—4 and also from some ascidians.5—7 In our continuing search for new bioactive marine natural products, we have isolated a new purine base from a Philippine sponge, Zyzzya fuliginosa (CARTER, 1879), together with the known metabolites makaluvamines A (2), C (3), and K (4) as well as 4-hydroxyphenylacetic acid (5), methyl ester of 4-hydroxyphenylacetic acid (6), 4-hydroxyphenethyl alcohol (7), L-phenylalanine (8) and L-tryptophan (9). In this paper, we describe the isolation and characterization of the new compound, 3,7-dimethylguanine (1).

Compound 1 was isolated as an amorphous white powder. Both FAB-MS and electrospray ionization (ESI)-MS spectra of 1 contained a pseudomolecular ion peak at m/z 180 ([M+H]+). A molecular formula of C7H9N5O derived from high resolution electron impact (HR-EI)-MS analysis (m/z 179.0803, Δ = −0.4 mmu) indicated the presence of six degrees of unsaturation. The 1H-NMR spectrum of 1 in D2O (Table 1) was deceptively simple, containing two N-methyl singlets at δ 3.53 and 3.80 and one methine singlet at δ 7.85. In dimethyl sulfoxide (DMSO)-d6, the latter signal shifted to δ 8.14 and a very broad singlet appeared at δ 8.66, indicating the presence of exchangeable protons. The 13C-NMR spectrum of 1 displayed four quaternary carbons (δ 110.5, 149.4, 152.4, 154.6), one methine (δ 145.3) and two methyl carbons (δ 32.4, 34.5). These data, in conjunction with characteristic UV absorptions (λmax 216, 268 nm), were suggestive of a guanine or an isoguanine structure. The 3,7-methylation pattern within 1 was determined by extensive 1H–15N and 1H–13C heteronuclear multiple bond connectivity (HMBC) experiments, both optimized for 8Hz coupling (Table 1). The methyl singal at δ 3.53 showed strong correlations to δN 111.1 (N-3) and δC 149.4 (C-4), δC 152.4 (C-2), δC 110.5 (C-5) and a weak correlation to δC 145.3 (C-8) which positioned it at N-3. An 1H-15N HMBC cross peak was observed from the other methyl function (δN 3.80) to a nitrogen atom at δN 158.2 (N-7). Similar correlations obtained between the methine function (δ 7.85) and δN 158.2 (N-7) and δN 224.5 (N-9) suggested that the second methyl group resided on the imidazole ring, either on N-7 or N-9. Crucial long range 1H–13C couplings from this methyl group to C-8 (δ 145.3), C-5 (δ 110.5), and C-6 (δ 154.6) unambiguously located it at N-7. The latter correlation (N-7–CH2/C-6) was also indicative of a guanine ring. Distinction between the two possible structures, 3,7-dimethylguanine (1) and 3,7-dimethylisoguanine (10) was made by MS. The fragmentation patterns of methylated purines have been investigated.8,9 The initial expulsion of neutral cyanamide fragments consisting of N-1, C-2, and their attached substituents is a very characteristic fragmentation of the molecular ion peak of these compounds. Since guanines contain an imino substituent and isoguanines have an oxygen substitution at the C-2 position, they can be easily distinguished by EI-MS due to a one mass-unit difference.1,3) Thus, 1 showed a diagnostic ion at m/z 137.0595 due to loss of CH3N2 (m/z 42) via a retro-Diels–Alder pathway. The positive mode tandem ESI-
MS \((n=2)\) of 1 also yielded an abundant ion at \(m/z\) 138 \([M-\text{CH}_2\text{N}_2+\text{H}]^+\). The corresponding EI fragmentation of 3,7-dimethylisoguanine \((10)\), previously isolated from an Agelas sponge, afforded a peak at \(m/z\) 136.0709 \((M-43)\). Figure 1 illustrates the predicted EI-MS fragmentation patterns of 1 and 10, obtained from the High Chem Mass Frontier computer program. Final comparison of the NMR data of 1 with those of 10 further proved these two compounds to be positional isomers. To the best of our knowledge, this is the first report of 3,7-dimethylguanine (1) as a natural product. Compound 1 has been prepared by methylation of guanine\((11)\) or \(O^\prime\)-methylguanine.\(^{12}\)

The cytotoxicity of 3,7-dimethylguanine (1) was evaluated in human T-cell leukemia “IA2” (CCRF CEM) and human colon carcinoma (HTC-116) cells. No significant activity was observed at the highest concentrations tested (100 and 10 \(\mu\)g/ml, respectively).

The known metabolites 2—4 were identified by comparison of their spectral data [one- and two-dimensional (1D, 2D) NMR, HR-MS] with those published.\(^{13,14}\) The structures of compounds 5—9 were elucidated by 1D and 2D NMR and confirmed by comparison of the EI-MS fragmentation patterns with the NIST library of known compounds.

The marine sponge Zyzzya fuliginosa has been extensively investigated for makaluvamine type pyrroloquinoline alka-
loids, some of which are substituted with 4-hydroxyphenethyl and \(\delta\)-tryptophan at N-9.\(^{11,12}\) It is interesting that compounds 5—9 were also isolated in this study. This is the first report of the isolation of a modified purine base from the genus Zyzzya. Although 1 did not demonstrate bioactivity in our test systems, the production of 3,7-dimethylguanine in high yields in the sponge material might indicate an ecological role for this metabolite.

**Experimental**

UV spectra were recorded in \(H_2O\) on a Hewlett-Packard 8452A diode array spectrophotometer. IR spectra were recorded on a Jasco FTIR-420 spectrophotometer, using a polyethylene IR card. NMR spectra were obtained on a Varian instrument, operating at 500 MHz for 1H- and 125 MHz for 13C-NMR spectra. NMR spectra were recorded in \(D_2O\) (containing three drops of CD\(_3\)OD) and DMSO-\(d_6\) using the residual signal of nondeuterated solvents as an internal reference. The \(\text{H}^+\text{C}^\text{N}\) HMBC experiment was optimized for \(J=8\) Hz and chemical shifts were referenced indirectly to liquid ammonia using CH\(_3\)NO\(_2\) (10 \(\mu\)l in 450 \(\mu\)l \(D_2O\)) as an internal standard. Mass spectra were taken on Finnigan MAT 95 (EI-MS, FAB-MS) and Finnigan LCQ DECA ion trap (ESI-MS) spectrometers. The NIST library for EI-MS was used to compare the fragmentation patterns of the known compounds 5—9. Prediction of EI-MS fragmentation patterns for compounds 1 and 10 were made by High Chem Mass Frontier program (version 2.0). C-18 material (J. T. Baker, 40 \(\mu\)m, 275 Å) was used for flash chromatography. Sephadex LH-20 gel (25—200 \(\mu\)m bead size) was purchased from Sigma. HPLC separations were performed on a Rainin Dynamax 60 Å semi-preparative column (10×250 mm, 8 \(\mu\)m, 4 \(\mu\)l/min) using a Beckman 168 photodiode array system.

**Animal Material**

The specimen of Zyzzya fuliginosa (phylum Porifera, order Pococelolidera) was collected by SCUBA (\(\sim 13\) m) in Batanes, Philippines, in 1999. A voucher specimen (ZMA POR. 16426) has been deposited in the Zoölogisch Museum, University of Amsterdam.

**Extraction and Isolation**

Frozen sponge material was soaked in MeOH for 24h and the solution decanted. This procedure was repeated two more times. The combined MeOH extracts were dried \(\text{in vacuo}\) to give a reddish residue. This residue was dissolved in 10% \(H_2O\) in MeOH (200 ml) and partitioned against hexane (3×200 ml). The water content of the MeOH phase was then adjusted to 30% by adding 80 ml water before partitioning against CHCl\(_3\). During the initial partition, an interphase formed between the hexane and aqueous MeOH phases. An aliquot (100 mg) of this suspension was dried and repartitioned between \(H_2O\) and EtOAc. The CHCl\(_3\) layer was applied to a C-18 flash column employing a multistep MeOH gradient (0—100% MeOH) in water [0.05% trifluoroacetic acid (TFA)]. 3,7-Dimethylguanine (1, 24.0 mg) and makaluvamine A (2, 20 mg) were eluted with 20 and 40% MeOH, respectively. The CHCl\(_3\)-soluble material was further partitioned between EtOAc and \(H_2O\). The EtOAc layer was applied to a C-18 flash column employing a MeOH in water step gradient. Fractions containing 4-hydroxyphenethyl alcohol (7) were eluted with 20 and 30% MeOH. Compound 7 (5.1 mg) was further purified by C-18 HPLC using 20% MeOH/80% aqueous TFA.

**Table 1**: \(\text{H}^\text{1}(500\text{MHz})\) and \(\text{C}^\text{13}(125\text{MHz})\) Data of 3,7-Dimethylguanine (1) in \(D_2O\)

<table>
<thead>
<tr>
<th>Position</th>
<th>(\text{H}^\text{1}-\text{NMR})</th>
<th>(\text{H}^\text{1}-\text{NMR}\text{a,b))</th>
<th>(\text{C}^\text{13}-\text{NMR})</th>
<th>(\text{N}^\text{15}-\text{C}^\text{13}\text{HMBC correlations})</th>
<th>(\text{H}^\text{1},\text{C}^\text{13}\text{HMBC correlations})</th>
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<tbody>
<tr>
<td>2</td>
<td>152.4 s</td>
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<td>111.1</td>
<td>149.4 (C-4), 110.5 (C-5), 154.6 (C-6), 34.5 (N-7-Me)</td>
<td>158.2 (N-7)</td>
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<tr>
<td>3</td>
<td></td>
<td></td>
<td>149.4 s</td>
<td>154.6 (C-6), 34.5 (N-7-Me)</td>
<td>224.5 (N-9)</td>
</tr>
<tr>
<td>4</td>
<td>149.4 s</td>
<td>110.5 s</td>
<td>158.2</td>
<td>152.4 (C-2), 149.4 (C-4), 110.5 (C-5), 145.3 (C-8)</td>
<td>111.1 (N-3)</td>
</tr>
<tr>
<td>5</td>
<td>110.5 s</td>
<td>154.6 s</td>
<td>158.2 (N-7)</td>
<td>145.3 (C-8), 110.5 (C-5), 154.6 (C-6)</td>
<td>158.2 (N-7)</td>
</tr>
<tr>
<td>6</td>
<td>154.6 s</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>7</td>
<td>8.66 s</td>
<td>8.14 s</td>
<td>145.3 d</td>
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<tr>
<td>8</td>
<td>7.85 s</td>
<td>8.14 s</td>
<td>158.2</td>
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<td>111.1 (N-3)</td>
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<td>32.4 q</td>
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<td>N-7-Me</td>
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<td>3.89 s</td>
<td>34.5 q</td>
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</table>

\(a)\) Measured in DMSO-\(d_6\). \(b)\) A broad exchangeable signal was also observed at \(\delta\) 8.66. \(c)\) \(\text{N}^\text{15}\) chemical shifts were determined by \(\text{H}^\text{1},\text{C}^\text{13}\text{HMBC experiment (8 Hz).}\)
(0.05%). The aqueous MeOH layer was repeatedly triturated with MeOH to remove salts before partitioning between EtOAc and H2O. The EtOAc-soluble material was fractionated by C-18 flash CC using 0 to 100% aqueous (0.05% TFA) MeOH followed by a MeOH (0.1% TFA) rinse. Fractions eluting with 40 and 60% MeOH were combined and purified by HPLC [C-18 column, 30% MeOH/70% aqueous TFA (0.05%)] to yield 4-hydroxyphenylacetic acid (5, 5 mg) and methyl ester of 4-hydroxyphenylacetic acid (6, 6.5 mg). The water-soluble portion of the initial MeOH layer was partitioned against n-BuOH. The n-BuOH layer was also separated by C-18 flash CC using the same procedure as above. t-Phenylalanine (8) and makaluvamine C (3) were eluted with 30% MeOH in aqueous TFA (0.05%). Fractions which eluted with 40 and 60% MeOH were further purified by a combination of Sephadex LH-20 chromatography (MeOH with 0.1% TFA) and C-18 HPLC eluted with 30% MeOH in aqueous TFA (0.05%). Fractions which eluted same procedure as above. L-Phenylalanine (n, 3 mg) and additional makaluvamine A (2, 19 mg).

3,7-Dimethylguaninone (1): White amorphous solid; UV (H2O, CD3OD) max 3590—3350 cm−1, 1H-NMR (500 MHz, D2O and DMSO-d6) δ 1.45 (3H, br), 2.91 (3H, t), 2.36 (2H, t), 3.46 (2H, t), 3.77 (2H, t), 5.68 (1H, d), 7.04 (1H, d), 7.15 (1H, d), 7.26 (1H, d), 7.68 (1H, d), 13C-NMR (125 MHz, D2O and DMSO-d6) δ 1.45, 26.2, 30.9, 62.2, 117.1, 127.3, 133.6, 156.6, 167.3, 198.1, ESI-MS m/z 180 [M+H]+, 179 (M−CH3)+, 178 (M−CH2)+, 177 (M−CH)+, 163 (M−NH2)+, 147 (37), 93 (72), 75 (31). EI-MS m/z 138 [M−CH3]+, 137 (100), 136 (8), 109 (20), 82 (14), 67 (18), 55 (19). ESI-MS/MS (positive) m/z 138 [M−CH3]+, 137 (100), 136 (8), 109 (20), 82 (14), 67 (18), 55 (19). ESI-MS (positive) m/z 138 [M−CH3]+, 137 (100), 136 (8), 109 (20), 82 (14), 67 (18), 55 (19). ESI-MS (positive) m/z 138 [M−CH3]+, 137 (100), 136 (8), 109 (20), 82 (14), 67 (18), 55 (19).

Cytotoxicity Assays The cytotoxic potential of 3,7-dimethylguaninone (1) against CCRF CEM (human T-cell leukemia) was measured as described by Matsumoto et al.11 An MTT assay16 was used to determine the activity in human colon carcinoma (HCT-116) cells.

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References