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

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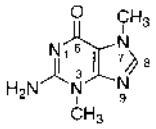
A new purine 3,7-dimethylguanine (1) has been isolated from the marine sponge *Zyzzya fuliginosa*, along with the known metabolites, makaluvamines A, C, K (2—4), 4-hydroxyphenylacetic acid (5), methyl ester of 4-hydroxyphenylacetic acid (6), 4-hydroxyphenethyl alcohol (7), L-phenylalanine (8) and L-tryptophan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

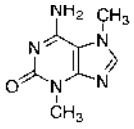
Key words marine sponge; *Zyzzya fuliginosa*; Poecilosclerida; 3,7-dimethylguanine; modified purine; makaluvamine A, C, K

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 sponges[1—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**).

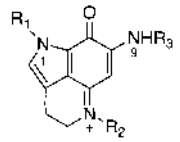
Compound **1** was isolated as an amorphous white powder. Both FAB-MS and electrospray ionization (ESI)-MS spectra of **1** contained a pseudomolecular ion peak at *m/z* 180 ($[M+H]^+$). A molecular formula of $C_7H_9N_5O$ derived from high resolution electron impact (HR-EI)-MS analysis (*m/z* 179.0803, $\Delta = -0.4$ mmu) indicated the presence of six degrees of unsaturation. The ^{1}H-NMR spectrum of **1** in D_2O (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)-d_6, 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 ^{13}C-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 (λ_{max} 216, 268 nm), were suggestive of a guanine or an isoguanine structure. The 3,7-methylation pattern within **1** was determined by extensive ^{1}H–^{15}N and ^{1}H–^{13}C heteronuclear multiple bond connectivity (HMBC) experiments, both optimized for 8 Hz coupling (Table 1). The methyl signal at δ_H 3.53 showed strong correlations to δ_N 111.1 (N-3) and δ_C 149.4 (C-4), δ_C 152.4 (C-2), δ_C 110.5 (C-5) and a weak correlation to δ_C 145.3 (C-8) which positioned it at N-3. An ^{1}H–^{15}N HMBC cross peak was observed from the other methyl function (δ_H 3.80) to a nitrogen atom at δ_N 158.2 (N-7). Similar correlations obtained between the methine function (δ 7.85) and δ_N 158.2 (N-7) and δ_N 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 ^{1}H–^{13}C 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-CH_3/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 CH_2N_2 (*m/z* 42) *via* a retro-Diels–Alder pathway. The positive mode tandem ESI-



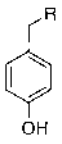
1



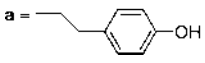
10



	R_1	R_2	R_3
2	CH_3	H	H
3	H	CH_3	H
4	CH_3	H	**a**



	R
5	COOH
6	$COOCH_3$
7	CH_2OH



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MS (n=2) of **1** also yielded an abundant ion at m/z 138 ($[M-CH_2N_2+H]^+$). 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).[10] 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^6-methylguanine.[12]

The cytotoxicity of 3,7-dimethylguanine (**1**) was evaluated in human T-cell leukemia "IA2" (CCRF CEM) and human colon carcinoma (HCT-116) cells. No significant activity was observed at the highest concentrations tested (100 and 10 μg/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 alkaloids, some of which are substituted with 4-hydroxyphenethyl and L-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.

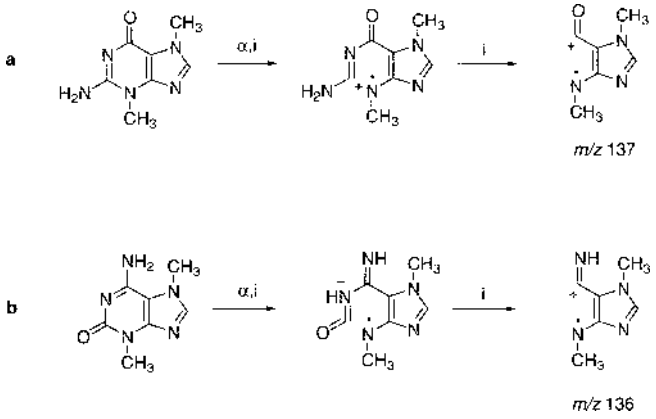

Fig. 1. Proposed EI-MS Fragmentation Pattern for Compounds **1** (a) and **10** (b)

α: α cleavage, i: inductive transfer of electrons.

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 ^{1}H- and 125 MHz for ^{13}C-NMR spectra. NMR spectra were recorded in D_2O (containing three drops of CD_3OD) and DMSO-d_6 using the residual signal of nondeuterated solvents as an internal reference. The ^{1}H–^{15}N HMBC experiment was optimized for J=8 Hz and chemical shifts were referenced indirectly to liquid ammonia using CH_3NO_2 (10 μl in 450 μl 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 μm, 275 Å) was used for flash chromatography. Sephadex LH-20 gel (25—200 μm bead size) was purchased from Sigma. HPLC separations were performed on a Rainin Dynamax 60 Å semi-preparative column (10×250 mm, 8 μm, 4 ml/min) using a Beckman 168 photodiode array system.

Animal Material The specimen of *Zyzzya fuliginosa* (phylum Porifera, order Poecilosclerida) was collected by SCUBA (−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 24 h and the solution decanted. This procedure was repeated two more times. The combined MeOH extracts were dried *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 H_2O phase was subjected to C-18 flash chromatography using 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% aqueous 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. ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) Data of 3,7-Dimethylguanine (**1**) in D_2O

Position	^{1}H-NMR	^{1}H-NMR[a,b]	^{13}C-NMR	^{15}N-NMR[c]	^{1}H–^{13}C HMBC correlations	^{1}H–^{15}N HMBC correlations[c]
2			152.4 s			
3				111.1		
4			149.4 s			
5			110.5 s			
6			154.6 s			
7				158.2		
8	7.85 s	8.14 s	145.3 d		149.4 (C-4), 110.5 (C-5), 154.6 (C-6), 34.5 (N-7-Me)	158.2 (N-7) 224.5 (N-9)
9				224.5		
N-3-Me	3.53 s	3.57 s	32.4 q		152.4 (C-2), 149.4 (C-4), 110.5 (C-5), 145.3 (C-8)	111.1 (N-3)
N-7-Me	3.80 s	3.89 s	34.5 q		145.3 (C-8), 110.5 (C-5), 154.6 (C-6)	158.2 (N-7)

a) Measured in DMSO-d_6. *b*) A broad exchangeable signal was also observed at δ 8.66. *c*) ^{15}N chemical shifts were determined by ^{1}H–^{15}N HMBC experiment (8 Hz).

(0.05%).

The aqueous MeOH layer was repeatedly triturated with MeOH to remove salts before partitioning between EtOAc and H_2O. 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. L-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 [MeOH : H_2O : TFA (20 : 80 : 0.05%)] to provide makaluvamine K (**4**, 4 mg), L-tryptophan (**9**, 3 mg) and additional makaluvamine A (**2**, 19 mg).

3,7-Dimethylguanine (**1**): White amorphous solid; UV (H_2O) λ_{max} (log ε) 216 (3.9), 268 (3.8) nm. IR (film, polyethylene card) ν_{max} 3500—3350 (broad), 2915, 2361, 1758, 1621, 1465, 1258 cm^{-1}. EI-MS *m/z* 179 $[M]^+$ (100), 137 (8), 109 (20), 82 (14), 67 (18), 55 (19). ESI-MS *m/z* 359 $[2M+H]^+$, 180 $[M+H]^+$, 163 $[M-NH_3+H]^+$. ESI-MS/MS (positive) *m/z* 138 $[M-CH_2N_2+H]^+$. FAB-MS (positive) 180 $[M+H]^+$, 149 (37), 93 (72), 75 (31). HR-EI-MS 179.0803 (Calcd for $C_7H_9N_5O$, 179.0807); 137.0595 (Calcd for $C_6H_7N_3O$, 137.0589), 109.0637 (Calcd for $C_5H_7N_3$, 109.0640). ^{1}H-NMR (500 MHz, D_2O and DMSO-d_6): Table 1; ^{13}C-NMR (125 MHz, D_2O with three drops of CD_3OD): Table 1.

Cytotoxicity Assays The cytotoxic potential of 3,7-dimethylguanine (**1**) against CCRF CEM (human T-cell leukemia) was measured as described by Matsumoto *et al*.[15] An MTT assay[16] was used to determine the activity in human colon carcinoma (HCT-116) cells.

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