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

Deniz TASDEMIR,*[,a,b] Gina C. MANGALINDAN,[c] Gisela P. CONCEPCIÓN,[c] Mary Kay HARPER,[a] and Chris M. IRELAND*[,a]

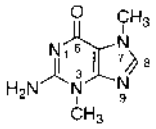
Department of Medicinal Chemistry, University of Utah,[a] Salt Lake City, Utah 84112, U.S.A., Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University,[b] TR-06100 Ankara, Turkey, and Marine Science Institute, University of the Philippines,[c] Diliman, Quezon City 1101, Philippines. Received April 5, 2001; accepted June 18, 2001

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).

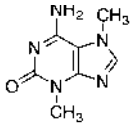
Key words marine sponge; *Zyzzya fuliginosa*; Poecilosclerida; 3,7-dimethylguanine; modified purine; makaluvamine A, C, K

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**).

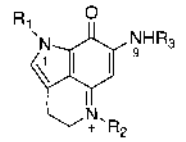
Compound **1** was isolated as an amorphous white powder. Both FAB-MS and electrospray ionization (ESI)-MS spectra of **1** contained a pseudomolecular ion peak at *m/z* 180 ($[M+H]^+$). A molecular formula of $C_7H_9N_5O$ derived from high resolution electron impact (HR-EI)-MS analysis (*m/z* 179.0803, $\Delta = -0.4$ 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-



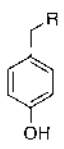
1



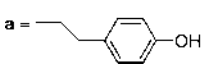
10



	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



* To whom correspondence should be addressed. e-mail: cireland@deans.pharm.utah.edu

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.

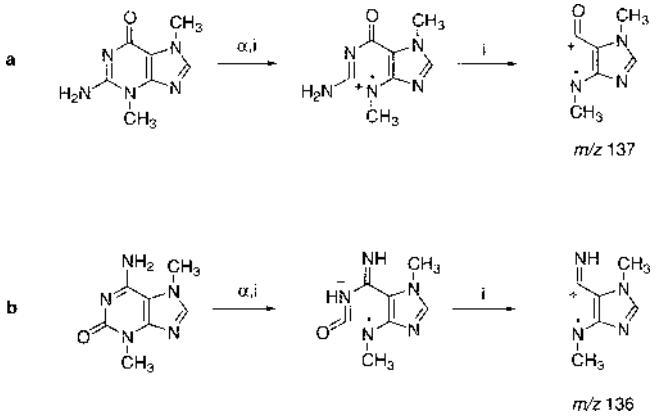


Fig. 1. Proposed EI-MS Fragmentation Pattern for Compounds **1** (a) and **10** (b)

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.

Acknowledgements This work was supported by NIH grant CA 36622 (C.M.I.). Funding for the Varian Unity 500 spectrometer was provided by NIH grant RR06262. We are grateful to Dr. E. Rachlin and Dr. V. Nanayakkara for recording mass spectra, T. S. Bugni and H. D. Fain for performing bioassays, and R. M. Van Wagoner for optimizing the ^{1}H–^{15}N HMBC experiment. We also thank Dr. R. W. M. van Soest, University of Amsterdam, for assistance with identification of the sponge. Deniz Tasdemir appreciates the leave given her by Hacettepe University, Department of Pharmacognosy, Ankara, Turkey.

References

1) Perry N. B., Blunt J. W., Munro M. H. G., *J. Nat. Prod*., **50**, 307—308 (1987).
2) Yagi H., Matsunaga S., Fusetani N., *J. Nat. Prod*., **57**, 837—838 (1994).
3) Mitchell S. S., Whitehill A. B., Trapido-Rosenthal H. G., Ireland C. M., *J. Nat. Prod.*, **60**, 727—728 (1997).
4) Capon R. J., Rooney F., Murray L. M., *J. Nat. Prod*., **63**, 261—262 (2000).
5) Lindsay B. S., Battershill C. N., Copp B. R., *J. Nat. Prod*., **62**, 638—639 (1999).
6) Lindsay B. S., Almeida A. M. P., Smith C. J., Berlinck R. G. S., da Rocha R. M., Ireland C. M., *J. Nat. Prod*., **62**, 1573—1575 (1999).
7) Copp B. R., Wassik C. M., Lambert G., Page M. J., *J. Nat. Prod*., **63**, 1168—1169 (2000).
8) Rice J. M., Dudek G. O., *J. Am. Chem. Soc*., **89**, 2719—2725 (1967).
9) Cook A. F., Bartlett R. T., Gregson R. P., Quinn R. J., *J. Org. Chem*., **45**, 4020—4025 (1980).
10) Cafieri F., Fattorusso E., Mangoni A., Taglialatela-Scafati O., *Tetrahedron Lett*., **36**, 7893—7896 (1995).
11) Yamauchi K., Tanabe T., Kinoshita M., *J. Org. Chem*., **41**, 3691—3696 (1976).
12) Kohda K., Baba K., Kawazoe Y., *Tetrahedron Lett*., **28**, 6285—6288 (1987).
13) Radisky D. C., Radisky E. S., Barrows L. R., Copp B. R., Ireland C. M., *J. Am. Chem. Soc*., **115**, 1632—1638 (1993).
14) Schmidt E. W., Harper M. K., Faulkner D. J., *J. Nat. Prod*., **58**, 1861—1867 (1995).
15) Matsumoto S. S., Haughey H. M., Schmehl D. M., Venables D. A., Ireland C. M., Holden J. A., Barrows L. R., *Anti-Cancer Drugs*, **10**, 39—45 (1999).
16) Park J. G., Kramer B. S., Steinberg S. M., Carmichael J., Collins J. M., Minna J. D., Gazdar A. F., *Cancer Res*., **47**, 5875—5879 (1987).