

1-ETHYL-3-METHYLISOGUANOSINE, A DORIDOSINE DERIVATIVE

Yong Hae Kim* and Sang Joa Jee

Department of Chemistry, Korea Advanced Institute of Science and Technology

P.O. Box 150 Chong-yang-ni, Seoul 131, Korea

Harry S. Mosher

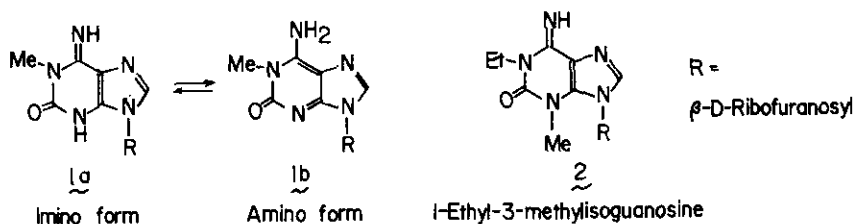
Department of Chemistry, Stanford University

Stanford, California 94305, U.S.A.

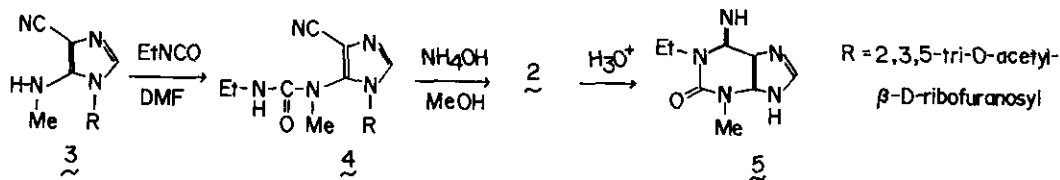
Abstract - 1-Ethyl-6-imino-3-methyl-2-oxo-9- β -D-ribofuranosylpurine (**2**, 1-ethyl-3-methylisoguanosine) has been synthesized from 5-(methylamino)-1- β -D-ribofuranosylimidazole-4-carbonitrile. This modified purine, by virtue of substituents on both N¹ and N³ nitrogens is frozen in the 6-imino form; its uv spectrum is compared to that of analogs which can exist in the tautomeric 6-amino \rightleftharpoons 6-imino forms. It is relatively stable to hydrolysis of the 9-ribosyl bond in contrast to other N³-substituted nucleosides

Recently 1-methylisoguanosine (**1**, doridosine,¹ 6-amino-1-methyl-2-oxo-9- β -D-ribofuranosylpurine) was independently isolated from three different marine animals (from a dorid nudibranch, *Anisodoris nobilis*, from the coast of California;¹ from a sponge, *Tedania digitata*, from the eastern coast of Australia;² and from a coral, *Madacis mirabilis*, from the Caribbean Sea³). Before these discoveries, N¹-substituted isoguanosines were conspicuously missing, either as naturally occurring or synthetic nucleosides. 1-Methylisoguanosine (**1**) causes sustained lowering of blood pressure in experimental animals.^{1,4} The prolonged activity presumably is made possible because it is not destroyed *in vivo* by adenosine deaminase. Among the rare modified nucleosides, the only ones which are substituted at both N¹ and N³ seem to be the unusual γ nucleosides, (wyosine,⁵ wybutosine⁶ and wybutoxosine⁷) which have a fused imidazole ring between C² and N¹ and are also methylated at N³. A characteristic of these N¹,N³-disubstituted nucleosides,¹¹ and of 3-substituted¹⁴⁻¹⁷ nucleosides in general, seems to be their unusually high susceptibility to acid hydrolysis of the glycosidic bond. The tautomeric structure of a nucleoside such as **1a** \rightleftharpoons **1b** under physiological conditions is an important factor in determining its intermolecular interactions via hydrogen bonding. In our report on the isolation of 1-methylisoguanosine **1** we represented it as the 6-imino form⁸ (**1a**), while Cook et al.² reported it as the 6-amino form (**1b**). The 6-amino form was convincingly supported by ¹³C nmr spin-lattice relaxation studies⁹ in dimethylsulfoxide (DMSO) solvent. We sought to obtain independent evidence in neutral aqueous solution via the uv spectra of model

compounds. We have synthesized 1-ethyl-3-methylisoguanosine (2) which must exist in the 6-imino form. We hoped that a comparison of the uv spectra of 1 and 2 might clarify this tautomerism question. A comparable study¹⁰ of the more complex tautomerism of isoguanosine (analogous to 1a \rightleftharpoons 1b but lacking the N¹-methyl group) favored the 6-amino form but was not conclusive, in part because of a lack of a suitable N¹,N³-disubstituted model compound.¹⁰ The X-ray crystal structure of the



hydrochloride salt of 9-methylisoguanine¹¹ does not answer the question of the tautomeric form in neutral solution since the protonated forms of 1a and 1b are resonance hybrids. Reaction of 3¹² with ethyl isocyanate in N,N-dimethylformamide (100°C, 6 h) followed by treatment with ammonium hydroxide in methanol at 0°C yielded 2 [76%, mp 165-167°C, m/e, 326 (M+1) uv $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ (pH 2), 282 nm (ϵ 5.4×10^3); $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ (pH 6), 249 (5.0×10^3), 292 (4.7×10^3); $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ (pH 10), 283 (5.6×10^3); pKa, 4.7; ¹H nmr (DMSO-d₆) 1.15 (3 H, t, CH₃), 3.2 (2 H, q, N¹-CH₂), 3.6 (3 H, s, N³-CH₃) 4.1 (2 H, d, -O-CH₂), 5.7 (1H, d, C_{1'}-H), 7.8 (1 H, s, C₈-H)]. The structure of 2 was assigned on the basis of its method of synthesis and its ¹H nmr, uv and mass spectral properties.



The ¹H nmr spectrum of the 6-imino form of 2 shows an equal intensity triplet (δ 6.60, $J_{14\text{N}-1\text{H}}$, 49 Hz) which agrees with the data^{1,8)} from 1a: namely 1 can exist in the imino form (1a) in DMSO. The uv spectrum of 1-ethyl-3-methylisoguanosine (2) in aqueous media at pH 6 is compared with the spectra of 1-methylisoguanosine (1), 1-ethylisoguanosine,¹³ N⁶,N⁶-9-trimethylisoguanine and the parent nucleoside, isoguanosine in Table I. These five purine derivatives have almost identical spectral curves with three common absorption maxima at approximately 210, 250, and 293 nm. Superficially one might assume that they all had the same basic chromophore. However, N⁶,N⁶-9-trimethyl-

isoguanine has the 6-amino structure analogous to 1b (it cannot exist in the 6-imino form) while the N¹,N³-disubstituted 2 has the 6-imino form analogous to 1a (it cannot exist in the amino form in neutral solution). Unfortunately, since the uv spectra of these two model compounds with different nuclear structures are essentially the same, no conclusion can be drawn concerning the tautomeric form of 1 in neutral solution based on uv spectra.

Table I. UV Spectra of Isoguanosine and Its Derivatives in Neutral Aqueous Solution.

Isoguanosine Derivative	λ_{\max} , nm ^a	$\epsilon \times 10^3$ ^b	Reference
Isoguanosine	293, 248, 208	11, 9, 23	10
1-Me-isoguanosine (<u>1</u>)	292, 248, --- ^c 294, 250, --- ^c	9, 7, -- ^c 11, 9, -- ^c	1 2
1-Et-isoguanosine (<u>5</u>)	293, 249, 209	10, 8, 18	13
1-Et-3-me-isoguanosine (<u>2</u>)	292, 249, 211	5, 5, 19	d
N ⁶ ,N ⁶ ,9-Trimethylisoguanine	295, 249, 212	11, 8, 17	10

a) Some of the values from the literature are taken from figures and are ± 2 nm.

b) Rounded to the nearest whole number. c) Not reported below 220 nm. d) This study.

The uv spectra of isoguanosine and 9-methylisoguanine in dioxane are strongly influenced by addition of water;¹⁰ however, under the same conditions, that of 2 is virtually unchanged (λ_{\max} 303, $\epsilon = 7 \times 10^3$), while that of N¹-ethylisoguanosine^{13a} shows only a slight shift in wavelength but no change in intensity (99:1 dioxane: H₂O, λ_{\max} 308; 93:7 dioxane: H₂O, λ_{\max} 302, $\epsilon = 10 \times 10^3$). We conclude that these spectral differences are caused by hydrogen bonding in the first two compounds and that such bonding has been blocked by N¹ and N³ substitution in the latter two compounds. The glucosidic bonds of N³-substituted nucleosides (3-methyladenosines,¹⁴ 3-methylguanosine,¹⁵ 3-methylxanthosine,¹⁶ and 3-methylisoguanosine¹⁷ are unusually susceptible to acid catalyzed hydrolysis; for example, 3-methyladenosine¹⁴ hydrolyzes one-thousand times faster than adenosine itself. However, we have found the N¹,N³-disubstituted nucleoside 2 is reasonably stable, hydrolyzing to 1-ethyl-3-methylisoguanine (5) at a rate only slightly faster than that of adenosine¹⁴. Pseudo-first order hydrolysis rate constants for 2 were obtained by measuring the decreasing peak area of 2 and increasing peak area for 5 in aliquots removed at intervals and analyzed by HPLC (μ -Bondapak C-18; H₂O:MeOH, 80:20 retention time for 2, 6.8 min; for 5, 8.2 min; uv detector 280 nm). The rate constant for 2 ($1.6 \times 10^{-4} \text{ min}^{-1}$, 0.1 HCl, 25°C; $3.2 \times 10^{-3} \text{ min}^{-1}$,

2.0 N HCl, 25°C; $1.4 \times 10^{-3} \text{ min}^{-1}$, 0.1 N HCl, 50°C) is about 1/105 that published for 3-methylisoguanosine,¹⁷ 1/6000 that for 3-methylguanosine¹⁵ and about 1/250 that for 3-methyladenosine.¹⁴

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