1-ETHYL-3-METHYLISOGUANOSINE, A DORIDOSINE DERIVATIVE

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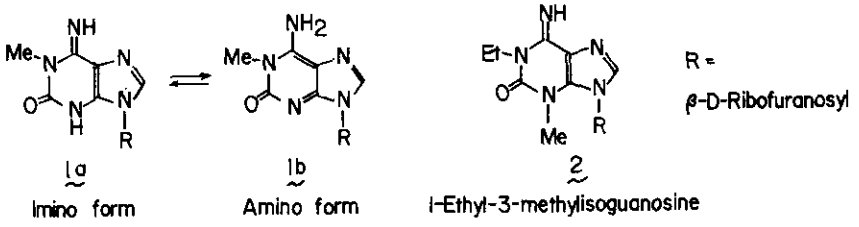
Abstract - 1-Ethyl-6-imino-3-methyl-2-oxo-9-β-D-ribofuranosylpurine (2, 1-ethyl-3-methylisoguanosine) has been synthesized from 5-(methylamino)-1-β-D-ribofuranosylimidazole-4-carbonitrile. This modified purine, by virtue of substituents on both N^1 and N^3 nitrogens is frozen in the 6-imino form; its uv spectrum is compared to that of analogs which can exist in the tautomeric 6-amino ⇌ 6-imino forms. It is relatively stable to hydrolysis of the 9-ribosyl bond in contrast to other N^3-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 Sea[3]). Before these discoveries, N^1-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 N^1 and N^3 seem to be the unusual Y nucleosides, (wyosine,[5] wybutosine[6] and wybutoxosine[7]) which have a fused imidazole ring between C^2 and N^1 and are also methylated at N^3. A characteristic of these N^1,N^3-disubstituted nucleosides,[11] and of 3-substituted[14-17] 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 ⇌ 1b under physiological conditions is in 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 form[8] (1a), while Cook et al.[2] reported it as the 6-amino form (1b). The 6-amino form was convincingly supported by ^{13}C nmr spin-lattice relaxation studies[9] 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 study[10] of the more complex tautomerism of isoguanosine (analogous to 1a ⇌ 1b but lacking the N^1-methyl group) favored the 6-amino form but was not conclusive, in part because of a lack of a suitable N^1,N^3-disubstituted model compound.[10] The X-ray crystal structure of the

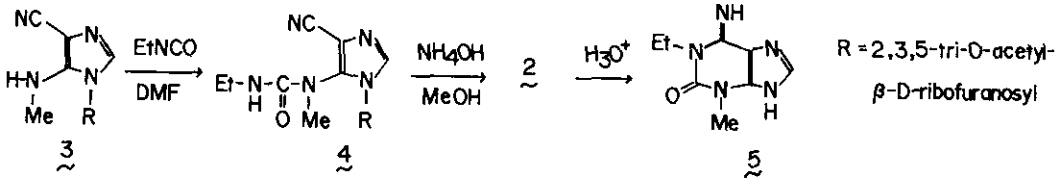


1a
Imino form

1b
Amino form

2
1-Ethyl-3-methylisoguanosine

hydrochloride salt of 9-methylisoguanine[11] 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 3[12] 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 $\lambda_{max}^{H_2O}$ (pH 2), 282 nm (ε 5.4 x 10^3); $\lambda_{max}^{H_2O}$ (pH 6), 249 (5.0 x 10^3), 292 (4.7 x 10^3); $\lambda_{max}^{H_2O}$ (pH 10), 283 (5.6 x 10^3); pKa, 4.7; ^{1}H nmr (DMSO-d_6) 1.15 (3 H, t, CH_3), 3.2 (2 H, q, N^1 - CH_2), 3.6 (3 H, s, N^3 - CH_3) 4.1 (2 H, d, -O-CH_2), 5.7 (1H, d, C_1'-H), 7.8 (1 H, s, C_8 - H)]. The structure of 2 was assigned on the basis of its method of synthesis and its ^{1}H nmr, uv and mass spectral properties.



3

4

2

5

The ^{1}H nmr spectrum of the 6-imino form of 2 shows an equal intensity triplet (δ6.60, $J_{^{14}N-^1H}$, 49 Hz) which agrees with the data[1,8] from 1a: namely 1 can exist in the imino from (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] N^6,N^6-9-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, N^6,N^6-9-trimethyl-

isoguanine has the 6-amino structure analogous to 1b (it cannot exist in the 6-imino form) while the N^1,N^3-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 (1)	292, 248, ---[c] 294, 250, ---[c]	9, 7, --[c] 11, 9, --[c]	1 2
1-Et-isoguanosine (6)	293, 249, 209	10, 8, 18	13
1-Et-3-me-isoguanosine (2)	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, ϵ 7 x 10^3), while that of N^1-ethylisoguanosine[13a] shows only a slight shift in wavelength but no change in intensity (99:1 dioxane: H_2O, λ_{max} 308; 93:7 dioxane: H_2O, λ_{max} 302, ϵ = 10 x 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 N^1 and N^3 substitution in the latter two compounds. The glucosidic bonds of N^3-substituted nucleosides (3-methyladenosines,[14] 3-methylguanosine,[15] 3-methylxanthosine,[16] and 3-methylisoguanosine[17] are unusually susceptible to acid catalyzed hydrolysis; for example, 3-methyladenosine[14] hydrolyzes one-thousand times faster than adenosine itself. However, we have found the N^1,N^3-disubstituted nucleoside 2 is reasonably stable, hydrolyzing to 1-ethyl-3-methylisoguanine (5) at a rate only slightly faster than that of adenosine[14]: 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; H_2O: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 x 10^{-4} min^{-1}, 0.1 HCl, 25°C; 3.2 x 10^{-3} min^{-1},

2.0 N HCl, 25°C; 1.4 x 10^{-3} min^{-1}, 0.1 N HCl, 50°C) is about 1/105 that published for 3-methylisoguanosine,[17] 1/6000 that for 3-methylguanosine[15] and about 1/250 that for 3-methyladensoine.[14]

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