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7-(β-D-RIBOFURANOSYL)IMIDAZO[1,2-c]PYRROLO[2,3-d]PYRIMIDINE (= 1,N^6-ETHENO-7-DEAZAADENOSINE). PROTONATION, QUATERNIZATION, AND FLUORESCENCE PROPERTIES

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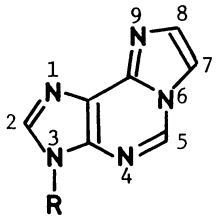
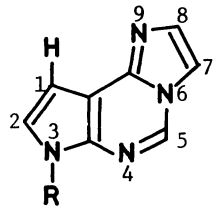
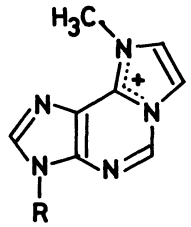
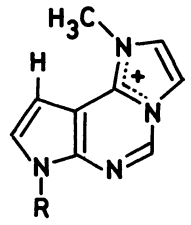
Tendency of protonated 1,N^6-ethenoadenosine to undergo deactivation of the fluorescence at 77 K is greatly decreased by the replacement of an N-1 nitrogen atom by a CH group. This phenomenon is discussed with reference to intramolecular proton transfer between N-1 and N-9 of the protonated 1,N^6-ethenoadenosine in the excited state even at 77 K.

Earlier work[1-3] on the fluorescence spectra of 1,N^6-ethenoadenosine (εAdo) and its quaternized methyl derivatives has now been extended to N(1)-deaza-εAdo (= 1,N^6-ethenotubercidin, i.e., compound named in the title), which differs from εAdo only in lacking one (N-1) of the nitrogen atoms in the ring, in order to see how N-1 atom in protonated εAdo ($H\varepsilon Ado^+$) affected fluorescence intensity in the protonated form at 77 K. At 293 K maximal fluorescence of N(9)-methyl-εAdo$^+$ ($m^9\varepsilon Ado^+$) occurs at 365 nm[2,3]. Fluorescence of $m^9\varepsilon Ado^+$ was found to increase strikingly and quantum yield as high as 0.89 was attained at 77 K while the quantum yield of $m^9\varepsilon Ado^+$ at 293 K was only 0.11. At 293 K fluorescence of protonated εAdo is not observed. However, as the ethylene glycol/water (EGW) solution of the protonated εAdo was cooled to 77 K, an emission originating from the excited $H\varepsilon Ado^+$ was detected at 334 nm with $\phi_F = 0.23$. As an explanation of this temperature effect, we considered that cooling might retard the rate required for prototropic collisional deactivation[3], which is significant at room temperature. Although the fluorescence behavior of $H\varepsilon Ado^+$ parallels that of $m^9\varepsilon Ado^+$, a comparison of the fluorescence intensity of $H\varepsilon Ado^+$ and $m^9\varepsilon Ado^+$ at 77 K strongly suggests that the diminished fluorescence intensity of $H\varepsilon Ado^+$ ($\phi_F = 0.23$), relative to $m^9\varepsilon Ado^+$ ($\phi_F = 0.89$), may be due to intramolecular proton transfer between N-1 and N-9 in $H\varepsilon Ado^+$ even at 77 K.

Table 1. Fluorescence Emission Characteristics of εAdo, N(1)-deaza-εAdo, and Related Quaternized Derivatives at 293 and 77 K[a]

	Compound[b]	Temp., K	Medium[c] (pH)	$\lambda_{max}^{emission}$, nm[d]	Quantum Yield (ϕ_F)
1		293	W or EGW (7.0)	406	0.56[e]
		77	EGW (7.0)	377	0.65
		77	EGW (2.0)	334	0.23
2		293	EGW (7.3)	406	0.71
		77	EGW (9.0)	379	ca.0.8
		77	EGW (2 ~ 6)	336	0.95
3		293	W or EGW (7.0)	365[f]	0.11[g]
		77	EGW (7.0)	337	0.89
4		293	EGW (7.5)	365	0.037
		77	EGW (3 ~ 7)	340	0.50

a. Data for εAdo and m^9εAdo$^+$ are taken from ref. 3.

b. R denotes β-D-ribofuranosyl residue.

c. W, 60 mM phosphate buffer; EGW, mixture of ethylene glycol and 60 mM phosphate buffer (1v/1v), and pH of EGW was adjusted by addition of HCl.

d. Fluorescence emission spectra were measured by excitation at 294 or 296 nm for εAdo and m^9εAdo$^+$, and at 280 nm for N(1)-deaza-εAdo and N(1)-deaza-m^9εAdo$^+$; Corrected values.

e. Value determined by Leonard et al. (ref. 5).

f. Reported, 358 nm (ref. 6).

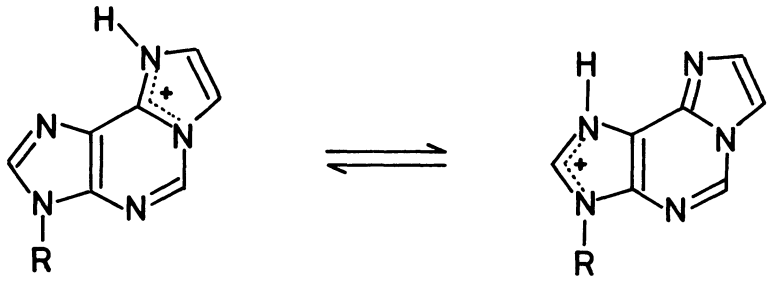
g. Reported, 0.06 (ref. 6).

As no relevant data for the effect of N-1 on the quenching of $H\varepsilon Ado^+$ at 77 K could be found in the literature, model substance was prepared: N(7)-deazaadenosine (= tubercidin) with chloroacetaldehyde at 30° for 18 h was reported[4] to give N(1)-deaza-εAdo (see **2** in Table 1 for numbering system). With dimethylsulfate N(1)-deaza-εAdo at pH 6 undergoes methylation on N-9 to give N(1)-deaza-N(9)-methyl-εAdo^+ (N(1)-deaza-$m^9\varepsilon Ado^+$). Similar treatment[1,3] of εAdo gave a mixture of two methyl derivatives, $m^1\varepsilon Ado^+$ and $m^9\varepsilon Ado^+$. Obviously, only a single methylation product was formed from N(1)-deaza-εAdo, namely, N(1)-deaza-$m^9\varepsilon Ado^+$ whose structure was confirmed by ^1H NMR spectroscopy [NMR in D_2O at pH 6.9 δ4.22(s, 3, CH_3), δ6.46(d, 1, J = 5.8 Hz, H1'), δ7.28(d, 1, J = 3.4 Hz, H1), δ7.88(d, 1, J = 2.7 Hz, H2), δ9.18(s, 1, H5), δ7.81(d, 1, J = 1.0 Hz, H7), δ8.16(d, 1, J = 1.0 Hz, H8); UV at pH 5.5 λ_{max} 244 nm(ε36,000), 283 nm(ε10,400)]. Protonation of εAdo has been rigorously proved[1,3] to occur mainly at N-9. The implication of N-9 as the most basic site is also upheld by the close similarity of the spectrum of N(1)-deaza-$H\varepsilon Ado^+$ to that of N(1)-deaza-$m^9\varepsilon Ado^+$. Concerning the ionization constants, εAdo (pK_a = 4.10)[7] is made more basic by replacement of N-1 by CH. By this means, N(1)-deaza-εAdo reaches pK_a 5.49. This shows that a hydrogen atom attached on C-1 does not interfere in the protonation of N(1)-deaza-εAdo at N-9 sterically.

The protonated εAdo and N(1)-deaza-εAdo have been shown to be practically non-fluorescent at room temperature. From the acidity dependence of fluorescence intensity for εAdo and N(1)-deaza-εAdo we found that the decrease in emission of the free base form occurred at lower acidities than the appearance of their protonated species[8]. Quenching of fluorescence in acidic solutions is quite common and is simply due to the radiationless formation of an encounter complex between the excited state molecules and H_3O^+. As observed previously[3] for $H^9\varepsilon Ado^+$, we measured the fluorescence spectra of N(1)-deaza-$H^9\varepsilon Ado^+$ and N(1)-deaza-$m^9\varepsilon Ado^+$ at 77 K, and compared them with those of $H^9\varepsilon Ado^+$ and $m^9\varepsilon Ado^+$ at 77 K. The results for N(1)-deaza-εAdo and its quaternized derivative are summarized in Table 1, where data for the corresponding available εAdo's are included for comparison.

By far the most remarkable property of N(1)-deaza-εAdo is that N(1)-deaza-$H^9\varepsilon Ado^+$ has an anomalously high fluorescence intensity at 77 K (Φ_F = 0.95), but $H^9\varepsilon Ado^+$ has not (Φ_F = 0.23). This marked difference in the fluorescence intensities of N(1)-deaza-$H^9\varepsilon Ado^+$ and $H^9\varepsilon Ado^+$ at 77 K indicates that the latter tends to undergo partial deactivation at 77 K. To account for this difference, the lower fluorescence intensity found for $H^9\varepsilon Ado^+$ entails partial deactivation by intramolecular proton transfer

between N-1 and N-9 even at 77 K:



In confirmation of this explanation, N(1)-deaza-$H^9\varepsilon Ado^+$ (ϕ_F = 0.95), with no acceptable nitrogen atom, and $m^9\varepsilon Ado^+$ (ϕ_F = 0.89), with no transferable proton, have been found to have practically identical fluorescence intensity, but not to behave similarly as $H^9\varepsilon Ado^+$ at 77 K.

Finally, the fluorescence intensities of $m^9\varepsilon Ado^+$ and N(1)-deaza-$m^9\varepsilon Ado^+$ are compared. On lowering temperature from 293 to 77 K the increase in fluorescence intensity of N(1)-deaza-$m^9\varepsilon Ado^+$ parallels that for $m^9\varepsilon Ado^+$. However, the diminished fluorescence intensity of the former relative to the latter may be associated with a slight bending of the aromatic ring(s) out of the plane because of the crowding of H-1 and CH_3 groups in N(1)-deaza-$m^9\varepsilon Ado^+$.

References

1) T. Kuramochi and Y. Inoue, *Chem. Lett.*, 1978, 1363.
2) T. Kuramochi, Y. Inoue, and K. Imakubo, *Chem. Lett.*, 1979, 115.
3) Y. Inoue, T. Kuramochi, and K. Imakubo, *Biopolymers*, 18, 2175 (1979).
4) K. H. Dchram and L. B. Townsend, *Tetrahedron Lett.*, 1974, 1345.
5) J. A. Secrist, III, J. R. Barrio, N. J. Leonard, and G. Weber, *Biochemistry*, 11, 3499 (1972).
6) P. D. Sattsangi, J. R. Barrio, and N. J. Leonard, *J. Am. Chem. Soc.*, 102, 770(1980).
7) T. Kuramochi and Y. Inoue, *Nucleic Acids Res.* Special Publication No. 3, s67 (1977).
8) S. Takahashi, Y. Nishimura, M. Tsuboi, T. Kuramochi, and Y. Inoue, *J. Chem. Phys.*, in press.

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