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FLUORESCENCE NATURE OF PROTONATED 1,N^6-ETHENOADENOSINE AND RELATED QUATERNIZED DERIVATIVES AT 293 AND 77°K

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Fluorescence properties of 1,N^6-ethenoadenosine(εAdo) at neutral and acidic pH's and its quaternized derivatives, 1-methyl- and 9-methyl-3-β-D-ribofuranosylimidazo[2,1-i]purinium cations(abbreviated as m^1εAdo$^+$ and m^9εAdo$^+$, respectively) have been examined at 293 and 77°K. We have found that m^1εAdo$^+$ and m^9εAdo$^+$ are both highly fluorescent at 293 and 77°K.

In previous publications[1,2] chloroacetaldehyde was introduced as a reagent for the selective modification of adenosine and cytidine to form fluorescent derivatives under mild conditions. These fluorescent moieties are especially useful in that fluorescence produces numerous parameters—emission wavelength, lifetime, quantum yield, and polarization. For this the chloroacetaldehyde modification reaction has been widely used in biological systems[3-14] such as coenzymes, NAD^+ and FAD^+, adenosine 3',5'-cyclic phosphate, and adenosine triphosphate.

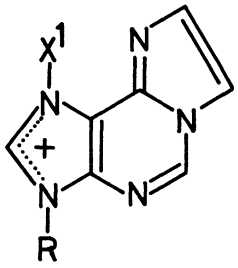
However, in connection with the controversy about the species responsible for the fluorescence of εAdo[9,15,16], a question which must be answered is whether the protonated εAdo is fluorescent or not: Penzer[15] has concluded that the protonated form of the εAdo base fluoresces whereas Leonard et al.[16] have suggested that protonated εAdo is nonfluorescent. We have thus investigated the fluorescence of εAdo with particular emphasis on the effect of protonation, and the studies described in the present communication provide a secure basis for describing the effect of protonation on the nature of fluorescence of εAdo.

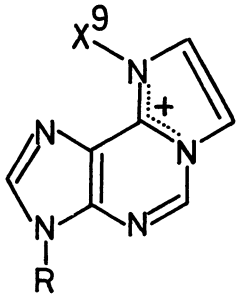
εAdo was prepared as described in the literature[2,9], examined chromatographically and spectrophotometrically and purified for analysis. εAdo and dimethylsulfate at room temperature for 12 hours yielded a mixture of the quaternized products. The conditions for methylation must be at pH 5-6 to prevent both loss of D-ribose by acid-hydrolysis and ring-opening of the methylated εAdo. The mixture was taken to dryness under reduced pressure at room temperature and the residue was first chromatographed on Dowex 50Wx2(NH_4^+ form) to separate methylated products from other materials including unreacted εAdo. The salt was removed by gel filtration using Sephadex G-10.

A mixture of $m^1\varepsilon Ado^+$ and $m^9\varepsilon Ado^+$ was then subjected to the twice repeated thin-layer chromatography on Eastman Chromagram cellulose sheets using n-butanol/ethanol/0.05% acetic acid(80/10/25) as developing agent. The two separated spots were marked by pencil in ultaviolet light and eluted with water. The structure of the products are based on 270 MHz ^1H NMR evidence: $m^1\varepsilon Ado^+$[D_2O, DSS] 9.43(s, 1, H5), 8.24(d, 1, J_{78} = 1.7 Hz, H7), 7.81(d, 1, J_{78} = 1.7 Hz, H8), 4.43(s, 3, CH_3), 6.43(d, 1, $J_{1'2'}$ = 3.3 Hz, H1');$m^9\varepsilon Ado^+$[D_2O, DSS] 8.82(s, 1, H2), 9.40(s, 1, H5), 8.31(d, 1, J_{78} = 2.2 Hz, H7), 7.94(d, 1, J_{78} = 2.2 Hz, H8), 4.36(s, 3, CH_3), 6.34(d, 1, $J_{1'2'}$ = 4.9 Hz, H1').

The emission and excitation spectra at 20°C and at liquid nitrogen temperature (77°K) were obtained. Fluorescence experimental method and apparatus used were similar to those described previously[17]. The quantum yield of each compound was determined from the observed absorbance at 276 nm and the area of the corrected emission spectrum. The results are all based on εAdo as standard with ϕ_F of 0.56[9].

Detailed knowledge of preferred protonation site(s) in εAdo in aqueous solution is necessary if we are to interpret properly the results of studies of the fluorescence titration of εAdo with acid. A preliminary account of protonation and quaternization has been given[18,19]. Arguing from ultraviolet absorption data for protonated εAdo and the two quaternized reference compounds, $m^1\varepsilon Ado^+$ and $m^9\varepsilon Ado^+$, at 25°C we have shown that the results are consistent with a protonation scheme for εAdo involving two basic sites, N1[the percentage of protonation, ca. 13%] and N9[ca. 87%], with N9 principal site of protonation. It is thus of interest to examine whether or not the fluorescence spectral measurements of $m^1\varepsilon Ado^+$ and $m^9\varepsilon Ado^+$ shed light on conflicting literature statements regarding the fluorescence nature of protonated εAdo.





R = D-ribosyl

X^1 = H, $H^1\varepsilon Ado^+$

X^1 = CH_3, $m^1\varepsilon Ado^+$

X^9 = H, $H^9\varepsilon Ado^+$

X^9 = CH_3, $m^9\varepsilon Ado^+$

It has been found that $m^1\varepsilon Ado^+$ and $m^9\varepsilon Ado^+$ are both highly fluorescent at 293°K (quantum yield: 0.43 and 0.11, respectively), and the fluorescence is the same over the range of pH 3 to 7. Moreover, a most interesting observation is that $m^1\varepsilon Ado^+$ exhibits nearly the same fluorescence characteristics as εAdo does. These findings alone strongly indicate that the protonated forms, $H^1\varepsilon Ado^+$ and $H^9\varepsilon Ado^+$, should be inherently fluorescent, and the former is expected to be a more efficient fluorescer than the latter species at room temperature because $m^1\varepsilon Ado^+$ is about 4 times more fluorescent than $m^9\varepsilon Ado^+$. As the ethylene glycol/water solution of $m^9\varepsilon Ado^+$ was cooled from 293°K to 77°K, fluorescence increased eightfold and quantum yield near 0.89 was attained. The excitation and emission maxima of εAdo, $m^1\varepsilon Ado^+$, and $m^9\varepsilon Ado^+$ are summarized in Table 1.

As an explanation of the previously observed effect of protonation on fluorescence

Table 1. Fluorescence Characteristics of εAdo and Related Quaternized Derivatives at 293° and 77°K

Compound	Temp., °K	Medium[a] (pH)	Absorption maximum, nm	Excitation maximum, nm[b]	Fluorescence maximum, nm[c]
εAdo	293	W or EGW (7.0)	228.5, 257.5(sh)[d], 265, 275, ca.300(sh)	257(sh), 266, 275 ca.300(sh)	406, ca.428(sh)
εAdo	77	EGW (7.0)	257(sh) 266, 275(sh) 296	-----	377
H^{x}εAdo^{+}	293	W or EGW (2.0)	222, 267.5(sh), 273.5	275	406
H^{x}εAdo^{+}	77	EGW (2.0)	266(sh), 273	-----	334, ca.350(sh)
m^{1}εAdo^{+}	293	W or EGW (7.0)	220, 276, 282, ca.310(sh)	ca.233, 275, 280	409.5, ca.430(sh)
m^{1}εAdo^{+}	77	EGW (7.0)	-----	ca.232, 272, 281, ca.310(sh)	ca.357(sh), 375, 394(sh)
m^{9}εAdo^{+}	293	W or EGW (7.0)	233, ca.269(sh), 277.5	275	ca.365, ca.380(sh)
m^{9}εAdo^{+}	77	EGW (7.0)	-----	269(sh), 277.5	ca.325(sh), 337, ca.353(sh)

a: W, 60 mM phosphate buffer(pH 7.0); EGW, mixture of ethylene glycol and 60 mM phosphate buffer(1v/1v), and pH of EGW was adjusted by addition of HCl.
b: Fluorescence excitation spectra were measured by fixing on the fluorescence emission maximum; Corrected values.
c: Fluorescence emission spectra were measured by excitation at 294 or 296 nm; Corrected values.
d: Here sh stands for shoulder or point of inflection.

intensity of εAdo without a change in the fluorescence maximum at room temperature, we imagine that N1 is the most basic site in the excited state, so that, on excitation of protonated εAdo, the H^{9}εAdo^{+} singlet form will tend to undergo the rapid protolytic deactivation, ending up in the ground state. However, at low temperature εAdo fluoresces maximally at 334 nm at pH 2, and this is apparently interpreted in terms of light emission from the Franck-Condon state of H^{9}εAdo^{+} presumably because the time required for the proton transfer may be longer than the lifetime of the excited singlet state at 77°K. The observed residual fluorescence of εAdo at pH 2 at room temperature must not be originating from the remaining free base form[the percentage of protonation, more than 99.2%; $pK_a(S_0) = 4.10 \pm 0.02$][20] but more likely from H^{1}εAdo^{+} which is present in the protonated εAdo as a minor tautomeric form[18].

The preparation and chemical properties of hitherto unknown methylated compounds used in this study and the results of their fluorescence studies will be reported in more details in a forthcomimg full paper[21].

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