# PROTONATION AND QUATERNIZATION OF 1,N$^6$-ETHENOADENOSINE(εADO)
## ——*SPECIES RESPONSIBLE FOR THE FLUORESCENCE OF εADO*——

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The present work has two principal objectives. The first is to obtain definite information as to which of the basic nitrogen atoms in εAdo is involved in protonation in aqueous solution because of the current interest in the protonation effect on the fluorescence intensity of the εAdo residue. For the dependence of the fluorescence nature of εAdo upon pH, two laboratories have published conflicting interpretation[1,2]. In order to circumvent a problem arising from a possible tautomeric equilibrium between $H^1\varepsilon\overset{+}{A}do$ and $H^9\varepsilon\overset{+}{A}do$, we have recourse to quaternization with an appropriate methylating reagent. The resulting hitherto unknown quaternized N-methyl derivatives, $m^1\varepsilon\overset{+}{A}do$ and $m^9\varepsilon\overset{+}{A}do$, are the nontautomerizable model compounds for $H^1\varepsilon\overset{+}{A}do$ and $H^9\varepsilon\overset{+}{A}do$, respectively, and have been found to be reasonably stable at pH 1-7 yet easily decomposed under mildly alkaline conditions. The uv absorption spectra of $m^1\varepsilon\overset{+}{A}do$ and $m^9\varepsilon\overset{+}{A}do$ have been found to be unaffected by the pH value over the range 1 to 7.

A question which clearly must be answered is whether $H^1\varepsilon\overset{+}{A}do$ and/or $H^9\varepsilon\overset{+}{A}do$ is fluorescent or not. We have now determined the effect of quaternization on the fluorescence spectrum. In a study which antedated ours, Leonard et al.[3] called attention to the use of quaternary methyl derivatives of εCyd in identifying the species responsible for fluorescence of εCyd. The high degree of coincidence between the fluorescence spectra of εAdo and $m^1\varepsilon\overset{+}{A}do$ at pH 6 is noted. However, the spectrum of $m^9\varepsilon\overset{+}{A}do$ greatly differs from the spectra of εAdo and $m^1\varepsilon\overset{+}{A}do$ at pH 6. The results of the fluorescence spectral measurements at room temperature alone provide sufficient evidence to support the assignment of molecular species $[H^1\varepsilon\overset{+}{A}do]^*$ rather than $[\varepsilon Ado]^*$ to the principal species responsible for the fluorescence of εAdo in aqueous solution. This evidence also suggests that at room temperature the prototropic reaction presumably reaches equilibrium within the lifetime of the excited state and N1 is the most basic site of the first excited singlet state of εAdo. The pK($S_1$) value for this reaction must be above 13.

## References

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