Tetrahedron Letters No. 33, pp 3087 - 3090, 1973. Pergamon Press. Printed in Great Britain.

SYNTHESIS OF FLUORESCENT ADENOSINE DERIVATIVES†

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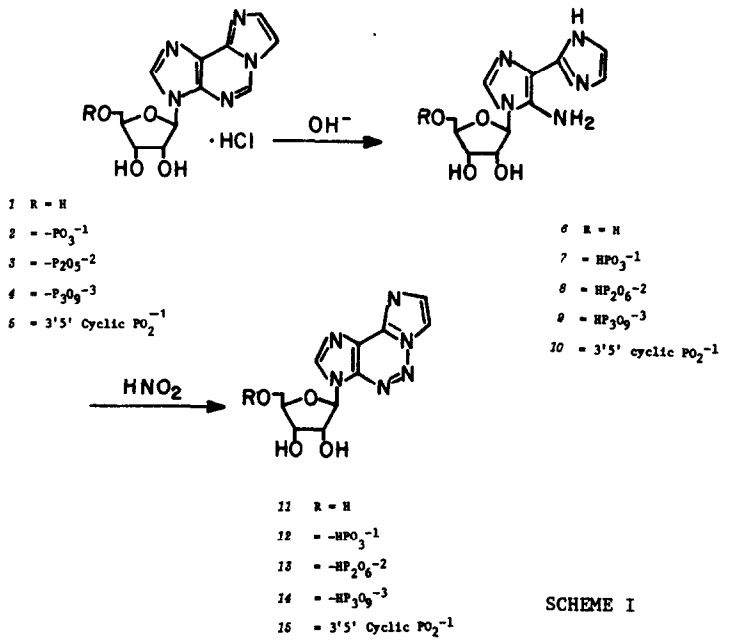
(Received in USA 14 May 1973; received in UK for publication 1 July 1973)

When 1,N^6-etheno-adenosine, *1*, was treated with sodium hydroxide, it lost fluorescence and gave 3-β-D-ribofuranosyl-4-amino-5-(imidazol-2-yl) imidazole *6*. Nitrosation of *6* yielded 2-aza-1,N^6-etheno-adenosine, *11* (Scheme I).



SCHEME I

Toxicity studies showed that *11* is selectively active in rat mammary tumor (AC-33) tissue culture system, although inactive in other tissue culture, (e.g., HeLa and Glioma 26).[1] In order to obtain further information concerning the unique properties of the 2-aza-1,N^6-etheno-adenosine in the biological system, the synthesis of other related 2-aza-1,N^6-etheno-adenosine derivatives was undertaken. Because of the unique fluorescent properties and the importance of the ε-adenosine [2,3] in biochemical studies, we wish to describe the

synthesis and some physical properties of these fluorescent adenosine derivatives. ε-5'-AMP, ε-ADP, ε-ATP and ε-3'5'-cyclic AMP[4] decomposed when treated with 0.1 N sodium hydroxide at room temperature, to form the diimidazole derivatives (*7-10*) in quantitative yield. All of the diimidazole derivatives reacted with equal molar of sodium nitrite in 80% acetic acid to give the 2-aza-ε-adenosine derivatives as expected (Table I).

Table I PREPARATION OF 2-AZA-1,N^6-ETHENO-ADENOSINE DERIVATIVES

Starting Material	Reagent	Reaction Time (Hr at 25° C)	Purification	Product (% Yield)	R_f[c] S_1	R_f[c] S_2
1	0.5 N NaOH, 20 ml/g	18	H_2O recry.	*6* (66)	0.69	0.25
2	0.1 N NaOH, 100 ml/g	18	EtOH ppt	*7* (95)	0.10	0.48
3	0.1 N NaOH, 100 ml/g	18	EtOH ppt	*8*(100)	0.07	0.52
4	0.1 N NaOH, 100 ml/g	18	EtOH ppt	*9*(100)	0.04	0.57
5	0.1 N NaOH, 100 ml/g	18	EtOH ppt	*10* (70)	0.48	0.16
6	$NaNO_2$/AcOH[a]	0.5	80% EtOH recry.	*11* (83)	0.73	0.11
7	$NaNO_2$/AcOH[a]	0.5	Sephadex A-25[b]	*12* (69)	0.18	0.30
8	$NaNO_2$/AcOH[a]	0.5	Sephadex A-25[b]	*13* (55)	0.08	0.32
9	$NaNO_2$/AcOH[a]	0.5	Sephadex A-25[b]	*14* (40)	0.06	0.37
10	$NaNO_2$/AcOH[a]	0.5	Sephadex A-25[b]	*15* (60)	0.57	0.08

a Equal molar of $NaNO_2$ in excess of 80% acetic acid; b Eluent NH_4HCO_3; c TLC was performed on Kodak Chromagram 6065. Solvent systems are S_1, ethanol: ammonium acetate 1 M (7:3, v); S_2, 0.1 M phosphate, pH = 6.8: ammonium sulfate: n-propanol (100:60:2).

The fluorescence of all of the 2-aza-ε-adenosine derivatives show maximum emission at 494 nm (Fig. I). The fluorescence intensity is detectable at concentrations of the order of 10^{-7} M. All of the 2-aza-ε-adenosine derivatives are indistinguishable by their fluorescent properties. At low pH, the fluorescence was quenched. The pKa of 2-aza-ε-adenosine was found to be 2.6 (Fig. II).

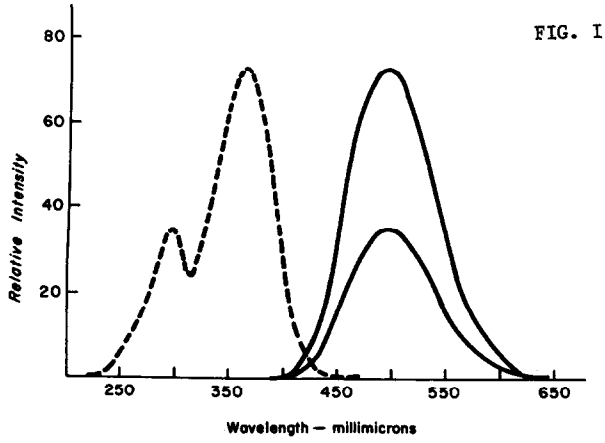
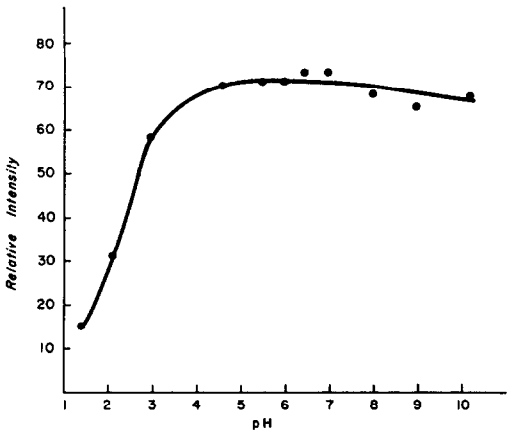
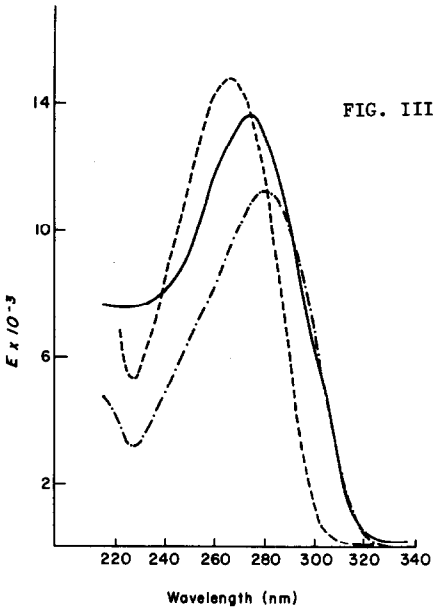


FIG. I Fluorescence spectra of *11* in citrate buffer (0.05 M, pH=7.0). —— Emission, (upper: excited at 358 nm; lower: excited at 295 nm); ---- Excitation.

Fig. II Variation in fluorescent intensity of *11* with pH. Excitation of 358 nm.



For the preparation of 2-aza-ε-ADP and 2-aza-ε-ATP, some free inorganic phosphate was present in the crude product as tested by the method of Berenblum and Chain.[5] Purification of the products can be successfully done using the Sephadex A-25 column (bicarbonate form) and linear gradients of ammonium bicarbonate. Traces of 2-aza-ε-AMP were found in both the crude 2-aza-ε-ADP and aza-ε-ATP, and about 12% of aza-ε-ADP was found in the crude 2-aza-ε-ATP. Both 2-aza-ε-5'-AMP and 2-aza-ε3'5'-cyclic-AMP can be purified on the same Sephadex A-25 column and isolated as the ammonium salt. Thus, the Sephadex A-25 column should be useful in the biochemical assay for all these aza-ε-adenosine derivatives. Details of the use of these novel substrates will be published elsewhere. Extension of the synthesis to FAD and NADH analogs was accomplished with low yield, and further purification is necessary.



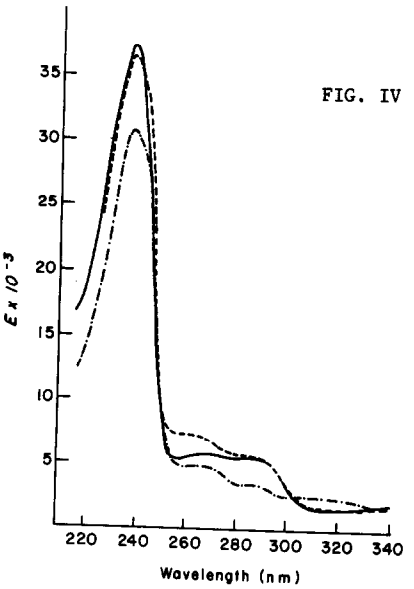


Fig. III Ultraviolet absorption spectrum of *6* at ---- pH = 14; ____ pH = 7; -·-·-·pH = 1.

Fig. IV Ultraviolet absorption spectrum of *11* at ---- pH = 14; ____pH = 7; -·-·-·pH = 1.

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† Supported by U.S.P.H.S. Grant CA 07339, from the National Institutes of Health.

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