FLUORESCENT ADENOSINE AND CYTIDINE DERIVATIVES

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SUMMARY

The reaction of chloroacetaldehyde with adenosine and cytidine produces a fluorescent product in each case. These products are easily distinguishable spectroscopically by their fluorescence emission maxima. Since the reaction is carried out in aqueous media under mild conditions of pH and temperature, it should prove extremely useful in nucleic acid chemistry. For example, reaction occurs between chloroacetaldehyde and E. coli tRNA, and the reaction can be followed by monitoring the increase in the fluorescence emission. Fluorescence lifetimes observed for the adenosine and cytidine products are close to 20 and 7 nsec, respectively.

In order to obtain further information concerning tRNA tertiary structure, we are engaged in a search for reagents which a) specifically modify the nucleosides found in tRNAs and b) produce a fluorescent derivative at the modification site.[3] Such reagents should prove generally useful in nucleic acid chemistry. The recent work of Kochetkov, Shibaev, and Kost on the reaction of chloroacetaldehyde with 9-methyladenine and 1-methylcytosine[4] led us to examine further the reaction of chloroacetaldehyde with the nucleosides adenosine and cytidine, with the idea that the products would certainly be fluorescent. This reaction system is potentially applicable to tRNAs since an aqueous medium is employed in conjunction with mild temperatures and a 3.5-4.5 pH range.

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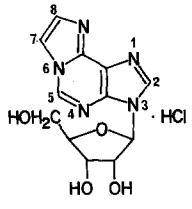
MATERIALS AND METHODS

Ultraviolet spectra were obtained in dilute aqueous solution on a Cary 15 spectrophotometer, fluorescence spectra on a Hitachi-Perkin MPF-2A fluorescence spectrophotometer, and proton magnetic resonance spectra on a Varian A-60A spectrometer with tetramethylsilane as internal standard. Fluorescence lifetimes were determined by phase and modulation using the cross-correlation fluorometer reported by Spencer and Weber.[5] Microanalyses were performed by Mr. Josef Nemeth and his staff, who also weighed samples for quantitative electronic absorption studies.

3-β-D-Ribofuranosylimidazo[2,1-i]purine Hydrochloride (1). A solution of 534 mg (2mmol) of adenosine in 20 ml of 2 M aqueous chloroacetaldehyde[6] (pH 4.0-4.5) was stirred at 37° for 24 hr[7] and then evaporated to dryness *in vacuo*. Recrystallization of the residue from H_2O-EtOH-Et_2O with decolorization furnished 620 mg (95%) of colorless needles, partial dec. at 178°, m.p. > 300°. The nmr spectrum in $(CD_3)_2SO$ exhibited a pair of doublets at δ 8.05 and 8.45, J = 2 Hz, corresponding to the 7- and 8-H's. Anal. Calcd for $C_{12}H_{14}ClN_5O_4$: C, 43.98; H, 4.30; N, 21.37. Found: C, 43.67; H, 4.57; N, 21.34.



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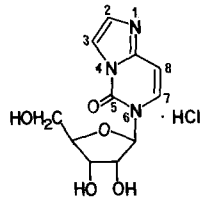

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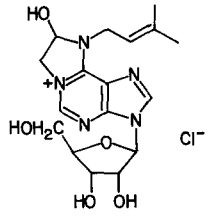

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5,6-Dihydro-5-oxo-6-β-D-ribofuranosylimidazo[1,2-c]pyrimidine hydrochloride (2) was prepared in a similar manner but at an optimum pH of 3.5. Recrystallization from H_2O-EtOH with decolorization afforded colorless needles, yield 90%, partial dec. at 182°, m.p. > 300°; nmr [$(CD_3)_2SO$], δ 7.80 (d, 1, J = 2 Hz,H-3), 8.00 (d of d, 1, J = 2 and 0.6 Hz, H-2). Anal. Calcd for $C_{11}H_{14}ClN_3O_5$: C, 43.49; H, 4.61; N, 13.83. Found: C, 43.16; H, 4.76; N, 13.81.

7,8-Dihydro-9-(3-methyl-2-butenyl)-8-hydroxy-3-β-D-ribofuranosylimidazo-[2,1-i]purine hydrochloride (3) was obtained from the reaction of chloroacetaldehyde with N^6-(Δ^2-isopentenyl)adenosine in the same manner as 1 and was purified by recrystallization from EtOH-Et_2O, softening below 90°, final dec. at 156-157°; nmr [$(CD_3)_2SO$] δ 1.78 (d, 6, $(CH_3)_2$), 3.00-5.93 (complex multiplet, ∿ 14), 6.03 (d, 1, J = 4 Hz, 1'-H), 8.01 (br d, 1, J = 8 Hz, 8-O-H), 8.91 (s, 2, 2,5-H's). Anal. Calcd for $C_{17}H_{24}ClN_5O_5 \cdot H_2O$: C, 47.28; H, 6.07; N, 16.22. Found: C, 47.40; H, 6.15; N, 15.95.

RESULTS AND DISCUSSION

Adenosine and cytidine reacted nearly quantitatively with chloroacetaldehyde[4] under mildly acidic conditions to form products 1 and 2, respectively, which were both fluorescent, as expected. Equally important is the additional feature that the two reaction products can be distinguished easily by their fluorescence maxima. Compound 1 shows maximum emission at 410 nm at pH 7 (Figure 1, Table I), while compound 2 shows maximum emission at 347 nm (Figure 2, Table II). The fluorescence intensity of 1 is extremely high, being readily visible at concentrations below 1 x 10^{-8} M. The less fluorescent cytidine derivative 2 is detectable down to concentrations of the order of 10^{-6} M.

We have also examined the fluorescence lifetimes of 1 in dimethylsulfoxide and water and of 2 in aqueous hydrochloric acid. The fluorescence lifetime of the 3,N^4-ethenocytidine derivative 2 in acidic solution, in which the emission was greatest, is close to 7 nanoseconds. By contrast, the 1,N^6-ethenoadenosine derivative 1 has a considerably longer lifetime,

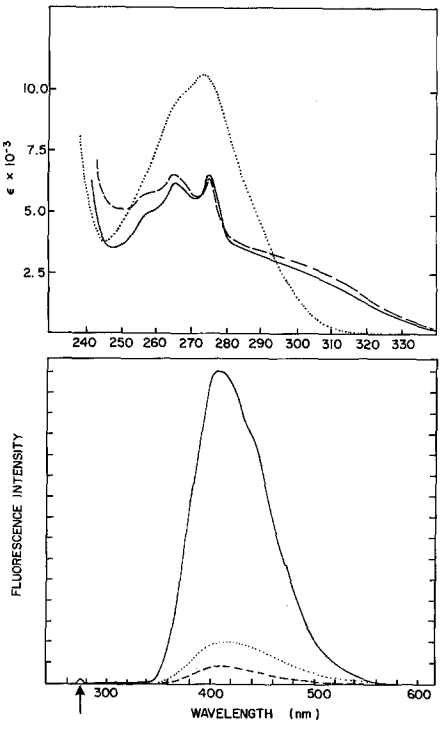

Figure 1. Top panel. Ultraviolet absorption spectra of 1 in aqueous solution. Bottom panel. Technical fluorescence spectra of 1 in aqueous solution. Neutral (————), 0.05 N HCl (·····), 0.05 N NaOH (— — —).

20 nsec in water and 18 nsec in dimethylsulfoxide, and promises to be of considerable value in further fluorescence studies. It is worth noting that 1 (cation) has the structure proposed for one of the major fragment ions observed in the mass spectrum of the modified ribonucleoside, 6-(4-hydroxy-3-methyl-cis-2-butenylamino)-9-β-D-ribofuranosylpurine (ribosyl-cis-zeatin).[8] The mass spectrum of 1 does in fact contain several major peaks which correspond closely to major peaks for ribosyl-cis-zeatin, thus lending additional support to the proposed fragmentation patterns not only for ribosyl-cis-zeatin, but for N^6-(Δ^2-isopentenyl)adenosine and some of its derivatives.[9-11]

The specificity of the reaction was checked not only with the major bases,[4] but also with several modified ribonucleosides. Under conditions similar to those used for adenosine and cytidine (pH 3.5-4.5, 37°) no reaction

TABLE I

Ultraviolet Absorption Data[a]

Compound	pH	λ_{max} (nm)	$\varepsilon \times 10^{-3}$
1	Neutral	300(sh)	2.6
		275	6.2
		265	5.9
		258(sh)	4.7
	.05 *N* HCl	274	10.3
	.05 *N* NaOH[b]	300(sh)	3.0
		275	6.3
		265	6.5
		258(sh)	5.7
2	Neutral	292(sh)	6.7
		281(sh)	11.1
		272	11.7
	.05 *N* HCl	302(sh)	7.0
		288	12.3
		248(sh)	4.4
	.05 *N* NaOH[b]	292(sh)	5.3
		281(sh)	10.9
		272	12.0
3	Neutral	265	16.4
		218	24.5
	.05 *N* HCl	265	16.4
		217	24.1
	.05 *N* NaOH[b]	268	13.1

[a]Determined in aqueous solution at concentrations on the order of 4×10^{-5} M.

[b]This compound decomposes quickly in basic solution, and the values obtained depend strongly on how soon after mixing the spectrum is determined.

was found with uridine, thymidine, guanosine, inosine, 3-methylcytidine, or 1-methyladenosine. However, N^6-(Δ^2-isopentenyl)adenosine was found to react quantitatively with chloroacetaldehyde to form product 3. The position of the hydroxyl group has not been fully confirmed, but since 1-methyladenosine was unreactive, this was suggestive that the mechanistic sequence involved

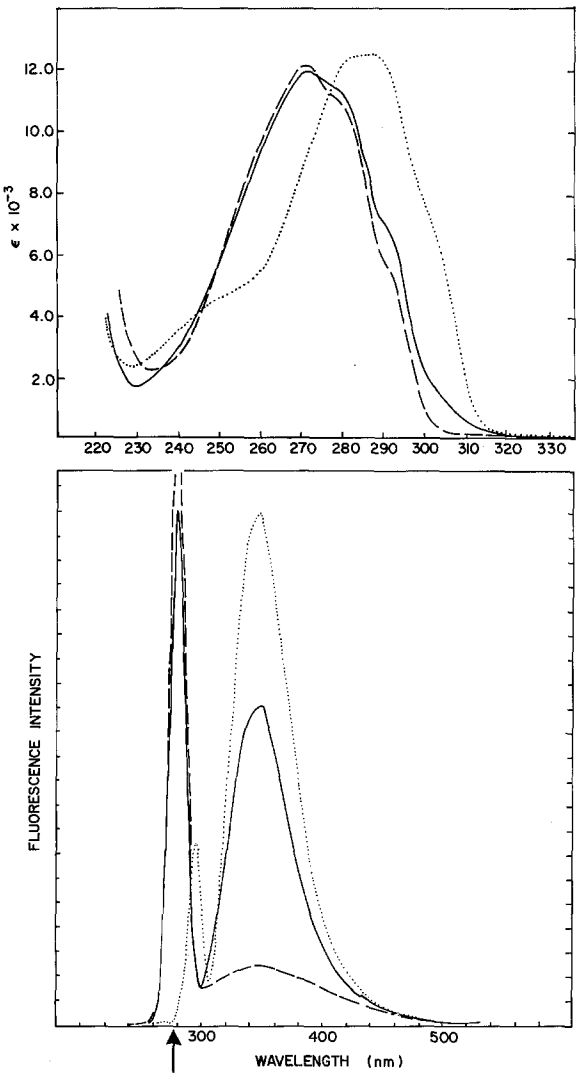

Figure 2. Top panel. Ultraviolet absorption spectra of 2 in aqueous solution. Bottom panel. Technical fluorescence spectra of 2 in aqueous solution. Neutral (————), 0.05 N HCl (·····), 0.05 N NaOH (— — —).

alkylation followed by ring closure and led us to the assignment shown in 3. This product was only weakly fluorescent, the nmr spectrum lacked the characteristic downfield pair of doublets present in 1 and 2, and the mass spectrum was also confirmatory (M-1 and M-29 peaks) for the structure assigned.

We have tested the reaction of chloroacetaldehyde with a tRNA preparation

TABLE II

Technical Fluorescence Data[a]

Compound	pH	Fluorescence Emission(nm)[b]			Fluorescence Excitation(nm)[c]
		λ_{max}	$\lambda+\frac{1}{2}$	$\lambda-\frac{1}{2}$	λ_{max}
1	Neutral	410	462	376	300,277,241
	.05 *N* HCl	412	467	378	278
	.05 *N* NaOH[d]	413	461	378	305,275,236
2	Neutral	347	379	320	300
	.05 *N* HCl	347	376	323	300
	.05 *N* NaOH[d]	Slight Fluorescence			—

[a] In water.

[b] Fluorescence emission spectra were taken by excitation at the longest wavelength ultraviolet maximum (Table I). Wavelengths representing half heights on each side of the maximum are also given.

[c] Fluorescence excitation spectra were taken by fixing on the fluorescence emission maximum.

[d] See footnote b of Table I.

from E. coli. The progress of the reaction can be followed easily by monitoring the increase in fluorescence of standard aliquots. In agreement with Kochetkov et al.[4], by varying the pH, reaction can be facilitated with either cytidine or adenosine residues. Near pH 3.5 the cytidine moiety reacts faster, while 4.5 is the optimum pH for reaction with adenosine.

The fluorescence data have shown that the chloroacetaldehyde reaction has several important advantages for application to purified-tRNA structure and function studies. The extremely intense emission of 1 upon excitation at 275 nm [12] allows ready detection of the modified adenosine at very low concentration. The relatively long fluorescence lifetime of 1 makes possible the use of additional fluorescence techniques, such as fluorescence polariza-

tion[13] and polarized decay,[14] to gain valuable information. Since the reagent used in the formation of 1 (and 3) is non-fluorescent and easily removable, no difficulties are encountered with respect to purification of the fluorescent products.

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