
The degradation of DNA by hydrazine: identification of 3-ureidopyrazole as a product of the hydrazinolysis of deoxycytidylic acid residues

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ABSTRACT

The reaction of hydrazine with the cytosine residues of DNA yields 3-ureidopyrazole, in approximately 50% yield, in addition to the expected 3-aminopyrazole. This product has been identified by ultraviolet, infrared, nuclear magnetic resonance and mass spectroscopy. A product of the reaction of hydrazine hydrate with cytosine, formulated by others as NN'-di-(3-pyrazolyl)hydrazine, has been identified as 3-ureidopyrazole.

INTRODUCTION

In a study of the degradation of DNA by hydrazine, we reported¹ that the cytosine residues in DNA reacted with hydrazine to yield 3-ureidopyrazole in approximately 50% yield, in addition to the expected² 3-aminopyrazole. We did not, however, give details of our positive identification of this product. A recent resurgence of interest in the reaction of hydrazine with pyrimidine nucleotides has been brought about through the introduction of elegant methods for the sequencing of DNA³ and RNA⁴. Since the positive identification of the products of the hydrazinolysis of pyrimidines is important for the full understanding of the chemical processes involved in the degradation of DNA by this reagent, we now give the experimental details supporting our identification of this major product of pyrimidine degradation.

MATERIALS AND METHODS

The preparation and purification of calf thymus DNA, of [2-¹⁴C]pyrimidine-labelled bacterial DNA and of anhydrous

hydrazine, the hydrazinolysis procedure and the general analytical techniques used in this study were detailed in our earlier paper¹.

The solvents used for descending paper chromatography on Whatman No. 1 paper were: solvent 1, isopropanol-conc.HCl-water (170:41:39, by vol); solvent 2, n-butanol-glacial acetic acid-water (120:30:50, by vol); solvent 3, n-butanol-ethanol-water (4:1:1, by vol).

Mass spectra were determined in the Associated Electrical Industries Model MS 9 mass spectrometer of Massey University, Palmerston North. NMR spectra were measured in the Varian Model H-60 instrument of DSIR, Chemistry Division, Gracefield. Infrared spectra were determined with a Perkin Elmer Infracord spectrometer Model 137 and ultraviolet absorption spectra were measured with a Beckman Model DU spectrophotometer or a Model DK-2A ratio recording instrument.

EXPERIMENTAL AND RESULTS

Calf thymus DNA (938 mg) and [2-¹⁴C]pyrimidine-labelled *Escherichia coli* DNA (0.20 mg) were mixed and treated with anhydrous hydrazine for 48 h at 30⁰. After removal of excess hydrazine by vacuum distillation, the residue was dissolved in distilled water and left for 2 h at room temperature. As was found in our earlier study¹, separation of the products of the reaction by gel filtration on Sephadex G-15 (Fig. 1) gave three radioactive peaks. The first, small peak, was identified¹ as the polymeric apyrimidinic acid with which traces of radioactive pyrimidine degradation products were still associated and the second, large peak, was identified as urea¹. Fractions 38 to 48, corresponding to the unknown component, were pooled and concentrated on a rotary evaporator to yield 33 mg of a crystalline compound.

This compound, which had m.p. 120-124⁰, gave the following spectral data:

Ultraviolet absorption spectrum

λ_{\max}	(in 0.1 M NaOH)	223 nm; ϵ_M , 8 000
λ_{\max}	(in 0.1 M HCl)	237 nm; ϵ_M , 15 000

NMR spectrum (in CD₃SOCD₃)

6.07	(doublet, coupling const = 2 cycles/sec, C-4 proton)
6.32	(singlet, NH ₂)
7.53	(doublet, coupling const = 2 cycles/sec, C-5 proton)
8.76	(singlet, -NH-)
12.05	(broad singlet, N-1 proton)

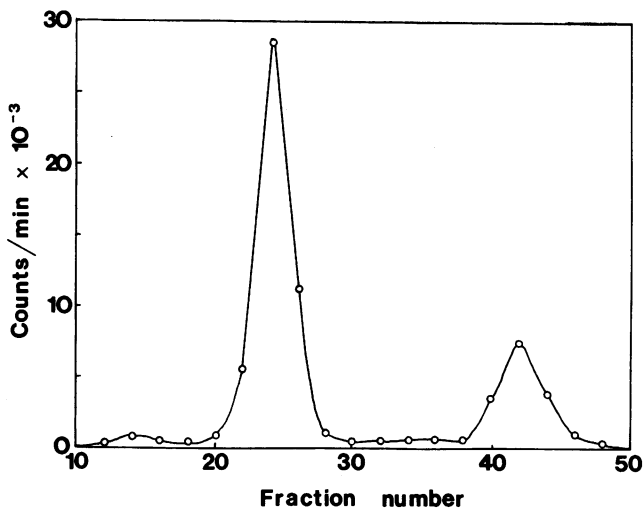


Figure 1. Chromatography of the products of hydrazinolysis of DNA. The products from the hydrazinolysis of [2-¹⁴C] pyrimidine-labelled DNA were separated on a column (4 cm x 50 cm) of Sephadex G-15, prepared in water. The column was eluted with water and 10ml fractions collected.

Infrared spectrum (in KBr)

ν_{\max} 1680 cm⁻¹ (C=O stretching), 2930, 3100, 3350 cm⁻¹ (N-H and C-H stretching).

Mass spectrum

m/e 126.0541 and 83, metastable peak at 54.8;

C₄H₆N₄O requires m/e 126.0542.

The behaviour of this compound on paper chromatography in three solvents is given in Table 1, where it is compared with the behaviour of urea, 3-aminopyrazole and the product of the degradation of cytosine by hydrazine hydrate, prepared as described by Hayes and Hayes-Baron⁵.

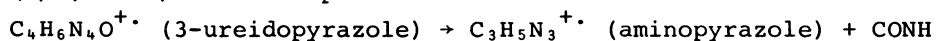
Table 1

Paper chromatography of hydrazinolysis products

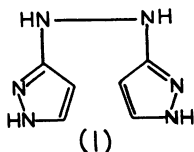
<u>Compound</u>	<u>Solvent, R_F</u>		
	<u>1</u>	<u>2</u>	<u>3</u>
Urea	-	0.53	0.39
3-Aminopyrazole	0.62	-	-
3-Ureidopyrazole	0.55	0.65	0.58
Cytosine hydrazinolysis product ⁵	0.55	-	0.58

DISCUSSION

The similarity of the ultraviolet absorption spectrum of the previously unidentified DNA-hydrazinolysis product to that of 3-aminopyrazole^{2,5}, suggested to us that the new compound is a pyrazole. The proton spectrum of pyrazole shows a characteristic low-field -NH- resonance at 12.52 δ and the C-4 and C-5 proton doublets at 6.33 δ and 7.62 δ , respectively⁶. Similar resonances (12.05, 6.32 and 7.53 δ) were found for the new compound. The identity of the compound as 3-ureidopyrazole (VI) was confirmed by the mass spectrum, which showed a parent peak corresponding to the molecular formula C₄H₆N₄O. A second peak (m/e, 83) and the corresponding metastable peak⁷ (m/e, 54.8) are interpreted as follows:



Hayes and Hayes-Baron⁵ have described a product of the reaction of cytosine with hydrazine hydrate which, on the basis of ultraviolet and elemental analysis, was concluded to be NN'-di-(3-pyrazolyl)hydrazine (I). The similarities in



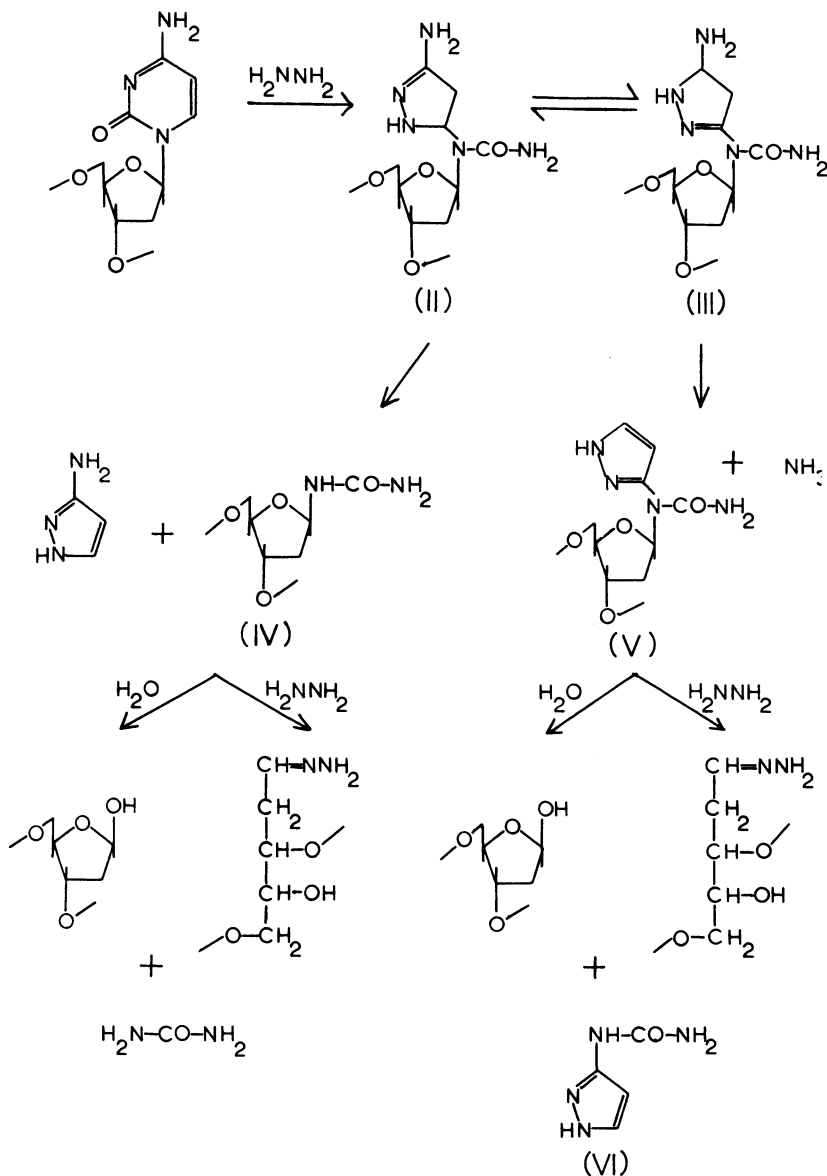


Figure 2. Proposed reaction sequence of the hydrazinolysis of deoxycytidylic acid residues in DNA.

origin, melting point and ultraviolet spectra suggested to us that this hydrazinolysis product was identical to the product that we have formulated as 3-ureidopyrazole (VI). We have confirmed this identity by treating cytosine with hydrazine

hydrate, as described by Hayes and Hayes-Baron⁵, and comparing the product obtained with 3-ureidopyrazole (Table 1). The structure proposed by Hayes and Hayes-Baron⁵ for this compound, while consistent with the ultraviolet absorption spectrum, can be discounted on the basis of the additional spectroscopic data that we have presented. It should also be noted that the hydrazinolysis of [2-¹⁴C]cytosine would not give rise to (I) as a radioactive compound.

The actual mechanism of hydrazinolysis of cytosine is of interest. By analogy with the reaction of hydroxylamine^{8,9} and methoxyamine¹⁰, initial attack is likely to be predominantly at C6 of the pyrimidine ring, followed by ring closure on to C4. The resulting intermediates (II and III, Figure 2) are substituted dihydropyrazoles and these can eliminate either the ureido group or ammonia. Under mild hydrazinolysis conditions, the ureido deoxyribose derivatives (IV and V) are not completely converted to the hydrazone, but they may be cleaved in aqueous solution¹, to yield either urea or 3-ureidopyrazole (VI).

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