

Identification of N¹-Glycolylbiuret in the Gamma Radiolysis of Aerated Aqueous Solution of Cytosine. Influence of the pH

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Cytosine, Gamma-Irradiation, N¹ Glycolylbiuret, pH-Effect

N¹ glycolylbiuret has been identified as a radiation product of cytosine in aerated aqueous solution at pH 4.5. When varying the pH of the solution before irradiation from acidic values towards neutral ones, G value of N¹ glycolylbiuret reached a maximum at pH 4.5.

In a previous work [1], the radiation-induced degradation of cytosine by gamma rays has mainly been described according to the classical hydroxyl radical attack on the 5,6 double bond leading to 5- and/or 6-pyrimidyl radicals which form peroxides when oxygen is present in the irradiated aqueous solution. These peroxides are known to be particularly unstable for cytosine [2]. The identification of biuret in the gamma radiolysis of cytosine in an aerated aqueous solution [3] and the subsequent characterization of *trans*-1-carbamoyl-4,5-dihydroxyimidazolidin-2-one as the most important radiolysis product of cytosine [4–7], has focused our attention on the ring concerted rearrangements occurring throughout the radiolytic oxidation processes.

In this paper, the identification of N¹-glycolylbiuret as one of the stable radiolysis product of cytosine is described.

Results

1. Irradiation of cytosine aerated aqueous solutions and chromatography of the degradation products

Acidified solutions of cytosine dissolved in tri-distilled water were submitted to gamma ray irradiation in aerated conditions.

Once taken up by a water-methanol mixture, the radiation products of cytosine were analyzed by two-dimensional thin-layer chromatography on silicagel plates.

After localization of radioactive spots by autoradiography, each product was identified by the combination of conventional spectroscopic methods.

Among the previously identified radiation products of cytosine (bibliography references in Table I), a new radiation product of cytosine was evidenced: N¹-glycolylbiuret.

Its elementar formula C₄H₇N₃O₄ was determined by high resolution mass spectrometry (M⁺ · m/e 161).

The interpretation of the mass fragmentation pattern is straightforward and is given in Fig. 1 and 2.

Table I. Chromatographic R_f values of radiation-induced degradation products of cytosine.

Radiation product	Solvent A	Solvent B	References (*)
Cytosine	0.30	0.13	–
5-Hydroxycytosine	0.21	0.09	– 1 –
Uracil	0.67	0.59	–
Cis-5,6-dihydroxy-5,6-dihydrouacil	0.22	0.33	– 3 –
Trans-5,6-dihydroxy-5,6-dihydrouacil	0.29	0.51	– 3 –
Urea	0.30	0.27	– 3 –
Biuret	0.59	0.65	– 3 –
N ¹ -formylurea	0.68	0.74	– 3 –
N ¹ -formylbiuret	0.78	0.80	– 5 –
N ¹ -glycolylbiuret	0.66	0.73	this paper
Trans-1-carbamoyl-4,5-dihydroxyimi-dazolidin-2-one	0.47	0.65	– 4, 7 –
1-carbamoyl-5-hydroxy-hydantoin	0.58	0.76	– 5 –
5-Hydroxyhydantoin	0.55	0.71	– 3 –
4-Amino-1-formyl-5-hydroxy-2-oxo-3-imidazolidin	0.55	0.48	– 7 –
Parabanic acid	0.69	0.87	– 3 –

* The references listed here refer to the identification of these products.

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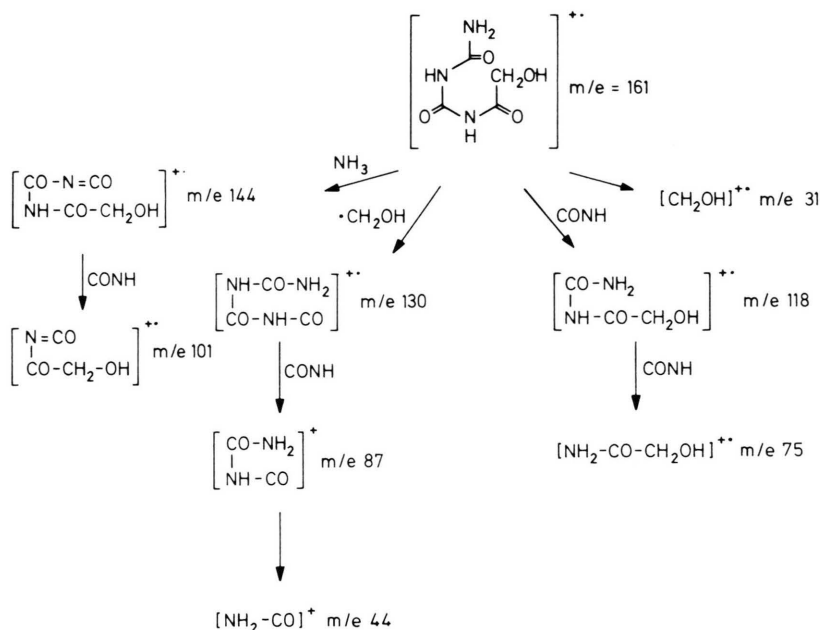


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Fig. 1. Mass fragmentation pattern of N¹-glycolylbiuret.

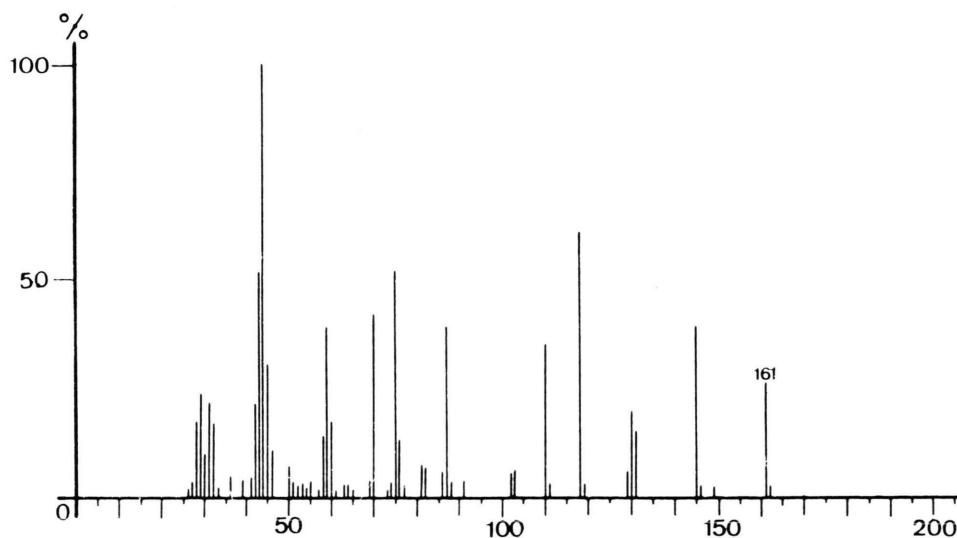
The mass spectrum of N¹-glycolylbiuret can be accounted in terms of three principal elimination processes: NH_3 , CH_2OH and CONH .

2. Quantitative analysis: Influence of the pH

After localization of radioactive spots by autoradiography, the silicagel was scrapped off the

plate, eluted at room temperature overnight with tridistilled water and the radioactivity was measured.

When aqueous solutions of cytosine were acidified before gamma irradiation (14,500 rad/min – 10^{-3} M – 10 ml – 0.5 μCi – 20 min), it was found that the G value of N¹-glycolylbiuret reached a maximum between pH 4 and 5, while that of the

Fig. 2. Mass spectrum of N¹-glycolylbiuret.

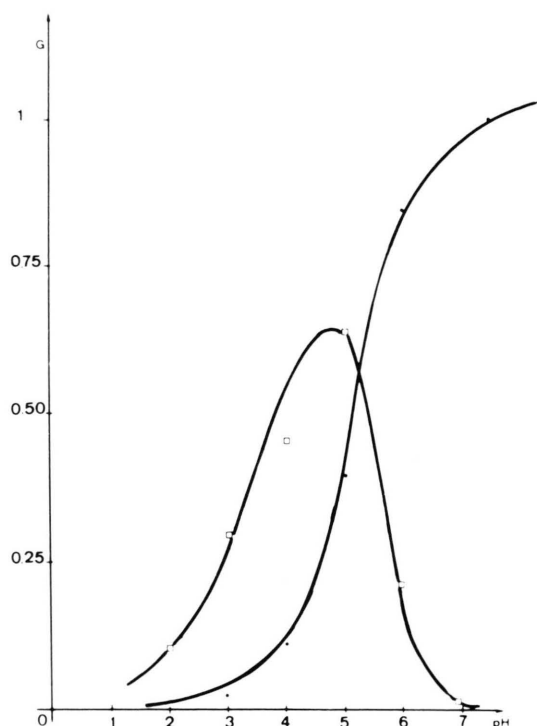


Fig. 3. *G* values of the *trans*-1-carbamoyl-4,5-dihydroxyimidazolidin-2-one (●) and of *N*¹-glycolylbiuret (□) vs the pH of the irradiated solution cytosine 10^{-3} M, dose $28 \cdot 10^4$ rad).

trans-1-carbamoyl-4,5-dihydroxyimidazolidin-2-one increased regularly from low pH values towards neutrality (Fig. 3).

It has to be emphasized that the *cis* form of the latter compound is unstable and gave rise to the *trans* isomer [8]. The acidification of the aqueous solution after gamma irradiation has no influence on the *G* values of the radiation products of cytosine namely: *N*¹-glycolylbiuret and *trans*-1-carbamoyl-4,5-dihydroxy-oxo-2-imidazolidin.

Discussion

Hydroxyl radical attack on pyrimidine bases is known to occur mainly on the C₅–C₆ double bond. The relative positions of this attack depend on the presence of substituents of the pyrimidine ring and of the pH [9–14].

The presence of *t*-butanol (50 mM) in aerated aqueous solution of cytosine (1 mM) prevented any decomposition to occur. This result implies the

main role of hydroxyl radical in the radiation decomposition of cytosine in aerated aqueous solution [10].

Experimental

Materials

Cytosine, biuret and uracil were supplied by Fluka. Radioactive [2-¹⁴C]cytosine (CMM-168-CEA-Saclay-France – 54.6 mCi/mM) was purified just before use by bidimensional thin-layer chromatography. The persulphate oxidation of cytosine was used to synthesize 5-hydroxycytosine [15].

The radiation products of uracil namely: 5,6-dihydroxy-5,6-dihydrouracil *cis* and *trans* and isodialuric acid were prepared by gamma radiolysis in aerated solution [15]. The radiation products of cytosine were prepared according to the previously published data [7].

Irradiation of cytosine

Cytosine was dissolved in tridistilled water and irradiated by Cobalt-60 gamma rays in a similar way to that described previously [3]. Acidic pH values were obtained by adding minute amounts of concentrated hydrochloric acid. Unless stated otherwise: 2 ml of cytosine 10^{-3} M, 0.5 μ Ci were irradiated at pH 4.5 with a dose rate of 9.4×10^3 rad/min at different exposure times.

Once brought to dryness, each irradiated residue was taken up with 0.2 ml of a water-methanol mixture (50/50, v/v) and deposited on a silicagel precoated plate.

Chromatography of radiation products of cytosine

From the irradiated mixture of radiation products of cytosine dissolved in the water-methanol mixture, 0.01 ml was deposited on a silicagel precoated glass plate (20 \times 20 cm) containing a fluorescent indicator (254 nm) (Schleicher and Schull).

Two-dimensional chromatographic separations were carried out with the following solvents:

Solvent A: Chloroform-methanol-water (4:2:1) (v/v/v) lower phase with 5% methanol added just before use. Two successive runs of seventy-five minutes each were carried out. A drying pause (fifteen minutes) was observed between these two runs.

Solvent B: Ethyl acetate-propan-2-ol-water (75:16:9) (v/v/v). One run was used. The chromatographic properties of these solvents have been previously described.

Quantitative analysis

The localization of radioactive spots on this layer plate was obtained by autoradiography with Kodirex (Kodak) or Structurix D.10 (Agfa) emulsions.

Once scrapped off the plates, the silicagel was poured in a counting vial (Packard glass vial) and eluted overnight (1 ml) with tridistilled water.

Twenty milliliters of Instagel (Packard) were added and after gentle mixing (1 min), radioactivity measurements were achieved on a Packard Tricarb liquid scintillation counter.

Dosimetry of the gamma ray source was achieved using quantitation of free radicals produced by gamma-irradiation of alanine by R.P.E.

Spectrometric analysis

¹H NMR spectra were recorded on a Varian Associates T.60 (60 MHz) spectrometer with DMSO-d₆ as solvent and TMS as internal reference. The potassium bromide pellet micromethod was used to record infrared spectra on a Perkin Elmer spectrophotometer (Model 257).

High resolution mass spectra (70 eV, 160 °C) were performed on a GEC AEI MS.9 mass spectrometer with sample introduction by direct probe. Mass measurements were obtained by computer.

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