Gamma Irradiation of Uridine and Uridylic Acid in Aerated Aqueous Solutions: Identification of the Major Product

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The major product of uridine gamma irradiation in aerated aqueous solution (10^{-3} M, pH ~6) has been identified: N-(β-D-ribofuranosyl)-formamide. Thin-layer silica gel chromatography and spectroscopic methods (mass spectrometry, NMR, IR, optical rotation) enabled us to isolate and characterize this product.

It may be assumed that this substance resulted from the hydrolytic degradation of unstable hydroxy-hydroperoxides. It was also prepared by uridine oxydation with pertrifluoracetic acid.

A similar major compound in the radiolysis of uridylic acid has been found.

The identification of the lesions produced by ionizing radiations on RNA or DNA [1–3] has been mostly based on that of nucleic acid constituents [4–6]. So far, only few investigations of uridine radiolysis have been performed [7–9].

N-(β-D-ribofuranosyl)-formamide appeared as the major product after the irradiation of uridine (pH ~$6 \cdot 10^{-3}$ M, aerated solution) with an yield close to 30%. This compound was isolated by thin-layer chromatography of the syrup which resulted from the evaporation at room temperature of the irradiated solution, (Macherey Nagel N/HR; *solvent A:* $CHCl_3 - CH_3OH - CH_3COOH - H_2O$ (50 : 30 : 4 : 5) $R_{F\mathrm{A}} = 0.49$; *solvent B:* $CH_3COOC_2H_5 - CH_3OH - CH_3COOH - H_2O$ (75 : 16 : 5 : 10) $R_{F\mathrm{B}} = 0.40$).

This substance was characterized by mass spectrometry, infrared, NMR. The infrared spectra exhibited a maximum at the stretching frequence of formamide carbonyle (1680 cm^{-1}). The NMR spectra recorded in DMSO d_6 (TMS, VARIAN A 60) showed the presence of two rotamers due to the steric hindrance of the C–N amidic bond free rotation [10]. These rotational conformers occurred in the ratio of 40 to 60. At room temperature, the NMR signal of formyl proton appeared as a doublet and that of the anomeric proton was split into two doublets. However, when the DMSO solution was warmed at 63 °C, the formyl doublet collapsed into a singlet. The doublet appeared again when the mixture was cooled.

Major conformer: Proton H-6 singlet at $\delta = 8.06$ ppm; proton H-1′ doublet centred at $\delta = 5.20$ ppm; $JH_{1'} - H_{2'} = 4.5$ cps; NH at $\delta = 8.35$ ppm; proton exchanged with D_2O.

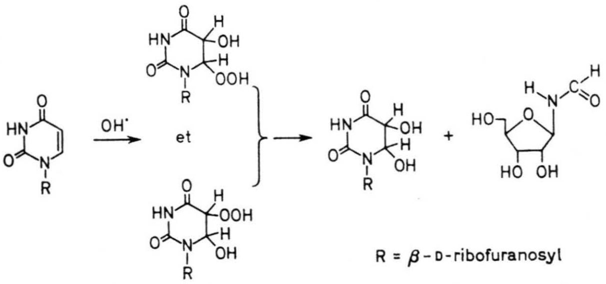
Minor conformer: proton H-6 singlet at $\delta = 8.15$ ppm; proton H-1′ doublet centred at $\delta = 5.35$ ppm; $JH_{1'} - H_{2'} = 4$ cps; NH at $\delta = 8.4$ ppm; proton exchanged with D_2O.

Hydroxyl protons for two conformers at $\delta = 4.83$ ppm.

In order to obtain a better volatility the tri-O-acetyl- derivative was studied in mass spectrometry (MS 9 instrument, direct introduction, 100 °C).

The molecular ion $M^{+\cdot}$ Tr: 303.0942; Calcd: 303.0954 was of low intensity. The major ions were the following: $m/e = 259$ ($C_{11}H_{15}O_7$) ($M^{+\cdot} - {}^{\cdot}NHCHO$); $m/e = 243$ ($C_{10}H_{13}NO_6$) ($M^{+\cdot} - AcOH$); $m/e = 230$ ($C_9H_{12}NO_6$) ($M^{+\cdot} - {}^{\cdot}CH_2OAc$); $m/e = 183$ ($M^{+\cdot} - 2\,AcOH$). Optical rotation: $[\alpha]_D^{25} = -53°$ (*c* 1.5, H_2O).

It may be assumed that N-(β-D-ribofuranosyl)-formamide resulted from the hydrolytic degradation of unstable hydroxyhydroperoxides in a similar way as occurred in thymidine radiolysis [11].



A mixture of pertrifluoracetic acid prepared *in situ* [12] and uridine, kept at room temperature for 20 hours, in molar concentration, also gave this compound. The two intermediate hydroxyhydroperoxides, stable in an acidic medium, were rapidly degradated at neutral pH. N-(β-D-ribofuranosyl)-formamide and *cis*(+) and (–)5,6-dihydroxy-5,6-dihydrouridine were produced by the hydrolytic degradation of these peroxides. They were separated by t.l.c. in solvents A and B.

We have found a similar major compound in the radiolysis of uridylic acid, namely N-(5-phosphoryl-β-D-ribofuranosyl)-formamide. This product was isolated by electrophoresis (cellulose thin-layer, $HCOONH_4 = 0.05$ M, 700 V, 45 min) and then dephosphorylated by acidic phosphatase, type I, into N-(β-D-ribofuranosyl)-formamide. The resulting product was compared to an authentic sample.

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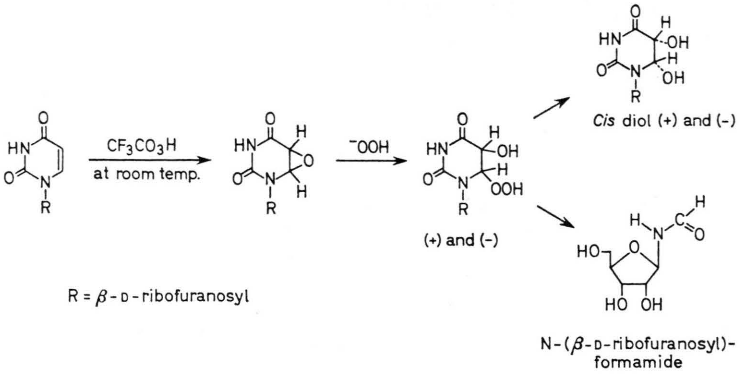

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