

SHORT COMMUNICATIONS

GAMMA-IRRADIATION OF URACIL IN DE-AERATED AQUEOUS SOLUTIONS*

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Weiss *et al.*¹ have shown that when the pyrimidine bases in aqueous solutions in the presence of oxygen are irradiated, they give high yields of peroxides at the 5-6 double bond. In the absence of oxygen this reaction apparently does not take place and the destruction of the bases is considerably reduced. This study is concerned with the case when the base is irradiated in de-aerated aqueous solutions, and with the identification of the radiolysis products.

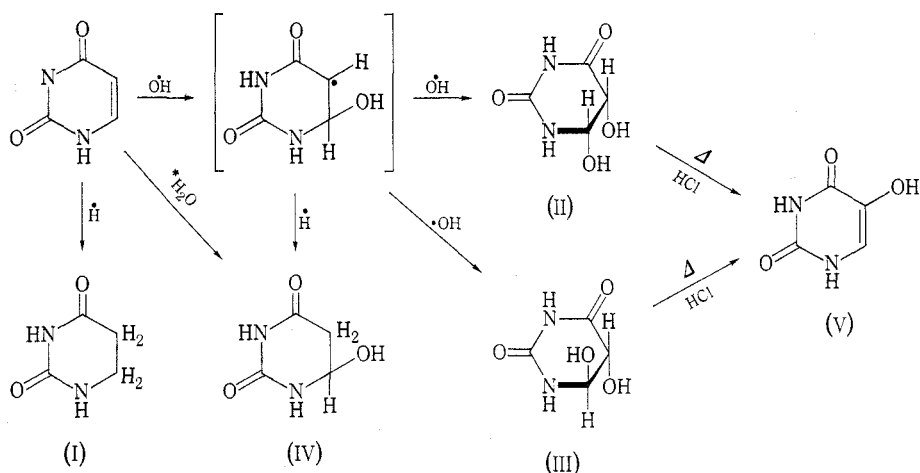


Fig. 1.—Degradation scheme.

Experiments were carried out by irradiating 5 ml de-aerated solutions (conc. 10^{-2}M) of the base in a ^{60}Co - γ -ray facility. The dose rate was about 1000 rads/min; total doses in the range of 10^5 – 10^6 rads were applied. The irradiated solutions were subsequently analysed by descending paper chromatography. The solvents found more suitable for separation were (a) propanol/water (10 : 3), (b) t-butanol/methyl ethyl ketone/water/ammonia (40 : 30 : 20 : 10), and (c) t-butanol/methyl ethyl ketone/water/formic acid (40 : 30 : 15 : 15). Ultraviolet-absorbing products were

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¹ Scholes, G., Weiss, J., and Wheeler, G. M., *Nature* 1956, **178**, 157; Scholes, G., Ward, J. F., and Weiss, J., *J. Mol. Biol.* 1960, **2**, 374.

located by viewing the chromatograms in u.v. light ($255\text{ m}\mu$) and identified by their R_F values and the u.v. spectra of their eluates. The pyrimidines saturated at the 5-6 double bond (dihydrouracil and uracil glycols) were found by spraying the chromatograms with 0.5N sodium hydroxide followed by a solution of *p*-dimethylaminobenzaldehyde (Fink's² reagent) and identified by their R_F values.

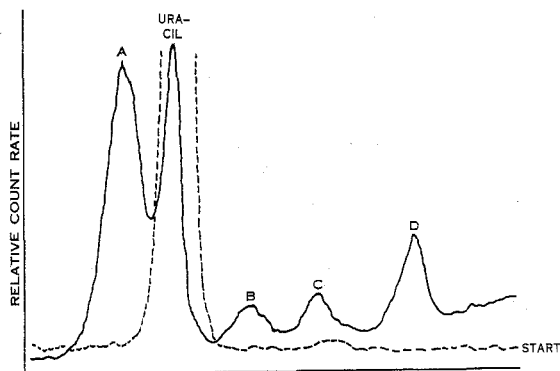


Fig 2.—Chromatogram of $[2-^{14}\text{C}]$ uracil.
 - - - Before irradiation.
 — After irradiation.

When the developed chromatograms using solvents (a) and (b), were viewed in u.v. light, two u.v.-absorbing spots appeared, locating their positions. On eluting from the papers and determining the u.v. spectra of the eluates one was identified

TABLE I
 COMPARISON OF R_F VALUES OF THE RADIOLYSIS PRODUCTS OF URACIL (SPOTS A-E) WITH THOSE OF KNOWN COMPOUNDS

Sample	Solvent Systems		
	(a)	(b)	(c)
Uracil	0.55	0.54	0.56
Dihydrouracil (I)	0.60	0.61	0.67
Spot A	0.60	0.60	0.67
Hydroxyhydrouracil (IV)	0.24	0.22	0.28
Spot D	0.24	0.22	0.24
<i>trans</i> -Uracil glycol (III)	0.5	0.42	0.44
<i>cis</i> -Uracyl glycol (II)	0.44	0.30	0.44
Spot B	0.5	0.42	0.45
Spot C	0.46	0.30	0.45
Isobarbituric acid (V)	0.45	0.53	0.38
Spot E	0.45	0.53	0.38

by its spectrum as uracil, the unchanged solute. The second product did not show any absorption maxima between 200 and $300\text{ m}\mu$ in neutral solution. When the eluate of this product was acidified and boiled for a few minutes, it showed the same spectrum as uracil. This reversible product is presumed to be 6-hydroxyhydrouracil (IV), resulting from addition of an excited water molecule at the 5-6 double bond or

² Fink, *et al.*, *J. Am. Chem. Soc.*, 1959, **81**, 2521.

addition of $H+OH$ radicals. (This derivative of uracil was prepared according to Moore³ from 5,5-dibromo-6-hydroxyuracil. The synthetic product was then compared chromatographically and spectroscopically with the product of irradiation of uracil.)

With Fink's reagent three coloured spots were found on the chromatograms. These were yellow in colour and varying in intensity. These three products had R_F values identical with those from authentic samples of dihydrouracil (I) and *cis*- and *trans*-uracil glycols (II and III). Dihydrouracil used for comparison was a Sigma (U.S.A.) product and the two glycols were prepared according to Baudish and Davidson.⁴ Further confirmation of the glycols was found when the irradiated uracil solution was boiled first with hydrochloric acid and then analysed. No glycols were found, but isobarbituric acid (V) was observed. Even when the eluates of (II and III) were heated to boiling in hydrochloric acid, the resulting solutions showed the same u.v. spectrum as isobarbituric acid. The formation of this acid was confirmed chromatographically. On mixing with known samples of isobarbituric acid and chromatographing on paper, it was found inseparable in the three solvent systems.

On the basis of these experimental observations, a possible scheme is shown in Figure 1 for degradation of uracil in de-aerated aqueous solution.

These experiments were repeated with irradiated $[2-^{14}C]$ uracil solution ($2\ \mu\text{c}/\text{ml}$). The developed chromatograms were scanned under a Geiger-Mueller tube connected with a ratemeter and recorder. Six different radioactive spots were located on the papers. A typical scan is shown in Figure 2. The radioactive spots (located from the scan) A, B, C, and D had R_F values identical with those of known samples (Table 1).

³ Moore, A. H., *Canad. J. Chem.*, 1958, **36**, 781.

⁴ Baudish, O., and Davidson, D. J., *J. Biol. Chem.*, 1925, **64**, 233.