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AN UNUSUAL REACTION OF CIS-6-HYDROPEROXY-5-HYDROXY-5,6-DIHYDROTHYMINE WITH ASCORBIC ACID

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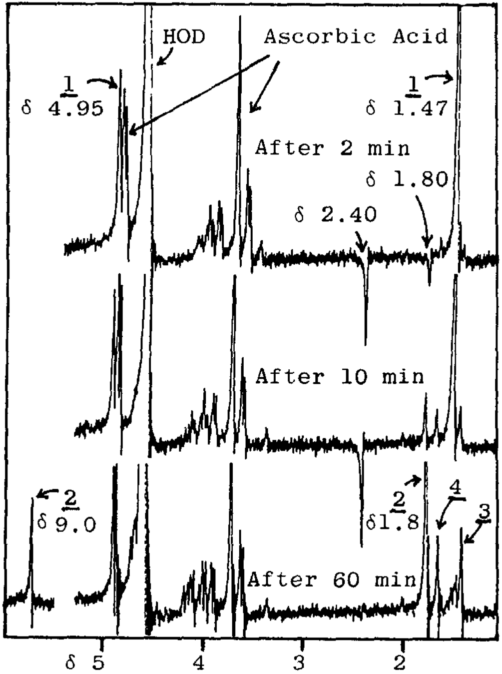
Summary: When two solutions of cis-6-hydroperoxy-5-hydroxy-5,6-dihydrothymine and ascorbic acid in deuterium oxide were mixed, chemically induced dynamic nuclear polarization was observed. The reaction gave N-formyl-N'-pyruvylurea.

Ionizing radiation of aqueous aerated solutions of DNA results in the formation of hydroperoxides of thymine residue in DNA.[1] One of the major products is cis-6-hydroperoxy-5-hydroxy-5,6-dihydrothymine (1),[2,3] which is known to have mutagenic activities.[4] In the studies of reaction of 1 with biomolecules, chemically induced dynamic nuclear polarization (CIDNP) was observed on treatment of 1 with ascorbic acid in D_2O by NMR spectroscopy. The reaction gave N-formyl-N'-pyruvylurea (2), although 2 was reported to be obtained from radiation[5] and oxidation with O_2 promoted with ascorbic acid and Cu(II) ion[6] of thymine.

Fig. 1. ^{1}H-CIDNP signals observed on reaction of 1 and ascorbic acid.

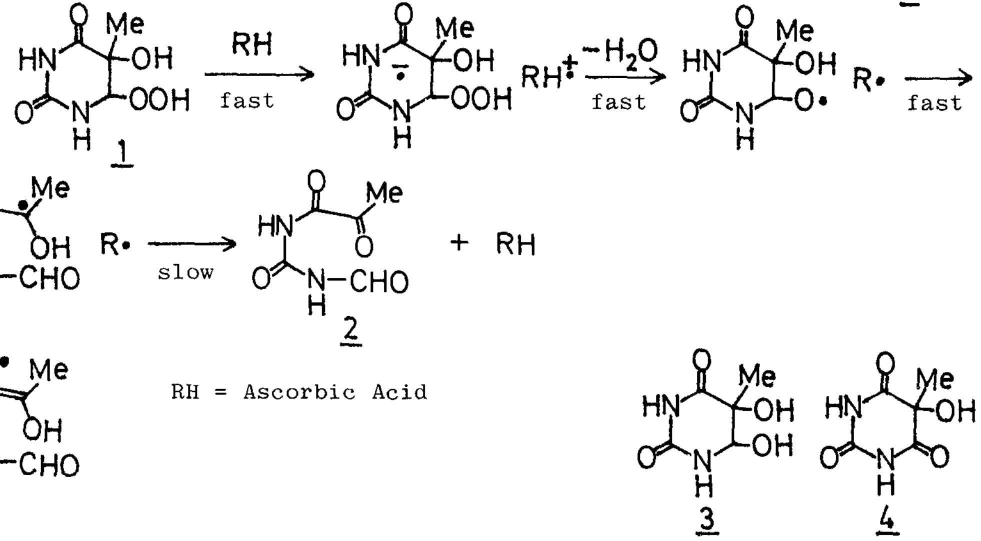


When a solution of 1[7] (10 mg) in D_2O (0.3 ml) added into a solution of ascorbic acid[8] (5, 10, 20, or 30 mg) in D_2O (0.3 ml), ^{1}H-CIDNP was observed by NMR spectroscopy. The CIDNP signals observed on treatment of 1 (10 mg) and ascorbic acid (10 mg) in D_2O at room temperature are illustrated in Fig. 1. The reaction gave 2 in 70 % yield. The structure of 2 was confirmed by comparing with IR spectrum[5] and ^{1}H-NMR data[9] reported for 2. Minor reaction products were cis-5,6-dihydroxy-5,6-dihydrothymine (3) and 5-hydroxy-5-methylbarbituric acid (4). The structures of 3 and 4 were confirmed by comparing with authentic samples prepared by oxidation of thymine with $KMnO_4$.[10]

In Fig. 1, two emission peaks at δ 1.80 and δ 2.40 were detected.

The emission peak at δ 1.80 was inverted within 5-8 min. On the other hand, the intensive emission peak at δ 2.40 was stable for more than 40 min and decreased with decreasing of the absorption peaks at δ 1.47 and δ 4.95 attributed to 1 and with increasing of the peaks at δ 1.80 and δ 8.90 attributed to 2. Furthermore, Fig. 1 shows that a large portion of ascorbic acid was intact after the reaction of 1. In view of the proposed mechanism for cleavage of C_5-C_6 bond of uracil by ionizing radiation[11] and our present results, the reaction of 1 and ascorbic acid may be explained as Scheme 1 and CIDNP observed may be attributed to a stable radical pair intermediate (5).

Scheme 1.



1 2 5 3 4

RH = Ascorbic Acid

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7. The compound 1 was prepared in 40 % yield by treatment of 3[10] with H_2O_2 in 0.1 M HCl (pH 1.0) at room temperature for 24 h according to the manner described by Hahn and Wang[3] and stable in water at room temperature for more than 3 days.
8. Guaranteed reagent from Wako Pure Chemicals or Specially Prepared Reagent for analysis of phenols from Nakarai Chemicals was used as ascorbic acid.
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