STUDIES ON CHEMICAL ALTERATIONS OF NUCLEIC ACIDS AND THEIR COMPONENTS. V.[1]

AMINATION REACTION OF 1,3-DIMETHYLURACIL WITH HYDROXYLAMINE-O-SULFONIC ACID

Mitsuaki Maeda and Yutaka Kawazoe

National Cancer Center Research Institute, Tokyo, Japan

We have paid attention to hydroxylamine-O-sulfonic acid on the basis of the assumption that it might be one of the simplest model compounds in carcinogenesis and mutagenesis of arylhydroxylamines.[1,2] In order to examine the reactivity of this agent toward pyrimidine bases of nucleic acids, this paper deals with the reaction with 1,3-dimethyluracil as a model system.

0.286 M of 1,3-dimethyluracil was treated with 4 molar equivalents of hydroxylamine-O-sulfonic acid at $37°$ for 40 hours in aqueous medium buffered with sodium phosphate to pH between 1.5 and 5.0, followed by being made alkaline and extracted with $CHCl_3$.   From the $CHCl_3$ extract 1,3-dimethyl-5-aminouracil(II) was obtained with a varying amount of the starting material(I), the yield depending on the pH of the medium.   At pH 2, the aminated product(II) was produced in almost quantitative yield.   The reaction did not proceed practically at pH below 1.0 nor above 5.0.   When the reaction was traced from the NMR spectra of the D_2O-based reaction mixture, it was found that the reaction proceeded via a considerably stable intermediate.   To compensate for the decrease in signals of the starting material(I) (δ_{NMe}, 3.12 and 3.45 ppm; δ_{5H}, 5.25 ppm; δ_{6H}, 7.66 ppm [3]), a new set of signals appeared and gradually became more intense (δ_{NMe}, 3.17 and 3.22 ppm; two doublets, 4.68 and 5.17 ppm and their coupling constant, 4.5 Hz [4]), until the intensity of the new set of signals reached its maximum after about 40 hours at room temperature.   After a lapse of several hours' reaction, the third set of signals due to the final product(II) had become observable (δ_{NMe}, 3.37 and 3.50 ppm;

δ_{6H}, 8.10 ppm [3]) but the intensity of these signals did not exceed 10% of the total intensity of the signals observed on the spectrum even after 40 hours' incubation.   The set of signals due to the reaction intermediate(III) was readily transfered to that of the final product(II) either by raising pH of the reaction mixture to nearly pH 7 or by heating the mixture to 80° for a few minutes.   Conversion of the intermediate(III) took place only to II but never back to the starting material(I).   When the reaction was carried out with the H_2O-based reaction mixture, the NMR spectrum of the intermediate showed two doublets due to 5-H and 6-H just the same as in the case of the D_2O-based one, revealing that no deuterium incorporation from the medium nor hydrogen exchange with the active hydrogens in the solvent took place during the reaction.   The reaction was traced also by UV spectroscopy, indicating that the intermediate has no appreciable absorption at around 260 nm. These results indicate that the structure of the intermediate is a 5,6-disubstituted 5,6-dihydrouracil derivative and that the carbon-5 must carry a nitrogen function leading to an amino group.

Taking into account the reactivities of the pyrimidine bases and this reagent in general, one may describe the reaction mechanism in the following two ways.

i) Initiated by nucleophilic attack of $NH_2OSO_3^-$ to the carbon-6.[5]
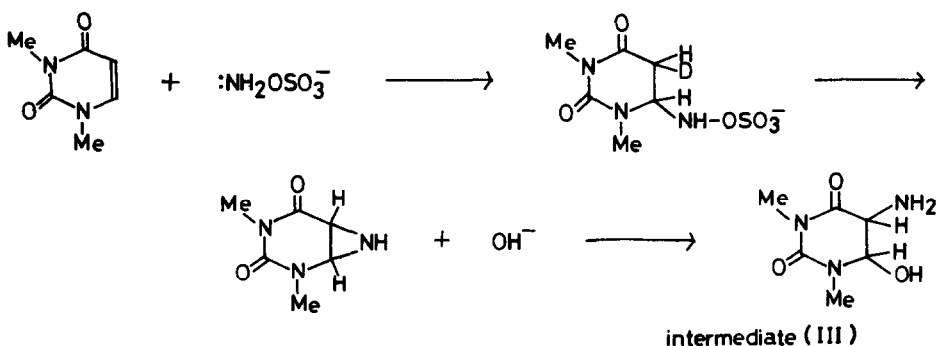
intermediate (III)

ii) Initiated by direct electrophilic attack of nitrenium ion (NH_2^+) produced from the reagent.[6]
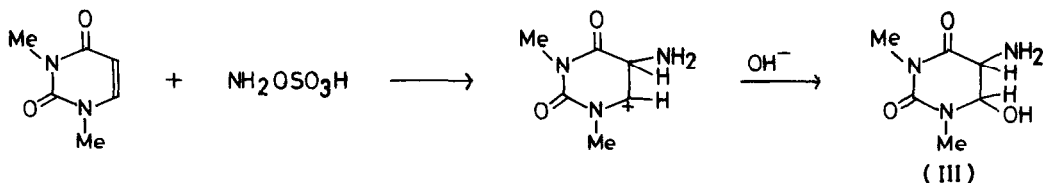
Another experimental evidence was obtained with regard to the reaction

mechanism.   Thus, the intermediate formation was greatly facilitated by the

presence of a catalytic amount of ferrous salts.   The rate was highly

dependent on the amount of the catalyst.   0.0142 M ferrous sulfate (0.05

molar equivalents to the substrate(I)) was effective enough for almost complete

conversion to the intermediate within a few hours and addition of 0.5 molar

equivalents of ferrous ion completed the reaction within one hour.   This

Fe^{++}-catalyzed reaction proceeded in acidic media from more than 10% sulfuric

acid to nearly neutral.   It is already known that hydroxylamine-O-sulfonic

acid is homolytically cleaved in the presence of ferrous ion to produce amino

radical ($NH_2 \cdot$ or $NH_3^{+\cdot}$) which is known to be electrophilic in nature.[7,8]

In addition, it has been suggested from ESR study that free radical addition of

amino radical takes place in the position-5 of uracil derivatives.[9]   It is,

therefore, concluded that the amination was initiated by a free radical addition

of amino radical to the position-5, followed by abstraction of an odd electron

from the carbon-6 by ferric ion to form the intermediate(III).[7]   This

mechanism can well explain the fact that the reaction required only a catalytic

amount of the ferrous salt.   The mechanism is formulated as follows.

$$NH_2OSO_3H \quad + \quad Fe^{++} \longrightarrow \quad \cdot NH_2 (\cdot NH_3^+) \quad + \quad Fe^{+++} + \quad HSO_4^-$$

(III)   (II)
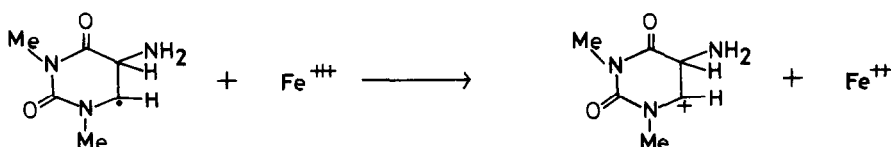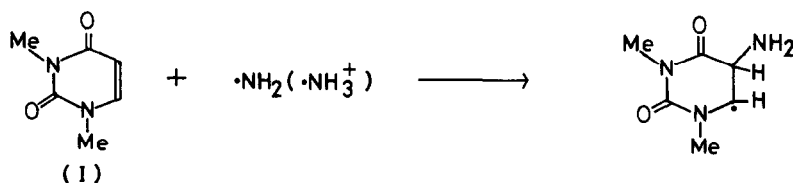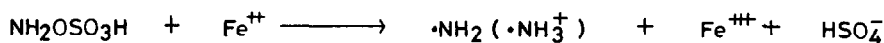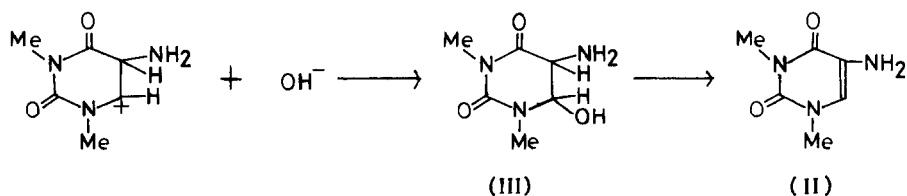
The result of the Fe^{++}-catalysis of the reaction may suggest that a free radical mechanism was involved also in the amination without the catalyst, because of the similarity in the intermediate formation between the experiments with and without ferrous ion.   If so, the reaction might have been catalyzed by a trace amount of metal ions which might have been contaminated in any of the reagents used for the experiment.   The strong pH dependence observed in this case may be related to both or either the catalytic efficiency of the ferrous ion and/or the pKa values (pKa_1, ?; pKa_2, 1.5 [8]) of the reagent.

Although the reaction mechanism is not clear yet in detail, the result obtained here may provide some promissing informations in exploring the chemical alteration of the nucleic acid bases from the chemical and biological interests. Studies along this line are now being pursued in our laboratory.

References

1) Part IV. : Y. Kawazoe, G.-F. Huang, Chem. Pharm. Bull., 20, 2073 (1972).
2) J. A. Miller, Cancer Res., 30, 559 (1970), and literatures cited therein.
3) Chemical shifts are presented in reference to the internal signal of DSS.
4) These values were read after addition of 60% D_2SO_4 to the reaction mixture upto the final D_2SO_4-concentration of 30% in order to move down field the water signal overlapping with the 6-H signal.   The resonance positions of other signals were not much affected by addition of sulfuric acid.
5) M. Hirobe, T. Ozawa, Tetrahedron Letters, 1971, 4493.
6) P. A. S. Smith, H. R. Alul, R. L. Baumgarten, J. Am. Chem. Soc., 86, 1139 (1964).
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