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STUDIES ON CHEMICAL ALTERATIONS OF NUCLEIC ACIDS AND THEIR COMPONENTS. VI.[1] N-AMINATION OF SOME NUCLEIC ACID BASES CONTAINING BASIC NITROGEN WITH HYDROXYLAMINE-O-ESTERS

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Our interest has recently been directed toward reactions of nucleic acid bases with hydroxylamine-O-esters which are assumed to be the simplest model reagent for induction of chemical damages of DNA among carcinogenic hydroxylamines.[1~3] Oxo-pyrimidines and -purines are readily aminated at lactam or lactim nitrogen by treatment with hydroxylamine-O-sulfonic acid (HAOS) in alkaline media probably through an electrophilic attack on anionic nitrogen of the conjugate base.[3~5] Amination of guanosine takes place at the carbon-8 with this reagent in acidic media.[2] As one of our series of studies along this line, we wish to report in this paper N-amination of the basic nitrogens of cytidine, adenosine, and their related compounds using HAOS or 2,4-dinitrophenoxyamine (DNPA).[6]

Adenosine or cytidine was treated with 3~10 equivalent moles of HAOS in phosphate buffer at around neutral pH at room temperature for several days. From the reaction mixture, only one product was isolated in each case in 10~40% yield. These products were much more conveniently prepared by using DNPA and dimethylformamide (DMF) instead of HAOS and aqueous solvents. Synthetic procedure and characterization of the products are as follows.

3-Aminocytidine Cytidine (1.0 mmole) and 1.2 mmoles of DNPA were dissolved

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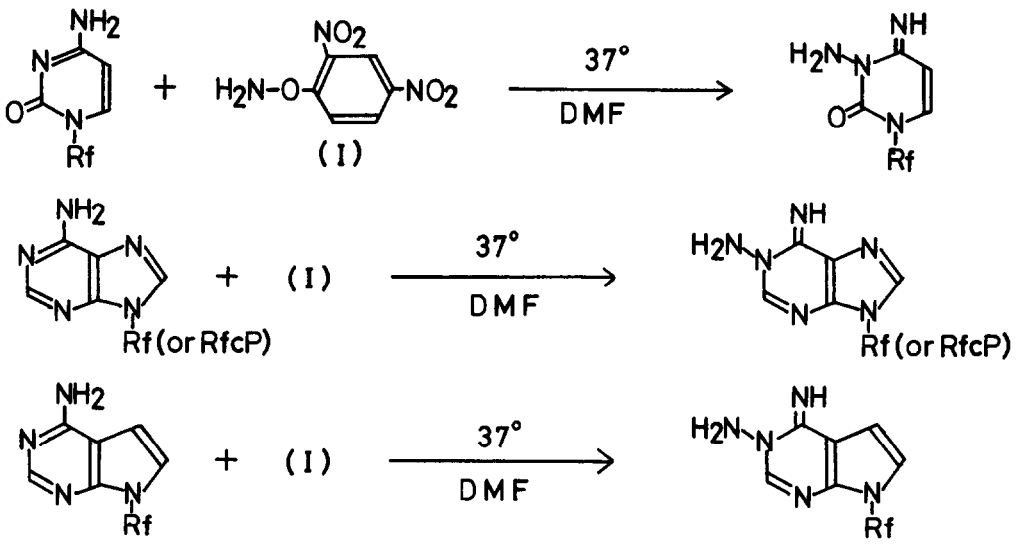
in 5 ml of DMF and kept standing at 37° for 12 hr. After evaporation of the solvent in vacuo, the resulting syrup was poured into ice-water and acidified by dil. HCl. Dinitrophenol thus freed was extracted with ether and then, 2~3 volumes of ethanol were added to the aqueous layer to form precipitates. They were dissolved in water and diluted with ethanol to give white needles in more than 90% yield. m.p. 181~196°(dec). Anal. Calcd. for $C_9H_{15}N_4O_5Cl$; C 36.64, H 5.09, N 19.00. Found; C 36.73, H 5.50, N 18.77. PMR(in D_2O): two doublets for two ring protons and signals for sugar protons. UV:λ max, 278 nm(pH 1), 278 nm(pH 7), 267 nm(pH 11). The maximum at pH 1 is identical with that of 3-methylcytidine (278 nm). The structure was proved to be 3-aminocytidine by, in addition to the spectroscopic data, the fact that this amination product was readily deaminated by $NaNO_2$-treatment in aqueous acetic acid to give cytidine quantitatively.

1-Aminoadenosine Adenosine (10 mmoles) and 15 mmoles of DNPA were dissolved in 70 ml of DMF and kept standing at 37° for 24 hr. After the solvent was evaporated in vacuo to a half volume, it was acidified with dil. HCl, washed with ether, and then diluted with appropriate amounts of ethanol and ether, when crystalline precipitates came out. The crude material (90% yield) was dissolved once in a small amount of water and reprecipitated by addition of ethanol and ether to white needles of monoaminated product. m.p. 192~193°(dec). Anal. Calcd. for $C_{10}H_{15}N_6O_4Cl$; C 37.67, H 4.70, N 26.37. Found; C 37.68, H 4.70 N 26.07. PMR(in D_2O): two singlets for two ring protons and signals for sugar protons. pKa: 9.0. UV:λ max, 257 nm(pH 1), 257 nm(pH 7), 257 and 290 nm(shoulder)(pH 11). This monoaminated product was readily deaminated to adenosine by treatment of a small excess of $NaNO_2$ in aqueous acetic acid. Thereby, N^6-deaminated product (inosine) was not detected. This finding indicates that the product is a derivative aminated at either N^1, N^3, or N^7 of adenosine. The structure was finally determined as 1-aminoadenosine from the following experimental evidence. 1) Since the glycosidic bond of the product was stable in acidic media, the position of the amino group is probably not N^7. 2) By analogy with protonation, methylation, and N-oxygenation of adenosine, N-amination is considered to take place at N^1 which is the most electron-rich

and sterically favorable for approach of the reagent. 3) The pKa of 9.0 seems to be smaller than that expected for N^7-aminoadenosine which is in equilibrium with ylide structure of N^+-NH^-. (For example, pKa of N-aminopyridine is 11.0.) The product did not show the UV red-shift characteristic of the aromatic ylide structure in strongly alkaline media.[7] Deprotonation must take place at the neighbouring NH_2 group through valence tautomerism but not at the introduced N-amino group. 4) UV absorption maximum of the product at pH 1 is almost the same as those of 1-methyl and -alkoxy derivatives of adenosine in acidic media (257 and 260 nm, respectively), whereas it considerably deviated from that of 3,5'-cyclic adenosinium iodide (272 nm). Although the product did not afford the 6-hydrazino derivative under the reaction condition for Dimroth rearrangement, the structure was conclusively suggested to be 1-aminoadenosine by these chemical and physical properties of the product.

N-Amination of Related Compounds In order to examine the applicability of this amination method to nucleic acid bases, the following two derivatives were chosen. 1-Aminoadenosine-3',5'-cyclic monophosphate: Triethylammonium salt of 3',5'-cyclic AMP (2.46 mmoles) and 3.96 mmoles of DNPA were dissolved in 10 ml of DMF and kept standing at 37° for one day. Dil. HCl was added to the reaction mixture, washed with ether, and the resulting precipitates in the aqueous layer were collected. As soon as they were dissolved in cold dil. NaOH, ethanol and then ether were added for precipitation of white needles. The yield was 36%. m.p. 262~263(dec). Anal. Calcd. for $C_{10}H_{12}N_6O_6PNa$; C 32.78, H 3.27. Found; C 32.48, H 3.87. The UV spectra of the product were superimposed in acidic and neutral media with those of 1-aminoadenosine, respectively. In addition, PMR data agree with the structure of N-aminoadenosine-cyclic monophosphate. 1-Aminotubercidin: Tubercidin (7-deazaadenosine)(3.9 mmoles)[8] was treated with 4.5 mmoles of DNPA in 25 ml of DMF and 20 ml of methanol. After a similar treatment to that for cytidine for purification of the product, 1-aminotubercidin was isolated as colorless needles in 93% yield. m.p. 230~232°. Anal. Calcd. for $C_{11}H_{16}N_5O_4Cl$; C 41.54, H 5.03, N 22.03. Found; C 41.52, H 4.97, N 21.82. UV:λmax, 271 nm(pH 1), 271 nm(pH 7), 263(shoulder), 269, and 292(shoulder) nm(pH 11). The UV absorption maximum in acidic media



Rf: β-D-ribofuranosyl
RfcP: β-D-(3,5-cyclic phospho)ribofuranosyl

was the same as that of 1-methyltubercidin. PMR data also supported the fact that the product was 1-aminotubercidin.

The amination method using hydroxylamine-O-esters [9] may be generally applicable to nucleosides, nucleotides, and the related derivatives containing basic nitrogens. It is worth noting that the N-aminated product undergoes unusual alkaline degradation. The details are to be published in a forthcoming paper.

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