Communications to the Editor

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SYNTHESIS OF 7-AMINOGUANOSINE AND ITS CONVERSION TO 8-SUBSTITUTED DERIVATIVES[1)]

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Guanosine is aminated with 2,4-dinitrophenoxyamine in DMF-H_2O to form 7-aminoguanosine which is readily converted mainly to 8,5′-O-cycloguanosine and 8-hydroxyguanosine by warming in H_2O. The aminated product from deoxyguanosine is 7-aminoguanine.

KEYWORDS —— amination; 7-aminoguanosine; 7-aminoguanine; 8-hydroxyguanosine; 8,5′-O-cycloguanosine; carcinogenesis

Genotoxic arylhydroxylamines are converted in cells to certain reactive metabolites capable of chemical modification of cellular DNA. The reactive species involved are considered to be O-acyl derivatives of arylhydroxylamines which aminate nucleic acid bases via the electrophilic nitrenium intermediates.[2)] In this connection, we have studied direct amination of nucleic acid components using hydroxylamine-O-sulfonic acid or 2,4-dinitrophenoxyamine (DNPA) as a simple electrophilic aminating agent. Some results were reported previously.[3)]

In this communication, we report the synthesis of 7-aminoguanosine by direct amination with DNPA and its conversion to 8,5′-O-cycloguanosine and 8-hydroxyguanosine. This is discussed in connection with the chemical lesions induced in cellular DNA exposed to carcinogenic arylhydroxylamines.[2,4)]

Aminations of Guanosine and Deoxyguanosine

Guanosine (I) was treated with 4 eq. mol of DNPA in DMF-H_2O (3:1, v/v) at 45°C for 4 days. After removal of the solvent, the residue was dissolved in aqueous dil. HCl and washed with AcOEt. The aqueous portion was evaporated under a reduced pressure into dryness, and subjected to chromatographic separations on a Whatman 3MM paper (developed with H_2O or iPrOH/1%$(NH_4)_2SO_4$ = 2/1, v/v) and/or in a Sephadex LH20 column (eluted with H_2O). A main product (II) in 35-45% yield, two minor products (III and IV) and the recovered starting material were obtained.[5)] The UV spectra of the main product II at various pH's (λ_{max}: 256 nm and 280(sh) in H_2O and at pH 1; 263 at pH 12) were very similar to those of 7-methylguanosine (λ_{max}: 256 and 280(sh) in H_2O and at pH 1; 264 at pH 12). The NMR spectrum measured in DMSO-d_6 (δ: 5.82 (s, 1′-H); 6.93 (s, 2-NH_2); 9.22 (s, 8-H)) showed the presence of the 8-H at δ 9.22 (much more deshielded than that of guanosine, δ 7.93) which was readily exchangable with a D atom upon addition of D_2O. This suggests that II may be 7-aminoguanosine which includes the ^{7}N-cationic structure.[6)] Isolation of II in a crystalline form failed because it was gradually converted to the minor products, III and IV, during purification procedures in aqueous media. Then,

when II was warmed in H_2O at 100°C for 1 h, it was completely converted mainly to III and IV in 30% and 65% yields, respectively. In the structural identification of these products, the NMR spectrum of III (δ: 3.5 - 4.5 (2'-, 3'-, 4'-, 5'-H's); 5.32 (d, 3'-OH); 5.54 (d, 2'-OH); 5.72 (s, 1'-H); 6.48 (s, 2-NH_2); 10.6 (s, 1-NH)) showed that the 8-position was substituted. III (mp 300°C) was relatively stable in neutral and alkaline media; no change took place when III was treated with 0.1 N NaOH at 100°C for 8 h. In contrast, when treated with 0.1 N HCl at 100°C for 8 h it was converted almost quantitatively into IV (8-hydroxyguanosine) as described later. The treatment of III with acetone and HCl gas in dimethoxypropane-DMF gave a product which was identical with the product obtained by the same treatment of 2',3'-O-isopropylidene-8,5'-O-cycloguanosine.[7] All of this, in addition to the mass spectral data (m/z 569 for M^++$(TMS)_4$) suggest that III is 8,5'-O- cycloguanosine (UV λ_{max}: 251 and 277(sh) in H_2O and at pH 1; 257 and 268(sh) at pH 12). In addition, III showed the positive Cotton effect in its CD spectrum and the fine-structural change of 1'-H signal (δ 5.72) into a singlet in its NMR spectrum. These are both known to be characteristic of 8,5'-O-cyclopurine ribosides.[8] It is worth noting that 8,5'-O-cycloguanosine is a new compound which is too unstable to be prepared by the partial hydrolysis of its isopropylidene derivative,[7] because of the concomitant hydrolysis of the O-cyclo function. Another product, IV, was identified with the authentic preparation of 8-hydroxyguanosine.[9] It is worth mentioning here that a trace amount of 8-aminoguanosine (V)[9] was identified on the chromatogram of the reaction mixture after the conversion of II. It is possible that V might be a conversion product produced by an intramolecular rearrangement of 7-NH_2 group to the 8-C position of II through the diaziridine intermediate. Alternatively, the preparations of II used for the hydrolytic conversion might have been contaminated with V which had been produced in the amination step. This is open to further investigation.

Next, deoxyguanosine (VI) was subjected to direct amination under the same conditions described for guanosine. From the reaction mixture, a monoaminated product, VII (Mass: m/z 454 for M^++$(TMS)_4$) was isolated in 20% yield with the recovered deoxyguanosine and deglycosylated guanine. The NMR spectrum of VII measured in DMSO-d_6 (δ: 6.09 (br s, 2-NH_2 and 7-NH_2); 7.70 (s, 8-H)) indicated the absence of the sugar moiety and the presence of the 8-H of the guanine moiety. Its UV spectra at various pH's (λ_{max}: 251 and 272(sh) at pH 1; 245 and 282 in H_2O; 241(sh) and 279 at pH 12) were almost identical with those of 7-methylguanine (λ_{max}: 251 and 272(sh) at pH 1; 245 and 282 in H_2O; 241(sh) and 279 at pH 12). The treatment of VII with $NaNO_2$ in AcOH gave guanine. Taking account of the fact that 7-substituted cationic deoxyguanosines are much more susceptible to acid-hydrolysis than the corresponding ribosides, VII is 7-aminoguanine which was produced by deglycosylation of 7-aminodeoxyguanosine. From these results, the reaction scheme can be formulated as follows.

With regard to the reactivity of guanosine, the first protonation takes place at the 7-N position and alkylation in neutral media also occur mainly at the same position. The present study revealed that the amination with DNPA occurred almost exclusively at the most basic and nucleophilic center of the 7-N position. ^{7}N-Aminoguanosine thus formed readily suffers from nucleophilic attacks on the 8-C position, followed by the elimination of NH_3. Thus, an intramolecular attack by

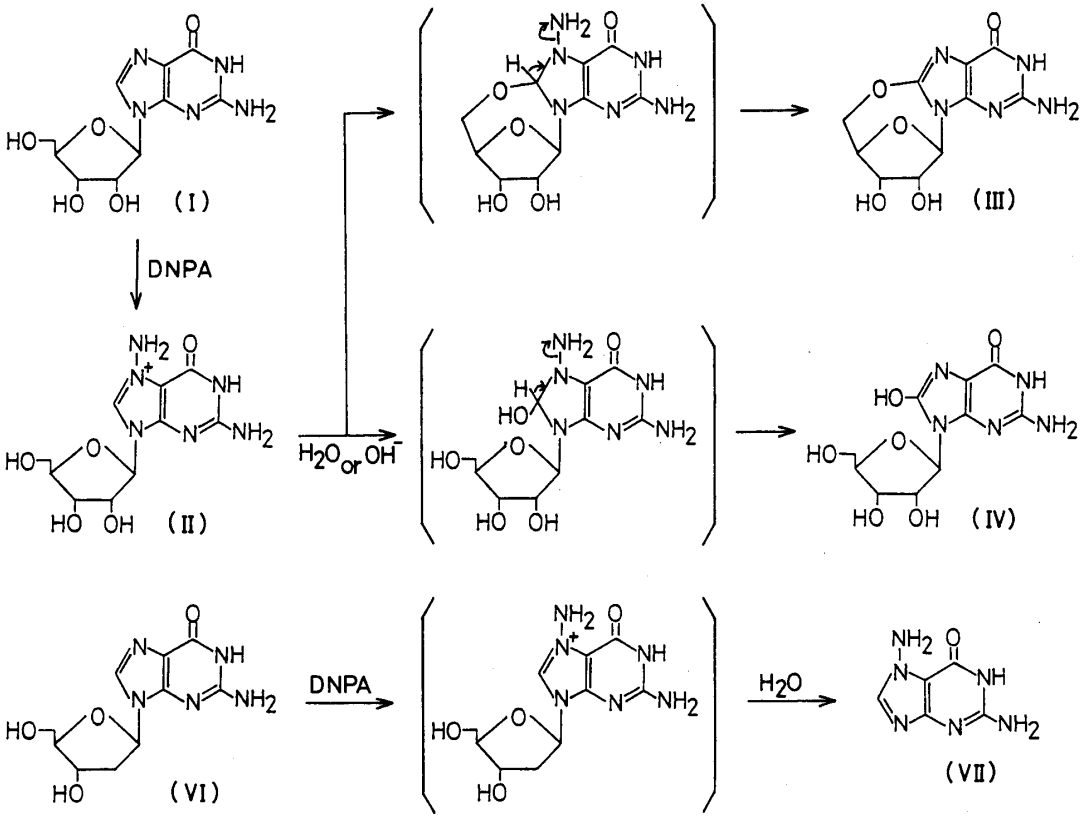

Chart 1. Amination with Dinitrophenoxyamine (DNPA) in Aqueous Dimethylformamide

the 5'-OH group of the sugar moiety and an intermolecular attack by a hydroxide ion or water molecule result in the formation of 8,5'-O-cycloguanosine and 8-hydroxyguanosine, respectively. This type of nucleophilic addition followed by re-aromatization through the deamination of N-aminoaromatics has not been reported except for the rearrangements of 1-amino-quinoline or -pyridine to cyano-quinoline or -pyridine in aqueous media.[10] With regard to deoxyguanosine, it is rational that deglycosylation takes place in the DMF-H_2O solvent system used for the amination prior to the nucleophilic attacks on the 8-C, so that 7-aminoguanine is the only product.

Implication in Chemical Modification of Cellular DNA Produced by Carcinogenic Arylhydroxylamines

Chemical modifications found in cellular DNA on exposure to carcinogenic arylhydroxylamines have been intensively studied and some covalently bound adducts of the carcinogens with nucleic acid bases are known: ^{8}C-, ^{2}N-, and ^{6}O-substituted guanine and ^{6}N-substituted adenine. However, no ^{7}N-substitutions of guanine moiety have been evidenced although alkylations are well known to occur predominantly at this position. The present study implies a possibility that electrophilic arylaminations might take place at the 7-N position of guanine and

that the ^{7}N-aminated products are readily rearranged to 8-hydroxyguanine, accompanied by loss of the once bound carcinogens. Most of studies on the carcinogen-bindings have so far been carried out by tracing the radioactivity of labeled carcinogens bound to nucleic acid bases. So that it is not surprizing that 8-hydroxyguanine has not been detected, even if it should have been produced in DNA at a certain frequency lower than 10^{-5} or so. On this working hypothesis, the authors have tried to determine whether 8-hydroguanine is produced in the cellular DNA treated with a representative carcinogenic arylhydroxylamine, 4-hydroxyaminoquinoline 1-oxide (4HAQO), in collaboration with Drs. Kasai and Nishimura who have previously proved that 8-hydroxyguanine was produced in the DNA after X-ray irradiation. Our recent result along this line definitely revealed that the treatment of DNA with 4HAQO produced 8-hydroxyguanine, in both in vivo and in vitro systems, up to about 30% of the total 4HAQO-bound bases.[4] It is worth emphasizing that the present study gives chemical evidence that formation of 8-hydroxyguanine is one of the major chemical injuries induced in the DNA exposed to carcingenic 4HAQO, and possibly to other carcinogenic arylhydroxylamines. Studies along this line are now being pursued.

REFERENCES AND NOTES

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