Direct C-Amination of Guanosine with Hydroxylamine-O-Sulfonic Acid. Simplest Model Reaction possibly involved in DNA Damage leading to Carcinogenesis by N-Arylhydroxylamines

An increasing number of studies have recently been done on chemical modifications of DNA with chemical carcinogens in connection with the cancerization mechanism. It has been proposed that a wide variety of carcinogens such as acetamidofluorenes, azo dyes, arylamines, and 4-nitroquinoline 1-oxides are metabolically activated to their corresponding O-acetylated arylhydroxylamines to induce cancer. In order to obtain basic information for this group of carcinogenesis, the present study deals with the reaction of guanosine, a target moiety of DNA presumably involved in certain chemical carcinogenesis, with hydroxylamine-O-sulfonic acid, one of the simplest examples of the hydroxylamine-O-acid conjugate.

Hydroxylamine-O-sulfonic acid (2.40 g, 21.2 mmole) in 25 ml of water was added in drops to a suspension of 0.60 g (2.12 mmole) of guanosine in 25 ml of water containing 2.70 g (27.5 mmole) of potassium acetate previously warmed to 70°. The combined reaction mixture became a clear solution instantaneously and remained at 70° for 2 hr. During this period, the pH of the mixture was adjusted to around 3.0 by dropwise addition of diluted potassium acetate. Consumption of hydroxylamine-O-sulfonic acid was quantitatively traced by titrating iodine liberated from potassium iodide added with 0.05 M sodium thiosulfate. At the end of a 2 hr reaction, 90% of the reagent was consumed. Thin-layer chromatography of the reaction mixture using an Avicel plate developed with isopropanol-water-1% ammonium sulfate (2:1) showed that the product was formed in 25% yield accompanied by 75% recovery of guanosine. For the preparative isolation of the product, the reaction mixture was cooled in an ice box. After the solid material (recovered guanosine) was filtered off, the filtrate was separated into the product and guanosine by paper or column chromatography. The product was preparatively isolated in more than 20% yield.

The product thus obtained was identified with an authentic specimen of 8-aminoguanosine, which was kindly supplied by Dr. Bunji Shimizu of Sankyo Co., Ltd., by UV spectroscopy ($\lambda_{\text{max}} (\varepsilon)$: 251 nm (16900) and 289 nm (9600); $\eta_{\text{max}} (\varepsilon)$: 258 nm (15500) and 280 nm (shoulder), and by co-chromatography using several solvent systems. The reaction seems to proceed without any by-product formation. Hydrolysis of glycosidic bond was not detected in either the product or guanosine by thin-layer chromatography. It is of interest that this reaction did not proceed in media of pH below 1.0 or above 4.0. At pH's below 1.0, 8-amino derivatives of guanosine or guanine was not detected at all. At pH's above 7.0, instead of 8-aminoguanosine, N3-aminoguanosine was produced in almost quantitative yield as previously reported by Broom and Robins. This characteristic pH-dependence of the C-8

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6) Esters of hydroxylamines and acids including sulfenic acid.
8) Decrease in the pH of the solution to below 2.5 produced glycoside bond fission to give 8-aminoguanine and guanine as by-products.
amination reaction suggests that the reaction involves either (i) a nucleophilic attack of NH$_2$OSO$_2$ to C-8 of N$^2$-protonated species of guanosine, (ii) an electrophilic attack of NH$_2$-OSO$_2$H to C-8 of neutral guanosine or to its N-7 followed by migration to C-8, (iii) an electrophilic attack of nitrene NH or nitrenium ion NH$_3^+$ produced from the anionic form of 
\[
\text{H}\text{N}-\text{O}^->\text{N}\text{H}^-
\]
(iv) a free radical attack of ·NH$_2$ or ·NH$_3^+$ to C-8 of neutral guanosine.

The details are now under investigation.

It is to be noted that adenosine did not give the 8-amino derivative under a similar reaction condition to that described here. Pending the elucidation of the reaction mechanism, this result may suggest a possibility that amination with hydroxylamine-O-sulfonic acid might be the simplest model for the DNA damage involved in carcinogenesis by N-arylhydroxylamines.$^{10}$

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$^{10}$ Another possibility of N$^2$-amination might be less probable in in vivo reactions with double stranded DNA in the cell nucleus.

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