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Reaction of 1-Naphthylhydroxylamine with Calf Thymus Deoxyribonucleic Acid. Isolation and Synthesis of N-(Guanin-C^8-yl)-1-naphthylamine

A possible activated form of 1-naphthylamine, N-hydroxy-1-naphthylamine, reacted with the C^8-position of guanine in deoxyribonucleic acid (DNA). Hydrolysis of the modified DNA with nuclease and acid gave N-(guanin-C^8-yl)-1-naphthylamine as a modified base. The C^8-position of guanine seems to be a common site of attack on DNA by activated muta-carcinogenic aromatic amines.

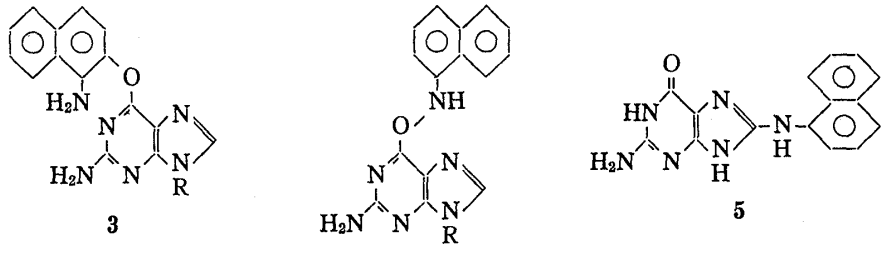
Keywords——1-naphthylamine; N-hydroxy-1-naphthylamine; aromatic hydroxylamine; modification of DNA; carcinogen; mutagen

In chemical carcinogenesis, metabolically activated carcinogens are thought to modify DNA. One important group of carcinogens such as 2-acetylaminofluorene (2-AAF), 4-dimethylaminoazobenzene (DAB), 3-amino-1-methyl-5H-pyrido[4,3-*b*]indole (Trp-P-2) and 2-amino-6-methyldipyrido[1,2-*a*: 3′,2′-*d*]imidazole (Glu-P-1) is an aromatic amine and the metabolically activated forms of all these amino carcinogens are electrophilic hydroxylamine derivatives.

1-Naphthylamine (**1**, NA) is known to be a carcinogen of the urinary bladder,[1] and its N-hydroxy derivative, N-hydroxy-1-naphthylamine (**2**, N-OH-1-NA), is thought to be its metabolically activated form.[2] It has been reported that **2** binds to DNA and ribonucleic acid covalently at a slightly acidic pH, and the structures of the major modified nucleosides were proposed to be 2-(deoxyguanosin-O^6-yl)-1-naphthylamine (**3**) and N-(deoxyguanosin-O^6-yl)-1-naphthylamine (**4**).[3]

However, activated forms (electrophilic hydroxylamine derivatives) of many muta-carcinogenic aromatic amines attack the nucleophilic C^8-position of guanine in DNA: the major bases in DNA modified by 2-AAF and DAB are derivatives of the C^8-position of guanine.[4,5] Recently the activated forms of Trp-P-2 and Glu-P-1 were found to modify native DNA almost entirely at the C^8-position of its guanine.[6,7] This finding prompted us to examine modification of the C^8-position of guanine of DNA by N-OH-1-NA *in vitro*.

N-OH-1-NA was prepared by reduction of 1-nitronaphthalene by the method of



3 **4** **5**

Chart 1

Willstaetter and Kubli.[8] N-(Guanin-C^8-yl)-1-naphthylamine (**5**) was prepared by nucleophilic substitution at the C^8-position of N^3-acetoxyguanine by 1-naphthylamine in DMSO-DMF (50: 5).[6,7,9] The reaction mixture was condensed to 1/5 of its volume, and water was added. Insoluble material was collected by centrifugation, washed successively with distilled water, CH_3OH and CH_2Cl_2, and dried. Crude crystals were recrystallised from 1 N aqueous HBr to give needles with mp>300°C in about 40% yield after purification. The structure of **5** was deduced by mass spectrometry [M^+(obs.) 292 ($C_{15}H_{12}N_6O$)], infrared spectroscopy and elemental analysis. The ^{1}H-NMR of **5** showed no signal of a guanine C^8-proton. Moreover, the ^{13}C-NMR showed that the signal of the guanine C^8-carbon, which is usually observed at about 138 ppm, is found at a lower magnetic field (148 ppm) as a singlet signal. Alkaline degradation in a sealed tube under degassed conditions gave 1-naphthylamine and uric acid as major products. These results indicate the presence of a bond between guanine C^8 and the nitrogen atom of NA.

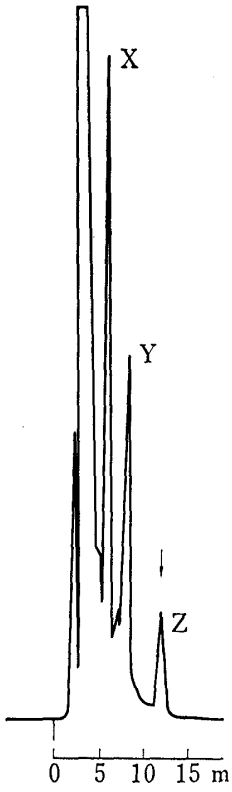


Fig. 1. High Performance Liquid Chromatography of the Modified Base Mixture obtained by Hydrolysis of Fluorescent DNA modified by N-Hydroxy-1-naphthylamine

Solvent: 25% CH_3OH- 1% NH_4OH- 74% H_2O.
Flow rate: 1.5 ml/min.
Column: Radial Pak A (a reversed phase column, Waters Associates).
Detector: Waters Model 440 at 254 nm.
The largest peak is a mixture of normal nucleotides.

Incubation of DNA with N-OH-1-NA was carried out under an argon atomosphere at 37°C in 10 mM potassium citrate buffer (pH 5) containing 0.1 mM EDTA, 5 mg/ml of N-OH-1-NA and 5 mg/ml of nucleic acid. Unreacted N-OH-1-NA and its degradation products were removed by CH_2Cl_2 extraction. Nucleic acids were isolated from the reaction mixture by adding ethanol, and dissolved in water. For removing compounds of low molecular weight, the DNA solution was subjected to Sephadex G-25 column chromatography, with water as solvent. Fractions with fluorescence were combined and lyophilised.

The NA-bound nucleic acids thus obtained were dissolved in water, and denatured by heating at 100°C for 10 minutes. The solution was adjusted to pH 5 with 0.05 M potassium acetate and NA-bound DNA was hydrolyzed enzymatically to NA-bound nucleotides with nuclease P_1 at 37°C. Sephadex G-25 column chromatography, in aqueous 0.3% NH_4Cl solution, gave NA-bound nucleotide mixtures, which were eluted after the normal nucleotides. The fractions of modified nucleotides were hydrolyzed with 1 N aqueous HCl at room temperature to NA-bound bases. Portions of the NA-bound bases thus obtained were identified by high performance liquid chromatography. Three major peaks were obtained, which were named X, Y and Z in order of elution (Fig. 1).

Compound (Z), which was eluted slowest, was identified as **5** by the following findings: (1) The retention time of Z was identical with that of synthetic **5**. (2) The fluorescence and excitation spectra of Z in neutral, acidic and basic media were identical with those of **5**. (In acidic conditions, the fluorescence of both was quenched.) (3) The ultraviolet spectrum of Z isolated by HPLC had maxima at 275 nm and 372 nm, as in **5** (Fig. 2). Thus, we concluded that Z is identical with synthetic N-(guanin-C^8-yl)-1-naphthylamine. The C^8 position of guanine is the most frequently modified by muta-carcinogenic aromatic amines, but the effect

of this modification in chemical carcinogenesis is open to debate. Structural studies on modified bases X and Y are in progress.

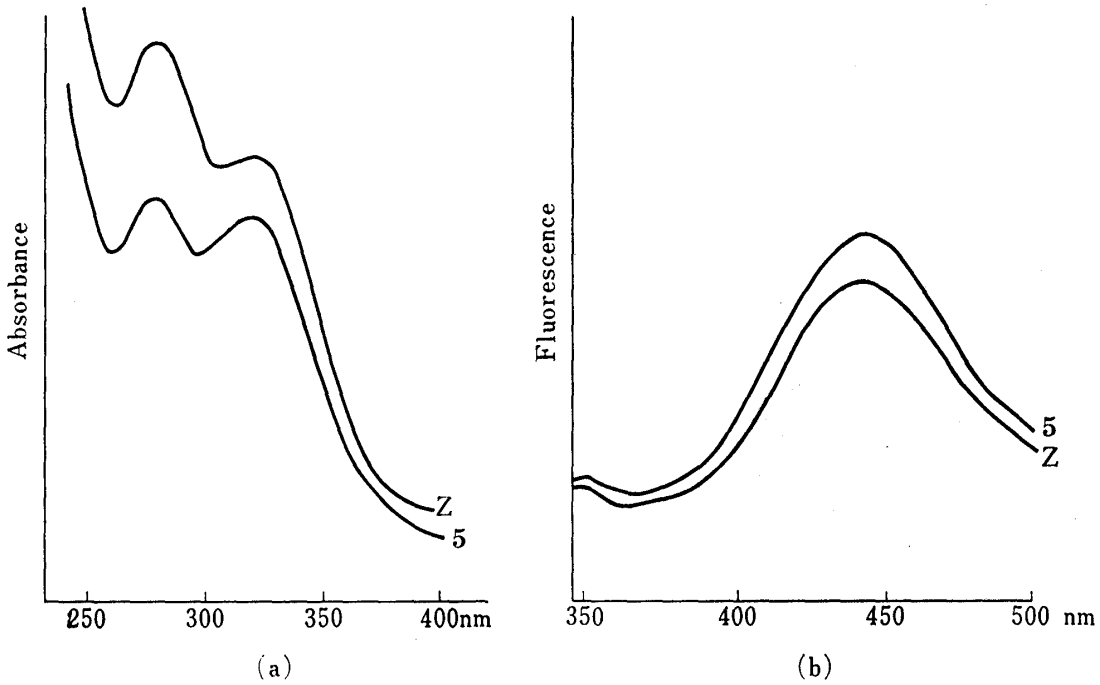


Fig. 2. Ultraviolet and Fluorescence Spectra of 1-Naphthylamine-bound Base

(a) UV spectra were obtained with a stopped flow ultraviolet absorption monitor, Shimadzu SPD-1 system (25% CH_3OH-1% NH_4OH- 74% H_2O).
(b) Fluorescence spectra were obtained in water with a Hitachi MPF-4 apparatus (excitation : 310 nm).

References and Notes

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