

Pharmaceutical Sciences,
Teikyō University,
Sagamiko-cho, Tsukui-gun,
Kanagawa 199-01, Japan

DEN-ICHI MIZUNO

Received May 6, 1981

[Chem. Pharm. Bull.]
29(9)2730-2732(1981)

Reaction of 1-Naphthylhydroxylamine with Calf Thymus Deoxyribonucleic Acid. Isolation and Synthesis of N-(Guanin-C⁸-yl)-1-naphthylamine

A possible activated form of 1-naphthylamine, N-hydroxy-1-naphthylamine, reacted with the C⁸-position of guanine in deoxyribonucleic acid (DNA). Hydrolysis of the modified DNA with nuclease and acid gave N-(guanine-C⁸-yl)-1-naphthylamine as a modified base. The C⁸-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-O⁶-yl)-1-naphthylamine (3) and N-(deoxyguanosin-O⁶-yl)-1-naphthylamine (4).³⁾

However, activated forms (electrophilic hydroxylamine derivatives) of many muta-carcinogenic aromatic amines attack the nucleophilic C⁸-position of guanine in DNA: the major bases in DNA modified by 2-AAF and DAB are derivatives of the C⁸-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 C⁸-position of its guanine.^{6,7)} This finding prompted us to examine modification of the C⁸-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

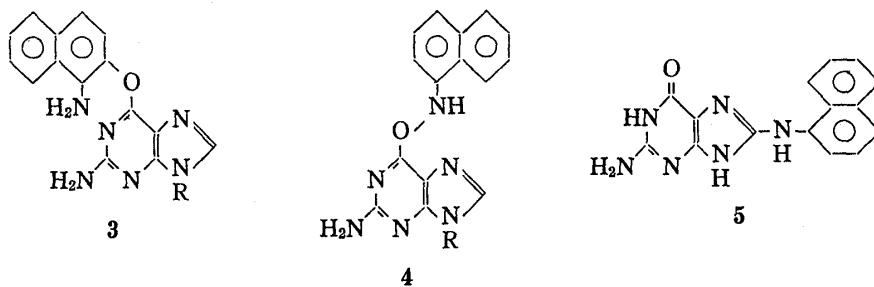


Chart 1

Willstaetter and Kubli.⁸⁾ N-(Guanin-C⁸-yl)-1-naphthylamine (**5**) was prepared by nucleophilic substitution at the C⁸-position of N³-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,

CH₃OH and CH₂Cl₂, 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 (C₁₅H₁₂N₆O)], infrared spectroscopy and elemental analysis. The ¹H-NMR of **5** showed no signal of a guanine C⁸-proton. Moreover, the ¹³C-NMR showed that the signal of the guanine C⁸-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 C⁸ and the nitrogen atom of NA.

Incubation of DNA with N-OH-1-NA was carried out under an argon atmosphere 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 CH₂Cl₂ 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 enzymati-

cally to NA-bound nucleotides with nuclease P₁ at 37°C. Sephadex G-25 column chromatography, in aqueous 0.3% NH₄Cl 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-C⁸-yl)-1-naphthylamine. The C⁸ position of guanine is the most frequently modified by muta-carcinogenic aromatic amines, but the effect

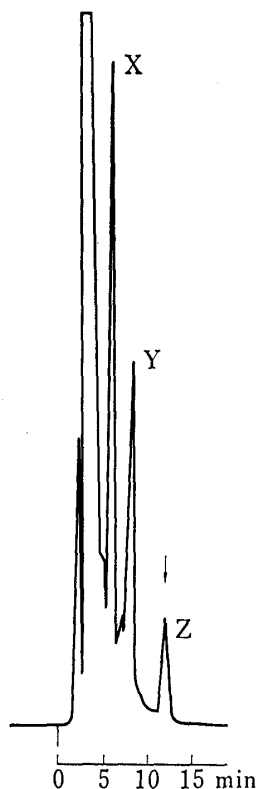


Fig. 1. High Performance Liquid Chromatography of the Modified Base Mixture obtained by Hydrolysis of Fluorescent DNA modified by N-Hydroxy-1-naphthylamine

Solvent: 25% CH₃OH-1% NH₄OH-74% H₂O.
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.

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

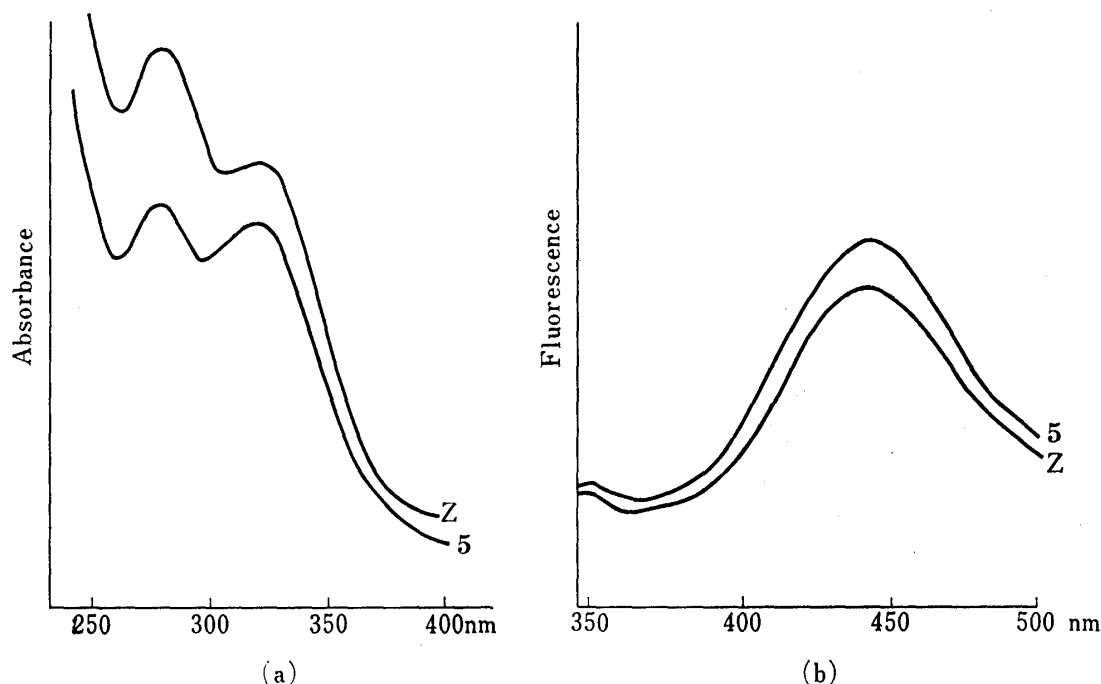


Fig. 2. Ultraviolet and Fluorescence Spectra of 1-Naphthylamine-bound Base

- (a) UV spectra were obtained with a stopped flow ultraviolet absorption monitor, Shimadzu SPD-1 system (25% CH₃OH-1% NH₄OH- 74% H₂O).
 (b) Fluorescence spectra were obtained in water with a Hitachi MPF-4 apparatus (excitation : 310 nm).

References and Notes

- 1) a) F.F. Kadlubar, J.A. Miller, and E.C. Miller, *Cancer Res.*, **37**, 805 (1977); b) *idem, ibid.*, **38**, 3628 (1978).
- 2) a) S. Belman, W. Troll, B. Teebor, and F. Mukai, *Cancer Res.*, **28**, 535 (1968); b) J.L. Radomski, E. Brill, *Science.*, **167**, 992 (1970).
- 3) F.F. Kadlubar, J.A. Miller, and E.C. Miller, *Cancer Res.*, **38**, 3628 (1978).
- 4) J.A. Miller, *Cancer Res.*, **30**, 559 (1970).
- 5) J.K. Lin, J.A. Miller, and E.C. Miller, *Cancer Res.*, **35**, 844 (1975).
- 6) Y. Hashimoto, K. Shudo, and T. Okamoto, *Chem. Pharm. Bull.*, **27**, 1058 (1979).
- 7) Y. Hashimoto, K. Shudo, and T. Okamoto, *Chem. Pharm. Bull.*, **27**, 2532 (1979).
- 8) R. Willstaetter and H. Kubli, *Chem. Ber.* **41**, 1936 (1908).
- 9) N.J.M. Bindsall, J.C. Parham, V. Woelke, and G.B. Brown, *Tetrahedron*, **28**, 3 (1972).

Faculty of Pharmaceutical Sciences,
 University of Tokyo,
 Bunkyo-ku, Tokyo

YOSHINOBU MUROFUSHI
 YUICHI HASHIMOTO
 KOICHI SHUDO
 TOSHIHIKO OKAMOTO*

Received June 29, 1981