Communications to the Editor

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Structure of a Base in DNA modified by Glu-P-1

A mutagen, 2-amino-6-methyldipyrido[1,2-*a*: 3′,2′-*d*]imidazole (Glu-P-1), was reacted with DNA in the presence of rat liver microsomes. The major modified base was identified as 2-(C^8-guanyl)amino-6-methyldipyrido[1,2-*a*: 3′,2′-*d*]imidazole (**1**).

Keywords——mutagen; carcinogen; microsomes; modified DNA; 2-amino-6-methyldipyrido[1,2-*a*: 3′,2′-*d*]imidazole; glutamic acid pyrolysate

Potent mutagens were isolated from a glutamic acid pyrolysate and identified as 2-amino-6-methyl- and 2-amino-dipyrido[1,2-*a*: 3′,2′-*d*]imidazoles (Glu-P-1 and 2, respectively).[1] These amines show quite potent mutagenicity on bacteria in the presence of rat liver microsomes. The induction of mutagenesis or carcinogenesis may be closely related to the binding of the activated mutagens or carcinogens to DNA. The structural identification of these modified DNA can be of some importance in understanding of a molecular basis and further study for mutagenesis or carcinogenesis. This paper reports the chemical structure of the major Glu-P-1 bound base formed by the hydrolysis of DNA modified by Glu-P-1 in the presence of rat liver microsomes.

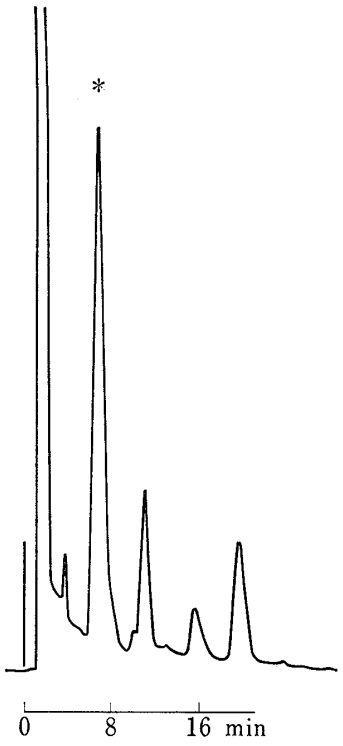

Fig. 1. High Performance Liquid Chromatogram of Glu-P-1 Bound Bases

Column: Polygosil $_5C_{18}$ 4.6ϕ×150 mm.
Solvent: 37% MeOH-1% conc. NH_4OHaq-62%H_2O.
Flow rate: 1.0 ml/min.
Detection: Absorbance at 254 nm.

Glu-P-1 bound DNA was obtained by three repeated incubations of calf thymus DNA (1 g) with Glu-P-1 (50 mg) in the presence of rat liver microsomes (300 mg protein) in bis(2-hydroxyethyl)-Tris-HCl buffer[2] (0.1 M, 500 ml, pH 7.5). Incubation mixture also included NADPH (0.5 mmol), glucose-6-phosphate (5 mmol), glucose-6-phosphate-dehydrogenase (100 units), $MgCl_2$ (1.5 mmol), Na_2SO_4 (1.5 mmol), and EDTA (50 μmol) in 500 ml. The amount of Glu-P-1 bound to DNA was roughly 200 μmol/mol P estimated from the intensity of fluorescence of the modified DNA. Glu-P-1 bound DNA thus obtained was hydrolysed in the usual way by 26×10^4 Kunits of DNase I, 100 units of phosphodiesterase from *Crotalus adamanteus venom*, and 2500 units of alkaline phosphatase, successively. The hydrolysate was subjected to Sephadex LH-20 column, eluted with water-methanol stepwise gradient. The fastest moving part was fluorescent, and therefore, modified nucleosides. The following, but partly superimposed with the fluorescent fractions, was normal nucleosides. Fractions eluted with 60—100% methanol were again fluorescent. The fastest fractions were hydrolysed by acid to a similar mixture to the last fluorescent fractions. Therefore the last fractions are bases modified by Glu-P-1. The hydrolysis to bases under the conditions may be interpreted by lability of glucosidyl bond of modified

1) T. Yamamoto, K. Tsuji, T. Kosuge, T. Okamoto, K. Shudo, K. Takeda, Y. Iitaka, K. Yamaguchi, Y. Seino, T. Yahagi, M. Nagao, and T. Sugimura, *Proc. Japan Acad.*, **54**, (B) 248, (1978).
2) F.F. Kadlubar, J.A. Miller, and E.C. Miller, *Cancer Res.*, **36**, 2350, (1976).

DNA or contamination of nucleotidase or nucleosidase in the enzymes employed. Figure 1 illustrates an example of a chromatographic pattern of a modified base mixture. The major peak (*) was now identified as 2-(C^8-guanyl)amino-6-methyldipyrido[1,2-*a*: 3′,2′-*d*]imidazole (**1**). The identification of the base with an authentic specimen synthesized below was performed by high performance liquid chromatography (HPLC) with use of three different column conditions. Good coincidence of fluorescence, excitation, and ultraviolet spectra of the isolated base with the authentic specimen supports the identification.

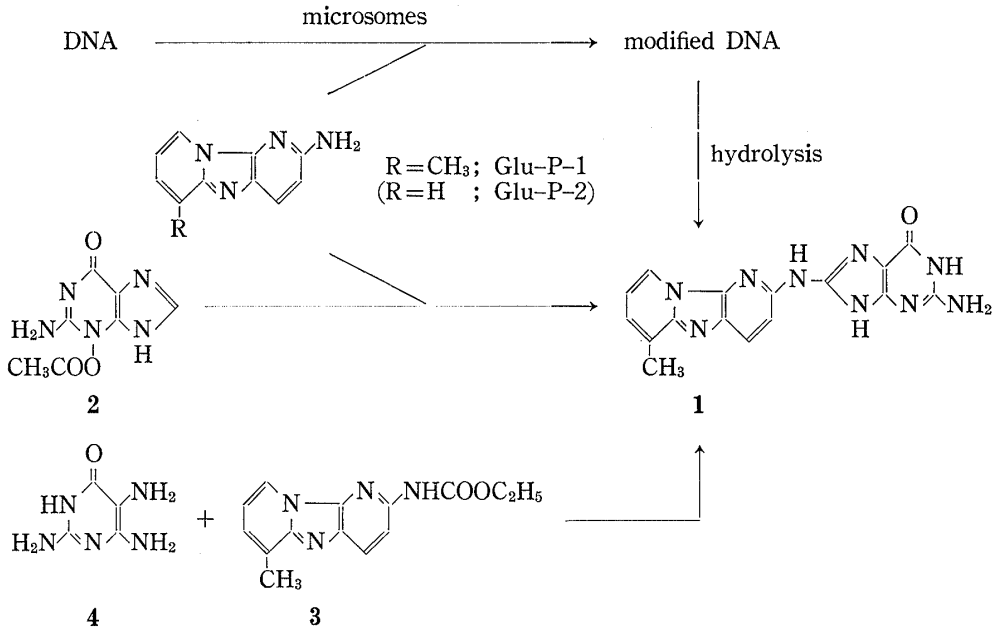

Chart 1

The authentic specimen was prepared by a reaction of N^3-acetoxyguanine (**2**)[3] with Glu-P-1. The mojor product was purified by HPLC, and the yield was about 60%. The structure was deduced by molecular weight, C^{13}-NMR (the presence of 16 carbons), and proton NMR (a complete assignment of all the hydrogens of Glu-P-1 moiety, and the absence of C^8-hydrogen of guanine moiety). The hydrolysis of the product as well as the isolated base from modified DNA gave Glu-P-1 and uric acid. The bonding site of Glu-P-1 nitrogen atoms was, however, ambiguous. Alkylation-hydrolysis experiment to determine the position was fruitless. The final proof was performed by another synthesis of the base from 2-carboethoxyamino-6-methyldipyrido[1,2-*a*: 3′,2′-*d*]imidazole (**3**) and 2,4,5-triamino-6-hydroxypyrimidine (**4**).

In summary, the major binding site of activated Glu-P-1 to DNA is the 8-position of guanine in DNA. The similar type of products were obtained as major bases modified by 2-acetylaminofluorene,[4] N-methylaminoazobenzene,[5] and 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]-indole (Trp-P-2).[6] The 8-position of guanine in DNA may be the most susceptible target by mutagenic polycyclic aromatic amines. The activated form leading to the modified base is plausibly 2-hydroxyamino-6-methyldipyrido[1,2-*a*: 3′,2′-*d*]imidazole (**5**).

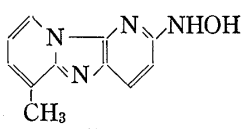

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Chart 2

3) N.J.M. Birdsall, J.C. Parham, U. Wölke, and G.B. Brown, *Tetrahedron*, **28**, 3 (1972).
4) J.A. Miller, *Cancer Res.*, **30**, 559 (1970), and references therein.
5) Jen-Kun Lin, J.A. Miller, and E.C. Miller, *Cancer Res.*, **35**, 884 (1975), and references therein.
6) Y. Hashimoto, K. Shudo, and T. Okamoto, *Chem. Pharm. Bull.* (Tokyo), **27**, 1058 (1979).

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Two New *Veratrum* Alkaloids, Hosukinidine and Epirubijervine from Illuminated *Veratrum* Plant

Hosukinidine, (20*R*,22*R*,25*S*)-veratra-5,12-dien-3β-ol, and epirubijervine, (22*R*,25*S*)-solanid-5-ene-3β,12β-diol, were isolated from illuminated *Veratrum* plant.

Keywords——Liliaceae; *Veratrum grandiflorum*; a new veratranine alkaloid; hosukinidine; a solanidanine alkaloid; epirubijervine; illuminated *Veratrum*

Concerning the biogenesis of C-nor-D-homo steroidal alkaloids, two new cevanine alkaloids, shinonomenine and veraflorizine, and a new cevanidane alkaloid, procevine, were isolated from a *Veratrum* plant cultivated under illumination with a red fluorescent light, after 10 days of etiolation, as described previously.[1] The isolation of these three alkaloids, in addition to isorubijervine, from illuminated *Veratrum* plant suggests the biogenesis of cevanine alkaloid via the formation of C-18-N bond from isorubijervine, before C-nor-D-homo rearrangement.

In continuation of our work on the separation of alkaloids which accumulate particularly in illuminated plants but not found in etiolated plants, two new alkaloids, hosukinidine (**1a**) from the rhizomes and epirubijervine (**2a**) from the aerial part, were isolated from hydrolytic fraction of the illuminated *Veratrum grandiflorum* (Max.) Loesen.

Hosukinidine (**1a**) named after Ainu name "Hosuki" for *Veratrum* plant: $C_{27}H_{43}NO$ (elementary analysis); mp 176.5—177.5°; $[\alpha]_D$ −56.5° (*c* 0.27, MeOH); IR: 3600, 1045 cm^{-1}; MS *m/e*: 397 (M^+), 125, 98 (base peak), afforded on acetylation in pyridine N,O-diacetate (**1b**): mp 195—197°; $[\alpha]_D$ −55.6° (*c* 0.23, $CHCl_3$); IR: 1715, 1615, 1235, 1030 cm^{-1}; PMR: δ 2.03 (3H, s, –OAc) 2.07 (3H, s, –NAc).

The PMR spectrum of **1a** exhibited a singlet at δ 0.98, indicative of C-19 methyl group of a steroidal ring system with Δ^5-double bond, two doublets at δ 0.80 and 0.84 (3H each, J=7 Hz), corresponding to two secondary methyl groups at C-21 and C-27, a singlet at δ 1.56 (3H) for a vinyl methyl, and a signal at δ 5.38 (1H) for an olefinic proton. Multiplet centered at δ 3.48 is associated with α-hydrogen at C-3 (bearing β-hydroxyl group) and this signal shifted downfield to δ 4.64 on acetylation.

In the mass spectrum of **1a**, the base peak at *m/e* 98 is assigned to the methyl piperidyl side chain moiety as a result of a bond fission between C-20 and C-22 of **1a**. In the light of these spectral data, **1a** was considered to be a C-nor-D-homo steroidal alkaloid having veratranine skeleton, and hosukinidine is represented by formula **1a**, except for the configurations at C-17, -20, -22, and -25.

The final structural proof of **1a** was elucidated by the X-ray crystal structure analysis of its hydrochloride (**1c**), colorless needles, mp 285° (dec.). Crystals of **1c** are orthorhombic, space group $P2_12_12_1$, a=33.75±0.07, b=9.43±0.02, c=7.84±0.03 Å, $\alpha=\beta=\gamma$ 90°, z=4. The

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