

### Structural Identification of a Modified Base in DNA covalently Bound with Mutagenic 3-Amino-1-methyl-5*H*-pyrido[4,3-*b*]indole

A mutagen, 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2, 1), was reacted with DNA in the presence of rat liver microsomes. One of the modified bases was identified as 3-(C<sup>8</sup>-guanyl)amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (2).

**Keywords**—mutagen; carcinogen; microsome; modified DNA; 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole; tryptophan pyrolysate

The induction of mutagenesis or carcinogenesis may be closely related to the binding of the activated mutagens or carcinogens to cellular macromolecules, in particular, to DNA. Elucidation of chemical structure of the modified DNA by the mutagens or carcinogens is essential in order to understand a molecular basis for alteration of gene expression in mutagenesis or carcinogenesis. Recently,<sup>1)</sup> we have reported rat liver microsomes-mediated binding to DNA of a potent mutagen, 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2,1),<sup>2)</sup> isolated from tryptophan pyrolysate. This communication describes the structural identification of one of Trp-P-2 bound bases formed by the hydrolysis of DNA modified by Trp-P-2 in the presence of rat liver microsomes.

The treatment of 1 gram of calf thymus DNA with Trp-P-2 in the presence of PCB-treated rat liver microsomes, performed as described previously,<sup>1)</sup> gave 850 mg of Trp-P-2 bound DNA. A portion of Trp-P-2 bound DNA was hydrolyzed similarly to the method described by Baird *et al.*<sup>3)</sup> Each mg of Trp-P-2 bound DNA was dissolved in 1 ml of a solution of 0.01 M Tris and 0.01 M MgCl<sub>2</sub> at pH 7.0, and then 260 Kunits of DNase I from beef pancreas were added and the mixture was incubated at 37° for 6 hr. After changing the pH of the solution to 9.0 by 1 ml of 0.1 M Tris buffer, 0.1 unit of phosphodiesterase from *Crotalus adamanteus* venom, Type II, was added. After the incubation at 37° for 60 hr, 2.5 units of alkaline phosphatase from calf intestinal mucosa, Type I, were added and digestion was allowed to proceed at 37° for 48 hr. The deoxyribonucleosides thus obtained were subjected to Sephadex LH-20 column chromatography (2φ × 100 cm), eluted with water-methanol stepwise gradient. Most of nonfluorescent (unmodified) nucleosides were eluted with 3 through 5% methanol-

TABLE I. Retention Times of Trp-P-2 Bound Bases and 2 in Several HPLC Systems

Column	Carrier	Flow rate (ml/min)	Retention time (min)			
			I	II	III	2
Zorbax ODS 4.6φ × 150 mm	MeOH-water-aq. NH <sub>4</sub> OH 45 : 55 : 1	1.5	10.0	18.8	27.0	10.0
Zorbax CN 4.6φ × 150 mm	MeOH-water-aq. NH <sub>4</sub> OH 35 : 65 : 1	1.5	9.6	14.6	16.8	9.6
Zorbax CN 4.6φ × 150 mm	CHCl <sub>3</sub> -CH <sub>3</sub> CN-aq. NH <sub>4</sub> OH 95 : 5 : sat.	1.5	10.0	8.2	6.6	10.0
Zorbax NH <sub>2</sub> 4.0φ × 200 mm	CHCl <sub>3</sub> -CH <sub>3</sub> CN-aq. NH <sub>4</sub> OH 85 : 15 : sat.	2.0	8.8	6.0	6.0	8.8

- 1) Y. Hashimoto, K. Takeda, K. Shudo, T. Okamoto, T. Sugimura, and T. Kosuge, *Chem.-Biol. Interact.*, **23**, 137 (1978).
- 2) T. Sugimura, T. Kawachi, M. Nagao, T. Yahagi, Y. Seino, T. Okamoto, K. Shudo, T. Kosuge, K. Tsuji, K. Wakabayashi, Y. Iitaka, and A. Itai, *Proc. Japan Acad.*, **53**, 58 (1977).
- 3) W.M. Baird and P. Brookes, *Cancer Res.*, **33**, 2378 (1973).

water. Fluorescent fractions (excited at 315 nm) were eluted later than the unmodified nucleosides with 5 through 20% methanol-water. All the fluorescent fractions were combined and lyophilized. Trp-P-2 bound bases were prepared by the acid hydrolysis of the fluorescent fractions by 0.1 N aqueous HCl at room temperature. Portions of the Trp-P-2 bound bases thus obtained were submitted to high performance liquid chromatography (HPLC). Three major peaks were obtained which were named I, II, and III (Fig. 1 and Table I).

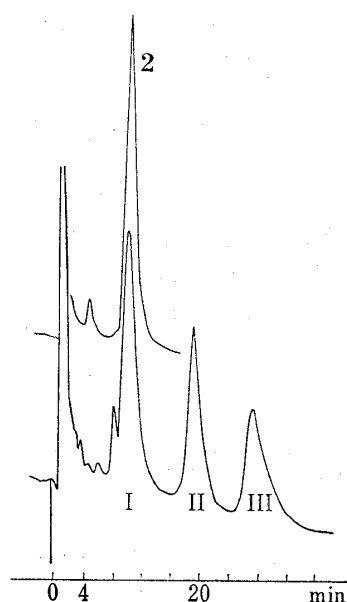


Fig. 1. High Performance Liquid Chromatography of Trp-P-2 Bound Bases and 2

Column; Zorbax ODS, 4.6 $\phi$ ×150 mm.  
Solvent; MeOH-water-aq. NH<sub>4</sub>OH  
(45:55:1).  
Flow rate; 1.5 ml/min.

One of the products, I, was now identified as 3-(C<sup>8</sup>-guanyl)amino-1-methyl-5H-pyrido[4,3-*b*]indole (2) which was prepared by the nucleophilic substitution by Trp-P-2 at the 8-position of 3-acetoxyguanine<sup>4</sup> in dimethylsulfoxide. The identification of I with 2 was performed by the following lines of evidence: (i) Retention times of I in various HPLC systems were identical with those of 2 (Fig. 1 and Table I). (ii) Fluorescence and excitation spectra of I at neutral, acidic, and basic media were identical with those of 2 (Fig. 2). (iii) Ultraviolet spectrum

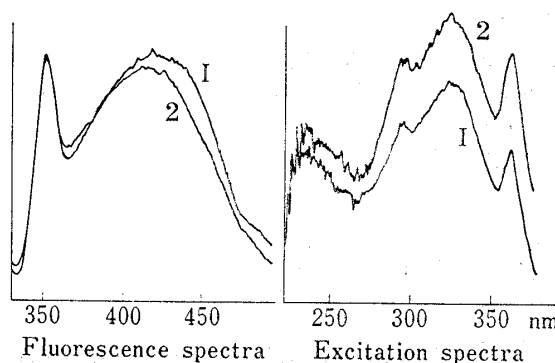
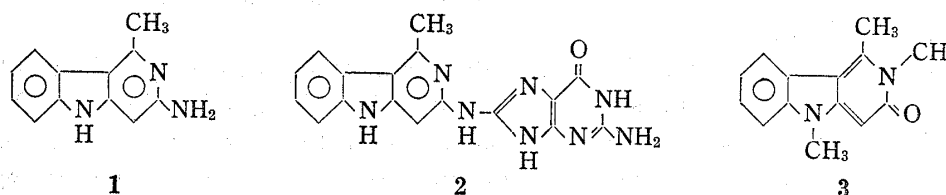


Fig. 2. Fluorescence and Excitation Spectra of I and 2 in 1 N NaOH aq.

of isolated I by HPLC had maxima at 320 nm and 333 nm. These were identical with the spectrum of 2 treated similarly by HPLC.<sup>5</sup> (iv) Dilute solutions of both I and 2 were unstable under alkaline condition in air. The HPLC of their decomposed products gave the same chromatographic pattern.

The structure of 2 was proved by its high resolution mass spectroscopy [ $M^+$ (obs.) 346.1225, (C<sub>17</sub>H<sub>14</sub>N<sub>8</sub>O)], CMR (a characteristic C-4 signal of Trp-P-2 bearing a hydrogen), and PMR (5 aromatic protons including a singlet one). Chemically, alkaline hydrolysis under degassed conditions gave Trp-P-2 as the major product. Uric acid and 8-hydroxyguanine were also identified in the hydrolyzate. Thus, the presence of a bond between guanine C<sup>8</sup> and Trp-P-2 nitrogen was confirmed. The bonding site of Trp-P-2 was also confirmed as the 3-amino



4) N. J. M. Birdsall, J. C. Parham, U. Wölke, and G. B. Brown, *Tetrahedron*, **28**, 3 (1972)

5) The shorter wave length part could not be compared because of contaminants in carrier for HPLC.

group by alkaline hydrolysis after the methylation of 2 by dimethyl sulfate, giving 3-oxo-1,2,5-trimethyl-3H-pyrido[4,3-*b*]indole (3) as a major product.

The ultraviolet, fluorescence and excitation spectra of II were similar to those of I (or 2). Those of III were very different from those of I or II.

In summary, one of the binding sites of activated Trp-P-2 to DNA is the 8-position of guanine in DNA. It is quite plausible that the activated form of Trp-P-2 is the corresponding hydroxylamine or its ester. N<sup>2</sup>-oxide would be an alternative activated form. We are trying to identify II and III, and to get information on the structure of the modified DNA by amino-dipyridoimidazoles,<sup>6)</sup> potent mutagens isolated from glutamic acid pyrolysate.

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

YUICHI HASHIMOTO  
KOICHI SHUDO  
TOSHIHIKO OKAMOTO

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### Behaviour of S-Substituted Cysteine Sulfoxide under Acidolytical Deprotecting Conditions

Treatment of S-*p*-methoxybenzylcysteine sulfoxide with hydrogen fluoride or methanesulfonic acid in the presence of anisole afforded S-*p*-methoxyphenylcysteine as a major product, while S-benzylcysteine sulfoxide resisted to the action of these deprotecting reagents in peptide synthesis. Thiophenol was found to be a powerful reducing reagent of the sulfoxides.

**Keywords**—S-*p*-methoxybenzylcysteine sulfoxide; S-benzylcysteine sulfoxide; hydrogen fluoride deprotection; methanesulfonic acid deprotection; sodium in liquid ammonia reduction; S-*p*-methoxyphenylcysteine; thiophenol reduction of sulfoxides

In 1977, Live *et al.*<sup>1)</sup> mentioned briefly the reduction of the sulfoxide of Cys(S-*p*-methylbenzyl) residue by acetone treatment in hydrogen bromide-acetic acid in the course of the solid phase synthesis of oxytocin. Little is known about the chemical nature of the sulfoxide of S-substituted cysteines, such as Cys(MBzl) and Cys(Bzl) [MBzl=S-*p*-methoxybenzyl, Bzl=S-benzyl], in peptide synthesis. Behaviour of these sulfoxides under acidolytical deprotecting conditions was examined.

Oxidation of Z(OMe)-Cys(MBzl)-OH [Z(OMe)=*p*-methoxybenzyloxycarbonyl] by sodium perborate gave the corresponding sulfoxide (I) [mp 148–151°,  $[\alpha]_D^{25}$  –72.8° in DMF. *Anal.* Calcd. for C<sub>20</sub>H<sub>23</sub>NO<sub>7</sub>S: C, 56.99; H, 5.50; N, 3.32. Found: C, 56.89; H, 5.53; N, 3.33] quantitatively. The product seems to be a mixture of two diastereoisomers concerning the configuration of the sulfoxide grouping, as predicted by the similar oxidation of N<sup>α</sup>-protected meth-

1) D. H. Live, W. C. Agosta and D. Cowburn, *J. Org. Chem.*, **42**, 3556 (1977).