Preliminary biological study on mutagenesis showed that compound **9a**, **9b**, **1a**, **2b** are mutagenic to *Salmonella typhimurium* TA 100.[7] Detailed biological studies are in progress.

Faculty of Pharmaceutical Sciences
University of Tokyo
Hongo, Bunkyo-ku, Tokyo, 113, Japan

Yoshiyasu Kitahara
Koichi Shudo
Toshihiko Okamoto

Received April 3, 1980

7) Co-worked with M. Nagao, and will be published.

[Chem. Pharm. Bull. 28(6)1961—1963(1980)]

Alkylation of 5′-Guanylic Acid by Reductively Activated Mitomycin C

Mitomycin C (MMC) was reduced by H_2-gas in the presence of Pd–C and 5′-guanylic acid (5′-GMP). The structure of the alkylated 5′-GMP by reductively activated MMC was determined as *cis*-2,7-diamino-1-(5′-guanylyl)mitosene.

Keywords——mitomycin C; reductively activated mitomycin C; 5′-guanylic acid; alkylated 5′-guanylic acid; antitumor agent

Mitomycin C (MMC), the potent antibiotic and clinically useful antitumor agent, is known to cross-link or alkylate cellular DNA.[1] Chemically, the DNA-binding and cross-linking effects of MMC could only be demonstrated if a reducing agent was also added.[2] Recently, an acid catalyzed alkylation of a series of phosphate compounds including 5′-uridylic acid by MMC was described by Tomasz *et al.*,[3] while MMC does not react with nucleotides under neutral conditions. However, no alkylation product by reductively activated MMC has been characterized. The redox chemistry of MMC itself is very complex and not well understood. The only product ever characterized from reaction of reduced MMC is the bisulfite adduct.[4] In this paper, binding of 5′-guanylic acid (5′-GMP) with reduced MMC and structural determination of MMC bound 5′-GMP are described.

MMC (200 mg) in bis(2-hydroxymethyl)-Tris-HCl buffer (0.05 M, pH 7.5, 80 ml) was reduced by H_2-gas in the presence of 10% Pd–C (80 mg) and 5′-GMP (4.0 g). Blue color of the mixture disappeared by first 5 min, and colored to deep red purple by another 10 min. The mixture was filtrated and subjected to Sephadex G-25 column chromatography (4.0×40.0 cm), eluted with 0.3% $(NH_4)_2CO_3$.[5] MMC bound 5′-GMP (**1**) was eluted after 5′-GMP, and subsequently hydrolysates of MMC were eluted. Fractions containing **1** which contaminated with 5′-GMP were rechromatographed. All fractions mainly containing **1** were combined,

1) J.W. Lown, "Interactions of selected antitumor antibiotics with nucleic acid," in "Bioorganic Chemistry," Vol. 3, ed. by E.E. van Tamelen, Academic press, and references therein.
2) H.S. Schwartz, J.E. Sodergren, and F.S. Philips, *Science*, **142**, 1181 (1963).
3) M. Tomasz and R. Lipman, *J. Am. Chem. Soc.*, **101**, 6063 (1979).
4) U. Horneman, Y.K. Ho, J.K. Mackey, Jr., and S.C. Srivastava, *J. Am. Chem. Soc.*, **98**, 7069 (1976).
5) All fractions of column chromatography were checked by HPLC. Polygosil $_5C_{18}$, 4.6×150 mm, 15% CH_3CN in 0.3% NH_4Cl, 1.0 ml/min. Retention times: 5′-GMP; 0.5 min, **1**; 3.2 min, **2**; 7.9 min, MMC; 12.0 min.

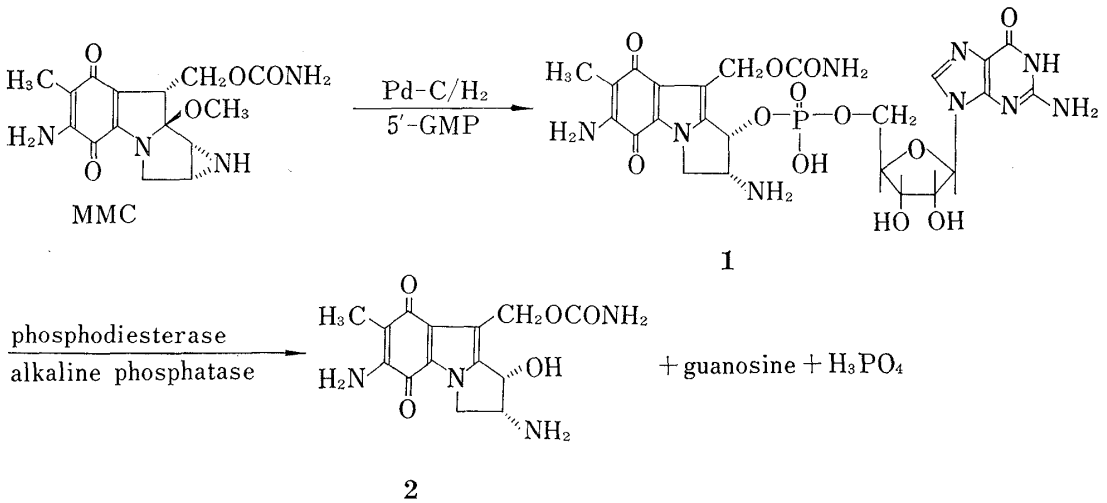

Chart 1

lyophilized, subjected to Sephadex LH-20 column chromatography (4.0×40.0 cm), and eluted with water. Sixteen mg of **1** (y: 4.3%) was obtained as a purple powder.

The structure of MMC bound 5′-GMP was now determined as represented as **1** in Chart 1. Its ultraviolet (UV) spectrum [247 nm (ε=14000), 272 nm (sh), 308 nm, and 345 nm (sh)] indicates the presence of mitosene chromophore. ^{1}H-NMR shows that **1** consists of a molecule of mitosene and a molecule of guanotide: [δ(DMSO-d_6): 1.76 (s, 3H) for 6-CH_3 of mitosene moiety, 5.70 (d, J=5 Hz, 1H) for 1′-H of ribose moiety, and 7.96 (d, J=0.7 Hz, 1H) for 8-H of guanine moiety].

1 was stable under a basic condition (pH 9.0, 37°, 3 hr), but **1** was hydrolyzed by phosphodiesterase from *Crotalus adamanteus* venom and alkaline phosphatase at pH 9.0, 37° within 1 hr completely to guanosine (y: 84%) and *cis*-2,7-diamino-1-hydroxymitosene (**2**, y: 56%). No *trans* isomer was found. **2** was identified by comparing its retention time of high performance liquid chromatography (HPLC) and UV-spectrum with authentic sample prepared by the method of Taylor *et al.*[6] Thus, the presence of a phosphodiester bond and the binding site of **1** and configuration of the site was established. Consequently, the structure of MMC bound 5′-GMP is determined as *cis*-2,7-diamino-1-(5′-guanylyl)mitosene (**1**).

The mechanism of formation of **1** under the conditions is not clear since even the structure(s) of reduced MMC is not unequivocally determined. However, it is very likely that

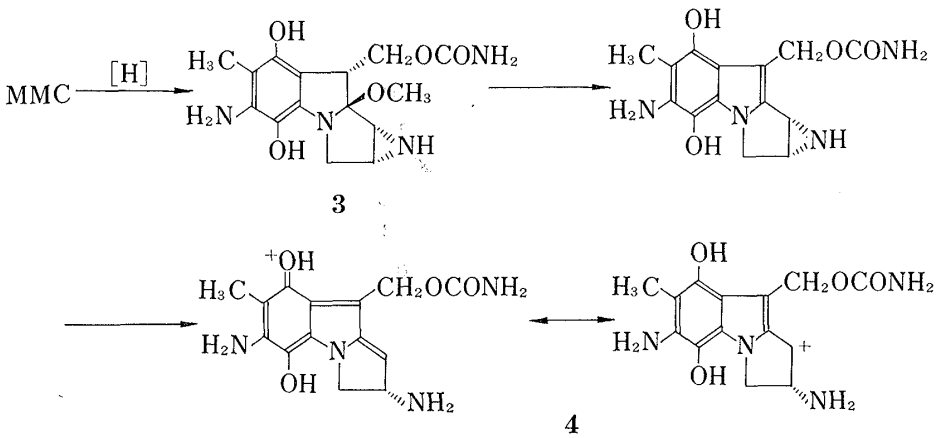

Chart 2

6) W.G. Taylor and W.A. Remers, *J. Med. Chem.*, **18**, 307 (1975).

reduction of MMC to hydroquinone (**3**) might facilitate a carbonium ion (**4**) formation by elimination of the methoxy group and the aziridine ring opening. This carbonium ion (**4**) might react with 5′-GMP. The product might be oxidized to **1** during work-up or an unknown redox reaction under the reaction conditions.

In conclusion, an alkylation product of a nucleotide by reductively activated MMC was characterized for the first time. This will contribute toward understanding of the molecular basis for the functioning of MMC as a reductively activated alkylating agent.

Acknowledgement We are very grateful to Kyowa Hakko Kogyo Co. for a generous supply of mitomycin C.

Faculty of Pharmaceutical Sciences
University of Tokyo
Hongo, Tokyo, Japan

Yuichi Hashimoto
Koichi Shudo
Toshihiko Okamoto

Received April 10, 1980

[Chem. Pharm. Bull. 28(6)1963—1965(1980)]

Isolation and Characterization of a New 16-Membered Lactone, Protylonolide, from a Mutant of Tylosin-Producing Strain, *Streptomyces fradiae* KA-427[1,2]

A new 16-membered lactone named protylonolide was isolated from the culture of the blocked mutant of *Streptomyces fradiae*, KA-427. Protylonolide was converted to tylosin by both parent strain and several tylosin-non-producing mutants of KA-427. Protylonolide is an important biosynthetic intermediate of tylosin.

Keywords——protylonolide (PTL); tylosin (TYL); 16-membered macrolide antibiotics; blocked mutants; bioconversion and biosynthesis

In the previous paper,[4] the authors reported the biosynthetic relationship between tylosin (TYL) and relomycin (20-dihydro-TYL) using enzyme inhibitor, cerulenin,[5] a specific inhibitor of fatty acid and polyketide biosynthesis. However, investigation on the biosynthetic route of TYL was hampered by the limited amounts of appropriate intermediates. We therefore explored suitable blocked mutants producing the intermediates of TYL, and obtained two mutant strains No. 261 and No. 551 producing 16-membered lactone ring named protylonolide (PTL) by the NTG treatment of the TYL-producing strain of *Sm. fradiae* KA-427.

The mutant strain was cultured in 50-l jar fermentor containing 30 l of the medium (1.0% glucose, 2.0% starch, 0.5% peptone, 0.5% meat extract, 0.3% L-asparagine, and 0.4% $CaCO_3$, pH 7.5) for 3 days at 27°. PTL was extracted with benzene and purified by the preparative silica gel TLC (Kieselgel 60 F_{254}, Merck, developed with $CHCl_3$/MeOH/1.5 N NH_4OH=2:1:1, bottom layer, *Rf* 0.59) to give about 200 mg of PTL as a white powder. PTL was crystallized

1) Bioconversion and biosynthesis of 16-membered macrolide antibiotics. Part XVI. See ref. 3) for Part XV.
2) Abbreviations: PTL, Protylonolide; TYL, Tylosin; NTG, N-methyl-N′-nitro-N-nitrosoguanidine.
3) C. Kitao, H. Hamada, H. Ikeda, and S. Ōmura, *J. Antibiotics*, **32**, 1055 (1979).
4) S. Ōmura, C. Kitao, J. Miyazawa, H. Imai, and H. Takeshima, *J. Antibiotics* **31**, 254 (1978).
5) S. Ōmura, *Bact. Rev.* **40**, 681 (1976).