TABLE III. Antitumor Activities and LD_{50} of **1** and **2**

	Sarcoma 180, (T/C) (ddY mouse)	Ehrlich carcinoma (T/C) (ICR mouse)	LD_{50} mg/kg
1	4.0	29.0	75
2	21.0	34.0	375

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Synthesis of Non-K-region Dihydrodiols and Epoxides of Carcinogenic Dibenz[*c,h*]acridine

Dibenz[*c,h*]acridine-1,2-oxide, dibenz[*c,h*]acridine-3,4-oxide, *trans*-1,2-dihydroxy-1,2-dihydrodibenz[*c,h*]acridine and *trans*-3,4-dihydroxy-3,4-dihydrodibenz[*c,h*]acridine, which are possible active metabolites of dibenz[*c,h*]acridine, were synthesized.

Keywords——arene oxide; aza-arene oxide; dibenz[*c,h*]acridine oxide; carcinogen; mutagen

For the carcinogenic polynuclear aromatic hydrocarbons including benzo[*a*]pyrene, metabolically formed arene oxides and diol epoxides have emerged as the most responsible molecules to account covalent binding to biomolecules such as nucleic acid proposed as a prerequisite for the chemical induction of cancer.1) Many aza-arenes such as dibenz[*c,h*]-acridine (DBA) are known to be carcinogenic and some of them are as quite highly active as benzo[*a*]pyrene.2) They were also found in tar, urban atmosphere and tobacco smoke.3)

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1) E.C. Miller and J.A. Miller, "Molecular Biology of Cancer," ed. by H. Busch, Academic Press, New York, 1972, p. 377.

2) A. Dipple, "Chemical Carcinogens," ed. by C.E. Searle, American Chemical Society, New York, 1976, p. 245.

3) E. Sawicki, J.A. Meeker, and M.J. Morgan, *Int. J. Air Water Pollution*, **9**, 291 (1965); E. Sawicki, S.P. McPherson, T.W. Stanley, J. Meeker, and W.C. Elbert, *ibid.*, **9**, 515 (1965); B.L. van Duuren, J.A. Bilbao, and C.A. Joseph, *J. Natl. Cancer Inst.*, **25**, 53 (1965).

We have tried to find active metabolites of DBA and synthesized K-region oxide of DBA. However, the K-region oxide was not mutagenic in *Salmonella typhimurium* TA 98 and TA 100.[4] This result indicates that non-K-region oxides may be active metabolites of DBA. We undertook, therefore, the synthesis of non-K-region dihydrodiols (**1a**, **b**) and epoxides (**2a**, **b**) of DBA as possible active (proximate) metabolites.

Oxidation of 1,2,3,4-tetrahydro-DBA (**3**), mp 131—132°, obtained by heating of an equimolar mixture of 5,6,7,8-tetrahydro-1-naphthylamine, 1-naphthol and paraformaldehyde (250°, 15 min), with lead tetraacetate in benzene-acetic acid (50—60°, 24 hr) afforded a mixture of two acetoxy isomers, 1-acetoxy- and 4-acetoxy-1,2,3,4-tetrahydro-DBA, in 31% yield.

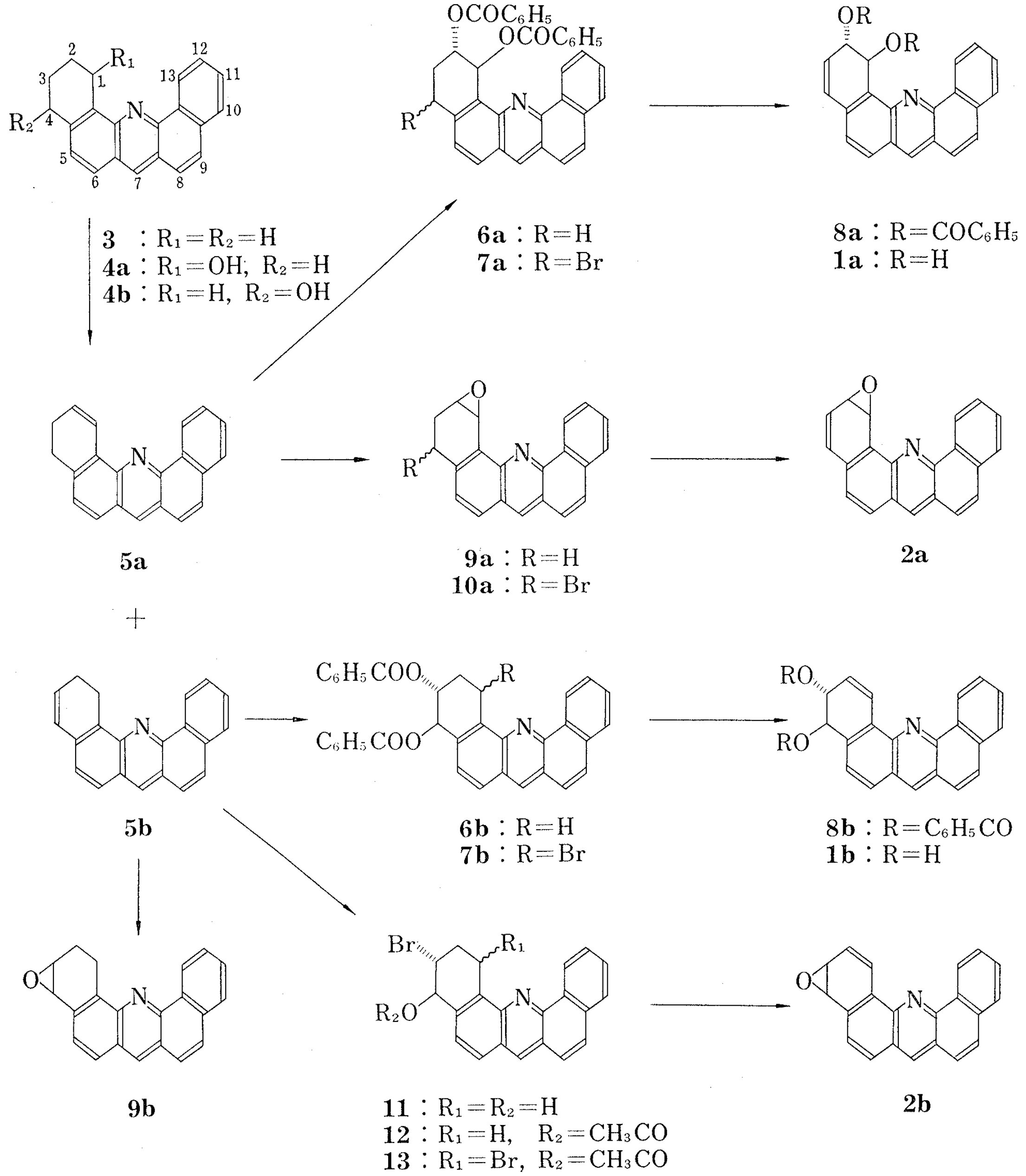

Chart 1

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4) Y. Kitahara, H. Okuda, K. Shudo, T. Okamoto, M. Nagao, Y. Seino, and T. Sugimura, *Chem. Pharm. Bull.*, **26**, 1950 (1978).

The isomeric mixture was hydrolyzed with 10% NaOH–ethanol at refluxing temperature. The reaction products were extracted with dichloromethane and chromatographed on silica gel with dichloromethane–hexane containing 1% ethanol as eluent to afford 1-hydroxy-(**4a**, mp 143—144°, 65%) and 4-hydroxy-1,2,3,4-tetrahydro-DBA (**4b**, mp 214—215°, 22%).[5]

Acid catalyzed dehydration of the alcohols **4a** and **4b** in 18% HCl–acetone at refluxing temperature for 8 hr afforded 3,4-dihydro-DBA (**5a**, mp 132—133°) and 1,2-dihydro-DBA (**5b**, mp 147—148°) in 68% and 67% yield, respectively. Assignment of isomers was based on analysis of the 100 MHz NMR spectra. The major isomer **5a** exhibited δ 2.46 (m, allylic $-C\underline{H}_2-$), 3.00 (t, benzylic $-C\underline{H}_2-$), 6.30 (d of t, olefinic H_2), 8.16 (d of t, olefinic H_1). Isomer **5b** had δ 2.60 (m, allylic $-C\underline{H}_2-$), 3.82 (t, benzylic $-C\underline{H}_2-$), 6.28 (d of t, olefinic H_3), 6.62 (d of t, olefinic H_4). The lower field chemical shifts of the olefinic proton H_1 of isomer **5a** and the benzylic protons of **5b** are indicative of anisotropic interaction with nitrogen, confirming the structural assignment.[6]

Prévost reaction of **5a** with silver benzoate–iodine (2:1) in dry benzene at refluxing temperature for 20 min furnished smoothly *trans*-1,2-dibenzoxy-1,2,3,4-tetrahydro-DBA (**6a**), mp 239—240°, in 97% yield. Similarly, *trans*-3,4-dibenzoxy-1,2,3,4-tetrahydro-DBA (**6b**), mp 217—218°, was obtained from **5b** in 84% yield. Bromination of **6a** and **6b** with *N*-bromosuccinimide (NBS) in refluxing CCl_4 afforded bromodibenzoates, which were dehydrobrominated with 1,5-diazabicyclo[4.3.0]-5-nonene (DBN) in tetrahydrofuran (THF) under argon (4°, 18 hr) to *trans*-1,2-dibenzoxy-1,2-dihydro-DBA (**8a**), mp 204—206° (28% yield from **6a**) and *trans*-3,4-dibenzoxy-3,4-dihydro-DBA (**8b**), mp 248—250° (23% yield from **6b**). Treatment of **8a** with sodium methoxide in methanol-THF under argon (60°, 10 min) cleaved the benzoate ester group to furnish the free dihydrodiol **1a** in 87% yield. NMR of **1a**: δ 1.6—2.0 ($O\underline{H}$), 5.00 (d of t, allylic H_2), 5.80 (d, benzylic H_1), 6.31 (d of d, olefinic H_3), 6.55 (d of d, olefinic H_4), $J_{1,2}=12$ Hz, $J_{3,4}=10$ Hz, $J_{2,3}=J_{2,4}=2.5$ Hz. Another benzoate ester **8b** was hydrolyzed with aqueous ammonia in methanol to *trans*-3,4-dihydroxy-3,4-dihydro-DBA (**1b**) in 80% yield. NMR of **1b**: δ 1.6—2.1 ($O\underline{H}$), 4.70 (d of t, allylic H_3), 5.08 (d, benzylic H_4), 6.27 (d of d, olefinic H_2), 8.16 (d of d, olefinic H_1), $J_{1,2}=11$ Hz, $J_{3,4}=12$ Hz, $J_{1,3}=J_{2,3}=2$ Hz.

Non-K-region epoxides **2a** and **2b** were synthesized from dihydroarenes. Epoxidation of 3,4-dihydro-DBA (**5a**) and 1,2-dihydro-DBA (**5b**) with *m*-chloroperbenzoic acid in dichloromethane at room temperature for 40—120 min afforded the corresponding epoxide **9a**, mp 135.5—136.5° (76% yield) and **9b**, mp 179—179.5° (61% yield), respectively. Bromination of the tetrahydro epoxide **9a** with NBS in CCl_4 at refluxing temperature for 30 min afforded a mixture of stereoisomeric bromo epoxide **10a**. The isomeric mixture was dehydrobrominated with DBN in THF under argon at 4° for 18 hr to DBA-1,2-oxide (**2a**), mp 123—125° (dec.) in 32% yield from **9a**. NMR of **2a**: δ 4.46 (m, epoxy H_2), 6.40 (d, epoxy H_1), 6.76 (d of d, olefinic H_3), 6.99 (d of d, olefinic H_4), $J_{1,2}=J_{2,3}=4$ Hz, $J_{2,4}=2$ Hz, $J_{3,4}=10$ Hz.

In contrast, attempted bromination of 3,4-epoxy-1,2,3,4-tetrahydro-DBA (**9b**) was completely without success. The epoxide **2b** was synthesized *via* halohydrin ester (**12**). A reaction of the dihydroarene **5b** with *N*-bromoacetamide in aqueous THF containing a drop of 36% HCl at room temperature for 2 hr afforded *trans*-3-bromo-4-hydroxy-1,2,3,4-tetrahydro-DBA (**11**), mp 179—180°, in 83% yield. Acetylation of **11** with acetic anhydride–pyridine afforded **12**, mp 158.5—159.5°, in 95% yield. The acetate **12** was brominated with NBS in CCl_4 to **13**, mp 137—139° (dec.) in 96% yield. A reaction of **13** with sodium methoxide in THF under argon at 4° for 16 hr produced DBA-3,4-oxide (**2b**), mp 233—236° (dec.), in 74% yield. NMR of **2b**: δ 4.42 (m, epoxy H_3), 4.76 (d, epoxy H_4), 6.81 (d of d, olefinic H_2), 8.76 (d of d, olefinic H_1), $J_{1,2}=10$ Hz, $J_{1,3}=2$ Hz, $J_{2,3}=J_{3,4}=4$ Hz.

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5) All new isolated compounds were analyzed and characterized by IR, NMR and mass spectra.

6) W. Brügel, "Handbook of NMR Spectral Parameters," Heyden and Son, London, 1979, p. 612; P.J. Collin, J.S. Shannon, H. Silberman, S. Sternhell, and G. Sugowdz, *Tetrahedron*, **24**, 3069 (1968).

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.

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

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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,

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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.