were dissected from larvae of the museum beetle, *Trogoderma versicolor*, and coned in a Syracuse watch glass. Each mouse received 5–10 cysticercoids by stomach tube. Sixteen to twenty days after infection, mice were placed in individual cages and those passing proglottids into the pans beneath the cages were used in the experiments.

During the test each mouse was kept in an individual cage, which contained a bottle of H_2O and a special feeding rack. Feces and worms were collected in a pan of H_2O beneath the cage. The test compound was prepared as a suspension made by grinding it in a mortar with Tween 80 and H_2O or, if sol, as a soln. It was then given in a single dose by stomach tube. The max dosage employed was 500 mg/kg, and at least 2 mice were used at each dose level.

Twenty-four hours after treatment the pan of H_2O was replaced by a clean one. The first pan was searched for worms or fragments and the findings were recorded. After another period of 24 hr the mouse was necropsied and any worms remaining were counted and recorded. Worms recovered from both pans and fragments of worms recovered from the cecum and large intestine were considered to have been dislodged by the treatment. The number of worms removed was expressed as a percentage of the total worm burden, and the results from all mice used in the experiment were averaged.

Mitomycin Derivatives. 1. Preparation of Mitosane and Mitosene Compounds and Their Biological Activities

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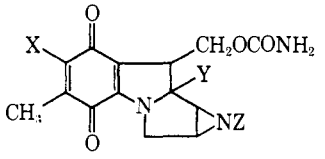
Several derivatives of mitomycin were prepared from natural mitomycins and subjected to antibacterial tests. The structure–activity relationship was investigated for enzymatic activation, inhibition of DNA synthesis, and prophage induction. Substituents X, Y, and Z in mitomycins (1) are closely related to the rate of activation, which has a great effect on the biological activity. The aziridine group is responsible for inhibition of DNA synthesis and also for alkylating actions of mitomycins.

Mitomycins (**1**) prossess strong activities against Gram-positive and Gram-negative bacteria, as well as against several kinds of tumors. These formulas[1,2] are



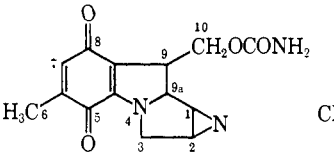
1

	X	Y	Z
1a, mitomycin A	CH_3O	OCH_3	H
b, mitomycin B	CH_3O	OH	CH_3
c, mitomycin C	NH_2	OCH_3	H
d, porifiromycin	NH_2	OCH_3	CH_3

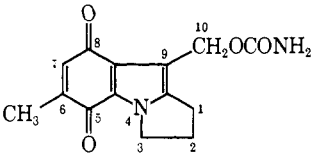
unique not only in natural products chemistry, but also among antitumor substances in that they have three carcinostatic groups—quinone, aziridine, and carbamoyloxymethyl—in their structures. It appeared important to elucidate the role of these 3 groups, and that of the X, Y, and Z substituents, on the biological activities of mitomycins.

Many derivatives were synthesized from the natural mitomycins in a search for less toxic and more effective substances. The present paper deals with their synthetic methods, biological activities, and structure–activity relationship.

A. Synthesis of Derivatives.—The derivatives of mitomycins in this report are classified in two groups, mitosane compounds **2** and mitosene compounds **3.**



2



3

I. Mitosane Compounds.—Several kinds of mitosane compounds were prepared from natural mitomycins as shown in Charts I and II.

Mitomycin A (**1a**) and C (**1c**) were acylated (**4**) with acyl chlorides in the presence of Et_3N. Using the same procedure 1a-sulfonyl derivatives **5** were also prepared. Alkylation of the 1a position was performed with alkyl iodide in the presence of K_2CO_3. Mitomycin A (**1a**) and B (**1b**) were treated with NH_3, and primary or secondary amines to give 7-aminomitosane compounds **7**.

Reduction with LAH of **1b** followed by oxidation with potassium nitrosodisulfonate (Fremy's salt) gave a new quinoid compound **8** which had the same chromophor as mitomycin B. In the ir and nmr spectra of **8**, no C=O ($\nu_{C=O}$1700 cm^{-1}) of CH_2OCONH_2 and MeO (3.7 ppm in $CDCl_3$) at C-9 was found, while those absorptions exist in mitomycin A and C. Compound **8** was converted into dehydroxymitomycin B (**10**) and demethoxyporfiromycin (**11**) through the phenoxycarbonyl derivative **9**. Compounds **10** and **11** were also prepared by $NaBH_4$ reduction of mitomycins. Compounds **8–11** were not converted into mitosene or decarbamoylmitosene by acid hydrolysis, and unchanged original products were recovered, while mitomycins gave mitosene by

(1) F. S. Webb, D. B. Cosulich, J. H. Mowat, R. W. Broschard, W. E. Meyer, R. P. William, C. F. Wolf, W. Fulmor, C. Pidacks, and J. E. Lancaster, *J. Amer. Chem. Soc.*, **84**, 3185 (1962).

(2) J. S. Webb, D. B. Cosulich, J. H. Mowat, R. W. Broschard, W. E. Meyer, R. P. William, C. F. Wolf, W. Fulmor, C. Pidacks, and J. E. Lancaster, *ibid.*, **84**, 3187 (1962).

CHART I
PREPARATION OF 1a-SUBSTITUTED AND 7-SUBSTITUTED MITOSANES

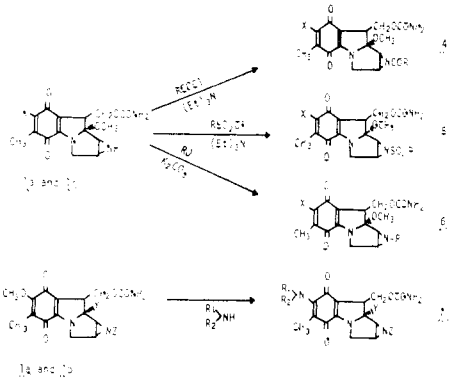
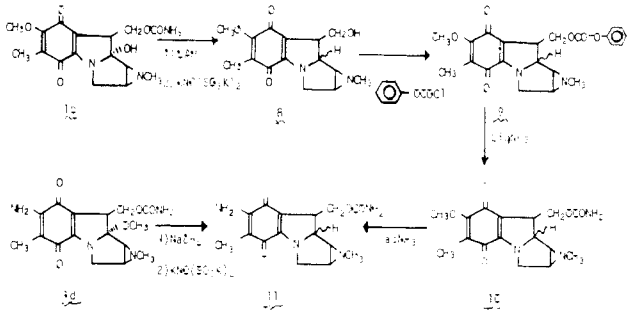


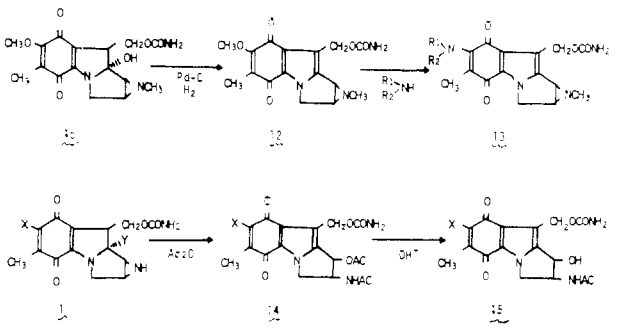
CHART II
PREPARATION OF DEMETHOXYMITOMYCIN



acid hydrolysis as described in a previous paper.[2,3] This result also supported the structures **8–11**.

II. Mitosene Compounds.—These were prepared by acid hydrolysis of mitomycins as described previously[3,4] (Chart III). Catalytic hydrogenation of **1b** followed by aerial oxidation gave 7-methoxyaziridinomitosene (**12**) as reported by Patrick, *et al.*[5] It was then converted into the 7-amino compound **13**. When mitomycins were refluxed in Ac_2O, diacetyl mitosene compounds **14** were obtained, which were hydrolyzed in alkaline solution to the monoacetate **15**. 7-Aminomitosenes (**15**, X = R_2N) were also prepared from 7-methoxymitosene (**14**, X = CH_3O).

CHART III
PREPARATION OF MITOSENE COMPOUNDS



B. Structure–Activity Relationship. 1. Effect of X, Y, and Z Substituents on the Biological Activity.—Tables I–III show the considerable effects of substituents X, Y, and Z of mitosane compounds upon the biological activities. Substituent X adjacent to a quinone CO affects the reduction potential of quinone, and the antibacterial activities of 7-substituted compounds seem to be related to their reduction potential. Substituent Y in the 9a position also played an important part on the biological action. Change of MeO to OH resulted in a great decrease of antibacterial activity. Moreover, it was very interesting to find that 9a-demethoxymitomycins in which Y was H, showed no antibacterial activity. Transformation of Z in the 1a position brought on the decrease of antibacterial activity. A strong decrease of activity was observed in the ortho-substituted benzoyl and sulfonyl derivatives. (Tables I–III).

2. Effect of Three Active Groups on the Biological Activities.—The influence of 3 active groups on the biological activities was investigated using the mitosene compounds (Table IV). Aziridinomitosene containing the 3 active groups showed the strongest activity in this series, while the cleavage of the aziridine ring resulted in a decrease of antitumor activity and toxicity.

When the aziridine group and either one of the carbamoyl or quinone groups were removed, the mitomycins lost all biological activities. These results indicate that two active centers are necessary for the appearance of their biological activities.

C. Relationship between Activity and Activation. 1. Mitosane Compound.—The influence of substituents X, Y, and Z in mitosanes on the enzymatic activation was investigated. Enzymatic activation has a close relationship to antibacterial activities and toxicities.

Mitomycins are metabolically activated *in vivo* to act upon DNA. The mechanism of the activation has been investigated by Schwarz,[6] Iyer,[7] and our group.[8] According to these studies, mitomycins **1** are reduced by TPNH-dependent enzyme to form hydroquinones **16** which are transformed to pyroloindole **17**. The pyroloindole **17** is an active form of mitomycin and reacts as an alkylating agent (**18**).

Reaction a is enzymatic, while reactions b and c proceed without enzymes. Substituents X, Y, and Z play important parts in these activation reactions and the biological activities. In general, the faster the activation the stronger the biological activity.

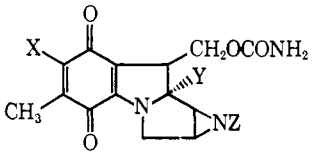
The rate of reaction a is a function of the reduction potential of the quinone which is affected by substituent X. Figure 1 shows the effect of X on the activation rate in liver homogenate. The higher the reduction potential, the faster the velocity of reaction a. The antibacterial activities also parallel the reduction potential and the rate of reaction a as shown in Table I.

Substituent Y has an effect on reaction b, which forms an indole nucleus. If Y is OMe, the rate of reaction b was much faster with Y = OH. Figure 2 shows the effect of substituent Y on the change of uv spectra of mitosanes in liver homogenate compared with chemical reduction by $Na_2S_2O_3$. Antibacterial activities were also proportional to the rate of reaction b. Moreover,

(3) K. Uzu, Y. Harada, S. Wakaki and Y. Yamada, *Agr. Biol. Chem. (Japan)*, **28**, 394 (1964).
(4) K. Uzu, Y. Harada, S. Wakaki, and Y. Yamada, *ibid.*, **28**, 388 (1964).
(5) J. B. Patrick, R. P. Williams, W. E. Meyer, W. Fulmor, Danna B. Cosulich, R. W. Broschard, and J. S. Webb, *J. Amer. Chem. Soc.*, **86**, 1889 (1964).
(6) H. S. Schwartz, J. E. Sodergren, and F. S. Philips, *Science*, **142**, 1181 (1963).
(7) V. N. Iyer and W. Szybalsky, *Science*, **145**, 55 (1964).
(8) M. Matsui, Y. Yamada, S. Miyamura, N. Shigeno, I. Usubuchi, Y. Sobajima, T. Hongo, T. Kawaguchi, M. Sugawara, S. Oboshi, S. Ishii, S. Masago, S. Wakaki, and K. Uzu, *Proc. Jap. Cancer Ass., 23rd General Meet.*, 104 (1964).

TABLE I

EFFECT OF X-SUBSTITUENTS OF THE BIOLOGICAL ACTIVITIES ON MITOSANE COMPOUNDS

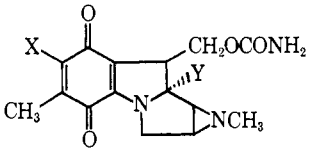


X	Y	Z	Reduction[a] potential	LD_{50} (mg/kg)[b]	MIC (μg/ml)[c] Gram-positive	MIC (μg/ml)[c] Gram-negative	Antitumor activity[d] Ascites Hirosaki sarcoma	Antitumor activity[d] Solid sarcoma 180
CH_3O	OCH_3	H	−0.19	2.0	0.01	0.57	+++	+
C_6H_5NH	OCH_3	H	−0.233	9.3	0.09	7.55	++	+
$(CH_3)_2N$	OCH_3	H	−0.25	9.38	0.33	7.29	+++	++
EtNH	OCH_3	H	−0.307	9.38	0.107	17.7	+++	+++
NH_2	OCH_3	H	−0.395	9.0	0.208	0.48	+++	+++
CH_3O	OH	CH_3	−0.21	3.0	0.65	50	±	±
C_6H_5NH	OH	CH_3	−0.23	5.62	0.47	>50		
$(CH_3)_2N$	OH	CH_3	−0.25	12.5	3.32	50		
EtNH	OH	CH_3	−0.32	>100	4.4	>50		
NH_2	OH	CH_3	−0.37	180	2.39	23.0	++	++

[a] These values were measured by polarographic method described by J. Ikeda [*Yakugaku Zasshi*, **75**, 1073 (1955)]. [b] DD male mice weighing 20 ± 1 g were used in this experiment. Test samples were administered by single iv injection. After 7 days observation, LD_{50} value was determined by Behren–Kaerber method. [c] Minimal inhibitory concn (MIC) of each compd against following 14 species of bacteria was measured by the plate dilution method using heart infusion agar. The mean value of MIC of each compd was calcd in the case of Gram-pos and Gram-neg bacteria: Gram-positive bacteria: *Staphylococcus aureus* 209 P; *Sarcina lutea* PCI 1001; *Bacillus subtilis* ATCC 6633; *Streptococcus haemolyticus* 68; *Deplococcus pneumoniae* 1-9; *Corynebacterium diphtheriae* 92; Gram-negative bacteria: *Salmonella typhi* 379; *Shigella flexneri 2a* 3196; *Klebsiella pneumoniae* 0/10; *Proteus vulgaris* X-19; *Escherichia coli* K-12; *Pseudomonas aeruginosa* 35. [d] These data were quoted from the studies conducted by I. Usubuchi [*Gann*, **58**, 301 (1967)] and S. Oboshi [*Gann*, **58**, 315 (1967)].

TABLE II

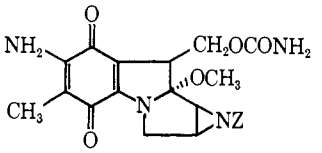
EFFECT OF Y-SUBSTITUENTS ON THE BIOLOGICAL ACTIVITIES OF MITOSANE COMPOUNDS

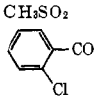
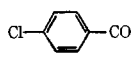


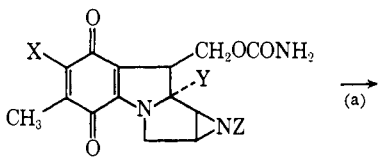
X	Y	LD_{50} (mg/kg)	MIC (μg/ml) Gram-positive bacteria	MIC (μg/ml) Gram-negative bacteria	Antitumor activity Ascites Hirosaki sarcoma	Antitumor activity Solid sarcoma 180
NH_2	OCH_3	55	0.27	11.4	+++	++
NH_2	OH	150	2.39	23.0	++	++
NH_2	H	>200	>50	>50	−	−
CH_3O	OCH_3	4.68	0.19	20	++	+
CH_3O	OH	3.4	0.65	50	±	±
CH_3O	H	>200	>50	>50	−	−

TABLE III

EFFECT OF Z-SUBSTITUENT ON THE BIOLOGICAL ACTIVITIES OF MITOSENE COMPOUNDS



Z	LD_{50} (mg/kg)	MIC (μg/ml) Gram-positive bacteria	MIC (μg/ml) Gram-negative bacteria	Antitumor activity Ascites Hirosaki sarcoma	Antitumor activity Solid sarcoma 180
H	9.0	0.20	0.42	+++	+++
C_2H_5	48.0	0.27	11.4	++	++
CH_3CO	27.0	0.39	2.5	++	++
CH_3SO_2	100	10	50	+++	−
	100	3.23	29.2		+
	18.75	0.965	13.5		+



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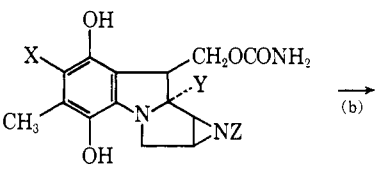

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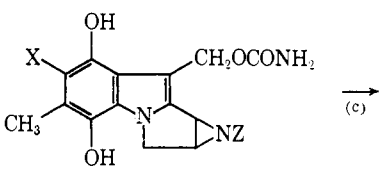

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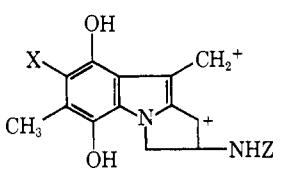
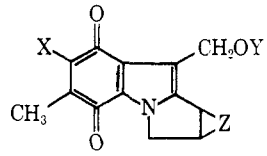


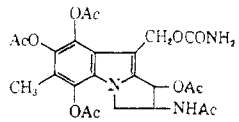
18

9a-demethoxymitomycin, in which Y is H, failed to give such a biological activity because this compound cannot be converted into compounds of formulas **17** and **18**. This finding provided the evidence for Szybalsky's hypothesis that the formation of activated mitomycins **17** which have an indole skeleton, is essential for the biological action of these mitosane compounds.

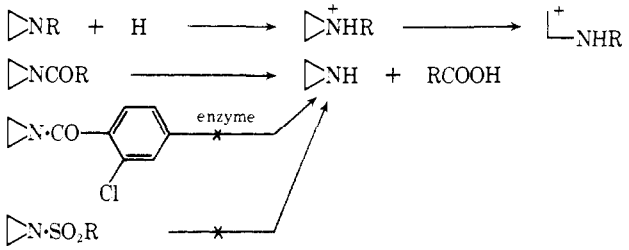
Group Z affects reaction c, which is the protonation

TABLE IV
BIOLOGICAL ACTIVITIES OF MITOSENE COMPOUNDS

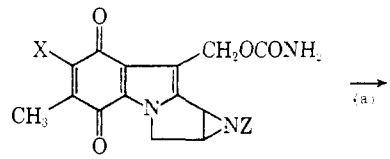


X	Y	Z	LD_{50} (mg/kg)	MIC (μg/ml) Gram-positive bacteria	MIC (μg/ml) Gram-negative bacteria	Antitumor activity: Ascites Hirosaki sarcoma	Antitumor activity: Solid sarcoma 180
CH_3O	$CONH_2$	NCH_3	18.7	1.05	2.5		+++
CH_3O	$CONH_2$	OH, $NHCH_3$	37.5	1.12	28.12		+
CH_3O	$CONH_2$	OH, NH	9.0	5.8	>50		++
CH_3O	H	OH, NHAc	>50	>50	>50	–	
NH_2	$CONH_2$	OH, NH_2	130	2.27	20.8		+
NH_2	H	OH, NHAc	>50	>50	>50		–
			>100	>100	>100	–	

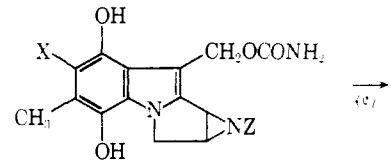
process of aziridine. A great decrease of activities was observed in ortho-substituted benzoyl and sulfonyl derivatives; this may be due to the difficulty of protonation. In general, acyl derivatives may be deacylated by enzymes, and protonation may occur. However, in the ortho-substituted benzoyl derivatives, deacylation by an enzyme seems to be inhibited by steric hindrance of the bulky ortho group. In the case of sulfonyl derivatives, such a hydrolytic enzyme is probably absent from the organisms.



2. Mitosene Compounds.—The enzymatic activation may be necessary for the biological activities of the mitosene compounds as shown in the following scheme. In this case, reaction b which is necessary for the formation of an indole nucleus in the case of mitosane, is not included. Table V summarizes the relationship between reduction potentials and antibacterial activities. Antibacterial activities are not parallel to reduction potential, thereby differing from the mitosanes. Where X was NH_2 or NHEt, the mitosene derivatives were less active, probably due to the lower reduction potential which hardly enables the enzymatic reduction (reaction a) to proceed.[9] On the other hand, low activity in the case of X = AcO may be due to the absence of a third active site of substituent X as suggested by Iyer and Szybalsky.[7]



13



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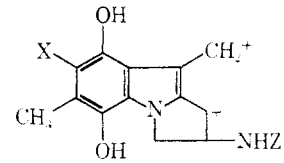


18

D. Inhibition of DNA Synthesis and Prophage Induction of Mitomycin Derivatives.—Mitomycin C exhibits selective inhibition of DNA synthesis.[10,11] Which is the group responsible for this inhibition? The effects of derivatives on nucleic acid and protein synthesis of *Escherichia coli* were investigated. The results for the principal compounds are shown in Table VI. Since compounds which have an aziridine group inhibited DNA synthesis, the aziridine was considered to be related to this selective inhibition. On the other hand, when the aziridine was removed in those compounds which have an aminoquinone moiety, they still showed selective inhibition. Therefore, the aminoquinone group in mitomycin C is also responsible for the selective inhibition of DNA synthesis.

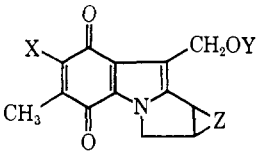
(9) Similar relationship has been observed in the indolequinone derivatives conducted by W. A. Remers and M. J. Weiss, *J. Med. Chem.*, **11**, 737 (1968).

(10) S. Shiba, A. Terawaki, T. Taguchi, and J. Kawamata, *Biken J.*, **1**, 179 (1958).

(11) M. Sekiguchi and Y. Takagi, *Biochim. Biophys. Acta*, **41**, 434 (1960).

TABLE V

EFFECT OF X-SUBSTITUENT ON THE BIOLOGICAL ACTIVITIES OF MITOSENE COMPOUNDS



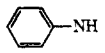
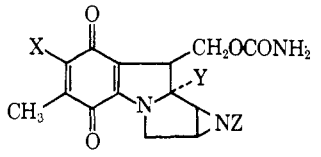
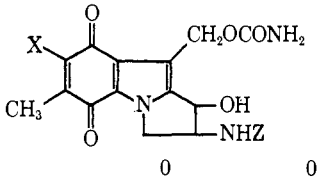
X	Y	Z	Reduction potential	Gram-positive bacteria	Gram-negative bacteria
CH_3COO	$CONH_2$	$[NCH_3$		>20	>20
		$[OH$ $[NHCH_3$	−0.320 −0.320	>50	>50
CH_3O	$CONH_2$	$[N—CH_3$	−0.39	1.10	12.0
		$[OH$ $[NHCH_3$	−0.39	1.12	28.12
$(CH_3)_2N$	$CONH_2$	$[N—CH_3$	−0.415	1.152	50
		$[OH$ $[NHCH_3$	−0.415	1.30	50
	$CONH_2$	$[OH$ $[NHCH_3$	−0.420	4.76	50
EtNH	$CONH_2$	$[NCH_3$	−0.495	>50	>50
		$[OH$ $[NHCH_3$	−0.495	>50	>50
NH_2	$CONH_2$	$[N—CH_3$	−0.529	>50	>50
		$[OH$ $[NHCH_3$	−0.529	>50	>50

TABLE VI

INHIBITION OF DNA SYNTHESIS AND PROPHAGE INDUCTIVITY OF MITOMYCIN DERIVATIVES



X	Y	Z	Prophage inductivity[a] 0.1 μg/ml	1 μg/ml	10 μg/ml	Inhibition of[b] DNA synthesis
OCH_3	OCH_3	H	0.25	0.5		+
NH_2	OCH_3	H	0.2	0.4		+
OCH_3	OH	CH_3	0	0.05	0.35	+
NH_2	OCH_3	CH_3	0.05	0.2		+
OH	OCH_3	H	0	0	0.20	+

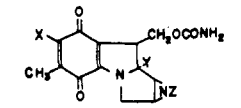


CH_3O	0	0	−
NH_2	0	0	+
OH	0	0	−

[a] This experiment was conducted by the method described by Sekiguchi, *et al.*:[11] (+) selective inhibition; (−): no inhibition. [b] This experiment was conducted by using *E. coli W3100* as lysogenic strain by the method described by Endo, *et al.*[12] Prophage inductivity was calculated as ratio of infective center and original bacteria.

Endo[12] employed the prophage-inductive action of various alkylating agents to the screening method of antitumor agents. This method was applied to mitomycin derivatives. The results are given in Table VI. Compounds containing an aziridine ring induced prophage, while the cleavage of the aziridine ring resulted in failure to produce such action. Thus the aziridine ring was one of active sites in mitomycin.

(12) H. Endo, M. Ishizawa, T. Kamiya, and S. Sonoda, *Nature (London)*, **198**, 258 (1963).



	X	Y	Z	reduction potential
1)	CH_3O	OCH_3	H	−0.195
2)	CH_3O	OH	CH_3	−0.200
3)	C_6H_5NH-	OCH_3	H	−0.233
4)	OH	OCH_3	H	−0.382
5)	EtNH	OCH_3	H	−0.307
6)	NH_2	OCH_3	H	−0.395
7)	NH_2	OH	H	−0.372

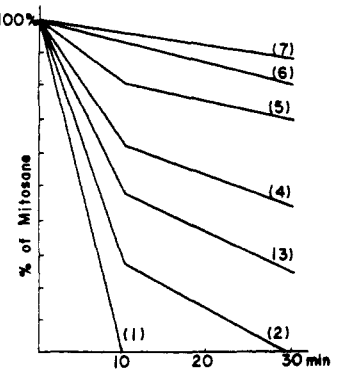


Figure 1.—Enzymatic activation rate of mitosane compounds in liver homogenate (effect of X substituents). To a soln of mitomycin derivatives (200 μg/ml) in 0.01 *M* phosphate buffer which contains 0.14 *M* KCl and 0.02 *M* nicotinamide was introduced N_2 and 3 ml of rat liver homogenate (1 g of fresh liver in 3 ml of buffer). After incubating at 37° for 1, 10, and 30 min, the reaction mixture was oxidized by air in an ice bath and immediately heated at 100° for 5 min to denaturate the enzyme. After centrifugation, the suppernatant was assayed by the cup method using *Bacillus subtilis ATCC* as test organism. Figure 1 shows the change of relative potency of derivatives against *Bacillus subtilis* after incubations of 10 and 30 min.

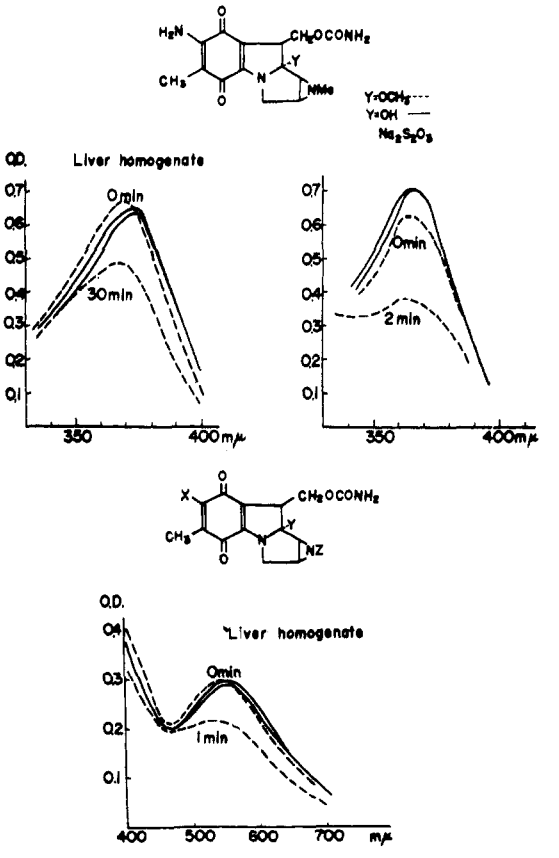


Figure 2.—Effect of Y substituent on activation by chemical and enzymatic reduction.

Experimental Section

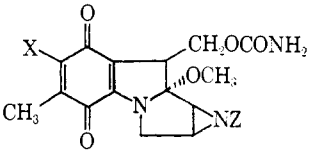
1a-Acylmitomycins (4).—To a suspension of 1 g of mitomycin in 100 ml of anhyd THF was added 2 ml of Et_3N. Acid chloride (3 mmoles) in C_6H_6 (5 ml) was then introduced dropwise into the soln with stirring, and the mixture was stirred for 30 min. The

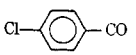
$Et_3NH^+Cl^-$ formed was removed by filtration, and the filtrate was evapd to dryness under reduced pressure. The residue was dissolved in EtOAc and chromatographed on silicic acid. Elution with EtOAc gave a purple band of **4** and a minor band of the starting material. The main purple band of **4** was eluted with Me_2CO-EtOAc (1:1), and the eluate was evapd to dryness. The residue was crystd from EtOAc. 1a-Acyl derivatives obtained by this procedure and their properties are shown in Table VII.

1a-Methanesulfonylmitomycins (5).—1a-Methanesulfonyl derivatives of mitomycins were prepared by the same procedure as 1a-acylmitomycins. (See Table VII).

TABLE VII

1a-SUBSTITUTED MITOMYCINS



X	Z	Color	Mp (°C)	Formula[a]
NH_2	H	Purple needles	>300	$C_{15}H_{18}N_4O_5$
NH_2	CH_3	Purple needles	199	$C_{16}H_{20}N_4O_5$
NH_2	$CH_3CH{=}CHCO$	Purple needles	>300	$C_{19}H_{22}N_4O_6$
NH_2		Purple needles	200	$C_{22}H_{21}ClN_4O_6$
NH_2		Purple needles	>300	$C_{22}H_{21}ClN_4O_6$
NH_2	CH_3SO_2	Purple needles		$C_{16}H_{20}N_4O_7S$
CH_3O	H	Reddish purple needles	170	$C_{16}H_{19}N_3O_6$
CH_3O	CH_3	Reddish purple needles	145	$C_{17}H_{21}N_3O_6$
CH_3O	$CH_3CH{=}CO$	Reddish purple needles	80–88	$C_{20}H_{23}N_3O_7$
CH_3O		Reddish purple needles	188–191	$C_{23}H_{22}ClN_3O_7$
CH_3O		Reddish purple needles	158–161	$C_{23}H_{23}N_3O_7$
CH_3O	CH_3SO_2	Reddish purple needles	198–199	$C_{17}H_{21}N_3O_8S$

[a] All compounds were analyzed for C, H, N.

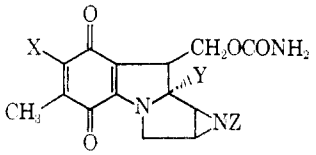
1a-Methylmitomycins (6).—To a soln of 200 mg of **1** in 25 ml of Me_2CO, 1 g of K_2CO_3 and 1 ml of MeI were added. The mixture was refluxed for 4 hr with vigorous stirring. The reaction mixture was filtered to remove K_2CO_3 and the filtrate was evapd to dryness under reduced pressure. The residual paste was dissolved in EtOAc and chromatographed on silicic acid using Me_2CO-EtOAc as a solvent. The first purple band was eluted and the eluate was evapd. The residue was crystd from Me_2CO or EtOAc.

7-Aminomitosane (7).—To a soln of 500 mg of **1a** or **1b** in 5 ml of MeOH, excess amine was added. After standing for 30 min–24 hr at room temp, the color of the soln changed to bluish purple or green from the reddish purple of the mitomycins. The end point of the reaction was checked by tlc. The reaction mixture was evapd under reduced pressure. The residue was crystd from Me_2CO or EtOAc. Table VIII shows compounds prepared by this method.

Decarbamoyldehydroxymitomycin B (8).—To a soln of mitomycin B (1 g) in 100 ml of anhyd THF was added gradually 800 mg of LAH with stirring under N_2. The reaction mixture became colorless immediately. After 1 hr moist EtOAc was added to the reaction mixture to decompose excess LAH. Then the reaction mixture was poured in the soln of potassium nitrosodisulfonate in 270 ml of H_2O and 140 ml of 0.166 *M* KH_2PO_4. The reaction mixture became purple; it was extracted with $CHCl_3$, and the extract was dried (Na_2SO_4) and evapd under reduced pressure. The pasty residue was dissolved in C_6H_6 and purified by partition chromatography of silicic acid using C_6H_6-Me_2CO-H_2O (8:2:5) as a solvent. The first bluish fraction was collected and evapd. The residue was crystd from Me_2CO and petr ether.

TABLE VIII

7-AMINOMITOSANE COMPOUND

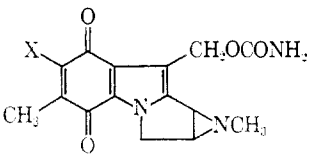


X	Y	Z	Color	Mp	Formula[a]
C_6H_5NH	OCH_3	H	Greenish needles	170 ~ 180	$C_{21}H_{22}N_4O_5$
$(CH_3)_2N$	OCH_3	H	Greenish needles	Not clear	$C_{17}H_{22}N_4O_5$
EtNH	OCH_3	H	Purple needles	Not clear	$C_{17}H_{22}N_4O_5$
C_6H_5NH	OH	CH_3	Greenish needles	Not clear	$C_{21}H_{22}N_4O_5$
$(CH_3)_2N$	OH	CH_3	Greenish needles	185 ~ 190	$C_{17}H_{22}N_4O_5$
EtNH	OH	CH_3	Bluish purple needles	200 ~ 203	$C_{17}H_{22}N_4O_5$
NH_2	OH	CH_3	Bluish purple needles	230 ~ 232	$C_{15}H_{18}N_4O_5$

[a] See footnote *a*, Table VI.

TABLE IX

7-SUBSTITUTED AZIRIDINOMITOSENE



X	Color	Mp (°C)	Formula[a]
CH_3O	Orange needles	Not clear	$C_{16}H_{17}N_3O_5$
NH_2	Reddish purple needles	Not clear	$C_{15}H_{16}N_4O_4$
NEt	Purple needles	Not clear	$C_{17}H_{20}N_4O_4$
$N(CH_3)_2$	Reddish purple needles	Not clear	$C_{17}H_{20}N_4O_4$

[a] See footnote *a*, Table VI.

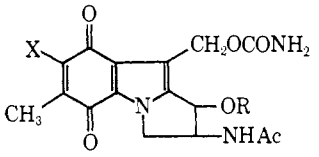
Dark purple needles (160 mg) were obtained, mp 148–150°. The product was identified as decarbamoyldehydroxymitomycin B (**8**) by its ir and nmr spectra. The second purple fraction gave 200 mg of an oily product which was identified as decarbamoylmitomycin B. The third fraction was identified as mitomycin B.

Dehydroxymitomycin B (10).—Phenyl chlorocarbonate (0.5 ml) was added dropwise to 70 mg of **8** in 2 ml of pyridine with mechanical stirring in an ice bath. After 2 hr, the reaction mixture was dild with H_2O and extd several times with $CHCl_3$. The organic layer was collected, washed with $NaHCO_3$ and H_2O, and dried (Na_2SO_4). The soln was evapd, and the residue was dissolved in $CHCl_3$. Purification by silicic acid chromatography gave an oily phenyl carbonate (**9**) of **8**, which was then dissolved in $CHCl_3$. Dry NH_3 was introduced to this soln under cooling in Dry Ice-Me_2CO. The solvent was gradually evapd at room temp with stirring. The residue was purified on silica gel and purple prisms were obtained. *Anal.* ($C_{16}H_{19}N_3O_5$) C, H, N.

Demethoxyporifiromycin (11).—Method A.—When dehydroxymitomycin B was treated with methanolic NH_3 for 1 hr, **11** was obtained quant.

Method B.—To a soln of 1d (100 mg) in 20 ml of MeOH, 200 mg of $NaBH_4$ was added. After stirring for 1 hr, the reaction mixture was introduced into a soln of potassium nitrosodisulfonate (500 mg) in 21 ml of H_2O and 14 ml of 0.166 KH_2PO_4. The soln was extd with $CHCl_3$ and the ext was dried (Na_2SO_4) and

TABLE X
7-AMINOMITOSENE COMPOUND



X	R	Color	Mp (°C)	Formula[a]
C_6H_5NH	H	Purple prism	250	$C_{22}H_{22}N_4O_6$
$(CH_3)_2N$	H	Reddish purple prisms	201–202	$C_{18}H_{22}N_4O_6$
EtNH	H	Reddish purple needles	223–226	$C_{18}H_{22}N_4O_6$
NH_2	H	Reddish prisms	174–178	$C_{16}H_{18}N_4O_6$
CH_3O	H	Orange needles	173–178	$C_{17}H_{19}N_3O_7$
C_6H_5NH	$COCH_3$	Purple prisms	271–275	$C_{24}H_{24}N_4O_7$
$(CH_3)_2N$	$COCH_3$	Purple prisms	164–169	$C_{20}H_{24}N_4O_7$
EtNH	$COCH_3$	Reddish purple prisms	240–250	$C_{20}H_{24}N_4O_7$
NH_2	$COCH_3$	Reddish purple needles	250	$C_{18}H_{20}N_4O_7$
CH_3O	$COCH_3$	Orange needles	227	$C_{19}H_{21}N_3O_8$

[a] See footnote *a*, Table VI.

evapd under reduced pressure. The residue was purified by silicic acid chromatography and crystd from Me_2CO. Greenish needles of mp 270–275° were obtained. *Anal.* ($C_{15}H_{18}N_4O_4$) C, H, N.

1a-Methyl-7-methoxyaziridinomitosene (12).—In 100 ml of EtOAc, **1b** (340 mg) was catalytically hydrogenated using 10% Pd-C. After 22 ml of H_2 was consumed, the mixture was filtered and the filtrate oxidized by aeration. Then the soln was evapd under reduced pressure, to produce 120 mg of orange plates which were recrystd from pyridine. Compound **12** showed no definite melting point. *Anal.* ($C_{16}H_{17}N_4O_5$) C, H, N.

1a-Methyl-7-alkylaminoaziridinomitosene (13).—To a DMF soln of **12** an excess of amine was added. After standing at room temperature for 2–5 days the yellowish soln changed to purple. It was evapd *in vacuo* to dryness. The residue was crystd from EtOH (Table IX).

1-Acetoxy-2-acetamino-7-substituted-mitosene (14).—A suspension of 7-substituted-9a-methoxymitosane (1 g) in Ac_2O was refluxed for 20–30 min. The reaction mixture was allowed to stand for 2 hr in the refrigerator. A crystalline product pptd was collected by filtration, yield 300–500 mg (Table X).

1-Hydroxy-2-acetamino-7-aminomitosene (15).—To a soln of 1-acetoxy-2-acetamino-7-methoxymitosene (900 mg) in 50 ml of MeOH an excess of amine was introduced. After standing at room temp overnight, the color of the reaction mixture had changed from yellow to reddish purple. The soln was evapd to dryness. The residue was crystd from EtOAc, yield 80–90% (Table X).

Mitomycin Derivatives. 2. Derivatives of Decarbamoylmitosane and Decarbamoylmitosene

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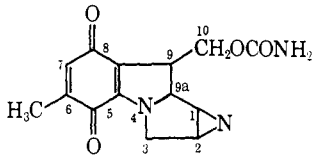
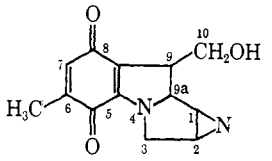
Several derivatives of decarbamoylmitosane (**2**) and decarbamoylmitosene (**3**) were prepared and subjected to *in vitro* antibacterial and prophage induction tests to investigate structure–activity relationship. The carbamoyl and aziridine groups are not essential for the biological action of mitomycin, but can be replaced by other acyl groups without loss of activity. The essential structure for the biological action is postulated to be the indolequinone.

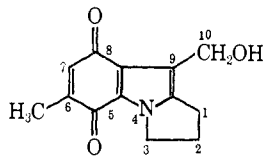
In the previous paper,[1] we reported on mitosane (**1**) and mitosene compounds having a carbamoyl group, and their biological activities, in order to study the structure–activity relationships of mitomycins. The present paper concerns decarbamoylmitosane (**2**) and decarbamoylmitosene (**3**) along with their biological activities.



1



2



3

Some of the mitosene derivatives which had no aziridine ring showed strong antibacterial and antitumor activities.[1] Iyer and Szybalsky[2] presented as a hypothesis for the chemical mechanism of the action of mitomycins that these compounds were bifunctionally masked alkylating agents due to protonation at the 1 and 10 positions of enzymatically reduced mitomycins. If this hypothesis were correct, the aziridine and carbamoyl groups in mitomycins would not be essential for their biological actions. In the present paper, the effects of substituents at the 10 position of decarbamoylmitosane (**2**) and decarbamoylmitosene (**3**) are described relating to their biological activities.

Decarbamoylmitosanes and Their Derivatives.—When mitomycins and their homologs were treated with NaOR, carbamoyl ($\nu_{C=O}$ 1700 cm^{-1}) was removed and decarbamoylmitosanes **4** were obtained. Then **4** was acetylated with acid anhydrides in pyridine to produce 10-acyloxy derivatives **5** (ester $\nu_{C=O}$ 1725 cm^{-1}). When Z was H, diacyl derivatives **6** (ester $\nu_{C=O}$ 1725 cm^{-1}, amide $\nu_{C=O}$ 1700 cm^{-1}) were obtained. They were partially deacylated in a weak alkaline condition to give 10-acyloxy derivatives (**7**) (ester $\nu_{C=O}$ 1725 cm^{-1}) (Scheme I).

Decarbamoylmitosene Derivatives.—Acid degradation of mitomycins gave mitomycinone as described

(1) S. Kinoshita, K. Uzu, K. Nakano, M. Shimizu and T. Takahashi, *J. Med. Chem.*, **14**, 103 (1971).

(2) V. N. Iyer and W. Szybalsky, *Science*, **145**, 55 (1964).