

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 H₂ 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. (C₁₆H₁₇N₄O₅) 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.



Some of the mitosene derivatives which had no aziridine ring showed strong antibacterial and antitumor ac-

 S. Kinoshita, K. Uzu, K. Nakano, M. Shimizu and T. Takahashi, J. Med. Chem., 14, 103 (1971). tivities.¹ Iyer and Szybalsky² 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 ($\nu_{C=0}$ 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 $\nu_{C=0}$ 1725 cm⁻¹). When Z was H, diacyl derivatives 6 (ester $\nu_{C=0}$ 1725 cm⁻¹, amide $\nu_{C=0}$ 1700 cm⁻¹) were obtained. They were partially deacylated in a weak alkaline condition to give 10-acyloxy derivatives (7) (ester $\nu_{C=0}$ 1725 cm⁻¹) (Scheme I).

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

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





х	Y	R	Z
$\rm NH_2$	OCH_3	Н	Н
$\rm NH_2$	OCH_3	COCH3	COCH3
$\rm NH_2$	OCH_3	Н	COCH_3
$\rm NH_2$	OCH_3	Н	CONH_2
NH_2	OCH_3	CH_3	\mathbf{H}
$\rm NH_2$	OCH_3	CH_3	$\rm COCH_3$

Staph. aureus. 209 P	Bacillus subtilis ATCC 6633	-MIC (µg/ml) Sarcina lutea ATCC 1001	E. coli K-12	Pseudo- monas aerogino s a
0.78	0.195	3.12	0.78	1.56
0.048	0.015	12.5	1.56	25.0
0.039	0.039	0.39	0.156	5.47
0.195	0.097	0.097	0.39	0.78
12.5	3.125	1.95	25.0	25.0
0.78	0.039	6.25	25.0	25.0

Scheme I

PREPARATION OF DECARBAMOYLMITOSANES AND THEIR ACETATES



previously.^{3,4} This compound was identified as 1-hydroxy-2-amino-7-hydroxydecarbamoylmitosene^{3,4} (8). Acylation of 8 followed by alkaline hydrolysis and methylation gave 1-hydroxy-2-acetamino-7-methoxydecarbamoylmitosene (9).³ It was acetylated to give triacetate 10, which was hydrolyzed with NH₃-MeOH to form a diacetyl derivative. It was identified as 11 by nmr (CH₂OAc, 5.2 ppm, 2 protons, doublet) and ir spectra (ester $\nu_{C=0}$, 1730 cm⁻¹). It was treated with ClCH₂COCl and C₅H₅N in dilute solution to give a mixed ester 12 (ester $\nu_{C=0}$, 1740 and 1760 cm⁻¹) (Scheme II).

Scheme II Preparation of Derivatives of Decarbamoylmitosenes



Biological Test and Structure-Activity Relationship. —The derivatives prepared as described above were subjected to antibacterial tests using *Bacillus subtilis*,

(3) K. UZU, Y. Harada, S. Wakaki and Y. Yamada, Agr. Biol. Chem., 28, 394 (1964). Sarcina lutea, Pseudomonas aeruginosa, and Staphylococcus aureus as test organisms. The minimal inhibitory concentration (MIC) of each compound was measured by agar plate dilution method. The prophage induction test was conducted by using Escherichia coli W. 3100 as test organism in the method employed by Endo, et al.⁵ The prophage inducing activity was calculated by plague index (sample's plague/control's plague).

Tables I and II show the antibacterial activities of the derivatives of decarbamoylmitosane and decarbamoylmitosene. In the mitosane and mitosene compounds, removal of the carbamoyl group resulted in a pronounced decrease of their antibacterial activities. It had been suggested that the carbamoyloxymethyl group (CH_2OCONH_2) was one of the active sites in mitomycins. Another interesting finding was that the 10-acyloxy derivatives of decarbamoylmitosane and decarbamoylmitosene showed almost the same antibacterial activity as mitosane and mitosene containing the carbamoyl group (Tables I, II). These phenomena indicated that the carbamoyl group was not essential for antibacterial activity, but can be replaced by other acyl groups without substantial loss of antibacterial activity. The protonation of C-10 is necessary for the appearance of the antibacterial activities. Mitosene compounds having no aziridine ring showed no prophage inducing activity as described previously.¹ Therefore the aziridine group is also one of the active sites of alkylating action in mitomycins. However, the aziridine group is not essential for the prophage inducing and alkylating actions. When an electron-withdrawing group was introduced at the oxygen of C-1 in mitosene compounds, they exhibited a strong prophage inducing action as shown in Table III.

These results gave partial evidence to Szybalsky's hypothesis that mitomycins are bifunctional alkylating agents. Moreover, they suggested that the carbamoyl and aziridine groups were not essential for the action of mitomycin, but can be replaced by other acyl groups without loss of biological activity.

Mechanism of Protonation of Reduced Mitomycin. —The following three factors seem to be necessary for the protonation of activated mitomycins.

(1) First, an indole fragment is formed by reduction.

(5) H. Endo, M. Ishizawa, and T. Kamiya, Nature (London), 198, 195 (1963).

⁽⁴⁾ 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. Laucartei, J. Amer. Chem. Soc., 84, 3185 (1962).

TABLE II ANTIBACTERIAL ACTIVITIES OF DECARBAMOYLMITOSENES AND THEIR DERIVATIVES









Two types of resonance should be possible as suggested by Iyer and Szybalsky.²

(2) The second is an electron-donating force by the substituents on ring A. Since OH, X, and Me groups

The Prophage Inductivity of Derivatives of Mitosene and Decarbamoylmitosene



are electron-donating groups, many types of resonance resulting from these groups are to be considered.



(3) The last is an electron-withdrawing action of V and W at the C-10 and the C-1 positions, respectively.

The cooperation of these three factors is necessary for the protonation at the C-1 and the C-10 of reduced mitomycin, and for the appearance of the biological activities of mitomycins. Essential structures for the biological action of mitomycins are therefore shown in the following formulas.



Experimental Section

Decarbamoylmitomycins (4).—To a mixture of 500 mg of mitomycins in 50 ml of MeOH and 85 ml of dry C_6H_6 was added a soln of 5 g of MeONa in 35 ml of MeOH. The mixture was vigorously stirred at room temp for 12 hr. The reaction mixture was neutralized with excess Dry Ice; the bluish color changed to purple. The reaction mixture was filtered, and the collected NaHCO₃ was washed with Me₂CO until the filtrate became colorless. The filtrate was combined and evapd under reduced pressure and the residue was chromatographed on a silica gel column using CHCl₃-Me₂CO (1:2) as an eluent. Conen of the purple eluate afforded crystals of 4, yield 60–70%.

Acetates 5 and 6 of Decarbamoylmitomycin (4).—In a mixture of 0.5 ml of Ac₂O and 1 ml of pyridine was dissolved 100 ml of decarbamoylmitomycin (4). After the mixture was allowed to stand for 16 hr in the refrigerator, it was poured into ice water. The resulting soln was extd with EtOAC, and the ext was collected, washed with aq NaHCO₃ and satd aq NaCl, and dried (Na₂SO₄). The solvent was evapd under reduced pressure to give a quant amt of acetates 5 and 6.

Monoacetyldecarbamoylmitomycin C (7, $X = NH_2$).—In a mixture of 3 ml of MeOH and 5 ml of 10% NaHCO₃ was dissolved 100 mg of diacetyldecarbamoylmitomycin C (6, $X = NH_2$). The mixture was allowed to stand for 2 days at room temp and then extd with EtOAc. The ext was dried (Na₂SO₄ and evapd to dryness under reduced pressure. The residue was chromatographed on silicic acid using CHCl₃-Me₂CO (1:1) as a solvent. The second main fraction was crystd as purple needles. Table IV shows the properties of decarbamoylmitosane and their derivatives.

1-Hydroxy-2-acetamino-7-methoxydecarbamoylmitosene (9) was prepared by acetylation of mitomycinone (8) followed by hydrolysis and methylation as described previously.³

Diacetate 10 of 1-Hydroxy-2-acetamino-7-methoxydecarbamoylmitosene (9).—To a mixture of 1 ml of Ac₂O and 1 ml of pyridine was added 100 mg of decarbamoylmitosene (9). After standing at room temp for 1 hr, the soln was evapd to dryness under reduced pressure, and the residue was chromatographed on silica gel using CHCl₃-Me₂CO (9:1) as a solvent. The main fraction was evapd and the residue was crystd from Me₂Co-Et₂O: ir $\nu_{C=0}$ 1725, cm⁻¹ (ester), 1700 cm⁻¹ (amide). The dipropionate was prepared by this procedure.

Monoacetates 11 of 1-Hydroxy-2-acetamino-7-methoxydecarbamoylmitosene (9).—In 80 ml of 0.25 N NH₃-MeOH, 340 mg of 10 was dissolved. After standing for 20 min, the reaction mixture was evapd to dryness, and the residue was chromatographed on silicic acid using Me₂CO-CHCl₃ (1:9). Evapn of the main fraction gave crystals of 11, yield 50-60%: ir $\nu_{C=0}$, 1730 cm⁻¹; nmr 5.2 ppm, 2 protons, doublet.





^a All compds were analyzed for C, H, N.



		O II		
	CH ₃ O	<u> </u>	CH ₂ OR ₁	
	H _a C			
\mathbf{R}_1	\mathbf{R}_2	M.P.	Color	Formula ^a
Н	Н	255	Orange	$\mathrm{C}_{16}\mathrm{H}_{18}\mathrm{N}_{2}\mathrm{O}_{6}$
COCH₃	$COCH_3$	222	Yellow	$C_{20}H_{22}N_2O_8$
$\mathrm{COC}_{2}\mathrm{H}_{5}$	$\mathrm{COC}_{2}\mathrm{H}_{5}$	209	Yellow	${\rm C}_{22}{\rm H}_{26}{\rm N}_{2}{\rm O}_{8}$
COCH ₂ Cl	$\rm COCH_2Cl$	208	Orange needles	$\mathrm{C_{20}H_{20}ClN_2O_8}$
$\rm COCH_3$	Н	178	Orange needles	${\rm C}_{18}{\rm H}_{20}{\rm N}_{2}{\rm O}_{7}$
COCH3	COCH ₂ Cl	164	Orange needles	$\mathrm{C}_{20}\mathrm{H}_{21}\mathrm{ClN}_{2}\mathrm{O}_{8}$

^a See footnote *a*, Table IV.

Mixed Ester (12, $\mathbf{R} = \mathbf{CH}_3$; $\mathbf{R}' = \mathbf{ClCH}_2$) of Decarbamoylmitosene (9).—To 100 mg of 11 in 5 ml of C_3H_3N , 450 mg of ClCH₂COCl in 5 ml of anhyd C_6H_6 was added dropwise in an ice bath with vigorous stirring. After 2 hr, a small amt of H₂O was added to the reaction mixture and extd with CHCl₃. The organic soln was washed with aq NaHCO₃ and H₂O and dried (Na₂SO₄). The solvent was evapd to dryness. The residue was chromatographed on silicic acid using Me₂CO-CHCl₃ (1:9). The main fraction was evapd to dryness under reduced pressure and the residue was crystd from Me₂CO to give 87 mg of needles: ir ester $\nu_{C=0}$, 1740, 1760 cm⁻¹. Table V shows the properties of derivatives of decarbamoylmitosene.