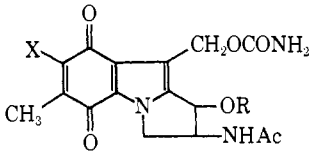
TABLE X
7-AMINOMITOSENE COMPOUND



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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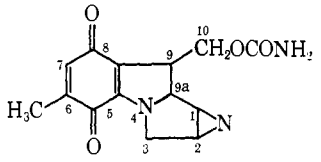
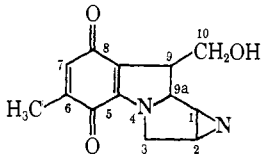
Several derivatives of decarbamoylmitosane (**2**) and decarbamoylmitosene (**3**) were prepared and subjected to *in vitro* antibacterial and prophage induction tests to investigate structure–activity relationship. The carbamoyl and aziridine groups are not essential for the biological action of mitomycin, 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.

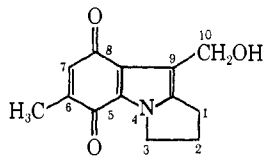
In the previous paper,[1] we reported on mitosane (**1**) and mitosene compounds having a carbamoyl group, and their biological activities, in order to study the structure–activity relationships of mitomycins. The present paper concerns decarbamoylmitosane (**2**) and decarbamoylmitosene (**3**) along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

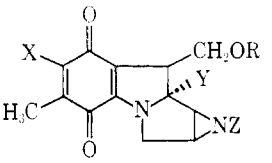
Decarbamoylmitosene Derivatives.—Acid degradation of mitomycins gave mitomycinone as described

(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).

TABLE I

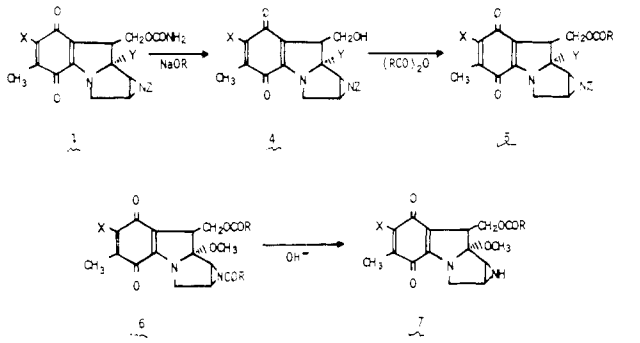
ANTIBACTERIAL ACTIVITIES OF DECARBAMOYLMITOSANES AND THEIR DERIVATIVES



X	Y	R	Z	MIC (μg/ml) *Staph. aureus* 209 P	*Bacillus subtilis* ATCC 6633	*Sarcina lutea* ATCC 1001	*E. coli* K-12	*Pseudomonas aeroginosa*
NH_2	OCH_3	H	H	0.78	0.195	3.12	0.78	1.56
NH_2	OCH_3	$COCH_3$	$COCH_3$	0.048	0.015	12.5	1.56	25.0
NH_2	OCH_3	H	$COCH_3$	0.039	0.039	0.39	0.156	5.47
NH_2	OCH_3	H	$CONH_2$	0.195	0.097	0.097	0.39	0.78
NH_2	OCH_3	CH_3	H	12.5	3.125	1.95	25.0	25.0
NH_2	OCH_3	CH_3	$COCH_3$	0.78	0.039	6.25	25.0	25.0

SCHEME I

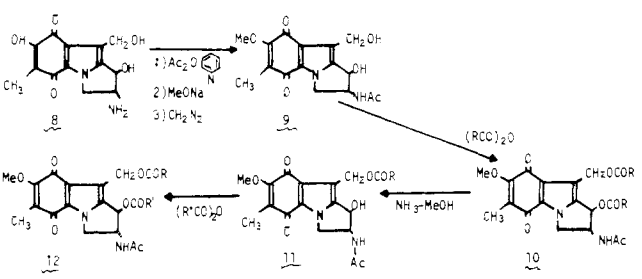
PREPARATION OF DECARBAMOYLMITOSANES AND THEIR ACETATES



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 NH_3–MeOH to form a diacetyl derivative. It was identified as **11** by nmr (CH_2OAc, 5.2 ppm, 2 protons, doublet) and ir spectra (ester $\nu_{C=O}$, 1730 cm^{-1}). It was treated with $ClCH_2COCl$ and C_5H_5N in dilute solution to give a mixed ester **12** (ester $\nu_{C=O}$, 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*, *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.

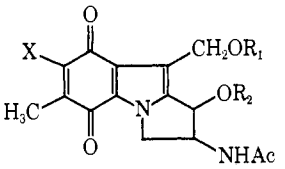
(3) K. Uzu, Y. Harada, S. Wakaki and Y. Yamada, *Agr. Biol. Chem.*, **28**, 394 (1964).

(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. Lancartei, *J. Amer. Chem. Soc.*, **84**, 3185 (1962).

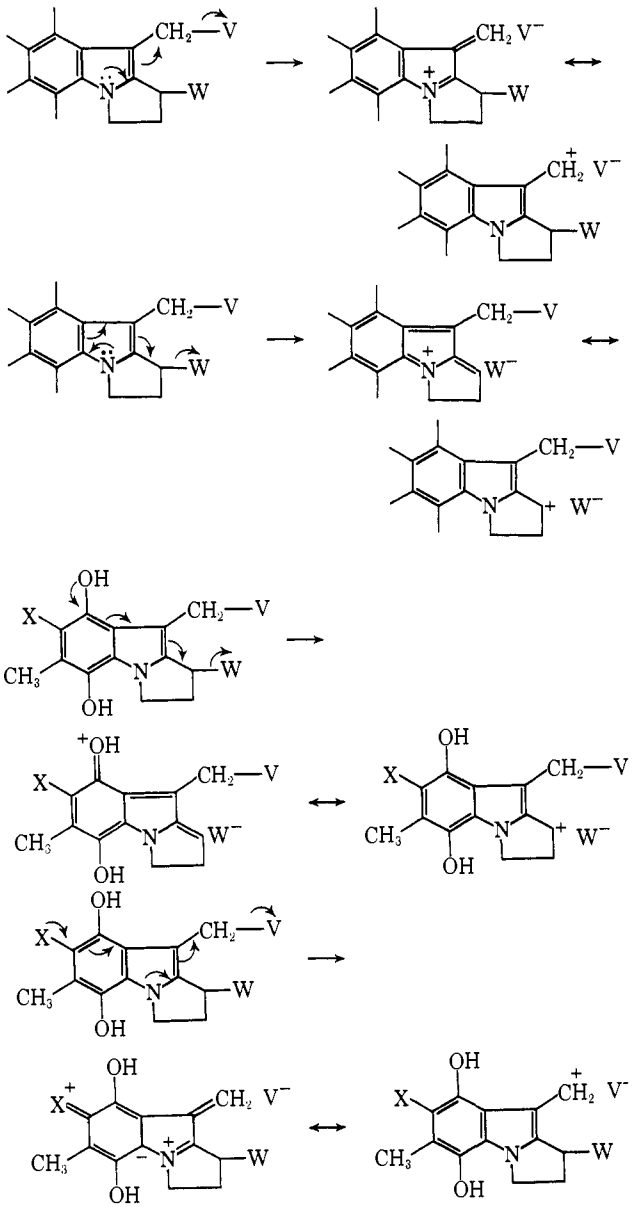
(5) H. Endo, M. Ishizawa, and T. Kamiya, *Nature (London)*, **198**, 195 (1963).

TABLE II

ANTIBACTERIAL ACTIVITIES OF DECARBAMOYLMITOSENES AND THEIR DERIVATIVES



			MIC (μg/ml)				
X	R_1	R_2	*Staph. aureus* 209 P	*Bacillus subtilis* ATCC	*Sarcina lutea* PCI	*E. coli* B (H)	*Pseudomonas aeruginosa*
CH_3O	H	H	>50	>50	>50	>50	>50
CH_3O	$COCH_3$	$COCH_3$	0.383	0.191	0.383	1.53	>50
CH_3O	COC_2H_5	COC_2H_5	0.766	0.766	1.53	6.12	>50
CH_3O	$COCH_2Cl$	$COCH_2Cl$	3.06	1.53	3.06	6.12	>50
CH_3O	$COCH_3$	H	0.38	0.38	0.76	3.06	>50
CH_3O	$COCH_3$	$COCH_2Cl$	0.38	0.38	0.76	6.12	>50
CH_3O	$CONH_2$	H	0.38	0.38	1.56	6.12	>50

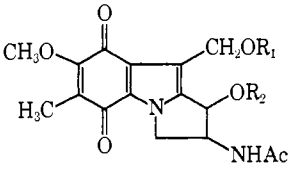


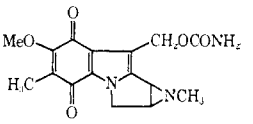
Two types of resonance should be possible as suggested by Iyer and Szybalsky.[2]

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

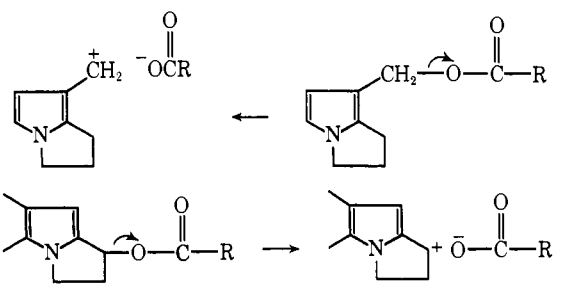
TABLE III

THE PROPHAGE INDUCTIVITY OF DERIVATIVES OF MITOSENE AND DECARBAMOYLMITOSENE



R_1	R_2	Prophage inductivity (1 mg/ml) Plague index (sample's plague)/(control's plague)
$CONH_2$	H	1.0
$CONH_2$	$COCH_3$	1.8
$CONH_2$	$COCH_2Cl$	5.6
$CONH_2$	$COCHCl_2$	6.6
H	H	1.0
$COCH_3$	$COCH_3$	1.8
$COCH_3$	H	1.0
$COCH_2Cl$	$COCH_2Cl$	7.2
$COCH_3$	$COCH_2Cl$	4.7
		14.0

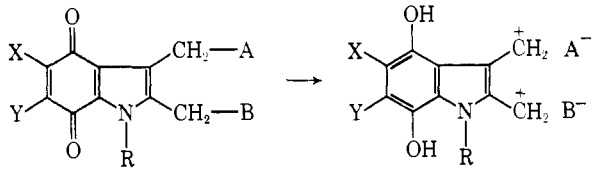
are electron-donating groups, many types of resonance resulting from these groups are to be considered.



(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 $NaHCO_3$ was washed with Me_2CO 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 $CHCl_3$-Me_2CO (1:2) as an eluent. Concn 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 Ac_2O 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 $NaHCO_3$ and satd aq NaCl, and dried (Na_2SO_4). 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% $NaHCO_3$ 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 (Na_2SO_4) and evapd to dryness under reduced pressure. The residue was chromatographed on silicic acid using $CHCl_3$-Me_2CO (1:1) as a solvent. The second main fraction was evapd to leave a residue, from which 59.5 mg of monoacetate **7** was crystd as purple needles. Table IV shows the properties of decarbamoylmitosane and their derivatives.

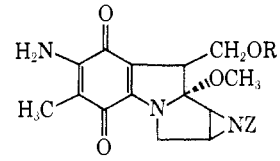
1-Hydroxy-2-acetamino-7-methoxydecarbamoylmitosene (9) was prepared by acetylation of mitomycinone (**8**) followed by hydrolysis and methylation as described previously.[3]

Diacetate 10 of 1-Hydroxy-2-acetamino-7-methoxydecarbamoylmitosene (9).—To a mixture of 1 ml of Ac_2O 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 $CHCl_3$-Me_2CO (9:1) as a solvent. The main fraction was evapd and the residue was crystd from Me_2Co-Et_2O: ir $\nu_{C=O}$ 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* NH_3-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 Me_2CO-$CHCl_3$ (1:9). Evapn of the main fraction gave crystals of 11, yield 50–60%: ir $\nu_{C=O}$, 1730 cm^{-1}; nmr 5.2 ppm, 2 protons, doublet.

TABLE IV

DECARBAMOYLMITOMYCINS AND THEIR DERIVATIVES

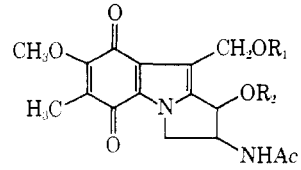


X	R	Mp, °C	Color	Formula[a]
H	H	250	Dark purple needles	$C_{14}H_{17}N_3O_4$
CH_3	H	158	Dark purple needles	$C_{15}H_{19}N_3O_4$
$COCH_3$	$COCH_3$		Dark purple needles	$C_{18}H_{21}N_3O_6$
H	$COCH_3$	178	Dark purple needles	$C_{16}H_{19}N_3O_5$
CH_3	$COCH_3$	174	Dark purple needles	$C_{17}H_{21}N_3O_6$

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

TABLE V

DECARBAMOYLMITOSENES AND THEIR DERIVATIVES



R_1	R_2	M.P.	Color	Formula[a]
H	H	255	Orange needles	$C_{16}H_{18}N_2O_6$
$COCH_3$	$COCH_3$	222	Yellow needles	$C_{20}H_{22}N_2O_8$
COC_2H_5	COC_2H_5	209	Yellow needles	$C_{22}H_{26}N_2O_8$
$COCH_2Cl$	$COCH_2Cl$	208	Orange needles	$C_{20}H_{20}ClN_2O_8$
$COCH_3$	H	178	Orange needles	$C_{18}H_{20}N_2O_7$
$COCH_3$	$COCH_2Cl$	164	Orange needles	$C_{20}H_{21}ClN_2O_8$

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

Mixed Ester (12, R = CH_3; R′ = $ClCH_2$) of Decarbamoylmitosene (9).—To 100 mg of 11 in 5 ml of C_5H_5N, 450 mg of $ClCH_2COCl$ 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 H_2O was added to the reaction mixture and extd with $CHCl_3$. The organic soln was washed with aq $NaHCO_3$ and H_2O and dried (Na_2SO_4). The solvent was evapd to dryness. The residue was chromatographed on silicic acid using Me_2CO-$CHCl_3$ (1:9). The main fraction was evapd to dryness under reduced pressure and the residue was crystd from Me_2CO to give 87 mg of needles: ir ester $\nu_{C=O}$, 1740, 1760 cm^{-1}. Table V shows the properties of derivatives of decarbamoylmitosene.