STUDIES ON MITOMYCINS. III

THE SYNTHESIS AND PROPERTIES OF MITOMYCIN DERIVATIVES

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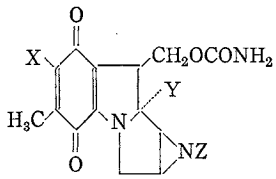
About sixty derivatives of mitomycin were synthesized from natural mitomycins and subjected to antibacterial and antitumor tests. Some of them were more effective than mitomycin C. The structure-activity relationship of derivatives were also investigated.

Mitomycins possess a strong activity against gram-positive and gram-negative bacteria as well as against EHRLICH ascites tumor cells and have been applied to the chemotherapy of cancer in Japan. The structures of mitomycin homologues were elucidated by WEBB *et al.*[1,2] as shown in the following formula.



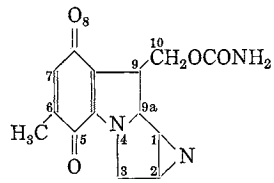
(I)

	X	Y	Z
Mitomycin A	CH_3O	OCH_3	H
Mitomycin B	CH_3O	OH	CH_3
Mitomycin C	NH_2	OCH_3	H
Porfiromycin	NH_2	OCH_3	CH_3

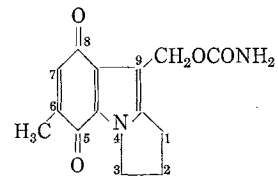
These structures are very unique not only for the natural product, but also the antitumor substance in respect that they have three carzinostatic groups——quinone, aziridine, and carbamoyloxymethyl——in their individual structures.

Authors synthesized several derivatives of them from the natural mitomycins to search less toxic and more effective substances. The present paper deals with synthetic methods, properties of these derivatives and their biological activities.

Derivatives of mitomycin[2] are classified into two main groups, the one is mitosane and the other metosene as shown in the following fomula.



Mitosane

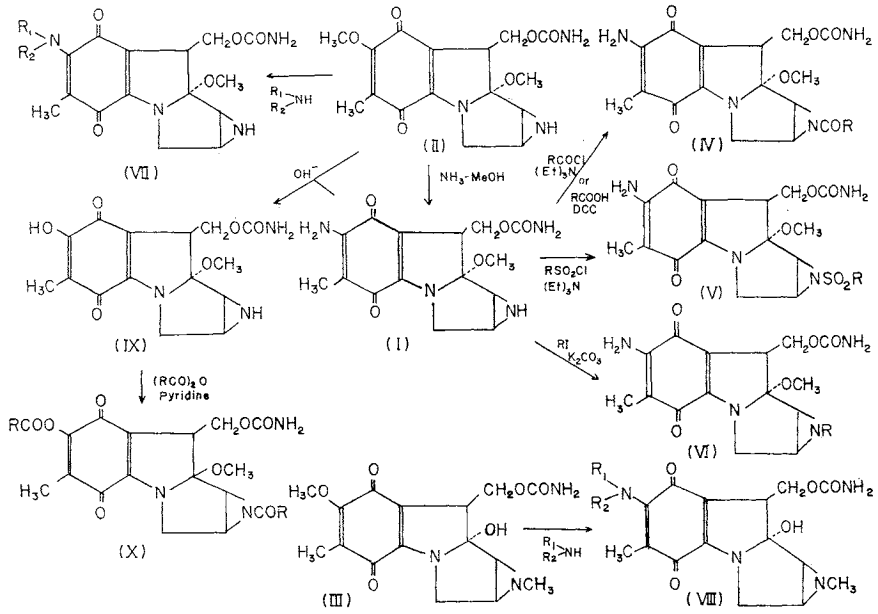


Mitosene

I. Preparation of Mitosane Compounds

Many kinds of mitosane compound were prepared from natural mitomycins as summarized in Chart 1.

Chart 1. Derivatives of mitosane



1a-Substituted mitosane compounds

Mitomycin C (I) were acylated with acylchloride in the presence of triethylamine to give 1a-monoacyl derivatives (IV) which showed almost the same ultraviolet spectra as I and a new infrared maxima at 5.84 μ. 1a-Sulfonyl derivatives (V) were prepared with sulfonyl chloride by the same method. 1a-Acyl derivatives (IV) were also prepared by the condensation of I with organic acid in the presence of dicyclohexylcarbodiimide. Alkylation of I with alkyl iodide in the presence of potassium carbonate gave 1a-alkyl derivatives (VI). 1a-Methyl derivative obtained by this method was identical with porifiromycin[3].

7-Substituted mitosane compounds

Treatment of mitomycin A (II) with ammonia gave mitomycin C (I) as described in the previous paper[4]. When treated with primary and secondary amines instead of ammonia, II should give several 7-alkylamino-mitosane compounds (VII). The mixture of alcoholic solution of II and amine changed its color to purple or green from reddish purple of II and gave IV having different color from II and almost the same ultra-violet spectra as I. Amination of mitomycin B (III) by the same procedure gave 7-alkylamino-9a-hydroxy compounds (VIII).

7-Hydroxy mitosane compound and its derivatives

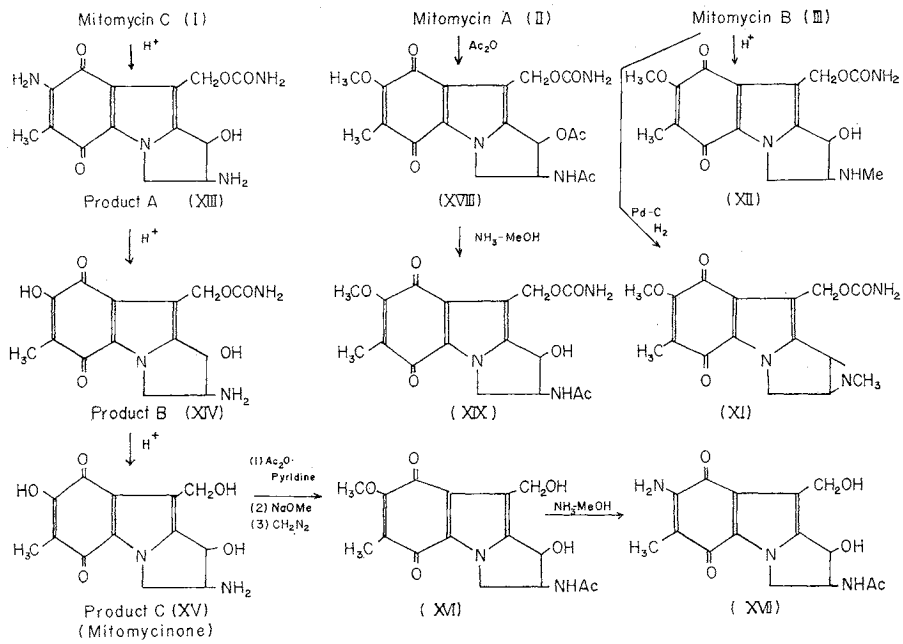
When mitomycin A (II) and C (I) were hydrolyzed with weak base, acidic quinone was obtained. This compound was methylated with diazomethane to yield mitomycin A. Then hydrolysate must be 7-hydroxy mitosane (IX). Acylation of (IX) with acyl chloride or acid anhydride resulted in diacyl-compound (X).

The properties of mitosane compounds prepared by the above method are shown in Tables 5, 6, 7 and 8.

II. Preparation of Mitosene

Mitosene compounds were prepared by acid hydrolysis as described in the previous report[4,5]. Products A, B and C in the previous paper are substances having the structures shown in Chart 2. 7-Amino-decarbamoylmitosene (XVII) were prepared by amination of 7-methoxy-decarbamoylmitosene (XVII), which is produced by acetylation followed by hydrolysis and methylation of mitomycinone as described in the previous reports[5]. Acetates (XVIII) and (XIX) of 7-methoxy-mitosene compounds were prepared by heating mitomycin A (II) in acetic anhydride and hydrolysis. Apomitomycin B (XII) and 7-methoxy-aziridinomitosene (XI) were preparaed by WEBB's method[2,5].

Chart 2. Derivatives of mitosene



III. Biological Test

Above described derivatives were subjected to the antibacterial and antitumor tests. Minimum inhibition concentration (M.I.C.) against 14 kinds of bacteria were estimated by the dilution method using heart infusion agar. The chemotherapeutic index was calculated by LD_{50}/M.I.C. Detailed data will be described by MIYAMURA *et al.*[6] in this Journal. 1a-Crotonyl-7-amino-9a-methoxy mitosane, 7-anilino-9a-methoxy-mitosane, and 1a-acetylmethionyl-7-amino-9a-methoxy-mitosane showed ten-fold chemotherapeutic index as mitomycin C against gram-positive bacteria.

Antitumor tests were conducted using Hirosaki ascites sarcoma and solid tumor of sarcoma 180. Detailed reports will be described by USUBUCHI *et al.* and OHOSHI *et al.* in the "Gann"[8,9].

Many of 1a-acyl, 1a-alkyl, and 7-alkylamino derivatives of mitomycin C showed same or greater effect against Hirosaki ascites sarcoma when compared with mitomycin C.

On the other hand, against solid tumor of sarcoma 180, 7-monoalkylamino derivatives and 1a chloroacyl derivatives gave good results. Above all, 7-isopropyl-amino-9a-methoxy-mitosane, 1a-chloroacetyl and 1a-dichloroacetyl derivatives showed excellent effects.

IV. Structure-activity Relationship

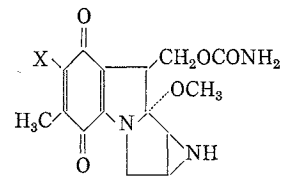
Tables 1, 2 and 3 show the effects of substituents X, Y and Z of mitosane compound against the biological activities. X, Y and Z substituents have a great influence against the biological activities of mitosane compounds.

Substituent X adjacent to quinone carbonyl affects reduction potential of quinone, and the biological activity of 7-substituted compound seems to be related with reduction potential.

Substituent Y in 9a-position also played an important part in the biological action. Change of methoxy group to hydroxyl resulted in a great decrease in antitumor and antibacterial activity.

Transformation of Z in 1a-position brought on the decrease in the biological activity. A remarkable decrease in activity was observed in the ortho-substituted benzoyl and the sulfonyl derivatives. This phenomenon may have occurred owing to the

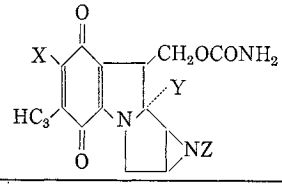
Table 1. Effect of X-substituent on the biological activities of mitosane compounds



X	Reduction* potential	LD_{50}	MIC		Antitumor activity	
			Gram-positive bacteria	Gram-negative bacteria	Ascites Hirosaki sarcoma	Solid sarcoma 180
CH_3	−0.195	2.0	0.01	0.57		
 -NH-	−0.233	38.5	0.09	7.55	++++	++
EtNH-	−0.307	9.38	0.109	17.7	++++	++
OH	−0.382	100	8.56	14.06		++
NH_2	−0.395	9.0	0.208	0.48	+++	+++

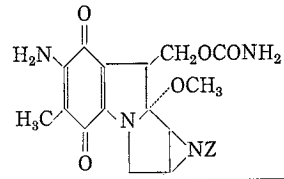
* Measured by polarographic method.

Table 2. Effect of Y-substituent on the biological activities of mitosane compounds



X	Y	Z	LD_{50} (mg/kg)	MIC		Antitumor activity	
				Gram-positive bacteria	Gram-negative bacteria	Ascites Hirosaki sarcoma	Solid sarcoma 180
NH_2	OCH_3	C_2H_5	55	0.27	11.4	++	++
CH_3O	OCH_3	CH_3	4.68	0.19	20		
NH_2	OH	CH_3	100	2.39	23.0		−
CH_3O	OH	CH_3	3.4	3.12	50	±	

Table 3. Effect of Z-substituent on the biological activities of mitosane compounds



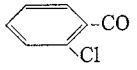
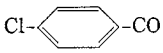
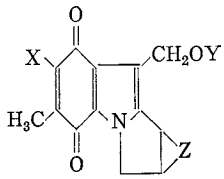
Z	LD_{50} (mg/kg)	MIC (mcg/ml)		Antitumor activity	
		Gram-positive bacteria	Gram-negative bacteria	Ascites Hirosaki sarcoma	Solid sarcoma 180
H	9.0	0.20	0.12	+++	+++
C_2H_5	48.0	0.27	11.4	++	++
CH_3CO	27.0	0.39	2.5	++	++
CH_3SO_2	100	10	50	++++	−
	100	3.23	29.2		+
	18.75	0.965	13.5		+

Table 4. Biological activities of mitosene compounds



X	Y	Z	LD_{50} (m/kg)	MIC* (mcg/ml)		Antitumor activity**	
				Gram-positive bacteria	Gram-negative bacteria	Ascites Hirosaki sarcoma	Solid sarcoma 180
CH_3O	$CONH_2$		18.7	1.05	2.5		+++
CH_3O	$CONH_2$		37.5	1.12	28.12		+
CH_3O	$CONH_2$		9.0	5.8	>50	++	++
CH_3O	H		>50	>50	>50		
NH_2	$CONH_2$		130	2.27	20.8	++	+
NH_2	H		>50	>50	>50		−

* MIC : Minimal inhibitory concentration, average value of MIC of gram-positive and gram-negative bacteria

** The detail data will be described in reference[2,3,4]

difficulty of protonation of aziridine as described by MIYAMURA *et al.*[7] Table 4 shows activities of various mitosene compounds prepared by acid hydrolysis which have no aziridine. Antitumor and antibacterial activities were observed in the mitosene compounds having carbamoyloxymethyl group.

These results indicate that the antibacterial and antitumor activity of mitomycins depend upon not only difunctional alkylating action[10] caused by protonation of carbamoyloxymethyl ($-CH_2OCONH_2$) and aziridine ($\rangle NH$). Thus it is suggested that mitomycins are the very unique antitumor substance different from the other alkylating agents.

Experimental

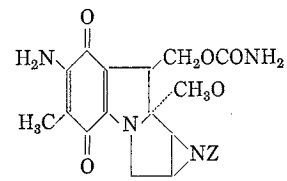
1a-Acyl-7-amino-9a-methoxy-mitosane (IV)

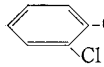
To a suspension of 1 g of mitomycin C (I) in 100 ml of anhydrous tetrahydrofuran was added 2 ml of triethylamine. Acid chloride (3 m mol) in benzene (5 ml) was then introduced dropwise into the solution under stirring. The mixture was stirred for 30 minutes. The resulting triethylamine hydrochloride precipitate was removed by filtration and the filtrate was evaporated under reduced pressure to dryness. The residue was dissolved in ethylacetate and chromatographed on silicic acid. Development with ethylacetate gave purple band of IV and minor band of original product (I). Main purple band of IV was eluted with acetone-ethylacetate (1 : 1) and eluate was evaporated to dryness. The residue was crystalized from ethylacetate.

1a-Acyl derivatives obtained by this procedure and their properties are shown in Table 5.

1a-Acetyl, 1a-chloroacetyl, and 1a-phthalylglutamyl derivatives were also prepared by the same method. They are chromatographically homogenous but were not obtained in crystalline form.

Table 5. 1a-Acyl-7-amino-9a-methoxy-mitosane



Z	Yield (%)	Color	Melting point (°C)	Formula	Analysis					
					Calcd.			Found		
					C	H	N	C	H	N
C_2H_5CO	80	purple needles	132	$C_{18}H_{22}O_6N_4$	55.38	5.68	13.86	55.25	5.75	14.02
nC_3H_7CO	74	〃	158	$C_{19}H_{24}O_6N_4$	56.46	5.98	13.86	56.50	5.80	13.95
nC_4H_9CO	73	〃	195	$C_{20}H_{26}O_6N_4$	57.40	6.26	13.39	57.66	6.38	13.51
$nC_5H_{11}CO$	75	〃	205	$C_{21}H_{28}O_6N_4$	58.32	6.53	12.96	58.52	6.78	12.87
$nC_6H_{13}CO$	77	〃	190	$C_{22}H_{30}O_6N_4$	59.18	6.77	12.51	58.85	6.72	12.58
$CH_3CH{=}CHCO$	53	〃	>300	$C_{19}H_{22}O_6N_4$	56.71	5.51	13.91	56.79	5.18	13.59
 -CO	75	〃	205	$C_{22}H_{22}O_6N_4$	60.27	5.66	12.78	60.00	5.20	12.50
 -CH=CHCO	93	〃	196	$C_{24}H_{24}O_6N_4$	62.06	5.21	12.06	61.86	5.05	12.30
 -CO	71	〃	200	$C_{22}H_{21}O_6N_4Cl$	55.84	4.48	11.85	55.68	4.49	12.21
 -CO	68	〃	205	$C_{23}H_{24}O_6N_4$	61.05	5.35	12.38	60.95	5.20	12.05
C_2H_5OCO	77	〃	185	$C_{18}H_{22}O_7N_4$	53.20	5.46	13.76	53.05	5.61	13.65

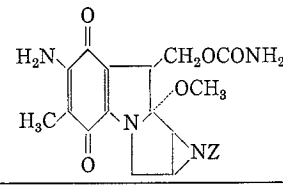
1a-Acyl-7-amino-9a-methoxy-mitosane (IV)

One gram of mitomycin C was dissolved in 100 ml of dry tetrahydrofuran and 630 mg of dicyclohexylcarbodiimide and equimolar corresponding acid were added to the solution, which was then allowed to stand for a few minutes or several hours neccessary for the completion of the reaction. The reaction mixture was filtered to remove dicylohexylurea and the filtrate was concentrated under reduced pressure at low temperature. The residue

was dissolved in ethylacetate and chromatographed on silicic acid using the solvent as acetone-ethylacetate (3 : 7). The first purple fraction was eluted and the eluate was concentrated to dryness. The residue was crystalized from acetone or ethylacetate. Table 6 shows derivatives prepared by this procedure.

1a-Bromoacetyl, 1a-α-chloropropionyl, 1a-fluroacetyl, 1a-β-chloropropionyl, 1a-dichloroacetyl and 1a-trichloroacetyl derivatives of mitomycin C were also prepared by the same procedure. They are chromatographically homogenous, but were not obtained in crystalline form.

Table 6. 1a-Acyl-7-amino-9a-methoxy-mitosane



Z	Yield (%)	Color	Melting point (°C)	Formula	Analysis					
					Calcd.			Found		
					C	H	N	C	H	N
$CH_3CONHCH_2CO$	45	purple amorphous	95~100	$C_{19}H_{23}O_7N_5$	52.65	5.35	16.16	52.54	5.72	15.21
	89	purple needles	not clear	$C_{22}H_{20}O_{10}N_6$	50.00	3.82	15.91	50.11	3.61	15.41
	90	〃	182	$C_{22}H_{21}O_8N_5$	54.66	4.39	14.39	54.31	4.69	13.87
	95	〃	193~195	$C_{22}H_{21}O_6N_4I$	46.81	3.72	10.01	46.56	3.80	10.18
	80	〃	not clear	$C_{22}H_{21}O_6N_4Cl$	55.98	4.44	11.82	56.02	4.57	11.16

1a-Alkyl-7-amino-9a-methoxy-mitosane (VI)

Two hundred mg of mitomycin C was dissolved in 25 ml of acetone, and 1 g of pottasium carbonate and 1 ml of alkyl iodide were added to the solution, which was refluxed for 4 hours stirring vigorously.

The reaction mixture was filtered to remove potassium carbonate and the filtrate was evaporated under reduced pressure to dryness. The residual paste was dissolved in ethylacetate and chromatographed on silicic acid using acetone-ethyl-acetate as solvent. First purple band was eluted and the eluate was evaporated. The residue was tried to crystalize from acetone or ethylacetate. 1a-Methyl, 1a-ethyl, 1a-propyl and 1a-butyl derivatives were prepared by this method.

1a-Methyl derivative was obtained as purple needles of m. p. 199°C and the others were chromatographically homogeneous although amorphous. In this series, the increase of the carbon number of alkyl radical resulted in the decrease of yields as follows.

$Z=CH_3$ 62 %, C_2H_5 42 %, C_3H_7 27 %, C_4H_9 19 %

Analysis of 1a-methyl derivative.

Calcd. for $C_{16}H_{20}O_5N_4$: C 55.16, H 5.79, N 16.08.
Found : C 55.16, H 5.70, N 16.20.

1a-Methyl-7-amino-9a-methoxy-mitosane was identified as porfiromycin by comparison of infrared spectra and mixed melting point.

1a-Methanesulfonyl derivative of mitomycin C (V R=CH_3)

One gram of mitomycin C was dissolved in 50 ml of dry tetrahydrofuran and 400 mg

of triethylamine was added to this solution, then 350 mg of methanesulfonyl chloride was introduced dropwise under stirring. After stirring for 30 minutes at room temperature, the reaction mixture was filtered to remove triethylaminehydrochloride and the filtrate was evaporated to dryness. The residual powder was dissolved in ethylacetate and chromatographed on silicic acid. The first purple band was eluted and the eluate was evaporated to dryness to obtain 950 mg of purple amorphous powder.

Anal. Calcd. for $C_{16}H_{70}O_7N_4S$: C 46.59, H 4.88.
Found : C 46.32, H 4.56.

1a-*p*-Toluensulfonyl derivative (V. R=CH_3-  -) was prepared by the same procedure.

7-Alkylamino-9a-methoxy-mitosane (VII)

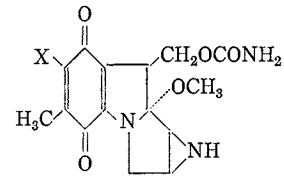
In 5 ml of methanol was dissolved 500 mg of mitomycin A (II) and excess amine was introduced to the solution. After standing for 30 minutes or 24 hours at room temperature, color of the solution changed to bluish purple or green from the reddish purple of (II). The end point of reaction was checked by thinlayer chromatography. The reaction mixture was evaporated under reduced pressure. The residue was crystalized from acetone or ethylacetate.

Table 7 shows compounds prepared by this method. 7-Butylamino (VII. R_1=H, R_2=nC_4H_9), 7-amylamino (VII. R_1=H, R_2=nC_5H_{11}), 7-lysino and 7-argino compounds were also prepared by the same procedure. They are chromatographically homogenous but amorphous and did not to crystalize.

1a-Methyl-7-alkylamino-9a-hydroxy-mitosane

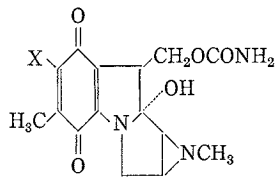
One gram of mitomycin B (III) was dissolved in 20 ml of methanol and excess amine was introduced to the solution. After standing for 15 minutes, the reaction mixture was

Table 7. 7-Alkylamino-9a-methoxy-mitosane



X	Yield (%)	Color	Melting point (°C)	Formula	Calcd.			Found		
					C	H	N	C	H	N
CH_3NH	69	bluish needles	not clear	$C_{16}H_{20}O_5N_4$	55.16	5.76	16.08	55.30	5.81	16.01
C_2H_5NH	73.5	//	//	$C_{17}H_{22}O_5N_4$	56.34	6.12	15.46	56.32	6.15	15.03
nC_3H_7NH	74	//	//	$C_{18}H_{24}O_5N_4$	57.43	6.34	14.89	57.10	6.20	14.95
iC_3H_7NH	80	//	//	$C_{18}H_{24}O_5N_4$	57.43	6.43	14.89	57.20	6.52	14.42
 -NH	65	//	//	$C_{21}H_{28}O_5N_4$	56.82	7.42	14.73	56.50	7.60	14.40
 -NH	68	//	//	$C_{21}H_{22}O_5N_4$	61.45	5.40	13.65	61.30	5.20	13.70
CH_2-CH_2-NH- | OH	62	//	//	$C_{17}H_{22}O_6N_4$	53.96	5.86	14.81	53.47	5.56	14.70
CH_3 CH_3>NH	71.5	greenish needles	//	$C_{17}H_{22}O_5N_4$	56.34	6.12	15.46	56.50	6.61	15.20
 N-	78	brownish needles	//	$C_{17}H_{20}O_5N_4$	56.66	5.59	15.53	56.40	5.40	15.30
 N-	92.5	greenish needles	//	$C_{19}H_{24}O_5N_4$	58.76	6.23	14.43	58.69	6.25	13.90
 N-	95	//	//	$C_{20}H_{22}O_6N_4$	59.69	6.51	13.92	59.72	6.77	13.67

Table 8. 1a-Methyl-7-alkylamino-9a-hydroxy-mitosane



X	Yield (%)	Color	Melting point (°C)	Formula	Calcd.			Found		
					C	H	N	C	H	N
NH_2	85	purple needles	235	$C_{15}H_{13}O_5N_4$	58.88	5.48	16.76	53.59	5.58	16.38
CH_3NH	76	//	155	$C_{16}H_{20}O_5N_4$	55.16	5.79	16.08	55.38	5.58	15.75
C_2H_5NH	80	//	188	$C_{17}H_{22}O_5N_4$	56.34	6.12	15.46	56.54	6.10	14.98
>N-	78	//	>300	$C_{19}H_{20}O_5N_4$	56.66	5.59	15.59	56.40	5.83	15.84

evaporated to dryness. The residue was crystalized from ethylacetate. Table 8 shows compounds prepared by this process.

7-Hydroxy-9a-methoxy-mitosane and its derivatives

One gram of mitomycin C (I) was dissolved in 0.1 N methanolic NaOH (50 %) and allowed to stand for 30 hours. The solution was then adjusted to pH 4.0 by mineral acid and extracted with ethylacetate until reddish color of the aqueous solution disappeared. The extract was evaporated under reduced pressure at low temperature to obtain 540 mg of the amorphous powder of 7-hydroxy-9a-methoxy-mitosane as the residue. This substance was very unstable, not to crystalize.

Methylation with diazomethane followed by chromatographic purification gave reddish purple needles of m. p. 160°C, which was identified as mitomycin A by comparison of infrared spectra and mixed melting point.

1a-Methyl-7-methoxy-aziridinomitosene (XI)

In 100 ml of ethyl acetate was dissolved 340 mg of mitomycin B and subjected to catalytic hydrogenation using paladium-charcoal (10 % Pd). When H_2-uptake was 22 ml, the suspension was filtered, and the filtrate was oxidized by the introduction of air. The solution was evaporated under reduced pressure, to produce 120 mg of orange plate, which were recrystalized from pyridine. This compound showed no definite melting point.

Anal. Calcd. for $C_{16}H_{17}O_5N_4$: C 58.00, H 5.17, N 12.78.
Found : C 58.95, H 5.53, N 12.78.

1-Hydroxy-2,7-diamino-mitosene (XIII)

Product I obtained by acid hydrolysis of mitomycin C as described in the previous paper[4] is 1-hydroxy-2,7-diaminomitosene. This compound was prepared by the same procedure.

1,7-Dihydroxy-2-amino-mitosene (XIV)

Product II, described in the previous report is 1,7-dihydroxy-2-amino-mitosene. This compound was prepared by the same procedure.

1-Hydroxy-2-methylamino-7-methoxy-mitosene (XII)

In 200 ml of 0.1 N hydrochloric acid was dissolved 1.7 g of mitomycin B (III) and allowed to stand for 1 hour at room temperature. The reaction mixture was adjusted to pH 8.5 with sodium bicarbonate and extracted with *n*-butanol. The extract was evaporated under reduced pressure to dryness. The residue was dissolved in acetone and chromatographed on silicic acid using acetone as solvent. After elution of first minor band, second, orange band was eluted. This eluate was evaporated and the residue was crystalized from methanol.

Anal. Calcd. for $C_{16}H_{19}O_6N_3$: C 55.01, H 5.48, N 12.03.
Found : C 54.90, H 5.54, N 67. m. p. 210°C.

1-Hydroxy-2-acetamino-7-methoxy-decarbomoylmitosene (XVI)

This compound was prepared by acetylation of mitomycinone (XV) followed by hydrolysis and methylation as described in the previous paper[5].

1-Hydroxy-2-acetamino-7-amino-decarbamoylmitosene (XVII)

In 100 ml of methanol was dissolved 200 mg of XVI and excess ammonia was add to the solution. After standing for 20 hours, the solution was evaporated under reduced pressure. The residue was crystalized from methanol.

Anal. Calcd. for $C_{16}H_{18}O_6N_4$: C 53.03, H 5.01, N 15.46.
Found : C 52.95, H 4.98, N 15.25.

this showed on definite melting point.

1-Acetoxy-2-acetamino-7-methoxy-mitosene (XVIII)

One gram of mitomycin A (II) was suspended in 20 ml of acetic anhydride and boiled for 20 minutes. When cooled in the refrigerator, the yellow needles were formed. Filtration gave 420 mg of yellow crystal of m. p. 227°C.

Anal. Calcd. for $C_{19}H_{21}O_8N_3$: C 54.41, H 5.05, N 10.02.
Found : C 53.90, H 4.96, N 10.05.

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References

1) WEBB, J. S. ; D. B. COSULICH, J. H. MOWAT, J. B. PATRICK, R. W. BROSCHARD, W. E. MEYER, R. P. WILLIAMS, C. F. WOLF, W. FULMOR, C. PIDACKS & J. E. LANCASTER : The structures of mitomycins A, B and C and porfiromycin. I. J. Am. Chem. Soc. 84 : 3185～3187, 1962
2) WEBB, J. S. ; D. B. COSULICH, J. H. MOWAT, J. B. PATRICK, R. W. BROSCHARD. W. E. MEYER, R. P. WILLIAMS, C. F. WOLF, W. FULMOR, C. PIDACKS & J. E. LANCASTER : The structures of mitomycins A, B and C and porfiromycin. II. J. Am. Chem. Soc. 84 : 3187～3188, 1962
3) HERR, R. R. ; M. E. BERGY, T. E. EBLE & H. K. JAHNKE : Porfiromycin, a new antibiotic. II. Isolation and characterization. Antimicrobial Agents Annual 1960 : 23～26, 1961
4) UZU, K. ; Y. HARADA & S. WAKAKI : Studies on mitomycins, carcinostatic antibiotics. I. Derivatives and acid hydrolysis of mitomycin A and C. Agr. Biol. Chem. (Japan) 28 : 388～393, 1964
5) UZU, K. ; Y. HARADA, S. WAKAKI & Y. YAMADA : Studies on mitomycins, carcinostatic antibiotics. II. Mitomycinone. Agr. Biol. Chem. 28 : 394～402, 1964
6) PATRICK, J. B. ; R. P. WILLIAMS, W. E. MEYER, W. FULMOR, D. B. COSULICH, R. W. BROSCHARD & J. S. WEBB : Aziridinomitosenes : A new class of antibiotics related to mitomycins. J. Am. Chem. Soc. 86 : 1889～1890, 1964
7) MIYAMURA, S. ; N. SHIGENO, M. MATSUI, S. WAKAKI & K. UZU : The biological studies on mitomycin derivatives. I. Antibacterial activities of mitomycin derivatives. J. Antibiotics, Ser. A 20 : 72～76, 1967
8) USUBUCHI, I. ; Y. SOBAJIMA, T. HONGO, T. KAWAGUCHI, M. SUGAWARA, M. MATSUI, S. WAKAKI & K. UZU : The antitumor studies on mitomycin derivatives. I. Effect on ascites Hirosaki Sarcoma. Gann 58, 1967 (in press)
9) OBOSHI, S. ; M. MATSUI, S. ISHII, N. MASAGO, S. WAKAKI & K. UZU : The antitumor studies on mitomycin derivatives. II. Effect on solid tumor of Sarcoma-180. Gann 58, 1967 (in press)