SYNTHESIS AND BIOLOGICAL ACTIVITIES OF MITOMYCIN DERIVATIVES

Kin-ichi Nakano
Tokyo Research Laboratory, Kyowa Hakko Kogyo Co., Ltd.
3-6-6 Asahicho, Machidashi, Tokyo, Japan

Abstract — The synthesis, biological activities and structure-activity relationships of mitomycin derivatives and analogs are described.

INTRODUCTION

The mitomycins are a group of alkylating antibiotics which shows strong activity against both gram-positive and gram-negative bacteria as well as various kinds of tumors.

Mitomycin A and B were isolated firstly from the culture broth of Streptomyces caespitosus by Hata and his co-workers[1] in 1956. Thereafter, mitomycin C was isolated from the same culture broth by Wakaki and his co-workers[2] and it showed the strongest activity against various types of tumors and has widely been used as a cancer chemotherapeutic agent.

After the discovery of the mitomycins, the structure elucidation, mode of action and synthesis of their derivatives have been studied by many groups and clinical applications have also been attempted.

In this review we will describe the advance of the studies of mitomycin derivatives, including their chemical and biological properties.

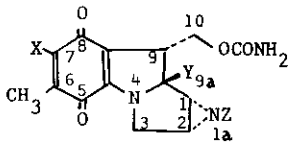
THE STRUCTURES OF MITOMYCINS

Several natural mitomycins were found after the first discovery of mitomycin A, B and C. Porfiromycin was isolated by Herr and his co-workers[3] and mitiromycin was found as a minor product of the culture broth of Streptomyces verticillatus by Lefemine and his co-workers[4].

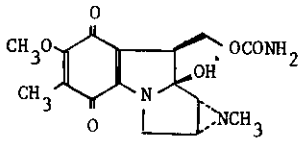
Although the studies of structural elucidation of natural mitomycins using chemical degradation techniques were initiated by our group soon after the discovery of mitomycin C, the complexity of its structure prevented rapid

elucidation[5,6]. Porfiromycin was shown to be a N-methyl derivative of mitomycin C in 1962[7]. In this same year, Webb and his co-workers[8,9] at the Lederle Laboratory reported the structure of the mitomycins and Tulinsky[10,11] showed the absoluted configuration of mitomycin A by using X-ray crystallographic methods of N-brosyl-mitomycin A as shown in Fig 1.

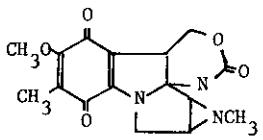
Fig 1 Structures of Natural Mitomycins



	X	Y	Z
Mitomycin A	CH_3O	CH_3O	H
Mitomycin C	NH_2	CH_3O	H
Porfiromycin	NH_2	CH_3O	CH_3



Mitomycin B



Mitiromycin

As mitomycin C and porfiromycin were derived from mitomycin A by known chemical reactions[12], the stereochemistry of mitomycin A, C and porfiromycin was decided to be the identical. The absolute configuration of mitomycin B was not clarified, since any attempt to derive mitomycin B from the other natural mitomycins was not successful. Recently, Yahashi and Matsubara[13] reported the absolute configuration of mitomycin B by using X-ray analysis of 7-demethoxy-7-bromoanilino-mitomycin B. The structure of mitomycin B (Fig 1) is distinguished from other natural mitomycins at the stereochemistry of position 9 .

Structure of mitiromycin was clarified by Morton and his co-workers[14], but the absolute configuration is not yet reported. (Fig 1)

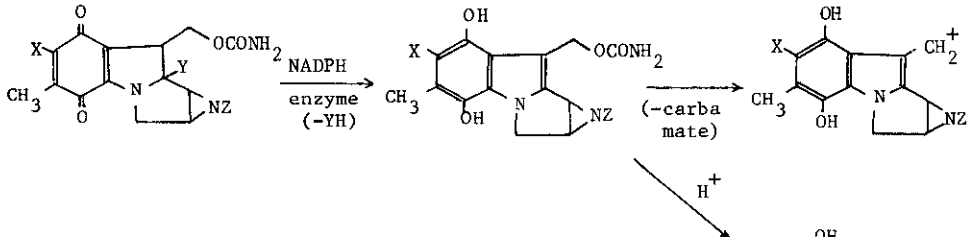
It is important to note that the mitomycins have unique structures which contain three anticancer functional groups; quinone, carbamate and aziridine in one molecule.

MODE OF ACTION

It was known by earlier research that mitomycin C inhibited DNA synthesis[15].

Iyer and Szybalsky[16] proposed a hypothesis concerning the mode of action of mitomycin in 1964. According to their hypothesis, the mitomycins are reduced with NADPH-dependent reductase in living cells and resulting hydrogenated mitomycins are protonated at position 10 and 1. Then, protonated mitomycins link covalently to the double strand of DNA and inhibit DNA synthesis as shown in Fig 2. This hypothesis was not disclosed directly for reason of instability of resulting hydrogenated mitomycins.

Fig 2 Activation Mechanisms of Mitomycins



A simple solution to the problem of mitomycin activation in vitro was provided by Tomasz[17]. She proved that mitomycin C semiquinone radicals involved initial binding to DNA. This interacts of activated mitomycin with DNA prevents subsequent DNA synthesis resulting the inhibition of cell division and loss of cell viability.

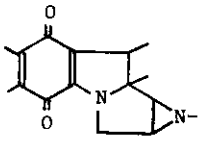
It is believed that binding sites on DNA is Guanine-O-6-position.

STUDIES OF MITOMYCIN DERIVATIVES

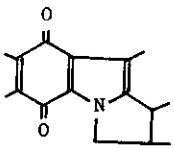
During the structural elucidation of the mitomycins, several chemical reactions or degradation methods were developed. These methods were used to prepare various mitomycin derivatives which were subsequently utilized to examine structure-activity relationships of the mitomycins.

Mitomycin derivatives can be classified in two types, mitosane type and mitosene type. (Fig 3)

Fig 3 Two Types of Mitomycin Derivatives



mitosane type



mitosene type

A. MITOSANE TYPE DERIVATIVES

Various mitosane derivatives have been prepared through several synthetic routes as shown in Fig 4, and their structure-activity relationships have been investigated.

Various 7-alkylaminomitosanes were synthesized by treating 7-methoxy-mitomycins with alkylamine in methanol[18,19]. These derivatives retained strong antibacterial and antitumor activities. The biological activities of 7-substituted mitosanes paralleled the reduction potential of the quinone as shown in Table I.

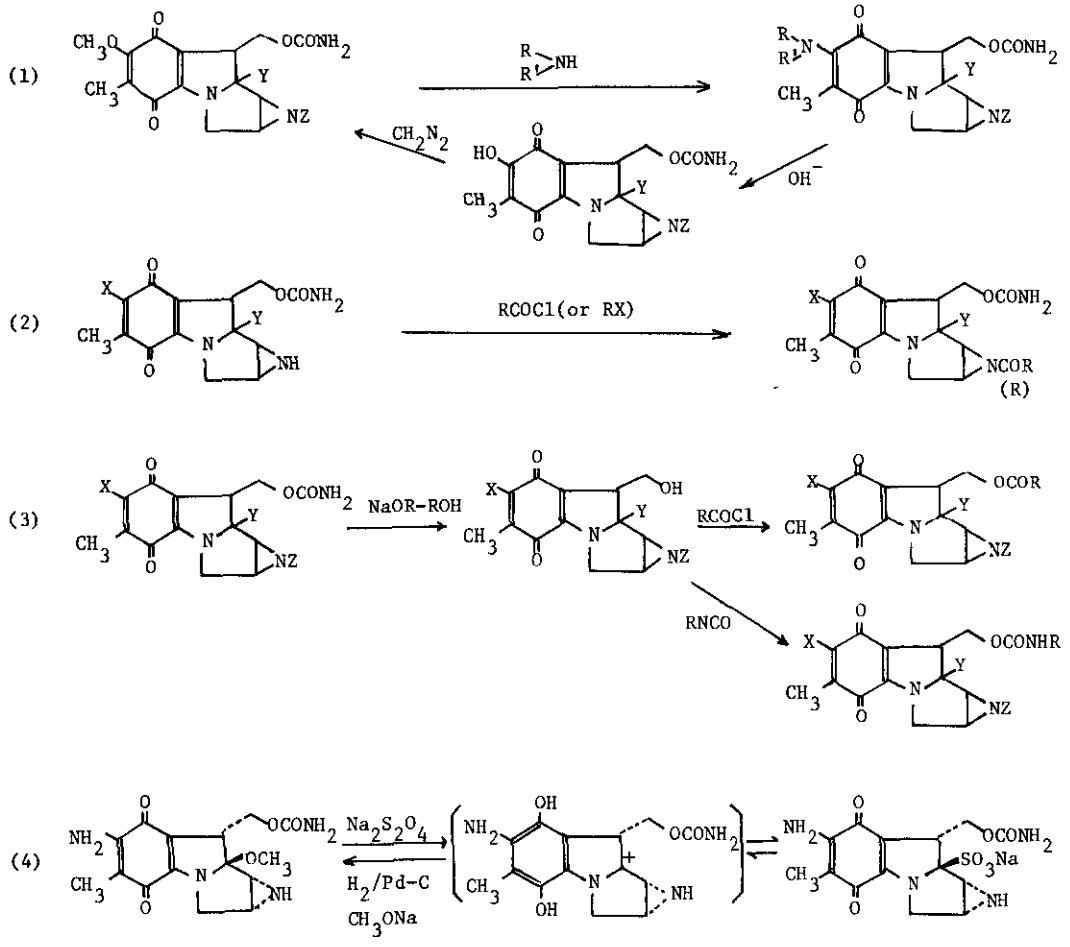
According to the activation mechanisms of mitomycins, the active form of the mitomycins is hydrogenated compounds which result from the enzymatic reduction of the mitomycins. Thus, it is anticipated that the most readily reduced compound should have the highest activity. As shown in Table I, this anticipation is realized in antibacterial activity, except for mitomycin C which shows the strongest activity against gram-negative bacteria. However, antitumor activity is inversely related to reduction potential.

This result suggests the presence of another factors which may effect to the biological activities of mitosanes.

1a-Acyl or alkyl mitosane derivatives were prepared by treating with acylating agents or alkyl halide in pyridine from 1a-unsubstituted mitosanes[19]. (mitomycin A or C), and were tested biological activities as shown in Table II. 1a-Substituted mitosanes having large alkyl or acyl groups diminish both antibacterial and antitumor activities. This indicates that the position 1a is one of the active sites of the mitomycins. On the other hand, derivatives having smaller alkyl or acyl groups still retained the biological activities. This may indicate that 1a-substituents can be eliminated enzymatically in living cells.

These results may suggest that 1a-substituted derivatives will include

Fig 4 Synthetic Routs of Mitosane Derivatives



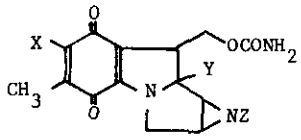
useful compounds as prodrugs.

Effects of 9a-substituents of mitosanes were also studied[19]. Elimination velocity of 9a-substituents was discussed by using 9a-methoxy and hydroxy groups of natural mitomycins. Although these 9a-hydroxy compounds were derivatives of mitomycin B, which had different stereochemistry from other natural mitomycins at position 9a, the fact that 9a-demethoxymitosane in which position 9a was hydrogen failed to show antibacterial activity suggests that the activation of mitosanes needed to form indole nucleous in which methanol or water was lost at position 9a of mitosanes.

9a-Sodium sulfonate derivatives of mitomycin C was synthesized, when

Table I

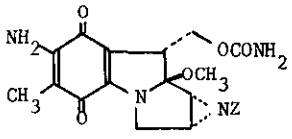
Effect of 7-Substituents of the Biological Activities of Mitosane Compounds

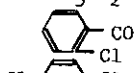
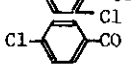


X	Y	Z	Reduction Potential	LD_{50} (mg/kg)	MIC(μg/ml) Gram-positive	MIC(μg/ml) Gram-negative	Antitumor Activity Sarcoma-180
CH_3O	OCH_3	H	-0.19	2.0	0.01	0.57	+
C_6H_5NH	OCH_3	H	-0.233	9.3	0.09	7.55	+
$(CH_3)_2N$	OCH_3	H	-0.25	9.38	0.03	7.29	++
EtNH	OCH_3	H	-0.307	9.38	0.107	17.7	+++
NH_2	OCH_3	H	-0.395	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	++

Table II

Effect of 1a-Substituents on the Biological Activities of Mitosane Compounds



Z	LD_{50} (mg/kg)	MIC(μg/ml) Gram-positive	MIC(μg/ml) Gram-negative	Antitumor Activity 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	+

mitomycin C was reduced with sodium dithionate and reoxidized with air. Mitomycin C was recovered from the 9a-sodiumsulfonate derivatives in MeONa-MeOH solution under reducing condition by Hornemann and his co-workers[20].

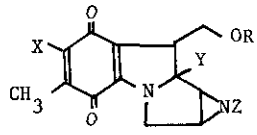
9a-Sodiumsulfonate derivatives of mitomycin C showed a substantially increased acid stability in comparison with mitomycin C and weaker antibacterial activity than mitomycin C[20].

Decarbamoyl mitosanes which were hydrolysed selectively at 10-carbamoyloxy group of mitosanes were obtained[21] by treating with natural mitomycins in MeONa-MeOH solution. Among the 10-substituted mitosane derivatives, 10-acetyloxy-decarbamoylmitomycin C and decarbamoylmitomycin C showed strong antibacterial activities against both gram-positive and negative bacteria as shown in Table III.

However, remarkable decrease of activities was observed in the presence of rabbit serum in the medium[22]. This observation may show that binding ability to serum protein may give a great influence to biological activities of mitomycin derivatives. In fact, binding ability of mitomycin C is less than that of the above derivatives.

Table III

Antibacterial Activities of Decarbamoylmitosanes and their Derivatives



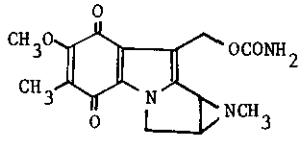
				MIC(μg/ml)				
X	Y	Z	R	Staph. aureus 209P	Bacillus subtilis ATCC6633	Sarcina lutea ATCC1001	E.coli K-12	Pseudo-monas aeruginosa
NH_2	OCH_3	H	H	0.78	0.195	3.12	0.78	1.56
NH_2	OCH_3	$COCH_3$	$COCH_3$	0.048	0.015	12.5	1.56	25.0
NH_2	OCH_3	H	$COCH_3$	0.039	0.039	0.39	0.156	5.47
NH_2	OCH_3	H	$CONH_2$	0.195	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

In contrast of 10-acetyloxydecarbamoylmitomycin C, some of the 10-alkylcarbamoyldecarbamoylmitosane derivatives showed strong antileukemia activity as comparable as mitomycin C against L-1210 murine Leukemia in vivo by Kojima and his co-workers[23].

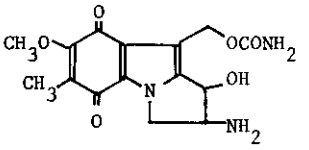
B. MITOSENE TYPE DERIVATIVES

Patrick and his co-workers[24] obtained a mitosene compound, aziridinomitosene (1), which was prepared from mitomycin B by catalytic reduction following reoxidation. This compound showed strong activities against gram-positive and negative bacteria and was active both by oral and subcutaneous routes against infection of Staphylococcus aureus (var Smith) and Streptococcus pyogenes C-203 in mice. Moreover, a mitosene compound (2) obtained from mitomycin A by acid hydrolysis[18] and a synthetic mitosene (3) prepared by Allen and his co-workers[25] showed antibacterial activities.

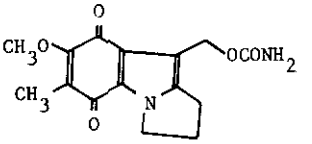
These observation that some of the mitosene derivatives showed strong biological activities have driven to the study of the relating mitosene analogs.



Aziridinomitosene (1)



(2)

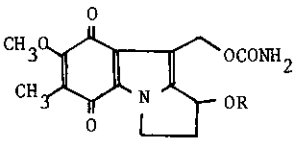


(3)

Synthetic routes of mitosene derivatives were studied as shown in Fig 5, and biological activities of these derivatives were also examined[19,21]. Antibacterial activities and quinone reduction potentials of 7-substituted mitosenes were determined as shown in Table Ⅳ.

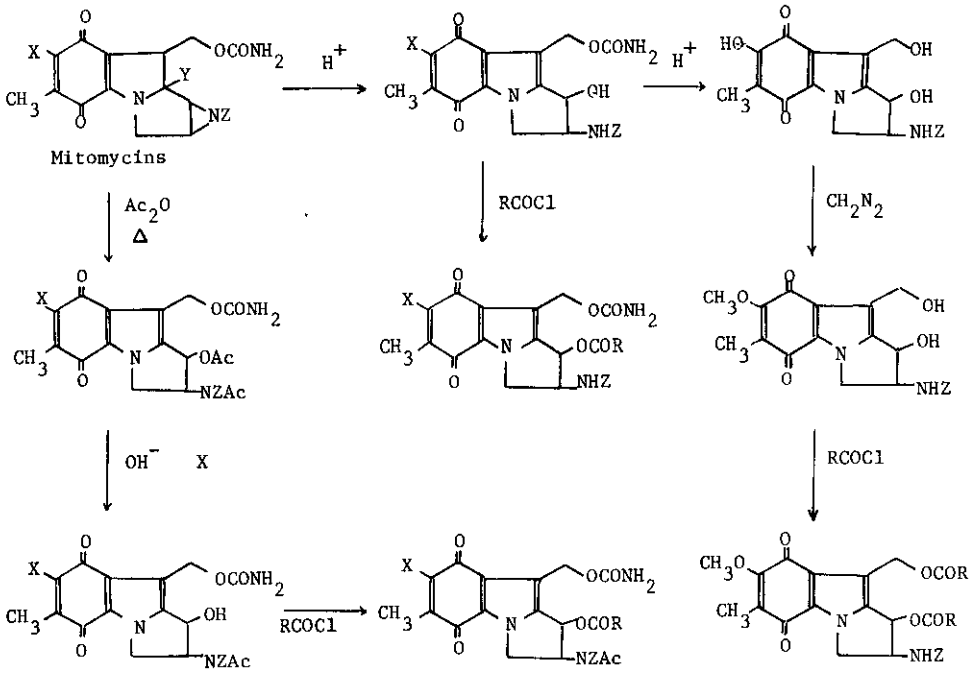
Although the aziridine ring is not essential for antibacterial activities, the quinone reduction potential gives strong influence to antibacterial activities.

Remers and Schepman[26] also reported concerning with biological activities of various synthetic analogs of mitosenes. They found that the synthetic analogs (4) and (5) were highly active against both gram-positive and negative bacteria in vitro, but less active in mice infected with some bacteria.



(4) R=H
(5) R=$COCH_3$

Fig 5 Preparation of Mitosene Derivatives from Mitomycins



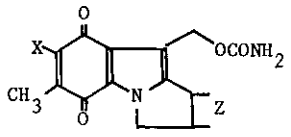
Generally, antitumor and antibacterial activities of mitosene derivatives are less than those of the mitosane from which they are derived. According to the hypothesis of activation mechanisms of mitomycins by Szybalsky[16], aziridine ring and carbamoyloxy group of mitomycins play an important role in binding to DNA. Then, mitosene analogs lacked aziridine ring cannot bind to double strands of DNA bifunctionally.

Otsuji[27] reported that decarbamoylmitomycin C and 7-methoxymitosene (2) bound to DNA monofunctionally. If another alkylating sites is introduced at position 1 or 2 of mitosene analogs, the more biological active mitosene compounds may be obtained from mitosenes without aziridine ring.

Endo and his co-workers[28] employed the prophage inducing ability of various alkylating agents for the screening of antitumor agents. Using this method, the

Table Ⅳ

Effect of 7-Substituted Mitosenes of the Biological Activities



X	Z	Reduction Potential	MIC(μg/ml) Gram-positive bacteria	Gram-negative bacteria
CH_3O	$\rangle NCH_3$	-0.39	1.10	12.0
CH_3O	$\begin{matrix}OH\\NHCH_3\end{matrix}$	-0.39	1.12	28.12
$(CH_3)_2N$	$\rangle NCH_3$	-0.415	1.152	50
$(CH_3)_2N$	$\begin{matrix}OH\\NHCH_3\end{matrix}$	-0.415	1.30	50
	$\begin{matrix}OH\\NHCH_3\end{matrix}$	-0.420	4.76	50
EtNH	$\rangle NHCH_3$	-0.495	>50	>50
EtNH	$\begin{matrix}OH\\NHCH_3\end{matrix}$	-0.495	>50	>50
NH_2	$\rangle NCH_3$	-0.529	>50	>50
NH_2	$\begin{matrix}OH\\NHCH_3\end{matrix}$	-0.529	>50	>50

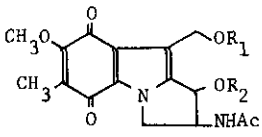
prophage inducing activities of mitosene derivatives were examined as shown in Table V[21]. When an electron-withdrawing group was introduced at the oxygen of C-1 in mitosene compounds, prophage inducing activities of mitosenes were enhanced. This result may suggest that bifunctional alkylating sites are reproduced by introducing electron-withdrawing group at position 1 of mitosenes.

Induction of λ-bacteriophage in E.coli of mitosenes were also examined by Taylor and his co-workers[29]. A few 1-substituted mitosene derivatives show significant activities. Especially, 1-acetoxymitosene (5) was most active and when tested against P-388 Leukemia in CDF, it gave 65% increase in the survival time. But it was less active than mitomycin A against λ-phage inductivity in E.coli and P-388 Leukemia in mice.

These studies may suggest that active sites of mitomycins, carbamoyloxy group and aziridine ring, are not essential and can be replaced by another substituents without the loss of biological activities.

Table V

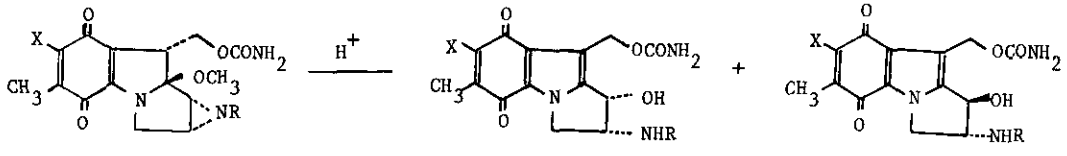
Prophage Inductivity and Antibacterial Activities of Mitosene Derivatives



R_1	R_2	Prophage Inductivity (1mg/ml) Plaque Index (Sample's plaque)/(Control's plaque)	MIC(μg/ml) Staph. aureus 209P	Bacillus subtilis ATCC	Sarcina lutea PCI	E.coli B(H)	Pseudomonas aeruginosa
$CONH_2$	H	1.0	0.38	0.38	1.56	6.12	50
$CONH_2$	$COCH_3$	1.8					
$CONH_2$	$COCH_2Cl$	5.6					
$CONH_2$	$COCHCl_2$	6.6					
H	H	1.0	>50	>50	>50	>50	>50
$COCH_3$	$COCH_3$	1.8	0.383	0.191	0.383	1.53	>50
$COCH_3$	H	1.0	0.38	0.38	0.76	3.06	>50
$COCH_2Cl$	$COCH_2Cl$	7.2	3.06	1.53	3.06	6.12	>50
$COCH_3$	$COCH_2Cl$	4.7	0.38	0.38	0.76	6.12	>50
Aziridinomitosene		14.0					

Remers and his co-workers[30,31,32] investigated the stereochemistry of 1,2-disubstituted mitosenes which were obtained from acid solvolysis of mitomycins. When natural mitomycins were hydrolysed with 0.05N-hydrochloric acid, two mitosene compounds were obtained. The major product was confirmed to be cis-2-amino-1-hydroxy mitosene (6) and the minor one was assigned to be trans-2-amino-1-hydroxy mitosene (7) as shown in Fig 6.

Fig 6



X=CH_3O or NH_2

R= H or CH_3

(6) (7)

Of the two mitosene compounds lacked aziridine ring, cis isomer has higher activity than the trans isomer against the induction of λ-bacteriophage in E.coli. This observation may anticipate an interesting feature of the binding process between DNA and the mitomycins. On the activation of the mitomycins, the reduced mitomycins bind to DNA with alkylation by opening of the aziridine ring. Thus the structure and stereochemistry of 1,2-substituted mitosene may play an important role in alkylation on DNA.

In earlier studies of mitosene derivatives, the stereochemistry of 1,2-position of mitosenes has never been discussed, and most of derivatives without aziridine used in earlier experiments would be mixtures of cis and trans isomers. Therfore, it is interesting to examine the role of 1,2-position for alkylation of DNA.

In the course of the studies of the biological activities of mitomycin derivatives, it is clear that active sites of mitomycins, carbamoyl group and aziridine ring, is not essential and can be replaced by the other appropriate substituents. Furthermore, indole nucleus of mitomycins may be replaced by the simpler molecule without the loss of the activity of mitomycins to act as bioreductive alkylating agents. Many investigators have tried to prepare the bioreductive alkylating agents having the simpler structures, for example, benzoquinone or naphthoquinone[32,33]. Some of these compounds shows the strong activities against tumor cells comparable to mitomycins.

CONCLUDING REMARKS

As we summerized in the foregoing section, the mode of action of the mitomycins has been clarified and many derivatives have been synthesized and tested for biological activities. In spite of these efforts, no superior derivatives than mitomycin C have been found as yet. The failure in developing analogs or derivatives may be due in part to the fact that the most analogs or derivatives lack one or more of the important physicochemical properties of mitomycin C. Namely, mitomycin C has a good water solubility, low lipophylicity, low binding to serum protein and low reduction potential of quinone enough to permit facile enzymatic reduction.

When we look back the past studies of the mitomycins, most investigations

have been concentrated on modification of active-sites of the mitomycins.

Since the total synthesis of the mitomycins and porfiromycin has been made by Kishi and his co-workers[35,36,37] and the rapid and elabolated advancement of the mitomycin chemistry have been achieved, the more complex and elabolated modifications will be performed successfully for developing clinically useful derivatives.

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