SYNTHESIS AND BIOLOGICAL ACTIVITIES OF 7-ALKOXYMITOSANES

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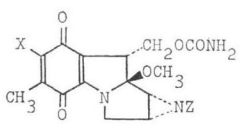
A facile alcoholysis of 7-methoxymitosanes and 5-methoxyindolequinone under basic conditions was discovered and a series of 7-alkoxymitosanes were synthesized from mitomycins A and B using this reaction. They showed strong antibacterial activity against various Gram-positive and Gram-negative bacteria and were potent inhibitors of cultivating HeLa S-3 cells *in vitro*. Among them, 7-*n*-propoxy-7-demethoxymitomycin A (**2**) showed the strongest antitumor activity against solid type Sarcoma-180 in mice.

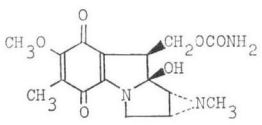
Since mitomycins A and B were found in a culture broth of *Streptomyces caespitosus* in 1956,[1] many studies have been carried out on a group of mitomycins which includes the well-known antitumor antibiotic, mitomycin C.[2] The mitomycins, including their derivatives, can be classified into two groups mitosanes and mitosenes.[3] Among the mitosane group, mitomycin A has the greatest antibacterial activity against various bacteria and is the most cytotoxic. Therefore, it has been suggested that mitomycin A analogs such as 7-alkoxymitosanes are interesting derivatives. Although there are some reports on the preparation of 7-alkoxymitosanes, those processes consist of two-steps and are low-yield synthetic methods due to the alkylation of unstable 7-hydroxymitosane intermediates by diazoalkanes. Furthermore, no biological activities of the 7-alkoxymitosanes have been investigated.[4,5]

The preparation of various 7-alkoxymitosanes might be a logical approach for the synthesis of potentially potent chemotherapeutic agents. With this goal in mind we have developed useful one-step synthesis of various 7-alkoxymitosanes from naturally occurring mitomycins having 7-methoxy substituents. The present report describes the synthesis and biological evaluation of various 7-alkoxymitosanes.

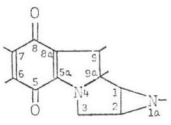
	X	Z
Mitomycin A	CH_3O	H
Mitomycin C	NH_2	H
Porfiromycin	NH_2	CH_3



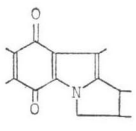
Mitomycin B



Mitosane structure



Mitosene structure



Chemistry

In the presence of small amounts of a base, 7-methoxymitosanes underwent alcoholysis and were converted to the corresponding 7-alkoxymitosanes. The reaction was carried out in a short period when the 7-methoxymitosanes were dissolved in alcohol in the presence of a base such as sodium alkoxide or potassium hydroxide with stirring at room temperature.

The reactions with mitomycin A or B and various alcohols yielded the corresponding 7-alkoxymitosanes. They are 7-ethoxy (**1**), 7-*n*-propoxy (**2**), 7-*i*-propoxy (**3**), 7-*n*-butoxy (**4**), 7-*i*-butoxy (**5**), 7-*sec*-butoxy (**6**), 7-*n*-amyloxy (**7**), 7-*i*-amyloxy (**8**), 7-*n*-hexyloxy (**9**), 7-cyclohexyloxy (**10**), 7-*n*-heptyloxy (**11**), 7-*n*-octyloxy (**12**), 7-*n*-decyloxy (**13**), 7-stearyloxy (**14**), 7-(2-methoxy)ethoxy (**15**), and 7-benzyloxy-7-demethoxymitomycin A (**16**) and 7-*i*-propoxy-7-demethoxymitomycin B (**17**).

Fig. 1. Alcoholysis of mitomycins A and B and indolequinone.

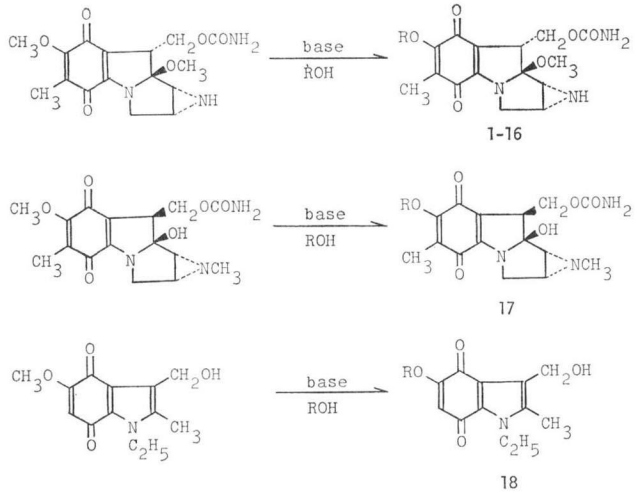


Table 1. Properties and yields of mitomycin 7-alkoxy derivatives and a related compound.

Product*		Base	Yield %	Mp °C (dec.)	M^+	
No.	RO				Calcd.	Found
1	CH_3CH_2O	KOH	97.3	143~150	363.1430 ($C_{17}H_{21}N_3O_6$)	363.1430
2	$CH_3(CH_2)_2O$	$CH_3(CH_2)_2ONa$	85.7	148~157	377.1587 ($C_{18}H_{23}N_3O_6$)	377.1541
3	$(CH_3)_2CHO$	$(CH_3)_2CHONa$	78.5	170~183	377.1587 ($C_{18}H_{23}N_3O_6$)	377.1557
4	$CH_3(CH_2)_3O$	KOH	69.6	130~140	391.1743 ($C_{19}H_{25}N_3O_6$)	391.1826
5	$(CH_3)_2CHCH_2O$	KOH	75.4	powder	391.1743 ($C_{19}H_{25}N_3O_6$)	391.1758
7	$CH_3(CH_2)_4O$	KOH	49.7	106~112	405.1900 ($C_{20}H_{27}N_3O_6$)	405.1826
8	$(CH_3)_2CHCH_2CH_2O$	KOH	71.7	powder	405.1900 ($C_{20}H_{27}N_3O_6$)	405.1758
9	$CH_3(CH_2)_5O$	KOH	36.6	105~116	419.2056 ($C_{21}H_{29}N_3O_6$)	419.2138
11	$CH_3(CH_2)_6O$	KOH	57.0	102~108	433.2212 ($C_{22}H_{31}N_3O_6$)	433.2190
12	$CH_3(CH_2)_7O$	KOH	56.1	110~112	447.2369 ($C_{23}H_{33}N_3O_6$)	447.2397
13	$CH_3(CH_2)_9O$	KOH	57.3	113~117	475.2682 ($C_{25}H_{37}N_3O_6$)	475.2680
15	$CH_3OCH_2CH_2O$	KOH	48.8	powder	393.1536 ($C_{18}H_{23}N_3O_7$)	393.1570
16	$C_6H_5CH_2O$	KOH	49.9	139~146	425.1587 ($C_{22}H_{23}N_3O_6$)	425.1611
17	$(CH_3)_2CHO$	$(CH_3)_2CHONa$	95.8	200~206	377.1586 ($C_{18}H_{23}N_3O_6$)	377.1550
18	$(CH_3)_2CHO$	KOH	67.4	210	277.1313 ($C_{15}H_{19}NO_4$)	277.1287

* Structures are shown in Fig. 1.

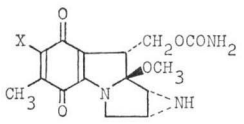
The reaction products were purified by chromatography on silica gel followed by crystallization from appropriate solvents. Yields, melting points and the molecular ion peaks by high resolution mass spectrum of 7-alkoxymitosanes are shown in Table 1. Furthermore, the same reaction could also be applied to an indolequinone. 1-Ethyl-3-hydroxymethyl-5-methoxy-2-methylindole-4,7-dione reacted with *i*-propanol to give 1-ethyl-3-hydroxymethyl-5-*i*-propoxy-2-methylindole-4,7-dione (**18**). These results suggest that the reaction may be applicable to the 7-alkoxymitosenes and *o*-alkoxy-*p*-quinones. Such transalkoxylation was not known in mitomycin chemistry or in general quinone chemistry. As shown in Table 1, alcoholysis of 7-methoxymitosanes was found to be more efficient with a lower alcohol than with a higher alcohol. One of the reasons may be due to the fact that solubility of the base is higher in a lower alcohol than in a higher alcohol. The yields were not affected by the kind of the base. It is known that mitomycins A and B are subjected to decarbamoylation by the action of excess sodium methoxide and that the corresponding decarbamoylmitosanes are given.[2c] But in the alcoholysis which is described in the present paper, no corresponding decarbamoylmitosane was obtained. It is well-known that 7-methoxy or 7-amino groups on mitosane undergo selective hydrolysis by treatment with aqueous sodium hydroxide, and 7-hydroxymitosane is obtained, and that treatment of 7-methoxymitosane such as mitomycin A or B with methanolic amines yields the corresponding 7-aminomitosane.[5] The reactivities of the 7-position of mitomycins may be explained by the conjugation system of the quinone ring. In hydrolysis, aminolysis and alcoholysis at the 7-position of mitosanes, it is reasonable to assume that nucleophiles such as hydroxides, amines or alkoxides attack at C7 by MICHAEL addition and then the original substituents on C7 are replaced by the shift of equilibrium. Thus, the amino group or methoxy group on C7 of the original mitosanes is lost as a leaving group. By conjugation of the quinoid system, 7-hydroxymitosane is acidic enough to make a salt with a weak base such as triethylamine.[4]

The p.m.r. spectra of the resulting 7-alkoxymitosanes were compatible with these structures. The methyl protons of 7-methoxy on mitomycins A and B were assigned to δ 4.03 ppm (3 H, s) and δ 4.05 ppm (3 H, s) respectively, while these peaks disappeared upon alcoholysis products and signals corresponding to newly introduced alkoxy protons were shown, at δ 1.33 ppm (3 H, t, J=6.5 Hz) and δ 4.93 ppm (2 H, q, J=6.5 Hz) in **1**, and at δ 5.40 ppm (2H, s) and δ 7.43 ppm (5 H, s) in **16**. The amino protons of the urethane group on these derivatives were assigned at *ca.* δ 5.2 ppm (2 H, bs). In the i.r. spectra of these compounds, the carbonyl absorption of the urethane group was observed *ca.* 1720 cm^{-1}. The mass spectra of these compounds gave the expected molecular ion peaks and produced fragmentation patterns similar to those of mitomycin A or B which were discussed in detail by VAN LEAR.[6]

Biological Activities

Antibacterial activities of the 7-alkoxymitosanes obtained as above are shown in Table 2 as minimum inhibition concentrations (MIC) by using the agar dilution method. Most of the compounds showed very strong antibacterial activities against Gram-positive and Gram-negative bacteria. Generally, the activities of these compounds were greater against Gram-positive bacteria than against Gram-negative bacteria. This type of antibacterial activity of these compounds is a general characteristic for mitomycin antibiotics and their derivatives. Antibacterial activities decreased when the chain length of the 7-alkoxy group increased. The activities of 7-alkoxymitosanes were greater than those of the corresponding 7-alkylaminomitosanes. It is well-known that a substituent group at the 7-position of mitosane strongly influences the activities, since activities are affected by oxidation-reduction potential in

Table 2. Antibacterial activities of 7-alkoxymitomycins.



No.	X	MIC (mcg/ml)*					
		(a)	(b)	(c)	(d)	(e)	(f)
	CH_3O	<0.025	0.196	<0.025	0.098	0.391	<0.025
1	CH_3CH_2O	<0.025	0.196	<0.025	0.098	0.391	<0.025
2	$CH_3CH_2CH_2O$	<0.025	0.782	<0.025	0.391	0.782	<0.025
3	$(CH_3)_2CHO$	<0.025	1.563	<0.025	0.391	1.563	0.049
4	$CH_3(CH_2)_3O$	<0.025	3.125	<0.025	1.563	1.563	<0.025
5	$(CH_3)_2CHCH_2O$	<0.025	1.563	<0.025	0.391	1.563	<0.025
6	$CH_3CH_2CH(CH_3)O$	<0.025	3.125	<0.025	1.563	3.125	0.049
7	$CH_3(CH_2)_4O$	<0.025	6.25	<0.025	3.125	12.5	0.098
8	$(CH_3)_2CHCH_2CH_2O$	<0.025	6.25	<0.025	1.563	6.25	0.049
9	$CH_3(CH_2)_5O$	<0.025	25	<0.025	12.5	25	0.391
10	$C_6H_{11}O$	<0.025	12.5	<0.025	12.5	12.5	0.196
11	$CH_3(CH_2)_6O$	0.049	>50	<0.025	25	50	0.391
12	$CH_3(CH_2)_7O$	<0.025	>50	<0.025	>50	>50	3.125
13	$CH_3(CH_2)_9O$	0.196	>50	<0.025	>50	>50	25
14	$CH_3(CH_2)_{17}O$	3.125	>50	3.125	>50	>50	25
15	$CH_3OCH_2CH_2O$			<0.025			<0.025
16	$C_6H_5CH_2O$	<0.025	3.125	<0.025	0.782	1.563	0.049
	NH_2	0.049	3.125	<0.025	3.125	3.125	<0.025
	CH_3NH	<0.025	12.5	<0.025	25	12.5	<0.025
	$CH_3CH_2CH_2NH$	<0.025	25	<0.025	>50	25	0.098
	$(CH_3)_2CHNH$	<0.025	>50	<0.025	50	>50	0.196
	$C_6H_{11}NH$	<0.025	>50	<0.025	25	25	0.196

* (a) *Staphylococcus aureus* ATCC 6538P (b) *Escherichia coli* No. 26 (c) *Bacillus subtilis* ATCC 10707 (d) *Shigella sonnei* ATCC 6897 (e) *Salmonella typhi* ATCC 9992 (f) *Klebsiella pneumoniae* ATCC 10031

the quinone-hydroquinone system which is influenced to a marked degree by the 7-substituent.[7]

Acute toxicity and antitumor activity of the 7-alkoxymitosanes are shown in Table 3. 7-Alkoxymitosanes with lower alkoxy group on C7 showed a greater toxicity in mice, while the toxicity of 7-alkoxymitosanes with higher alkoxy group on C7 decreased. All 7-alkoxymitosanes showed a strong inhibition on the growth of HeLa S-3 cells *in vitro* (IC_{50}; $<0.01 \sim 0.1$ mcg/ml). Most of the 7-alkoxymitosanes suppressed the growth of transplanted Sarcoma-180 (solid type) in mice. 7-*n*-Propoxy-7-demethoxymitomycin A (**2**) was equal to mitomycin C in antitumor activity. A more detailed report on the antitumor activities of the 7-alkoxymitosanes will be published elsewhere.

Experimental Section

Melting points were determined on a Yanagimoto melting point apparatus and were uncorrected. Infrared spectra were determined in KBr disk on a Hitachi 215 spectrophotometer and nuclear magnetic resonance spectra were recorded on a Varian T-60 spectrometer using tetramethylsilane as an internal standard. Mass spectra were determined on a JEOL-OISG-2 mass spectrometer. Each compound

Table 3. Biological activities of 7-alkoxymitosanes.

Compounds	LD_{50}(i.p.)*1 (mg/kg)	Antitumor activity*2 (Sarcoma-180 solid tumor) (T/C)
Mitomycin A	2.1	Toxic
Mitomycin C	8.4	0.3~0.4
1	2.9	Toxic
2	3.35	0.35
3	4.5	0.53
4	5.25	0.60
5	3.7	0.49
7	10.5	0.51
8	6.75	0.62
9	22.5	0.62
11	87.5	Toxic
12	95	0.45
13	87.5	0.61
16	5.94	0.61
17	9.75	0.82

*1 LD_{50} was calculated by BEHRENS-KÄRBER method. Mice (*ddY*-strain, male) weighing 20±1 g were used and were observed for 2 weeks after injection (i.p.) of drugs.

*2 Mice (*ddY*-strain, male) weighing 19±1 g were used. The drug dose was one-sixth of each LD_{50}. Other details of the antitumor test followed the paper described by OBOSHI[8].

showed i.r., p.m.r. and mass spectra compatible with its structure. Thin-layer chromatography on silica gel (Eastman Chromagram No 6061) with the solvent system $CHCl_3$ - Me_2CO (1: 1) was used for identification of the products and tracing of the reactions.

7-Ethoxy-7-demethoxymitomycin A (**1**)

To a stirred mixture of 100 mg of mitomycin A and 10 ml of ethanol at room temperature was added 240 mg of 1.6% KOH ethanol solution in portions over 30 minutes. After excess dry-ice was added to the reaction mixture, the precipitate was filtered off and the filtrate was evaporated under reduced pressure and the residue was subjected to gradient chromatography on silica gel with acetone - chloroform (0: 1~1: 1). From the main reddish-purple elution, 103 mg (97.3% yield) of reddish-purple powder was obtained. It was crystallized from acetone as reddish-purple needles.

A similar method offered the following derivatives; 7-*n*-butoxy (**4**) (69.6% yield), 7-*i*-butoxy (**5**) (75.4% yield), 7-*n*-amyloxy (**7**) (49.7% yield), 7-*i*-amyloxy (**8**) (71.7% yield), 7-*n*-hexyloxy (**9**) (36.6% yield), 7-*n*-heptyloxy (**11**) (57.0% yield), 7-*n*-octyloxy (**12**) (56.1% yield), 7-*n*-decanoyloxy (**13**) (57.3% yield), 7-(2-methoxy)-ethoxy (**15**) (48.8% yield), and 7-benzyloxy-7-demethoxymitomycin A (**16**) (49.4% yield).

7-*i*-Propoxy-7-demethoxymitomycin A (**3**)

To a stirred mixture of 1 g of mitomycin A and 22.5 ml of *i*-propanol was added 257 mg of 18.7% sodium *i*-propoxide *i*-propanol solution in portions over 1 hour at room temperature. After excess dry-ice was added to the reaction mixture, the precipitate was filtered off and the filtrate was evaporated under reduced pressure and the residue was chromatographed on silica gel with acetone - chloroform (0: 1~1: 1) in a gradient fashion. From the main reddish-purple elution, 887.9 mg (75.8% yield) of powder was obtained. It was crystallized from acetone as reddish-purple needles.

A similar method offered the following derivatives; 7-*n*-propoxy (**2**) (85.7% yield), 7-*sec*-butoxy (**6**) (68.4% yield), 7-cyclohexyloxy (**10**) (27.8% yield) and 7-stearyloxy-7-demethoxymitomycin A (**14**) (11.0% yield). In these compounds, only **14** was obtained when tetrahydrofuran was used as a solvent.

7-*i*-Propoxy-7-demethoxymitomycin B (**17**)

To a stirred mixture of 100 mg of mitomycin B and 4.5 ml of *i*-propanol was added 44 mg of 12.7% sodium *i*-propoxide *i*-propanol solution in portions over 1 hour at room temperature. After excess dry-ice was added to the reaction mixture, the precipitate was filtered off and the filtrate was evaporated under reduced pressure and the residue was chromatographed on silica gel with acetone - chloroform (0: 1~1: 1) in a gradient manner. From the principal purple colored fraction, 101 mg (95.8% yield) of deep-purple powder was obtained. It was crystallized from acetone as deep-purple needles.

1-Ethyl-3-hydroxymethyl-5-*i*-propoxy-2-methylindole-4,7-dione (**18**)

To a stirred mixture of 200 mg of 1-ethyl-3-hydroxymethyl-5-methoxy-2-methylindole-4,7-dione[9] and 20 ml of *i*-propanol was added 150 mg of saturated KOH *i*-propanol solution in portions over 1 hour at room temperature. After excess dry-ice was added to the reaction mixture, the precipitate was filtered off and the filtrate was evaporated under reduced pressure. The residue was crystallized from ethanol

and 150 mg (67.4% yield) of reddish-orange needles were obtained.

Acknowledgments

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