

PREPARATION AND BIOLOGICAL ACTIVITIES OF  
10-DECARBAMOYLOXY-9-DEHYDROMITOMYCIN B  
AND ITS ANALOGS

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A new naturally occurring mitomycin, 10-decarbamoxyloxy-9-dehydromitomycin B (**1**), was prepared by treating mitomycin B with sodium hydride. Its analogs having an exo-cyclic double bond in their structure were also synthesized. These compounds showed antibacterial and cytotoxic activities. Among them, 7-amino-10-decarbamoxyloxy-9-dehydro-7-demethoxymitomycin B (**2**) was the most potent growth inhibitor of KB cells *in vitro* and, accordingly, it appears to be a potential antitumor agent.

It has previously been reported<sup>1)</sup> that new mitomycin, 10-decarbamoxyloxy-9-dehydromitomycin B (**1**), was isolated from a mitomycin B rich fraction of the mitomycin fermentation products of *Streptomyces caespitosus*. It was the first example of a mitomycin differing from hitherto known naturally occurring mitomycins by having an exo-cyclic double bond in its structure. The present paper describes the preparation and biological evaluation of **1** and its analogs. These compounds were prepared because we were interested in the mode of action of mitomycin antibiotics<sup>2)</sup> and also wished to study the chemical conversion of a naturally occurring mitomycin to compound **1**.

#### Chemistry

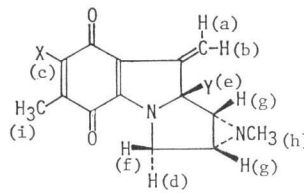
Compound **1** and its analogs were prepared from mitomycin B as shown in Fig. 1. Bluish needles were obtained by treating mitomycin B with an excess of sodium hydride or potassium *tert*-butoxide in tetrahydrofuran, followed by purification. The compound obtained was identified as **1** by PMR, IR, TLC and mass spectrum. The yield was 5~10% from mitomycin B.

The similar treatment of 7-amino-7-demethoxymitomycin B<sup>3)</sup> gave 7-amino-10-decarbamoxyloxy-9-dehydro-7-demethoxymitomycin B (**2**) in a 10~20% yield. Since tar-like by-products formed in these reactions, an appropriate amount of silica gel was added in the reaction system to adsorb the tar. Under these conditions **1** and **2** were obtained in 17.0% and 42.8% yield, respectively. The difference between the yields might be due to the difference in the stability of 7-methoxymitosane and 7-aminomitosane.

Mitosane structure is known to be fairly stable under basic conditions. For instance, hydrolysis<sup>3)</sup> of the 7-amino or 7-methoxy group, aminolysis<sup>3)</sup> or alcoholysis<sup>4)</sup> of the 7-methoxy group and decarbamylation<sup>5)</sup> of the urethane group can be carried out with retention of the mitosane structure. In the present case the reaction conditions were basic and the removal of carbamoxyloxy group probable pro-

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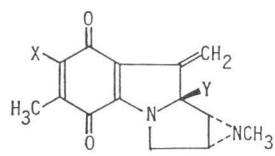
Table 1.  $^1\text{H}$  Chemical shifts of 10-decarbamoxy-9-dehydromitomycin B and its analogs.\*


	X	Y	$\delta$ from TMS as internal standard
1	$\text{CH}_3\text{O}$ ( $\text{CDCl}_3$ )	OH	(a) 6.13 (1H, s) (b) 5.53 (1H, s) (c) 4.05 (3H, s) (d) 3.98 (1H, d, $J=13$ Hz) (e) 3.00~4.20 (1H, broad) (f) 3.45 (1H, d, $J=13$ Hz) (g) 2.27 (2H, s) (h) 2.18 (3H, s) (i) 1.75 (3H, s)
2	$\text{NH}_2$ ( $\text{DMSO}-d_6$ - $\text{CDCl}_3$ )	OH	(a) 5.90 (1H, s) (b) 5.32 (1H, s) (c) 6.47 (2H, bs) (d) 4.22 (1H, d, $J=13$ Hz) (e) 6.33 (1H, s) (f) 3.43 (1H, d, $J=13$ Hz) (g) 2.23 (2H, s) (h) 2.17 (3H, s) (i) 1.70 (3H, s)
3	$\text{CH}_3\text{O}$ ( $\text{CDCl}_3$ )	$\text{OCH}_3$	(a) 6.32 (1H, s) (b) 5.50 (1H, s) (c) 4.08 (3H, s) (d) 4.08 (1H, d, $J=13$ Hz) (e) 3.07 (3H, s) (f) 3.41 (1H, dd, $J=1.5$ Hz, $J=13$ Hz) (g) 2.25 (2H, bs) (h) 2.22 (3H, s) (i) 1.85 (3H, s)
4	$\text{NH}_2$ ( $\text{CD}_3\text{OD}$ )	$\text{OCH}_3$	(a) 6.08 (1H, d, $J=0.7$ Hz) (b) 5.34 (1H, d, $J=0.7$ Hz) (c) 4.81 (2H, s, as $\text{CD}_3\text{OH}$ ) (d) 4.26 (1H, d, $J=13.1$ Hz) (e) 3.06 (3H, s) (f) 3.43 (1H, dd, $J=1.3$ Hz, $J=13.1$ Hz) (g) 2.43 (2H, bs) (h) 2.21 (3H, s) (i) 1.77 (3H, s)

\* 1~3: Varian T-60 spectrometer

4: JEOL FX-100 NMR spectrometer

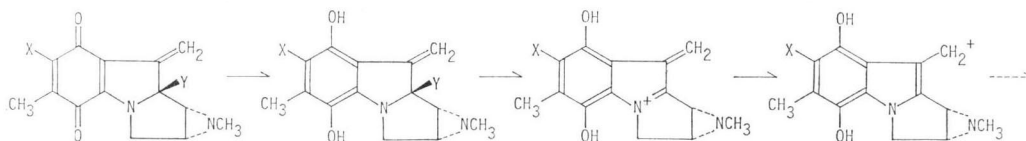
Table 2. Antibacterial activity of 10-decarbamoxy-9-dehydromitomycin B and its analogs.



1	X: $\text{CH}_3\text{O}$	Y: OH
2	X: $\text{NH}_2$	Y: OH
3	X: $\text{CH}_3\text{O}$	Y: $\text{OCH}_3$
4	X: $\text{NH}_2$	Y: $\text{OCH}_3$

	MIC(mcg/ml)				
	1	2	3	4	Mitomycin B
<i>Vibrio percolans</i> MB 1272	0.196	0.782	3.125	6.25	<0.025
<i>Pseudomonas alcaligenes</i> ATCC 12825	>50	>50	>50	>50	3.125
<i>Erwinia aroideae</i> ATCC 15390	50	25	>50	>50	1.563
<i>Staphylococcus aureus</i> ATCC 6538P	0.782	3.125	6.25	50	<0.025
<i>Escherichia coli</i> No 26	50	>50	>50	>50	6.25
<i>Bacillus subtilis</i> ATCC 10707	0.098	0.098	0.391	0.196	<0.025
<i>Proteus vulgaris</i> ATCC 6897	6.25	1.563	50	50	0.098
<i>Shigella sonnei</i> ATCC 9290	>50	25	>50	>50	3.125
<i>Salmonella typhosa</i> ATCC 9992	50	>50	>50	>50	12.5
<i>Klebsiella pneumoniae</i> ATCC 10031	12.5	3.125	12.5	12.5	0.196

Fig. 3.



concentrations (MIC) by the agar dilution method. They showed weaker antibacterial activities than mitomycin B, although their antibacterial spectra were similar to that of mitomycin B. Compounds **1** and **2** showed stronger antibacterial activities than **3** and **4**. It seems that in this series the 9a-hydroxy substituent provides stronger activity than the 9a-methoxy substituent. On the other hand, it appears that the effect of 7-substituent in the compounds upon antibacterial activity varies according to the kinds of bacteria.

As shown in Table 3, these compounds were strong growth inhibitors of KB cells. Among them, **2** was the most potent inhibitor. The LD<sub>50</sub> value of **2** in mice (i.p.) was 210 mg/kg and its ED<sub>50</sub> value to transplanted Sarcoma-180 A (solid type) in mice (i.p.-i.p.) was 82 mg/kg. Therefore, these compounds are potential antitumor agents.

The mode of activation of these compounds, as shown in Fig. 3 could be similar to that of known mitomycins.<sup>2)</sup>

Table 3. Inhibition on the growth of KB cells *in vitro* by 10-decarbamoxy-9-dehydromitomycin B and its analogs.\*

	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	Mitomycin C
IC <sub>50</sub> (mcg/ml)	0.05	0.03	0.25	0.07	0.01

\* Structure: see Table 2.

### Experimental

Infrared spectra were determined in KBr disks on a Hitachi 215 spectrophotometer and proton nuclear magnetic resonance spectra were recorded on a Varian T-60 spectrometer and a JEOL FX-100 NMR spectrometer with tetramethylsilane as an internal standard. Mass spectra were determined on a JEOL-OISG-2 mass spectrometer. Thin-layer chromatography on silica gel (Merck Art. 5719) was carried out with a solvent system CHCl<sub>3</sub> - Me<sub>2</sub>CO (1:1). The products were purified by column chromatography on silica gel (Merck Art. 7729).

#### 10-Decarbamoxy-9-dehydromitomycin B (**1**)

To a solution of 100 mg (0.287 mmole) of mitomycin B in 5 ml of dry tetrahydrofuran was added 300 mg of silica gel (Merck Art. 7729) and 55 mg (1.146 mmole) of sodium hydride (50% dispersion in mineral oil), and the reaction mixture was stirred at room temperature for two days. After addition of excess dry-ice, the suspension was filtered and the filtrate was evaporated under reduced pressure. The residue was subjected to gradient chromatography on silica gel with acetone - chloroform (0:1 ~ 1:4). From the main blue fraction eluted a crude solid was obtained which was crystallized from acetone yielding 14 mg (17.0%) of dark purple needles; Rf 0.70; IR  $\nu_{\max}$  3450, 1660, 1625, 1560; mass spectrum M<sup>+</sup> 288.1086 (288.1110 calcd. for C<sub>15</sub>H<sub>16</sub>N<sub>2</sub>O<sub>4</sub>).

#### 7-Amino-10-decarbamoxy-9-dehydro-7-demethoxymitomycin B (**2**)

To a stirred mixture of 1 g (2.994 mmole) of 7-amino-7-demethoxymitomycin B<sup>3)</sup> and 3 g of silica gel (Merck Art. 7729) in 50 ml of dry tetrahydrofuran was added 480 mg (10 mmole) of sodium hydride (50% dispersion in mineral oil) at room temperature. The reaction mixture was stirred at room temperature for two days. After addition of excess dry-ice, the suspension was filtered and the filtrate was evaporated under reduced pressure. The residue was chromatographed on silica gel with acetone - chloro-

form (1 : 4). The main dark green fractions were combined. Evaporation gave 350 mg (42.8%) of dark green needles; Rf 0.42; IR  $\nu_{\max}$  3400, 1610, 1535; mass spectrum  $M^+$  273.1118 (273.1113 calcd. for  $C_{14}H_{15}N_3O_3$ ).

10-Decarbamoyloxy-9-dehydro-N-methylmitomycin A (3)

To a stirred solution of 20 mg (0.069 mmole) of **1** in 0.3 ml of dry dimethylformamide and 1 ml of dry benzene was added 20 mg (0.417 mmole) of sodium hydride (50% dispersion in mineral oil), followed by addition of 0.035 ml of dimethyl sulfate at room temperature. After 2 minutes, to the reaction mixture were added 1 ml of ethyl acetate saturated with water and an excess amount of dry-ice. The suspension was filtered and the filtrate was diluted with 10 ml of ethyl acetate. The solution was washed with water and dried over anhydrous sodium sulfate. After removal of the solvent under reduced pressure, the residue was chromatographed over silica gel with acetone - chloroform (1 : 9). From the main bluish-purple fraction eluted were obtained dark purple needles, 8.7 mg (41.5%); Rf 0.80; IR  $\nu_{\max}$  3450, 2950, 1650, 1560; mass spectrum  $M^+$  302.1280 (302.1266 calcd. for  $C_{16}H_{18}N_2O_4$ ).

10-Decarbamoyloxy-9-dehydroporfiromycin (4)

To 5 ml of methanol saturated with ammonia was added 10 mg (0.033 mmole) of **3** and the solution was stirred at room temperature for 18 hours. The solution was evaporated under reduced pressure and crystallization of the residue from acetone - petroleum ether gave 6.2 mg (65.2%) of green needles; Rf 0.63; IR  $\nu_{\max}$  3400, 2950, 1650, 1610; mass spectrum  $M^+$  287.1252 (287.1269 calcd. for  $C_{15}H_{17}N_3O_3$ ).

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