Communication to the Editor

ONE STEP PREPARATION OF 6-DEMETHYL-6-FORMYLMITOMYCIN C AND CYTOTOXICITY ACTIVITY OF ITS DERIVATIVES

Sir:

Mitomycin C is a clinically useful antineoplastic antibiotic compound[1,2], but its use is limited by side effects, such as severe bone marrow suppression or gastrointestinal damage. In the hope of obtaining compounds with improved therapeutic properties, a number of derivatives have been prepared. Preparation of new mitomycin analogues have involved substituents on the aziridine ring[3,4], and carbamoyloxymethyl side chain[5], and replacement of 7-substituent in the quinone ring with other functional groups, especially substituted amines[6~10]. But little is known concerning derivatives of 6-substituent of mitomycins[11,12].

In this communication we report the synthesis of 6-demethyl-6-formylmitomycin C (**1**) which could be a good starting material for various kinds of 6-position derivatives, and also report the cytotoxic activity of **1** and its several derivatives.

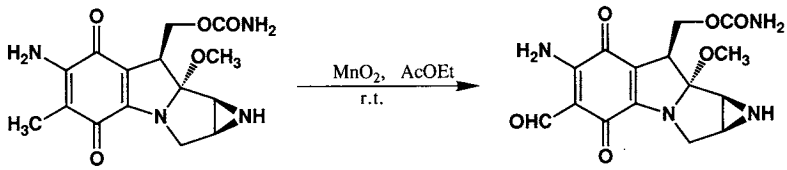
After testing many oxidizing agents, activated manganese(IV) oxide was found to be the best for oxidation of the 6-methyl group of mitomycin C. Compound **1** was prepared from mitomycin C by oxidation with activated manganese(IV) oxide as follows. To a solution of mitomycin C in ethyl

Table 1. Physico-chemical properties of **1**, **2**, **3**, **4** and **5**.

	1	**2**	**3**	**4**	**5**
Appearance	Red purple powder	Red purple powder	Red purple powder	Orange powder	Red purple powder
Molecular formula	$C_{15}H_{16}N_4O_6$	$C_{21}H_{29}N_4O_5$	$C_{21}H_{21}N_4O_5$	$C_{21}H_{20}N_4O_5$	$C_{26}H_{27}N_6O_6$
MP (dec)	95~96°C	135~138°C	160~165°C	132~135°C	170~175°C
FAB-MS (*m/z*)	349 $(M+H)^+$	432 $(M+H)^+$	425 $(M+2H)^+$	503 $(M+H)^+$	535 $(M+2H)^+$
UV λ_{max}^{MeOH} nm ($\log\varepsilon$)	212 (4.30), 240 sh (3.96), 302 (4.23), 350 (4.34), 504 (3.21)	196 (4.31), 325 (4.51), 460 (3.13)	203 (4.35), 360 (4.58), 469 (3.23)	198 (4.52), 234 (4.29), 352 (4.44)	198 (4.55), 270 (4.37), 345 (4.53), 472 (4.12)
IR cm^{-1} (KBr)	3300, 3180, 1712, 1620	3320, 1730, 1680	3300, 1714, 1642	3370, 1730, 1680	3350, 1730, 1660

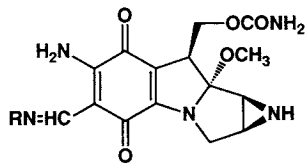
Table 2. ^1H and ^{13}C NMR chemical shifts (δ ppm) of **1** in $CDCl_3$.

Assignment	^{13}C	^1H
1	53.95	2.94 (1H, d, J=4.5 Hz)
2	32.47	2.89 (1H, dd, J=2.0, 4.5 Hz)
3	49.69	3.57 (1H, dd, J=2.0, 13.0 Hz), 4.36 (1H, d, J=13.0 Hz)
4a	106.11	
5	177.62	
6	155.43	
6-CHO	189.60	10.04 (1H, s)
7	155.33	
7-NH_2		7.48 (1H, br s), 10.46 (1H, br s)
8	171.42	
8a	114.35	
9	42.67	3.69 (1H, dd, J=4.5, 10.5 Hz)
9a	106.11	
9a-OMe	50.04	3.23 (1H, s)
10	61.95	4.53 (1H, t, J=10.5 Hz), 4.72 (1H, dd, J=4.5, 10.5 Hz)
10-$OCONH_2$	156.29	4.79 (1H, br s)



Mitomycin C

1



2 R=$-CH_2(CH_2)_4CH_3$

3 R=

4 R=

5 R=

acetate, an excess of activated manganese(IV) oxide was added. After stirring the reaction mixture for 2 hours at room temperature, **1** was separated as an almost single compound from the reaction mixture by washing manganese(IV) oxide with ethyl acetate several times. Thus **1** was easily prepared by a one step reaction in 77% yield (Table 1). Mitomycin A, however, was not oxidized under the same condition as that of mitomycin C. The 7-amino group of mitomycin C appears to enable the conversion of the neighboring 6-methyl group to the formyl group.

The structure of **1** was elucidated by detailed analyses of the ^1H NMR and ^{13}C NMR (400 MHz, $CDCl_3$) spectral data (Table 2). The presence of the formyl group at δ 10.04 ppm and a disappearance of the C-6 methyl group whose signal was at δ 1.90 ppm in case of mitomycin C proved that the 6-methyl group was converted into the 6-formyl group. All of the other signals corresponded to the rest of the structure of 6-demethyl-6-formylmitomycin C.

For further confirmation of the structure of **1**, several Schiff base (**2**, **3**, **4** and **5**) of **1** were prepared in the usual method. The structures of these compounds (**2**, **3**, **4** and **5**) were established by comparing their ^1H NMR spectral data with that of **1**. One of the major differences is that compounds **2**, **3**, **4** and **5** lack a formyl proton and a new peak due to the imino group (–N=CH–) appeared at δ 8.24, 8.82, 8.58 and 9.38 ppm corresponding to compounds **2**, **3**, **4** and **5**, respectively.

The cytotoxicity and antimicrobial activity of **1** and its derivatives (**2**, **3**, **4** and **5**) against B-16 melanoma cells and two strains of bacteria are shown in Table 3. Cytotoxicity of these compounds generally correlates to antibacterial activity. Compound **5** was relatively potent among the compounds tested. While these compounds had less activity than mitomycin C, compound **1** is a promising starting material for further derivatives.

Table 3. Comparison of *in vitro* cytotoxicity and antimicrobial activity of mitomycin derivatives.

Compound	IC_{50} value[a] (μM)	Inhibitory zone (mm)[b]	
		Micrococcus luteus	*Escherichia coli*
1	10.6	6.4	15.4
2	18.8	—	—
3	26.6	—	12.7
4	>49.8	—	—
5	5.8	11.4	31.0
MMC	1.5	18.6	39.6

[a] B-16 melanoma cell was exposed to the compounds for 3 days, and growth rates were examined as reported previously[13].

[b] Antimicrobial activities of these compounds at a concentration of 25 μg/ml were examined on agar plate by paper disc method (6 mm)[13].

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(Received June 8, 1992)

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