THE DERIVATION OF 1a-DEMETHYLMITOMYCIN G FROM MITOMYCIN C

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Mitomycin G (**2**) was derived from porfiromycin (**10b**) in 3 steps *via* the methanesulfonate (**14b**) in an overall yield of 39%. On the basis of the established method for the introduction of an exo-methylene group in mitomycins with a 9a-methoxy group, the preparation of biologically more important 1a-demethylmitomycin G (**5**) from mitomycin C (**1**) was accomplished by the use of a protective acetyl group on the aziridine in an overall yield of 57%. 1a-Demethylmitomycin K (**6**) was obtained from **5** in a yield of 42%. In a preliminary evaluation of their antitumor activity, compound **5** showed superior activity against sarcoma 180 (sc-ip) to its 1a-methyl congener, *i.e.*, mitomycin G (**2**).

Mitomycin C (**1**) is an antitumor antibiotic which is clinically used against a wide range of tumors.[1] Considerable research efforts have been made to clarify the mechanism of action of **1** and have revealed that the activated mitomycin could bind bifunctionally to nucleotide bases of DNA.[2~6] Recently great attention has been given to the study of mitomycin analogs.[7~12] As part of our effort to obtain more potent mitomycin derivatives, we have screened the minor components from the fermentation broth, and have identified mitomycins D, E, F, G,[13] H,[14] I, J, K, L, M,[13] albomitomycin A, and isomitomycin A.[15] Among them, mitomycins G (**2**), H (**3**), and K (**4**) have an exo-methylene group at the C-9 position, and are therefore potentially biologically significant molecules in a sense that the C-10 position could be an electrophilic center as in **1**.[2~6] We herein, report the first synthesis of **2** from porfiromycin (**10b**) and mitomycin E (**10a**). In other words, these are the first examples of introduction of an exo-methylene functionality in mitomycins with a 9a-methoxy group. In addition the introduction of the exo-methylene from **1** was achieved to afford 1a-demethylmitomycins G (**5**) and K (**6**), which attracted our interest from the viewpoint of biological activity due to their structural similarity to the unsubstituted aziridine of **1**.

In previous studies[16,17] on the introduction of double bond at the C-9 and C-10 positions in mitomycins with 9a-hydroxy group, the conversions of mitomycin B (**8**) to **3** and mitomycin D (**9**) to 9a-*O*-demethylmitomycin G (**7**) have been achieved. However the fermentation yields of naturally occurring **8** and **9** were quite low†† compared to that of **1**. Accordingly, in order to extend the availability of **2**, we employed **1** as a starting material. Firstly, we attempted the introduction of an exo-methylene bond in **10b** (with 9β configuration), which was readily derived from **1**.[19] However application of the same condition

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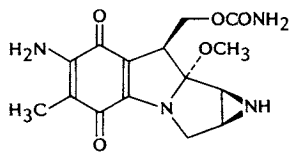
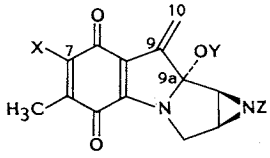
†† Recently ARAI reported[18] the efforts to increase the fermentation yield of **8**.

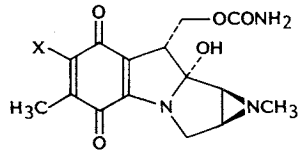
Fig. 1. The structure of mitomycins.



1

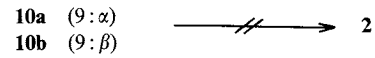


2 X=NH_2 Y=Z=CH_3
3 X=OCH_3 Y=H Z=CH_3
4 X=OCH_3 Y=Z=CH_3
5 X=NH_2 Y=CH_3 Z=H
6 X=OCH_3 Y=CH_3 Z=H
7 X=NH_2 Y=H Z=CH_3

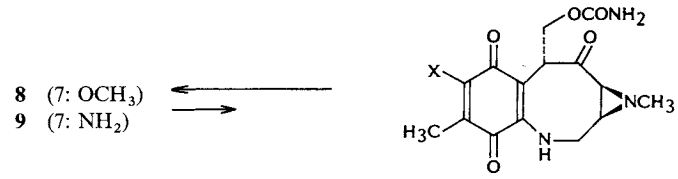


8 X=OCH_3
9 X=NH_2

Equation 1:



Equation 2:



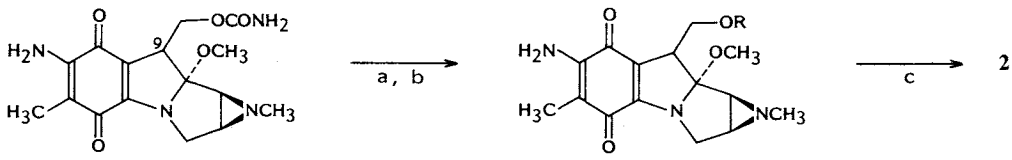
11 X=OCH_3
12 X=NH_2

(DBU/THF, described in our previous article[17]) to **10b** failed to afford the desired compound and resulted in the recovery of **10b**. Next we tried to apply the same condition to **10a**,[†††] since the β elimination in **10a** might be facilitated by the steric hindrance between *cis* C-9 and C-9a substituents. Nevertheless such a trial also ended in failure resulting in the recovery of **10a** (equation 1). These results suggested that in compounds having 9a-hydroxy group like **8** and **9**, a strong base imparted a carbonyl character to the 9a position due to the resonance structures **11** and **12** (equation 2). The resultant acidic C-9 proton could be abstracted by a base leading to a smooth β elimination to give the exo-methylene compound. In contrast, in 9a-methoxy compounds there would be no contribution of resonance structure mentioned above and thus the acidity of the C-9 proton would be negligible.

To circumvent the difficulty of introducing an exo-methylene bond in **10b**, the carbamate was converted to the methanesulfonate (**14b**) in a conventional way to increase the leaving ability. The reaction of **10b** with sodium alkoxide afforded decarbamoylporfiromycin (**13b**),[20] and subsequent methanesulfonylation with methanesulfonyl chloride gave the desired sulfonate **14b**. This change of leaving group resulted in a successful β elimination in **14b** by DBU to afford the desired product **2** in good yield. Thus we obtained **2** from **10b** by 3 steps in an overall yield of 39%. Next we examined **10a** with the same process, and also succeeded in the introduction of exo-methylene bond at the C-9 position.

††† The fermentation yield of **10a** was also quite low. Consequently, mitomycin E (**10a**) was derived from **8** by 2 steps: (1) Methylation at the 9a-OH position[17] and (2) amination at the C-7 position[19] in an overall yield of 44%.

Scheme 1.

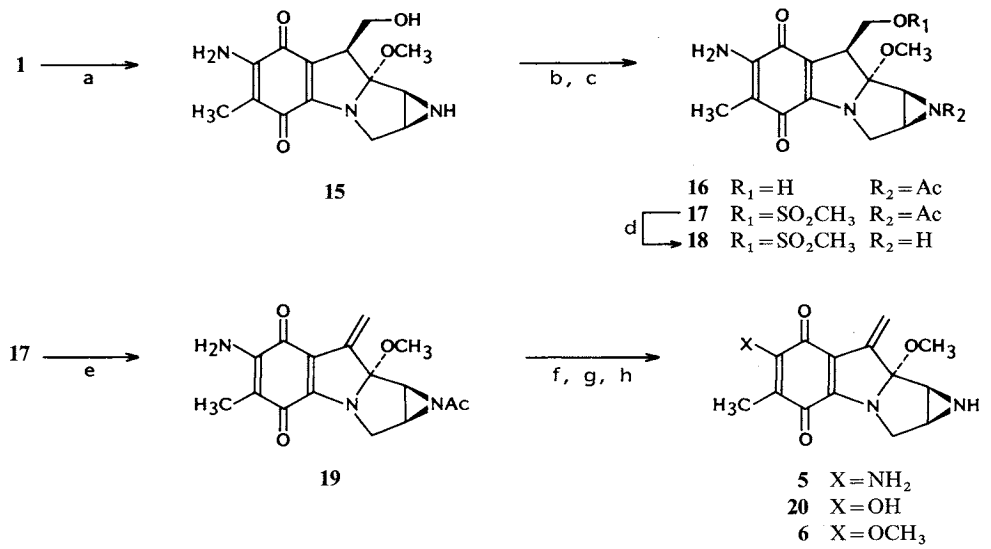


10a (9: α)
10b (9: β)

13a, 13b R=H
14a, 14b R=SO_2CH_3

a: 2-PrONa, 2-PrOH, room temperature, **10a**→**13a**; 58%, **10b**→**13b**; 68%. b: CH_3SO_2Cl, pyridine, room temperature, **13a**→**14a**; 98%, **13b**→**14b**; 98%. c: DBU, THF, reflux, **14a**→**2**; 65%, **14b**→**2**; 59%.

Scheme 2.



15

16 R_1=H R_2=Ac
17 R_1=SO_2CH_3 R_2=Ac
18 R_1=SO_2CH_3 R_2=H

19

5 X=NH_2
20 X=OH
6 X=OCH_3

a: 2-PrONa, 2-PrOH, room temperature; 78%. b: Ac_2O, pyridine, 0°C; 97%. c: CH_3SO_2Cl, pyridine, −15°C; 97%. d: $NaHCO_3$, $(CH_3)_2CO$-H_2O, room temperature; 41%. e: DBU, dimethoxyethane, reflux; 82%. f: pyrrolidine, MeOH, room temperature; 94%. g: NaOH, MeOH-H_2O, room temperature; 66%. h: CH_2N_2; 63%.

In the next stage of efforts, we extended this approach to prepare biologically more important 1a-demethylmitomycin derivatives, since generally they showed higher antitumor activities than 1a-methyl relatives.[21] The protection of the aziridine was needed to derive a 1a-demethylmitomycin with exo-methylene from **1**. The acrolein adduct and its derivatives were used in the KISHI's total synthesis of **1**.[22,23] Through the course of our reactions, we found the 1a-acetate was an excellent protecting group of the aziridine.[24] The mild deprotection was proceeded from 1a-acetate on treatment with secondary amines (C. URAKAWA; personal communication) or sodium hydrogen carbonate.[24]

Mitomycin C (**1**) was treated with sodium 2-propoxide to give the alcohol (**15**).[20] The protection of the aziridine was proceeded by using acetic anhydride, and subsequent methanesulfonylation afforded the methanesulfonyl acetate (**17**). These two steps could be conducted in one pot reaction in a yield of 90%. Exo-methylene was induced from **17** on treatment with DBU in dimethoxyethane at 82°C in a better yield than in THF at 66°C. Finally the clean and mild deprotection of **19** was accomplished on treatment with

Table 1. Antitumor activity of mitomycin derivatives.

No.	Sarcoma 180 (sc-ip)			
	LD_{50} ip (mg/kg)	ED_{50} ip (mg/kg)	CI (LD_{50}/ED_{50})	WBC_{4000} (mg/kg)
2	131	102	1.3	>100
3[a]	11.7	6.8	0.72	>12.5
4[a]	22.4	35	0.66	30
7[b]	210	82	2.6	>200
5	75	26	2.9	>50
6	13	7.5	1.8	14
18	>200	>200	—	>200
1	8.4	3.7	2.3	2.7

[a] The preparation of the compound was described in the ref 17.

[b] The values were quoted from the ref 16.

The LD_{50} values were determined in *dd*Y mice (5 mice/group) after 14 days of observation and were calculated by Behrens-Karber analysis. Sarcoma 180 cells (5×10^6/mouse) were implanted sc into *dd*Y mice and the drug was administered ip respectively on day 1. ED_{50} values were doses which gave 50% inhibition of tumor growth on day 7. WBC_{4000} values were doses to give a WBC number of 4000/mm^3 on day 4.

secondary amine, *i.e.*, pyrrolidine or diisopropylamine in excellent yields. Thus **5** was obtained from **1** in an overall yield of 57% *via* **17**. In addition, the conversion of the amino group at the C-7 position in **5** to the methoxy group was carried out by the known method,[25,26] hydrolysis of **5** afforded **20** and subsequent methylation gave the expected **6** in a yield of 42% from **5**.

Antitumor Activity

The potentially informative methanesulfonate analog (**18**), which had a high electrophilic center at the C-10 position, was obtained from **17** on treatment with sodium hydrogen carbonate. The activity of **18** was of interest in comparison with that of **1**. Antitumor effects against sarcoma 180 (sc-ip) of **2**, **3**, **4**, **5**, **6**, **7**, and **18** were evaluated according to the method described in the literature.[27] Mitomycin C (**1**) was used as a control compound. 1a-Demethylmitomycin G (**5**) showed wide range of effective dosage against sarcoma 180 (sc-ip) and appeared to have reduced myelosuppression in comparison to **1**. As expected the CI value of **5** was superior to that of **2**. A similar relationship between **6** and **4** was also observed. Although the higher reactivity of the C-10 substitution of **18** than that of **1**, **18** showed no antitumor activity. This implied premature quenching of **18** by nucleophiles in the living system before it reached the DNA target. These results will aid in the design of future mitomycin analogs and the interpretation of the structure-activity relationship among naturally occurring mitomycins and their related compounds.

Experimental

MP's were recorded on a Yanagimoto melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Jeol FX-100 and a JNM-PS-PFT-100 spectrometers. MS spectra were recorded on a JMS-O1SG-2 spectrometer. IR spectra were recorded on a Shimadzu IR-27-G spectrometer. Electronic spectra were recorded on a Shimadzu Spectrophotometer MPS-50L.

10-*O*-Decarbamoyl-10-*O*-methanesulfonylporfiromycin (**14b**)

Compound **13b**[20] (100 mg) was dissolved in anhydrous pyridine (2 ml), to which was added methanesulfonyl chloride (50 μl) and the reaction mixture was stirred at room temperature for 20 minutes. Then saturated aqueous solution (10 ml) of sodium hydrogen carbonate was added to the reaction mixture

to quench the reaction. The mixture was extracted with ethyl acetate and dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel with chloroform - methanol (95 : 5). The purple fractions were collected and concentrated *in vacuo* to give 123 mg of purple solids **14b** (yield; 98%). **14b**: EI-MS *m/z* 383 (M^+, $C_{16}H_{21}N_3O_6S$); ^1H NMR (pyridine-d_5) δ 2.03 (3H, s), 2.23 (1H, dd, J=4.6 and 2.0 Hz), 2.32 (3H, s), 2.64 (1H, d, J=4.6 Hz), 3.19 (3H, s), 3.30 (3H, s), 3.52 (1H, dd, J=12.9 and 2.0 Hz), 3.96 (1H, dd, J=11.0 and 4.2 Hz), 4.47 (1H, d, J=12.9 Hz), 4.84 (1H, dd, J=11.0 and 9.5 Hz), 5.31 (1H, dd, J=9.5 and 4.2 Hz), 7.64 (2H, br).

10-*O*-Decarbamoyl-10-*O*-methanesulfonylmitomycin E (**14a**)

Compound **14a** was obtained from **13a**[20] by the same procedure as the preparation of **14b** in a yield of 98%. **14b**: EI-MS *m/z* 383 (M^+, $C_{16}H_{21}N_3O_6S$); ^1H NMR ($CDCl_3$) δ 1.73 (3H, s), 2.34 (3H, s), 2.45 (1H, d, J=4.6 Hz), 2.64 (1H, dd, J=4.6 and 2.0 Hz), 3.10 (3H, s), 3.38 (3H, s), 3.64 (1H, dd, J=12.9 and 2.0 Hz), 3.84 (1H, dd, J=10.5 and 3.7 Hz), 3.93 (1H, d, J=12.9 Hz), 4.50 (1H, dd, J=10.5 and 9.8 Hz), 4.92 (1H, dd, J=9.8 and 3.7 Hz).

Mitomycin G (**2** from **14b** or **14a**)

The compound **14b** (17 mg) was dissolved in anhydrous tetrahydrofuran (1 ml), to which was added 1,5-diazabicyclo[5.4.0]undec-5-ene (54 mg) and the reaction mixture was refluxed under nitrogen atmosphere for 5 hours. The solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel with chloroform - acetone (4 : 1). The green fractions were collected and concentrated *in vacuo* to give 7.5 mg of dark green needles of **2** (yield; 59%). **2**: MP 238~241°C (dec). All the spectroscopic data were identical with those of reported ones.[16] Compound **2** was obtained from **14a** according to the same procedure as mentioned above in a yield of 65%.

10-*O*-Decarbamoylmitomycin C (**15**)

Compound **15** was prepared according to the procedure described in the literature[20] in a yield of 78%.

1a-Acetyl-10-*O*-decarbamoylmitomycin C (**16**)

Compound **15** (138 mg) was dissolved in anhydrous pyridine (1 ml), to which was added acetic anhydride (60 μl) at 0°C and the reaction mixture was stirred under nitrogen atmosphere for 1 hour. Then the reaction mixture was poured into a saturated aqueous sodium hydrogen carbonate solution and the mixture was extracted with ethyl acetate. The extract was washed with water and dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel using chloroform - methanol (95 : 5). The purple fractions were collected and concentrated *in vacuo* to give 153 mg of purplish black solids of **16** (yield; 97%). **16**: EI-MS *m/z* 333 (M^+, $C_{16}H_{19}N_3O_5$); ^1H NMR (pyridine-d_5) δ 2.05 (3H, s), 2.23 (3H, s), 3.23 (3H, s), 3.57 (1H, dd, J=4.6 and 2.0 Hz), 3.66 (1H, dd, J=13.2 and 2.0 Hz), 3.94 (1H, dd, J=9.5 and 5.7 Hz), 3.98 (1H, d, J=4.6 Hz), 4.33 (1H, dd, J=10.6 and 9.5 Hz), 4.76 (1H, d, J=13.2 Hz), 4.84 (1H, dd, J=10.6 and 5.7 Hz), 7.57 (2H, br); IR (KBr) cm^{-1} 3435, 3335, 1694, 1605, 1555.

1a-Acetyl-10-*O*-decarbamoyl-10-*O*-methanesulfonylmitomycin C (**17**)

Compound **16** (62 mg) was dissolved in anhydrous pyridine (0.33 ml), to which was added methanesulfonyl chloride (15 μl) under nitrogen atmosphere at −15°C and the reaction mixture was stirred for 2 hours. Then the reaction mixture was poured into a saturated aqueous solution of sodium hydrogen carbonate and the mixture was extracted with ethyl acetate. The extract was washed with water and dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel using chloroform - methanol (96 : 4). The purple fractions were collected and concentrated *in vacuo* to give 74 mg of purplish black solids of **17** (yield; 97%). **17**: ^1H NMR (pyridine-d_5) δ 2.04 (3H, s), 2.11 (3H, s), 3.20 (3H, s), 3.46 (3H, s), 3.60 (1H, dd, J=4.5 and 1.6 Hz), 3.61 (1H, dd, J=13.7 and 1.6 Hz), 3.79 (1H, d, J=4.5 Hz), 4.09 (1H, dd, J=10.7 and 4.2 Hz), 4.84 (1H, d, J=13.7 Hz), 4.84 (1H, dd, J=10.7 and 9.8 Hz), 5.58 (1H, dd, J=9.8 and 4.2 Hz), 7.71 (2H, br); IR (KBr) cm^{-1} 3445, 3235, 1697, 1605, 1563, 1350, 1173.

1a-Demethyl-1a-acetylmitomycin G (**19**)

Compound **17** (38 mg) was dissolved in anhydrous ethylene glycol dimethyl ether (8 ml), to which was added 1,5-diazabicyclo[5.4.0]undec-5-ene (150 mg) and the reaction mixture was refluxed under nitrogen atmosphere for 2 hours. A saturated aqueous sodium hydrogen carbonate solution was added to the reaction mixture and the mixture was extracted with ethyl acetate. The extract was washed with water and dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel using chloroform - acetone (6 : 4). The bluish green fractions were collected and concentrated *in vacuo* to give 24 mg of bluish green solids of **19** (yield; 82%). **19**: ^{1}H NMR (pyridine-d_5) δ 2.03 (3H, s), 2.05 (3H, s), 3.17 (3H, s), 3.57 (1H, dd, J=4.5 and 1.6 Hz), 3.64 (1H, dd, J=13.2 and 1.6 Hz), 3.78 (1H, d, J=4.5 Hz), 4.84 (1H, d, J=13.2 Hz), 5.61 (1H, d, J=0.7 Hz), 6.55(1H, d, J=0.7 Hz), 7.76 (2H, br); IR (KBr) cm^{-1} 3435, 3330, 1695, 1656, 1694, 1537.

1a-Demethylmitomycin G (**5**)

Compound **19** (143 mg) was dissolved in methanol (4.5 ml), to which was added pyrrolidine (0.3 ml) and the reaction mixture was stirred at room temperature for 30 minutes. The solvent was removed *in vacuo* and the residue was purified by column chromatography on silica gel with chloroform - acetone (1 : 1). The bluish green fractions were collected and concentrated *in vacuo* to give 116 mg of dark green prisms of **5** (yield; 94%). **5**: MP 149~151°C; EI-MS (HR) calcd for $C_{14}H_{15}N_3O_3$: m/z 273.1113, found: m/z 273.1099; UV λ_{max}^{MeOH} nm (log ε) 222 (4.15), 290 (3.96), 377 (4.17); ^{1}H NMR (pyridine-d_5) δ 2.00 (3H, s), 2.77 (1H, dd, J=4.4 and 1.7 Hz), 3.04 (1H, d, J=4.4 Hz), 3.17 (3H, s), 3.59 (1H, dd, J=12.7 and 1.7 Hz), 4.67 (1H, d, J=12.7 Hz), 5.50 (1H, d, J=1.1 Hz), 6.50 (1H, d, J=1.1 Hz), 7.57 (2H, br); ^{13}C NMR (pyridine-d_5) δ 8.9 (q), 33.9 (d), 38.8 (d), 49.8 (t), 50.2 (q), 105.7 (s, two carbons), 108.7 (dd), 112.3 (s), 140.0 (s), 149.5 (s), 158.3 (s), 176.2 (s), 178.1 (s); IR (KBr) cm^{-1} 3420, 3320, 3285, 1651, 1592, 1532.

1a-Demethyl-7-*O*-demethylmitomycin K (**20**)

Compound **5** (18 mg) was dissolved in 0.1 N aqueous sodium hydroxide solution (3.75 ml) and the reaction mixture was stirred at room temperature for 45 minutes. The reaction mixture was adjusted to pH 4.0 with diluted hydrochloric acid and extracted with ethyl acetate. The extract was washed with water and dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel with chloroform - methanol (9 : 1). The bluish purple fractions were collected and concentrated *in vacuo* to give 12 mg of purplish black solids of **20** (yield; 66%). **20**: EI-MS m/z 274 (M^+, $C_{14}H_{14}N_2O_4$); ^{1}H NMR (pyridine-d_5) δ 2.05 (3H, s), 2.80 (1H, dd, J=4.5 and 1.7 Hz), 3.08 (1H, d, J=4.5 Hz), 3.19 (3H, s), 3.58 (1H, dd, J=12.6 and 1.7 Hz), 4.50 (1H, d, J=12.6 Hz), 5.55 (1H, d, J=0.9 Hz), 6.52 (1H, d, J=0.9 Hz); IR (KBr) cm^{-1} 3285, 1644, 1630, 1548.

1a-Demethylmitomycin K (**6**)

Compound **20** (12 mg) was dissolved in ethyl acetate (3 ml). An excess amount of ethyl ether solution of diazomethane was added dropwise to the solution while ice cooling and the mixture was allowed to stand for 10 minutes. The mixture was then concentrated *in vacuo* and the residue was purified by column chromatography on silica gel using chloroform - acetone (6 : 4). The bluish purple fractions were collected and concentrated *in vacuo* to give 8 mg of blackish purple prisms of **6** (yield; 63%). **6**: MP 65~67.5°C; EI-MS (HR) calcd for $C_{15}H_{16}N_2O_4$: m/z 288.1110, found: m/z 288.1085; UV λ_{max}^{MeOH} nm (log ε) 223 (4.11), 288 (4.05), 322 (sh, 3.98); ^{1}H NMR (pyridine-d_5) δ 1.83 (3H, s), 2.80 (1H, dd, J=4.3 and 1.5 Hz), 3.07 (1H, d, J=4.3 Hz), 3.16 (3H, s), 3.53 (1H, dd, J=12.6 and 1.5 Hz), 4.01 (3H, s), 4.33 (1H, d, J=12.5 Hz), 5.59 (1H, d, J=1.0 Hz), 6.57 (1H, d, J=1.0 Hz); IR (KBr) cm^{-1} 3285, 1644, 1630, 1548.

10-*O*-Decarbamoyl-10-*O*-methanesulfonylmitomycin C (**18**)

Compound **17** (14.8 mg) was dissolved in acetone (1 ml), to which was added aqueous solution of 10% sodium hydrogen carbonate (1 ml). The reaction mixture was stirred at room temperature for 50 hours. The reaction mixture was extracted with ethyl acetate. The extract was washed with water and brine and dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel using ethyl acetate - methanol (98 : 2). The purple fractions (Rf 0.56) were collected and concentrated *in vacuo* to give 5.0 mg of purple solids of **18** (yield; 41%). From the

second purple fractions (Rf 0.31) 1.2 mg of **17** was recovered. **18**: ^{1}H NMR (pyridine-d_5) δ 2.04 (3H, s), 2.82 (1H, dd, J=4.4 and 2.0 Hz), 3.22 (3H, s), 3.23 (1H, d, J=4.4 Hz), 3.29 (3H, s), 3.63 (1H, dd, J=12.9 and 2.0 Hz), 4.02 (1H, dd, J=10.5 and 4.8 Hz), 4.52 (1H, d, J=12.9 Hz), 5.18 (1H, dd, J=10.5 and 9.4 Hz), 5.32 (1H, dd, J=9.4 and 4.8 Hz), 7.66 (2H, br); IR (KBr) cm^{-1} 3440, 3315, 1604, 1555, 1352, 1174.

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