THE DERIVATION OF A NOVEL MITOMYCIN SKELETON: 3α-ALKOXYMITOMYCIN

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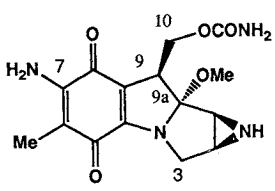
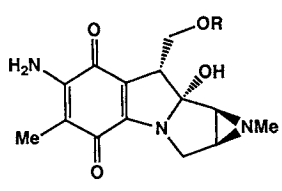
The first example of C-3 alkoxylation in mitomycins has been achieved. 3α-*iso*-Propoxy-10-*O*-decarbamoylmitomycin D (**4**) and 3α-*iso*-propoxymitomycin D (**5**) were derived from mitomycin D (**3**) under decarbamoylation conditions with *iso*-propoxide. Under similar conditions 3α-*iso*-propoxy-10-*O*-decarbamoylporfiromycin (**8**) and 3α-methoxy-10-*O*-decarbamoylmitomycin B (**11**) were also derived from porfiromycin (**6**) and mitomycin B (**9**), respectively. The mechanism of generation of these novel analogs was based on the premise that the key intermediate of hydroquinone iminium salt (**14**) was led through the iminium salt (**13**), followed by alkoxide addition and oxidation.

Mitomycin C (**1**) is known as one of the most potent antitumor antibiotics and has been widely used in clinical chemotherapy.[1] The molecular mechanism of action of **1** was interpreted as the reduction of quinone and subsequent activation of C-1 and C-10 with elimination of methanol.[2] As part of our approach to obtain more potent and less toxic mitomycin analogs than **1**, we have been screening the minor constituents from the fermentation broth of mitomycins since 1977. We have found several mitomycin congeners[3] including new skeletons[4] and developed the syntheses of compounds considered to be "missing links"[5,6] in the mitomycin family. During these studies, the decarbamoylation step required was investigated in detail. In an attempt to convert mitomycin D (**2**) to 10-*O*-decarbamoylmitomycin D (**3**), we have found unexpected novel 3α-alkoxymitomycin analogs. These skeletons represent novel evolution in mitomycin chemistry from the viewpoint of the mode of action, *viz.*, they carry the third latent reacting site; the aminal at C-3. Antitumor antibiotics often have an aminal group, *e.g.*, in the anthramycin family[7] the aminal group plays an important role in the molecular mechanism of action.[8] They also show new aspects of the reactivity at C-3 in the mitomycin family, which have never been reported. Hence we applied this method to other mitomycins and obtained a variety of 3α-alkoxymitomycins. We herein describe the introduction of novel 3α-alkoxymitomycins from conventional mitomycins.

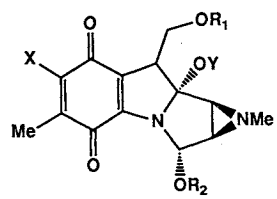
Fig. 1. Structures of mitomycins and 3α-alkoxymitomycins (**1**).



Mitomycin C (**1**)



2 R=$CONH_2$
3 R=H



3α-Alkoxymitomycins

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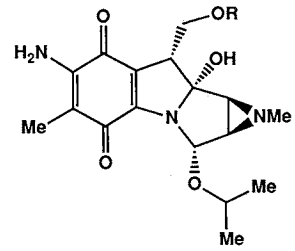
Decarbamoylation of mitomycins played a fundamental role in the introduction of exo-methylene at C-9[5] and the derivation of 9-*epi*-mitomycins B and D.[6] In our first synthetic efforts to obtain 9-*epi*-mitomycin D, we employed 10-*O*-decarbamoylmitomycin D (**3**) as a substrate for the epimerization at C-9. However the application of the known decarbamoylation method (2-PrONa - 2-PrOH)[9] to mitomycin D (**2**) resulted in a low yield of the desired **3** and afforded many byproducts. In order to elucidate the complexity in this reaction, we separated two byproducts **4** and **5** (silica gel TLC, $CHCl_3$ - MeOH (9 : 1), $Rf_4 = 0.52$ and $Rf_5 = 0.40$) which had similar hue with that of **3** (same TLC condition, $Rf_3 = 0.32$). In the IR (KBr) spectrum of **4**, a carbamoyl carbonyl stretching band at about 1710 cm^{-1} disappeared, therefore **4** was suggested to be a decarbamoylmitomycin. The EI-MS spectrum of **4** showed a remarkable molecular ion peak at *m/z* 349 (68%), which increased by 58 mass units in comparison with 10-*O*-decarbamoylmitomycin D (**3**) (*m/z* 291). The characteristic *m/z* 70 (observed in mitomycin B[10]) was also exchanged for *m/z* 128 (70 + 58), suggesting the presence of *iso*-propoxy group in the aziridinopyrrolidine moiety. ^1H NMR (pyridine-d_5) of **4** showed the presence of *iso*-propyl group, *i.e.*, δ 1.20 (3H, d, $J = 6.4$ Hz) and 1.42 (3H, d, $J = 6.4$ Hz). While the chemical shifts of 9-H (δ 3.95) and 10-Ha, 10-Hb (δ 4.49 and 4.82) were consistent with those of **3**, geminal protons of the 3-Hα, 3-Hβ methylene disappeared and a novel 1H singlet (δ 5.84) emerged. The chemical shift of this singlet should be assigned reasonably to the α-proton of the ether oxygen. Accordingly **4** was proved to have *iso*-propoxy group at C-3. Generally the coupling between 2-H and 3-Hβ could not be observed in mitomycins.[4,11] Therefore, δ 5.84 was assigned to 3-Hβ and *iso*-propoxy group was determined to be substituted at α position of C-3. Thus, the structure of **4** was unambiguously determined as 10-*O*-decarbamoyl-3α-*iso*-propoxymitomycin D (yield 9%).

Fig. 2. Structures of 3α-alkoxymitomycins derived from mitomycin D.

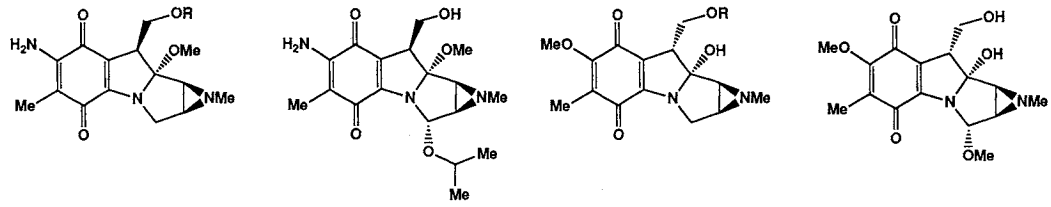


4 R = H
5 R = $CONH_2$

The EI-MS spectrum of **5** showed a molecular ion peak at *m/z* 392, which exceeded the molecular number of **2** (334) by oxypropylene units (58), and also showed the same fragment ion (*m/z* 128) as **4**. The ^1H NMR of **5** (pyridine-d_5) also exhibited the presence of an *iso*-propoxy group, *i.e.*, δ 1.17 (3H, d, $J = 6.1$ Hz), 1.42 (3H, d, $J = 5.9$ Hz), and 4.66 (1H, heptad, $J = 6.0$ Hz) instead of 3-Hα of **2**, supporting consistently the C-3α substitution of the *iso*-propoxy group in **5**. Accordingly the structure of **5** was unambiguously assigned to 3α-*iso*-propoxymitomycin D (yield; 5%). Neither substitution reaction at C-3 in mitomycins nor naturally occurring mitomycins substituted at C-3 have been reported. The unexpected discovery of 3α-alkoxymitomycins prompted us to apply this reaction condition to porfiromycin (**6**) and mitomycin C (**1**) in order to explicate the reactivity of the C-3 in 9a-methoxymitomycin. The decarbamoylation condition (2-PrONa - 2-PrOH)[9] afforded both 10-*O*-decarbamoylporfiromycin (**7**, yield; 62.7%) and expected 10-*O*-decarbamoyl-3α-*iso*-propoxyporfiromycin (**8**, yield; 0.7%). However **1** did not afford such a 3-alkoxy analog on treatment with sodium *iso*-propoxide (2-PrONa - 2-PrOH). In addition, we extended this approach to 7-methoxymitomycin, and obtained 10-*O*-decarbamoyl-3α-methoxymitomycin B (**11**, yield; 0.8%) from mitomycin B (**9**) on treatment with sodium methoxide (MeONa - MeOH-benzene).[12]

The mechanism of the unexpected 3α-alkoxylation including oxidation process was supposed to be

Fig. 3. Structures of mitomycins and 3α-alkoxymitomycins (**2**).



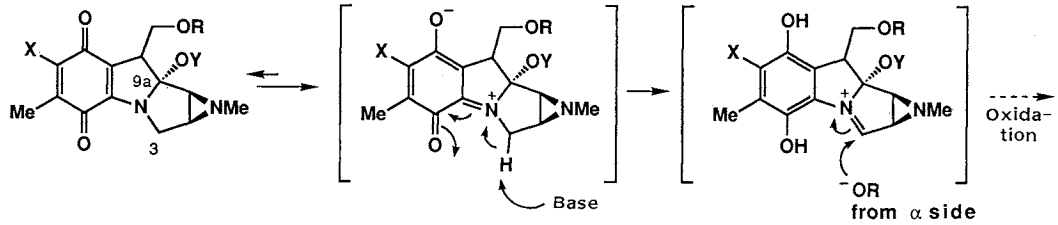
6 R=$CONH_2$
7 R=H

8

9 R=$CONH_2$
10 R=H

11

Scheme 1. Proposed mechanism of 3α-alkoxylation in mitomycins.



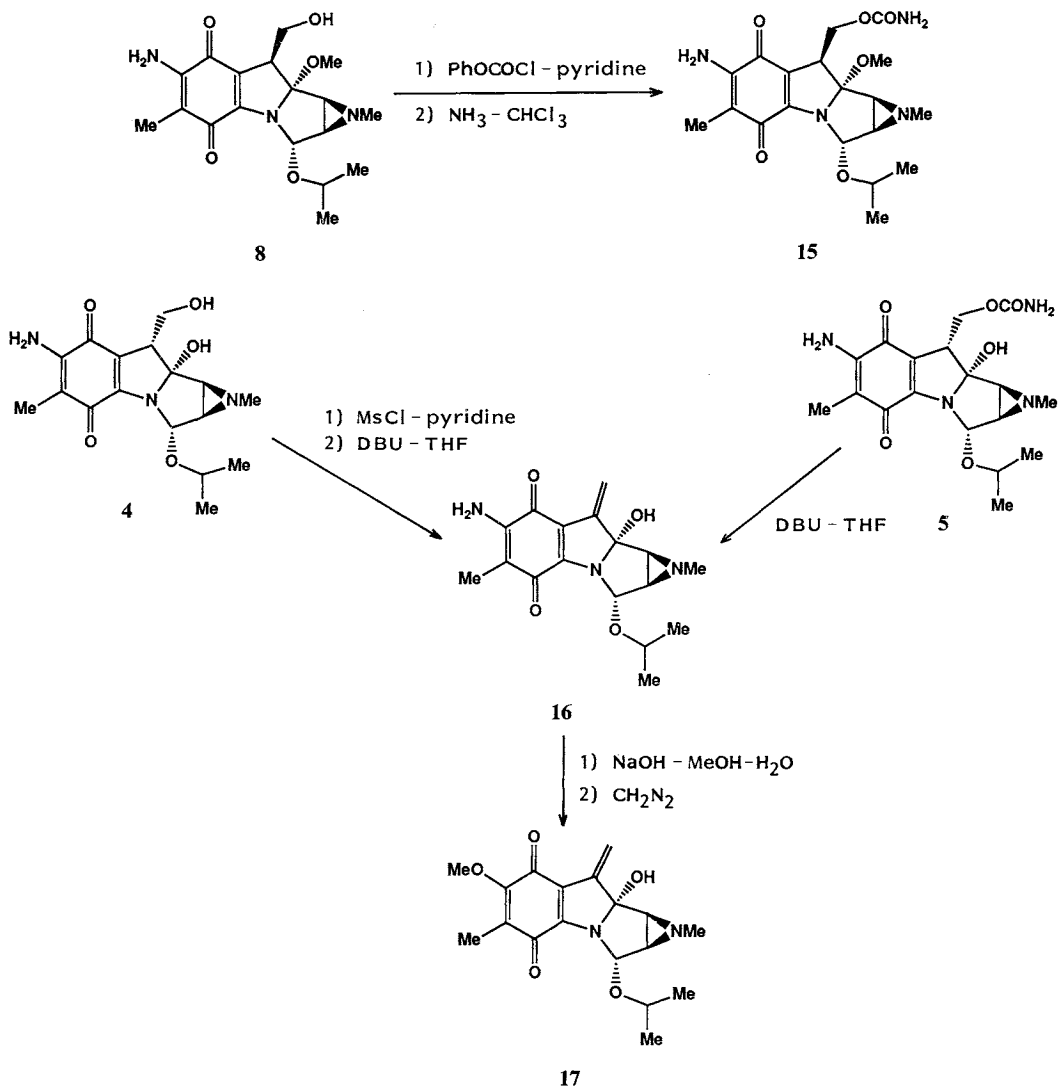
12 **13** **14**

mediated by hydroquinone (**14**), which would be generated through the α-proton abstraction of an iminium cation (**13**) by a base. Subsequent 1,2-addition of alkoxide from the less hindered α side and oxidation would readily lead the 3α-alkoxymitomycins. In 9a-hydroxy compounds, apparently the ketonic character at C-9a could stabilize the key intermediate (**13**). While when a methoxy group was present in the 9a position, **13** might be less stable, resulting in a low yield of 3α-alkoxymitomycin compared to those of 9a-hydroxy compounds. Substituents at C-7 also might control the yield of 3α-alkoxymitomycins through the modifications of stability of **13**. In the case of **2** with the 7-amino group, strong electron donation or contribution of the 7-imine tautomer could help the promotion from **13** to **14**. On the other hand, the 7-methoxy group in **9** could not contribute to such efficient progression.

We then examined the possible derivatization of the obtained novel mitomycins in order to prepare the mimics of naturally occurring mitomycins. The conversion of C-10 hydroxy group in **8** to carbamoyloxy group was proceeded by phenoxycarbonylation (PhOCOCl - pyridine) and succeeding ammonolysis to give **15** (NH_3 - $CHCl_3$; yield; 87% from **8**).[6] The introduction of exo-methylene at C-9 in **4** was carried out by methanesulfonylation (MsCl - pyridine) and subsequent β-elimination (DBU - THF)[5] to afford **16** (yield; 61% from **4**). The same **16** was also derived from **5** (DBU - THF)[13] in a higher yield (yield; 69%). The 7-amino group in **16** was converted to a methoxy group by hydrolysis (NaOH - MeOH-H_2O) and succeeding methylation (CH_2N_2)[14] to afford **17** (yield; 56% from **10**).

The novel reactivity of C-3 in 3α-alkoxymitomycins led to an investigation of their antitumor activity. A preliminary evaluation of the antimicrobial and antitumor effects of these compounds was made against several lines of pathogenic bacteria and sarcoma 180 (sc-ip) according to the method described in the literature.[15] However these compounds did not show antimicrobial and antitumor activity at all. These results implied the premature quenching of these compounds in the living system before they reached the

Scheme 2. Derivatization of 3α-alkoxymitomycins.



DNA target or their structural inconvenience in their interaction with the DNA. Although the first alkoxylation in C-3 of mitomycins resulted in inactive compounds, these results will aid in the design of future mitomycin analogs.

Experimental

MP's were recorded on a Yanagimoto melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Jeol FX-100 and a JNM-PS-PFT-100 spectrometers. MS spectra were recorded on a JMS-01SG-2 spectrometer. IR spectra were recorded on a Shimadzu IR-27-G spectrometer.

Preparation of 10-*O*-Decarbamoylmitomycin D (**3**), 10-*O*-Decarbamoyl-3α-*iso*-propoxymitomycin D (**4**), and 3α-*iso*-Propoxymitomycin D (**5**)

Mitomycin D (**2**, 1.93 g) was dissolved in anhydrous 2-propanol (225 ml), to which was added sodium 2-propoxide (19.4 g) and the reaction mixture was stirred at ambient temperature for 23 hours. Then excess

dry ice was added to the reaction mixture while stirring. Copious salt was removed by filtration and the filtrate was concentrated under reduced pressure to dryness. The residue was dissolved in ethyl acetate (400 ml). The solution was washed with brine and dried over anhydrous sodium sulfate and concentrated under reduced pressure to dryness. The residue was applied to column chromatography on silica gel with chloroform - methanol (95 : 5 ~ 9 : 1) to give 4 fractions including mitomycins. The first fractions containing **4** were concentrated under reduced pressure to afford a paste (261 mg). From the 2nd fractions, a paste (130 mg) containing **5** was obtained. From the 3rd fraction, a paste (272 mg) containing **3** was obtained. From the 4th fractions, dark bluish solids of **2** (164 mg) were recovered. The paste containing **4** was purified by repeating column chromatography; (1) silica gel with chloroform - methanol (97 : 3), (2) alumina (activity I + 5% water) with chloroform - methanol (97 : 3), (3) silica gel with chloroform - methanol (98 : 2). Reddish purple solids of **4** were obtained (165.3 mg, yield; 9.0%). The paste containing **5** was purified by column chromatography on silica gel with chloroform - acetone (1 : 1) to give reddish purple solids of **5** (103.8 mg, yield; 5.0%). The paste containing **3** was purified by repeating column chromatography; (1) silica gel with chloroform - methanol (95 : 5), (2) alumina (activity I + 5% water) with chloroform - methanol (95 : 5), (3) silica gel with chloroform - methanol (95 : 5). Dark green solids of **3** were obtained (125.2 mg, yield; 8.1%). **3**: Greenish brown powders (from benzene - acetone) mp 118 ~ 123°C; IR (KBr) cm^{-1} 3320, 1586, 1537, 1440, 1350; ^1H NMR (100 MHz, pyridine-d_5) δ 1.90 (3H, s), 2.19 (3H, s), 2.21 (1H, dd, J = 4.6 and 2.0 Hz), 2.49 (1H, d, J = 4.6 Hz), 3.60 (1H, dd, J = 12.9 and 2.0 Hz), 3.98 (1H, t, J = 6.6 Hz), 4.41 (1H, d, J = 12.9 Hz), 4.51 (1H, dd, J = 11.1 and 6.6 Hz), 4.69 (1H, dd, J = 11.1 and 6.6 Hz); EI-MS m/z 392 (M^+), 349, 331 (base peak), 288, 272, 242, 128. **4**: Dark reddish purple prisms (from n-hexane - acetone) mp 230 ~ 235°C (dec); IR (KBr) cm^{-1} 3410, 1609, 1582, 1564, 1420, 1342, 1090, 1080; ^1H NMR (100 MHz, pyridine-d_5) δ 1.20 (3H, d, J = 6.4 Hz), 1.42 (3H, d, J = 6.4 Hz), 1.94 (3H, s), 2.18 (3H, s), 2.49 (3H, d, J = 4.3 Hz), 2.58 (1H, d, J = 4.3 Hz), 3.95 (1H, dd, J = 5.2 and 4.9 Hz), 4.49 ~ 4.82 (3H, m), 5.84 (1H, s); EI-MS m/z 349 (M^+), 289, 272, 258, 242 (base), 230, 128. **5**. Dark bluish green prisms (from n-hexane - acetone) mp 119 ~ 124°C (dec); IR (KBr) cm^{-1} 3430, 3350, 1705, 1558, 1411, 1339, 1057; ^1H NMR (100 MHz, pyridine-d_5) δ 1.17 (3H, d, J = 6.1 Hz), 1.42 (3H, d, J = 5.9 Hz), 1.96 (3H, s), 2.12 (3H, s), 2.48 (2H, s), 4.13 (1H, dd, J = 9.0 and 3.9 Hz), 4.66 (1H, heptad, J = 6.0 Hz), 5.19 (1H, dd, J = 10.5 and 9.0 Hz), 5.38 (1H, dd, J = 10.5 and 3.9 Hz), 5.84 (1H, s); EI-MS m/z 392 (M^+), 349, 331 (base), 288, 272, 242, 128.

Preparation of 10-*O*-Decarbamoylporfiromycin (**7**) and 3α-*iso*-Propoxy-10-*O*-decarbamoylporfiromycin (**8**)

Porfiromycin (**6**, 5.27 g) was dissolved in anhydrous 2-propanol (800 ml), to which was added sodium 2-propoxide (62 g) and the reaction mixture was stirred at ambient temperature for 19 hours. Then excess dry ice was added to the reaction mixture while stirring. Copious salt was removed by filtration and the filtrate was concentrated under reduced pressure to dryness. The residue was dissolved in ethyl acetate (1,000 ml) and the solution was washed with brine and dried over anhydrous sodium sulfate and concentrated under reduced pressure to dryness. The residue was applied to column chromatography on silica gel with chloroform - methanol (96 : 4 ~ 9 : 1) to give 3 fractions including mitomycins. The 1st fractions were concentrated under reduced pressure and the residue was purified by column chromatography on silica gel with ethyl acetate - acetone (95 : 5) to give a reddish purple paste of **8** (39.5 mg, yield; 0.73%). The 2nd fractions were concentrated under reduced pressure to give brownish purple prisms of **7** (2.91 g, yield; 62.7%). The 3rd fractions were concentrated under reduced pressure to recover purple solids of **6** (238 mg). **8**: A reddish purple paste; ^1H NMR (100 MHz, pyridine-d_5) δ 1.26 (3H, d, J = 6.1 Hz), 1.46 (3H, d, J = 5.9 Hz), 2.02 (3H, s), 2.24 (3H, s), 2.41 (1H, d, J = 4.4 Hz), 2.85 (1H, d, J = 4.4 Hz), 3.33 (3H, s), 3.80 (1H, dd, J = 8.3 and 5.6 Hz), 4.28 (1H, dd, J = 10.3 and 8.2 Hz), 4.54 (1H, heptad, J = 6.1 Hz), 4.77 (1H, dd, J = 10.3 and 5.6 Hz), 5.90 (1H, s); EI-MS m/z 363 (M^+), 333, 242 (base).

Preparation of 10-*O*-Decarbamoylmitomycin B (**10**) and 3α-Methoxy-10-*O*-decarbamoylmitomycin B (**11**)

Mitomycin B (**9**, 1 g) was dissolved in anhydrous benzene - methanol (1 : 1, 150 ml), to which was added sodium methoxide (6.2 g) and the reaction mixture was stirred at ambient temperature for 72 hours. Then excess dry ice was added to the reaction mixture while stirring. Copious salt was removed by filtration and the filtrate was concentrated under reduced pressure to dryness. The residue was dissolved in ethyl

acetate (200 ml). The solution was washed with brine and dried over sodium sulfate and concentrated under reduced pressure to dryness. The residue was applied to column chromatography on silica gel with chloroform - acetone (7 : 3 ~ 1 : 1) to give 4 fractions containing mitomycins. From the 1st fractions, a dark bluish purple paste of mitomycin H, *i.e.*, 10-decarbamoyloxy-9-dehydromitomycin B was obtained (27.3 mg, yield; 4.8%). From the 2nd fractions, a reddish purple paste containing **11** was obtained (13.6 mg) and purified by column chromatography on silica gel with chloroform - methanol (98 : 2). Reddish purple needles were obtained (5.9 mg, yield; 0.8%). The 3rd fractions were concentrated under reduced pressure to dryness to give a bluish purple paste of **10** (138.1 mg, yield; 20.6%). From the 4th fractions, a purple paste of **9** was recovered in the same way (234 mg). **11**: Reddish purple needles (from *n*-hexane - acetone) mp 165 ~ 167°C; IR (KBr) cm^{-1} 3400, 1640, 1577, 1281; ^1H NMR (100 MHz, pyridine-d_5) δ 1.77 (3H, s), 2.18 (3H, s), 2.56 (1H, d, J=4.4 Hz), 2.65 (1H, d, J=4.4 Hz), 3.68 (3H, s), 3.91 (3H, s), 3.97 (1H, m), 4.70 (2H, m), 5.39 (1H, s); EI-MS m/z 336 (M^+), 318, 304, 287, 275, 257, 245, 100 (base).

Preparation of 3α-*iso*-Propoxyporfiromycin (**15**)

3α-*iso*-Propoxy-10-*O*-decarbamoylporfiromycin (**8**, 85 mg) was dissolved in anhydrous methylene chloride - pyridine (5 : 1, 12 ml), to which was added phenyl chloroformate (35 μl) and the reaction mixture was stirred at ambient temperature for 90 minutes. Saturated aqueous sodium bicarbonate solution (10 ml) was added to the reaction mixture and the mixture was extracted with ethyl acetate (20 ml). The solution was washed with brine and dried over anhydrous sodium sulfate and concentrated under reduced pressure to dryness. The residue was purified by column chromatography on silica gel with chloroform - methanol (98 : 2) to give reddish purple solids of 10-decarbamoyloxy-3α-*iso*-propoxy-10-phenoxycarbonyloxyporfiromycin (65 mg, yield; 57.7%). 10-Decarbamoyloxy-3α-*iso*-propoxy-10-phenoxycarbonyloxyporfiromycin (39.5 mg) was dissolved in methylene chloride (1 ml), to which was added ammonia - methanol solution (6%, 5 ml) and the reaction mixture was stirred at ambient temperature for 37 hours. The solution was concentrated under reduced pressure to dryness. The residue was purified by column chromatography on silica gel with chloroform - acetone (7 : 3) to give reddish purple solids of **15** (29.0 mg, yield; 87.3%). 10-Decarbamoyloxy-3α-*iso*-propoxy-10-phenoxycarbonyloxyporfiromycin: ^1H NMR (100 MHz, pyridine-d_5) δ 1.24 (3H, d, J=6.4 Hz), 1.43 (3H, d, J=5.9 Hz), 1.99 (3H, s), 2.23 (3H, s), 2.42 (1H, d, J=4.4 Hz), 2.63 (1H, d, J=4.4 Hz), 3.31 (3H, s), 3.96 (1H, dd, J=10.7 and 4.6 Hz), 4.51 (1H, m), 4.73 (1H, dd, J=10.7 and 10.5 Hz), 5.33 (1H, J=10.5 and 4.6 Hz), 5.82 (1H, s), 7.30 (5H, m). **15**: Reddish purple solids; IR (KBr) cm^{-1} 3450, 3345, 1718, 1608, 1570, 1414, 1340, 1323, 1060; ^1H NMR (100 MHz, pyridine-d_5) δ 1.24 (3H, d, J=6.1 Hz), 1.44 (3H, d, J=5.9 Hz), 2.01 (3H, s), 2.25 (3H, s), 2.36 (1H, d, J=4.4 Hz), 2.66 (1H, d, J=4.4 Hz), 3.30 (3H, s), 3.94 (1H, dd, J=11.1 and 4.4 Hz), 4.53 (1H, heptad, J=6.0 Hz), 4.71 (1H, dd, J=11.5 and 10.5 Hz), 5.31 (1H, dd, J=10.5 and 4.4 Hz), 5.83 (1H, s); EI-MS m/z 406 (M^+), 374, 363, 345, 330, 315 (base), 302, 262, 256, 242.

Preparation of 9a-*O*-Demethyl-3α-*iso*-propoxymitomycin G (**16**)

(a) 3α-*iso*-Propoxymitomycin D (**5**, 29.5 mg) was dissolved in anhydrous tetrahydrofuran (2 ml), to which was added diazabicyclo[5.4.0]undec-7-ene (62.1 mg) and the reaction mixture was stirred under nitrogen atmosphere at ambient temperature for 5 days. The reaction mixture was concentrated under reduced pressure to dryness. The residue was purified by column chromatography on silica gel with chloroform - acetone (3 : 1 ~ 1 : 1) to give dark green needles of **16** (12.9 mg, yield; 69.1% based on consumed **5**) and recovered **5** (7.4 mg). (b) 3α-*iso*-Propoxy-10-*O*-decarbamoylmitomycin D (**4**, 36.0 mg) was dissolved in anhydrous pyridine (0.5 ml), to which was added methanesulfonylchloride (12.0 μl) under nitrogen atmosphere and the reaction mixture was stirred at 0°C for 4 hours. The reaction mixture was poured into saturated aqueous sodium bicarbonate solution while ice cooling and extracted with ethyl acetate (40 ml). The solution was washed with water and dried over anhydrous sodium sulfate and concentrated under reduced pressure to dryness. The residue was purified by column chromatography on silica gel with chloroform - acetone (7 : 3) to give reddish purple solids of 10-*O*-decarbamoyl-3α-*iso*-propoxy-10-*O*-methanesulfonylmitomycin D (29.5 mg, yield; 97.1% based on consumed **4**) and recovered **4** (11.1 mg). 10-*O*-Decarbamoyl-3α-*iso*-propoxy-10-*O*-methanesulfonylmitomycin D (12.4 mg) was dissolved in anhydrous tetrahydrofuran (1 ml), to which was added diazabicyclo[5.4.0]undec-7-ene (8.9 mg) and the reaction mixture was stirred at ambient temperature under nitrogen atmosphere for 18 hours. The reaction

mixture was concentrated under reduced pressure to dryness. The residue was purified by column chromatography on silica gel with chloroform - acetone (4 : 1) to give dark green fine needles of **16** (8.0 mg, yield; 82.7%). 10-*O*-Decarbamoyl-3α-*iso*-propoxy-10-*O*-methanesulfonylmitomycin D: ^{1}H NMR (100 MHz, pyridine-d_5) δ 1.16 (3H, d, J=6.1 Hz), 1.42 (3H, d, J=5.9 Hz), 2.00 (3H, s), 2.54 (1H, d, J=4.4 Hz), 2.61 (1H, d, J=4.4 Hz), 3.36 (3H, s), 4.18 (1H, dd, J=9.5 and 3.2 Hz), 4.66 (1H, heptad, J=6.1 Hz), 5.17 (1H, dd, J=9.5 and 9.3 Hz), 5.41 (1H, dd, J=9.3 and 3.2 Hz), 5.86 (1H, s); EI-MS m/z 427 (M^+), 331 (base), 288, 272, 242, 230, 128. **16**: Dark green fine needles (from chloroform - acetone) mp 208~214°C (dec); IR (KBr) cm^{-1} 3460, 3410, 1603, 1543, 1200, 1144, 1115; ^{1}H NMR (100 MHz, pyridine-d_5) δ 1.28 (3H, d, J=6.3 Hz), 1.46 (3H, d, J=5.9 Hz), 1.93 (3H, s), 2.14 (3H, s), 2.50 (1H, d, J=4.4 Hz), 2.65 (1H, d, J=4.4 Hz), 4.65 (1H, heptad, J=6.1 Hz), 5.76 (1H, d, J=1.1 Hz), 5.97 (1H, s), 6.46 (1H, d, J=1.1 Hz); EI-MS m/z 331 (M^+, base), 288, 272, 260, 242, 230, 128 (base).

Preparation of 3α-*iso*-Propoxymitomycin H (**17**)

9a-*O*-Demethyl-3α-*iso*-propoxymitomycin G (**16**, 4.6 mg) was dissolved in aqueous sodium hydroxide solution (0.1 N, 2 ml) and the reaction mixture was stirred at ambient temperature for 3.3 hours. The pH of the reaction mixture was adjusted to 3.3 by adding dropwise diluted hydrochloric acid. The mixture was extracted with ethyl acetate. The solution was washed with water and dried over anhydrous sodium sulfate and concentrated under reduced pressure to dryness. The residue was dissolved in methanol (5 ml) at 0°C, to which was added excess diazomethane (ether solution) while stirring. The reaction mixture was stirred at ambient temperature for 3 hours and concentrated under reduced pressure to dryness. The residue was purified by column chromatography on silica gel with chloroform - acetone (9 : 1) to give reddish purple solids of **17** (2.7 mg, yield; 56.1%). **17**: Reddish purple fine needles (from *n*-hexane - methylene chloride) mp 177~180°C; IR (KBr) cm^{-1} 1644, 1557, 1080; ^{1}H NMR (pyridine-d_5) δ 1.32 (3H, d, J=6.4 Hz), 1.46 (3H, d, J=5.9 Hz), 1.80 (3H, s), 2.14 (3H, s), 2.56 (1H, d, J=4.2 Hz), 2.71 (1H, d, J=4.2 Hz), 3.93 (3H, s), 4.57 (1H, m), 5.72 (1H, s), 5.91 (1H, d, J=1.0 Hz), 6.59 (1H, d, J=1.0 Hz); EI-MS m/z 346 (M^+), 287, 275, 257, 232, 128 (base), 86.

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