THE CONFIGURATION OF MITIROMYCIN AND ITS DERIVATION FROM MITOMYCIN B

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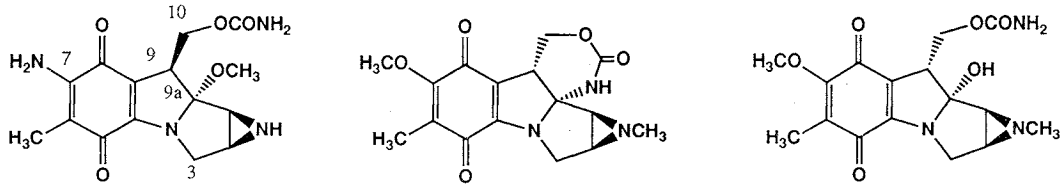
The configuration of mitiromycin (**2**) was first determined by NMR experiments including the NOE technique. The absolute structure of **2** was related to mitomycin B (**3**) because **2** was derived from **3** under basic conditions. The ketonic form **4** was presumed to be involved in the generation of **2**.

The antitumor antibiotic mitomycin family has been represented by mitomycin C (**1**) which has been widely used in clinical chemotherapy.[1] This compound was isolated from the fermentation broth of a *Streptomyces caespitosus* strain,[2] which produces other mitomycins as minor constituents.[3] These naturally occurring mitomycins are ordinarily classified into 3 groups (besides mitiromycin (**2**) produced by *Streptomyces verticillatus*[4,5]) according to the substituents at C-9 as follows: (1) 9β-carbamoyloxymethyl, such as mitomycin A and **1**, (2) 9α-carbamoyloxymethyl, such as mitomycins B (**3**) and D, and (3) 9-exo-methylene, such as mitomycin G. As part of our approach to synthesize mitomycin congeners considered to be "missing links" of these naturally occurring mitomycins, we have been successful with the syntheses of 1a-demethylmitomycins G, K,[6] 9-*epi*-mitomycins B, and D.[7] During these studies we discovered several novel reactions of mitomycins. In the preceding account[8] we described the oxidative alkoxide substitution at C-3 of mitomycins under basic decarbamoylation conditions (2-PrONa - 2-PrOH or MeONa - MeOH-benzene).[9] In addition, we found the formation of **2** under the same decarbamoylation conditions (MeONa - MeOH-benzene)[9] when applied to **3**. Among mitomycins discovered from *Streptomyces*, **2** has an unusual oxazinone ring structure fused at C-9 and C-9a, as elucidated by the early structural study of **2**.[5] Despite the structural interest, the stereochemistry of **2** has not yet been reported to date. However the incorrect configuration of **2** was proposed without evidence in previous articles.[10,11] We clarified the complete structure of **2** on the basis of NMR. This report deals with the derivation and the configuration of **2** and a novel reaction of **3**.

Fig. 1. Structures of mitomycins B, C, and mitiromycin.



Mitomycin C (**1**) Mitiromycin (**2**) Mitomycin B (**3**)

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Table 1. ^{13}C NMR ($CDCl_3$) chemical shifts of mitiromycin (**2**) and mitomycin B (**3**).

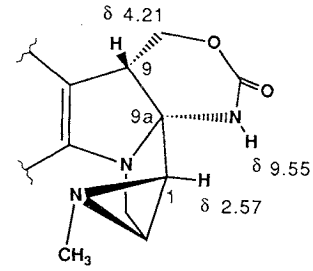
	2	3		2	3
1	44.8 (d)	43.9 (d)	7	157.6 (s)	157.6 (s)
1a-NCH_3	43.8 (q)	44.0 (q)	7-OCH_3	61.2 (q)	61.3 (q)
2	46.9 (d)	49.4 (d)	8	178.2 (s)	178.2 (s)
3	48.3 (t)	48.9 (t)	8a	113.0 (s)	114.7 (s)
4a	152.0 (s)	151.6 (s)	9	40.9 (d)	45.1 (d)
5	182.2 (s)	182.9 (s)	10	65.6 (t)	61.2 (t)
6	124.7 (s)	123.9 (s)	CON	157.0 (s)	157.0 (s)
6-CH_3	8.2 (q)	8.2 (q)	9a	86.6 (s)	100.7 (s)

^{13}C NMR spectra of **2** and **3** were recorded at 100.7 MHz.

^{13}C chemical shifts are from TMS signal. Multiplicities were determined by DEPT experiments. Assignments of the signals were done by LSPD experiments.

Decarbamoylation of **3** was the essential step for the syntheses of 9-*epi*-mitomycins B and D,[7] hence we used this established method[9] to obtain 10-*O*-decarbamoylmitomycin B from **3** during the course of the study. Once, for reasons undetermined, reaction of **3** under normal decarbamoylation conditions (MeONa - MeOH-benzene)[9] resulted in decomposition of almost all mitomycins. However we succeeded in isolating dark purple prisms (72 mg) crystallized in $CHCl_3$ - MeOH (mp 83~88°C) from the reaction mixture which had the molecular formula $C_{16}H_{17}N_3O_5$ based on HREI-MS (calcd for $C_{16}H_{17}N_3O_5$ *m/z* 331.1166, found *m/z* 331.1159). The ^1H NMR ($CDCl_3$) and EI-MS spectra were consistent with those of **2**[5] (the mp was different from the reported 124~126°C, suggesting a difference of crystal structure). Thus, the compound was identified as mitiromycin (**2**). Since no trace of **2** was found by HPLC in the starting lot of **3**, **2** was uncontroversially generated from **3**.

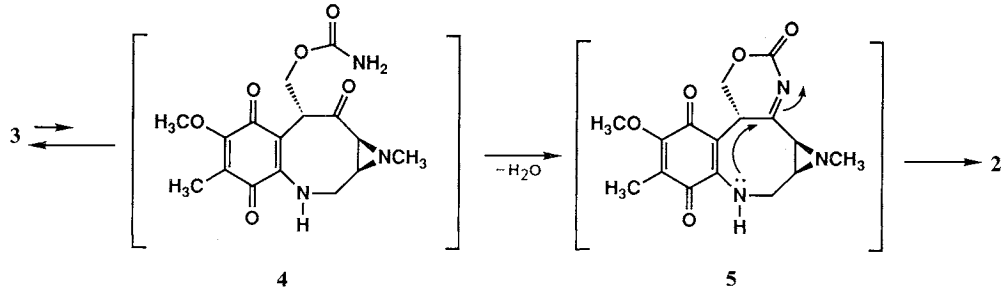
Fig. 2. NOE effects in pyridine-d_5.



Irradiation	NOE effects	
1-H	CONH-H	2.4%
	9-H	3.1%
CONH-H	1-H	6.9%
9-H	1-H	3.2%

Our search for the unreported configuration of **2** initially focused on the ^{13}C NMR data. Comparison of ^{13}C NMR resonances for C-1 through C-3 around the aziridine of **2** with those of **3** (Table 1) showed excellent correspondence, supporting the preservation of the stereochemistry around the aziridine ring. Concerning C-9, it was suggested that the stereochemistry was in accordance with the starting compound of **3**. In consideration of the viable epimerization at C-9 under basic conditions,[7] further NOE experiments (in pyridine-d_5) were needed to clarify the stereochemistry at C-9 and C-9a. The relative configuration between 1-H and the aminocarbonyloxy moiety was determined to be *cis* by the significant NOE between 1-H and 9a-NH (Fig. 2). The connectivity between the oxazinone ring moiety and the dihydroindoloquinone partial structure at C-9 and C-9a was then shown to be *cis* by the presence of meaningful NOE between 1-H and 9-H (Fig. 2). Thus these results of NOE showed unambiguously the complete configuration of **2**. Later the X-ray crystallographic study confirmed the relative structure of **2**.[12] The absolute structure of mitomycin B (**3**) had been confused by inadequate X-ray analyses[13] before it was revised by a new discreet X-ray analysis[14] (concerning the absolute configuration of mitomycin C (**1**), there had been also

Fig. 3. Proposed mechanism of generation of mitiromycin.



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confused before it was revised by a X-ray analysis[15]). Therefore, the absolute structure of **2** was determined according to the revised structure of **3**.

Under basic conditions, it was suggested that 8 membered ketonic form **4** interchanged with 9a-hydroxymitomycin, resulting in epimerization at C-9 and exo-methylene introduction at C-9.[16,17] Similarly the production of **2** might be interpreted by a ketonic form **4** generated by a base, followed by cyclodehydration to afford the cyclic imine (**5**) (Fig. 3). This precursor **5** having a 8 membered ring would be interchanged to the mitosane skeleton through the *trans*-annulation reaction.

The profile of biological activity of **2** makes it important to develop an analog of **1** by modification of the 9a functional group. While the preliminary antimicrobial effects were weak compared to **1**, the program to study the potency of **2** against several tumor lines is now in progress.

Experimental

MP's were recorded on a Yanagimoto melting point apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Bruker AM-400 spectrometer. MS spectra were recorded on a JMS-01SG-2 spectrometer. IR spectra were recorded on a Shimadzu IR-27-G spectrometer. Electronic spectra were recorded on a Hitachi 200-20 spectrophotometer.

Preparation of Mitiromycin (**2**)

Small pieces of sodium (29.1 g) were dissolved in anhydrous methanol (485 ml) while ice cooling under nitrogen atmosphere, to which was added anhydrous benzene (445 ml) and mitomycin B (**3**, 9.27 g) in small portions. The reaction mixture was stirred under nitrogen atmosphere at ambient temperature for 48 hours. Then excess amount of dry ice was added to the reaction mixture and the resultant copious precipitate was separated by filtration on Celite. The filtrate was concentrated under reduced pressure to dryness. The residue was purified by repeating column chromatography; (1) silica gel with chloroform - methanol (4 : 1), (2) silica gel with chloroform - methanol (98 : 2 ~ 95 : 5), (3) silica gel with ethyl acetate - methanol (97 : 3). The obtained reddish purple paste was purified by preparative TLC on silica gel with ethyl acetate - methanol (97 : 3) to give a reddish purple paste of **2** (91.4 mg). The paste was further purified by column chromatography on neutral silica gel with chloroform - methanol (93 : 7) to give dark purple prisms of **2** (72.0 mg). **2**: MP 83 ~ 88°C; EI-MS *m/z* 331 (M^+, base peak), 316, 257, 243, 70; IR (KBr) cm^{-1} 3270, 2960, 1720, 1654, 1629, 1578, 1440, 1210, 1097, 753; UV-Vis λ_{max}^{MeOH} nm (log ε) 227 (3.95), 322 (3.88), 530 (3.01); ^1H NMR (400 MHz, $CDCl_3$) δ 1.81 (3H, s), 2.29 (1H, d, J=4.6 Hz), 2.31 (3H, s), 2.42 (1H, dd, J=4.6 and 2.0 Hz), 3.43 (1H, dd, J=13.0 and 2.0 Hz), 3.92 (1H, d, J=13.0 Hz), 3.98 (1H, dd, J=4.1 and 2.4 Hz), 4.06 (3H, s), 4.48 (1H, dd, J=11.3 and 4.1 Hz), 4.78 (1H, dd, J=11.3 and 2.4 Hz), 6.36 (1H, s), (400 MHz, pyridine-d_5) δ 1.80 (3H, s), 2.25 (3H, s), 2.42 (1H, dd, J=4.6 and 2.0 Hz), 2.57 (1H, d, J=4.6 Hz), 3.39 (1H, dd, J=12.8 and 2.0 Hz), 4.00 (3H, s), 4.08 (1H, d, J=12.8 Hz), 4.21 (1H, dd, J=4.0 and 2.1 Hz), 4.65 (1H, dd, J=11.2 and 4.0 Hz), 5.01 (1H, dd, J=11.2 and 2.1 Hz), 9.55 (1H, s).

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