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Absolute Configuration of Kigamicins A, C and D

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Abstract The stereochemistry of kigamicins A (**1**), C (**2**) and D (**3**) were elucidated by a combination of X-ray crystallographic analysis and degradation studies. The absolute structures of kigamicins thus determined were depicted as shown in Fig. 2.

Keywords kigamicin, natural products, antitumor antibiotics, absolute configuratioin, X-ray crystallography

Introduction

In the course of screening for new antitumor antibiotics, we have isolated five new antibiotics, kigamicins [1, 2], from the culture broth of *Amycolatopsis* sp. ML630-mF1 by their selective killing activities against PANC-1 cells only under a nutrient starvation condition. Among them, kigamicin D, the major compound in the cultured broth showed antitumor activities [1, 3] *in vitro* and *in vivo*. Kigamicins also showed antimicrobial activities against Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA). The planar structures of kigamicins were elucidated by NMR and MS spectral analyses [2]. The structures of kigamicins were found to be composed of an aglycon of fused octacyclic ring and deoxy sugars. However, the relative and absolute configuration of kigamicins has not been determined based on the NMR studies alone due to the lack of NOE information. In this paper, we describe the absolute structures of kigamicins A, C and D determined by NMR analysis, chemical degradation studies and X-ray crystallographic analyses.

Results and Discussion

Determination of stereochemistry was conducted at first for kigamicin A (**1**), because other members of the antibiotics could not be crystallized in all solvents so far used. Compound **1** was crystallized from hot MeOH/H_2O to give yellow plate crystals. The relative stereochemistry of **1** was thus determined by X-ray analysis as shown in Fig. 1.

In order to determine the absolute structure of **1**, the configuration of amicetose was examined by measuring its optical rotation value after hydrolysis of **1** as shown in Scheme 1. Treatment of **1** with 1 N HCl in THF at room temperature for 18 hours gave an aglycon (**4**) in 76% yield and amicetose (**5**) in 90% yield. The aglycon part was proved to be identical with those derived from the other kigamicins in all spectroscopic properties. The optical rotation value of **5** was $[\alpha]_D^{22}$ +42.5° (*c* 0.7, Me_2CO), which is identical to the reported value of D-amicetose; $[\alpha]_D^{22}$ +43.6° (*c* 1.0, Me_2CO) [4, 5]. Therefore, amicetose (**5**) in **1** was determined to be D-form. Taking the configuration of amicetose into consideration, the absolute stereochemistry of **1** was determined as shown in Fig. 2 having 12*S*, 14*R*, 15*S*, 20*R*, 26*R* configurations as an aglycon. In addition, the coupling constant of anomeric proton (J=2.0, 9.0 Hz) [2] in **1** indicated the presence of β-

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D amicetoside, which is consistent with the results obtained by X-ray analysis.

As reported in a previous paper [2], kigamicin D contained one amicetose and two oleandrose moieties. Since there are discrepancies between the reported optical rotation values of oleandrose [6～8], and since the complete separation of amicetose and oleandrose in the hydrolysate of kigamicin D was difficult, we attempted to obtain di- or tri-saccharides containing amicetose and oleandrose as crystals. As shown in Scheme 2, mild acid hydrolysis of **3** yielded amicetose, oleandrose, disaccharide (**6**) and trisaccharide (**7**) as well as aglycon (**4**), kigamicin A (**1**) and kigamicin C (**2**). This result indicated that the absolute configurations of aglycon and amicetose moieties in **1**, **2** and **3** were identical. Compounds **6** and **7** were crystallized from EtOAc/*n*-hexane and ether/*n*-hexane to give colorless crystals with melting point of 133～136°C and 161～163°C, respectively. The X-ray structural analysis of **7** exhibited the presence of anomeric mixture (α-anomer : β-anomer=55 : 45). Fig. 3 shows the ORTEP drawing of **7** (α-anomer) by a single crystal X-ray analysis. Since the absolute configuration of amicetose had been determined to be D, two oleandrose moieties were established to be both D-forms. On the basis of the above observation, the absolute structure of kigamicin D (**3**) was depicted as shown in Fig. 2 having 12*S*, 14*R*, 15*S*, 20*R*, 26*R* configurations as an aglycon and D-amicetose and D-oleandrose as deoxy sugar moieties. Coincidentally, the

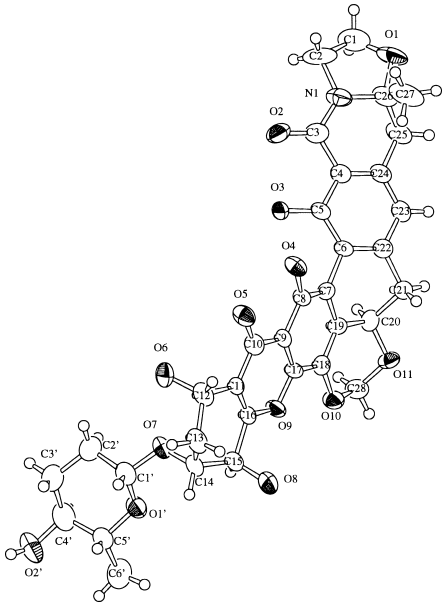


Fig. 1 X-ray crystal structure of kigamicin A.

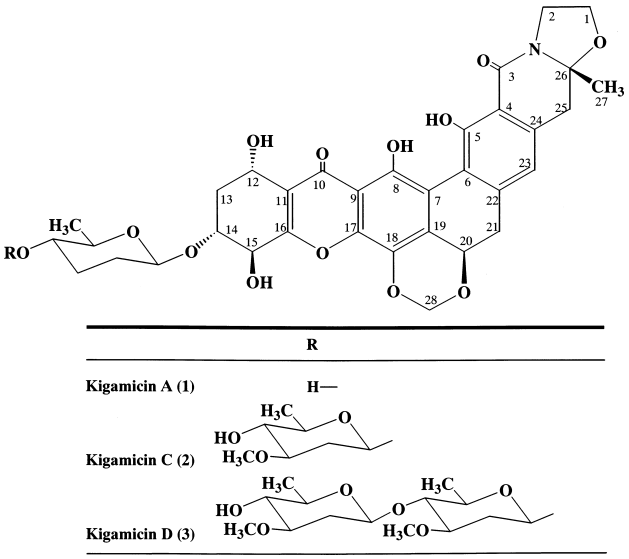
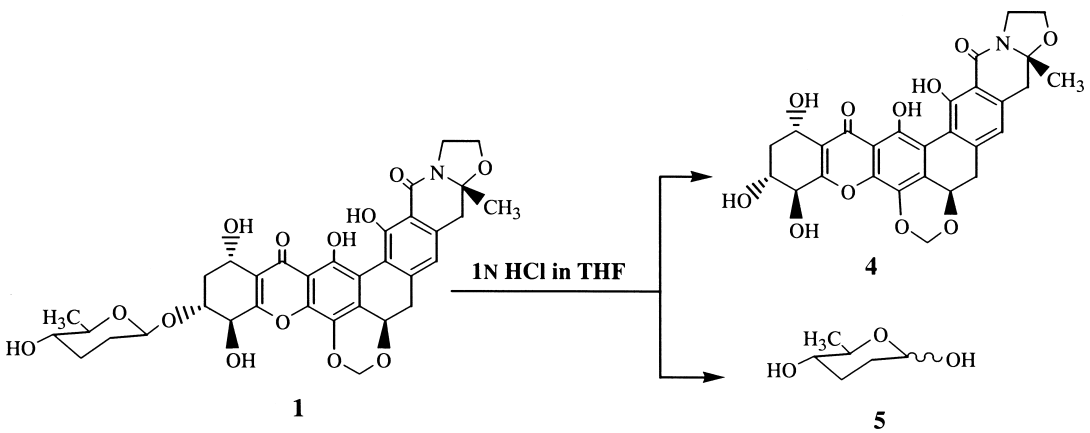
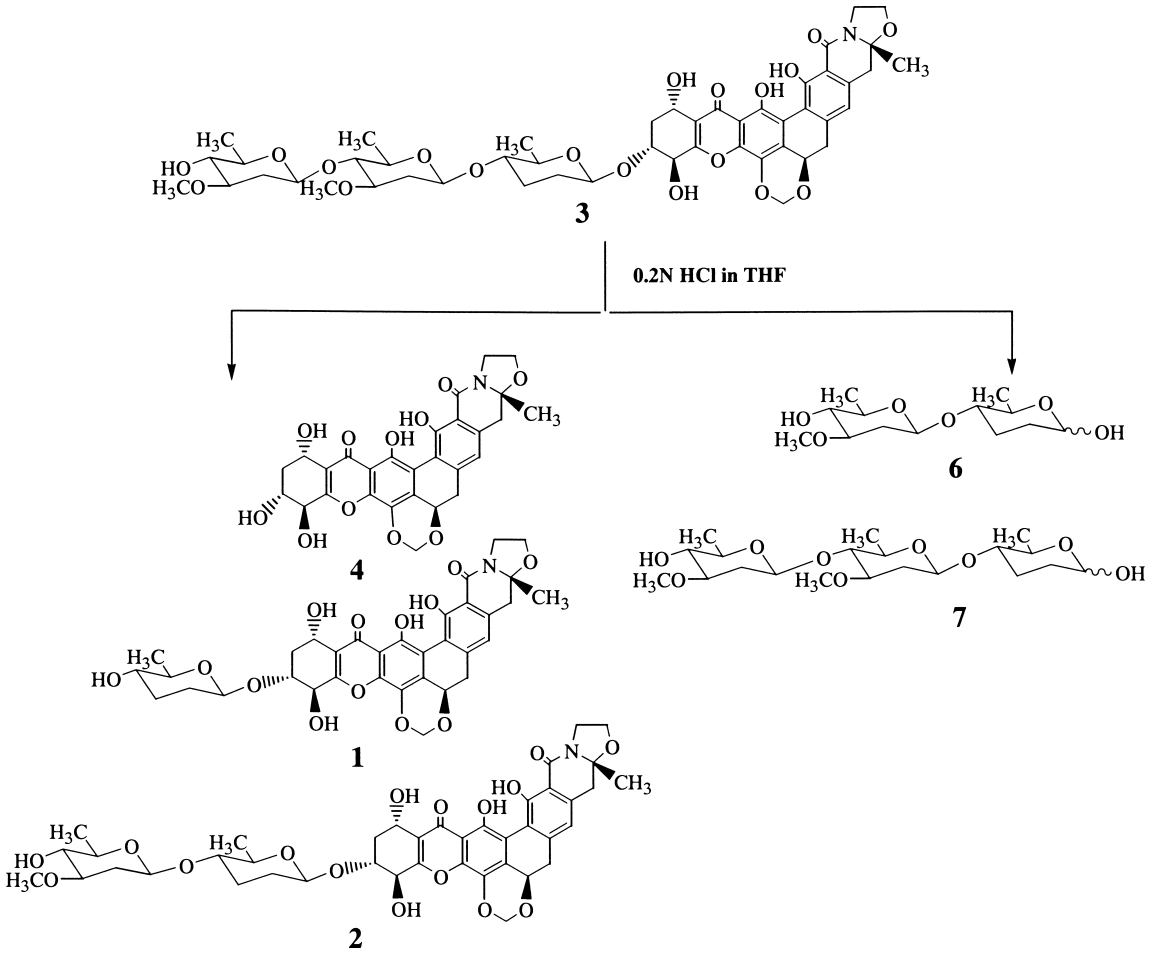


Fig. 2 Structures of kigamicins A (**1**), C (**2**) and D (**3**).



Scheme 1



Scheme 2

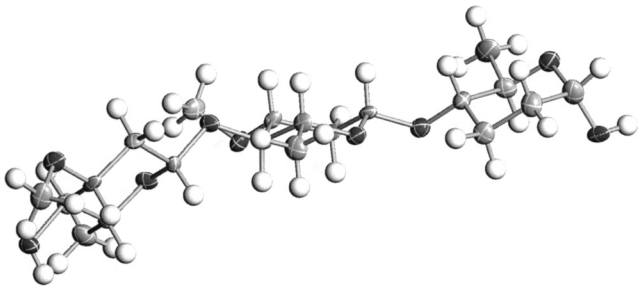


Fig. 3 X-ray crystal structure of trisaccharide (**7**).

absolute structure of kigamicin C (**2**) could be determined as shown in Fig. 2.

As described in this paper, the absolute structures of kigamicins A, C and D were determined. The structures of kigamicins are unique in that mono-, di-, tri- and tetrasaccharide moieties are attached to the polycyclic xanthone moiety. Other members of this family include cervinomycin [9], actinoplanones [10], LL-E19085α [11], LL-D42067 [12], BE-13793X [13], MS 901809 [14] and FD-594 [15]. Among them, MS 901809, FD-594 and BE-13793X were glycosides. Kondo *et al.* [15] reported an attractive biosynthetic pathway of FD-594 and MS901809, in which the glycosidic position of both compounds are C-13 and C-15, respectively. They postulated that the same benzo[*a*]naphthacenequinone chromophore may be derived at an early stage. Then, Baeyer-Villiger type oxidation occurs at a quinone carbonyl group. After the production of ring-opened intermediate, recyclization via different hydroxyl group results in two structurally related compounds. Kigamicins may be biosynthesized in the same manner. However, it is noteworthy from the viewpoint of biosynthesis that the glycosidic position of kigamicins is C-14 instead of C-15 and C-13. Although the limited supply of kigamicin B and E prevented the determination of their stereochemistry, the absolute configuration of both compounds may be identical with kigamicin D due to the kigamicin biosynthesis.

Up to now, there are few X-ray crystallographic data on

polycyclic xanthones due to the difficulty in obtaining suitable single crystals for X-ray analysis [9] and there are very few polycyclic xanthones whose absolute structures have been determined. Fortunately, we could obtain single crystals of **1** and using them could successfully carry out the absolute structure determination of kigamicins.

Further biological evaluation of kigamicins is in progress.

Experimental

General

Melting points were determined with a Yanagimoto micro melting point apparatus. UV spectra were recorded on a Hitachi U-3210 spectrometer. IR spectra were recorded on a HORIBA FT-210 fourier transform infrared spectrometer. HRESI-MS spectra were recorded on a JEOL JMS-T100LC spectrometer. NMR spectra were recorded on a JEOL JNM-A400 spectrometer using TMS as an internal reference. Optical rotations were measured with a Perkin-Elmer 241 polarimeter.

Preparation of D-Amicetose (5)

Kigamicin A (81.1 mg, 0.122 mmol) in THF (2.5 ml) and 1 N-HCl (1.0 ml) was stirred at room temperature for 18 hours. After removal of THF by evaporation, the residue was dissolved with water (30 ml) and ethyl acetate (30 ml) and shaken vigorously. The aqueous layer was neutralized with Ag_2CO_3. The resulting precipitate was filtered off and the filtrate was concentrated *in vacuo* to afford oily material. This material was subjected to silica gel column chromatography using $CHCl_3/Me_2CO=1/1$ as an eluent. The fractions showing positive color reaction to anisaldehyde-H_2SO_4 at Rf 0.33 ($CHCl_3/Me_2CO=1/1$) on a TLC were collected to afford 14.5 mg (90% yield) of colorless syrup. HRESI-MS *m/z* found 155.0701 $(M+Na)^+$, calcd for $C_6H_{12}O_3Na$ 155.0684: $[\alpha]_D^{22}$ +42.5° (*c* 0.7, Me_2CO), lit. [4]; $[\alpha]_D^{22}$ +43.6° (*c* 1.2, Me_2CO): TLC (silica gel) Rf 0.33 (solvent system; $CHCl_3/Me_2CO=1/1$).

Preparation of Aglycon (4)

The organic layer above-mentioned was concentrated *in vacuo* and subjected to Sephadex LH-20 column chromatography using $MeOH/CHCl_3=5/1$ as an eluent. Fractions containing **4** were collected and evaporated *in vacuo* to afford 51 mg (76%) of yellowish powders. Yellow crystals were obtained from $MeOH/H_2O$. mp 230～235°C (dec.): $[\alpha]_D^{24}$ −438° (*c* 0.3, $CHCl_3$): UV λ_{max} (MeOH) nm (ε) 219 (32,900), 253 (32,200), 355 (14,300): IR ν_{max} cm^{-1} (KBr) 3495, 2885, 1645, 1610, 1465, 1440, 1275, 1200, 1090: HRESI-MS *m/z* found 574.1336 $(M+Na)^+$, calcd for $C_{28}H_{25}NO_{11}Na$ 574.1325: ^{13}C NMR ($CDCl_3/CD_3OD=2/1$) 182.8 (C-10), 164.6 (C-3), 163.1 (C-16), 158.1 (C-5), 149.8 (C-8), 143.9 (C-17), 140.5 (C-22), 135.7 (C-24), 130.6 (C-18), 129.8 (C-19), 118.7 (C-11), 118.5 (C-23), 117.8 (C-4), 111.7 (C-9), 110.4 (C-6), 110.0 (C-7), 92.1 (C-26), 90.9 (C-28), 72.6 (C-20), 70.1 (C-14), 69.7 (C-15), 64.0 (C-1), 61.6 (C-12), 41.6 (C-2), 39.9 (C-25), 36.0 (C-21), 32.2 (C-13), 22.0 (C-27).

Preparation of Disaccharide (6)

Kigamicin D (53.3 mg, 0.056 mmol) in THF (1.25 ml) and 0.2 N-HCl (0.5 ml) was stirred at room temperature for 20 hours. After removal of THF by evaporation, the residue was dissolved with water (20 ml) and ethyl acetate (20 ml) and the mixture was shaken vigorously. The aqueous layer was neutralized with Ag_2CO_3. The resulting precipitates were filtered off and the filtrate was concentrated *in vacuo* to afford 12 mg of crude powder. This material was subjected to silica gel column chromatography using $CHCl_3/Me_2CO=1/1$ as an eluent. The fractions showing positive color reaction to anisaldehyde-H_2SO_4 at Rf 0.50 ($CHCl_3/Me_2CO=1/1$) on a TLC were collected and concentrated to afford 3.1 mg of white powders. Colorless needles were obtained from EtOAc/*n*-hexane. mp 133～136°C: $[\alpha]_D^{22}$ +32.5° (*c* 0.2, Me_2CO): HRESI-MS *m/z* found 299.1464 $(M+Na)^+$, calcd for $C_{13}H_{24}O_6Na$ 299.1471.

Preparation of Trisaccharide (7)

Kigamicin D (100 mg) in THF (2.5 ml) and 0.2 N-HCl (0.5 ml) was stirred at room temperature for 48 hours. After removal of THF by evaporation, the residue was dissolved with water (40 ml) and ethyl acetate (40 ml). The aqueous layer was neutralized with Ag_2CO_3. The resulting precipitates were filtered off and the filtrate was concentrated *in vacuo* to afford 20 mg of crude powder. This material was subjected to silica gel column chromatography using toluene/$Me_2CO=3/2$ as an eluent. The fractions showing positive color reaction to anisaldehyde-H_2SO_4 at Rf 0.28 (toluene/$Me_2CO=3/2$) on a TLC were collected and concentrated to afford 11 mg of white powders. Colorless needles were obtained from ether/n-hexane. mp 161～163°C: $[\alpha]_D^{22}$ +6.2° (*c* 0.2, Me_2CO): HRESI-MS *m/z* found 443.2244 $(M+Na)^+$, calcd for $C_{20}H_{36}O_9Na$ 443.2257.

X-Ray Structure Analysis of 1

Crystals of **1** were obtained from a hot MeOH/H_2O solution. A yellow plate crystal of 0.01×0.15×0.30 mm was mounted on a glass fiber. All measurements were made on a Rigaku AFC7R diffractometer with graphite monochromated Cu-Kα radiation. Crystal data: Empirical formula; $C_{34}H_{35}NO_{13}$, Formula weight; 665.65, Crystal system; orthorhombic, Space group; $P2_12_12_1$, Lattice parameters; a=12.097(2) Å, b=32.337(3) Å, c=8.053(2) Å, Volume; 31501(1) Å^3, Z value; 4, D_{calc}; 1.403 g/cm^3, μ(CuKα); 9.2 cm^{-1}, T; 293 K. The structure was solved by a direct method (SIR92). Final R and wR were 0.06 and 0.157 for 2572 observed reflections, respectively.

X-Ray Structure Analysis of 7

A colorless needle crystal of **7** (0.46×0.11×0.07 mm) was mounted in a loop. All measurements were made on a Bruker SMART APEX diffractometer with graphite monochromated Cu-Kα radiation. Crystal data: Empirical formula; $C_{20}H_{36}O_9$, Formula weight; 420.49, Crystal system; monoclinic, Space group; C2, Lattice parameters; a=60.606(6) Å, b=5.0208(5) Å, c=14.9319(14) Å, β=101.659(4)°, Volume; 4449.9(7) Å^3, Z value; 8, D_{calc}; 1.255 g/cm^3, (CuKα); 0.819 mm^{-1}. The reflection data were collected at 90 K using the ω scans. The structure was solved by a direct method (SHELXS-97). Final R and wR were 0.088 and 0.2163 for 5771 observed reflections, respectively.

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