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Kigamicins, Novel Antitumor Antibiotics

II. Structure Determination

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Kigamicin A (**1**), B (**2**), C (**3**), D (**4**) and E (**5**) are novel antitumor antibiotics. Their structures were determined by spectroscopic analyses including various NMR measurements. Kigamicins have a unique aglycone of fused octacyclic ring system containing seven of six-membered rings and one oxazolidine. The aglycone links a sugar chain composed of one to four deoxysugars. These sugars were found to be amicetose and oleandrose.

Kigamicins have been isolated from a culture broth of *Amicolatopsis* sp. ML630-mF1 as a result of their selective killing activity against PANC-1 cells only under a nutrient starvation condition[1]. They have antimicrobial activity against Gram-positive bacteria including Methicillin resistant *Staphylococcus aureus* (MRSA) and *in vitro* antitumor activity[1]. Kigamicin D showed good *in vivo* therapeutic activity against PANC-1 cells implanted into nude mice by subcutaneous or oral administration[2]. We herein describe the structure determination of kigamicins.

Results and Discussion

The structural studies were carried out first for kigamicin D (**4**), the major component of the antibiotics. The structures of other components were subsequently determined by comparing their spectral data with those of **4**.

Structure Determination of Kigamicin D (4)

The molecular formula of kigamicin D (**4**) was established as $C_{48}H_{59}NO_{19}$ (MW953) on the basis of HRESI-MS [*m/z*, found 976.3531 $(M+Na)^+$, calcd. 976.3579] and NMR data (Tables 1 and 2). The UV spectrum of **4** showed characteristic absorption maxima at 227, 253, 306 and 384 nm suggesting the presence of a polycyclic xanthone chromophore[3]. IR absorption bands implied the presence of hydroxyl (~3450, 1062 cm^{-1}), conjugated carbonyl (1650 cm^{-1}, broad sh.) and γ-pyrone (1620 cm^{-1}) functions in the molecule.

The ^{13}C NMR, DEPT and HMQC spectra of **4** in $CDCl_3$ revealed the presence of 48 carbon signals consisting of six methyl, ten methylene, sixteen methine and sixteen quaternary carbons. The ^1H NMR spectrum indicated the presence of five deuterium exchangeable protons (δ 2.45, 4.53, 5.22, 12.58 and 13.01). The signals at δ 12.58 and 13.01 were deduced to be chelated phenolic protons based on their chemical shifts and a bathochromic shift in alkaline solution in the UV spectrum. The ^1H-^1H COSY spectrum revealed the seven spin systems; from 1-H_2 to 2-H_2, 12-H to 15-H, 20-H to 21-H_2, 28H*ax* to 28-H*eq*, 1′-H to 6′-H_3,1″-H to 6″-H_3 and 1‴-H to 6‴-H_3. Long-range couplings were observed between 21-H_2 and 23-H, and between 23-H and 25-H_2, and thus the spin systems could be extended from 20-H to 25-H_2. The spin system corresponding to 1′-H to 6′-H_3 confirmed the presence of a 2,3,6-trideoxyhexose (amicetose) moiety. Other two spin systems corresponding to 1″-H to 6″-H_3 and 1‴-H to 6‴-H_3 confirmed the presence

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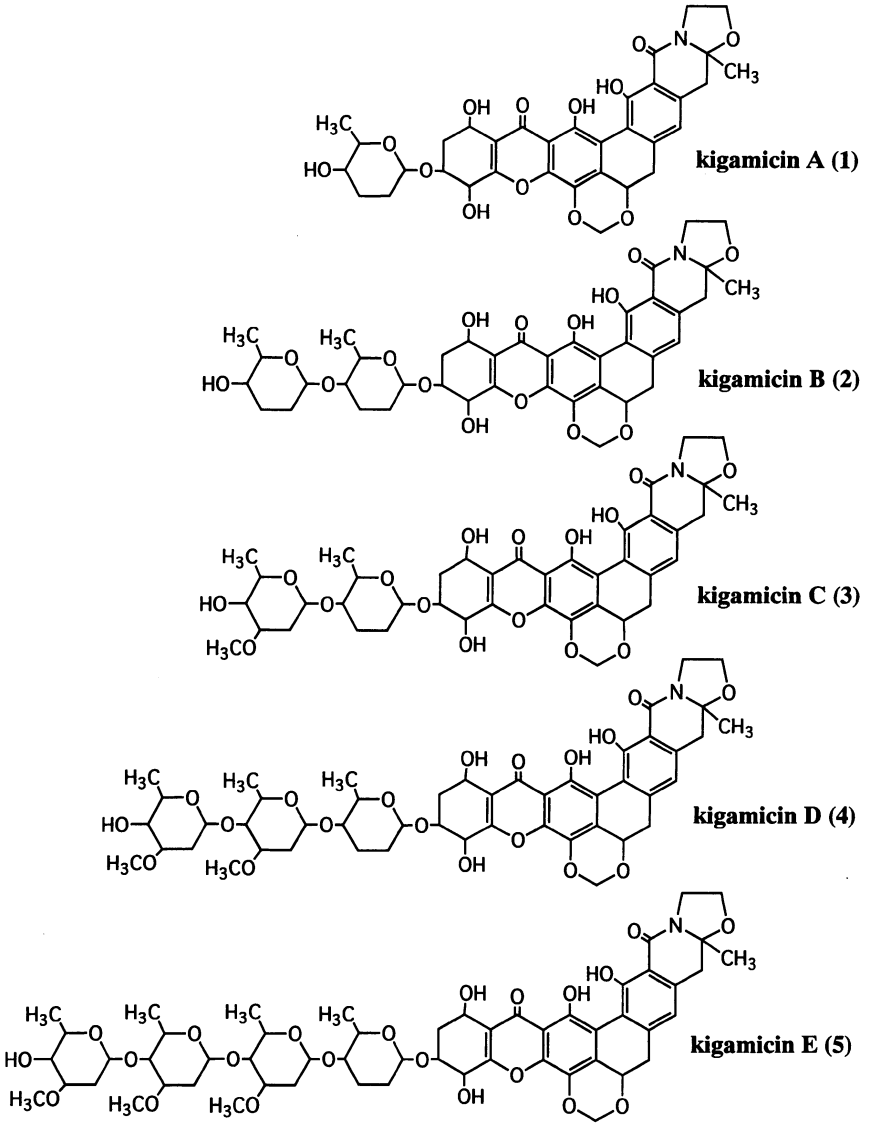
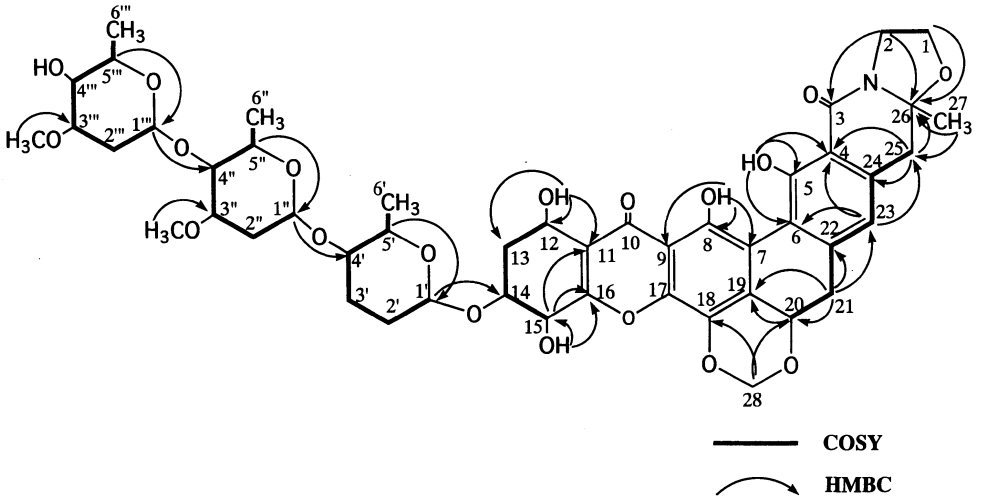
Fig. 1. Structures of kigamicins.



of two 2,6-dideoxyhexose (oleandrose) moieties from the analyses of ^{1}H-^{1}H COSY and HMQC-TOCSY spectra. The analysis of HMBC spectrum of **4** revealed the connectivity between proton signals of the above described spin systems (*i.e.* from 1-H to 27-H_3) and quaternary carbons, and thus confirmed the aglycone moiety (Fig. 2). The aglycone moiety was found to be similar to albofungin type compound. However, a five-membered hetero ring in **4** was replaced by the six-membered hetero ring in albofungin type compound. This five-membered ring was further supported by the ^{1}H-^{15}N HMBC spectrum of **4** showing correlation between ^{15}N signal at δ 130.6 and following proton signals; 1-methylene (δ 4.23), 25-methylene (δ 3.08) and 27-methyl (δ 1.38), respectively. The same situation of five-membered ring was reported in the structure of cervinomycin A_2[4] Long-range couplings from 1′-H to C-14, that from 1″-H to C-4′ and that from 1‴-H to C-4″ in the HMBC spectrum confirmed the position of glycosidic linkage. Further evidence supporting the glycosidic linkage was provided by the mutual NOE observation between 14-H and the anomeric proton 1′-H of amicetose. NOE cross peaks were also observed between 1″-H and 4′-H, and between 1‴-H and 4″-H. Thus, the structure of **4** was determined as shown in Fig. 1. The NMR data of **4** and other kigamicins are shown in Tables 1 and 2.

Fig. 2. ^{1}H-^{1}H COSY and HMBC correlations of kigamicin D (**4**).



Structure of Kigamicin A (**1**)

The molecular formula of kigamaicin A (**1**) was elucidated as $C_{34}H_{35}NO_{13}$ (MW 665) from the HRESI-MS [*m/z*, found 688.2055 $(M+Na)^+$, calcd. 688.2006] and NMR data. The UV and IR spectra of **1** were closely similar to those of **4**. The ^{1}H and ^{13}C NMR spectral data of **1** indicated the presence of common aglycone moieties in **1** and **4**, and the presence of one amicetose moiety in **1**. An anomeric proton (δ 4.63) observed as double of doublets (J=9 and 2 Hz) was coupled to C-14 of aglycone moiety in the HMBC spectrum. Thus, the structure of **1** was determined as shown in Fig. 1.

Structure of Kigamicin B (**2**)

The molecular formula of **2** was determined to be $C_{40}H_{45}NO_{15}$ (MW 779) from the HRESI-MS [*m/z*, found 802.2647 $(M+Na)^+$, calcd. 802.2687] and NMR data. The UV and IR spectra of **2** were very similar to those of **4**. The ^{1}H and ^{13}C NMR data of **2** indicated that the aglycone moiety of **2** was the same as that of **4**. Just like **1** and **4**, the difference between **2** and **4** exists in the sugar moieties. In the HMBC spectrum of **2**, 1′-H of amicetose moiety was coupled to C-14 of aglycone moiety and 1″-H of another amicetose moiety was coupled to C-4′. Thus the structure of **2** was determined as shown in Fig. 1.

Structure of Kigamicin C (**3**)

The molecular formula of **3** was determined to be $C_{41}H_{47}NO_{16}$ (MW 809) from the HRESI-MS [*m/z*, found 832.2763 $(M+Na)^+$, calcd. 832.2793] and NMR data. The UV and IR spectra of **3** were also very similar to those of **4**. The ^{1}H and ^{13}C NMR data indicated the presence of common aglycone moieties in **3** and **4**, and the presence of one amicetose and one oleandrose moieties in **3**. In the HMBC spectrum of **3**, an anomeric proton of amicetose moiety was coupled to C-14 and an anomeric proton of oleandrose moiety was coupled to C-4′ of amicetose moiety. Thus, the structure of **3** is proposed as shown in Fig. 1.

Structure of Kigamicin E (**5**)

The molecular formula of **5** was determined to be $C_{55}H_{71}NO_{22}$ (MW 1097) from the HRESI-MS [*m/z*, found 1120.4357 $(M+Na)^+$, calcd. 1120.4365] and NMR data. The UV and IR spectra of **5** were also very similar to those of **4**. The ^{1}H and ^{13}C NMR data of **4** indicated the presence of common aglycone moieties in **4** and **5**, and the presence of one amicetose and three oleandrose moieties in **5**. In the ^{1}H NMR spectrum, proton signals were very difficult to assign due to the severe overlapping in oleandrose moieties. The HMBC spectrum of **5** revealed that an anomeric proton of amicetose moiety was coupled to C-14 of aglycone moiety. Other anomeric protons in three oleandrose

Table 1. ^{1}H NMR data of kigamicins.

Position	δ (multiplicity, J (Hz)) A	B	C	D	E
1 CH_2	4.23 (t,7.0)	4.23 (t, 7.0)	4.23 (t, 7.0)	4.23 (t, 7.0)	4.23 (t, 7.0)
2 CH_2	3.68 (dt, 7.0, 11.0)	3.68 (dt, 7.0, 11.0)	3.69 (dt, 7.0, 11.0)	3.68 (dt, 7.0,11.0)	3.68 (dt, 7.0,11.0)
	4.00 (dt, 7.0, 11.0)	4.00 (dt, 7.0, 11.0)	4.00 (dt, 7.0, 11.0)	4.00 (dt, 7.0, 11.0)	4.00 (dt, 7.0, 11.0)
12 CH	5.10 (m)	5.11 (m)	5.11 (m)	5.10 (m)	5.10 (m)
13 CH_2	1.95 (dt, 10.0, 12.0)	1.94 (m)	1.94 (dt, 10.0, 12.0)	1.93 (m)	1.94 (m)
	2.50 (m)	2.50 (m)	2.49 (m)	2.50 (m)	2.50 (m)
14 CH	3.79 (ddd, 3.0, 8.0, 12.0)	3.76 (ddd, 3.0, 8.0, 12.0)	3.77 (ddd, 3.0, 8.0, 12.0)	3.77 (m)	3.77 (m)
15 CH	4.72 (dd, 1.0, 8.0)	4.72 (dd, 1.0, 8.0)	4.72 (dd, 1.0, 8.0)	4.71 (dd, 1.0, 8.0)	4.71 (m)
20 CH	4.84 (dd, 5.0, 13.5)	4.84 (dd, 5.0, 13.0)	4.84 (dd,5.0, 13.0)	4.84 (dd, 4.5, 13.5)	4.84 (dd, 5.0, 13.5)
21 CH_2	2.83 (t, 13.5)	2.83 (t, 13.0)	2.83 (t, 13.0)	2.83 (t, 13.5)	2.83 (t, 13.5)
	3.08 (dd, 5.0, 13.5)	3.08 (dd,5.0, 13.0)	3.08 (dd, 5.0, 13.0)	3.07 (m)	3.07 (dd, 5.0, 13.5)
23 CH	6.67 (s)	6.67 (s)	6.67 (s)	6.67 (s)	6.67 (s)
25 CH_2	3.09 (d, 15.0)	3.10 (d, 15.0)	3.12 (d, 15.0)	3.08 (d, 15.0)	3.08 (d, 15.0)
	3.16 (d, 15.0)	3.17 (d, 15.0)	3.18 (d, 15.0)	3.16 (d, 15.0)	3.16 (d, 15.0)
27 CH_3	1.38 (s)	1.38 (s)	1.38 (s)	1.38 (s)	1.38 (s)
28 CH_2	5.32 (d, 6.0)	5.32 (d, 6.0)	5.32 (d, 6.0)	5.32 (d, 6.0)	5.32 (d, 6.0)
	5.60 (d, 6.0)	5.61 (d, 6.0)	5.60(d, 6.0)	5.60 (d, 6.0)	5.60 (d, 6.0)
5 OH	13.00 (s)	13.01 (s)	13.00 (s)	13.01 (s)	13.01 (s)
8 OH	12.58 (s)	12.58 (s)	12.58 (s)	12.58 (s)	12.58 (s)
12 OH	4.53 (s)	4.54 (br s)	4.54 (s)	4.53 (d, ≦1)	4.54 (br s)
15 OH	5.26 (s)	5.27 (br s)	5.24 (s)	5.22 (br s)	5.23 (br s)
1' CH	4.63 (dd, 2.0, 9.0)	4.60 (dd, 2.0, 9.0)	4.61 (dd, 2.0, 9.0)	4.60 (dd, 2.0, 9.0)	4.60 (dd, 2.0, 9.0)
2' CH_2	1.70 (m)	1.69 (m)	1.68 (m)	1.67 (m)	1.67 (m)
	2.00 (m)	1.99 (m)	1.98 (m)	1.97 (m)	~2.38 (m)
3' CH_2	1.52 (m)	1.60 (m)	1.62 (m)	1.60 (m)	1.60 (m)
	2.12 (m)	2.30 (m)	2.29 (m)	2.27 (m)	2.27 (m)
4' CH	3.33 (m)	3.24 (m)	3.25 (m)	3.23 (m)	3.23 (m)
5' CH	3.41 (dq, 6.0, 10.0)	3.49 (m)	3.50 (m)	3.49 (m)	3.49 (m)
6' CH_3	1.36 (d, 6.0)	1.32 (d, 6.0)	1.32 (d, 6.0)	1.32 (d, 7.0)	1.32 (d, 6.0)
4' OH	1.75 (br s)				
1'' CH		4.51 (dd, 2.0, 9.0)	4.52 (dd, 2.0, 9.0)	4.46 (dd, 2.0, 9.5)	4.48 (dd, 2.0, 9.0)
2'' CH_2		1.60 (m)	1.44 (q, 11.0)	1.48 (m)	1.48 (m)
		1.87 (m)	2.31 (m)	2.30 (m)	2.30 (m)
3'' CH or CH_2		1.46 (m)	3.18 (m)	3.38 (m)	3.38 (m)
		2.07 (m)			
4'' CH		3.29 (m)	3.33 (m)	3.16 (m)	3.16 (m)
5'' CH		3.29 (m)	3.20 (m)	3.34 (m)	3.34 (m)
6'' CH_3		1.31 (d, 6.0)	1.34 (d,6.0)	1.31 (d, 7.0)	1.31 (d, 6.0)
3'' OCH_3			3.40 (s)	3.42 (s)	3.40 (s)
4'' OH		1.60 (br s)	2.51 (br s)		
1''' CH				4.73 (dd, 2.0, 9.0)	4.72 (dd, 2.0, 9.0)
2''' CH_2				1.44 (m)	1.44 (m)
				2.33 (m)	2.33 (m)
3''' CH_2				3.18 (m)	3.17 (m)
				3.18 (m)	3.17 (m)
4''' CH				3.16 (m)	3.16 (m)
5''' CH				3.32 (m)	3.32 (m)
6''' CH_3				1.35 (d , 6.0)	1.35 (d , 6.0)
3''' OCH_3				3.41 (s)	3.39 (s)
4''' OH				2.45 (br s)	
1'''' CH					4.67 (dd, 2.0, 9.0)
2'''' CH_2					1.48 (m)
					2.30 (m)
3'''' CH_2					3.17 (m)
					3.17 (m)
4'''' CH					3.18 (m)
5'''' CH					~3.33 (m)
6'''' CH_3					1.33 (d, 6.0)
3'''' OCH_3					3.42 (s)
4'''' OH					2.44 (br s)

Table 2. ^{13}C NMR data of kigamicins.

	A	B	C	D	E
1	64.32	64.32	64.31	64.31	64.31
2	41.90	41.90	41.88	41.88	41.89
3	164.87	164.88	164.87	164.87	164.87
4	118.12	118.14	118.12	118.13	118.13
5	158.83	158.85	158.83	158.84	158.84
6	110.34	110.36	110.35	110.35	110.35
7	110.35	110.36	110.35	110.35	110.35
8	150.11	150.12	150.12	150.10	150.10
9	112.24	112.23	112.23	112.23	112.23
10	183.69	183.72	183.68	183.69	183.69
11	119.20	119.21	119.20	119.19	119.19
12	63.71	63.79	63.73	63.75	63.75
13	34.58	34.74	34.65	34.69	34.69
14	80.24	80.36	80.26	80.32	80.32
15	71.14	71.22	71.15	71.18	71.18
16	161.35	161.35	161.30	161.29	161.29
17	144.19	144.21	144.18	144.19	144.19
18	131.14	131.17	131.12	131.15	131.15
19	130.19	130.17	130.17	130.17	130.17
20	72.81	72.82	72.81	72.80	72.80
21	36.37	36.38	36.36	36.37	36.37
22	140.61	140.60	140.58	140.59	140.59
23	118.46	118.44	118.43	118.43	118.43
24	135.76	135.74	135.75	135.74	135.74
25	40.29	40.31	40.29	40.30	40.30
26	92.26	92.26	92.24	92.24	92.24
27	22.54	22.55	22.54	22.54	22.54
28	91.16	91.16	91.15	91.15	91.15
1'	102.15	102.25	102.16	102.17	102.18
2'	30.76	30.71	30.66	30.66	30.65
3'	31.02	29.80	29.73	29.73	29.72
4'	70.96	79.47	79.83	79.76	79.76
5'	76.55	75.23	75.07	75.08	75.08
6'	17.99	18.15	17.95	18.15	~18
1''		103.08	101.13	101.01	101.00
2''		30.91	35.33	36.28	36.30
3''		31.20	80.59	79.16	79.11
4''		75.85	75.37	82.32	82.54
5''		71.37	71.63	71.01	71.04
6''		18.15	18.17	18.41	~18
3''-OCH_3			56.38	56.75	~56
1'''				100.25	100.27
2'''				35.49	35.49
3'''				80.70	80.72
4'''				75.48	82.34
5'''				71.69	71.67
6'''				18.01	~18
3'''-OCH_3				56.37	~18
1''''					100.14
2''''					36.46
3''''					79.26
4''''					75.49
5''''					71.11
6''''					~18
3''''-OCH_3					~56

moieties, 1″-H (δ 4.47), 1‴-H (δ 4.72) and 1⁗-H (δ 4.67) were coupled to C-4′ (δ 79.76), C-4″ (δ 82.54) and C-4‴ (δ 82.34) of oleandrose moieties, respectively. Thus, the structure of **5** is shown in Fig. 1.

As described in this paper, kigamicins are new antitumor antibiotics possessing a polycyclic xanthone moiety and sugar moieties in the molecule. Other members of this family include cervinomycin[4], actinoplanones[5], LL-E19085α[6], LL-D42067[7]. However, most of the polycyclic xanthone antibiotics do not possess a sugar moiety.

The determination of stereochemistry of kigamicins is now in progress. The relative and absolute stereochemistry of kigamicins will be demonstrated on the basis of NMR studies, chemical degradation studies and a single crystal X-ray diffraction analyses.

Materials and Methods

UV spectra were measured on a Hitachi U-3210 spectrometer. IR spectra were recorded on a HORIBA FT-210 fourier transform infrared spectrometer. HRESI-MS spectra were measured with a JEOL JMS-T100LC spectrometer. ^{1}H, ^{13}C, and ^{15}N NMR spectra were measured on a JEOL JNM-A500 spectrometer using TMS as an internal reference. ^{15}N-chemical shifts were given in ppm using $CH_3NO_2/CDCl_3$ (1 : 1) solution at 379.6 ppm as an external standard.

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