Kalimantacin A, B, and C, Novel Antibiotics Produced by *Alcaligenes* sp. YL-02632S

II. Physico-chemical Properties and Structure Elucidation

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(Received for publication August 30, 1995)

Kalimantacin A , B and C are new antibiotics produced by *Alcaligenes* sp. YL-02632S. Their structures were elucidated to be novel long chain structure compounds containing *O*-carbamoyl, amide and carboxylic acid moieties based on various 2D NMR experiments and MS analysis.

In the screening program for new antibiotics, we discovered novel antibiotics named kalimantacin A (**1**), B (**2**) and C (**3**) from the cultute broth of *Alcaligenes* sp. YL-02632S. Details of the taxonomy, fermentation, isolation and biological activities of kalimantacins are reported in the preceding paper.[1)] In the present article we describe the physico-chemical properties and the structural elucidation of **1**, **2** and **3**.

Kalimantacin A (**1**) was obtained as a white to pale yellow powder from the fermentation broth of *Alcaligenes* sp. YL-02632S by isolation procedures described in the preceding paper[1)]. The molecular formula of **1** was determined to be $C_{30}H_{48}N_2O_7$ on the basis of positive-ion high resolution FAB-MS data $(M+H)^+$ *m/z* calcd: 549.3540, found: 549.3535). The IR spectral data had absorption bands at 3365 cm^{-1} indicating the presence of –OH and/or –NH. An amide function was suggested by the absorption band at 1640 cm^{-1}, which clearly separated from a large carbonyl band at 1700 cm^{-1}. The physico-chemical properties of **1** are summarized in Table 1.

The ^1H and ^{13}C NMR spectra of 1 in $CDCl_3$ are shown in Figs. 1 and 2. The ^{13}C NMR spectrum of **1** showed 30 carbon signals which were assigned to five methyl, nine methylene, ten methine and six quaternary carbons by a DEPT experiment. The ^1H and ^{13}C NMR spectral data of **1** are summarized in Tables 2 and 3.

From the analysis of the ^1H-^1H DQF COSY and

Table 1. Physico-chemical properties of kalimantacin A (**1**), B (**2**) and C (**3**).

	1	2	3
Appearance	white to pale yellow powder	white to pale yellow powder	white to pale yellow powder
$[\alpha]_D^{25}$	+56.3 (*c* 1.0, MeOH)	ND	ND
Molecular formula	$C_{30}H_{48}N_2O_7$	$C_{30}H_{48}N_2O_7$	$C_{29}H_{46}N_2O_7$
FAB-MS (m/z)	549 $(M+H)^+$	549 $(M+H)^+$	535 $(M+H)^+$
HRFAB-MS (m/z)			
Found:	549.3535 $(M+H)^+$	ND	ND
Calcd:	549.3540		
UV λ_{max} nm(ε) (in MeOH)	228.5 (41,200)	230.0 (40,500)	234.0 (43,200)
IR ν (KBr) cm^{-1}	3365, 2935, 1700, 1640, 1380	3365, 2935, 1700, 1640, 1380	3365, 2935, 1700, 1640, 1380
Solubility	Soluble in MeOH, acetone, AcOEt benzene, $CHCl_3$ Insoluble in hexane, H_2O	Soluble in MeOH, acetone, AcOEt benzene, $CHCl_3$ Insoluble in hexane, H_2O	Soluble in MeOH, acetone, AcOEt benzene, $CHCl_3$ Insoluble in hexane, H_2O

ND: Not determined.

Fig. 1. ^{1}H NMR spectrum of kalimantacin A ($CDCl_3$, 500 MHz).

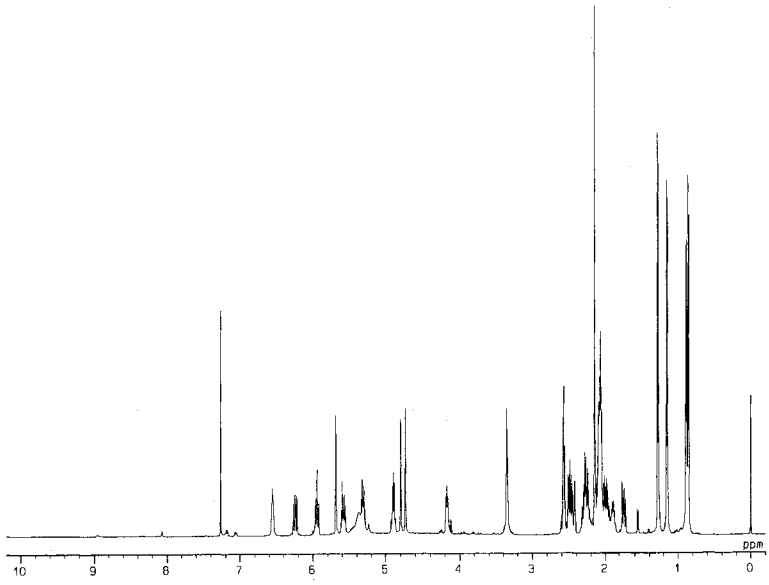
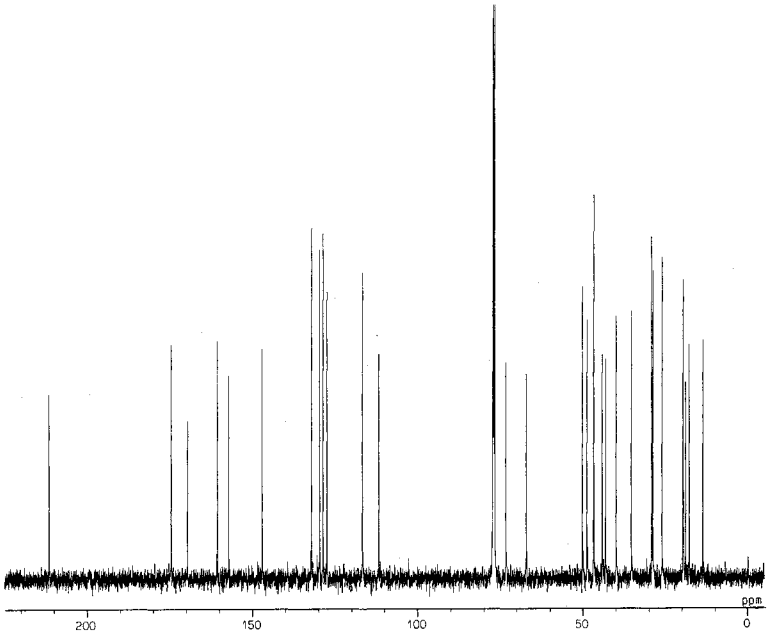


Fig. 2. ^{13}C NMR spectrum of kalimantacin A ($CDCl_3$, 500 MHz).



HOHAHA spectral data of **1**, four proton sequences, from 4-H to 6-H and 22-H, from 8-H to 16-H and 24-H, from 18-H to 20-NH, and from 29-H to 28-H, were established (Fig. 3). The ^{1}H-^{13}C long-range couplings were observed from 2-H (δ_H 5.69) to C-1 (δ_C 169.6), C-3 (δ_C 160.7), C-4 (δ_C 48.9) and C-21 (δ_C 18.9), from 21-H (δ_H 2.15) to C-2 (δ_C 116.7), C-3 (δ_C 160.7), C-4 (δ_C 48.9) in the HMBC[2] spectrum (Fig. 3). These data revealed the presence of a butenoic acid structure connected to C-4. The ^{1}H-^{13}C long-range couplings were observed from the terminal methylene protons 23-H (δ_H 4.74 and 4.80) to C-6 (δ_C 43.2), C-8 (δ_C 35.4) and C-7 (δ_C 147.1), and from 6-H (δ_H 1.75, 2.08) to C-7, C-23 (δ_C 111.8) and C-8, thereby showing the presence of a vinylidene moiety bound to C-6 and C-8. The ^{1}H-^{13}C long-range couplings observed from 16-H (δ_H 2.27) and 18-H (δ_H 2.57) to C-17 (δ_C 211.5) suggested that a carbonyl group attached to C-16 and C-18.

Table 2. ^{1}H NMR data[a] of kalimantacin A (**1**), B (**2**) and C (**3**).

Position	1[b]	2[c]	3[c]
1-OH	---[d]		
2	5.69 (s)	5.65 (s)	5.65 (s)
4	2.01 (m), 2.10(m)	1.91 (m), 2.20 (m)	1.93 (m), 2.20 (m)
5	1.89 (m)	1.93 (m)	1.87 (m)
6	1.75 (dd, *J*=13.5Hz, 8.6Hz), 2.08 (m)	1.88 (m), 2.07 (m)	2.10 (m)
8	2.07 (m)	2.20 (m)	2.10 (m)
9	2.24 (m), 2.30 (m)	2.00 (m)	2.10 (m), 2.29 (m)
10	5.31 (dt, *J*=11.0Hz, 7.3Hz)	5.52 (dt, *J*=14.4Hz, 7.3Hz)	5.30 (dt, *J*=11.0Hz, 7.3Hz)
11	5.95 (t, *J*=11.0Hz)	5.97 (dd,*J*=14.4Hz, 10.4Hz)	5.95 (t, *J*=11.0Hz)
12	6.25 (dd, *J*=15.3Hz, 11.0Hz)	6.03 (dd, *J*=14.4Hz, 10.4Hz)	6.32 (dd, *J*=15.3Hz, 11.0Hz)
13	5.58 (dt, *J*=15.3Hz, 7.3Hz)	5.57 (dt, *J*=14.4Hz, 7.3Hz)	5.61 (dt, *J*=15.3Hz, 7.3Hz)
14	1.98 (m), 2.07 (m)	1.90 (m), 2.08 (m)	1.92 (m), 2.05 (m)
15	2.08 (m)	2.06 (m)	2.09 (m)
16	2.27 (m), 2.44 (dd, *J*=16.5Hz, 5.0Hz)	2.28 (dd, *J*=16.5Hz, 7.3Hz), 2.48 (m)	2.30 (m), 2.51 (m)
18	2.57 (m)	2.55 (m)	2.52 (m)
19	4.17 (m)	4.14 (m)	4.13 (m)
20	3.35 (m)	3.16 (m), 3.28 (m)	3.22 (m)
20-NH	6.56 (br)		
21	2.15 (s)	2.12 (s)	2.12 (s)
22	0.86 (d, *J*=6.7Hz)	0.84 (d, *J*=5.5Hz)	0.85 (d, *J*=6.7Hz)
23	4.74 (s), 4.80 (s)	4.75 (s), 4.90 (s)	4.75 (s), 4.80 (s)
24	0.89 (d, *J*=6.7Hz)	0.89 (d, *J*=6.7Hz)	0.90 (d, *J*=6.7Hz)
26	2.49 (m)	2.50 (m)	2.39 (m), 2.48 (m)
27	4.90 (m)	4.90 (m)	5.07 (m)
28	1.28 (d, *J*=6.1Hz)	1.23 (d, *J*=6.7Hz)	1.28 (d, *J*=6.7Hz)
29	1.15 (d, *J*=7.5Hz)	1.10 (d, *J*=6.7Hz)	
30-NH_2	5.37 (br)		

[a] ^{1}H NMR spectra were recorded at 500 MHz.
[b] $CDCl_3$ as solvent.
[c] CD_3OD as solvent.
[d] Not detected.

Table 3. ^{13}C NMR data[a] of kalimantacin A (**1**), B (**2**) and C (**3**).

Position	1[b]	2[c]	3[c]	Position	1[b]	2[c]	3[c]
1	169.6	170.1	170.1	16	50.3	51.1	51.2
2	116.7	118.3	118.4	17	211.5	211.7	211.5
3	160.7	160.5	160.4	18	46.9	48.6	48.3
4	48.9	49.7	49.1	19	67.2	67.8	67.6
5	28.9	30.1	30.2	20	44.2	45.9	46.1
6	43.2	44.9	44.9	21	18.9	18.8	18.9
7	147.1	148.7	148.7	22	19.7	19.8	19.8
8	35.4	36.5	36.7	23	111.8	112.0	112.2
9	26.1	32.0	27.1	24	19.6	20.1	20.1
10	129.7	131.1	130.5	25	174.6	177.1	173.2
11	128.7	133.7	130.0	26	46.8	47.5	43.9
12	127.4	132.0	128.7	27	73.4	73.7	69.8
13	132.2	132.9	133.6	28	17.9	18.1	20.3
14	40.0	40.9	41.2	29	13.7	14.1	---
15	29.2	30.5	30.5	30	157.2	159.1	159.2

[a] ^{13}C NMR spectra were recorded at 125 MHz.
[b] $CDCl_3$ as solvent.
[c] CD_3OD as solvent.

The ^{1}H-^{13}C long-range couplings observed from 20-NH (δ_H 6.56) and 29-H (δ_H 1.15) to C-25 (δ_C 174.6) showed the presence of an amide group connected to C-20 and C-26. Taking the molecular formula in consideration, the remaining quaternary carbon C-30 (δ_C 157.2) which have ^{1}H-^{13}C long-range coupling with 27-H (δ_H 4.90) was suggested to be a carbamoyl or carboxy carbon. The elimination of fragment ion peak at *m/z* 61 in **1** by means of the B/E linked scan method of the FAB-MS indicated that the elements of carbamic acid have been lost[3]. Therefore, the quaternary carbon C-30 was determined to be a carbamoyl carbon. As a result the quaternary carbon C-1 was decided to be a carboxylic carbon.

The geometry of trisubstituted double bond (C-2) was assigned as *E* form because of the presence of NOE between 2-H (δ_H 5.69) and 4-Ha (δ_H 2.10) observed in the NOESY spectrum and the chemical shift of methyl carbon C-21 (δ_C 18.9) of **1**[4,5]. The geometries of the two disubstituted double bonds, C-10 and C-12, were determined to be 10*Z* and 12*E* by the coupling constants, $J_{10,11}=11.0$ Hz and $J_{12,13}=15.3$ Hz. The planar structure of **1** was thus elucidated as shown in Fig. 4.

Kalimantacin B (**2**) has the same molecular weight of 548 as that of **1** confirmed by the observation peak at *m/z* 549 $(M+H)^+$, *m/z* 547 $(M-H)^-$ and *m/z* 571 $(M+Na)^+$ by addition of NaCl in FAB-MS. The connectivities between protons and carbons by 2D NMR experiments of **2** coincided with those of **1**, except for the differences of the chemical shifts (^{1}H, ^{13}C) of conjugated diene moiety (C-10 and C-12) and the adjacent methylene carbon (C-9). The deshielded ^{13}C chemical shift (δ_C 32.0) of C-9 of **2** comparing with that (δ_C 26.1) of **1** and the coupling constant ($J=14.4$ Hz) between 10-H and 11-H showed the geometrical change of the disubstituted double bond (C-10) from *Z* to *E*. Thus, the structure of **2** was elucidated as shown in Fig. 4 with the *E*-*E* configuration in the conjugated diene moiety.

The molecular weight of kalimantacin C (**3**) was determined to be 535 by the observation peak at *m/z* 535 $(M+H)^+$, 533 $(M-H)^-$ and 557 $(M+Na)^+$ by addition of NaCl in FAB-MS. The structure of **3** was elucidated to be 26 demethyl derivative of **1** because of the decrease of the molecular weight by 14 mass units, the replacement of one methyl signal in the ^{13}C NMR spectrum by a methylene carbon (δ_C 43.7) and the ^{1}H-^{13}C long-range coupling between its appending proton and C-28. The structures of both **2** and **3** are shown in Fig. 4. The ^{1}H and ^{13}C NMR spectral data of **2** and **3** are summarized in Tables 2 and 3. Further studies on the absolute stereochemistry are in progress.

Fig. 3. ^{1}H-^{1}H DQF COSY, HOHAHA and HMBC experiments of kalimantacin A.

—: ^{1}H-^{1}H couplings obtained from ^{1}H-^{1}H DQF COSY and HOHAHA. →: ^{1}H-^{13}C long-range couplings obtained from HMBC.

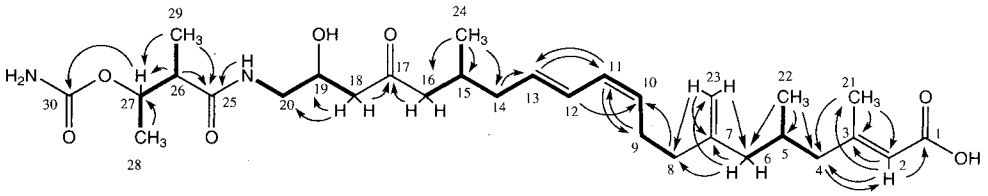
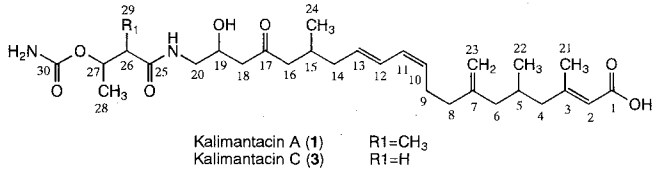
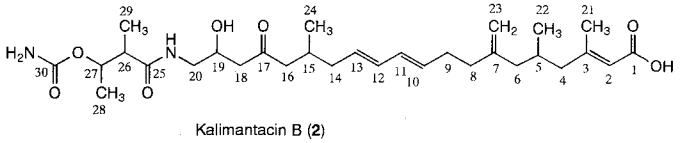


Fig. 4. The structure of kalimantacin A, B and C.



Kalimantacin A (**1**) R1=CH_3
Kalimantacin C (**3**) R1=H



Kalimantacin B (**2**)

Experimental

General procedures

IR spectra were recorded on a Hitachi 260-50 infrared spectrophotometer. Fast atom bombardment mass spectra (FAB-MS) were obtained with a VG ZAB-VSE and a JEOL DX300 mass spectrometer using nitrobenzyl alcohol-DMSO (positive ion) as matrix. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-ALPHA500 FT NMR spectrometer.

Acknowledgments

We thank Prof. H. SETO, Insutitute of Molecular and Cellular Biosciences, the University of Tokyo, for helpful discussion.

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