VOL. 52, NO. 3, MAR. 1999 THE JOURNAL OF ANTIBIOTICS pp. 325 — 328

NOTES

Kitamycins, New Antimycin Antibiotics Produced by *Streptomyces* sp.

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(Received for publication November 16, 1998)

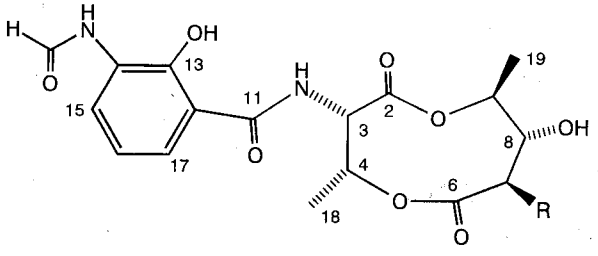
In the course of our screening program for plant growth inhibitors, *Streptomyces* sp. K385 isolated from a soil sample collected in Okayama Japan, was found to produce kitamycin A (**1**) and B (**2**), new antimycin type antibiotics, together with known antimycin type antibiotics, antimycin A_1, A_{2a}, A_3, A_{4a}[1,2], urauchimycin B (**3**)[3], and deisovalerylblastmycin (**4**).[4,5] This study describes the fermentation, isolation, structure elucidation and biological activity of **1** and **2** and stereochemistry of **3**. The strain K385 was cultivated in the medium consisting of dextrin 1.5%, soybean meal 0.4%, peptone 0.1%, meat extract 0.1%, yeast extract 0.1% and corn steep liquor 0.1% (pH 7.2) for 2 days at 28°C. This seed culture (200 ml) was inoculated into a 10-liter jar fermenter containing of 7 liters of the same medium above mentioned. Fermentation was carried out for 48 hours at 28°C with aeration (0.8 v/v/m) under constant agitation (300 rpm). The fermentation broth (13 liter) was filtered to remove mycelia, and the filtrate was adjusted to pH 4.0 with 6 N HCl and extracted with EtOAc. The organic layer was dried over Na_2SO_4 and evaporated to give an oily residue (2.3 g). The oily residue was chromatographed on silica gel column eluting successively with $CHCl_3$-MeOH and benzene-acetone. The active principle was separated into two fractions. Antimycin A_1, A_{2a}, A_3 and A_{4a} were isolated from an active fraction by HPLC and identified by spectroscopic analyses.[2] The other active fraction was further purified by Sephadex LH-20 column chromatography ($CHCl_3$: MeOH = 1 : 1) and HPLC using Nova pak ODS column (Waters, RCM 8 × 10) developed with 60% MeOH. **1** (0.8 mg) and **2** (0.2 mg) were obtained as a white powder together with urauchimycin B (**3**) and deisovalerylblastmycin (**4**).

The physico-chemical properties of **1** and **2** were as follows.

1: White powder; MP 158 ~ 160°C; $[\alpha]_D$ +47° (*c* 0.04, $CHCl_3$); UV λ_{max} (E tOH) nm 228 (64000), 315 (21000); IR ν_{max} ($CHCl_3$) 3414, 2930, 1744, 1644, 1530 cm^{-1}; FAB-MS *m/z* 465 $(M+H)^+$, 487 $(M+Na)^+$; HRFAB-MS *m/z* 487.2069 calcd. for $C_{23}H_{32}O_8N_2Na$ (Δ +1.2 mmu); ^1H and ^{13}C NMR data (Table 1). **2**: White powder; $[\alpha]_D$ +45° (*c* 0.01, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3412, 2928, 1740, 1641, 1530 cm^{-1}; UV λ_{max} (EtOH) nm 227 (64000), 315 (21000); FAB-MS *m/z* 465 $(M+H)^+$, 487 $(M+Na)^+$; HRFAB-MS *m/z* 465.2242 calcd. for $C_{23}H_{33}O_8N_2$ (Δ +0.5 mmu); ^1H NMR data (Table 1). ^{13}C NMR data could not be obtained due to a limited amount of **2**.

The molecular formula of **1** was determined to be $C_{23}H_{33}O_8N_2$ with HRFAB-MS spectrum. The ^1H NMR spectrum of **1** indicated two doublet methyl protons at δ 1.30 and 1.45, and three oxymethine protons at δ 3.59, 4.88 and 5.71, and triplet methine proton attached to nitrogen at δ 5.24. An aliphatic protons and aromatic protons (δ 6.92, 7.24 and 8.55) implied the presence of alkyl side chain and a 3-trisubstituted benzene ring, respectively. In addition, a phenolic proton, aldehyde proton and two amide protons were observed. In the ^{13}C NMR spectrum, 23 carbon signals were appeared including an aldehyde, two amide carbonyl, two ester carbonyl, three oxymethine and six aromatic carbons.

Fig. 1. Structures of kitamycin A and B.



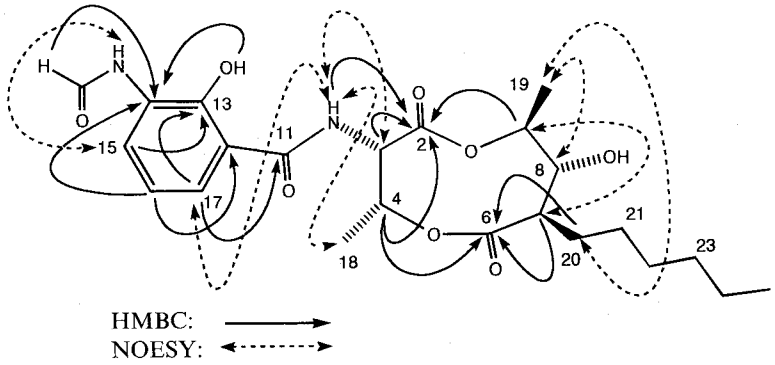
Kitamycin A (**1**)	$R=(CH_2)_5CH_3$
Kitamycin B (**2**)	$R=(CH_2)_3CH(CH_3)_2$
Urauchimycin B (**3**)	$R=(CH_2)_2CH(CH_3)_2$
Deisovalerylblastomycin (**4**)	$R=(CH_2)_3CH_3$

Table 1. NMR spectral data of **1** and **2**.

Position	**1** (δ_C)	**1** (δ_H)	**2** (δ_H)	Position	**1** (δ_C)	**1** (δ_H)	**2** (δ_H)
2	170.2			18	15.0	1.30 (d, 6.7)	1.31 (d, 6.3)
3	53.7	5.24 (t, 7.4)	5.24 (t, 7.6)	19	18.4	1.45 (d, 6.2)	1.46 (d, 6.7)
4	70.7	5.71 (m)	5.71 (m)	20	28.8	1.58 (m)	1.62 (m)
6	173.9					1.75 (m)	1.71 (m)
7	52.5	2.36 (m)	2.34 (m)	21	22.5	1.26 (m)	1.26 (m)
8	77.2	3.59 (m)	3.61 (m)	22	27.2	1.26 (m)	1.26 (m)
9	76.3	4.88 (m)	4.85 (m)	23	31.6	1.26 (m)	1.26 (m)
11	169.4			24	29.1	1.26 (m)	0.86 (d, 7.1)
12	112.6			25	14.0	0.87 (t, 7.5)	0.86 (d, 7.1)
13	150.6			8-OH		1.94 (brs)	1.94 (brs)
14	127.4			10-NH		7.09 (d, 7.6)	7.09 (d, 7.6)
15	124.8	8.55 (d, 8.5)	8.55 (d, 8.0)	13-OH		12.66 (brs)	12.66 (brs)
16	119.0	6.92 (t, 8.5)	6.92 (t, 8.2)	14-NH		7.91 (s)	7.91 (s)
17	120.1	7.24 (d,8.1)	7.24 (d, 8.2)	14-NHCHO	158.8	8.50 (d, 1.7)	8.50 (d, 1.7)

All spectra were recorded at 400 MHz for ^1H and 100 MHz for ^{13}C in $CDCl_3$, coupling constant (Hz).

Fig. 2. HMBC and NOESY spectrum of **1**.



The DQF-COSY spectrum of **1** revealed a trisubstituted benzene moiety and the sequence from an amide proton (H-10) to methyl proton (H-18) and from methyl proton (H-19) to alkyl side chain proton (H-20). The long range coupling between H-9 and C-2 and between H-4 and C-6 were detected by HMBC spectrum. The connectivity of H-3 to C-2 and H-7 to C-6 and the trisubstituted benzene moiety was also confirmed by the HMBC and NOESY spectrum as indicated in Fig. 2. NOESY cross peaks also made the connection between aromatic proton (H-17) and amide proton (H-10). These NMR signals of **1** are characteristic of the 9-membered dilactone of the antimycin type antibiotics and are in good agreement with those of deisovalerylblastmycin (**4**), except for the alkyl side chain. The alkyl moiety of **1** was determined to be hexyl by the NMR signals and the molecular formula of **1**. The configurations of **1** was deduced from the NOESY spectrum and the comparison of NMR data between **1** and **4**. The NOESY correlations of an amide proton (H-10) to H-18, of H-19 to H-8 (and H-20) and of H-7 to H-9 were observed. ^1H, ^{13}C NMR signals and NOESY spectrum of **1** except for alkyl side chain were nearly equal to those of **4**. These data indicate that the configurations of **1** are same as known antimycins including **4**. As the results, the structure of **1** was determined as shown in Fig. 1. The ^1H NMR and DQF-COSY spectrum of **2** displayed the same signals as observed on **1** except for alkyl moiety, which

Fig. 3. DQF-COSY and mass fragmentation of **2**.

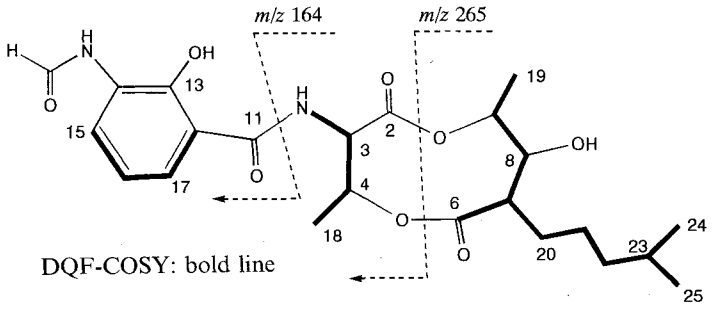


Table 2. Plant growth inhibitory activities of **1** and **2** against lettuce.

Compound	50 μ g/ml	25 μ g/ml	12.5 μ g/ml	Control
1	4.3 ± 0.3 [a]	8.1 ± 0.2	11.2 ± 0.3	12.7 ± 0.2
2	4.5 ± 0.4	8.3 ± 0.3	11.4 ± 0.3	12.8 ± 0.2

[a] Five individuals were grown in each well and wet weight (mg). Lettuce was cultured in Murashige-Skoog medium without plant hormone.

suggests that **2** is very similar to **1** except for the alkyl moiety. DQF-COSY spectrum of **2** showed the cross peak between two doublet methyl at δ 0.86 (J=7.1 Hz, H-24 and H-25) and methine proton at δ 1.26 (H-23). The alkyl side chain of **2** was proved to be 2-methylpentyl from this evidence, which was further supported by molecular formula and fragment ions at m/z 265 and 164 in FAB-MS spectrum (Fig. 3). The stereochemistry of **2** was elucidated to be same configuration as **1** due to very good agreement between the chemical shifts of **1** and **2**. Thus, the structure of **2** was determined as indicated in Fig. 1. The assignments of NMR signals of **1** and **2** are listed in Table 1.

IMAMURA *et al.* have been reported the isolation of **3** from the broth of *Streptomyces* sp. Ni-80.[3] However, the stereochemistry of **3** have not been described. NOESY spectrum of **3** showed the same correlations as observed on **1**. Therefore, the configurations of **3** are elucidated as shown in Fig. 1.

The plant growth inhibition activities of **1**, **2**, **3** and **4** were tested against the lettuce (*Grand rapid*). The seed were germinated in a 12-well micro titerplate for 2 days at 25°C under light. The sample was diluted with Murashige-Skoog medium and placed into the well after germination. The plate was incubated at 25°C for 3 days under light condition. All experiments were carried out triple and wet weight of plant was measured. **1** and **2** showed plant growth inhibitory activity at the concentration of 12.5 μg/ml (Table 2). **3** and **4** also exhibited inhibitory activities at the same concentration. On the other hand, antimycins A_1, A_{2a}, A_3, and A_{4a} showed plant growth inhibitory activity at 100 μg/ml. **1** and **2** have antimicrobial activity against *Candida albicans* IFO 1061 at 25 μg/ml.

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