Notes

3-*O*-ISOBUTYRYLKINAMYCIN C AND 4-DEACETYL-4-*O*-ISOBUTYRYLKINAMYCIN C, NEW ANTIBIOTICS PRODUCED BY A *SACCHAROTHRIX* SPECIES

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During the course of a screening program for antibiotics produced by a *Saccharothrix*, two new kinamycins **1** and **2** have been isolated from cultured broth of strain MI293-N4. They inhibit the growth of Gram-positive bacteria and neoplastic cells. This paper describes the production, isolation, purification and the structure elucidation of the antibiotics. Some physicochemical properties and biological activities of these compounds are also reported.

The strain was cultured at 27°C for 96 hours on a rotatory shaker (180 rpm) in 500-ml Erlenmeyer flasks containing 110 ml of medium: Potato starch 3.0%, corn steep liquor 0.5%, yeast extract (Oriental) 0.2%, toast soya 1.5%, NaCl 0.3%, $MgSO_4 \cdot 7H_2O$ 0.05%, $CaCO_3$ 0.3% and $CoCl_2 \cdot 6H_2O$ 0.001% in deionized water. After centrifuging the cultured broth (10 liters), the mycelial cake was extracted with methanol and the extract was concentrated. The concentrate and supernatant were combined and extracted with ethyl acetate. The organic layer was evaporated under reduced pressure to dryness. The crude extract was purified by chromatography on Silica gel 60 (Merck Art. No. 7734, 40 g) developed with a mixture of toluene and ethyl acetate (50 : 1). The active fraction was further separated into five fractions by preparative silica gel TLC with a solvent system of toluene - ethyl acetate (2 : 1). The two main fractions, which contained **1** and **2**, respectively, were subjected to Sephadex LH-20 column chromatography and eluted with methanol to give pure compounds **1** (5.2 mg) and **2** (9.2 mg).

Physico-chemical properties of **1** and **2** are summarized in Table 1. The UV and visible spectra of **1** and **2** bear close resemblance to those of kinamycins.[1] This was supported by the characteristic IR absorption at 2145 cm^{-1} (compound **1**), 2140 cm^{-1} (compound **2**) and 1745 cm^{-1} (compounds **1** and **2**) which were attributed to nitrile and ester carbonyl groups. Each ^1H NMR spectrum of **1** and **2** (Table 2) showed the presence of an isopropyl group, which was assigned to an isobutyrate residue from the chemical shift of the methine proton (δ 2.51 or 2.70). That was also revealed that **1** has three acetyl groups (δ 2.11, 2.12 and 2.17) and **2** has two acetyl groups (δ 2.12 and 2.18). From this data and the high resolution (HR)-MS of **1** and **2**, these compounds were recognized as isobutyryl derivatives of kinamycins. The positions of these acyl groups on the kinamycin structure were assigned as shown in Fig. 1 by two dimensional ^1H-^{13}C long-range correlation spectra (HMBC). That is, the three acetyl carbonyl carbons in **1** at δ 170.1, 170.4 and 170.6 had long-range coupling with the 1-H, 2-H and 4-H protons respectively. In **2**, the two acetyl carbonyl carbons at δ 172.4 and 172.6 had long-range coupling with the 1-H and 2-H protons, respectively. In addition, the isobutyryl carbonyl carbon (δ 178.8) was coupled with the 4-H proton. It was thus concluded that **1** is 3-*O*-isobutyrylkinamycin C and **2** is 4-deacetyl-4-*O*-isobutyrylkinamycin C. Though, the coupling constant between 1-H and 2-H (J=7.0 Hz) of **1** and **2** suggested that they have the same conformation as kinamycin C ($J_{1,2}$=

Table 1. Physico-chemical properties for **1** and **2**.

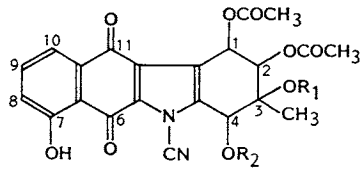
	1	**2**
Appearance	Yellow powder	Yellow powder
Molecular formula	$C_{28}H_{26}N_2O_{11}$	$C_{26}H_{24}N_2O_{10}$
HR-MS (*m/z*)		
Found:	566.1519	524.1435
Calcd:	566.1536	524.1431
MP (°C)	137~140	125~128
$[\alpha]_D^{26}$ (*c* 0.17, MeOH)	−85°	−126°
UV λ_{max}^{MeOH} nm (ε)	209 (21,300), 243 (32,200), 275 (18,700), 292 (sh, 12,000), 305 (sh, 9,900), 393 (11,500), 435 (11,300)	209 (19,300), 243 (29,000), 273 (15,400), 292 (sh, 10,500), 304 (sh, 8,700), 390 (9,800), 440 (10,000)
$\lambda_{max}^{MeOH-NaOH}$ nm (ε)	210 (27,100), 234 (26,800), 273 (25,400), 316 (sh, 9,300), 375 (8,200), 510 (8,200)	212 (30,500), 235 (30,200), 270 (23,000), 314 (sh, 13,600), 378 (11,600), 530 (10,300)
IR ν_{max}^{KBr} cm^{-1}	3425, 2970, 2145, 1745, 1660, 1620, 1460, 1225	3420, 2974, 2140, 1745, 1655, 1620, 1455, 1230

Table 2. ^{1}H NMR spectral data of **1** and **2** at 400 MHz.

Assignment	**1**[a]	**2**[a]
$(CH_3)_2C$	1.13, 1.14 (d, *J*=7.0 Hz)	1.21, 1.14 (d, *J*=7.0 Hz)
3-CH_3	1.58 (s)	1.27 (s)
$COCH_3$	2.11, 2.12, 2.17 (s)	2.12, 2.18 (s)
3-OH	—	2.68 (s)
COCH	2.51 (dq, *J*=7.0 Hz)	2.70 (dq, *J*=7.0 Hz)
1-H	6.24 (d, *J*=7.0 Hz)	6.23 (d, *J*=7.0 Hz)
2-H	5.88 (d, *J*=7.0 Hz)	5.61 (d, *J*=7.0 Hz)
4-H	6.58 (s)	5.49 (s)
8-H	7.18 (dd, *J*=1.0, 8.0 Hz)	7.17 (dd, *J*=1.7, 8.5 Hz)
9-H	7.55 (t, *J*=8.0 Hz)	7.55 (t, *J*=8.5 Hz)
10-H	7.67 (dd, *J*=1.0, 8.0 Hz)	7.67 (dd, *J*=1.7, 8.5 Hz)
7-OH	12.13 (s)	12.12 (s)

[a] Chemical shift δ (ppm) from internal TMS and multiplicity in $CDCl_3$.

Fig. 1. Structures of **1** and **2**.



1	R_1=COCH$(CH_3)_2$	R_2=$COCH_3$
2	R_1=H	R_2=COCH$(CH_3)_2$
Kinamycin C	R_1=H	R_2=$COCH_3$

7.2 Hz),[2] the stereochemistry could not be determined by the spectroscopy. Recently, SATO *et al.*[3] had reported the assignment of the ^{13}C NMR spectrum of kinamycin D for biosynthetic studies. The ^{13}C NMR spectra of **1** and **2** (Table 3) are very similar to that of kinamycin D. The nitrile carbon was not observed in the spectra of **1** and **2** and had not been assigned in the kinamycin D study.[3] It is also not observed in the spectrum of kinamycin A under the same spectroscopic conditions used for compounds **1** and **2**.

MICs against various bacteria on Müller-Hinton agar (Difco) were determined. Compounds **1** and **2** inhibited the growth of Gram-positive bacteria as shown in Table 4 and also showed cytotoxicity against L1210 leukemia (IC_{50} **1**: 0.72, **2**: 0.38 μg/ml) and IMC carcinoma cells (IC_{50} **1**: 0.88, **2**: 0.72 μg/ml) in suspension culture. In a clonogenic assay,[4] compounds

Table 3. ^{13}C NMR spectral data of **1** and **2** in ppm from internal TMS at 100 MHz.

Assignment	Chemical shifts δ (ppm) in $CDCl_3$ (CD_3OD)	
	1	**2**
C-1	68.1 d	67.9 (70.8) d
C-2	73.5 d	75.4 (76.8) d
C-3	80.8 s	73.8 (73.9) s
3-CH_3	16.1 q	18.6 (19.1) q
C-4	66.8 d	70.6 (71.6) d
C-4a	126.2 s	126.7 (127.1) s
C-5a	132.6 s	132.5 (133.1) s
C-6	184.1 s	184.1 (185.1) s
C-6a	115.6 s	115.6 (116.8) s
C-7	162.1 s	162.0 (163.1) s
C-8	123.8 d	123.8 (124.5) d
C-9	136.2 d	136.2 (137.2) d
C-10	119.9 d	119.9 (120.4) d
C-10a	134.3 s	134.3 (135.6) s
C-11	178.2 s	178.2 (179.5) s
C-11a	128.8 s	129.2 (129.7) s
C-11b	129.3 s	130.3 (133.1) s
COCH_3	170.1 s	170.2 (172.4) s
	170.4 s	172.1 (172.6) s
	170.6 s	—
CO*C*H_3	20.7 q	20.8 (20.9) q
	20.7 q	21.0 (21.1) q
	20.9 q	—
C$OCH(CH_3)_2$	175.7 s	176.9 (178.8) s
CO*C*$H(CH_3)_2$	34.8 d	34.0 (35.0) d
$COCH(CH_3)_2$	18.7 q	18.8 (19.4) q
	18.9 q	19.0 (20.0) q

Table 4. Antimicrobial spectra of **1** and **2**.

Test organism	MIC (μg/ml)	
	1	**2**
Staphylococcus aureus FDA 209P	0.78	0.39
S. aureus Smith	0.78	0.39
Micrococcus luteus PCI 1001	0.78	0.78
Bacillus subtilis PCI 219	0.39	0.20
Escherichia coli NIHJ	>50	>50
Salmonella typhi T-63	>50	>50
Serratia marcescens	>50	>50
Pseudomonas aeruginosa A3	>25	>25
Mycobacterium smegmatis ATCC 607	3.12	3.12

1 and **2** also showed cytotoxicity against LX-1 human lung carcinoma (IC_{50} **1**: 0.542, **2**: 0.790 μg/ml) and SC-6 human stomach carcinoma (IC_{50} **1**: 2.50, **2**: 0.760 μg/ml). Compound **2** was lethal in mice at 0.5 mg per mouse by intraperitoneal administration, and at 0.25 mg, it showed no toxicity.

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