Communications to the Editor

KOBUTIMYCINS A AND B, NEW ALKALOID ANTIBIOTICS PRODUCED BY A *Streptomyces* STRAIN

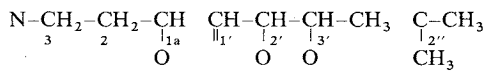
Sir:

New alkaloid antibiotics named kobutimycins A (**1**) and B (**2**) (Fig. 1) were isolated from the culture broth of a streptomycete strain No. TA3341, which was isolated from a soil sample collected at Kobutizawa, Yamanashi Prefecture, Japan. The strain has been classified as *Streptomyces* sp. TA3341. In this communication we wish to report the fermentation, isolation, structural elucidation and biological properties of kobutimycins.

A loopful of slant culture of strain TA3341 was inoculated into a 500-ml Erlenmeyer flask containing 110 ml of production medium consisting of glycerol 2%, Bacto-Soytone 1%, $CaCO_3$ 0.4% (pH 7.0 before autoclaving), and cultured at 27°C on a rotary shaker (180 rpm) until the production reached maximum in 2 days. The broth filtrate (5 liters) was extracted twice with ethyl acetate (5 liters × 2 times). The extract was concentrated to dryness under reduced pressure, and the crude residue (1.25 g) was applied to a slica gel column (100 ml) equilibrated with $CHCl_3$. The column was developed with 300 ml of $CHCl_3$ - MeOH (100 : 1). The active fractions were pooled and concentrated to give 57 mg of oily residue. From the residue, two components **1** and **2** were purified by preparative HPLC (Capcell Pak C18 SG120, 20 × 250 mm, Shiseido) using MeOH - H_2O (7 : 3) as solvent system with detection at 254 nm. These active eluates were concentrated to dryness yielding 23 mg of **1** and 28 mg of **2**.

The physico-chemical properties of both substances are summarized in Table 1. Compounds **1** and **2** were obtained as colorless oily substances. They were labile as such oils but stable in organic solvents such as MeOH, EtOAc and CH_3Cl. The molecular formulae were established as $C_{19}H_{25}NO_5$ for **1** and $C_{20}H_{27}NO_5$ for **2** by HRFAB-MS spectra and ^{13}C NMR spectra. In their UV spectra, λ_{max} at 257 nm in their neutral solution was shifted to longer wavelengths (*ca.* 65 nm) in their acidic solution. This result suggested that **1** and **2** have the conjugated imino moiety in their structure[1]. Their IR spectra exhibited characteristic absorption at 1740 cm^{-1} which is attributed to the ester bond. The ^1H and ^{13}C NMR spectral data of **1** and **2** are summarized in Table 2. The multiplicities of carbon signals were determined by the distortionless enhancement by polarization transfer (DEPT) experiment.

In the ^1H NMR spectrum of **1**, five methyl proton signals and two methylene proton signals were easily recognizable. Furthermore, oxygen-bearing methine proton signals (δ 4.42, 4.91 and 5.74) and olefinic proton signals (δ 5.46 and 6.82) were observed. In addition to these signals, the ^1H-^1H shift correlation spectroscopy (COSY) experiment indicated the presence of the following 3 fragments:



The ^1H-^{13}C long range connectivities of kobutimycins were determined by the ^1H-detected heteronuclear multiple-bond ^1H-^{13}C correlation spectroscopy (HMBC) experiment. In compound **1**, an oxygen-bearing methine proton (1a-H, δ 4.42) was coupled to an oxygen-bearing quaternary carbon (C-7a, δ 55.3) and imino carbon (C-4a, δ 171.2). Methylene protons (3-Ha, δ 3.66 and 3-Hb, δ 3.80) were coupled to the imino carbon (C-4a). Methyl protons (5-CH_3, δ 1.98) were coupled to the imino carbon (C-4a) and to two olefinic carbons (C-5, δ 114.4 and C-6, δ 143.1). An olefinic proton (6-H, δ 6.82) was coupled to the imino carbon (C-4a), an oxygen-bearing quaternary carbon (C-7a) and an olefinic carbon (C-5). These long range coupling data suggested the presence of a pyrindine skeleton. An oxygen-bearing methine proton (2′-H, δ 5.74) and two methyl protons (2″-CH_3, δ 1.16 and 3″-H, δ 1.14) were coupled to an ester carbonyl carbon (C-1″, δ 175.5). Furthermore, an oxygen-bearing methine proton (3′-H, δ 4.91) and methyl protons at δ 2.00 were coupled to an ester carbonyl carbon at δ 170.5. A summary of the HMBC experiment of **1** is shown in Fig. 2. NOEs of **1** were observed between

Fig. 1. Structures of kobutimycins A (**1**) and B (**2**).

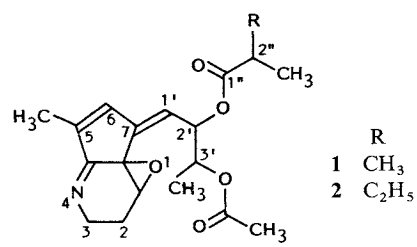


Table 1. Physico-chemical properties of kobutimycins A (**1**) and B (**2**).

	Kobutimycin A	Kobutimycin B
Appearance	Colorless oily substance	Colorless oily substance
Molecular formula	$C_{19}H_{25}NO_5$	$C_{20}H_{27}NO_5$
HRFAB-MS (*m/z*)		
Found:	348.1805 $(M+H)^+$	362.1967 $(M+H)^+$
Calcd:	348.1811	362.1968
$[\alpha]_D^{22}$ (*c* 1.0, $CHCl_3$)	+88.2	+107.2
UV λ_{max}^{MeOH} nm (ε)	212.0 (5,600), 256.8 (14,500)	207.6 (7,100), 256.8 (16,100)
$\lambda_{max}^{MeOH-HCl}$ nm (ε)	230.0 (11,700), 321.8 (11,500)	232.0 (12,900), 322.8 (12,800)
$\lambda_{max}^{MeOH-NaOH}$ nm (ε)	219.6 (sh, 6,800), 258.4 (16,300)	211.2 (7,000), 257.6 (15,400)
IR (KBr) cm^{-1}	2980, 2950, 1740, 1660, 1380, 1240, 1160, 1060, 920	2980, 2950, 1740, 1660, 1380, 1240, 1180, 1150, 1070, 920
Solubility		
Soluble:	*n*-Hexane, $CHCl_3$, EtOAc, MeOH, CH_3COCH_3	*n*-Hexane, $CHCl_3$, EtOAc, MeOH, CH_3COCH_3
Insoluble:	H_2O	H_2O
TLC[a] (Rf value) (I)	0.20	0.21
(II)	0.48	0.50
HPLC[b] (Rt, minutes)	4.82	6.42
HVPE[c]		
(Rm value, alanine = 1.0)	0.82	0.80

[a] Silica gel TLC (Merck Art 5715), solvent system (I) $CHCl_3$-MeOH (100:1) (II) EtOAc.
[b] Column, Capcell Pak C18 SG120 4.6×150 mm (Shiseido); mobile phase, MeOH-H_2O (70:30); flow rate, 1.0 ml/minute; detection, UV 254 nm.
[c] HVPE in HCOOH-CH_3COOH-H_2O (1:3:36) under 2000 V for 30 minutes.

Table 2. ^1H and ^{13}C NMR spectral data of kobutimycins A (**1**) and B (**2**) (400 MHz and 100 MHz, respectively, in $CDCl_3$).

Position	Kobutimycin A		Kobutimycin B	
	δ_C (ppm)	δ_H (ppm, *J* in Hz)	δ_C (ppm)	δ_H (ppm, *J* in Hz)
1a	60.6	4.42 br s*	60.6	4.42 br s
2	22.2	1.63, dt, *J*=5.6, 12.6, 14.8, 2.24 m, *J*=5.5, 14.8	22.2	1.63 m, 2.24 m, *J*=14.8
3	44.0	3.66 dt, *J*=5.5, 12.6, 3.80 dd, *J*=5.5, 15.1	44.0	3.66 dt, *J*=5.6, 12.4, 3.80 dd, *J*=5.6, 15.4
4a	171.2		171.2	
5	144.4		144.4	
5-CH_3	11.9	1.98 s	11.9	1.98 s
6	143.1	6.82 br s	143.1	6.82 br s
7	140.1		140.1	
7a	55.3		55.2	
1′	118.4	5.46 d, *J*=10.0	118.5	5.46 d, *J*=10.0
2′	68.4	5.74 dd, *J*=3.8, 10.0	68.3	5.75 dd, *J*=4.0, 10.0
3′	70.9	4.91 m, *J*=3.8, 6.6	71.0	4.91 m, *J*=4.0, 6.0
4′	14.5	1.24 d, *J*=6.6	14.5	1.24 d, *J*=6.0
3′-OC(=O)	170.5		170.5	
3′-OC(=O)-CH_3	21.0	2.00 s	21.0	2.00 s
1″	175.5		175.1	
2″	34.0	2.53 m, *J*=6.9	41.1	2.36 m, *J*=7.2
3″	18.7	1.14 d, *J*=6.9	16.6	1.13 d, *J*=7.2
2″-CH_3	19.0	1.16 d, *J*=6.9	—	
2″-CH_2	—		26.6	1.45 m, *J*=6.6, 1.66 m
2″-CH_2CH_3	—		11.5	0.89 t, *J*=7.8

* Multiplicity.

Fig. 2. Summary of the CH long range couplings (→) observed in the HMBC spectrum of kobutimycin A.

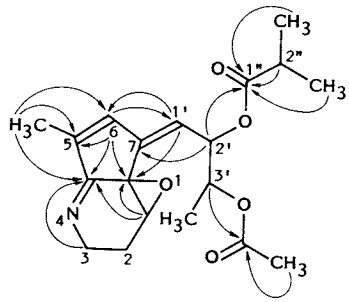


Table 3. Antimicrobial spectrum of kobutimycins A (**1**) and B (**2**).

Test organisms	Medium*	MIC (μg/ml) **1**	**2**
Staphylococcus aureus FDA 209P	a	25	25
Micrococcus luteus IFO 3333	a	12.5	12.5
Bacillus subtilis PCI 219	a	25	25
Escherichia coli K-12	a	>100	>100
Klebsiella pneumoniae PCI 602	a	>100	>100
Candida albicans 3147	b	>100	>100
Saccharomyces cerevisiae F-7	b	50	50
Pyricularia oryzae	b	50	50
Rhizocutonia solani	b	12.5	50
Rosellinia necatrix	c	3.1	12.5
Phomopsis fukushii PF-6B-1	c	50	50

* a: Mueller-Hinton agar (Difco), 37°C, 18 hours.
b: Nutrient agar+glucose 1%, 27°C, 42 hours.
c: Potato-sucrose agar, 27°C, 4 days.

6-H (δ 6.82) and 1'-H (δ 5.46). These NOEs revealed that relative position between the chromophore and the side chain.

Based on these results, the total planar structure of **1** was determined to be (7*Z*)-5-methyl-7-[2'-(2''-methylpropionyloxy)-3'-acetoxy]butylidene-1a,2,3,7-tetrahydrocyclopent[*b*]oxireno[*c*]pyridine (Fig. 1). The ^{1}H and ^{13}C NMR spectra of **2** were quite similar to those of **1** except the proton signals at high field region. The NMR experiments of **2** suggested the presence of a 2-methylbutyryl group instead of the 2-methylpropionyl group of **1**. Thus, the structure of **2** was determined as shown in Fig. 1.

Kobutimycins are structurally similar to the known alkaloid antibiotics such as abikoviromycin (latumcidin)[1~3], dihydrolatumcidin[4], *n*-hydroxy dihydroabikoviromycin[5] and hatomamicin[6].

The antimicrobial activity of kobutimycins were tested according to the agar dilution method with two-fold dilution. The results are summarized in Table 3. Kobutimycins showed weak activity against Gram-positive bacteria, and some phytopathogenic fungi. Cytotoxicities against lymphoid leukemia L1210 with IC_{50} value were 0.19 μg/ml in **1** and 0.11 μg/ml in **2**.

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