Communications to the Editor

A83016A, A NEW KINAMYCIN TYPE ANTIBIOTIC

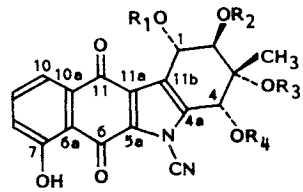
Sir:

As part of a screening program for new antibiotics, a new member of the kinamycin family has been isolated from the culture broth of an unidentified actinomycete designated A83016[1]. This paper describes the isolation, structure elucidation, and antimicrobial activity of this compound.

A83016 whole broth (212 liters) was extracted with an equal volume of ethyl acetate, forming a heavy emulsion. The emulsion was separated by passage through a continuous-flow type centrifuge, and the organic layer concentrated to an oil which was dissolved in 400 ml $CHCl_3$ - EtOAc (1 : 1). This solution was added slowly to 4 liters of hexane. The resulting precipitate was removed by filtration and the filtrate concentrated *in vacuo* to an oil (99 g). A 25 g aliquot of the oil was chromatographed over Sephadex LH-20 in methanol. Fractions which showed antimicrobial activity vs. *Staphylococcus aureus* were combined and partitioned between isooctane and MeOH - H_2O (95 : 5). The MeOH - H_2O layer was evaporated and the solids rechromatographed on Sephadex LH-20 in toluene - MeOH (10 : 1). This column separated A83016A from the known compounds 4-deacetyl-4-*O*-isobutyrylkinamycin C (**2**) and 3-*O*-isobutyrylkinamycin C (**3**)[2], which are also produced by this culture. The fractions from this column containing A83016A were evaporated, and the residue (853 mg) was chromatographed on an Ito coil planet centrifugal countercurrent apparatus using a solvent system of hexane - EtOAc - MeOH - 0.2% aqueous AcOH (pH adjusted to 5.7 with sodium hydroxide) (80 : 20 : 25 : 6), with the upper phase used as the mobile phase. The final purification step, preparative TLC on silica gel with toluene - EtOH (9 : 1) as the solvent, afforded 54 mg of pure A83016A (**1**).

Comparison of the UV, IR, and mass spectral

Fig. 1. Structures of A83016A (**1**), 4-deacetyl-4-*O*-isobutyrylkinamycin C (**2**), 3-*O*-isobutyrylkinamycin C (**3**), and kinamycin C (**4**).



	R_1	R_2	R_3	R_4
A83016A (**1**)	$COCH_3$	$COCH(CH_3)_2$	H	$COCH(CH_3)_2$
2	$COCH_3$	$COCH_3$	H	$COCH(CH_3)_2$
3	$COCH_3$	$COCH_3$	$COCH(CH_3)_2$	$COCH(CH_3)_2$
Kinamycin C (**4**)	$COCH_3$	$COCH_3$	H	$COCH_3$

Table 1. Physico-chemical properties for **1** and **2**[a].

	1		**2**
Molecular formula	$C_{28}H_{28}N_2O_{10}$		$C_{26}H_{24}N_2O_{10}$
MS (*m/z*) Found:	553.1799 $(M+H)^+$		524.1435 $(M)^+$
Calcd:	553.1822		524.1431
UV λ_{max}^{MeOH} (ε)	244 (37,300), 273 (27,300), 391 (10,300), 441 (11,700)		243 (29,000), 273 (15,400), 390 (9,800), 440 (10,000)
$\lambda_{max}^{MeOH-NaOH}$ nm (ε)	237 (37,500), 274 (28,200), 377 (8,500), 530 (6,800)		235 (30,200), 270 (23,000), 378 (11,600), 530 (10,300)
IR ν_{max} ($CHCl_3$) cm^{-1}	2978, 2150, 1743, 1626, 1460	IR ν_{max} (KBr) cm^{-1}	2974, 2140, 1745, 1620, 1455

[a] Selected data for **2** from Table 1 of ISSHIKI *et al.*, ref 2.

Table 2. ^{13}C and ^{1}H NMR data of **1** and **4**.

Assignment	**1**[a]		**4**[b]
	^{13}C	^{1}H	^{13}C
C-1	69.4	6.13	68.1
C-2	76.1	5.67	75.4
C-3	73.8		73.5
3-CH_3	19.8	1.33	18.6
C-4	71.3	5.86	70.9
C-4a	127.4		126.6
C-5a	132.6		132.5
C-6	184.8		184.0
C-6a	116.4		115.5
C-7	162.8	12.07	162.0
C-8	124.1	7.13	123.7
C-9	137.1	7.61	136.2
C-10	120.1	7.51	119.9
C-10a	135.4		134.2
C-11	178.5		178.0
C-11a	129.7		128.9
C-11b	133.2		130.1
CN	78.7		
$COCH_3$	170.1		172.0, 171.1, 170.2
$COCH_3$	20.8	2.02	21.1, 21.0, 20.9
$COCH(CH_3)_2$	177.8, 177.0		176.9
$COCH(CH_3)_2$	34.8, 34.6	2.62, 2.79	34.0
$COCH(CH_3)_2$	19.1, 19.3, 19.2 (2C)	1.24, 1.20, 1.19 (6H)	18.8, 19.0

[a] Chemical shifts, δ, $(CD_3)_2CO$.
[b] Chemical shifts, δ, $CDCl_3$; from ref 6.

data of A83016A with those of **2** (see Table 1) shows that A83016A is a member of the kinamycin family of antibiotics, being larger than **2** by C_2H_4. Examination of the ^{1}H NMR spectrum (see Table 2) indicates the presence of one acetyl and two isobutyryl esters on the kinamycin nucleus. Structures for the kinamycins have been determined by chemical degradation and spectroscopy[3,4], and their absolute stereochemistry has been determined by X-ray crystallography[5].

The ^{1}H and ^{13}C NMR spectra of A83016A were examined in $(CD_3)_2CO$ (see Table 2); assignments were made using homonuclear and heteronuclear correlations and by comparison with the ^{13}C NMR assignments of kinamycin C (**4**) derived from biosynthetic studies[6]. A long-range ^{1}H-^{13}C COSY experiment[7] provided a distinction between the carbon pair C-4a and C-11b (127.4 and 133.2 ppm), which had identical long-range correlations, and a pair C-5a and C-11a (132.6 and 129.7 ppm), which had no correlations; assignments were made within each pair by comparison with **4**. The signal at 78.7 ppm was assigned to the cyanamide carbon[8].

The remaining structural problems were the placement of the acetyl and two isobutyryl groups, and the determination of the relative stereochemistry. The placement of the esters was solved by the long range ^{1}H-^{13}C COSY experiment. Since the chemical shifts of 1-H, 2-H, 4-H and the C-3 methyl are consistent with ester functionality at C-1, C-2 and C-4,[3] the correlation between the acetate carbonyl (170.1 ppm) and the proton at 1-H (6.13 ppm) places the acetyl group at the 1 position and therefore the isobutyryl groups at positions 2 and 4.

Table 3. Antimicrobial activity of **1**.

Test organism	MIC (μg/ml)
Staphylococcus aureus	0.06
S. epidermidis	0.06
Streptococcus pyogenes	0.06
S. pneumoniae PARK	0.06
Enterococcus faecium	4.0
Salmonella sp. 514	>128
Pseudomonas sp. 258	>128
Serratia marcescens	>128
Haemophilus influenzae	>128

The relative stereochemistry of the cyclohexene ring was determined by the use of coupling constants and a difference NOE experiment. Irradiation of the C-3 methyl group resulted in enhancement of the 1-H and 4-H signals. Assuming a chair-like conformation, the enhancement of 1-H establishes a 1~3 diaxial or *cis* orientation between the methyl and 1-H. The enhancement of 4-H places this hydrogen equatorial or *cis* to the C-3 methyl, while the absence of enhancement for 2-H suggests a *trans* diaxial arrangement of 2-H and the C-3 methyl. The 7.2 Hz coupling constant between 1-H and 2-H also suggests that they are *trans*. This relative stereochemistry corresponds to that of kinamycin C[4].

A83016A showed MICs of 0.06~4.0 μg/ml vs. Gram-positive bacteria, but was inactive vs. Gram-negative organisms (see Table 3), consistent with other members of the kinamycin family.

Acknowledgments

We would like to thank LAVERNE BOECK, ROGER WETZEL, PAT BAKER, DICK CLARK, JAMES GILLIAM, JOHN OCCOLOWITZ, LESTER ROUSE, HELEN MICHAELS, LOUISE CRANDALL and the Physical Chemistry group for technical assistance during this work.

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(Received August 19, 1991)

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