SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF 1-N-(1,3-DIHYDROXY-2-PROPYL)KANAMYCIN B (UK-31,214)

K. RICHARDSON*, K. W. BRAMMER, S. JEVONS, R. M. PLEWS and J. R. WRIGHT

Pfizer Central Research, Pfizer Limited,
Sandwich, Kent, U.K.

(Received for publication July 30, 1979)

1-N-(1,3-Dihydroxy-2-propyl)kanamycin B was prepared and its *in vitro* activity against aminoglycoside-sensitive and aminoglycoside-resistant organisms was compared with that of kanamycin B and gentamicin. This kanamycin B derivative (code No. UK-31,214) demonstrated potent activity in all of these tests and gave good protection in experimental infections in mice.

The antibacterial activity of some 1-N-alkyl derivatives of kanamycin A has been reported[1]. The most potent of these, UK-18,892 (**2**), was shown to be highly active against both aminoglycoside-sensitive and aminoglycoside-resistant bacteria. We undertook a program of 1-N-alkyl modifications of both kanamycins A and B to investigate structure-activity relationships in this area, and this paper describes the synthesis and antibacterial properties of one of the kanamycin B derivatives prepared during this study, namely UK-31,214 [1-N-(1,3-dihydroxy-2-propyl)kanamycin B, Fig. 1, compound **1**].

Materials and Methods

1-N-(1,3-Dihydroxy-2-propyl)-2′,3,3″,6′-tetra-N-formylkanamycin B (Fig. 1, **4**)

2′,3,3″,6′-Tetra-N-formylkanamycin B[2] (**3**) (5.0 g as $\frac{1}{2}H_2CO_3 \cdot H_2O$; 0.0069 mole) dissolved in dimethylsulfoxide (250 ml) was treated with 1,3-dihydroxy-acetone (2.27 g) and 2 N hydrochloric acid (1 ml) at 20°C. To this solution was added sodium cyanoborohydride (1.73 g) and this reaction mixture was then stirred at 60～65°C for 20 hours. After evaporation under reduced pressure the residue was dissolved in water (100 ml), adjusted to pH 5.35 with 5 N hydrochloric acid and then chromatographed on a column of Sephadex CM-25 (950 ml) in the ammonium ion form. The column was eluted with water (2.4 litres) followed by 0.005 N ammonia solution (5 liters). The fractions containing the product were combined and evaporated to dryness under reduced pressure to yield the product as an amorphous solid. 3.91 g (84.5%), m.p. 180～185°C (Table 1).

1-N-(1,3-Dihydroxy-2-propyl)kanamycin B, UK-31,214 (Fig. 1, **1**)

1-N-(1,3-Dihydroxy-2-propyl)-2′,3,3″,6′-tetra-N-formylkanamycin B (**4**) (4.69 g) dissolved in 1 N sodium hydroxide solution (141 ml) was heated at 55～56°C for 3.5 hours. The solution was cooled to 20°C and adjusted to pH 5.5 using 6 N hydrochloric acid. The solution was chromatographed on a column of Sephadex CM-25 (2.1 litres) in the ammonium ion form. The column was eluted successively with water (6 litres), 0.025 N ammonia solution (7 litres), 0.033 N ammonia solution (21 litres) and finally 0.04 N ammonia solution (9 litres). The fractions containing the product were combined and evaporated under reduced pressure to give an amorphous solid. 2.418 g (59.9%), m.p. 165～170°C (Table 1). The structures of the products prepared in the present study have been confirmed by ^{13}C-n.m.r. Microanalyses of the products reported are in agreement with the expected formulae (compound **1** calculated as the monohydrate).

Micro-organisms

The bacteria designated 'aminoglycoside-sensitive' were pathogenic strains well adapted to growth under laboratory conditions. The aminoglycoside-resistant organisms were bacteria resistant to one or more commercially available aminoglycosides and were obtained either from Prof. J. DAVIES, University of Wisconsin, or from hospitals in Europe[3].

M.I.C. Determinations

Minimum inhibitory concentrations (MIC) of the compounds were determined by a standard dilution method in Diagnostic Sensitivity Test Agar (DSTA-Oxoid). Serial dilutions (in 2-fold steps) of the compounds were prepared in the agar plates over the required concentration range and, after the surface moisture had been removed by drying at 28 °C, the organisms were inoculated on the surface of the agar using a multipoint inoculator (Denley Instruments Ltd.). The standard inoculum consisted of a 1 in 100 dilution of an overnight broth culture (Brain-Heart Infusion) and subsequent incubation was for 18 hours at 37°C. The MIC was the lowest concentration of compound to completely inhibit growth of the organism.

Experimental Infections in Mice

Acute systemic infections in mice were produced by intraperitoneal inoculation of standardized bacterial cultures suspended in 5% hog gastric mucin. The challenge dose was generally 1 to 10 lethal doses, *i.e.* 1 to 10 times the number of organisms needed to kill 100% of the mice within 72 hours, depending on the organism. The dosage regimen for all experimental infections was 0.5 and 4 hours post-infection and all compounds were administered subcutaneously. After 72 hours, a 50% protective dose value (PD_{50}), expressed in mg of compound per kg body-weight, was calculated by a probit method. The PD_{50} values quoted were based on the dose of compound given in each injection. All mice used were female, strain CD-1 with an average weight of 20 g and were obtained from the Charles River Company.

Fig. 1.

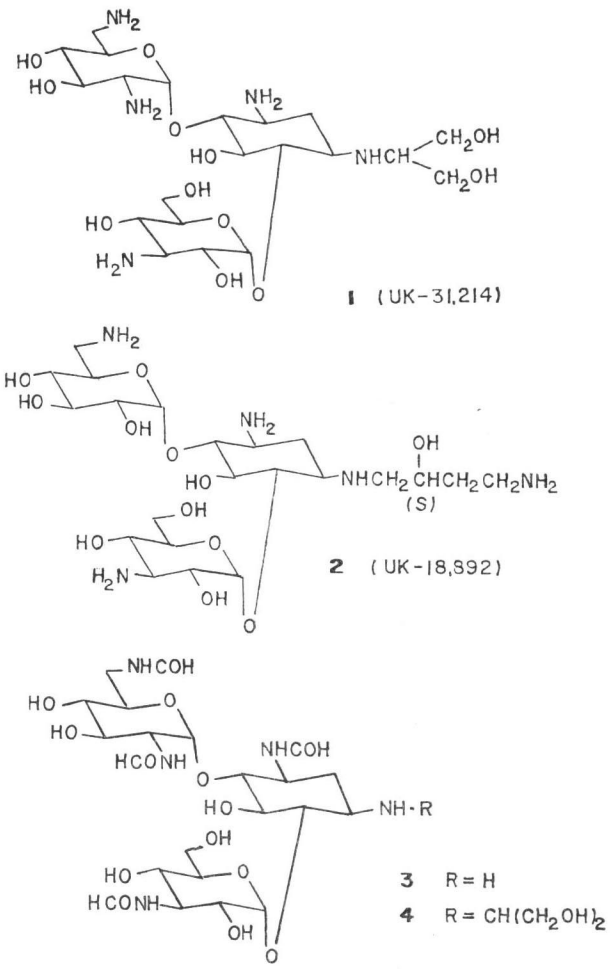

Table 1. Electrophoretic behaviour of compounds **1** and **4**.

Compound	Electrophoretic mobility relative to 2′,3,3″,6′-tetraformyl kanamycin B*
1	0.91
4	0.40

* Thin-layer electrophoresis on glass plates coated with silica (0.25-mm thick). Using 1 : 1.5 mixture of 2 M formic acid and 2 M acetic acid and a potential of 900 volts for 90 minutes. Detection was by means of hypochlorite-starch iodide and under these conditions 2′,3,3″,6′-tetraformylkanamycin B (**3**) had a relative mobility of 1.0.

Results

1-N-(1,3-Dihydroxy-2-propyl)kanamycin B (UK-31,214, **1**, Fig. 1) was prepared from a derivative of kanamycin B in which all of the amino groups were protected except for the 1-amine. Thus 2′,3,3″,6′-tetra-N-formylkanamycin B was reductively alkylated at the 1-position, using 1,3-dihydroxyacetone and sodium cyanoborohydride in dimethylsulfoxide, in 84.5% yield. The formyl protecting groups were removed by treatment with 1 N sodium hydroxide solution at 55°C for 3.5 hours to give UK-31,214 (**1**) in 59.9% yield. The *in vitro* activity of UK-31,214 against 25 aminoglycoside-sensitive strains of bacteria are shown in Table 2 in comparison with gentamicin and kanamycin B.

From the data shown in Table 2 it is apparent that UK-31,214 has an antibacterial spectrum similar to that of gentamicin with good activity against *Staphylococcus aureus* and a wide range of Gram-negative bacteria. Overall, UK-31,214 is approximately one third as potent as gentamicin against these aminoglycoside-sensitive bacteria. It is about half as potent as kanamycin B against most of these bacteria with the notable exception of *Pseudomonas aeruginosa* where UK-31,214 is four to eight times more potent than kanamycin B. Good activity is also shown against several aminoglycoside-resistant bacteria possessing known inactivating enzymes (Table 3). These bacteria were chosen because they contain the inactivating enzymes that are most frequently encountered in aminoglycoside-resistant bacteria found in clinical practice[3]. It appears from the data shown in Table 3 that UK-31,214 may be resistant to inactivation by enzymes capable of 3′-phosphorylation, 3-acetylation or 2″-adenylylation. Kanamycin B has poor activity against all of these bacteria whilst gentamicin has weak activity against those strains possessing enzymes capable of 3-acetylation or 2″-adenylylation.

Table 2. Activity *in vitro* against aminoglycoside-sensitive bacteria (MIC in mcg/ml).

Organism		Gentamicin*	**1** (UK-31,214)	Kanamycin B*
E. coli	E104	0.8	3.1	1.6
	E110	0.8	3.1	1.6
	E116	0.8	1.6	1.6
	E172	0.8	3.1	1.6
	E10	0.8	3.1	>100
	E36	1.6	3.1	1.6
	E51	1.6	3.1	>100
	E173	0.8	1.6	0.8
Pr. mirabilis	P133	1.6	6.2	3.1
	P8	1.6	3.1	1.6
Pr. vulgaris	P136	0.8	6.2	1.6
	P124	0.8	0.8	3.1
Ps. aeruginosa	Ps56	0.8	1.6	12.5
	Ps52	0.8	1.6	12.5
	Ps48	0.8	1.6	12.5
	Ps169	1.6	6.2	25
Klebsiella	K37	0.8	1.6	1.6
	K38	0.4	0.8	0.4
K. aerogenes	K33	0.8	1.6	0.8
	K39	0.8	1.6	12.5
	K40	0.8	1.6	0.8
S. aureus	S223	0.2	0.8	0.2
	S222	0.2	0.8	0.2
	S202	0.8	6.2	1.6
	S208	0.2	0.8	0.4

* The gentamicin used in this study was obtained commercially as gentamicin sulphate (Roussel). The kanamycin B used was obtained commercially as Kanendomycin (Meiji).

The activity of UK-31,214 has been further evaluated against recent clinical isolates of aminoglycoside-resistant strains of *Escherichia coli, P. aeruginosa, Enterobacter* spp., *Klebsiella pneumoniae* and *Serratia* spp. and the results are shown in Table 4 in comparison with amikacin. This data confirms that UK-31,214 has potent activity against aminoglycoside-resistant bacteria.

The efficacy of UK-31,214 has also been examined in mouse-protection tests against lethal bacterial infections, and the results from these studies are shown in Table 5. It is clear

Table 3. Activity *in vitro* against aminoglycoside-resistant bacteria (MIC in mcg/ml).

Organism	Resistance mechanism*	Gentamicin	Kanamycin B	UK-31,214 (1)
P. aeruginosa (130)	AAC(3)	12.5	25	3.1
P. aeruginosa (209)	AAC(3)/AP(3′)**	12.5	25	3.1
E. coli (JR 35/25)	AP(3′)-I	1.6	>100	6.2
E. coli (JR66/W677)	AAD(2″)/AP(3′)-II	12.5	>100	3.1

* AAC(3): 3-acetyltransferase, AP(3′): 3′-phosphotransferase, AAD(2″): 2″-adenylsynthetase.
** Classification as AP(3′)-I or -II not proven.

Table 4. Activity of UK-31,214 against aminoglycoside-resistant bacteria*.

Organism	Amikacin**	UK-31,214
E. coli (6)	3.9	4.4
P. aeruginosa (3)	3.9	3.9
Enterobacter spp. (4)	1.6	1.9
K. pneumoniae (4)	1.9	1.9
S. marcescens (3)	3.9	3.9

( ) indicates number of isolates evaluated.
* Numbers indicate geometric mean MIC values. (All strains were resistant to gentamicin and/or kanamycin A).
** Amikacin was generously provided by Dr. K. E. PRICE, Bristol Laboratories, U.S.A.

Table 5. PD_{50} values (mg/kg body weight) of UK-31,214 against acute bacterial infections in mice.

Organism	PD_{50}
E. coli E172	2.67
P. aeruginosa Ps48	7.83
S. aureus S223	3.98
K. aerogenes K33	1.54

from the data that UK-31,214 offers good protection against these infections due to *E. coli*, *P. aeruginosa*, *S. aureus* and *Klebsiella aerogenes*.

Discussion

Several 1-N-alkyl derivatives of kanamycin A have been reported recently by RICHARDSON *et al.*[1] and shown to have potent activity against both aminoglycoside-sensitive and aminoglycoside-resistant bacteria. These studies have been extended to include 1-N-alkyl derivatives of kanamycin B and this paper describes the properties of one of these compounds, namely UK-31,214 which is the 1-N-(1,3-dihydroxy-2-propyl) derivative of kanamycin B.

It is clear from the data in Table 2 that UK-31,214 is approximately half as potent as kanamycin B against the aminoglycoside-sensitive strains of *E. coli, Proteus* spp., *Klebsiella* spp. and *S. aureus,* but it is approximately 8-fold more potent against the isolates of *P. aeruginosa.* This increase in potency against isolates of *P. aeruginosa* is to be expected since we have previously shown[1] that several 1-N-ω-amino-α-hydroxyalkyl derivatives of kanamycin A have potent activity against *P. aeruginosa* whereas kanamycin A is only weakly active[3]. It has been suggested[4] that *P. aeruginosa* strains in general possess enzymes capable of 3′-phosphorylation and it therefore seemed possible that UK-31,214 is resistant to this mode of inactivation. To evaluate this possibility we examined UK-31,214 against bacterial isolates possessing known aminoglycoside-inactivating enzymes (Table 3). UK-31,214 demonstrated good activity against bacterial isolates possessing enzymes capable of 3′-phosphorylation, 3-acetylation and 2″-adenylylation. In contrast kanamycin B was either inactive or only weakly active and gentamicin has poor activity against those bacteria possessing either 3-acetylating or 2″-adenylylating ability. In view of its good activity against these four aminoglycoside-resistant organisms, UK-31,214 was evaluated against twenty recent clinical isolates of aminoglycoside-resistant bacteria (Table 4). Against these isolates of *E. coli, P. aeruginosa, Enterobacter* spp., *Klebsiella* spp. and *Serratia* spp., UK-31,214 demonstrated activity similar to that shown by amikacin. UK-31,214 was also effective against lethal bacterial infections in mice (Table 5). Preliminary results from animal studies indicate that UK-31,214 may have reduced potential for ototoxicity and nephrotoxicity compared with that of

commercially available aminoglycosides[5]. In view of these favourable properties UK-31,214 is being progressed to more extensive pre-clinical evaluation.

Acknowledgement

We acknowledge the excellent technical assistance provided by H. E. CHEESEMAN and W. A. MILLION.

References

1) RICHARDSON, K.; S. JEVONS, J. W. MOORE, B. C. ROSS & J. R. WRIGHT: Synthesis and antibacterial activities of 1-N-(S)-ω-amino-2-hydroxyalkyl kanamycin A derivatives. J. Antibiotics 30: 843~846, 1977
2) Takeda Chemical Industries Ltd.: Belgian Patent No. 817,546, Jan. 13, 1975
3) JEVONS, S.; H. E. CHEESEMAN & K. W. BRAMMER: *In vitro* studies of UK-18,892, a new aminoglycoside antibiotic. Antimicr. Agents & Chemoth. in press.
4) UMEZAWA, H.; O. DOI, M. OGURA, S. KONDO & N. TANAKA: Phosphorylation and inactivation of kanamycin by *Pseudomonas aeruginosa*. J. Antibiotics 21: 154~155, 1968
5) BAIRD, J. R. C.; A. J. CARTER, D. COWLEY & M. GREGORY: paper in preparation.