MUTATIONAL BIOSYNTHESIS OF BUTIROSIN ANALOGS

Sir:

Butirosins[1] are broad-spectrum antibiotics with activity against *Pseudomonas* strains and are less toxic than other aminoglycoside antibiotics. Studies[2] on the inactivating mechanism of aminoglycoside antibiotics prompted us to prepare new butirosin analogs effective against resistant Gram-negative bacteria.

We now communicate the preparation of 6′-N-methyl and 6′-C-methyl derivatives of butirosins *via* the technique of "mutational biosynthesis".[3~6]

A number of non-butirosins-producing mutants were derived from *Bacillus circulans* MCRL 5001, an isolate in our laboratory and a producer of butirosins and 6′-deamino-6′-hydroxybutirosins,[7] by treatment with N-methyl-N′-nitro-N-nitrosoguanidine (1,000 μg/ml for 30 minutes) using an agar-plug technique[8] with *Pseudomonas aeruginosa* as a test organism. Two of these non-producers, strains MCRL 5003 and MCRL 5004, were found to produce butirosins when the culture medium was supplemented with neamine. These strains were designated neamine-negative mutants. One of these mutants, MCRL 5004, also produced butirosins in the presence of exogenous 2-deoxystreptamine (DOS). In contrast, strain MCRL 5003 produced DOS in the fermentation broth, but could not utilize DOS for biosynthesis of the butirosins, as described in a succeeding communication.[9]

The neamine analogs (100~250 μg/ml) were added to actively growing culture (1~2 days) of the neamine-negative mutants, MCRL 5003 and MCRL 5004, and the culture was further incubated for 4~6 days until antibacterial potency reached a maximum. Both strains converted neamine analogs such as 6′-N-methylneamine (**I**), 3′,4′-dideoxyneamine (**II**), 3′,4′-dideoxy-6′-N-methylneamine (**III**) and 3′,4′-dideoxy-6′-C-methylneamine (**IV**) to the corresponding analogs of butirosins A (*xylo*-isomer) and B (*ribo*-isomer). 6′-N-Methylbutirosins (NMB-A and -B), 3′,4′-dideoxybutirosins,[10,11] 3′,4′-dideoxy-6′-N-methylbutirosins (DMB-A and -B) and 3′,4′-dideoxy-6′-C-methylbutirosins (DCB-A and -B) were produced in the cultured broth supplemented with **I**, **II**, **III** and **IV**, respectively. In the case of **II**, **III** and **IV**, the production of *ribo*-isomers was predominant, whereas bioconversion of **I** gave mainly a *xylo*-isomer.

These butirosin analogs were isolated from the cultured broths by adsorption on Amberlite IRC-50 (NH_4^+ form) resin followed by elution with 1.0 N ammonia. The products were then separated into *xylo*- and *ribo*-isomers by repeating column chromatography on Amberlite CG-50 (NH_4^+ form) and CM-Sephadex C-25 (NH_4^+ form) eluted with dilute ammonia. Further purification was accomplished by column chromatography on Dowex 1×2 (OH^- form) resin developed with water. Four new butirosin analogs thus obtained showed the following physicochemical properties:

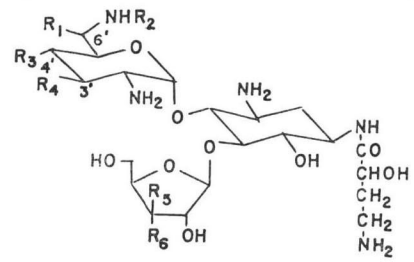
NMB-A, m.p. 188~192°C (dec.); $[\alpha]_D^{25}$ +24.3° (*c* 0.7, H_2O); IR (KBr), 1635 and 1560 cm^{-1} (amide);[Calcd. for $C_{22}H_{43}N_5O_{12}\cdot 2H_2CO_3\cdot 2H_2O$: C 39.51, H 7.05, N 9.60. Found: C 39.62, H 6.58, N 9.71].

DMB-A, m.p. 175~180°C (dec.); $[\alpha]_D^{25}$ +20.7° (*c* 0.3, H_2O); IR (KBr), 1630 and 1560 cm^{-1} (amide);[Calcd. for $C_{22}H_{43}N_5O_{10}\cdot 2H_2CO_3\cdot 2H_2O$: C 41.32, H 7.37, N 10.04. Found: C 41.08, H 6.98, N 10.01].

DMB-B, m.p. 190~195°C (dec.); $[\alpha]_D^{25}$ +25.2° (*c* 0.5, H_2O); IR (KBr), 1630 and 1560 cm^{-1} (amide);[Calcd. for $C_{22}H_{43}N_5O_{10}\cdot 2H_2CO_3\cdot 2H_2O$: C 41.32, H 7.37, N 10.04. Found: C 41.10, H 6.90, N 9.73].

DCB-B, m.p. 188~192°C (dec.); $[\alpha]_D^{25}$ +23.0° (*c* 0.3, H_2O); IR (KBr), 1630 and 1540 cm^{-1} (amide); [Calcd. for $C_{22}H_{43}N_5O_{10}\cdot H_2CO_3\cdot H_2O$: C 44.73, H 7.67, N 11.34. Found: C 44.44, H 7.27, N 11.29]. The structures of these antibiotics shown in Fig. 1 were confirmed by mass,

Fig. 1. Structures of new butirosin analogs



	R_1	R_2	R_3	R_4	R_5	R_6
NMB – A	H	CH_3	OH	OH	OH	H
DMB – A	H	CH_3	H	H	OH	H
DMB – B	H	CH_3	H	H	H	OH
DCB – B	CH_3	H	H	H	H	OH
[Butirosin A	H	H	OH	OH	OH	H
Butirosin B]	H	H	OH	OH	H	OH

Table 1. Antibacterial activities of NMB-A, DMB-A, DMB-B, DCB-B and butirosin A against aminoglycoside-resistant bacteria

Test organisms	Inactivating enzymes[12,13]	Minimal inhibitory concentration (μg/ml)*				
		NMB-A	DMB-A	DMB-B	DCB-B	Butirosin A
Escherichia coli K-12		0.4	0.4	0.8	0.8	0.4
E. coli K-12 ML1630	APH(3′)-I	1.6	1.6	1.6	1.6	1.6
E. coli JR35/C600	APH(3′)-I	0.8	0.8	0.8	0.8	0.4
E. coli K-12 R5	AAC(6′)-1	0.4	0.4	0.8	6.3	100
E. coli JR66/W677	APH(3′)-II AAD(2″)	>100	0.8	1.6	1.6	>100
E. coli A20107	APH(3′)-II	100	1.6	1.6	1.6	100
E. coli A20732	AAD(2″)	0.8	0.8	0.8	0.8	0.8
E. coli A20895	AAC(3)	1.6	1.6	1.6	1.6	0.8
Providencia stuartii #164 A20894	AAC(2′)	>100	25	25	12.5	>100
Pseudomonas aeruginosa A_8		1.6	0.2	0.2	0.2	0.4
P. aeruginosa GN315	AAC(6′)-4	>100	1.6	1.6	6.3	>100

* Agar dilution method; Heart infusion agar (Eiken), 37°C, 18 hours.

^{1}H-NMR, ^{13}C-NMR spectra and chemically by periodate oxidation and analyses of the acid hydrolyzates.

The *in vitro* antibacterial activities of new butirosin analogs NMB-A, DMB-A, DMB-B and DCB-B are shown in Table 1. Among them, DMB-A and -B were broadly active against various types of aminoglycoside-resistant bacteria including the strains having 6′-N-acetylating enzymes, AAC(6′)-1 and AAC(6′)-4, as expected.

The details of the present work will be published elsewhere.

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