AMINOGLYCOSIDE ANTIBIOTICS. III

BIO-ACTIVE DEGRADATION PRODUCTS FROM BUTIROSINS AND SEMI-SYNTHESIS OF BUTIROSIN ANALOGS

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In the course of our search for new antibiotics, a strain of *Bacillus circulans* No. YQW-B6 was isolated from a soil sample collected in Taiwan. This organism produced a complex of aminoglycoside antibiotics designated Bu-1709, and the two major components, A_1 and A_2, were identified as butirosins A (I) and B (II)[2~4], respectively. This paper reports the bioactive degradation products from butirosins and the semi-synthesis of butirosin analogs.

Bio-active Degradation Products from Butirosins

The amide bond of **I** and **II** was cleaved by mild alkaline hydrolysis (0.5 N NaOH, 100°C, 1 hour) to give 5-β-D-xylofuranosylneamine (**III**) and 5-β-D-ribofuranosylneamine (**IV**), respectively, the latter identical with the *Streptomyces* antibiotic ribostamycin[5,6] (Fig. 1).

The furanosyl linkage of **I** and **II** was hydrolyzed by methanolic hydrogen chloride (0.5 N HCl, 25°C, 18 hours) to yield 1-N-(L-γ-amino-α-hydroxybutyryl) neamine (**V**), a new antimicrobial compound (Fig. 2).

Fig. 1.

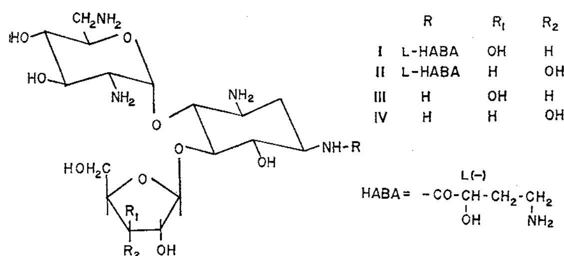
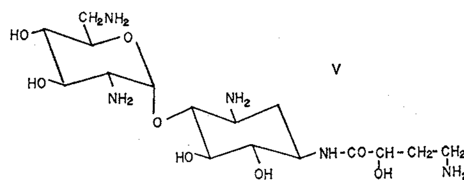


Fig. 2.



The physico-chemical properties of these fragments, **III**, **IV** and **V**, are shown in Table 1 along with those of **I** and **II**. It is interesting to note that the NMR spectra of **I** and **II** show differences in the chemical shift of the anomeric proton of the neamine part (δ 6.10 and 5.98 ppm, respectively) and the same difference is also seen between **III** and **IV** (δ 6.20 and 6.08 ppm, respectively).

The antibacterial spectra of **I**, **II**, **III**, **IV** and **V** are shown in Table 2 comparatively with kanamycin and neamine. Several strains of aminoglycoside-resistant organisms, for which the mechanism of inactivation of antibiotics has been reported, are included in the spectrum. The activities and spectra of **III** and **IV** are essentially the same and in agreement with those published

Table 1. Physico-chemical properties of butirosin and the degradation products

Compound	I	II	III	IV	V
	Butirosin A ($Bu-1709A_1$)	Butirosin B ($Bu-1709A_2$)	5-β-D-Xylofuranosylneamine	5-β-D-Ribofuranosylneamine (Ribostamycin)	1-N-(γ-Amino-α-hydroxybutyryl) neamine
M. p. (°C)	166~168 (dec.)	172~173 (dec.)	134~137 (dec.)	133~137 (dec.)	138~150 (dec.)
$[\alpha]_D$ in H_2O	+25.7° (*c* 1.5)	+37.5° (*c* 1.5)	+51° (*c* 1.0)	+61° (*c* 1.0)	+28.5 (*c* 1.0)
NMR (δ ppm) in D_2O+H^+	1.2~2.2 (m, 4H) 2.5~4.3 (m, 18H) 5.16 (s, 1H) 6.10 (d, J=3.6, 1H)	1.2~2.2 (m, 4H) 2.5~4.3 (m, 18H) 5.16 (s, 1H) 5.98 (d, J=3.6, 1H)	1.7~2.8 (m, 2H) 3.2~4.4 (m, 16H) 5.40 (s, 1H) 6.20 (d, J=3.6, 1H)	1.6~2.9 (m, 2H) 3.2~4.4 (m, 16H) 5.40 (s, 1H) 6.08 (d, J=3.6, 1H)	1.5~2.4 (m, 4H) 3.0~4.4 (m, 14H) — 5.84 (d, J=3.6, 1H)
TLC* (Rf)					
S-106	0.23	0.37	0.46	0.56	—
S-108	0.27	0.27	0.20	0.20	0.26
S-110	0.21	0.21	0.43	0.43	0.22
S-111	0.07	0.18	0.36	0.50	—
S-115	0.30	0.08	0.24	0.05	0.13
Relative potency**	1,000 u/mg	980 u/mg	800 u/mg	750 u/mg	480 u/mg

* S-106: N/50 H_2SO_4-treated carbon plate, developed by N/2 H_2SO_4
S-108: silica gel plate, Me_2CO-AcOH-H_2O (20 : 6 : 74)
S-110: silica gel plate, $CHCl_3$-MeOH-28% NH_4OH-H_2O (1 : 4 : 2 : 1)
S-111: silica gel treated with pH 2 borate buffer, $CHCl_3$-MeOH-28% NH_4OH-5% H_3BO_3 (1 : 4 : 2 : 1)
S-115: alumina plate, upper phase of $CHCl_3$-MeOH-17% NH_4OH (2 : 1 : 1)
** Agar diffusion assay on *B. subtilis* plate. Standard: Butirosin A (1,000 units/mg)

for ribostamycin[5]. When assayed by the agar diffusion method on a *Bacillus subtilis* plate with butirosin A (**I**) as standard (1,000 u/mg), **III** and **IV** showed a potency of 750~800 u/mg. The antibacterial spectra of **III** and **IV** are similar to those of kanamycin and neamine, with no activity against the aminoglycoside-resistant organisms.

Compound **V**, which is a new neamine derivative acylated at the C-1 amino group with L(—)-γ-amino-α-hydroxybutyric acid (L-HABA), exhibits an interesting antibacterial spectrum. It inhibits growth of *Escherichia coli* A 20363 (ML-1630)[7] and *E. coli* JR 35/C 600[9] which have been reported to inactivate kanamycin and neomycin by 3′-phosphorylation (neomycin phosphotransferase I[15]). However, **V** is not active against *E. coli* JR 66/W677[10] and *Klebsiella pneumoniae* type 22 # 3038[10] which are known to produce the neomycin phosphotransferase II[15]. Recently YAGISAWA *et al.*[16] reported that the enzyme obtained from *E. coli* JR 66/W 677 phosphorylated the 3′-hydroxyl group of butirosin A, kanamycin, neamine and ribostamycin. Thus, the antibacterial features of **V** are quite similar to those of butirosin, but **V** is different from butirosin in that it is much less active than butirosin against *E. coli* NR 79/W 677 and *S. aureus* A 20239. *E. coli* NR 79/W 677 has been reported to acetylate the aminoglycoside antibiotics at the 6′-amino group[8]. On the other hand, it is interesting that **V** is relatively more active than butirosin against *Pseudomonas aeruginosa* strain 130 which is known to inactivate gentamicin C by acetylation at the C-3 amino group of 2-deoxystreptamine part[14].

The strucrure-activity relationship observed with butirosin and its bio-active fragments suggests that a pentose moiety at the C-5 position of neamine increases the intrinsic activity but does not

Table 2. Antibacterial spectra of butirosins and the degradation products

Test organism	Code #	MIC (mcg/ml)							Inactivating enzyme	
		KM*	NA*	I	II	III	IV	V	Substrate*	Mechanism
Staphylococcus aureus FDA 209P	Sa-1	0.8	3.1	0.8	0.8	1.6	1.6	1.6		
〃 〃 Smith	Sa-2	0.4	1.6	0.8	0.8	1.6	1.6	1.6		
〃 〃 A20239	Sa-10	50	>100	6.3	6.3	>100	>100	100		
Escherichia coli NIHJ	Ec-1	0.8	3.1	0.8	0.8	1.6	1.6	3.1		
〃 〃 Juhl	Ec-3	1.6	6.3	0.8	0.8	3.1	6.3	6.3		
〃 〃 A20363 (ML-1630)	Ec-5	100	>100	0.8	0.8	>100	>100	6.3	KM	3′-Phosphorylation[7]
〃 〃 K 12	Ec-8	0.8	6.3	0.4	0.4	0.4	0.4	1.6		
〃 〃 NR79/W677	Ec-9	6.3	50	3.1	3.1	12.5	12.5	50	KM	6′-Acetylation[8]
〃 〃 JR35/C600	Ec-10	100	>100	0.2	0.2	100	100	1.6	KM, NM	3′-Phosphorylation[9]
〃 〃 W677	Ec-52	0.8	6.3	0.8	0.8	0.8	1.6	6.3		
〃 〃 JR66/W677	Ec-53	100	>100	50	50	>100	>100	>100	KM, NM, GM, DKB	3′-Phosphorylation[10] 2′′-Adenylation[10,11]
Klebsiella pneumoniae D-11	Kp-1	0.2	0.8	0.1	0.1	0.4	0.4	0.4		
〃 〃 Type 22, #3038	Kp-8	>100	>100	50	100	>100	>100	>100	KM GM	3′-Phosphorylation[10] 2′′-Adenylation[10]
Pseudmouas aeruginosa D-15	Pa-1	25	>100	3.1	3.1	>100	100	3.1		
〃 〃 A9930	Pa-3	6.3	100	1.6	1.6	100	100	6.3		
〃 〃 H 9	Pa-4	100	100	100	100	100	100	100	KM, NM	3′-Phosphorylation[12,13]
〃 〃 A20718 (strain 130)	Pa-16	50	>100	12.5	12.5	>100	>100	6.3	GM	3-Acetylation[14]
Proteus vulgaris A9436	Pv-1	0.4	6.3	0.8	0.8	1.6	1.6	1.6		
Proteus mirabilis A9554	Pm-1	1.6	6.3	1.6	1.6	3.1	3.1	6.3		
Proteus morganii A9553	Pg-1	0.8	3.1	1.6	1.6	3.1	3.1	6.3		
Proteus rettgeri A15167	Pr-1	0.8	3.1	1.6	1.6	3.1	3.1	6.3		
Mycobacterium 607	M6-1	0.4	12.5	0.8	0.4	3.1	3.1	6.3		
〃 *phlei*	Mp-1	0.4	12.5	0.1	0.1	0.8	0.8	6.3		
〃 *ranae*	Mr-1	0.4	12.5	0.4	0.4	1.6	1.6	6.3		

* KM: kanamycin, NA: neamine, NM: neomycin, GM: gentamicin C, DKB: 3′, 4′-dideoxykanamycin B

change the spectrum appreciably as seen in the comparison of **I** (or **II**) *vs.* **V** and **III** (or **IV**) *vs.* neamine, and that N-acylation with L-HABA at the C-1 amino group of neamine moiety plays an important role in potentiating the activity and broadening the spectrum as shown in the comparisons of **I** (or **II**) *vs.* **III** (or **IV**), and **V** *vs.* neamine.

Semi-synthesis of Butirosin Analogs

The synthesis of butirosin analogs from compounds **III** and **IV** blocked at all amino groups except C-1 amino with dimedone was reported by HASKELL *et al.* recently[17]. Independently we have prepared several analogs of butirosin by selective acylation of **III** or **IV***. The synthetic procedure was essentially the same as that reported previously[1]. The 6′-amino group of **III** was found to be the most reactive among the four amino groups of compound **III**, and the 6′-N-acylation products of **III** shown in Fig. 3 have been prepared by reacting **III** with an equimolar amount of the N-hydroxysuccinimide (NOS) ester of N-protected amino acids. The N-protection of amino acid was generally made by carbobenzoxylation, with subsequently deblocking by hydrogenolysis over palladium-charcoal.

Compound **3** is a position isomer of butirosin A (**I**) but has only weak biological activity (about 1/50 of **I**). The other three derivatives in this class are also very weakly active. The properties and activities of these compounds are shown in Table 3. In the NMR spectrum of compound **3**,

Fig. 3.

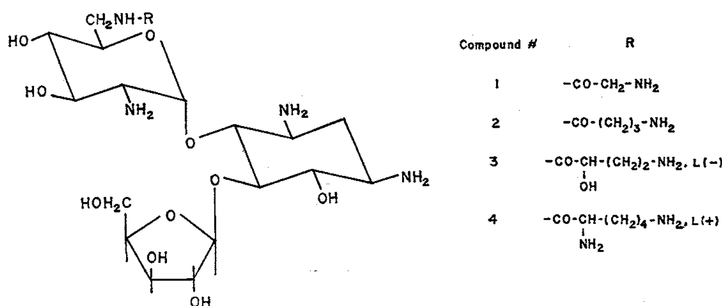
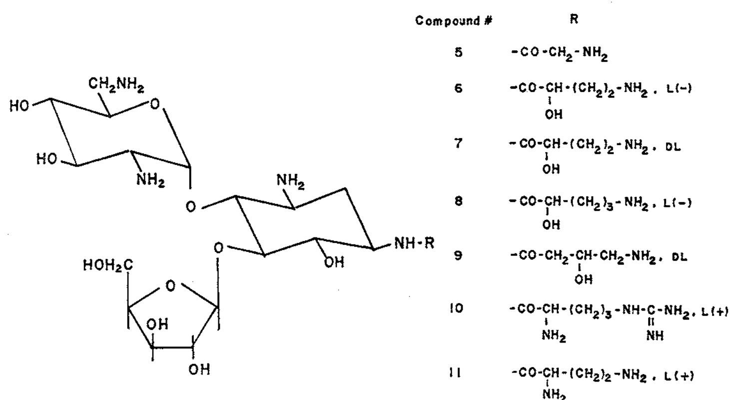


Fig. 4.



* Compound **IV** gave acylated derivatives with essentially the same activity as those obtained from compound **III**. The following descriptions are therefore confined to the derivatives of III.

Table 3. Properties and activities of semi-synthetic butirosin analogs

Compound #	Acylation site	Acyl residue	Melting point (°C)	TLC* (Rf)	Potency** (u/mg)	MIC*** (mcg/ml)					
						Sa-1	Sa-10	Ec-1	Ec-5	Pv-1	Pa-1
1	6′	Glycyl	177 (dec.)	0.51	51	50	>100	25	>100	25	>100
2	6′	γ-Aminobutyryl	153 (dec.)	0.30	20	50	>100	50	>100	50	>100
3	6′	L(−)-γ-Amino-α-hydroxybutyryl	154~158 (dec.)	0.40	22	25	>100	50	>100	25	>100
4	6′	L(+)-Lysyl	173 (dec.)	0.25	6	50	>100	50	>100	50	>100
5	1	Glycyl	175~178 (dec.)	0.46	110	12.5	>100	3.1	25	3.1	100
6	1	L(−)-γ-Amino-α-hydroxybutyryl	175~179 (dec.)	0.20	960	0.8	6.3	0.4	0.8	0.4	3.1
7	1	DL-γ-Amino-α-hydroxybutyryl	174~178 (dec.)	0.20	550	1.6	12.5	0.8	1.6	0.8	6.3
8	1	L(−)-δ-Amino-α-hydroxyvaleryl	173~176 (dec.)	0.20	709	1.6	12.5	0.8	1.6	0.8	12.5
9	1	DL-γ-Amino-β-hydroxybutyryl	172~176 (dec.)	0.36	82	6.3	50	3.1	12.5	3.1	50
10	1	L(+)-Arginyl	203~210 (dec.)	0.03	117	12.5	50	3.1	12.5	12.5	50
11	1	L(+)-α, γ-Diaminobutyryl	>250 (dec.)	0.20	60	25	100	3.1	12.5	6.3	50
12	3 or 2′	L(−)-α-Amino-α-hydroxybutyryl	198~203 (dec.)	0.18	10	100	>100	100	>100	100	100
III	—	—	134~137 (dec.)	0.42	800	1.6	>100	1.6	>100	0.8	100

* TLC system: silica gel plate, $CHCl_3$-MeOH-28% NH_4OH-H_2O (1:4:2:1)

** agar plate assay on *B. subtilis* plate, assay standard: Butirosin A (1,000 u/mg)

*** agar dilution method, nutrient agar medium. Code No. of test organisms shown in Table 2.

the signal of the anomeric proton of the neamine moiety appeared at δ 5.87 ppm (d), about 0.23 ppm higher than in butirosin A (δ 6.10 ppm). Such shielding effect was seen in all the other 6′-N-acyl derivatives, suggesting that the 6′-N-substitution is sterically closer to the anomeric proton of neamine than the 1-N-substitution.

The second most reactive amino function of **III** was found to be the C-1 amino group of the deoxystreptamine moiety. The 6′-amino group of **III** was protected by carbobenzoxylation and the product was then reacted with an equimolar amount of acylating agents to give the series of 1-N-acyl derivatives shown in Fig 4. Compound **6** was identical with butirosin A obtained by fermentation. Butirosin B has recently been synthesized by IKEDA *et al.*[18] from **IV** through an elegant sequence involving partial deblocking of fully blocked ribostamycin.

The properties and activities of the 1-N-acyl derivatives of **III** are shown in Table 3. This series of compounds have in general much greater activity than the 6′-N-acyl derivatives. Acylating agents which have both an α-hydroxy and an ω-amino group gave superior derivatives (compounds **6**, **7** and **8**). Furthermore, the configuration of the α-hydroxy group seems to be important for biological activity since compound **7** from racemic amino acid is about half as active as compound **6**, semi-synthetic butirosin A.

The N-acylation of **III** at the third most reactive amino function was achieved in a similar fashion to that described above, except that 1, 6′-di-N-carbobenzoxy derivative of **III** was used as the starting material. Acylation with a mole of L-HABA gave compound **12** which was practically inactive as shown in Table 3. Whether compound **12** is acylated at C-3 or C-2″ has not been determined.

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