NOTES

AMINOGLYCOSIDE ANTIBIOTICS. IV
BU-1709 E_1 AND E_2, NEW AMINOGLYCOSIDE ANTIBIOTICS RELATED TO THE BUTIROSINS

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In a previous paper[1] we reported that *Bacillus circulans*, strain No. YQW-B 6, produced a complex of aminoglycoside antibiotics designated Bu-1709, of which the two major components, A_1 and A_2, were identified as butirosins A and B[2]. Two additional components, E_1 and E_2, have now been isolated and characterized. They have antibacterial spectra similar to the butirosins but with substantially less intrinsic activity. Bu-1709 E_1 and E_2 contain the paromamine moiety in place of the neamine moiety of the butirosins.

Isolation and Characterization

The components of the Bu-1709 complex were separated by ion-exchange chromatography on Amberlite CG-50 (NH_4^+). The column was developed with an increasing concentration of aqueous ammonium hydroxide. Components E_1 and E_2 were eluted with N/5 NH_4OH and butirosins with N/2 NH_4OH. The crude mixture of E_1 and E_2 was purified by Dowex 1×2 (OH^-) chromatography and the components separated with Amberlite CG-50 (NH_4^+), E_2 being eluted with N/8 NH_4OH followed by E_1 with N/6 NH_4OH.

Bu-1709 E_1 and E_2 were isolated as white amorphous powders. They are basic water-soluble antibiotics giving positive reactions with ninhydrin, ELSON-MORGAN, EHRLICH and anthrone reagents but negative with TOLLENS and SAKAGUCHI. The molecular formula of $C_{21}H_{40}N_4O_{13}$ was assigned to both components E_1 and E_2. The specific rotation determined in water was +28° (*c* 1.47) for E_1 and +33° (*c* 1.30) for E_2. The presence of two anomeric protons was indicated by NMR signals at 5.35 (s) and 5.85 ppm (d, J=6 Hz) for E_1, and at δ 5.38 (s) and 5.80 ppm (d, J=6 Hz) for E_2. Both components showed the same Rf value (0.35) in TLC system S-110* but they were differentiated by system S-115,** the Rf being 0.25 for E_1 and 0.05 for E_2. Butirosins A and B showed similar behavior with an Rf of 0.21 with system S-110 but were differentiated by system S-115 (A, 0.25; B, 0.05).

The antibacterial activity of Bu-1709 E_1 and E_2 are essentially the same. Table 1 shows the antibacterial spectrum of component E_1 compared with butirosin A. Component E_1 inhibits, as does butirosin, some of the kanamycin-neomycin resistant organisms (Ec-5, Ec-7 and Ec-10 in Table 1) which are known to produce neomycin phosphotransferase I[3], but the intrinsic activity of E_1 is approximately 1/8 to 1/16 that of butirosin A against most of the test organisms. However, Bu-1709 E_1 is as active as butirosin A against Ec-9 (*Escherichia coli* NR 79/W 677) which is known to produce kanamycin 6′-acetyltransferase[5].

Structure of Bu-1709 E_1 and E_2

Bu-1709 E_1 was hydrolyzed in methanolic hydrogen chloride (0.5 N) at room temperature for 27 hours. The hydrolyzate was diluted with ether to precipitate a weakly bioactive fragment (**I**) which was purified by Dowex 1×2 (OH^-). Compound **I** was analyzed as $C_{16}H_{32}N_4O_9 \cdot H_2CO_3$, mp 196°C (dec.), $[\alpha]_D^{21.3}$ +68° (*c* 0.88, H_2O). The IR spectrum of **I** showed amide absorption bands at 1640 and 1550 cm^{-1}. The presence of one anomeric proton, δ 5.73 ppm (d, J=6 Hz), was indicated by NMR. Evaporation of the mother liquor gave a methyl glycoside (**II**) which was identified as methyl D-xyloside by TLC and also by gas chromatography of the trimethylsilyl derivative of **II**. Likewise, methanolysis of component E_2

* silica gel plate, $CHCl_3$-MeOH-28% NH_4OH-H_2O (1:4:2:1).
** alumina plate, upper phase of $CHCl_3$-MeOH-17% NH_4OH (2:1:1)

Table 1. Antibacterial spectra of Bu-1709 E_1 and butirosin A (Agar dilution method on nutrient agar)

Test organism	Code #	Resistant to	MIC (mcg/ml)		MIC ratio
			Bu-1709 E_1	Butirosin A	
Staphylococcus aureus Smith	Sa-2		6.3	0.4	16
〃 〃 R 4	Sa-4	SM	50	6.3	8
〃 〃 A 20239	Sa-10	KM, NM	25	3.1	8
Escherichia coli NIHJ	Ec-1		6.3	0.8	8
〃 〃 Juhl	Ec-3		12.5	0.8	16
〃 〃 ML-1630	Ec-5	KM, NM	25	1.6	16
〃 〃 A20365	Ec-7	KM, NM	3.1	0.4	8
〃 〃 K 12	Ec-8		12.5	0.8	16
〃 〃 NR79/W677	Ec-9	KM	6.3	6.3	1
〃 〃 JR35/C600	Ec-10	KM, NM	6.3	0.4	16
〃 〃 W677	Ec-52		12.5	0.8	16
〃 〃 JR66/W677	Ec-53	KM, NM, GM	>100	50	—
Klebsiella pneumoniae D-11	Kp-1		0.8	0.2	4
〃 〃 Type 22, # 3038	Kp-8	KM, NM, GM	>100	50	—
Serratia marcescens A 20019	Sm-1		6.3	3.1	2
Pseudomonas aeruginosa D-15	Pa-1		50	6.3	8
〃 〃 A9930	Pa-3		6.3	0.8	8
〃 〃 H9	Pa-4	KM, NM	>100	>100	—
〃 〃 strain 130	Pa-16	GM	100	12.5	8
Proteus vulgaris A9436	Pv-1		3.1	0.4	8
Proteus mirabilis A9554	Pm-1		6.3	0.8	8
Proteus morganii A9553	Pg-1		6.3	0.8	8
My*cobacterium* 607	M6-1		12.5	0.4	32
〃 *phlei*	Mp-1		3.1	0.2	16
〃 *ranae*	Mr-1		6.3	0.4	16

* KM: kanamycin, NM: neomycin, GM: gentamicin C, SM: streptomycin.

liberated **I** and methyl D-riboside (**III**). Thus the difference between E_1 and E_2 appeared to reside in the pentose moiety as is the case with butirosins A and B.

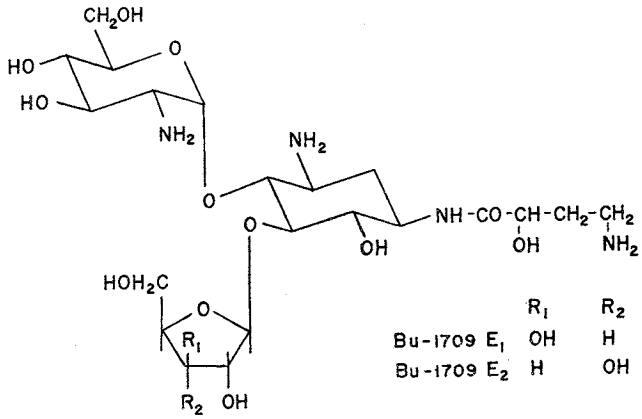
Alternatively, alkaline hydrolysis (0.5 N NaOH, 120°C, 1 hour) of Bu-1709 E_2 and chromatographic fractionation (Amberlite CG-50, NH_4^+ type, developed with water and N/10 NH_4OH) yielded an amino acid (**IV**) and a bioactive product (**V**). The crude amino acid fraction was purified by Amberlite IR-120 (H^+) and crystallized from 90 % aqueous ethanol to give colorless needles, $C_4H_9NO_3$, mp 213~215°C, $[\alpha]_D^{23}$ −28° (*c* 0.45, H_2O), which was identical by IR and NMR spectra to L(−)-γ-amino-α-hydroxybutyric acid obtained from butirosins. Compound **V** was isolated as a white amorphous powder, $C_{17}H_{33}N_3O_{11} \cdot H_2CO_3$, mp 168~170°C, $[\alpha]_D^{20.5}$ +42° (*c* 0.5, H_2O). The NMR spectrum of **V** indicated two anomeric protons with signals at δ 5.24 (s) and 5.78 (d, J=3.7 Hz) ppm.

Acid methanolysis of compound **V** (0.5 N HCl, 100°C, 2 hours) yielded a product which was recrystalized from 80 % aqueous ethanol to give colorless needles (**VI**). Microanalysis of **VI** was consistent with the formula $C_{12}H_{25}N_3O_7 \cdot 3HCl$, mp>240°C (dec.), $[\alpha]_D^{20.5}$ +81.3° (*c* 0.8, H_2O). These data along with the IR and NMR spectra established the identity of compound **VI** with paromamine, a degradation product of paromomycin. The filtrate of the above methanolyzate was neutralized with Amberlite IR-4 B (OH^-) and evaporated *in vacuo* to leave a neutral solid which was purified and then identified as methyl D-riboside (**III**).

Thus, compound **V** is D-ribosyl paromamine and hence Bu-1709 E_2 is D-ribosyl paromamine acylated by the amino acid (**IV**) at one of the

three amino groups.

Mild periodate oxidation of component E_2 (M/100 $NaIO_4$, 4°C, 3 days in the dark), followed by acid hydrolysis (1 N HCl, 80°C, 30 min.) and subsequent chromatographic fractionation (Amberlite CG-50) and purification (Dowex 1× 2) yielded a white amorphous solid (**VII**) which was identical with an authentic sample of 1-N-L(−)-γ-amino-α-hydroxybutyryl-2-deoxystreptamine[4] obtained from butirosin A by a similar reaction sequence. A quantitative periodate oxidation study on component E_1, butirosin A and compound **V** showed consumption of 2.6, 2.6 and 4.1 moles, respectively, of the periodate reagent at 3 hours, and 3.0, 2.8 and 4.4 moles, respectively, at 7 hours, indicating the site of the pentose substitution in compound **I** to be the C-5 hydroxyl of 2-deoxystreptamine. The structure shown below was therefore established for Bu-1709 E_1 and E_2.



	R_1	R_2
Bu-1709 E_1	OH	H
Bu-1709 E_2	H	OH

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