SYNTHESIS OF 3′, 4′-DIDEOXYBUTIROSIN B

Sir:

In the foregoing communication[1] we reported the synthesis of 1-N-((s)-4-amino-2-hydroxybutyryl)-3′, 4′-dideoxyneamine and it was shown that the attachment of (s)-4-amino-2-hydroxybutyryl residue to the amino group at C-1 of 3′, 4′-dideoxyneamine enhanced the activity of the parent antibiotic. As an extension of this work, the synthesis of 3′, 4′-dideoxybutirosin B was planned to examine the effect of an (s)-4-amino-2-hydroxybutyryl residue at 1-NH_2 on the antibacterial activity, because the attachment of this group markedly enhanced the antibacterial activity of ribostamycin as reported in a previous paper[2].

When 3, 2′, 6′-tri-N-benzyloxycarbonyl-3′, 4′; 2″, 3″-di-O-cyclohexylidene-1-N-((s)-2-hydroxy-4-phthalimidobutyryl)-5″-O-(1-methoxycyclohexyl) ribostamycin[2] (**1**), previously prepared as an intermediate in the synthesis of butirosin B, was treated with 40 % acetic acid-acetone (1:5) at room temperature for 6 hours, the 5″-O-blocking group was selectively removed to give the di-O-cyclohexylidene-trihydroxy derivative (**2**) in a yield of 71 %, mp 135~138°C (reprecipitated from chloroform-*n*-hexane), $[\alpha]_D^{28}$ + 16.2° (*c* 2, chloroform). [Calcd. for $C_{65}H_{77}N_5O_{20}$: C 62.54, H 6.22, N 5.61; Found: C 62.34, H 6.33, N 5.55].

Acetylation of **2** with acetic anhydride in pyridine gave the tri-O-acetyl derivative (**3**) in a yield of 88 %, mp 114~118°C (from benzene-*n*-hexane), $[\alpha]_D^{25}$+8.9° (*c* 1.7, chloroform). NMR (in $CDCl_3$): τ 7.94, 7.85 and 7.78 (each 3H s, OAc). [Calcd. for $C_{71}H_{88}N_5O_{23}$: C 62.04 H 6.09, N 5.10; Found: C 62.22, H 6.27, N 4.99]. Treatment of **3** with 60 % acetic acid-acetone (4:5) at 60°C for 75 minutes selectively removed the cyclohexylidene group at 3′ and 4′ and tri-O-acetyl-mono-O-cyclohexylidene derivative (**4**) was obtained in a yield of 90 %, mp 117~121°C (from chloroform-*n*-hexane), $[\alpha]_D^{25}$+1.5° (*c* 1.7, chloroform). [Calcd. for $C_{65}H_{75}N_5O_{23}$: C 60.32, H 5.84, N 5.41; Found: C 60.69, H 6.11, N 5.15]. Mesylation of **4** gave the 3′, 4′-di-O-mesyl derivative (**5**) in a yield of 96 %, mp 120~123°C (chloroform-*n*-hexane), $[\alpha]_D^{20}$—8.8° (*c* 2, chloroform). NMR (in $CDCl_3$): τ 7.93, 7.86 and 7.74 (each 3H s, OAc), 7.23 and 6.96 (each 3H s, Ms). [Calcd. for $C_{67}H_{79}N_5O_{27}S_2$: C 55.48, H 5.49, N 4.83, S 4.42; Found: C 55.82, H 5.63, N 4.79, S 4.27].

3′, 4′-Unsaturation of **5** was carried out as described in the previous papers[3] by use of sodium iodide and zinc dust in DMF in the presence of molecular sieve (Union Carbide Co., grade 3A) at 90°C for 1 hour and 5″, 6-di-O-acetyl-1-N-((s)-2-acetoxy-4-phthalimidobutyryl)-3, 2′, 6′-tri-N-benzyloxycarbonyl-2″, 3″-O-cyclohexylidene-3′, 4′-dideoxy-3′-enoribostamycin (**6**) was obtained in a yield of 73 %, mp 99~

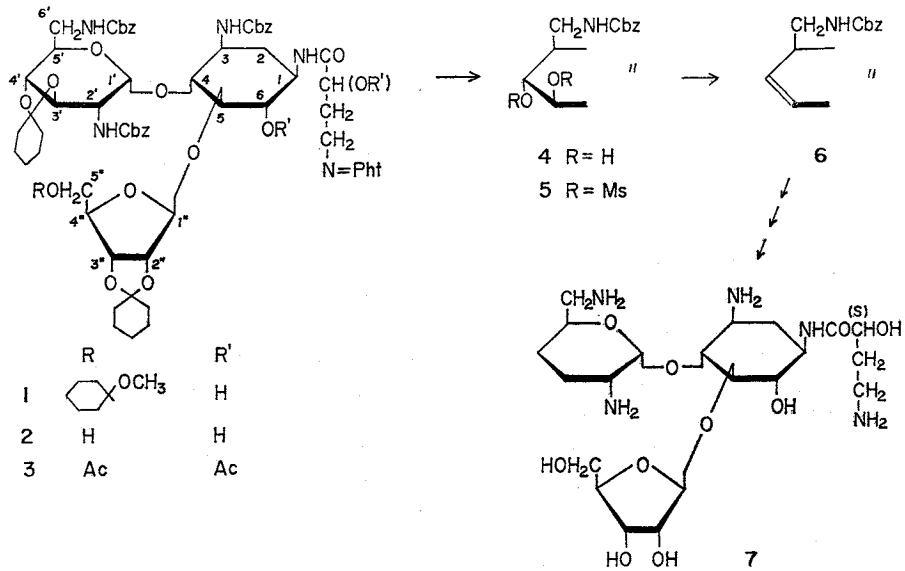

Table 1. Antibacterial spectra of 3′, 4′-dideoxybutirosin B, butirosin B, 3′, 4′-dideoxyribostamycin and ribostamycin

Test organisms*	Minimal inhibitory concentration (mcg/ml)			
	3′, 4′-Dideoxy-butirosin B	Butirosin B	3′, 4′-Dideoxy-ribostamycin	Ribostamycin
Staphylococcus aureus FDA 209P	1.56	1.56	3.12	3.12
Sarcina lutea PCI 1001	25	50	>100	100
Bacillus subtilis NRRL B-558	<0.39	0.39	1.56	3.12
Klebsiella pneumoniae PCI 602	0.78	0.78	3.12	1.56
〃 type 22 #3038	3.12	>100	6.25	>100
Salmonella typhosa T-63	0.39	0.39	1.56	1.56
Escherichia coli NIHJ	1.56	3.12	6.25	6.25
〃 K-12	1.56	0.78	3.12	3.12
〃 〃 R-5	6.25	6.25	100	50
〃 〃 ML 1629	1.56	1.56	>100	>100
〃 〃 ML 1630	0.78	1.56	>100	>100
〃 〃 ML 1410	0.78	0.78	6.25	3.12
〃 〃 〃 R 81	1.56	3.12	>100	>100
〃 〃 LA 290 R 55	1.56	0.78	3.12	3.12
〃 〃 〃 R 56	<0.39	0.78	1.56	3.12
〃 〃 〃 R 64	1.56	0.78	3.12	1.56
〃 〃 C 600 R 135	0.78	0.78	3.12	1.56
〃 〃 W 677	0.78	0.39	3.12	1.56
〃 〃 JR 66/W 677	3.12	>100	6.25	>100
〃 J 5 R 11-2	<0.39	1.56	100	>100
Pseudomonas aeruginosa A 3	6.25	3.12	6.25	>100
〃 No. 12	6.25	6.25	12.5	>100
〃 GN 315	>100	>100	>100	>100
〃 TI-13	12.5	25	25	>100
〃 99	25	50	50	>100
Proteus rettgeri GN 311	12.5	6.25	6.25	12.5
〃 GN 466	3.12	3.12	6.25	6.25
Mycobacterium smegmatis ATCC 607**	<0.39	0.78	3.12	6.25

* Agar dilution streak method (nutrient agar, 37°C, 18 hours).
** 48 hours.

102°C (chloroform-*n*-hexane), $[\alpha]_D^{23}$—24.5° (*c* 2, chloroform). NMR (in $CDCl_3$ at 60 MHz): τ 4.38 (2H broadened singlet, H-3′, 4′). Calcd. for $C_{65}H_{78}N_5O_{21}$: C 61.94, H 5.84, N 5.56: Found: C 61.77, H.5.71, N 5.56].

Compound **6** (70 mg) was successively treated with hydrazine (hydrazine hydrate 0.9 g in 1.8 ml 80 % ethanol)* at 60°C for 2 hours to remove the acetyl and phthaloyl groups, with palladium black and hydrogen to hydrogenate the double bond and to remove the benzyloxycarbonyl groups and with 1N hydrochloric acid at 60°C for 1 hour to remove the cyclohexylidene group to give the deblocked product, which was purified by a column of CM-Sephadex C-25 (NH_4^+ form) with ammonia (0~0.5 N). At the concentration of 0.5 N ammonia, the desired product was eluted, and 1-N-((s)-4-amino-2-hydroxybutyryl)-3′, 4′-dideoxyribostamycin, namely 3′, 4′-dideoxybutirosin B (**7**) was obtained as a monohydrate, 24 mg (80 %), $[\alpha]_D^{16}$+25° (*c* 1.8, water). $Rf_{\text{butirosin B}}$ 1.73 (on paper chromatography with 1-butanol-pyridine-water-acetic acid (6:4:3:1)), Rf 0.24 (on thin-layer chroma-

* If the amount of hydrazine was reduced, a large proportion of 1-N-(4-acetamido-2-hydroxybutyryl) derivative was formed rendering the purification of **7** difficult.

tography with silica gel and chloroform-methanol-17 % ammonia (1:4:3) (Solvent A)). [Calcd. for $C_{21}H_{41}N_5O_{10}\cdot H_2O$: C 46.57, H 8.00, N 12.93; Found: C 46.74, H 7.70, N 13.13].

Hydrolysis of 7 (Rf 0.24 with Solvent A) with 0.4 N hydrogen chloride in methanol at 70°C overnight gave 1-N-((s)-4-amino-2-hydroxybutyryl)-3′, 4′-dideoxyneamine[1] (Rf 0.32, major), 3′, 4′-dideoxyneamine[4] (Rf 0.59) and (s)-4-amino-2-hydroxybutyric acid (Rf 0.59).

The synthetic 3′, 4′-dideoxybutirosin B showed strongly enhanced antibacterial activity (Table 1) as compared with that of ribostamycin and 3′, 4′-dideoxyribostamycin and was comparable to that of butirosin B. Moreover it was effective against *Klebsiella pneumoniae* type 22 #3038 and *Escherichia coli* K-12 JR 66/W 677, which were resistant to butirosin B. *E. coli* K-12 JR 66/W 677 is known[5] to produce an enzyme phosphorylating 3′-hydroxyl group of butirosin A.

These results indicate that combination of 3′, 4′-dideoxygenation and the attachment of (s)-4-amino-2-hydroxybutyryl residue to the 1-NH_2 group of ribostamycin gives a derivative markedly effective against both sensitive and resistant bacteria.

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