SYNTHESIS OF BUTIROSIN B

Sir:

Butirosins[1] are new aminoglycosidic antibiotics active against some kanamycin-resistant bacteria, and butirosin B is, structurally, the 1-N-((s)-4-amino-2-hydroxybutyryl) derivative of ribostamycin,[2] against which the above bacteria are resistant. The present paper deals with the synthesis of butirosin B from ribostamycin.

Tetra-N-benzyloxycarbonyl-3′,4′; 2″,3″-di-O-cyclohexylidene-5″-O-(1-methoxycyclo-hexyl) ribostamycin (**1**), which was an intermediate in the synthesis of 3′,4′-dideoxyribostamycin,[3] was dissolved in dry DMF and after displacement of the air in the reaction vessel with nitrogen, approximately 1~3 molecular equivalents of sodium hydride were added to the solution. The mixture was agitated at room temperature for 2 hours. The resulting pale yellow solution was neutralized with acetic acid, evaporated, and extracted with chloroform. The product, mp 125-128°C, $[\alpha]_D^{25}$ +14.4° (*c* 2, chloroform) had an absorption peak at 1770 cm^{-1} characteristic of *trans*-fused cyclic carbamates and proved to be tri-N-benzyloxycarbonyl-3′,4′; 2″,3″-di-O-cyclohexylidene-5″-O-(1-methoxycyclohexyl)ribostamycin-1,6-carbamate (**2**); ir, 1770, 1720, 1535 cm^{-1}. [Found: C 63.55, H 7.03, N 4.68. Calcd. for $C_{61}H_{78}N_4O_{18}$: C 63.42, H 6.80, N 4.85].

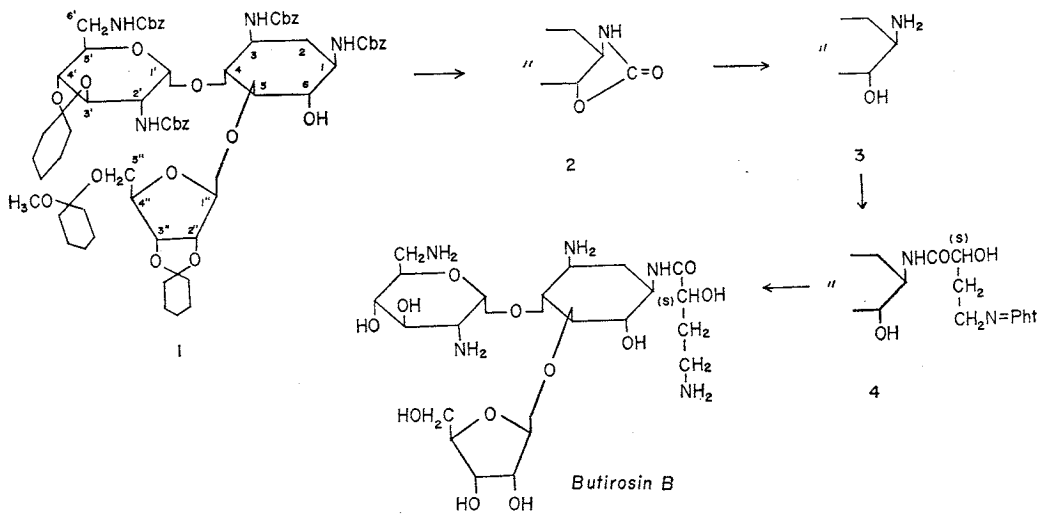
The formation of the cyclic carbamate can be interpreted by initial attack of sodium hydride on the C-6 hydroxyl followed by an anchimeric attack of the resulting alkoxide ion on the urethane carbonyl at C-1. In the past, *trans*-fused cyclic carbamates were prepared by MIYAI and GROSS[4] using N,N′-carbonyldiimidazole and by us[5] using *p*-nitrophenoxycarbonyl chloride or phenoxycarbonyl chloride. The latter reaction occurs even in aqueous media.

Compound **2** was then dissolved in aqueous dioxane and 1.1 molecular equivalents of barium hydroxide was gradually added to the solution, at about 80°C. A weakly ninhydrin-positive product, 3, 2′, 6′-tri-N-benzyloxycarbonyl-3′,4′; 2″,3″-di-O-cyclohexylidene-5″-O-(1-methoxycyclohexyl)ribostamycin (**3**) was isolated in 70% yield, mp 103~106°C, $[\alpha]_D^{27}$ +16.7° (*c* 2.1, chloroform). The absorption peak at 1770 cm^{-1} characteristic of the cyclic carbamate had disappeared; ir, 1720, 1530 cm^{-1}. [Calcd. for $C_{60}H_{80}N_4O_{17}$: C 63.81, H 7.14, N 4.96. Found: C 63.69, H 7.28, N 4.90].

Thus, the cyclic carbamate was selectively hydrolyzed into free amino and hydroxyl groups, retaining the other three benzyloxycarbonylamino groups intact.

The monoamino derivative (**3**) was then condensed with (s)-2-hydroxy-4-phthalimido-butyric acid. A mixture of the acid (1.3 molecular equivalents), N-hydroxysuccinimide (1.3 m. eq.) and dicyclohexylcarbo-

Fig. 1.



diimide (1.3 m. eq.) in THF was stirred in an ice bath for 1 hour. To the resulting suspension containing dicyclohexylurea, **3** (1 m. eq.) was added and the mixture was stirred overnight at room temperature. The 1-N-((S)-2-hydroxy-4-phthalimidobutyryl) derivative (**4**) was obtained in a yield of 67 %, mp 156~158°C, $[\alpha]_D^{27}$ +9.9° (*c* 2, chloroform), ir, 1710, 1655, 1530 cm^{-1}. [Calcd. for $C_{72}H_{89}N_5O_{21}$: C 63.56, H 6.59, N 5.15. Found: C 63.58, H 6.56, N 5.04].

Compound **4** was then treated successively with dilute hydrazine in 80 % ethanol to remove the phthaloyl group, with palladium black and hydrogen to remove the benzyloxycarbonyl groups and with 1 N hydrochloric acid to remove the cyclohexylidene groups to give a 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 0.4 N ammonia, 1-N-((S)-4-amino-2-hydroxybutyryl)ribostamycin, namely butirosin B was eluted in a yield of 63 % from **4** as a monohydrate, $[\alpha]_D^{27}$ +34° (*c* 2, water) (lit.[1] +33° (*c* 1.5, water)). $Rf_{ribostamycin}$ 0.53 (on paper chromatography with 1-butanol - pyridine - water - acetic acid (6 : 4 : 3 : 1)), the value being identical with that of butirosin B of natural origin, ir; 1650, 1560 cm^{-1}. [Calcd. for $C_{21}H_{41}N_5O_{12}\cdot H_2O$: C 43.97, H 7.56, N 12.21. Found: C 43.65, H 7.49, N 12.08].

The antibacterial spectrum is shown in Table 1.

Table 1. Antibacterial spectrum of synthetic butirosin B*

Test organisms**	Minimal inhibitory concentration (mcg/ml)
Staphylococcus aureus FDA 209P	1.56
Escherichia coli K-12	0.78
〃 ML 1629	1.56
〃 ML 1630	1.56
〃 ML 1410	0.78
〃 〃 R 81	3.12
〃 R 5	6.25
〃 LA 290 R 55	0.78
〃 〃 R 56	0.78
〃 〃 R 64	0.78
〃 C 600 R 135	0.78
〃 J 5 R 11-2	1.56
Escherichia coli W 677	0.39
〃 JR 66/W 677	>100
Klebsiella pneumoniae type 22 #3038	>100
Pseudomonas aeruginosa A3	3.12
〃 No. 12	6.25
〃 H 9	3.12
〃 H 11	25
〃 TI 13	25
〃 GN 315	>100
〃 99	50
Mycobacterium smegmatis ATCC 607***	0.78

* The activity for the respective strain was quite identical with that of natural origin.
** Nutrient agar, 37°C, 17 hours
*** Nutrient agar, 37°C, 42 hours

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