5″-AMINO-5″-DEOXYBUTIROSIN, A NEW SEMI-SYNTHETIC AMINOGLYCOSIDE ANTIBIOTIC

Sir:

We wish to report the preparation of 5″-amino-5″-deoxybutirosin, a new synthetic analog of butirosin.[1] It has been reported that resistance development among certain bacteria results from the production of phosphotransferase and acetyl transferase enzymes[2,3] which phosphorylate and N-acetylate these basic antibiotics resulting in biological inactivation. One such phosphotransferase phosphorylates the primary hydroxyl group in the pentofuranose portion of lividomycin.[4] The C-5 hydroxyl of the pentose moiety of butirosins A and B has been substituted with an amino group, which, in the case of the xylo (A) isomer, results in enhanced *in vitro* and *in vivo* biological activity compared with the butirosins.

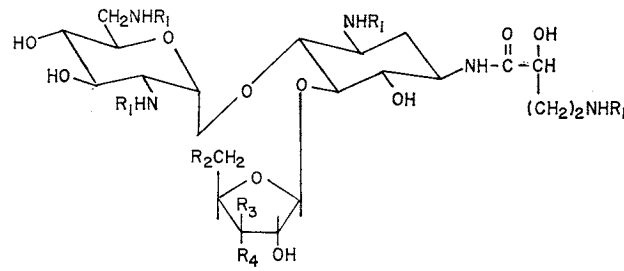
The synthesis was carried out on butirosins A and B as well as on an AB mixture (85:15) by selective tosylation or mesitylenesulfonylation of the tetra N-(trifluoroacetyl) or tetra N-(benzyloxycarbonyl) derivatives. Subsequent azide displacement, hydrogenation and base hydrolysis in the case of the trifluoroacetyl analog afforded the amino compounds.

Butirosin base (**1**) was partially N-trifluoroacetylated by refluxing 3 hours in a mixture of methanol and ethyl trifluoroacetate. The residue obtained by evaporation was dissolved in pyridine, treated first with hexamethyldisilazane and trimethylchlorosilane, then cooled to $<10°C$ and treated with trifluoroacetic anhydride. Evaporation afforded a product which was hydrolyzed by refluxing 3 hours in ethanol-2N acetic acid (2 : 1) to give tetra [N-(trifluoroacetyl)]-butirosin (**2**), $[\alpha]_D^{25}$ +32.7° (*c* 1.0, MeOH), IR: 1710, 1660, 1555 cm^{-1}. [Calcd. for $C_{29}H_{37}F_{12}N_5O_{16}$: C 37.07, H 3.97, N 7.45, F 24.27; Found: C 37.29, H 4.18, N 7.54, F 23.84].

Tosylation of (**2**) was carried out in pyridine solution at 0°C with a 50 % excess of *p*-toluenesulfonyl chloride. Chromatographic purification with silica gel and benzene-methanol (4 : 1) afforded the tosyl ester (**3**) in 32 % yield. $[\alpha]_D^{25}$ +18.5° (*c* 0.94, MeOH), IR: 1710, 1655, 1555, 1350 cm^{-1}. [Calcd. for $C_{36}H_{43}F_{12}N_5O_{18}S$: C 39.53, H 3.96, N 6.40, S 2.93; Found: C 39.01, H 3.79, N 6.14, S 2.89].

By using 2-mesitylenesulfonyl chloride a monomesisyl derivative (**4**) was isolated in 43% yield. $[\alpha]_D^{25}$ +13.1° (*c* 1.0, MeOH). [Calcd. for $C_{38}H_{47}F_{12}N_5O_{18}S$: C 40.68, H 4.22, F 20.32, N 6.24, S 2.86; Found: C 40.23, H 4.30, F 20.30, N 5.95, S 3.26].

Heating (**3**) or (**4**) in DMF solution with an excess of NaN_3 yielded after chromatographic purification the monoazido derivative (**5**) in



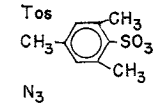
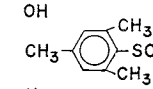
	R_1	R_2	R_3	R_4
1	H	OH	OH(H)	H(OH)
2	TFA	OH	OH(H)	H(OH)
3	TFA	Tos	OH(H)	H(OH)
4	TFA		OH(H)	H(OH)
5	TFA	N_3	OH(H)	H(OH)
6	H	NH_2	OH(H)	H(OH)
7	TFA	N-TFA	OH(H)	H(OH)
8	Cbz	OH	OH	H
9	Cbz		OH	H
10	Cbz	N_3	OH	H
11	H	NH_2	OH	H
12	TFA	N-TFA		
13	TFA	OH	H	OH
14	H	NH_2	H	OH
15	TFA	N-TFA	H	OH

Table 1.

Test organism		Minimal inhibitory concentration (mcg/ml)		
		Butirosin (1)	5-NH_2 "A" (6)	5-NH_2 "B" (12)
Staphylococcus aureus	S-18713	200	12.5	100
Klebsiella pneumoniae	MGH-1	6.3	6.3	100
Serratia marcescens	IMM-5	12.5	3.1	12.5
Enterobacter aerogenes	IMM-50	3.1	6.3	25
Pseudomonas aeruginosa	28	6.3	3.1	12.5
〃	CB-CS	12.5	6.3	12.5
〃	CB-CR	6.3	3.1	12.5
〃	VAD 12-7-7	6.3	1.6	12.5
〃	74 C-1	12.5	3.1	12.5
〃	733	6.3	6.3	12.5
〃	LA 3399	25	3.1	6.3
〃	UI 18	6.3	3.1	6.3

Microtitration broth dilution tests.

Table 2.

Challenge microorganism		Mouse protective activity*(mg/kg)	
		Butirosin (1)	Aminobutirosin (6)
Enterobacter aerogenes	IMM-11	1.9	1.9
Klebsiella pneumoniae	MGH-2	4.2	2.9
Proteus vulgaris	UC-232	4.8	3.0
Pseudomonas aeruginosa	UI-18	46.2	19
〃	12-7-7	58.2	21
Serratia marcescens	IMM-5	71	17.5
Diplococcus pneumoniae	SVI	99.4	77
Staphylococcus aureus	UC-76	2.8	1.1
Streptococcus pyogenes	C203	28.4	16.0

* Compounds administered subcutaneously in saline concurrent with lethal intraperitoneal challenge (100 LD_{50} in 0.3 ml mucin).

80% yield. $[\alpha]_D^{25}$+32.4° (*c* 0.56, MeOH) IR: 2100, 1710, 1660, 1550 cm^{-1}. [Calcd. for $C_{29}H_{36}F_{12}N_6O_{15}\cdot H_2O$: C 35.45, H 3.90, F 23.20, N 11.40; Found: C 35.59, H 3.82, F 22.92, N 10.89].

Compound (**5**) was hydrogenated with 20% Pd/C catalyst and then hydrolyzed with 0.5 N NaOH for 3 hours at room temperature. 5″-Amino-5″-deoxybutirosin (**6**) was isolated from Amberlite IRC-50 (NH_4^+) by elution with 1 N ammonium hydroxide and lyophilization, $[\alpha]_D^{25}$ +26° (*c* 1, H_2O). [Calcd. for $C_{21}H_{42}N_6O_{11}\cdot 2H_2O$: C 42.70, H 7.85, N 14.23; Found: C 42.55, H 7.65, N 14.02].

The penta [N-(trifluoroacetyl)]-5″-amino-5″-deoxybutirosin (**7**) was prepared as described for (**2**). [Calcd. for $C_{21}H_{37}F_{15}N_6O_{16}$: C 35.98, H 3.60, F 27.54, N 8.12; Found C 35.61, H 3.77, F 27.74, N 7.94].

In order to establish the position of the new amino group, a sample was hydrolyzed with 1 N HCl at 80°C for 1 hour. Paper chromatography* showed no spot for xylose or ribose and TLC with $CHCl_3$–MeOH (4:1) on silica gel showed a single spot Rf 0.5 identical with 3-hydroxypyridine.[5] The UV spectrum of the hydrolysate

* Whatman #1 filter paper with EtOAc, pyridine, H_2O (36 : 10 : 11.5) and $AgNO_3$ spray.

also corresponded to that for 3-hydroxypyridine, λ_{max} 283 nm, made basic with KOH λ_{max} 235, 294 nm.

The synthesis of 5''-amino-5''-deoxybutirosin was also carried out *via* the tetra [N-(benzyloxycarbonyl)] butirosin A (**8**) which was prepared by alternate addition of benzyl chloroformate and $NaHCO_3$ to an aqueous methanol solution at 5°C, $[\alpha]_D^{25}$+14.6° (*c* 1, MeOH). [Calcd. for $C_{53}H_{65}N_5O_{20}$: C 58.28, H 6.00, N 6.41; Found: C 57.99, H 6.26, N 6.40].

Treatment of (**8**) with 2-mesitylenesulfonyl chloride in pyridine gave (**9**) in 34 % yield following chromatography on silica gel and $CHCl_3$–MeOH (15 : 1), $[\alpha]_D^{25}$+10.7° (*c* 1.0, MeOH). [Calcd. for $C_{62}H_{75}N_5O_{22}S$: C 58.43, H 5.93, N 5.50, S 2.52; Found: C 57.85; H 5.86, N 5.48, S 2.56]. Treatment of (**9**) with NaN_3 in DMF overnight at 100°C afforded the azido derivative (**10**) in 45 % yield after purification on silica gel, IR: 2100 cm^{-1}, $[\alpha]_D^{25}$+13.7° (*c* 1.0, MeOH). [Calcd. for $C_{53}H_{64}N_8O_{20}$: C 56.18, H 5.69, N 9.89; Found: C 55.97, H 5.70, N 9.69].

Hydrogenation of (**10**) with 20 % Pd/C gave the 5''-amino-5''-deoxybutirosin A (**11**), $[\alpha]_D^{25}$ +25.4° (*c* 1.0, H_2O). [Calcd. for $C_{21}H_{42}N_6O_{11}\cdot 1/2\,H_2O\cdot 1/2\,H_2CO_3$: C 43.43, H 7.56, N 14.14; Found: C 43.45, H 7.64, N 14.10].

The preparation of tetra [N-(trifluoroacetyl)] butirosin B (**13**) was the same as for the xylo isomer, $[\alpha]_D^{25}$+35° (*c* 1.0, MeOH). [Calcd. for $C_{29}H_{37}F_{12}N_5O_{16}$: C 37.07, H 3.97, N 7.45, F 24.27; Found: C 36.77, H 4.16, N 7.31, F 23.85]. However, selective sulfonylation was considerably more difficult. The same sequence of reactions described for the butirosin AB mixture was utilized and afforded 5''-amino-5''-deoxybutirosin B (**14**) in 3 % yield, $[\alpha]_D^{25}$+32° (*c* 1.0, H_2O). [Calcd. for $C_{21}H_{42}N_6O_{11}\cdot H_2CO_3$: C 42.85, H 7.19, N 13.63; Found: C 42.92, H 7.11, N 12.22].

Penta [N-(trifluoroacetyl)]-5''-amino-5''-deoxybutirosin B (**15**) was prepared as described for (**2**). [Calcd. for $C_{21}H_{37}F_{15}N_6O_{16}$: C 35.98, H 3.60, F 27.54, N 8.12; Found: C 36.14, H 3.82; F 25.33, N 7.48] and TLC with $CHCl_3$–MeOH (7 : 2) showed an Rf 0.40 compared with Rf 0.55 for the A component (**12**). Compound (**7**) prepared from the 85:15 mixture of A and B showed no detectable quantity of (**15**) by TLC which suggests the feasibility of preparing compound (**11**) from such a mixture.

In vitro tests (Table 1) show that 5''-amino-5''-deoxybutirosin A (**11**) in contrast to the B analog (**14**) has enhanced inhibitory activity relative to butirosin towards *Serratia marcescens* and *Pseudomonas aeruginosa* strains. This superiority was also demonstrated by *in vivo* mouse experiments shown in Table 2. Further details of the chemistry and biological properties of aminobutirosin are in preparation.

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