AMINOGLYCOSIDE ANTIBIOTICS. VI

STRUCTURE DETERMINATION OF 4′-DEOXYBUTIROSINS (BU-1975C_1 and C_2)

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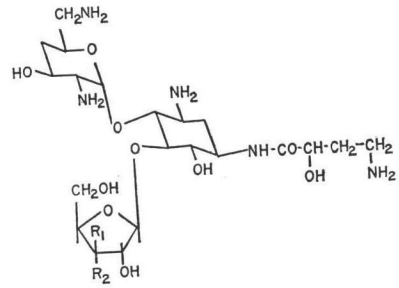
The structures of Bu-1975C_1 and C_2 have been determined to be 4′-deoxybutirosins A and B. They contain the new deoxyaminosugar, 2,6-diamino-2,4,6-trideoxy-α-D-*xylo*-hexopyranose (4-deoxyneosamine C).

Antibiotic Bu-1975 is a complex of at least five active components (A_1, A_2, B, C_1, C_2) produced by strains of *Bacillus circulans*. The fermentation, isolation and characterization of the antibiotics have been described in a companion paper[1]. Components C_1 and C_2 are new aminoglycoside antibiotics similar to butirosins A and B[2]. The structural studies on Bu-1975C_1 and C_2 have shown that they are 4′-deoxybutirosins A and B with the structures **Ia** and **Ib**.



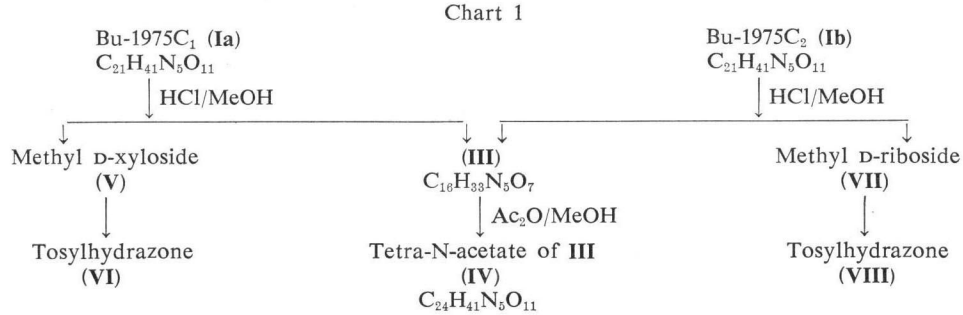
Bu-1975C_1(4′-Deoxybutirosin A) (**Ia**)
R_1=OH, R_2=H
Bu-1975C_2(4′-Deoxybutirosin B) (**Ib**)
R_1=H, R_2=OH

General Structural Characteristics

Bu-1975C_1 (**Ia**) and C_2 (**Ib**) are basic compounds, both having a titration equivalent of 154 (pKa′=8.37). The molecular formulae of the free bases were both determined to be $C_{21}H_{41}N_5O_{11}$ from the analyses of the sulfate (**Ia**), carbonate (**Ib**) and the tetra-N-acetates (**IIa** and **IIb**)[1]. The IR spectra of **Ia**[1] and **Ib** were almost the same showing the presence of multi-hydroxyl (3200~3500 and 1040 cm^{-1}) and amide (1645 and 1570 cm^{-1}) groups. Both components C_1 and C_2 are positive to ninhydrin and anthrone, but negative to SAKAGUCHI, TOLLENS and FEHLING reactions indicating the absence of a reactive reducing sugar moiety.

The NMR spectrum of **Ia** hydrochloride in deuterium oxide[1] showed two anomeric protons

Chart 1



at δ 6.10 (doublet, J=3.5 Hz) and 5.28 (broad singlet) ppm, 18 protons on carbons linked to oxygen or ammonium group at around δ 2.8~4.4 ppm, and 6 protons on carbon atoms not bonded with heteroatoms at around δ 1.2~2.4 ppm. The NMR spectrum of **Ib** hydrochloride was almost the same as that of **Ia** except that the doublet anomeric proton of **Ib** appeared at a slightly higher field at δ 5.98 ppm. The difference in chemical shift for the anomeric protons of **Ia** and **Ib** was quite similar to that of butirosins A and B which are isomeric only in the pentose moiety[12].

Degradation Studies of Bu-1975C_1 (Ia) and C_2 (Ib)

Methanolysis of Ia and Ib (Chart 1)

By refluxing in a methanolic hydrogen chloride solution, **Ia** was split into a new biologically active fragment (**III**) and a methyl glycoside (**V**). After trimethylsilylation of **V** by the published method[3], GLC determination indicated **V** to be a mixture of methyl α- and β-D-xylosides. For further confirmation, **V** was hydrolyzed by dil.sulfuric acid to D-xylose which was reacted with tosylhydrazide[4] to yield the tosylhydrazone (**VI**). **VI** was identical with authentic tosylhydrazone of D-xylose by m.p., $[\alpha]_D$ and IR spectra[5]. Likewise, methanolysis of **Ib** liberated **III** and methyl D-riboside (**VII**). Thus, the difference between **Ia** and **Ib** resides only in the pentose part as in the case of butirosins A and B.

The common fragment, **III**, was purified by column chromatography on Amberlite CG-50 and obtained as a white amorphous solid. The IR spectrum of **III** was similar to that of the parent antibiotics exhibiting absorptions due to poly-hydroxyl and amide carbonyl groups. The NMR spectrum of **III** (Fig. 1) showed one anomeric proton at δ 5.86 ppm (doublet, J=3.5 Hz), 13 protons on carbons linked to heteroatom and 6 protons on carbons not bonded with heteroatom. Consequently, the anomeric proton of the pentose moiety in **Ia** and **Ib** was assignable to the broad singlet at δ 5.28 ppm and the lack of splitting suggested a β-furanosyl configuration for the pentoses[9]. Compound **III** gave a crystalline tetra-N-acetate (**IV**) by acetylation in absolute methanol. Microanalysis of **IV** was consistent with the formula $C_{24}H_{41}N_5O_{11}\cdot H_2O$ which established a molecular formula of $C_{16}H_{33}N_5O_7$ for compound **III**.

Hydrolysis of III and Isolation of X (Charts 2 and 3)

Compound **III** was hydrolyzed by refluxing for one hour in 0.5 N NaOH solution. The hydrolyzate was chromatographed on a column of Amberlite CG-50 and separated into an amino acid (**IX**) and a biologically active fragment (**X**). The amino acid, **IX**, which was not absorbed on the resin, was purified by column chromatography on Amberlite IR-120 and

Chart 2

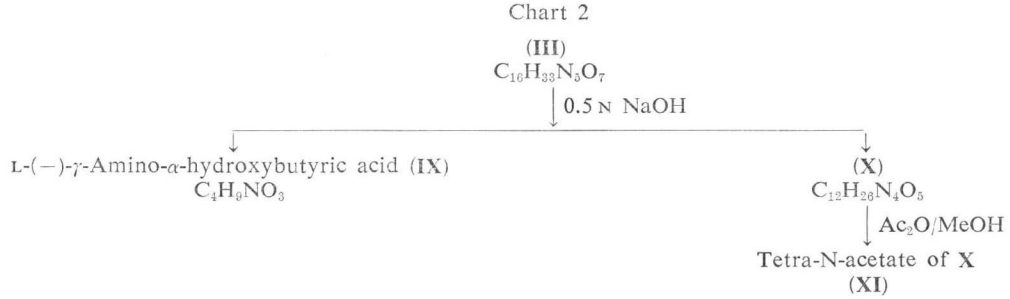
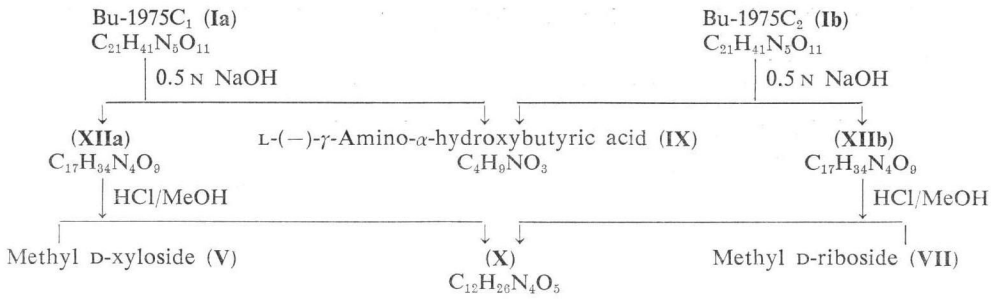


Chart 3

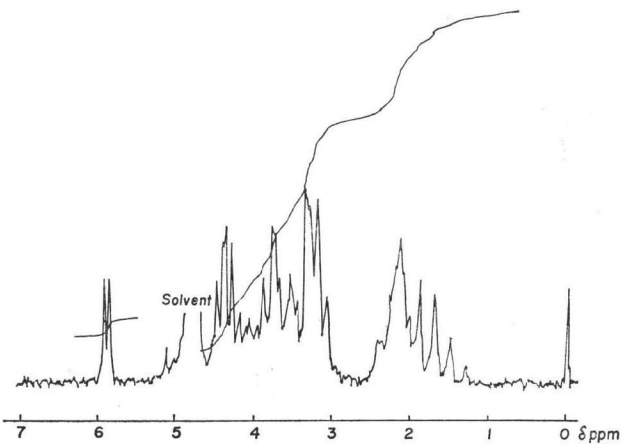


crystallized from aqueous ethanol. **IX** was identified as L-(−)-γ-amino-α-hydroxybutyric acid[9] by IR, NMR, $[\alpha]_D$ and mixed melting point determination.

The bio-active fragment **X** which was eluted from the CG-50 resin with 0.3 N NH_4OH was obtained as a homogeneous crystalline solid. The IR spectrum of **X** lacked the amide carbonyl absorption in the 1650 cm^{-1} region. Treatment of **X** with acetic anhydride in absolute methanol gave colorless crystals of the tetra-N-acetate (**XI**). Elemental analysis of **X** (sulfate) and **XI** were in agreement with a formula for the free base of $C_{12}H_{26}N_4O_5$. The NMR spectrum of **X** (Fig. 2) showed an anomeric proton at δ 5.87 ppm (J=3.5 Hz), 10 protons at δ 3.0~4.4 ppm and 4 protons at δ 1.2~2.6 ppm. These properties suggested a close similarity to nebramine[7], a degradation product of tobramycin. However, the two compounds were clearly differentiated by direct comparison of TLC*, NMR and antibacterial spectra.

Compound **X** was alternatively obtained as illustrated in Chart 3. Alkaline hydrolysis of **Ia** and **Ib** yielded new bio-active fragments **XIIa** and **XIIb**, respectively, and a common fragment **IX**, identified as L-(−)-γ-amino-α-hydroxybutyric acid. Compounds **XIIa** and **XIIb** showed no amide absorption in the IR spectra and were given the same molecular formula of $C_{17}H_{34}N_4O_9$ based on micro analyses of the crystalline tetra-N-acetates (**XIIIa** and **XIIIb**). The NMR spectrum of **XIIa** exhibited two anomeric protons at δ 5.36 ppm (broad singlet) and 6.10 ppm (doublet, J=3.5 Hz).

Fig. 1. NMR spectrum of **III**·HCl (60 MHz, in D_2O)

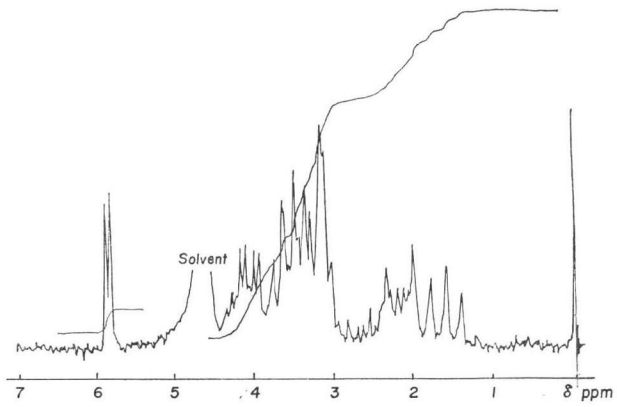


* Silica gel TLC, system S-117 ($CHCl_3$-MeOH-28% NH_4OH, 1:3:2), Rf of **X**: 0.61, nebramine : 0.58.

On acid methanolysis **XIIa** was cleaved to methyl D-xyloside (**V**) and compound **X** which was identified as that obtained previously. Similarly, compound **XIIb** yielded methyl D-riboside (**VII**) and fragment **X**.

These experiments illustrated in Charts 1, 2 and 3 indicated that both the amino acid (**IX**) and pentose moieties (D-xylose or D-ribose) are attached directly to the fragment **X** in the structure of Bu-1975C_1 and C_2.

Fig. 2. NMR spectrum of **X**·HCl (60 MHz, in D_2O)

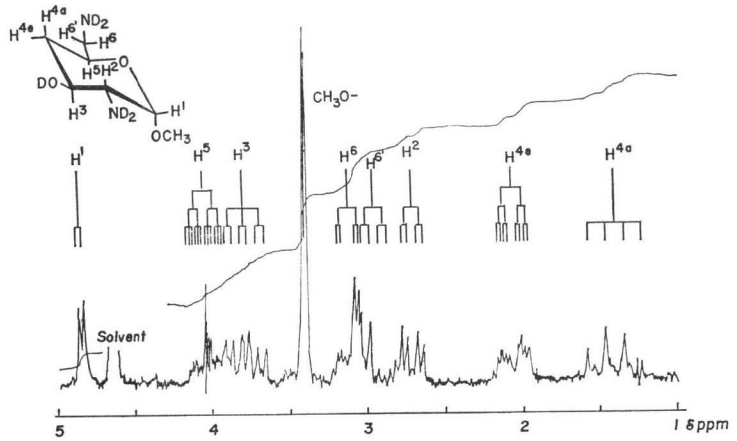


Hydrolysis of X

Fragment **X** resisted further acid hydrolysis because of the presence of a positively charged ammonium group near the glycoside bond[8] as was later shown. The hydrolysis was however successfully achieved starting from the tetra-N-acetate (**XI**).

A methanolic hydrogen chloride solution of **XI** was refluxed for 3 hours and then concentrated under reduced pressure to isolate 2-deoxystreptamine (**XIV**) as the crystalline hydrochloride, identical with an authentic sample prepared from kanamycin A[10]. The mother liquor was concentrated and chromatographed on a column of CG-50 to yield **XVb** (minor component, eluted with 0.1 N NH_4OH) and **XVa** (major component, eluted with 0.3 N NH_4OH).

Fig. 3. NMR spectrum of **XVa** (100 MHz, D_2O)



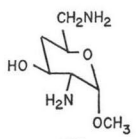
Structure of XVa and XVb

Compounds **XVa** and **XVb** were purified by further column chromatography with CG-50 resin and obtained as hygroscopic colorless solids. Both **XVa** and **XVb** were positive to ninhydrin and anthrone but negative to FEHLING and TOLLENS reagents. Acetylation of **XVa** in methanol gave a crystalline di-N-acetate (**XVI**), and in pyridine the N, O-triacetate (**XVII**). Both **XVa** and **XVb** consumed one mole of periodate. The molecular formula of $C_7H_{16}N_2O_3$ was assigned to **XVa** from the mass spectrum and microanalysis of **XVII**.

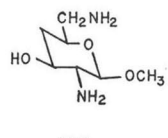
XVa was determined to be methyl 2,6-diamino-2,4,6-trideoxy-α-D-*xylo*-hexopyranoside by analyzing the 100 MHz NMR spectrum. As shown in Fig. 3, a doublet of quartets centered at δ 2.07 ppm and a quartet centered at δ 1.41 ppm were respectively assigned to an equatorial and an axial proton of a methylene group in a six-membered ring. The resonance patterns indicated that both vicinal protons of the methylene group had an axial orientation. The proton doublet appearing at δ 4.85 ppm (J=3.5 Hz) was assignable to an equatorial anomeric proton since the anomeric proton of **XIVb** (60 MHz) was located at higher field (δ 4.51 ppm) with a larger coupling constant (J=8.0 Hz). It is apparent from these data that the proton on C-2 had an axial orientation.

All the other protons of **XVa** were also assignable as illustrated in Fig. 3, which was further supported by decoupling studies. Irradiation of H_1 collapsed the doublet of doublets signal of H_2 (δ 2.72 ppm) to a doublet (J=10.5 Hz) indicating that H_2 was still coupled with a vicinal axial proton, and irradiation of H_{4e} converted the splitting of H_3 to a clear triplet (J=10.5 Hz). Additional support for the structure of **XVa** was provided by the NMR spectrum of its N,O-triacetate (**XVII**). Acetylation resulted in a shift to lower field of three protons: H_2 appeared at δ 3.94 ppm (doublet of doublets), H_6 at around δ 3.2~3.3 ppm and H_3 at δ 5.00 ppm (sextet).

The configuration at the C-2 and C-3 substitutions in **XV** was determined by the copper complex method developed by S. UMEZAWA *et al.*[11] The difference between the molecular rotation of **XVa** in CuAm (1.6 % cuprous chloride in 15 N NH_4OH) and in water was −690°, and this negative value was consistent with those reported for the compounds having a pair of adjacent amino and hydroxy groups in a projection angle of −60°. Thus, the structure of **XVa** is methyl 2,6-diamino-2,4,6-trideoxy-α-D-*xylo*-hexopyranoside and **XVb** is the β-isomer.



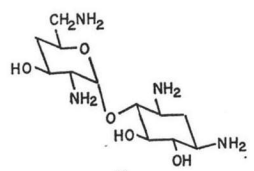
XVa



XVb

Structure of X

Since compound **X** showed no reducing reaction and its N-acetate (**XI**) consumed one mole of periodate, the sugar **XV** should be glycosidically linked to the C-4 or C-6 position of 2-deoxystreptamine (**XIV**). The NMR spectrum of **X** showed the anomeric proton at δ 5.90 ppm as a sharp doublet with a spacing of 3.5 Hz which, along with its rotational value, allowed definitive assignment of an α-glucopyranoside configuration for sugar **XV**. The site of attachment of **XV** to deoxystreptamine was determined to be C-4 by employing the copper complex method. The $\Delta[M]_{CuAm}$ value measured for its tetra-N-acetate (**XI**) was −1910°. This high negative value indicated that the projection angle between the two adjacent hydroxy groups



X

was about −60°, and accordingly that the C-5 and C-6 hydroxyl groups of deoxystreptamine were unsubstituted. Thus, the structure of **X** is 4-(2,6-diamino-2,4,6-trideoxy-α-D-*xylo*-hexopyranosyl)-2-deoxystreptamine.

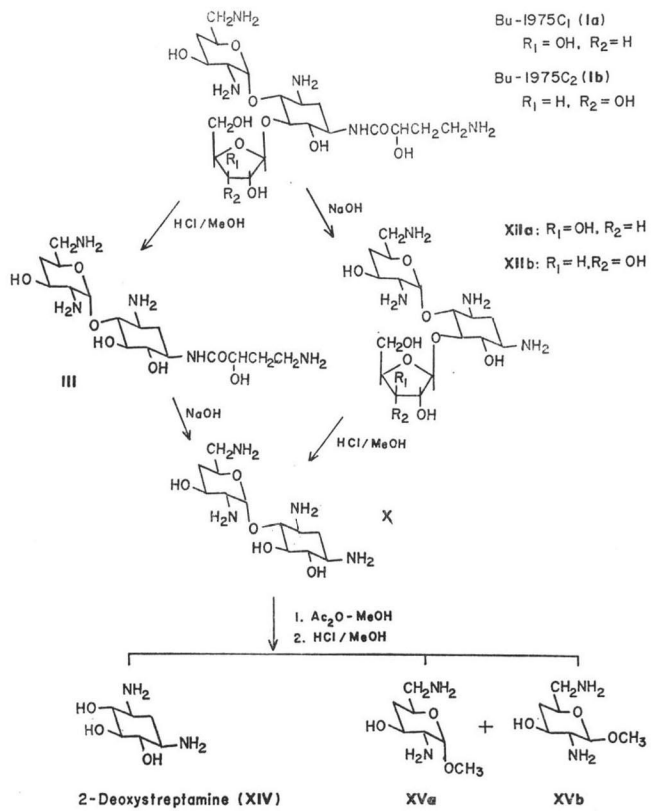
Structure of **Ia** and **Ib**

As described before, both the hydroxy-amino acid (**IX**) and the pentose moieties should be attached directly to fragment **X** through the amide and glycoside linkages, respectively. Although the butirosin-like conformation was thought to be most probable for **Ia** and **Ib** from biosynthetic considerations, the site of attachment of the two moieties to **X** has been established unequivocally by the sequence of experiments described below.

In order to determine the position of the amide linkage, **Ia** was oxidized by periodate and then hydrolyzed with 1 N HCl to yield a basic compound (**XVIII**). Alkaline hydrolysis of **XVIII** gave 2-deoxystreptamine (**XIV**) and the amino acid (**IX**). Acetylation of **XVIII** gave crystalline di-N-acetate (**XIX**) which was identical with the N^3, $N^{3'}$-diacetate of 1-[L(−)-γ-amino-α-hydroxybutyryl]-2-deoxystreptamine obtained from butirosin A[12]. Therefore, the site of substitution of **IX** was determined to be the C-1 amino group of 2-deoxystreptamine.

Isolation of **XVIII** after periodate oxidation also indicated that the pentose moiety must be attached to one of the two hydroxy groups at C-5 or C-6 of the deoxystreptamine moiety. If the pentose were on the C-3′-hydroxy of the 4′-deoxysugar part, the 2-deoxystreptamine

Chart 4



ring would have been destroyed by the periodate oxidation. The site of pentose substitution was determined by the following methylation experiment[14].

A mixture of **Ia** and **Ib** was N-acetylated in methanol, then the product was treated with excess of methyl iodide and silver oxide in anhydrous dimethyl formamide solution to give tetra-N-acetyl-hexa-O-methyl derivative **XX**. Acid hydrolysis of **XX** yielded γ-amino-α-methoxybutyric acid (**XXI**, m.p. 241~242°C)[12] and 6-O-methyl-2-deoxystreptamine (**XXII**), the latter and its crystalline N, N′-diacetate (**XXIII**, m.p. 287~289°C) being identical with authentic specimens[12] prepared from butirosins. The $\Delta[M]_{CuAm}$ measurement for N, N′-diacetyl-6-O-methyldeoxystreptamine (**XXIII**) by the copper complex method gave a high positive value (+1690°) which was close to the $\Delta[M]_{Cupra\ B}$ value reported for the compound[15] having vicinal free hydroxyl groups at C-4 and C-5 in the 2-deoxystreptamine. Thus, it was determined that the pentose moiety was substituted at the C-5 hydroxyl of 2-deoxystreptamine.

Therefore, the complete structures of **Ia**, **Ib** and their degradation products described above are as shown in Chart 4.

Discussion

The structures of Bu-1975C_1 and C_2 are closely related to those of butirosins A and B, having a new deoxy-amino-sugar of 2,6-diamino-2,4,6-trideoxy-α-D-*xylo*-hexopyranose in place of 2,6-diamino-2,6-dideoxy-D-glucose (neosamine C) in butirosins. There have been several deoxy-aminosugars disclosed as a constituent of the aminoglycoside antibiotics, *e.g.* 2,6-diamino-2,3,6-trideoxy-α-D-*ribo*-hexopyranose[7] from tobramycin, 2-amino-2,3-dideoxy-α-D-*ribo*-hexopyranose[14] from lividomycin A, and purpurosamines[16] from gentamicin C's. The deoxy-aminosugar component of Bu-1975C is a new addition to the chemistry of aminoglycoside antibiotics.

As reported in a companion paper[1], Bu-1975C_1 and C_2 possess a broader antibacterial spectrum than butirosins, inhibiting some butirosin-resistant organisms and showing improved activity against *Pseudomonas* species. This superiority in activity of Bu-1975C over butirosin is undoubtedly attributable to the lack of 4′-hydroxyl group. Compound **III** in the text, which is a partial degradation product of Bu-1975C and lacks the pentose moiety from the parent antibiotic, retained considerable antibiotic activity. The antibacterial spectrum of **III** is similar to that of Bu-1975C and broader than that of 1-[L(−)-γ-amino-α-hydroxybutyryl]-neamine[17] which is a corresponding degradation product from butirosins, supporting the 4′-deoxy effect on the antibacterial spectra of this class of aminoglycoside antibiotics.

However, it is interesting that other bio-active fragments from Bu-1975C, *i.e.* **X**, **XIIa** and **XIIb**, all of which do not contain the α-hydroxy-γ-amino acid unit, are no longer active against the resistant organisms and *Pseudomonas* species. The antibacterial spectra of **X** and **XII** (**a** or **b**) are nearly the same as those of neamine and ribostamycin[18] (or its xylosyl congener), respectively, which are the corresponding degradation products from butirosins. Thus, it may be that the 4′-dehydroxylation effect improves the spectrum of ribostamycin-butirosin class of antibiotics only when combined with the amino acid substitution.

Experimental

Tetra-N-acetyl Bu-1975C_1 (**IIa**)

A solution of **Ia** (200 mg) in 1 ml of acetic anhydride and 15 ml of absolute methanol was stirred for 3 hours at room temperature. Insoluble material was removed by filtration and the filtrate was poured into 200 ml of ether with stirring. The white crystalline acetate (**IIa**) was collected by filtration and dried *in vacuo*, yield 216 mg. m.p. >250°C, $[\alpha]_D^{24.5}$+26° (*c* 0.5, H_2O).

NMR $\delta_{DSS}^{D_2O}$ in ppm: 5.19 (1 H, d, J=3.5 Hz), 5.25 (1H, br-s), 3.2~4.3 (18H) and 1.3~2.2 (18H, including 4 $\underline{CH_3}CO$ singlet protons).

Anal. Calcd. for $C_{29}H_{49}N_5O_{15}\cdot\frac{3}{2}H_2O$: C 47.40, H 7.13, N 9.53.
Found : C 47.49, H 7.37, N 9.65.

Tetra-N-acetyl Bu-1975C_2 (**IIb**)

By the procedure described above, **Ib** (200 mg) was reacted with acetic anhydride in absolute methanol to yield 199 mg of **IIb**. m.p. >250°C, $[\alpha]_D^{24.5}$+43° (*c* 0.5, H_2O).

Anal. Calcd. for $C_{29}H_{49}N_5O_{15}\cdot H_2O$: C 47.99, H 7.08, N 9.70.
Found : C 48.08, H 7.27, N 9.50.

Isolation of **III** and Methyl D-Xyloside (**V**) from **Ia**

A solution of **Ia** (1.27 g) in 250 ml of 1 N methanolic hydrogen chloride was stirred for 20 hours at room temperature. The solution was then concentrated to 20 ml *in vacuo* and poured into 250 ml of ether. The white precipitate which deposited was collected by filtration and thoroughly dried *in vacuo*. The crude **III** hydrochloride was dissolved in 5 ml of water and applied to a column of Amberlite CG-50 (NH_4^+ form, 150 ml). The column was washed with water and eluted succesively with 0.1, 0.25 and 0.5 N NH_4OH. The effluent was collected in fractions and the bioactive fractions which were eluted with 0.5 N NH_4OH were pooled, concentrated *in vacuo* and lyophylized to give 690 mg of **III**. m.p. 178~181°C. TLC*: Rf 0.35 (S-117). IR ν_{max}^{KBr} in cm^{-1}: 3200~3500, 1645 and 1570. NMR $\delta_{DSS}^{D_2O+DCl}$ in ppm: 5.86 (1 H, d, J=3.5 Hz), 3.0~4.0 (13H), 1.3~2.5 (6H). The sulfate was prepared by dissolving **III** in dil. sulfuric acid, followed by precipitation with addition of methanol.

Anal. Calcd. for $C_{16}H_{33}N_5O_7\cdot 2H_2SO_4\cdot H_2O$: C 30.91, H 6.33, N 12.69.
Found : C 30.87, H 6.70, N 12.14.

The filtrate of the above methanolyzate was concentrated to 100 ml and treated with Amberlite IR-4B (OH^- form, washed with methanol) to remove hydrochloric acid. The solution was evaporated *in vacuo* to afford 480 mg of oily mixture of anomeric methyl D-xylosides (**V**). TLC: Rf 0.31 (SG-104).

V was converted to trimethylsilyl derivatives. GLC** Rt in minute: 3.2 (major) and 4.2 (minor). Identical with authentic trimethylsilylated methyl D-xyloside.

Isolation of **III** and Methyl D-Ribosides (**VII**) from **Ib**

By the same procedure as above, **Ib** was hydrolyzed to give a mixture of anomeric methyl D-ribosides (**VII**) and **III**. TLC of **VII**: Rf 0.50 (system SG-104). GLC of trimethylsilyl derivative of **VII** Rt: 2.1 (main) and 2.4 (minor). Identical with authentic trimethylsilylated methyl D-riboside.

Tosylhydrazones (**VI** and **VIII**)

A solution of **V** (1.036 g) in 15 ml of 10 % sulfuric acid was heated for 4 hours at 120°C and then neutralized with barium hydroxide solution. Barium sulfate formed was removed by filtration and the mother liquor was evaporated *in vacuo* to give 770 mg of syrupy material which was identified as D-xylose by TLC. To a solution of the syrup (470 mg) in 25 ml of methanol was added 700 mg of *p*-toluenesulfonylhydrazide (Tosylhydrazide)[6] and the mixture

* The TLC systems used in Experimental are:

System	Plate	Solvent system
S-117	silica gel	$CHCl_3$ - MeOH - 28% NH_4OH (1:3:2)
S-115	alumina	$CHCl_3$ - MeOH - 28% NH_4OH (2:1:1)
SG-104	silica gel	MEK - HCOOH - aq. sat'd H_3BO_4 (9:0.5:1.5)
S-114	silica gel	MeOAc-*n*PrOH-28% NH_4OH (45:105:60)

MEK: methyl ethyl ketone

** Column OV-1 (3%), flow rate of He 60 ml/min., column temp. 172.5°C.

Table 1. Periodate oxidation of Bu-1975 and the related compounds

Compounds	Periodate consumption*			Theoretical
	1 hr	3 hrs	8 hrs	
Bu-1975C_1 (**Ia**)	1.40	1.95	2.09	2 moles
Tetra-N-acetyl Bu-1975C_1 (**IIa**)	0.75	0.80	0.80	1
Des-xylosyl Bu-1975C_1 (**III**)	1.71	2.02	2.19	2
Tetra-N-acetyl **III** (**IV**)	0.57	0.72	0.85	1
4′-Deoxyneamine (**X**)	2.10	2.74	3.04	3
Tetra-N-acetyl **X** (**XI**)	0.68	0.75	0.82	1
Des-HABA Bu-1975C_1 (**XIIa**)	2.57	3.06	3.23	3
Tetra-N-acetyl **XIIa** (**XIIIa**)	0.65	0.72	0.91	1
Methyl β-4-deoxyneosamine C (**XVb**)	0.84	1.10	1.18	1
Butirosin A	1.97	2.58	2.88	3

* moles of periodate/mole of compound

was heated under reflux for half an hour. On evaporation of the solvent *in vacuo*, D-xylose tosyl hydrazone (**VI**) was obtained as colorless needles[7]. Yield, 240 mg. m.p. 150~151°C. The crystals showed identical IR spectrum and melting point with those of authentic D-xylose tosylhydrazone.

Anal. Calcd. for $C_{12}H_{18}N_2O_6S$: C 45.27, H 5.70, N 8.80, S 10.07.
Found : C 45.21, H 5.60, N 8.79, S 9.91.

D-Ribose tosylhydrazone (**VIII**) was obtained as pale yellow needles from **VII**. m.p. 159°C. The crystals showed no depression on mixed melting point determination with authentic D-ribose tosylhydrazone[7].

Anal. Calcd. for $C_{12}H_{18}N_2O_6S$: C 45.27, H 5.70, N 8.80, S 10.07.
Found : C 44.73, H 5.74, N 8.57, S 9.80.

Tetra-N-acetyl Derivative of **III** (**IV**)

A solution of **III** (200 mg) and acetic anhydride (1 ml) in 25 ml of absolute methanol was stirred for 3 hours at room temperature. White crystals which separated were collected by filtration and dried *in vacuo*. Yield 118 mg. m.p. >250°C, $[\alpha]_D^{24.5}$+77° (*c* 0.5, H_2O).

Anal. Calcd. for $C_{24}H_{41}N_5O_{11}\cdot H_2O$: C 48.56, H 7.30, N 11.80.
Found : C 48.45, H 7.20, N 11.39.

Isolation of L-(−)-γ-Amino-α-hydroxybutyric Acid (**IX**) and **XIIa** from **Ia**

A solution of **Ia** (300 mg) in 12 ml of 0.5 N NaOH solution was refluxed for 1 hour. The solution was cooled, adjusted to pH 7.0 and loaded on a column of Amberlite CG-50 (NH_4^+ form, 20 ml). The column was washed with water (500 ml) until the washings became negative to ninhydrin reagent and then eluted with 0.2 N NH_4OH. Bio-active eluates were pooled and concentrated *in vacuo* to give 218 mg of white solid, **XIIa**. Crystallization from aq. methanol gave colorless prisms of the methanol solvate. m.p. 102~105°C, $[\alpha]_D^{25}$+63° (*c* 0.5, H_2O). TLC: Rf 0.43 (S-117) and 0.33 (S-115). IR: no absorption at around 1650 cm^{-1}.

Anal. Calcd. for $C_{17}H_{34}N_4O_9\cdot CH_3OH\cdot 2H_2O$: C 42.68, H 8.36, N 11.06.
Found : C 42.70, H 8.39, N 10.94.

The washing from the above column chromatography was adjusted to pH 7.0 and absorbed on a column of Amberlite IR-120 (H^+ form, 20 ml). The column was washed with water and then eluted with 0.5 N NH_4OH. Ninhydrin-positive fractions were combined, decolorized with activated carbon and neutralized with Amberlite IRC-50 (H^+ form). The solution was concentrated *in vacuo* to 2 ml, diluted with ethanol to cloudiness and kept in the cold to deposit 39 mg of **IX** as colorless needles. m.p. 215~216°C, $[\alpha]_D^{23}$−27.5° (*c* 1.0, H_2O). **IX** showed identical IR

and NMR with those of L-(−)-γ-amino-α-hydroxybutyric acid.

Anal. Calcd. for $C_4H_9NO_3$: C 40.33, H 7.62, N 11.76.
Found : C 40.39, H 7.90, N 11.69.

Isolation of **IX** and **XIIb** from **Ib**

By essentially the same procedure as above, **Ib** gave **IX** and **XIIb**. TLC of **XIIb**: Rf 0.43 (S-117) and 0.06 (S-115).

Isolation of **X** and **IX** from **III**

A solution of **III** (780 mg) in 40 ml of 0.5 N NaOH was heated under reflux for 1 hour. The reaction mixture was adjusted to pH 7.0 with 6 N HCl and applied to a column of Amberlite CG-50 (NH_4^+ form, 120 ml). The column was washed with water (600 ml) and then eluted with 0.3 N NH_4OH. The bioactive fractions were pooled, concentrated *in vacuo* and lyophylized to give 491 mg of white powder (**X**). m.p. 208~209°C (dec.), $[\alpha]_D^{25}$+90° (*c* 1.0, H_2O). IR: no absorption in 1650 cm^{-1} region. NMR $\delta_{DSS}^{D_2O+DCl}$ in ppm: 5.84 (1H, d, J=3.5 Hz), 3.0~4.4 (10H) and 1.25~2.7 (4H). TLC: Rf 0.57 (S-117). The sulfate of **X** was prepared by the usual manner for analysis.

Anal. Calcd. for $C_{12}H_{26}N_4O_5 \cdot 2H_2SO_4 \cdot 2H_2O$: C 26.76, H 6.36, N 10.40.
Found : C 26.42, H 6.01, N 10.46.

The water wash from the above chromatography was concentrated *in vacuo* and chromatographed on Amberlite IR-120 (H^+ form). Elution with 0.5 N NH_4OH, followed by crystallization from aqueous ethanol afforded 160 mg of L-(−)-γ-amino-α-hydroxybutyric acid (**IX**).

Isolation of **X** and **V** from **XIIa**

A solution of **XIIa** (170 mg) in 30 ml of 0.4 N methanolic hydrogen chloride was stirred at room temperature overnight. Upon addition of 200 ml of ether, 170 mg of **X** hydrochloride precipitated. Further purification by chromatography (Amberlite CG-50, NH_4^+ form) gave 120 mg of free base of **X**. From the etherial mother liquor 54 mg of **V** was obtained as a syrupy material.

Tetra-N-acetate of **X** (**XI**)

A mixture of **X** (230 mg) in 10 ml of absolute methanol and 1 ml of acetic anhydride was stirred at room temperature. White crystals deposited in a few minutes and stirring was continued for 3 hours. The crystals were collected by filtration and dried *in vacuo* to yield 242 mg of **XI**. m.p. >280°C, $[\alpha]_D^{25}$+117° (*c* 0.5, H_2O), $\Delta[M]_{CuAm}$=−1910°.

Anal. Calcd. for $C_{20}H_{34}N_4O_9$: C 50.62, H 7.22, N 11.81.
Found : C 50.41, H 7.34, N 11.71.

Isolation of 2-Deoxystreptamine (**XIV**) and **XV** from **XI**

A solution of 1.4 g of **XI** in 350 ml of 2.5 N methanolic hydrogen chloride was heated under reflux for 16 hours. The solution was concentrated to 30 ml to deposit 2-deoxystreptamine hydrochloride (**XIV**). The solid was crystallized from aqueous ethanol to give 413 mg of colorless prisms. m.p. 260~265°C (dec.), TLC: Rf 0.17 (S-117).

Anal. Calcd. for $C_6H_{14}N_2O_3 \cdot 2HCl$: C 30.65, H 6.86, N 11.91.
Found : C 30.71, H 7.04, N 11.85.

The filtrate from the above methanolyzate was concentrated *in vacuo* to dryness. The residual solid was dissolved in 2 ml of water, neutralized with dil. NaOH and adsorbed on a column of Amberlite CG-50 (NH_4^+ form, 150 ml). The column was washed with water and eluted with 0.1 N, 0.3 N and 0.5 N NH_4OH, successively. The elution was followed by ninhydrin test on silica gel plate. **XVb** and then **XVa** were eluted with 0.3 N NH_4OH, collected, concentrated *in vacuo* and lyophylized to give 50 mg of **XVb** and 250 mg of **XVa**.

XVb: white crystalline powder, m.p. 129~130°C, $[\alpha]_D^{25}$−22° (*c* 0.5, H_2O), TLC: Rf 0.80 (S-117).

XVa: very hygroscopic powder, $[\alpha]_D^{25.5}$+151° (*c* 0.5, H_2O), $\Delta[M]_{CuAm}$=−686.4°, TLC: Rf

0.83 (S-117).

Triacetate of XVa (XVII)

XVa (30 mg) was dissolved in a mixture of acetic anhydride (0.5 ml) and pyridine (0.5 ml) and the solution was kept at room temperature for 40 hours. A trace of insoluble material was removed by filtration and the filtrate was concentrated *in vacuo* to dryness. To the residue was added 3 ml of water and the solution was again evaporated *in vacuo* to a syrup which was crystallized from a mixture of acetone and *n*-hexane to yield 33 mg of **XVII**. m.p. 155~157°C, IR ν_{max}^{KBr} in cm^{-1}: 1735 (AcO-), 1660 and 1540 (Ac-NH-).

Anal. Calcd. for $C_{13}H_{22}N_2O_6 \cdot \frac{1}{2}H_2O$: C 50.15, H 7.45, N 9.00.
Found : C 50.20, H 7.35, N 8.85.

1-[L-(—)-γ-Amino-α-hydroxybutyryl]-2-deoxystreptamine (XVIII) and its N, N'-Diacetate(XIX)

In a solution of 0.01 M sodium metaperiodate in 450 ml of acetate buffer (pH 4.5) was dissolved 450 mg of **Ia** and the mixture was allowed to stand for 5 days at 5°C. The reaction mixture, which had consumed 3.6 moles of periodate per mole of **Ia**, was treated with 0.5 ml of propylene glycol for 1 hour to destroy the excess of periodate and evaporated *in vacuo* to dryness. The residue was heated with 40 ml of 1 N hydrochloric acid at 80°C for 30 minutes and the hydrolyzate was adjusted to pH 7 with ammonia and chromatographed on a column of CG-50 (NH_4^+, 20 ml). The column was washed with water, developed with 0.1 N and 0.25 N aqueous ammonia and the elution was followed by TLC (S-117). The desired fractions were pooled, concentrated *in vacuo* and lyophylized to give 56 mg of amorphous white solid (**XVIII**). **XVIII** was identical with 1-[L(—)-γ-amino-α-hydroxybutyryl]-2-deoxystreptamine obtained from butirosins. TLC: Rf 0.16 (S-117).

A solution of **XVIII** (12 mg) and acetic anhydride (0.1 ml) in 1 ml of absolute methanol was stirred for 2.5 hours at room temperature. The reaction mixture was concentrated *in vacuo* and the residue was crystallized from a mixture of methanol and ethyl acetate to yield 6 mg of **XIX**. m.p. 213~214°C. No depression by the mixed melting point determination with an authentic sample.

Anal. Calcd. for $C_{14}H_{25}N_3O_7 \cdot \frac{1}{2}H_2O$: C 47.18, H 7.35, N 11.80.
Found : C 47.60, H 7.48, N 11.58.

γ-Amino-α-methoxybutyric acid (XXI), 6-O-Methyl-2-deoxystreptamine (XXII) and 1,3-Di-N-acetyl-6-O-methyl-2-deoxystreptamine (XXIII)

To a stirred solution of a mixture of **IIa** and **IIb** (3.05 g) and methyl iodide (40 ml) in 90 ml of anhydrous dimethylformamide, 7.0 g of silver oxide was added gradually over an hour. The inorganic salt formed was removed by filtration and the filtrate was evaporated to an oily residue which was extracted with 150 ml of methanol. The methanolic extract was concentrated to 10 ml and added dropwise to 300 ml of ether under stirring. The tetra-N-acetyl-hexa-O-methyl derivative (**XX**) which precipitated was collected by filtration and dried *in vacuo* to yield 3.016 g of amorphous solid. Without further purification, 3.0 g of **XX** was refluxed with 100 ml of 3 N HCl for 3 hours. The hydrolyzate was concentrated *in vacuo*, adjusted to pH 7.0 and applied to a column of Amberlite CG-50 (NH_4^+ form). The column was developed with 600 ml of water followed with 2 liters of 0.1 N aqueous ammonia, and the chromatogram was monitored by TLC (S-114) and ninhydrin test. The watery eluated which contained **XXI** were pooled, concentrated *in vacuo* and charged on a column of Amberlite IR-120 (H^+ form). Elution with 0.5 N ammonia afforded 110 mg of crystalline **XXI** which was recrystallized from a mixture of water, methanol and acetone. m.p. 241~242°C, $[\alpha]_D^{24}$—62° (*c* 0.5, H_2O), TLC: Rf 0.21 (S-114).

Anal. Calcd. for $C_5H_{11}NO_3$: C 45.10, H 8.33, N 10.52.
Found : C 44.81, H 8.63, N 10.83.

From the ammoniacal eluates, 380 mg of **XXII** was obtained as an amorphous solid. TLC: Rf 0.36 (S-114).

A solution of **XXII** (95 mg) and 0.6 ml of acetic anhydride in 6 ml of absolute methanol was stirred for 2.5 hours at room temperature. The solvent was evaporated *in vacuo* and the residue was crystallized from ethanol to yield 68 mg of colorless needles (**XXIII**). m.p. 287.5~289°C, $[\alpha]_D^{24}+3°$ (*c* 1.1, H_2O), NMR $\delta_{DSS}^{D_2O}$ in ppm: 3.54 (CH_3O-) and 2.30 ($2CH_3CO$-), $\Delta[M]_{CuAm}=+1690°$.

Anal. Calcd. for $C_{11}H_{20}N_2O_5$: C 50.75, H 7.75, N 10.76.
Found : C 50.34, H 7.77, N 10.75.

Periodate Oxidation of Bu-1975C_1 (**Ia**) and its Degradation Products

Periodate oxidation experiments were carried out on Bu-1975C_1 and its degradation products (**IIa**, **III**, **IV**, **X**, **XI**, **XIIa**, **XIII** a and **XVb**) by essentially the same procedure as published by OGAWA *et al*[10]. The results are shown in Table 1.

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