1-N-ALKYL ANALOGS OF BUTIROSIN

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Butirosin (**Ia**), 5″-amino-5″-deoxy-(**Ic**), 3′,4′-dideoxy-(**Ie**), and 5″-amino-3′,4′,5″-trideoxybutirosin A (**If**) were converted into the corresponding 1‴-deoxo derivatives, **Ib**, **Id**, **Ig**, and **Ih** by borane reduction. In addition, xylostasin (**IIIa**) was converted into its 1-N-ethyl derivative (**IIIb**) by reductive ethylation. Their antibacterial activities were discussed.

Butirosin is an aminoglycoside antibiotic complex mainly consisting of butirosin A (**Ia**) with structural characteristics due to the (*S*)-4-amino-2-hydroxybutanoyl group attached to the C-1* amino group *via* an amide bond and exhibits broad inhibitory activity against Gram-positive and Gram-negative bacteria with rather low toxicity.** The importance of the acyl side chain in the structure-activity relationship in butirosin has been discussed[1], but it is still not known whether the acyl side chain is necessary for eliciting the characteristic activities of butirosin or is exchangeable with other alkyl groups. The present paper deals with the synthesis of 1-N-alkyl analogs of butirosin and their antibacterial activities.

In 1977, RICHARDSON *et al.*[2] synthesized 1-N-alkyl derivatives of kanamycin A by borane reduction of the corresponding 1-N-acyl derivatives and discussed their antibacterial activities. Accordingly, we applied this method to reduction of the amide bond of butirosin and its derivatives as described below.*** A mixture of butirosin A (**Ia**), trifluoroacetic acid and borane-tetrahydrofuran complex was heated at about 60~70°C with stirring and gave 1‴-deoxobutirosin A (**Ib**). The contaminated starting material was removed by saponification of the reaction mixture with baryta and successive chromatography on Amberlite CG-50. Alternatively, butirosin was converted into the tetra-N-benzoyl (**IIa**) or the tetra-N-benzylidene derivative whose borane reduction was similarly carried out to give a tetra-N-benzyl derivative of **Ib** (**IIb**). Hydrogenolysis of **IIb** over palladium-charcoal afforded a better yield of 1‴-deoxobutirosin A (**Ib**).

Analogously, borane reduction of 5″-amino-5″-deoxybutirosin A[3] (**Ic**) was carried out and gave 5″-amino-1‴-deoxo-5″-deoxybutirosin A (**Id**). An alternate synthesis of **Id** starting from tetra-N-benzoylbutirosin (**IIa**) was performed in the following way. Treatment of **IIa** with 2,2-dimethoxypropane in the presence of an acid gave a 3″,5″-O-isopropylidene derivative which was converted into tetra-N-benzoyl-5″-azido-5″-deoxy derivative (**IIc**) by the following sequence of reactions: acetylation of the remaining hydroxy functions, removal of the 3″,5″-isopropylidene group by acid-hydrolysis, selective tosylation of the resultant 3″,5″-dihydroxy derivative at the 5″-position, substitution of the 5″-tosyloxy group by an azide group and removal of the remaining acetyl group with sodium

* The numbering of the carbons is shown in the chart.
** See the foot-notes cited in the reference 1.
*** After completion of this manuscript, a similar application was published[10].

Table 1. ^{13}C-NMR Chemical shifts of butirosin and its derivatives.

Carbons	Butirosin A (**Ia**)* (sulfate, pD 7)	1‴-Deoxo-butirosin A (**Ib**) (sulfate)	5″-Amino-5″-deoxy-butirosin A (**Ic**)** (pD 5.5)	5″-Amino-1‴-deoxo-5″-deoxybutirosin A (**Id**) (sulfate)	Xylostasin (**IIIa**) (sulfate)	1-N-Ethyl-xylostasin (**IIIb**)*** (sulfate)
1	51.7[a]	52.5[a]	52.1[a]	52.7[a]	51.4	56.9
2	32.9	29.5	32.7	29.6	31.0	28.5
3	51.3[a]	51.4[a]	51.7[a]	51.8[a]	52.7	52.8
4	83.3	83.4	81.1	81.4	83.4	83.4
5	88.7	88.1	86.1	85.4	88.1	87.5
6	78.1	74.5	78.0	74.4	75.2	75.2
1′	97.3	97.2	97.1	59.3	97.3	97.1
2′	56.3	56.4	56.1	56.2	56.4	56.3
3′	70.7	70.7	71.4	71.3	70.7	70.9
4′	73.7	73.8	73.1	73.5	73.8	73.3
5′	72.2[b]	72.0	72.4[b]	72.5	72.1	72.2
6′	43.0	43.1	42.9	43.1	43.2	42.7
1″	114.5	114.6	112.1	112.1	114.7	114.2
2″	77.0[c]	77.3[b]	75.9[c]	77.4[b]	77.4[a]	76.9[a]
3″	76.4[c]	77.1[b]	77.4[c]	77.0[b]	77.1[a]	76.8[a]
4″	85.5	85.6	83.7	85.4	85.7	85.7
5″	63.2	63.3	41.8	41.9	63.3	63.3
1‴	178.2	59.0	178.3	59.3	—	—
2‴	71.8[b]	68.1	72.3[b]	68.3	—	—
3‴	33.4	34.2	33.6	34.3	—	—
4‴	39.2	39.1	39.4	39.2	—	—

a, b, and c may be reversed within the vertical column.

* See reference 9.

** Clear spectrum was obtained at low pH values.

*** 42.7 for $-\overset{|}{N}-\underline{C}H_2CH_3$; 13.7 for $-\overset{|}{N}-CH_2\underline{C}H_3$.

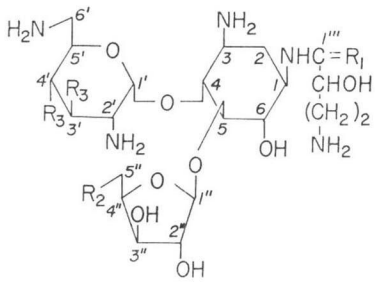
methoxide. Analogous borane reduction of the 5″-azido derivative (**IIc**) gave a 1‴-deoxo compound **IId** which on removal of the benzyl function by hydrogenolysis afforded **Id**.

The conversion of the amide group into the alkylamine in these deoxo compounds, **Ib** and **Id**, was established by infrared spectrometry and inertness to basic saponification. In addition, carbon-13 nuclear magnetic resonance spectra (^{13}C-NMR) were used for further confirmation of their structures as shown in Table 1. In the spectrum of 1‴-deoxobutirosin A (**Ib**), compared with that of butirosin A (**Ia**), the C-1‴ signal at 178.2 ppm of **Ia** shifts to 59.0 ppm. In addition, a large up-field shift of the neighboring carbons, C-2‴ (-3.7 ppm), C-2 (-3.4 ppm) and C-6 (-3.6 ppm) was observed, verifying the C-1‴ deoxo structure. Similar differences were observed in the spectra of **Id** and the parent compound **Ic**. The C-1‴ signal resonates at 59.3 ppm and the neighboring carbons, C-2‴, C-2 and C-6 shift up-field to values of -4.0, -3.1 and -3.6 ppm, respectively, reflecting the structure **Id**.

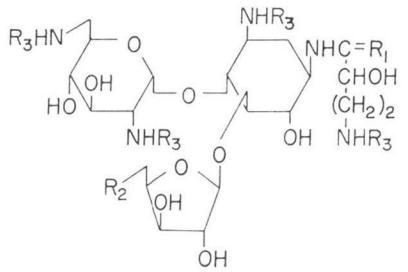
Borane reduction of 3′,4′-dideoxybutirosin A[4] (**Ie**) and 5″-amino-3′,4′,5″-trideoxybutirosin A[5] (**If**) was also carried out and gave 1‴-deoxo-3′,4′-dideoxybutirosin A (**Ig**) and 5″-amino-1‴-deoxo-3′,4′,5″-trideoxybutirosin A (**Ih**), respectively.

Utilizing the lower basicity of the C-1 amino group in aminoglycoside antibiotics, the Schering researchers were recently successful in selective ethylation of C-1 amino group of sisomicin by reductive ethylation with acetaldehyde and borohydride at a fixed pH[6]. 1-Ethyl-sisomicin (netilmicin) thus

Chart 1.



	R_1	R_2	R_3
Ia :	O	OH	OH
Ib :	H_2	OH	OH
Ic :	O	NH_2	OH
Id :	H_2	NH_2	OH
Ie :	O	OH	H
If :	O	NH_2	H
Ig :	H_2	OH	H
Ih :	H_2	NH_2	H

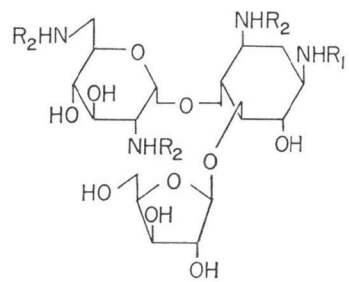


	R_1	R_2	R_3
IIa :	O	OH	COC_6H_5
IIb :	H_2	OH	$CH_2C_6H_5$
IIc :	O	N_3	COC_6H_5
IId :	H_2	NH_2	$CH_2C_6H_5$

obtained revealed broad activities, especially against some resistant strains proved to be inactivators of 3″-O-adenylating-type[7]. Following this method, xylostasin (**IIIa**) was treated with acetaldehyde in a buffer solution at pH 5.3 to give a 1-N-ethylidene derivative whose subsequent reduction with sodium borohydride afforded 1-N-ethylxylostasin (**IIIb**) in about 20% yield. The position of the alkyl group introduced was ascertained by ^{13}C-NMR analysis as shown in Table 1. The spectrum of **IIIb** is almost superimposable with that of the parent compound **IIIa**, showing additional peaks at 42.7 and 13.7 ppm which are assignable to the introduced ethyl group. The attachment of the ethyl group to the C-1 amino function can be deduced by the down-field shift of C-1 (+5.5 ppm) and up-field shift of C-2 (−2.5 ppm) compared with those of xylostasin (**IIIa**), although an expected down-field shift of C-6 was not observed. The structure of **IIIb** was further confirmed by direct comparison with the sample which was synthesized by an unambiguous path as follows. All amino functions of butirosin (**Ia**) were protected with the benzenesulfonyl group in the usual way[8] and the resulting tetra-N-benzenesulfonylbutirosin A was saponified with hydrazine hydrate to give tri-N-benzenesulfonylxylostasin (**IIIc**). Reductive ethylation of **IIIc** with acetaldehyde and sodium borohydride gave a 1-N-ethyl compound **IIId** whose treatment with sodium in liquid ammonia afforded 1-N-ethylxylostasin (**IIIb**).

As shown in Table 2, 1-N-alkyl analogs of butirosins are less active than the parent antibiotics. Thus the 1-N-amide bond in the side chain seems to be necessary for maximal antimicrobial activity in the series of butirosin derivatives. Further, it was found that 1-N-ethylxylostasin (**IIIb**) synthesized as above lost all activity.

Chart 2.



	R_1	R_2
IIIa :	H	H
IIIb :	CH_2CH_3	H
IIIc :	H	$SO_2C_6H_5$
IIId :	CH_2CH_3	$SO_2C_6H_5$

Table 2. Comparative minimal inhibitory concentrations of 1-N-alkyl analogs of butirosin [mcg (as free base)/ml, Heart infusion agar].

Organisms	**Ia** (Sulf.)	**Ib** (Carb.)	**Ic** (Sulf.)	**Id** (Carb.)	**Ie** (Sulf.)	**Ig** (Carb.)	**If** (Carb.)	**Ih** (Carb.)
Staphylococcus aureus FDA 209P	0.2	0.4	0.2	0.2	0.2	0.4	≤0.1	0.2
Staphylococcus aureus 109	25	100	25	25	0.8	1.5	0.2	0.4
Escherichia coli NIHJ	0.8	3.1	3.1	6.2	3.1	3.1	3.1	6.2
Escherichia coli 665	>200	>200	50	200	6.2	12.5	6.2	12.5
Pseudomonas aeruginosa 1055	200	>200	12.5	12.5	6.2	25	3.1	12.5
Pseudomonas aeruginosa 1001	12.5	12.5	3.1	3.1	6.2	12.5	1.5	3.1
Klebsiella pneumoniae 806	1.5	3.1	1.5	3.1	1.5	3.1	1.5	3.1
Klebsiella sp. 857	3.1	6.2	3.1	6.2	12.5	12.5	12.5	25
Proteus vulgaris	1.5	3.1	1.5	3.1	6.2	6.2	3.1	3.1
Proteus mirabilis	12.5	25	25	25	12.5	25	25	25

Sulf.: sulfate salt, Carb.: carbonate salt

Experimental

General Procedures

Melting points are not corrected. Infrared absorption spectra (IR) were recorded on a JASCO A-2 spectrometer (Japan Spectroscopic Co., Ltd.), proton magnetic resonance spectra (NMR) on a Hitachi-Perkin-Elmer R-24 spectrometer at 60 MHz and ^{13}C-NMR on a Varian XL 100A-15 spectrometer at 25.2 MHz, respectively. ^{13}C-Chemical shifts were given in ppm down-field from DSS as an external reference ($\delta^{TMS} = \delta^{DSS} - 2.9$ ppm) and FOURIER analysis was carried out on a Varian 620L-100 computer. Amberlite CG-50 (type I) for ion-exchange chromatography was used in NH_4^+ form unless otherwise stated. Wakogel C-200 (Wako Pure Chemical Industries, Ltd.) was used for silica-gel column chromatography. Amounts of absorbant used and developing solvents are shown in parentheses.

1‴-Deoxobutirosin A (**Ib**)

(1) To a stirred mixture of butirosin base (**Ia**, 557 mg), tetrahydrofuran (THF, 15 ml) and trifluoroacetic acid (0.3 ml) was added 1 M solution of borane in THF (Aldrich Chemical Co., Inc., 20 ml) at 60°C (bath temperature) under nitrogen atmosphere. The mixture was heated at 60°C with stirring for 6 hours and cooled. After the excess reagent was decomposed by careful addition of water, the resultant clear solution was evaporated *in vacuo* to dryness. Methanol containing acetic acid was added to the residue and the solvent was evaporated *in vacuo*. The same procedures were repeated several times. Then, the residue was dissolved in saturated aqueous $Ba(OH)_2$ (50 ml) and the mixture was heated at 100°C for 2 hours. After cooling, the mixture was neutralized with 2 N H_2SO_4 and centrifuged for removal of the formed inorganic precipitates. The supernatant solution was chromatographed on Amberlite CG-50 (120 ml, linear gradient: 0.3~1.0 N NH_4OH), giving **Ib** (271 mg, 50%) along with xylostasin (**IIIa**, 110 mg) as fast-running fractions. The sulfate salt of **Ib** was obtained in the usual way to give an amorphous powder, $[\alpha]_D^{30}$ +18.3° (*c* 0.53, H_2O).

Anal. Calcd. for $C_{21}H_{43}N_5O_{11}\cdot 2.5H_2SO_4\cdot 2H_2O$: C, 30.65; H, 6.37; N, 8.51; S, 9.74.
Found : C, 30.77; H, 6.47; N, 8.37; S, 9.47.

The carbonate salt of **Ib**, amorphous powder, $[\alpha]_D^{30}$ +27.7° (*c* 0.47, H_2O).

Anal. Calcd. for $C_{21}H_{43}N_5O_{11}\cdot 2.5H_2CO_3$: C, 40.51; H, 6.94; N, 10.05.
Found : C, 40.77; H, 6.82; N, 9.94.

(2) A mixture of butirosin base (1.7 g), benzaldehyde (2.7 g) and water (22 ml) was stirred at room temperature for 5 hours, and then evaporated *in vacuo* to dryness. The residual solid was washed

with hexane several times to give a tetra-N-benzylidene derivative as a colorless powder (2.5 g, 90%). The SCHIFF base (1.0 g) thus obtained was dissolved in THF (15 ml) and 1 M solution of borane in THF (20 ml) was added dropwise under nitrogen atmosphere. The mixture was gently refluxed with stirring for 5 hours and was worked-up as described above, giving tetra-N-benzyl-1‴-deoxobutirosin which, without purification, was dissolved in a mixture of EtOH (60 ml) and water (20 ml). After addition of 10% palladium-charcoal (1.0 g) and cyclohexene (10 ml), the mixture was refluxed with stirring for 4 hours and, then cooled, diluted with water and filtered. The catalyst was rinsed with water and the combined filtrate and washings were evaporated *in vacuo* to give a syrup which upon saponification with $Ba(OH)_2$ and successive chromatography (CG-50, 100 ml) as described above gave **Ib** (420 mg, 70% based on the SCHIFF base).

(3) In an analogous way, tetra-N-benzoylbutirosin A (**IIa**) was prepared as described below and converted into **Ib** in 23.4% yield.

5″-Amino-1‴-deoxo-5″-deoxybutirosin A (**Id**)

(1) A mixture of 5″-amino-5″-deoxybutirosin A base[3] (**Ic**, 437 mg), trifluoroacetic acid (0.3 ml), THF (12 ml) and 1 M solution of borane in THF (18 ml) was heated at 70°C with stirring under nitrogen atmosphere for 6 hours and worked-up as described for the preparation of **Ib**, giving **Id** (197 mg, 46%) along with 5″-amino-5″-deoxyxylostasin (146 mg). The sulfate salt (amorphous powder): $[\alpha]_D^{30}$ +19.4° (*c* 0.50, H_2O).

Anal. Calcd. for $C_{21}H_{44}N_6O_{10}\cdot 3H_2SO_4\cdot 5H_2O$: C, 27.27; H, 6.49; N, 6.54; S, 10.40.
Found : C, 26.75; H, 6.20; N, 8.70; S, 10.86.

The carbonate salt: $[\alpha]_D^{30}$ +17.1° (*c* 0.51, H_2O).

Anal. Calcd. for $C_{21}H_{44}N_6O_{10}\cdot 3.5H_2CO_3\cdot H_2O$: C, 37.93; H, 6.89; N, 10.83.
Found : C, 37.84; H, 6.61; N, 10.84.

(2) To a stirred mixture of 5″-azido-tetra-N-benzoyl-5″-deoxybutirosin A (**IIc**, 1.00 g) described below and THF (10 ml) was added 1 M solution of borane in THF (50 ml) at 60°C over a period of 20 minutes under nitrogen atmosphere. The mixture was stirred at 60°C for 4 hours and was worked-up as described above to give 5″-amino-tetra-N-benzyl-1‴-deoxo-5″-deoxybutirosin A (**IId**) as an amorphous solid. Hydrogenolysis of the product with 10% palladium-charcoal (2.0 g) and cyclohexene (7 ml) in a mixture of ethanol (45 ml) and water (10 ml), successive baryta hydrolysis at 100°C for 2 hours and chromatography (CG-50, 150 ml, linear gradient: 0.3~1.5 N NH_4OH/1,200 ml and then 450 ml of 1.5 N NH_4OH) afforded **Id** (217 mg, 40%) as an amorphous powder.

1‴-Deoxo-3′,4′-dideoxybutirosin A (**Ig**)

Similarly, treatment of 3′,4′-dideoxybutirosin A[4] base (**Ie**, 418 mg) with trifluoroacetic acid (0.22 ml) and 1 M borane in THF (15 ml) followed by saponification with $Ba(OH)_2$ and chromatography (CG-50, 60 ml, 0.3~1.2 N NH_4OH/1,200 ml, 1.5 N NH_4OH/300 ml) gave **Ig** (146 mg, 40%) along with 3′,4′-dideoxyxylostasin (109 mg). The carbonate salt: $[\alpha]_D^{30}$ +7.8° (*c* 0.68, H_2O).

Anal. Calcd. for $C_{21}H_{43}N_5O_9\cdot 3H_2CO_3\cdot 3H_2O$: C, 38.45; H, 7.39; N, 9.34.
Found : C, 38.75; H, 6.39; N, 9.10.

5″-Amino-1‴-deoxo-3′,4′,5″-trideoxybutirosin A (**Ih**)

Treatment of the carbonate salt of 5″-amino-3′,4′,5″-trideoxybutirosin A[5] (246 mg) with trifluoroacetic acid (0.1 ml) and 1 M borane in THF (10 ml), followed by saponification and chromatography on CG-50 (50 ml, 0.3~1.5 N NH_4OH/600 ml, 200 ml of 1.7 N NH_4OH) gave **Ih** (51 mg, 28%) along with 5″-amino-3′,4′,5″-trideoxyxylostasin. The carbonate salt: $[\alpha]_D^{30}$ +3.9° (*c* 0.56, H_2O).

Anal. Calcd. for $C_{21}H_{44}N_6O_8\cdot 3.5H_2CO_3\cdot 3H_2O$: C, 37.74; H, 7.37; N, 10.76.
Found : C, 37.75; H, 6.56; N, 10.14.

1-N-Ethylxylostasin (**IIIb**)

(1) To a solution of xylostasin base (**IIIa**, 2.0 g) in an acetate buffer solution (pH 5.3, 100 ml) was added 80% aqueous acetaldehyde (1 ml) and the mixture was stirred for 5 minutes at room temperature. After addition of $NaBH_4$ (50 mg), the mixture was stirred for 30 minutes. Further aqueous acetaldehyde (1 ml) and $NaBH_4$ (50 mg) were added in the same manner. The resultant solution was

charged onto a column of CG-50 (150 ml) and the column was washed with water (300 ml) and eluted with NH_4OH (linear gradient: 0.3~0.6 N/1,600 ml) to give **IIIb** (425 mg, 20%) along with the unchanged **IIIa** (1.22 g, 50% recovery). The sulfate salt: $[\alpha]_D^{30}$ +38.6° (*c* 0.48, H_2O), mp > 250°C.

Anal. Calcd. for $C_{19}H_{36}N_4O_{10}\cdot 2H_2SO_4\cdot 3H_2O$: C, 31.15; H, 6.55; N, 7.65; S, 8.74.
Found : C, 30.80; H, 6.64; N, 7.12; S, 9.23.

(2) Aqueous acetaldehyde (80%, 2.5 ml) was added to a solution of tri-N-benzenesulfonylxylostasin, **IIIc** (500 mg) in 75% methanol (75 ml) at room temperature. After stirring for 10 minutes, $NaBH_4$ (50 mg) was added portionwise to the mixture. After stirring for another 20 minutes, acetic acid (250 mg), acetaldehyde (2.5 ml) and $NaBH_4$ (50 mg) were added successively and the solution was stirred for 1 hour at room temperature. During that time the pH of the solution was kept between 6 and 7. The mixture was concentrated *in vacuo* and diluted with water. The resultant solid was collected and chromatographed on silica gel (10 g, MeOH-$CHCl_3$, 1: 10~2: 5, v/v). The 1-N-ethyl derivative, **IIId** (300 mg, 58%), mp 153~156°, was obtained with the unchanged **IIIc** (300 mg). IR (KBr, cm^{-1}): 3400br., 1440, 1320, 1160, 1090. NMR (60 MHz, CD_3OD) δ: 6.0 (6H, m), 7.7 (9H, m), 1.20 (3H, t, J=7 Hz).

Anal. Calcd. for $C_{37}H_{50}N_4O_{16}S_3\cdot 2H_2O$: C, 47.33; H, 5.54; N, 5.97; S, 10.23.
Found : C, 47.78; H, 5.71; N, 5.93; S, 10.35.

To a stirred solution of **IIId** (300 mg) in liquid ammonia (5 ml) were added pieces of sodium (250 mg) at −78°C and the mixture was stirred at −70~−50°C for 30 minutes. After treatment with MeOH (5 ml) and successive evaporation, the residue was dissolved in water and treated with CG-50 (H^+ form) until the supernatant solution became negative to ninhydrin test. The resin was transferred into a column, washed with water and then eluted with NH_4OH (linear gradient: 0.3~0.6 N). **IIIb** was isolated in 50% yield based on **IIId**.

Tetra-N-benzoylbutirosin A (**IIa**)

To a solution of butirosin sulfate hydrate (**Ia**, base content *ca.* 70%, 2.0 g) and Na_2CO_3 (0.7 g) in water (10 ml) was added benzoyl chloride (0.4 ml) with cooling in an ice-bath and stirring. The stirring was continued for 5 minutes and additional Na_2CO_3 (1.3 g), MeOH (25 ml) and benzoyl chloride (1.4 ml) were added. After stirring for 30 minutes at room temperature the mixture was diluted with MeOH (25 ml) and evaporated to dryness below 45°C. The residue was triturated with cold water and the resulting solid was collected, washed with water and ether and then dissolved again in MeOH. The cloudy solution was passed through carbon powder and diluted with ether, yielding 2.36 g (95.7%) of **IIa**, mp *ca.* 170°C, amorphous solid. A pure sample was obtained by chromatography on silica gel (× 20 by weight, 10~20% MeOH - $CHCl_3$, v/v) followed by precipitation from MeOH with ether, mp 175~200°, amorphous solid.

Anal. Calcd. for $C_{49}H_{57}N_5O_{16}$: C, 60.55; H, 5.91; N, 7.21.
Found : C, 59.98; H, 6.24; N, 7.03.

5″-Azido-tetra-N-benzoyl-5″-deoxybutirosin A (**IIc**)

A solution of **IIa** (4.0 g), 2,2-dimethoxypropane (4 ml) and *p*-toluenesulfonic acid hydrate (200 mg) in dimethyl formamide (40 ml) was allowed to stand at room temperature for 5 hours and then treated with Amberlite IR-45 (OH^- form, 6 g). After evaporation of the solvent, the residual amorphous substance was acetylated with pyridine (40 ml) and acetic anhydride (20 ml) in the usual manner. Usual work-up gave 3″,5″-O-isopropylidene (colorless powder) (4.96 g) which was dissolved in MeOH (60 ml) and *p*-toluenesulfonic acid hydrate (80 mg) was added. The mixture was kept at 50~55°C (bath temp.) for 1.5 hours, cooled, neutralized with pyridine and evaporated to give a syrup which was chromatographed on silica gel (100 g packed with $CHCl_3$, 2, 3 and 5% MeOH - $CHCl_3$, v/v) and solidified with hexane, giving 6,3′,4′,2″,2‴-penta-O-acetyl-3,2′,6′,4‴-tetra-N-benzoylbutirosin A (3.6 g, 69% from **IIa**), mp 153~177°C, amorphous powder.

Anal. Calcd. for $C_{59}H_{67}N_5O_{21}\cdot C_6H_{14}$(hexane) : C, 61.55; H, 6.44; N, 5.52.
Found : C, 61.31; H, 6.34; N, 5.47.

p-Toluenesulfonyl chloride (709 mg) was added to the solution of the O-acetyl-N-benzoylbutirosin (2.20 g) obtained as above in pyridine (15 ml) with cooling and stirring. The resultant solution was

allowed to stand at room temperature for 5 hours. Work-up in the usual manner gave a 5″-*p*-toluenesulfonate [2.25 g, IR(KBr, cm^{-1}): 1600, 1180, 1175(OTs). NMR (60 MHz, $CDCl_3$) δ: 2.43 (3H, s, CH_3 of Ts), 2.25~1.75 (15H, Ac)]. A mixture of the sulfonate (2.18 g), NaN_3 (0.5 g) and dimethyl sulfoxide (12 ml) was heated at 100°C for 1 hour and, after cooling, poured into ice-water to afford a 5″-azide [1.71 g, IR(KBr, cm^{-1}): 2080 (N_3)]. Removal of the acetyl group was carried out with $NaOCH_3$ in MeOH in the usual manner, giving **IIc** (1.27 g, 51% from **IIa**) as a slightly hygroscopic powder, mp 153°(softened)~170°C.

Anal. Calcd. for $C_{48}H_{56}N_8O_{15}$: C, 59.03; H, 5.66; N, 11.24.
Found : C, 58.92; H, 5.88; N, 10.84.

3,2′,6′-Tri-N-benzenesulfonylxylostasin (**IIIc**)

Benzenesulfonyl chloride (7.1 ml) in acetone (6 ml) was added dropwise to a stirred solution of butirosin sulfate (**Ia**, 7.9 g) and Na_2CO_3 (6.3 g) in 30% aqueous acetone (100 ml) with cooling in an ice-bath. The acetone (30 ml) was added and the mixture was stirred for 1.5 hours at room temperature overnight. After acidification with acetic acid to pH 5, the precipitates formed on addition of ice-water were collected and dried, yielding tetra-N-benzenesulfonylbutirosin (10 g). Chromatography on silica gel (10~20% MeOH - $CHCl_3$, v/v) gave a pure sample, mp 168~173°C.

Anal. Calcd. for $C_{45}H_{57}N_5O_{20}S_4$: C, 48.42; H, 5.15; N, 6.27; S, 11.49.
Found : C, 47.65; H, 5.24; N, 6.04; S, 11.31.

A solution of tetra-N-benzenesulfonylbutirosin (2.9 g) in 80% hydrazine hydrate (10 ml) was refluxed for 7 hours with stirring, then evaporated *in vacuo* to give a thick oil which on trituration with MeOH followed by filtration and washing with ether afforded **IIIc** (2.0 g, 16% from **Ia**). Recrystallization from 95% MeOH gave a pure sample, mp 241~244°C(decomposed). IR (KBr, cm^{-1}): 3500, 3350, 3200, 1440, 1320, 1160, 1090.

Anal. Calcd. for $C_{35}H_{46}N_4O_{16}S_3 \cdot H_2O$: C, 47.08; H, 5.38; N, 6.28; S, 10.76.
Found : C, 46.90; H, 5.12; N, 6.39; S, 11.52.

3,2′,6′-Tri-N-benzenesulfonyl-1-N-ethylxylostasin (**IIId**)

80% Aqueous acetaldehyde (2.5 ml) was added to a solution of **IIIc** (500 mg) in 75% MeOH (75 ml) at room temperature. After stirring for 10 minutes, $NaBH_4$ (50 mg) was added portionwise to the mixture. The mixture was stirred for 20 minutes and, after addition of acetic acid (250 mg), acetaldehyde (2.5 ml) and $NaBH_4$ (50 mg), was further stirred for 1 hour at room temperature. During that time the pH of the solution was kept between 6 and 7. The solvent was evaporated *in vacuo* and the residue was treated with Amberlite CG-50 (H^+ form) in MeOH and chromatographed on silica gel (1:10~2:5 MeOH - $CHCl_3$, v/v), giving **IIId** (300 mg, 58%), mp 153~156°C, with the unchanged **IIIc** (300 mg). IR (KBr, cm^{-1}); 3500br., 1440, 1320, 1160, 1090. NMR (60 MHz, CD_3OD) δ: 8.0 (6H, m), 7.7 (9H, m), 1.20 (3H, t, J=7 Hz).

Anal. Calcd. for $C_{37}H_{50}N_4O_{16}S_3 \cdot 2H_2O$: C, 47.33; H, 5.54; N, 5.97; S, 10.23.
Found : C, 47.78; H, 5.71; N, 5.93; S, 10.35.

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