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Synthesis of 3′,4′-Dideoxybutirosin A, Active against Butirosin Resistant Bacteria[1]

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In order to overcome butirosin-resistant microorganisms of 3′-O-phosphorylation type, 3′,4′-dideoxybutirosin A (2) was synthesized according to the method of Umezawa, *et al.*[3] The synthesized 3′,4′-dideoxy derivative (2) exhibited significant activities agaisnt both butirosin-sensitive and resistant strains *in vitro* and *in vivo* and its acute toxicity was found to be at a similar level to that of butirosin.

Butirosin, an aminoglycoside antibiotic complex obtained from fermentation filtrates of *Bacillus circulans* (NRRL B-3312 and B-3313),[4,5] has been shown to exhibit broad inhibitory activity against gram-positive and -negative bacteria and notably against *Pseudomonas aeruginosa* including gentamicin-resistant clinical isolates.[6] The complex has been resolved into two bases, butirosin A (**1a**) and butirosin B (**1b**),[4,5] and chemical studies have elucidated their structures as shown in the Chart.[5,7] In succession, it was found that, similarly to other aminoglycoside antibiotics,[8] butirosin was inactivated enzymatically in the media of several clinically isolated strains of *Escherichia* and *Pseudomonas* through being phosphorylated at the 3′[9]-hydroxy group.[10]

In the recent semi-synthetic studies on 3′-deoxy or 3′,4′-dideoxy derivatives of aminoglycoside antibiotics, Umezawas and their groups verified that removal of the hydroxy group amenable to enzymatic phosphorylation gives compounds active against the organisms resistant to the parent antibiotics.[3,11] Consequently, we directed our attention towards a syn-

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[2] Location: *Hiromachi 1-2-58, Shinagawa-ku, Tokyo.*

[3] 3′,4′-Dideoxykanamycin B: S. Umezawa, H. Umezawa, Y. Okazaki, and T. Tsuchiya, *Bull. Chem. Soc. Japan*, **45**, 3624 (1972). 3′,4′-Dideoxyneamine: S. Umezawa, T. Tsuchiya, T. Jikihara, and H. Umezawa, *J. Antibiotics*, **24**, 711 (1971). 3′,4′-Dideoxyribostamycin: S. Umezawa, T. Tsuchiya, D. Ikeda, and H. Umezawa, *ibid.*, **25**, 613 (1972).

[4] H.W. Dion, P.W.K. Woo, N.E. Willer, D.L. Kern, J.Onaga, and S.A. Fusari, *Antimicrob. Ag. Chemother.*, **2**, 84 (1972).

[5] P.W.K. Woo, H.W. Dion, and Q.R. Bartz, *Tetrahedron Letters*, **1971**, **2617**.

[6] J.D. Howell, L.E. Anderson, G.L. Coffey, G.D. Senos, M.A. Underhill, D.L. Vogler, and J. Ehrlich, *Antimicrob. Ag. Chemother.*, **2**, 73 (1972); C.L. Heifetz, M.W. Fisher, J.A. Chodubski, and M.O′. DeCarlo, *ibid.*, **2**, 89 (1972).

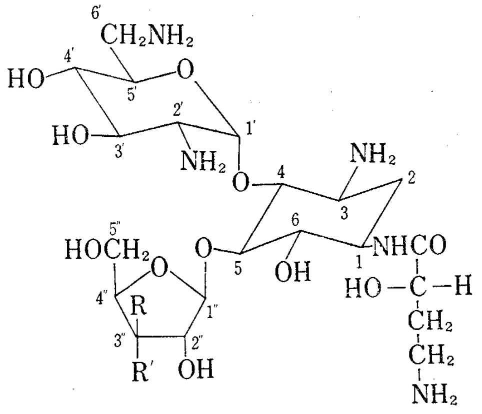
[7] P.W.K. Woo, *Tetrahedron Letters*, **1971**, 2621; P.W.K. Woo, H.W. Dion, and Q.R. Bartz, *ibid.*, **1971**, 2625.

[8] H. Umezawa, *Progress in Antimicrobial and Anticancer Chemotherapy*, **2**, 567 (1970) and the related references cited therein.

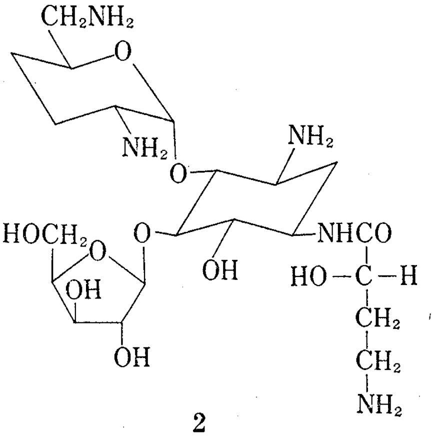
[9] The conventional numbering of carbons in butirosin is as follows: That of 2-deoxystreptamine is according to the proposal of Hichens and Rinehart (M. Hichens and K.L. Rinehart, Jr., *J. Am. Chem. Soc.*, **85**, 1547 (1963)]. Single prime on the number indicates carbons of a sugar attached to the 4-position of 2-deoxystreptamine and double primed carbons of a sugar attached to the 5-position. See Chart 1.

[10] M. Brzezinska and J. Davies, *Antimicrob. Ag. Chemother.*, **3**, 266 (1973); M. Yagisawa, H. Yamamoto, H. Naganawa, S. Kondo, T. Takeuci, and H. Umezawa, *J. Antibiotics*, **25**, 748 (1972).

[11] 3′-Deoxykanamycin A: S. Umezawa, Y. Nishimura, H. Hineno, K. Watanabe, S. Koike, T. Tsuchiya, and H. Umezawa, *Bull. Chem. Soc. Japan*, **45**, 2847 (1972).



1a : R=OH, R'=H
1b : R=H, R'=OH



2

Chart 1

thesis of butirosin derivatives lacking the hydroxy group at the 3′ position which would be inert to the resistance mechanism; subsequently by applying the method developed by Umezawa, *et al.*,[3] we attempted a synthesis of 3′,4′-dideoxybutirosin A (**2**)[12] in the following way.

The starting material, butirosin A (**1a**) was isolated from the complex by means of Dowex 1X2 (borate form) chromatography according to the method of Dion, *et al.*[4] Tetra-N-benzyloxycarbonyl-butirosin A (**3**) obtained from **1a** was treated with 1,1-dimethoxycyclohexane in the presence of *p*-toluenesulfonic acid in N,N-dimethylformamide, giving a 3′,4′: 3″,5″-di-O-cyclohexylidene derivative (**4a**), along with 3″,5″-mono-O-cyclohexylidene derivative.[13] The structure of **4a** was verified by its inertness to periodate oxidation. Acetylation of **4a** in the usual manner gave a triacetate (**4b**) which was dissolved in 70% aqueous acetic acid at room temperature to afford a 3′,4′-diol (**5a**) with removal of one cyclohexylidene group. The diol (**5a**) was treated with methanesulfonyl chloride in pyridine, giving a di-O-methanesulfonyl compound (**5b**). The sulfonate (**5b**) was heated at 90—95° in N,N-dimethylformamide with an excess amount of sodium iodide and zinc powder and the resulting product was treated with methanolic sodium methoxide to give a deacetylated product which was, without purification, hydrolyzed with hot aqueous acetic acid for a short time to yield a crude 3′,4′-dideoxy-3′-eno compound (**6**). The 3′-eno compound (**6**) was purified by chromatography on silica gel and was hydrogenated over 10% palladized charcoal in a mixture of methanol and dilute hydrochloric acid, giving crude 3′,4′-dideoxybutirosin A (**2**) as its tetrahydrochloride. The pure sample was obtained by means of chromatography over Amberlite CG-50 (NH_4^+ form) using 0.3—1.0N ammonium hydroxide as eluant.

As illustrated in Table I, 3′,4′-dideoxybutirosin A (**2**) thus synthesized showed outstanding activities against not only butirosin sensitive strains but also butirosin resistant strains which have been proved to be resistant by a phosphorylating mechanism such as *Escherichia coli* 620, *E. coli* 665, *Pseudomonas aeruginosa* 1055, and so on.[14] These facts are also reflected

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12) Synthesis of 3′,4′-dideoxybutirosin B from ribostamycin by introducing the side chain and successive analogous deoxylation was announced during this study. See, D. Ikeda, T. Tsuchiya, S. Umezawa, H. Umezawa, and M. Hamada, *J. Antibiotics*, 26, 307 (1973).

13) The prolonged treatment of 3 with 1,1-dimethoxycyclohexane afforded tri-cyclohexylidene derivatives as by-products whose structure are still uncertain, but on treatment with acid, these by-products reverted to the starting material (3) and 3″,5″-mono-O-cyclohexylidene derivative. The structure of the mono-O-cyclohexylidene derivative was verified by the fact that its periodate oxidation followed by methanolysis afforded a mixture of methyl xylosides.

14) Inactivation of butirosin by these strains clinically isolated, and the isolation and characterization of 3′-O-phosphoryl-butirosin will be published elsewhere.

in protecting effects of **2** against bacterial infections in mice as compared with those of butirosin and kanamycin. The results are shown in Table II. In addition, Table III shows susceptibility of clinical isolates (*Escherichia* and *Pseudomonas*) to **2**, indicating that the numbers of

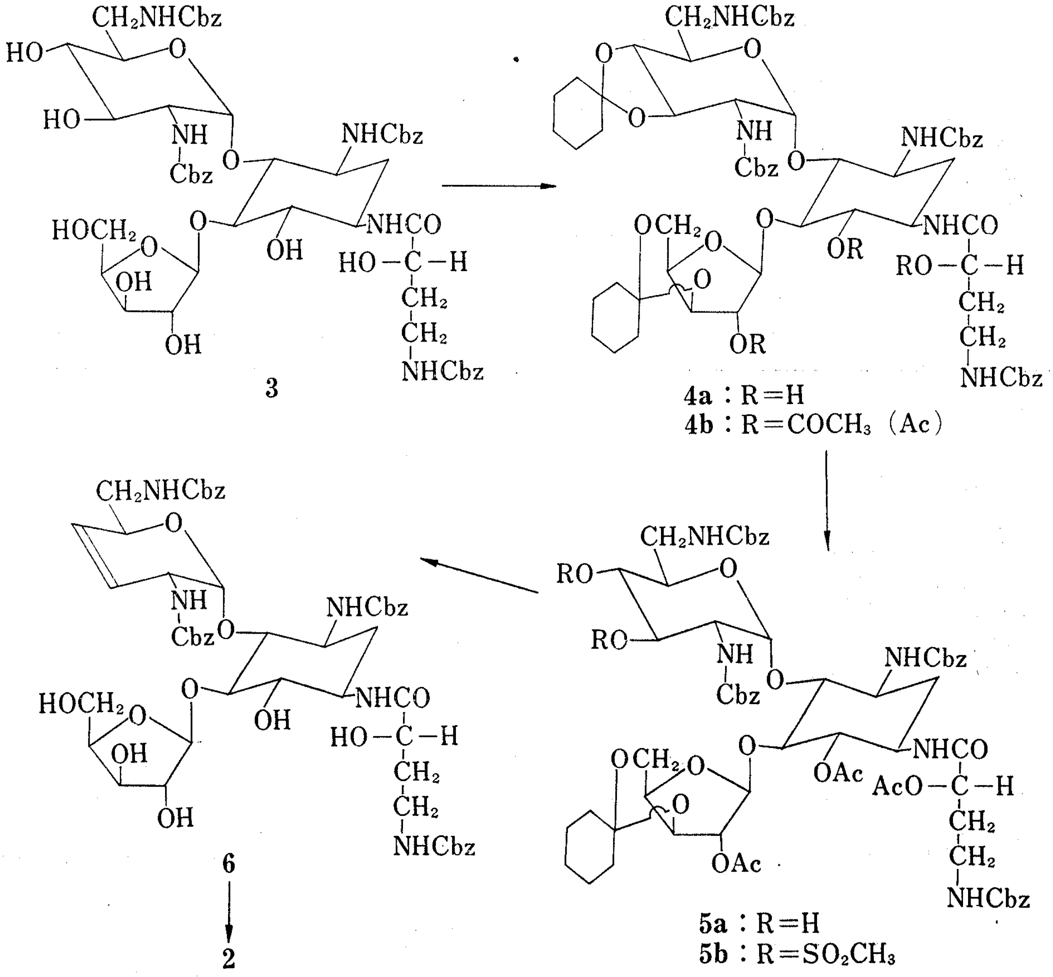

Chart 2

$Cbz=COOCH_2C_6H_5$

Table I. Minimum Inhibitory Concentration of Butirosin and 3′,4′-Dideoxybutirosin A (in mcg/ml, heart infusion agar)

Test organisms	Butirosin	3′,4′-Dideoxybutirosin A
Bacillus subtilis PCI-219	≦0.1	≦0.1
Staphylococcus aureus 209 P	≦0.1	≦0.1
Staphylococcus aureus 56	0.8	0.4
Escherichia coli NIHJ	0.8	0,4
Escherichia coli 620 ^{x}	100	3.1
Escherichia coli 665 ^{x}	200	3.1
Klebsiella 806	0.8	0.8
Klebsiella 813	50	50
Proteus mirabilis 1306	50	50
Proteus vulgaris 025	0.8	0.8
Salmonella enteritidis	0.8	1.5
Shigella flexneri 2 a	3.1	3.1
Pseudomonas aeruginosa Scr.	6.2	3.1
Pseudomonas aeruginosa 1055 ^{x}	400	6.2

x: strains proved to be inactivators of phosphorylation type highly resistant strains (≥100 mcg/ml) to butirosin decrease. As shown in Table IV, the acute toxicity of **2** seems to be at a similar level as butirosin and therefore **2** is much safer than other 3′-deoxy-type aminoglycoside antibiotics.

Table II. Protecting Effects of 3′,4′-Dideoxybutirosin A

Organisms challenged intraperitonially	3′,4′-Dideoxy-butirosin A	Butirosin	Kanamycin
Escherichia coli 704–1	2.30	2.47	4.60
Escherichia coli 665	2.43	146.3	>200

ED_{50}: mg/kg/dose (2 doses subcutaneously at 0 and 4 hr post-infection)

Table III. Susceptibility of Clinical Isolates to Butirosin and 3′,4′-Dideoxybutirosin A

MIC mcg/ml	>200	200	100	50	25	12.5	6.2	3.1	1.5	0.8
Escherichia coli (125 strains)										
Butirosin	5	0	2	1	9	68	39	1	0	0
3′,4′-Dideoxy butirosin A	0	0	2	2	15	100	6	0	0	0
Pseudomonas aeruginosa (102 strains)										
Butirosin	7	1	7	21	37	16	8	3	2	0
3′,4′-Dideoxy butirosin A	1	2	5	3	21	41	20	5	3	1

Table IV. Acute Toxicities of 3′,4′-Dideoxybutirosin A and Butirosin

Route	Dose (mg/kg)	Death incidence	
		Butirosin	3′,4′-Dideoxybutirosin A
i.v.	500	2/2	1/2
i.v.	250	0/3	0/3
s.c.	4000	4/4	not tested
s.c.	2000	0/4	0/3

mice: ddY strain, male, 20±0.2 g

Experimental

Melting points are not corrected. Infrared spectra were recorded on a Hitachi EPI-G3 spectrometer and nuclear magnetic resonance spectra (NMR) on a Varian A-60 spectrometer. Optical rotations were measured on a Perkin-Elmer Model 141 automatic polarimeter in 1 dm tubes. Thin-layer chromatography (TLC) was performed on TLC-plates, Silica gel F_{254} precoated, layer thickness 0.25 mm (E. Merck AG) and spots were visualized by UV-irradiation and/or spraying with vanadic acid–sulfuric acid reagent.[15] Amino compounds were detected by ninhydrin reagent. For column chromatography on silica gel, Wakogel C-200 (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and commercial $CHCl_3$ stabilized with about 1% EtOH were used. Solvents were removed by a rotating flash evaporator at diminished pressure and usually at 35—50°.

Tetra-N-benzyloxycarbonyl-butirosin A (3)——Butirosin A sulfate was prepared from butirosin complex by means of Dowex 1×2 (borate form) chromatography according to Dion, *et al.*[3] To an ice-cold solution of 9.4 g of butirosin A sulfate (**1a**) thus obtained and 2.1 g of Na_2CO_3 in 50 ml of H_2O, 7.5 ml (*ca.* 9 g) of benzyloxycarbonyl chloride and a solution of 2.49 g of Na_2CO_3 in 20 ml of H_2O were added alternately with stirring. Then, 30 ml of MeOH and 30 ml of acetone were added to the mixture and stirring was continued at room temperature for 5.5 hr. Concentration of the mixture *in vacuo* below 45° caused precipitation of

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[15] M. Ishidate, M. Matsui, and M. Okada, *Anal. Biochem.*, **11**, 176 (1965).

an oil. The oil was collected by decantation, washed with H_2O twice and dissolved in EtOH. The solution was filtered and evaporated *in vacuo* to dryness, leaving 10.1 g of **3** as amorphous powder. $[\alpha]^{27}_{D}+16.6°$ ($c=1.85$, $CHCl_3$). *Anal.* Calcd. for $C_{53}H_{65}O_{20}N_5$: C, 58.29; H, 6.00; N, 6.41. Found: C, 58.67; H, 6.14; N, 6.42.

Tetra-N-benzyloxycarbonyl-3′,4′: 3″,5″-di-O-cyclohexylidenebutirosin A (4a)——A solution of 10 g of **3**, 6 ml of 1,1-dimethoxycyclohexane, and 0.35 g of *p*-toluenesulfonic acid hydrate in 30 ml of N,N-dimethylformamide was refluxed at 35° (bath temp.) under diminished pressure of 4 mmHg with vigorous stirring for 1—2 hr and aliquots of the mixture were monitored by TLC (solvent system: MeOH: $CHCl_3=1:19$ or 1: 9, v/v) several times. After cooling an excess of K_2CO_3 (solid) was added and the mixture was stirred for 20 min and then filtered. The filtrate was evaporated *in vacuo* below 50° and a solution of the residue in $CHCl_3$ was charged on a column of 200 g of silica gel packed with $CHCl_3$. The column was eluted stepwise with $CHCl_3$, 2%, 3%, and 5% (v/v) MeOH–$CHCl_3$ and the fraction were examined by TLC and evaporated *in vacuo*. First, 1.8 g of tricyclohexylidene derivative, next 6.5 g of the desired di-O-cyclohexylidene derivative (**4a**) and finally 1.7 g of mono-O-cyclohexylidene derivative emerged. The di-O-cyclohexylidene derivative (**4a**) thus obtained was amorphous and its NMR spectrum (60 MHz, $CDCl_3$) exhibited a broad absorption due to protons (20H) of the cyclohexylidene groups at δ 1.0—2.0 ppm. $[\alpha]^{27}_{D}+11°$ ($c=1.4$, $CHCl_3$). *Anal.* Calcd. for $C_{65}H_{81}O_{20}N_5$: C, 62.34; H, 6.52; N, 5.59. Found: C, 61.99; H, 6.45; N, 5.31.

Tri-O-acetyl-tetra-N-benzyloxycarbonyl-3′,4′: 3″,5″-di-O-cyclohexylidene-butirosin A (4b)——Working up in the usual manner, 3.7 g of **4a** was acetylated with 20 ml of pyridine and 8 ml of Ac_2O, giving 4.2 g of the crude acetate (**4b**). The analytical sample was obtained by chromatography on silica gel. $[\alpha]^{27}_{D}+7.8°$ ($c=2.8$, $CHCl_3$). *Anal.* Calcd. for $C_{71}H_{87}O_{23}N_5$: C, 61.86; H, 6.39; N, 5.08. Found: C, 61.27; H, 6.34; N, 4.99.

Tri-O-acetyl-tetra-N-benzyloxycarbonyl-3″,5″-O-cyclohexylidene-butirosin A (5a) and Its 3′,4′-Di-O-methanesulfonyl Derivative (5b)——A solution of 4.2 g of **4b** in a mixture of 40 ml of AcOH and 12 ml of H_2O was left standing for 1—2 hr at room temperature. Progress of the reaction was examined by TLC (solvent system: MeOH: $CHCl_3=1:19$, v/v). Then, after cooling, the mixture was almost neutralized with 1 N NaOH or solid K_2CO_3 and extracted with $CHCl_3$. The extract was washed with sat. aq. $NaHCO_3$ exhaustively and, after washing with H_2O, dried over anhyd. $MgSO_4$. Evaporation of the solvent left 3.6 g of the 3′,4′-dihydroxy derivative (**5a**) as amorphous powder.

To an ice-cold solution of 3.4 g of the crude **5a** in 30 ml of pyridine, 6 ml of MsCl was added with stirring. The mixture was kept at room temperature for 1 hr, then diluted with ice-water, and neutralized with aq. $NaHCO_3$. The oily precipitates were collected by decantation, washed with H_2O and dissolved in $CHCl_3$. The supernatant was extracted with a small amount of $CHCl_3$. The combined $CHCl_3$ solution was washed successively with 2 N HCl, H_2O, sat. aq. $NaHCO_3$, and H_2O, dried over anhyd. $MgSO_4$, and evaporated to give 3.24 g of yellow powder. Purification of this powder was carried out by column chromatography on 65 g of silica gel, using $CHCl_3$ and 2% (v/v) MeOH–$CHCl_3$ as eluant; thus, 2.64 g of **5b** was obtained as powder, mp 108—125° (from benzene–hexane). $[\alpha]^{27}_{D}-14.3°$ ($c=1.5$, $CHCl_3$). IR $\nu^{\text{Nujol}}_{\text{max}}$: 1180 cm^{-1} ($-OSO_2CH_3$). NMR (60 MHz, $CDCl_3$) δ ppm: 2.81 (3H, s), 3.07 (3H, s). *Anal.* Calcd. for $C_{66}H_{83}O_{27}N_5S_2$: C, 54.95; H, 5.80; N, 4.86; S, 4.45. Found: C, 55.41; H, 5.80; N, 4.80; S, 4.40.

Tetra-N-benzyloxycarbonyl-3′,4′-dideoxy-3′-eno-butirosin A (6)——A mixture of 10 g of **5b**, 80 ml of N,N-dimethylformamide 26.7 g of zinc powder, 50 g of NaI was heated at 95—100° (bath temp.) with vigorous stirring for 2—3 hr. To the mixture were added 560 ml of $CHCl_3$ and 100 ml of 10% aq. $Na_2S_2O_3$ solution and undissolved zinc powder was removed by filtration. The filtrate was shaken with 200 ml of 10% aq. $Na_2S_2O_3$ solution and the $CHCl_3$ layer was washed with H_2O, dried over $MgSO_4$, and evaporated *in vacuo* to afford 8.8 g of a brown thick syrup. A solution of the syrup thus obtained in 70 ml of MeOH containing 2 ml of 2 N NaOMe was kept at room temperature overnight, neutralized with AcOH, concentrated *in vacuo* to a half volume, diluted with $CHCl_3$, washed with sat. aq. NaCl solution and H_2O, dried with $MgSO_4$ and evaporated to give 8 g of a syrup. A solution of this syrup in a mixture of 80 ml of AcOH and 30 ml of H_2O was heated on a steam bath for 15 min, then evaporated below 50° (bath temp.) under diminished pressure. The residue was dissolved in EtOH and treated with active carbon. The crude product obtained by evaporation of EtOH was dissolved in AcOEt and poured onto a column of 80 g of silica gel packed with $CHCl_3$. The column was first washed with $CHCl_3$ and then eluted with 4%, 5%, and 7% (v/v) MeOH–$CHCl_3$ successively. From earlier eluates, 0.57 g of contaminant (probably originating from unchanged di-O-methanesulfonate (**5b**), IR $\nu^{\text{Nujol}}_{\text{max}}$: 1180 cm^{-1}) was obtained. The successive eluates which contained the major component (TLC: MeOH: $CHCl_3=3:17$, v/v, *Rf ca.* 0.4, slightly smaller than that of the above contaminant) were collected and evaporated to leave 1.69 g of a thick syrup of **6**, which was dissolved in AcOEt. The precipitates, amorphous hygroscopic powder, obtained by addition of ether were collected and dried. The infrared spectrum showed no absorption at 1180 cm^{-1} (sulfonyloxy). NMR (60 MHz, pyridine-d_5) δ ppm: 5.83 (broad singlet, 2H, olefinic protons). $[\alpha]^{27}_{D}$ $-23.5°$ ($c=0.75$, $CHCl_3$). *Anal.* Calcd. for $C_{53}H_{63}O_{18}N_5\cdot 1.5H_2O$: C, 58.66; H, 6.13; N, 6.45. Found: C, 58.51; H, 5.94; N, 6.51.

3′,4′-Dideoxybutirosin A (2)——Hydrogen was passed through a suspension of 2.3 g of 10% Pd-C on a solution of 1 g of **6** and 1.2 ml of 2 N HCl in a mixture of 40 ml of MeOH and 15 ml of H_2O at room temperature with stirring for 1.5 hr. Then, after addition of 0.7 ml of 2 N HCl, the hydrogenation was continued for another 30 min. The mixture was diluted with H_2O and the catalyst was removed by filtration. The filtrate was adjusted to pH 5—6 with Amberlite IR-45 (OH^- form) and concentrated below 45° (bath temp.) to a small volume. The resultant solution was charged on a column of 300 ml of Amberlite CG-50 (NH_4^+ form), and the column was washed with H_2O, eluted with 1800 ml of 0.3 N NH_4OH, affording contaminant. Then, the column was eluted with 200 ml of 1 N NH_4OH. The fractions which contained a substance showing *Rf* value slightly larger than that of butirosin on TLC (solvent system: upper layer of $CHCl_3$: MeOH: 17%-NH_4OH=2: 1: 1 or MeOH: conc. NH_4OH=1: 1, v/v) were collected and concentrated below 55° (bath temp.) until ammonia was removed. After saturation with carbon dioxide, the resultant solution was freeze-dried, and gave 360 mg of hydrogen carbonate salt of 2 as powder, mp 130—145° (with gas evolution at 168°), $[\alpha]_D^{25}+15.7°$ (c=0.79, H_2O). NMR (60 MHz, D_2O, DSS as internal standard) δ ppm: 1.3—2.4 (broad multiplet, 8H, 2,3′,4′-methylenes and β-methylene of the acyl moiety), 5.25 (broad singlet, 1H, H-1″), 5.55 (multiplet, 1H, H-1′), no signals due to olefinic protons. *Anal.* Calcd. for $C_{21}H_{41}O_{10}N_5 \cdot 2H_2CO_3 \cdot 2H_2O$: C, 40.40; H, 7.22; N, 10.24. Found: C, 40.51; H, 7.52; N, 10.58.

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