SYNTHESIS OF AMINOTRIDEOXYBUTIROSIN A, A CHEMICALLY MODIFIED ANTIBIOTIC ACTIVE AGAINST BUTIROSIN-RESISTANT BACTERIA

HIROMICHI SAEKI, YOSHIKAZU SHIMADA, EIJI OHKI and SHINICHI SUGAWARA

Central Research Laboratories, Sankyo Co., Ltd.,
Hiromachi, Shinagawa-ku, Tokyo, Japan

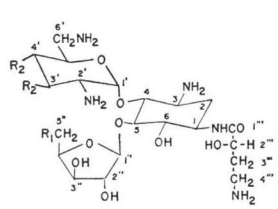
(Received for publication March 31, 1975)

5''-Amino-3',4',5''-trideoxybutirosin A (**4**) was synthesized by two routes starting from the known tri-O-acetyl-tetra-N-benzyloxycarbonyl-3'',5''-O-cyclohexylidene-3',4'-di-O-mesylbutirosin A (**5**). Introduction of amino function at C-5'' was carried out by displacement of 5''-tosyloxy group with sodium azide either before or after 3',4'-deoxygenation. Compound **4** shows outstanding activities against strains including *Pseudomonas aeruginosa* and *Escherichia coli* which are highly resistant to butirosin and 5''-amino-5''-deoxybutirosin A (**2**).

Butirosin is an aminoglycosidic antibiotic complex obtained from culture filtrates of *Bacillus circulans* and has a marked activity against gram-positive and -negative bacteria as well as a low toxicity in mammals.[1~7] Recently, CULBERTSON *et al.*[8] reported that butirosin A* (**1**) was chemically converted into 5''-amino-5''-deoxybutirosin A (**2**) which exhibited an enhanced activity especially against *Pseudomonas* species including gentamicin-resistant strains *in vitro* and *in vivo*. Meanwhile, susceptibility tests to butirosin and its analog (**2**) with various clinical isolates have revealed that there already exist a few highly resistant strains among various genera such as *Escherichia*, *Proteus*, *Pseudomonas* and *Staphylococcus*. Biochemical studies on the mechanisms of resistance with these bacteria have shown that many species effect inactivation through a phosphorylation mechanism at 3'-position.[9~12] In addition, we previously reported[13] that 3',4'-dideoxybutirosin A (**3**) was prepared and showed significant activities against both sensitive and resistant strains *in vitro* and *in vivo*, indicating that removal of the 3'- and 4'-hydroxy groups confers a capability of overcoming butirosin resistant microorganisms of the 3'-O-phosphorylation type on the butirosin molecule. As a sequel to the preceding paper,[13] this paper deals with synthesis of 5''-amino-3',4',5''-trideoxybutirosin A (**4**), a 3',4'-dideoxy analog of **2**, and its biological properties.

A synthetic intermediate for 3',4'-dideoxybutirosin A (**3**), 6,2'',2'''-tri-O-acetyl-tetra-N-benzyloxycarbonyl-3'',5''-O-cyclohexylidene-3',4'-di-O-mesylbutirosin A (**5**), was used as the starting material for the preparation of **4**. Removal of the 3'',5''-cyclohexylidene group in **5** was effected by treatment with aqueous acetic acid to give a 3'',5''-diol (**6**) which yielded a 5''-*p*-toluenesulfonate (**7**) in good yield on selective tosylation in pyridine. The sulfonate (**7**) was converted into an azide (**8**) with sodium azide in dimethyl sulfoxide. TIPSON-COHEN reaction of the 3',4'-di-O-mesyl-5''-azide (**8**) thus obtained was carried out by treatment with zinc and sodium iodide in N,N-dimethylformamide; however, partial reduction of the 5''-azido group to the amino group was observed and rendered the isolation of the desired product

* The numbering of the carbons is shown in the chart.

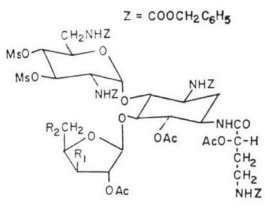


1: $R_1 = R_2 = OH$

2: $R_1 = NH_2$, $R_2 = OH$

3: $R_1 = OH$, $R_2 = H$

4: $R_1 = NH_2$, $R_2 = H$

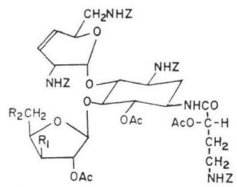


5: R_1, R_2 = cyclohexylidene

6: $R_1 = R_2 = OH$

7: $R_1 = OH$, $R_2 = OTs$

8: $R_1 = OH$, $R_2 = N_3$

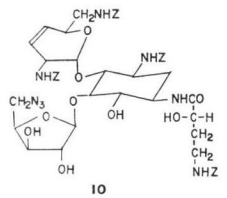


9: $R_1 = OH$, $R_2 = N_3$

11: R_1, R_2 = cyclohexylidene

12: $R_1 = R_2 = OH$

13: $R_1 = OH$, $R_2 = OTs$



10

difficult. Subsequently, the azide (**8**) was treated with an excess amount of sodium iodide in the absence of zinc at a higher temperature. The reaction mixture immediately became fairly colored and darkened; but the desired 3′-eno-5″-azido derivative (**9**) was obtained in a moderate yield without notable reduction of the azido group. The 3′-eno compound (**9**) was de-O-acetylated in methanol with a small amount of sodium methoxide and purified by column chromatography, giving a deacetylated 3′-eno-5″-azide (**10**) as a colorless powder, wherein the NMR spectrum revealed a broad singlet corresponding to the 3′,4′-protons at δ 5.58 ppm as reported for other 3′-eno derivatives of protected aminoglycoside antibiotics.[14,15]

Meanwhile, we examined an alternate route for the intermediate (**10**) including prior formation of the 3′,4′-double bond before introducing an azide group at the 5″-position, because the presence of the 5″-azido group led to difficulties in the 3′,4′-deoxygenation as mentioned earlier. Thus, TIPSON-COHEN reaction of the 3′,4′-dimethanesulfonate (**5**) was effected with zinc and sodium iodide as described in our preceding paper.[13] During this reaction, some of the O,N-protected groups were partially removed; accordingly, the reaction product was once again N-benzyloxycarbonylated and successively acetylated and purified by column chromatography over silica gel, giving the 3′-eno derivative (**11**). Treatment of **11** with methanol containing a small amount of *p*-toluenesulfonic acid gave a de-O-cyclohexylidene compound (**12**) which without purification was converted into its 5″-*p*-toluenesulfonate (**13**). Analogously, treatment of **13** with sodium azide, followed by deacetylation of the resulting azide (**14**) gave the intermediate (**10**).

The 3′-eno-5″-azido compound (**10**) thus obtained was hydrogenated over palladium on charcoal in aqueous methanol in the presence of hydrochloric acid, giving 5″-amino-3′,4′,5″-trideoxybutirosin A (**4**) which was purified by ion-exchange column chromatography with Amberlite CG-50 (NH_4^+ form) in a usual manner. The NMR spectrum of **4** thus obtained revealed eight protons assignable to four methylenes corresponding to the 2,-3′-, and 4′-positions and the β-position of the acyl side chain. On high-voltage paper electrophoresis at pH 1.74, **4** moved toward the cathode similar to 5″-amino-5″-deoxybutirosin A (**2**) and faster than butirosin A or 3′,4′-dideoxybutirosin A (**3**). On a thin-layer chromatogram using silica gel with conc. ammonia-methanol (1 : 1, v/v), **4** showed the same Rf value as butirosin, while with *n*-propanol-acetic acid-conc. ammonia (2 : 1 : 2, v/v) a smaller value than that of butirosin and a similar Rf to that of 5″-amino-5″-deoxybutirosin A (**2**). Thus, the new semisynthetic antibiotic (**4**) was distinguishable from the other butirosin analogs.

As illustrated in Tables 1 and 2, 5″-amino-3′,4′,5″-trideoxybutirosin A (**4**) showed out-

Table 1. Minimal inhibitory concentrations* of butirosin, 5″-amino-5″-deoxybutirosin A (**2**), and 5″-amino-3′,4′,5″-trideoxybutirosin A (**4**)

Test organisms	Butirosin**	Aminodeoxybutirosin (**2**)	Aminotrideoxybutirosin (**4**)
Bacillus subtilis PCI-219	≦0.1	≦0.1	≦0.1
Staphylococcus aureus 209 P	≦0.1	≦0.1	≦0.1
Staphylococcus aureus 109	25	12.5	≦0.1
Escherichia coli NIHJ	1.5	0.8	0.4
Escherichia coli 665***	200	50	1.5
Klebsiella pneumoniae 806	1.5	0.8	0.8
Proteus vulgaris 025	0.8	0.8	0.8
Proteus rettgeri 1602	50	3.1	1.5
Salmonella enteritidis	1.5	1.5	1.5
Shigella flexneri 2a	3.1	3.1	3.1
Pseudomonas aeruginosa Scr.	6.2	1.5	0.8
Pseudomonas aeruginosa 1055***	200	6.2	1.5

* in mcg/ml, heart infusion agar.
** Butirosin complex was used.
*** Strains proved to be inactivators of phosphorylation type. See references 9 and 12.

Table 2. Susceptibility of clinical isolates to butirosin, 5″-amino-5″-deoxybutirosin A (**2**), and 5″-amino-3′,4′,5″-trideoxybutirosin A (**4**)

		MIC* mcg/ml								
		>100	100	50	25	12.5	6.2	3.1	1.5	0.8
Escherichia coli (130 strains)	Butirosin**	6	2	5	17	87	13			
	Aminodeoxybutirosin (**2**)	5	1	3	13	78	29	1		
	Aminotrideoxybutirosin (**4**)		1	2	12	69	43	3		
Pseudomonas aeruginosa (138 strains)	Butirosin**	25	21	49	25	14	2	1	1	
	Aminodeoxybutirosin (**2**)	6	3	8	4	13	41	56	7	
	Aminotrideoxybutirosin (**4**)		3	6	3	11	30	68	15	2

* Heart infusion agar.
** Butirosin complex was used.

standing activities against not only butirosin-sensitive strains but also strains resistant to butirosin or 5″-amino-5″-deoxybutirosin A (**2**). Protective effects and acute toxicities of **4** and related compounds in mice are published separately.[12]

Experimental*

6,2″,2‴-Tri-O-acetyl-3,2′,6′,4‴-tetra-N-benzyloxycarbonyl-3′,4′-di-O-mesylbutirosin A (**6**):

A solution of 1 g of 6,2″,2‴-tri-O-acetyl-3,2′,6′,4‴-tetra-N-benzyloxycarbonyl-3″,5″-O-cyclohexylidene-3′,4′-di-O-mesylbutirosin A (**5**)[13] in a mixture of 10 ml of acetic acid and 4 ml

* Melting points are not corrected. Infrared (IR) spectra were recorded on a JASCO A-2 spectrophotometer (Japan Spectroscopic Co., Ltd.) and nuclear magnetic resonance (NMR) spectra on a Varian A-60 or a Hitachi-Perkin-Elmer R-24 spectrometer. Optical rotations were measured on a Perkin-Elmer Model 141 automatic polarimeter in 10 cm tubes. Thin-layer chromatography (TLC) was performed on TLC-plates, Silica Gel_{254} precoated, thickness 0.25 mm (E. Merck) and spots were visualized by UV-irradiation and/or spraying with vanadic acid-sulfuric acid reagent. For column chromatography on silica gel, Wakogel C-200 (Wako Pure Chemical Industries, Ltd.) and commercial chloroform stabilized with about 1% ethanol were used.

of water was heated on a steam bath for 10 minutes. After evaporation of the solvent at a low temperature, the residue was dissolved in chloroform, and the solution was washed with aqueous $NaHCO_3$ solution and water, dried with $MgSO_4$ and evaporated. The crude product was dissolved in benzene and hexane was added to the resulting solution. The powder of **6** (0.9 g) thus obtained was used in the next reaction. Analytical sample was obtained by chromatography of the crude powder (0.1 g) on silica gel using 2% (v/v) methanol-chloroform as eluant and succesively by precipitation from benzene-hexane. Yield, 90 mg, amorphous powder, $[\alpha]_D^{25}$ +4.5° (*c* 2.5, $CHCl_3$).

Anal. Calcd. for $C_{61}H_{75}O_{27}N_5S_2$: C 53.30, H 5.50, N 5.10, S 4.67.
Found: C 53.14, H 5.48, N 5.22, S 4.24.

6,2″,2‴-Tri-O-acetyl-3,2′,6′,4‴-tetra-N-benzyloxycarbonyl-3′,4′-di-O-mesyl-5″-O-tosylbutirosin A (**7**):

To a solution of 1 g of **6** in 4 ml of pyridine, 0.5 g of *p*-toluenesulfonyl chloride was added with stirring and cooling at 0~5°C. The mixture was stirred at 20~25°C for 2 hours and then poured into ice-water. Working up in the usual manner by means of chloroform as solvent for extraction gave a crude powder of **7** (1.1 g). The powder was chromatographed on silica gel column (silica gel 20 g) using chloroform as eluant and gave 144 mg of a 3″,5″-di-O-tosyl derivative [NMR ($CDCl_3$) δ ppm: 2.44 (6H, s, CH_3 of tosyl)] and 660 mg of **7** as a colorless powder, mp 94~105°C, $[\alpha]_D^{25}$ −4.3° (*c* 1.25, $CHCl_3$). NMR ($CDCl_3$) δ ppm: 3.10 (3H, s, mesyl), 2.75 (3H, s, mesyl), 2.43 (3H, s, tosyl), 2.12 (6H, s, acetyl), 2.05 (3H, s, acetyl).

Anal. Calcd. for $C_{68}H_{81}O_{29}N_5S_3$: C 53.43, H 5.34, N 4.58, S 6.29.
Found: C 53.67, H 5.34, N 4.60, S 6.10.

6,2″,2‴-Tri-O-acetyl-5″-azido-3,2′,6′,4‴-tetra-N-benzyloxycarbonyl-5″-deoxy-3′,4′-di-O-mesylbutirosin A (**8**):

A mixture of 5.75 g of **7**, 500 mg of sodium azide, and 20 ml of dimethyl sulfoxide was heated at 100°C (bath temp.) with stirring in an atmosphere of nitrogen for 2 hours and diluted with ice-water. The resultant precipitates were collected, washed with water and dried. Reprecipitation from ethyl acetate-hexane gave a crude powder of **8** (5.0 g). Analytical sample was obtained by chromatography of the powder (250 mg) over silica gel (3.5 g) using 0.5% (v/v) methanol-chloroform as eluant. Yield, 215 mg, mp 80~101°C (from ethyl acetate-hexane), $[\alpha]_D^{25}$ −1.8° (*c* 2.5, $CHCl_3$). IR ν_{max}^{Nujol}: 2100 cm^{-1} (azido). NMR ($CDCl_3$) δ ppm: 2.77 (3H, s, mesyl), 3.05 (3H, s, mesyl).

Anal. Calcd. for $C_{61}H_{74}O_{26}N_8S_2$: C 52.34, H 5.33, N 8.01, S 4.58.
Found: C 52.49, H 5.18, N 8.13, S 4.76.

6,2″,2‴-Tri-O-acetyl-3,2′,6′,4‴-tetra-N-benzyloxycarbonyl-3″,5″-O-cyclohexylidene-3′,4′-dideoxy-3′-enobutirosin A (**11**):

To a vigorously stirred suspension of 26 g of zinc powder in a solution of 5 g of **5** in 80 ml of N,N-dimethylformamide, 50 g of sodium azide was added. The mixture was heated at 100°C (bath temp.) with vigorous stirring for 1.5 hours, diluted with 400 ml of chloroform and 100 ml of water and then filtered. The filtrate was shaken with saturated aqueous NaCl solution. The organic layer was collected, washed with 10% aqueous $Na_2S_2O_3$ solution and successively with saturated NaCl solution, dried with $MgSO_4$ and evaporated to give about 4.0 g of a syrup. To a stirred solution of the syrup and 1 ml of benzyloxycarbonyl chloride in 40 ml of methanol, saturated aqueous $NaHCO_3$ solution was slowly added at room temperature until the solution became alkaline (pH 7~8). The reaction mixture was diluted with water and extracted with chloroform. The extract was dried, evaporated, and gave a syrup which was acetylated with a mixture of 8 ml of acetic anhydride and 30 ml of pyridine at room temperature for 30 minutes. The product (4.2 g) obtained by the usual manner was chromatographed on a column of silica gel (100 g) packed with chloroform. The column was first washed with 400 ml of chloroform and then eluted with 800 ml of 1% (v/v) methanol-chloroform.

Fractions were examined by TLC and gave successively 590 mg of a mixture of the starting material (**5**) and a small amount of **11**, 1.37 g of **11** and 600 mg of a mixture of **11** and an unidentified substance. Rechromatography of the latter mixture gave a further crop of **11** (0.16 g). Total yield, 1.53 g. Re-precipitation from benzene-hexane gave a powder of **11**, mp 91~110°C, $[\alpha]_D^{20}$ −16.3° (*c* 1.12, $CHCl_3$). NMR ($CDCl_3$) δ ppm: 7.30, 7.27 (15H, 5H, each s, phenyl), 5.58 (2H, br. s, 3′,4′-H), 2.10 (3H, s, acetyl), 2.08 (6H, s, acetyl), 2.2~1.2 (14H, m).

Anal. Calcd. for $C_{83}H_{77}O_{21}N_5 \cdot H_2O$: C 60.88, H 6.21, N 5.46.
Found: C 60.56, H 6.13, N 5.38.

6,2″,2‴-Tri-O-acetyl-3,2′,6′,4‴-tetra-N-benzyloxycarbonyl-3′,4′-dideoxy-3′-eno-5″-O-tosylbutirosin A (**13**):

A solution of 1.22 g of **11** and 60 mg of *p*-toluenesulfonic acid hydrate in 24 ml of methanol was allowed to stand overnight at room temperature, then diluted with 60 ml of chloroform and shaken with saturated aqueus $NaHCO_3$ solution. The organic layer was collected, washed with water, dried and evaporated to leave 1.16 g of **12** as a thick syrup. The syrup was treated at room temperature with 0.6 g of *p*-toluenesulfonyl chloride in 8 ml of pyridine for 1.5 hours. Working up as usual afforded 1.30 g of the crude **13**. The product was chromatographed on silica gel (24 g) with chloroform (200 ml) and 1% (v/v) methanol-chloroform (300 ml) for elution. First, 150 mg of di-O-tosyl compound [NMR ($CDCl_3$): δ 2.30 ppm (6H, s, tosyl)] emerged, successively, 600 mg of **13** and 160 mg of uncharacterized material. Re-precipitation of **13** from benzene-hexane gave a powder, mp 81~86°C, $[\alpha]_D^{20}$ −13.1° (*c* 1.23, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 1170, 1185 (sulfonyl). NMR ($CDCl_3$) δ ppm: 7.37, 7.35, 7.30 (10H, 5H, 5H, each s, phenyl), 5.70 (2H, s, 3′,4′-H), 2.45 (3H, s, tosyl), 2.15 (6H, s, acetyl), 2.10 (3H, s, acetyl).

Anal. Calcd. for $C_{66}H_{75}O_{23}N_5S \cdot H_2O$: C 58.44, H 5.72, N 5.16, S 2.36.
Found: C 58.35, H 5.80, N 4.97, S 2.34.

5″-Azido-3,2′,6′,4‴-tetra-N-benzyloxycarbonyl-3′,4′,5″-trideoxy-3′-enobutirosin A (**10**)

(**1**) A solution of 3.77 g of **8** and 25 g of sodium iodide in 35 ml of N,N-dimethylformamide was heated at 130°C (bath temp.) with vigorous stirring for 2 hours. Then, to the solution, a large amount of chloroform was added and the resulting suspension was washed with 10% aqueous $Na_2S_2O_3$ solution and successivly with NaCl solution, dried with $MgSO_4$ and evaporated, affording a brown powder (*ca.* 2.8 g). This powder was dissolved in 15 ml of methanol containing 0.7 ml of 2 N methanolic sodium methoxide solution and allowed to stand at room temperature for 1 hour. After neutralization with acetic acid, the mixture was evaporated. A solution of the residue in chloroform was charged on a column of silica gel (30 g). Elution was carried out stepwise with chloroform, and 2%, 3%, 4% and 5% methanol-chloroform. The fractions were monitored by TLC (silica gel, methanol-chloroform (1 : 9, v/v)). Fractions which contained a substance having a Rf value slightly smaller than that of the de-O-acetylated derivative of **8** were collected and evaporated, giving a powder. To a solution of the powder in ethyl acetate, ether was added. The amorphous solid thus deposited was dried and gave 685 mg of **10**, mp 86~94°C (preliminary softening at 80°C), $[\alpha]_D^{20}$ −29.2° (*c* 1.20, methanol) IR ν_{max}^{Nujol} cm^{-1}: 2150 (azido), no absorption at 1180 corresponding to sulfonate. NMR ($CDCl_3$) δ ppm: 7.40 (20H, s, phenyl), 5.58 (2H, s, 3′,4′-H), 5.17 (8H, s, benzylic protons).

Anal. Calcd. for $C_{53}H_{62}O_{17}N_5 \cdot H_2O$: C 57.81, H 5.86, N 10.18.
Found: C 58.09, H 5.52, N 9.89.

(**2**) A mixture of 0.58 g of **13**, 0.25 g of sodium azide and 2.5 ml of dimethyl sulfoxide was stirred at 100°C (bath temp.) under nitrogen atmosphere for 2 hours, and then diluted with ice-water. A chloroform solution of the resulting precipitates was washed with water, dried with $MgSO_4$ and evaporated to leave 0.52 g of a powder [IR ν_{max}^{Nujol} cm^{-1}: 2100 (azido), no absorption at 1170 or 1185]. The powder was dissolved in 5 ml of methanol and 0.1 ml of 2 N methanolic sodium methoxide solution was added. After 30-minute standing, the reaction mixture was neutralized with Dowex 50 W×4 (H^+ form) and filtered. The filtrate was decolorized with activated carbon, and evaporated to leave a powder which was reprecipitated from ethyl

acetate-ether, dried and gave 0.42 g of **10**.

Anal. Calcd. for $C_{53}H_{82}O_{17}N_3 \cdot H_2O$: C 57.81, H 5.86, N 10.18.
Found: C 57.75, H 5.83, N 9.60.

5''-Amino-3',4',5''-trideoxybutirosin A (**4**):

Hydrogen was passed through a mixture of 1.37 g of **10**, 60 ml of methanol, 20 ml of water, 4 ml of 2 N hydrochloric acid and 5.0 g of 10 % palladium-charcoal with stirring at room temperature for 2 hours. The reaction mixture was diluted with water and filtered. The filtrate was neutralized with Amberlite IR-45 (OH^- form) and concentrated for removal of methanol. The resultant aqueous solution was poured onto a column of Amberlite CG-50 (NH_4^+ form) (100 ml) and the column was washed with water and 0.3 N NH_4OH. Next, elution was carried out with 1 N and 1.5 N NH_4OH. Fractions which contain TLC-pure and ninhydrin-positive substance were collected, concentrated for complete removal of ammonia. The resulting solution was saturated with CO_2, freeze-dried and gave a powder (280 mg), mp *ca.* 175°C (with evolution of CO_2 gas), $[\alpha]_D^{21}$ +12.8° (*c* 1.09, water). NMR (D_2O, DSS as internal standard) δ ppm: 5.43 (1H, br. d, 1'-H), 5.20 (1H, br. s, 1''-H), 4.4~2.8 (17H, unresolved m), 2.3~1.2 (8H, complex m, methylene protons). High-voltage paper electrophoresis [Toyo Roshi No 51, 3,300 V, 30 minutes, formic acid-acetic acid-water (1 : 3 : 36, v/v), pH 1.74] $Rm_{(alanine)}$: butirosin or 3',4'-dideoxybutirosin A (**3**) 1.75, 5''-amino-5''-deoxybutirosin A (**2**) 2.00, **4** 2.04. TLC on silica gel (methanol-conc·NH_4OH (1 : 1, v/v)) Rf: butirosin 0.40, 5''-amino-5''-deoxybutirosin A (**2**) 0.33, **4** 0.40; (*n*-propanol-acetic acid-conc·NH_4OH (2 : 1 : 2, v/v) $Rf_{butirosin}$: **2** or **4** 0.80~0.83.

Anal. Calcd. for $C_{21}H_{42}O_9N_3 \cdot 2.5H_2CO_3 \cdot H_2O$: C 40.57, H 7.10, N 12.08.
Found: C 20.23, H 6.88, N 11.97.

Acknowledgement

The authors with to express their appreciation to Mr. ISAMU IGARASHI for skillful technical assistance rendered during this study.

References

1) WOO, P. W. K.; H. W. DION & Q. R. BARTZ: Butirosins A and B, aminoglycoside antibiotics. I. Structural units. Tetrahedron Letters 1971: 2617~2620, 1971
2) WOO, P. W. K.: Butirosins A and B, aminoglycoside antibiotics. II. Mass spectrometric study. Tetrahedron Letters 1971: 2621~2624, 1971
3) WOO, P. W. K.; H. W. DION & Q. R. BARTZ: Butirosins A and B, aminoglycoside antibiotics. III. Structures. Tetrahedron Letters 1971: 2625~2628, 1971
4) HOWELLS, J. D.; L. E. ANDERSON, G. L. COFFEY, G. D. SENOS, M. A. UNDERHILL, D. L. VOGLER & J. EHRLICH: Butirosin, a new aminoglycosidic antibiotic complex: Bacterial origin and some microbiological studies. Antimicr. Agents & Chemoth. 2: 79~83, 1972
5) DION, H. W.; P. W. K. WOO, N. E. WILLMER, D. L. KERN, J. ONAGA & S. A. FUSARI: Butirosin, a new aminoglycosidic antibiotic complex: Isolation and characterization. Antimicr. Agents & Chemoth. 2: 84~88, 1972
6) HEIFETZ, C. L.; M. W. FISHER, J. A. CHODUBSKI & M. O'. DECARLO: Butirosin, a new aminoglycosidic antibiotic complex: Antibacterial activity *in vitro* and in mice. Antimicr. Agents & Chemoth. 2: 89~94, 1972
7) HEIFETZ, C. L.; J. A. CHODUBSKI, I. A. PEARSON, C. A. SILVERMAN & M. W. FISHER: Butirosin compared with gentamicin *in vitro* and *in vivo*. Antimicr. Agents & Chemoth. 6: 124~135, 1974
8) CULBERTSON, T. P.; D. R. WATSON & T. H. HASKELL: 5''-Amino-5''-deoxybutirosin, a new semisynthetic aminoglycoside antibiotic. J. Antibiotics 26: 790~793, 1973
9) SUGAWARA, S.; S. INABA, M. MADATE & H. SAEKI: Bacterial inactivation of butirosin. Ann. Sankyo Res. Lab. 25: 56~61, 1973
10) BRZEZINSKA, M. & J. DAVIES: Two enzymes which phosphorylate neomycin and kanamycin in *Escherichia coli* strains carrying R-factors. Antimicr. Agents & Chemoth. 3: 266~269, 1972
11) YAGISAWA, M.; H. YAMAMOTO, H. NAGANAWA, S. KONDO, T. TAKEUCHI & H. UMEZAWA: A

new enzyme in *Escherichia coli* carrying R-factor phosphoylating 3′-hydroxy of butirosin A, kanamycin, neamine and ribostamycin. J. Antibiotics 25: 748~750, 1972

12) SUGAWARA, S.; M. TAJIMA, H. SAEKI & E. OHKI: Dideoxybutirosin A and amino-trideoxybutirosin A, chemically modified antibiotics active against butirosin-resistant bacteria. Proc. 1st Intersect. Congr. Internat. Assoc. Microbiol. Soc. Sept. 1974 (Tokyo): in press

13) SAEKI, H.; Y. SHIMADA, Y. OHASHI, M. TAJIMA, S. SUGAWARA & E. OHKI: Synthesis of 3′,4′-dideoxybutirosin A, active against butirosin resistant bacteria. Chem. Pharm. Bull. (Tokyo) 22: 1145~1150, 1974

14) IKEDA, D.; T. SUZUKI, T. TSUCHIYA, S. UMEZAWA & H. UMEZAWA: Studies on aminosugars. XXXVI: Synthesis of 3′,4′-dideoxy and 3′,4′,5″-trideoxyribostamycin. Bull. Chem. Soc. Japan 46: 3210~3213, 1973

15) IKEDA, D.; T. TSUCHIYA, S. UMEZAWA & H. UMEZAWA: Synthesis of butirosin B and its 3′, 4′-dideoxy derivative. Bull. Chem. Soc. Japan 47: 3136~3138, 1974; and references cited therein.