Communication to the editor

THE SYNTHESIS OF BUTIROSIN B AND RELATED COMPOUNDS BY AN ACYL MIGRATION METHOD

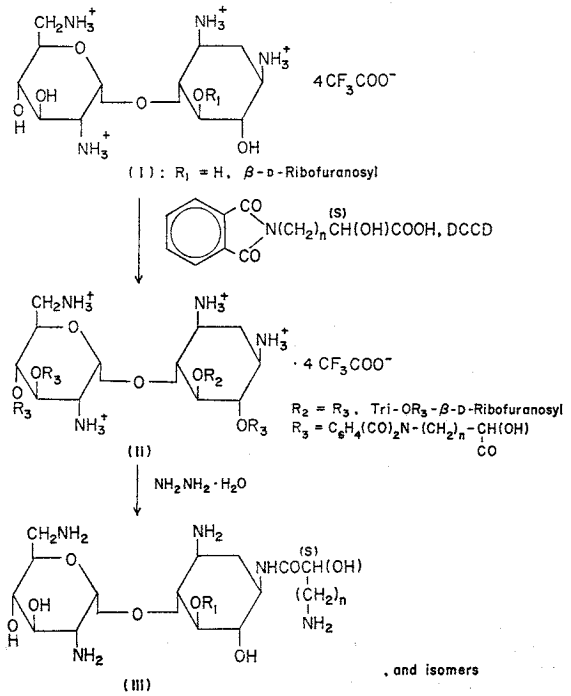
Sir:

Ribostamycin[1] and neamine are the components of butirosin B, 1-N-((S)-α-hydroxy-γ-amino-*n*-butyryl)-ribostamycin.[2] Recently, HASKELL *et al.* reported some new butirosin A homologs by acylation of a blocked butirosin derivative.[3] IKEDA *et al.* reported the synthesis of butirosin B from ribostamycin through a cyclic carbamate.[4] The present paper deals with another synthesis of butirosin B and related compounds by O → N acyl migration of the amino acid moiety.[5] Amino groups were blocked as ammonium ions with trifluoroacetic acid which also gave an acidic environment for O-acylation[6] with the desired amino acids. With subsequent alkaline treatment the aminoacyl group at the 6-hydroxyl migrated to the 1-amino group.

Ribostamycin base was dissolved in trifluoroacetic acid at 0°C and the ribostamycin trifluoroacetic acid salt was precipitated with ethyl ether. Twenty grams of the salt was dissolved in tetrahydrofuran and condensed with 16.4 g of (S)-α-hydroxy-γ-N-phthaloyl-amino-*n*-butyric acid at 0°C with dicyclohexylcarbodiimide. The crude condensate was dissolved in a mixture of ethanol and 80% aqueous hydrazine hydrate (10:1) and heated at 80~100°C for 1~2 hours. The reaction product was evaporated, washed with ethanol and extracted with water. The aqueous solution was adjusted to pH 8.0~8.4 and passed through a column of CM-Sephadex C-25(NH_4^+). The column was washed with water and 0.1N NH_4OH and 5,303 mg of resinous material was recovered from this fraction. Four hundred and two milligrams of ribostamycin and 1,703 mg of crude butirosin B contaminated with 2′- or 6′-acylated ribostamycin were successively eluted with 0.2N NH_4OH. The column was further developed with 1N NH_4OH and 1,376 mg of diacylated ribostamycin mixture was obtained. The crude butirosin B fraction was again passed through the same column and purified to yield 1,336 mg of butirosin B (10.3%, based on ribostamycin). Colorless powder; m.p. 130~191°C (dec.); $[\alpha]_D$+34° (*c* 0.2, H_2O); CD negative COTTON effect at 214 mμ; $R_{ribostamycin}$ 0.47 (*n*-BuOH-Pyr-AcOH-H_2O (6 : 4 : 1 : 3), PPC); Anal. Calcd. for $C_{21}H_{41}N_5O_{12}\cdot 2H_2O$: C 42.7, H 7.63, N 11.85 %, Found: C 43.39, H 6.89, N 11.50 %. The sample gave an intact 2-deoxystreptamine nucleus on periodate oxidation.[2]

Neamine base was dissolved in trifluoroacetic acid as in the preceding instance and the ether precipitate was collected. Six hundred and thirty six milligrams of the salt was condensed with 615 mg of (S)-α-hydroxy-γ-N-phthaloyl-amino-*n*-butyric acid in a mixture of tetrahydrofuran and dimethylformamide (23:2) with dicyclohexylcarbodiimide at 0°C. The crude condensation product was treated with ethanol and 80 % aqueous hydrazine hydrate mixture (10:1) at 80~100°C for 1~2 hours. The aqueous extract of the reaction mixture was passed through a column of CM-Sephadex

Chart 1. The course of synthesis of butirosin B, 1-N-((S)-α-hydroxy-γ-amino-*n*-butyryl)-neamine and 1-N-((S)-α-hydroxy-δ-amino-*n*-valeryl)-ribostamycin



C-25(NH_4^+), washed with water and 0.1N NH_4OH and a mixture containing neamine was recovered from this fraction. 2′- or 6′-Acylated neamine mixture and 1-N-((S)-α-hydroxy-γ-amino-*n*-butyryl)-neamine were eluted separately with 0.2N NH_4OH. The column was further developed with 1N NH_4OH to give a diacylated neamine mixture. Fourty seven milligrams of 1-N-((S)-α-hydroxy-γ-amino-*n*-butyryl)-neamine was obtained (13.0 %, based on neamine) as a colorless powder; m.p. 138 ~ 198°C(dec.); $[\alpha]_D$+42°(*c* 0.2, H_2O); CD negative COTTON effect at 218 mμ; $R_{neamine}$ 0.44 (*n*-BuOH-Pyr-AcOH-H_2O(6:4:1:3), PPC); Anal. Calcd. for $C_{16}H_{33}N_5O_8 \cdot H_2O$: C 43.5, H 7.93, N 15.86 %, Found: C 42.80, H 7.50, N 14.86 %. The sample gave an intact 2-deoxystreptamine nucleus on periodate oxidation.[2]

Two grams of ribostamycin trifluoroacetic acid salt was condensed with 1.74 g of (S)-α-hydroxy-δ-N-phthaloylamino-*n*-valeric acid in a mixed solvent of tetrahydrofuran and dimethylformamide (20:1), with dicyclohexylcarbodiimide at 0°C. The condensed ion product was treated with ethanol-80 % aqueous hydrazine hydrate mixture (10:1) at 80~100°C for 1~2 hours. The aqueous extract of the reaction mixture was passed through a column of CM-Sephadex C-25 (NH_4^+), washed with water and 0.1N NH_4OH and 786 mg of a mixture containing ribostamycin was recovered from this fraction. Eighty two milligrams of crude material contaminated with 2′- or 6′-acylated ribostamycin was eluted with 0.2N NH_4OH. The column was further developed with 1N NH_4OH to give a diacylated ribostamycin mixture. The crude material was rechromatographed on the same column to give 66.8 mg of 1-N-((S)-α-hydroxy-δ-amino-*n*-valeryl)-ribostamycin (5.0 %, based on ribostamycin); colorless powder; m.p. 130~195°C (dec.); $[\alpha]_D$+33° (*c* 0.2, H_2O); CD negative COTTON effect at

Table 1. Antibacterial spectra of synthetic butirosin B, 1-N-((S)-α-hydroxy-γ-amino-*n*-butyryl)-neamine and 1-N-((S)-α-hydroxy-δ-amino-*n*-valeryl)-ribostamycin

Test organisms*			Minimal inhibitory concentration (mcg/ml)		
			Butirosin B	1-N-((S)-α-Hydroxy-γ-amino-*n*-butyryl)-neamine	1-N-((S)-α-Hydroxy-δ-amino-*n*-valeryl)-ribostamycin
Staphylococcus aureus		FDA 209P	1.56	12.5	3.12
Escherichia coli	K-12		1.56	6.25	3.12
〃	〃	ML 1629	3.12	50	6.25
〃	〃	ML 1630	3.12	100	25
〃	〃	ML 1410	3.12	12.5	3.12
〃	〃	〃 R81	6.25	100	12.5
〃	〃	R5 R64	12.5	>100	50
〃	〃	LA 290 R55	1.56	6.25	3.12
〃	〃	〃 R56	1.56	6.25	3.12
〃	〃	〃 R64	1.56	12.5	3.12
〃	W 677		0.78	25	6.25
〃	JR 66/W 677		>100	>100	>100
Klebsiella pneumoniae		type 22 3038	>100	>100	>100
Pseudomonas aeruginosa		A3	6.25	12.5	100
〃	〃	No. 12	12.5	12.5	50
〃	〃	H 9	3.12	6.25	12.5
〃	〃	H 11	50	25	>100
〃	〃	TI 13	50	25	>100
〃	〃	GN 315	>100	>100	>100
〃	〃	99	100	100	>100
Mycobacterium smegmatis		ATCC 607**	1.56	50	6.25

* Nutrient agar, 37°C, 17 hours. ** Nutrient agar, 37°C, 42 hours.

213 mμ; $R_{ribostamycin}$ 0.47 (*n*-BuOH-Pyr-AcOH-H_2O(6 : 4 : 1 : 3), PPC); Anal. Calcd. for $C_{22}H_{43}N_5O_{12}\cdot 2H_2O$: C 43.63, H 7.82, N 11.56%, Found: C 44.34, H 7.28, N 11.67 %. The sample gave an intact 2-deoxystreptamine nucleus on periodate oxidation.[2]

Antibacterial spectra of the synthetic butirosin B, 1-N-((S)-α-hydroxy-γ-amino-*n*-butyryl)-neamine and 1-N-((S)-α-hydroxy-δ-amino-*n*-valeryl)-ribostamycin are shown in Table 1.

The ionized amino groups in Chart 1 (**I**) are resistant to N-acylation,[7] and trifluoroacetic anion gives an acidic condition for O-acylation. Thus the first product is the poly-O-acyl derivative (**II**). In the next step, the poly-O-acyl salt was treated with hydrazine hydrate and the hydrolysis to free amine was followed by O→N migration to give **III**, 2′-acylated, 6′-acylated or diacylated antibiotic, the amino group at 3-position of 2-deoxystreptamine moiety remaining intact. Thus by this method, the most difficult separation of 1-N-aminoacyl from 3-N-aminoacyl derivative was successfully avoided.

Acknowledgements

The authors wish to express their deep thanks to Prof. Dr. S. UMEZAWA and Dr. T. TSUCHIYA, Keio University, for the important suggestion about the present study.

They gratefully express their thanks to Prof. Dr. H. UMEZAWA, Tokyo University and Institute of Microbial Chemistry, for important advice and encouragement concerning the present work, and they also wish to extend their gratitude to Dr. M. HAMADA, Institute of Microbial Chemistry, for microbiological assay, and to Dr. S. KONDO for useful advices.

EIICHI AKITA
YUKIO HORIUCHI
SHUNTARO YASUDA

Central Research Laboratories,
Meiji Seika Kaisha Ltd.
Morooka-cho, Kohoku-ku,
Yokohama, Japan

(Received March 8, 1973)

References

1) AKITA, E.; T. TSURUOKA, N. EZAKI & T. NIIDA: Studies on antibiotic SF-733, a new antibiotic. II. J. Antibiotics 23: 173～183, 1970
2) WOO, P. K. W.; H. DION & Q. R. BARTZ: Butirosins A and B, aminoglycoside antibiotics. I. Structural units. Tetrahedron Letters 1971: 2617～2620, 1971; WOO, P. K. W.: II. Mass spectroscopic study. *ibid.* 1971: 2621～2624, 1971; WOO, P. K. W.; H. DION & Q. R. BARTZ: III. Structures. *ibid.* 1971: 2625～2628, 1971
3) HASKELL, T. H.; R. RODEBAUGH, N. PLESSAS, D. WATSON & R. D. WESTLAND: Aminoglycoside antibiotics. The preparation and biological activity of novel aminoacid analogs of butirosin. The 164th National Meeting of American Chemical Society, Division of Carbohydrate Chemistry, New York City, N. Y., Aug. 28～31, 1972
4) IKEDA, D.; T. TSUCHIYA, S. UMEZAWA & H. UMEZAWA: Synthesis of butirosin B by a new method. J. Antibiotics 24: 741～742, 1972
5) UMEZAWA, S.; Y. NISHIMURA, T. KURISU & T. TSUCHIYA: Details will be published elsewhere.
6) ALBERTSON, N. F.: Synthesis of peptides with mixed anhydrides. Scope and limitations. Organic Reactions 12: 172～179, John Wiley & Sons, 1962
7) BELLO, J. & J. R. VINOGRAD: Selective acetylation of hydroxyl groups in gelatin. J. Amer. Chem. Soc. 78: 1369～1372, 1956