[Chem. Pharm. Bull. 25(6)1302—1305(1977)]

UDC 547.466.1.04.09 : 615.282.011.5.076.7

Studies on the β-Lysine Peptide. IV.[1,2] Preparation of Semi-synthetic Racemomycins and Their Antimicrobial Activities

Yosuke Sawada and Hyozo Taniyama

Faculty of Pharmaceutical Sciences, Nagasaki University[3]

(Received September 17, 1976)

Racemomycin-C was semi-synthesized from racemomycin-A by condensation with diZ-β-lysine-OSU followed by catalytic hydrogenation. Racemomycins B, D and E were also prepared in the similar manner. Among these antibiotics, racemomycin-B containing three β-lysine residues in one molecule showed the strongest antibacterial activity against *B. subtilis*.

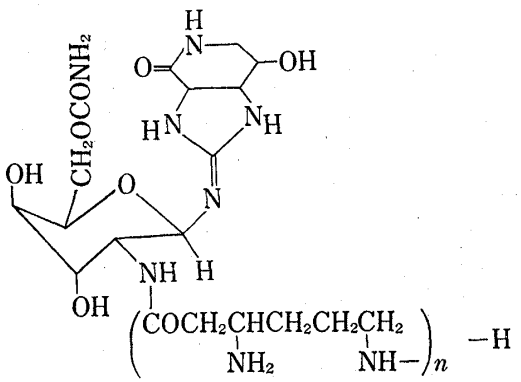
Keywords——semi-synthetic racemomycins; racemomycin-E; β-lysine; β-lysine tri-peptide; diZ-β-lysine; diZ-β-lysine-OSU or diZ-β-lysine N-hydroxysuccinimide ester; N-hydroxysuccinimide ester procedure; disk assay method; antimicrobial activity; ε-amino function

Racemomycins[4] belonging to a water-soluble, basic streptothricin group antibiotic, contain a number of β-lysine[5] residues(n) in their molecules (Chart 1). In a previous paper,[6] we have speculated that the antimicrobial activity and the toxicity of racemomycin components[7] increase correlating with the numbers of the β-lysine residues in their molecules and that they reach the maximum at a certain number of the β-lysine residues in a molecule. In the other paper,[8] we have reported the synthesis of amino acid derivatives of racemomycin-A, in which ε-amino function of β-lysine moiety was reactive to active esters.[9,10] These amino acid derivatives exhibited a lower antimicrobial activity and a weaker toxicity than the original antibiotic. However, a β-lysine peptide derivative of racemomycin-A, which was identified as racemomycin-C,[11] showed a higher antimicrobial activity than the original one.

In the present paper, we describe the synthesis of racemomycins C,B,D and E starting from racemomycin-A and compare their antimicrobial activity.

The synthesis of β-lysine peptide derivatives was carried out practically in the similar manner as that in the preparations of the amino acid derivatives.[8] That is, racemomycin-A was condensed with diZ-β-lysine in aqueous alkaline solution (pH 10) by the N-hydroxysuccinimide ester procedure.[12] The ε-amino function of the β-lysine moiety in racemomycin-A was

1) Part III: H. Taniyama, Y. Sawada, K. Miyazeki, and F. Miyoshi, *Chem. Pharm. Bull.* (Tokyo), **20**, 601 (1972).
2) Abbreviations used are those recommended by IUPAC-IUB Commission of Biochemical Nomenclature: *Biochem.*, **5**, 2485 (1966), **6**, 362 (1967), **11**, 1726 (1972). Z=benzyloxycarbonyl, OSU=N-hydroxysuccinimide ester, DCC=dicyclohexylcarbodiimide.
3) Location: *1–14, Bunkyo-machi, Nagasaki, 852, Japan.*
4) Racemomycin is abbreviated as RM.
5) β-Lysine(β,ε-diaminocaproic acid) is of the L-configuration, E.E. van Tamelen and E.E. Smissman, *J. Am. Chem. Soc.*, **75**, 2031 (1953).
6) H. Taniyama, Y. Sawada, and T. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **19**, 1627 (1971).
7) Samples were examined about racemomycins A, C and B.
8) Y. Sawada and H. Taniyama, *Yakugaku Zasshi*, **94**, 858 (1974).
9) Y. Sawada, H. Sakamoto, and H. Taniyama, *Yakugaku Zasshi*, **94**, 176 (1974).
10) Y. Sawada and H. Taniyama, *Yakugaku Zasshi*, **94**, 264 (1974).
11) H. Taniyama, Y. Sawada, K. Miyazeki, S. Tanaka, F. Miyoshi, and K. Hiraoka, *Chem. Pharm. Bull.* (Tokyo), **20**, 1432 (1972).
12) G.W. Anderson, J.E. Zimmerman, and F. Callahan, *J. Am. Chem. Soc.*, **85**, 3039 (1963).



racemomycins A: n 1
C: n 2
B: n 3
D: n 4
E: n 5

Chart 1

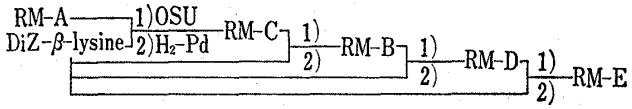


Chart 2. Synthetic Route of the Semi-synthetic Recemomycins

Table I. *Rf* Values of the Protected Racemomycins

DiZ-β-lysine derivative of	PPC, *Rf* value
RM–A	0.72(0.37)
RM–C	0.62(0.32)
RM–B	0.53(0.27)
RM–D	0.42(0.22)

( ): *Rf* value of the original antibiotic
solvent system: A (BuOH–pyridine–AcOH–H_2O–*tert*–BuOH=15:10:3:12:4)
detection: Rydon-Smith reagent
paper: Toyo-Roshi No. 51 UH

Table II. Elemental Analysis and Yields of the Protected Racemomycins

DiZ-β-lysine derivative of	Formula	*Anal.* (%) Calcd. (Found) C	H	N	Yield (mg)
RM–A	$C_{41}H_{58}O_{13}N_{10}2CH_3COOH$	53.00 (52.83)	6.52 (6.73)	13.74 (13.54)	80
RM–C	$C_{47}H_{70}O_{14}N_{12}3CH_3COOH$	52.73 (52.63)	6.85 (6.98)	13.92 (13.76)	50
RM–B	$C_{53}H_{82}O_{15}N_{14}4CH_3COOH$	52.50 (52.38)	7.08 (7.24)	14.05 (14.00)	20
RM–D	$C_{59}H_{94}O_{16}N_{16}5CH_3COOH$	52.33 (52.21)	7.26 (7.42)	14.15 (13.86)	15

Table III. Chromatographic Comparison of Natural and Semi-synthetic Racemomycins

Antibiotic	*Rf* value[a] PPC[b]	TLC[c]	Amino acid analysis[d] β-lysine/streptolidine
RM–A	0.59	0.32	1.43
RM–C	0.51	0.22	2.75
Synthetic RM–C (II)	0.50	0.22	3.10
RM–B	0.46	0.15	4.25
Synthetic RM–B (III)	0.45	0.16	3.90
RM–D	0.42	0.09	5.95
Synthetic RM–D (IV)	0.42	0.08	5.45
RM–E[13]	0.38	0.05	7.30
Synthetic RM–E (V)	0.37	0.05	7.70

a) solvent system: B (PrOH–pyridine–AcOH–H_2O=15:10:3:13), detection: ninhydrin and Rydon-Smith reagents and *B. subtilis*
b) Toyo-Roshi No. 51 UH paper
c) Avicel SF plate (Funakoshi)
d) Hitachi KLA-3 type apparatus

13) Y. Sawada, H. Taniyama, and A. Seino, *J. Ferment. Technol.*, **55**, 290 (1977).

preferentially masked by diZ-β-lysine. The protected racemomycin-A was isolated by use of column chromatography on cellulose and Sephadex LH-20. Its homogeneity was further assessed by paper chromatography (Table I) and by elemental analysis (Table II). The Z group of this compound was removed by catalytic hydrogenation to give racemomycin-C. The semi-synthetic racemomycin-C was converted to the corresponding hydrochloride salt by 0.3N hydrochloric acid and fifty volumes of acetone, and purified by column chromatography on Sephadex LH–20 which was eluted with water. Lyophilization on the homogeneous fractions gave a white, hygroscopic powder of semi-synthetic racemomycin-C. Semi-synthetic racemomycins B,D and E were also prepared from racemomycins C,B and D according to the schema described in Chart 2. Table II shows the yields of the protected racemomycins. They were characterized by paper and thin-layer chromatography, and amino acid analysis (Table III).

Antimicrobial activity of natural and semi-synthetic antibiotics against *B. subtilis* was measured by disk assay method (serial double dilutions) as shown in Fig. 1. Inhibited patterns between natural and semi-synthetic antibiotics resemble each other. Among these antibiotics, racemomycin-B containing three β-lysine residues in one molecule exhibited the strongest antimicrobial activity against bacterium (Fig. 2). These facts confirmed that the increase of antimicrobial activity is correlated with the

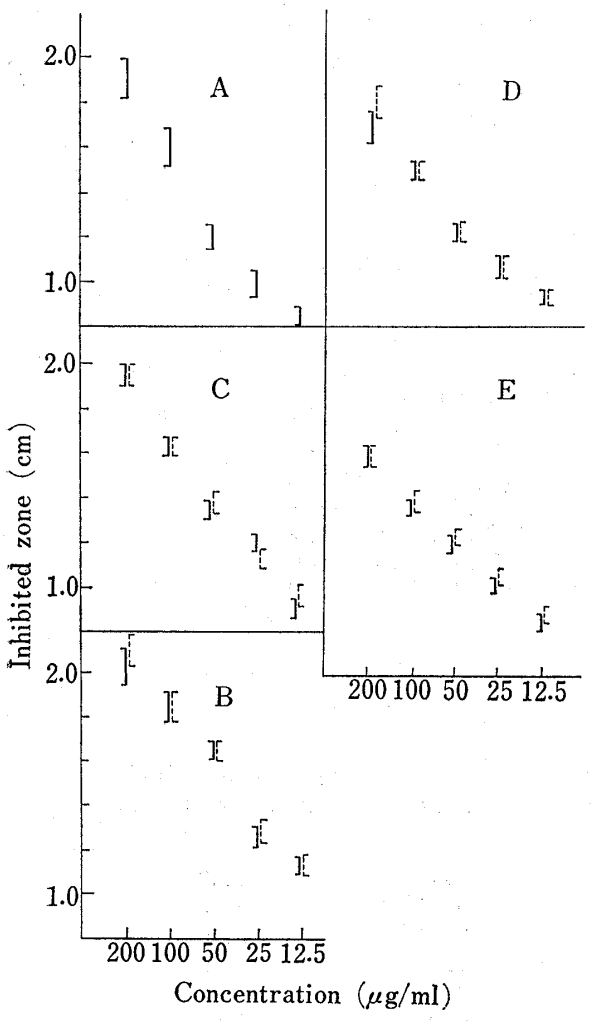


Fig. 1. Antimicrobial Activity of Natural and Semi-synthetic Racemomycins

: natural
: semi-synthetic
test strain: *B. subtilis* PCI-219

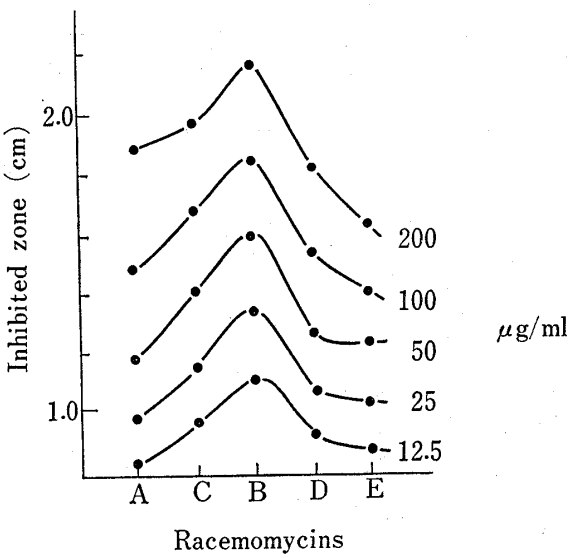


Fig. 2. Comparison of Antimicrobial Activity among Racemomycin Components

numbers of the β-lysine residues in the antibiotic. A β-lysine tri-peptide unit in racemomycin-B may participate in its antibacterial mechanism more importantly than other β-lysine peptide units.

Experimental

Individual racemomycins (sulfate) were isolated from racemomycin mixture by dextran-gel chromatography.[14] Hydrolysis of antibiotics was carried out in 6N HCl at 110—120° for 24 hr in a sealed tube.

DiZ-β-lysine-OSU (I)——To a solution of diZ-β-lysine[15,16] (470 mg) and N-hydroxysuccinimide (120 mg) in dry-tetrahydrofuran (THF) (30 ml), dicyclohexylcarbodiimide (DCC) (280 mg) in THF (5 ml) was added in an ice-bath and the mixture was stirred for 3 hr. After filtration, the filtrate was condensed *in vacuo* and the residue was treated with AcOEt, to give ester (I), which was crystallized from AcOEt and ether in a 98% yield, mp 73—74° (uncorr.), IR $\nu(C=O)_{max}^{KBr}$ cm^{-1}: 1818, 1796, 1740, 1700. *Anal.* Calcd. for $C_{26}H_{29}O_8N_3$: C, 61.05; H, 5.71; N, 8.21. Found: C, 61.33; H, 6.00; N, 8.20.

Racemomycin-C (II)——To a solution of racemomycin-A (520 mg) in 30 ml of 1/20M Na_2CO_3—1/10M $NaHCO_3$ (pH 10) was added I (620 mg) in THF (10 ml), and the mixture was stirred at room temperature for 3 hr. The solvent was evaporated and the residue was dissolved in H_2O (5 ml), to which acetone (50 ml) was added to give a white precipitation. The supernatant solution was decanted and the residue was suspended in a solvent system of A (5 ml, BuOH–pyridine–AcOH–H_2O–*tert*-BuOH=15: 10: 3: 12: 4). The suspention was applied on a column (2×20 cm) of cellulose, which was eluted with the same solvent system. The eluates containing the substance of *Rf* 0.72 were combined and evaporated. The solution of the residue in H_2O (5 ml) was further purified by column (2×140 cm) chromatography on Sephadex LH-20, which was eluted with H_2O. The homogeneous fractions were pooled and lyophilized to give a white powder. Table II shows the results of elemental analysis and yield of the protected racemomycin-C. This compound was hydrogenated in H_2O–AcOH (9: 1) over a Pd catalyst for 12 hr in the usual way. The deblocking reaction was monitored by positive ninhydrin and Rydon-Smith reagents after paper chromatography. The solution was filtered, and the filtrate was condensed *in vacuo* and the residue was treated with 0.3N HCl and fifty volumes of acetone. The acetone powder obtained was dissolved in a few ml of H_2O and applied on a column (2×140 cm) of Sephadex LH-20 eluted with H_2O. Table III shows *Rf* value and amino acid analysis of acid hydrolysate of semi-synthetic racemomycin-C.

Racemomycin-B (III)——To a solution of racemomycin-C (650 mg) in 40 ml of the buffer was added I (620 mg) in THF (10 ml). The subsequent procedures were carried out in a similar manner to that described above.

Racemomycin-D (IV)——Racemomycin-B (790 mg) in 80 ml of the buffer was used.

Racemomycin-E (V)——Racemomycin-D (940 mg) in 100 ml of the buffer was used.

Antimicrobial Activity——A medium (Heart infusion agar, Eiken) was used for the assay of antimicrobial activity. *B. subtilis* PCI-219 was used as an indicator strain (inoculam size: 10^6 cells/ml). Inhibited zone was obtained after 18 hr cultivation at 27°.

Acknowledgement This study was aided by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

14) H. Taniyama, Y. Sawada, and T. Kitagawa, *J. Chromatog.*, **56**, 360 (1971).
15) H. Taniyama, Y. Sawada, K. Miyazeki, S. Tanaka, and F. Miyoshi, *Chem. Pharm. Bull.* (Tokyo), **19**, 2645 (1971).
16) T. Wakamiya, H. Uratani, T. Teshima, and T. Shiba, *Bull. Chem. Soc. Japan*, **48**, 2401 (1975).