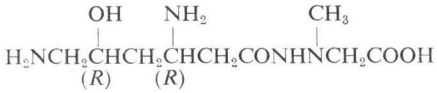
SYNTHESES AND PROPERTIES OF NEGAMYCIN ANALOGS MODIFIED THE δ-HYDROXY-β-LYSINE MOIETY

Sir:

Negamycin produced by *Streptomyces purpeofuscus* inhibits the growth of Gram-positive and Gram-negative bacteria including pseudomonas[1] and causes inhibition of protein synthesis.[2~5] *In vitro*, it shows also a miscoding effect on bacterial ribosome system. Negamycin has a unique hydrazide structure,[6] [2-{(3*R*, 5*R*)-3,6-diamino-5-hydroxyhexanoyl}-1-methylhydrazino] acetic acid, which has been confirmed by total synthesis.[7] We report herein the synthesis and antibacterial activity of negamycin analogs which are modified in the (*R*, *R*)-δ-hydroxy-β-lysine moiety.



Nagamycin

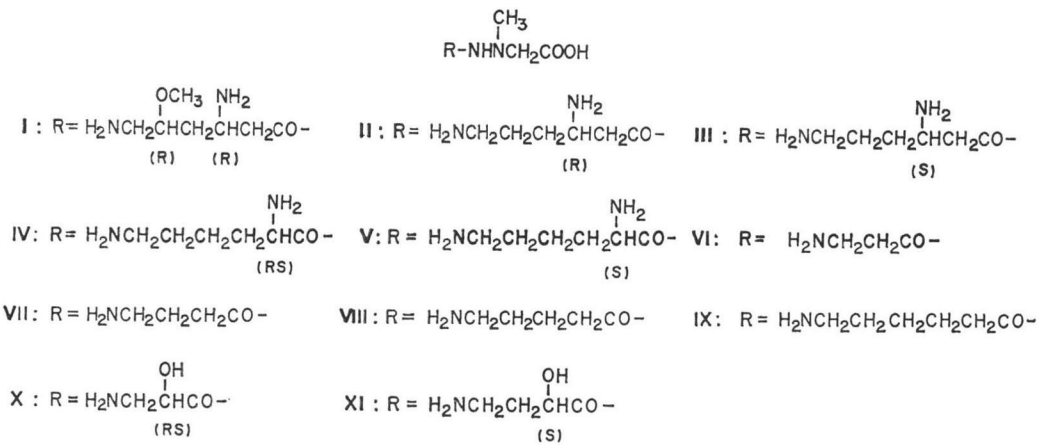
As reported in previous papers, a (*S*, *S*)-δ-hydroxy-β-lysine derivative (antipode of negamycin)[7] has far weaker activity against bacteria than does negamycin, and leucylnegamycin[8] (6-N-L-leucyl derivative), a direct intermediate in the biosynthesis of negamycin, exhibits 42 % of the activity of negamycin, when assayed by the cylinder plate method using *Escherichia coli* K-12 as the test organism. Recently, STREICHER and REINSHAGEN[9] reported the synthesis of 3-aza-analogs which had no activity.

O-Methylnegamycin (**I**), deoxynegamycin (**II**) and 3-*epi*-deoxynegamycin (**III**) were synthesized from di-N-benzyloxycarbonyl derivatives of (3*R*, 5*R*)-3,6-diamino-5-methoxyhexanoic acid, (*R*)-3, 6-diaminohexanoic acid (D-β-lysine) and (*S*)-3,6-diaminohexanoic acid (L-β-lysine) in 44, 47 and 39 % yield respectively by coupling with 1-methylhydrazinoacetic acid by the active ester method using N-hydroxysuccinimide,[10] followed by removal of the protecting groups by catalytic hydrogenation with 5 % palladium on carbon in a mixture of methanol, acetic acid and water for 2~5 hours at atmospheric pressure. These compounds were purified by column chromatography on Amberlite CG-50 (NH_4^+) resin eluted with 0.1 % aqueous ammonia. (3*R*, 5*R*)-3,6-Dibenzyloxycarbonylamido - 5 - methoxyhexanoic acid was synthesized from (3*R*, 5*R*)-3,6-dibenzyloxycarbonylamido-5-hydroxy-hexanoic acid methyl ester[6] in 32 % yield by methylation[11] with diazomethane in a mixture of dichloromethane and ethyl ether in the presence of boron trifluoride etherate followed by hydrolysis of the methyl ester with sodium hydroxide in aqueous ethanol solution. D-β-Lysine ($[\alpha]_D^{25}$ −22.5° (*c* 0.8, 1 N HCl)) was obtained by direct dehydroxylation of (*R*, *R*)-δ-hydroxy-β-lysine[6] with red phosphorus in hydriodic acid at 150°C for 20 hours in a sealed tube. L-β-Lysine[12] was obtained from acid hydrolysis of the antibiotic viomycin.[13]

O-Methylnegamycin (**I**) and deoxynegamycin (**II**) were also synthesized from negamycin as follows: Di-N-benzyloxycarbonylnegamycin, mp 110~113°C (dec.), $[\alpha]_D^{26}$ +4.2° (*c* 6.3, methanol), was prepared from negamycin in 92 % yield with benzyl S-4,6-dimethylpyrimidin-2-ylthiolcarbonate (Kokusan Chemical Works, Tokyo) in the presence of triethylamine in 50 % aqueous dioxane at room temperature for 17 hours. Methylation of di-N-benzyloxycarbonylnegamycin with diazomethane in the presence of boron trifluoride etherate gave di-N-benzyloxycarbonyl-O-methylnegamycin methly ester. The ester was treated with 25 % HBr in acetic acid, and purified by column chromatography on Amberlite CG-50 (NH_4^+) eluted with 0.9 % aqueous ammonia and on silica gel developed with a mixture of butanol, ethanol, chloroform and 17 % aqueous ammonia (4:5:2:3 in volume) to give **I** in 13 % yield from di-N-benzyloxycarbonylnegamycin.

Di-N-benzyloxycarbonylnegamycin methyl ester, mp 107~112°C (dec.), $[\alpha]_D^{26}$ +4.0° (*c* 5.0, methanol), was prepared by esterification of di-N-benzyloxycarbonylnegamycin with diazomethane in a mixture of methanol, ethanol and ethyl ether in 97 % yield. The hydroxyl group of the methyl ester was sulfonylated with mesyl chloride in pyridine at room temperature for 5 hours and treated with NaI in acetone at 65°C overnight to give an isomeric mixture of iodo derivatives (22 % yield) which were purified by column chromatography on silica gel with a mixture of benzene and methyl ethyl ketone (1:1 in volume). Hy-

Table 1. The properties of negamycin analogs



Compound	mp (dec.)	$[\alpha]_D$ in H_2O	Formula[a]	Rf on tlc[b]	Activity (%)[c]
I	137~140°C	−3° at 22°C	$C_{10}H_{22}N_4O_4 \cdot H_2O$	0.33	33
II	120~125°C	−5° at 23°C	$C_9H_{20}N_4O_3 \cdot H_2O$	0.14	29
III	89~100°C	+7° at 26°C	$C_9H_{20}N_4O_3 \cdot H_2O$	0.14	2.5
IV	100~129°C		$C_9H_{20}N_4O_3 \cdot H_2O$	0.20	<0.6
V	127~129°C	+11° at 26°C	$C_9H_{20}N_4O_3 \cdot H_2O$	0.20	<0.6
VI	119~127°C		$C_6H_{13}N_3O_3 \cdot H_2O$	0.27	<0.6
VII	98~115°C		$C_7H_{15}N_3O_3 \cdot H_2O$	0.23	<0.6
VIII	105~115°C		$C_8H_{17}N_3O_3 \cdot H_2O$	0.25	<0.6
IX	105~120°C		$C_9H_{19}N_3O_3 \cdot H_2O$	0.32	<0.6
X	113~138°C		$C_6H_{13}N_3O_4 \cdot H_2O$	0.27	<0.6
XI	120~138°C	−20° at 26°C	$C_7H_{15}N_3O_4 \cdot H_2O$	0.19	<0.6

[a] Satisfactory elemental analyses were obtained for all compounds.

[b] Thin-layer chromatography on Silica gel G (Merck, Art. 5721) developed with butanol-ethanol-chloroform-17% aqueous ammonia (4:5:2:5).

[c] The activities were compared with those of negamycin (100%) by the cylinder plate method using *Escherichia coli* K-12 as a test organism.

drogenolysis of the iodo derivatives with 5% palladium on $BaCO_3$ in aqueous methanol in a PARR apparatus followed by column chromatography on Amberlite CG-50 (NH_4^+) eluted with 0.2% aqueous ammonia afforded **II** in 28% yield.

(2-DL- or L-Lysyl-1-methylhydrazino) acetic acid (**IV** or **V**), (2-β-alanyl-1-methylhydrazino) acetic acid (**VI**), [2-(4-aminobutyryl)-1-methylhydrazino] acetic acid (**VII**), [2-(5-amino-*n*-valeryl)-1-methylhydrazino] acetic acid (**VIII**) and [2-(6-aminocaproyl)-1-methylhydrazino] acetic acid (**IX**) were synthesized from N-benzyloxycarbonyl derivatives of their respective amino acids in 25~57% yield by coupling with 1-methylhydrazinoacetic acid. Two analogs having α-hydroxyl amino acids, (2-DL-isoseryl-1-methylhydrazino) acetic acid (**X**) and [2-{(*S*)-4-amino-2-hydroxybutyryl}-1-methylhydrazino] acetic acid (**XI**) were synthesized from N-*tert*-butoxycarbonyl-DL-isoserine[14] and (*S*)-5-*tert*-butoxycarbonylamido-2-hydroxybutyric acid[15] in 29 and 53% yield, respectively, by coupling with 1-methylhydrazinoacetic acid, followed by removal of the protecting group with 90% trifluoroacetic acid.

The properties of the eleven negamycin analogs described above are summarized in Table 1. The structures of these compounds were confirmed by their NMR spectra and by mass spectra of their methyl ester, N-acetyl derivatives. Among these analogs, only two

Table 2. Antimicrobial spectra of O-methylnegamycin (**I**), deoxynegamycin (**II**), and negamycin

Test organisms	Minimum inhibitory concentrations* (mcg/ml)		
	I	**II**	Negamycin
Staphylococcus aureus FDA209P	12.5	25	12.5
Staphylococcus aureus Smith	6.25	6.25	3.13
Sarcina lutea PCI1001	25	>100	50
Micrococcus flavus FDA16	50	50	25
Bacillus subtilis NRRL B-558	50	100	25
Mycobacterium smegmatis ATCC 607	25	50	50
Escherichia coli NIHJ	6.25	6.25	3.13
Escherichia coli K-12	3.13	3.13	3.13
Escherichia coli K-12 ML1629	1.56	3.13	1.56
Salmonella typhi T-63	1.56	0.78	1.56
Proteus vulgaris OX-19	1.56	3.13	1.56
Proteus rettgeri GN311	6.25	6.25	3.13
Proteus rettgeri GN466	3.13	3.13	3.13
Serratia marcescens	25	25	12.5
Klebsiella pneumoniae PCI602	6.25	6.25	3.13
Pseudomonas fluorescens	1.56	3.13	0.78
Pseudomonas aeruginosa A3	12.5	25	12.5
Pseudomonas aeruginosa No. 12	25	50	6.25

* Minimum inhibitory concentrations were determined on a 0.5% peptone agar by incubation at 37°C for 17 hours.

compounds, **I** and **II**, showed strong antibacterial activity (Table 2), indicating that the δ-hydroxyl group in the (*R*, *R*)-δ-hydroxy-β-lysine moiety is not essential for activity. Furthermore, it is noteworthy that the *R*-configuration of the β-amino group is essential for the activity. **I** and **II** were stable in aqueous solution or in 0.02 N HCl solution at 37°C for 1 month, while the activity of negamycin against *Escherichia coli* K-12 was reduced to 63% or 50%, respectively under these conditions.

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