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Chemical Modification of Streptothricin Group Antibiotics. II.[1] Some Amino Derivatives in Racemomycin-A and Their Biological Activity[2]

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Racemomycin-A, one of streptothricin-group antibiotics, was condensed with aromatic aldehydes. These N-phenylalkylidene derivatives were active against gram-positive and -negative bacteria and Mycobacterium, and showed a tendency of lower delayed toxicity in mice. N-Phenylalkyl and N-acetyl derivatives of Racemomycin-A showed poor antimicrobial activity but with weaken delayed toxicity.

Racemomycins,[4] one of the streptothricin-group antibiotics, show growth inhibition against gram-positive and gram-negative bacteria, and Mycobacterium. However, these antibiotics reveal a typical delayed toxicity which prevents their clinical use. We have been working with new natural or semisynthetic streptothricin-like antibiotics to overcome this toxicity.

In this paper, some amino derivatives of Racemomycin-A involving one mole of β-lysine are described for studying the structure-activity relation.

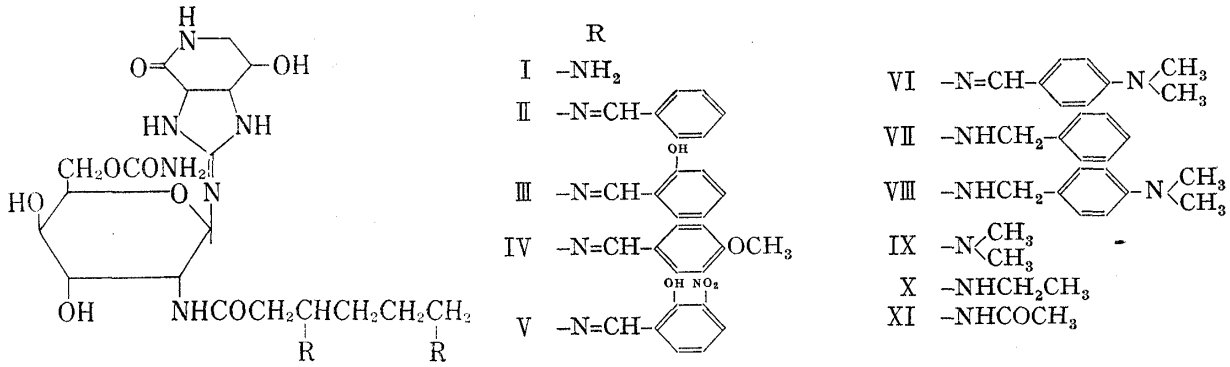

Chart 1

Chemistry

In streptothricin group antibiotics, unstability[5] to acidic or especially to basic solution and insolubility in organic solvents have prevented their chemical modification and this point was first examined.

Condensation products of aromatic aldehydes with five amino groups in Aminosidin, an antibiotic of the amino-glycoside group, have been reported.[6] Similar condensation

1) Part I: H. Taniyama, Y. Sawada, and K. Hashimoto, *Yakugaku Zasshi*, **92**, 182 (1972).
2) A part of paper read at the 77th Kyushu Local Meeting of the Pharmaceutical Society of Japan, May 27, 1972.
3) Location: *1–14, Bunkyo-machi, Nagasaki, 852, Japan.*
4) H. Taniyama, Y. Sawada, and T. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **19**, 1627 (1971).
5) H. Taniyama, Y. Sawada, and T. Kitagawa, *J. Antibiotics*, **24**, 662 (1971).
6) H. Taniyama, Y. Sawada, and S. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **21**, 609, (1973).

products with Kanamycin[7] and with Gentamicin-C_2[8] has independently been described by other workers. These amino derivatives exhibited varied antimicrobial activity and different toxicity. Therefore, amino derivatives of Racemomycin-A was prepared in order to decrease its delayed toxicity.

The aqueous methanol solution of Racemomycin-A hydrochloride was neutralized by triethylamine and an aromatic aldehyde was added to the solution with cooling under stirring. The reaction mixture was concentrated and dried in a desiccator. The residue was reprecipitated with abs. methanol and ether to give the objective products, phenylalkylidene derivatives (II—VI). Compound (V) is sparingly soluble in methanol and was purified from hexamethylphosphoric triamide and ether. Other phenylalkylidene derivatives were freely soluble in methanol and sparingly soluble in ethanol, dimethylformamide, dioxane, and tetrahydrofuran.

Phenylalkyl derivatives, II and VI, were hydrogenated in the presence of platinum dioxide to give the corresponding compounds, VII and VIII. In the hydrogenation of these compounds, sodium borohydride used for the synthesis of phenylalkyl derivatives of Kanamycin[9] and Gentamicin-C_2[8] was not convenient for later purification.

Compounds (IX and X) were directly produced by hydrogenation of Racemomycin-A with formaldehyde and acetaldehyde in the presence of platinum dioxide. Two N-alkyl derivatives were purified by reprecipitation and their reineckate was prepared for microanalysis.

N-Acetyl derivative, XI, was prepared by the reaction of Racemomycin-A with N-acetoxysuccinimide and purification by Sephadex G-10 column chromatography.

Table I. Antimicrobial Activity of Racemomycin-A Derivatives

Microorganisms	MIC (μg/ml) Compounds									
	I[a,b]	II[c]	III[c]	IV[c]	V[c]	VI[c]	VII[c]	VIII[c]	IX[a,d]	X[a,d]	XI[a]
Staphylococcus aureus FDA 209-P	25	50	16	30	50	100	125	>100	>400	>500	>500
Micrococcus luteus IFO-3763	3	6	<4	<4	>50	>100	60	>100	>400	500	—
Sarcina lutea NIHJ	—	100	60	30	>50	>100	>500	>100	>400	>500	—
Bacillus subtilis PCI-219	25	25	<4	16	12.5	12.5	250	>100	>400	>500	—
Escherichia coli NIHJ	12.5	12.5	60	30	12.5	12.5	250	>100	>400	>500	>500
Klebsiella pneumoniae PCI-602	50	50	250	500	25	25	500	>100	>400	>500	—
Proteus vulgaris OX-19	25	25	250	250	6	6	500	>100	>400	500	—
Serratia marcescens var kiliensis IFO-3736	>400	>100	>500	>500	>50	>100	>500	>100	>400	>500	—
Pseudomonas aeruginosa IFO-3901	>200	>100	>500	>500	>50	>100	>500	>100	>400	>500	—
Xanthomonas citri NIAS	—	6	8	30	6	<3	>100	100	>400	250	—
Mycobacterium avium IFO-3153	6	12.5	125	125	6	12.5	>100	>100	>400	>500	—
Mycobacterium smegmatis ATCC-607	6	12.5	125	125	6	12.5	>100	>100	>400	>500	—
Mycobacterium phlei CCM-1889	3	6	125	125	6	6	100	>100	>400	>500	—

solvent: *a*) H_2O, *c*) MeOH
salt: *b*) sulfate, *d*) hydrochloride

Biological Activity

The minimum inhibitory concentration (MIC) of the derivatives was determined by a two-fold dilution method as shown in Table I. It is evident that the spectrum of activity

7) K. Miyaki and T. Yasuda, *Annu. Rep. Inst. Food Microbiol. Chiba Univ.*, **14**, 39 (1961).
8) D.J. Cooper, J. Weinstein, and J.A. Waitz, *J. Med. Chem.*, **14**, 1118 (1971).
9) A. Fujii, K. Maeda, and H. Umezawa, *J. Antibiotics*, **21**, 340 (1968).

and potency of phenylalkylidene derivatives are similar to those of the parent antibiotic. All possess *in vitro* antimicrobial activity comparable to the parent Racemomycin-A, presumably because they can undergo facile hydrolysis to the parent antibiotic in solution. On the other hand, phenylalkyl derivatives, alkyl derivatives, and an acetyl derivative were inactive *in vitro*. This fact shows that the free amino group in β-lysine moiety is essential for the antimicrobial activity.

Table II. Toxicity Pattern of Racemomycin-A Derivatives in Mice

Compound	Route	Dose (mg/kg)	Day after administration							
			0	1	2	3	4	5	6	7
I[a]	*i.v.*	100	0/6	0/6	0/6	0/6	1/6	1/6	1/6	1/6
		200	0/6	0/6	0/6	1/6	2/6	2/6	2/6	2/6
II	*i.v.*	100	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
		150	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
		200	5/5	—	—	—	—	—	—	—
III	*i.v.*	100	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
IV	*i.v.*	100	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
V	*i.p.*	200	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
VI	*i.v.*	100	1/3	1/3	1/3	1/3	1/3	1/3	1/3	1/3
VII	*i.p.*	100	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
		200	1/5	1/5	1/5	1/5	1/5	1/5	1/5	1/5
		300	3/5	3/5	3/5	3/5	3/5	3/5	3/5	3/5
		400	4/5	4/5	4/5	4/5	4/5	4/5	4/5	4/5
VIII	*i.p.*	200	3/3	—	—	—	—	—	—	—

a) sulfate
i.v.: intravenous injection
i.p.: intrapenetoneal injection

Toxicity of the derivatives in mice by single intravenous or intraperitoneal injection is shown in Table II. Acute toxicity of N,N′-dibenzylidene racemomycin-A (II) was increased but no delayed toxicity was observed with a dose of 150 mg/kg. Compounds (III, IV, and V) had no toxicity in a dose of 100 mg/kg, but compound (VI) showed increased acute toxicity compared to the parent antibiotic. Mice which died from the dosage of VI showed bleeding in the chest, and mice dead of acute toxicity showed convulsion and dyspnea after injection (1—1.5 min, compounds (II and VIII)). Abnormality was not seen at autopsy in mice given other derivatives. Further studies on the toxicity of phenylalkylidene derivatives in mice are in progress.

Experimental

Melting points were measured by an automatic melting point apparatus and are uncorrected. Thin-layer chromatography (TLC) on Silica gel G was developed with the solvent system of BuOH–pyridine–HOAc–H_2O–*t*-BuOH (15:10:3:12:4) and the results are given in *Rf* values. Racemomycin-A hydrochloride (I) used herein was isolated from Racemomycin complex by a carbon chromatography.

N,N′-Dibenzylideneracemomycin-A (II)——To a solution of 1.0 g (0.0011 mole) of I in 20 ml of H_2O and 10 ml of MeOH, 0.3 g (0.003 mole) of Et_3N was added. To the stirred solution, 0.5 g (0.005 mole) of BzH was added under cooling. After 1 hr, the reaction mixture was concentrated to produce an oily substance which was washed three times with ether and then dried in a desiccator. The residue was dissolved in 20 ml of abs. MeOH and then filtered. To the filtrate excess of ether was added to give a white powder. Then reprecipitation with MeOH–ether was repeated three times to give a white powder in yield of 1.1 g. *Rf* 0.34 (I: 0.21), infrared (IR) absorption in KBr (cm^{-1}): 3140, 3020, 1605, 1520, 1460, 1350, 1285, 1150, 1105, 1065, 1020, 930, 905, 835, 820, 740, 710. Melting point, optical rotation and elemental analysis shown in Table III.

N,N′-Disalicylideneracemomycin-A (III)——Compound (III) was prepared by condensation of I with salicylaldehyde (0.36 g, 0.003 mole) and by reprecipitation as mentioned above as a yellow powder (1.1 g

Table III. Physico-chemical Data of Racemomycin-A Derivatives

Compound	mp (decomp.)	α_D (18°)	Formula	Analysis					
				Calcd.			Found		
				C	H	N	C	H	N
II	190—200°	−38±2° c=1, MeOH	$C_{33}H_{42}O_8N_8 \cdot 3H_2O$	54.09	6.60	15.29	53.50	6.58	15.01
III	185—190°	−24±2° c=1, MeOH	$C_{33}H_{42}O_{10}N_8 \cdot 3H_2O$	51.83	6.33	14.65	52.25	6.31	14.98
IV	195—205°	−25±2° c=1, MeOH	$C_{35}H_{46}O_{10}N_8 \cdot 3H_2O$	53.02	6.61	14.13	52.85	6.60	14.46
V	180—190°	−125±5° c=0.5, MeOH	$C_{33}H_{40}O_{14}N_{10} \cdot 3H_2O$	46.37	5.42	16.39	46.73	5.00	16.15
VI	180°—	−40±2° c=1, MeOH	$C_{37}H_{52}O_8N_{10} \cdot 3H_2O$	54.27	7.14	17.10	54.09	6.79	17.36

yield). *Rf* 0.30. Ultraviolet (UV) λ_{max}^{MeOH} nm (ε): 255 (16000), 318 (6100), λ_{min}^{MeOH} nm (ε): 290 (2600), IR ν_{max}^{KBr} (cm^{-1}): 3325, 1630, 1540, 1380, 1300, 1275, 1145, 1070, 970, 760.

N,N′-Dianisylideneracemomycin-A (IV)——Compound (IV) was prepared by the condensation of I with anisaldehyde (0.37 g, 0.003 mole) in yield of 1.1 g as a white powder. *Rf* 0.37. UV λ_{max}^{MeOH} nm (ε): 272 (13000), λ_{min}^{MeOH} nm (ε): 235 (2700), IR ν_{max}^{KBr} (cm^{-1}): 3340, 1650, 1600, 1540, 1380, 1300, 1250, 1160, 1070, 1020, 950, 835.

N,N′-Bis(2-hydroxy-3-nitrobenzylidene)racemomycin-A (V)——To a solution of 1.0 g of I in 20 ml of H_2O and 10 ml of MeOH, 0.3 g of Et_3N was added, followed by methanolic solution of 2-hydroxy-3-nitrobenzaldehyde (0.4 g, 0.003 mole) with stirring. The reaction mixture was stirred for 1 hr, evaporated to dryness, and the residue was recrystallized three times from hexamethylphosphoric triamide and ether to a yellow-brownish powder; yield, 1.1 g. *Rf* 0.35, UV λ_{max}^{MeOH} nm (ε): 255 (28000), 430 (19600), IR ν_{max}^{KBr} (cm^{-1}): 3360, 1635, 1510, 1450, 1345, 1285, 1220, 1140, 1065, 945, 865, 750.

N,N′-Bis(4-dimethylaminobenzylidene)racemomycin-A——Compound (VI) was prepared by condensation of I with 4-dimethylaminobenzaldehyde (0.45 g, 0.003 mole), 1.0 g yield, as a white powder. *Rf* 0.31. UV λ_{max}^{MeOH} nm (ε): 334 (7700), IR ν_{max}^{KBr} (cm^{-1}): 3300, 1640, 1530, 1380, 1300, 1180, 1130, 1065, 945, 820.

N,N′-Dibenzylracemomycin-A (VII)——Compound (II) (0.5 g) was dissolved in 40 ml of MeOH and hydrogenated over PtO_2 (0.2 g) for 24 hr. The filtrate after filtration of the catalyst was evaporated to dryness. The residue was reprecipitated with abs. MeOH–ether to give a hygroscopic white powder, in 0.45 g yield, mp above 200° (decomp.), $[\alpha]_D^{18}$ −24 ±2° (c=1, MeOH), *Rf* 0.65 using solvent system of BuOH–pyridine–H_2O (15 : 10 : 12) and detecting with the Pauly reagent. Reineckate was prepared from H_2O and three recrystallizations from H_2O to give red-purple crystals, mp 230—240° (decomp.). *Anal.* Calcd. for $C_{45}H_{64}O_8N_{26}S_{12}Cr_3 \cdot 3H_2O$: C, 31.95; H, 4.17; N, 21.52; Cr, 9.22. Found: C, 31.61; H, 3.78; N, 21.47; Cr, 9.53. UV λ_{max}^{MeOH} nm (ε): 258 (3000), IR ν_{max}^{KBr} (cm^{-1}): 3340, 1660, 1550, 1380, 1155, 1070, 1020, 940, 930, 750.

N,N′-Bis(4-dimethylaminobenzyl)racemomycin-A (VIII)——Compound (VIII) was prepared by hydrogenation of VI in the presence of PtO_2 as for the preparation of VII. The concentrated reaction mixture was charged on a column of cellulose (3×25 cm) and the column was eluted with a solvent system of BuOH–pyridine–H_2O. Fractions with a component of *Rf* 0.66 was concentrated and then reprecipitated with MeOH–EtOH–ether to give a white powder in a quantitative yield; mp 210—220° (decomp.), $[\alpha]_D^{18}$ −20±2° (c=1, MeOH). *Anal.* Calcd. for $C_{37}H_{56}O_8N_{10} \cdot 3H_2O$: C, 54.00; H, 7.59; N, 17.02. Found: C, 53.76; H, 7.87; N, 16.81, UV λ_{max}^{MeOH} nm (ε): 266 (20800), IR ν_{max}^{KBr} (cm^{-1}): 3440, 3000, 1640, 1450, 1375, 1300, 1125, 1020, 945, 880, 800.

N,N,N′,N′-Tetramethylracemomycin-A (IX)——To the solution of I (100 mg), 38% HCHO (1 ml) and PtO_2 (100 mg) were added and the mixture was hydrogenated until it became negative to the Ninhydrin reaction. Filtrate of the reaction mixture was concentrated to a small volume and acetone was added to precipitate a white powder. Reprecipitation was repeated three times with H_2O–MeOH–acetone to give a hygroscopic powder in a quantitative yield, mp above 210° (decomp.). $[\alpha]_D^{20}$ −12° (c=1, H_2O). IR ν_{max}^{KBr} (cm^{-1}): 3400, 2800, 1660, 1540, 1470, 1400, 1380, 1310, 1240, 1100, 1025, 970. Nuclear magnetic resonance (NMR) in D_2O δ from DSS: 2.87 (12H, 2×$-N\langle^{CH_3}_{CH_3}$). Hydrolysis with 6N HCl gave products which showed no β-lysine by automatic amino acid analyzer. Reineckate was prepared in H_2O. *Anal.* Calcd. for $C_{35}H_{60}O_8N_{26}S_{12}Cr_3 \cdot 3H_2O$: C, 26.81; H, 4.24; N, 23.23; Cr, 9.95. Found: C, 26.53; H, 6.48; N, 23.02; Cr, 10.22.

N,N′-Diethylracemomycin-A (X)——To a solution of I (400 mg) in 15 ml of H_2O, Et_3N (0.1 g), MeCHO (1.5 ml), and PtO_2 (100 mg) were added and the mixture was hydrogenated for 24 hr. Filtrate of the reaction mixture was concentrated to a small volume and reprecipitation with H_2O–acetone was repeated three times to give 350 mg of white hygroscopic powder, mp 175—182° (decomp.), $[\alpha]_D^{18}$ −21±2° (c=1, H_2O), *Rf*

0.58. Reineckate was prepared in H_2O. *Anal.* Calcd. for $C_{35}H_{60}O_8N_{26}S_{12}Cr_3 \cdot 3H_2O$: C, 26.81; H, 4.24; N, 23.23; Cr, 9.95. Found: C, 26.40; H, 4.34; N, 22.94; Cr, 10.33. IR ν_{max}^{KBr} (cm^{-1}): 3440, 2990, 1625, 1550, 1400, 1330, 1075, NMR (D_2O) δ: 1.27 (6H, t, $J=7$ Hz $2\times-C\underline{H}_3$), 3.21 (4H, q, $J=7$ Hz, $2\times-C\underline{H}_3-$).

N,N'-Diacetylracemomycin-A (XI)——To a solution of I (400 mg) in 15 ml of H_2O, 0.1 g of Et_3N and 350 mg of N-acetoxysuccinimide in pyridine (5 ml) were added. After stirring for a few minutes, the mixture was concentrated to a small volume and acetone was added to give a white powder. After standing in a refrigerator, the precipitate was collected by filtration and the aqueous solution of the precipitate was charged on a column of Sephadex G-10 (2×150 cm). The column was eluted with H_2O, fractions positive to the Rydon–Smith reagent were collected, and lyophylized to give a white powder, in 360 mg yield, mp above 210° (decomp.). $[\alpha]_D^{18}$ −45° ($c=1$, H_2O). *Anal.* Calcd. for $C_{23}H_{38}O_{10}N_8 \cdot 3H_2O$: C, 43.12; H, 6.92; N, 17.49. Found: C, 42.88; H, 6.98; N, 17.20. NMR (D_2O) δ: 1.94 (s. $2\times-COC\underline{H}_3$). IR ν_{max}^{KBr} (cm^{-1}): 3340, 1660, 1500, 1320, 1225, 1080.

Antimicrobial Activity——The MIC against bacteria was determined by the agar dilution streak method using Brain Heart Infusion agar (Difco), pH 7.4 at 37°. Mycobacterium was cultured in a medium containing meat extract (1%), peptone (1%), glycerol (4%), NaCl (0.2%), and Tween 80 (0.4%), pH 7.3, at 37°. Potato–sucrose medium (mash potato 4.5%, sucrose 2%, pH 6.0) for fungi was used at 27°. Xanthomonas was cultured in a medium (peptone 1%, meat extract 1%, NaCl 0.2%, pH 7.0) at 27°.

Toxicity Test——Female mice of ICR strain, weighing 22—25 g were used. Compound (V) was injected as a solution dissolved by Tween 80 (1%), and other compounds were dissolved in saline.

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