Moreover, the absorption of heparin from the large intestine was extremely promoted and this can be seen in Table I, (B) where a remarkable increase in the clearing factor activity by monoolein mixed micelles was occured although the administered dose was half of that of the small intestine. Therefore, the gastrointestinal absorption of heparin which is practically not absorbed can be induced if the drug was administered in the form of monoolein bile salts mixed micelles.

Also, taurocholate and glycocholate had been found to be extremely less harmful to the gastrointestinal tract contrary to dihydroxy bile acids such as deoxycholate and chenodeoxycholate.[11] Moreover, the presence of monoolein taurocholate mixture could modify a number of the toxic effect of deoxycholate on jejunal function.[12] Therefore, the adjuvants used in our experiment are considered to be safe for the gastrointestinal tract. The mechanism of action of the monoolein bile salts mixed micelles is not clear and our studied are proceeding forward to elucidate the real mechanism.

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Biosynthesis of Streptothricin Antibiotics. IV.[1] On the Incorporation of L-Arginine into Streptolidine Moiety by *Streptomyces lavendulae* OP-2

Carbon atom from L-arginine-U-^{14}C was preferentially incorporated into racemomycin-D which was produced by strain of *S. lavendulae* OP-2. 51% of the activity was located in the streptolidine moiety.

Keywords——*S. lavendulae*; *S. noursei*; Streptothricin; Nourseothricin; Racemomycin; streptolidine moiety; lysine pathway

Through the studies of streptothricin antibiotics, we have described previously the physico-chemical and biological properties of racemomycins A, B and C.[2]

Recently, a strain OP-2 which belongs to *S. lavendulae* was found to produce a racemomycin-D as a main component.[3] Working with this strain, we examined the efficiencies of various amino acids on the fermentative production of racemomycin-D. It was confirmed that several amino acids stimulated the antibiotic production and only arginine was specifically incorporated into streptolidine moiety.

In order to investigate the influence of amino acids on the yield of antibiotic, the supplementation of the synthetic medium containing 3% glucose, 1% ammonium tartarate,

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3) Taxonomical respects of this strain and physico-chemical and biological properties of racemomycin-D will be published elsewhere.

0.5% NaCl, 0.2% $NaNO_3$, 0.1% K_2HPO_4, 0.05% KCl, 0.05% $MgSO_4 \cdot 7H_2O$, 0.04% $CaSO_4 \cdot 2H_2O$ and 0.001% $FeSO_4 \cdot 7H_2O$ with them was carried out. As shown in Table I, stimulative effects were observed when proline, alanine, leucine or arginine was added. And tyrosine, lysine, isoleucine, phenylalanine and aspartic acid are also stimulative to some extent. These facts suggest that these amino acids may play an important role in the biosynthesis of racemomycin-D. Therefore, the uptake of these amino acids into the antibiotic was examined. The incorporation of aspartic acid and its family including lysine into β-lysine moiety will be described elsewhere.

Table I. Effect of Certain Amino Acids on the Antibiotic Production in The Synthetic Medium

Amino acids added[a] (10^{-2} M)	Antibiotic[b] (μg/ml)	Amino acids added[a] (10^{-2} M)	Antibiotic[b] (μg/ml)
Proline	53.0	Glutamic acid	3.1
Alanine	50.0	Tryptophan	2.5
Leucine	17.0	Histidine	1.9
Arginine	16.0	Serine	1.5
Tyrosine	7.8	Glycine	1.4
Isoleucine	7.5	Methionine	1.1
Hydroxy-proline	7.5	Cysteine	0
Lysine	7.5	Cystine	0
Phenylalanine	7.4	Valine	0
Aspartic acid	7.1	None	2.0
Threonine	3.5		

a) Amino acid (L-form) was added to the medium at the begining of the fermentation and cultivation was done at 30° for 48 hr.
b) Antibiotic content was determined by a disk method by using *B.subtilis* PCI-219 as an indicator strain.

Six amino acids-U-^{14}C were added, respectively, to the fermentation medium consisting of 3.5% glycerin, 0.5% NaCl, 0.5% polypeptone and 0.5% beef extract, pH 7.0. Racemomycin-D produced was isolated from the culture filtrate (500 ml) by column chromatography on Amberlite IRC-50 (Na^+, 2×15 cm) and following Sephadex LH-20 (3×140 cm). As shown in Table II, arginine was incorporated efficiently into the antibiotic molecule and alanine was also incorporated to some extent.

Table II. Incorporation of Amino Acids-U-^{14}C into Racemomycin-D

^{14}C-Compounds	Amount of ^{14}C added[a] (μCi)	Racemomycin-D recovered		Incorp. rate (%)
		Spec. act.[b] (dpm/μM)	Total act.[c] (×10^4 dpm)	
Arginine	25	15003.5	260.1	4.68
Alanine	25	2628.5	45.6	0.82
Proline	25	1303.4	23.8	0.43
Leucine	25	1110.7	20.3	0.37
Phenylalanine	25	409.5	7.5	0.13
Tyrosine	25	246.0	4.3	0.08

Isotope was added at 4 hr cultivation, flask was shaked for further 44 hr.
a) Specific activity of all amino acids were 10mCi/mM.
b) Radioactivity was determined by a liquid scintillation counter.
c) Antibiotic potency at about 250μg/ml was obtained.

Labeled racemomycin-D was hydrolyzed (6N HCl, 110—120°, 69 hr) and the hydrolyzate was chromatographed on paper (Toyo-Roshi No. 51). After developed with a solvent system of *n*-BuOH–pyridine–AcOH–H_2O–*t*-BuOH (75: 50: 191: 236: 548), the radioactivity of the

paper strip was scanned. The comparison of individual radioactivities of streptolidine, β-lysine and D-gulosamine indicates that arginine is a direct precursor of the streptolidine moiety (51% of total radioactivity) and other amino acids had little radioactivity. A part of arginine seemed to be converted to β-lysine moiety (11%) maybe *via* the lysine pathway. Therefore, the stimulating effect of several amino acids, which were not incorporated, on racemomycin–D production seemed to be correlate with cell wall synthesis.[4]

Gräfe, *et al.*[5] reported that streptolidine moiety of nourseothricin produced by *S. noursei* JA 3890b was biosynthesized specifically from arginine. Before this publication, we had reported[6] that racemomycin–A from the fermentation broth of *S. lavendulae* ISP–5069 could not be formed from this amino acid, but be formed from the acetic acid pathway. From the results of *S. lavendulae* OP–2, biosynthesis of streptolidine moiety in streptothricins by *S. lavendulae* strains seemed to be due to, at least, two different pathways.

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