Chemical Synthesis, Isolation and Characterization of α -N-Fattyacyl Colistin Nonapeptide with Special Reference to the Correlation between Antimicrobial Activity and Carbon Number of Fattyacyl Moiety[†]

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Five normal and two isofattyacyl colistin nonapeptides, of which carbon numbers of acyl moiety ranged in 9~14, were synthesized in addition to those as previously reported, and characterized on their chemical, physicochemical and biological natures. These derivatives obtained in hydrochloride salt indicated clear melting point at 200~225°C, easily dissolved in water and their optical activities were levorotatory like colistin nonapeptide and colistin. Antimicrobial spectra of the *n*-fattyacyl derivatives were extended in kinds of susceptible organisms to gram positive bacteria, thus *n*-undecanoyl, *n*-dodecanoyl and *n*-tetradecanoyl derivatives inhibited all of test organisms except *Serratia marcescens* and four *Proteus* species. As compared with antimicrobial activity of colistin, that of α -N-fattyacyl derivatives was considerably weakened to extents of 1/4~1/8 against gram negative bacteria while enforced 2~8 times against gram positive bacteria. In two isofattyacyl derivatives synthesized, their antimicrobial activities were inferior to those of corresponding normal fattyacyl derivatives.

Moreover, correlation between antimicrobial activity against each test organism employed and carbon number of acyl moiety in ten α -N-fattyacyl derivatives synthesized hitherto, was investigated, and normal fattyacyl derivatives in their fattyacyl moieties of carbon number in 8~12, or rather in the range more than 10 apparently exhibited a characteristic antimicrobial profiles against gram negative and positive bacteria. Against colistin-insensitive gram negative bacteria such as *S. marcescens* and four *Proteus* species, all of α -N-fattyacyl colistin nonapeptides so far were inactive at the concentration of 100 μ g/ml.

As previously reported,¹⁾ eight α -N-acyl colistin nonapeptides including three aliphatic fattyacyl derivatives were synthesized and characterized in their chemical and biological natures. Since all the derivatives synthesized exhibited definite antimicrobial spectra, it was evidenced that colistin nonapeptide,²⁾ the antimicrobially inactive peptide fragment of colistin, acquired specific antimicrobial activity by means of the introduction of an acyl moiety to α -NH₂ group in its molecule. In three fattyacyl derivatives synthesized, such as *n*-hexanoyl, isoheptanoyl and *n*-octanoyl colistin nonapeptides, they were very similar to each

other in antimicrobial spectrum while their antimicrobial activity was considerably lowered as compared with that of colistin.

Among natural polymyxin antibiotics,³⁾ the kind of fatty acid component linked to α -NH₂ group of terminous α,γ -diaminobutyric acid (Dab) is restricted only to 6-methyloctanoic acid (MOA) or isooctanoic acid (IOA) like that colistin A and B are composed of MOA and IOA, respectively. Although MOA and IOA were distinctive in carbon number and substitution group at C-6 position, antimicrobial activity of colistin A and B³⁾ against *Escherichia coli* hardly differentiated to each other. Indeed, the biological significance of acyl moiety in colistin molecule has not yet

[†] Studies on Colistin Nonapeptide Derivatives. Part II.

been clarified at least on the basis of experimental results.

Along with the previous line of studies, five normal and two isofattyacyl colistin nonapeptides were newly synthesized, and all of the α -N-fattyacyl derivatives synthesized hitherto including those newly synthesized were subjected to investigate correlation between antimicrobial activity against test organisms employed and carbon number of acyl moiety.

Such an effect has been often studied on penicillin⁴⁾ and coumermycin⁵⁾ derivatives. In the α -N-fattyacyl colistin nonapeptides, an elongation of carbon chain of their acyl moiety gave rise to a characteristic broadening of the extent in antimicrobial spectrum and to an enhanced inclination to attack gram positive bacteria. Moreover, in a series of normal fattyacyl derivatives, the maximal antimicrobial activity against gram negative bacteria tested was shown by the fattyacyl moiety of carbon number in 10~12, while against gram positive bacteria, it was in the range of more than 10. Isofattyacyl derivatives manifested a weakened antimicrobial activity as compared with normal fattyacyl ones.

This paper is concerned with chemical synthesis of seven α -N-aliphatic fattyacyl colistin nonapeptides, which were characterized on chemical, physicochemical and biological properties to reveal a possible correlation between antimicrobial activity and carbon number of normal and isofattyacyl moieties.

MATERIALS AND METHODS

Colistin nonapeptide. Colistin nonapeptide was prepared by enzymatic cleavage of colistin as described in the previous paper.²⁾

Acid chlorides. The following acid chlorides were synthesized by chlorination with thionyl chloride from each corresponding fatty acid preparation purchased from Tokyo Kasei Kogyo Co. Ltd.; *n*-nonanoyl chloride bp 139°C/80 mm Hg, isononanoyl chloride bp 154°C/90 mm Hg, *n*-decanoyl chloride bp 90~91°C/7 mm Hg, isodecanoyl chloride bp 98~101°C/10 mm Hg, *n*-undecanoyl chloride bp 161°C/55 mm Hg, *n*-dodecanoyl chloride bp 119~121°C/8 mm Hg and *n*-tetradecanoyl chloride bp 156°C/3 mm Hg.

Reaction conditions for the synthesis of α -N-fattyacyl colistin nonapeptides. In order to selectively acylate only α -NH₂ group in colistin nonapeptide molecule, synthetic reaction was carried out in a restricted range of pH value as precisely described in the previous paper.¹⁾ Reaction conditions for each preparation were summarized in Table I.

Isolation and purification of α -N-fattyacyl colistin nonapeptides. After acylation was stopped at pH 1.0 with the addition of 1 N HCl, coexisting acetone or dioxane was removed *in vacuo* and unreacted acid chloride was extracted by ether. The residual aqueous layer was saturated with NaCl and repeatedly extracted with *n*-butanol at pH 7.5. Reaction products transferred to *n*-butanol layer was reextracted with a small volume of dil. HCl, and concentrated *in vacuo* at pH 5.0, desalted by column chromatography of Biogel P-2 and then followed by lyophilization. Crude powder obtained was subjected to counter current distribution using the solvent system of *n*-butanol, *sec*-butanol, and 0.1 N HCl in 6: 30: 40. After transferred for 200 times, distribution pattern of reaction

TABLE I. REACTION CONDITIONS FOR THE SYNTHESIS OF EACH SEVEN α -N-FATTYACYL COLISTIN NONAPEPTIDE DERIVATIVES

	C.N.P. (HCl salt)	Acid chloride	Solvent ^{b)}	Reaction time	Reaction temp.
α -N- <i>n</i> -Nonanoyl C.N.P. ^{a)}	1 mm	5 mm	60% A ^{c)}	2.5 hr	25°C
α -N-Isononanoyl C.N.P.	1	5	60% A	3	25
α -N- <i>n</i> -Decanoyl C.N.P.	1	10	60% A	3	25
α -N-Isodecanoyl C.N.P.	1	10	60% A	3	25
α -N- <i>n</i> -Undecanoyl C.N.P.	1	10	50% A	3	25
α -N- <i>n</i> -Dodecanoyl C.N.P.	1	5	60% D	3	25
α -N- <i>n</i> -Tetradecanoyl C.N.P.	1	4	73% A	3.5	30

^{a)} C.N.P., colistin nonapeptide.

^{b)} Solvent content in 1000 ml of bufferized (1/5 M acetate buffer, pH 5.0) aqueous solvent excepting the case of *n*-tetradecanoyl derivative of which total volume was 750 ml.

^{c)} A, acetone; D, dioxane.

products was tested colorimetrically with ninhydrin reagent, where the aqueous layer corresponding to the objective peak was collected and adjusted to pH 5.0 by addition of a small volume of IRA 410 (OH type) resin. With concentration *in vacuo* following lyophilization, purified preparation of α -N-fattyacyl colistin nonapeptide hydrochloride was obtained.

Quantitative determination of amino acid and fatty acid component constituting α -N-fattyacyl colistin nonapeptides. Each purified preparation of the synthesized α -N-fattyacyl colistin nonapeptide derivatives was hydrolyzed with 6 N HCl at 110°C for 24 hr in a sealed tube, then repeatedly extracted with ether to separate fatty acid. After removal of ether, the extract was esterified with diazomethane and subjected to a Hitachi gas liquid chromatograph type K-53 under the conditions described previously.²¹ The remaining aqueous layer was dried up *in vacuo* and amino acids containing were analyzed by a Japan Electron Optics Laboratory full automatic amino acid analyzer type JLC-6AS.

Paper chromatography and bioautography of α -N-fattyacyl colistin nonapeptides. Toyo Roshi No. 50 paper was used and each sample was ascendingly developed by the following solvent system; *n*-butanol, pyridine, acetic acid and water, 15:10:3:12. α -N-Fattyacyl colistin nonapeptides were detected by ninhydrin reagent and their bioautography were performed on the nutrient agar plate sowed *E. coli* NIHJ or *Bacillus subtilis* PCI 219 as test organism.

Determination of antimicrobial spectrum and acute toxicity of α -N-fattyacyl colistin nonapeptides. Antimicrobial spectrum was determined by agar dilution method streaking 19 bacterial strains on 0.5% glucose nutrient agar. Acute toxicity was estimated by ad-

ministration through the intravenous injection to male mice as described previously.²¹

RESULTS AND DISCUSSION

Physical, chemical and biological characteristics of seven α -N-fattyacyl colistin nonapeptide derivatives

Seven derivatives containing five *n*-fattyacyl, *i.e.* α -N-*n*-nonanoyl-, α -N-*n*-decanoyl-, α -N-*n*-undecanoyl-, α -N-*n*-dodecanoyl- and α -N-*n*-tetradecanoyl- and two isofattyacyl, *i.e.* α -N-*n*-isononanoyl- and α -N-isodecanoyl- colistin nonapeptide were synthesized under reaction conditions given in Table I. All preparations as hydrochloride salt were obtained in white amorphous powder and clear melting points were observed between 200~225°C. They were easily soluble in water, and considerably soluble in lower alcohol, aqueous acetone and aqueous dioxane. Optical activity of these derivatives was levorotatory like other α -N-acyl derivatives previously synthesized,¹⁾ colistin nonapeptide²⁾ and colistin.^{6,7)} On a paper chromatogram, each preparation showed a ninhydrin positive spot apparently distinguished from that of colistin nonapeptide²⁾ in respective *R_f* values, which coincided to an inhibition zone on a bioautogram using *E. coli* NIHJ or *B. subtilis* PCI 219 as test organism.

Analytical data of amino acids and fatty acid composing each of seven derivatives synthesiz-

TABLE II. PHYSICOCHEMICAL CHARACTERISTICS OF SEVEN α -N-FATTYACYL COLISTIN NONAPEPTIDE DERIVATIVES

	Appearance	mp	$[\alpha]_D^{25}$ (in water)	<i>R_f</i>
α -N- <i>n</i> -Nonanoyl C.N.P. ^{a)}	White Amorphous	210~214°C	-25.38°	0.63
α -N-Isononanoyl C.N.P.	White Amorphous	218~224	-20.78	0.57
α -N- <i>n</i> -Decanoyl C.N.P.	White Amorphous	205	-39.16	0.61
α -N-Isodecanoyl C.N.P.	White Amorphous	215~220	-18.91	0.55
α -N- <i>n</i> -Undecanoyl C.N.P.	White Amorphous	211~218	-31.92	0.63
α -N- <i>n</i> -Dodecanoyl C.N.P.	White Amorphous	212~217	-33.50	0.69
α -N- <i>n</i> -Tetradecanoyl C.N.P.	White Amorphous	202~214	-24.55	0.73

^{a)} C.N.P., colistin nonapeptide.

TABLE III. MOLAR RATIO OF AMINO ACIDS AND FATTY ACID IN EACH OF SEVEN α -N-FATTYACYL COLISTIN NONAPEPTIDES

	Molar ratio			
	Leu	Thr	Dab	Fatty acid
α -N- <i>n</i> -Nonanoyl C.N.P. ^{a)}	2.00	1.68	4.73	1.19
α -N-Isononanoyl C.N.P.	2.00	1.85	4.76	0.73
α -N- <i>n</i> -Decanoyl C.N.P.	2.00	1.88	4.64	1.14
α -N-Isodecanoyl C.N.P.	2.00	1.61	4.46	0.70
α -N- <i>n</i> -Undecanoyl C.N.P.	2.00	1.86	4.55	0.80
α -N- <i>n</i> -Dodecanoyl C.N.P.	2.00	2.02	4.57	0.82
α -N- <i>n</i> -Tetradecanoyl C.N.P.	2.00	1.72	4.85	0.99

^{a)} C.N.P., colistin nonapeptide.

ed are shown in Table III. After each purified preparation was hydrolyzed in 6N HCl at 110°C for 24 hr, acid hydrolyzate was repeatedly extracted with ether, where the fatty acid contained in ether layer and amino acids remaining in acidic aqueous layer were quantitatively estimated by gas liquid chromatograph or automatic amino acid analyzer. Molar ratio of amino acid composition of all the synthesized derivatives, showing a slightly lowered

analytical value of Dab in contrast to the theoretical one, was as follows, Leu: Thr: Dab, 2: 1.61~2.02: 4.46~4.85 (*Theo.*, Leu: Thr: Dab, 2: 2: 5). Fatty acids found in the above seven preparations ranged in molar ratio to colistin nonapeptide from 0.7 in isodecanoic acid to 1.19 in *n*-nonanoic acid, and thus it is evident that in all the synthesized derivatives the fattyacyl moiety linked to colistin nonapeptide is in equimolar amounts within experimen-

TABLE IV. ANTIMICROBIAL SPECTRA OF SEVEN α -N-FATTYACYL COLISTIN NONAPEPTIDES

Antimicrobial spectra were investigated by agar dilution method using 19 bacterial strains, of which MIC values were determined after 24 hr incubation at 37°C.

Test organism	<i>n</i> -Nonanoyl (C 9) C.N.P. ^{a)}	Iso-nonanoyl (C 9) C.N.P.	<i>n</i> -Decanoyl (C 10) C.N.P.	Iso-decanoyl (C 10) C.N.P.	<i>n</i> -Undecanoyl (C 11) C.N.P.	<i>n</i> -Dodecanoyl (C 12) C.N.P.	<i>n</i> -Tetradecanoyl (C 14) C.N.P.
<i>M. lysodeikticus</i>	100 ^{b)}	50	50	50	25	50	25
<i>St. aureus</i> FDA 209P	>100	>100	100	>100	50	50	25
<i>S. lutea</i> PCI 1001	>100	100	25	>100	25	12	12
<i>B. subtilis</i> PCI 219	50	25	12	50	6	12	12
<i>B. cereus</i> IFO 3001	100	50	>100	100	12	50	12
<i>E. coli</i> NIHJ	25	50	3	50	12	3	25
<i>E. coli</i> K-12	12	50	3	25	3	3	12
<i>A. aerogenes</i> IAM 1063	>100	>100	12	>100	50	12	100
<i>Ser. marcescens</i> IAM 1022	>100	>100	>100	>100	>100	>100	>100
<i>Pr. vulgaris</i> IFO 3045	>100	>100	>100	>100	>100	>100	>100
<i>Pr. mirabilis</i> IFO 3849	>100	>100	>100	>100	>100	>100	>100
<i>Pr. morganii</i> IFO 3848	>100	>100	>100	>100	>100	>100	>100
<i>Pr. rettgeri</i>	>100	>100	>100	>100	>100	>100	>100
<i>Sal. enteritidis</i>	>100	>100	12	>100	100	12	100
<i>Sal. infantis</i>	100	25	12	50	25	12	25
<i>Sh. flexneri</i>	12	12	6	25	6	6	12
<i>Ps. aeruginosa</i> IFO 3901	100	100	25	100	50	25	100
<i>Ps. desmolytica</i>	12	25	12	50	50	12	12
<i>X. oryzae</i>	12	12	12	12	12	6	6

^{a)} C.N.P., colistin nonapeptide.^{b)} MIC value, μ g/ml.

tal errors. Chemical purity was confirmed by quantitative estimation of N-terminal amino acids of peptide moiety in these seven preparations, as reported in the previous paper.¹¹ The concept is fundamentally based upon the fact that α -NH₂ acylated derivative is assessed as impurity since all of fatty acid molecules when introduced under given experimental conditions are to be linked to α -NH₂ group of the terminous Thr of colistin nonapeptide. From the analytical data concerning DNP-Thr and γ -DNP-Dab, amounts of the impurity ranging from 20 to 30% were considered negligible and compatible with those of the fattyacyl derivatives previously synthesized.¹¹ Subsequently, the following antimicrobial test was performed with these seven preparations.

Throughout a series of α -N-fattyacyl colistin nonapeptides, either previously or newly synthesized, they belong substantially to gram-negative type antibiotic because of their common antimicrobial profiles towards gram-negative bacteria such as *E. coli*, *Salmonella infantis*, *Shigella flexneri*, *Pseudomonas desmolytica* and *Xanthomonas oryzae*, whereas they were as inactive as colistin against *Serratia marcescens* and four *Proteus* species. Antimicrobial activity against gram-negative bacteria of all these fattyacyl derivatives was weaker than that of colistin while the activity against gram-positive bacteria exhibited by higher fattyacyl derivatives were fairly enforced. For example, colistin could not inhibit the growth of *Staphylococcus aureus* and *Bacillus cereus* at the concentration of 100 μ g/ml while *n*-undecanoyl, *n*-dodecanoyl and *n*-tetradecanoyl derivatives inhibited them at concentrations of 25~50 μ g/ml and 12~50 μ g/ml, respectively. Thus, these higher fattyacyl derivatives were recognized to belong rather to the broader spectrum type antibiotic than colistin.

In comparison of isofattyacyl derivatives with the corresponding *n*-fattyacyl ones, both nonanoyl derivatives were hardly discriminated in their antimicrobial activity against each test organism, whereas in case of decanoyl derivatives antimicrobial activity of isofattyacyl

derivative was apparently inferior to that of normal fattyacyl one as exemplified in activities against *Sarcina lutea*, *Salmonella enteritidis* and *Pseudomonas aeruginosa*.

The correlationship between antimicrobial activity and carbon number of fattyacyl group of α -N-fattyacyl colistin nonapeptides

When antimicrobial activities of α -N-*n*- or α -N-isofattyacyl colistin nonapeptides were compared with each other, considerable differences were observed in inhibitory profiles against each test organism. However, in this connection, a close correlationship was recognized to reside in the increase of carbon number of fattyacyl moiety and broadening of antimicrobial spectrum. To visualize such a correlationship more distinctly, a graphical analysis was performed as indicated in Figs. 1~3, where values of minimum inhibitory concentration (MIC) of the fattyacyl nonapeptides in logarithm were taken as longitude while carbon number of fattyacyl moieties as abscissa. On the basis of characteristic curves depicted by seven *n*-fattyacyl derivatives, all test organisms were differentiated into three groups, *i.e.* type I, II and III.

Type I includes colistin-sensitive gram-negative bacteria, for which the most effective carbon numbers of *n*-fattyacyl moiety is limited in very narrow range of 10~12. Type II includes gram-positive bacteria and one gram-negative bacterium, *X. oryzae*, for which effective carbon numbers more than 10 in *n*-fattyacyl moieties facilitate antimicrobial activity with their carbon increase, C-14 or possibly more carbon number being most effective. Type III includes colistin-insensitive gram-negative bacteria, for which all the normal and isofattyacyl derivatives are entirely ineffective.

Against type I bacteria, throughout effective *n*-fattyacyl nonapeptides, rather lower antimicrobial levels were usually observed, as compared with MIC values of colistin, and even the most effective fattyacyl nonapeptide, showed an antimicrobial activity, which was compatible with 1/4~1/8 of colistin level in

FIG. 1. The Correlation between Antimicrobial Activity (MIC) against Type I Bacteria and Carbon Number of Acyl Moiety of α -N-Fattyacyl Colistin Nonapeptides.

In each figure, log of MIC was expressed on the longitude while the carbon number on the abscissa.

●—●, *n*-fattyacyl derivative; ○—○, isofattyacyl derivative; —, colistin sulfate.

general other than 1/16 in activity against *P. aeruginosa*. Of *n*-fattyacyl moieties in most active range of carbon number, *n*-undecanoyl (C-11) nonapeptide was distinguished from other two, *n*-decanoyl (C-10) and *n*-dodecanoyl (C-12) nonapeptides in lower antimicrobial activity against six bacterial strains of type I other than *E. coli* K-12 and *Sh. flexneri*. The latter C-10 and C-12 *n*-fattyacyl nonapeptides inhibited *Sal. enteritidis* at the concentration of 12 μ g/ml while the former C-11 derivative did so at the concentration of 100 μ g/ml. Thus, the fact that against gram negative bacteria belonging to type I the most efficient carbon number of *n*-fattyacyl moiety was restricted to even number such as 10 or 12 was noticeably mentioned.

Furthermore, the most characteristic aspect of this *n*-fattyacyl series was exhibited in the

antimicrobial spectra against gram-positive bacteria of type II. As shown in Fig. 2, the longer carbon chain than C-8 or C-10 in *n*-fattyacyl moiety, the higher antimicrobial activity against the type II bacteria was indicated by these *n*-fattyacyl colistin nonapeptides, attaining to the level of two to eight times superiority to the antimicrobial activity of colistin. Two antimicrobial peaks appeared at C-11, *n*-undecanoyl nonapeptide, and C-14, *n*-tetradecanoyl one. The former C-11 *n*-fattyacyl nonapeptide showed the highest antimicrobial activity especially against *Micrococcus lysodeikticus*, *B. subtilis* and *B. cereus*, though not covering all other gram-positive bacteria of type II. This C-11 fattyacyl derivative, as above mentioned, indicated characteristically weaker antimicrobial activity against the type I bacteria, thus making a trough in

FIG. 2. The Correlationship between Antimicrobial Activity (MIC) against Type II Bacteria and Carbon Number of Acyl Moiety of α -N-Fattyacyl Colistin Nonapeptides.

●—●, *n*-fattyacyl derivative; ○—○, isofattyacyl derivative; —, colistin sulfate.

FIG. 3. Antimicrobial Activity (MIC) against Type III Bacteria and Carbon Number of Acyl Moiety of α -N-Fattyacyl Colistin Nonapeptides.

●—●, *n*-fattyacyl derivative; ○—○, isofattyacyl derivative; —, colistin sulfate.

profiles between C-10 and C-12 fattyacyl nonapeptides. Such an alternative antimicrobial tendency against the type I and the type II bacteria was emphasized in C-14 *n*-fattyacyl derivative, *n*-tetradecanoyl nonapeptide.

As for three isofattyacyl colistin nonapeptides such as isoheptanoyl (C-7), isononanoyl (C-9) and isodecanoyl (C-10) peptides, so far synthesized, their antimicrobial activity varied so irregularly due to kinds of these isofattyacyl moiety as well as of susceptible bacteria, that a clear-cut correlationship as in the above normal series of derivatives was not obtained in their effectivenesses. For examples, two strains of *E. coli* were most susceptible to isoheptanoyl nonapeptide while *Sh. flexneri*, *Sal. infantis* and *Ps. desmolytica* were inhibited most seriously by isononanoyl nonapeptide and none of *Aerobacter aerogenes*, *Sal. enteritidis* and *Ps. aeruginosa*, all of which in turn were susceptible to *n*-decanoyl nonapeptide, received efficient inhibition by any of these isofattyacyl colistin nonapeptides including isodecanoyl peptide even at the concentration of 100 μ g/ml. Despite such irregularities, it is pointed out as aspects characterizing isofattyacyl nonapeptides that antimicrobial activities of these isofattyacyl derivatives against gram-negative bacteria of type I are much inferior to those of *n*-fattyacyl nonapeptides, showing more limited antimicrobial spectra, and that antimicrobial peak concerned with isofattyacyl moieties, as seen from Fig. 1, tends to shift towards less carbon number side such as C-7 in contrast to C-10 or more in peaks shown by *n*-fattyacyl nonapeptides. Against the gram-positive bacteria of type II such as *M. lysodeikticus*, *B. subtilis* and *B. cereus* the antimicrobial peak appeared at C-9 in isofattyacyl moiety, *i.e.* isononanoyl nonapeptide, which was odd carbon number, same as in the case of *n*-fattyacyl nonapeptides in this respect, but less by 2 carbons than C-11, *i.e.* *n*-undecanoyl moiety at the peak.

Effects of increased carbon number of acyl moiety on antimicrobial profiles of the antibiotics were observed in semisynthetic penicillin⁴⁾ and coumermycin.⁵⁾ Including the

results obtained with the synthetically acylated colistin nonapeptides, the effects caused by the increased carbon number in acyl moiety in each case seem apparently to be mainly governed by the given acyl group, possibly raising hydrophobicity especially in the case of variously fattyacylated colistin nonapeptides here reported, in which colistin nonapeptide itself almost eliminated in antimicrobial activity turns effective with acylation at a site of N-terminal threonine. However, in view of the difference in antimicrobial activity exhibited by isofattyacyl and the corresponding *n*-fattyacyl nonapeptides, the events brought about by different carbon chain lengths of fattyacyl moiety are not entirely accounted for as the alteration in hydrophobicity. Indeed, a slight diversity of chemical structure in aliphatic fattyacyl groups such as normal or iso type and fattyacyl moieties of carbon numbers between 9 and 14, which often showed a trough in antimicrobial activity-carbon number curves, rendered to afford qualitative and quantitative alterations in antimicrobial spectra. If markedly lower antimicrobial activity against the gram negative type I bacteria of these fattyacyl colistin nonapeptides is compared with much stronger one of colistin or its homologs, it is unexplainable as a result due to a difference merely in chemical structure of acyl groups. Rather, the terminous Dab existing in the peptide part of colistin may be more significantly responsible for its stronger antimicrobial activity. Further, that normal and isofattyacyl colistin nonapeptides, all were inactive against the gram-negative and colistin-insensitive bacteria belonging to type III may possibly be based upon inadequacy of chemical structure in their peptide moieties including peptide part of colistin.

In conclusion, in α -N-fattyacyl colistin nonapeptides, so far synthesized, the increase of carbon number in their fattyacyl moieties especially of normal series seems very likely to give rise to a reduction in antimicrobial activity against sensitive gram-negative bacteria like *E. coli* while a facilitation in the activity against gram positive bacteria. Furthermore, these

α -N-fattyacyl colistin nonapeptides are evidently discriminated from colistin or other known colistin derivatives^{8~11)} in antimicrobial spectrum and chemical structure. Hence, the series of fattyacylated colistin nonapeptides may be regarded as a new group of fattyacyl peptide antibiotic synthetically obtained from colistin nonapeptide.

Acute toxicity of seven α -N-fattyacyl colistin nonapeptides

Acute toxicity of seven α -N-fattyacyl colistin

TABLE V. ACUTE TOXICITY OF SEVEN α -N-FATTYACYL COLISTIN NONAPEPTIDE DERIVATIVES

Each preparation was intravenously injected into male mice.

	LD ₅₀ (mg/kg)
α -N- <i>n</i> -Nonanoyl C.N.P. ^{a)}	90
α -N-Isononanoyl C.N.P.	92
α -N- <i>n</i> -Decanoyl C.N.P.	33
α -N-Isodecanoyl C.N.P.	125
α -N-Undecanoyl C.N.P.	65
α -N- <i>n</i> -Dodecanoyl C.N.P.	50
α -N- <i>n</i> -Tetradecanoyl C.N.P.	70

^{a)} C.N.P., colistin nonapeptide.

FIG. 4. Correlation between Acute Toxicity and Carbon Number of Acyl Moiety in α -N-Fattyacyl Colistin Nonapeptide Derivatives.

●—●, *n*-fattyacyl derivative; ○—○, isofattyacyl derivative; —, colistin sulfate; ----, colistin nonapeptide.

nonapeptides was tested by administration through intravenous injection to male mice. As shown in Table V, the most toxic derivative, α -N-*n*-decanoyl colistin nonapeptide was 33 mg/kg in LD₅₀ and other fattyacyl derivatives than isodecanoyl nonapeptide showing 125 mg/kg in LD₅₀ were within 50~90 mg/kg in LD₅₀.

Correlationship between LD₅₀ and carbon number of acyl moiety in all α -N-fattyacyl colistin nonapeptides hitherto examined was indicated in Fig. 4. In a series of *n*-fattyacyl colistin nonapeptides, the most toxic one was shown by both derivatives of carbon 8 and 10 in normal fattyacyl moiety, although a derivative of carbon number 9 was fairly lowered in toxicity as compared with the above two. However, all the *n*-fattyacyl nonapeptides having acyl carbon number within the range of 6~14, so far examined, were of less toxicity than colistin but of higher one than colistin nonapeptide. Considering the acute toxicity of colistin nonapeptide in LD₅₀ of 145 mg/kg, it seems likely that acute toxicity of *n*-fattyacyl derivatives is more seriously governed by the peptide moiety than the acyl moiety in their molecule.

Acute toxicity of isofattyacyl colistin nonapeptides such as isoheptanoyl, isononanoyl and isodecanoyl derivatives were gradually reduced with the increased carbon number of acyl

moiety (Fig. 4).

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