Chemical Structures of Polymyxin Series Antibiotics

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Many reports on the chemical structures of polymyxins have been published to date, but the structures proposed were not satisfactory to account for all the properties of the natural substances. The authors elucidated the structures of colistin, polymyxin B, polymyxin E and circulin. This review deals with the chemical structures of antibiotics of polymyxin series which are closely related each other in its structure.

INTRODUCTION

An antibiotic substance designated as polymyxin was first isolated from the culture fluid of Bacillus polymyxa, a spore-forming rod occurring in soil, by Stansly, Shepherd and White. Brownlee and his colleagues, and Benedict and Langlykke independently reported the discovery, in a different strain of Bacillus polymyxa, of an antibiotic designated as aerosporin which appeared to be similar to polymyxin in its antibacterial spectrum, but which exhibited certain differences in pharmacological properties. Evidence has been obtained to confirm both the pharmacological and chemical differences between polymyxin and aerosporin. Furthermore, it was known that different strains of Bacillus polymyxa are capable of producing a number of related antibacterial substances which differ chemically, and pharmacologically from each other. Thus it seemed that the nomenclature of this group of antibiotics should be unified in order to emphasize the relationship between its members. Consequently, it was agreed to use the generic term "Polymyxin" for these antibiotics, the individual members being named polymyxin A, B, C, D etc. Polymyxin A was formerly called aerosporin and polymyxin D was formerly polymyxin.

Jones describes a polymyxin E, the qualitative composition of which is identical with that of polymyxin A but which moves at the same rate as polymyxin B on partition chromatography. On the other hand, in 1950, Koyama et al. reported the isolation of an antibiotic from the culture fluid of a new species named

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** The abbreviations used in this paper are ; Dab : α,γ-diaminobutyric acid residue, MOA : (−)-6-methyloctanoic acid residue, IOA : isoolctanoic acid residue, DNP : 2,4-dinitrophenyl residue, α-DNP-Dab : α-2,4-dinitrophenylamino-γ-amino acid residue, γ-DNP-Dab : γ-2,4-dinitrophenylamino-α-amino acid residue, Di-DNP-Dab : α,γ-2,4-dinitrophenyl-diamino acid, 8α : cyclic octapeptide with a side chain of MOA→(α)Dab→Thr connected in the α-position, 8γ : cyclic octapeptide with a side chain of MOA→(α)Dab→Thr connected in the γ-position, 7α : cyclic heptapeptide with a side chain of MOA→(α)Dab→Thr connected in the α-position, 7γ : cyclic heptapeptide with a side chain of MOA→(α)Dab→Thr connected in the γ-position, (α) : → Dab, (γ) : → Dab.

γ-NH₂ α-NH₂
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*Bacillus polymyxa var. colistinus* (*Aerobacillus colistinus*). This antibiotic was called colistin. Chemical investigations showed that colistin was a cyclic basic peptide which, on hydrolysis, gave α,γ-diaminobutyric acid, leucine, threonine, and (+)-6-methyloctanoic acid, so it was classified as a polymyxin13-15. Colistin differs from polymyxins B and C, which contain phenylalanine, and it is insoluble in water, unlike polymyxins A and D. The composition of colistin is qualitatively identical with that of polymyxin E15.

In 1948 Tetrault et al.18,19 reported that *Bacillus circulans* Q-19 produces an antibiotic polypeptide which is active against gram negative bacteria and that, from its composition, it appears to be related to the polymyxins20. This substance has been named circulin. Furthermore, it was recently reported by Khokhlov et al.21 that a strain of *Bacillus polymyxa* isolated from soil near Moscow yielded a further member of the series, polymyxin M.

A summary of the constituents, and references to these polymyxins are given in Table 1.

It can be seen that each polymyxin consists of only three or four kinds of amino acids, and that α,γ-diaminobutyric acid is common to them all. This amino acid does not occur in proteins and is not known to be a constituent of any other natural products. The fatty acids, (+)-6-methyloctanoic acid and iso-octanoic acid, are in the form of amides attached to the α-amino group of one of the α,γ-diaminobutyric acid residues.

### Table 1. Literature on the constitutions of polymyxins.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Dab</th>
<th>Leu</th>
<th>Thr</th>
<th>Phe</th>
<th>Ser</th>
<th>Fatty Acid</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymyxin A</td>
<td>L and D</td>
<td>D</td>
<td>L</td>
<td>—</td>
<td>—</td>
<td>+</td>
<td>22</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>L and D</td>
<td>L</td>
<td>L</td>
<td>D</td>
<td>—</td>
<td>+</td>
<td>23</td>
</tr>
<tr>
<td>Polymyxin B₁</td>
<td>5L and 1D</td>
<td>1L</td>
<td>2L</td>
<td>1D</td>
<td>—</td>
<td>1</td>
<td>24</td>
</tr>
<tr>
<td>Polymyxin B₂</td>
<td>6L</td>
<td>1L</td>
<td>2L</td>
<td>1D</td>
<td>—</td>
<td>1MOA</td>
<td>25, 26</td>
</tr>
<tr>
<td>Polymyxin B₃</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>—</td>
<td>10A</td>
<td>24, 27</td>
</tr>
<tr>
<td>Polymyxin B₄</td>
<td>6L</td>
<td>1L</td>
<td>2L</td>
<td>1D</td>
<td>—</td>
<td>110A</td>
<td>27, 28</td>
</tr>
<tr>
<td>Polymyxin C</td>
<td>+</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>—</td>
<td>+</td>
<td>6</td>
</tr>
<tr>
<td>Polymyxin D</td>
<td>5L</td>
<td>1D</td>
<td>3L</td>
<td>—</td>
<td>1D</td>
<td>1MOA</td>
<td>5</td>
</tr>
<tr>
<td>Polymyxin E</td>
<td>6L</td>
<td>1L and 1D</td>
<td>2L</td>
<td>—</td>
<td>—</td>
<td>1MOA</td>
<td>30, 31</td>
</tr>
<tr>
<td>Polymyxin E₁</td>
<td>6L</td>
<td>1L and 1D</td>
<td>2L</td>
<td>—</td>
<td>—</td>
<td>1MOA</td>
<td>29, 32</td>
</tr>
<tr>
<td>Polymyxin E₂</td>
<td>6L</td>
<td>1L and 1D</td>
<td>2L</td>
<td>—</td>
<td>—</td>
<td>110A</td>
<td>29, 32</td>
</tr>
<tr>
<td>Polymyxin M</td>
<td>6L</td>
<td>1L</td>
<td>3</td>
<td>—</td>
<td>—</td>
<td>MOA</td>
<td>21, 33, 34</td>
</tr>
<tr>
<td>Colistin</td>
<td>5L</td>
<td>1L and 1D</td>
<td>1L</td>
<td>—</td>
<td>—</td>
<td>+</td>
<td>35, 36, 37</td>
</tr>
<tr>
<td>Colistin</td>
<td>4L</td>
<td>1L</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>+</td>
<td>38</td>
</tr>
<tr>
<td>Colistin</td>
<td>6L</td>
<td>1L and 1D</td>
<td>2L</td>
<td>—</td>
<td>—</td>
<td>+</td>
<td>31, 39</td>
</tr>
<tr>
<td>Colistin A</td>
<td>6L</td>
<td>1L and 1D</td>
<td>2L</td>
<td>—</td>
<td>—</td>
<td>1MOA</td>
<td>40, 41, 42</td>
</tr>
<tr>
<td>Colistin B</td>
<td>6L</td>
<td>1L and 1D</td>
<td>2L</td>
<td>—</td>
<td>—</td>
<td>110A</td>
<td>43</td>
</tr>
<tr>
<td>Circulin A</td>
<td>6L</td>
<td>1Ll and 1D</td>
<td>2L</td>
<td>—</td>
<td>—</td>
<td>1MOA</td>
<td>20, 44, 45</td>
</tr>
<tr>
<td>Circulin B</td>
<td>6L</td>
<td>1Ll and 1D</td>
<td>2L</td>
<td>—</td>
<td>—</td>
<td>1MOA</td>
<td>46</td>
</tr>
</tbody>
</table>

In the figures, (+) indicates the presence of optically uncharacterized amino acid and L and D indicate the optical form of the amino acid. Numbers in the columns of amino acids indicate the molar ratios of these constituents.
Chemical Structures of Polymyxin Series Antibiotics

The authors have separated colistin, circulin, polymyxin E and polymyxin B into colistin's A and B, circulin's A and B, polymyxin's E and E2, and polymyxin's B1 and B2 respectively, and have determined the molar ratios of their constituents to be as shown in Table 1. Moreover, it has been confirmed that the fatty acid in colistin B is isooctanoic acid 44. The configuration of the α,γ-diaminobutyric acid was shown to be that of the L-isomer.

Polymyxins are stable at physiological pH values and temperatures either in aqueous solution or as powders. Polymyxin D, and presumably the other polymyxins, also is stable in acid but unstable in alkali, its destruction rate depending upon the pH and temperature.

The chemical structures of these antibiotics have been studied by many workers, but no systematic observations have yet been made. The present authors have deduced the chemical structures of colistin's A and B, circulin A and polymyxin's B, and B2 and also confirmed that the structure of polymyxin E1 is identical with that of colistin A while that of polymyxin E2 is the same as that of colistin B.

Chemical Structure of Colistin A

In 1953 Oda et al.14,15 reported the separation of commercial colistin into three components. In 1957, K. Suzuki16 reported that the amino acid composition of unfractionated colistin was threonine: leucine: α,γ-diaminobutyric acid=1:2:5 and they proposed the tentative structure for it shown in Fig. 1 on the basis of sequential analyses of the products obtained by partial acid hydrolysis. This cyclic structure with no side chain seemed to be very similar to that proposed by Biserte17 for polymyxin B from his earlier studies, though later Biserte and Dautrevaux18, and Hausmann19, independently amended this structure. In 1957, in a preliminary communication, Dautrevaux and Biserte20 reported the amino acid composition of colistin as threonine: leucine: α,γ-diaminobutyric acid=1:1:4. These discrepancies led us to re-examine the chemical structure of colistin.

We first examined the amino acid sequence of colistin. Commercial colistin was fractionated into colistin's A, B, and C by countercurrent distribution, using a mixture of n-butanol: sec-butanol: 0.1 N HCl = 6: 30: 40 (v/v) as solvent (Fig. 2). The content of colistin C in the commercial colistin was usually too small to investigate.

Then the molecular weight of colistin A was determined21 by partial DNP-substitution22 and by spectrophotometric measurement23 of the picrate. The molecular weight of the pentahydrochloride of colistin A was found to be about 1,360.

For analysis of its constitution, purified colistin A was hydrolyzed with HCl and the hydrolyzate was evaporated in vacuo. The quantitative analysis of its amino

Fig. 1. Chemical structure of colistin previously proposed by K. Suzuki et al. in 1957.
Solvent system; n-BuOH: sec-BuOH: 0.1 N HCI = 6:3:0:4 Phase; 10 ml. upper, 10 ml. lower. Charge; 5 g. in tubes 0-12 transfers; 1,332

Fig. 2. Countercurrent distribution of commercial colistin.

Assay was carried out by the method of Yemm and Cocking\textsuperscript{53}, except that 0.05 ml. aliquots were taken for analysis and 3.0 ml. of buffer was used and total volume was 4.2 ml.

acid was carried out both by the ninhydrin method of Yemm and Cocking\textsuperscript{53}, and also with a Hitachi Automatic Aminoacid Analyzer. The data obtained are given in Table 2, which shows that the amino acid composition was found to be threonine: leucine: \(\alpha,\gamma\)-diaminobutyric acid = 1:1:3.

The fractions containing threonine and leucine were estimated microbiologically with \textit{Streptococcus faecalis} R for L-threonine\textsuperscript{54} and \textit{Leuconostoc mesenteroides} P-60 for L-leucine\textsuperscript{55}. From these experiments, it was demonstrated that 2 moles of threonine and 1 mole of leucine were present as the L-isomers. Furthermore, since using \(\text{D-}\)amino acid oxidase (\(\text{D-}\)amino acid : O\(_2\) oxidoreductase, EC 1.4.4.3) K. Suzuki\textsuperscript{57} had shown that colistin contains \(\text{D-}\)leucine, it was evident that 1 mole of leucine is in the \(\text{D-}\)configuration. The configuration of \(\alpha,\gamma\)-diaminobutyric acid was exam-

<table>
<thead>
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<th>Tube No.</th>
</tr>
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<tbody>
<tr>
<td>100</td>
</tr>
<tr>
<td>150</td>
</tr>
<tr>
<td>200</td>
</tr>
<tr>
<td>250</td>
</tr>
<tr>
<td>300</td>
</tr>
<tr>
<td>350</td>
</tr>
</tbody>
</table>

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Table 2. Amino acid analysis of colistin A.

<table>
<thead>
<tr>
<th></th>
<th>Thr Found ((\mu) moles)</th>
<th>Leu Found ((\mu) moles)</th>
<th>Dab Found ((\mu) moles)</th>
<th>Molar ratio (Thr: Leu: Dab)</th>
</tr>
</thead>
<tbody>
<tr>
<td>By ninhydrin method</td>
<td>2.44</td>
<td>2.64</td>
<td>7.78</td>
<td>0.92 : 1.0 : 2.94</td>
</tr>
<tr>
<td>By amino acid analyzer</td>
<td>0.62</td>
<td>0.67</td>
<td>2.13</td>
<td>0.93 : 1.0 : 3.15</td>
</tr>
</tbody>
</table>

Table 3. Physicochemical data on Dab-HCl preparation obtained from colistin A.

<table>
<thead>
<tr>
<th>mp°C</th>
<th>([\alpha]\text{D}(\text{Temp.}))</th>
<th>c</th>
<th>Solvent</th>
<th>Elemental analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C</td>
</tr>
<tr>
<td>229-230</td>
<td>+22.6(20.5)</td>
<td>2.17</td>
<td>5 (\text{N}) HCl</td>
<td>Calcd. 31.08 7.17 18.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Found 31.49 7.37 17.72</td>
</tr>
</tbody>
</table>

(262)
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Table 4. Amino acids and peptides from partial acid hydrolyzate of colistin A.

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Thr</th>
<th>Leu</th>
<th>Dab</th>
<th>L-Leu</th>
<th>N-terminal</th>
<th>C-terminal</th>
<th>Amino acid sequences</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Thr</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Leu</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>D-Leu→L-Leu</td>
</tr>
<tr>
<td>4a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Dab</td>
</tr>
<tr>
<td>4b</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Thr→(γ)Dab</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>MOA→(α)Dab</td>
</tr>
<tr>
<td>6</td>
<td>0.8</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Thr→(α)Dab</td>
</tr>
<tr>
<td>7</td>
<td>1.0</td>
<td>1.2</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td>L-Leu→(α)Dab</td>
</tr>
<tr>
<td>8</td>
<td>1.0</td>
<td>1.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Dab→D-Leu</td>
</tr>
<tr>
<td>9</td>
<td>2.0</td>
<td>1.0</td>
<td>1.2</td>
<td></td>
<td></td>
<td></td>
<td>D-Leu→L-Leu→(α)Dab</td>
</tr>
<tr>
<td>10</td>
<td>0.9</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Thr→(α)Dab→(α)Dab (γ)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Thr</td>
</tr>
<tr>
<td>11</td>
<td>0.9</td>
<td>2.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Thr→(γ)Dab→(α)Dab</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Dab)α</td>
</tr>
<tr>
<td>13</td>
<td>0.8</td>
<td>1.0</td>
<td>2.0</td>
<td></td>
<td></td>
<td></td>
<td>Thr→(γ)Dab→(α)Dab→D-Leu</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L-Leu→(α)Dab→(α)Dab</td>
</tr>
<tr>
<td>15</td>
<td>1.0</td>
<td>2.2</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td>Leu</td>
</tr>
</tbody>
</table>

Fig. 3. Chromatogram of partial acid hydrolyzate of colistin A (1.0 g.) on a column (1.8×180 cm.) of Dowex 50×2 (200 to 400 mesh).

Gradient elution with ammonium formate and ammonium acetate was carried out by inserting a mixing chamber (1,000 ml.) between the reservoir and the top of the column. The column was maintained at 38°C by circulating water through a jacket. The effluent was collected in 10 ml. fractions at a flow rate of 20 ml. per hour and 0.2 ml. aliquots of every other tube were subjected to analysis by the ninhydrin method.

The pH of effluent is indicated by the dotted line.

Ined by measurement of its optical rotation and the results are given in Table 3. The optical rotation of the isolated material agreed with that of the authentic L-isomer.
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The ester derivative of the fatty acid isolated from colistin A was prepared, and from it the fatty acid in colistin A was identified as 6-methyloctanoic acid by gas chromatography. The data obtained by elemental analysis of the amide derivative of the fatty acid were identical with the theoretical values. The 6-methyloctanoic acid extracted from a hydrolyzate of colistin A had an optical rotation of \( \left( \alpha \right)_{D,58}^{\theta} = +7.50 \) (c=3.57 in \( n \)-heptane), and was determined to be (+)-6-methyloctanoic acid. The molar ratio of colistin A was thus found to be L-threonine: L-leucine: D-leucine: L-\( \alpha,\gamma \)-diaminobutyric acid: (+)-6-methyloctanoic acid = 2 : 1 : 1 : 6 : 1.

Colistin A was then partially hydrolyzed with 6\( \text{N} \) HCl and the resulting peptides were separated by gradient column chromatography. The 15 fragments shown in Fig. 3 were isolated in apparently pure states as judged by paper chromatography and paper electrophoresis. The amino acid sequences of these peptides are given in Table 4. From the amino acid sequences of the five key peptides, the open chain nonapeptide shown in Table 5 was deduced. However, it was uncertain,

<table>
<thead>
<tr>
<th>Peak</th>
<th>Sequence</th>
</tr>
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<tbody>
<tr>
<td>5</td>
<td>MOA→L-(( \alpha ))Dab</td>
</tr>
<tr>
<td>11</td>
<td>L-Thr→L-(( \alpha ))Dab→L-(( \alpha ))Dab(( \gamma ))</td>
</tr>
<tr>
<td>13</td>
<td>L-(( \alpha ))Dab→L-(( \alpha ))Dab→D-Leu(( \gamma ))</td>
</tr>
<tr>
<td>3</td>
<td>D-Leu→L-Leu</td>
</tr>
<tr>
<td>15</td>
<td>MOA→L-(( \alpha ))Dab→L-Thr→L-(( \alpha ))Dab→L-(( \alpha ))Dab→D-Leu→L-Leu→L-(( \alpha ))Dab→L-(( \alpha ))Dab(( \gamma ))</td>
</tr>
</tbody>
</table>

Table 5. Key peptides utilized to deduce partial structure of colistin A.

Fig. 4. Two possible structures for colistin A.
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to which threonine residue in the nonapeptide the \( \text{MOA} \rightarrow (\alpha) \text{Dab} \) was bound. Thus, both the structures for colistin A shown in Fig. 4 fit the data obtained. The structure on the right of the figure consists of a large ring made up of eight amino acids and a tail ending with the \((\pm)-6\)-methyl octanoic acid. The junction between the ring and the tail is made by a completely covered residue of \(\alpha,\gamma\)-diaminobutyric acid. The other structure shown in the figure is very similar, the only difference being that one \(\alpha,\gamma\)-diaminobutyric acid residue is located in the tail instead of in the ring.

The main difficulty during partial acid hydrolysis is the random splitting of each of the peptide bonds. Moreover, the peptide bonds involving the amino group of threonine are very labile in mineral acid and no Thr-peptide in which this amino acid was not N-terminal could be found.

It was thought that enzymatic hydrolysis would be much more selective, so the enzymatic hydrolysis of colistin was investigated using three proteolytic enzymes, that is, Nagarse (\textit{Subtilopeptidase} A, EC 3.4.4.16), Pronase (\textit{Streptomyces} peptidase), and Proteinase (\textit{Carica papaya} peptidase). In this way, the peptide,

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Molar ratio of amino acid</th>
<th>N-terminal amino acid</th>
<th>DNP-amino acid</th>
<th>C-terminal amino acid</th>
<th>Fatty acid</th>
<th>Amino acid sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1*</td>
<td>0.9</td>
<td>1.0</td>
<td>7-DNP-Dab</td>
<td>Thr</td>
<td>MOA→Dab→Thr</td>
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</tr>
<tr>
<td>N2</td>
<td>1.0</td>
<td>2.0</td>
<td>7-DNP-Dab</td>
<td>Dab</td>
<td>MOA→Dab→Thr→Dab</td>
<td></td>
</tr>
<tr>
<td>N3</td>
<td></td>
<td></td>
<td>7-DNP-Dab</td>
<td>Dab</td>
<td>MOA→Dab→Thr→Dab</td>
<td></td>
</tr>
<tr>
<td>N4</td>
<td></td>
<td></td>
<td>α-DNP-Dab</td>
<td>-</td>
<td>MOA→Dab→Thr→Dab</td>
<td></td>
</tr>
<tr>
<td>N5</td>
<td>0.9</td>
<td>2.0</td>
<td>3.8</td>
<td>α-DNP-Dab</td>
<td>MOA→Dab→Thr→Dab</td>
<td></td>
</tr>
<tr>
<td>Pr1</td>
<td>0.9</td>
<td>2.0</td>
<td>7-DNP-Dab</td>
<td>Thr</td>
<td>MOA→Dab→Thr→Dab</td>
<td></td>
</tr>
<tr>
<td>Pr2</td>
<td></td>
<td></td>
<td>7-DNP-Dab</td>
<td>Dab</td>
<td>MOA→Dab→Thr→Dab</td>
<td></td>
</tr>
<tr>
<td>Pr3</td>
<td>1.7</td>
<td>2.0</td>
<td>4.7</td>
<td>Thr</td>
<td>MOA→Dab→Thr→Dab</td>
<td></td>
</tr>
<tr>
<td>Pr4</td>
<td></td>
<td></td>
<td>DNP-Thr</td>
<td>DNP-Dab</td>
<td>MOA→Dab→Thr→Dab</td>
<td></td>
</tr>
<tr>
<td>Pr5</td>
<td>0.8</td>
<td>2.0</td>
<td>3.8</td>
<td>DNP-Dab</td>
<td>MOA→Dab→Thr→Dab</td>
<td></td>
</tr>
<tr>
<td>Pt1</td>
<td>0.9</td>
<td>2.0</td>
<td>7-DNP-Dab</td>
<td>Dab</td>
<td>MOA→Dab→Thr→Dab</td>
<td></td>
</tr>
<tr>
<td>Pt2</td>
<td>1.6</td>
<td>2.0</td>
<td>5.6</td>
<td>7-DNP-Dab</td>
<td>MOA→Dab→Thr→Dab</td>
<td></td>
</tr>
<tr>
<td>Pt3</td>
<td>1.0</td>
<td>2.0</td>
<td>4.0</td>
<td>α-DNP-Dab</td>
<td>MOA→Dab→Thr→Dab</td>
<td></td>
</tr>
</tbody>
</table>

* Peptide P1, P2, P3, P4, and P5 were obtained from the hydrolyzate of colistin A with Nagarse and peptides Pr 1, Pr 2, Pr 3, Pr 4, and Pr 5 from the hydrolyzate with Pronase and Peptides Pt1, Pt2 and Pt3 from the hydrolyzate with \textit{Papaya} Proteinase, respectively.

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Kyozo HAYASHI, and Tomoji SUZUKI

which was essential for determination of the full structure of colistin A, namely MOA→Dab→Thr→Dab, was obtained from the Nagarse, Pronase, and Proteinase hydrolyzates of colistin A (Table 6). Thus using the partial acid hydrolysis method in combination with the enzymatic hydrolysis method the chemical structure of colistin A was concluded to be that shown in Fig. 4-(I)\textsuperscript{41,42}. The synthetic product with the structure proposed by the authors was prepared by Vogler\textsuperscript{43} and was found to be identical with natural colistin A.

**Chemical Structure of Colistin B**

Acid and enzymatic hydrolyzates of colistin B gave almost the same spots by paper chromatography and paper electrophoresis as those obtained from colistin A, so that it was considered that the structure of colistin B might be the same as that of colistin A, except for having isooctanoic acid instead of (+)-6-methyloctanoic acid. This was supported by an experiment in which pure deacylated colistin, giving a single peak in countercurrent distribution, was obtained from commercial colistin with an 78\% yield using the acylase\textsuperscript{43} of a Pseudomonas soil bacterium, isolated by our group. The same fragments as those obtained with colistin A were obtained from enzymatic and partial acid hydrolyzates of colistin B, except that (+)-6-methyloctanoic acid was replaced by isooctanoic acid. Thus the structure of this antibiotic was determined\textsuperscript{44} to be that shown in Fig. 5.

![Diagram of colistin B structure](image)

**Identity of Polymyxin E\textsubscript{1} with Colistin A and of Polymyxin E\textsubscript{2} with Colistin B**

While this study was in progress, Dautrevaux and Biserte\textsuperscript{45} reported that the composition of colistin was threonine : leucine : a,\gamma-diaminobutyric acid = 2 : 2 : 6. These workers used colimycin\textsuperscript{*} produced by Kagaku Antibiotic Research Co. Ltd., Japan. They were unable to confirm either the amino acid composition or the structure proposed by K. Suzuki\textsuperscript{46} On the contrary, they found that the amino acid sequences of the products isolated by partial hydrolysis of colimycin and its penta-DNP-derivative were indicative of one of the structures shown in Fig. 6.

In 1963, on the basis of studies on the amino acid compositions, elementary analyses, infrared spectra, and the amino acid sequences of the peptides of a partial

Chemical Structures of Polymyxin Series Antibiotics

\[
\begin{align*}
\text{(γ)NH}_2 \\
\text{Dab} & \rightarrow \text{Thr} \\
\end{align*}
\]

MOA → Dab → D-Leu → Leu → Dab → Dab → (γ)Dab

\[
\begin{align*}
\text{(γ)NH}_2 \\
\text{[Thr→Dab]} \\
\text{(γ)NH}_2 \\
\end{align*}
\]

Fig. 6. Chemical structure of colimycin (polymyxin E) proposed by Biserte et al., and Wilkinson previously. The amino acid sequence bracketed in the formula has not been decided.

acid hydrolyzate of polymyxin E, Wilkinson reported that polymyxin E was indistinguishable from colimycin, and deduced structures for them identical to those proposed by Dautrevaux and Biserte (Fig. 6).

As described above, the present authors demonstrated that the structures of colistin's A and B were those shown in Figs. 4–(I) and 5, respectively. In this connection, the authors re-examined the chemical structure of polymyxin E produced by Burroughs Wellcome & Co., Ltd. After fractionating it into polymyxin's E₁ and E₂ by countercurrent distribution, enzymatic studies were carried out on its structure in the same way as for colistin A. The results obtained showed that polymyxin's E₁ and E₂ are identical with colistin's A and B, respectively. Furthermore, after fractionation of colimycin M (Kagaku Antibiotics Research Co., Ltd., Japan) into colimycin's A and B by countercurrent distribution, studies were made on its structure in the same way as those described for polymyxin E. The results obtained showed that colimycin's A and B are identical with colistin's A and B, respectively. On the other hand, referring to our reports on colistin and polymyxin E, Wilkinson re-examined the structures of polymyxin E₁ and E₂, and arrived to the same results as those obtained by us.

**The Chemical Structure of Polymyxin's B₁ and B₂**

In 1956 a tentative structure (Fig. 7) for polymyxin B was proposed by Biserte and Dautrevaux, from analysis of the peptides obtained from it by partial acid hydrolysis. Subsequently, Hausmann and Craig, Hausmann, and Biserte and Dautrevaux concluded that crude polymyxin B could be separated into polymyxin's B₁ and B₂ by countercurrent distribution and that possibilities for the structure of polymyxin B₁ were limited to one of the four formulae, 8α, 8γ, 7α and 7γ shown in Fig. 8. With regard to the configuration of the α,γ-diaminobutyric acid present in polymyxin B₁, Hausmann reported that one of the six moles of α,γ-diaminobutyric acid in polymyxin B₁ might be the d-isomer, because of the value for the

\[
\begin{align*}
\text{NH}_2(\gamma) \\
\text{Thr} \rightarrow \text{Phe} \rightarrow \text{Dab} \rightarrow \text{Leu} \\
\text{(γ)HN-Dab} \\
\text{Dab-NH}_2(\gamma) \\
\text{(γ)HN-Dab} \leftarrow \text{D-Dab} \leftarrow \text{Thr} \\
\text{(γ)NH}_2 \\
\text{Fatty Acid} \\
\end{align*}
\]

Fig. 7. Chemical structure of polymyxin B proposed by Biserte and Dautrevaux in their earlier work.

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specific rotation of the $\alpha,\gamma$-diaminobutyric acid monohydrochloride isolated from polymyxin B. Later, Biserte and Dautrevaux$^{46}$ hydrolyzed polymyxin B with acid and obtained MOA->($\alpha$)Dab, and from this fragment isolated $\alpha,\gamma$-diaminobutyric acid which they oxidized with $\alpha$-amino acid oxidase from hog kidney. From the result obtained they proposed that the $\alpha$-$\alpha,\gamma$-diaminobutyric acid in polymyxin B, was situated in the position adjacent to the fatty acid. Subsequently, Vogler et al. attempted to deduce the proper constitution of polymyxin B, by total synthesis$^{66-66}$. They prepared the four compounds shown in Fig. 8 in which only the mole of $\alpha,\gamma$-diaminobutyric acid attached to (+)-6-methyloctanoic acid was the $\alpha$-isomer. Among these synthetic peptides, $8\alpha$ and $8\gamma$ were found to have lower antimicrobial activities than the natural product,$^{47}$ and $7\alpha$ and $7\gamma$, although they had the same antimicrobial activity with that of natural polymyxin B, had lower optical rotations than polymyxin B,$^{66,66}$. Since colistin A, which has a very similar structure to polymyxin B, contained no $\alpha$-$\alpha,\gamma$-diaminobutyric acid, the presence of the $\alpha$-isomer of $\alpha,\gamma$-diaminobutyric acid in polymyxin B, became doubtful. To elucidate this point, all the preparations of $\alpha,\gamma$-diaminobutyric acid obtained from each peptide and from intact polymyxin B, were examined with a Rudolph Automatically Recording Spectropolarimeter. The specific optical rotations and the optical rotatory dispersion curves of all the $\alpha,\gamma$-diaminobutyric acid preparations obtained from all the peptides agreed with that of authentic $L-\alpha,\gamma$-diaminobutyric acid monohydrochloride$^{43}$. As
shown in Fig. 9, the application of infrared spectrophotometry to the examination of the configuration was also useful. The spectra of all the \( \alpha,\gamma \)-diaminobutyric acid monohydrochloride preparations from all the peptides and intact polymyxin B₁ were identical with that of authentic \( L-\alpha,\gamma \)-diaminobutyric acid monohydrochloride. \( \alpha \)-Amino acid oxidase was applied to all these \( \alpha,\gamma \)-diaminobutyric acid preparations but no oxygen consumption was observed. These results show that all the \( \alpha,\gamma \)-diaminobutyric acid present in polymyxin B₁ is in the \( L \)-form.

The configurations of the other constituent amino acids in polymyxin B₁ were also examined microbiologically or manometrically, and the results agreed with those given by Hausmann⁶⁵.

On enzymatic hydrolysis of polymyxin B₁ with Nagarse, two straight chain
Table 7. Peptides from enzymatic hydrolysis of polymyxin B1 by Nagarse.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Yield</th>
<th>Molar ratio of amino acid</th>
<th>MOA</th>
<th>DNP-amino acid</th>
<th>C-terminal amino acid</th>
<th>Amino acid sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>P 1</td>
<td>9</td>
<td>1.1</td>
<td>1.0</td>
<td>γ-DNP-Dab</td>
<td>Thr</td>
<td>MOA→(α)L-Dab→L-Thr</td>
</tr>
<tr>
<td>P 2</td>
<td>15</td>
<td>1.0</td>
<td>2.0</td>
<td>γ-DNP-Dab</td>
<td>Dab</td>
<td>MOA→(α)L-Dab→L-Thr→</td>
</tr>
<tr>
<td>P 3</td>
<td>93</td>
<td>1.9 (1.7)</td>
<td>0.9 (1.0)</td>
<td>5.6</td>
<td>γ-DNP-Dab</td>
<td>unreacted polymyxin B1</td>
</tr>
<tr>
<td>P 4</td>
<td>48</td>
<td>0.9 (1.1)</td>
<td>1.0 (1.0)</td>
<td>0.9 (1.1)</td>
<td>α-DNP-Dab</td>
<td>γ-DNP-Dab</td>
</tr>
</tbody>
</table>

1) The molar ratio of amino acids was determined by the ninhydrin method in combination with ion exchange column chromatography. The configurations of leucine and threonine were determined microbiologically and that of phenylalanine manometrically using D-amino acid oxidase, and the values in parenthesis were determined by these methods. The values of α,γ-diaminobutyric acid were taken as 1.0 to express the data for P1 and P2 and those of leucine were taken as 1.0 for P3 and P4.

peptides, MOA→(α)L-Dab→L-Thr and MOA→(α)L-Dab→L-Thr→(α)L-Dab and a cyclic peptide, cyclo-(γ)L-Dab→(α)L-Dab→D-Phe→L-Leu→(α)L-Dab→(α)L-Dab→L-Thr→, were obtained (Table 7). From the sequences of these peptides, it was concluded that the side chain of polymyxin B1 is linked to the α-amino group of one residue of α,γ-diaminobutyric acid in the cyclic peptide portion and that the γ-amino group of the residue is involved in the ring formation. The structure was thus established to be the 7α type (Fig. 10).

Subsequently, Vogler et al. have synthesized the compound with the structure proposed by the authors, and by thin-layer chromatography, amino acid analysis and measurement of its specific rotation, its optical rotatory dispersion of the nickel-complex and the microbiological activity, the synthetic product was found to be identical with natural polymyxin B1.

In 1964, from experiments on the incorporation of radioactive D-α,γ-diaminobutyric acid into polymyxin B1, Pauls and Gray reported that D-isomer is more efficiently incorporated into the position adjacent to (+)-6-methyloctanoic acid than its enantiomorph.
However, after the authors' report on the structure of polymyxin B, Pauls undertook to re-examine the configuration of the terminal \(\alpha,\gamma\)-diaminobutyric acid residue in polymyxin B, using an amino acid activating enzyme which is specific for \(\text{L-}\alpha,\gamma\text{-diaminobutyric acid}.\) From the result, he concluded that the \(\text{L-}\alpha,\gamma\text{-diaminobutyric acid}\) connected to \((+)-6\text{-methyl octanoic acid}\) has the \textit{levor} configuration. Thus the structure of polymyxin B, is now established unequivocally.

By similar experiments on polymyxin B, it was established that polymyxin B, has a similar structure to polymyxin B, except that \((+)-6\text{-methyl octanoic acid}\) is replaced by iso-octanoic acid (Fig. 11).\(^{290}\)

\begin{center}
\begin{tikzpicture}
\node (A) at (0,0) {L-Leu \rightarrow L-Dab-NH$_2$(\(\gamma\))};
\node (B) at (1,0) {D-Phe \rightarrow L-Dab-NH$_2$(\(\gamma\))};
\node (C) at (2,0) {L-(\(\gamma\))H$_2$N-Dab \rightarrow L-Thr (\(\gamma\))};
\node (D) at (3,0) {L-Dab (\(\alpha\)) \rightarrow L-Dab-NH$_2$(\(\gamma\))};
\node (E) at (4,0) {L-Thr \rightarrow L-Dab-NH$_2$(\(\gamma\))};
\node (F) at (5,0) {I0A};
\end{tikzpicture}
\end{center}

\textit{Fig. 11. Concluded structure of polymyxin B.}

\textbf{The Chemical Structure of Circulin A}

Peterson and Reineke,\(^{289}\) and Dowling \textit{et al.}\(^{199}\) separated circulin into two main components, namely, circulin A and circulin B. Wilkinson\(^{299}\) reported that purified circulin A has the same composition as polymyxin A and polymyxin E, containing L-threonine, D-leucine, L-\(\alpha,\gamma\)-diaminobutyric acid, and \((+)-6\text{-methyl octanoic acid}\) in the molar ratio of 1:1:5:1. Subsequently, Koffler\(^{499}\) reported that circulin A contains L-threonine, D-leucine, L-isoleucine, L-\(\alpha,\gamma\)-diaminobutyric acid, and \((+)-6\text{-methyl octanoic acid}\) in the molar proportions of 2:1:1:6:1, and from quantitative analysis of the fragments obtained from a partial acid hydrolyzate, Koffler and Kobayashi tentatively deduced the chemical structure of circulin A\(^{299}\) as that of the cyclic decapeptide shown in Fig. 12.

This cyclic structure with no side chain seemed to be rather strange because of the close biological and chemical relationships between colistin A and circulin A. The amino acid composition of circulin closely resembles that of colistin and so it was of interest to re-examine the chemical structure of circulin. \textit{Bacillus circulans} ATCC 14040 was grown in flasks in which the medium was aerated and

\begin{center}
\begin{tikzpicture}
\node (A) at (0,0) {NH$_2$(\(\gamma\)) \rightarrow Dab \rightarrow Thr \rightarrow Dab \rightarrow Thr \rightarrow Dab \rightarrow D-Leu \rightarrow Ile};
\node (B) at (1,0) {\(\gamma\)-NH \rightarrow Dab \rightarrow Dab \rightarrow Dab \rightarrow Dab \rightarrow D-Leu \rightarrow Ile};
\node (C) at (2,0) {NH$_2$(\(\gamma\)) \rightarrow Dab \rightarrow Dab \rightarrow Dab \rightarrow Dab \rightarrow D-Leu \rightarrow Ile};
\node (D) at (3,0) {NH$_2$(\(\gamma\)) \rightarrow Dab \rightarrow Dab \rightarrow Dab \rightarrow Dab \rightarrow D-Leu \rightarrow Ile};
\node (E) at (4,0) {NH$_2$(\(\gamma\)) \rightarrow Dab \rightarrow Dab \rightarrow Dab \rightarrow Dab \rightarrow D-Leu \rightarrow Ile};
\node (F) at (5,0) {NH$_2$(\(\gamma\)) \rightarrow Dab \rightarrow Dab \rightarrow Dab \rightarrow Dab \rightarrow D-Leu \rightarrow Ile};
\end{tikzpicture}
\end{center}

\textit{Fig. 12. Chemical structure of circulin A previously proposed by Koffler and Kobayashi in 1958.}

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agitated as described by Nelson et al

Crude circulin was obtained from the culture fluid using a column of Amberlite IRC-50 (H+ form). It was then fractionated by countercurrent distribution using a mixture of n-butanol : sec-butanol : 2N acetic acid = 2:1:5 (v/v) as solvent, and the peaks were obtained by the method described for colistin. Using Nagarse the following fragments were obtained from circulin A: (i) MOA → (α)Dab → Thr (ii) MOA → (α)Dab → Thr → (α)Dab (iii) Dab (iv) a cyclic peptide with the molar ratio of threonine: leucine: isoleucine: α,γ-diaminobutyric acid = 1:1:1:4. In the hydrolyzate of the DNP derivative of the cyclic peptide, α-DNP-Dab was found, though it could not be found in completely dinitrophenylated circulin A. From the above results, the structure of circulin A was deduced to be MOA → (α)Dab → Thr → (α)Dab → (α) cyclo-heptapeptide. To elucidate the full structure of circulin A, it was partially hydrolyzed with 6N HCl, and the amino acid sequences of fourteen fragments in the hydrolyzate were analyzed by the usual method. Among these, five key peptides were used to deduce the open chain nonapeptide as shown in Table 8. Referring to the results of the enzymatic hydrolysis described above, the chemical structure of circulin A was established to be as shown in Fig. 13. Thus it was confirmed that circulin A belongs to the polymyxin family.

| Key peptide 1 | MOA → L-(α)Dab |
| Key peptide 2 | L-Thr → L-(α)Dab → L-(α)Dab (γ) |
| Key peptide 3 | L-(α)Dab → L-(α)Dab → D-Leu |
| Key peptide 4 | D-Leu → L-Ile |
| Key peptide 5 | D-Leu → L-Ile → L(α)Dab → L(α)Dab |
| Deduced partial structure | MOA → L-(α)Dab → L-Thr → L-(α)Dab → L-(α)Dab → L-(α)Dab → D-Leu → L-Ile → L-Thr |

Fig. 13. Concluded structure of circulin A.
Chemical Structure of Polymyxin D

In 1949, Bell et al.\(^1\) reported that polymyxin D is a basic polypeptide containing L-threonine, D-leucine, L-\(\alpha,\gamma\)-diaminobutyric acid, D-serine and a C\(_9\) fatty acid = 3:1:5:1:1 and has a deca-cyclic peptide structure. The authors fractionated crude polymyxin D obtained from the culture broth of Bacillus polymyxa ATCC 10401, by countercurrent distribution, and named the main biologically active entity polymyxin D\(_1\). By analysis of its composition, it was found that upon total hydrolysis of polymyxin D\(_1\), six moles of predominantly the L-form of \(\alpha,\gamma\)-diaminobutyric acid, three moles of L-threonine, one mole of D-serine and one mole of D-leucine, were liberated. This result agrees with that of Bell et al. Furthermore, the fatty acid obtained from the hydrolyzate was identified as (\(+\))-6-methyloctanoic acid by gas chromatography. Purified polymyxin D\(_1\) was then partially hydrolyzed with 6 N HCl and the resulting hydrolyzate was fractionated by gradient column chromatography. Sequential analysis was applied to the nineteen peptides and amino acids separated from the hydrolyzate. From the key peptides in acid hydrolyzate, four possible structures were deduced, and the proper structure was deduced by N\(\rightarrow\)O rearrangement and enzymatic hydrolysis\(^*\).

Chemical Structure of Polymyxin M

It has been indicated that the chemical composition of Polymyxin M is not identical with those of other known polymyxins. Chromatographic analysis of a hydrolyzate of this substance showed that it contained threonine, leucine, \(\alpha,\gamma\)-diaminobutyric acid and a fatty acid which was probably similar or identical with (\(+\))-6-methyloctanoic acid. Later, it was reported that the amino acid composition of polymyxin M was threonine : leucine : \(\alpha,\gamma\)-diaminobutyric acid = 3:1:6,75-77 but the configurations of the constituent amino acids have not yet been established. From terminal analysis, it is known that, since this antibiotic has no free \(\alpha\)-amino or carboxyl group, it must have a cyclic peptide structure\(^78-80\). Work on the chemical structure of polymyxin M is in progress in Moscow.

Relationship between the Chemical Structures of Polymyxins

Colistin A has a similar structure to polymyxin B\(_1\) and circulin A, and colistin A only differs from polymyxin B\(_1\) in the presence of D-leucine in place of D-phenylalanine and from circulin A in the presence of L-leucine in place of L-isoleucine. Colistin's A and B\(_1\) and Polymyxin's B\(_1\) and B\(_2\) differ from each other only in the nature of their fatty acid component, namely, colistin A and polymyxin B\(_1\) contain (\(+\))-6-methyloctanoic acid while colistin B and polymyxin B\(_2\) contain (\(+\))-6-methyloctanoic acid while colistin B and polymyxin B\(_2\) contain iso-octanoic acid. The comparison of the structures of colistin, polymyxin B and circulin were shown in Fig. 14 in which the different points were emphasized with thick letters.

No report has been published on the chemical structures of polymyxin A and C since the reports on polymyxin A of Catch et al.\(^2\) and on polymyxin C of Jones\(^3\) in 1949. Therefore, in addition to the studies on polymyxin D and M, the studies on polymyxin A and C should be undertaken with the pure substances in future. Polymyxin D contains D-leucine and D-serine, which is not found in other polymyxin series antibiotics, and is characterized by containing two kinds of D-amino acid.

\(^*\) This report will be published in J. Biochem in near future.
Polymyxin M contains three moles of threonine and one mole of leucine, and it may be speculated that polymyxin M differs from colistin in the presence of threonine in place of leucine.

Table 9. Comparative antibiotic potencies of polymyxins.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>units per mg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymyxin B₁</td>
<td>18,780</td>
</tr>
<tr>
<td>Polymyxin B₂</td>
<td>17,480</td>
</tr>
<tr>
<td>Polymyxin D₁</td>
<td>16,140</td>
</tr>
<tr>
<td>Polymyxin E₁</td>
<td>19,770</td>
</tr>
<tr>
<td>Polymyxin E₂</td>
<td>19,200</td>
</tr>
<tr>
<td>Colistin A</td>
<td>20,550</td>
</tr>
<tr>
<td>Colistin B</td>
<td>18,180</td>
</tr>
<tr>
<td>Circulin A</td>
<td>22,830</td>
</tr>
</tbody>
</table>

A unit is that defined in the "Official Assay Methods of Antibiotic Preparations" issued by the Department of Public Welfare, Japan. (274)
Comparative Antibiotic Potencies of Polymyxins, Colistins and Circulin

Table 9 gives the relative potencies of what are believed to be pure preparations of the natural compounds, based on the standard assay method for colistin. It is interesting that the synthetic polymyxin B₁ analogues having $7\alpha$ and $7\gamma$ structures shown in Fig. 8, have the same antibiotic activities as that of natural polymyxin B₁.

Speculation on Fatty Acid Biosynthesis in Polymyxins

As mentioned above, (+)-6-methyloctanoic acid and isooctanoic acid are usually isolated from polymyxin series antibiotics. It is well known that four final steps in the synthesis of valine and isoleucine are catalyzed by common enzymes via the corresponded keto acids, $\alpha$-keto isovaleric acid and $\alpha$-keto-$\beta$-methylvaleric acid. From these facts, it is probable that (+)-6-methyloctanoic acid and isooctanoic acid are synthesized by the successive condensation of malonyl CoA with the decarboxylated products of the keto acids (Fig. 15). The fact that, the configuration of the asymmetric carbon in (+)-6-methyloctanoic acid is identical with that of the $\beta$-carbon of L-isoleucine, also supports the above speculation.

Fig. 15. Suggested biosynthetic pathway of fatty acid formed in polymyxin series antibiotics.

Biosynthetic Pathway of D-Amino Acids in Bacteria

As can be seen in Table 1, polymyxin series antibiotics, like other some antibiotics, contain one or two moles of D-amino acid.

In 1955, Thorne et al. have reported that D-glutamic acid is synthesized biologically by a transamination reaction between $\alpha$-ketoglutaric acid and D-alanine which is obtained by racemization of L-alanine. Later, Kuramitsu and Snoke reported that D-ornithine, D-aspartic acid, D-asparagine and D-phenylalanine are formed by transamination of the appropriate $\alpha$-keto acids with D-glutamic acid.

Using a cell-free extract from Bacillus brevis Nagano, Kurahashi et al. demonstrated recently that L-phenylalanine is converted to D-phenylalanine, and that ATP or ADP is essential for this conversion.

The biosynthesis of polymyxin series antibiotics and the formation of D-amino acids in Bacillus polymyxa still require intensive investigation.

REFERENCES

(37) K. Suzuki, Tohoku Yakka Daigaku Kiyo, (Japan), 4, 117 (1957).
Chemical Structures of Polymyxin Series Antibiotics

(56) K. Vogler, personal communication.
(57) Application for Japanese Patent is pending.
(71) H. Pauls, private communication.