

the complex sequence of thermally-induced rearrangements in $4\text{CaO}\cdot\text{Al}_2\text{O}_3\cdot 13\text{H}_2\text{O}$ which were reported by Buttler, Dent-Glasser and Taylor⁷.

It has been determined that a reaction somewhat similar to that described above takes place between wetted mixtures of hydrous alumina and magnesium hydroxide. In this case the reaction is not rapid, the X-ray diffraction peaks for the new phase becoming evident only after several hours and slowly increasing in intensity over a period of several days. This product is apparently a double hydroxide of magnesium and aluminium. The X-ray pattern observed (Fig. 2) is very nearly identical with that given by Feitknecht and Gerber⁸. The present product yields a (001) spacing of 8.2 Å, which is slightly higher than the 7.9 Å spacing reported by these authors and the 7.95 Å and 7.63 Å spacings reported for different magnesium-aluminium double hydroxide preparations by Mortland and Gastuche⁹.

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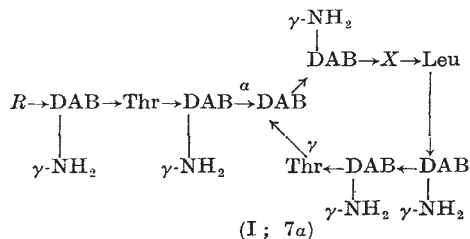
SIDNEY DIAMOND

U.S. Department of Commerce,
Bureau of Public Roads,
Washington, D.C.

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Structures of Polymyxin B2 and Polymyxin E1

FROM the degradative investigations of Suzuki *et al.*¹, and finally by its synthesis², the structure of polymyxin B1 has now been established as (I): ($R = (+)$ -6-methylheptanoyl, $X = \text{D-Phe}$), in which each of the six $\alpha\gamma$ -diaminobutyric acid units is of the L-configuration^{1,3}.



Suzuki *et al.*⁴ have also shown that the structure of colistin A is identical with that of polymyxin B1, except that the D-Phe in the latter is replaced by D-Leu (that is, 1; $R = (+)$ -6-methyloctanoyl, $X = \text{D-Leu}$).

From the partial acid hydrolysate of polymyxin B2 by fractionation on 'Dowex 50', using gradient elution with ammonium formate-ammonium acetate buffers, we have now isolated and identified the following peptides:

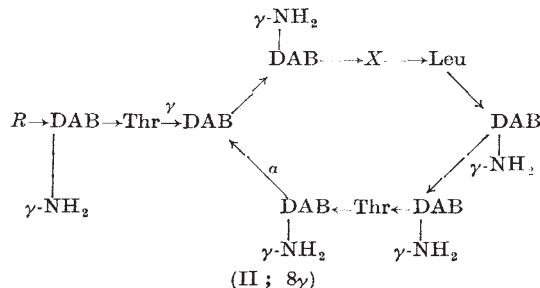
- 6-methylheptanoyl $\xrightarrow{\alpha}$ L-DAB; Thr $\xrightarrow{\gamma}$ DAB; Thr $\xrightarrow{\alpha}$ DAB;
- D-Phe \rightarrow L-Leu; Leu $\xrightarrow{\alpha}$ DAB; DAB \rightarrow Phe;
- Thr $\xrightarrow{\alpha}$ DAB \rightarrow DAB $\xrightarrow{\gamma}$ Thr; D-Phe \rightarrow Leu $\xrightarrow{\alpha}$ DAB;
- Thr $\xrightarrow{\gamma}$ DAB $\xrightarrow{\alpha}$ DAB; DAB \rightarrow Phe \rightarrow Leu; DAB $\xrightarrow{\alpha}$ (DAB)_n;
- Thr $\xrightarrow{\gamma}$ DAB $\xrightarrow{\alpha}$ DAB \rightarrow Phe and Leu $\xrightarrow{\alpha}$ DAB $\xrightarrow{\alpha}$ DAB

With the exception of the first of these peptides, which is replaced by (+)-6-methyloctanoyl $\xrightarrow{\alpha}$ DAB, they are identical with those obtained from a comparable hydrolysate of polymyxin B1.

We have also re-examined more thoroughly the partial acid hydrolysate of polymyxin E1 and by the procedure outlined above have isolated and characterized the following peptides:

- D-Leu \rightarrow L-Leu; (+)-6-methyloctanoyl $\xrightarrow{\alpha}$ L-DAB; Thr $\xrightarrow{\gamma}$ DAB;
 - Thr $\xrightarrow{\alpha}$ DAB; L-Leu $\xrightarrow{\alpha}$ DAB; Leu \rightarrow Leu $\xrightarrow{\alpha}$ DAB;
 - DAB $\xrightarrow{\alpha}$ DAB \rightarrow Leu \rightarrow Leu; DAB \rightarrow Leu \rightarrow Leu;
 - Thr $\xrightarrow{\alpha}$ DAB \rightarrow DAB $\xleftarrow{\gamma}$ Thr; Thr $\xrightarrow{\gamma}$ DAB $\xrightarrow{\alpha}$ DAB \rightarrow D-Leu;
 - Thr $\xrightarrow{\gamma}$ DAB $\xrightarrow{\alpha}$ DAB; Thr $\xrightarrow{\gamma}$ DAB $\xrightarrow{\alpha}$ DAB \rightarrow Leu \rightarrow Leu;
 - DAB $\xrightarrow{\alpha}$ (DAB)_n; DAB $\xrightarrow{\alpha}$ DAB \rightarrow Leu; Thr \rightarrow DAB \rightarrow DAB
- $$\begin{array}{c}
 \uparrow \\
 \text{DAB} \leftarrow \text{Thr} \\
 \uparrow \\
 \text{Thr} \rightarrow \text{DAB} \rightarrow \text{DAB} \rightarrow \text{Leu} \rightarrow \text{Leu}; \text{Leu} \xrightarrow{\alpha} \text{DAB} \xrightarrow{\alpha} \text{DAB}
 \end{array}$$
- and Leu \rightarrow Leu $\xrightarrow{\alpha}$ DAB $\xrightarrow{\alpha}$ DAB

The re-assembling of each of these two series of peptides in turn indicates that the structure of polymyxin B2 is restricted to either (I) or (II) ($R = 6$ -methylheptanoyl and $X = \text{D-Phe}$) and that of polymyxin E1 to either (I) or (II) ($R = (+)$ -6-methyloctanoyl and $X = \text{D-Leu}$).



In the determinations of the unequivocal structures of both polymyxin B1 and colistin A by Suzuki *et al.*^{1,4}, the differentiation between formulae (I) and (II) was not forthcoming from the products of partial acid hydrolyses but in each case only by the isolation of the peptide $R \xrightarrow{\alpha}$ DAB \rightarrow Thr $\xrightarrow{\alpha}$ DAB by enzymatic hydrolysis. The close relationship between the physical properties of polymyxins B1 and B2 and their identities in the case of polymyxin E and colistin have already been described⁵ so that fundamental differences between the structures of these pairs of antibiotics would not be expected, particularly taking into consideration, for example, the difference in optical rotation between the 7 α and 8 γ -type peptides synthesized by Vogler *et al.*⁶. It is intended to subject polymyxin E1 and polymyxin B2 to enzymatic degradation to confirm the correctness of structure (I).

S. WILKINSON
L. A. LOWE

Wellcome Research Laboratories,
Beckenham, Kent.

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