Synthesis of a Decapeptide from the Polymyxin Series

During the course of structural studies with the group of polypeptide antibiotics known as polymyxins, HAUSMANN and CRAIG[1] were able to separate a product of polymyxin B into two fractions B_1 and B_2, which differed only in the nature of the fatty acid component. According to their degradation studies, HAUSMANN[2] and later BISERTE and DAUTREVAUX[3] independently proposed for polymyxin B_1 two alternative structures A et B (Fig. 1)[4] with the only difference that one L-Dab residue was located in the side chain instead of in the ring.

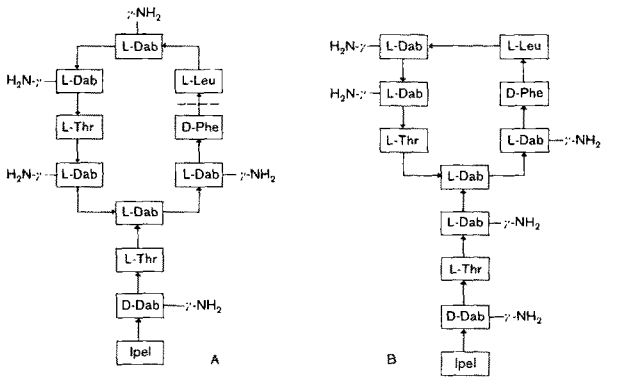

Fig. 1

On Dr. CRAIG's suggestion we are trying to assign the proper constitution to polymyxin B_1 by total synthesis. For this purpose we chose quite arbitrarily two years ago structure A assuming γ-junction between ring and side chain of the completely covered L-Dab residue. In the meantime we performed the synthesis of a decapeptide with the probable structure A in the following manner.

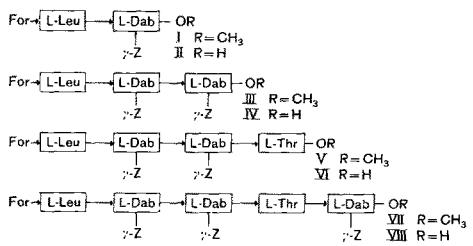

Fig. 2

Preliminary studies showed that the open branched decapeptide XXI (Fig. 4) corresponding to structure A (the ring being opened as indicated by the dotted line in Fig. 1), might be a useful intermediate for its synthesis. XXI was built up from the two protected pentapeptides VIII (Fig. 2) and XX (Fig. 3) exclusively by the carbodiimide procedure[5] preferably in dimethylformamide as a solvent at low temperature[6]. Ester hydrolysis was achieved by one mole of aqueous alkali in dioxane or dimethyl sulfoxide.

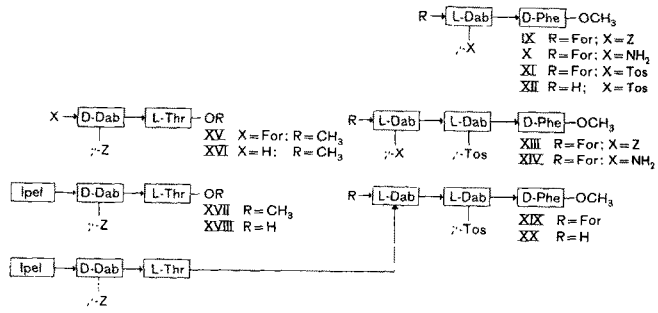

Fig. 3

As protecting groups we needed three residues, which can be removed selectively without interfering with each other. These protecting groups are represented by formyl[7] benzyloxycarbonyl[8], and *p*-toluenesulfonyl[9]. The intermediate formyl-L-leucine[10] has been known for a long time and the N^{γ}-benzyloxycarbonyl-α,γ-L-diaminobutyric acid methylester (m.p. 164–166°C) was obtained via the corresponding acid[11] in the usual manner. The L-threonine methylester (free base) has been synthesized in crystalline form (m.p. 70–71°C). D-Phenylalanine methylester (free base) was obtained from the hydrochloride[12] by using the chloroform/NH_3 procedure of HILLMANN[13], whereas N^{α}-formyl-N^{γ}-benzyloxycarbonyl-α, γ-L-diaminobutyric acid (m.p. 98–100°C) was synthesized from the unformylated precursor. (+)-6-Methyloctanoic acid[14] (Ipel) was synthesized according to a new method[15].

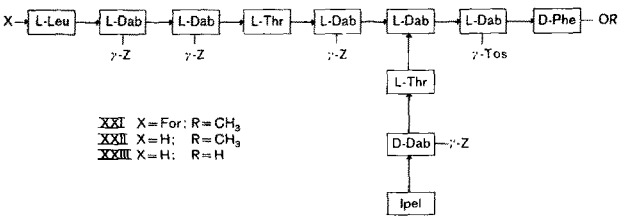

Fig. 4

The other intermediates in Fig. 2, 3, and 4 are new and are characterized in the following Table by their physical constants.

XXI (Fig. 4) after deformylation[7] led to XXII and subsequent hydrolysis to XXIII. Cyclization by dicyclohexylcarbodiimide[5] in dioxane/dimethylformamide gave

[1] W. HAUSMANN and L. C. CRAIG, J. Amer. chem. Soc. *76*, 4892 (1954).

[2] W. HAUSMANN, J. Amer. chem. Soc. *78*, 3663 (1956).

[3] G. BISERTE and M. DAUTREVAUX, Bull. Soc. Chim. biol. *39*, 795 (1957).

[4] Abbreviations for amino acids in this and the following figures have been made in the usual manner according to E. BRAND and J. T. EDSALL, Ann. Rev. Biochem. *16*, 224 (1947); Z = benzyloxycarbonyl, Tos = tosyl and For = formyl, Ipel = isopelargonic acid ((+)-6-methyloctanoic acid); → = C to N bond in –CONH–.

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[7] A. HILLMANN and G. HILLMANN, Z. Naturf. *6b*, 340 (1951). – G. VALEY, Chem. & Ind. *72*, 107 (1953). In the meantime the ability of the formyl-group for peptide synthesis could be demonstrated independently from us by J. C. SHEEHAN and DING DJUNG H. YANG, J. Amer. chem. Soc. *80*, 1154 (1958).

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[10] E. FISCHER and O. WARBURG, Ber. dtsch. chem. Ges. *38*, 4000 (1901).

[11] M. ZAORAL, J. RUDINGER, and F. ŠORM, Chem. Listy *47*, 427 (1953); Chem. Abstr. *49*, 179 (1953).

[12] B. F. ERLANGER, H. SACHS, and E. BRAND, J. Amer. chem. Soc. *76*, 1806 (1954).

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[14] L. CROMBIE and ST. H. HARPER, J. Chem. Soc. *1950*, 2685.

[15] To be published.

Table

Product	Crystallized or precipitated from	Melting points uncorrected	$[\alpha]_D^{21} \pm 2°$ (in dimethylformamide)	ε at 257 mμ
I	Acetic acid ester	130–131°	– 38,1°	
II	Alcohol/ether	167–168°	– 33,4°	
III	Alcohol	189–190°	– 35,2°	
IV	Methanol	203–205°	– 31,1°	
V	Methanol/ether	227–228°	– 26,1°	
VI	Methanol	211–212°	– 23,6°	
VII	Dimethylformamide/acetic acid ester	205–207°	– 32,8°	
VIII	Methanol/ether	198–200°	– 26,9°	640 (acetic acid)
IX	Alcohol	149–150°	+ 5,8°	
XI	Alcohol	147–148°	+ 4,7°	612 (alcohol)
XIII	Dioxane	194–196°	– 9,2°	818 (alcohol)
XV	Dimethylformamide/acetic acid ester	187–188°	+ 20,3°	
XVI	Acetone	173–174°		
XVII	Acetic acid ester/petroleum ether	146–147°	+ 29,4°	
XVIII	Acetone	160–161°	+ 35,2°	
XIX	Dimethylformamide/acetone	226–227°	– 4,1°	810 (acetic acid)
XXI	Dimethylformamide/methanol	246–249°	– 15,1°	1507 (acetic acid)

Intermediates X, XII, XIV, and XX have only been obtained as crude materials. All other products were subjected to elementary analysis which agreed with the calculated values. XXI was completely hydrolyzed and assayed (STEIN and MOORE[16]) for constituent amino acids, which were found to conform exactly with the proposed structure.

a ninhydrine negative product XXIV (containing all constituent amino acids) from which the free cyclic decapeptide (A) was obtained by removing the protecting groups with sodium in liquid ammonia. Precipitation of the free base from the aqueous salt solution by ammonia at low temperature and redissolving in 0,5 *n* HCl followed by freezedrying gave the hydrochloride of A, which was used for paper chromatography and microbiological examination.

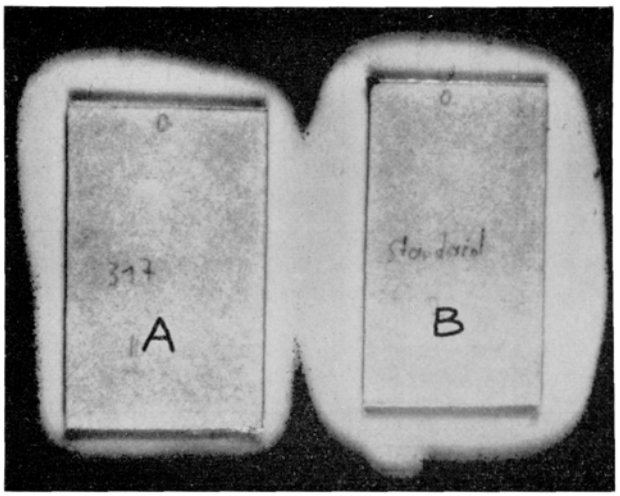

Fig. 5. – A = crude synthetic XXV (A Fig. 1), approx. 300 γ
B = Polymyxin-B-sulfate (Pfizer), approx. 150 γ

Descending paper chromatography carried out as outlined by JONES[17] in the system *n*-butanol/15% aqueous acetic acid, 1:1 (upper phase) for 16 h at 22°C using Whatman No. 4 paper, gave a running distance of 31,5 cm (solvent front off the leading edge of the paper) for the synthetic A (hydrochloride), whereas commercial Polymyxin-B-sulfate (Pfizer) of natural origin, had a running distance of 33 cm and the open decapeptide (XXIII, without any protecting groups, Fig. 4) of 24 cm.

Microbiological determinations[18] carried out in our Department of Experimental Medicine by Prof. B. FUST and Dr. ERIKA BÖHNI against *Brucella bronchiseptica* with the paper strip directly on the agar, showed a remarkable inhibition zone for the synthetic material, which was used in about twice the concentration of the commercial product (Fig. 5).

The open decapeptide (XXIII, without any protecting groups, Fig. 4) showed less activity against the same microorganism. Further purification of the synthetic material as well as comparison with natural polymyxin B_1 are under way.

Full details will be published in Helvetica Chimica Acta.

K. VOGLER, P. LANZ, and W. LERGIER

Chemical Research Department of F. Hoffmann-La Roche & Co. Ltd., Basle, July 1, 1959.

Zusammenfassung

Das offenkettige Dekapeptid XXI (Fig. 4) wurde aus den beiden geschützten Pentapeptiden VIII und XX (Fig. 2 und 3) aufgebaut. Die Zyklisierung von XXI führte zu einem mikrobiologisch aktiven Produkt der Formel A (Fig. 1).

[16] We are indebted to Prof. M. BRENNER and Dr. R. WEBER from the Institute of Organic Chemistry, University of Basle, for this analysis.

[17] T. S. G. JONES, Ann. N. Y. Acad. Sci. *51*, 909 (1949).

[18] Brucella bronchiseptica ATCC 4617, Polymyxin assay plate according to FDA, Washington, see also R. G. BENEDICT and F. H. STODOLA, Ann. N. Y. Acad. Sci. *51*, 866 (1949).