Tetrahedron Letters,Vol.28,No.40,pp 4705-4706,1987
Printed in Great Britain
0040-4039/87 $3.00 + .00
Pergamon Journals Ltd.

TOTAL SYNTHESIS OF LEPIDOPTERAN A, SELF-DEFENCE SUBSTANCE PRODUCED BY SILKWORM.

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Summary: Lepidopteran, self-defence substance produced by silkworm (Bombyx mori), was synthesized by the solution method for confirmation of the proposed structure.

Silkworm (Bombyx mori) produces an immune response substances, i.e., lepidopterans, in its hemolymph when it is vaccinated with killed E. coli. Structures of lepidopteran A, B, and C were determined as shown in Fig. 1.[1] They showed wide antibacterial spectra against Gram-negative as well as Gram-positive bacteria.

The total synthesis of lepidopteran A was carried out by the solution method applying a maximum protection procedure. Protecting groups of functional groups in the side chain of amino acid residues were as follows: p-toluenesulfonyl for Arg, formyl for Trp, 2-chlorobenzyloxycarbonyl for Lys, benzyl for Glu and Ser, and cyclohexyl for Asp. The molecule of lepidopteran was attempted to be built up from six fragments. Protecting groups used for the α-amino group and the carboxyl group were t-butoxycarbonyl (Boc) and phenacyl (Pac) group respectively. The fragments were constructed stepwise from the carboxyl-terminal mainly by the N-hydroxysuccinimide active ester method except for Asn residue which was introduced by its nitrophenyl ester. After removal of the Boc or the Pac group, each fragment was coupled together by 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (WSCI)—1-hydroxybenzotriazole (HOBt) in the manner as shown in Fig. 2.

Lys^6 was an only amino acid residue which had the possibility of racemization during fragment condensation, because all carboxyl-terminal amino acids of the other fragments were Gly. In order to estimate the content of D-Lys^6 in the coupling product of Boc-(1-6)-OH with H-(7-12)-OPac, the ratio of [D-Lys^6]-(1-12)[2] vs. [L-Lys^6]-(1-12) was measured by HPLC after removal of all protecting groups. Observed racemization degree was 1.6%, which means that

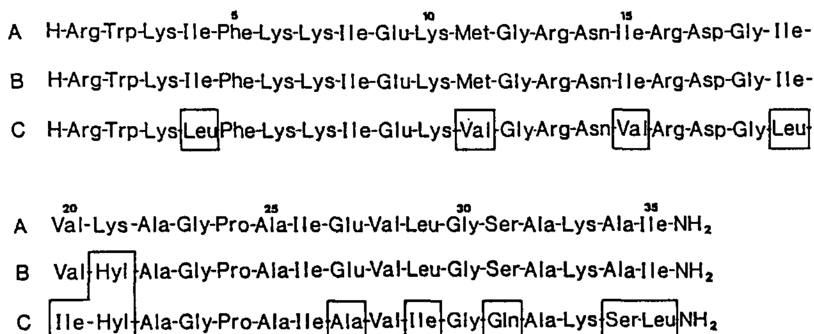

Fig.1. Structures of Lepidopteran A, B, and C. Hyl: δ-hydroxylysine

racemization in the actual synthesis was practically negligible.

The final coupling product was deprotected by three steps as follows. After removal of the N^{α}-Boc group with TFA, all other protecting groups except the formyl group of Trp were then removed with HF in the presence of anisole at 0°C for 60 min. The formyl group was removed by treatment with HF-1,2-ethanedithiol (1:1) at 0°C for 30 min[3]. The crude product was purified by HPLC, and the purity of synthetic lepidopteran was examined by HPLC. The synthetic lepidopteran[4] was completely identical with natural one in respects tested so far including retention time of HPLC, peptide mapping of trypsin digests, specific rotation, UV spectrum and antibacterial activity. From these results, the structure of lepidopteran A was now confirmed unequivocally.

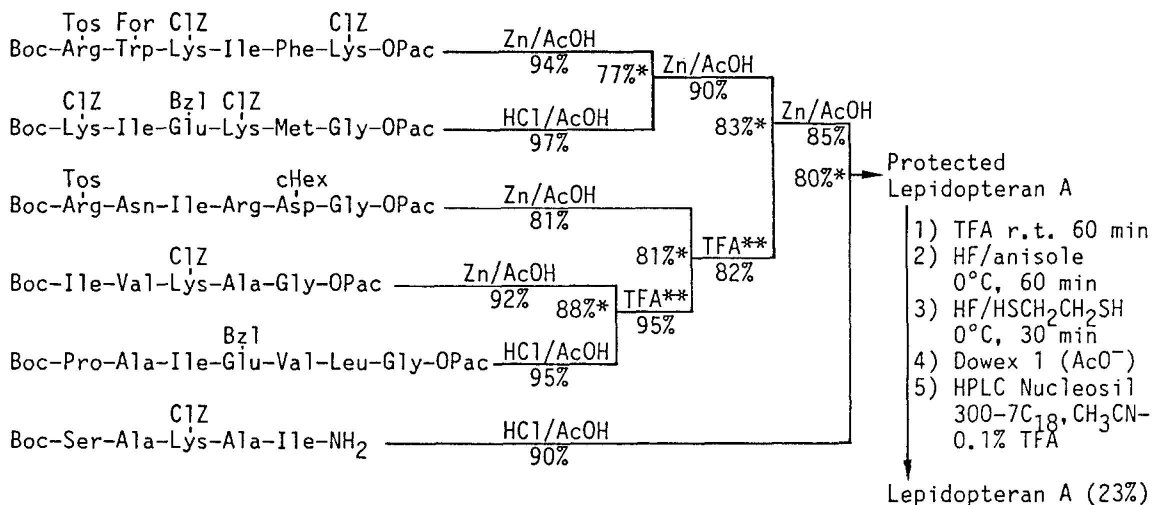

Fig. 2. Synthetic Scheme of Lepidopteran A. Abbreviations; ClZ: 2-chlorobenzyloxycarbonyl, cHex: cyclohexyl. *: Yield of fragment condensation which was carried out by WSCI-HOBt method, **: TFA salt of deblocking product was changed to HCl salt before next fragment condensation.

References and Notes

1) a) T. Shiba, Y. Ueki, I. Kubota, T. Teshima, Y. Sugiyama, Y. Oba, and M. Kikuchi, Peptide Chemistry 1983, 209 (1984). b) T. Teshima, Y. Ueki, T. Nakai, T. Shiba, and M. Kikuchi, Tetrahedron, 42, 829 (1986). c) The structure of lepidopteran C was presented at the 52nd National Meeting of the Chemical Society of Japan, Kyoto, April, 1986, Abstr., No. 2N44.

2) The reference fragment [D-Lys^6]-(1-12) was prepared by treatment of Boc-(1-6)-OH with WSCI and HOBt followed by fragment condensation and deprotection. The racemization condition using WSCI and HOBt was informed by the private communication from Dr. T. Kimura, Peptide Institute Inc., Protein Research Foundation.

3) The proceeding of Akabori Conference, 1st German-Japanese Symposium on Peptide Chemistry, Munich, June, 1985, p.10.

4) Amino acid analysis (molar ratio): Asp(2.09), Ser(0.85), Glu(2.03), Pro(1.16), Gly(4.00), Ala(4.05), Val(1.59), Met(1.05), Ile(5.68), Leu(1.03), Phe(0.96), Lys(6.67), Trp(1.05*), Arg(3.07), Hydrolysis conditions: 6M HCl containing 4% thioglycolic acid, 110°C, 90h(*24h); Retention time of HPLC: 30.0 min (Nucleosil 300-7C_{18}, 4x250 mm, CH_3CN-0.1% TFA, linear gradient 10-60% CH_3CN (50 min), flow rate 0.8 ml/min); Peptide mapping by HPLC (min)(peptide fragment): 12.9(34-35), 15.1(11-13), 16.7(7-10), 17.4(14-16), 22.5(2-3), 23.8(1-3), 27.3(17-21), 29.5(4-6), 49.3(22-33) (Cosmosil 5C_{18}, 4x250 mm, CH_3CN-0.01M phosphate buffer (pH 2.6) containing 0.05M Na_2SO_4, 1% CH_3CN (5 min), linear gradient 1-21% CH_3CN (40 min), 21-50% CH_3CN (29 min), flow rate 1 ml/min); $[\alpha]_D^{25}$ -55° (c 0.1, 4% CH_3COOH); UV: λ_{max} 279 nm (ε 4,000)

(Received in Japan 13 June 1987)