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RAPID PEPTIDE SYNTHESIS IN LIQUID PHASE. PREPARATION OF ANGIOTENSIN II AS AN EXAMPLE

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A rapid method of peptide synthesis is proposed. In this method a peptide chain is stepwise elongated in a water-immiscible organic solvent without isolation of the intermediates. Protected angiotensin II was prepared by the method in an overall yield of 74% in a short period of time.

The liquid phase method for peptide synthesis is superior to the solid phase method in many points, but is quite time-consuming. Several attempts have been made out to diminish tedious work in the liquid phase method.[1-3] The present paper reports a method of peptide elongation using the coupling reaction in a wet or two-layer solvent system consisting of water and water-immiscible organic solvent. In this method, all procedures, that is, coupling, washing, and deblocking of the N-terminal protecting group, are accomplished successively in a single vessel, although a coupling reagent which will give a good yield even in such a wet condition is required.

First the preparation of Boc-Ala-Gly-OEt[4] was examined by the use of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSCD·HCl) as a coupling reagent. The addition of 1-hydroxybenzotriazole (HOBt) to the reaction mixture was effective in improving the yield of the peptide (Table 1).

Next the rapid preparation of protected angiotensin II, Z-Asp(OBzl)-Arg(NO_2)-Val-Tyr(Bzl)-Ile-His(Bzl)-Pro-Phe-OBzl (1)[4], was tried. 1 was derived from H-Phe-OBzl by successive coupling with Boc-Pro-OH, Boc-His(Bzl)-OH, Boc-Ile-OH, Boc-Tyr(Bzl)-OH, Boc-Val-OH, Boc-Arg(NO_2)-OH[5], and Z-Asp(OBzl)-OH. Each residue was introduced to a growing peptide chain by repeating the following five-step cycle. (1) Acylation of an amine component (0.5 mM) in 1,2-dichloroethane (ca. 10 ml) at room temperature for 2 h with Boc-amino acid (Z-derivative for the final acylation) (2 eq.), WSCD·HCl (2 eq. in 1.5 ml of water) and HOBt (2 eq.). (2) Washing with 0.1 M HCl, water, 5% Na_2CO_3 and water; each washing was removed by pipetting. (3) Cleavage of the Boc-group by HCl/dioxane at room temperature for 0.5 h. (4) Addition of a slight excess of 2 M Na_2CO_3 and removal of the aqueous phase. (5) Washing with water. In every cycle, the completion of acylation (Step 1) and the purity of the resulting peptide benzyl ester (Step 5) were confirmed by TLC. Throughout the whole procedure, no intermediate was isolated. When an interme-

Table 1. Effect of HOBt on the Coupling Reaction in Two-Layer Solvent System

HOBt (eq.)	Yield of Boc-Ala-Gly-OEt (%)
0	9
1	79
2	78

In each run, Boc-Ala-OH, H-Gly-OEt·HCl, triethylamine, and WSCD·HCl (0.5 mM, each) were added in a two-layer solvent consisting of 10 ml each of 1,2-dichloroethane and water with or without HOBt. Reaction time: 2 h at room temperature. The product (oil) was isolated by the usual method.

diate precipitated in the reaction mixture, it was dissolved by the addition of a small amount of N,N-dimethylformamide (DMF). Finally 1 was isolated by evaporating the organic layer and reprecipitating from DMF-water. The overall yield based on H-Phe-OBzl was 74% and the total time required for the synthesis was 34 h in one case. Found: C, 63.53; H, 6.16; N, 12.08%. Calcd for $C_{86}H_{100}N_{16}O_{14}\cdot 2H_2O$: C, 63.69; H, 6.46; N, 12.09%.

Angiotensin II was obtained in a theoretical yield by catalytic hydrogenation of 1 in DMF-acetic acid-water. TLC, R_f (Avicel-cellulose, 1-butanol-pyridine-acetic acid-water, 16:10:3:12): 0.56 with trace spots at 0.17 and 0.60. Amino acid ratio in acid hydrolysate (with const. boiling HCl, 110°C, 16 h): His 0.93, Arg 1.03, Asp 1.04, Pro 1.09, Val 0.95, Ile 0.91, Tyr 1.04, Phe 1.01. From these results, 1 seemed to be almost pure.

The product was further purified by partition chromatography on a Sephadex G-25 column (equilibrated with two layers of 1-butanol-acetic acid-water, 5:1:5). TLC, R_f (in the same way as above): 0.56, single spot. Amino acid ratio in acid-hydrolysate: His 0.95, Arg 1.04, Asp 0.94, Pro 1.08, Val 1.05, Ile 0.95, Tyr 0.97, Phe 1.03. Amino acid ratio in aminopeptidase M digest: His 1.01, Arg 0.86, Asp 0.90, Pro 1.08, Val 0.98, Ile 1.06, Tyr 1.07, Phe 1.03. The values of elemental analyses agreed with the calculated ones for the monoacetate hexahydrate of 1. $[\alpha]_D^{24}$ -58.5° (c 0.16, 1 M HCl); lit. $[\alpha]_D^{21}$ -66.98° (1 M HCl, as the monoacetate).[6] The biological potency (oxytocic activity of the isolated rat uterus) was the same as that of an authentic sample (purchased from the Protein Research Foundation, Osaka). The present method, proposed as a 'hold-in-solution' method, is expected to be applicable to syntheses of other oligopeptides.

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References and Note

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