Use of Anhydrous Hydrogen Fluoride in Peptide Synthesis. III. Synthesis of Bradykinin

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During repeated synthesis of bradykinin following a recently published procedure,[1] the final stage of removal of the nitro group from the nitroarginine residue by catalytic hydrogenolysis was found to be unsatisfactory; that is, the biological activity of the final product varied within a range of 100 to 60%, with the quality of the palladium black catalyst used, even though the same protected nona-peptide was subjected to each reaction. This variance in the biological activity of the product was probably due to the formation of some side products during the reduction of the nitro group, as pointed out for general cases by Iselin.[2] Furthermore, removal of the nitro group from large arginine peptides by catalytic hydrogenolysis is known to be a time-consuming reaction. Recently,[3,4] anhydrous hydrogen fluoride (HF) has been shown to be useful for removal of various protective groups from peptides, and it was noted especially that a nitro group can be removed quickly from nitroarginine peptides in the presence of anisole.[3-5] This procedure was applied in the present study for removal of protecting groups from a nonapeptide, tricarbobenzoxy-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanyl-N^G-nitro-L-arginine benzyl ester (V). Synthesis of the starting material V was carried out following the original procedure[1] with several improvements as described in the experimental part of this paper. This material V was treated with HF at 0°C for 30 min in the

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4) S. Sakakibara, Y. Kishida, R. Nishizawa and Y. Shimonishi, *ibid.*, **41**, 438 (1968).

5) J. Lenard, *J. Org. Chem.*, **32**, 250 (1967).

presence of anisole as described in a preceding paper.[4] The HF which contaminated the final reaction product was eliminated by passing the aqueous solution through a column of Dowex 1 (OH^- form). Then the HF free product was converted to the acetate form, and was lyophilized. If a faint pink color remained in the product, it will be removed easily by passing the aqueous solution through a short column of Sephadex G 10. The biological activities of three different batches of bradykinin acetate synthesized in this way were assayed by the contraction test with guinea-pig ileum,*2 and they were found to show essentially the same biological activity which was as strong as that of an available authentic sample.[6] The purity of bradykinin synthesized by the HF procedure was also compared with that of another sample which was synthesized by catalytic hydrogenolysis of the same protected nonapeptide V. Both samples were converted to the DNS-derivatives,[7] and were analyzed by thin-layer chromatography with Silica-gel H.*3 The chromatogram clearly indicated the great advantage of the HF method, as shown in Fig. 1. Thus, it was concluded that homogeneous bradykinin can be obtained easily by the present HF method without the use of any additional purification procedure, while catalytic hydrogenolysis of the nitroarginine peptide may result in contamination with undesirable compounds.

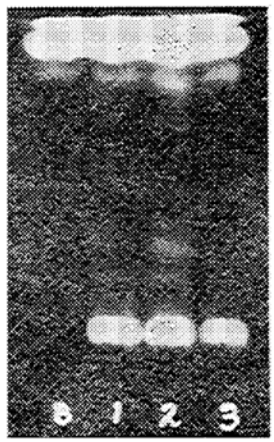

Fig. 1. Thin-layer chromatogram of DNS-bradykinin (10^{-8} mol) with Silica-gel H.
The solvent system was methyl acetate: isopropanol: 28% ammonia (9 : 7 : 4) containing mercaptoethanol (1%).
B: Control. 1: An available sample as the standard bradykinin. 2: Bradykinin synthesized by catalytic hydrogenolysis. 3: Bradykinin synthesized by the HF method.

Lenard and Robinson[8] have mentioned recently in a short communication that the HF method is also useful to release bradykinin from the Merrifield's polymer support. Although they reported the presence of four extra peaks in a distribution curve of their sample when it was analyzed by the counter current method, formation of such by-products did not occur on careful treatment of this peptide with HF.

Experimental

***t*-Amyloxycarbonyl-L-phenylalanyl-N^G-nitro-L-arginine Benzyl Ester (I).** This material was synthesized as described previously.[1] The melting point of the final product increased to 132—135°C on repeated recrystallization from ethyl acetate and petroleum ether; $[\alpha]_D^{24}$ −15.6° (*c* 3, ethanol), $[\alpha]_D^{25}$ −14,2° (*c* 1.6, pyridine). Cited values[1] were mp 120—124°C, $[\alpha]_D$ −15.8° (*c* 3, ethanol).

***p*-Methoxybenzyloxycarbonyl-L-prolyl-L-phenylalanyl-N^G-nitro-L-arginine Benzyl Ester (II).** The same procedure was followed for its synthesis as that described in the previous paper[1] for the preparation of *t*-amyloxycarbonyl-L-prolyl-L-phenylalanyl-N^G-nitro-L-arginine benzyl ester. *p*-Methoxybenzyloxycarbonyl-L-proline[9] was used instead of *t*-amyloxycarbonyl-L-proline. This *p*-methoxybenzyloxycarbonyl derivative was more easily crystallizable than the former *t*-amyloxycarbonyl compound. The yield of this compound after recrystallization from a mixture of methanol and chloroform by the addition of ether was 79%; mp 179—182°C, $[\alpha]_D^{25}$ −40.8° (*c* 1, dimethylformamide).

Found: C, 60.46; H, 6.30; N, 13.65%. Calcd for $C_{36}H_{43}O_9N_7$: C, 60.24; H, 6.04; N, 13.67%.

***t*-Amyloxycarbonyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanyl-N^G-nitro-L-arginine Benzyl Ester (III).** This material was prepared as described in the previous paper,[1] but compound II was used instead of the *t*-amyloxycarbonyl derivative. Although the compound was reported to be an amorphous powder, it was crystallized in this case from hot ethyl acetate or methanol by the addition of ether; yield 80%, mp 175—177°C, $[\alpha]_D^{24}$ −37.1° (*c* 1, dimethylformamide). Reported value[1] mp 100—115°C, $[\alpha]_D^{22}$ −38.2° (*c* 0.5, dimethylformamide).

***t*-Amyloxycarbonyl-L-prolyl-L-prolylglycine *p*-Nitrophenyl Ester (IV).** Although this material was used without purification in the previous case,[1] it was crystallized in the present study by trituration with ether; the yield of crude product was 85%. The crude crystals were recrystallized from ethyl acetate and petroleum ether; yield 80%, mp 191—192°C, $[\alpha]_D^{19}$ −92.7° (*c* 1, dimethylformamide).

*2 The biological tests were performed by Professor Tomoji Suzuki of Osaka University and his colleagues, to whom the authors are deeply grateful.

6) Obtained from Sandoz AG., Basel, Switzerland, (Batch No. 65226, BRS 640) as 0.1 mg/m*l* solution.

7) W. R. Gray and B. S. Hartley, *Biochem. J.*, **89**, 59p (1963); DNS-=1-Dimethylamino-naphthalene-5-sulfonyl-

*3 The authors wish to express their thanks to Professor Zenzo Tamura of the University of Tokyo for carrying out this procedure skillfully. *Cf.* Z. Tamura and T. Nakajima, *Protein, Nucleic Acid, Enzyme (Tampakushitsu, Kakusan, Kohso)*, **12**, 729 (1967).

8) J. Lenard and A. B. Robinson, *J. Am. Chem. Soc.*, **89**, 181 (1967).

9) F. Weygand and K. Hunger, *Chem. Ber.*, **95**, 1 (1962).

Found: C, 57.11; H, 6.34; N, 11.10%. Calcd for $C_{24}H_{32}O_8N_4$: C, 57.13; H, 6.39; N, 11.11%.

Bradykinin Triacetate. A protected nonapeptide V (402 mg, 0.25 mmol), which was synthesized by following a previously published procedure[1] with compound III and IV, was placed in an HF-reaction cylinder together with anisole (0.28 m*l*, 10 eq).[3] HF (5 m*l*) was added into the cylinder using an HF-reaction apparatus,[3] and the mixture was allowed to react at 0°C for 30 min with stirring. Excess HF was removed under reduced pressure at 0°C, and the residue was kept at reduced pressure (3 mmHg) for 5 hr at room temperature. The product was dissolved in water (10 m*l*), insoluble material was removed by filtration with Hyflo Supercel, and the clear solution thus obtained was passed through a column of Dowex 1 (X 2, OH^- form, 1 cm × 15 cm). The column was washed with distilled water (about 100 m*l*) until the pH of the washing became 7. The eluate and washing were collected together in a receiver which contained acetic acid (0.5 mol), and the solution was lyophilized. After drying in a desiccator over phosphorus pentoxide *in vacuo* for 24 hr, the yield of the product was 257 mg (77% as triacetate pentahydrate); mp 170—171°C; $[\alpha]_D^{13}$ −80.6° (*c* 0.28, water).[*4]

Found: C, 50.45; H, 7.30; N, 16.26%. Calcd for $C_{50}H_{73}O_{11}N_{15}\cdot 3CH_3COOH\cdot 5H_2O$: C, 50.57; H, 7.15; N, 15.80%.

After drying this compound at 110°C for 18 hr *in vacuo*, the anhydrous compound was obtained.

Found: C, 54.34; H, 7.10; N, 17.73%. Calcd for $C_{50}H_{73}O_{11}N_{15}\cdot 3CH_3COOH$: C, 54.22; H, 6.91; N, 16.94%.

[*4] The concentration was calculated as dry bradykinin triacetate.