SHORT COMMUNICATIONS

A New Method for Releasing Oxytocin from Fully-protected Nona-peptides Using Anhydrous Hydrogen Fluoride

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Methods of the synthesis of oxytocin[1] are generally based on the principle that the *S*-benzyl group is stable against hydrogen bromide or trifluoroacetic acid and can only be removed by treatment with sodium in liquid ammonia. This procedure, however, has several defects; for instance, the product is inevitably contaminated with sodium salts, and it may be partially decomposed by the strong alkali used during the procedure. The *S*-*p*-methoxybenzyl group is also stable against hydrogen bromide, but it can be removed by boiling trifluoroacetic acid in contrast with the *S*-benzyl group.[2] However, it has been shown[3] that it is more difficult to remove, with trifluoroacetic acid, the *S*-methoxybenzyl group in a large peptide such as oxytocin than that in a small peptide. Therefore, a more effective method for the removal of these protecting groups is required. Katz[4] first showed that anhydrous hydrogen fluoride (HF) is a good solvent for proteins, and that the biological activity of proteins is not irreversibly affected by this solvent. Moreover, anhydrous HF is known to be a very strong acid, although it becomes a weak acid in the presence of water.[5] These facts were effectively utilized by Hess et al.[6] in an *N*, *O*-acyl migration reaction with serine or threonine peptides. The above-mentioned facts also suggested that this solvent might be an effective reagent for the acidolysis of various protecting groups. The boiling point of the solvent (19.5°C) was also considered to give it an additional advantage. The basic idea of the present project arose from these considerations.

First, cysteine formation from *S*-benzylcysteine and from *S*-*p*-methoxybenzylcysteine in anhydrous HF was tested by iodometry, and the quantitative removal of the protecting groups was observed during one-hour reactions at room temperature in both cases. These experiments indicated clearly, as expected, that anhydrous HF is the first effective reagent for the acidolysis of *S*-benzyl groups. Then, the pre-oxytocin nona-peptides, *N*-carbobenzyloxy-*S*-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-*S*-benzyl-L-cysteinyl-L-prolyl-L-leucylglycine amide (I)[1] and the *N*-carbobenzyloxy-*S*-*p*-methoxybenzylpeptide of the same sequence (II),[3] were each subjected to the HF reaction under the same conditions. The addition of anisole to the reaction mixture effectively prevented the succeeding polymerization of the benzyl fluoride which may be formed in the solution; it also protected the tyrosyl residue from possible modification by benzyl fluoride. After the peptides had been oxidized by air to cystinyl peptides, the hormonal activities of the resulting products were determined by the rat-uterine contraction test; 133 units per mg. of I and 148 units per mg. of II were observed (about 370 units per mg. would correspond to quantitative recovery),[7] whereas the acidolysis of II with trifluoroacetic acid gave only 33 units per mg.[3]

Further possibilities for the use of HF in general peptide synthesis are being studied.

Experimental.—*Reaction in Anhydrous HF.*—The procedure was, in principle, the same as that for the *N*, *O*-acyl migration reaction in HF.[6] Compound I or II was dried over phosphorous pentoxide in vacuo at 80°C for 3 hr. before the reaction. The dried material (50

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2) S. Akabori, S. Sakakibara, Y. Shimonishi and Y. Nobuhara, This Bulletin, **37**, 433 (1964).
3) S. Sakakibara, Y. Nobuhara, Y. Shimonishi and R. Kiyoi, ibid., **38**, 120 (1965).
4) J. J. Katz, *Arch. Biochem. Biophys.*, **51**, 293 (1954).
5) J. H. Simons, "Fluorine Chemistry," Vol. I, Academic Press, New York (1950), p. 225.
6) S. Sakakibara, K. H. Shin and G. P. Hess, *J. Am. Chem. Soc.*, **84**, 4921 (1962); J. Lenard and G. P. Hess, *J. Biol. Chem.*, **239**, 3275 (1964).

7) The authors wish to express their thanks to Dr. Keiji Nakamura of the Dai Nippon Pharmaceutical Co. for determining the oxytocic activities.

mg.) was placed in the reaction vessel, together with anisole (0.1 ml.); anhydrous HF (ca. 2 ml.) was then distilled into the mixture, which was then kept for one hr. at room temperature before the solvent was removed by distillation. The remaining HF was completely removed by letting the material stand over sodium hydroxide in vacuo, and the residue was dissolved in water (50 ml.). The solution was adjusted to pH 6.5 by adding dilute ammonia, and then carbon dioxide-free air was bubbled through the solution for about 2 hr. until the nitroprusside test became negative. Then the oxidized solution was brought to pH 4 with acetic acid, and the insoluble materials formed were removed by filtration. An aliquot of this filtrate was subjected to the biological test.

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