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Use of Anhydrous Hydrogen Fluoride in Peptide Synthesis. I. Behavior of Various Protective Groups in Anhydrous Hydrogen Fluoride*[1]

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The properties of anhydrous hydrogen fluoride (HF) as a reagent for the acidolysis of various protective groups have been studied. Amino acids with various protective groups were each treated with HF at 0 or 20°C in the presence of anisole, and the reaction products were tested. Thus, HF was found to be much better than the other known reagents, such as hydrogen bromide and trifluoroacetic acid. In the present study, the following groups were shown, for the first time, to be removed safely: the nitro group in nitroarginine, the diphenylmethyl group in diphenylmethylamide, the *t*-butyl group in *S*-*t*-butylcysteine, the isopropyloxycarbonyl group, and the isopropyl ester group. A new and convenient apparatus was designed for the safe handling of HF for peptide synthesis.

Anhydrous hydrogen fluoride (HF) is the first reagent used in the acidolysis of the *S*-benzyl group in cysteine peptides. To illustrate the procedure, a new route for the release of oxytocin from fully-protected parent peptides was described in a preceeding paper.[1] The results suggested that HF might be the best reagent for the acidolysis of protective groups, and that the procedure might be useful in the chemical synthesis of complicated peptides. The present investigation is on the general reactivity of HF with various protective groups which are known to be, or expected to be, useful in peptide synthesis.

One difficulty in the use of HF is the caustic action of this reagent on glass. In the literature[2,3] it has been mentioned that an apparatus suitable for the reaction of HF should be made of nickel, monel, silver, or various synthetic resins such as poly-fluorothene, and that of these, poly-trifluoromonochloroethylene is the best because it is semi-transparent and is sufficiently stable. Katz[3] has used a monel vacuum line with poly-trifluoromonochloroethylene reaction vessels for HF reactions, but there remains the possibility that the product might be contaminated by metal ions. Therefore, in the present work, reactions were

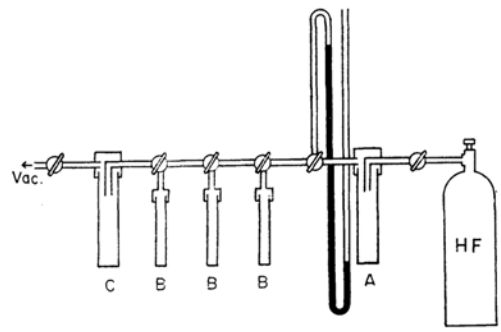

Fig. 1. HF-reaction apparatus suitable for samples of less than one gram.
A Cylinder for purification and measurement of volume of HF: 30 mm×200 mm
B Cylinder for HF reaction: 20 mm×150 mm
C Trap: 30 mm×200 mm

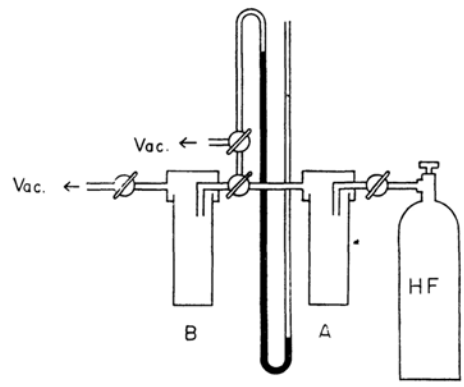

Fig. 2. HF-reaction apparatus suitable for samples of about 10 g.
A Cylinder for purification and measurement of volume of HF: 50 mm×200 mm
B Cylinder for HF reaction: 50 mm×200 mm

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1) S. Sakakibara and Y. Shimonishi, This Bulletin, **38**, 1412 (1965).

2) J. H. Simons, "Fluorine Chemistry," Vol. I, Academic Press, Publishers, New York, N. Y. (1950), p. 225.

3) J. J. Katz, *Arch. Biochem. Biophys.*, **51**, 293 (1954).

TABLE 1. CONDITIONS FOR REMOVAL OF VARIOUS PROTECTIVE GROUPS WITH HF

Protective group	Compound	Reaction conditions	
		Temp., °C	Time, min
$(CH_3)_3C$-O-CO-	*t*-BOC-Ala	0	<30
$(CH_3)_2(C_2H_5)C$-O-CO-	*t*-AOC-Pro[e]	0	<30
$(CH_3)_2CH$-O-CO-	*i*-POC-Leu (DCHA)	20	30
	Lys(*i*-POC)	20	>120[a]
 -CH_2-O-CO-	Z-Ala	0	<30
	Lys(Z)	0	30
R-COO-$C(CH_3)_3$	Gly-OBu^t (TosH)	0	<30
R-COO-$CH(CH_3)_2$	Phe-OPr^i (TosH)	0	120
	Asp(OPr^i)	0	90
	Glu(OPr^i)	20	90
R-COO-CH_2- 	Leu-OBZL (TosH)	0	30
	Asp(OBZL)	0	30
	Glu(OBZL)	0	60
R-COO-CH_2-  -NO_2	Gly-ONB (HBr)	20	>60[b]
R-O-CH_2- 	Ser(BZL)	0	<30
	Tyr(BZL)	0	60[c]
N^G-NO_2	Arg(NO_2)	0	<30
R-S-$C(CH_3)_3$	Cys(Bu^t)[f]	20	60
R-S-CH_2- 	Cys(BZL)	0	>60[d]
		20	30
R-S-CH_2-  -OMe	Cys(MBZL)[g]	0	<30
R-S-CH()$_2$	Cys(DPM)[h]	0	30
R-CO-NH-CH()$_2$	Leu-NH(DPM) (HBr)	0	30
	Z-Glu(NH-DPM)	0	60

a) Recovery of Lys, 64%.
b) Recovery of Gly, 15%.
c) Recovery of Tyr, 70%.
d) Recovery of SH groups, 72%.
e) S. Sakakibara, M. Shin, M. Fujino, Y. Shimonishi, S. Inouye and N. Inukai, This Bulletin, **38**, 1522 (1965).
f) G. H. L. Nefkens, G. I. Tesser and R. J. F. Nivard, *Rec. trav. chim.*, **79**, 688 (1960).
g) A. Akabori, S. Sakakibara, Y. Shimonishi and Y. Nobuhara, This Bulletin, **37**, 433(1964).
h) L. Zervas and I. Photaki, *J. Am. Chem. Soc.*, **84**, 3887 (1962).

carried out using an apparatus made entirely from Daiflon resin, as is shown in Fig. 1 for samples of less than one gram and in Fig. 2 for larger-scale reactions. When a carbobenzoxyamino acid was placed in HF at room temperature, the evolution of carbon dioxide was observed, and then an amorphous precipitate appeared in the reaction mixture. This phenomenon is probably due to the formation of benzyl cations, which may polymerize with each other in HF. The cations formed may also modify aromatic amino acids or methionine residues during the reaction. To prevent these side reactions, an equimolar amount of anisole was used in all reactions, as this had been recommended by Weygand and Steglich[4] for similar reactions with trifluoroacetic acid. Generally, the solution became red when the cleavage reaction occurred, and the color disappeared after the HF had been completely removed from the reaction mixture.

Various protected amino acids were each subjected to the HF reaction together with anisole, and the products were analyzed by thin-layer chromatography, paper chromatography, paper electrophoresis, and/or with an automatic amino

4) F. Weygand and W. Steglich, *Z. Natuuforsch.*, **14b**, 472 (1959).

acid analyzer. The results are summarized in Table 1. Many protective groups, which were either not removed or were only slightly removed under mild conditions by the usual method of acidolysis, were found to be removed within one hour at 0°C. The removal of the nitro group from N^G-nitroarginine is a big advantage of the procedure, because the HF reaction is not affected by sulfur compounds which disturb the catalytic hydrogenation of the nitro group. The nitro group may be transferred to anisole in HF *via* the nitronium cation. The removal of *S*-*t*-butyl and of *N*-diphenylmethyl groups was also noteworthy. The latter group especially may be useful in the protection of the amide groups from nitril formation of a glutamine or asparagine residue.[5] With regard to *S*-protection, *S*-*p*-methoxybenzyl and *S*-diphenylmethyl groups were found to be removed more smoothly than *S*-benzyl and *S*-*t*-butyl groups.

When *N*-carbobenzoxy-L-tyrosine was tested in the HF reaction, the recovery of tyrosine was less than 75% and appreciable amounts of nuclear-substituted compounds were detected in the reaction mixture by paper chromatography. Their formation was almost entirely prevented, however, by dissolving the starting material in trifluoroacetic acid, together with five equivalents of anisole, before adding HF; in this way the recovery of tyrosine was increased to about 90%. The recovery of phenylalanine, tryptophan, and methionine, which might be destroyed or modified in HF, was determined after their reaction with HF in the presence of one equivalent of anisole at 0°C. Under these conditions no appreciable side reaction was observed, and the recoveries were more than 90%. No oxidation reaction followed the removal of the nitro group from nitroarginine; this was confirmed by the facts that no methionine sulfoxide was formed from methionine in the reaction mixture, and that the recovery of cysteine from *S*-*p*-methoxybenzyl-L-cysteine in the reaction mixture was 85%.

The following protective groups were found to be stable in HF at 20°C under the standard reaction conditions used: carboethoxy, benzoyl, tosyl, formyl, phthalyl, methyl ester, ethyl ester, *N*-benzyl, *N*-*p*-methoxybenzyl, *S*-methyl, *S*-ethyl, and *S*-isopropyl. These stable groups may be useful for the selective protection of functional groups during peptide synthesis.

Details of the conditions used in applying the HF procedure to actual peptide synthesis will be published later.

Experimental

Materials. Anhydrous hydrogen fluoride of a 99.5% purity was obtained from the Matheson Chemical Co., U. S. A. It was used for the reactions without further drying. Amino acid derivatives known from the literature were prepared according to standard procedures.

Apparatus. All parts of the apparatus shown in Figs. 1 and 2 were made from Daiflon,*4 and the apparatus was assembled by the Toho-Kasei Co., Ltd.*5 Viton*6 O-rings were used for the vacuum-seal.

Reaction with HF. The amino acid derivative to be tested (100 mg) and an equimolar amount of anisole were placed in the reaction cylinder, B (Fig. 1), equipped with a Teflon-coated stirring bar, and the vessel was attached to a vacuum line, as is shown in Fig. 1. HF was initially accumulated in the first cylinder, A, to purify it and to measure the volume. Then, about 5 m*l* of this HF were redistilled into the reaction cylinder. Distillations were carried out under a vacuum using a methanol - dry ice bath, and then the reaction mixture was kept at 0°C or 20°C with an ice-water bath or a water bath at 20 ± 1°C. When reaction cylinder B, which had been chilled in a methanol - dry ice bath, was put in a water bath at 20°C, it took about 10 min for the inside temperature to rise to 20°C. Therefore, the reaction time was measured 10 min after changing baths. After the reaction was over, the HF was removed by vacuum distillation, and the residue was analyzed by the standard procedures of paper chromatography, paper electropholesis, thin-layer chromatography, and/or automatic amino acid analysis. The final cylinder, C, was a trap for HF cooled with liquid nitrogen. When this was used, the HF-line could be evacuated using a mechanical pump with a trap containing sodium hydroxide pellets. When liquid nitrogen was not available, the excess HF could be removed with a plastic water pump; then the residue was dried thoroughly under a vacuum using a mechanical pump and a sodium hydroxide trap.

Isopropyloxycarbonyl Chloride. This reagent was prepared directly from isopropanol and phosgene in the same way as carbobenzoxy chloride.[6] After the removal of the hydrogen chloride formed and of the excess phosgene *in vacuo* at room temperature this reagent was used in the following reactions without further purification.

Isopropyloxycarbonyl-L-leucine Dicyclohexylamine Salt. Isopropyloxycarbonyl-L-leucine was prepared from L-leucine by the standard Schotten-Baumann reaction with isopropyloxycarbonyl chloride. The final oily product was neutralized with dicyclohexylamine, and the salt was recrystallized from petroleum ether (60—80°C); yield 95% mp 110—111°C, $[\alpha]_D^{16}$ −13.6° (*c* 2.7, pyridine). Found: C, 65.99; H, 10.57; N, 7.21%. Calcd for $C_{22}H_{42}O_4N_2$: C, 66.29; H, 10.62; N, 7.03%.

ε-Isopropyloxycarbonyl-L-lysine. A solution of the L-lysine Cu-complex, which had been prepared from L-lysine hydrochloride (18.3 g, 0.1 mol) and

5) D. T. Gish, P. G. Katsoyannis, G. P. Hess and R. J. Stedman, *J. Am. Chem. Soc.*, **78**, 5954 (1956); C. Ressler, *ibid.*, **78**, 5956 (1956).

*4 The commercial name of poly-trifluoro-monochloroethylene manufactured by the Daikin Kogyo Co. Ltd., Osaka.

*5 Kamifukuido 2, Higashi-sumiyoshi, Osaka

*6 The commercial name of the poly-fluorocarbon elastomer manufactured by E. I. du Pont de Nemours Co., Inc., U. S. A.

6) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. II, John Wiley & Sons, New York, N. Y. (1961), p. 890.

cupric carbonate (14.3 g, 0.06 mol) in water (150 ml), was treated with isopropyloxycarbonyl chloride (about 15 g, 0.12 mol) at 0—3°C under stirring. The mixture was kept at pH 8.5—9.5 during the reaction with sodium carbonate. After 4 hr at 0°C, the precipitate formed was collected by filtration and washed successively with water, ethanol, and ether, and dried; the yield of the product, the ε-isopropyloxycarbonyl-L-lysine Cu-complex, was 17.4 g. The Cu-complex was then dissolved in N hydrochloric acid (50 ml), and hydrogen sulfide was bubbled through the solution until the formation of copper sulfide was complete. The precipitate was filtrered off, and the filtrate was concentrated to dryness. The residue was recrystallized from a mixture of water and ethanol; yield, 7.4 g (over-all 32%) mp 235—241°C (decomp.), $[\alpha]_D^{15}$ +17.7° (*c* 1.1, N HCl). Found: C, 51.51.; H, 8.72; N, 12.00%. Calcd for $C_{10}H_{20}O_4N_2$: C, 51.70; H, 8.68; N, 12.06%.

L-Phenylalanine Isopropyl Ester Tosylate. L-Phenylalanine (16.5 g, 0.1 mol), isopropanol (50 ml), and *p*-toluenesulfonic acid (21 g, 0.11 mol) in chloroform (200 ml) were refluxed for about 2 days using an apparatus described in the literature.[7] The reaction mixture was then concentrated to dryness, and the residue was recrystallized from isopropanol and ether; yield, 33.1 g (87.4%), mp 138—140°C, $[\alpha]_D^{16}$ +25.1° (*c* 3, ethanol). Found: C, 60.24; H, 6.70; N, 3.63%. Calcd for $C_{19}H_{25}O_5NS$: C, 60.14; H, 6.64; N, 3.69%.

L-Aspartic Acid β-Isopropyl Ester. A suspension of L-aspartic acid (13.3 g, 0.1 mol) in isopropanol (200 ml) containing hydrogen chloride (7.3 g, 0.2 mol) was refluxed for one hour, and then the solution was concentrated to dryness. The residue was suspended in water (about 20 ml), and the suspension was neutralized with triethylamine. The crude product which precipitated was collected by filtration, and then recrystallized from water at pH 7; yield, 6.1 g (34.9%). mp 213—218°C (decomp.), $[\alpha]_D^{17}$ +28.2° (*c* 4, N HCl). Found: C, 47.82; H, 7.51; N, 7.95%. Calcd for $C_7H_{13}O_4N$: C, 47.99; H, 7.48; N, 8.00%.

L-Glutamic Acid γ-Isopropyl Ester. A suspension of L-glutamic acid (14.7 g, 0.1 mol) in a mixture of isopropanol (200 ml) and concentrated hydrochloric acid (17 ml, 0.2 mol) was placed in a flask equipped with a reflux condenser and shaken in a boiling water bath for about 90 min. Then the reaction mixture was neutralized with triethylamine. The precipitate formed was collected by filtration and recrystallized from water at pH 7; yield, 7.7 g (40.7%), mp 157—161°C (decomp.), $[\alpha]_D^{17}$ +32.2° (*c* 4, N HCl). Found: C, 50.55; H, 8.13; N, 7.71%. Calcd for $C_8H_{15}O_4N$: C, 50.78; H, 7.99; N, 7.40%.

Carbobenzoxy-L-leucine Diphenylmethyl Amide. A mixture of carbobenzoxy-L-leucine (5.3 g, 0.02 mol), diphenylmethylamine hydrochloride (4.4 g, 0.02 mol), and triethylamine (2.8 ml, 0.02 mol) in chloroform (35 ml) was treated with dicyclohexyl carbodiimide (4.2 g, 0.02 mol) at −5°C. After about 3 hr the temperature was brought to 20°C, and reaction was continued for a further 10 hr. The solvent was then replaced with ethyl acetate, and the dicyclohexylurea was removed by filtration. The filtrate was washed successively with N hydrochloric acid, 5% sodium bicarbonate, and water, and dried over sodium sulfate. The concentration of the dried solution gave crystals, which were recrystallized from a mixture of ethyl acetate and petroleum ether; yield, 6.2 g (72%), mp 122—123°C, $[\alpha]_D^{17}$ +4.7° (*c* 4, dimethylformamide). Found: C, 75.08; H, 7.01; N, 6.68%. Calcd for $C_{27}H_{30}O_3N_2$: C, 75.32; H, 7.02; N, 6.51%.

L-Leucine Diphenylmethylamide Hydrobromide. Carbobenzoxy-L-leucine diphenylmethylamide (3.87 g, 0.009 mol) was treated with a 25% solution of hydrogen bromide in acetic acid (9 ml). After a 45-min reaction at room temperature, dry ether (about 200 ml) was added to the mixture in order to precipitate the product as crystals, which were recrystallized from ethanol and ether; yield, 2.16 g (64%), mp 204—206°C (decomp.), $[\alpha]_D^{17}$ +12.9° (*c* 2, methanol). Found: C, 60.36; H, 6.79; N, 7.36%. Calcd for $C_{19}H_{25}ON_2Br$: C, 60.47; H, 6.69; N, 7.42%.

Carbobenzoxy-L-glutamic Acid γ-Diphenylmethylamide. A solution of carbobenzoxy-L-glutamic acid γ-hydrazide[8] (10.3 g, 0.035 mol) and 6 N hydrochloric acid (11.7 ml) in tetrahydrofuran (100 ml) was treated with a saturated solution of sodium nitrite (2.42 g, 0.035 mol) in water at −5—−10°C. After about 15 min, diphenylmethylamine hydrochloride (7.69 g, 0.035 mol) was added to the reaction mixture, and the solution was adjusted to pH 8 with triethylamine (about 16 ml was used). The mixture was allowed to react at 4°C for 2 days with stirring, and then the tetrahydrofuran was removed by evaporation. The residue was dissolved in ethyl acetate, and the solution was washed with N hydrochloric acid, 5% sodium bicarbonate, N hydrochloric acid, and water, and dried. The removal of the solvent gave crystals, which were recrystallized from ethyl acetate; yield, 7.9 g (50.4%), mp 162—164°C, $[\alpha]_D^{17}$ −5.5° (*c* 2, dimethylformamide). Found: C, 69.91; H, 5.58; N, 6.41%. Calcd for $C_{26}H_{26}O_5N_2$: C, 69.94; H, 5.87; N, 6.27%.

7) S. Sakakibara and N. Inukai, This Bulletin, **39**, 1567 (1966).

8) B. Hegedüs, *Helv. Chim. Acta*, **31**, 737 (1948).