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Ethyl Thioltrifluoroacetate as an Acetylating Agent with Particular Reference to Peptide Synthesis[1]

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Recent developments in fluorine and sulfur chemistry have led us to investigate the use of ethyl thioltrifluoroacetate as an acetylating agent for amino acids and peptides in aqueous solution. The intense electrophilic property of the trifluoroacyl group is combined with the unusual aminophilicity of the sulfur atom in this thiol ester. The hydrolytic stability of the ester is in sharp contrast to the highly reactive trifluoroacetic anhydride which has been used to prepare several N-trifluoroacetylamino acids and simple peptide derivatives. These acyl-amino acids are stable in acidic media, but the ease with which the trifluoroacylamide bond undergoes hydrolysis at a *p*H greater than 10 distinguishes this protective group from others used in peptide chemistry. Ethyl thioltrifluoroacetate acetylates the amino acid anion in aqueous solution in good yield, giving crystalline products which are easily purified. The applicability to peptide synthesis was demonstrated by the preparation of N-trifluoroacetylglycyl-D,L-phenylalanine. Synthesis of the dipeptide was effected in 50% aqueous tetrahydrofuran at room temperature by treatment of D,L-phenylalanyl anion with N-trifluoroacetylglycine thiophenyl ester. The optical integrity was verified by studying the properties of N-trifluoroacetyl-L-phenylalanine. Hydrolytic cleavage of the trifluoroacyl–nitrogen bond yielded the optically active amino acid of unchanged rotation. Conversion of the acylamino acid to the anilide, followed by mild hydrolysis, led to the isolation of L-phenylalanylanilide. These observations indicate that thiol esters of N-trifluoroacetylamino acids may find application in the controlled formation of the peptide bond in aqueous media.

Synthetic methods applicable to the controlled formation of the peptide bond in general require the preparation of an amino acid derivative in which the reactivity of one of the functional groups is masked. A requisite imposed on the practicality of such functional derivatives is that regeneration be effected under mild conditions.

The preparation of a number of N-trifluoroacetylamino acids has realized the exceptional character of this protective group.[2] The acetylating agent employed by Weygand and co-workers was the highly reactive trifluoroacetic anhydride. Some undesirable features were associated with the use of this reagent: the reactivity of the reagent, coupled with an unusual solubility in hydrolytic solvents, prohibited applications in Schotten–Baumann-type acylation reactions; unsymmetrical anhydrides were formed between the N$^{\alpha}$-trifluoroacetylamino acids and the generated trifluoroacetic acid; optically active centers were racemized in the presence of excess anhydride.

However, developments in organic sulfur chemistry led to a novel approach to the problem of trifluoroacetylation. Various esters of thiol acids have attained a chemical significance in recent years following the elucidation of the structure of Coenzyme A, which established the biochemical importance of the thiol ester.[3] The intense electrophilic property of the trifluoroacyl group combined with the unusual aminophilicity of the sulfur atom in a thiol ester suggested an investigation of the acetylating properties of ethyl thioltrifluoroacetate.[4] Acetyl transfer from the ethyl mercaptide radical to an amino nitrogen atom would be analogous to the ammonolysis–aminolysis reaction of the oxygen-esters.

Direct acetylation of amino acids and peptides in an aqueous solution was demonstrated by the isolation of N-trifluoroacetylamino acids from aqueous solutions of the amino acid anions following treatment with ethyl thioltrifluoroacetate; saturation of the aqueous phase with respect to the thiol ester was maintained. In general, the products were precipitated on acidification of the reaction mixture. The extreme volatility of ethyl mercaptan obviated any tedious purification scheme. Acetylation was effected under mild conditions in aqueous solution (*p*H 8–9). Hydrolytic cleavage of the protecting group occurred on increasing the basicity of the solution to a *p*H of 10 or greater; dilute aqueous sodium hydroxide or concentrated aqueous ammonia was employed. Under such conditions, the fragile peptide linkage was not ruptured. The applications to peptide chemistry are manifest.

This paper reports the preparation of a number of N-trifluoroacetylamino acids which had not been characterized thus far, describes the preparation of a simple dipeptide, and presents evidence for the optical integrity of these compounds during chemical manipulations. Some properties of the acylamino acids are collected in Table I.

The Nα-trifluoroacetylamino acids are stable crystalline compounds; no evidence of decomposition could be detected after two years. The compounds are appreciably soluble in aqueous media; the trifluoromethyl group also promotes increased solubility in a number of organic reagents, especially benzene. Titration with aqueous alkali indicated that the trifluoroacyl–nitrogen bond is labile at a *p*H greater than 10. Qualitative hydrolytic rates of N-trifluoroacetylglycine and several other amino acids were determined at *p*H 12; the data are summarized in Fig. 1.

Evidence that the trifluoroacetyl groups in the mono-acetylated D,L-lysine and D,L-ornithine were in the terminal positions, and not on the α-amino groups, was obtained from a study of the visible absorption spectra of aqueous solutions of the

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(2) (a) F. Weygand and E. Csendes, *Angew. Chem.*, **64**, 136 (1952); (b) F. Weygand and E. Leising, *Ber.*, **87**, 248 (1954).

(3) "Chemistry and Functions of Coenzyme A," Symposium, *Fed. Proc.*, **12**, 673 (1953), particularly F. Lipmann, G. D. Novelli and F. Lynen.

(4) M. Hauptschein, C. S. Stokes and E. A. Nodiff, THIS JOURNAL, **74**, 4005 (1952).

TABLE I

SOME PROPERTIES OF N-TRIFLUOROACETYLAMINO ACIDS

N-Trifluoroacetyl derivative of	Yield, %	Solvent for recryst.	M.p., °C.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
4-Aminobenzoic acid[a]	92.3	Ethanol–water (5:3)	274 (sublim.)	$C_9H_6F_3NO_3$	46.36	46.41	2.59	2.66	6.01	6.09
ε-Amino-*n*-caproic acid	71.3	Benzene–hexane	88.6–90.6	$C_8H_{12}F_3NO_3$	42.29	42.54	5.33	5.48	6.17	6.12
L(+)-Arginine dihydrate	62.9	Water	140–142	$C_8H_{13}F_3N_4O\cdot 2H_2O$	31.37	31.54	5.60	5.88	18.30	18.41
L-Asparagine	64.5	Methanol–water	163.8–165.2	$C_6H_7F_3N_2O_4$	31.59	31.72	3.09	3.27	12.28	11.98
Glycine[b]	54.8	Benzene	114–116.4	$C_4H_4F_3NO_3$	28.08	28.59	2.36	2.31	8.19	8.23
D,L-Lysine (N$^\epsilon$)	70.0	Water–ethanol (2:3)	226–231 dec.	$C_8H_{13}F_3N_2O_3$	39.67	39.39	5.41	5.54	11.57	11.35
D,L-Methionine	70.2	Benzene–pet. ether (2:1)	94.2–96.5	$C_7H_{10}F_3NO_3S$	39.28	34.85	4.11	3.88	5.71	5.78
D,L-Norleucine	48.4	Benzene	79.0–82.5	$C_8H_{12}F_3NO_3$	42.29	42.59	5.33	5.31	6.17	5.86
D,L-Ornithine (N$^\delta$)	53.5	Water–ethanol (1:1)	228–232 dec.	$C_7H_{11}F_3NO_3$	36.84	36.84	4.86	4.96	12.27	12.48
D,L-Phenylalanine	80.4	Benzene–hexane (1:1)	125.6–126.8	$C_{11}H_{10}F_3NO_3$	50.58	50.52	3.86	4.15	5.36	5.18
L(−)-Phenylalanine	76.2	Benzene–hexane (1:1)	119.4–120.6	$C_{11}H_{10}F_3NO_3$	50.58	50.69	3.86	4.09	5.36	5.22
D,L-Tyrosine ethyl ester[c]	99	Ethyl acetate–pet. ether	172.5–174	$C_{13}H_{14}F_3NO_4$	51.15	51.18	4.62	4.85	4.59	4.48
L(−)-Tryptophan hydrate	48.4	Water	Sinter 95 melt 162–164	$C_{13}H_{11}F_3N_2O_3\cdot H_2O$	49.06	49.41	4.12	3.93	8.80	9.18
D,L-Valine	64.6	Benzene–pet. ether (4:3)	117.6–120.6	$C_7H_{10}F_3NO_3$	39.44	39.69	4.73	4.83	6.57	6.49

[a] Reported for N-trifluoroacetyl-4-aminobenzoic acid, m.p. 285° (ref. 1b). [b] Reported for N-trifluoroacetylglycine, m.p. 120–121° (ref. 1a). [c] Reported for N-trifluoroacetyl-D,L-tyrosine ethyl ester, m.p. 175–176° (ref. 9).

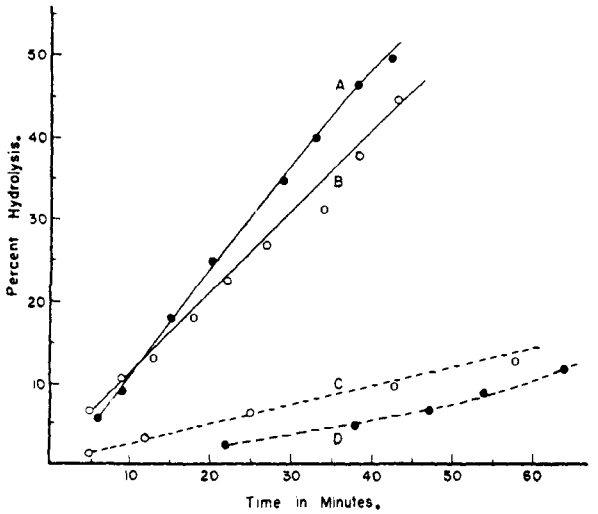


Fig. 1.—Qualitative hydrolytic rates determined in 40% aqueous ethanol at *p*H 12.0–12.1 and room temperature: A, N-trifluoroacetylglycine; B, N-trifluoroacetyl-D,L-lysine; C, N-trifluoroacetyl-D,L-norleucine; D, N-trifluoroacetyl-D,L-phenylalanine.

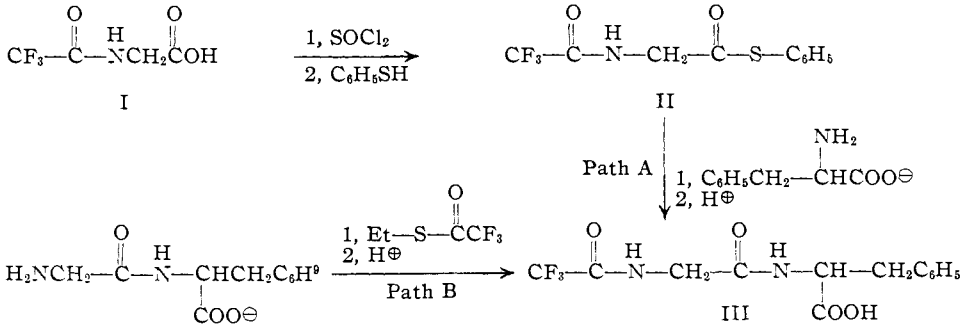
compounds in the presence of cupric ion (Table II). The spectrum of N-trifluoroacetyl-D,L-ornithine in acidic media has absorption bands of wave length and molar extinction characteristic of the cupric chelates of α-amino acids.[5]

TABLE II

VISIBLE ABSORPTION SPECTRA OF THE CUPRIC CHELATES OF SEVERAL AMINO ACIDS AND TRIFLUOROACETYLAMINO ACIDS

Compound	*p*H	λmax, mμ	εmax
α-D,L-Aminobutyric acid	3.20	745	23.6
	4.46	672	37.2
	7.72	620	51.0
	9.42	615	60.4
ε-Aminocaproic acid	4.70	>790	..
	5.96	>790	..
D,L-Ornithine hydrochloride	3.02	755	20.8
	3.72	715	29.8
	4.88	640	43.4
	9.22	630	60.4
Nδ-Trifluoroacetyl-D,L-ornithine	3.06	750	23.6
	3.78	690	33.6
Trifluoroacetylglycine	2.45	>790	..
	5.65	>790	..
	11.5 (hydrolysis)	635	..

indicated by the synthesis of N-trifluoroacetylglycyl-D,L-phenylalanine (III) *via* the thiolphenyl ester method (path A) developed by Th. Wieland in Germany and R. Schwyzer in Switzerland.[6] The acetylation of the dipeptide anion also could be effected by the use of ethylthioltrifluoroacetate (path B). The reaction scheme is outlined below.

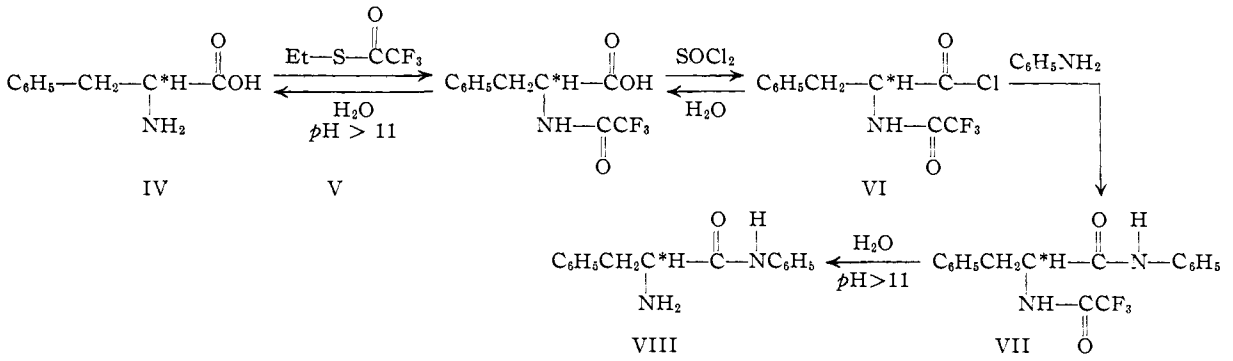


The applicability of thiol ester derivatives of the trifluoroacylamino acids to peptide chemistry was

The retention of optical asymmetry was demon-

(5) (a) H. Borsook and K. V. Thimann, *J. Biol. Chem.*, **98**, 671 (1932); (b) I. M. Klotz, I. L. Falles and J. M. Urquhart, *J. Phys. Colloid Chem.*, **54**, 18 (1950).

(6) (a) Th. Wieland, W. Schäfer and E. Bokelmann, *Ann.*, **573**, 99 (1951); (b) Th. Wieland and W. Schäfer, *ibid.*, **576**, 104 (1952); (c) Th. Wieland and H. Bernhard, *ibid.*, **582**, 218 (1953); (d) R. Schwyzer, *Helv. Chim. Acta*, **36**, 414 (1953); **37**, 647 (1954); (e) R. Schwyzer and Ch. Hürlimann, *ibid.*, **37**, 155 (1954).



strated by several hydrolytic experiments on derivatives of L-phenylalanine. Isolation of L-phenylalanylanilide (VIII), whose properties agreed with those reported earlier,[7] indicated that activation of the carboxyl group of the asymmetric acylamino acid did not alter the special configuration.

Experimental[8,9]

A. N-Trifluoroacetyl Derivatives.—The same procedure was used in the preparation of most of the derivatives whose properties are described in Table I. Modifications in the method are indicated below for the particular amino acids.

General Procedure.—The amino acid was dissolved in 1.00 equivalent of *N* sodium hydroxide in a flask possessing a ground glass joint and stopper fitted with a stopcock open to the atmosphere. Ethyl mercaptan, the by-product of the acetylation, was allowed to escape from the system; the reactions were run in a well-ventilated hood. Ethyl thioltrifluoroacetate was added (0.20 ml./mmole amino acid, *i.e.*, 1.6:1 molar ratio), and the heterogeneous reaction mixture was shaken mechanically for 24 hours. Upon acidification with 1 ml. of concentrated hydrochloric acid, the mixture was cooled in an ice-water bath and the precipitated product was collected by filtration.

N-Trifluoroacetylglycine (I).—A solution of 378.2 mg. of (5.04 mmoles) glycine in 5 ml. of distilled water was titrated to *p*H 10 with 3.02 ml. of 1.00 *N* sodium hydroxide. Upon the addition of 1.50 ml. (1.82 g., 11.5 mmoles) of ethyl thioltrifluoroacetate[4] the heterogeneous solution was placed on a mechanical shaker for 18 hours. The solution was acidified with 2 ml. of 1 *N* hydrochloric acid and extracted with three 10-ml. portions of ethyl ether. The ethereal extract was taken to dryness under reduced pressure. The crystalline residue, after drying to constant weight in a vacuum desiccator, weighed 476.6 mg. (54.8%), m.p. 110.5–116.5°. A sample was recrystallized several times from benzene, m.p. 114–116.5°; reported m.p. 116°.[6] A sample was dried at 50° for analysis.

Anal. Calcd. for $C_4H_4F_3NO_3$: C, 28.08; H, 2.36; N, 8.19; neut. equiv., 171. Found: C, 28.59; H, 2.31; N, 8.23; neut. equiv., 174; *pK*, 3.05.[9]

In a second experiment the acetylation of glycine was carried out in a sodium borate buffer solution (a saturated aqueous solution of sodium tetraborate, *p*H 9.2). To a solution of 750.7 mg. (10.0 mmoles) of glycine in 10.0 ml. of 1.00 *N* sodium hydroxide was added 40.0 ml. of borate buffer solution and 2.00 ml. (2.47 g., 15.6 mmoles) of ethyl thioltrifluoroacetate. The heterogeneous solution was placed on a mechanical shaker; at specified time intervals 10.0-ml. aliquots were withdrawn, acidified with 1 *N* hydrochloric acid, and extracted with three 15-ml. portions of ether. After drying over anhydrous magnesium sulfate the ethereal extracts were evaporated in tared flasks and the weight of crystalline residue determined after drying to constant weight *in vacuo*. The results are tabulated in Table III.

TABLE III

RECOVERY OF TRIFLUOROACETYLGLYCINE AS A FUNCTION OF TIME

Time, hr.	Wt. residue, mg.	Cor. wt., mg.	Product, %	
0	19.7	...	...	
2	85.8	66.1	19.3	(Based on 342.8 mg. theoretical)
4	136.7	117.0	34.1	(Based on 342.8 mg. theoretical)
6	277.7	258.0	75.3	(Based on 342.8 mg. theoretical)
8[a]	176.3	156.6	91.3	(Based on 171.4 mg. theoretical)
8[b]	197.5	177.8	103	(Based on 171.5 mg. theoretical)

[a] 10.0 ml. of buffer solution added immediately before withdrawal of aliquot. [b] Ethereal extractions contained unreacted thiol ester.

N$^{\epsilon}$-Trifluoroacetyl-D,L-lysine.—To a solution of 1.83 g. (10.0 mmoles) of D,L-lysine monohydrochloride in 10.0 ml. of 1 *N* sodium hydroxide was added 2.0 ml. of ethyl thioltrifluoroacetate. The heterogeneous mixture was shaken for six hours. A precipitate slowly separated and finally filled the solution. The reaction mixture was cooled in ice-water and the solid was collected by filtration; yield 1.81 g. (75%), m.p. 224–230° with decomposition. The crude material was dissolved in 10 ml. of hot water and the solution was diluted with 15 ml. of hot ethanol. White, rectangular crystals separated on cooling; yield 1.25 g. (69% recovery), m.p. 226–231° dec. A second recrystallization yielded an analytical sample which was dried at 100° *in vacuo*.

Anal. Calcd. for $C_8H_{13}N_2O_3F_3$: C, 39.67; H, 5.41; N, 11.57; neut. equiv., 242. Found: C, 39.39; H, 5.54; N, 11.35; neut. equiv., 240; *pK*, 9.47.

N-Trifluoroacetyl-D,L-tyrosine Ethyl Ester.—To a suspension of 2.09 g. (10.0 mmoles) of D,L-tyrosine ethyl ester in 10 ml. of ethyl acetate was added 2.0 ml. of ethyl thioltrifluoroacetate. The heterogeneous system was shaken and the solid gradually went into solution. After 24 hours, a crystalline solid had separated. The reaction mixture was taken to dryness under reduced pressure. The residue weighed 3.02 g. (99%), m.p. 166–172°. The crude material was recrystallized from 20 ml. of ethyl acetate by the addition of 60 ml. of petroleum ether (b.p. 30–60°); yield 2.08 g. (69% recovery), m.p. 170–172°. A sample was recrystallized twice from ethyl acetate–petroleum ether for analysis, m.p. 172.6–174°; reported m.p. 175–176°.[10]

Anal. Calcd. for $C_{13}H_{14}F_3NO_4$: C, 51.15; H, 4.62; N, 4.59. Found: C, 51.18; H, 4.85; N, 4.48.

N-Trifluoroacetyl-D,L-norleucine.—The acetylation was effected in the usual manner. However, crystallization of the organic phase could not be induced on acidification of the reaction mixture. The crude product was extracted with three 10-ml. portions of ethyl acetate. The combined extracts were dried over $MgSO_4$. The solution was filtered and concentrated under reduced pressure. The oily residue was dissolved in 40 ml. of hot benzene; slow cooling and

(7) J. C. Sheehan, D. W. Chapman and R. W. Roth, THIS JOURNAL, **74**, 3822 (1952).

(8) Melting points are uncorrected; microanalyses were performed by the Microchemical Laboratory, University of California.

(9) The neutral equivalents were determined by dissolving samples of the respective amino acids in distilled water or 50% aqueous ethanol and titrating with 1.00 *N* sodium hydroxide. The titration curves were constructed by plotting moles of base combined *versus* the apparent *p*H as determined by the glass electrode. The apparent *pK* values were obtained directly from these plots.

(10) A. Taurog, S. Abraham and I. L. Chaikoff, THIS JOURNAL, **75**, 3473 (1953).

vigorous scratching eventually led to the separation of a crystalline solid; yield 1.20 g. (53%), m.p. 77.5–82°. After two recrystallizations from benzene the melting point was 79.0–82.5°. A sample was dried at 40° for analysis.

Anal. Calcd. for $C_8H_{12}F_3NO_3$: C, 42.29; H, 5.33; N, 6.17; neut. equiv., 227. Found: C, 42.59; H, 5.31; N, 5.86; neut. equiv., 222; *pK*, 4.36 (50% aqueous ethanol).

B. Hydrolytic Experiments.—The hydrolytic experiments were performed in 40% aqueous ethanol at *p*H 12 and at room temperature. A typical run was performed as follows: A solution of 54.6 mg. (0.241 mmole) of N-trifluoroacetyl-D,L-norleucine in 10.0 ml. of 50% aqueous ethanol was titrated to *p*H 12.0 by the addition of 2.93 ml. of standard 0.1 *N* sodium hydroxide. The *p*H of the solution was maintained at 12.0–12.1 by the addition of standard alkali from a buret; measurements were made against a Beckman Type "E" glass electrode. The addition of alkali was recorded as a function of time; and the percentage hydrolysis was determined from a plot of base consumed *versus* time. The data are summarized in Fig. 1.

C. Spectral Studies of the Cupric Chelates of Some Amino Acids and N-Trifluoroacetyl Derivatives.—Solutions were prepared of the respective amino acids and N-trifluoroacetyl derivatives which were of 0.040 *M* concentration. A 0.0100 *M* solution of cupric chloride was prepared by dissolving 1.706 g. of cupric chloride dihydrate in one liter of distilled water. Equi-volume mixtures of the cupric ion solution and solutions of the amino acids were prepared immediately before the spectra were determined: final concentrations—0.0050 *M* cupric ion, 0.020 *M* amino acid. The visible absorption spectra were determined on a Cary recording spectrophotometer, Model 11. The solvent blank was distilled water; 5.0 cm. quartz cells were employed. The *p*H of the solutions were determined with a Beckman glass electrode; the solutions were basified with 6 *N* sodium hydroxide, and concd. hydrochloric acid was used to acidify the solution of ε-aminocaproic acid. At a *p*H greater than 6, solutions of ε-aminocaproic acid and N-trifluoroacetylglycine deposited a pale blue gelatinous precipitate of cupric hydroxide. The characteristic cupric chelate absorption band at 635 mμ appeared in the solution containining N-trifluoroacetylglycine after standing at *p*H 11.5 for 8 hours. The data are tabulated (Table II).

D. Peptide Synthesis.—N-Trifluoroacetylglycyl-D,L-phenylalanine was prepared by two distinct methods: (1) a direct synthesis *via* the thiophenyl ester of the acetylated glycine, and (2) acetylation of a sample of a commercial preparation of the dipeptide.

N-Trifluoroacetylglycine Thiophenyl Ester (II).—In a 50-ml. pear-shaped flask fitted with a reflux condenser and a calcium chloride tube was placed a suspension of 4.28 g. (0.025 mole) of N-trifluoroacetylglycine. The mixture was heated under reflux for 2.5 hours with 3.0 ml. (4.96 g., 0.041 mole) of purified thionyl chloride. On concentration under reduced pressure (dry nitrogen atmosphere), the solution of the acid chloride was taken up in 10 ml. of dry benzene and concentrated a second time. A solution of 3.50 ml. (3.77 g., 0.034 mole) of thiophenol in 10 ml. of dry benzene was added. The reaction mixture was heated under reflux for 4 hours and allowed to stand at room temperature overnight. Solvent and excess thiophenol were removed under reduced pressure. The solid residue was dissolved in 15 ml. of benzene, treated with Norite, filtered and diluted with 15 ml. of hexane. The yellow crystalline product weighed 5.42 g. (82.4%), m.p. 70–77°. Two recrystallizations from benzene–hexane yielded colorless crystals, m.p. 80.2–81.5°. A sample was dried at 60° *in vacuo* for analysis.

Anal. Calcd. for $C_{10}H_8F_3NO_2S$: C, 45.63; H, 3.06; N, 5.32. Found: C, 45.62; H, 3.45; N, 5.19.

N-Trifluoroacetylglycyl-D,L-phenylalanine (III).—To a solution of 166.5 mg. (1.01 mmoles) of D,L-phenylalanine in 4.0 ml. of distilled water containing one equivalent sodium hydroxide was added a solution of 264.8 mg. (1.01 mmoles) of N-trifluoroacetylglycine thiophenyl ester in 4.0 ml. of tetrahydrofuran. Two phases were present and the mixture was shaken mechanically for 48 hours at room temperature. At intervals, the *p*H of the system was measured with the Beckman glass electrode. The initial *p*H was 9.45; after 24 hours the *p*H fell to 7.65, and remained essentially constant during the second 24-hour period. The solution was evaporated to dryness *in vacuo*. The residue was taken up in 3.0 ml. of 1 *N* HCl by warming and the solution placed in the refrigerator overnight. A crystalline product was collected by filtration; recovered, 96 mg. (30%), m.p. 151.5–154.5°. The solid was soluble in ethanol but sparingly soluble in cold water. Recrystallization from 1.5 ml. of water yielded 44.2 mg. (46% recovery), m.p. 152.5–155°. The material was dried at 80° *in vacuo* for analysis.

Anal. Calcd. for $C_{13}H_{13}F_3N_2O_4$: C, 49.06; H, 4.12; N, 8.80. Found: C, 48.80; H, 4.26; N, 8.85.

Acetylation of Glycyl-D,L-phenylalanine.—To a solution of 222.1 mg. (1.00 mmoles) of glycyl-D,L-phenylalanine (Mann Assayed Biochemicals, C.P. grade) in 1.00 ml. of 1 *N* sodium hydroxide was added 0.25 ml. of ethyl thioltrifluoroacetate. The suspension was shaken mechanically at room temperature for 5 hours. The solution was acidified with 0.50 ml. of 6 *N* HCl, cooled in ice-water, and the solid was collected by filtration; yield 312.2 mg. (98%), m.p. 152–155°. A mixed melting point with the product isolated from the direct synthesis showed no depression.

E. Optical Integrity. N-Trifluoroacetyl-L-phenylalanine (V).—The acylated amino acid was prepared according to the general procedure. The crude material was recrystallized from 50 ml. of hot benzene by diluting with 30 ml. of hexane. The product crystallized as colorless needles; yield 2.02 g. (78.2% of theory), m.p. 119.4–120.6°. A second recrystallization from benzene–hexane yielded material for analysis, m.p. 119–120.6°, optical rotation: in 95% ethanol $[\alpha]^{24.8}D$ +13.8° (0.0208 g. in 5.00 ml. of 95% ethanol); in glacial acetic acid $[\alpha]^{25}D$ +36.4° (0.0187 g. in 5.00 ml. of glacial acetic acid). A sample was dried at 80° for analysis.

Anal. Calcd. for $C_{11}H_{10}F_3NO_3$: C, 50.58; H, 3.86; N, 5.36. Found: C, 50.69; H, 4.09; N, 5.22.

Hydrolysis of N-Trifluoroacetyl-L-phenylalanine.—A solution of 262.4 mg. (1.00 mmole) of N-trifluoroacetyl-L-phenylalanine in 5 ml. of 95% ethanol was titrated with standard 1 *N* sodium hydroxide. Excess alkali (5.00 ml. total) was added and the solution was allowed to stand at room temperature for 24 hours. The solution was back titrated with standard 1 *N* hydrochloric acid and evaporated to dryness. The residue was taken up in 3 ml. of water, the insoluble material collected by filtration and crystallized from 2 ml. of water; yield 73.0 mg. (44%). The optical rotation was determined in distilled water, $[\alpha]^{22}D$ −32.6° (0.0302 g. in 5.00 ml. of water). A sample of L-phenylalanine used as starting material had a specific rotation $[\alpha]^{21.6}D$ −34.2°.

N-Trifluoroacetyl-L-phenylalanyl Chloride (VI).—To a suspension of 261 mg. (1.00 mmole) of N-trifluoroacetyl-L-phenylalanine in 5 ml. of dry benzene was added 0.20 ml. (0.32 g., 2.7 mmoles) of purified thionyl chloride. The mixture was heated under reflux for 2.5 hours in a system protected from atmospheric moisture by a calcium chloride tube. Solvent and excess thionyl chloride were removed under reduced pressure (dry nitrogen atmosphere). The crude product was washed with 5 ml. of dry benzene and again taken to dryness. The residue was dissolved in 15 ml. of dry benzene; the hot solution was filtered, and, upon cooling, was diluted with 10 ml. of petroleum ether (b.p. 30–60°). After storing the solution in a refrigerator overnight, the crystalline solid was collected on a sintered glass filter and washed with several portions of petroleum ether. The fine, colorless, silky needles were stored in a vacuum desiccator; yield 172 mg. (60.3%), m.p. 105–107°. Two recrystallizations from benzene–petroleum ether (2:1) gave crystals, m.p. 109.5–111.5°. A sample was dried at 50° for analysis.

Anal. Calcd. for $C_{11}H_9ClF_3NO_2$: C, 47.24; H, 3.24; N, 5.01. Found: C, 47.07; H, 3.48; N, 5.12; $[\alpha]^{28.2}D$ +15.5° (0.0081 g. in 5.00 ml. of glacial acetic acid).

Hydrolysis of N-Trifluoroacetyl-L-phenylalanyl Chloride.—To a solution of the acid chloride, prepared from 130.8 mg. of N-trifluoroacetyl-L-phenylalanine in 5 ml. of acetone was added 0.20 ml. of water. The solution was allowed to stand at room temperature for 6 hours, and then evaporated to dryness under reduced pressure. The residue was recrystallized from 10 ml. of benzene diluted with hexane. Needle-shaped crystals which separated were collected by filtration; yield 96.3 mg. (73.7%), m.p. 115.6–117.6°, $[\alpha]^{26}D$ +15.5° (0.0247 g. in 5.00 ml. of 95% ethanol).

N-Trifluoroacetyl-L-phenylalanylanilide (VII).—The acid chloride of N-trifluoroacetyl-L-phenylalanine was prepared from 264.6 mg. (0.946 mmole) of the acetylated amino acid in the usual manner. The crude acid chloride was taken up in 5 ml. of dry benzene and to the cold solution was slowly added a solution of 0.21 ml. (0.20 g., 2.4 mmoles) of aniline in 5 ml. of dry benzene. A heavy white precipitate separated. The reaction mixture was heated under reflux for one hour and the solvent was removed under reduced pressure. The solid residue was extracted with three 4-ml. portions of water, and the crude product was crystallized from 10 ml. of 70% aqueous ethanol. The white, fine, silky needles were filtered from the cold ethanolic solution, washed with two 5-ml. portions of water and dried *in vacuo;* yield 275.6 mg. (80.8%), m.p., 195.5–198.5°. A sample was recrystallized from 70% aqueous ethanol and dried at 100° *in vacuo* for analysis.

Anal. Calcd. for $C_{17}H_{15}F_3N_2O_2$: C, 60.71; H, 4.50; N, 8.33. Found: C, 60.89; H, 4.47; N, 8.10; $[\alpha]^{27.2}D$ +54.3° (0.0196 g. in 5.00 ml. of 95% ethanol).

Isolation of L-Phenylalanylanilide (VIII).—To a solution of 91.1 mg. (0.271 mmole) of N-trifluoroacetyl-L-phenylalanylanilide in 5.0 ml. of 95% ethanol was added 1.0 ml. of 1 *N* sodium hydroxide. The basic solution was allowed to stand for 48 hours at room temperature and then acidified with 1 *N* hydrochloric acid. On evaporation to dryness, the solid residue was extracted with 2 ml. of dilute aqueous ammonia and the crude product was crystallized from 4 ml. of 50% aqueous ethanol; yield 37.0 mg. (56.9%), m.p. 72.6–74.2°, $[\alpha]^{25.5}D$ +22.1° (0.0102 g. in 1.00 ml. of abs. ethanol); reported for L-phenylalanylanilide m.p. 72–74°, $[\alpha]^{25}D$ +19°.[7]

BERKELEY, CALIFORNIA

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF FLORIDA]

Free Radical Additions Involving Fluorine Compounds. IV. The Addition of Dibromodifluoromethane to Some Fluoroölefins[1]

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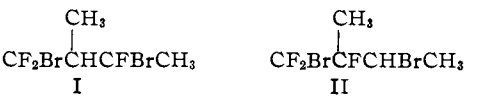
Dibromodifluoromethane has been found to react readily with fluoroölefins containing relatively few fluorine atoms to give the one-to-one addition product in the presence of benzoyl peroxide. The presence of greater number of fluorine atoms in the olefins leads to two-to-one and higher addition products. The effect of the position of the fluorine on the course of the reaction is discussed. The addition products from dibromodifluoromethane and various olefins have been converted into other fluorine-containing molecules such as olefins, dienes and cyclic compounds. In certain cases replacement of bromine by hydrogen can be carried out.

It was reported in the first paper of this series[2] that dibromodifluoromethane reacts with a variety of hydrocarbon olefins in the presence of benzoyl peroxide to give one-to-one addition products which could be converted to 1,1-difluorobutadienes. Chloroölefins were found to be unreactive toward the dibromomethane.

It seemed of interest to study the reaction of dibromodifluoromethane with fluoroölefins to determine the effect of fluorine on the course and extent of the reaction. Furthermore, should the reaction proceed satisfactorily to give simple addition products, it seemed that a convenient method of synthesis of polyfluorobutadienes could be developed. The present paper presents the results of research carried out with these objectives in view.

In most cases, the reaction of dibromodifluoromethane with fluoroölefins proceeded in the manner similar to that described for the hydrocarbon olefins to give one-to-one addition products. In two cases, with 2-trifluoromethylpropene and 2-H-pentafluoropropene, no reaction took place.

It appears that the addition of dibromodifluoromethane to a fluoroölefin takes place in such a manner that the difluorobromomethyl group becomes attached to the carbon of the double bond which contains the fewer number of fluorine atoms. This situation is well represented in the reaction with 2-fluorobutene-2, where the compound represented by I or II could be obtained.



I II

The adduct was shown to have structure I by conversion to a methyltrifluorobutadiene by dehydrohalogenation.

Reactions with fluoroethylenes also follow the same course outlined above. For example, 1,1-difluoroethylene gave the symmetrical product $CF_2BrCH_2CF_2Br$ from which hydrogen bromide could be removed quite readily. Trifluoroethylene also gave some of the one-to-one addition product from which hydrogen bromide was removed to give the known[3] perfluoroallyl bromide. In neither case would it be possible to remove the halogen acid had the difluorobromomethyl group attacked the carbon containing two fluorine atoms.

Haszeldine[4] has shown that fluoroethylene reacts with trifluoromethyl iodide in the presence of ultraviolet radiation to give 1,1,1-tetrafluoro-3-iodopropane. Dibromodifluoromethane has been found to follow the same course with fluoroethylene to give 1,3-dibromo-1,1,3-trifluoropropane. The structure of the adduct was established by its fluorination to a bromotetrafluoropropane which gave the known 1,1,1,3-tetrafluoropropane when reduced with zinc and hydrochloric acid.

It should be noted that the use of the fluoroethylenes and 2-fluorobutene-2 gives products with dibromodifluoromethane in which the order of the free

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(2) P. Tarrant and A. M. Lovelace, THIS JOURNAL, **76**, 3466 (1954).

(3) A. H. Fainberg and W. T. Miller, Jr., p. 7K, Abstracts of Papers, 120th Meeting of the American Chemical Society, New York, N. Y., September, 1951.

(4) R. N. Haszeldine, *J. Chem. Soc.*, 1199 (1953).