UCRL-2632
Unclassified-chemistry distribution

UNIVERSITY OF CALIFORNIA

Radiation Laboratory

Contract No. W-7405-eng-48

# ETHYL THIOLTRIFLUOROACETATE AS AN ACETYLATING AGENT WITH PARTICULAR REFERENCE TO PEPTIDE SYNTHESIS

Elmer E. Schallenberg and M. Calvin

June, 1954

Berkeley, California

# ETHYL THIOLTRIFLUOROACETATE AS AN ACETYLATING AGENT WITH PARTICULAR REFERENCE TO PEPTIDE SYNTHESIS

Elmer E. Schallenberg and M. Calvin

Department of Chemistry and Radiation Laboratory*
University of California, Berkeley 4, California

* The work described in this paper was sponsored by the U. S. Atomic Energy Commission.

## ABSTRACT

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-trifluoroacetyl-amino acids and simple peptide derivatives.[1] These acyl-amino acids are stable in acidic media, but the ease with which the trifluoroacyl-amine bond undergoes hydrolysis at a pH greater than 10 distinguishes this protective group from others used in peptide chemistry.

Ethyl thioltrifluoroacetate[2] acetylates the amino acid anion in aqueous

(1) F. Weygand and co-workers, Angew. Chem., 64, 136 (1952); Ber., 87, 248 (1954).

(2) H. Hauptschein, et al., J. Am. Chem. Soc., 74, 4005 (1952).

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 50% aqueous tetrahydrofuran at room temperature by treatment of D,L-phenylalanyl anion with N-trifluoroacetyl-glycine thiophenyl ester. The application of phenyl thiol esters of acyl-amino acids to peptide chemistry has been described by T. Wieland and co-workers.[3]

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 acyl-amino 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.

---

(3) T. Wieland and co-workers, Ann. 573, 99 (1951) and subsequent papers.

# ETHYL THIOLTRIFLUOROACETATE AS AN ACETYLATING AGENT WITH PARTICULAR REFERENCE TO PEPTIDE SYNTHESIS

Elmer E. Schallenberg and M. Calvin

Department of Chemistry and Radiation Laboratory*
University of California, Berkeley 4, California

---

* The work described in this paper was sponsored by the U. S. Atomic Energy Commission.

---

The rather unusual acetylating properties of free thiol acids were first described by Pawlewski,[1] and recently the thiol analogs of N-acyl amino acids were reported to be active acetylating agents for amines and amino acid derivatives under mild conditions.[2]

Various esters of thiol acids have attained a chemical significance in recent years because of the elucidation of the structure of Coenzyme A.[3] It was found by F. Lipmann, and others, that the coenzyme, which is an important participant in metabolic reactions, is an N-acyl derivative of mercaptoethylamine. Acylation of the thiol group in the coenzyme yields Acetyl Coenzyme A, which in the appropriate environment, rapidly transfers its acyl group to other substrates.

---

(1) B. Pawlewski, Ber., 31, 661 (1898); 34, 657 (1901); 35, 110 (1902). See also H. L. Wheeler, J. Am. Chem. Soc., 23, 444 (1901).

(2) M. W. Cronyn and J. Jiu, J. Am. Chem. Soc., 74, 4726 (1952); J. C. Sheehan and D. A. Johnson, ibid., 74, 4726 (1952).

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

It seemed reasonable, therefore, to investigate the trans-acetylating properties of ethyl thioltrifluoroacetate.[4] The intense electrophilic property of the trifluoroacyl group is combined with the unusual animophilicity of the sulfur atom in this thiol ester; and acetyl transfer from the ethyl mercaptide radical to an amino nitrogen atom would be analogous to the ammonolysis-aminolysis reaction of esters. The ester is of very low water solubility; it is stable in water and dilute acids, but is slowly hydrolyzed by hot dilute aqueous alkali. The transacetylating properties of the thiol ester were demonstrated by the isolation of N-trifluoroacetyl-amino acids from aqueous solutions of the amino acid anions in which saturation of the aqueous phase with respect to the thiol ester was maintained.

We have found that the N-trifluoroacetyl derivatives of racemic and optically active amino acids can be prepared in good yields by this method. The compounds are stable, easily crystallizable products and, in general, are precipitated by acidification of the aqueous reaction mixture. Also, the extreme volatility of ethyl mercaptan obviates any tedious purification scheme. However, the volatility of the mercaptan does require the use of a well-ventilated hood. This acetylation is effected under very mild conditions in aqueous solution; and hydrolytic cleavage of the protective group also can be effected under conditions mild enough so that the fragile peptide linkage is not ruptured, i.e., ammonical solution or dilute aqueous alkali, pH 11-12. Therefore, the applicability to peptide chemistry becomes manifest.

---

(4) M. Hauptschein, C. S. Stokes, and E. A. Nodiff, J. Am. Chem. Soc., 74, 4005 (1952).

The preparation and some properties of N-trifluoroacetyl amino acids have been reported by F. Weygand and co-workers.[5] Acetylation was effected on treatment of the amino acid with the highly reactive trifluoroacetic anhydride. These experimenters found that a racemic product was obtained from an optically active amino acid in the presence of excess anhydride. Also mixed anhydrides were formed when the amino acids were treated with a molar excess of trifluoroacetic anhydride. These unsymmetrical anhydrides, though not isolated, were effective in the acetylation of aromatic amines and amino acid esters. The symmetrical anhydride of the acyl amino acid could be isolated by treating the reaction mixture, containing the mixed anhydride, with triethyl amine.

We wish to report the preparation of a number of N-trifluoroacetyl amino acids which have not been characterized thus far, describe the preparation of a simple dipeptide, and present evidence for the optical integrity of these compounds during chemical manipulations. Some properties of these new acyl amino acids are tabulated (Table I). Preliminary evidence that the trifluoroacetyl group in the compounds called $N^{\varepsilon}$-trifluoroacetyl-D,L-lysine and $N^{\delta}$-trifluoroacetyl-D,L-ornithine is on the terminal amino nitrogen, and not the α-amino group, was obtained from a study of the visible absorption spectra of aqueous solutions of the acyl amino acids in the presence of cupric ion, (Table II). The spectrum in acidic media of N-trifluoroacetyl-D,L-ornithine possess an absorption band of wave length and molar extinction characteristic of the cupric chelates

(5) F. Weygand and E. Scendes, Angew. Chem., 64, 136 (1952); F. Weygand and E. Leising, Ber., 87, 248 (1954).

Table I, Section A

Some Properties of N-Trifluoroacetylamino Acids

| N-Trifluoroacetyl Derivative of | Yield | Solvent for Recryst. | m.p., °C. | Formula |
|---|---|---|---|---|
| 4-Aminobenzoic acid[a] | 92.3 | ethanol water (5:3) | 274° (sublim.) | $C_9H_6F_3NO_3$ |
| L(+) Arginine dihydrate | 62.9 | water | 140-142 | $C_8H_{13}F_3N_4O_3 \cdot 2H_2O$ |
| D,L-Aspartic acid | 45.6 | methanol ether (1:1) | 163.8-165.2 | |
| Glycine[b] | 54.8 | benzene | 114-116.5 | $C_4H_4F_3NO_3$ |
| D,L-Lysine ($N^{\epsilon}$) | 70.0 | water ethanol (2:3) | 226-231 (dec.) | $C_8H_{13}F_3N_2O_3$ |
| D,L-Methionine | 70.2 | benzene pet. ether (2:1) | 94.2-96.5 | $C_7H_{10}F_3NO_3S$ |
| D,L-Norleucine | 48.4 | benzene | 79.0-82.5 | $C_8H_{12}F_3NO_3$ |
| D,L-Ornithine ($N^{\Delta}$) | 53.5 | water ethanol (1:1) | 228-232 (dec.) | $C_7H_{11}F_3NO_3$ |
| D,L-Phenylalanine | 80.4 | benzene hexane (1:1) | 125.6-126.8 | $C_{11}H_{10}F_3NO_3$ |
| L(-) Phenylalanine | 76.2 | benzene hexane (1:1) | 119.4-120.6 | $C_{11}H_{10}F_3NO_3$ |
| D,L-Tryosine ethyl ester[c] | 99 | ethylacetate pet. ether | 172.6-174 | $C_{13}H_{14}F_3NO_4$ |
| L(-) Tryptophan hydrate | 48.4 | water | sinter 95 melt 162-164 | $C_{13}H_{11}F_3N_2O_3 \cdot H_2O$ |
| D,L-Valine | 64.6 | benzene pet. ether (4:3) | 117.6-120.6 | $C_7H_{10}F_3NO_3$ |

Table I, Section B

| N-Trifluoroacetyl Derivative of | Carbon | | Hydrogen | | Nitrogen | |
|---|---|---|---|---|---|---|
| | Calcd. | Found | Calcd. | Found | Calcd. | Found |
| 4-Aminobenzoic acid[a] | 46.36 | 46.41 | 2.59 | 2.66 | 6.01 | 6.09 |
| L(+) Arginine dihydrate | 31.37 | 31.54 | 5.60 | 5.88 | 18.30 | 18.41 |
| D,L-Aspartic acid | | | | | | |
| Glycine[b] | 28.08 | 28.59 | 2.36 | 2.31 | 8.19 | 8.23 |
| D,L-Lysine ($N^{\epsilon}$) | 39.67 | 39.39 | 5.41 | 5.54 | 11.57 | 11.35 |
| D,L-Methionine | 39.28 | 34.85 | 4.11 | 3.88 | 5.71 | 5.78 |
| D,L-Norleucine | 42.29 | 42.59 | 5.33 | 5.31 | 6.17 | 5.86 |
| D,L-Ornithine ($N^{\Delta}$) | 36.84 | 36.84 | 4.86 | 4.96 | 12.27 | 12.48 |
| D,L-Phenylalanine | 50.58 | 50.52 | 3.86 | 4.15 | 5.36 | 5.18 |
| L(-) Phenylalanine | 50.58 | 50.69 | 3.86 | 4.09 | 5.36 | 5.22 |
| D,L-Tryosine ethyl ester[c] | 51.15 | 51.18 | 4.62 | 4.85 | 4.59 | 4.48 |
| L(-) Tryptophan hydrate | 49.06 | 49.41 | 4.12 | 3.93 | 8.80 | 9.18 |
| D,L-Valine | 39.44 | 39.69 | 4.73 | 4.83 | 6.57 | 6.49 |

(a) Reported for N-Trifluoroacetyl-4-Aminobenzoic acid, m.p. 285°, (Ref. 5).
(b) Reported for N-Trifluoroacetyl glycine, m.p. 120-121°, (Ref. 5).
(c) Reported for N-Trifluoroacetyl-D,L-Tyrosine ethyl ester, m.p. 175-176°, (Ref. 8).

Table II

Visible Absorption Spectra of the Cupric Chelates of Several Amino Acids and Trifluoroacetylamino Acids*

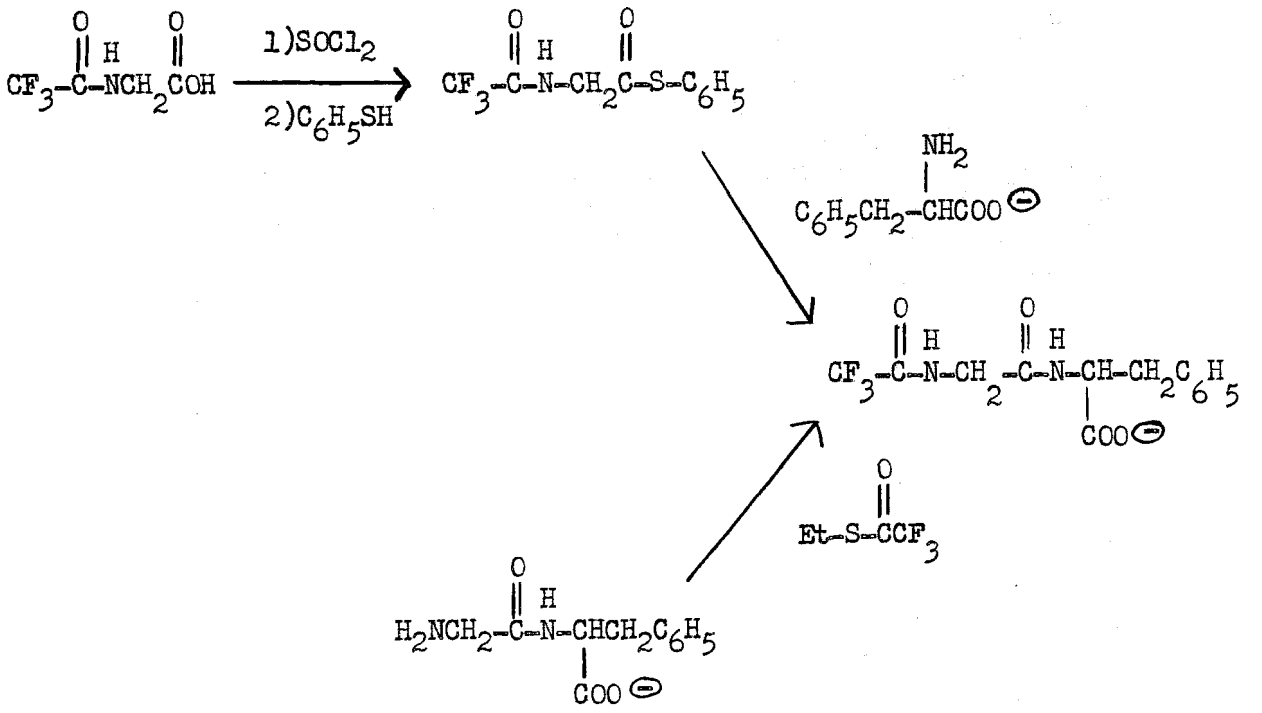
| Compound | pH | $\lambda_{max}$ | $\epsilon_{max}$ |
|---|---|---|---|
| α-D,L-Aminobutyric acid | 3.20 | 745 mμ | 23.6 |
| | 4.46 | 672 | 37.2 |
| | 7.72 | 620 | 51.0 |
| | 9.42 | 615 | 60.4 |
| ε-Aminocaproic acid | 4.70 | >790 | - |
| | 5.96 | >790 | - |
| D,L-Ornithine hydrochloride | 3.02 | 755 | 20.8 |
| | 3.72 | 715 | 29.8 |
| | 4.88 | 640 | 43.4 |
| | 9.22 | 630 | 60.4 |
| $N^{\Delta}$-Trifluoroacetyl-D,L-Ornithine | 3.06 | 750 | 23.6 |
| | 3.78 | 690 | 33.6 |
| Trifluoroacetyl glycine | 2.45 | >790 | - |
| | 5.65 | >790 | - |
| | 11.5 (hydrolysis) | 635 | - |

* Initial concentrations: amino acid, 0.02 M; cupric ion, 0.005 M.

pH measurements made with the Beckman glass electrode; 6 N sodium hydroxide used in titrations.

of α-amino acids.[6] Additional evidence was obtained through semi-quantitative studies on the hydrolytic rates of several N-trifluoroacetyl amino acid derivatives in 50% aqueous ethanol, (Table III).

The applicability of thiol ester derivatives of the trifluoroacyl-amino acids to peptide chemistry was indicated by the synthesis of N-trifluoroacetylglycyl-D,L-phenylalanine via the thiolphenyl ester method developed by Th. Wieland in Germany and R. Schwyzer in Switzerland.[7] The acetylation of the dipeptide anion could also be effected by the use of ethyl thiol trifluoroacetate. The reaction scheme is outlined below.




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

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

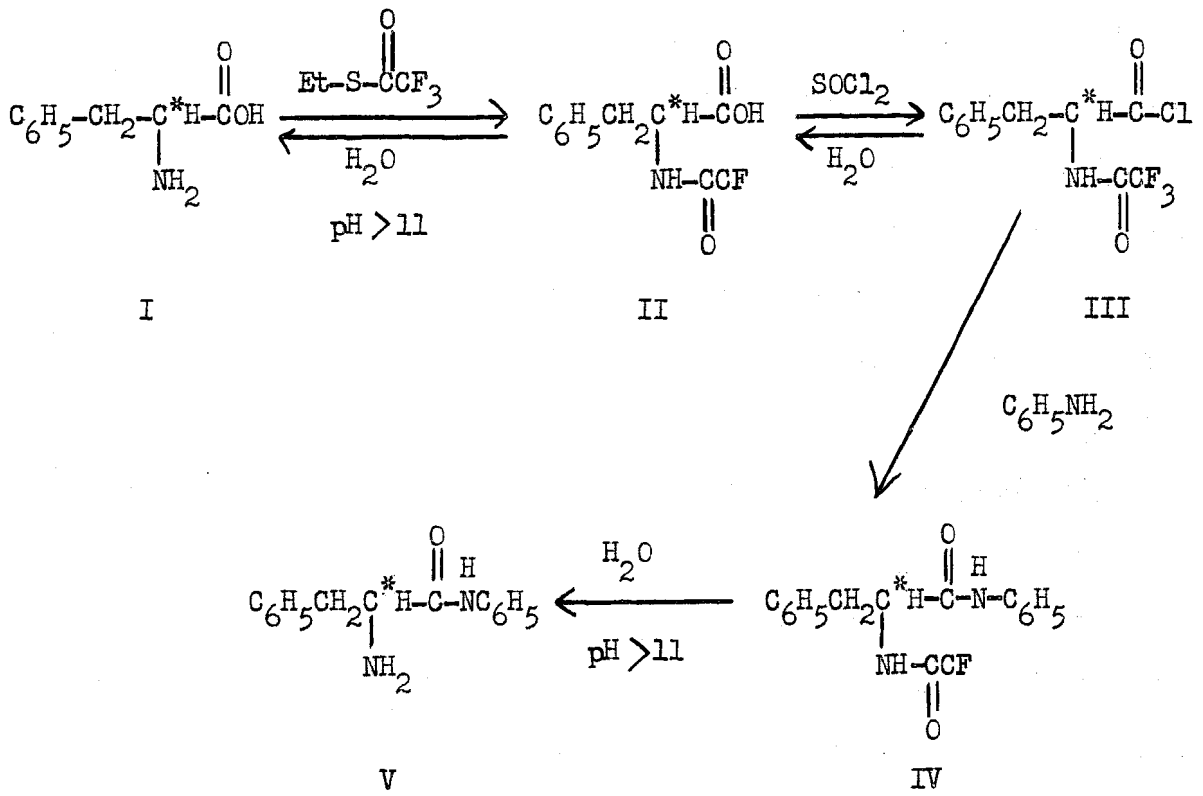
Table III

Hydrolytic Experiments* in 50% Aqueous Ethanol at pH 12.0-12.1

| Compound | Hydrolysis % | Time (min.) |
|---|---|---|
| Trifluoroacetylglycine | 100 | 116 |
| | 50 | 42 |
| | 35 | 29 |
| | 10 | 8 |
| Trifluoroacetyl-D,L-Lysine | 35 | 34 |
| | 10 | 8 |
| Trifluoroacetyl-D,L-Norleucine | 10 | 40 |

* A typical run was made as follows: A solution of 42.2 mg. (2.47 mmoles) N-trifluoroacetylglycine in 10.0 ml. 50% aqueous ethanol was titrated to pH 12.0 by the addition of 1.00 N sodium hydroxide from a buret. pH measurements were made against a Beckman Type "E" glass electrode, and the pH of the solution was maintained at 12.0 ± 0.1 by the addition of alkali. The percent hydrolysis was determined from a plot of base consumed versus time.

The retention of optical asmmetry was demonstrated by several hydrolytic experiments on derivatives of L-phenylalanine and the isolation of L-phenylanilide (V) whose properties agreed with those reported earlier.[8]




Hence we have experimentally verified the optical stability of a trifluoroacetyl amino acid and, further, have demonstrated the applicability of the trifluoroacyl protecting group in peptide chemistry.

## Experimental*

### N-Trifluoroacetyl Derivatives.

Essentially the same procedure was used in the preparation of the derivatives whose properties are described in Table I. Modifications in

(8) J. C. Sheehan, D. W. Chapman, and R. W. Roth, J. Am. Chem. Soc., 74, 3822 (1952).

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

the procedure are indicated below for the particular amino acids.

General Procedure. - The amino acid was dissolved in 1.00 equivalents of 1 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. concentrated hydrochloric acid, the mixture was cooled in an ice-water bath and the precipitated product was collected by filtration.

N-Trifluoroacetyl Glycine. - A solution of 378.2 mg. (5.04 mmole) glycine in 5 ml. distilled water was titrated to pH 10 with 3.02 ml. 1.00 N sodium hydroxide. Upon the addition of 1.50 ml. (1.82 g., 11.5 mmole) ethylthioltrifluoroacetate[4] the heterogeneous solution was placed on a mechanical shaker for 18 hours. The solution was acidified with 2 ml. 1 N hydrochloric acid and extracted with three 10 ml. portions ethyl ether. The etheral extract was taken to dryness under reduced pressure. The crystalline residue, after drying to constant weight in a vacuum dessicator, 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° C.[5] 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. eq., 171. Found: C, 28.59; H, 2.31; N, 8.23; neut. eq., 174; pK, 3.05.

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 pH as determined by the glass electrode. The apparent pK values were obtained directly from these plots.

In a second experiment the acetylation of glycine was carried out in a sodium borate buffer solution (a saturated aqueous solution of sodium tetraborate, pH 9.2). To a solution of 750.7 mg. (10.0 mmole) glycine in 10.0 ml. 1.00 N sodium hydroxide was added 40.0 ml. of borate buffer solution and 2.00 ml. (2.47 g., 15.6 mmole) 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 ether. After drying over anhydrous magnesium sulfate the etheral extracts were evaporated in tared flasks and the weight of crystalline residue determined after drying to constant weight *in vacuo*.

Table IV

Recovery of Trifluoroacetylglycine as a Function of Time

| Time | Wt. residue | Corrected Weight | % Product | |
|---|---|---|---|---|
| 0 hrs. | 19.7 mg. | - | - | |
| 2 | 85.8 | 66.1 mg. | 19.3% | (based on 342.8 mg. theoretical) |
| 4 | 136.7 | 117.0 | 34.1 | " |
| 6 | 277.7 | 258.0 | 75.3 | " |
| 8* (a) | 176.3 | 156.6 | 91.3 | (based on 171.4 mg. theoretical) |
| (b) | 197.5 | 177.8 | 103 | " |

* 10.0 ml. buffer solution added immediately before withdrawal of aliquot. 8(b) etheral extractions contained unreacted thiol ester.

$N^{\epsilon}$-Trifluoroacetyl-D,L-Lysine. - To a solution of 1.83 g. (10.0 mmole) D,L-lysine monohydrochloride in 10.0 ml. 1 N sodium hydroxide was added 2.0 ml. 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. hot water and the solution was diluted with 15 ml. 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. eq., 242. Found: C, 39.39; H, 5.54; N, 11.35; neut. eq., 240; pK, 9.47.

N-Trifluoroacetyl-D,L-Tyrosine Ethyl Ester. - To a suspension of 2.09 g. (10.0 mmole) D,L-tyrosine ethyl ester in 10 ml. ethyl acetate was added 2.0 ml. ethylthioltrifluoroacetate. 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. ethyl acetate by the addition of 60 ml. petroleum ether (b.p. 30-60°). Yield, 2.08 g. (69% recovery), m.p. 170-172°. A sample was recrystallized twice from ethylacetate-petroleum ether for analysis. m.p. 172.6-174°. Reported, m.p. 175-176°.[9]

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 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. hot benzene, slow cooling and 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. eq., 227. Found: C, 42.59; H, 5.31; N, 5.86; neut. eq., 222; pK, 4.36 (50% aqueous ethanol).

---

(9) A. Taurog, S. Abraham, and I. L. Chaikoff, J. Am. Chem. Soc., 75, 3473 (1953).

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. - 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) N-trifluoroacetyl glycine. The mixture was heated under reflux for 2.5 hours with 3.0 ml. (4.96 g., 0.041 mole) purified thionyl chloride. The reaction mixture was concentrated under reduced pressure (dry nitrogen atmosphere). The solution of the acid chloride was taken up in 10 ml. dry benzene and concentrated a second time. A solution of 3.50 ml. (3.77 g., 0.034 mole) thiophenol in 10 ml. 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. benzene, treated with Norite, filtered and diluted with 15 ml. 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. - To a solution of 166.5 mg. (1.01 mmole) D,L-phenylalanine in 4.0 ml. distilled water containing one equivalent sodium hydroxide was added a solution of 264.8 mg. (1.01 mmole) N-trifluoroacetyl glycine thiophenyl ester in 4.0 ml. tetrahydrofuran. Two phases

were present and the mixture was shaken mechanically for 48 hours at room temperature. The pH of the system was measured at intervals by use of the electrode. The initial pH was 9.45; after 24 hours the pH had fallen to 7.65, and 43mained 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. 1 N HCl by warming and the solution placed in the refrigerator overnight. The 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 water. Recrystallization from 1.5 ml. 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 mmole) glycyl-D,L-phenylalanine (Mann Assayed Biochemicals, C.P. grade) in 1.00 ml. 1 N sodium hydroxide was added 0.25 ml. ethyl thioltrifluoroacetate. The suspension was shaken mechanically at room temperature for 5 hours. The solution was acidified with 0.50 ml. 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.

Optical Integrity.

N-Trifluoroacetyl-L-Phenylalanine. - The acylated amino acid was prepared according to the general procedure. The crude material was recrystallized from 50 ml. hot benzene by diluting with 30 ml. 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 $[\alpha]_D^{24.8}$ + 13.8° (0.0208 g. in 5.00 ml. 95% ethanol); in glacial acetic acid $[\alpha]_D^{25}$ + 36.4° (0.0187 g. in 5.00 ml. 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) N-trifluoroacetyl-L-phenylalanine in 5 ml. 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. water and the insoluble material collected by filtration. The residue was crystallized from 2 ml. water. Yield, 73.0 mg. (44%). The optical rotation was determined in distilled water. $[\alpha]_D^{22}$ - 32.6° (0.0302 g. in 5.00 ml. water). A sample of L-phenylalanine used as starting material had a specific rotation $[\alpha]_D^{21.6}$ - 34.2°.

N-Trifluoroacetyl-L-Phenylalanylchloride. - To a suspension of 261 mg. (1.00 mmole) N-trifluoroacetyl-L-phenylalanine in 5 ml. dry benzene added 0.20 ml. (0.32 g., 2.7 mmole 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. The solvent and excess thionyl chloride were removed under reduced pressure (dry nitrogen atmosphere). The crude product was washed with 5 ml. dry benzene and again taken to dryness. The residue was dissolved in 15 ml. dry benzene; the hot solution

was filtered, and, upon cooling, was diluted with 10 ml. petroleum ether. (b.p. 30-60°.) The solution was stored 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 dessicator. 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, 32.4; N, 5.01. Found: C, 47.07; H, 3.48; N, 5.12. $[\alpha]_D^{28.2}$ + 15.5° (0.0081 g. in 5.00 ml. glacial acetic acid).

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

N-Trifluoroacetyl-L-Phenylalanylanilide. - The acid chloride of N-trifluoroacetyl-L-phenylalanine was prepared from 264.6 mg. (0.946 mmole) of the acetylated amine acid in the usual manner. The crude acid chloride was taken up in 5 ml. dry benzene and to the cold solution was slowly added a solution of 0.21 ml. (0.20 g., 2.4 mmole) aniline in 5 ml. 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 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. $[\alpha]_D^{27.2}$ + 54.3° (0.0196 g. in 5.00 ml. 95% ethanol).

*Isolation of L-Phenylalanylanilide.* - To a solution of 91.1 mg. (0.271 mmole) N-trifluoroacetyl-L-phenylalanylanilide in 5.0 ml. 95% ethanol was added 1.0 ml. 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. The acidic solution was evaporated to dryness and the solid residue was extracted with 2 ml. dilute aqueous ammonia. The crude product was crystallized from 4 ml. 50% aqueous ethanol. Yield, 37.0 mg. (56.9%); m.p. 72.6-74.2°; $[\alpha]_D^{25.5}$ + 22.1° (0.0102 g. in 1.00 ml. abs. ethanol). Reported for L-phenylalanylanilide: m.p., 72-74°; $\alpha_D^{26}$ + 19°.[8]