Pauly's reagent) the crude hydrochloride gave three red spots with R_f 0.74 (strong), R_f 0.83 (strong) and R_f 0.95 (weak). By comparison, histamine under the same conditions gave a spot at R_f 0.65, as reported by Ames and Mitchell.[7]

The crude hydrochloride described above was dissolved in 300 cc. of water. To this solution was added a hot solution of 20 g. of picric acid in 250 cc. of water. A black oil separated immediately. The mixture was refluxed for a short time, decanted from an insoluble precipitate which was again extracted with 250 cc. of boiling water. Upon conversion to the hydrochloride the residue gave only a slight pink color with Pauly's reagent and was discarded. Upon cooling the combined aqueous layers, yellow crystals separated, which were filtered and washed with water and ether in order to remove picric acid; yield nearly pure picrate, 1.7 g., m.p. 223–226° (dec.); this corresponds to 0.4 g. of dimethylhistamine or about 0.06% of the original weight of the seeds used. Four recrystallizations from water with very little loss of material raised the m.p. to 229–230°, either alone or in mixture with an authentic sample of synthetic N^{α},N^{α}-dimethylhistamine dipicrate.[9]

Anal. Calcd. for $C_7H_{18}N_3.2C_6H_3N_3O_7$: C, 38.20; H, 3.21; N, 21.10. Found: C, 38.12; H, 3.03; N, 20.86.

A sample of the picrate was dissolved in a small amount of 50% ethanol and shaken with sufficient anion exchange resin Dowex 1-X4 in the chloride form to decolorize the solution. Upon evaporation the dihydrochloride was obtained, m.p. 182–184°, either alone or in mixture with an authentic sample of synthetic N^{α},N^{α}-dimethylhistamine dihydrochloride.[9]

Anal. Calcd. for $C_7H_{13}N_3$ + 2HCl: C, 39.63; H, 7.13; N, 19.81; Cl, 33.43. Found: C, 39.50; H, 7.14; N, 19.40; Cl, 33.31.

The infrared spectra of both salts and of the corresponding authentic synthetic salts were superimposable. With paper chromatography both the isolated material and the synthetic sample gave a single spot at R_f 0.81 under the conditions described above.

The mother liquor from the crude picrate, above, was evaporated to dryness, washed with ether to remove excess picric acid and the amorphous picrate which remained was converted into the hydrochloride, yield 5.0 g. of black resin. On paper chromatography a sample showed a single spot at R_f 0.73. This product is now being investigated.

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Addition of Dibutyltin Hydride to Tetrafluoroethylene

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Silanes have been reported to add to fluoro-olefins *via* the inorganic free radicals, and the possibility of extending this type of reaction to various metal hydrides has been considered.[1] However, no such addition of a metal hydride to a fluoro-olefin to form a fluoro-organometallic compound has been reported up to this time.

Tin hydrides have previously been added to a number of olefins such as styrene and acrylonitrile to form new organotin compounds.[2] This reaction evidently does not involve free radicals since it occurs in the presence of radical inhibitors. Extension of the reaction to include tetrafluoroethylene as the olefin has now been accomplished with di-*n*-butyltin hydride at 90°. Like the other adducts from tin hydrides, this fluoroalkyltin compound is readily formed without the aid of a free radical initiator.

$$(C_4H_9)_2SnH_2 + 2CF_2{=}CF_2 \longrightarrow (C_4H_9)_2Sn(CF_2CF_2H)_2$$

EXPERIMENTAL

Di-*n*-butyltin hydride, b.p. 56–59° (5 mm.), was prepared by reduction of di-*n*-butyltin dichloride with lithium aluminum hydride.[3] A mixture of 4.0 g. (0.017 mole) of di-*n*-butyltin hydride and 10 g. (0.10 mole) of tetrafluoroethylene was heated in an 80-ml. shaker tube at 90° under autogenous pressure for 4 hr. Distillation of the liquid product gave 2.1 g. (28% conversion based on the hydride) of di-*n*-butylbis(1,1,2,2-tetrafluorethyl)tin, b.p. 46–47° (0.2 mm.).

Anal. Calcd. for $C_{12}H_{20}F_8Sn$: C, 33.13; H, 4.64; F, 34.94, Sn, 27.29. Found: C, 33.14, H, 4.39; F, 34.52, Sn, 27.88. The structure was confirmed by determination of the F^{19} and H^1 spectra by nuclear magnetic resonance. The expected doublet for fluorine and triplet for hydrogen, characteristic of the CF_2H group, were found. The presence of the Sn—CF_2 group was established by the observation that this fluorine peak had satellites attributable to splitting by tin isotopes.

There was no evidence that the product, a clear colorless oil, decomposed to tin fluorides and trifluoroethylene under the conditions of the reaction.

CONTRIBUTION NO. 472 FROM THE CENTRAL RESEARCH DEPARTMENT, EXPERIMENTAL STATION, E. I. DU PONT DE NEMOURS & CO., WILMINGTON, DEL.

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Formation of an *N*-Acylamide in Peptide Synthesis

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The reaction between mixed carbonic-carboxylic anhydrides and amines has by now been successfully applied to the synthesis of a large number of

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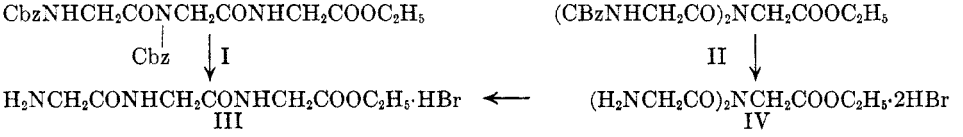
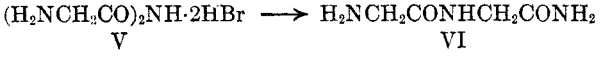
peptides.[2] We report here a side reaction, the formation of an *N*-acylamide, which occurs on coupling carbobenzoxyglycine and glycine ethyl ester by this method.

Under commonly used reaction conditions, there is obtained from the above starting materials a product which contains two carbobenzoxyglycyl residues per glycine ester portion; yields of about 30%, based on carbobenzoxyglycine, are obtained. Saponification of this product results in a mixture from which carbobenzoxyglycylglycine is isolated. Treatment of the substance with hydrazine in ethanol produces carbobenzoxyglycylglycine hydrazide. Removal of both carbobenzoxy groups, by means of hydrogen bromide in acetic acid, leads to an aminoester monohydrobromide which is converted to *N*-(2,4-dinitrophenyl)*di*glycylglycine by saponification and treatment with dinitrofluorobenzene.

Either of the two possible *N*-acylamides, I or II, might conceivably undergo the reactions described above. I, on removal of the blocking groups, would lead directly to the linear tripeptide ester III. From compound II, however, there would be expected *N*,*N*-diglycylglycine ester (IV), since the analogous *N*,*N*-diglycylamide (V) is stable to anhydrous acid.[3] Although the fact that a monohydrobromide is formed on decarbobenzoxylation indicates structure III, additional evidence is readily adduced.



N,*N*-diglycyl amide (V), stable in acid, rearranges cleanly to linear glycylglycine amide (VI) in basic solution;[3] IV could be expected to rearrange to linear triglycine ester under similar conditions.



The decarbobenzoxylated product is, in fact, recovered unchanged after storage in basic solution and reconversion to its hydrobromide, and is therefore linear diglycylglycine ester hydrobromide (III). Structure I, then, can be assigned to the initial coupling product. On the assumption that the diacylamide is formed by reaction of mixed anhydride with carbobenzoxyglycylglycine ester, structure I would have been predicted, for the urethane nitrogen is the more nucleophilic of the two available in the latter reagent.

When excess glycine ester (two moles per mole of anhydride) is employed in the coupling reaction, only the normal product, carbobenzoxyglycylglycine ester, is obtained.

That *N*-acylamide formation during mixed anhydride coupling of amino acids has not previously been reported may indicate that in most cases such reaction is slowed by steric effects. Wieland[3] has indeed reported, although without experimental data, failure to obtain *N*-acylamides on reaction of mixed anhydrides of carbobenzoxy amino acids with carbobenzoxy amino amides, or on fusion of carbobenzoxyglycyl chloride with carbobenzoxyalanine amide.

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EXPERIMENTAL

Coupling of carbobenzoxyglycine and glycine ethyl ester. To 250 ml. of toluene contained in a one-liter, three-neck flask fitted with stirrer, dropping funnel and drying tubes was added 33.4 g. (0.16 mole) of carbobenzoxyglycine and 22 ml. (0.16 mole) of triethylamine. The resulting solution was thoroughly cooled by an ice-salt bath before 16 ml. of ethyl chloroformate (0.16 mole) in 50 ml. of toluene was added. Ten minutes later 125 ml. of chloroform containing 22.2 g. (0.16 mole) of glycine ethyl ester hydrochloride and 22 ml. of triethylamine was dropped in over a period of minutes. The stirred contents of the flask were held at −5° for an additional hour.

After storage at room temperature overnight, the reaction mixture was washed with water, 3% bicarbonate, and again water; on acidification, the bicarbonate washings yielded 3.5 g. of carbobenzoxyglycine. The organic phase was dried over magnesium sulfate and evaporated at reduced pressure. Crystallization began after about one half of the solvent had been removed; 10 g. of product, m.p. 130°, was collected by filtration. (Carbobenzoxyglycylglycine ethyl ester is reported to have m.p. 82–83°.[4]) Although carbobenzoxyglycylglycine ethyl ester was not isolated from the products of this run, other experiments resulted in its identification as an important product.

On recrystallization from ethanol or ethyl acetate there was obtained an analytical sample of the *N*-acylamide, m.p. 131.5–132.0°. In the carbonyl region of the infrared spectrum this product absorbed at 1750, 1735, 1710, 1695, and 1665 cm.$^{-1}$ (potassium bromide pellet). It gave negative tests with ninhydrin and aqueous-alcoholic ferric chloride.

Anal.[5] Calcd. for $C_{24}H_{27}O_8N_3$: C, 59.37; H, 5.61; N, 8.66. Found: C, 59.60; H, 5.58; N, 8.76.

When a portion of the above product was warmed with aqueous alkali, solution occurred. On acidification this solution deposited a crystalline product, m.p. 151–162°. Two recrystallizations from ethanol afforded pure carbobenzoxyglycylglycine, m.p. 177°.

Addition of hydrazine to a solution of the same product in ethanol led to overnight crystallization of crude carbobenzoxyglycylglycine hydrazide, m.p. 159–163°. Two recrystallizations from water led to pure material, m.p. 165–167°.

Decarbobenzoxylation of I. Anhydrous hydrogen bromide was bubbled for 10 min. into 12 ml. of glacial acetic acid

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(5) Microanalyses were performed by Mr. William Saschek of this laboratory.

containing 500 mg. of the *N*-acylamide. After removal of acetic acid under reduced pressure, the residue was crystallized readily from absolute ethanol-ether to give 180 mg. (60%) of hydrobromide, m.p. 179°. Recrystallizations from absolute ethanol yielded an analytically pure sample, m.p. 193–193.5°. This product gave a positive test with ninhydrin and a negative test with ferric chloride.

Anal.[5] Calcd. for $C_8H_{16}O_4N_3Br$: C, 32.23; H, 5.41; Br, 26.87. Found: C, 32.31; H, 5.22; Br, 26.90.

A sample of this product, dissolved in absolute ethanol containing 2% triethylamine, was allowed to remain overnight. After removal of solvent, the residue was taken up in fresh ethanol and treated with anhydrous hydrogen bromide. The product which crystallized on cooling was identical with the starting material in melting point and infrared spectrum.

Another portion of the product was titrated with dilute aqueous alkali to a phenolphthalein end point stable for 2 min. at 50°. The resulting solution was buffered with bicarbonate and treated with dinitrofluorobenzene under the usual conditions.[6] The crude dinitrophenyl derivative so prepared was chromatographed on *p*H 6 buffered paper using a benzyl alcohol–ethanol-water system.[7] Under these conditions *N*-dinitrophenyl glycine had R_f = 0.30; *N*-dinitrophenylglycyl glycine had R_f = 0.40; and *N*-dinitrophenylglycylglycylglycine had R_f = 0.19. The unknown sample ran with the tripeptide derivative.

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5-Fluoronorvaline and 6-Fluoronorleucine

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Discovery of the toxicity of fluoroacetic acid stimulated the investigation of many monofluoro compounds during World War II.[1] The toxicity of these compounds was usually dependent on whether they could be metabolized to fluoroacetic acid. Thus, straight chain ω-monofluoro alkanoic acids with an even number of carbon atoms were found to be toxic because beta-oxidation in living organisms converts them to fluoroacetic acid.[2] R. A. Peters[3] provided evidence that fluoroacetic acid is metabolized in the tricarboxylic acid cycle to fluorocitric acid which combines with aconitase, blocks the cycle, and causes accumulation of citric acid. These studies provided further impetus to the investigation of monofluoro compounds as a means of elucidating biological pathways.[4] The synthesis of two ω-fluoro-α-amino acids, with odd and even numbers of carbon atoms, and their toxicities are reported in this note.

Synthesis. The amino acids were synthesized by the reaction of an ω-fluoroalkyl bromide with the sodio derivative of diethyl acetamidomalonate and hydrolysis of the diethyl acetamido(ω-fluoroalkyl)-malonate with hydrofluoric acid.

$$F(CH_2)_nBr + NaC(COOC_2H_5)_2 \longrightarrow \underset{\displaystyle NHCOCH_3}{\overset{\displaystyle |}{}}$$

$$F(CH_2)_nC(COOC_2H_5)_2 \longrightarrow F(CH_2)_nCHCOOH$$
$$\underset{\displaystyle NHCOCH_3}{\overset{\displaystyle |}{}} \qquad \underset{\displaystyle NH_2}{\overset{\displaystyle |}{}}$$

The procedure was successful when n = 3 or 4, to form 5-fluoronorvaline and 6-fluoronorleucine, but when n = 2, most of the fluorine was lost during the hydrolysis step.

Toxicity data. Toxicities were determined on white Swiss mice by intraperitoneal injection:

Compound	LD_{50}, mg./kg.
5-Fluoronorvaline	1.08
6-Fluoronorleucine	>215 (no deaths)
Sodium fluoroacetate	14.7

5-Fluoronorvaline is highly toxic, whereas 5-fluoronorleucine is relatively nontoxic. Moreover, 5-fluoronorvaline is about 10 times as toxic on a molar basis as sodium fluoroacetate. A toxicity considerably greater than that of sodium fluoroacetate has also been exhibited by other monofluoro compounds such as 6-fluorohexanoic acid and 6-fluorohexylamine.[4] The latter is reported to be about 14 times as toxic as sodium fluoroacetate on a molar basis, but the evidence points to a similar mechanism of action. The compound produces similar symptoms and citric acid accumulation.[4] The cause of toxicities greater than that of sodium fluoroacetate has not been experimentally established, but it has been suggested that the highly toxic compounds are excreted less rapidly or metabolized more efficiently.[4]

The results, in combination with the data in the literature on ω-fluoro compounds, indicate that in ω-monofluoro amino acids, the compounds with an odd number of carbon atoms are toxic; whereas in ω-monofluoro alkanoic acids, the acids with an even number of carbon atoms are the toxic ones. This difference can be explained by the gross mechanism whereby the amino acid is oxidatively deaminated, oxidatively decarboxylated, and β-oxidized, leading eventually to fluoroacetic acid if the original amino acid contained an odd number of carbon atoms.

$$F(CH_2)_3CH(NH_2)COOH \longrightarrow F(CH_2)_3COCOOH$$
$$\longrightarrow F(CH_2)_3COOH \longrightarrow FCH_2COOH$$

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