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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It should be noted in this connection, however, that fluoropyruvic acid, which would be derivable from 3-fluoroalanine, has the relatively low toxicity to rats and mice of 80 mg./kg.[5] and evidence has been presented that this compound is not converted to fluoroacetic acid in the rat.[6]

EXPERIMENTAL[7]

Diethyl acetamido(3-fluoropropyl)malonate. Twenty-three grams (1 mole) of sodium was dissolved in 1 l. of absolute ethyl alcohol and to this solution were added 217 g. (1 mole) of diethyl acetamidomalonate and 141 g. (1 mole) of 1-bromo-3-fluoropropane.[8] The mixture was refluxed for 15 hr., filtered from sodium bromide, and evaporated. The crystalline residue was washed with water and recrystallized from ethyl alcohol. There was obtained 174 g. (63% yield) of diethyl acetamido(3-fluoropropyl)malonate in three crops; m.p. 75–77°.

Anal. Calcd. for $C_{12}H_{20}FNO_5$: F, 6.85; N, 5.05. Found: F, 6.85; N, 5.02 (K).

5-Fluoronorvaline. Diethyl acetamido(3-fluoropropyl)-malonate (140 g.), 325 g. of 48% hydrofluoric acid, and 100 ml. of water were heated at 105–112° for 5 hr. in a "Monel" pressure vessel. The vessel was cooled in ice, opened, and 300 g. of calcium hydroxide was added in portions to bring the *p*H to 3.5. The calcium fluoride was filtered off, washed with water, and the filtrate was evaporated to dryness under reduced pressure. The residue was extracted with ethyl alcohol to remove soluble material. The ethyl alcohol-insoluble residue was recrystallized from ethyl alcohol-water to give 18.5 g. of shining plates of 5-fluoronorvaline; m.p. 190°; yield 27%.

Anal. Calcd. for $C_5H_{10}FNO_2$: F, 14.06; N, 10.37. Found: F, 14.1; N, 10.14 (K).

Diethyl acetamido(4-fluorobutyl)malonate. This compound was prepared by the same procedure used for the 3-fluoropropyl derivative from 1-bromo-4-fluorobutane.[8] It was obtained as a sirup.

6-Fluoronorleucine. The crude diethyl acetamido(4-fluorobutyl)malonate (180 g.) was heated with 360 g. of 48% hydrofluoric acid in a polyethylene bottle in a steam bath for 15 hr. The solution was diluted with 1.5 l. of water, neutralized to *p*H 6 with calcium hydroxide, filtered from calcium fluoride, and evaporated to dryness. The residue was recrystallized 3 times from ethyl alcohol-water to give 43 g. (47% yield) of 6-fluoronorleucine. The product still contained a trace of inorganic material. This was removed by heating the amino acid in water with basic copper carbonate to form the insoluble copper salt, decomposing the copper salt with hydrogen sulfide, and again recrystallizing the amino acid which then melted at 244°.

Anal. Calcd. for $C_6H_{12}FNO_2$: F, 12.74; N, 9.39. Found: F, 12.1; N, 9.13 (K).

Diethyl acetamido(2-fluoroethyl)malonate. This compound was prepared from 1-bromo-2-fluoroethane[8] as described for the 3-fluoropropyl homolog. It was recrystallized from ethyl alcohol to give a 34% yield in 3 crops; m.p. 74–75°.

Anal. Calcd. for $C_{11}H_{18}FNO_5$: F, 7.22; N, 5.32. Found: F, 7.5; N, 5.24 (K).

In hydrolysis experiments, the amino acid produced contained from 8 to 39% of the theoretical amount of fluorine. It was not completely purified.

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DL-3-*p*-Aminophenylalanine. Nitrogen Mustard and Other Derivatives[1]

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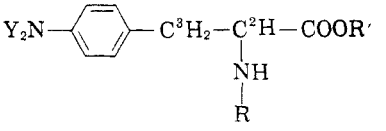
In 1956 Larionov[2] reported that DL-phenylalanine nitrogen mustard ("Sarkolysin") (I) caused the regression of a well established rodent tumor. The need for large amounts of this drug for further experimental tumor studies led us to develop an improved synthesis, since the previous synthetic routes of Bergel *et al.*[3] were not too satisfactory for preparative purposes.

After successful completion of our new synthesis (which is based on the well known azlactone approach to aromatic amino acids), we learned of a recent publication by Pedrazzoli,[4] which describes a similar synthesis of the L- and D- (but not the DL-) forms of phenylalanine nitrogen mustard. Our synthesis also provides a new route to DL-3-*p*-



(I, Y = CH_2CH_2Cl, R = R′ = H)
(II, Y = R = R′ = H)
(III, Y = O, R = C_6H_5CO, R′ = H, 2,3-didehydro, azlactone)
(IV, Y = O, R = C_6H_5CO, R′ = C_2H_5, 2,3-didehydro)
(V, Y = H, R = C_6H_5CO, R′ = C_2H_5)
(VI, Y = R′ = H, R = C_6H_5CO)

(1) Presented before the Organic Division at the San Francisco Meeting of the American Chemical Society, April 1958.

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