that previously obtained with white paraffin oil. No definite relationships appeared evident when comparisons were made of chain length, amount of unsaturation, and functional group. The greatest improvement in the correlation between biological response and R_m for all stationary phases investigated was obtained with either *cis*-9,*cis*-12,*cis*-15-octadecatrienoic acid (linoleic acid) or hexadecanoic acid (palmitic acid).

Although no definite trend resulted from comparison between the stationary phase material and improvement of correlation, the following general relationship was found between functional group and improvement of correlation: fatty acids = alcohols > ethyl esters > white paraffin oil.

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Amino Acid Analogs IV: 4-Fluoroisoleucine

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Received April 28, 1977, from the **Boyce Thompson Institute for Plant Research, Yonkers, NY 10701*, and the ‡*Department of Chemistry, Fordham University, Bronx, NY 10458.* Accepted for publication August 23, 1977.

Abstract □ 4-Fluoroisoleucine was produced by ammonolysis of 2-bromo-4-fluoro-3-methylpentanoic acid, which resulted from the bromofluorination of 4-methyl-2-pentenoic acid. It did not inhibit *Plasmodium berghei* in mice at 640 mg/kg and was not toxic to the animals. The fluoroamino acid inhibited *Aspergillus niger, Trichoderma viride, Myrothecium verrucaria, Trichophyton mentagrophytes,* and *Mucor mucedo* in Czapek solution agar at a concentration between 10^4 and 10^3 μg/ml. Growth of *Escherichia coli* was inhibited 25% at 900 μg/ml in a defined medium.

Keyphrases □ 4-Fluoroisoleucine—synthesized, evaluated for antimicrobial activity □ Antimicrobial activity—4-fluoroisoleucine evaluated □ Amino acid analogs—4-fluoroisoleucine synthesized and evaluated for antimicrobial activity

The preparation of straight chain 3-fluoroamino acids with three to seven carbon atoms as well as 3-fluorovaline was reported (1). The general approach employed started with the bromofluorination of the corresponding 2-alkenoic acid followed by ammonolysis.

DISCUSSION

In attempting to prepare 3-fluoroleucine, 4-methyl-2-pentenoic acid (2) was dissolved in liquid hydrofluoric acid with the subsequent addition of *N*-bromoacetamide. The expected product was not obtained. The resulting mixture was composed of two major products: 2-bromo-4-fluoro-3-methylpentanoic acid and 2-bromo-4,4-dimethyl-4-butyrolactone. The 2-bromo-4-fluoro-3-methylpentanoic acid was converted to 4-fluoroisoleucine by ammonlysis in liquid ammonia.

Identification of these compounds was made by elemental analysis and NMR spectroscopy (see *Experimental*). IR spectra of the three compounds were obtained (Fig. 1).

The neutral product obtained from the addition of the bromo and fluoro elements to 4-methyl-2-pentenoic acid contained no fluorine and showed two singlets in the NMR spectrum corresponding to three protons each at δ 1.48 and 1.63 ppm. This result indicated the fragment $(CH_3)_2C(-X)C$, in which X is an electronegative group that shifts the methyl groups from their original position near δ 1.10 ppm. The classic *ABX* spectrum located between δ 2.47–2.79 (*AB* portion) and 4.7 (*X* portion) ppm, with J_{AB} = 14.5 Hz, indicated geminal coupling of the protons on a saturated carbon atom. This result suggested the presence of the fragment CH_2CHBr. The IR spectrum of the compound (Fig. 1A) showed a strong peak at 1780 cm^{-1}. The spectral data were in agreement with the structure of the compound being 2-bromo-4,4-dimethylbutyrolactone.

The structures of the bromofluoro and fluoroamino acids were deduced from the NMR and IR spectra as follows. That fluorine was attached to a carbon atom bearing a proton was evident from the 49-Hz coupling to the proton centered at δ 5.2 ppm. This coupling was expected for the anticipated product, 2-bromo-3-fluoro-4-methylpentanoic acid. Instead of a doublet near δ 1.10 ppm (J = 7 Hz) of an area corresponding to six protons, characteristic of the isopropyl group as in the starting acid, the product of bromofluorination of 4-methyl-2-pentenoic acid showed a doublet at δ 1.12 ppm (J = 7 Hz) and a doublet of doublets centered at δ 1.37 ppm (J_{HH} = 6 Hz; J_{HF} = 29 Hz).

A comparison of the 60- and 100-MHz spectra indicated that the 29-Hz separation was a coupling constant rather than a chemical shift. The NMR spectrum showed that a rearrangement involving the isopropyl group of the starting acid had taken place. The doublet of doublets is indicative of the fragment CH_3CHF and was reported previously in the product of the addition of the bromo and fluoro elements to crotonic acid (1). Further confirmation that fluorine was present in the molecule and was coupled to the methyl group at δ 1.37 ppm and to the protons at C-3

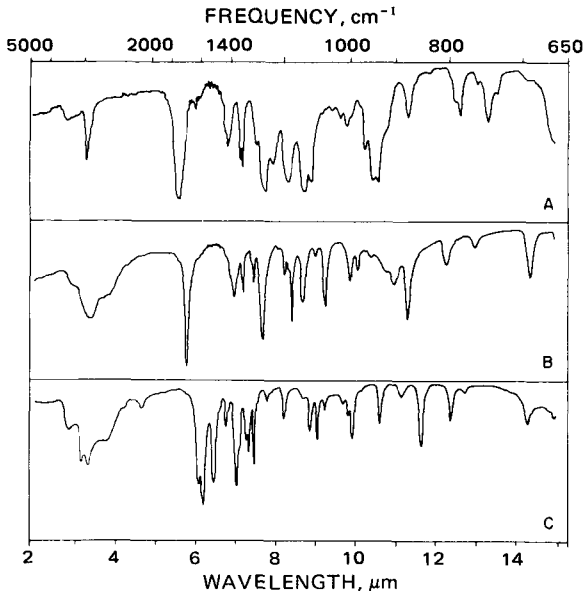
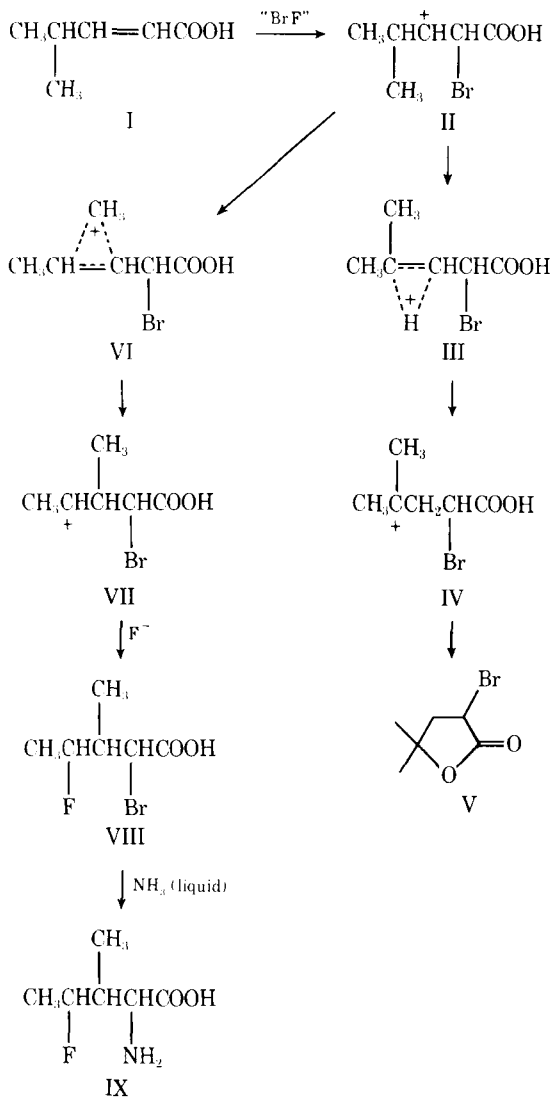


Figure 1—*IR spectra (neat) of: A, 2-bromo-4,4-dimethyl-4-butyrolactone; B, 2-bromo-4-fluoro-3-methylpentanoic acid (KBr); and C, 4-fluoroisoleucine.*



Scheme I

and C-4 was obtained from the ^{19}F-NMR spectrum of the product at 94 MHz. The IR spectrum (Fig. 1B) showed characteristic peaks at 1735 (C=O), 1128 (C—F), and 658 (C—Br) cm^{-1}. It was concluded from these spectra that the structure of the bromofluoro acid was 2-bromo-4-fluoro-3-methylpentanoic acid.

Replacement of the bromo substituent of 2-bromo-4-fluoro-3-methylpentanoic acid with amino yielded an amino acid with NMR characteristics similar to those of the bromofluoro acid. The fact that all NMR peaks could be assigned in a straightforward manner indicated that a single isomer of the amino acid had been obtained. The precise configuration could not be specified from the available data. The IR spectrum showed peaks at 1631 and 1597 (CO_2^-) and 1115 (CF) cm^{-1}. The spectral data were consistent with the structure 2-amino-4-fluoroisoleucine.

A synthetic sequence based on the nonclassical bridged carbonium ion (3), which could explain the formation of the identified compounds, is shown in Scheme I. 2-Bromo-4,4-dimethylbutyrolactone (V) would result from the hydride shift in the initial carbonium ion (II) to yield a tertiary carbonium ion (IV), which should be a lower energy intermediate. Thus, V is the major product isolated from the reaction mixture (60%). Carbonium ion II, which should yield 2-bromo-3-fluoro-4-methylpentanoic acid, would be expected to be of similar stability to the rearranged carbonium ion (VII) involving methyl migration. Why none of the expected product was obtained is not understood.

The methyl, ethyl, and *tert*-butyl esters of 4-fluoroisoleucine have been reported but not the free amino acid (4)[1].

The fluoroamino acid was tested against *Aspergillus niger* (ATCC 1004), *Trichoderma viride* (ATCC 8678), and *Myrothecium verrucaria* (ATCC 9095C) in Czapek solution agar[2] at pH 7.3 and against *Trichophyton mentagrophytes* (ATCC 9129) and *Mucor mucedo* (ATCC 7941) in the same medium in the presence of 10% beef serum[3] according to published methods (5). All fungi were completely inhibited at concentrations between 10^3 and 10^4 μg/ml. Growth of *Escherichia coli* (ATCC 9723) was inhibited 25% in a defined medium (6) at a level of 900 μg of fluoroamino acid/ml.

L-Isoleucine is essential for the growth of erythrocytic forms of *Plasmodium knowlesi* (7). Therefore, 4-fluoroisoleucine was tested against *Plasmodium berghei* in mice. Neither toxicity nor antiplasmodial activity was observed at 640 mg/kg, the highest level administered[4].

EXPERIMENTAL[5]

Bromofluorination of 4-Methyl-2-pentenoic Acid—4-Methyl-2-pentenoic acid (2) (89 g, 0.78 mole) was dissolved in 300 ml of liquid hydrogen fluoride kept at from −30 to −10° in a polyethylene bottle. To the mixture was added *N*-bromoacetamide (118 g, 0.85 mole) in small portions, with stirring, over 0.5 hr. Stirring was continued overnight, allowing the mixture to come to room temperature. Excess hydrogen fluoride was removed under a stream of air, and the residue was poured into a slurry of ice and water. The product was extracted with ether (3 × 150 ml).

2-Bromo-4-fluoro-3-methylpentanoic Acid (VIII)—The ether solution was extracted with 6 *N* NH_4OH (3 × 150 ml), and the aqueous phase was back-extracted with ether (2 × 100 ml). The ether extracts were combined for subsequent workup. The aqueous solution was acidified with hydrochloric acid and extracted with ether (3 × 100 ml). The ether solution, dried with sodium sulfate, was treated with cyclohexylamine dissolved in ether, with stirring, until a slightly basic reaction was detected.

After stirring for 2 hr, the mixture was refrigerated overnight. The cyclohexylamine salt was obtained by filtration (yield of 29.2 g, 12.8%). Then the salt was crystallized several times from an acetone–ether mixture, mp 132–133° (acetone).

Anal.—Calc. for $C_{12}H_{22}BrFNO_2$: C, 46.31; H, 7.13; Br, 25.68. Found: C, 46.44; H, 7.34; Br, 25.42.

[1] M. Hudlicky supplied an NMR spectrum of 4-fluoroisoleucine prepared in his laboratory. The details of his work will be published.

[2] Difco.

[3] Miles Laboratories.

[4] Testing was done by the Rane Laboratory, University of Miami, Miami, Fla.

[5] Melting points were taken in a Thomas-Hoover melting-point apparatus and are uncorrected. GLC was performed on a Varian Aerograph model 1200 gas chromatograph with a flame-ionization detector to which was attached a Varian Aerograph model 20 recorder. The purity of the acids was established by GLC of the trimethylsilyl esters on a column containing 1% Apiezon L on acid-washed Chromosorb W (80–100 mesh), previously treated with dimethyldichlorosilane. IR spectra were obtained with a Perkin-Elmer model 221 spectrophotometer. The 60-MHz NMR spectra were taken with a Varian A-60A spectrometer, and 100-MHz spectra were taken with a Varian XL-100 spectrometer.

Cyclohexylammonium 2-bromo-4-fluoro-3-methylpentanoate (29.0 g, 0.093 mole) was dissolved in 150 ml of 5% HCl with cooling. The mixture was extracted twice with 50-ml portions of chloroform. The solution was dried over sodium sulfate, and the solvent was evaporated under vacuum. The solid residue was crystallized from pentane (18.0 g, 10.9%), mp 72–74°. The analytical sample was crystallized from pentane, mp 74–76°; NMR (100 MHz, deuterochloroform, tetramethylsilane): δ 1.12 [d, C-3 CH_3, $J_{C\text{-}3}$ (H)—C-3 CH_3 = 7 Hz], 1.37 [dd, C-4 CH_3, $J_{C\text{-}4}$ (CH_3)—C-4 H = 6 Hz, $J_{C\text{-}4}$ (CH_3)—C-4 F = 29 Hz], 1.62–2.4 (m, C-3 H), 4.25 [d, C-2 H, $J_{C\text{-}2}$ (H)—C-3 H = 10 Hz], and 4.8–5.6 [m, C-4 H, $J_{C\text{-}4}$ (H)—C-4 F = 49 Hz, $J_{C\text{-}4}$ (H)—C-4 CH_3 = 7 Hz, $J_{C\text{-}4}$ (H)—C-3 H = 2 Hz] ppm.

Anal.—Calc. for $C_6H_{10}BrFO_2$: C, 33.82; H, 4.73; Br, 37.51; F, 8.92. Found: C, 33.59; H, 4.72; Br, 37.67; F, 8.92.

2-Bromo-4,4-dimethyl-4-butyrolactone (V)—The ether extract remaining after removal of 2-bromo-4-fluoro-3-methylpentanoic acid was dried over sodium sulfate and freed of solvent. The residue was distilled and yielded 90 g (60%) of product, bp 110–118°/5.0 mm. An analytical sample boiled at 88–90°/0.75 mm; NMR (60 MHz, deuterochloroform, tetramethylsilane): δ 1.48 [s, C-4 $(CH_3)_2$], 1.63 [s, C-4 $(CH_3)_2$], 2.47–2.79 (C-3 H_2, *AB* portions of *ABX* spectrum), and 4.7 (C-2 H, *X* portion of *ABX* spectrum, J_{AX} = 9 Hz, J_{BX} = 7 Hz, J_{AB} = 14.5 Hz) ppm.

Anal.—Calc. for $C_6H_9BrO_2$: C, 37.33; H, 4.70; Br, 41.40; O, 16.58. Found: C, 37.57; H, 4.72; Br, 41.48; O, 16.30.

4-Fluoroisoleucine (IX)—2-Bromo-4-fluoro-3-methylpentanoic acid (30 g, 0.14 mole) was dissolved in 175 ml of liquid ammonia and sealed in a stainless steel pressure vessel. After remaining at room temperature for 3 days, the excess ammonia was removed. The residue was dissolved in a small volume of water and adjusted to pH 5 with hydrobromic acid. The solution was evaporated under reduced pressure below 40°, and the residue was slurried repeatedly with methanol until a negative halogen test was obtained with silver nitrate.

The residue then was removed by filtration and dried under vacuum. The yield of product was 9.1 g (43.5%), mp 195.5–196° dec. An analytical sample was crystallized from a mixture of water and acetone, mp 202–202.5° dec.; NMR (100 MHz, deuterium oxide, sodium 2,2-dimethyl-2-silapentane-5-sulfonate): δ 1.13 [d, C-3 CH_3, $J_{C\text{-}3}$ (H)—C-3 CH_3 = 7 Hz], 1.36 [dd, C-4 CH_3, $J_{C\text{-}4}$ (CH_3)—C-4 H = 7 Hz, $J_{C\text{-}4}$ CH_3—C-4 F = 25 Hz], 1.9–2.4 (m, C-3 H), 3.78 [d, C-2 H, $J_{C\text{-}2}$ (H)—C-3 H = 4 Hz], and 4.4–5.2 [m, C-4 H, $J_{C\text{-}4}$ (H)—C-4 F = 50 Hz] ppm[6].

Anal.—Calc. for $C_6H_{12}FNO_2$: C, 48.31; H, 8.11; F, 12.74; N, 9.39. Found: C, 48.09; H, 8.10; F, 12.73; N, 9.30.

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[6] The NMR spectrum of 3-fluorovaline as reported in Ref. 1 is now corrected to: δ 1.56 [d, C-3 CH_3, $J_{C\text{-}3}$ (CH_3)—F = 23 Hz] and 1.70 [d, C-3 $(CH_3)_2$, $J_{C\text{-}3}$ $(CH_3)_2$—F = 23 Hz] ppm. These results are based on a comparison of the 60- and 100-MHz spectra.

Modified NF Method for Quantitative Determination of Pentaerythritol Tetranitrate

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Received July 11, 1977, from the *College of Pharmacy, University of Houston, Houston, TX 77004.* Accepted for publication August 11, 1977.

Abstract □ A modified NF method for the quantitative determination of pentaerythritol tetranitrate is reported. A solution of powder is made directly from the dosage form in glacial acetic acid and is then reacted with phenoldisulfonic acid TS. The proposed method saves approximately 75% of the time required with the NF method. The results on six different commercial dosage forms with four different colors and three other active ingredients are reported.

Keyphrases □ Pentaerythritol tetranitrate—colorimetric analysis in dosage forms, NF method modified □ Colorimetry—analysis, pentaerythritol tetranitrate in dosage forms, NF method modified □ Vasodilators—pentaerythritol tetranitrate, colorimetric analysis in dosage forms, NF method modified

The NF method (1) for the quantitative determination of pentaerythritol tetranitrate (I) in dilutions and tablets is lengthy and tedious. The method requires boiling the powder in acetone at 60°, cooling it, centrifuging, and then evaporating the decanted solution at 35°. In the hands of inexperienced analysts, the recovery may not be quantitative. By eliminating these steps, 75% of the time required can be saved. This paper reports a modified NF method for the quantitative determination of I in dosage forms.

EXPERIMENTAL

Reagents and Chemicals—All chemicals and reagents were USP, NF, or ACS grade and were used without further purification.

Solutions—All solutions were prepared according to NF directions (1).

Assay—The dosage form was ground to a fine powder in a mortar. An appropriate quantity (accurately weighed), representing 0.5 mg of I, was transferred to a dry, clean, 150-ml beaker. Then 1 ml of glacial acetic acid was added, and the mixture was stirred for several minutes. A 2-ml quantity of phenoldisulfonic acid TS was added, and the mixture was allowed to stand for 5 min. Then 25 ml of water and 25 ml of ammonia TS were added, and the solution was allowed to cool.

The mixture was transferred to a 100-ml volumetric flask and brought to volume with water. The absorbance value of the clear solution was measured[1] at 409 nm against a reagent blank. For increased accuracy, the quantity of powder may be doubled. If so, all volumes should be doubled.

The modified method was tried on six commercial dosage forms containing four different colors; some contained another active ingredient. The results were calculated according to the NF formula (1) (Table I). The results obtained by the NF method (1) are also presented in Table I.

[1] Bausch & Lomb Spectronic 20.