and dried to yield 0.67 g of material melting at 232–237°. Two recrystallizations from EtOH provided **9** as white needles: mp 265–267°; λ_{max} 231, 285 mμ (ϵ 26160, 9940); nmr (CF_3COOH) at τ 2.45 (singlet, proton assigned to position 8), 3.07 (singlet, proton in position 5), 5.93 and 5.99 (two singlets, six protons of 6,7-OCH_3 groups). The proton in position 2 (NH) is believed to be covered by the solvent signal.

Anal. Calcd for $C_9H_9ClN_2O_4S$: C, 39.06; H, 3.28; Cl, 12.81; N, 10.12; S, 11.60. Found: C, 38.79; H, 3.47; Cl, 12.77; N, 10.10; S, 11.69.

B.—A suspension of 10.2 g (0.04 mole) of **8** in 100 ml of $POCl_3$ was cooled to 5°. Pyridine (6.2 g, 0.08 mole) was then added dropwise at such a rate that the temperature did not exceed 10°. The gummy mixture was heated to reflux, and after 18 hr the solution was cooled and concentrated *in vacuo* to a black oil. This was added to 400 ml of ice–H_2O. The resulting mixture was stirred for 1 hr, and the brown solids were collected by filtration. These were washed with ice–H_2O and dried to furnish 10.2 g of material melting at 142–154°. Crystallization from hot EtOH furnished 5.1 g (47%) of **9**, mp 252–256°, identical in infrared spectrum and mobility on thin layer chromatography with the material described under A.

3-Dimethylamino-6,7-dimethoxy-2H-1,2,4-benzothiadiazine 1,1-Dioxide (2a).—A mixture of 4.7 g (0.02 mole) of **9** and 2.3 g (0.05 mole) of Me_2NH in 75 ml of ethanol was heated in a stainless steel, pressure bomb at 140° for 5 hr. The solvent was then evaporated and the residue was triturated in H_2O. The solids were filtered (3.8 g, mp 309–311°) and recrystallized from $CHCl_3$–EtOH to give 2.7 g (58%) of **2a**: mp 318–320°; λ_{max} 225, 260 and 295 mμ (ϵ 40,110, 13,560, 4237); nmr (CF_3COOH), τ 0.67 (singlet, NH), 5.93 and 5.98 (two singlets, six protons of 6,7-OCH_3 groups), 6.60 (singlet, six protons of $N(CH_3)_2$ group).

Anal. Calcd for $C_{11}H_{15}N_3O_4S$: C, 46.30; H, 5.30; N, 14.73; S, 11.24. Found: C, 46.29; H, 5.42; N, 14.78; S, 11.46.

3-Diethylamino-6,7-dimethoxy-2H-1,2,4-benzothiadiazine 1,1-dioxide (2b) was obtained similarly in 76% yield: mp 193–195° (from ethanol-H_2O); nmr ($CDCl_3$), τ 1.0 (singlet, NH, exchanged with D_2O), 6.18 (singlet, six protons of 6,7-OCH_3 groups), 6.55 (quartet, four CH_2 protons of $N(CH_2CH_3)_2$, J = 7 cps), 8.83 (triplet, six CH_3 protons of $N(CH_2CH_3)_2$, J = 7 cps).

Anal. Calcd for $C_{13}H_{19}N_3O_4S$: C, 49.83; H, 6.11; N, 13.41; S, 10.23. Found: C, 49.59; H, 6.06; N, 13.14; S, 10.13.

3-(4-Methyl-1-piperazinyl)-6,7-dimethoxy-2H-1,2,4-benzothiadiazine 1,1-Dioxide (2c).—A mixture of 3.0 g (0.01 mole) of **9** and 2.2 g (0.02 mole) of N-methylpiperazine in 40 ml of *i*-AmOH was refluxed for 90 min. A solution was obtained at the beginning, followed by a precipitate toward the end of the reaction time. The mixture was chilled, and the solids were collected. Washing of the filtered material with isoamyl alcohol and ether gave 3.2 g (85%) of the desired product, mp 262–263°. The analytical sample (from EtOH–H_2O) melted at 264–266°.

Anal. Calcd for $C_{14}H_{20}N_4O_4S$: C, 49.40; H, 5.92; N, 16.46; S, 9.42. Found: C, 49.56; H, 5.75; N, 16.20; S, 9.49.

3-Diallylamino-6,7-dimethoxy-2H-1,2,4-benzothiadiazine 1,1-dioxide (2d) was prepared similarly in 92% yield, mp 154–156° (from EtOH).

Anal. Calcd for $C_{15}H_{19}N_3O_4S$: C, 53.39; H, 5.68; N, 12.46; S, 9.50. Found: C, 53.56; H, 5.58; N, 12.46; S, 9.61.

Acknowledgment.—The authors are grateful to Dr. Jay W. Constantine for kindly providing the pharmacological results.

Fluorinated Analogs of Leucine, Methionine, and Valine

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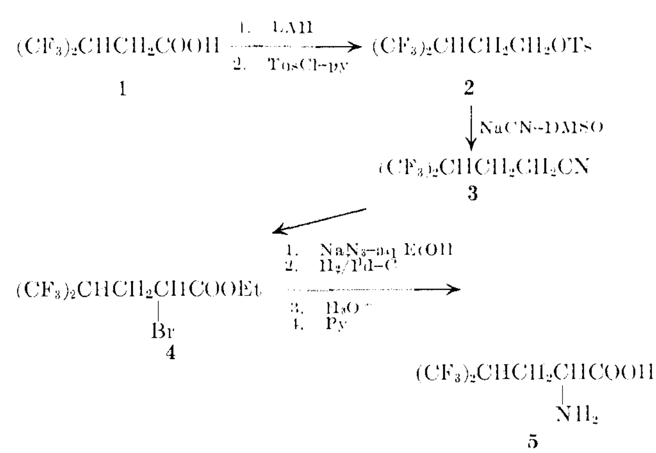
Received July 14, 1967

Many analogs of amino acids have been prepared and studied in a variety of biological systems,[1] but very few have been found that can effectively function like normal amino acids. Since a trifluoromethyl group appears to be approximately the same size as a methyl group, amino acids with CH_3 replaced by CF_3 groups should have approximately the same steric requirements. In addition, the trifluoromethyl group is chemically inert and nontoxic relative to the mono- or difluoromethyl groups as substituents; however, the strong electron-withdrawing effect of CF_3 will alter the acidity of the amino acid function (unless substituted in a remote position in the molecule) which could influence the function of the amino acid in the biological system. A study of fluorinated amino acids (with trifluoromethyl groups) has been reported,[2] but the biological studies were usually limited simply to observations of growth effects on microorganisms. We undertook to prepare selected fluorinated analogs of amino acids and to substitute them for naturally occurring ones in enzymatically active proteins of microorganisms. The fluorine would also be useful as a marker and a probe in elucidation of the structure of proteins and in studying mechanisms of enzyme action. *p*-Fluorophenylalanine has been reported to be incorporated into normal strains of *Escherichia coli* in place of phenylalanine but not into mutant strains with altered phenylalanine, ribonucleic acid synthetase.[3] Recently 4-(trifluoromethyl)-2-aminopentanoic acid (trifluoroleucine) was claimed to replace leucine in certain leucine auxotrophs of *E. coli* without adversely influencing the growth of these microorganisms.[4]

Three of the fluorinated amino acid analogs (trifluorovaline, hexafluorovaline, and trifluoromethionine) are known and were prepared[2a,5,6] by literature procedures. Hexafluoroleucine was prepared by the procedure shown in Chart I.

CHART I



An interesting side reaction was observed during the displacement of the *p*-toluenesulfonate group of **2** by cyanide ion; in all instances equimolar amounts of

(1) W. Shive and C. G. Skinner in "Metabolic Inhibitors," Vol. 1, R. M. Hochster and J. H. Quastel, Ed., Academic Press Inc., New York, N. Y., 1963, Chapter 1, pp 2–73.

(2) (a) D. F. Loncrini and H. M. Walborsky, *J. Med. Chem.*, **7**, 369 (1964); (b) H. M. Walborsky and M. Baum, *J. Org. Chem.*, **21**, 538 (1956); (c) H. M. Walborsky, M. Baum, and D. F. Loncrini, *J. Am. Chem. Soc.*, **77**, 3637 (1955); (d) H. M. Walborsky and M. Schwarz, *ibid.*, **75**, 3241 (1953).

(3) (a) R. Munier and G. N. Cohen, *Biochim. Biophys. Acta*, **21**, 347, 378 (1959); (b) W. L. Fangman and F. C. Neidhardt, *J. Biol. Chem.*, **239**, 1839, 1844 (1964).

(4) O. M. Rennert and H. S. Anker, *Biochemistry*, **2**, 471 (1963).

(5) I. L. Knunyants and Yu. A. Cherburkov, *Izv. Akad. Nauk. SSSR, Otd. Khim. Nauk*, 2162 (1960).

(6) R. L. Dannley and R. G. Taborsky, *J. Org. Chem.*, **22**, 1275 (1957).

Table I
Comparison of pK_A Values of Fluorinated and Natural Amino Acids

Compd	pK_a[a]	pK_a[b]
Leucine	2.34	2.33
5,5,5-Trifluoroleucine		2.05
Hexafluoroleucine	1.81	
Valine	2.29	2.29
4,4,4-Trifluorovaline	1.54	1.54
Hexafluorovaline	1.21	
Methionine	2.16	2.28[c]
Trifluoromethionine	2.05	

[a] Measured in this laboratory by method given in ref 7. [b] Values from ref 7. [c] Value from O. H. Emerson, P. L. Kirk, and C. L. A. Schmidt, *J. Biol. Chem.*, **72**, 449 (1931).

recovered starting material **2** and methyl thiocyanate were obtained in addition to the desired nitrile **3**. Methyl thiocyanate does not form from sodium cyanide and dimethyl sulfoxide alone. The mechanism of this unusual side reaction is not known.

The pK_a values of the four fluorinated amino acid analogs and the corresponding naturally occurring amino acids are listed in Table I. For the leucine and valine analogs, the fluorine has a marked effect in decreasing the pK_a from 0.5 to 0.9 unit. This increased acidity is explained by the electron-withdrawing effect of the fluorines and has been discussed in some detail by Walborsky and Lang.[7] An apparent consequence of the increased acidity is the behavior of these fluorinated amino acids in analysis. They are eluted much faster than their natural counterparts on the automatic amino acid analyzer.

Trifluoromethionine and methionine have almost the same pK_a and cannot be separated by the normal amino acid analysis procedure; both are oxidized with hydrogen peroxide to a mixture of the corresponding sulfoxides and sulfones which can be separated in amino acid analyses. However, the strongly electron-withdrawing trifluoromethyl group adjacent to the sulfur does retard the rate of oxidation of trifluoromethionine relative to methionine.

Biological Stuidies.—The growth of *E. coli* B-14 Leu$^-$ was not supported by hexafluoroleucine, nor did trifluorovaline, hexafluorovaline, and trifluoromethionine support the growth of valine and methionine auxotrophs of *E. coli* K_{12}. Growth of wild type *E. coli* B and K_{12} was not inhibited by the analogs. Complete amino acid analyses of total cell-protein hydrolysates from all growth experiments indicated the absence of these fluorinated analogs in protein.

The lack of growth effects in *E. coli* and of incorporation into protein of four amino acid analogs shows the high specificity requirements for protein synthesis. Although steric requirements of the fluorinated amino acids are close to those of the natural analogs, small changes in physical properties (particularly pK_a) prevent them from being taken into the cell and transported to the site of protein synthesis and/or accepted by the s-RNA. If the fluorine substitution is at a sufficient distance from the amino acid functionality and gives a stable substituted analog such as in *p*-fluorophenylalanine[3] or trifluoroleucine,[4] the analog can replace the natural amino acid in protein synthesis under certain conditions.

(7) H. M. Walborsky and J. H. Lang, *J. Am. Chem. Soc.*, **78**, 4314 (1956).

Experimental Section

Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ± 0.4 of the theoretical values.

4,4,4-Trifluoro-3-(trifluoromethyl)butyric acid (1) was obtained in 63% yield by hydrogenating a solution of 11.9 g (0.057 mole) of 4,4,4-trifluoro-3-(trifluoromethyl)crotonic acid[8] in 50 ml of 95% EtOH (1 g of 10% Pd-C, 3.51-2.1 kg/cm^2 of H_2); bp 94-95° (48 mm), $n^{25}D$ 1.3250-1.3252. *Anal.* ($C_5H_4F_6O_2$) C, H, F.

Ethyl 4,4,4-trifluoro-3-(trifluoromethyl)butyrate was obtained in 70% yield from the corresponding acid (1) by direct esterification with a refluxing mixture of EtOH and H_2SO_4; bp 126-127°, $n^{25}D$ 1.3294-1.3296. *Anal.* Calcd for $C_7H_8F_6O_2$: C, 35.3; H, 3.39; F, 48.5. Found: C, 36.0; H, 3.61; F, 47.8.

4,4,4-Trifluoro-3-(trifluoromethyl)butan-1-ol was obtained by the $LiAlH_4$ reduction of **1** or the corresponding ethyl ester in 54-85% yield, bp 128°, $n^{25}D$ 1.3254. *Anal.* ($C_5H_5F_6O$) C, H, F.

The *p*-toluenesulfonate (2) was prepared in 93% yield by keeping a mixture of 80.0 g (0.37 mole) of **4**, 140 g (0.74 mole) of *p*-toluenesulfonyl chloride, and 950 ml of anhydrous pyridine in the cold room for 48 hr, then extracting the product with C_6H_6. The solvent was removed under reduced pressure, and the product was distilled; bp 115-120° (0.5-0.6 mm), mp 33-34°. *Anal.* ($C_{12}H_{12}F_6O_3S$) C, H; F: calcd, 32.1; found, 31.0.

5,5,5-Trifluoro-4-(trifluoromethyl)valeronitrile (3) was prepared according to Cope and Mehta.[9] To a solution of 118.2 g (0.4 mole) of **2** in 1 l. of freshly distilled, anhydrous DMSO was added a total of 18.2 g (0.37 mole) of dry NaCN in 3-g portions over 3 days. The mixture was stirred at room temperature for 14 days, then poured into ice-H_2O and extracted with CH_2Cl_2; the organic extracts were combined, washed with several portions of saturated brine, and dried. The solvent was removed by distillation through a Vigreux column, and the residue was fractionated on a spinning-band column under reduced pressure. Two fractions, bp 85-95° (45 mm) and bp 110-115° (0.3 mm), weighing 24.7 g, were obtained. The higher boiling material was identified as **2** (*ca.* 21% recovery). Gas chromatographic analysis of the lower boiling fraction [tris(cyanoethyoxy)propane column, 100°] revealed the presence of five components. Careful fractionation of this material on a spinning-band column resulted in the separation of 7.8 g (11% yield) of the desired nitrile **3**, bp 72° (27 mm), $n^{25}D$ 1.3400. *Anal.* ($C_6H_5F_6N$) C, F; H: calcd, 2.46; found, 2.94.

Another fraction, bp 43-52° (27 mm), weighed 4.95 g. This material exhibited very strong infrared absorption at 2150 cm^{-1}, a region that is characteristic of isonitriles, isocyanates, and thiocyanates. An analytical sample, prepared by several distillations (bp 127-130°, at least 99% pure by gas chromatographic analysis, conditions as above) had composition: C, 34.23; H, 4.92. Mass spectrometric analysis as well as comparison of the infrared spectrum with that of an authentic sample identified the compound as methyl thiocyanate. The yield of this material, based on NaCN as the only source of nitrogen, was 18%. In another experiment conducted similarly but on a smaller scale, the nitrile **3** was obtained in 30% yield together with 28% of **2**.

5,5,5-Trifluoro-4-(trifluoromethyl)valeric acid was obtained by refluxing a mixture of 24.8 g (0.12 mole) of **3** and 125 ml of contrated HCl for 24 hr, pouring on ice, and extracting with CH_2Cl_2. After removal of the solvent, the residue was distilled under reduced pressure to give 18.3 g (68% yield) of product, bp 105° (26 mm), $n^{25}D$ 1.3473. *Anal.* ($C_6H_6F_6O_2$) C, H, F.

Ethyl 5,5,5-Trifluoro-4-(trifluoromethyl)-2-bromovalerate (4).—A mixture of 20.38 g (0.091 mole) of 5,5,5-trifluoro-4-(trifluoromethyl)valeric acid, 15.5 g (0.097 mole) of Br_2, and 31 ml of $SOCl_2$ was heated at 75° for 10 hr and at 90° for 4 hr. Excess Br_2 and $SOCl_2$ were then removed under reduced pressure, and 20 ml of absolute EtOH was added to the residue, dropwise, and with stirring. To the resulting mixture, 200 ml of CH_2Cl_2 was added, and the solution was washed with saturated $NaHCO_3$ followed by saturated brine. After removal of the solvent, the residue was fractionated on a spinning-band column, and 11.6 g (38% yield) of **4** was isolated, bp 95-98° (46 mm).

Anal. ($C_8H_9F_6BrO_2$): H, F; C: calcd, 29.0; found, 29.8.

Hexafluoroleucine (5).—A mixture of 10.5 g (0.032 mole) of **4**, 40 g of NaN_3, 20 ml of EtOH, and 100 ml of H_2O was refluxed for 6 days and then steam distilled. The distillate was saturated

(8) W. J. Linn (to Du Pont), U. S. Patent 3,271,419 (1966).
(9) A. C. Cope and A. Mehta, personal communication.

with salt and extracted with CH_2Cl_2. The organic solution was washed with saturated brine, and the solvent was removed under reduced pressure: 3.4 g (37%) of crude ethyl 5,5,5-trifluoro-4-(trifluoromethyl)-2-azidovalerate was obtained. This material was dissolved in 100 ml of absolute EtOH and hydrogenated for 18 hr (1 g of 5% Pd-C). The filtered solution was saturated with HCl and evaporated to dryness. The residue was dissolved in 50 ml of concentrated HCl; the solution was refluxed for 4 hr and evaporated to dryness. The residue was dissolved in 50 ml of absolute EtOH, 10 ml of dry pyridine was added, and the mixture was kept in the refrigerator overnight. The precipitated white crystals were collected by filtration and recrystallized from H_2O-EtOH to give 0.92 g (13%) of **5**, mp 234-237° dec (sealed tube). *Anal.* ($C_6H_7F_6NO_2$) C, H, N.

Oxidation of Methionine and Trifluoromethionine.—To a mixture of 15.0 mg of methionine and 12.5 mg of trifluoromethionine were added 0.022 ml of concentrated HCl, 0.15 ml of H_2O, 0.20 ml of MeOH, and 0.10 ml of 30% H_2O_2. The mixture was left at room temperature for 3 hr, then freeze-dried. The dry residue was taken up in 25 ml of citrate buffer (pH 2.2), and appropriate aliquots were analyzed on a Phoenix Scientific Co. automatic amino acid analyzer. Under these conditions, all the methionine was converted to the corresponding sulfoxide and sulfone, whereas approximately 50% of the trifluoromethionine was converted into a derivative assumed to be the sulfoxide. All products now could be clearly distinguished from one another.

pK_a Measurements.—The pK_a measurements were carried out as described by Walborsky and Lang.[7]

Growth Experiments with Various Strains of *E. coli*.—The following strains of *E. coli* were used in our growth studies: *E. coli* B-14 Leu⁻, *E. coli* K_{12} W-3100 (standard wild type ATCC 15153), *E. coli* K_{12} Val⁻ (isolated at Du Pont), *E. coli* B (wild type ATCC 11303), and *E. coli* $K_{12}W_6$ 58-141 (RC^{rel} Met⁻). Bacteria were grown in a chemically defined medium containing per liter, 7.0 g of Na_2HPO_4, 3.0 g of KH_2PO_4, 1.0 g of NH_4Cl, 0.13 g of $MgSO_4 \cdot 7H_2O$, at pH 7.0, and supplemented as required for the various auxotrophs with 20 μg/ml of the amino acids or their fluorinated analogs. Growth was followed by determinations of cells/ml of bacterial density with a Klett colorimeter using a green filter. For total amino acid analyses of bacterial proteins, the bacteria were pelletted by centrifugation, and the pellets were washed successively with 10% trichloroacetic acid and H_2O. The samples were then lyophilized, hydrolyzed with 10 *N* HCl, and analyzed in the usual manner.

Acknowledgments.—The authors express their thanks to Mr. D. W. Krause for the pK_a measurements.

Iodinated Phenylalanines. Tests for Selective Localization in Pancreas and Preparation of 3,4,5-Triiodophenylalanine[1a]

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Received August 17, 1967

Amino acids are among the very few classes of compounds which show any significant selective localization in pancreatic tissue.[2,3] A radioactive amino acid derivative, ^{75}Se-selenomethionine, has been used with limited success to visualize the pancreas of the human being and the dog by isotope scanning techniques.[4-6] Roentgenographic visualization of the pancreas would be a powerful diagnostic tool and in theory might be achieved with a heavily iodinated amino acid provided it retained its affinity for the pancreas after iodination. There is an excellent review of the literature by Peskin and Johnson.[3]

We have prepared two iodinated phenylalanine derivatives and have tested them for selective localization in pancreatic tissue of the rat. Initial work was done with the known[7] 4-iodophenylalanine, which we prepared from 4-iodobenzyl bromide by the diethyl acetamidomalonate method. Tests on two rats showed that the administration of 4-iodophenylalanine did cause an increase in the iodine content of the rat pancreatic tissue (Table I), although, as anticipated, this iodine level was too low to produce radiopacity.

Table I

Iodine Content of Rat Tissue

4-Iodophenylalanine, mg iv or sc	Tissue	Total iodine found, mg	mg of iodine/g of tissue
Controls (av of 11 samples)	Pancreas	1.9 ± 0.57[a]	3.9 ± 1.1[a]
10.0	Pancreas	2.9	...
66.25	Pancreas	4.3	6.1
Controls (av of 10 samples)	Liver	4.1 ± 1.2[a]	1.7 ± 0.4[a]
10.0	Liver	10.3	...
66.25	Liver	8.0	3.2

[a] Average deviation.

In theory a triiodinated phenylalanine should offer a better chance of obtaining the required iodine concentration. However, none of the possible di- or triiodophenylalanine isomers has been reported up to the present time. We succeeded in preparing one of these, 3,4,5-triiodophenylalanine, from a condensation of diethyl acetamidomalonate with the previously unknown 3,4,5-triiodobenzyl bromide followed by work-up in acid medium.

By far the most difficult step in this synthesis was the preparation of 3,4,5-triiodobenzyl bromide (TIBB). Low yields (15%) of TIBB were finally obtained by treating 3,4,5-triiodotoluene with N-bromosuccinimide in the presence of relatively large amounts of the catalyst, dibenzoyl peroxide, added throughout the course of the reaction. The product was identified as the desired isomer by means of its chemical properties and nmr spectrum. Once sufficient quantities of TIBB were on hand, preparation of 3,4,5-triiodophenylalanine (TIPA) proceeded smoothly with good yields.

TIPA was tested for selective localization in pancreatic tissue of rats in the form of its somewhat more soluble hydrochloride salt (TIPA·HCl). Young adult female rats were starved for 24 hr, then fed and injected either intravenously or subcutaneously with 35.3-66.0 mg of TIPA·HCl in propylene glycol solution. Each animal was sacrificed 1 hr after injection, and the pancreas and liver were analyzed separately for iodine. Control animals were treated by exactly the same

(1) (a) This work was supported by a grant (C-2981) from the U. S. Public Health Service, National Institutes of Health. (b) To whom requests for reprints should be sent.

(2) V. B. Schatz, B. C. O'Brien, W. M. Chaddock, A. M. Kanter, A. Borger, and W. R. Sandusky, *J. Med. Pharm. Chem.*, **2**, 425 (1960).

(3) G. W. Peskin and C. Johnson, *Am. J. Med. Sci.*, **249**, 223 (1965).

(4) M. Blau and R. F. Manske, *J. Nucl. Med.*, **2**, 102 (1961).

(5) M. Blau and M. A. Bender, *Radiology*, **78**, 974 (1962).

(6) M. C. Anderson, J. M. Beal, D. N. Sim, M. K. Copass, and J. L. Quinn, Department of Surgery, Northwestern University Medical School, Chicago, Ill., personal communication.

(7) (a) H. L. Wheeler and S. H. Clapp, *Am. Chem. J.*, **40**, 458 (1908); (b) E. H. Zeller, G. Ramachander, G. A. Fleisher, T. Ishimaru, and V. Zeller, *Biochem. J.*, **95**, 262 (1965).