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Amino Acid Analogs. 2. 3-Fluoroamino Acids. 1. Chain Length Three to Seven Carbon Atoms[1]

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The synthesis of 3-fluoroamino acids has presented an intriguing challenge for many years. 3-Fluoroalanine as the hydrochloride was first reported in 1959,[2] but the validity of the synthesis was questioned.[3] It was disclosed in 1972 that the compound was prepared by the same as well as other methods, but none of the products or intermediates were characterized;[4a] however, the same author prepared and characterized 3-fluoro-D-alanine from D-alanine by photofluorination with trifluoromethyl hypofluorite in liquid HF.[4b] In 1967 a synthesis of 3-fluoroalanine was reported which was carried out by ammonolysis of 2-bromo-3-fluoropropionic acid obtained from the bromination of 3-fluoropropionic acid.[5] 3-Fluoro-2-methylalanine was also synthesized in 1963 by two independent methods.[6,7] None of these methods were readily adaptable for the preparation of other 3-fluoroamino acids.

A general approach to the preparation of 2-bromo-3-fluorocarboxylic acids was devised, which upon ammonolysis yielded a series of 3-fluoroamino acids. By extending the method of bromo fluorination of methyl acrylate[8] to substituted acrylic acids with subsequent ammonolysis, the following amino acids were prepared: 3-fluoroalanine, 2-amino-3-fluorobutyric acid, 3-fluorovaline, 3-fluoronorvaline, 3-fluoronorleucine, and 2-amino-3-fluoroheptanoic acid.

The bromo fluorination of acrylic, crotonic, and 3-methylcrotonic acids was carried out by dissolving the substrate in liquid HF, followed by addition of *N*-bromoacetamide (NBA). The reaction proceeded smoothly without formation of excessive complicating side products, and the three bromofluoro compounds were purified easily by distillation. Upon bromo fluorination of 2-pentenoic, 2-hexenoic, and 2-heptenoic acids, mixtures of products were obtained which could not be separated conveniently by distillation. Purification of the desired products was achieved by formation of cyclohexylamine salts followed by repeated recrystallizations until the required degree of purity was obtained, as indicated by gas chromatographic analysis of the trimethyl silyl esters of the acids.[9] The salts were subsequently decomposed by mineral acid, and the bromofluoro acids were distilled. No attempt was made at this time to determine structures of by-products produced in these reactions.

3-Fluoroamino acids were obtained by ammonolysis of the bromofluoro acids in liquid ammonia by modifications of the method of Lettré and Wölcke.[5] 3-Fluoroalanine and 2-amino-3-fluorobutyric acid were obtained by ammonolysis at room temperature. The remaining four 2-amino acids were prepared by reaction with liquid ammonia at 65°. Although it is recognized that four of the described fluoroamino acids contain two centers of asymmetry, no attempt was made at separating the erythro and threo diastereoisomers.

The data characterizing the bromofluoro acids and the fluoroamino acids are contained in Table I, and the 60-MHz nmr spectral features of the compounds are listed in Table II. Infrared spectra for the 3-fluoroamino acids have been obtained.†

All of the 3-fluoroamino acids were tested against *Aspergillus niger, Trichoderma viride,* and *Myrothecium verrucaria* in Sabouraud dextrose agar (Difco) at pH 4.0 and 5.6 and against *Trichophyton mentagrophytes* in the same medium at pH 5.6 and 7.0, according to published methods.[10] Very little fungitoxic activity was observed under those conditions. 3-Fluoroalanine inhibited *A. niger* and *M. verrucaria* at concentrations between 10^3 and 10^4 ppm at pH 4.0 and *T. mentagrophytes* at a concentration of between 10^2 and 10^3 ppm at pH values of 5.6 and 7.0. 3-Fluorovaline was less active and inhibited *M. verrucaria* at a concentration between 10^3 and 10^4 ppm at pH values of 4.0 and 5.6 and *T. mentagrophytes* at the same concentration at pH values of 5.6 and 7.0. Of the remaining compounds, 3-fluoronorvaline inhibited *M. verrucaria* at pH 4.0 at a concentration between 10^3 and 10^4 ppm, and 3-fluoronorleucine inhibited *T. mentagrophytes* at concentrations between 10^2 and 10^3 ppm at pH 5.6 and 10^3 and 10^4 ppm at pH 7.0.

Experimental Section‡

2-Bromo-3-fluoropropionic Acid. Acrylic acid (18 g, 0.25 mol) was dissolved in 25 ml of liquid HF kept at −30 to −10° in a polyethylene bottle. To the mixture was added NBA (37.3 g, 0.27 mol) in small portions with stirring during the course of 0.5 hr. Stirring was continued overnight, allowing the mixture to come to room temperature. The excess HF was removed under a stream of air, and the residue was poured into a slurry of ice and H_2O. The product was extracted with Et_2O, dried (Na_2SO_4), and distilled under vacuum.

2-Bromo-3-fluorobutyric acid was prepared from crotonic acid in the same manner as 2-bromo-3-fluoropropionic acid.

2-Bromo-3-fluoro-4-methylbutyric acid was prepared from 3,3-dimethylacrylic acid in the same manner as 2-bromo-3-fluoropropionic acid.

2-Bromo-3-fluoropentanoic Acid. 2-Pentenoic acid (89 g, 0.89 mol) was bromo fluorinated in 200 ml of liquid HF with NBA (124 g, 0.9 mol) in the same manner as acrylic acid. The aqueous solution of product was extracted with ether which in turn was extracted three times with 200-ml portions of 10% NaOH. The basic extract was acidified with HCl and extracted with $CHCl_3$. Upon evaporation of the solvent, 120 g of residue remained which was then dissolved in 250 ml of ether. A solution of cyclohexylamine (60 g, 0.6 mol) in 200 ml of ether was added slowly to the bromofluoro compound with stirring. After stirring for 2 hr, the mixture was refrigerated overnight. The cyclohexylamine salt was obtained by filtration (yield 113 g, 42%). It was recrystallized sever-

†See paragraph at end of paper regarding supplementary material.

‡Melting points were taken in a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra were obtained with a Perkin-Elmer Model 221 spectrophotometer, and nmr spectra were taken with a Jeolco JNM-C-6HL spectrometer. Gas chromatography 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 bromofluoro acids was established by gas chromatographing the trimethyl silyl esters[8] on a column containing 1% Apiezon L on acid-washed Chromosorb W (80–100 mesh), previously treated with dimethyldichlorosilane.

Table I. 2-Bromo-3-fluoro Fatty Acids and 3-Fluoroamino Acids

R_1	R_2	Yield, %	Bp (mm) or mp, °C[a]	Formula	Analyses
			$R_1R_2CFCHBrCOOH$		
H	H	44	66–69 (0.2)[b]		
CH_3	H	70	86–88 (0.6)	$C_4H_6BrFO_2$	C, H, Br, F
CH_3	CH_3	63	91 (1.0)[c]	$C_5H_8BrFO_2$	C, H, Br, F
C_2H_5	H	19	80–82 (0.25)	$C_5H_8BrFO_2$	C, H, Br, F
C_3H_7	H	6.4	110 (2.25)	$C_6H_{10}BrFO_2$	C, H, Br, F
C_4H_9	H	30	112–114 (1.0)	$C_7H_{12}BrFO_2$	C, H, Br, F
			$R_1R_2CFCH(NH_2)COOH$		
H	H	30	151–152 dec[d]		
CH_3	H	36	204.5–205 dec[e]	$C_4H_8FNO_2$	C, H, F, N
CH_3	CH_3	62	205 dec[f]	$C_5H_{10}FNO_2$	C, H, F, N
C_2H_5	H	16	182 dec[e]	$C_5H_{10}FNO_2$	C, H, F, N
C_3H_7	H	36	180–181 dec[g]	$C_6H_{12}FNO_2$	C, H, F, N
C_4H_9	H	10	180–181 dec[g]	$C_7H_{14}FNO_2$	C, H, F, N

[a] Analytical sample. [b] Lit.[6] bp 72.5° (2 mm). [c] Crystallized from hexane, mp 59–59.5°. [d] Lit.[6] mp 152° dec. [e] From H_2O–MeOH. [f] From H_2O–Me_2CO. [g] From dilute HBr adjusted to pH 5 with NH_4OH, wash H_2O, and Me_2CO.

Table II. Proton Chemical Shifts (τ, ppm) for 2-Bromo-3-fluoro Fatty Acids and 3-Fluoroamino Acids

		Proton[c]					
R_1	R_2	α	β	γ	δ	ϵ	ζ
				$R_1R_2CFCHBrCOOH$[a]			
H	H	4.1–5.5 (un m)					
CH_3	H	4.24 (t, J_{23} = J_{2F} = 8)	5.03 (m, J_{32} = 8, J_{34} = 6, J_{3F} = 46)	1.56 (2 d, J_{43} = 6, J_{4F} = 24)			
CH_3	CH_3	4.38 (d, J_{2F} = 9)		1.63 (d, J_{4F} = 22)			
C_2H_5	H	4.21 (t, J_{23} = J_{2F} = 8)	4.79 (m, J_{32} = 8, J_{34} = 3, J_{3F} = 46)	1.5–2.5 (un m)	1.04 (t, J_{54} = 7)		
C_3H_7	H	4.21 (t, J_{25} = J_{2F} = 8)	4.80 (m, J_{32} = 8, J_{34} = 3, $J_{3F} \simeq$ 50)	1.4–2.5 (un m)		1.10 (t, J_{65} = 7)	
C_4H_9	H	4.25 (t, J_{23} = J_{2F} = 8)	4.85 (m, J_{32} = 8, J_{34} = 3, $J_{3F} \simeq$ 50)	1.1–2.5 (un m)			0.89 (pr t, $J_{76} \simeq$ 5)
				$R_1R_2CFCH(NH_2)COOH$[b]			
H	H	4.63 (m, J_{23} = 3, J_{2F} = 30)	5.08 (2 d, J_{32} = 3, J_{3F} = 47)				
CH_3	H	4.40 (2 d, J_{23} = 4, J_{2F} = 25)	5.41 (m, J_{32} = 3, J_{34} = 7, J_{3F} = 50)	1.60 (2 d, J_{43} = 7, J_{4F} = 32)			
CH_3	CH_3	4.39 (d, J_{2F} = 12)		1.44 and 1.82 (2 d, J_{4F} = 8, J_{4F} = 9)			
C_2H_5	H	4.52 (2 d, J_{23} = 3, J_{2F} = 24)	5.05 (m, J_{32} = 3, J_{34} = 7, $J_{3F} \simeq$ 50)	1.4–2.4 (un m)	1.04 (t, J_{54} = 7)		
C_3H_7	H	4.52 (2 d, J_{2F} = 25, J_{23} = 3)	5.18 (m, J_{32} = 3, J_{34} = 7, $J_{3F} \simeq$ 50)	1.2–2.3 (un m)		0.92 (t, J_{65} = 6)	
C_4H_9	H	4.5 (un m)	5.2 (un m)	1.1–2.4 (un m)			0.91 (pr t, J_{76} = 5)

[a] Spectra taken on 5% solutions in $CDCl_3$ with tetramethylsilane (TMS) as internal standard. [b] Spectra taken on 5% solutions in D_2O–D_2SO_4 (50:50 w/w) with sodium 2,2-dimethyl-2-silapentane-5-sulfonate as internal standard. [c] J values in hertz. un m = unresolved multiplet; d = doublet; t = triplet; m = multiplet; pr t = poorly resolved triplet.

al times from a mixture of ether and hexane until its trimethyl silyl ester was nearly pure by gas chromatographic analysis. An analytical sample was crystallized from a mixture of MeOH and Me_2CO, mp 112–113°. *Anal.* ($C_{11}H_{21}BrFNO_2$) C, H, Br, F, N.

Cyclohexylammonium 2-bromo-3-fluoropentanoate (112 g, 0.38 mol) was added to 300 ml of 20% H_2SO_4 with cooling and was extracted with three 100-ml portions of $CHCl_3$. The solution was dried (Na_2SO_4), the solvent evaporated, and the residue distilled under vacuum. The yield of product was 50% based on the salt and 19% based on 2-pentenoic acid.

2-Bromo-3-fluorohexanoic Acid. 2-Hexenoic acid (100 g, 0.88 mol) was bromo fluorinated in 200 ml of liquid HF with NBA (121 g, 0.88 mol) in the same manner as acrylic acid. The ether extract was dried (Na_2SO_4), freed of solvent, and vacuum distilled. A fraction was collected [69.6 g, bp 102–120° (1.75 mm)]. The impure acid (0.33 mol) in 500 ml of ether was treated with 32.6 g (0.33 mol) of cyclohexylamine in 200 ml of ether. The crystalline product obtained was recrystallized several times from a mixture of ether and hexane until a silylated sample of acid appeared nearly pure by gas chromatographic analysis. The purified salt was obtained in 27 g (26%) yield, and an analytical sample was crystallized from a mixture of MeOH and Me_2CO, mp 78–80°. *Anal.* ($C_{12}H_{23}BrFNO_2$) C, H, Br, F, N.

Cyclohexylammonium 2-bromo-3-fluorohexanoate (27 g, 0.087 mol) was mixed with 150 ml of 5% HCl with cooling. The free acid was extracted twice with 50-ml portions of $CHCl_3$. The solu-

tion was dried (Na_2SO_4), freed of solvent, and vacuum distilled. The yield of product was 12 g (65% based on the cyclohexylamine salt and 6.4% based on 2-hexenoic acid).

2-Bromo-3-fluoroheptanoic Acid. 2-Heptenoic acid (91 g, 0.7 mol) was bromo fluorinated in 150 ml of liquid HF with NBA (104 g, 0.75 mol), and the product was purified in the same manner as 2-bromo-3-fluorohexanoic acid except that a dicyclohexylamine salt was used for purification. The yield of salt was 226 g (79%), and an analytical sample was crystallized from an ether-petroleum ether mixture: mp 117.5–118°. *Anal.* ($C_{19}H_{35}BrFNO_2$) C, H, Br, N.

Dicyclohexylammonium 2-bromo-3-fluoroheptanoate (226 g, 0.55 mol) was dissolved in 200 ml of concentrated H_2SO_4, and 50 ml of H_2O was added dropwise with cooling and stirring. The mixture was extracted twice with 100-ml portions of $CHCl_3$. The $CHCl_3$ solution was dried (Na_2SO_4), freed of solvent, and vacuum distilled. The yield of product was 47.7 g (38% based on the dicyclohexylamine salt and 30% based on 2-heptenoic acid).

3-Fluoroalanine. 2-Bromo-3-fluoropropionic acid (30.8 g, 0.18 mol) was dissolved in 75 ml of liquid NH_3 and sealed in a stainless steel pressure vessel. After remaining at room temperature for 3 days, the NH_3 was removed, and the residue was dissolved in a small volume of H_2O and brought to pH 5 with HBr. The solution was evaporated under vacuum below 40°, and the residue was slurried repeatedly with MeOH until a negative test was obtained with $AgNO_3$. A yield of 5.4 g of product was obtained.

2-Amino-3-fluorobutyric Acid. 2-Bromo-3-fluorobutyric acid (52 g, 0.28 mol) was treated with 100 ml of liquid NH_3 in the same manner as for the preparation of 3-fluoroalanine. A yield of 12.2 g of product was obtained.

3-Fluorovaline was prepared in the same manner as 3-fluoroalanine except that the amination was carried out for 3 days at 65°. 2-Bromo-3-fluoro-3-methylbutyric acid (31 g, 0.31 mol) in 80 ml of liquid NH_3 yielded 10 g of product.

3-Fluoronorvaline was prepared in the same manner as 3-fluorovaline. 2-Bromo-3-fluoropentanoic acid (39 g, 0.2 mol) in 150 ml of liquid NH_3 yielded 4.4 g of product.

3-Fluoronorleucine was prepared in the same manner as 3-fluorovaline. 2-Bromo-3-fluorohexanoic acid (10.8 g, 0.05 mol) was aminated in 50 ml of liquid NH_3. The yield of product was 2.7 g.

2-Amino-3-fluoroheptanoic Acid was prepared in the same manner as 3-fluorovaline. 2-Bromo-3-fluoroheptanoic acid (30 g, 0.13 mol) was aminated in 100 ml of liquid NH_3, and the yield of product was 2.2 g.

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Supplementary Material Available. Infrared spectra will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 20× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JMED-73-1407.

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Probiotics.† Antistaphylococcal and Antifibrinolytic Activities of ω-Guanidino Acids and ω-Guanidinoacyl-L-histidines[2,‡]

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Following the discovery of the antistaphylococcal activity of a series of ω-amino acids,[3] ω-aminoacyl-L-histidines,[3b] N^{α}-(ω-aminoacyl)-L-lysines,[4] and ω-amino-β-hydroxyacyl-L-histidines,[5] we have now prepared a series of ω-guanidino acids and ω-guanidinoacyl-L-histidines in order to compare their antistaphylococcal activity. The antifibrinolytic activity of both ω-guanidino acids and ω-guanidinoacyl-L-histidines was also determined because of the relationship discussed previously.[3b,4]

The compounds described in this paper are ω-guanidino acids, $H_2NC(=NH)NH(CH_2)_nCOOH$ [where n = 1, guanidinoacetic acid (**1**); n = 2, β-guanidinopropionic acid (**2**); n = 3, γ-guanidinobutyric acid (**3**); n = 4, δ-guanidinovaleric acid (**4**); n = 5, ε-guanidinohexanoic acid (**5**)], and ω-guanidinoacyl-L-histidines, $H_2NC(=NH)NH(CH_2)_nCO$-His [where n = 1, guanidinoacetyl-L-histidine (**6**); n = 2, β-guanidinopropionyl-L-histidine (**7**); n = 3, γ-guandinobutyryl-L-histidine (**8**); n = 4, δ-guanidinovaleryl-L-histidine (**9**); n = 5, ε-guanidinohexanoyl-L-histidine (**10**)].

Chemistry. Compounds 4–10 were prepared from the corresponding ω-amino acids and ω-aminoacyl-L-histidines[3b] by treating with *S*-ethylisothiourea. This preparative procedure is a modification of the one described by Takahashi, *et al.*[6] Compounds **4** and **5** were synthesized under strong basic conditions,[6] but **6–10** were formed at mild basic pH of 8–9. Previous references indicated the use of strong basic conditions, such as concentrated NH_4OH,[7,8] 1 *N* MeONa,[9] and 2 *N* NaOH.[6] We found that weak basic conditions (pH 8–9) are also applicable, an advantage for the synthesis of the compounds which are not stable under strong basic conditions. The reaction products were purified by means of ion-exchange chromatography, using aqueous pyridine for **4** and **5** and pyridine–NH_4OH for **6–10.** The yields and physical and analytical data for the compounds are given in Table I. R_f values on tlc were determined in the five solvents used previously.[3b] All compounds were homogeneous by tlc.

Testing Procedure. *In vivo* antistaphylococcal activity was determined by the method described before.[3b,4] γ-Aminobutyryl-L-histidine was used as the positive control. Antistaphylococcal activity *in vitro* was determined by the paper disk method. Approximately 0.2 mg of the sample was placed on the paper disk which rested upon the surface of a plate of Bacto Staphylococcus Medium 110 (Difco) which had been inoculated with *Staphylococcus aureus.* The plates were examined after incubation for 24 hr at 37°. The lysis time procedure for antifibrinolytic activity *in vitro* was described previously[3b] with ε-aminohexanoic acid as a positive control. The lysis area procedure for antifibrinolytic activity was as follows.[10,§] Bovine fibrinogen (4%, 10 ml) in pH 7.4 Palitzsch's buffer was poured into a 10-cm (i.d.) petri dish. Bovine thrombin (5

†The term probiotics has been proposed to designate these compounds and those described previously which build resistance to infection in the host but do not inhibit the growth of microorganisms *in vitro.* The term first was used in 1953.[1a] Sperti[1b] describes the earlier history and particularly the biological aspects. The isolation and identification of probiotics from natural sources was recently summarized by Cook and Tanaka.[1c]

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§A. Fujii, Ph.D. Dissertation, St. Thomas Institute, Cincinnati, Ohio, 1970.