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MONOFLUORINATED ANALOGUES OF SOME ALIPHATIC BASIC AMINO ACIDS+

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SUMMARY

Monofluorinated derivatives of ornithine, citrulline, arginine and lysine were synthesized and their growth-inhibiting power determined, using E. coli. Bromofluorination of methyl 4-pentenoate led to complete lactonization of the ester, while the homologous 5-hexenoate reacted normally.

INTRODUCTION

Fluorinated derivatives of amino acids are highly interesting from both biochemical and medical points of view. Large number of these compounds have been synthesized, as was recently reviewed [1]. Most of them are derivatives of neutral amino acids, while in the acidic group only a few examples are known. As far as we know, nobody has tried to prepare fluorinated analogues of amino acids, mentioned in the Title. It is the more surprising the more we realize that we may reasonably presume biological activity of such compounds. These reasons led us to synthesize some members of this very interesting group.

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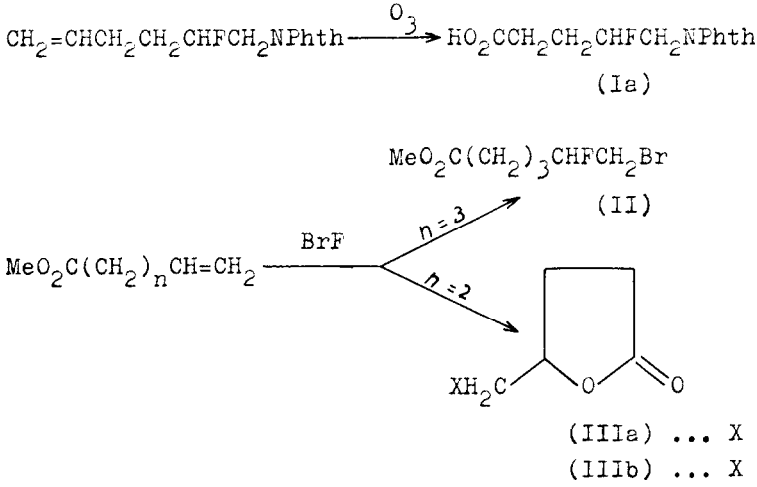
RESULTS AND DISCUSSION

In the preparation of 4-fluoroornithine and of 5-fluorolysine, the key intermediates, acids (Ia) and (Ib) were needed. For this purpose, bromofluorination [2] of the corresponding terminal olefinic methyl esters was attempted at first. This effort was succesfull in the case of methyl 5-hexenoate, which gave the normal addition product, methyl 6-bromo-5-fluorohexanoate (II); transformation of (II) into (Ib) needs no detailed description (see the Scheme). On the other side, bromofluorination of methyl 4-pentenoate did not lead to the expected bromofluoro derivative; as a sole product there was isolated a fluorine-free material, characterized as impure 5-bromo-4--valerolactone (IIIa) by conversion into the 5-phthalimido--4-valerolactone (IIIb). The requisite acid (Ia) was finally prepared by oxidation of 5-fluoro-6-phthalimido-1-hexene, the synthesis of which was described previously [3]. Ozonolytical oxidation was found to be most convenient for this purpose.

Acids (Ia) and (Ib) were brominated in position 2, giving, after the usual workup, methyl esters (Va) and (Vb), respectively. These were then converted, by reaction with potassium phthalimide, into the fully protected derivatives of the two desired amino fluoro acids, i.e. methyl 2,5-diphthalimido-4-fluorovalerate (VIa) and methyl 2,6-diphthalimido-5-fluorohexanoate (VIb). Deblocking was carried out by hydrazinolysis and mild acid hydrolysis; (VIa) gave 4-fluoroornithine monohydrochloride (VIIa), while deprotecting of (VIb) led to 5-fluorolysine monohydrochloride (VIIb).

Transformations of the terminal amino group in (VIIa) were realized using conventional methods [4;5]. Thermal lability of the 4-fluoroornithine copper complex forced us to prepare it at ambient temperature; nevertheless, 100% complexation was reached after a prolonged reaction time. Reaction of the complex with potassium cyanate [4] or with O--methylisourea [5], resp., followed by removal of copper by hydrogen sulphide, led to 4-fluorocitrulline (VIII) or 4-fluoroarginine monohydrochloride (IX). In contrast to citrulline and arginine, both (VIII) and (IX) gave yellow ninhydrin spots, gradually turning violet.



(Ia)

(II)

(IIIa) ... X = Br
(IIIb) ... X = Phth

(IV)

(Ib)

(Ia) ... n = 2
(Ib) ... n = 3

(Va) ... m = 1
(Vb) ... m = 2

(VIa) ... m = 1
(VIb) ... m = 2

(VIIa) ... m = 1
(VIIb) ... m = 2

(VIII)

(IX)

Biological activity of the prepared amino acids was tested on Escherichia coli. It was found that (VIIb) and (IX) in 10^{-3} molar concentrations stronghly inhibited its growth during 3,5 h incubation (84%, resp. 74% inhibition), while (VIIa) and (VIII) were not too active under the same conditions.

EXPERIMENTAL

Temperature data are uncorrected. Melting points were determined on a Kofler apparatus. PMR spectra were measured on Tesla BS 487 (80 MHz), using hexamethyldisiloxane as internal standard; chemical shifts were correlated with the δ-scale by equation $\delta_{TMS} = \delta_{HMDS} + 0.06$ ppm. Mass spectrum was recorded on Varian MAT - 311 at 70 eV. Chromatography of amino acids was carried out on Whatman 1 paper, the solvents systems being n-butanol - acetic acid - water 4:1:5 (system A) and phenol - ethanol - water 2:1:1 (atmosphere saturated with ammonia) (system B). Detection by 0,25% ninhydrin in acetone.

Oxidation of 5-fluoro-6-phthalimido-1-hexene

5-Fluoro-6-phthalimido-1-hexene (7.0 g), dissolved in ethyl acetate (100 ml), was ozonized at -80° for 11 h, evaporated in vacuo and the evaporation repeated after addition of ether (30 ml). The ozonide was dissolved in 85% formic acid (35 ml), 30% hydrogen peroxide (24 ml) added and the mixture was gently heated on the water bath. When the reaction started (vigorous evolution of oxygen!), heating was removed and the mixture was allowed to decompose spontaneously. The resulting solution was heated on boiling water bath for 0.5 h, concentrated in vacuo up to crystallization and chilled overnight. The acid (Ia) was filtered, washed with cold water and dried. Yield, 6.3 g (84%), m.p. 130-135° (unchanged by recrystallization from ethanol). Analysis: Found: C, 58.7; H, 4.5; F, 7.4; N, 5.1%. $C_{13}H_{12}FNO_4$ requires C, 58.9; H, 4.6; F, 7.2; N, 5.3%.

Mother liquors were evaporated, the residue again dissolved in ethyl acetate and a slight excess of N,N-dicyclohexylamine (DCHA) was added under cooling. The DCHA salt of (Ia) separated; yield, 1.3 g (10%), m.p. 127-145° (from ethyl acetate). Analysis: Found: C, 67.0; H, 8.0; F, 4.4; N, 6.3%. $C_{25}H_{35}FN_2O_4$ requires C, 67.2; H, 7.9; F, 4.3; N, 6.3%.

Preparation of methyl 2-bromo-4-fluoro-5-phthalimido-valerate (Va)

Acid (Ia) (12.6 g) and red phosphorus (0.85 g) were well homogenized and mixed with tetrachloromethane (40 ml). Bromine (9.4 ml) was slowly added under stirring, the mixture was refluxed for 4 h and evaporated in vacuo. Dichloromethane (20 ml) was added and evaporation repeated, the crude bromoacyl bromide was carefully decomposed with methanol (50 ml) and after refluxing for 1 h the solution was allowed to stand overnight. The crude ester (Va), which separated, was crystallized from methanol (charcoal); yield, 10.0 g (57.5%), m.p. 102-111°. Further 2.4 g (14%) of somewhat less pure second crop were obtained after workup of the mother liquor. A pure sample had m.p. 105-115° (methanol). Analysis: Found: C, 47.0; H, 3.6; Br, 22.4; F, 5.1; N, 3.7%. $C_{14}H_{13}BrFNO_4$ requires C, 47.0; H, 3.7; Br, 22.3; F, 5.3; N, 3.9%.

Preparation of methyl 2,5-diphthalimido-4-fluorovalerate (VIa)

Ester (Va) (10.7 g), potassium phthalimide (5.52 g) and dry DMF (100 ml) were stirred at 90-95° for 45 min. The neutral mixture was evaporated at 0.1 Torr, the residue triturated with water (2 x 200 ml) and finally shaken with methanol (50 ml) for 1 h, during which time it became a sandy solid. Yield, 10.7 g (85%), m.p. 153-169° (sintering at 135°). Recrystallization from acetone - methanol (1:1) raised the m.p. to 177-183° (sintering at 115°). Analysis: Found: C, 60.0; H, 4.4; F, 3.7; N, 5.4%. $C_{25}H_{21}FN_2O_8$ requires C, 60.5; H, 4.3; F, 3.8; N, 5.7%.

Deblocking of the ester (VIa)

Ester (VIa) (11.6 g), 98% hydrazine hydrate (2.8 ml) and ethanol (200 ml) were stirred under reflux for 1 h. The ester went into solution and a new precipitate emerged. The whole mixture was evaporated in vacuo, 5 mol l^{-1} hydrochloric acid (160 ml) added and heating continued at 60° for 1 h. After

cooling, the insoluble material was filtered, washed with water, the solution evaporated and, after dissolution in water (25 ml) and filtering, the evaporation repeated. The solid was taken into hot methanol (20 ml) and pyridine added until the mixture was neutral to congo red. The resulting suspension of 4-fluoroornithine monohydrochloride (VIIa) was cooled overnight and the product washed with cold methanol. Yield, 5.4 g (66.5%) of chromatographically pure (VIIa), m.p. 211-216°. Recrystallization of a sample from aqueous ethanol gave m.p. 235-240°. Analysis: Found: C, 32.1; H, 7.0; Cl, 19.0; F, 10.2; N, 14.8%. $C_5H_{12}ClFN_2O_2$ requires C, 32.0; H, 7.0; Cl, 18.9; F, 10.1; N, 14.9%. R_{ChN} values: 0.86 (system A); 0.87 (system B).

Attempted bromofluorination of methyl 4-pentenoate

N-Bromoacetamide (23.5 g) was dissolved in a mixture of hydrogen fluoride (30.0 g) and chloroform (140 ml) at -60°. After 10 min., methyl 4-pentenoate (19.0 g) was slowly added and the mixture was stirred at the same temperature for 3 h, whereupon it was poured into a solution of sodium carbonate (80 g) in water containing ice. The organic matter was extracted with chloroform, the extract washed with slightly acidified sodium nitrite, then with sodium bicarbonate, water and dried. Distillation afforded 25.0 g of a fluorine-free fraction, b.p. 68-72°/0.2 Torr. A sample was redistilled, b.p. 116-119°/10 Torr; for (IIIa) cited [6] : 101°/20 Torr. Analysis: Found: C, 33.6; H, 4.1; Br, 43.6%. $C_5H_7BrO_2$ requires C, 33.7; H, 3.4; Br, 44.8%.

Preparation of 5-phthalimido-4-valerolactone (IIIb)

Compound (IIIa) (6.49 g), potassium phthalimide (5.63 g) and DMF (50 ml) were stirred at 100° for 1 h and the neutral mixture was evaporated at 0.1 Torr. The residue was treated with water, giving a solid, which was thoroughly washed with water and dried. Yield, 5.6 g (62.5%), m.p. 169° (methanol); cited [7] : 161-164°. PMR spectrum (in $CDCl_3$ + d_6-DMSO):

1.94-2.82 mt (~ 4H), 3.66 mt (nonstochiometric), 3.82 d (J = = 2.6 Hz, 1H), 3.91 d (J = 4.8 Hz, 1H), 4.81 mt (~ 1H), 7.79 mt (4H). Mass spectrum, quoted as m/e (relative intensity in %): 44 (16.7), 50 (52.1), 74 (19.8), 75 (16.7), 77 (100), 78 (10.4), 85 (17.7), 103 (32.3), 104 ([illegible]), 147 ([illegible]), [illegible] ([illegible]), 217 (0.3, M − 28), 245 (0.1, M^+). Interpretation: phthalimide: 147 (+H), 103, 104, 74 - 78, 50.

Bromofluorination of methyl 5-hexenoate

In the same manner as described for methyl 4-pentenoate, this reaction was carried out using N-bromoacetamide (16.2 g), hydrogen fluoride (15.0 g), chloroform (100 ml) and methyl 5-hexenoate (14.8 g). Fraction 70-110°/0.5 Torr was redistilled and gave 13.1 g (50%) of methyl 6-bromo-5-fluorohexanoate (II), b.p. 110-120°/15 Torr. A purified sample had b.p. 115--116°/15 Torr. Analysis: Found: C, 36.9; H, 5.2; Br, 35.1; F, 8.3%. $C_7H_{12}BrFO_2$ requires C, 37.0; H, 5.3; Br, 35.2; F, 8.4%. PMR spectrum (in $CDCl_3$): 1.76 mt (4H), 2.34 t (J = = 6 Hz, 2H), 3.47 dd (J_{HH} = 5 Hz, J_{HF} = 19 Hz, 2H, CH_2Br), 3.67 s (3H, $COOCH_3$), 4.65 dmt (J_{HF} = 48 Hz, 1H, $CH_2BrC\underline{H}FCH_2$).

Preparation of methyl 5-fluoro-6-phthalimidohexanoate (IV)

Ester (II) (5.79 g), potassium phthalimide (4.82 g) and DMF (20 ml) were stirred at 100° for 1 h and the solvent evaporated at 0.1 Torr. The residue was thoroughly triturated with water (30 ml), yielding 7.2 g (95%) of slightly brownish (IV), m.p. 50-65°. Recrystallization from methanol raised the m.p. to 68-70°; however, the crude product could be used in the next step without purification. Analysis: Found: C, 61.5; H, 5.2; F, 6.2; N, 4.5%. $C_{15}H_{16}FNO_4$ requires C, 61.4; H, 5.5; F, 6.5; N, 4.8%.

Hydrolysis of ester (IV)

The crude ester (7.1 g) was shaken with concentrated hydrochloric acid (40 ml) for 20 h, the precipitate of 5-fluoro-6--phthalimidohexanoic acid (Ib) washed with cold water and

dried in a dessicator. Yield, 5.8 g (86%), m.p. 120-130° (water). Analysis: Found: C, 60.4; H, 5.0; F, 6.6; N, 5.2%. $C_{14}H_{14}FNO_4$ requires C, 60.2; H, 5.1; F, 6.8; N, 5.0%.

Preparation of methyl 2-bromo-5-fluoro-6-phthalimido-hexanoate (Vb)

Acid (Ib) (5.0 g) was slowly added to thionyl chloride (30 ml), containing pyridine (4 drops) and refluxed for 1 h. After cooling, red phosphorus (100 mg), iodine (25 mg) and bromine (1.0 ml) were successively added and refluxing was continued for 16 h. Thionyl chloride was perfectly evaporated, then methanol (20 ml) was dropped in and refluxing repeated for 1 h. Evaporation afforded a semisolid mass, which was dissolved in ethyl acetate (50 ml), washed with sodium bisulfite, sodium bicarbonate, water and dried. The solvent was removed and the crude product crystallized from methanol (15 ml). Yield, 4.2 g (63%), m.p. 62-75°. A recrystallized sample had m.p. 68-75°. Analysis: Found: C, 48.7; H, 4.1; Br, 21.6; F, 5.2; N, 3.9%. $C_{15}H_{15}BrFNO_4$ requires C, 48.4; H, 4.1; Br, 21.5; F, 5.1; N, 3.8%.

Preparation of (impure) methyl 2,6-diphthalimido-5-fluoro-hexanoate (VIb)

Ester (Vb) (1.86 g), potassium phthalimide (0.92 g) and DMF (17 ml) were stirred at laboratory temperature for 4 days and the neutral mixture evaporated at 0.1 Torr. The residue was taken into ethyl acetate, washed with water, dried and the solvent removed, leaving 2.25 g of a yellow, semisolid mass, which was not further purified.

Deblocking of the ester (VIb)

The reaction was carried out in the same manner as in the case of ester (VIa). Crude (VIb) (1.21 g) gave 0.31 g (56%) of 5-fluorolysine monohydrochloride (VIIb), m.p. 218-220°. A sample, recrystallized from aqueous ethanol, had m.p. 237-240°.

Analysis: Found: C, 35.7; H, 7.0; Cl, 17.8; F, 9.4; N, 14.0%. $C_6H_{14}ClFN_2O_2$ requires C, 35.9; H, 7.0; Cl, 17.7; F, 9.5; N, 14.0%. R_{LYS} values: 0.74 (system A); 0.95 (system B).

Preparation of 4-fluorocitrulline (VIII)

The suspension of basic cupric carbonate (450 mg) in a solution of (VIIa) (187 mg) in water (2 ml) was stirred overnight, filtered and the precipitate thoroughly washed with water. TLC (Silufol®, 60% dioxane in water) showed complete conversion of the amino acid into the complex. The solution was lyophylized, the complex redissolved in water (2 ml) and potassium cyanate (170 mg) was added. After 1 week at laboratory temperature, the copper complex of (VIII) was filtered, washed and dried; yield, 181 mg (80%). Copper was removed by saturating the suspension of the complex in water (5 ml) with hydrogen sulfide and the filtrate lyophylized, giving 121 mg (63%) of chromatographically pure (VIII), m.p. 230-240° (aqueous ethanol). Analysis: Found: C, 37.4; H, 6.2; F, 10.0; N, 21.6%. $C_6H_{12}FN_3O_3$ requires C, 37.3; H, 6.3; F, 9.9; N, 21.8%. R_{CIT} values: 0.88 (system A); 0.85 (system B).

Preparation of 4-fluoroarginine (IX)

(a) The diflavianate of (IX)

The complex, prepared as above from (VIIa) (561 mg) and cupric carbonate (1.0 g) in water (10 ml), was dissolved in water (7 ml) and O-methylisourea hydrochloride (636 mg) was added at 0°, followed by 1 mol l^{-1} sodium hydroxide (6 ml). After 1 week at laboratory temperature, the solution was acidified to p_H 0 by hydrochloric acid, then saturated with hydrogen sulfide and the copper-free filtrate was lyophylized to about 5 ml volume. Flavianic acid (2.2 g) in water (10 ml) was added and the whole chilled. The 4-fluoroarginine diflavianate amounted 2.37 g (96%). A sample (50 mg) was dissolved in water (1 ml) containing 28% ammonia (1 drop), acidified with hydrochloric acid to p_H 1 and cooled in ice for several days. 40 mg of a pure salt were recovered, m.p.

206-212° (dec.). Analysis: Found: F, 2.3; N, 13.4%. $C_{26}H_{25}FN_8O_{18}S_2$ requires F, 2.3; N, 13.7%.

(b) The monoflavianate of (IX)

The diflavianate (2.32 g) was recrystallized from boiling water (20 ml), yielding 1.11 g (77%) of 4-fluoroarginine monoflavianate, m.p. 233-236° (water). Analysis: Found: C, 38.2; H, 3.9; F, 3.5; N, 16.3%. $C_{16}H_{19}FN_6O_{10}S$ requires C, 37.9; H, 3.8; F, 3.8; N, 16.6%.

(c) The monohydrochloride of (IX)

The diflavianate (1.9 g) was suspended in water (25 ml) and 28% ammonia was carefully dropped in just to achieve complete solution of the salt. Barium hydroxide octahydrate (1.2 g) in water (30 ml) was then added, the precipitate of barium flavianate was thoroughly washed with cold water and the yellow filtrate stirred with Dowex 1 untill the yellow colour disappeared. The solution was lyophylized to about 5 ml volume, acidified with hydrochloric acid to p_H 1, shortly boiled with charcoal and lyophylized to dryness. The solid was redissolved in water (1 ml), ethanol (10 ml) added and the p_H was adjusted to 6 using pyridine. After cooling overnight, 200 mg (38%) of 4-fluoroarginine monohydrochloride was collected, m.p. 175-178° (aqueous ethanol). Analysis: Found: C, 31.2; H, 6.4; Cl, 15.4; F, 8.1; N, 24.5%. $C_6H_{14}ClFN_4O_2$ requires C, 31.5; H, 6.2; Cl, 15.5; F, 8.3; N, 24.5%. R_{ARG} values: 0.92 (system A); 0.91 (system B).

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REFERENCES

1 F. Weygand and W. Oettmeier, Usp. Khim. (Advances in Chemistry), 39 (1970) 622.
2 F.L.M. Pattison, D.A.V. Peters and F.H. Dean, Can. J. Chem., 43 (1965) 1689.
3 V. Tolman and K. Vereš, Coll. Czech. Chem. Commun., 37 (1972) 2962.
4 L.H. Smith, J. Amer. Chem. Soc., 77 (1955) 6691.
5 A.C. Kurtz, J. Biol. Chem., 180 (1949) 1253.
6 A. Hrubesch, Ger. Pat. 860,203 (1952); Chem. Abstr., 52 (1958) 16206a.
7 B.R. Baker, M.V. Querry, R. Polikoff, R.E. Schaub and J.H. Williams, J. Org. Chem., 17 (1952) 68.