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# THE SYNTHESIS OF DL- AND L-2-AMINO-3-FLUOROBUTYRIC ACID

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## SUMMARY

DL- and L-Threonine were esterified to their methyl and ethyl esters which on treatment with sulfur tetrafluoride in anhydrous hydrogen fluoride gave DL- and L-methyl and ethyl 2-amino-3-fluorobutyrate, respectively. Both fluorinated esters afforded on hydrolysis DL- and L-2-amino-3-fluorobutyric acid, respectively.

## INTRODUCTION

In our study of fluorinated amino acids as potential cancerostatics we noticed that some of the fluorinated amino acids which showed cancerostatic activity had, at the same time, a strong tendency to eliminate hydrogen fluoride. This was the case of  $\beta$ -fluoro- $\alpha$ -alanine [1],  $\beta$ -fluoromethyl- $\gamma$ -fluorovaline [2],  $\gamma$ -fluoromethyl- $\beta$ -fluoroleucine [2], and  $\omega$ -fluoro-isoleucine [3].

With this view in mind we prepared a homolog of  $\beta$ -fluoro- $\alpha$ -alanine, 2-amino-3-fluorobutyric acid in which fluorine atom should be rather labile.

Two other monofluorinated derivatives of aminobutyric acid have been prepared earlier: 2-amino-4-fluorobutyric acid [4] and 4-amino-2-fluorobutyric acid [5]. However, none of them seems to have been tested as a cancerostatic.

The most promising starting material for the synthesis of 2-amino-3-fluorobutyric acid is threonine which is available both as natural L-acid and synthetic DL-racemate.

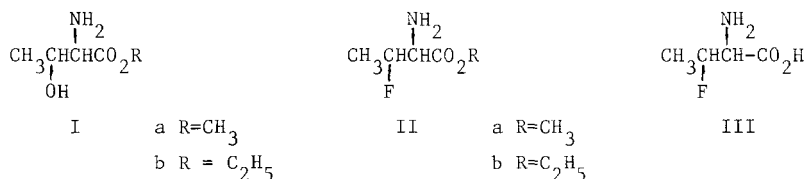
## RESULTS AND DISCUSSION

The most simple approach - treatment of threonine with a mixture of anhydrous hydrogen fluoride and pyridine which was successfully used for the replacement of hydroxyl groups by fluorine [6] - failed, and unchanged starting material was recovered.

Also, diethylaminosulfur trifluoride [7] which has been recently used for replacement of hydroxyls by fluorine proved unsuccessful when applied to methyl ester of N-acetyl threonine.

On the other hand, sulfur tetrafluoride effected the replacement of hydroxyl smoothly in the reaction of threonine methyl or ethyl esters (Ia,b) in anhydrous hydrogen fluoride at 60°. Under these conditions the amino group is protected by protonation [8], and the ester group is unreactive.

In this way methyl DL-2-amino-3-fluorobutyrate (IIa) and ethyl DL- and L-2-amino-3-fluorobutyrate (IIb) were obtained in good yields. Hydrolysis on boiling with water or hydrochloric acid afforded DL- or L-2-amino-3-fluorobutyric acid (III).



According to the NMR spectra the compound contains predominantly (80-88%) one diastereomer. Based on the results in the field of steroids [9] where replacement of hydroxyl by fluorine with retention of configuration showed in the change of the sign of optical rotation, we are tentatively assigning threo-configuration to our L-2-amino-3-fluorobutyric acid since the laevorotatory L-threonine ( $[\alpha]_D -28.5^\circ [\text{H}_2\text{O}]$ ) gave dextrorotatory L-2-amino-3-fluorobutyric acid ( $[\alpha]_D +11.5^\circ [\text{H}_2\text{O}]$ ). The erythro isomer, allothreonine, on the other hand, shows positive rotation ( $[\alpha]_D +10^\circ [\text{H}_2\text{O}]$ ).

## EXPERIMENTAL

Melting points are not corrected. Samples for analysis were dried at 0.02-0.05 mm Hg at 55°. NMR spectra were measured on a JEOL PS 100 spectrometer using TMS, HFB, and TFA as standards. Paper chromatography was carried out in the system BuOH:AcOH:H<sub>2</sub>O 4:1:5.

Preparation of methyl DL-2-amino-3-fluorobutyrate (DL-IIa)

Freshly distilled crystalline DL-threonine methyl ester, b.p. 83-85°/1.3 mm Hg, m.p. 35-36° (13.5 g, 0.10 mole) was placed in a 100 ml Teflon bottle cooled to -78°; 21 g (1.05 mole) of anhydrous hydrogen fluoride was added, the mixture was protected from moisture with a calcium chloride drying tube, and stirred at -78° overnight until a clear solution was obtained. This was added to a stainless steel autoclave cooled to -78° in which 26 g (0.24 mole) of sulfur tetrafluoride had been condensed. The autoclave was sealed and heated for 5 hours at 60°. After cooling with Dry Ice, the autoclave was opened and the excess of hydrogen fluoride, sulfur tetrafluoride, and thionyl fluoride was vented off. The residue was poured into a Teflon evaporating dish and allowed to stand for 1 day at room temperature. The pasty mass was washed with a minimum amount of water into a 100 ml flask, the solution was filtered and evaporated in vacuo to a fine dry powder. The salt was suspended in dry chloroform and neutralized with a solution of ammonia in chloroform. After filtering off ammonium fluoride and evaporating chloroform, 12.5 g (91%) of crude methyl 2-amino-3-fluoro-butyrate was obtained. Attempts to purify and distil the ester led to fluorine-free decomposition products.

$^1\text{H}$  NMR ( $\text{CHCl}_3$ ) (ppm):  $\text{CH}_3\text{CHF}$ -(3) 1.32 (dd);  $J(\text{H,H})=7$  Hz;  $J(\text{H,F})_{\text{vic}}=24$  Hz;  $-\text{CHF}$ -(1) 4.78 (d-quintet);  $J(\text{H,H})=6$  Hz;  $J(\text{H,F})_{\text{gem}}=46$  Hz;  $-\text{CH}(\text{NH}_2)$ -(1) 3.58(d)(partly covered by  $\text{CH}_3\text{O}$ );  $J(\text{H,H})=5$  Hz;  $-\text{NH}_2$ (2) 2.05(s);  $\text{CH}_3\text{O}$ -(3) 3.70(s).  $^{19}\text{F}$  NMR ( $\text{CHCl}_3$ ) (ppm): two multiplets (ratio 88:12) at -18.5 and -25.0 ppm (upfield from  $\text{C}_6\text{F}_6$  external);  $J(\text{H,F})_{\text{gem}}=49$  Hz;  $J(\text{H,F})_{\text{vic}}=24.5$  Hz.

Preparation of ethyl DL-2-amino-3-fluorobutyrate (DL-IIb)

The title compound was prepared similarly to the methyl ester in 91% yield (crude) as a thick oil, b.p. 26-27°/0.05 mm, decomposing above 50° and in v.p.c. at 200°. Elementary analysis gave no satisfactory results; however, the NMR spectrum showed only slight contamination and was identical with that of the L-ester (vide infra).

Preparation of L-ethyl-2-amino-3-fluorobutyrate (L-IIb)

The L-ester IIb was prepared in 88% yield from 18.5 g L-threonine ethyl ester (L-Ib, m.p. 56°) following the same procedure. Although distilling uniformly at 20-21°/0.05 mm and giving clear-cut NMR spectra the compound did not give a satisfactory elemental analysis.

$^1\text{H}$  NMR ( $\text{CHCl}_3$ ) (ppm):  $\beta\text{-CH}_3\text{CHF}$ -(3) (partly overlapped by  $\text{CH}_3\text{CH}_2\text{O}$ -) 1.50; (dd);  $\text{J}(\text{H,H})=7$  Hz;  $\text{J}(\text{H,F})_{\text{vic}}=18-22$  Hz;  $\beta\text{-CHF}$ -(1) 4.96 (d quintets);  $\text{J}(\text{H,H})=7$  Hz;  $\text{J}(\text{H,F})_{\text{gem}}=48$  Hz;  $\beta\text{-CH}(\text{NH}_2)$ - 3.80 (dd);  $\text{J}(\text{H,H})=6$  Hz;  $\text{J}(\text{H,F})_{\text{vic}}=14$  Hz;  $\beta\text{-NH}_2$  1.90(s);  $\beta\text{-OCH}_2\text{CH}_3$  4.34(q),  $\text{J}(\text{H,H})=7$  Hz;  $\beta\text{-OCH}_2\text{CH}_3$  1.46 ppm(t);  $\text{J}(\text{H,H})=7$  Hz.  $^{19}\text{F}$  NMR ( $\text{CHCl}_3$ ): two multiplets in the ratio of (84:16), -19.3 and -25.8 ppm upfield from  $\text{C}_6\text{F}_6$ .

Preparation of DL-2-amino-3-fluorobutyric acid (DL-III)(a) By hydrolysis of the methyl ester IIa with water

The fluoroester (DL-IIa) (12.5 g) was refluxed with a twenty fold amount of water for 30 minutes. The unhydrolyzed ester was extracted with ether, the extract was evaporated, and the residue was hydrolyzed with water once more. The combined aqueous solutions were evaporated in vacuo to a syrup which on trituration with ethanol gave a crystalline product. Decoloration with activated charcoal and two recrystallizations from aqueous ethanol afforded 0.45 g (4%) of analytically pure DL-2-amino-3-fluorobutyric acid III, m.p. 194-195° (decompn.). The compound was uniform in paper chromatography ( $R_F$  0.26-0.27 in  $\text{BuOH}:\text{AcOH}:\text{H}_2\text{O} = 4:1:5$ ) and in Moore-Stein ion exchanger analysis.

$^1\text{H}$  NMR ( $\text{CF}_3\text{CO}_2\text{H}$ ) (ppm):  $\beta\text{-CH}_3\text{CHF}$ -(3) 1.70(dd);  $\text{J}(\text{H,H})=7$  Hz;  $\text{J}(\text{H,F})_{\text{vic}}=25$  Hz;  $\beta\text{-CHF}$ -(1) 5.38(d of multiplets of unresolved fine structure);  $\text{J}(\text{H,H})=7$  Hz;  $\text{J}(\text{H,F})_{\text{gem}}=48$  Hz;  $\beta\text{-CH}(\text{NH}_2)$ -(1) 4.68(d, fine structure unresolved);  $\text{J}(\text{H,F})_{\text{vic}}=21$  Hz;  $\beta\text{-NH}_3^+$  7.65(s) (broad);  $\beta\text{-OH}$  11.44(s).  $^{19}\text{F}$  NMR ( $\text{CF}_3\text{CO}_2\text{H}$ ): a multiplet at -109 ppm upfield from  $\text{CF}_3\text{CO}_2\text{H}$ ;  $\text{J}(\text{H,F})_{\text{gem}}=47$  Hz;  $\text{J}(\text{H,F})_{\text{vic}}=23.5$  Hz.

(b) By hydrolysis of the DL-ethyl ester IIb with hydrochloric acid

Ethyl DL-2-amino-3-fluorobutyrate (28 g, 0.188 mole) was refluxed with 135 ml of 10% hydrochloric acid for 2 hours. The unreacted ester was removed by extraction with ether, and the aqueous layer was evaporated in vacuo until a salt started to crystallize. A solution of triethylamine in

chloroform was added until the aqueous solution was neutral, the chloroform layer was separated, and the aqueous solution was evaporated in vacuo at a temperature below 40° to a thick orange oil. The oily residue was boiled with 50 ml of chloroform for 10 minutes, the chloroform layer was decanted, and the washing with chloroform was repeated to remove all the triethylamine hydrochloride. After the evaporation of the residual chloroform in vacuo the oily residue was triturated with absolute ethanol to give a white solid. Boiling with activated charcoal and two crystallizations from 70-80% ethanol gave 2.86 g (12.5%) of analytically pure DL-2-amino-3-fluorobutyric acid, m.p. 194-195°.

#### Preparation of L-2-amino-3-fluorobutyric acid

Ethyl L-2-amino-3-fluorobutyrate (12.3 g, 0.0825 mole) was refluxed with 61.5 ml of 10% hydrochloric acid for 2 hours. The treatment with triethylamine in chloroform as described for the preparation of the DL-acid gave 5 g (50%) of crude L-2-amino-3-fluorobutyric acid which, after two crystallizations from dilute ethanol, afforded 3.3 g (33%) of the acid L-III, m.p. 195.5-197° (decompn.).  $R_F$  0.27-0.28 (BuOH: AcOH:H<sub>2</sub>O 4:1:5);  $[\alpha]_D + 11.5^\circ$ . Both DL- and L-2-fluoro-3-aminobutyric acids were found uniform in paper chromatography and in Moore-Stein analysis.

Analysis: Found: C, 39.45; 39.87%; H, 6.59, 6.87%; F, 15.82%; N, 11.80; 11.42%. Calculated for C<sub>4</sub>H<sub>8</sub>FN<sub>2</sub>O<sub>2</sub> (121.1): C, 39.67%; H, 6.66%; F, 15.69%; N, 11.57%.

<sup>1</sup>H NMR (CF<sub>3</sub>CO<sub>2</sub>H) (ppm):  $\alpha$ -CH<sub>3</sub>-(3) 1.75(dd); J(H,H)=7 Hz; J(H,F)<sub>vic</sub>=21 Hz;  $\alpha$ -CHF-(1) 5.40 (d, fine structure unresolved), J(H,H)=7 Hz; J(H,F)<sub>gem</sub>=47 Hz;  $\alpha$ -CH(NH<sub>2</sub>)(1) 4.68 (d, fine structure unresolved), J(H,F)<sub>vic</sub>=21 Hz; -NH<sub>3</sub><sup>+</sup>(3) 7.73(s)(broad). <sup>19</sup>F NMR (CF<sub>3</sub>CO<sub>2</sub>H) (ppm): a seven line multiplet (ratio 1:4:7:8:7:4:1) 105 ppm upfield from trifluoroacetic acid; J(H,F)<sub>vic</sub>=23.5 Hz; J(H,F)<sub>gem</sub>=47 Hz.

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